Introduction to Metabolism

In the preceding chapters, we described the structures and functions of the major components of living cells from small molecules to polymers to larger aggregates such as membranes. The next nine chapters focus on the biochemical activities that assimilate, transform, synthesize, and degrade many of the nutrients and cellular components already described. The biosynthesis of proteins and nucleic acids, which represent a significant proportion of the activity of all cells, will be described in Chapters 20–22.

We now move from molecular structure to the dynamics of cell function. Despite the marked shift in our discussion, we will see that metabolic pathways are governed by basic chemical and physical laws. By taking a stepwise approach that builds on the foundations established in the first two parts of this book, we can describe how metabolism operates. In this chapter, we discuss some general themes of metabolism and the thermodynamic principles that underlie cellular activities.
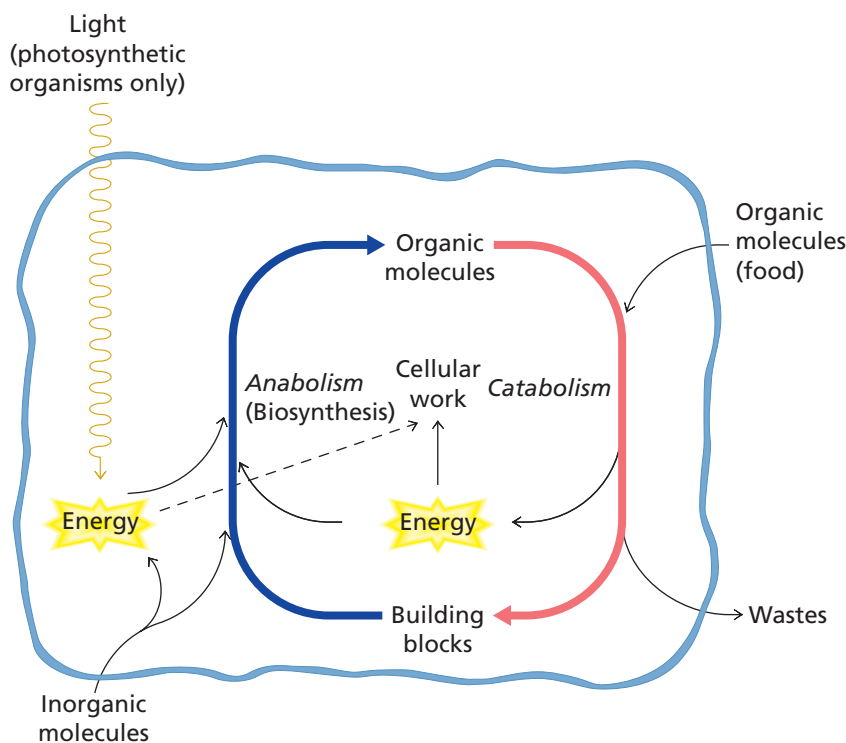
For most metabolic sequences neither the substrate concentration nor the product concentration changes significantly, even though the flux through the pathway may change dramatically.

—Jeremy R. Knowles (1989)

10.1 Metabolism Is a Network of Reactions

Metabolism is the entire network of chemical reactions carried out by living cells. **Metabolites** are the small molecules that are intermediates in the degradation or biosynthesis of biopolymers. The term *intermediary metabolism* is applied to the reactions involving these low-molecular-weight molecules. It is convenient to distinguish between reactions that synthesize molecules (anabolic reactions) and reactions that degrade molecules (catabolic reactions).

Anabolic reactions are those responsible for the synthesis of all compounds needed for cell maintenance, growth, and reproduction. These biosynthesis reactions make simple metabolites such as amino acids, carbohydrates, coenzymes, nucleotides, and

Top: The fundamental principles of metabolism are the same in animals and plants and in all other organisms.

fatty acids. They also produce larger molecules such as proteins, polysaccharides, nucleic acids, and complex lipids (Figure 10.1).

In some species, all of the complex molecules that make up a cell are synthesized from inorganic precursors (carbon dioxide, ammonia, inorganic phosphates, etc.)(Section 10.3). Some species derive energy from these inorganic molecules or from the creation of membrane potential (Section 9.11). Photosynthetic organisms use light energy to drive biosynthesis reactions (Chapter 15).

Catabolic reactions degrade large molecules to liberate smaller molecules and energy. All cells carry out degradation reactions as part of their normal cell metabolism but some species rely on them as their only source of energy. Animals, for example, require organic molecules as food. The study of these energy-producing catabolic reactions in mammals is called *fuel metabolism*. The ultimate source of these fuels is a biosynthetic pathway in another species. Keep in mind that all catabolic reactions involve the breakdown of compounds that were synthesized by a living cell—either the same cell, a different cell in the same individual, or a cell in a different organism.

There is a third class of reactions called **amphibolic reactions**. They are involved in both anabolic and catabolic pathways.

Whether we observe bacteria or large multicellular organisms, we find a bewildering variety of biological adaptations. More than 10 million species may be living on Earth and several hundred million species may have come and gone throughout the course of evolution. Multicellular organisms have a striking specialization of cell types or tissues. Despite this extraordinary diversity of species and cell types the biochemistry of living cells is surprisingly similar not only in the chemical composition and structure of cellular components but also in the metabolic routes by which the components are modified. These universal pathways are the key to understanding metabolism. Once you've learned about the fundamental conserved pathways you can appreciate the additional pathways that have evolved in some species.

The complete sequences of the genomes of a number of species have been determined. For the first time we are beginning to have a complete picture of the entire metabolic network of these species based on the sequences of the genes that encode metabolic enzymes. *Escherichia coli*, for example, has about 900 genes that encode enzymes used in intermediary metabolism and these enzymes combine to create about 130 different pathways.

KEY CONCEPT

Most of the fundamental metabolic pathways are present in all species.
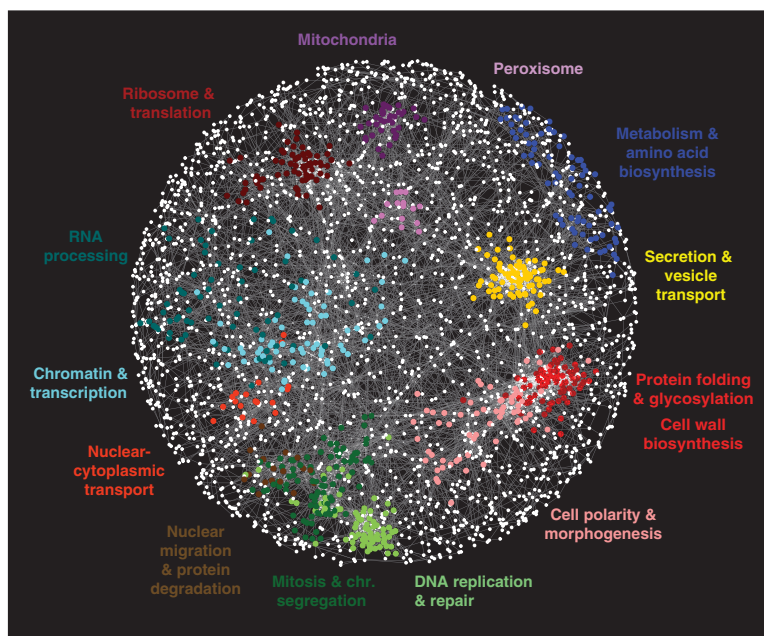
These metabolic genes account for 21% of the genes in the genome. Other species of bacteria have a similar number of enzymes that carry out the basic metabolic reactions. Some species contain additional pathways. The bacterium that causes tuberculosis, *Mycobacterium tuberculosis*, has about 250 enzymes involved in fatty acid metabolism—five times as many as *E. coli*.

The yeast *Saccharomyces cerevisiae* is a single-celled member of the fungus kingdom. Its genome contains 5900 protein-encoding genes. Of these, 1200 (20%) encode enzymes involved in intermediary and energy metabolism (Figure 10.2). The nematode *Caenorhabditis elegans* is a small, multicellular animal with many of the same specialized cells and tissues found in larger animals. Its genome encodes 19,100 proteins of which 5300 (28%) are thought to be required in various pathways of intermediary metabolism. In the fruit fly, *Drosophila melanogaster*, approximately 2400 (17%) of its 14,100 genes are predicted to be involved in intermediary metabolic pathways and bioenergetics. The exact number of genes required for basic metabolism in humans is not known but it's likely that about 5000 genes are needed. (The human genome has approximately 22,000 genes.)

There are five common themes in metabolism.

1. Organisms or cells maintain specific internal concentrations of inorganic ions, metabolites, and enzymes. Cell membranes provide the physical barrier that segregates cell components from the environment.

2. Organisms extract energy from external sources to drive energy-consuming reactions. Photosynthetic organisms derive energy from the conversion of solar energy to chemical energy. Other organisms obtain energy from the ingestion and catabolism of energy-yielding compounds.

3. The metabolic pathways in each organism are specified by the genes it contains in its genome.

4. Organisms and cells interact with their environment. The activities of cells must be geared to the availability of energy, organisms grow and reproduce. When the supply of energy from the environment is plentiful. When the supply of energy from the environment is limited, energy demands can be temporarily met by using internal stores or by slowing metabolic rates as in hibernation, sporulation, or seed formation. If the shortage is prolonged, organisms die.

5. The cells of organisms are not static assemblies of mtneylecules. Many cell components are continually synthesized and degraded, that is, they undergo *turnover*, even

though their concentrations may remain virtually constant. The concentrations of other compounds change in response to changes in external or internal conditions.

The metabolism section of this book describes metabolic reactions that operate in most species. For example, enzymes of glycolysis (the degradation of sugar) and of gluconeogenesis (biosynthesis of glucose) are present in almost all species. Although most cells possess the same set of central metabolic reactions, cell and organism differentiation is possible because of additional enzymatic reactions specific to the tissue or species.
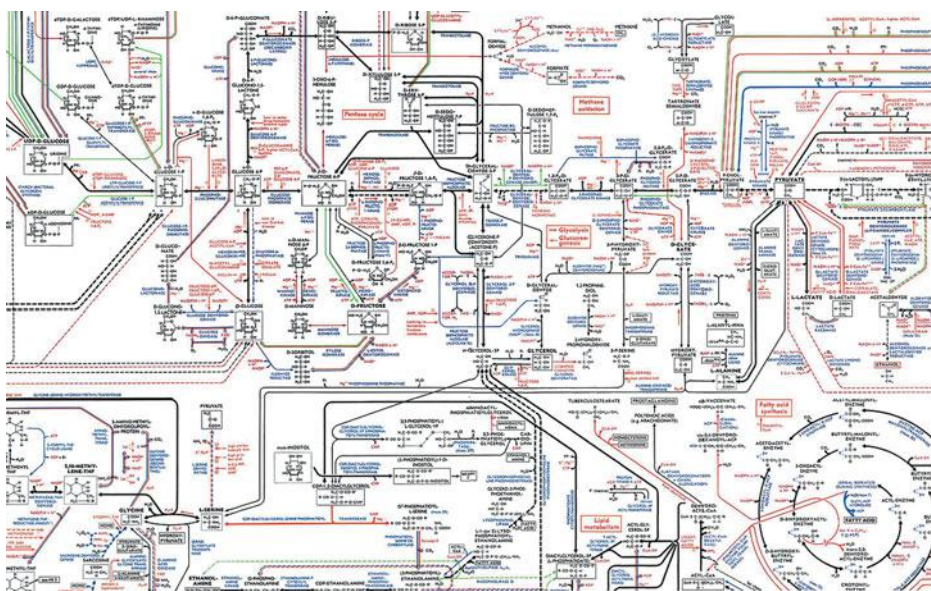
10.2 Metabolic Pathways

The vast majority of metabolic reactions are catalyzed by enzymes so a complete description of metabolism includes not only the reactants, intermediates, and products of cellular reactions but also the characteristics of the relevant enzymes. Most cells can perform hundreds to thousands of reactions. We can deal with this complexity by systematically subdividing metabolism into segments or branches. In the following chapters, we begin by considering separately the metabolism of the four major groups of biomolecules: carbohydrates, lipids, amino acids, and nucleotides. Within each of the four areas of metabolism, we recognize distinct sequences of metabolic reactions, called pathways.
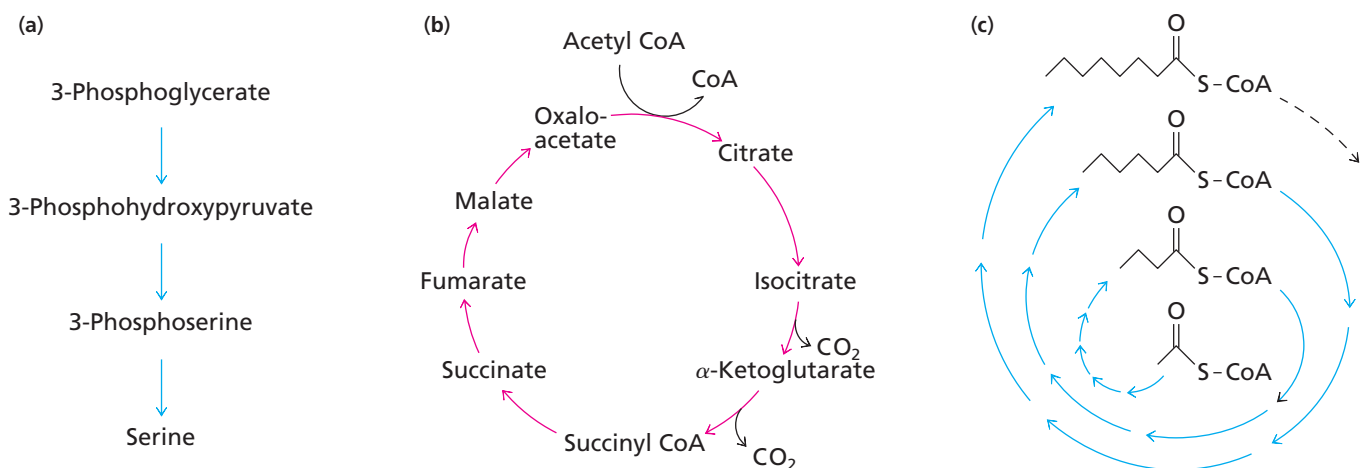
A. Pathways Are Sequences of Reactions

A **metabolic pathway** is the biological equivalent of a synthesis scheme in organic chemistry. A metabolic pathway is a series of reactions where the product of one reaction becomes the substrate for the next reaction. Some metabolic pathways may consist of only two steps while others may be a dozen steps in length.

It's not easy to define the limits of a metabolic pathway. In the laboratory, a chemical synthesis has an obvious beginning substrate and an obvious end product but cellular pathways are interconnected in ways that make it difficult to pick a beginning and an end. For example, in the catabolism of glucose (Chapter 11), where does glycolysis begin and end? Does it begin with polysaccharides (such as glycogen and starch), extracellular glucose, glucose 6-phosphate, or intracellular glucose? Does the pathway end with pyruvate, acetyl CoA, lactate, or ethanol? Start and end points can be assigned somewhat arbitrarily, often according to tradition or for ease of study, but keep in mind that reactions and pathways can be linked to form extended metabolic routes. This network is very obvious when you examine the large metabolic charts that are sometimes posted on the walls outside professors' offices (Figure 10.3).

Individual metabolic pathways can take different forms. A linear metabolic pathway, such as the biosynthesis of serine, is a series of independent enzyme-catalyzed reactions

◄ Figure 10.3
Part of a large metabolic chart published by Roche Applied Science.

(a)

3-Phosphoglycerate

↓

3-Phosphohydroxypyruvate

↓

3-Phosphoserine

↓

Serine

(b)

(c)

▲ **Figure 10.4**
Forms of metabolic pathways. (a) The biosynthesis of serine is an example of a linear metabolic pathway. The product of each step is the substrate for the next step. **(b)** The sequence of reactions in a cyclic pathway forms a closed loop. In the citric acid cycle, an acetyl group is metabolized via reactions that regenerate the intermediates of the cycle. **(c)** In fatty acid biosynthesis, a spiral pathway, the same set of enzymes catalyzes a progressive lengthening of the acyl chain.

KEY CONCEPT

The limitations of chemistry and physics dictate that metabolic pathways consist of many small steps.

in which the product of one reaction is the substrate for the next reaction in the pathway (Figure 10.4a). A cyclic metabolic pathway, such as the citric acid cycle, is also a sequence of enzyme-catalyzed steps, but the sequence forms a closed loop, so the intermediates are regenerated with every turn of the cycle (Figure 10.4b). In a spiral metabolic pathway, such as the biosynthesis of fatty acids (Section 16.6), the same set of enzymes is used repeatedly for lengthening or shortening a given molecule (Figure 10.4c).

Each type of pathway may have branch points where metabolites enter or leave. In most cases, we don't emphasize the branching nature of pathways because we want to focus on the main routes followed by the most important metabolites. We also want to focus on the pathways that are commonly found in all species. These are the most fundamental pathways. Don't be misled by this simplification. A quick glance at any metabolic chart will show that pathways have many branch points and that initial substrates and final products are often intermediates in other pathways. The serine pathway in Figure 10.3 is a good example. Can you find it?

B. Metabolism Proceeds by Discrete Steps

Intracellular environments don't change very much. Reactions proceed at moderate temperatures and pressures, at rather low reactant concentrations, and at close to neutral pH. We often refer to this as homeostasis at the cellular level.

These conditions require a multitude of efficient enzymatic catalysts. Why are so many distinct reactions carried out in living cells? In principle, it should be possible to carry out the degradation and the synthesis of complex organic molecules with far fewer reactions.
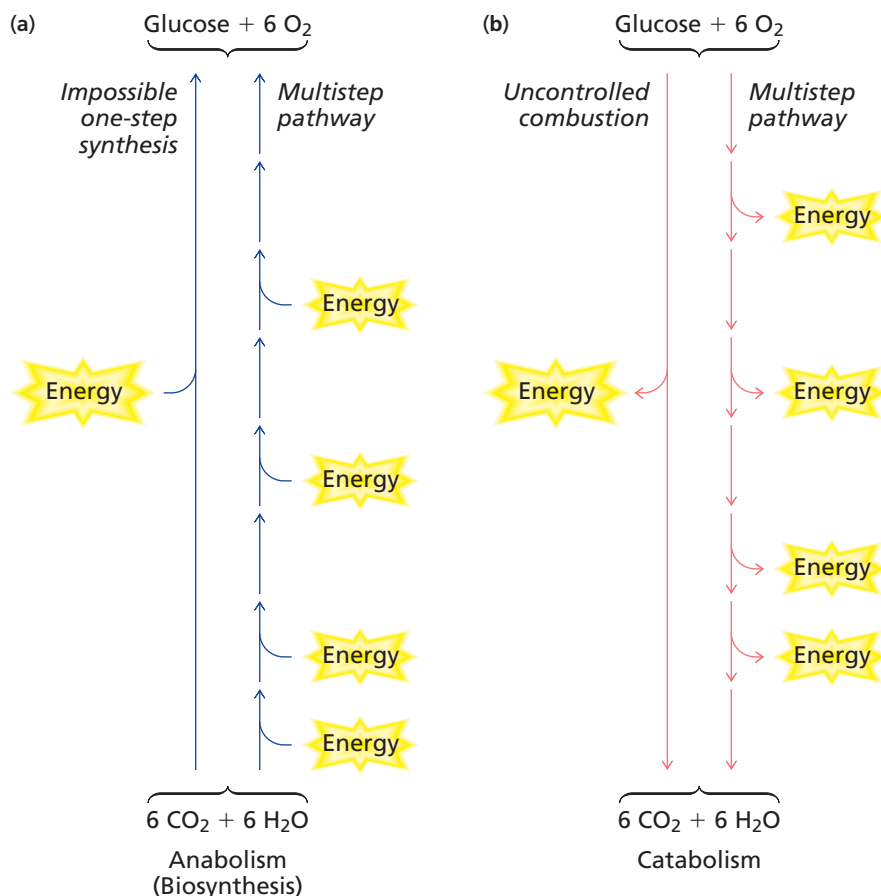
One reason for multistep pathways is the limited reaction specificity of enzymes. Each active site catalyzes only a single step of a pathway. The synthesis of a molecule— or its degradation—therefore follows a metabolic route defined by the availability of suitable enzymes. As a general rule, a single enzyme-catalyzed reaction can only break or form a few covalent bonds at a time. Often the reaction involves the transfer of a single chemical group. Thus, the large number of reactions and enzymes is due, in part, to the limitations of enzymes and chemistry.

Another reason for multiple steps in metabolic pathways is to control energy input and output. Energy flow is mediated by energy donors and acceptors that carry discrete quanta of energy. As we will see, the energy transferred in a single reaction seldom exceeds 60 kJ mol^{-1}. Pathways for the biosynthesis of molecules require the transfer of energy at multiple points. Each energy-requiring reaction corresponds to a single step in the reaction sequence.

The synthesis of glucose from carbon dioxide and water requires the input of ~2900 kJ mol^{-1} of energy. It is not thermodynamically possible to synthesize glucose in a single step (Figure 10.5). Similarly, much of the energy released during a catabolic process (such as the oxidation of glucose to carbon dioxide and water, which releases the same 2900 kJ mol^{-1}) is transferred to individual acceptors one step at a time rather

(a)

Glucose + 6 O_2

Impossible one-step synthesis   *Multistep pathway*

Energy

Energy

Energy

Energy

Energy

Energy

6 CO_2 + 6 H_2O

Anabolism
(Biosynthesis)

(b)

Glucose + 6 O_2

Uncontrolled combustion   *Multistep pathway*

Energy

Energy   Energy

Energy

Energy

6 CO_2 + 6 H_2O

Catabolism

◄ **Figure 10.5**
Single-step versus multistep pathways. **(a)** The synthesis of glucose cannot be accomplished in a single step. Multistep synthesis is coupled to the input of small quanta of energy from ATP and NADH. **(b)** The uncontrolled combustion of glucose releases a large amount of energy all at once. A multistep enzyme-catalyzed pathway releases the same amount of energy but conserves much of it in a manageable form.

than being released in one grand, inefficient explosion. The efficiency of energy transfer at each step is never 100%, but a considerable percentage of the energy is conserved in manageable form. Energy carriers that accept and donate energy, such as adenine nucleotides (ATP) and nicotinamide coenzymes (NADH), are found in all life forms.
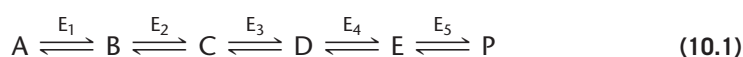
A major goal of learning about metabolism is to understand how these "quanta" of energy are used. ATP and NADH—and other coenzymes—are the "currency" of metabolism. This is why metabolism and bioenergetics are so closely linked.

C. Metabolic Pathways Are Regulated

Metabolism is highly regulated. Organisms react to changing environmental conditions such as the availability of energy or nutrients. Organisms also respond to genetically programmed instructions. For example, during embryogenesis or reproduction, the metabolism of individual cells can change dramatically.

The responses of organisms to changing conditions range from small changes to drastically reorganizing the metabolic processes that govern the synthesis or degradation of biomolecules and the generation or consumption of energy. Control processes can affect many pathways or only a few, and the response time can range from less than a second to hours or longer. The most rapid biological responses, occurring in milliseconds, include changes in the passage of small ions (e.g., $Na^\oplus$, $K^\oplus$, and $Ca^{2\oplus}$) through cell membranes. Transmission of nerve impulses and muscle contraction depend on ion movement. The most rapid responses are also the most short-lived; slower responses usually last longer.

It is important to understand some basic concepts of pathways in order to see how they are regulated. Consider a simple linear pathway that begins with substrate A and ends with product P.

$$A \underset{}{\overset{E_1}{\rightleftharpoons}} B \underset{}{\overset{E_2}{\rightleftharpoons}} C \underset{}{\overset{E_3}{\rightleftharpoons}} D \underset{}{\overset{E_4}{\rightleftharpoons}} E \underset{}{\overset{E_5}{\rightleftharpoons}} P \qquad \textbf{(10.1)}$$

Each of the reactions is catalyzed by an enzyme and they are all reversible. Most reactions in living cells have reached equilibrium so the concentrations of B, C, D, and E do not change very much. This is similar to the **steady state** condition we encountered in Section 5.3A. The steady state condition can be visualized by imagining a series of beakers of different sizes (Figure 10.6). Water flows into the first beaker from a tap and when it fills up the water spills over into another beaker. After filling up a series of beakers, there will be a steady flow of water from the tap onto the floor. The rate of flow is analogous to the **flux** through a metabolic pathway. The flux can vary from a trickle to a gusher but the steady state levels of water in each beaker don't change. (Unfortunately, this analogy doesn't allow us to see that in a metabolic pathway the flux could also be in the opposite direction.)

Flux through a metabolic pathway will decrease if the concentration of the initial substrate falls below a certain threshold. It will also decrease if the concentration of the final product rises. These are changes that affect all pathways. However, in addition to these normal concentration effects, there are special regulatory controls that affect the activity of particular enzymes in the pathway. It is tempting to visualize regulation of a pathway by the efficient manipulation of a single rate limiting enzymatic reaction, sometimes likened to the narrow part of an hourglass. In many cases, however, this is an oversimplification. Flux through most pathways depends on controls at several steps. These steps are special reactions in the pathways where the steady state concentrations of substrates and products are far from the equilibrium concentrations so the flux tends to go only in one direction. A regulatory enzyme contributes a particular degree of control over the overall flux of the pathway in which it participates. Because intermediates or cosubstrates from several sources can feed into or out of a pathway, the existence of multiple control points is normal; an isolated, linear, pathway is rare.

There are two common patterns of metabolic regulation: feedback inhibition and feed-forward activation. **Feedback inhibition** occurs when a product (usually the end product) of a pathway controls the rate of its own synthesis through inhibition of an early step, usually the first committed step (the first reaction that is unique to the pathway).

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} E \xrightarrow{E_5} P \qquad \textbf{(10.2)}$$
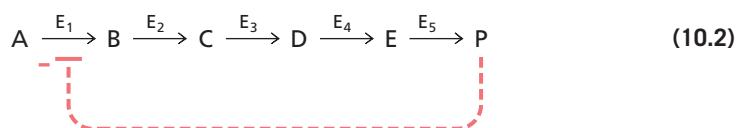
The advantage of such a regulatory pattern in a biosynthetic pathway is obvious. When the concentration of P rises above its steady state level, the effect is transmitted back through the pathway and the concentrations of each intermediate also rise. This causes flux to reverse in the pathway, leading to a net increase in the production of product A from reactant P. Flux in the normal direction is restored when P is depleted. The pathway is inhibited at an early step; otherwise, metabolic intermediates would accumulate unnecessarily. The important point in Reaction 10.2 is that the reaction catalyzed by enzyme E1 is not allowed to reach equilibrium. It is a metabolically irreversible reaction because the enzyme is regulated. Flux through this point is not allowed to go in the opposite direction.

Feed-forward activation occurs when a metabolite produced early in a pathway activates an enzyme that catalyzes a reaction further down the pathway.

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} E \xrightarrow{E_5} P \qquad \textbf{(10.3)}$$
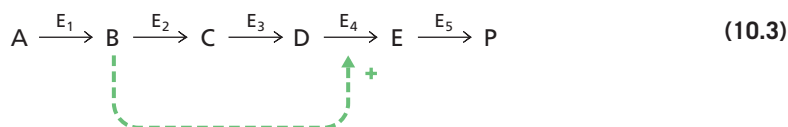
In this example, the activity of enzyme E_1 (which converts A to B) is coordinated with the activity of enzyme E_4 (which converts D to E). An increase in the concentration of metabolite B increases flux through the pathway by activating E4. (E4 would normally be inactive in low concentrations of B.)

In Section 5.10, we discussed the modulation of individual regulatory enzymes. Allosteric activators and inhibitors, which are usually metabolites, can rapidly alter the

> The precise technical term for the condition where cellular pathways are not in a dynamic steady-state condition is . . . dead.
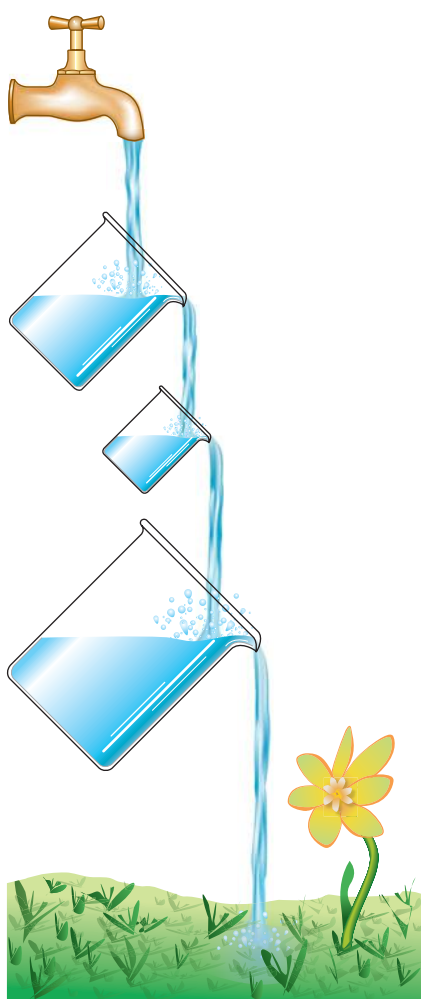
▲ **Figure 10.6**
Steady state and flux in a metabolic pathway. The rate of flow is equivalent to the flux in a pathway, and the constant amount of water in each beaker is analogous to the steady state concentrations of metabolites in a pathway.

activity of many of these enzymes by inducing conformational changes that affect catalytic activity. We will see many examples of allosteric modulation in the coming chapters. The allosteric modulation of regulatory enzymes is fast but not as rapid in cells as it can be with isolated enzymes.
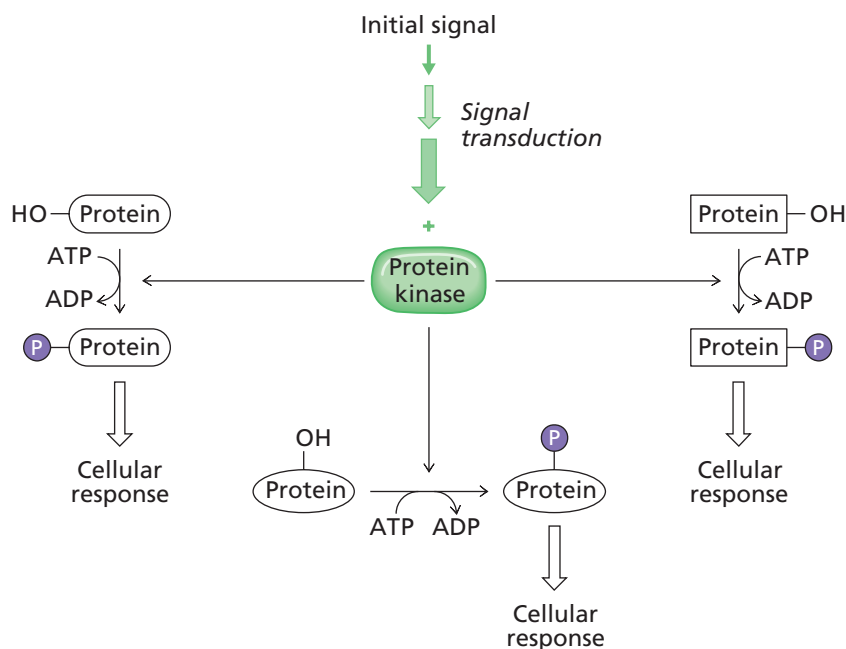
The activity of interconvertible enzymes can also be rapidly and reversibly altered by covalent modification, commonly by the addition and removal of phosphoryl groups as described in Section 5.9D. Recall that phosphorylation, catalyzed by protein kinases at the expense of ATP, is reversed by the action of protein phosphatases, which catalyze the hydrolytic removal of phosphoryl groups. Individual enzymes differ in whether their response to phosphorylation is activation or deactivation. Interconvertible enzymes in catabolic pathways are generally activated by phosphorylation and deactivated by dephosphorylation; most interconvertible enzymes in anabolic pathways are inactivated by phosphorylation and reactivated by dephosphorylation. The activation of kinases with multiple specificities allows coordinated regulation of more than one metabolic pathway by one signal. The cascade nature of intracellular signaling pathways, described in Section 9.12, also means that the initial signal is amplified (Figure 10.7).

The amounts of specific enzymes can be altered by increasing the rates of specific protein synthesis or degradation. This is usually a slow process relative to allosteric or covalent activation and inhibition. However, the turnover of certain enzymes may be rapid. Keep in mind that several modes of regulation can operate simultaneously within a metabolic pathway.

D. Evolution of Metabolic Pathways

The evolution of metabolic pathways is an active area of biochemical research. These studies have been greatly facilitated by the publication of hundreds of complete genome sequences, especially prokaryotic genomes. Biochemists can now compare pathway enzymes in a number of species that show a diverse variety of pathways. Many of these pathways provide clues to the organization and structure of the primitive pathways that were present in the first cells.

There are many possible routes to the formation of a new metabolic pathway. The simplest case is the addition of a new terminal step to a preexisting pathway. Consider the hypothetical pathway in Equation 10.1. The original pathway might have terminated with the production of metabolite E after a four-step transformation from substrate A. The availability of substantial quantities of metabolite E might favor the evolution of a new enzyme (E_5 in this case) that could use E as a substrate to make P. The pathways

> **In Part 4 of this book, we examine more closely the regulation of gene expression and protein synthesis.**

◄ **Figure 10.7**
Regulatory role of a protein kinase. The effect of the initial signal is amplified by the signaling cascade. Phosphorylation of different cellular proteins by the activated kinase results in coordinated regulation of different metabolic pathways. Some pathways may be activated, whereas others are inhibited. —Ⓟ represents a protein-bound phosphate group.

leading to synthesis of asparagine and glutamine from aspartate and glutamate pathways are examples of this type of pathway evolution. This *forward evolution* is thought to be a common mechanism of evolution of new pathways.

In other cases, a new pathway can form by evolving a branch to a preexisting pathway. For example, consider the conversion of C to D in the Equation 10.1 pathway. This reaction is catalyzed by enzyme E_3. The primitive E_3 enzyme might not have been as specific as the modern enzyme. In addition to producing product D, it might have synthesized a smaller amount of another metabolite, X. The availability of product X might have conferred some selective advantage to the cell favoring a duplication of the E_3 gene. Subsequent divergence of the two copies of the gene gave rise to two related enzymes that specifically catalyzed $C \rightarrow D$ and $C \rightarrow X$. There are many examples of *evolution by gene duplication and divergence* (e.g., lactate dehydrogenase and malate dehydrogenase, Section 4.7). (We have mostly emphasized the extreme specificity of enzyme reactions but, in fact, many enzymes can catalyze several different reactions using structurally similar substrates and products.)

Some pathways might have evolved "backwards." A primitive pathway might have utilized an abundant supply of metabolite E in the environment in order to make product P. As the supply of E became depleted over time there was selective pressure to evolve a new enzyme (E_4) that could make use of metabolite D to replenish metabolite E. When D became rate limiting, cells could gain a selective advantage by utilizing C to make more metabolite D. In this way the complete modern pathway evolved by *retroevolution*, successively adding simpler precursors and extending the pathway.

Sometimes an entire pathway can be duplicated and subsequent adaptive evolution leads to two independent pathways with homologous enzymes that catalyze related reactions. There is good evidence that the pathways leading to biosynthesis of tryptophan and histidine evolved in this manner. Enzymes can also be recruited from one pathway for use in another without necessarily duplicating an entire pathway. We'll encounter several examples of homologous enzymes that are used in different pathways.
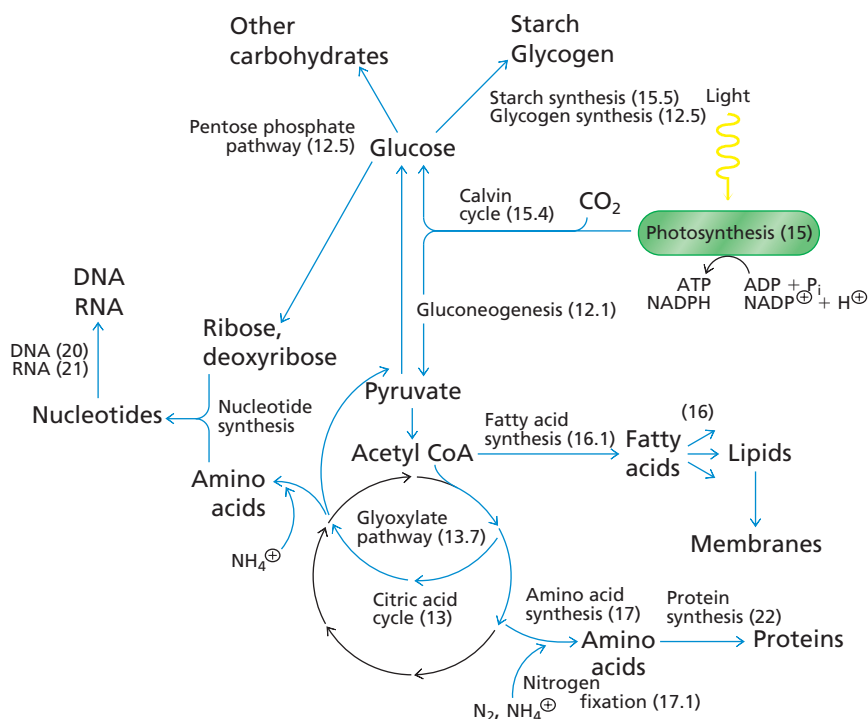
Finally, a new pathway can evolve by "reversing" an existing pathway. In most cases, there is one step in a pathway that is essentially irreversible. Let's assume that the third step in our hypothetical pathway ($C \rightarrow D$) is unable to catalyze the conversion of D to C because the normal reaction is far from equilibrium. The evolution of a new enzyme that can catalyze $D \rightarrow C$ would allow this entire pathway to reverse direction, converting P to A. This is how the glycolysis pathway evolved from the glucose biosynthesis (gluconeogenesis) pathway. There are many other examples of *evolution by pathway reversal*.

All of these possibilities play a role in the evolution of new pathways. Sometimes a new pathway evolves by a combination of different mechanisms of adaptive evolution. The evolution of the citric acid cycle pathway, which took place several billion years ago, is an example (Section 12.9). New metabolic pathways are evolving all the time in response to pesticides, herbicides, antibiotics, and industrial waste. Organisms that can metabolize these compounds, thus escaping their toxic effects, have evolved new pathways and enzymes by modifying existing ones.

10.3 Major Pathways in Cells

This section provides an overview of the organization and function of some central metabolic pathways that are discussed in subsequent chapters. We begin with the anabolic, or biosynthetic, pathways since these pathways are the most important for growth and reproduction. A general outline of biosynthetic pathways is shown in Figure 10.8. All cells require an external source of carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur plus additional inorganic ions (Section 1.2). Some species, notably bacteria and plants, can grow and reproduce by utilizing inorganic sources of these essential elements. These species are called **autotrophs**. There are two distinct categories of autotrophic species. **Heterotrophs**, such as animals, need an organic carbon source (e.g., glucose).

Biosynthetic pathways require energy. The most complex organisms (from a biochemical perspective!) can generate useful metabolic energy from sunlight or by oxidizing inorganic molecules such as $NH_4^{\oplus}$, H_2, or H_2S. The energy from these reactions is

Other carbohydrates

Starch
Glycogen

Pentose phosphate pathway (12.5)   Glucose

Starch synthesis (15.5)   Light
Glycogen synthesis (12.5)

Calvin cycle (15.4) CO_2

Photosynthesis (15)

ATP   ADP + P_i
NADPH   NADP$^{\oplus}$ + H$^{\oplus}$

DNA
RNA

Gluconeogenesis (12.1)

DNA (20)
RNA (21)

Ribose, deoxyribose

Nucleotides

Nucleotide synthesis

Pyruvate

Amino acids

$NH_4^{\oplus}$

Acetyl CoA

Fatty acid synthesis (16.1)   Fatty acids

(16)

Lipids

Glyoxylate pathway (13.7)

Membranes

Citric acid cycle (13)

Amino acid synthesis (17)   Protein synthesis (22)

Amino acids   Proteins

Nitrogen fixation (17.1)

N_2, $NH_4^{\oplus}$

◀ **Figure 10.8**
Overview of anabolic pathways. Large molecules are synthesized from smaller ones by adding carbon (usually in the form of CO_2) and nitrogen (usually as $NH_4^{\oplus}$). The main pathways include the citric acid cycle, which supplies the intermediates in amino acid biosynthesis, and gluconeogenesis, which results in the production of glucose. The energy for biosynthetic pathways is supplied by light in photosynthetic organisms or by the breakdown of inorganic molecules in other autotrophs. (Numbers in parentheses refer to the chapters and sections of this book.)

used to synthesize the energy-rich compound ATP and the reducing power of NADH. These cofactors transfer their energy to biosynthetic reactions.

There are two types of autotrophic species. **Photoautotrophs** obtain most of their energy by photosynthesis and their main source of carbon is CO_2. This category includes photosynthetic bacteria, algae, and plants. **Chemoautotrophs** obtain their energy by oxidizing inorganic molecules and utilizing CO_2 as a carbon source. Some bacterial species are chemoautotrophs but there are no eukaryotic examples.

Heterotrophs can be split into two categories. **Photoheterotrophs** are photosynthetic organisms that require an organic compound as a carbon source. There are several groups of bacteria that are capable of capturing light energy but must rely on some organic molecules as a carbon source. **Chemoheterotrophs** are nonphotosynthetic organisms that require organic molecules as carbon sources. Their metabolic energy is usually derived from the breakdown of the imported organic molecules. We are chemoheterotrophs, as are all animals, most protists, all fungi, and many bacteria.

The main catabolic pathways are shown in Figure 10.9. As a general rule, these degradative pathways are not simply the reverse of biosynthesis pathways. Note that the citric acid cycle is a major pathway in both anabolic and catabolic metabolism. The main roles of catabolism are to eliminate unwanted molecules and to generate energy for use in other processes.

We will examine metabolism in the next few chapters. Our discussion of metabolic pathways begins in Chapter 11 with glycolysis, a ubiquitous pathway for glucose catabolism. There is a long-standing tradition in biochemistry of introducing students to glycolysis before any other pathways are encountered. We know a great deal about the reactions in this pathway and they will illustrate many of the fundamental principles of biochemistry. In glycolysis, the hexose is split into two three-carbon metabolites. This pathway can generate ATP in a process called *substrate level phosphorylation*. Often, the product of glycolysis is pyruvate, which can be converted to acetyl CoA for further oxidation.

Chapter 12 describes the synthesis of glucose, or gluconeogenesis. This chapter also covers starch and glycogen metabolism and outlines the pathway by which glucose is oxidized to produce NADPH for biosynthetic pathways and ribose for the synthesis of nucleotides.

The citric acid cycle (Chapter 13) facilitates complete oxidation of the acetate carbons of acetyl CoA to carbon dioxide. The energy released from this oxidation is conserved in

▲ **Chemoautotrophs in Yellowstone National Park.** There are many species of *Thiobacillus* that derive their energy from the oxidation of iron or sulfur. They do not require any organic molecules. The orange and yellow colors surrounding this hot spring in Yellowstone National Park are due to the presence of *Thiobacillus*. See Chapter 14 for an explanation of how such organisms generate energy from inorganic molecules.

Figure 10.9 ▶
Overview of catabolic pathways. Amino acids, nucleotides, monosaccharides, and fatty acids are formed by enzymatic hydrolysis of their respective polymers. They are then degraded in oxidative reactions and energy is conserved in ATP and reduced coenzymes (mostly NADH). (Numbers in parentheses refer to the chapters and sections of this book.)
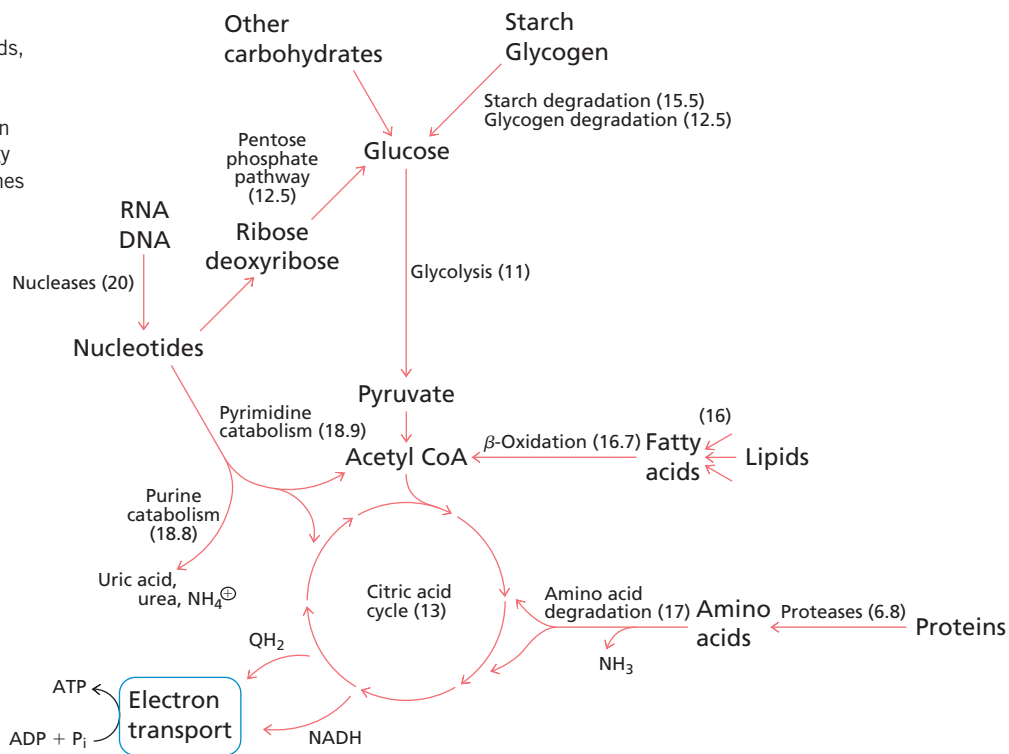
the formation of NADH and ATP. As mentioned above, the citric acid cycle is an essential part of both anabolic and catabolic metabolism.

The production of ATP is one of the most important reactions in metabolism. The synthesis of most ATP is coupled to membrane-associated electron transport (Chapter 14). In electron transport, the energy of reduced coenzymes such as NADH is used to generate an electrochemical gradient of protons across a cell membrane. The potential energy of this gradient is harnessed to drive the phosphorylation of ADP to ATP.

$$ADP + P_i \longrightarrow ATP + H_2O \qquad \textbf{(10.4)}$$

We will see that the reactions of membrane-associated electron transport and coupled ATP synthesis are similar in many ways to the reactions that capture light energy during photosynthesis (Chapter 15).

Three additional chapters examine the anabolism and catabolism of lipids, amino acids, and nucleotides. Chapter 16 discusses the storage of nutrient material as triacylglycerols and the subsequent oxidation of fatty acids. This chapter also describes the synthesis of phospholipids and isoprenoid compounds. Amino acid metabolism is discussed in Chapter 17. Although amino acids were introduced as the building blocks of proteins, some also play important roles as metabolic fuels and biosynthetic precursors. Nucleotide biosynthesis and degradation are considered in Chapter 18. Unlike the other three classes of biomolecules, nucleotides are catabolized primarily for excretion rather than for energy production. The incorporation of nucleotides into nucleic acids and of amino acids into proteins are major anabolic pathways. Chapters 20 to 22 describe these biosynthetic reactions.

10.4 Compartmentation and Interorgan Metabolism

Some metabolic pathways are localized to particular regions within a cell. For example, the pathway of membrane-associated electron transport coupled to ATP synthesis takes place within the membrane. In bacteria this pathway is located in the plasma membrane and in eukaryotes it is found in the mitochondrial membrane. Photosynthesis is another example of a membrane-associated pathway in bacteria and eukaryotes.

Golgi apparatus P
(end-on view)
sorting and secretion
of some proteins

Mitochondria:
citric acid cycle,
electron transport +
ATP synthesis, fatty
acid degradation

Lysosome:
degradation of proteins,
lipids, etc.

Plasma membrane

Cytosol:
fatty acid synthesis,
glycolysis, most
gluconeogme:s reaction
pentose phosphase
pathwwary

Nucleus:
nucleic acid synthesis

Endoplasmic reticulum:
delivery of proteins and
synthesis of lipids for
membranes

Nuclear membranes

Figure 10.10 ▲
Compartmentation of metabolic processes within a eukaryotic cell. This is a colored electron micrograph of a cell showing the nucleus (green), mitochondria (purple), lysosomes (brown), and extensive endoplasmic reticulum (blue). (Not all pathways and organelles are shown.)

In eukaryotes, metabolic pathways are localized within several membrane-bound compartments (Figure 10.10). For example, the enzymes that catalyze fatty acid synthesis are located in the cytosol, whereas the enzymes that catalyze fatty acid breakdown are located inside mitochondria. One consequence of compartmentation is that separate pools of metabolites can be found within a cell. This arrangement permits the simultaneous operation of opposing metabolic pathways. Compartmentation can also offer the advantage of high local concentrations of metabolites and coordinated regulation of enzymes. Some of the enzymes that catalyze reactions in mitochondria (which have evolved from a symbiotic prokaryote) are encoded by mitochondrial genes; this origin explains their compartmentation.

There is also compartmentation at the molecular level. Enzymes that catalyze some pathways are physically organized into multienzyme complexes (Section 5.11). With these complexes, channeling of metabolites prevents their dilution by diffusion. Some enzymes catalyzing adjacent reactions in pathways are bound to membranes and can diffuse rapidly in the membrane for interaction.

Individual cells of multicellular organisms maintain different concentrations of metabolites, depending in part on the presence of specific transporters that facilitate the entry and exit of metabolites. In addition, depending on the cell-surface receptors and signal-transduction mechanisms present, individual cells respond differently to external signals.

In multicellular organisms, compartmentation can also take the form of specialization of tissues. The division of labor among tissues allows site-specific regulation of metabolic processes. Cells from different tissues are distinguished by their complement of enzymes. We are very familiar with the specialized role of muscle tissue, red blood cells, and brain cells but cell compartmentation is a common feature even in simple species. In cyanobacteria, for example, the pathway for nitrogen fixation is sequestered in special cells called heterocysts (Figure 10.11). This separation is necessary because nitrogenase is inactivated by oxygen and the cells that carry out photosynthesis produce lots of oxygen.

▲ Figure 10.11
Anabaena spherica. Many species of cyanobacteria form long, multicellular filaments. Some specialized cells have adapted to carry out nitrogen fixation. These heterocysts have become rounded and are surrounded by a thickened cell wall. The heterocysts are connected to adjacent cells by internal pores. The formation of heterocysts is an example of compartmentation of metabolic pathways.

10.5 Actual Gibbs Free Energy Change, Not Standard Free Energy Change, Determines the Direction of Metabolic Reactions

The Gibbs free energy change is a measure of the energy available from a reaction (Section 1.4B). The *standard* Gibbs free energy change for any given reaction ($\Delta G^{\circ\prime}_{reaction}$) is the change under standard conditions of pressure (1 atm), temperature (25°C = 298 K), and hydrogen ion concentration (pH = 7.0). The concentration of every reactant and product is 1 M under standard conditions. For biochemical reactions, the concentration of water is assumed to be 55 M.

The standard Gibbs free energy change in a reaction can be determined by using tables that list the Gibbs free energies of formation ($\Delta_f G^{\circ\prime}$) of important biochemical molecules.

$$\Delta G^{\circ\prime}_{reaction} = \Delta_f G^{\circ\prime}_{products} - \Delta_f G^{\circ\prime}_{reactants} \tag{10.5}$$

Keep in mind that Equation 10.5 only applies to the free energy change under standard conditions where the concentrations of products and reactants are 1 M. It's also important to use tables that apply to biochemical reactions. These tables correct for pH and ionic strength. The Gibbs free energies of formation under cellular conditions are often quite different from the ones used in chemistry and physics.

The *actual* Gibbs free energy change (ΔG) for a reaction depends on the real concentrations of reactants and products, as described in Section 1.4B. The relationship between the standard free energy change and the actual free energy change is given by

$$\Delta G_{reaction} = \Delta G^{\circ\prime}_{reaction} + RT \ln \frac{[products]}{[reactants]} \tag{10.6}$$

For a chemical or physical process, the free energy change is expressed in terms of the changes in enthalpy (heat content) and entropy (randomness) as the reactants are converted to products at constant pressure and volume.

$$\Delta G = \Delta H - T\Delta S \tag{10.7}$$

ΔH is the change in enthalpy, ΔS is the change in entropy, and T is the temperature in degrees Kelvin.

When ΔG for a reaction is negative, the reaction will proceed in the direction it is written. When ΔG is positive, the reaction will proceed in the reverse direction—there will be a net conversion of products to reactants. For such a reaction to proceed in the direction written, enough energy must be supplied from outside the system to make the free energy change negative. When ΔG is zero, the reaction is at equilibrium and there is no net synthesis of product.

Because changes in both enthalpy and entropy contribute to ΔG, the sum of these contributions at a given temperature (as indicated in Equation 10.7) must be negative for a reaction to proceed. Thus, even if ΔS for a particular process is negative (i.e., the products are more ordered than the reactants), a sufficiently negative ΔH can overcome the decrease in entropy, resulting in a ΔG that is less than zero. Similarly, even if ΔH is positive (i.e., the products have a higher heat content than the reactants), a sufficiently positive ΔS can overcome the increase in enthalpy, resulting in a negative ΔG. Reactions that proceed because of a large positive ΔS are said to be entropy driven. Examples of entropy-driven processes include protein folding (Section 4.10) and the formation of lipid bilayers (Section 9.8A), both of which depend on the hydrophobic effect (Section 2.5D). The processes of protein folding and lipid-bilayer formation result in states of decreased entropy for the protein molecule and bilayer components, respectively. However, the decrease in entropy is offset by a large increase in the entropy of surrounding water molecules.

For any enzymatic reaction within a living organism, the actual free energy change (the free energy change under cellular conditions) must be less than zero in order for

the reaction to occur in the direction it is written. Many metabolic reactions have *standard* Gibbs free energy changes ($\Delta G^{\circ\prime}_{\text{reaction}}$) that are positive. The difference between ΔG and $\Delta G^{\circ\prime}$ depends on cellular conditions. The most important condition affecting free energy change in cells is the concentrations of substrates and products of a reaction. Consider the reaction

$$A + B \rightleftharpoons C + D \tag{10.8}$$

At equilibrium, the ratio of substrates and products is by definition the equilibrium constant (K_{eq}) and the Gibbs free energy change under these conditions is zero.

$$\text{(at equilibrium)} \qquad K_{\text{eq}} = \frac{[C][D]}{[A][B]} \quad \Delta G = 0 \tag{10.9}$$

When this reaction is not at equilibrium, a different ratio of products to substrates is observed and the Gibbs free energy change is derived using Equation 10.6.

$$\Delta G_{\text{reaction}} = \Delta G^{\circ\prime}_{\text{reaction}} + RT \ln \frac{[C][D]}{[A][B]} = \Delta G^{\circ\prime}_{\text{reaction}} + RT \ln Q$$

$$\left(\text{where } Q = \frac{[C][D]}{[A][B]} \right) \tag{10.10}$$

Q is the **mass action ratio**. The difference between this ratio and the ratio of products to substrates at equilibrium determines the actual Gibbs free energy change for a reaction. In other words, the free energy change is a measure of how far from equilibrium the reacting system is operating. Consequently, ΔG, not $\Delta G^{\circ\prime}$, is the criterion for assessing the direction of a reaction in a biological system.

We can divide metabolic reactions into two types. Let Q represent the steady-state ratio of product and reactant concentrations in a living cell. Reactions for which Q is close to K_{eq} are called **near-equilibrium reactions**. The free energy changes associated with near-equilibrium reactions are small, so these reactions are readily reversible. Reactions for which Q is far from K_{eq} are called **metabolically irreversible reactions**. These reactions are greatly displaced from equilibrium, with Q usually differing from K_{eq} by two or more orders of magnitude. Thus, ΔG is a large negative number for metabolically irreversible reactions.

When flux through a pathway changes by a large amount, there may be short-term perturbations of metabolite concentrations in the pathway. The intracellular concentrations of metabolites vary, but usually over a range of not more than two- or threefold and equilibrium is quickly restored. As mentioned above, this is called the steady state condition and it's typical of most of the reactions in a pathway. Most enzymes in a pathway catalyze near-equilibrium reactions and have sufficient activity to quickly restore concentrations of substrates and products to near-equilibrium conditions. They can accommodate flux in either direction. The Gibbs free energy change for these reactions is effectively zero.

In contrast, the activities of enzymes that catalyze metabolically irreversible reactions are usually insufficient to achieve near-equilibrium status for the reactions. Metabolically irreversible reactions are generally the control points of pathways, and the enzymes that catalyze these reactions are usually regulated in some way. In fact, the regulation maintains metabolic irreversibility by preventing the reaction from reaching equilibrium. Metabolically irreversible reactions can act as bottlenecks in metabolic traffic, helping control the flux through reactions further along the pathway.

Near-equilibrium reactions are not usually suitable control points. Flux through a near-equilibrium step cannot be significantly increased since it is already operating under conditions where the concentrations of products and reactants are close to the equilibrium values. The direction of near-equilibrium reactions can be controlled by changes in substrate and product concentrations. In contrast, flux through metabolically irreversible reactions is relatively unaffected by changes in metabolite concentration; flux through these reactions must be controlled by modulating the activity of the enzyme.

KEY CONCEPT

Metabolically irreversible reactions are catalyzed by enzymes whose activity is regulated in order to prevent the reaction from reaching equilibrium.
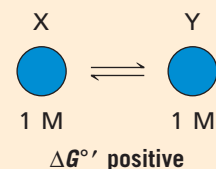
Consider a sample reaction X = Y under standard conditions of pressure, temperature, and concentration. Assume that $\Delta G^{\circ\prime}$ is negative.
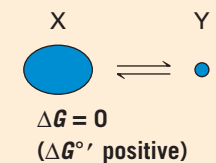

X   Y

1 M   1 M

$\Delta G^{\circ\prime}$ **negative**
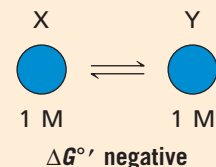
Inside the cell, the reaction will likely be at equilibrium and $\Delta G = 0$

X   Y

$\Delta G = 0$
($\Delta G^{\circ\prime}$ **negative**)
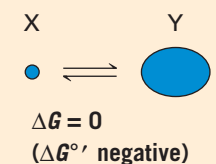
For a reaction in which $\Delta G^{\circ\prime}$ is positive,

X   Y

1 M   1 M

$\Delta G^{\circ\prime}$ **positive**

at equilibrium, the concentration of reactant will be higher than that of the product.

X   Y

$\Delta G = 0$
($\Delta G^{\circ\prime}$ **positive**)

The standard Gibbs free energy change does not predict whether a reaction will proceed in one direction or another. Instead, it predicts the steady state concentrations of reactants and products in near-equilibrium reactions.

Because so many metabolic reactions are near-equilibrium reactions, we have chosen not to emphasize $\Delta G°'$ values in our discussions of most reactions. Those values are not relevant except when they are used to calculate steady state concentrations.

SAMPLE CALCULATION 10.1 Calculating Standard Gibbs Free Energy Change from Energies of Formation

For any reaction, the standard Gibbs free energy change for the reaction is given by

$$\Delta G°'_{reaction} = \Delta_f G°'_{products} - \Delta_f G°'_{reactants}$$

For the oxidation of glucose,

$$(CH_2O)_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$
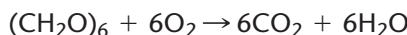
you obtain the standard Gibbs free energies of formation from biochemical tables.

$\Delta_f G°'(glucose) = -426 \text{ kJ mol}^{-1}$
$\Delta_f G°'(O_2) = 0$
$\Delta_f G°'(CO_2) = -394 \text{ kJ mol}^{-1}$
$\Delta_f G°'(H_2O) = -156 \text{ kJ mol}^{-1}$
$\Delta G°'_{reaction} = 6(-394) + 6(-156) - (-426)$
$\qquad\qquad = -2874 \text{ kJ mol}^{-1}$

Glucose is an energy-rich organic molecule and its oxidation releases a great deal of energy. Nevertheless, all living cells routinely synthesize glucose from simple precursors. In many cases, the precursors are CO_2 and H_2O in the reverse of the reaction shown here. How do they do it?

Section 7.2 A described the structure and functions of nucleoside triphosphates.

Another example of the role of pyrophosphate is discussed in Section 10.7C. Hydrolysis of pyrophosphate is often counted as one ATP equivalent in terms of energy currency.

Table 10.1 Free Energies of Formation ($\Delta_f G°'$)

|	kJ mol^{-1}
ATP	−2102
ADP	−1231
AMP	−360
P_i	−1059
H_2O	−156

(1 mM Mg$^{\oplus}$, ionic strength of 0.25 M)

10.6 The Free Energy of ATP Hydrolysis

ATP contains one phosphate ester formed by linkage of the α-phosphoryl group to the $5'$-oxygen of ribose and two phosphoanhydrides formed by the α,β and β,γ linkages between phosphoryl groups (Figure 10.12). ATP is a donor of several metabolic groups, usually a phosphoryl group, leaving ADP, or an AMP group, leaving inorganic pyrophosphate (PPi). Both reactions require the cleavage of a phosphoanhydride linkage. Although the various groups of ATP are not transferred directly to water, hydrolytic reactions provide useful estimates of the Gibbs free energy changes involved. Table 10.1 lists the free energies of formation of the various reactants and products under standard conditions, 1 mM Mg^{2+}, and an ionic strength of 0.25 M. Table 10.2 lists the standard Gibbs free energies of hydrolysis ($\Delta G°'_{hydrolysis}$) for ATP and AMP, and Figure 10.9 shows the hydrolytic cleavage of each of the phosphoanhydrides of ATP. Note from Table 10.2 that cleavage of the ester releases only 13 kJ mol^{-1} under standard conditions but cleavage of either of the phosphoanhydrides releases at least 30 kJ mol^{-1} under standard conditions.

Table 10.2 also gives the standard Gibbs free energy change for hydrolysis of pyrophosphate. All cells contain an enzyme called pyrophosphatase that catalyzes this reaction. The cellular concentration of pyrophosphate is maintained at a very low concentration as a consequence of this highly favorable reaction. This means that the hydrolysis of ATP to AMP + pyrophosphate will always be associated with a negative Gibbs free energy change even when the AMP concentration is significant.

Nucleoside diphosphates and triphosphates in both aqueous solution and at the active sites of enzymes are usually present as complexes with magnesium (or sometimes manganese) ions. These cations coordinate with oxygen atoms of the phosphate groups, forming six-membered rings. A magnesium ion can form several different complexes with ATP; the complexes involving the α and β and the β and γ phosphate groups are shown in Figure 10.13. Formation of the β,γ complex is favored in aqueous solutions. We will see later that nucleic acids are also usually complexed with counterions such as
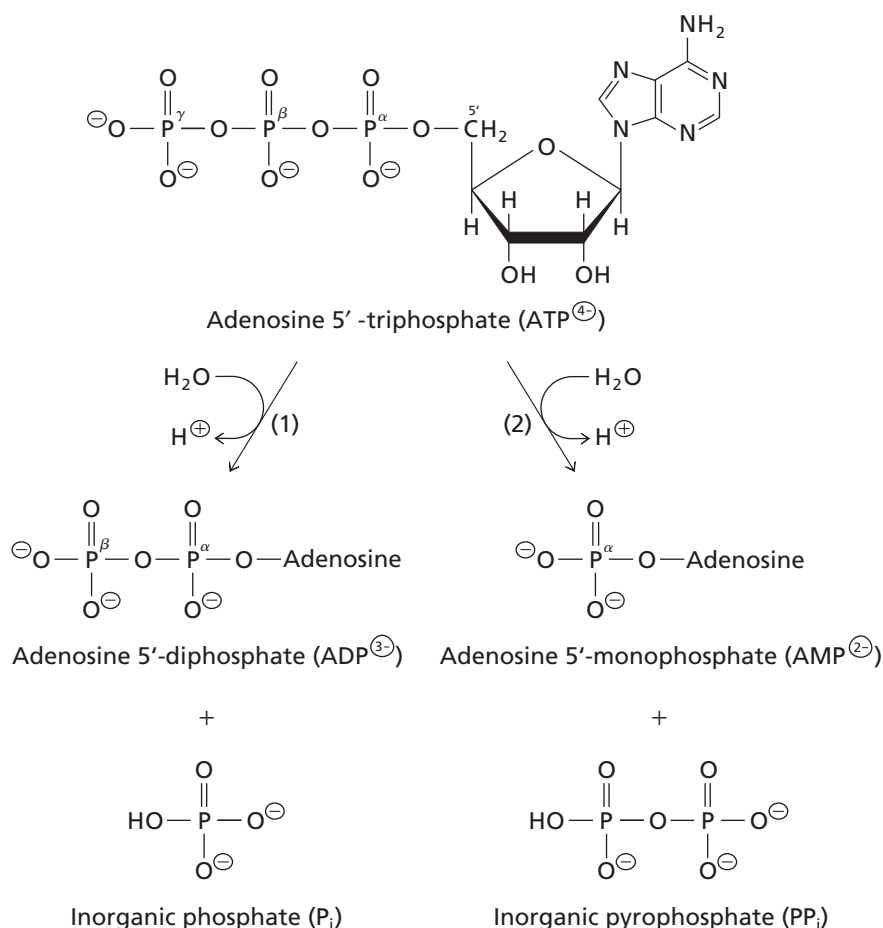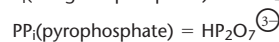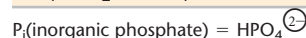
Hydrolysis of ATP to (1) ADP and inorganic phosphate (P_i) and (2) AMP and inorganic pyrophosphate (PP_i).

Adenosine 5′ -triphosphate (ATP^{4-})

Adenosine 5′-diphosphate (ADP^{3-})

Adenosine 5′-monophosphate (AMP^{2-})

+

+

Inorganic phosphate (P_i)

Inorganic pyrophosphate (PP_i)

> The release of a free proton in these reactions depends on the conditions since the pKa values of the various components are close to the value inside cells (see Figure 2.19).
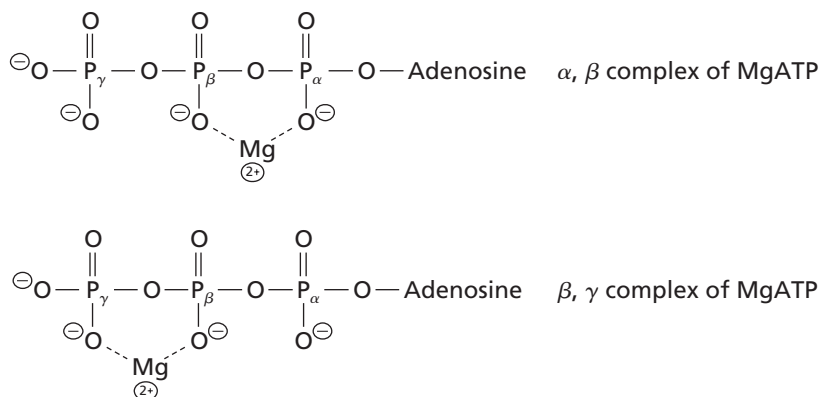
Table 10.2 Standard Gibbs free energies of hydrolysis for ATP, AMP, and pyrophosphate

Reactants and products	$\Delta G^{\circ\prime}_{hydrolysis}$ (kJ mol^{-1})
ATP + H_2O → ADP + P_i + $H^{\oplus}$	−32
ATP + H_2O → AMP + PP_i + $H^{\oplus}$	−45
AMP + H_2O → Adenosine + P_i + $H^{\oplus}$	−13
PP_i + H_2O → $2P_i$	−29

P_i(inorganic phosphate) = HPO_4^{2-}

PP_i(pyrophosphate) = $HP_2O_7^{3-}$

Mg^{2+} or cationic proteins. For convenience, we usually refer to the nucleoside triphosphates as adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), and uridine triphosphate (UTP), but remember that these molecules actually exist as complexes with Mg^{2+} in cells.

