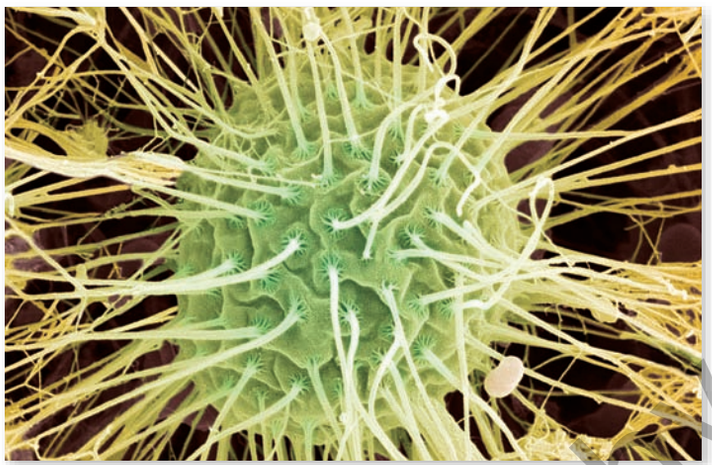
multicellular and some unicellular eukaryotes no longer possess flagella and are nonmotile. Other structures, called **cilia** (singular, *cilium*), with an organization similar to the 9 + 2 arrangement of microtubules can still be found within them. Cilia are short cellular projections that are often organized in rows. They are more numerous than flagella on the cell surface, but have the same internal structure.

In many multicellular organisms, cilia carry out tasks far removed from their original function of propelling cells through water. In several kinds of vertebrate tissues, for example, the beating of rows of cilia move water over the tissue surface. The sensory cells of the vertebrate ear also contain conventional cilia surrounded by actin-based stereocilia; sound waves bend these structures and provide the initial sensory input for hearing. Thus, the 9 + 2 structure of flagella and cilia appears to be a fundamental component of eukaryotic cells (figure 4.24).



a.

b.

Figure 4.24 Flagella and cilia. *a.* A green alga with numerous flagella that allow it to move through the water. *b.* Paramecia are covered with many cilia, which beat in unison to move the cell. The cilia can also be used to move fluid into the paramecium's mouth to ingest material.

Inquiry question

The passageways of the human trachea (the path of airflow into and out of the lungs) are known to be lined with ciliated cells. What function could these cilia perform?

Plant cell walls provide protection and support

The cells of plants, fungi, and many types of protists have cell walls, which protect and support the cells. The cell walls of these eukaryotes are chemically and structurally different from prokaryotic cell walls. In plants and protists, the cell walls are composed of fibers of the polysaccharide cellulose, whereas in fungi, the cell walls are composed of chitin.

In plants, **primary walls** are laid down when the cell is still growing. Between the walls of adjacent cells a sticky substance, called the **middle lamella,** glues the cells together (figure 4.25). Some plant cells produce strong **secondary walls,** which are deposited inside the primary walls of fully expanded cells.

Animal cells secrete an extracellular matrix

Animal cells lack the cell walls that encase plants, fungi, and most protists. Instead, animal cells secrete an elaborate mixture of glycoproteins into the space around them, forming

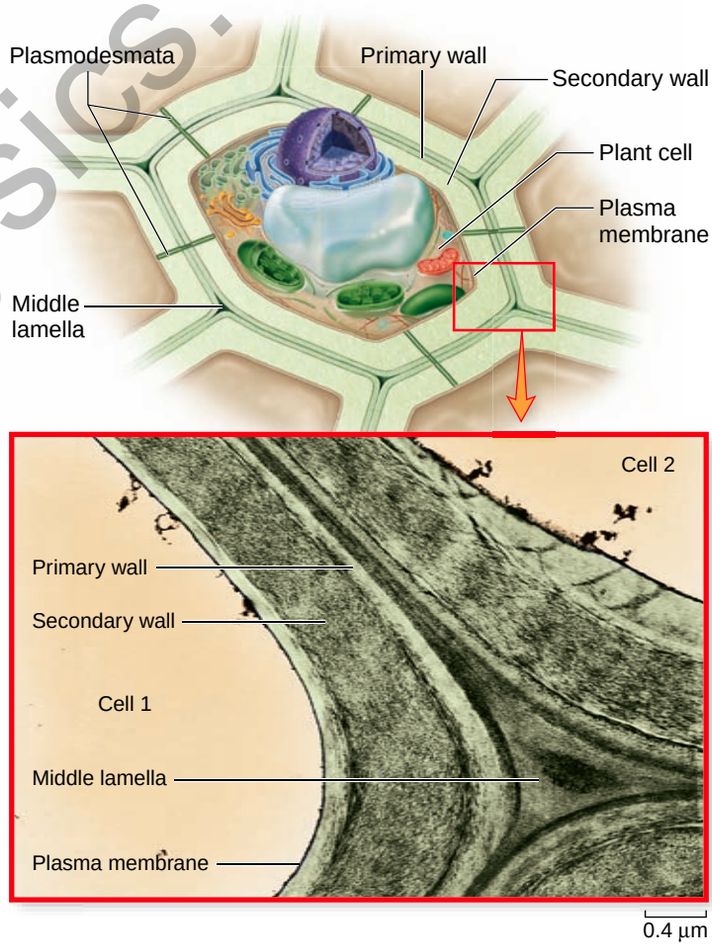


Figure 4.25 Cell walls in plants. Plant cell walls are thick, strong, and rigid. Primary cell walls are laid down when the cell is young. Thicker secondary cell walls may be added later when the cell is fully grown.

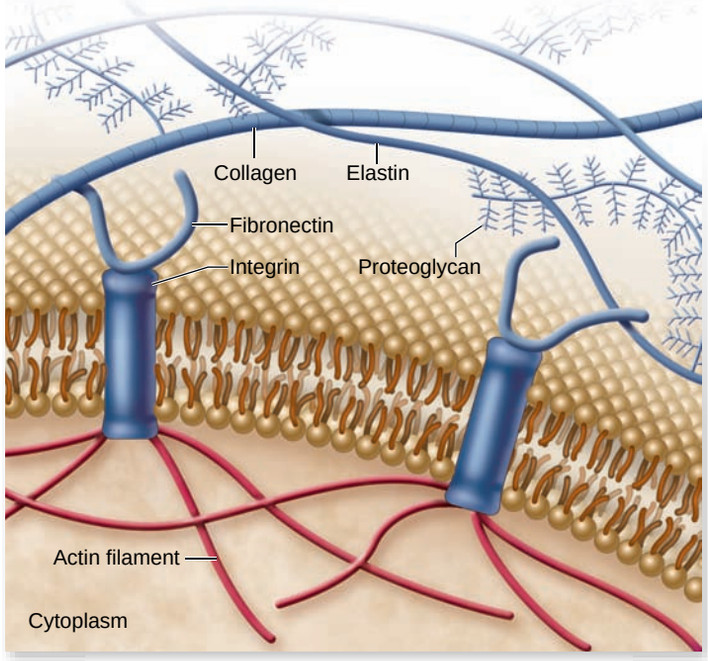


Figure 4.26 The extracellular matrix. Animal cells are surrounded by an extracellular matrix composed of various glycoproteins that give the cells support, strength, and resilience.

the *extracellular matrix (ECM)* (figure 4.26). The fibrous protein collagen, the same protein found in cartilage, tendons, and ligaments may be abundant in the ECM. Strong fibers of collagen and another fibrous protein, elastin, are embedded within a complex web of other glycoproteins, called proteoglycans, that form a protective layer over the cell surface.

The ECM of some cells is attached to the plasma membrane by a third kind of glycoprotein, *fibronectin*. Fibronectin molecules bind not only to ECM glycoproteins but also to proteins called **integrins.** Integrins are an integral part of the plasma membrane, extending into the cytoplasm, where they are attached to the microfilaments and intermediate filaments of the cytoskeleton. Linking ECM and cytoskeleton, integrins allow the ECM to influence cell behavior in important ways. They can alter gene expression and cell migration patterns by a combination of mechanical and chemical signaling pathways. In this way, the ECM can help coordinate the behavior of all the cells in a particular tissue.

Table 4.3 compares and reviews the features of three types of cells.

TABLE 4.3 A Comparison of Prokaryotic, Animal, and Plant Cells

	Prokaryote	Animal	Plant
EXTERIOR STRUCTURES			
Cell wall	Present (protein-polysaccharide)	Absent	Present (cellulose)
Cell membrane	Present	Present	Present
Flagella/cilia	Flagella may be present	May be present (9 + 2 structure)	Absent except in sperm of a few species (9 + 2 structure)
INTERIOR STRUCTURES			
Endoplasmic reticulum	Absent	Usually present	Usually present
Ribosomes	Present	Present	Present
Microtubules	Absent	Present	Present
Centrioles	Absent	Present	Absent
Golgi apparatus	Absent	Present	Present
Nucleus	Absent	Present	Present
Mitochondria	Absent	Present	Present
Chloroplasts	Absent	Absent	Present
Chromosomes	Single; circle of DNA	Multiple; DNA–protein complex	Multiple; DNA–protein complex
Lysosomes	Absent	Usually present	Present
Vacuoles	Absent	Absent or small	Usually a large single vacuole

Learning Outcomes Review 4.7

Cell movement involves proteins. These can either be internal in the case of crawling cells that use actin and myosin, or external in the case of cells powered by cilia or flagella. Eukaryotic cilia and flagella are different from prokaryotic flagella because they are composed of bundles of microtubules in a 9 + 2 array. They undulate rather than rotate.

Plant cells have a cellulose-based cell wall. Animal cells lack a cell wall. In animal cells, the cytoskeleton is linked to a web of glycoproteins called the extracellular matrix.

- ***What cellular roles are performed by microtubules and microfilaments and not intermediate filaments?***

4.8 Cell-to-Cell Interactions

Learning Outcomes

1. *Differentiate between types of cell junctions.*
2. *Describe the roles of surface proteins.*

In multicellular organisms, not only must cells be able to communicate with one another, they must also be organized in specific ways. With the exception of a few primitive types of organisms, the hallmark of multicellular life is the organization of highly specialized groups of cells into *tissues*, such as blood and muscle. Remarkably, each cell within a tissue performs the functions of that tissue and no other, even though all cells of the body are derived from a single fertilized cell and contain the same genetic information—all of the genes found in the genome.

This kind of tissue organization requires that cells have both identity and specific kinds of cell-to-cell connections. As an organism develops, the cells acquire their identities by carefully controlling the *expression* of those genes, turning on the specific set of genes that encode the functions of each cell type. How do cells sense where they are? How do they "know" which type of tissue they belong to? Table 4.4 provides a summary of the kinds of connections seen between cells that are explored in the following sections.

Surface proteins give cells identity

One key set of genes functions to mark the surfaces of cells, identifying them as being of a particular type. When cells make contact, they "read" each other's cell surface markers and react accordingly. Cells that are part of the same tissue type recognize each other, and they frequently respond by forming connections between their surfaces to better coordinate their functions.

Glycolipids

Most tissue-specific cell surface markers are glycolipids, that is, lipids with carbohydrate heads. The glycolipids on the surface of red blood cells are also responsible for the A, B, and O blood types.

MHC proteins

One example of the function of cell surface markers is the recognition of "self" and "nonself" cells by the immune system. This function is vital for multicellular organisms, which need to defend themselves against invading or malignant cells. The immune system of vertebrates uses a particular set of markers to distinguish self from nonself cells, encoded by genes of the

TABLE 4.4 Cell-to-Cell Connections and Cell Identity

Type of Connection	Structure	Function	Example
Surface markers	Variable, integral proteins or glycolipids in plasma membrane	Identify the cell	MHC complexes, blood groups, antibodies
Tight junctions	Tightly bound, leakproof, fibrous protein seal that surrounds cell	Organizing junction; holds cells together such that materials pass *through* but not *between* the cells	Junctions between epithelial cells in the gut
Anchoring junction (Desmosome)	Intermediate filaments of cytoskeleton linked to adjoining cells through cadherins	Anchoring junction; binds cells together	Epithelium
Anchoring junction (Adherens junction)	Transmembrane fibrous proteins	Anchoring junction; connects extracellular matrix to cytoskeleton	Tissues with high mechanical stress, such as the skin
Communicating junction (Gap junction)	Six transmembrane connexon proteins creating a pore that connects cells	Communicating junction; allows passage of small molecules from cell to cell in a tissue	Excitable tissue such as heart muscle
Communicating junction (Plasmodesmata)	Cytoplasmic connections between gaps in adjoining plant cell walls	Communicating junction between plant cells	Plant tissues

major histocompatibility complex (*MHC*). Cell recognition in the immune system is covered in chapter 52.

Cell connections mediate cell-to-cell adhesion

Most cells in a multicellular organism are in physical contact with other cells at all times, usually as members of organized tissues such as those in a leaf or those in your lungs, heart, or gut. These cells and the mass of other cells clustered around them form long-lasting or permanent connections with one another called *cell junctions*.

The nature of the physical connections between the cells of a tissue in large measure determines what the tissue is like. Indeed, a tissue's proper functioning often depends critically on how the individual cells are arranged within it. Just as a house cannot maintain its structure without nails and cement, so a tissue cannot maintain its characteristic architecture without the appropriate cell junctions.

Cell junctions are divided into three categories, based on their functions: tight, anchoring, and communicating junctions (figure 4.27).

Tight junctions

Tight junctions connect the plasma membranes of adjacent cells in a sheet. This sheet of cells acts as a wall within the organ, keeping molecules on one side or the other (figure 4.27*a*).

Creating sheets of cells. The cells that line an animal's digestive tract are organized in a sheet only one cell thick. One surface of the sheet faces the inside of the tract, and the other

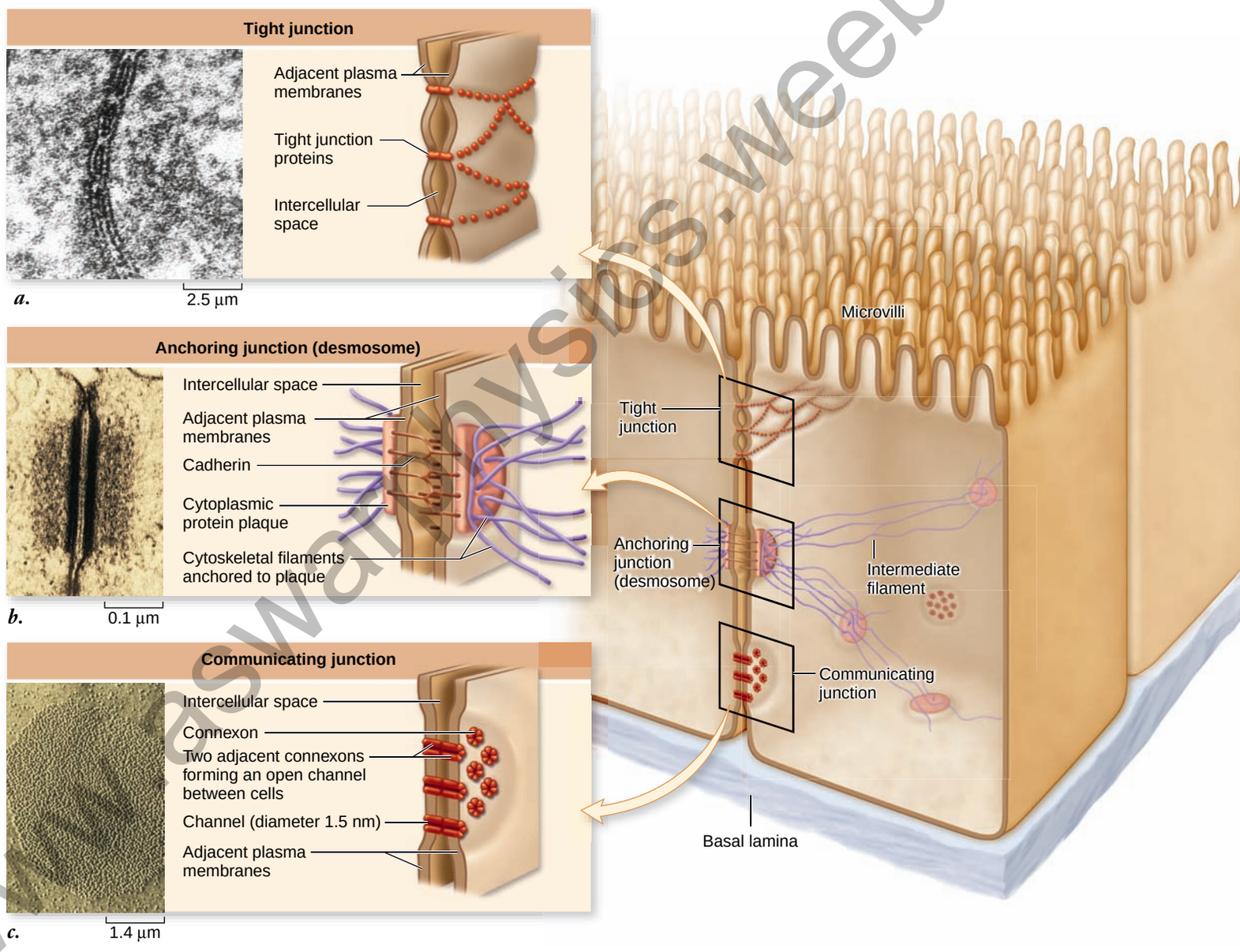


Figure 4.27 An overview of cell junction types. Here, the diagram of gut epithelial cells on the right illustrates the comparative structures and locations of common cell junctions. The detailed models on the left show the structures of the three major types of cell junctions: ***(a)*** tight junction; ***(b)*** anchoring junction, the example shown is a desmosome; ***(c)*** communicating junction, the example shown is a gap junction.

faces the extracellular space, where blood vessels are located. Tight junctions encircle each cell in the sheet, like a belt cinched around a person's waist. The junctions between neighboring cells are so securely attached that there is no space between them for leakage. Hence, nutrients absorbed from the food in the digestive tract must pass directly through the cells in the sheet to enter the bloodstream because they cannot pass through spaces between cells.

Partitioning the sheet. The tight junctions between the cells lining the digestive tract also partition the plasma membranes of these cells into separate compartments. Transport proteins in the membrane facing the inside of the tract carry nutrients from that side to the cytoplasm of the cells. Other proteins, located in the membrane on the opposite side of the cells, transport those nutrients from the cytoplasm to the extracellular fluid, where they can enter the bloodstream.

For the sheet to absorb nutrients properly, these proteins must remain in the correct locations within the fluid membrane. Tight junctions effectively segregate the proteins on opposite sides of the sheet, preventing them from drifting within the membrane from one side of the sheet to the other. When tight junctions are experimentally disrupted, just this sort of migration occurs.

Anchoring junctions

Anchoring junctions mechanically attach the cytoskeleton of a cell to the cytoskeletons of other cells or to the extracellular matrix. These junctions are most common in tissues subject to mechanical stress, such as muscle and skin epithelium.

Cadherin and intermediate filaments. *Desmosomes* connect the cytoskeletons of adjacent cells (figure 4.27*b*), and *hemidesmosomes* anchor epithelial cells to a basement membrane. Proteins called **cadherins,** most of which are single-pass transmembrane glycoproteins, create the critical link. Proteins link the short cytoplasmic end of a cadherin to the intermediate filaments in the cytoskeleton. The other end of the cadherin molecule projects outward from the plasma membrane, joining directly with a cadherin protruding from an adjacent cell similar to a firm handshake, binding the cells together. Connections between proteins tethered to the intermediate filaments are much more secure than connections between free-floating membrane proteins.

Cadherin and actin filaments. Cadherins can also connect the actin frameworks of cells in cadherin-mediated junctions (figure 4.28). When they do, they form less stable links between cells than when they connect intermediate filaments. Many kinds of actin-linking cadherins occur in different tissues. For example, during vertebrate development, the migration of neurons in the embryo is associated with changes in the type of cadherin expressed on their plasma membranes.

Integrin-mediated links. Anchoring junctions called **adherens junctions** connect the actin filaments of one cell with those of neighboring cells or with the extracellular matrix. The linking proteins in these junctions are members of a large superfamily of cell-surface receptors called integrins that bind to a protein component of the extracellular matrix. At least 20 different integrins exist each with a differently shaped binding domain.

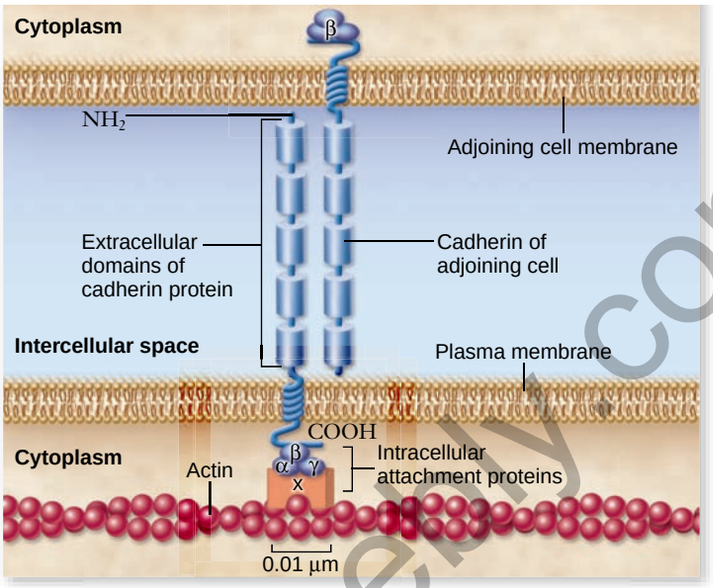


Figure 4.28 A cadherin-mediated junction. The cadherin molecule is anchored to actin in the cytoskeleton and passes through the membrane to interact with the cadherin of an adjoining cell.

Communicating junctions

Many cells communicate with adjacent cells through direct connections called *communicating junctions.* In these junctions, a chemical or electrical signal passes directly from one cell to an adjacent one. Communicating junctions permit small molecules or ions to pass from one cell to the other. In animals, these direct communication channels between cells are called *gap junctions*, and in plants, *plasmodesmata*.

Gap junctions in animals. Gap junctions are composed of structures called connexons, complexes of six identical transmembrane proteins (see figure 4.27*c*). The proteins in a connexon are arranged in a circle to create a channel through the plasma membrane that protrudes several nanometers from the cell surface. A gap junction forms when the connexons of two cells align perfectly, creating an open channel that spans the plasma membranes of both cells.

Gap junctions provide passageways large enough to permit small substances, such as simple sugars and amino acids, to pass from one cell to the next. Yet the passages are small enough to prevent the passage of larger molecules, such as proteins.

Gap junction channels are dynamic structures that can open or close in response to a variety of factors, including Ca^{2+} and H^+ ions. This gating serves at least one important function. When a cell is damaged, its plasma membrane often becomes leaky. Ions in high concentrations outside the cell, such as Ca^{2+}, flow into the damaged cell and close its gap junction channels. This isolates the cell and so prevents the damage from spreading to other cells.

Plasmodesmata in plants. In plants, cell walls separate every cell from all others. Cell–cell junctions occur only at holes or gaps in the walls, where the plasma membranes of adjacent cells can come into contact with one another. Cytoplasmic connections that form across the touching plasma membranes are called **plasmodesmata** (singular, *plasmodesma*) (figure 4.29). The majority of living cells within a higher plant are connected to their neighbors by these junctions.

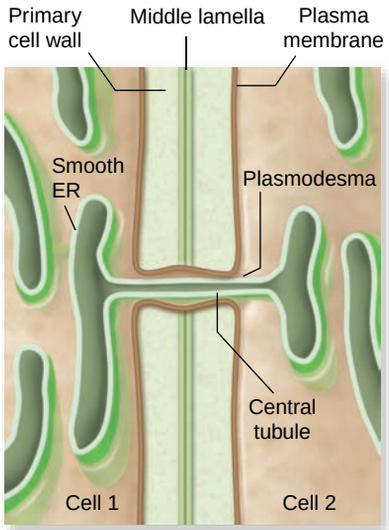


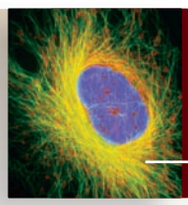
Figure 4.29 Plasmodesmata. Plant cells can communicate through specialized openings in their cell walls, called plasmodesmata, where the cytoplasm of adjoining cells are connected.

Plasmodesmata function much like gap junctions in animal cells, although their structure is more complex. Unlike gap junctions, plasmodesmata are lined with plasma membrane and contain a central tubule that connects the endoplasmic reticulum of the two cells.

Learning Outcomes Review 4.8

Cell connections fall into three basic categories: (1) Tight junctions help to make sheets of cells that form watertight seals; (2) anchoring junctions provide strength and flexibility; and (3) communicating junctions, including gap junctions in animals and plasmodesmata in plants, allow passage of some materials between cells. Cells in multicellular organisms are usually organized into tissues, requiring that cells have distinct identity and connections. Cell identity is conferred by surface glycoproteins, which include the MHC proteins that are important in the immune system.

- ***How do cell junctions help to form tissues?***



Chapter Review

4.1 Cell Theory

Cell theory is the unifying foundation of cell biology.
All organisms are composed of one or more cells. Cells arise only by division of preexisting cells.

Cell size is limited.
Cell size is constrained by the diffusion distance. As cell size increases, diffusion becomes inefficient.

Microscopes allow visualization of cells and components.
Magnification gives better resolution than is possible with the naked eye. Staining with chemicals enhances contrast of structures.

All cells exhibit basic structural similarities.
All cells have centrally located DNA, a semifluid cytoplasm, and an enclosing plasma membrane.

4.2 Prokaryotic Cells (see figure 4.3)

Prokaryotic cells have relatively simple organization.
Prokaryotic cells contain DNA and ribosomes, but they lack a nucleus, an internal membrane system, and membrane-bounded organelles. A rigid cell wall surrounds the plasma membrane.

Bacterial cell walls consist of peptidoglycan.
Peptidoglycan is composed of carbohydrate cross-linked with short peptides.

Archaea lack peptidoglycan.
Archaeal cell walls do not contain peptidoglycan, and they have unique plasma membranes.

Some prokaryotes move by means of rotating flagella.
Prokaryotic flagella rotate because of proton transfer across the plasma membrane.

4.3 Eukaryotic Cells (see figures 4.6 and 4.7)

Eukaryotic cells have a membrane-bounded nucleus, an endomembrane system, and many different organelles.

The nucleus acts as the information center.
The nucleus is surrounded by an envelope of two phospholipid bilayers; the outer layer is contiguous with the ER. Pores allow exchange of small molecules. The nucleolus is a region of the nucleoplasm where rRNA is transcribed and ribosomes are assembled.

In most prokaryotes, DNA is organized into a single circular chromosome. In eukaryotes, numerous chromosomes are present.

Ribosomes are the cell's protein synthesis machinery.
Ribosomes translate mRNA to produce polypeptides. They are found in all cell types.

4.4 The Endomembrane System

The endoplasmic reticulum (ER) creates channels and passages within the cytoplasm (see figure 4.10).

The rough ER is a site of protein synthesis.
The rough ER (RER), studded with ribosomes, synthesizes and modifies proteins and manufactures membranes.

The smooth ER has multiple roles.
The smooth endoplasmic reticulum (SER) lacks ribosomes; it is involved in carbohydrate and lipid synthesis and detoxification.

The Golgi apparatus sorts and packages proteins.
The Golgi apparatus receives vesicles from the ER, modifies and packages macromolecules, and transports them (see figure 4.11).

Lysosomes contain digestive enzymes.
Lysosomes break down macromolecules and recycle the components of old organelles (see figure 4.13).

Microbodies are a diverse category of organelles.

Plants use vacuoles for storage and water balance.

4.5 Mitochondria and Chloroplasts: Cellular Generators

Mitochondria and chloroplasts have a double-membrane structure, contain their own DNA, and can divide independently.

Mitochondria metabolize sugar to generate ATP.
The inner membrane of mitochondria is extensively folded into layers called cristae. Proteins on the surface and in the inner membrane carry out metabolism to produce ATP (see figure 4.16).

Chloroplasts use light to generate ATP and sugars.
Chloroplasts capture light energy via thylakoid membranes arranged in stacks called grana, and use it to synthesize glucose (see figure 4.17).

Mitochondria and chloroplasts arose by endosymbiosis.
The endosymbiont theory proposes that mitochondria and chloroplasts were once prokaryotes engulfed by another cell.

4.6 The Cytoskeleton

The cytoskeleton consists of crisscrossed protein fibers that support the shape of the cell and anchor organelles (see figure 4.19).

Three types of fibers compose the cytoskeleton.
Actin filaments, or microfilaments, are long, thin polymers involved in cellular movement. Microtubules are hollow structures that move materials within a cell. Intermediate filaments serve a wide variety of functions.

Centrosomes are microtubule-organizing centers.
Centrosomes help assemble the nuclear division apparatus of animal cells (see figure 4.20).

The cytoskeleton helps move materials within cells.
Molecular motors move vesicles along microtubules, like a train on a railroad track. Kinesin and dynein are two motor proteins.

4.7 Extracellular Structures and Cell Movement

Some cells crawl.
Cell crawling occurs as actin polymerization forces the cell membrane forward, while myosin pulls the cell body forward.

Flagella and cilia aid movement.
Eukaryotic flagella have a 9 + 2 structure and arise from a basal body. Cilia are shorter and more numerous than flagella.

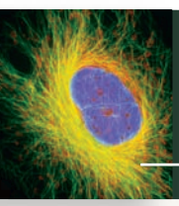
Plant cell walls provide protection and support.
Plants have cell walls composed of cellulose fibers. The middle lamella, between cell walls, holds adjacent cells together.

Animal cells secrete an extracellular matrix.
Glycoproteins are the main component of the extracellular matrix (ECM) of animal cells.

4.8 Cell-to-Cell Interactions (see figure 4.27)

Surface proteins give cells identity.
Glycolipids and MHC proteins on cell surfaces help distinguish self from nonself.

Cell connections mediate cell-to-cell adhesion.
Cell junctions include tight junctions, anchoring junctions, and communicating junctions. In animals, gap junctions allow the passage of small molecules between cells. In plants, plasmodesmata penetrate the cell wall and connect cells.



Review Questions

UNDERSTAND

1. Which of the following statements is NOT part of the cell theory?
   a. All organisms are composed of one or more cells.
   b. Cells come from other cells by division.
   c. Cells are the smallest living things.
   d. Eukaryotic cells have evolved from prokaryotic cells.
2. All cells have all of the following except
   a. plasma membrane.
   b. genetic material.
   c. cytoplasm.
   d. cell wall.
3. Eukaryotic cells are more complex than prokaryotic cells. Which of the following are found only in a eukaryotic cell?
   a. Cell wall
   b. Plasma membrane
   c. Endoplasmic reticulum
   d. Ribosomes
4. Which of the following are differences between bacteria and archaea?
   a. The molecular architecture of their cell walls
   b. The type of ribosomes found in each
   c. Archaea have an internal membrane system that bacteria lack.
   d. Both a and b
5. The cytoskeleton includes
   a. microtubules made of actin filaments.
   b. microfilaments made of tubulin.
   c. intermediate filaments made of twisted fibers of vimentin and keratin.
   d. smooth endoplasmic reticulum.
6. The smooth endoplasmic reticulum is
   a. involved in protein synthesis.
   b. a site of protein glycosylation.
   c. used to store a variety of ions.
   d. the site of lipid and membrane synthesis.

7. Plasmodesmata in plants and gap junctions in animals are functionally similar in that
   a. each is used to anchor layers of cells.
   b. they form channels between cells that allow diffusion of small molecules.
   c. they form tight junctions between cells.
   d. they are anchored to the extracellular matrix.

APPLY

1. The most important factor that limits the size of a cell is the
   a. quantity of proteins and organelles a cell can make.
   b. rate of diffusion of small molecules.
   c. surface area-to-volume ratio of the cell.
   d. amount of DNA in the cell.
2. All eukaryotic cells possess each of the following except
   a. mitochondria.
   b. cell wall.
   c. cytoskeleton.
   d. nucleus.
3. Which of these organelles is NOT associated with the production or sorting of proteins in a cell?
   a. Ribosomes
   b. Smooth endoplasmic reticulum (SER)
   c. Rough endoplasmic reticulum (RER)
   d. Golgi apparatus
4. Different motor proteins like kinesin and myosin are similar in that they can
   a. interact with microtubules.
   b. use energy from ATP to produce movement.
   c. interact with actin.
   d. do both a and b.
5. The protein sorting pathway involves the following organelles/compartments in order:
   a. SER, RER, transport vesicle, Golgi.
   b. RER, lysosome, Golgi.
   c. RER, transport vesicle, Golgi, final destination.
   d. Golgi, transport vesicle, RER, final destination.
6. Chloroplasts and mitochondria have many common features because both
   a. are present in plant cells.
   b. arose by endosymbiosis.
   c. function to oxidize glucose.
   d. function to produce glucose.
7. Eukaryotic cells are composed of three types of cytoskeletal filaments. How are these three filaments similar?
   a. They contribute to the shape of the cell.
   b. They are all made of the same type of protein.
   c. They are all the same size and shape.
   d. They are all equally dynamic and flexible.

SYNTHESIZE

1. The smooth endoplasmic reticulum is the site of synthesis of the phospholipids that make up all the membranes of a cell—especially the plasma membrane. Use the diagram of an animal cell (see figure 4.6) to trace a pathway that would carry a phospholipid molecule from the SER to the plasma membrane. What endomembrane compartments would the phospholipids travel through? How can a phospholipid molecule move between membrane compartments?
2. Use the information provided in table 4.3 to develop a set of predictions about the properties of mitochondria and chloroplasts if these organelles were once free-living prokaryotic cells. How do your predictions match with the evidence for endosymbiosis?
3. In evolutionary theory, homologous traits are those with a similar structure and function derived from a common ancestor. Analogous traits represent adaptations to a similar environment, but from distantly related organisms. Consider the structure and function of the flagella found on eukaryotic and prokaryotic cells. Are the flagella an example of a homologous or analogous trait? Defend your answer.
4. The protist, *Giardia intestinalis*, is the organism associated with water-borne diarrheal diseases. *Giardia* is an unusual eukaryote because it seems to lack mitochondria. Provide two possible evolutionary scenarios for this in the context of the endosymbiotic theory.

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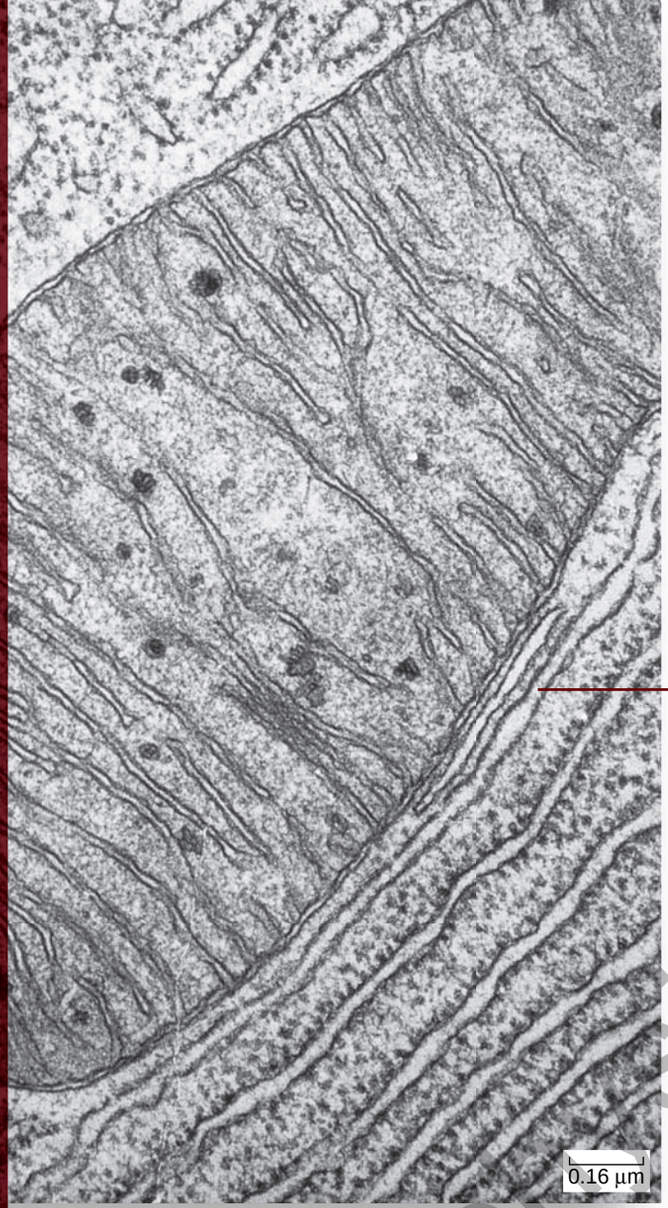


Chapter 5

Membranes

Chapter Outline

Introduction

A cell's interactions with the environment are critical, a give-and-take that never ceases. Without it, life could not exist. Living cells are encased within a lipid membrane through which few water-soluble substances can pass. The membrane also contains protein passageways that permit specific substances to move into and out of the cell and allow the cell to exchange information with its environment. Eukaryotic cells also contain internal membranes like those of the mitochondrion and endoplasmic reticulum pictured here. We call the delicate skin of lipids with embedded protein molecules that encase the cell a plasma membrane. *This chapter examines the structure and function of this remarkable membrane.*

5.1 The Structure of Membranes

Learning Outcomes

1. *Describe the components of biological membranes.*
2. *Explain the fluid mosaic model of membrane structure.*

The membranes that encase all living cells are two phospholipid sheets that are only 5–10 nanometers thick; more than 10,000 of these sheets piled on one another would just equal the thickness of this sheet of paper. Biologists established the components of membranes—not only lipids, but also proteins and other molecules—through biochemical assays, but the organization of the membrane components remained elusive.

We begin by considering the theories that have been advanced about membrane structure. We then look at the individual components of membranes more closely.

The fluid mosaic model shows proteins embedded in a fluid lipid bilayer

The lipid layer that forms the foundation of a cell's membranes is a bilayer formed of **phospholipids** (figure 5.1). For many years, biologists thought that the protein components of the cell membrane covered the inner and outer surfaces of the phospholipid bilayer like a coat of paint. An early model portrayed the membrane as a sandwich; a phospholipid bilayer between two layers of globular protein.

In 1972, S. Jonathan Singer and Garth J. Nicolson revised the model in a simple but profound way: They proposed that the globular proteins are *inserted* into the lipid bilayer, with their nonpolar segments in contact with the nonpolar interior of the bilayer and their polar portions protruding out from the membrane surface. In this model, called the *fluid mosaic model*, a mosaic of proteins floats in or on the fluid lipid bilayer like boats on a pond (figure 5.2).

We now recognize two categories of membrane proteins based on their association with the membrane. *Integral membrane proteins* are embedded in the membrane, and *peripheral proteins* are associated with the surface of the membrane.

Cellular membranes consist of four component groups

A eukaryotic cell contains many membranes. Although they are not all identical, they share the same fundamental architecture. Cell membranes are assembled from four components (table 5.1):

1. **Phospholipid bilayer.** Every cell membrane is composed of phospholipids in a bilayer. The other components of the membrane are embedded within the bilayer, which provides a flexible matrix and, at the same time, imposes a barrier to permeability. Animal cell membranes also contain cholesterol, a steroid with a polar hydroxyl group (—OH). Plant cells have a much lower cholesterol content.
2. **Transmembrane proteins.** A major component of every membrane is a collection of proteins that float in the lipid bilayer. These proteins have a variety of functions, including transport and communication across the membrane. Many integral membrane proteins are not fixed in position. They can move about, just as the phospholipid molecules do. Some membranes are

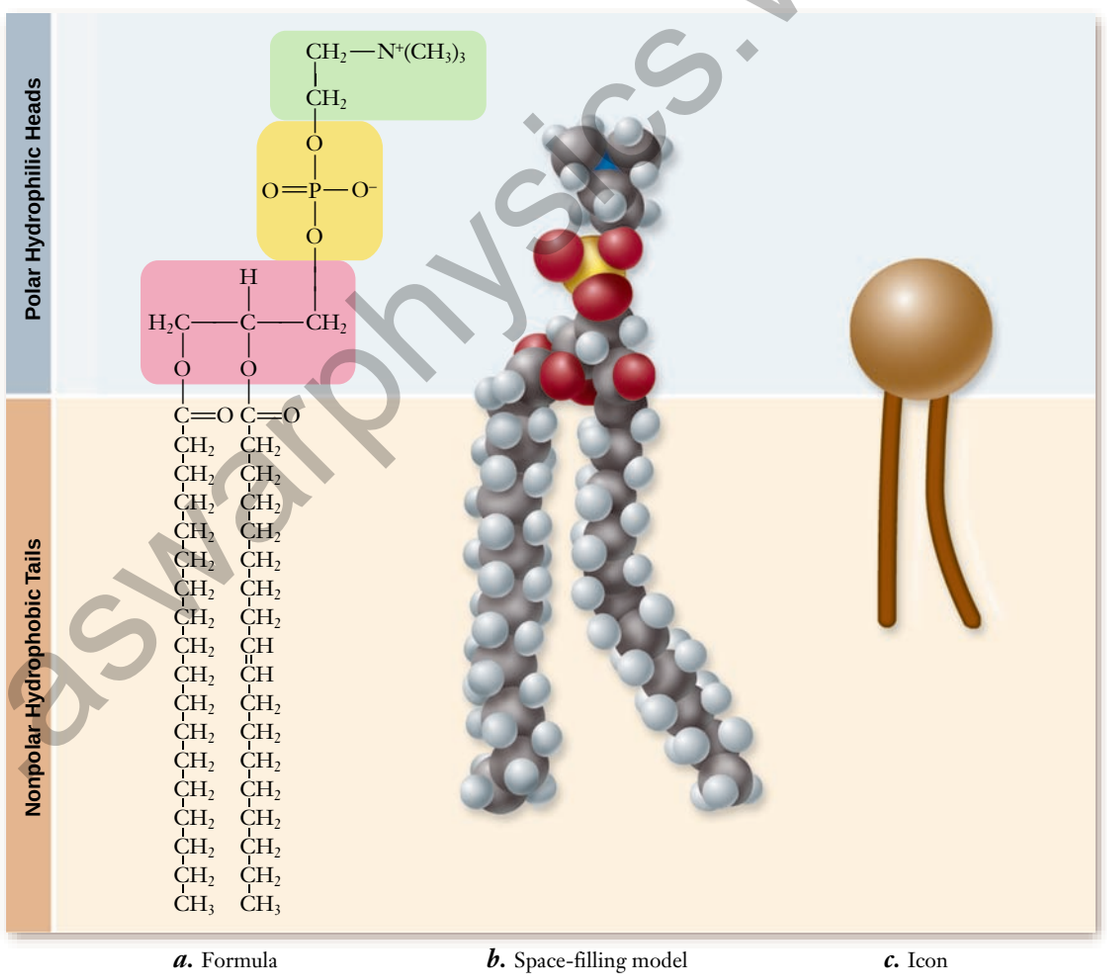


Figure 5.1 Different views of phospholipid structure. Phospholipids are composed of glycerol *(pink)* linked to two fatty acids and a phosphate group. The phosphate group *(yellow)* can have additional molecules attached, such as the positively charged choline *(green)* shown. Phosphatidylcholine is a common component of membranes, it is shown in ***(a)*** with its chemical formula, ***(b)*** as a space-filling model, and ***(c)*** as the icon that is used in most of the figures in this chapter.

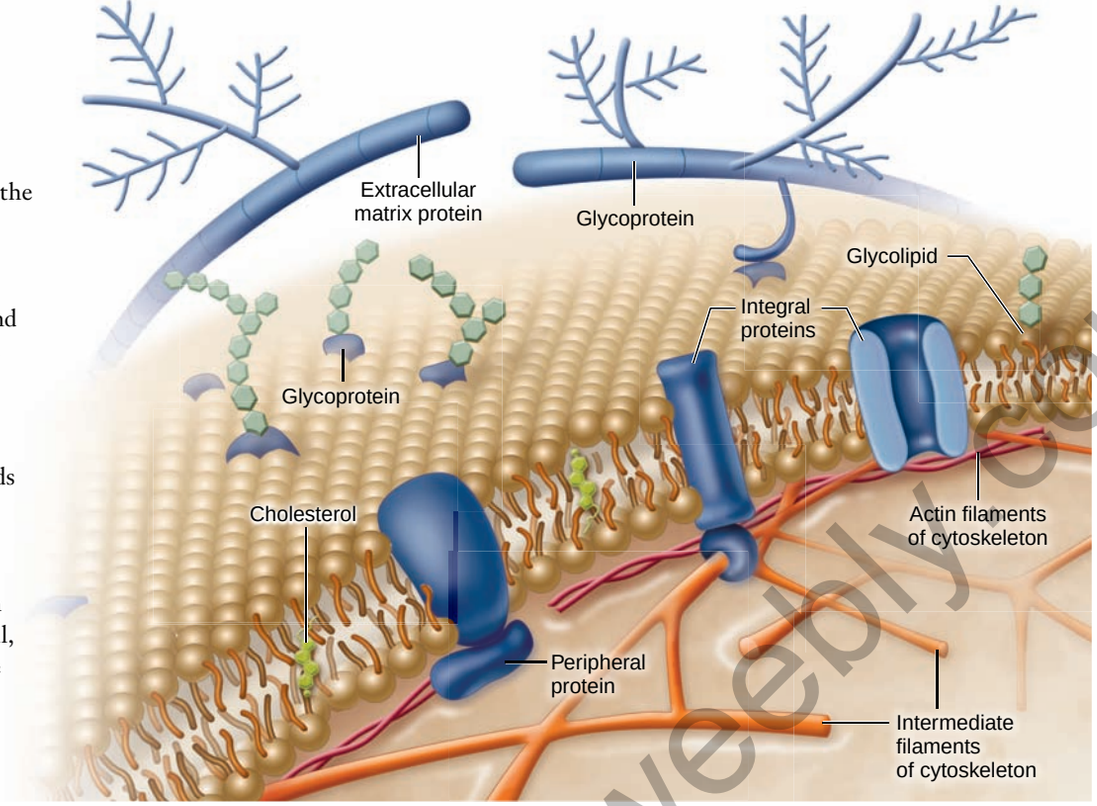


Figure 5.2 The fluid mosaic model of cell membranes.
Integral proteins protrude through the plasma membrane, with nonpolar regions that tether them to the membrane's hydrophobic interior. Carbohydrate chains are often bound to the extracellular portion of these proteins, forming glycoproteins. Peripheral membrane proteins are associated with the surface of the membrane. Membrane phospholipids can be modified by the addition of carbohydrates to form glycolipids. Inside the cell, actin filaments and intermediate filaments interact with membrane proteins. Outside the cell, many animal cells have an elaborate extracellular matrix composed primarily of glycoproteins.

