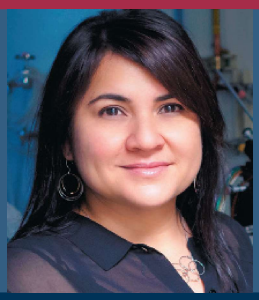
Unit 2 THE CELL



Dr. Diana Bautista is an Associate Professor of Molecular and Cell Biology at the University of California, Berkeley, and a Howard Hughes Medical Institute Faculty Scholar. Recipient of the Gill Transformative Investigator Award and the Society for Neuroscience Young Investigator Award, she received a B.S. in Biology from the University of Oregon and a Ph.D. in Neuroscience from Stanford University. Dr. Bautista grew up in Chicago, the first in her family to attend college and to pursue a career in science. She and her laboratory members investigate the cell-signaling pathways associated with pain and itch and how their misregulation can result in chronic pain and chronic itch. Her research is interdisciplinary: Dr. Bautista collaborates with computational biologists, immunologists, and physiologists. She believes that science benefits most from a diverse group of colleagues, each bringing a different perspective.

AN INTERVIEW WITH

Diana M. Bautista

What got you interested in biology, and in neuroscience in particular?

I always liked science as a kid, but it was never a big focus in my life. I went to college interested in art, then took some time off because I was a terrible artist. I ended up working with an environmental group helping low-income communities oppose a hazardous waste incinerator. That got me excited about science because it was at the interface of the environment and community empowerment, so I decided to return to college and major in biology. Once I returned to school, at the University of Oregon, I found out you could get a work-study job in a lab. I ended up working in Peter O'Day's lab studying vision in fruit flies by recording electrical signals in response to light. For me, shining light on a fly eye and recording an electrical signal from their nervous system in real time was just the coolest thing I had ever seen. The lab was a really inclusive, warm environment, and Peter was an amazing mentor—if it weren't for him, I wouldn't be in science. I went to graduate school at Stanford in neuroscience, where I worked with Dr. Richard Lewis on a newly discovered calcium channel and how calcium signaling pathways drive cellular behaviors.

▼ Dr. Bautista studies cell responses to stimuli using equipment that measures electrical signals.

Why did you focus your research on pain?

After my Ph.D., I wanted to continue working on cell signaling but from a more organismal viewpoint, so I did my postdoctoral research with Dr. David Julius at the University of California, San Francisco. His lab had recently identified the cellular protein (TRPV1) that is responsive to capsaicin—the molecule that gives chili peppers their "hotness"—and showed that this protein is also activated by painful heat. That was really exciting to me because it involved thinking about proteins involved in cell signaling, but in the bigger context of pain. Proteins like TRPV1 serve important protective functions—for example, by stopping us from grabbing a hot frying pan that could cause a burn. One of my projects was to identify the wasabi receptor, a protein on the surface of nerve cells that plays a key role in inflammatory pain hypersensitivity. Once you activate this receptor protein—with irritants such as wasabi, mustard oil, or inflammatory mediators produced in the body by tissue injury such as a burn—it activates and makes pain cells more sensitive: Warmth triggers sensations of burning heat, and light touch triggers pain. Normally, pain hypersensitivity dies down after a few days as the tissue heals, but in some patients, it becomes a chronic debilitating disease.

What does your lab work on now?

We are interested in the sense of touch. Touch-sensitive neurons innervate the skin and mediate gentle touch sensations and texture preferences: Do you like wool sweaters, or do you find them itchy? Touch-sensitive neurons also innervate our musculature, allowing us to detect limb position and mediating coordinated movements, such as our ability to text or to walk in a straight line without staring at our feet. In someone suffering from chronic pain, a gentle touch feels incredibly painful, while a chronic itch sufferer experiences light touch as itchy and pain as pleasure. When you scratch an itch, you're scratching really hard—if you didn't have an itch it would hurt, but when you do have an itch it feels good. We have no idea how our body processes the same type of stimulus under different injury or disease conditions, and that's what really excited me when I started my own lab.

What is your advice to an undergraduate considering a career in biology?

I'm an advisor for undergraduates, so I talk to a lot of students. I like to tell them about my path and let them know that it doesn't matter what you did before or where you come from—anybody can be a scientist. I think it's sometimes tough early on in biology, because at first you're taught facts, but as you progress, biology is about figuring out the unknown. And if you can get into a lab early on, then you really see that side of science—that it's about learning what we know, and then going beyond that, doing experiments to identify new mechanisms.

"It doesn't matter what you did before or where you come from—anybody can be a scientist."

6 A Tour of the Cell

KEY CONCEPTS

6.1 **Biologists use microscopes and biochemistry to study cells** *p. 94*

6.2 **Eukaryotic cells have internal membranes that compartmentalize their functions** *p. 97*

6.3 **The eukaryotic cell's genetic instructions are housed in the nucleus and carried out by the ribosomes** *p. 102*

6.4 **The endomembrane system regulates protein traffic and performs metabolic functions** *p. 104*

6.5 **Mitochondria and chloroplasts change energy from one form to another** *p. 109*

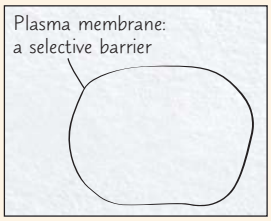
6.6 **The cytoskeleton is a network of fibers that organizes structures and activities in the cell** *p. 112*

6.7 **Extracellular components and connections between cells help coordinate cellular activities** *p. 118*

6.8 **A cell is greater than the sum of its parts** *p. 121*

Study Tip

Draw animal and plant cells: Draw an outline of an animal cell and add structures, labels, and functions. Draw a plant cell labeled with structures unique to plant cells.



Go to Mastering Biology

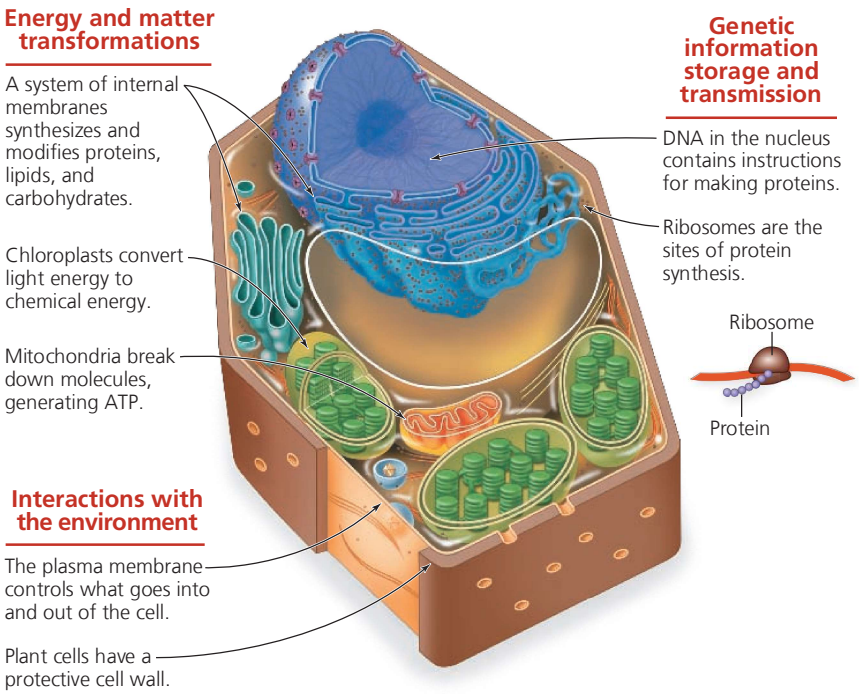
For Students (in eText and Study Area)
- Get Ready for Chapter 6
- Figure 6.7 Walkthrough: Geometric Relationships between Surface Area and Volume
- BioFlix® Animations: Tour of an Animal Cell and Tour of a Plant Cell

For Instructors to Assign (in Item Library)
- Tutorial: Tour of an Animal Cell: The Endomembrane System
- Tutorial: Tour of a Plant Cell: Structures and Functions

Figure 6.1 The cell is an organism's basic unit of structure and function. Many forms of life exist as single-celled organisms, such as the *Paramecium* shown here. Larger, more complex organisms, including plants and animals, are multicellular. In this chapter, we focus mainly on eukaryotic cells—cells with a nucleus.

How does the internal organization of eukaryotic cells allow them to perform the functions of life?

Internal membranes divide a cell, such as this plant cell, into compartments where specific chemical reactions occur.



CONCEPT 6.1

Biologists use microscopes and biochemistry to study cells

How can cell biologists investigate the inner workings of a cell, usually too small to be seen by the unaided eye? Before we tour the cell, it will be helpful to learn how cells are studied.

Microscopy

The development of instruments that extend the human senses allowed the discovery and early study of cells. Microscopes were invented in 1590 and further refined during the 1600s. Cell walls were first seen on dead cells of oak bark by Robert Hooke in 1665 and living cells by Antoni van Leeuwenhoek a few years later.

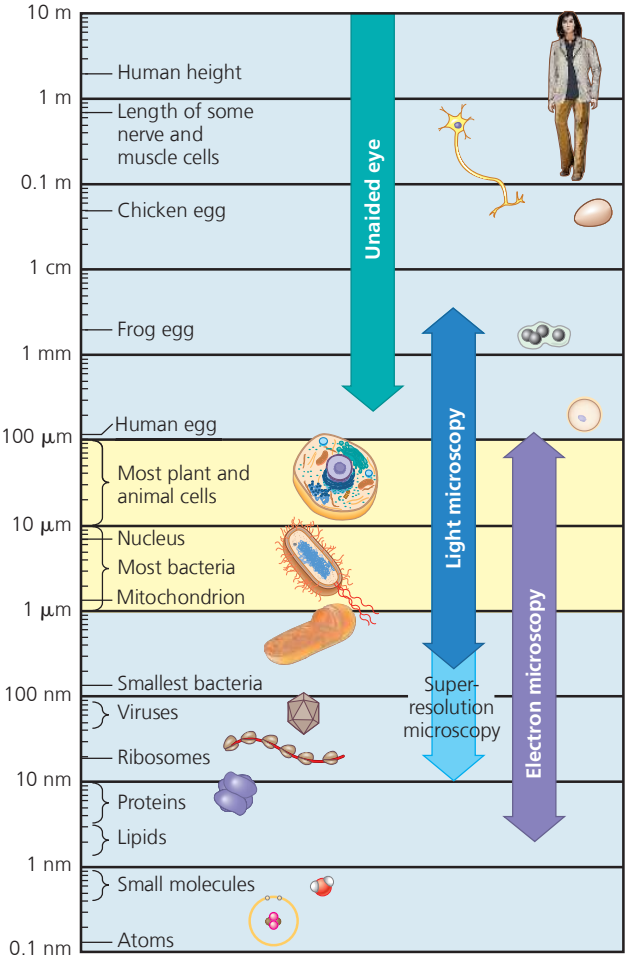
The microscopes first used by Renaissance scientists, as well as the microscopes you are likely to use in the laboratory, are all light microscopes. In a **light microscope (LM)**, visible light is passed through the specimen and then through glass lenses. The lenses refract (bend) the light in such a way that the image of the specimen is magnified as it is projected into the eye or into a camera (see Appendix C).

Three important parameters in microscopy are magnification, resolution, and contrast. *Magnification* is the ratio of an object's image size to its real size. Light microscopes can magnify effectively to about 1,000 times the actual size of the specimen; at greater magnifications, additional details cannot be seen clearly. *Resolution* is a measure of the clarity of the image; it is the minimum distance two points can be separated and still be distinguished as separate points. For example, what appears to the unaided eye as one star in the sky may be resolved as twin stars with a telescope, which has a higher resolving ability than the eye. Similarly, using standard techniques, the light microscope cannot resolve detail finer than about 0.2 micrometer (μm), or 200 nanometers (nm), regardless of the magnification **(Figure 6.2)**. The third parameter, *contrast*, is the difference in brightness between the light and dark areas of an image. Methods for enhancing contrast include staining or labeling cell components to stand out visually. **Figure 6.3** shows some different types of microscopy; study this figure as you read this section.

Until recently, the resolution barrier prevented cell biologists from using standard light microscopy when studying **organelles**, the membrane-enclosed structures within eukaryotic cells. To see these structures in any detail required the development of a new instrument. In the 1950s, the electron microscope was introduced to biology. Rather than focusing light, the **electron microscope (EM)** focuses a beam of electrons through the specimen or onto its surface (see Appendix C). Resolution is inversely related to the wavelength of the light (or electrons) a microscope uses for imaging, and electron beams have much shorter wavelengths than visible light. Modern electron microscopes can theoretically achieve a resolution of about 0.002 nm, though in practice they usually cannot resolve structures smaller than about 2 nm across. Still, this is a 100-fold improvement over the standard light microscope.

The **scanning electron microscope (SEM)** is especially useful for detailed study of the topography of a specimen (see Figure 6.3). The electron beam scans the surface of the sample, usually coated with a thin film of gold. The beam excites

▼ Figure 6.2 The size range of cells. Most cells are between 1 and 100 μm in diameter (yellow region of chart), and their components are even smaller (see Figure 6.32), as are viruses. Notice that the scale along the left side is logarithmic, to accommodate the range of sizes shown. Starting at the top of the scale with 10 m and going down, each reference measurement marks a tenfold decrease in diameter or length. For a complete table of the metric system, see the back of the book.



1 centimeter (cm) = 10^{-2} meter (m) = 0.4 inch
1 millimeter (mm) = 10^{-3} m
1 micrometer (μm) = 10^{-3} mm = 10^{-6} m
1 nanometer (nm) = 10^{-3} μm = 10^{-9} m

Mastering Biology Animation: Metric System Review

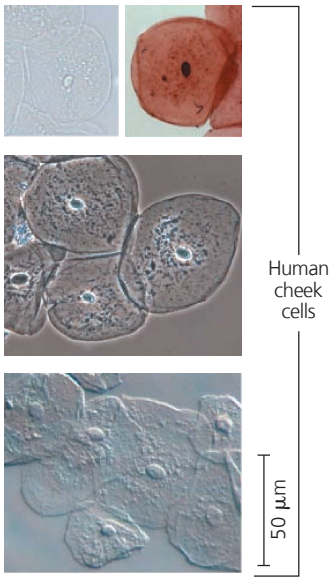
▼ Figure 6.3 Exploring Microscopy

Light Microscopy (LM)

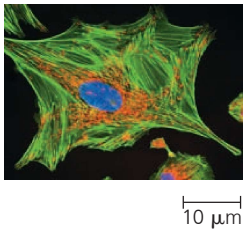
Brightfield. Light passes directly through the specimen. Unstained (left), the image has little contrast. Staining with dyes (right) enhances contrast. Most stains require cells to be preserved, which kills them.

Phase-contrast. Variations in density within the specimen are amplified to enhance contrast in unstained cells; this is especially useful for examining living, unstained cells.

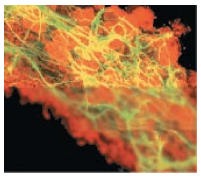
Differential interference contrast (Nomarski). As in phase-contrast microscopy, optical modifications are used to exaggerate differences in density; the image appears almost 3-D.



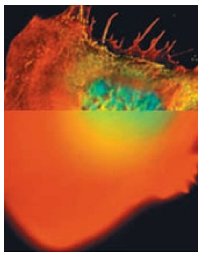
Fluorescence. Locations of specific molecules are revealed by labeling the molecules with fluorescent dyes or antibodies, which absorb ultraviolet radiation and emit visible light. In this fluorescently labeled uterine cell, DNA is blue, organelles called mitochondria are orange, and part of the cell's "skeleton" (called the *cytoskeleton*) is green.



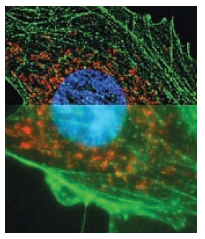
Confocal. This image shows two types of fluorescence micrographs: confocal (top) and standard (bottom). (Nerve cells are green, support cells orange, areas of overlap yellow.) In confocal microscopy, a laser is used to create a single plane of fluorescence; out-of-focus light from other planes is eliminated. By capturing sharp images at many different planes, a 3-D reconstruction can be created. A standard fluorescence micrograph is blurry because out-of-focus light is not excluded.



Deconvolution. The top of this image of a white blood cell was reconstructed from many blurry fluorescence images at different planes, each processed using deconvolution software. This process digitally removes out-of-focus light and reassigns it to its source, creating a much sharper 3-D image. The bottom is a compilation of standard fluorescent micrographs through the same cell.

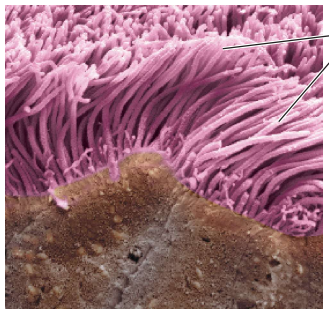


Super-resolution. To make this super-resolution image of a cow aorta cell (top), individual fluorescent molecules were excited by UV light and their position recorded. (DNA is blue, mitochondria red, and part of the cytoskeleton green.) Combining information from many molecules in different places "breaks" the resolution limit, resulting in the sharp image on top. The size of each dot is well below the 200-nm resolution of a standard light microscope, as seen in the confocal image (bottom) of the same cell.

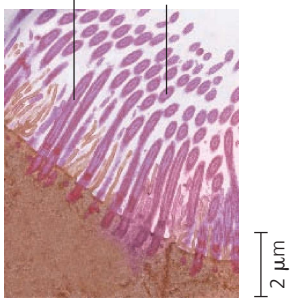


Electron Microscopy (EM)

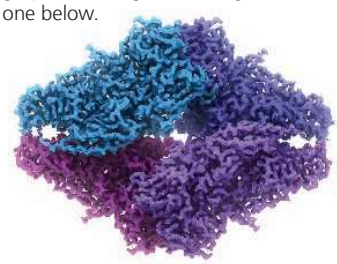
Scanning electron microscopy (SEM). Micrographs taken with a scanning electron microscope show a 3-D image of the surface of a specimen. This SEM shows the surface of a cell from a trachea (windpipe) covered with cell projections called cilia. Electron micrographs are black and white but are often artificially colorized to highlight particular structures, as has been done with all three electron micrographs shown here.



Transmission electron microscopy (TEM). A transmission electron microscope profiles a thin section of a specimen. This TEM shows a section through a tracheal cell, revealing its internal structure. In preparing the specimen, some cilia were cut along their lengths, creating longitudinal sections, while other cilia were cut straight across, creating cross sections.



Cryo-electron microscopy (cryo-EM). Specimens of tissue or aqueous solutions of proteins are frozen rapidly at temperatures less than –160°C, locking the molecules into a rigid state. A beam of electrons is passed through the sample to visualize the molecules by electron microscopy, and software is used to merge a series of such micrographs, creating a 3-D image like the one below.



Computer-generated image of the bacterial enzyme β-galactosidase, which breaks down lactose. This image was compiled from more than 90,000 cryo-EM images.

Abbreviations used in figure legends in this text:
LM = Light Micrograph
SEM = Scanning Electron Micrograph
TEM = Transmission Electron Micrograph

VISUAL SKILLS *When the tissue was sliced, what was the orientation of the cilia in the lower portion of the TEM? The upper portion? Explain how the orientation of the cilia determined the type of sections we see.*

electrons on the surface, and these secondary electrons are detected by a device that translates the pattern of electrons into an electronic signal sent to a video screen. The result is an image of the specimen's surface that appears three-dimensional.

The **transmission electron microscope (TEM)** is used to study the internal structure of cells (see Figure 6.3). The TEM aims an electron beam through a very thin section of the specimen, much as a light microscope aims light through a sample on a slide. For the TEM, the specimen has been stained with atoms of heavy metals, which attach to certain cellular structures, thus enhancing the electron density of some parts of the cell more than others. The electrons passing through the specimen are scattered more in the denser regions, so fewer are transmitted. The image displays the pattern of transmitted electrons. Instead of using glass lenses, both the SEM and TEM use electromagnets as lenses to bend the paths of the electrons, ultimately focusing the image onto a monitor for viewing.

Electron microscopes have revealed many subcellular structures that were impossible to resolve with the light microscope. But the light microscope offers advantages, especially in studying living cells. A disadvantage of electron microscopy is that the methods customarily used to prepare the specimen kill the cells and can introduce artifacts, structural features seen in micrographs that do not exist in the living cell.

In the past several decades, light microscopy has been revitalized by major technical advances (see Figure 6.3). Labeling individual cellular molecules or structures with fluorescent markers has made it possible to see such structures with increasing detail. In addition, both confocal and deconvolution microscopy have produced sharper images of three-dimensional tissues and cells. Finally, a group of new techniques and labeling molecules developed in recent years, called *super-resolution microscopy*, has allowed researchers to "break" the resolution barrier and distinguish subcellular structures as small as 10–20 nm across.

A recently developed new type of TEM called cryo-electron microscopy (cryo-EM) (see Figure 6.3) allows specimens to be preserved at extremely low temperatures. This avoids the use of preservatives, allowing visualization of structures in their cellular environment. This method is increasingly used to complement X-ray crystallography in revealing protein complexes and subcellular structures like ribosomes, described later. Cryo-EM has even been used to resolve some individual proteins. The Nobel Prize for Chemistry was awarded in 2017 to the developers of this valuable technique.

Microscopes are the most important tools of *cytology*, the study of cell structure. Understanding the function of each structure, however, required the integration of cytology and *biochemistry*, the study of the chemical processes (metabolism) of cells.

Cell Fractionation

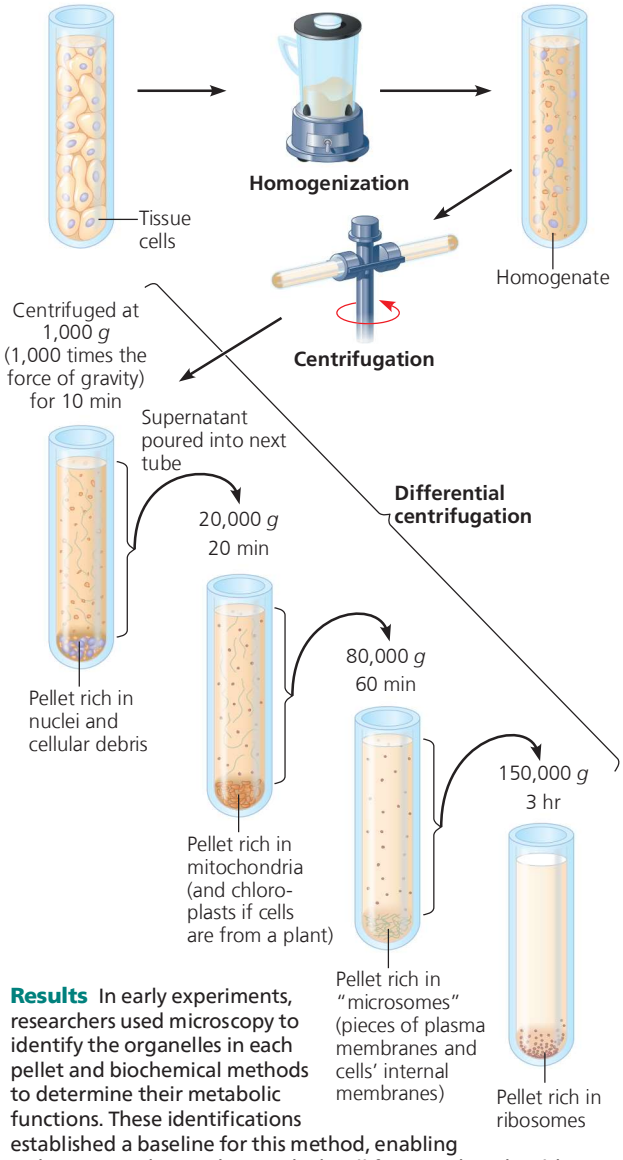
A useful technique for studying cell structure and function is **cell fractionation** (Figure 6.4), which takes cells apart

▼ Figure 6.4 Research Method

Cell Fractionation

Application Cell fractionation is used to separate (fractionate) cell components based on size and density.

Technique Cells are homogenized in a blender to break them up. The resulting mixture (*homogenate*) is centrifuged. The liquid above the pellet (*supernatant*) is poured into another tube and centrifuged at a higher speed for a longer period. This process is repeated several times. This process, called *differential centrifugation*, results in a series of pellets, each containing different cell components.



Results In early experiments, researchers used microscopy to identify the organelles in each pellet and biochemical methods to determine their metabolic functions. These identifications established a baseline for this method, enabling today's researchers to know which cell fraction they should collect in order to isolate and study particular organelles.

MAKE CONNECTIONS *If you wanted to study the process of translation of proteins from mRNA, which part of which fraction would you use? (See Figure 5.22.)*

and separates major organelles and other subcellular structures from one another. The piece of equipment that is used for this task is the centrifuge, which spins test tubes holding mixtures of disrupted cells at a series of increasing speeds, a process called *differential centrifugation*. At each speed, the resulting force causes a subset of the cell components to settle to the bottom of the tube, forming a pellet. At lower speeds, the pellet consists of larger components, and higher speeds result in a pellet with smaller components.

Cell fractionation enables researchers to prepare specific cell components in bulk and identify their functions, a task not usually possible with intact cells. For example, on one of the cell fractions, biochemical tests showed the presence of enzymes involved in cellular respiration, while electron microscopy revealed large numbers of the organelles called mitochondria. Together, these data helped biologists determine that mitochondria are the sites of cellular respiration. Biochemistry and cytology thus complement each other in correlating cell function with structure.

CONCEPT CHECK 6.1

1. How do stains used for light microscopy compare with those used for electron microscopy?
2. **WHAT IF?** Which type of microscope would you use to study (a) the changes in shape of a living white blood cell? (b) the details of surface texture of a hair?

For suggested answers, see Appendix A.

CONCEPT 6.2

Eukaryotic cells have internal membranes that compartmentalize their functions

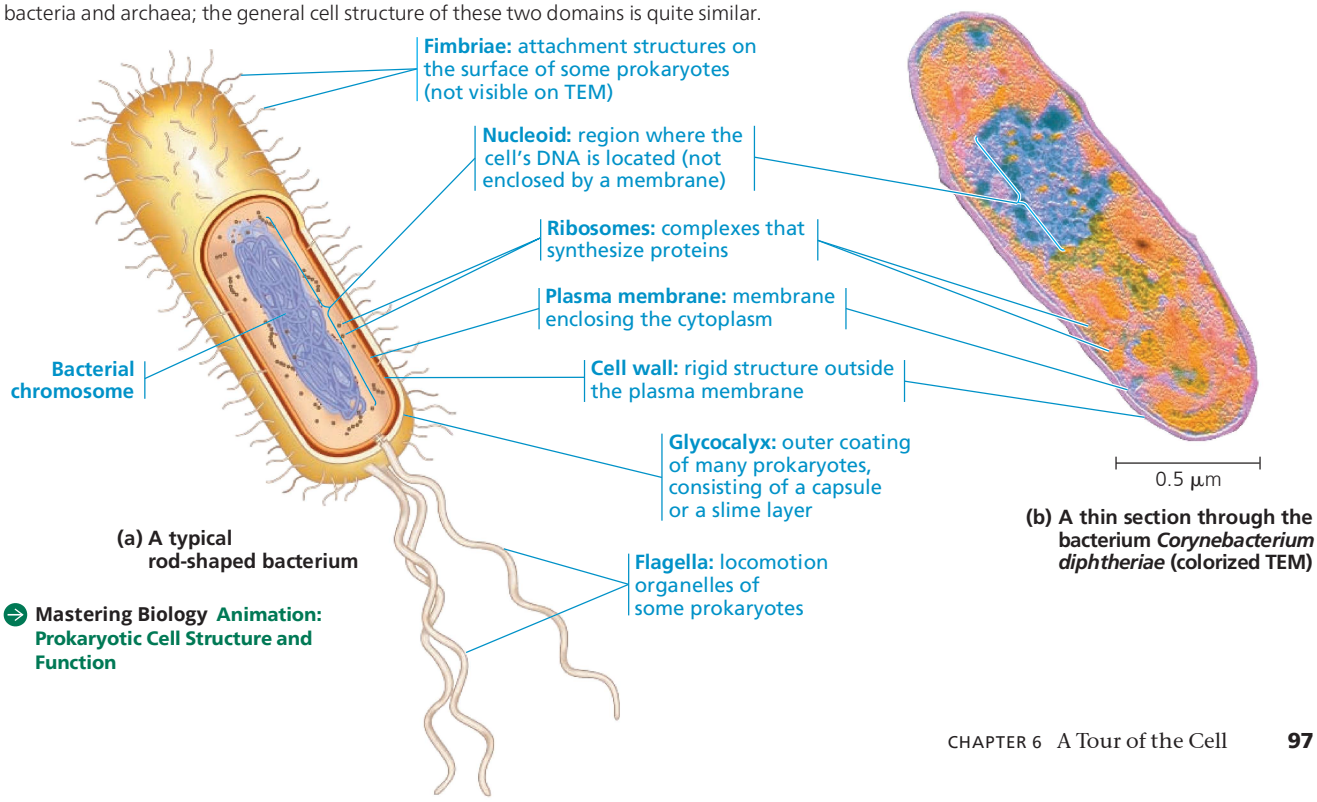
Cells—the basic structural and functional units of every organism—are of two distinct types: prokaryotic and eukaryotic. Organisms of the domains Bacteria and Archaea consist of prokaryotic cells. Organisms of the domain Eukarya—protists, fungi, animals, and plants—all consist of eukaryotic cells. ("Protist" is an informal term referring to a diverse group of mostly unicellular eukaryotes.)

Comparing Prokaryotic and Eukaryotic Cells

All cells share certain basic features: They are all bounded by a selective barrier, called the *plasma membrane* (or the cell membrane). Inside all cells is a semifluid, jellylike substance called **cytosol**, in which subcellular components are suspended. All cells contain *chromosomes*, which carry genes in the form of DNA. And all cells have *ribosomes*, tiny complexes that make proteins according to instructions from the genes.

A major difference between prokaryotic and eukaryotic cells is the location of their DNA. In a **eukaryotic cell**, most of the DNA is in an organelle called the *nucleus*, which is bounded by a double membrane (see Figure 6.8). In a **prokaryotic cell**, the DNA is concentrated in a region that is not membrane-enclosed, called the **nucleoid (Figure 6.5)**.

▼ Figure 6.5 A prokaryotic cell. Lacking a true nucleus and the other membrane-enclosed organelles of the eukaryotic cell, the prokaryotic cell appears much simpler in internal structure. Prokaryotes include bacteria and archaea; the general cell structure of these two domains is quite similar.



(a) A typical rod-shaped bacterium

(b) A thin section through the bacterium *Corynebacterium diphtheriae* (colorized TEM)

Mastering Biology **Animation: Prokaryotic Cell Structure and Function**

Eukaryotic means "true nucleus" (from the Greek *eu*, true, and *karyon*, kernel, referring to the nucleus), and *prokaryotic* means "before nucleus" (from the Greek *pro*, before), reflecting the earlier evolution of prokaryotic cells.

The interior of either type of cell is called the **cytoplasm**; in eukaryotic cells, this term refers only to the region between the nucleus and the plasma membrane. Within the cytoplasm of a eukaryotic cell, suspended in cytosol, are a variety of organelles of specialized form and function. These membrane-bounded structures are absent in almost all prokaryotic cells, another distinction between prokaryotic and eukaryotic cells. In spite of the absence of organelles, though, the prokaryotic cytoplasm is not a formless soup. For example, some prokaryotes contain regions surrounded by proteins (not membranes), within which specific reactions take place.

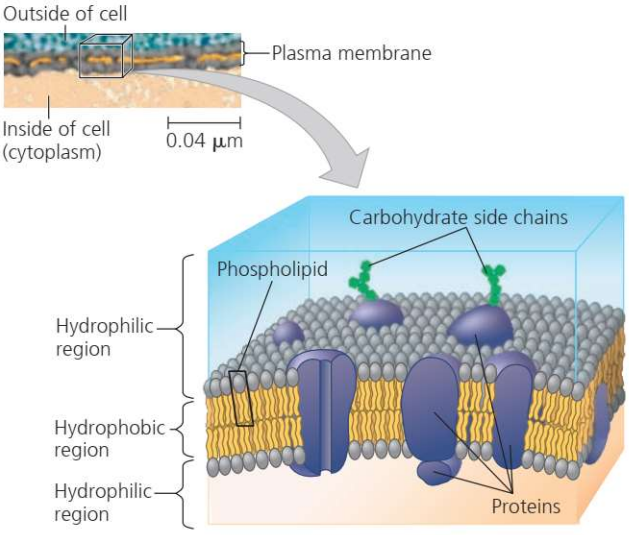
Eukaryotic cells are generally much larger than prokaryotic cells (see Figure 6.2). Size is a general feature of cell structure that relates to function. The logistics of carrying out cellular metabolism sets limits on cell size. At the lower limit, the smallest cells known are bacteria called mycoplasmas, which have diameters between 0.1 and 1.0 µm. These are perhaps the smallest packages with enough DNA to program metabolism and enough enzymes and other cellular equipment to carry out the activities necessary for a cell to sustain itself and reproduce. Typical bacteria are 1–5 µm in diameter, about ten times the size of mycoplasmas. Eukaryotic cells are typically 10–100 µm in diameter.

Metabolic requirements also impose theoretical upper limits on the size that is practical for a single cell. At the boundary of every cell, the **plasma membrane** functions as a selective barrier that allows passage of enough oxygen, nutrients, and wastes to service the entire cell **(Figure 6.6)**. For each square micrometer of membrane, only a limited amount of a particular substance can cross per second, so the ratio of surface area to volume is critical. As a cell (or any other object) increases in size, its surface area grows proportionately less than its volume. (Area is proportional to a linear dimension squared, whereas volume is proportional to the linear dimension cubed.) Thus, a smaller cell has a greater ratio of surface area to volume: Compare the calculations for the first two "cells" in **Figure 6.7**. The **Scientific Skills Exercise** gives you a chance to calculate the volumes and surface areas of two actual cells—a mature yeast cell and a cell budding from it. To explore different ways that the surface area of cells is maximized in various organisms, see Make Connections Figure 33.8.

The need for a surface area large enough to accommodate the volume helps explain the microscopic size of most cells and the narrow, elongated shapes of some cells, such as nerve cells. Larger organisms do not generally have *larger* cells than smaller organisms—they simply have *more* cells (see the far right of Figure 6.7). A sufficiently high ratio of surface area to volume is especially important in cells that exchange a lot of material with their surroundings, such as intestinal cells. Such cells may have many long, thin projections from their

▼ Figure 6.6 The plasma membrane. The plasma membrane and the membranes of organelles consist of a double layer (bilayer) of phospholipids with various proteins attached to or embedded in it. The hydrophobic parts of phospholipids and membrane proteins are found in the interior of the membrane, while the hydrophilic parts are in contact with aqueous solutions on either side. Carbohydrate side chains may be attached to proteins or lipids on the outer surface of the plasma membrane.

(a) Colorized TEM of a plasma membrane. The plasma membrane appears as a pair of dark bands separated by a gold band.

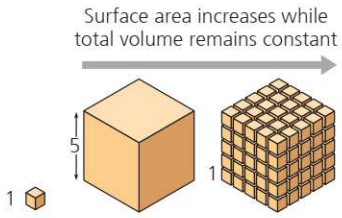


(b) Structure of the plasma membrane

VISUAL SKILLS *What parts of the membrane diagram in (b) correspond to the dark bands in the TEM in (a)? What parts correspond to the gold band? (Review Figure 5.11.)*

Mastering Biology BioFlix® Animation: Membranes

▼ Figure 6.7 Geometric relationships between surface area and volume. In this diagram, cells are represented as cubes. Using arbitrary units of length, we can calculate the cell's surface area (in square units, or units^2), volume (in cubic units, or units^3), and ratio of surface area to volume. A high surface area-to-volume ratio facilitates the exchange of materials between a cell and its environment.



Total surface area [(height × width of 1 side) × 6 sides × number of cells]	6 units^2	150 units^2	750 units^2
Total volume [(height × width × length of 1 cell) × number of cells]	1 unit^3	125 units^3	125 units^3
Surface area-to-volume ratio [surface area ÷ volume]	6	1.2	6

Mastering Biology Figure Walkthrough

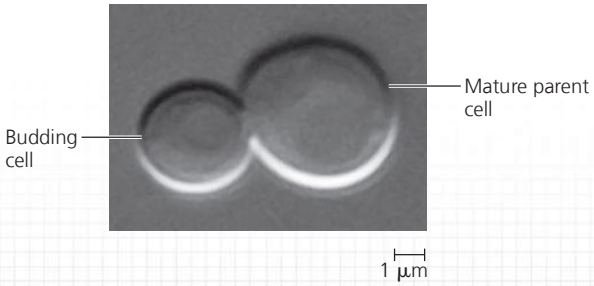
Scientific Skills Exercise

Using a Scale Bar to Calculate Volume and Surface Area of a Cell

How Much New Cytoplasm and Plasma Membrane Are Made by a Growing Yeast Cell? The unicellular yeast *Saccharomyces cerevisiae* divides by budding off a small new cell that then grows to full size (see the yeast cells at the bottom of Figure 6.8). During its growth, the new cell synthesizes new cytoplasm, which increases its volume, and new plasma membrane, which increases its surface area. In this exercise, you will use a scale bar to determine the sizes of a mature parent yeast cell and a cell budding from it. You will then calculate the volume and surface area of each cell. You will use your calculations to determine how much cytoplasm and plasma membrane the new cell needs to synthesize to grow to full size.

How the Experiment Was Done Yeast cells were grown under conditions that promoted division by budding. The cells were then viewed with a differential interference contrast light microscope and photographed.

Data from the Experiment This light micrograph shows a budding yeast cell about to be released from the mature parent cell:

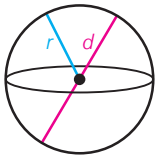


Micrograph from K. Tatchell, using yeast cells grown for experiments described in L. Kozubowski et al., Role of the septin ring in the asymmetric localization of proteins at the mother-bud neck in *Saccharomyces cerevisiae*, *Molecular Biology of the Cell* 16:3455–3466 (2005).

INTERPRET THE DATA

1. Examine the micrograph of the yeast cells. The scale bar under the photo is labeled 1 μm. The scale bar works in the same way as a scale on a map, where, for example, 1 inch equals 1 mile. In this case the bar represents one thousandth of a millimeter. Using the scale bar as a basic unit, determine the diameter of the mature parent cell and the new cell. Start by measuring the scale bar and the diameter of each cell. The units you use are irrelevant, but working in millimeters is convenient. Divide each diameter by the length of the scale bar and then multiply by the scale bar's length value to give you the diameter in micrometers.
2. The shape of a yeast cell can be approximated by a sphere. **(a)** Calculate the volume of each cell using the formula for the volume of a sphere:

$$V = \frac{4}{3}\pi r^3$$



Note that π (the Greek letter pi) is a constant with an approximate value of 3.14, *d* stands for diameter, and *r* stands for radius, which is half the diameter. **(b)** What volume of new cytoplasm will the new cell have to synthesize as it matures? To determine this, calculate the difference between the volume of the full-sized cell and the volume of the new cell.

3. As the new cell grows, its plasma membrane needs to expand to contain the increased volume of the cell. **(a)** Calculate the surface area of each cell using the formula for the surface area of a sphere: $A = 4\pi r^2$. **(b)** How much area of new plasma membrane will the new cell have to synthesize as it matures?
4. When the new cell matures, it will be approximately how many times greater in volume and how many times greater in surface area than its current size?

Instructors: A version of this Scientific Skills Exercise can be assigned in **Mastering Biology**.

surface called *microvilli*, which increase surface area without an appreciable increase in volume.

The evolutionary relationships between prokaryotic and eukaryotic cells will be discussed later in this chapter, and prokaryotic cells will be described in detail elsewhere (see Chapter 27). Most of the discussion of cell structure that follows in this chapter applies to eukaryotic cells.

A Panoramic View of the Eukaryotic Cell

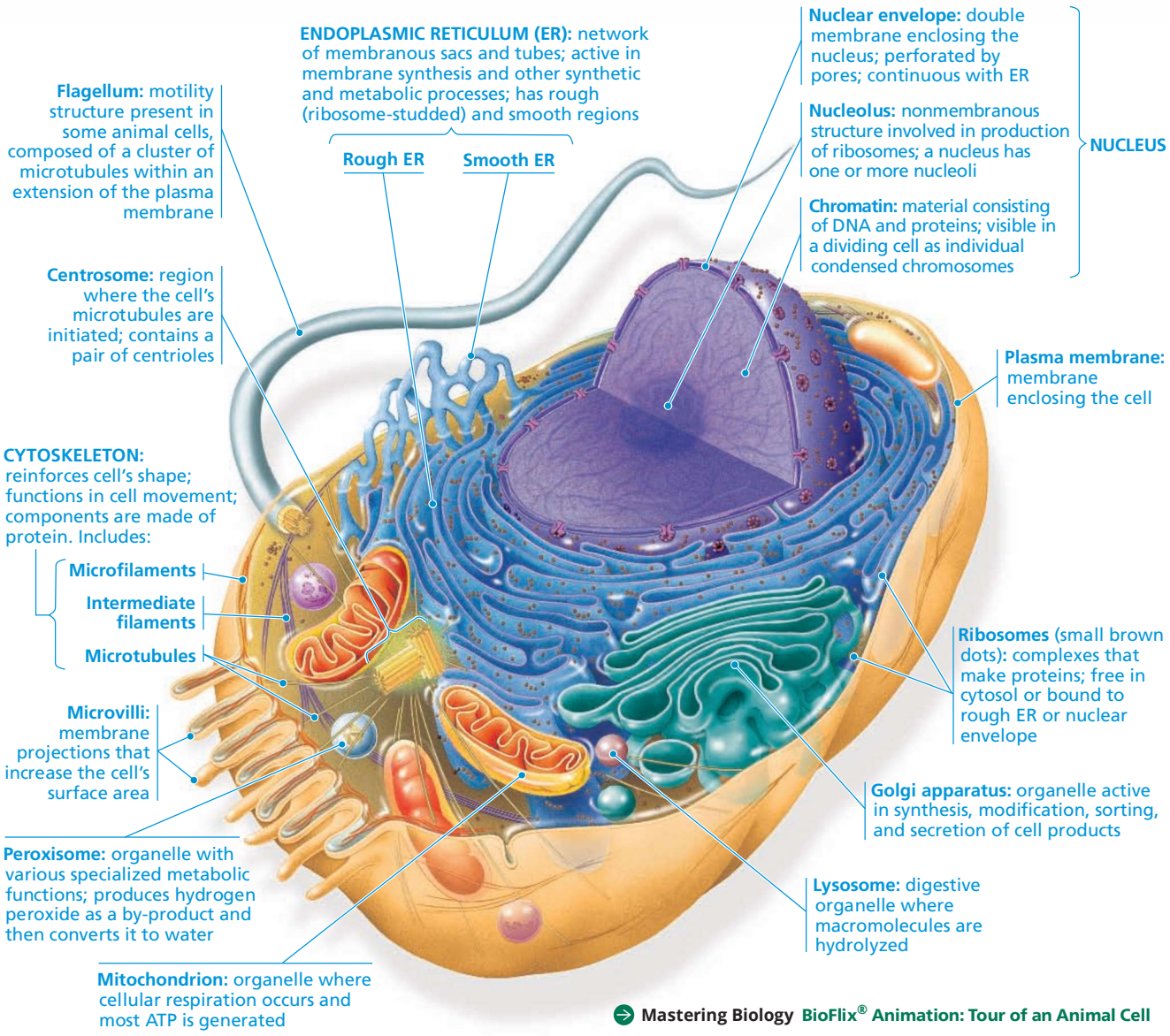
In addition to the plasma membrane at its outer surface, a eukaryotic cell has extensive, elaborately arranged internal membranes that divide the cell into compartments—the organelles mentioned earlier. The cell's compartments provide different local environments that support specific metabolic functions, so incompatible processes can occur simultaneously in a single cell. The plasma membrane and organelle membranes also participate directly in the cell's metabolism because many enzymes are built right into the membranes.

The basic fabric of most biological membranes is a double layer of phospholipids and other lipids. Embedded in this lipid bilayer or attached to its surfaces are diverse proteins (see Figure 6.6). However, each type of membrane has a unique composition of lipids and proteins suited to that membrane's specific functions. For example, enzymes embedded in the membranes of the organelles called mitochondria function in cellular respiration. Because membranes are so fundamental to the organization of the cell, Chapter 7 will discuss them in detail.

Before continuing with this chapter, examine the eukaryotic cells in **Figure 6.8**. The generalized diagrams of an animal cell and a plant cell introduce the various organelles and show the key differences between animal and plant cells. The micrographs at the bottom of the figure give you a glimpse of cells from different types of eukaryotic organisms.

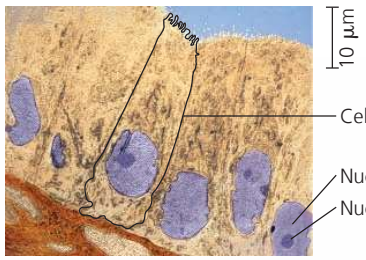
▼ Figure 6.8 Exploring Eukaryotic Cells

Animal Cell (cutaway view of generalized cell)



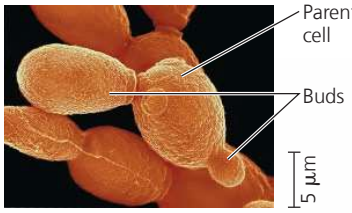
Mastering Biology **BioFlix® Animation: Tour of an Animal Cell**

Animal Cells

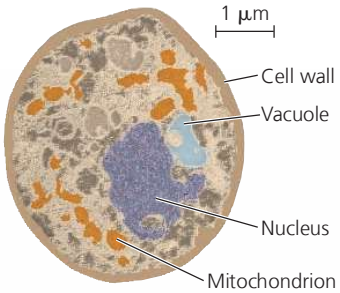


Human cells from lining of uterus (colorized TEM)

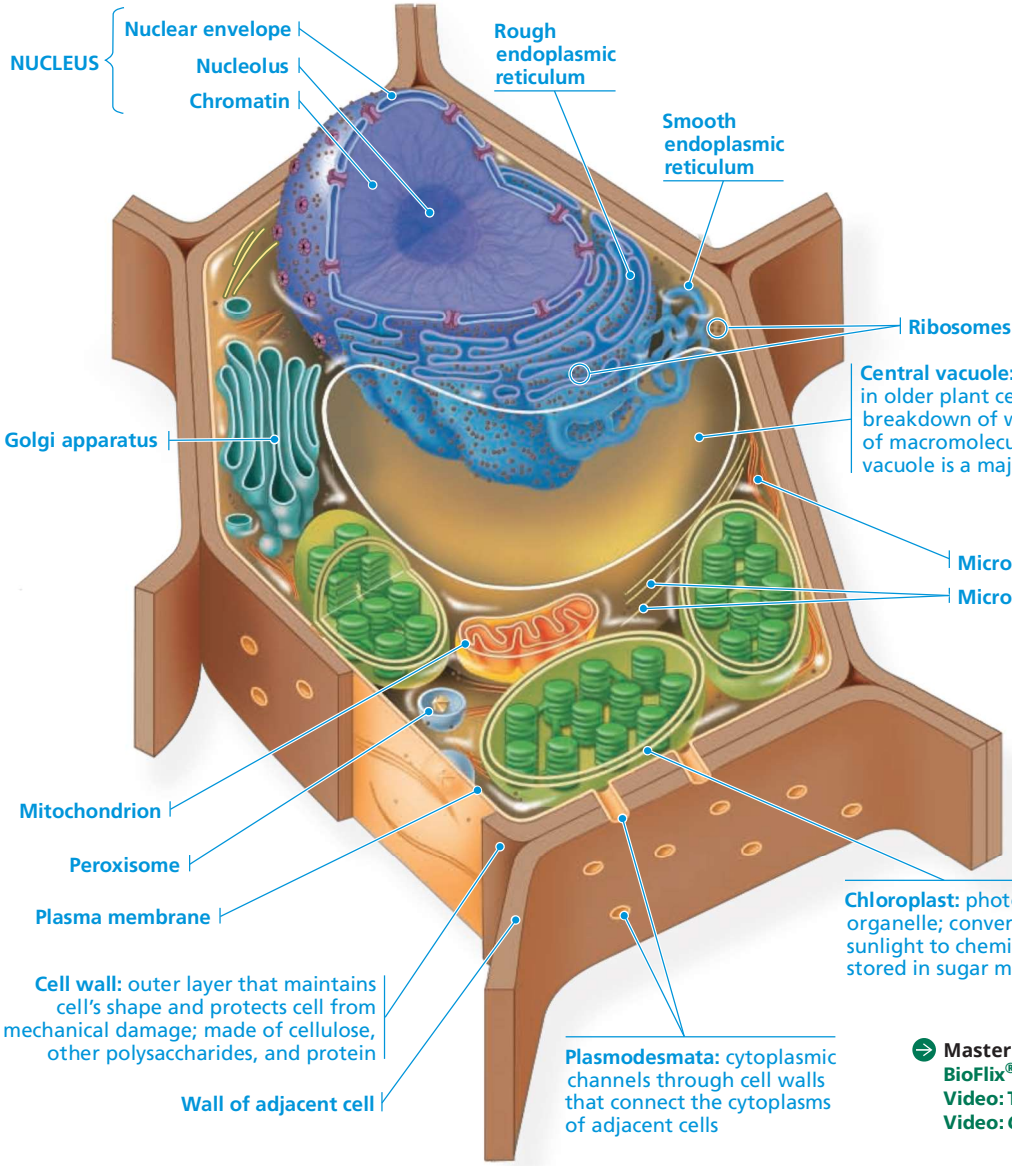
Unicellular Fungi



Yeast cells: reproducing by budding (above, colorized SEM) and a single cell (right, colorized TEM)

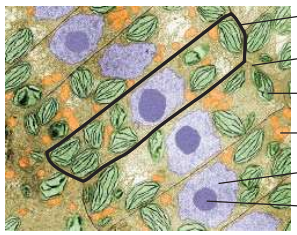


Plant Cell (cutaway view of generalized cell)



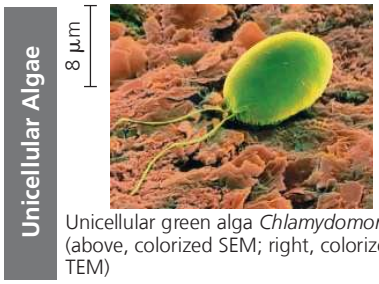
Mastering Biology
BioFlix® Animation: Tour of a Plant Cell
Video: Turgid *Elodea*
Video: *Chlamydomonas*

Plant Cells



Cells from duckweed (*Spirodela oligorrhiza*), a floating plant (colorized TEM)

Unicellular Algae



Unicellular green alga *Chlamydomonas* (above, colorized SEM; right, colorized TEM)

CONCEPT CHECK 6.2

1. Briefly describe the structure and function of the nucleus, the mitochondrion, the chloroplast, and the endoplasmic reticulum.
2. **DRAW IT** Draw a simplified elongated cell that measures $125 \times 1 \times 1$ arbitrary units. A nerve cell would be roughly this shape. Predict how its surface-to-volume ratio would compare with those in Figure 6.7. Then calculate the ratio and check your prediction.

For suggested answers, see Appendix A.

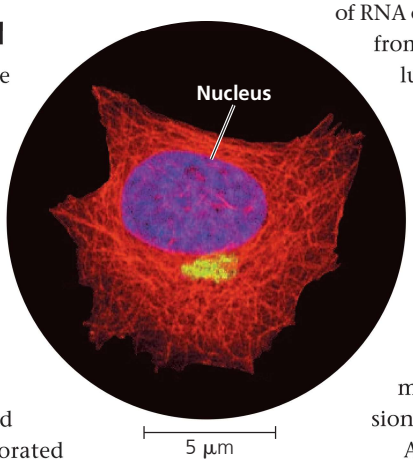
CONCEPT 6.3

The eukaryotic cell's genetic instructions are housed in the nucleus and carried out by the ribosomes

On the first stop of our detailed tour of the eukaryotic cell, let's look at two cellular components involved in the genetic control of the cell: the nucleus, which houses most of the cell's DNA, and the ribosomes, which use information from the DNA to make proteins.

The Nucleus: Information Central

The **nucleus** contains most of the genes in the eukaryotic cell. (Some genes are located in mitochondria and chloroplasts.) It is usually the most conspicuous organelle (see the purple structure in the fluorescence micrograph), averaging about 5 µm in diameter. The **nuclear envelope** encloses the nucleus **(Figure 6.9)**, separating its contents from the cytoplasm.



The nuclear envelope is a *double* membrane. The two membranes, each a lipid bilayer with associated proteins, are separated by a space of 20–40 nm. The envelope is perforated by pore structures that are about 100 nm in diameter. At the lip of each pore, the inner and outer membranes of the nuclear envelope are continuous. An intricate protein structure called a *pore complex* lines each pore and plays an important role in the cell by regulating the entry and exit of proteins and RNAs, as well as large complexes of macromolecules. Except at the pores, the nuclear side of the envelope is lined by the **nuclear lamina**, a netlike array of protein filaments (in animal cells, called *intermediate filaments*) that maintains the shape of the nucleus by mechanically supporting the nuclear envelope. There is also much evidence for a *nuclear matrix*, a framework of protein fibers extending throughout the nuclear interior. The nuclear lamina and matrix may help organize the genetic material so it functions efficiently.

Within the nucleus, the DNA is organized into discrete units called **chromosomes**, structures that carry the genetic information. Each chromosome contains one long DNA molecule associated with many proteins, including small basic proteins called histones. Some of the proteins help coil the DNA molecule of each chromosome, reducing its length and allowing it to fit into the nucleus. The complex of DNA and proteins making up chromosomes is called **chromatin**. When a cell is not dividing, stained chromatin appears as a diffuse mass in micrographs, and the chromosomes cannot be distinguished from one another, even though discrete chromosomes are present. As a cell prepares to divide, however, the chromosomes form loops and coil, condensing and becoming thick enough to be distinguished under a microscope as separate structures (see Figure 16.23). Each eukaryotic species has a characteristic number of chromosomes. For example, a typical human cell has 46 chromosomes in its nucleus; the exceptions are human sex cells (eggs and sperm), which have only 23 chromosomes. A fruit fly cell has 8 chromosomes in most cells and 4 in the sex cells.

A prominent structure within the nondividing nucleus is the **nucleolus** (plural, *nucleoli*), which appears through the electron microscope as a mass of densely stained granules and fibers adjoining part of the chromatin. Here a type of RNA called *ribosomal RNA* (rRNA) is synthesized from genes in the DNA. Also in the nucleolus, proteins imported from the cytoplasm are assembled with rRNA into large and small subunits of ribosomes. These subunits then exit the nucleus through the nuclear pores to the cytoplasm, where a large and a small subunit can assemble into a ribosome. Sometimes there are two or more nucleoli; the number depends on the species and the stage in the cell's reproductive cycle. The nucleoli may also play a role in controlling cell division and the life span of a cell.

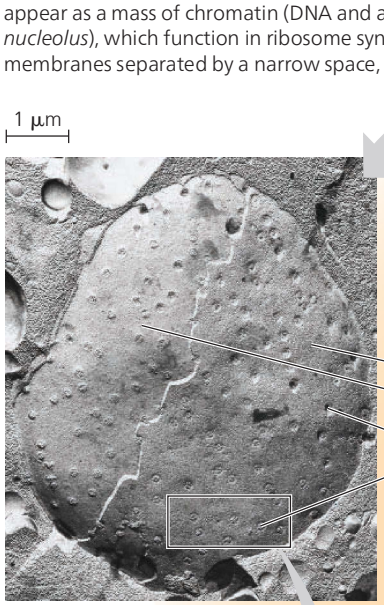
As we saw in Figure 5.22, the nucleus directs protein synthesis by synthesizing messenger RNA (mRNA) that carries information from the DNA. The mRNA is then transported to the cytoplasm via nuclear pores. Once an mRNA molecule reaches the cytoplasm, ribosomes translate the mRNA's genetic message into the primary structure of a specific polypeptide. (This process of transcribing and translating genetic information is described in detail in Chapter 17.)

Mastering Biology **BioFlix® Animation: Nucleus and Ribosomes**

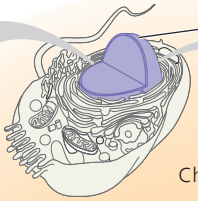
Ribosomes: Protein Factories

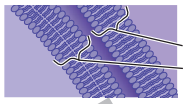
Ribosomes, which are complexes made of ribosomal RNAs and proteins, are the cellular components that carry out protein synthesis **(Figure 6.10)**. (Note that ribosomes are not membrane bounded and thus are not considered organelles.)

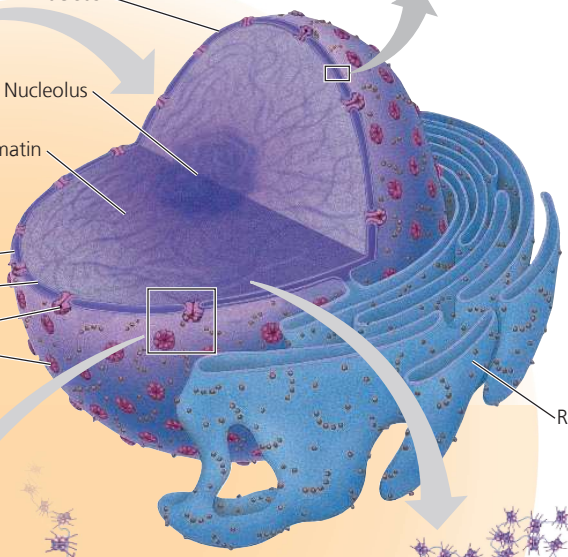
▼ Figure 6.9 The nucleus and its envelope. Within the nucleus are the chromosomes, which appear as a mass of chromatin (DNA and associated proteins) and one or more nucleoli (singular, *nucleolus*), which function in ribosome synthesis. The nuclear envelope, which consists of two membranes separated by a narrow space, is perforated with pores and lined by the nuclear lamina.

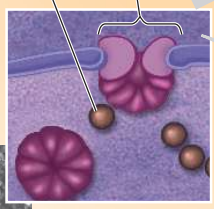


▲ **Surface of nuclear envelope (TEM).** This specimen was prepared by a technique known as freeze-fracture, which cuts from the outer membrane to the inner membrane, revealing both.

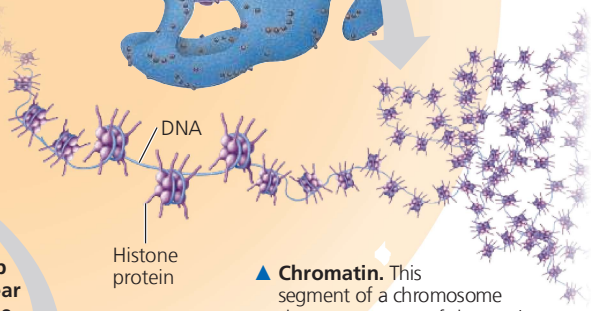




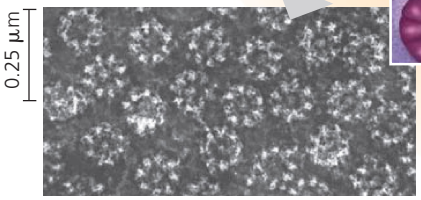




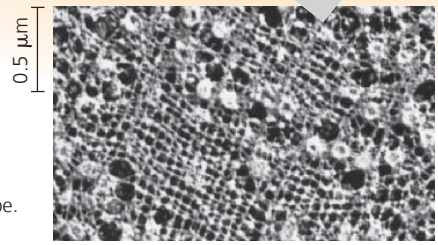
◀ **Close-up of nuclear envelope**



▲ **Chromatin.** This segment of a chromosome shows two states of chromatin (DNA—blue—wrapped around histone proteins--purple) in a nondividing cell. In preparation for cell division, the chromatin will become more condensed.



▲ **Pore complexes (TEM).** Each pore is ringed by protein particles.

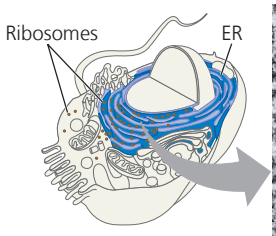


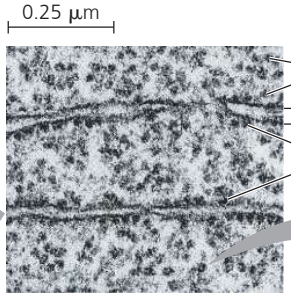
► **Nuclear lamina (TEM).** The netlike lamina lines the inner surface of the nuclear envelope. (The light circular spots are nuclear pores.)

MAKE CONNECTIONS *Chromosomes contain the genetic material and reside in the nucleus. How does the rest of the cell get access to the information they carry? (See Figure 5.22.)*

▼ Figure 6.10 Ribosomes. This electron micrograph of a pancreas cell shows both free and bound ribosomes. The simplified diagram and computer model show the two subunits of a ribosome.

Mastering Biology
Interview with Venki Ramakrishnan: Studying ribosome structure





TEM showing ER and ribosomes

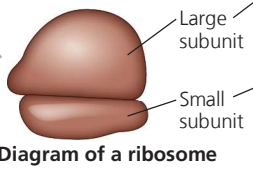
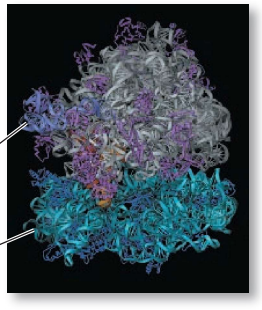


Diagram of a ribosome



Computer model of a ribosome

DRAW IT *After you have read the section on ribosomes, circle a ribosome in the micrograph that might be making a protein that will be secreted.*

Cells with high rates of protein synthesis have particularly large numbers of ribosomes as well as prominent nucleoli, which makes sense, given the role of nucleoli in ribosome assembly. For example, a human pancreas cell, which makes many digestive enzymes, has a few million ribosomes.

Ribosomes build proteins in two cytoplasmic regions: At any given time, *free ribosomes* are suspended in the cytosol, while *bound ribosomes* are attached to the outside of the endoplasmic reticulum or nuclear envelope (see Figure 6.10). Bound and free ribosomes are structurally identical, and ribosomes can play either role at different times. Most of the proteins made on free ribosomes function within the cytosol; examples are enzymes that catalyze the first steps of sugar breakdown. Bound ribosomes generally make proteins that are destined for insertion into membranes, for packaging within certain organelles such as lysosomes (see Figure 6.8), or for export from the cell (secretion). Cells that specialize in protein secretion—for instance, the cells of the pancreas that secrete digestive enzymes—frequently have a high proportion of bound ribosomes. (You will learn more about ribosome structure and function in Concept 17.4.)

CONCEPT CHECK 6.3

1. What role do ribosomes play in carrying out genetic instructions?
2. Describe the molecular composition of nucleoli and explain their function.
3. **WHAT IF?** As a cell begins the process of dividing, its chromosomes become shorter, thicker, and individually visible in an LM (light micrograph). Explain what is happening at the molecular level.

For suggested answers, see Appendix A.

CONCEPT 6.4

The endomembrane system regulates protein traffic and performs metabolic functions

Many of the different membrane-bounded organelles of the eukaryotic cell are part of the **endomembrane system**, which includes the nuclear envelope, the endoplasmic reticulum, the Golgi apparatus, lysosomes, various kinds of vesicles and vacuoles, and the plasma membrane. This system carries out a variety of tasks in the cell, including synthesis of proteins, transport of proteins into membranes and organelles or out of the cell, metabolism and movement of lipids, and detoxification of poisons. The membranes of this system are related either through direct physical continuity or by the transfer of membrane segments as tiny **vesicles** (sacs made of membrane). Despite these relationships, the various membranes are not identical in structure and function. Moreover, the thickness, molecular composition, and types of chemical reactions carried out in a given membrane are not fixed, but may be modified several times during the membrane's life.

Having already looked at the nuclear envelope, we will now focus on the endoplasmic reticulum and the other endomembranes to which the endoplasmic reticulum gives rise.

The Endoplasmic Reticulum: Biosynthetic Factory

The **endoplasmic reticulum (ER)** is such an extensive network of membranes that it accounts for more than half the total membrane in many eukaryotic cells. (The word *endoplasmic* means "within the cytoplasm," and *reticulum* is Latin for "little net.") The ER consists of a network of membranous tubules and sacs called cisternae (from the Latin *cisterna*, a reservoir for a liquid). The ER membrane separates the internal compartment of the ER, called the *ER lumen* (cavity) or cisternal space, from the cytosol. And because the ER membrane is continuous with the nuclear envelope, the space between the two membranes of the envelope is continuous with the lumen of the ER **(Figure 6.11)**.

There are two distinct, though connected, regions of the ER that differ in structure and function: smooth ER and rough ER. **Smooth ER** is so named because its outer surface lacks ribosomes. **Rough ER** is studded with ribosomes on the outer surface of the membrane and thus appears rough through the electron microscope. As already mentioned, ribosomes are also attached to the cytoplasmic side of the nuclear envelope's outer membrane, which is continuous with rough ER.

Functions of Smooth ER

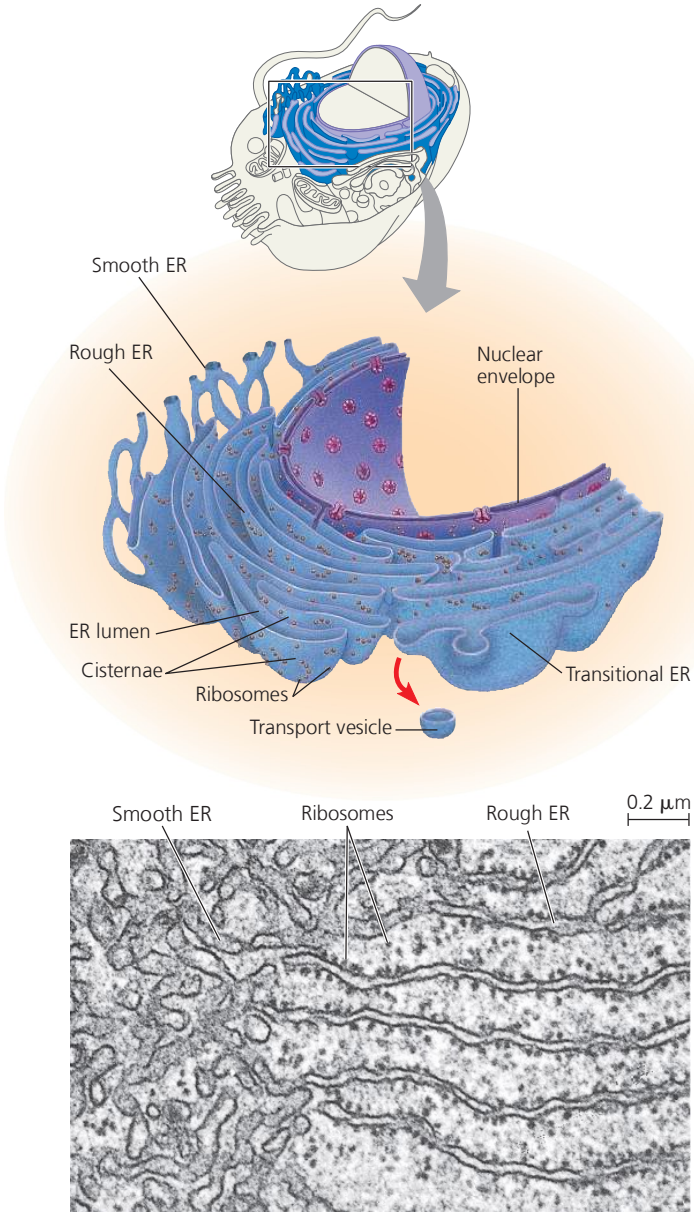
The smooth ER functions in diverse metabolic processes, which vary with cell type. These processes include synthesis of lipids, metabolism of carbohydrates, detoxification of drugs and poisons, and storage of calcium ions.

Enzymes of the smooth ER are important in the synthesis of lipids, including oils, steroids, and new membrane phospholipids. Among the steroids produced by the smooth ER in animal cells are the sex hormones of vertebrates and the various steroid hormones secreted by the adrenal glands. The cells that synthesize and secrete these hormones—in the testes and ovaries, for example—are rich in smooth ER, a structural feature that fits the function of these cells.

Other enzymes of the smooth ER help detoxify drugs and poisons, especially in liver cells. Detoxification usually involves adding hydroxyl groups to drug molecules, making them more water-soluble and easier to flush from the body. The sedative phenobarbital and other barbiturates are examples of drugs metabolized in this manner by smooth ER in liver cells. In fact, barbiturates, alcohol, and many other drugs induce the proliferation of smooth ER and its associated detoxification enzymes, thus increasing the rate of detoxification. This, in turn, increases tolerance to the drugs, meaning that higher doses are required to achieve a particular effect, such as sedation. Also, because some of the detoxification

enzymes have relatively broad action, the proliferation of smooth ER in response to one drug can increase the need for higher dosages of other drugs as well. Barbiturate abuse, for instance, can decrease the effectiveness of certain antibiotics and other useful drugs.

▼ Figure 6.11 Endoplasmic reticulum (ER). A membranous system of interconnected tubules and flattened sacs called cisternae, the ER is also continuous with the nuclear envelope, as shown in the cutaway diagram at the top. The membrane of the ER encloses a continuous compartment called the ER lumen (or cisternal space). Rough ER, which is studded on its outer surface with ribosomes, can be distinguished from smooth ER in the electron micrograph (TEM). Transport vesicles bud off from a region of the rough ER called transitional ER and travel to the Golgi apparatus and other destinations.



The smooth ER also stores calcium ions. In muscle cells, for example, the smooth ER membrane pumps calcium ions from the cytosol into the ER lumen. When a muscle cell is stimulated by a nerve impulse, calcium ions rush back across the ER membrane into the cytosol and trigger contraction of the muscle cell. In other cell types, release of calcium ions from the smooth ER triggers different responses, such as secretion of vesicles carrying newly synthesized proteins.

Functions of Rough ER

Many cells secrete proteins that are produced by ribosomes attached to rough ER. For instance, certain pancreatic cells synthesize the protein insulin in the ER and secrete this hormone into the bloodstream. As a polypeptide chain grows from a bound ribosome, the chain is threaded into the ER lumen through a pore formed by a protein complex in the ER membrane. The new polypeptide folds into its functional shape as it enters the ER lumen. Most secretory proteins are **glycoproteins**, proteins with carbohydrates covalently bonded to them. The carbohydrates are attached to the proteins in the ER lumen by enzymes built into the ER membrane.

After secretory proteins are formed, the ER membrane keeps them separate from proteins in the cytosol, which are produced by free ribosomes. Secretory proteins depart from the ER wrapped in the membranes of vesicles that bud like bubbles from a specialized region called transitional ER (see Figure 6.11). Vesicles in transit from one part of the cell to another are called **transport vesicles**; we will examine their fate shortly.

In addition to making secretory proteins, rough ER is a membrane factory for the cell; it grows in place by adding membrane proteins and phospholipids to its own membrane. As polypeptides destined to be membrane proteins grow from the ribosomes, they are inserted into the ER membrane itself and anchored there by their hydrophobic portions. Like the smooth ER, the rough ER also makes membrane phospholipids; enzymes built into the ER membrane assemble phospholipids from precursors in the cytosol. The ER membrane expands, and portions of it are transferred in the form of transport vesicles to other components of the endomembrane system.

The Golgi Apparatus: Shipping and Receiving Center

After leaving the ER, many transport vesicles travel to the **Golgi apparatus**. We can think of the Golgi as a warehouse for receiving, sorting, shipping, and even some manufacturing. Here, products of the ER, such as proteins, are modified and stored and then sent to other destinations. Not surprisingly, the Golgi apparatus is especially extensive in cells specialized for secretion.

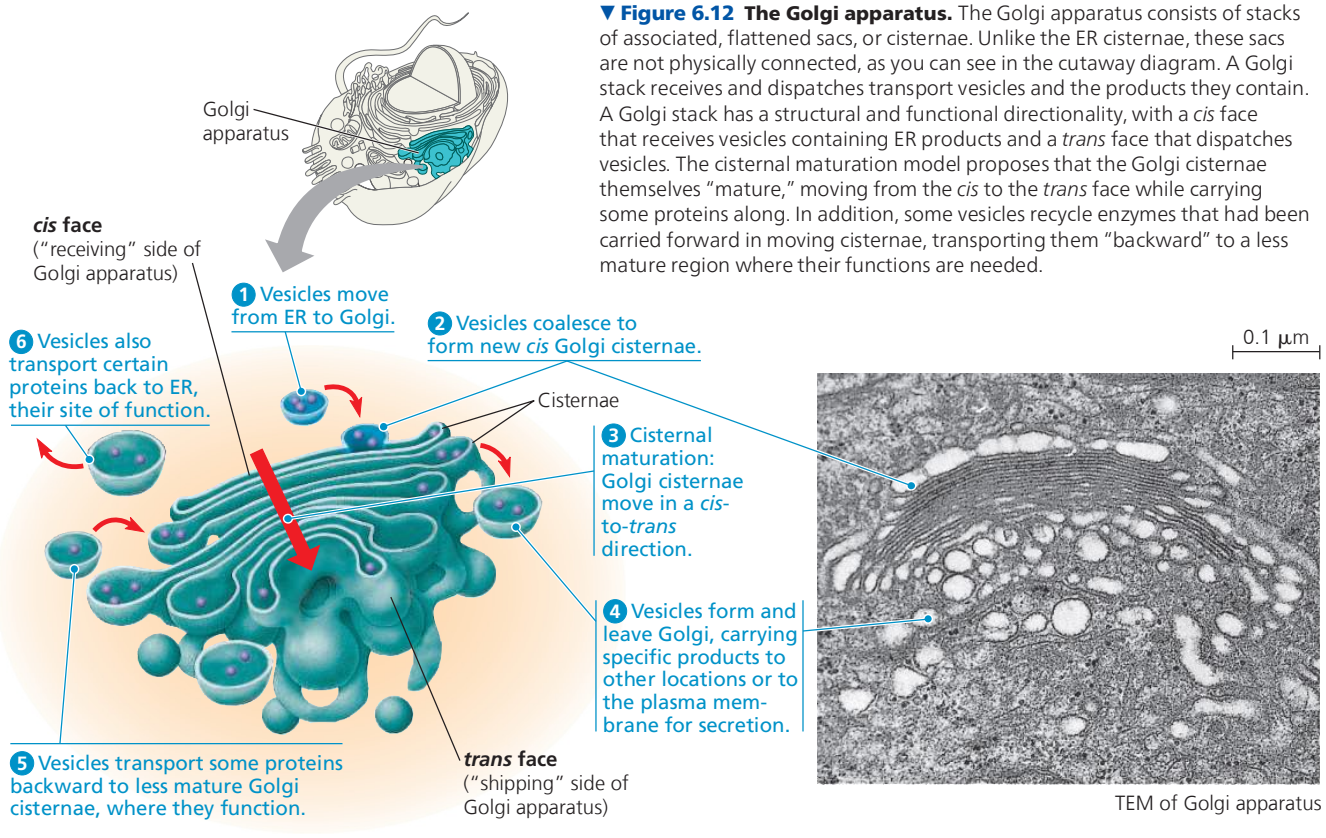
The Golgi apparatus consists of a group of associated, flattened membranous sacs—cisternae—looking like a stack of pita bread **(Figure 6.12)**. A cell may have many, even hundreds, of these stacks. The membrane of each cisterna in a stack separates its internal space from the cytosol. Vesicles concentrated in the vicinity of the Golgi apparatus are engaged in the transfer of material between parts of the Golgi and other structures.

A Golgi stack has a distinct structural directionality, with the membranes of cisternae on opposite sides of the stack differing in thickness and molecular composition. The two sides of a Golgi stack are referred to as the *cis* face and the *trans* face; these act, respectively, as the receiving and shipping departments of the Golgi apparatus. The term *cis* means "on the same side," and the *cis* face is usually located near the ER. Transport vesicles move material from the ER to the Golgi apparatus. A vesicle that buds from the ER can add its membrane and the contents of its lumen to the *cis* face by fusing with a Golgi membrane on that side. The *trans* face ("on the opposite side") gives rise to vesicles that pinch off and travel to other sites.

Products of the endoplasmic reticulum are usually modified during their transit from the *cis* region to the *trans* region of the Golgi apparatus. For example, glycoproteins formed in the ER have their carbohydrates modified, first in the ER itself, and then as they pass through the Golgi. The Golgi removes some sugar monomers and substitutes others, producing a large variety of carbohydrates. Membrane phospholipids may also be altered in the Golgi.

In addition to its finishing work, the Golgi apparatus also manufactures some macromolecules. Many polysaccharides secreted by cells are Golgi products. For example, pectins and certain other noncellulose polysaccharides are made in the Golgi of plant cells and then incorporated along with cellulose into their cell walls. Like secretory proteins, nonprotein Golgi products that will be secreted depart from the *trans* face of the Golgi inside transport vesicles that eventually fuse with the plasma membrane. The contents are released and the vesicle membrane is incorporated into the plasma membrane, adding to the surface area.

The Golgi manufactures and refines its products in stages, with different cisternae containing unique teams of enzymes. Until recently, biologists viewed the Golgi as a static structure, with products in various stages of processing transferred from one cisterna to the next by vesicles. While this may occur, research from several labs has given rise to a new model of the Golgi as a more dynamic structure. According to the *cisternal maturation model*, the cisternae of the Golgi actually progress forward from the *cis* to the *trans* face, carrying and modifying their cargo as they move. Figure 6.12 shows the details of this model. The reality probably lies somewhere between the two models; recent research suggests the central



▼ **Figure 6.12 The Golgi apparatus.** The Golgi apparatus consists of stacks of associated, flattened sacs, or cisternae. Unlike the ER cisternae, these sacs are not physically connected, as you can see in the cutaway diagram. A Golgi stack receives and dispatches transport vesicles and the products they contain. A Golgi stack has a structural and functional directionality, with a *cis* face that receives vesicles containing ER products and a *trans* face that dispatches vesicles. The cisternal maturation model proposes that the Golgi cisternae themselves "mature," moving from the *cis* to the *trans* face while carrying some proteins along. In addition, some vesicles recycle enzymes that had been carried forward in moving cisternae, transporting them "backward" to a less mature region where their functions are needed.

regions of the cisternae may remain in place, while the outer ends are more dynamic.

Before a Golgi stack dispatches its products by budding vesicles from the *trans* face, it sorts these products and targets them for various parts of the cell. Molecular identification tags, such as phosphate groups added to the Golgi products, aid in sorting by acting like zip codes on mailing labels. Finally, transport vesicles budded from the Golgi may have external molecules on their membranes that recognize "docking sites" on the surface of specific organelles or on the plasma membrane, thus targeting the vesicles appropriately.

Lysosomes: Digestive Compartments

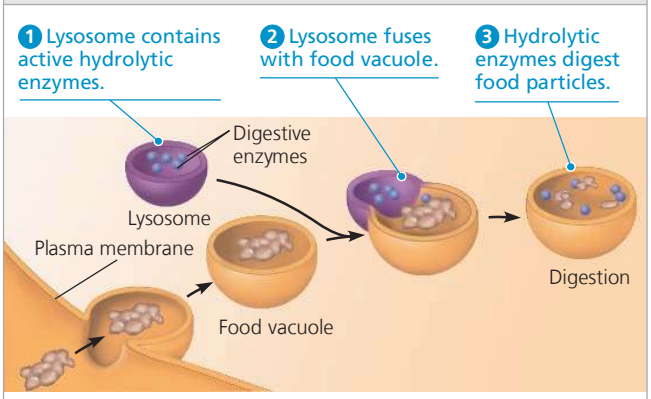
A **lysosome** is a membranous sac of hydrolytic enzymes that many eukaryotic cells use to digest (hydrolyze) macromolecules. Lysosomal enzymes work best in the acidic environment found in lysosomes. If a lysosome breaks open or leaks its contents, the released enzymes are not very active because the cytosol has a near-neutral pH. However, excessive leakage from a large number of lysosomes can destroy a cell by self-digestion.

Hydrolytic enzymes and lysosomal membrane are made by rough ER and then transferred to the Golgi apparatus for further processing. At least some lysosomes probably arise by budding from the *trans* face of the Golgi apparatus (see Figure 6.12). How are the proteins of the inner surface of the lysosomal membrane and the digestive enzymes themselves spared from destruction? Apparently, the three-dimensional shapes of these proteins protect vulnerable bonds from enzymatic attack.

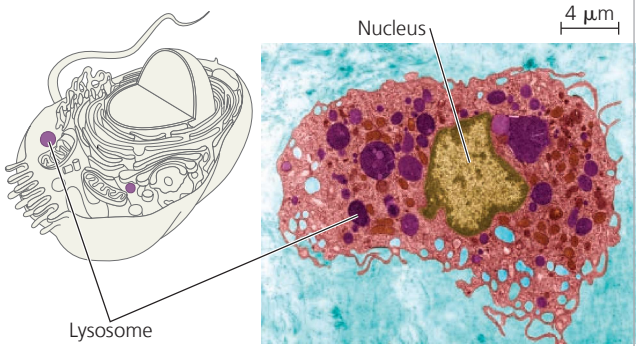
Lysosomes carry out intracellular digestion in a variety of circumstances. Amoebas and many other unicellular protists eat by engulfing smaller organisms or food particles, a process called **phagocytosis** (from the Greek *phagein*, to eat, and *kytos*, vessel, referring here to the cell). The *food vacuole* formed in this way then fuses with a lysosome, whose enzymes digest the food (**Figure 6.13a**, top). Digestion products, including simple sugars, amino acids, and other monomers, pass into the cytosol and become nutrients for the cell. Some human cells also carry out phagocytosis. Among them are macrophages, a type of white blood cell that helps defend the body by engulfing and destroying bacteria and other invaders (see Figure 6.13a, bottom, and Figure 6.31).

▼ Figure 6.13 Lysosomes.

(a) Phagocytosis

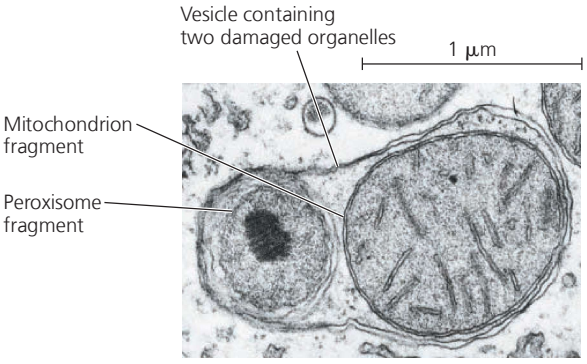


A lysosome fuses with a food vacuole during the process of phagocytosis by a unicellular protist.

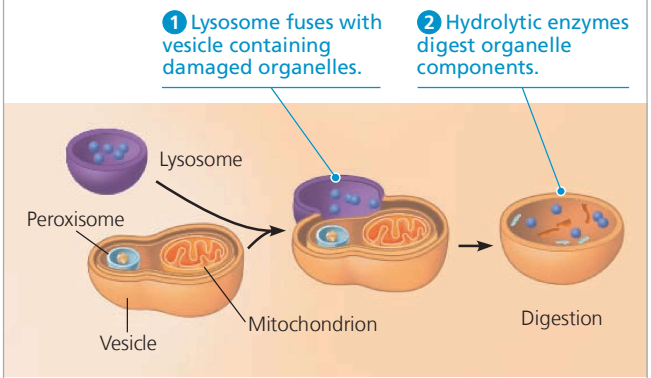


In this colorized TEM of a macrophage (a type of white blood cell), lysosomes are purple. They contain enzymes that digest foreign particles such as bacteria and pollen.

(b) Autophagy



This vesicle containing two damaged organelles is in the cytoplasm of a rat liver cell (TEM).



The vesicle with damaged organelles fuses with a lysosome. The organelles are then digested and their components recycled.

Lysosomes also use their hydrolytic enzymes to recycle the cell's own organic material, a process called *autophagy*. During autophagy, a damaged organelle or small amount of cytosol becomes surrounded by a double membrane (of unknown origin), and a lysosome fuses with the outer membrane of this vesicle **(Figure 6.13b)**. The lysosomal enzymes dismantle the inner membrane and the enclosed material, and the resulting small organic compounds are released to the cytosol for reuse. With the help of lysosomes, the cell continually renews itself. A human liver cell, for example, recycles half of its macromolecules each week.

The cells of people with inherited lysosomal storage diseases lack a functioning hydrolytic enzyme normally present in lysosomes. The lysosomes become engorged with indigestible material, which begins to interfere with other cellular activities. In Tay-Sachs disease, for example, a lipid-digesting enzyme is missing or inactive, and the brain becomes impaired by an accumulation of lipids in the cells. Fortunately, lysosomal storage diseases are rare in the general population.

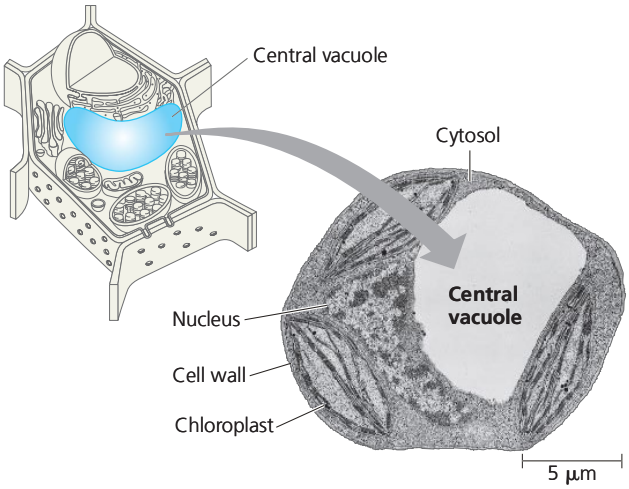
Vacuoles: Diverse Maintenance Compartments

Vacuoles are large vesicles derived from the endoplasmic reticulum and Golgi apparatus. Thus, vacuoles are an integral part of a cell's endomembrane system. Like all cellular membranes, the vacuolar membrane is selective in transporting solutes; as a result, the solution inside a vacuole differs in composition from the cytosol.

Vacuoles perform a variety of functions in different kinds of cells. **Food vacuoles**, formed by phagocytosis, have already been mentioned (see Figure 6.13a). Many unicellular protists living in fresh water have **contractile vacuoles** that pump excess water out of the cell, thereby maintaining a suitable concentration of ions and molecules inside the cell (see Figure 7.13). In plants and fungi, certain vacuoles carry out enzymatic hydrolysis, a function shared by lysosomes in animal cells. (In fact, some biologists consider these hydrolytic vacuoles to be a type of lysosome.) In plants, small vacuoles can hold reserves of important organic compounds, such as the proteins stockpiled in the storage cells in seeds. Vacuoles may also help protect the plant against herbivores by storing compounds that are poisonous or unpalatable to animals. Some plant vacuoles contain pigments, such as the red and blue pigments of petals that help attract pollinating insects to flowers.

Mature plant cells generally contain a large **central vacuole (Figure 6.14)**, which develops by the coalescence of smaller vacuoles. The solution inside the central vacuole, called cell sap, is the plant cell's main repository of inorganic ions, including potassium and chloride. The central vacuole plays a major role in the growth of plant cells, which enlarge as the vacuole absorbs water, enabling the cell to become larger with a minimal investment in new cytoplasm. The cytosol often occupies only a thin layer between the central vacuole and the plasma membrane, so the ratio of plasma membrane surface to cytosolic volume is sufficient, even for a large plant cell.

▼ **Figure 6.14 The plant cell vacuole.** The central vacuole is usually the largest compartment in a plant cell; the rest of the cytoplasm is often confined to a narrow zone between the vacuolar membrane and the plasma membrane (TEM).



Mastering Biology **BioFlix® Animation: Central Vacuole**

The Endomembrane System: *A Review*

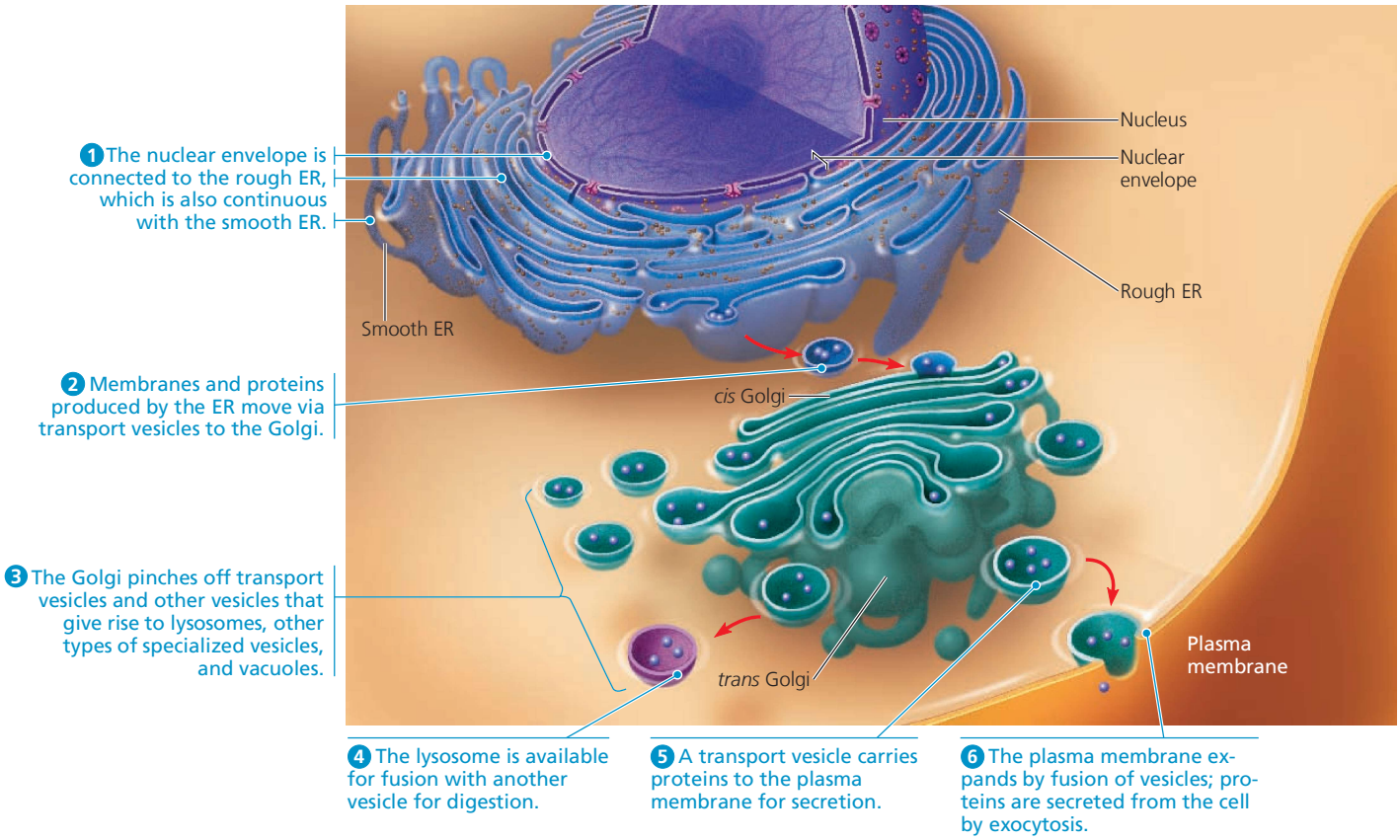
Figure 6.15 reviews the endomembrane system, showing the flow of membrane lipids and proteins through the various organelles. As the membrane moves from the ER to the Golgi and then elsewhere, its molecular composition and metabolic functions are modified, along with those of its contents. The endomembrane system is a complex and dynamic player in the cell's compartmental organization.

We'll continue our tour of the cell with some organelles that are not closely related to the endomembrane system but play crucial roles in the energy transformations carried out by cells.

CONCEPT CHECK 6.4

1. Describe the structural and functional distinctions between rough and smooth ER.
2. Describe how transport vesicles integrate the endomembrane system.
3. **WHAT IF?** Imagine a protein that functions in the ER but requires modification in the Golgi apparatus before it can achieve that function. Describe the protein's path through the cell, starting with the mRNA molecule that specifies the protein.

For suggested answers, see Appendix A.



▲ Figure 6.15 Review: Relationships among organelles of the endomembrane system.
The red arrows show some of the migration pathways for membranes and the materials they enclose.

Mastering Biology
BioFlix® Animation: Endomembrane System

CONCEPT 6.5

Mitochondria and chloroplasts change energy from one form to another

Organisms transform the energy they acquire from their surroundings. In eukaryotic cells, mitochondria and chloroplasts are the organelles that convert energy to forms that cells can use for work. **Mitochondria** (singular, *mitochondrion*) are the sites of cellular respiration, the metabolic process that uses oxygen to drive the generation of ATP by extracting energy from sugars, fats, and other fuels. **Chloroplasts**, found in plants and algae, are the sites of photosynthesis. This process in chloroplasts converts solar energy to chemical energy by absorbing sunlight and using it to drive the synthesis of organic compounds such as sugars from carbon dioxide and water.

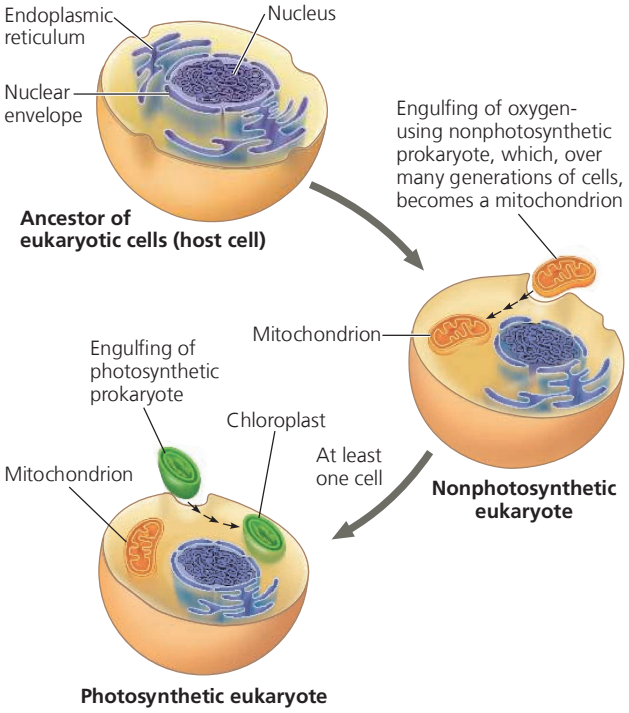
In addition to having related functions, mitochondria and chloroplasts share similar evolutionary origins, which we'll look at briefly before examining their structures. In this section, we'll also consider the peroxisome, an oxidative organelle. The evolutionary origin of the peroxisome, as well as its relation to other organelles, is still a matter of some debate.

The Evolutionary Origins of Mitochondria and Chloroplasts

EVOLUTION Mitochondria and chloroplasts display similarities with bacteria that led to the **endosymbiont theory**, illustrated in **Figure 6.16**. This theory states that an early ancestor of eukaryotic cells (a *host* cell) engulfed an oxygen-using nonphotosynthetic prokaryotic cell. Eventually, the engulfed cell formed a relationship with the host cell in which it was enclosed, becoming an *endosymbiont* (a cell living within another cell). Indeed, over the course of evolution, the host cell and its endosymbiont merged into a single organism, a eukaryotic cell with the endosymbiont having become a mitochondrion. At least one of these cells may have then taken up a photosynthetic prokaryote, becoming the ancestor of eukaryotic cells that contain chloroplasts.

This is a widely accepted theory, which we will examine in more detail in Concept 25.3. This theory is consistent with many structural features of mitochondria and chloroplasts. First, rather than being bounded by a single membrane like organelles of the endomembrane system, mitochondria and typical chloroplasts have two membranes surrounding them. (Chloroplasts also have an internal system of membranous sacs.) There is evidence that the ancestral engulfed prokaryotes had two outer membranes, which became the double

▼ Figure 6.16 The endosymbiont theory of the origins of mitochondria and chloroplasts in eukaryotic cells. According to this theory, the proposed ancestors of mitochondria were oxygen-using nonphotosynthetic prokaryotes that were taken into host cells, while the proposed ancestors of chloroplasts were photosynthetic prokaryotes. The large arrows represent change over evolutionary time; the small arrows inside the cells show the process of the endosymbiont becoming an organelle, also over long periods of time.



membranes of mitochondria and chloroplasts. Second, like prokaryotes, mitochondria and chloroplasts contain ribosomes, as well as circular DNA molecules—like bacterial chromosomes—associated with their inner membranes. The DNA in these organelles programs the synthesis of some organelle proteins on ribosomes that have been synthesized and assembled there as well. Third, also consistent with their probable evolutionary origins as cells, mitochondria and chloroplasts are autonomous (somewhat independent) organelles that grow and reproduce within the cell.

Next, we focus on the structures of mitochondria and chloroplasts, while providing an overview of their structures and functions. (In Chapters 9 and 10, we will examine their roles as energy transformers.)

Mitochondria: Chemical Energy Conversion

Mitochondria are found in nearly all eukaryotic cells, including those of plants, animals, fungi, and most protists. Some cells have a single large mitochondrion, but more often a cell has hundreds or even thousands of mitochondria; the number correlates with the cell's level of metabolic activity. For example, cells that move or contract have proportionally more mitochondria per volume than less active cells.

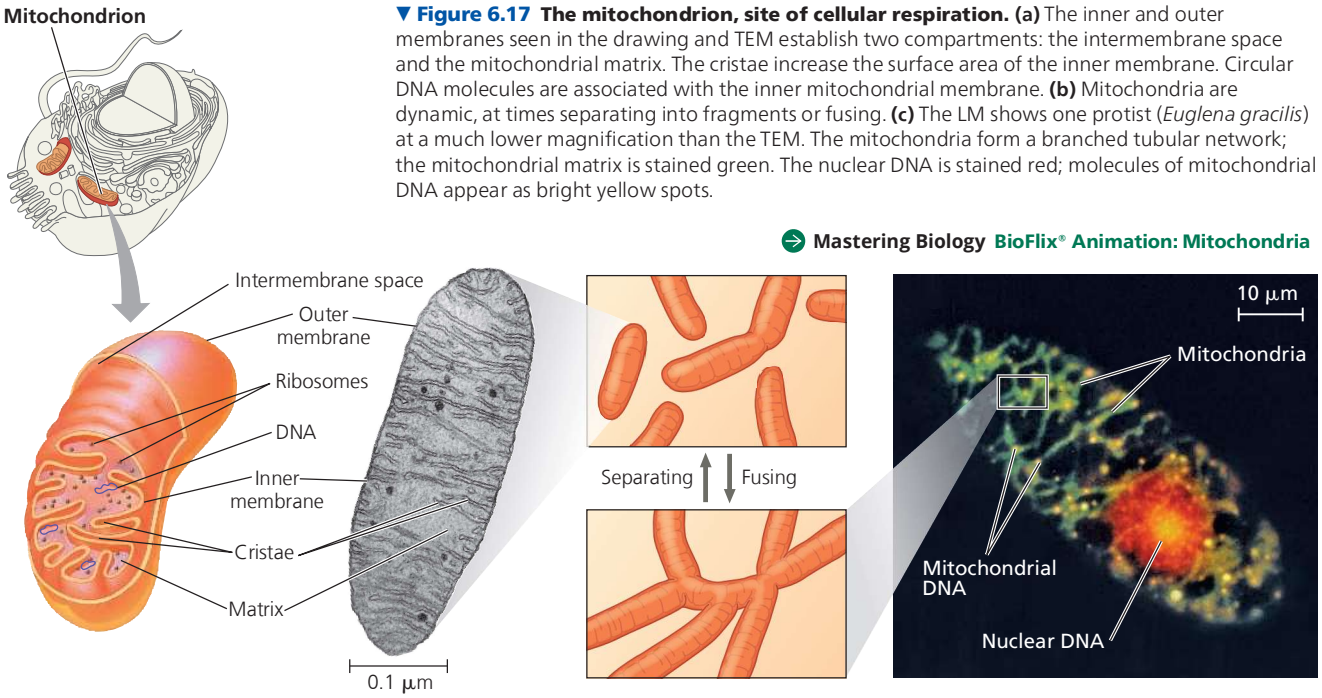
Each of the two membranes enclosing the mitochondrion is a phospholipid bilayer with a unique collection of embedded proteins. The outer membrane is smooth, but the inner membrane is convoluted, with infoldings called **cristae** (**Figure 6.17a**). The inner membrane divides the mitochondrion into two internal compartments. The first is the intermembrane space, the narrow region between the inner and outer membranes. The second compartment, the **mitochondrial matrix**, is enclosed by the inner membrane. The matrix contains many different enzymes as well as the mitochondrial DNA and ribosomes. Enzymes in the matrix catalyze some of the steps of cellular respiration. Other proteins that function in respiration, including the enzyme that makes ATP, are built into the inner membrane. As highly folded surfaces, the cristae give the inner mitochondrial membrane a large surface area, thus enhancing the productivity of cellular respiration. This is another example of structure fitting function. (Chapter 9 discusses cellular respiration in detail.)

Mitochondria are generally in the range of 1–10 µm long. Time-lapse films of living cells reveal mitochondria moving around, changing their shapes, and fusing or dividing into separate fragments, unlike the static structures seen in most diagrams and electron micrographs. These studies helped cell biologists understand that mitochondria in a living cell form a branched tubular network that is in a dynamic state of flux (see **Figure 6.17b** and **c**). In skeletal muscle, this network has been referred to by researchers as a "power grid."

Chloroplasts: Capture of Light Energy

Chloroplasts contain the green pigment chlorophyll, along with enzymes and other molecules that function in the photosynthetic production of sugar. These lens-shaped organelles, about 3–6 µm in length, are found in leaves and other green organs of plants and in algae (**Figure 6.18**; see also Figure 6.26c).

The contents of a chloroplast are partitioned from the cytosol by an envelope consisting of two membranes separated by a very narrow intermembrane space. Inside the chloroplast is another membranous system in the form of flattened, interconnected sacs called **thylakoids**. In some regions, thylakoids are stacked like poker chips; each stack is called a **granum** (plural, *grana*). The fluid outside the thylakoids is the **stroma**, which contains the chloroplast DNA and ribosomes as well as many enzymes. The membranes of the chloroplast divide the chloroplast space into three compartments: the intermembrane space, the stroma, and the thylakoid space. This compartmental organization enables the chloroplast to convert light energy to chemical energy



(a) Diagram and TEM of mitochondrion

(b) Dynamic nature of mitochondrial networks

(c) Network of mitochondria in *Euglena* (LM)

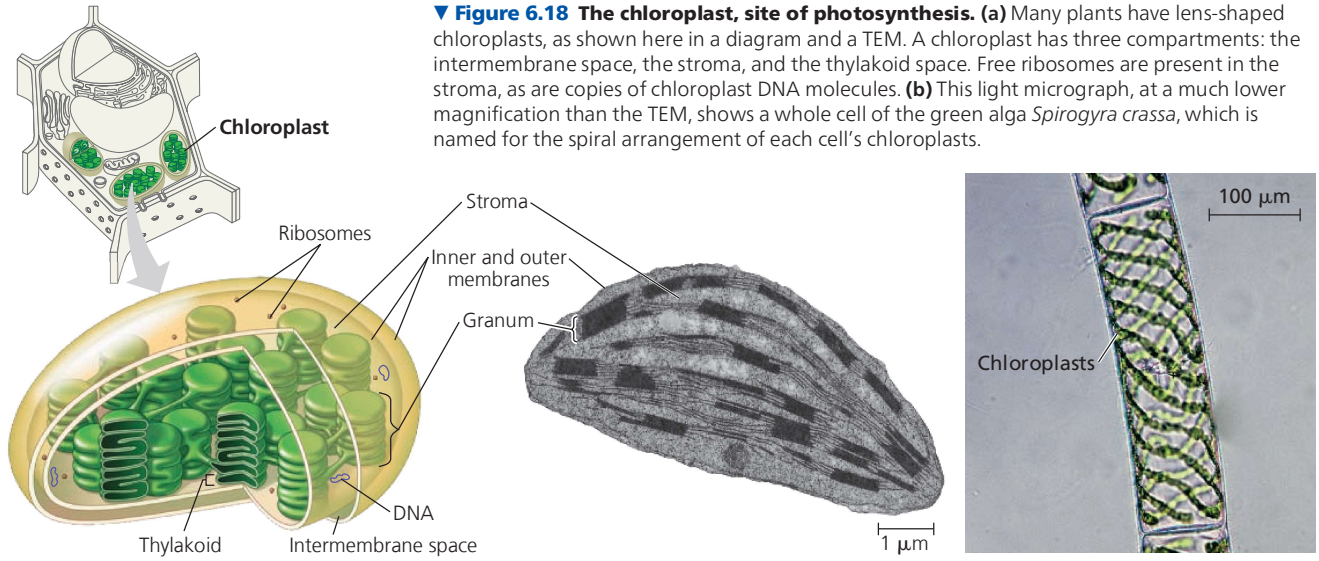
▼ Figure 6.17 The mitochondrion, site of cellular respiration. (a) The inner and outer membranes seen in the drawing and TEM establish two compartments: the intermembrane space and the mitochondrial matrix. The cristae increase the surface area of the inner membrane. Circular DNA molecules are associated with the inner mitochondrial membrane. **(b)** Mitochondria are dynamic, at times separating into fragments or fusing. **(c)** The LM shows one protist (*Euglena gracilis*) at a much lower magnification than the TEM. The mitochondria form a branched tubular network; the mitochondrial matrix is stained green. The nuclear DNA is stained red; molecules of mitochondrial DNA appear as bright yellow spots.