Several factors contribute to the large amount of energy released during hydrolysis of the phosphoanhydride linkages of ATP.

1. **Electrostatic repulsion.** Electrostatic repulsion among the negatively charged oxygen atoms of the phosphoanhydride groups of ATP is less after hydrolysis. [In cells, $\Delta G^{\circ\prime}_{hydrolysis}$ is actually increased (made more positive) by the presence of Mg^{2+}, which partially neutralizes the charges on the oxygen atoms of ATP and diminishes electrostatic repulsion.]

2. **Solvation effects.** The products of hydrolysis, ADP and inorganic phosphate, or AMP and inorganic pyrophosphate, are better solvated than ATP itself. When ions

Complexes between ATP and Mg^{2+}.

α, β complex of MgATP

β, γ complex of MgATP

are solvated, they are electrically shielded from each other. Solvation effects are probably the most important factor contributing to the energy of hydrolysis.

3. **Resonance stabilization.** The products of hydrolysis are more stable than ATP. The electrons on terminal oxygen atoms are more delocalized than those on bridging oxygen atoms. Hydrolysis of ATP replaces one bridging oxygen atom with two new terminal oxygen atoms.

Because of the free energy change associated with the cleavage of their phosphoanhydrides, ATP and the other nucleoside triphosphates (UTP, GTP, and CTP) are often referred to as **energy-rich compounds**, but keep in mind that it's the system, not the molecule, that contributes free energy to biochemical reactions. ATP, by itself, is not really a high energy compound. It can only work if the system (reactants and products) is far from equilibrium. The ATP currency becomes worthless if the reaction reaches equilibrium and $\Delta G = 0$. We will find it useful to refer to "energy-rich" or "**high energy**" molecules in the jargon of biochemistry but we will put the terms in quotation marks to remind you that it is jargon.

All the phosphoanhydrides of nucleoside triphosphates have nearly equal standard Gibbs free energies of hydrolysis. We occasionally express the consumption or formation of the phosphoanhydride linkages of nucleoside triphosphates in terms of ATP equivalents.

ATP is usually the phosphoryl group donor when nucleoside monophosphates and diphosphates are phosphorylated. Of course, the intracellular concentrations of individual nucleoside mono-, di-, and triphosphates differ, depending on metabolic needs. For example, the intracellular levels of ATP are far greater than deoxythymidine triphosphate (dTTP) levels. ATP is involved in many reactions, whereas dTTP has fewer functions and is primarily a substrate for DNA synthesis.

A series of kinases (phosphotransferases) catalyze interconversions of nucleoside mono-, di-, and triphosphates. Phosphoryl group transfers between nucleoside phosphates have equilibrium constants close to 1.0. Nucleoside monophosphate kinases are a group of enzymes that catalyze the conversion of nucleoside monophosphates to nucleoside diphosphates. For example, guanosine monophosphate (GMP) is converted to guanosine diphosphate (GDP) by the action of guanylate kinase. GMP or its deoxy analog dGMP is the phosphoryl group acceptor in the reaction, and ATP or dATP is the phosphoryl group donor.

$$\text{GMP} + \text{ATP} \rightleftharpoons \text{GDP} + \text{ADP} \qquad (10.11)$$
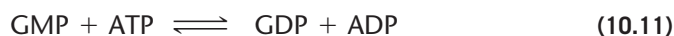
Nucleoside diphosphate kinase acts in the conversion of nucleoside diphosphates to nucleoside triphosphates. This enzyme, present in both the cytosol and mitochondria of eukaryotes, is much less specific than nucleoside monophosphate kinases. All nucleoside diphosphates, regardless of the purine or pyrimidine base, are substrates for nucleoside diphosphate kinase. Nucleoside monophosphates are not substrates. Because of its relative abundance, ATP is usually the phosphoryl-group donor in cells:

$$\text{GDP} + \text{ATP} \rightleftharpoons \text{GTP} + \text{ADP} \qquad (10.12)$$

Although the concentration of ATP varies among cell types, the intracellular ATP concentration fluctuates very little within a particular cell, and the sum of the concentrations of the adenine nucleotides remains nearly constant. Intracellular ATP concentrations are maintained in part by the action of adenylate kinase that catalyzes the following near-equilibrium reaction:

$$\text{AMP} + \text{ATP} \rightleftharpoons 2\,\text{ADP} \qquad (10.13)$$

When the concentration of AMP increases, AMP can react with ATP to form two molecules of ADP. These ADP molecules can be converted to two molecules of ATP. The overall process is

$$\text{AMP} + \text{ATP} + 2\,\text{P}_i \rightleftharpoons 2\,\text{ATP} + 2\,\text{H}_2\text{O} \qquad (10.14)$$

ATP concentrations in cells are greater than ADP or AMP concentrations, and relatively minor changes in the concentration of ATP can result in large changes in the concentrations of the di- and monophosphates. Table 10.3 shows the theoretical increases in

---

A quantitative definition of a "high energy" compound is presented in Section 10.7A.

KEY CONCEPT

The large free energy change associated with hydrolysis of ATP is only possible if the system is far from equilibrium.

Table 10.3 Theoretical changes in concentrations of adenine nucleotides

ATP	ADP	AMP
(mM)	(mM)	(mM)
4.8	0.2	0.004
4.5	0.5	0.02
3.9	1.0	0.11
3.2	1.5	0.31

[Adapted from Newsholme. E. A., and Leech, A. R. (1986). *Biochemistry for the Medical Science* (New York: John Wiley & Sons), p. 315.]

[ADP] and [AMP] under conditions in which ATP is consumed, assuming that the total adenine nucleotide concentration remains 5.0 mM. Note that when the ATP concentration decreases from 4.8 mM to 4.5 mM (a decrease of about 6%), the ADP concentration increases 2.5-fold and the AMP concentration increases 5-fold. In fact, when cells are well supplied with oxidizable fuels and oxygen, they maintain a balance of adenine nucleotides in which ATP is present at a steady concentration of 2 to 10 mM, [ADP] is less than 1 mM, and [AMP] is even lower. As we will see, ADP and AMP are often effective allosteric modulators of some energy-yielding metabolic processes. ATP, whose concentration is relatively constant, is generally not an important modulator under physiological conditions.

One important consequence of the concentrations of ATP and its hydrolysis products *in vivo* is that the free energy change for ATP hydrolysis is actually greater than the standard value of -32 kJ mol^{-1}. This is illustrated in Sample Calculation 10.2 using measured concentrations of ATP, ADP, and P$_i$ from rat liver cells. The calculated Gibbs free energy change is close to the value determined in many other types of cells.

As mentioned above, ATP hydrolysis is an example of a metabolically irreversible reaction. The activities of various enzymes are regulated so they become inactive as ATP concentrations fall below a minimal threshold. Thus, the reverse of the hydrolysis reaction, leading to ATP synthesis, does not occur except under special circumstances (Chapter 14). We will see in Chapter 14 that ATP is synthesized by another pathway.

The importance of maintaining a high concentraion of ATP cannot be overemphasized. It is required in order to get a large free energy change from ATP hydrolysis. Cells will die if the reactants and products reach equilibrium.

10.7 The Metabolic Roles of ATP

The energy produced by one biological reaction or process, such as the synthesis of X—Y in Reaction 10.15, is often coupled to a second reaction, such as the hydrolysis of ATP. The first reaction would not otherwise occur spontaneously.

$$X + Y \rightleftharpoons X—Y$$
$$ATP + H_2O \rightleftharpoons ADP + P_i + H^\oplus \qquad \textbf{(10.15)}$$
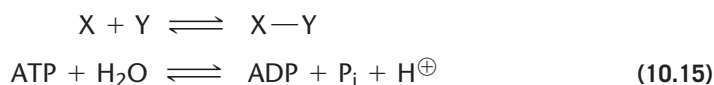
SAMPLE CALCULATION 10.2 Gibbs Free Energy Change

Q: In a rat hepatocyte, the concentrations of ATP, ADP, and P$_i$ are 3.4 mM, 1.3 mM, and 4.8 mM, respectively. Calculate the Gibbs free energy change for hydrolysis of ATP in this cell. How does this compare to the standard free energy change?

A: The actual Gibbs free energy change is calculated according to Equation 10.10.

$$\Delta G_{reaction} = \Delta G^{\circ\prime}_{reaction} + RT \ln \frac{[ADP][P_i]}{[ATP]} = \Delta G^{\circ}_{reaction} + 2.303\ RT \log \frac{[ADP][P_i]}{[ATP]}$$

When known values and constants are substituted (with concentrations expressed as molar values), assuming pH 7.0 and 25°C.

$$\Delta G = -32000\ \text{J mol}^{-1} + (8.31\ \text{JK}^{-1}\text{mol}^{-1})(298\ \text{K})\left[2.303 \log \frac{(1.3 \times 10^{-3})(4.8 \times 10^{-3})}{(3.4 \times 10^{-3})} \right]$$

$$\Delta G = -32000\ \text{J mol}^{-1} + (2480\ \text{J mol}^{-1})[2.303\ \log(1.8 \times 10^{-3})]$$

$$\Delta G = -32000\ \text{J mol}^{-1} - 16000\ \text{J mol}^{-1}$$

$$\Delta G = -48000\ \text{J mol}^{-1} = -48\ \text{kJ mol}^{-1}$$

The actual free energy change is about $1\frac{1}{2}$ times the standard free energy change.

The sum of the Gibbs free energy changes for the coupled reactions must be negative for the reactions to proceed. This does not mean that both of the individual reactions have to be favored in isolation ($\Delta G < 0$). The advantage of coupled reactions is that the energy released from one of them can be used to drive the other even when the second reaction is unfavorable by itself ($\Delta G > 0$). (Recall that the ability to couple reactions is one of the key properties of enzymes.)

Energy flow in metabolism depends on many coupled reactions involving ATP. In many cases, the coupled reactions are linked by a shared intermediate such as a phosphorylated derivative of reactant X.

$$X + ATP \rightleftharpoons X—P + ADP$$
$$X—P + Y + H_2O \rightleftharpoons X—Y + P_i + H^{\oplus} \qquad \textbf{(10.16)}$$
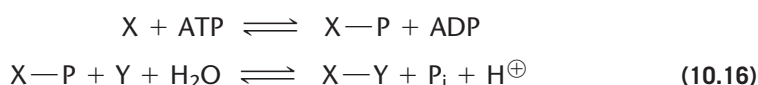
Transfer of either a phosphoryl group or a nucleotidyl group to a substrate activates that substrate (i.e., prepares it for a reaction that has a large negative Gibbs free energy change). The activated compound ($X—P$), can be either a metabolite or the side chain of an amino acid residue in the active site of an enzyme. The intermediate then reacts with a second substrate to complete the reaction.

A. Phosphoryl Group Transfer

The synthesis of glutamine from glutamate and ammonia illustrates how the "high energy" compound ATP drives a biosynthetic reaction. This reaction, catalyzed by glutamine synthetase, allows organisms to incorporate inorganic nitrogen into biomolecules as carbon-bound nitrogen. In this synthesis of an amide bond, the γ-carboxyl group of the substrate is activated by synthesis of an anhydride intermediate.

Glutamine synthetase catalyzes the nucleophilic displacement of the γ-phosphoryl group of ATP by the γ-carboxylate of glutamate. ADP is released, producing enzyme-bound γ-glutamyl phosphate as an intermediate (Figure 10.14). γ-Glutamyl phosphate is unstable in aqueous solution but is protected from water in the active site of glutamine synthetase. In the second step of the mechanism, ammonia acts as a nucleophile, displacing the phosphate (a good leaving group) from the carbonyl carbon of γ-glutamyl phosphate to generate the product, glutamine. Overall, one molecule of ATP is converted to ADP + P_i for every molecule of glutamine formed from glutamate and ammonia.


BOX 10.1 THE SQUIGGLE

Fritz Lipmann (1899–1986) won the Nobel Prize in Physiology and Medicine in 1953 for discovering coenzyme A. He also made important contributions to our understanding of ATP as an energy currency. In 1941 he introduced the idea of a high energy bond in ATP by drawing it as a squiggle (~). For the next several decades, biochemistry textbooks often depicted ATP with two high energy bonds.
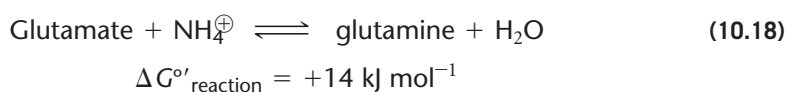
AMP~P~P

We know now that this depiction is misleading since there's nothing special about the covalent bonds in phosphoanhydride linkages. It's the overall system of reactants and products that makes the ATP currency so valuable and not the energy of individual bonds. However, it's true that the three main explanations for the high energy of ATP (electrostatic repulsion, solvation effects, and resonance stabilization) are due mostly to the phosphoanhydride linkages so the focus on that particular linkage isn't entirely wrong. The squiggle used to be very common in the older scientific literature and in textbooks but it's much less common today.

Source: Lipmann, F. (1941) Metabolic generation and utilization of phosphate bond energy. *Advances in Enzymology* 1:99–162.

γ-Glutamyl phosphate   **(10.17)**
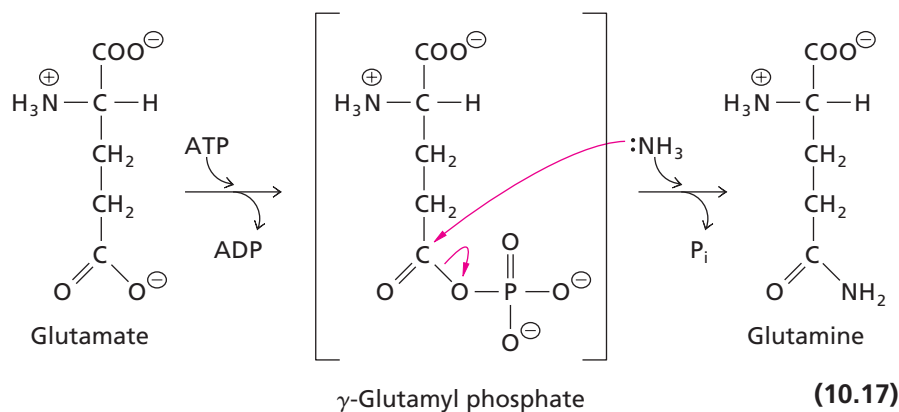
We can calculate the predicted standard Gibbs free energy change for the reaction that is not coupled to ATP hydrolysis.

$$\text{Glutamate} + NH_4^{\oplus} \rightleftharpoons \text{glutamine} + H_2O \qquad \textbf{(10.18)}$$

$$\Delta G^{\circ\prime}_{reaction} = +14 \text{ kJ mol}^{-1}$$

This is a standard free energy change so it doesn't necessarily reflect the actual Gibbs free energy change given cellular concentrations of glutamate, glutamine, and ammonia. The hypothetical Reaction 10.18 might be associated with a negative free energy change inside the cell if the concentrations of glutamate and ammonia were high relative to the concentration of glutamine. But this is not the case. The steady-state concentrations of glutamate and glutamine must be kept nearly equivalent in order to support protein synthesis and other metabolic pathways. This means that the Gibbs free energy change for the hypothetical Reaction 10.18 cannot be negative. Furthermore, the concentration of ammonia is very low relative to glutamate and glutamine. In both bacteria and eukaryotes, ammonia must be efficiently incorporated into glutamine even when the concentration of free ammonia is very low. Thus Reaction 10.18 is not possible in living cells due to the requirement for a high steady-state concentration of glutamine and due to a limiting supply of ammonia. Glutamine synthesis must be coupled to hydrolysis of ATP in order to drive it in the right direction.

Glutamine synthetase catalyzes a phosphoryl group transfer reaction in which the phosphorylated compound is a transient intermediate (Reaction 10.17). There are other reactions that produce a stable phosphorylated product. As we have seen, kinases catalyze
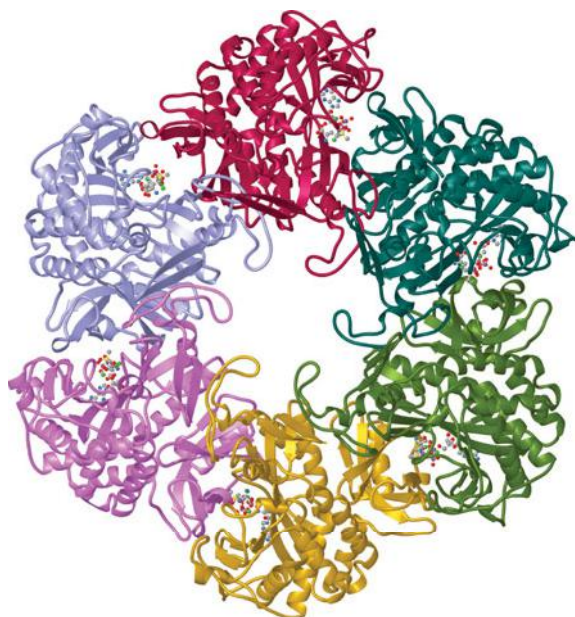
◄ **Figure 10.14**
Glutamine synthetase bound to ADP and a transition state analog. Glutamine synthetase from *Mycobacterium tuberculosis* is a complex enzyme consisting of two hexameric rings on top of each other. Only one ring is shown in this figure. The active site is occupied by ADP and a transition state analog (L-methionine-S-sulfoximine phosphate) that resembles γ-glutamyl phosphate. [PDB 2BVC]

Table 10.4 Standard Gibbs free energies of hydrolysis for common metabolites

Metabolite	$\Delta G^{\circ\prime}_{hydrolysis}$ (kJ mol^{-1})
Phosphoenolpyruvate	−62
1, 3-*Bis*phosphoglycerate	−49
ATP to AMP + PP$_i$	−45
Phosphocreatine	−43
Phosphoarginine	−32
Acetyl CoA	−32
Acyl CoA	−31
ATP to ADP + P$_i$	−32
Pyrophosphate	−29
Glucose 1-phosphate	−21
Glucose 6-phosphate	−14
Glycerol 3-phosphate	−9

KEY CONCEPT

Many phosphorylated metabolites have group transfer potentials similar to that of ATP.

transfer of the γ-phosphoryl group from ATP (or, less frequently, from another nucleoside triphosphate) to another substrate. Kinases typically catalyze metabolically irreversible reactions. A few kinase reactions, however, such as those catalyzed by adenylate kinase (Reaction 10.13) and creatine kinase (Section 10.7B), are near equilibrium reactions. Although the reactions they catalyze are sometimes described as phosphate group transfer reactions, kinases actually transfer a phosphoryl group (—PO$_3^{\textcircled{2}}$—)to their acceptors.

The ability of a phosphorylated compound to transfer its phosphoryl group(s) is termed its **phosphoryl group transfer potential**, or simply group transfer potential. Some compounds, such as phosphoanhydrides, are excellent phosphoryl group donors. They may have a group transfer potential equal to or greater than that of ATP. Other compounds, such as phosphoesters, are poor phosphoryl group donors. They have a group transfer potential less than that of ATP. Under standard conditions, group transfer potentials have the same values as the standard free energies of hydrolysis but are opposite in sign. Thus, the group transfer potential is a measure of the free energy required for formation of the phosphorylated compound. In Table 10.4 we list the standard Gibbs free energy of hydrolysis for a number of phosphorylated compounds.

B. Production of ATP by Phosphoryl Group Transfer

Often, one kinase catalyzes transfer of a phosphoryl group from an excellent donor to ADP to form ATP, which then acts as a donor for a different kinase reaction. Phosphoenolpyruvate and 1,3-bisphosphoglycerate are two examples of common metabolites that have higher energy than ATP even under conditions found inside the cell ($\Delta G <$ −50 kJ mol^{-1}). Some of these compounds are intermediates in catabolic pathways; others are energy storage compounds.

Phosphoenolpyruvate, an intermediate in the glycolytic pathway, has the highest phosphoryl group transfer potential known. The standard free energy of phosphoenolpyruvate hydrolysis is −62 kJ mol^{-1} and the actual Gibbs free energy change is comparable to that of ATP. The free energy of hydrolysis for phosphoenolpyruvate can be understood by picturing the molecule as an enol whose structure is locked by attachment of the phosphoryl group. When the phosphoryl group is removed, the molecule spontaneously forms the much more stable keto tautomer (Figure 10.15). Transfer of the phosphoryl group from phosphoenolpyruvate to ADP is catalyzed by the enzyme pyruvate kinase. Because the $\Delta G^{\circ\prime}$ for the reaction is about −30 kJ mol^{-1}, the equilibrium for this reaction under standard conditions lies far in the direction of transfer of the phosphoryl group from phosphoenolpyruvate to ADP. In cells, this metabolically irreversible reaction is an important source of ATP.

Phosphagens, including phosphocreatine and phosphoarginine, are "high energy" phosphate storage molecules found in animal muscle cells. Phosphagens are phosphoamides (rather than phosphoanhydrides) and have higher group transfer potentials than ATP. In the muscles of vertebrates, large amounts of phosphocreatine are formed during times of ample ATP supply. In resting muscle, the concentration of phosphocreatine is about fivefold higher than that of ATP. When ATP levels fall, creatine kinase catalyzes rapid replenishment of ATP through transfer of the activated phosphoryl group from phosphocreatine to ADP.

$$\text{Phosphocreatine + ADP} \underset{}{\overset{\text{Creatine kinase}}{\rightleftharpoons}} \text{creatine + ATP} \qquad \textbf{(10.19)}$$
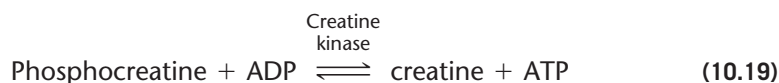
The supply of phosphocreatine is adequate for 3- to 4-second bursts of activity, long enough for other metabolic processes to begin restoring the ATP supply. Under cellular conditions, the creatine kinase reaction is a near-equilibrium reaction. In many invertebrates—notably mollusks and arthropods—phosphoarginine is the source of the activated phosphoryl group.

Because ATP has an intermediate phosphoryl group transfer potential, it is thermodynamically suited as a carrier of phosphoryl groups. (Figure 10.15) ATP is also kinetically stable under physiological conditions until acted on by an enzyme so it can carry chemical potential energy from one enzyme to another without being hydrolyzed. Not surprisingly, ATP mediates most chemical energy transfers in all organisms.
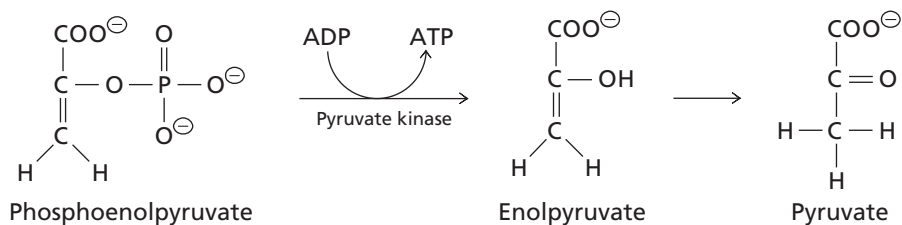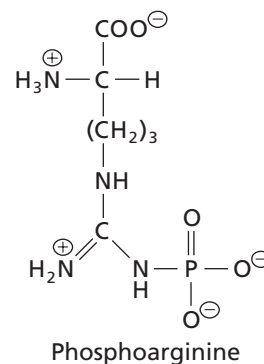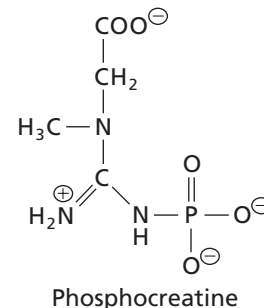
◄ Figure 10.15
Transfer of the phosphoryl group from phospho-enolpyruvate to ADP.

C. Nucleotidyl Group Transfer

The other common group transfer reaction involving ATP is transfer of the nucleotidyl group. An example is the synthesis of acetyl CoA, catalyzed by acetyl-CoA synthetase. In this reaction, the AMP moiety of ATP is transferred to the nucleophilic carboxylate group of acetate to form an acetyl–adenylate intermediate (Figure 10.16). Note that pyrophosphate (PP$_i$) is released in this step. Like the glutamyl–phosphate intermediate in Reaction 10.17, the reactive intermediate is shielded from nonenzymatic hydrolysis by tight binding within the active site of the enzyme. The reaction is completed by transfer of the acetyl group to the nucleophilic sulfur atom of coenzyme A, leading to the formation of acetyl CoA and AMP.

The synthesis of acetyl CoA also illustrates how the removal of a product can cause a metabolic reaction to approach completion, just as the formation of a precipitate or a gas can drive an inorganic reaction toward completion. The standard Gibbs free energy for the formation of acetyl CoA from acetate and CoA is about -13 kJ mol^{-1} ($\Delta G^{\circ\prime}_{\text{hydrolysis}}$ of acetyl CoA = -32 kJ mol^{-1}). But note that the product PP$_i$ is hydrolyzed to two molecules of P$_i$ by the action of pyrophosphatase (Section 10.6). Almost all cells have high levels of activity of this enzyme, so the concentration of PP$_i$ in cells is generally very low (less than 10^{-6} M). Cleavage of PP$_i$ contributes to the negative value of the standard Gibbs free energy change for the overall reaction. The additional hydrolytic reaction adds the energy cost of one phosphoanhydride linkage to the overall synthetic process. In reactions such as this, we say that the cost is two ATP equivalents in order to emphasize that two "high energy" compounds are hydrolyzed. Hydrolysis of pyrophosphate accompanies many synthetic reactions in metabolism.


Phosphocreatine

Phosphoarginine

▲ Structures of phosphocreatine and phosphoarginine.
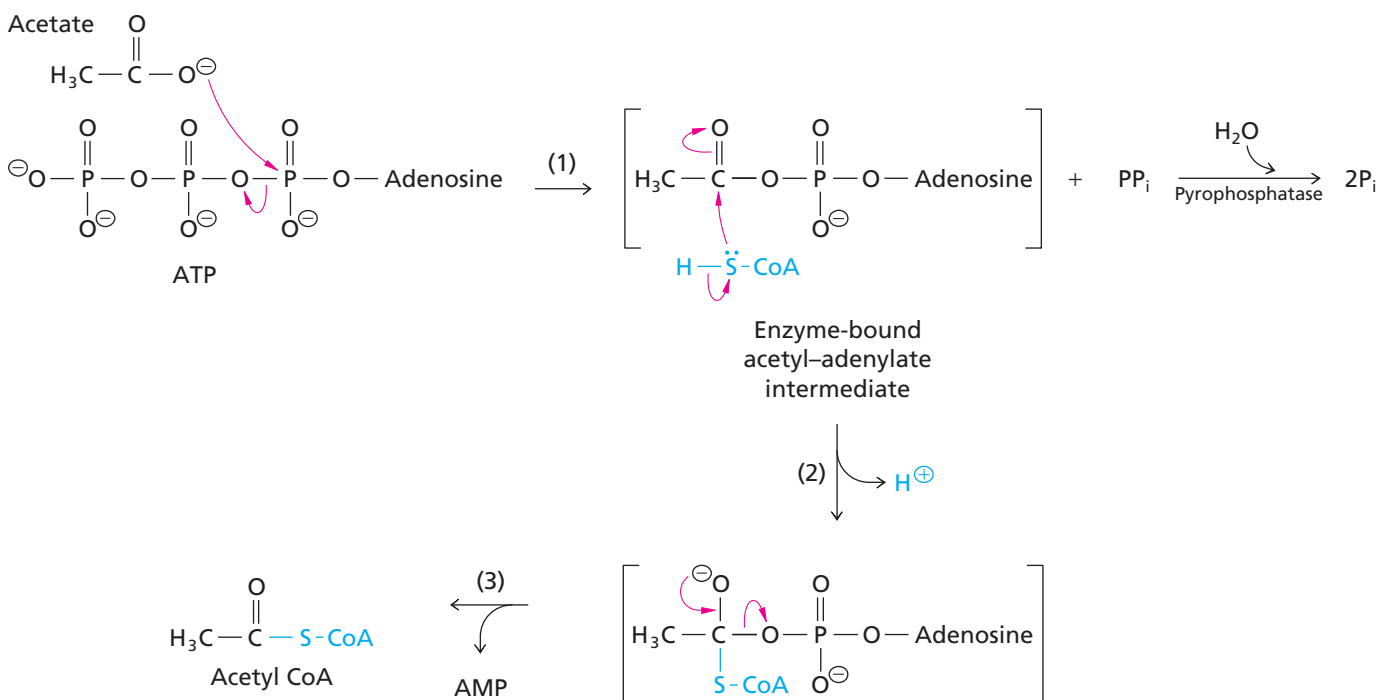
▲ Figure 10.16
Synthesis of acetyl CoA from acetate, catalyzed by acetyl-CoA synthetase.

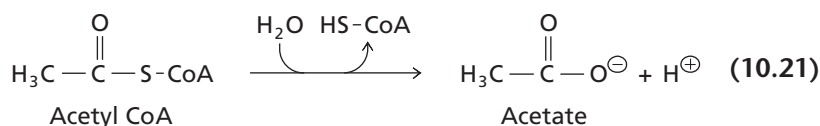## 10.8 Thioesters Have High Free Energies of Hydrolysis

Thioesters are another class of "high energy" compounds forming part of the currency of metabolism. Acetyl CoA is one example. It occupies a central position in metabolism (Figures 10.8 and 10.9). The high energy of thioester reactions can be used in generating ATP equivalents or in transferring the acyl groups to acceptor molecules. Recall that acyl groups are attached to coenzyme A (or acyl carrier protein) via a thioester linkage (Section 7.6 and Figure 7.13).

$$R-\overset{\overset{\displaystyle O}{\|}}{C}-S-\text{Coenzyme A} \tag{10.20}$$
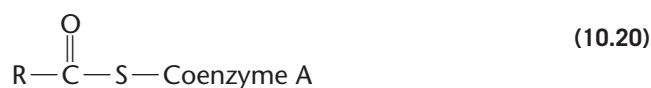
Unlike oxygen esters of carboxylic acids, thioesters resemble carboxylic acid anhydrides in reactivity. Sulfur is in the same group of the periodic table as oxygen but thioesters are less stable than typical esters because the unshared electrons of the sulfur atom are not as effectively delocalized in a thioester as the unshared electrons in an oxygen ester. The energy associated with hydrolyzing the thioester linkage is similar to the energy of hydrolysis of the phosphoanhydride linkages in ATP. The standard Gibbs free energy change for hydrolysis of acetyl CoA is -31 kJ mol^{-1}, and the actual change may somewhat smaller (more negative) under conditions inside the cell.

KEY CONCEPT

Reactions involving thioesters, such as acetyl CoA, release amounts of energy comparable to that of ATP hydrolysis.

$$\underset{\text{Acetyl CoA}}{H_3C-\overset{\overset{\displaystyle O}{\|}}{C}-S\text{-CoA}} \xrightarrow{\quad H_2O \;\; HS\text{-CoA} \quad} \underset{\text{Acetate}}{H_3C-\overset{\overset{\displaystyle O}{\|}}{C}-O^{\ominus} + H^{\oplus}} \tag{10.21}$$

Despite its high free energy of hydrolysis, a CoA thioester resists nonenzymatic hydrolysis at neutral pH values. In other words, it is kinetically stable in the absence of appropriate catalysts.

The high energy of hydrolysis of a CoA thioester is used in the fifth step of the citric acid cycle, when the thioester succinyl CoA reacts with GDP (or sometimes ADP) and P_i to form GTP (or ATP).
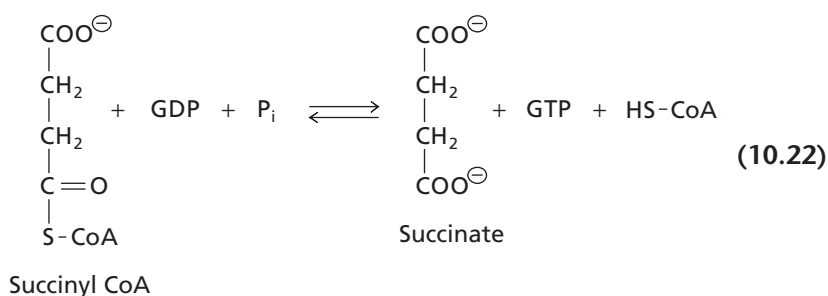
We discuss succinyl CoA synthetase in Section 13.4, part 5, and fatty acid synthesis in Section 16.5.

$$
\underset{\text{Succinyl CoA}}{
\begin{array}{c}
COO^{\ominus}\\
|\\
CH_2\\
|\\
CH_2\\
|\\
C=O\\
|\\
S\text{-CoA}
\end{array}}
+ \; GDP \; + \; P_i \;\; \rightleftharpoons \;\;
\underset{\text{Succinate}}{
\begin{array}{c}
COO^{\ominus}\\
|\\
CH_2\\
|\\
CH_2\\
|\\
COO^{\ominus}
\end{array}}
\; + \; GTP \; + \; HS\text{-CoA}
\tag{10.22}
$$

This substrate-level phosphorylation conserves energy used in the formation of succinyl CoA as ATP equivalents. The energy of thioesters also drives the synthesis of fatty acids.

10.9 Reduced Coenzymes Conserve Energy from Biological Oxidations

In Section 14.11 we will learn that NADH is equivalent to 2.5 ATPs and QH$_2$ is equivalent to 1.5 ATPs.

Many reduced coenzymes are "high energy" compounds in the sense we described earlier (i.e., part of a system). Their high energy (or reducing power) can be donated in oxidation-reduction reactions. The energy of reduced coenzymes may be represented as ATP equivalents since their oxidation can be coupled to the synthesis of ATP.

As described in Section 6.1C, the oxidation of one molecule must be coupled with the reduction of another molecule. A molecule that accepts electrons and is reduced is an oxidizing agent. A molecule that loses electrons and is oxidized is a reducing agent. The net oxidation–reduction reaction is

$$A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red} \tag{10.23}$$

The electrons released in biological oxidation reactions are transferred enzymatically to oxidizing agents, usually a pyridine nucleotide ($NAD^\oplus$ or sometimes $NADP^\oplus$), a flavin coenzyme (FMN or FAD), or ubiquinone (Q). When $NAD^\oplus$ and $NADP^\oplus$ are reduced, their nicotinamide rings accept a hydride ion (Figure 7.8). One electron is lost when a hydrogen atom (composed of one proton and one electron) is removed and two electrons are lost when a hydride ion (composed of one proton and two electrons) is removed. (Remember that oxidation is loss of electrons.)

NADH and NADPH, along with QH_2, supply reducing power. $FMNH_2$ and $FADH_2$ are reduced enzyme-bound intermediates in some oxidation reactions.

> The structures and functions of $NAD^\oplus$ and $NADP^\oplus$ are discussed in Section 7.4, of FMN and FAD in Section 7.5, and of ubiquinone in Section 7.14.

A. Gibbs Free Energy Change Is Related to Reduction Potential

The **reduction potential** of a reducing agent is a measure of its thermodynamic reactivity. Reduction potential can be measured in electrochemical cells. An example of a simple inorganic oxidation–reduction reaction is the transfer of a pair of electrons from a zinc atom (Zn) to a copper ion (Cu^{2+}).

$$Zn + Cu^{2+} \rightleftharpoons Zn^{2+} + Cu \tag{10.24}$$

This reaction can be carried out in two separate solutions that divide the overall reaction into two half-reactions (Figure 10.17). At the zinc electrode, two electrons are given up by each zinc atom that reacts (the reducing agent). The electrons flow through a wire to the copper electrode, where they reduce Cu^{2+} (the oxidizing agent) to metallic copper. A salt bridge, consisting of a tube with a porous partition filled with electrolyte, preserves electrical neutrality by providing an aqueous path for the flow of nonreactive counterions between the two solutions. The flow of ions and the flow of electons are separated in such an electrochemical cell and electron flow through the wire (i.e., electric energy) can be measured using a voltmeter.

The direction of the current through the circuit in Figure 10.17 indicates that Zn is more easily oxidized than Cu (i.e., Zn is a stronger reducing agent than Cu). The reading on the voltmeter represents a potential difference, the difference between the reduction potential of the reaction on the left and that on the right. The measured potential difference is the **electromotive force**.

◄ **Figure 10.17**
Diagram of an electrochemical cell. Electrons flow through the external circuit from the zinc electrode to the copper electrode. The salt bridge permits the flow of counterions (sulfate ions in this example) without extensive mixing of the two solutions. The electromotive force is measured by the voltmeter connected across the two electrodes. (Two other kinds of salt bridges are shown in Section 2.5A.)

It is useful to have a reference standard for measurements of reduction potentials just as in measurements of Gibbs free energy changes. For reduction potentials, the reference is not simply a set of reaction conditions, but a reference half-reaction to which all other half-reactions can be compared. The reference half-reaction is the reduction of $H^{\oplus}$ to hydrogen gas (H_2). The reduction potential of this half-reaction under standard conditions ($E°$) is arbitrarily set at 0.0 V. The standard reduction potential of any other half-reaction is measured with an oxidation–reduction coupled reaction in which the reference half-cell contains a solution of 1 M $H^{\oplus}$ and 1 atm H_2 (gaseous), and the sample half-cell contains 1 M each of the oxidized and reduced species of the substance whose reduction potential is to be determined. Under standard conditions for biological measurements, the hydrogen ion concentration in the sample half-cell is (10^{-7}M). The voltmeter across the oxidation–reduction couple measures the electromotive force, or the difference in the reduction potential, between the reference and sample half-reactions. Since the standard reduction potential of the reference half-reaction is 0.0 V, the measured potential is that of the sample half-reaction.

Table 10.5 gives the standard reduction potentials at pH 7.0 ($E°'$) of some important biological half-reactions. Electrons flow spontaneously from the more readily oxidized

Table 10.5 Standard reduction potentials of some important biological half-reactions

Reduction half-reaction	$E°'$(V)
Acetyl CoA + CO_2 + $H^{\oplus}$ + $2e^{\ominus}$ → Pyruvate + CoA	−0.48
Ferredoxin (spinach). Fe^{3+} + $e^{\ominus}$ → Fe^{2+}	−0.43
2 $H^{\oplus}$ + $2e^{\ominus}$ → H_2 (at pH 7.0)	−0.42
α-Ketoglutarate + CO_2 + 2 $H^{\oplus}$ + $2e^{\ominus}$ → Isocitrate	−0.38
Lipoyl dehydrogenase (FAD) + 2 $H^{\oplus}$ + $2e^{\ominus}$ → Lipoyl dehydrogenase ($FADH_2$)	−0.34
$NADP^{\oplus}$ + $H^{\oplus}$ + $2e^{\ominus}$ → NADPH	−0.32
$NAD^{\oplus}$ + $H^{\oplus}$ + $2e^{\ominus}$ → NADH	−0.32
Lipoic acid + 2 $H^{\oplus}$ + $2e^{\ominus}$ → Dihydrolipoic acid	−0.29
Thioredoxin (oxidized) + $2H^{\oplus}$ + $2e$ → Thioredoxin (reduced)	−0.28
Glutathione (oxidized) + 2 $H^{\oplus}$ + $2e^{\ominus}$ → 2 Glutathione (reduced)	−0.23
FAD + 2 $H^{\oplus}$ + $2e^{\ominus}$ → $FADH_2$	−0.22
FMN + 2 $H^{\oplus}$ + $2e^{\ominus}$ → $FMNH_2$	−0.22
Acetaldehyde + 2 $H^{\oplus}$ + $2e^{\ominus}$ → Ethanol	−0.20
Pyruvate + 2 $H^{\oplus}$ + $2e^{\ominus}$ → Lactate	−0.18
Oxaloacetate + 2 $H^{\oplus}$ + $2e^{\ominus}$ → Malate	−0.17
Cytochrome b_5 (microsomal). Fe^{3+} + $e^{\ominus}$ → Fe^{2+}	0.02
Fumarate + 2 $H^{\oplus}$ + $2e^{\ominus}$ → Succinate	0.03
Ubiquinone (Q) + 2 $H^{\oplus}$ + $2e^{\ominus}$ → QH_2	0.04
Cytochrome b (mitochondrial), Fe^{3+} + $e^{\ominus}$ → Fe^{2+}	0.08
Cytochrome c_1, Fe^{3+} + $e^{\ominus}$ → Fe^{2+}	0.22
Cytochrome c, Fe^{3+} + $e^{\ominus}$ → Fe^{2+}	0.23
Cytochrome a, Fe^{3+} + $e^{\ominus}$ → Fe^{2+}	0.29
Cytochrome f, Fe^{3+} + $e^{\ominus}$ → Fe^{2+}	0.36
Plastocyanin, Cu^{2+} + $e^{\ominus}$ → Cu^{+}	0.37
$NO_3^{\ominus}$ + 2 $H^{\oplus}$ + $2e^{\ominus}$ → $NO_2^{\ominus}$ + H_2O	0.42
Photosystem I (P700)	0.43
Fe^{3+} + $e^{\ominus}$ → Fe^{2+}	0.77
$^1/_2 O_2$ + 2 $H^{\oplus}$ + $2e^{\ominus}$ → H_2O	0.82
Photosystem II (P680)	1.1

substance (the one with the more negative reduction potential) to the more readily reduced substance (the one with the more positive reduction potential). Therefore, more negative potentials are assigned to reaction systems that have a greater tendency to donate electrons (i.e., systems that tend to oxidize more easily).

The standard reduction potential for the transfer of electrons from one molecular species to another is related to the standard free energy change for the oxidation–reduction reaction by the equation

$$\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime} \tag{10.25}$$

where n is the number of electrons transferred and F is Faraday's constant (96.48 kJ V^{-1} mol^{-1}). Note that Equation 10.25 resembles Equation 9.5 except that here we are dealing with reduction potential and not membrane potential. $\Delta E^{\circ\prime}$ is defined as the difference in volts between the standard reduction potential of the electron-acceptor system and that of the electron donor system.

$$\Delta E^{\circ\prime} = E^{\circ\prime}_{\text{electron acceptor}} - E^{\circ\prime}_{\text{electron donor}} \tag{10.26}$$

Recall from Equation 10.6 that $\Delta G^{\circ\prime} = -RT \ln K_{\text{eq}}$. Combining this equation with Equation 10.25, we have

$$\Delta E^{\circ\prime} = \frac{RT}{nF} \ln K_{\text{eq}} \tag{10.27}$$

Under biological conditions, the reactants in a system are not present at standard concentrations of 1 M. Just as the actual Gibbs free energy change for a reaction is related to the standard Gibbs free energy change by Equation 10.6, an observed difference in reduction potentials (ΔE) is related to the difference in the standard reduction potentials ($\Delta E^{\circ\prime}$) by the Nernst equation. For Reaction 10.23, the Nernst equation is

$$\Delta E = \Delta E^{\circ\prime} - \frac{RT}{nF} \ln \frac{[A_{\text{ox}}][B_{\text{red}}]}{[A_{\text{red}}][B_{\text{ox}}]} \tag{10.28}$$

At 298 K, Equation 10.28 reduces to

$$\Delta E = \Delta E^{\circ\prime} - \frac{0.026}{n} \ln Q \tag{10.29}$$

where Q represents the actual concentrations of reduced and oxidized species. To calculate the electromotive force of a reaction under nonstandard conditions, use the Nernst equation and substitute the actual concentrations of reactants and products. Keep in mind that a *positive* ΔE value indicates that an oxidation–reduction reaction will have a *negative* standard Gibbs free energy change.

B. Electron Transfer from NADH Provides Free Energy

$NAD^{\oplus}$ is reduced to NADH in coupled reactions where electrons are transferred from a metabolite to $NAD^{\oplus}$. The reduced form of the coenzyme (NADH) becomes a source of electrons in other oxidation–reduction reactions. The Gibbs free energy changes associated with the overall oxidation–reduction reaction under standard conditions can be calculated from the standard reduction potentials of the two half-reactions using Equation 10.25. As an example, let's consider the reaction where NADH is oxidized and molecular oxygen is reduced. This represents the available free energy change during membrane-associated electron transport. This free energy is recovered in the form of ATP synthesis (Chapter 14).

The two half reactions from Table 10.5 are

$$NAD^{\oplus} + H^{\oplus} + 2\,e^{\ominus} \longrightarrow NADH \qquad E^{\circ\prime} = -0.32\,V \tag{10.30}$$

and

$$\tfrac{1}{2}\,O_2 + 2\,H^{\oplus} + 2\,e^{\ominus} \longrightarrow H_2O \qquad E^{\circ\prime} = 0.82\,V \tag{10.31}$$

Since the $NAD^{\oplus}$ half-reaction has the more negative standard reduction potential, NADH is the electron donor and oxygen is the electron acceptor. Note that the values in Table 10.5 are for half-reactions written as reductions (gain of electrons). That's because $E^{\circ\prime}$ is a reduction potential. In an oxidation–reduction reaction, two of these half-reactions are combined. One of them will be an oxidation reaction, so the equation in Table 10.5 must be reversed. The reduction potentials tell you which way the electrons will flow. They flow from the half-reaction near the top of the table (more negative $E^{\circ\prime}$) to the one nearer the bottom of the table (less negative $E^{\circ\prime}$) (Figure 10.18). What this means is that the overall $\Delta E^{\circ\prime}$ for the complete reaction will be positive according to Equation 10.26. (This is the American convention. The European convention uses a different way of arriving at the same answer.)

The net oxidation–reduction reaction is Reaction 10.31 plus the reverse of Reaction 10.30.

$$NADH + {}^1\!/_2\, O_2 + H^{\oplus} \longrightarrow NAD^{\oplus} + H_2O \qquad \textbf{(10.32)}$$

and $\Delta E^{\circ\prime}$ for the reaction is

$$\Delta E^{\circ\prime} = E^{\circ\prime}_{O_2} - E^{\circ\prime}_{NADH} = 0.82\ \text{V} - (-0.32\ \text{V}) = 1.14\ \text{V} \qquad \textbf{(10.33)}$$

Using Equation 10.25,

$$\Delta G^{\circ\prime} = -(2)(96.48\ \text{kJ V}^{-1}\ \text{mol}^{-1})(1.14\ \text{V}) = -220\ \text{kJ mol}^{-1} \qquad \textbf{(10.34)}$$

KEY CONCEPT

The standard Gibbs free energy change of an oxidation–reduction reaction is calculated from the reduction potentials of the two half-reactions.

The standard Gibbs free energy change for the formation of ATP from ADP + P_i is +32 kJ mol^{-1} (the actual free energy change is about +48 kJ mol^{-1} under the conditions of the living cell, as noted earlier). The energy released during the oxidation of NADH under cellular conditions is sufficient to drive the formation of several molecules of ATP. We will learn in Chapter 14 that the actual energy yield of an NADH molecule is about 2.5 ATP equivalents (Section 14.11).

Figure 10.18 ▶
Electron flow in oxidation–reduction reactions. Half-reactions can be plotted on a chart where the standard reduction potentials are on the x axis, arranged so that the most negative values are at the top of the chart. Using this convention, electrons flow from the half-reaction at the top of the chart to the one nearer the bottom of the chart.

BOX 10.2 NAD$^{\oplus}$ AND NADH DIFFER IN THEIR ULTRAVIOLET ABSORPTION SPECTRA

The differing absorption spectra of NAD$^{\oplus}$ and NADH are useful in experimental work. NAD$^{\oplus}$ (and NADP$^{\oplus}$) absorbs maximally at 260 nm. This absorption is due to both the adenine and nicotinamide moieties. When NAD$^{\oplus}$ is reduced to NADH (or NADP$^{\oplus}$ to NADPH), the absorbance at 260 nm decreases and an absorption band centered at 340 nm appears (adjacent figure). The 340-nm band comes from the formation of the reduced nicotinamide ring. The spectra of NAD$^{\oplus}$ and NADH do not change in the pH range 2 to 10 in which most enzymes are active. In addition, few other biological molecules undergo changes in light absorption near 340 nm.

In a suitably prepared enzyme assay, one can determine the rate of formation of NADH by measuring an increase in the absorbance at 340 nm. Similarly, in a reaction proceeding in the opposite direction, the rate of NADH oxidation is indicated by the rate of decrease in absorbance at 340 nm. Many dehydrogenases can be directly assayed by this procedure. In addition, the concentrations of a product formed in a nonoxidative reaction can often be determined by oxidizing the product in a dehydrogenase– NAD$^{\oplus}$ system. Such a measurement of concentrations of NAD$^{\oplus}$ or NADH by their absorption of ultraviolet light is used not only in the research laboratory but also in many clinical analyses.
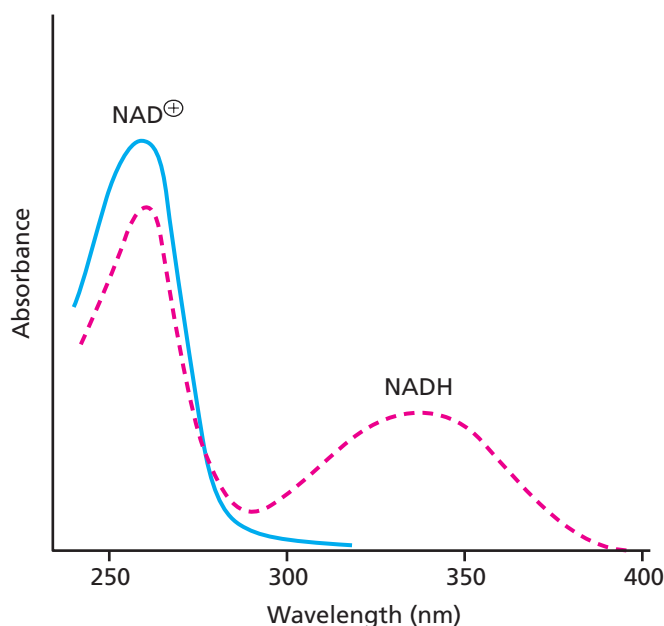
▲ Ultraviolet absorption spectra of NAD$^{\oplus}$ and NADH.

10.10 Experimental Methods for Studying Metabolism

The complexity of many metabolic pathways makes them difficult to study. Reaction conditions used with isolated reactants in the test tube (*in vitro*) are often very different from the reaction conditions in the intact cell (*in vivo*). The study of the chemical events of metabolism is one of the oldest branches of biochemistry, and many approaches have been developed to characterize the enzymes, intermediates, flux, and regulation of metabolic pathways.

A classical approach to unraveling metabolic pathways is to add a substrate to preparations of tissues, cells, or subcellular fractions and then follow the emergence of intermediates and end products. The fate of a substrate is easier to trace when the substrate has been specifically labeled. Since the advent of nuclear chemistry, isotopic tracers have been used to map the transformations of metabolites. For example, compounds containing atoms of radioactive isotopes such as ^{3}H or ^{14}C can be added to cells or other preparations, and the radioactive compounds produced by anabolic or catabolic reactions can be purified and identified. Nuclear magnetic resonance (NMR) spectroscopy can trace the reactions of certain isotopes. It can also be employed to study the metabolism of whole animals (including humans) and is being used for clinical analysis.

Verification of the steps of a particular pathway can be accomplished by reproducing the separate reactions *in vitro* using isolated substrates and enzymes. Individual enzymes have been isolated for almost all known metabolic steps. By determining the substrate specificity and kinetic properties of a purified enzyme, it is possible to draw some conclusions regarding the regulatory role of that enzyme. This reductionist approach has led to many of the key concepts in this book. It's the approach that allows us to understand the relationship between structure and function. However, a complete assessment of the regulation of a pathway requires analysis of metabolite concentrations in the intact cell or organism under various conditions.

Valuable information can be acquired by studying mutations in single genes associated with the production of inactive or defective individual enzyme forms. Whereas some mutations are lethal and not transmitted to subsequent generations, others can be tolerated by the descendants. The investigation of mutant organisms has helped identify enzymes and intermediates of numerous metabolic pathways. Typically, a defective enzyme results in a deficiency of its product and the accumulation of its substrate or a product derived from the substrate by a branch pathway. This approach has been extremely successful in identifying metabolic pathways in simple organisms such as bacteria, yeast, and *Neurospora* (Box 7.4). In humans, enzyme defects are manifested in metabolic diseases. Hundreds of single-gene diseases are known. Some are extremely rare, and others are fairly common; some are tragically severe. In cases where a metabolic disorder produces only mild symptoms, it appears that the network of metabolic reactions contains enough overlap and redundancy to allow near-normal development of the organism.

In instances where natural mutations are not available, mutant organisms can be generated by treatment with radiation or chemical mutagens (agents that cause mutation). Biochemists have characterized entire pathways by producing a series of mutants, isolating them, and examining their nutritional requirements and accumulated metabolites. More recently, site-directed mutagenesis (Box 6.1) has proved valuable in defining the roles of enzymes. Bacterial and yeast systems have been the most widely used for introducing mutations because large numbers of these organisms can be grown in a short period of time. It is possible to produce animal models—particularly insects and nematodes—in which certain genes are not expressed. It is also possible to delete certain genes in vertebrates. "Gene knockout" mice, for instance, provide an experimental system for investigating the complexities of mammalian metabolism.

In a similar fashion, investigating the actions of metabolic inhibitors has helped identify individual steps in metabolic pathways. The inhibition of one step of a pathway affects the entire pathway. Because the substrate of the inhibited enzyme accumulates, it can be isolated and characterized more easily. Intermediates formed in steps preceding the site of inhibition also accumulate. The use of inhibitory drugs not only helps in the study of metabolism but also determines the mechanism of action of the drug, often leading to improved drug variations.

Summary

1. The chemical reactions carried out by living cells are collectively called metabolism. Sequences of reactions are called pathways. Degradative (catabolic) and synthetic (anabolic) pathways proceed in discrete steps.

2. Metabolic pathways are regulated to allow an organism to respond to changing demands. Individual enzymes are commonly regulated by allosteric modulation or reversible covalent modification.

3. The major catabolic pathways convert macromolecules to smaller, energy-yielding metabolites. The energy released in catabolic reactions is conserved in the form of ATP, GTP, and reduced coenzymes.

4. Within a cell or within a multicellular organism, metabolic processes are sequestered.

5. Metabolic reactions are in a steady state. If the steady state concentration of reactants and products is close to the equilibrium ratio the reaction is said to be a near-equilibrium reaction. If the steady state concentrations are far from equilibrium the reaction is said to be metabolically irreversible.

6. The actual free energy change (ΔG) of a reaction inside a cell differs from the standard free energy change ($\Delta G°'$).

7. Hydrolytic cleavage of the phosphoanhydride groups of ATP releases large amounts of free energy.

8. The energy of ATP is made available when a terminal phosphoryl group or a nucleotidyl group is transferred. Some metabolites with high phosphoryl group transfer potentials can transfer their phosphoryl groups to ADP to produce ATP. Such metabolites are called energy-rich compounds.

9. Thioesters, such as acyl coenzyme A, can donate acyl groups and can sometimes also generate ATP equivalents.

10. The free energy of biological oxidation reactions can be captured in the form of reduced coenzymes. This form of energy is measured as the difference in reduction potentials.

11. Metabolic pathways are studied by characterizing their enzymes, intermediates, flux, and regulation.

Problems

1. A biosynthetic pathway proceeds from compound A to compound E in four steps and then branches. One branch is a two-step pathway to G, and the other is a three-step pathway to J. Substrate A is a feed-forward activator of the enzyme that catalyzes the synthesis of E. Products G and J are feedback inhibitors of the initial enzyme in the common pathway, and they also inhibit the first enzyme after the branch point in their own pathways.

   (a) Draw a diagram showing the regulation of this metabolic pathway.

   (b) Why is it advantageous for each of the two products to inhibit two enzymes in the pathway?

2. Glucose degradation can be accomplished by a combination of the glycolytic and citric acid pathways. The enzymes for glycolysis are located in the cytosol, while the enzymes for the citric acid cycle are located in the mitochondria. What are two advantages in separating the enzymes for these major carbohydrate degradation pathways in different cellular compartments?

3. In bacteria, the glycolytic and citric acid cycle pathways are both cytosolic. Why don't the "advantages" in Question 2 apply to bacteria?

4. In multistep metabolic pathways, enzymes for successive steps may be associated with each other in multienzyme complexes or be bound in close proximity on membranes. Explain the major advantage of having enzymes organized in either of these associations.

5. (a) Calculate the K_{eq} at 25°C and pH 7.0 for the following reaction using the data in Table 10.4.

   $$\text{Glycerol 3-phosphate} + H_2O \rightarrow \text{glycerol} + P_i.$$
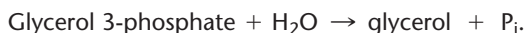
   (b) The final step in the pathway for the synthesis of glucose from lactate (gluconeogenesis) is:

   $$\text{Glucose 6-P} + H_2O \rightarrow \text{glucose} + P_i.$$

   When glucose 6-P is incubated with the proper enzyme and the reaction runs until equilibrium has been reached, the final concentrations are found to be: glucose 6-P (0.035 mM), glucose (100 mM), and P_i (100 mM). Calculate $\Delta G^{o\prime}$ at 25°C and pH 7.0.

6. $\Delta G^{o\prime}$ for the hydrolysis of phosphoarginine is -32 kJ mol^{-1}.

   (a) What is the actual free energy change for the reaction at 25°C and pH 7.0 in resting lobster muscle, where the concentrations of phosphoarginine, arginine, and P_i are 6.8 mM, 2.6 mM, and 5 mM, respectively?

   (b) Why does this value differ from $\Delta G^{o\prime}$?

   (c) High-energy compounds have large negative free energies of hydrolysis, indicating that their reactions with water proceed almost to completion. How can millimolar concentrations of acetyl CoA, whose $\Delta G^{o\prime}_{hydrolysis}$ is -32 kJ mol^{-1}, exist in cells?

7. Glycogen is synthesized from glucose-1-phosphate. Glucose-1-phosphate is activated by a reaction with UTP, forming UDP-glucose and pyrophosphate (PP_i).

   $$\text{Glucose-1-phosphate} + UTP \rightarrow \text{UDP-glucose} + PP_i$$

   UDP-glucose is the substrate for the enzyme glycogen synthase which adds glucose molecules to the growing carbohydrate chain. The $\Delta G^{o\prime}$ value for the condensation of UTP with glucose-1-phosphate to form UDP-glucose is approximately 0 kJ mol^{-1}.

The PP_i that is released is rapidly hydrolyzed by inorganic pyrophosphatase. Determine the overall $\Delta G^{o\prime}$ value if the formation of UDP-glucose is coupled to the hydrolysis of PP_i.

8. (a) A molecule of ATP is usually consumed within a minute after synthesis, and the average human adult requires about 65 kg of ATP per day. Since the human body contains only about 50 grams of ATP and ADP combined, how it is possible that so much ATP can be utilized?

   (b) Does ATP have a role in energy storage?

9. Phosphocreatine is produced from ATP and creatine in mammalian muscle cells at rest. What ATP/ADP ratio is necessary to maintain a phosphocreatine/creatine ratio of 20:1? (To maintain the coupled reaction at equilibrium, the actual free energy change must be zero.)

10. Amino acids must be covalently attached to a ribose hydroxyl group on the correct tRNA (transfer RNA) prior to recognition and insertion into a growing polypeptide chain. The overall reaction carried out by the amino acyl tRNA synthetase enzymes is:

    $$\text{Amino acid} + \text{HO-tRNA} + \text{ATP} \longrightarrow$$
    $$\text{amino acyl-O-tRNA} + \text{AMP} + 2P_i$$

    Assuming this reaction proceeds through an acyl adenylate intermediate, write all the steps involved in this enzyme-catalyzed reaction.

11. When a mixture of glucose 6-phosphate and fructose 6-phosphate is incubated with the enzyme phosphohexose isomerase, the final mixture contains twice as much glucose 6-phosphate as fructose 6-phosphate. Calculate the value of $\Delta G^{o\prime}$.

    $$\text{Glucose 6-phosphate} \longleftrightarrow \text{fructose 6-phosphate}$$
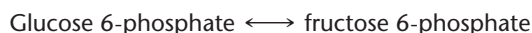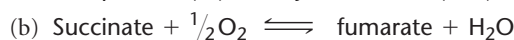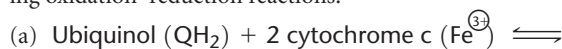
12. Coupling ATP hydrolysis to a thermodynamically unfavorable reaction can markedly shift the equilibrium of the reaction.

    (a) Calculate K_{eq} for the energetically unfavorable biosynthetic reaction $A \rightarrow B$ when $\Delta G^{o\prime} = +25$ kJ mol^{-1} at 25°C.

    (b) Calculate K_{eq} for the reaction $A \rightarrow B$ when it is coupled to the hydrolysis of ATP. Compare this value to the value in Part (a).

    (c) Many cells maintain [ATP]/[ADP] ratios of 400 or more. Calculate the ratio of [B] to [A] when [ATP]:[ADP] is 400:1 and [P_i] is constant at standard conditions. How does this ratio compare to the ratio of [B] to [A] in the uncoupled reaction?
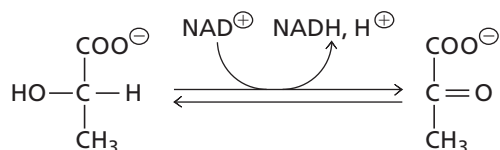
13. Using data from Table 10.5, write the coupled reaction that would occur spontaneously for the following pairs of molecules under standard conditions:

    (a) Cytochrome f and cytochrome b_5

    (b) Fumarate/succinate and ubiquinone/ubiquinol (Q/QH_2)

   (c) α-ketoglutarate/isocitrate and $NAD^{\oplus}/NADH$

14. Using data from Table 10.5, calculate the standard reduction potential and the standard free energy change for each of the following oxidation–reduction reactions:

    (a) $\text{Ubiquinol } (QH_2) + 2 \text{ cytochrome c } (Fe^{3+}) \Longleftrightarrow$
    $$\text{ubiquinone } (Q) + 2 \text{ cytochrome c } (Fe^{2+}) + 2 H^{\oplus}$$

    (b) $\text{Succinate} + \frac{1}{2}O_2 \Longleftrightarrow \text{fumarate} + H_2O$

15. Lactate dehydrogenase is an NAD-dependent enzyme that catalyzes the reversible oxidation of lactate.

$$HO-\overset{\displaystyle COO^{\ominus}}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{C}}}}-H \quad \xrightleftharpoons[]{NAD^{\oplus} \quad NADH, H^{\oplus}} \quad \overset{\displaystyle COO^{\ominus}}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{C}}}}=O$$

Initial reaction rates are followed spectrophotometrically at 340 nm after addition of lactate, NAD$^{\oplus}$, lactate dehydrogenase, and buffer to the reaction vessel. When the change in absorbance at 340 nm is monitored over time, which graph is representative of the expected results? Explain.
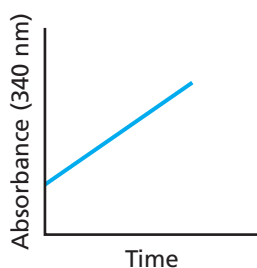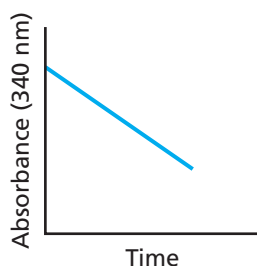
16. Using the standard reduction potentials for Q and FAD in Table 10.5, show that the oxidation of $FADH_2$ by Q liberates enough energy to drive the synthesis of ATP from ADP and P_i under cellular conditions where $[FADH_2] = 5$ mM, $[FAD] = 0.2$ mM, $[Q] = 0.1$ mM, and $[QH_2] = 0.05$ mM. Assume that ΔG for ATP synthesis from ADP and P_i is +30 kJ mol^{-1}.

Selected Readings

Alberty, R. A. (1996). Recommendations for nomenclature and tables in biochemical thermodynamics. *Eur. J. Biochem.* 240:1–14.

Alberty, R. A. (2000). Calculating apparent equilibrium constants of enzyme-catalyzed reactions at pH 7. *Biochem. Educ.* 28:12–17.

Burbaum, J. J., Raines, R. T., Albery, W. J., and Knowles, J. R. (1989). Evolutionary optimization of the catalytic effectiveness of an enzyme. *Biochem.* 28:9293–9305.

Edwards, R. A. (2001). The free energies of metabolic reactions (ΔG) are not positive. *Biochem. Mol. Bio. Educ.* 29:101–103.

Hayes, D. M., Kenyon, G. L., and Kollman, P. A. (1978). Theoretical calculations of the hydrolysis energies of some "high-energy" molecules. 2. A survey of some biologically important hydrolytic reactions. *J. Am. Chem. Soc.* 100:4331–4340.

Schmidt. S., Sunyaev, S., Bork. P., and Dandekar, T. (2003). Metabolites: a helping hand for pathway evolution? *Trends Biochem. Sci.* 28:336–341.

Silverstein, T. (2005). Redox redox: a response to Feinman's "Oxidation–reduction calculations in the biochemistry course." *Biochem. Mol. Bio. Educ.* 33:252–253.

Tohge, T., Nunes-Nesi, A., and Fernie, A. R. (2009). Finding the paths: metabolomics and approaches to metabolic flux analysis. *The Biochem. Soc.* (June 2009):8–12.

Yus, E., et al. (2009). Impact of genome reduction on bacterial metabolism and its regulation. *Science* 326:1263–1272.
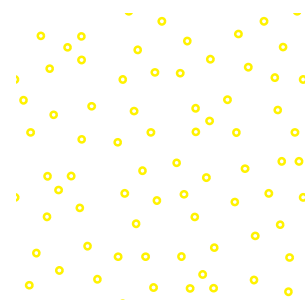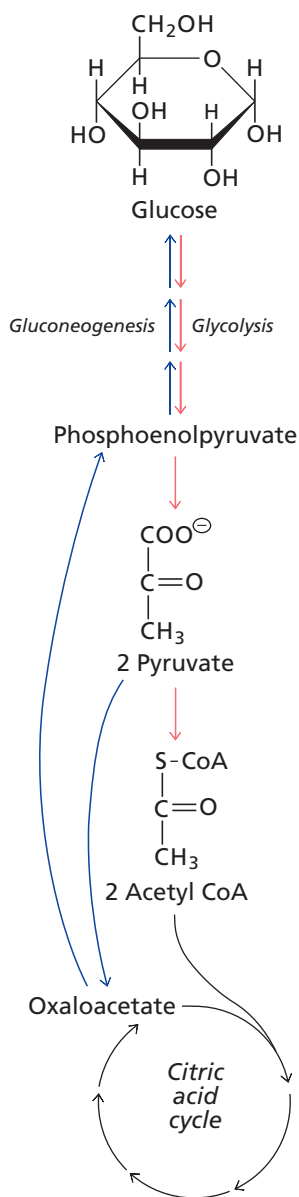
11 CHAPTER

Glycolysis

The first three metabolic pathways we examine are central to both carbohydrate metabolism and energy generation. Gluconeogenesis is the main pathway for synthesis of hexoses from three carbon precursors. As the name of the pathway indicates, glucose is the primary end product of gluconeogenesis. This biosynthetic pathway will be described in the next chapter. Glucose, and other hexoses, can be the precursors for synthesis of many complex carbohydrates. Glucose can also be degraded in a catabolic glycolytic pathway with recovery of the energy used in its synthesis. In glycolysis, the subject of this chapter, glucose is converted to the three-carbon acid pyruvate. Pyruvate has several possible fates, one of which is oxidative decarboxylation to form acetyl CoA. The third pathway is the citric acid cycle, described in Chapter 13. This is the route by which the acetyl group of acetyl CoA is oxidized to carbon dioxide and water. One of the important intermediates in the citric acid cycle, oxaloacetate, is also an intermediate in the synthesis of glucose from pyruvate. Figure 11.1 shows the relationship among the three pathways. All three pathways play a role in the formation and degradation of noncarbohydrate molecules such as amino acids and lipids.