TABLE 5.1 Components of the Cell Membrane

Component	Composition	Function	How It Works	Example
Phospholipid bilayer	Phospholipid molecules	Provides permeability barrier, matrix for proteins	Excludes water-soluble molecules from nonpolar interior of bilayer and cell	Bilayer of cell is impermeable to large water-soluble molecules, such as glucose
Transmembrane proteins	Carriers	Actively or passively transport molecules across membrane	Move specific molecules through the membrane in a series of conformational changes	Glycophorin carrier for sugar transport; sodium–potassium pump
	Channels	Passively transport molecules across membrane	Create a selective tunnel that acts as a passage through membrane	Sodium and potassium channels in nerve, heart, and muscle cells
	Receptors	Transmit information into cell	Signal molecules bind to cell-surface portion of the receptor protein. This alters the portion of the receptor protein within the cell, inducing activity	Specific receptors bind peptide hormones and neurotransmitters
Interior protein network	Spectrins	Determine shape of cell	Form supporting scaffold beneath membrane, anchored to both membrane and cytoskeleton	Red blood cell
	Clathrins	Anchor certain proteins to specific sites, especially on the exterior plasma membrane in receptor-mediated endocytosis	Proteins line coated pits and facilitate binding to specific molecules	Localization of low-density lipoprotein receptor within coated pits
Cell-surface markers	Glycoproteins	"Self" recognition	Create a protein/carbohydrate chain shape characteristic of individual	Major histocompatibility complex protein recognized by immune system
	Glycolipid	Tissue recognition	Create a lipid/carbohydrate chain shape characteristic of tissue	A, B, O blood group markers

crowded with proteins, but in others, the proteins are more sparsely distributed.

3. **Interior protein network.** Membranes are structurally supported by intracellular proteins that reinforce the membrane's shape. For example, a red blood cell has a characteristic biconcave shape because a scaffold made of a protein called spectrin links proteins in the plasma membrane with actin filaments in the cell's cytoskeleton.

   Membranes use networks of other proteins to control the lateral movements of some key membrane proteins, anchoring them to specific sites.

4. **Cell-surface markers.** As you learned in the preceding chapter, membrane sections assemble in the endoplasmic reticulum, transfer to the Golgi apparatus, and then are transported to the plasma membrane. The ER adds chains of sugar molecules to membrane proteins and lipids, converting them into **glycoproteins** and **glycolipids.** Different cell types exhibit different varieties of these glycoproteins and glycolipids on their surfaces, which act as cell identity markers.

Originally, it was believed that because of its fluidity, the plasma membrane was uniform, with lipids and proteins free to diffuse rapidly in the plane of the membrane. However, in the last decade evidence has accumulated suggesting the plasma membrane is not homogeneous and contains microdomains with distinct lipid and protein composition. One type of microdomain, the *lipid raft*, is heavily enriched with cholesterol, which fills space between the phospholipids, packing them more tightly together than the surrounding membrane.

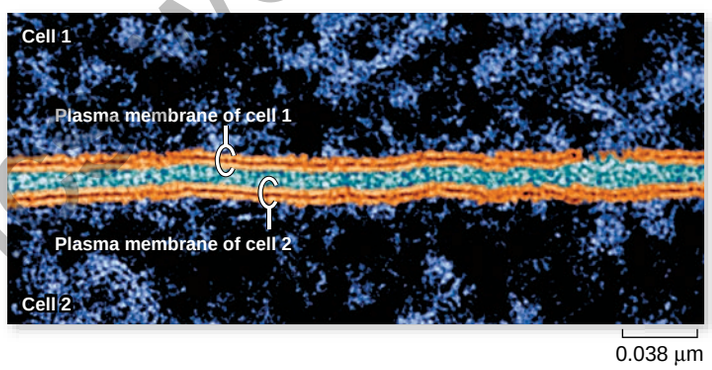
Although the distribution of membrane lipids is symmetrical in the ER where they are synthesized, this distribution is asymmetrical in the plasma membrane, Golgi apparatus, and endosomes. This is accomplished by enzymes that transport lipids across the bilayer from one face to the other.

Electron microscopy has provided structural evidence

Electron microscopy allows biologists to examine the delicate, filmy structure of a cell membrane. We discussed two types of electron microscopes in chapter 4: the transmission electron microscope (TEM) and the scanning electron microscope (SEM). Both provide illuminating views of membrane structure.

When examining cell membranes with electron microscopy, specimens must be prepared for viewing. In one method of preparing a specimen, the tissue of choice is embedded in a hard epoxy matrix. The epoxy block is then cut with a microtome, a machine with a very sharp blade that makes incredibly thin, transparent "epoxy shavings" less than 1 μm thick that peel away from the block of tissue.

These shavings are placed on a grid, and a beam of electrons is directed through the grid with the TEM. At the high magnification an electron microscope provides, resolution is good enough to reveal the double layers of a membrane. False color can be added to the micrograph to enhance detail.



Freeze-fracturing a specimen is another way to visualize the inside of the membrane (figure 5.3). The tissue is embedded

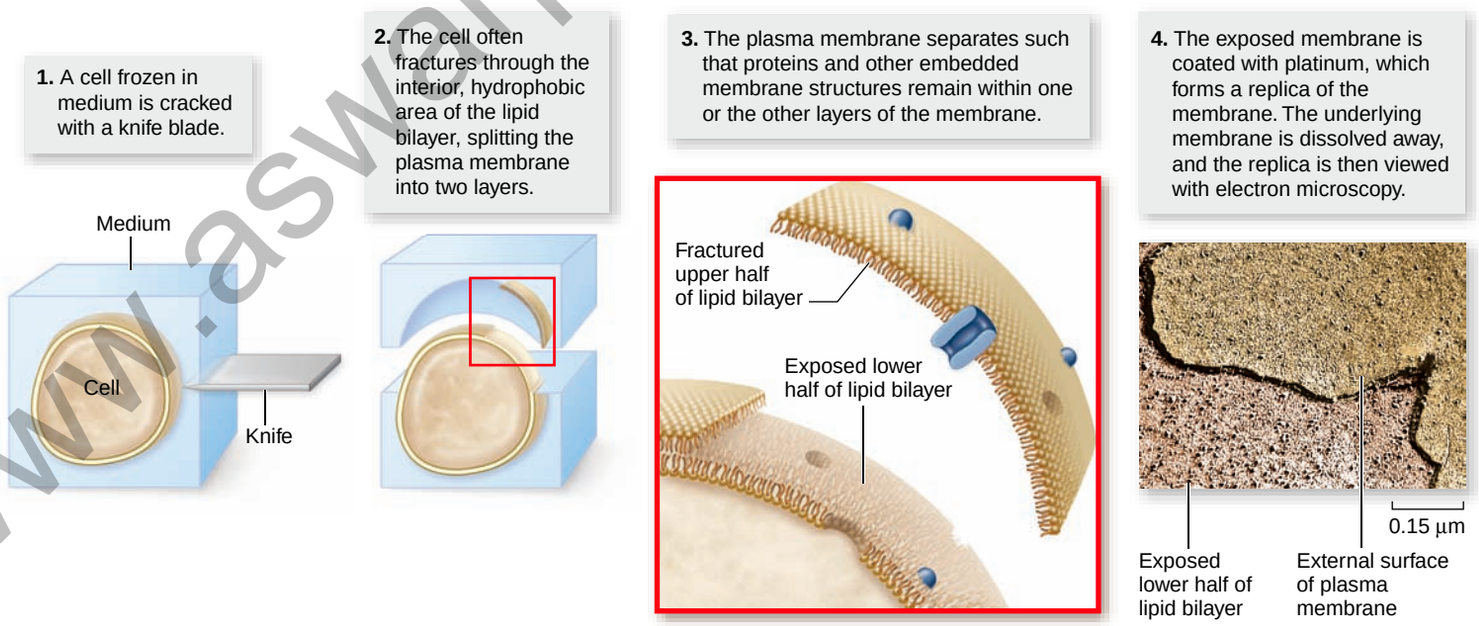


Figure 5.3 **Viewing a plasma membrane with freeze-fracture microscopy.**

in a medium and quick frozen with liquid nitrogen. The frozen tissue is then "tapped" with a knife, causing a crack between the phospholipid layers of membranes. Proteins, carbohydrates, pits, pores, channels, or any other structure affiliated with the membrane will pull apart (whole, usually) and stick with one or the other side of the split membrane.

Next, a very thin coating of platinum is evaporated onto the fractured surface, forming a replica or "cast" of the surface. After the topography of the membrane has been preserved in the cast, the actual tissue is dissolved away, and the cast is examined with electron microscopy, creating a textured and three-dimensional view of the membrane.

Learning Outcomes Review 5.1

Cellular membranes contain four components: (1) a phospholipid bilayer, (2) transmembrane proteins, (3) an internal protein network providing structural support, and (4) cell-surface markers composed of glycoproteins and glycolipids. The fluid mosaic model of membrane structure includes both the fluid nature of the membrane and the mosaic composition of proteins floating in the phospholipid bilayer. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have provided evidence supporting the fluid mosaic model.

- ***If the plasma membrane were just a phospholipid bilayer, how would this affect its function?***

5.2 *Phospholipids: The Membrane's Foundation*

Learning Outcomes

1. ***List the different components of phospholipids.***
2. ***Explain how membranes form spontaneously.***
3. ***Describe the factors involved in membrane fluidity.***

Like the fat molecules (triglycerides) described in chapter 3, a phospholipid has a backbone derived from the three-carbon polyalcohol *glycerol.* Attached to this backbone are one to three fatty acids, long chains of carbon atoms ending in a carboxyl (—COOH) group. A triglyceride molecule has three such chains, one attached to each carbon in the backbone. Because these chains are nonpolar, they do not form hydrogen bonds with water, and triglycerides are not water-soluble.

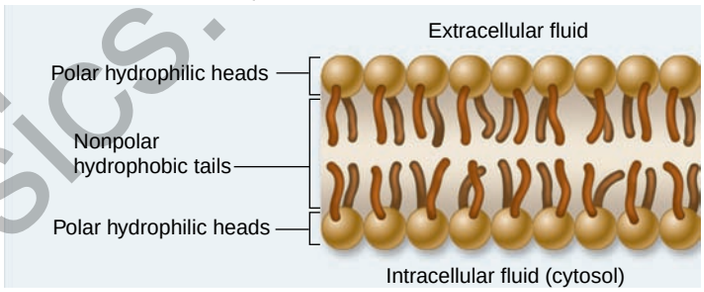
A phospholipid, by contrast, has only two fatty acid chains attached to its backbone. The third carbon of the glycerol carries a phosphate group, thus the name *phospho*lipid. An additional polar organic molecule is often added to the phosphate group as well.

From this simple molecular framework, a large variety of lipids can be constructed by varying the polar organic group attached to the phosphate and the fatty acid chains attached to the glycerol. Mammalian membranes, for example, contain hundreds of chemically distinct species of lipids.

Phospholipids spontaneously form bilayers

The phosphate groups are charged, and other molecules attached to them are polar or charged. This creates a huge change in the molecule's physical properties compared with a triglyceride. The strongly polar phosphate end is hydrophilic, or "water-loving," while the fatty acid end is strongly nonpolar and hydrophobic, or "water-fearing." The two nonpolar fatty acids extend in one direction, roughly parallel to each other, and the polar phosphate group points in the other direction. To represent this structure, phospholipids are often diagrammed as a polar head with two dangling nonpolar tails, as in figure 5.1*c*.

What happens when a collection of phospholipid molecules is placed in water? The polar water molecules repel the long, nonpolar tails of the phospholipids while seeking partners for hydrogen bonding. Because of the polar nature of the water molecules, the nonpolar tails of the phospholipids end up packed closely together, sequestered as far as possible from water. Every phospholipid molecule is oriented with its polar head toward water and its nonpolar tails away. When *two* layers form with the tails facing each other, no tails ever come in contact with water. The resulting structure is the phospholipid bilayer. Phospholipid bilayers form spontaneously, driven by the tendency of water molecules to form the maximum number of hydrogen bonds.



The nonpolar interior of a lipid bilayer impedes the passage of any water-soluble substances through the bilayer, just as a layer of oil impedes the passage of a drop of water. This barrier to water-soluble substances is the key biological property of the lipid bilayer.

The phospholipid bilayer is fluid

A lipid bilayer is stable because water's affinity for hydrogen bonding never stops. Just as surface tension holds a soap bubble together, even though it is made of a liquid, so the hydrogen bonding of water holds a membrane together. Although water continually drives phospholipid molecules into the bilayer configuration, it does not have any effect on the mobility of phospholipids relative to their lipid and nonlipid neighbors in the bilayer. Because phospholipids interact relatively weakly with one another, individual phospholipids and unanchored proteins are comparatively free to move about within the membrane. This can be demonstrated vividly by fusing cells and watching their proteins intermix with time (figure 5.4).

Membrane fluidity can change

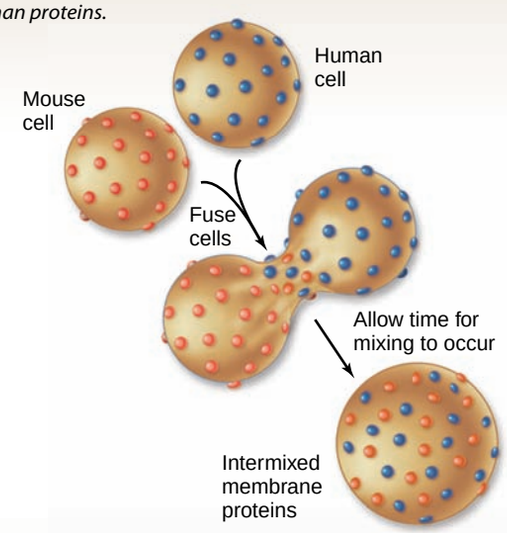
The degree of membrane fluidity changes with the composition of the membrane itself. Much like triglycerides can be solid or liquid at room temperature, depending on their fatty acid

SCIENTIFIC THINKING

Hypothesis: *The plasma membrane is fluid, not rigid.*

Prediction: *If the membrane is fluid, membrane proteins may diffuse laterally.*

Test: *Fuse mouse and human cells, then observe the distribution of membrane proteins over time by labeling specific mouse and human proteins.*



Result: *Over time, hybrid cells show increasingly intermixed proteins.*

Conclusion: *At least some membrane proteins can diffuse laterally in the membrane.*

Further Experiments: *Can you think of any other explanation for these observations? What if newly synthesized proteins were inserted into the membrane during the experiment? How could you use this basic experimental design to rule out this or other possible explanations?*

Figure 5.4 **Test of membrane fluidity.**

composition, membrane fluidity can be altered by changing the membrane's fatty acid composition.

Saturated fats tend to make the membrane less fluid because they pack together well. Unsaturated fats make the membrane more fluid—the "kinks" introduced by the double bonds keep them from packing tightly. You saw this effect on fats and oils earlier in chapter 3. Most membranes also contain sterols such as cholesterol, which can either increase or decrease membrane fluidity, depending on the temperature.

Changes in the environment can have drastic effects on the membranes of single-celled organisms such as bacteria. Increasing temperature makes a membrane more fluid, and decreasing temperature makes it less fluid. Bacteria have evolved mechanisms to maintain a constant membrane fluidity despite fluctuating temperatures. Some bacteria contain enzymes called *fatty acid desaturases* that can introduce double bonds into fatty acids in membranes. Genetic studies, involving either the inactivation of these enzymes or the introduction of them into cells that normally lack them, indicate that the action of these enzymes confers cold tolerance. At colder temperatures, the double bonds introduced by fatty acid desaturase make the membrane more fluid, counteracting the environmental effect of reduced temperature.

Learning Outcomes Review 5.2

Biological membranes consist of a phospholipid bilayer. Each phospholipid has a hydrophilic (phosphate) head and a hydrophobic (lipid) tail. In water, phospholipid molecules spontaneously form a bilayer, with phosphate groups facing out toward the water and lipid tails facing in, where they are sequestered from water. Membrane fluidity varies with composition and conditions: unsaturated fats disturb packing of the lipid tails and make the membrane more fluid, as do higher temperatures.

- ***Would a phospholipid bilayer form in a nonpolar solvent?***

5.3 *Proteins: Multifunctional Components*

Learning Outcomes

1. ***List the functions of membrane proteins.***
2. ***Explain how proteins can associate with the membrane.***
3. ***Identify a transmembrane domain.***

Cell membranes contain a complex assembly of proteins enmeshed in the fluid soup of phospholipid molecules. This very flexible organization permits a broad range of interactions with the environment, some directly involving membrane proteins.

Proteins and protein complexes perform key functions

Although cells interact with their environment through their plasma membranes in many ways, we will focus on six key classes of membrane protein in this chapter and in chapter 9 (figure 5.5).

1. **Transporters.** Membranes are very selective, allowing only certain solutes to enter or leave the cell, either through channels or carriers composed of proteins.
2. **Enzymes.** Cells carry out many chemical reactions on the interior surface of the plasma membrane, using enzymes attached to the membrane.
3. **Cell-surface receptors.** Membranes are exquisitely sensitive to chemical messages, which are detected by receptor proteins on their surfaces.
4. **Cell-surface identity markers.** Membranes carry cell-surface markers that identify them to other cells. Most cell types carry their own ID tags, specific combinations of cell-surface proteins and protein complexes such as glycoproteins that are characteristic of that cell type.
5. **Cell-to-cell adhesion proteins.** Cells use specific proteins to glue themselves to one another. Some act by forming temporary interactions, and others form a more permanent bond. (See chapter 9.)
6. **Attachments to the cytoskeleton.** Surface proteins that interact with other cells are often anchored to the cytoskeleton by linking proteins.

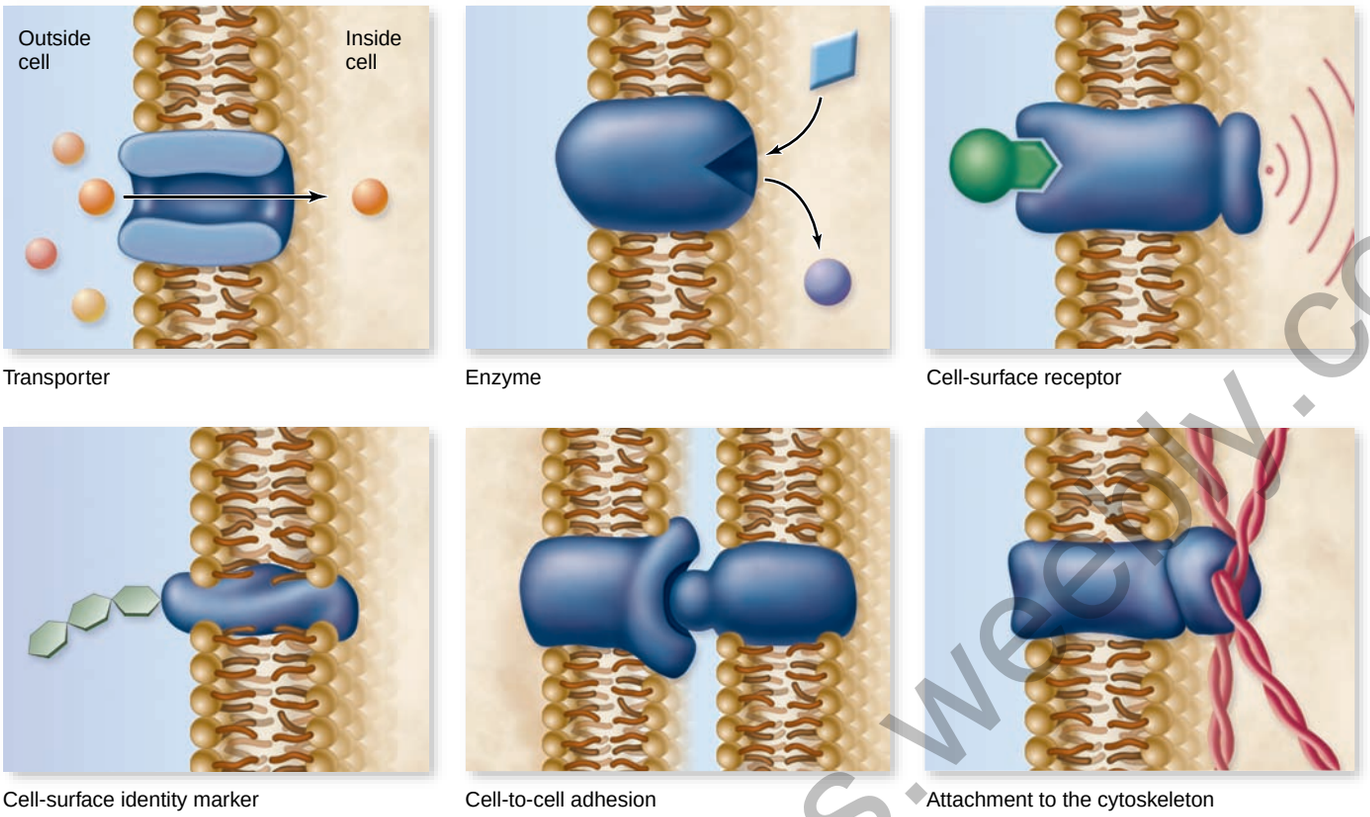


Figure 5.5 Functions of plasma membrane proteins. Membrane proteins act as transporters, enzymes, cell-surface receptors, and cell-surface identity markers, as well as aiding in cell-to-cell adhesion and securing the cytoskeleton.

Inquiry question

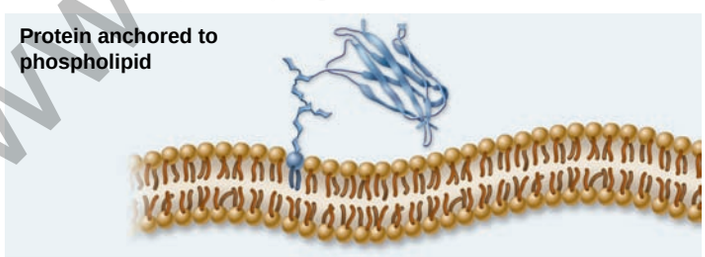
According to the fluid mosaic model, membranes are held together by hydrophobic interactions. Considering the forces that some cells may experience, why do membranes not break apart every time an animal moves?

Structural features of membrane proteins relate to function

As we've just detailed, membrane proteins can serve a variety of functions. These diverse functions arise from the diverse structures of these proteins, yet they also have common structural features related to their role as membrane proteins.

The anchoring of proteins in the bilayer

Some membrane proteins are attached to the surface of the membrane by special molecules that associate strongly with phospholipids. Like a ship tied to a floating dock, these anchored proteins are free to move about on the surface of the membrane tethered to a phospholipid. The anchoring molecules are modified lipids that have (1) nonpolar regions that insert into the internal portion of the lipid bilayer and (2) chemical bonding domains that link directly to proteins.

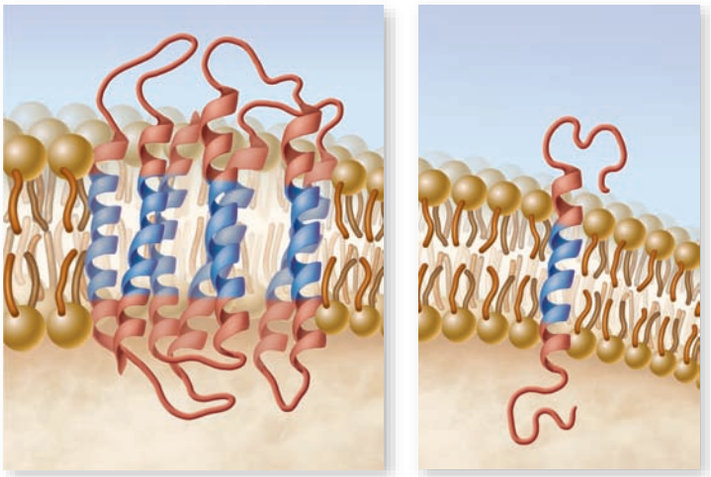


In contrast, other proteins actually span the lipid bilayer (transmembrane proteins). The part of the protein that extends through the lipid bilayer and that is in contact with the nonpolar interior are α-helices or β-pleated sheets (see chapter 3) that consist of nonpolar amino acids. Because water avoids nonpolar amino acids, these portions of the protein are held within the interior of the lipid bilayer. The polar ends protrude from both sides of the membrane. Any movement of the protein out of the membrane, in either direction, brings the nonpolar regions of the protein into contact with water, which "shoves" the protein back into the interior. These forces prevent the transmembrane proteins from simply popping out of the membrane and floating away.

Transmembrane domains

Cell membranes contain a variety of different transmembrane proteins, which differ in the way they traverse the lipid bilayer. The primary difference lies in the number of times that the protein crosses the membrane. Each membrane-spanning region is called a **transmembrane domain.** These domains are composed of hydrophobic amino acids usually arranged into α helices (figure 5.6).

Proteins need only a single transmembrane domain to be anchored in the membrane, but they often have more than one such domain. An example of a protein with a single transmembrane domain is the linking protein that attaches the spectrin network of the cytoskeleton to the interior of the plasma membrane.



a. *b.*

Figure 5.6 Transmembrane domains. Integral membrane proteins have at least one hydrophobic transmembrane domain (shown in blue) to anchor them in the membrane. ***a.*** Receptor protein with seven transmembrane domains. ***b.*** Protein with single transmembrane domain.

Biologists classify some types of receptors based on the number of transmembrane domains they have, such as G protein–coupled receptors with seven membrane-spanning domains (chapter 9). These receptors respond to external molecules, such as epinephrine, and initiate a cascade of events inside the cell.

Another example is bacteriorhodopsin, one of the key transmembrane proteins that carries out photosynthesis in halophilic (salt-loving) archaea. It contains seven nonpolar helical segments that traverse the membrane, forming a structure within the membrane through which protons pass during the light-driven pumping of protons (figure 5.7).

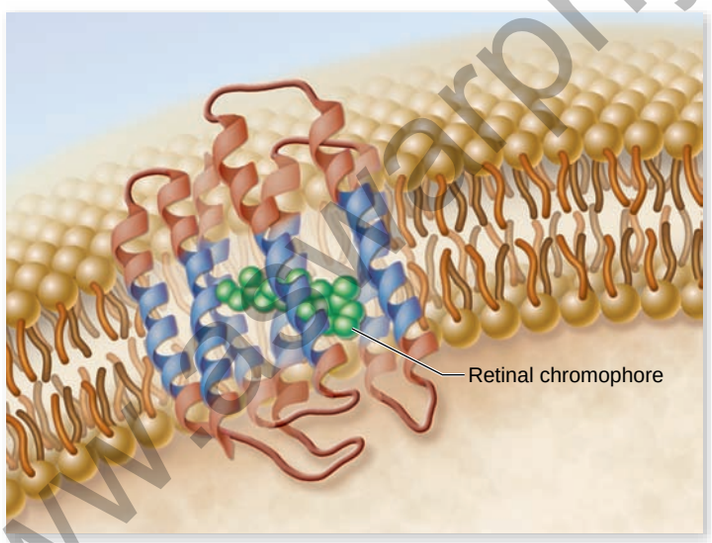


Figure 5.7 Bacteriorhodopsin. This transmembrane protein mediates photosynthesis in the archaean *Halobacterium salinarium*. The protein traverses the membrane seven times with hydrophobic helical strands that are within the hydrophobic center of the lipid bilayer. The helical regions form a structure across the bilayer through which protons are pumped by the retinal chromophore *(green)* using energy from light.

Pores

Some transmembrane proteins have extensive nonpolar regions with secondary configurations of β-pleated sheets instead of α helices (chapter 3). The β sheets form a characteristic motif, folding back and forth in a cylinder so the sheets arrange themselves like a pipe through the membrane. This forms a polar environment in the interior of the β sheets spanning the membrane. This so-called *β barrel*, open on both ends, is a common feature of the porin class of proteins that are found within the outer membrane of some bacteria. The openings allow molecules to pass through the membrane (figure 5.8).

Learning Outcomes Review 5.3

Proteins in the membrane confer the main differences between membranes of different cells. Their functions include transport, enzymatic action, reception of extracellular signals, cell-to-cell interactions, and cell identity markers. Peripheral proteins can be anchored in the membrane by modified lipids. Integral membrane proteins span the membrane and have one or more hydrophobic regions, called transmembrane domains, that anchor them.

- ***Why are transmembrane domains hydrophobic?***

Inquiry question

Based only on amino acid sequence, how would you recognize an integral membrane protein?

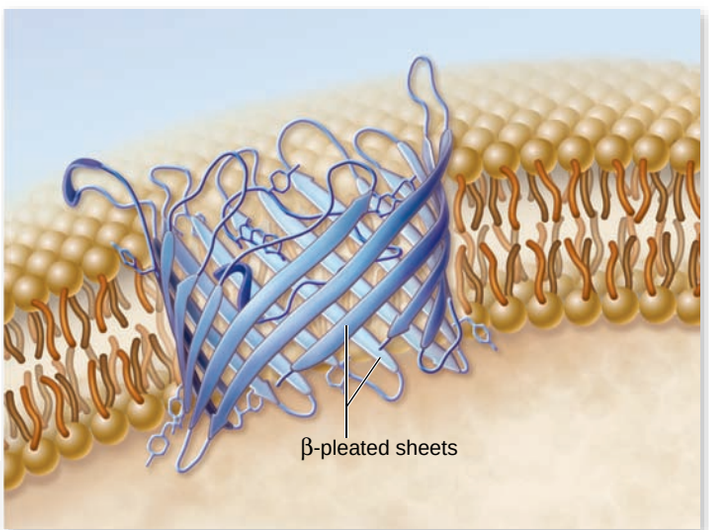


Figure 5.8 A pore protein. The bacterial transmembrane protein porin creates large open tunnels called pores in the outer membrane of a bacterium. Sixteen strands of β-pleated sheets run antiparallel to one another, creating a so-called β barrel in the bacterial outer cell membrane. The tunnel allows water and other materials to pass through the membrane.

5.4 Passive Transport Across Membranes

Learning Outcomes

1. *Compare simple diffusion and facilitated diffusion.*
2. *Differentiate between channel proteins and carrier proteins.*
3. *Explain the movement of water by osmosis.*

Many substances can move in and out of the cell without the cell's having to expend energy. This type of movement is termed **passive transport.** Some ions and molecules can pass through the membrane fairly easily and do so because of a *concentration gradient*—a difference between the concentration on the inside of the membrane and that on the outside. Some substances also move in response to a gradient, but do so through specific channels formed by proteins in the membrane.

Transport can occur by simple diffusion

Molecules and ions dissolved in water are in constant random motion. This random motion causes a net movement of these substances from regions of high concentration to regions of lower concentration, a process called **diffusion** (figure 5.9).

Net movement driven by diffusion will continue until the concentration is the same in all regions. Consider what happens when you add a drop of colored ink to a bowl of water. Over time the ink becomes dispersed throughout the solution. This is due to diffusion of the ink molecules. In the context of cells, we are usually concerned with differences in concentration of molecules across the plasma membrane. We need to consider the relative concentrations both inside and outside the cell, as well as how readily a molecule can cross the membrane.

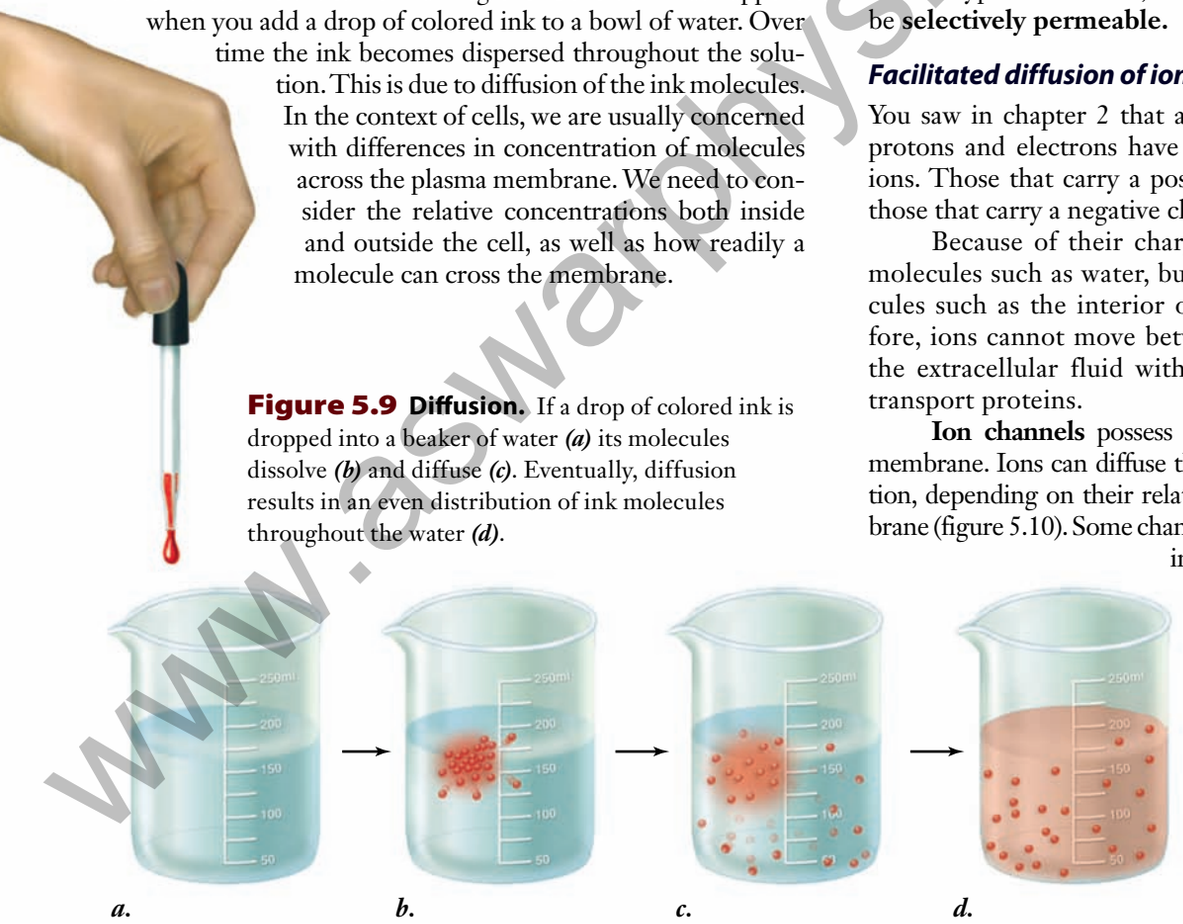


Figure 5.9 Diffusion. If a drop of colored ink is dropped into a beaker of water *(a)* its molecules dissolve *(b)* and diffuse *(c)*. Eventually, diffusion results in an even distribution of ink molecules throughout the water *(d)*.

The major barrier to crossing a biological membrane is the hydrophobic interior that repels polar molecules but not nonpolar molecules. If a concentration difference exists for a nonpolar molecule, it will move across the membrane until the concentration is equal on both sides. At this point, movement in both directions still occurs, but there is no net change in either direction. This includes molecules like O_2 and nonpolar organic molecules such as steroid hormones.

The plasma membrane has limited permeability to small polar molecules and very limited permeability to larger polar molecules and ions. The movement of water, one of the most important polar molecules, is discussed in its own section later on.

Proteins allow membrane diffusion to be selective

Many important molecules required by cells cannot easily cross the plasma membrane. These molecules can still enter the cell by diffusion through specific channel proteins or carrier proteins embedded in the plasma membrane, provided there is a higher concentration of the molecule outside the cell than inside. We call this process of diffusion mediated by a membrane protein **facilitated diffusion. Channel proteins** have a hydrophilic interior that provides an aqueous channel through which polar molecules can pass when the channel is open. **Carrier proteins,** in contrast to channels, bind specifically to the molecule they assist, much like an enzyme binds to its substrate. These channels and carriers are usually selective for one type of molecule, and thus the cell membrane is said to be **selectively permeable.**

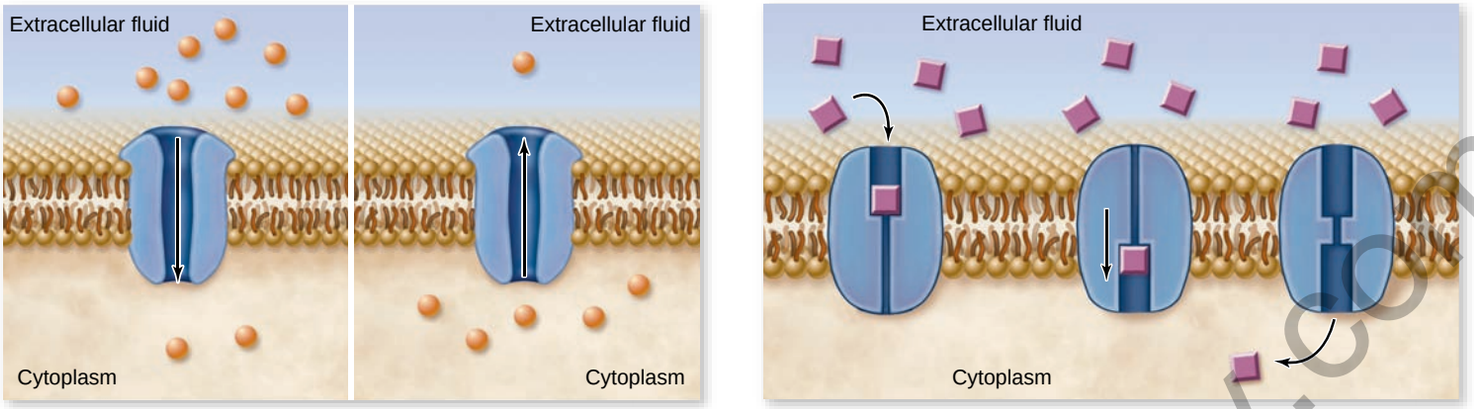
Facilitated diffusion of ions through channels

You saw in chapter 2 that atoms with an unequal number of protons and electrons have an electric charge and are called ions. Those that carry a positive charge are called *cations* and those that carry a negative charge are called *anions.*

Because of their charge, ions interact well with polar molecules such as water, but are repelled by nonpolar molecules such as the interior of the plasma membrane. Therefore, ions cannot move between the cytoplasm of a cell and the extracellular fluid without the assistance of membrane transport proteins.

Ion channels possess a hydrated interior that spans the membrane. Ions can diffuse through the channel in either direction, depending on their relative concentration across the membrane (figure 5.10). Some channel proteins can be opened or closed in response to a stimulus. These channels are called *gated channels*, and depending on the nature of the channel, the stimulus can be either chemical or electrical.

Three conditions determine the direction of net movement of the ions: (1) their relative concentrations on either side of the membrane, (2) the voltage difference across the membrane and for the gated channels, and



a. *b.*

Figure 5.10 Facilitated diffusion. Diffusion can be facilitated by membrane proteins. ***a.*** The movement of ions through a channel is shown. On the left the concentration is higher outside the cell, so the ions move into the cell. On the right the situation is reversed. In both cases, transport continues until the concentration is equal on both sides of the membrane. At this point, ions continue to cross the membrane in both directions, but there is no net movement in either direction. ***b.*** Carrier proteins bind specifically to the molecules they transport. In this case, the concentration is higher outside the cell, so molecules bind to the carrier on the outside. The carrier's shape changes, allowing the molecule to cross the membrane. This is reversible, so net movement continues until the concentration is equal on both sides of the membrane.

(3) the state of the gate (open or closed). A voltage difference is an electrical potential difference across the membrane called a *membrane potential.* Changes in membrane potential form the basis for transmission of signals in the nervous system and some other tissues. (We discuss this topic in detail in chapter 45.) Each type of channel is specific for a particular ion, such as calcium (Ca^{2+}), sodium (Na^{+}), potassium (K^{+}), or chloride (Cl^{-}), or in some cases, for more than one cation or anion. Ion channels play an essential role in signaling by the nervous system.

Facilitated diffusion by carrier proteins

Carrier proteins can help transport both ions and other solutes, such as some sugars and amino acids, across the membrane. Transport through a carrier is still a form of diffusion and therefore requires a concentration difference across the membrane.

Carriers must bind to the molecule they transport, so the relationship between concentration and rate of transport differs from that due to simple diffusion. As concentration increases, transport by simple diffusion shows a linear increase in rate of transport. But when a carrier protein is involved, a concentration increase means that more of the carriers are bound to the transported molecule. At high enough concentrations all carriers will be occupied, and the rate of transport will be constant. This means that the carrier exhibits *saturation.*

This situation is somewhat like that of a stadium (the cell) where a crowd must pass through turnstiles to enter. If there are unoccupied turnstiles, you can go right through, but when all are occupied, you must wait. When ticket holders are passing through the gates at maximum speed, the rate at which they enter cannot increase, no matter how many are waiting outside.

Facilitated diffusion in red blood cells

Several examples of facilitated diffusion can be found in the plasma membrane of vertebrate red blood cells (RBCs). One RBC carrier protein, for example, transports a different molecule in each direction: chloride ion (Cl^{-}) in one direction and bicarbonate ion (HCO_3^{-}) in the opposite direction. As you will learn in chapter 51, this carrier is important in the uptake and release of carbon dioxide.

The glucose transporter is a second vital facilitated diffusion carrier in RBCs. Red blood cells keep their internal concentration of glucose low through a chemical trick: They immediately add a phosphate group to any entering glucose molecule, converting it to a highly charged glucose phosphate that can no longer bind to the glucose transporter, and therefore cannot pass back across the membrane. This maintains a steep concentration gradient for unphosphorylated glucose, favoring its entry into the cell.

The glucose transporter that assists the entry of glucose into the cell does not appear to form a channel in the membrane. Instead, this transmembrane protein appears to bind to a glucose molecule and then to flip its shape, dragging the glucose through the bilayer and releasing it on the inside of the plasma membrane. After it releases the glucose, the transporter reverts to its original shape and is then available to bind the next glucose molecule that comes along outside the cell.

Osmosis is the movement of water across membranes

The cytoplasm of a cell contains ions and molecules, such as sugars and amino acids, dissolved in water. The mixture of these substances and water is called an *aqueous solution.* Water is termed the **solvent,** and the substances dissolved in the water are **solutes.** Both water and solutes tend to diffuse from regions of high concentration to ones of low concentration; that is, they diffuse down their concentration gradients.

When two regions are separated by a membrane, what happens depends on whether the solutes can pass freely through that membrane. Most solutes, including ions and sugars, are not lipid-soluble and, therefore, are unable to cross the lipid bilayer. The concentration gradient of these solutes can lead to the movement of water.

Osmosis

Water molecules interact with dissolved solutes by forming hydration shells around the charged solute molecules. When a membrane separates two solutions with different concentrations of solutes, the concentrations of *free* water molecules on the two sides of the membrane also differ. The side with higher solute concentration has tied up more water molecules in hydration shells and thus has fewer free water molecules.

As a consequence of this difference, free water molecules move down their concentration gradient, toward the higher solute concentration. This net diffusion of water across a membrane toward a higher solute concentration is called **osmosis** (figure 5.11).

The concentration of *all* solutes in a solution determines the **osmotic concentration** of the solution. If two solutions have unequal osmotic concentrations, the solution with the higher concentration is **hypertonic** (Greek *hyper*, "more than"), and the solution with the lower concentration is **hypotonic** (Greek *hypo*, "less than"). When two solutions have the same osmotic concentration, the solutions are **isotonic** (Greek *iso*, "equal"). The terms *hyperosmotic*, *hypoosmotic*, and *isosmotic* are also used to describe these conditions.

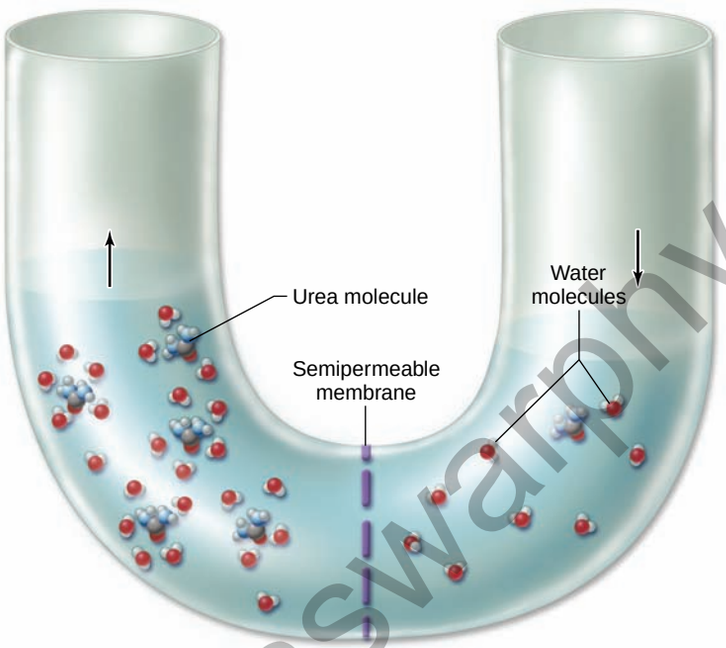


Figure 5.11 Osmosis. Concentration differences in charged or polar molecules that cannot cross a semipermeable membrane result in movement of water, which can cross the membrane. Water molecules form hydrogen bonds with charged or polar molecules creating a hydration shell around them in solution. A higher concentration of polar molecules (urea) shown on the left side of the membrane leads to water molecules gathering around each urea molecule. These water molecules are no longer free to diffuse across the membrane. The polar solute has reduced the concentration of free water molecules, creating a gradient. This causes a net movement of water by diffusion from right to left in the U-tube, raising the level on the left and lowering the level on the right.