Mastering Biology **BioFlix® Animation: Mitochondria**

during photosynthesis. (You will learn more about photosynthesis in Chapter 10.)

As with mitochondria, the static and rigid appearance of chloroplasts in micrographs or schematic diagrams cannot accurately depict their dynamic behavior in the living cell. Their shape is changeable, and they grow and occasionally pinch in two, reproducing themselves. They are mobile and, with mitochondria and other organelles, move around the cell along tracks of the cytoskeleton, a structural network we will consider in Concept 6.6.

The chloroplast is a specialized member of a family of closely related plant organelles called **plastids**. One type of plastid, the *amyloplast*, is a colorless organelle that stores starch (amylose), particularly in roots and tubers. Another is the *chromoplast*, which has pigments that give fruits and flowers their orange and yellow hues.



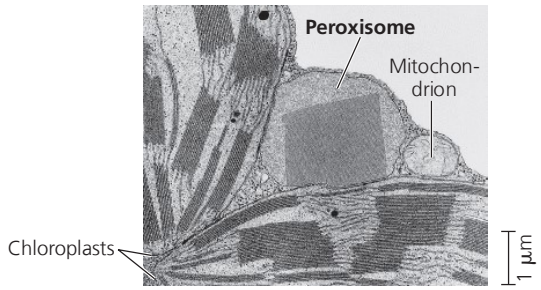
(a) Diagram and TEM of chloroplast

(b) Chloroplasts in an algal cell

▼ Figure 6.18 The chloroplast, site of photosynthesis. (a) Many plants have lens-shaped chloroplasts, as shown here in a diagram and a TEM. A chloroplast has three compartments: the intermembrane space, the stroma, and the thylakoid space. Free ribosomes are present in the stroma, as are copies of chloroplast DNA molecules. **(b)** This light micrograph, at a much lower magnification than the TEM, shows a whole cell of the green alga *Spirogyra crassa*, which is named for the spiral arrangement of each cell's chloroplasts.

Mastering Biology **BioFlix® Animation: Chloroplasts and Mitochondria**

▼ **Figure 6.19** **A peroxisome.** Peroxisomes are roughly spherical and often have a granular or crystalline core that is thought to be a dense collection of enzyme molecules. Chloroplasts and mitochondria cooperate with peroxisomes in certain metabolic functions (TEM).



Peroxisomes: Oxidation

The **peroxisome** is a specialized metabolic compartment bounded by a single membrane **(Figure 6.19)**. Peroxisomes contain enzymes that remove hydrogen atoms from various substrates and transfer them to oxygen (O_2), producing hydrogen peroxide (H_2O_2) as a by-product (from which the organelle derives its name). These reactions have many different functions. Some peroxisomes use oxygen to break fatty acids down into smaller molecules that are transported to mitochondria and used as fuel for cellular respiration. Peroxisomes in the liver detoxify alcohol and other harmful compounds by transferring hydrogen from the poisonous compounds to oxygen. The H_2O_2 formed by peroxisomes is itself toxic, but the organelle also contains an enzyme that converts H_2O_2 to water. This is an excellent example of how the cell's compartmental structure is crucial to its functions: The enzymes that produce H_2O_2 and those that dispose of this toxic compound are sequestered away from other cellular components that could be damaged.

Specialized peroxisomes called *glyoxysomes* are found in the fat-storing tissues of plant seeds. These organelles contain enzymes that initiate the conversion of fatty acids to sugar, which the emerging seedling uses as a source of energy and carbon until it can produce its own sugar by photosynthesis.

Peroxisomes grow larger by incorporating proteins made in the cytosol and ER, as well as lipids made in the ER and within the peroxisome itself. But how peroxisomes increase in number and how they arose in evolution—as well as what organelles they are related to—are still open questions.

CONCEPT CHECK 6.5

1. Describe two characteristics shared by chloroplasts and mitochondria. Consider both function and membrane structure.
2. Do plant cells have mitochondria? Explain.
3. **WHAT IF?** A classmate proposes that mitochondria and chloroplasts should be classified in the endomembrane system. Argue against the proposal.

For suggested answers, see Appendix A.

CONCEPT 6.6

The cytoskeleton is a network of fibers that organizes structures and activities in the cell

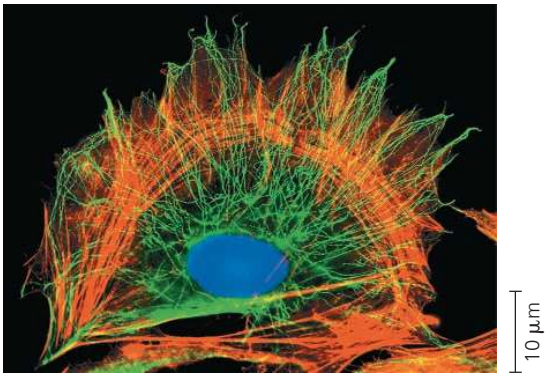
In the early days of electron microscopy, biologists thought that the organelles of a eukaryotic cell floated freely in the cytosol. But improvements in both light microscopy and electron microscopy have revealed the **cytoskeleton**, a network of fibers extending throughout the cytoplasm **(Figure 6.20)**. Bacterial cells also have fibers that form a type of cytoskeleton, constructed of proteins similar to eukaryotic ones, but here we will concentrate on eukaryotes. The eukaryotic cytoskeleton plays a major role in organizing the structures and activities of the cell.

Roles of the Cytoskeleton: Support and Motility

The most obvious function of the cytoskeleton is to give mechanical support to the cell and maintain its shape. This is especially important for animal cells, which lack walls. The remarkable strength and resilience of the cytoskeleton as a whole are based on its architecture. Like a dome tent, the cytoskeleton is stabilized by a balance between opposing forces exerted by its elements. And just as the skeleton of an animal helps fix the positions of other body parts, the cytoskeleton provides anchorage for many organelles and even cytosolic enzyme molecules. The cytoskeleton is more dynamic than an animal skeleton, however. It can be quickly dismantled in one part of the cell and reassembled in a new location, changing the shape of the cell.

Some types of cell motility (movement) also involve the cytoskeleton. The term *cell motility* includes both changes in

▼ **Figure 6.20** **The cytoskeleton.** As shown in this fluorescence micrograph, the cytoskeleton extends throughout the cell. The cytoskeletal elements have been tagged with different fluorescent molecules: green for microtubules and reddish-orange for microfilaments. A third component of the cytoskeleton, intermediate filaments, is not evident here. (The blue color tags the DNA in the nucleus.)



Mastering Biology **BioFlix® Animation: Cytoskeleton**

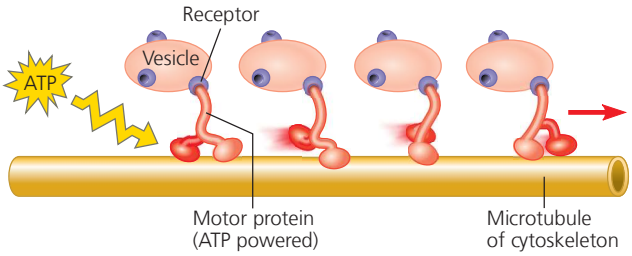
cell location and movements of cell parts. Cell motility generally requires interaction of the cytoskeleton with **motor proteins**. There are many such examples: Cytoskeletal elements and motor proteins work together with plasma membrane molecules to allow whole cells to move along fibers outside the cell. Inside the cell, vesicles and other organelles often use motor protein "feet" to "walk" to their destinations along a track provided by the cytoskeleton. For example, this is how vesicles containing neurotransmitter molecules migrate to the tips of axons, the long extensions of nerve cells that release these molecules as chemical signals to adjacent nerve cells **(Figure 6.21)**. The cytoskeleton also manipulates the plasma membrane, bending it inward to form food vacuoles or other phagocytic vesicles.

Mastering Biology
Interview with George Langford: Investigating how motor proteins move cargo

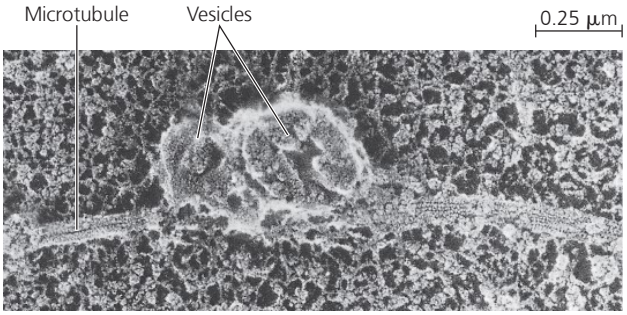
Components of the Cytoskeleton

Now let's look more closely at the three main types of fibers that make up the cytoskeleton: *Microtubules* are the thickest of the three types; *microfilaments* (also called actin filaments) are the thinnest; and *intermediate filaments* are fibers with diameters in a middle range **(Table 6.1)**.

▼ Figure 6.21 Motor proteins and the cytoskeleton.

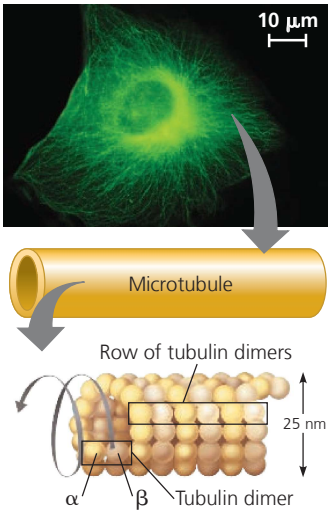
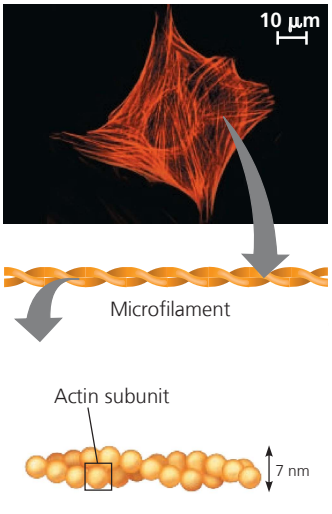
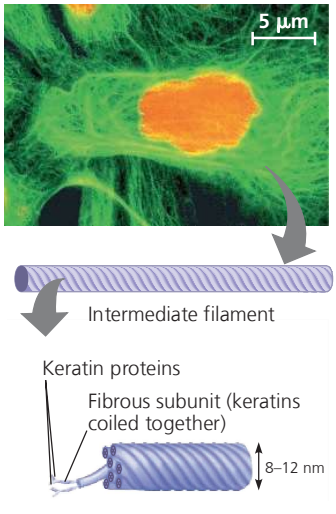


(a) A motor protein that attaches to a receptor on a vesicle can "walk" the vesicle along a microtubule or microfilament.



(b) Two vesicles containing neurotransmitters move along a microtubule toward the tip of a nerve cell extension called an axon (SEM).

Table 6.1 The Structure and Function of the Cytoskeleton

Property	Microtubules (Tubulin Polymers)	Microfilaments (Actin Filaments)	Intermediate Filaments
Structure	Hollow tubes	Two intertwined strands of actin	Fibrous proteins coiled into cables
Diameter	25 nm with 15-nm lumen	7 nm	8–12 nm
Protein subunits	Tubulin, a dimer consisting of an α-tubulin and a β-tubulin	Actin	One of several different proteins (including keratins)
Main functions	Maintenance of cell shape; cell motility; chromosome movements in cell division; organelle movements	Maintenance of cell shape; changes in cell shape; muscle contraction; cytoplasmic streaming (plant cells); cell motility; cell division (animal cells)	Maintenance of cell shape; anchorage of nucleus and certain other organelles; formation of nuclear lamina
Fluorescence micrographs of fibroblasts. Connective tissue cells called fibroblasts are a favorite cell type for cell biology studies because they spread out flat and their internal structures are easy to see. In each fibroblast shown here, the structure of interest has been tagged with fluorescent molecules. In the third micrograph, the DNA in the nucleus has also been tagged (orange).			

VISUAL SKILLS *How many tubulin dimers are in the boxed row?*

Microtubules

All eukaryotic cells have **microtubules**, hollow rods constructed from globular proteins called tubulins. Each tubulin protein is a *dimer*, a molecule made up of two components. A tubulin dimer consists of two slightly different polypeptides, α-tubulin and β-tubulin. Microtubules grow in length by adding tubulin dimers; they can also be disassembled and their tubulins used to build microtubules elsewhere in the cell. Because of the orientation of tubulin dimers, the two ends of a microtubule are slightly different. One end can accumulate or release tubulin dimers at a much higher rate than the other, thus growing and shrinking significantly during cellular activities. (This is called the "plus end," not because it can only add tubulin proteins but because it's the end where both "on" and "off" rates are much higher.)

Microtubules shape and support the cell and also serve as tracks along which organelles equipped with motor proteins can move. In addition to the example in Figure 6.21, microtubules guide vesicles from the ER to the Golgi apparatus and from the Golgi to the plasma membrane. Microtubules are also involved in the separation of chromosomes during cell division, as shown in Figure 12.7.

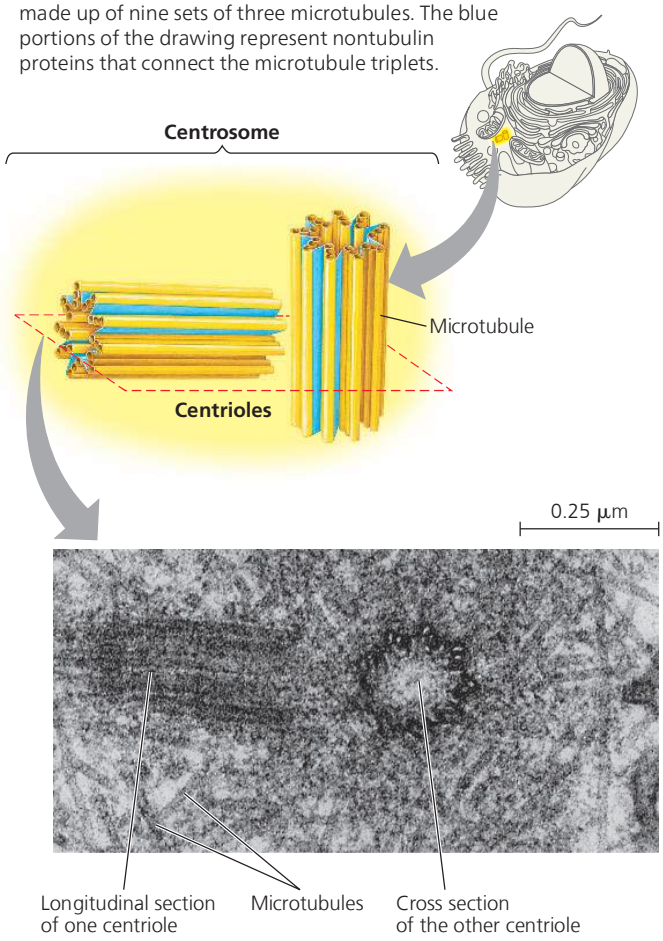
Centrosomes and Centrioles In animal cells, microtubules grow out from a **centrosome**, a region that is often located near the nucleus. These microtubules function as compression-resisting girders of the cytoskeleton. Within the centrosome is a pair of **centrioles**, each composed of nine sets of triplet microtubules arranged in a ring **(Figure 6.22)**. Although centrosomes with centrioles may help organize microtubule assembly in animal cells, many other eukaryotic cells lack centrosomes with centrioles and instead organize microtubules by other means.

Cilia and Flagella Some eukaryotic cells have **flagella** (singular, *flagellum*) and **cilia** (singular, *cilium*), cellular extensions that contain microtubules. A specialized arrangement of the microtubules is responsible for the beating of these structures. (The bacterial flagellum, shown in Figure 6.5, has a completely different structure.) Many unicellular protists are propelled through water by cilia or flagella that act as locomotor appendages, and the sperm of animals, algae, and some plants have flagella. When cilia or flagella extend from cells that are attached tightly together in a sheet that is part of a tissue layer, they can move fluid over the surface of the tissue. For example, the ciliated lining of the trachea (windpipe) sweeps mucus containing trapped debris out of the lungs (see the EMs in Figure 6.3). In a woman's reproductive tract, the cilia lining the oviducts help move an egg toward the uterus.

Motile cilia usually occur in large numbers on the cell surface. Flagella are usually limited to just one or a few per cell, and they are longer than cilia. Flagella and cilia differ in their beating patterns. A flagellum has an undulating motion like the tail of a fish. In contrast, cilia have alternating power and recovery strokes, much like the oars of a racing crew boat **(Figure 6.23)**.

A cilium may also act as a signal-receiving "antenna" for the cell. Cilia that have this function are generally nonmotile, and there is only one per cell. (In fact, in vertebrate animals, it appears that almost all cells have such a cilium, which is called a *primary cilium*.) Membrane proteins on this kind of cilium transmit molecular signals from the cell's environment to its interior, triggering signaling pathways that may lead to changes in the cell's activities. Cilium-based signaling appears to be crucial to brain function and to embryonic development.

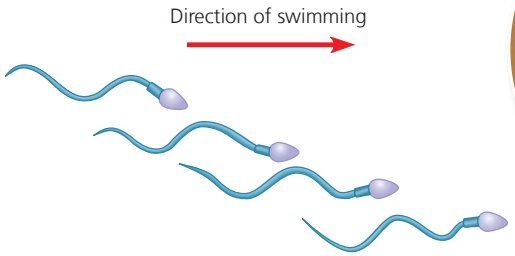
▼ Figure 6.22 Centrosome containing a pair of centrioles. Most animal cells have a centrosome, a region near the nucleus where the cell's microtubules are initiated. Within the centrosome is a pair of centrioles, each about 250 nm (0.25 μm) in diameter. The two centrioles are at right angles to each other, and each is made up of nine sets of three microtubules. The blue portions of the drawing represent nontubulin proteins that connect the microtubule triplets.



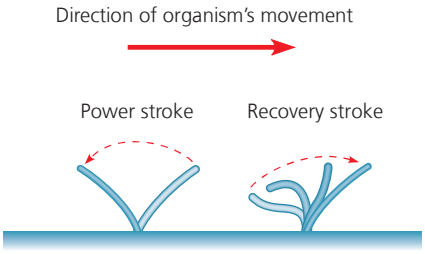
VISUAL SKILLS *How many microtubules are in a centrosome? In the drawing, circle and label one microtubule and describe its structure. Circle and label a triplet.*

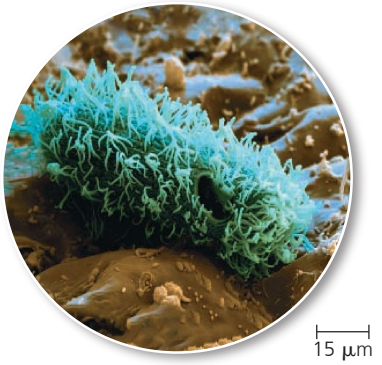
▼ Figure 6.23 A comparison of the beating of flagella and motile cilia.

(a) Motion of flagella. A flagellum usually undulates, its snakelike motion driving a cell in the same direction as the axis of the flagellum. Propulsion of a human sperm cell is an example of flagellate locomotion (LM).



(b) Motion of cilia. Cilia have a back-and-forth motion. The rapid power stroke moves the cell in a direction perpendicular to the axis of the cilium. Then, during the slower recovery stroke, the cilium bends and sweeps sideways, closer to the cell surface. A dense nap of cilia, beating at a rate of about 40 to 60 strokes a second, covers this *Colpidium*, a freshwater protist (colorized SEM).





Mastering Biology
Videos: Flagellum Movement in Swimming Sperm
Flagellum Beating with ATP
***Paramecium* Cilia**

Though different in length, number per cell, and beating pattern, motile cilia and flagella share a common structure. Each motile cilium or flagellum has a group of microtubules sheathed in an extension of the plasma membrane **(Figure 6.24a)**. Nine doublets of microtubules are arranged in a ring with two single microtubules in its center **(Figure 6.24b)**. This arrangement, referred to as the "9 + 2" pattern, is found in nearly all eukaryotic flagella and motile cilia. (Nonmotile primary cilia have a "9 + 0" pattern, lacking the central pair of microtubules.) The microtubule assembly of a cilium or flagellum is anchored in the cell by a **basal body**, which is structurally very similar to a centriole, with microtubule triplets in a "9 + 0" pattern **(Figure 6.24c)**. In fact, in many animals (including humans), the basal body of the fertilizing sperm's flagellum enters the egg and becomes a centriole.

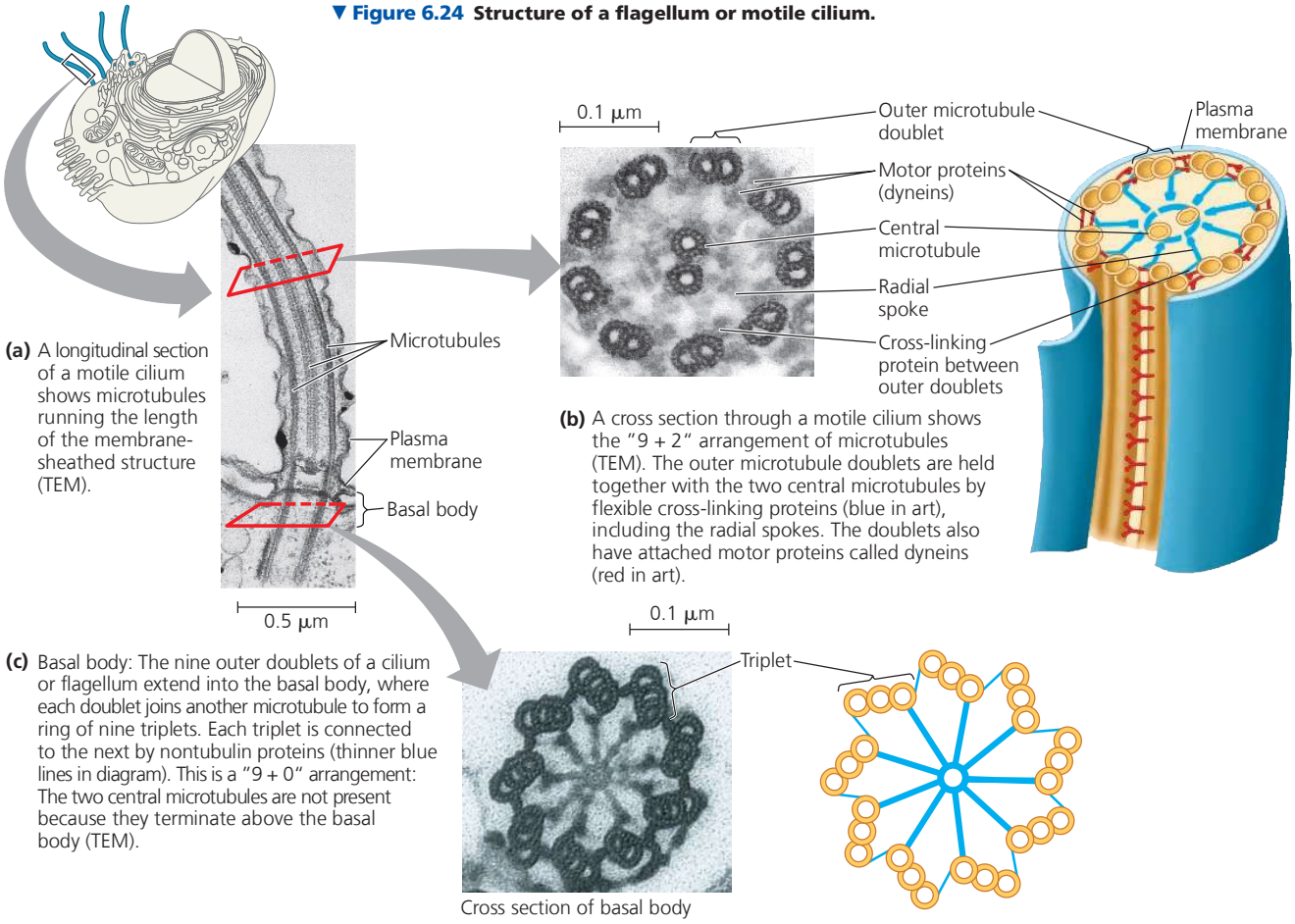
How does the microtubule assembly produce the bending movements of flagella and motile cilia? Bending involves large motor proteins called **dyneins** (red in the diagram in Figure 6.24) that are attached along each outer microtubule doublet. A typical dynein protein has two "feet" that "walk" along the microtubule of the adjacent doublet, using ATP for energy. One foot maintains contact, while the other releases and reattaches one step farther along the microtubule (see Figure 6.21). The outer doublets and two central microtubules are held together by flexible cross-linking proteins (blue in the diagram in Figure 6.24), and the walking movement is coordinated so that it happens on one side of the circle at a time. If the doublets were not held in place, the walking action would make them slide past each other. Instead, the movements of the dynein feet cause the microtubules—and the organelle as a whole—to bend.

Microfilaments (Actin Filaments)

Microfilaments are thin solid rods. They are also called actin filaments because they are built from molecules of **actin**, a globular protein. A microfilament is a twisted double chain of actin subunits (see Table 6.1). Besides occurring as linear filaments, microfilaments can form structural networks when certain proteins bind along the side of such a filament and allow a new filament to extend as a branch. Like microtubules, microfilaments seem to be present in all eukaryotic cells.

In contrast to the compression-resisting role of microtubules, the structural role of microfilaments in the cytoskeleton is to bear tension (pulling forces). A three-dimensional

▼ Figure 6.24 Structure of a flagellum or motile cilium.



(a) A longitudinal section of a motile cilium shows microtubules running the length of the membrane-sheathed structure (TEM).

(b) A cross section through a motile cilium shows the "9 + 2" arrangement of microtubules (TEM). The outer microtubule doublets are held together with the two central microtubules by flexible cross-linking proteins (blue in art), including the radial spokes. The doublets also have attached motor proteins called dyneins (red in art).

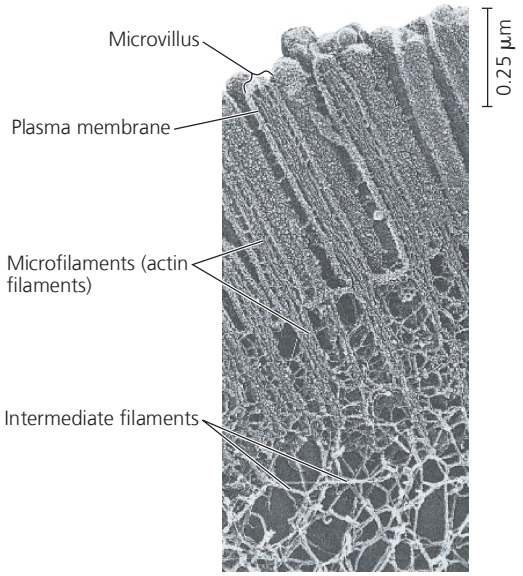
(c) Basal body: The nine outer doublets of a cilium or flagellum extend into the basal body, where each doublet joins another microtubule to form a ring of nine triplets. Each triplet is connected to the next by nontubulin proteins (thinner blue lines in diagram). This is a "9 + 0" arrangement: The two central microtubules are not present because they terminate above the basal body (TEM).

DRAW IT *In (a) and (b), circle and label the central pair of microtubules. In (a), show where they terminate, and explain why they aren't seen in the cross section of the basal body in (c).*

Mastering Biology Animation: Cilia and Flagella

network formed by microfilaments just to the inside of the plasma membrane (*cortical microfilaments*) helps support the cell's shape (see Figure 6.8). This network gives the outer cytoplasmic layer of a cell, called the **cortex**, the semisolid consistency of a gel, in contrast with the more fluid state of the interior cytoplasm. In some kinds of animal cells, such as nutrient-absorbing intestinal cells, bundles of microfilaments make up the core of microvilli, delicate projections that increase the cell's surface area **(Figure 6.25)**.

Microfilaments are well known for their role in cell motility. Thousands of actin filaments and thicker filaments made



▶ Figure 6.25 A structural role of microfilaments. The surface area of this nutrient-absorbing intestinal cell is increased by its many microvilli (singular, *microvillus*), cellular extensions reinforced by bundles of microfilaments. These actin filaments are anchored to a network of intermediate filaments (TEM).

of a protein called **myosin** interact to cause contraction of muscle cells **(Figure 6.26a)**; muscle contraction is described in detail in Concept 50.5. In the unicellular protist *Amoeba* and some of our white blood cells, localized contractions brought about by actin and myosin are involved in the amoeboid (crawling) movement of the cells. The cell crawls along a surface by extending cellular extensions called **pseudopodia** (from the Greek *pseudes*, false, and *pod*, foot) and moving toward them **(Figure 6.26b)**. In plant cells, actin-protein interactions contribute to **cytoplasmic streaming**, a circular flow of cytoplasm within cells **(Figure 6.26c)**. This movement, which is especially common in large plant cells, speeds the movement of organelles and the distribution of materials within the cell.

Intermediate Filaments

Intermediate filaments are named for their diameter, which is larger than the diameter of microfilaments but smaller than that of microtubules (see Table 6.1). While microtubules and microfilaments are found in all eukaryotic cells, intermediate filaments are only found in the cells of some animals, including vertebrates. Specialized for bearing tension (like microfilaments), intermediate filaments are a diverse class of cytoskeletal elements. Each type is constructed from a particular molecular subunit belonging to a family of proteins whose members include the keratins. Microtubules and microfilaments, in contrast, are consistent in diameter and composition in all eukaryotic cells.

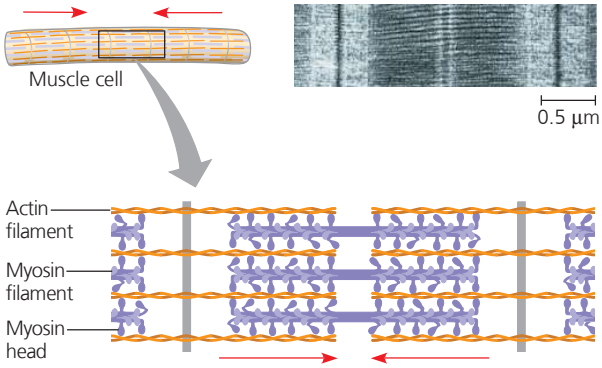
Intermediate filaments are more permanent fixtures of cells than are microfilaments and microtubules, which are often disassembled and reassembled in various parts of a cell. Even after cells die, intermediate filament networks often persist; for example, the outer layer of our skin consists of dead skin cells full of keratin filaments. Intermediate filaments are especially sturdy and play an important role in reinforcing the shape of a cell and fixing the position of certain organelles. For instance, the nucleus typically sits within a cage made of intermediate filaments. Other intermediate filaments make up the nuclear lamina, which lines the interior of the nuclear envelope (see Figure 6.9). In general, the various kinds of intermediate filaments seem to function together as the permanent framework of the entire cell.

CONCEPT CHECK 6.6

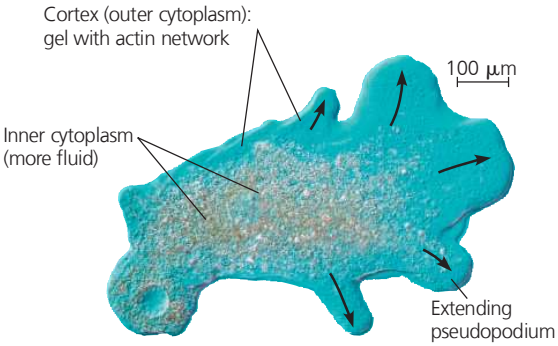
1. Describe how cilia and flagella bend.
2. **WHAT IF?** Males afflicted with Kartagener's syndrome are sterile because of immotile sperm, and they tend to suffer from lung infections. This disorder has a genetic basis. Suggest what the underlying defect might be.

For suggested answers, see Appendix A.

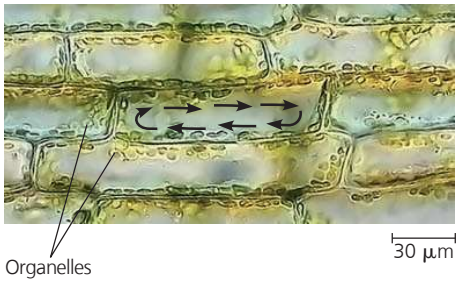
▼ Figure 6.26 Microfilaments and motility. In these three examples, interactions between actin filaments and motor proteins bring about cell movement.



(a) Myosin motors in muscle cell contraction. The "walking" of myosin projections (the so-called heads) drives the parallel myosin and actin filaments past each other so that the actin filaments approach each other in the middle (red arrows). This shortens the muscle cell. Muscle contraction involves the shortening of many muscle cells at the same time (TEM).



(b) Amoeboid movement. Interaction of actin filaments with myosin causes contraction of the cell, pulling the cell's trailing end (at left) forward (to the right) (LM).



(c) Cytoplasmic streaming in plant cells. A layer of cytoplasm cycles around the cell, moving over tracks of actin filaments. Myosin motors attached to some organelles drive the streaming by interacting with the actin (LM).

Mastering Biology **BioFlix® Animation: Actin and Myosin in Muscle Contraction • Video: *Amoeba* Pseudopodia • Video: Cytoplasmic Streaming**

CONCEPT 6.7

Extracellular components and connections between cells help coordinate cellular activities

Having crisscrossed the cell to explore its inner components, we complete our tour of the cell by returning to the surface of this microscopic world, where there are additional structures with important functions. The plasma membrane is usually regarded as the boundary of the living cell, but most cells synthesize and secrete materials extracellularly (to the outside of the cell). Although these materials and the structures they form are outside the cell, their study is important to cell biology because they are involved in a great many essential cellular functions.

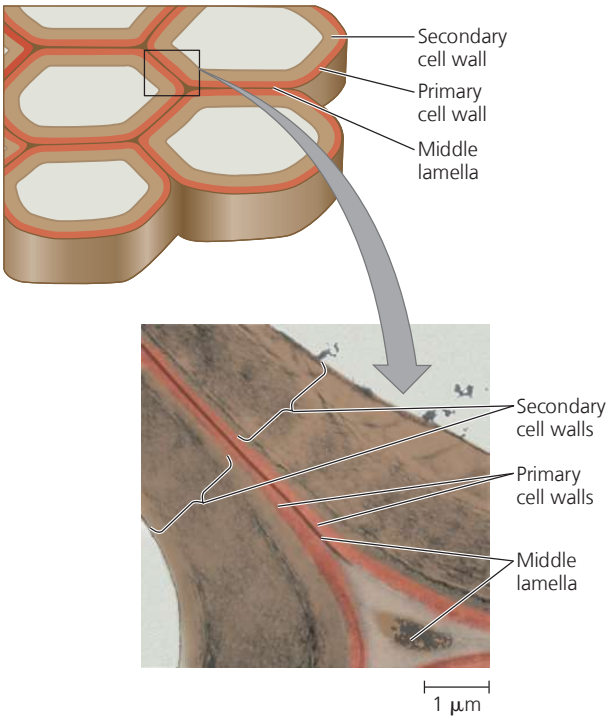
Cell Walls of Plants

The **cell wall** is an extracellular structure of plant cells **(Figure 6.27)**. This is one of the features that distinguishes plant cells from animal cells. The wall protects the plant cell, maintains its shape, and prevents excessive uptake of water. At the level of the whole plant, the strong walls of specialized cells hold the plant up against the force of gravity. Prokaryotes, some protists, and fungi also have cell walls, as you saw in Figures 6.5 and 6.8. Those organisms will be discussed in Chapters 27, 28, and 31.

Plant cell walls are much thicker than the plasma membrane, ranging from 0.1 µm to several micrometers. The exact chemical composition of the wall varies from species to species and even from one cell type to another in the same plant, but the basic design of the wall is consistent. Microfibrils made of the polysaccharide cellulose (see Figure 5.6) are synthesized by an enzyme called cellulose synthase and secreted to the extracellular space, where they become embedded in a matrix of other polysaccharides and proteins. This combination of materials, strong fibers in a "ground substance" (matrix), is the same basic architectural design found in steel-reinforced concrete and in fiberglass.

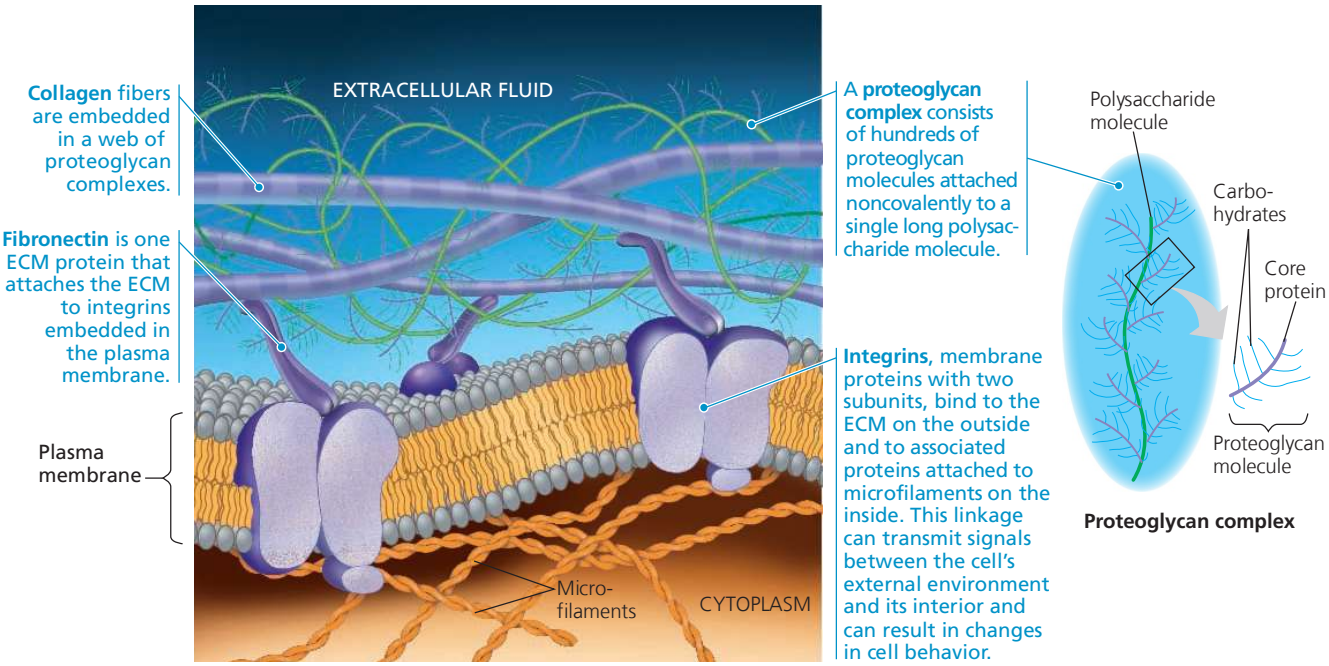
A young plant cell first secretes a relatively thin and flexible wall called the **primary cell wall** (see the micrograph in Figure 6.27). Between primary walls of adjacent cells is the **middle lamella**, a thin layer rich in sticky polysaccharides called pectins. The middle lamella glues adjacent cells together. (Pectin is used in cooking as a thickening agent in jams and jellies.) When the cell matures and stops growing, it strengthens its wall. Some plant cells do this simply by secreting hardening substances into the primary wall. Other cells add a **secondary cell wall** between the plasma membrane and the primary wall. The secondary wall, often deposited in several laminated layers, has a strong and durable matrix that affords the cell protection and support. Wood, for example, consists mainly of secondary walls.

▼ **Figure 6.27 Plant cell walls.** The drawing shows the relationship between primary and secondary cell walls in several mature plant cells. (Organelles aren't shown because many cells with secondary walls, such as the water-conducting cells, lack organelles.) The TEM shows the cell walls where two cells come together. The multilayered partition between plant cells consists of adjoining walls individually secreted by the cells. Adjacent cells are glued together by a very thin layer called the middle lamella.



The Extracellular Matrix (ECM) of Animal Cells

Although animal cells lack walls akin to those of plant cells, they do have an elaborate **extracellular matrix (ECM)**. The main ingredients of the ECM are glycoproteins and other carbohydrate-containing molecules secreted by the cells. (Recall that glycoproteins are proteins with covalently bonded carbohydrates.) The most abundant glycoprotein in the ECM of most animal cells is **collagen**, which forms strong fibers outside the cells (see Figure 5.18). In fact, collagen accounts for about 40% of the total protein in the human body. The collagen fibers are embedded in a network woven out of **proteoglycans** secreted by



▲ Figure 6.28 Extracellular matrix (ECM) of an animal cell. The molecular composition and structure of the ECM vary from one cell type to another. In this example, three different types of ECM molecules are present: collagen, fibronectin, and proteoglycans.

cells **(Figure 6.28)**. A proteoglycan molecule consists of a small core protein with many carbohydrate chains covalently attached, so that it may be up to 95% carbohydrate. Large proteoglycan complexes can form when hundreds of proteoglycan molecules become noncovalently attached to a single long polysaccharide molecule, as shown in Figure 6.28. Some cells are attached to the ECM by ECM glycoproteins such as **fibronectin**. Fibronectin and other ECM proteins bind to cell-surface receptor proteins called **integrins** that are built into the plasma membrane. Integrins span the membrane and bind on their cytoplasmic side to associated proteins attached to microfilaments of the cytoskeleton. The name *integrin* is based on the word *integrate*: Integrins are in a position to transmit signals between the ECM and the cytoskeleton and thus to integrate changes occurring outside and inside the cell.

Current research on fibronectin, other ECM molecules, and integrins reveals the influential role of the ECM in the lives of cells. By communicating with a cell through integrins, the ECM can regulate a cell's behavior. For example, some cells in a developing embryo migrate along specific pathways by matching the orientation of their microfilaments to the "grain" of fibers in the extracellular matrix. Researchers have also learned that the extracellular matrix around a cell can influence the activity of genes in the nucleus. Information about the ECM probably reaches the nucleus by a combination of mechanical and chemical signaling pathways. Mechanical signaling involves fibronectin, integrins, and microfilaments of the cytoskeleton. Changes in the cytoskeleton may in turn trigger signaling pathways inside the cell, leading to changes in the set of proteins being made by the cell and therefore changes in the cell's function. In this way, the extracellular matrix of a particular tissue may help coordinate the behavior of all the cells of that tissue. Direct connections between cells also function in this coordination, as we'll explore next.

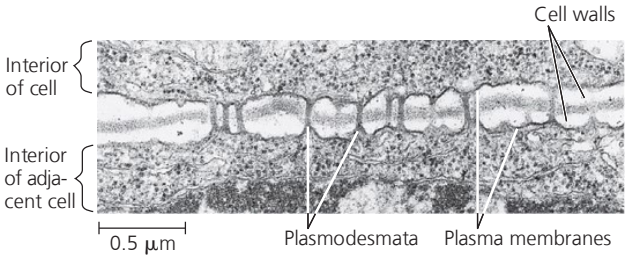
Cell Junctions

Cells in an animal or plant are organized into tissues, organs, and organ systems. Neighboring cells often adhere, interact, and communicate via sites of direct physical contact.

Plasmodesmata in Plant Cells

It might seem that the nonliving cell walls of plants would isolate plant cells from one another. But in fact, as shown in **Figure 6.29**, many plant cell walls are perforated with **plasmodesmata** (singular, *plasmodesma*; from the Greek *desma*, bond), channels that connect cells. The plasma

▼ Figure 6.29 Plasmodesmata between plant cells. The cytoplasm of one plant cell is continuous with the cytoplasm of its neighbors via plasmodesmata, cytoplasmic channels through the cell walls (TEM).

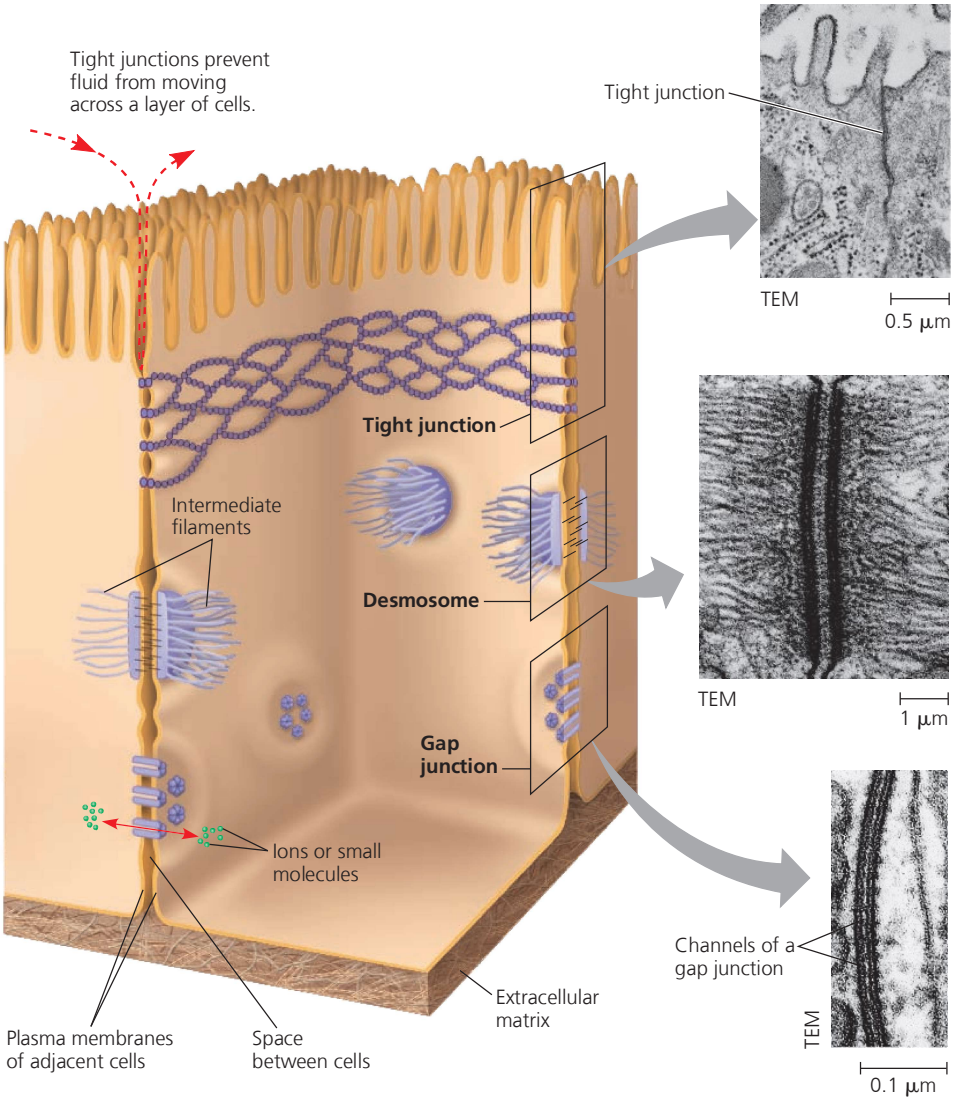


membranes of adjacent cells line the channel of each plasmodesma and thus are continuous. Because the channels are filled with cytosol, the cells share the same internal chemical environment. By joining adjacent cells, plasmodesmata unify most of the plant into one living continuum. Water and small solutes can pass freely from cell to cell, and several experiments have shown that in some circumstances, certain proteins and RNA molecules can do this as well (see Concept 36.6). The macromolecules transported to neighboring cells appear to reach the plasmodesmata by moving along fibers of the cytoskeleton.

Tight Junctions, Desmosomes, and Gap Junctions in Animal Cells

In animals, there are three main types of cell junctions: *tight junctions, desmosomes*, and *gap junctions*. (Gap junctions are most like the plasmodesmata of plants, although gap junction pores are not lined with membrane—rather, they consist of proteins extending from each cell's membrane that form a connecting pore.) All three types of cell junctions are especially common in epithelial tissue, which lines the external and internal surfaces of the body. **Figure 6.30** uses epithelial cells of the intestinal lining to illustrate these junctions.

▼ Figure 6.30 Exploring Cell Junctions in Animal Tissues



Tight Junctions

At **tight junctions**, the plasma membranes of neighboring cells are very tightly pressed against each other, bound together by specific proteins. Forming continuous seals around the cells, tight junctions establish a barrier that prevents leakage of extracellular fluid across a layer of epithelial cells (see red dashed arrow). For example, tight junctions between skin cells make us watertight.

Desmosomes

Desmosomes (one type of *anchoring junction*) function like rivets, fastening cells together into strong sheets. Intermediate filaments made of sturdy keratin proteins anchor desmosomes in the cytoplasm. Desmosomes attach muscle cells to each other in a muscle. Some "muscle tears" involve the rupture of desmosomes.

Gap Junctions

Gap junctions (also called *communicating junctions*) provide cytoplasmic channels from one cell to an adjacent cell and in this way are similar in their function to the plasmodesmata in plants. Gap junctions consist of membrane proteins extending from the membranes of the two cells. These proteins create pores through which ions, sugars, amino acids, and other small molecules may pass. Gap junctions are necessary for communication between cells in many types of tissues, such as heart muscle, and in animal embryos.

Mastering Biology Animation: Cell Junctions

CONCEPT CHECK 6.7

1. In what way are the cells of plants and animals structurally different from single-celled eukaryotes?
2. **WHAT IF?** If the plant cell wall or the animal extracellular matrix were impermeable, what effect would this have on cell function?
3. **MAKE CONNECTIONS** The polypeptide chain that makes up a tight junction weaves back and forth through the membrane four times, with two extracellular loops and one loop plus short C-terminal and N-terminal tails in the cytoplasm. Looking at Figure 5.14, what would you predict about the amino acid sequence of the tight junction protein?

For suggested answers, see Appendix A.

CONCEPT 6.8

A cell is greater than the sum of its parts

From our panoramic view of the cell's compartmental organization to our close-up inspection of each organelle's architecture, this tour of the cell has shown the correlation of structure with function. (See Figure 6.8 to review cell structure.)

Mastering Biology **BioFlix® Animations: Tour of an Animal Cell • Tour of a Plant Cell**

Remember that none of a cell's components work alone. For example, consider the microscopic scene in **Figure 6.31**. The large cell is a macrophage (see Figure 6.13a). It helps defend the mammalian body against infections by ingesting bacteria (the smaller cells) into phagocytic vesicles. The macrophage crawls along a surface and reaches out to the bacteria with thin pseudopodia (specifically, filopodia). Actin filaments interact with other elements of the cytoskeleton in these movements. After the macrophage engulfs the bacteria, they are destroyed by lysosomes produced by the elaborate endomembrane system. The digestive enzymes of the lysosomes and the proteins of the cytoskeleton are all made by ribosomes. And the synthesis of these proteins is programmed by genetic messages dispatched from the DNA in the nucleus. All these processes require energy, which mitochondria supply in the form of ATP.

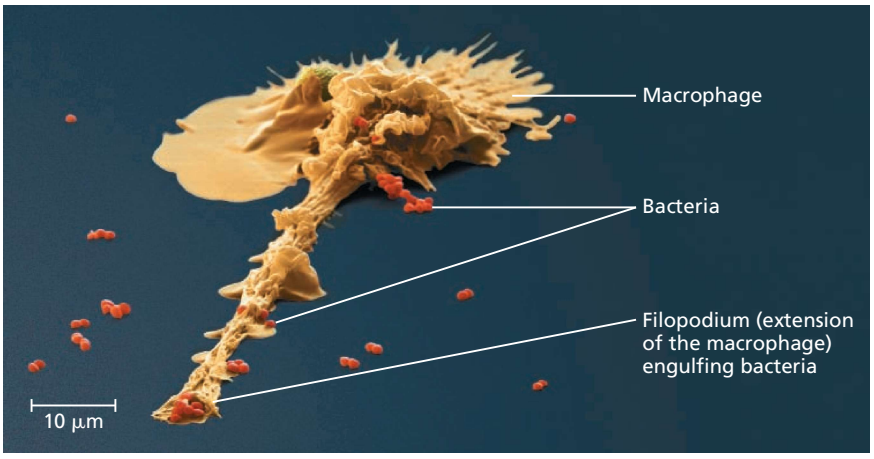
Cellular functions arise from cellular order: The cell is a living unit greater than the sum of its parts. The cell in Figure 6.31 is a good example of integration of cellular processes, seen from the outside. But what about the internal organization of a cell? As you proceed in your study of biology to consider different cellular processes, it will be helpful to try to visualize the architecture and furnishings inside a cell. **Figure 6.32** is designed to introduce you to some important biological molecules and molecules and to help you get a sense of their relative sizes and organization in the context of cellular structures and organelles. As you study this figure, see if you can shrink yourself down to the size of a protein and contemplate your surroundings.

CONCEPT CHECK 6.8

1. *Colpidium colpoda* is a unicellular protist that lives in freshwater, eats bacteria, and moves by cilia (see Figure 6.23b). Describe how the parts of this cell work together in the functioning of *C. colpoda*, including as many organelles and other cell structures as you can.

For suggested answers, see Appendix A.

▼ Figure 6.31 Coordination of activities in a cell. The ability of this macrophage to recognize, apprehend, and destroy bacteria involves coordination among components such as the cytoskeleton, lysosomes, and plasma membrane (colorized SEM).

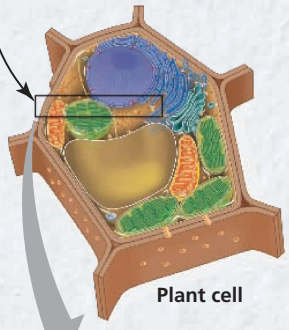


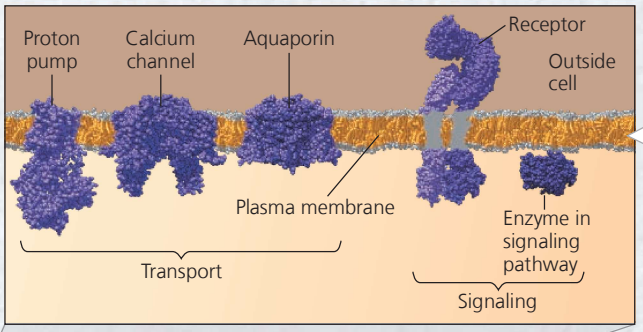
Mastering Biology **Animation: Review of Animal Cell Structure and Function**

▼ Figure 6.32 Visualizing the Scale of the Molecular Machinery in a Cell

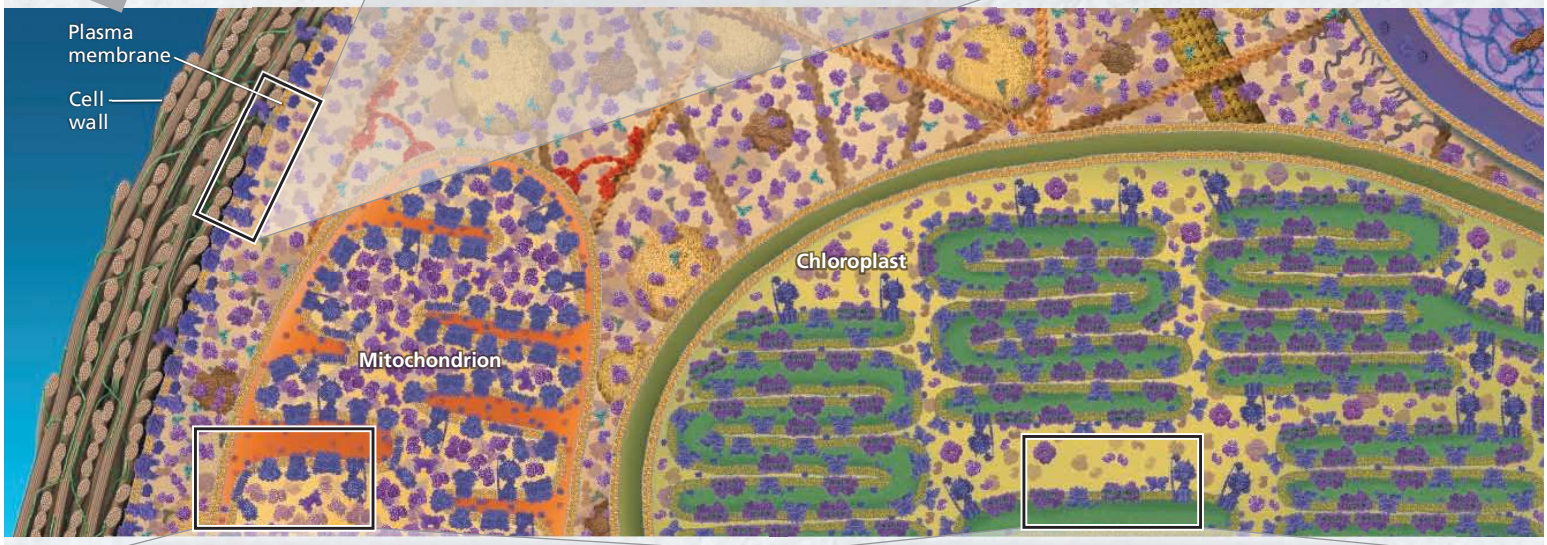
A slice of a plant cell's interior is illustrated in the center panel, with all structures and molecules drawn to scale. Selected molecules and structures are shown above and below, all enlarged by the same factor so you can compare their sizes. All protein and nucleic acid structures are based on data from the Protein Data Bank; regions whose structure has not yet been determined are shown in gray.

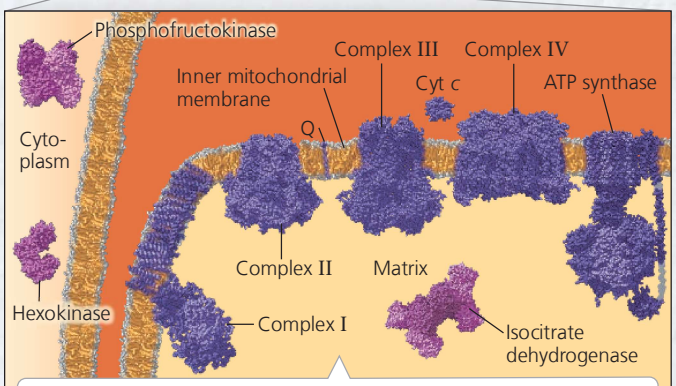
This figure introduces a cast of characters that you will learn more about as you study biology. Refer back to this figure as you encounter these molecules in your studies.



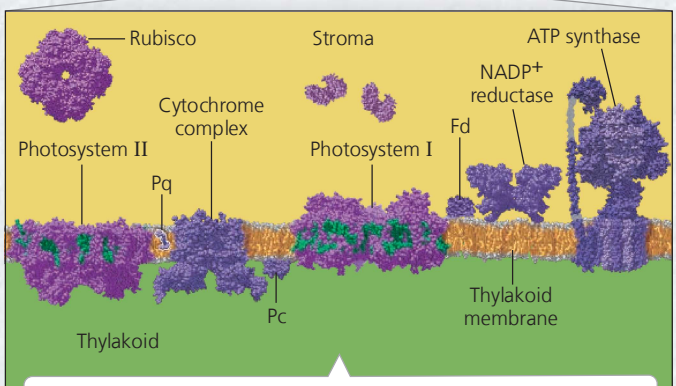


(a) Membrane proteins (Chapter 7) Proteins embedded in cellular membranes help transport substances and conduct signals across membranes. They also participate in other crucial cellular functions. Many proteins are able to move within the membrane.



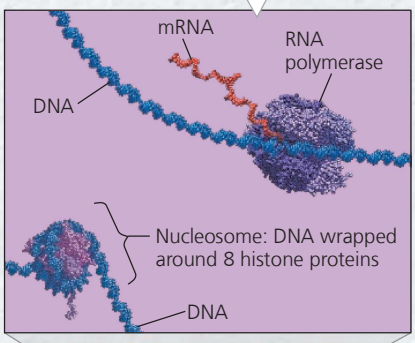


(b) Cellular respiration (Chapter 9) Cellular respiration, a multi-step process, generates ATP from food molecules. The first two stages are carried out by enzymes in the cytoplasm and mitochondrial matrix; a few of these enzymes (pinkish-purple) are shown. The final stage is carried out by proteins (bluish-purple) that form a "chain" in the inner mitochondrial membrane.



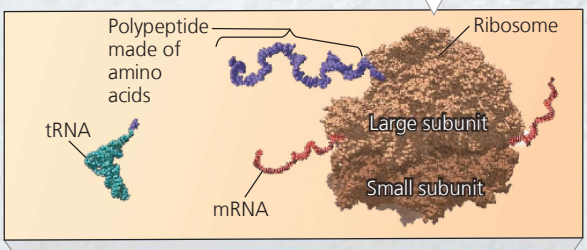
(c) Photosynthesis (Chapter 10) Photosynthesis produces sugars that provide food for all life on the planet. The process begins with large complexes of proteins and chlorophyll (green) embedded in the thylakoid membranes. These complexes trap light energy in molecules that are used by rubisco and other proteins in the stroma to make sugars.

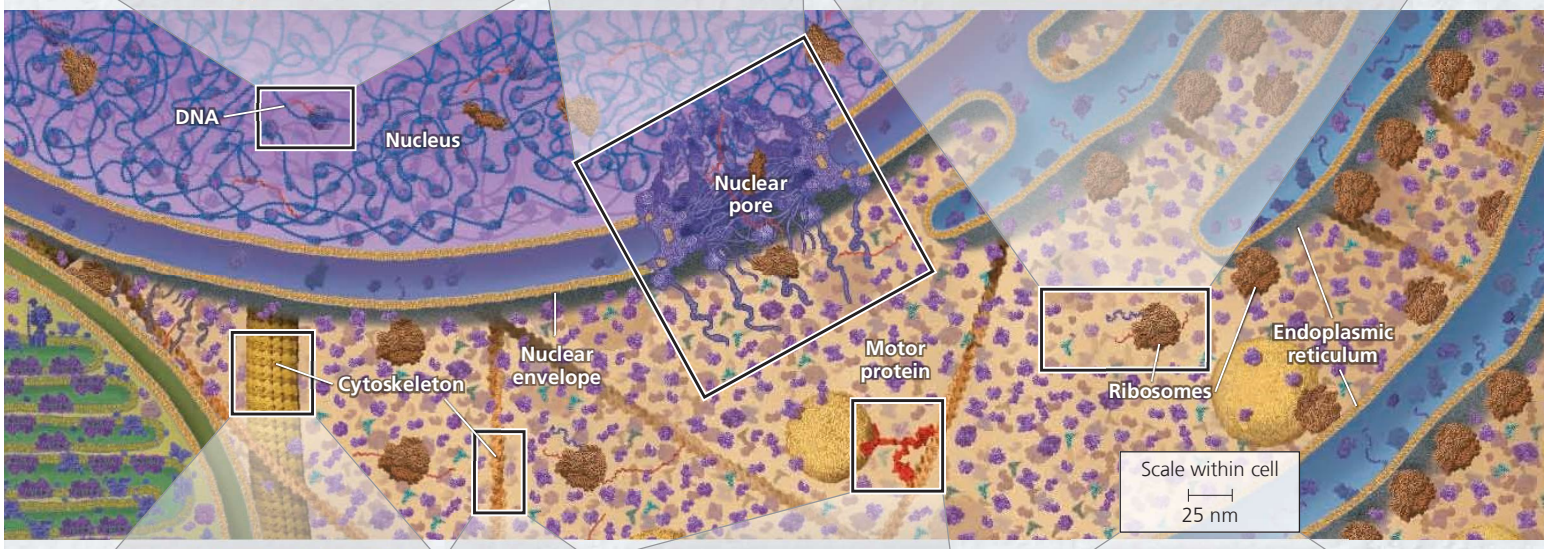
(d) Transcription (Chapter 17) In the nucleus, the information contained in a DNA sequence is transferred to messenger RNA (mRNA) by an enzyme called RNA polymerase. After their synthesis, mRNA molecules leave the nucleus via nuclear pores.

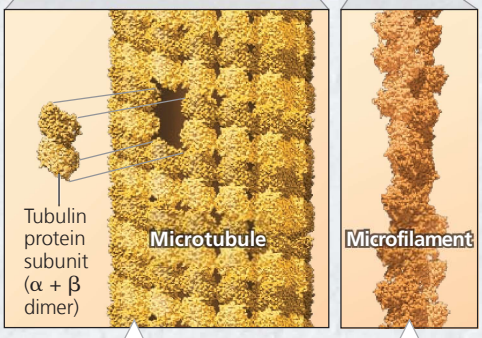


(e) Nuclear pore (Concept 6.3) The nuclear pore complex regulates molecular traffic in and out of the nucleus, which is bounded by a double membrane. Among the largest structures that pass through the pore are the ribosomal subunits, which are built in the nucleus.

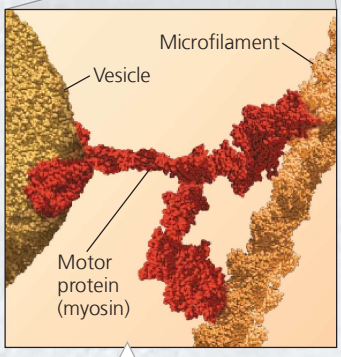
(f) Translation (Chapter 17) In the cytoplasm, the information in mRNA is used to assemble a polypeptide with a specific sequence of amino acids. Both transfer RNA (tRNA) molecules and ribosomes play a role. The eukaryotic ribosome, which includes a large subunit and a small subunit, is a colossal complex composed of four large ribosomal RNA (rRNA) molecules and more than 80 proteins. Through transcription and translation, the nucleotide sequence of DNA in a gene determines the amino acid sequence of a polypeptide, via the intermediary mRNA.







(g) Cytoskeleton (Concept 6.6)
Cytoskeletal structures are polymers of protein subunits. Microtubules are hollow structural rods made of tubulin protein subunits, while microfilaments are cables that have two chains of actin proteins wound around each other.



(h) Motor proteins (Concept 6.6) Motor proteins, such as myosin, are responsible for transport of vesicles and movement of organelles within the cell.

25 nm
Scale of enlarged structures

1. ***List the following structures from largest to smallest: proton pump, nuclear pore, cyt c, ribosome.***

2. ***Considering the structures of a nucleosome and of RNA polymerase, speculate about what must happen before RNA polymerase can transcribe the DNA that is wrapped around the histone proteins of a nucleosome.***

3. ***Find another myosin motor protein walking on a microfilament in this figure. What organelle is being moved by that myosin protein?***

Instructors: Additional questions related to this Visualizing Figure can be assigned in **Mastering Biology**.

6 Chapter Review

Go to **Mastering Biology** for Assignments, the eText, the Study Area, and Dynamic Study Modules.

SUMMARY OF KEY CONCEPTS

To review key terms, go to the **Vocabulary Self-Quiz** in the **Mastering Biology** eText or Study Area, or go to goo.gl/zkjz9t.

CONCEPT 6.1

Biologists use microscopes and biochemistry to study cells *(pp. 94–97)*

- Improvements in microscopy that affect the parameters of magnification, resolution, and contrast have catalyzed progress in the study of cell structure. **Light microscopy** (LM) and **electron microscopy** (EM), as well as other types, remain important tools.
- Cell biologists can obtain pellets enriched in particular cellular components by centrifuging disrupted cells at sequential speeds, a process known as **cell fractionation**.

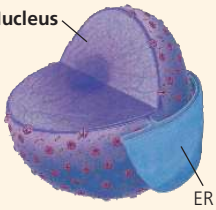
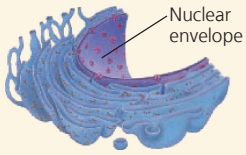
? *How do microscopy and biochemistry complement each other to reveal cell structure and function?*

CONCEPT 6.2

Eukaryotic cells have internal membranes that compartmentalize their functions *(pp. 97–102)*

- All cells are bounded by a **plasma membrane**.
- **Prokaryotic cells** lack nuclei and other membrane-enclosed **organelles**, while **eukaryotic cells** have internal membranes that compartmentalize cellular functions.
- The surface-to-volume ratio is an important parameter affecting cell size and shape.
- Plant and animal cells have most of the same organelles: a nucleus, endoplasmic reticulum, Golgi apparatus, and mitochondria. Chloroplasts are present only in cells of photosynthetic eukaryotes.

? *Explain how the compartmental organization of a eukaryotic cell contributes to its biochemical functioning.*

	Cell Component	Structure	Function
CONCEPT 6.3 **The eukaryotic cell's genetic instructions are housed in the nucleus and carried out by the ribosomes** *(pp. 102–104)* ? *Describe the relationship between the nucleus and ribosomes.*	**Nucleus** 	Surrounded by nuclear envelope (double membrane) perforated by nuclear pores; nuclear envelope is continuous with endoplasmic reticulum (ER)	Houses chromosomes, which are made of chromatin (DNA and proteins); contains nucleoli, where ribosomal subunits are made; pores regulate entry and exit of materials
	Ribosome	Two subunits made of ribosomal RNAs and proteins; can be free in cytosol or bound to ER	Protein synthesis
CONCEPT 6.4 **The endomembrane system regulates protein traffic and performs metabolic functions** *(pp. 104–109)* ? *Describe the key role played by transport vesicles in the endomembrane system.*	**Endoplasmic reticulum (ER)** 	Extensive network of membrane-bounded tubules and sacs; ER membrane separates lumen from cytosol and is continuous with nuclear envelope	**Smooth ER:** synthesis of lipids, metabolism of carbohydrates, storage of calcium ions, detoxification of drugs and poisons **Rough ER:** aids in synthesis of secretory and other proteins on bound ribosomes; adds carbohydrates to proteins to make glycoproteins; produces new membrane
	Golgi apparatus	Stacks of flattened membranous sacs; has polarity (*cis* and *trans* faces)	Modification of proteins, carbohydrates on proteins, and phospholipids; synthesis of many polysaccharides; sorting of Golgi products, which are then released in vesicles
	Lysosome	Membranous sac of hydrolytic enzymes (in animal cells)	Breakdown of ingested substances, cell macromolecules, and damaged organelles for recycling
	Vacuole	Large membrane-bounded vesicle	Digestion, storage, waste disposal, water balance, cell growth, and protection

	Cell Component	Structure	Function
CONCEPT 6.5 **Mitochondria and chloroplasts change energy from one form to another** *(pp. 109–112)* ? *What does the endosymbiont theory propose as the origin for mitochondria and chloroplasts? Explain.*	Mitochondrion	Bounded by double membrane; inner membrane has infoldings	Cellular respiration
	Chloroplast	Typically two membranes around fluid stroma, which contains thylakoids stacked into grana	Photosynthesis (chloroplasts are present in cells of photosynthetic eukaryotes, including plants)
	Peroxisome	Specialized metabolic compartment bounded by a single membrane	Contains enzymes that transfer H atoms from substrates to oxygen, producing H_2O_2 (hydrogen peroxide), which is converted to H_2O

CONCEPT 6.6

The cytoskeleton is a network of fibers that organizes structures and activities in the cell *(pp. 112–117)*

- The **cytoskeleton** functions in structural support for the cell and in motility and signal transmission.
- **Microtubules** shape the cell, guide organelle movement, and separate chromosomes in dividing cells. **Cilia** and **flagella** are motile appendages containing microtubules. Primary cilia also play sensory and signaling roles. **Microfilaments** are thin rods that function in muscle contraction, amoeboid movement, **cytoplasmic streaming**, and support of microvilli. **Intermediate filaments** support cell shape and fix organelles in place.

? *Describe the role of motor proteins inside the eukaryotic cell and in whole-cell movement.*

CONCEPT 6.7

Extracellular components and connections between cells help coordinate cellular activities *(pp. 118–121)*

- Plant **cell walls** are made of cellulose fibers embedded in other polysaccharides and proteins.
- Animal cells secrete glycoproteins and **proteoglycans** that form the **extracellular matrix (ECM)**, which functions in support, adhesion, movement, and regulation.
- Cell junctions connect neighboring cells. Plants have **plasmodesmata** that pass through adjoining cell walls. Animal cells have **tight junctions, desmosomes**, and **gap junctions**.

? *Compare the structure and functions of a plant cell wall and the extracellular matrix of an animal cell.*

CONCEPT 6.8

A cell is greater than the sum of its parts *(pp. 121–123)*

Many components work together in a functioning cell.

? *When a cell ingests a bacterium, what role does the nucleus play?*

TEST YOUR UNDERSTANDING

For more multiple-choice questions, go to the **Practice Test** in the **Mastering Biology** eText or Study Area, or go to goo.gl/GruWRg.

Levels 1-2: Remembering/Understanding

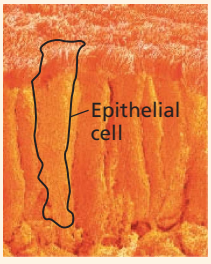
1. Which structure is part of the endomembrane system?
   (A) mitochondrion (C) chloroplast
   (B) Golgi apparatus (D) centrosome
2. Which structure is common to plant *and* animal cells?
   (A) chloroplast (C) mitochondrion
   (B) central vacuole (D) centriole
3. Which of the following is present in a prokaryotic cell?
   (A) mitochondrion (C) nuclear envelope
   (B) ribosome (D) chloroplast

Levels 3-4: Applying/Analyzing

4. Cyanide binds to at least one molecule involved in producing ATP. In a cell exposed to cyanide, most of the cyanide will be in
   (A) mitochondria. (C) peroxisomes.
   (B) ribosomes. (D) lysosomes.
5. Which cell would be best for studying lysosomes?
   (A) muscle cell (C) bacterial cell
   (B) nerve cell (D) phagocytic white blood cell
6. **DRAW IT** Draw two eukaryotic cells. Label the structures listed here and show any physical connections between the structures of each cell: nucleus, rough ER, smooth ER, mitochondrion, centrosome, chloroplast, vacuole, lysosome, microtubule, cell wall, ECM, microfilament, Golgi apparatus, intermediate filament, plasma membrane, peroxisome, ribosome, nucleolus, nuclear pore, vesicle, flagellum, microvilli, plasmodesma.

Levels 5-6: Evaluating/Creating

7. **EVOLUTION CONNECTION** (a) What cell structures best reveal evolutionary unity? (b) Give an example of diversity related to specialized cellular modifications.
8. **SCIENTIFIC INQUIRY** Imagine protein X, destined to span the plasma membrane; assume the mRNA carrying the genetic message for protein X has been translated by ribosomes in a cell culture. If you fractionate the cells (see Figure 6.4), in which fraction would you find protein X? Explain by describing its transit.
9. **WRITE ABOUT A THEME: ORGANIZATION** Write a short essay (100–150 words) on this topic: Life is an emergent property that appears at the level of the cell. (See Concept 1.1.)
10. **SYNTHESIZE YOUR KNOWLEDGE**



The cells in this SEM are epithelial cells from the small intestine. Discuss how their cellular structure contributes to their specialized functions of nutrient absorption and as a barrier between the intestinal contents and the blood supply on the other side of the sheet of epithelial cells.

For selected answers, see Appendix A.

Explore Scientific Papers with Science in the Classroom AAAS
How do actin networks affect myosin motors?
Go to "Fussy about Filaments" at www.scienceintheclassroom.org.
Instructors: Questions can be assigned in Mastering Biology.

7 Membrane Structure and Function

KEY CONCEPTS

7.1 Cellular membranes are fluid mosaics of lipids and proteins *p. 127*

7.2 Membrane structure results in selective permeability *p. 131*

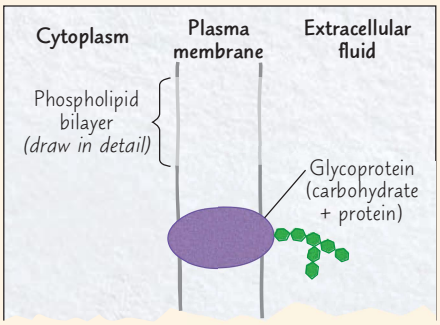
7.3 Passive transport is diffusion of a substance across a membrane with no energy investment *p. 132*

7.4 Active transport uses energy to move solutes against their gradients *p. 136*

7.5 Bulk transport across the plasma membrane occurs by exocytosis and endocytosis *p. 139*

Study Tip

Make a visual study guide: Draw a plasma membrane (two lines) all the way down a piece of paper (or digitally). Label the cytoplasm and the extracellular fluid. At the top, draw the phospholipid bilayer in detail. As you read the chapter, draw and label membrane proteins that you encounter and diagram the different ways materials can enter or leave a cell.



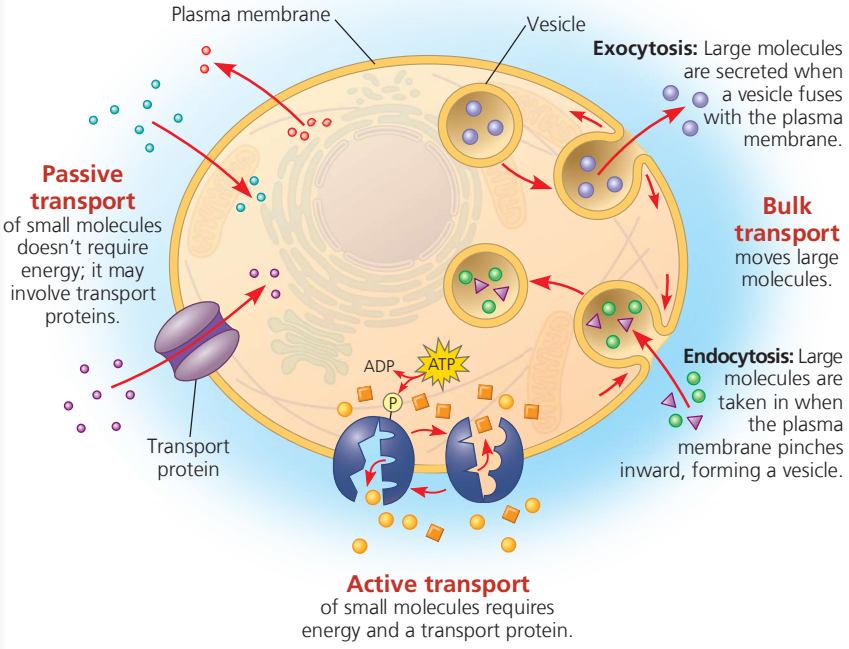
Go to Mastering Biology

For Students (in eText and Study Area)
- Get Ready for Chapter 7
- Figure 7.12 Walkthrough: Osmosis
- BioFlix® Animation: Membrane Transport

For Instructors (in Item Library)
- Tutorial: Membrane Transport: Diffusion and Passive Transport
- Tutorial: Membrane Transport: Bulk Transport

Figure 7.1 Successful learning relies on communication between brain cells. Here, the vesicles fusing with the plasma membrane of the top cell release molecules (yellow) that bind to membrane proteins (light green) on the surface of the bottom cell, triggering the proteins to change shape. The plasma membrane that surrounds each cell regulates its exchanges with its environment and surrounding cells.

How does the plasma membrane regulate inbound and outbound traffic?



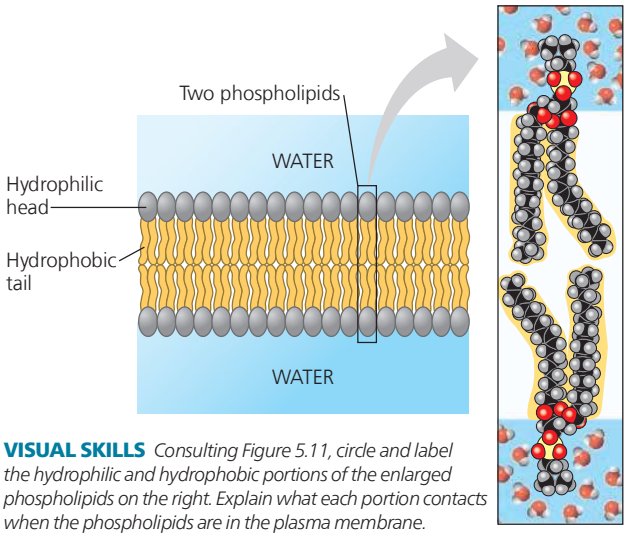
CONCEPT 7.1

Cellular membranes are fluid mosaics of lipids and proteins

Lipids and proteins are the staple ingredients of membranes, although carbohydrates are also important. The most abundant lipids in most membranes are phospholipids. Their ability to form membranes is inherent in their molecular structure. A phospholipid is an **amphipathic** molecule, meaning it has both a hydrophilic ("water-loving") region and a hydrophobic ("water-fearing") region (see Figure 5.11). A phospholipid bilayer can exist as a stable boundary between two aqueous compartments because the molecular arrangement shelters the hydrophobic tails of the phospholipids from water while exposing the hydrophilic heads to water **(Figure 7.2)**.

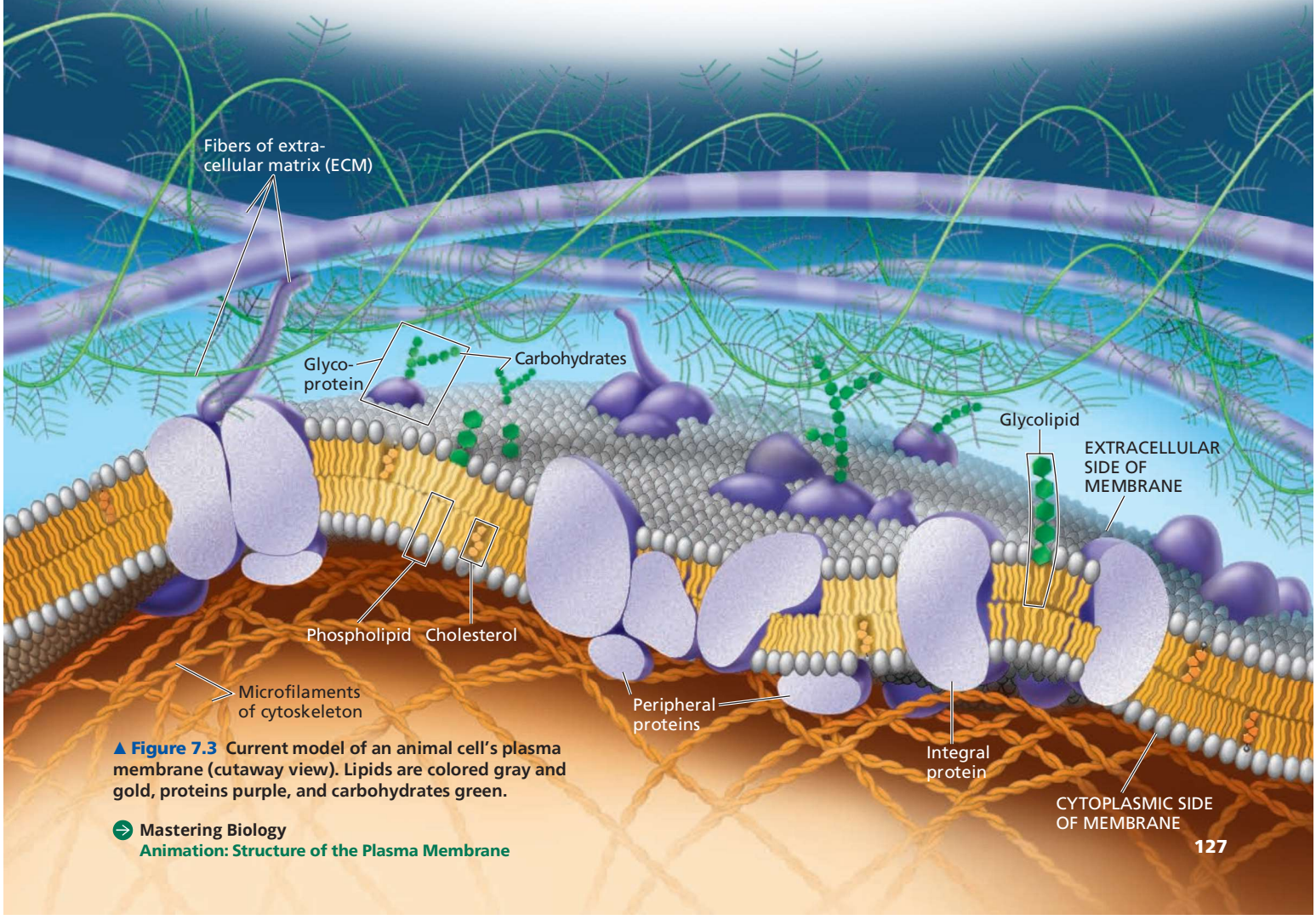
Like membrane lipids, most membrane proteins are amphipathic. Such proteins can reside in the phospholipid bilayer with their hydrophilic regions protruding. This molecular orientation maximizes contact of hydrophilic regions of a protein with water in the cytosol and extracellular fluid, while providing their hydrophobic parts with a nonaqueous environment.

▼ **Figure 7.2** **Phospholipid bilayer (cross section).**



VISUAL SKILLS *Consulting Figure 5.11, circle and label the hydrophilic and hydrophobic portions of the enlarged phospholipids on the right. Explain what each portion contacts when the phospholipids are in the plasma membrane.*

Figure 7.3 shows the currently accepted model of the arrangement of molecules in the plasma membrane. In this **fluid mosaic model**, the membrane is a mosaic of protein molecules bobbing in a fluid bilayer of phospholipids.



▲ **Figure 7.3** **Current model of an animal cell's plasma membrane (cutaway view). Lipids are colored gray and gold, proteins purple, and carbohydrates green.**

Mastering Biology
Animation: Structure of the Plasma Membrane

The proteins are not randomly distributed in the membrane, however. Groups of proteins are often associated in long-lasting, specialized patches, where they carry out common functions. Researchers have found specific lipids in these patches as well and have proposed naming them *lipid rafts*; however, the debate continues about whether such structures exist in living cells or are an artifact of biochemical techniques. In some regions, the membrane may be much more tightly packed with proteins than shown in Figure 7.3. Like all models, the fluid mosaic model is continually being refined as new research reveals more about membrane structure.

The Fluidity of Membranes

Membranes are not static sheets of molecules locked rigidly in place. A membrane is held together mainly by hydrophobic interactions, which are much weaker than covalent bonds (see Figure 5.18). Most of the lipids and some proteins can shift about sideways—that is, in the plane of the membrane, like partygoers elbowing their way through a crowded room. Very rarely, also, a lipid may flip-flop across the membrane, switching from one phospholipid layer to the other.

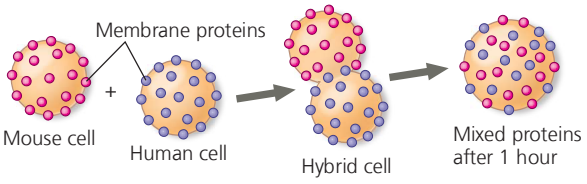
The sideways movement of phospholipids within the membrane is rapid. Adjacent phospholipids switch positions about 10^7 times per second, which means that a phospholipid can travel about 2 μm—the length of a typical bacterial cell—in 1 second. Proteins are much larger than lipids and move more slowly, when they do move. Many membrane proteins seem to be held immobile by their attachment to the cytoskeleton or to the extracellular matrix (see Figure 7.3).

▼ Figure 7.4 Inquiry

Do membrane proteins move?

Experiment Larry Frye and Michael Edidin, at Johns Hopkins University, labeled the plasma membrane proteins of a mouse cell and a human cell with two different markers and fused the cells. Using a microscope, they observed the markers on the hybrid cell.

Results



Conclusion The mixing of the mouse and human membrane proteins indicates that at least some membrane proteins move sideways within the plane of the plasma membrane.

Data from L. D. Frye and M. Edidin, The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons, *Journal of Cell Science* 7:319 (1970).

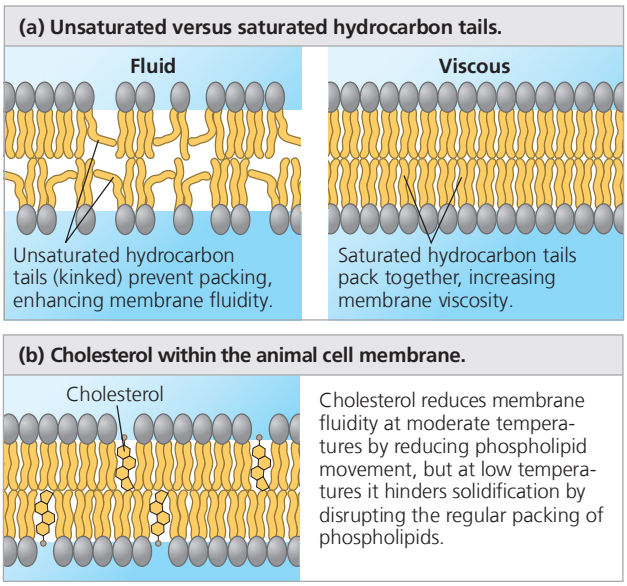
WHAT IF? *Suppose the proteins did not mix in the hybrid cell, even many hours after fusion. Could you conclude that proteins don't move within the membrane? What other explanation could there be?*

Some membrane proteins seem to move in a highly directed manner, perhaps driven along cytoskeletal fibers by motor proteins. And other proteins simply drift in the membrane, as shown the classic experiment described in **Figure 7.4**.

A membrane remains fluid as temperature decreases until the phospholipids settle into a closely packed arrangement and the membrane solidifies, much as bacon grease forms lard when it cools. The temperature at which a membrane solidifies depends on the types of lipids it is made of. As the temperature decreases, the membrane remains fluid to a lower temperature if it is rich in phospholipids with unsaturated hydrocarbon tails (see Figures 5.10 and 5.11). Because of kinks in the tails where double bonds are located, unsaturated hydrocarbon tails cannot pack together as closely as saturated hydrocarbon tails, making the membrane more fluid **(Figure 7.5a)**.

The steroid cholesterol, which is wedged between phospholipid molecules in the plasma membranes of animal cells, has different effects on membrane fluidity at different temperatures **(Figure 7.5b)**. At relatively high temperatures—at 37°C, the body temperature of humans, for example—cholesterol makes the membrane less fluid by restraining phospholipid movement. However, because cholesterol also hinders the close packing of phospholipids, it lowers the temperature required for the membrane to solidify. Thus, cholesterol can be thought of as a "fluidity buffer" for the membrane, resisting changes in membrane fluidity that can be caused by changes in temperature. Compared to animals, plants have very low levels of cholesterol; rather, related steroid lipids buffer membrane fluidity in plant cells.

▼ Figure 7.5 Factors that affect membrane fluidity.



Membranes must be fluid to work properly; the fluidity of a membrane affects both its permeability and the ability of membrane proteins to move to where their function is needed. Usually, membranes are about as fluid as olive oil. When a membrane solidifies, its permeability changes, and enzymatic proteins in the membrane may become inactive if their activity requires movement within the membrane. However, membranes that are too fluid cannot support protein function either. Therefore, extreme environments (for example, those with extreme temperatures) pose a challenge for life, resulting in evolutionary adaptations that include differences in membrane lipid composition.

Evolution of Differences in Membrane Lipid Composition

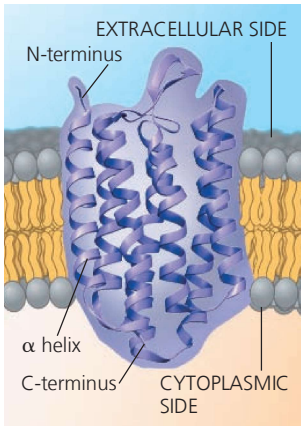
EVOLUTION Variations in the cell membrane lipid compositions of many species appear to be evolutionary adaptations that maintain the appropriate membrane fluidity under specific environmental conditions. For instance, fishes that live in extreme cold have membranes with a high proportion of unsaturated hydrocarbon tails, enabling their membranes to remain fluid in spite of the low temperature (see Figure 7.5a). At the other extreme, some bacteria and archaea thrive at temperatures greater than 90°C (194°F) in thermal hot springs and geysers. Their membranes include unusual lipids that may prevent excessive fluidity at such high temperatures.

The ability to change the lipid composition of cell membranes in response to changing temperatures has evolved in organisms that live where temperatures vary. In many plants that tolerate extreme cold, such as winter wheat, the percentage of unsaturated phospholipids increases in autumn, an adjustment that keeps the membranes from solidifying during winter. Some bacteria and archaea also exhibit different proportions of unsaturated phospholipids in their cell membranes, depending on the temperature at which they are growing. Overall, natural selection has apparently favored organisms whose mix of membrane lipids ensures an appropriate level of membrane fluidity for their environment.

Membrane Proteins and Their Functions

Now we come to the *mosaic* aspect of the fluid mosaic model. Somewhat like a tile mosaic (shown here), a membrane is a collage of different proteins, often clustered together in groups, embedded in the fluid matrix of the lipid bilayer (see Figure 7.3). More than 50 kinds of proteins have been found in the plasma membrane of red blood cells, for example. Phospholipids form the main fabric of the membrane, but proteins determine most of the membrane's functions. Different types of cells contain different sets of membrane proteins, and the various membranes within a cell each have a unique collection of proteins.

◀ **Tile mosaic.**



▶ **Figure 7.6 The structure of a transmembrane protein.** Bacteriorhodopsin (a bacterial transport protein) has a distinct orientation in the membrane, with its N-terminus outside the cell and its C-terminus inside. This ribbon model highlights the secondary structure of the hydrophobic parts, including seven transmembrane α helices, which lie mostly within the hydrophobic interior of the membrane. The nonhelical hydrophilic segments are in contact with the aqueous solutions on the extracellular and cytoplasmic sides of the membrane.

Notice in Figure 7.3 that there are two major populations of membrane proteins: integral proteins and peripheral proteins. **Integral proteins** penetrate the hydrophobic interior of the lipid bilayer. The majority are **transmembrane proteins**, which span the membrane; other integral proteins extend only partway into the hydrophobic interior. The hydrophobic regions of an integral protein consist of one or more stretches of nonpolar amino acids (see Figure 5.14), typically 20–30 amino acids in length, usually coiled into α helices **(Figure 7.6)**. The hydrophilic parts of the molecule are exposed to the aqueous solutions on either side of the membrane. Some proteins also have one or more hydrophilic channels that allow passage through the membrane of hydrophilic substances (even of water itself). **Peripheral proteins** are not embedded in the lipid bilayer at all; they are loosely bound to the surface of the membrane, often to exposed parts of integral proteins (see Figure 7.3).

On the cytoplasmic side of the plasma membrane, some membrane proteins are held in place by attachment to the cytoskeleton. And on the extracellular side, certain membrane proteins may attach to materials outside the cell. For example, in animal cells, membrane proteins may be attached to fibers of the extracellular matrix (see Figure 6.28; *integrins* are one type of integral, transmembrane protein). These attachments combine to give animal cells a stronger framework than the plasma membrane alone could provide.

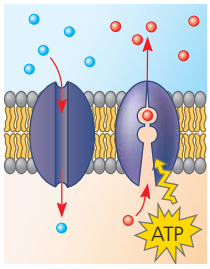
Figure 7.7 illustrates six major functions performed by proteins of the plasma membrane. A single cell may have different membrane proteins that carry out various functions, and one protein may itself carry out multiple functions. Thus, the membrane is a functional mosaic as well as a structural one.

Mastering Biology **Animation: Functions of the Plasma Membrane**

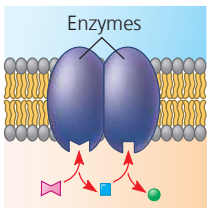
Proteins on a cell's surface are important in the medical field. For example, a protein called CD4 on the surface

▼ Figure 7.7 Some functions of membrane proteins. In many cases, a single protein performs multiple tasks.

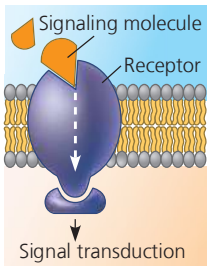
(a) Transport. *Left:* A protein that spans the membrane may provide a hydrophilic channel across the membrane that is selective for a particular solute (see Figures 6.32a and 7.15a). *Right:* Other transport proteins shuttle a substance from one side to the other by changing shape (see Figure 7.15b). Some of these proteins hydrolyze ATP as an energy source to actively pump substances across the membrane.



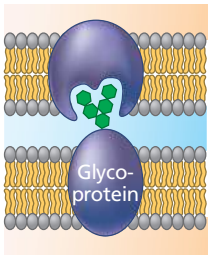
(b) Enzymatic activity. A protein built into the membrane may be an enzyme with its active site (where the reactant binds) exposed to substances in the adjacent solution. In some cases, several enzymes in a membrane are organized as a team that carries out sequential steps of a metabolic pathway.



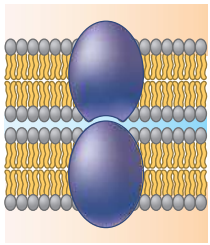
(c) Signal transduction. A membrane protein (receptor) may have a binding site with a specific shape that fits the shape of a chemical messenger, such as a hormone. The external messenger (signaling molecule) may cause the protein to change shape, allowing it to relay the message to the inside of the cell, usually by binding to a cytoplasmic protein (see Figures 6.32a and 11.6).



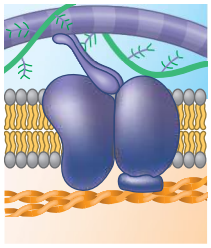
(d) Cell-cell recognition. Some glycoproteins serve as identification tags that are specifically recognized by membrane proteins of other cells. This type of cell-cell binding is usually short-lived compared with that shown in (e).



(e) Intercellular joining. Membrane proteins of adjacent cells may hook together in various kinds of junctions, such as gap junctions or tight junctions (see Figure 6.30). This type of binding is more long-lasting than that shown in (d).



(f) Attachment to the cytoskeleton and extracellular matrix (ECM). Microfilaments or other elements of the cytoskeleton may be noncovalently bound to membrane proteins, a function that helps maintain cell shape and stabilizes the location of certain membrane proteins. Proteins that can bind to ECM molecules can coordinate extracellular and intracellular changes (see Figure 6.28).



VISUAL SKILLS *Some transmembrane proteins can bind to a particular ECM molecule and, when bound, transmit a signal into the cell. Use the proteins shown in (c) and (f) to explain how this might occur.*

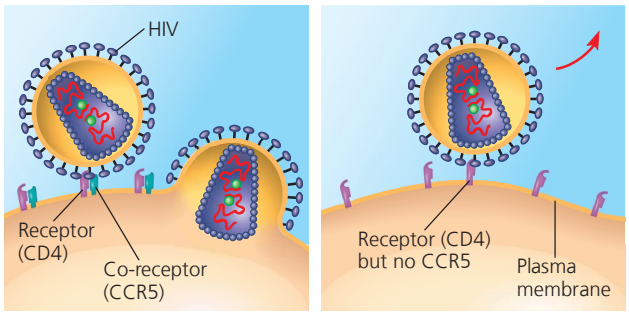
of immune cells helps the human immunodeficiency virus (HIV) infect these cells, leading to acquired immune deficiency syndrome (AIDS). Despite multiple exposures to HIV, however, a small number of people do not develop AIDS and show no evidence of HIV-infected cells. Comparing their genes with the genes of infected individuals, researchers learned that resistant people have an unusual form of a gene that codes for an immune cell-surface protein called CCR5. Further work showed that although CD4 is the main HIV receptor, HIV must also bind to CCR5 as a "co-receptor" to infect most cells **(Figure 7.8a)**. An absence of CCR5 on the cells of resistant individuals, due to the gene alteration, prevents the virus from entering the cells **(Figure 7.8b)**.

This information has been key to developing a treatment for HIV infection. Interfering with CD4 causes dangerous side effects because of its many important functions in cells. Discovery of the CCR5 co-receptor provided a safer target for development of drugs that mask this protein and block HIV entry. One such drug, maraviroc (brand name Selzentry), was approved for treatment of HIV in 2007; ongoing trials to determine its ability to prevent HIV infection in uninfected, at-risk patients have been disappointing.

The Role of Membrane Carbohydrates in Cell-Cell Recognition

Cell-cell recognition, a cell's ability to distinguish one type of neighboring cell from another, is crucial to the functioning of an organism. It is important, for example, in the sorting of cells into tissues and organs in an animal embryo. It is also the basis for the rejection of foreign cells by the immune system, an important line of defense in vertebrate animals (see Concept 43.1). Cells recognize other cells by binding to molecules, often containing carbohydrates, on the extracellular surface of the plasma membrane (see Figure 7.7d).

▼ Figure 7.8 The genetic basis for HIV resistance.



(a) HIV can infect a cell with CCR5 on its surface, as in most people.

(b) HIV cannot infect a cell lacking CCR5 on its surface, as in resistant individuals.

MAKE CONNECTIONS *Study Figures 2.16 and 5.17; each shows pairs of molecules binding to each other. What would you predict about CCR5 that would allow HIV to bind to it? How could a drug molecule interfere with this binding?*

Membrane carbohydrates are usually short, branched chains of fewer than 15 sugar units. Some are covalently bonded to lipids, forming molecules called **glycolipids**. (Recall that *glyco* refers to carbohydrate.) However, most are covalently bonded to proteins, which are thereby **glycoproteins** (see Figure 7.3).

The carbohydrates on the extracellular side of the plasma membrane vary from species to species, among individuals of the same species, and even from one cell type to another in a single individual. The diversity of the molecules and their location on the cell's surface enable membrane carbohydrates to function as markers that distinguish one cell from another. For example, the four human blood types designated A, B, AB, and O reflect variation in the carbohydrate part of glycoproteins on the surface of red blood cells.

Synthesis and Sidedness of Membranes

Membranes have distinct inside and outside faces. The two lipid layers may differ in lipid composition, and each protein has directional orientation in the membrane (see Figure 7.6). **Figure 7.9** shows how membrane sidedness arises: The asymmetrical arrangement of proteins, lipids, and their associated carbohydrates in the plasma membrane is determined as the membrane is being built.

CONCEPT CHECK 7.1

1. **VISUAL SKILLS** Carbohydrates are attached to plasma membrane proteins in the ER (see Figure 7.9). On which side of the vesicle membrane are the carbohydrates during transport to the cell surface?
2. **WHAT IF?** How might the membrane lipid composition of a native grass found in very warm soil around hot springs differ from that of a native grass found in cooler soil? Explain.

For suggested answers, see Appendix A.