We present the reactions of glycolysis, gluconeogenesis, and the citric acid cycle in more detail than those of other metabolic pathways in this book but the same principles apply to all pathways. We introduce many biomolecules and enzymes, some of which appear in more than one pathway. Keep in mind that the chemical structures of the metabolites prompt the enzyme names and that the names of the enzymes reflect the substrate specificity and the type of reaction catalyzed. A confident grasp of terminology will prepare you to enjoy the chemical elegance of metabolism. However, do not lose sight of the major concepts and general strategies of metabolism while memorizing the details. The names of particular enzymes might fade over time but we hope you will retain an understanding of the patterns and purposes behind the interconversion of metabolites in cells.

In this book we follow the tradition of presenting glycolysis as our first metabolic pathway. The catabolism of glucose is a major source of energy in animals. The details of the various reactions, and their regulation, are well known.

The glycolytic sequence of reactions is perhaps the best understood and most studied multi-enzyme system of the cell. The pattern of interplay between enzymes and substrates in this relatively simple multi-enzyme system applies to all the multi-enzyme systems of the cell, especially the very complex systems involved in respiration and photosynthesis.

—Albert Lehninger (1965), Bioenergetics, p. 75

Top: Wine, beer and bread. For centuries, wineries, breweries, and bakeries have exploited the basic biochemical pathway of glycolysis where glucose is converted to ethanol and CO_2.

▲ Figure 11.1
Gluconeogenesis, glycolysis, and the citric acid cycle. Glucose is synthesized from pyruvate via oxaloacetate and phosphoenolpyruvate. In glycolysis, glucose is degraded to pyruvate. Many (but not all) of the steps in glycolysis are the reverse of the gluconeogenesis reactions. The acetyl group of pyruvate is transferred to coenzyme A (CoA) and oxidized to carbon dioxide by the citric acid cycle. Energy in the form of ATP equivalents is required for the synthesis of glucose. Some of this energy is recovered in glycolysis but much more is recovered as a result of the citric acid cycle.

KEY CONCEPT

The main energy gain in glycolysis is due to production of NADH molecules.

11.1 The Enzymatic Reactions of Glycolysis

Glycolysis is a sequence of ten enzyme-catalyzed reactions by which glucose is converted to pyruvate (Figure 11.2 on page 328). The conversion of one molecule of glucose to two molecules of pyruvate is accompanied by the net conversion of two molecules of ADP to two molecules of ATP and the reduction of two molecules of $NADH^{\oplus}$ to two molecules NADH. The enzymes of this pathway are found in most living species and are located in the cytosol. The glycolytic pathway is active in all differentiated cell types in multicellular organisms. In some mammalian cells (such as those in the retina and some brain cells), it is the only ATP-producing pathway.

The net reaction of glycolysis is shown in Reaction 11.1.

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{NAD}^{\oplus} + 2\ P_i \rightarrow$$
$$2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ H^{\oplus} + 2\ H_2O \qquad \textbf{(11.1)}$$
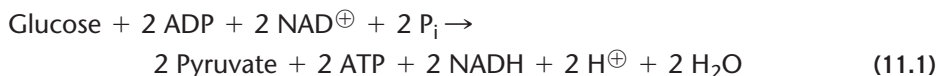
The ten reactions of glycolysis are listed in Table 11.1. They can be divided into two stages: the hexose stage and the triose stage. The left page of Figure 11.2 shows the hexose stage. At Step 4, the C-3—C-4 bond of the hexose is cleaved to produce two trioses. From that point on the intermediates of the pathway are triose phosphates. Two triose phosphates are formed from fructose 1,6-*bis*phosphate. Dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate in Step 5 and glyceraldehyde 3-phosphate continues through the pathway. All subsequent steps of the triose stage of glycolysis (right page of Figure 11.2) are traversed by two molecules for each molecule of glucose metabolized.

Two molecules of ATP are converted to ADP in the hexose stage of glycolysis. In the triose stage, four molecules of ATP are formed from ADP for each molecule of glucose metabolized. Thus, glycolysis has a net yield of two molecules of ATP per molecule of glucose.

ATP consumed per glucose:	2 (hexose stage)	
ATP produced per glucose:	4 (triose stage)	**(11.2)**
Net ATP production per glucose:	2	

The first and third reactions of glycolysis are coupled to the utilization of ATP. These priming reactions help drive the pathway in the direction of glycolysis since the reverse reactions are thermodynamically favored in the absence of ATP. Two later intermediates of glycolysis have sufficient group transfer potentials to allow the transfer of a phosphoryl group to ADP producing ATP (Steps 7 and 10). Step 6 is coupled to the synthesis of reducing equivalents in the form of NADH. Each molecule of NADH is equivalent to several molecules of ATP (Section 10.9) so the net energy gain in glycolysis is mostly due to production of NADH.

11.2 The Ten Steps of Glycolysis

Now we examine the chemistry and enzymes of each glycolytic reaction. As you read, pay attention to the chemical logic and economy of the pathway. Consider how each chemical reaction prepares a substrate for the next step in the process. Note, for example, that a cleavage reaction converts a hexose to two trioses, not to a two-carbon compound and a tetrose. The two trioses rapidly interconvert allowing both products of the cleavage reaction to be further metabolized by the action of one set of enzymes, not two. Finally, be aware of how ATP is both consumed and produced in glycolysis. We have already seen a number of examples of the transfer of the chemical potential energy of ATP (e.g., in Section 10.7) but the reactions in this chapter are our first detailed examples of how the energy released by oxidation reactions is captured for use in other biochemical pathways.

1. Hexokinase

In the first reaction of glycolysis, the γ-phosphoryl group of ATP is transferred to the oxygen atom at C-6 of glucose producing glucose 6-phosphate and ADP (Figure 11.3 on

Table 11.1 The reactions and enzymes of glycolysis

1. Glucose + ATP ⟶ Glucose 6-phosphate + ADP + H$^\oplus$	Hexokinase, glucokinase
2. Glucose 6-phosphate ⇌ Fructose 6-phosphate	Glucose-6-phosphate isomerase
3. Fructose 6-phosphate + ATP ⟶ Fructose 1,6-*bis*phosphate + ADP + H$^\oplus$	Phosphofructokinase-1
4. Fructose 1,6-*bis*phosphate ⇌ Dihydroxyacetone phosphate + Glyceraldehyde 3-phosphate	Aldolase
5. Dihydroxyacetone phosphate ⇌ Glyceraldehyde 3-phosphate	Triose phosphate isomerase
6. Glyceraldehyde 3-phosphate + NAD$^\oplus$ + P$_i$ ⇌ 1,3-*Bis*phosphoglycerate + NADH + H$^\oplus$	Glyceraldehyde 3-phosphate dehydrogenase
7. 1,3-*Bis*phosphoglycerate + ADP ⇌ 3-Phosphoglycerate + ATP	Phosphoglycerate kinase
8. 3-Phosphoglycerate ⇌ 2-Phosphoglycerate	Phosphoglycerate mutase
9. 2-Phosphoglycerate ⇌ Phosphoenolpyruvate + H$_2$O	Enolase
10. Phosphoenolpyruvate + ADP + H$^\oplus$ ⟶ Pyruvate + ATP	Pyruvate kinase

page 330). This phosphoryl group transfer reaction is catalyzed by hexokinase. Kinases catalyze four reactions in the glycolytic pathway—Steps 1, 3, 7, and 10.

The hexokinase reaction is regulated making it a metabolically irreversible reaction. Cells need to maintain a relatively high concentration of glucose 6-phosphate and a low internal concentration of glucose. As we'll see in Section 11.5B, the reverse reaction is inhibited by glucose 6-phosphate. Hexokinases from yeast and mammalian tissues have been thoroughly studied. These enzymes have a broad substrate specificity; they catalyze the phosphorylation of glucose and mannose, and of fructose when it is present at high concentrations.

Multiple forms, or **isozymes**, of hexokinase occur in many eukaryotic cells. (Isozymes are different proteins from one species that catalyze the same chemical reaction.) Four hexokinase isozymes have been isolated from mammalian liver. All four are found in varying proportions in other mammalian tissues. These isozymes catalyze the same reaction but have different K_m values for glucose. Hexokinases I, II, and III have K_m values of about 10^{-6} to 10^{-4} M, whereas hexokinase IV, also called glucokinase, has a much higher K_m value for glucose (about 10^{-2} M). In eukaryotes, glucose is taken up and secreted by passive transport using various glucose transporters (GLUT). The concentration of glucose in the blood and the cell cytoplasm is usually below the K_m of glucokinase for glucose. At these low concentrations the other hexokinase isozymes catalyze the phosphorylation of glucose. With high glucose levels, glucokinase is active. Because glucokinase is never saturated with glucose, the liver can respond to large increases in blood glucose by phosphorylating it for entry into glycolysis or the glycogen synthesis pathway.

In most bacteria, the uptake of glucose is coupled to the phosphorylation of glucose to glucose 6-phosphate via the phosphoenolpyruvate sugar transport system (Section 21.7B). The phosphoryl group is donated by phosphoenolpyruvate. Hexokinases and glucokinases can be found in bacteria but they play a minor role in glycolysis because, unlike the situation in eukaryotic cells, the bacterial enzymes rarely encounter free glucose in their cytoplasm.

2. Glucose 6-Phosphate Isomerase

In the second step of glycolysis, glucose 6-phosphate isomerase catalyzes the conversion of glucose 6-phosphate (an aldose) to fructose 6-phosphate (a ketose), as shown in Figure 11.4. The enzyme is also known as phosphoglucose isomerase (PGI). Isomerases interconvert aldoses and ketoses that have identical configurations at all other chiral atoms.

The α anomer of glucose 6-phosphate (α-D-glucopyranose 6-phosphate) preferentially binds to glucose 6-phosphate isomerase. The open-chain form of glucose 6-phosphate is then generated within the active site of the enzyme, and an aldose-to-ketose conversion occurs. The open-chain form of fructose 6-phosphate cyclizes to form α-D-fructofuranose 6-phosphate. The mechanism of glucose 6-phosphate isomerase is similar to the mechanism of triose phosphate isomerase (Section 6.4A).
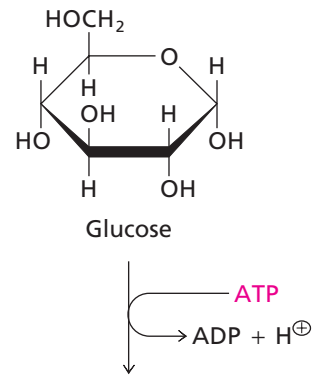
Glucose 6-phosphate isomerase is highly stereospecific. For example, in the reverse reaction catalyzed by this enzyme fructose 6-phosphate (in which C-2 is not chiral) is

Conversion of glucose to pyruvate by glycolysis. At Step 4, the hexose molecule is split in two, and the remaining reactions of glycolysis are traversed by two triose molecules. ATP is consumed in the hexose stage and generated in the triose stage.
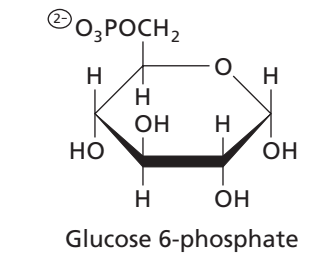

Glucose

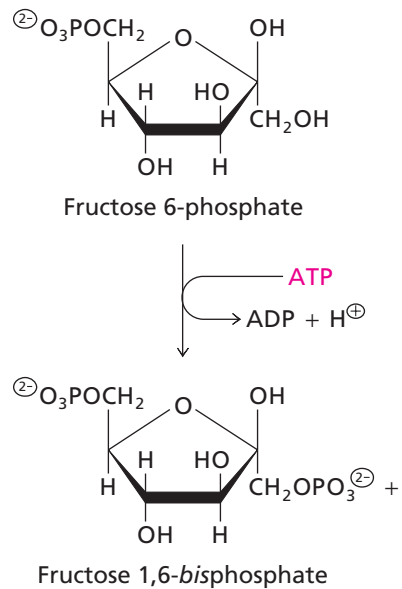*Transfer of a phosphoryl group from ATP to glucose*   ①   Hexokinase, glucokinase

ATP

ADP + H$^{\oplus}$

Glucose 6-phosphate

Isomerization   ②   Glucose 6-phosphate isomerase

Fructose 6-phosphate

Transfer of a second phosphoryl group from ATP to fructose 6-phosphate   ③   Phosphofructokinase-1

ATP

ADP + H$^{\oplus}$

Fructose 1,6-*bis*phosphate

C-3—C-4 bond cleavage, yielding two triose phosphates   ④   Aldolase

Dihydroxyacetone phosphate   Glyceraldehyde 3-phosphate

CH₂OH

$|$

C=O

$|$

CH₂OPO₃$^{2-}$

Dihydroxyacetone phosphate

$\Longleftrightarrow$

$$\underset{O}{\overset{}{\underset{\|}{C}}}\!\!\diagup H$$

$|$

H—C—OH

$|$

CH₂OPO₃$^{2-}$

Glyceraldehyde 3-phosphate

Triose phosphate isomerase ⑤

Rapid interconversion of triose phosphates

— NAD$^{⊕}$ + P$_i$

→ NADH + H$^{⊕}$

Glyceraldehyde 3-phosphate dehydrogenase ⑥

Oxidation and phosphorylation, yielding a high-energy mixed-acid anhydride

O   OPO₃$^{2-}$

C

$|$

H—C—OH

$|$

CH₂OPO₃$^{2-}$

1,3-*Bis*phosphoglycerate

— ADP

→ ATP

Phosphoglycerate kinase ⑦

Transfer of a high-energy phosphoryl group to ADP, yielding ATP

COO$^{⊖}$

$|$

H—C—OH

$|$

CH₂OPO₃$^{2-}$

3-Phosphoglycerate

Phosphoglycerate mutase ⑧

Intramolecular phosphoryl-group transfer

COO$^{⊖}$

$|$

H—C—OPO₃$^{2-}$

$|$

CH₂OH

2-Phosphoglycerate

H₂O —↗   ↘— H₂O

Enolase ⑨

Dehydration to an energy-rich enol ester

COO$^{⊖}$

$|$

C—OPO₃$^{2-}$

$\|$

CH₂

Phosphoenolpyruvate

— ADP + H$^{⊕}$

→ ATP

Pyruvate kinase ⑩

Transfer of a high-energy phosphoryl group to ADP, yielding ATP

COO$^{⊖}$

$|$

C=O

$|$

CH₃

Pyruvate

Phosphoryl group transfer reaction catalyzed by hexokinase. This reaction occurs by attack of the C-6 hydroxyl oxygen of glucose on the γ-phosphorus of MgATP$^{\ominus}$. MgADP$^{\ominus}$ is displaced, and glucose 6-phosphate is generated. All four kinases in glycolysis catalyze direct nucleophilic attack of a hydroxyl group on the terminal phosphoryl group of ATP (and/or its reverse under cellular conditions). (Mg$^{\oplus}$, shown explicitly here, is also required in the other kinase reactions in this chapter, although it is not shown for those reactions.)

The hexokinase mechanism is a classic example of induced fit (Section 6.5C).

We discuss the regulation of glycolysis in detail in Section 11.5.

We explore glycogen synthesis in Section 12.5.

converted almost exclusively to glucose 6-phosphate. Only traces of mannose 6-phosphate, the C-2 epimer of glucose 6-phosphate, are formed.

The glucose 6-phosphate isomerase reaction is a near-equilibrium reaction. The reverse reaction is part of the pathway for the biosynthesis of glucose.

3. Phosphofructokinase-1

Phosphofructokinase-1 (PFK-1) catalyzes the transfer of a phosphoryl group from ATP to the C-1 hydroxyl group of fructose 6-phosphate producing fructose 1,6-*bis*phosphate. The "*bis*" in *bis*phosphate indicates that the two phosphoryl groups are attached to different carbon atoms (cf. diphosphate).

Fructose 6-phosphate → (ATP, ADP, Phosphofructokinase-1) → Fructose 1,6-*bis*phosphate + H$^+$
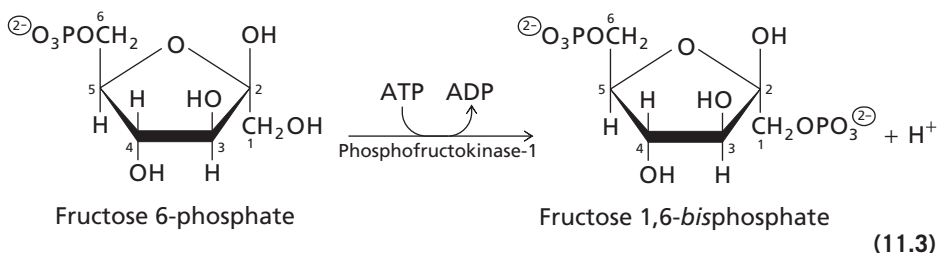
(11.3)

Note that the reaction catalyzed by glucose 6-phosphate isomerase produces α-D-fructose 6-phosphate. However, it is the β-D anomer that is the substrate for the next step in glycolysis—the one catalyzed by phosphofructokinase-1. The α and β anomers of fructose 6-phosphate equilibrate spontaneously (Section 8.2). This interconversion is extremely rapid in aqueous solution and has no effect on the overall rate of glycolysis.

The reaction catalyzed by PFK-1 is metabolically irreversible indicating that the activity of the enzyme is regulated. In fact, this step is a critical control point for the regulation of glycolysis in most species. The PFK-1 catalyzed reaction is the first *committed* step of glycolysis because some substrates other than glucose can enter the glycolytic pathway by direct conversion to fructose 6-phosphate, thus bypassing the steps catalyzed by hexokinase and glucose 6-phosphate isomerase (Section 11.6C). (The metabolically irreversible reaction catalyzed by hexokinase is *not* the first committed step.) Another reason for regulating PFK-1 activity has to do with the competing glycolysis and gluconeogenesis pathways (Figure 11.1). PFK-1 activity must be inhibited when glucose is being synthesized.

PFK-1 is one of the classic allosteric enzymes. Recall that the bacterial enzyme is activated by ADP and allosterically inhibited by phosphoenolpyruvate (Section 5.10A). The activity of the mammalian enzyme is regulated by AMP and citrate (Section 11.6C).

PFK-1 has the suffix "1" because there is a second phosphofructokinase that catalyzes the synthesis of fructose 2,6-*bis*phosphate instead of fructose 1,6-*bis*phosphate. This second enzyme, which we will encounter later in this chapter, is known as PFK-2.

4. Aldolase

The first three steps of glycolysis prepare the hexose for cleavage into two triose phosphates, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.
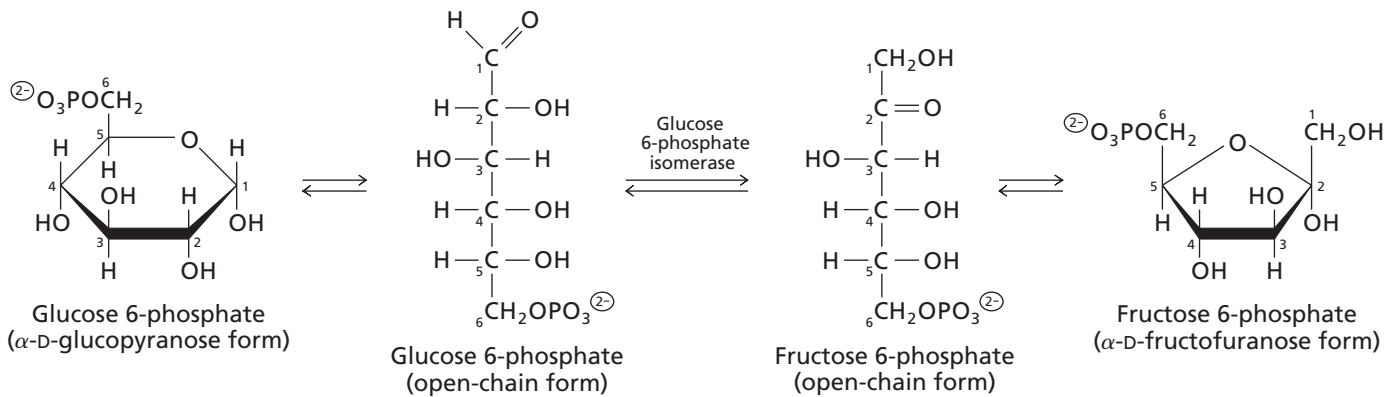
▲ **Figure 11.4**
Conversion of glucose 6-phosphate to fructose 6-phosphate. This aldose-ketose isomerization is catalyzed by glucose 6-phosphate isomerase.

Dihydroxyacetone phosphate (DHAP) is derived from C-1 to C-3 of fructose 1,6-*bis*phosphate, and glyceraldehyde 3-phosphate (GAP) is derived from C-4 to C-6. The enzyme that catalyzes the cleavage reaction is fructose 1,6-*bis*phosphate aldolase, commonly shortened to aldolase. Aldol cleavage is a common mechanism for cleaving C—C bonds in biological systems and for C—C bond formation in the reverse direction.

BOX 11.1 A BRIEF HISTORY OF THE GLYCOLYTIC PATHWAY

Glycolysis was one of the first metabolic pathways to be elucidated. It played an important role in the development of biochemistry. In 1897, Eduard Buchner (Section 1.1) discovered that bubbles of carbon dioxide were released from a mixture of sucrose and a cell-free yeast extract. He concluded that fermentation was occurring in his cell-free extract. More than 20 years earlier, Louis Pasteur had shown that yeast cells ferment sugar to alcohol (i.e., produce ethanol and CO_2) but Buchner showed that intact cells were not required. Buchner named the fermenting activity zymase. Today, we recognize that the zymase of yeast extracts is not a single enzyme but a mixture of enzymes that together catalyze the reactions of glycolysis.

The steps of the glycolytic pathway were gradually discovered by analyzing the reactions catalyzed by extracts of yeast or muscle. In 1905, Arthur Harden and William John Young found that when the rate of glucose fermentation by yeast extract decreased it could be restored by adding inorganic phosphate. Harden and Young assumed that phosphate derivatives of glucose were being formed. They succeeded in isolating fructose 1,6-*bis*phosphate and showed that it is an intermediate in the fermentation of glucose because it too is fermented by cell-free yeast extracts. Harden was awarded the Nobel Prize in Chemistry in 1929 for his work on glycolysis.

By the 1940s, the complete glycolytic pathway in eukaryotes—including its enzymes, intermediates, and coenzymes—was known. The further characterization of individual enzymes and studies of the regulation of glycolysis and its integration with other pathways have taken many more years. In bacteria, the classic glycolytic pathway is called the Embden–Meyerhof–Parnas pathway after Gustav Embden (1874–1933), Otto Meyerhof (1884–1951), and Jacob Parnas (1884–1949). The bacterial pathway differs in some minor ways from the eukaryotic pathway. In 1922 Meyerhof was awarded the Nobel Prize in Physiology or Medicine for his work on the production of lactic acid in muscle cells.

▲ Louis Pasteur (1822–1895).

▲ Arthur Harden (1865–1940).

Fructose 1,6-*bis*phosphate   Dihydroxyacetone phosphate   Glyceraldehyde 3-phosphate

$$(11.4)$$
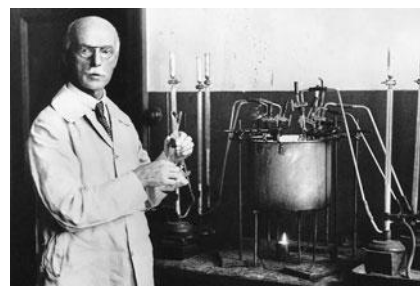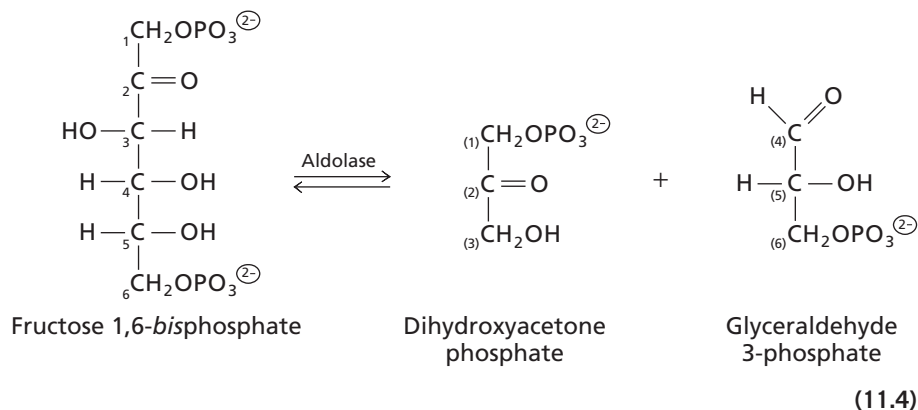
There are two distinct classes of aldolases. class I enzymes are found in plants and animals; class II enzymes are more common in bacteria, fungi, and protists. Many species have both types of enzyme. Class I and class II aldolases are unrelated. The enzymes have very different structures and sequences in spite of the fact that they catalyze the same reaction. This is an example of convergent evolution.

The two classes of aldolase have slightly different mechanisms. Class I aldolases involve formation of a covalent Schiff base between lysine and pyruvate derivatives (Section 6.3) and class II aldolases use a metal ion cofactor (Figures 11.5 and 11.6).

The standard Gibbs free energy change for this reaction is strongly positive ($\Delta G^{\circ\prime} = +28$ kJ mol^{-1}). Nevertheless, the aldolase reaction is a near-equilibrium reaction (actual $\Delta G \cong 0$) in cells where glycolysis is an important catabolic pathway. This means that the concentration of fructose 1,6-*bis*phosphate is very high relative to the two trioses. (But see Problem 10).

The key to understanding the strategy of glycolysis lies in appreciating the significance of the aldolase reaction. It's best to think of this as a near-equilibrium biosynthesis reaction and not a degradation reaction. Aldolases evolved originally as enzymes that could catalyze the synthesis of fructose 1,6-*bis*phosphate. This reaction occurred at the end of a biosynthesis pathway leading from pyruvate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

During glycolysis, flux in the triose stage is in the opposite direction—toward pyruvate synthesis. The first steps of glycolysis—the hexose stage—are directed toward formation of fructose 1,6-*bis*phosphate so that it can serve as substrate for the reversal of the pathway leading to its synthesis. Keep in mind that the glucose biosynthesis pathway (gluconeogenesis) evolved first. It was only after glucose became readily available that pathways for its degradation evolved.

5. Triose Phosphate Isomerase

Of the two molecules produced by the splitting of fructose 1,6-*bis*phosphate, only glyceraldehyde 3-phosphate is a substrate for the next reaction in the glycolytic pathway.
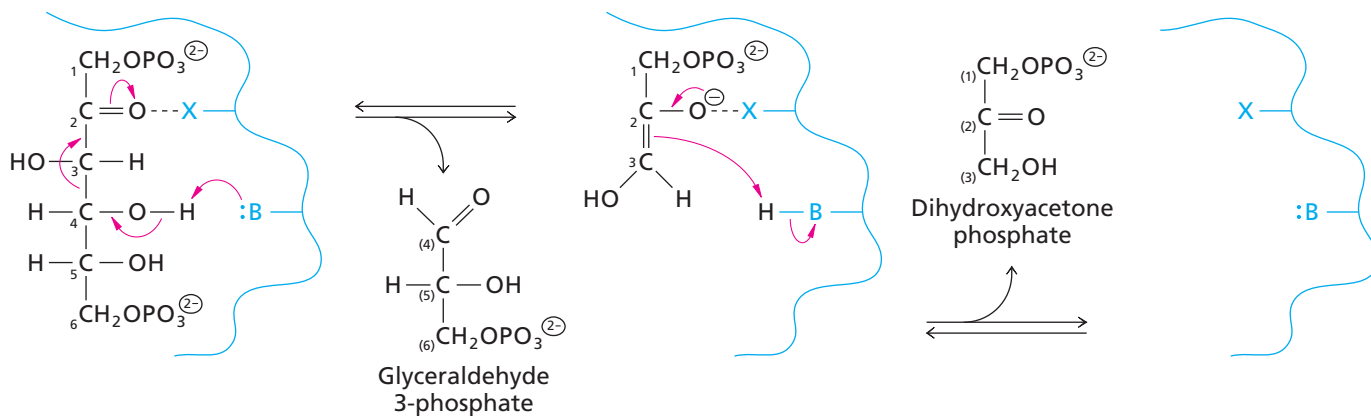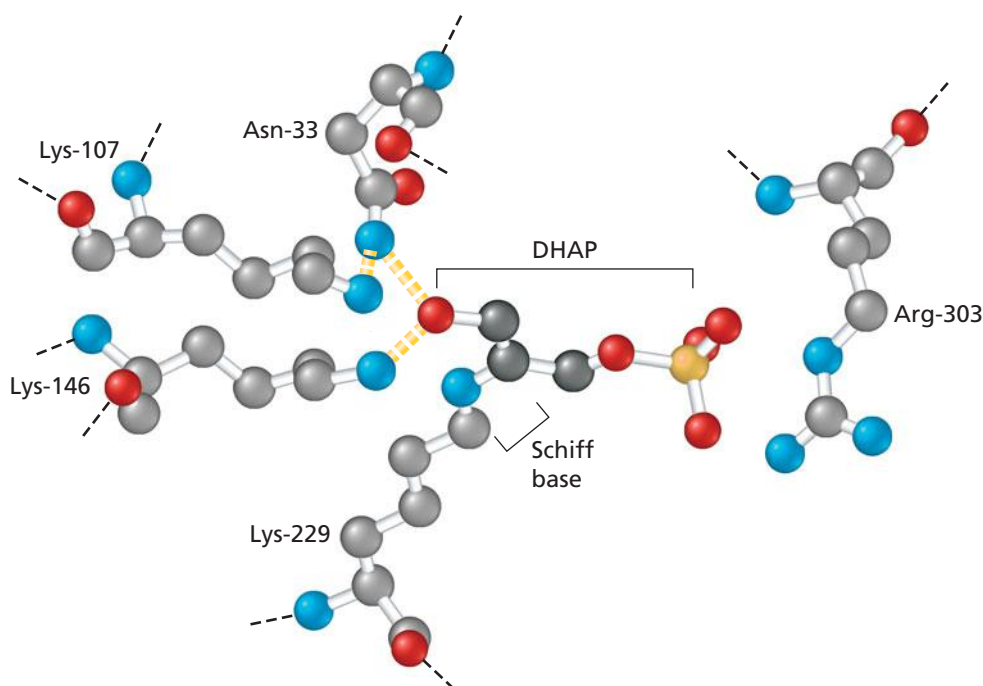
▼ **Figure 11.5**
Mechanism of aldol cleavage catalyzed by aldolases. Fructose 1,6-*bis*phosphate is the aldol substrate. Aldolases have an electron-withdrawing group (—X) that polarizes the C-2 carbonyl group of the substrate. Class I aldolases use the amino group of a lysine residue at the active site, and the other class II aldolases use Zn⊕ for this purpose. A basic residue (designated —B:) removes a proton from the C-4 hydroxyl group of the substrate.

The other product, dihydroxyacetone phosphate, is converted to glyceraldehyde 3-phosphate in a near-equilibrium reaction catalyzed by triose phosphate isomerase.

$$
\begin{array}{ccc}
\text{CH}_2\text{OH} & & \text{H}\diagdown\diagup\text{O} \\
| & \xrightarrow{\text{Triose phosphate isomerase}} & \text{C} \\
\text{C}=\text{O} & & \text{H}-\text{C}-\text{OH} \\
| & & | \\
\text{CH}_2\text{OPO}_3^{2-} & & \text{CH}_2\text{OPO}_3^{2-}
\end{array} \tag{11.5}
$$

Dihydroxyacetone phosphate   Glyceraldehyde 3-phosphate
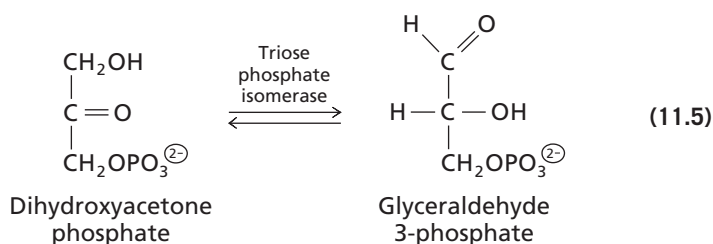
As glyceraldehyde 3-phosphate is consumed in Step 6, its steady state concentration is maintained by flux from dihydroxyacetone phosphate. In this way, two molecules of glyceraldehyde 3-phosphate are supplied to glycolysis for each molecule of fructose 1,6-*bis*phosphate split. Triose phosphate isomerase catalyzes a stereospecific reaction so that only the D isomer of glyceraldehyde 3-phosphate is formed.

Triose phosphate isomerase, like glucose 6-phosphate isomerase, catalyzes an aldose-to-ketose conversion. The mechanism of the triose phosphate isomerase reaction is described in Section 6.4A. The catalytic mechanisms of aldose–ketose isomerases have been studied extensively, and the formation of an enzyme-bound enediolate intermediate appears to be a common feature.

The fate of the individual carbon atoms of a molecule of glucose is shown in Figure 11.7. This distribution has been confirmed by radioisotopic tracer studies in a variety of organisms. Note that carbons 1, 2, and 3 of one molecule of glyceraldehyde 3-phosphate are derived from carbons 4, 5, and 6 of glucose, whereas carbons 1, 2, and 3 of the second molecule of glyceraldehyde 3-phosphate (converted from dihydroxyacetone phosphate) originate as carbons 3, 2, and 1 of glucose. When these molecules of glyceraldehyde 3-phosphate mix to form a single pool of metabolites, a carbon atom from C-1 of glucose can no longer be distinguished from a carbon atom from C-6 of glucose.

The rate of the triose phosphate isomerase reaction is close to the theoretical limit for a diffusion controlled reaction.

6. Glyceraldehyde 3-Phosphate Dehydrogenase

The recovery of energy from triose phosphates begins with the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase. In this step, glyceraldehyde 3-phosphate is oxidized and phosphorylated to produce 1,3-*bis*phosphoglycerate.

$$
\begin{array}{ccc}
\underset{\substack{\text{Glyceraldehyde} \\ \text{3-phosphate}}}{
\begin{array}{c}
\text{O} \diagdown \text{H} \\
\text{C} \\
\text{H}-\text{C}-\text{OH} \\
\text{CH}_2\text{OPO}_3^{(2-)}
\end{array}}
& + \text{NAD}^{\oplus} + \text{P}_i \rightleftharpoons
&
\underset{\text{1,3-\textit{Bis}phosphoglycerate}}{
\begin{array}{c}
\text{O} \diagdown \text{OPO}_3^{(2-)} \\
\text{C} \\
\text{H}-\text{C}-\text{OH} \\
\text{CH}_2\text{OPO}_3^{(2-)}
\end{array}} + \text{NADH} + \text{H}^{\oplus}
\end{array}
$$

Glyceraldehyde 3-phosphate dehydrogenase

(11.6)

This is an oxidation–reduction reaction; the oxidation of glyceraldehyde 3-phosphate is coupled to the reduction of $\text{NAD}^{\oplus}$ to NADH. In some species the coenzyme is $\text{NADP}^{\oplus}$.

The oxidation of the aldehyde group of glyceraldehyde 3-phosphate proceeds with a large negative Gibbs standard free energy change, and some of this energy is conserved in the acid–anhydride linkage of 1,3-*bis*phosphoglycerate. In the next step of glycolysis, the C-1 phosphoryl group of 1,3-*bis*phosphoglycerate is transferred to ADP to form ATP. The remaining energy is conserved in the form of reducing equivalents (NADH). As we saw in the previous chapter, each molecule of NADH is equivalent to several molecules of ATP. Thus, this step of glycolysis is the main energy-producing step in the entire pathway.

The overall standard Gibbs free energy change (oxidation of the aldehyde and reduction of $\text{NAD}^{\oplus}$) for this reaction is positive ($\Delta G^{\circ\prime} = +6.7 \text{ kJ mol}^{-1}$), which means that the 1,3-*bis*phosphate concentration should be much lower than that of glyceraldehyde 3-phosphate at the near-equilibium conditions that exist inside the cell. However, glyceraldehyde 3-phosphate dehydrogenase associates with the next enzyme in the pathway (phosphoglycerate kinase), to form a complex. The product of the first reaction, 1,3-*bis*phosphoglycerate, appears to be channeled directly into the active site of phospoglycerate kinase. In this way the two reactions are effectively linked to form a single reaction and the effective concentration of 1,3-*bis*phosphoglycerate is close to zero.

The NADH formed in the glyceraldehyde 3-phosphate dehydrogenase reaction is reoxidized, either by the membrane-associated electron transport chain (Chapter 14) or in other reactions where NADH serves as a reducing agent, such as the reduction of acetaldehyde to ethanol or of pyruvate to lactate (Section 11.3B). The concentration of $\text{NAD}^{\oplus}$ in most cells is low. Thus, it is essential to replenish it by reoxidizing NADH or glycolysis will stop at this step. We will see in Section 11.3 that there are several different ways of accomplishing this goal.

▼ **Figure 11.7**
Fate of carbon atoms from the hexose stage to the triose stage of glycolysis. All numbers refer to the carbon atoms in the original glucose molecule.
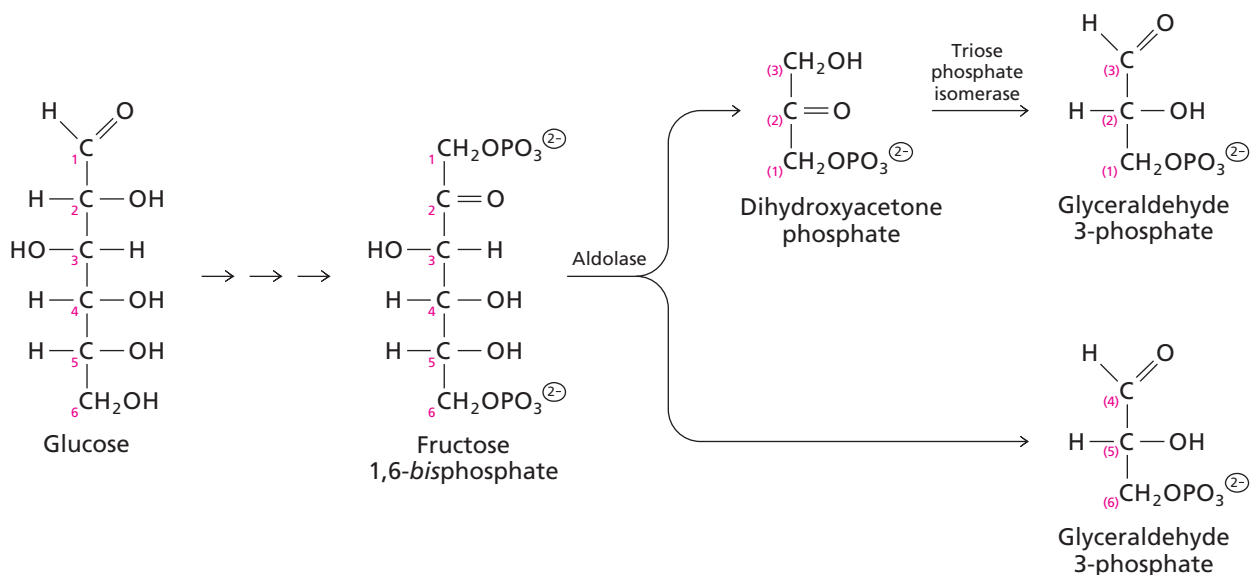
7. Phosphoglycerate Kinase

Phosphoglycerate kinase catalyzes phosphoryl group transfer from the "high-energy" mixed anhydride 1,3-*bis*phosphoglycerate to ADP, generating ATP and 3-phosphoglycerate. The enzyme is called a kinase because of the reverse reaction in which 3-phosphoglycerate is phosphorylated.

$$
\begin{array}{ccc}
\underset{\text{1,3-}\textit{Bis}\text{phosphoglycerate}}{
\begin{array}{c}
O \quad OPO_3^{2-} \\
\diagdown \diagup \\
C \\
| \\
H-C-OH \\
| \\
CH_2OPO_3^{2-}
\end{array}} + \text{ADP}
&
\underset{\substack{\text{Phosphoglycerate} \\ \text{kinase}}}{\rightleftharpoons}
&
\underset{\text{3-Phosphoglycerate}}{
\begin{array}{c}
COO^{-} \\
| \\
H-C-OH \\
| \\
CH_2OPO_3^{2-}
\end{array}} + \text{ATP}
\end{array}
$$

(11.7)
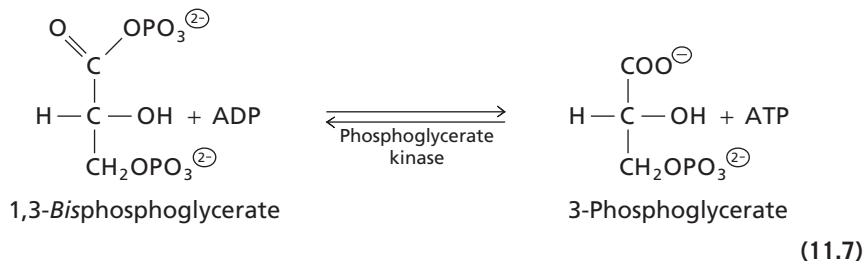
Steps 6 and 7 together couple the oxidation of an aldehyde to a carboxylic acid with the phosphorylation of ADP to ATP and the formation of a reducing equivalent.

$$\text{Glyceraldehyde 3-phosphate} + \text{NAD}^{\oplus} \longrightarrow \text{1,3-}\textit{Bis}\text{phosphoglycerate} + \text{NADH} + \text{H}^{\oplus}$$

$$\text{1,3-}\textit{Bis}\text{phosphoglycerate} + \text{ADP} \longrightarrow \text{3-Phosphoglycerate} + \text{ATP}$$

$$\text{Glyceraldehyde 3-phosphate} + \text{NAD}^{\oplus} + \text{P}_i + \text{ADP} \longrightarrow \text{3-Phosphoglycerate} + \text{NADH} + \text{H}^{\oplus} + \text{ATP}$$

(11.8)
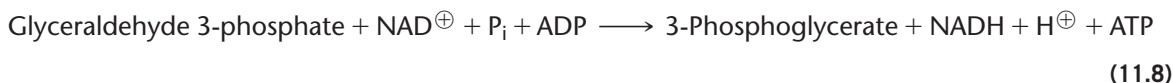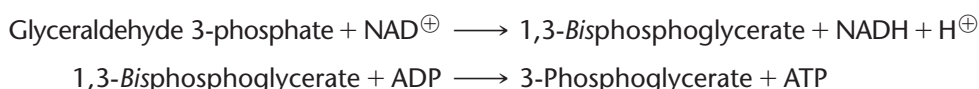
BOX 11.2 FORMATION OF 2,3-*BIS*PHOSPHOGLYCERATE IN RED BLOOD CELLS

An important function of glycolysis in red blood cells is the production of 2,3-*bis*phosphoglycerate, an allosteric inhibitor of the oxygenation of hemoglobin (Section 4.13C). This metabolite is a reaction intermediate and cofactor in Step 8 of glycolysis.

Erythrocytes contain *bis*phosphoglycerate mutase. This enzyme catalyzes the transfer of a phosphoryl group from C-1 to C-2 of 1,3-*bis*phosphoglycerate, to form 2,3-*bis*phosphoglycerate. As shown in the reaction scheme, 2,3-*bis*phosphoglycerate phosphatase catalyzes the hydrolysis of excess 2,3BPG to 3-phosphoglycerate, which can reenter glycolysis and be converted to pyruvate.

The shunting of 1,3-*bis*phosphoglycerate through these two enzymes bypasses phosphoglycerate kinase, which catalyzes Step 7 of glycolysis, one of the two ATP-generating steps. However, only a portion of the glycolytic flux in red blood cells—about 20%—is diverted through the mutase and phosphatase. Accumulation of free 2,3BPG (i.e., 2,3BPG not bound to hemoglobin) inhibits *bis*phosphoglycerate mutase. In exchange for diminished ATP generation, this bypass provides a regulated supply of 2,3BPG, which is necessary for the efficient release of O$_2$ from oxyhemoglobin.
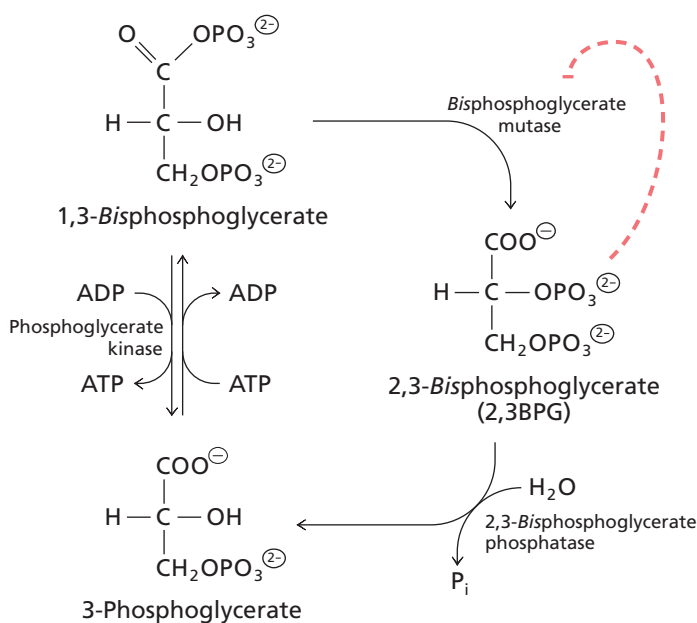
▲ Formation of 2,3-*bis*phosphoglycerate (2,3BPG) in red blood cells.

BOX 11.3 ARSENATE POISONING

Arsenic, like phosphorus, is in Group V of the periodic table. Arsenate ($AsO_4^{\ominus}$) therefore, is an analog of inorganic phosphate. Arsenate competes with phosphate for its binding site in glyceraldehyde 3-phosphate dehydrogenase. Like phosphate, arsenate cleaves the energy-rich thioacyl–enzyme intermediate. However, arsenate produces an unstable analog of 1,3-*bis*phosphoglycerate, called 1-arseno-3-phosphoglycerate, which is rapidly hydrolyzed on contact with water. This nonenzymatic hydrolysis produces 3-phosphoglycerate and regenerates inorganic arsenate, which can again react with a thioacyl–enzyme intermediate. In the presence of arsenate, glycolysis can proceed from 3-phosphoglycerate, but the ATP-producing reaction involving 1,3-*bis*phosphoglycerate is bypassed. As a result, there is no net formation of ATP from glycolysis. Arsenate is a poison because it can replace phosphate in many phosphoryl transfer reactions.

Arsenite, ($AsO_2^{\ominus}$) is much more toxic than arsenate. Arsenite poisons by an entirely different mechanism than arsenate. The arsenic atom of arsenite binds tightly to the two sulfur atoms of lipoamide (Section 7.12), thereby inhibiting the enzymes that require this coenzyme.
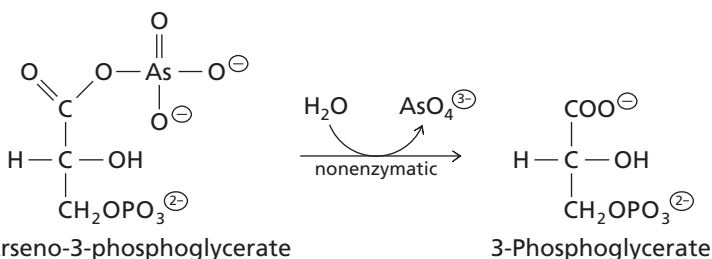
▲ Spontaneous hydrolysis of 1-arseno-3-phosphoglycerate. Inorganic arsenate can replace inorganic phosphate as a substrate for glyceraldehyde 3-phosphate dehydrogenase, forming the unstable 1-arseno analog of 1,3-*bis*phosphoglycerate.

▲ **Cary Grant learned about the effects of arsenic in a popular 1944 movie.**

The formation of ATP by the transfer of a phosphoryl group from a "high energy" compound (such as 1,3-*bis*phosphoglycerate) to ADP is termed **substrate level phosphorylation**. This reaction is the first ATP-generating step of glycolysis. It operates at substrate and product concentrations that are close to the equilibrium concentrations. This is not surprising since the reverse reaction is important in gluconeogenesis, where ATP is utilized. Flux can proceed easily in either direction.

8. Phosphoglycerate Mutase

Phosphoglycerate mutase catalyzes the near-equilibrium interconversion of 3-phosphoglycerate and 2-phosphoglycerate.

3-Phosphoglycerate   2-Phosphoglycerate   (11.9)
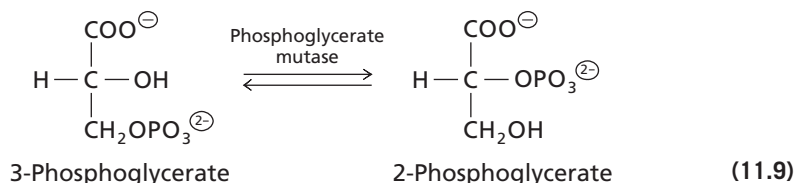
Mutases are isomerases that catalyze the transfer of a phosphoryl group from one part of a substrate molecule to another. There are two different types of phosphoglycerate mutase enzymes. In one type, the phosphoryl group is first transferred to an amino

acid side chain of the enxyme. The enzyme phosphoryl group is then transferred to the second site of the substrate molecule. The dephosphorylated intermediate remains bound in the active site during this process.

Another type of phosphoglycerate mutase makes use of a 2,3-*bis*phosphoglycerate (2,3BPG) intermediate as shown in Figure 11.8. This mechanism also involves a phosphorylated enzyme intemediate but it differs from the other type of enzyme because at no time is there a dephosphorylated metabolite during the reaction. Small amounts of 2,3-*bis*phosphoglycerate are required for full activity of this second type of enzyme. This is because 2,3BPG is required to phosphorylate the enzyme if it becomes dephosphorylated. The enzyme will lose its phosphate group whenever 2,3BPG is released from the active site before it can be converted to 2-phosphoglycerate or 3-phosphoglycerate. The second type of phosphoglycerate mutase is called cofactor-dependent PGM, or dPGM. The first type of enzyme is called cofactor-independent PGM, or iPGM.

dPGM and iPGM are not evolutionarily related. The cofactor-dependent enzyme (dPGM) belongs to a family of enzymes that include acid phosphatases and fructose 2,6-*bis*phosphatase. It is the major form of phosphoglycerate mutase in fungi, some bacteria, and most animals. The cofactor-independent enzyme (iPGM) belongs to the alkaline phosphatase family of enzymes. This version of phosphoglycerate mutase is found in plants and some bacteria. Some species of bacteria have both types of enzyme.
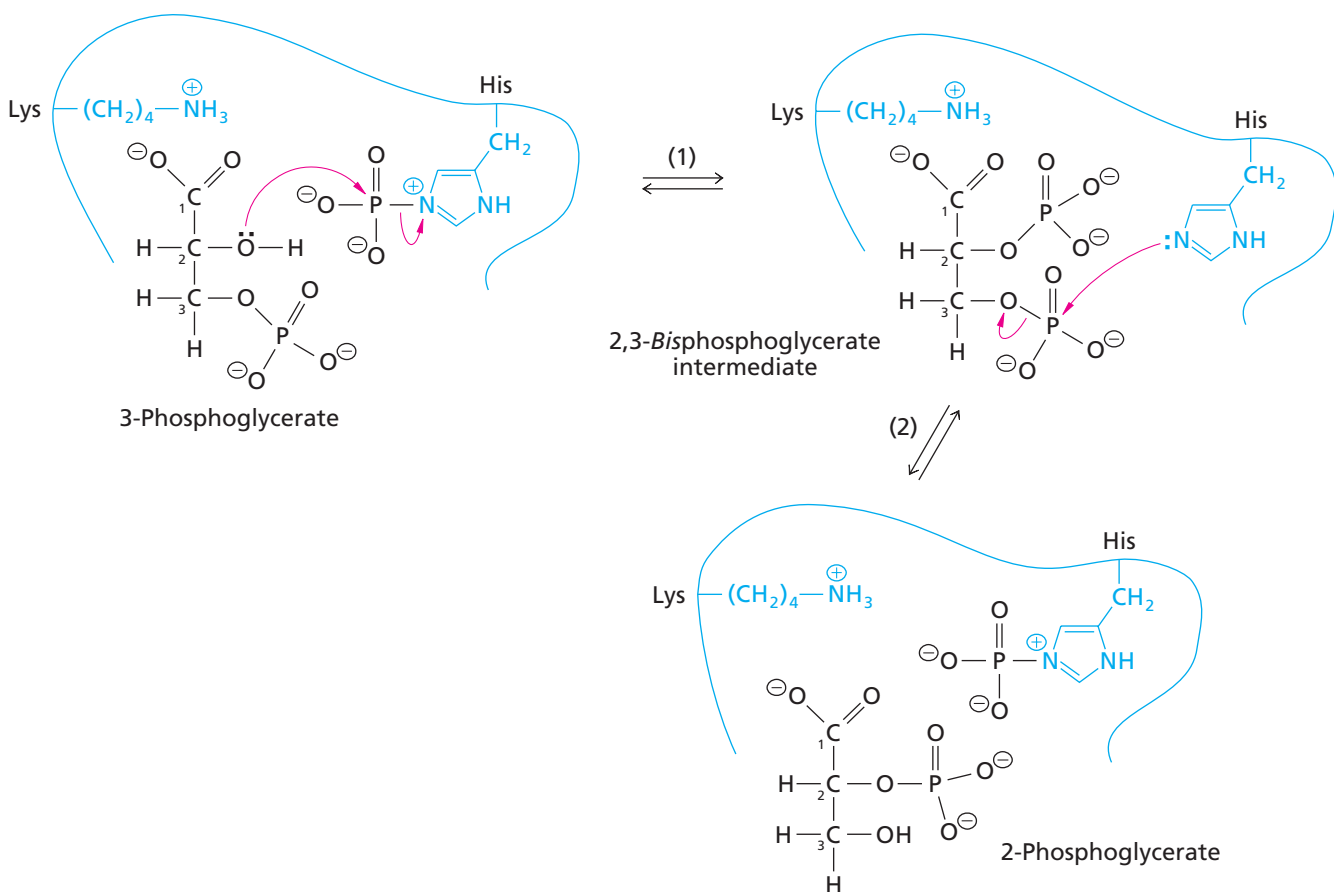
▲ **Figure 11.8**
Mechanism of the conversion of 3-phosphoglycerate to 2-phosphoglycerate in animals and fungi. (1) A lysine residue at the active site of phosphoglycerate mutase binds the carboxylate anion of 3-phosphoglycerate. A histidine residue, which is phosphorylated before the substrate binds, donates its phosphoryl group to form the 2,3-*bis*phosphoglycerate intermediate. (2) Rephosphorylation of the enzyme with a phosphoryl group from the C-3 position of the intermediate yields 2-phosphoglycerate.

9. Enolase

2-Phosphoglycerate is dehydrated to phosphoenolpyruvate in a near-equilibrium reaction catalyzed by enolase. The systematic name of enolase is 2-phosphoglycerate dehydratase.

$$\begin{array}{c} COO^{\ominus} \\ | \\ H-\underset{2}{C}-OPO_3^{\textcircled{2-}} \\ | \\ H-\underset{3}{C}-OH \\ | \\ H \end{array} \quad \xrightleftharpoons[\text{Mg}^{\textcircled{2+}}]{\text{Enolase,}} \quad \begin{array}{c} COO^{\ominus} \\ | \\ C-OPO_3^{\textcircled{2-}} \; + \; H_2O \\ \| \\ CH_2 \end{array}$$

2-Phosphoglycerate   Phosphoenolpyruvate   **(11.10)**
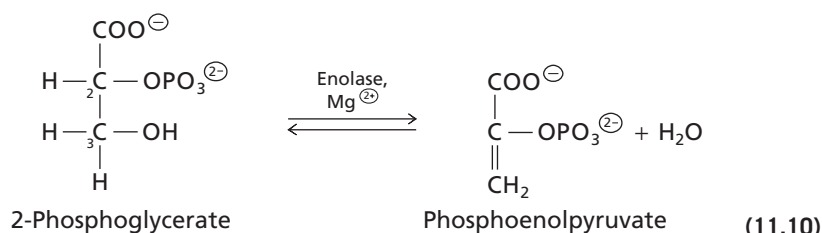
In this reaction, the phosphomonoester 2-phosphoglycerate is converted to an enol–phosphate ester, phosphoenolpyruvate, by the reversible elimination of water from C-2 and C-3. Phosphoenolpyruvate has an extremely high phosphoryl group transfer potential because the phosphoryl group holds pyruvate in its unstable enol form (Section 10.7B).

Enolase requires $Mg^{\textcircled{2+}}$ for activity. Two magnesium ions participate in this reaction: a "conformational" ion binds to the carboxylate group of the substrate, and a "catalytic" ion participates in the dehydration reaction.

10. Pyruvate Kinase

The second substrate level phosphorylation of glycolysis is catalyzed by pyruvate kinase. Phosphoryl group transfer to ADP generates ATP in this metabolically irreversible reaction. The unstable enol tautomer of pyruvate is an enzyme-bound intermediate.

$$\begin{array}{c} COO^{\ominus} \\ | \\ C-OPO_3^{\textcircled{2-}} \; + \; ADP + H^{\oplus} \\ \| \\ CH_2 \end{array} \quad \xrightleftharpoons[\text{kinase}]{\text{Pyruvate}} \quad \left[\begin{array}{c} COO^{\ominus} \\ | \\ C-OH \\ \| \\ CH_2 \end{array}\right] \quad \longrightarrow \quad \begin{array}{c} COO^{\ominus} \\ | \\ C=O \; + \; ATP \\ | \\ CH_3 \end{array}$$

Phosphoenolpyruvate   Enolpyruvate   Pyruvate

(11.11)
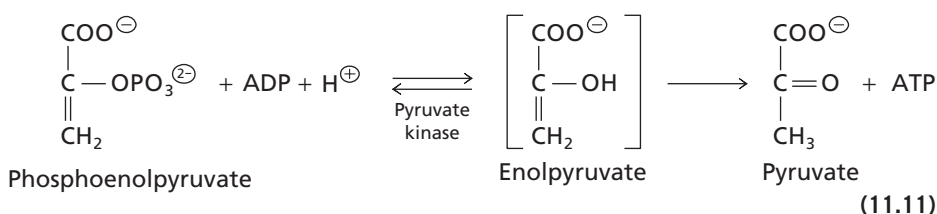
Transfer of the phosphoryl group from phosphoenolpyruvate to ADP is the third regulated reaction of glycolysis. Pyruvate kinase is regulated both by allosteric modulators and by covalent modification. In addition, expression of the pyruvate kinase gene in mammals is regulated by various hormones and nutrients. Recall from Chapter 10 that phosphoenolpyruvate hydrolysis has a higher standard Gibbs free energy change than ATP hydrolysis (Table 10.3). Because pyruvate kinase is regulated, the concentration of phosphoenolpyruvate is maintained at a high enough level to drive ATP formation during glycolysis.

11.3 The Fate of Pyruvate

The formation of pyruvate from phosphoenolpyruvate is the last step of glycolysis. Further metabolism of pyruvate typically takes one of five routes (Figure 11.9).

1. Pyruvate can be converted to acetyl CoA and acetyl CoA can be used in a number of metabolic pathways. In one important pathway it is completely oxidized to CO_2 in the citric acid cycle. This fate of pyruvate is described in Chapter 13. This is a route that operates efficiently in the presence of oxygen.
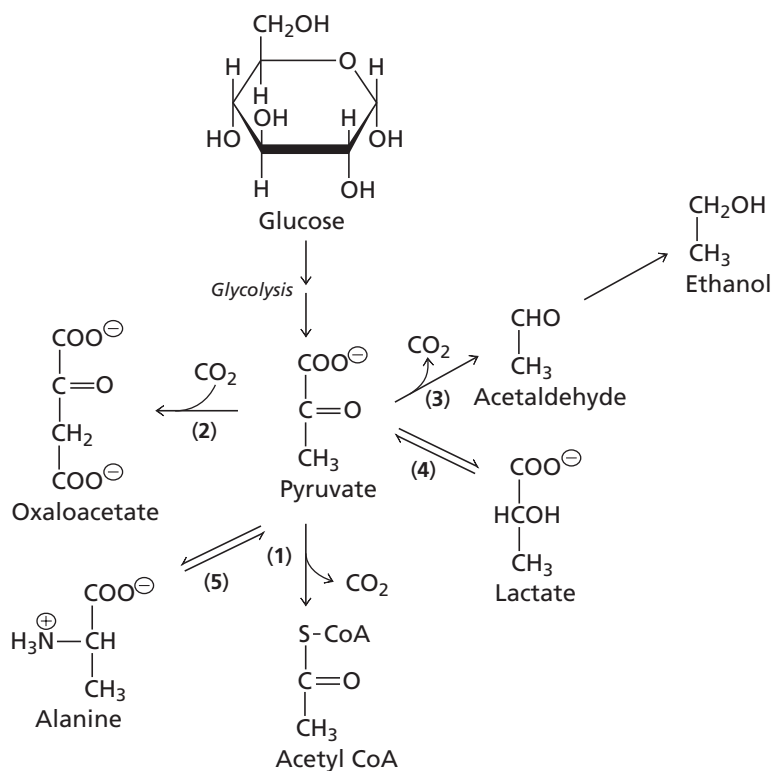
Five major fates of pyruvate: (1) Under aerobic conditions, pyruvate is oxidized to the acetyl group of acetyl CoA, which can enter the citric acid cycle for further oxidation. **(2)** Pyruvate can be converted to oxaloacetate, which can be a precursor in gluconeogenesis. **(3)** Under anaerobic conditions, certain microorganisms ferment glucose to ethanol via pyruvate. **(4)** Glucose undergoes anaerobic glycolysis to lactate in vigorously exercising muscles, red blood cells, and certain other cells. **(5)** Pyruvate is converted to alanine.

2. Pyruvate can be carboxylated to produce oxaloacetate. Oxaloacetate is one of the citric acid cycle intermediates but it is also an intermediate in the synthesis of glucose. The fate of pyruvate as a precursor in gluconeogenesis is covered in Chapter 12.

3. In some species, pyruvate can be reduced to ethanol, which is then excreted from cells. This reaction normally takes place under anaerobic conditions where entry of acetyl CoA into the citric acid cycle is unfavorable.

4. In some species, pyruvate can be reduced to lactate. Lactate can be transported to cells that convert it back to pyruvate for entry into one of the other pathways. This is also an anaerobic pathway.

5. In all species, pyruvate can be converted to alanine.

   During glycolysis, $NAD^{\oplus}$ is reduced to NADH at the glyceraldehyde 3-phosphate dehydrogenase reaction (Step 6). In order for glycolysis to operate continuously, the cell must be able to regenerate $NAD^{\oplus}$. Otherwise, all the coenzyme would rapidly accumulate in the reduced form, and glycolysis would stop. Under aerobic conditions, NADH can be oxidized by the membrane-associated electron transport system (Chapter 14), which requires molecular oxygen. Under anaerobic conditions, the synthesis of ethanol or lactate consumes NADH and regenerates the $NAD^{\oplus}$ essential for continued glycolysis.

A. Metabolism of Pyruvate to Ethanol

Many bacteria, and some eukaryotes, are capable of surviving in the absence of oxygen. They convert pyruvate to a variety of compounds that are secreted. Ethanol is one of these compounds. It assumes significance in biochemistry because the synthesis of ethanol by highly selected strains of yeast is important in the production of beer and wine. Yeast cells convert pyruvate to ethanol and CO_2 and oxidize NADH to $NAD^{\oplus}$. Two reactions are required. First, pyruvate is decarboxylated to acetaldehyde in a reaction catalyzed by pyruvate decarboxylase. This enzyme requires the coenzyme thiamine diphosphate (TDP); its mechanism was described in the coenzymes chapter (Section 7.7).

   Alcohol dehydrogenase catalyzes the reduction of acetaldehyde to ethanol. This oxidation–reduction reaction is coupled to the oxidation of NADH. These reactions and

The fate of pyruvate as a precursor in amino acid biosynthesis is discussed in Chapter 17.

In some species, pyruvate can be converted to phosphoenolpyruvate (Section 12.1B).

KEY CONCEPT

In the absence of oxygen, eukaryotes have to give up the net gain of 2 NADH molecules in order to make lactate or ethanol.
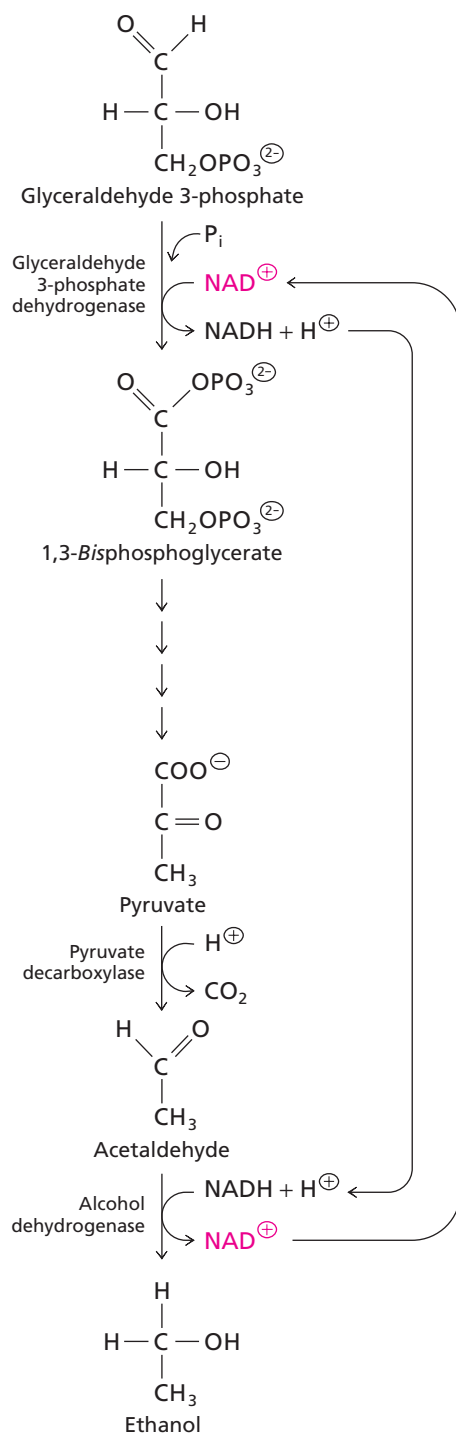
▲ **Figure 11.10**
Anaerobic conversion of pyruvate to ethanol in yeast.

the cycle of $NAD^{\oplus}$/NADH reduction and oxidation in alcoholic fermentation are shown in Figure 11.10. *Fermentation* refers to a process where electrons from glycolysis—in the form of NADH—are passed to an organic molecule such as ethanol instead of being passed on to the membrane-associated electron transport chain and ultimately oxygen (*respiration*).