A cell in any environment can be thought of as a plasma membrane separating two solutions: the cytoplasm and the extracellular fluid. The direction and extent of any diffusion of water across the plasma membrane is determined by comparing the osmotic strength of these solutions. Put another way, water diffuses out of a cell in a hypertonic solution (that is, the cytoplasm of the cell is hypotonic, compared with the extracellular fluid). This loss of water causes the cell to shrink until the osmotic concentrations of the cytoplasm and the extracellular fluid become equal.

Aquaporins: Water channels

The transport of water across the membrane is complex. Studies on artificial membranes show that water, despite its polarity, can cross the membrane, but this flow is limited. Water flow in living cells is facilitated by **aquaporins,** which are specialized channels for water.

A simple experiment demonstrates this. If an amphibian egg is placed in hypotonic spring water (the solute concentration in the cell is higher than that of the surrounding water), it does not swell. If aquaporin mRNA is then injected into the egg, the channel proteins are expressed and appear in the egg's plasma membrane. Water can now diffuse into the egg, causing it to swell.

More than 11 different kinds of aquaporins have been found in mammals. These fall into two general classes: those that are specific for only water, and those that allow other small hydrophilic molecules, such as glycerol or urea, to cross the membrane as well. This latter class explains how some membranes allow the easy passage of small hydrophilic substances.

The human genetic disease, hereditary (nephrogenic) diabetes insipidus (NDI), has been shown to be caused by a nonfunctional aquaporin protein. This disease causes the excretion of large volumes of dilute urine, illustrating the importance of aquaporins to our physiology.

Osmotic pressure

What happens to a cell in a hypotonic solution? (That is, the cell's cytoplasm is hypertonic relative to the extracellular fluid.) In this situation, water diffuses into the cell from the extracellular fluid, causing the cell to swell. The pressure of the cytoplasm pushing out against the cell membrane, or hydrostatic pressure, increases. The amount of water that enters the cell depends on the difference in solute concentration between the cell and the extracellular fluid. This is measured as **osmotic pressure,** defined as the force needed to stop osmotic flow.

If the membrane is strong enough, the cell reaches an equilibrium, at which the osmotic pressure, which tends to drive water into the cell, is exactly counterbalanced by the hydrostatic pressure, which tends to drive water back out of the cell. However, a plasma membrane by itself cannot withstand large internal pressures, and an isolated cell under such conditions would burst like an overinflated balloon (figure 5.12).

Accordingly, it is important for animal cells, which only have plasma membranes, to maintain osmotic balance. In contrast, the cells of prokaryotes, fungi, plants, and many protists are surrounded by strong cell walls, which can withstand high internal pressures without bursting.

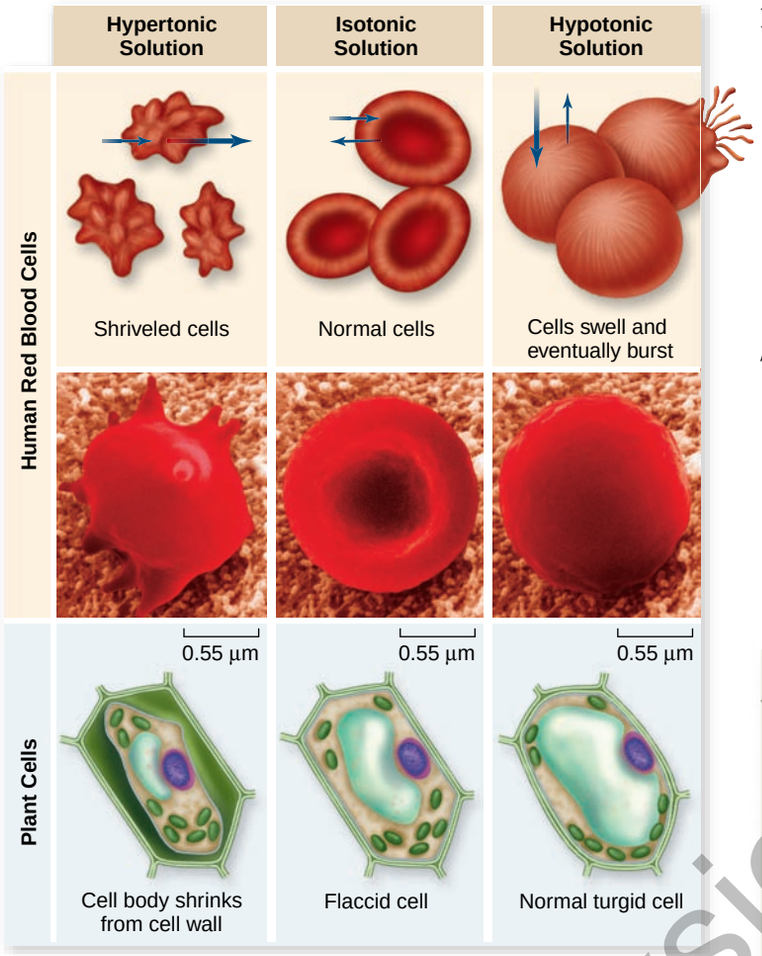


Figure 5.12 How solutes create osmotic pressure. In a hypertonic solution, water moves out of the cell, causing the cell to shrivel. In an isotonic solution, water diffuses into and out of the cell at the same rate, with no change in cell size. In a hypotonic solution, water moves into the cell. Direction and amount of water movement is shown with blue arrows *(top)*. As water enters the cell from a hypotonic solution, pressure is applied to the plasma membrane until the cell ruptures. Water enters the cell due to osmotic pressure from the higher solute concentration in the cell. Osmotic pressure is measured as the force needed to stop osmosis. The strong cell wall of plant cells can withstand the hydrostatic pressure to keep the cell from rupturing. This is not the case with animal cells.

Maintaining osmotic balance

Organisms have developed many strategies for solving the dilemma posed by being hypertonic to their environment and therefore having a steady influx of water by osmosis.

Extrusion. Some single-celled eukaryotes, such as the protist *Paramecium*, use organelles called contractile vacuoles to remove water. Each vacuole collects water from various parts of the cytoplasm and transports it to the central part of the vacuole, near the cell surface. The vacuole possesses a small pore that opens to the outside of the cell. By contracting rhythmically, the vacuole pumps out (extrudes) through this pore the water that is continuously drawn into the cell by osmotic forces.

Isosmotic Regulation. Some organisms that live in the ocean adjust their internal concentration of solutes to match that of the surrounding seawater. Because they are isosmotic with respect to their environment, no net flow of water occurs into or out of these cells.

Many terrestrial animals solve the problem in a similar way, by circulating a fluid through their bodies that bathes cells in an isotonic solution. The blood in your body, for example, contains a high concentration of the protein albumin, which elevates the solute concentration of the blood to match that of your cells' cytoplasm.

Turgor. Most plant cells are hypertonic to their immediate environment, containing a high concentration of solutes in their central vacuoles. The resulting internal hydrostatic pressure, known as **turgor pressure,** presses the plasma membrane firmly against the interior of the cell wall, making the cell rigid. Most green plants depend on turgor pressure to maintain their shape, and thus they wilt when they lack sufficient water.

Learning Outcomes Review 5.4

Passive transport involves diffusion, which requires a concentration gradient. Hydrophobic molecules can diffuse directly through the membrane (simple diffusion). Polar molecules and ions can also diffuse through the membrane, but only with the aid of a channel or carrier protein (facilitated diffusion). Channel proteins assist by forming a hydrophilic passageway through the membrane, whereas carrier proteins bind to the molecule they assist. Water passes through the membrane and through aquaporins in response to solute concentration differences inside and outside the cell. This process is called osmosis.

- ***If you require intravenous (IV) medication in the hospital, what should the concentration of solutes in the IV solution be relative to your blood cells?***

5.5 *Active Transport Across Membranes*

Learning Outcomes

1. ***Differentiate between active transport and diffusion.***
2. ***Describe the function of the Na^+/K^+ pump.***
3. ***Explain the energetics of coupled transport.***

Diffusion, facilitated diffusion, and osmosis are passive transport processes that move materials down their concentration gradients, but cells can also actively move substances across a cell membrane *up* their concentration gradients. This process requires the expenditure of energy, typically from ATP, and is therefore called **active transport.**

Active transport uses energy to move materials against a concentration gradient

Like facilitated diffusion, active transport involves highly selective protein carriers within the membrane that bind to the transported substance, which could be an ion or a simple molecule, such as a sugar, an amino acid, or a nucleotide. These carrier proteins are called **uniporters** if they transport a single type of molecule and symporters or antiporters if they transport two different molecules together. **Symporters** transport two molecules in the same direction, and **antiporters** transport two molecules in opposite directions. These terms can also be used to describe facilitated diffusion carriers.

Active transport is one of the most important functions of any cell. It enables a cell to take up additional molecules of a substance that is already present in its cytoplasm in concentrations higher than in the extracellular fluid. Active transport also enables a cell to move substances out of its cytoplasm and into the extracellular fluid, despite higher external concentrations.

The use of energy from ATP in active transport may be direct or indirect. Let's first consider how ATP is used directly to move ions against their concentration gradients.

The sodium–potassium pump runs directly on ATP

More than one-third of all of the energy expended by an animal cell that is not actively dividing is used in the active transport of sodium (Na^+) and potassium (K^+) ions. Most animal cells have a low internal concentration of Na^+, relative to their surroundings, and a high internal concentration of K^+. They maintain these concentration differences by actively pumping Na^+ out of the cell and K^+ in.

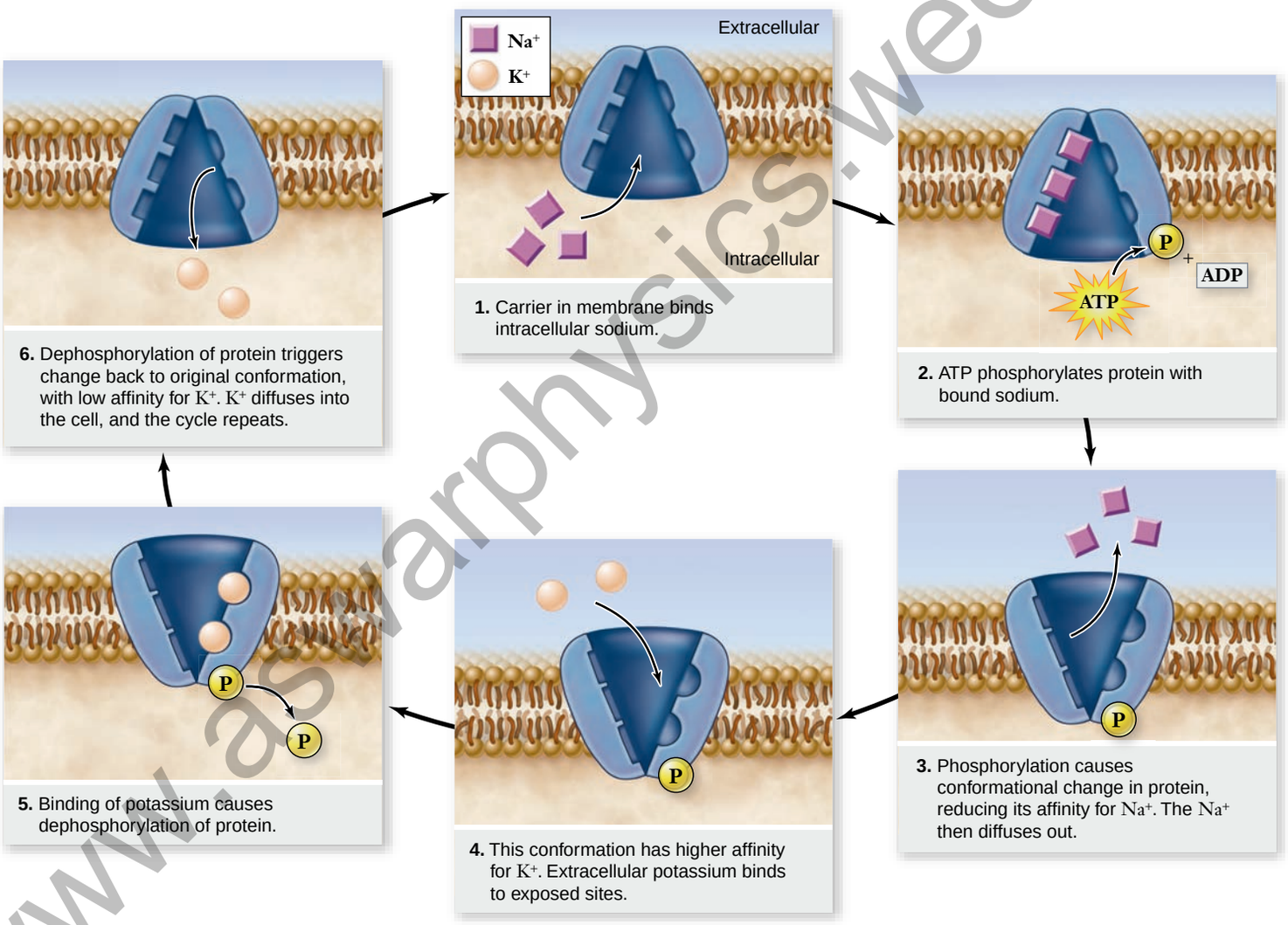


Figure 5.13 The sodium–potassium pump. The protein carrier known as the sodium–potassium pump transports sodium (Na^+) and potassium (K^+) across the plasma membrane. For every three Na^+ transported out of the cell, two K^+ are transported into it. The sodium–potassium pump is fueled by ATP hydrolysis. The affinity of the pump for Na^+ and K^+ is changed by adding or removing phosphate (P), which changes the conformation of the protein.

The remarkable protein that transports these two ions across the cell membrane is known as the **sodium–potassium pump** (figure 5.13). This carrier protein uses the energy stored in ATP to move these two ions. In this case, the energy is used to change the conformation of the carrier protein, which changes its affinity for either Na^+ ions or K^+ ions. This is an excellent illustration of how subtle changes in the structure of a protein affect its function.

The important characteristic of the sodium–potassium pump is that it is an active transport mechanism, transporting Na^+ and K^+ from areas of low concentration to areas of high concentration. This transport is the opposite of passive transport by diffusion; it is achieved only by the constant expenditure of metabolic energy. The sodium–potassium pump works through the following series of conformational changes in the transmembrane protein (summarized in figure 5.13):

Step 1. Three Na^+ bind to the cytoplasmic side of the protein, causing the protein to change its conformation.

Step 2. In its new conformation, the protein binds a molecule of ATP and cleaves it into adenosine diphosphate (ADP) and phosphate (P_i). ADP is released, but the phosphate group is covalently linked to the protein. The protein is now phosphorylated.

Step 3. The phosphorylation of the protein induces a second conformational change in the protein. This change translocates the three Na^+ across the membrane, so they now face the exterior. In this new conformation, the protein has a low affinity for Na^+, and the three bound Na^+ break away from the protein and diffuse into the extracellular fluid.

Step 4. The new conformation has a high affinity for K^+, two of which bind to the extracellular side of the protein as soon as it is free of the Na^+.

Step 5. The binding of the K^+ causes another conformational change in the protein, this time resulting in the hydrolysis of the bound phosphate group.

Step 6. Freed of the phosphate group, the protein reverts to its original shape, exposing the two K^+ to the cytoplasm. This conformation has a low affinity for K^+, so the two bound K^+ dissociate from the protein and diffuse into the interior of the cell. The original conformation has a high affinity for Na^+. When these ions bind, they initiate another cycle.

In every cycle, three Na^+ leave the cell and two K^+ enter. The changes in protein conformation that occur during the cycle are rapid, enabling each carrier to transport as many as 300 Na^+ per second. The sodium–potassium pump appears to exist in all animal cells, although cells vary widely in the number of pump proteins they contain.

Coupled transport uses ATP indirectly

Some molecules are moved against their concentration gradient by using the energy stored in a gradient of a different molecule. In this process, called *coupled transport*, the energy released as one molecule moves down its concentration gradient is captured and used to move a different molecule against its gradient. As you just saw, the energy stored in ATP molecules can be used to create a gradient of Na^+ and K^+ across the membrane. These gradients can then be used to power the transport of other molecules across the membrane.

As one example, let's consider the active transport of glucose across the membrane in animal cells. Glucose is such an important molecule that there are a variety of transporters for it, one of which was discussed earlier under passive transport. In a multicellular organism, intestinal epithelial cells can have a higher concentration of glucose inside the cell than outside, so these cells need to be able to transport glucose against its concentration gradient. This requires energy and a different transporter than the one involved in facilitated diffusion of glucose.

The active glucose transporter uses the Na^+ gradient produced by the sodium–potassium pump as a source of energy to power the movement of glucose into the cell. In this system, both glucose and Na^+ bind to the transport protein, which allows Na^+ to pass into the cell down its concentration gradient, capturing the energy and using it to move glucose into the cell. In this kind of cotransport, both molecules are moving in the same direction across the membrane; therefore the transporter is a symporter (figure 5.14).

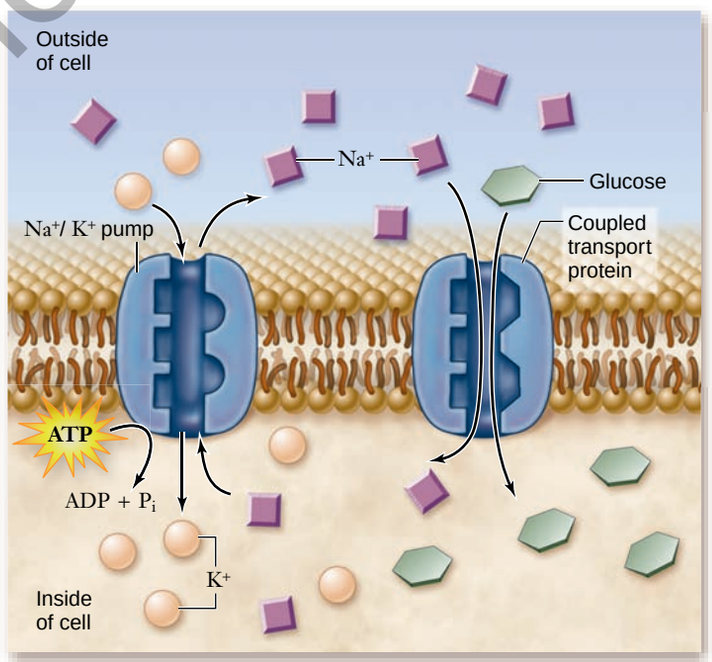


Figure 5.14 Coupled transport. A membrane protein transports Na^+ into the cell, down its concentration gradient, at the same time it transports a glucose molecule into the cell. The gradient driving the Na^+ entry allows sugar molecules to be transported against their concentration gradient. The Na^+ gradient is maintained by the Na^+/K^+ pump. ADP = adenosine diphosphate; ATP = adenosine triphosphate; P_i = inorganic phosphate

In a related process, called *countertransport*, the inward movement of Na^+ is coupled with the outward movement of another substance, such as Ca^{2+} or H^+. As in cotransport, both Na^+ and the other substance bind to the same transport protein, which in this case is an antiporter, as the substances bind on opposite sides of the membrane and are moved in opposite directions. In countertransport, the cell uses the energy released as Na^+ moves down its concentration gradient into the cell to eject a substance against its concentration gradient. In both cotransport and countertransport, the potential energy in the concentration gradient of one molecule is used to transport another molecule against its concentration gradient. They differ only in the direction that the second molecule moves relative to the first.

Learning Outcomes Review 5.5

Active transport requires both a carrier protein and energy, usually in the form of ATP, to move molecules against a concentration gradient. The sodium–potassium pump uses ATP to moved Na^+ in one direction and K^+ in the other to create and maintain concentration differences of these ions. In coupled transport, a favorable concentration gradient of one molecule is used to move a different molecule against its gradient, such as in the transport of glucose by Na^+.

- ***Can active transport involve a channel protein. Why or why not?***

5.6 Bulk Transport by Endocytosis and Exocytosis

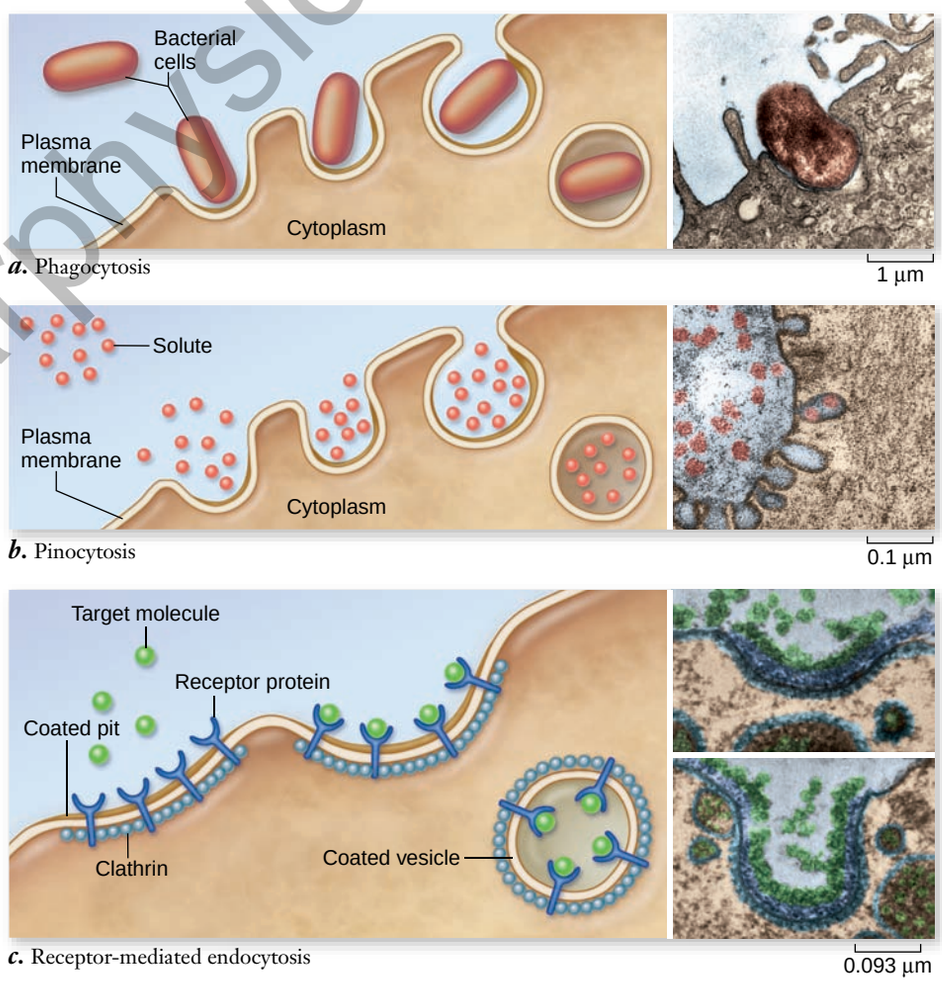
Learning Outcomes

1. ***Distinguish between endocytosis and exocytosis.***
2. ***Explain how endocytosis can be specific.***

The lipid nature of cell plasma membranes raises a second problem. The substances cells require for growth are mostly large, polar molecules that cannot cross the hydrophobic barrier a lipid bilayer creates. How do these substances get into cells? Two processes are involved in this **bulk transport:** *endocytosis* and *exocytosis*.

Bulk material enters the cell in vesicles

In **endocytosis,** the plasma membrane envelops food particles and fluids. Cells use three major types of endocytosis: phagocytosis, pinocytosis, and receptor-mediated endocytosis (figure 5.15). Like active transport, these processes also require energy expenditure.



a. Phagocytosis

b. Pinocytosis

c. Receptor-mediated endocytosis

Figure 5.15 Endocytosis. Both *(a)* phagocytosis and *(b)* pinocytosis are forms of endocytosis. *c.* In receptor-mediated endocytosis, cells have pits coated with the protein clathrin that initiate endocytosis when target molecules bind to receptor proteins in the plasma membrane. Photo inserts (false color has been added to enhance distinction of structures): *(a)* A TEM of phagocytosis of a bacterium, *Rickettsia tsutsugamushi*, by a mouse peritoneal mesothelial cell. The bacterium enters the host cell by phagocytosis and replicates in the cytoplasm. *(b)* A TEM of pinocytosis in a smooth muscle cell. *(c)* A coated pit appears in the plasma membrane of a developing egg cell, covered with a layer of proteins. When an appropriate collection of molecules gathers in the coated pit, the pit deepens and will eventually seal off to form a vesicle.

Phagocytosis and pinocytosis

If the material the cell takes in is particulate (made up of discrete particles), such as an organism or some other fragment of organic matter (figure 5.15*a*), the process is called **phagocytosis** (Greek *phagein*, "to eat," + *cytos*, "cell"). If the material the cell takes in is liquid (figure 5.15*b*), the process is called **pinocytosis** (Greek *pinein*, "to drink"). Pinocytosis is common among animal cells. Mammalian egg cells, for example, "nurse" from surrounding cells; the nearby cells secrete nutrients that the maturing egg cell takes up by pinocytosis.

Virtually all eukaryotic cells constantly carry out these kinds of endocytotic processes, trapping particles and extracellular fluid in vesicles and ingesting them. Endocytosis rates vary from one cell type to another. They can be surprisingly high; some types of white blood cells ingest up to 25% of their cell volume each hour.

Receptor-mediated endocytosis

Molecules are often transported into eukaryotic cells through **receptor-mediated endocytosis.** These molecules first bind to specific receptors in the plasma membrane—they have a conformation that fits snugly into the receptor. Different cell types contain a characteristic battery of receptor types, each for a different kind of molecule in their membranes.

The portion of the receptor molecule that lies inside the membrane is trapped in an indented pit coated on the cytoplasmic side with the protein *clathrin*. Each pit acts like a molecular mousetrap, closing over to form an internal vesicle when the right molecule enters the pit (figure 5.15*c*). The trigger that releases the trap is the binding of the properly fitted target molecule to the embedded receptor. When binding occurs, the cell reacts by initiating endocytosis; the process is highly specific and very fast. The vesicle is now inside the cell carrying its cargo.

One type of molecule that is taken up by receptor-mediated endocytosis is low-density lipoprotein (LDL). LDL molecules bring cholesterol into the cell where it can be incorporated into membranes. Cholesterol plays a key role in determining the stiffness of the body's membranes. In the human genetic disease familial hypercholesterolemia, the LDL receptors lack tails, so they are never fastened in the clathrin-coated pits and as a result, do not trigger vesicle formation. The cholesterol stays in the bloodstream of affected individuals, accumulating as plaques inside arteries and leading to heart attacks.

It is important to understand that endocytosis in itself does not bring substances directly into the cytoplasm of a cell. The material taken in is still separated from the cytoplasm by the membrane of the vesicle.

Material can leave the cell by exocytosis

The reverse of endocytosis is **exocytosis**, the discharge of material from vesicles at the cell surface (figure 5.16). In plant cells, exocytosis is an important means of exporting the materials needed to construct the cell wall through the plasma membrane. Among protists, contractile vacuole discharge is considered a form of exocytosis. In animal cells, exocytosis provides a mechanism for secreting many hormones, neurotransmitters, digestive enzymes, and other substances.

The mechanisms for transport across cell membranes are summarized in table 5.2.

Learning Outcomes Review 5.6

Large molecules and other bulky materials can enter a cell by endocytosis and leave the cell by exocytosis. These processes require energy. Endocytosis may be mediated by specific receptor proteins in the membrane that trigger the formation of vesicles.

- ***What feature unites transport by receptor-mediated endocytosis, transport by a carrier, and catalysis by an enzyme?***

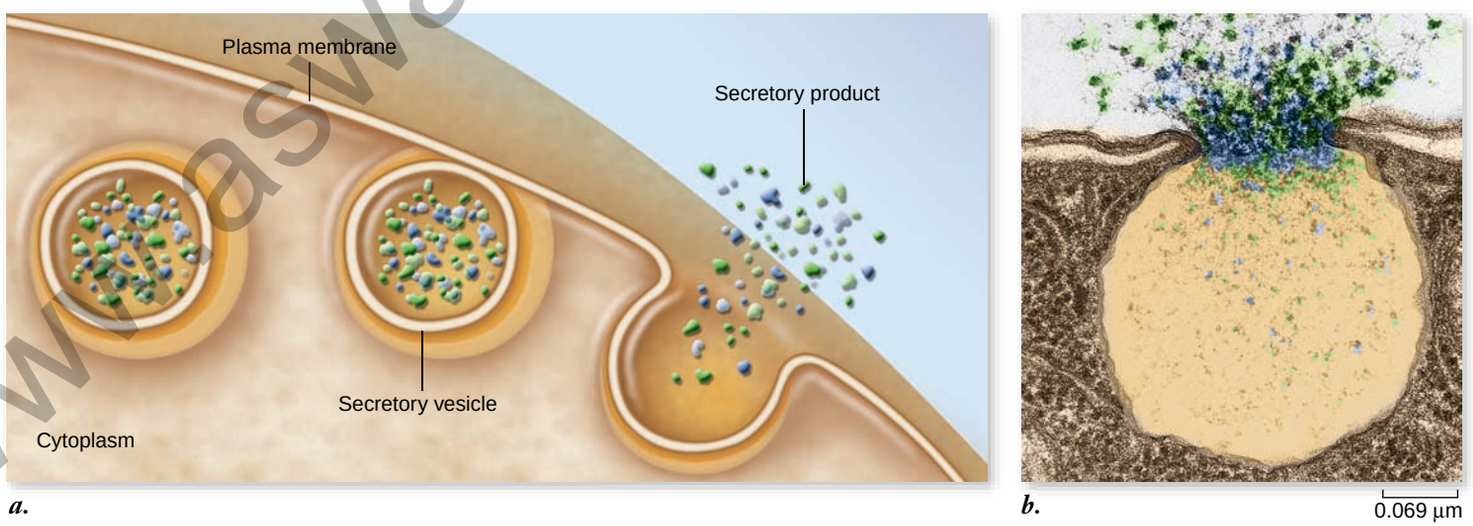
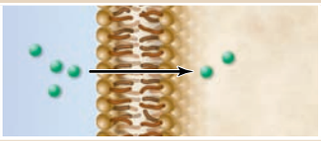
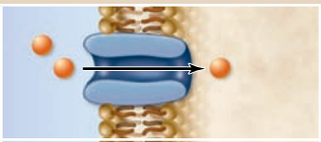
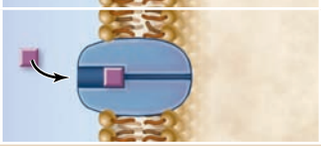
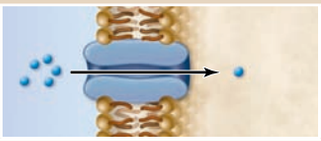
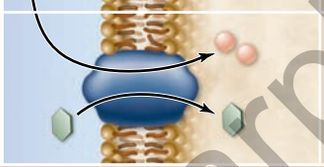
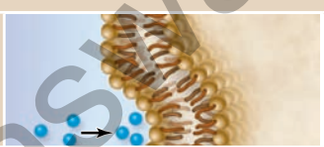
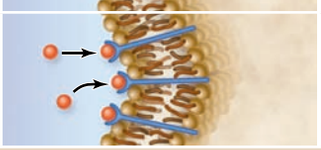
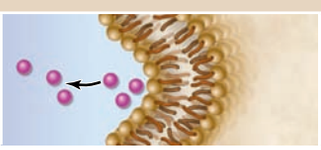
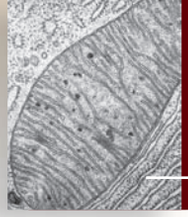


Figure 5.16 Exocytosis. ***a.*** Proteins and other molecules are secreted from cells in small packets called vesicles, whose membranes fuse with the plasma membrane, releasing their contents outside the cell. ***b.*** A false-colored transmission electron micrograph showing exocytosis.

TABLE 5.2 Mechanisms for Transport Across Cell Membranes

Process		How It Works	Example
PASSIVE PROCESSES			
Diffusion			
Direct		Random molecular motion produces net migration of nonpolar molecules toward region of lower concentration	Movement of oxygen into cells
Facilitated Diffusion			
Protein channel		Polar molecules or ions move through a protein channel; net movement is toward region of lower concentration	Movement of ions in or out of cell
Protein carrier		Molecule binds to carrier protein in membrane and is transported across; net movement is toward region of lower concentration	Movement of glucose into cells
Osmosis			
Aquaporins		Diffusion of water across the membrane via osmosis; requires osmotic gradient	Movement of water into cells placed in a hypotonic solution
ACTIVE PROCESSES			
Active Transport			
Protein carrier			
Na^+/K^+ pump		Carrier uses energy to move a substance across a membrane against its concentration gradient	Na^+ and K^+ against their concentration gradients
Coupled transport		Molecules are transported across a membrane against their concentration gradients by the cotransport of sodium ions or protons down their concentration gradients	Coupled uptake of glucose into cells against its concentration gradient using a Na^+ gradient
Endocytosis			
Membrane vesicle			
Phagocytosis		Particle is engulfed by membrane, which folds around it and forms a vesicle	Ingestion of bacteria by white blood cells
Pinocytosis		Fluid droplets are engulfed by membrane, which forms vesicles around them	"Nursing" of human egg cells
Receptor-mediated endocytosis		Endocytosis triggered by a specific receptor, forming clathrin-coated vesicles	Cholesterol uptake
Exocytosis			
Membrane vesicle		Vesicles fuse with plasma membrane and eject contents	Secretion of mucus; release of neurotransmitters



Chapter Review

5.1 The Structure of Membranes

The fluid mosaic model shows proteins embedded in a fluid lipid bilayer.

Membranes are sheets of phospholipid bilayers with associated proteins (figure 5.2). Hydrophobic regions of a membrane are oriented inward and hydrophilic regions oriented outward. In the fluid mosaic model, proteins float on or in the lipid bilayer.

Cellular membranes consist of four component groups.

In eukaryotic cells, membranes have four components: a phosopholipid bilayer, transmembrane proteins (integral membrane proteins), an interior protein network, and cell-surface markers. The interior protein network is composed of cytoskeletal filaments and peripheral membrane proteins, which are associated with the membrane but are not an integral part. Membranes contain glycoproteins and glycolipids on the surface that act as cell identity markers.

Electron microscopy has provided structural evidence.

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have confirmed the structure predicted by the fluid mosaic model.

5.2 Phospholipids: The Membrane's Foundation

Phospholipids are composed of two fatty acids and a phosphate group linked to a three-carbon glycerol molecule.

Phospholipids spontaneously form bilayers.

The phosphate group of a phospholipid is polar and hydrophilic; the fatty acids are nonpolar and hydrophobic, and they orient away from the polar head of the phospholipids. The nonpolar interior of the lipid bilayer impedes the passage of water and water-soluble substances.

The phospholipid bilayer is fluid.

Hydrogen bonding of water keeps the membrane in its bilayer configuration; however, phospholipids and unanchored proteins in the membrane are loosely associated and can diffuse laterally.

Membrane fluidity can change.

Membrane fluidity depends on the fatty acid composition of the membrane. Unsaturated fats tend to make the membrane more fluid because of the "kinks" of double bonds in the fatty acid tails. Temperature also affects fluidity. Some bacteria have enzymes that alter the fatty acids of the membrane to compensate for temperature changes.

5.3 Proteins: Multifunctional Components

Proteins and protein complexes perform key functions.

Transporters are integral membrane proteins that carry specific substances through the membrane. Enzymes often occur on the interior surface of the membrane. Cell-surface receptors respond to external chemical messages and change conditions inside the cell; cell identity markers on the surface allow recognition of the body's cells as "self." Cell-to-cell adhesion proteins glue cells together; surface proteins that interact with other cells anchor to the cytoskeleton.

Structural features of membrane proteins relate to function.

Surface proteins are attached to the surface by nonpolar regions that associate with polar regions of phospholipids. Transmembrane proteins may cross the bilayer a number of times, and each membrane-spanning region is called a transmembrane domain. Such a domain is composed of hydrophobic amino acids usually arranged in α-helices. In certain proteins, β-pleated sheets in the nonpolar region form a pipelike passageway having a polar environment. An example is the porin class of proteins.

5.4 Passive Transport Across Membranes

Transport can occur by simple diffusion.

Simple diffusion is the passive movement of a substance along a chemical or electrical gradient. Biological membranes pose a barrier to hydrophilic polar molecules, while they allow hydrophobic substances to diffuse freely.

Proteins allow membrane diffusion to be selective.

Ions and large hydrophilic molecules cannot cross the phospholipid bilayer. Diffusion can still occur with the help of proteins, thus we call this facilitated diffusion. These proteins can be either channels, or carriers. Channels allow the diffusion of ions based on concentration and charge across the membrane. They are specific for different ions, but form an aqueous pore in the membrane. Carrier proteins bind to the molecules they transport, much like an enzyme. The rate of transport by a carrier is limited by the number of carriers in the membrane.

Osmosis is the movement of water across membranes.

The direction of movement due to osmosis depends on the solute concentration on either side of the membrane (figure 5.12). Solutions can be isotonic, hypotonic, or hypertonic. Cells in an isotonic solution are in osmotic balance; cells in a hypotonic solution will gain water; and cells in a hypertonic solution will lose water. Aquaporins are water channels that facilitate the diffusion of water.

5.5 Active Transport Across Membranes

Active transport uses energy to move materials against a concentration gradient.

Active transport uses specialized protein carriers that couple a source of energy to transport. They are classified based on the number of molecules and direction of transport. Uniporters transport a specific molecule in one direction; symporters transport two molecules in the same direction; and antiporters transport two molecules in opposite directions.

The sodium–potassium pump runs directly on ATP.

The sodium–potassium pump moves Na^+ out of the cell and K^+ into the cell against their concentration gradients using ATP. In every cycle of the pump, three Na^+ leave the cell and two K^+ enter it. This pump appears to be almost universal in animal cells.

Coupled transport uses ATP indirectly.

Coupled transport occurs when the energy released by a diffusing molecule is used to transport a different molecule against its concentration gradient in the same direction. Countertransport is similar to coupled transport, but the two molecules move in opposite directions.

5.6 Bulk Transport by Endocytosis and Exocytosis

Bulk transport moves large quantities of substances that cannot pass through the cell membrane.

Bulk material enters the cell in vesicles.

In endocytosis, the cell membrane surrounds material and pinches off to form a vesicle. In receptor-mediated endocytosis, specific molecules bind to receptors on the cell membrane.

Material can leave the cell by exocytosis.

In exocytosis, material in a vesicle is discharged when the vesicle fuses with the membrane.

Review Questions

UNDERSTAND

1. The fluid mosaic model of the membrane describes the membrane as
   a. containing a significant quantity of water in the interior.
   b. composed of fluid phospholipids on the outside and protein on the inside.
   c. composed of protein on the outside and fluid phospholipids on the inside.
   d. made of proteins and lipids that can freely move.
2. What chemical property characterizes the interior of the phospholipid bilayer?
   a. It is hydrophobic.
   b. It is hydrophilic.
   c. It is polar.
   d. It is saturated.
3. The transmembrane domain of an integral membrane protein
   a. is composed of hydrophobic amino acids.
   b. often forms an α-helical structure.
   c. can cross the membrane multiple times.
   d. is all of the above.
4. The specific function of a membrane within a cell is determined by the
   a. degree of saturation of the fatty acids within the phospholipid bilayer.
   b. location of the membrane within the cell.
   c. presence of lipid rafts and cholesterol.
   d. type and number of membrane proteins.
5. The movement of water across a membrane is dependent on
   a. the solvent concentration.
   b. the solute concentration.
   c. the presence of carrier proteins.
   d. membrane potential.
6. If a cell is in an isotonic environment, then
   a. the cell will gain water and burst.
   b. no water will move across the membrane.
   c. the cell will lose water and shrink.
   d. osmosis still occurs, but there is no net gain or loss of cell volume.
7. Which of the following is NOT a mechanism for bringing material into a cell?
   a. Exocytosis
   b. Endocytosis
   c. Pinocytosis
   d. Phagocytosis

APPLY

1. A bacterial cell that can alter the composition of saturated and unsaturated fatty acids in its membrane lipids is adapted to a cold environment. If this cell is shifted to a warmer environment, it will react by
   a. increasing the amount of cholesterol in its membrane.
   b. altering the amount of protein present in the membrane.
   c. increasing the degree of saturated fatty acids in its membrane.
   d. increasing the percentage of unsaturated fatty acids in its membrane.
2. What variable(s) influence(s) whether a nonpolar molecule can move across a membrane by passive diffusion?
   a. The structure of the phospholipids bilayer
   b. The difference in concentration of the molecule across the membrane
   c. The presence of transport proteins in the membrane
   d. All of the above
3. Which of the following does NOT contribute to the selective permeability of a biological membrane?
   a. Specificity of the carrier proteins in the membrane
   b. Selectivity of channel proteins in the membrane
   c. Hydrophobic barrier of the phospholipid bilayer
   d. Hydrogen bond formation between water and phosphate groups
4. How are *active* transport and *coupled* transport related?
   a. They both use ATP to move molecules.
   b. Active transport establishes a concentration gradient, but coupled transport doesn't.
   c. Coupled transport uses the concentration gradient established by active transport.
   d. Active transport moves one molecule, but coupled transport moves two.
5. A cell can use the process of facilitated diffusion to
   a. concentrate a molecule such as glucose inside a cell.
   b. remove all of a toxic molecule from a cell.
   c. move ions or large polar molecules across the membrane regardless of concentration.
   d. move ions or large polar molecules from a region of high concentration to a region of low concentration.

SYNTHESIZE

1. Figure 5.4 describes a classic experiment demonstrating the ability of proteins to move within the plane of the cell's plasma membrane. The following table outlines three different experiments using the fusion of labeled mouse and human cells.

Experiment	Conditions	Temperature (°C)	Result
1	Fuse human and mouse cells	37	Intermixed membrane proteins
2	Fuse human and mouse cells in presence of ATP inhibitors	37	Intermixed membrane proteins
3	Fuse human and mouse cells	4	No intermixing of membrane proteins

   What conclusions can you reach about the movement of these proteins?
2. Each compartment of the endomembrane system of a cell is connected to the plasma membrane. Create a simple diagram of a cell including the RER, Golgi apparatus, vesicle, and the plasma membrane. Starting with the RER, use two different colors to represent the inner and outer halves of the bilayer for each of these membranes. What do you observe?
3. The distribution of lipids in the ER membrane is symmetric, that is, it is the same in both leaflets of the membrane. The Golgi apparatus and plasma membrane do not have symmetric distribution of membrane lipids. What kinds of processes could achieve this outcome?

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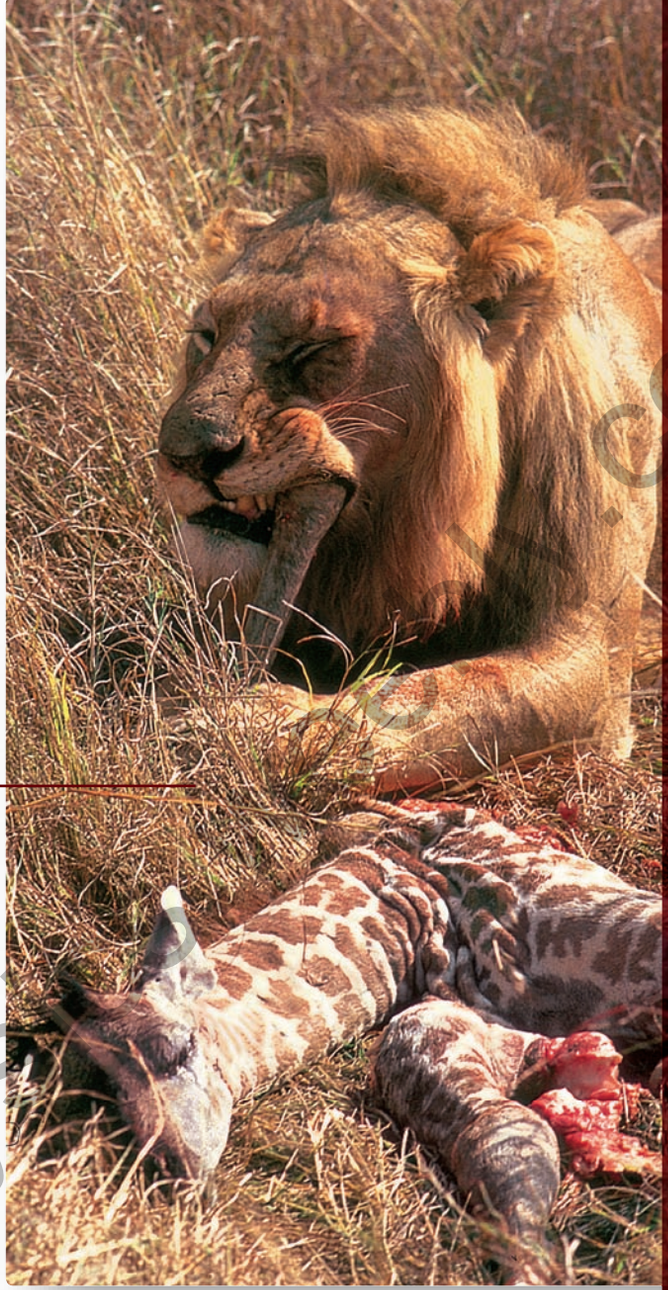
Chapter 6

Energy and Metabolism



Chapter Outline

Introduction

Life can be viewed as a constant flow of energy, channeled by organisms to do the work of living. Each of the significant properties by which we define life—order, growth, reproduction, responsiveness, and internal regulation—requires a constant supply of energy. Both the lion and the giraffe need to eat to provide energy for a wide variety of cellular functions. Deprived of a source of energy, life stops. Therefore, a comprehensive study of life would be impossible without discussing bioenergetics, *the analysis of how energy powers the activities of living systems. In this chapter, we focus on energy—what it is and how it changes during chemical reactions.*

6.1 The Flow of Energy in Living Systems

Learning Outcomes

1. *Explain what energy is and describe its different forms.*
2. *Identify the source of energy for the biosphere.*
3. *Contrast oxidation and reduction reactions.*

Thermodynamics is the branch of chemistry concerned with energy changes. Cells are governed by the laws of physics and chemistry, so we must understand these laws in order to understand how cells function.