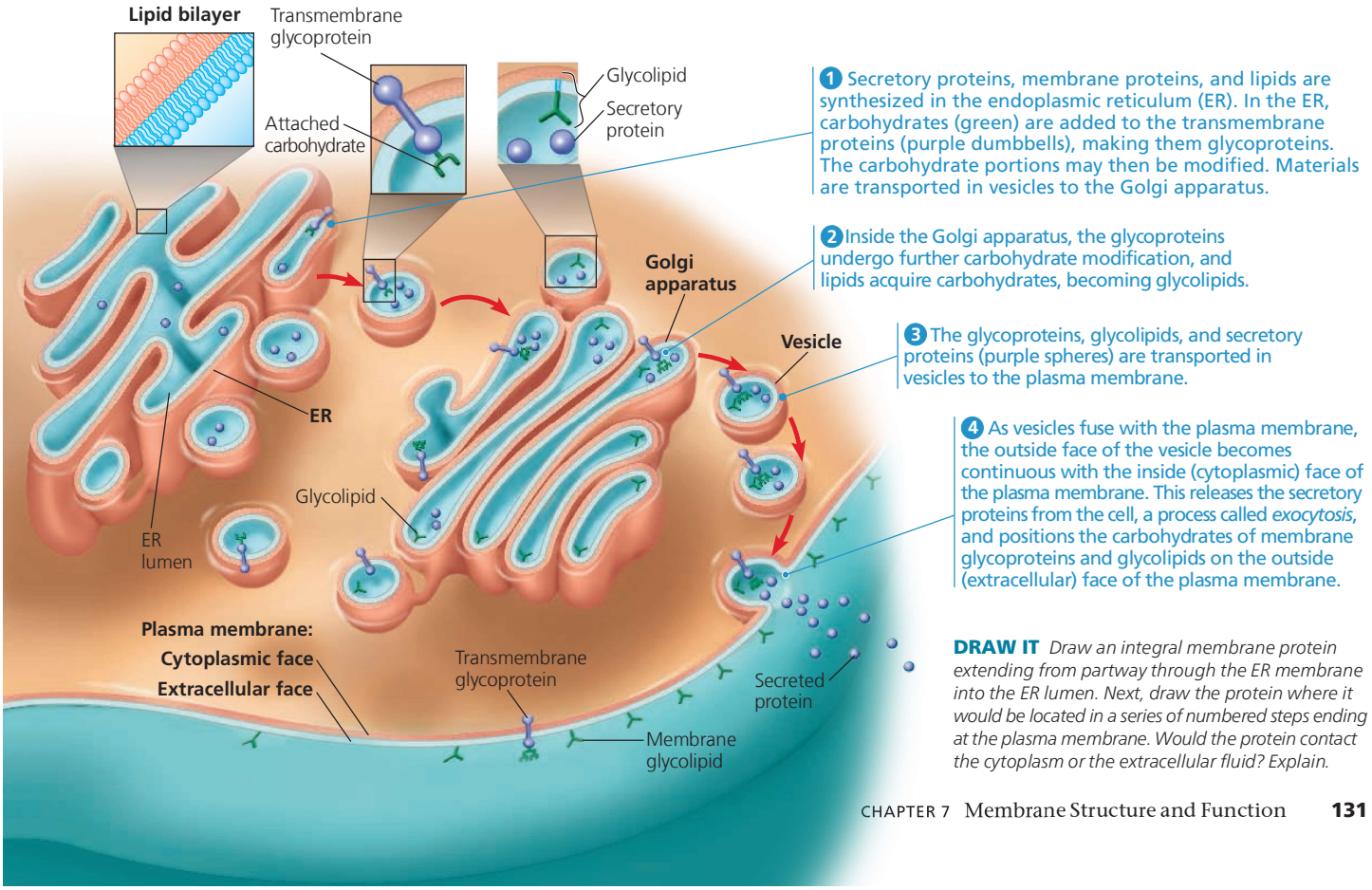
CONCEPT 7.2

Membrane structure results in selective permeability

The biological membrane has emergent properties beyond those of the many individual molecules that make it up. The remainder of this chapter focuses on one of those properties: A membrane exhibits **selective permeability**; that is, it allows some substances to cross more easily than others. The ability to regulate transport across cellular boundaries is essential to the cell's existence. We will see once again that form fits function: The fluid mosaic model helps explain how membranes regulate the cell's molecular traffic.

A steady traffic of small molecules and ions moves across the plasma membrane in both directions. Consider the chemical

▼ Figure 7.9 Synthesis of membrane components and their orientation in the membrane.
The cytoplasmic (orange) face of the plasma membrane differs from the extracellular (aqua) face. The latter arises from the inside face of ER, Golgi, and vesicle membranes.



DRAW IT *Draw an integral membrane protein extending from partway through the ER membrane into the ER lumen. Next, draw the protein where it would be located in a series of numbered steps ending at the plasma membrane. Would the protein contact the cytoplasm or the extracellular fluid? Explain.*

exchanges between a muscle cell and the extracellular fluid that bathes it. Sugars, amino acids, and other nutrients enter the cell, and metabolic waste products leave it. The cell takes in O_2 for use in cellular respiration and expels CO_2. Also, the cell regulates its concentrations of inorganic ions, such as Na^+, K^+, Ca^{2+}, and Cl^-, by shuttling them one way or the other across the plasma membrane. In spite of heavy traffic through them, cell membranes are selective in their permeability: Substances do not cross the barrier indiscriminately. The cell is able to take up some small molecules and ions and exclude others.

The Permeability of the Lipid Bilayer

Nonpolar molecules, such as hydrocarbons, CO_2, and O_2, are hydrophobic, as are lipids. They can all therefore dissolve in the lipid bilayer of the membrane and cross it easily, without the aid of membrane proteins. However, the hydrophobic interior of the membrane impedes direct passage through the membrane of ions and polar molecules, which are hydrophilic. Polar molecules such as glucose and other sugars pass only slowly through a lipid bilayer, and even water, a very small polar molecule, does not cross rapidly relative to nonpolar molecules. A charged atom or molecule and its surrounding shell of water (see Figure 3.8) are even less likely to penetrate the hydrophobic interior of the membrane. Furthermore, the lipid bilayer is only one aspect of the gatekeeper system responsible for a cell's selective permeability. Proteins built into the membrane play key roles in regulating transport.

Transport Proteins

Specific ions and a variety of polar molecules can't move through cell membranes on their own. However, these hydrophilic substances can avoid contact with the lipid bilayer by passing through **transport proteins** that span the membrane.

Some transport proteins, called *channel proteins*, function by having a hydrophilic channel that certain molecules or ions use as a tunnel through the membrane (see Figure 7.7a, left). For example, the passage of water molecules through the membrane in certain cells is greatly facilitated by channel proteins known as **aquaporins** (Figure 7.10). Most aquaporin proteins consist of four identical polypeptide subunits. Each polypeptide forms a channel that water molecules pass through, single-file, overall allowing entry of up to *3 billion* water molecules per second. Without aquaporins, only a tiny fraction of these water molecules would pass through the same area of the cell membrane in a second. Other transport proteins, called *carrier proteins*, hold on to their passengers and change shape in a way that shuttles them across the membrane (see Figure 7.7a, right).

A transport protein is specific for the substance it translocates (moves), allowing only a certain substance (or a small group of related substances) to cross the membrane. For example, a glucose carrier protein in the plasma membrane of red blood cells transports glucose across the membrane 50,000 times faster than glucose can pass through on its own. This "glucose transporter" is so selective that it even rejects fructose, a structural isomer of glucose (see Figure 5.3). Thus, the selective permeability of a membrane depends on both the discriminating barrier of the lipid bilayer and the specific transport proteins built into the membrane.

Mastering Biology Animation: Selective Permeability of Membranes

What establishes the *direction* of traffic across a membrane? And what mechanisms drive molecules across membranes? We will address these questions next as we explore two modes of membrane traffic: passive transport and active transport.

CONCEPT CHECK 7.2

1. What property allows O_2 and CO_2 to cross a lipid bilayer without the aid of membrane proteins?
2. **VISUAL SKILLS** Examine Figure 7.2. Why is a transport protein needed to move many water molecules rapidly across a membrane?
3. **MAKE CONNECTIONS** Aquaporins exclude passage of hydronium ions (H_3O^+), but some aquaporins allow passage of glycerol, a three-carbon alcohol (see Figure 5.9), as well as H_2O. Since H_3O^+ is closer in size to water than glycerol is, yet cannot pass through, what might be the basis of this selectivity?

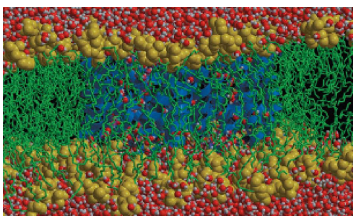
For suggested answers, see Appendix A.

CONCEPT 7.3

Passive transport is diffusion of a substance across a membrane with no energy investment

Molecules have a type of energy called thermal energy, due to their constant motion (see Concept 3.2). One result of this motion is **diffusion**, the movement of particles of any substance so that they spread out into the available space. Each molecule moves randomly, yet diffusion of a *population* of molecules may be directional. Imagine a synthetic membrane separating pure water from a solution of a dye in water. Study **Figure 7.11a** carefully to see how diffusion would result in equal concentrations of dye molecules in both solutions. At that point, a dynamic equilibrium will exist, with as many dye molecules crossing per second in one direction as in the other.

Here is a simple rule of diffusion: In the absence of any other forces, a substance will diffuse from where it is more concentrated



▶ Figure 7.10 An aquaporin. This computer model shows water molecules (red and gray) passing through an aquaporin (blue ribbons), in a lipid bilayer (yellow, hydrophilic heads; green, hydrophobic tails).

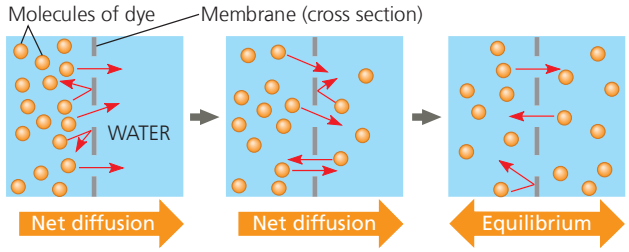
Mastering Biology
Interview with Peter Agre: Discovering aquaporins
Animation: Aquaporin

to where it is less concentrated. Put another way, a substance diffuses down its **concentration gradient**, the region along which the density of a chemical substance increases or decreases (in this case, decreases). Diffusion is a spontaneous process, needing no input of energy. Each substance diffuses down its *own* concentration gradient, unaffected by the concentration gradients of other substances **(Figure 7.11b)**.

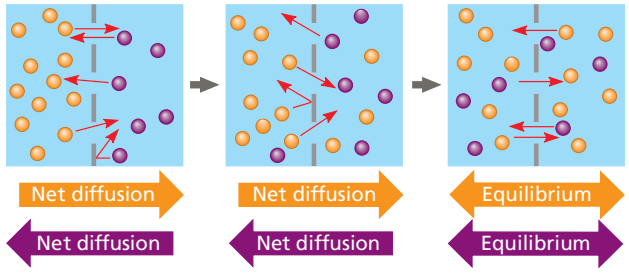
Much of the traffic across cell membranes occurs by diffusion. When a substance is more concentrated on one side of a membrane than on the other, there is a tendency for it to diffuse across, down its concentration gradient (assuming that the membrane is permeable to that substance). One important example is the uptake of oxygen by a cell performing cellular respiration. Dissolved oxygen diffuses into the cell across the plasma membrane. As long as cellular respiration consumes the O_2 as it enters, diffusion into the cell will continue because the concentration gradient favors movement in that direction.

The diffusion of a substance across a biological membrane is called **passive transport** because it requires no energy. The concentration gradient itself represents potential energy

▼ Figure 7.11 Diffusion of solutes across a synthetic membrane. Each large arrow shows net diffusion of dye molecules of that color.



(a) Diffusion of one solute. Molecules of dye can pass through membrane pores. Random movement of dye molecules will cause some to pass through the pores; this happens more often on the side with more dye molecules. The dye diffuses from the more concentrated side to the less concentrated side (called diffusing down a concentration gradient). A dynamic equilibrium results: Solute molecules still cross, but at roughly equal rates in both directions.



(b) Diffusion of two solutes. Solutions of two different dyes are separated by a membrane that is permeable to both. Each dye diffuses down its own concentration gradient. There will be a net diffusion of the purple dye toward the left, even though the *total* solute concentration was initially greater on the left side.

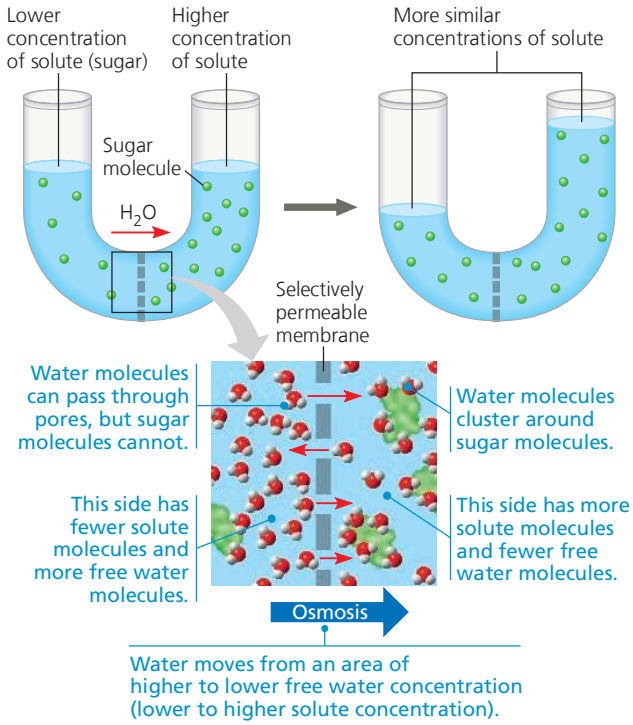
Mastering Biology Animation: Diffusion

(see Concept 2.2 and Figure 8.5b) and drives diffusion. Remember, though, that membranes are selectively permeable and therefore have different effects on the rates of diffusion of various molecules. Water can diffuse very rapidly across the membranes of cells with aquaporins, compared with diffusion in the absence of aquaporins. The movement of water across the plasma membrane has important consequences for cells.

Effects of Osmosis on Water Balance

To see how two solutions with different solute concentrations interact, picture a U-shaped glass tube with a selectively permeable artificial membrane separating two sugar solutions **(Figure 7.12)**. Pores in this synthetic membrane are too small for sugar molecules to pass through but large enough for water molecules. However, tight clustering of water molecules around the hydrophilic solute molecules makes some

▼ Figure 7.12 Osmosis. Two sugar solutions of different concentrations are separated by a membrane that the solvent (water) can pass through but the solute (sugar) cannot. Water molecules move randomly and may cross in either direction, but overall, water diffuses from the solution with less concentrated solute to that with more concentrated solute. This passive transport of water, or osmosis, makes the sugar concentrations on both sides roughly equal.



VISUAL SKILLS *If an orange dye capable of passing through the membrane was added to the left side of the tube above, how would it be distributed at the end of the experiment? (See Figure 7.11.) Would the final solution levels in the tube be affected? What cellular component does the membrane represent in this experiment?*

Mastering Biology Figure Walkthrough

of the water unavailable to cross the membrane. As a result, the solution with a higher solute concentration has a lower *free* water concentration. Water diffuses across the membrane from the region of higher free water concentration (lower solute concentration) to that of lower free water concentration (higher solute concentration) until the solute concentrations on both sides of the membrane are more nearly equal. The diffusion of free water across a selectively permeable membrane, whether artificial or cellular, is called **osmosis**. The movement of water across cell membranes and the balance of water between the cell and its environment are crucial to organisms. Let's now apply what we've learned about osmosis in this system to living cells.

Water Balance of Cells Without Cell Walls

To explain the behavior of a cell in a solution, we must consider both solute concentration and membrane permeability. Both factors are taken into account in the concept of **tonicity**, the ability of a surrounding solution to cause a cell to gain or lose water. The tonicity of a solution depends in part on its concentration of solutes that cannot cross the membrane (nonpenetrating solutes) relative to that inside the cell. If there is a higher concentration of nonpenetrating solutes in the surrounding solution, water will tend to leave the cell, and vice versa.

If a cell without a cell wall, such as an animal cell, is immersed in an environment that is **isotonic** to the cell (*iso* means "same"), there will be no *net* movement of water across the plasma membrane. Water diffuses across the membrane, but at the same rate in both directions. In an isotonic environment, the volume of an animal cell is stable **(Figure 7.13a)**.

Let's transfer the cell to a solution that is **hypertonic** to the cell (*hyper* means "more," in this case referring to nonpenetrating solutes). The cell will lose water, shrivel, and probably die. This is why an increase in the salinity (saltiness) of a lake can kill the animals there; if the lake water becomes hypertonic to the animals' cells, they might shrivel and die. However, taking up too much water can be just as hazardous to a cell as losing water. If we place the cell in a solution that is **hypotonic** to the cell (*hypo* means "less"), water will enter the cell faster than it leaves, and the cell will swell and lyse (burst) like an overfilled water balloon.

A cell without rigid cell walls can't tolerate either excessive uptake or excessive loss of water. This problem of water balance is automatically solved if such a cell lives in isotonic surroundings. Seawater is isotonic to many marine invertebrates. The cells of most terrestrial (land-dwelling) animals are bathed in an extracellular fluid that is isotonic to the cells. In hypertonic or hypotonic environments, however, organisms that lack rigid cell walls must have other adaptations for **osmoregulation**, the control of solute concentrations and water balance. For example, the unicellular protist *Paramecium caudatum* lives in pond water, which is hypotonic to the cell. *Paramecium* has a plasma membrane that is much less permeable to water than the membranes of most other cells, but this only slows the uptake of water, which continually enters the cell. The reason the *Paramecium* cell doesn't burst is that it has a contractile vacuole, an organelle that functions as a pump to force water out of the cell as fast as it enters by osmosis **(Figure 7.14)**. In contrast, the bacteria and archaea that live in hypersaline (excessively salty) environments (see Figure 27.1) have cellular mechanisms that balance the internal and external solute concentrations to ensure that water does not move out of the cell. We'll examine other evolutionary adaptations for osmoregulation by animals in Concept 44.1.

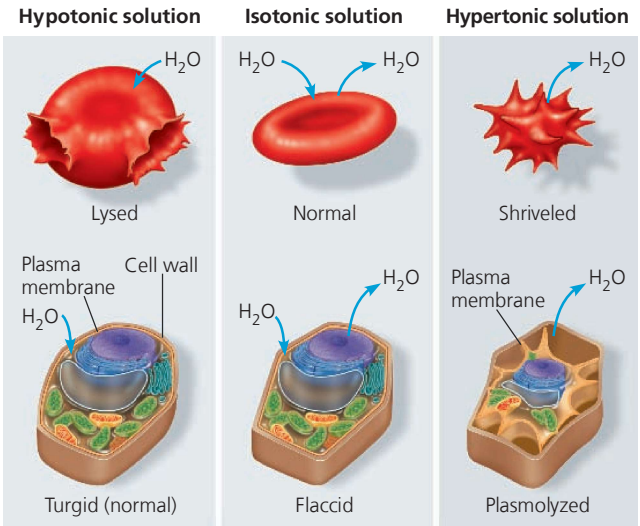
Water Balance of Cells with Cell Walls

The cells of plants, prokaryotes, fungi, and some protists are surrounded by cell walls (see Figure 6.27). When such a cell is immersed in a hypotonic solution—bathed in rainwater, for example—the cell wall helps maintain the cell's water balance. Consider a plant cell. Like an animal cell, the plant cell swells as water enters by osmosis **(Figure 7.13b)**. However, the relatively inelastic cell wall will expand only so much before it exerts a back pressure on the cell, called *turgor pressure*, that

▼ Figure 7.13 The water balance of living cells. How living cells react to changes in the solute concentration of their environment depends on whether or not they have cell walls. **(a)** Animal cells, such as this red blood cell, do not have cell walls. **(b)** Plant cells do have cell walls. (Arrows indicate net water movement after the cells were first placed in these solutions.)

(a) Animal cell. An animal cell, such as this red blood cell, does not have a cell wall. Animal cells fare best in an isotonic environment unless they have special adaptations that offset the osmotic uptake or loss of water.

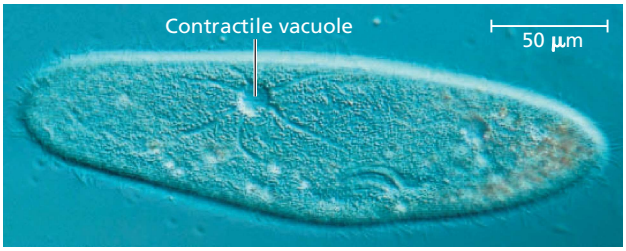
(b) Plant cell. Plant cells are turgid (firm) and generally healthiest in a hypotonic environment, where the uptake of water is eventually balanced by the wall pushing back on the cell.



? *Why do limp celery stalks become crisp when placed in a glass of water?*

Mastering Biology **Animation: Osmosis and Water Balance in Cells**
Video: Turgid *Elodea*
Video: Plasmolysis in *Elodea*

▼ Figure 7.14 The contractile vacuole of *Paramecium*. The vacuole collects fluid from canals in the cytoplasm. When full, the vacuole and canals contract, expelling fluid from the cell (LM).



Mastering Biology **Video: *Paramecium* Vacuole**

opposes further water uptake. At this point, the cell is **turgid** (very firm), which is the healthy state for most plant cells. Plants that are not woody, such as most houseplants, depend for mechanical support on cells kept turgid by a surrounding hypotonic solution. If a plant's cells and surroundings are isotonic, there is no net tendency for water to enter and the cells become **flaccid** (limp); the plant wilts.

However, a cell wall is of no advantage if the cell is immersed in a hypertonic environment. In this case, a plant cell, like an animal cell, will lose water to its surroundings and shrink. As the plant cell shrivels, its plasma membrane pulls away from the cell wall at multiple places. This phenomenon, called **plasmolysis**, causes the plant to wilt and can lead to plant death. The walled cells of bacteria and fungi also plasmolyze in hypertonic environments.

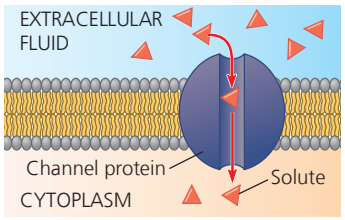
Facilitated Diffusion: Passive Transport Aided by Proteins

Let's look more closely at how water and certain hydrophilic solutes cross a membrane. As mentioned earlier, many polar molecules and ions blocked by the lipid bilayer of the membrane diffuse passively with the help of transport proteins that span the membrane. This phenomenon is called **facilitated diffusion**. Cell biologists are still trying to learn exactly how various transport proteins facilitate diffusion. Most transport proteins are very specific: They transport some substances but not others.

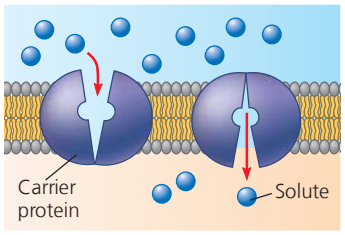
As mentioned earlier, the two types of transport proteins are channel proteins and carrier proteins. Channel proteins simply provide corridors that allow specific molecules or ions to cross the membrane **(Figure 7.15a)**. The hydrophilic passageways provided by these proteins can allow water molecules or small ions to diffuse very quickly from one side of the membrane to the other. Aquaporins, the water channel proteins, facilitate the massive levels of diffusion of water (osmosis) that occur in plant cells and in animal cells such as red blood cells (see Figure 7.13). Certain kidney cells also have a high number of aquaporins, allowing them to reclaim water from urine before it is excreted. If the kidneys didn't perform this function, you would excrete about 180 L of urine per day—and have to drink an equal volume of water!

▼ Figure 7.15 Two types of transport proteins that carry out facilitated diffusion. In both cases, the protein can transport the solute in either direction, but the net movement is down the concentration gradient of the solute.

(a) A channel protein has a channel through which water molecules or a specific solute can pass.



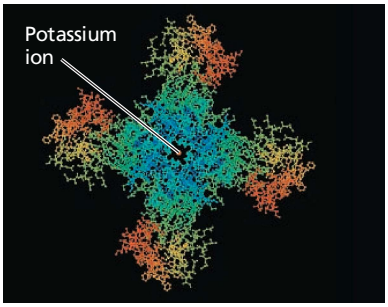
(b) A carrier protein alternates between two shapes, moving a solute across the membrane during the shape change.



Mastering Biology **Animation: Facilitated Diffusion**

Channel proteins that transport ions are called **ion channels**. Many ion channels function as **gated channels**, which open or close in response to a stimulus (see Figure 11.8). For some gated channels, the stimulus is electrical. In a nerve cell, for example, a potassium ion channel protein (see computer model) opens in response to an electrical stimulus, allowing a stream of potassium ions to leave the cell. This restores the cell's ability to fire again. Other gated channels have a chemical stimulus: They open or close when a specific substance (not the one to be transported) binds to the channel. Ion channels are important in the functioning of the nervous system, as you'll learn in Chapter 48.

▼ Potassium ion channel protein (See also the side view of a calcium channel protein in Figure 6.32a.)



Mastering Biology
Interview with Elba Serrano: Investigating how ion channels enable you to hear

Carrier proteins, such as the glucose transporter mentioned earlier, seem to undergo a subtle change in shape that somehow translocates the solute-binding site across the membrane **(Figure 7.15b)**. Such a change in shape may be triggered by the binding and release of the transported molecule. Like ion channels, carrier proteins involved in facilitated diffusion result in the net movement of a substance down

Scientific Skills Exercise

Interpreting a Scatter Plot with Two Sets of Data

Is Glucose Uptake into Cells Affected by Age? Glucose, an important energy source for animals, is transported into cells by facilitated diffusion using protein carriers. In this exercise, you will interpret a graph with two sets of data from an experiment that examined glucose uptake over time in red blood cells from guinea pigs of different ages. You will determine if the cells' rate of glucose uptake depended on the age of the guinea pig.

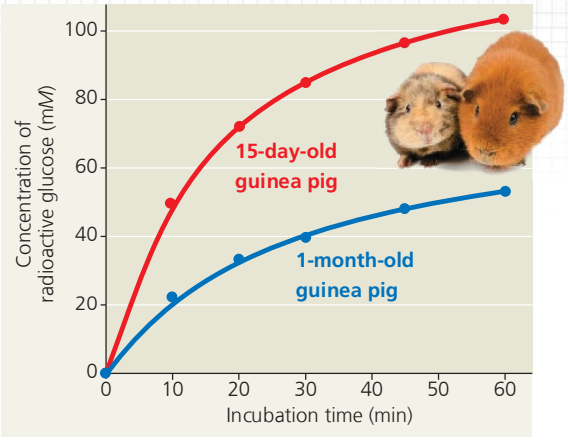
How the Experiment Was Done Researchers incubated guinea pig red blood cells in a 300 m*M* (millimolar) radioactive glucose solution at pH 7.4 at 25°C. Every 10 or 15 minutes, they removed a sample of cells and measured the concentration of radioactive glucose inside those cells. The cells came from either a 15-day-old or a 1-month-old guinea pig.

Data from the Experiment When you have multiple sets of data, it can be useful to plot them on the same graph for comparison. In the graph here, each set of dots (of the same color) forms a *scatter plot*, in which every data point represents two numerical values, one for each variable. For each data set, a curve that best fits the points has been drawn to make it easier to see the trends. (For additional information about graphs, see the Scientific Skills Review in Appendix D.)

INTERPRET THE DATA

1. First make sure you understand the parts of the graph. **(a)** Which variable is the independent variable—the variable controlled by the researchers? **(b)** Which variable is the dependent variable—the variable that depended on the treatment and was measured by the researchers? **(c)** What do the red dots represent? **(d)** The blue dots?

▼ Glucose Uptake over Time in Guinea Pig Red Blood Cells.



Data from T. Kondo and E. Beutler, Developmental changes in glucose transport of guinea pig erythrocytes, *Journal of Clinical Investigation* 65:1–4 (1980).

2. From the data points on the graph, construct a table of the data. Put "Incubation Time (min)" in the left column of the table.
3. What does the graph show? Compare and contrast glucose uptake in red blood cells from 15-day-old and 1-month-old guinea pigs.
4. Develop a hypothesis to explain the difference between glucose uptake in red blood cells from 15-day-old and 1-month-old guinea pigs. (Think about how glucose gets into cells.)
5. Design an experiment to test your hypothesis.

Instructors: A version of this Scientific Skills Exercise can be assigned in **Mastering Biology**.

its concentration gradient. No energy input is thus required: This is passive transport. The **Scientific Skills Exercise** gives you an opportunity to work with data from an experiment related to glucose transport.

Mastering Biology **BioFlix® Animation: Passive Transport**

CONCEPT CHECK 7.3

1. Speculate about how a cell performing cellular respiration might rid itself of the resulting CO_2.
2. **WHAT IF?** If a *Paramecium* swims from a hypotonic to an isotonic environment, will its contractile vacuole become more active or less? Why?

For suggested answers, see Appendix A.

CONCEPT 7.4

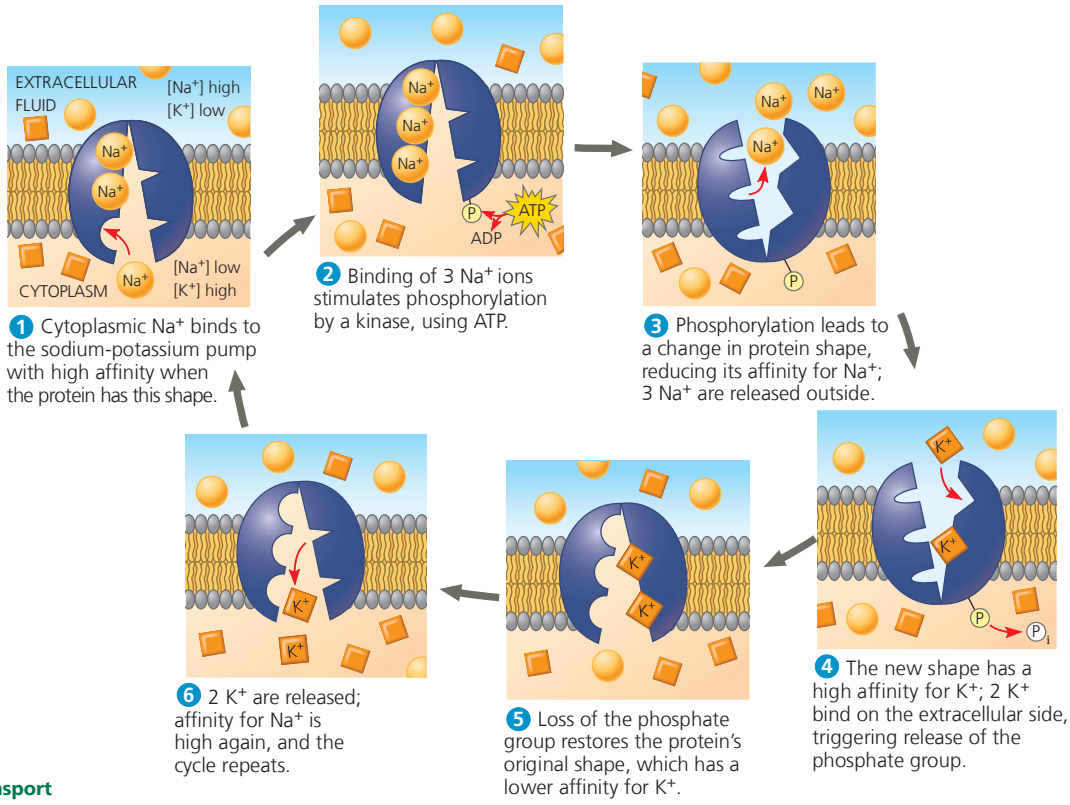
Active transport uses energy to move solutes against their gradients

Despite the help of transport proteins, facilitated diffusion is considered passive transport because the solute is moving down its concentration gradient, a process that requires no energy. Facilitated diffusion speeds transport of a solute by providing efficient passage through the membrane, but it does not alter the direction of transport. Some other transport proteins, however, can use energy to move solutes *against* their concentration gradients, across the plasma membrane from the side where they are less concentrated (whether inside or outside) to the side where they are more concentrated.

The Need for Energy in Active Transport

To pump a solute across a membrane against its gradient requires work; the cell must expend energy. Therefore, this type of membrane traffic is called **active transport**. The transport proteins that move solutes against their concentration gradients are all carrier proteins rather than channel proteins. This makes sense because when channel proteins are open, they merely allow solutes to diffuse down their concentration gradients rather than picking them up and transporting them against their gradients.

Active transport enables a cell to maintain internal concentrations of small solutes that differ from concentrations in its environment. For example, compared with its surroundings,



▶ Figure 7.16 The sodium-potassium pump: a specific case of active transport. This transport system pumps ions against steep concentration gradients. The pump oscillates between two shapes in a cycle that moves Na^+ out of the cell (steps 1 – 3) and K^+ into the cell (steps 4 – 6). The two shapes have different binding affinities for Na^+ and K^+. ATP hydrolysis powers the shape change by transferring a phosphate group to the transport protein (phosphorylating the protein).

VISUAL SKILLS *For each ion (Na^+ and K^+), describe its concentration inside the cell relative to outside. How many Na^+ are moved out of the cell and how many K^+ moved in per cycle?*

Mastering Biology
Animation: Active Transport

an animal cell has a much higher concentration of potassium ions (K^+) and a much lower concentration of sodium ions (Na^+). The plasma membrane helps maintain these steep gradients by pumping Na^+ out of the cell and K^+ into the cell.

As in other types of cellular work, ATP hydrolysis supplies the energy for most active transport. One way ATP can power active transport is when its terminal phosphate group is transferred directly to the transport protein. This can induce the protein to change its shape in a manner that translocates a solute bound to the protein across the membrane. One transport system that works this way is the **sodium-potassium pump**, which exchanges Na^+ for K^+ across the plasma membrane of animal cells **(Figure 7.16)**. The distinction between passive transport and active transport is reviewed in **Figure 7.17**.

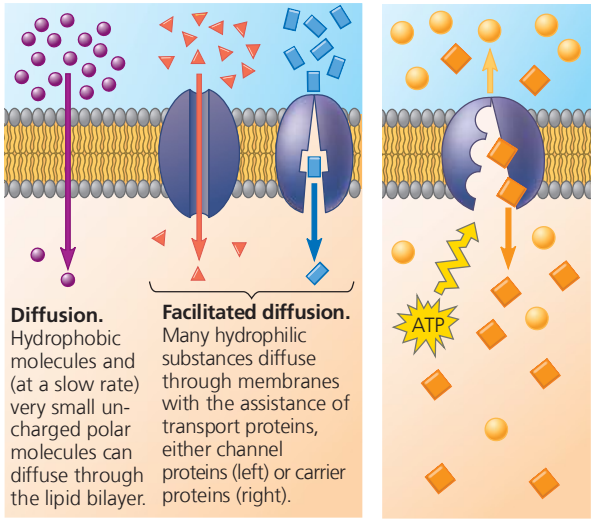
How Ion Pumps Maintain Membrane Potential

All cells have voltages across their plasma membranes. Voltage is electrical potential energy (see Concept 2.2)—a separation of opposite charges. The cytoplasmic side of the membrane is negative in charge relative to the extracellular side because of an unequal distribution of anions and cations on the two sides. The voltage across a membrane, called a **membrane potential**, ranges from about −50 to −200 millivolts (mV). (The minus sign indicates that the inside of the cell is negative relative to the outside.)

▼ Figure 7.17 Review: passive and active transport.

Passive transport. Substances diffuse spontaneously down their concentration gradients, crossing a membrane with no expenditure of energy by the cell. The rate of diffusion can be greatly increased by transport proteins in the membrane.

Active transport. Some transport proteins expend energy and act as pumps, moving substances across a membrane against their concentration (or electrochemical) gradients. Energy is usually supplied by ATP hydrolysis.



Diffusion. Hydrophobic molecules and (at a slow rate) very small uncharged polar molecules can diffuse through the lipid bilayer.

Facilitated diffusion. Many hydrophilic substances diffuse through membranes with the assistance of transport proteins, either channel proteins (left) or carrier proteins (right).

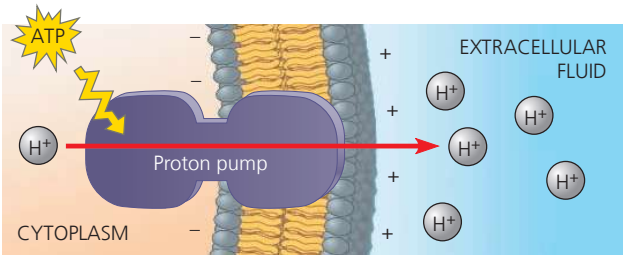
VISUAL SKILLS *For each solute in the right panel, describe its direction of movement, and state whether it is moving with or against its concentration gradient.*

The membrane potential acts like a battery, an energy source that affects the traffic of all charged substances across the membrane. Because the inside of the cell is negative compared with the outside, the membrane potential favors the passive transport of cations into the cell and anions out of the cell. Thus, *two* forces drive the diffusion of ions across a membrane: a chemical force (the ion's concentration gradient, which has been our sole consideration thus far in the chapter) and an electrical force (the effect of the membrane potential on the ion's movement). This combination of forces acting on an ion is called the **electrochemical gradient**.

In the case of ions, then, we must refine our concept of passive transport: An ion diffuses not simply down its *concentration* gradient but, more exactly, down its *electrochemical* gradient. For example, the concentration of Na^+ inside a resting nerve cell is much lower than outside it. When the cell is stimulated, gated channels open that facilitate Na^+ diffusion. Sodium ions then "fall" down their electrochemical gradient, driven by the concentration gradient of Na^+ and by the attraction of these cations to the negative side (inside) of the membrane. In this example, both electrical and chemical contributions to the electrochemical gradient act in the same direction across the membrane, but this is not always so. In cases where electrical forces due to the membrane potential oppose the simple diffusion of an ion down its concentration gradient, active transport may be necessary. In Concepts 48.2 and 48.3, you'll learn about the importance of electrochemical gradients and membrane potentials in the transmission of nerve impulses.

Some membrane proteins that actively transport ions contribute to the membrane potential. Study Figure 7.16 to see if you can see why the sodium-potassium pump is a good example. Notice that the pump does not translocate Na^+ and K^+ one for one, but pumps *three* sodium ions out of the cell for every *two* potassium ions it pumps into the cell. With each "crank" of the pump, there is a net transfer of one positive charge from the cytoplasm to the extracellular fluid, a process that stores energy as voltage. A transport protein that generates voltage across a membrane is called an **electrogenic pump**.

▼ Figure 7.18 A proton pump. Proton pumps are electrogenic pumps that store energy by generating voltage (charge separation) across membranes. A proton pump translocates positive charge in the form of hydrogen ions (H^+, or protons). The voltage and H^+ concentration gradient represent a dual energy source that can drive other processes, such as the uptake of nutrients. Most proton pumps are powered by ATP hydrolysis. (See Figure 6.32a.)

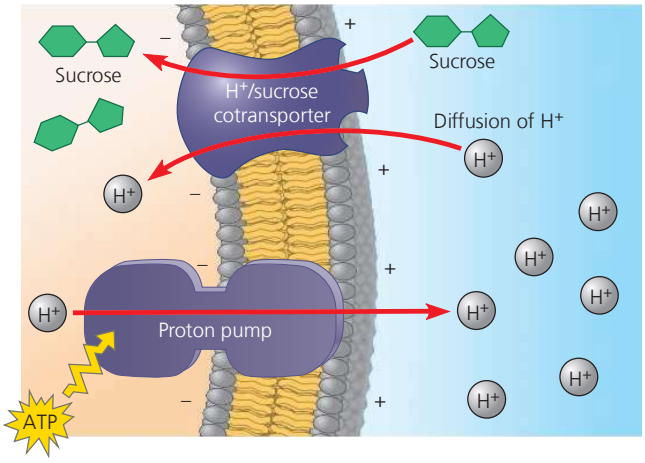


The sodium-potassium pump appears to be the major electrogenic pump of animal cells. The main electrogenic pump of plants, fungi, and bacteria is a **proton pump**, which actively transports protons (hydrogen ions, H^+) out of the cell. The pumping of H^+ transfers positive charge from the cytoplasm to the extracellular solution **(Figure 7.18)**. By generating voltage across membranes, electrogenic pumps help store energy that can be tapped for cellular work. One important use of proton gradients in the cell is for ATP synthesis during cellular respiration, as you will see in Concept 9.4. Another is a type of membrane traffic called cotransport.

Cotransport: Coupled Transport by a Membrane Protein

A solute that exists in different concentrations across a membrane can do work as it moves across that membrane by diffusion down its concentration gradient. This is analogous to water that has been pumped uphill and performs work as it flows back down. In a mechanism called **cotransport**, a transport protein (a cotransporter) can couple the "downhill" diffusion of the solute to the "uphill" transport of a second substance against its own concentration gradient. For instance, a plant cell uses the gradient of H^+ generated by its ATP-powered proton pumps to drive the active transport of amino acids, sugars, and several other nutrients into the cell. In the example shown in **Figure 7.19**, a cotransporter couples the return of H^+ to the transport of sucrose into the cell. This protein can translocate sucrose into the cell against

▼ Figure 7.19 Cotransport: active transport driven by a concentration gradient. A carrier protein, such as this H^+/sucrose cotransporter in a plant cell (top), is able to use the diffusion of H^+ down its electrochemical gradient into the cell to drive the uptake of sucrose. (The cell wall is not shown.) Although not technically part of the cotransport process, an ATP-driven proton pump is shown here (bottom), which concentrates H^+ outside the cell. The resulting H^+ gradient represents potential energy that can be used for active transport—of sucrose, in this case. Thus, ATP hydrolysis indirectly provides the energy necessary for cotransport.



its concentration gradient, but only if the sucrose molecule travels in the company of an H^+. The H^+ uses the transport protein as an avenue to diffuse down its own electrochemical gradient, which is maintained by the proton pump. Plants use H^+/sucrose cotransport to load sucrose produced by photosynthesis into cells in the veins of leaves. The vascular tissue of the plant can then distribute the sugar to roots and other nonphotosynthetic organs that do not make their own sugars.

A similar cotransporter in animals transports Na^+ into intestinal cells together with glucose, which is moving down its concentration gradient into the cell. (The Na^+ is then pumped out of the cell into the blood on the other side by Na^+/K^+ pumps; see Figure 7.16.) Our understanding of Na^+/glucose cotransporters has helped us find more effective treatments for diarrhea, a serious problem in developing countries. Normally, sodium in waste is reabsorbed in the colon, maintaining constant levels in the body, but diarrhea expels waste so rapidly that reabsorption is not possible, and sodium levels fall precipitously. To treat this life-threatening condition, patients are given a solution to drink containing high concentrations of salt (NaCl) and glucose. The solutes are taken up by Na^+/glucose cotransporters on the surface of intestinal cells and passed through the cells into the blood. This simple treatment has lowered infant mortality worldwide.

Mastering Biology BioFlix® Animation: Active Transport

CONCEPT CHECK 7.4

1. Na^+/K^+ pumps help nerve cells establish a voltage across their plasma membranes. Do these pumps use ATP or produce ATP? Explain.
2. **VISUAL SKILLS** Compare the Na^+/K^+ pump in Figure 7.16 with the cotransporter in Figure 7.19. Explain why the Na^+/K^+ pump would not be considered a cotransporter.
3. **MAKE CONNECTIONS** Review the characteristics of the lysosome in Concept 6.4. Given the internal environment of a lysosome, what transport protein might you expect to see in its membrane?

For suggested answers, see Appendix A.

CONCEPT 7.5

Bulk transport across the plasma membrane occurs by exocytosis and endocytosis

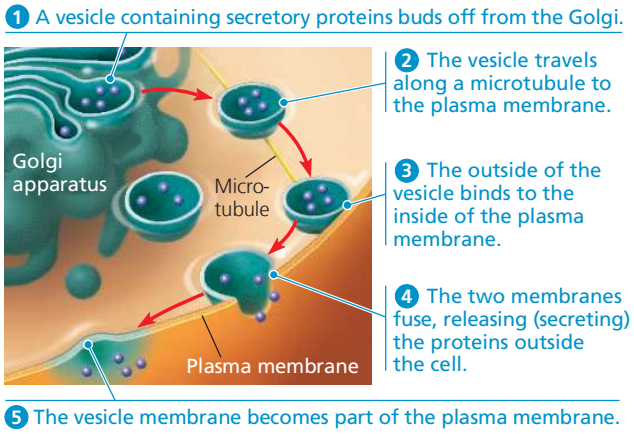
Large molecules, such as proteins and polysaccharides, generally don't cross the membrane by diffusion or transport proteins. Instead, they usually enter and leave the cell in bulk, packaged in vesicles.

Mastering Biology BioFlix® Animation: Exocytosis and Endocytosis

Exocytosis

The cell secretes certain molecules by the fusion of vesicles with the plasma membrane; this process is called **exocytosis**

▼ Figure 7.20 Exocytosis.



(Figure 7.20). A transport vesicle that has budded from the Golgi apparatus moves along a microtubule of the cytoskeleton to the plasma membrane. When the vesicle membrane and plasma membrane come into contact, specific proteins in both membranes rearrange the lipid molecules of the two bilayers so that the two membranes fuse. The contents of the vesicle spill out of the cell, and the vesicle membrane becomes part of the plasma membrane.

Many secretory cells use exocytosis to export products. For example, cells in the pancreas that make insulin secrete it into the extracellular fluid by exocytosis. In another example, nerve cells use exocytosis to release neurotransmitters that signal other neurons or muscle cells (see Figure 7.1). When plant cells are making cell walls, exocytosis delivers some of the necessary proteins and carbohydrates from Golgi vesicles to the outside of the cell.

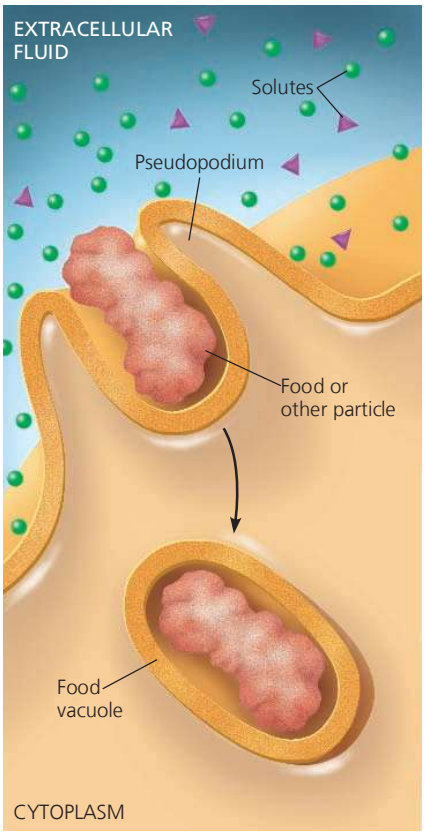
Endocytosis

In **endocytosis**, the cell takes in molecules and particulate matter by forming new vesicles from the plasma membrane. Although the proteins involved in the processes are different, the events of endocytosis look like the reverse of exocytosis. First, a small area of the plasma membrane sinks inward to form a pocket. Then, as the pocket deepens, it pinches in, forming a vesicle containing material that had been outside the cell. Study **Figure 7.21** carefully to understand the three types of endocytosis: phagocytosis ("cellular eating"), pinocytosis ("cellular drinking"), and receptor-mediated endocytosis.

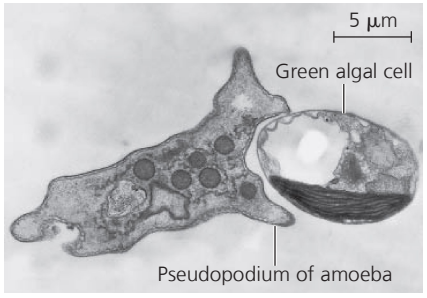
Human cells use receptor-mediated endocytosis to take in cholesterol for membrane synthesis and the synthesis of other steroids. Cholesterol travels in the blood in particles called low-density lipoproteins (LDLs), each a complex of lipids and a protein. LDLs bind to LDL receptors on plasma membranes and then enter the cells by endocytosis. In the inherited disease familial hypercholesterolemia, characterized by a very high level of cholesterol in the blood, LDLs cannot enter cells because the LDL receptor proteins are defective or missing. Cholesterol thus accumulates in the blood, contributing to

▼ Figure 7.21 Exploring Endocytosis in Animal Cells

Phagocytosis

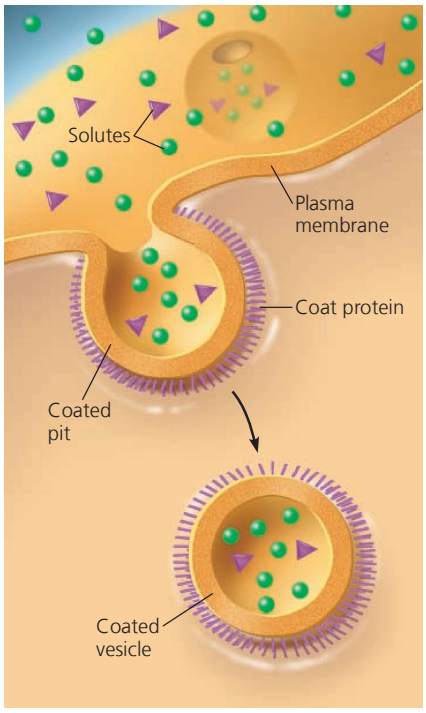


In **phagocytosis**, a cell engulfs a particle by extending pseudopodia (singular, *pseudopodium*) around it and packaging it within a membranous sac called a food vacuole. The particle will be digested after the food vacuole fuses with a lysosome containing hydrolytic enzymes (see Figure 6.13a).

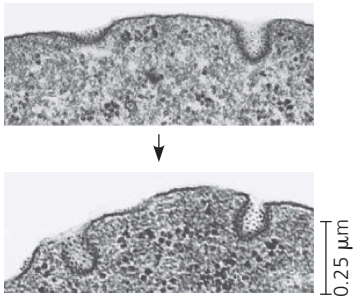


An amoeba engulfing a green algal cell via phagocytosis (TEM).

Pinocytosis

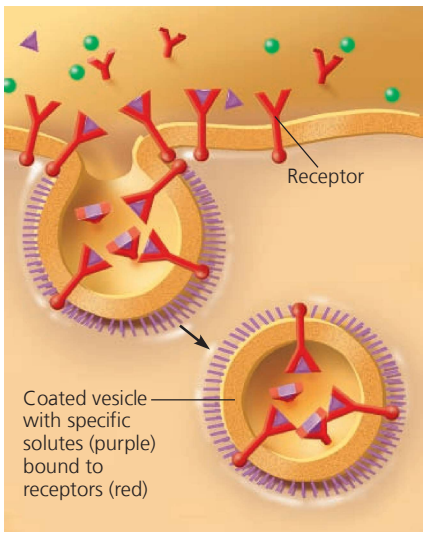


In **pinocytosis**, a cell continually "gulps" droplets of extracellular fluid into tiny vesicles, formed by infoldings of the plasma membrane. In this way, the cell obtains molecules dissolved in the droplets. Because any and all solutes are taken into the cell, pinocytosis as shown here is nonspecific for the substances it transports. In many cases, the parts of the plasma membrane that form vesicles are lined on their cytoplasmic side by a fuzzy layer of coat protein; the "pits" and resulting vesicles are called *coated pits*.

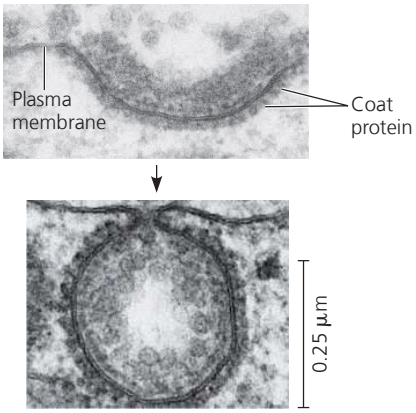


Pinocytotic vesicles forming (TEMs).

Receptor-Mediated Endocytosis



Receptor-mediated endocytosis is a specialized type of pinocytosis that enables the cell to acquire bulk quantities of specific substances, even though those substances may not be very concentrated in the extracellular fluid. Embedded in the plasma membrane are proteins with receptor sites exposed to the extracellular fluid. Specific solutes bind to the receptors. The receptor proteins then cluster in coated pits, and each coated pit forms a vesicle containing the bound molecules. The diagram shows only bound molecules (purple triangles) inside the vesicle, but other molecules from the extracellular fluid are also present. After the ingested material is liberated from the vesicle, the emptied receptors are recycled to the plasma membrane by the same vesicle (not shown).



Top: A coated pit. *Bottom*: A coated vesicle forming during receptor-mediated endocytosis (TEMs).

VISUAL SKILLS *Use the scale bars to estimate the diameters of (a) the food vacuole that will form around the algal cell (left micrograph; measure the length, not the width) and (b) the coated vesicle (lower right micrograph). (c) Which is larger, and by what factor?*

Mastering Biology **Animation: Exocytosis and Endocytosis**

early atherosclerosis, the buildup of lipids within blood vessel walls. This narrows the space in the vessels and impedes blood flow, potentially resulting in heart damage and stroke.

Endocytosis and exocytosis also provide mechanisms for rejuvenating or remodeling the plasma membrane. These processes occur continually in most eukaryotic cells, yet the amount of plasma membrane in a nongrowing cell remains fairly constant. The addition of membrane by one process appears to offset the loss of membrane by the other.

Mastering Biology **BioFlix® Animation: Membrane Transport**

CONCEPT CHECK 7.5

1. As a cell grows, its plasma membrane expands. Does this involve endocytosis or exocytosis? Explain.
2. **DRAW IT** Return to Figure 7.9, and circle a patch of plasma membrane that is coming from a vesicle involved in exocytosis.
3. **MAKE CONNECTIONS** In Concept 6.7, you learned that animal cells make an extracellular matrix (ECM). Describe the cellular pathway of synthesis and deposition of an ECM glycoprotein.

For suggested answers, see Appendix A.

7 Chapter Review

Go to **Mastering Biology** for Assignments, the eText, the Study Area, and Dynamic Study Modules.

SUMMARY OF KEY CONCEPTS

To review key terms, go to the **Vocabulary Self-Quiz** in the **Mastering Biology** eText or Study Area, or go to goo.gl/zkjz9t.

CONCEPT 7.1

Cellular membranes are fluid mosaics of lipids and proteins *(pp. 127–131)*

- In the **fluid mosaic model, amphipathic** proteins are embedded in the phospholipid bilayer.
- Phospholipids and some proteins move sideways within the membrane. The unsaturated hydrocarbon tails of some phospholipids keep membranes fluid at lower temperatures, while cholesterol helps membranes resist changes in fluidity caused by temperature changes.
- Membrane proteins function in transport, enzymatic activity, signal transduction, cell-cell recognition, intercellular joining, and attachment to the cytoskeleton and extracellular matrix. Short chains of sugars linked to proteins (in **glycoproteins**) and lipids (in **glycolipids**) on the exterior side of the plasma membrane interact with surface molecules of other cells.
- Membrane proteins and lipids are synthesized in the ER and modified in the ER and Golgi apparatus. The inside and outside faces of membranes differ in molecular composition.

? *In what ways are membranes crucial to life?*

CONCEPT 7.2

Membrane structure results in selective permeability *(pp. 131–132)*

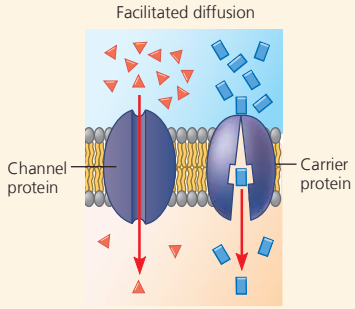
- A cell must exchange molecules and ions with its surroundings, a process controlled by the **selective permeability** of the plasma membrane. Hydrophobic substances are soluble in lipids and pass through membranes rapidly, whereas polar molecules and ions generally require specific **transport proteins**.

? *How do aquaporins affect the permeability of a membrane?*

CONCEPT 7.3

Passive transport is diffusion of a substance across a membrane with no energy investment *(pp. 132–136)*

- **Diffusion** is the spontaneous movement of a substance down its **concentration gradient**. Water diffuses out of a cell (**osmosis**) if the solution outside has a higher concentration of nonpenetrating solutes (**hypertonic**); water enters if the solution has a lower solute concentration (**hypotonic**). If the concentrations are equal (**isotonic**), no net osmosis occurs. Cell survival depends on balancing water uptake and loss.
- In **facilitated diffusion**, a transport protein speeds water or solute movement down its concentration gradient across a membrane. **Ion channels** facilitate the diffusion of ions across a membrane. Carrier proteins can undergo changes in shape that translocate bound solutes across the membrane.

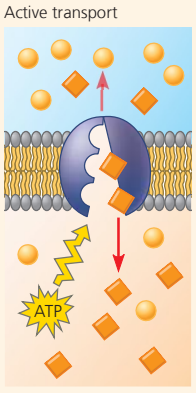


? *What happens to a cell placed in a hypertonic solution? Describe the free water concentration inside and out.*

CONCEPT 7.4

Active transport uses energy to move solutes against their gradients *(pp. 136–139)*

- Specific membrane proteins use energy, usually in the form of ATP, to do the work of **active transport**.
- Ions can have both a concentration (chemical) gradient and an electrical gradient (voltage). These gradients combine in the **electrochemical gradient**, which determines the net direction of ionic diffusion.
- **Cotransport** of two solutes occurs when a membrane protein enables the "downhill" diffusion of one solute to drive the "uphill" transport of the other.



? *ATP is not directly involved in the functioning of a cotransporter. Why, then, is cotransport considered active transport?*

CONCEPT 7.5

Bulk transport across the plasma membrane occurs by exocytosis and endocytosis *(pp. 139–141)*

- In **exocytosis**, transport vesicles migrate to the plasma membrane, fuse with it, and release their contents. In **endocytosis**, molecules enter cells within vesicles that pinch inward from the plasma membrane. The three types of endocytosis are **phagocytosis, pinocytosis**, and **receptor-mediated endocytosis**.

? *Which type of endocytosis involves the binding of specific substances in the extracellular fluid to membrane proteins? What does this type of transport enable a cell to do?*

TEST YOUR UNDERSTANDING

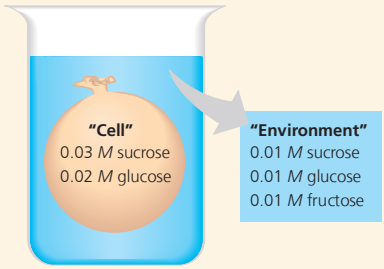
For more multiple-choice questions, go to the **Practice Test** in the **Mastering Biology** eText or Study Area, or go to goo.gl/GruWRg.

Levels 1-2: Remembering/Understanding

1. In what way do the membranes of a eukaryotic cell vary?
   (A) Phospholipids are found only in certain membranes.
   (B) Certain proteins are unique to each membrane.
   (C) Only certain membranes of the cell are selectively permeable.
   (D) Only certain membranes are constructed from amphipathic molecules.
2. According to the fluid mosaic model of membrane structure, proteins of the membrane are mostly
   (A) spread in a continuous layer over the inner and outer surfaces of the membrane.
   (B) confined to the hydrophobic interior of the membrane.
   (C) embedded in a lipid bilayer.
   (D) randomly oriented in the membrane, with no fixed inside-outside polarity.
3. Which of the following factors would tend to increase membrane fluidity?
   (A) a greater proportion of unsaturated phospholipids
   (B) a greater proportion of saturated phospholipids
   (C) a lower temperature
   (D) a relatively high protein content in the membrane

Levels 3-4: Applying/Analyzing

4. Which of the following processes includes all the others?
   (A) osmosis
   (B) diffusion of a solute across a membrane
   (C) passive transport
   (D) transport of an ion down its electrochemical gradient
5. Based on Figure 7.19, which of these experimental treatments would increase the rate of sucrose transport into a plant cell?
   (A) decreasing extracellular sucrose concentration
   (B) decreasing extracellular pH
   (C) decreasing cytoplasmic pH
   (D) adding a substance that makes the membrane more permeable to hydrogen ions
6. **DRAW IT** An artificial "cell" consisting of an aqueous solution enclosed in a selectively permeable membrane is immersed in a beaker containing a different solution, the "environment," as shown in the accompanying diagram. The membrane is permeable to water and to the simple sugars glucose and fructose but impermeable to the disaccharide sucrose.
   (a) Draw solid arrows to indicate the net movement of solutes into and/or out of the cell.
   (b) Is the solution outside the cell isotonic, hypotonic, or hypertonic?
   (c) Draw a dashed arrow to show the net osmosis, if any.
   (d) Will the artificial cell become more flaccid, more turgid, or stay the same?
   (e) Eventually, will the two solutions have the same or different solute concentrations?



Levels 5-6: Evaluating/Creating

7. **EVOLUTION CONNECTION** *Paramecium* and other protists that live in hypotonic environments have cell membranes that limit water uptake, while those living in isotonic environments have membranes that are more permeable to water. Describe what water regulation adaptations might have evolved in protists in hypertonic habitats such as the Great Salt Lake and in habitats with changing salt concentration.
8. **SCIENTIFIC INQUIRY** An experiment is designed to study the mechanism of sucrose uptake by plant cells. Cells are immersed in a sucrose solution, and the pH of the solution is monitored. Samples of the cells are taken at intervals, and their sucrose concentration is measured. The pH is observed to decrease until it reaches a steady, slightly acidic level, and then sucrose uptake begins. (a) Evaluate these results and propose a hypothesis to explain them. (b) Predict what would happen if an inhibitor of ATP regeneration by the cell were added to the beaker once the pH was at a steady level. Explain.
9. **SCIENCE, TECHNOLOGY, AND SOCIETY** Extensive irrigation in arid regions causes salts to accumulate in the soil. (When water evaporates, salts that were dissolved in the water are left behind in the soil.) Based on what you learned about water balance in plant cells, explain why increased soil salinity (saltiness) might be harmful to crops.
10. **WRITE ABOUT A THEME: INTERACTIONS** A human pancreatic cell obtains O_2—and necessary molecules such as glucose, amino acids, and cholesterol—from its environment, and it releases CO_2 as a waste product. In response to hormonal signals, the cell secretes digestive enzymes. It also regulates its ion concentrations by exchange with its environment. Based on what you have just learned about the structure and function of cellular membranes, write a short essay (100–150 words) to describe how such a cell accomplishes these interactions with its environment.
11. **SYNTHESIZE YOUR KNOWLEDGE**

In the supermarket, lettuce and other produce are often sprayed with water. Explain why this makes vegetables crisp.

For selected answers, see Appendix A.

8 An Introduction to Metabolism

KEY CONCEPTS

8.1 An organism's metabolism transforms matter and energy *p. 144*

8.2 The free-energy change of a reaction tells us whether or not the reaction occurs spontaneously *p. 147*

8.3 ATP powers cellular work by coupling exergonic reactions to endergonic reactions *p. 150*

8.4 Enzymes speed up metabolic reactions by lowering energy barriers *p. 153*

8.5 Regulation of enzyme activity helps control metabolism *p. 159*

Study Tip

Make a table: Fill in the following table for each process you read about in this chapter, such as water spilling over a dam or a chemical reaction.

Process	Starting Materials; Relative Energy Level	Ending Materials; Relative Energy Level	Will this process occur spontaneously (without an input of energy)?
Water spilling over a dam	Water at the top of a dam; higher energy level	Water at the bottom of a dam; lower energy level	Yes
Hydrolysis of ATP (Figure 8.9)			

Go to Mastering Biology

For Students (in eText and Study Area)
- Get Ready for Chapter 8
- Animation: Exergonic and Endergonic Reactions
- Figure 8.10 Walkthrough: How ATP Drives Chemical Work

For Instructors to Assign (in Item Library)
- Activity: Energy Transformations
- Tutorial: How Enzymes Function

Figure 8.1 The green glowing spots on the outside of this Brazilian termite mound are larvae of the click beetle, *Pyrophorus nyctophanus*. These larvae convert the energy stored in organic molecules to light, a process called bioluminescence, which attracts termites that the larvae eat. Bioluminescence and other metabolic activities in a cell are energy transformations that are subject to physical laws.

How do the laws of thermodynamics relate to biological processes?

The first law of thermodynamics:

Energy can be transferred and transformed, but it cannot be created or destroyed.
For example:

Light energy from the sun

Transformed by plants to

Chemical energy in organic molecules in plants

Transformed by termites to

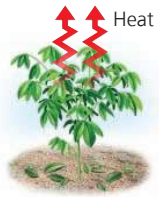
Chemical energy in organic molecules in termites

Transformed by click beetle larvae to

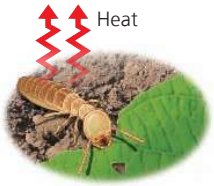
Light energy produced by bioluminescence

The second law of thermodynamics:

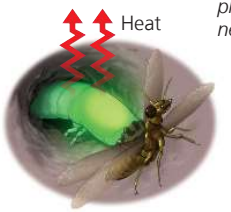
Every energy transfer or transformation increases the entropy (disorder) of the universe. For example, during every energy transformation, some energy is converted to thermal energy and released as heat:



Plant using light to make organic molecules



Termite digesting plant and making new molecules



Click beetle larva digesting termite and emitting light

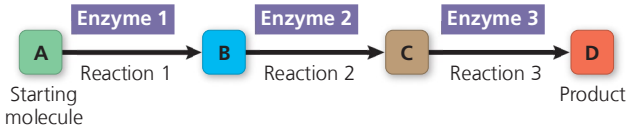
CONCEPT 8.1

An organism's metabolism transforms matter and energy

The totality of an organism's chemical reactions is called **metabolism** (from the Greek *metabole*, change). Metabolism is an emergent property of life that arises from orderly interactions between molecules.

Metabolic Pathways

We can picture a cell's metabolism as an elaborate road map of many chemical reactions, arranged as intersecting metabolic pathways. In a **metabolic pathway**, a specific molecule is altered in a series of defined steps, resulting in a certain product. Each step is catalyzed by a specific *enzyme*, a macromolecule that speeds up a chemical reaction:



The mechanisms that regulate these enzymes balance metabolic supply and demand, just like traffic lights control the flow of traffic.

Metabolism as a whole manages the material and energy resources of the cell. Some metabolic pathways release energy by breaking down complex molecules to simpler compounds. These degradative processes are called **catabolic pathways**, or breakdown pathways. One major catabolic pathway is cellular respiration, which breaks down glucose and other organic fuels in the presence of oxygen to carbon dioxide and water. (Pathways can have more than one starting molecule and/or product.) Energy stored in the organic molecules becomes available to do cellular work, such as ciliary beating or membrane transport. **Anabolic pathways**, in contrast, consume energy to build complicated molecules from simpler ones; they are sometimes called biosynthetic pathways. Examples of anabolism are synthesis of an amino acid from simpler molecules and synthesis of a protein from amino acids. Catabolic and anabolic pathways are the "downhill" and "uphill" avenues of the metabolic landscape. Energy released from downhill reactions of catabolic pathways can be stored and then used to drive uphill reactions of anabolic pathways.

In this chapter, we will focus on mechanisms common to metabolic pathways. Because energy is fundamental to all metabolic processes, a basic knowledge of energy is necessary to understand how the living cell works. Although we'll look at some nonliving examples here to consider energetic principles, the concepts demonstrated by these examples also apply to **bioenergetics**, the study of how energy flows through living organisms.

Forms of Energy

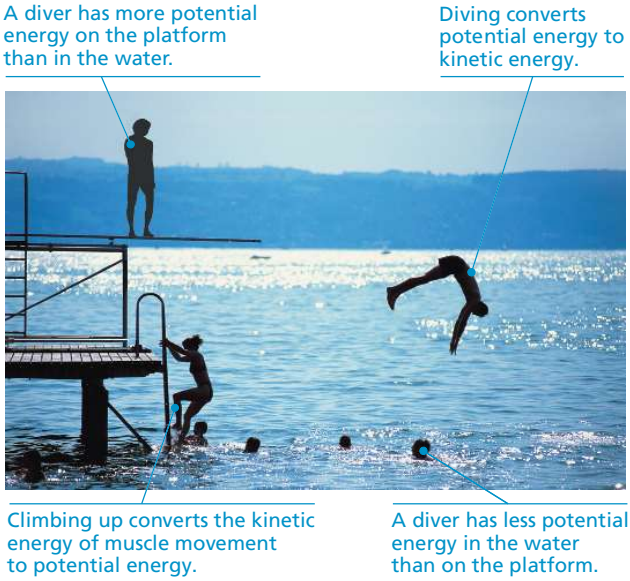
Energy is the capacity to cause change. In everyday life, energy is important because some forms of energy can be used to do work—that is, to move matter against opposing forces, such as gravity and friction. Put another way, energy is the ability to rearrange a collection of matter. For example, you expend energy to turn the pages of this book, and your cells expend energy in transporting certain substances across membranes. Energy exists in various forms, and the work of life depends on the ability of cells to transform energy from one form to another.

Energy can be associated with the relative motion of objects; this energy is called **kinetic energy**. Moving objects can perform work by imparting motion to other matter: A pool player uses the motion of the cue stick to push the cue ball, which in turn moves the other balls; water gushing through a dam turns turbines; and the contraction of leg muscles pushes bicycle pedals. **Thermal energy** is kinetic energy associated with the random movement of atoms or molecules; thermal energy in transfer from one object to another is called **heat**. Light is also a type of energy that can be harnessed to perform work, such as powering photosynthesis in green plants.

An object not presently moving may still possess energy. Energy that is not kinetic is called **potential energy**; it is energy that matter possesses because of its location or structure. Water behind a dam, for instance, possesses energy because of its altitude above sea level. Molecules possess energy because of the arrangement of electrons in the bonds between their atoms. **Chemical energy** is a term used by biologists to refer to the potential energy available for release in a chemical reaction. Recall that catabolic pathways release energy by breaking down complex molecules. Biologists say that these complex molecules, such as glucose, are high in chemical energy. During a catabolic reaction, some bonds are broken and others are formed, releasing energy and resulting in lower-energy breakdown products. This transformation also occurs in the engine of a car when the hydrocarbons of gasoline react explosively with oxygen, releasing the energy that pushes the pistons and producing exhaust. Although less explosive, a similar reaction of food molecules with oxygen provides chemical energy in biological systems, producing carbon dioxide and water as waste products. Biochemical pathways, carried out in the context of cellular structures, enable cells to release chemical energy from food molecules and use the energy to power life processes.

How is energy converted from one form to another? Consider **Figure 8.2**. The woman climbing the ladder to the diving platform is releasing chemical energy from the food she ate for lunch and using some of that energy to perform

▼ Figure 8.2 Transformations between potential energy and kinetic energy.



Mastering Biology **Animation: Energy Transformations**

the work of climbing. The kinetic energy of muscle movement is thus being transformed into potential energy due to her increasing height above the water. The man diving is converting his potential energy to kinetic energy, which is then transferred to the water as he enters it, resulting in splashing, noise, and increased movement of water molecules. A small amount of energy is lost as heat due to friction.

Now let's consider the original source of the organic food molecules that provided the necessary chemical energy for these divers to climb the steps. This chemical energy was itself derived from light energy absorbed by plants during photosynthesis. Organisms are energy transformers.

The Laws of Energy Transformation

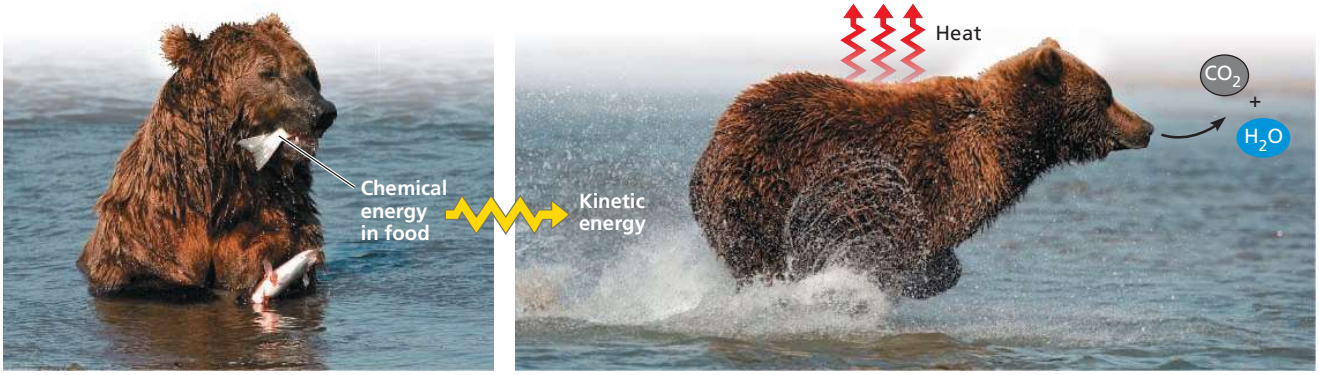
The study of the energy transformations that occur in a collection of matter is called **thermodynamics**. Scientists use the word *system* to denote the matter under study; they refer to the rest of the universe—everything outside the system—as the *surroundings*. An *isolated system*, such as that approximated by liquid in a thermos bottle, is unable to exchange either energy or matter with its surroundings outside the thermos. In an *open system*, energy and matter can be transferred between the system and its surroundings. Organisms are open systems. They absorb energy—for instance, light energy or chemical energy in the form of organic molecules—and release heat and metabolic waste products, such as carbon dioxide, to the surroundings. Two laws of thermodynamics govern energy transformations in organisms and all other collections of matter.

The First Law of Thermodynamics

According to the **first law of thermodynamics**, the energy of the universe is constant: *Energy can be transferred and transformed, but it cannot be created or destroyed.* The first law is also known as the *principle of conservation of energy*. The electric company does not make energy, but merely converts it to a form that is convenient for us to use. By converting sunlight to chemical energy, a plant acts as an energy transformer, not an energy producer.

The brown bear in **Figure 8.3a** will convert the chemical energy of the organic molecules in its food to kinetic and other forms of energy as it carries out biological processes. What happens to this energy after it has performed work? The second law of thermodynamics helps to answer this question.

▼ Figure 8.3 The first two laws of thermodynamics.



(a) First law of thermodynamics: Energy can be transferred or transformed but neither created nor destroyed. For example, chemical reactions in this brown bear will convert the chemical (potential) energy in the fish into the kinetic energy of running.

(b) Second law of thermodynamics: Every energy transfer or transformation increases the disorder (entropy) of the universe. For example, as the bear runs, disorder is increased around its body by the release of heat and small molecules that are the by-products of metabolism. A brown bear can run at speeds up to 35 miles per hour (56 km/hr)—as fast as a racehorse.

Mastering Biology **MP3 Tutor: Basic Energy Concepts**

The Second Law of Thermodynamics

If energy cannot be destroyed, why can't organisms simply recycle their energy over and over again? It turns out that during every energy transfer or transformation, some energy is converted to thermal energy and released as heat, becoming unavailable to do work. Only a small fraction of the chemical energy from the food in Figure 8.3a is transformed into the motion of the brown bear shown in **Figure 8.3b**; most is lost as heat, which dissipates rapidly through the surroundings.

A system can put thermal energy to work only when there is a temperature difference that results in thermal energy flowing as heat from a warmer location to a cooler one. If temperature is uniform, as it is in a living cell, then the heat generated during a chemical reaction will simply warm a body of matter, such as the organism. (This can make a room crowded with people uncomfortably warm, as each person is carrying out a multitude of chemical reactions!)

A consequence of the loss of usable energy as heat to the surroundings is that each energy transfer or transformation makes the universe more disordered. We are all familiar with the word "disorder" in the sense of a messy room or a run-down building. The word "disorder" as used by scientists, however, has a specific molecular definition related to how dispersed the energy is in a system and how many different energy levels are present. For simplicity, we use "disorder" in the following discussion because our common understanding (the messy room) is a good analogy for molecular disorder.

Scientists use a quantity called **entropy** as a measure of molecular disorder, or randomness. The more randomly arranged a collection of matter is, the greater its entropy. We can now state the **second law of thermodynamics**: *Every energy transfer or transformation increases the entropy of the universe.* Although order can increase locally, there is an unstoppable trend toward randomization of the universe as a whole.

The physical disintegration of a system's organized structure is a good analogy for an increase in entropy. For example, you can observe increasing entropy in the gradual decay of an unmaintained building over time. Much of the increasing entropy of the universe is more abstract, however, because it takes the form of increasing amounts of heat and less ordered forms of matter. As the bear in Figure 8.3b converts chemical energy to kinetic energy, it is also increasing the disorder of its surroundings by producing heat and small molecules, such as the CO_2 it exhales, that are the breakdown products of food.

The concept of entropy helps us understand why certain processes are energetically favorable and occur on their own. It turns out that if a given process, by itself, leads to an increase in entropy, that process can proceed without requiring an input of energy. Such a process is called a **spontaneous process**. Note that as we're using it here, the word *spontaneous* does not imply that the process would occur quickly; rather, the word signifies that it is energetically favorable. (In fact, it may be helpful for you to think of the phrase "energetically favorable" when you read the formal term *spontaneous*, the word preferred by chemists.) Some spontaneous processes, such as an explosion, may be virtually instantaneous, while others, such as the rusting of an old car over time, are much slower.

A process that, on its own, leads to a decrease in entropy is said to be nonspontaneous: It will happen only if energy is supplied. We know from experience that certain events occur spontaneously and others do not. For instance, we know that water flows downhill spontaneously but moves uphill only with an input of energy, such as when a machine pumps the water against gravity. Some of that energy is inevitably lost as heat, increasing entropy in the surroundings, so usage of energy during a nonspontaneous process also leads to an increase in the entropy of the universe as a whole.

Biological Order and Disorder

Living systems increase the entropy of their surroundings, as predicted by thermodynamic law. You may wonder, then, how cells create ordered structures from less organized starting materials. For example, simpler molecules are ordered into the more complex structure of an amino acid, and amino acids are ordered into polypeptide chains. At the organismal level as well, complex and beautifully ordered structures result from biological processes that use simpler starting materials **(Figure 8.4)**. How can this happen, if entropy must constantly increase?

▼ Figure 8.4 Order as a characteristic of life. Order is evident in the detailed structures of **(a)** the Venus flower basket glass sponge, which inspired the Spanish architect Antoni Gaudi in his design of **(b)** the towers of La Sagrada Família church in Barcelona, Spain.

(a) Glass sponge **(b)** La Sagrada Família towers

This increase in order is balanced by an organism's taking in organized forms of matter and energy from the surroundings and replacing them with less ordered forms. For example, an animal obtains starch, proteins, and other complex molecules from the food it eats. As catabolic pathways break these molecules down, the animal releases CO_2 and H_2O —small molecules that possess less chemical energy than the food did (see Figure 8.3b). The depletion of chemical energy is accounted for by heat generated during metabolism. On a larger scale, energy flows into most ecosystems in the form of light and exits in the form of heat (see Figure 1.9).

During the early history of life, complex organisms evolved from simpler ancestors. For instance, we can trace the ancestry of the plant kingdom from much simpler organisms called green algae to more complex flowering plants. However, this increase in organization over time in no way violates the second law. The entropy of a particular system, such as an organism, may actually decrease as long as the total entropy of the *universe*—the system plus its surroundings—increases. Thus, organisms are islands of low entropy in an increasingly random universe. The evolution of biological order is perfectly consistent with the laws of thermodynamics.

CONCEPT CHECK 8.1

1. **MAKE CONNECTIONS** How does the second law of thermodynamics help explain the diffusion of a substance across a membrane? (See Figure 7.11.)
2. Describe the forms of energy found in an apple as it grows on a tree, then falls, then is digested by someone who eats it.
3. **WHAT IF?** If you place a teaspoon of sugar in the bottom of a glass of water, it will dissolve completely over time. Left longer, eventually the water will disappear and the sugar crystals will reappear. Explain these observations in terms of entropy.

For suggested answers, see Appendix A.

CONCEPT 8.2

The free-energy change of a reaction tells us whether or not the reaction occurs spontaneously

The laws of thermodynamics that we've just explored apply to the universe as a whole. As biologists, we want to understand the chemical reactions of life—for example, which reactions occur spontaneously and which ones require some input of energy from outside. But how can we know this without assessing the energy and entropy changes in the entire universe for each separate reaction?

Free-Energy Change, ΔG

Recall that the universe is really equivalent to "the system" plus "the surroundings." In 1878, J. Willard Gibbs, a professor at Yale, defined a very useful function called the Gibbs free energy of a system (without considering its surroundings), symbolized by the letter G. We'll refer to the Gibbs free energy simply as free energy. **Free energy** is the portion of a system's energy that can perform work when temperature and pressure are uniform throughout the system, as in a living cell. Let's consider how we determine the free-energy change that occurs when a system changes—for example, during a chemical reaction.

The change in free energy, ΔG, can be calculated for a chemical reaction by applying the following equation:

$$\Delta G = \Delta H - T\Delta S$$

This equation uses only properties of the system (the reaction) itself: ΔH symbolizes the change in the system's *enthalpy* (in biological systems, equivalent to total energy); ΔS is the change in the system's entropy; and T is the absolute temperature in Kelvin (K) units ($K = °C + 273$; see the back of the book).

Using chemical methods, we can measure ΔG for any reaction. (The value will depend on conditions such as pH, temperature, and concentrations of reactants and products.) Once we know the value of ΔG for a process, we can use it to predict whether the process will be spontaneous (that is, whether it is energetically favorable and will occur without an input of energy). More than a century of experiments has shown that only processes with a negative ΔG are spontaneous. For ΔG to be negative, ΔH must be negative (the system gives up enthalpy and H decreases) or $T\Delta S$ must be positive (the system gives up order and S increases), or both: When ΔH and $T\Delta S$ are tallied, ΔG has a negative value ($\Delta G < 0$) for all spontaneous processes. In other words, every spontaneous process decreases the system's free energy, and processes that have a positive or zero ΔG are never spontaneous.

This information is immensely interesting to biologists, for it allows us to predict which kinds of change can happen without an input of energy. Such spontaneous changes can be harnessed by the cell to perform work. This principle is very important in the study of metabolism, where a major goal is to determine which reactions can supply energy for cellular work.

Free Energy, Stability, and Equilibrium

As we saw in the previous section, when a process occurs spontaneously in a system, we can be sure that ΔG is negative. Another way to think of ΔG is to realize that it represents the

difference between the free energy of the final state and the free energy of the initial state:

$$\Delta G = G_{\text{final state}} - G_{\text{initial state}}$$

For a reaction to have a negative ΔG, the system must lose free energy during the change from initial state to final state. Because it has less free energy, the system in its final state is less likely to change and is therefore more stable than it was previously.

We can think of free energy as a measure of a system's instability—its tendency to change to a more stable state. Unstable systems (higher G) tend to change in such a way that they become more stable (lower G). For example, a diver on top of a platform is less stable (more likely to fall) than when floating in the water; a drop of concentrated dye is less stable (more likely to disperse) than when the dye is spread randomly through the liquid; and a glucose molecule is less stable (more likely to break down) than the simpler molecules into which it can be split **(Figure 8.5)**. Unless something prevents it, each of these systems will move toward greater stability: The diver falls, the solution becomes uniformly colored, and the glucose molecule is broken down into smaller molecules.

Another term that describes a state of maximum stability is *equilibrium*, which you learned about in Concept 2.4 in connection with chemical reactions. At equilibrium, the forward and reverse reactions occur at the same rate, and there is no further net change in the relative concentration of products and reactants. For a system at equilibrium, G is at its lowest possible value in that system. Free energy increases when a reaction is somehow pushed away from equilibrium, perhaps by removing some of the products (and thus changing their concentration relative to that of the reactants). We can think of the equilibrium state as a free-energy valley. Any change from the equilibrium position will have a positive ΔG and will not be spontaneous. For this reason, systems never spontaneously move away from equilibrium. Because a system at equilibrium cannot spontaneously change, it can do no work. *A process is spontaneous and can perform work only when it is moving toward equilibrium.*

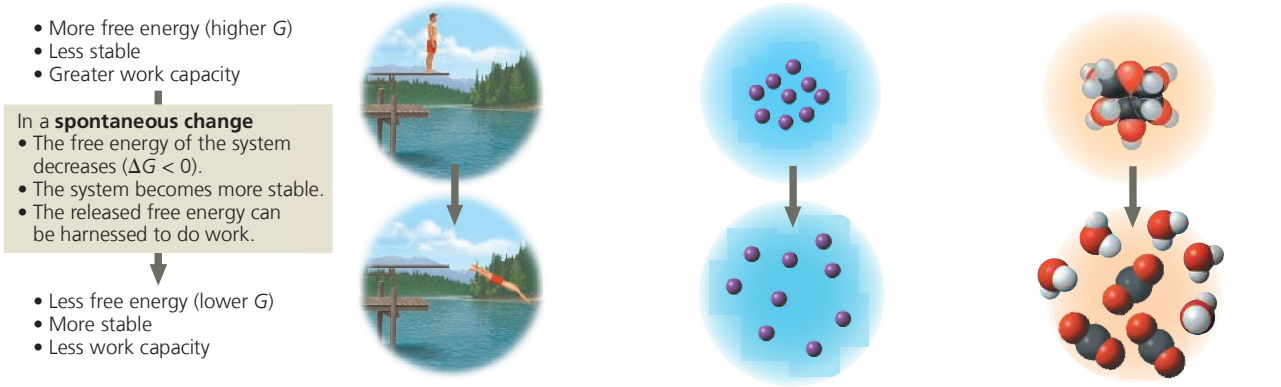
Free Energy and Metabolism

We can now apply the free-energy concept more specifically to the chemistry of life's processes.

Exergonic and Endergonic Reactions in Metabolism

Based on their free-energy changes, chemical reactions can be classified as either exergonic ("energy outward") or endergonic ("energy inward"). An **exergonic reaction** proceeds with a net release of free energy **(Figure 8.6a)**. Because the chemical mixture loses free energy (G decreases), ΔG is negative for an exergonic reaction. Using ΔG as a standard for spontaneity, exergonic reactions are those that occur spontaneously. (Remember, the word *spontaneous* implies that it is energetically favorable, not that it will occur rapidly.) The magnitude of ΔG for an exergonic reaction

▼ Figure 8.5 The relationship of free energy to stability, work capacity, and spontaneous change. Unstable systems (top) are rich in free energy, G. They tend to change spontaneously to a more stable state (bottom). This "downhill" change can be harnessed to perform work.



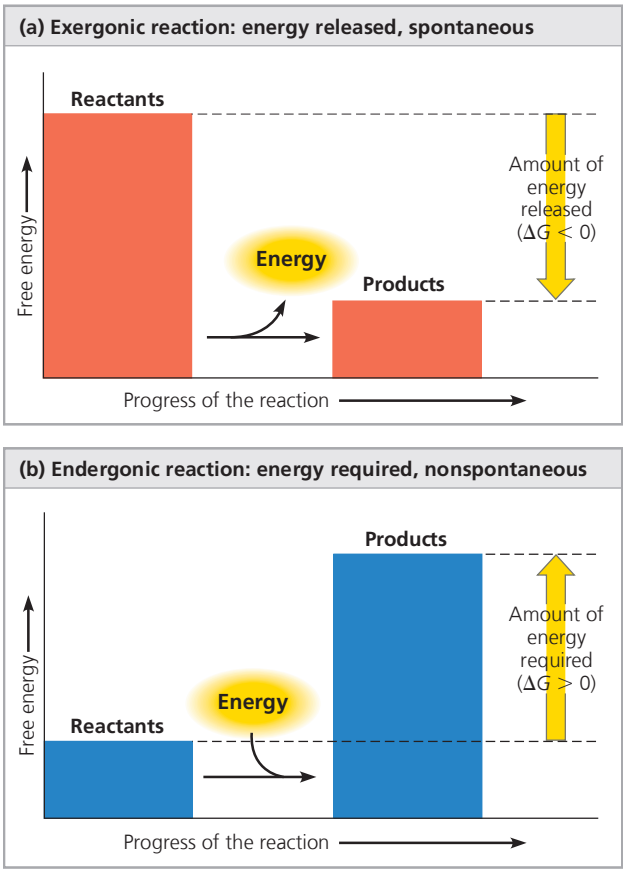
(a) Gravitational motion. Objects move spontaneously from a higher altitude to a lower one.

(b) Diffusion. Molecules in a drop of dye diffuse until they are randomly dispersed.

(c) Chemical reaction. In a cell, a glucose molecule is broken down into simpler molecules.

MAKE CONNECTIONS *Compare the redistribution of molecules shown in (b) to the transport of hydrogen ions (H^+) across a membrane by a proton pump, creating a concentration gradient (see Figure 7.18). Which process(es) result(s) in higher free energy? Which system(s) can do work?*

▼ Figure 8.6 Free energy changes (ΔG) in exergonic and endergonic reactions.



Mastering Biology **Animation: Exergonic and Endergonic Reactions**

represents the maximum amount of work the reaction can perform.* The greater the decrease in free energy, the greater the amount of work that can be done.

We can use the overall reaction for cellular respiration as an example:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$
$$\Delta G = -686 \text{ kcal/mol } (-2{,}870 \text{ kJ/mol})$$

For each mole (180 g) of glucose broken down by respiration under what are called "standard conditions" (1 *M* of each reactant and product, 25°C, pH 7), 686 kcal (2,870 kJ) of energy is made available for work. Because energy must be conserved, the chemical *products* of respiration store 686 kcal less free energy per mole than the *reactants*. The products are, in a sense, the spent exhaust of a process that tapped the free energy stored in the bonds of the sugar molecules.

It is important to realize that the breaking of bonds does not release energy; on the contrary, as you will soon see, it requires energy. The phrase "energy stored in bonds" is shorthand for the potential energy that can be released when new bonds are formed after the original bonds break, as long as the products are of lower free energy than the reactants.

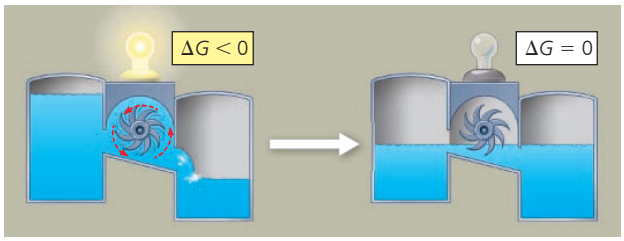
An **endergonic reaction** is one that absorbs free energy from its surroundings **(Figure 8.6b)**. Because this kind of reaction essentially *stores* free energy in molecules (*G* increases), ΔG is positive. Such reactions are non-spontaneous, and the magnitude of ΔG is the quantity of energy required to drive the reaction. If a chemical process is exergonic (downhill), releasing energy in one direction, then the reverse process must be endergonic (uphill), using energy. A reversible process cannot be downhill in both directions. If $\Delta G = -686$ kcal/mol for respiration, which converts glucose and oxygen to CO_2 and H_2O, then the reverse process—the conversion of CO_2 and H_2O to glucose and oxygen (O_2)—must be strongly endergonic, with $\Delta G = +686$ kcal/mol. Such a reaction would never happen by itself.

How, then, do plants make sugar? They get the required energy (686 kcal to make a mole of glucose) by capturing light from the sun and converting its energy to chemical energy. Next, in a long series of exergonic steps, they gradually spend that chemical energy to assemble glucose molecules.

Equilibrium and Metabolism

Reactions in an isolated system eventually reach equilibrium and can then do no work, as illustrated by the isolated hydroelectric system in **Figure 8.7**. The chemical reactions of metabolism are reversible, and they, too, would reach equilibrium if they occurred in the isolation of a test tube. Because systems at equilibrium are at a minimum of *G* and can do no work, a cell that has reached metabolic equilibrium is dead! *The fact that metabolism as a whole is never at equilibrium is one of the defining features of life.*

▼ Figure 8.7 Equilibrium and work in an isolated hydroelectric system. Water flowing downhill turns a turbine that drives a generator providing electricity to a lightbulb, but only until the system reaches equilibrium.

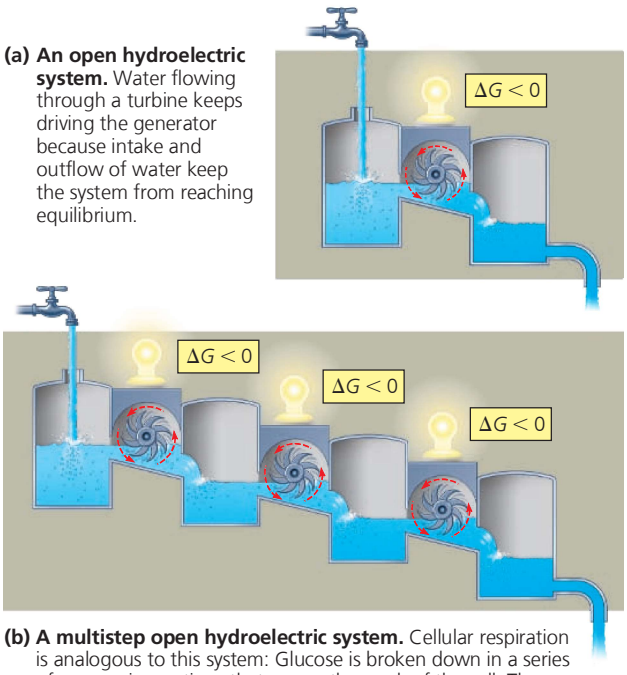


*The word *maximum* qualifies this statement because some of the free energy is released as heat and cannot do work. Therefore, ΔG represents a theoretical upper limit of available energy.

Like most systems, a living cell is not in equilibrium. Materials flow in and out, keeping metabolic pathways from ever reaching equilibrium, and the cell continues to do work throughout its life. This principle is illustrated by the open (and more realistic) hydroelectric system in **Figure 8.8a**. However, unlike this simple single-step system, a catabolic pathway in a cell releases free energy in a series of reactions. An example is cellular respiration, illustrated by analogy in **Figure 8.8b**. Some of the reversible reactions of respiration are constantly "pulled" in one direction—that is, they are kept out of equilibrium. The key to maintaining this lack of equilibrium is that the product of a reaction does not accumulate but instead becomes a reactant in the next step; finally, waste products are expelled from the cell. The overall sequence of reactions is kept going by the huge free-energy difference between glucose and O_2 at the top of the energy "hill" and CO_2 and H_2O at the "downhill" end. As long as our cells have a steady supply of glucose or other fuels and oxygen and are able to expel waste products to the surroundings, their metabolic pathways never reach equilibrium and can continue to do the work of life.

Organisms are open systems. Sunlight provides a daily source of free energy for an ecosystem's plants and other photosynthetic organisms. Animals and other nonphotosynthetic organisms in an ecosystem must have a source of free energy—the organic products of photosynthesis. Now we are ready to see how a cell actually performs the work of life.

▼ Figure 8.8 Equilibrium and work in open systems.



(a) An open hydroelectric system. Water flowing through a turbine keeps driving the generator because intake and outflow of water keep the system from reaching equilibrium.

(b) A multistep open hydroelectric system. Cellular respiration is analogous to this system: Glucose is broken down in a series of exergonic reactions that power the work of the cell. The product of each reaction is used as the reactant for the next, so no reaction reaches equilibrium.

CONCEPT CHECK 8.2

1. Cellular respiration uses glucose and O_2, which have high levels of free energy, and releases CO_2 and H_2O, which have low levels of free energy. Is cellular respiration spontaneous or not? Is it exergonic or endergonic? What happens to the energy released from glucose?
2. **VISUAL SKILLS** How would the processes of catabolism and anabolism relate to Figure 8.5c?
3. **WHAT IF?** Some partygoers wear glow-in-the-dark necklaces that start glowing once they are "activated" by snapping the necklace. This allows two chemicals to react and emit light in the form of chemiluminescence. Is the reaction exergonic or endergonic? Explain.

For suggested answers, see Appendix A.

CONCEPT 8.3

ATP powers cellular work by coupling exergonic reactions to endergonic reactions

A cell does three main kinds of work:

- *Chemical work*, the pushing of endergonic reactions that would not occur spontaneously, such as the synthesis of polymers from monomers (chemical work will be discussed further here; examples are shown in Chapters 9 and 10)
- *Transport work*, the pumping of substances across membranes against the direction of spontaneous movement (see Concept 7.4)
- *Mechanical work*, such as the beating of cilia (see Concept 6.6), the contraction of muscle cells, and the movement of chromosomes during cellular reproduction

A key feature in the way cells manage their energy resources to do this work is **energy coupling**, the use of an exergonic process to drive an endergonic one. ATP is responsible for mediating most energy coupling in cells, and in most cases it acts as the immediate source of energy that powers cellular work.

Mastering Biology Animation: Energy Coupling

The Structure and Hydrolysis of ATP

ATP (adenosine triphosphate; see Concept 4.3) contains the sugar ribose, with the nitrogenous base adenine and a chain of three phosphate groups (the triphosphate group) bonded to it **(Figure 8.9a)**. In addition to its role in energy coupling, ATP is also one of the nucleoside triphosphates used to make RNA (see Figure 5.23).

The bonds between the phosphate groups of ATP can be broken by hydrolysis. When the terminal phosphate bond is broken by addition of a water molecule, a molecule of inorganic phosphate ($HOPO_3^{2-}$, abbreviated as $\textcircled{P}_i$ throughout this book) leaves the ATP, which becomes adenosine diphosphate, or ADP **(Figure 8.9b)**. The reaction is exergonic and releases 7.3 kcal of energy per mole of ATP hydrolyzed:

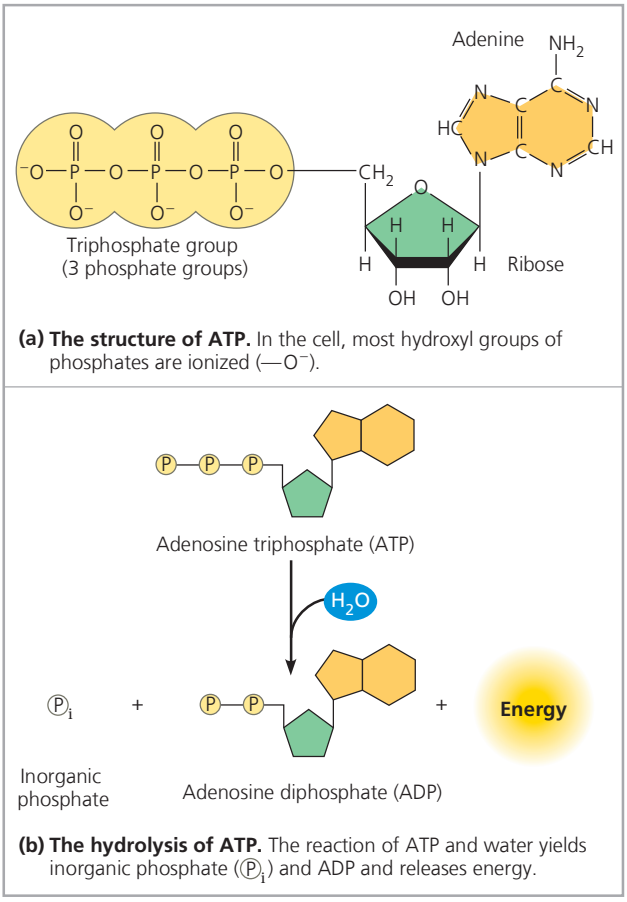
$$ATP + H_2O \rightarrow ADP + \textcircled{P}_i$$
$$\Delta G = -7.3 \text{ kcal/mol } (-30.5 \text{ kJ/mol})$$

This is the free-energy change measured under standard conditions. In the cell, conditions do not conform to standard conditions, primarily because reactant and product concentrations differ from 1 *M*. For example, when ATP hydrolysis occurs under typical cellular conditions, the actual ΔG is about −13 kcal/mol, 78% greater than the energy released by ATP hydrolysis under standard conditions.

Because their hydrolysis releases energy, the phosphate bonds of ATP are sometimes referred to as high-energy phosphate bonds, but the term is misleading. The phosphate bonds of ATP are not unusually strong bonds, as "high-energy" may imply; rather, the reactants (ATP and water) themselves have high energy relative to the energy of the products (ADP and $\textcircled{P}_i$). The release of energy during the hydrolysis of ATP comes from the chemical change of the system to a state of lower free energy, not from the phosphate bonds themselves.

ATP is useful to the cell because the energy it releases on losing a phosphate group is somewhat greater than the energy most other molecules could deliver. But why does this hydrolysis release so much energy? If we reexamine the ATP molecule in Figure 8.9a, we can see that all three phosphate groups are negatively charged. These like charges are crowded together, and their mutual repulsion contributes to the instability of this region of the ATP molecule. The triphosphate tail of ATP is the chemical equivalent of a compressed spring.

▼ Figure 8.9 The structure and hydrolysis of adenosine triphosphate (ATP). Throughout this book, the chemical structure of the triphosphate group seen in **(a)** will be represented by the three joined yellow circles shown in **(b)**.



(a) The structure of ATP. In the cell, most hydroxyl groups of phosphates are ionized (—O^-).

(b) The hydrolysis of ATP. The reaction of ATP and water yields inorganic phosphate ($\textcircled{P}_i$) and ADP and releases energy.

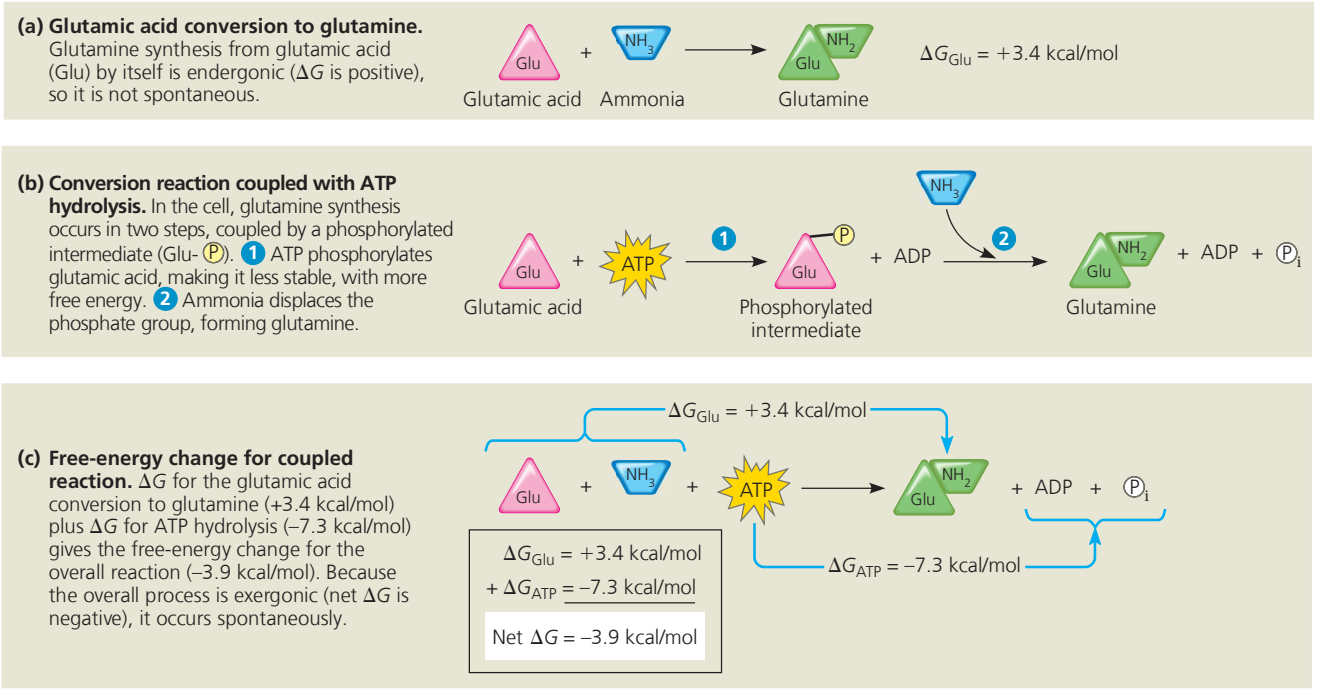
Mastering Biology Animation: The Structure of ATP
Animation: Space-Filling Model of ATP
Animation: Stick Model of ATP

How ATP Provides Energy That Performs Work

When ATP is hydrolyzed in a test tube, the release of free energy merely heats the surrounding water. In an organism, this same generation of heat can sometimes be beneficial. For instance, the process of shivering uses ATP hydrolysis during muscle contraction to warm the body. In most cases in the cell, however, the generation of heat alone would be an inefficient (and potentially dangerous) use of a valuable energy resource. Instead, the cell's proteins harness the energy released during ATP hydrolysis in several ways to perform the three types of cellular work—chemical, transport, and mechanical.

For example, with the help of specific enzymes, the cell is able to use the high free energy of ATP to drive chemical reactions that, by themselves, are endergonic. If the ΔG of an endergonic reaction is less than the amount of energy released by ATP hydrolysis, then the two reactions can be coupled so that, overall, the coupled reactions are exergonic. This usually involves phosphorylation, the transfer of a phosphate group from ATP to some other molecule, such as the reactant. The recipient molecule with the phosphate group covalently bonded to it is then called a **phosphorylated intermediate**. The key to coupling exergonic and endergonic reactions is the formation of this phosphorylated intermediate, which is more

▼ Figure 8.10 How ATP drives chemical work: energy coupling using ATP hydrolysis. In this example, the exergonic process of ATP hydrolysis drives an endergonic process—synthesis of the amino acid glutamine.