The sum of the glycolytic reactions and the conversion of pyruvate to ethanol is

$$\text{Glucose} + 2\ P_i^{\,\textcircled{\scriptsize 2-}} + 2\ ADP^{\textcircled{\scriptsize 3-}} + 2\ H^{\oplus} \longrightarrow$$
$$2\ \text{Ethanol} + 2\ CO_2 + 2\ ATP^{\textcircled{\scriptsize 4-}} + 2\ H_2O \qquad \textbf{(11.12)}$$
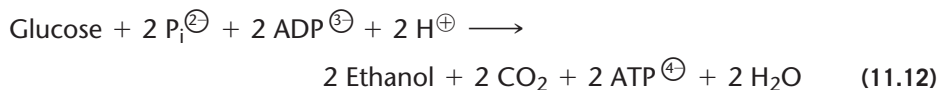
These reactions have familiar commercial roles in the manufacture of beer and bread. In the brewery, the carbon dioxide produced during the conversion of pyruvate to ethanol can be captured and used to carbonate the final alcoholic brew; this gas produces the foamy head. In the bakery, carbon dioxide is the agent that causes bread dough to rise.

B. Reduction of Pyruvate to Lactate

Pyruvate is reduced to lactate in a reversible reaction catalyzed by lactate dehydrogenase. This reaction is common in anaerobic bacteria and also in mammals.

$$\underset{\text{Pyruvate}}{\overset{\displaystyle COO^{\ominus}}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{C=O}}}}} + NADH + H^{\oplus} \xrightleftharpoons[\text{dehydrogenase}]{\text{Lactate}} \underset{\text{L-Lactate}}{\overset{\displaystyle COO^{\ominus}}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{HO-C-H}}}}} + NAD^{\oplus} \qquad \textbf{(11.13)}$$
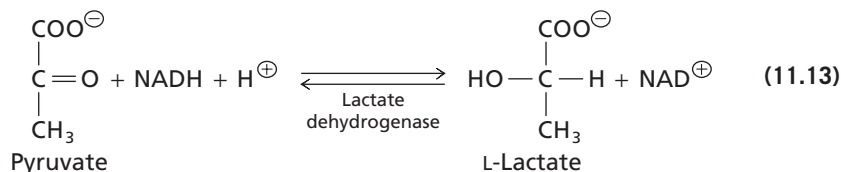
Lactate dehydrogenase is a classic dehydrogenase using $NAD^{\oplus}$ as a coenzyme; the mechanism was presented in Section 7.4. This is an oxidation–reduction reaction in which pyruvate is reduced to lactate by transfer of a hydride ion from NADH.

The lactate dehydrogenase reaction oxidizes the reducing equivalents generated in the glyceraldehyde 3-phosphate reaction and lowers the potential energy gain of glycolysis. It plays the same role that ethanol production accomplishes in other species (Figure 11.10). The net effect is to maintain flux in the glycolytic pathway and the production of ATP. In bacteria, lactate is secreted or converted to other end products, such as propionate. In mammals, lactate can only be reconverted to pyruvate.

The production of lactate in mammalian cells is essential in tissues where glucose is the main carbon source and reducing equivalents (NADH) are not needed in biosynthesis reactions or cannot be used to generate ATP by oxidative phosphorylation. A good example is the formation of lactate in skeletal muscle cells during vigorous exercise. Lactate formed in muscle cells is transported out of cells and carried via the bloodstream to the liver, where it is converted to pyruvate by the action of hepatic lactate dehydrogenase (see Cori cycle, Section 12.2A). Further metabolism of pyruvate requires oxygen. When the supply of oxygen to tissues is inadequate, all tissues produce lactate by anaerobic glycolysis.

The overall reaction for glucose degradation to lactate is

$$\text{Glucose} + 2\ P_i^{\,\textcircled{\scriptsize 2-}} + 2\ ADP^{\textcircled{\scriptsize 3-}} \longrightarrow 2\ \text{Lactate}^{\ominus} + 2\ ATP^{\textcircled{\scriptsize 4-}} + 2\ H_2O \qquad \textbf{(11.14)}$$
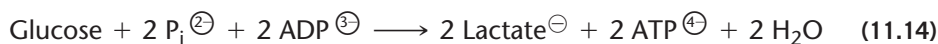
Lactic acid is also produced by *Lactobacillus* and certain other bacteria when they ferment the sugars in milk. The acid denatures the proteins in milk, causing the curdling necessary for cheese and yogurt production.

Regardless of the final product—ethanol or lactate—glycolysis generates two molecules of ATP per molecule of glucose consumed. Oxygen is not required in either case. This feature is essential not only for anaerobic organisms but also for some specialized cells in multicellular organisms. Some tissues (such as kidney medulla and parts of the brain), termed obligatory glycolytic tissues, rely on glycolysis for all their energy. In the

BOX 11.4 THE LACTATE OF THE LONG-DISTANCE RUNNER

Most of you have heard stories about lactate buildup during strenuous exercise. It all sounds so plausible. When muscle cells are working hard they use up glucose to generate ATP, which is required for muscle contraction. During very strenuous activity, the production of pyruvate may outstrip its ability to be oxidized by the citric acid cycle. If muscle cells aren't getting enough oxygen, then pyruvate is converted to lactic acid and the accumulation of lactic acid causes acidosis leading to muscle pain and reduced efficiency.

It's a nice story, but it's wrong.

Lactate concentration in muscle cells and in the bloodstream does increase but lactate is not an acid. It cannot donate a proton, so the increase in protons (acidosis) must come from another source. Lactate really is the product of the lactate dehydrogenase reaction, not lactic acid (which can donate a proton).

There is no net production of protons in the pathway leading from glucose to lactate. The acidosis seen after strenuous exercise is mostly due to the release of protons during ATP hydrolysis associated with muscle contraction. This is a temporary imbalance since ATP is soon regenerated in order to maintain a high steady state concentration. Lactate may indirectly contribute to some acidosis because, as a potent anion, it may affect buffering capacity but the effect is not large. Lactate has been getting a bum rap for decades, including previous editions of this textbook.

cornea of the eye, for example, oxygen availability is limited by poor blood circulation. Anaerobic glycolysis provides the necessary ATP for such tissues.

11.4 Free Energy Changes in Glycolysis

When the glycolytic pathway is operating, the flow of metabolites is from glucose to pyruvate. Under these conditions, the Gibbs free energy change for every single reaction must be either negative or zero. It is interesting to compare the *standard* Gibbs free energy changes ($\Delta G^{\circ\prime}$) and the *actual* Gibbs free energy changes (ΔG) under conditions where flux through the glycolytic pathway is high. Such conditions occur in erythrocytes where blood glucose is the main source of energy and there is very little synthesis of carbohydrates (or any other molecules). The actual concentrations of the intermediates in glycolysis have been measured and the Gibbs free energy changes have been calculated. The standard Gibbs free energy changes for each of the ten reactions of glycolysis are shown in Table 11.2, The first column lists $\Delta G^{\circ\prime}$ values under typical standard conditions (25°C and zero ionic strength) and the second column corrects those standard Gibbs free energy changes to mammalian physiological conditions (37°C in the presence of Mg^{2+}, Ca^{2+}, Na^{+} and K^{+}).

Figure 11.11 shows the cumulative standard Gibbs free energy changes and actual free energy changes for the glycolytic reactions in erythrocytes. The vertical axis indicates cumulative Gibbs free energy changes for each of the steps of glycolysis. The figure illustrates the difference between the Gibbs free energy changes under standard physiological conditions ($\Delta G^{\circ\prime}$) and actual free energy changes under cellular conditions (ΔG).

The blue plot tracks the actual cumulative free energy changes. It shows that each reaction has a Gibbs free energy change that is either negative or zero. This is an essential requirement for conversion of glucose to pyruvate. It follows that the overall pathway, which is the sum of the individual reactions, must also have a negative free energy change. The overall Gibbs free energy change for glycolysis is about -72 kJ mol^{-1} under the conditions found in erythrocytes.

Table 11.2 Standard Gibbs free energies for reactions of glycolysis

Glycolysis reaction	$\Delta G°'$ (kJ mol^{-1}) (standard conditions)	$\Delta G°'$ (kJ mol^{-1}) (physiological conditions)
1	−17.2	−19.4
2	+2.0	+2.8
3	−18.0	−15.6
4	+28.0	+24.6
5	+7.9	+7.6
6	+6.7	+2.6
7	−18.8	−16.4
8	+4.4	+6.4
9	−2.7	−4.5
10	−25.5	−27.2

Data from Minakami and de Verdier (1976) and Li et al. (2010).

The actual Gibbs free energy changes are large only for Steps 1, 3, and 10, which are catalyzed by hexokinase, phosphofructokinase-1, and pyruvate kinase, respectively—the steps that are both metabolically irreversible and regulated. The ΔG values for the other steps are very close to zero. In other words, these other steps are near-equilibrium reactions in cells.

In contrast, the standard Gibbs free energy changes for the same ten reactions exhibit no consistent pattern. Although the three reactions with large negative Gibbs free energy changes in cells also have large standard Gibbs free energy changes, this may be coincidental since some of the near-equilibrium reactions in cells also have large values for $\Delta G°'$. Furthermore, some of the $\Delta G°'$ values for the reactions of glycolysis are positive, indicating that under standard conditions, flux through these reactions occurs toward substrate rather than product. This is especially obvious in Step 4 (aldolase) and Step 6 (glyceraldehyde 3-phosphate dehydrogenase). In other types of cells these near-equilibrium reactions might operate in the opposite direction during glucose synthesis.

KEY CONCEPT

The net production of product in a metabolic pathway (flux) will only occur if: (a) the overall Gibbs free energy change is negative, and (b) the Gibbs free energy change of each step in the pathway is either negative or zero.
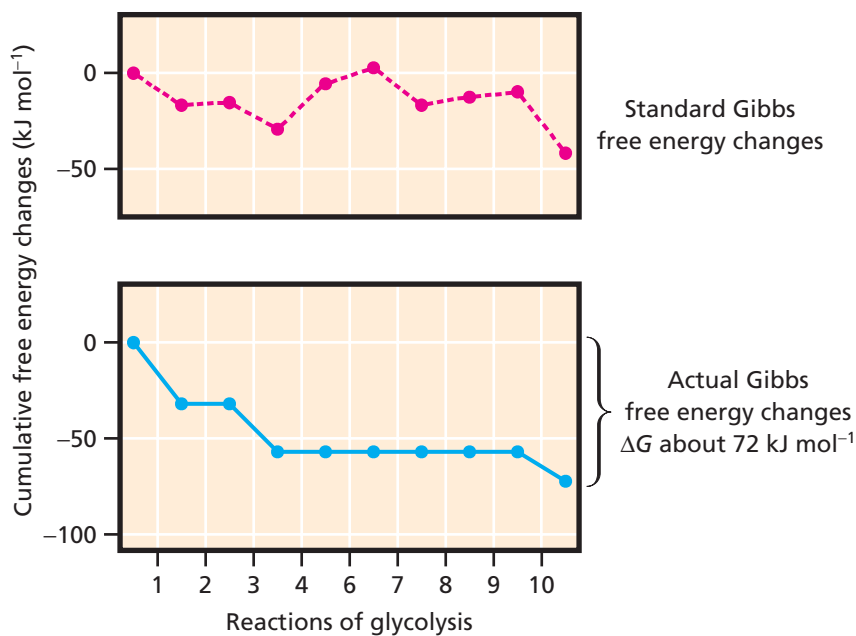
Figure 11.11 ▶
Cumulative standard and actual Gibbs free energy changes for the reactions of glycolysis. The vertical axis indicates free energy changes in kJ mol^{-1}. The reactions of glycolysis are plotted in sequence horizontally. The upper plot (red) tracks the standard free energy changes, and the bottom plot (blue) shows actual free energy changes in erythrocytes. The interconversion reaction catalyzed by triose phosphate isomerase (Reaction 5) is not shown. [Adapted from Hamori, E. (1975). Illustration of free energy changes in chemical reactions. *J. Chem. Ed.* 52:370–373.]

11.5 Regulation of Glycolysis

The regulation of glycolysis has been examined more thoroughly than that of any other pathway. Data on regulation come primarily from two types of biochemical research: enzymology and metabolic biochemistry. In enzymological approaches, metabolites are tested for their effects on isolated enzymes and the structure and regulatory mechanisms of individual enzymes are studied. Metabolic biochemistry analyzes the concentrations of pathway intermediates *in vivo* and stresses pathway dynamics under cellular conditions. We sometimes find that *in vitro* studies are deceptive as indicators of pathway dynamics *in vivo*. For instance, a compound may modulate enzyme activity *in vitro*, but only at concentrations not found in the cell. Accurate interpretation of biochemical data greatly benefits from a combination of enzymological and metabolic expertise.

In this section, we examine each regulatory site of glycolysis. Our primary focus is on the regulation of glycolysis in mammalian cells—in particular, those cells where glycolysis is an important pathway. Variations on the regulatory themes presented here can be found in other species.

The regulatory effects of metabolites on glycolysis are summarized in Figure 11.12. The activation of glycolysis is desirable when ATP is required by processes such as muscle contraction. Hexokinase is inhibited by excess glucose 6-phosphate, and PFK-1 is inhibited by the accumulation of ATP and citrate (an intermediate in the energy-producing citric acid cycle). ATP and citrate both signal an adequate energy supply. Consumption of ATP leads to the accumulation of AMP, which relieves the inhibition of PFK-1 by ATP. Fructose 2,6-*bis*phosphate also relieves this inhibition. The rate of formation of fructose 1,6-*bis*phosphate then increases, which in certain tissues activates pyruvate kinase. Glycolytic activity decreases when its products are no longer required.

A. Regulation of Hexose Transporters

The first potential step for regulating glycolysis is the transport of glucose into the cell. In most mammalian cells, the intracellular glucose concentration is far lower than the blood glucose concentration, and glucose moves into the cells, down its concentration gradient, by passive transport. All mammalian cells possess membrane-spanning
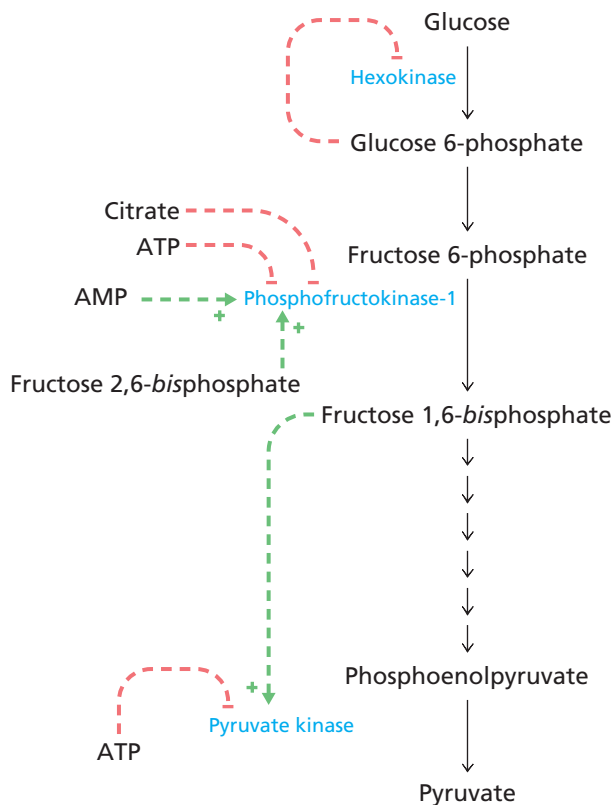
◀ **Figure 11.12**
Summary of the metabolic regulation of the glycolytic pathway in mammals. Not shown are the effects of ADP on PFK-1, which vary among species.

Membrane transport systems are described in Section 9.11.

glucose transporters. Intestinal and kidney cells have a $Na^{\oplus}$-dependent cotransport system called SGLT1 for absorbing dietary glucose and urinary glucose, respectively. Other mammalian cells contain transporters from the GLUT family of passive hexose transporters. Each of the six members of the GLUT family has unique properties suitable for the metabolic activities of the tissues in which it is found.

The hormone insulin stimulates high rates of glucose uptake into skeletal and heart muscle cells and adipocytes via the transporter GLUT4. When insulin binds to receptors on the cell surface, intracellular vesicles that have GLUT4 embedded in their membranes fuse with the cell surface by exocytosis (Section 9.11D), thereby increasing the capacity of the cells to transport glucose (Figure 11.13). Because GLUT4 is found at high levels only in striated muscle and adipose tissue, insulin-regulated uptake of glucose occurs only in these tissues.

In most tissues, a basal level of glucose transport in the absence of insulin is maintained by GLUT1 and GLUT3. GLUT2 transports glucose into and out of the liver, and GLUT5 transports fructose in the small intestine. GLUT7 transports glucose 6-phosphate from the cytoplasm into the endoplasmic reticulum.

Once inside a cell, glucose is rapidly phosphorylated by the action of hexokinase. This reaction traps the glucose inside the cell since phosphorylated glucose cannot cross the plasma membrane. As we will see, phosphorylated glucose can also be used in glycogen synthesis or in the pentose phosphate pathway (Chapter 12).

B. Regulation of Hexokinase

The reaction catalyzed by mammalian hexokinase is metabolically irreversible (because it is regulated) but in bacteria and many other eukaryotes hexokinase is not regulated. In those species, the concentrations of reactants and products reach equilibrium. In mammals, the various forms of hexokinase are subject to complex regulation.

At physiological concentrations, the enzyme product, glucose 6-phosphate, allosterically inhibits hexokinase isozymes I, II, and III, but not glucokinase (isozyme IV). Glucokinase is more abundant than the other hexokinases in the liver and the insulin-secreting cells of the pancreas. The concentration of glucose 6-phosphate increases when glycolysis is inhibited at sites further along the pathway. The inhibition of hexokinases I, II, and III by glucose 6-phosphate therefore coordinates the activity of hexokinase with the activity of subsequent enzymes of glycolysis.

Glucokinase is suited to the physiological role of the liver in managing the supply of glucose for the entire body. In most cells, glucose concentrations are maintained far

BOX 11.5 GLUCOSE 6-PHOSPHATE HAS A PIVOTAL METABOLIC ROLE IN THE LIVER

Glucose 6-phosphate is an initial substrate for several metabolic pathways (figure below). We have already seen that it is the initial intermediate in glycolysis. Glucose 6-phosphate is formed rapidly in liver cells from dietary glucose or newly synthesized glucose (from gluconeogenesis in liver cells; Section 12.1).

The principal use of liver glucose 6-phosphate is to maintain a constant concentration of blood glucose. Glucose 6-phosphatase is the enzyme responsible for catalyzing hydrolysis of glucose 6-phosphate to glucose. (This reaction is also the last step in gluconeogenesis.)

Glucose 6-phosphate that is not required for blood glucose is stored as liver glycogen (Section 12.6). Glycogen is subsequently degraded when a supply of glucose is needed. Hormones regulate both the synthesis and degradation of glycogen.

In addition to using it for balancing the blood glucose concentration, the liver metabolizes glucose 6-phosphate by the pentose phosphate pathway (Section 12.5) to produce ribose 5-phosphate (for nucleotides) and NADPH (for synthesis of fatty acids). We have seen in this chapter that glucose 6-phosphate can also enter the glycolytic pathway, where it is converted initially to pyruvate, which leads to another major metabolite—acetyl CoA.

▼ **Glucose 6-phosphate is at a pivotal position in carbohydrate metabolism in the liver.**
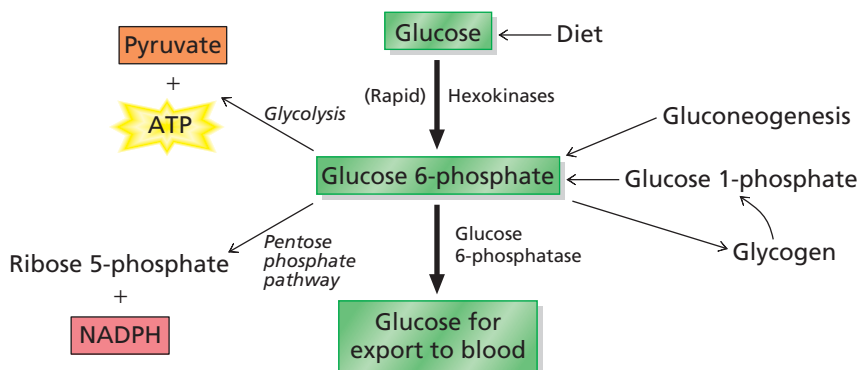
below the concentration in blood. However, glucose freely enters the liver via GLUT2, and the concentration of glucose in liver cells matches the concentration in blood. The blood glucose concentration is typically 5 mM, though after a meal it can rise as high as 10 mM. Most hexokinases have K_m values for glucose of about 0.1 mM or less. In contrast, glucokinase has a K_m of 2 to 5 mM for glucose; in addition, it is not significantly inhibited by glucose 6-phosphate. Therefore, liver cells can form glucose 6-phosphate (for glycogen synthesis) by the action of glucokinase when glucose is abundant and other tissues have sufficient glucose.

The activity of glucokinase is modulated by fructose phosphates. In liver cells, a regulatory protein inhibits glucokinase in the presence of fructose 6-phosphate, lowering its affinity for glucose to about 10 mM (Figure 11.14). Note that the v_0 vs. [S] curves for glucokinase are sigmoidal and not the hyperbolic curves expected for an enzyme obeying Michaelis–Menten kinetics. This is a common feature of allosterically regulated proteins. It means that there is no true K_m value for glucokinase. We can say that the effect of the regulatory protein is to raise the *apparent* K_m of the enzyme. Flux through glucokinase is usually low because liver cells always contain considerable fructose 6-phosphate. The flux can increase after a meal, when fructose 1-phosphate—derived only from dietary fructose—relieves the inhibition of glucokinase by the regulatory protein. Therefore, the liver can respond to increases in blood carbohydrate concentrations with proportionate increases in the rate of phosphorylation of glucose.

C. Regulation of Phosphofructokinase-1

The second site of allosteric regulation is the reaction catalyzed by phosphofructokinase-1. PFK-1 is a large, oligomeric enzyme with a molecular weight ranging in different species from about 130,000 to 600,000. The quaternary structure of PFK-1 also varies among species. The bacterial and mammalian enzymes are both tetramers; the yeast
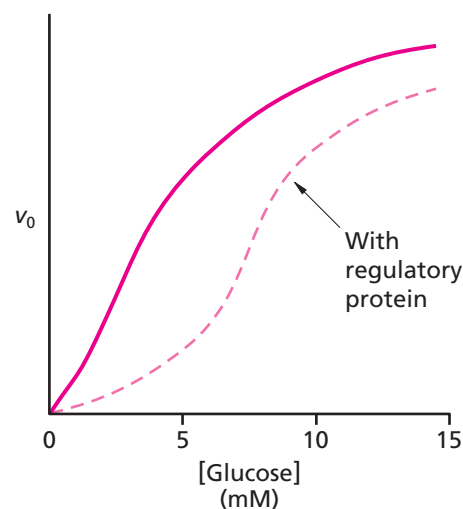
▲ **Figure 11.14**
Plot of initial velocity (v_0) versus glucose concentration for glucokinase. The addition of a regulatory protein lowers the enzyme's affinity for glucose. The blood glucose concentration is 5 to 10 mM.

Figure 11.15 ▶
Regulation of PFK-1 by ATP and AMP. In the absence of AMP, PFK-1 is almost completely inhibited by physiological concentrations of ATP. In the range of AMP concentrations found in the cell, the inhibition of PFK-1 by ATP is almost completely relieved. [Adapted from Martin, B. R. (1987). *Metabolic Regulation: A Molecular Approach* (Oxford: Blackwell Scientific Publications), p. 222.]
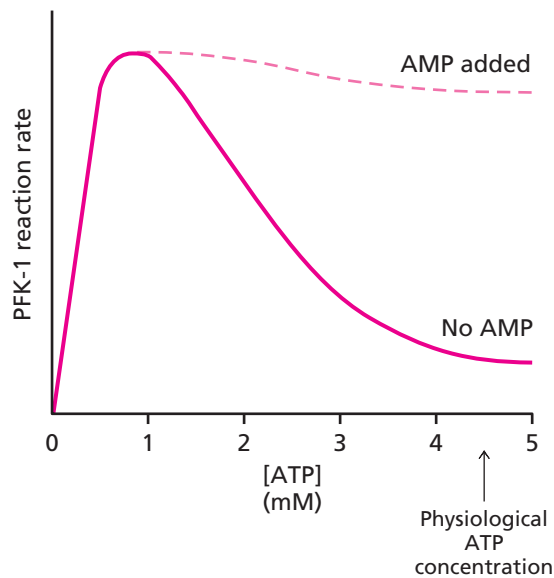
enzyme is an octamer. This complex enzyme has several regulatory sites. The regulatory properties of the *Escherichia coli* phosphofructokinase-1 are described in Section 5.10A.

ATP is both a substrate and, in most species, an allosteric inhibitor of PFK-1. ATP increases the apparent K_m of PFK-1 for fructose 6-phosphate. The bacterial enzyme is activated by ADP but in mammals AMP is the allosteric activator of PFK-1. AMP acts by relieving the inhibition caused by ATP (Figure 11.15). ADP activates mammalian PFK-1 but inhibits the plant kinase; in bacteria, protists, and fungi, the regulatory effects of purine nucleotides vary among species.

The concentration of ATP does not change very much in most mammalian cells despite large changes in the rate of its formation and utilization. However, as discussed in Section 10.6, significant changes in the concentrations of ADP and AMP do occur because these molecules are present in cells in much lower concentrations than ATP and small changes in the level of ATP cause proportionally larger changes in the levels of ADP and AMP. The steady state concentrations of these compounds are therefore able to control flux through PFK-1.

Recall that activation by ADP (or AMP) makes sense in light of the net production of ATP in glycolysis. Elevated levels of ADP or AMP indicate a deficiency of ATP that can be offset by increasing the rate of degradation of glucose (Section 5.9A).

Citrate, an intermediate of the citric acid cycle, is another physiologically important inhibitor of mammalian PFK-1. An elevated concentration of citrate indicates that the citric acid cycle is blocked and further production of pyruvate would be pointless. The regulatory effect of citrate on PFK-1 is an example of feedback inhibition that regulates the supply of pyruvate to the citric acid cycle. (Phosphoenolpyruvate, not citrate, inhibits the bacterial enzyme.)

As shown in Figure 11.12, fructose 2,6-*bis*phosphate is a potent activator of PFK-1, effective in the micromolar range. This compound is present in mammals, fungi, and plants, but not prokaryotes. We will return to the role of fructose 2,6-*bis*phosphate in the next chapter after we have described gluconeogenesis and glycogen metabolism.

D. Regulation of Pyruvate Kinase

The third site of allosteric regulation of glycolysis is the reaction catalyzed by pyruvate kinase. Single-cell species, such as bacteria, and protists, have a single pyruvate kinase gene. The enzyme is allosterically regulated in a simple manner—its activity is affected by pyruvate and/or fructose 1,6-*bis*phosphate. Regulation is much more complex in mammals because different organs have different requirements for glucose and glycolysis.

Four different isozymes of pyruvate kinase are present in mammalian tissues. The isozymes found in liver, kidney, and red blood cells yield a sigmoidal curve when initial velocity is plotted against phosphoenolpyruvate concentration (Figure 11.16a). This indicates that PEP is an allosteric activator. These enzymes are also allosterically

(a)

(b)
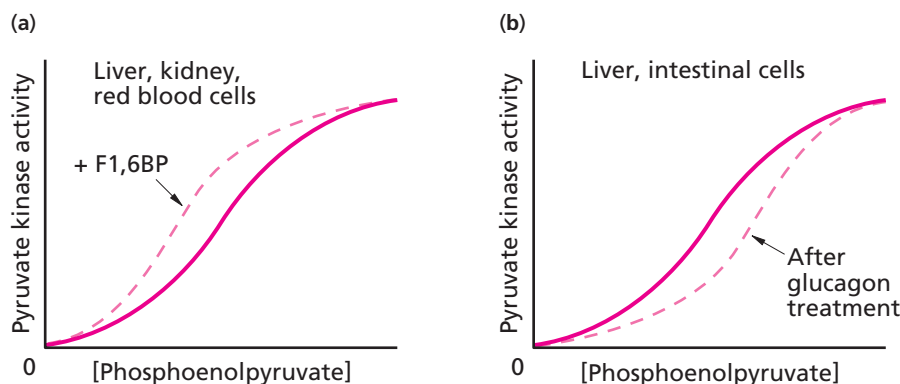
Plots of initial velocity (v_0) versus phospho-enolpyruvate concentration for pyruvate kinase. (a) For isozymes in some cells, the presence of fructose 1,6-*bis*phosphate shifts the curve to the left, indicating that fructose 1,6-*bis*phosphate is an activator of the enzymes. **(b)** When liver or intestinal cells are incubated with glucagon, pyruvate kinase is phosphorylated by the action of protein kinase A. The curve shifts to the right, indicating less activity for pyruvate kinase.

activated by fructose 1,6-*bis*phosphate and inhibited by ATP. In the absence of fructose 1,6-*bis*phosphate, physiological concentrations of ATP almost completely inhibit the isolated enzyme. The presence of fructose 1,6-*bis*phosphate—probably the most important modulator *in vivo*—shifts the curve to the left. With sufficient fructose 1,6-*bis*phosphate, the curve becomes hyperbolic. Figure 11.16a shows that for a range of substrate concentrations, enzyme activity is greater in the presence of the allosteric activator. Recall that fructose 1,6-*bis*phosphate is the product of the reaction catalyzed by PFK-1. Its concentration increases when the activity of PFK-1 increases. Since fructose 1,6-*bis*phosphate activates pyruvate kinase, the activation of PFK-1 (which catalyzes Step 3 of the glycolytic pathway) causes subsequent activation of pyruvate kinase (the last enzyme in the pathway). This is an example of feed-forward activation.

The predominant isozyme of pyruvate kinase found in mammalian liver and intestinal cells is subject to an additional type of regulation, covalent modification by phosphorylation. Protein kinase A, which also catalyzes the phosphorylation of PFK-2 (Figure 11.17), catalyzes the phosphorylation of pyruvate kinase. Pyruvate kinase is less active in the phosphorylated state. The change in kinetic behavior is shown in Figure 11.16b, which depicts a plot of pyruvate kinase activity in liver and intestinal cells in the presence and absence of glucagon, a stimulator of protein kinase A. Dephosphorylation of pyruvate kinase is catalyzed by a protein phosphatase.

The pyruvate kinase activity of liver cells decreases on starvation and increases on ingestion of a diet high in carbohydrate. These long term changes are due to changes in the rate of synthesis of pyruvate kinase and not allosteric regulation or covalent modification.

▲ **Figure 11.17**
Pyruvate kinase from the yeast *Saccharomyces cerevisiae*, with the activator fructose 1,6-*bis*phosphate (red). The active site is in the large central domain. [PDB 1A3W]

E. The Pasteur Effect

Louis Pasteur observed that when yeast cells grow anaerobically, they produce much more ethanol and consume much more glucose than when they grow aerobically. Similarly, skeletal muscle accumulates lactate under anaerobic conditions but not when it metabolizes glucose aerobically. In both yeast and muscle, the rate of conversion of glucose to pyruvate is much higher under anaerobic conditions. The slowing of glycolysis in the presence of oxygen is called the **Pasteur effect**. As we will see in Chapter 13, the complete aerobic metabolism of a glucose molecule produces much more ATP than the two molecules of ATP produced by glycolysis alone. Therefore, for any given ATP requirement, fewer glucose molecules must be consumed under aerobic conditions. Cells sense the state of ATP supply and demand, and they modulate glycolysis by several mechanisms. For example, the availability of oxygen leads to the inhibition of PFK-1 (and thus glycolysis), probably through an increase in the ATP/AMP ratio.

11.6 Other Sugars Can Enter Glycolysis

Glucose and glucose 6-phosphate are the most common substrates for glycolysis, especially in vertebrates where glucose is circulated in the bloodstream. However, a variety of other sugars can be degraded by the glycolytic pathway. In this section, we will see how sucrose, fructose, lactose, galactose, and mannose can be metabolized.

▲ **Invertase from the yeast *Schwanniomyces occidentalis*.** The active form of the enzyme is a dimer of identical subunits. Fructose (space-filling representation) is bound at the active site. [PDB 3KF3]

## A. Sucrose Is Cleaved to Monosaccharides

The disaccharide sucrose can be degraded to its two component monosaccharides: fructose and glucose. This cleavage is catalyzed by a class of enzymes called sucrases. Invertase (β-fructofuranosidease) is one of the most common sucrases. It catalyzes a hydrolytic cleavage of the glycosidic linkage between the oxygen and the glucose residue, producing fructose and glucose (Figure 11.18). The glucose residues are then phosphorylated by hexokinase and the fructose residues enter the pathway as described below.

Some bacteria have a very interesting enzyme called sucrose phosphorylase. It cleaves sucrose in the presence of inorganic phosphate converting it to a molecule of fructose and a molecule of glucose 1-phosphate (Figure 11.18). All sugars entering glycolysis need to be phosphorylated at some stage and this step almost always involves the expenditure of one ATP equivalent. Sucrose phosphorylase is an important exception because it produces glucose 1-phosphate without spending any ATP currency.

B. Fructose Is Converted to Glyceraldehyde 3-Phosphate

Fructose is phosphorylated to fructose 1-phosphate by the action of a specific ATP-dependent fructokinase (Figure 11.19). In mammals, this step occurs in the liver after fructose has been absorbed in the intestine and transferred in the bloodstream. Fructose 1-phosphate aldolase catalyzes the cleavage of fructose 1-phosphate to dihydroxyacetone phosphate and glyceraldehyde. The glyceraldehyde is then phosphorylated to glyceraldehyde 3-phosphate in a reaction catalyzed by triose kinase, consuming a second molecule of ATP. Dihydroxyacetone phosphate is converted to a second molecule of glyceraldehyde 3-phosphate by the action of triose phosphate isomerase.

Figure 11.18 ▶
Entry of other sugars into glycolysis.
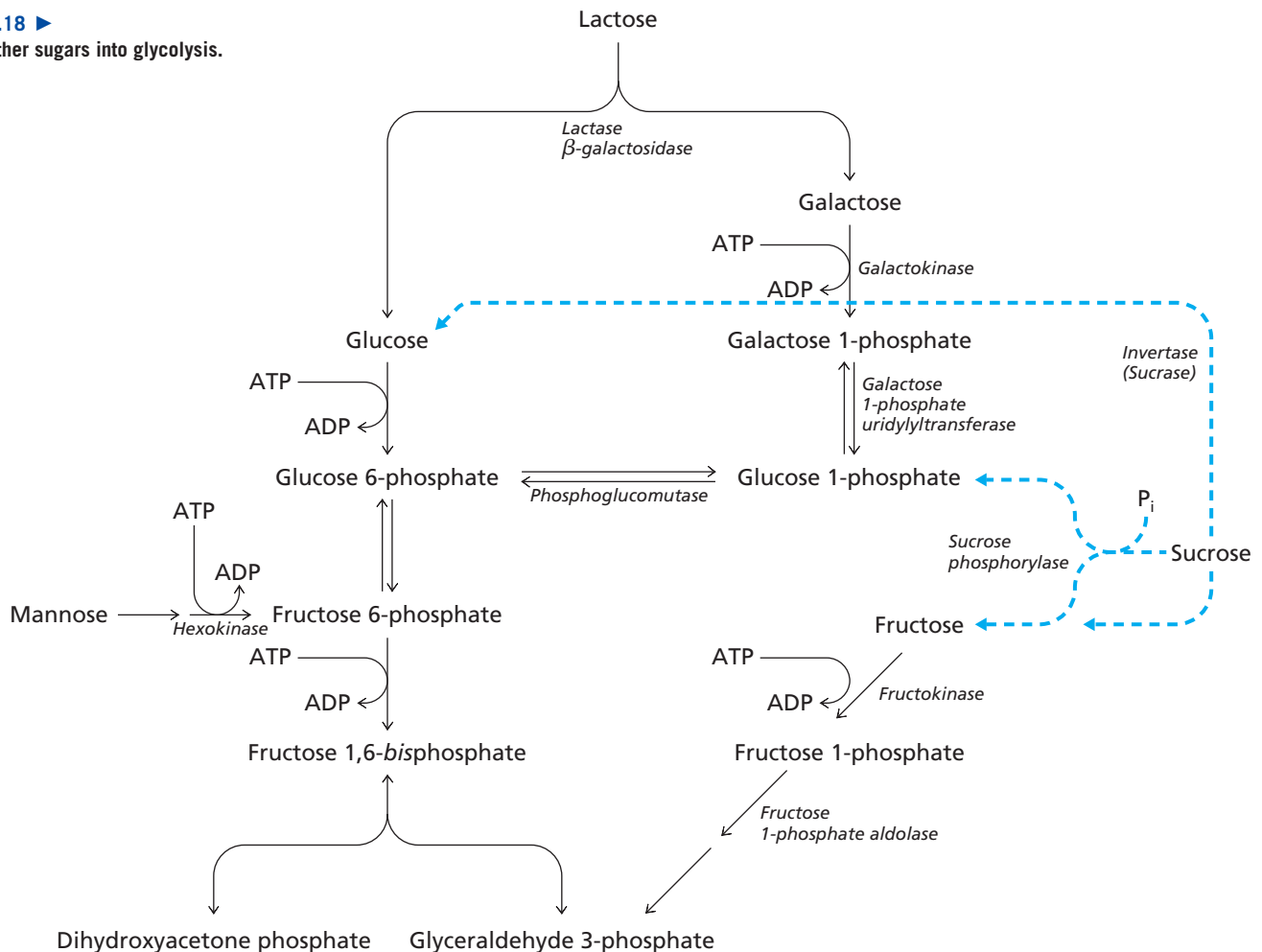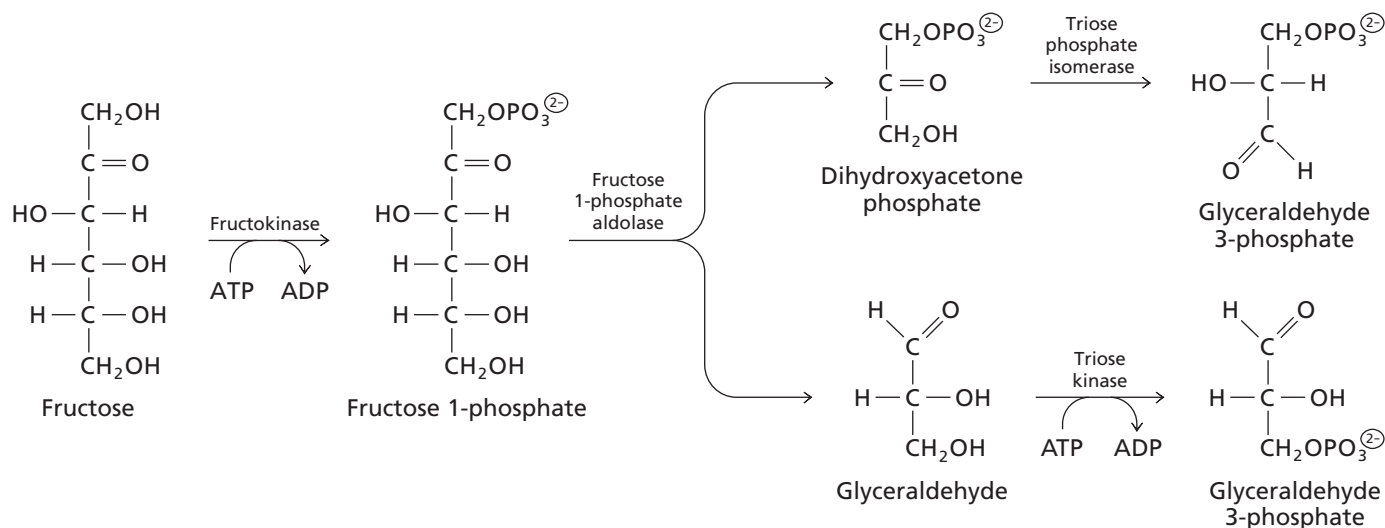
▲ **Figure 11.19**
Conversion of fructose to two molecules of glyceraldehyde 3-phosphate.

The two molecules of glyceraldehyde 3-phosphate produced can then be metabolized to pyruvate by the remaining steps of glycolysis. The metabolism of one molecule of fructose to two molecules of pyruvate produces two molecules of ATP and two molecules of NADH. This is the same yield as the conversion of glucose to pyruvate. Fructose catabolism bypasses phosphofructokinase-1 and its associated regulation. Regulation of pyruvate kinase can still control flux in the pathway.

C. Galactose Is Converted to Glucose 1-Phosphate

The disaccharide lactose, present in milk, is a major source of energy for nursing mammals. In newborns, intestinal lactase catalyzes the hydrolysis of lactose to its components, glucose and galactose, both of which are absorbed from the intestine and transported in the bloodstream.

As shown in Figure 11.20, galactose—the C-4 epimer of glucose—can be converted to glucose 1-phosphate by a pathway in which the nucleotide sugar UDP-glucose (Section 7.2A) is recycled. In the liver, galactokinase catalyzes transfer of a phosphoryl group from ATP to galactose. The galactose 1-phosphate formed in this reaction exchanges with the glucose 1-phosphate moiety of UDP-glucose by cleavage of the pyrophosphate bond of UDP-glucose. This reaction is catalyzed by galactose 1-phosphate uridylyltransferase and produces glucose 1-phosphate and UDP-galactose. Glucose

---

BOX 11.6 A SECRET INGREDIENT

Purified invertase is frequently used in the candy industry to convert sucrose to fructose and glucose. Fructose is sweeter than sucrose and therefore more appealing in some food. The liquid, creamy centers of some chocolates are produced by adding invertase—purified from yeast—to a sucrose mixture. In addition to tasting sweeter, fructose is much less likely to form crystals. The catalytic breakdown of sucrose inside the chocolate usually takes several days or weeks at room temperature.

Look for "invertase" on the labels of food to see more examples of this industrial application of biochemistry, but keep in mind that not all liquid centers in chocolates are due to added invertase.

▶ **Cherry Blossom by Lowney's (Hershey Canada).** The liquid center is due to the presence of added invertase.
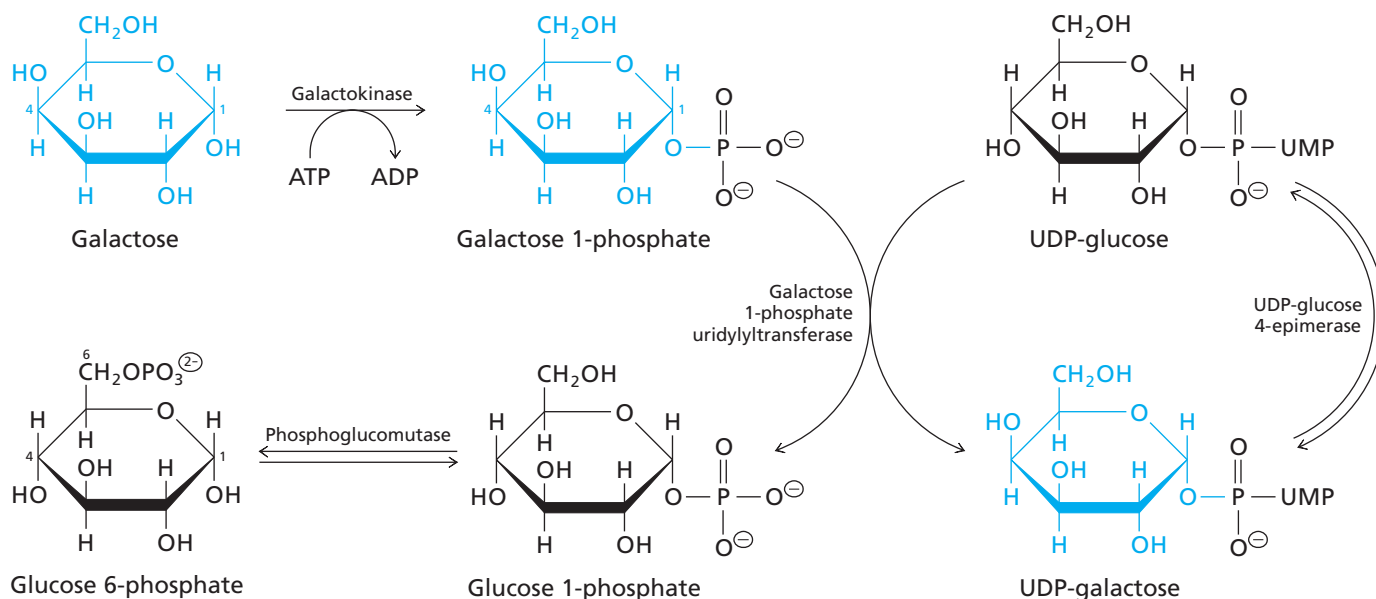
▲ **Figure 11.20**
Conversion of galactose to glucose 6-phosphate. The metabolic intermediate UDP-glucose is recycled in the process. The overall stoichiometry for the pathway is galactose + ATP –> glucose 6-phosphate + ADP.

> **UDP-Galactose is required for biosynthesis of gangliosides (Section 16.11).**

1-phosphate can enter glycolysis after conversion to glucose 6-phosphate in a reaction catalyzed by phosphoglucomutase. UDP-galactose is recycled to UDP-glucose by the action of UDP-glucose 4-epimerase.

The conversion of one molecule of galactose to two molecules of pyruvate produces two molecules of ATP and two molecules of NADH, the same yield as the conversions of glucose and fructose. The required UDP-glucose is formed from glucose and the ATP equivalent UTP, but only small (catalytic) amounts of it are needed since it is recycled.

Infants fed an exclusive diet of milk rely on galactose metabolism for about 20% of their caloric intake. In the most common form of the genetic disorder galactosemia (the inability to properly metabolize galactose), infants are deficient in galactose 1-phosphate uridylyltransferase. In such cases, galactose 1-phosphate accumulates in the cells and this can lead to a compromise in liver function, recognized by the appearance of jaundice (yellowing of the skin). The liver damage is potentially fatal. Other effects include damage to the central nervous system. Screening for galactose 1-phosphate uridylyltransferase in the red blood cells of the umbilical cord allows detection of galactosemia at birth. Many of the most severe effects of this genetic deficiency can be mitigated by a special diet that contains very little galactose and lactose.

The majority of humans undergo a reduction in the level of lactase at about 5 to 7 years of age. This is the normal situation found in most other primates. It parallels the switch from childhood, where mother's milk is a major source of nourishment, to adulthood, where milk is not consumed. In some human populations the production of lactase is not turned off during adolescence. These populations have acquired a mutant gene that continues to synthesize lactase in adults. As a result, individuals in these populations can consume milk products throughout their lives. Northern European populations and their descendants have high proportions of lactase-producing adults.

In normal adults, lactose is metabolized by bacteria in the large intestine, with the production of gases such as CO_2 and H_2 and short-chain acids. The acids can cause diarrhea by increasing the ionic strength of the intestinal fluid. Milk and milk products are usually avoided by people who do not synthesize lactase. Since they do not tolerate diets rich in milk products, they are said to be lactose intolerant although it's worth keeping in mind that this is the normal condition in most mammals, and most humans. Some lactose-intolerant individuals can eat yogurt, in which the lactose has been partially hydrolyzed by the action of an endogenous β-galactosidase of the microorganism in the yogurt culture. A commercially prepared enzyme supplement that contains β-galactosidase from a microorganism can be used to pretreat milk to reduce the lactose content or can be taken when milk products are ingested by lactase-deficient individuals.
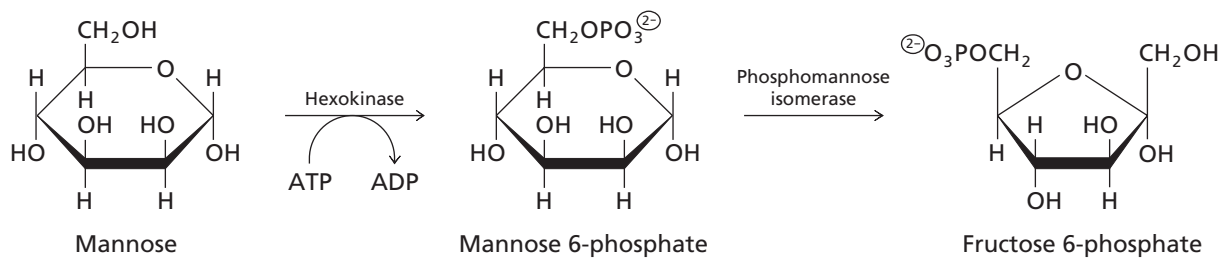
▲ Figure 11.21
Conversion of mannose to fructose 6-phosphate.

D. Mannose Is Converted to Fructose 6-Phosphate

The aldohexose mannose is obtained in the diet from glycoproteins and certain polysaccharides. Mannose is converted to mannose 6-phosphate by the action of hexokinase. In order to enter the glycolytic pathway, mannose 6-phosphate undergoes isomerization to fructose 6-phosphate in a reaction catalyzed by phosphomannose isomerase. These two reactions are depicted in Figure 11.21.

11.7 The Entner–Doudoroff Pathway in Bacteria

The classic glycolysis pathway is also called the Embden–Meyerhof–Parnas pathway. This pathway is found in all eukaryotes and many species of bacteria. However, a large number of bacterial species do not have phosphofructokinase-1 and cannot convert glucose 6-phosphate to fructose 1,6-*bis*phosphate in the hexose stage of glycolysis.

The hexose stage of classic glycolysis can be bypassed by the Entner–Doudoroff pathway. This pathway begins with the conversion of glucose 6-phosphate to 6-phosphogluconate, a reaction that is catalyzed by two enzymes: glucose 6-phosphate dehdrogenase and 6-phosphogluconolactonase (Figure 11.22). The oxidation of glucose 6-phosphate by glucose 6-phosphate dehydrogenase is coupled to the reduction of NADP$^\oplus$. The dehydrogenase and 6-phosphogluconolactonase enzymes are common in almost all species since they are required in the pentose phosphate pathway (Section 12.5). The Entner–Doudoroff pathway is the earliest pathway for glucose degradation. The classic glycolysis pathway (EMP) evolved later.

6-Phosphogluconate is converted to 2-keto-3-deoxy-6-phosphogluconate (KDPG) in an unusual dehydration (dehydratase) reaction. KDPG is then split by the action of KDPG aldolase to one molecule of pyruvate and one molecule of glyceraldehyde 3-phosphate. Pyruvate is the end product of glycolysis and glyceraldehyde 3-phosphate can be converted to another molecule of pyruvate by the triose stage of glycolysis. The enzymes of the triose stage of the EMP pathway are found in all species since they are essential for glucose synthesis as well as glycolysis. Note that only one molecule of glyceraldehyde 3-phosphate passes down the bottom half of the glycolytic pathway for every glucose 6-phosphate molecule that enters the Entner–Doudoroff pathway. This means that only one molecule of ATP is produced for every glucose molecule degraded, whereas two ATP molecules are synthesized during glycolysis. Two reducing equivalents (NADH) are produced during glycolysis and two in the ED pathway (NADPH in the first reaction and one molecule of NADH when glyceraldehyde 3-phosphate is converted to 1,3-*bis*phosphoglycerate).

In addition to being the main pathway for glucose degradation in some species, the Entner–Doudoroff pathway is also important in species that possess a complete Embden–Meyeroff–Parnas pathway. The Entner–Doudoroff pathway is used in the metabolism of gluconate and other related organic acids. These metabolites cannot be shunted into the normal glycolytic pathway. Many bacterial species, including *E. coli*, can grow on gluconate as their sole carbon source. Under these conditions the main energy-producing degradation pathway is the Entner–Doudoroff pathway. The first reaction in the ED pathway produces NADPH instead of NADH and many species use the glucose 6-phosphate dehydrogenase reaction as an important source of NADPH reducing equivalents (Section 12.4).

KEY CONCEPT

The classic glycolysis pathway evolved millions of years after the Entner–Douderoff and the gluconeogenesis pathways.

Figure 11.22 ▶
The Entner–Doudoroff pathway.

In Box 12.2 we discuss metabolic diseases associated with glucose 6-phosphate dehydrogenase in humans.
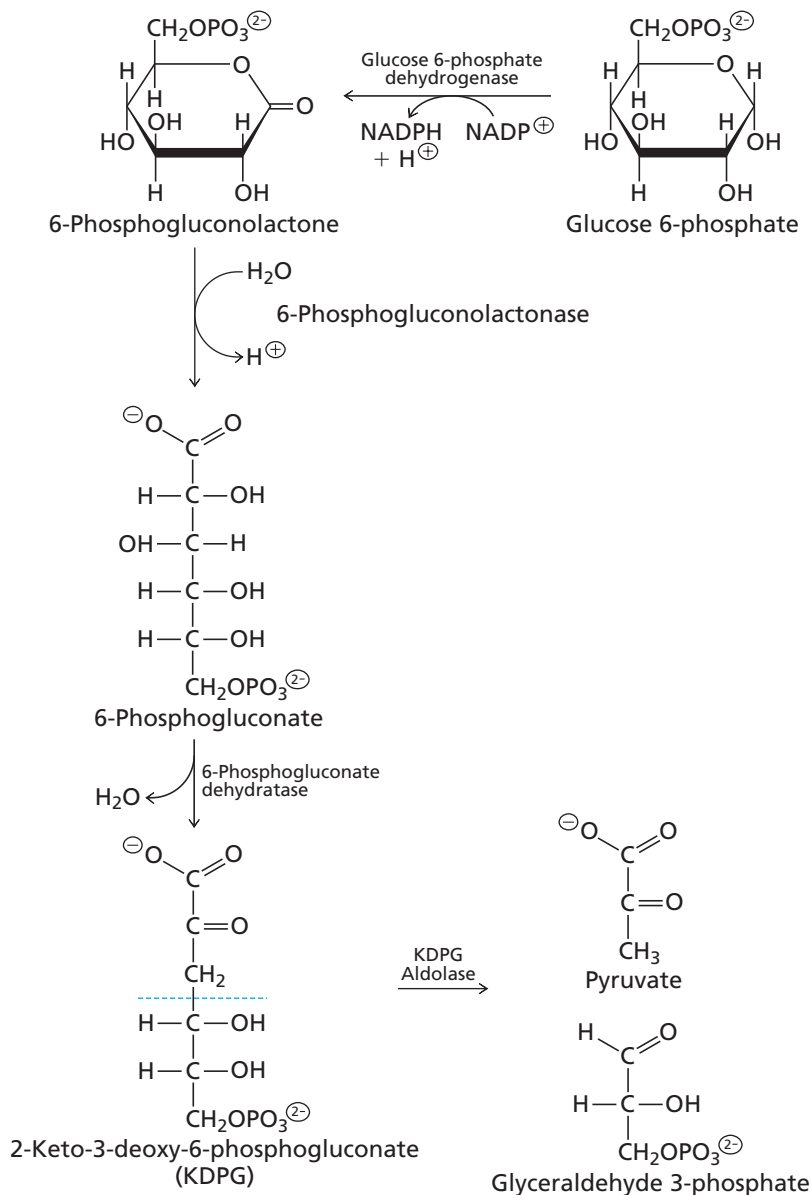
Aldolases cleave hexoses to two 3-carbon compounds. KDPG is the third aldolase we have described.

Summary

1. Glycolysis is a ten-step pathway in which glucose is catabolized to pyruvate. Glycolysis can be divided into a hexose stage and a triose stage. The products of the hexose stage are glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The triose phosphates interconvert, and glyceraldehyde 3-phosphate is metabolized to pyruvate.

2. For each molecule of glucose converted to pyruvate, there is a net production of two molecules of ATP from ADP + P_i and two molecules of $NAD^\oplus$ are reduced to NADH.

3. Under anaerobic conditions in yeast, pyruvate is metabolized to ethanol and CO_2. In some other organisms, pyruvate can be converted to lactate under anaerobic conditions. Both processes use NADH and regenerate $NAD^\oplus$.

4. The overall Gibbs free energy change for glycolysis is negative. The steps catalyzed by hexokinase, phosphofructokinase-1, and pyruvate kinase are metabolically irreversible.

5. Glycolysis is regulated at four steps: the transport of glucose into some cells and the reactions catalyzed by hexokinase, phosphofructokinase-1, and pyruvate kinase.

6. Fructose, galactose, and mannose can enter the glycolytic pathway via conversion to glycolytic metabolites.

7. The Entner–Doudoroff pathway is an alternate pathway for glucose catabolism in some bacteria.

Problems

1. Calculate the number of ATP molecules obtained from the anaerobic conversion of each of the following sugars to lactate: (a) glucose, (b) fructose, (c) mannose, and (d) sucrose.

2. (a) Show the positions of the six glucose carbons in the two lactate molecules formed by anaerobic glycolysis. (b) Under aerobic conditions, pyruvate can be decarboxylated to yield acetyl CoA and CO_2. Which carbons of glucose must be labeled with ^{14}C to yield $^{14}CO_2$?

3. If ^{32}P (i.e., isotopically labeled phosphorus) is added to a cell-free liver preparation undergoing glycolysis, will this label be directly incorporated in any glycolytic intermediate or pathway product?

4. Huntington's disease is a member of the "glutamine-repeat" family of diseases. In middle-aged adults the disease causes neurodegenerative conditions, including involuntary movements and dementia. The mutated protein (Huntington protein) contains a polyglutamine region with 40 to 120 glutamines that is thought to mediate a tight binding of this protein to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). If the brain relies almost solely on glucose as an energy source, suggest a role for the Huntington protein in this disease.

5. Fats (triacylglycerols) are a significant source of stored energy in animals and are metabolized initially to fatty acids and glycerol. Glycerol can be phosphorylated by the action of a kinase to produce glycerol 3-phosphate, which is oxidized to produce dihydroxyacetone phosphate.

   (a) Write the reactions for the conversion of glycerol to dihydroxyacetone phosphate.

   (b) The kinase that acts on the prochiral molecule glycerol is stereospecific, leading to production of L-glycerol 3-phosphate. Which carbons of glycerol 3-phosphate must be labeled with ^{14}C so that aerobic glycolysis yields acetyl CoA with both carbons labeled?

$$\begin{array}{c} _1CH_2OH \\ | \\ HO-_2C-H \\ | \\ _3CH_2OH \end{array}$$

Glycerol
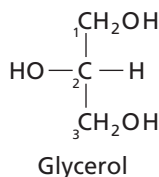
6. Tumor cells often lack an extensive capillary network and must function under conditions of limited oxygen supply. Explain why these cancer cells take up far more glucose and may overproduce some glycolytic enzymes.

7. Rapid glycolysis during strenuous exercise provides the ATP needed for muscle contraction. Since the lactate dehydrogenase reaction does not produce any ATP, would glycolysis be more efficient if pyruvate rather than lactate were the end product?

8. Why are both hexokinase and phosphofructokinase-1 inhibited by an ATP analog in which the oxygen atom joining the β- and γ-phosphorus atoms is replaced by a methylene group ($—CH_2—$)?

9. The $\Delta G^{\circ\prime}$ for the aldolase reaction in muscle is $+22.8$ kJ mol^{-1}. In view of this, why does the aldolase reaction proceed in the direction of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate during glycolysis?

10. For the aldolase reaction, calculate the concentration of fructose 1,6-*bis*phosphate if the concentrations of DHAP and G3P were each: (a) 5 μM, (b) 50 μM, (c) 500 μM.

11. The following plot shows the rate of mammalian phosphofructokinase-1 (PFK-1) activity versus fructose 6-phosphate (F6P) concentration in (a) the presence of ATP, AMP, or both and (b) in the absence or presence of fructose 2,6-*bis*phosphate (F26P). Explain these effects on the reaction rates of PFK-1.
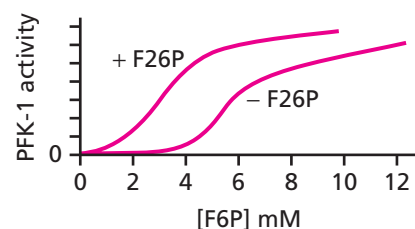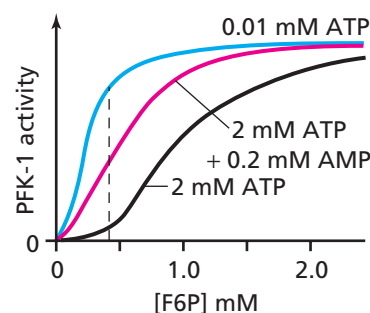
12. Draw a diagram showing how increased intracellular [cAMP] affects the activity of pyruvate kinase in mammalian liver cells.

13. In response to low levels of glucose in the blood, the pancreas produces glucagon, which triggers the adenylyl cyclase signaling pathway in liver cells. As a result, flux through the glycolytic pathway decreases.

    (a) Why is it advantageous for glycolysis to decrease in the liver in response to low blood glucose levels?

    (b) How are the effects of glucagon on glycolysis reversed when the level of glucagon decreases in response to adequate blood glucose levels?

14. Chemoautotrophs growing in the ocean will sometimes have all the enzymes needed for glycolysis even though they will never encounter external glucose. Why?

Selected Readings

Metabolism of Glucose

Alberty, R. A. (1996) Recommendations for nomenclature and tables in biochemical thermo-dynamics. *Eur. J. Biochem.* 240:1–14.

Cullis, P. M. (1987). Acyl group transfer–phosphoryl group transfer. In *Enzyme Mechanisms*, M. I. Page and A. Williams, eds. (London: Royal Society of Chemistry), pp. 178–220.

Hamori, E. (1975). Illustration of free energy changes in chemical reactions. *J. Chem. Ed.* 52:370–373.

Hoffmann-Ostenhof, O., ed. (1987). *Intermediary Metabolism* (New York: Van Nostrand Reinhold).

Li X, Dash RK, Pradhan RK, Qi F, Thompson M, Vinnakota KC, Wu F, Yang F, Beard DA. (2010) A database of thermodynamic quantities for the reactions of glycolysis and the tricarboxylic acid cycle. J Phys Chem B. 114:16068–16082.

Minakami S. and de Verdier, C-H. (1976) Colorimetric study on human erythrocyte glycolysis. Eur. J. Biochem. 65: 451–460.

Ronimus, R. S., and Morgan, H. W. (2003), Distribution and phylogenies of enzymes of the Embden–Meyerof–Parnas pathway from archaea and hyperthermophilic bacteria support a gluconeogenic origin of metabolism. *Archaea* 1:199–221.

Seeholzer, S. H., Jaworowski, A., and Rose, I. A. (1991). Enolpyruvate: chemical determination as a pyruvate kinase intermediate. *Biochem.* 30:727–732.

St-Jean, M., Blonski, C., and Sygush, J. (2009). Charge stabilization and entropy reduction of central lysine residues in fructose-*bis*phosphate aldolase. *Biochem.* 48:4528–4537.

Regulation of Glycolysis

Depré, C., Rider, M. H., and Hue, L. (1998). Mechanisms of control of heart glycolysis. *Eur. J. Biochem.* 258:277–290.

Engström, L., Ekman, P., Humble, E., and Zetterqvist, Ö. (1987). Pyruvate kinase. In *The Enzymes*, Vol. 18, P. D. Boyer and E. Krebs, eds. (San Diego: Academic Press), pp. 47–75.

Gould, G. W., and Holman, G. D. (1993). The glucose transporter family: structure, function and tissue-specific expression. *Biochem. J.* 295:329–341.

Pessin, J. E., Thurmond, D. C., Elmendorf, J. S., Coker, K. J., and Okada, S. (1999). Molecular basis of insulin-stimulated GLUT4 vesicle trafficking. Location! Location! Location! *J. Biol. Chem.* 274:2593–2596.

Pilkis, S. J., Claus, T. H., Kurland, I. J., and Lange, A. J. (1995). 6-Phosphofructo-2-kinase/fructose-2,6-*bis*phosphatase: a metabolic signaling enzyme. *Annu. Rev. Biochem.* 64:799–835.

Pilkis, S. J., El-Maghrabi, M. R., and Claus, T. H. (1988). Hormonal regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Biochem.* 57:755–783.

Pilkis, S. J., and Granner, D. K. (1992). Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Physiol.* 54:885–909.

Van Schaftingen, E. (1993). Glycolysis revisited. *Diabetologia* 36:581–588.

Yamada, K., and Noguchi, T. (1999). Nutrient and hormonal regulation of pyruvate kinase gene expression. *Biochem. J.* 337:1–11.

Metabolism of Other Sugars

Álvaro-Benito, M., Polo, A., González, B., Fernández-Lobato, M., and Sanz-Aparicio, J. (2010). Structural and kinetic analysis of *Schwanniomyces occidentalis* invertase reveals a new oligomerization pattern and the role of its supplementary domain in substrate binding. *J. Biol. Chem.* 285:13930–13941; doi:10.1074/jbc.M109.095430

Frey, P. A. (1996). The Leloir pathway: a mechanistic imperative for three enzymes to change the stereochemical configuration of a single carbon in galactose. *FASEB J.* 10:461–470.

Itan, Y., Jones, B. L., Ingram, C. J. E., Swallow, D. M., and Thomas, M. G. (2010). A worldwide correlation of lactase persistence phenotypes and genotypes. *BMC Evol. Biol.* 10:36; www.biomedcentral.com/1471-2148/10/36
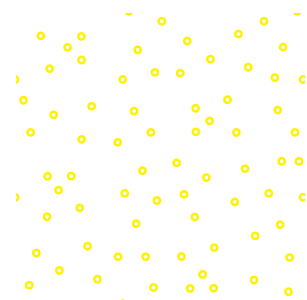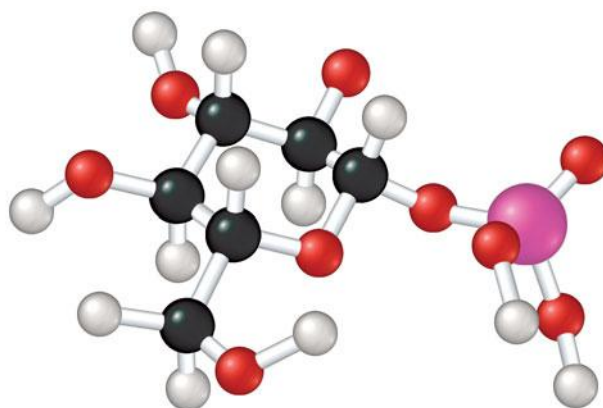
Gluconeogenesis, The Pentose Phosphate Pathway, and Glycogen Metabolism

We have seen that the catabolism of glucose is central to energy metabolism in some cells. In contrast, all species can synthesize glucose from simple two-carbon and three-carbon precursors by gluconeogenesis (literally, the formation of new glucose). Some species, notably photosynthetic organisms, can make these precursors by fixing carbon dioxide leading to the net synthesis of glucose from inorganic compounds. In our discussion of gluconeogenesis in this chapter we must keep in mind that every glucose molecule used in glycolysis had to be synthesized in some species.

The pathway for gluconeogenesis shares some steps with glycolysis, the pathway for glucose degradation, but four reactions specific to the gluconeogenic pathway are not found in the degradation pathway. These reactions replace the metabolically irreversible reactions of glycolysis. These opposing sets of reactions are an example of separate, regulated pathways for synthesis and degradation (Section 10.2).

In addition to fueling the production of ATP (via glycolysis and the citric acid cycle), glucose is also a precursor of the ribose and deoxyribose moieties of nucleotides and deoxynucleotides. The pentose phosphate pathway is responsible for the synthesis of ribose as well as the production of reducing equivalents in the form of NADPH.

Glucose availability is controlled by regulating the uptake and synthesis of glucose and related molecules and by regulating the synthesis and degradation of storage polysaccharides composed of glucose residues. Glucose is stored as glycogen in bacteria and animals and as starch in plants. Glycogen and starch can be degraded to release glucose monomers that can fuel energy production via glycolysis or serve as precursors in biosynthesis reactions. The metabolism of glycogen will illustrate another example of opposing, regulated pathways.

Although the reaction we had found would be viewed today as utterly trivial, it came nevertheless as a great surprise, because, at that time, nobody could imagine that the phosphorylation of an enzyme could be involved in its regulation.

—Eddy Fischer, *Memories of Ed Krebs* (2010)

Top: The Cori ester, α-D-glucopyranose 1-phosphate.