Energy can take many forms

Energy is defined as the capacity to do work. We think of energy as existing in two states: kinetic energy and potential energy (figure 6.1). **Kinetic energy** is the energy of motion. Moving objects perform work by causing other matter to move. **Potential energy** is stored energy. Objects that are not actively moving but have the capacity to do so possess potential energy. A boulder perched on a hilltop has gravitational potential energy. As it begins to roll downhill, some of its potential energy is converted into kinetic energy. Much of the work that living organisms carry out involves transforming potential energy into kinetic energy.

Energy can take many forms: mechanical energy, heat, sound, electric current, light, or radioactivity. Because it can exist in so many forms, energy can be measured in many ways. Heat is the most convenient way of measuring energy because all other forms of energy can be converted into heat. In fact, the term *thermodynamics* means "heat changes."

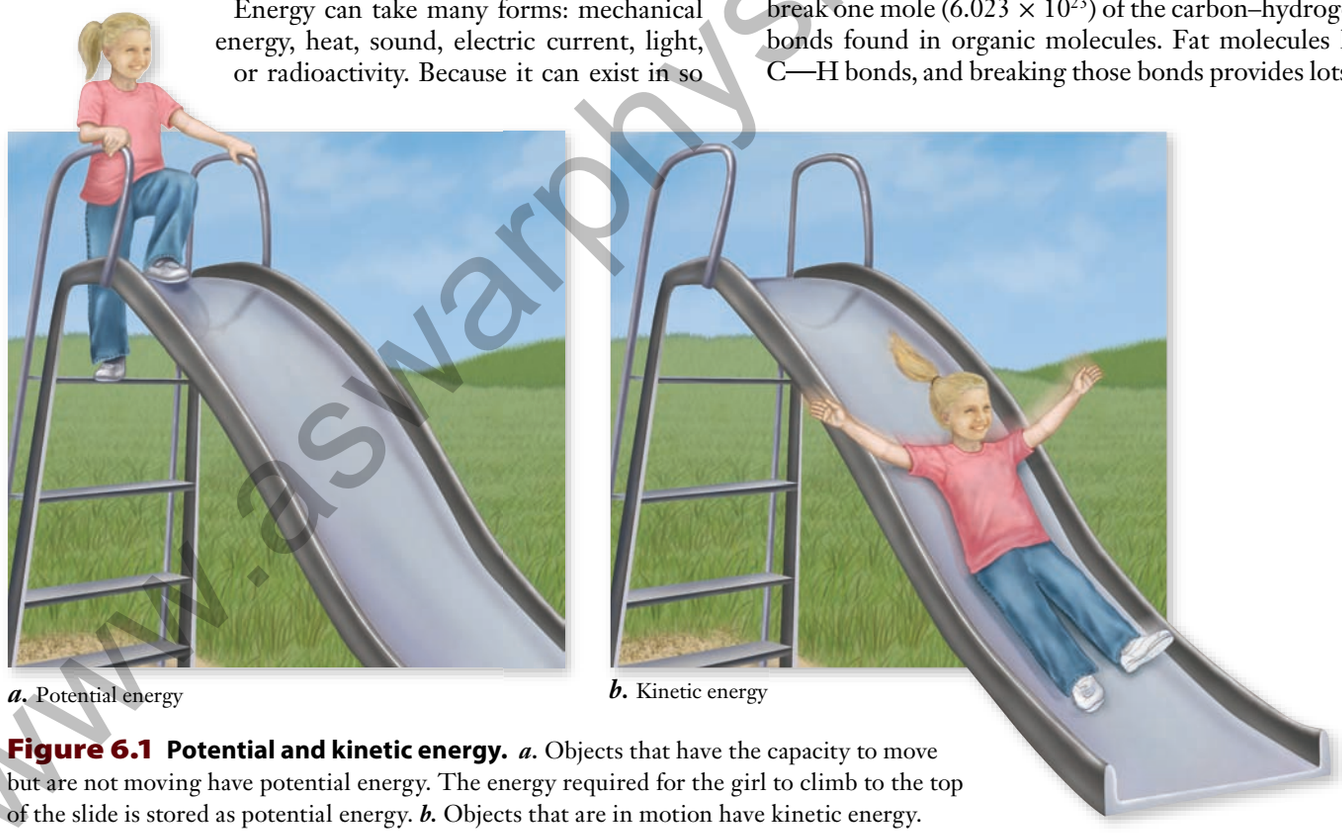
The unit of heat most commonly employed in biology is the kilocalorie (kcal). One kilocalorie is equal to 1000 calories (cal). One calorie is the heat required to raise the temperature of one gram of water one degree Celsius (°C). (You are probably more used to seeing the term *Calorie* with a capital C. This is used on food labels and is actually the same as kilocalorie.) Another energy unit, often used in physics, is the *joule;* one joule equals 0.239 cal.

The sun provides energy for living systems

Energy flows into the biological world from the Sun. It is estimated that the Sun provides the Earth with more than 13×10^{23} calories per year, or 40 million billion calories per second! Plants, algae, and certain kinds of bacteria capture a fraction of this energy through photosynthesis.

In photosynthesis, energy absorbed from sunlight is used to combine small molecules (water and carbon dioxide) into more complex ones (sugars). This process converts carbon from an inorganic to an organic form. In the process, energy from the Sun is stored as potential energy in the covalent bonds between atoms in the sugar molecules.

Breaking the bonds between atoms requires energy. In fact, the strength of a covalent bond is measured by the amount of energy required to break it. For example, it takes 98.8 kcal to break one mole (6.023×10^{23}) of the carbon–hydrogen (C—H) bonds found in organic molecules. Fat molecules have many C—H bonds, and breaking those bonds provides lots of energy.



a. Potential energy

b. Kinetic energy

Figure 6.1 Potential and kinetic energy. ***a.*** Objects that have the capacity to move but are not moving have potential energy. The energy required for the girl to climb to the top of the slide is stored as potential energy. ***b.*** Objects that are in motion have kinetic energy. The stored potential energy is released as kinetic energy as the girl slides down.

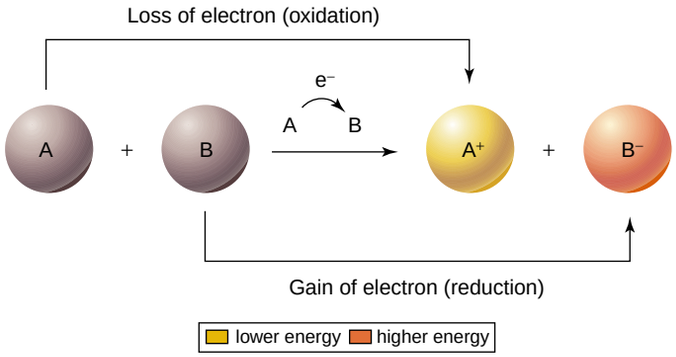


Figure 6.2 Redox reactions. Oxidation is the loss of an electron; reduction is the gain of an electron. In this example, the charges of molecules A and B appear as superscripts in each molecule. Molecule A loses energy as it loses an electron, and molecule B gains that energy as it gains an electron.

This is one reason animals store fat. The oxidation of one mole of a 16-carbon fatty acid that is completely saturated with hydrogens yields 2340 kcal.

Oxidation–reduction reactions transfer electrons while bonds are made or broken

During a chemical reaction, the energy stored in chemical bonds may be used to make new bonds. In some of these reactions, electrons actually pass from one atom or molecule to another. An atom or molecule that loses an electron is said to be oxidized, and the process by which this occurs is called **oxidation.** The name comes from the fact that oxygen is the most common electron acceptor in biological systems. Conversely, an atom or molecule that gains an electron is said to be reduced, and the process is called *reduction.* The reduced form of a molecule has a higher level of energy than the oxidized form (figure 6.2).

Oxidation and reduction always take place together, because every electron that is lost by one atom through oxidation is gained by another atom through reduction. Therefore, chemical reactions of this sort are called **oxidation–reduction,** or **redox,** *reactions.* Oxidation–reduction reactions play a key role in the flow of energy through biological systems.

In the next two chapters, you will learn the details of how organisms derive energy from the oxidation of organic compounds via respiration, as well as from the energy in sunlight via photosynthesis.

Learning Outcomes Review 6.1

Energy is defined as the capacity to do work. The two forms of energy are kinetic energy, or energy of motion, and potential energy, or stored energy. The ultimate source of energy for living systems is the Sun. Organisms derive their energy from oxidation–reduction reactions. In oxidation, a molecule loses an electron; in reduction, a molecule gains an electron.

- ***What energy source might ecosystems at the bottom of the ocean use?***

6.2 The Laws of Thermodynamics and Free Energy

Learning Outcomes

1. *Explain the laws of thermodynamics.*
2. *Recognize how free energy can be used to predict the outcome of chemical reactions.*
3. *Contrast the course of a reaction with and without an enzyme catalyst.*

All activities of living organisms—growing, running, thinking, singing, reading these words—involve changes in energy. A set of two universal laws we call the laws of thermodynamics govern all energy changes in the universe, from nuclear reactions to a bird flying through the air.

The First Law states that energy cannot be created or destroyed

The **First Law of Thermodynamics** concerns the amount of energy in the universe. Energy cannot be created or destroyed; it can only change from one form to another (from potential to kinetic, for example). The total amount of energy in the universe remains constant.

The lion eating a giraffe at the beginning of this chapter is acquiring energy. Rather than creating new energy or capturing the energy in sunlight, the lion is merely transferring some of the potential energy stored in the giraffe's tissues to its own body, just as the giraffe obtained the potential energy stored in the plants it ate while it was alive.

Within any living organism, chemical potential energy stored in some molecules can be shifted to other molecules and stored in different chemical bonds. It can also be converted into other forms, such as kinetic energy, light, or electricity. During each conversion, some of the energy dissipates into the environment as **heat,** which is a measure of the random motion of molecules (and therefore a measure of one form of kinetic energy). Energy continuously flows through the biological world in one direction, with new energy from the Sun constantly entering the system to replace the energy dissipated as heat.

Heat can be harnessed to do work only when there is a heat gradient—that is, a temperature difference between two areas. Cells are too small to maintain significant internal temperature differences, so heat energy is incapable of doing the work of cells. Instead, cells must rely on chemical reactions for energy.

Although the total amount of energy in the universe remains constant, the energy available to do work decreases as more of it is progressively lost as heat.

The Second Law states that some energy is lost as disorder increases

The **Second Law of Thermodynamics** concerns the transformation of potential energy into heat, or random molecular motion. It states that the disorder in the universe, more formally called **entropy,** is continuously increasing. Put simply, disorder is more likely than order. For example, it is much more likely that a column of bricks will tumble over than that a pile of bricks will arrange themselves spontaneously to form a column.

In general, energy transformations proceed spontaneously to convert matter from a more ordered, less stable form to a less ordered, but more stable form. For this reason, the second law is sometimes called "time's arrow." Looking at the photographs in figure 6.3, you could put the pictures into correct sequence using the information that time had elapsed with only natural processes occurring. Although it might be great if our rooms would straighten themselves up, we know from experience how much work it takes to do so.

The Second Law of Thermodynamics can also be stated simply as "entropy increases." When the universe formed, it held all the potential energy it will ever have. It has become progressively more disordered ever since, with every energy exchange increasing the amount of entropy.

Chemical reactions can be predicted based on changes in free energy

It takes energy to break the chemical bonds that hold the atoms in a molecule together. Heat energy, because it increases atomic motion, makes it easier for the atoms to pull apart. Both chemical bonding and heat have a significant influence on a molecule. Chemical bonding reduces disorder; heat increases it. The net effect, the amount of energy actually available to break and subsequently form other chemical bonds, is called the *free energy* of that molecule. In a more general sense, **free energy** is defined as the energy available to do work in any system.

For a molecule within a cell, where pressure and volume usually do not change, the free energy is denoted by the symbol G (for "Gibbs free energy"). G is equal to the energy contained in a molecule's chemical bonds (called **enthalpy** and designated H) together with the energy term (TS) related to the degree of disorder in the system, where S is the symbol for *entropy* and T is the absolute temperature expressed in the Kelvin scale (K = °C + 273):

$$G = H - TS$$

Chemical reactions break some bonds in the reactants and form new ones in the products. Consequently, reactions can produce changes in free energy. When a chemical reaction occurs under conditions of constant temperature, pressure, and volume—as do most biological reactions—the change symbolized by the Greek capital letter delta, Δ, in free energy (ΔG) is simply:

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

We can use the change in free energy, or ΔG, to predict whether a chemical reaction is spontaneous or not. For some reactions, the ΔG is positive, which means that the products of the reaction contain *more* free energy than the reactants; the bond energy (H) is higher, or the disorder (S) in the system is lower. Such reactions do not proceed spontaneously because they require an input of energy. Any reaction that requires an input of energy is said to be **endergonic** ("inward energy").

For other reactions, the ΔG is negative. In this case, the products of the reaction contain less free energy than the reactants; either the bond energy is lower, or the disorder is higher, or both. Such reactions tend to proceed spontaneously. These reactions release the excess free energy as heat and are thus said to be **exergonic** ("outward energy"). Any chemical reaction tends to proceed spontaneously if the difference in disorder ($T\Delta S$) is *greater* than the difference in bond energies between reactants and products (ΔH).

Note that *spontaneous* does not mean the same thing as *instantaneous*. A spontaneous reaction may proceed very slowly. Figure 6.4 sums up endergonic and exergonic reactions.

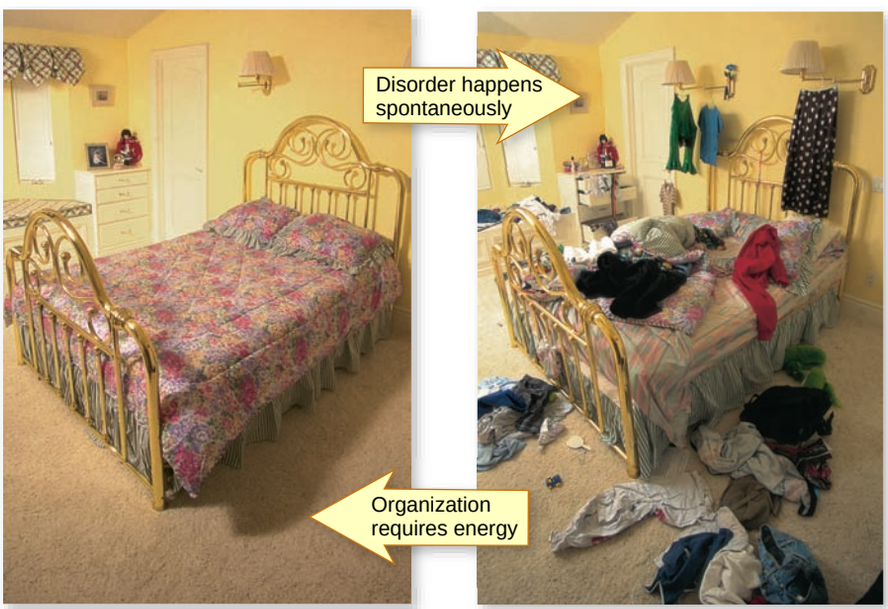
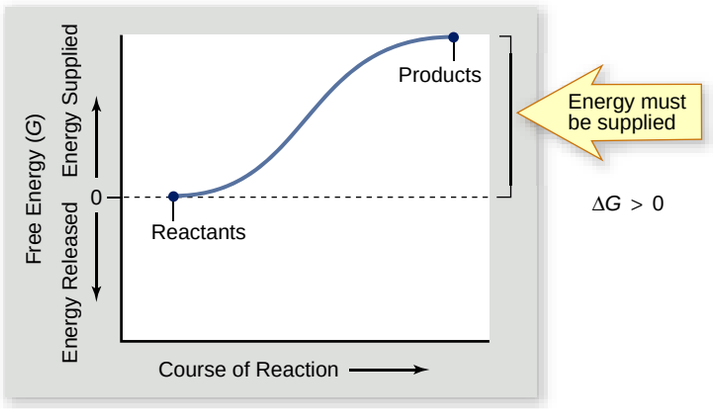
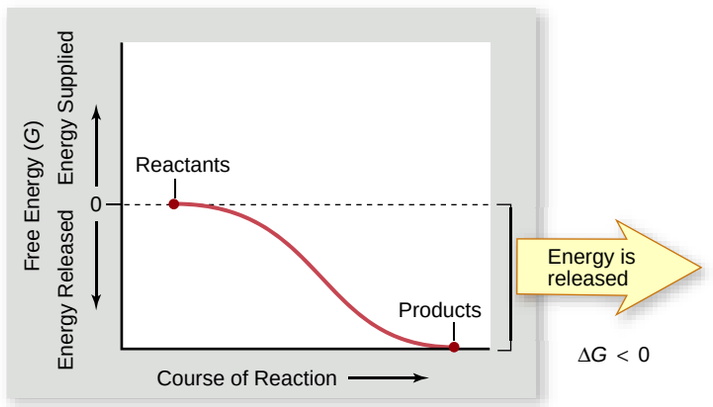


Figure 6.3 **Entropy in action.** As time elapses, the room shown at right becomes more disorganized. Entropy has increased in this room. It takes energy to restore it to the ordered state shown at left.



a.



b.

Figure 6.4 Energy in chemical reactions. ***a.*** In an endergonic reaction, the products of the reaction contain more energy than the reactants, and the extra energy must be supplied for the reaction to proceed. ***b.*** In an exergonic reaction, the products contain less energy than the reactants, and the excess energy is released.

Because chemical reactions are reversible, a reaction that is exergonic in the forward direction will be endergonic in the reverse direction. For each reaction, an equilibrium exists at some point between the relative amounts of reactants and products. This equilibrium has a numeric value and is called the *equilibrium constant*. This characteristic of reactions provides us with another way to think about free energy changes: an exergonic reaction has an equilibrium favoring the products, and an endergonic reaction has an equilibrium favoring the reactants.

Spontaneous chemical reactions require activation energy

If all chemical reactions that release free energy tend to occur spontaneously, why haven't all such reactions already occurred? Consider the gasoline tank of your car: The oxidation of the hydrocarbons in gasoline is an exergonic reaction, but your gas tank does not spontaneously explode. One reason is that most reactions require an input of energy to get started. In the case of your car, this input consists of the electrical sparks in the engine's cylinders, producing a controlled explosion.

Activation energy

Before new chemical bonds can form, even bonds that contain less energy, existing bonds must first be broken, and that requires energy input. The extra energy needed to destabilize existing chemical bonds and initiate a chemical reaction is called **activation energy** (figure 6.5).

The rate of an exergonic reaction depends on the activation energy required for the reaction to begin. Reactions with larger activation energies tend to proceed more slowly because fewer molecules succeed in getting over the initial energy hurdle. The rate of reactions can be increased in two ways: (1) by increasing the energy of reacting molecules or (2) by lowering activation energy. Chemists often drive important industrial reactions by increasing the energy of the reacting molecules, which is frequently accomplished simply by heating up the reactants. The other strategy is to use a catalyst to lower the activation energy.

How catalysts work

Activation energies are not constant. Stressing particular chemical bonds can make them easier to break. The process of influencing chemical bonds in a way that lowers the activation energy needed to initiate a reaction is called **catalysis,** and substances that accomplish this are known as *catalysts* (see figure 6.5).

Catalysts cannot violate the basic laws of thermodynamics; they cannot, for example, make an endergonic reaction proceed spontaneously. By reducing the activation energy, a catalyst accelerates both the forward and the reverse reactions by exactly the same amount. Therefore, a catalyst does not alter the proportion of reactant that is ultimately converted into product.

To understand this, imagine a bowling ball resting in a shallow depression on the side of a hill. Only a narrow rim of dirt below the ball prevents it from rolling down the hill. Now imagine digging away that rim of dirt. If you remove enough dirt from below the ball, it will start to roll down the hill—but

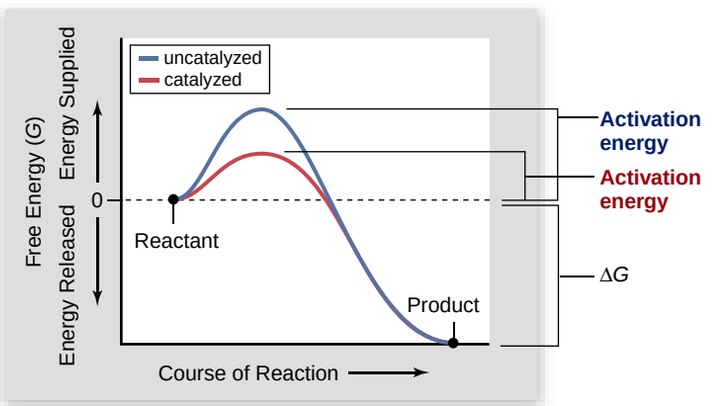


Figure 6.5 Activation energy and catalysis. Exergonic reactions do not necessarily proceed rapidly because activation energy must be supplied to destabilize existing chemical bonds. Catalysts accelerate particular reactions by lowering the amount of activation energy required to initiate the reaction. Catalysts do not alter the free-energy change produced by the reaction.

removing dirt from below the ball will *never* cause the ball to roll up the hill. Removing the lip of dirt simply allows the ball to move freely; gravity determines the direction it then travels.

Similarly, the direction in which a chemical reaction proceeds is determined solely by the difference in free energy between reactants and products. Like digging away the soil below the bowling ball on the hill, catalysts reduce the energy barrier that is preventing the reaction from proceeding. Only exergonic reactions can proceed spontaneously, and catalysts cannot change that. What catalysts *can* do is make a reaction proceed much faster. In living systems, enzymes act as catalysts.

Learning Outcomes Review 6.2

The First Law of Thermodynamics states that energy cannot be created or destroyed. The Second Law states that the loss of energy results in greater disorder, or entropy. Free-energy changes (ΔG) can predict whether chemical reactions take place. Reactions with a negative ΔG occur spontaneously, and those with a positive ΔG do not. Energy needed to initiate a reaction is termed activation energy. Catalysts, such as enzymes in living systems, lower this activation energy to speed up reactions.

- ***Can an enzyme make an endergonic reaction exergonic?***

6.3 ATP: The Energy Currency of Cells

Learning Outcomes

1. ***Describe the role of ATP in short-term energy storage.***
2. ***Explain what "high-energy" bonds are in ATP.***

The chief "currency" all cells use for their energy transactions is the nucleotide *adenosine triphosphate (ATP)*. ATP powers almost every energy-requiring process in cells, from making sugars, to supplying activation energy for chemical reactions, to actively transporting substances across membranes, to moving through the environment and growing.

Cells store and release energy in the bonds of ATP

You saw in chapter 3 that nucleotides serve as the building blocks for nucleic acids, but they play other cellular roles as well. ATP is used as a building block for RNA molecules, and it also has a critical function as a portable source of energy on demand for endergonic cellular processes.

The structure of ATP

Like all nucleotides, ATP is composed of three smaller components (figure 6.6). The first component is a five-carbon sugar, ribose, which serves as the framework to which the other two subunits are attached. The second component is adenine, an organic molecule composed of two carbon–nitrogen rings. Each of the nitrogen atoms in the ring has an unshared pair of electrons and weakly attracts hydrogen ions, making adenine chemically a weak base. The third component of ATP is a chain of three phosphates.

How ATP stores energy

The key to how ATP stores energy lies in its triphosphate group. Phosphate groups are highly negatively charged, and thus they strongly repel one another. This electrostatic repulsion makes the covalent bonds joining the phosphates unstable. The molecule is often referred to as a "coiled spring," with the phosphates straining away from one another.

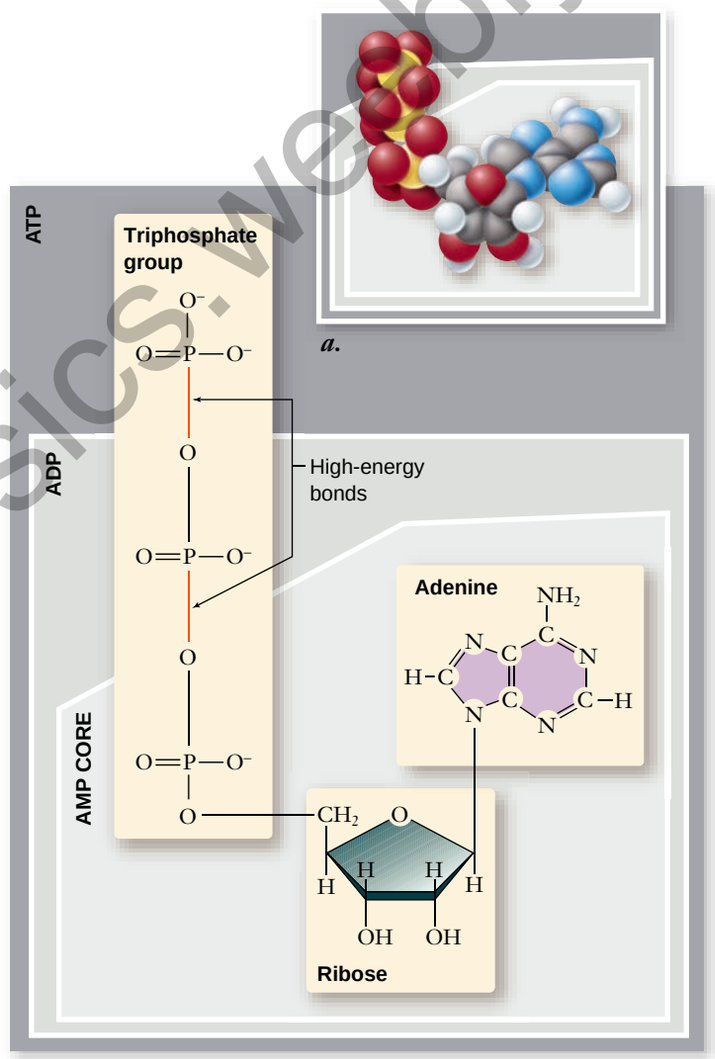


Figure 6.6 The ATP molecule. The model *(a)* and the structural diagram *(b)* both show that ATP has a core of AMP. Addition of one phosphate to AMP yields ADP, and addition of a second phosphate yields ATP. These two terminal phosphates are attached by high-energy bonds so that removing either by hydrolysis is an exergonic reaction that releases energy. ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate.

The unstable bonds holding the phosphates together in the ATP molecule have a low activation energy and are easily broken by hydrolysis. When they break, they can transfer a considerable amount of energy. In other words, the hydrolysis of ATP has a negative ΔG, and the energy it releases can be used to perform work.

In most reactions involving ATP, only the outermost high-energy phosphate bond is hydrolyzed, cleaving off the phosphate group on the end. When this happens, ATP becomes *adenosine diphosphate (ADP)* plus an **inorganic phosphate (P_i),** and energy equal to 7.3 kcal/mol is released under standard conditions. The liberated phosphate group usually attaches temporarily to some intermediate molecule. When that molecule is dephosphorylated, the phosphate group is released as P_i.

Both of the two terminal phosphates can be hydrolyzed to release energy, leaving *adenosine monophosphate (AMP)*, but the third phosphate is not attached by a high-energy bond. With only one phosphate group, AMP has no other phosphates to provide the electrostatic repulsion that makes the bonds holding the two terminal phosphate groups high-energy bonds.

ATP hydrolysis drives endergonic reactions

Cells use ATP to drive endergonic reactions. These reactions do not proceed spontaneously because their products possess more free energy than their reactants. However, if the cleavage of ATP's terminal high-energy bond releases more energy than the other reaction consumes, the two reactions can be coupled so that the energy released by the hydrolysis of ATP can be used to supply the endergonic reaction with the energy it needs. Coupled together, these reactions result in a net release of energy ($-\Delta G$) and are therefore exergonic and proceed spontaneously. Because almost all the endergonic reactions in cells require less energy than is released by the cleavage of ATP, ATP can provide most of the energy a cell needs.

Inquiry question

When ATP hydrolysis is coupled with an endergonic reaction and supplies more than enough energy, is the overall process endergonic or exergonic? Would the ΔG for the overall process be negative or positive?

ATP cycles continuously

The same feature that makes ATP an effective energy donor—the instability of its phosphate bonds—prevents it from being a good long-term energy-storage molecule. Fats and carbohydrates serve that function better.

The use of ATP can be thought of as a cycle: Cells use exergonic reactions to provide the energy needed to synthesize ATP from ADP + P_i; they then use the hydrolysis of ATP to provide energy to drive the endergonic reactions they need (figure 6.7).

Most cells do not maintain large stockpiles of ATP. Instead, they typically have only a few seconds' supply of ATP at any given time, and they continually produce more from ADP and P_i. It is estimated that even a sedentary individual turns over an amount of ATP in one day roughly equal to his body weight. This statistic makes clear the importance of ATP synthesis. In the next two chapters we will explore in detail the cellular mechanisms for synthesizing ATP.

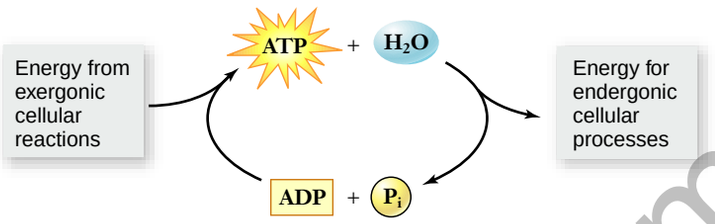


Figure 6.7 The ATP cycle. ATP is synthesized and hydrolyzed in a cyclic fashion. The synthesis of ATP from ADP + P_i is endergonic and is powered by exergonic cellular reactions. The hydrolysis of ATP to ADP + P_i is exergonic, and the energy released is used to power endergonic cellular functions such as muscle contraction. ADP, adenosine diphosphate; ATP, adenosine triphosphate; P_i, inorganic phosphate.

Learning Outcomes Review 6.3

ATP is a nucleotide with three phosphate groups. Endergonic cellular processes can be driven by coupling to the exergonic hydrolysis of the two terminal phosphates. The bonds holding the terminal phosphate groups together are easily broken, releasing energy like a coiled spring. The cell is constantly building ATP using exergonic reactions and breaking it down to drive endergonic reactions.

- ***If the molecular weight of ATP is 507.18 g/mol, and the ΔG for hydrolysis is –7.3 kcal/mol how much energy is released over the course of the day by a 100-kg man?***

6.4 Enzymes: Biological Catalysts

Learning Outcomes

1. *Discuss the specificity of enzymes.*
2. *Explain how enzymes bind to their substrates.*
3. *List the factors that influence the rate of enzyme-catalyzed reactions.*

The chemical reactions within living organisms are regulated by controlling the points at which catalysis takes place. Life itself, therefore, can be seen as regulated by catalysts. The agents that carry out most of the catalysis in living organisms are called enzymes. Most enzymes are proteins, although increasing evidence indicates that some enzymes are actually RNA molecules, as discussed later in this chapter.

An enzyme alters the activation energy of a reaction

The unique three-dimensional shape of an enzyme enables it to stabilize a temporary association between **substrates—**the molecules that will undergo the reaction. By bringing two substrates together in the correct orientation or by stressing particular chemical bonds of a substrate, an enzyme lowers the

activation energy required for new bonds to form. The reaction thus proceeds much more quickly than it would without the enzyme.

The enzyme itself is not changed or consumed in the reaction, so only a small amount of an enzyme is needed, and it can be used over and over.

As an example of how an enzyme works, let's consider the reaction of carbon dioxide and water to form carbonic acid. This important enzyme-catalyzed reaction occurs in vertebrate red blood cells:

$$\underset{\text{carbon dioxide}}{CO_2} + \underset{\text{water}}{H_2O} \rightleftharpoons \underset{\text{carbonic acid}}{H_2CO_3}$$

This reaction may proceed in either direction, but because it has a large activation energy, the reaction is very slow in the absence of an enzyme: Perhaps 200 molecules of carbonic acid form in an hour in a cell in the absence of any enzyme. Reactions that proceed this slowly are of little use to a cell. Vertebrate red blood cells overcome this problem by employing an enzyme within their cytoplasm called *carbonic anhydrase* (enzyme names usually end in "–ase"). Under the same conditions, but in the presence of carbonic anhydrase, an estimated 600,000 molecules of carbonic acid form every *second!* Thus, the enzyme increases the reaction rate by more than one million times.

Thousands of different kinds of enzymes are known, each catalyzing one or a few specific chemical reactions. By facilitating particular chemical reactions, the enzymes in a cell determine the course of metabolism—the collection of all chemical reactions—in that cell.

Different types of cells contain different sets of enzymes, and this difference contributes to structural and functional variations among cell types. For example, the chemical reactions taking place within a red blood cell differ from those that occur within a nerve cell, in part because different cell types contain different arrays of enzymes.

Active sites of enzymes conform to fit the shape of substrates

Most enzymes are globular proteins with one or more pockets or clefts, called **active sites**, on their surface (figure 6.8). Substrates bind to the enzyme at these active sites, forming an **enzyme–substrate complex** (figure 6.10). For catalysis to occur within the complex, a substrate molecule must fit precisely into an active site. When that happens, amino acid side groups of the enzyme end up very close to certain bonds of the substrate. These side groups interact chemically with the substrate, usually stressing or distorting a particular bond and consequently lowering the activation energy needed to break the bond. After the bonds of the substrates are broken, or new bonds are formed, the substrates have been converted to products. These products then dissociate from the enzyme, leaving the enzyme ready to bind its next substrate and begin the cycle again.

Proteins are not rigid. The binding of a substrate induces the enzyme to adjust its shape slightly, leading to a better *induced fit* between enzyme and substrate (see figure 6.9).

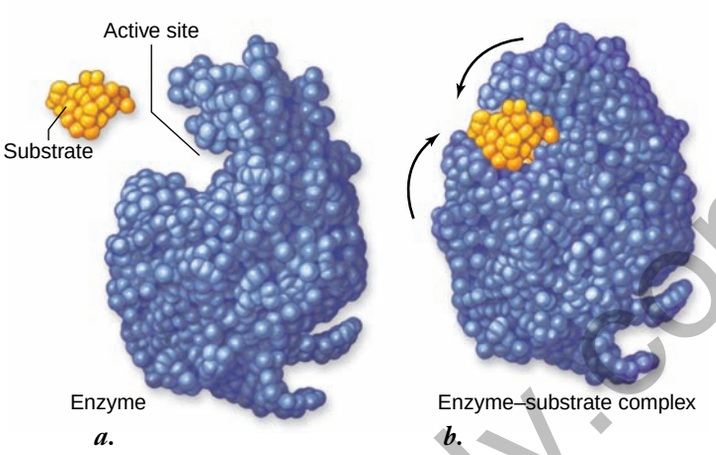


Figure 6.8 Enzyme binding its substrate. ***a.*** The active site of the enzyme lysozyme fits the shape of its substrate, a peptidoglycan that makes up bacterial cell walls. ***b.*** When the substrate, indicated in yellow, slides into the groove of the active site, the protein is induced to alter its shape slightly and bind the substrate more tightly. This alteration of the shape of the enzyme to better fit the substrate is called induced fit.

This interaction may also facilitate the binding of other substrates; in such cases, one substrate "activates" the enzyme to receive other substrates.

Enzymes occur in many forms

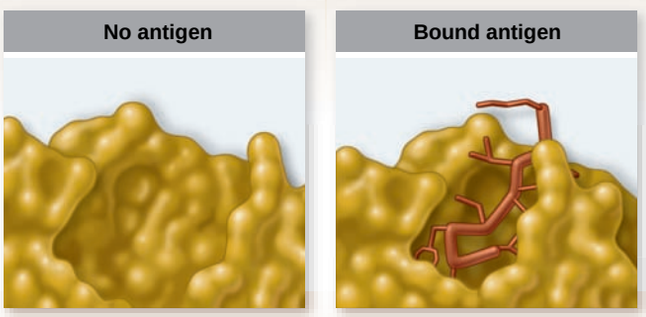
Although many enzymes are suspended in the cytoplasm of cells, not attached to any structure, other enzymes function as

SCIENTIFIC THINKING

Hypothesis: *Protein structure is flexible not rigid.*

Prediction: *Antibody–antigen binding can involve a change in protein structure.*

Test: *Determine crystal structure of a fragment of a specific antibody with no antigen bound, and with antigen bound for comparison.*



Result: *After binding, the antibody folds around the antigen forming a pocket.*

Conclusion: *In this case, binding involves an induced-fit kind of change in conformation.*

Further Experiments: *Why is this experiment easier to do with an antibody than with an enzyme? Can this experiment be done with an enzyme?*

Figure 6.9 Induced-fit binding of antibody to antigen.

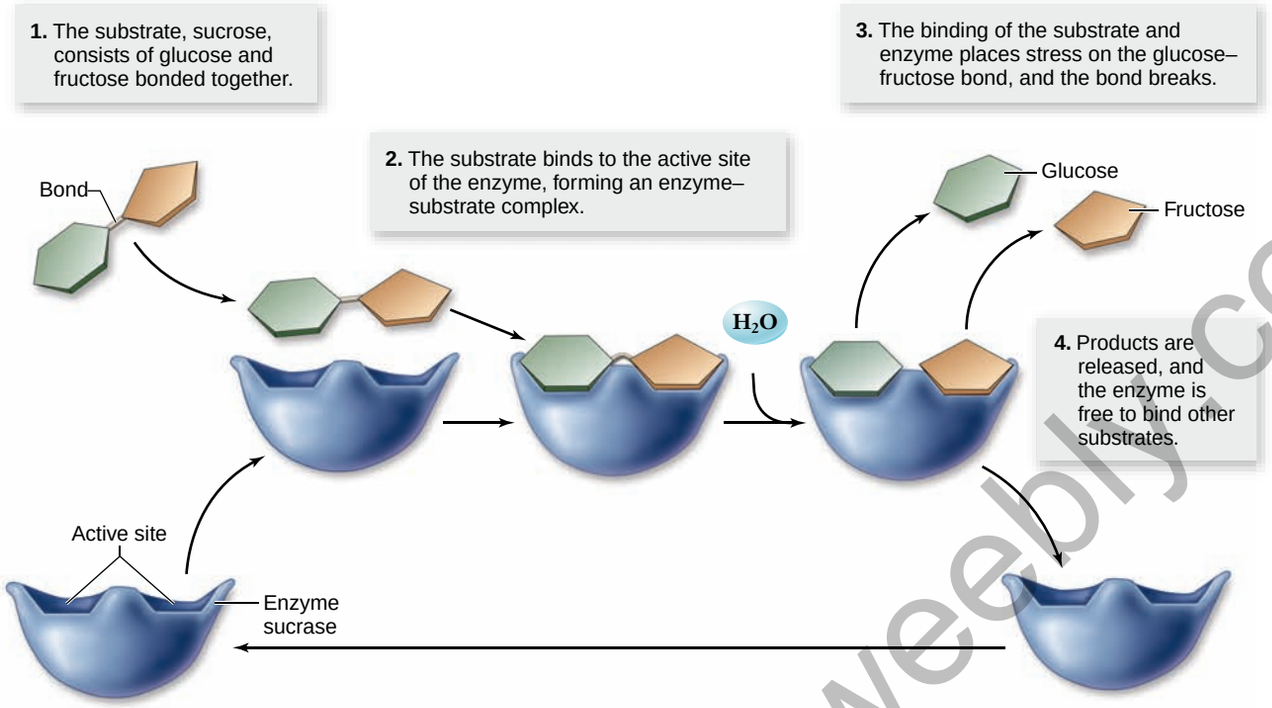


Figure 6.10 The catalytic cycle of an enzyme. Enzymes increase the speed at which chemical reactions occur, but they are not altered permanently themselves as they do so. In the reaction illustrated here, the enzyme sucrase is splitting the sugar sucrose into two simpler sugars: glucose and fructose.

integral parts of cell membranes and organelles. Enzymes may also form associations called *multienzyme complexes* to carry out reaction sequences. And, as mentioned earlier, evidence exists that some enzymes may consist of RNA rather than being only protein.

Multienzyme complexes

Often several enzymes catalyzing different steps of a sequence of reactions are associated with one another in noncovalently bonded assemblies called **multienzyme complexes.** The bacterial pyruvate dehydrogenase multienzyme complex, shown in figure 6.11, contains enzymes that carry out three sequential reactions in oxidative metabolism. Each complex has multiple copies of each of the three enzymes—60 protein subunits in all. The many subunits work together to form a molecular machine that performs multiple functions.

Multienzyme complexes offer the following significant advantages in catalytic efficiency:

1. The rate of any enzyme reaction is limited by how often the enzyme collides with its substrate. If a series of sequential reactions occurs within a multienzyme complex, the product of one reaction can be delivered to the next enzyme without releasing it to diffuse away.
2. Because the reacting substrate doesn't leave the complex while it goes through the series of reactions, unwanted side reactions are prevented.
3. All of the reactions that take place within the multienzyme complex can be controlled as a unit.

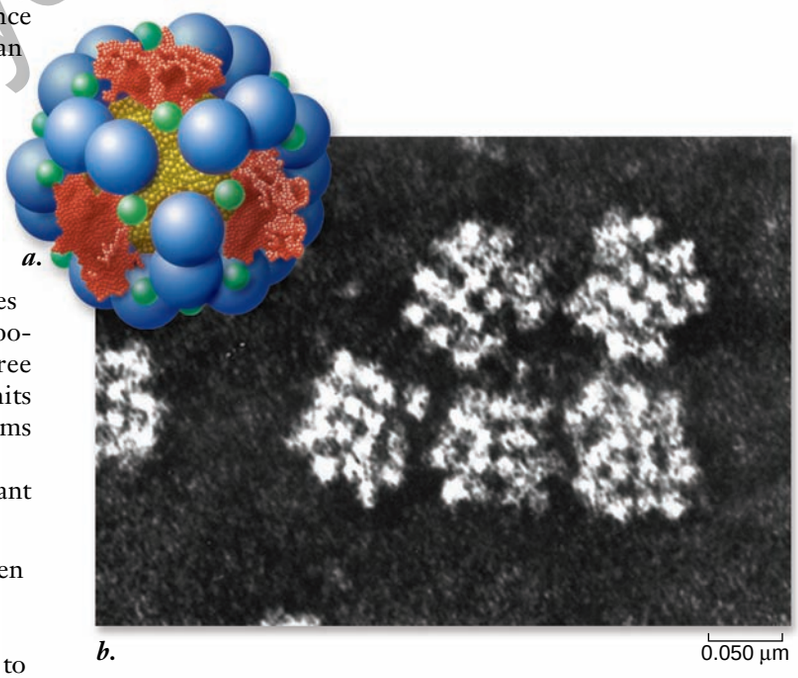


Figure 6.11 A complex enzyme: pyruvate dehydrogenase. Pyruvate dehydrogenase, which catalyzes the oxidation of pyruvate, is one of the most complex enzymes known. ***a.*** A model of the enzyme showing the arrangement of the 60 protein subunits. ***b.*** Many of the protein subunits are clearly visible in the electron micrograph.

In addition to pyruvate dehydrogenase, which controls entry to the Krebs cycle during aerobic respiration (see chapter 7), several other key processes in the cell are catalyzed by multienzyme complexes. One well-studied system is the fatty acid synthetase complex that catalyzes the synthesis of fatty acids from two-carbon precursors. Seven different enzymes make up this multienzyme complex, and the intermediate reaction products remain associated with the complex for the entire series of reactions.

Nonprotein enzymes

Until a few years ago, most biology textbooks contained statements such as "Proteins called enzymes are the catalysts of biological systems." We can no longer make that statement without qualification.

Thomas J. Cech and colleagues at the University of Colorado reported in 1981 that certain reactions involving RNA molecules appear to be catalyzed in cells by RNA itself, rather than by enzymes. This initial observation has been corroborated by additional examples of RNA catalysis. Like enzymes, these RNA catalysts, which are loosely called "ribozymes," greatly accelerate the rate of particular biochemical reactions and show extraordinary substrate specificity.

Research has revealed at least two sorts of ribozymes. Some ribozymes have folded structures and catalyze reactions on themselves, a process called *intra*molecular catalysis. Other ribozymes act on other molecules without being changed themselves, a process called *inter*molecular catalysis.

The most striking example of the role of RNA as enzyme is emerging from recent work on the structure and function of the ribosome. For many years it was thought that RNA was a structural framework for this vital organelle, but it is now clear that ribosomal RNA plays a key role in ribosome function. The ribosome itself is a ribozyme.

The ability of RNA, an informational molecule, to act as a catalyst has stirred great excitement because it seems to answer the question—Which came first, the protein or the nucleic acid? It now seems at least possible that RNA evolved first and may have catalyzed the formation of the first proteins.