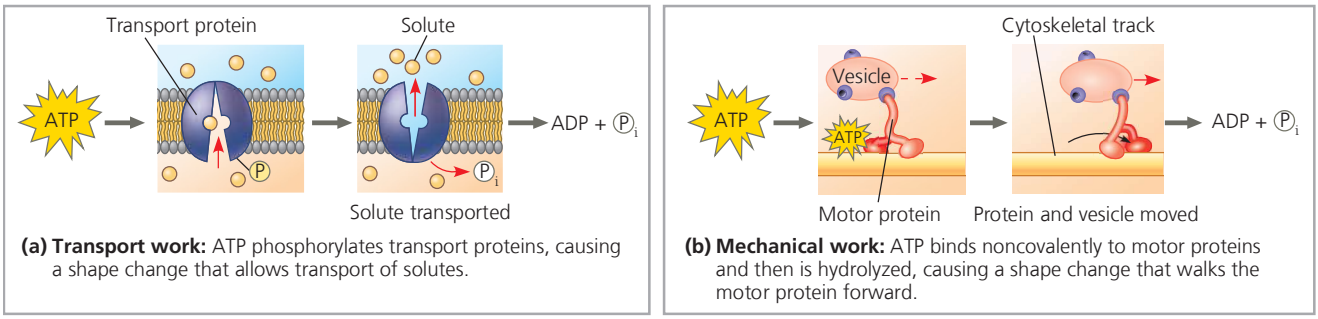
MAKE CONNECTIONS *Referring to Figure 5.14, explain why glutamine (Gln) is drawn in this figure as a glutamic acid (Glu) with an amino group attached.*

Mastering Biology Figure Walkthrough

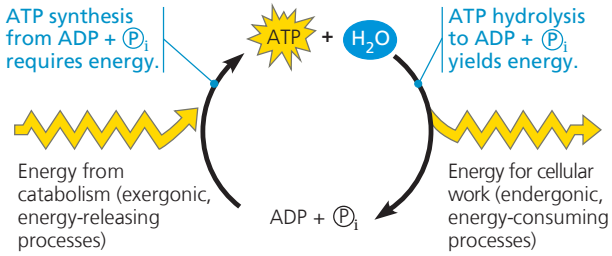
reactive (less stable, with more free energy) than the original unphosphorylated molecule **(Figure 8.10)**.

Transport and mechanical work in the cell are also nearly always powered by the hydrolysis of ATP. In these cases, ATP hydrolysis leads to a change in a protein's shape and often its ability to bind another molecule. Sometimes this occurs via a phosphorylated intermediate, as seen for the transport protein in **Figure 8.11a**. In most instances of mechanical work involving motor proteins "walking" along cytoskeletal elements **(Figure 8.11b)**, a cycle occurs in which ATP is first bound noncovalently to the motor protein. Next, ATP is hydrolyzed, releasing ADP and Ⓟi. Another ATP molecule can then bind. At each stage, the motor protein changes its shape and ability to bind the cytoskeleton, resulting in movement of the protein along the cytoskeletal track. Phosphorylation and dephosphorylation promote crucial protein shape changes during many other important cellular processes as well.

▼ Figure 8.11 How ATP drives transport and mechanical work. ATP hydrolysis causes changes in the shapes and binding affinities of proteins. This can occur either **(a)** directly, by phosphorylation, as shown for a membrane protein carrying out active transport of a solute (see also Figure 7.16 and the proton pump in Figure 6.32, upper left), or **(b)** indirectly, via noncovalent binding of ATP and its hydrolytic products, as is the case for motor proteins that move vesicles (and other organelles) along cytoskeletal "tracks" in the cell (see also Figures 6.21 and 6.32, lower right).



▼ Figure 8.12 The ATP cycle. Energy released by breakdown reactions (catabolism) in the cell is used to phosphorylate ADP, regenerating ATP. Chemical potential energy stored in ATP drives most cellular work.



Mastering Biology **Animation: Metabolism Overview**

The Regeneration of ATP

An organism at work uses ATP continuously, but ATP is a renewable resource that can be regenerated by the addition of phosphate to ADP **(Figure 8.12)**. The free energy required to phosphorylate ADP comes from exergonic breakdown reactions (catabolism) in the cell. This shuttling of inorganic phosphate and energy is called the ATP cycle, and it couples the cell's energy-yielding (exergonic) processes to the energy-consuming (endergonic) ones. The ATP cycle proceeds at an astonishing pace. For example, a working muscle cell recycles its entire pool of ATP in less than a minute. That turnover represents 10 million molecules of ATP consumed and regenerated per second per cell. If ATP could not be regenerated by the phosphorylation of ADP, humans would use up nearly their body weight in ATP each day.

Because both directions of a reversible process cannot be downhill, the regeneration of ATP from ADP and Ⓟi is necessarily endergonic:

$$ADP + Ⓟ_i \rightarrow ATP + H_2O$$

$$\Delta G = +7.3 \text{ kcal/mol } (+30.5 \text{ kJ/mol}) \text{ (standard conditions)}$$

Since ATP formation from ADP and Ⓟi is not spontaneous, free energy must be spent to make it occur. Catabolic (exergonic) pathways, especially cellular respiration, provide the energy for the endergonic process of making ATP. Plants also use light energy to produce ATP. Thus, the ATP cycle is a key player in bioenergetics, functioning as a revolving door through which energy passes during its transfer from catabolic to anabolic pathways.

CONCEPT CHECK 8.3

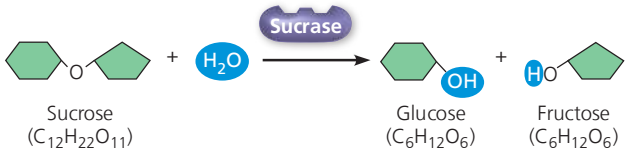
1. How does ATP typically transfer energy from an exergonic to an endergonic reaction in the cell?
2. Which combination has more free energy: glutamic acid + ammonia + ATP or glutamine + ADP + Ⓟi? Explain.
3. **MAKE CONNECTIONS** Does Figure 8.11a show passive or active transport? Explain. (See Concepts 7.3 and 7.4.)

For suggested answers, see Appendix A.

CONCEPT 8.4

Enzymes speed up metabolic reactions by lowering energy barriers

The laws of thermodynamics tell us what will and will not happen under given conditions but say nothing about the rate of these processes. A spontaneous chemical reaction occurs without any requirement for outside energy, but it may occur so slowly that it is imperceptible. For example, even though the hydrolysis of sucrose (table sugar) to glucose and fructose is exergonic, occurring spontaneously with a release of free energy ($\Delta G = -7$ kcal/mol), a solution of sucrose dissolved in sterile water will sit for years at room temperature with no appreciable hydrolysis. However, if we add a small amount of the enzyme sucrase to the solution, then all the sucrose may be hydrolyzed within seconds, as shown here:



How does the enzyme do this? An **enzyme** is a macromolecule that acts as a **catalyst**, a chemical agent that speeds up a reaction without being consumed by the reaction. In this chapter, we focus on enzymes that are proteins. (Some RNA molecules, called ribozymes, can function as enzymes; these will be discussed in Concepts 17.3 and 25.1.) Without regulation by enzymes, chemical traffic through the pathways of metabolism would become terribly congested because many chemical reactions would take such a long time. In the next two sections, we will see why spontaneous reactions can be slow and how an enzyme changes the situation.

The Activation Energy Barrier

Every chemical reaction between molecules involves both bond breaking and bond forming. For example, the hydrolysis of sucrose involves breaking the bond between glucose and fructose and one of the bonds of a water molecule and then forming two new bonds, as shown above. Changing one molecule into another generally involves contorting the starting molecule into a highly unstable state before the reaction can proceed. This contortion can be compared to the bending of a metal key ring when you pry it open to add a new key. The key ring is highly unstable in its opened form but returns to a stable state once the key is threaded all the way onto the ring. To reach the contorted state where bonds can change, reactant molecules must absorb energy from their surroundings. When the new bonds of the product molecules form, energy is released as heat, and the molecules return to stable shapes with lower energy than the contorted state.

The initial investment of energy for starting a reaction—the energy required to contort the reactant molecules so the bonds can break—is known as the *free energy of activation*, or **activation energy**, abbreviated E_A in this book. We can think of activation energy as the amount of energy needed to push the reactants to the top of an energy barrier, or "uphill," so that the "downhill" part of the reaction can begin. Activation energy is often supplied by heat in the form of thermal energy that the reactant molecules absorb from the surroundings. The absorption of thermal energy accelerates the reactant molecules, so they collide more often and more forcefully. It also agitates the atoms within the molecules, making the breakage of bonds more likely. When the molecules have absorbed enough energy for the bonds to break, the reactants are in an unstable condition known as the *transition state*.

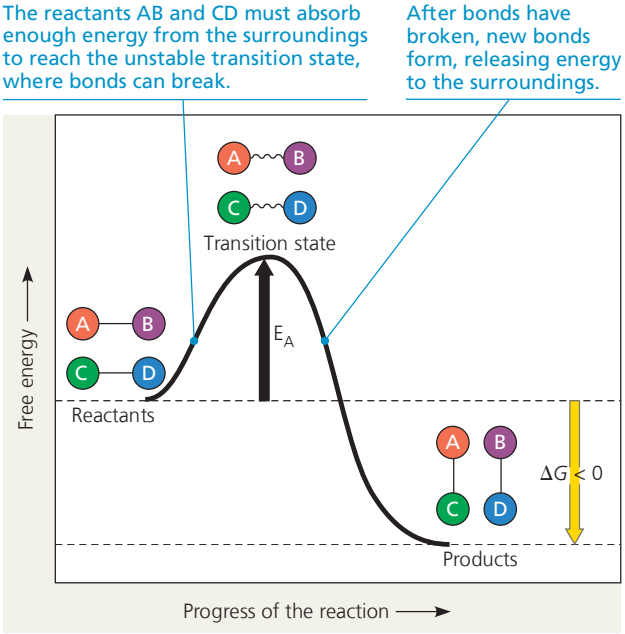
Figure 8.13 graphs the energy changes for a hypothetical exergonic reaction that swaps portions of two reactant molecules:

$$\underset{\text{Reactants}}{AB + CD} \rightarrow \underset{\text{Products}}{AC + B}$$

The activation of the reactants is represented by the uphill portion of the graph, in which the free-energy content of the reactant molecules is increasing. At the summit, when energy equivalent to E_A has been absorbed, the reactants are in the transition state: They are activated, and their bonds can be broken. As the atoms then settle into their new, more stable bonding arrangements, energy is released to the surroundings. This corresponds to the downhill part of the curve, which shows the loss of free energy by the molecules. The overall decrease in free energy means that E_A is repaid with dividends, as the formation of new bonds releases more energy than was invested in the breaking of old bonds.

The reaction shown in Figure 8.13 is exergonic and occurs spontaneously ($\Delta G < 0$). However, the activation energy provides a barrier that determines the rate of the reaction. The reactants must absorb enough energy to reach the top of the activation energy barrier before the reaction can occur. For some reactions, E_A is modest enough that even at room temperature there is sufficient thermal energy for many of the reactant molecules to reach the transition state in a short time. In most cases, however, E_A is so high and the transition state is reached so rarely that the reaction will hardly proceed at all. In these cases, the reaction will occur at a noticeable rate only if energy is provided, usually by heat. For example, the reaction of gasoline and oxygen is exergonic and will occur spontaneously, but energy is required for the molecules to reach the transition state and react. Only when the spark plugs fire in an automobile engine can there be the explosive release of energy that pushes the pistons. Without a spark, a mixture of gasoline hydrocarbons and oxygen will not react because the E_A barrier is too high.

▼ Figure 8.13 Energy profile of an exergonic reaction. The "molecules" are hypothetical, with A, B, C, and D representing portions of the molecules. Thermodynamically, this is an exergonic reaction, with a negative ΔG, and the reaction occurs spontaneously. However, the activation energy (E_A) provides a barrier that determines the rate of the reaction.



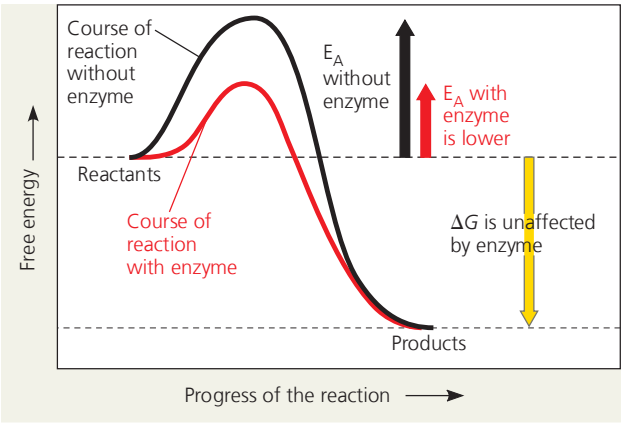
DRAW IT *Graph the progress of an endergonic reaction in which EF and GH form products EG and FH, assuming that the reactants must pass through a transition state.*

How Enzymes Speed Up Reactions

Proteins, DNA, and other complex cellular molecules are rich in free energy and have the potential to decompose spontaneously; that is, the laws of thermodynamics favor their breakdown. These molecules only persist because at temperatures typical for cells, few molecules can make it over the hump of activation energy. The barriers for selected reactions must occasionally be surmounted, however, for cells to carry out the processes needed for life. Heat can increase the rate of a reaction by allowing reactants to attain the transition state more often, but this would not work well in biological systems. First, high temperature denatures proteins and kills cells. Second, heat would speed up *all* reactions, not just those that are needed. Instead of heat, organisms carry out **catalysis**, the process by which a catalyst selectively speeds up a reaction without itself being consumed. (You learned about catalysts earlier in this section.)

An enzyme catalyzes a reaction by lowering the E_A barrier **(Figure 8.14)**, enabling the reactant molecules to absorb enough energy to reach the transition state even at moderate temperatures, as we'll see shortly. It is crucial to note that *an enzyme cannot change the ΔG for a reaction; it cannot make an endergonic reaction exergonic*. Enzymes can only

▼ Figure 8.14 The effect of an enzyme on activation energy. Without affecting the free-energy change (ΔG) for a reaction, an enzyme speeds the reaction by reducing its activation energy (E_A).



Mastering Biology **Animation: How Enzymes Work**

hasten reactions that would eventually occur anyway, but this enables the cell to have a dynamic metabolism, routing chemicals smoothly through metabolic pathways. Also, enzymes are very specific for the reactions they catalyze, so they determine which chemical processes will be going on in the cell at any given time.

Substrate Specificity of Enzymes

The reactant an enzyme acts on is referred to as the enzyme's **substrate**. The enzyme binds to its substrate (or substrates, when there are two or more reactants), forming an **enzyme-substrate complex**. While enzyme and substrate are joined, the catalytic action of the enzyme converts the substrate to the product (or products) of the reaction. The overall process can be summarized as follows:

$$\text{Enzyme} + \text{Substrate(s)} \rightleftharpoons \text{Enzyme-substrate complex} \rightleftharpoons \text{Enzyme} + \text{Product(s)}$$

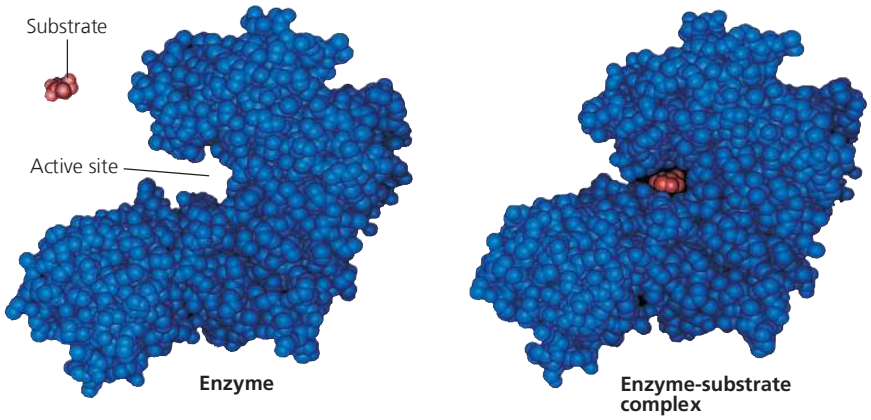
Most enzyme names end in *-ase*. (See if you can find three examples of enzymes in the lower left of Figure 6.32.) For example, the enzyme sucrase catalyzes the hydrolysis of the disaccharide sucrose into its two monosaccharides, glucose and fructose (see the diagram at the beginning of Concept 8.4):

$$\text{Sucrase} + \text{Sucrose} + H_2O \rightleftharpoons \text{Sucrase-sucrose-}H_2O\text{ complex} \rightleftharpoons \text{Sucrase} + \text{Glucose} + \text{Fructose}$$

The reaction catalyzed by each enzyme is very specific; an enzyme can recognize its specific substrate even among closely related compounds. For instance, sucrase will act only on sucrose and will not bind to other disaccharides, such as maltose. What accounts for this molecular recognition? Recall that most enzymes are proteins, and that proteins are macromolecules with unique 3-D configurations. The specificity of an enzyme results from its shape, which is a consequence of its amino acid sequence.

Only a restricted region of the enzyme molecule actually binds to the substrate. This region, called the **active site**, is typically a pocket or groove on the surface of the enzyme where catalysis occurs (**Figure 8.15a**; see also Figure 5.16). Usually, the active site is formed by only a few of the enzyme's amino acids, with the rest of the protein molecule providing a framework that determines the shape of the active site. The specificity of an enzyme is attributed to a complementary fit between the shape of its active site and the shape of the substrate.

▼ Figure 8.15 Induced fit between an enzyme and its substrate.



(a) In this space-filling model of the enzyme hexokinase (blue), the active site forms a groove on the surface. The enzyme's substrate is glucose (red).

(b) When the substrate enters the active site, it forms weak bonds with the enzyme, inducing a change in the shape of the protein. This change allows additional weak bonds to form, causing the active site to enfold the substrate and hold it in place.

An enzyme is not a stiff structure locked into a given shape. In fact, recent work by biochemists has shown that enzymes (and other proteins) seem to "dance" between subtly different shapes in a dynamic equilibrium, with slight differences in free energy for each "pose." The shape that best fits the substrate isn't necessarily the one with the lowest energy, but during the very short time the enzyme takes on this shape, its active site can bind to the substrate. The active site itself is also not a rigid receptacle for the substrate. As shown in **Figure 8.15b**, when the substrate enters the active site, the enzyme changes shape slightly due to interactions between the substrate's chemical groups and chemical groups

on the side chains of the amino acids that form the active site. This shape change makes the active site fit even more snugly around the substrate. The tightening of the binding after initial contact—called **induced fit**—is like a clasping handshake. Induced fit brings chemical groups of the active site into positions that enhance their ability to catalyze the chemical reaction.

Catalysis in the Enzyme's Active Site

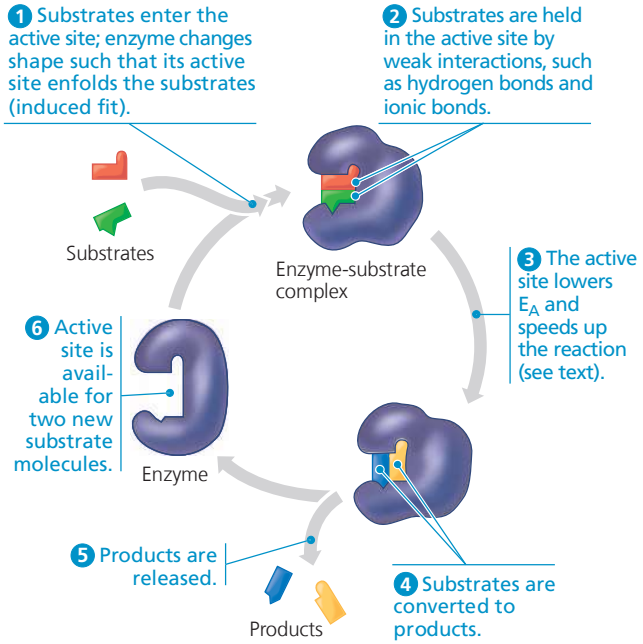
In most enzymatic reactions, the substrate is held in the active site by so-called weak interactions, such as hydrogen bonds and ionic bonds. The R groups of a few of the amino acids that make up the active site catalyze the conversion of substrate to product, and the product departs from the active site. The enzyme is then free to take another substrate molecule into its active site. The entire cycle happens so fast that a single enzyme molecule typically acts on about 1,000 substrate molecules per second, and some enzymes are even faster. Enzymes, like other catalysts, emerge from the reaction in their original form. Therefore, very small amounts of enzyme can have a huge metabolic impact by functioning over and over again in catalytic cycles. **Figure 8.16** shows a catalytic cycle involving two substrates and two products.

Most metabolic reactions are reversible, and an enzyme can catalyze either the forward or the reverse reaction, depending on which direction has a negative ΔG. This in turn depends mainly on the relative concentrations of reactants and products. The net effect is always in the direction of equilibrium.

Enzymes use a variety of mechanisms that lower activation energy and speed up a reaction (see Figure 8.16, step 3):

- In reactions involving two or more reactants, the active site provides a template on which the substrates can come together in the proper orientation for a reaction to occur between them.
- As the active site of an enzyme clutches the bound substrates, the enzyme may stretch the substrate molecules toward their transition state form, stressing and bending critical chemical bonds to be broken during the reaction. Because E_A is proportional to the difficulty of breaking the bonds, distorting the substrate helps it approach the transition state and thus reduces the amount of free energy that must be absorbed to achieve that state.
- The active site may also provide a microenvironment that is more conducive to a certain reaction than the solution itself would be without the enzyme. For example, if the active site has amino acids with acidic R groups, it may provide a pocket of low pH in an otherwise neutral cell. Here, an acidic amino acid may facilitate H^+ transfer to the substrate as a key step in catalyzing the reaction.
- Amino acids in the active site may directly participate in the chemical reaction. Sometimes this process involves brief covalent bonding between the substrate and the side chain of an amino acid of the enzyme. Subsequent steps restore the side chains to their original states so that the active site is the same after the reaction as it was before.

▼ Figure 8.16 The active site and catalytic cycle of an enzyme. An enzyme can convert one or more reactant molecules to one or more product molecules. The enzyme shown here converts two substrate molecules to two product molecules.



VISUAL SKILLS *The enzyme-substrate complex passes through a transition state (see Figure 8.13). Label the part of the cycle where the transition state occurs.*

Mastering Biology **Animation: Enzymes: Steps in a Reaction**

The rate at which a particular amount of enzyme converts substrate to product is partly a function of the initial concentration of the substrate: The more substrate molecules that are available, the more frequently they access the active sites of the enzyme molecules. However, there is a limit to how fast the reaction can be pushed by adding more substrate to a fixed concentration of enzyme. At some point, the concentration of substrate will be high enough that all enzyme molecules will have their active sites engaged. As soon as the product exits an active site, another substrate molecule enters. At this substrate concentration, the enzyme is said to be *saturated*, and the rate of the reaction is determined by the speed at which the active site converts substrate to product. When an enzyme population is saturated, the only way to increase the rate of product formation is to add more enzyme. Cells often increase the rate of a reaction by producing more enzyme molecules. You can graph the overall progress of an enzymatic reaction in the **Scientific Skills Exercise**.

Scientific Skills Exercise

Making a Line Graph and Calculating a Slope

Does the Rate of Glucose 6-Phosphatase Activity Change over Time in Isolated Liver Cells? Glucose 6-phosphatase, which is found in mammalian liver cells, is a key enzyme in control of blood glucose level. The enzyme catalyzes the breakdown of glucose 6-phosphate into glucose and inorganic phosphate ($Ⓟ_i$). These products are transported out of liver cells into the blood, increasing the blood glucose level. In this exercise, you will graph data from a time-course experiment that measured $Ⓟ_i$ concentration in the buffer outside isolated liver cells, thus indirectly measuring glucose 6-phosphatase activity inside the cells.

How the Experiment Was Done Isolated rat liver cells were placed in a dish with buffer at physiological conditions (pH 7.4, 37°C). Glucose 6-phosphate (the substrate) was added so it could be taken up by cells and broken down by glucose 6-phosphatase. A sample of buffer was removed every 5 minutes and the concentration of $Ⓟ_i$ that had been transported out of the cells was determined.

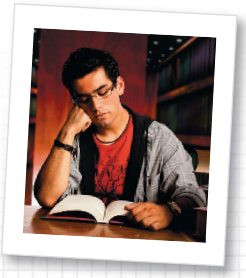
Data from the Experiment

Time (min)	Concentration of $Ⓟ_i$ (μmol/mL)
0	0
5	10
10	90
15	180
20	270
25	330
30	355
35	355
40	355

Data from S. R. Commerford et al., Diets enriched in sucrose or fat increase gluconeogenesis and G-6-Pase but not basal glucose production in rats, *American Journal of Physiology-Endocrinology and Metabolism* 283:E545–E555 (2002).

INTERPRET THE DATA

1. To see patterns in the data from a time-course experiment like this, it is helpful to graph the data. First, determine which set of data goes on each axis. **(a)** What did the researchers intentionally vary in the experiment? This is the independent variable, which goes on the *x*-axis. **(b)** What are the units (abbreviated) for the independent variable? Explain in words what the abbreviation stands for. **(c)** What was measured by the researchers? This is the dependent variable, which goes on the *y*-axis. **(d)** What does the units abbreviation stand for? Label each axis, including the units.
2. Next, mark off the axes with just enough evenly spaced tick marks to accommodate the full set of data. Determine the span of data values for each axis. **(a)** What is the largest value to go on the *x*-axis? What is a reasonable spacing for the tick marks, and what should be the highest one? **(b)** What is the largest value to go on the *y*-axis? What is a reasonable spacing for the tick marks, and what should be the highest one?
3. Plot the data points on your graph. Match each *x*-value with its partner *y*-value and place a point on the graph at that coordinate. Draw a line that connects the points. (For additional information about graphs, see the Scientific Skills Review in Appendix D.)
4. Examine your graph and look for patterns in the data. **(a)** Does the concentration of $Ⓟ_i$ increase evenly throughout the course of the experiment? To answer this question, describe the pattern you see in the graph. **(b)** What part of the graph shows the highest rate of enzyme activity? Consider that the rate of enzyme activity is related to the slope of the line, $\Delta y/\Delta x$ (the "rise" over the "run"), in μmol/(mL · min), with the steepest slope indicating the highest rate of enzyme activity. Calculate the rate of enzyme activity (slope) where the graph is steepest. **(c)** Can you think of a biological explanation for the pattern you see?
5. If your blood glucose level is low from skipping lunch, what reaction (discussed in this exercise) will occur in your liver cells? Write out the reaction and put the name of the enzyme over the reaction arrow. How will this reaction affect your blood glucose level?



Instructors: A version of this Scientific Skills Exercise can be assigned in **Mastering Biology**.

Effects of Local Conditions on Enzyme Activity

The activity of an enzyme—how efficiently the enzyme functions—is affected by general environmental factors, such as temperature and pH. It can also be affected by chemicals that specifically influence that enzyme. In fact, researchers have learned much about enzyme function by employing such chemicals.

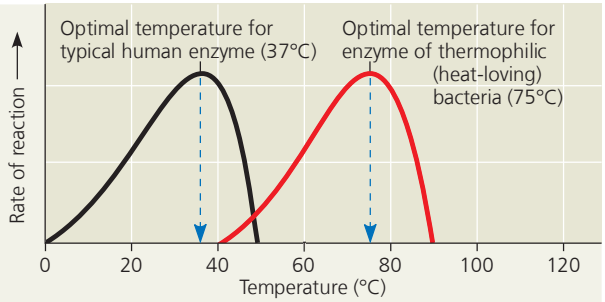
Effects of Temperature and pH

The 3-D structures of proteins are sensitive to their environment (see Figure 5.20). As a consequence, each enzyme works better under some conditions than others, because these *optimal conditions* favor the most active shape for the enzyme.

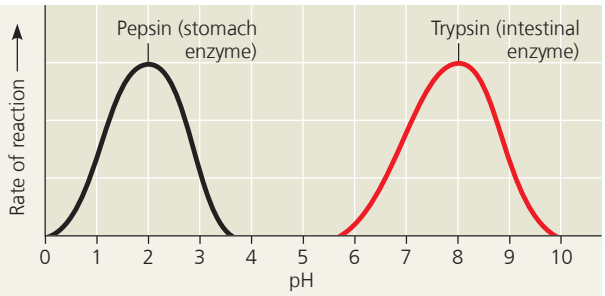
Temperature and pH are environmental factors important in the activity of an enzyme. Up to a point, the rate of an enzymatic reaction increases with increasing temperature, partly because substrates collide with active sites more frequently when the molecules move rapidly. Above that temperature, however, the speed of the enzymatic reaction drops sharply. The thermal agitation of the enzyme molecule disrupts the hydrogen bonds, ionic bonds, and other weak interactions that stabilize the active shape of the enzyme, and the protein molecule eventually denatures. Each enzyme has an optimal temperature at which its reaction rate is greatest. Without denaturing the enzyme, this temperature allows the greatest number of molecular collisions and the fastest conversion of the reactants to product molecules. Most human

enzymes have optimal temperatures of about 35–40°C (close to human body temperature). The thermophilic bacteria that live in hot springs contain enzymes with optimal temperatures of 70°C or higher **(Figure 8.17a)**.

▼ Figure 8.17 Environmental factors affecting enzyme activity. Each enzyme has an optimal **(a)** temperature and **(b)** pH that favor the most active shape of the protein molecule.



(a) The photo shows thermophilic cyanobacteria (green) thriving in the hot water of a Nevada geyser. The graph compares the optimal temperatures for an enzyme from the thermophilic bacterium *Thermus oshimai* (75°C) and human enzymes (37°C, body temperature).



(b) This graph shows the rate of reaction for two digestive enzymes over a range of pH values.

INTERPRET THE DATA *Looking at the graph in (b), what is the optimal pH for pepsin activity? Explain why natural selection might have resulted in the optimal pH for pepsin, a stomach enzyme (see Figure 3.11). What is the optimal pH for trypsin?*

Just as each enzyme has an optimal temperature, it also has a pH at which it is most active. The optimal pH values for most enzymes fall in the range of pH 6–8, but there are exceptions. For example, pepsin, a digestive enzyme in the human stomach, works best at a very low pH. Such an acidic environment denatures most enzymes, but pepsin is well-adapted evolutionarily to maintain its functional 3-D structure in the acidic environment of the stomach. In contrast, trypsin, a digestive enzyme residing in the more alkaline environment of the human intestine would be denatured in the stomach **(Figure 8.17b)**.

Cofactors

Many enzymes require nonprotein helpers for catalytic activity, often for chemical processes like electron transfers that cannot easily be carried out by the amino acids in proteins. These adjuncts, called **cofactors**, may be bound tightly to the enzyme as permanent residents, or they may bind loosely and reversibly along with the substrate. The cofactors of some enzymes are inorganic, such as the metal atoms zinc, iron, and copper in ionic form. If the cofactor is an organic molecule, it is referred to, more specifically, as a **coenzyme**. Most vitamins are important in nutrition because they act as coenzymes or raw materials from which coenzymes are made.

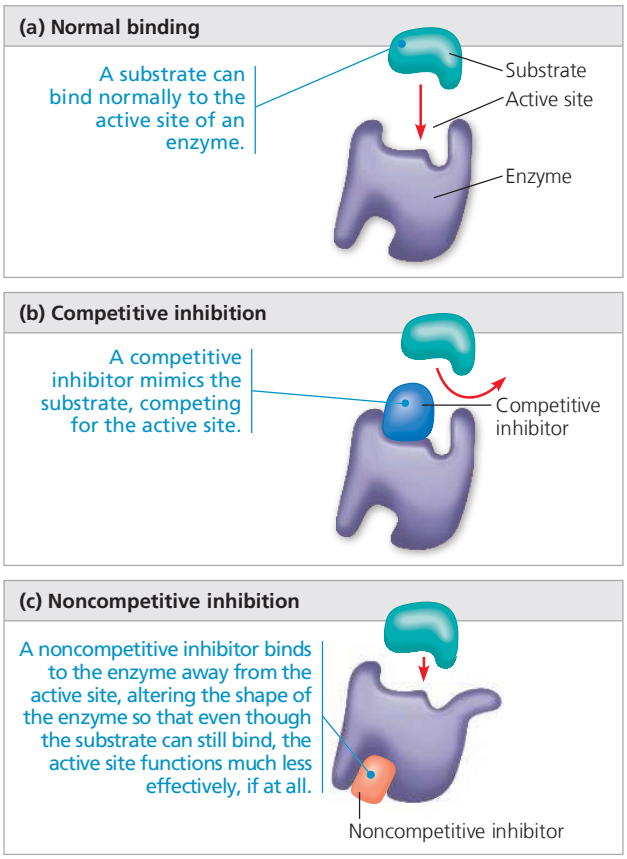
Enzyme Inhibitors

Certain chemicals selectively inhibit the action of specific enzymes. Sometimes the inhibitor attaches to the enzyme by covalent bonds, in which case the inhibition is usually irreversible. Many enzyme inhibitors, however, bind to the enzyme by weak interactions, and when this occurs the inhibition is reversible. Some reversible inhibitors resemble the normal substrate molecule and compete for admission into the active site **(Figure 8.18a** and **b)**. These mimics, called **competitive inhibitors**, reduce the productivity of enzymes by blocking substrates from entering active sites. This kind of inhibition can be overcome by increasing the concentration of substrate so that as active sites become available, more substrate molecules than inhibitor molecules are around to gain entry to the sites.

In contrast, **noncompetitive inhibitors** do not directly compete with the substrate to bind to the enzyme at the active site **(Figure 8.18c)**. Instead, they impede enzymatic reactions by binding to another part of the enzyme. This interaction causes the enzyme molecule to change its shape in such a way that the active site becomes much less effective at catalyzing the conversion of substrate to product.

Toxins and poisons are often irreversible enzyme inhibitors. An example is sarin, a nerve gas. In 2017, sarin was used in a chemical attack in Syria, killing about 100 people and injuring hundreds more. This small molecule binds covalently to the R group on the amino acid serine, which is found in the active site of acetylcholinesterase, an enzyme important in the nervous system. Other examples include the pesticides DDT and parathion, inhibitors of key enzymes in the nervous system. Finally, many antibiotics are inhibitors of specific

▼ Figure 8.18 Inhibition of enzyme activity.



Mastering Biology **Animation: Enzymes: Competitive Inhibition • Animation: Enzymes: Noncompetitive Inhibition**

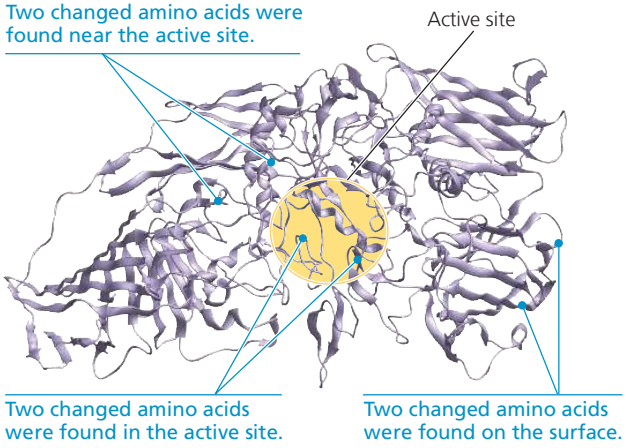
enzymes in bacteria. For instance, penicillin blocks the active site of an enzyme that many bacteria use to make cell walls.

Citing enzyme inhibitors that are metabolic poisons may give the impression that enzyme inhibition is generally abnormal and harmful. In fact, molecules naturally present in the cell often regulate enzyme activity by acting as inhibitors. Such regulation—selective inhibition—is essential to the control of cellular metabolism, as you'll see in Concept 8.5.

The Evolution of Enzymes

EVOLUTION Thus far, biochemists have identified more than 4,000 different enzymes in various species, most likely a very small fraction of all enzymes. How did this grand profusion of enzymes arise? Recall that most enzymes are proteins, and proteins are encoded by genes. A permanent change in a gene, known as a *mutation*, can result in a protein with one or more changed amino acids. In the case of an enzyme, if the changed amino acids are in the active site or some other crucial region, the altered enzyme might have a novel activity or might bind to a different substrate. Under environmental conditions where the new function benefits the organism, natural selection would

▼ Figure 8.19 Mimicking evolution of an enzyme with a new function. Researchers tested whether the function of an enzyme called β-galactosidase, which breaks down the sugar lactose, could change over time in populations of the bacterium *Escherichia coli*. After seven rounds of mutation and selection in the lab, β-galactosidase evolved into an enzyme specialized for breaking down a sugar other than lactose. This ribbon model shows one subunit of the altered enzyme; six amino acids (blue dots) were different.



tend to favor the mutated form of the gene, causing it to persist in the population. This simplified model is generally accepted as the main way in which the multitude of different enzymes arose over the past few billion years of life's history. Data supporting this model have been collected by researchers using a lab procedure that mimics evolution in natural populations **(Figure 8.19)**.

CONCEPT CHECK 8.4

1. Many spontaneous reactions occur very slowly. Why don't all spontaneous reactions occur instantly?
2. Say you are using a Bunsen burner in the lab. Why doesn't the flame creep back into the gas line and set the gas supply on fire?
3. **WHAT IF?** Malonate is an inhibitor of the enzyme succinate dehydrogenase. How would you determine whether malonate is a competitive or noncompetitive inhibitor?
4. **DRAW IT** A mature lysosome has an internal pH of around 4.5. Using Figure 8.17b as a guide, draw a graph showing what you would predict for the rate of reaction for a lysosomal enzyme. Label its optimal pH, assuming its optimal pH matches its environment.

For suggested answers, see Appendix A.

CONCEPT 8.5

Regulation of enzyme activity helps control metabolism

Chemical chaos would result if all of a cell's metabolic pathways were operating simultaneously. Intrinsic to life's processes is a cell's ability to tightly regulate its metabolic pathways by controlling when and where its various enzymes are active. It does this either by switching on and off the genes that encode specific enzymes (which will be discussed in

Concepts 18.1 and 18.2) or, as discussed here, by regulating the activity of enzymes once they are made.

Allosteric Regulation of Enzymes

In many cases, the molecules that naturally regulate enzyme activity in a cell behave something like reversible noncompetitive inhibitors (see Figure 8.18c): These regulatory molecules change an enzyme's shape and the functioning of its active site by binding to a site elsewhere on the molecule, via noncovalent interactions. **Allosteric regulation** is the term used to describe any case in which a protein's function at one site is affected by the binding of a regulatory molecule to a separate site. It may result in either inhibition or stimulation of an enzyme's activity.

Allosteric Activation and Inhibition

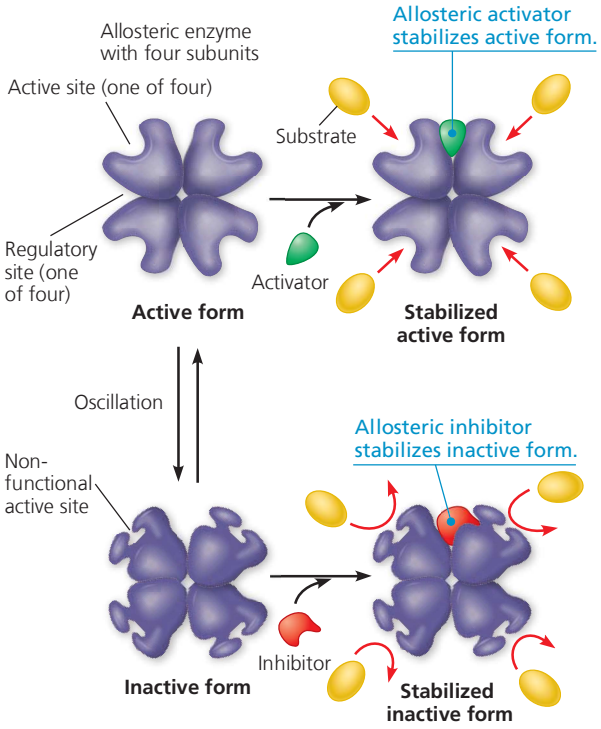
Most enzymes known to be allosterically regulated are constructed from two or more subunits, each composed of a polypeptide chain with its own active site. The entire complex oscillates between two different shapes, one catalytically active and the other inactive **(Figure 8.20a)**. In the simplest kind of allosteric regulation, an activating or inhibiting regulatory molecule binds to a regulatory site (sometimes called an allosteric site), often located where subunits join. The binding of an *activator* to a regulatory site stabilizes the shape that has functional active sites, whereas the binding of an *inhibitor* stabilizes the inactive form of the enzyme. The subunits of an allosteric enzyme fit together in such a way that a shape change in one subunit is transmitted to all others. Through this interaction of subunits, a single activator or inhibitor molecule that binds to one regulatory site will affect the active sites of all subunits.

Fluctuating concentrations of regulators can cause a sophisticated pattern of response in the activity of cellular enzymes. The products of ATP hydrolysis (ADP and $\textcircled{P}_i$), for example, play a complex role in balancing the flow of traffic between anabolic and catabolic pathways by their effects on key enzymes. ATP binds to several catabolic enzymes allosterically, lowering their affinity for substrate and thus inhibiting their activity. ADP, however, functions as an activator of the same enzymes. This is logical because catabolism functions in regenerating ATP. If ATP production lags behind its use, ADP accumulates and activates the enzymes that speed up catabolism, producing more ATP. If the supply of ATP exceeds demand, then catabolism slows down as ATP molecules accumulate and bind to the same enzymes, inhibiting them. (You'll see specific examples of this type of regulation when you learn about cellular respiration in the next chapter; see, for example, Figure 9.19.) ATP, ADP, and other related molecules also affect key enzymes in anabolic pathways. In this way, allosteric enzymes control the rates of important reactions in both sorts of metabolic pathways.

In another kind of allosteric activation, a *substrate* molecule binding to one active site in a multisubunit enzyme triggers a shape change in all the subunits, thereby increasing catalytic activity at the other active sites **(Figure 8.20b)**.

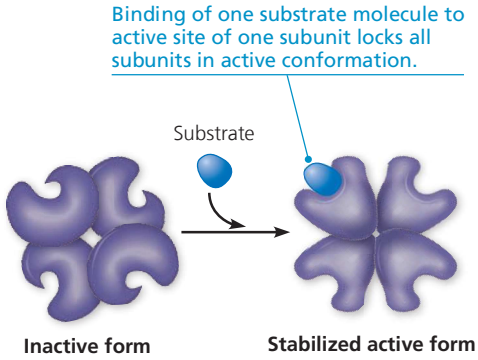
▼ Figure 8.20 Allosteric regulation of enzyme activity.

(a) Allosteric activators and inhibitors



At low concentrations, activators and inhibitors dissociate from the enzyme. The enzyme can then oscillate again.

(b) Cooperativity: another type of allosteric activation



The inactive form shown on the left oscillates with the active form when the active form is not stabilized by substrate.

Called **cooperativity**, this mechanism amplifies the response of enzymes to substrates: One substrate molecule primes an enzyme to act on additional substrate molecules more readily. Cooperativity is considered allosteric regulation because even though substrate is binding to an active site, its binding affects catalysis in another active site.

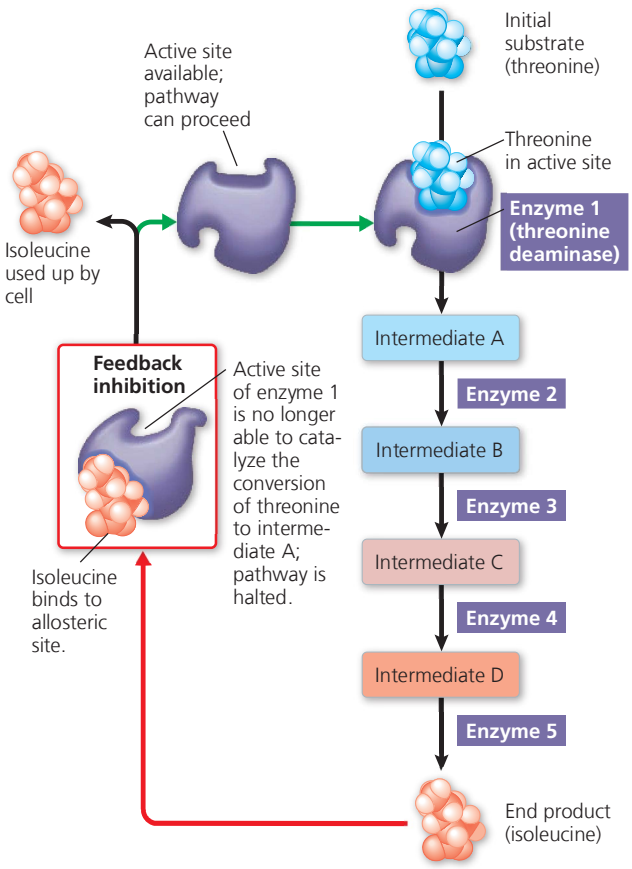
Although hemoglobin is not an enzyme (it carries O_2 rather than catalyzing a reaction), classic studies of hemoglobin

have elucidated the principle of cooperativity. Hemoglobin is made up of four subunits, each with an O_2-binding site (see Figure 5.18). The binding of an O_2 to one binding site increases the affinity for O_2 of the remaining binding sites. Thus, where O_2 is at a high level, such as in the lungs or gills, hemoglobin's affinity for O_2 increases as more binding sites are filled. In O_2-deprived tissues, however, the release of each O_2 molecule decreases the O_2 affinity of the other binding sites, resulting in the release of O_2 where it is most needed. Cooperativity works similarly in multisubunit enzymes that have been studied.

Feedback Inhibition

Earlier, we looked at the allosteric inhibition of an enzyme in an ATP-generating pathway by ATP itself. This is a common mode of metabolic control, called **feedback inhibition**, in which a metabolic pathway is halted by the inhibitory binding of its end product to an enzyme that acts early in the pathway. **Figure 8.21** shows an example of feedback inhibition operating on an anabolic pathway. Some cells use this five-step pathway to synthesize the amino acid isoleucine from threonine, another amino acid. As isoleucine accumulates, it slows down its own synthesis by allosterically inhibiting the enzyme for the first step of the pathway. Feedback inhibition thereby prevents the cell from making more isoleucine than is necessary and thus wasting chemical resources.

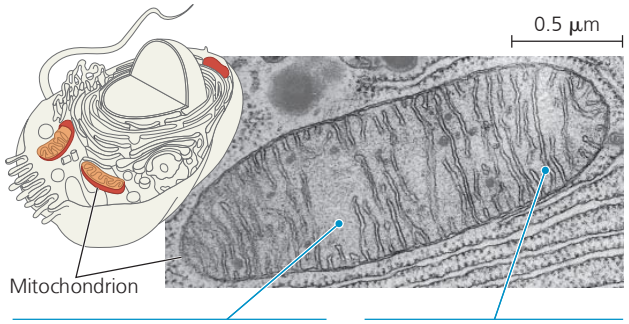
▼ Figure 8.21 Feedback inhibition in isoleucine synthesis.



Localization of Enzymes Within the Cell

The cell is not just a bag of chemicals with thousands of different kinds of enzymes and substrates in a random mix. The cell is compartmentalized, and cellular structures help bring order to metabolic pathways. In some cases, a team of enzymes for several steps of a metabolic pathway are assembled into a multienzyme complex. The arrangement facilitates the sequence of reactions, with the product from the first enzyme becoming the substrate for an adjacent enzyme in the complex, and so on, until the end product is released. Some enzymes and enzyme complexes have fixed locations within the cell and act as structural components of particular membranes. Others are in solution within particular membrane-enclosed eukaryotic organelles, each with its own internal chemical environment. For example, in eukaryotic cells, the enzymes for the second and third stages of cellular respiration reside in specific locations within mitochondria (**Figure 8.22**; see also Figure 6.32b).

▼ Figure 8.22 Organelles and structural order in metabolism. Organelles such as the mitochondrion (TEM) contain enzymes that carry out specific functions, in this case the second and third stages of cellular respiration. (See also Figure 6.32b, lower left.)



In this chapter, you have learned about the laws of thermodynamics that govern metabolism, the intersecting set of chemical pathways characteristic of life. We have explored the bioenergetics of breaking down and building up biological molecules. To continue the theme of energy flow, and specifically of bioenergetics, we will next examine cellular respiration. This is the major catabolic pathway that breaks down organic molecules and releases energy that can be used for the crucial processes of life.

CONCEPT CHECK 8.5

1. How do an activator and an inhibitor have different effects on an allosterically regulated enzyme?
2. **WHAT IF?** Regulation of isoleucine synthesis is an example of feedback inhibition of an anabolic pathway. With that in mind, explain how ATP might be involved in feedback inhibition of a catabolic pathway.

For suggested answers, see Appendix A.

8 Chapter Review

Go to **Mastering Biology** for Assignments, the eText, the Study Area, and Dynamic Study Modules.

SUMMARY OF KEY CONCEPTS

To review key terms, go to the **Vocabulary Self-Quiz** in the **Mastering Biology** eText or Study Area, or go to goo.gl/zkjz9t.

CONCEPT 8.1

An organism's metabolism transforms matter and energy *(pp. 144–147)*

- **Metabolism** is the collection of chemical reactions that occur in an organism. Enzymes catalyze reactions in intersecting **metabolic pathways**, which may be **catabolic** (breaking down molecules, releasing energy) or **anabolic** (building molecules, consuming energy). **Bioenergetics** is the study of the flow of energy through living organisms.
- **Energy** is the capacity to cause change; some forms of energy do work by moving matter. **Kinetic energy** is associated with motion and includes **thermal energy** associated with random motion of atoms or molecules. **Heat** is thermal energy in transfer from one object to another. **Potential energy** is related to the location or structure of matter and includes **chemical energy** possessed by a molecule due to its structure.
- The **first law of thermodynamics**, conservation of energy, states that energy cannot be created or destroyed, only transferred or transformed. The **second law of thermodynamics** states that **spontaneous processes**, those requiring no outside input of energy, increase the **entropy** (molecular disorder) of the universe.

? *Explain how the highly ordered structure of a cell does not conflict with the second law of thermodynamics.*

CONCEPT 8.2

The free-energy change of a reaction tells us whether or not the reaction occurs spontaneously *(pp. 147–150)*

- A living system's **free energy** is energy that can do work under cellular conditions. The change in free energy (ΔG) during a biological process is related directly to enthalpy change (ΔH) and to the change in entropy (ΔS): $\Delta G = \Delta H - T\Delta S$. Organisms live at the expense of free energy. A spontaneous process occurs with no energy input; during such a process, free energy decreases and the stability of a system increases. At maximum stability, the system is at equilibrium and can do no work.
- In an **exergonic** (spontaneous) chemical reaction, the products have less free energy than the reactants ($-\Delta G$). **Endergonic** (nonspontaneous) reactions require an input of energy ($+\Delta G$). The addition of starting materials and the removal of end products prevent metabolism from reaching equilibrium.

? *Explain the meaning of each component in the equation for the change in free energy of a spontaneous chemical reaction. Why are spontaneous reactions important in the metabolism of a cell?*

CONCEPT 8.3

ATP powers cellular work by coupling exergonic reactions to endergonic reactions *(pp. 150–153)*

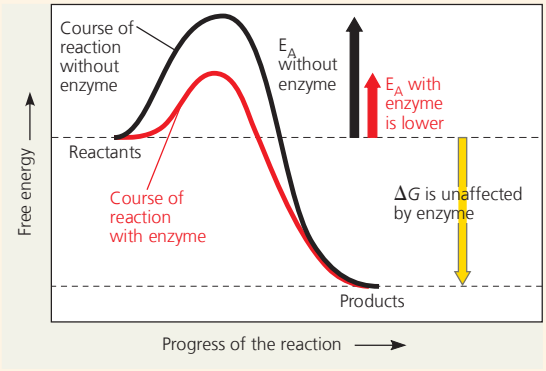
- **ATP** is the cell's energy shuttle. Hydrolysis of its terminal phosphate yields ADP and Ⓟ_i and releases free energy.
- Through **energy coupling**, the exergonic process of ATP hydrolysis drives endergonic reactions by transfer of a phosphate group to specific reactants, forming a **phosphorylated intermediate** that is more reactive. ATP hydrolysis (sometimes with protein phosphorylation) also causes changes in the shape and binding affinities of transport and motor proteins.
- Catabolic pathways drive regeneration of ATP from ADP + Ⓟ_i.

? *Describe the ATP cycle: How is ATP used and regenerated in a cell?*

CONCEPT 8.4

Enzymes speed up metabolic reactions by lowering energy barriers *(pp. 153–159)*

- In a chemical reaction, the energy necessary to break the bonds of the reactants is the **activation energy**, E_A.
- **Enzymes** lower the E_A barrier:



- Each enzyme has a unique **active site** that binds one or more **substrate(s)**, the reactants on which it acts. It then changes shape, binding the substrate(s) more tightly (**induced fit**).
- The active site can lower an E_A barrier by orienting substrates correctly, straining their bonds, providing a favorable microenvironment, or even covalently bonding with the substrate.
- Each enzyme has an optimal temperature and pH.
- Inhibitors reduce enzyme function. A **competitive inhibitor** binds to the active site, whereas a **noncompetitive inhibitor** binds to a different site on the enzyme.
- Natural selection, acting on organisms with variant enzymes, is responsible for the diversity of enzymes found in organisms.

? *How do both activation energy barriers and enzymes help maintain the structural and metabolic order of life?*

CONCEPT 8.5

Regulation of enzyme activity helps control metabolism *(pp. 159–161)*

- Many enzymes are subject to **allosteric regulation**: Regulatory molecules, either activators or inhibitors, bind to specific regulatory sites, affecting the shape and function of the enzyme. In **cooperativity**, binding of one substrate molecule can stimulate binding or activity at other active sites. In **feedback inhibition**, the end product of a metabolic pathway allosterically inhibits the enzyme for a previous step in the pathway.
- Some enzymes are grouped into complexes, some are incorporated into membranes, and some are contained inside organelles, increasing the efficiency of metabolic processes.

? *What roles do allosteric regulation and feedback inhibition play in the metabolism of a cell?*

TEST YOUR UNDERSTANDING

For more multiple-choice questions, go to the **Practice Test** in the **Mastering Biology** eText or Study Area, or go to goo.gl/GruWRg.

Levels 1-2: Remembering/Understanding

1. Choose the pair of terms that correctly completes this sentence: Catabolism is to anabolism as _____ is to _____.
   (A) exergonic; spontaneous
   (B) exergonic; endergonic
   (C) free energy; entropy
   (D) work; energy

2. Most cells cannot harness heat to perform work because
   (A) heat does not involve a transfer of energy.
   (B) cells do not have much thermal energy; they are relatively cool.
   (C) temperature is usually uniform throughout a cell.
   (D) heat can never be used to do work.

3. Which of the following metabolic processes can occur without a net influx of energy from some other process?
   (A) ADP + $\textcircled{P}_i$ + $\rightarrow$ ATP + H_2O
   (B) $C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$
   (C) $6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2$
   (D) Amino acids $\rightarrow$ Protein

4. If an enzyme in solution is saturated with substrate, the most effective way to obtain a faster yield of products is to
   (A) add more of the enzyme.
   (B) heat the solution to 90°C.
   (C) add more substrate.
   (D) add a noncompetitive inhibitor.

5. Some bacteria are metabolically active in hot springs because
   (A) they are able to maintain a lower internal temperature.
   (B) high temperatures make catalysis unnecessary.
   (C) their enzymes have high optimal temperatures.
   (D) their enzymes are completely insensitive to temperature.

Levels 3-4: Applying/Analyzing

6. If an enzyme is added to a solution where its substrate and product are in equilibrium, what will occur?
   (A) Additional substrate will be formed.
   (B) The reaction will change from endergonic to exergonic.
   (C) The free energy of the system will change.
   (D) Nothing; the reaction will stay at equilibrium.

Levels 5-6: Evaluating/Creating

7. **DRAW IT** Using a series of arrows, draw the branched metabolic reaction pathway described by the following statements, and then answer the question at the end. Use red arrows and minus signs to indicate inhibition.

   L can form either M or N.

   M can form O.

   O can form either P or R.

   P can form Q.

   R can form S.

   O inhibits the reaction of L to form M.

   Q inhibits the reaction of O to form P.

   S inhibits the reaction of O to form R.

   Which reaction would prevail if both Q and S were present in the cell in high concentrations?
   (A) L $\rightarrow$ M
   (B) M $\rightarrow$ O
   (C) L $\rightarrow$ N
   (D) O $\rightarrow$ P

8. **EVOLUTION CONNECTION** Some people argue that biochemical pathways are too complex to have evolved because all intermediate steps in a given pathway must be present to produce the final product. Critique this argument. How could you use the diversity of metabolic pathways that produce the same or similar products to support your case?

9. **SCIENTIFIC INQUIRY • DRAW IT** A researcher has developed an assay to measure the activity of an important enzyme present in pancreatic cells growing in culture. She adds the enzyme's substrate to a dish of cells and then measures the appearance of reaction products. The results are graphed as the amount of product on the *y*-axis versus time on the *x*-axis. The researcher notes four sections of the graph. For a short period of time, no products appear (section A). Then (section B) the reaction rate is quite high (the slope of the line is steep). Next, the reaction gradually slows down (section C). Finally, the graph line becomes flat (section D). Draw and label the graph, and propose a model to explain the molecular events occurring at each stage of this reaction profile.

10. **WRITE ABOUT A THEME: ENERGY AND MATTER** Life requires energy. In a short essay (100–150 words), describe the basic principles of bioenergetics in an animal cell. How are the flow and transformation of energy different in a photosynthesizing cell? Include the role of ATP and enzymes in your discussion.

11. **SYNTHESIZE YOUR KNOWLEDGE**

Explain what is happening in this photo in terms of kinetic energy and potential energy. Include the energy conversions that occur when the penguins eat fish and climb back up on the glacier. Describe the role of ATP and enzymes in the underlying molecular processes, including what happens to the free energy of some of the molecules involved.

For selected answers, see Appendix A.

9 Cellular Respiration and Fermentation

KEY CONCEPTS

9.1 Catabolic pathways yield energy by oxidizing organic fuels *p. 165*

9.2 Glycolysis harvests chemical energy by oxidizing glucose to pyruvate *p. 170*

9.3 After pyruvate is oxidized, the citric acid cycle completes the energy-yielding oxidation of organic molecules *p. 171*

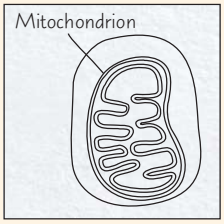
9.4 During oxidative phosphorylation, chemiosmosis couples electron transport to ATP synthesis *p. 174*

9.5 Fermentation and anaerobic respiration enable cells to produce ATP without the use of oxygen *p. 179*

9.6 Glycolysis and the citric acid cycle connect to many other metabolic pathways *p. 182*

Study Tip

Make a visual study guide: Draw a cell with a large mitochondrion, labeling the parts of the mitochondrion. As you go through the chapter, add key reactions for each stage of cellular respiration, linking the stages together. Label the carbon molecule(s) with the most energy and the carbon molecule(s) with the least energy. Your cell can be a simple sketch, as shown here.



Go to Mastering Biology

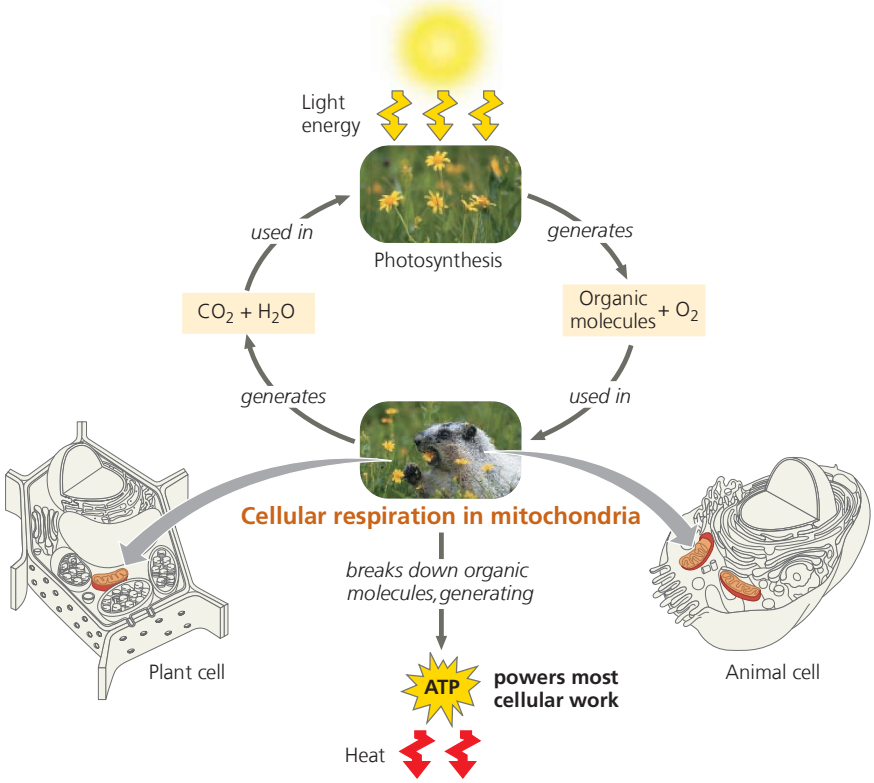
For Students (in eText and Study Area)
- Get Ready for Chapter 9
- BioFlix® Animation: Cellular Respiration
- Figure 9.12 Walkthrough: Free-Energy Change During Electron Transport

For Instructors to Assign (in Item Library)
- BioFlix® Tutorial: Glycolysis
- BioFlix® Tutorial: Cellular Respiration: Inputs and Outputs

Ready-to-Go Teaching Module (in Instructor Resources)
- Oxidative Phosphorylation (Concept 9.4)

Figure 9.1 This hoary marmot (*Marmota caligata*) obtains energy for its cells by feeding on plants. In the process of cellular respiration, mitochondria in the cells of animals, plants, and other organisms break down organic molecules, generating ATP and waste products: carbon dioxide, water, and heat. Note that energy flows one way, but chemicals are recycled.

How is the chemical energy stored in food used to generate ATP, the molecule that drives most cellular work?



CONCEPT 9.1

Catabolic pathways yield energy by oxidizing organic fuels

Living cells require transfusions of energy from outside sources to perform their many tasks—for example, assembling polymers, pumping substances across membranes, moving, and reproducing. The outside source of energy is food, and the energy stored in the organic molecules of food ultimately comes from the sun. As shown in **Figure 9.1**, energy flows into an ecosystem as sunlight and exits as heat; in contrast, the chemical elements essential to life are recycled. Photosynthesis generates oxygen, as well as organic molecules used by the mitochondria of eukaryotes as fuel for cellular respiration. Respiration breaks this fuel down, using oxygen (O_2) and generating ATP. The waste products of this type of respiration, carbon dioxide (CO_2) and water (H_2O), are the raw materials for photosynthesis.

Mastering Biology Animation: Energy Flow and Chemical Recycling

Let's consider how cells harvest the chemical energy stored in organic molecules and use it to generate ATP, the molecule that drives most cellular work. Metabolic pathways that release stored energy by breaking down complex molecules are called catabolic pathways (see Concept 8.1). Transfer of electrons from food molecules (like glucose) to other molecules plays a major role in these pathways. In this section, we consider these processes, which are central to cellular respiration.

Catabolic Pathways and Production of ATP

Organic compounds possess potential energy as a result of the arrangement of electrons in the bonds between their atoms. Compounds that can participate in exergonic reactions can act as fuels. Through the activity of enzymes (see Concept 8.4), a cell systematically degrades complex organic molecules that are rich in potential energy to simpler waste products that have less energy. Some of the energy taken out of chemical storage can be used to do work; the rest is dissipated as heat.

One catabolic process, **fermentation**, is a partial degradation of sugars or other organic fuel that occurs without the use of oxygen. However, the most efficient catabolic pathway is **aerobic respiration**, in which oxygen is consumed as a reactant along with the organic fuel (*aerobic* is from the Greek *aer*, air, and *bios*, life). The cells of most eukaryotic and many prokaryotic organisms can carry out aerobic respiration. Some prokaryotes use substances other than oxygen as reactants in a similar process that harvests chemical energy without oxygen; this process is called *anaerobic respiration* (the prefix *an-* means "without"). Technically, the term **cellular respiration** includes both aerobic and anaerobic processes. However, it originated as a synonym for aerobic respiration because of the relationship of that process to organismal respiration, in which an animal breathes in oxygen. Thus, *cellular respiration* is often used to refer to the aerobic process, a practice we follow in most of this chapter.

Although very different in mechanism, aerobic respiration is in principle similar to the combustion of gasoline in an automobile engine after oxygen is mixed with the fuel (hydrocarbons). Food provides the fuel for respiration, and the exhaust is carbon dioxide and water. The overall process can be summarized as follows:

$$\text{Organic compounds} + \text{Oxygen} \rightarrow \text{Carbon dioxide} + \text{Water} + \text{Energy}$$

Carbohydrates, fats, and proteins from food can all be processed and consumed as fuel. In animal diets, a major source of carbohydrates is starch, a storage polysaccharide that can be broken down into glucose ($C_6H_{12}O_6$) subunits. Here, we will learn the steps of cellular respiration by tracking the degradation of the sugar glucose:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + \text{Energy (ATP + heat)}$$

Mastering Biology BioFlix® Animation: Introduction to Cellular Respiration

This breakdown of glucose is exergonic, having a free-energy change of −686 kcal (−2,870 kJ) per mole of glucose decomposed ($\Delta G = -686$ kcal/mol). Recall that a negative ΔG ($\Delta G < 0$) indicates that the products of the chemical process store less energy than the reactants and that the reaction can happen spontaneously—in other words, without an input of energy (see Concept 8.2).

Catabolic pathways do not directly move flagella, pump solutes across membranes, polymerize monomers, or perform other cellular work. Catabolism is linked to work by a chemical drive shaft—ATP (see Concept 8.3). To keep working, the cell must regenerate its supply of ATP from ADP and Ⓟ_i (see Figure 8.12). To understand how cellular respiration accomplishes this, let's examine the fundamental chemical processes known as oxidation and reduction.

Redox Reactions: Oxidation and Reduction

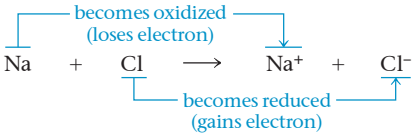
How do the catabolic pathways that decompose glucose and other organic fuels yield energy? The answer is based on the transfer of electrons during the chemical reactions. The relocation of electrons releases energy stored in organic molecules, and this energy ultimately is used to synthesize ATP.

The Principle of Redox

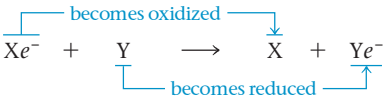
In many chemical reactions, there is a transfer of one or more electrons (e^-) from one reactant to another. These electron transfers are called oxidation-reduction reactions, or **redox reactions** for short. In a redox reaction, the loss of electrons

from one substance is called **oxidation**, and the addition of electrons to another substance is known as **reduction**. (Note that *adding* electrons is called *reduction*; adding negatively charged electrons to an atom *reduces* the amount of positive charge of that atom.)

To take a simple, nonbiological example, consider the reaction between the elements sodium (Na) and chlorine (Cl) that forms table salt:



We could generalize a redox reaction this way:



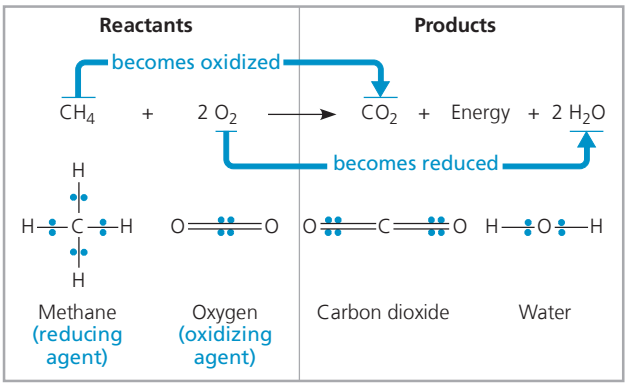
In the generalized reaction, substance Xe^-, the electron donor, is called the **reducing agent**; it reduces Y, which accepts the donated electron. Substance Y, the electron acceptor, is the **oxidizing agent**; it oxidizes Xe^- by removing its electron. Because an electron transfer requires both an electron donor and an acceptor, oxidation and reduction always go hand in hand.

Not all redox reactions involve the complete transfer of electrons from one substance to another; some change the degree of electron sharing in covalent bonds. Methane combustion, shown in **Figure 9.2**, is an example. The covalent electrons in methane are shared nearly equally between the bonded atoms because carbon and hydrogen have about the same affinity for valence electrons; they are about equally electronegative (see Concept 2.3). But when methane reacts with O_2, forming CO_2, electrons end up shared less equally between the carbon atom and its new covalent partners, the oxygen atoms, which are very electronegative. In effect, the carbon atom has partially "lost" its shared electrons; thus, methane has been oxidized.

Now let's examine the fate of the reactant O_2. The two atoms of O_2 share their electrons equally. But after the reaction with methane, when each O atom is bonded to two H atoms in H_2O, the electrons of those covalent bonds spend more time near the oxygen (see Figure 9.2). In effect, each O atom has partially "gained" electrons, so the oxygen molecule (O_2) has been reduced. Because the O atom is so electronegative, O_2 is one of the most powerful of all oxidizing agents.

Energy must be added to pull an electron away from an atom, just as energy is required to push a ball uphill. The more electronegative the atom (the stronger its pull on electrons), the more energy is required to take an electron away from it. An electron loses potential energy when it shifts from a less electronegative atom toward a more electronegative one, just as a ball loses potential energy when it rolls downhill. A redox reaction that moves electrons closer to an O atom, such as the burning (oxidation) of methane, therefore releases chemical energy that can be put to work.

▼ Figure 9.2 Methane combustion as an energy-yielding redox reaction. The reaction releases energy to the surroundings because the electrons lose potential energy when they end up being shared unequally, spending more time near electronegative atoms such as oxygen.

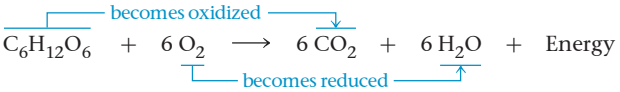


VISUAL SKILLS *Is the carbon atom oxidized or reduced during this reaction? Explain.*

Mastering Biology **Animation: Redox Reactions**

Oxidation of Organic Fuel Molecules During Cellular Respiration

The oxidation of methane by O_2 is the main combustion reaction that occurs at the burner of a gas stove. The combustion of gasoline in an automobile engine is also a redox reaction; the energy released pushes the pistons. But the energy-yielding redox process of greatest interest to biologists is respiration: the oxidation of glucose and other molecules in food. Examine again the summary equation for cellular respiration, but this time think of it as a redox process:



As in the combustion of methane or gasoline, the fuel (glucose) is oxidized and O_2 is reduced. The electrons lose potential energy along the way, and energy is released.

In general, organic molecules that have an abundance of hydrogen are excellent fuels because their bonds are a source of "hilltop" electrons, whose energy may be released as these electrons "fall" down an energy gradient during their transfer to oxygen. The summary equation for respiration indicates that hydrogen is transferred from glucose to the O atoms in O_2. But the important point, not visible in the summary equation, is that the energy state of the electron changes as hydrogen (with its electron) is transferred to oxygen. In respiration, the oxidation of glucose transfers electrons to a lower energy state, liberating energy that becomes available for ATP synthesis. So, in general, we see fuels with multiple C—H bonds oxidized into products with multiple C—O bonds.

The main energy-yielding foods—carbohydrates and fats—are reservoirs of electrons associated with hydrogen, often in the form of C — H bonds. Only the barrier of activation energy holds back the flood of electrons to a lower energy state (see Figure 8.13). Without this barrier, a food substance like glucose would combine almost instantaneously with O_2. If we supply the activation energy by igniting glucose, it burns in air, releasing 686 kcal (2,870 kJ) of heat per mole of glucose (about 180 g). Body temperature is not high enough to initiate burning, of course. Instead, if you swallow some glucose, enzymes in your cells will lower the barrier of activation energy, allowing the sugar to be oxidized in a series of steps.

Stepwise Energy Harvest via NAD$^+$ and the Electron Transport Chain

If energy is released from a fuel all at once, it cannot be harnessed efficiently for constructive work. For example, if a gasoline tank explodes, it cannot drive a car very far. Cellular respiration does not oxidize glucose (or any other organic fuel) in a single explosive step either. Rather, glucose is broken down in a series of steps, each one catalyzed by an enzyme. At key steps, electrons are stripped from the glucose. As is often the case in oxidation reactions, each electron travels with a proton—thus, as a hydrogen atom. The hydrogen atoms are not transferred directly to O_2, but instead are usually passed first to an electron carrier, a coenzyme called nicotinamide adenine dinucleotide, a derivative of the vitamin niacin. This coenzyme is well suited as an electron carrier because it can cycle easily between its oxidized form, **NAD$^+$**, and its reduced form, **NADH**. As an electron acceptor, NAD$^+$ functions as an oxidizing agent during respiration.

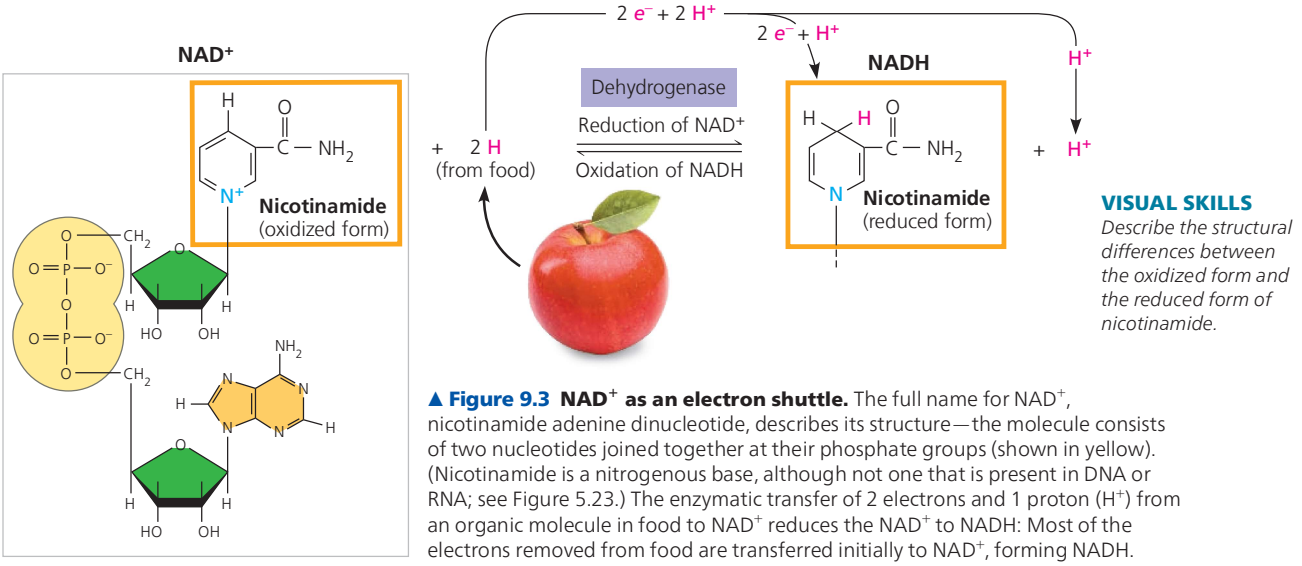
How does NAD$^+$ trap electrons from glucose and the other organic molecules in food? Enzymes called dehydrogenases remove a pair of hydrogen atoms (2 electrons and 2 protons) from the substrate (glucose, in the preceding example), thereby oxidizing it. The enzyme delivers the 2 electrons along with 1 proton to its coenzyme, NAD$^+$, forming NADH **(Figure 9.3)**. The other proton is released as a hydrogen ion (H^+) into the surrounding solution:

$$H{-}\overset{|}{\underset{|}{C}}{-}OH + NAD^+ \xrightarrow{\text{Dehydrogenase}} \overset{|}{\underset{|}{C}}{=}O + NADH + H^+$$

By receiving 2 negatively charged electrons but only 1 positively charged proton, the nicotinamide portion of NAD$^+$ has its charge neutralized when NAD$^+$ is reduced to NADH. The name NADH shows the hydrogen that has been received in the reaction. NAD$^+$ is the most versatile electron acceptor in cellular respiration and functions in several of the redox steps during the breakdown of glucose.

Electrons lose very little of their potential energy when they are transferred from glucose to NAD$^+$. Each NADH molecule formed during respiration represents stored energy that can be tapped to make ATP when the electrons complete their "fall" down an energy gradient from NADH to O_2.

How do electrons that are extracted from glucose and stored as potential energy in NADH finally reach oxygen? It will help to compare the redox chemistry of cellular respiration to a much simpler reaction: the reaction between hydrogen and oxygen to form water **(Figure 9.4a)**. Mix H_2 and O_2, provide a spark for activation energy, and the gases combine explosively. In fact, combustion of liquid H_2 and O_2 is harnessed to help power the rocket engines that boost satellites into orbit and launch spacecraft. The explosion represents a release of energy as the electrons of hydrogen "fall" closer to the electronegative oxygen atoms. Cellular respiration also brings hydrogen and oxygen together to form water, but there are two important differences. First, in cellular respiration, the hydrogen that reacts with oxygen



VISUAL SKILLS
Describe the structural differences between the oxidized form and the reduced form of nicotinamide.

▲ Figure 9.3 NAD$^+$ as an electron shuttle. The full name for NAD$^+$, nicotinamide adenine dinucleotide, describes its structure—the molecule consists of two nucleotides joined together at their phosphate groups (shown in yellow). (Nicotinamide is a nitrogenous base, although not one that is present in DNA or RNA; see Figure 5.23.) The enzymatic transfer of 2 electrons and 1 proton (H^+) from an organic molecule in food to NAD$^+$ reduces the NAD$^+$ to NADH: Most of the electrons removed from food are transferred initially to NAD$^+$, forming NADH.

is derived from organic molecules rather than H_2. Second, instead of occurring in one explosive reaction, respiration uses an electron transport chain to break the fall of electrons to oxygen into several energy-releasing steps **(Figure 9.4b)**. An **electron transport chain** consists of a number of molecules, mostly proteins, built into the inner membrane of the mitochondria of eukaryotic cells (and the plasma membrane of respiring prokaryotes). Electrons removed from glucose are shuttled by NADH to the "top," higher-energy end of the chain. At the "bottom," lower-energy end, O_2 captures these electrons along with hydrogen nuclei (H^+), forming water. (Anaerobically respiring prokaryotes have an electron acceptor at the end of the chain that is different from O_2.)

Electron transfer from NADH to oxygen is an exergonic reaction with a free-energy change of −53 kcal/mol (−222 kJ/mol). Instead of this energy being released and wasted in a single explosive step, electrons cascade down the chain from one carrier molecule to the next in a series of redox reactions, losing a small amount of energy with each step until they finally reach oxygen, the terminal electron acceptor, which has a very great affinity for electrons. Each "downhill" carrier has a greater affinity for electrons than, and is thus capable of accepting electrons from (oxidizing), its "uphill" neighbor, with O_2 at the bottom of the chain. Therefore, the electrons transferred from glucose to NAD^+, reducing it to NADH, fall down an energy gradient in the electron transport chain to a far more stable location in an electronegative oxygen atom from O_2. Put another way, O_2 pulls electrons down the chain in an energy-yielding tumble analogous to gravity pulling objects downhill.

In summary, during cellular respiration, most electrons travel the following "downhill" route: glucose → NADH → electron transport chain → oxygen. Later in this chapter, you will learn more about how the cell uses the energy released from this exergonic electron fall to regenerate its supply of ATP. For now, having covered the basic redox mechanisms of cellular respiration, let's look at the entire process by which energy is harvested from organic fuels.

The Stages of Cellular Respiration: *A Preview*

The harvesting of energy from glucose by cellular respiration is a cumulative function of three metabolic stages. We list them here along with a color-coding scheme we will use throughout the chapter to help you keep track of the big picture:

1. GLYCOLYSIS (color-coded blue throughout the chapter)
2. PYRUVATE OXIDATION (light orange) and the CITRIC ACID CYCLE (dark orange)
3. OXIDATIVE PHOSPHORYLATION: Electron transport and chemiosmosis (purple)

Biochemists usually reserve the term *cellular respiration* for stages 2 and 3 together. In this text, however, we include glycolysis as a part of cellular respiration because most respiring cells deriving energy from glucose use glycolysis to produce the starting material for the citric acid cycle.

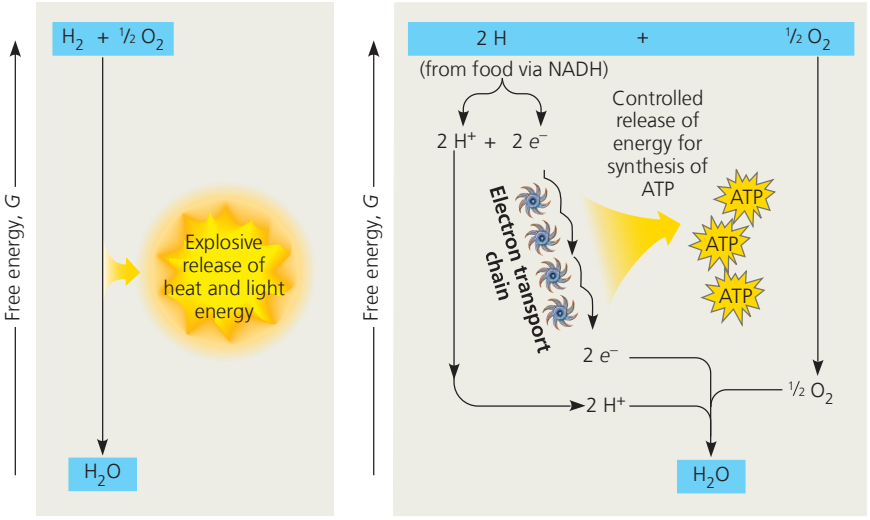
As diagrammed in **Figure 9.5**, glycolysis and then pyruvate oxidation and the citric acid cycle are the catabolic pathways that break down glucose and other organic fuels. **Glycolysis**, which occurs in the cytosol, begins the degradation process by breaking glucose into two molecules of a compound called pyruvate. In eukaryotes, pyruvate enters the mitochondrion and is oxidized to a compound called acetyl CoA, which enters the **citric acid cycle**. There, the breakdown of glucose to carbon dioxide is completed. (In prokaryotes, these processes take place in the cytosol.) Thus, the carbon dioxide produced by respiration represents fragments of oxidized organic molecules.

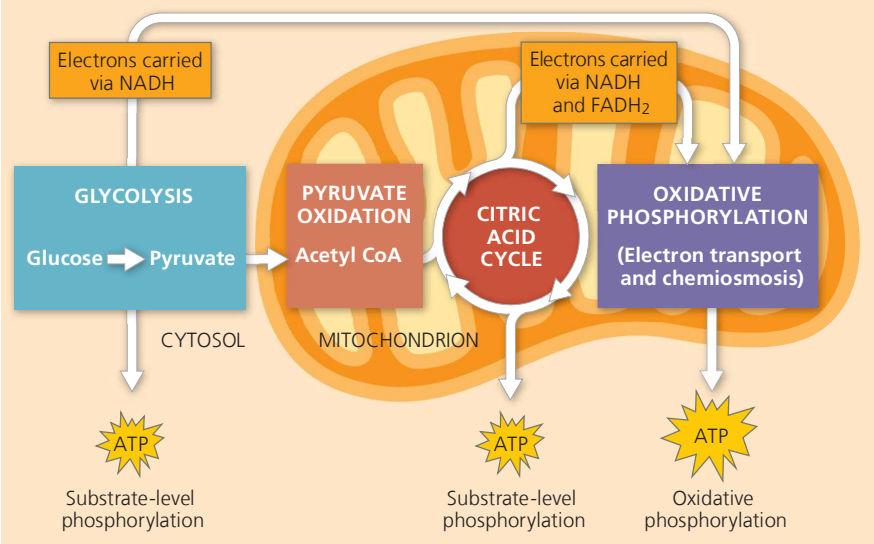
Some of the steps of glycolysis and the citric acid cycle are redox reactions in which dehydrogenases transfer electrons from substrates to NAD^+ or the related electron carrier FAD, forming NADH or $FADH_2$. (You'll learn more about FAD and $FADH_2$ later.) In the third stage of respiration, the electron transport chain accepts

▼ Figure 9.4 An introduction to electron transport chains.

(a) Uncontrolled reaction. The one-step exergonic reaction of hydrogen with oxygen to form water releases a large amount of energy in the form of heat and light: an explosion.

(b) Cellular respiration. In cellular respiration, the same reaction occurs in stages: An electron transport chain breaks the "fall" of electrons in this reaction into a series of smaller steps and stores some of the released energy in a form that can be used to make ATP. (The rest of the energy is released as heat.)





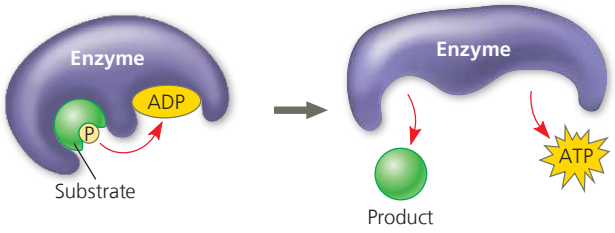
▶ Figure 9.5 An overview of cellular respiration. During glycolysis, each glucose molecule is broken down into two molecules of pyruvate. In eukaryotic cells, as shown here, the pyruvate enters the mitochondrion. There it is oxidized to acetyl CoA, which will be further oxidized to CO_2 in the citric acid cycle. The electron carriers NADH and $FADH_2$ transfer electrons derived from glucose to electron transport chains. During oxidative phosphorylation, electron transport chains convert the chemical energy to a form used for ATP synthesis in the process called chemiosmosis. (During earlier steps of cellular respiration, a few molecules of ATP are synthesized in a process called substrate-level phosphorylation.) To visualize these processes in their cellular context, see Figure 6.32b.

Mastering Biology Animation: Overview of Cellular Respiration

electrons from NADH or $FADH_2$ generated during the first two stages and passes these electrons down the chain. At the end of the chain, the electrons are combined with molecular oxygen (O_2) and hydrogen ions (H^+), forming water (see Figure 9.4b). The energy released at each step of the chain is stored in a form the mitochondrion (or prokaryotic cell) can use to make ATP from ADP. This mode of ATP synthesis is called **oxidative phosphorylation** because it is powered by the redox reactions of the electron transport chain.

In eukaryotic cells, the inner membrane of the mitochondrion is the site of electron transport and another process called *chemiosmosis*, together making up oxidative phosphorylation. (In prokaryotes, these processes take place in the plasma membrane.) Oxidative phosphorylation accounts for almost 90% of the ATP generated by respiration. A smaller amount of ATP is formed directly in a few reactions of glycolysis and the citric acid cycle by a mechanism called **substrate-level phosphorylation (Figure 9.6)**. This mode of ATP synthesis occurs when an enzyme transfers a phosphate group from a substrate molecule to ADP, rather than adding an inorganic phosphate to ADP as in oxidative phosphorylation. "Substrate molecule" here refers to an organic molecule generated as an intermediate during the catabolism of glucose. You'll see examples of substrate-level phosphorylation later in the chapter, in both glycolysis and the citric acid cycle.

▼ Figure 9.6 Substrate-level phosphorylation. Some ATP is made by direct transfer of a phosphate group from an organic substrate to ADP by an enzyme. (For examples in glycolysis, see Figure 9.8, steps 7 and 10.)



MAKE CONNECTIONS *Review Figure 8.9. In the reaction shown above, is the potential energy higher for the reactants or for the products? Explain.*

You can think of the whole process this way: When you withdraw a relatively large sum of money from an ATM, it is not delivered to you in a single bill of a large denomination. Instead, the machine dispenses a number of smaller-denomination bills that you can spend more easily. This is analogous to ATP production during cellular respiration. For each molecule of glucose degraded to CO_2 and H_2O by respiration, the cell makes up to about 32 molecules of ATP, each with 7.3 kcal/mol of free energy. Respiration cashes in the large denomination of energy banked in a single molecule of glucose (686 kcal/mol under standard conditions) for the small change of many molecules of ATP, which is more practical for the cell to spend on its work.

This preview has introduced you to how glycolysis, the citric acid cycle, and oxidative phosphorylation fit into the process of cellular respiration so you can keep the big picture in mind as you take a closer look at each of these three stages of respiration. As you read about the chemical reactions, remember that each reaction is catalyzed by a specific enzyme, some of which are shown in Figure 6.32b.

CONCEPT CHECK 9.1

1. Compare and contrast aerobic and anaerobic respiration, including the processes involved.
2. **WHAT IF?** If the following redox reaction occurred, which compounds would be oxidized? Reduced?

$$C_4H_6O_5 + NAD^+ \rightarrow C_4H_4O_5 + NADH + H^+$$

For suggested answers, see Appendix A.

CONCEPT 9.2

Glycolysis harvests chemical energy by oxidizing glucose to pyruvate

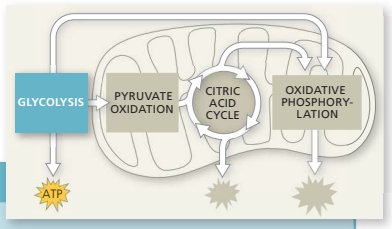
The word *glycolysis* means "sugar splitting," and that is exactly what happens during this pathway. Glucose, a six-carbon sugar, is split into two three-carbon sugars. These smaller sugars are then oxidized and their remaining atoms rearranged to form two molecules of pyruvate. (Pyruvate is the ionized form of pyruvic acid.)

As summarized in **Figure 9.7**, glycolysis can be divided into two phases: the energy investment phase and the energy payoff phase. During the energy investment phase, the cell actually spends ATP. This investment is repaid with interest during the energy payoff phase, when ATP is produced by substrate-level phosphorylation and NAD^+ is reduced to NADH by electrons released from the oxidation of glucose. The net energy yield from glycolysis, per glucose molecule, is 2 ATP plus 2 NADH. The ten steps of the glycolytic pathway are shown in **Figure 9.8**.

All of the carbon originally present in glucose is accounted for in the two molecules of pyruvate; no carbon is released as CO_2 during glycolysis. Glycolysis occurs whether or not O_2 is present. However, if O_2 *is* present, the chemical energy stored in pyruvate and NADH can be extracted by pyruvate oxidation, the citric acid cycle, and oxidative phosphorylation.

▼ Figure 9.7 The inputs and outputs of glycolysis.

Mastering Biology
Animation: Glycolysis



GLYCOLYSIS:

Energy Investment Phase

Glucose

2 ATP used → 2 ADP + 2 P

Energy Payoff Phase

4 ADP + 4 P → 4 ATP formed

2 NAD^+ + 4 e^- + 4 H^+ → 2 NADH + 2 H^+

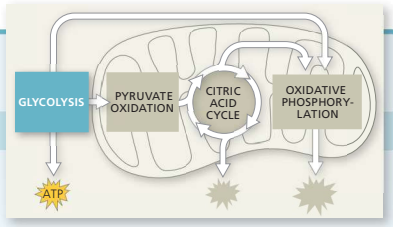
2 Pyruvate + 2 H_2O

Net Inputs and Outputs

Glucose → 2 Pyruvate + 2 H_2O

4 ATP formed – 2 ATP used → 2 ATP

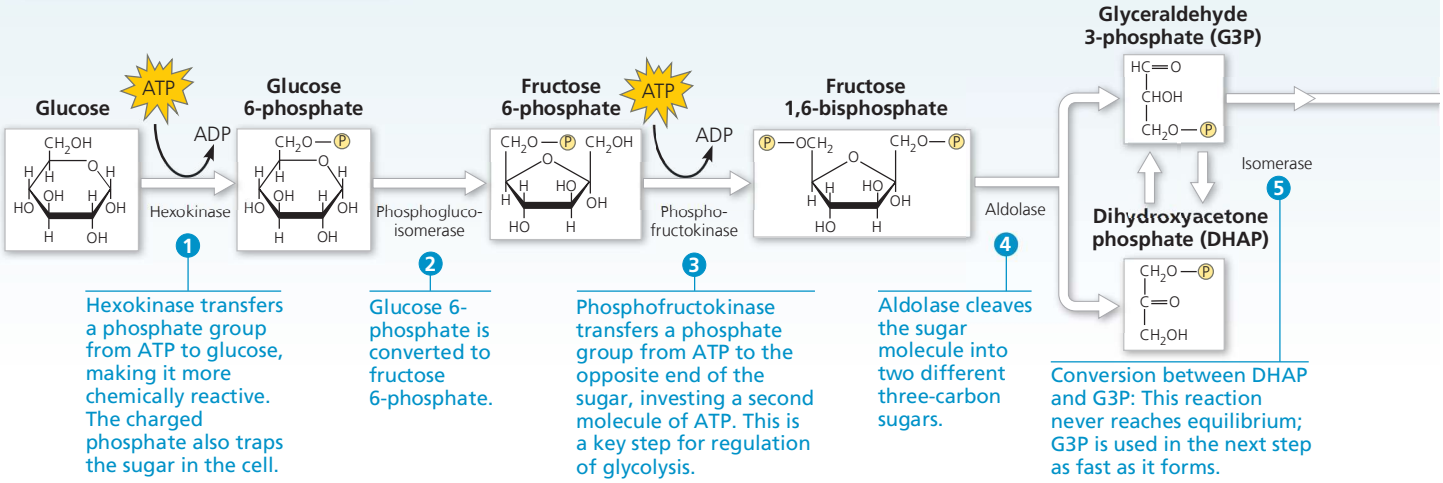
2 NAD^+ + 4 e^- + 4 H^+ → 2 NADH + 2 H^+



▼ Figure 9.8 The steps of glycolysis. Glycolysis, a source of ATP and NADH, takes place in the cytosol. Two of the enzymes (in steps 1 and 3) are shown in Figure 6.32b.

GLYCOLYSIS: Energy Investment Phase

WHAT IF? *What would happen if you removed the dihydroxyacetone phosphate generated in step 4 as fast as it was produced?*



CONCEPT CHECK 9.2

1. **VISUAL SKILLS** During the redox reaction in glycolysis (see step 6 in Figure 9.8), which one of the molecules acts as the oxidizing agent? The reducing agent?

For suggested answers, see Appendix A.

CONCEPT 9.3

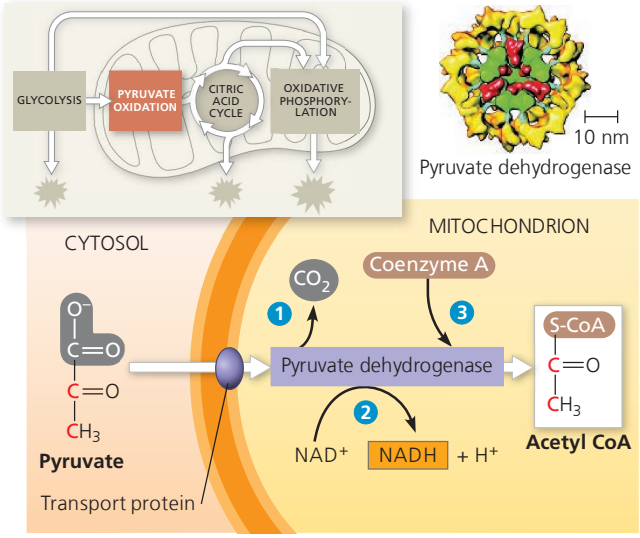
After pyruvate is oxidized, the citric acid cycle completes the energy-yielding oxidation of organic molecules

Glycolysis releases less than a quarter of the chemical energy in glucose that can be harvested by cells; most of the energy remains stockpiled in the two molecules of pyruvate. When O_2 is present, the pyruvate in eukaryotic cells enters a mitochondrion, where the oxidation of glucose is completed. In aerobically respiring prokaryotic cells, this process occurs in the cytosol. (Later in the chapter, we'll examine other fates for pyruvate—for example, when O_2 is unavailable or in a prokaryote that is unable to use O_2.)

Oxidation of Pyruvate to Acetyl CoA

Upon entering the mitochondrion via active transport, pyruvate is first converted to a compound called acetyl coenzyme A, or **acetyl CoA (Figure 9.9)**. This step, linking glycolysis and the citric acid cycle, is carried out by a multienzyme complex that catalyzes three reactions: 1 Pyruvate's carboxyl group

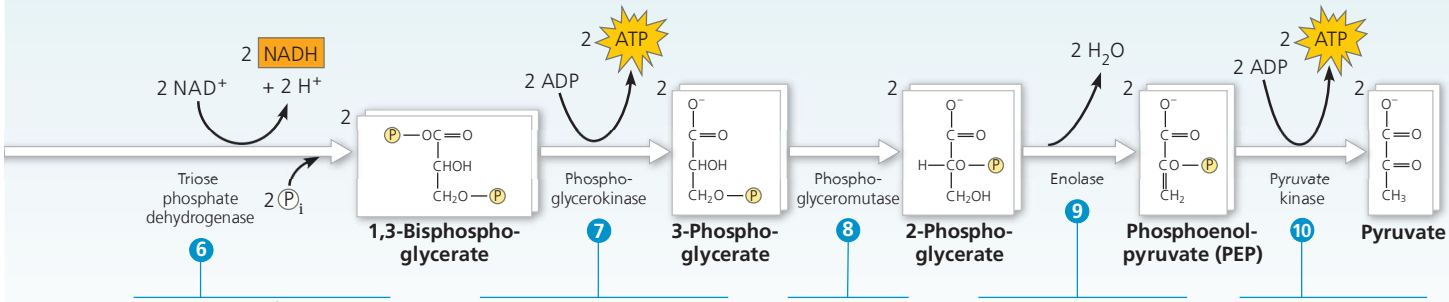
▼ Figure 9.9 Oxidation of pyruvate to acetyl CoA, the step before the citric acid cycle. Pyruvate enters the mitochondrion through a transport protein and is processed by a complex of several enzymes known as pyruvate dehydrogenase (shown in the computer-generated image based on cryo-EMs). This complex catalyzes the three numbered steps, which are described in the text. The CO_2 molecule will diffuse out of the cell. The NADH will be used in oxidative phosphorylation. The acetyl group of acetyl CoA will enter the citric acid cycle. (Coenzyme A is abbreviated S-CoA when it is attached to a molecule, emphasizing its sulfur atom, S.)



Mastering Biology **BioFlix® Animation: Acetyl CoA**

The energy payoff phase occurs after glucose is split into two three-carbon sugars. Thus, the coefficient 2 precedes all molecules in this phase.

GLYCOLYSIS: Energy Payoff Phase



Two sequential reactions: (1) G3P is oxidized by the transfer of electrons to NAD^+, forming NADH. (2) Using energy from this exergonic redox reaction, a phosphate group is attached to the oxidized substrate, making a high-energy product.

The phosphate group is transferred to ADP (substrate-level phosphorylation) in an exergonic reaction. The carbonyl group of G3P has been oxidized to the carboxyl group ($—COO^-$) of an organic acid (3-phosphoglycerate).

This enzyme relocates the remaining phosphate group.

Enolase causes a double bond to form in the substrate by extracting a water molecule, yielding phosphoenolpyruvate (PEP), a compound with a very high potential energy.

The phosphate group is transferred from PEP to ADP (a second example of substrate-level phosphorylation), forming pyruvate.

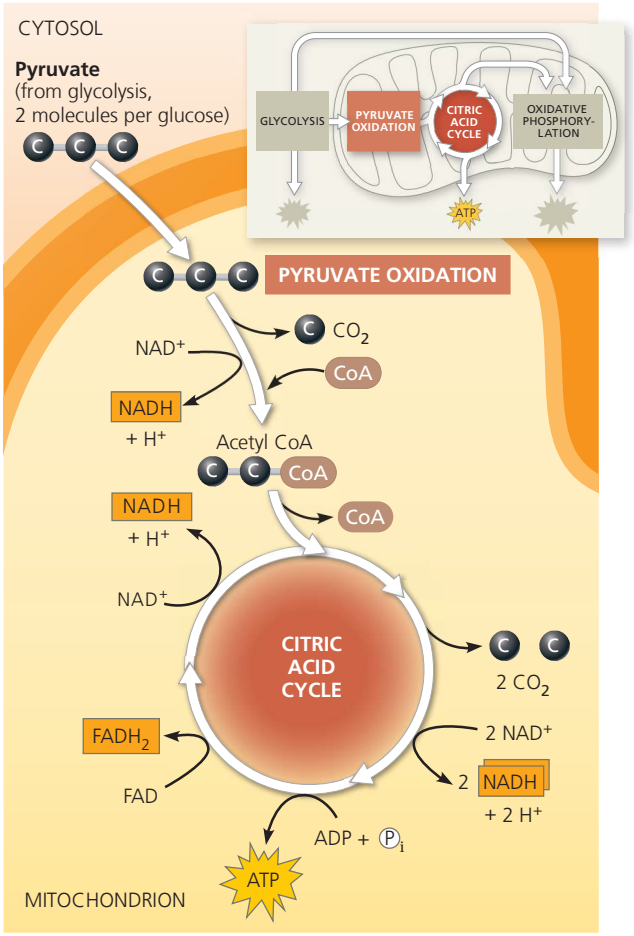
Mastering Biology **BioFlix® Animation: Glycolysis**

(—COO^-), already somewhat oxidized and thus carrying little chemical energy, is now fully oxidized and given off as a molecule of CO_2. This is the first step in which CO_2 is released during respiration. (2) Next, the remaining two-carbon fragment is oxidized and the electrons transferred to NAD^+, storing energy in the form of NADH. (3) Finally, coenzyme A (CoA), a sulfur-containing compound derived from a B vitamin, is attached via its sulfur atom to the two-carbon intermediate, forming acetyl CoA. Acetyl CoA has a high potential energy, which is used to transfer the acetyl group to a molecule in the citric acid cycle, a reaction that is therefore highly exergonic.

The Citric Acid Cycle

The citric acid cycle functions as a metabolic furnace that further oxidizes organic fuel derived from pyruvate. **Figure 9.10** summarizes the inputs and outputs as

▼ Figure 9.10 An overview of pyruvate oxidation and the citric acid cycle. The inputs and outputs per pyruvate molecule are shown with a focus on the carbon atoms involved. To calculate on a per-glucose basis, multiply by 2 because each glucose molecule is split during glycolysis into two pyruvate molecules.



Mastering Biology **Animation: The Citric Acid Cycle**

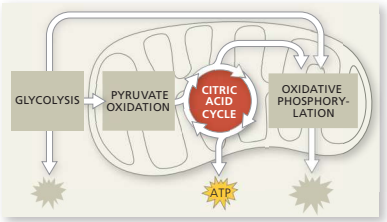
pyruvate is broken down to three CO_2 molecules, including the molecule of CO_2 released during the conversion of pyruvate to acetyl CoA. The cycle generates 1 ATP per turn by substrate-level phosphorylation, but most of the chemical energy is transferred to NAD^+ and FAD during the redox reactions. The reduced coenzymes, NADH and $FADH_2$, shuttle their cargo of high-energy electrons into the electron transport chain. The citric acid cycle is also called the tricarboxylic acid cycle or the Krebs cycle, the latter honoring Hans Krebs. Krebs was the German-British scientist largely responsible for working out the pathway in the 1930s.

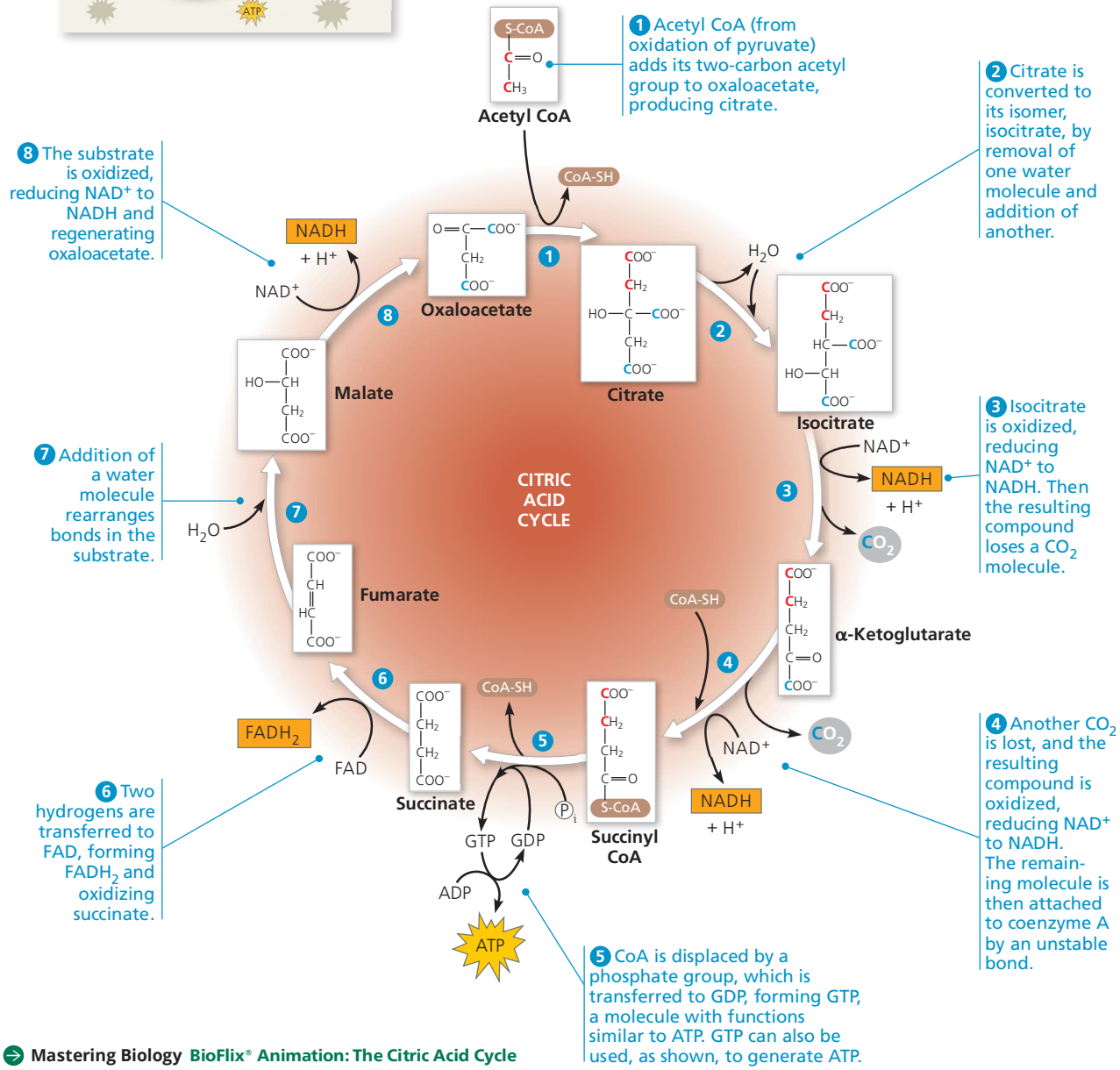
Now let's look at the citric acid cycle in more detail. The cycle has eight steps, each catalyzed by a specific enzyme. You can see in **Figure 9.11** that for each turn of the citric acid cycle, two carbons (red) enter in the relatively reduced form of an acetyl group (step 1), and two different carbons (blue) leave in the completely oxidized form of CO_2 molecules (steps 3 and 4). The acetyl group of acetyl CoA joins the cycle by combining with the compound oxaloacetate, forming citrate (step 1). Citrate is the ionized form of citric acid, for which the cycle is named. The next seven steps decompose the citrate back to oxaloacetate. It is this regeneration of oxaloacetate that makes the process a *cycle*.