In mammals, gluconeogenesis, the pentose phosphate pathway, and glycogen metabolism are closely and coordinately regulated in accordance with the moment-to-moment requirements of the organism. In this chapter, we review these pathways and examine some of the mechanisms for regulating glucose metabolism in mammalian cells. The regulation of glucose and glycogen metabolism in mammals is important from a historical perspective because it was the first example of a signal transduction mechanism.

12.1 Gluconeogenesis

As stated in the introduction, all organisms have a pathway for glucose biosynthesis, or gluconeogenesis. This is true even for animals that use exogenous glucose as an important energy source because glucose may not always be available from external sources or intracellular stores. For example, large mammals that have not eaten for 16 to 24 hours have depleted their liver glycogen reserves and need to synthesize glucose to stay alive because glucose is required for the metabolism of certain tissues, for example, brain. Some mammalian tissues, primarily liver and kidney, can synthesize glucose from simple precursors such as lactate and alanine. Under fasting conditions, gluconeogenesis supplies almost all of the body's glucose. When exercising under anaerobic conditions, muscle converts glucose to pyruvate and lactate, which travel to the liver and are converted to glucose. Brain and muscle consume much of the newly formed glucose. Bacteria can convert many nutrients to phosphate esters of glucose and to glycogen.

It is convenient to consider pyruvate as the starting point for the synthesis of glucose. The pathway for gluconeogenesis from pyruvate is compared to the glycolytic pathway in Figure 12.1. Note that many of the intermediates and enzymes are identical. All seven of the near-equilibrium reactions of glycolysis proceed in the reverse direction during gluconeogenesis. Enzymatic reactions unique to gluconeogenesis are required for the three metabolically irreversible reactions of glycolysis. These irreversible glycolytic reactions are catalyzed by pyruvate kinase, phosphofructokinase-1, and hexokinase. In the biosynthesis direction these reactions are catalyzed by different enzymes.

Although all species have a gluconeogenesis pathway, they don't all have the glycolysis pathway (Section 11.7). This is especially true of bacterial species that diverged very early in the evolution of prokaryotes. Thus, it seems like gluconeogenesis is the more ancient pathway, which makes sense since there has to be a source of glucose before pathways for its degradation can evolve. Since the biosynthesis pathway evolved first, it is appropriate to think of the glycolytic enzymes as bypass enzymes. These enzymes, especially phosphofructokinase-1, evolved in order to bypass the metabolically irreversible reactions of gluconeogenesis.

The synthesis of one molecule of glucose from two molecules of pyruvate requires four ATP and two GTP molecules as well as two molecules of NADH. The net equation for gluconeogenesis is

$$2 \text{ Pyruvate} + 2 \text{ NADH} + 4 \text{ ATP} + 2 \text{ GTP} + 6 \text{ H}_2\text{O} + 2 \text{ H}^\oplus \longrightarrow$$
$$\text{Glucose} + 2 \text{ NAD}^\oplus + 4 \text{ ADP} + 2 \text{ GDP} + 6 \text{ P}_i \qquad \textbf{(12.1)}$$
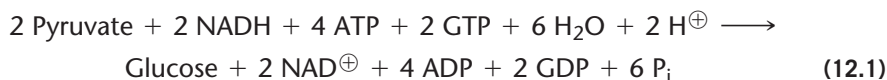
Four ATP equivalents are needed to overcome the thermodynamic barrier to the formation of two molecules of the energy-rich compound phosphoenolpyruvate from two molecules of pyruvate. Recall that in glycolysis the conversion of phosphoenolpyruvate to pyruvate is a metabolically irreversible reaction catalyzed by pyruvate kinase. In the catabolic direction this reaction is coupled to the synthesis of ATP. Two ATP molecules are required to carry out the reverse of the glycolytic reaction catalyzed by phosphoglycerate kinase. In the hexose stage of gluconeogenesis, no energy is recovered in the steps that convert fructose 1,6-*bis*phosphate to glucose because fructose 1,6-*bis*phosphate is not a "high energy" intermediate. Recall that glycolysis consumes two ATP molecules and generates four, for a net yield of two ATP equivalents and two molecules of NADH. Contrast this with the synthesis of one molecule of glucose by gluconeogenesis consuming a total of six ATP equivalents and two molecules of NADH. As expected, the biosynthesis of glucose requires energy and its degradation releases energy.
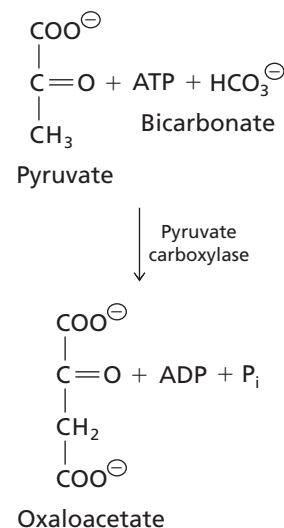
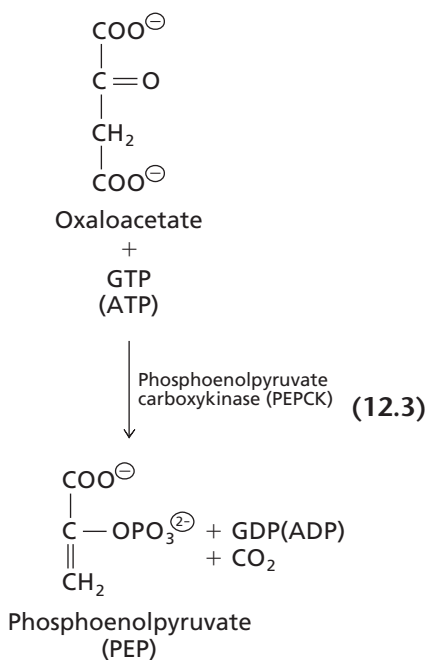**In the next section, we discuss how other precursors enter the pathway.**

Comparison of gluconeogenesis and glycolysis. There are four metabolically irreversible reactions of gluconeogenesis (blue). These are the reactions catalyzed by three different enzymes in glycolysis (red). Both pathways include a triose stage and a hexose stage. Two molecules of pyruvate are therefore required to produce one molecule of glucose.

Pyruvate carboxylase is a biotin-containing enzyme. The reaction mechanism was described in Section 7.10.

▲ **Figure 12.2**
Pyruvate carboxylase reaction.

A. Pyruvate Carboxylase

We begin our examination of the individual steps in the conversion of pyruvate to glucose with the two enzymes required for synthesis of phosphoenolpyruvate. The two steps involve a carboxylation followed by decarboxylation. In the first step, pyruvate carboxylase catalyzes the conversion of pyruvate to oxaloacetate. The reaction is coupled to the hydrolysis of one molecule of ATP (Figure 12.2).

Pyruvate carboxylase is a large, complex, enzyme composed of four identical subunits. Each subunit has a biotin prosthetic group covalently linked to a lysine residue. The biotin is required for the addition of bicarbonate to pyruvate. Pyruvate carboxylase catalyzes a metabolically irreversible reaction—it can be allosterically activated by acetyl CoA. This is the only regulatory mechanism known for the enzyme. Accumulation of

$$
\begin{array}{c}
COO^{\ominus} \\
| \\
C = O \\
| \\
CH_2 \\
| \\
COO^{\ominus}
\end{array}
$$

Oxaloacetate
+
GTP
(ATP)

↓ Phosphoenolpyruvate carboxykinase (PEPCK) **(12.3)**

$$
\begin{array}{c}
COO^{\ominus} \\
| \\
C - OPO_3^{\,2\ominus} \;\; + \; GDP(ADP) \\
\| \qquad\qquad + \; CO_2 \\
CH_2
\end{array}
$$

Phosphoenolpyruvate
(PEP)

▲ **Figure 12.3**
Phosphoenolpyruvate carboxykinase reaction.
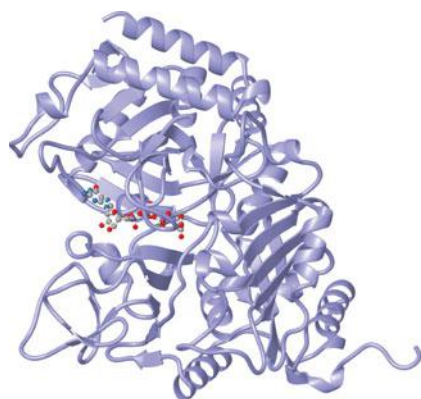
▲ **Phosphoenolpyruvate carboxykinase from rat (*Rattus norvegicus*).** The closed active site contains a bound GTP molecule, a molecule of oxaloacetate, and two $Mn^{2\oplus}$ ions (pink). [PDB 3DT4]

acetyl CoA indicates that it is not being efficiently metabolized by the citric acid cycle. Under these conditions, pyruvate carboxylase is stimulated in order to direct pyruvate to oxaloacetate instead of acetyl CoA. Oxaloacetate can enter the citric acid cycle or serve as a precursor for glucose biosynthesis.

Bicarbonate is one of the substrates in the reaction shown in Figure 12.2. Bicarbonate is formed when carbon dioxide dissolves in water so the reaction is sometimes written with CO_2 as a substrate. The pyruvate carboxylase reaction plays an important role in fixing carbon dioxide in bacteria and some eukaryotes. This role may not be so obvious when we examine gluconeogenesis since the carbon dioxide is released in the very next reaction; however, much of the oxaloacetate that is made is not used for gluconeogenesis. Instead, it replenishes the pool of citric acid cycle intermediates that serve as precursors to the biosynthesis of amino acids and lipids (Section 13.7).

B. Phosphoenolpyruvate Carboxykinase

Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate (Figure 12.3). This is a well-studied enzyme with an induced-fit binding mechanism similar to that described for yeast hexokinase (Section 6.5C) and citrate synthase (Section 13.3A).

There are two different versions of PEPCK. The enzyme found in bacteria, protists, fungi, and plants uses ATP as the phosphoryl group donor in the decarboxylation reaction. The animal version uses GTP. In most species, the enzyme displays no allosteric kinetic properties and has no known physiological modulators. Its activity is most often affected by controls at the level of transcription of its gene. The level of PEPCK activity in cells influences the rate of gluconeogenesis. This is especially true in mammals where gluconeogenesis is mostly confined to cells in the liver, kidneys, and small intestine. During fasting in mammals, prolonged release of glucagon from the pancreas leads to continued elevation of intracellular cAMP, that triggers increased transcription of the PEPCK gene in the liver and increased synthesis of PEPCK. After several hours, the amount of PEPCK rises and the rate of gluconeogenesis increases. Insulin, abundant in the fed state, acts in opposition to glucagon at the level of the gene reducing the rate of synthesis of PEPCK.

The two-step synthesis of phosphoenolpyruvate from pyruvate is common in most eukaryotes, including humans. This is the main reason why it's usually shown when the gluconeogenesis pathway is described (Figure 12.1). However, many species of bacteria can convert pyruvate directly to phosphoenolpyruvate in an ATP-dependent reaction catalyzed by phosphoenolpyruvate synthetase (Figure 12.4). The products of this reaction include AMP and P_i. The second phosphoryl from ATP is transferred to pyruvate. Thus, two ATP equivalents are used in the conversion of pyruvate to phosphoenolpyruvate. This is a much more efficient route than the eukaryotic two-step pathway catalyzed by pyruvate carboxylase and PEPCK. The presence of phosphoenolpyruvate synthetase in bacterial cells is due to the fact that efficient gluconeogenesis is much more important in bacteria than in eukaryotes.

C. Fructose 1,6-*bis*phosphatase

The reactions of gluconeogenesis between phosphoenolpyruvate and fructose 1,6-*bis*phosphate are simply the reverse of the near-equilibrium reactions of glycolysis. The next reaction in the glycolysis pathway—catalyzed by phosphofructokinase-1—is metabolically irreversible. In the biosynthesis direction, this reaction is catalyzed by the third enzyme specific to gluconeogenesis, fructose 1,6-*bis*phosphatase. This enzyme catalyzes the conversion of fructose 1,6-*bis*phosphate to fructose 6-phosphate.

Fructose 1,6-*bis*phosphate $\quad + H_2O \quad$ Fructose 1,6-*bis*phosphatase $\quad$ Fructose 6-phosphate $\quad + P_i$

(12.2)
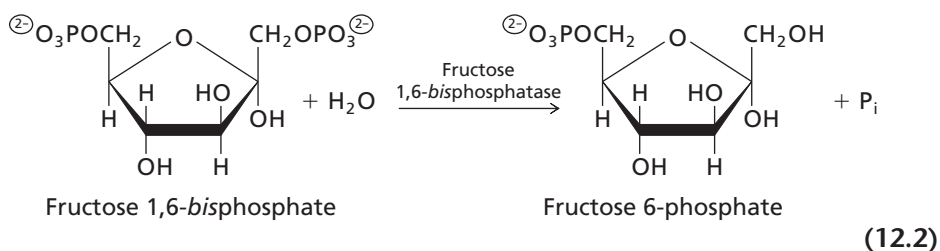
BOX 12.1 SUPERMOUSE

Richard Hanson's group at Case Western Reserve University in Cleveland, Ohio, USA, created a form of supermouse by adding extra copies of the cytoplasmic phosphoenopyruvate carboxykinase gene. The homozygous transgenic mice expressed $10\times$ more PEPCK in their skeletal muscle. They were hyperactive, aggressive, and capable of running for extended periods of time on a mouse treadmill (up to 5 km without stopping!). They ate more than control mice but were significantly smaller.

The rodent athletes converted prodigious amounts of oxaloacetate into phosphoenolpyruvate and subsequently to intermediates in the gluconeogenesis pathway, including glucose. Their muscle cells had many more mitochondria than the cells of normal mice.

The biochemical explanation of this hyperactivity is not completely understood. It's probably due to effects on the citric acid cycle (Chapter 13). This allows increased flux in that pathway leading ultimately to higher levels of ATP. When asked whether this genetic modification would be a good way of creating superior human athletes, Hanson and Hakimi (2008) replied, "The PEKCK-C^{mus} mice are very aggressive; the world needs *less*, not more aggression," besides, the creation of such transgenic humans is ". . . neither ethical nor possible."

Watch the video at: youtube.com/watch?v=4PXC_mctsgY

▲ **Mighty Mouse © CBS Operations.**

As you might expect, hydrolysis of the phosphate ester in this reaction is associated with a large negative standard Gibbs free energy change ($\Delta G^{\circ\prime}$). The actual Gibbs free energy change *in vivo* is also negative because this reaction is metabolically irreversible. The mammalian enzyme displays sigmoidal kinetics and is allosterically inhibited by AMP and by the regulatory molecule fructose 2,6-*bis*phosphate. Thus, the reaction cannot reach equilibrium. Recall that fructose 2,6-*bis*phosphate is a potent activator of phosphofructokinase-1, the enzyme that catalyzes the formation of fructose 1,6-*bis*phosphate in glycolysis (Section 11.5C). The two enzymes that catalyze the interconversion of fructose 6-phosphate and fructose 1,6-*bis*phosphate are reciprocally controlled by the concentration of fructose 2,6-*bis*phosphate (see Section 12.6C).

D. Glucose 6-phosphatase

The final step of gluconeogenesis is the hydrolysis of glucose 6-phosphate to form glucose. The enzyme is glucose 6-phosphatase.

Glucose 6-phosphate + H$_2$O → (Glucose 6-phosphatase) → Glucose + P$_i$ (12.3)

Glucose 6-phosphate   Glucose
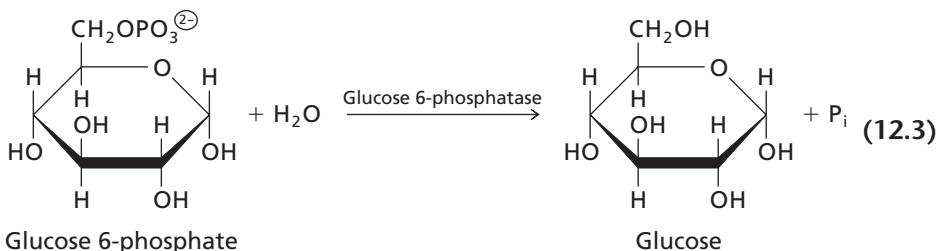
Although we present glucose as the final product of gluconeogenesis, this is not true in all species. In most cases, the biosynthetic pathway ends with glucose 6-phosphate. This product is an activated form of glucose. It becomes the substrate for additional carbohydrate pathways leading to synthesis of glycogen (Section 12.6), starch and sucrose (Section 15.11), pentose sugars (Section 12.5), and other hexoses.

In mammals, glucose is an important end product of gluconeogenesis since it serves as an energy source for glycolysis in many tissues. Glucose is made in the cells of the liver, kidneys, and small intestine and exported to the bloodstream. In these cells, glucose 6-phosphatase is bound to the endoplasmic reticulum with its active site in the lumen. The enzyme is part of a complex that includes a glucose 6-phosphate transporter (G6PT) and a phosphate transporter. G6PT moves glucose 6-phosphate from the

▼ **Figure 12.4**
Phosphoenolpyruvate synthetase reaction.

COO$^{\ominus}$
|
C=O + ATP
|
CH$_3$
    Pyruvate

Phosphoenolpyruvate synthetase

COO$^{\ominus}$
|
C—OPO$_3^{\text{2-}}$ + ATP + AMP
‖   + P$_i$
CH$_2$

Phosphoenolpyruvate
(PEP)
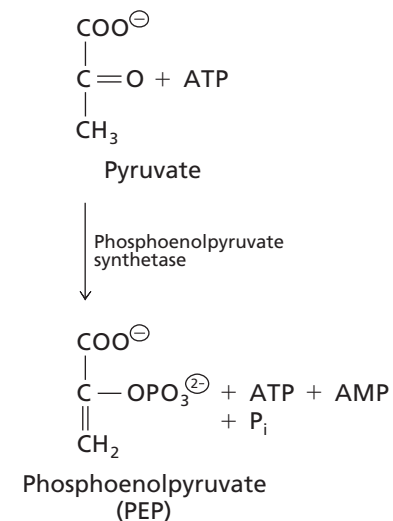
Additional effects of glucagon and insulin are described in Section 12.6C.

> Defects in the activities of glucose 6-phosphatase or glucose 6-phosphate transporter cause von Gierke disease (Section 12.8).

KEY CONCEPT

Mammalian fuel metabolism is an important subset of biochemistry because it helps us to understand our own bodies.

cytosol to the interior of the ER where it is hydrolyzed to glucose and inorganic phosphate. Phosphate is returned to the cytosol and glucose is transported to the cell surface (and the bloodstream) via the secretory pathway.

The other enzymes required for gluconeogenesis are found, at least in small amounts, in many mammalian tissues. Glucose 6-phosphatase is found only in cells from the liver, kidneys, and small intestine, so only these tissues can synthesize free glucose. Cells of tissues that lack glucose 6-phosphatase retain glucose 6-phosphate for internal carbohydrate metabolism.

12.2 Precursors for Gluconeogenesis

The main substrates for glucose 6-phosphate synthesis are pyruvate, citric acid cycle intermediates, three-carbon intermediates in the pathway (e.g. glyceraldehyde 3-phosphate), and two-carbon compounds such as acetyl CoA. Acetyl CoA is converted to oxaloacetate in the glyoxylate cycle, that operates in bacteria, protists, fungi, plants, and some animals (Section 13.8). Some organisms can fix inorganic carbon by incorporating it into two-carbon and three-carbon organic compounds (e.g., Calvin cycle, Section 15.4). These compounds enter the gluconeogenesis pathway resulting in net synthesis of glucose from CO_2.

Mammalian biochemistry is focused on fuel metabolism and biosynthesis of glucose from simple precursors and is it usually discussed in that context. The major gluconeogenic precursors in mammals are lactate and most amino acids, especially alanine. Glycerol, which is produced from the hydrolysis of triacylglycerols, is also a substrate for gluconeogenesis. Glycerol enters the pathway after conversion to dihydroxyacetone phosphate. Precursors arising in nongluconeogenic tissues must first be transported to the liver to be substrates for gluconeogenesis.

A. Lactate

Glycolysis generates large amounts of lactate in active muscle and red blood cells. Lactate from these and other sources enters the bloodstream and travels to the liver where it is converted to pyruvate by the action of lactate dehydrogenase. Pyruvate can then be a substrate for gluconeogenesis. Glucose produced by the liver enters the bloodstream for delivery to peripheral tissues, including muscle and red blood cells. This sequence is known as the **Cori cycle** (Figure 12.5). The conversion of lactate to glucose requires energy, most of which is derived from the oxidation of fatty acids in the liver. Thus, the Cori cycle transfers chemical potential energy in the form of glucose from the liver to the peripheral tissues.

B. Amino Acids

The carbon skeletons of most amino acids are catabolized to pyruvate or intermediates of the citric acid cycle. The end products of these catabolic pathways can serve directly as precursors for synthesis of glucose 6-phosphate in cells that are capable of gluconeogenesis. In peripheral mammalian tissues, pyruvate formed from glycolysis or amino acid catabolism
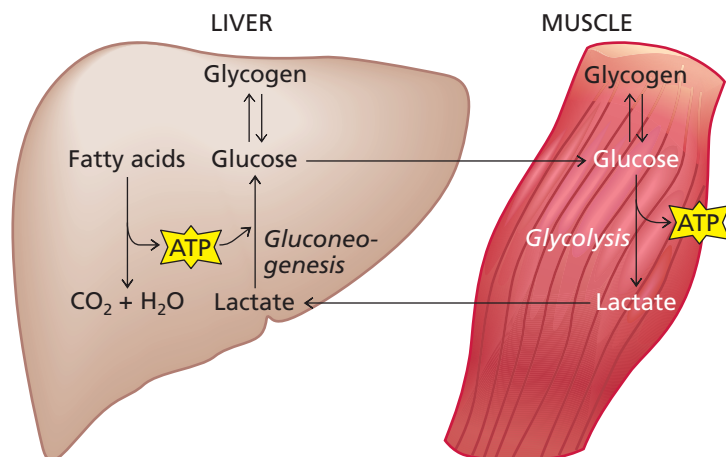
Figure 12.5 ▶
Cori cycle. Glucose is converted to ʟ-lactate in muscle cells. Some of this lactate is secreted and passes via the bloodstream to the liver. Lactate is converted to glucose in the liver and the glucose is secreted into the bloodstream where it is taken up by muscle cells. Both tissues are capable of synthesizing glycogen and mobilizing it.

must be transported to the liver before it can be used in glucose synthesis. The Cori cycle is one way of accomplishing this transfer by converting pyruvate to lactate in muscle and re-converting it to pyruvate in liver cells. The glucose–alanine cycle is a similar transport system (Section 17.9B). Pyruvate can also accept an amino group from an α-amino acid, such as glutamate, forming alanine by the process of transamination (Section 7.2B) (Figure 12.6).

Alanine travels to the liver, where it undergoes transamination with α-ketoglutarate to re-form pyruvate for gluconeogenesis. Amino acids become a major source of carbon for gluconeogenesis during fasting when glycogen supplies are depleted.

The carbon skeleton of aspartate is also a precursor of glucose. Aspartate is the amino group donor in the urea cycle, a pathway that eliminates excess nitrogen from the cell (Section 17.9B). Aspartate is converted to fumarate in the urea cycle and then fumarate is hydrated to malate that is oxidized to oxaloacetate. In addition, the transamination of aspartate with α-ketoglutarate directly generates oxaloacetate.

C. Glycerol

The catabolism of triacylglycerols produces glycerol and acetyl CoA. As mentioned earlier, acetyl CoA contributes to the net formation of glucose through reactions of the glyoxylate cycle (Section 13.8). The glyoxylate cycle does not contribute to net synthesis of glucose from lipids in mammalian cells. Glycerol, however, can be converted to glucose by a route that begins with phosphorylation to glycerol 3-phosphate, catalyzed by glycerol kinase (Figure 12.7). Glycerol 3-phosphate enters gluconeogenesis after conversion to dihydroxyacetone phosphate. This oxidation can be catalyzed by a flavin containing glycerol 3-phosphate dehydrogenase complex embedded in the inner mitochondrial membrane. The outer face of this enzyme binds glycerol 3-phosphate and electrons are passed to ubiquinone (Q) and subsequently to the rest of the membrane-associated electron transport chain. The oxidation of glycerol 3-phosphate can also be catalyzed by the $NAD^{\oplus}$ requiring cytosolic glycerol 3-phosphate dehydrogenase, although this enzyme is usually associated with the reverse reaction for making glycerol. Both enzymes are found in the liver, the site of most gluconeogenesis in mammals.

D. Propionate and Lactate

In ruminants—cattle, sheep, giraffes, deer, and camels—the propionate and lactate produced by the microorganisms in the rumen (chambered stomach) are absorbed and
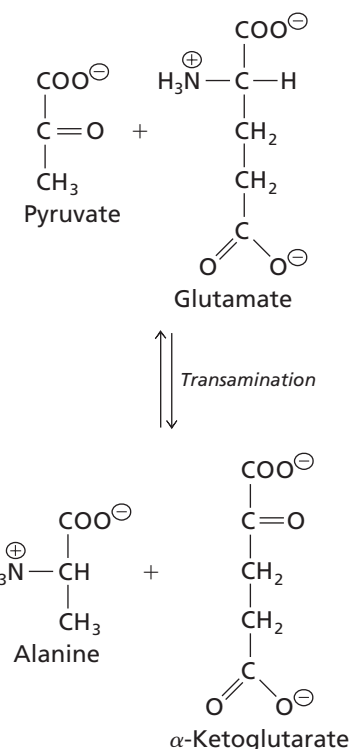
▲ **Figure 12.6**
Conversion of pyruvate to alanine. Pyruvate can be converted to alanine in peripheral tissues. Alanine is secreted into the bloodstream where it is taken up by liver cells and converted back to pyruvate by the same transamination reaction. Pyruvate then serves as a precursor for gluconeogenesis.

▲ **Figure 12.7**
Gluconeogenesis from glycerol. Glycerol 3-phosphate can be oxidized by a glycerol 3-phosphate dehydrogenase complex in the mitochondrial membrane. A cytoplasmic version of this enzyme interconverts dihydroxyacetone phosphate and glycerol 3-phosphate.

▲ **Glycerol 3-phosphate dehydrogenase.** This is the human (*Homo sapiens*) version of the cytosolic enzyme containing DHAP and $NAD^{\oplus}$ at the active site. The structure of the membrane-bound version is not known. [PDB 1WPQ]

▶ **Precursors for gluconeogenesis.** The glyoxylate pathway, the Calvin cycle, and fixation of CO_2 into acetate, do not occur in mammals. Propionate is produced by microorganisms in the rumen of ruminants.
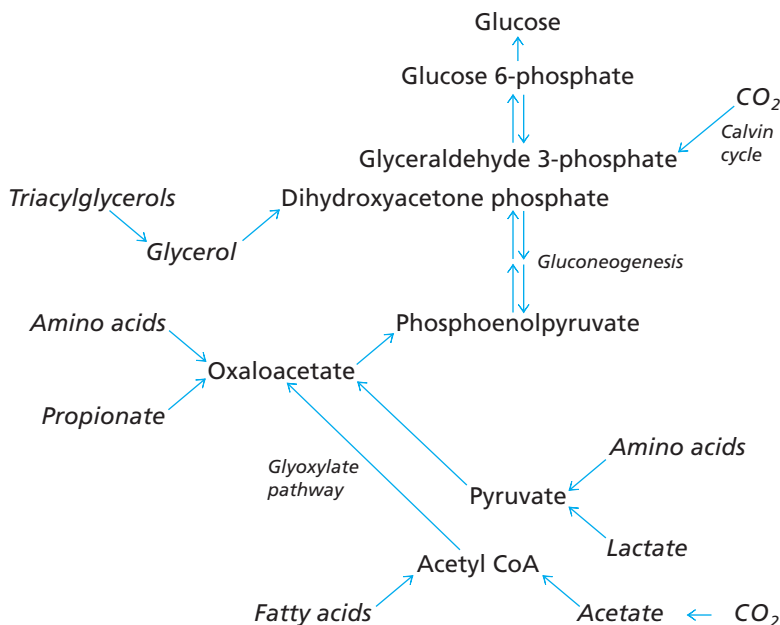
enter the gluconeogenesis pathway. Propionate is converted to propionyl CoA and then to succinyl CoA. These reactions will be covered in the chapter on lipid metabolism (Section 16.3). Succinyl CoA is an intermediate of the citric acid cycle that can be metabolized to oxaloacetate. Lactate from the rumen is oxidized to pyruvate.

E. Acetate

Many species can utilize acetate as their main source of carbon. They can convert acetate to acetyl CoA that serves as the precursor to oxaloacetate. Bacteria and

---

BOX 12.2 GLUCOSE IS SOMETIMES CONVERTED TO SORBITOL

In most animals, glucose—whether from gluconeogenesis, food, or glycogenolysis—is usually oxidized or reincorporated into glycogen. However, in some mammalian tissues (including, testes, pancreas, brain and the lens of the eye), glucose can be converted to fructose as shown in the pathway below. Aldose reductase catalyzes the reduction of glucose to produce sorbitol and polyol dehydrogenase catalyzes the oxidation of sorbitol to fructose. This short pathway supplies essential fructose for some cells. For example, fructose is the main fuel for sperm cells.

Aldose reductase has a high K_m value for glucose so flux through this pathway is normally low and glucose is usually metabolized by glycolysis. When the concentration of glucose is higher than usual (e.g., in individuals with diabetes), increased amounts of sorbitol are produced in tissues such as the lens. There is less polyol dehydrogenase activity than aldose reductase activity so sorbitol can accumulate. Since membranes are relatively impermeable to sorbitol, the resulting change in the osmolarity of the cells causes aggregation and precipitation of lens proteins leading to cataracts—opaque regions in the lens.

▼ **Production of sorbitol from glucose.**
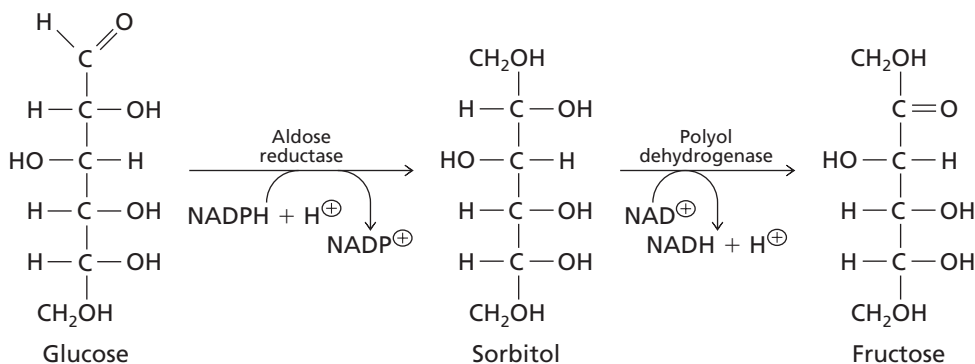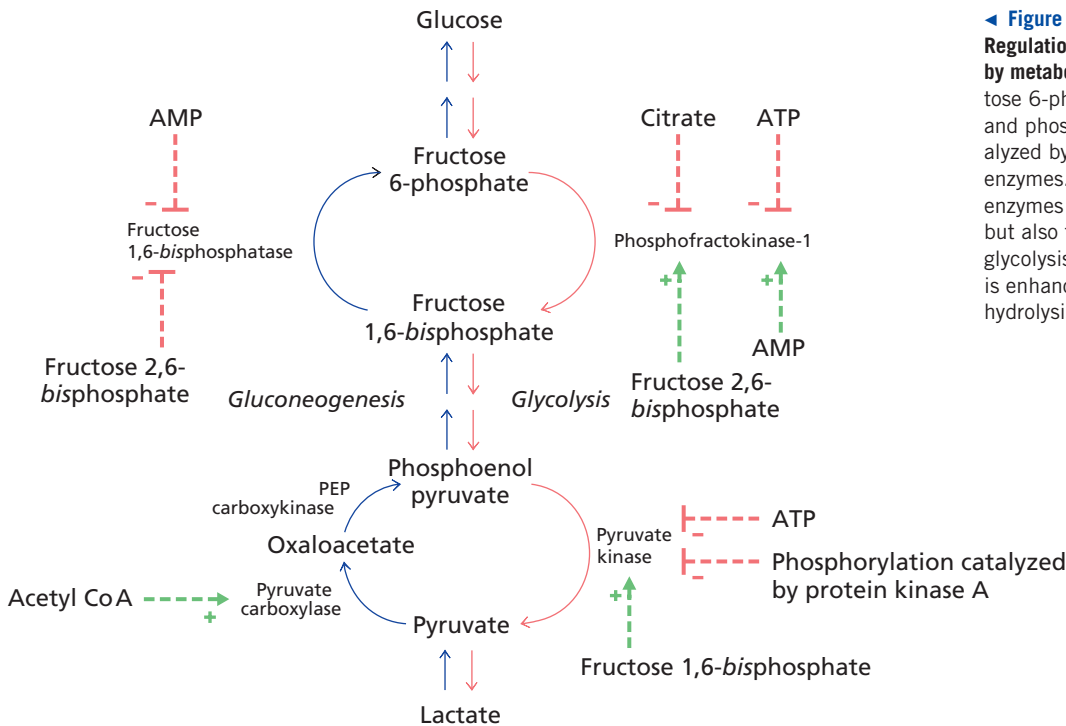
single-celled eukaryotes such as yeast utilize acetate as a precursor for gluconeogenesis. Some species of bacteria can synthesize acetate directly from CO_2. In those species the gluconeogenesis pathway provides a route for the synthesis of glucose from inorganic substrates.

12.3 Regulation of Gluconeogenesis

Gluconeogenesis is carefully regulated *in vivo*. Glycolysis and gluconeogenesis are opposing catabolic and anabolic pathways that share some enzymatic steps but certain reactions are unique to each pathway. For example, phosphofructokinase-1 catalyzes a reaction in glycolysis and fructose 1,6-*bis*phosphatase catalyzes the opposing reaction in gluconeogenesis; both reactions are metabolically irreversible. Usually, only one of the enzymes is active at any given time.

Short-term regulation of gluconeogenesis (regulation that occurs within minutes and does not involve the synthesis of new protein) is exerted at two sites—the reactions involving pyruvate and phosphoenolpyruvate and those that interconvert fructose 1,6-*bis*phosphate and fructose 6-phosphate (Figure 12.8). When there are two enzymes catalyzing the same reaction (in different directions), modulating the activity of either enzyme can alter the flux through the two opposing pathways. For example, inhibiting phosphofructokinase-1 stimulates gluconeogenesis since more fructose 6-phosphate enters the pathway leading to glucose rather than being converted to fructose 1,6-*bis*phosphate. Simultaneous control of fructose 1,6-*bis*phosphatase also regulates the flux of fructose 1,6-*bis*phosphate toward either glycolysis or gluconeogenesis.

We've encountered phosphofructokinase-1 (PFK-1) several times, most notably in the previous chapter (Section 11.5C) and in our discussion of allostery (Section 5.9). Now it's time to examine the effect of the allosteric effector, fructose 2,6-*bis*phosphate, on the activity of PFK-1.

Fructose 2,6-*bis*phosphate is formed from fructose 6-phosphate by the action of the enzyme phosphofructokinase-2 (PFK-2) (Figure 12.9). In mammalian liver, a different
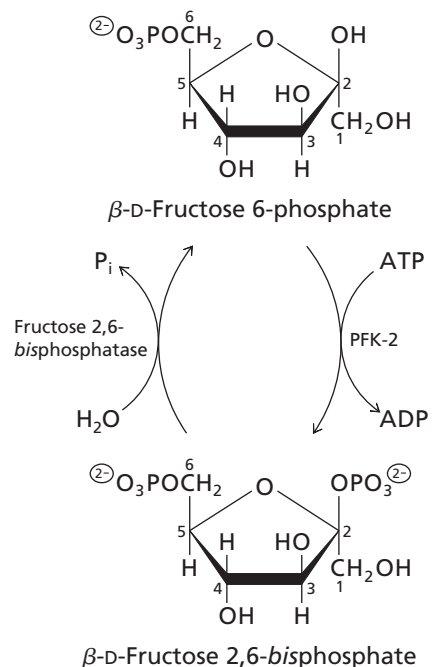
▲ **Figure 12.9**
Interconversion of β-ᴅ-fructose 6-phosphate and β-ᴅ-fructose 2,6-*bis*phosphate.

BOX 12.3 THE EVOLUTION OF A COMPLEX ENZYME

Bacterial versions of phosphofructokinase-1 are homotetramers (Figure 5.19). The functional unit is a head-to-tail dimer with two active sites and two regulatory sites in the interface between the monomers. Phosphoenolpyruvate (PEP) inhibits the enzyme.

In eukaryotes, a tandem gene duplication occurred in the fungi/animal lineage. This was followed by a fusion of the two genes leading to a monomer that was twice the size of the bacterial version. This larger monomer resembled the bacterial dimer with two active sites and two regulatory sites. Over a period of millions of years these sites became modified. One of the active sites continued to bind fructose 6-phosphate and ATP catalyzing the formation of fructose 1,6-*bis*phosphate. In the reverse reaction it binds fructose 1,6-*bis*phosphate. The other active site evolved to bind fructose 2,6-*bis*phosphate, which became an allosteric activator.

The two original regulatory sites also evolved to accommodate new ligands. Citrate became the new inhibitor at one of the sites and the other site became the allosteric site for regulation by ATP (inhibitor) or AMP (activator).

▼ **Evolution of the fungal and animal versions of phosphofructokinase-1.**

active site on the same protein catalyzes the hydrolytic dephosphorylation of fructose 2,6-*bis*phosphate, re-forming fructose 6-phosphate. This activity of the enzyme is called fructose 2,6-*bis*phosphatase. The dual activities of this bifunctional enzyme control the steady state concentration of fructose 2,6-*bis*phosphate and, ultimately, the switch between glycolysis and gluconeogenesis.

As shown in Figure 12.8, the allosteric effector fructose 2,6-*bis*phosphate activates PFK-1 and inhibits fructose 1,6-*bis*phosphatase. Note that an increase in fructose 2,6-*bis*phosphate has reciprocal effects: it stimulates glycolysis and inhibits gluconeogenesis. Similarly, AMP affects the two enzymes in a reciprocal manner; inhibiting fructose 1,6-*bis*phosphatase and activating phosphofructokinase-1. The regulation of the bifunctional enzyme PFK-2/fructose 2,6-*bis*phosphatase will be described after we cover glycogen metabolism.

12.4 The Pentose Phosphate Pathway

The pentose phosphate pathway is a pathway for the synthesis of three pentose phosphates: ribulose 5-phosphate, ribose 5-phosphate, and xylulose 5-phosphate. Ribose 5-phosphate is required for the synthesis of RNA and DNA. The complete pathway has two stages: an oxidative stage and a nonoxidative stage (Figure 12.10). In the oxidative stage, NADPH is produced when glucose 6-phosphate is converted to the five-carbon compound ribulose 5-phosphate.
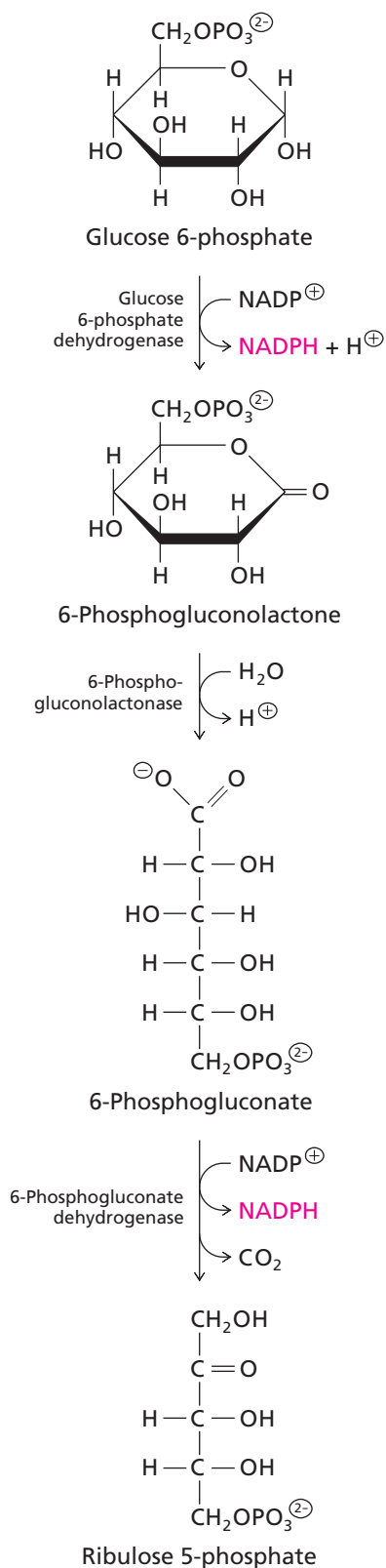

▲ **T conformation (inactive) of fructose 1,6-*bis*phosphatase.** This is the tetrameric enzyme from human (Homo sapiens) bound to the allosteric inhibitor AMP (space-filling) at the regulatory sites between the two dimers. The competitive inhibitor fructose 2,6-*bis*phosphate (ball-and-stick) is bound at the active sites of each monomer. [PDB 1EYJ]

Glucose 6-phosphate $+$ 2 NADP$^{\oplus}$ $+$ H$_2$O $\longrightarrow$

Ribulose 5-phosphate $+$ 2 NADPH $+$ CO$_2$ $+$ 2 H$^{\oplus}$ **(12.4)**

(a)

Glucose 6-phosphate

Glucose 6-phosphate dehydrogenase
— NADP$^{\oplus}$
↘ **NADPH** + H$^{\oplus}$

6-Phosphogluconolactone

6-Phosphogluconolactonase
— H$_2$O
↘ H$^{\oplus}$

6-Phosphogluconate

6-Phosphogluconate dehydrogenase
— NADP$^{\oplus}$
↘ **NADPH**
↘ CO$_2$

Ribulose 5-phosphate

Oxidative stage

Ribulose 5-phosphate 3-epimerase

Ribose 5-phosphate isomerase

Xylulose 5-phosphate

Ribose 5-phosphate

Transketolase

Sedoheptulose 7-phosphate

Glyceraldehyde 3-phosphate

Transaldolase

Erythrose 4-phosphate

Non-oxidative stage

Transketolase

Glyceraldehyde 3-phosphate

Fructose 6-phosphate

Fructose 6-phosphate

(b)

6C (3)

↘ 1C (3)

5C (3)

5C (2)   5C (1)

7C (1)   3C (1)

4C (1)   6C (1)

3C (1) 6C (1)

▲ **Figure 12.10**
Pentose phosphate pathway. (a) The oxidative stage of the pathway produces a five-carbon sugar phosphate, ribulose 5-phosphate, with concomitant production of NADPH. The nonoxidative stage produces the glycolytic intermediates glyceraldehyde 3-phosphate and fructose 6-phosphate. **(b)** The path of carbon in the pentose phosphate pathway. In the oxidative stage, three molecules of a six-carbon compound are converted to three molecules of a five-carbon sugar (ribulose 5-phosphate) with release of three molecules of CO$_2$. In the nonoxidative stage, three molecules of five-carbon sugars are interconverted to produce two molecules of a six-carbon sugar (fructose 6-phosphate) and one molecule of a three-carbon compound (glyceraldehyde 3-phosphate).

If a cell requires both NADPH and nucleotides then all the ribulose 5-phosphate is isomerized to ribose 5-phosphate and the pathway is completed at this stage. In some cases, more NADPH than ribose 5-phosphate is needed and most of the pentose phosphates are converted to intermediates in the gluconeogenesis pathway.

The nonoxidative stage of the pentose phosphate pathway disposes of the pentose phosphate formed in the oxidative stage by providing a route to gluconeogenesis

$CH_2OPO_3^{\text{(2-)}}$

Glucose 6-phosphate

Glucose
6-phosphate
dehydrogenase

NADP$^{\oplus}$

NADPH + H$^{\oplus}$

$CH_2OPO_3^{\text{(2-)}}$

6-Phosphogluconolactone

6-Phospho-
gluconolactonase

H$_2$O

H$^{\oplus}$

6-Phosphogluconate

6-Phosphogluconate
dehydrogenase

NADP$^{\oplus}$

NADPH

CO$_2$

CH_2OH
$C=O$
$H-C-OH$
$H-C-OH$
$CH_2OPO_3^{\text{(2-)}}$

Ribulose 5-phosphate

▲ **Figure 12.11**
Oxidative stage of the pentose phosphate pathway. Two molecules of NADP$^{\oplus}$ are reduced to two molecules of NADPH for each molecule of glucose 6-phosphate that enters the pathway.

or glycolysis. In this stage, ribulose 5-phosphate is converted to the intermediates fructose 6-phosphate and glyceraldehyde 3-phosphate. If all the pentose phosphate were converted to these intermediates, the sum of the nonoxidative reactions would be the conversion of three pentose molecules to two hexose molecules plus one triose molecule.

$$3 \text{ Ribulose 5-phosphate} \longrightarrow$$
$$2 \text{ Fructose 6-phosphate } + \text{ Glyceraldehyde 3-phosphate} \quad \textbf{(12.5)}$$

Both fructose 6-phosphate and glyceraldehyde 3-phosphate can be metabolized by glycolysis or gluconeogenesis.

Let's take a closer look at the individual reactions of the pentose phosphate pathway.

A. Oxidative Stage

The three reactions of the oxidative stage of the pentose phosphate pathway are shown in Figure 12.11. The first two steps are the same as those in the bacterial Entner–Doudoroff pathway (Section 11.7). The first reaction, catalyzed by glucose 6-phosphate dehydrogenase (G6PDH), is the oxidation of glucose 6-phosphate to 6-phosphogluconolactone. This step is the major regulatory site for the entire pentose phosphate pathway. Glucose 6-phosphate dehydrogenase is allosterically inhibited by NADPH (feedback inhibition). This simple regulatory feature ensures that the production of NADPH by the pentose phosphate pathway is self-limiting.

The next enzyme of the oxidative phase is 6-phosphogluconolactonase that catalyzes the hydrolysis of 6-phosphogluconolactone to the sugar acid 6-phosphogluconate. Finally, 6-phosphogluconate dehydrogenase catalyzes the oxidative decarboxylation of 6-phosphogluconate. This reaction produces a second molecule of NADPH, ribulose 5-phosphate, and CO$_2$. In the oxidative stage, therefore, a six-carbon sugar is oxidized to a five-carbon sugar plus CO$_2$ and two molecules of NADP$^{\oplus}$ are reduced to two molecules of NADPH.

B. Nonoxidative Stage

The nonoxidative stage of the pentose phosphate pathway consists entirely of near equilibrium reactions. This stage of the pathway provides five-carbon sugars for biosynthesis and introduces sugar phosphates into glycolysis or gluconeogenesis. Ribulose 5-phosphate has two fates: an epimerase can catalyze the formation of xylulose 5-phosphate, or an isomerase can catalyze the formation of ribose 5-phosphate (Figure 12.12). (Note the difference between an epimerase and an isomerase.) Ribose 5-phosphate is the precursor of the ribose (or deoxyribose) portion of nucleotides. The remaining steps of the pathway convert the five-carbon sugars into glycolytic intermediates. Rapidly dividing cells that require both ribose 5-phosphate (as a precursor of ribonucleotide and deoxyribonucleotide residues) and NADPH (for the reduction of ribonucleotides to deoxyribonucleotides) generally have high pentose phosphate pathway activity.

The overall pentose phosphate pathway (Figure 12.10) shows that in the nonoxidative stage two molecules of xylulose 5-phosphate and one molecule of ribose 5-phosphate are interconverted to generate one three-carbon molecule (glyceraldehyde 3-phosphate) and two six-carbon molecules (fructose 6-phosphate). Thus, the carbon-containing products from the passage of three molecules of glucose through the pentose phosphate pathway are glyceraldehyde 3-phosphate, fructose 6-phosphate, and CO$_2$. The balanced equation for this process is

$$3 \text{ Glucose 6-phosphate} + 6 \text{ NADP}^{\oplus} + 3 \text{ H}_2\text{O} \longrightarrow 2 \text{ Fructose 6-phosphate} +$$
$$\text{Glyceraldehyde 3-phosphate} + 6 \text{ NADPH} + 3 \text{ CO}_2 + 6 \text{ H}^{\oplus} \quad \textbf{(12.6)}$$
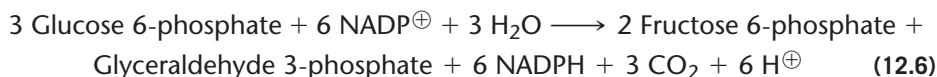
BOX 12.4 GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN HUMANS

The genetics of human glucose 6-phosphate dehydrogenase has been the subject of much research. There are two different enzymes that can catalyze the reaction shown in Figure 12.11. One of the genes (G6PDH) is found on the X chromosome (Xq28) and it is expressed almost exclusively in red blood cells. The other gene (H6PDH) encodes an enzyme that is less specific; it can use other hexose substrates. Hexose 6-phosphate dehydrogenase is synthesized in many cells where it serves as the first enzyme in the oxidative stage of the pentose phosphate pathway.

The glucose 6-phosphate dehydrogenase reaction is the only reaction capable of reducing $NADP^{\oplus}$ in red blood cells; consequently, deficiencies of this enzyme have drastic effects on the metabolism of these cells. Other cells are not affected since they contain H6PDH. G6PDH deficiency in humans causes hemolytic anemia.

There are hundreds of different alleles of the X chromosome G6PDH gene. The variants produce lower amounts of the enzyme or they alter its catalytic efficiency. There are no known null mutants in the human population because the complete absence of G6PDH activity is lethal. Note that males are more likely to be affected since they have only a single copy of the gene on their one X chromosome.

It is estimated that 400 million people have some form of G6PDH deficiency and suffer from mild forms of hemolytic anemia. The symptoms can be life threatening if the patient is treated with certain drugs that are normally prescribed for other diseases. Many of these individuals have an increased resistance to malaria because the malarial parasite does not survive well in red blood cells that produce lowered amounts of NADPH. This explains why there are so many deficiency alleles

segregating in the human population in spite of the fact that the pentose phosphate pathway is inefficient. It's an example of balanced selection like the familiar sickle cell anemia example.

Human genome database entries for these genes can be viewed on the Entrez Gene website [ncbi.nlm.nih.gov/gene]. Type in the entries for the G6PDH gene (2531) or the H6PDH gene (9563). The Online Mendelian Inheritance in Man (OMIM) webpage is at ncbi.nlm.nih.gov/omim. The entry for G6PDH is MIM=305900 and the entry for H6PDH is MIM=138090.
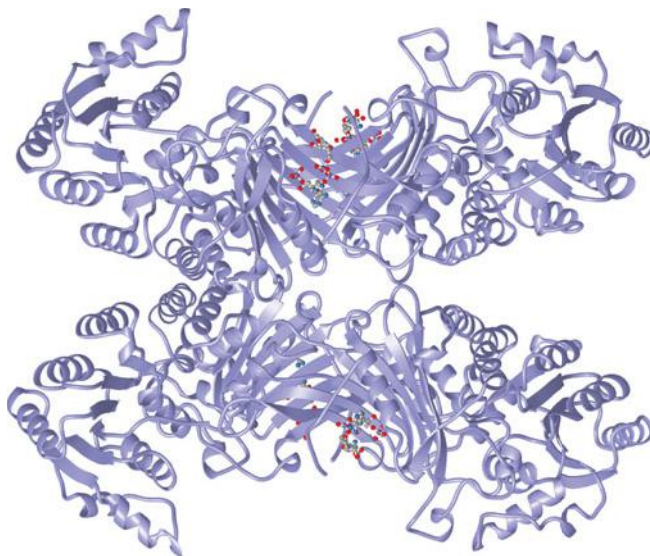
▲ **Human glucose 6-phosphate dehydrogenase, variant Canton R459L.** The enzyme is a dimer of dimers (tetramer). Two molecules of $NADP^{\oplus}$ are bound at the active sites in each dimer. [PDB 1QK1]
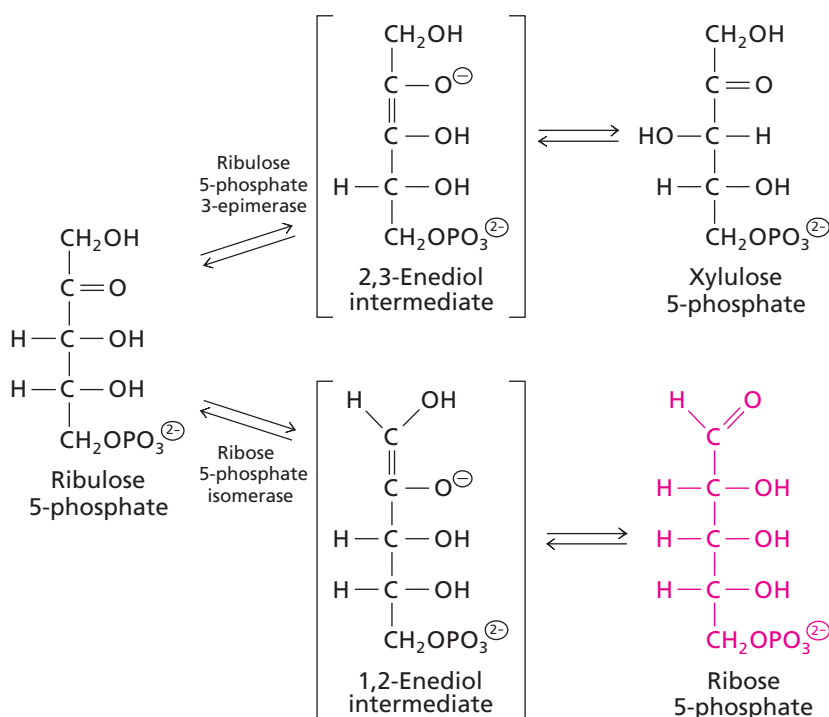
> The reactions of the nonoxidative stage of the pentose phosphate pathway are similar to those of the regeneration stage of the reductive pentose phosphate cycle of photosynthesis (Section 15.8).

◀ **Figure 12.12**
Conversion of ribulose 5-phosphate to xylulose 5-phosphate or ribose 5-phosphate. In either case, the removal of a proton forms an enediol intermediate. Reprotonation forms either the ketose xylulose 5-phosphate or the aldose ribose 5-phosphate.
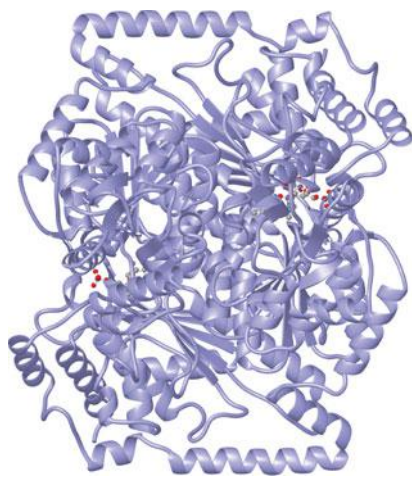
▲ **Transketolase from *Escherichia coli*.**
The active site of each monomer contains
one molecule of xylulose 5-phosphate
(space-filling) and the TDP cofactor. [PDN
2R8O]

Figure 12.13 ▼
Reaction catalyzed by transketolase. The
reversible transfer of a glycoaldehyde group
(shown in red) from xylulose 5-phosphate
to ribose 5-phosphate generates glyceralde-
hyde 3-phosphate and sedoheptulose 7-
phosphate. Note that the ketose–phosphate
substrate (in either direction) is shortened
by two carbon atoms, whereas the
aldose–phosphate substrate is lengthened
by two carbon atoms. In this example,
5C + 5C ⇌ 3C + 7C.

In most cells, the glyceraldehyde 3-phosphate and fructose 6-phosphate produced
by the pentose phosphate pathway are used to resynthesize glucose 6-phosphate. This
glucose 6-phosphate molecule can reenter the pentose phosphate pathway. In that case,
the equivalent of one molecule of glucose is completely oxidized to CO_2 by six passages
through the pathway. After six molecules of glucose 6-phosphate are oxidized, the six
ribulose 5-phosphates produced can be rearranged by the reactions of the pentose phos-
phate pathway and part of the gluconeogenic pathway to form five glucose 6-phosphate
molecules. (Recall that two glyceraldehyde 3-phosphate molecules are equivalent to one
fructose 1,6-*bis*phosphate molecule.) If we disregard H_2O and $H^{\oplus}$, the overall stoi-
chiometry for this process is

$$6 \text{ Glucose 6-phosphate} + 12 \text{ NADP}^{\oplus} \longrightarrow$$

$$5 \text{ Glucose 6-phosphate} + 12 \text{ NADPH} + 6 \text{ } CO_2 + P_i \qquad \textbf{(12.7)}$$
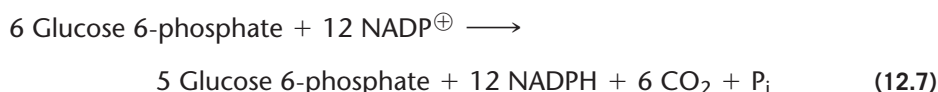
This net reaction emphasizes that most of the glucose 6-phosphate entering the pentose
phosphate pathway could be recycled; one-sixth is converted to CO_2 and P_i. Indeed, an
alternate name for the pathway is the *pentose phosphate cycle*.

C. Interconversions Catalyzed by Transketolase and Transaldolase

The interconversions of the nonoxidative stage of the pentose phosphate pathway are
catalyzed by two enymes called transketolase and transaldolase. These enzymes have
broad substrate specificities.

Transketolase is also called glycoaldehydetransferase. It is a thiamine diphosphate
(TDP)-dependent enzyme that catalyzes the transfer of a two-carbon glycoaldehyde
group from a ketose phosphate to an aldose phosphate. The ketose phosphate is
shortened by two carbons and the aldose phosphate is lengthened by two carbons
(Figure 12.13).

Transaldolase is also called dihydroxyacetonetransferase. It catalyzes the transfer of
a three-carbon dihydroxyacetone group from a ketose phosphate to an aldose phosphate.
The transaldolase reaction of the pentose phosphate pathway converts sedoheptulose
7-phosphate and glyceraldehyde 3-phosphate to erythrose 4-phosphate and fructose
6-phosphate (Figure 12.14).

| Xylulose 5-phosphate | Ribose 5-phosphate | Glyceraldehyde 3-phosphate | Sedoheptulose 7-phosphate |

12.5 Glycogen Metabolism

Glucose is stored as the intracellular polysaccharides starch and glycogen. In Chapter 15
we discuss starch metabolism, which occurs mostly in plants. Glycogen is an important
storage polysaccharide in bacteria, protists, fungi and animals. Large glycogen particles
can be easily seen in the cytoplasm of these organisms. Most of the glycogen in verte-
brates is found in muscle and liver cells. Muscle glycogen appears in electron micrographs
as cytosolic granules with a diameter of 10 to 40 nm, about the size of ribosomes.
Glycogen particles in liver cells are about three times larger. The glycogen particles in
bacteria are smaller.

▲ **Figure 12.14**
Reaction catalyzed by transaldolase. The reversible transfer of a three-carbon dihydroxyacetone group (shown in red) from sedoheptulose 7-phosphate, to C-1 of glyceraldehyde 3-phosphate generates a new ketose phosphate, fructose 6-phosphate, and releases a new aldose phosphate, erythrose 4-phosphate. Note that the carbon atoms balance: $7C + 3C \rightleftharpoons 6C + 4C$.

A. Glycogen Synthesis

De novo glycogen synthesis requires a preexisting primer of four to eight α-$(1 \rightarrow 4)$-linked glucose residues. This primer is attached to a specific tyrosine residue of a protein called glycogenin (Figure 12.15) via the 1-hydroxyl group of the reducing end of the short polysaccharide. The primer is formed in two steps. The first glucose residue is attached to glycogenin by the action of a glucosyltransferase activity that requires UDP-glucose. Glycogenin itself catalyzes this reaction as well as the extension of the primer by up to seven more glucose residues. Thus, glycogenin is both a protein scaffold for glycogen and an enzyme. Each glycogen molecule (which can contain thousands of glucose residues) contains a single glycogenin protein at its center.

Further glycogen addition reactions begin with glucose 6-phosphate that can be converted to glucose 1-phosphate. We saw in Section 11.5 that glucose 6-phosphate can enter a number of pathways, including glycolysis and the pentose phosphate pathway. Glycogen synthesis and degradation is mostly a way of storing glucose 6-phosphate until it is needed by the cell. The synthesis and degradation of glycogen require separate enzymatic steps. We have already noted that it is a general rule of metabolism that biosynthesis pathways and degradation pathways follow different routes.

Three separate enzyme-catalyzed reactions are required to incorporate a molecule of glucose 6-phosphate into glycogen (Figure 12.16). First, phosphoglucomutase catalyzes the conversion of glucose 6-phosphate to glucose 1-phosphate. Glucose 1-phosphate is then activated by reaction with UTP, forming UDP-glucose and pyrophosphate (PP$_i$). In the third step, glycogen synthase catalyzes the addition of glucose residues from UDP-glucose to the nonreducing end of glycogen.

Phosphoglucomutase is a ubiquitous enzyme. It catalyzes a near-equilibrium reaction that converts α-D-glucose 6-phosphate to α-D-glucose 1-phosphate Glucose 1-phosphate is the famous "Cori ester" discovered by Gerty Cori and Carl Cori in the 1930s when the reactions of glycogen metabolism were first being elucidated.
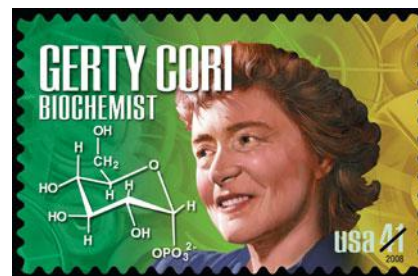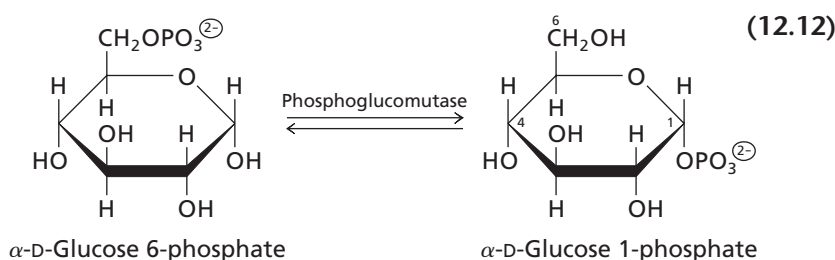
▲ **Gerty Cori, (1896–1957) biochemist.** Carl Cori and Gerty Cori won the Nobel Prize in 1947 "for their discovery of the course of the catalytic conversion of glycogen." This stamp depicts the "Cori ester" but it's slightly different than the structure we usually see in textbooks. Can you spot the difference?
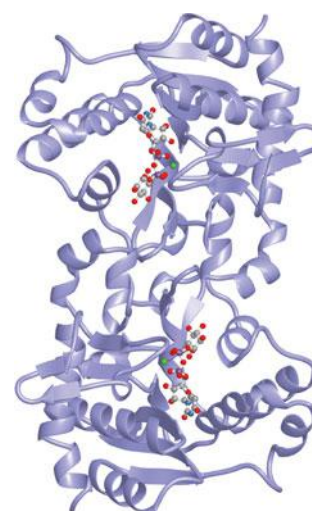
▲ **Figure 12.15**
Glycogenin from rabbit (*Oryctolagus cuniculus*). The molecule is a homodimer and each of the active sites contains a bound molecule of UDP-glucose. [PDB 1LL2]

α-D-Glucose 6-phosphate $\xrightarrow{\text{Phosphoglucomutase}}$ α-D-Glucose 1-phosphate   **(12.12)**
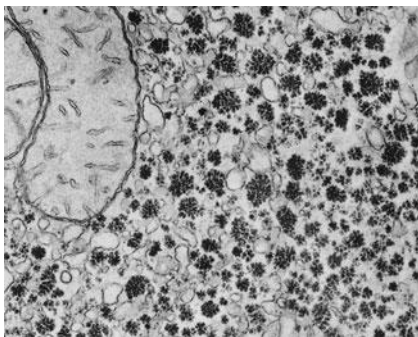
▲ **Large glycogen particles in a section of a liver cell.** (Electron micrograph.)

▲ **Stained glycogen granules in bacteria** (*Candidatus* spp.)
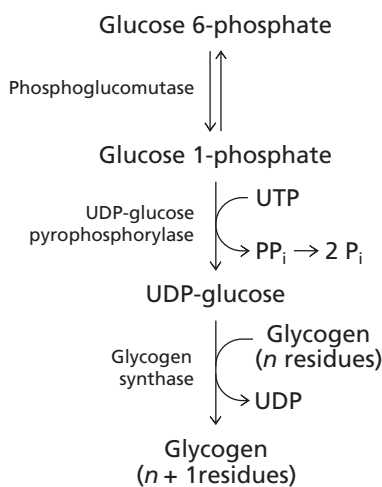
▲ **Figure 12.16**
Synthesis of glycogen in eukaryotes.

The mechanism of this reaction is similar to that of cofactor-dependent phosphoglycerate mutase (Section 11.2 8). Glucose 6-phosphate binds to the phosphoenzyme, and glucose 1,6-*bis*phosphate is formed as an enzyme-bound intermediate. Transfer of the C-6 phosphate to the enzyme leaves glucose 1-phosphate.

Glucose 1-phosphate is activated by formation of UDP-glucose in the second step of glycogen synthesis. In this reaction a UMP group from UTP is transferred to the phosphate at C-1 with release of pyrophosphate (see Figure 7.6). The enzyme that catalyzes this reaction is called UDP glucose pyrophosphorylase and it is present in most eukaryotic species. Note that the activation of glucose requires UTP. The energy is stored in UDP-glucose where it can be used in many biosynthesis reactions. We saw in Section 11.6 that UDP-glucose can be a substrate for synthesis of UDP-galactose. (UDP-galactose is used in the synthesis of gangliosides.) The standard Gibbs free energy change in the UDP glucose pyrophospholylase reaction is close to zero. Under the steady state, near-equilibrium conditions found *in vivo*, $\Delta G = 0$ and the concentrations of glucose 1-phosphate and UDP-glucose are nearly equal. Flux in the direction of UDP-glucose synthesis is driven by subsequent hydrolysis of pyrophosphate (Section 10.6). Two ATP equivalents (UTP and PP_i) are used in the activation of glucose.

Glycogen synthesis is a polymerization reaction where glucose units are added one at a time to a growing polysaccharide chain. This reaction is catalyzed by glucogen synthase (Figure 12.17). Many polymerization reactions are **processive**—the enzyme remains bound to the end of the growing chain and addition reactions are very rapid (see Section 20.2B). The glycogen synthase reaction is **distributive**—the enzyme releases the growing glycogen chain after each reaction.

Glycogen synthases that use UDP-glucose as their substrate are present in protists, animals, and fungi. Some bacteria synthesize glycogen using ADP-glucose. Starch synthesis in plants also requires ADP-glucose. The glycogen synthase reaction is the major regulatory step of glycogen synthesis. In animals, there are hormones that control the rate of glycogen synthesis by altering the activity of glycogen synthase. We will describe regulation in the next section.

Another enzyme, amylo-$(1,4 \rightarrow 1,6)$-transglycosylase, catalyzes branch formation in glycogen. This enzyme, also known as the branching enzyme, removes an oligosaccharide of at least six residues from the nonreducing end of an elongated chain and attaches it by an α-$(1 \rightarrow 6)$ linkage to a position at least four glucose residues from the nearest α-$(1 \rightarrow 6)$ branch point. These branches provide many sites for adding or removing glucose residues, thereby contributing to the speed with which glycogen can be synthesized or degraded.

The complete glycogen molecule has many layers of polysaccharide chains extending out from the glycogenin core (Figure 12.18). The large granules in liver cells, for example, have glycogen molecules with up to 120,000 glucose residues. There are usually two branches per chain and each chain is 8–14 residues in length. The molecule has about 12 layers of chains. If there were on average two branches per chain then each polysacharide unit would have thousands of free ends.