Environmental and other factors affect enzyme function

The rate of an enzyme-catalyzed reaction is affected by the concentrations of both the substrate and the enzyme that works on it. In addition, any chemical or physical factor that alters the enzyme's three-dimensional shape—such as temperature, pH, and the binding of regulatory molecules—can affect the enzyme's ability to catalyze the reaction.

Temperature

Increasing the temperature of an uncatalyzed reaction increases its rate because the additional heat increases random molecular movement. This motion can add stress to molecular bonds and affect the activation energy of a reaction.

The rate of an enzyme-catalyzed reaction also increases with temperature, but only up to a point called the *optimum temperature* (figure 6.12*a*). Below this temperature, the hydrogen bonds and hydrophobic interactions that determine the enzyme's shape are not flexible enough to permit the induced fit that is optimum for catalysis. Above the optimum temperature, these forces are too weak to maintain the enzyme's shape against the increased random movement of the atoms in the enzyme. At higher temperatures, the enzyme denatures, as described in chapter 3.

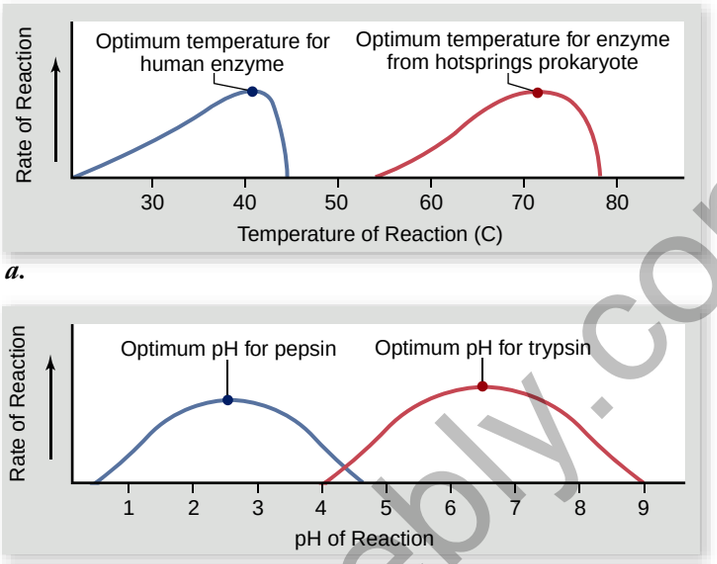


Figure 6.12 Enzyme sensitivity to the environment. The activity of an enzyme is influenced by both *(a)* temperature and *(b)* pH. Most human enzymes, such as the protein-degrading enzyme trypsin, work best at temperatures of about 40°C and within a pH range of 6 to 8. The hot springs prokaryote tolerates a higher environmental temperature and a correspondingly higher temperature optimum for enzymes. Pepsin works in the acidic environment of the stomach and has a lower optimum pH.

Most human enzymes have an optimum temperature between 35°C and 40°C—a range that includes normal body temperature. Prokaryotes that live in hot springs have more stable enzymes (that is, enzymes held together more strongly), so the optimum temperature for those enzymes can be 70°C or higher. In each case the optimal temperature for the enzyme corresponds to the "normal" temperature usually encountered in the body or the environment, depending on the type of organism.

pH

Ionic interactions between oppositely charged amino acid residues, such as glutamic acid (–) and lysine (+), also hold enzymes together. These interactions are sensitive to the hydrogen ion concentration of the fluid in which the enzyme is dissolved, because changing that concentration shifts the balance between positively and negatively charged amino acid residues. For this reason, most enzymes have an *optimum pH* that usually ranges from pH 6 to 8.

Enzymes able to function in very acidic environments are proteins that maintain their three-dimensional shape even in

the presence of high hydrogen ion concentrations. The enzyme pepsin, for example, digests proteins in the stomach at pH 2, a very acidic level (figure 6.12*b*).

Inhibitors and activators

Enzyme activity is also sensitive to the presence of specific substances that can bind to the enzyme and cause changes in its shape. Through these substances, a cell is able to regulate which of its enzymes are active and which are inactive at a particular time. This ability allows the cell to increase its efficiency and to control changes in its characteristics during development. A substance that binds to an enzyme and *decreases* its activity is called an **inhibitor.** Very often, the end product of a biochemical pathway acts as an inhibitor of an early reaction in the pathway, a process called *feedback inhibition* (discussed later in this chapter).

Enzyme inhibition occurs in two ways: **Competitive inhibitors** compete with the substrate for the same active site, occupying the active site and thus preventing substrates from binding; **noncompetitive inhibitors** bind to the enzyme in a location other than the active site, changing the shape of the enzyme and making it unable to bind to the substrate (figure 6.13).

Many enzymes can exist in either an active or inactive conformation; such enzymes are called *allosteric enzymes.* Most noncompetitive inhibitors bind to a specific portion of the enzyme called an **allosteric site.** These sites serve as chemical on/off switches; the binding of a substance to the site can switch the enzyme between its active and inactive configurations. A substance that binds to an allosteric site and reduces enzyme activity is called an **allosteric inhibitor** (figure 6.13*b*).

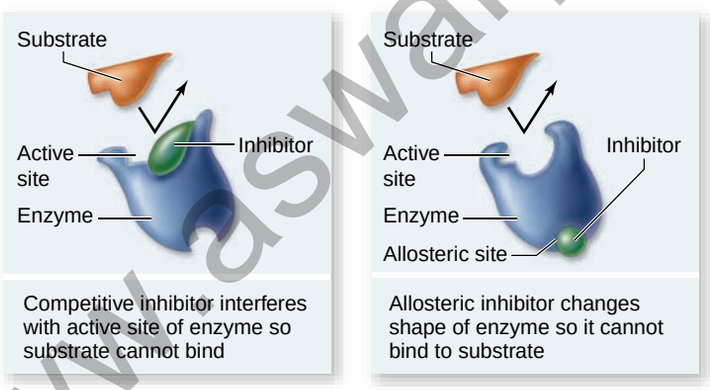
This kind of control is also used to activate enzymes. An **allosteric activator** binds to allosteric sites to keep an enzyme in its active configuration, thereby *increasing* enzyme activity.

Enzyme cofactors

Enzyme function is often assisted by additional chemical components known as **cofactors.** These can be metal ions that are often found in the active site participating directly in catalysis. For example, the metallic ion zinc is used by some enzymes, such as protein-digesting carboxypeptidase, to draw electrons away from their position in covalent bonds, making the bonds less stable and easier to break. Other metallic elements, such as molybdenum and manganese, are also used as cofactors. Like zinc, these substances are required in the diet in small amounts.

When the cofactor is a nonprotein organic molecule, it is called a **coenzyme.** Many of the small organic molecules essential in our diets that we call vitamins function as coenzymes. For example the B vitamins B_6 and B_{12}, both function as coenzymes for a number of different enzymes. Modified nucleotides are also used as coenzymes.

In numerous oxidation–reduction reactions that are catalyzed by enzymes, the electrons pass in pairs from the active site of the enzyme to a coenzyme that serves as the electron acceptor. The coenzyme then transfers the electrons to a different enzyme, which releases them (and the energy they bear) to the substrates in another reaction. Often, the electrons combine with protons (H^+) to form hydrogen atoms. In this way, coenzymes shuttle energy in the form of hydrogen atoms from one enzyme to another in a cell. The role of coenzymes and the specifics of their action will be explored in detail in the following two chapters.



a. Competitive inhibition ***b.*** Noncompetitive inhibition

Figure 6.13 How enzymes can be inhibited. ***a.*** In competitive inhibition, the inhibitor has a shape similar to the substrate and competes for the active site of the enzyme. ***b.*** In noncompetitive inhibition, the inhibitor binds to the enzyme at the allosteric site, a place away from the active site, effecting a conformational change in the enzyme, making it unable to bind to its substrate.

Learning Outcomes Review 6.4

Enzymes are biological catalysts that accelerate chemical reactions inside the cell. Enzymes bind to their substrates based on molecular shape, which allows them to be highly specific. Enzyme activity is affected by conditions such as temperature and pH and the presence of inhibitors or activators. Some enzymes also require an inorganic cofactor or an organic coenzyme.

- ***Why do proteins and RNA function as enzymes but DNA does not?***

6.5 *Metabolism: The Chemical Description of Cell Function*

Learning Outcomes

1. ***Explain the kinds of reactions that make up metabolism.***
2. ***Discuss what is meant by a metabolic pathway.***
3. ***Recognize that metabolism is a product of evolution.***

Living chemistry, the total of all chemical reactions carried out by an organism, is called **metabolism.** Those chemical reactions that expend energy to build up molecules are called *anabolic* reactions, or **anabolism.** Reactions that harvest energy by breaking down molecules are called *catabolic* reactions, or **catabolism.** This section presents a general overview of metabolic processes that will be described in much greater detail in later chapters.

Biochemical pathways organize chemical reactions in cells

Organisms contain thousands of different kinds of enzymes that catalyze a bewildering variety of reactions. Many of these reactions in a cell occur in sequences called **biochemical pathways.** In such pathways, the product of one reaction becomes the substrate for the next (figure 6.14). Biochemical pathways are the organizational units of metabolism—the elements an organism controls to achieve coherent metabolic activity.

Many sequential enzyme steps in biochemical pathways take place in specific compartments of the cell; for example, the steps of the Krebs cycle (see chapter 7) occur in the matrix inside mitochondria in eukaryotes. By determining where many of the enzymes that catalyze these steps are located, we can "map out" a model of metabolic processes in the cell.

Biochemical pathways may have evolved in stepwise fashion

In the earliest cells, the first biochemical processes probably involved energy-rich molecules scavenged from the environment. Most of the molecules necessary for these processes are thought to have existed independently in the "organic soup" of the early oceans.

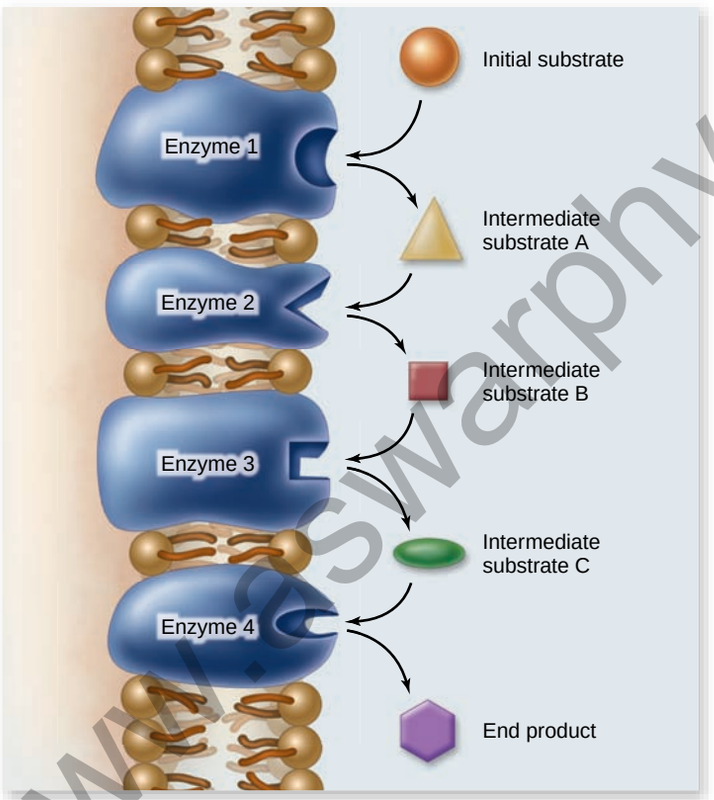


Figure 6.14 A biochemical pathway. The original substrate is acted on by enzyme 1, changing the substrate to a new intermediate, substrate A, recognized as a substrate by enzyme 2. Each enzyme in the pathway acts on the product of the previous stage. These enzymes may be either soluble or arranged in a membrane as shown.

The first catalyzed reactions were probably simple, one-step reactions that brought these molecules together in various combinations. Eventually, the energy-rich molecules became depleted in the external environment, and only organisms that had evolved some means of making those molecules from other substances could survive. Thus, a hypothetical reaction,

$$\begin{matrix} F \\ + \\ G \end{matrix} \longrightarrow H$$

where two energy-rich molecules (F and G) react to produce compound H and release energy, became more complex when the supply of F in the environment ran out.

A new reaction was added in which the depleted molecule, F, is made from another molecule, E, which was also present in the environment:

$$E \longrightarrow \begin{matrix} F \\ + \\ G \end{matrix} \longrightarrow H$$

When the supply of E was in turn exhausted, organisms that were able to make E from some other available precursor, D, survived. When D was depleted, those organisms in turn were replaced by ones able to synthesize D from another molecule, C:

$$C \longrightarrow D \longrightarrow E \longrightarrow \begin{matrix} F \\ + \\ G \end{matrix} \longrightarrow H$$

This hypothetical biochemical pathway would have evolved slowly through time, with the final reactions in the pathway evolving first and earlier reactions evolving later.

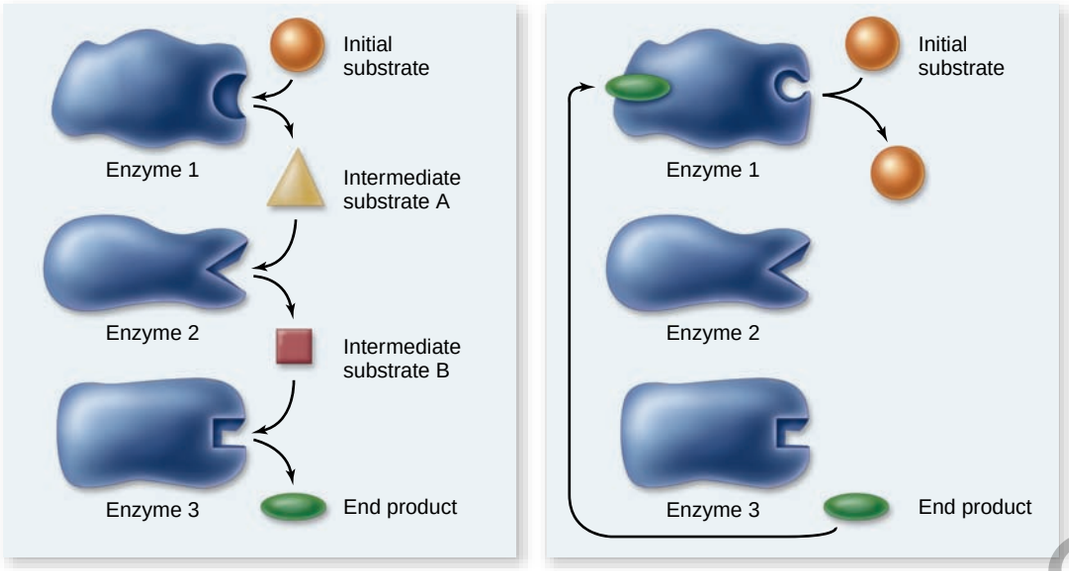
Looking at the pathway now, we would say that the "advanced" organism, starting with compound C, is able to synthesize H by means of a series of steps. This is how the biochemical pathways within organisms are thought to have evolved—not all at once, but one step at a time, backwards.

Feedback inhibition regulates some biochemical pathways

For a biochemical pathway to operate efficiently, its activity must be coordinated and regulated by the cell. Not only is it unnecessary to synthesize a compound when plenty is already present, but doing so would waste energy and raw materials that could be put to use elsewhere. It is to the cell's advantage, therefore, to temporarily shut down biochemical pathways when their products are not needed.

The regulation of simple biochemical pathways often depends on an elegant feedback mechanism: The end-product of the pathway binds to an allosteric site on the enzyme that catalyzes the first reaction in the pathway. This mode of regulation is called **feedback inhibition** (figure 6.15).

In the hypothetical pathway we just described, the enzyme catalyzing the reaction C $\longrightarrow$ D would possess an allosteric site for H, the end-product of the pathway. As the pathway churned out its product and the amount of H in the cell increased, it would become more likely that an H molecule would encounter



a. *b.*

Figure 6.15 Feedback inhibition. ***a.*** A biochemical pathway with no feedback inhibition. ***b.*** A biochemical pathway in which the final end-product becomes the allosteric inhibitor for the first enzyme in the pathway. In other words, the formation of the pathway's final end-product stops the pathway. The pathway could be the synthesis of an amino acid, a nucleotide, or another important cellular molecule.

the allosteric site on the C ⟶ D enzyme. Binding to the allosteric site would essentially shut down the reaction C ⟶ D and in turn effectively shut down the whole pathway.

In this chapter we have reviewed the basics of energy and its transformations as carried out in living systems. Chemical bonds are the primary location of energy storage and release, and cells have developed elegant methods of making and breaking chemical bonds to create the molecules they need. Enzymes facilitate these reactions by serving as catalysts. In the following chapters you will learn the details of the mechanisms by which organisms harvest, store, and utilize energy.

Learning Outcomes Review 6.5

Metabolism is the sum of all chemical reactions in a cell. Anabolic reactions use energy to build up molecules. Catabolic reactions release energy by breaking down molecules. In a metabolic pathway, the end-product of one reaction is the substrate for the next reaction. Evolution may have favored organisms that could use precursor molecules to synthesize a nutrient. Over time, more reactions would be linked together as novel enzymes arose by mutation.

- ***Is a catabolic pathway likely to be subject to feedback inhibition?***

Chapter Review

6.1 The Flow of Energy in Living Systems

Thermodynamics is the study of energy changes.

Energy can take many forms.

Energy is the capacity to do work. Potential energy is stored energy, and kinetic energy is the energy of motion. Energy can take many forms: mechanical, heat, sound, electric current, light, or radioactive radiation. Energy is measured in units of heat known as kilocalories.

The Sun provides energy for living systems.

Photosynthesis stores light energy from the Sun as potential energy in the covalent bonds of sugar molecules. Breaking these bonds in living cells releases energy for use in other reactions.

Oxidation–reduction reactions transfer electrons while bonds are made or broken.

Oxidation is a reaction involving the loss of electrons. Reduction is the gain of electrons (see figure 6.2). These two reactions take place together and are therefore termed redox reactions.

6.2 The Laws of Thermodynamics and Free Energy

The First Law states that energy cannot be created or destroyed.

Virtually all activities of living organisms require energy. Energy changes form as it moves through organisms and their biochemical systems, but it is not created or destroyed.

The Second Law states that some energy is lost as disorder increases.

The disorder, or entropy, of the universe is continuously increasing. In an open system like the Earth, which is receiving energy from the Sun, this may not be the case. To increase order however, energy must be expended. In energy conversions, some energy is always lost as heat.

Chemical reactions can be predicted based on changes in free energy.

Free energy (G) is the energy available to do work in any system. Changes in free energy (ΔG) predict the direction of reactions. Reactions with a negative ΔG are spontaneous (exergonic) reactions, and reactions with a positive ΔG are not spontaneous (endergonic).

Endergonic chemical reactions absorb energy from the surroundings, whereas exergonic reactions release energy to the surroundings.

Spontaneous chemical reactions require activation energy.

Activation energy is the energy required to destabilize chemical bonds and initiate chemical reactions (see figure 6.5). Even exergonic reactions require this activation energy. Catalysts speed up chemical reactions by lowering the activation energy.

6.3 ATP: The Energy Currency of Cells

Adenosine triphosphate (ATP) is the molecular currency used for cellular energy transactions.

Cells store and release energy in the bonds of ATP.

The energy of ATP is stored in the bonds between its terminal phosphate groups. These groups repel each other due to their negative charge and therefore the covalent bonds joining these phosphates are unstable.

ATP hydrolysis drives endergonic reactions.

Enzymes hydrolyze the terminal phosphate group of ATP to release energy for reactions. If ATP hydrolysis is coupled to an endergonic reaction with a positive ΔG with magnitude less than that for ATP hydrolysis, the two reactions together will be exergonic.

ATP cycles continuously.

ATP hydrolysis releases energy to drive endergonic reactions, and it is synthesized with energy from exergonic reactions (see figure 6.7).

6.4 Enzymes: Biological Catalysts

An enzyme alters the activation energy of a reaction.

Enzymes lower the activation energy needed to initiate a chemical reaction.

Active sites of enzymes conform to fit the shape of substrates.

Substrates bind to the active site of an enzyme. Enzymes adjust their shape to the substrate so there is a better fit (see figure 6.8).

Enzymes occur in many forms.

Enzymes can be free in the cytosol or exist as components bound to membranes and organelles. Enzymes involved in a biochemical pathway can form multienzyme complexes. While most enzymes are proteins, some are actually RNA molecules, called ribozymes.

Environmental and other factors affect enzyme function.

An enzyme's functionality depends on its ability to maintain its three-dimensional shape, which can be affected by temperature and pH. The activity of enzymes can be affected by inhibitors. Competitive inhibitors compete for the enzyme's active site, which leads to decreased enzyme activity (see figure 6.13). Enzyme activity can be controlled by effectors. Allosteric enzymes have a second site, located away from the active site, that binds effectors to activate or inhibit the enzyme. Noncompetitive inhibitors and activators bind to the allosteric site, changing the structure of the enzyme to inhibit or activate it. Cofactors are nonorganic metals necessary for enzyme function. Coenzymes are nonprotein organic molecules, such as certain vitamins, needed for enzyme function. Often coenzymes serve as electron acceptors.

6.5 Metabolism: The Chemical Description of Cell Function

Metabolism is the sum of all biochemical reactions in a cell. Anabolic reactions require energy to build up molecules, and catabolic reactions break down molecules and release energy.

Biochemical pathways organize chemical reactions in cells.

Chemical reactions in biochemical pathways use the product of one reaction as the substrate for the next.

Biochemical pathways may have evolved in stepwise fashion.

In the primordial "soup" of the early oceans, many reactions were probably single-step reactions combining two molecules. As one of the substrate molecules was depleted, organisms having an enzyme that could synthesize the substrate would have a selective advantage. In this manner, biochemical pathways are thought to have evolved "backward" with new reactions producing limiting substrates for existing reactions.

Feedback inhibition regulates some biochemical pathways.

Biosynthetic pathways are often regulated by the end product of the pathway. Feedback inhibition occurs when the end-product of a reaction combines with an enzyme's allosteric site to shut down the enzyme's activity (see figure 6.15).

Review Questions

UNDERSTAND

1. A covalent bond between two atoms represents what kind of energy?
   a. Kinetic energy
   b. Potential energy
   c. Mechanical energy
   d. Solar energy
2. During a redox reaction the molecule that gains an electron has been
   a. reduced and now has a higher energy level.
   b. oxidized and now has a lower energy level.
   c. reduced and now has a lower energy level.
   d. oxidized and now has a higher energy level.
3. An endergonic reaction has the following properties
   a. $+\Delta G$ and the reaction is spontaneous.
   b. $+\Delta G$ and the reaction is not spontaneous.
   c. $-\Delta G$ and the reaction is spontaneous.
   d. $-\Delta G$ and the reaction is not spontaneous.
4. A spontaneous reaction is one in which
   a. the reactants have a higher free energy than the products.
   b. the products have a higher free energy than the reactants.
   c. an input of energy is required.
   d. entropy is decreased.

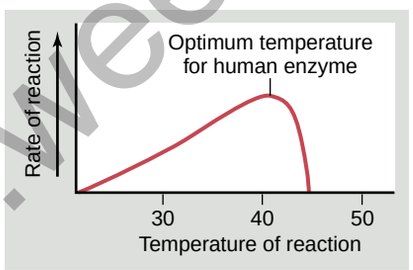
5. What is *activation energy*?
   a. The thermal energy associated with random movements of molecules
   b. The energy released through breaking chemical bonds
   c. The difference in free energy between reactants and products
   d. The energy required to initiate a chemical reaction
6. Which of the following is NOT a property of a catalyst?
   a. A catalyst reduces the activation energy of a reaction.
   b. A catalyst lowers the free energy of the reactants.
   c. A catalyst does not change as a result of the reaction.
   d. A catalyst works in both the forward and reverse directions of a reaction.
7. Where is the energy stored in a molecule of ATP?
   a. Within the bonds between nitrogen and carbon
   b. In the carbon-to-carbon bonds found in the ribose
   c. In the phosphorus-to-oxygen double bond
   d. In the bonds connecting the two terminal phosphate groups

APPLY

1. Cells use ATP to drive endergonic reactions because
   a. ATP is the universal catalyst.
   b. energy released by ATP hydrolysis makes ΔG for coupled reactions more negative.
   c. energy released by ATP hydrolysis makes ΔG for coupled reactions more positive.
   d. the conversion of ATP to ADP is also endergonic.
2. Which of the following statements is NOT true about enzymes?
   a. Enzymes use the three-dimensional shape of their active site to bind reactants.
   b. Enzymes lower the activation energy for a reaction.
   c. Enzymes make ΔG for a reaction more negative.
   d. Enzymes can catalyze the forward and reverse directions of a reaction.
3. What is the function of the *active site* of an enzyme?
   a. Bind the substrate, forming an enzyme–substrate complex
   b. Side groups within the active site interact with the substrate
   c. Bind to regulatory molecules, thereby altering the enzymes conformation
   d. Both a and b
4. The discovery of ribozymes meant that
   a. only proteins have catalytic function.
   b. only nucleic acids have catalytic function.
   c. RNAs can act as enzymes.
   d. RNA has the same function as protein.
5. Enzymes have similar responses to both changes in temperature and pH. The effect of both is on the
   a. rate of movement of the substrate molecules.
   b. strength of the chemical bonds within the substrate.
   c. three-dimensional shape of the enzyme.
   d. rate of movement of the enzyme.
6. In feedback inhibition, the
   a. first enzyme in a pathway is inhibited by its own product.
   b. last enzyme in a pathway is inhibited by its own product.
   c. first enzyme in a pathway is inhibited by the end-product of the pathway.
   d. last enzyme in a pathway is inhibited by the end-product of the pathway.

SYNTHESIZE

1. Examine the graph showing the rate of reaction versus temperature for an enzyme–catalyzed reaction in a human.
   a. Describe what is happening to the enzyme at around 40°C.
   b. Explain why the line touches the *x*-axis at approximately 20°C and 45°C.
   c. Average body temperature for humans is 37°C. Suggest a reason why the temperature optimum of this enzyme is greater than 37°C.



2. Phosphofructokinase functions to add a phosphate group to a molecule of fructose-6-phosphate. This enzyme functions early in glycolysis, an energy-yielding biochemical pathway discussed in chapter 7. The enzyme has an active site that binds fructose and ATP. An allosteric inhibitory site also binds ATP when cellular levels of ATP are very high.
   a. Predict the rate of the reaction if the levels of cellular ATP are low.
   b. Predict the rate of the reaction if levels of cellular ATP are very high.
   c. Describe what is happening to the enzyme when levels of ATP are very high.

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Chapter 7

How Cells Harvest Energy

Chapter Outline

Introduction

Life is driven by energy. All the activities organisms carry out—the swimming of bacteria, the purring of a cat, your thinking about these words—use energy. In this chapter, we discuss the processes all cells use to derive chemical energy from organic molecules and to convert that energy to ATP. Then, in chapter 8, we will examine photosynthesis, which uses light energy to make chemical energy. We consider the conversion of chemical energy to ATP first because all organisms, both the plant, a photosynthesizer, and the caterpillar feeding on the plant, pictured in the photo are capable of harvesting energy from chemical bonds. Energy harvest via respiration is a universal process.

7.1 Overview of Respiration

Learning Outcomes

1. *Characterize oxidation–dehydrogenation reactions in biological systems.*
2. *Understand the role of electron carriers in energy metabolism.*
3. *Describe the role of ATP in biological systems.*

Plants, algae, and some bacteria harvest the energy of sunlight through photosynthesis, converting radiant energy into chemical energy. These organisms, along with a few others that use chemical energy in a similar way, are called **autotrophs** ("self-feeders"). All other organisms live on the organic compounds autotrophs produce, using them as food, and are called **heterotrophs** ("fed by others"). At least 95% of the kinds of organisms on Earth—all animals and fungi, and most protists and prokaryotes—are heterotrophs. Autotrophs also extract energy from organic compounds—they just have the additional capacity to use the energy from sunlight to synthesize these compounds. The process by which energy is harvested is **cellular respiration**—the oxidation of organic compounds to extract energy from chemical bonds.

Cells oxidize organic compounds to drive metabolism

Most foods contain a variety of carbohydrates, proteins, and fats, all rich in energy-laden chemical bonds. Carbohydrates and fats, as you recall from chapter 3, possess many carbon–hydrogen (C—H) bonds, as well as carbon–oxygen (C—O) bonds.

The job of extracting energy from the complex organic mixture in most foods is tackled in stages. First, enzymes break down the large molecules into smaller ones, a process called digestion (see chapter 48). Then, other enzymes dismantle these fragments a bit at a time, harvesting energy from C—H and other chemical bonds at each stage.

The reactions that break down these molecules share a common feature: They are oxidations. Energy metabolism is therefore concerned with redox reactions, and to understand the process we must follow the fate of the electrons lost from the food molecules.

These reactions are not the simple transfer of electrons, however; they are also **dehydrogenations.** That is, the electrons lost are accompanied by protons, so that what is really lost is a hydrogen atom, not just an electron.

Cellular respiration is the complete oxidation of glucose

In chapter 6, you learned that an atom that loses electrons is said to be *oxidized*, and an atom accepting electrons is said to be *reduced.* Oxidation reactions are often coupled with reduction reactions in living systems, and these paired reactions are called *redox reactions.* Cells utilize enzyme-facilitated redox reactions to take energy from food sources and convert it to ATP.

Redox reactions

Oxidation–reduction reactions play a key role in the flow of energy through biological systems because the electrons that pass from one atom to another carry energy with them. The amount of energy an electron possesses depends on its orbital position, or energy level, around the atom's nucleus. When this electron departs from one atom and moves to another in a redox reaction, the electron's energy is transferred with it.

Figure 7.1 shows how an enzyme catalyzes a redox reaction involving an energy-rich substrate molecule, with the help of a cofactor, **nicotinamide adenosine dinucleotide (NAD^+).** In this reaction, NAD^+ accepts a pair of electrons from the substrate, along with a proton, to form **NADH** (this process is described in more detail shortly). The oxidized product is now released from the enzyme's active site, as is NADH.

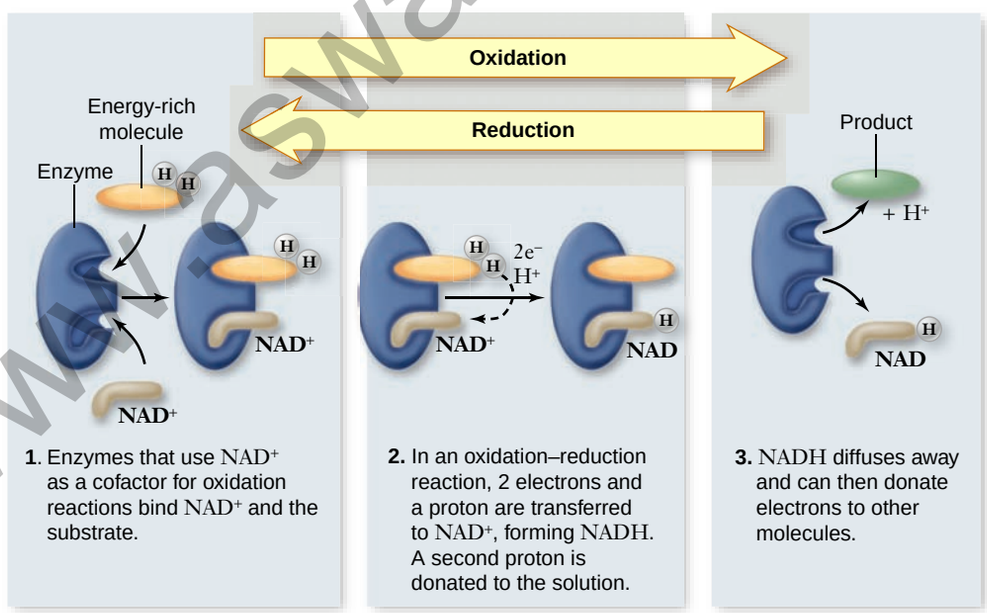


Figure 7.1 Oxidation–reduction reactions often employ cofactors. Cells use a chemical cofactor called nicotinamide adenosine dinucleotide (NAD^+) to carry out many oxidation–reduction reactions. Two electrons and a proton are transferred to NAD^+ with another proton donated to the solution. Molecules that gain electrons are said to be reduced, and ones that lose energetic electrons are said to be oxidized. NAD^+ oxidizes energy-rich molecules by acquiring their electrons (in the figure, this proceeds 1 ⟶ 2 ⟶ 3) and then reduces other molecules by giving the electrons to them (in the figure, this proceeds 3 ⟶ 2 ⟶ 1). NADH is the reduced form of NAD^+.

In the overall process of cellular energy harvest dozens of redox reactions take place, and a number of molecules, including NAD^+, act as electron acceptors. During each transfer of electrons energy is released. This energy may be captured and used to make ATP or to form other chemical bonds; the rest is lost as heat.

At the end of this process, high-energy electrons from the initial chemical bonds have lost much of their energy, and these depleted electrons are transferred to a final electron acceptor (figure 7.2). When this acceptor is oxygen, the process is called **aerobic respiration.** When the final electron acceptor is an inorganic molecule other than oxygen, the process is called **anaerobic respiration,** and when it is an organic molecule, the process is called **fermentation.**

"Burning" carbohydrates

Chemically, there is little difference between the catabolism of carbohydrates in a cell and the burning of wood in a fireplace. In both instances, the reactants are carbohydrates and oxygen, and the products are carbon dioxide, water, and energy:

$$\underset{\text{glucose}}{C_6H_{12}O_6} + 6\,\underset{\text{oxygen}}{O_2} \longrightarrow 6\,\underset{\text{carbon dioxide}}{CO_2} + 6\,\underset{\text{water}}{H_2O} + \text{energy (heat and ATP)}$$

The change in free energy in this reaction is −686 kcal/mol (or −2870 kJ/mol) under standard conditions (that is, at room temperature, 1 atm pressure, and so forth). In the conditions that exist inside a cell, the energy released can be as high as −720 kcal/mol (−3012 kJ/mol) of glucose. This means that under actual cellular conditions, more energy is released than under standard conditions.

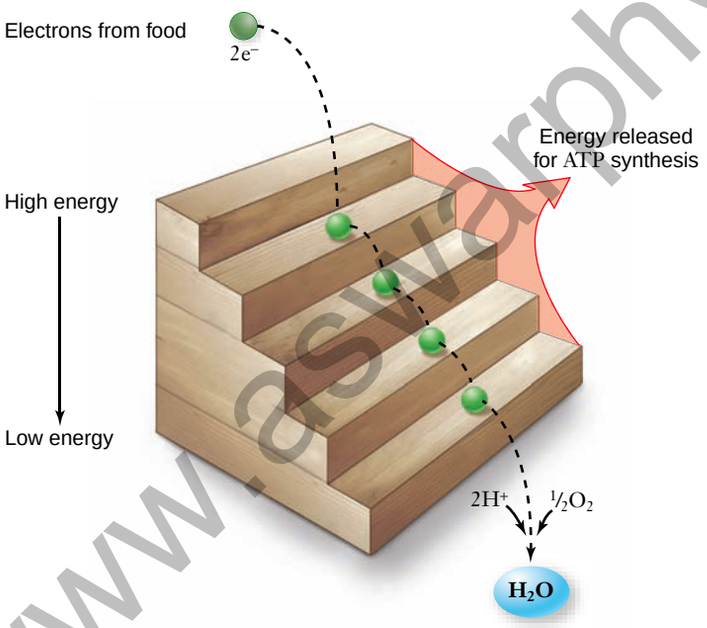


Figure 7.2 How electron transport works. This diagram shows how ATP is generated when electrons transfer from one energy level to another. Rather than releasing a single explosive burst of energy, electrons "fall" to lower and lower energy levels in steps, releasing stored energy with each fall as they tumble to the lowest (most electronegative) electron acceptor, O_2.

The same amount of energy is released whether glucose is catabolized or burned, but when it is burned, most of the energy is released as heat. Cells harvest useful energy from the catabolism of glucose by using a portion of the energy to drive the production of ATP.

Electron carriers play a critical role in energy metabolism

During respiration, glucose is oxidized to CO_2. If the electrons were given directly to O_2, the reaction would be combustion, and cells would burst into flames. Instead, as you have just seen, the cell transfers the electrons to intermediate electron carriers, then eventually to O_2.

Many forms of electron carriers are used in this process: (1) soluble carriers that move electrons from one molecule to another, (2) membrane-bound carriers that form a redox chain, and (3) carriers that move within the membrane. The common feature of all of these carriers is that they can be reversibly oxidized and reduced. Some of these carriers, such as the iron-containing cytochromes, can carry just electrons, and some carry both electrons and protons.

NAD^+ is one of the most important electron (and proton) carriers. As shown on the left in figure 7.3, the NAD^+ molecule is composed of two nucleotides bound together. The two nucleotides that make up NAD^+, nicotinamide monophosphate (NMP) and adenosine monophosphate (AMP), are joined head-to-head by their phosphate groups. The two nucleotides serve different functions in the NAD^+ molecule: AMP acts as the core, providing a shape recognized by many enzymes; NMP is the active part of the molecule, because it is readily reduced, that is, it easily accepts electrons.

When NAD^+ acquires two electrons and a proton from the active site of an enzyme, it is reduced to NADH, shown on the right in figure 7.3. The NADH molecule now carries the two energetic electrons and can supply them to other molecules and reduce them.

This ability to supply high-energy electrons is critical to both energy metabolism and to the biosynthesis of many organic molecules, including fats and sugars. In animals, when ATP is plentiful, the reducing power of the accumulated NADH is diverted to supplying fatty acid precursors with high-energy electrons, reducing them to form fats and storing the energy of the electrons.

Metabolism harvests energy in stages

It is generally true that the larger the release of energy in any single step, the more of that energy is released as heat, and the less is available to be channeled into more useful paths. In the combustion of gasoline, the same amount of energy is released whether all of the gasoline in a car's gas tank explodes at once, or burns in a series of very small explosions inside the cylinders. By releasing the energy in gasoline a little at a time, the harvesting efficiency is greater, and more of the energy can be used to push the pistons and move the car.

The same principle applies to the oxidation of glucose inside a cell. If all of the electrons were transferred to oxygen in one explosive step, releasing all of the free energy at once, the cell

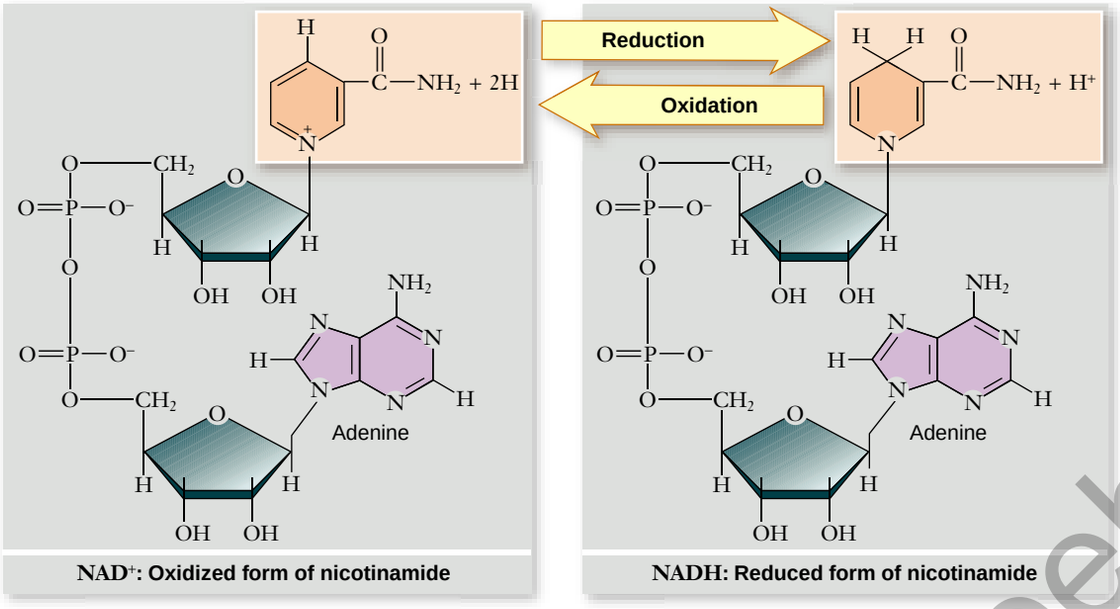


Figure 7.3 NAD⁺ and NADH. This dinucleotide serves as an "electron shuttle" during cellular respiration. NAD^+ accepts a pair of electrons and a proton from catabolized macromolecules and is reduced to NADH.

would recover very little of that energy in a useful form. Instead, cells burn their fuel much as a car does, a little at a time.

The electrons in the C—H bonds of glucose are stripped off in stages in the series of enzyme-catalyzed reactions collectively referred to as glycolysis and the Krebs cycle. The electrons are removed by transferring them to NAD^+, as described earlier, or to other electron carriers.

The energy released by all of these oxidation reactions is also not all released at once (see figure 7.2). The electrons are passed to another set of electron carriers called the **electron transport chain,** which is located in the mitochondrial inner membrane. Movement of electrons through this chain produces potential energy in the form of an electrochemical gradient. We examine this process in more detail later in this chapter.

ATP plays a central role in metabolism

The previous chapter introduced ATP as the energy currency of the cell. Cells use ATP to power most of those activities that require work—one of the most obvious of which is movement. Tiny fibers within muscle cells pull against one another when muscles contract. Mitochondria can move a meter or more along the narrow nerve cells that extend from your spine to your feet. Chromosomes are pulled apart by microtubules during cell division. All of these movements require the expenditure of energy by ATP hydrolysis. Cells also use ATP to drive endergonic reactions that would otherwise not occur spontaneously (see chapter 6).

How does ATP drive an endergonic reaction? The enzyme that catalyzes a particular reaction has two binding sites on its surface: one for the reactant and another for ATP. The ATP site splits the ATP molecule, liberating over 7 kcal (ΔG = −7.3 kcal/mol) of chemical energy. This energy pushes the reactant at the second site "uphill," reaching the activation energy and driving the endergonic reaction. Thus endergonic reactions coupled to ATP hydrolysis become favorable.

The many steps of cellular respiration have as their ultimate goal the production of ATP. ATP synthesis is itself an endergonic reaction, which requires cells to perform exergonic reactions to drive this synthesis.

Cells make ATP by two fundamentally different mechanisms

The synthesis of ATP can be accomplished by two distinct mechanisms: one that involves chemical coupling with an intermediate bound to phosphate, and another that relies on an electrochemical gradient of protons for the potential energy to phosphorylate ADP.

1. In *substrate-level phosphorylation*, ATP is formed by transferring a phosphate group directly to ADP from a phosphate-bearing intermediate, or substrate (figure 7.4). During **glycolysis,** the initial breakdown of glucose (discussed later), the chemical bonds of glucose are shifted

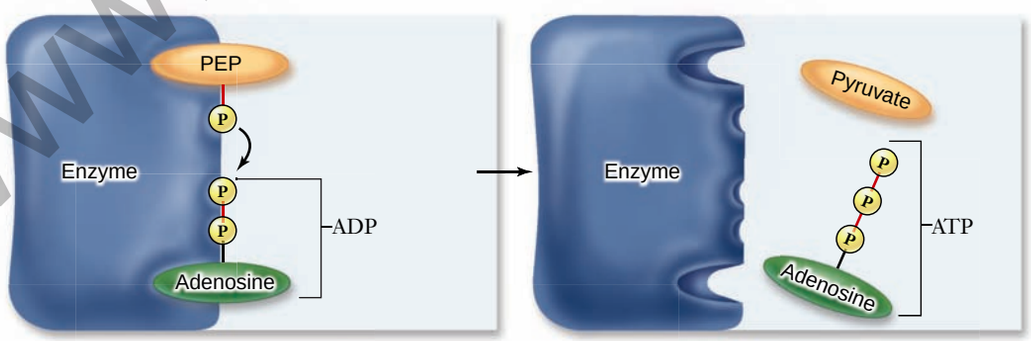


Figure 7.4 Substrate-level phosphorylation. Some molecules, such as phosphoenolpyruvate (PEP), possess a high-energy phosphate (P) bond similar to the bonds in ATP. When PEP's phosphate group is transferred enzymatically to ADP, the energy in the bond is conserved, and ATP is created.

around in reactions that provide the energy required to form ATP by substrate-level phosphorylation.

2. In **oxidative phosphorylation,** ATP is synthesized by the enzyme **ATP synthase,** using energy from a proton (H^+) gradient. This gradient is formed by high-energy electrons from the oxidation of glucose passing down an electron transport chain (described later). These electrons, with their energy depleted, are then donated to oxygen, hence the term *oxidative phosphorylation*. ATP synthase uses the energy from the proton gradient to catalyze the reaction:

$$ADP + P_i \longrightarrow ATP$$

Eukaryotes and aerobic prokaryotes produce the vast majority of their ATP this way.

In most organisms, these two processes are combined. To harvest energy to make ATP from glucose in the presence of oxygen, the cell carries out a complex series of enzyme-catalyzed reactions that remove energetic electrons via oxidation reactions. These electrons are then used in an electron transport chain that passes the electrons down a series of carriers while translocating protons into the intermembrane space. The final electron acceptor in aerobic respiration is oxygen, and the resulting proton gradient provides energy for the enzyme ATP synthase to phosphorylate ADP to ATP (figure 7.5). The details of this complex process will be covered in the remainder of this chapter.

Learning Outcomes Review 7.1

Cells acquire energy from the complete oxidation of glucose. In these redox reactions, protons as well as electrons are transferred, and thus they are dehydrogenation reactions. Electron carriers aid in the gradual, stepwise release of the energy from oxidation, rather than rapid combustion. The result is the synthesis of ATP, a portable source of energy. ATP synthesis can occur by two mechanisms: substrate level phosphorylation and oxidative phosphorylation.