Referring to Figure 9.11, we can tally the energy-rich molecules produced by the citric acid cycle. For each acetyl group entering the cycle, 3 NAD^+ are reduced to NADH (steps 3, 4, and 8). In step 6, electrons are transferred not to NAD^+, but to FAD, which accepts 2 electrons and 2 protons to become $FADH_2$. In many animal tissue cells, the reaction in step 5 produces a guanosine triphosphate (GTP) molecule by substrate-level phosphorylation. GTP is a molecule similar to ATP in its structure and cellular function. This GTP may be used to make an ATP molecule (as shown) or directly power work in the cell. In the cells of plants, bacteria, and some animal tissues, step 5 forms an ATP molecule directly by substrate-level phosphorylation. The output from step 5 represents the only ATP generated during the citric acid cycle. Recall that each glucose gives rise to two molecules of acetyl CoA that enter the cycle. Because the numbers noted earlier are obtained from a single acetyl group entering the pathway, the total yield per glucose from the citric acid cycle turns out to be doubled, or 6 NADH, 2 $FADH_2$, and the equivalent of 2 ATP.

Most of the ATP produced by respiration is generated later, from oxidative phosphorylation, when the NADH and $FADH_2$ produced by the citric acid cycle and earlier steps relay the electrons extracted from food to the electron transport chain. In the process, they supply the necessary energy for the phosphorylation of ADP to ATP. We will explore this process in the next section.

▼ Figure 9.11 A closer look at the citric acid cycle. In the chemical structures, red type traces the fate of the two carbon atoms that enter the cycle via acetyl CoA (step 1), and blue type indicates the two carbons that exit the cycle as CO_2 in steps 3 and 4. (The red type goes only through step 5 because the succinate molecule is symmetrical; the two ends cannot be distinguished from each other.) Notice that the carbon atoms that enter the cycle from acetyl CoA do not leave the cycle in the same turn. They remain in the cycle, occupying a different location in the molecules on their next turn, after another acetyl group is added. Therefore, the oxaloacetate regenerated at step 8 is made up of different carbon atoms each time around. Carboxylic acids are represented in their ionized forms, as —COO^-, because the ionized forms prevail at the pH within the mitochondrion. In eukaryotic cells, all the citric acid cycle enzymes are located in the mitochondrial matrix except for the enzyme that catalyzes step 6, which resides in the inner mitochondrial membrane. (The enzyme that catalyzes step 3, isocitrate dehydrogenase, is shown in Figure 6.32b.)





Mastering Biology **BioFlix® Animation: The Citric Acid Cycle**

CONCEPT CHECK 9.3

1. **VISUAL SKILLS** In the citric acid cycle shown in Figure 9.11, what molecules capture energy from the redox reactions? How is ATP produced?
2. What processes in your cells produce the CO_2 that you exhale?
3. **VISUAL SKILLS** The conversions shown in Figure 9.9 and step 4 of Figure 9.11 are each catalyzed by a large multienzyme complex. What similarities are there in the reactions that occur in these two cases?

For suggested answers, see Appendix A.

CONCEPT 9.4

During oxidative phosphorylation, chemiosmosis couples electron transport to ATP synthesis

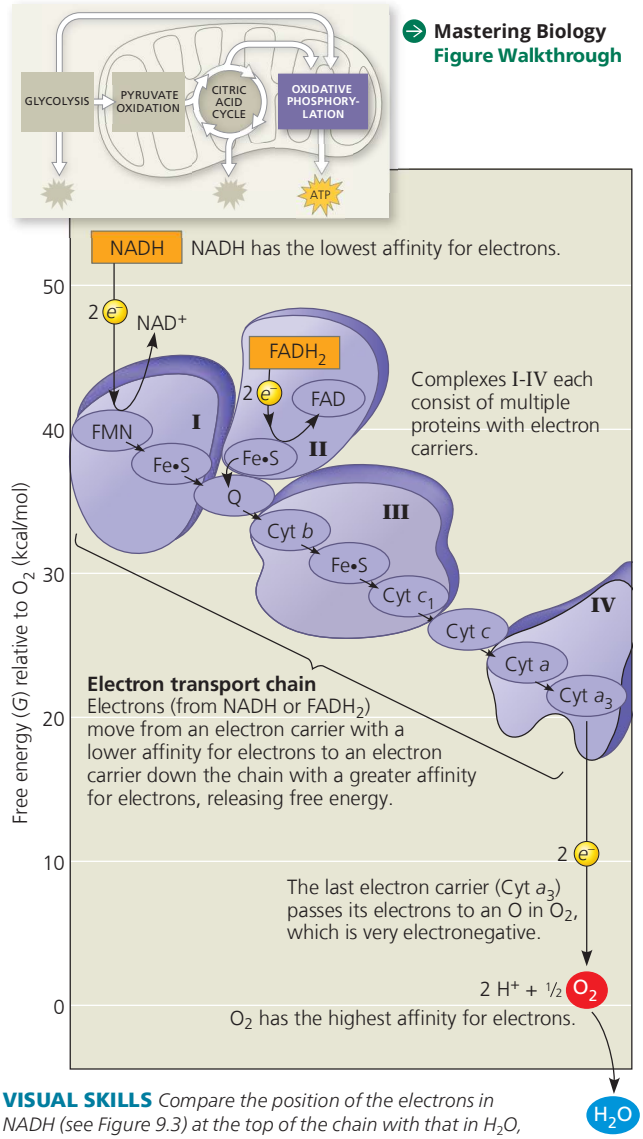
Our main objective in this chapter is to learn how cells harvest the energy of glucose and other nutrients in food to make ATP. But the metabolic components of respiration we have examined so far, glycolysis and the citric acid cycle, produce only 4 ATP molecules per glucose molecule, all by substrate-level phosphorylation: 2 net ATP from glycolysis and 2 ATP from the citric acid cycle. At this point, molecules of NADH (and $FADH_2$) account for most of the energy extracted from each glucose molecule. These electron escorts link glycolysis and the citric acid cycle to the machinery of oxidative phosphorylation, which uses energy released by the electron transport chain to power ATP synthesis. In this section, you will learn first how the electron transport chain works and then how electron flow down the chain is coupled to ATP synthesis.

The Pathway of Electron Transport

The electron transport chain is a collection of molecules embedded in the inner membrane of the mitochondrion in eukaryotic cells. (In prokaryotes, these molecules reside in the plasma membrane.) The folding of the inner membrane to form cristae increases its surface area, providing space for thousands of copies of each component of the electron transport chain in a mitochondrion. Structure fits function: The infolded membrane with its concentration of electron carrier molecules is well-suited for the series of sequential redox reactions that take place along the electron transport chain. Most components of the chain are proteins, which exist in multiprotein complexes numbered I through IV. Tightly bound to these proteins are *prosthetic groups*, nonprotein components such as cofactors and coenzymes essential for the catalytic functions of certain enzymes.

Figure 9.12 shows the sequence of electron carriers in the electron transport chain and the drop in free energy as electrons travel down the chain. During this electron transport, electron carriers alternate between reduced and oxidized states as they accept and then donate electrons. Each component

▼ Figure 9.12 Free-energy change during electron transport. The overall energy drop (ΔG) for electrons traveling from NADH to oxygen is 53 kcal/mol, but this "fall" is broken up into a series of smaller steps by the electron transport chain. (An oxygen atom is represented here as $\frac{1}{2}$ O_2 to show that O_2 is reduced, not individual oxygen atoms.) To view these proteins in their cellular context, see Figure 6.32b.



VISUAL SKILLS *Compare the position of the electrons in NADH (see Figure 9.3) at the top of the chain with that in H_2O, at the bottom. Describe why the electrons in H_2O have less potential energy, using the term electronegativity.*

of the chain becomes reduced when it accepts electrons from its "uphill" neighbor, which has a lower affinity for electrons. It then returns to its oxidized form as it passes electrons to its "downhill" neighbor, which has a higher affinity for electrons.

Now let's take a closer look at the electrons as they drop in energy level, passing through the components of the electron transport chain in Figure 9.12. We'll first look at the passage of

electrons through complex I in some detail as an illustration of the general principles involved in electron transport. Electrons acquired from glucose by NAD^+ during glycolysis and the citric acid cycle are transferred from NADH to the first molecule of the electron transport chain in complex I. This molecule is a flavoprotein, so named because it has a prosthetic group called flavin mononucleotide (FMN). In the next redox reaction, the flavoprotein returns to its oxidized form as it passes electrons to an iron-sulfur protein (Fe · S in complex I), one of a family of proteins with both iron and sulfur tightly bound. The iron-sulfur protein then passes the electrons to a compound called ubiquinone (Q in Figure 9.12). This electron carrier is a small hydrophobic molecule, the only member of the electron transport chain that is not a protein. Ubiquinone is individually mobile within the membrane rather than residing in a particular complex. (Another name for ubiquinone is coenzyme Q, or CoQ; you may have seen it sold as a nutritional supplement.)

Most of the remaining electron carriers between ubiquinone and oxygen are proteins called **cytochromes**. Their prosthetic group, called a heme group, has an iron atom that accepts and donates electrons. (The heme group in a cytochrome is similar to the heme group in hemoglobin, the protein of red blood cells, except that the iron in hemoglobin carries oxygen, not electrons.) The electron transport chain has several types of cytochromes, each named "cyt" with a letter and number to distinguish it as a different protein with a slightly different electron-carrying heme group. The last cytochrome of the chain, cyt a_3, passes its electrons to oxygen (in O_2), which is *very* electronegative. Each O also picks up a pair of hydrogen ions (protons) from the aqueous solution, neutralizing the −2 charge of the added electrons and forming water.

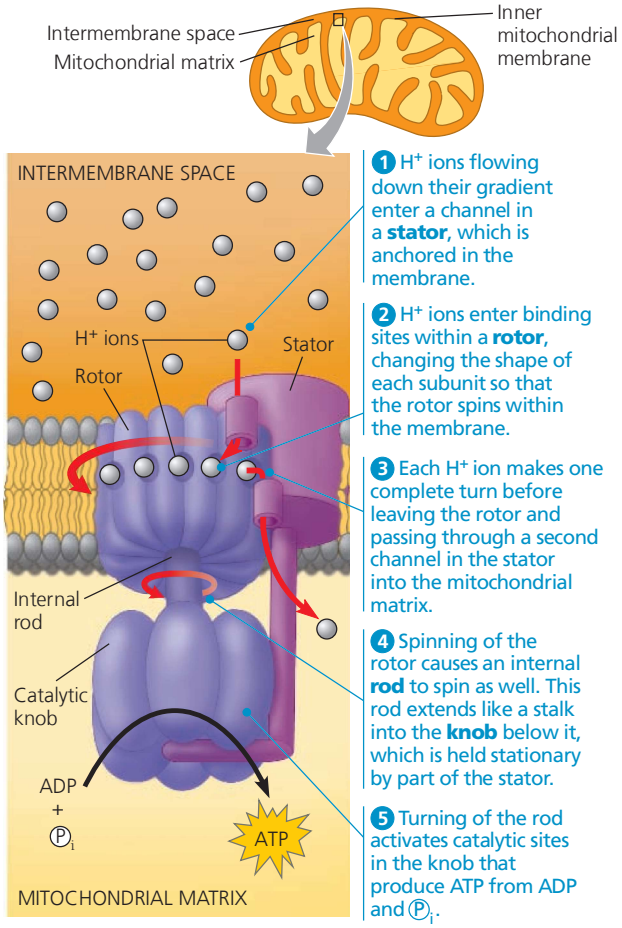
Another source of electrons for the electron transport chain is $FADH_2$, the other reduced product of the citric acid cycle. Notice in Figure 9.12 that $FADH_2$ adds its electrons from within complex II, at a lower energy level than NADH does. Consequently, although NADH and $FADH_2$ each donate an equivalent number of electrons (2) for oxygen reduction, the electron transport chain provides about one-third less energy for ATP synthesis when the electron donor is $FADH_2$ rather than NADH. We'll see why in the next section.

The electron transport chain makes no ATP directly. Instead, it eases the fall of electrons from food to oxygen, breaking a large free-energy drop into a series of smaller steps that release energy in manageable amounts, step by step. How does the mitochondrion (or the plasma membrane in prokaryotes) couple this electron transport and energy release to ATP synthesis? The answer is a mechanism called chemiosmosis.

Chemiosmosis: The Energy-Coupling Mechanism

Populating the inner membrane of the mitochondrion or the prokaryotic plasma membrane are many copies of a protein complex called **ATP synthase**, the enzyme that makes ATP from ADP and inorganic phosphate **(Figure 9.13)**. ATP synthase works like an ion pump running in reverse. Ion pumps usually use ATP as an energy source to transport ions against their gradients. Enzymes can catalyze a reaction in either direction, depending on the ΔG for the reaction, which is affected by the local concentrations of reactants and products (see Concepts 8.2 and 8.3). Under the conditions of cellular respiration, rather than hydrolyzing ATP to pump protons against their concentration gradient, ATP synthase uses the energy of an existing ion gradient to power ATP synthesis. The power source for ATP synthase is a difference in the concentration of H^+ (a pH difference) on opposite sides of the inner mitochondrial membrane. This process, in which energy stored in the form of a hydrogen ion gradient across a membrane is used to drive cellular work such as the synthesis of ATP, is

▼ Figure 9.13 ATP synthase, a molecular mill. Multiple ATP synthases reside in eukaryotic mitochondrial and chloroplast membranes and in prokaryotic plasma membranes. (See Figure 6.32b and c.)



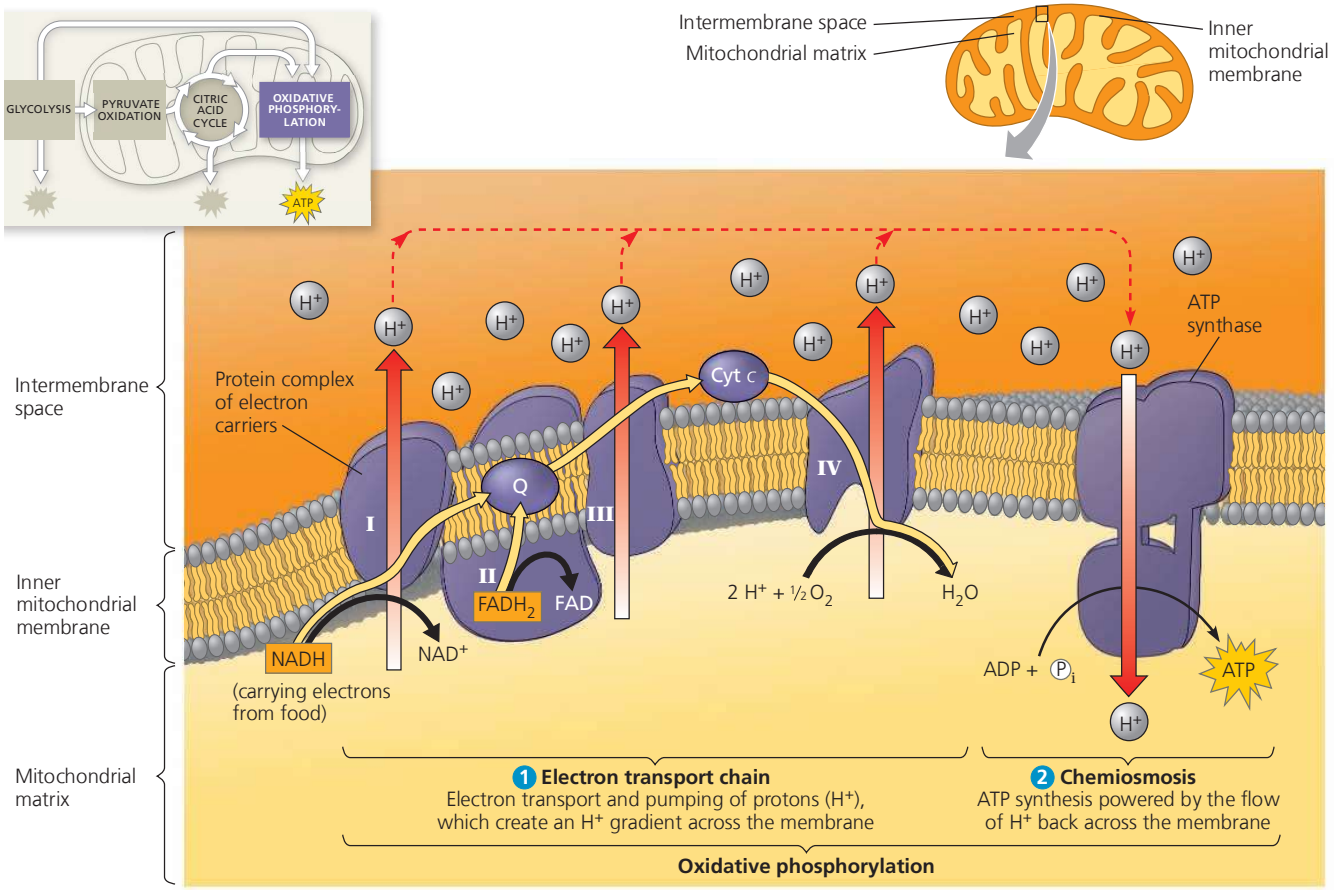
Mastering Biology BioFlix® Animation: ATP Synthase
Animation: Rotating ATP Synthase

called **chemiosmosis** (from the Greek *osmos*, push). The word *osmosis* was previously used in discussing water transport, but here it refers to the flow of H^+ across a membrane.

From studying the structure of ATP synthase, scientists have learned how the flow of H^+ through this enzyme powers ATP generation. ATP synthase is a multisubunit complex with four main parts, each made up of multiple polypeptides (see Figure 9.13). Protons move one by one into binding sites on the rotor, causing it to spin in a way that catalyzes ATP production from ADP and $\textcircled{P}_i$. The flow of protons thus behaves somewhat like a rushing stream that turns a waterwheel. ATP synthase is the smallest molecular rotary motor known in nature.

How does the inner mitochondrial membrane (or the prokaryotic plasma membrane) generate and maintain the H^+ gradient that drives ATP synthesis by the ATP synthase protein complex? Establishing the H^+ gradient is a major function of the electron transport chain, which is shown in its mitochondrial location in **Figure 9.14**. The chain is an energy converter that uses the exergonic flow of electrons from NADH and $FADH_2$ to pump H^+ across the membrane, from the mitochondrial matrix into the intermembrane space. The H^+ has a tendency to move back across the membrane, diffusing down its gradient. And the ATP synthases are the only sites that provide a route through the membrane for H^+. As we described previously, the passage

▼ Figure 9.14 Chemiosmosis couples the electron transport chain to ATP synthesis. 1 NADH and $FADH_2$ shuttle high-energy electrons extracted from food during glycolysis and the citric acid cycle into an electron transport chain built into the inner mitochondrial membrane. (See Figure 6.32b.) The gold arrows trace the transport of electrons, which are finally passed to a terminal acceptor (O_2, in the case of aerobic respiration) at the "downhill" end of the chain, forming water. Most of the electron carriers of the chain are grouped into four complexes (I–IV). Two mobile carriers, ubiquinone (Q) and cytochrome *c* (Cyt *c*), move rapidly, ferrying electrons between the large complexes. As the complexes shuttle electrons, they pump protons from the mitochondrial matrix into the intermembrane space. $FADH_2$ deposits its electrons via complex II and so results in fewer protons being pumped into the intermembrane space than occurs with NADH. Chemical energy originally harvested from food is transformed into a proton-motive force, a gradient of H^+ across the membrane. 2 During chemiosmosis, the protons flow back down their gradient via ATP synthase, which is built into the membrane nearby. The ATP synthase harnesses the proton-motive force to phosphorylate ADP, forming ATP. Together, electron transport and chemiosmosis make up oxidative phosphorylation.



WHAT IF? *If complex IV were nonfunctional, could chemiosmosis produce any ATP, and if so, how would the rate of synthesis differ?*

Mastering Biology Animation: Electron Transport
BioFlix® Animation: Electron Transport

of H^+ through ATP synthase uses the exergonic flow of H^+ to drive the phosphorylation of ADP. Thus, the energy stored in an H^+ gradient across a membrane couples the redox reactions of the electron transport chain to ATP synthesis.

At this point, you may be wondering how the electron transport chain pumps hydrogen ions into the intermembrane space. Researchers have found that certain members of the electron transport chain accept and release protons (H^+) along with electrons. (The aqueous solutions inside and surrounding the cell are a ready source of H^+.) At certain steps along the chain, electron transfers cause H^+ to be taken up and released into the surrounding solution. In eukaryotic cells, the electron carriers are spatially arranged in the inner mitochondrial membrane in such a way that H^+ is accepted from the mitochondrial matrix and deposited in the intermembrane space (see Figure 9.14). The H^+ gradient that results is referred to as a **proton-motive force**, emphasizing the capacity of the gradient to perform work. The force drives H^+ back across the membrane through the H^+ channels provided by ATP synthases.

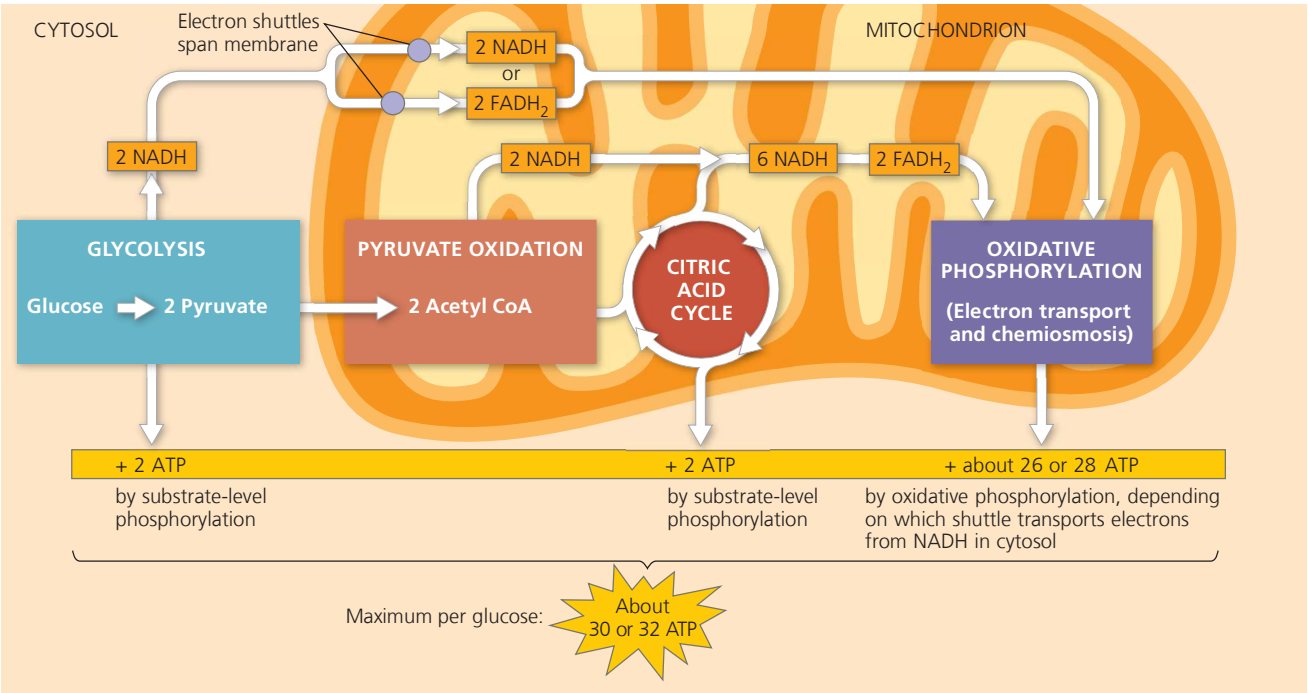
In general terms, *chemiosmosis is an energy-coupling mechanism that uses energy stored in the form of an H^+ gradient across a membrane to drive cellular work*. In mitochondria, the energy for gradient formation comes from exergonic redox reactions along the electron transport chain, and ATP synthesis is the work performed. But chemiosmosis also occurs elsewhere and in other variations. Chloroplasts use chemiosmosis to generate ATP during photosynthesis; in these organelles, light (rather than chemical energy) drives both electron flow down an electron transport chain and the resulting H^+ gradient formation. Prokaryotes, as already mentioned, generate H^+ gradients across their plasma membranes. They then tap the proton-motive force not only to make ATP inside the cell but also to rotate their flagella and to pump nutrients and waste products across the membrane. Because of its central importance to energy conversions in prokaryotes and eukaryotes, chemiosmosis has helped unify the study of bioenergetics. Peter Mitchell was awarded the Nobel Prize in 1978 for originally proposing the chemiosmotic model.

An Accounting of ATP Production by Cellular Respiration

In the last few sections, we have looked rather closely at the key processes of cellular respiration. Now let's take a step back and remind ourselves of its overall function: harvesting the energy of glucose for ATP synthesis.

During respiration, most energy flows in this sequence: glucose → NADH → electron transport chain → proton-motive force → ATP. We can do some bookkeeping to calculate the ATP profit when cellular respiration oxidizes a molecule of glucose to six molecules of carbon dioxide. The three main departments of this metabolic enterprise are glycolysis, pyruvate oxidation and the citric acid cycle, and the electron transport chain, which drives oxidative phosphorylation. **Figure 9.15** gives a detailed accounting of the ATP yield for

▼ Figure 9.15 ATP yield per molecule of glucose at each stage of cellular respiration.



VISUAL SKILLS *After reading the discussion in the text, explain exactly how the total of 26 or 28 ATP from oxidative phosphorylation was calculated (see the yellow bar).*

Mastering Biology Animation: ATP Yield from Cellular Respiration

each glucose molecule that is oxidized. The tally adds the 4 ATP produced directly by substrate-level phosphorylation during glycolysis and the citric acid cycle to the many more molecules of ATP generated by oxidative phosphorylation. Each NADH that transfers a pair of electrons from glucose to the electron transport chain contributes enough to the proton-motive force to generate a maximum of about 3 ATP.

Why are the numbers in Figure 9.15 inexact? There are three reasons we cannot state an exact number of ATP molecules generated by the breakdown of one molecule of glucose. First, phosphorylation and the redox reactions are not directly coupled to each other, so the ratio of the number of NADH molecules to the number of ATP molecules is not a whole number. We know that 1 NADH results in 10 H^+ being transported out across the inner mitochondrial membrane, but the exact number of H^+ that must reenter the mitochondrial matrix via ATP synthase to generate 1 ATP has long been debated. Based on experimental data, however, most biochemists now agree that the most accurate number is 4 H^+. Therefore, a single molecule of NADH generates enough proton-motive force for the synthesis of 2.5 ATP. The citric acid cycle also supplies electrons to the electron transport chain via $FADH_2$, but since its electrons enter later in the chain, each molecule of this electron carrier is responsible for transport of only enough H^+ for the synthesis of 1.5 ATP. These numbers also take into account the slight energetic cost of moving the ATP formed in the mitochondrion out into the cytosol, where it will be used.

Second, the ATP yield varies slightly depending on the type of shuttle used to transport electrons from the cytosol into the mitochondrion. The mitochondrial inner membrane is not permeable to NADH, so NADH in the cytosol is segregated from the machinery of oxidative phosphorylation. The 2 electrons of NADH captured in glycolysis must be conveyed into the mitochondrion by one of several electron shuttle systems. Depending on the kind of shuttle in a particular cell type, the electrons are passed either to NAD^+ or to FAD in the mitochondrial matrix (see Figures 9.14 and 9.15). If the electrons are passed to FAD, as in brain cells, only about 1.5 ATP can result from each NADH that was originally generated in the cytosol. If the electrons are passed to mitochondrial NAD^+, as in liver cells and heart cells, the yield is about 2.5 ATP per NADH.

A third variable that reduces the yield of ATP is the use of the proton-motive force generated by the redox reactions of respiration to drive other kinds of work. For example, the proton-motive force powers the mitochondrion's uptake of pyruvate from the cytosol (see Figure 9.9). However, if *all* the proton-motive force generated by the electron transport chain were used to drive ATP synthesis, one glucose molecule could generate a maximum of 28 ATP produced by oxidative phosphorylation plus 4 ATP (net) from substrate-level phosphorylation to give a total yield of about 32 ATP (or only about 30 ATP if the less efficient shuttle were functioning).

Mastering Biology **Animation: Electron Transport Chain: Factors Affecting ATP Yield**

We can now roughly estimate the efficiency of respiration—that is, the percentage of chemical energy in glucose that has been transferred to ATP. Recall that the complete oxidation of a mole of glucose releases 686 kcal of energy under standard conditions ($\Delta G = -686$ kcal/mol). Phosphorylation of ADP to form ATP stores at least 7.3 kcal per mole of ATP. Therefore, the efficiency of respiration is 7.3 kcal per mole of ATP times 32 moles of ATP per mole of glucose divided by 686 kcal per mole of glucose, which equals 0.34. Thus, about 34% of the potential chemical energy in glucose has been transferred to ATP; the actual percentage is bound to vary as ΔG varies under different cellular conditions. Cellular respiration is remarkably efficient in its energy conversion. By comparison, even the most efficient automobile converts only about 25% of the energy stored in gasoline to energy that moves the car.

The rest of the energy stored in glucose is lost as heat. We humans use some of this heat to maintain our relatively high body temperature (37°C), and we dissipate the rest through sweating and other cooling mechanisms.

Surprisingly, perhaps, it may be beneficial under certain conditions to reduce the efficiency of cellular respiration. A remarkable adaptation is shown by hibernating mammals, which overwinter in a state of inactivity and lowered metabolism. Although their internal body temperature is lower than normal, it still must be kept significantly higher than the external air temperature. One type of tissue, called brown fat, is made up of cells packed full of mitochondria. The inner mitochondrial membrane contains a channel protein called the uncoupling protein that allows protons to flow back down their concentration gradient without generating ATP. Activation of these proteins in hibernating mammals results in ongoing oxidation of stored fuel (fats), generating heat without any ATP production. In the absence of such an adaptation, the buildup of ATP would eventually cause cellular respiration to be shut down by regulatory mechanisms that will be discussed later. Brown fat is also used for heat generation in humans. In the **Scientific Skills Exercise**, you can work with data in a related but different case where a decrease in metabolic efficiency in cells is used to generate heat.

Mastering Biology **BioFlix® Animation: Cellular Respiration**

CONCEPT CHECK 9.4

1. **WHAT IF?** What effect would an absence of O_2 have on the process shown in Figure 9.14?
2. **WHAT IF?** In the absence of O_2, as in question 1, what do you think would happen if you decreased the pH of the intermembrane space of the mitochondrion? Explain your answer.
3. **MAKE CONNECTIONS** Membranes must be fluid to function properly (see Concept 7.1). How does the operation of the electron transport chain support that assertion?

For suggested answers, see Appendix A.

Scientific Skills Exercise

Making a Bar Graph and Evaluating a Hypothesis

Does Thyroid Hormone Level Affect O_2 Consumption in Cells? Some animals, such as mammals and birds, maintain a relatively constant body temperature, above that of their environment, by using heat produced as a by-product of metabolism. When the core temperature of these animals drops below an internal set point, their cells are triggered to reduce the efficiency of ATP production by the electron transport chains in mitochondria. At lower efficiency, extra fuel must be consumed to produce the same number of ATPs, generating additional heat. This response is moderated by the endocrine system, and researchers hypothesized that it might be triggered by thyroid hormone. In this exercise, you will use a bar graph to visualize data from an experiment that compared the metabolic rates (by measuring O_2 consumption) in mitochondria of cells from animals with different levels of thyroid hormone.

How the Experiment Was Done Liver cells were isolated from sibling rats that had low, normal, or elevated thyroid hormone levels. The oxygen consumption rate due to activity of the mitochondrial electron transport chains of each type of cell was measured under controlled conditions.

Data from the Experiment

Thyroid Hormone Level	Oxygen Consumption Rate [nmol O_2/(min · mg cells)]
Low	4.3
Normal	4.8
Elevated	8.7

Data from M. E. Harper and M. D. Brand, The quantitative contributions of mitochondrial proton leak and ATP turnover reactions to the changed respiration rates of hepatocytes from rats of different thyroid status, *Journal of Biological Chemistry* 268:14850–14860 (1993).

INTERPRET THE DATA

1. To visualize any differences in O_2 consumption between cell types, it will be useful to graph the data in a bar graph. First, set up the axes. **(a)** What is the independent variable (intentionally varied by the researchers), which goes on the *x*-axis? List the categories along the *x*-axis; because they are discrete rather than continuous, you can list them in any order. **(b)** What is the dependent variable (measured by the researchers), which goes on the *y*-axis? **(c)** What units (abbreviated) should go on the *y*-axis? Label the *y*-axis, including the units specified in the data table. Determine the range of values of the data that will need to go on the *y*-axis. What is the largest value? Draw evenly spaced tick marks and label them, starting with 0 at the bottom.
2. Graph the data for each sample. Match each *x*-value with its *y*-value and place a mark on the graph at that coordinate, then draw a bar from the *x*-axis up to the correct height for each sample. Why is a bar graph more appropriate than a scatter plot or line graph? (For additional information about graphs, see the Scientific Skills Review in Appendix D.)
3. Examine your graph and look for a pattern in the data. **(a)** Which cell type had the highest rate of O_2 consumption, and which had the lowest? **(b)** Does this support the researchers' hypothesis? Explain. **(c)** Based on what you know about mitochondrial electron transport and heat production, predict which rats had the highest, and which had the lowest, body temperature.

Instructors: A version of this Scientific Skills Exercise can be assigned in **Mastering Biology**.

CONCEPT 9.5

Fermentation and anaerobic respiration enable cells to produce ATP without the use of oxygen

Because most of the ATP generated by cellular respiration is due to the work of oxidative phosphorylation, our estimate of ATP yield from aerobic respiration depends on an adequate supply of O_2 to the cell. Without the electronegative oxygen atoms in O_2 to pull electrons down the transport chain, oxidative phosphorylation eventually ceases. However, there are two general mechanisms by which certain cells can oxidize organic fuel and generate ATP *without* the use of O_2: anaerobic respiration and fermentation. The distinction between these two is that an electron transport chain is used in anaerobic respiration but not in fermentation. (The electron transport chain is also called the respiratory chain because of its role in both types of cellular respiration.)

We have already mentioned anaerobic respiration, which takes place in certain prokaryotic organisms that live in environments without O_2. These organisms have an electron transport chain but do not use O_2 as a final electron acceptor at the end of the chain. O_2 performs this function very well because it consists of two extremely electronegative atoms, but other substances can also serve as final electron acceptors. Some "sulfate-reducing" marine bacteria, for instance, use the sulfate ion (SO_4^{2-}) at the end of their respiratory chain. Operation of the chain builds up a proton-motive force used to produce ATP, but H_2S (hydrogen sulfide) is made as a

by-product rather than water. The rotten-egg odor you may have smelled while walking through a salt marsh or a mudflat signals the presence of sulfate-reducing bacteria.

Fermentation is a way of harvesting chemical energy without using either O_2 or any electron transport chain—in other words, without cellular respiration. How can food be oxidized without cellular respiration? Remember, oxidation simply refers to the loss of electrons to an electron acceptor, so it does not need to involve O_2. Glycolysis oxidizes glucose to two molecules of pyruvate. The oxidizing agent of glycolysis is NAD^+, and neither O_2 nor any electron transfer chain is involved. Overall, glycolysis is exergonic, and some of the energy made available is used to produce 2 ATP (net) by substrate-level phosphorylation. If O_2 *is* present, then additional ATP is made by oxidative phosphorylation when NADH passes electrons removed from glucose to the electron transport chain. But glycolysis generates 2 ATP whether oxygen is present or not—that is, whether conditions are aerobic or anaerobic.

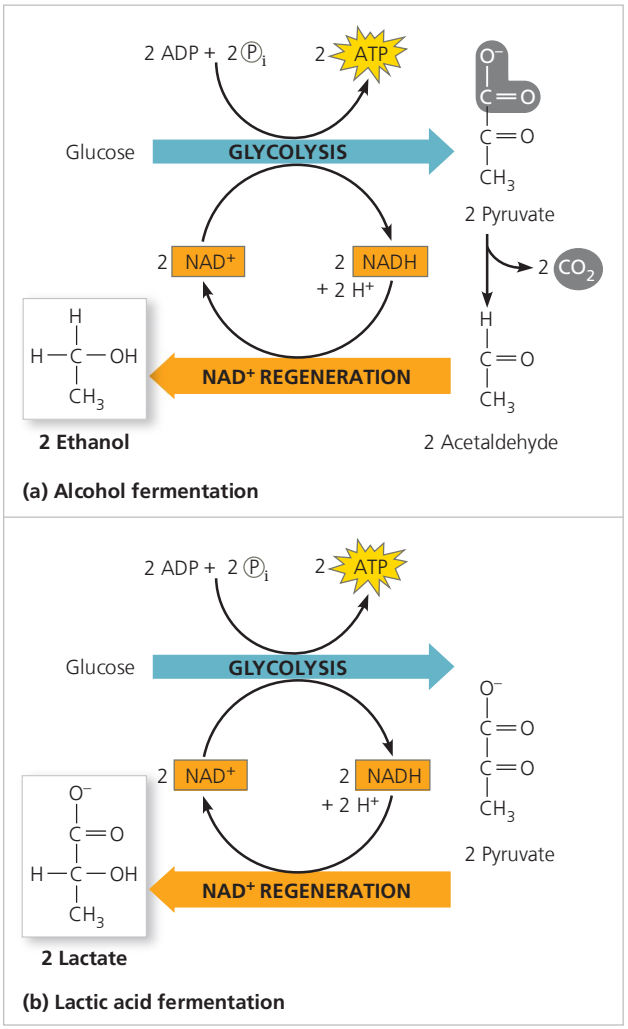
As an alternative to respiratory oxidation of organic nutrients, fermentation is an extension of glycolysis that allows continuous generation of ATP by the substrate-level phosphorylation of glycolysis. For this to occur, there must be a sufficient supply of NAD^+ to accept electrons during the oxidation step of glycolysis. Without some mechanism to recycle NAD^+ from NADH, glycolysis would soon deplete the cell's pool of NAD^+ by reducing it all to NADH and would shut itself down for lack of an oxidizing agent. Under aerobic conditions, NAD^+ is recycled from NADH by the transfer of electrons to the electron transport chain. An anaerobic alternative is to transfer electrons from NADH to pyruvate, the end product of glycolysis.

Types of Fermentation

Fermentation consists of glycolysis plus reactions that regenerate NAD^+ by transferring electrons from NADH to pyruvate or derivatives of pyruvate. The NAD^+ can then be reused to oxidize sugar by glycolysis, which nets two molecules of ATP by substrate-level phosphorylation. There are many types of fermentation, differing in the end products formed from pyruvate. Two types are alcohol fermentation and lactic acid fermentation, and both are harnessed by humans for food and industrial production.

In **alcohol fermentation** (Figure 9.16a), pyruvate is converted to ethanol (ethyl alcohol) in two steps. The first step releases CO_2 from the pyruvate, which is converted to the two-carbon compound acetaldehyde. In the second step, acetaldehyde is reduced by NADH to ethanol. This regenerates the supply of NAD^+ needed for the continuation of glycolysis. Many bacteria carry out alcohol fermentation under anaerobic conditions. Yeast (a fungus), in addition to aerobic respiration, also carries out alcohol fermentation. For thousands of years, humans have used yeast in brewing, winemaking, and baking. The CO_2 bubbles generated by baker's yeast during alcohol fermentation allow bread to rise.

▼ Figure 9.16 Fermentation. In the absence of oxygen, many cells use fermentation to produce ATP by substrate-level phosphorylation. NAD^+ is regenerated for use in glycolysis when pyruvate, the end product of glycolysis, serves as an electron acceptor for oxidizing NADH. Two of the common end products formed from fermentation are **(a)** ethanol and **(b)** lactate, the ionized form of lactic acid.



Mastering Biology Animation: Fermentation

During **lactic acid fermentation** (Figure 9.16b), pyruvate is reduced directly by NADH to form lactate as an end product, regenerating NAD^+ with no release of CO_2. (Lactate is the ionized form of lactic acid.) Lactic acid fermentation by certain fungi and bacteria is used in the dairy industry to make cheese and yogurt. A complex series of fermentation and aerobic respiration pathways carried out by yeasts and bacteria on cacao beans is responsible for the production of chocolate.

▼ Cacao beans and fruits

What about lactate production in humans? Previously, we thought that human muscle cells only produced lactate when O_2 was in short supply, such as during intense exercise. Research done over the last few decades, though, indicates that the lactate story, in mammals at least, is more complicated. There are two types of skeletal muscle fibers. One (red muscle) preferentially oxidizes glucose completely to CO_2; the other (white muscle) produces significant amounts of lactate from the pyruvate made during glycolysis, even under aerobic conditions, offering fast but energetically inefficient ATP production. The lactate product is then mostly oxidized by red muscle cells in the vicinity, with the remainder exported to liver or kidney cells for glucose formation. Because this lactate production is not anaerobic, but the result of glycolysis in these cells, exercise physiologists prefer not to use the term *fermentation*.

During strenuous exercise, when carbohydrate catabolism outpaces the supply of O_2 from the blood to the muscle, lactate can't be oxidized to pyruvate. The lactate that accumulates was once thought to cause muscle fatigue during intense exercise and pain a day or so later. However, research suggests that, contrary to popular opinion, lactate production actually improves performance during exercise! Furthermore, within an hour, excess lactate is shuttled to other tissues for oxidation or to the liver and kidneys for production of glucose or its storage molecule, glycogen. (Nextday muscle soreness is more likely caused by trauma to cells in small muscle fibers, which leads to inflammation and pain.)

Comparing Fermentation with Anaerobic and Aerobic Respiration

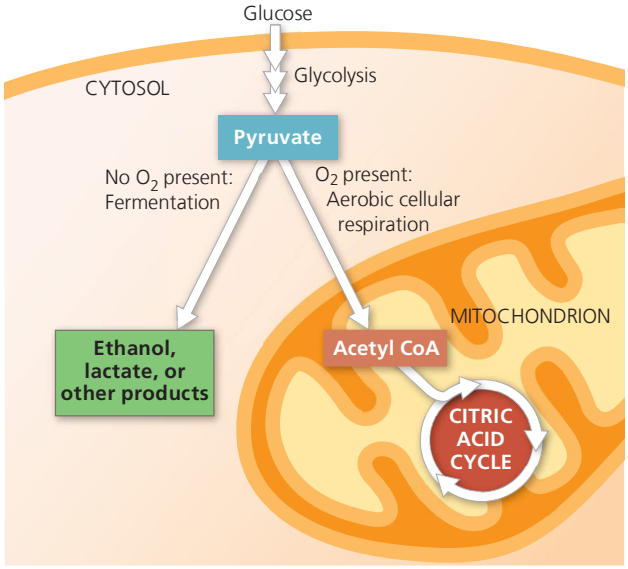
Fermentation, anaerobic respiration, and aerobic respiration are three alternative cellular pathways for producing ATP by harvesting the chemical energy of food. All three use glycolysis to oxidize glucose and other organic fuels to pyruvate, with a net production of 2 ATP by substrate-level phosphorylation. And in all three pathways, NAD^+ is the oxidizing agent that accepts electrons from food during glycolysis.

A key difference is the contrasting mechanisms for oxidizing NADH back to NAD^+, which is required to sustain glycolysis. In fermentation, the final electron acceptor is an organic molecule such as pyruvate (lactic acid fermentation) or acetaldehyde (alcohol fermentation). In cellular respiration, by contrast, electrons carried by NADH are transferred to an electron transport chain, which regenerates the NAD^+ required for glycolysis.

Another major difference is the amount of ATP produced. Fermentation yields two molecules of ATP, produced by substrate-level phosphorylation. In the absence of an electron transport chain, the energy stored in pyruvate is unavailable. In cellular respiration, however, pyruvate is completely oxidized in the mitochondrion. Most of the chemical energy from this process is shuttled by NADH and $FADH_2$ in the form of electrons to the electron transport chain. There, the electrons move stepwise down a series of redox reactions to a final electron acceptor. (In aerobic respiration, the final electron acceptor is O_2; in anaerobic respiration, the final acceptor is another molecule with a high affinity for electrons, although less so than O_2.) Stepwise electron transport drives oxidative phosphorylation, yielding ATP. Thus, cellular respiration harvests much more energy from each sugar molecule than fermentation can. In fact, aerobic respiration yields up to 32 molecules of ATP per glucose molecule—up to 16 times as much as does fermentation.

Some organisms, called **obligate anaerobes**, carry out only fermentation or anaerobic respiration. In fact, these organisms cannot survive in the presence of oxygen, some forms of which can actually be toxic if protective systems are not present in the cell. A few cell types, such as cells of the vertebrate brain, can carry out only aerobic oxidation of pyruvate, and need O_2 to survive. Other organisms, including yeasts and many bacteria, can make enough ATP to survive using either fermentation or respiration. Such species are called **facultative anaerobes**. In yeast cells, for example, pyruvate is a fork in the metabolic road that leads to two alternative catabolic routes **(Figure 9.17)**. Under aerobic conditions, pyruvate can be converted to acetyl CoA, and oxidation continues in the citric acid cycle via aerobic respiration. Under anaerobic conditions, lactic acid fermentation occurs. Pyruvate is diverted from the citric acid cycle, serving instead

▼ Figure 9.17 Pyruvate as a key juncture in catabolism. Glycolysis is common to fermentation and cellular respiration. The end product of glycolysis, pyruvate, represents a fork in the catabolic pathways of glucose oxidation. In a facultative anaerobe, capable of both aerobic cellular respiration and fermentation, pyruvate is committed to one of those two pathways, usually depending on whether or not oxygen is present.



as an electron acceptor to recycle NAD^+. To make the same amount of ATP, a facultative anaerobe has to consume sugar at a much faster rate when fermenting than when respiring.

The Evolutionary Significance of Glycolysis

EVOLUTION The role of glycolysis in both fermentation and respiration has an evolutionary basis. Ancient prokaryotes are thought to have used glycolysis to make ATP long before oxygen was present in Earth's atmosphere. The oldest known fossils of bacteria date back 3.5 billion years, but appreciable quantities of oxygen probably did not begin to accumulate in the atmosphere until about 2.7 billion years ago. Cyanobacteria produced this O_2 as a by-product of photosynthesis. Therefore, early prokaryotes may have generated ATP exclusively from glycolysis. The fact that glycolysis is today the most widespread metabolic pathway among Earth's organisms suggests that it evolved very early in the history of life. The cytosolic location of glycolysis also implies great antiquity; the pathway does not require any of the membrane-enclosed organelles of the eukaryotic cell, which evolved approximately 1 billion years after the first prokaryotic cell. Glycolysis is a metabolic heirloom from early cells that continues to function in fermentation and as the first stage in the breakdown of organic molecules by respiration.

CONCEPT CHECK 9.5

1. Consider the NADH formed during glycolysis. What is the final acceptor for its electrons during fermentation? During aerobic respiration? During anaerobic respiration?
2. **WHAT IF?** A glucose-fed yeast cell is moved from an aerobic environment to an anaerobic one. How would its rate of glucose consumption change if ATP were to be generated at the same rate?

For suggested answers, see Appendix A.

CONCEPT 9.6

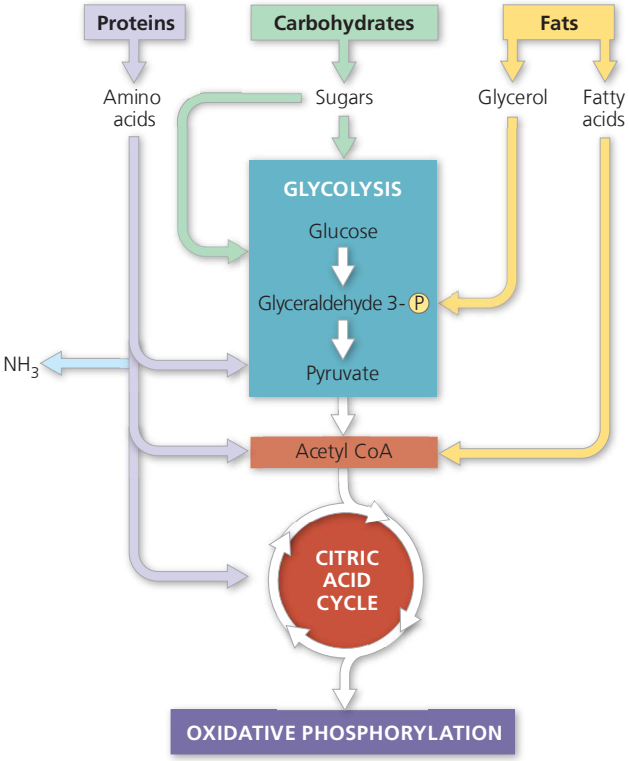
Glycolysis and the citric acid cycle connect to many other metabolic pathways

So far, we have looked at the oxidative breakdown of glucose in isolation from the cell's overall metabolic economy. In this section, you will learn that glycolysis and the citric acid cycle are major intersections of the cell's catabolic (breakdown) and anabolic (biosynthetic) pathways.

The Versatility of Catabolism

Throughout this chapter, glucose has been used as an example of a fuel for cellular respiration. But free glucose molecules are not common in the diets of humans and other animals. We obtain most of our calories in the form of fats, proteins, and carbohydrates such as sucrose and other disaccharides, and starch, a polysaccharide. All these organic molecules in food can be used by cellular respiration to make ATP **(Figure 9.18)**.

▼ Figure 9.18 The catabolism of various molecules from food. Carbohydrates, fats, and proteins can all be used as fuel for cellular respiration. Monomers of these molecules enter glycolysis or the citric acid cycle at various points. Glycolysis and the citric acid cycle are catabolic funnels through which electrons from all kinds of organic molecules flow on their exergonic fall to oxygen.



Glycolysis can accept a wide range of carbohydrates for catabolism. In the digestive tract, starch is hydrolyzed to glucose, which is broken down in cells by glycolysis and the citric acid cycle. Glycogen, the polysaccharide that humans and many other animals store in their liver and muscle cells, can be hydrolyzed to glucose between meals as fuel for respiration. Digestion of disaccharides, including sucrose, provides glucose and other monosaccharides as fuel for respiration.

Proteins can also be used for fuel, but first they must be digested to their constituent amino acids. Many of the amino acids are used by the organism to build new proteins. Amino acids present in excess are converted by enzymes to intermediates of glycolysis and the citric acid cycle. Before amino acids can feed into glycolysis or the citric acid cycle, their amino groups must be removed, a process called *deamination*. The nitrogenous waste is excreted from the animal in the form of ammonia (NH_3), urea, or other waste products.

Catabolism can also harvest energy stored in fats obtained either from food or from fat cells in the body. After fats are digested to glycerol and fatty acids, the glycerol is converted to glyceraldehyde 3-phosphate, an intermediate of glycolysis. Most of the energy of a fat is stored in the fatty acids. A metabolic sequence called **beta oxidation** breaks the fatty acids down to two-carbon fragments, which enter the citric acid cycle as acetyl CoA. NADH and $FADH_2$ are also generated during beta oxidation; they can enter the electron transport chain, leading to further ATP production. Fats make excellent fuels, in large part due to their chemical structure and the high energy level of their electrons (present in many C—H bonds, equally shared between C and H) compared to those of carbohydrates. A gram of fat oxidized by respiration produces more than twice as much ATP as a gram of carbohydrate. Unfortunately, this also means that a person trying to lose weight must work hard to use up fat stored in the body because so many calories are stockpiled in each gram of fat.

Biosynthesis (Anabolic Pathways)

Cells need substance as well as energy. Not all the organic molecules of food are destined to be oxidized as fuel to make ATP. In addition to calories, food must also provide the carbon skeletons that cells require to make their own molecules. Some organic monomers obtained from digestion can be used directly. For example, as previously mentioned, amino acids from the hydrolysis of proteins in food can be incorporated into the organism's own proteins. Often, however, the body needs specific molecules that are not present as such in food. Compounds formed as intermediates of glycolysis and the citric acid cycle can be diverted into anabolic pathways as precursors from which the cell can synthesize the molecules it requires. For example, humans can make about half of the 20 amino acids in proteins by modifying compounds siphoned away from the citric acid cycle; the rest are "essential amino acids" that must be obtained in the diet. Also, glucose can be made from pyruvate, and fatty acids can be synthesized from acetyl CoA. Of course, these anabolic, or biosynthetic, pathways do not generate ATP, but instead consume it.

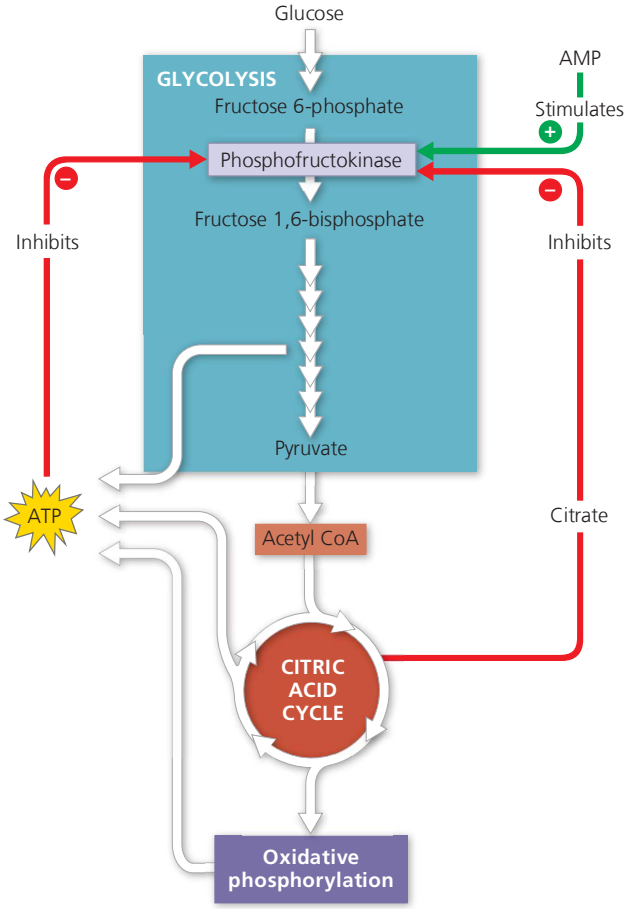
In addition, glycolysis and the citric acid cycle function as metabolic interchanges that enable our cells to convert some kinds of molecules to others as we need them. For example, an intermediate compound generated during glycolysis, dihydroxyacetone phosphate (see Figure 9.8, step 5), can be converted to one of the major precursors of fats. If we eat more food than we need, we store fat even if our diet is fat-free. Metabolism is remarkably versatile and adaptable.

Regulation of Cellular Respiration via Feedback Mechanisms

Basic principles of supply and demand regulate the metabolic economy. The cell does not waste energy making more of a particular substance than it needs. If there is a surplus of a certain amino acid, for example, the anabolic pathway that synthesizes that amino acid from an intermediate of the citric acid cycle is switched off. The most common mechanism for this control is feedback inhibition: The end product of the anabolic pathway inhibits the enzyme that catalyzes an early step of the pathway (see Figure 8.21). This prevents the needless diversion of key metabolic intermediates from uses that are more urgent.

The cell also controls its catabolism. If the cell is working hard and its ATP concentration begins to drop, cellular respiration speeds up. When there is plenty of ATP to meet demand, respiration slows down, sparing valuable organic molecules for other functions. Again, control is based mainly on regulating the activity of enzymes at strategic points in the catabolic pathway. As shown in **Figure 9.19**, one important switch is phosphofructokinase, the enzyme that catalyzes step 3 of glycolysis (see Figure 9.8). That is the first step that

▼ Figure 9.19 The control of cellular respiration. Allosteric enzymes at certain points in the respiratory pathway respond to inhibitors and activators that help set the pace of glycolysis and the citric acid cycle. Phosphofructokinase, which catalyzes an early step in glycolysis (see Figure 9.8, step 3 and Figure 6.32b), is one such enzyme. It is stimulated by AMP (derived from ADP) but is inhibited by ATP and by citrate. This feedback regulation adjusts the rate of respiration as the cell's catabolic and anabolic demands change.



commits the substrate irreversibly to the glycolytic pathway. By controlling the rate of this step, the cell can speed up or slow down the entire catabolic process. Phosphofructokinase can thus be considered the pacemaker of cellular respiration.

Phosphofructokinase is an allosteric enzyme with receptor sites for specific inhibitors and activators. It is inhibited by ATP and stimulated by AMP (adenosine monophosphate), which the cell derives from ADP. As ATP accumulates, inhibition of the enzyme slows down glycolysis. The enzyme becomes active again as cellular work converts ATP to ADP (and AMP) faster than ATP is being regenerated. Phosphofructokinase is also sensitive to citrate, the first product of the citric acid cycle. If citrate accumulates in mitochondria, some of it passes into the cytosol and inhibits phosphofructokinase. This mechanism helps synchronize the rates of glycolysis and the citric acid cycle. As citrate accumulates, glycolysis slows down, and the supply of pyruvate and thus acetyl groups to the citric acid cycle decreases. If citrate consumption increases, either because of a demand for more ATP or because anabolic pathways are draining off intermediates of the citric acid cycle, glycolysis accelerates and meets the demand. Metabolic balance is augmented by the control of enzymes that catalyze other key steps of glycolysis and the citric acid cycle. Cells are thrifty, expedient, and responsive in their metabolism.

Review the first page of this chapter to put cellular respiration into the broader context of energy flow and chemical cycling in ecosystems. The energy that keeps us alive is *released*, not *produced*, by cellular respiration. We are tapping energy that was stored in food by photosynthesis, which captures light and converts it to chemical energy, a process you will learn about next, in Chapter 10.

CONCEPT CHECK 9.6

1. **MAKE CONNECTIONS** Compare the structures of a carbohydrate and a fat (see Figures 5.3 and 5.9). What features make fat a much better fuel?
2. Under what circumstances might your body synthesize fat molecules?
3. **VISUAL SKILLS** What will happen in a muscle cell that has used up its supply of O_2 and ATP? (Review Figures 9.17 and 9.19.)
4. **VISUAL SKILLS** During intense exercise, can a muscle cell use fat as a concentrated source of chemical energy? Explain. (Review Figures 9.17 and 9.18.)

For suggested answers, see Appendix A.

9 Chapter Review

Go to **Mastering Biology** for Assignments, the eText, the Study Area, and Dynamic Study Modules.

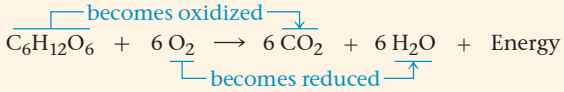
SUMMARY OF KEY CONCEPTS

To review key terms, go to the **Vocabulary Self-Quiz** in the **Mastering Biology** eText or Study Area, or go to goo.gl/zkjz9t.

CONCEPT 9.1

Catabolic pathways yield energy by oxidizing organic fuels *(pp. 165–169)*

- Cells break down glucose and other organic fuels to yield chemical energy in the form of ATP. **Fermentation** is a process that results in the partial degradation of glucose without the use of oxygen (O_2). The process of **cellular respiration** is a more complete breakdown of glucose. In **aerobic respiration**, O_2 is used as a reactant; in *anaerobic respiration*, other substances are used in place of O_2.
- The cell taps the energy stored in food molecules through **redox reactions**, in which one substance partially or totally shifts electrons to another. **Oxidation** is the total or partial loss of electrons, while **reduction** is the total or partial addition of electrons. During aerobic respiration, glucose ($C_6H_{12}O_6$) is oxidized to CO_2, and O_2 is reduced to H_2O:



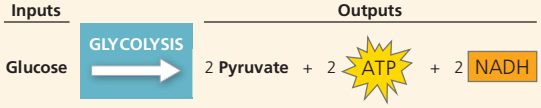
- Electrons lose potential energy during their transfer from glucose or other organic compounds to O_2. Electrons are usually passed first to **NAD^+**, reducing it to **NADH**, and are then passed from NADH to an **electron transport chain**, which conducts the electrons to O_2 in energy-releasing steps. The energy that is released is used to make ATP.
- Aerobic respiration occurs in three stages: (1) **glycolysis**, (2) pyruvate oxidation and the **citric acid cycle**, and (3) **oxidative phosphorylation** (electron transport and chemiosmosis).

? *Describe the difference between the two processes in cellular respiration that produce ATP: oxidative phosphorylation and substrate-level phosphorylation.*

CONCEPT 9.2

Glycolysis harvests chemical energy by oxidizing glucose to pyruvate *(pp. 170–171)*

- Glycolysis ("splitting of sugar") is a series of reactions that breaks down glucose into two pyruvate molecules, which may go on to enter the citric acid cycle, and nets 2 ATP and 2 NADH per glucose molecule.

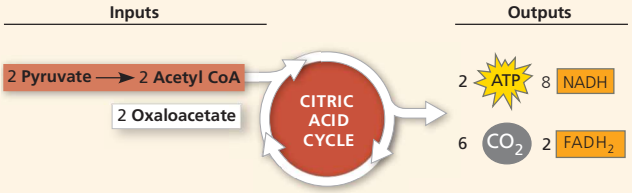


? *Which reactions in glycolysis are the source of energy for the formation of ATP and NADH?*

CONCEPT 9.3

After pyruvate is oxidized, the citric acid cycle completes the energy-yielding oxidation of organic molecules *(pp. 171–174)*

- In eukaryotic cells, pyruvate enters the mitochondrion and is oxidized to **acetyl CoA**, which is further oxidized in the citric acid cycle.

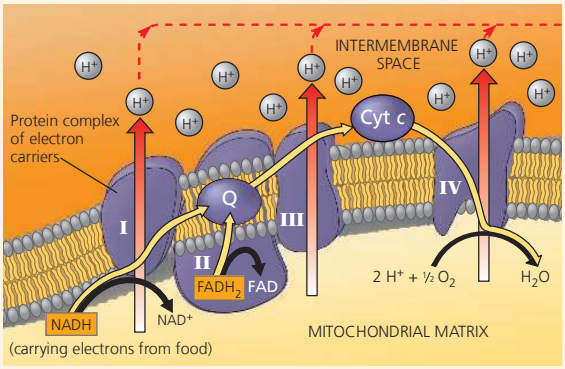


? *What molecular products indicate the complete oxidation of glucose during cellular respiration?*

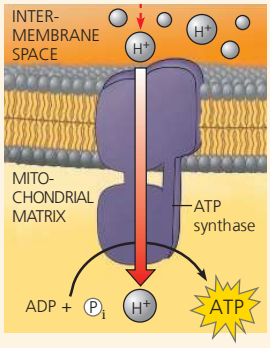
CONCEPT 9.4

During oxidative phosphorylation, chemiosmosis couples electron transport to ATP synthesis *(pp. 174–179)*

- NADH and $FADH_2$ transfer electrons to the electron transport chain. Electrons move down the chain, losing energy in several energy-releasing steps. Finally, electrons are passed to O_2, reducing it to H_2O.



- Along the electron transport chain, electron transfer causes protein complexes to move H^+ from the mitochondrial matrix (in eukaryotes) to the intermembrane space, storing energy as a **proton-motive force** (H^+ gradient). As H^+ diffuses back into the matrix through **ATP synthase**, its passage drives the phosphorylation of ADP to form ATP, called **chemiosmosis**.
- About 34% of the energy stored in a glucose molecule is transferred to ATP during cellular respiration, producing a maximum of about 32 ATP.



? *Briefly explain the mechanism by which ATP synthase produces ATP. List three locations in which ATP synthases are found.*

CONCEPT 9.5

Fermentation and anaerobic respiration enable cells to produce ATP without the use of oxygen *(pp. 179–182)*

- Glycolysis nets 2 ATP by substrate-level phosphorylation, whether O_2 is present or not. Under anaerobic conditions, anaerobic respiration or fermentation can take place. In anaerobic respiration, an electron transport chain is present with a final electron acceptor other than oxygen. In fermentation, the electrons from NADH are passed to pyruvate or a derivative of pyruvate, regenerating the NAD^+ required to oxidize more glucose. Two common types of fermentation are **alcohol fermentation** and **lactic acid fermentation**.
- Fermentation, anaerobic respiration, and aerobic respiration all use glycolysis to oxidize glucose, but they differ in their final electron acceptor and whether an electron transport chain is used (respiration) or not (fermentation). Respiration yields more ATP; aerobic respiration, with O_2 as the final electron acceptor, yields about 16 times as much ATP as does fermentation.
- Glycolysis occurs in nearly all organisms and is thought to have evolved in ancient prokaryotes before there was O_2 in the atmosphere.

? *Which process yields more ATP, fermentation or anaerobic respiration? Explain.*

CONCEPT 9.6

Glycolysis and the citric acid cycle connect to many other metabolic pathways *(pp. 182–184)*

- Catabolic pathways funnel electrons from many kinds of organic molecules into cellular respiration. Many carbohydrates can enter glycolysis, most often after conversion to glucose. Amino acids of proteins must be deaminated before being oxidized. The fatty acids of fats undergo **beta oxidation** to two-carbon fragments and then enter the citric acid cycle as acetyl CoA. Anabolic pathways can use small molecules from food directly or build other substances using intermediates of glycolysis or the citric acid cycle.
- Cellular respiration is controlled by allosteric enzymes at key points in glycolysis and the citric acid cycle.

? *Describe how the catabolic pathways of glycolysis and the citric acid cycle intersect with anabolic pathways in the metabolism of a cell.*

TEST YOUR UNDERSTANDING

For more multiple-choice questions, go to the **Practice Test** in the **Mastering Biology** eText or Study Area, or go to goo.gl/GruWRg.

Levels 1-2: Remembering/Understanding

1. The *immediate* energy source that drives ATP synthesis by ATP synthase during oxidative phosphorylation is the
   (A) oxidation of glucose and other organic compounds.
   (B) flow of electrons down the electron transport chain.
   (C) H^+ concentration gradient across the membrane holding ATP synthase.
   (D) transfer of phosphate to ADP.
2. Which metabolic pathway is common to both fermentation and cellular respiration of a glucose molecule?
   (A) the citric acid cycle
   (B) the electron transport chain
   (C) glycolysis
   (D) reduction of pyruvate to lactate

3. The final electron acceptor of the electron transport chain that functions in aerobic oxidative phosphorylation is
   (A) O_2.
   (B) water.
   (C) NAD^+.
   (D) pyruvate.

4. In mitochondria, exergonic redox reactions
   (A) are the source of energy driving prokaryotic ATP synthesis.
   (B) provide the energy that establishes the proton gradient.
   (C) reduce carbon atoms to carbon dioxide.
   (D) are coupled via phosphorylated intermediates to endergonic processes.

Levels 3-4: Applying/Analyzing

5. What is the oxidizing agent in the following reaction?

$$\text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{Lactate} + NAD^+$$

   (A) oxygen
   (B) NADH
   (C) lactate
   (D) pyruvate

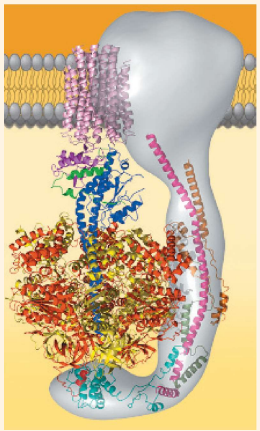
6. When electrons flow along the electron transport chains of mitochondria, which of the following changes occurs?
   (A) The pH of the matrix increases.
   (B) ATP synthase pumps protons by active transport.
   (C) The electrons gain free energy.
   (D) NAD^+ is oxidized.

7. Most CO_2 from catabolism is released during
   (A) glycolysis.
   (B) the citric acid cycle.
   (C) lactate fermentation.
   (D) electron transport.

8. **MAKE CONNECTIONS** Step 3 in Figure 9.8 is a major point of regulation of glycolysis. The enzyme phosphofructokinase is allosterically regulated by ATP and related molecules (see Concept 8.5). Considering the overall result of glycolysis, would you expect ATP to inhibit or stimulate activity of this enzyme? Explain. (Hint: Make sure you consider the role of ATP as an allosteric regulator, not as a substrate of the enzyme.)

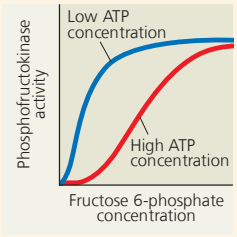
9. **MAKE CONNECTIONS** The proton pump shown in Figures 7.18 and 7.19 is a type of ATP synthase like that in Figure 9.13. Compare the processes shown in the three figures, and say whether they are involved in active or passive transport (see also Concepts 7.3 and 7.4).

10. **VISUAL SKILLS** This computer model shows the four parts of ATP synthase, each part consisting of a number of polypeptide subunits (the solid gray part is still an area of active research). Using Figure 9.13 as a guide, label the rotor, stator, internal rod, and catalytic knob of this molecular motor.

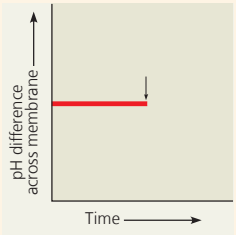


Levels 5-6: Evaluating/Creating

11. **INTERPRET THE DATA** Phosphofructokinase is an enzyme that acts on fructose 6-phosphate at an early step in glucose breakdown. Regulation of this enzyme controls whether the sugar will continue on in the glycolytic pathway. Considering this graph, under which condition is phosphofructokinase more active? Given what you know about glycolysis and regulation of metabolism by this enzyme, explain the mechanism by which phosphofructokinase activity differs depending on ATP concentration. Explain why it makes sense that regulation of this enzyme has evolved so that it works this way.



12. **DRAW IT** The graph here shows the pH difference across the inner mitochondrial membrane over time in an actively respiring cell. At the time indicated by the vertical arrow, a metabolic poison is added that specifically and completely inhibits all function of mitochondrial ATP synthase. Draw what you would expect to see for the rest of the graphed line over the next short period of time. Explain.



13. **EVOLUTION CONNECTION** ATP synthases are found in the prokaryotic plasma membrane and in mitochondria and chloroplasts. (a) Propose a hypothesis to account for an evolutionary relationship of these eukaryotic organelles and prokaryotes. (b) Explain how the amino acid sequences of the ATP synthases from the different sources might either support or fail to support your hypothesis.

14. **SCIENTIFIC INQUIRY** In the 1930s, some physicians prescribed low doses of a compound called dinitrophenol (DNP) to help patients lose weight. This unsafe method was abandoned after some patients died. DNP uncouples the chemiosmotic machinery by making the lipid bilayer of the inner mitochondrial membrane leaky to H^+. Explain how this could cause weight loss and death.

15. **WRITE ABOUT A THEME: ORGANIZATION** In a short essay (100–150 words), explain how oxidative phosphorylation—production of ATP using energy from the redox reactions of a spatially organized electron transport chain followed by chemiosmosis—is an example of how new properties emerge at each level of the biological hierarchy.

16. **SYNTHESIZE YOUR KNOWLEDGE**

Coenzyme Q (CoQ) is sold as a nutritional supplement. One company uses this marketing slogan for CoQ: "Give your heart the fuel it craves most." Considering the role of coenzyme Q, critique this claim. How do you think this product might function to benefit the heart? Is CoQ used as a "fuel" during cellular respiration?

For selected answers, see Appendix A.

10 Photosynthesis

KEY CONCEPTS

10.1 **Photosynthesis feeds the biosphere** *p. 188*

10.2 **Photosynthesis converts light energy to the chemical energy of food** *p. 189*

10.3 **The light reactions convert solar energy to the chemical energy of ATP and NADPH** *p. 192*

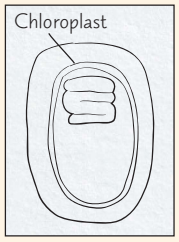
10.4 **The Calvin cycle uses the chemical energy of ATP and NADPH to reduce CO_2 to sugar** *p. 201*

10.5 **Alternative mechanisms of carbon fixation have evolved in hot, arid climates** *p. 203*

10.6 **Photosynthesis is essential for life on Earth: a review** *p. 206*

Study Tip

Make a visual study guide: Draw a cell with a large chloroplast, labeling the parts of the chloroplast. As you go through the chapter, add key reactions for each stage of photosynthesis, linking the stages together. Label the carbon molecule(s) with the most energy and the carbon molecule(s) with the least energy.



Go to Mastering Biology

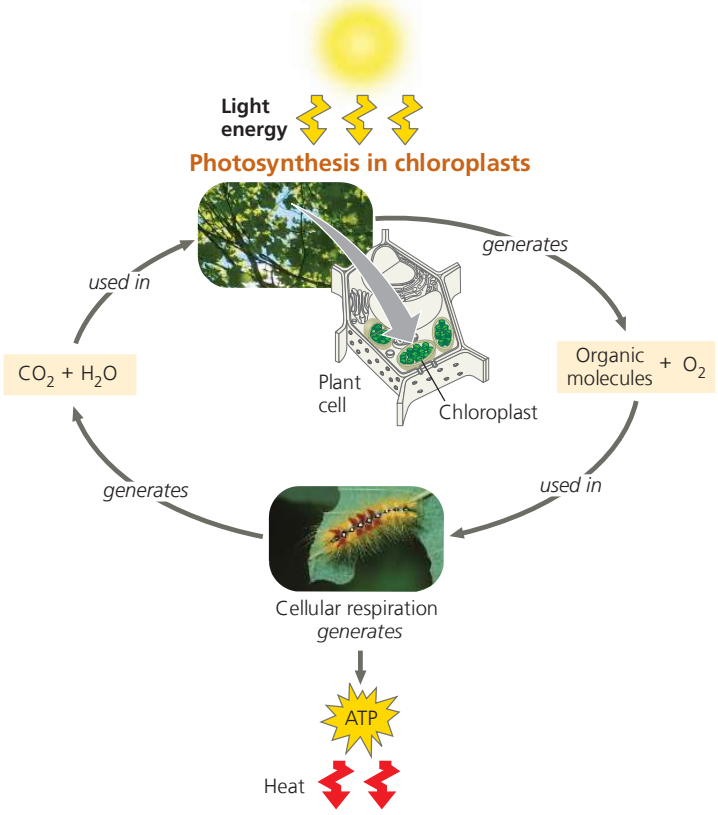
For Students (in eText and Study Area)
- Get Ready for Chapter 10
- BioFlix® Animation: Photosynthesis
- Figure 10.13 Walkthrough: How Linear Electron Flow During the Light Reactions Generates ATP and NADPH

For Instructors to Assign (in Item Library)
- Activity: The Light Reactions
- BioFlix® Tutorial: Photosynthesis

Ready-to-Go Teaching Module (in Instructor Resources)
- The Light Reactions (Concept 10.3)

Figure 10.1 Each leaf of this tree is harvesting energy from sunlight and using it to convert CO_2 and H_2O into chemical energy stored in sugar and other organic molecules. The tree uses these sugars for energy and to build its own trunk, branches, and leaves. Remarkably, enough sugars are left over to feed other organisms (like moth larvae, shown below) that cannot carry out this extraordinary transformation.

How do photosynthetic cells use light to change carbon dioxide and water into organic molecules and oxygen?



CONCEPT 10.1

Photosynthesis feeds the biosphere

The conversion process that transforms the energy of sunlight into chemical energy stored in sugars and other organic molecules is called **photosynthesis**. Let's begin by placing photosynthesis in its ecological context.

Photosynthesis nourishes almost the entire living world directly or indirectly. An organism acquires the organic compounds it uses for energy and carbon skeletons by one of two major modes: autotrophic nutrition or heterotrophic nutrition. **Autotrophs** are "self-feeders" (*auto-* means "self," and *trophos* means "feeder"); they sustain themselves without eating anything derived from other living beings. Autotrophs produce their organic molecules from CO_2 and other inorganic raw materials obtained from the environment. They are the ultimate sources of organic compounds for all nonautotrophic organisms, and for this reason, biologists refer to autotrophs as the *producers* of the biosphere.

Almost all plants are autotrophs; the only nutrients they require are water and minerals from the soil and CO_2 from the air. Specifically, plants are *photo*autotrophs, organisms that use light as a source of energy to synthesize organic substances **(Figure 10.1)**. Photosynthesis also occurs in algae, certain other protists, and some prokaryotes **(Figure 10.2)**. In this chapter, we will touch on these other groups in passing, but our emphasis will be on plants. Variations in autotrophic nutrition that occur in prokaryotes and algae will be described in Concept 27.3.

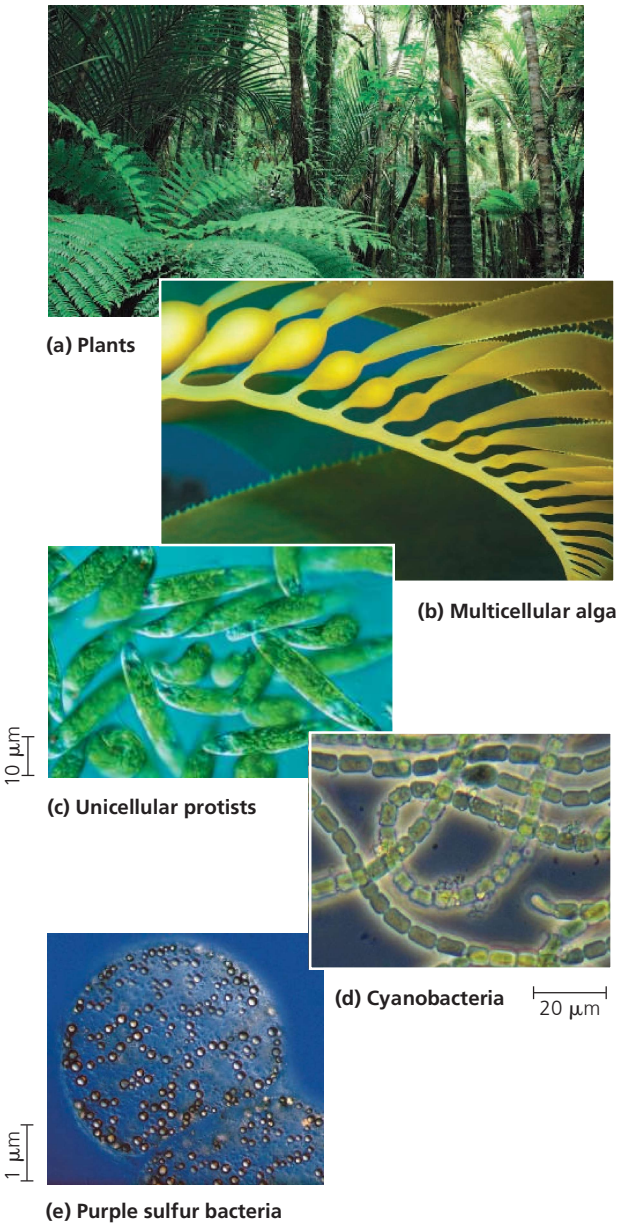
Heterotrophs are unable to make their own food; they live on compounds produced by other organisms (*hetero-* means "other"). Heterotrophs are the biosphere's *consumers*. The most obvious "other-feeding" occurs when an animal eats plants or other organisms, but heterotrophic nutrition may be more subtle. Some heterotrophs consume the remains of other organisms and organic litter such as feces, and fallen leaves; these types of heterotrophs are known as *decomposers*. Most fungi and many types of prokaryotes get their nourishment this way. Earth's supply of fossil fuels was formed from remains of organisms that died hundreds of millions of years ago. In a sense, then, fossil fuels represent stores of the sun's energy from the distant past. Almost all heterotrophs, including humans, are completely dependent, either directly or indirectly, on photoautotrophs for food—and also for oxygen, a by-product of photosynthesis.

CONCEPT CHECK 10.1

1. Why are heterotrophs dependent on autotrophs?
2. **WHAT IF?** Fossil fuels are being depleted faster than they are replenished. Therefore, researchers are developing ways to produce "biodiesel" from the products of photosynthetic algae. They have proposed placing containers of these algae near industrial plants or highly congested city streets. Considering the process of photosynthesis, how does this arrangement make sense?

For suggested answers, see Appendix A.

▼ Figure 10.2 Photoautotrophs. These organisms use light energy to drive the synthesis of organic molecules from carbon dioxide and (in most cases) water. They feed themselves and the entire living world. On land, **(a)** plants are the predominant producers of food. In aquatic environments, photoautotrophs include unicellular and **(b)** multicellular algae, such as this kelp; **(c)** some non-algal unicellular protists, such as *Euglena*; **(d)** the prokaryotes called cyanobacteria; and **(e)** other photosynthetic prokaryotes, such as these purple sulfur bacteria, which produce sulfur (the yellow globules within the cells) (c–e, LMs).



Mastering Biology **BioFlix® Animation: The Flow of Carbon Atoms in Producers, Consumers, and Decomposers**

CONCEPT 10.2

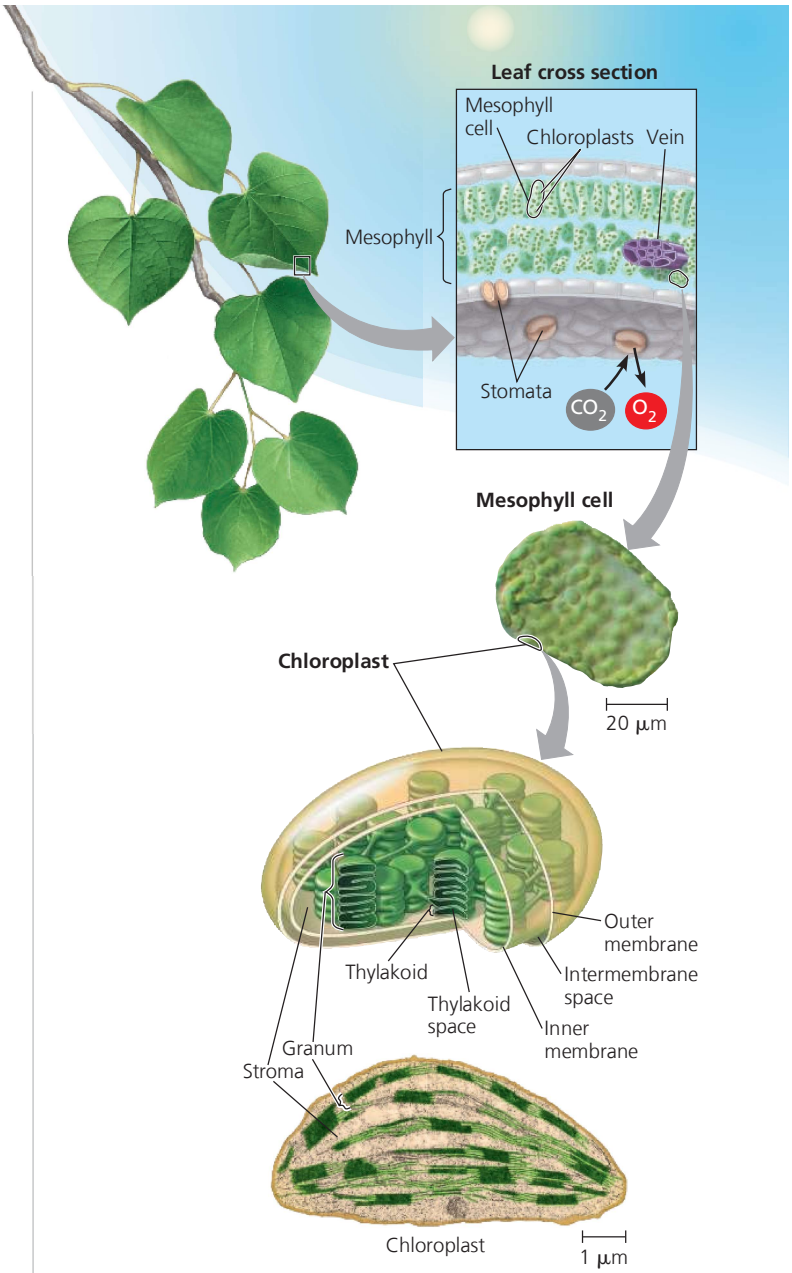
Photosynthesis converts light energy to the chemical energy of food

The remarkable ability of an organism to harness light energy and use it to drive the synthesis of organic compounds emerges from structural organization in the cell: Photosynthetic enzymes and other molecules are grouped together as specialized molecular complexes in a biological membrane, enabling the necessary series of chemical reactions to be carried out efficiently. The process of photosynthesis most likely originated in a group of bacteria that had infolded regions of the plasma membrane containing clusters of such molecules. In existing photosynthetic bacteria, infolded photosynthetic membranes function similarly to the internal membranes of the **chloroplast**, the eukaryotic organelle that absorbs energy from sunlight and uses it to drive the synthesis of organic compounds from carbon dioxide (CO_2) and water (H_2O). According to what has come to be known as the *endosymbiont theory*, the original chloroplast was a photosynthetic prokaryote that lived inside an ancestor of eukaryotic cells. (You learned about this theory in Concept 6.5, and it will be described more fully in Concept 25.3.) Chloroplasts are present in a variety of photosynthesizing organisms (see some examples in Figure 10.2); in this chapter we focus on chloroplasts in plants, while mentioning prokaryotes from time to time.

Chloroplasts: The Sites of Photosynthesis in Plants

All green parts of a plant, including green stems and unripened fruit, have chloroplasts, but the leaves are the major sites of photosynthesis in most plants **(Figure 10.3)**. There are about half a million chloroplasts in a chunk of leaf with a top surface area of 1 mm^2. Chloroplasts are found mainly in the cells of the **mesophyll**, the tissue in the interior of the leaf. CO_2 enters the leaf, and O_2 exits, by way of microscopic pores called **stomata** (singular, *stoma*; from the Greek, meaning "mouth"). Water absorbed by the roots is delivered to the leaves in veins. Leaves also use veins to export sugar to roots and other nonphotosynthetic parts of the plant.

A typical mesophyll cell has about 30–40 chloroplasts, each measuring about 2–4 µm by 4–7 µm. A chloroplast has two membranes surrounding a dense fluid called the **stroma**. Suspended within the stroma is a third membrane system, made up of sacs called **thylakoids**, which segregates the stroma from the *thylakoid space* inside these sacs. In some places, thylakoid sacs are stacked in columns called *grana* (singular, *granum*). **Chlorophyll**, the green pigment that gives leaves their color, resides in the thylakoid membranes of the chloroplast. (The internal photosynthetic membranes of some prokaryotes are also called thylakoid membranes; see Figure 27.8b.) It is the light energy absorbed by chlorophyll that drives the synthesis of organic molecules in the chloroplast. Now that we have looked at the sites of photosynthesis in plants, we are ready to look more closely at the process itself.



▲ **Figure 10.3 Zooming in on the location of photosynthesis in a plant.** Leaves are the major organs of photosynthesis in plants. These images take you into a leaf, then into a cell, and finally into a chloroplast, the organelle where photosynthesis occurs (middle, LM; bottom, TEM).

Mastering Biology Video: Chloroplasts in Motion

Tracking Atoms Through Photosynthesis

Scientists have tried for centuries to piece together the process by which plants make food. Although some of the steps are still not completely understood, the overall photosynthetic equation has been known since the 1800s: In the presence of light, the green parts of plants produce organic compounds and O_2 from CO_2

and H_2O. We can summarize the complex series of chemical reactions in photosynthesis with this chemical equation:

$$6\,CO_2 + 12\,H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\,O_2 + 6\,H_2O$$

We use glucose ($C_6H_{12}O_6$) here to simplify the relationship between photosynthesis and respiration, but the direct product of photosynthesis is actually a three-carbon sugar that can be used to make glucose. Water appears on both sides of the equation because 12 molecules are consumed and 6 molecules are newly formed during photosynthesis. We can simplify the equation by indicating only the net consumption of water:

$$6\,CO_2 + 6\,H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\,O_2$$

Writing the equation in this form, we can see that the overall chemical change during photosynthesis is the reverse of the one that occurs during cellular respiration (see Concept 9.1). It is important to realize that *both* of these metabolic processes occur in plant cells. However, as you will soon learn, chloroplasts do not synthesize sugars by simply reversing the steps of respiration.

Now let's divide the photosynthetic equation by 6 to put it in its simplest possible form:

$$CO_2 + H_2O \rightarrow [CH_2O] + O_2$$

Here, the brackets indicate that CH_2O is not an actual sugar but represents the general formula for a carbohydrate (see Concept 5.2). In other words, we are imagining the synthesis of a sugar molecule one carbon at a time (with six repetitions theoretically adding up to a glucose molecule: $C_6H_{12}O_6$). Let's now see how researchers tracked the elements C, H, and O from the reactants of photosynthesis to the products.

The Splitting of Water: Scientific Inquiry

One of the first clues to the mechanism of photosynthesis came from the discovery that the O_2 given off by plants is derived from H_2O and not from CO_2. The chloroplast splits water into hydrogen and oxygen atoms. Before this discovery, the prevailing hypothesis was that photosynthesis split carbon dioxide ($CO_2 \rightarrow C + O_2$) and then added water to the carbon ($C + H_2O \rightarrow [CH_2O]$). That hypothesis predicted that the O_2 released during photosynthesis came from CO_2, an idea challenged in the 1930s by C. B. van Niel, of Stanford University. Van Niel was investigating photosynthesis in bacteria that make their carbohydrate from CO_2 but do not release O_2. He concluded that, at least in these bacteria, CO_2 is not split into carbon and oxygen. One group of bacteria used hydrogen sulfide (H_2S) rather than water for photosynthesis, forming yellow globules of sulfur as a waste product (these globules are visible in **Figure 10.2e**). Here is the chemical equation for photosynthesis in these sulfur bacteria:

$$CO_2 + 2\,H_2S \rightarrow [CH_2O] + H_2O + 2\,S$$

Van Niel reasoned that the bacteria split H_2S and used the hydrogen atoms to make sugar. He then generalized that idea, proposing that all photosynthetic organisms require a hydrogen source but that the source varies:

$$\text{Sulfur bacteria: } CO_2 + 2\,H_2S \rightarrow [CH_2O] + H_2O + 2\,S$$

$$\text{Plants: } CO_2 + 2\,H_2O \rightarrow [CH_2O] + H_2O + O_2$$

$$\text{General: } CO_2 + 2\,H_2X \rightarrow [CH_2O] + H_2O + 2\,X$$

Thus, van Niel hypothesized that plants split H_2O as a source of electrons from hydrogen atoms, releasing O_2 as a by-product.

Nearly 20 years later, scientists confirmed van Niel's hypothesis by using oxygen-18 (^{18}O), a heavy isotope, as a tracer to follow the path of oxygen atoms during photosynthesis. The experiments showed that the O_2 produced by plants was labeled with ^{18}O *only* if water was the source of the tracer (experiment 1). If the ^{18}O was introduced to the plant in the form of CO_2, the label did not turn up in the released O_2 (experiment 2). In the following summary, green denotes labeled atoms of oxygen (^{18}O):

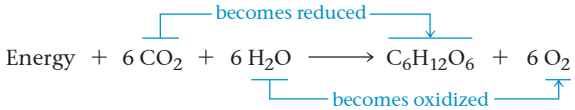
$$\text{Experiment 1: } CO_2 + 2\,H_2\mathbf{O} \rightarrow [CH_2O] + H_2O + \mathbf{O_2}$$

$$\text{Experiment 2: } C\mathbf{O_2} + 2\,H_2O \rightarrow [CH_2\mathbf{O}] + H_2\mathbf{O} + O_2$$

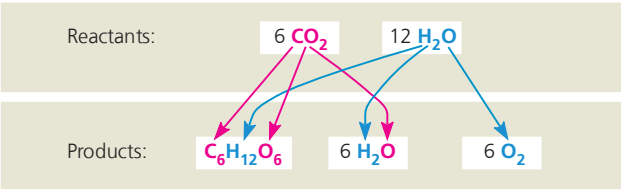
A significant result of the shuffling of atoms during photosynthesis is the extraction of hydrogen from water and its incorporation into sugar. The waste product of photosynthesis, O_2, is released to the atmosphere. **Figure 10.4** shows the fates of all atoms in photosynthesis.

Photosynthesis as a Redox Process

Let's briefly compare photosynthesis with cellular respiration. Both processes involve redox reactions. During cellular respiration, energy is released from sugar when electrons associated with hydrogen are transported by carriers to oxygen, forming water as a by-product (see Concept 9.1). The electrons lose potential energy as they "fall" down the electron transport chain toward electronegative oxygen, and the mitochondrion harnesses that energy to synthesize ATP (see Figure 9.14). Photosynthesis reverses the direction of electron flow. Water is split, and its electrons are transferred along with hydrogen ions (H^+) from the water to carbon dioxide, reducing it to sugar.



▼ Figure 10.4 Tracking atoms through photosynthesis. The atoms from CO_2 are shown in magenta, and the atoms from H_2O are shown in blue.



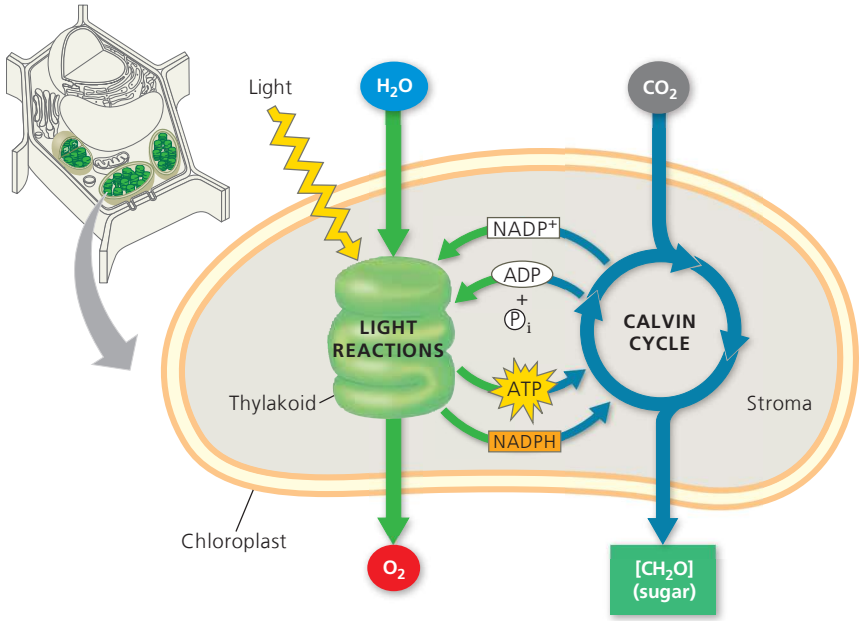
Because the electrons increase in potential energy as they move from water to sugar, this process requires energy—in other words, it is endergonic. This energy boost that occurs during photosynthesis is provided by light.

The Two Stages of Photosynthesis: *A Preview*

The equation for photosynthesis is a deceptively simple summary of a very complex process. Actually, photosynthesis is not a single process, but two processes, each of which has multiple steps. These two stages of photosynthesis are known as the **light reactions** (the *photo* part of photosynthesis) and the **Calvin cycle** (the *synthesis* part) **(Figure 10.5)**.

The light reactions are the steps of photosynthesis that convert solar energy to chemical energy. Water is split, providing a source of electrons and protons (hydrogen ions, H^+) and giving off O_2 as a by-product. Light absorbed by chlorophyll drives a transfer of the electrons and hydrogen ions from water to an acceptor called **NADP$^+$** (nicotinamide adenine dinucleotide phosphate), where they are temporarily stored. (The electron acceptor $NADP^+$ is first cousin to NAD^+, which functions as an electron carrier in cellular respiration; the two molecules differ only by the presence of an extra phosphate group in the $NADP^+$ molecule.) The light reactions use solar energy to reduce $NADP^+$ to **NADPH** by adding a pair of electrons along with an H^+. The light reactions also generate ATP, using chemiosmosis to power the addition of a phosphate group to ADP, a process called **photophosphorylation**. Thus, light energy is initially converted to chemical energy in the form of two compounds: NADPH and ATP. NADPH, a source of electrons, acts as "reducing power" that can be passed along to an electron acceptor, reducing it, while ATP is the versatile energy currency of cells. Notice that the light reactions produce no sugar; that happens in the second stage of photosynthesis, the Calvin cycle.

The Calvin cycle is named for Melvin Calvin, who, along with his colleagues James Bassham and Andrew Benson, began to elucidate its steps in the late 1940s. The cycle begins by incorporating CO_2 from the air into organic molecules already present in the chloroplast. This initial incorporation of carbon into organic compounds is known as **carbon fixation**. The Calvin cycle then reduces the fixed carbon to carbohydrate by the addition of electrons. The reducing power is provided by NADPH, which acquired its cargo of electrons in the light reactions. To convert CO_2 to carbohydrate, the Calvin cycle also requires chemical energy in the form of ATP, which is also generated by the light reactions. Thus, it is the Calvin cycle that makes sugar, but it can do so only with the help of the NADPH and ATP produced by the light reactions. The metabolic steps of the Calvin cycle are sometimes referred to as the dark reactions, or light-independent reactions, because none of the steps requires light *directly*. Nevertheless, the Calvin cycle in most plants occurs during daylight, for only then can the light reactions provide the NADPH and ATP that the Calvin cycle requires. In essence, the chloroplast uses light energy to make sugar by coordinating the two stages of photosynthesis.



▶ Figure 10.5 An overview of photosynthesis: cooperation of the light reactions and the Calvin cycle. In the chloroplast, the thylakoid membranes (green) are the sites of the light reactions, whereas the Calvin cycle occurs in the stroma (gray). The light reactions use solar energy to make ATP and NADPH, which supply chemical energy and reducing power, respectively, to the Calvin cycle. The Calvin cycle incorporates CO_2 into organic molecules, which are converted to sugar. (Recall that most simple sugars have formulas that are some multiple of CH_2O.) To visualize these processes in their cellular context, see Figure 6.32.

Mastering Biology
BioFlix® Animation: Photosynthesis

As Figure 10.5 indicates, the thylakoids of the chloroplast are the sites of the light reactions, while the Calvin cycle occurs in the stroma. On the outside of the thylakoids, during the light reactions, molecules of $NADP^+$ and ADP pick up electrons and phosphate, respectively, and NADPH and ATP are then released to the stroma, where they play crucial roles in the Calvin cycle. The two stages of photosynthesis are treated in this figure as metabolic modules that take in ingredients and crank out products. In the next two sections, we'll look more closely at how the two stages work, beginning with the light reactions.

CONCEPT CHECK 10.2

1. **MAKE CONNECTIONS** How do the CO_2 molecules used in photosynthesis reach and enter the chloroplasts inside leaf cells? (See Concept 7.2.)
2. Explain how the use of an oxygen isotope helped elucidate the chemistry of photosynthesis.
3. **WHAT IF?** The Calvin cycle requires ATP and NADPH, products of the light reactions. If a classmate asserted that the light reactions don't depend on the Calvin cycle and, with continual light, could just keep on producing ATP and NADPH, how would you respond?

For suggested answers, see Appendix A.

CONCEPT 10.3

The light reactions convert solar energy to the chemical energy of ATP and NADPH

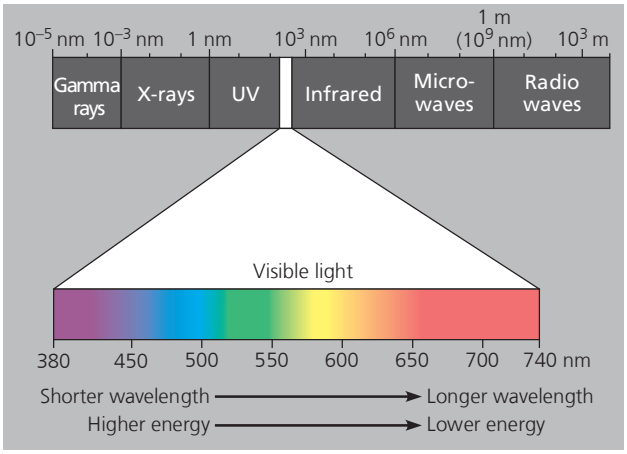
Chloroplasts are chemical factories powered by the sun. Their thylakoids transform light energy into the chemical energy of ATP and NADPH. To better understand the conversion of light to chemical energy, we need to know about some important properties of light.

The Nature of Sunlight

Light is a form of energy known as electromagnetic energy, also called electromagnetic radiation. Electromagnetic energy travels in rhythmic waves analogous to those created by dropping a pebble into a pond. Electromagnetic waves, however, are disturbances of electric and magnetic fields rather than disturbances of a material medium such as water.

The distance between the crests of electromagnetic waves is called the **wavelength**. Wavelengths range from less than a nanometer (for gamma rays) to more than a kilometer (for radio waves). This entire range of radiation is known as the **electromagnetic spectrum** (Figure 10.6). The segment most important to life is the narrow band from about 380 nm to 740 nm in wavelength. This radiation is known as

▼ **Figure 10.6 The electromagnetic spectrum.** White light is a mixture of all wavelengths of visible light. A prism can sort white light into its component colors by bending light of different wavelengths at different angles. (Droplets of water in the atmosphere can act as prisms, causing a rainbow to form.) Visible light drives photosynthesis.



visible light because it can be detected as various colors by the human eye.

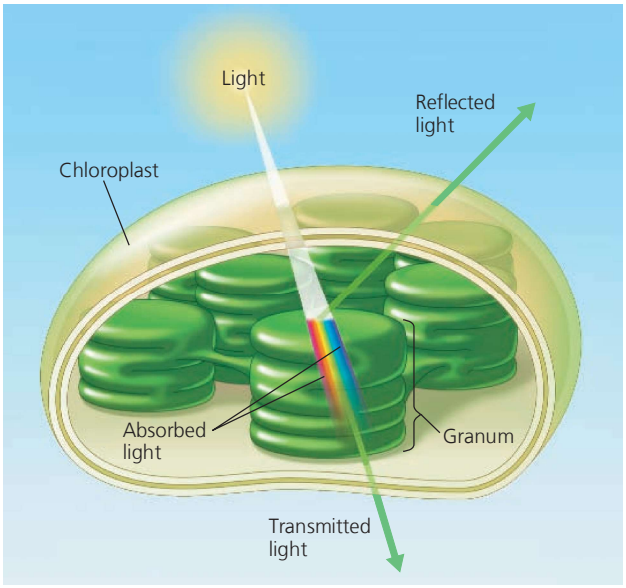
The model of light as waves explains many of light's properties, but in certain respects light behaves as though it consists of discrete particles, called **photons**. Photons are not tangible objects, but they act like objects in that each of them has a fixed quantity of energy. The amount of energy is inversely related to the wavelength of the light: The shorter the wavelength, the greater the energy of each photon of that light. Thus, a photon of violet light packs nearly twice as much energy as a photon of red light (see Figure 10.6).

Although the sun radiates the full spectrum of electromagnetic energy, the atmosphere acts like a selective window, allowing visible light to pass through while screening out a substantial fraction of other radiation. The part of the spectrum we can see—visible light—is also the radiation that drives photosynthesis.