B. Glycogen Degradation

The glucose residues of starch and glycogen are released from storage polymers through the action of enzymes called polysaccharide phosphorylases: starch phosphorylase (in plants) and glycogen phosphorylase (in other organisms). These enzymes catalyze the removal of glucose residues from the nonreducing ends of starch or glycogen, provided the monomers are attached by α-$(1 \rightarrow 4)$ linkages. As the name implies, the enzymes catalyze phosphorolysis—cleavage of a bond by group transfer to an oxygen atom of phosphate. In contrast to hydrolysis (group transfer to water), phosphorolysis produces phosphate esters. Thus, the first product of polysaccharide breakdown is α-D-glucose 1-phosphate (the Cori ester), not free glucose.

$$\text{Polysaccharide} \atop (n \text{ residues}) + P_i \xrightarrow{\text{Polysaccharide phosphorylase}} \text{Polysaccharide} \atop (n\text{-1 residues}) + \text{Glucose 1-phosphate}$$

(12.9)
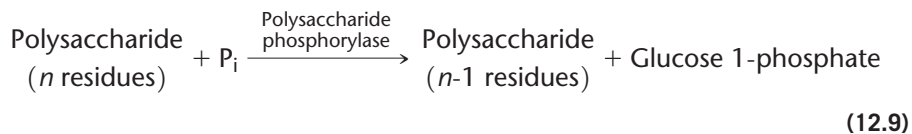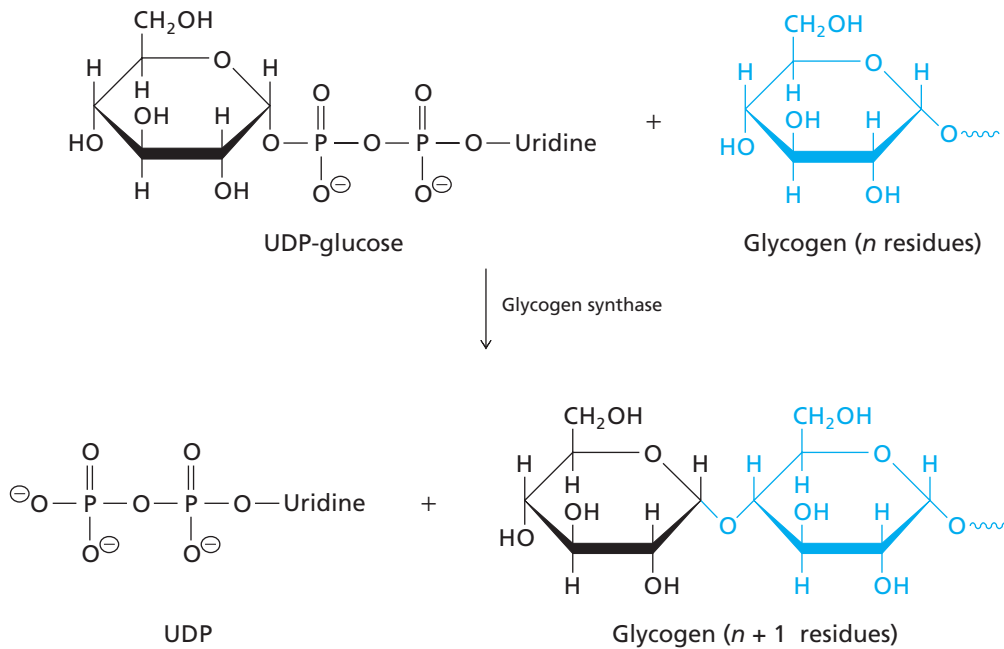
▲ **Figure 12.17**
The glycogen synthase reaction.

The phosphorolysis reaction catalyzed by glycogen phosphorylase is shown in Figure 12.19. Pyridoxal phosphate (PLP) is a prosthetic group in the active site of the enzyme. The phosphate group of PLP appears to relay a proton to the substrate phosphate to help cleave the scissile C—O bond of glycogen. Note that glycogen phosphorylase catalyzes a remarkable reaction since it only uses glycogen and inorganic phosphates as substrates in a reaction that produces a relatively "high energy" compound, glucose 1-phosphate (Table 10.1).

Glycogen phosphorylase is a dimer of identical subunits. The catalytic sites lie in the middle of each subunit. It binds phosphate and the end of a glycogen chain (Figure 12.20). The large glycogen particle binds to a nearby site and the chain being degraded passes along a groove on the surface of the enzyme. Four or five glucose residues can be cleaved sequentially before the enzyme has to release a glycogen particle and re-bind. Thus, in contrast to glycogen synthase, glycogen phosphorylase is partially processive.

The enzyme stops four glucose residues from a branch point (an α-(1 → 6) glucosidic bond) leaving a limit dextrin. The limit dextrin can be further degraded by the action of the bifunctional glycogen debranching enzyme (Figure 12.21). A glucanotransferase activity of the debranching enzyme catalyzes the relocation of a chain of three glucose residues from a branch to a free 4-hydroxyl end of the glycogen molecule. Both the original linkage and the new one are α-(1 → 4). The other activity of glycogen debranching enzyme, amylo-1,6-glucosidase, catalyzes hydrolytic (not phosphorolytic) removal of the remaining α-(1 → 6)-linked glucose residue. The products are one free glucose molecule and an elongated chain that is again a substrate for glycogen phosphorylase. When a glucose molecule released from glycogen by the action of the debranching enzyme enters glycolysis, two ATP molecules are produced (Section 11.1). In contrast, each glucose molecule mobilized by the action of glycogen phosphorylase (representing about 90% of the residues in glycogen) yields three ATP molecules. The energy yield from glycogen is higher than from free glucose because glycogen phosphorylase catalyzes phosphorolysis rather than hydrolysis—no ATP is consumed as in the hexokinase-catalyzed phosphorylation of free glucose.

The product of glycogen degradation, glucose 1-phosphate, is rapidly converted to glucose 6-phosphate by phosphoglucomutase.
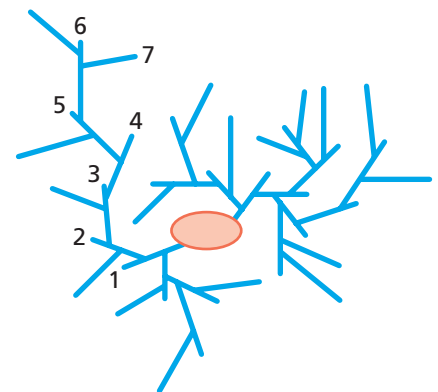
▲ **Figure 12.18**
A glycogen molecule. Two polysaccharides (blue) are attached to each core glycogenin molecule. Each chain core has 8–14 residues and two branches. Not all branches are shown. Seven layers are numbered but typical glycogen molecules have 8–12 layers, depending on the species.

There's no magical net gain of energy by storing glucose as glycogen since the cost of incorporating glucose 6-phosphate into glycogen is two ATP equivalents (Figure 12.16).
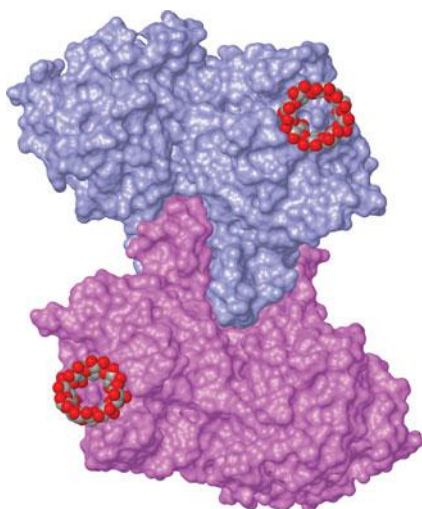
▲ **Inhibiting glycogen phosphorylase.**
The action of glycogen phosphorylase produces glucose in the liver. Insulin controls this activity by inactivating glycogen phosphorylase but in the absence of insulin (e.g., Type II diabetes), excess production of glucose can be dangerous. Many inhibitors of glycogen phosphorylase have been developed as possible treatments for diabetes. One of them is a cyclic maltose molecule shown here bound to the active sites of the rabbit (*Oryctolagus cuniculis*) enzyme. [PDB1P2G]

▲ **Figure 12.20**
Binding and catalytic sites on glycogen phosphorylase.
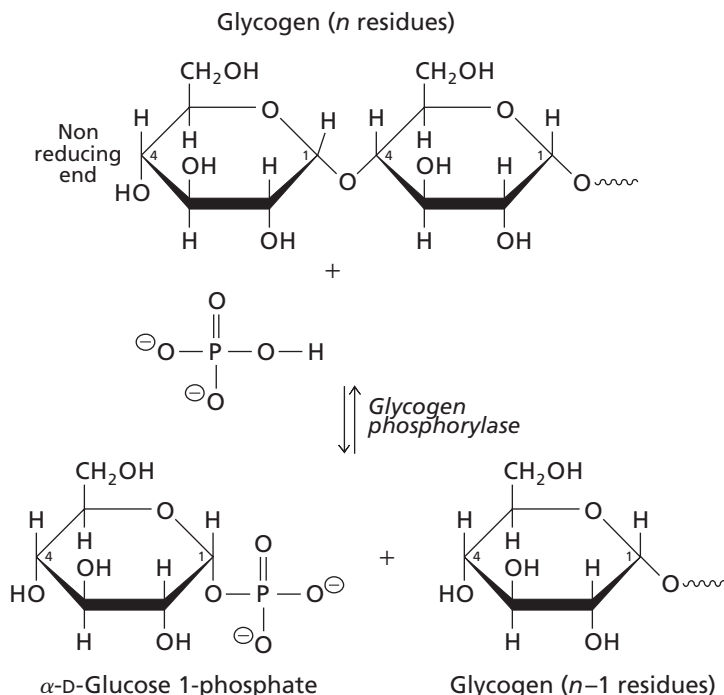
▲ **Figure 12.19**
Cleavage of a glucose residue from the nonreducing end of a glycogen chain, catalyzed by glycogen phosphorylase.

12.6 Regulation of Glycogen Metabolism in Mammals

Mammalian glycogen stores glucose in times of plenty (after feeding, a time of high glucose levels) and supplies glucose in times of need (during fasting or in "fight or flight" situations). In muscle, glycogen provides fuel for muscle contraction. In contrast, liver glycogen is largely converted to glucose that exits liver cells and enters the bloodstream for transport to other tissues that require it. Both the mobilization and synthesis of glycogen are regulated by hormones.

A. Regulation of Glycogen Phosphorylase

Glycogen phosphoryase is responsible for the breakdown of glycogen to produce glucose 1-phosphate. In muscle cells, glucose 1-phosphate is converted to glucose 6-phosphate that is used in glycolysis to produce ATP. In liver cells, glucose 6-phosphate is hydrolyzed to free glucose that is secreted into the bloodstream where it can be taken up by other tissues.

The activity of glycogen phosphorylase is regulated by several allosteric effectors and by covalent modification (phosphorylation). Let's take a few minutes to study the regulation of glycogen phosphorylase because not only is it important in glycogen metabolism, it's also historically important.

The enzyme exists in four different forms as shown in Figure 12.22. The unphosphorylated form is called glycogen phosphorylase b (GPb) and the phosphorylated form is called glycogen phosphorylase a (GPa). The enzyme is phosphorylated by a kinase enzyme and dephosphorylated by a phosphatase.

Like other allosterically regulated enzymes, glycogen phosphorylase adopts two conformations; the R conformation is the active conformation and the T conformation is much less active. This is depicted in Figure 12.22 as a change in the shape of the catalytic site: In the R conformation, inorganic phosphate (a substrate of the reaction) can bind and in the T conformation binding of inorganic phosphate is inhibited.

Unphosphorylated GPb can exist in both inactive T conformations and active R conformations. The allosteric site of the enzyme binds several effectors that cause a
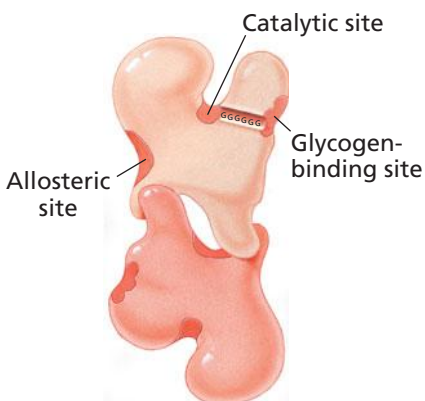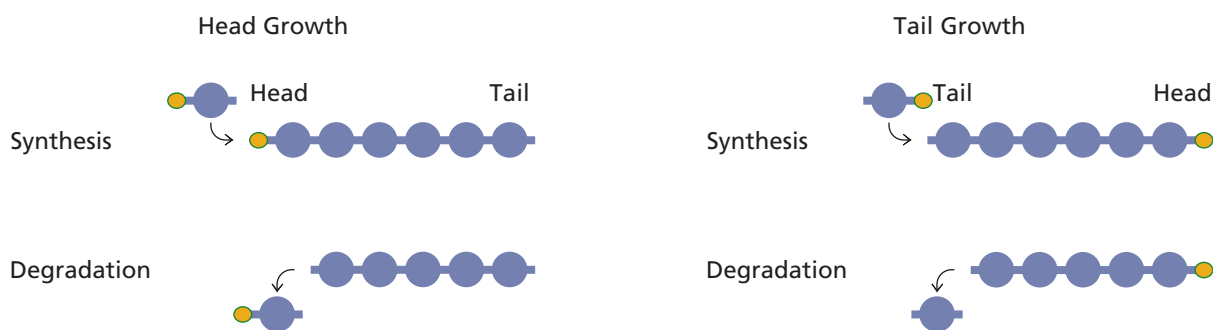
BOX 12.5 HEAD GROWTH AND TAIL GROWTH

Polymerization reactions can be described as either head growth or tail growth. In a head growth mechanism, the growing end of the chain is "activated" and cleavage of the "high energy" linkage at the head of the molecule provides the energy for the next addition of a monomer. In a tail growth mechanism, the growing end does not contain the high energy linkage; instead, the energy for the addition reaction comes from the activated monomer.

Glycogen synthesis is an example of a tail growth mechanism. The incoming monomer (UDP-glucose) is activated and, when the reaction is complete, the end of the glycogen chain is a simple hydroxyl group at the 4-carbon atom of a glucose residue. DNA and RNA synthesis are also examples of a tail growth mechanism. Protein synthesis and fatty acid synthesis are examples of head growth mechanism.

The differences between the two mechanisms become clear when you think of the reverse reaction: degradation.

Glycogen and nucleic acids can be degraded by chopping off a single residue. In the case of glycogen, synthesis and degradation are part of an ongoing process since the glycogen particle serves as a storage molecule for glucose. In the case of nucleic acids, especially DNA, the degradation reaction is an essential part of DNA repair and proofreading that ensures DNA replication is extremely accurate (Section 20.2C). Removal of single residues does not prevent the polymer from serving immediately as a substrate for further addition reactions.

Protein synthesis and fatty acid synthesis utilize head growth mechanisms for synthesis. In this case, removal of an end residue also removes the activated head so further addition reactions are not possible without an additional step to "reactivate" the head. This is one reason why protein synthesis errors can't be repaired and one reason why fatty acid chains aren't used as energy storage molecules in the same way that glycogen is used.

▲ **Head and tail growth.** In a head growth mechanism (left), incoming activated monomers are added to the "head" of the growing polymer. (The end that contains the activated residue.) After the addition reaction, the polymer still contains an activated residue at the growing end. In tail growth (right), the incoming activated monomer is added to the "tail" end of the growing polymer. The monomer substrate carries the energy for its own addition reaction. When the polymer is degraded, a single residue is removed. Polymers that use a head growth mechanism will no longer be a substrate for addition reactions following degradation because the activated head has been removed. Polymers that employ a tail growth mechanism are still able to act as substrates for addition reactions.

shift in conformation. The allosteric site is close to the dimer interface between the two monomers and both subunits change conformation simultaneously—a result that conforms to the *concerted model* of Monod, Wyman, and Changeux (Section 5.9C).

When ATP is bound, the activity of the enzyme is inhibited (T state). This is the normal state of activity since physiological concentrations of ATP are high and relatively constant. When the AMP concentration rises, it displaces ATP from the allosteric site causing a shift to the active R conformation and activation of glycogen breakdown. In muscle cells, increasing AMP concentration results from strenuous muscle activity and signals the need for more glucose 1-phosphate to stimulate ATP production by glycolysis. The enzyme is inhibited by glucose 6-phosphate (feedback inhibition). There's no need to continue glycogen breakdown if glucose 6-phosphate concentration is sufficient to fuel glycolysis.

The main difference between the R conformation and the T conformation is the position of a loop containing Asp-283 and nearby residues (the 280s loop). In the T conformation, the negatively charged side chain of Asp-283 lies close to the pyridoxal 5-phosphate (PLP) cofactor at the catalytic site. This proximity prevents binding of inorganic phosphate, inhibiting the reaction. In the R conformation, the position of this loop shifts allowing inorganic phosphate to enter the active site.
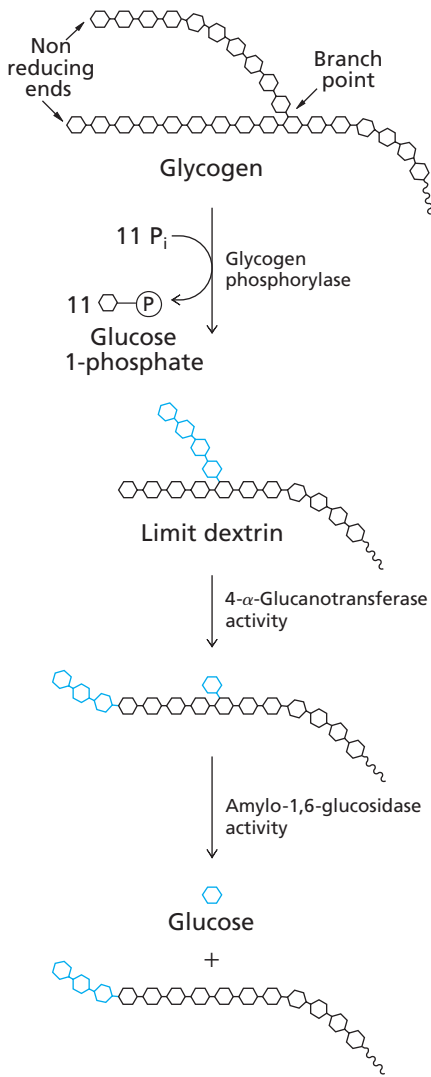
▲ **Figure 12.21**
Degradation of glycogen. Glycogen phosphorylase catalyzes the phosphorolysis of glycogen chains, stopping four residues from an α-$(1 \rightarrow 6)$ branch point and producing one molecule of glucose 1-phosphate for each glucose residue mobilized. Further degradation is accomplished by the two activities of the glycogen debranching enzyme. The 4-α-glucanotransferase activity catalyzes the transfer of a trimer from a branch of the limit dextrin to a free end of the glycogen molecule. The amylo-1,6-glucosidase activity catalyzes hydrolytic release of the remaining α-$(1 \rightarrow 6)$-linked glucose residue.

Phosphofructokinase-1 (PFK-1) is regulated in a similar manner by ATP and AMP.

The structures of GPa and GPb are shown in Figure 12.23 in order to illustrate the structural changes that take place when the enzyme is phosphorylated and dephosphorylated. The phosphoryl group is covalently attached to serine residue 14 (Ser-14) near the N-terminal end of the protein.

In the unphosphorylated state (GPb), the N-terminal residues, including Ser-14, associate with the surface near the catalytic site. In the phosphorylated state (GPa), phosphoserine-14 interacts with two positively charged arginine residues near the allosteric site. The remarkable shift in the location of the N-terminal end of the chain cause other conformation changes in the enzyme; notably, a reorientation of two α helices, the tower helices, on the other side of the dimer interface. This, in turn, affects the position of the 280s loop controlling the transition between the active R conformation and the inactive T conformation.

The equilibrium between T and R is greatly shifted in favor of the R conformation (active) when glycogen phosphorylase is phosphorylated (GPa). GPa is relatively insensitive to ATP, AMP, and glucose 6-phosphate. In muscle cells, GPa will be formed in response to hormones that signal the need for glucose and strenuous muscle activity. This promotes rapid mobilization of glycogen. In liver cells, the liver version of glycogen phosphorylase responds to the same hormones but in this case glycogen breakdown leads to excretion of glucose that can be taken up by muscle cells. Liver glycogen phosphorylase a is inhibited by glucose by shifting GPa to the T conformation. This makes sense since the presence of a high concentration of free glucose means that it's not necessary to continue producing glucose from glycogen.

The muscle version of glycogen phosphorylase is not inhibited by glucose since muscle cells rarely see significant concentrations of free glucose. Muscle cells don't convert glucose 6-phosphate to glucose and any glucose taken up from the bloodstream is quickly phosphoryated by hexokinase to glucose 6-phosphate.
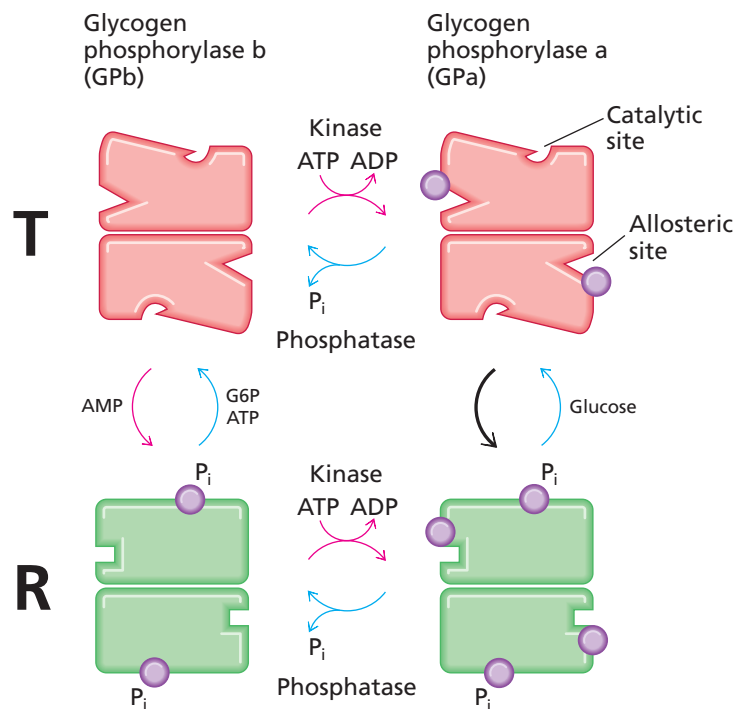
▲ **Figure 12.22**
Regulation of glycogen phosphorylase. Glycogen phosphorylase b is the unphosphorylated form of the enzyme. Glycogen phosphorylase a is phosphorylated at a position near the allosteric site. Phosphorylation is indicated by a purple ball at that site. The T conformation (red) is mostly inactive and the R conformation (green) is active in glycogen breakdown as shown by binding of inorganic phosphate (purple ball) to the catalytic site. The R conformation is greatly favored when the enzyme is phosphorylated (glycogen phosphorylase a).

T state

Catalytic site
PLP′

Ser-14′

Tower
helices

Catalytic site
PLP

R state

Catalytic site
PLP′

subunit
rotation
10°

Ser-14′ shift
34Å

Arg-69′

Ser14-P′

Arg-14

Tower
helices
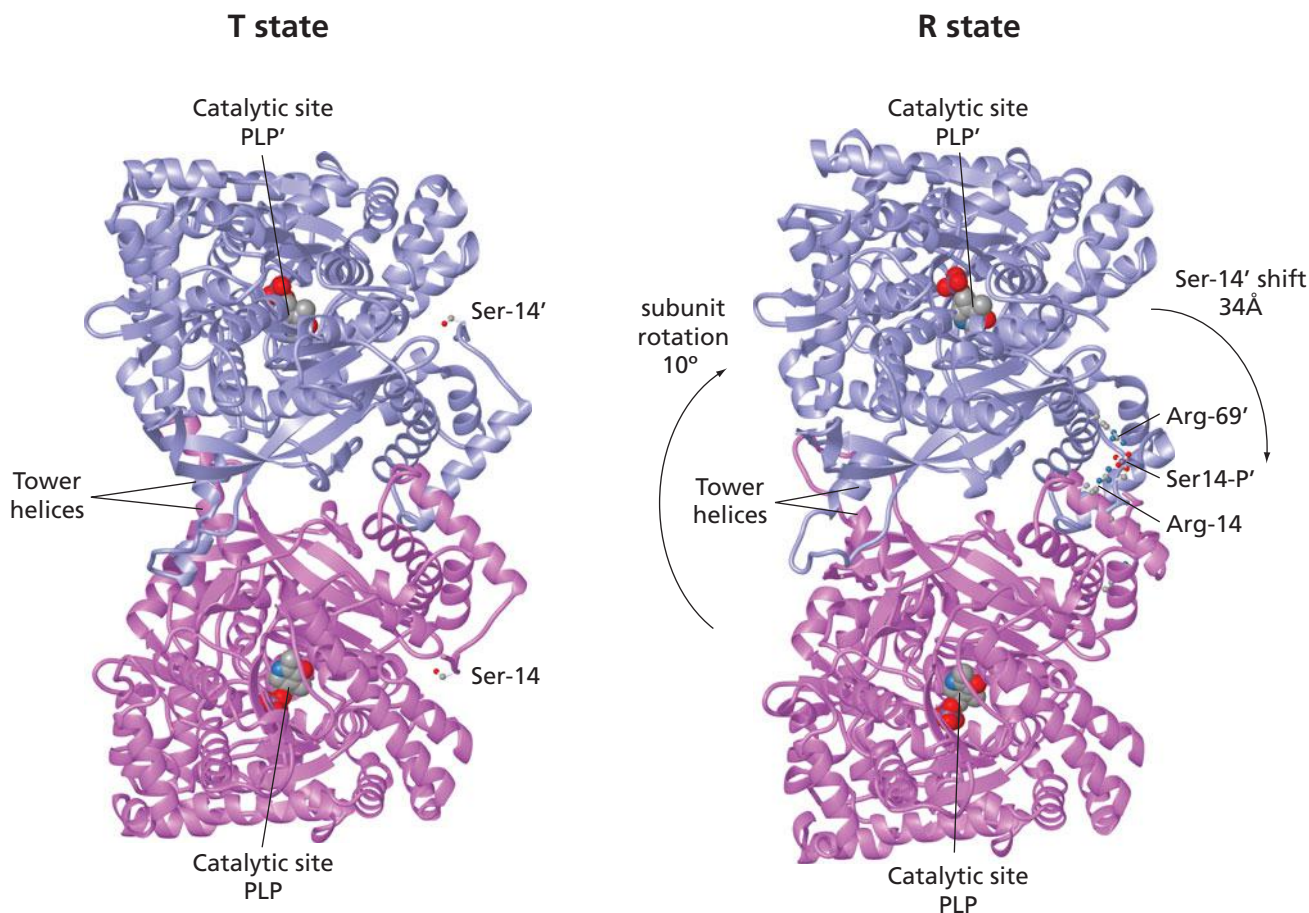
Catalytic site
PLP

▲ **Figure 12.23**

Phosphorylated and unphosphoylated forms of glycogen phosphorylase. PLP at the catalytic site is shown as a space-filling molecule. The large shift in position of Ser-14 upon phospharylation to Ser-14-P causes a conformational change that allows access to the catalytic site [PDB 3CEH, 1Z8D].

Gerty Cori and Carl Cori discovered in 1938 that glycogen phosphorylase activity was regulated by AMP. Since then, glycogen phosphorylase has been one of the prime examples of allosterically regulated enzymes, exciting three generations of biochemistry students. Glycogen phosphorylase was the very first enzyme whose regulation by covalent modification was demonstrated. Eddy Fischer and Edwin Krebs published their result in 1956 and for a long time regulation by phosphorylation was thought to be an unusual form of regulation confined to glycogen metabolism. Today, we know that phosphorylation is a very common form of regulation in eukaryotes and it is the most important part of many signal transduction pathways. There are hundreds of labs studying signal transduction.

B. Hormones Regulate Glycogen Metabolism

Insulin, glucagon, and epinephrine are the principal hormones that control glycogen metabolism in mammals. Insulin, a 51-residue protein synthesized by the β cells of the pancreas, is secreted when the concentration of glucose in the blood increases. High levels of insulin are associated with the fed state of an animal. Insulin increases the rate of glucose transport into muscle and adipose tissue via the GLUT4 glucose transporter (Section 11.5A). Insulin also stimulates glycogen synthesis in the liver.

Glucagon, a peptide hormone containing 29 amino acid residues, is secreted by the α cells of the pancreas in response to a low blood glucose concentration. Glucagon restores the blood glucose concentration to a steady state level by stimulating glycogen

▲ Edmond ("Eddy") H. Fischer (1920–) (left) and Edwin G. Krebs (1918–2009) (right) received the Nobel Prize in Physiology or Medicine in 1992 "for their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism."

degradation. Glucagon is extremely selective in its target because only liver cells are rich in glucagon receptors. The effect of glucagon is opposite that of insulin and an elevated glucagon concentration is associated with the fasted state.

The adrenal glands release the catecholamine epinephrine (also known as adrenaline), in response to neural signals that trigger the fight or flight response (Figure 3.5c). The epinephrine precursor, norepinephrine, also has hormone activity. Epinephrine stimulates the breakdown of glycogen. It triggers a response to a sudden energy requirement whereas glucagon and insulin act over longer periods to maintain a relatively constant concentration of glucose in the blood. Epinephrine binds to β-adrenergic receptors of liver and muscle cells and to α_1-adrenergic receptors of liver cells. The binding of epinephrine to β-adrenergic receptors or of glucagon to its receptors activates the adenylyl cyclase signaling pathway. The second messenger, cyclic AMP (cAMP), then activates protein kinase A (PKA).

PKA phosphorylates a number of other proteins causing significant changes in metabolism. Let's look first at the regulation of glycogen metabolism by glucagon (Figure 12.24). When glucagon binds to its receptor it stimulates adenylate cyclase causing an increase in cAMP that leads to activation of PKA. PKA phosphorylates glycogen synthase converting the "a" form to the inactive "b" form. This blocks glycogen synthesis. PKA also phosphorylates another kinase called phosphorylase kinase. As the name implies, this is the kinase that phosphorylates glycogen phosphorylase. PKA activates phosphorylase kinase leading to conversion of glycogen phosphorylase b to the the active form, glycogen phosphorylase a. The result is an increase in the rate of degradation of glycogen.

The net effect of glucagon (or epinephrine) is to block synthesis of glycogen and stimulate its breakdown. The reciprocal regulation of these two enzymes is an important feature of regulation in this pathway.

Glycogen synthase and glycogen phosphorylase are dephosphorylated by phosphoprotein phosphatase-1, an enzyme that acts on many other substrates. As shown in Figure 12.25, dephosphorylation leads to reciprocal inactivation of glycogen phosphorylase and activation of glycogen synthase. This results in synthesis of glycogen from UDP-glucose and inhibition of glycogen breakdown. Insulin stimulates the activity of phosphoprotein phosphatase-1, thus causing the uptake of glucose into glycogen and its depletion in the bloodstream. Prosphoprotein phosphatase-1 also acts on phosphorylase kinase blocking further activation of glycogen phosphorylase.

C. Hormones Regulate Gluconeogenesis and Glycolysis

Now it's time to return to our discussion of the regulation of gluconeogenesis and glycolysis. Fructose 1,6-*bis*phosphatase (FBPase) and phosphofructokinase-1 (PFK-1) are the key enzymes involved in the decision to either degrade glucose or synthesize it (Section 12.3). Recall that these two enzymes are reciprocally regulated by the effector fructose 2,6-*bis*phosphate (Figure 12.8). This effector molecule is synthesized from fructose 6-phosphate by phosphofructokinase-2 (PFK-2) and it is dephosphorylated back to fructose 6-phosphate by fructose 2,6-*bis*phosphatase (F2,6BPase) (Figure 12.9). These two enzymatic activities are located on the same bifunctional protein. The relationship among the four enzymes and their products is summarized in Figure 12.26.

The F2,6BPase and PFK-2 activities in the bifunctional enzyme are regulated by phosphorylation in a reciprocal manner. When the protein is phosphorylated, the enyme acts as a fructose 2,6-*bis*phosphatase and the phosphofructokinase activity is inhibited. Conversely, when the enzyme is unphosphorylated it acts as a phosphofructokinase and the fructose 2,6-*bis*phosphatase activity is inhibited.

This is the same mode of reciprocal regulation we encountered with glycogen phosphorylase and glycogen synthase, except this time the two enzyme activities are on the same molecule. In the presence of glucagon, protein kinase A (PKA) is active and it phosphorylates the bifunctional enzyme (Figure 12.27). Thus, glucagon stimulates gluconeogenesis and inhibits glycolysis in liver cells causing glucose levels in the bloodstream to rise. At the same time, epinephrine can stimulate glycogen degradation and inhibit glycogen synthesis in muscle cells. The result is more glucose for muscle cells and more ATP from glycolysis.
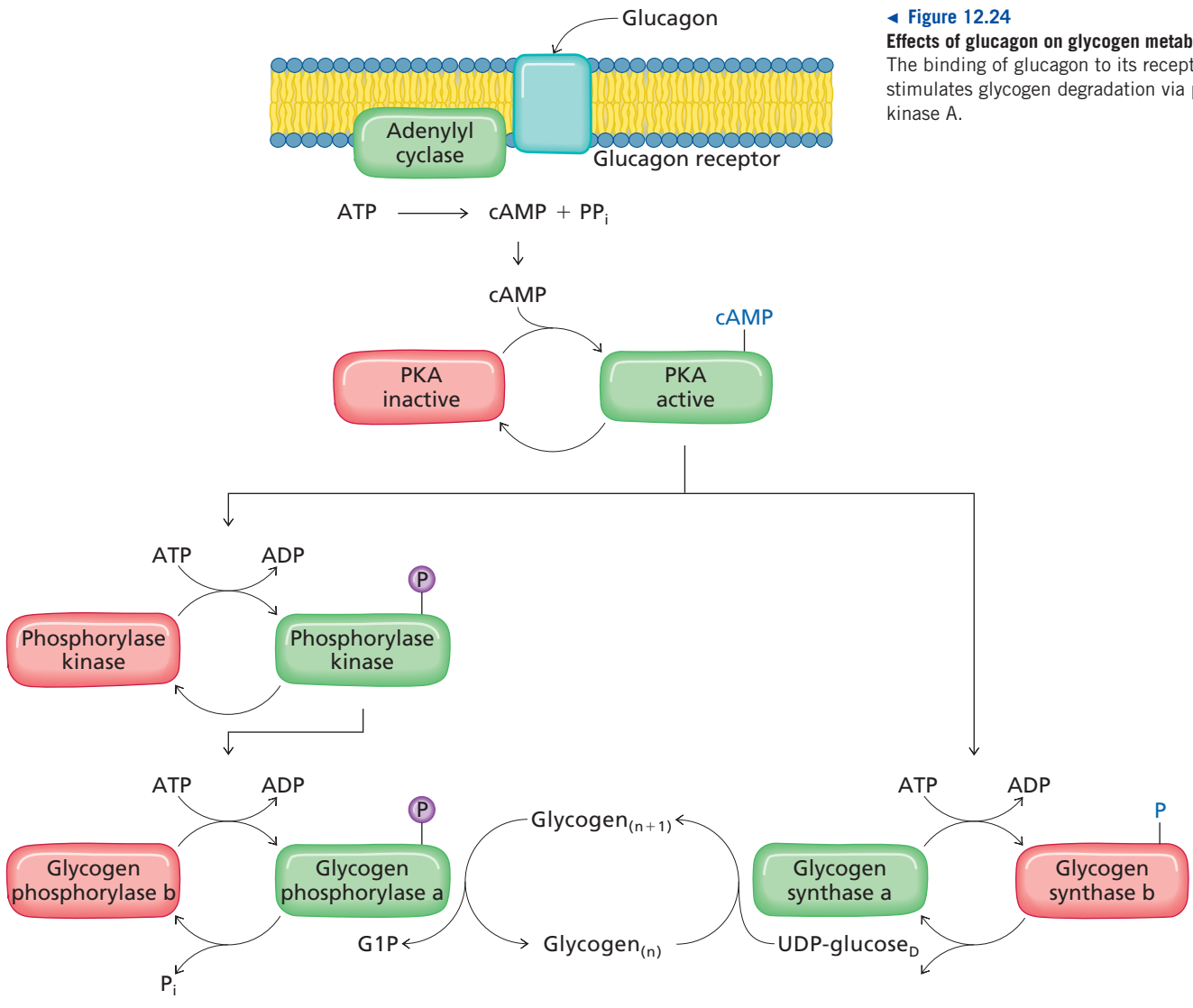
Effects of glucagon on glycogen metabolism. The binding of glucagon to its receptors stimulates glycogen degradation via protein kinase A.

▼ **Figure 12.25**
Effect of insulin on glycogen metabolism. Insulin simulates the phosphatase activity of phosphoprotein phosphatase-1, leading to inactivation of glycogen phosphorylase and activation of glycogen synthase.
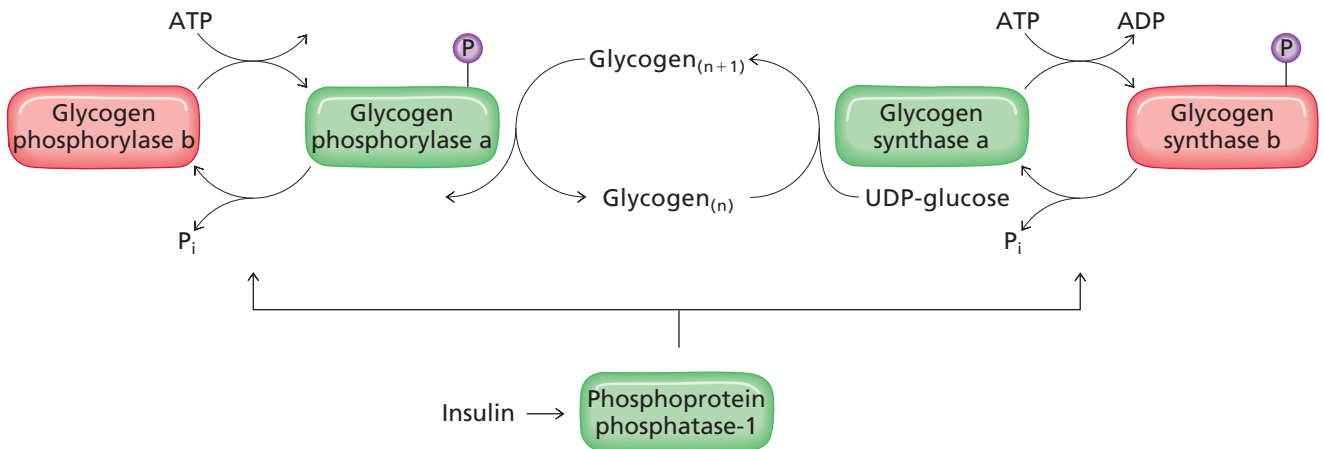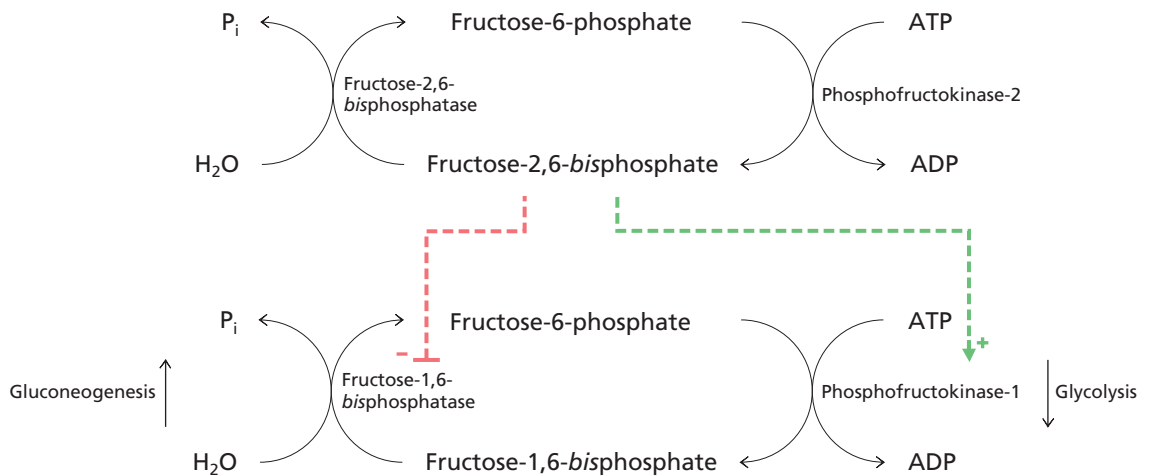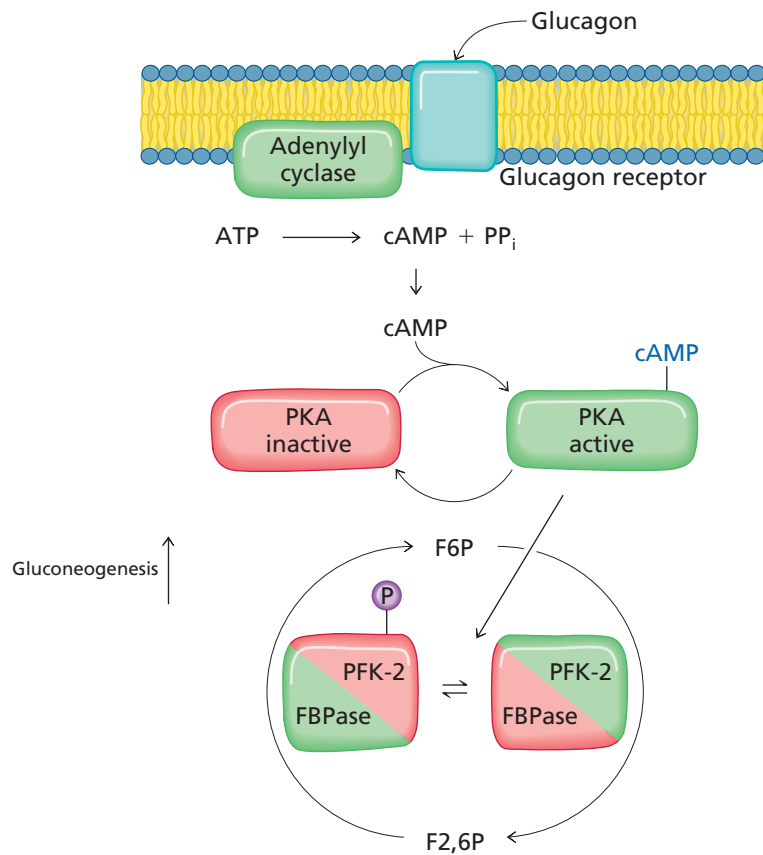
Figure 12.26 ▲
The role of fructose 2,6-*bis*phosphate in regulating glycolysis and gluconeogenesis.

12.7 Maintenance of Glucose Levels in Mammals

Mammals maintain blood glucose levels within strict limits by regulating both the synthesis and degradation of glucose. Glucose is the major metabolic fuel in the body. Some tissues, such as brain, rely almost entirely on glucose for their energy needs. The concentration of glucose in the blood seldom drops below 3 mM or exceeds 10 mM. When the concentration of glucose in the blood falls below 2.5 mM, glucose uptake into the brain is compromised, with severe consequences. Conversely, when blood glucose levels are very high, glucose is filtered out of the blood by the kidneys accompanied by osmotic loss of water and electrolytes.

The liver plays a unique role in energy metabolism participating in the interconversions of all types of metabolic fuels: carbohydrates, amino acids, and fatty acids.

Figure 12.27 ▶

Effect of glucagon on gluconeogenesis.
Glucagon binds to its receptor, causing activation of adenylate cyclase. Increased levels of cAMP activate protein kinase A, which phosphorylates the bifunctional enzyme leading to activation of fructose 2,6-*bis*phosphatase activity. In the absence of the effector fructose 2,6-*bis*phosphate, fructose 1,6-*bis*phosphatase is activated and this increases flux in the gluconeogenesis pathway.

Anatomically, the liver is centrally located in the circulatory system (Figure 12.28). Most tissues are perfused in parallel with the arterial system supplying oxygenated blood and the venous circulation returning blood to the lungs for oxygenation. The liver, however, is perfused in series with the visceral tissues (gastrointestinal tract, pancreas, spleen, and adipose tissue); blood from these tissues drains into the portal vein and then flows to the liver. This means that after the products of digestion are absorbed by the intestine, they pass immediately to the liver. Using its specialized complement of enzymes, the liver regulates the distribution of dietary fuels and supplies fuel from its own reserves when dietary supplies are exhausted.

The consumption of glucose by tissues removes dietary glucose from the blood. When glucose levels fall, liver glycogen and gluconeogenesis become the sources of glucose. However, since these sources are limited, hormones act to restrict the use of glucose to those cells and tissues that absolutely depend on glycolysis for generating ATP (kidney medulla, retina, red blood cells, and parts of the brain). Other tissues can generate ATP by oxidizing fatty acids mobilized from adipose tissue (Sections 16.1C and 16.2).

The complexity of carbohydrate metabolism in mammals is evident from the changes that occur on feeding and starvation. In the 1960s, George Cahill examined the glucose utilization of obese patients as they underwent therapeutic starvation. After an initial feeding of glucose, the subjects received only water, vitamins, and minerals. Cahill noted that glucose homeostasis (maintenance of constant levels in the circulation) proceeds through five phases. Figure 12.29, based on Cahill's observations, summarizes the metabolic changes in the five phases.

1. During the initial absorptive phase (the first four hours), dietary glucose enters the liver via the portal vein and most tissues use glucose as the primary fuel. Under these conditions, the pancreas secretes insulin, which stimulates glucose uptake by muscle and adipose tissue via GLUT4. The glucose taken up by these tissues is
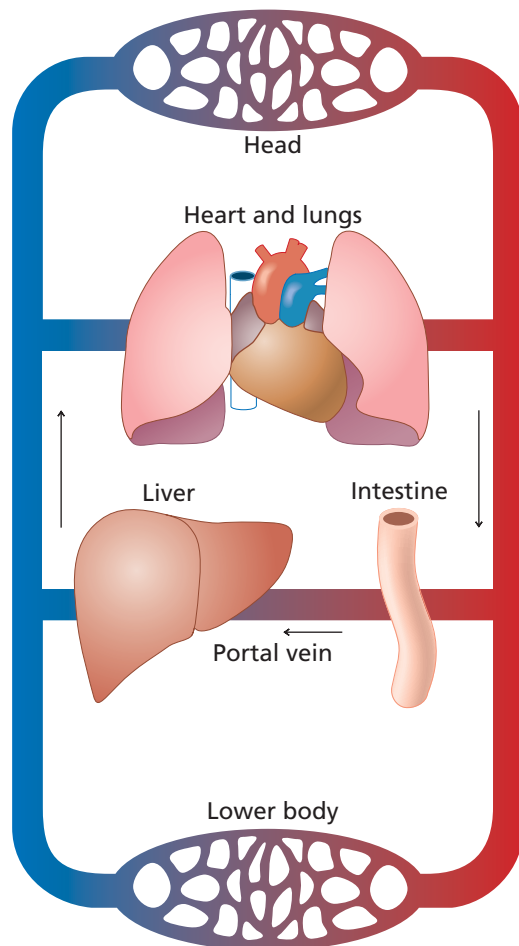
◄ **Figure 12.28**
Placement of the liver in the circulatory system. Most tissues are perfused in parallel. However, the liver is perfused in series with visceral tissues. Blood that drains from the intestine and other visceral tissues flows to the liver via the portal vein. The liver is therefore ideally placed to regulate the passage of fuels to other tissues.

Five phases of glucose homeostasis. The graph, based on observations of a number of individuals, illustrates glucose utilization in a 70 kg man who consumed 100 g of glucose and then fasted for 40 days.
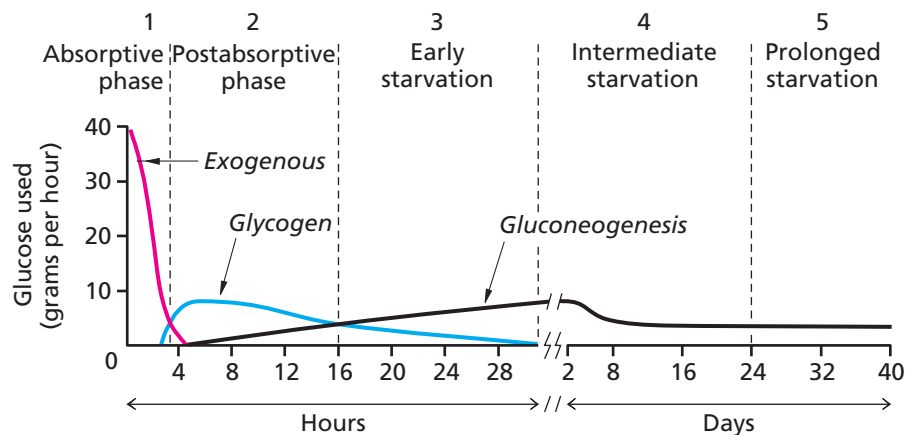
phosphorylated to glucose 6-phosphate, which cannot diffuse out of the cells. Liver cells also absorb glucose and convert it to glucose 6-phosphate. Excess glucose is stored as glycogen in liver and muscle cells.

2. When the dietary glucose is consumed, the body mobilizes liver glycogen to maintain circulating glucose levels. In the liver, glucose 6-phosphatase catalyzes the hydrolysis of glucose 6-phosphate to glucose, which exits the liver via glucose transporters. Glycogen in muscle (which lacks glucose 6-phosphatase) is metabolized to lactate to produce ATP for contraction; the lactate is used by other tissues as a fuel or by the liver for gluconeogenesis.

3. After about 24 hours, liver glycogen is depleted, and the only source of circulating glucose is gluconeogenesis in the liver, using lactate, glycerol, and alanine as precursors. Fatty acids mobilized from adipose tissue become an alternate fuel for most tissues. The obligatory glycolytic tissues continue to use glucose and produce lactate, which is converted to glucose in the liver by the Cori cycle; this cycle makes energy, not carbon, from fatty acid oxidation in the liver available to other tissues.

4. Gluconeogenesis in the liver continues at a high rate for a few days, then decreases. As starvation progresses, gluconeogenesis in the kidney becomes proportionately more significant. Proteins in peripheral tissues are broken down to provide gluconeogenic precursors. In this phase, the body adapts to several alternate fuels.

5. In prolonged starvation, there is less gluconeogenesis and lipid stores are depleted. If refeeding does not occur, death will follow. On refeeding, metabolism is quickly restored to the conditions of the fed state.

We have seen how glucose, a major fuel, can be stored in polysaccharide form and mobilized as needed. Glucose can also be synthesized from noncarbohydrate precursors by the reactions of gluconeogenesis. We have seen that glucose can be oxidized by the pentose phosphate pathway to produce NADPH or transformed by glycolysis into pyruvate.

The effect of insulin and diabetes on the production of ketone bodies is described in Section 16.11 (Box 16.6).

Diabetes mellitus (DM) is a metabolic disease that results from improper regulation of carbohydrate and lipid metabolism. Despite an ample supply of glucose, the body behaves as though starved and glucose is overproduced by the liver and underused by other tissues. As a result, the concentration of glucose in the blood is extremely high. The levels of glucose in the blood often exceed the capacity of the kidney to reabsorb glucose so some of it spills into the urine. The high concentration of glucose in urine draws water osmotically from the body.

There are two types of diabetes both of which arise from faulty control of fuel metabolism by the hormone insulin. In Type 1 diabetes mellitus (also called insulin-dependent diabetes mellitus, or IDDM) damage to the β cells of the pancreas, where insulin is synthesized, results in diminished or absent secretion of insulin. This autoimmune disease is characterized by early onset (usually before age 15). Patients are thin and exhibit hyperglycemia (high blood glucose levels), dehydration, excessive urination, hunger, and thirst. In Type 2 (also called non-insulin-dependent diabetes, or NIDDM), chronic hyperglycemia results from insulin resistance—decreased

sensitivity to insulin possibly caused by a shortage or decreased activity of insulin receptors. Insulin secretion may be normal and circulating levels of insulin may even be elevated. This type is also known as adult-onset diabetes (although its incidence is increasing among children) and it is usually associated with obesity. Type 2 diabetes affects about 5% of the population and Type 1 affects about 1%. In addition, about 2% to 5% of pregnant women develop a form of diabetes. Most women who exhibit gestational diabetes return to normal after giving birth but are at risk for developing Type 2 diabetes.

To understand diabetes, we must consider the functions of insulin. Insulin stimulates the synthesis of glycogen, triacylglycerols, and proteins and inhibits the breakdown of these compounds. Insulin also stimulates glucose transport into muscle cells and adipocytes. When insulin levels are low in IDDM, glycogen is broken down in the liver and gluconeogenesis occurs regardless of the glucose supply. In addition, glucose uptake and its use in peripheral tissues are restricted.

12.8 Glycogen Storage Diseases

Several metabolic diseases are related to the storage of glycogen. The general rule about metabolic diseases is that they usually affect the activity of nonessential genes and enzymes. Defects in essential genes are usually lethal and don't show up as metabolic diseases.

Many metabolic enzymes in humans are encoded by gene families. Different versions are expressed in different tissues. In the case of enzymes involved in glycogen metabolism, the most common versions are found in liver and muscle. A deficiency in one of these enzymes will produce severe symptoms but may not be lethal. There are nine types of glycogen storage diseases resulting from defects in glycogen metabolism.

Type 0: In type 0a, the activity of liver glycogen synthase is affected. The gene for this enzyme is on the short arm of chromosome 12 at locus 12p12.2 (MIM = 240600). This is a severe disease causing early death in cases where the activity is very low. Type 0b affects the muscle version of glycogen synthase whose gene is on the long arm of chromosome 19 at 19q13.3 (MIM = 611556). Patients have no muscle glycogen and are unable to engage in strenuous physical activity.

Type I: The most common glycogen storage disease is called von Gierke disease. It is caused by a deficiency in glucose 6-phosphatase (Type 1a, MIM = 23220) whose gene is on chromosome 17 (17q21). Defects in the complex that transports glucose across the endoplasmic reticulum (Section 21.1D) also cause von Gierke's disease. Type 1b affects the glucose 6-phosphate transporter (chromosome 11 (11q23), MIM = 232220) and type 1c affects the phosphate transporter (chromosome 6 (6p21.3), MIM = 232240). Patients are unable to secrete glucose leading to accumulation of glycogen in the liver and kidneys.

Type II: Patients suffering from type II disease, known as Pompe's disease, suffer from reduced activity of α-1,4-glucosidase, or acid maltase, an enzyme required for glycogen breakdown in lysozomes (MIM = 232300). The gene is on chromosome 17 (17q25.2). The defect causes glycogen to accumulate in lysosomes leading to problems with muscle tissue, especially in the heart. In the most severe forms, children die within the first few years of life.

Type III: Type III is Cori disease, characterized by defects in the gene encoding the glycogen debranching enzyme in liver and muscle (chromosome 1 (1p21), MIM = 232400). People suffering from this disease have weakened muscles because they are unable to mobilize all of the stored glycogen. Some defects have very mild symptoms.

Type IV: Often called Anderson's disease, the mutations occur in the gene for liver branching enzyme found on chromosome 3 (3p12, MIM = 232500). Long-chain polysaccharides accumulate in patients with these mutations, resulting in death within a few years from heart failure or liver failure.

MIM numbers refer to the Online Mendelian Inheritance in Man (OMIM) database at: *ncbi.nlm.nih.gov/omim*

Type V: McArdle's disease (type V glycogen storage disease) is caused by a deficiency of muscle glycogen phosphorylase (MIM = 232600). The gene is on chromosome 11 (11q13). Individuals having this genetic disease cannot perform strenuous exercise and suffer painful muscle cramps.

Type VI: Hers' disease (type VI) is a mild form of glycogen storage disease due to a deficiency in liver glycogen phosphorylase (MIM = 232700). Several mutant alleles interfere with proper splicing of the primary transcript from the gene on chromosome 14 (14q21).

Type VII: Mutations in the gene for muscle phosphofructokinase-1 cause Tarui's disease, characterized by inability to exercise and muscle cramps (MIM = 232800). The gene for this isozyme is on chromosome 12 (12q13.3).

Type VIII: Now recognized as a subtype of type IX.

Type IX: This form of glycogen storage disease manifests as muscle weakness and/or muscle cramps. The symptoms are usually mild. All subtypes are due to mutations in the genes for the various subunits of glycogen phosphorylase kinase. Type IXa: liver α subunit gene on the X chromosome at Xp20 (MIM = 300798). Type IXb: β subunit gene at 16q12 (MIM = 172490). Type IXc: liver γ subunit gene at 16p12 (MIM = 172471). Type IXd: muscle α subunit gene on the X chromosome at Xq13 (MIM = 311870).

Summary

1. Gluconeogenesis is the pathway for glucose synthesis from non-carbohydrate precursors. The seven near-equilibrium reactions of glycolysis proceed in the reverse direction in gluconeogenesis. Four enzymes specific to gluconeogenesis catalyze reactions that bypass the three metabolically irreversible reactions of glycolysis.

2. Noncarbohydrate precursors of glucose include pyruvate, lactate, alanine, and glycerol.

3. Gluconeogenesis is regulated by glucagon, allosteric modulators, and the concentrations of its substrates.

4. The pentose phosphate pathway metabolizes glucose 6-phosphate to generate NADPH and ribose 5-phosphate. The oxidative stage of the pathway generates two molecules of NADPH per molecule of glucose 6-phosphate converted to ribulose 5-phosphate and CO_2. The nonoxidative stage includes isomerization of ribulose 5-phosphate to ribose 5-phosphate. Further metabolism of pentose phosphate molecules can convert them to glycolytic intermediates. The combined activities of transketolase and transaldolase convert pentose phosphates to triose phosphates and hexose phosphates.

5. Glycogen synthesis is catalyzed by glycogen synthase, using a glycogen primer and UDP-glucose.

6. Glucose residues are mobilized from glycogen by the action of glycogen phosphorylase. Glucose 1-phosphate is then converted to glucose 6-phosphate.

7. Glycogen degradation and glycogen synthesis are reciprocally regulated by hormones. Kinases and phosphatases control the activities of the interconvertible enzymes glycogen phosphorylase and glycogen synthase.

8. Mammals maintain a nearly constant concentration of glucose in the blood. The liver regulates the amount of glucose supplied by the diet, glycogenolysis, and other fuels.

9. Glycogen storage diseases result from defects in genes required for glycogen metabolism.

Problems

1. Write a balanced equation for the synthesis of glucose from pyruvate. Assuming that the oxidation of NADH is equal to 2.5 ATP equivalents (Section 14.11), how many ATP equivalents are required in this pathway? Convert this to kJ mol^{-1} and explain how this value compares to the total energy required to synthesize glucose from CO_2 and H_2O.

2. What important products of the citric acid cycle are required for gluconeogenesis from pyruvate?

3. Epinephrine promotes the utilization of stored glycogen for glycolysis and ATP production in muscles. How does epinephrine promote the use of liver glycogen stores for generating the energy needed by contracting muscles?

4. (a) In muscle cells, insulin stimulates a protein kinase that catalyzes phosphorylation of protein phosphatase-1, thereby activating it. How does this affect glycogen synthesis and degradation in muscle cells?

   (b) Why does glucagon selectively regulate enzymes in the liver but not in other tissues?

   (c) How does glucose regulate the synthesis and degradation of liver glycogen via protein phosphatase-1?

5. The polypeptide hormone glucagon is released from the pancreas in response to low blood glucose levels. In liver cells, glucagon plays a major role in regulating the rates of the opposing glycolysis

and gluconeogenesis pathways by influencing the concentrations of fructose 2,6-*bis*phosphate (F2,6 BP). If glucagon causes a decrease in the concentrations of F2,6 BP, how does this result in an increase in blood glucose levels?

6. When the concentration of glucagon rises in the blood, which of the following enzyme activities is decreased? Explain.

> Adenylyl cyclase
> Protein kinase A
> PFK-2 (kinase activity)
> Fructose 1, 6-*bis*phosphatase

7. (a) Is the energy required to synthesize glycogen from glucose 6-phosphate greater than the energy obtained when glycogen is degraded to glucose 6-phosphate?

   (b) During exercise, glycogen in both muscle and liver cells can be converted to glucose metabolites for ATP generation in the muscles. Do liver glycogen and muscle glycogen supply the same amount of ATP to the muscles?

8. Individuals with a total deficiency of muscle glycogen phosphorylase (McArdle's disease) cannot exercise strenuously due to muscular cramping. Exertion in these patients leads to a much greater than normal increase in cellular ADP and P_i. Furthermore, lactic acid does not accumulate in the muscles of these patients, as it does in normal individuals. Explain the chemical imbalances in McArdle's disease.

9. Compare the number of ATP equivalents generated in the breakdown of one molecule of glucose 1-phosphate into two molecules of lactate with the number of ATP equivalents required for the synthesis of one molecule of glucose 1-phosphate from two molecules of lactate. (Assume anaerobic conditions.)

10. (a) How does the glucose–alanine cycle allow muscle pyruvate to be used for liver gluconeogenesis and subsequently returned to muscles as glucose?

    (b) Does the glucose–alanine cycle ultimately provide more energy for muscles than the Cori cycle does?

11. Among other effects, insulin is a positive modulator of the enzyme glucokinase in liver cells. If patients with diabetes mellitus produce insufficient insulin, explain why these patients cannot properly respond to increases in blood glucose.

12. Glycogen storage diseases (GSDs) due to specific enzyme deficiencies can affect the balance between glycogen stores and blood glucose. Given the following diseases, predict the effects of each on (1) the amount of liver glycogen stored and (2) blood glucose levels.

    (a) Von Gierke disease (GSD-1a), defective enzyme: glucose 6-phosphatase.

    (b) Cori's disease (GSD III), defective enzyme: amylo-1,6 glucosidase (debranching enzyme).

    (c) Hers' disease (GSD VI), defective enzyme: liver phosphorylase

13. The pentose phosphate pathway and the glycolytic pathway are interdependent, since they have in common several metabolites whose concentrations affect the rates of enzymes in both pathways. Which metabolites are common to both pathways?

14. In many tissues, one of the earliest responses to cellular injury is a rapid increase in the levels of enzymes in the pentose phosphate pathway. Ten days after an injury, heart tissue has levels of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase that are 20 to 30 times higher than normal, whereas the levels of glycolytic enzymes are only 10% to 20% of normal. Suggest an explanation for this phenomenon.

15. (a) Draw the structures of the reactants and products for the second reaction catalyzed by transketolase in the pentose phosphate pathway. Show which carbons are transferred.

    (b) When 2-[^{14}C]-glucose 6-phosphate enters the pathway, which atom of fructose 6-phosphate produced by the reaction in Part (a) is labeled?

Selected Readings

Gluconeogenesis

Hanson, R. W., and Hakimi, P. (2008). Born to run. *Biochimie.* 90:838–842.

Hanson, R. W., and Reshef, L. (1997). Regulation of phosphenolpyruvate carboxykinase (GTP) gene expression. *Annu. Rev. Biochem.* 66:581–611. Describes the metabolic control of gene expression.

Hines, J. K., Chen, X., Nix, J. C., Fromm, H. J., and Honzatko, R. B. (2007). Structures of mammalian and bacterial fructose-1,6-bisphosphatase reveal the basis for synergism in AMP/fructose 2,6-bisphosphate inhibition. *J. Biol. Chem.* 282:36121–36131.

Jitrapakdee, S., and Wallace, J. C. (1999). Structure, function and regulation of pyruvate carboxylase. *Biochem. J.* 340:1–16.

Kemp, R. G. and Gunasekera, D. (2002). Evolution of the allosteric ligand sites of mammalian phosphofructo-1-kinase. *Biochem.* Biochemistry 41:9426–9430.

Ou, X., Ji, C., Han, X., Zhao, X., Li, X., Mao, Y., Wong, L-L., Bartlam, M., and Rao, Z. (2006).

Crystal structure of human glycerol 3-phosphate dehydrogenase (GPD1). *J. Mol. Biol.* 357:858–869.

Pilkis, S. J., and Granner, D. K. (1992). Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Physiol.* 57:885–909.

Rothman, D. L., Magnusson, I., Katz, L. D., Shulman, R. G., and Shulman, G. I. (1991). Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with ^{13}C NMR. *Science.* 254:573–576. Describes the continuous operation of the pathway of gluconeogenesis in humans.

Sullivan, S. M., and Holyoak (2008). Enzymes with lid-gated active sites must operate by an induced fit mechanism instead of conformational selection. *Proc. Natl. Acad. Sci. (USA)* 105:13829–13834.

van de Werve, G., Lange, A., Newgard, C., Méchin, M.-C., Li, Y., and Berteloot, A. (2000). New lessons in the regulation of glucose metabolism taught by the glucose 6-phosphatase system. *Eur. J. Biochem.* 267:1533–1549. Explains why there is still much to

learn about the catalytic site and the transporter associated with this enzyme.

Xue, Y., Huang, S., Liang, J. Y., Zhang, Y., and Lipscomb, W. N. (1994). Crystal structure of fructose-1,6-bisphosphatase complexed with fructose 2,6-bisphosphate, AMP, and Zn2+ at 2.0-A resolution: aspects of synergism between inhibitors. *Proc. Natl. Acad. Sci. (USA)* 91:12482–12486.

Pentose Phosphate Pathway

Au, S.W.N., Gover, S., Lam, V.M.S., and Adams, M.J. (2000) Human glucose-6-phospate dehydrogenase: the crystal structure reveals a structural NADP$^+$ molecule and provides insights into enzyme deficiency. Structure 8:293–303.

Wood, T. (1985). *The Pentose Phosphate Pathway.* (Orlando: Academic Press).

Wood, T. (1986). Physiological functions of the pentose phosphate pathway. *Cell Biochem. Func.* 4:241–247.

Glycogen Metabolism

Barford, D. Hu, S-H., and Johnson, L. N. (1991). Structural mechanisms for glycogen phosphorylase control by phosphorylation and AMP. *J. Mol. Biol.* 218:233–260.

Chou, J. Y., Matern, D., Mansfield, B. C., and Chen, Y. T. (2002). Type I glycogen storage diseases: disorders of the glucose 6-phosphate complex. *Curr. Mol. Med.* 2:121–143.

Cohen, P., Alessi, D. R., and Cross, D. A. E. (1997). PDK1, one of the missing links in insulin signal transduction? *FEBS Lett.* 410:3–10.

Fischer, E. (2010). Memories of Ed Krebs. *J. Biol. Chem.* 285:4267.

Johnson, L. N. (2009). Novartis Medal Lecture: The regulation of protein phosphorylation. *Biochem. Soc. Trans.* 37:627–641.

Johnson, L. N., and Barford, D. (1990). Glycogen phosphorylase: the structural basis of the allosteric response and comparison with other allosteric proteins. *J. Biol. Chem.* 265:2409–2412.

Johnson, L. N., Lowe, E. D., Noble, M. E. M., and Owen, D. J. (1998). The structural basis for substrate recognition and control by protein kinases. *FEBS Lett.* 430:1–11.

Larner, J. (1990). Insulin and the stimulation of glycogen synthesis: the road from glycogen synthase to cyclic AMP-dependent protein kinase to insulin mediators. *Adv. Enzymol. Mol. Biol.* 63:173–231.

Meléndez-Hevia, E., Waddell, T. G., and Shelton, E. D. (1993). Optimization of molecular design in the evolution of metabolism: the glycogen molecule. *Biochem. J.* 295:477–483.