- ***Why don't cells just link the oxidation of glucose directly to cellular functions that require the energy?***

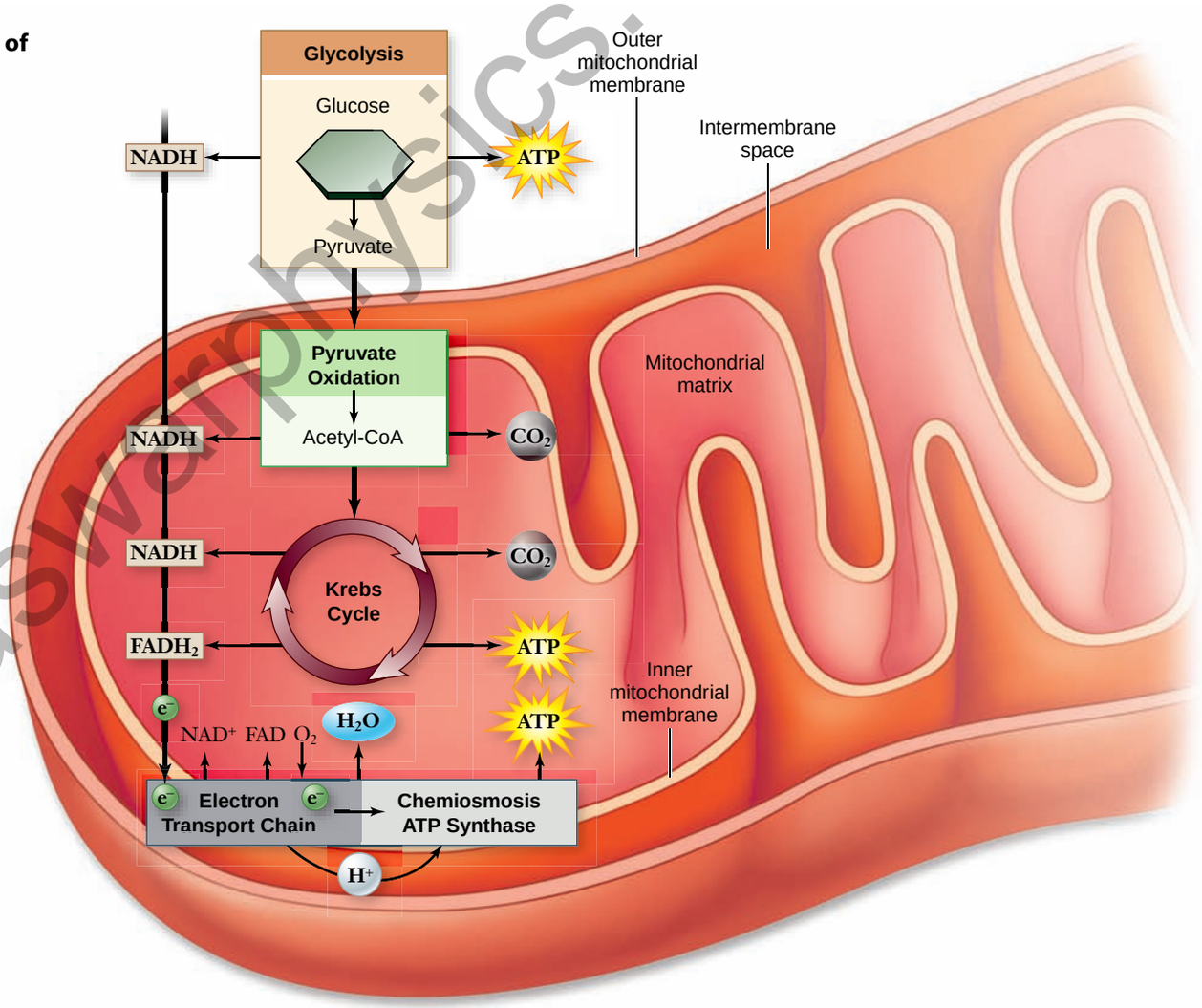


Figure 7.5 **An overview of aerobic respiration.**

7.2 Glycolysis: Splitting Glucose

Learning Outcomes

1. *Describe the process of glycolysis.*
2. *Calculate the energy yield from glycolysis.*
3. *Distinguish between aerobic respiration and fermentation.*

Glucose molecules can be dismantled in many ways, but primitive organisms evolved a glucose-catabolizing process that releases enough free energy to drive the synthesis of ATP in enzyme-coupled reactions. Glycolysis occurs in the cytoplasm and converts glucose into two 3-carbon molecules of pyruvate (figure 7.6). For each molecule of glucose that passes through this transformation, the cell nets two ATP molecules.

Priming changes glucose into an easily cleaved form

The first half of glycolysis consists of five sequential reactions that convert one molecule of glucose into two molecules of the 3-carbon compound *glyceraldehyde 3-phosphate (G3P)*. These reactions require the expenditure of ATP, so they are an endergonic process.

Step A: Glucose priming Three reactions "prime" glucose by changing it into a compound that can be cleaved readily into two 3-carbon phosphorylated molecules. Two of these reactions transfer a phosphate from ATP, so this step requires the cell to use two ATP molecules.

Step B: Cleavage and rearrangement In the first of the remaining pair of reactions, the 6-carbon product of step A is split into two 3-carbon molecules. One is G3P, and the other is then converted to G3P by the second reaction (figure 7.7).

ATP is synthesized by substrate-level phosphorylation

In the second half of glycolysis, five more reactions convert G3P into pyruvate in an energy-yielding process that generates ATP.

Step C: Oxidation Two electrons (and one proton) are transferred from G3P to NAD^+, forming NADH. A molecule of P_i is also added to G3P to produce 1,3-bisphosphoglycerate (BPG). The phosphate incorporated will later be transferred to ADP by substrate-level phosphorylation to allow a net yield of ATP.

Step D: ATP generation Four reactions convert BPG into pyruvate. This process generates two ATP molecules per G3P (see figures 7.4 and 7.7) produced in Step B.

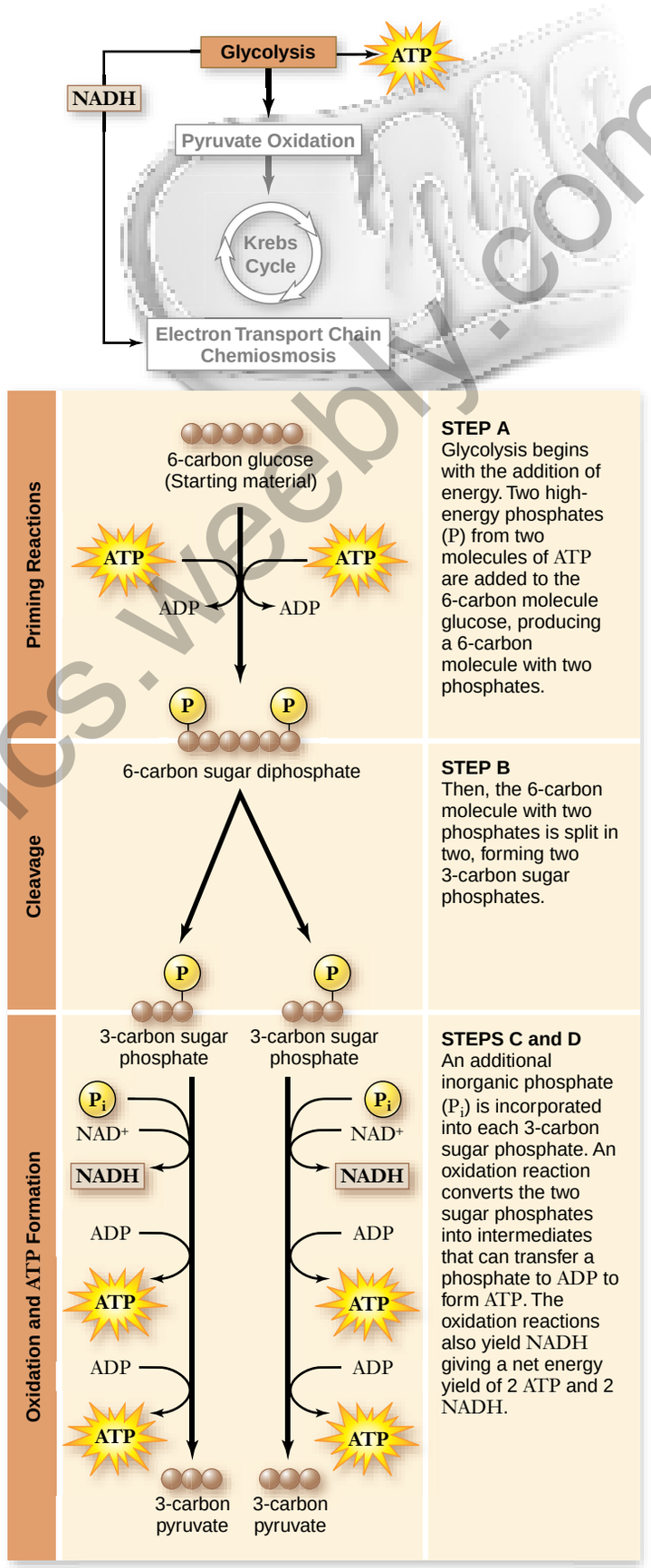
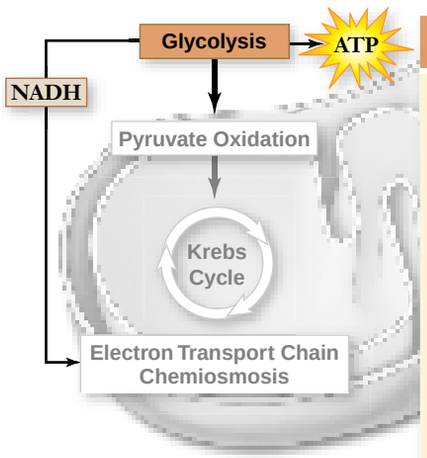


Figure 7.6 How glycolysis works.



1. Phosphorylation of glucose by ATP.

2–3. Rearrangement, followed by a second ATP phosphorylation.

4–5. The 6-carbon molecule is split into two 3-carbon molecules—one G3P, another that is converted into G3P in another reaction.

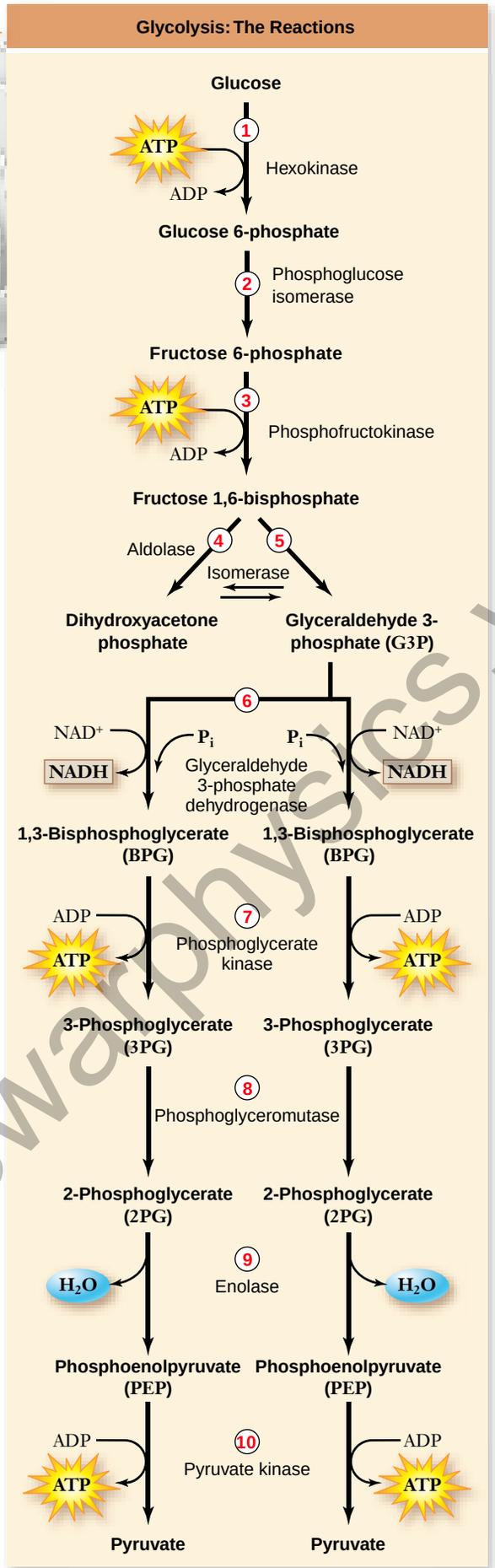
6. Oxidation followed by phosphorylation produces two NADH molecules and two molecules of BPG, each with one high-energy phosphate bond.

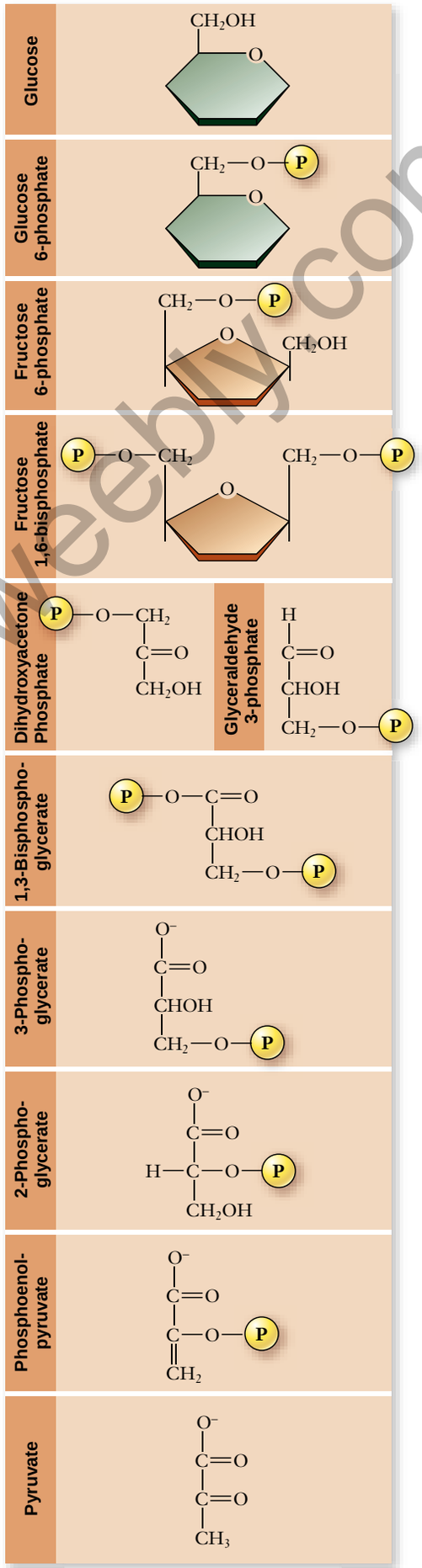
7. Removal of high-energy phosphate by two ADP molecules produces two ATP molecules and leaves two 3PG molecules.

8–9. Removal of water yields two PEP molecules, each with a high-energy phosphate bond.

10. Removal of high-energy phosphate by two ADP molecules produces two ATP molecules and two pyruvate molecules.

Figure 7.7
The glycolytic pathway.
The first five reactions convert a molecule of glucose into two molecules of G3P. The second five reactions convert G3P into pyruvate.





Because each glucose molecule is split into two G3P molecules, the overall reaction sequence has a net yield of two molecules of ATP, as well as two molecules of NADH and two of pyruvate:

4 ATP (2 ATP for each of the 2 G3P molecules in step D)
− 2 ATP (used in the two reactions in step A)

2 ATP (net yield for entire process)

The hydrolysis of one molecule of ATP yields a ΔG of −7.3 kcal/mol under standard conditions. Thus cells harvest a maximum of 14.6 kcal of energy per mole of glucose from glycolysis.

A brief history of glycolysis

Although far from ideal in terms of the amount of energy it releases, glycolysis does generate ATP. For more than a billion years during the anaerobic first stages of life on Earth, glycolysis was the primary way heterotrophic organisms generated ATP from organic molecules.

Like many biochemical pathways, glycolysis is believed to have evolved backward, with the last steps in the process being the most ancient. Thus, the second half of glycolysis, the ATP-yielding breakdown of G3P, may have been the original process. The synthesis of G3P from glucose would have appeared later, perhaps when alternative sources of G3P were depleted.

Why does glycolysis take place in modern organisms, since its energy yield in the absence of oxygen is comparatively little? The answer is that evolution is an incremental process: Change occurs by improving on past successes. In catabolic metabolism, glycolysis satisfied the one essential evolutionary criterion—it was an improvement. Cells that could not carry out glycolysis were at a competitive disadvantage, and only cells capable of glycolysis survived. Later improvements in catabolic metabolism built on this success. Metabolism evolved as one layer of reactions added to another. Nearly every present-day organism carries out glycolysis, as a metabolic memory of its evolutionary past.

The last section of this chapter discusses the evolution of metabolism in more detail.

NADH must be recycled to continue respiration

Inspect for a moment the net reaction of the glycolytic sequence:

$$\text{glucose} + 2\ \text{ADP} + 2\ P_i + 2\ \text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ \text{H}^+ + 2\ \text{H}_2\text{O}$$

You can see that three changes occur in glycolysis: (1) Glucose is converted into two molecules of pyruvate; (2) two molecules of ADP are converted into ATP via substrate-level phosphorylation; and (3) two molecules of NAD^+ are reduced to NADH. This leaves the cell with two problems: extracting the energy that remains in the two pyruvate molecules, and regenerating NAD^+ to be able to continue glycolysis.

Recycling NADH

As long as food molecules that can be converted into glucose are available, a cell can continually churn out ATP to drive its activities. In doing so, however, it accumulates NADH and depletes the pool of NAD^+ molecules. A cell does not contain a large amount of NAD^+, and for glycolysis to continue, NADH must be recycled into NAD^+. Some molecule other than NAD^+ must ultimately accept the electrons taken from G3P and be reduced. Two processes can carry out this key task (figure 7.8):

1. **Aerobic respiration.** Oxygen is an excellent electron acceptor. Through a series of electron transfers, electrons taken from G3P can be donated to oxygen, forming water. This process occurs in the mitochondria of eukaryotic cells in the presence of oxygen. Because air is rich in oxygen, this process is also referred to as *aerobic metabolism*. A significant amount of ATP is also produced.
2. **Fermentation.** When oxygen is unavailable, an organic molecule, such as acetaldehyde in wine fermentation, can accept electrons instead. This reaction plays an important role in the metabolism of most organisms, even those capable of aerobic respiration.

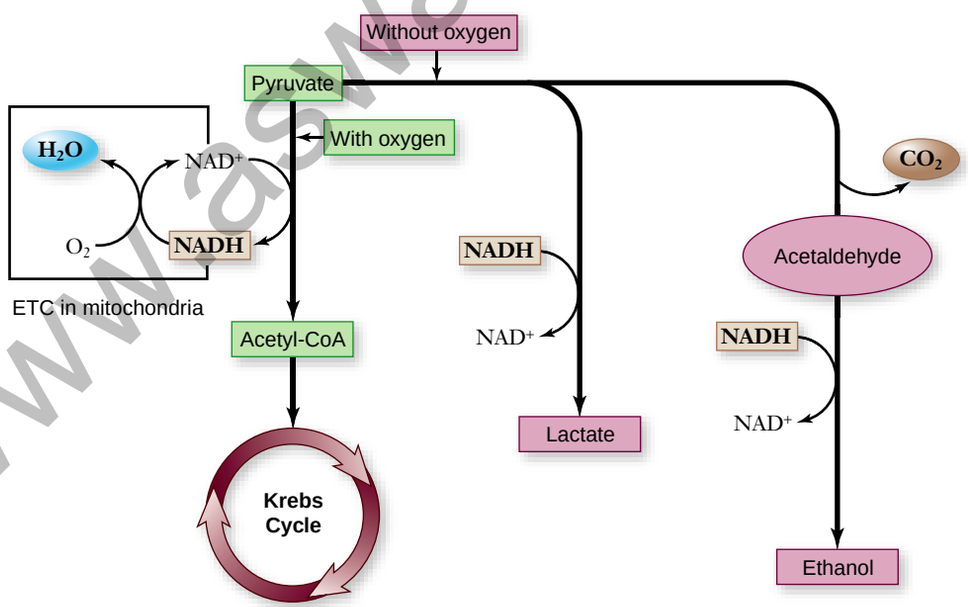


Figure 7.8 The fate of pyruvate and NADH produced by glycolysis. In the presence of oxygen, NADH is oxidized by the electron transport chain (ETC) in mitochondria using oxygen as the final electron acceptor. This regenerates NAD^+, allowing glycolysis to continue. The pyruvate produced by glycolysis is oxidized to acetyl-CoA, which enters the Krebs cycle. In the absence of oxygen, pyruvate is instead reduced, oxidizing NADH and regenerating NAD^+ thus allowing glycolysis to continue. Direct reduction of pyruvate, as in muscle cells, produces lactate. In yeast, carbon dioxide is first removed from pyruvate, producing acetaldehyde, which is then reduced to ethanol.

The fate of pyruvate

The fate of the pyruvate that is produced by glycolysis depends on which of these two processes takes place. The aerobic respiration path starts with the oxidation of pyruvate to produce acetyl coenzyme A (acetyl-CoA), which is then further oxidized in a series of reactions called the Krebs cycle. The fermentation path, by contrast, uses the reduction of all or part of pyruvate to oxidize NADH back to NAD^+. We examine aerobic respiration next; fermentation is described in detail in a later section.

Learning Outcomes Review 7.2

Glycolysis splits the 6-carbon molecule glucose into two 3-carbon molecules of pyruvate. This process uses two ATP molecules in "priming" reactions and eventually produces four molecules of ATP per glucose for a net yield of two ATP. The oxidation reactions of glycolysis require NAD^+ and produce NADH. When oxygen is abundant, NAD^+ is regenerated in the electron transport chain using O_2 as an acceptor. When oxygen is absent, NAD^+ is regenerated in a fermentation reaction using an organic molecule as an electron receptor.

- ***Does glycolysis taking place in the cytoplasm argue for or against the endosymbiotic origin of mitochondria?***

7.3 The Oxidation of Pyruvate to Produce Acetyl-CoA

Learning Outcome

1. ***Explain how the oxidation of pyruvate joins glycolysis with the Krebs cycle.***

In the presence of oxygen, the oxidation of glucose that begins in glycolysis continues where glycolysis leaves off—with pyruvate. In eukaryotic organisms, the extraction of additional energy from pyruvate takes place exclusively inside mitochondria. In prokaryotes similar reactions take place in the cytoplasm and at the plasma membrane.

The cell harvests pyruvate's considerable energy in two steps. First, pyruvate is oxidized to produce a two-carbon compound and CO_2, with the electrons transferred to NAD^+ to produce NADH. Next, the two-carbon compound is oxidized to CO_2 by the reactions of the Krebs cycle.

Pyruvate is oxidized in a "decarboxylation" reaction that cleaves off one of pyruvate's three carbons. This carbon departs as CO_2 (figure 7.9). The remaining 2-carbon compound, called an acetyl group, is then attached to coenzyme A; this entire molecule is called *acetyl-CoA*. A pair of electrons and one associated proton is transferred to the electron carrier NAD^+, reducing it to NADH, with a second proton donated to the solution.

The reaction involves three intermediate stages, and it is catalyzed within mitochondria by a *multienzyme complex*. As chapter 6 noted, a multienzyme complex organizes a series of enzymatic steps so that the chemical intermediates do not diffuse away or undergo other reactions. Within the complex, component polypeptides pass

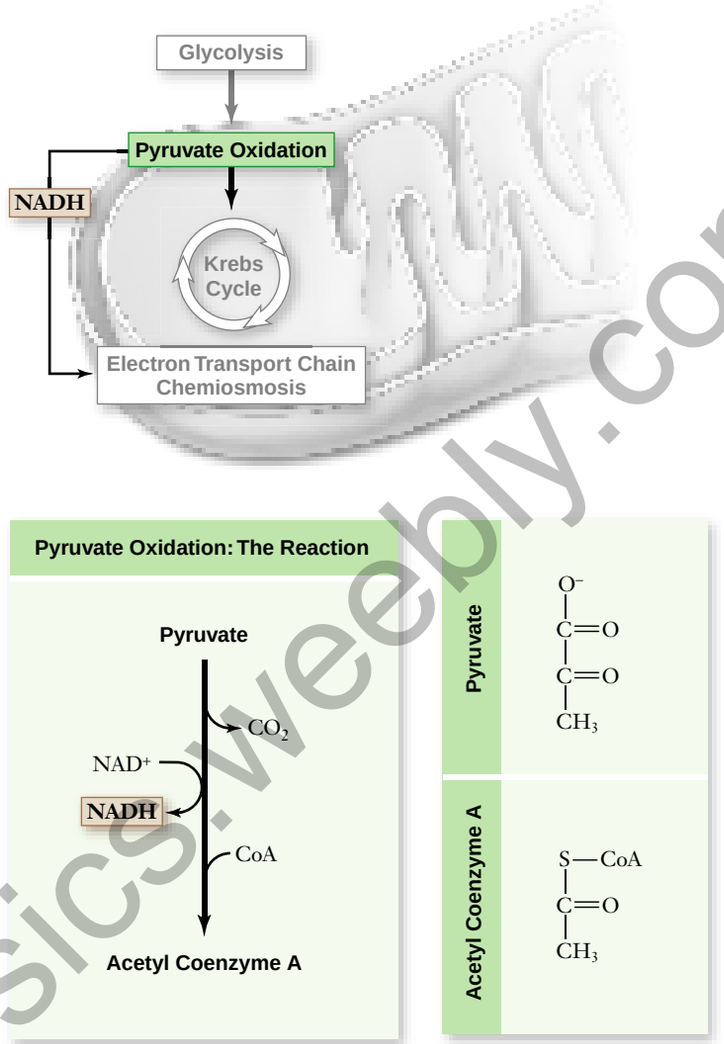


Figure 7.9 The oxidation of pyruvate. This complex reaction uses NAD^+ to accept electrons, reducing it to NADH. The product, acetyl coenzyme A (acetyl-CoA), feeds the acetyl unit into the Krebs cycle, and the CoA is recycled for another oxidation of pyruvate. NADH provides energetic electrons for the electron transport chain.

the substrates from one enzyme to the next without releasing them. *Pyruvate dehydrogenase*, the complex of enzymes that removes CO_2 from pyruvate, is one of the largest enzymes known; it contains 60 subunits! The reaction can be summarized as:

$$\text{pyruvate} + NAD^+ + \text{CoA} \longrightarrow \text{acetyl-CoA} + \text{NADH} + CO_2 + H^+$$

The molecule of NADH produced is used later to produce ATP. The acetyl group is fed into the Krebs cycle, with the CoA being recycled for another oxidation of pyruvate. The Krebs cycle then completes the oxidation of the original carbons from glucose.

Learning Outcome Review 7.3

Pyruvate is oxidized in the mitochondria to produce acetyl-CoA and CO_2. Acetyl-CoA is the molecule that links glycolysis and the reactions of the Krebs cycle.

- ***What are the advantages and disadvantages of a multienzyme complex?***

7.4 The Krebs Cycle

Learning Outcomes

1. *Describe the three segments and nine reactions of the Krebs cycle.*
2. *Explain the fate of the electrons produced by the Krebs cycle.*

In this third stage, the acetyl group from pyruvate is oxidized in a series of nine reactions called the *Krebs cycle*. These reactions occur in the matrix of mitochondria.

In this cycle, the 2-carbon acetyl group of acetyl-CoA combines with a 4-carbon molecule called oxaloacetate. The resulting 6-carbon molecule, citrate, then goes through a several-step sequence of electron-yielding oxidation reactions, during which two CO_2 molecules split off, restoring oxaloacetate. The regenerated oxaloacetate is used to bind to another acetyl group for the next round of the cycle.

In each turn of the cycle, a new acetyl group is added and two carbons are lost as two CO_2 molecules, and more electrons are transferred to electron carriers. These electrons are then used by the electron transport chain to drive *proton pumps* that generate ATP.

The Krebs cycle has three segments: An overview

The nine reactions of the Krebs cycle can be grouped into three overall segments. These are described in the following sections and summarized in figure 7.10.

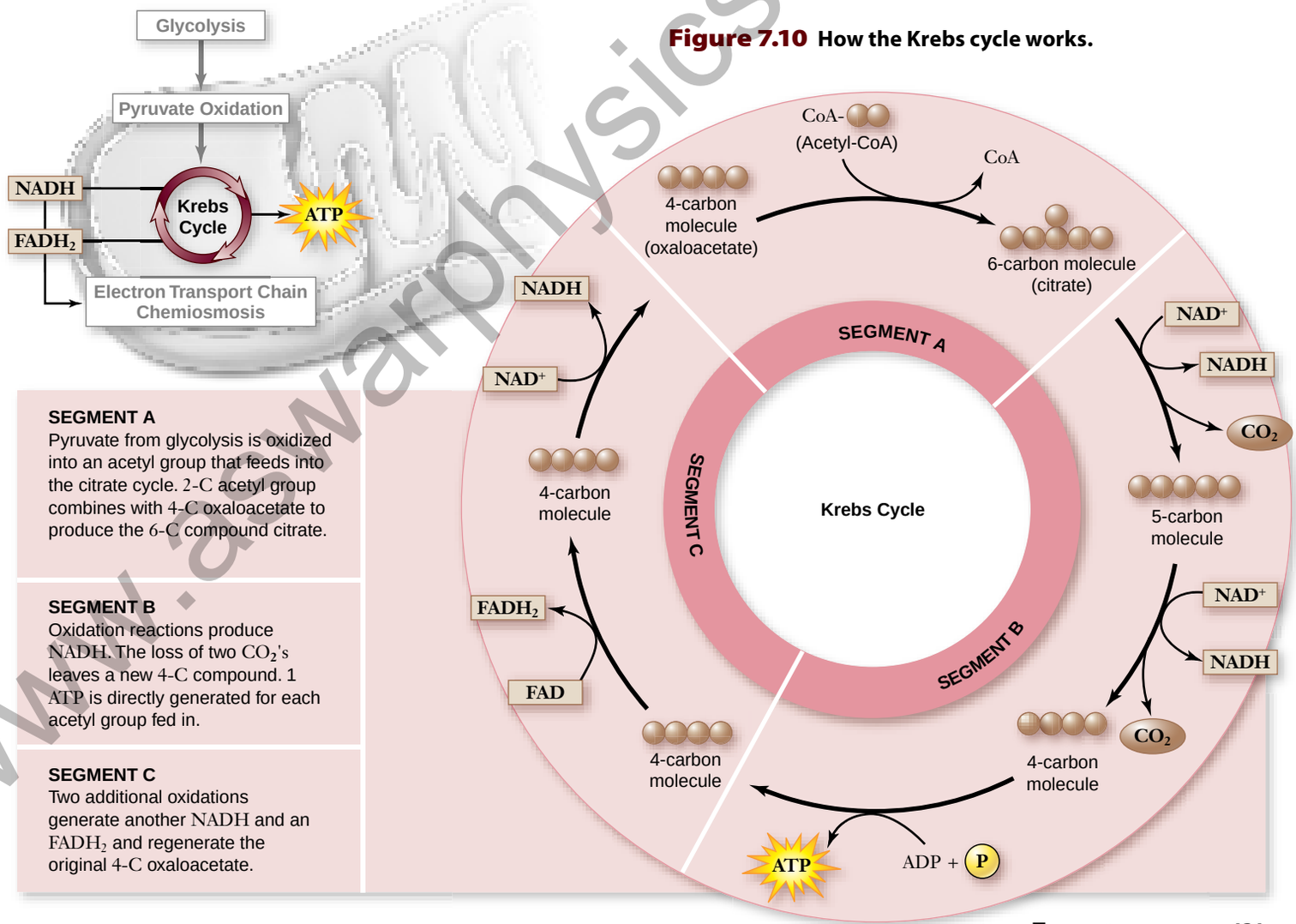
Segment A: Acetyl-CoA plus oxaloacetate This reaction produces the 6-carbon citrate molecule.

Segment B: Citrate rearrangement and decarboxylation Five more steps, which have been simplified in figure 7.10, reduce citrate to a 5-carbon intermediate and then to 4-carbon succinate. During these reactions, two NADH and one ATP are produced.

Segment C: Regeneration of oxaloacetate Succinate undergoes three additional reactions, also simplified in the figure, to become oxaloacetate. During these reactions, one NADH is produced; in addition, a molecule of flavin adenine dinucleotide (FAD), another cofactor, becomes reduced to $FADH_2$.

The specifics of each reaction are described next.

Figure 7.10 How the Krebs cycle works.



The Krebs cycle extracts electrons and synthesizes one ATP

Figure 7.11 summarizes the sequence of the Krebs cycle reactions. A 2-carbon group from acetyl-CoA enters the cycle at the beginning, and two CO_2 molecules, one ATP, and four pairs of electrons are produced.

Reaction 1: Condensation Citrate is formed from acetyl-CoA and oxaloacetate. This condensation reaction is irreversible, committing the 2-carbon acetyl group to the Krebs cycle. The reaction is inhibited when the cell's ATP concentration is high and stimulated when it is low. The result is that when the cell possesses ample amounts of ATP, the Krebs cycle shuts down, and acetyl-CoA is channeled into fat synthesis.

Reactions 2 and 3: Isomerization Before the oxidation reactions can begin, the hydroxyl (—OH) group of citrate must be repositioned. This rearrangement is done in two steps: First, a water molecule is removed from one carbon; then water is added to a different carbon. As a result, an —H group and an —OH group change positions. The product is an isomer of citrate called *isocitrate*. This rearrangement facilitates the subsequent reactions.

Reaction 4: The First Oxidation In the first energy-yielding step of the cycle, isocitrate undergoes an oxidative decarboxylation reaction. First, isocitrate is oxidized, yielding a pair of electrons that reduce a molecule of NAD^+ to NADH. Then the oxidized intermediate is decarboxylated; the central carboxyl group splits off to form CO_2, yielding a 5-carbon molecule called *α-ketoglutarate*.

Reaction 5: The Second Oxidation Next, α-ketoglutarate is decarboxylated by a multienzyme complex similar to pyruvate dehydrogenase. The succinyl group left after the removal of CO_2 joins to coenzyme A, forming *succinyl-CoA*. In the process, two electrons are extracted, and they reduce another molecule of NAD^+ to NADH.

Reaction 6: Substrate-Level Phosphorylation The linkage between the 4-carbon succinyl group and CoA is a high-energy bond. In a coupled reaction similar to those that take place in glycolysis, this bond is cleaved, and the energy released drives the phosphorylation of guanosine diphosphate (GDP), forming guanosine triphosphate (GTP). GTP can transfer a phosphate to ADP converting it into ATP. The 4-carbon molecule that remains is called *succinate*.

Reaction 7: The Third Oxidation Next, succinate is oxidized to *fumarate* by an enzyme located in the inner mitochondrial membrane. The free-energy change in this reaction is not large enough to reduce NAD^+. Instead, FAD is the electron acceptor. Unlike NAD^+, FAD is not free to diffuse within the mitochondrion; it is tightly associated with its enzyme in the inner mitochondrial membrane. Its reduced form, $FADH_2$, can only contribute electrons to the electron transport chain in the membrane.

Reactions 8 and 9: Regeneration of Oxaloacetate In the final two reactions of the cycle, a water molecule is added to fumarate, forming *malate*. Malate is then oxidized, yielding a 4-carbon molecule of *oxaloacetate* and two electrons that reduce a molecule of NAD^+ to NADH. Oxaloacetate, the molecule that began the cycle, is now free to combine with another 2-carbon acetyl group from acetyl-CoA and begin the cycle again.

Glucose becomes CO_2 and potential energy

In the process of aerobic respiration, glucose is entirely consumed. The 6-carbon glucose molecule is cleaved into a pair of 3-carbon pyruvate molecules during glycolysis. One of the carbons of each pyruvate is then lost as CO_2 in the conversion of pyruvate to acetyl-CoA. The two other carbons from acetyl-CoA are lost as CO_2 during the oxidations of the Krebs cycle.

All that is left to mark the passing of a glucose molecule into six CO_2 molecules is its energy, some of which is preserved in four ATP molecules and in the reduced state of 12 electron carriers. Ten of these carriers are NADH molecules; the other two are $FADH_2$.

Following the electrons in the reactions reveals the direction of transfer

As you examine the changes in electrical charge in the reactions that oxidize glucose, a good strategy for keeping the transfers clear is always to *follow the electrons*. For example, in glycolysis, an enzyme extracts two hydrogens—that is, two electrons and two protons—from glucose and transfers both electrons and one of the protons to NAD^+. The other proton is released as a hydrogen ion, H^+, into the surrounding solution. This transfer converts NAD^+ into NADH; that is, two negative electrons ($2e^-$) and one positive proton (H^+) are added to one positively charged NAD^+ to form NADH, which is electrically neutral.

As mentioned earlier, energy captured by NADH is not harvested all at once. The two electrons carried by NADH are passed along the electron transport chain, which consists of a series of electron carriers, mostly proteins, embedded within the inner membranes of mitochondria.

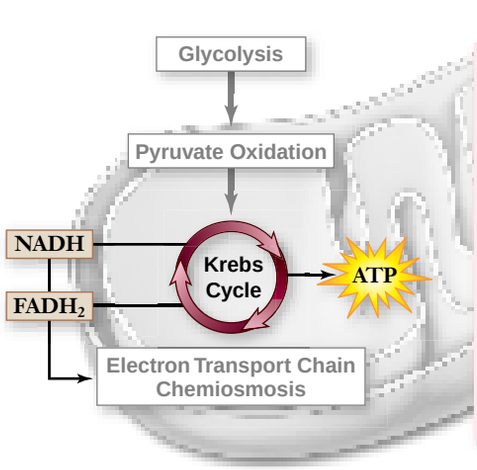
NADH delivers electrons to the beginning of the electron transport chain, and oxygen captures them at the end. The oxygen then joins with hydrogen ions to form water. At each step in the chain, the electrons move to a slightly more electronegative carrier, and their positions shift slightly. Thus, the electrons move *down* an energy gradient.

The entire process of electron transfer releases a total of 53 kcal/mol (222 kJ/mol) under standard conditions. The transfer of electrons along this chain allows the energy to be extracted gradually. Next, we will discuss how this energy is put to work to drive the production of ATP.

Learning Outcomes Review 7.4

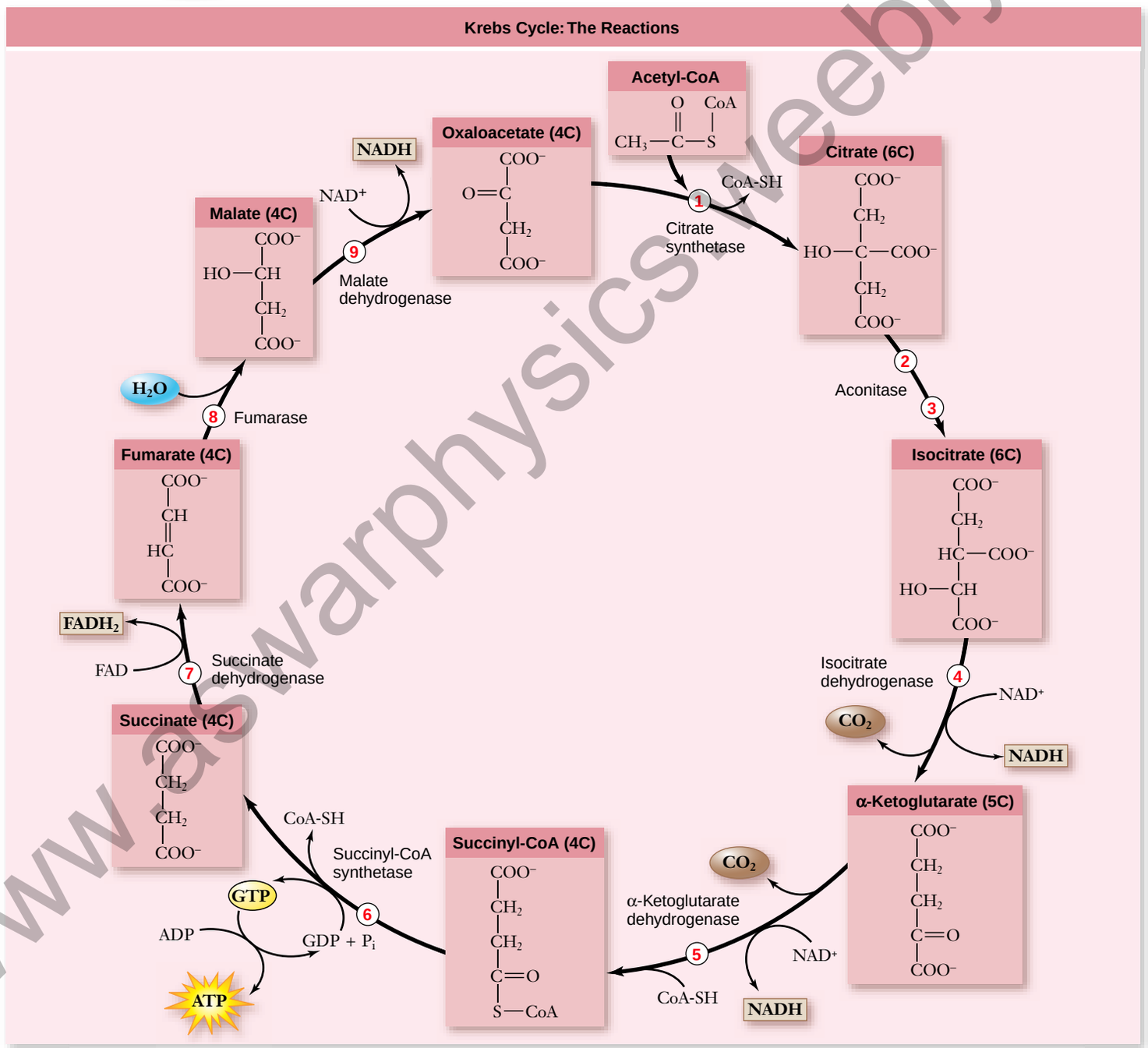
The Krebs cycle completes the oxidation of glucose begun with glycolysis. In the first segment, acetyl-CoA is added to oxaloacetate to produce citrate. In the next segment, five reactions produce succinate, two NADH from NAD^+, and one ATP. Finally, succinate undergoes three more reactions to regenerate oxaloacetate, producing one more NADH and one $FADH_2$ from FAD.

- ***What happens to the electrons removed from glucose at this point?***



1. Reaction 1: Condensation
2–3. Reactions 2 and 3: Isomerization
4. Reaction 4: The first oxidation
5. Reaction 5: The second oxidation
6. Reaction 6: Substrate-level phosphorylation
7. Reaction 7: The third oxidation
8–9. Reactions 8 and 9: Regeneration of oxaloacetate and the fourth oxidation

Figure 7.11 The Krebs cycle. This series of reactions takes place within the matrix of the mitochondrion. For the complete breakdown of a molecule of glucose, the two molecules of acetyl-CoA produced by glycolysis and pyruvate oxidation each have to make a trip around the Krebs cycle. Follow the different carbons through the cycle, and notice the changes that occur in the carbon skeletons of the molecules and where oxidation reactions take place as they proceed through the cycle.



7.5 The Electron Transport Chain and Chemiosmosis

Learning Outcomes

1. *Describe the structure and function of the electron transport chain.*
2. *Understand how the proton gradient connects electron transport with ATP synthesis.*

The NADH and $FADH_2$ molecules formed during aerobic respiration each contain a pair of electrons that were gained when NAD^+ and FAD were reduced. The NADH molecules carry their electrons to the inner mitochondrial membrane, where they transfer the electrons to a series of membrane-associated proteins collectively called the *electron transport chain*.

The electron transport chain produces a proton gradient

The first of the proteins to receive the electrons is a complex, membrane-embedded enzyme called **NADH dehydrogenase.** A carrier called *ubiquinone* then passes the electrons to a protein–cytochrome complex called the *bc_1 complex*. Each complex in the chain operates as a proton pump, driving a proton out across the membrane into the intermembrane space (figure 7.12*a*).