Photosynthetic Pigments: The Light Receptors

When light meets matter, it may be reflected, transmitted, or absorbed. Substances that absorb visible light are known as *pigments*. Different pigments absorb light of different wavelengths, and the wavelengths that are absorbed disappear. If a pigment is illuminated with white light, the color we see is the color most reflected or transmitted by the pigment. (If a pigment absorbs all wavelengths, it appears black.) We see green when we look at a leaf because chlorophyll absorbs violet-blue and red light while transmitting and reflecting green light

▼ Figure 10.7 Why leaves are green: interaction of light with chloroplasts. The chlorophyll molecules of chloroplasts absorb violet-blue and red light (the colors most effective in driving photosynthesis) and reflect or transmit green light. This is why leaves appear green.



Mastering Biology
Animation: Light Energy and Pigments

(Figure 10.7). The ability of a pigment to absorb various wavelengths of light can be measured with an instrument called a **spectrophotometer**. This machine directs beams of light of different wavelengths through a solution of the pigment and measures the fraction of the light transmitted at each wavelength. A graph plotting a pigment's light absorption versus wavelength is called an **absorption spectrum (Figure 10.8)**.

The absorption spectra of chloroplast pigments provide clues to the relative effectiveness of different wavelengths for driving photosynthesis since light can perform work in chloroplasts only if it is absorbed. **Figure 10.9a** shows the absorption spectra of three types of pigments in chloroplasts: **chlorophyll *a***, the key light-capturing pigment that participates directly in the light reactions; the accessory pigment **chlorophyll *b***; and a separate group of accessory pigments called carotenoids. The spectrum of chlorophyll *a* suggests that violet-blue and red light work best for photosynthesis, since they are absorbed, while green is the least effective color. This is confirmed by an **action spectrum** for photosynthesis **(Figure 10.9b)**, which profiles the relative effectiveness of different wavelengths of radiation in driving the process. An action spectrum is prepared by illuminating chloroplasts with light of different colors and then plotting wavelength against some measure of photosynthetic rate, such as CO_2 consumption or O_2 release. The action spectrum for photosynthesis was first demonstrated by Theodor W. Engelmann, a German botanist, in 1883.

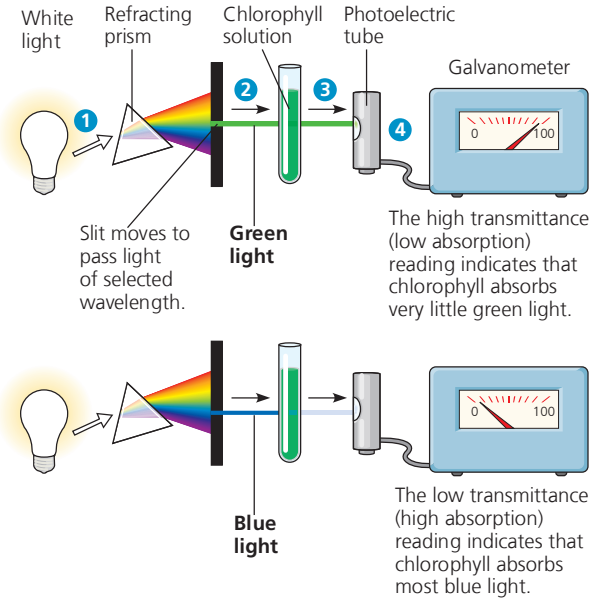
▼ Figure 10.8 Research Method

Determining an Absorption Spectrum

Application An absorption spectrum is a visual representation of how well a particular pigment absorbs different wavelengths of visible light. Absorption spectra of various chloroplast pigments help scientists decipher the role of each pigment in a plant.

Technique A spectrophotometer measures the relative amounts of light of different wavelengths absorbed and transmitted by a pigment solution.

1. White light is separated into colors (wavelengths) by a prism.
2. One by one, the different colors of light are passed through the sample (chlorophyll in this example). Green light and blue light are shown here.
3. The transmitted light strikes a photoelectric tube, which converts the light energy to electricity.
4. The electric current is measured by a galvanometer. The meter indicates the fraction of light transmitted through the sample, from which we can determine the amount of light absorbed.



Results See Figure 10.9a for absorption spectra of three types of chloroplast pigments.

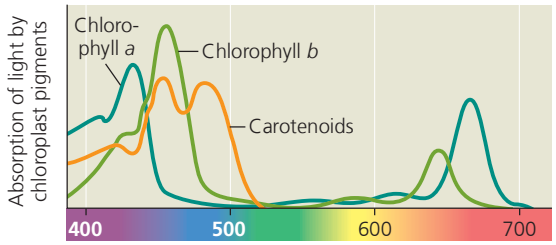
Before equipment for measuring O_2 levels had even been invented, Engelmann performed a clever experiment in which he used O_2-requiring bacteria to measure rates of photosynthesis in filamentous algae **(Figure 10.9c)**. His results are a striking match to the modern action spectrum shown in Figure 10.9b.

▼ Figure 10.9 Inquiry

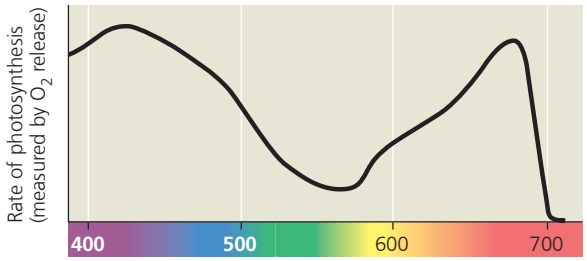
Which wavelengths of light are most effective in driving photosynthesis?

Experiment Absorption and action spectra, along with a classic experiment by Theodor W. Engelmann, reveal which wavelengths of light are photosynthetically important.

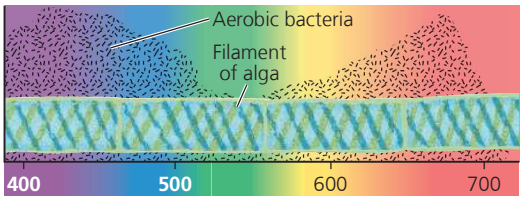
Results



(a) Absorption spectra. The three curves show the wavelengths of light best absorbed by three types of chloroplast pigments.



(b) Action spectrum. This graph plots the rate of photosynthesis versus wavelength. The resulting action spectrum resembles the absorption spectrum for chlorophyll *a* but does not match exactly (see part a). This is partly due to the absorption of light by accessory pigments such as chlorophyll *b* and carotenoids.



(c) Engelmann's experiment. In 1883, Theodor W. Engelmann shined light through a prism onto an alga, exposing different segments of the alga to different wavelengths. He used aerobic bacteria, which congregate near an oxygen source, to determine which segments of the alga were releasing the most O_2 and thus photosynthesizing most. Bacteria congregated in greatest numbers around the parts of the alga illuminated with violet-blue or red light.

Conclusion The action spectra, confirmed by Engelmann's experiment, show which portions of the spectrum are most effective in driving photosynthesis.

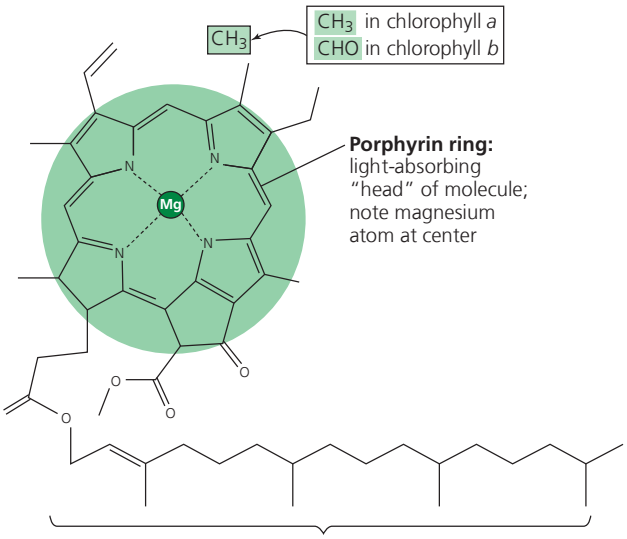
Data from T. W. Engelmann, *Bacterium photometricum*. Ein Beitrag zur vergleichenden Physiologie des Licht-und Farbensinnes, *Archiv. für Physiologie* 30:95–124 (1883).

Instructors: A related Experimental Inquiry Tutorial can be assigned in Mastering Biology.

INTERPRET THE DATA *According to the graph, which wavelengths of light drive the highest rates of photosynthesis?*

▼ Figure 10.10 Structure of chlorophyll *a* and *b*.

Chlorophyll *a* and *b* differ in only one functional group:



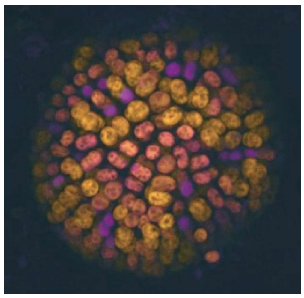
Porphyrin ring: light-absorbing "head" of molecule; note magnesium atom at center

Hydrocarbon tail: interacts with hydrophobic regions of proteins inside thylakoid membranes of chloroplasts

Mastering Biology
Animation: Space-Filling Model of Chlorophyll

Notice by comparing Figures 10.9a and 10.9b that the action spectrum for photosynthesis is much broader than the absorption spectrum of chlorophyll *a*. The absorption spectrum of chlorophyll *a* alone underestimates the effectiveness of certain wavelengths in driving photosynthesis. This is partly because accessory pigments with different absorption spectra—including chlorophyll *b* and carotenoids—broaden the spectrum of colors that can be used for photosynthesis. **Figure 10.10** shows the structure of chlorophyll *a* compared with that of chlorophyll *b*. A slight structural difference between them is enough to cause the two pigments to absorb at slightly different wavelengths in the red and blue parts of the spectrum (see Figure 10.9a). As a result, chlorophyll *a* appears blue green and chlorophyll *b* olive green under visible light.

In the last decade, researchers have identified two other forms of chlorophyll—chlorophyll *d* and chlorophyll *f*—that absorb higher wavelengths of light. In 2018, researchers grew a species of cyanobacterium called *Chroococcidiopsis thermalis*



▶ Colony of the cyanobacteria *Chroococcidiopsis thermalis*. In this micrograph of cells becoming acclimated to higher wavelengths of light, cells that haven't yet acclimated and are still using chlorophyll *a* for photosynthesis appear purplish-pink, and cells that have acclimated and are using chlorophyll *f* appear yellow.

under only far-red light with a wavelength of 750 nm. They concluded that chlorophyll *f* was functioning in place of chlorophyll *a*, enabling this cyanobacterium to flourish in very shaded conditions. Higher-wavelength light has less energy, so this observation extends the lower limit of energy (the "red limit") needed for photosynthesis to occur.

Other accessory pigments include **carotenoids**, hydrocarbons that are various shades of yellow and orange because they absorb violet and blue-green light (see Figure 10.9a). Carotenoids may broaden the spectrum of colors that can drive photosynthesis. However, a more important function of at least some carotenoids seems to be *photoprotection*: These compounds absorb and dissipate excessive light energy that would otherwise damage chlorophyll or interact with oxygen, forming reactive oxidative molecules that are dangerous to the cell. Interestingly, carotenoids similar to the photoprotective ones in chloroplasts have a photoprotective role in the human eye. (Carrots, known for aiding night vision, are rich in carotenoids.) These and related molecules are, of course, found naturally in many vegetables and fruits. They are also often advertised in health food products as "phytochemicals" (from the Greek *phyton*, plant), some of which have antioxidant properties. Plants can synthesize all the antioxidants they require, but humans and other animals must obtain some of them from their diets.

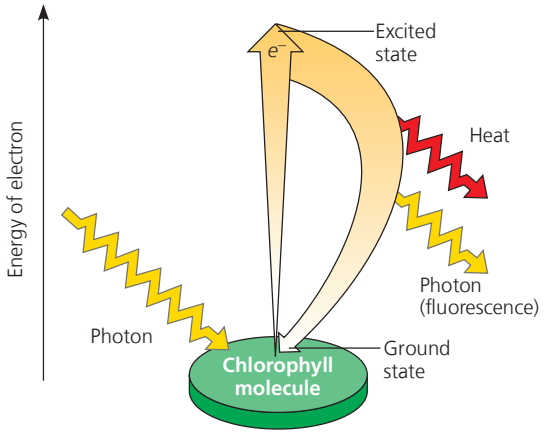
Excitation of Chlorophyll by Light

What exactly happens when chlorophyll and other pigments absorb light? The colors corresponding to the absorbed wavelengths disappear from the spectrum of the transmitted and reflected light, but energy cannot disappear. When a molecule absorbs a photon of light, one of the molecule's electrons is elevated to an orbital where it has more potential energy (see Figure 2.6b). When the electron is in its normal orbital, the pigment molecule is said to be in its ground state. Absorption of a photon boosts an electron to an orbital of higher energy, and the pigment molecule is then said to be in an excited state. The only photons absorbed are those whose energy is exactly equal to the energy difference between the ground state and an excited state, and this energy difference varies from one kind of molecule to another. Thus, a particular compound absorbs only photons corresponding to specific wavelengths, which is why each pigment has a unique absorption spectrum.

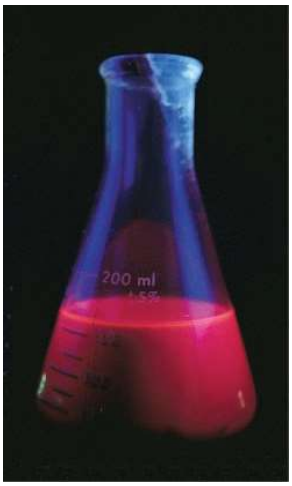
Once absorption of a photon raises an electron to an excited state, the electron cannot stay there long **(Figure 10.11a)**. The excited state, like all high-energy states, is unstable. Generally, when isolated pigment molecules absorb light, their excited electrons drop back down to the ground-state orbital in a billionth of a second, releasing their excess energy as heat. This conversion of light energy to heat is what makes the top of an automobile so hot on a sunny day. (White cars are coolest because their paint reflects all wavelengths of visible light.) In isolation, some pigments, including chlorophyll, emit light as well as heat after absorbing photons. As excited electrons fall back to the ground state, photons are given off, an afterglow called fluorescence. An illuminated solution of chlorophyll isolated from chloroplasts will fluoresce in the red part of the spectrum and also give off heat **(Figure 10.11b)**. This is best seen by illuminating with ultraviolet light, which chlorophyll can also absorb (see Figures 10.6 and 10.9a). Viewed under visible light, the fluorescence would be difficult to see against the green of the solution.

A Photosystem: A Reaction-Center Complex Associated with Light-Harvesting Complexes

Chlorophyll molecules excited by the absorption of light energy produce very different results in an intact chloroplast than they do in isolation (see Figure 10.11). In their native environment of the thylakoid membrane, chlorophyll



(a) Excitation of isolated chlorophyll molecule



(b) Fluorescence

▶ Figure 10.11 Excitation of isolated chlorophyll by light. (a) Absorption of a photon causes a transition of the chlorophyll molecule from its ground state to its excited state. The photon boosts an electron to an orbital where it has more potential energy. If the illuminated molecule exists in isolation, its excited electron immediately drops back down to the ground-state orbital, and its excess energy is given off as heat and fluorescence (light). **(b)** A chlorophyll solution excited with ultraviolet light fluoresces with a red-orange glow.

WHAT IF? *If a leaf containing the same concentration of chlorophyll as in the solution was exposed to the same ultraviolet light, no fluorescence would be seen. Propose an explanation for the difference in fluorescence emission between the solution and the leaf.*

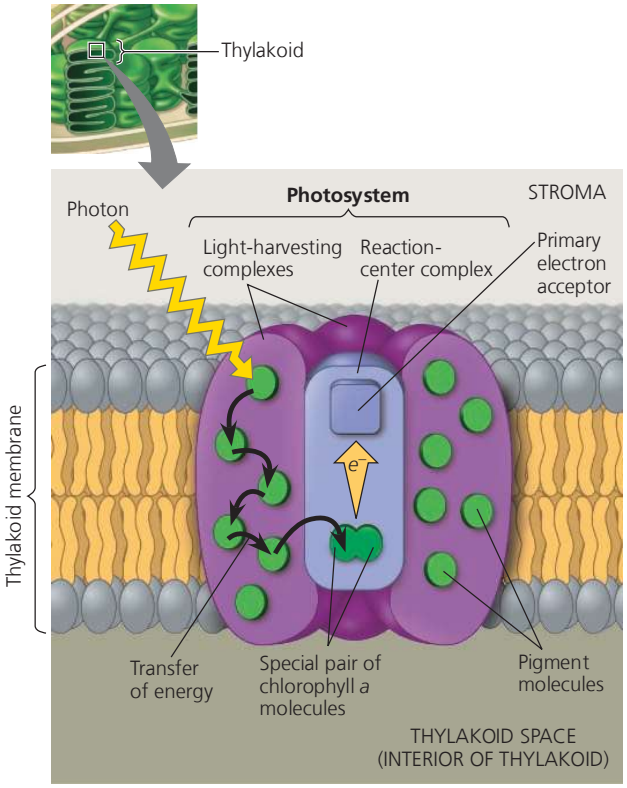
molecules are organized along with other small organic molecules and proteins into complexes called photosystems.

A **photosystem** is composed of a reaction-center complex surrounded by several light-harvesting complexes **(Figure 10.12)**. The **reaction-center complex** is an organized association of proteins holding a special pair of chlorophyll *a* molecules and a primary electron acceptor. Each **light-harvesting complex** consists of various pigment molecules (which may include chlorophyll *a*, chlorophyll *b*, and multiple carotenoids) bound to proteins. The number and variety of pigment molecules enable a photosystem to harvest light over a larger surface area and a larger portion of the spectrum than could any single pigment molecule alone. Together, these light-harvesting complexes act as an antenna for the reaction-center complex. When a pigment molecule absorbs a photon, the energy is transferred from pigment molecule to pigment molecule within a light-harvesting complex, like a human "wave" at a sports arena, until it is passed to the pair of chlorophyll *a* molecules in the reaction-center complex. This pair of chlorophyll *a* molecules is special because their molecular environment—their location and the other molecules with which they are associated—enables them to use the energy from light not only to boost one of their electrons to a higher energy level, but also to transfer it to a different molecule—the **primary electron acceptor**, which is a molecule capable of accepting electrons and becoming reduced.

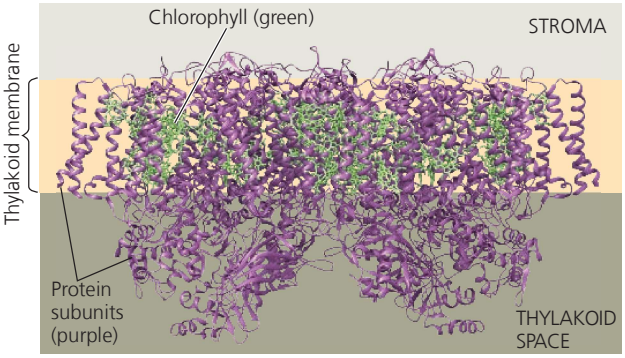
The solar-powered transfer of an electron from the reaction-center chlorophyll *a* pair to the primary electron acceptor is the first step of the light reactions. As soon as the chlorophyll electron is excited to a higher energy level, the primary electron acceptor captures it; this is a redox reaction. In the flask shown in Figure 10.11b, isolated chlorophyll fluoresces because there is no electron acceptor, so electrons of photoexcited chlorophyll drop right back to the ground state. In the structured environment of a chloroplast, however, an electron acceptor is readily available, and the potential energy represented by the excited electron is not dissipated as light and heat. Thus, each photosystem—a reaction-center complex surrounded by light-harvesting complexes—functions in the chloroplast as a unit. It converts light energy to chemical energy, which will ultimately be used for the synthesis of sugar.

The thylakoid membrane is populated by two types of photosystems that cooperate in the light reactions of photosynthesis: **photosystem II (PS II)** and **photosystem I (PS I)**. (They were named in order of their discovery, but photosystem II functions first in the light reactions.) Each has a characteristic reaction-center complex—a particular kind of primary electron acceptor next to a special pair of chlorophyll *a* molecules associated with specific proteins. The reaction-center chlorophyll *a* of photosystem II is known as P680 because this pigment is best at absorbing light having a wavelength of 680 nm (in the red part of the spectrum). The chlorophyll *a* at the reaction-center complex of photosystem I is called P700 because it most effectively absorbs light of wavelength 700 nm (in the far-red part of the spectrum). These two pigments, P680 and P700, are nearly identical chlorophyll *a* molecules. However, their association with different proteins in the thylakoid membrane affects the

▼ Figure 10.12 The structure and function of a photosystem.



(a) How a photosystem harvests light. When a photon strikes a pigment molecule in a light-harvesting complex, the energy is passed from molecule to molecule until it reaches the reaction-center complex. Here, an excited electron from the special pair of chlorophyll *a* molecules is transferred to the primary electron acceptor.



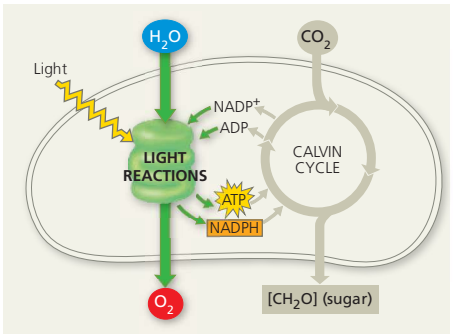
(b) Structure of a photosystem. This computer model, based on X-ray crystallography, shows two photosystem complexes side by side. Chlorophyll molecules (bright green ball-and-stick models within the membrane; the tails are not shown) are interspersed with protein subunits (purple ribbons; notice the many α helices spanning the membrane). For simplicity, a photosystem will be shown as a single complex in the rest of the chapter.

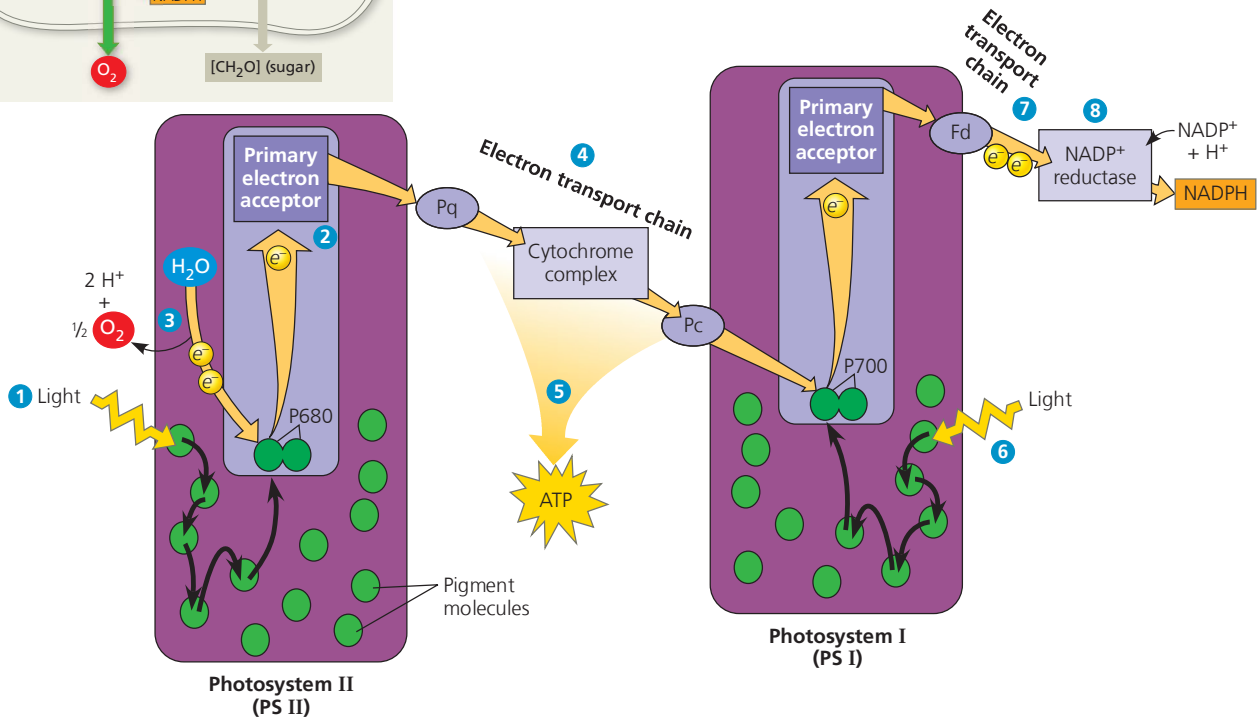
electron distribution in the two pigments and accounts for the slight differences in their light-absorbing properties. Now let's see how the two types of photosystems work together in using light energy to generate ATP and NADPH, the two main products of the light reactions.

Linear Electron Flow

Light drives the synthesis of ATP and NADPH by energizing the two types of photosystems embedded in the thylakoid membranes of chloroplasts. The key to this energy transformation is a flow of electrons through the photosystems and other molecular components built into the thylakoid membrane. This is called **linear electron flow**, and it occurs during the light reactions of photosynthesis, as shown in **Figure 10.13**. The numbered steps in the text correspond to the numbered steps in the figure.

1. A photon of light strikes one of the pigment molecules in a light-harvesting complex of PS II, boosting one of its electrons to a higher energy level. As this electron falls back to its ground state, an electron in a nearby pigment molecule is simultaneously raised to an excited state. The process continues, with the energy being relayed to other pigment molecules until it reaches the P680 pair of chlorophyll *a* molecules in the PS II reaction-center complex. It excites an electron in this pair of chlorophylls to a higher energy state.
2. This electron is transferred from the excited P680 to the primary electron acceptor. We can refer to the resulting form of P680, missing the negative charge of an electron, as $P680^+$.
3. An enzyme catalyzes the splitting of a water molecule into two electrons, two hydrogen ions (H^+), and an oxygen atom. The electrons are supplied one by one to the $P680^+$ pair, each electron replacing one transferred to the primary electron acceptor. ($P680^+$ is the strongest biological oxidizing agent known; its electron "hole" must be filled. This greatly facilitates the transfer of electrons from the split water molecule.) The H^+ are released into the thylakoid space (interior of the thylakoid). The oxygen atom immediately combines with an oxygen atom generated by the splitting of another water molecule, forming O_2.





▼ Figure 10.13 How linear electron flow during the light reactions generates ATP and NADPH. The gold arrows trace the flow of light-driven electrons from water to NADPH. The black arrows trace the transfer of energy from pigment molecule to pigment molecule. To see these proteins in their cellular context, see Figure 6.32b.

Mastering Biology Figure Walkthrough

4. Each photoexcited electron passes from the primary electron acceptor of PS II to PS I via an electron transport chain, the components of which are similar to those of the electron transport chain that functions in cellular respiration. The electron transport chain between PS II and PS I is made up of the electron carrier plastoquinone (Pq), a cytochrome complex, and a protein called plastocyanin (Pc). Each component carries out redox reactions as electrons flow down the electron transport chain, releasing free energy that is used to pump protons (H^+) into the thylakoid space, contributing to a proton gradient across the thylakoid membrane.

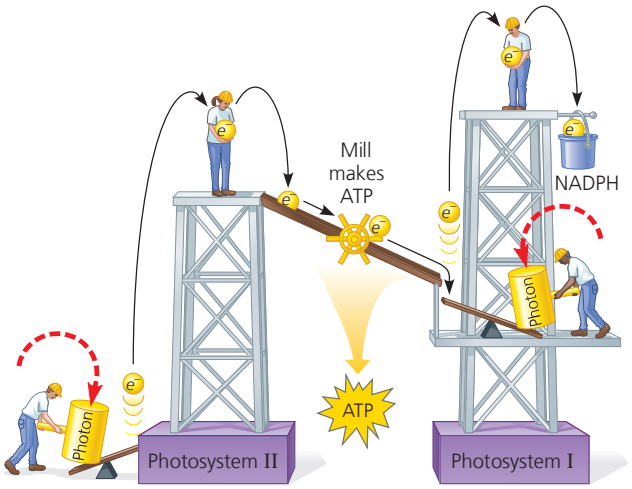
5. The potential energy stored in the proton gradient is used to make ATP in a process called chemiosmosis, to be discussed shortly.

6. Meanwhile, light energy has been transferred via light-harvesting complex pigments to the PS I reaction-center complex, exciting an electron of the P700 pair of chlorophyll *a* molecules located there. The photoexcited electron is then transferred to PS I's primary electron acceptor, creating an electron "hole" in the P700—which we now can call $P700^+$. In other words, $P700^+$ can now act as an electron acceptor, accepting an electron that reaches the bottom of the electron transport chain from PS II.

7. Photoexcited electrons are passed in a series of redox reactions from the primary electron acceptor of PS I down a second electron transport chain through the protein ferredoxin (Fd). (This chain does not create a proton gradient and thus does not produce ATP.)

8. The enzyme $NADP^+$ reductase catalyzes the transfer of electrons from Fd to $NADP^+$. Two electrons are required for its reduction to NADPH. Electrons in NADPH are at a higher energy level than they are in water (where they started), so they are more readily available for the reactions of the Calvin cycle. This process also removes an H^+ from the stroma.

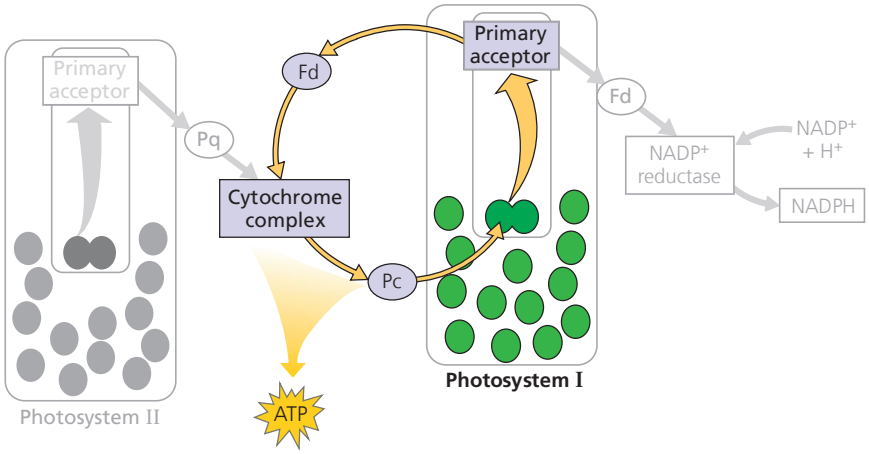
▼ Figure 10.14 A mechanical analogy for linear electron flow during the light reactions.



The energy changes of electrons during their linear flow through the light reactions are shown in a mechanical analogy in **Figure 10.14**. Although the scheme shown in Figures 10.13 and 10.14 may seem complicated, do not lose track of the big picture: The light reactions use solar power to generate ATP and NADPH, which provide chemical energy and reducing power, respectively, to the carbohydrate-synthesizing reactions of the Calvin cycle.

Cyclic Electron Flow

In certain cases, photoexcited electrons can take an alternative path called **cyclic electron flow**, which uses photosystem I but not photosystem II. You can see in **Figure 10.15** that cyclic flow is a short circuit: The electrons cycle back from ferredoxin (Fd) to the cytochrome complex, then via a plastocyanin molecule (Pc) to a P700 chlorophyll in the PS I reaction-center complex. There is



◀ Figure 10.15 Cyclic electron flow. Photoexcited electrons from PS I are occasionally shunted back from ferredoxin (Fd) to chlorophyll via the cytochrome complex and plastocyanin (Pc). This electron shunt supplements the supply of ATP (via chemiosmosis) but produces no NADPH. The "shadow" of linear electron flow is included in the diagram for comparison with the cyclic route. The two Fd molecules in this diagram are actually one and the same—the final electron carrier in the electron transport chain of PS I—although it is depicted twice to clearly show its role in two parts of the process.

VISUAL SKILLS *Look at Figure 10.14 and explain how you would alter it to show a mechanical analogy for cyclic electron flow.*

no production of NADPH and no release of oxygen that results from this process. On the other hand, cyclic flow does generate ATP.

Rather than having both PS II and PS I, several of the currently existing groups of photosynthetic bacteria are known to have a single photosystem related to either PS II or PS I. For these species, which include the purple sulfur bacteria (see Figure 10.2e) and the green sulfur bacteria, cyclic electron flow is the one and only means of generating ATP during the process of photosynthesis. Evolutionary biologists hypothesize that these bacterial groups are descendants of ancestral bacteria in which photosynthesis first evolved, in a form similar to cyclic electron flow.

Cyclic electron flow can also occur in photosynthetic species that possess both photosystems; this includes some prokaryotes, such as the cyanobacteria shown in Figure 10.2d, as well as the eukaryotic photosynthetic species that have been tested thus far. Although the process is probably in part an "evolutionary leftover," research suggests it plays at least one beneficial role for these organisms. Plants with mutations that render them unable to carry out cyclic electron flow are capable of growing well in low light, but do not grow well where light is intense. This is evidence for the idea that cyclic electron flow may be photoprotective. Later you'll learn more about cyclic electron flow as it relates to a particular adaptation of photosynthesis (C_4 plants; see Concept 10.5).

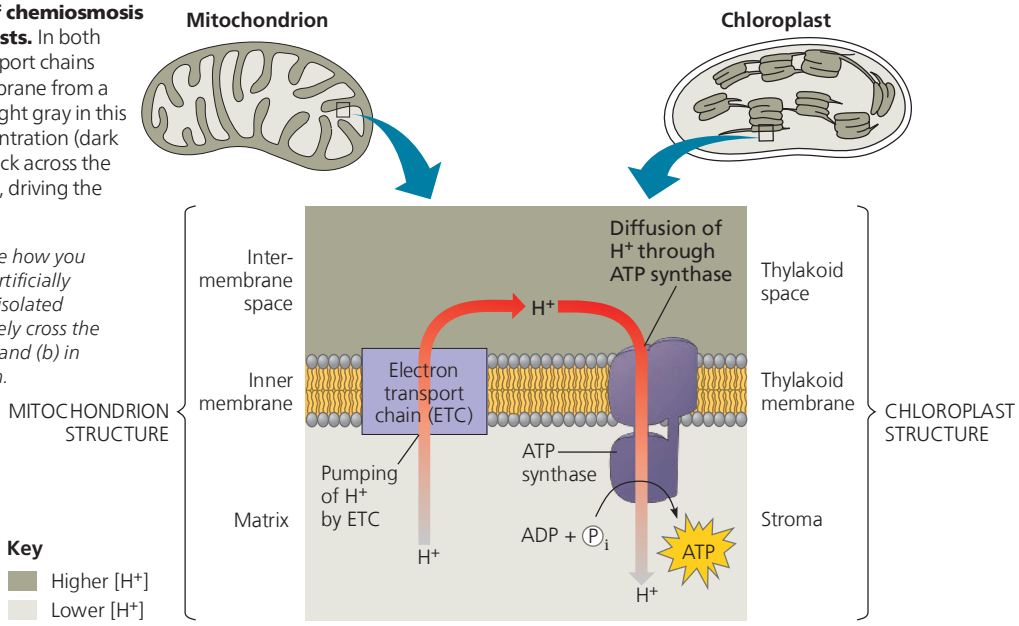
Whether ATP synthesis is driven by linear or cyclic electron flow, the actual mechanism is the same. Before we move on to consider the Calvin cycle, let's review chemiosmosis, the process that uses membranes to couple redox reactions to ATP production.

A Comparison of Chemiosmosis in Chloroplasts and Mitochondria

Chloroplasts and mitochondria generate ATP by the same basic mechanism: chemiosmosis (see Figure 9.14). An electron transport chain pumps protons (H^+) across a membrane as electrons are passed through a series of carriers that have progressively more affinity for electrons. Thus, electron transport chains transform redox energy to a proton-motive force, potential energy stored in the form of an H^+ gradient across a membrane. An ATP synthase complex in the same membrane couples the diffusion of hydrogen ions down their gradient to the phosphorylation of ADP, forming ATP.

Some of the electron carriers, including the iron-containing proteins called cytochromes, are very similar in mitochondria and chloroplasts (see Figure 6.32b and c). The ATP synthase complexes of the two organelles are also quite similar. But there are noteworthy differences between photophosphorylation in chloroplasts and oxidative phosphorylation in mitochondria. Both work by way of chemiosmosis, but in chloroplasts, the high-energy electrons dropped down the transport chain come from water, while in mitochondria, they are extracted from organic molecules (which are thus oxidized). Chloroplasts do not need molecules from food to make ATP; their photosystems capture light energy and use it to drive the electrons from water to the top of the transport chain. In other words, mitochondria use chemiosmosis to transfer chemical energy from food molecules to ATP, whereas chloroplasts use it to transform light energy into chemical energy in ATP.

Although the spatial organization of chemiosmosis differs slightly between chloroplasts and mitochondria, it is easy to see similarities in the two **(Figure 10.16)**. Electron transport



▶ Figure 10.16 Comparison of chemiosmosis in mitochondria and chloroplasts. In both kinds of organelles, electron transport chains pump protons (H^+) across a membrane from a region of low H^+ concentration (light gray in this diagram) to one of high H^+ concentration (dark gray). The protons then diffuse back across the membrane through ATP synthase, driving the synthesis of ATP.

MAKE CONNECTIONS *Describe how you would change the pH in order to artificially cause ATP synthesis (a) outside an isolated mitochondrion (assume H^+ can freely cross the outer membrane; see Figure 9.14) and (b) in the stroma of a chloroplast. Explain.*

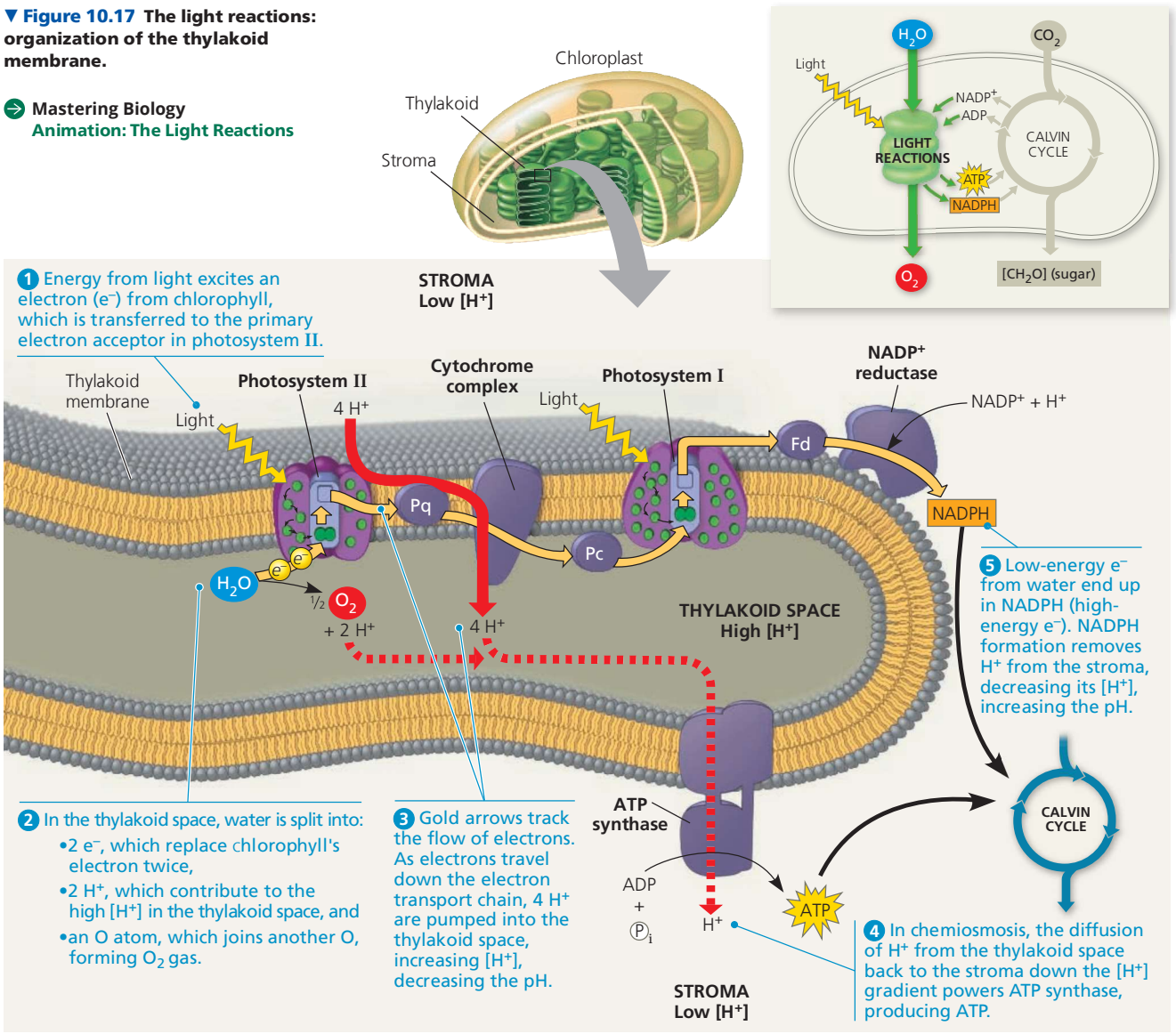
chain proteins in the inner membrane of the mitochondrion pump protons from the mitochondrial matrix out to the intermembrane space, which then serves as a reservoir of hydrogen ions. Similarly, electron transport chain proteins in the thylakoid membrane of the chloroplast pump protons from the stroma into the thylakoid space, which functions as the H^+ reservoir. If you imagine the cristae of mitochondria pinching off from the inner membrane, this may help you see how the thylakoid space and the intermembrane space are comparable spaces in the two organelles, while the mitochondrial matrix is analogous to the stroma of the chloroplast.

In the mitochondrion, protons diffuse down their concentration gradient from the intermembrane space through ATP synthase to the matrix, driving ATP synthesis. In the chloroplast, ATP is synthesized as the hydrogen ions diffuse from the thylakoid space back to the stroma through ATP synthase complexes **(Figure 10.17)**, whose catalytic knobs are on the stroma side of the membrane. Thus, ATP forms in the stroma, where it is used to help drive sugar synthesis during the Calvin cycle.

The proton (H^+) gradient, or pH gradient, across the thylakoid membrane is substantial. When chloroplasts in an

▼ Figure 10.17 The light reactions: organization of the thylakoid membrane.

Mastering Biology
Animation: The Light Reactions



VISUAL SKILLS *Which three steps in the figure contribute to the [H⁺] gradient across the thylakoid membrane?*

experimental setting are illuminated, the pH in the thylakoid space drops to about 5 (the H^+ concentration increases), and the pH in the stroma increases to about 8 (the H^+ concentration decreases). This gradient of three pH units corresponds to a thousandfold difference in H^+ concentration. If the lights are then turned off, the pH gradient is abolished, but it can quickly be restored by turning the lights back on. Experiments such as this provided strong evidence in support of the chemiosmotic model.

The currently accepted model for the organization of the light-reaction "machinery" within the thylakoid membrane is based on several research studies. Each of the molecules and molecular complexes in Figure 10.17 is present in numerous copies in each thylakoid. Notice that NADPH, like ATP, is produced on the side of the membrane facing the stroma, where the Calvin cycle reactions take place.

Let's step back and see the big picture by summarizing the light reactions. Electron flow pushes electrons from water, where they are at a state of low potential energy, ultimately to NADPH, where they are stored at a state of high potential energy. The light-driven electron flow also generates ATP. Thus, the equipment of the thylakoid membrane converts light energy to chemical energy stored in ATP and NADPH. O_2 is produced as a by-product. Let's now see how the enzymes of the Calvin cycle use the products of the light reactions to synthesize sugar from CO_2.

Mastering Biology **BioFlix® Animation: The Light Reactions**

CONCEPT CHECK 10.3

1. What color of light is *least* effective in driving photosynthesis? Explain.
2. In the light reactions, what is the initial electron donor? Where do the electrons finally end up?
3. **WHAT IF?** In an experiment, isolated chloroplasts placed in an illuminated solution with the appropriate chemicals can carry out ATP synthesis. Predict what would happen to the rate of synthesis if a compound is added to the solution that makes membranes freely permeable to hydrogen ions.

For suggested answers, see Appendix A.

CONCEPT 10.4

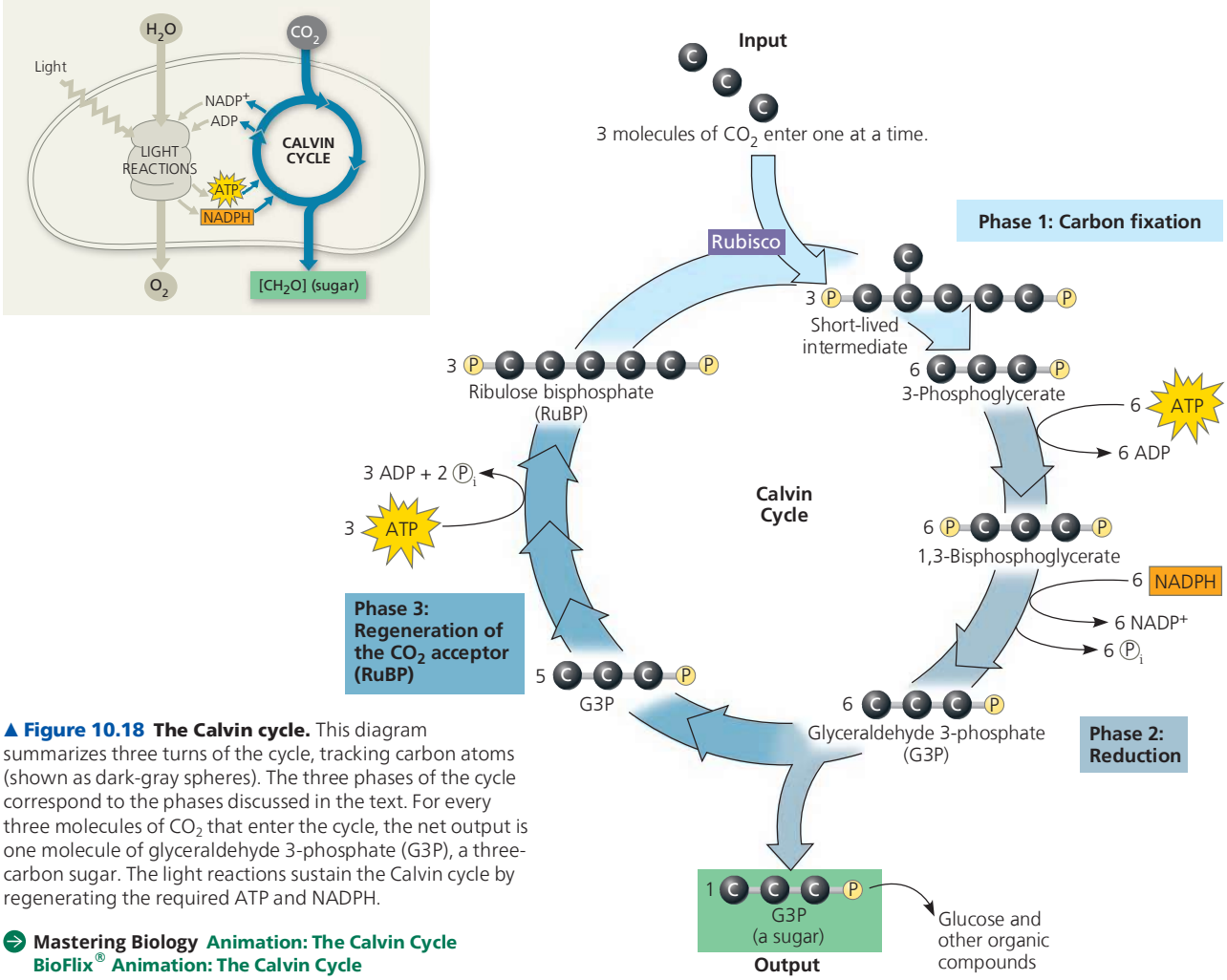
The Calvin cycle uses the chemical energy of ATP and NADPH to reduce CO_2 to sugar

The Calvin cycle takes place in the stroma; it is similar to the citric acid cycle in that a starting material is regenerated after some molecules enter and others exit the cycle. However, the citric acid cycle is catabolic, oxidizing acetyl CoA and using the energy to synthesize ATP (see Figure 9.11), while the Calvin cycle is anabolic, building carbohydrates from smaller molecules and consuming energy. Carbon enters the Calvin cycle in the form of CO_2 and leaves in the form of sugar. The cycle spends ATP as an energy source and consumes NADPH as reducing power for adding high-energy electrons to make the sugar.

As we mentioned in Concept 10.2, the carbohydrate produced directly from the Calvin cycle is not glucose. It is actually a three-carbon sugar called **glyceraldehyde 3-phosphate (G3P)**. For the net synthesis of *one* molecule of G3P, the cycle must take place three times, fixing *three* molecules of CO_2—one per turn of the cycle. (Recall that the term *carbon fixation* refers to the initial incorporation of CO_2 into organic material.) As we trace the steps of the Calvin cycle, keep in mind that we are following three molecules of CO_2 through the reactions. **Figure 10.18** divides the Calvin cycle into three phases: carbon fixation, reduction, and regeneration of the CO_2 acceptor.

Phase 1: Carbon fixation. The Calvin cycle incorporates each CO_2 molecule, one at a time, by attaching it to a five-carbon sugar named ribulose bisphosphate (which is abbreviated RuBP). The enzyme that catalyzes this first step is RuBP carboxylase-oxygenase, or **rubisco** (see Figure 6.32c). (This is the most abundant protein in chloroplasts and is also thought to be the most abundant protein on Earth.) The product of the reaction is a six-carbon intermediate that is short-lived because it is so energetically unstable that it immediately splits in half, forming two molecules of 3-phosphoglycerate (for each CO_2 fixed).

Phase 2: Reduction. Each molecule of 3-phosphoglycerate receives an additional phosphate group from ATP, becoming 1,3-bisphosphoglycerate. Next, a pair of electrons donated from NADPH reduces 1,3-bisphosphoglycerate, which also loses a phosphate group in the process, becoming glyceraldehyde 3-phosphate (G3P). Specifically, the electrons from NADPH reduce a carboxyl group on 1,3-bisphosphoglycerate to the aldehyde group of G3P, which stores more potential energy. G3P is a sugar—the same three-carbon sugar formed in glycolysis by the splitting of glucose (see Figure 9.8). Notice in Figure 10.18 that for every *three* molecules of CO_2 that enter the cycle, there are *six* molecules of G3P formed. But only one molecule of this three-carbon sugar can be counted as a net gain of carbohydrate because the rest are required to complete the cycle. The cycle began with 15 carbons' worth of carbohydrate in the form of three molecules of the five-carbon sugar RuBP. Now there are 18 carbons' worth of carbohydrate in the form of six molecules of G3P. One molecule exits the cycle to be used by the plant cell, but the other five molecules must be recycled to regenerate the three molecules of RuBP.



▲ Figure 10.18 The Calvin cycle. This diagram summarizes three turns of the cycle, tracking carbon atoms (shown as dark-gray spheres). The three phases of the cycle correspond to the phases discussed in the text. For every three molecules of CO_2 that enter the cycle, the net output is one molecule of glyceraldehyde 3-phosphate (G3P), a three-carbon sugar. The light reactions sustain the Calvin cycle by regenerating the required ATP and NADPH.

Mastering Biology Animation: The Calvin Cycle
BioFlix® Animation: The Calvin Cycle

Phase 3: Regeneration of the CO_2 acceptor (RuBP). In a complex series of reactions, the carbon skeletons of five molecules of G3P are rearranged by the last steps of the Calvin cycle into three molecules of RuBP. To accomplish this, the cycle spends three more molecules of ATP. The RuBP is now prepared to receive CO_2 again, and the cycle continues.

For the net synthesis of one G3P molecule, the Calvin cycle consumes a total of nine molecules of ATP and six molecules of NADPH. The light reactions regenerate the ATP and NADPH. The G3P spun off from the Calvin cycle becomes the starting material for metabolic pathways that synthesize other organic compounds, including glucose (from two molecules of G3P), sucrose, and other carbohydrates. Neither the light reactions nor the Calvin cycle alone can make sugar from CO_2. Photosynthesis is an emergent property of the intact chloroplast, which integrates the two stages of photosynthesis.

CONCEPT CHECK 10.4

1. To synthesize one glucose molecule, the Calvin cycle uses _____ molecules of CO_2, _____ molecules of ATP, and _____ molecules of NADPH.
2. How are the large numbers of ATP and NADPH molecules used during the Calvin cycle consistent with the high value of glucose as an energy source?
3. **WHAT IF?** Consider a poison that inhibits an enzyme of the Calvin cycle. Do you think such a poison will also inhibit the light reactions? Explain.
4. **DRAW IT** Draw a simple version of the Calvin cycle that shows only the number of carbon atoms at each step, using numerals rather than gray spheres (for example, "3 × 1C = 3C" at the beginning of the cycle). Explain how the total number of carbon atoms remains constant for every three turns of the cycle, noting the forms in which the carbon atoms enter and leave the cycle.
5. **MAKE CONNECTIONS** Review Figures 9.8 and 10.18, and then discuss the roles of intermediate and product played by glyceraldehyde 3-phosphate (G3P) in the two processes shown in these figures.

For suggested answers, see Appendix A.

CONCEPT 10.5

Alternative mechanisms of carbon fixation have evolved in hot, arid climates

EVOLUTION Ever since plants first moved onto land about 475 million years ago, they have been adapting to the problems of terrestrial life, particularly the problem of dehydration. In Concept 36.4, we will consider anatomical adaptations that help plants conserve water, while in this chapter we are concerned with metabolic adaptations. The solutions often involve trade-offs. An important example is the balance between photosynthesis and the prevention of excessive water loss from the plant. The CO_2 required for photosynthesis enters a leaf (and the resulting O_2 exits) via stomata, the pores on the leaf surface (see Figure 10.3). However, stomata are also the main avenues of transpiration, the evaporative loss of water from leaves. On a hot, dry day, most plants close their stomata, a response that conserves water but also reduces CO_2 levels. With stomata even partially closed, CO_2 concentrations begin to decrease in the air spaces within the leaf, and the concentration of O_2 released from the light reactions begins to increase. These conditions within the leaf favor an apparently wasteful process called photorespiration.

Photorespiration: An Evolutionary Relic?

In most plants, initial fixation of carbon occurs via rubisco, the Calvin cycle enzyme that adds CO_2 to ribulose bisphosphate. Such plants are called **C_3 plants** because the first organic product of carbon fixation is a three-carbon compound, 3-phosphoglycerate (see Figure 10.18). C_3 plants include important agricultural plants such as rice, wheat, and soybeans. When their stomata close on hot, dry days, C_3 plants produce less sugar because the declining level of CO_2 in the leaf starves the Calvin cycle. In addition, rubisco is capable of binding O_2 in place of CO_2. As CO_2 becomes scarce within the air spaces of the leaf and O_2 builds up, rubisco adds O_2 to the Calvin cycle instead of CO_2. The product splits, and a two-carbon compound leaves the chloroplast. Peroxisomes and mitochondria within the plant cell rearrange and split this compound, releasing CO_2. The process is called **photorespiration** because it occurs in the light (*photo*) and consumes O_2 while producing CO_2 (*respiration*). However, unlike normal cellular respiration, photorespiration uses ATP rather than generating it. And unlike photosynthesis, photorespiration produces no sugar. In fact, photorespiration *decreases* photosynthetic output by siphoning organic material from the Calvin cycle and releasing CO_2 that would otherwise be fixed. This CO_2 can eventually be fixed if it is still in the leaf once the CO_2 concentration builds up to a high enough level. In the meantime, though, the process is energetically costly, much like a hamster running on its wheel.

How can we explain the existence of a metabolic process that seems to be counterproductive for the plant? According to one hypothesis, photorespiration is evolutionary baggage—a metabolic relic from a much earlier time when the atmosphere had less O_2 and more CO_2 than it does today. In the ancient atmosphere that prevailed when rubisco first evolved, the ability of the enzyme's active site to bind O_2 would have made little difference. The hypothesis suggests that modern rubisco retains some of its chance affinity for O_2, which is now so concentrated in the atmosphere that a certain amount of photorespiration is inevitable. There is also some evidence that photorespiration may provide protection against the damaging products of the light reactions, which build up when the Calvin cycle slows due to low CO_2.

In many types of plants—including a significant number of crop plants—photorespiration drains away as much as 50% of the carbon fixed by the Calvin cycle. Indeed, if photorespiration could be reduced in certain plant species without otherwise affecting photosynthetic productivity, crop yields and food supplies might increase.

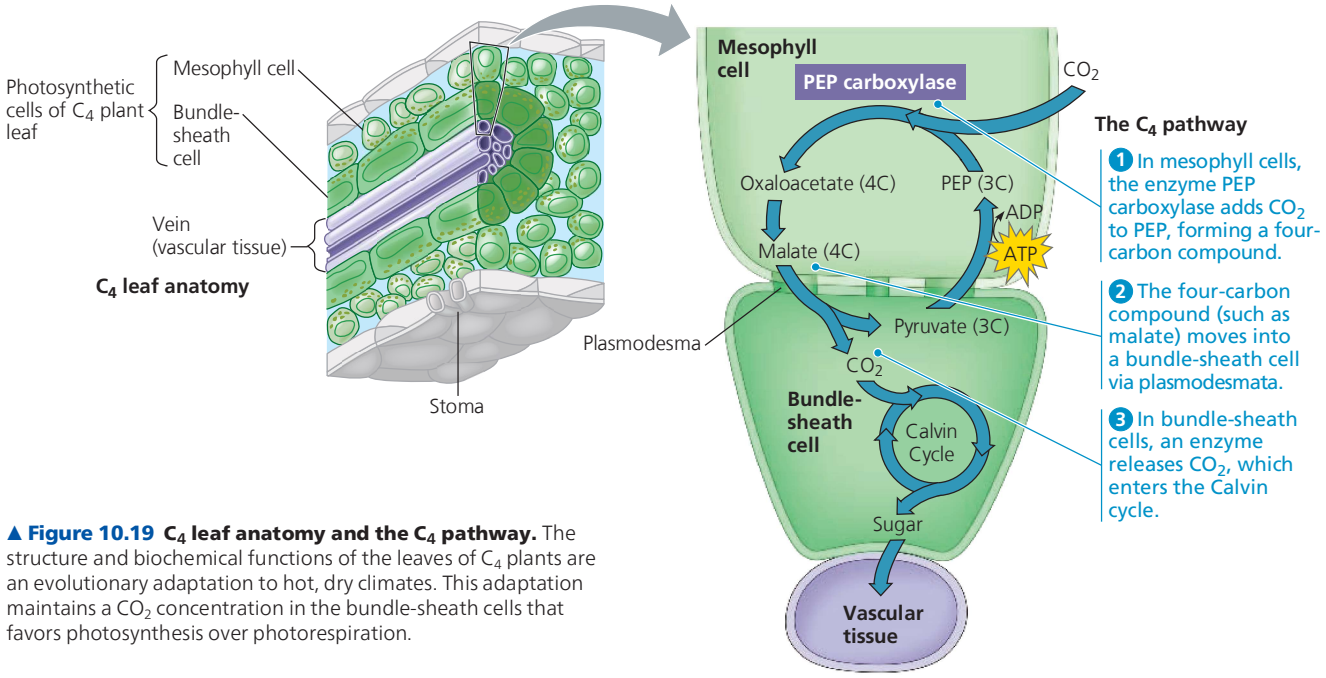
In some plant species, alternate modes of carbon fixation have evolved that minimize photorespiration and optimize the Calvin cycle—even in hot, arid climates. The two most important of these photosynthetic adaptations are C_4 photosynthesis and crassulacean acid metabolism (CAM).

C_4 Plants

The **C_4 plants** are so named because they preface the Calvin cycle with an alternate mode of carbon fixation that forms a four-carbon compound as its first product. The C_4 pathway is believed to have evolved independently at least 45 separate times and is used by several thousand species in at least 19 plant families. Among the C_4 plants important to agriculture are sugarcane and corn, members of the grass family.

When the weather is hot and dry, a C_4 plant partially closes its stomata, conserving water but reducing the CO_2 concentration in the leaves. However, sugar is still made because C_4 plants use a multistep process that operates even under low CO_2 conditions. Photosynthesis begins in mesophyll cells but is completed in **bundle-sheath cells**, cells that are arranged into tightly packed sheaths around the veins of the leaf **(Figure 10.19)**. In C_4 leaves, the loosely arranged mesophyll cells are located between the bundle-sheath cells and the leaf surface, no more than two to three cells away from the bundle-sheath cells. In mesophyll cells, CO_2 is incorporated into organic compounds that then move into the bundle-sheath cells, where the Calvin cycle takes place. See the numbered steps in Figure 10.19, which are also described here:

1. The first step is carried out by an enzyme present only in mesophyll cells called **PEP carboxylase**, which has a much higher affinity for CO_2 than does rubisco, and no affinity for O_2. This enzyme adds CO_2 to



▲ Figure 10.19 C_4 leaf anatomy and the C_4 pathway. The structure and biochemical functions of the leaves of C_4 plants are an evolutionary adaptation to hot, dry climates. This adaptation maintains a CO_2 concentration in the bundle-sheath cells that favors photosynthesis over photorespiration.

phosphoenolpyruvate (PEP), forming the four-carbon product oxaloacetate; it does this even under conditions of lower CO_2 concentration and relatively higher O_2 concentration.

2. After the CO_2 is fixed in the mesophyll cells, the four-carbon products (malate in the example shown in Figure 10.19) are exported to bundle-sheath cells through plasmodesmata (see Figure 6.29).

3. Within the bundle-sheath cells, an enzyme releases CO_2 from the four-carbon compounds; the CO_2 is re-fixed into organic material by rubisco and the Calvin cycle. The same reaction regenerates pyruvate, which is transported to mesophyll cells. There, ATP is used to convert pyruvate to PEP, which can then accept addition of another CO_2, allowing the reaction cycle to continue. This ATP can be thought of, in a sense, as the "price" of concentrating CO_2 in the bundle-sheath cells. To generate this extra ATP, bundle-sheath cells carry out cyclic electron flow, the process described earlier in this chapter (see Figure 10.15). In fact, these cells contain PS I but no PS II, so cyclic electron flow is their only photosynthetic mode of generating ATP.

In effect, the mesophyll cells of a C_4 plant pump CO_2 into the bundle-sheath cells, keeping the CO_2 concentration in those cells high enough for rubisco to bind CO_2 rather than O_2. The cyclic series of reactions involving PEP carboxylase and the regeneration of PEP can be thought of as an ATP-powered pump that concentrates CO_2 where it can be fixed. In this way, C_4 photosynthesis spends ATP energy to minimize photorespiration and enhance sugar production. This adaptation is especially advantageous in hot regions with intense sunlight, and it is in such environments that C_4 plants evolved and thrive today.

The concentration of CO_2 in the atmosphere has drastically increased since the Industrial Revolution began in the 1800s, and it continues to rise today due to human activities such as the burning of fossil fuels (see Concept 1.1). The resulting global climate change, including an increase in average temperatures around the planet, may have far-reaching effects on plant species. Scientists are concerned that increasing CO_2 concentration and temperature may affect C_3 and C_4 plants differently, thus changing the relative abundance of these species in a given plant community.

Which type of plant would stand to gain more from increasing CO_2 levels? Recall that in C_3 plants, the binding of O_2 rather than CO_2 by rubisco leads to photorespiration, lowering the efficiency of photosynthesis. C_4 plants overcome this problem by concentrating CO_2 in the bundle-sheath cells at the cost of ATP. Rising CO_2 levels should benefit C_3 plants by lowering the amount of photorespiration that occurs. At the same time, rising temperatures have the opposite effect, increasing photorespiration. (Other factors such as water availability may also come into play.) In contrast, many C_4 plants could be largely unaffected by increasing CO_2 levels or temperature. Researchers have investigated aspects of this question in several studies; you can work with data from one such experiment in the **Scientific Skills Exercise**. In different regions, the particular combination of CO_2 concentration and temperature is likely to alter the balance of C_3 and C_4 plants in varying ways. The effects of such a widespread and variable change in community structure are unpredictable and thus a cause of legitimate concern.

Scientific Skills Exercise

Making Scatter Plots with Regression Lines

Does Atmospheric CO_2 Concentration Affect the Productivity of Agricultural Crops? The atmospheric concentration of CO_2 has been rising globally, and scientists wondered whether this would affect C_3 and C_4 plants differently. In this exercise, you will make a scatter plot to examine the relationship between CO_2 concentration and growth of both corn (maize), a C_4 crop plant, and velvetleaf, a C_3 weed found in cornfields.

How the Experiment Was Done For 45 days, researchers grew corn and velvetleaf plants under controlled conditions, where all plants received the same amounts of water and light. The plants were divided into three groups, and each was exposed to a different concentration of CO_2 in the air: 350, 600, or 1,000 ppm (parts per million).

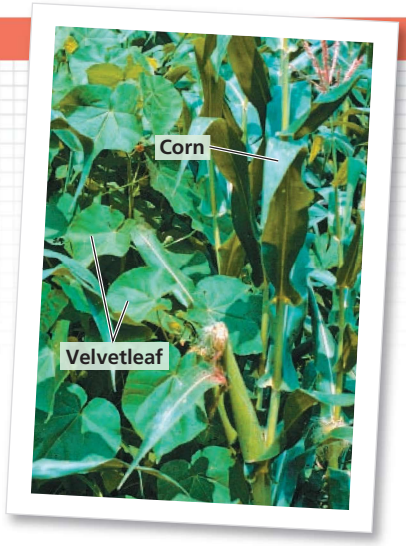
Data from the Experiment The table shows the dry mass (in grams) of corn and velvetleaf plants grown at the three concentrations of CO_2. The dry mass values are averages calculated from the leaves, stems, and roots of eight plants.

	350 ppm CO_2	600 ppm CO_2	1,000 ppm CO_2
Average dry mass of one corn plant (g)	91	89	80
Average dry mass of one velvetleaf plant (g)	35	48	54

Data from D. T. Patterson and E. P. Flint, Potential effects of global atmospheric CO_2 enrichment on the growth and competitiveness of C_3 and C_4 weed and crop plants, *Weed Science* 28(1):71–75 (1980).

INTERPRET THE DATA

1. To explore the relationship between the two variables, it is useful to graph the data in a scatter plot, and then draw a regression line. **(a)** First, place labels for the dependent and independent variables on the appropriate axes. Explain your choices. **(b)** Plot the data points for corn and velvetleaf using different symbols for each set of data, and add a key for the two symbols. (For additional information about graphs, see the Scientific Skills Review in Appendix D.)
2. Draw a "best-fit" line for each set of points. A best-fit line does not necessarily pass through all or even most points. Instead, it is a straight line that passes as close as possible to all data points from that set. Draw a best-fit line for each set of data. Because placement of the line is a matter of judgment, two individuals may draw two slightly different lines for a given set of points. The line that actually fits best, a regression line, can be identified by squaring the distances of all points to any candidate line, then selecting the line that minimizes the sum of the squares. (See the graph in the Scientific Skills Exercise in Chapter 3 for an example of a linear regression line.) Excel or other software programs, including those on a graphing calculator, can plot a regression line once data points are entered. Using either Excel or a graphing calculator, enter the data points for each data set and have the program draw the two regression lines. Compare them to the lines you drew.
3. Describe the trends shown by the regression lines in your scatter plot. **(a)** Compare the relationship between increasing concentration of CO_2 and the dry mass of corn to that for velvetleaf. **(b)** Considering that velvetleaf is a weed invasive to cornfields, predict how increased CO_2 concentration may affect interactions between the two species.
4. Based on the data in the scatter plot, estimate the percentage change in dry mass of corn and velvetleaf plants if atmospheric CO_2 concentration increased from 390 ppm (current levels) to 800 ppm. **(a)** What is the estimated dry mass of corn and velvetleaf plants at 390 ppm? 800 ppm? **(b)** To calculate the percentage change in mass for each plant, subtract the mass at 390 ppm from the mass at 800 ppm (change in mass), divide by the mass at 390 ppm (initial mass), and multiply by 100. What is the estimated percentage change in dry mass for corn? For velvetleaf? **(c)** Do these results support the conclusion from other experiments that C_3 plants grow better than C_4 plants under increased CO_2 concentration? Why or why not?



Corn plant surrounded by invasive velvetleaf plants.

Instructors: A version of this Scientific Skills Exercise can be assigned in **Mastering Biology.**

C_4 photosynthesis is considered more efficient than C_3 photosynthesis because it uses less water and resources. On our planet today, the world population and demand for food are rapidly increasing. At the same time, the amount of land suitable for growing crops is decreasing due to the effects of global climate change, which include an increase in sea level as well as a hotter, drier climate in many regions. To address issues of food supply, scientists in the Philippines have been working on genetically modifying rice—an important food staple that is a C_3 crop—so that it can instead carry out C_4 photosynthesis. Results so far seem promising, and these researchers estimate that the yield of C_4 rice might be 30–50% higher than C_3 rice with the same input of water and resources.

CAM Plants

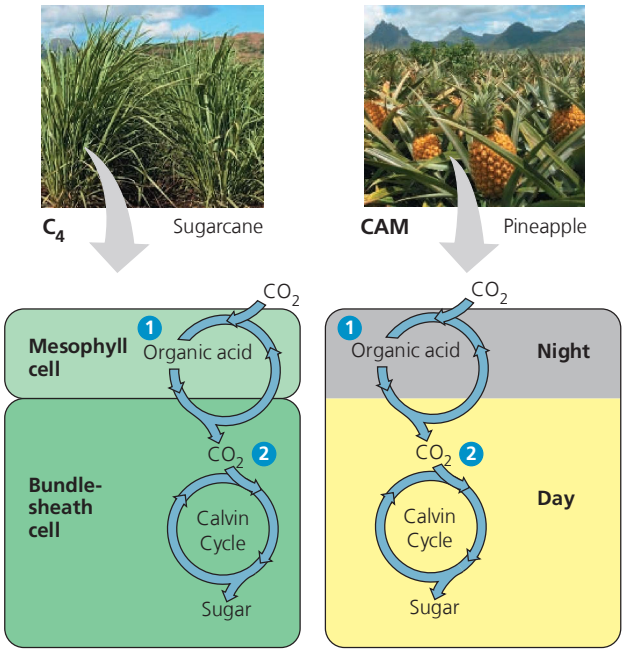
A second photosynthetic adaptation to arid conditions has evolved in pineapples, many cacti, and other succulent (water-storing) plants, such as aloe and jade plants. These plants open their stomata at night and close them during the day, just the reverse of how other plants behave. Closing stomata during the day helps desert plants conserve water, but it

also prevents CO_2 from entering the leaves. During the night, when their stomata are open, these plants take up CO_2 and incorporate it into a variety of organic acids. This mode of carbon fixation is called **crassulacean acid metabolism**, or **CAM**, after the plant family Crassulaceae, the succulents in which the process was first discovered. The mesophyll cells of **CAM plants** store the organic acids they make during the night in their vacuoles until morning, when the stomata close. During the day, when the light reactions can supply ATP and NADPH for the Calvin cycle, CO_2 is released from the organic acids made the night before to become incorporated into sugar in the chloroplasts.

Notice in **Figure 10.20** that the CAM pathway is similar to the C_4 pathway in that CO_2 is first incorporated into organic intermediates before it enters the Calvin cycle. The difference is that in C_4 plants, the initial steps of carbon fixation are separated structurally from the Calvin cycle, whereas in CAM plants, the two steps occur within the same cell but at separate times. (Keep in mind that CAM, C_4, and C_3 plants all eventually use the Calvin cycle to make sugar from carbon dioxide.)

Mastering Biology Animation: Photosynthesis in Dry Climates

▼ Figure 10.20 C_4 and CAM photosynthesis compared. The C_4 and CAM pathways are two evolutionary solutions to the problem of maintaining photosynthesis with stomata partially or completely closed on hot, dry days. Both adaptations are characterized by: 1 preliminary incorporation of CO_2 into organic acids, followed by 2 transfer of CO_2 to the Calvin cycle.



(a) Spatial separation of steps. In C_4 plants, carbon fixation and the Calvin cycle occur in different types of cells.

(b) Temporal separation of steps. In CAM plants, carbon fixation and the Calvin cycle occur in the same cell at different times.

CONCEPT CHECK 10.5

1. Describe how photorespiration lowers photosynthetic output for plants.
2. The presence of only PS I, not PS II, in the bundle-sheath cells of C_4 plants has an effect on O_2 concentration. What is that effect, and how might that benefit the plant?
3. **MAKE CONNECTIONS** Refer to the discussion of ocean acidification in Concept 3.3. Ocean acidification and changes in the distribution of C_3 and C_4 plants may seem to be two very different problems, but what do they have in common? Explain.
4. **WHAT IF?** How would you expect the relative abundance of C_3 versus C_4 and CAM species to change in a geographic region whose climate becomes much hotter and drier, with no change in CO_2 concentration?

For suggested answers, see Appendix A.

CONCEPT 10.6

Photosynthesis is essential for life on Earth: a review

Mastering Biology BioFlix® Animation: Photosynthesis

In this chapter, we have followed photosynthesis from photons to food. The light reactions capture solar energy and use it to make ATP and transfer electrons from H_2O to $NADP^+$, forming NADPH. The Calvin cycle uses the ATP and NADPH to produce sugar from CO_2. The energy that enters the chloroplasts as sunlight becomes stored as chemical energy in organic compounds. The entire process is reviewed visually in **Figure 10.21**, where photosynthesis is also put in its natural context.

As for the fates of photosynthetic products, enzymes in the chloroplast and cytosol convert the G3P made in the Calvin cycle to many other organic compounds. In fact, the sugar made in the chloroplasts supplies the entire plant with chemical energy and carbon skeletons for the synthesis of all the major organic molecules of plant cells. About 50% of the organic material made by photosynthesis is consumed as fuel for cellular respiration in plant cell mitochondria.

Technically, green cells are the only autotrophic parts of the plant. The rest of the plant depends on organic molecules exported from leaves through veins (see Figure 10.21, top). In most plants, carbohydrate is transported out of the leaves to the rest of the plant in the form of sucrose, a disaccharide. After arriving at nonphotosynthetic cells, the sucrose provides raw material for cellular respiration and a multitude of anabolic pathways that synthesize proteins, lipids, and other products. A considerable amount of sugar in the form of glucose is linked together to make the polysaccharide cellulose (see Figure 5.6c), especially in plant cells that are still growing and maturing. Cellulose, the main ingredient of cell walls, is the most abundant organic molecule in the plant—and probably on the surface of the planet.

Most plants and other photosynthesizers make more organic material per day than they need to use as respiratory

fuel and precursors for biosynthesis. For example, flowering plants stockpile the extra sugar in the form of starch, storing some in the chloroplasts and some in cells of roots, tubers, seeds, and fruits. In accounting for the use of food molecules produced by photosynthesis, note that most flowering plants lose leaves, roots, stems, fruits, and often their entire bodies to heterotrophs, including humans.

On a global scale, photosynthesis is the process responsible for the presence of O_2 in our atmosphere. Furthermore, although each chloroplast is minuscule, their collective productivity in terms of food production is enormous: Photosynthesis makes an estimated 150 billion metric tons of carbohydrate per year (a metric ton is 1,000 kg, about 1.1 tons). That's organic matter equivalent in mass to a stack of about 60 trillion biology textbooks—and the stack would reach 17 times the distance from Earth to the sun! No chemical process is more important than photosynthesis to the welfare of life on Earth.

In Chapters 5 through 10, you have learned about many activities of cells. **Figure 10.22** integrates these cellular processes into the context of a working plant cell. As you study the figure, reflect on how each process fits into the big picture: As the most basic unit of living organisms, a cell performs all functions characteristic of life.

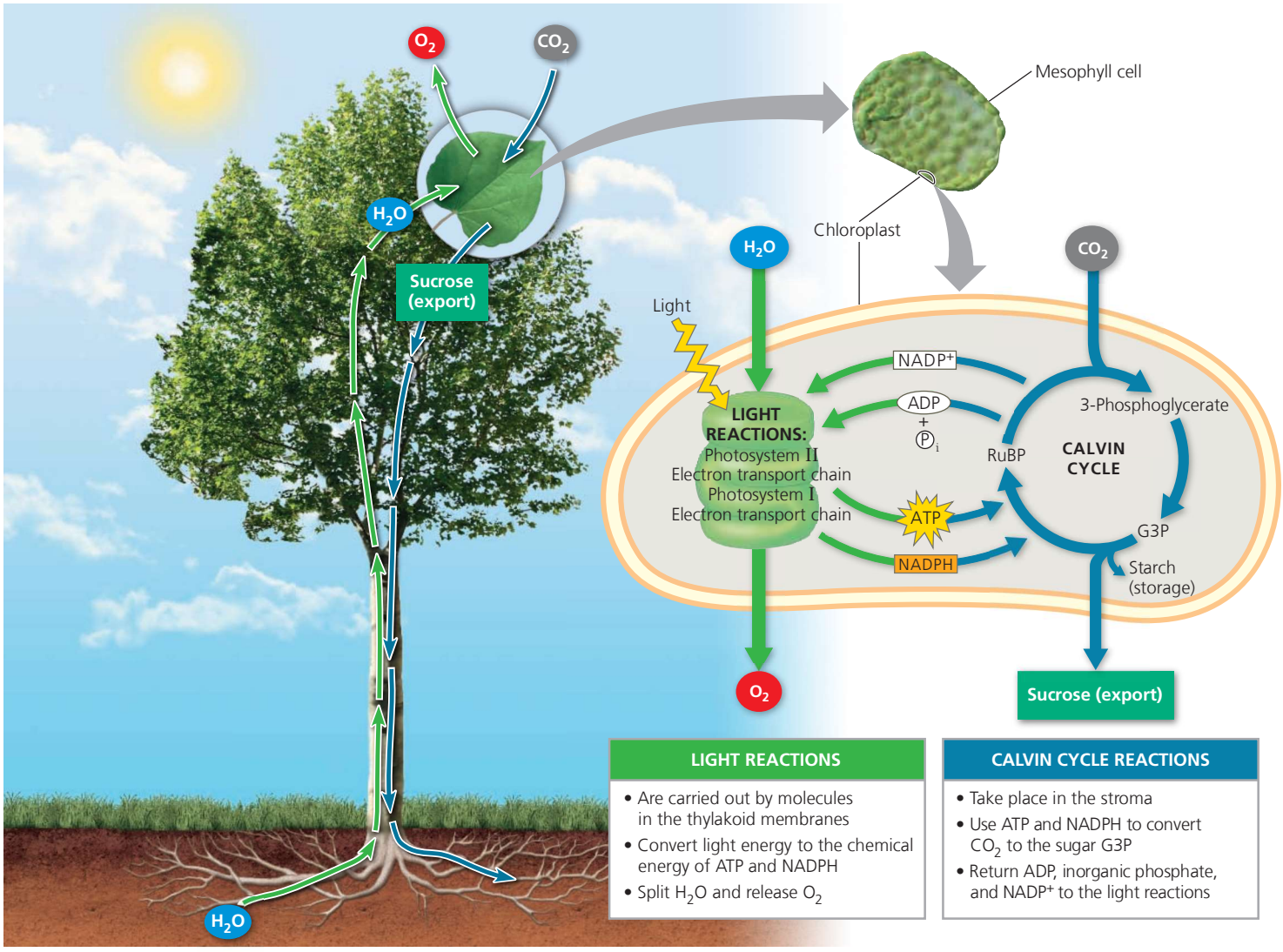
CONCEPT CHECK 10.6

1. **MAKE CONNECTIONS** How do plants use the sugar they produce during photosynthesis to directly power the work of the cell? Provide some examples of cellular work. (See Figures 8.10, 8.11, and 9.5.)

For suggested answers, see Appendix A.

▼ Figure 10.21 A review of photosynthesis. This diagram shows the main reactants and products of photosynthesis as they move through the tissues of a tree (left) and a chloroplast (right).

Mastering Biology HHMI Animation: Photosynthesis
MP3 Tutor: Photosynthesis

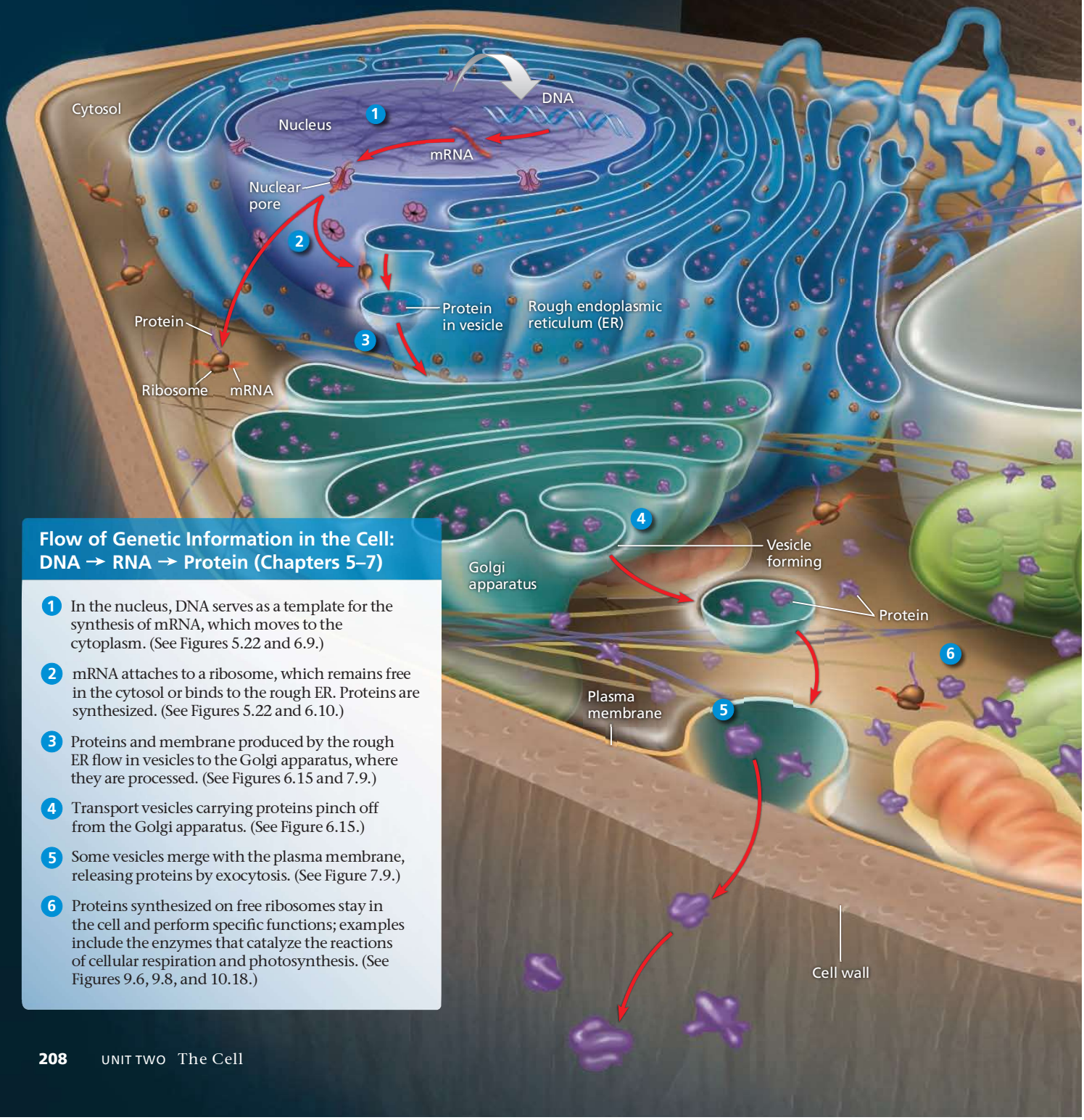


▼ Figure 10.22

MAKE CONNECTIONS

The Working Cell

This figure illustrates how a generalized plant cell functions, integrating the cellular activities you learned about in Chapters 5–10. To see some of the enzymes involved, look at Figure 6.32.



Flow of Genetic Information in the Cell: DNA → RNA → Protein (Chapters 5–7)

1. In the nucleus, DNA serves as a template for the synthesis of mRNA, which moves to the cytoplasm. (See Figures 5.22 and 6.9.)
2. mRNA attaches to a ribosome, which remains free in the cytosol or binds to the rough ER. Proteins are synthesized. (See Figures 5.22 and 6.10.)
3. Proteins and membrane produced by the rough ER flow in vesicles to the Golgi apparatus, where they are processed. (See Figures 6.15 and 7.9.)
4. Transport vesicles carrying proteins pinch off from the Golgi apparatus. (See Figure 6.15.)
5. Some vesicles merge with the plasma membrane, releasing proteins by exocytosis. (See Figure 7.9.)
6. Proteins synthesized on free ribosomes stay in the cell and perform specific functions; examples include the enzymes that catalyze the reactions of cellular respiration and photosynthesis. (See Figures 9.6, 9.8, and 10.18.)

Movement Across Cell Membranes (Chapter 7)

7 Water diffuses into and out of the cell directly through the plasma membrane and by facilitated diffusion through aquaporins. (See Figure 7.10.)

8 By passive transport, the CO_2 used in photosynthesis diffuses into the cell, and the O_2 formed as a by-product of photosynthesis diffuses out of the cell. Both solutes move down their concentration gradients. (See Figures 7.11 and 10.21.)

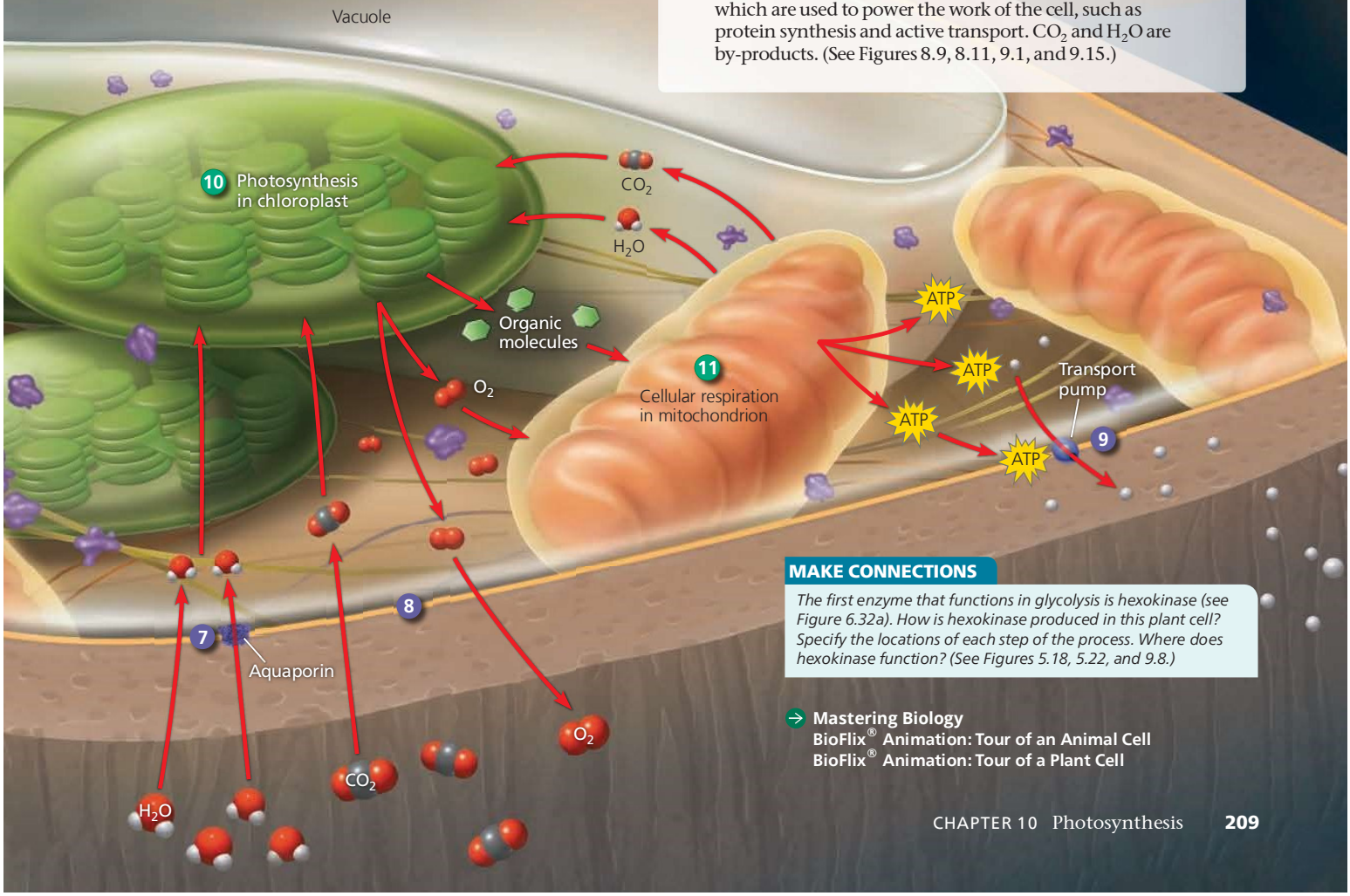
9 In active transport, energy (usually supplied by ATP) is used to transport a solute against its concentration gradient. (See Figure 7.17.)

Exocytosis (shown in step 5) and endocytosis move larger materials out of and into the cell. (See Figures 7.20 and 7.21.)

Energy Transformations in the Cell: Photosynthesis and Cellular Respiration (Chapters 8–10)

10 In chloroplasts, the process of photosynthesis uses the energy of light to convert CO_2 and H_2O to organic molecules, with O_2 as a by-product. (See Figures 10.1 and 10.21.)

11 In mitochondria, organic molecules are broken down by cellular respiration, capturing energy in molecules of ATP, which are used to power the work of the cell, such as protein synthesis and active transport. CO_2 and H_2O are by-products. (See Figures 8.9, 8.11, 9.1, and 9.15.)



MAKE CONNECTIONS

The first enzyme that functions in glycolysis is hexokinase (see Figure 6.32a). How is hexokinase produced in this plant cell? Specify the locations of each step of the process. Where does hexokinase function? (See Figures 5.18, 5.22, and 9.8.)

Mastering Biology
BioFlix® Animation: Tour of an Animal Cell
BioFlix® Animation: Tour of a Plant Cell

10 Chapter Review

Go to **Mastering Biology** for Assignments, the eText, the Study Area, and Dynamic Study Modules.

SUMMARY OF KEY CONCEPTS

To review key terms, go to the **Vocabulary Self-Quiz** in the **Mastering Biology** eText or Study Area, or go to goo.gl/zkjz9t.

CONCEPT 10.1

Photosynthesis feeds the biosphere *(p. 188)*

- **Photosynthesis** converts the energy of sunlight into chemical energy stored in sugars.
- **Autotrophs** ("producers") produce their organic molecules from CO_2 and other inorganic raw materials; plants are *photoautotrophs* that use light energy to do this. **Heterotrophs** (consumers, including decomposers) are unable to make their own food and live on compounds produced by others.

? *Using the terms producers, consumers, and decomposers, explain how photosynthesis feeds organisms, either directly or indirectly.*

CONCEPT 10.2

Photosynthesis converts light energy to the chemical energy of food *(pp. 189–192)*

- In plants and other eukaryotic autotrophs, photosynthesis occurs in **chloroplasts**, organelles containing **thylakoids**. Stacks of thylakoids form grana. Photosynthesis is summarized as

  $6\ CO_2 + 12\ H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O.$

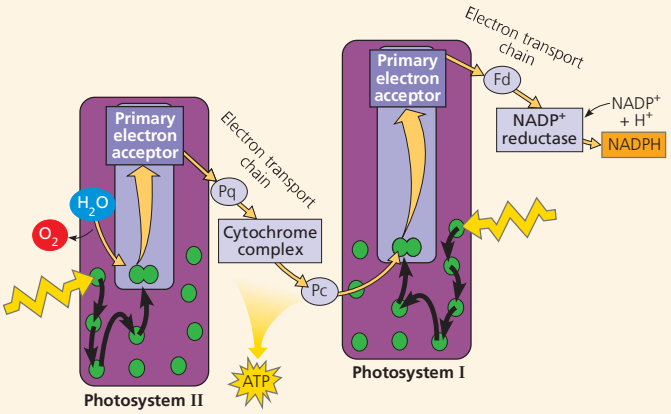
  Chloroplasts split water into hydrogen and oxygen, incorporating the electrons of hydrogen into sugar molecules. Photosynthesis is a redox process: H_2O is oxidized, and CO_2 is reduced. The **light reactions** in the thylakoid membranes split water, releasing O_2, producing ATP, and forming **NADPH**. The **Calvin cycle** in the **stroma** forms sugar from CO_2, using ATP for energy and NADPH for reducing power.

? *Compare the roles of CO_2 and H_2O in cellular respiration and photosynthesis.*

CONCEPT 10.3

The light reactions convert solar energy to the chemical energy of ATP and NADPH *(pp. 192–201)*

- Light is a form of electromagnetic energy. The colors we see as **visible light** include those **wavelengths** that drive photosynthesis. A pigment absorbs light of specific wavelengths; **chlorophyll *a*** is the main photosynthetic pigment in plants. Other accessory pigments absorb different wavelengths of light and pass the energy on to chlorophyll *a*.
- A pigment goes from a ground state to an excited state when a **photon** of light boosts one of the pigment's electrons to a higher-energy orbital. This excited state is unstable. Electrons from isolated pigments tend to fall back to the ground state, giving off heat and/or light.
- A **photosystem** is composed of a **reaction-center complex** surrounded by **light-harvesting complexes** that funnel the energy of photons to the reaction-center complex. When a special pair of reaction-center chlorophyll *a* molecules absorbs energy, one of its electrons is boosted to a higher energy level and transferred to the **primary electron acceptor. Photosystem II** contains P680 chlorophyll *a* molecules in the reaction-center complex; **photosystem I** contains P700 molecules.
- **Linear electron flow** during the light reactions uses both photosystems and produces NADPH, ATP, and oxygen:



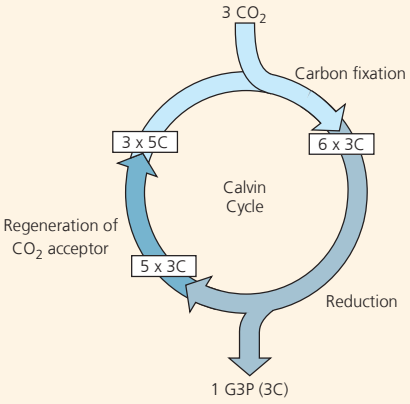
- **Cyclic electron flow** employs only one photosystem, producing ATP but no NADPH or O_2.
- During chemiosmosis in both mitochondria and chloroplasts, electron transport chains generate an H^+ gradient across a membrane. ATP synthase uses this proton-motive force to make ATP.

? *The absorption spectrum of chlorophyll a differs from the action spectrum of photosynthesis. Explain this observation.*

CONCEPT 10.4

The Calvin cycle uses the chemical energy of ATP and NADPH to reduce CO_2 to sugar *(pp. 201–202)*

- The Calvin cycle occurs in the stroma, using electrons from NADPH and energy from ATP.
- One molecule of **G3P** exits the cycle per three CO_2 molecules fixed and is converted to glucose and other organic molecules.



DRAW IT *On the diagram above, draw where ATP and NADPH are used and where rubisco functions. Describe these steps.*

CONCEPT 10.5

Alternative mechanisms of carbon fixation have evolved in hot, arid climates *(pp. 203–206)*

- On dry, hot days, **C_3 plants** close their stomata, conserving water but keeping CO_2 out and O_2 in. Under these conditions, **photorespiration** can occur: **Rubisco** binds O_2 instead of CO_2, consuming ATP and releasing CO_2 without producing ATP or carbohydrate. Photorespiration may be an evolutionary relic, and it may play a photoprotective role.
- C_4 and CAM plants are adopted to hot, dry climates.

- **C_4 plants** minimize the cost of photorespiration by incorporating CO_2 into four-carbon compounds in mesophyll cells. These compounds are exported to **bundle-sheath cells**, where they release carbon dioxide for use in the Calvin cycle.
- **CAM plants** open their stomata at night, incorporating CO_2 into organic acids, which are stored in mesophyll cells. During the day, the stomata close, and the CO_2 is released from the organic acids for use in the Calvin cycle.
- Organic compounds produced by photosynthesis provide the energy and building material for Earth's ecosystems.

? *Why are C_4 and CAM photosynthesis more energetically expensive than C_3 photosynthesis? What climate conditions would favor C_4 and CAM plants?*

CONCEPT 10.6

Photosynthesis is essential for life on Earth: a review

(pp. 206–209)

- Organic compounds produced by photosynthesis provide the energy and building material for Earth's ecosystems.

? *How do plants use the products of photosynthesis?*

TEST YOUR UNDERSTANDING

For more multiple-choice questions, go to the **Practice Test** in the **Mastering Biology** eText or Study Area, or go to goo.gl/GruWRg.

Levels 1-2: Remembering/Understanding

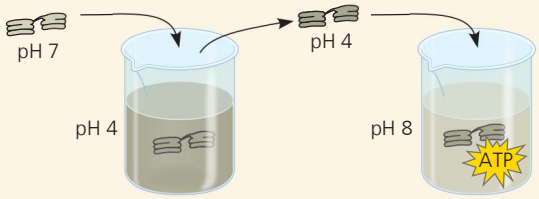
1. The light reactions supply the Calvin cycle with
   (A) light energy.
   (B) CO_2 and ATP.
   (C) H_2O and NADPH.
   (D) ATP and NADPH.
2. Which of the following sequences correctly represents the flow of electrons during photosynthesis?
   (A) NADPH → O_2 → CO_2
   (B) H_2O → NADPH → Calvin cycle
   (C) H_2O → photosystem I → photosystem II
   (D) NADPH → electron transport chain → O_2
3. How is photosynthesis similar in C_4 plants and CAM plants?
   (A) In both cases, only photosystem I is used.
   (B) Both types of plants make sugar without the Calvin cycle.
   (C) In both cases, rubisco is not used to fix carbon initially.
   (D) Both types of plants make most of their sugar in the dark.
4. Which of the following statements is a correct distinction between autotrophs and heterotrophs?
   (A) Autotrophs, but not heterotrophs, can nourish themselves beginning with CO_2 and other nutrients that are inorganic.
   (B) Only heterotrophs require chemical compounds from the environment.
   (C) Cellular respiration is unique to heterotrophs.
   (D) Only heterotrophs have mitochondria.
5. Which of the following occurs during the Calvin cycle?
   (A) carbon fixation
   (B) reduction of $NADP^+$
   (C) release of oxygen
   (D) generation of CO_2

Levels 3-4: Applying/Analyzing

6. In mechanism, photophosphorylation is most similar to
   (A) substrate-level phosphorylation in glycolysis.
   (B) oxidative phosphorylation in cellular respiration.
   (C) carbon fixation.
   (D) reduction of $NADP^+$.
7. Which process is most directly driven by light energy?
   (A) creation of a pH gradient by pumping protons across the thylakoid membrane
   (B) reduction of $NADP^+$ molecules
   (C) transfer of energy from pigment molecule to pigment molecule
   (D) ATP synthesis

Levels 5-6: Evaluating/Creating

8. **SCIENCE, TECHNOLOGY, AND SOCIETY** Scientific evidence indicates that the CO_2 added to the air by the burning of wood and fossil fuels is contributing to global warming, a rise in global temperature. Tropical rain forests are estimated to be responsible for approximately 20% of global photosynthesis, yet the consumption of large amounts of CO_2 by living trees is thought to make little or no *net* contribution to reduction of global warming. Explain why this might be the case. (*Hint*: What processes in both living and dead trees produce CO_2?)
9. **EVOLUTION CONNECTION** Photorespiration can decrease soybeans' photosynthetic output by about 50%. Would this figure be higher or lower in wild relatives of soybeans? Why?
10. **SCIENTIFIC INQUIRY • DRAW IT** The following diagram represents an experiment with isolated thylakoids. The thylakoids were first made acidic by soaking them in a solution at pH 4. After the thylakoid space reached pH 4, the thylakoids were transferred to a basic solution at pH 8. The thylakoids then made ATP in the dark. (See Concept 3.3 to review pH.)



Draw an enlargement of part of the thylakoid membrane in the beaker with the solution at pH 8. Draw ATP synthase. Label the areas of high H^+ concentration and low H^+ concentration. Show the direction protons flow through the enzyme, and show the reaction where ATP is synthesized. Would ATP end up in the thylakoid or outside of it? Explain why the thylakoids in the experiment were able to make ATP in the dark.

11. **WRITE ABOUT A THEME: ENERGY AND MATTER** In a short essay (100–150 words), describe how photosynthesis transforms solar energy into the chemical energy of sugar molecules.
12. **SYNTHESIZE YOUR KNOWLEDGE**

"Watermelon snow" in Antarctica is caused by a certain species of photosynthetic green algae that thrives in subzero temperatures (*Chlamydomonas nivalis*). These algae are also found in high-altitude, year-round snowfields. In both locations, UV light levels tend to be high. Propose an explanation for why this alga appears reddish-pink.

For selected answers, see Appendix A.

Explore Scientific Papers with Science in the Classroom AAAS
Can we capture the sun's energy by mimicking plants?
Go to "Replicating Photosynthesis"
at www.scienceintheclassroom.org.
Instructors: Questions can be assigned in Mastering Biology.

11 Cell Communication

KEY CONCEPTS

11.1 External signals are converted to responses within the cell *p. 213*

11.2 Signal reception: A signaling molecule binds to a receptor, causing it to change shape *p. 217*

11.3 Signal transduction: Cascades of molecular interactions transmit signals from receptors to relay molecules in the cell *p. 221*

11.4 Cellular response: Cell signaling leads to regulation of transcription or cytoplasmic activities *p. 226*

11.5 Apoptosis requires integration of multiple cell-signaling pathways *p. 229*

Study Tip

Make a table: As you read about examples of cell signaling in this chapter, make a table and classify the events of each example into three stages: signal reception, signal transduction, and cellular response.

Example of Cell Signaling	Signal Reception	Signal Transduction	Cellular Response
Epinephrine	Epinephrine binds cell-surface receptor.	Relay molecules each activate the next molecule.	An enzyme is activated that breaks down glycogen into glucose for energy to fight or flee.

Go to Mastering Biology

For Students (in eText and Study Area)
- Get Ready for Chapter 11
- Animation: Overview of Cell Signaling
- Animation: Signal Transduction Pathways

For Instructors to Assign (in Item Library)
- Problem-Solving Exercise: Can a Skin Wound Turn Deadly?
- Tutorial: Cell-Signaling: Transduction and Response

Figure 11.1 This impala is fleeing for its life, racing to escape the predatory cheetah nipping at its heels. The impala is breathing rapidly, its heart pounding and its legs pumping furiously. These physiological functions are all part of the impala's "fight-or-flight" response, driven by hormones released from its adrenal glands at times of stress—in this case, upon sensing the cheetah.

How does cell signaling fuel the desperate flight of an impala?

The impala senses a cheetah.

↓

Its brain signals the adrenal glands to release epinephrine into the blood.

↓

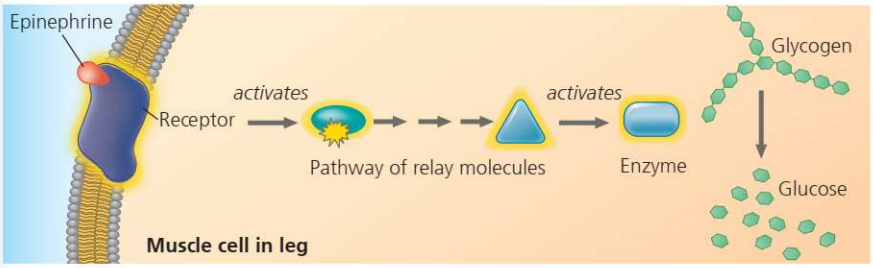
Signal reception
An epinephrine molecule binds to a receptor on a muscle cell.

→

Signal transduction
Relay molecules transmit the signal, ultimately activating an enzyme.

→

Cellular response
The enzyme breaks down glycogen, releasing glucose that fuels the leg muscles.



CONCEPT 11.1

External signals are converted to responses within the cell

Scientists think that signaling mechanisms first evolved hundreds of millions of years ago in ancient prokaryotes and single-celled eukaryotes and then were adapted for new uses in their multicellular descendants. So let's begin by considering signaling in some examples of single-celled organisms: bacteria and yeasts.