Murray, R. K., Bender, D. A., Kennelly, P. J., Rodwell, V. W., and Weil. P. A. (2009). *Harper's Illustrated Biochemistry*, 28th ed. (New York: McGraw-Hill).

Pinotsis, N., Leonidas, D. D., Chrysina, E. D., Oikonomakos, N. G., and Mavridis, I. M. (2003). The binding of β- and γ-cyclodextrins to glycogen phosphorylase b: kinetic and crystallographic studies. *Prot. Sci.* 12:1914–1924.

Shepherd, P. R., Withers, D. J., and Siddle, K. (1998). Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. *Biochem. J.* 333:471–490.

Smythe, C., and Cohen, P. (1991). The discovery of glycogenin and the priming mechanism for glycogen biosynthesis. *Eur. J. Biochem.* 200:625–631.

Villar-Palasi, C., and Guinovart, J. J. (1997). The role of glucose 6-phosphate in the control of glycogen synthase. *FASEB J.* 11:544–558.
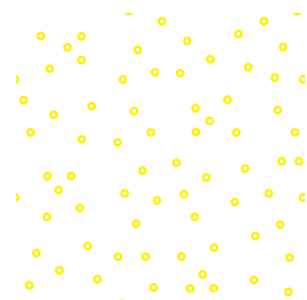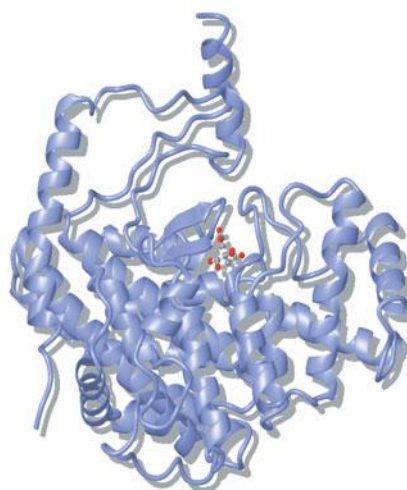
The Citric Acid Cycle

In the last two chapters we were mainly concerned with the synthesis and degradation of complex carbohydrates such as glucose. We saw that the biosynthetic pathway leading to glucose began with pyruvate and oxaloacetate and that pyruvate was the end product of glycolysis. In this chapter we will describe pathways that interconvert a number of simple organic acids. Several of these compounds are essential precursors for the biosynthesis of amino acids, fatty acids, and porphyrins.

Acetyl CoA is one of the key intermediates in the interconversion of small organic acids. Acetyl CoA is formed by the oxidative decarboxylation of pyruvate with the release of CO_2. This reaction is catalyzed by pyruvate dehydrogenase, an enzyme that we briefly encountered in Section 11.3 when we discussed the fate of pyruvate. We begin this chapter with a more detailed description of this important enzyme.

The acetyl group (a two-carbon organic acid) from acetyl CoA can be transferred to the four-carbon dicarboxylic acid, oxaloacetate, to form a new six-carbon tricarboxylic acid known as citrate (citric acid). Citrate can then be oxidized in a seven-step pathway to regenerate oxaloacetate and release two molecules of CO_2. Oxaloacetate can recombine with another molecule of acetyl CoA and the citrate oxidation reactions are repeated. The net effect of this eight-enzyme cyclic pathway is the complete oxidation of an acetyl group to CO_2 and the transfer of electrons to several cofactors to form reducing equivalents. The pathway is known as the **citric acid cycle**, the tricarboxylic acid cycle (TCA cycle), or the Krebs cycle, after Hans Krebs who discovered it in the 1930s.

The citric acid cycle lies at the hub of energy metabolism in eukaryotic cells—especially in animals. The energy released in the oxidations of the citric acid cycle is largely conserved as reducing power when the coenzymes $NAD^{\oplus}$ and ubiquinone (Q) are reduced to form NADH and QH_2. This energy is ultimately derived from pyruvate (via acetyl CoA). Since pyruvate is the end product of glycolysis, we can think of the citric acid cycle as a series of reactions that complete the oxidation of glucose. NADH and QH_2 are substrates in the reactions of membrane-associated electron transport leading to the formation of a proton gradient that drives the synthesis of ATP (Chapter 14).

Since citric acid reacts catalytically in the tissue it is probable that it is removed by a primary reaction but regenerated by a subsequent reaction. In the balance sheet no citrate disappears and no intermediate products accumulate.

—H. A. Krebs and W. A. Johnson (1937)

Top: Citrate synthase with its product citrate in the active site. This enzyme catalyzes the first step of the citric acid cycle. [PDB 1CTS]

Hans Krebs and W. A. Johnson proposed the citric acid cycle in 1937 in order to explain several puzzling observations. They were interested in understanding how the oxidation of glucose in muscle cells was coupled to the uptake of oxygen. Albert Szent-Györgyi had previously discovered that adding a four-carbon dicarboxylic acid—succinate, fumarate, or oxaloacetate—to a suspension of minced muscle stimulated the consumption of O_2. The substrate of the oxidation was carbohydrate, either glucose or glycogen. Especially intriguing was the observation that adding small amounts of four-carbon dicarboxylic acids caused larger amounts of oxygen to be consumed than were required for their own oxidation. This indicated that these four-carbon organic acids had catalytic effects.

Krebs and Johnson observed that citrate, a six-carbon tricarboxylic acid, and α-ketoglutarate, a five-carbon compound, also had a catalytic effect on the uptake of O_2. They proposed that citrate was formed from a four-carbon intermediate and an unknown two-carbon derivative of glucose (later shown to be acetyl CoA). The cyclic nature of the pathway explained how its intermediates could act catalytically without being consumed. Albert Szent-Györgyi received the Nobel Prize in Physiology or Medicine in 1937 for his work on respiration, including the catalytic role of fumarate in biological combustion processes. Hans Krebs was awarded the Nobel Prize in Physiology or Medicine in 1953 for discovering the citric acid cycle.

In muscle cells, the intermediates in the citric acid cycle are almost exclusively used in the cyclic pathway of energy metabolism. In these cells, the metabolic machinery is mainly devoted to extracting energy from glucose in the form of ATP. This is why it was possible to recognize the cyclic nature of the pathway by carrying out experiments on muscle extracts. In other cells, the intermediates of the citric acid cycle are the starting points for many biosynthetic pathways. Thus, the enzymes of the citric acid cycle play a key role in both anabolic and catabolic reactions.

Many of these same enzymes are found in prokaryotes although few bacteria possess a complete citric acid cycle. In this chapter, we examine the reactions of the citric acid cycle as they occur in eukaryotic cells. We will explore how these enzymes are regulated. Next we will introduce the various biosynthetic pathways that require citric acid cycle intermediates and examine the relationship of these pathways to the main reactions of the cyclic pathway in eukaryotes and the partial pathways in bacteria. We will also look at pathways involving glyoxylate, specifically the glyoxylate shunt and the glyoxylate cycle. These are pathways that are closely related to the citric acid cycle. Finally, we will discuss the evolution of the citric acid cycle enzymes.

BOX 13.1 AN EGREGIOUS ERROR

In 1937, Krebs and Johnson submitted a paper to *Nature* outlining their discovery of citric acid as a catalyst in the oxidation of glucose by muscle tissue. The journal declined to publish the paper on the grounds that it had too many papers in press. Krebs writes in his memoirs, "This was the first time in my career, after having published more than fifty papers, that I experienced a rejection or semi-rejection."

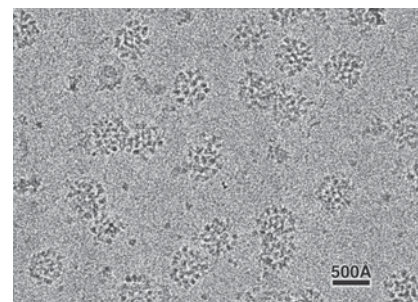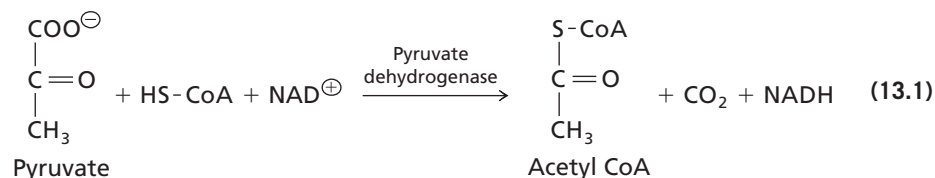Krebs and Johnson published the paper in the journal *Enzymologia* and Krebs went on to win the Nobel Prize based largely on this paper. It took *Nature* 51 years to publically recognize the mistake it made. An editor wrote in the October 28, 1988 issue, "An editor's nightmare is to reject a Nobel-prizewinning paper. . . . Rejection of Hans Krebs' discovery of the tricarboxylic (or Krebs') cycle, a pivot of biochemical metabolism, remains *Nature's* most egregious error (as far as we know)."

▶ **Hans Krebs (1900–1981).** Krebs was awarded the Nobel Prize in Physiology or Medicine in 1953 for his discovery of the citric acid cycle. He is shown here beside a Warburg apparatus for measuring oxygen consumption in metabolizing tissue. Krebs worked with Otto Warburg in the 1920s.

13.1 Conversion of Pyruvate to Acetyl CoA

Pyruvate is a key substrate in a number of reactions, as described in Section 11.3. In this chapter we are concerned with the conversion of pyruvate to acetyl CoA since acetyl CoA is the main substrate of the citric acid cycle. The reaction is catalyzed by a large complex of enzymes and cofactors known as the pyruvate dehydrogenase complex (Figure 13.1). The stoichiometry of the complete reaction is
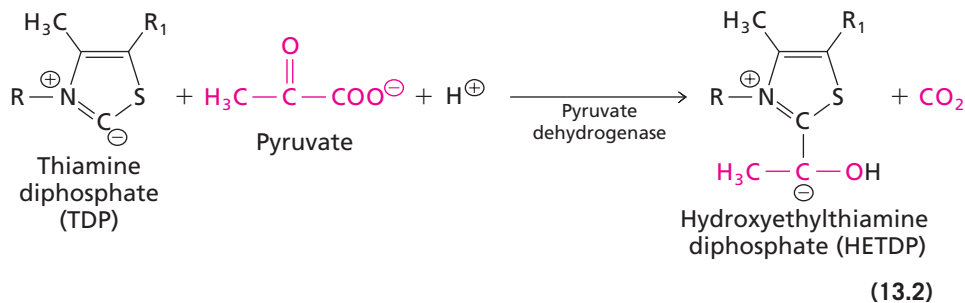
$$
\begin{array}{c}
\text{COO}^{\ominus} \\
| \\
\text{C}=\text{O} \\
| \\
\text{CH}_3 \\
\text{Pyruvate}
\end{array}
+ \text{HS-CoA} + \text{NAD}^{\oplus}
\xrightarrow[\text{dehydrogenase}]{\text{Pyruvate}}
\begin{array}{c}
\text{S-CoA} \\
| \\
\text{C}=\text{O} \\
| \\
\text{CH}_3 \\
\text{Acetyl CoA}
\end{array}
+ \text{CO}_2 + \text{NADH} \quad \textbf{(13.1)}
$$

▲ **Figure 13.1**
Electron micrograph of pyruvate dehydrogenase complexes from *E. coli*.

where HS-CoA is coenzyme A. This is the first step in the oxidation of pyruvate and the products of the reaction are acetyl CoA, one molecule of carbon dioxide, and one molecule of reducing equivalent (NADH). The pyruvate dehydrogenase reaction is an oxidation–reduction reaction. In this case, the oxidation of pyruvate to CO_2 is coupled to the reduction of $NAD^{\oplus}$ to NADH. The net result is the transfer of two electrons from pyruvate to NADH.

The pyruvate dehydrogenase complex is a multienzyme complex containing multiple copies of three distinct enzymatic activities: pyruvate dehydrogenase (E_1 subunits), dihydrolipoamide acetyltransferase (E_2 subunits), and dihydrolipoamide dehydrogenase (E_3 subunits). The oxidative decarboxylation of pyruvate can be broken down into five steps. (In each step of the following reactions the fates of the atoms from pyruvate are shown in red.)

The systematic names of the enzymes in the complex are: pyruvate lipoamide 2-oxidoreductase (E_1); acetyl CoA:dihydrolipoamide *S*-acetyltransferase (E_2); and dihydrolipoamide:NAD$^{\oplus}$ oxidoreductase (E_3).

1. The E_1 component contains the prosthetic group thiamine diphosphate (TDP). As we saw in Chapter 7, TDP (vitamin B_1) plays a catalytic role in a number of decarboxylase reactions. The initial reaction results in the formation of a hydroxyethyl–TDP intermediate and the release of CO_2.

$$
\begin{array}{c}
\text{H}_3\text{C} \quad\quad \text{R}_1 \\
\diagdown\!\!\!\!\diagup \\
\text{R}-\overset{\oplus}{\text{N}}\diagdown\!\!\diagup\text{S} \\
\text{C} \\
\ominus \\
\text{Thiamine} \\
\text{diphosphate} \\
\text{(TDP)}
\end{array}
+
\begin{array}{c}
\text{O} \\
\| \\
\text{H}_3\text{C}-\text{C}-\text{COO}^{\ominus} \\
\text{Pyruvate}
\end{array}
+ \text{H}^{\oplus}
\xrightarrow[\text{dehydrogenase}]{\text{Pyruvate}}
\begin{array}{c}
\text{H}_3\text{C} \quad\quad \text{R}_1 \\
\diagdown\!\!\!\!\diagup \\
\text{R}-\overset{\oplus}{\text{N}}\diagdown\!\!\diagup\text{S} \\
\text{C} \\
| \\
\text{H}_3\text{C}-\text{C}-\text{OH} \\
\ominus
\end{array}
+ \text{CO}_2
$$

Hydroxyethylthiamine
diphosphate (HETDP)

(13.2)

Note that the reactive form of TDP is the carbanion or ylid form. The carbanion form is relatively stable because of the unique environment of the coenzyme bound to the protein (Section 7.6). The product of the first step is the carbanion form of hydroxyethyl–TDP. The mechanism is similar to the pyruvate decarboxylase mechanism (Section 7.7).

2. In the second step, the two-carbon hydroxylethyl group is transferred to the lipoamide group of E_2. The lipoamide group consists of lipoic acid covalently bound by an amide linkage to a lysine residue of an E_2 subunit (Figure 7.29). This particular coenzyme is only found in pyruvate dehydrogenase and related enzymes.

The transfer reaction is catalyzed by the E_1 component of the pyruvate dehydrogenase complex.

HETDP   Lipoamide   Ylid   Acetyl-dihydrolipoamide

$$(13.3)$$
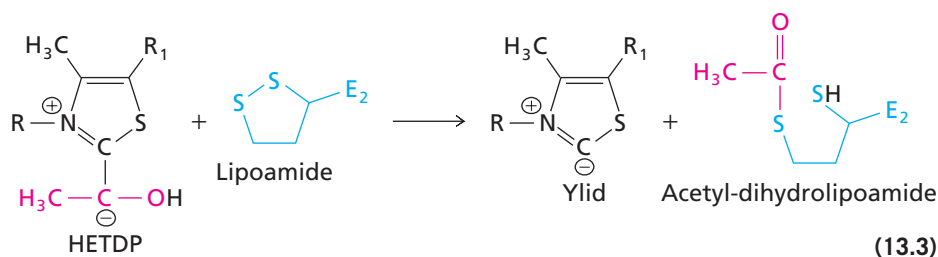
In this reaction, the oxidation of hydroxyethyl–TDP is coupled to the reduction of the disulfide of lipoamide and the acetyl group is transferred to one of the sulfhydryl groups of the coenzyme regenerating the ylid form of TDP.

3. The third step involves the transfer of the acetyl group to HS-CoA, forming acetyl CoA and leaving the lipoamide in the reduced dithiol form. This reaction is catalyzed by the E_2 component of the complex.

Acetyl-dihydrolipoamide   Dihydrolipoamide   Acetyl CoA

$$(13.4)$$
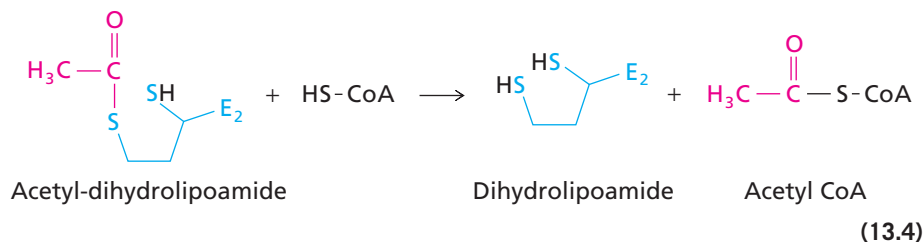
4. The reduced lipoamide of E_2 must be reoxidized in order to regenerate the prosthetic group for additional reactions. This is accomplished in step 4 by transferring two protons and two electrons from the dithiol form of lipoamide to FAD. FAD is the prosthetic group of E_3 and the redox reaction produces the reduced coenzyme ($FADH_2$). (Recall from Section 7.5 that $FADH_2$ carries two electrons and two protons that are usually acquired as a single proton and a hydride ion.)

Dihydrolipoamide   Lipoamide

$$(13.5)$$
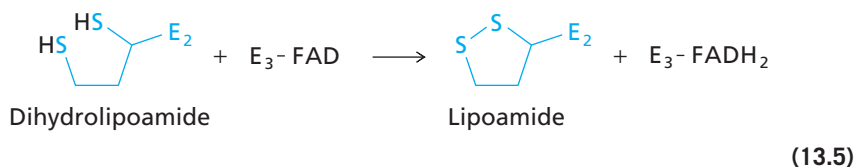
5. In the final step, E_3–$FADH_2$ is reoxidized to FAD. This reaction is coupled to the reduction of $NAD^{\oplus}$.

$$E_3\text{---}FADH_2 + NAD^{\oplus} \longrightarrow E_3\text{---}FAD + NADH + H^{\oplus} \qquad (13.6)$$

The oxidation of E_3–$FADH_2$ regenerates the original pyruvate dehydrogenase complex, completing the catalytic cycle. Step 5 produces NADH and $H^{\oplus}$. Note that one proton is released in step 5 and one proton is taken up in step 1 so that the overall stoichiometry of the pyruvate dehydrogenase reaction shows no net gain or loss of protons (Reaction 13.1).

The interplay of five coenzymes in the pyruvate dehydrogenase complex illustrates the importance of coenzymes in metabolic reactions. Two of the coenzymes are cosubstrates (HS-CoA and $NAD^{\oplus}$), and three are prosthetic groups (TDP, lipoamide, and FAD—one

cofactor is bound to each type of subunit). The lipoamide groups bound to E_2 are primarily responsible for transferring reactants from one active site in the complex to another. A lipoamide picks up a two-carbon unit from hydroxyethyl–TDP in step 2 to form the acetyl–dihydrolipoamide intermediate. This intermediate is repositioned in the active site of dihydrolipoamide acetyltransferase where the two-carbon group is transferred to coenzyme A in step 3. The reduced lipoamide produced in that reaction is then moved to the active site of dihydrolipoamide dehydrogenase in E_3. Lipoamide is reoxidized in step 4 and the regenerated coenzyme is repositioned in the active site of E_1 where it is ready to receive a new two-carbon group. In these reactions, the lipoamide prosthetic group acts as a swinging arm that visits the three active sites in the pyruvate dehydrogenase complex (Figure 13.2). The swinging arm portion of the E_2 subunit consists of a flexible polypeptide chain that includes the lysine residue to which lipoamide is covalently bound.

The various subunits of the complex are arranged in a way that facilitates the swinging arm mechanism of lipoamide. The mechanism ensures that the product of one reaction does not diffuse into the medium but is immediately acted on by the next component of the system. This is a form of channeling where the product of one reaction becomes the substrate of a second reaction but it differs from other examples because, in this case, the two-carbon intermediate is covalently bound to the flexible lipoamide group of E_2.

The entire pyruvate dehydrogenase reaction is a series of coupled oxidation–reduction reactions in which electrons are transported from the initial substrate (pyruvate) to the final oxidizing agent ($NAD^\oplus$). The four half reactions are. . . .

> **Channeling and multienzyme complexes were discussed in Section 5.11.**

$$E^{\circ\prime}$$

1. $\text{acetyl CoA} + CO_2 + H^\oplus + 2e^\ominus \longrightarrow \text{pyruvate} + \text{CoA}$ -0.48
2. $E_2\text{—lipoamide} + 2H^\oplus + 2e^\ominus \longrightarrow E_2\text{—dihydrolipoamide}$ -0.29
3. $E_3\text{—FAD} + 2H^\oplus + 2e^\ominus \longrightarrow E_3\text{—FADH}_2$ -0.34
4. $NAD^\oplus + 2H^\oplus + 2e^\ominus \longrightarrow NADH + H^\oplus$ -0.32

$$(13.7)$$
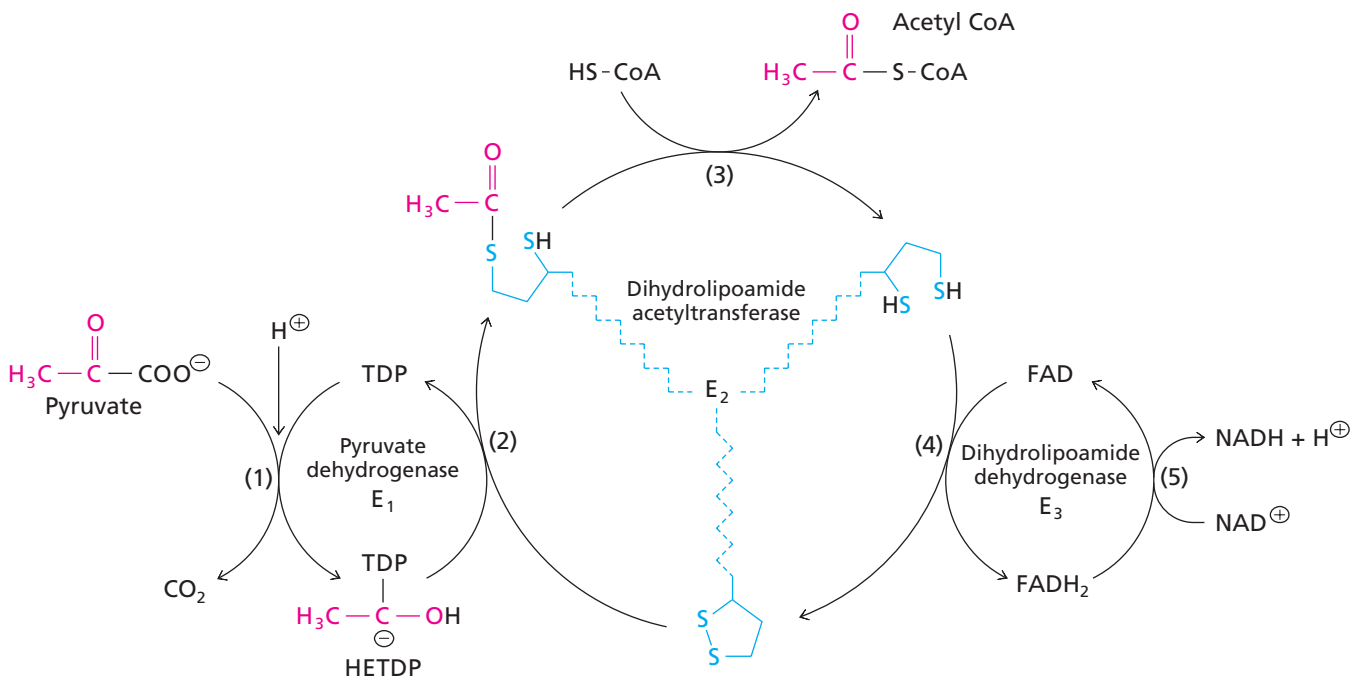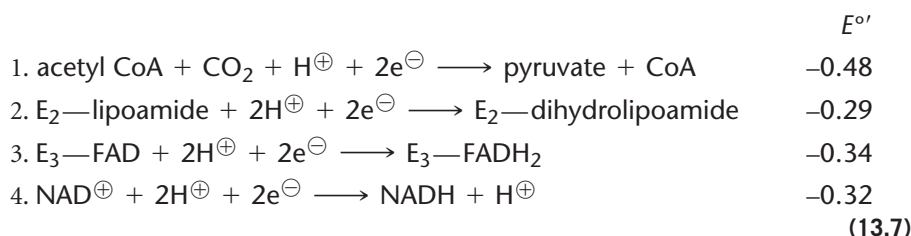
▲ **Figure 13.2**
Reactions of the pyruvate dehydrogenase complex. The lipoamide prosthetic group (blue) is attached by an amide linkage between lipoic acid and the side chain of a lysine residue of E_2. This prosthetic group is a swinging arm that carries the two-carbon unit from the pyruvate dehydrogenase active site to the dihydrolipoamide acetyltransferase active site. The arm then carries hydrogen to the dihydrolipoamide dehydrogenase active site.

(a)

(b)

▲ **Figure 13.3**
Structural model of the pyruvate dehydrogenase complex. **(a)** The inner core consists of 60 E_2 enzymes arranged in the shape of a pentagonal dodecahedron with one E_2 trimer at each of the 20 vertices. A single trimer is outlined by a yellow box. The center of the pentagon shape is indicated by the orange pentagon. Note the linker regions projecting upward from the surface of the core structure. **(b)** Cutaway view of the complete complex showing the outer E_1 enzymes (yellow) and the BP–E_3 enzymes (red) located in the space between the E_2 enzymes of the inner core.

[From Zhou, H. Z. et al. (2001). The remarkable structural and functional organization of the eukaryotic pyruvate dehydrogenase complexes. *Proc. Natl. Acad. Sci.* (USA) 98:14082–14087.]

▲ **A biochemistry laboratory.**

Each half-reaction has a characteristic standard reduction potential (Table 10.4) that provides some indication of the direction of electron flow. (Recall from Section 10.9 that the actual reduction potentials depend on the concentrations of reducing agents and oxidizing agents.) Electron transport begins with pyruvate, which gives up two electrons in the reverse of half-reaction 1. These electrons are taken up by E_2–lipoamide. Subsequent electron flow is from E_2–lipoamide to E_3–FAD to $NAD^\oplus$. The final product is NADH, which carries a pair of electrons. There are many examples of metabolic pathway enzymes with simple electron transport systems such as this one. They should not be confused with the much more complex membrane-associated electron transport system covered in the next chapter.

The pyruvate dehydrogenase complex is enormous. It is several times bigger than a ribosome. In bacteria these complexes are located in the cytosol, and in eukaryotic cells they are found in the mitochondrial matrix. Pyruvate dehydrogenase complexes are also present in chloroplasts.

The eukaryotic pyruvate dehydrogenase complex is the largest multienzyme complex known. The core of the complex is formed from 60 E_2 subunits arranged in the shape of a pentagonal dodecahedron (12 pentagons joined at their edges to form a ball). This shape has 20 vertices and each vertex is occupied by an E_2 trimer (Figure 13.3A). Each of the E_2 subunits has a linker region projecting upward from the surface. This linker contacts an outer ring of E_1 subunits that surround the inner core (Figure 13.3B). The linker region contains the lipoamide swinging arm.

The outer shell has 60 E_1 subunits. Each E_1 enzyme contacts one of the underlying E_2 enzymes and makes additional contacts with its neighbors. The E_1 enzyme consists of two α subunits and two β subunits ($\alpha_2\beta_2$), so it is considerably larger than the E_2 enzyme of the core. The E_3 enzyme (an α_2 dimer) lies in the center of the pentagon formed by the core E_2 enzymes. There are 12 E_3 enzymes in the complete complex, corresponding to the 12 pentagons in the pentagonal dodecahedron shape. In eukaryotes, the E_3 enzymes are associated with a small binding protein (BP) that's part of the complex.

The model shown in Figure 13.3 has been constructed from high resolution electron microscopy images of pyruvate dehydrogenase complexes at low temperature (cryo-EM) (Figure 13.1). In this technique, a large number of individual images are combined and a three-dimensional image is built with the help of a computer.

Sample Calculation 13.1

Q. Calculate the standard Gibbs free energy change for the pyruvate dehydrogenase reaction.

A. From Equation 10.26, the overall change in standard reduction potential is

$$\Delta E^{\circ\prime} = \Delta E^{\circ\prime}_{\text{electron acceptor}} - \Delta E^{\circ\prime}_{\text{electron donor}}$$
$$= -0.32 - (-0.48) = 0.16 \text{ V}$$

from Equation 10.25,

$$\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime}$$
$$= -(2)(96.5)(0.16)$$
$$= -31 \text{ kJ mol}^{-1}$$

The model is then matched with the structures of any of the individual subunits that have been solved by X-ray crystallography or NMR. So far, it has not been possible to grow large crystals of the entire pyruvate dehydrogenase complex on Earth and experiments to grow crystals on the International Space Station in the absence of gravity were also unsuccessful.

A similar pyruvate dehydrogenase complex is present in many species of bacteria although some, such as gram-negative bacteria, have a smaller version where there are only 24 E_2 enzymes in the core. In these bacteria, the core enzymes are arranged as a cube with one trimer at each of the eight vertices. The E_2 subunits of the two different bacterial enzymes and the eukaryotic mitochondrial and chloroplast versions are all closely related. However, the gram-negative bacterial enzymes contain E_1 enzymes that are unrelated to the eukaryotic versions.

Pyruvate dehydrogenase is a member of a family of multienzyme complexes known as the 2-oxo acid dehydrogenase family. (Pyruvate is the smallest 2-oxo organic acid.) We will encounter two other 2-oxo (or α-keto) acid dehydrogenases that closely resemble pyruvate dehydrogenase in structure and function. One is a citric acid cycle enzyme, α-ketoglutarate dehydrogenase (Section 13.3#4), and the other is branched chain α-keto acid dehydrogenase, used in amino acid metabolism (Section 17.10E). All members of the family catalyze essentially irreversible reactions in which an organic acid is oxidized to CO_2 and a coenzyme A derivative is formed.

The reverse reactions are catalyzed in some bacteria by entirely different enzymes. These reactions form part of a pathway for fixing carbon dioxide in anaerobic bacteria. Some bacteria and some anaerobic eukaryotes convert pyruvate to acetyl CoA and CO_2 using pyruvate:ferredoxin 2-oxidoreductase, an enzyme that is unrelated to pyruvate dehydrogenase.

$$\text{Pyruvate} + \text{CoA} + 2\,\text{Fd}_{ox} \rightarrow \text{acetyl CoA} + 2\,\text{Fd}_{red} + 2\,\text{H}^{\oplus} \qquad \textbf{(13.8)}$$

The terminal electron carrier in this case is reduced ferredoxin (Fd_{red}) and not NADH, as with pyruvate dehydrogenase. The pyruvate:ferredoxin oxidoreductase reaction is reversible and may be used to fix CO_2 by reductive carboxylation. Bacterial species that have diverged very early in the history of life often contain pyruvate:ferredoxin oxidoreductase and not pyruvate dehydrogenase suggesting that the former enzyme is more primitive and pyruvate dehydrogenase evolved later.

13.2 The Citric Acid Cycle Oxidizes Acetyl CoA

Acetyl CoA formed from pyruvate or other compounds (such as fatty acids or some amino acids) can be oxidized by the citric acid cycle. The eight reactions of the citric acid cycle are listed in Table 13.1. Before examining each of the reactions individually, we should consider two general features of the pathway; the flow of carbon and the production of "high energy" molecules.

The fates of the carbon atoms are depicted in Figure 13.4. In the first reaction of the citric acid cycle, the two-carbon acetyl group of acetyl CoA is transferred to the four-carbon dicarboxylic acid oxaloacetate to form citrate, a six-carbon tricarboxylic acid. The cycle proceeds with oxidative decarboxylation of a six-carbon acid and a five-carbon acid. This releases two molecules of CO_2 and produces succinate, a four-carbon dicarboxylic acid. The remaining steps of the cycle convert succinate to oxaloacetate, the original reactant that began the cycle.

The complete reactions are shown in Figure 13.5 where the two carbons of the acetyl group are also colored green so their fate can be followed. Note that the two carbon atoms entering the cycle as the acetyl group on acetyl CoA are not the same carbon atoms that are lost as CO_2. However, the carbon balance in the overall reaction pathway is such that for each two-carbon group from acetyl CoA that enters the cycle, two carbon atoms are released during one complete turn of the cycle. The two carbon atoms of acetyl CoA become half of the symmetric four-carbon dicarboxylic acid (succinate) in the fifth step of the cycle. The two halves of this symmetric molecule are chemically equivalent so carbons arising from acetyl CoA become evenly distributed in molecules formed from succinate.

Acetyl CoA is a "high energy" molecule (Section 10.8). The thioester linkage conserves some of the energy gained from the decarboxylation of pyruvate by the pyruvate dehydrogenase complex. The net equation of the citric acid cycle (Table 13.1) tends to

KEY CONCEPT

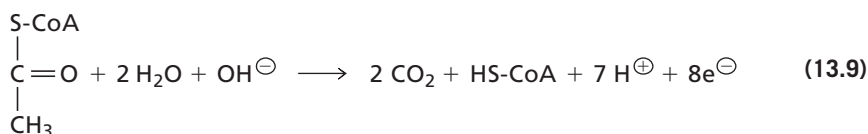**Large multienzyme complexes improve efficiency by channeling substrates and products.**
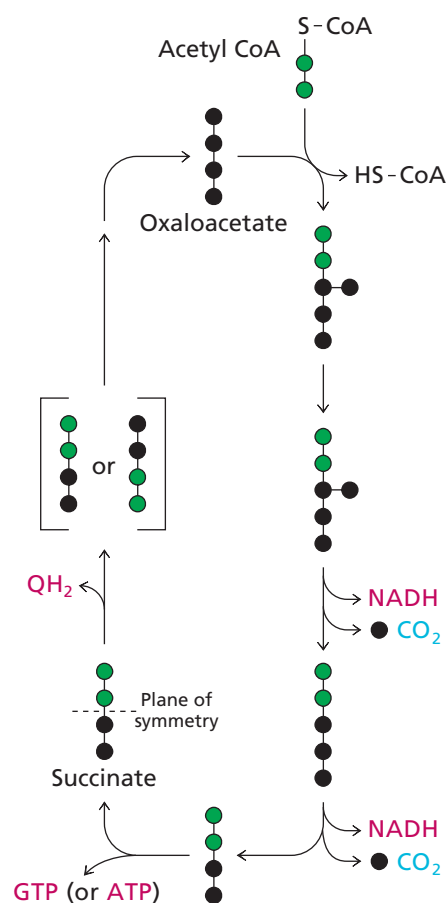
▲ **Figure 13.4**
Fates of the carbon atoms from oxaloacetate and acetyl CoA during one turn of the citric acid cycle. The plane of symmetry of succinate means that the two halves of the molecule are chemically equivalent; thus, carbon atoms from acetyl CoA (green) are uniformly distributed in the four-carbon intermediates leading to oxaloacetate. Carbon atoms from acetyl CoA that enter in one turn of the cycle are thus lost as CO_2 only in the second and subsequent turns. Energy is conserved in the reduced coenzymes NADH and QH_2 and in one GTP (or ATP) produced by substrate level phosphorylation.

Table 13.1 The enzymatic reactions of the citric acid cycle

Reaction	Enzyme
1. Acetyl CoA + Oxaloacetate + $H_2O \longrightarrow$ Citrate + HS−CoA + $H^\oplus$	Citrate synthase
2. Citrate $\rightleftharpoons$ Isocitrate	Aconitase (Aconitate hydratase)
3. Isocitrate + $NAD^\oplus \longrightarrow$ α-Ketoglutarate + NADH + CO_2	Isocitrate dehydrogenase
4. α-Ketoglutarate + HS-CoA + $NAD^\oplus \longrightarrow$ Succinyl CoA + NADH + CO_2	α-Ketoglutarate dehydrogenase complex
5. Succinyl CoA + GDP (or ADP) + P_i $\rightleftharpoons$ Succinate + GTP(or ATP) + HS−CoA	Succinyl-CoA synthetase
6. Succinate + Q $\rightleftharpoons$ Fumarate + QH_2	Succinate dehydrogenase complex
7. Fumarate + H_2O $\rightleftharpoons$ L-Malate	Fumarase (Fumarate hydratase)
8. L-Malate + $NAD^\oplus$ $\rightleftharpoons$ Oxaloacetate + NADH + $H^\oplus$	Malate dehydrogenase

Net equation:

Acetyl CoA + 3 $NAD^\oplus$ + Q + GDP (or ADP) + P_i + 2 H_2O $\longrightarrow$ HS−CoA + 3 NADH + QH_2 + GTP (or ATP) + 2 CO_2 + 2 $H^\oplus$

obscure the fact that the citric acid cycle is equivalent to the oxidation of an acetyl CoA molecule with release of electrons. The overall reaction sequence can be simplified to

$$\begin{array}{c} \text{S-CoA} \\ | \\ \text{C}=\text{O} + 2\,H_2O + OH^\ominus \longrightarrow 2\,CO_2 + \text{HS-CoA} + 7\,H^\oplus + 8e^\ominus \\ | \\ \text{CH}_3 \end{array} \qquad (13.9)$$

where the hydroxyl group is donated by inorganic phosphate in Reaction 5 and some of the products are shown as free protons and free electrons. This form of the net equation reveals that eight electrons are released during the oxidation. (Recall that oxidation reactions release electrons and reduction reactions take up electrons.) Six of the electrons are transferred to three molecules of $NAD^\oplus$ along with three of the protons depicted in Reaction 13.9. The remaining two electrons are transferred to one molecule of ubiquinone (Q) along with two of the protons. Two free protons are produced in each turn of the cycle. (Keep in mind that the carbon dioxide molecules released during the citric acid cycle do not actually come directly from acetyl CoA. Reaction 13.9 is a simplified version that emphasizes the net oxidation.)

BOX 13.2 WHERE DO THE ELECTRONS COME FROM?

Chemical reaction equations, such as Reaction 13.9, aren't very helpful in understanding where electrons are released and taken up. In order to see the electron balance in such reactions it's often useful to redraw the structures with the valence electrons replacing the lines that represent the chemical bonds in most drawings. Each covalent bond involves a shared pair of electrons and each of the standard atoms (C, O, N, S) requires eight valence electrons. Covalently bonded hydrogen atoms have only a single pair of electrons in their single shell.

The oxidation of acetyl CoA from Equation 13.8 is shown in this form in the figure. Note that only the electrons in the outer shells of the atoms are shown. These are the ones removed by oxidations or added in reduction reactions. There are 42 electrons (21 pairs) in the reactants and 34 electrons (17 pairs) in the products: CO_2 and Coenzyme A. Thus, 8 electrons are released in the oxidation. Most of the time, electrons are released when double bonds are formed (as in

carbon dioxide) since this results in the sharing of an extra electron pair.
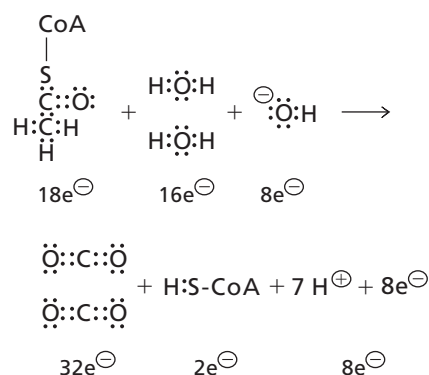
▲ The oxidation of an acetyl CoA equivalent by the citric acid cycle showing the valence electrons in the reactants and products.

▶ **Figure 13.5**
Citric acid cycle. For each acetyl group that enters the pathway, two molecules of CO_2 are released, the mobile coenzymes $NAD^\oplus$ and ubiquinone (Q) are reduced, one molecule of GDP (or ADP) is phosphorylated, and the acceptor molecule (oxaloacetate) is re-formed.

S-CoA
C=O
CH_3
Acetyl CoA

Entry of substrate by condensation with oxaloacetate

$COO^\ominus$
C=O
CH_2
$COO^\ominus$
Oxaloacetate

① Citrate synthase H_2O → $HS-CoA + H^\oplus$

Oxidation   ⑧ Malate dehydrogenase $NADH + H^\oplus$ ← $NAD^\oplus$

$COO^\ominus$
CH_2
$HO-C-COO^\ominus$
CH_2
$COO^\ominus$
Citrate

② Aconitase   *Rearrangement*

$COO^\ominus$
$HO-C-H$
CH_2
$COO^\ominus$
L-Malate

Hydration   ⑦ Fumarase H_2O

$COO^\ominus$
CH_2
$H-C-COO^\ominus$
$HO-C-H$
$COO^\ominus$
Isocitrate

$COO^\ominus$
$H-C$
$C-H$
$COO^\ominus$
Fumarate

Oxidation   ⑥ Succinate dehydrogenase complex   FAD QH_2 ← Q

③ Isocitrate dehydrogenase $NAD^\oplus$ → NADH → CO_2 *First oxidative decarboxylation*

$COO^\ominus$
CH_2
CH_2
$COO^\ominus$
Succinate

$COO^\ominus$
CH_2
CH_2
C=O
$COO^\ominus$
α-Ketoglutarate

Substrate-level phosphorylation   ⑤ Succinyl-CoA synthetase   HS-CoA ← GTP (or ATP) ← GDP (or ADP) ← P_i

$COO^\ominus$
CH_2
CH_2
C=O
S-CoA
Succinyl CoA

④ α-Ketoglutarate dehydrogenase complex   HS-CoA → $NAD^\oplus$ → NADH → CO_2 *Second oxidative decarboxylation*

Most of the energy released in the citric acid cycle reactions is conserved in the form of electrons transferred from organic acids to generate the reduced coenzymes NADH and QH_2 (Figure 13.5). NADH is formed by the reduction of $NAD^{\oplus}$ at three oxidation–reduction steps—two of these are oxidative decarboxylations. QH_2 is formed when succinate is oxidized to fumarate. Subsequent oxidation of the reduced coenzymes by membrane-associated electron transport leads to the transfer of electrons from NADH and QH_2 to a terminal electron acceptor. In the case of most eukaryotes (and many prokaryotes), this terminal electron acceptor is oxygen, which is reduced to water. Membrane-associated electron transport is coupled to the production of ATP from ADP and P_i. The entire process (electron transport + phosphorylation of ADP) is often referred to as oxidative phosphorylation when oxygen is present (Chapter 14). In addition to the formation of reducing equivalents, the citric acid cycle produces a nucleotide triphosphate directly by substrate level phosphorylation. The product can be either ATP or GTP, depending on the cell type or species.

KEY CONCEPT

The citric acid cycle is a mechanism for the oxidation of the acetyl group of acetyl CoA.

13.3 The Citric Acid Cycle Enzymes

The citric acid cycle can be viewed as a multistep catalytic reaction returning to its original state after an acetyl CoA molecule is oxidized. This view is based on the fact that when the reactions operate as a cycle the original reactant, oxaloacetate, is regenerated. By definition, a catalyst increases the rate of a reaction without itself undergoing net transformation. All enzymatic reactions, in fact all catalytic reactions, can be represented as cycles. An enzyme goes through a cyclic series of conversions and finally returns to the form in which it began. In this sense, the citric acid cycle fits the description of a catalyst.

Taken as a whole, the citric acid cycle is a mechanism for oxidizing the acetyl group of acetyl CoA to CO_2 by $NAD^{\oplus}$ and ubiquinone. When the citric acid cycle operates in isolation its intermediates are re-formed with each full turn of the cycle. As a result, the citric acid cycle doesn't appear to be a pathway for net synthesis or degradation of any of the intermediates in the pathway unlike, for example, the gluconeogenesis pathway or the glycolysis pathway. However, we will see later on (Section 13.6) that the citric acid pathway doesn't always operate in isolation and appearances can be deceiving. Some of the intermediates are shared with other pathways. Let's first examine the catalytic aspect of the citric acid cycle by examining each of the eight enzymatic steps.

1. Citrate Synthase

In the first reaction of the citric acid cycle, acetyl CoA reacts with oxaloacetate and water to form citrate, HS-CoA, and a proton. This reaction is catalyzed by citrate synthase and results in the formation of an enzyme-bound intermediate called citryl CoA (Figure 13.6).

Citrate is the first of two tricarboxylic acids that are part of the cycle. The standard Gibbs free energy change for the citrate synthase reaction is -31.5 kJ·mol^{-1} ($\Delta G°' = -31.5$ kJ·mol^{-1}) due to the hydrolysis of the high energy thioester bond in the citryl CoA intermediate. Normally you might expect that such a large negative Gibbs free energy change

▼ **Figure 13.6**

Reaction catalyzed by citrate synthase. In the first step, acetyl CoA combines with oxaloacetate to form an enzyme-bound intermediate, citryl CoA. The thioester is hydrolyzed to release the products, citrate and HS-CoA.
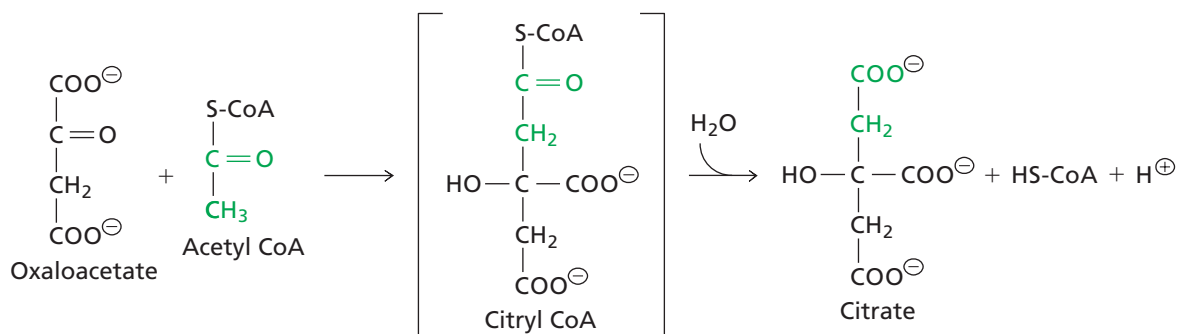
would be coupled to synthesis of ATP—keeping in mind that the actual Gibbs free energy change inside the cell might be very different. Indeed, the hydrolysis of the similar thioester bond in succinyl CoA (Reaction 5 of the citric acid cycle) is coupled to synthesis of GTP (or ATP). However, in the case of the citrate synthase reaction, the available energy is used for a different purpose. It ensures that the reaction proceeds in the direction of citrate synthesis when the concentration of oxaloacetate is very low (Figure 13.7). This appears to be the normal situation when the citric acid cycle is operating. In the presence of only small (catalytic) amounts of oxaloacetate the equilibrium of the reaction depicted in Figure 13.6 still favors citrate synthesis. In other words, the actual Gibbs free energy change inside the cell is close to zero. The reaction is a near-equilibrium reaction. The thermodynamics ensures that the citric acid cycle operates in the direction of acetyl CoA oxidation even under conditions where the concentration of oxaloacetate is very low.

Citrate synthase is a transferase—one of the six categories of enzymes described in Section 5.1. Transferases catalyze transfer reactions, in this case transfer of an acetyl group. The term "synthase" is used for transferases that do not use ATP as a cofactor. "Synthetases," on the other hand, are members of the ligase category of enzymes (Section 5.1). The reactions catalyzed by synthetases must be coupled to ATP (or GTP) hydrolysis. It's important to remember the difference between synthases and synthetases since the words look very similar and since the citric acid cycle contains an example of each type of enzyme. (For some reason, it's easier to pronounce "synthetase" and it's tempting to throw in the extra syllable when you should be saying "synthase.")

In gram-positive bacteria, archaebacteria, and eukaryotes, citrate synthase is a dimeric protein composed of two identical subunits. In gram-negative bacteria, the enzymes are hexameric complexes of identical subunits.

In animals each subunit of the enzyme has two distinct domains: a small flexible domain on the outer surface and a larger domain that forms the core of the protein (Figure 13.8). The two subunits associate by interactions between four α helices in each of the large domains to form an α helix sandwich. Citrate synthase undergoes a large conformational change on binding oxaloacetate as shown in Figure 13.8. The binding site lies at the base of a deep cleft between the small domain of one subunit and the large domain of the other subunit. When oxaloacetate is bound, the small domain rotates by 20° relative to the large domain. This closure creates the binding site for acetyl CoA—a site which is formed by amino acid side chains from both large and small domains. When the reaction is complete coenzyme A is released. The enzyme then reverts to the open conformation when citrate is released.

The structure of the enzyme requires that oxaloacetate and acetyl CoA bind sequentially. This reduces the chance of binding acetyl CoA in the absence of oxaloacetate and
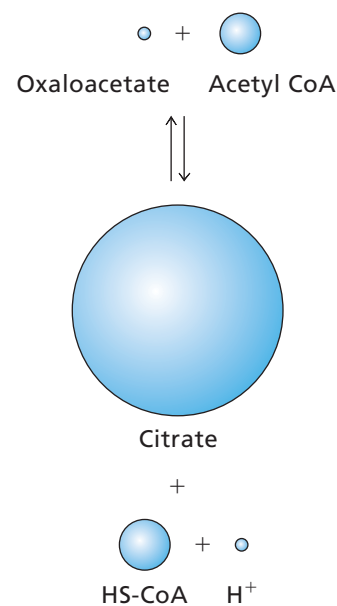
▲ **Figure 13.7**
Representation of the relative ratios of products and reactants in the citrate synthase reaction. The equilibrium constant (K_{eq}) for the citrate synthase reaction can be calculated from standard Gibbs free energy change according to Equation 1.12, $K_{eq} = 2.7 \times 10^5$, meaning that, at equilibrium, the concentrations of products are more than 200,000 times that of the reactants. [Not to scale.]

▼ **Figure 13.8**
Citrate synthase induced fit mechanism. The two identical subunits are colored blue and purple. Each is composed of a small and a large domain. **(a)** Open conformation. The substrate binding site is located in the deep cleft between the small domain of one subunit and the large domain of the other. [PDB 5CSC] **(b)** Closed conformation. The small domain has shifted relative to the large domain in order to close off the large binding cleft seen in the open conformation. Substrate analogues are shown as space-filling models. This version of the enzyme is from chicken (*Gallus gallus*). [PDB 6CSC]

(a)

Small domain

Large domain

(b)

20° rotation
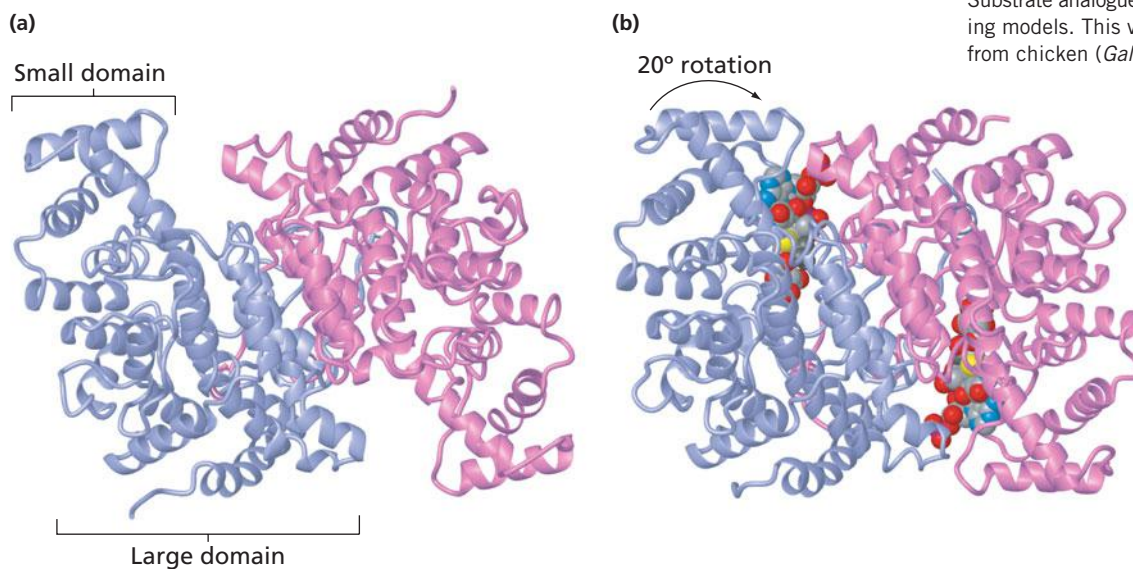
BOX 13.3 CITRIC ACID

The discovery of citric acid is usually attributed to Abu Musa Jābir ibn Hayyān (~721–~815), known as Geber in Europe. He worked in Kufa in modern-day Iraq and is recognized as the father of modern chemistry. Jābir identified citric acid as a major component of citrus fruits such as lemons and limes. We know now that the level of citric acid in these fruits is related to its ability to act as a preservative and a reservoir of carbon. This is unrelated to the role of citrate in the citric acid cycle.

Citric acid is a weak organic acid (pK_{a1}= 3.2, pK_{a2}= 4.8, pK_{a3}= 6.4). The sodium salt is sometimes used as a buffer in biochemistry labs and in drugs but its most important application is as a food additive, especially in soft drinks.

▲ Citric acid is an important natural preservative in citrus fruits.

---

the possibility of catalyzing hydrolysis of the thioester bond of acetyl CoA in a wasteful reaction. This potential side reaction is a very real danger since the thioester bond of acetyl CoA is near the active site for hydrolysis of the citryl CoA thioester and since the concentration of oxaloacetate may be very low relative to that of acetyl CoA. Our previous examples of an induced fit mechanism involved protecting ATP from inappropriate hydrolysis but the same principle applies here. We will encounter several other examples of important structure–function relationships in this chapter and the next one.

2. Aconitase

Aconitase (systematic name: aconitate hydratase) catalyzes a near-equilibrium conversion of citrate to isocitrate. Citrate is a tertiary alcohol and thus cannot be oxidized directly to a keto acid. The formation of a keto acid intermediate is required for the oxidative decarboxylation reaction that occurs in step 3 of the citric acid cycle. The step catalyzed by aconitase creates a secondary alcohol in preparation for step 3. The name of the enzyme is derived from *cis*-aconitate, an enzyme-bound intermediate of the reaction. The reaction proceeds by the elimination of water from citrate to form a carbon–carbon double bond. This is followed by stereospecifc addition of water to form isocitrate.

KEY CONCEPT

Stereospecific reactions occur because substrates bind to enzymes in specific orientations.
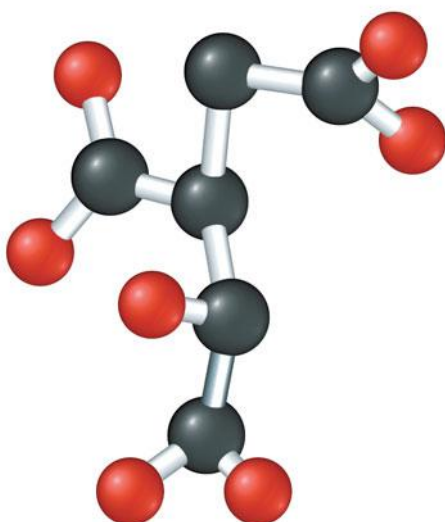
▲ Figure 13.9
Structure of 2R,3S-isocitrate.

(13.10)
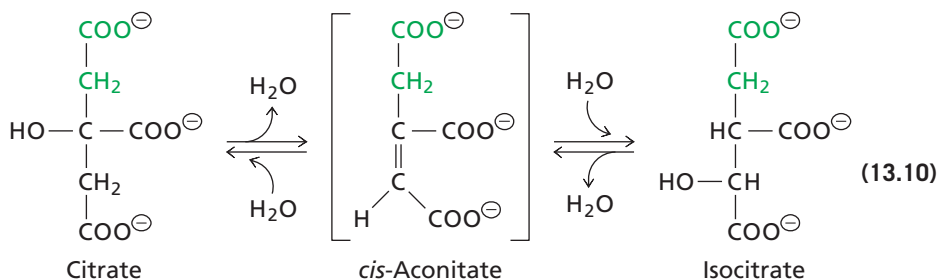
The aconitase gene is a member of a complex gene family. The family encodes distinct mitochondrial and cytoplasmic versions of aconitase, a regulatory protein with no catalytic activity, and an enzyme involved in the synthesis of amino acids (Sections 13.8 and 17.3C). Bacteria contain two distantly related enzymes, aconitase A and aconitase B. All family members contain a characteristic [4 Fe–4 S] iron–sulfur cluster. In the next chapter we will encounter many oxidation–reduction enzymes with iron–sulfur clusters. In most of these oxidation–reduction enzymes, the iron–sulfur clusters participate in electron transport but members of the aconitase family are unusual because the role of the iron–sulfur cluster is to aid in the binding of citrate. The aconitase reaction is an isomerization reaction and not an oxidation-reduction reaction.

Note that citrate is not a chiral molecule because none of the carbon atoms is bonded to four different groups. However, the product of the reaction, isocitrate, has two chiral centers, C2 and C3. Each of these carbon atoms has four different constituents

BOX 13.4 THREE POINT ATTACHMENT OF PROCHIRAL SUBSTRATES TO ENZYMES

When the citric acid cycle was first proposed by Krebs, the inclusion of the citrate-to-isocitrate reaction was a major barrier to its acceptance because labeling studies indicated that only one of the two possible forms of 2R,3S-isocitrate was produced in cells. The "problem" was not that a chiral molecule was produced from a non-chiral molecule—this is easily understood. The difficulty was in understanding why formation of the double bond of *cis*-aconitate, and subsequent addition of water to form isocitrate, occurred only in the moiety contributed originally by oxaloacetate and not in the group derived from acetyl CoA. When isotopically labeled acetate was added to cells the ^{14}C-labeled carbon atoms appeared in citrate as shown in green in Reaction 13.10. Since citrate is a symmetric molecule, the labeled carbon atoms were expected to show up equally in the two versions of isocitrate shown in the figure on the right.

Instead, only the left-hand form was produced. At the time, conversion of a non-chiral molecule to a single form of chiral isomer was unknown but in 1948, Alexander Ogston showed how the active site of an enzyme could distinguish between chemically equivalent groups on the citrate molecule. Ogston envisioned citrate binding in a manner he called three point attachment, with nonidentical groups involved in the enzyme–substrate binding (see figure). Once citrate is correctly bound to the asymmetric binding site, the two —CH_2—$COO^\ominus$ groups of citrate have specific orientations and thus are no longer equivalent. Formation of the carbon–carbon double bond can only take place in the group contributed by oxaloacetate.

Citrate is a *prochiral* molecule because it can react asymmetrically in spite of the fact that it is chemically symmetric. There are now many examples of such reactions in metabolic pathways.

reactive group

no reaction
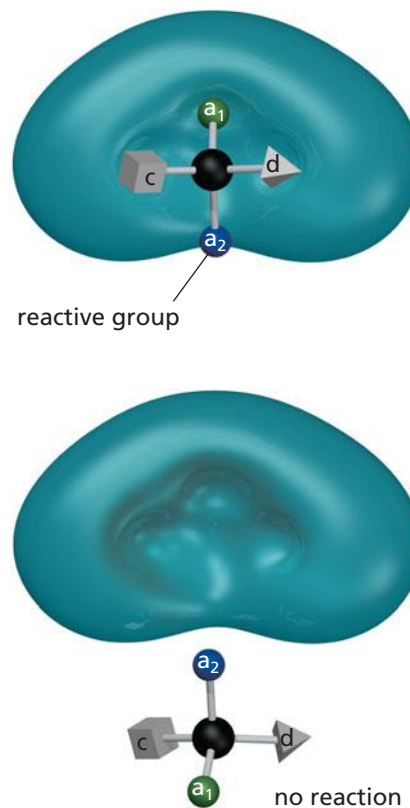
▲ **Binding of citrate to the active site of aconitase.** The central carbon atom of the citrate molecule is shown with four attached groups: the hydroxyl group (—OH) is represented by a square; the carboxyl group (—COOH) by a triangle; the two —CH2—COO— groups are shown as spheres. The two —CH2—COO— groups are chemically indistinguishable, but the one derived from acetyl CoA is shown as a green sphere and the one derived from oxaolacetate is colored blue. A cartoon of aconitase is depicted as an asymmetric molecule with three-point attachments sites for the hydroxyl group, the carboxyl group, and one of the —CH2—COO— groups. When citrate is oriented as shown in the top figure, it can bind to aconitase and the reaction takes place in the moiety derived from oxaloacetate. The other orientation (bottom) cannot bind to the enzyme and the reaction cannot take place in the group derived from acetyl CoA.

▲ **Two forms of isocitrate.** The green carbon atoms represent the group originally derived from acetyl CoA. The reaction catalyzed by aconitase was expected to yield two forms of isocitrate in equal quantities because the substrate (citrate) is symmetric. Only the left-hand form was produced.

and in each case the four groups can be arranged in two different orientations. There are four different stereoisomers of isocitrate but only one of them is produced in the reaction catalyzed by aconitase. The formal name of this product is 2R,3S-isocitrate (Figure 13.9) using the RS nomenclature described in Box 3.2. This is one of the few times when this nomenclature is useful in introductory biochemistry.

3. Isocitrate Dehydrogenase

Isocitrate dehydrogenase catalyzes the oxidative decarboxylation of isocitrate to form α-ketoglutarate (Figure 13.10). This reaction is the first of four oxidation–reduction reactions in the citric acid cycle. The reaction is coupled to the reduction of $NAD^\oplus$ and occurs in two steps involving an enzyme-bound oxalosuccinate intermediate.

> The regulation of isocitrate dehydrogenase in prokaryotes is described in Section 13.8.
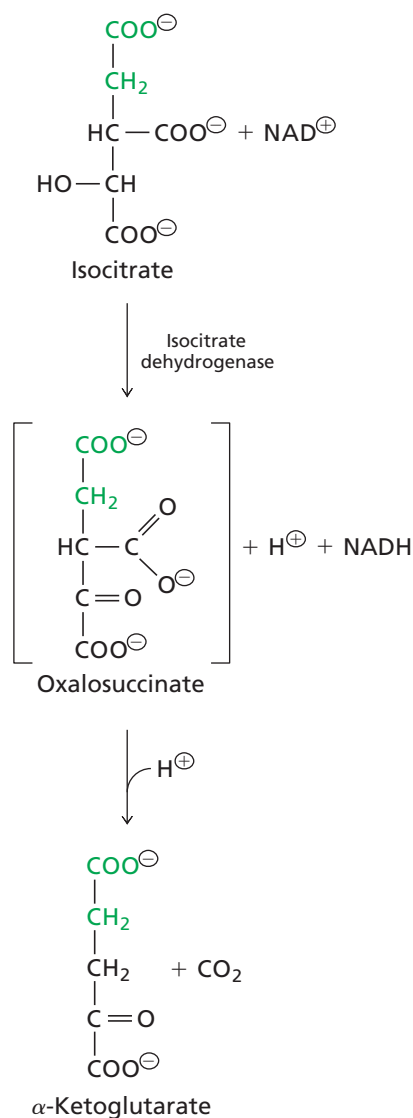
▲ **Figure 13.10**
Isocitrate dehydrogenase reaction. The enzyme catalyzes an oxidation–reduction reaction using $NAD^{\oplus}$ as the electron acceptor. Oxalosuccinate is an unstable intermediate that is rapidly decarboxylated to CO_2 and α-ketoglutarate. This is the first decarboxylation step in the citric acid cycle.

KEY CONCEPT

The important "pay off" reactions of the citric acid cycle are those that produce reducing equivalents such as NADH and QH_2.

In the first step, the alcohol group of isocitrate is oxidized by removal of two hydrogens to form a —C═O double bond. This is a typical dehydrogenase reaction. One of the hydrogens (the one bound to the carbon atom) is transferred to $NAD^{\oplus}$ as a hydride ion carrying two electrons and the other (the one on the —OH group) is incorporated into the final product. This is the first of the reactions that result in the loss of electrons (i.e., oxidation of an organic acid).

Oxalosuccinate, an unstable keto acid, is the product of the first step in the overall reaction catalyzed by α-ketoglutarate dehydrogenase. Before it is released from the enzyme, the intermediate undergoes decarboxylation to form α-ketoglutarate in the second step of the reaction. The decarboxylation reaction is associated with the release of CO_2 and uptake of a proton. The overall stoichiometry of the reaction is

$$\text{Isocitrate} + NAD^{\oplus} \longrightarrow \alpha\text{-Ketoglutarate} + NADH + CO_2 \qquad \textbf{(13.11)}$$

There are several different versions of isocitrate dehydrogenase. Bacteria contain both an $NAD^{\oplus}$-dependent enzyme and an $NADP^{\oplus}$-dependent enzyme. Eukaryotes also have both types but, in addition, the $NADP^{\oplus}$-dependent enzymes form several subclasses. In general, the $NAD^{\oplus}$-dependent enzyme is localized to the mitochondria and plays the major role in the citric acid cycle. The $NADP^{\oplus}$-dependent enzymes are found in the cytoplasm, chloroplasts, and other membrane compartments. All forms of the enzymes are homologous by sequence similarity and they share a common ancestor with an enzyme in the leucine biosynthesis pathway (Section 13.9, Section 17.3C).

4. The α-Ketoglutarate Dehydrogenase Complex

Oxidative decarboxylation of α-ketoglutarate is analogous to the reaction catalyzed by pyruvate dehydrogenase. In both cases, the reactants are an α-keto acid and HS-CoA and the products are CO_2 and a "high energy" thioester compound. Step 4 of the citric acid cycle is catalyzed by α-ketoglutarate dehydrogenase (also known as 2-oxoglutarate dehydrogenase) (Figure 13.11)

α-Ketoglutarate dehydrogenase is a large complex that resembles pyruvate dehydrogenase in both structure and function. The same coenzymes are involved and the reaction mechanism is the same. The three component enzymes of the α-ketoglutarate dehydrogenase complex are α-ketoglutarate dehydrogenase (E_1, containing TDP), dihydrolipoamide succinyl transferase (E_2, containing a lipoamide swinging arm), and dihydrolipoamide dehydrogenase (E_3, the same flavoprotein found in the pyruvate dehydrogenase complex). The overall reaction is the second of the two CO_2 producing reactions in the citric acid cycle and the second reaction that generates reducing equivalents. In the four remaining reactions of the cycle, the four-carbon succinyl group of succinyl CoA is converted back to oxaloacetate.

Eukaryotic cells have a single mitochondrial α-ketoglutarate dehydrogenase. Archaebacteria, and some other species of bacteria, do not have α-ketoglutarate dehydrogenase. Instead, they convert α-ketoglutarate to succinyl CoA using an entirely different enzyme called 2-oxoglutarate:ferredoxin oxidoreductase.

5. Succinyl CoA Synthetase

The conversion of succinyl CoA to succinate is catalyzed by succinyl CoA synthetase, sometimes called succinate thiokinase. The reaction couples hydrolysis of the thioester linkage in succinyl CoA to formation of a nucleoside triphosphate—either GTP or ATP, depending on the species. The complicated IUPAC names of these two related enzymes are: succinate-CoA ligase, ADP-forming (E.C. 6.2.1.5); and succinate-CoA ligase, GDP-forming (E.C. 6.2.1.4).

Inorganic phosphate is one of the reactants and the reaction takes place in three steps (Figure 13.12).

The first step generates succinyl phosphate as an intermediate and releases coenzyme A. In the second step, the phosphoryl group is transferred to a histidine side chain in the active site of the enzyme to form a stable phosphoenzyme intermediate. The third step transfers the phosphoryl group to GDP to form GTP. This reaction is the only example of *substrate level phosphorylation* in the citric acid cycle. (Recall from Section 10.8

that the standard Gibbs free energy change for hydrolysis of the thioester linkage in succinyl CoA is approximately equivalent to that of ATP hydrolysis.) The overall stoichiometry of the succinyl CoA synthetase reaction is

$$\text{Succinyl CoA} + P_i + \text{GDP} \longrightarrow \text{Succinate} + \text{HS-CoA} + \text{GTP} \qquad \textbf{(13.12)}$$

Inorganic phosphate contributes the phosphoryl group to GDP, plus an oxygen to form succinate and a hydrogen to form HS-CoA. Note that the enzyme is named for the reverse reaction where succinyl CoA is synthesized from succinate at the expense of GTP or ATP. It is called a synthetase because the reaction combines two molecules and it is coupled to hydrolysis of nucleoside triphosphate.