The electrons are then carried by another carrier, *cytochrome c*, to the cytochrome oxidase complex. This complex uses four electrons to reduce a molecule of oxygen. Each oxygen then combines with two protons to form water:

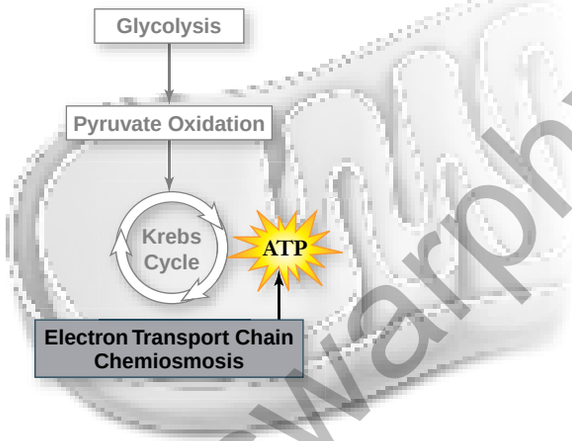
$$O_2 + 4\,H^+ + 4\,e^- \longrightarrow 2\,H_2O$$

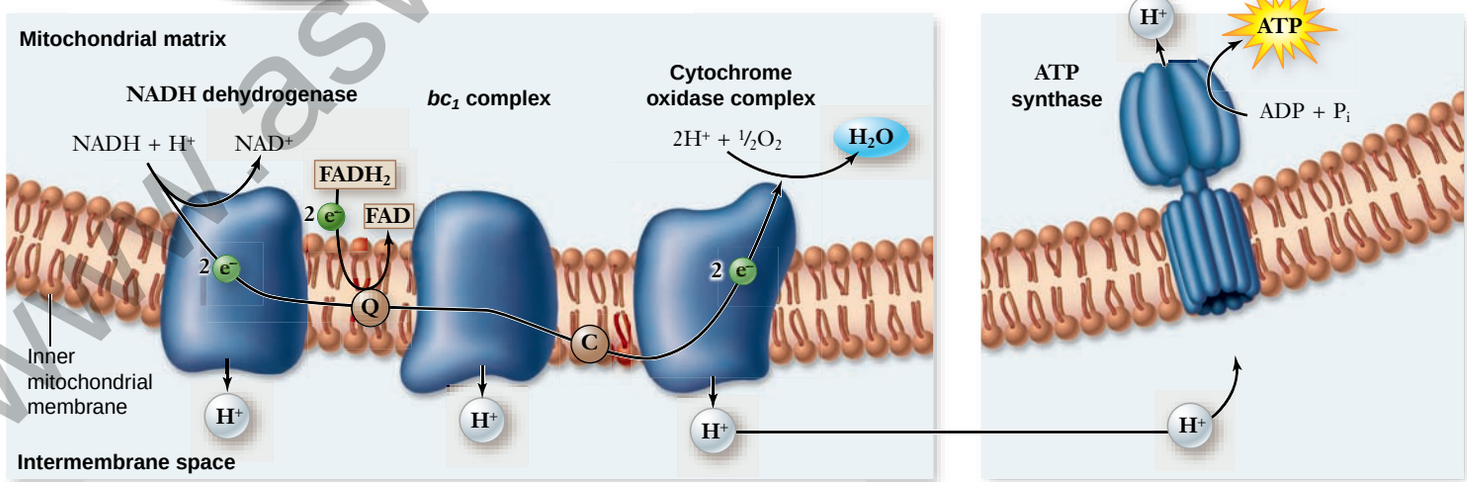
In contrast to NADH, which contributes its electrons to NADH dehydrogenase, $FADH_2$, which is located in the inner mitochondrial membrane, feeds its electrons to ubiquinone, which is also in the membrane. Electrons from $FADH_2$ thus "skip" the first step in the electron transport chain.

The plentiful availability of a strong electron acceptor, oxygen, is what makes oxidative respiration possible. As you'll see in chapter 8, the electron transport chain used in aerobic respiration is similar to, and may well have evolved from, the chain employed in photosynthesis.

The gradient forms as electrons move through electron carriers

Respiration takes place within the mitochondria present in virtually all eukaryotic cells. The internal compartment, or matrix, of a mitochondrion contains the enzymes that carry out the reactions of the Krebs cycle. As mentioned earlier, protons (H^+) are produced when electrons are transferred to NAD^+. As the electrons harvested





a. The electron transport chain

b. Chemiosmosis

Figure 7.12 The electron transport chain and chemiosmosis. ***a.*** High-energy electrons harvested from catabolized molecules are transported by mobile electron carriers (ubiquinone, marked Q, and cytochrome c, marked C) between three complexes of membrane proteins. These three complexes use portions of the electrons' energy to pump protons out of the matrix and into the intermembrane space. The electrons are finally used to reduce oxygen, forming water. ***b.*** This creates a concentration gradient of protons across the inner membrane. This electrochemical gradient is a form of potential energy that can be used by ATP synthase. This enzyme couples the reentry of protons to the phosphorylation of ADP to form ATP.

by oxidative respiration are passed along the electron transport chain, the energy they release transports protons out of the matrix and into the outer compartment called the intermembrane space.

Three transmembrane complexes of the electron transport chain in the inner mitochondrial membrane actually accomplish the proton transport (see figure 7.12*a*). The flow of highly energetic electrons induces a change in the shape of pump proteins, which causes them to transport protons across the membrane. The electrons contributed by NADH activate all three of these proton pumps, whereas those contributed by $FADH_2$ activate only two because of where they enter the chain. In this way a proton gradient is formed between the intermembrane space and the matrix.

Chemiosmosis utilizes the electrochemical gradient to produce ATP

Because the mitochondrial matrix is negative compared with the intermembrane space, positively charged protons are attracted to the matrix. The higher outer concentration of protons also tends to drive protons back in by diffusion, but because membranes are relatively impermeable to ions, this process occurs only very slowly. Most of the protons that reenter the matrix instead pass through ATP synthase, an enzyme that uses the energy of the gradient to catalyze the synthesis of ATP from ADP and P_i. Because the chemical formation of ATP is driven by a diffusion force similar to osmosis, this process is referred to as *chemiosmosis* (figure 7.12*b*). The newly formed ATP is transported by facilitated diffusion to the many places in the cell where enzymes require energy to drive endergonic reactions. This chemiosmotic mechanism for the coupling of electron transport and ATP synthesis was controversial when it was proposed. Over the years, experimental evidence accumulated to support this hypothesis (figure 7.13).

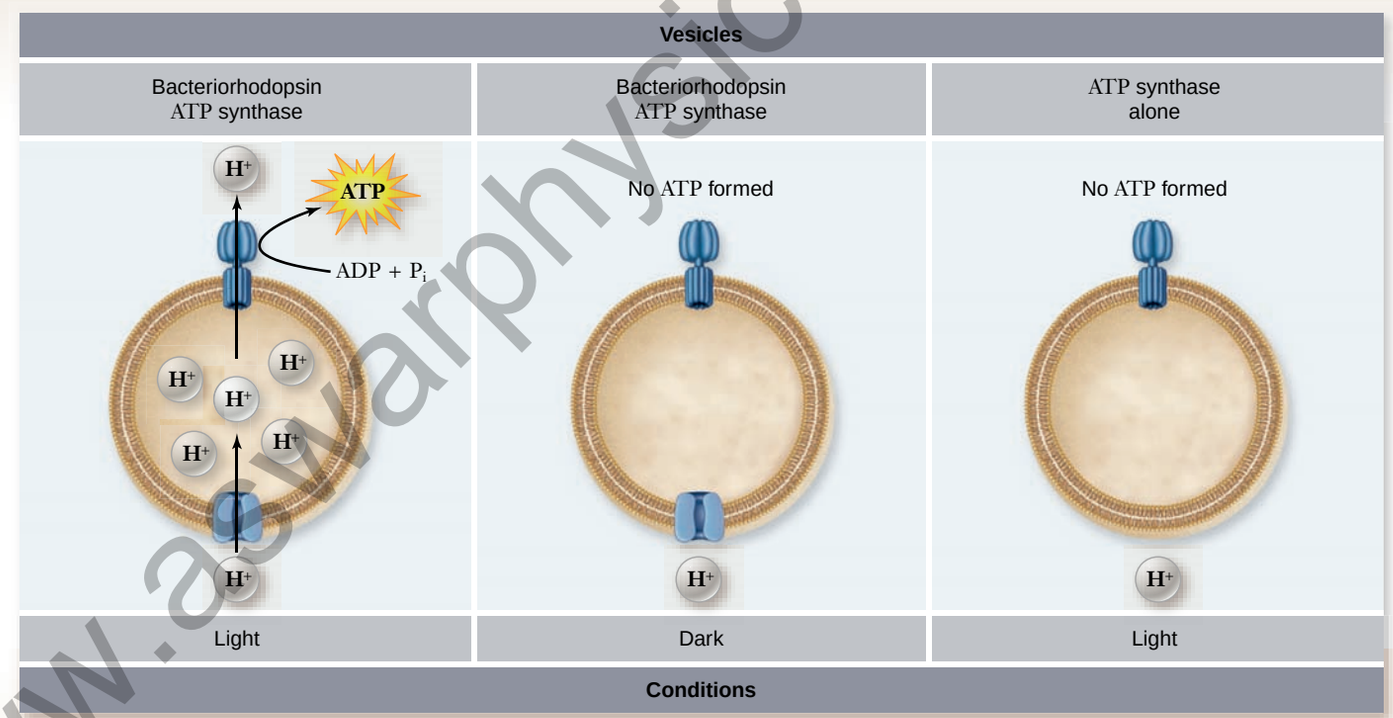
The energy released by the reactions of cellular respiration ultimately drives the proton pumps that produce the

SCIENTIFIC THINKING

Hypothesis: *ATP synthase enzyme uses a proton gradient to provide energy for phosphorylation reaction.*

Prediction: *The source of the proton gradient should not matter. A proton gradient formed by the light-driven pump bacteriorhodopsin should power phosphorylation in the light but not in the dark.*

Test: *Artificial vesicles are made with bacteriorhodopsin and ATP synthase, and ATP synthase alone. These are illuminated with light and assessed for ATP production.*



Result: *The vesicle with both bacteriorhodopsin and ATP synthase can form ATP in the light but not in the dark. The vesicle with ATP synthase alone cannot form ATP in the light.*

Conclusion: *ATP synthase is able to utilize a proton gradient for energy to form ATP.*

Further Experiments: *What other controls would be appropriate for this type of experiment? Why is this experiment a better test of the chemiosmotic hypothesis than the acid bath experiment in Jangendorf/chapter 8 (see figure 8.16)?*

Figure 7.13 **Evidence for the chemiosmotic synthesis of ATP by ATP synthase.**

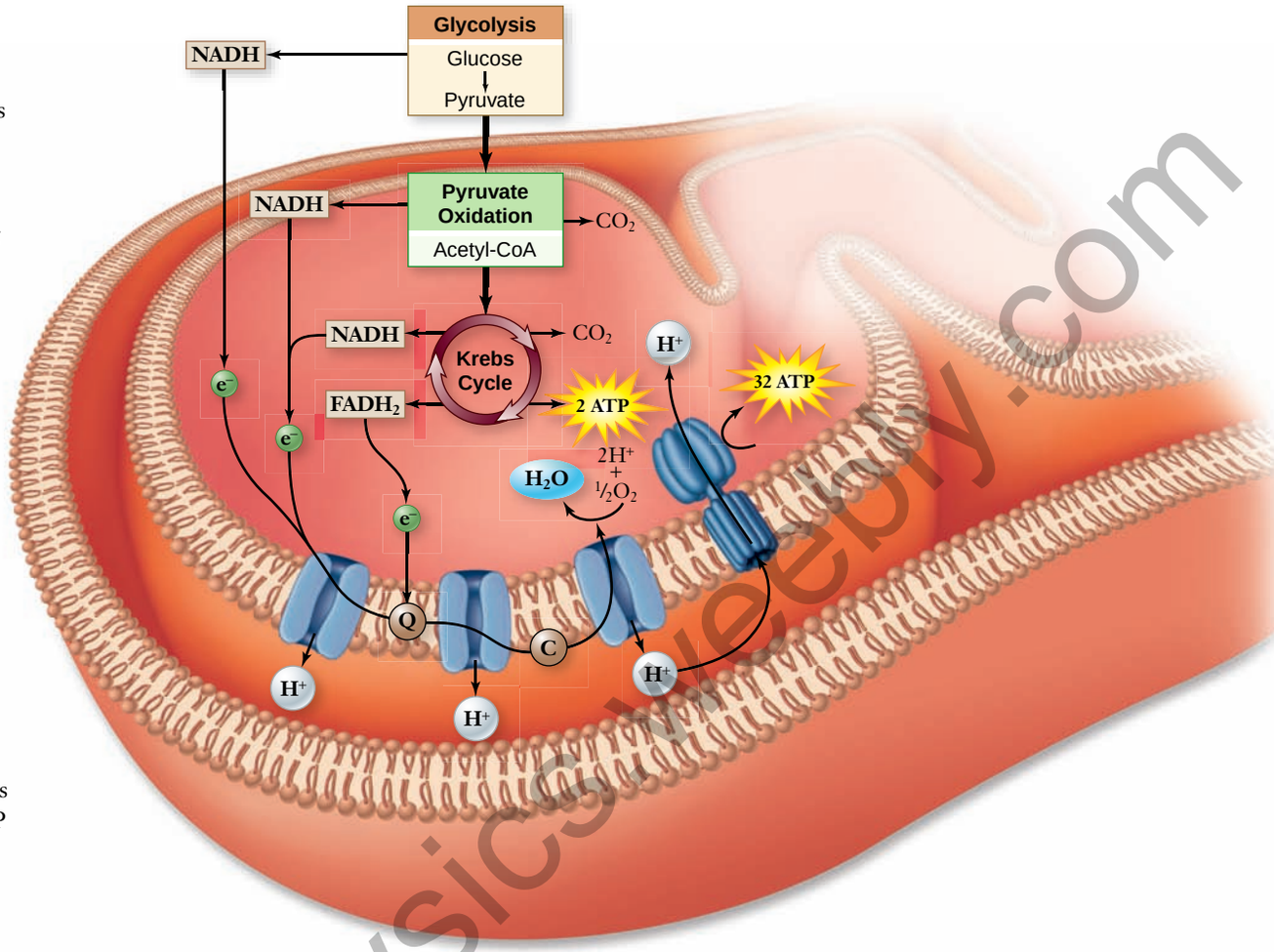


Figure 7.14 Aerobic respiration in the mitochondria. The entire process of aerobic respiration is shown in cellular context. Glycolysis occurs in the cytoplasm with the pyruvate and NADH produced entering the mitochondria. Here, pyruvate is oxidized and fed into the Krebs cycle to complete the oxidation process. All the energetic electrons harvested by oxidations in the overall process are transferred by NADH and $FADH_2$ to the electron transport chain. The electron transport chain uses the energy released during electron transport to pump protons across the inner membrane. This creates an electrochemical gradient that contains potential energy. The enzyme ATP synthase uses this gradient to phosphorylate ADP to form ATP.

proton gradient. The proton gradient provides the energy required for the synthesis of ATP. Figure 7.14 summarizes the overall process.

ATP synthase is a molecular rotary motor

ATP synthase uses a fascinating molecular mechanism to perform ATP synthesis (figure 7.15). Structurally, the enzyme has a membrane-bound portion and a narrow stalk that connects the membrane portion to a knoblike catalytic portion. This complex can be dissociated into two subportions: the F_0 membrane-bound complex, and the F_1 complex composed of the stalk and a knob, or head domain.

The F_1 complex has enzymatic activity. The F_0 complex contains a channel through which protons move across the membrane down their concentration gradient. As they do so, their movement causes part of the F_0 complex and the stalk to rotate relative to the knob. The mechanical energy of this rotation is used to change the conformation of the catalytic domain in the F_1 complex.

Thus, the synthesis of ATP is achieved by a tiny rotary motor, the rotation of which is driven directly by a gradient of protons. The flow of protons is like that of water in a hydroelectric power plant. Like the flow of water driven by gravity causes a turbine to rotate and generate electrical current, the proton gradient produces the energy that drives the rotation of the ATP synthase generator.

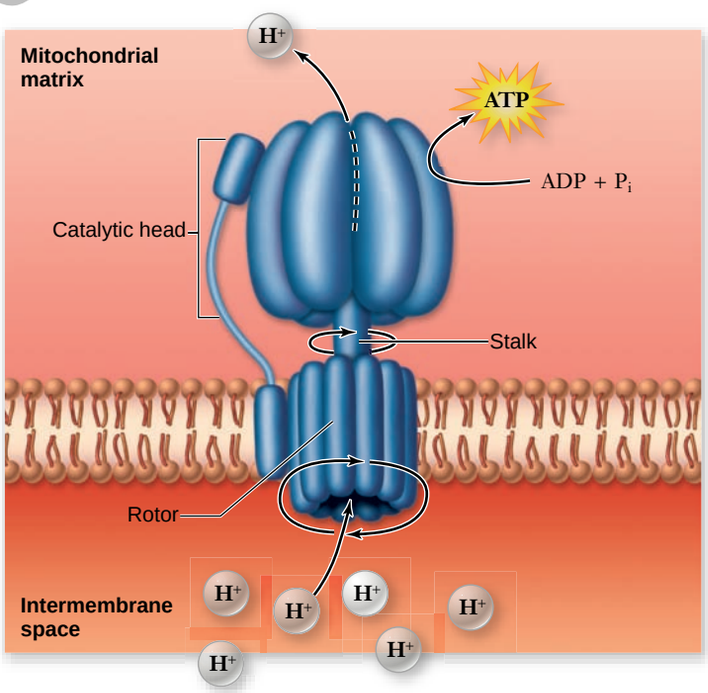


Figure 7.15 The ATP rotary engine. Protons move across the membrane down their concentration gradient. The energy released causes the rotor and stalk structures to rotate. This mechanical energy alters the conformation of the ATP synthase enzyme to catalyze the formation of ATP.

Learning Outcomes Review 7.5

The electron transport chain receives electrons from NADH and $FADH_2$ and passes them down the chain to oxygen. The protein complexes of the electron transport chain, in the inner membrane of mitochondria, use the energy from electron transfer to pump protons across the membrane, creating an electrochemical gradient. The enzyme ATP synthase uses this gradient to drive the endergonic reaction of phosphorylating ADP to ATP.

- *How would poking a small hole in the outer membrane affect ATP synthesis?*

7.6 Energy Yield of Aerobic Respiration

Learning Outcome

1. *Calculate the number of ATP molecules produced by aerobic respiration.*

How much metabolic energy in the form of ATP does a cell gain from aerobic breakdown of glucose? Knowing the steps involved in the process, we can calculate the theoretical yield of ATP and compare it with the actual yield.

The theoretical yield for eukaryotes is 36 molecules of ATP per glucose molecule

The chemiosmotic model suggests that one ATP molecule is generated for each proton pump activated by the electron transport chain. Because the electrons from NADH activate three pumps and those from $FADH_2$ activate two, we would expect each molecule of NADH and $FADH_2$ to generate three and two ATP molecules, respectively.

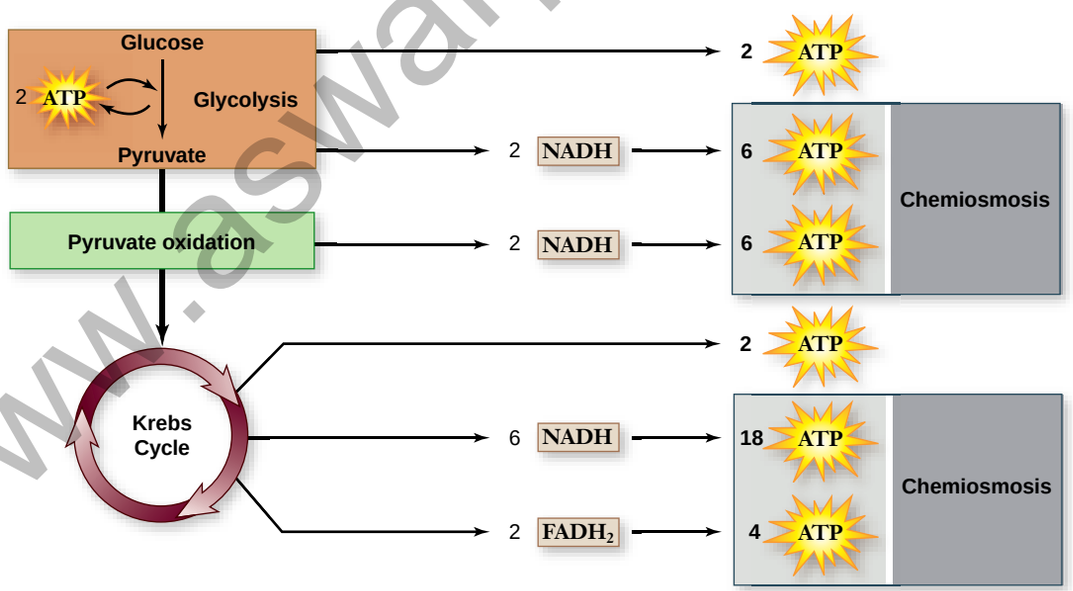
In doing this accounting, remember that everything downstream of glycolysis must be multiplied by 2 because two pyruvates are produced per molecule of glucose. A total of 10 NADH molecules is generated by respiration: 2 from glycolysis, 2 from the oxidation of pyruvate (1×2), and another 6 from the Krebs cycle (3×2). Also, two $FADH_2$ are produced (1×2). Finally, two ATP are generated directly by glycolysis and another two ATP from the Krebs cycle (1×2). This gives a total of $10 \times 3 = 30$ ATP from NADH, plus $2 \times 2 = 4$ ATP from $FADH_2$, plus 4 ATP, for a total of 38 ATP (figure 7.16).

This number is accurate for bacteria, but it does not hold for eukaryotes because the NADH produced in the cytoplasm by glycolysis needs to be transported into the mitochondria by active transport, which costs one ATP per NADH transported. This reduces the predicted yield for eukaryotes to 36 ATP.

The actual yield for eukaryotes is 30 molecules of ATP per glucose molecule

The amount of ATP actually produced in a eukaryotic cell during aerobic respiration is somewhat lower than 36, for two reasons. First, the inner mitochondrial membrane is somewhat "leaky" to protons, allowing some of them to reenter the matrix without passing through ATP synthase. Second, mitochondria often use the proton gradient generated by chemiosmosis for purposes other than ATP synthesis (such as transporting pyruvate into the matrix).

Consequently, the actual measured values of ATP generated by NADH and $FADH_2$ are closer to 2.5 for each NADH, and 1.5 for each $FADH_2$. With these corrections, the overall harvest of ATP from a molecule of glucose in a eukaryotic cell is calculated as: 4 ATP from substrate-level



Total net ATP yield = **38**
(36 in eukaryotes)

Figure 7.16 Theoretical ATP yield. The theoretical yield of ATP harvested from glucose by aerobic respiration totals 38 molecules. In eukaryotes this is reduced to 36 because it takes 1 ATP to transport each molecule of NADH that is generated by glycolysis in the cytoplasm into the mitochondria.

phosphorylation + 25 ATP from NADH (2.5 × 10) + 3 ATP from $FADH_2$ (1.5 × 2) − 2 ATP for transport of glycolytic NADH = 30 molecules of ATP.

We mentioned earlier that the catabolism of glucose by aerobic respiration, in contrast to that by glycolysis alone, has a large energy yield. Aerobic respiration in a eukaryotic cell harvests about (7.3 × 30)/686 = 32% of the energy available in glucose. (By comparison, a typical car converts only about 25% of the energy in gasoline into useful energy.)

The higher yield of aerobic respiration was one of the key factors that fostered the evolution of heterotrophs. As this mechanism for producing ATP evolved, nonphotosynthetic organisms could more successfully base their metabolism on the exclusive use of molecules derived from other organisms. As long as some organisms captured energy by photosynthesis, others could exist solely by feeding on them.

Learning Outcome Review 7.6

Passage of electrons down the electron transport chain produces roughly three ATP per NADH (two ATP per $FADH_2$). This process plus the ATP generated by substrate-level phosphorylation could yield a maximum of 38 ATP for the complete oxidation of glucose. But NADH generated in the cytoplasm yields only two ATP/NADH because transporting the NADH into the mitochondria uses ATP. Therefore the theoretical total is 36 ATP per glucose in eukaryotes.

- ***Why is the expected yield not necessarily the same as the actual yield in a cell?***

7.7 Regulation of Aerobic Respiration

Learning Outcome

1. ***Understand the control points for cellular respiration.***

When cells possess plentiful amounts of ATP, the key reactions of glycolysis, the Krebs cycle, and fatty acid breakdown are inhibited, slowing ATP production. The regulation of these biochemical pathways by the level of ATP is an example of feedback inhibition. Conversely, when ATP levels in the cell are low, ADP levels are high, and ADP activates enzymes in the pathways of carbohydrate catabolism to stimulate the production of more ATP.

Control of glucose catabolism occurs at two key points in the catabolic pathway, namely at a point in glycolysis and at the beginning of the Krebs cycle (figure 7.17). The control point in glycolysis is the enzyme phosphofructokinase, which catalyzes the conversion of fructose phosphate to fructose bisphosphate. This is the first reaction of glycolysis that is not readily reversible, committing the substrate to the glycolytic sequence. ATP itself is an allosteric inhibitor (see chapter 6) of phosphofructokinase, as is the Krebs cycle intermediate citrate. High levels of both ATP and citrate inhibit phosphofructokinase. Thus, under conditions when

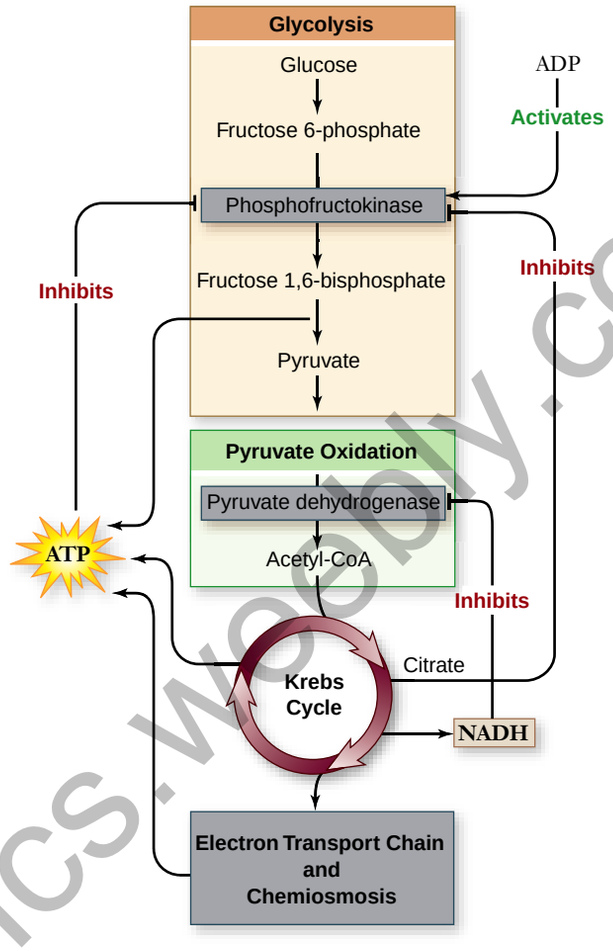


Figure 7.17 Control of glucose catabolism. The relative levels of ADP and ATP and key intermediates NADH and citrate control the catabolic pathway at two key points: the committing reactions of glycolysis and the Krebs cycle.

ATP is in excess, or when the Krebs cycle is producing citrate faster than it is being consumed, glycolysis is slowed.

The main control point in the oxidation of pyruvate occurs at the committing step in the Krebs cycle with the enzyme pyruvate dehydrogenase, which converts pyruvate to acetyl-CoA. This enzyme is inhibited by high levels of NADH, a key product of the Krebs cycle.

Another control point in the Krebs cycle is the enzyme citrate synthetase, which catalyzes the first reaction, the conversion of oxaloacetate and acetyl-CoA into citrate. High levels of ATP inhibit citrate synthetase (as well as phosphofructokinase, pyruvate dehydrogenase, and two other Krebs cycle enzymes), slowing down the entire catabolic pathway.

Learning Outcome Review 7.7

Respiration is controlled by levels of ATP in the cell and levels of key intermediates in the process. The control point for glycolysis is the enzyme phosphofructokinase, which is inhibited by ATP or citrate (or both). The main control point in oxidation of pyruvate is the enzyme pyruvate dehydrogenase, inhibited by NADH.

- ***How does feedback inhibition ensure economic production of ATP?***

7.8 Oxidation Without O_2

Learning Outcomes

1. *Compare anaerobic and aerobic respiration.*
2. *Distinguish the role of fermentation in anaerobic metabolism.*

In the presence of oxygen, cells can use oxygen to produce a large amount of ATP. But even when no oxygen is present to accept electrons, some organisms can still respire *anaerobically*, using inorganic molecules as final electron acceptors for an electron transport chain.

For example, many prokaryotes use sulfur, nitrate, carbon dioxide, or even inorganic metals as the final electron acceptor in place of oxygen (figure 7.18). The free energy released by using these other molecules as final electron acceptors is not as great as that using oxygen because they have a lower affinity for electrons. The amount of ATP produced is less, but the process is still respiration and not fermentation.

Methanogens use carbon dioxide

Among the heterotrophs that practice anaerobic respiration are Archaea such as thermophiles and methanogens. Methanogens use carbon dioxide (CO_2) as the electron acceptor, reducing CO_2 to CH_4 (methane). The hydrogens are derived from organic molecules produced by other organisms. Methanogens are found in diverse environments, including soil and the digestive systems of ruminants like cows.

Sulfur bacteria use sulfate

Evidence of a second anaerobic respiratory process among primitive bacteria is seen in a group of rocks about 2.7 BYA, known as the Woman River iron formation. Organic material in these rocks is enriched for the light isotope of sulfur, ^{32}S, relative to the heavier isotope, ^{34}S. No known geochemical process produces such enrichment, but biological sulfur reduction does, in a process still carried out today by certain prokaryotes.

In this sulfate respiration, the prokaryotes derive energy from the reduction of inorganic sulfates (SO_4) to hydrogen sulfide (H_2S). The hydrogen atoms are obtained from organic molecules other organisms produce. These prokaryotes thus are similar to methanogens, but they use SO_4 as the oxidizing (that is, electron-accepting) agent in place of CO_2.

The early sulfate reducers set the stage for the evolution of photosynthesis, creating an environment rich in H_2S. As discussed in chapter 8, the first form of photosynthesis obtained hydrogens from H_2S using the energy of sunlight.

Fermentation uses organic compounds as electron acceptors

In the absence of oxygen, cells that cannot utilize an alternative electron acceptor for respiration must rely exclusively on

Figure 7.18 Sulfur-respiring prokaryote. *a.* The micrograph shows the archaeal species *Thermoproteus tenax*. This organism can use elemental sulfur as a final electron acceptor for anaerobic respiration. ***b.*** *Thermoproteus* is often found in sulfur-containing hot springs such as the Norris Geyser Basin in Yellowstone National Park shown here.

glycolysis to produce ATP. Under these conditions, the electrons generated by glycolysis are donated to organic molecules in a process called *fermentation*. This process recycles NAD^+, the electron acceptor that allows glycolysis to proceed.

Bacteria carry out more than a dozen kinds of fermentation reactions, often using pyruvate or a derivative of pyruvate to accept the electrons from NADH. Organic molecules other than pyruvate and its derivatives can be used as well; the important point is that the process regenerates NAD^+:

$$\text{organic molecule} + \text{NADH} \longrightarrow \text{reduced organic molecule} + NAD^+$$

Often the reduced organic compound is an organic acid—such as acetic acid, butyric acid, propionic acid, or lactic acid—or an alcohol.

Ethanol fermentation

Eukaryotic cells are capable of only a few types of fermentation. In one type, which occurs in yeast, the molecule that accepts electrons from NADH is derived from pyruvate, the end-product of glycolysis.

Yeast enzymes remove a terminal CO_2 group from pyruvate through decarboxylation, producing a 2-carbon molecule called acetaldehyde. The CO_2 released causes bread made with yeast to rise. The acetaldehyde accepts a pair of electrons from NADH, producing NAD^+ and ethanol (ethyl alcohol) (figure 7.19).

This particular type of fermentation is of great interest to humans, because it is the source of the ethanol in wine and beer. Ethanol is a by-product of fermentation that is actually toxic to yeast; as it approaches a concentration of about 12%, it begins to kill the yeast. That explains why naturally fermented wine contains only about 12% ethanol.

Lactic acid fermentation

Most animal cells regenerate NAD^+ without decarboxylation. Muscle cells, for example, use the enzyme lactate dehydrogenase to transfer electrons from NADH back to the pyruvate that is produced by glycolysis. This reaction converts pyruvate into lactic acid and regenerates NAD^+ from NADH (see figure 7.19). It therefore closes the metabolic circle, allowing glycolysis to continue as long as glucose is available.

Circulating blood removes excess lactate, the ionized form of lactic acid, from muscles, but when removal cannot keep pace with production, the accumulating lactic acid interferes with muscle function and contributes to muscle fatigue.

Learning Outcomes Review 7.8

Nitrate, sulfur, and CO_2 are all used as terminal electron acceptors in anaerobic respiration of different organisms. Organic molecules can also accept electrons in fermentation reactions that regenerate NAD^+. Fermentation reactions produce a variety of compounds, including ethanol in yeast and lactic acid in humans.

- ***In what kinds of ecosystems would you expect to find anaerobic respiration?***

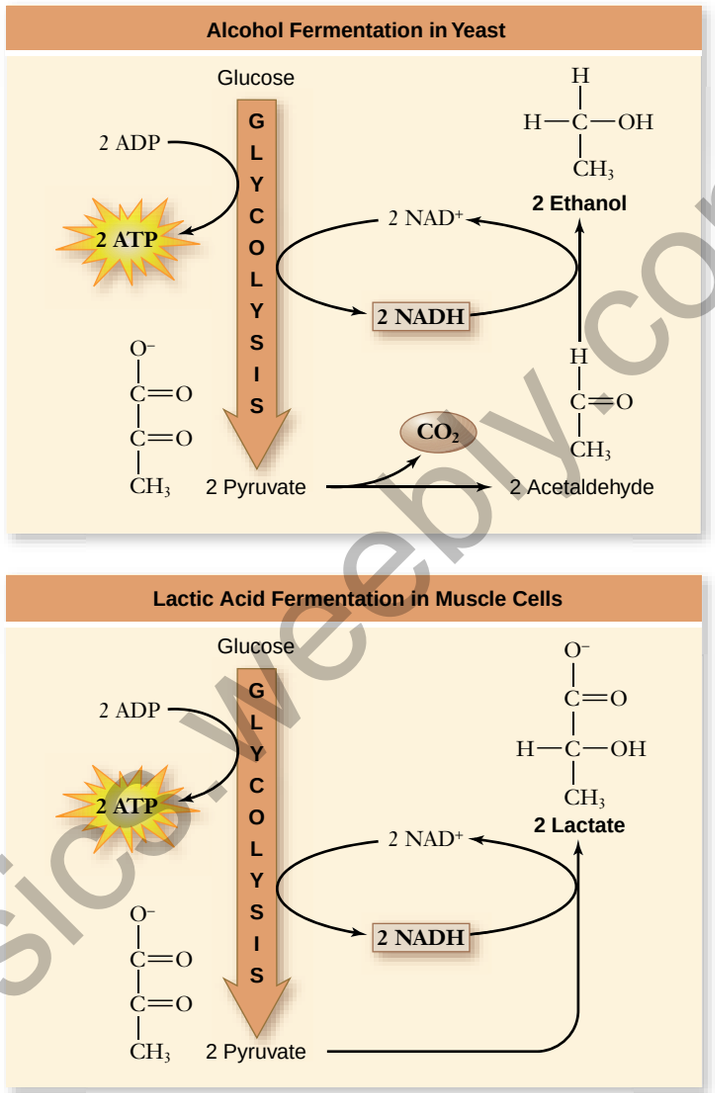


Figure 7.19 Fermentation. Yeasts carry out the conversion of pyruvate to ethanol. Muscle cells convert pyruvate into lactate, which is less toxic than ethanol. In each case, the reduction of a metabolite of glucose has oxidized NADH back to NAD^+ to allow glycolysis to continue under anaerobic conditions.

7.9 Catabolism of Proteins and Fats

Learning Outcomes

1. ***Identify the points at which proteins and fats enter energy metabolism.***
2. ***Describe the linkages between catabolic and anabolic pathways.***

Thus far we have focused on the aerobic respiration of glucose, which organisms obtain from the digestion of carbohydrates or from photosynthesis. Organic molecules other than glucose,

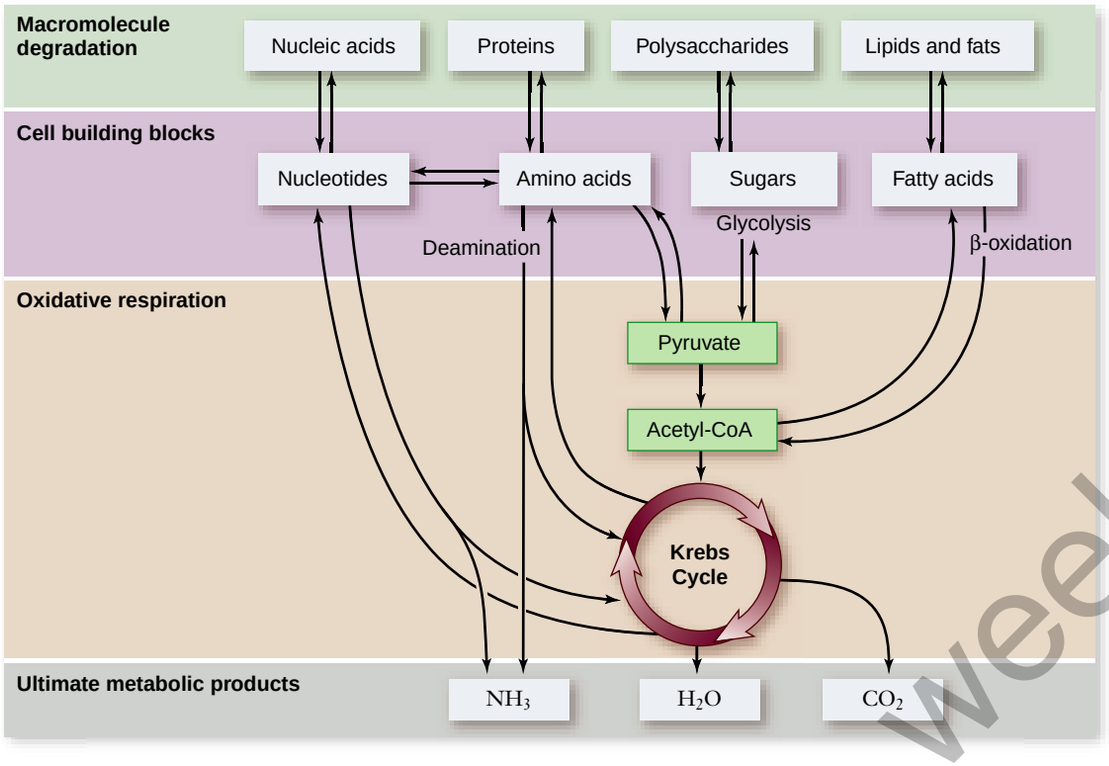


Figure 7.20 How cells extract chemical energy. All eukaryotes and many prokaryotes extract energy from organic molecules by oxidizing them. The first stage of this process, breaking down macromolecules into their constituent parts, yields little energy. The second stage, oxidative or aerobic respiration, extracts energy, primarily in the form of high-energy electrons, and produces water and carbon dioxide. Key intermediates in these energy pathways are also used for biosynthetic pathways, shown by reverse arrows.

particularly proteins and fats, are also important sources of energy (figure 7.20).

Catabolism of proteins removes amino groups

Proteins are first broken down into their individual amino acids. The nitrogen-containing side group (the amino group) is then removed from each amino acid in a process called **deamination.** A series of reactions converts the carbon chain that remains into a molecule that enters glycolysis or the Krebs cycle. For example, alanine is converted into pyruvate, glutamate into α-ketoglutarate (figure 7.21), and aspartate into oxaloacetate. The reactions of glycolysis and the Krebs cycle then extract the high-energy electrons from these molecules and put them to work making ATP.

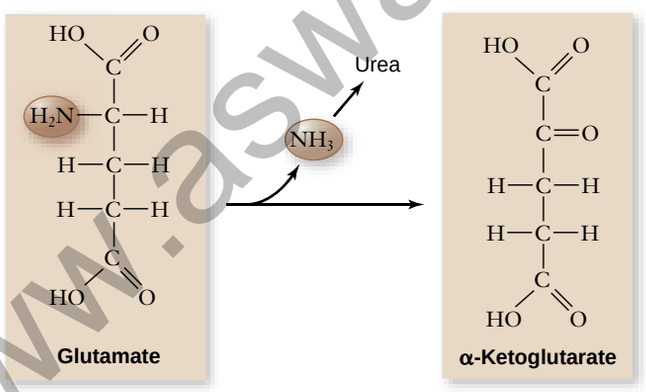


Figure 7.21 Deamination. After proteins are broken down into their amino acid constituents, the amino groups are removed from the amino acids to form molecules that participate in glycolysis and the Krebs cycle. For example, the amino acid glutamate becomes α-ketoglutarate, a Krebs cycle intermediate, when it loses its amino group.

Catabolism of fatty acids produces acetyl groups

Fats are broken down into fatty acids plus glycerol. Long-chain fatty acids typically have an even number of carbons, and the many C—H bonds provide a rich harvest of energy. Fatty acids are oxidized in the matrix of the mitochondrion. Enzymes remove the 2-carbon acetyl groups from the end of each fatty acid until the entire fatty acid is converted into acetyl groups (figure 7.22). Each acetyl group is combined with coenzyme A to form acetyl-CoA. This process is known as **β oxidation.** This process is oxygen-dependent, which explains why aerobic exercise burns fat, but anaerobic exercise does not.

How much ATP does the catabolism of fatty acids produce? Let's compare a hypothetical 6-carbon fatty acid with the 6-carbon glucose molecule, which we've said yields about 30 molecules of ATP in a eukaryotic cell. Two rounds of β oxidation would convert the fatty acid into three molecules of acetyl-CoA. Each round requires one molecule of ATP to prime the process, but it also produces one molecule of NADH and one of $FADH_2$. These molecules together yield four molecules of ATP (assuming 2.5 ATPs per NADH, and 1.5 ATPs per $FADH_2$).

The oxidation of each acetyl-CoA in the Krebs cycle ultimately produces an additional 10 molecules of ATP. Overall, then, the ATP yield of a 6-carbon fatty acid is approximately: 8 (from two rounds of β oxidation) − 2 (for priming those two rounds) + 30 (from oxidizing the three acetyl-CoAs) = 36 molecules of ATP. Therefore, the respiration of a 6-carbon fatty acid yields 20% more ATP than the respiration of glucose.

Moreover, a fatty acid of that size would weigh less than two thirds as much as glucose, so a gram of fatty acid contains more than twice as many kilocalories as a gram of glucose. You can see from this fact why fat is a storage molecule for excess

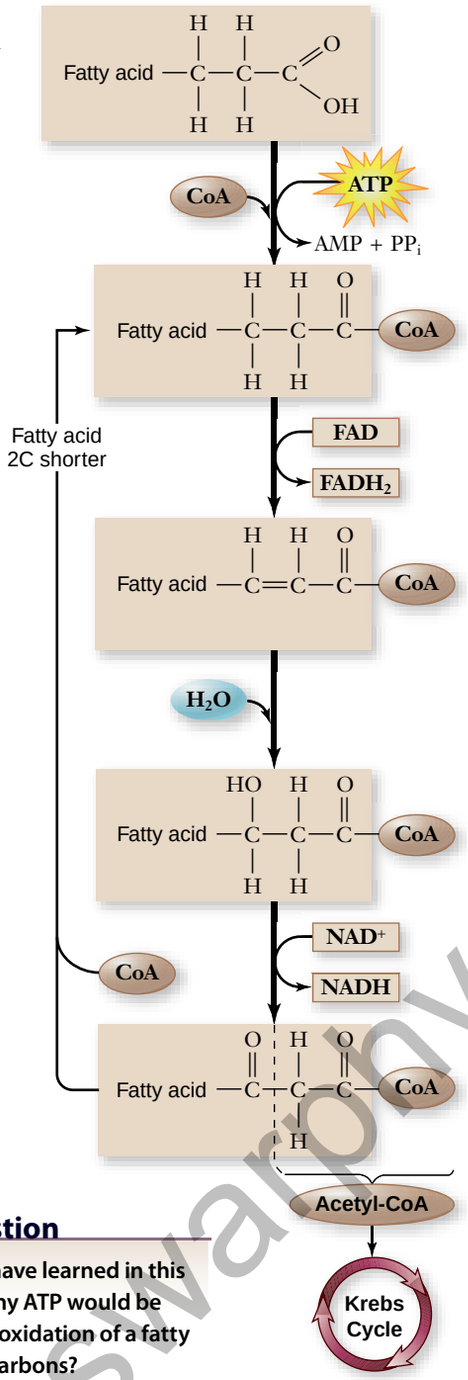


Figure 7.22
β oxidation. Through a series of reactions known as β oxidation, the last two carbons in a fatty acid combine with coenzyme A to form acetyl-CoA, which enters the Krebs cycle. The fatty acid, now two carbons shorter, enters the pathway again and keeps reentering until all its carbons have been used to form acetyl-CoA molecules. Each round of β oxidation uses one molecule of ATP and generates one molecule each of $FADH_2$ and NADH.

Inquiry question

Given what you have learned in this chapter, how many ATP would be produced by the oxidation of a fatty acid that has 16 carbons?

energy in many types of animals. If excess energy were stored instead as carbohydrate, as it is in plants, animal bodies would have to be much bulkier.

A small number of key intermediates connect metabolic pathways

Oxidation pathways of food molecules are interrelated in that a small number of key intermediates, such as pyruvate and acetyl-CoA, link the breakdown from different starting points. These key intermediates allow the interconversion of different types of molecules, such as sugars and amino acids (see figure 7.20).

Cells can make glucose, amino acids, and fats, as well as getting them from external sources. They use reactions similar to those that break down these substances. In many cases, the reverse pathways even share enzymes if the free-energy changes are small. For example, gluconeogenesis, the process of making new glucose, uses all but three enzymes of the glycolytic pathway. Thus, much of glycolysis runs forward or backward, depending on the concentrations of the intermediates—with only three key steps having different enzymes for forward and reverse directions.

Acetyl-CoA has many roles

Many different metabolic processes generate acetyl-CoA. Not only does the oxidation of pyruvate produce it, but the metabolic breakdown of proteins, fats, and other lipids also generates acetyl-CoA. Indeed, almost all molecules catabolized for energy are converted into acetyl-CoA.

Acetyl-CoA has a role in anabolic metabolism as well. Units of two carbons derived from acetyl-CoA are used to build up the hydrocarbon chains in fatty acids. Acetyl-CoA produced from a variety of sources can therefore be channeled into fatty acid synthesis or into ATP production, depending on the organism's energy requirements. Which of these two options is taken depends on the level of ATP in the cell.