Evolution of Cell Signaling

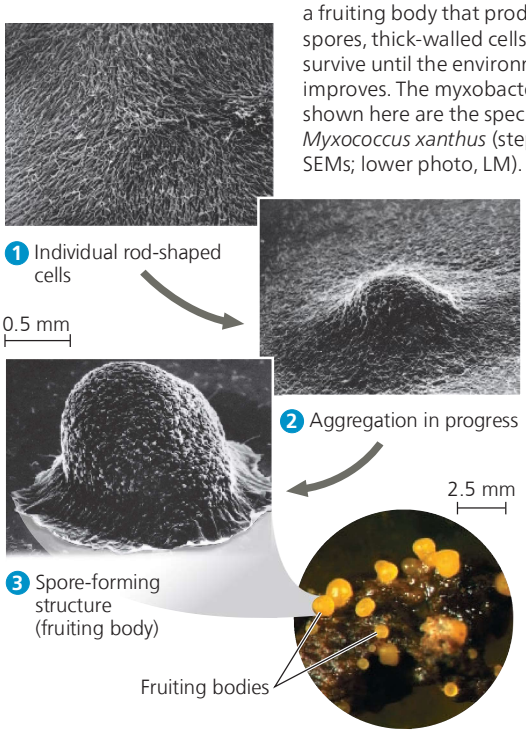
EVOLUTION Research during the 1970s suggested that bacterial cells—somewhat surprisingly, since they are single-celled organisms—were capable of signaling to each other. Since then, we have come to understand that cell signaling is critical among prokaryotes. Bacterial cells secrete molecules that can be detected by other bacterial cells **(Figure 11.2)**. Sensing the concentration of such signaling molecules allows bacteria to monitor their own local cell density, a phenomenon called *quorum sensing*.

Quorum sensing allows bacterial populations to coordinate the behavior of all cells in a population in activities that require a given density of cells acting at the same time. One example is formation of a *biofilm*, an aggregation of bacterial cells attached to a surface by molecules secreted by the cells, but only after the cells have reached a certain density. The biofilm protects the cells in it, and they often derive nutrition from the surface they are on. Biofilms are believed to be involved in up to 80% of all human bacterial infections. You have probably encountered biofilms many times, perhaps without realizing it. The slimy coating on a fallen log or on leaves lying on a forest path, and even the film on your teeth each morning, are examples of bacterial biofilms. In fact, tooth-brushing and flossing disrupt biofilms that would otherwise cause cavities and gum disease.

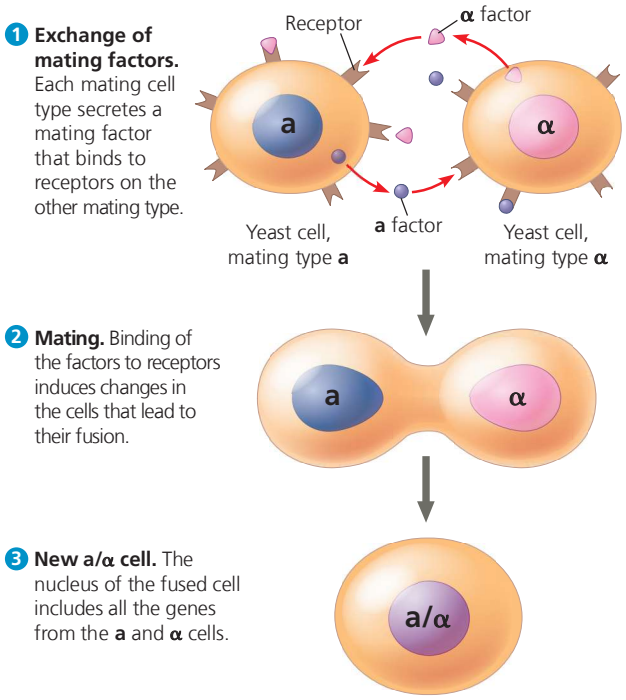
Another example of bacterial behavior coordinated by quorum sensing is the secretion of toxins by infectious bacteria, which has serious medical implications. Sometimes treatment by antibiotics doesn't work with such infections because of antibiotic resistance that has evolved in a particular strain of bacteria. A promising alternative treatment would be to disrupt toxin production by interfering with the signaling pathways used in quorum sensing. In the **Problem-Solving Exercise**, you can participate in the process of scientific thinking involved in this novel approach.

Now let's look at an example of cell signaling in yeasts (single-celled fungi). Cells of the yeast *Saccharomyces cerevisiae*—which are used to make bread, wine, and beer—identify their sexual mates by chemical signaling when they reproduce sexually. There are two sexes, or mating types, called **a** and **α** **(Figure 11.3)**. Each type secretes a specific

▼ Figure 11.2 Communication among bacteria. Soil-dwelling bacteria called myxobacteria ("slime bacteria") use chemical signals to share information about nutrient availability. When food is scarce, starving cells secrete a signaling molecule that stimulates neighboring cells to aggregate. The cells form a structure called a fruiting body that produces spores, thick-walled cells that can survive until the environment improves. The myxobacteria shown here are the species *Myxococcus xanthus* (steps 1–3, SEMs; lower photo, LM).



▼ Figure 11.3 Communication between mating yeast cells. *Saccharomyces cerevisiae* cells use chemical signaling to identify cells of the opposite mating type and initiate the mating process. The two mating types and their corresponding chemical signaling molecules, or mating factors, are called **a** and **α**.



PROBLEM-SOLVING EXERCISE

Can a skin wound turn deadly?

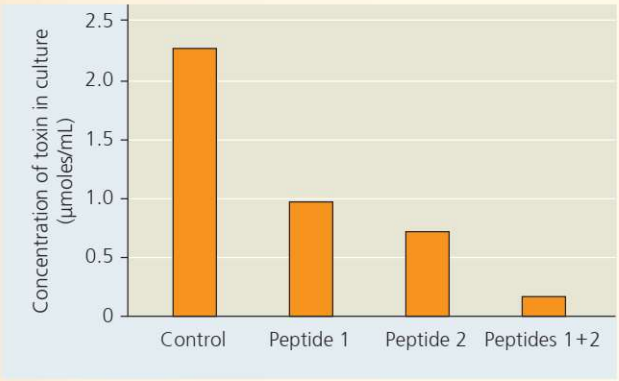
Staphylococcus aureus (S. aureus) is a common bacterial species found on the surface of healthy skin that can turn into a serious pathogen if introduced into tissue through a cut or abrasion. Once *S. aureus* cells enter the body and reach a certain density, they secrete a toxin that kills body cells and contributes significantly to inflammation and damage. Because about one in 100 people carry a strain of *S. aureus* that is resistant to common antibiotics, a minor infection can turn permanently harmful or even deadly.

Cells sense their own population density by *quorum sensing*, and at a certain density they start to secrete toxin. In this exercise, you will analyze whether blocking quorum sensing can stop *S. aureus* from producing toxin.

Your Approach In *S. aureus,* quorum sensing involves two separate signal transduction pathways. Two candidate synthetic peptides (short proteins), called peptides 1 and 2, have been proposed to interfere with *S. aureus* quorum-sensing pathways. Your job is to test each potential inhibitor of quorum sensing to see if it blocks either or both of the pathways that lead to toxin production.

For your experiment, you grow four cultures of *S. aureus* to a standardized high density and measure the concentration of toxin in the culture. The control culture contains no peptide. The other cultures have one or both candidate inhibitory peptides mixed into the growth medium before starting the cultures.

Your Data



Data from N. Balaban et al., Treatment of *Staphylococcus aureus* biofilm infection by the quorum-sensing inhibitor RIP, *Antimicrobial Agents and Chemotherapy* 51(6):2226–2229 (2007).

Your Analysis

1. Rank the cultures according to toxin production, from most to least.
2. Which, if any, of the cultures with peptide(s) resulted in a toxin concentration similar to the control culture? What is your evidence for this?
3. Was there an additive effect on toxin production when peptides 1 and 2 were both present in the growth medium? What is your evidence for this?
4. Based on these data, would you hypothesize that peptides 1 and 2 act on the same quorum-sensing pathway leading to toxin production or on two different pathways? What is your reasoning?
5. Do these data suggest a possible treatment for antibiotic-resistant *S. aureus* infections? What else would you want to know to investigate this further?

Instructors: A version of this Problem-Solving Exercise can be assigned in **Mastering Biology**. Or a more extensive investigation called "Solve It: Is It Possible to Treat Bacterial Infections Without Traditional Antibiotics?" can be assigned.

Mastering Biology
HHMI Video: Interview with Bonnie Bassler
BBC Video: Brushing Your Teeth Can Save Your Life

Mastering Biology **Interview with Bonnie Bassler: Exploring how bacteria communicate with each other**

factor that binds only to receptors on the other type of cell. When exposed to each other's mating factors, a pair of cells of opposite type change shape, grow toward each other, and fuse (mate). The new **a**/**α** cell contains all the genes of both original cells, providing advantages to the cell's descendants, which arise by subsequent cell divisions.

The unique match between mating factor and receptor is key to ensuring mating only between cells of the same species of yeast. How does the binding of a mating factor by the yeast cell-surface receptor initiate a signal that brings about the cellular response of mating? This occurs in a series of three major steps—signal reception, signal transduction, and cellular response—called a *signal transduction pathway*. Many such pathways exist in the cells of both unicellular and multicellular organisms. In fact, the molecular details of signal transduction in yeasts and mammals are strikingly similar, even though it's been over a billion years since they shared a common ancestor. This similarity suggests that early versions of cell-signaling mechanisms evolved well before the first multicellular organisms appeared on Earth.

Local and Long-Distance Signaling

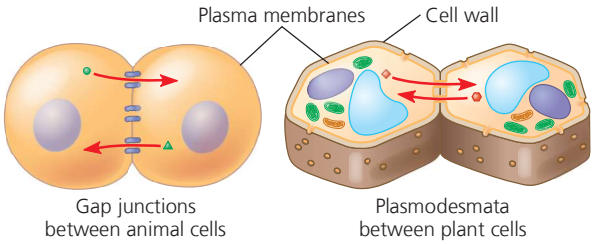
Like bacteria or yeast cells, cells in a multicellular organism communicate by way of a signaling molecules targeted for cells that may or may not be immediately adjacent. As we saw in Concepts 6.7 and 7.1, eukaryotic cells may communicate by direct contact, which is one type of local signaling. Many animal and plant cells have cell junctions that directly connect the cytoplasms of adjacent cells **(Figure 11.4a)**. In these cases, signaling substances dissolved in the cytosol can pass between neighboring cells. Moreover, some animal cells may communicate by direct contact between cell-surface molecules, as shown in **Figure 11.4b**. This type of local signaling is especially important in embryonic development, the immune response, and in maintaining adult stem cell populations.

In many other cases of local signaling, signaling molecules are secreted by the signaling cell. Some molecules travel only short distances; such local regulators influence cells that are nearby. This type of local signaling in animals is called *paracrine signaling* **(Figure 11.5a)**. One class of local regulators in animals, *growth factors*, consists of compounds that stimulate nearby target cells to grow and divide. Numerous cells can simultaneously receive and respond to the growth factors produced by a single cell in their vicinity.

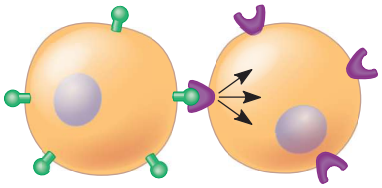
A highly specialized type of local signaling called *synaptic signaling* occurs in the animal nervous system **Figure 11.5b**; see Concept 48.4. An electrical signal along a nerve cell triggers the secretion of neurotransmitter molecules. These molecules act as chemical signals, diffusing across the synapse—the narrow space between the nerve cell and its target cell—triggering a response in the target cell. Drugs to treat depression, anxiety, and post-traumatic stress disorder (PTSD) affect this signaling process.

Both animals and plants use molecules called **hormones** for long-distance signaling. In hormonal signaling in animals, also known as *endocrine signaling*, specialized cells release hormones, which travel through the circulatory system to other parts of the body, where they reach target cells that can recognize and respond to them **(Figure 11.5c)**. Many

▼ Figure 11.4 Communication requiring contact between cells.



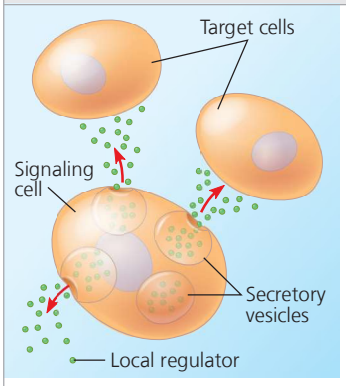
(a) Cell junctions. Both animals and plants have cell junctions that allow molecules, including signaling molecules, to pass readily between adjacent cells without crossing plasma membranes.



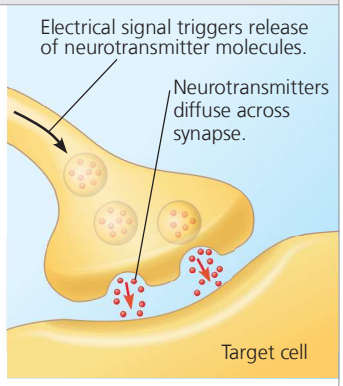
(b) Cell-surface molecules. In many animal cells, cell-surface molecules on adjacent cells interact with each other, resulting in a signal passing between the cells.

▼ Figure 11.5 Local and long-distance cell signaling by secreted molecules in animals. In both local and long-distance signaling, only specific target cells that can recognize a given signaling molecule will respond to it.

Local signaling: up to a few cells' distance

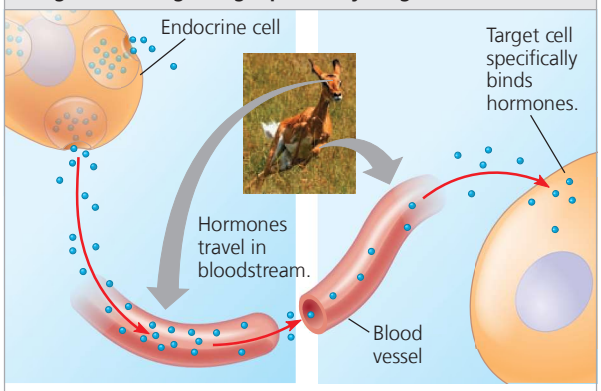


(a) Paracrine signaling. A signaling cell acts on nearby target cells by secreting molecules of a local regulator (a growth factor, for example).



(b) Synaptic signaling. A nerve cell releases neurotransmitter molecules into a synapse, stimulating the target cell, such as a muscle or another nerve cell.

Long-distance signaling: up to body-length distance



(c) Endocrine (hormonal) signaling. Specialized endocrine cells secrete hormones into body fluids, often blood. Hormones reach most body cells, but are bound by and affect only some cells.

plant hormones reach distant targets by traveling through cells (see Concept 39.2). Like local regulators, plant hormones vary widely in size and type. For instance, the plant hormone ethylene, which promotes fruit ripening and helps regulate growth, is a hydrocarbon of only six atoms (C_2H_4), small enough to diffuse through the air and pass through cell walls. (Small signaling molecules probably evolved early on among single-celled organisms.) In contrast, the mammalian hormone insulin, which regulates sugar levels in the blood, is a protein with thousands of atoms.

What happens when a potential target cell is exposed to a secreted signaling molecule? The ability of a cell to respond is determined by whether it has a specific receptor molecule that can bind to the signaling molecule. The information conveyed by this binding, the signal, must then be changed into another form—transduced—inside the cell before the cell can respond. The remainder of the chapter discusses this process, primarily as it occurs in animal cells. (Two signaling pathway proteins are shown in their cellular context in Figure 6.32a.)

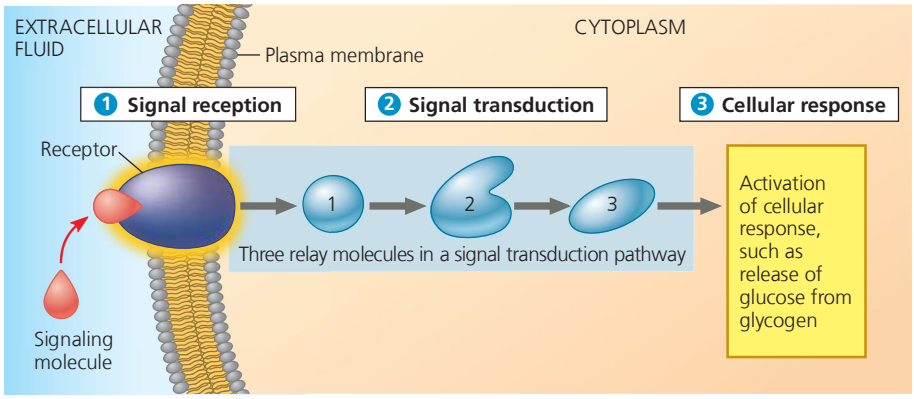
The Three Stages of Cell Signaling: *A Preview*

Our current understanding of how signaling molecules act through signal transduction pathways had its origins in the pioneering work of Earl W. Sutherland, whose research led to a Nobel Prize in 1971. Sutherland and his colleagues at Vanderbilt University were investigating how the animal hormone epinephrine (also called adrenaline) triggers the "fight-or-flight" response in animals like the impala in Figure 11.1. One effect of epinephrine is to mobilize fuel reserves, which can be used by the animal to either defend itself (fight) or try to escape (flight). Epinephrine stimulates the breakdown of the storage polysaccharide glycogen within liver cells and skeletal muscle cells. The breakdown of glycogen releases the sugar glucose 1-phosphate, which the cell converts to glucose 6-phosphate. The liver or muscle cell can then use this compound, an early intermediate in glycolysis, for energy production (see Figure 9.8). Alternatively, the compound can be stripped of phosphate and released from the liver cell into the blood as glucose, which can fuel cells throughout the body.

But how, exactly, does epinephrine mobilize glucose for use? Sutherland's research team discovered that epinephrine outside the cell stimulates glycogen breakdown by somehow activating an enzyme, glycogen phosphorylase, inside the cell. However, when epinephrine was added to a solution containing the enzyme and its substrate, glycogen, no breakdown occurred. Glycogen phosphorylase could be activated by epinephrine only when the hormone was added to intact cells. This result told Sutherland two things. First, epinephrine does not interact directly with the enzyme responsible for glycogen breakdown; an intermediate step or series of steps must be occurring in the cell. Second, an intact, membrane-bound cell must be present for transmission of the signal to take place.

Sutherland's work suggested that the process going on at the receiving end of a cellular communication can be dissected into three stages: signal reception, signal transduction, and cellular response **(Figure 11.6)**:

1. **Signal reception.** Reception is the target cell's detection of a signaling molecule coming from outside the cell. A chemical signal is "detected" when the signaling molecule binds to a receptor protein located at the cell's surface (or inside the cell, to be discussed later).
2. **Signal transduction.** The binding of the signaling molecule changes the receptor protein in some way, initiating the process of transduction. The transduction stage converts the signal to a form that can bring about a specific cellular response. In Sutherland's system, the binding of epinephrine to a receptor protein in a liver cell's plasma membrane leads to activation of glycogen phosphorylase in the cytosol. Transduction sometimes occurs in a single step but more often requires a sequence of changes in a series of different molecules—a **signal transduction pathway**. The molecules in the pathway are often called relay molecules; three are shown as an example.



▶ Figure 11.6 Overview of cell signaling. From the perspective of the cell receiving the message, cell signaling can be divided into three stages: signal reception, signal transduction, and cellular response. When reception occurs at the plasma membrane, as shown here, the transduction stage is usually a pathway of several steps (three are shown as an example), with each specific relay molecule in the pathway bringing about a change in the next molecule. The final molecule in the pathway triggers the cell's response.

VISUAL SKILLS *Where would the epinephrine in Sutherland's experiment fit into this diagram of cell signaling?*

Mastering Biology **Animation: Overview of Cell Signaling**

3 **Cellular response.** The transduced signal finally triggers a specific cellular response. The response may be almost any imaginable cellular activity—such as catalysis by an enzyme (for example, glycogen phosphorylase), rearrangement of the cytoskeleton, or activation of specific genes in the nucleus. The cell-signaling process helps ensure that crucial activities like these occur in the right cells, at the right time, and in proper coordination with the activities of other cells of the organism. We'll now explore the mechanisms of cell signaling in more detail, including a discussion of regulation and termination of the process.

CONCEPT CHECK 11.1

1. Explain how signaling is involved in ensuring that yeast cells fuse only with cells of the opposite mating type.
2. In liver cells, glycogen phosphorylase acts in which of the three stages of the signaling pathway associated with an epinephrine-initiated signal?
3. **WHAT IF?** If epinephrine were mixed with glycogen phosphorylase and glycogen in a cell-free mixture in a test tube, would glucose 1-phosphate be generated? Why or why not?

For suggested answers, see Appendix A.

CONCEPT 11.2

Signal reception: A signaling molecule binds to a receptor, causing it to change shape

A wireless router may broadcast its network signal indiscriminately, but only computers with the correct password can connect to it: Reception of the signal depends on the receiver. Similarly, the signals emitted by an **a** mating type yeast cell are "heard" only by its prospective mates, **α** cells. In the case of the epinephrine circulating throughout the bloodstream of the impala in Figure 11.1, the hormone encounters many types of cells, but only certain target cells, those with the corresponding receptor protein, detect and respond to the epinephrine molecule. The signaling molecule is complementary in shape to a specific site on the receptor and attaches there, like a hand in a glove. The signaling molecule acts as a **ligand**, the term for a molecule that specifically binds to another (often larger) molecule. Ligand binding generally causes a receptor protein to undergo a change in shape. For many receptors, this shape change directly activates the receptor, enabling it to interact with other molecules in or on the cell. For other receptors, the immediate effect of ligand binding is to cause the aggregation of two or more receptor proteins, which leads to further molecular events inside the cell. Most signal receptors are plasma membrane proteins, but others are located inside the cell. We'll look at both of these types next.

Mastering Biology Animation: Reception

Receptors in the Plasma Membrane

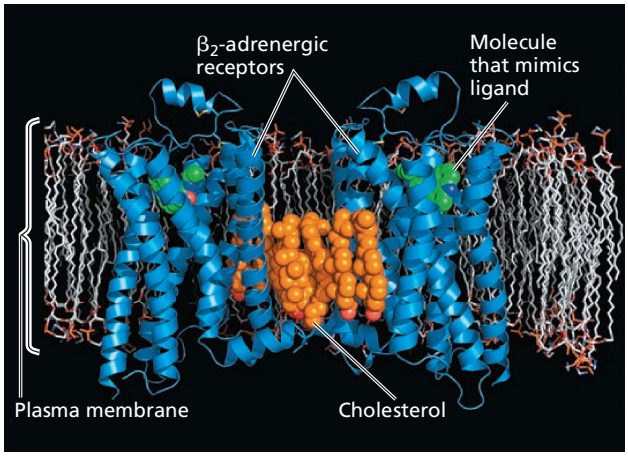
Cell-surface transmembrane receptors play crucial roles in the biological systems of animals. The largest family of human cell-surface receptors is that of the G protein-coupled receptors (GPCRs). There are more than 800 GPCRs; an example is shown in **Figure 11.7**. Another example is the co-receptor hijacked by HIV to enter immune cells (see Figure 7.8); this GPCR is the target of the drug maraviroc, which has shown some success at treating AIDS.

Most water-soluble signaling molecules bind to specific sites on transmembrane receptor proteins that transmit information from the extracellular environment to the inside of the cell. We can see how cell-surface transmembrane receptors work by looking at three major types: G protein-coupled receptors (GPCRs), receptor tyrosine kinases, and ion channel receptors. These receptors are discussed and illustrated in **Figure 11.8**; study this figure before going on.

Given the many important functions of cell-surface receptors, it is not surprising that their malfunctions are associated with many human diseases, including cancer, heart disease, and asthma. To better understand and treat these conditions, a major focus of both university research teams and the pharmaceutical industry has been to analyze the structure of these receptors.

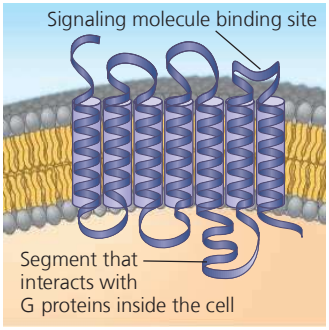
Although cell-surface receptors (half of which are GPCRs) represent 30% of all human proteins, determining their structures by X-ray crystallography (see Figure 5.21) has proved challenging. For one thing, cell-surface receptors tend to be flexible and inherently unstable, thus difficult to crystallize. It took years of persistent efforts for researchers to determine the first few of these structures, such as the GPCR

▼ Figure 11.7 The structure of a G protein-coupled receptor (GPCR). This is a model of the human β_2-adrenergic receptor, which binds epinephrine (adrenaline). The receptor was crystallized (discussed later in this section) in the presence of both a molecule that mimics epinephrine (green in the model) and cholesterol in the membrane (orange). Two receptors (blue) are shown as ribbon models in a side view. Caffeine can also bind to this receptor; see question 10 at the end of the chapter.



▼ Figure 11.8 Exploring Cell-Surface Transmembrane Receptors

G Protein-Coupled Receptors



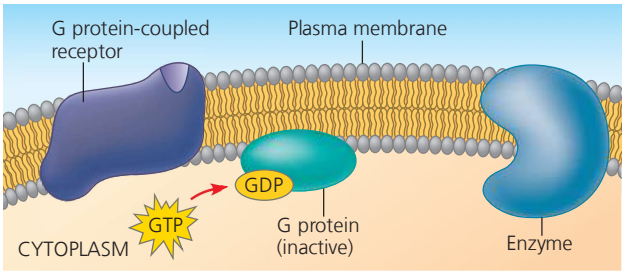
G protein-coupled receptor

A **G protein-coupled receptor** (GPCR) is a cell-surface transmembrane receptor that works with the help of a **G protein**, a protein that binds the energy-rich molecule GTP. Many different signaling molecules—including yeast mating factors, neurotransmitters, and epinephrine (adrenaline) and many other hormones—use GPCRs.

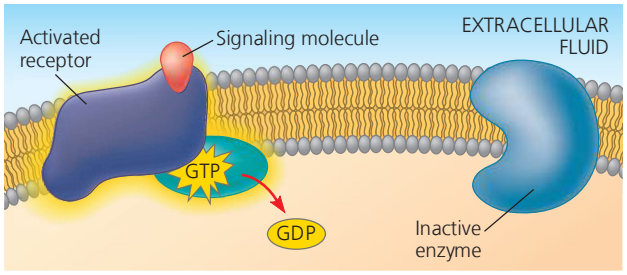
G protein-coupled receptors vary in the binding sites for their ligands and also for different types of G proteins inside the cell. Nevertheless, GPCR proteins are all remarkably similar in structure. In fact, they make up a large family of eukaryotic receptor proteins with a secondary structure in which the single polypeptide, represented here in a ribbon model, has seven transmembrane α helices (outlined with cylinders and depicted in a row for clarity). Specific loops between the helices (here, the loops on the right) form binding sites for signaling molecules (outside the cell) and G proteins (on the cytoplasmic side).

GPCR-based signaling systems are extremely widespread and diverse in their functions, including roles in embryonic development and sensory reception. In humans, for example, vision, smell, and taste depend on GPCRs (see Concept 50.4). Similarities in the structures of G proteins and GPCRs in diverse organisms suggest that G proteins and their associated receptors evolved very early among eukaryotes.

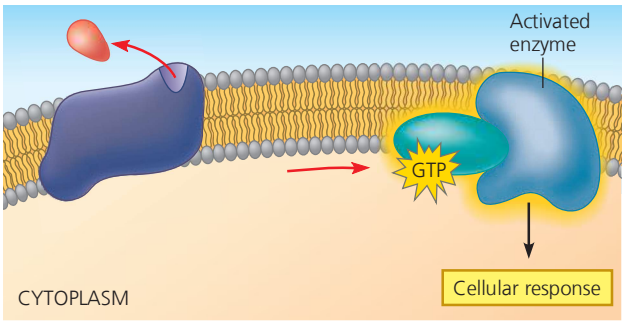
Malfunctions of the associated G proteins themselves are involved in many human diseases, including bacterial infections. The bacteria that cause cholera, pertussis (whooping cough), and botulism, among others, make their victims ill by producing toxins that interfere with G protein function. Up to 60% of all medicines used today exert their effects by influencing G protein pathways.



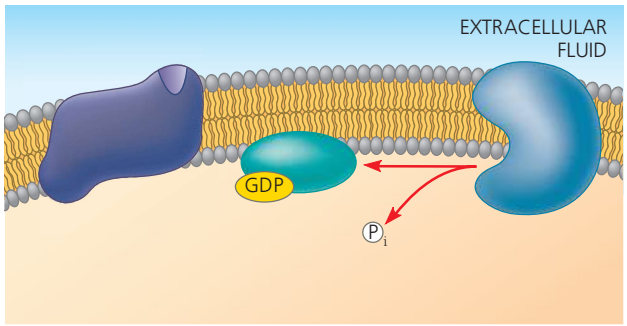
1 Attached but able to move along the cytoplasmic side of the membrane, a G protein functions as a molecular switch that is either on or off, depending on whether GDP or GTP is attached—hence the term *G protein*. (GTP, or guanosine triphosphate, is similar to ATP.) When GDP is bound to the G protein, as shown above, the G protein is inactive. The receptor and G protein work together with another protein, usually an enzyme.



2 When the appropriate signaling molecule binds to the extracellular side of the receptor, the receptor is activated and changes shape. Its cytoplasmic side then binds an inactive G protein, causing a GTP to displace the GDP. This activates the G protein.



3 The activated G protein dissociates from the receptor, diffuses along the membrane, and then binds to an enzyme, altering the enzyme's shape and activity. Once activated, the enzyme can trigger the next step leading to a cellular response. Binding of signaling molecules is reversible: Like other ligands, they bind and dissociate many times. The ligand concentration outside the cell determines how often a ligand is bound and initiates signaling.

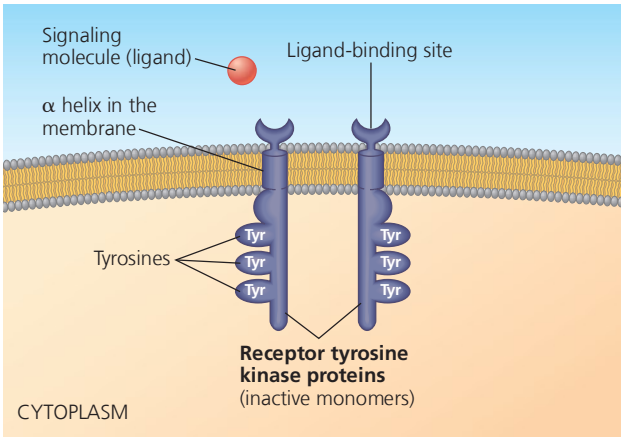


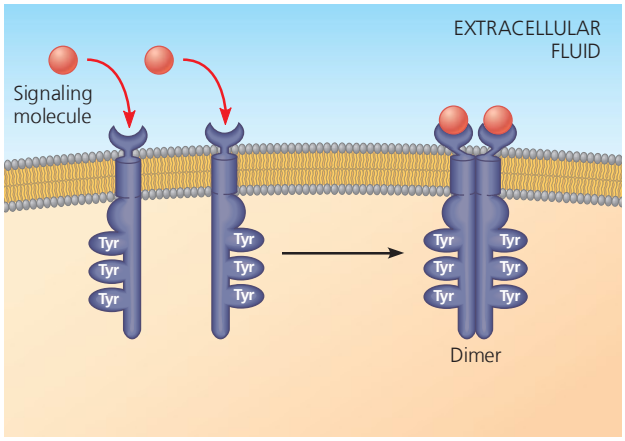
4 The changes in the enzyme and G protein are only temporary: The G protein also acts as a GTPase, hydrolyzing its bound GTP to GDP and P_i; as a result, it can no longer activate the enzyme. The G protein leaves the enzyme, which returns to its inactive state. The G protein is now available for reuse. Its GTPase function allows the pathway shut down rapidly when the signaling molecule is no longer present.

Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs) belong to a major class of plasma membrane receptors characterized by having enzymatic activity. An RTK is a *protein kinase*—an enzyme that catalyzes the transfer of phosphate groups from ATP to another protein. The part of the receptor protein extending into the cytoplasm functions more specifically as a tyrosine kinase, an enzyme that catalyzes the transfer of a phosphate group from ATP to the amino acid tyrosine of a substrate protein. Thus, RTKs are membrane receptors that attach phosphates to tyrosines.

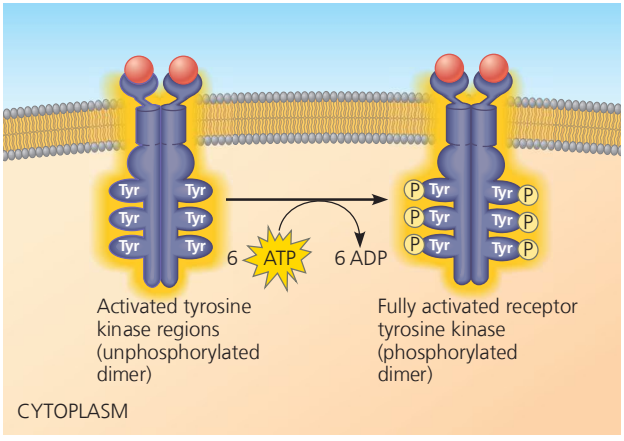
Upon binding a ligand such as a growth factor, one RTK may activate ten or more different transduction pathways and cellular responses. Often, more than one signal transduction pathway can be triggered at once, helping the cell regulate and coordinate many aspects of cell growth and cell reproduction. The ability of a single ligand-binding event to trigger so many pathways is a key difference between RTKs and GPCRs; GPCRs generally activate a single transduction pathway. Abnormal RTKs that function even in the absence of signaling molecules are associated with many kinds of cancer.

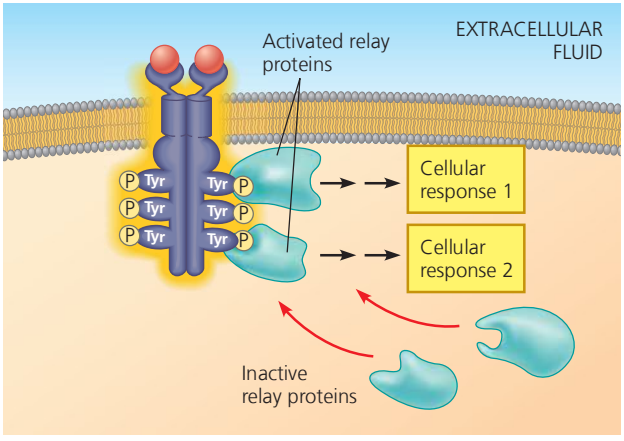




1 Many receptor tyrosine kinases have the structure depicted schematically here. Before the signaling molecule binds, the receptors exist as individual units referred to as monomers. Notice that each monomer has an extracellular ligand-binding site, an α helix spanning the membrane, and an intracellular tail containing multiple tyrosines.

2 The binding of a signaling molecule (such as a growth factor) causes two receptor monomers to associate closely with each other, forming a complex known as a dimer, a process called dimerization. (In some cases, larger clusters form. The details of monomer association are a focus of current research.)





3 Dimerization activates the tyrosine kinase region of each monomer; each tyrosine kinase adds a phosphate from an ATP molecule to a tyrosine that is part of the tail of the other monomer.

4 Now that the receptor is fully activated, it is recognized by specific relay proteins inside the cell. Each such protein binds to a specific phosphorylated tyrosine, undergoing a resulting structural change that activates the bound relay protein. Each activated protein triggers a transduction pathway, leading to a cellular response.

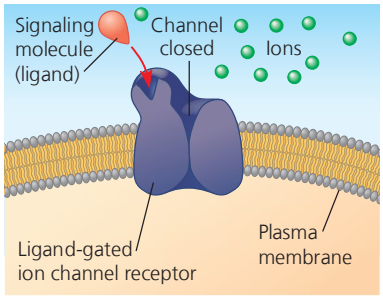
Continued on next page

▼ **Figure 11.8 (continued)**

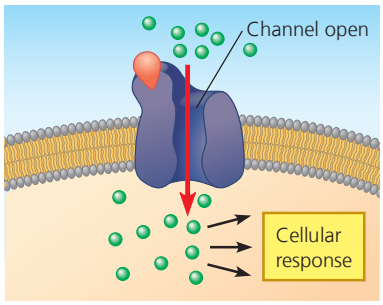
Ion Channel Receptors

A **ligand-gated ion channel** is a type of membrane channel receptor containing a region that can act as a "gate," opening or closing the channel when the receptor changes shape. When a signaling molecule binds as a ligand to the channel receptor, the channel opens or closes, allowing or blocking the flow of specific ions, such as Na^+ or Ca^{2+}. Like the other receptors we have discussed, these proteins bind the ligand at a specific site on their extracellular sides.

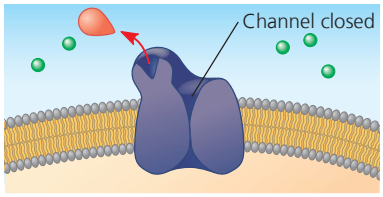
1 Here we show a ligand-gated ion channel receptor in which the channel remains closed until a ligand binds to the receptor.



2 When the ligand binds to the receptor and the channel opens, specific ions can flow through the channel and rapidly change the local concentration of that ion inside the cell. This change may directly affect the activity of the cell in some way.



3 When the ligand dissociates from this receptor, the channel closes and ions no longer enter the cell.



Ligand-gated ion channels are very important in the nervous system. For example, the neurotransmitter molecules released at a synapse between two nerve cells (see Figure 11.5b) bind as ligands to some ion channels on the receiving cell, causing the channels to open. Ions flow in (or, in some cases, out), triggering an electrical signal that propagates down the length of the receiving cell. Some of the gated ion channels are controlled by electrical signals instead of ligands; these voltage-gated ion channels are crucial to the functioning of the nervous system, as you'll see in Chapter 48. Some ion channels are present on membranes of organelles, such as the ER.

MAKE CONNECTIONS *Is the flow of ions through a ligand-gated channel an example of active or passive transport? (Review Concepts 7.3 and 7.4.)*

Mastering Biology **Animation: Acetylcholine Receptor**

shown in Figure 11.7. In that case, the β-adrenergic receptor was stable enough to be crystallized only while it was among membrane molecules and in the presence of a molecule mimicking its ligand. Newer techniques that do not require crystallization, like cryo-EM (see Figure 6.3), have shown promise in determining the structures of cell-surface receptors.

Abnormal functioning of receptor tyrosine kinases (RTKs) is associated with many types of cancers. For example, some breast cancer patients have tumor cells with excessive levels of a receptor tyrosine kinase called HER2 (see Concept 12.3 and Figure 18.27). Using molecular biological techniques, researchers have developed a protein called Herceptin that binds to HER2 on cells and inhibits cell division, thus thwarting further tumor development. In some clinical studies, treatment with Herceptin improved patient survival rates by more than one-third. One goal of ongoing research into these cell-surface receptors and other cell-signaling proteins is development of additional successful treatments.

Mastering Biology **Interview with Diana Bautista: How cell signaling leads to itch and pain (see the interview before Chapter 6)**

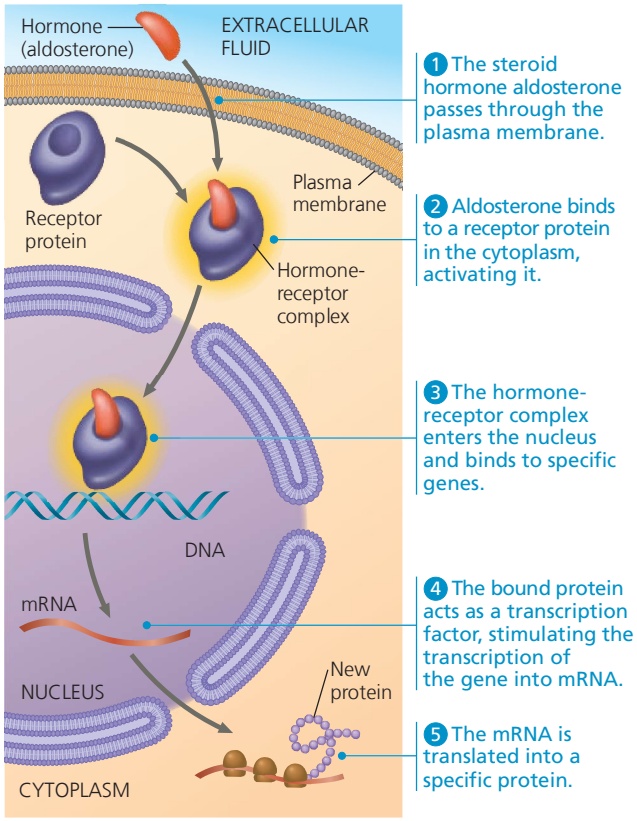
Intracellular Receptors

Intracellular receptor proteins are found in either the cytoplasm or nucleus of target cells. To reach such a receptor, a signaling molecule passes through the target cell's plasma membrane. A number of important signaling molecules can do this because they are either hydrophobic enough or small enough to cross the hydrophobic interior of the membrane (see Concept 7.1). Hydrophobic signaling molecules include steroid hormones and thyroid hormones of animals. Another chemical signaling molecule that possesses an intracellular receptor is nitric oxide (NO), a gas; this very small molecule readily passes between the membrane phospholipids. Once a hormone or other signaling molecule has entered a cell, its binding to an intracellular receptor changes the receptor into a hormone-receptor complex that is able to cause a response—in many cases, the turning on or off of particular genes.

The behavior of aldosterone is a representative example of how steroid hormones work. This hormone is secreted by cells of the adrenal gland (a gland that lies above the kidney), then travels through the blood and enters cells all over the body. However, a response occurs only in kidney cells because they alone contain receptors for aldosterone. In these cells, the hormone binds to and activates the receptor protein. With aldosterone attached, the active form of the receptor protein then enters the nucleus and turns on specific genes that control water and sodium flow in kidney cells, ultimately affecting blood volume **(Figure 11.9)**.

How does the activated hormone-receptor complex turn on genes? Recall that the genes in a cell's DNA

▼ Figure 11.9 Steroid hormone interacting with an intracellular receptor.



MAKE CONNECTIONS *Why is a cell-surface receptor protein not required for this steroid hormone to enter the cell? (See Concept 7.2.)*

Mastering Biology **Animation: Steroid Hormone Pathway**

function by being transcribed and processed into messenger RNA (mRNA), which leaves the nucleus and is translated into a specific protein by ribosomes in the cytoplasm (see Figure 5.22). Special proteins called *transcription factors* control which genes are turned on—that is, which genes are transcribed into mRNA—in a particular cell at a particular time. When the aldosterone receptor is activated, it acts as a transcription factor that turns on specific genes. (You'll learn more about transcription factors in Chapters 17 and 18.)

By acting as a transcription factor, the aldosterone receptor itself carries out two parts of the signaling pathway, as receptor and transducer. Most other intracellular receptors function in the same way, although many of them, such as the thyroid hormone receptor, are already in the nucleus before the signaling molecule reaches them. Interestingly, many of these intracellular receptor proteins are structurally similar, suggesting an evolutionary kinship.

CONCEPT CHECK 11.2

1. Nerve growth factor (NGF) is a water-soluble signaling molecule. Would you expect the receptor for NGF to be intracellular or in the plasma membrane? Explain.
2. **WHAT IF?** What would the effect be if a cell made defective receptor tyrosine kinase proteins that were unable to dimerize?
3. **MAKE CONNECTIONS** How is ligand binding similar to the process of allosteric regulation of enzymes? (See Figure 8.20.)

For suggested answers, see Appendix A.

CONCEPT 11.3

Signal transduction: Cascades of molecular interactions transmit signals from receptors to relay molecules in the cell

When receptors for signaling molecules are plasma membrane proteins, like most of those we have discussed, the transduction stage of cell signaling is usually a multistep pathway involving many molecules. Steps often include activation of proteins by addition or removal of phosphate groups or release of other small molecules or ions that act as signaling molecules. One benefit of using multiple steps is that a signal caused by a small number of signaling molecules can be greatly amplified. If each molecule transmits the signal to numerous molecules at the next step in the series, the result is a geometric increase in the number of activated molecules by the end (see Figure 11.16). A second benefit of using multistep pathways is that they provide more opportunities for coordination and control than do simpler systems. This allows regulation of the response, as we'll see later in the chapter.

Signal Transduction Pathways

The binding of a specific signaling molecule to a receptor in the plasma membrane triggers the first step in the signal transduction pathway—the chain of molecular interactions that leads to a particular response within the cell. Like falling dominoes, the signal-activated receptor activates another molecule, which activates yet another molecule, and so on, until the protein that produces the final cellular response is activated. The molecules that relay a signal from receptor to response, which we call relay molecules in this book, are often proteins. Protein-protein interactions are a major theme of cell signaling—indeed, a unifying theme of all cellular activities.

Mastering Biology **Animation: Signal Transduction Pathways**

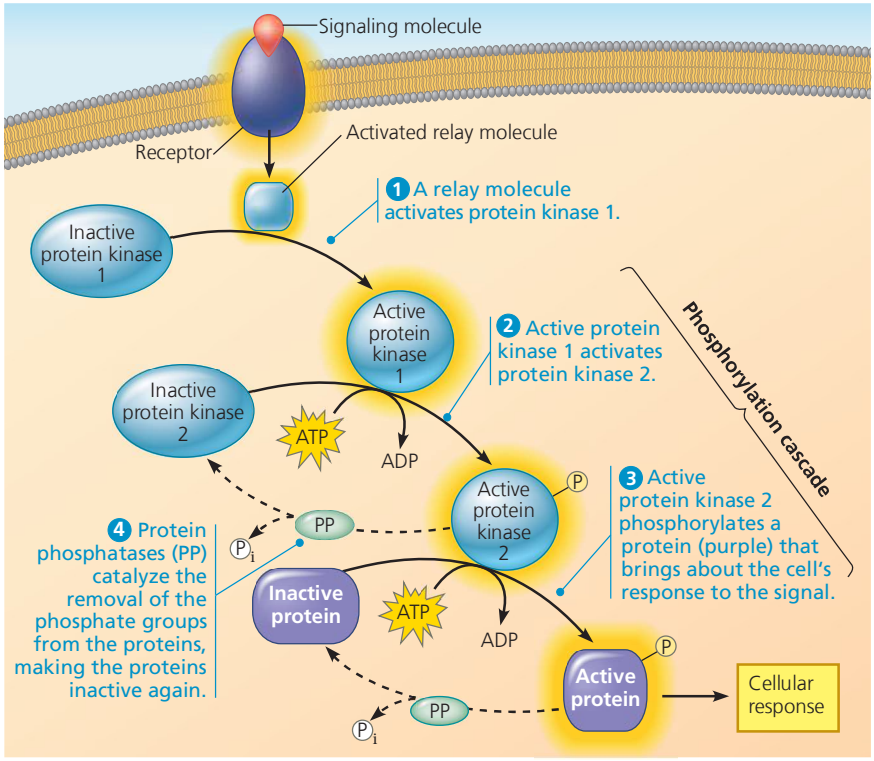
Keep in mind that the original signaling molecule is not physically passed along a signaling pathway; in most cases, it never even enters the cell. When we say that the signal is relayed along a pathway, we mean that certain information is passed on. At each step, the signal is transduced into a different form, commonly a shape change in the next protein. Very often, the shape change is brought about by phosphorylation.

Protein Phosphorylation and Dephosphorylation

Previous chapters introduced the concept of activating a protein by adding one or more phosphate groups to it (see Figure 8.11a). In Figure 11.8, you have already seen how phosphorylation is involved in the activation of receptor tyrosine kinases. In fact, phosphorylation of proteins and its reverse, dephosphorylation, are commonly used in cells to regulate protein activity. An enzyme that transfers phosphate groups from ATP to a protein is generally known as a **protein kinase**. Recall that a receptor tyrosine kinase (RTK) is a specific kind of protein kinase that phosphorylates tyrosines on the other RTK in a dimer. Most cytoplasmic protein kinases, however, act on proteins different from themselves. Another distinction is that most cytoplasmic protein kinases phosphorylate either of two other amino acids, serine or threonine, rather than tyrosine. Serine/threonine kinases are widely involved in signaling pathways in animals, plants, and fungi.

Many signal transduction pathways use relay molecules that are protein kinases, and they often act on other protein kinases in the pathway. **Figure 11.10** depicts a hypothetical pathway containing two different protein kinases that create a **phosphorylation cascade**. The sequence of steps shown in the figure is similar to many known pathways, including those triggered in yeast by mating factors and in animal cells by many growth factors. The signal is transmitted by a cascade of protein phosphorylations, each causing a shape change in the phosphorylated protein. The shape change results from the interaction of the newly added phosphate groups with charged or polar amino acids on the protein being phosphorylated (see Figure 5.14). The shape change in turn alters the function of the protein, most often activating it. (In some cases, though, phosphorylation instead *decreases* the activity of the protein.)

A significant percentage of our own genes—about 2%—is thought to code for protein kinases. A single cell may have hundreds of different kinds, each specific for a different substrate protein. Together, protein kinases probably regulate the activity of a large proportion of the thousands of proteins in a cell. Among these are most of the proteins that, in turn, regulate cell division. Abnormal activity of such a



▶ Figure 11.10 A phosphorylation cascade. In a phosphorylation cascade, a series of different proteins in a pathway are phosphorylated in turn, each protein adding a phosphate group to the next one in line. Here, phosphorylation activates each protein, and dephosphorylation returns it to its inactive form. The active and inactive forms of each protein are represented by different shapes to remind you that activation is usually associated with a change in molecular shape.

WHAT IF? *What would happen if a mutation in protein kinase 2 made it incapable of being phosphorylated?*

kinase can cause abnormal cell division and contribute to the development of cancer.

Equally important in the phosphorylation cascade are the **protein phosphatases**, enzymes that can rapidly remove phosphate groups from proteins, a process called *dephosphorylation*. By dephosphorylating and thus inactivating protein kinases, phosphatases provide the mechanism for turning off the signal transduction pathway when the initial signal is no longer present. Phosphatases also make the protein kinases available for reuse, enabling the cell to respond again to an extracellular signal. The phosphorylation-dephosphorylation system acts as a molecular switch in the cell, turning activities on or off, or up or down, as required. At any given moment, the activity of a protein regulated by phosphorylation depends on the balance in the cell between active kinase molecules and active phosphatase molecules.

Small Molecules and Ions as Second Messengers

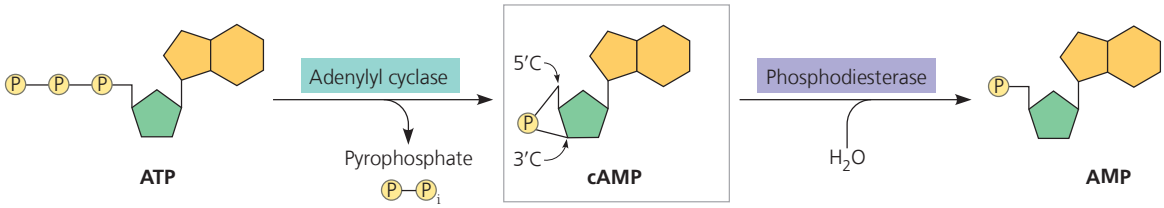
Not all components of signal transduction pathways are proteins. Many signaling pathways also involve small, nonprotein, water-soluble molecules or ions called **second messengers**. (The pathway's "first messenger" is considered to be the extracellular signaling molecule—the ligand—that binds to the membrane receptor.) Because they are small and water-soluble, they can readily spread through regions of the cell by diffusion. For example, as we'll see shortly, a second messenger called cyclic AMP carries the signal initiated by epinephrine from the plasma membrane of a liver or muscle cell into the cell's interior, where the signal eventually brings about glycogen breakdown. Second messengers participate in pathways that are initiated by both G protein-coupled receptors and receptor tyrosine kinases. The two most common second messengers are cyclic AMP and calcium ions, Ca^{2+}. A large variety of relay proteins respond to changes in the cytosolic concentration of one or the other of these second messengers.

Cyclic AMP

Having discovered that epinephrine somehow causes glycogen breakdown within cells, Earl Sutherland next looked for a second messenger that transmits the signal from the plasma membrane to the metabolic machinery in the cytoplasm. He found that binding of epinephrine to the G protein-coupled receptor (GPCR) in the plasma membrane results in a rise in the cytosolic concentration of **cyclic AMP (cAMP**; cyclic adenosine monophosphate), a small molecule produced from ATP. As shown in **Figure 11.11**, an enzyme embedded in the plasma membrane, **adenylyl cyclase** (also known as adenylate cyclase), converts ATP to cAMP in response to an extracellular signal—in this case, provided by epinephrine. When epinephrine outside the cell binds to a GPCR, it activates a G protein that in turn activates adenylyl cyclase. Adenylyl cyclase can then catalyze the synthesis of many molecules of cAMP. In this way, the normal cellular concentration of cAMP can be boosted 20-fold in a matter of seconds. The cAMP broadcasts the signal to the cytoplasm. It does not persist for long in the absence of the hormone because a different enzyme, called phosphodiesterase, converts cAMP to AMP. Another surge of epinephrine is needed to boost the cytosolic concentration of cAMP again.

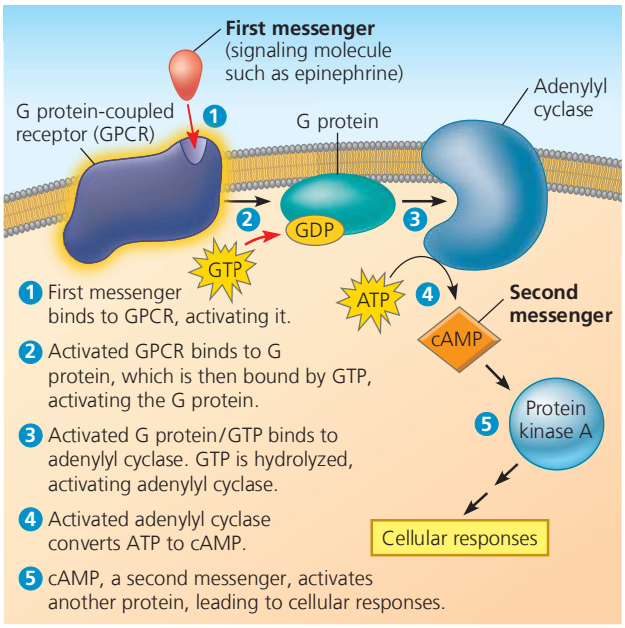
Subsequent research has revealed that epinephrine is only one of many hormones and many other signaling molecules that lead to activation of adenylyl cyclase by G proteins and formation of cAMP **(Figure 11.12)**. The immediate effect of an elevation in cAMP level is usually the activation of a serine/threonine kinase called *protein kinase A*. The activated protein kinase A then phosphorylates various other proteins, depending on the cell type. (The complete pathway for epinephrine's stimulation of glycogen breakdown is shown later, in Figure 11.16.)

▼ Figure 11.11 Cyclic AMP. The second messenger cyclic AMP (cAMP) is made from ATP by adenylyl cyclase, an enzyme embedded in the plasma membrane. The phosphate group in cAMP is attached to both the 5′ and the 3′ carbons; cAMP's cyclic arrangement is the basis for its name. cAMP is inactivated by phosphodiesterase, an enzyme that converts it to AMP.



WHAT IF? *What would happen if a molecule that inactivated phosphodiesterase were introduced into the cell?*

▼ Figure 11.12 cAMP as a second messenger in a G protein signaling pathway.



DRAW IT *The bacterium that causes the disease cholera produces a toxin that locks the G protein in its activated state. Review Figure 11.8, then draw this figure as it would be if cholera toxin were present. (You do not need to draw the cholera toxin molecule.)*

Further regulation of cell metabolism is provided by other G protein systems that *inhibit* adenylyl cyclase. In these systems, a different signaling molecule activates a different receptor, which in turn activates an *inhibitory* G protein that blocks activation of adenylyl cyclase. Cell activities can be fine-tuned by the balance between these systems.

Now that we know about the role of cAMP in G protein signaling pathways, we can explain in molecular detail how certain microorganisms cause disease. Consider cholera, a disease that is frequently epidemic in places where the water supply is contaminated with human feces. People acquire the cholera bacterium, *Vibrio cholerae*, by drinking contaminated water. The bacteria form a biofilm on the lining of the small intestine and produce a toxin. The cholera toxin is an enzyme that chemically modifies a G protein involved in regulating salt and water secretion. Because the modified G protein is unable to hydrolyze GTP to GDP, it remains stuck in its active form, continuously stimulating adenylyl cyclase to make cAMP (see the question with Figure 11.12). The resulting high concentration of cAMP causes the intestinal cells to secrete large amounts of salts into the intestines, with water following by osmosis. An infected person quickly develops profuse diarrhea and if left untreated can soon die from the loss of water and salts.

Our understanding of signaling pathways involving cyclic AMP or related messengers has allowed us to develop treatments for certain conditions in humans such as erectile dysfunction. In one pathway, the gas nitric oxide (NO) is released by a cell and enters a neighboring muscle cell, where it causes production of a molecule similar to cAMP called *cyclic GMP* (*cGMP*). The cGMP then acts as a second messenger that causes relaxation of muscles, such as those in the walls of arteries. A compound that prolongs the signal (by inhibiting the hydrolysis of cGMP to GMP) was originally prescribed for chest pains because it relaxed blood vessels and increased blood flow to the heart muscle. Under the trade name Viagra, this compound is now widely used as a treatment for erectile dysfunction in human males. Because Viagra leads to dilation of blood vessels, it also allows increased blood flow to the penis, optimizing physiological conditions for penile erections.

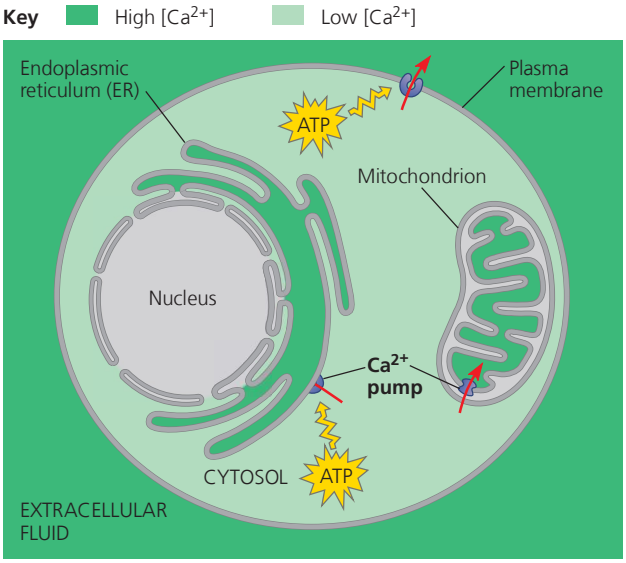
Calcium Ions and Inositol Trisphosphate (IP_3)

Many of the signaling molecules that function in animals—including neurotransmitters, growth factors, and some hormones—induce responses in their target cells through signal transduction pathways that increase the cytosolic concentration of calcium ions (Ca^{2+}). Calcium is even more widely used than cAMP as a second messenger. Increasing the local cytosolic concentration of Ca^{2+} causes many responses in animal cells, including muscle cell contraction, exocytosis of molecules (secretion), and cell division. In plant cells, a wide range of hormonal and environmental stimuli can cause brief increases in cytosolic Ca^{2+} concentration, triggering various signaling pathways, such as the pathway for greening in response to light (see Figure 39.4). Cells use Ca^{2+} as a second messenger in pathways triggered by both G protein-coupled receptors and receptor tyrosine kinases.

Although cells always contain some Ca^{2+}, this ion can function as a second messenger because its concentration in the cytosol is normally much lower than the concentration outside the cell **(Figure 11.13)**. In fact, the level of Ca^{2+} in the blood and extracellular fluid of an animal is often more than 10,000 times higher than that in the cytosol. Calcium ions are actively transported out of the cell and are actively imported from the cytosol into the endoplasmic reticulum (and, under some conditions, into mitochondria and chloroplasts) by various protein pumps. As a result, the calcium concentration in the ER is usually much higher than that in the cytosol. Because the cytosolic calcium level is low, a small change in absolute numbers of ions represents a relatively large percentage change in local calcium concentration.

In response to a signal relayed by a signal transduction pathway, the cytosolic calcium level may rise, usually by a

▼ Figure 11.13 The maintenance of calcium ion concentrations in an animal cell. The Ca^{2+} concentration in the cytosol is usually much lower (light green) than in the extracellular fluid and ER (dark green). Protein pumps in the plasma membrane and the ER membrane, driven by ATP, move Ca^{2+} from the cytosol into the extracellular fluid and into the lumen of the ER. Mitochondrial pumps, driven by chemiosmosis (see Concept 9.4), move Ca^{2+} into mitochondria when the calcium level in the cytosol rises significantly.



mechanism that releases Ca^{2+} from the cell's ER. The pathways leading to calcium release involve two other second messengers, **inositol trisphosphate (IP_3)** and **diacylglycerol (DAG)**. These two messengers are produced by cleavage of a certain kind of phospholipid in the plasma membrane. **Figure 11.14** shows the complete picture of how a signal causes IP_3 to stimulate the release of calcium from the ER. Because IP_3 acts before calcium in these pathways, calcium could be considered a "*third* messenger." However, scientists use the term *second messenger* for all small, nonprotein components of signal transduction pathways.

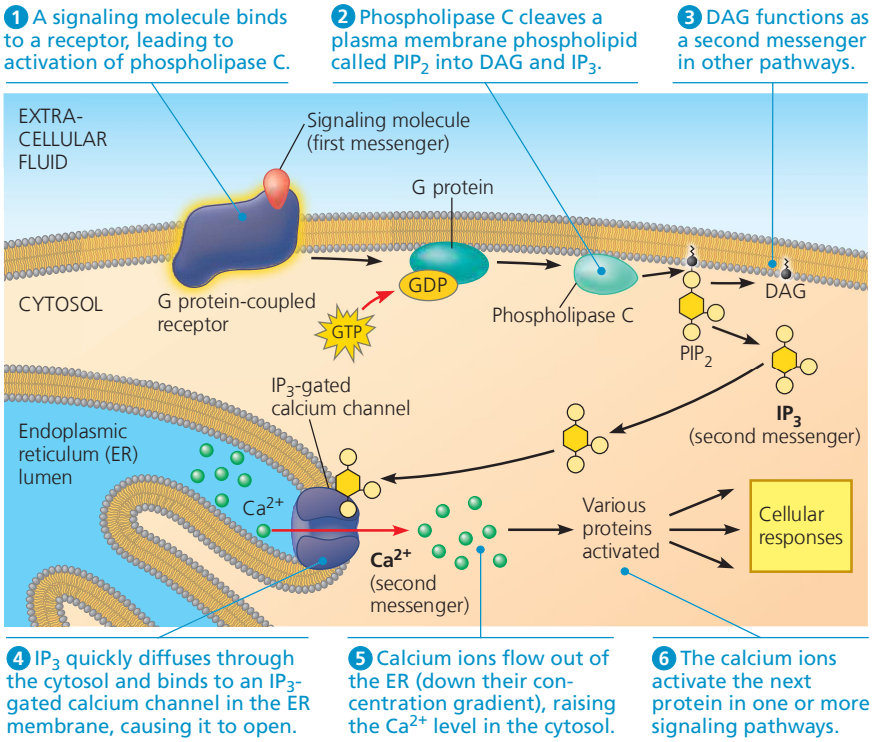
CONCEPT CHECK 11.3

1. What is a protein kinase, and what is its role in a signal transduction pathway?
2. When a signal transduction pathway involves a phosphorylation cascade, how does the cell's response get turned off?
3. What is the actual "signal" that is being transduced in any signal transduction pathway, such as those shown in Figures 11.6 and 11.10? In what way is this information being passed from the exterior to the interior of the cell?
4. **WHAT IF?** If you exposed a cell to a ligand that binds to a receptor and activates phospholipase C, predict the effect the IP_3-gated calcium channel would have on Ca^{2+} concentration in the cytosol.

For suggested answers, see Appendix A.

▶ Figure 11.14 Calcium and IP_3 in signaling pathways. Calcium ions (Ca^{2+}) and inositol trisphosphate (IP_3) function as second messengers in many signal transduction pathways. In this figure, the process is initiated by the binding of a signaling molecule to a G protein-coupled receptor. A receptor tyrosine kinase could also initiate this pathway by activating phospholipase C.

MAKE CONNECTIONS *Explain the difference in function (in terms of ion transport) between the Ca^{2+} pump in Figure 11.13 and the Ca^{2+} channel protein shown here. (See Figure 7.17.)*



CONCEPT 11.4

Cellular response: Cell signaling leads to regulation of transcription or cytoplasmic activities

We now take a closer look at the cell's subsequent response to an extracellular signal—what some researchers call the "output response." What is the nature of the final step in a signaling pathway?

Nuclear and Cytoplasmic Responses

Ultimately, a signal transduction pathway leads to the regulation of one or more cellular activities. The response may occur in the nucleus or in the cytoplasm of the cell.

Many signaling pathways ultimately regulate protein synthesis, usually by turning specific genes on or off in the nucleus. Like an activated steroid receptor (see Figure 11.9), the final activated molecule in a signaling pathway may function as a transcription factor. **Figure 11.15** shows an example in which a signaling pathway activates a transcription factor that turns a gene on: The response to this growth factor signal is transcription, the synthesis of one or more specific mRNAs, which will be translated in the cytoplasm into specific proteins. In other cases, the transcription factor might regulate a gene by turning it off. Often, a transcription factor regulates several different genes.

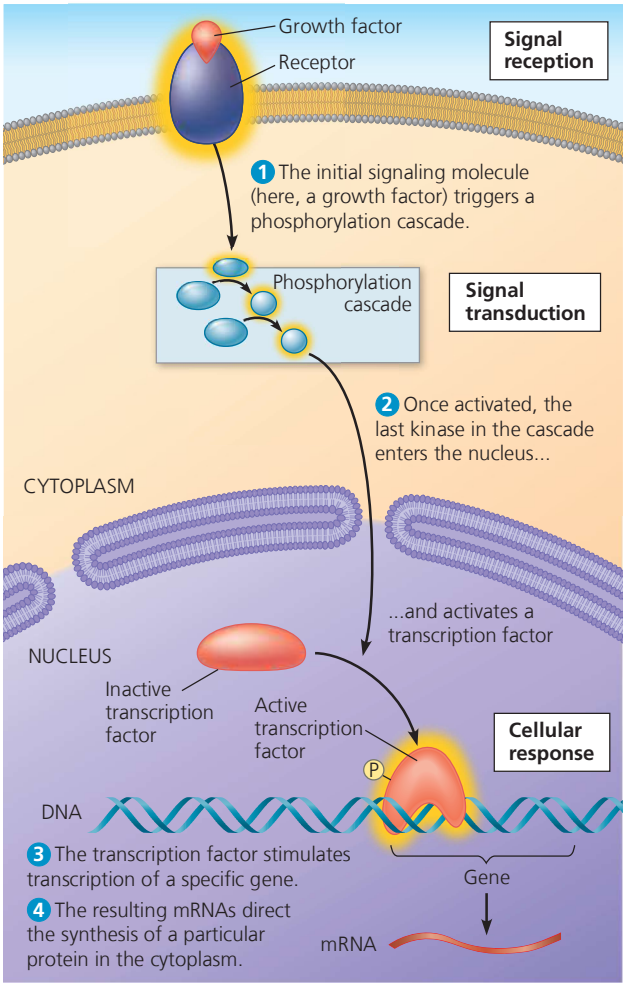
Sometimes a signaling pathway may regulate the *activity* of proteins rather than causing their synthesis by activating gene expression. This directly affects proteins that function outside the nucleus. For example, a signal may cause the opening or closing of an ion channel in the plasma membrane or a change in the activity of a metabolic enzyme. As we have seen, the response of liver cells to the hormone epinephrine helps regulate cellular energy metabolism by affecting the activity of an enzyme. The final step in the epinephrine signaling pathway activates the enzyme that catalyzes the breakdown of glycogen. **Figure 11.16** shows the complete pathway leading to the release of glucose 1-phosphate molecules from glycogen. Notice that as each molecule is activated, the response is amplified, a subject we'll return to shortly.

Signal receptors, relay molecules, and second messengers participate in a variety of pathways, leading to both nuclear and cytoplasmic responses, including cell division. Malfunctioning of growth factor pathways like the one in Figure 11.15 can contribute to abnormal cell division and the development of cancer, as we'll see in Concept 18.5.

Regulation of the Response

Whether the response occurs in the nucleus or in the cytoplasm, it is not simply turned "on" or "off." Rather, the extent and specificity of the response are regulated in multiple ways.

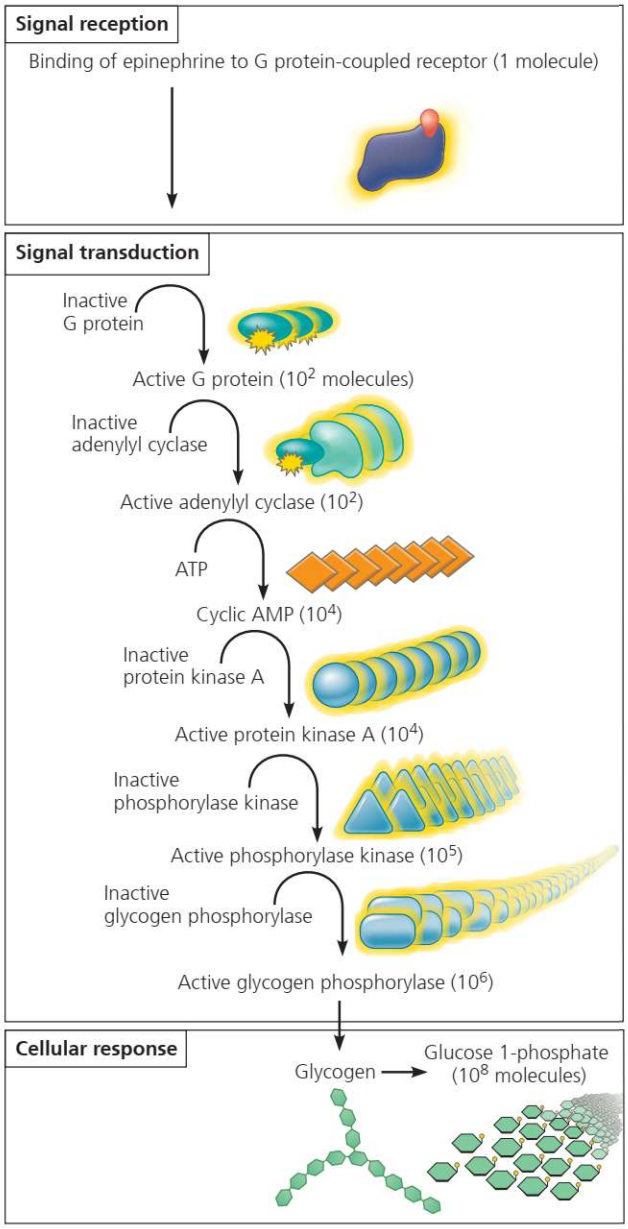
▼ Figure 11.15 Nuclear responses to a signal: the activation of a specific gene by a growth factor. This diagram shows a typical signaling pathway that leads to regulation of gene activity in the cell nucleus. The initial signaling molecule, in this case a growth factor, triggers a phosphorylation cascade, as in Figure 11.10. (The ATP molecules and phosphate groups are not shown.) Once phosphorylated, the last kinase in the sequence enters the nucleus and activates a transcription factor, which stimulates transcription of a specific gene (or genes). The resulting mRNAs then direct the synthesis of a particular protein.



Mastering Biology **Animation: Nuclear Response: Activating a Gene**

Here we'll consider four aspects of this regulation. First, as mentioned earlier, signaling pathways generally amplify the cell's response to a single signaling event. The degree of amplification depends on the function of the specific molecules in the pathway. Second, the many steps in a multistep pathway provide control points at which the cell's response can be further regulated, contributing to the specificity of the response and allowing coordination with other signaling pathways. Third, the overall efficiency of the response is enhanced by the presence of proteins known as scaffolding

▼ Figure 11.16 Cytoplasmic response to a signal: the stimulation of glycogen breakdown by epinephrine (adrenaline). In this signaling system, the hormone epinephrine acts through a G protein-coupled receptor to activate a succession of relay molecules, including cAMP and two protein kinases (see also Figure 11.12). The final protein activated is the enzyme glycogen phosphorylase, which uses inorganic phosphate to release glucose monomers from glycogen in the form of glucose 1-phosphate molecules. This pathway amplifies the hormonal signal: One receptor protein can activate approximately 100 molecules of G protein, and each enzyme in the pathway, once activated, can act on many molecules of its substrate, the next molecule in the cascade. The number of activated molecules given for each step is approximate.



VISUAL SKILLS *In the figure, how many glucose 1-phosphate molecules are released in response to one signaling molecule? Calculate the factor by which the response is amplified in going from each step to the next.*

Mastering Biology **Animation: Cytoplasmic Response: Glycogen Breakdown**

proteins. Finally, a crucial point in regulating the response is the termination of the signal.

Signal Amplification

Elaborate enzyme cascades amplify the cell's response to a signal. At each catalytic step in the cascade, the number of activated products can be much greater than in the preceding step. For example, in the epinephrine-triggered pathway in Figure 11.16, each adenylyl cyclase molecule catalyzes the formation of 100 or so cAMP molecules, each molecule of protein kinase A phosphorylates about 10 molecules of the next kinase in the pathway, and so on. The amplification effect stems from the fact that these proteins persist in their active form long enough to process multiple molecules of substrate before they become inactive again. As a result of the signal's amplification, a small number of epinephrine molecules binding to receptors on the surface of a liver cell or muscle cell can lead to the release of hundreds of millions of glucose molecules from glycogen.

The Specificity of Cell Signaling and Coordination of the Response

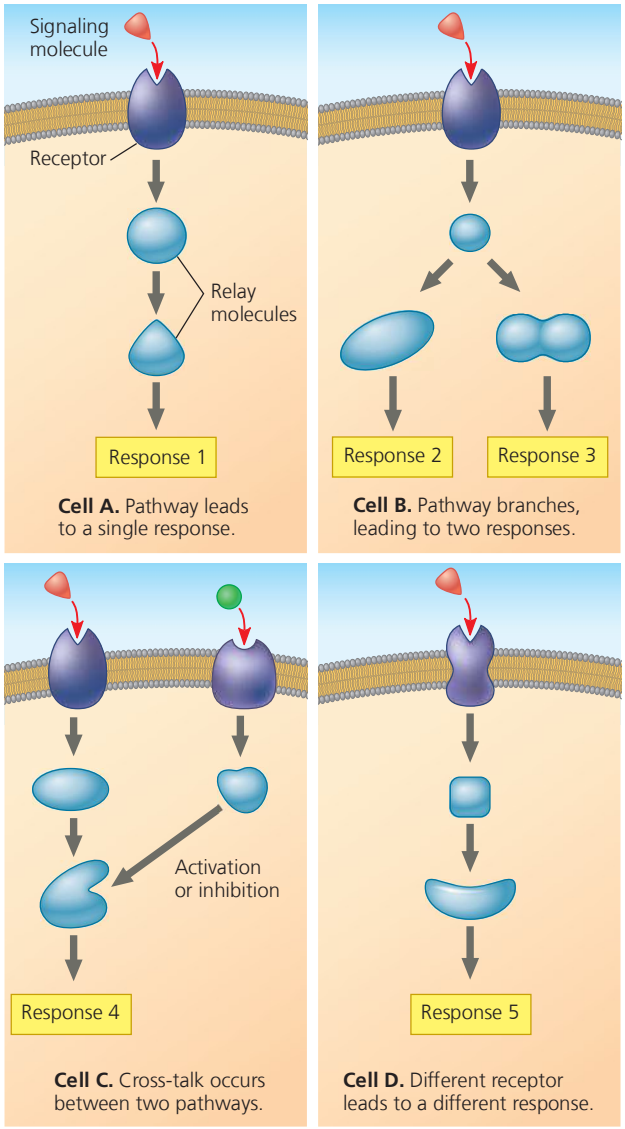
Consider two different cells in your body—a liver cell and a heart muscle cell, for example. Both are in contact with your bloodstream and are therefore constantly exposed to many different hormone molecules, as well as to local regulators secreted by nearby cells. Yet the liver cell responds to some signals but ignores others, and the same is true for the heart cell. And some kinds of signals trigger responses in both cells—but different responses. For instance, epinephrine stimulates the liver cell to break down glycogen, but the main response of the heart cell to epinephrine is contraction, leading to a more rapid heartbeat. How do we account for this difference?

The explanation for the specificity exhibited in cellular responses to signals is the same as the basic explanation for virtually all differences between cells: Because different kinds of cells turn on different sets of genes, *different kinds of cells have different collections of proteins*. The response of a cell to a signal depends on its particular collection of signal receptor proteins, relay proteins, and proteins needed to carry out the response. A liver cell, for example, is poised to respond appropriately to epinephrine by having the proteins listed in Figure 11.16 as well as those needed to manufacture glycogen.

Thus, two cells that respond differently to the same signal differ in one or more proteins that respond to the signal. Also, within some cells, there are more complex pathways that branch or converge, as shown in **Figure 11.17**. Notice, though, that these different pathways still have some molecules in common. For example, cells A, B, and C all use the same receptor protein for the red signaling molecule; differences in other proteins account for their differing responses. In cell D, a different receptor protein is used for the same signaling molecule, leading to yet another response. In cell B,

a pathway triggered by one signal diverges to produce two responses; such branched pathways often involve receptor tyrosine kinases (which can activate multiple relay proteins) or second messengers (which can regulate numerous proteins). In cell C, two pathways triggered by separate signals converge to modulate a single response. Branching of pathways and "cross-talk" (interaction) between pathways are important in regulating and coordinating a cell's responses to information coming in from different sources in the body. (You'll learn more about this coordination in Concept 11.5.) Moreover, the use of some of the same proteins in more than one pathway allows the cell to economize on the number of different proteins it must make.

▼ Figure 11.17 The specificity of cell signaling. The particular proteins a cell possesses determine what signaling molecules it responds to and the nature of the response. The four cells in these diagrams respond to the same signaling molecule (red) in different ways because each has a different set of proteins (purple and teal). Note, however, that the same kinds of molecules can participate in more than one pathway.



Cell A. Pathway leads to a single response.

Cell B. Pathway branches, leading to two responses.

Cell C. Cross-talk occurs between two pathways.

Cell D. Different receptor leads to a different response.

VISUAL SKILLS *Study the signaling pathway shown in Figure 11.14, and explain how the situation pictured for cell B in Figure 11.17 could apply to that pathway.*

Instructors: The Scientific Skills Exercise "Using Experiments to Test a Model" can be assigned in **Mastering Biology**. It explores the cellular response of a yeast cell to the signal initiated by a mating factor from a cell of the opposite mating type.

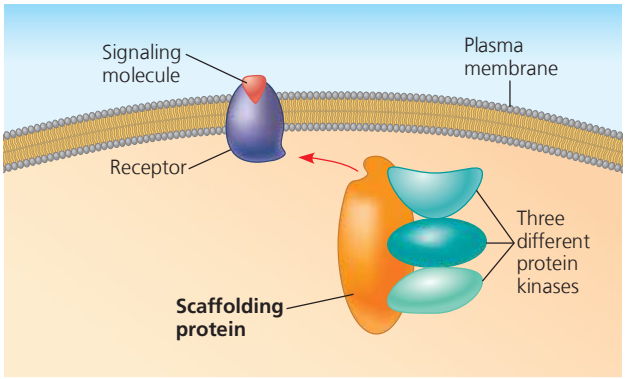
Signaling Efficiency: Scaffolding Proteins and Signaling Complexes

The illustrations of signaling pathways in Figure 11.17 (as well as diagrams of other pathways in this chapter) are greatly simplified. The diagrams show only a few relay molecules and, for clarity's sake, display these molecules spread out in the cytosol. If this were true in the cell, signaling pathways would operate very inefficiently because most relay molecules are proteins, and proteins are too large to diffuse quickly through the viscous cytosol. How does a given protein kinase, for instance, find its protein substrate?

In many cases, the efficiency of signal transduction is apparently increased by the presence of **scaffolding proteins**, large relay proteins to which several other relay proteins are simultaneously attached **(Figure 11.18)**. Researchers have found scaffolding proteins in brain cells that *permanently* hold together networks of signaling pathway proteins at synapses. This hardwiring enhances the speed and accuracy of signal transfer between cells because the rate of protein-protein interaction is not limited by diffusion. Furthermore, in some cases the scaffolding proteins themselves may directly activate relay proteins.

The importance of the relay proteins that serve as points of branching or intersection in signaling pathways is highlighted by the problems arising when these proteins are

▼ Figure 11.18 A scaffolding protein. The scaffolding protein shown here (orange) simultaneously binds to a specific activated membrane receptor and three different protein kinases. This physical arrangement facilitates signal transduction by these molecules.



defective or missing. For instance, in an inherited disorder called Wiskott-Aldrich syndrome (WAS), the absence of a single relay protein leads to such diverse effects as abnormal bleeding, eczema, and a predisposition to infections and leukemia. These symptoms are thought to arise primarily from the absence of the protein in cells of the immune system. By studying normal cells, scientists found that the WAS protein is located just beneath the immune cell surface. The protein interacts both with microfilaments of the cytoskeleton and with several different components of signaling pathways that relay information from the cell surface, including pathways regulating immune cell proliferation. This multifunctional relay protein is thus both a branch point and an important intersection point in a complex signal transduction network that controls immune cell behavior. When the WAS protein is absent, the cytoskeleton is not properly organized and signaling pathways are disrupted, leading to the WAS symptoms.

Termination of the Signal

In the interest of keeping Figure 11.17 simple, we did not show the *inactivation* mechanisms that are an essential aspect of any cell-signaling pathway. For a cell of a multicellular organism to remain capable of responding to incoming signals, each molecular change in its signaling pathways must last only a short time. As we saw in the cholera example, if a signaling pathway component becomes locked into one state, whether active or inactive, consequences for the organism can be serious.

The ability of a cell to receive new signals depends on reversibility of the changes produced by prior signals. The binding of signaling molecules to receptors is reversible. As the external concentration of signaling molecules falls, fewer receptors are bound at any given moment, and the unbound receptors revert to their inactive form. The cellular response occurs only when the concentration of receptors with bound signaling molecules is above a certain threshold. When the number of active receptors falls below that threshold, the cellular response ceases. Then, by a variety of means, the relay molecules return to their inactive forms: The GTPase activity intrinsic to a G protein hydrolyzes its bound GTP; the enzyme phosphodiesterase converts cAMP to AMP; protein phosphatases inactivate phosphorylated kinases and other proteins; and so forth. As a result, the cell is soon ready to respond to a fresh signal.

Mastering Biology BioFlix® Animation: Mechanism of Hormone Action: Second Messenger cAMP

In this section, we explored the complexity of signaling initiation and termination in a single pathway, and we saw the potential for pathways to intersect with each other. In the next section, we'll consider one especially important network of interacting pathways in the cell.

CONCEPT CHECK 11.4

1. How can a target cell's response to a single hormone molecule result in a response that affects a million other molecules?
2. **WHAT IF?** If two cells have different scaffolding proteins, explain how they might behave differently in response to the same signaling molecule.
3. **WHAT IF?** Some human diseases are associated with malfunctioning protein phosphatases. How would such proteins affect signaling pathways? (Review the discussion of protein phosphatases in Concept 11.3 and see Figure 11.10.)
4. Epinephrine affects heart muscle cells by causing them to mobilize glucose, contract faster, and increase heart rate. The muscle cells around lungs and airways, on the other hand, have the opposite response to epinephrine: They relax, allowing more air to be breathed in. What might explain why respiratory (breathing-related) muscle cells can respond so differently from heart muscle cells?

For suggested answers, see Appendix A.

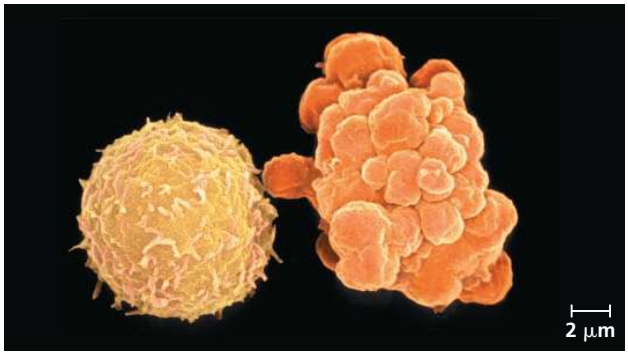
CONCEPT 11.5

Apoptosis requires integration of multiple cell-signaling pathways

When signaling pathways were first discovered, they were thought to be linear, independent pathways. Our understanding of cellular communication has benefited from the realization that signaling pathway components interact with each other in various ways. For a cell to carry out the appropriate response, cellular proteins often must integrate multiple signals. Let's consider an important cellular process—cellular suicide—as an example.

Cells that are infected, are damaged, or have reached the end of their functional life span often undergo "programmed cell death" **(Figure 11.19)**. The best-understood type of this controlled cell suicide is **apoptosis** (from the Greek, meaning "falling off," and used in a classic Greek poem to refer to leaves falling from a tree). During this process, cellular agents chop up

▼ Figure 11.19 Apoptosis of a human white blood cell. On the left is a normal white blood cell, while on the right is a white blood cell undergoing apoptosis. The apoptotic cell is shrinking and forming lobes ("blebs"), which eventually are shed as membrane-bounded cell fragments (colorized SEMs).



Mastering Biology Video: Phosphate-Induced Apoptosis

the DNA and fragment the organelles and other cytoplasmic components. The cell shrinks and becomes lobed (a change called "blebbing"), and the cell's parts are packaged up in vesicles that are engulfed and digested by specialized scavenger cells, leaving no trace. Apoptosis protects neighboring cells from damage that they would otherwise suffer if a dying cell merely leaked out all its contents, including its many digestive enzymes.

The signal that triggers all of the complex events that occur during apoptosis can come from either outside or inside the cell. Outside the cell, signaling molecules released from other cells can initiate a signal transduction pathway that activates the genes and proteins responsible for carrying out cell death. Within a cell whose DNA has been irretrievably damaged, a series of protein-protein interactions can pass along a signal that similarly triggers cell death. Considering some examples of apoptosis can help us to see how signaling pathways are integrated in cells.

Apoptosis in the Soil Worm *Caenorhabditis elegans*

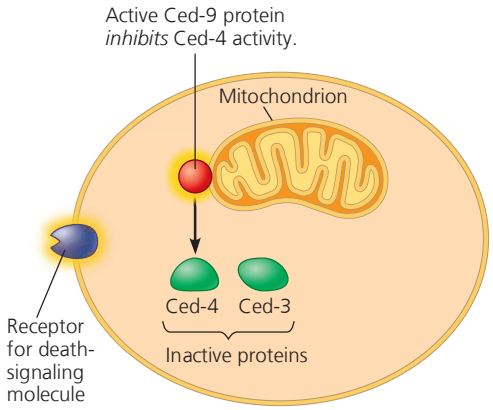
The molecular mechanisms of apoptosis were worked out by researchers studying embryonic development of a small soil worm, a nematode called *Caenorhabditis elegans*. Because the adult worm has only about 1,000 cells, the researchers were able to work out the entire ancestry of each cell. The timely suicide of cells occurs exactly 131 times during normal development of *C. elegans*, at precisely the same points in the cell lineage of each worm. In worms and other species, apoptosis is triggered by signals that activate a cascade of "suicide" proteins in the cells destined to die.

Genetic research on *C. elegans* initially revealed two key apoptosis genes, called *ced-3* and *ced-4* (*ced* stands for "cell death"), which encode proteins essential for apoptosis. The proteins are called Ced-3 and Ced-4, respectively. These and most other proteins involved in apoptosis are continually present in cells, but in inactive form; thus, regulation in this case occurs at the level of protein activity rather than through gene activity and protein synthesis. In *C. elegans*, a protein in the outer mitochondrial membrane, called Ced-9 (the product of the *ced-9* gene), serves as a master regulator of apoptosis, acting as a brake in the absence of a signal promoting apoptosis **(Figure 11.20a)**. When a death signal is received by the cell, signal transduction involves a change in Ced-9 that disables the brake, and the apoptotic pathway activates proteases and nucleases, enzymes that cut up the proteins and DNA of the cell. The main proteases of apoptosis are called *caspases*; in the nematode, the chief caspase is the Ced-3 protein.

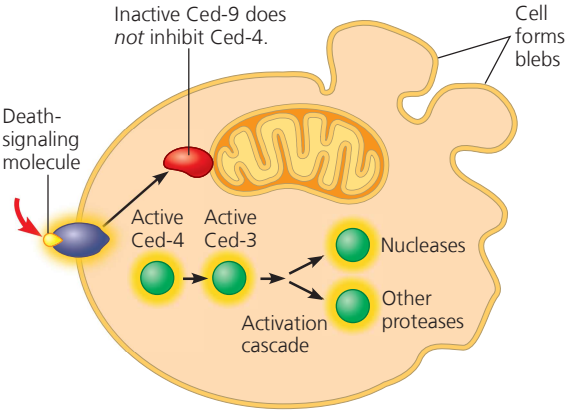
Apoptotic Pathways and the Signals That Trigger Them

In humans and other mammals, several different pathways, involving about 15 different caspases, can carry out

▼ Figure 11.20 Molecular basis of apoptosis in *C. elegans*. Three proteins, Ced-3, Ced-4, and Ced-9, are critical to apoptosis and its regulation in the nematode. Apoptosis is more complicated in mammals but involves proteins similar to those in *C. elegans*.



(a) No death signal. As long as Ced-9, located in the outer mitochondrial membrane, is active, apoptosis is inhibited, and the cell remains alive.



(b) Death signal. When a cell receives a death signal, Ced-9 is inactivated, relieving its inhibition of Ced-4. Active Ced-4 activates Ced-3, a protease, which triggers a cascade of reactions leading to the activation of nucleases and other proteases. The action of these enzymes causes the changes seen in apoptotic cells and eventually leads to cell death.

apoptosis. The pathway that is used depends on the type of cell and on the particular signal that initiates apoptosis. One major pathway involves certain mitochondrial proteins that are triggered to form molecular pores in the mitochondrial outer membrane, causing it to leak and release other proteins that promote apoptosis. Perhaps surprisingly, these latter include cytochrome *c*, which functions in mitochondrial electron transport in healthy cells (see Figure 9.14) but acts as a cell death factor when released from mitochondria. The process of mitochondrial apoptosis

in mammals uses proteins similar to the nematode proteins Ced-3, Ced-4, and Ced-9. These can be thought of as relay proteins capable of transducing the apoptotic signal.

Mastering Biology **Animation: Apoptosis**

At key gateways into the apoptotic program, relay proteins integrate signals from several different sources and can send a cell down an apoptotic pathway. Often, the signal originates outside the cell, like the death-signaling molecule depicted in **Figure 11.20b**, which presumably was released by a neighboring cell. When a death-signaling ligand occupies a cell-surface receptor, this binding leads to activation of caspases and other enzymes that carry out apoptosis, without involving the mitochondrial pathway. This process of signal reception, transduction, and response is similar to what we have discussed throughout this chapter. In a twist on the classic scenario, two other types of alarm signals that can lead to apoptosis originate from *inside* the cell rather than from a cell-surface receptor. One signal comes from the nucleus, generated when the DNA has suffered irreparable damage, and a second comes from the endoplasmic reticulum when excessive protein misfolding occurs. Mammalian cells make life-or-death "decisions" by somehow integrating the death signals and life signals they receive from these external and internal sources.

A built-in cell suicide mechanism is essential to development and maintenance in all animals. The similarities between apoptosis genes in nematodes and those in mammals, as well as the observation that apoptosis occurs in multicellular fungi and even in single-celled yeasts, indicate that the basic mechanism evolved early in the evolution of eukaryotes. In vertebrates, apoptosis is essential for normal development of the nervous system, for normal operation of the immune system, and for normal morphogenesis of hands and feet in humans and paws in other mammals **(Figure 11.21)**. The level of apoptosis between the developing digits is lower in the webbed feet of ducks and other water birds than in the nonwebbed feet of land birds, such as chickens. In the case of humans, the failure of appropriate apoptosis can result in webbed fingers and toes.

Significant evidence points to the involvement of apoptosis in certain degenerative diseases of the nervous system, such as Parkinson's disease and Alzheimer's disease. In Alzheimer's disease, an accumulation of aggregated proteins in neuronal cells activates an enzyme that triggers apoptosis, resulting in the loss of brain function seen in these patients. Furthermore, cancer can result from a failure of cell suicide; some cases of human melanoma, for example, have been linked to faulty forms of the human version of the *C. elegans* Ced-4 protein. It is not surprising, therefore, that the signaling pathways feeding into apoptosis are quite elaborate. After all, the life-or-death question is the most fundamental one imaginable for a cell.

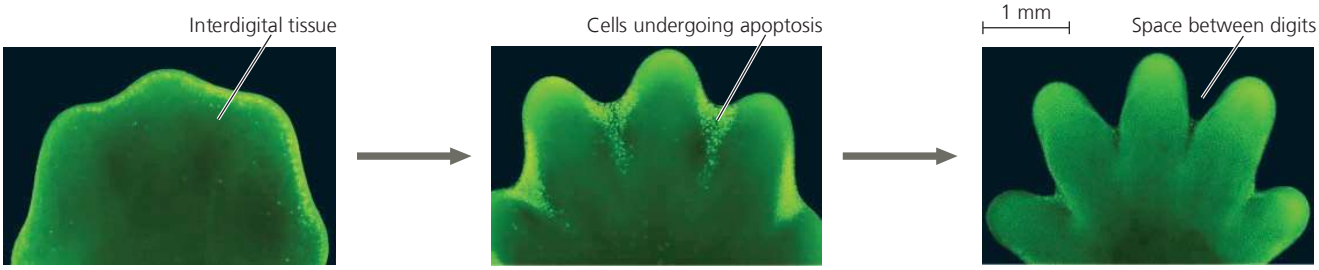
This chapter has introduced you to many of the general mechanisms of cell communication, such as ligand binding, protein-protein interactions and shape changes, cascades of interactions, and protein phosphorylation. Throughout your study of biology, you will encounter numerous examples of cell signaling.

CONCEPT CHECK 11.5

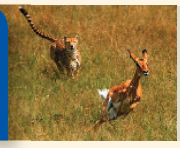
1. Give an example of apoptosis during embryonic development, and explain its function in the developing embryo.
2. **WHAT IF?** If apoptosis occurred when it should not, what types of protein defects might be the cause? What types could result in apoptosis not occurring when it should?

For suggested answers, see Appendix A.

▼ Figure 11.21 Effect of apoptosis during paw development in the mouse. In mice, humans, other mammals, and land birds, the embryonic region that develops into feet or hands initially has a solid, platelike structure. Apoptosis eliminates the cells in the interdigital regions, thus forming the digits. The embryonic mouse paws shown in these fluorescence light micrographs are stained so that cells undergoing apoptosis appear a bright yellowish green. Apoptosis of cells begins at the margin of each interdigital region (left), peaks as the tissue in these regions is reduced (middle), and is no longer visible when the interdigital tissue has been eliminated (right).



11 Chapter Review



Go to **Mastering Biology** for Assignments, the eText, the Study Area, and Dynamic Study Modules.

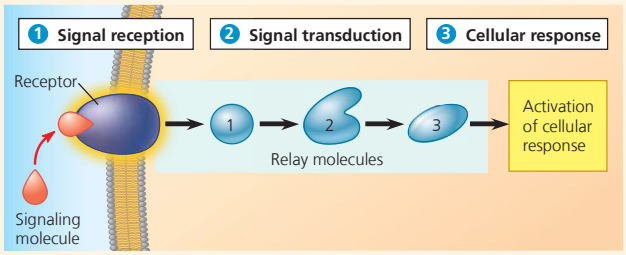
SUMMARY OF KEY CONCEPTS

To review key terms, go to the **Vocabulary Self-Quiz** in the **Mastering Biology** eText or Study Area, or go to goo.gl/zkjz9t.

CONCEPT 11.1

External signals are converted to responses within the cell *(pp. 213–217)*

- **Signal transduction pathways** are crucial for many processes. Bacterial cells can sense the local density of bacterial cells (quorum sensing). Signaling during yeast cell mating has much in common with processes in multicellular organisms, suggesting an early evolutionary origin of signaling mechanisms.
- Local signaling by animal cells involves direct contact or the secretion of local regulators. For long-distance signaling, animal and plant cells use **hormones**; animals also pass signals electrically.
- Like epinephrine, other hormones that bind to membrane receptors trigger a three-stage cell-signaling pathway:



? *What determines* whether *a cell responds to a hormone such as epinephrine? What determines* how *a cell responds to such a hormone?*

CONCEPT 11.2

Signal reception: A signaling molecule binds to a receptor, causing it to change shape *(pp. 217–221)*

- The binding between signaling molecule (**ligand**) and receptor is highly specific. A specific shape change in a receptor is often the initial transduction of the signal.
- Three of the major types of cell-surface transmembrane receptors are the following: (1) **G protein-coupled receptors (GPCRs)** work with cytoplasmic **G proteins**. Ligand binding activates the receptor, which then activates a specific G protein, which activates yet another protein, thus propagating the signal. (2) **Receptor tyrosine kinases (RTKs)** react to the binding of signaling molecules by forming dimers and then adding phosphate groups to tyrosines on the cytoplasmic part of the other monomer making up the dimer. Relay proteins in the cell can then be activated by binding to different phosphorylated tyrosines, allowing this receptor to trigger several pathways at once. (3) **Ligand-gated ion channels** open or close in response to binding by specific signaling molecules, regulating the flow of specific ions across the membrane.
- The activity of all three types of receptors is crucial; abnormal GPCRs and RTKs are associated with many human diseases.
- Intracellular receptors are cytoplasmic or nuclear proteins. Signaling molecules that are hydrophobic or small enough to cross the plasma membrane bind to these receptors inside the cell.

? *How are the structures of a GPCR and an RTK similar? How does initiation of signal transduction differ for these two types of receptors?*

CONCEPT 11.3

Signal transduction: Cascades of molecular interactions transmit signals from receptors to relay molecules in the cell *(pp. 221–225)*

- At each step in a signal transduction pathway, the signal is transduced into a different form, which commonly involves a shape change in a protein. Many signal transduction pathways include **phosphorylation cascades**, in which a series of **protein kinases** each add a phosphate group to the next one in line, activating it. Enzymes called **protein phosphatases** remove the phosphate groups. The balance between phosphorylation and dephosphorylation regulates the activity of proteins involved in the sequential steps of a signal transduction pathway.
- **Second messengers**, such as the small molecule **cyclic AMP (cAMP)** and the ion Ca^{2+}, diffuse readily through the cytosol and thus help broadcast signals quickly. Many G proteins activate **adenylyl cyclase**, which makes cAMP from ATP. Cells use Ca^{2+} as a second messenger in GPCR and RTK pathways. The tyrosine kinase pathways can also involve two other second messengers, **diacylglycerol (DAG)** and **inositol trisphosphate** (IP_3). IP_3 can trigger a subsequent increase in Ca^{2+} level.

? *What is the difference between a protein kinase and a second messenger? Can both operate in the same signal transduction pathway?*

CONCEPT 11.4

Cellular response: Cell signaling leads to regulation of transcription or cytoplasmic activities *(pp. 226–229)*

- Some pathways lead to a nuclear response: Specific genes are turned on or off by activated transcription factors. In others, the response involves cytoplasmic regulation.
- Cellular responses are not simply on or off; they are regulated at many steps. Each protein in a signaling pathway amplifies the signal by activating multiple copies of the next component; for long pathways, the total amplification may be over a millionfold. The combination of proteins in a cell confers specificity in the signals it detects and the responses it carries out. **Scaffolding proteins** increase signaling efficiency. Pathway branching further helps the cell coordinate signals and responses. Signal response can be terminated quickly because ligand binding is reversible.

? *What mechanisms in the cell terminate its response to a signal and maintain its ability to respond to new signals?*

CONCEPT 11.5

Apoptosis requires integration of multiple cell-signaling pathways *(pp. 229–231)*

- **Apoptosis** is a type of programmed cell death in which cell components are disposed of in an orderly fashion. Studies of the soil worm *Caenorhabditis elegans* clarified molecular details of the relevant signaling pathways. A death signal leads to activation of caspases and nucleases, the main enzymes involved in apoptosis.
- Several apoptotic signaling pathways with related proteins exist in the cells of humans and other mammals, triggered in different ways. Signals eliciting apoptosis can originate from outside or inside the cell.

? *What is an explanation for the similarities between genes in yeasts, nematodes, and mammals that control apoptosis?*

TEST YOUR UNDERSTANDING

For more multiple-choice questions, go to the **Practice Test** in the **Mastering Biology** eText or Study Area, or go to goo.gl/GruWRg.

Levels 1-2: Remembering/Understanding

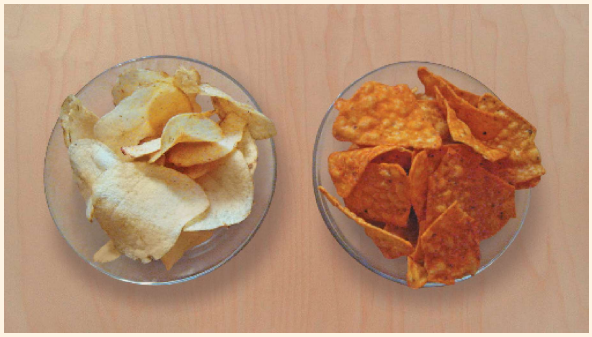
1. Binding of a signaling molecule to which type of receptor leads directly to a change in the distribution of substances on opposite sides of the membrane?
   (A) intracellular receptor
   (B) G protein-coupled receptor
   (C) phosphorylated receptor tyrosine kinase dimer
   (D) ligand-gated ion channel
2. The activation of receptor tyrosine kinases is characterized by
   (A) dimerization and phosphorylation.
   (B) dimerization and IP_3 binding.
   (C) a phosphorylation cascade.
   (D) GTP hydrolysis.
3. Lipid-soluble signaling molecules, such as aldosterone, cross the membranes of all cells but affect only target cells because
   (A) only target cells retain the appropriate DNA segments.
   (B) intracellular receptors are present only in target cells.
   (C) only target cells have enzymes that break down aldosterone.
   (D) only in target cells is aldosterone able to initiate the phosphorylation cascade that turns genes on.
4. Consider this pathway: epinephrine → G protein-coupled receptor → G protein → adenylyl cyclase → cAMP. Identify the second messenger.
   (A) cAMP
   (B) G protein
   (C) GTP
   (D) adenylyl cyclase
5. Which of the following occurs during apoptosis?
   (A) lysis of the cell
   (B) direct contact between signaling cells
   (C) fragmentation of the DNA
   (D) release of proteases outside the cell

Levels 3-4: Applying/Analyzing

6. Which observation suggested to Sutherland the involvement of a second messenger in epinephrine's effect on liver cells?
   (A) Enzymatic activity was proportional to the amount of calcium added to a cell-free extract.
   (B) Receptor studies indicated that epinephrine was a ligand.
   (C) Glycogen breakdown was observed only when epinephrine was administered to intact cells.
   (D) Glycogen breakdown was observed only when epinephrine and glycogen phosphorylase were mixed.
7. Protein phosphorylation is commonly involved with which of the following?
   (A) ligand binding by receptor tyrosine kinases.
   (B) activation of G protein-coupled receptors.
   (C) activation of protein kinase molecules.
   (D) release of Ca^{2+} from the ER lumen.