When ATP levels are high, the oxidative pathway is inhibited, and acetyl-CoA is channeled into fatty acid synthesis. This explains why many animals (humans included) develop fat reserves when they consume more food than their activities require. Alternatively, when ATP levels are low, the oxidative pathway is stimulated, and acetyl-CoA flows into energy-producing oxidative metabolism.

Learning Outcomes Review 7.9

Proteins can be broken into their constituent amino acids, which are then deaminated and can enter metabolism at glycolysis or different steps of the Krebs cycle. Fats can be broken into units of acetyl-CoA by β oxidation and then fed into the Krebs cycle. Many metabolic processes can be used reversibly, to either build up (anabolism) or break down (catabolism) the major biological macromolecules. Key intermediates, such as pyruvate and acetyl-CoA, connect these processes.

- ***Can fats be oxidized in the absence of O_2?***

7.10 *Evolution of Metabolism*

Learning Outcome

1. ***Describe one possible hypothesis for the evolution of metabolism.***

We talk about cellular respiration as a continuous series of stages, but it is important to note that these stages evolved over time, and metabolism has changed a great deal in that time.

Both anabolic processes and catabolic processes evolved in concert with each other. We do not know the details of this biochemical evolution, or the order of appearance of these processes. Therefore the following timeline is based on the available geochemical evidence and represents a hypothesis rather than a strict timeline.

The earliest life forms degraded carbon-based molecules present in the environment

The most primitive forms of life are thought to have obtained chemical energy by degrading, or breaking down, organic molecules that were abiotically produced, that is, carbon-containing molecules formed by inorganic processes on the early Earth.

The first major event in the evolution of metabolism was the origin of the ability to harness chemical bond energy. At an early stage, organisms began to store this energy in the bonds of ATP.

The evolution of glycolysis also occurred early

The second major event in the evolution of metabolism was glycolysis, the initial breakdown of glucose. As proteins evolved diverse catalytic functions, it became possible to capture a larger fraction of the chemical bond energy in organic molecules by breaking chemical bonds in a series of steps.

Glycolysis undoubtedly evolved early in the history of life on Earth, because this biochemical pathway has been retained by all living organisms. It is a chemical process that does not appear to have changed for more than 2 billion years.

Anoxygenic photosynthesis allowed the capture of light energy

The third major event in the evolution of metabolism was anoxygenic photosynthesis. Early in the history of life, a different way of generating ATP evolved in some organisms. Instead of obtaining energy for ATP synthesis by reshuffling chemical bonds, as in glycolysis, these organisms developed the ability to use light to pump protons out of their cells and to use the resulting proton gradient to power the production of ATP through chemiosmosis.

Photosynthesis evolved in the absence of oxygen and works well without it. Dissolved H_2S, present in the oceans of the early Earth beneath an atmosphere free of oxygen gas, served as a ready source of hydrogen atoms for building organic molecules. Free sulfur was produced as a by-product of this reaction.

Oxygen-forming photosynthesis used a different source of hydrogen

The substitution of H_2O for H_2S in photosynthesis was the fourth major event in the history of metabolism. Oxygen-forming photosynthesis employs H_2O rather than H_2S as a source of hydrogen atoms and their associated electrons. Because it garners its electrons from reduced oxygen rather than from reduced sulfur, it generates oxygen gas rather than free sulfur.

More than 2 BYA, small cells capable of carrying out this oxygen-forming photosynthesis, such as cyanobacteria, became the dominant forms of life on Earth. Oxygen gas began to accumulate in the atmosphere. This was the beginning of a great transition that changed conditions on Earth permanently. Our atmosphere is now 20.9% oxygen, every molecule of which is derived from an oxygen-forming photosynthetic reaction.

Nitrogen fixation provided new organic nitrogen

Nitrogen is available from dead organic matter, and from chemical reactions that generated the original organic molecules. For life to expand, a new source of nitrogen was needed. Nitrogen fixation was the fifth major step in the evolution of metabolism. Proteins and nucleic acids cannot be synthesized from the products of photosynthesis because both of these biologically critical molecules contain nitrogen. Obtaining nitrogen atoms from N_2 gas, a process called *nitrogen fixation*, requires breaking an $N{\equiv}N$ triple bond.

This important reaction evolved in the hydrogen-rich atmosphere of the early Earth, where no oxygen was present. Oxygen acts as a poison to nitrogen fixation, which today occurs only in oxygen-free environments or in oxygen-free compartments within certain prokaryotes.

Aerobic respiration utilized oxygen

Respiration is the sixth and final event in the history of metabolism. Aerobic respiration employs the same kind of proton pumps as photosynthesis and is thought to have evolved as a modification of the basic photosynthetic machinery.

Biologists think that the ability to carry out photosynthesis without H_2S first evolved among purple nonsulfur bacteria, which obtain their hydrogens from organic compounds instead. It was perhaps inevitable that among the descendants of these respiring photosynthetic bacteria, some would eventually do without photosynthesis entirely, subsisting only on the energy and electrons derived from the breakdown of organic molecules. The mitochondria within all eukaryotic cells are thought to be descendants of these bacteria.

The complex process of aerobic metabolism developed over geological time, as natural selection favored organisms with more efficient methods of obtaining energy from organic molecules. The process of photosynthesis, as you have seen in this concluding section, has also developed over time, and the rise of photosynthesis changed life on Earth forever. The next chapter explores photosynthesis in detail.

Learning Outcome Review 7.10

Major milestones in the evolution of metabolism include the evolution of pathways to extract energy from organic compounds, the pathways of photosynthesis, and those of nitrogen fixation. Photosynthesis began as an anoxygenic process that later evolved to produce free oxygen, thus allowing the evolution of aerobic metabolism.

- ***What evidence can you cite for this hypothesis of the evolution of metabolism?***

Chapter Review

7.1 Overview of Respiration

Cells oxidize organic compounds to drive metabolism.

Cellular respiration is the complete oxidation of glucose.

Aerobic respiration uses oxygen as the final electron acceptor for redox reactions. Anaerobic respiration utilizes inorganic molecules as acceptors, and fermentation uses organic molecules.

Electron carriers play a critical role in energy metabolism.

Electron carriers can be reversibly oxidized and reduced. For example, NAD^+ is reduced to NADH by acquiring two electrons; NADH supplies these electrons to other molecules to reduce them.

Metabolism harvests energy in stages.

Mitochondria of eukaryotic cells move electrons in steps via the electron transport chain to capture energy efficiently.

ATP plays a central role in metabolism.

The ultimate goal of cellular respiration is synthesis of ATP, which is used to power most of the cell's activities.

Cells make ATP by two fundamentally different mechanisms.

Substrate-level phosphorylation transfers a phosphate directly to ADP (see figure 7.4). Oxidative phosphorylation generates ATP via the enzyme ATP synthase, powered by a proton gradient.

7.2 Glycolysis: Splitting Glucose (see figure 7.6)

Glycolysis converts glucose into two 3-carbon molecules of pyruvate. Each molecule of glucose yields two net ATP molecules.

Priming changes glucose into an easily cleaved form.

Priming reactions add two phosphates to glucose; this is cleaved into two 3-carbon molecules of glyceraldehyde 3-phosphate (G3P).

ATP is synthesized by substrate-level phosphorylation.

Oxidation of G3P transfers electrons to NAD^+, yielding NADH. After four more reactions, the final product is two molecules of pyruvate. Glycolysis produces 2 net ATP, 2 NADH, and 2 pyruvate.

NADH must be recycled into NAD^+ to continue respiration.

In the presence of oxygen, NADH passes electrons to the electron transport chain. In the absence of oxygen, NADH passes the electrons to an organic molecule such as acetaldehyde (fermentation).

7.3 The Oxidation of Pyruvate to Produce Acetyl-CoA

Pyruvate is oxidized to yield 1 CO_2, 1 NADH, and 1 acetyl-CoA. Acetyl-CoA enters the Krebs cycle as 2-carbon acetyl units.

7.4 The Krebs Cycle

The Krebs cycle extracts electrons and synthesizes one ATP.

The first reaction is an irreversible condensation that produces citrate; it is inhibited when ATP is plentiful. The second and third reactions rearrange citrate to isocitrate. The fourth and fifth reactions are oxidations; in each reaction, one NAD^+ is reduced to NADH. The sixth reaction is a substrate-level phosphorylation producing GTP, and from that ATP. The seventh reaction is another oxidation that reduces FAD to $FADH_2$. Reactions eight and nine regenerate oxaloacetate, including one final oxidation that reduces NAD^+ to NADH.

Glucose becomes CO_2 and potential energy.

As a glucose molecule is broken down to CO_2, some of its energy is preserved in 4 ATPs,10 NADH, and 2 $FADH_2$.

Following the electrons in the reactions reveals the direction of transfer.

7.5 The Electron Transport Chain and Chemiosmosis (see figure 7.12)

The electron transport chain produces a proton gradient.

In the inner mitochondrial membrane, NADH is oxidized to NAD^+ by NADH dehydrogenase. Electrons move through ubiquinone and the bc_1 complex to cytochrome oxidase, where they join with H^+ and O_2 to form H_2O. This results in three protons being pumped into the intermembrane space. For $FADH_2$, electrons are passed directly to ubiquinone. Thus only two protons are pumped into the intermembrane space.

The gradient forms as electrons move through electron carriers

Chemiosmosis utilizes the electrochemical gradient to produce ATP

ATP synthase is a molecular rotary motor.

Protons diffuse back into the mitochondrial matrix via the ATP synthase channel. The enzyme uses this energy to synthesize ATP (see figure 7.15).

7.6 Energy Yield of Aerobic Respiration

The theoretical yield for eukaryotes is 36 molecules of ATP per glucose molecule.

The actual yield for eukaryotes is 30 molecules of ATP per glucose molecule (see figure 7.16).

7.7 Regulation of Aerobic Respiration

Glucose catabolism is controlled by the concentration of ATP molecules and intermediates in the Krebs cycle (see figure 7.17).

7.8 Oxidation Without O_2 (see figure 7.8)

In the absence of oxygen other final electron acceptors can be used for respiration.

Methanogens use carbon dioxide.

Sulfur bacteria use sulfate.

Fermentation uses organic compounds as electron acceptors.

Fermentation is the regeneration of NAD^+ by oxidation of NADH and reduction of an organic molecule. In yeast, pyruvate is decarboxylated, then reduced to ethanol. In animals, pyruvate is reduced directly to lactate.

7.9 Catabolism of Proteins and Fats

Catabolism of proteins removes amino groups (see figure 7.20).

Catabolism of fatty acids produces acetyl groups.

Fatty acids are converted to acetyl groups by successive rounds of β-oxidation (see figure 7.22). These acetyl groups feed into the Krebs cycle to be oxidized and generate NADH for electron transport.

A small number of key intermediates connect metabolic pathways.

Acetyl-CoA has many roles.
With high ATP, acetyl-CoA is converted into fatty acids.

7.10 Evolution of Metabolism

Major milestones are recognized in the evolution of metabolism; the order of events is hypothetical.

The earliest life forms degraded carbon-based molecules present in the environment.

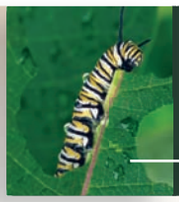
The evolution of glycolysis also occurred early.

Anoxygenic photosynthesis allowed the capture of light energy.

Oxygen-forming photosynthesis used a different source of hydrogen.

Nitrogen fixation provided new organic nitrogen.

Aerobic respiration utilized oxygen.



Review Questions

UNDERSTAND

1. An *autotroph* is an organism that
   a. extracts energy from organic sources.
   b. converts energy from sunlight into chemical energy.
   c. relies on the energy produced by other organisms as an energy source.
   d. does both a and b.
2. Which of the following processes is (are) required for the complete oxidation of glucose?
   a. The Krebs cycle
   b. Glycolysis
   c. Pyruvate oxidation
   d. All of the above
3. Which of the following is NOT a product of glycolysis?
   a. ATP
   b. Pyruvate
   c. CO_2
   d. NADH
4. Glycolysis produces ATP by
   a. phosphorylating organic molecules in the priming reactions.
   b. the production of glyceraldehyde 3-phosphate.
   c. substrate-level phosphorylation.
   d. the reduction of NAD^+ to NADH.
5. What is the role of NAD^+ in the process of cellular respiration?
   a. It functions as an electron carrier.
   b. It functions as an enzyme.
   c. It is the final electron acceptor for anaerobic respiration.
   d. It is a nucleotide source for the synthesis of ATP.
6. The reactions of the Krebs cycle occur in the
   a. inner membrane of the mitochondria.
   b. intermembrane space of the mitochondria.
   c. the cytoplasm.
   d. matrix of the mitochondria.
7. The electrons carried by NADH and $FADH_2$ can be
   a. pumped into the intermembrane space.
   b. transferred to the ATP synthase.
   c. moved between proteins in the inner membrane of the mitochondrion.
   d. transported into the matrix of the mitochondrion.

APPLY

1. Which of the following is NOT a true statement regarding cellular respiration?
   a. Enzymes catalyze reactions that transfer electrons.
   b. Electrons have a higher potential energy at the end of the process.
   c. Carbon dioxide gas is a by-product.
   d. The process involves multiple redox reactions.
2. The direct source of energy for the ATP produced by ATP synthase comes from
   a. the electron transport chain.
   b. the proton gradient.
   c. substrate-level phosphorylation.
   d. the oxidation reactions occurring during respiration.
3. Can cellular respiration occur in the absence of O_2?
   a. No, O_2 is necessary as the final electron acceptor.
   b. No, anaerobic organisms only need glycolysis and fermentation.
   c. Yes, because oxygen can be generated by splitting H_2O.
   d. Yes, but it requires an alternative to O_2 as a final electron acceptor.
4. Why is fermentation an important metabolic function in cells?
   a. It generates glucose for the cell in the absence of O_2.
   b. It oxidizes NADH to NAD^+.
   c. It oxidizes pyruvate.
   d. It produces ATP.
5. Which of the following statements is NOT true about the oxidation of pyruvate?
   a. Pyruvate oxidation occurs in the cytoplasm.
   b. Pyruvate oxidation only occurs if oxygen is present.
   c. Pyruvate is converted into acetyl-CoA.
   d. Pyruvate oxidation results in the production of NADH.
6. A chemical agent that makes holes in the inner membrane of the mitochondria would
   a. stop the movement of electrons down the electron transport chain.
   b. stop ATP synthesis.
   c. stop the Krebs cycle.
   d. all of the above.

7. Yeast cells that have mutations in genes that encode enzymes in glycolysis can still grow on glycerol. They are able to utilize glycerol because it
   a. enters glycolysis after the step affected by the mutation.
   b. can feed into the Krebs cycle and generate ATP via electron transport and chemiosmosis.
   c. can be utilized by fermentation.
   d. can donate electrons directly to the electron transport chain.

SYNTHESIZE

1. Use the following table to outline the relationship between the molecules and the metabolic reactions.

Molecules	Glycolysis	Cellular Respiration
Glucose		
Pyruvate		
Oxygen		
ATP		
CO_2		

2. Human babies and hibernating or cold-adapted animals are able to maintain body temperature (a process called *thermogenesis*) due to the presence of brown fat. Brown fat is characterized by a high concentration of mitochondria. These brown fat mitochondria have a special protein located within their inner membranes. *Thermogenin* is a protein that functions as a passive proton transporter. Propose a likely explanation for the role of brown fat in thermogenesis based on your knowledge of metabolism, transport, and the structure and function of mitochondria.

3. Recent data indicate a link between colder temperatures and weight loss. If adults retain brown fat, how could this be explained?

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Chapter 8

Photosynthesis

Chapter Outline

Introduction

The rich diversity of life that covers our Earth would be impossible without photosynthesis. Almost every oxygen atom in the air we breathe was once part of a water molecule, liberated by photosynthesis. All the energy released by the burning of coal, firewood, gasoline, and natural gas, and by our bodies' burning of all the food we eat—directly or indirectly—has been captured from sunlight by photosynthesis. It is vitally important, then, that we understand photosynthesis. Research may enable us to improve crop yields and land use, important goals in an increasingly crowded world. In chapter 7, we described how cells extract chemical energy from food molecules and use that energy to power their activities. In this chapter, we examine photosynthesis, the process by which organisms such as the aptly named sunflowers in the picture capture energy from sunlight and use it to build food molecules that are rich in chemical energy.

8.1 Overview of Photosynthesis

Learning Outcomes

1. *Explain the reaction for photosynthesis.*
2. *Describe the structure of the chloroplast.*

Life is powered by sunshine. The energy used by most living cells comes ultimately from the Sun and is captured by plants, algae, and bacteria through the process of photosynthesis.

The diversity of life is only possible because our planet is awash in energy streaming Earthward from the Sun. Each day, the radiant energy that reaches Earth equals the power from about 1 million Hiroshima-sized atomic bombs. Photosynthesis captures about 1% of this huge supply of energy (an amount equal to 10,000 Hiroshima bombs) and uses it to provide the energy that drives all life.

Photosynthesis combines CO_2 and H_2O, producing glucose and O_2

Photosynthesis occurs in a wide variety of organisms, and it comes in different forms. These include a form of photosynthesis that does not produce oxygen (anoxygenic) and a form that does (oxygenic). Anoxygenic photosynthesis is found in four different bacterial groups: purple bacteria, green sulfur bacteria, green nonsulfur bacteria, and heliobacteria. Oxygenic photosynthesis is found in cyanobacteria, seven groups of algae, and essentially all land plants. These two types of photosynthesis share similarities in the types of pigments they use to trap light energy, but they differ in the arrangement and action of these pigments.

In the case of plants, photosynthesis takes place primarily in the leaves. Figure 8.1 illustrates the levels of organization in a plant leaf. As you learned in chapter 4, the cells of plant leaves contain organelles called chloroplasts, which carry out the photosynthetic process. No other structure in a plant cell is able to carry out photosynthesis (figure 8.2). Photosynthesis takes place in three stages:

1. capturing energy from sunlight;
2. using the energy to make ATP and to reduce the compound $NADP^+$, an electron carrier, to NADPH; and
3. using the ATP and NADPH to power the synthesis of organic molecules from CO_2 in the air.

The first two stages require light and are commonly called the **light-dependent reactions.**

The third stage, the formation of organic molecules from CO_2, is called **carbon fixation.** This process takes place via a cyclic series of reactions. As long as ATP and NADPH are available, the carbon fixation reactions can occur either in the presence or in the absence of light, and so these reactions are also called the **light-independent reactions.**

The following simple equation summarizes the overall process of photosynthesis:

$$\underset{\textit{carbon dioxide}}{6\ CO_2} + \underset{\textit{water}}{12\ H_2O} + \text{light} \longrightarrow \underset{\textit{glucose}}{C_6H_{12}O_6} + \underset{\textit{water}}{6\ H_2O} + \underset{\textit{oxygen}}{6\ O_2}$$

You may notice that this equation is the reverse of the reaction for respiration. In respiration, glucose is oxidized to CO_2 using O_2 as an electron acceptor. In photosynthesis, CO_2 is reduced to glucose using electrons gained from the oxidation of water. The oxidation of H_2O and the reduction of CO_2 requires energy that is provided by light. Although this statement is an oversimplification, it provides a useful "global perspective."

In plants, photosynthesis takes place in chloroplasts

In the preceding chapter, you saw that a mitochondrion's complex structure of internal and external membranes contribute to its function. The same is true for the structure of the chloroplast.

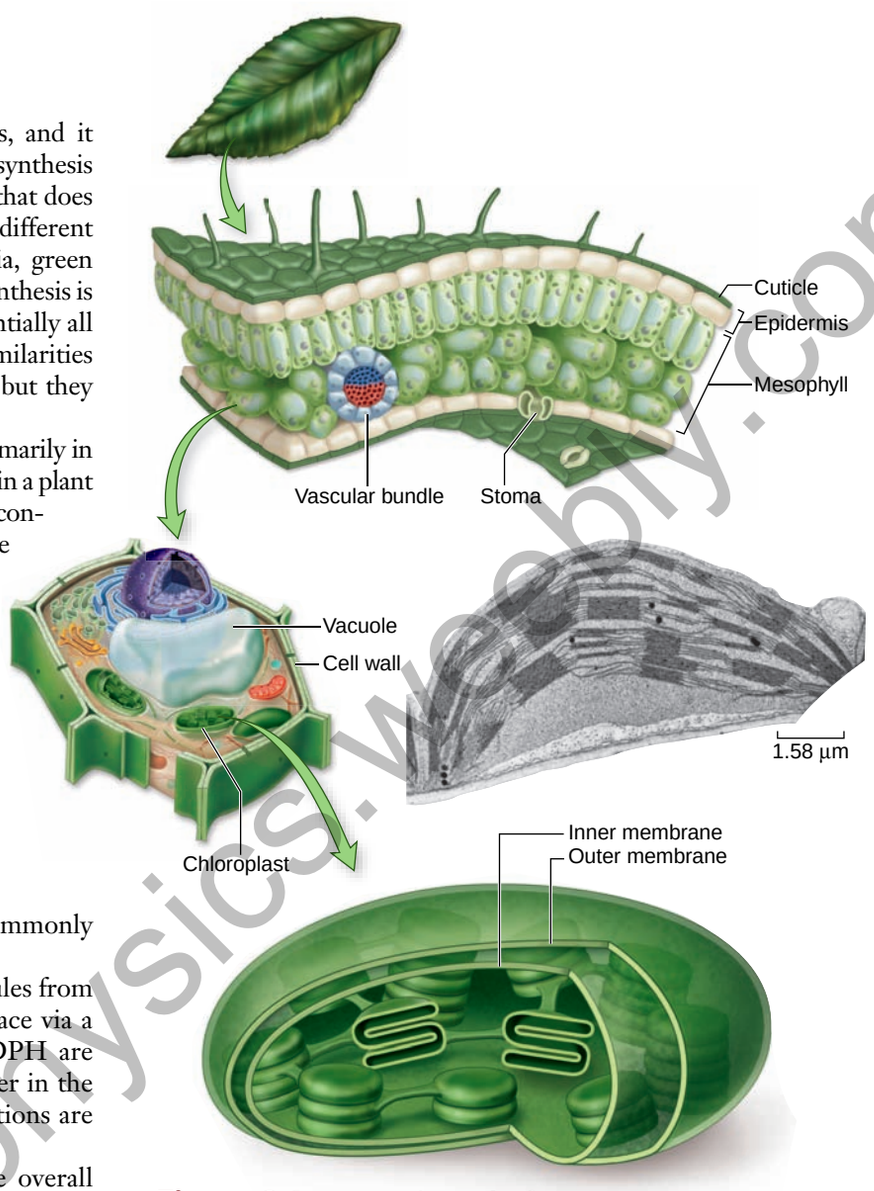

Figure 8.1 Journey into a leaf. A plant leaf possesses a thick layer of cells (the mesophyll) rich in chloroplasts. The inner membrane of the chloroplast is organized into flattened structures called thylakoid disks, which are stacked into columns called grana. The rest of the interior is filled with a semifluid substance called stroma.

The internal membrane of chloroplasts, called the *thylakoid membrane*, is a continuous phospholipid bilayer organized into flattened sacs that are found stacked on one another in columns called *grana* (singular, *granum*). The thylakoid membrane contains **chlorophyll** and other photosynthetic pigments for capturing light energy along with the machinery to make ATP. Connections between grana are termed *stroma lamella.*

Surrounding the thylakoid membrane system is a semiliquid substance called **stroma.** The stroma houses the enzymes needed to assemble organic molecules from CO_2 using energy from ATP coupled with reduction via NADPH. In the thylakoid membrane, photosynthetic pigments are clustered together to form **photosystems,** which show distinct organization within the thylakoid.

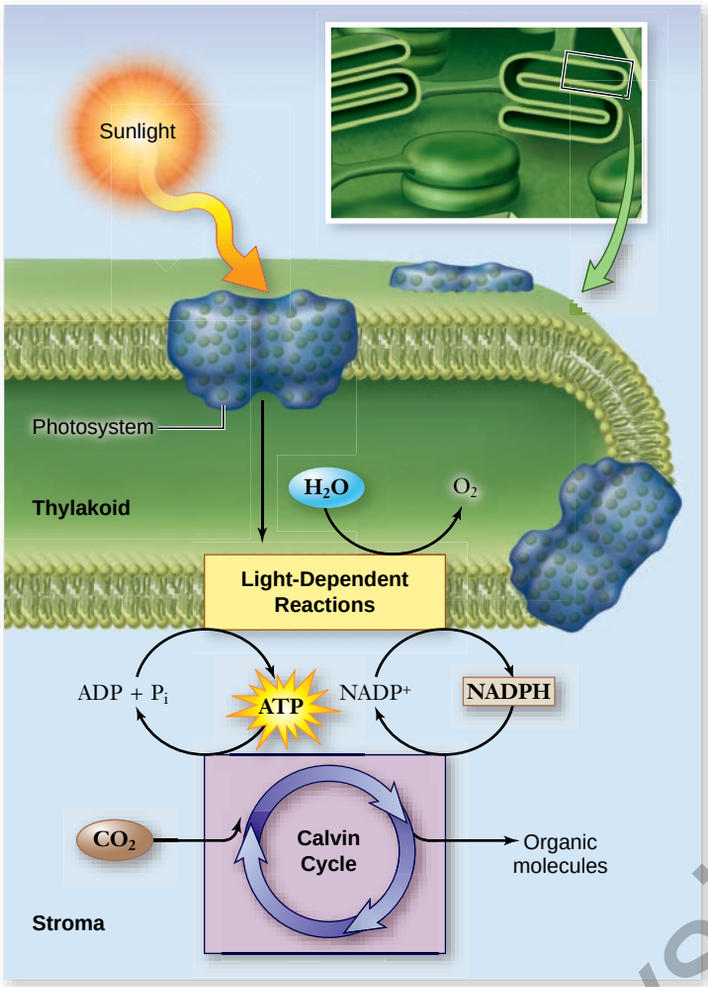

Figure 8.2 Overview of photosynthesis. In the light-dependent reactions, photosystems in the thylakoid absorb photons of light and use this energy to generate ATP and NADPH. Electrons lost from the photosystems are replaced by the oxidation of water, producing O_2 as a by-product. The ATP and NADPH produced by the light reactions is used during carbon fixation via the Calvin cycle in the stroma.

Each pigment molecule within the photosystem is capable of capturing photons, which are packets of energy. When light of a proper wavelength strikes a pigment molecule in the photosystem, the resulting excitation passes from one pigment molecule to another.

The excited electron is not transferred physically—rather, its *energy* passes from one molecule to another. The passage is similar to the transfer of kinetic energy along a row of upright dominoes. If you push the first one over, it falls against the next, and that one against the next, and so on, until all of the dominoes have fallen down.

Eventually, the energy arrives at a key chlorophyll molecule in contact with a membrane-bound protein that can accept an electron. The energy is transferred as an excited electron to that protein, which passes it on to a series of other membrane proteins that put the energy to work making ATP and NADPH. These compounds are then used to build organic molecules. The photosystem thus acts as a large antenna, gathering the light energy harvested by many individual pigment molecules.

Learning Outcomes Review 8.1

Photosynthesis consists of light-dependent reactions that require sunlight, and others that convert CO_2 into organic molecules. The overall reaction is essentially the reverse of respiration and produces O_2 as a by-product. The chloroplast's inner membrane, the thylakoid, is the site in which photosynthetic pigments are clustered, allowing passage of energy from one molecule to the next. The thylakoid membrane is organized into flattened sacs stacked in columns called grana.

- ***How is the structure of the chloroplast similar to the mitochondria?***

8.2 The Discovery of Photosynthetic Processes

Learning Outcomes

1. ***Describe experiments that support our understanding of photosynthesis.***
2. ***Explain the difference between the light-dependent and light-independent reactions.***

The story of how we learned about photosynthesis begins over 300 years ago, and it continues to this day. It starts with curiosity about how plants manage to grow, often increasing their organic mass considerably.

Plants do not increase mass from soil and water alone

From the time of the Greeks, plants were thought to obtain their food from the soil, literally sucking it up with their roots. A Belgian doctor, Jan Baptista van Helmont (1580–1644) thought of a simple way to test this idea.

He planted a small willow tree in a pot of soil, after first weighing the tree and the soil. The tree grew in the pot for several years, during which time van Helmont added only water. At the end of five years, the tree was much larger, its weight having increased by 74.4 kg. However, the soil in the pot weighed only 57 g less than it had five years earlier. With this experiment, van Helmont demonstrated that the substance of the plant was not produced only from the soil. He incorrectly concluded, however, that the water he had been adding mainly accounted for the plant's increased biomass.

A hundred years passed before the story became clearer. The key clue was provided by the English scientist Joseph Priestly (1733–1804). On the 17th of August, 1771, Priestly put

a living sprig of mint into air in which a wax candle had burnt out. On the 27th of the same month, Priestly found that another candle could be burned in this same air. Somehow, the vegetation seemed to have restored the air. Priestly found that while a mouse could not breathe candle-exhausted air, air "restored" by vegetation was not "at all inconvenient to a mouse." The key clue was that *living vegetation adds something to the air.*

How does vegetation "restore" air? Twenty-five years later, the Dutch physician Jan Ingenhousz (1730–1799) solved the puzzle. He demonstrated that air was restored only in the presence of sunlight and only by a plant's green leaves, not by its roots. He proposed that the green parts of the plant carry out a process that uses sunlight to split carbon dioxide into carbon and oxygen. He suggested that the oxygen was released as O_2 gas into the air, while the carbon atom combined with water to form carbohydrates. Other research refined his conclusions, and by the end of the nineteenth century, the overall reaction for photosynthesis could be written as:

$$CO_2 + H_2O + \text{light energy} \longrightarrow (CH_2O) + O_2$$

It turns out, however, that there's more to it than that. When researchers began to examine the process in more detail in the twentieth century, the role of light proved to be unexpectedly complex.

Photosynthesis includes both light-dependent and light-independent reactions

At the beginning of the twentieth century, the English plant physiologist F. F. Blackman (1866–1947) came to the startling conclusion that photosynthesis is in fact a multistage process, only one portion of which uses light directly.

Blackman measured the effects of different light intensities, CO_2 concentrations, and temperatures on photosynthesis. As long as light intensity was relatively low, he found photosynthesis could be accelerated by increasing the amount of light, but not by increasing the temperature or CO_2 concentration (figure 8.3). At high light intensities, however, an increase in temperature or CO_2 concentration greatly accelerated photosynthesis.

Blackman concluded that photosynthesis consists of an initial set of what he called "light" reactions, that are largely independent of temperature but depend on light, and a second set of "dark" reactions (more properly called light-independent reactions), that seemed to be independent of light but limited by CO_2.

Do not be confused by Blackman's labels—the so-called "dark" reactions occur in the light (in fact, they require the products of the light-dependent reactions); his use of the word *dark* simply indicates that light is not *directly* involved in those reactions.

Blackman found that increased temperature increased the rate of the light-independent reactions, but only up to about 35°C. Higher temperatures caused the rate to fall off rapidly. Because many plant enzymes begin to be denatured at 35°C, Blackman concluded that enzymes must carry out the light-independent reactions.

O_2 comes from water, not from CO_2

In the 1930s, C. B. van Niel (1897–1985) working at the Hopkins Marine Station at Stanford, discovered that purple sulfur bacteria do not release oxygen during photosynthesis; instead, they convert hydrogen sulfide (H_2S) into globules of pure elemental sulfur that accumulate inside them. The process van Niel observed was:

$$CO_2 + 2\ H_2S + \text{light energy} \longrightarrow (CH_2O) + H_2O + 2\ S$$

The striking parallel between this equation and Ingenhousz's equation led van Niel to propose that the generalized process of photosynthesis can be shown as:

$$CO_2 + 2\ H_2A + \text{light energy} \longrightarrow (CH_2O) + H_2O + 2\ A$$

In this equation, the substance H_2A serves as an electron donor. In photosynthesis performed by green plants, H_2A is water, whereas in purple sulfur bacteria, H_2A is hydrogen sulfide. The product, A, comes from the splitting of H_2A. Therefore, the O_2 produced during green plant photosynthesis results from splitting water, not carbon dioxide.

When isotopes came into common use in the early 1950s, van Niel's revolutionary proposal was tested. Investigators examined photosynthesis in green plants supplied with water containing heavy oxygen (^{18}O); they found that the ^{18}O label ended up in oxygen gas rather than in carbohydrate, just as van Niel had predicted:

$$CO_2 + 2\ H_2{}^{18}O + \text{light energy} \longrightarrow (CH_2O) + H_2O + {}^{18}O_2$$

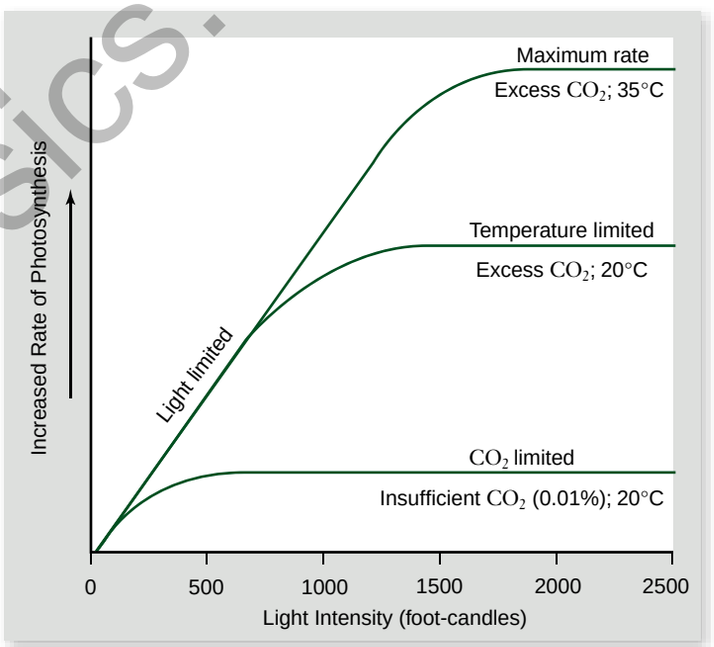

Figure 8.3 Discovery of the light-independent reactions. Blackman measured photosynthesis rates under differing light intensities, CO_2 concentrations, and temperatures. As this graph shows, light is the limiting factor at low light intensities, but temperature and CO_2 concentration are the limiting factors at higher light intensities. This implies the existence of reactions using CO_2 that involve enzymes.

Inquiry question

Blackman found that increasing light intensity above 2000 foot-candles did not lead to any further increase in the rate of photosynthesis. Can you suggest a hypothesis that would explain this?

In algae and green plants, the carbohydrate typically produced by photosynthesis is glucose. The complete balanced equation for photosynthesis in these organisms thus becomes:

$$6\ CO_2 + 12\ H_2O + \text{light energy} \longrightarrow C_6H_{12}O_6 + 6\ H_2O + 6\ O_2$$

ATP and NADPH from light-dependent reactions reduce CO_2 to make sugars

In his pioneering work on the light-dependent reactions, van Niel proposed that the H^+ ions and electrons generated by the splitting of water were used to convert CO_2 into organic matter in a process he called *carbon fixation*. In the 1950s, Robin Hill (1899–1991) demonstrated that van Niel was right, light energy could be harvested and used in a reduction reaction. Chloroplasts isolated from leaf cells were able to reduce a dye and release oxygen in response to light. Later experiments showed that the electrons released from water were transferred to $NADP^+$ and that illuminated chloroplasts deprived of CO_2 accumulate ATP. If CO_2 is introduced, neither ATP nor NADPH accumulate, and the CO_2 is assimilated into organic molecules.

These experiments are important for three reasons: First, they firmly demonstrate that photosynthesis in plants occurs within chloroplasts. Second, they show that the light-dependent reactions use light energy to reduce $NADP^+$ and to manufacture ATP. Third, they confirm that the ATP and NADPH from this early stage of photosynthesis are then used in the subsequent reactions to reduce carbon dioxide, forming simple sugars.

Learning Outcomes Review 8.2

Early experiments indicated that plants "restore" air to usable form, that is, produce oxygen—but only in the presence of sunlight. Further experiments showed that there are both light-dependent and independent reactions. The light-dependent reactions produce O_2 from H_2O, and generate ATP and NADPH. The light-independent reactions synthesize organic compounds through carbon fixation.

- ***Where does the carbon in your body come from?***

8.3 Pigments

Learning Outcomes

1. *Explain how pigments are important to photosynthesis.*
2. *Relate the absorption spectrum of a pigment to its color.*

For plants to make use of the energy of sunlight, some biochemical structure must be present in chloroplasts and the thylakoids that can absorb this energy. Molecules that absorb light energy in the visible range are termed **pigments.** We are most familiar with them as dyes that impart a certain color to clothing or other materials. The color that we see is the color that is not absorbed—that is, it is reflected. To understand how plants use pigments to capture light energy, we must first review current knowledge about the nature of light.

Light is a form of energy

The wave nature of light produces an electromagnetic spectrum that differentiates light based on its wavelength (figure 8.4). We are most familiar with the visible range of this spectrum because we can actually see it, but visible light is only a small part of the entire spectrum. Visible light can be divided into its separate colors by the use of a prism, which separates light based on wavelength.

A particle of light, termed a **photon,** acts like a discrete bundle of energy. We use the wave concept of light to understand different colors of light and the particle nature of light to understand the energy transfers that occur during photosynthesis. Thus, we will refer both to wavelengths of light and to photons of light throughout the chapter.

The energy in photons

The energy content of a photon is inversely proportional to the wavelength of the light: Short-wavelength light contains photons of higher energy than long-wavelength light (see figure 8.4). X-rays, which contain a great deal of energy, have very short wavelengths—much shorter than those of visible light.

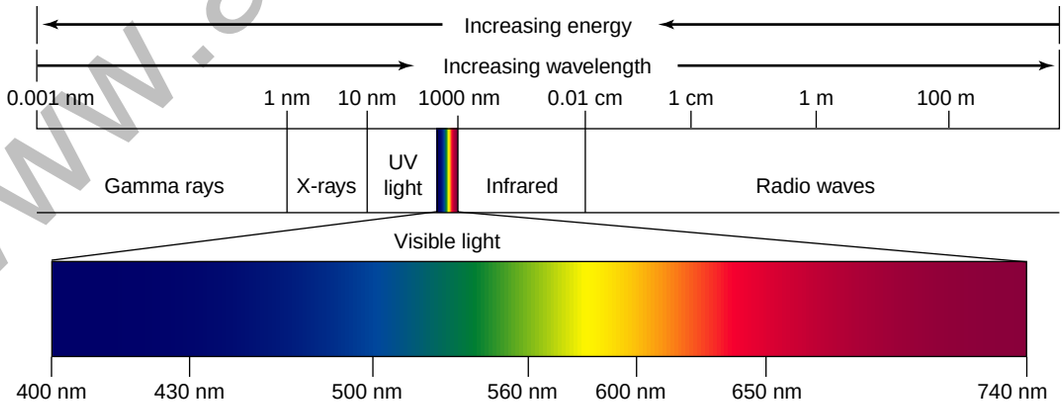

Figure 8.4 The electromagnetic spectrum. Light is a form of electromagnetic energy conveniently thought of as a wave. The shorter the wavelength of light, the greater its energy. Visible light represents only a small part of the electromagnetic spectrum between 400 and 740 nm.

A beam of light is able to remove electrons from certain molecules, creating an electrical current. This phenomenon is called the **photoelectric effect,** and it occurs when photons transfer energy to electrons. The strength of the photoelectric effect depends on the wavelength of light; that is, short wavelengths are much more effective than long ones in producing the photoelectric effect because they have more energy.

In photosynthesis, chloroplasts are acting as photoelectric devices: They absorb sunlight and transfer the excited electrons to a carrier. As we unravel the details of this process, it will become clear how this process traps energy and uses it to synthesize organic compounds.

Each pigment has a characteristic absorption spectrum

When a photon strikes a molecule with the amount of energy needed to excite an electron, then the molecule will absorb the photon raising the electron to a higher energy level. Whether the photon's energy is absorbed depends on how much energy it carries (defined by its wavelength), and also on the chemical nature of the molecule it hits.

As described in chapter 2, electrons occupy discrete energy levels in their orbits around atomic nuclei. To boost an electron into a different energy level requires just the right amount of energy, just as reaching the next rung on a ladder requires you to raise your foot just the right distance. A specific atom, therefore, can absorb only certain photons of light—namely, those that correspond to the atom's available energy levels. As a result, each molecule has a characteristic **absorption spectrum,** the range and efficiency of photons it is capable of absorbing.

As mentioned earlier, pigments are good absorbers of light in the visible range. Organisms have evolved a variety of different pigments, but only two general types are used in green plant photosynthesis: chlorophylls and carotenoids. In some organisms, other molecules also absorb light energy.

Chlorophyll absorption spectra

Chlorophylls absorb photons within narrow energy ranges. Two kinds of chlorophyll in plants, chlorophyll *a* and chlorophyll *b*, preferentially absorb violet-blue and red light (figure 8.5). Neither of these pigments absorbs photons with wavelengths between about 500 and 600 nm; light of these wavelengths is reflected. When these reflected photons are subsequently absorbed by the retinal pigment in our eyes, we perceive them as green.

Chlorophyll *a* is the main photosynthetic pigment in plants and cyanobacteria and the only pigment that can act directly to convert light energy to chemical energy. **Chlorophyll *b*,** acting as an **accessory pigment,** or secondary light-absorbing pigment, complements and adds to the light absorption of chlorophyll *a*.

Chlorophyll *b* has an absorption spectrum shifted toward the green wavelengths. Therefore, chlorophyll *b* can absorb photons that chlorophyll *a* cannot, greatly increasing the proportion of the photons in sunlight that plants can harvest. In addition, a variety of different accessory pigments are found in plants, bacteria, and algae.

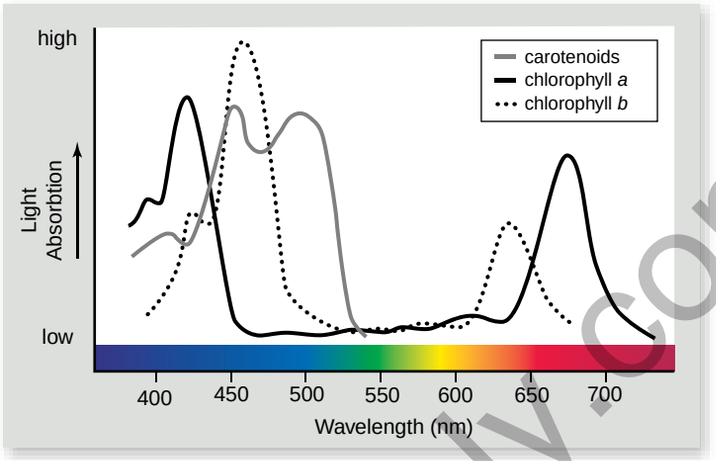

Figure 8.5 Absorption spectra for chlorophyll and carotenoids. The peaks represent wavelengths of light of sunlight absorbed by the two common forms of photosynthetic pigment, chlorophylls *a* and *b*, and the carotenoids. Chlorophylls absorb predominantly violet-blue and red light in two narrow bands of the spectrum and reflect green light in the middle of the spectrum. Carotenoids absorb mostly blue and green light and reflect orange and yellow light.

Structure of chlorophylls

Chlorophylls absorb photons by means of an excitation process analogous to the photoelectric effect. These pigments contain a complex ring structure, called a *porphyrin ring*, with alternating single and double bonds. At the center of the ring is a magnesium atom (figure 8.6).

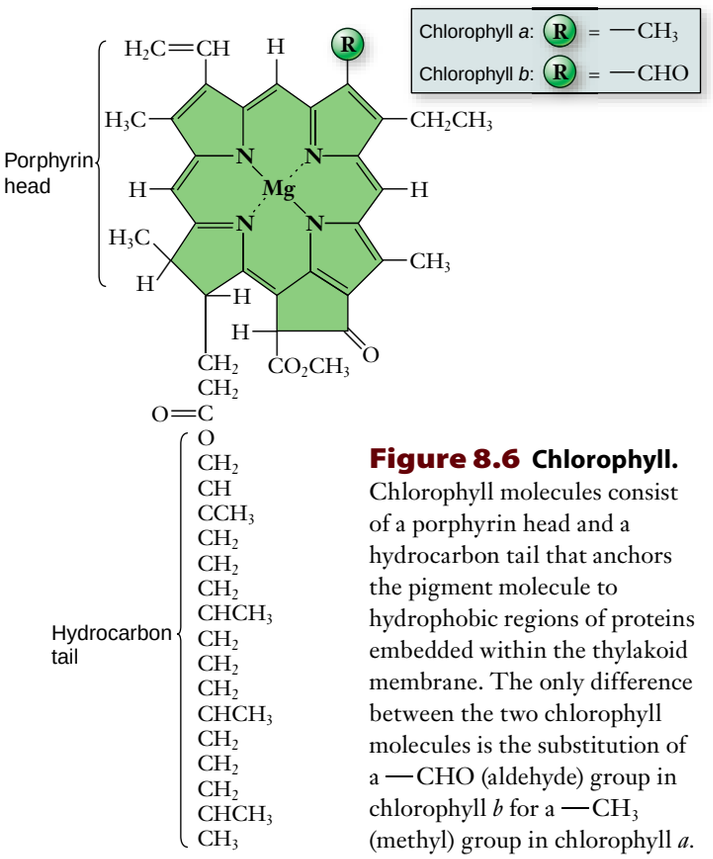

Figure 8.6 Chlorophyll. Chlorophyll molecules consist of a porphyrin head and a hydrocarbon tail that anchors the pigment molecule to hydrophobic regions of proteins embedded within the thylakoid membrane. The only difference between the two chlorophyll molecules is the substitution of a —CHO (aldehyde) group in chlorophyll *b* for a —CH_3 (methyl) group in chlorophyll *a*.