Levels 5-6: Evaluating/Creating

8. **DRAW IT** Draw the following apoptotic pathway, which operates in human immune cells. A death signal is received when a molecule called Fas binds its cell-surface receptor. The binding of many Fas molecules to receptors causes receptor clustering. The intracellular regions of the receptors, when together, bind proteins called adaptor proteins. These in turn bind to inactive molecules of caspase-8, which become activated and then activate caspase-3. Once activated, caspase-3 initiates apoptosis.
9. **EVOLUTION CONNECTION** Identify the evolutionary mechanisms that might account for the origin and persistence of cell-to-cell signaling systems in prokaryotes.
10. **SCIENTIFIC INQUIRY** Epinephrine initiates a signal transduction pathway that produces cyclic AMP (cAMP) and leads to the breakdown of glycogen to glucose, a major energy source for cells. But glycogen breakdown is only part of the fight-or-flight response that epinephrine brings about; the overall effect on the body includes an increase in heart rate and alertness, as well as a burst of energy. Given that caffeine blocks the activity of cAMP phosphodiesterase, propose a mechanism by which caffeine ingestion leads to heightened alertness and sleeplessness.
11. **SCIENCE, TECHNOLOGY, AND SOCIETY** The aging process is thought to be initiated at the cellular level. Among the changes that can occur after a certain number of cell divisions is the loss of a cell's ability to respond to growth factors and other signals. Much research into aging is aimed at understanding such losses, with the ultimate goal of extending the human life span. Not everyone, however, agrees that this is a desirable goal. If life expectancy were greatly increased, discuss what might be the social and ecological consequences.
12. **WRITE ABOUT A THEME: ORGANIZATION** The properties of life emerge at the biological level of the cell. The highly regulated process of apoptosis is not simply the destruction of a cell; it is also an emergent property. Write a short essay (about 100–150 words) that briefly explains the role of apoptosis in the development and proper functioning of an animal, and describe how this form of programmed cell death is a process that emerges from the orderly integration of signaling pathways.
13. **SYNTHESIZE YOUR KNOWLEDGE**



There are five basic tastes—sour, salty, sweet, bitter, and "umami." Salt is detected when the concentration of salt outside of a taste bud cell is higher than that inside of it, and ion channels allow the passive leakage of Na^+ into the cell. The resulting change in membrane potential (see Concept 7.4) sends the "salty" signal to the brain. Umami is a savory taste generated by glutamate (glutamic acid, found in monosodium glutamate, or MSG), which is used as a flavor enhancer in foods such as taco-flavored tortilla chips. The glutamate receptor is a GPCR, which, when bound, initiates a signaling pathway that ends with a cellular response, perceived by you as "taste." If you eat a regular potato chip and then rinse your mouth, you will no longer taste salt. But if you eat a flavored tortilla chip and then rinse, the taste persists. (Try it!) Propose a possible explanation for this difference.

For selected answers, see Appendix A.

12 The Cell Cycle

KEY CONCEPTS

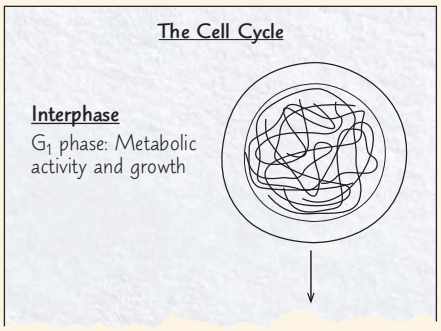
12.1 **Most cell division results in genetically identical daughter cells** *p. 235*

12.2 **The mitotic phase alternates with interphase in the cell cycle** *p. 237*

12.3 **The eukaryotic cell cycle is regulated by a molecular control system** *p. 244*

Study Tip

Make a visual study guide: Figure 12.1 presents the events of the cell cycle as a simplified linear diagram. As you learn more about the cell cycle, draw a detailed linear diagram of the stages of interphase, mitosis, and cytokinesis. Include explanatory labels. Add the circular chart from Figure 12.6 and show how the two diagrams are related.



Go to Mastering Biology

For Students (in eText and Study Area)
- Get Ready for Chapter 12
- BioFlix® Animation: Mitosis
- Animation: Microtubule Depolymerization

For Instructors to Assign (in Item Library)
- BioFlix® Tutorial: Mitosis (2 of 3): Mechanisms of Mitosis
- Coaching Activity: Evaluating Science in the Media: Tanning and Skin Cancer

Ready-to-Go Teaching Module (in Instructor Resources)
- Mitosis (Concept 12.2)

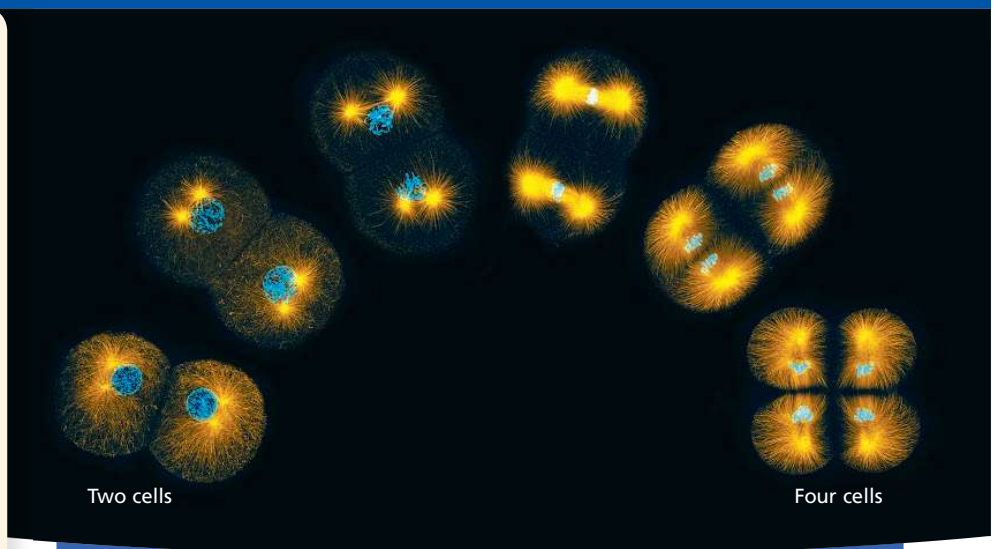
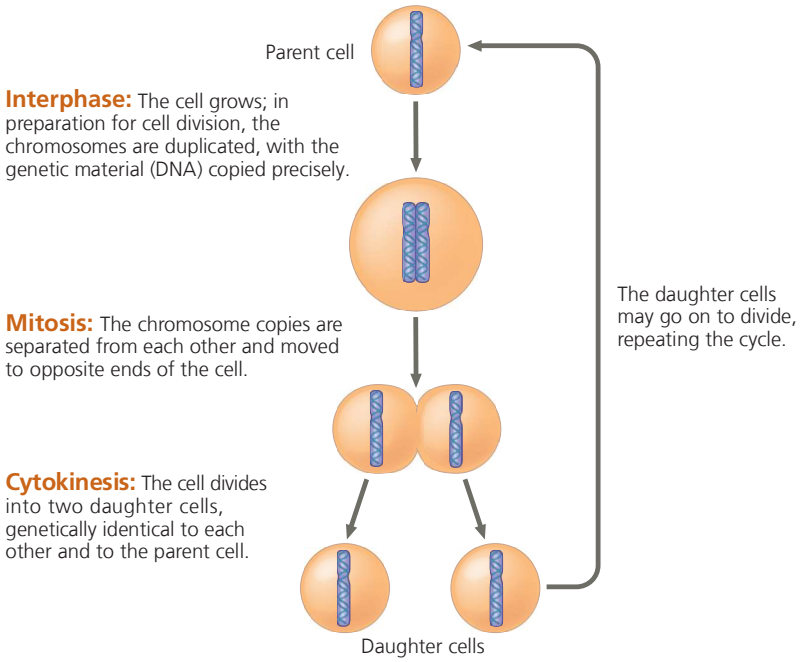


Figure 12.1 A multicellular organism starts out as a single cell that divides into two. Those two cells then divide into four, as shown in these fluorescence micrographs of a marine worm embryo. Cell division continues throughout an organism's life, for growth or to replace worn-out or damaged cells. Each time a cell divides in this way, it is crucial that the daughter cells be genetically identical to the parent cell.

How does one parent cell give rise to two genetically identical daughter cells?



CONCEPT 12.1

Most cell division results in genetically identical daughter cells

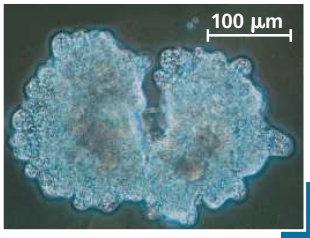
The ability of organisms to produce more of their own kind is the one characteristic that best distinguishes living things from nonliving matter. This unique capacity to procreate, like all biological functions, has a cellular basis. The continuity of life is based on the reproduction of cells, or **cell division**.

Key Roles of Cell Division

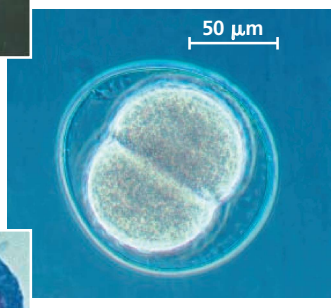
Cell division plays several important roles in life. When a prokaryotic cell divides, it is actually reproducing because the process gives rise to a new organism (another cell). The same is true of any unicellular eukaryote, such as the amoeba shown in **Figure 12.2a**. As for multicellular eukaryotes, cell division enables each of these organisms to develop from a single cell—the fertilized egg. A two-celled embryo, the first stage in this process, is shown in **Figure 12.2b**. And cell division continues to function in renewal and repair in fully grown multicellular eukaryotes, replacing cells that die from accidents or normal wear and tear. For example, dividing cells in your bone marrow continuously make new blood cells **(Figure 12.2c)**.

The reproduction of a cell, with all of its complexity, cannot occur by a mere pinching in half; a cell is not like a soap bubble that simply enlarges and splits in two. In both prokaryotes and eukaryotes, a crucial function of most cell divisions is the distribution of identical genetic material—DNA—to two daughter cells. (The exception is meiosis, the special type of eukaryotic cell division that can produce sperm and eggs.) What is most remarkable about cell division is the accuracy with which the DNA is passed from one generation of cells to the next. A dividing cell replicates its DNA, distributes the two copies to opposite ends of the cell, and then splits into daughter cells.

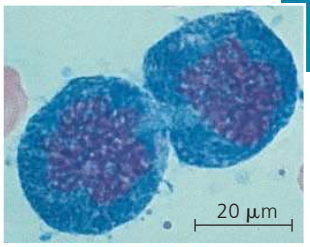
▼ Figure 12.2 The functions of cell division.



◀ **(a) Asexual reproduction.** An amoeba, a single-celled eukaryote, is dividing into two cells. Each new cell will be an individual organism (LM).



▶ **(b) Growth and development.** This micrograph shows a sand dollar embryo shortly after the fertilized egg divided, forming two cells (LM).



◀ **(c) Tissue renewal.** These dividing bone marrow cells will give rise to new blood cells (LM).

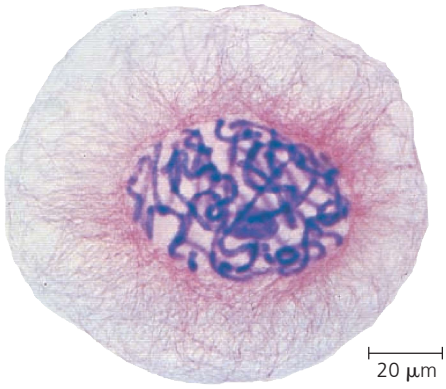
Mastering Biology Video: Cell Division in a Sea Urchin Embryo

Cellular Organization of the Genetic Material

A cell's DNA, its genetic information, is called its **genome**. Although a prokaryotic genome is often a single DNA molecule, eukaryotic genomes usually consist of a number of DNA molecules. The overall length of DNA in a eukaryotic cell is enormous. A typical human cell, for example, has about 2 m of DNA—a length about 250,000 times greater than the cell's diameter. Before the cell can divide to form genetically identical daughter cells, all of this DNA must be copied, or replicated, and then the two copies must be separated so that each daughter cell ends up with a complete genome.

The replication and distribution of so much DNA are manageable because the DNA molecules are packaged into structures called **chromosomes** (from the Greek *chroma*, color, and *soma*, body), so named because they take up certain dyes used in microscopy **(Figure 12.3)**. Each eukaryotic chromosome consists of one very long, linear DNA molecule associated with many proteins (see Figure 6.9). The DNA molecule carries several hundred to a few thousand genes, the units of information that specify an organism's inherited traits. The associated proteins maintain the structure of the chromosome and help control the activity of the genes. Together, the entire complex of DNA and proteins that is the building material of chromosomes is referred to as **chromatin**. As you will soon see, the chromatin of a chromosome varies in its degree of condensation during the process of cell division.

▼ Figure 12.3 Eukaryotic chromosomes. Chromosomes (stained purple) are visible within the nucleus of this cell from an African blood lily. The thinner red threads in the surrounding cytoplasm are the cytoskeleton. The cell is preparing to divide (LM).



Every eukaryotic species has a characteristic number of chromosomes in each cell's nucleus. For example, the nuclei of human **somatic cells** (all body cells except the reproductive cells) each contain 46 chromosomes, made up of two sets of 23, one set inherited from each parent. Reproductive cells, or **gametes**—such as sperm and eggs—have half as many chromosomes as somatic cells; in our example, human gametes have one set of 23 chromosomes. The number of chromosomes in somatic cells varies widely among species: 18 in cabbage plants, 48 in chimpanzees, 56 in elephants, 90 in hedgehogs, and 148 in one species of alga. We'll now consider how these chromosomes behave during cell division.

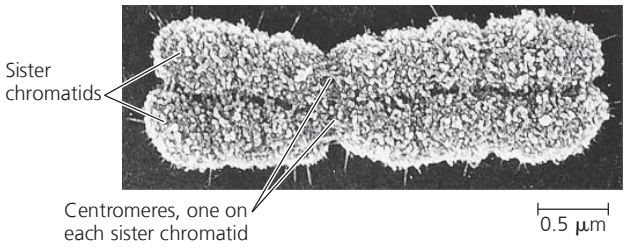
Distribution of Chromosomes During Eukaryotic Cell Division

When a cell is not dividing, and even as it replicates its DNA in preparation for cell division, each chromosome is in the form of a long, thin chromatin fiber. After DNA replication, however, the chromosomes condense as a part of cell division: Each chromatin fiber becomes densely coiled and folded, making the chromosomes much shorter and so thick that we can see them with a light microscope.

Each duplicated chromosome consists of two **sister chromatids**, which are joined copies of the original chromosome **(Figure 12.4)**. The two chromatids, each containing an identical DNA molecule, are often attached all along their lengths by protein complexes called *cohesins*; this attachment is known as *sister chromatid cohesion*. Each sister chromatid has a **centromere**, a region made up of repetitive sequences in the chromosomal DNA where the chromatid is attached most closely to its sister chromatid. This attachment is mediated by proteins that recognize and bind to the centromeric DNA; other bound proteins condense the DNA, giving the duplicated chromosome a narrow "waist." The portion of a chromatid to either side of the centromere is referred to as an *arm* of the chromatid. (An unduplicated chromosome has a single centromere, distinguished by the proteins that bind there, and two arms.)

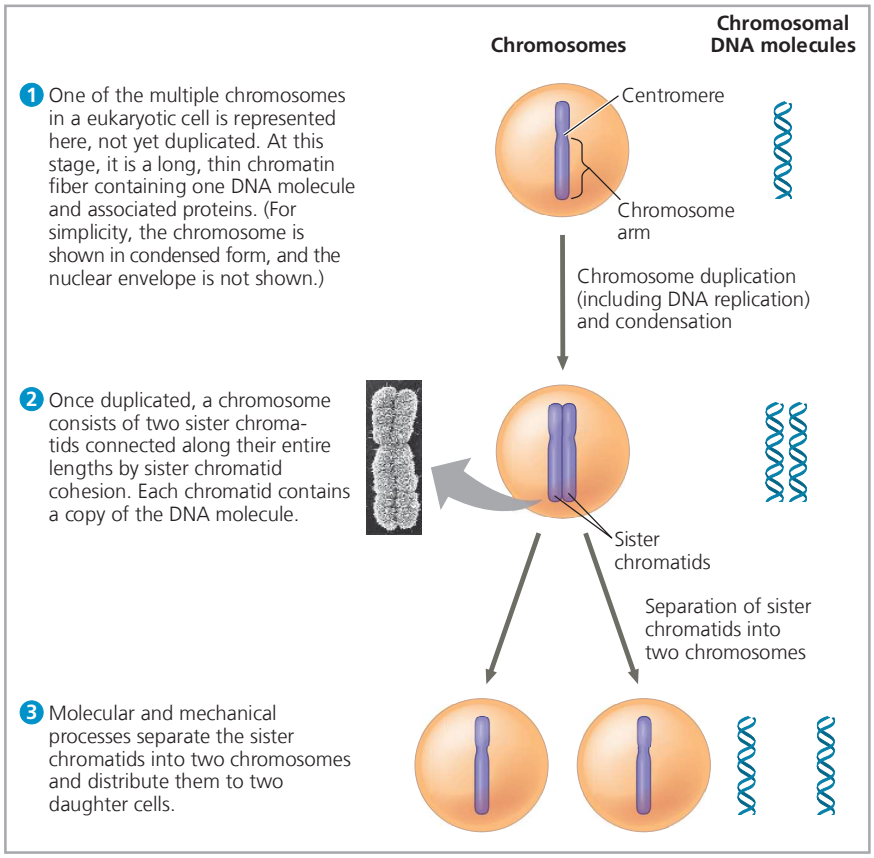
Later in the cell division process, the two sister chromatids of each duplicated chromosome separate and move into two new nuclei, one forming at each end of the cell. Once the sister chromatids separate, they are no longer called sister chromatids but are considered individual chromosomes; this is the step that essentially doubles the number of chromosomes during cell division. Thus, each new nucleus receives a collection of chromosomes identical to that of the parent cell **(Figure 12.5)**. **Mitosis**, the division of the genetic material in the nucleus, is usually followed immediately by **cytokinesis**, the division of the cytoplasm. One cell has become two, each the genetic equivalent of the parent cell.

▼ Figure 12.4 A highly condensed, duplicated human chromosome. (SEM)



DRAW IT *Circle one sister chromatid of this chromosome.*

▼ Figure 12.5 Chromosome duplication and distribution during cell division.



? *How many chromatid arms does the chromosome in 2 have? Identify the point in the figure where one chromosome becomes two.*

Mastering Biology BioFlix® Animation: Chromosome Duplication

From a fertilized egg, mitosis and cytokinesis produced the 37 trillion somatic cells that now make up your body, and the same processes continue to generate new cells to replace dead and damaged ones. In contrast, you produce gametes—eggs or sperm—by a variation of cell division called *meiosis*, which yields daughter cells with only one set of chromosomes, half as many chromosomes as the parent cell. Meiosis in humans occurs only in special cells in the ovaries or testes (the gonads). Generating gametes, meiosis reduces the chromosome number from 46 (two sets) to 23 (one set). Fertilization fuses two gametes together and returns the chromosome number to 46 (two sets). Mitosis then conserves that number in every somatic cell nucleus of the new human individual. In Chapter 13, we will examine the role of meiosis in reproduction and inheritance in more detail. In the remainder of this chapter, we focus on mitosis and the rest of the cell cycle in eukaryotes.

CONCEPT CHECK 12.1

1. How many chromosomes are drawn in each part of Figure 12.5? (Ignore the micrograph in step 2.)
2. **WHAT IF?** A chicken has 78 chromosomes in its somatic cells. How many chromosomes did the chicken inherit from each parent? How many chromosomes are in each of the chicken's gametes? How many chromosomes will be in each somatic cell of the chicken's offspring?

For suggested answers, see Appendix A.

CONCEPT 12.2

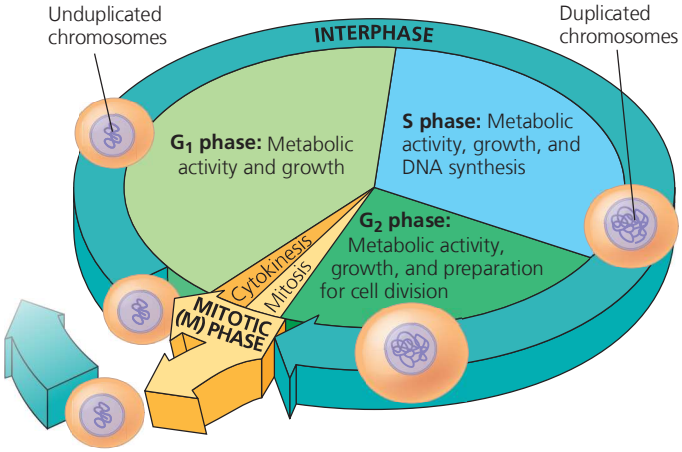
The mitotic phase alternates with interphase in the cell cycle

In 1882, a German anatomist named Walther Flemming developed dyes that allowed him to observe, for the first time, the behavior of chromosomes during mitosis and cytokinesis. (In fact, Flemming coined the terms *mitosis* and *chromatin*.) During the period between one cell division and the next, it appeared to Flemming that the cell was simply growing larger. But we now know that many critical events occur during this stage in the life of a cell.

Phases of the Cell Cycle

Mitosis is just one part of the **cell cycle**, the life of a cell from the time it is first formed during division of a parent cell until its own division into two daughter cells **(Figure 12.6)**. (Biologists use the words *daughter* or *sister* in relation to cells, but this is not meant to imply gender.) In fact, the **mitotic (M) phase**, which includes both mitosis and cytokinesis, is usually the shortest part of the cell cycle. The mitotic phase alternates with a much longer stage called **interphase**, which often accounts for about 90% of the cycle. Interphase can be divided into three phases: the **G_1 phase** ("first gap"), the **S phase** ("synthesis"), and the **G_2 phase** ("second gap").

▼ Figure 12.6 The cell cycle. In a dividing cell, the mitotic (M) phase alternates with interphase, a growth period.



MITOTIC (M) PHASE:
Mitosis: Distribution of chromosomes into two daughter nuclei
Cytokinesis: Division of cytoplasm, producing two daughter cells. Each daughter cell can start a new cell cycle.

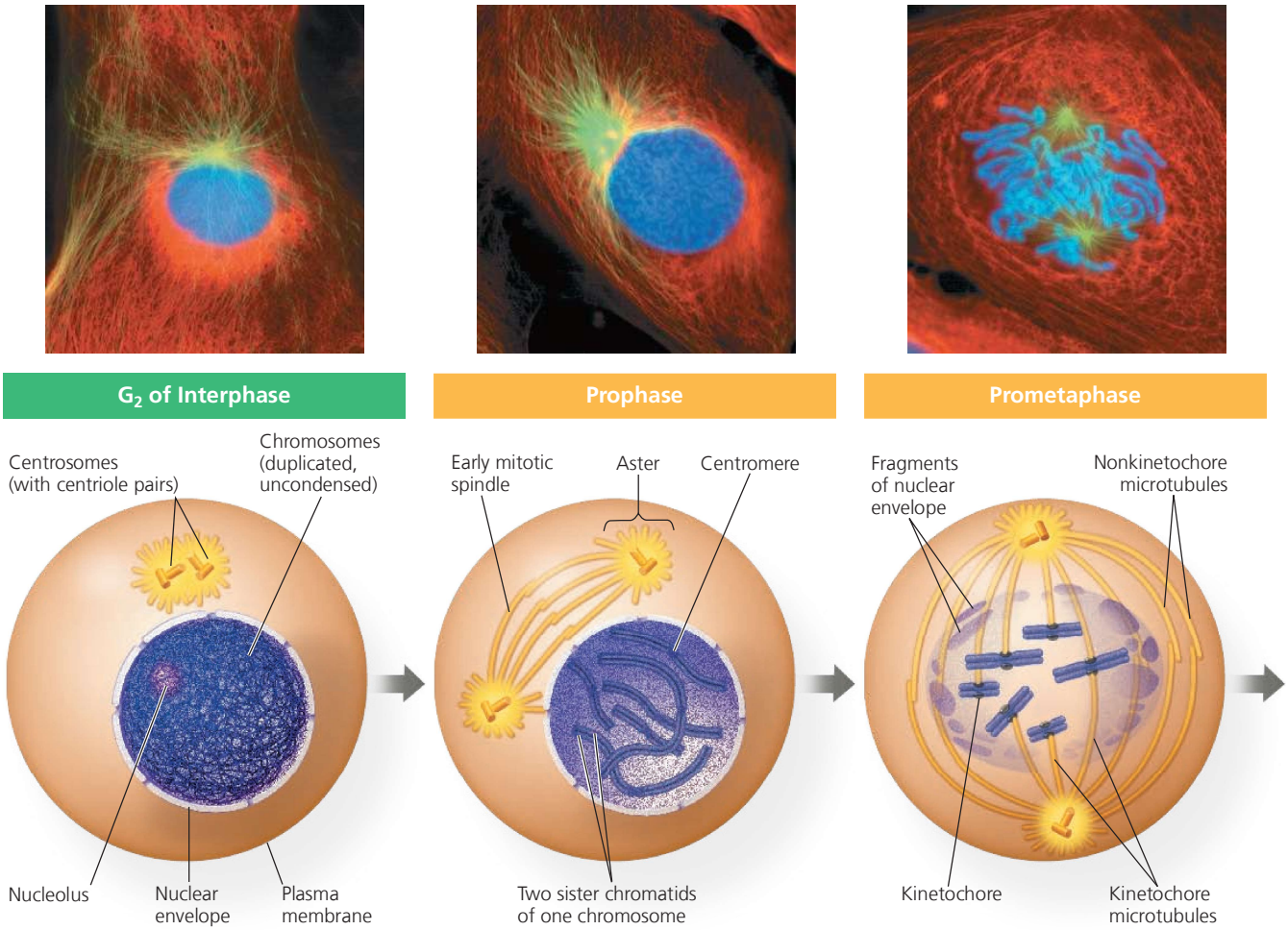
Mastering Biology **Animation: The Cell Cycle**

The G phases were misnamed as "gaps" when they were first observed because the cells appeared inactive, but we now know that intense metabolic activity and growth occur throughout interphase. During all three phases of interphase, actually, a cell grows by producing proteins and cytoplasmic organelles such as mitochondria and endoplasmic reticulum. Duplication of the chromosomes, crucial for eventual division of the cell, occurs entirely during the S phase. (We'll explore synthesis, or replication, of DNA in Concept 16.2.) Thus, a cell grows (G_1), continues to grow as it copies its chromosomes (S), grows more as it completes preparations for cell division (G_2), and divides (M). The daughter cells may then repeat the cycle.

A particular human cell might undergo one division in 24 hours. Of this time, the M phase would occupy less than 1 hour, while the S phase might occupy 10–12 hours, or about half the cycle. The rest of the time would be apportioned between the G_1 and G_2 phases. The G_2 phase usually takes 4–6 hours; in our example, G_1 would occupy about 5–6 hours. G_1 is the most variable in length in different types of cells. Some cells in a multicellular organism divide very infrequently or not at all. These cells spend their time in G_1 (or a related phase called G_0, to be discussed later in the chapter) doing their job in the organism—a cell of the pancreas secretes digestive enzymes, for example.

Mitosis is conventionally broken down into five stages: **prophase**, **prometaphase**, **metaphase**, **anaphase**, and **telophase**. Overlapping with the latter stages of mitosis, cytokinesis completes the mitotic phase. **Figure 12.7** describes these stages in an animal cell. Study this figure thoroughly before proceeding to the next two sections, which examine mitosis and cytokinesis more closely.

▼ Figure 12.7 Exploring Mitosis in an Animal Cell



G_2 of Interphase

- A nuclear envelope encloses the nucleus.
- The nucleus contains one or more nucleoli (singular, *nucleolus*).
- Two centrosomes have formed by duplication of a single centrosome. Centrosomes are regions in animal cells that organize the microtubules of the spindle. Each centrosome contains two centrioles.
- Chromosomes, duplicated during S phase, cannot be seen individually because they have not yet condensed.

The fluorescence micrographs show dividing lung cells from a newt; this species has 22 chromosomes. Chromosomes appear blue, microtubules green, and intermediate filaments red. For simplicity, the drawings show only 6 chromosomes.

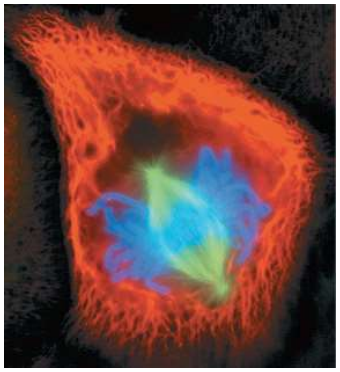
Prophase

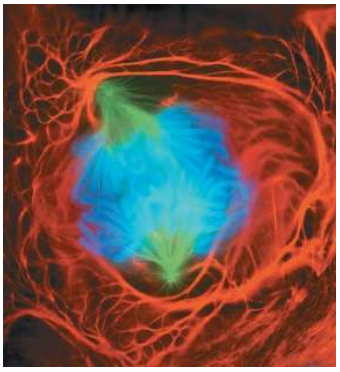
- The chromatin fibers become more tightly coiled, condensing into discrete chromosomes observable with a light microscope.
- The nucleoli disappear.
- Each duplicated chromosome appears as two identical sister chromatids joined at their centromeres and, often, all along their arms by cohesins, resulting in sister chromatid cohesion.
- The mitotic spindle (named for its shape) begins to form. It is composed of the centrosomes and the microtubules that extend from them. The radial arrays of shorter microtubules that extend from the centrosomes are called asters ("stars").
- The centrosomes move away from each other, propelled partly by the lengthening microtubules between them.

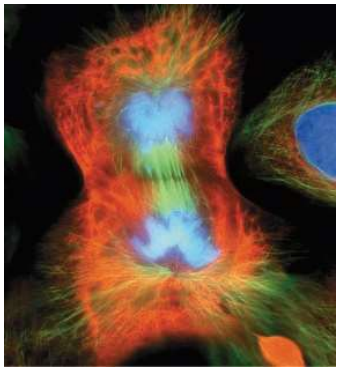
Prometaphase

- The nuclear envelope fragments.
- The microtubules extending from each centrosome can now invade the nuclear area.
- The chromosomes have become even more condensed.
- A kinetochore, a specialized protein structure, has now formed at the centromere of each chromatid (thus, two per chromosome).
- Some of the microtubules attach to the kinetochores, becoming "kinetochore microtubules," which jerk the chromosomes back and forth.
- Nonkinetochore microtubules interact with those from the opposite pole of the spindle, lengthening the cell.

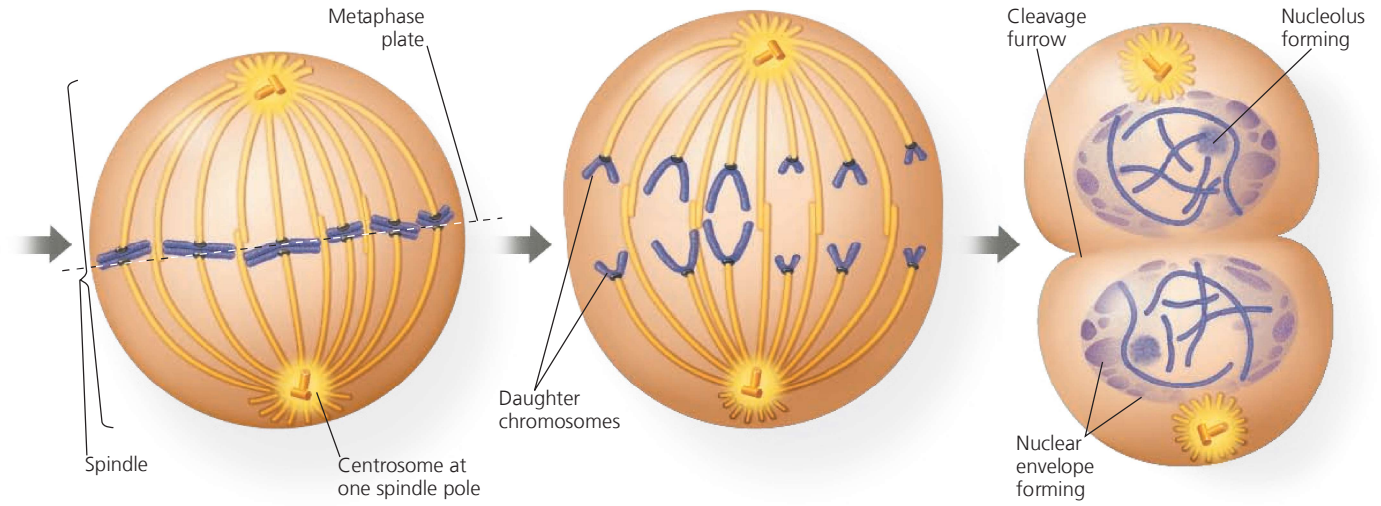
? *How many molecules of DNA are in the prometaphase drawing? How many molecules per chromosome? How many double helices are there per chromosome? Per chromatid?*







Metaphase | **Anaphase** | **Telophase and Cytokinesis**



Metaphase

- The centrosomes are now at opposite poles of the cell.
- The chromosomes have all arrived at the *metaphase plate*, a plane that is equidistant between the spindle's two poles. The chromosomes' centromeres lie at the metaphase plate.
- For each chromosome, the kinetochores of the sister chromatids are attached to kinetochore microtubules coming from opposite poles.

Anaphase

- Anaphase is the shortest stage of mitosis, often lasting only a few minutes.
- Anaphase begins when the cohesin proteins are cleaved. This allows the two sister chromatids of each pair to part suddenly. Each chromatid thus becomes an independent chromosome.
- The two new daughter chromosomes begin moving toward opposite ends of the cell as their kinetochore microtubules shorten. Because these microtubules are attached at the centromere region, the centromeres are pulled ahead of the arms, moving at a rate of about 1 µm/min.
- The cell elongates as the nonkinetochore microtubules lengthen.
- By the end of anaphase, the two ends of the cell have identical—and complete—collections of chromosomes.

Telophase

- Two daughter nuclei form in the cell. Nuclear envelopes arise from the fragments of the parent cell's nuclear envelope and other portions of the endomembrane system.
- Nucleoli reappear.
- The chromosomes become less condensed.
- Any remaining spindle microtubules are depolymerized.
- Mitosis, the division of one nucleus into two genetically identical nuclei, is now complete.

Cytokinesis

- The division of the cytoplasm is usually well under way by late telophase, so the two daughter cells appear shortly after the end of mitosis.
- In animal cells, cytokinesis involves the formation of a cleavage furrow, which pinches the cell in two.

Mastering Biology BioFlix® Animation: Mitosis
Video: Animal Mitosis (time-lapse)

The Mitotic Spindle: *A Closer Look*

Many of the events of mitosis depend on the **mitotic spindle**, which begins to form in the cytoplasm during prophase. This structure consists of fibers made of microtubules and associated proteins. While the mitotic spindle assembles, the other microtubules of the cytoskeleton partially disassemble, providing the material used to construct the spindle. The spindle microtubules elongate (polymerize) by incorporating more subunits of the protein tubulin (see Table 6.1) and shorten (depolymerize) by losing subunits.

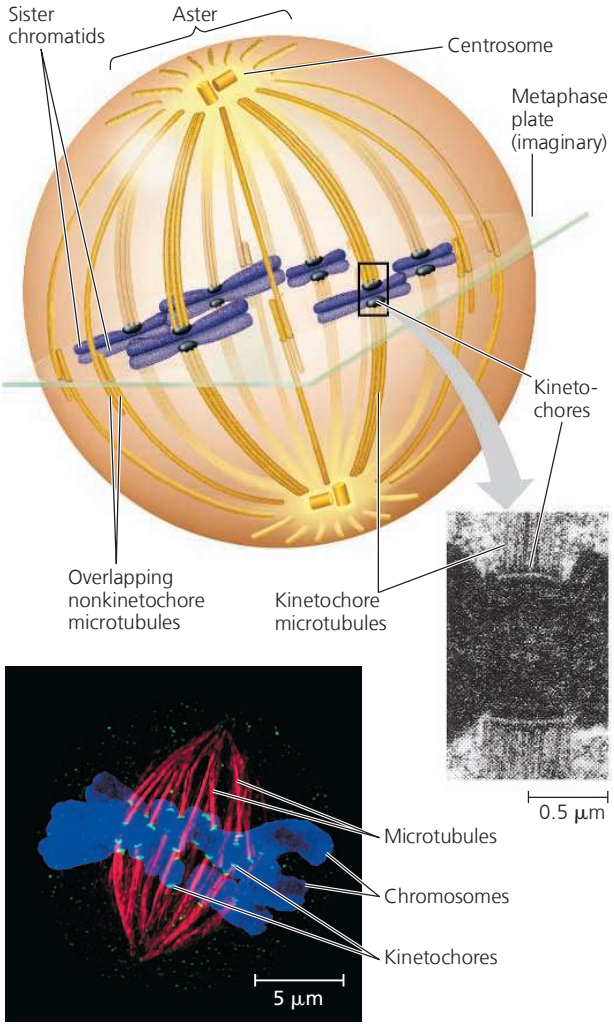
In animal cells, the assembly of spindle microtubules starts at the **centrosome**, a subcellular region containing material that functions throughout the cell cycle to organize the cell's microtubules. (It is also a type of *microtubule-organizing center*.) A pair of centrioles is located at the center of the centrosome, but they are not essential for cell division: If the centrioles are destroyed with a laser microbeam, a spindle nevertheless forms during mitosis. In fact, centrioles are not even present in plant cells, which do form mitotic spindles.

During interphase in animal cells, the single centrosome duplicates, forming two centrosomes, which remain near the nucleus. The two centrosomes move apart during prophase and prometaphase of mitosis as spindle microtubules grow out from them. By the end of prometaphase, the two centrosomes, one at each pole of the spindle, are at opposite ends of the cell. An *aster*, a radial array of short microtubules, extends from each centrosome. The spindle includes the centrosomes, the spindle microtubules, and the asters.

Each of the two sister chromatids of a duplicated chromosome has a **kinetochore**, a structure made up of proteins that have assembled on specific sections of DNA at each centromere. The chromosome's two kinetochores face in opposite directions. During prometaphase, some of the spindle microtubules attach to the kinetochores; these are called *kinetochore microtubules*. (The number of microtubules attached to a kinetochore varies among species, from one microtubule in yeast cells to 40 or so in some mammalian cells.) When one of a chromosome's kinetochores is "captured" by microtubules, the chromosome begins to move toward the pole from which those microtubules extend. However, this movement comes to a halt as soon as microtubules from the opposite pole attach to the kinetochore on the other chromatid. What happens next is like a tug-of-war that ends in a draw. The chromosome moves first in one direction and then in the other, back and forth, finally settling midway between the two ends of the cell. At metaphase, the centromeres of all the duplicated chromosomes are on a plane midway between the spindle's two poles. This plane is called the **metaphase plate**, which is an imaginary plate rather than an actual cellular structure **(Figure 12.8)**. Meanwhile, microtubules that do not attach to kinetochores have been elongating, and by metaphase they overlap and interact with other *nonkinetochore microtubules* from the opposite pole of the spindle. By metaphase, the microtubules of the asters have also grown and are in contact with the plasma membrane. The spindle is now complete.

The structure of the spindle correlates well with its function during anaphase. Anaphase begins suddenly when the cohesins holding together the sister chromatids of each chromosome are cleaved by an enzyme called *separase*. Once separated, the chromatids become individual chromosomes that move toward opposite ends of the cell.

▼ Figure 12.8 The mitotic spindle at metaphase. The kinetochores of each chromosome's two sister chromatids face in opposite directions. Here, each kinetochore is attached to a cluster of kinetochore microtubules (see TEM) extending from the nearest centrosome. Nonkinetochore microtubules overlap at the metaphase plate. The fluorescent micrograph is a rat kangaroo cell at metaphase.



DRAW IT *On the lower micrograph, draw a line indicating the position of the metaphase plate. Draw arrows showing the directions of chromosome movement when anaphase begins.*

Mastering Biology **Video: Spindle Formation During Mitosis**
Animation: Mitosis

How do the kinetochore microtubules function in this poleward movement of chromosomes? Apparently, two mechanisms are in play, both involving motor proteins. (To review how motor proteins move an object along a microtubule, see Figure 6.21.) Results of a cleverly designed experiment suggested that motor proteins on the kinetochores "walk" the chromosomes along the microtubules, which depolymerize at their kinetochore ends after the motor proteins have passed **(Figure 12.9)**. (This is referred to as the "Pac-man" mechanism because of its resemblance to the arcade game character that moves by eating all the dots in its path.) However, other researchers, working with different cell types or cells from other species, have shown that chromosomes are "reeled in" by motor proteins at the spindle poles and that the microtubules depolymerize after they pass by these motor proteins at the poles. The general consensus now is that both mechanisms are used and that their relative contributions vary among cell types.

In a dividing animal cell, the nonkinetochore microtubules are responsible for elongating the whole cell during anaphase. Nonkinetochore microtubules from opposite poles overlap each other extensively during metaphase (see Figure 12.8). During anaphase, the region of overlap is reduced as motor proteins attached to the microtubules walk them away from one another, using energy from ATP. As the microtubules push apart from each other, their spindle poles are pushed apart, elongating the cell. At the same time, the microtubules lengthen somewhat by the addition of tubulin subunits to their overlapping ends. As a result, the microtubules continue to overlap.

At the end of anaphase, duplicate groups of chromosomes have arrived at opposite ends of the elongated parent cell. Nuclei re-form during telophase. Cytokinesis generally begins during anaphase or telophase, and the spindle eventually disassembles by depolymerization of microtubules.

Cytokinesis: *A Closer Look*

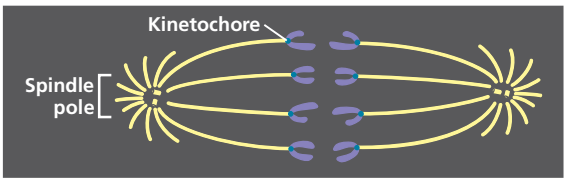
In animal cells, cytokinesis occurs by a process known as **cleavage**. The first sign of cleavage is the appearance of a **cleavage furrow**, a shallow groove in the cell surface near the old metaphase plate **(Figure 12.10a)**. On the cytoplasmic side of the furrow is a contractile ring of actin microfilaments associated with molecules of the protein myosin. The actin microfilaments interact with the myosin molecules, causing the ring to contract. The contraction of the dividing cell's ring of microfilaments is like the pulling of a drawstring. The cleavage furrow deepens until the parent cell is pinched in two, producing two completely separated cells, each with its own nucleus and its own share of cytosol, organelles, and other subcellular structures.

Cytokinesis in plant cells, which have cell walls, is markedly different. There is no cleavage furrow. Instead, during telophase, vesicles derived from the Golgi apparatus move along microtubules to the middle of the cell, where they coalesce, producing a **cell plate**. Cell wall materials carried in the

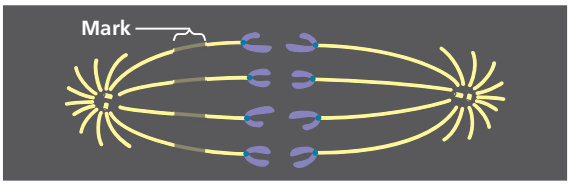
▼ Figure 12.9 Inquiry

At which end do kinetochore microtubules shorten during anaphase?

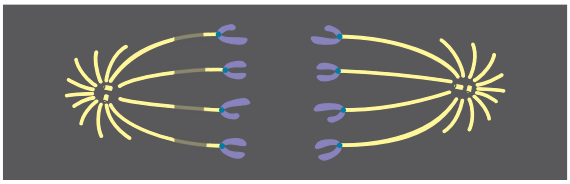
Experiment Gary Borisy and colleagues at the University of Wisconsin wanted to determine whether kinetochore microtubules depolymerize at the kinetochore end or the pole end as chromosomes move toward the poles during mitosis. First they labeled the microtubules of a pig kidney cell in early anaphase with a yellow fluorescent dye. (Nonkinetochore microtubules are not shown.)



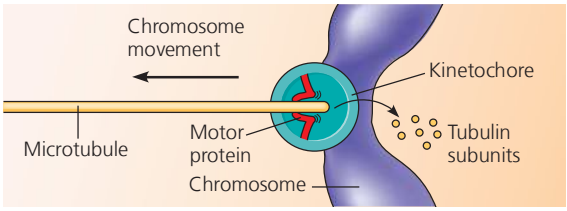
Then they marked a region of the kinetochore microtubules between one spindle pole and the chromosomes by using a laser to eliminate the fluorescence from that region, while leaving the microtubules intact (see below). As anaphase proceeded, they monitored the changes in microtubule length on either side of the non-fluorescent mark.



Results As the chromosomes moved poleward, the microtubule segments on the kinetochore side of the mark shortened, while those on the spindle pole side stayed the same length.



Conclusion During anaphase in this cell type, chromosome movement is correlated with kinetochore microtubules shortening at their kinetochore ends and not at their spindle pole ends. This experiment supports the hypothesis that during anaphase, a chromosome is walked along a microtubule as the microtubule depolymerizes at its kinetochore end, releasing tubulin subunits.



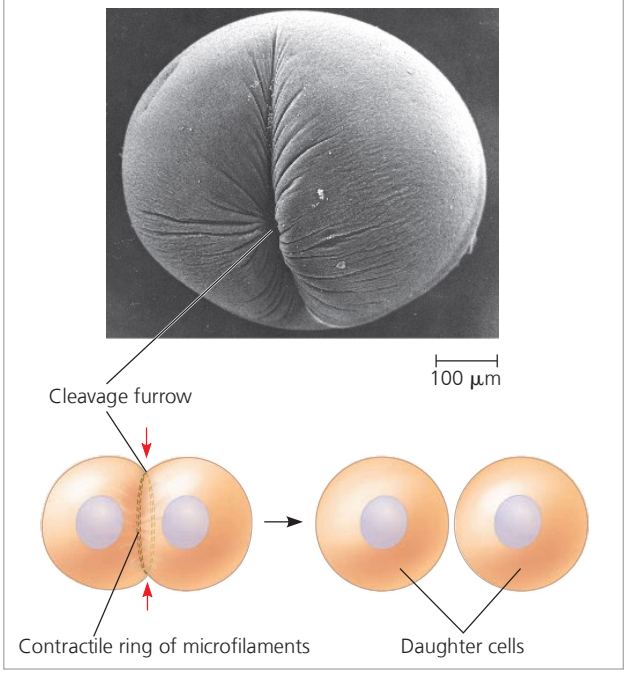
Data from G. J. Gorbsky, P. J. Sammak, and G. G. Borisy, Chromosomes move poleward in anaphase along stationary microtubules that coordinately disassemble from their kinetochore ends, *Journal of Cell Biology* 104:9–18 (1987).

WHAT IF? *If this experiment had been done on a cell type in which "reeling in" at the poles was the main cause of chromosome movement, how would the mark have moved relative to the poles? How would the microtubule portions on either side of the mark have changed?*

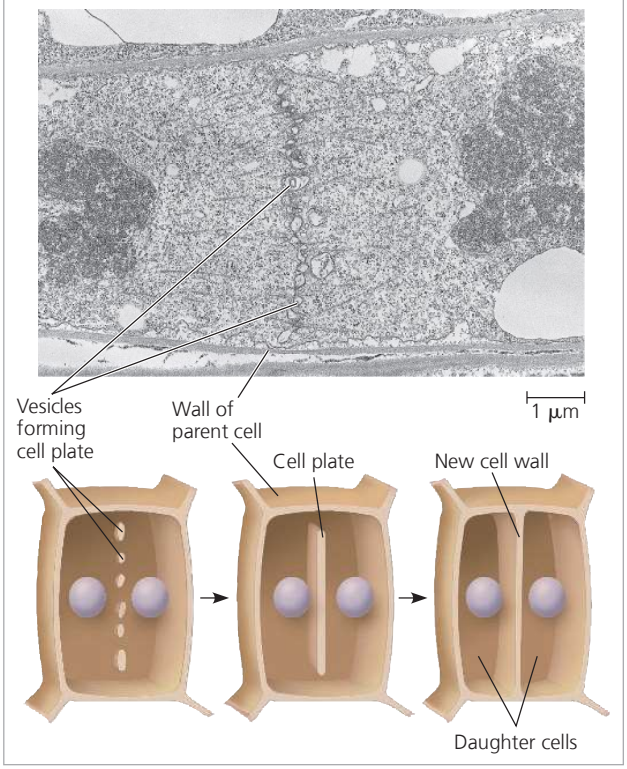
Mastering Biology Animation: Microtubule Depolymerization

▼ **Figure 12.10 Cytokinesis in animal and plant cells.**

(a) Cleavage of an animal cell (SEM)



(b) Cell plate formation in a plant cell (TEM)



Mastering Biology **Animation: Cytokinesis**
Video: Cytokinesis in an Animal Cell

vesicles collect inside the cell plate as it grows **(Figure 12.10b)**. The cell plate enlarges until its surrounding membrane fuses with the plasma membrane along the perimeter of the cell. Two daughter cells result, each with its own plasma membrane. Meanwhile, a new cell wall arising from the contents of the cell plate forms between the daughter cells.

Figure 12.11 is a series of micrographs of a dividing plant cell. Examining this figure will help you review mitosis and cytokinesis.

Binary Fission in Bacteria

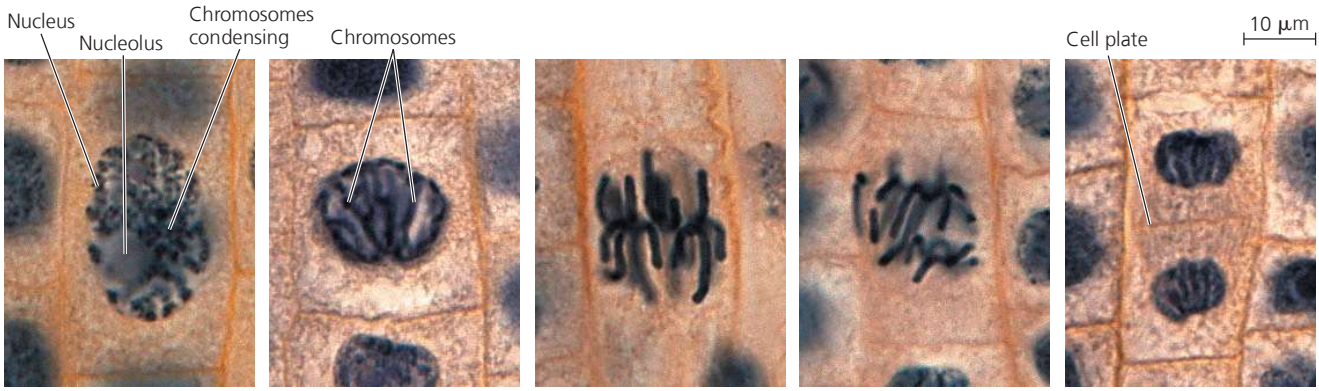
Prokaryotes (bacteria and archaea) can undergo a type of reproduction in which the cell grows to roughly double its size and then divides to form two cells. The term **binary fission**, meaning "division in half," refers to this process and to the asexual reproduction of single-celled eukaryotes, such as the amoeba in Figure 12.2a. However, the process in eukaryotes involves mitosis, while that in prokaryotes does not.

In bacteria, most genes are carried on a single bacterial chromosome that consists of a circular DNA molecule and associated proteins. Although bacteria are smaller and simpler than eukaryotic cells, the challenge of replicating their genomes in an orderly fashion and distributing the copies equally to two daughter cells is still formidable. For example, when it is fully stretched out, the chromosome of the bacterium *Escherichia coli* is about 500 times as long as the cell. For such a long chromosome to fit within the cell, it must be highly coiled and folded.

In some bacteria, the process of cell division is initiated when the DNA of the bacterial chromosome begins to replicate at a specific place on the chromosome called the **origin of replication**, producing two origins. As the chromosome continues to replicate, one origin moves rapidly toward the opposite end of the cell **(Figure 12.12)**. While the chromosome is replicating, the cell elongates. When replication is complete and the bacterium has reached about twice its initial size, proteins cause its plasma membrane to pinch inward, dividing the parent bacterial cell into two daughter cells. In this way, each cell inherits a complete genome.

Using the techniques of modern DNA technology to tag the origins of replication with molecules that glow green in fluorescence microscopy (see Figure 6.3), researchers have directly observed the movement of bacterial chromosomes. This movement is reminiscent of the poleward movements of the centromere regions of eukaryotic chromosomes during anaphase of mitosis, but bacteria don't have visible mitotic spindles or even microtubules. In most bacterial species studied, the two origins of replication end up at opposite ends of the cell or in some other very specific location, possibly anchored there by one or more proteins. How bacterial chromosomes move and how their specific location is established and maintained are active areas of research. Several proteins that play important roles have been identified. Polymerization of one protein resembling eukaryotic actin apparently functions

▼ Figure 12.11 Mitosis in a plant cell. These light micrographs show mitosis in cells of an onion root.



1 **Prophase.** The chromosomes are condensing and the nucleolus is beginning to disappear. Although not yet visible in the micrograph, the mitotic spindle is starting to form.

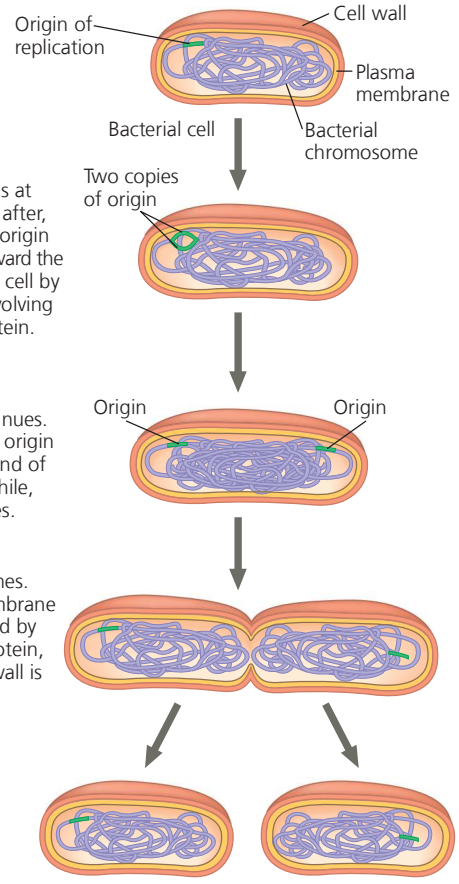
2 **Prometaphase.** Discrete chromosomes are now visible; each consists of two aligned, identical sister chromatids. Later in prometaphase, the nuclear envelope will fragment.

3 **Metaphase.** The spindle is complete, and the chromosomes, attached to microtubules at their kinetochores, are all at the metaphase plate.

4 **Anaphase.** Chromatids of each chromosome have separated. The daughter chromosomes are moving to the ends of the cell as their kinetochore microtubules shorten.

5 **Telophase.** Daughter nuclei are forming. Cytokinesis has started: The cell plate, which will divide the cytoplasm in two, grows toward the perimeter of the parent cell.

▼ Figure 12.12 Bacterial cell division by binary fission. The bacterium *E. coli*, shown here, has a single, circular chromosome.



1 Chromosome replication begins at the origin. Soon after, one copy of the origin moves rapidly toward the other end of the cell by a mechanism involving an actin-like protein.

2 Replication continues. One copy of the origin is now at each end of the cell. Meanwhile, the cell elongates.

3 Replication finishes. The plasma membrane is pinched inward by a tubulin-like protein, and a new cell wall is deposited.

4 Two daughter cells result.

Mastering Biology Animation: Cell Division in Bacteria

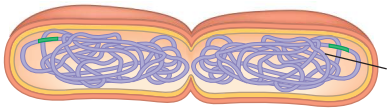
in bacterial chromosome movement during cell division, and another protein that is related to tubulin helps pinch the plasma membrane inward, separating the two bacterial daughter cells.

The Evolution of Mitosis

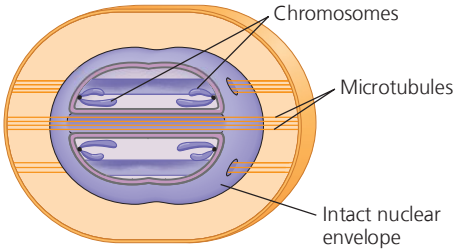
EVOLUTION Given that prokaryotes preceded eukaryotes on Earth by more than a billion years, we might hypothesize that mitosis evolved from simpler prokaryotic mechanisms of cell reproduction. The fact that some of the proteins involved in bacterial binary fission are related to eukaryotic proteins that function in mitosis supports that hypothesis.

As eukaryotes with nuclear envelopes and larger genomes evolved, the ancestral process of binary fission, seen today in bacteria, somehow gave rise to mitosis. Variations on cell division exist in different groups of organisms. These variant processes may be similar to mechanisms used by ancestral species and thus may resemble steps in the evolution of mitosis from a binary fission-like process presumably carried out by very early bacteria. Possible intermediate stages are suggested by two unusual types of nuclear division found today in certain unicellular eukaryotes—dinoflagellates, diatoms, and some yeasts **(Figure 12.13)**. These two modes of nuclear division are thought to be cases where ancestral mechanisms have remained relatively unchanged over evolutionary time. In both types, the nuclear envelope remains intact, in contrast to what happens in most eukaryotic cells. Keep in mind, however, that we can't observe cell division in cells of extinct species. This hypothesis uses only currently existing species as some possible examples of intermediate mechanisms. Other mechanisms may have existed in species that have gone extinct; we simply have no way of knowing.

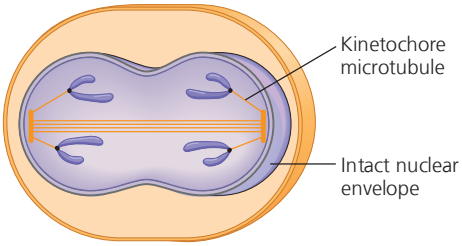
▼ Figure 12.13 Mechanisms of cell division in several groups of organisms. Some unicellular eukaryotes existing today have mechanisms of cell division that may resemble intermediate steps in the evolution of mitosis. Except for **(a)**, cell walls are not shown.



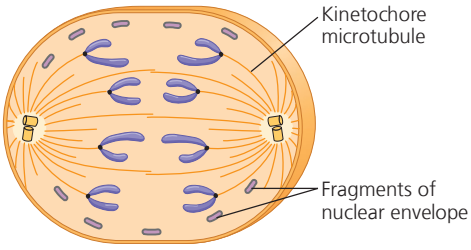
(a) Bacteria. During binary fission in bacteria, the origins of the daughter chromosomes move to opposite ends of the cell. The mechanism involves polymerization of actin-like molecules and possibly proteins that may anchor the daughter chromosomes to specific sites on the plasma membrane.



(b) Dinoflagellates. In unicellular eukaryotes called dinoflagellates, the chromosomes attach to the nuclear envelope, which remains intact during cell division. Microtubules pass through the nucleus inside cytoplasmic tunnels, reinforcing the spatial orientation of the nucleus, which then divides in a process reminiscent of bacterial binary fission.



(c) Diatoms and some yeasts. In these two other groups of unicellular eukaryotes, the nuclear envelope also remains intact during cell division. In these organisms, the microtubules form a spindle *within* the nucleus. Microtubules separate the chromosomes, and the nucleus splits into two daughter nuclei.



(d) Most eukaryotes. In most other eukaryotes, including plants and animals, the spindle forms outside the nucleus, and the nuclear envelope breaks down during mitosis. Microtubules separate the chromosomes, and two nuclear envelopes then form.

Mastering Biology **Video: Nuclear Envelope Breakdown and Formation During Mitosis in *C. elegans*, a Eukaryote**

CONCEPT CHECK 12.2

1. How many chromosomes are shown in the illustration in Figure 12.8? Are they duplicated? How many chromatids are shown?
2. Compare cytokinesis in animal cells and plant cells.
3. During which stages of the cell cycle does a chromosome consist of two identical chromatids?
4. Compare the roles of tubulin and actin during eukaryotic cell division with the roles of tubulin-like and actin-like proteins during bacterial binary fission.
5. A kinetochore has been compared to a coupling device that connects a motor to the cargo that it moves. Explain.
6. **MAKE CONNECTIONS** What other functions do actin and tubulin carry out? Name the proteins they interact with to do so. (Review Figures 6.21a and 6.26a.)

For suggested answers, see Appendix A.

CONCEPT 12.3

The eukaryotic cell cycle is regulated by a molecular control system

The timing and rate of cell division in different parts of a plant or animal are crucial to normal growth, development, and maintenance. The frequency of cell division varies with the type of cell. For example, human skin cells divide frequently throughout life, whereas liver cells maintain the ability to divide but keep it in reserve until an appropriate need arises—say, to repair a wound. Some of the most specialized cells, such as fully formed nerve cells and muscle cells, do not divide at all in a mature human. These cell cycle differences result from regulation at the molecular level. The mechanisms of this regulation are of great interest, not only to understand the life cycles of normal cells but also to learn how cancer cells manage to escape the usual controls.

The Cell Cycle Control System

What controls the cell cycle? In the early 1970s, a variety of experiments led to the hypothesis that the cell cycle is driven by specific signaling molecules present in the cytoplasm. Some of the first strong evidence for this hypothesis came from experiments with mammalian cells grown in culture. In these experiments, two cells in different phases of the cell cycle were fused to form a single cell with two nuclei **(Figure 12.14)**. If one of the original cells was in the S phase and the other was in G_1, the G_1 nucleus immediately entered the S phase, as though stimulated by signaling molecules present in the cytoplasm of the first cell. Similarly, if a cell undergoing mitosis (M phase) was fused with another cell in any stage of its cell cycle, even G_1, the second nucleus immediately entered mitosis, with condensation of the chromatin and formation of a mitotic spindle.

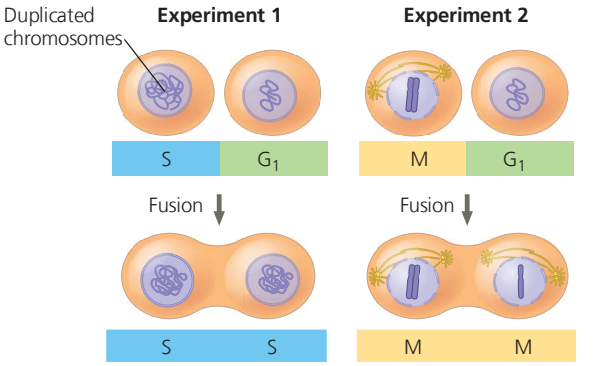
The experiment shown in Figure 12.14 and other experiments on animal cells and yeasts demonstrated that the

▼ Figure 12.14 Inquiry

Do molecular signals in the cytoplasm regulate the cell cycle?

Experiment Researchers at the University of Colorado wondered whether a cell's progression through the cell cycle is controlled by cytoplasmic molecules. They induced cultured mammalian cells at different phases of the cell cycle to fuse. Two experiments are shown.

Results



When a cell in S phase was fused with a cell in G_1, the G_1 nucleus immediately entered S phase—DNA was synthesized.

When a cell in M phase was fused with a cell in G_1, the G_1 nucleus immediately began mitosis—a spindle formed and the chromosomes condensed, even though the chromosomes had not been duplicated.

Conclusion The results of fusing a G_1 cell with a cell in the S or M phase of the cell cycle suggest that molecules present in the cytoplasm during the S or M phase control the progression to those phases.

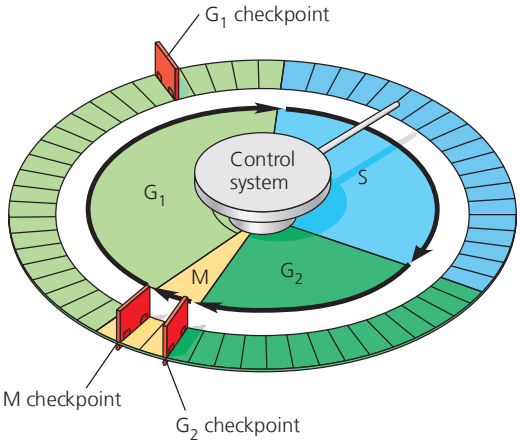
Data from R. T. Johnson and P. N. Rao, Mammalian cell fusion: Induction of premature chromosome condensation in interphase nuclei, *Nature* 226:717–722 (1970).

WHAT IF? *If the progression of phases did not depend on cytoplasmic molecules and each phase began when the previous one was complete, how would the results have differed?*

sequential events of the cell cycle are directed by a distinct **cell cycle control system**, a cyclically operating set of molecules in the cell that both triggers and coordinates key events in the cell cycle **(Figure 12.15)**. The cell cycle control system has been compared to the control device of a washing machine. Like the washer's timing device, the cell cycle control system proceeds on its own, according to a built-in clock. However, just as a washer's cycle is subject to both internal control (such as the sensor that detects when the tub is filled with water) and external adjustment (such as starting or stopping the machine), the cell cycle is regulated at certain checkpoints by both internal and external signals. A **checkpoint** in the cell cycle is a control point where stop and go-ahead signals can regulate the cycle. Three important checkpoints are found in the G_1, G_2, and M phases (red gates in Figure 12.15), which will be discussed shortly.

To understand how cell cycle checkpoints work, we'll first identify some of the molecules that make up the cell cycle control system (the molecular basis for the cell cycle clock) and describe how a cell progresses through the cycle. We'll then consider the internal and external checkpoint signals that can make the clock either pause or continue.

▼ Figure 12.15 Mechanical analogy for the cell cycle control system. In this diagram, the flat "stepping stones" around the perimeter represent sequential events. Like the control device of a washing machine, the cell cycle control system proceeds on its own, driven by a built-in clock. However, the system is subject to internal and external regulation at various checkpoints; three important checkpoints are shown (red).



Mastering Biology Animation: Control of the Cell Cycle

The Cell Cycle Clock: Cyclins and Cyclin-Dependent Kinases

Rhythmic fluctuations in the abundance and activity of cell cycle control molecules pace the sequential events of the cell cycle. These regulatory molecules are mainly proteins of two types: protein kinases and cyclins. As you learned in Concept 11.3, protein kinases are enzymes that activate or inactivate other proteins by phosphorylating them.

Many of the kinases that drive the cell cycle are actually present at a constant concentration in the growing cell, but much of the time they are in an inactive form. To be active, such a kinase must be attached to a **cyclin**, a protein that gets its name from its cyclically fluctuating concentration in the cell. Because of this requirement, these kinases are called **cyclin-dependent kinases**, or **Cdks**. The activity of a Cdk rises and falls with changes in the concentration of its cyclin partner. **Figure 12.16a** shows the fluctuating activity of **MPF**, the cyclin-Cdk complex that was discovered first (in frog eggs). Note that the peaks of MPF activity correspond to the peaks of cyclin concentration. The cyclin level rises during the S and G_2 phases and then falls abruptly during M phase.

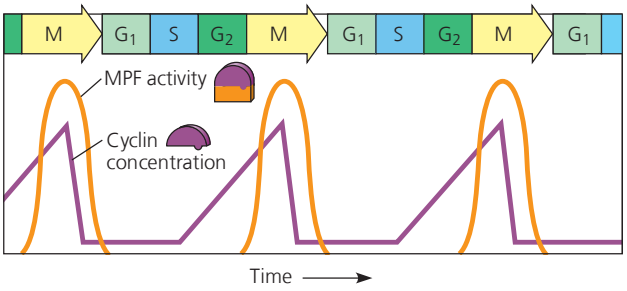
The initials MPF stand for "maturation-promoting factor," but we can think of MPF as "M-phase-promoting factor" because it triggers the cell's passage into the M phase, past the G_2 checkpoint. When cyclins that accumulate during G_2 associate with Cdk molecules, the resulting MPF complex

is active—it phosphorylates a variety of proteins, initiating mitosis **(Figure 12.16b)**. MPF acts both directly as a kinase and indirectly by activating other kinases. For example, MPF causes phosphorylation of various proteins of the nuclear lamina (see Figure 6.9), which promotes fragmentation of the nuclear envelope during prometaphase of mitosis. There is also evidence that MPF contributes to molecular events required for chromosome condensation and spindle formation during prophase.

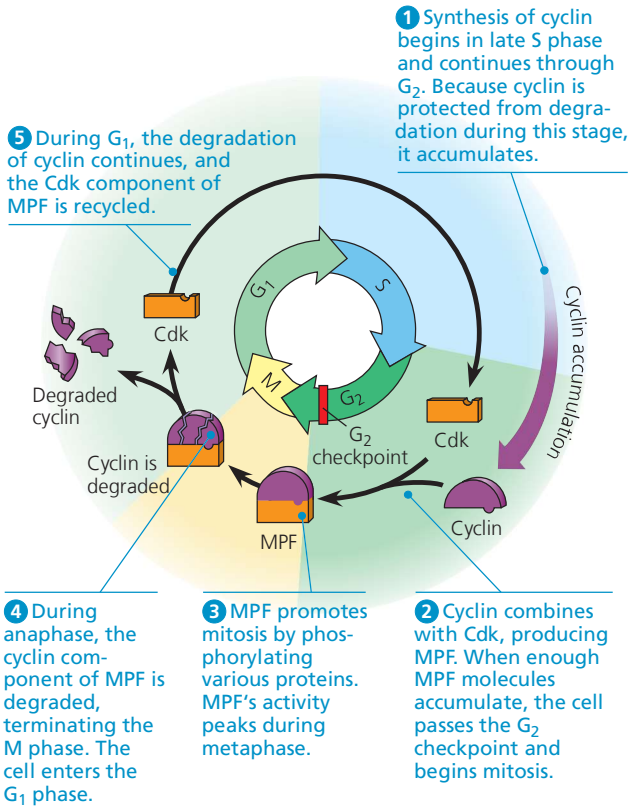
During anaphase, MPF helps switch itself off by initiating a process that leads to the destruction of its own cyclin. The noncyclin part of MPF, the Cdk, persists in the cell, inactive until it becomes part of MPF again by associating with new cyclin molecules synthesized during the S and G_2 phases of the next round of the cycle.

The fluctuating activities of different cyclin-Cdk complexes are of major importance in controlling all the stages of the cell cycle; they also give the go-ahead signals at some checkpoints. As mentioned above, MPF controls the cell's passage through the G_2 checkpoint. Cell behavior at the G_1 checkpoint is also regulated by the activity of cyclin-Cdk protein complexes. Animal cells appear to have at least three Cdk proteins and several different cyclins that operate at this checkpoint. Next, let's consider checkpoints in more detail.

▼ Figure 12.16 Molecular control of the cell cycle at the G_2 checkpoint. The steps of the cell cycle are timed by rhythmic fluctuations in the activity of cyclin-dependent kinases (Cdks). Here we focus on a cyclin-Cdk complex in animal cells called MPF, which acts at the G_2 checkpoint as a go-ahead signal, triggering the events of mitosis.



(a) Fluctuation of MPF activity and cyclin concentration during the cell cycle



(b) Molecular mechanisms that help regulate the cell cycle

VISUAL SKILLS *Explain how the events in the diagram in (b) are related to the "Time" axis of the graph in (a), beginning at the left.*

Mastering Biology
Interview with Paul Nurse: Discovering how protein kinases control the cell cycle

Stop and Go Signs: Internal and External Signals at the Checkpoints

Animal cells generally have built-in stop signals that halt the cell cycle at checkpoints until overridden by go-ahead signals. Many signals registered at checkpoints come from cellular surveillance mechanisms inside the cell. These signals report whether crucial cellular processes that should have occurred by that point have in fact been completed correctly and thus whether or not the cell cycle should proceed. Checkpoints also register signals from outside the cell. The signals are transmitted within the cell by signal transduction pathways (see Figure 11.6). Three important checkpoints are those in the G_1, G_2, and M phases (see Figure 12.15).

For many cells, the G_1 checkpoint seems to be the most important. If a cell receives a go-ahead signal at the G_1 checkpoint, it will usually complete the G_1, S, G_2, and M phases and divide. If it does not receive a go-ahead signal at that point, it may exit the cycle, switching into a nondividing state called the **G_0 phase (Figure 12.17a)**. Most cells of the human body are actually in the G_0 phase. As mentioned earlier, mature nerve cells and muscle cells never divide. Other cells, such as liver cells, can be "called back" from the G_0 phase to the cell cycle by external cues, such as growth factors released during injury.

Biologists are currently working out the pathways that link signals originating inside and outside the cell with the responses by cyclin-dependent kinases and other proteins. An example of an internal signal occurs at the third important checkpoint, the M checkpoint **(Figure 12.17b)**. Anaphase, the separation of sister chromatids, does not begin until all the chromosomes are properly attached to the spindle at the metaphase plate. Researchers have learned that as long as some kinetochores are unattached to spindle microtubules, the sister chromatids remain together, delaying anaphase. Only when

the kinetochores of all the chromosomes are properly attached to the spindle does the appropriate regulatory protein complex become activated. (In this case, the regulatory molecule is not a cyclin-Cdk complex but, instead, a different complex made up of several proteins.) Once activated, the complex sets off a chain of molecular events that activates the enzyme separase, which cleaves the cohesins, allowing the sister chromatids to separate. This mechanism ensures that daughter cells do not end up with missing or extra chromosomes.

There are checkpoints in addition to those in G_1, G_2, and M. For instance, a checkpoint in S phase stops cells with DNA damage from proceeding in the cell cycle. And, in 2014, researchers presented evidence for another checkpoint between anaphase and telophase that ensures anaphase is completed and the chromosomes are well separated before cytokinesis can begin, thus avoiding chromosomal damage.

What about the stop and go-ahead signals themselves—what are the signaling molecules? Studies using animal cells in culture have led to the identification of many external factors, both chemical and physical, that can influence cell division. For example, cells fail to divide if an essential nutrient is lacking in the culture medium. (This is analogous to trying to run a washing machine without the water supply hooked up; an internal sensor won't allow the machine to continue past the point where water is needed.) And even if all other conditions are favorable, most types of mammalian cells divide in culture only if the growth medium includes specific growth factors. As mentioned in Concept 11.1, a **growth factor** is a protein released by certain cells that stimulates other cells to divide. Different cell types respond specifically to different growth factors or combinations of growth factors.

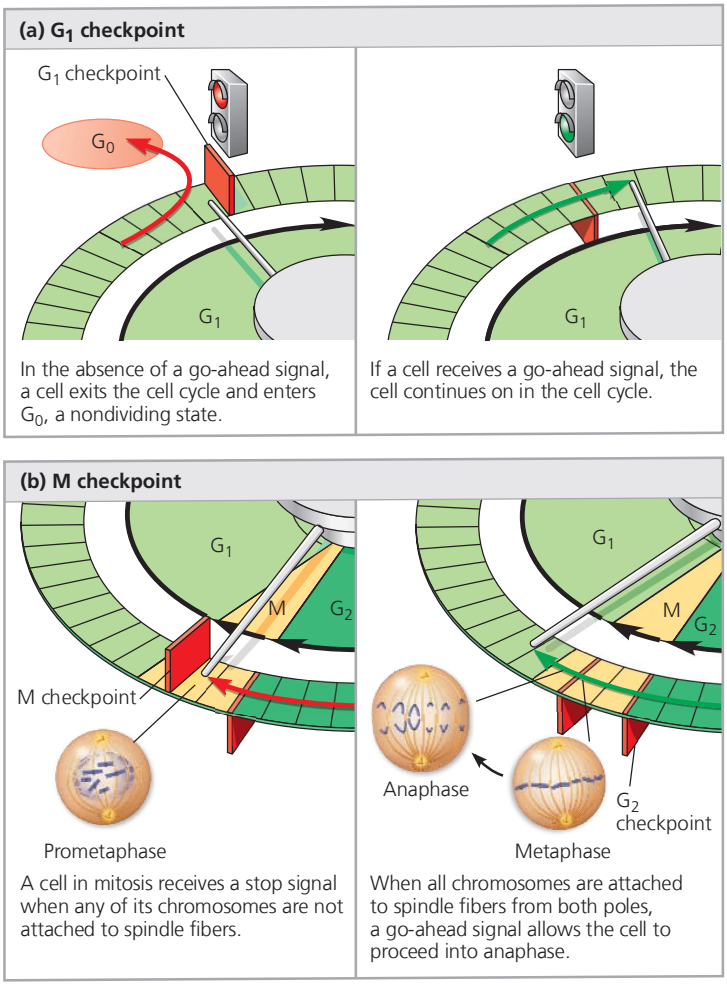
Consider, for example, *platelet-derived growth factor* (*PDGF*), which is made by blood cell fragments called platelets. When an injury occurs, platelets release PDGF in the vicinity. The experiment illustrated in **Figure 12.18** demonstrates that PDGF is required for the division of cultured fibroblasts, a type of connective tissue cell. Fibroblasts have PDGF receptors on their plasma membranes. The binding of PDGF molecules to these receptors (which are receptor tyrosine kinases; see Figure 11.8) triggers a signal transduction pathway that allows the cells to pass the G_1 checkpoint and divide. PDGF stimulates fibroblast division not only in the artificial conditions of cell culture but also in an animal's body. Thus, injury results in a proliferation of fibroblasts that helps heal the wound.

The effect of an external physical factor on cell division is clearly seen in **density-dependent inhibition**, a phenomenon in which crowded cells stop dividing **(Figure 12.19a)**. Studies done many years ago showed that cultured cells normally divide until they form a single layer of cells on the inner surface of a culture flask, at which point the cells stop dividing. If some cells are removed, those bordering the open space begin dividing again and continue until the vacancy is filled. Follow-up studies revealed that the binding of a cell-surface protein to its counterpart on an adjoining cell sends a signal to both cells that inhibits cell division, preventing them from moving forward in the cell cycle, even in the presence of growth factors.

Most animal cells also exhibit **anchorage dependence** (see Figure 12.19a). To divide, they must be attached to something, such as the inside of a culture flask or the extracellular matrix of a tissue. Experiments suggest that, like cell density, anchorage is signaled to the cell cycle control system via pathways involving plasma membrane proteins and elements of the cytoskeleton linked to them.

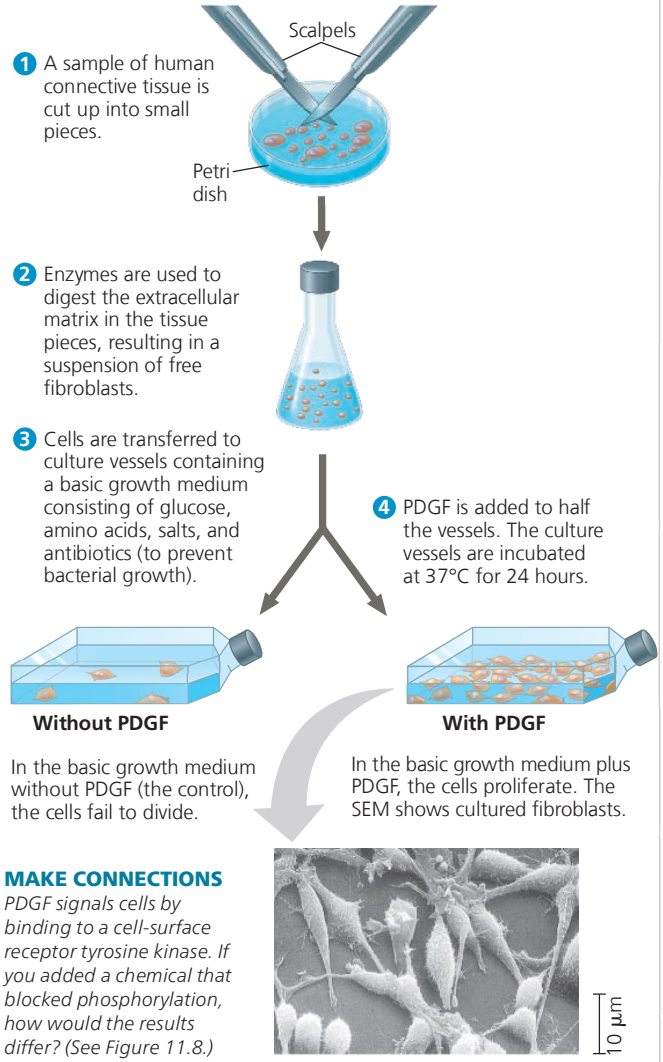
Density-dependent inhibition and anchorage dependence appear to function not only in cell culture but also in the

▼ Figure 12.17 Two important checkpoints. At certain checkpoints in the cell cycle (red gates), cells do different things depending on the signals they receive. Events of the **(a)** G_1 and **(b)** M checkpoints are shown. In **(b)**, the G_2 checkpoint has already been passed by the cell.



WHAT IF? *What might be the result if the cell ignored either checkpoint and progressed through the cell cycle?*

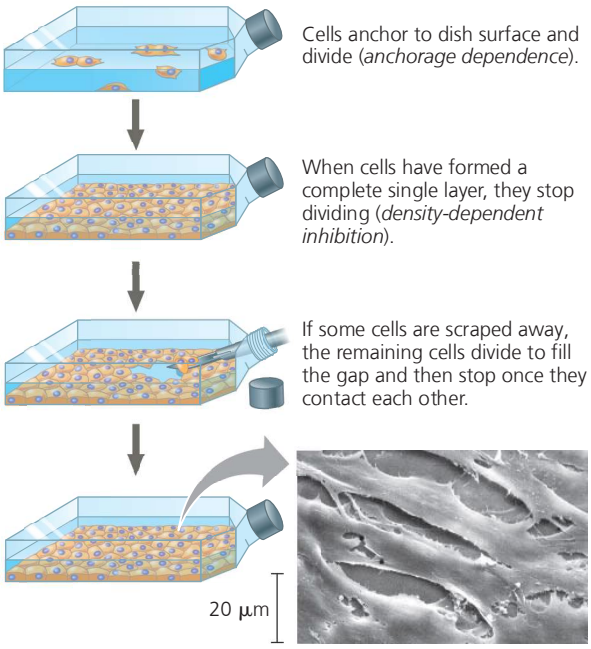
▼ Figure 12.18 The effect of platelet-derived growth factor (PDGF) on cell division.



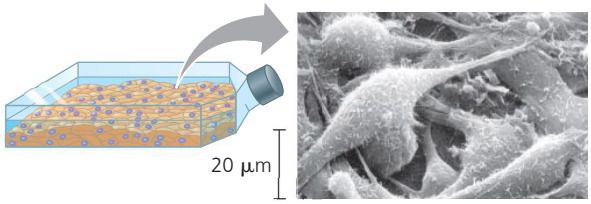
MAKE CONNECTIONS

PDGF signals cells by binding to a cell-surface receptor tyrosine kinase. If you added a chemical that blocked phosphorylation, how would the results differ? (See Figure 11.8.)

▼ Figure 12.19 Density-dependent inhibition and anchorage dependence of cell division. Individual cells are shown disproportionately large in the drawings.



(a) Normal mammalian cells. Cell density is limited to a single layer by contact with neighboring cells and the availability of nutrients, growth factors, and a substratum for attachment.



(b) Cancer cells. Cancer cells usually continue to divide well beyond a single layer, forming a clump of overlapping cells. They do not exhibit anchorage dependence or density-dependent inhibition.

body's tissues, checking the growth of cells at some optimal density and location during embryonic development and throughout an organism's life. Cancer cells, which we examine next, exhibit neither density-dependent inhibition nor anchorage dependence **(Figure 12.19b)**.

Loss of Cell Cycle Controls in Cancer Cells

Cancer cells do not heed the normal signals that regulate the cell cycle. In culture, they do not stop dividing when growth factors are depleted. A logical hypothesis is that cancer cells do not need growth factors in their culture medium to grow and divide. They may make a required growth factor themselves, or they may have an abnormality in the signaling pathway that conveys the growth factor's signal to the cell cycle control system even in the absence of that factor. Another possibility is an abnormal cell cycle control system. In these scenarios, the underlying basis of the abnormality is almost always a change in one or more genes (for example, a mutation) that alters the function of their protein products, resulting in faulty cell cycle control. We'll explore the molecular basis for such changes in Concept 18.5.

There are other important differences between normal cells and cancer cells that reflect malfunctions of the cell cycle. If and when they stop dividing, cancer cells do so at random points in the cycle, rather than at the normal checkpoints. Moreover, cancer cells can go on dividing indefinitely in culture if they are given a continual supply of nutrients; in essence, they are "immortal." A striking example is a cell line that has been reproducing in culture since 1951. Cells of this line are called HeLa cells because their original source was a tumor removed from a woman named Henrietta Lacks. (Neither Ms. Lacks nor her family gave permission or even

knew about the propagation and use of her cells, which have helped biologists make countless significant discoveries over the years.) Cells in culture that acquire the ability to divide indefinitely are said to have undergone a process called **transformation**, causing them to behave (in cell division, at least) like cancer cells. By contrast, nearly all normal, non-transformed mammalian cells growing in culture divide only about 20 to 50 times before they stop dividing, age, and die. Finally, cancer cells evade the normal controls that trigger a cell to undergo apoptosis when something is wrong—for example, when an irreparable mistake has occurred during DNA replication preceding mitosis.

Abnormal cell behavior in the body can be catastrophic. The problem begins when a single cell in a tissue undergoes the first of many steps that convert a normal cell to a cancer cell. Such a cell often has altered proteins on its surface, and the body's immune system normally recognizes the cell as "nonself"—an insurgent—and destroys it. However, if the cell evades destruction, it may proliferate and form a tumor, a mass of abnormal cells within otherwise normal tissue. The abnormal cells may remain at the original site if their genetic and cellular changes don't allow them to move to or survive at another site. In that case, the tumor is called a **benign tumor**. Most benign tumors do not cause serious problems (depending on their location) and can be removed by surgery. In contrast, a **malignant tumor** includes cells whose genetic and cellular changes enable them to spread to new tissues and impair the functions of one or more organs; these cells are also sometimes called *transformed* cells (although usage of this term is generally restricted to cells in culture). An individual with a malignant tumor is said to have cancer **(Figure 12.20)**.

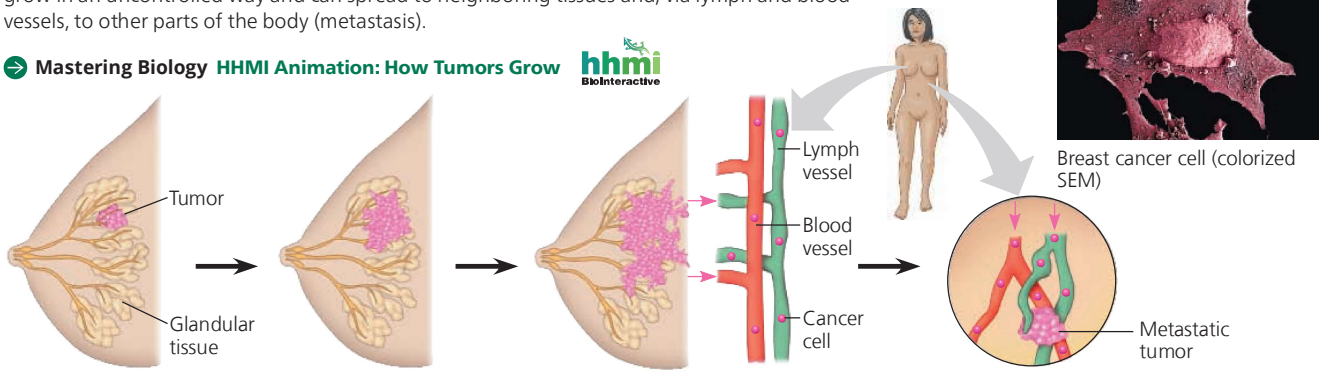
The changes that have occurred in cells of malignant tumors show up in many ways besides excessive proliferation. These cells may have unusual numbers of chromosomes, though whether this is a cause or an effect of tumor-related changes is an ongoing debate. Their metabolism may be altered, and they may cease to function in any constructive way. Abnormal changes on the cell surface cause cancer cells to lose attachments to neighboring cells and the extracellular matrix, allowing them to spread into nearby tissues. Cancer cells may also secrete signaling molecules that cause blood vessels to grow toward the tumor. A few tumor cells may separate from the original tumor, enter blood vessels and lymph vessels, and travel to other parts of the body. There, they may proliferate and form a new tumor. This spread of cancer cells to locations distant from their original site is called **metastasis** (see Figure 12.20).

A tumor that appears to be localized may be treated with high-energy radiation, which damages DNA in cancer cells much more than DNA in normal cells, apparently because the majority of cancer cells have lost the ability to repair such damage. To treat known or suspected metastatic tumors, chemotherapy is used, in which drugs that are toxic to actively dividing cells are administered through the circulatory system. As you might expect, chemotherapeutic drugs interfere with specific steps in the cell cycle. For example, the drug Taxol freezes the mitotic spindle by preventing microtubule depolymerization, which stops actively dividing cells from proceeding past metaphase and leads to their destruction. The side effects of chemotherapy are due to the effects of the drugs on normal cells that divide frequently, due to the function of that cell type in the organism. For example, nausea results from chemotherapy's effects on intestinal cells, hair loss from effects on hair follicle cells, and susceptibility to infection from effects on immune system cells. You can work with data from an experiment involving a potential chemotherapeutic agent in the **Scientific Skills Exercise**.

Over the past several decades, researchers have produced a flood of valuable information about cell-signaling pathways and how their malfunction contributes to the development of cancer through effects on the cell cycle. Coupled with new

▼ Figure 12.20 The growth and metastasis of a malignant breast tumor. A series of genetic and cellular changes contribute to a tumor becoming malignant (cancerous). The cells of malignant tumors grow in an uncontrolled way and can spread to neighboring tissues and, via lymph and blood vessels, to other parts of the body (metastasis).

Mastering Biology **HHMI Animation: How Tumors Grow**



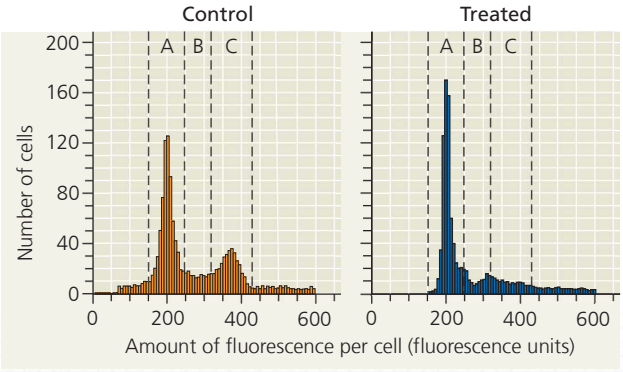
Scientific Skills Exercise

Interpreting Histograms

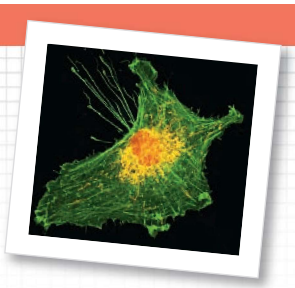
At What Phase Is the Cell Cycle Arrested by an Inhibitor? Many medical treatments are aimed at stopping cancer cell proliferation by blocking the cell cycle of cancerous tumor cells. One potential treatment is a cell cycle inhibitor derived from human umbilical cord stem cells. In this exercise, you will compare two histograms to determine where in the cell cycle the inhibitor blocks the division of cancer cells.

How the Experiment Was Done In the treated sample, human glioblastoma (brain cancer) cells were grown in tissue culture in the presence of the inhibitor, while a control sample of glioblastoma cells was grown in its absence. After 72 hours of growth, the two cell samples were harvested. To get a "snapshot" of the phase of the cell cycle each cell was in at that time, the samples were treated with a fluorescent chemical that binds to DNA and then run through a flow cytometer, an instrument that records the fluorescence level of each cell. Computer software then graphed the number of cells in each sample with a particular fluorescence level, as shown below.

Data from the Experiment



The data are plotted in a type of graph called a histogram (above), which groups the values for a numeric variable on the *x*-axis into intervals. A histogram allows you to see how all the experimental subjects (cells, in this case) are distributed along a continuous variable (amount of fluorescence). In these histograms, the bars are so narrow that the data appear to follow a curve for which you can detect peaks and dips. Each narrow bar represents the number of cells observed to have a level of fluorescence in the range of that interval. This in turn indicates the relative amount of DNA in those cells. Overall, comparing the two histograms allows you to see how the DNA content of this cell population is altered by the treatment.



▶ **Human brain cancer cell**

INTERPRET THE DATA

1. Study the data in the histograms. **(a)** Which axis indirectly shows the relative amount of DNA per cell? Explain your answer. **(b)** In the control sample, compare the first peak in the histogram (in region A) to the second peak (in region C). Which peak shows the population of cells with the higher amount of DNA per cell? Explain. (For additional information about histograms, see the Scientific Skills Review in Appendix D.)
2. **(a)** In the control sample histogram, identify the phase of the cell cycle (G_1, S, or G_2) of the population of cells in each region delineated by vertical lines. Label the histogram with these phases and explain your answer. **(b)** Does the S phase population of cells show a distinct peak in the histogram? Why or why not?
3. The histogram representing the treated sample shows the effect of growing the cancer cells alongside human umbilical cord stem cells that produce the potential inhibitor. **(a)** Label the histogram with the cell cycle phases. Which phase of the cell cycle has the greatest number of cells in the treated sample? Explain. **(b)** Compare the distribution of cells among G_1, S, and G_2 phases in the control and treated samples. What does this tell you about the cells in the treated sample? **(c)** Based on what you learned in Concept 12.3, propose a mechanism by which the stem cell–derived inhibitor might arrest the cancer cell cycle at this stage. (More than one answer is possible.)

Instructors: A version of this Scientific Skills Exercise can be assigned in **Mastering Biology**.

Data from K. K. Velpula et al., Regulation of glioblastoma progression by cord blood stem cells is mediated by downregulation of cyclin D1, *PLoS ONE* 6(3):e18017 (2011).

molecular techniques, such as the ability to rapidly sequence the DNA of cells in a particular tumor, medical treatments for cancer are beginning to become more "personalized" to a particular patient's tumor.

Mastering Biology **BBC Video: Are Fruit Flies the Key in the Fight Against Cancer?**

For example, the cells of roughly 20% of breast cancer tumors show abnormally high amounts of a cell-surface receptor tyrosine kinase called HER2, and many show an increase in the number of estrogen receptor (ER) molecules, intracellular receptors that can trigger cell division. Based on lab findings, a physician can prescribe chemotherapy with a molecule that blocks the function of the specific protein (Herceptin for HER2 and tamoxifen for ERs). Treatment using these agents, when appropriate, has led to increased survival rates and fewer cancer recurrences (see Make Connections Figure 18.27).

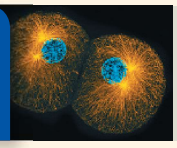
Mastering Biology
Interview with Bruce Alberts: Cancer control and careers in science

CONCEPT CHECK 12.3

1. In Figure 12.14, why do the nuclei resulting from experiment 2 contain different amounts of DNA?
2. How does MPF allow a cell to pass the G_2 phase checkpoint and enter mitosis? (See Figure 12.16.)
3. **MAKE CONNECTIONS** Explain how receptor tyrosine kinases and intracellular receptors might function in triggering cell division. (Review Figures 11.8 and 11.9 and Concept 11.2.)

For suggested answers, see Appendix A.

12 Chapter Review



Go to **Mastering Biology** for Assignments, the eText, the Study Area, and Dynamic Study Modules.

SUMMARY OF KEY CONCEPTS

To review key terms, go to the **Vocabulary Self-Quiz** in the **Mastering Biology** eText or Study Area, or go to goo.gl/zkjz9t.

CONCEPT 12.1

Most cell division results in genetically identical daughter cells *(pp. 235–237)*

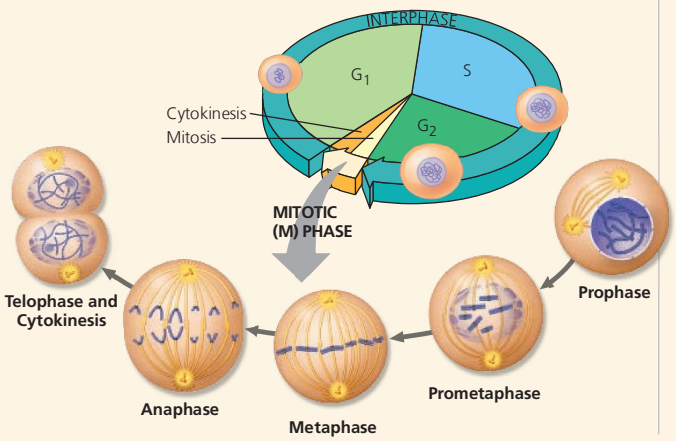
- Unicellular organisms reproduce by **cell division**; multicellular organisms depend on cell division for their development from a fertilized egg and for growth and repair.
- The genetic material (DNA) of a cell—its **genome**—is partitioned among **chromosomes**. Each eukaryotic chromosome consists of one DNA molecule associated with many proteins. Together, the complex of DNA and associated proteins is called **chromatin**. The chromatin of a chromosome exists in different states of condensation at different times. In animals, **gametes** have one set of chromosomes and **somatic cells** have two sets.
- Cells replicate their genetic material before they divide, each daughter cell receiving a copy of the DNA. Prior to cell division, chromosomes are duplicated. Each one then consists of two identical **sister chromatids** joined along their lengths by sister chromatid cohesion and held most tightly together at a constricted region at the **centromeres**. When this cohesion is broken, the chromatids separate during cell division, becoming the chromosomes of the daughter cells. Eukaryotic cell division consists of **mitosis** (division of the nucleus) and **cytokinesis** (division of the cytoplasm).

? *Differentiate between these terms: chromosome, chromatin, and chromatid.*

CONCEPT 12.2

The mitotic phase alternates with interphase in the cell cycle *(pp. 237–244)*

- Cell division is part of the **cell cycle**, an ordered sequence of events in the life of a cell.
- Between divisions, a cell is in **interphase**: the **G_1, S**, and **G_2** phases. The cell grows throughout interphase, with DNA being replicated during the synthesis (S) phase. Mitosis and cytokinesis make up the **mitotic (M) phase** of the cell cycle.



- The **mitotic spindle**, made up of microtubules, controls chromosome movement during mitosis. In animal cells, it arises from the **centrosomes** and includes spindle microtubules and asters. Some spindle microtubules attach to the **kinetochores** of chromosomes and move the chromosomes to the **metaphase plate**. After sister chromatids separate, motor proteins move them along kinetochore microtubules toward opposite ends of the cell. The cell elongates when motor proteins push nonkinetochore microtubules from opposite poles away from each other.
- Mitosis is usually followed by cytokinesis. Animal cells carry out cytokinesis by **cleavage**, and plant cells form a **cell plate**.
- During **binary fission** in bacteria, the chromosome replicates and the daughter chromosomes actively move apart. Some of the proteins involved in bacterial binary fission are related to eukaryotic actin and tubulin.
- Since prokaryotes preceded eukaryotes by more than a billion years, it is likely that mitosis evolved from prokaryotic cell division. Certain unicellular eukaryotes exhibit mechanisms of cell division that may be similar to those of ancestors of existing eukaryotes. Such mechanisms might represent intermediate steps in the evolution of mitosis.

? *In which of the three phases of interphase and the stages of mitosis do chromosomes exist as single DNA molecules?*

CONCEPT 12.3

The eukaryotic cell cycle is regulated by a molecular control system *(pp. 244–250)*

- Signaling molecules present in the cytoplasm regulate progress through the cell cycle.
- In the **cell cycle control system**, cyclic changes in regulatory proteins—including **cyclins** and **cyclin-dependent kinases (Cdks)**—work as a cell cycle clock. The cell cycle stops at specific **checkpoints** until a go-ahead signal is received; important checkpoints occur in G_1, G_2, and M phases. Internal and external signals control the cell cycle checkpoints via signal transduction pathways. Most cells exhibit **density-dependent inhibition** of cell division as well as **anchorage dependence**.
- Cancer cells elude normal cell cycle regulation and divide unchecked, forming tumors. **Malignant tumors** invade nearby tissues and can undergo **metastasis**, exporting cancer cells to other sites, where they may form secondary tumors.

? *Explain the significance of the G_1, G_2, and M checkpoints and the go-ahead signals involved in the cell cycle control system.*

TEST YOUR UNDERSTANDING

For more multiple-choice questions, go to the **Practice Test** in the **Mastering Biology** eText or Study Area, or go to goo.gl/GruWRg.

Levels 1-2: Remembering/Understanding

1. Through a microscope, you can see a cell plate beginning to develop across the middle of a cell and nuclei forming on either side of the cell plate. This cell is most likely
   (A) an animal cell in the process of cytokinesis.
   (B) a plant cell in the process of cytokinesis.
   (C) a bacterial cell dividing.
   (D) a plant cell in metaphase.

2. Vinblastine is a standard chemotherapeutic drug used to treat cancer. Because it interferes with the assembly of microtubules, its effectiveness must be related to
   (A) disruption of mitotic spindle formation.
   (B) suppression of cyclin production.
   (C) myosin denaturation and inhibition of cleavage furrow formation.
   (D) inhibition of DNA synthesis.

3. One difference between cancer cells and normal cells is that cancer cells
   (A) are unable to synthesize DNA.
   (B) are arrested at the S phase of the cell cycle.
   (C) continue to divide even when they are tightly packed together.
   (D) cannot function properly because they are affected by density-dependent inhibition.

4. The decline of MPF activity at the end of mitosis is due to
   (A) the destruction of the protein kinase Cdk.
   (B) decreased synthesis of Cdk.
   (C) the degradation of cyclin.
   (D) the accumulation of cyclin.

5. In the cells of some organisms, mitosis occurs without cytokinesis. This will result in
   (A) cells with more than one nucleus.
   (B) cells that are unusually small.
   (C) cells lacking nuclei.
   (D) cell cycles lacking an S phase.

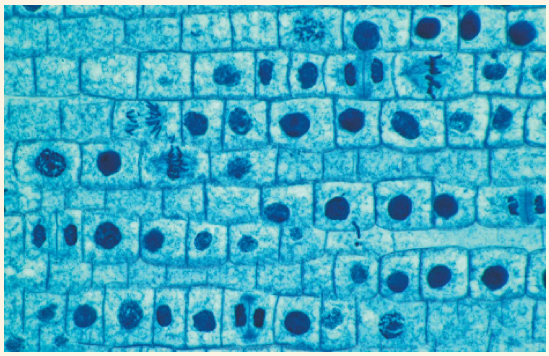
6. Which of the following occurs during S phase?
   (A) condensation of the chromosomes
   (B) replication of the DNA
   (C) separation of sister chromatids
   (D) spindle formation

Levels 3-4: Applying/Analyzing

7. Cell A has half as much DNA as cells B, C, and D in a mitotically active tissue. Cell A is most likely in
   (A) G_1.
   (B) G_2.
   (C) prophase.
   (D) metaphase.

8. The drug cytochalasin B blocks the function of actin. Which of the following aspects of the animal cell cycle would be most disrupted by cytochalasin B?
   (A) spindle formation
   (B) spindle attachment to kinetochores
   (C) cell elongation during anaphase
   (D) cleavage furrow formation and cytokinesis

9. **VISUAL SKILLS** The light micrograph shows dividing cells near the tip of an onion root. Identify a cell in each of the following stages: prophase, prometaphase, metaphase, anaphase, and telophase. Describe the major events occurring at each stage.



10. **DRAW IT** Draw one eukaryotic chromosome as it would appear during interphase, during each of the stages of mitosis, and during cytokinesis. Also draw and label the nuclear envelope and any microtubules attached to the chromosome(s).

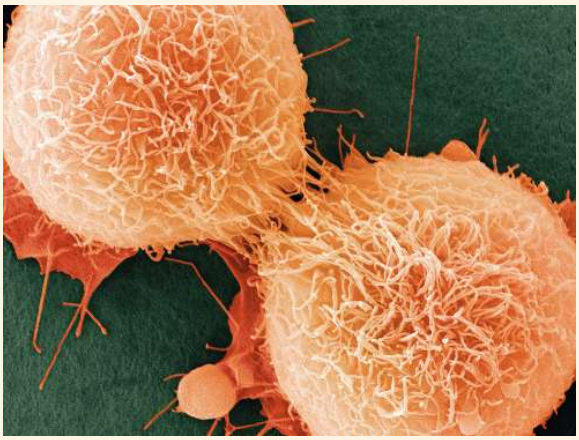
Levels 5-6: Evaluating/Creating

11. **EVOLUTION CONNECTION** The result of mitosis is that the daughter cells end up with the same number of chromosomes that the parent cell had. Another potential way to maintain the number of chromosomes would be to carry out cell division first and then duplicate the chromosomes in each daughter cell. Assess whether this would be an equally good way of organizing the cell cycle. Explain why evolution has not led to this alternative.

12. **SCIENTIFIC INQUIRY** Although both ends of a microtubule can gain or lose subunits, one end (called the plus end) polymerizes and depolymerizes at a higher rate than the other end (the minus end). For spindle microtubules, the plus ends are in the center of the spindle, and the minus ends are at the poles. Motor proteins that move along microtubules specialize in walking either toward the plus end or toward the minus end; the two types are called plus end–directed and minus end–directed motor proteins, respectively. Given what you know about chromosome movement and spindle changes during anaphase, predict which type of motor proteins would be present on (a) kinetochore microtubules and (b) nonkinetochore microtubules.

13. **WRITE ABOUT A THEME: INFORMATION** The continuity of life is based on heritable information in the form of DNA. In a short essay (100–150 words), explain how the process of mitosis faithfully parcels out exact copies of this heritable information in the production of genetically identical daughter cells.

14. **SYNTHESIZE YOUR KNOWLEDGE**



Shown here are two HeLa cancer cells that are just completing cytokinesis. Explain how the cell division of cancer cells like these is misregulated. Identify genetic and other changes that might have caused these cells to escape normal cell cycle regulation.

For selected answers, see Appendix A.