The enzyme is composed of two α and two β subunits ($\alpha_2\beta_2$). The β subunits contain the binding site for the nucleotide. Bacterial versions use ATP while animals often have two versions of the enzyme—one that uses GTP and one that uses ATP. They differ in their β subunits. The GTP-dependent versions clearly have evolved from the ATP-dependent versions. It's not clear why animal mitochondria have two versions of succinyl CoA synthetase in their mitochondria but one possibility is that the ATP-dependent version is used in the citric acid cycle and the GTP-dependent version primarily catalyzes the reverse reaction in some cells. Archaebacteria, and some other bacteria, do not have succinyl CoA synthetase. They carry out a similar reaction using an entirely different enzyme.

6. Succinate Dehydrogenase Complex

Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate forming a carbon–carbon double bond with the loss of two protons and two electrons (Figure 13.13). The protons and electrons are passed to a quinone, which is reduced to QH_2. (Ubiquinone is the preferred substrate in almost all cases but some bacteria use menaquinone.) The enzyme is present in all species and FAD is an essential bound cofactor.

One important feature of this reaction is the scrambling of the original acetyl carbon atoms. They can no longer be specifically identified (i.e., green) in the symmetrical
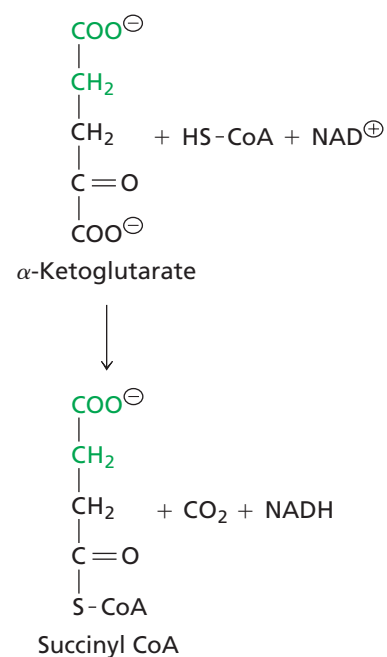
▲ **Figure 13.11**
Reaction catalyzed by α-ketoglutarate dehydrogenase. This is similar to the reaction catalyzed by pyruvate dehydrogenase.

The structure of menaquinone is shown in Figure 14.21.

BOX 13.5 WHAT'S IN A NAME?

α-Ketoglutarate is clearly named after the five-carbon dicarboxylic acid glutarate ($^{\ominus}$OOC—CH_2—CH_2—CH_2—COO$^{\ominus}$). The keto group is on the α carbon or the first carbon after one of the carboxyl groups. This naming convention is similar to the one we encountered in naming α-amino acids (Section 3.1). As is the case with amino acids, the correct chemical name, or systematic name, for α-ketoglutarate could be "2-ketoglutarate." However, the formal name is actually 2-oxoglutarate since according to the IUPAC/IUBMB rules of nomenclature the term "keto" should now be avoided.

It is perfectly acceptable to refer to organic molecules by their common (trivial) names if these common names are well known. For example, if you look back to step 1 of the citric acid cycle you can see that the systematic name for oxaloacetate is 2-oxosuccinate since it is a derivative of the four-carbon dicarboxylic acid, succinate. "Oxaloacetate" is the well-known and accepted common name for this compound and it would be confusing to use any other name. When it comes to the correct name for α-ketoglutarate, the situation is more complicated because α-ketoglutarate is the old-fashioned systematic name of the molecule and the new

rules say that the systematic name should be 2-oxoglutarate. The new name is becoming more and more popular in the scientific literature. Here, we continue to use the well-known name α-ketoglutarate on the grounds that it has become an acceptable common name for this compound. It's very likely that this will change in future editions.
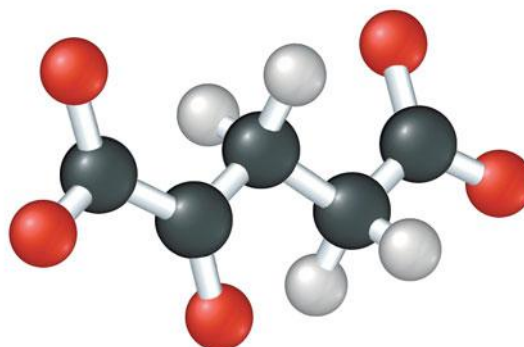
▶ 2-oxoglutarate, aka α-ketoglutarate.
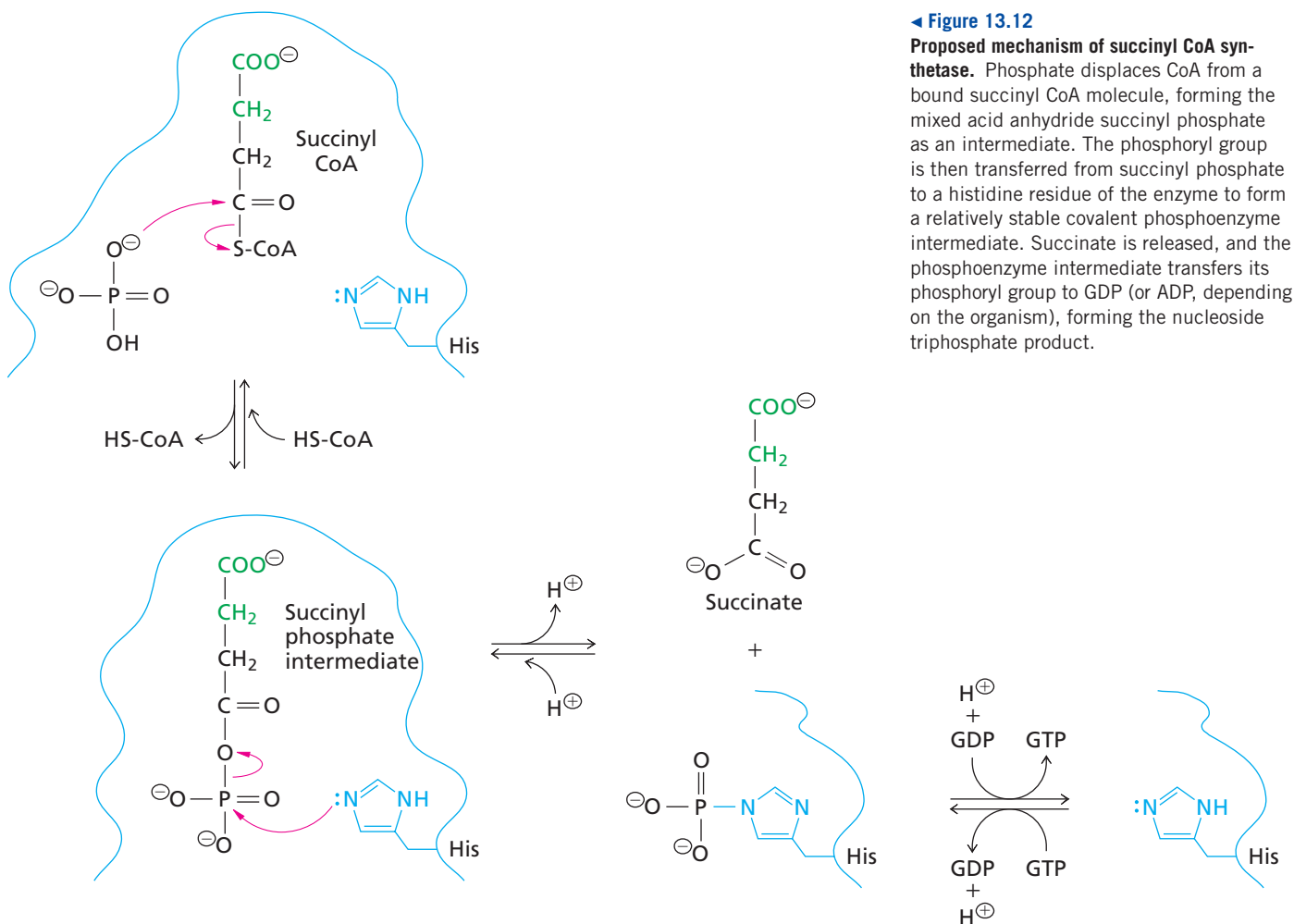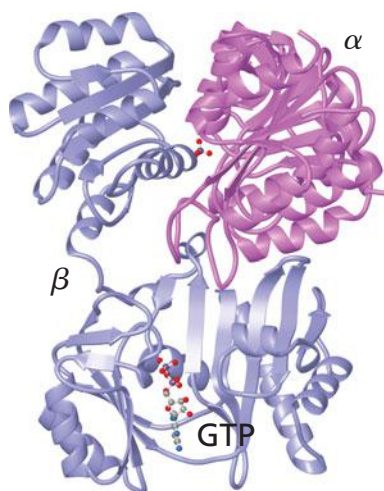
▲ **GTP-dependent succinyl CoA synthetase.** The structure of one unit of the dimer is shown with the α and β subunits in different colors. A molecule of GTP is bound at the active site within the β subunit. This is the pig (*Sus scrofa*) version of the enzyme. [PDB 2FPG]

reactant, succinate, or in the product, fumarate. This has interesting consequences (see Problem #6).

The active site of the enzyme is formed from two different subunits. One subunit contains iron–sulfur clusters and the other is a flavoprotein with covalently bound FAD. The succinate dehydrogenase dimer is bound to two membrane polypeptides to form a larger complex. The membrane components consist of a cytochrome *b*, with its associated heme group, and a quinone binding site. The electron transport cofactors participate in the transfer of electrons from succinate to FAD to several iron–sulfur clusters to heme to the quinone.

Recall that $FADH_2$ in subunit E_3 of pyruvate dehydrogenase is reoxidized by $NAD^{\oplus}$ to complete the catalytic cycle of that enzyme. In the succinate dehydrogenase reaction, $FADH_2$ is reoxidized by Q to regenerate FAD. In the past, it was very common to show $FADH_2$ as the redox product of this reaction but since FAD is covalently bound to the enzyme, the catalytic cycle is not completed until bound $FADH_2$ is reoxidized and the mobile product QH_2 is released.

The succinate dehydrogenase reaction is unusual for a dehydrogenase because it uses ubiquinone as an electron acceptor (oxidizing agent) instead of $NAD^{\oplus}$. It is also unusual in many other ways, as we will see in the next chapter. The succinate dehydrogenase complex is part of the electron transport system located in the plasma membrane of prokaryotes and in the inner mitochondrial membrane in eukaryotic cells. We will discuss this enzyme in more detail in Section 14.6 and examine its structure (Figure 14.9). In bacteria, the bulk of the enzyme complex projects into the cytoplasm where it can bind succinate and release fumarate as part of the citric acid cycle. In mitochondria, the active site is on the matrix side of the membrane where the other citric acid cycle enzymes are located.

The substrate analog malonate is a competitive inhibitor of the succinate dehydrogenase complex as described in Section 5.7A. Malonate, like succinate, is a dicarboxylate that binds to cationic amino acid residues in the active site of the succinate dehydrogenase complex. However, malonate cannot undergo oxidation because it lacks the —CH_2—CH_2— group necessary for dehydrogenation. In experiments with isolated mitochondria or cell homogenates, the presence of malonate caused succinate, α-ketoglutarate, and citrate to accumulate. Such experiments provided some of the original evidence for the sequence of reactions in the citric acid cycle.

7. Fumarase

Fumarase (systematic name: fumarate hydratase) catalyzes the near-equilibrium conversion of fumarate to malate through the stereospecific *trans* addition of water to the double bond of fumarate.

(13.13)
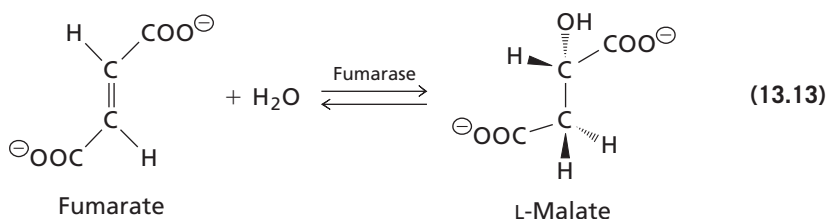
Fumarate is a prochiral molecule. When fumarate is positioned in the active site of fumarase, the double bond of the substrate can be attacked from only one direction. The product of the reaction is exclusively the L stereoisomer of the hydroxy acid malate.

There are two unrelated fumarases that can catalyze the same reaction. The class I enzyme is found in most bacteria. The class II enzyme is present in some bacteria and all eukaryotes. Some bacteria, such as *E. coli*, have both forms of the enzyme. One form is active in the normal citric acid cycle pathway and the other usually specializes in the reverse reaction to convert malate to fumarate.

8. Malate Deydrogenase

The last step in the citric acid cycle is the oxidation of malate to regenerate oxaloacetate, with formation of a molecule of NADH.

(13.14)

▲ **Figure 13.13**
The succinate dehydrogenase reaction.

▲ **Green (unripe) apples.** The sour taste of unripe apples is mostly due to the presence of malate. Malic acid was first isolated from apple juice and it was named after the Latin word for apple (*malum*).

We consider the transfer of reducing equivalents to Q again in Chapter 14, where we will see the role of the succinate dehydrogenase complex in membrane-associated electron transport.

The evolutionary origin of fumarase and the significance of the reverse reaction in bacteria are described in Section 13.8.

BOX 13.6 ON THE ACCURACY OF THE WORLD WIDE WEB

There's lots of good stuff on the web but everyone should be cautious about the quality of some webpages. The citric acid cycle is a fun test case for accuracy. Most sites get the basics correct but students are often challenged to find a website that accurately depicts every reaction of the pathway with no errors—including balancing every equation. Can you find such a website? The most common errors are leaving out protons and QH_2.

The one site you can rely on is the IUBMB Enzyme Nomenclature site that lists the correct reactions for each enzyme in the citric acid cycle: **www.chem.qmul.ac.uk/iubmb/enzyme/**

Some instructors have been known to give extra marks to students who can find a completely accurate website. Some students have been known to create their own webpages.

This reaction is catalyzed by $NAD^{\oplus}$-dependent malate dehydrogenase. The near-equilibrium interconversion of the α-hydroxy acid L-malate and the keto acid oxaloacetate is analogous to the reversible reaction catalyzed by lactate dehydrogenase (Sections 7.3 and 11.3B). This is not surprising since lactate dehydrogenase and malate dehydrogenase are homologous—they share a common ancestor.

The standard Gibbs free energy change for this reaction is +30 kJ mol^{-1} ($\Delta G^{\circ\prime}$ = 30 kJ mol^{-1}). Since this is a near-equilibrium reaction it means that under the conditions found inside the cell, the concentration of malate is very much higher than that of oxaloacetate. We've seen in the case of the citrate synthase reaction that the low concentration of oxaloacetate explains the Gibbs free energy change of that reaction. In the next section we'll see how the low concentration of oxaloacetate relative to that of malate explains some transport pathways.

> The structures of malate dehydrogenase and lactate dehydrogenase are compared in Figure 4.22.

13.4 Entry of Pyruvate Into Mitochondria

In bacterial cells, pyruvate is converted to acetyl CoA in the cytosol but in eukaryotic cells the pyruvate dehydrogenase complex is located in mitochondria (and in chloroplasts). Since glycolysis takes place in the cytoplasm, pyruvate must first be imported into the mitochondria (or chloroplasts) so that it can serve as a substrate in the reaction. The mitochondrion is enclosed by a double membrane. Small molecules such as

---

BOX 13.7 CONVERTING ONE ENZYME INTO ANOTHER

Despite having a low sequence identity, lactate dehydrogenase and malate dehydrogenase are closely related in three-dimensional structure and they clearly have evolved from a common ancestor. These enzymes catalyze reversible oxidation of 2-hydroxy acids that differ by only one carbon (malate has an additional carboxylate attached to C-3 of lactate). Both enzymes are highly specific for their own substrates. However, site specific mutation of a single amino acid residue of the lactate dehydrogenase of *Bacillus stearothermophilus* changes this enzyme to a malate dehydrogenase (see figure). Conversion of Gln-102 to Arg-102 completely reverses the specificity of the dehydrogenase. The positively charged side chain of the arginine forms an ion pair with the 4-carboxylate group of malate, and the mutant enzyme becomes inactive with lactate.
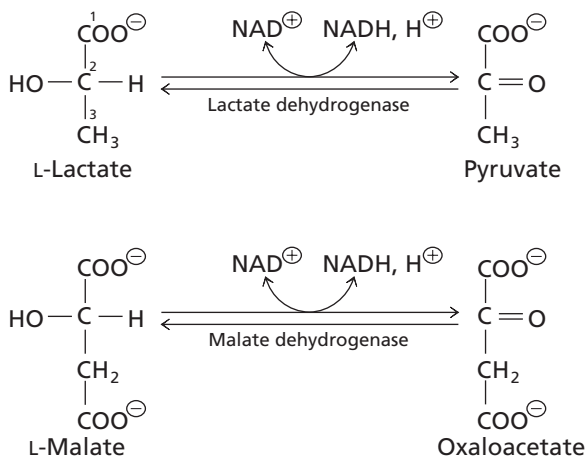
▲ Orientation of the substrate molecule in the active site of lactate dehydrogenase from *Bacillus stearmothermophilus*. **(a)** The three-carbon substrate pyruvate bound to the native enzyme. Neither oxaloacetate nor malate can bind at this site. **(b)** The four-carbon substrate oxaloacetate bound to the Gln-to-Arg mutant (position 102).
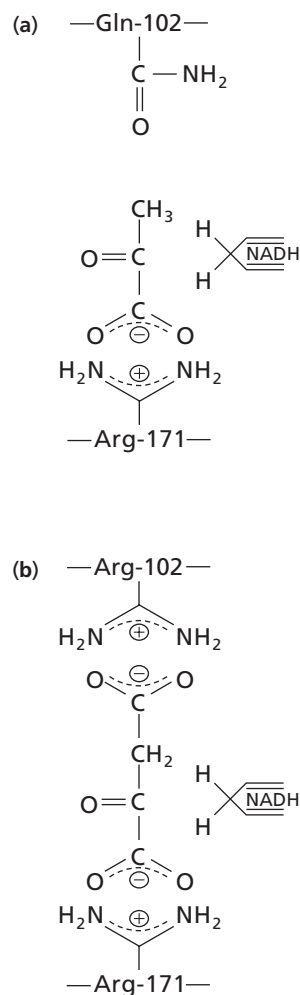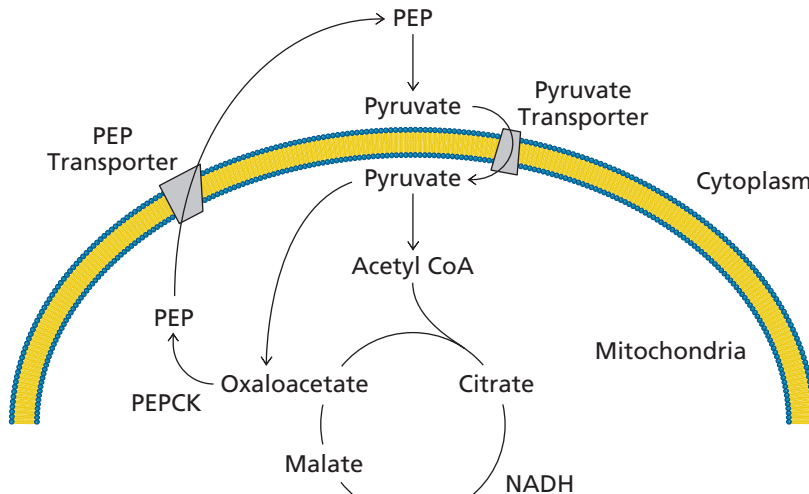
pyruvate pass through the outer membrane via aqueous channels formed by transmembrane proteins called porins (Section 9.11A). These channels allow free diffusion of molecules with molecular weights less than 10,000. However, in order to pass through the inner membrane a specific transport protein is required for most metabolites. Pyruvate translocase specifically transports pyruvate in symport with $H^{\oplus}$. Once inside the mitochondrion, pyruvate can be converted to acetyl CoA and CO_2. In eukaryotic cells the enzymes of the citric acid cycle are also located in the mitochondria (Figure 13.14).

Recall that one of the intermediates in the citric acid cycle is oxaloacetate and it can also be a substrate for gluconeogenesis. Since gluconeogenesis is a cytoplasmic pathway, it's necessary to move oxaloacetate, or its equivalent, from the mitochondria to the cytoplasm. In mammals this is accomplished using a mitochondrial version of phosphoenolpyruvate carboxykinase (PEPCK), that converts oxaloacetate to phosphoenolpyruvate (PEP). Mitochondria possess a PEP transporter that moves PEP to the cytoplasm (Figure 13.14). It would be very inefficient to transport oxaloacetate directly because its concentration in the mitochondria is very low compared to its concentration in the cytoplasm. (Deficiencies in the human mitochondrial PEPCK lead to death within the first two years of life.)

There are two other problems associated with the compartmentation of the citric acid cycle in mitochondria. Acetyl CoA is required for fatty acid synthesis in the cytoplasm, so there has to be a mechanism for transporting acetyl CoA from the mitochondria to the cytoplasm. This is accomplished using a tricarboxylic acid transporter that exports citrate. Once in the cytoplasm, citrate has to be reconverted to oxaloacetate and acetyl CoA and this is accomplished by a cytoplasmic enzyme called ATP-citrate lyase (Figure 13.15). ATP-citrate lyase doesn't just catalyze the reverse of the citrate synthase reaction. The enzyme has to be coupled to hydrolysis of ATP in order to drive the synthesis of "high energy" acetyl CoA in the cytoplasm. The mitochondrial enzyme can catalyze the same reaction (reversing the citric acid cycle reaction) because the concentration of citrate is so high relative to oxaloacetate (see Figure 13.7). In the cytoplasm, on the other hand, the steady state concentrations of citrate and oxaloacetate are comparable, so coupling to ATP hydrolysis is necessary.

Some species don't have a mitochondrial version of PEPCK so they have to use an alternative method of exporting oxaloacetate. The malate–aspartate shuttle is a common transport system, present even in species that have a mitochondrial PEPCK. A simplifed version of this shuttle is shown in Figure 13.16. We will describe it in more detail in Section 14.12.

Oxaloacetate is converted to malate by the reaction catalyzed by malate dehydrogenase. This is the same enzyme used in the citric acid cycle. Recall that the equilibrium concentrations of reactants and products in this reaction result in a very much higher concentration of malate than oxaloacetate. Thus, a malate transporter is much more efficient than an oxaloacetate transporter could be.

$$\text{Citrate} + \text{ATP} + \text{HS-CoA} \underset{\text{ATP-citrate lyase}}{\rightleftharpoons} \text{Oxaloacetate} + \text{Acetyl CoA} + \text{ADP} + P_i$$
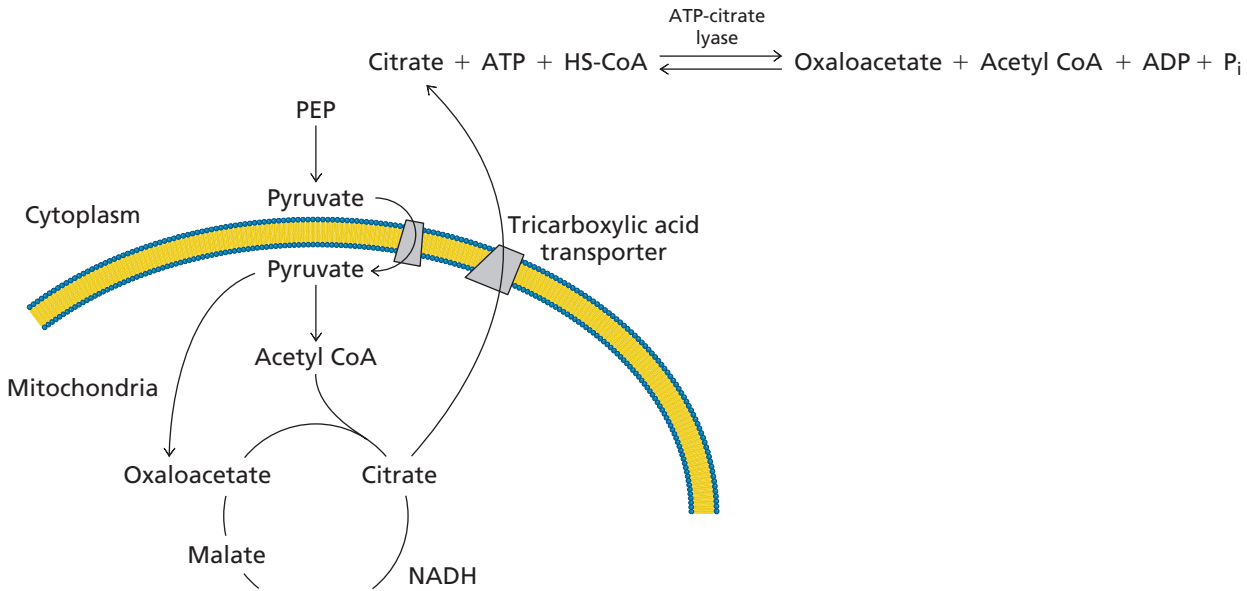
Figure 13.15 ▲
Export of acetyl CoA from mitochondria. Citrate is exported via the tricarboxylic acid transporter. Citrate is subsequently converted to acetyl CoA by cytoplasmic ATP-citrate lyase.

Malate is converted back to oxaloacetate by a cytoplasmic version of malate dehydrogenase. The net effect is that oxaloacetate from mitochondria can serve as a substrate for gluconeogenesis as described in the previous chapter.

The other part of the shuttle achieves the same goal by using a mitochondrial aminotransferase to convert oxaloacetate to aspartate. Aspartate is transported across the mitochondrial membrane by an aspartate transporter. In the cytoplasm, oxaloacetate can

Figure 13.16 ▶
Transport of oxaloacetate via the malate–aspartate shuttle.
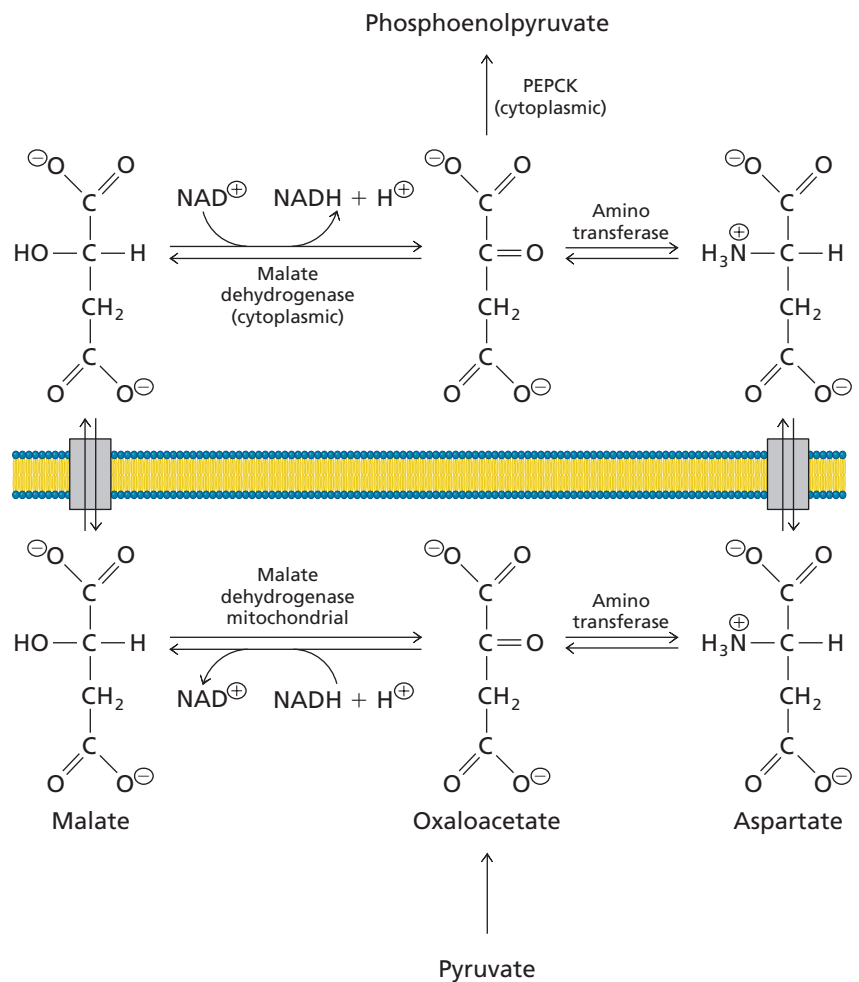
be re-formed by the action of a cytoplasmic aminotransferase. As you might guess, this pathway normally operates in the opposite direction, since the low concentration of oxaloacetate in the mitochondria means that the conversion of oxaloacetate to aspartate is unlikely.

13.5 Reduced Coenzymes Can Fuel Production of ATP

In the net reaction of the citric acid cycle, three molecules of NADH, one molecule of QH_2, and one molecule of GTP or ATP are produced for each molecule of acetyl CoA entering the pathway.

$$\text{Acetyl CoA} + 3\text{ NAD}^{\oplus} + \text{Q} + \text{GDP (or ADP)} + P_i + 2\text{ H}_2\text{O} \longrightarrow$$

$$\text{HS-CoA} + 3\text{ NADH} + QH_2 + \text{GTP (or ATP)} + 2\text{ CO}_2 + 2\text{ H}^{\oplus} \qquad \textbf{(13.15)}$$
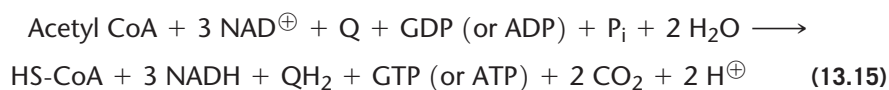
As mentioned earlier, NADH and QH_2 can be oxidized by the membrane-asscociated electron transport chain that is coupled to the the production of ATP. As we will see when we examine these reactions in Chapter 14, approximately 2.5 molecules of ATP are generated for each molecule of NADH oxidized to $NAD^{\oplus}$, and up to 1.5 molecules of ATP are produced for each molecule of QH_2 oxidized to Q. The complete oxidation of one molecule of acetyl CoA by the citric acid cycle and subsequent reactions is therefore associated with the production of approximately ten ATP equivalents (Table 13.2).

The citric acid cycle is the final stage in the catabolism of many major nutrients. It is the pathway for oxidation of all acetyl CoA molecules produced by the degradation of carbohydrates, lipids, and amino acids. Having covered glycolysis in Chapter 11, we can now give a complete accounting of the ATP produced from the degradation of one molecule of glucose.

Recall that glycolysis converts glucose to two molecules of pyruvate with a net gain of two molecules of ATP. There are two molecules of NADH produced in the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase. This corresponds to a combined yield of seven ATP equivalents from glycolysis. The conversion of both pyruvate molecules to acetyl CoA by the pyruvate dehydrogenase complex yields two NADH molecules, which correspond to about five additional molecules of ATP. When these are combined with the ATP equivalents from the citric acid cycle via the oxidation of two molecules of acetyl CoA, the total yield is about 32 molecules of ATP per molecule of glucose (Figure 13.17).

In bacteria, the two molecules of NADH produced by glycolysis in the cytosol can be directly reoxidized by the membrane-associated electron transport system in the plasma membrane. Thus, the theoretical maximum yield from complete oxidation of glucose (32 ATP equivalents) is achieved in bacteria cells.

In eukaryotic cells, glycolysis produces NADH in the cytosol but the membrane-associated electron transport complex is located in mitochondria membranes. The reducing equivalents from cytosolic NADH can be transported into the mitochondrion by shuttle

Table 13.2 Energy production in the citric acid cycle

Reaction	Energy-yielding product	ATP equivalents
Isocitrate dehydrogenase	NADH	2.5
α-Ketoglutarate dehydrogenase complex	NADH	2.5
Succinyl-CoA synthetase	GTP or ATP	1.0
Succinate dehydrogenase complex	QH_2	1.5
Malate dehydrogenase	NADH	2.5
Total		10.0

Figure 13.17 ▶
ATP production from the catabolism of one molecule of glucose by glycolysis, the citric acid cycle, and reoxidation of NADH and QH$_2$. The complete oxidation of glucose leads to the formation of up to 32 molecules of ATP.
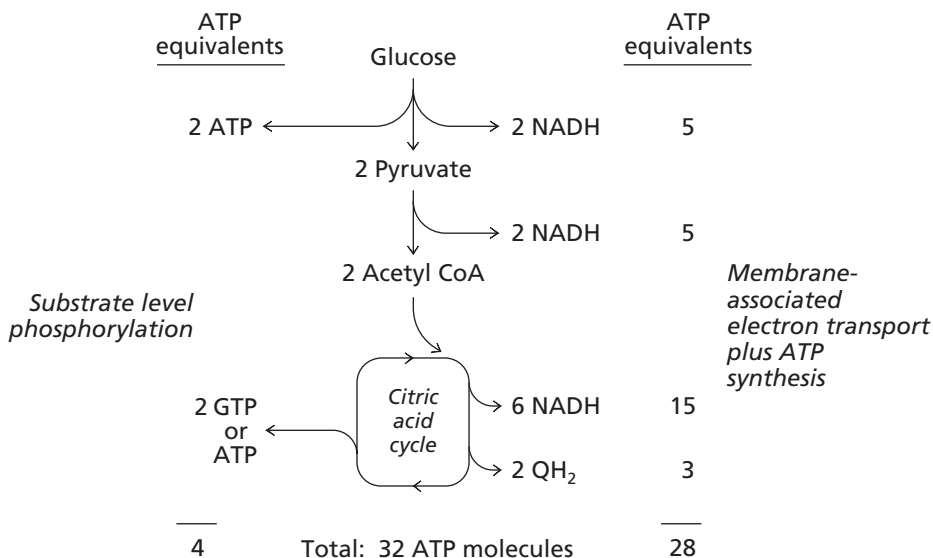
mechanisms such as the malate–aspartate shuttle described in Section 13.4. The transport of reducing equivalents of NADH will be described in more detail in Section 14.12.

It's interesting to compare this pathway (Figure 13.17) for complete oxidation of glucose to the pentose phosphate cycle described in Section 12.4. That pathway also results in the complete oxidation of one molecule of glucose. The result is production of 12 NADPH molecules that are equal to 30 ATP equivalents.

13.6 Regulation of the Citric Acid Cycle

Because the citric acid cycle occupies a central position in cellular metabolism, it's not surprising to find that the pathway is controlled. Regulation is mediated by allosteric modulators and by covalent modification of the citric acid cycle enzymes. Flux through the pathway is further controlled by the supply of acetyl CoA.

As noted earlier, acetyl CoA arises from several sources, including pathways for the degradation of carbohydrates, lipids, and amino acids. The activity of the pyruvate dehydrogenase complex controls the supply of acetyl CoA produced from pyruvate and hence from the degradation of carbohydrates. In general, substrates of the pyruvate dehydrogenase complex activate the complex and products inhibit it. In most species, the activities of the E$_2$ and E$_3$ components of the pyruvate dehydrogenase complex (dihydrolipoamide acetyltransferase and dihydrolipoamide dehydrogenase, respectively) are controlled by simple mass action effects when their products accumulate. The activity of the acetyltransferase (E$_2$) is inhibited when the concentration of acetyl CoA is high, whereas the dehydrogenase (E$_3$) is inhibited by a high NADH/NAD$^{\oplus}$ ratio (Figure 13.18). In general, the inhibitors are likely to be present in high concentrations when energy resources are plentiful, and the activators predominate when energy resources are scarce.

Figure 13.18 ▶
Regulation of the the pyruvate dehydrogenase complex. Accumulation of the products acetyl CoA and NADH decreases flux through the reversible reactions catalyzed by E$_2$ and E$_3$.
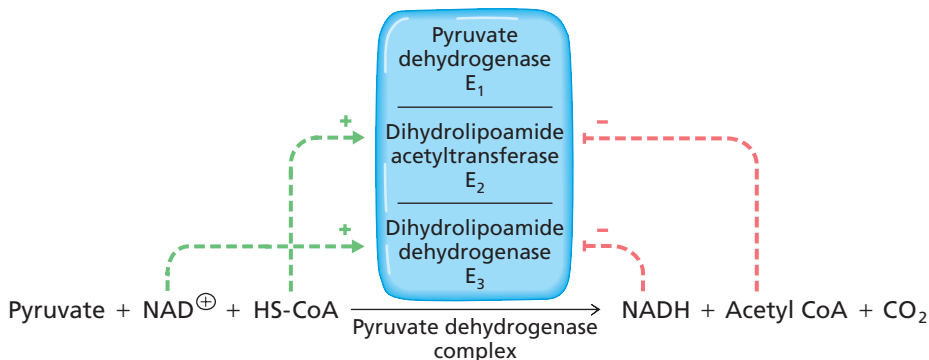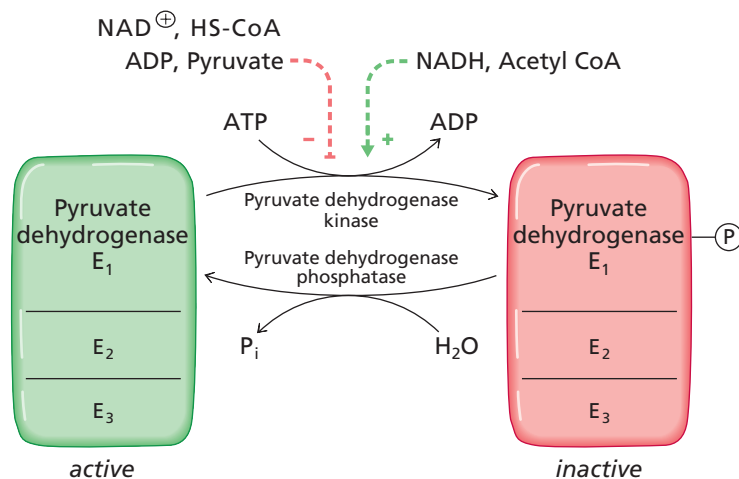
Mammalian (but not prokaryotic) pyruvate dehydrogenase complexes are further regulated by covalent modification. A protein kinase and a protein phosphatase are associated with the mammalian multienzyme complex. Pyruvate dehydrogenase kinase (PDK) catalyzes the phosphorylation of E_1, thereby inactivating the enzyme. Pyruvate dehydrogenase phosphatase (PDP) catalyzes the dephosphorylation and activation of pyruvate dehydrogenase (Figure 13.19). Control of E_1 activity controls the rate of reaction of the entire complex.

Pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase are themselves regulated. The kinase is allosterically activated by NADH and acetyl CoA, products of pyruvate oxidation. The accumulation of NADH and acetyl CoA signals energy availability and leads to an increase in phosphorylation of the pyruvate dehydrogenase subunit and inhibition of the further oxidation of pyruvate. Conversely, pyruvate, $NAD^{\oplus}$, HS-CoA, and ADP inhibit the kinase, leading to activation of the pyruvate dehydrogenase subunit.

Three enzymes of the citric acid cycle are regulated: citrate synthase, isocitrate dehydrogenase, and the α-ketoglutarate dehydrogenase complex. Citrate synthase catalyzes the first reaction of the citric acid cycle. This would seem to be a suitable control point for regulation of the entire cycle. ATP inhibits the enzyme *in vitro*, but significant changes in ATP concentration are unlikely *in vivo*; therefore, ATP may not be a physiological regulator. Some bacterial citrate synthases are activated by α-ketoglutarate and inhibited by NADH.

Mammalian isocitrate dehydrogenase is allosterically activated by Ca^{2+} and ADP and inhibited by NADH. In mammals, the enzyme is not subject to covalent modification. In bacteria, however, isocitrate dehydrogenase is regulated by phosphorylation. We will discuss this in more detail in Section 13.8.

Although the α-ketoglutarate dehydrogenase complex resembles the pyruvate dehydrogenase complex, the enzymes have quite different regulatory features. No kinase or phosphatase is associated with the α-ketoglutarate dehydrogenase complex. Instead, calcium ions bind to E_1 of the complex and decrease the K_m of the enzyme for α-ketoglutarate, thereby increasing the rate of formation of succinyl CoA. NADH and succinyl CoA are inhibitors of the α-ketoglutarate complex *in vitro*, but it has not been established that they have a significant regulatory role in living cells.

13.7 The Citric Acid Cycle Isn't Always a "Cycle"

The citric acid cycle is not exclusively a catabolic pathway for the oxidation of acetyl CoA. It also plays a central role in metabolism at the intersection of several other pathways. Some intermediates of the citric acid cycle are important anabolic precursors in biosynthesis pathways, and some catabolic pathways produce citric acid cycle intermediates. Pathways that are both catabolic and anabolic are said to be *amphibolic* (Section 10.1). The citric acid cycle is an excellent example.

BOX 13.8 A CHEAP CANCER DRUG?

In the absence of oxygen, the glycolytic pathway terminates at lactate and the citric acid cycle is not used in the oxidation of acetyl CoA. Under these conditions, pyruvate dehydrogenase is inactivated by phosphorylation. Many cancer cells grow anaerobically and pyruvate dehydrogenase is not active in these cells.

The activity of pyruvate dehydrogenase phosphorylase kinase (PDHK) can be inhibited by dichloroacetate (DCA). DCA binds to the active site of the enzyme preventing phosphorylation of pyruvate dehydrogenase. The net effect of DCA is activation of pyruvate dehydrogenase and this, in turn, causes major disruptions in cancer cell metabolism leading to death of the cancer cells. The chemical has been effective in a few trial studies with cancer cells *in vitro*. That's a good thing.

Unfortunately, the effectiveness of DCA as a cancer drug has not been demonstrated in clinical trials. Medical researchers are in a difficult position. The biochemistry is sound. It makes sense that cancer cells grow anaerobically (the Warburg effect) and it makes sense that DCA might be an effective cancer drug based on its ability to inhibit PDHK. However most physicians are reluctant to prescribe DCA in the absence of evidence of its effectiveness.

DCA has been around for a long time and it cannot be patented. This has provoked the claim that major drug companies are conspiring to suppress evidence of DCA's effectiveness on the grounds that they cannot make any money by selling DCA. A cottage industry of suppliers has sprung up on the Internet for people who want to treat themselves with this cheap "miracle" drug. The Food and Drug Administration in the United States has been forced to shut down some websites because they were making unsubstantiated claims about its ability to cure cancer. There was also concern about self-medication because high dosages of DCA are toxic. There's bound to be more publicity surrounding this complicated issue in the future. The blog *Respectful Insolence* (scienceblogs.com/insolence) is a good source of scientific and medical information on the controversy.
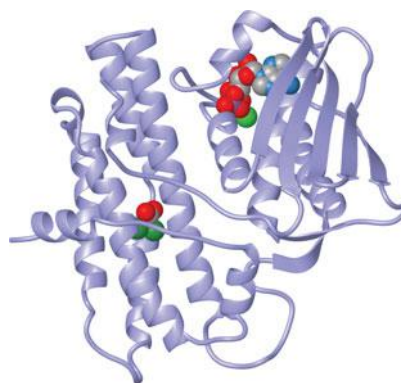
▲ **Pyruvate dehydrogenase kinase with dichloroacetate bound at the active site.** The human (*Homo sapiens*) PDHK is a dimer, only one subunit is shown here. The bound ligands are shown as space-filling molecules. ADP (top) is bound at the allosteric site, and dichloroacetate (left) is bound at the active site. [PDB 2BU8]

As shown in Figure 13.20, citrate, α-ketoglutarate, succinyl CoA, and oxaloacetate all lead to biosynthetic pathways. Citrate is part of a pathway for the formation of fatty acids and steroids. It undergoes cleavage to form acetyl CoA, the precursor of the lipids. In eukaryotes, this reaction takes place in the cytosol, and citrate must be transported from the mitochondria to the cytosol to support fatty acid biosynthesis. One major metabolic fate of α-ketoglutarate is reversible conversion to glutamate, which can then be incorporated into proteins or used for the synthesis of other amino acids or nucleotides. We will see in Chapter 17 that α-ketoglutarate pools are important in nitrogen metabolism. Succinyl CoA can condense with glycine to initiate the biosynthesis of porphyrins such as the heme groups of cytochromes. As we saw in the previous chapter, oxaloacetate is a precursor of carbohydrates formed by gluconeogenesis. Oxaloacetate also interconverts with aspartate, which can be used in the synthesis of urea, amino acids, and pyrimidine nucleotides.

When the citric acid cycle functions as a multistep catalyst, only small amounts of each intermediate are needed to convert large quantities of acetyl CoA to products. Therefore, the rate at which the citric acid cycle metabolizes acetyl CoA is extremely sensitive to changes in the concentrations of its intermediates. Thus, citric acid cycle intermediates that are removed by entry into biosynthetic pathways must be replenished by **anaplerotic** (Greek, "filling up") reactions. Because the pathway is cyclic, replenishing any of the cycle intermediates results in a greater concentration of all intermediates. Depletion of citric acid cycle intermediates is an example of a **cataplerotic** reaction. It's just as important as the filling up reactions.

The production of oxaloacetate by pyruvate carboxylase is an important anaplerotic reaction (Figure 13.20). This reaction is also part of the gluconeogenesis pathway (Section 12.1A). Pyruvate carboxylase is allosterically activated by acetyl CoA. The accumulation of acetyl CoA indicates a low concentration of oxaloacetate and a need for
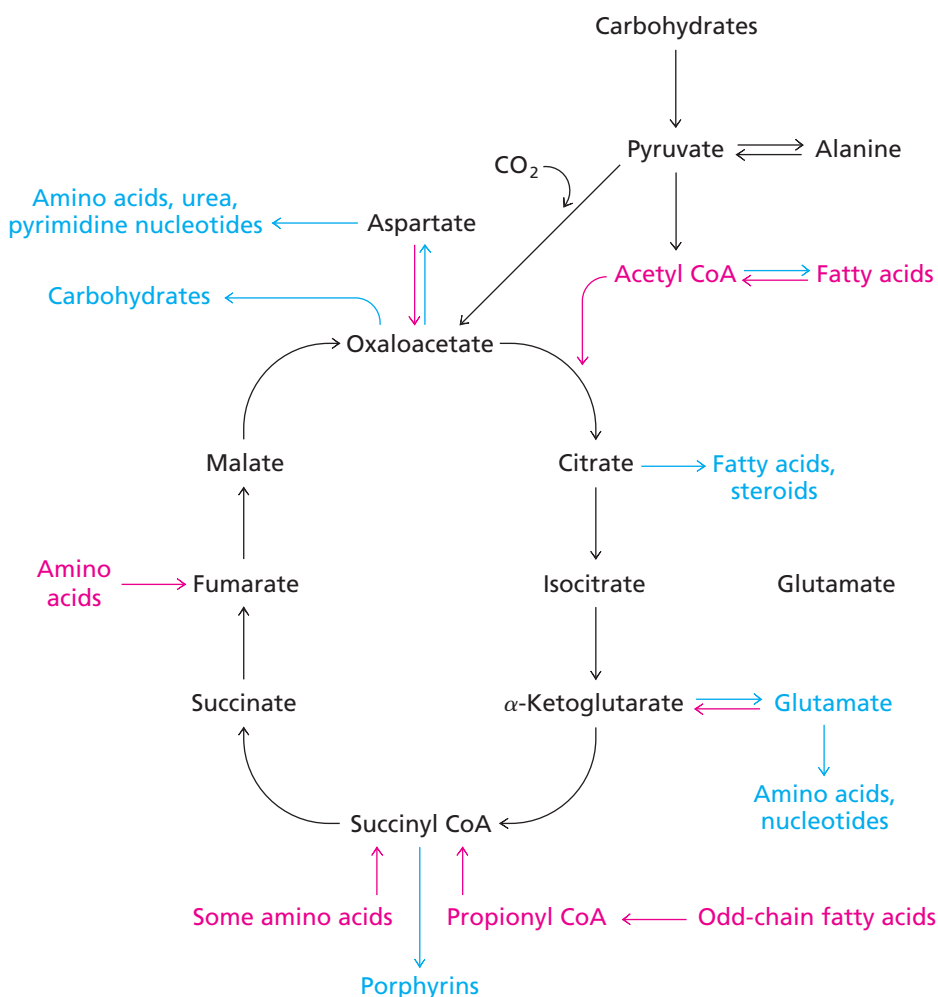
Routes leading to and from the citric acid cycle. Intermediates of the citric acid cycle are precursors of carbohydrates, lipids, and amino acids, as well as nucleotides and porphyrins. Reactions feeding into the cycle replenish the pool of cycle intermediates. Anabolic pathways are colored blue and catabolic pathways are colored red.

more citric acid cycle intermediates. The activation of pyruvate carboxylase supplies oxaloacetate for the cycle.

Many species use a variety of different reactions to keep the intake and output of citric acid cycle intermediates in a delicate balance. For example, many plants and some bacteria supply oxaloacetate to the citric acid cycle via a reaction catalyzed by phosphoenolpyruvate carboxylase.

$$\text{Phosphoenolpyruvate} + \text{HCO}_3^{\ominus} \rightleftharpoons \text{Oxaloacetate} + \text{P}_i \qquad \textbf{(13.16)}$$
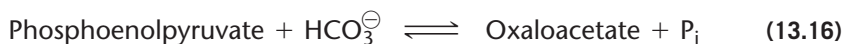
Pathways for degrading some amino acids and fatty acids can contribute succinyl CoA to the citric acid cycle. The interconversion of oxaloacetate and aspartate and of α-ketoglutarate and glutamate can either supply or remove intermediates of the cycle.

The interplay of all these reactions—the entry of acetyl CoA from glycolysis and other sources, the entry of intermediates from catabolic pathways and anaplerotic reactions, and the exit of intermediates to anabolic pathways—means that the citric acid cycle doesn't always operate as a simple cycle devoted to oxidizing acetyl CoA. In fact, most bacteria don't have all of the classic enzymes of the citric acid cycle so there is no "cycle" in these species. Instead, the enzymes that are present are used mostly in biosynthesis pathways where the intermediates become precursors for the synthesis of amino acids and porphyrins (Section 13.9).

13.8 The Glyoxylate Pathway

The glyoxylate pathway is a route that bypasses some of the reactions of the citric acid cycle. The pathway is named after the two-carbon molecule glyoxylate, an essential

intermediate in the pathway. There are only two reactions. In the first reaction, a six-carbon tricarboxylic acid (isocitrate) is split into a two-carbon molecule (glyoxylate) and a four-carbon dicarboxylic acid (succinate). This reaction is catalyzed by isocitrate lyase (Figure 13.21). In the second reaction, the two-carbon glyoxylate molecule combines with a two-carbon acetyl CoA molecule to make a four-carbon dicarboxylic acid (malate). The enzyme for the second reaction is malate synthase.

The glyoxylate pathway was first discovered in bacteria. Subsequently it was found in plants and later in fungi, protists, and some animals. The pathway is often called the glyoxylate shunt, the glyoxylate bypass, or the glyoxylate cycle. The glyoxylate pathway provides an anabolic alternative for the metabolism of acetyl CoA, leading to the formation of glucose from acetyl CoA via four-carbon compounds. Cells that contain glyoxylate pathway enzymes can synthesize all their required carbohydrates from any substrate that is a precursor of acetyl CoA. For example, yeast can grow on ethanol because yeast cells can oxidize ethanol to form acetyl CoA, which can be metabolized via the glyoxylate pathway to form malate. Similarly, many bacteria use the glyoxylate pathway to sustain growth on acetate, which can be incorporated into acetyl CoA in a reaction catalyzed by acetyl CoA synthetase.

$$H_3C-COO^{\ominus} + HS\text{-}CoA \xrightarrow[\substack{\text{Acetyl CoA} \\ \text{synthetase}}]{\substack{\text{ATP} \quad \text{AMP, PP}_i}} H_3C-\overset{\overset{\displaystyle O}{\|}}{C}-S\text{-}CoA \qquad (13.17)$$
$$\text{Acetate} \hspace{9cm} \text{Acetyl CoA}$$
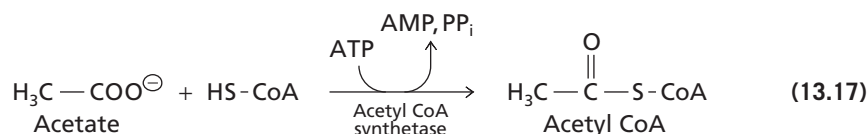
The glyoxylate pathway is a fundamental metabolic pathway in bacteria, protists, fungi, and plants. It is especially active in oily seed plants. In these plants, stored seed oils (triacylglycerols) are converted to carbohydrates that provide fuel during germination. In contrast, genes for the two enzymes of the pathway are present in most animals but the pathway is not actively used. Consequently, in humans acetyl CoA does not serve as the precursor for the net formation of either pyruvate or oxaloacetate; therefore, acetyl CoA is not a carbon source for the net production of glucose. (The carbon atoms of acetyl CoA are incorporated into oxaloacetate by the reactions of the citric acid cycle, but for every two carbon atoms incorporated, two other carbon atoms are released as CO_2.)

The glyoxylate pathway can be regarded as a shunt within the citric acid cycle, as shown in Figure 13.21. The two reactions provide a bypass around the CO_2-producing reactions of the citric acid cycle. No carbon atoms of the acetyl group of acetyl CoA are released as CO_2 during operation of the glyoxylate shunt, and the net formation of a four-carbon molecule from two molecules of acetyl CoA supplies a precursor that can be converted to glucose by gluconeogenesis. Succinate is oxidized to malate and oxaloacetate by the citric acid cycle to maintain the catalytic amounts of citric acid cycle intermediates. You can think of the glyoxylate shunt as part of a cycle that includes the upper portion of the citric acid cycle. In this case, the net reaction includes the formation of oxaloacetate for gluconeogenesis and the cyclic oxidation of succinate. Two molecules of acetyl CoA are consumed.

$$2 \text{ Acetyl CoA} + 2 \text{ NAD}^{\oplus} + Q + 3 H_2O \longrightarrow$$
$$\text{Oxaloacetate} + 2 \text{ HS-CoA} + 2 \text{ NADH} + QH_2 + 4 H^{\oplus} \qquad (13.18)$$
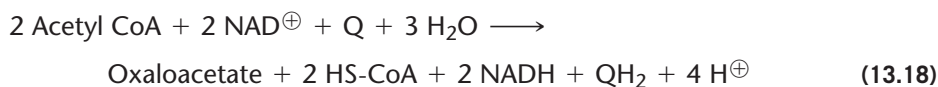
In eukaryotes, the operation of the glyoxylate cycle requires the transfer of metabolites between the mitochondria, where the citric acid cycle enzymes are located, and the cytosol, where isocitrate lyase and malate synthase are found. Thus, the actual pathway is more complicated than the diagram in Figure 13.21. In plants, the glyoxylate pathway enzymes are localized to a special membrane-bound organelle called the glyoxysome. Glyoxysomes contain some special versions of the citric acid cycle enzymes, but some metabolites still have to be transferred between compartments in order for the pathway to operate as a cycle.

NAD$^\oplus$ NADH, H$^\oplus$

COO$^\ominus$
|
C=O
|
CH$_2$
|
COO$^\ominus$

Acetyl CoA

Malate dehydrogenase

Oxaloacetate

Citrate synthase

H$_2$O

HS‑CoA, H$^\oplus$

COO$^\ominus$
|
HO—C—H
|
CH$_2$
|
COO$^\ominus$

L-Malate

CH$_3$
|
C=O
|
S‑CoA

Acetyl CoA

COO$^\ominus$
|
CH$_2$
|
HO—C—COO$^\ominus$
|
CH$_2$
|
COO$^\ominus$

Citrate

Fumarase

Malate synthase

H$_2$O

H$_2$O

HS‑CoA, H$^\oplus$

COO$^\ominus$
|
H—C
‖
C—H
|
COO$^\ominus$

Fumarate

Aconitase

COO$^\ominus$
|
CH$_2$
|
H—C—COO$^\ominus$
|
HO—C—H
|
COO$^\ominus$

Isocitrate

O   H
\ /
C
|
COO$^\ominus$

Glyoxylate

Succinate dehydrogenase complex

QH$_2$

Q

Isocitrate lyase

COO$^\ominus$
|
CH$_2$
|
CH$_2$
|
COO$^\ominus$

Succinate

Isocitrate dehydrogenase

CO$_2$

α-Ketoglutarate

CO$_2$

Succinyl CoA

◄ **Figure 13.21**
Glyoxylate pathway. Isocitrate lyase and malate synthase are the two enzymes of the pathway. When the pathway is functioning, the acetyl carbon atoms of acetyl CoA are converted to malate rather than oxidized to CO_2. Malate can be converted to oxaloacetate, which is a precursor in gluconeogenesis. The succinate produced in the cleavage of isocitrate is oxidized to oxaloacetate to replace the four-carbon compound consumed in glucose synthesis.

(a)

(b)
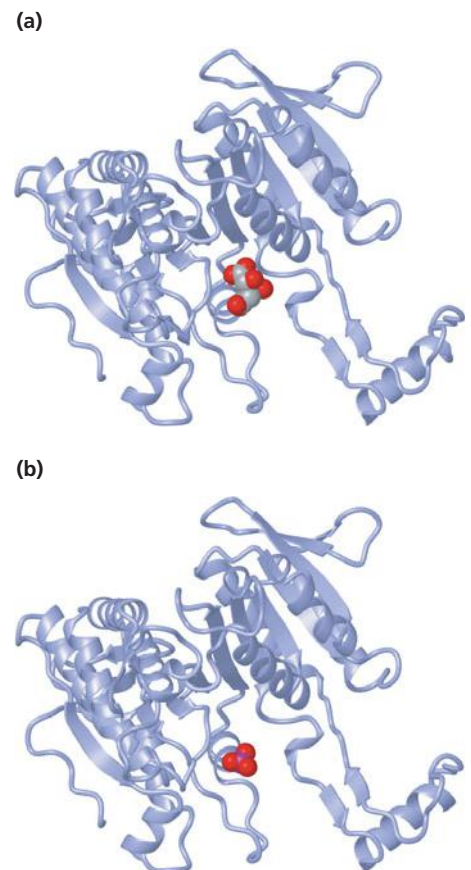
▲ **Figure 13.22**
Phosphorylated and dephosphorylated forms of *E. coli* isocitrate dehydrogenase. (a) The dephosphorylated enzyme is active; isocitrate binds to the active site. [PDB 5ICD] **(b)** The phosphorylated enzyme is inactive because the negatively charged phosphoryl group (red) electrostatically repels the substrate, preventing it from binding. [PDB 4ICD]

In bacteria, the glyoxylate pathway is often used to replenish citric acid cycle metabolites that are diverted into a number of biosynthesis pathways. Since all of the reactions take place in the cytosol in bacteria, it is important to regulate the flow of metabolites. The key regulated enzyme is isocitrate dehydrogenase. Its activity is regulated by covalent modification. Kinase-catalyzed phosphorylation of a serine residue abolishes isocitrate dehydrogenase activity. In the dephosphorylated form of the enzyme, the serine residue forms a hydrogen bond with a carboxylate group of isocitrate. Phosphorylation inhibits enzyme activity by causing electrostatic repulsion of the substrate rather than by causing an R-to-T conformational change (Figure 13.22). The same protein molecule that contains the kinase activity also has a separate domain with phosphatase activity that catalyzes hydrolysis of the phosphoserine residue, reactivating isocitrate dehydrogenase.

The kinase and phosphatase activities are reciprocally regulated; isocitrate, oxaloacetate, pyruvate, and the glycolytic intermediates 3-phosphoglycerate and phosphoenolpyruvate allosterically activate the phosphatase and inhibit the kinase

Regulation of *E. coli* isocitrate dehydrogenase by covalent modification. A bifunctional enzyme catalyzes phosphorylation and dephosphorylation of isocitrate dehydrogenase. The two activities of the bifunctional enzyme are reciprocally regulated allosterically by intermediates of glycolysis and the citric acid cycle.
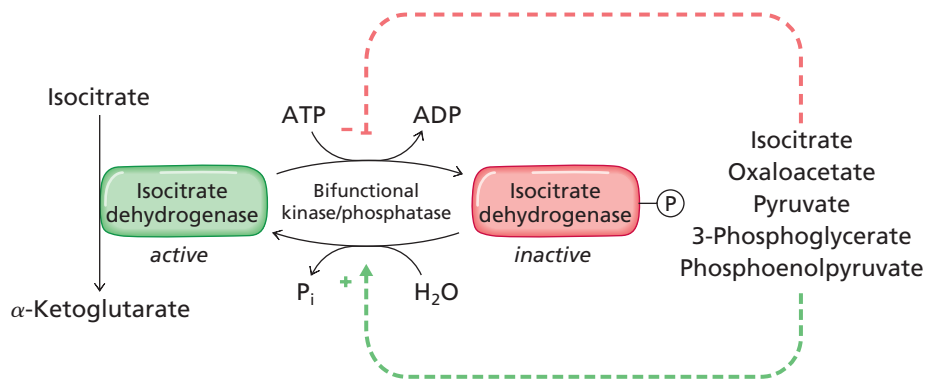
(Figure 13.23). Thus, when the concentrations of glycolytic and citric acid cycle intermediates in *E. coli* are high, isocitrate dehydrogenase is active. When phosphorylation abolishes the activity of isocitrate dehydrogenase, isocitrate is diverted to the glyoxylate pathway.

13.9 Evolution of the Citric Acid Cycle

The reactions of the citric acid cycle were first discovered in mammals and many of the key enzymes were purified from liver extracts. As we have seen, the citric acid cycle can be viewed as the end stage of glycolysis because it results in the oxidation of acetyl CoA produced as one of the products of glycolysis. However, there are many organisms that do not encounter glucose as a major carbon source and the production of ATP equivalents via glycolysis and the citric acid cycle is not an important source of metabolic energy in such species.

We need to examine the function of the citric acid cycle enzymes in bacteria in order to understand their role in simple single-celled organisms. These roles might allow us to deduce the pathways that could have existed in the primitive cells that eventually gave rise to complex eukaryotes. Fortunately, the sequences of several hundred prokaryotic genomes are now available as a result of the huge technological advances in recombinant DNA technology and DNA sequencing methods. We can now examine the complete complement of metabolic enzymes in many diverse species of bacteria and ask whether they possess the pathways that we have discussed in this chapter. These analyses are greatly aided by developments in the fields of comparative genomics, molecular evolution, and bioinformatics.

Most species of bacteria do not have a complete citric acid cycle. The most common versions of an incomplete cycle include part of the left-hand side. This short linear pathway leads to production of succinate or succinyl CoA or α-ketoglutarate by a reductive process using oxaloacetate as a starting point. This reductive pathway is the reverse of the traditional cycle that functions in the mitochondria of eukaryotes. In addition, many species of bacteria also have enzymes from part of the right-hand side of the citric acid cycle, especially citrate synthase and aconitase. This allows them to synthesize citrate and isocitrate from oxaloacetate and acetyl CoA. The presence of a forked pathway (Figure 13.24) results in the synthesis of all the precursors of amino acids, porphyrins, and fatty acids.

There are hundreds of diverse species of bacteria that can survive and grow in the complete absence of oxygen. Some of these species are obligate anaerobes—for them, oxygen is a lethal poison! Others are facultative anaerobes—they can survive in oxygen free environments as well as oxygen-rich environments. *E. coli* is one example of a species that can survive in both types of environment. When growing anaerobically, *E. coli* uses a forked version of the pathway to produce the necessary metabolic precursors and avoid the accumulation of reducing equivalents that cannot be reoxidized by the oxygen requiring electron transport system. Bacteria such as *E. coli* can grow in environments where acetate is the only source of organic carbon. In this case, they employ the glyoxylate pathway to convert acetate to malate and oxaloacetate for glucose synthesis.

Acetyl CoA

Oxaloacetate ——————→ Citrate

Citrate synthase

Oxidative pathway

Malate dehydrogenase

Aconitase

Malate

Malate synthase

Acetyl CoA

Isocitrate

Reductive pathway

Fumarase

Glyoxylate ←

Isocitrate dehydrogenase

Fumarate

CO_2

Fumarate reductase | Succinate dehydrogenase

α-Ketoglutarate

Isocitrate lyase

Succinate ←

Succinyl CoA synthetase
Succinyl CoA: acetoacetate
CoA transferase

Succinyl CoA

α-Ketoglutarate CO_2
Dehydrogenase
α-Ketoglutarate:
Ferredoxin oxidoreductase

α-Ketoglutarate

◄ **Figure 13.24**
Forked pathway found in many species of bacteria. The left-hand side of the fork is a reductive pathway leading to the synthesis of succinate or α-ketoglutarate in reactions that proceed in the reverse direction from those in the classic citric acid cycle. The right-hand branch is an oxidative pathway similar to the first few reactions of the classic citric acid cycle.

The first living cells arose in an oxygen-free environment over three billion years ago. These primitive cells undoubtedly possessed most of the enzymes that interconverted acetate, pyruvate, citrate, and oxaloacetate, since these enzymes are present in most modern bacteria. The development of the main branches of the forked pathway possibly began with the evolution of malate dehydrogenase from a duplication of the lactate dehydrogenase gene. Aconitase and isocitrate dehydrogenase evolved from enzymes that are used in the synthesis of leucine (isopropylmalate dehydratase and isopropylmalate dehydrogenase, respectively). (Note that the leucine biosynthesis pathway is more ubiquitous and more primitive than the citric acid cycle.)

Extension of the reductive branch continued with the evolution of fumarase from aspartase. Aspartase is a common bacterial enzyme that synthesizes fumarate from L-aspartate. L-aspartate, in turn, is synthesized by amination of oxaloacetate in a reaction catalyzed by aspartate transaminase (Section 17.3). It is likely that primitive cells used the pathway oxaloacetate → aspartate → fumarate to produce fumarate before the evolution of malate dehydrogenase and fumarase. The reduction of fumarate to succinate is catalyzed by fumarate reductase in many bacteria. The evolutionary origin of this complex enzyme is highly speculative but at least one of the subunits is related to another enzyme of amino acid metabolism. Succinate dehydrogenase, the enzyme that preferentially catalyzes the reverse reaction in the citric acid cycle, is likely to have evolved later on from fumarate reductase via a gene duplication event.

The synthesis of α-ketoglutarate can occur in either branch of the forked pathway. The reductive branch uses α-ketoglutarate:ferredoxin oxidoreductase, an enzyme found in many species of bacteria that don't have a complete citric acid cycle. The reaction catalyzed by this enzyme is not readily reversible. With the evolution of α-ketoglutarate dehydrogenase the two forks can be joined to create a cyclic pathway. It is clear that α-ketoglutarate dehydrogenase and pyruvate dehydrogenase share a common ancestor and it is likely that this was the last enzyme to evolve.

Some bacteria have a complete citric acid cycle but it is used in the reductive direction to fix CO_2 in order to build more complex organic molecules. This could have been one of the selective pressures leading to a complete pathway. The cycle requires a terminal electron acceptor to oxidize NADH and QH_2 when it operates in the more normal oxidative direction seen in eukaryotes. Originally, this terminal electron acceptor was sulfur or various sulfates, and these reactions still occur in many anaerobic bacterial species. Oxygen levels began to rise about 2.5 billion years ago with the evolution of photosynthesis reactions in cyanobacteria. Some bacteria, notably proteobacteria, exploited the availability of

oxygen when the membrane-associated electron transport reactions evolved. One species of proteobacteria entered into a symbiotic relationship with a primitive eukaryotic cell about two billion years ago. This led to the evolution of mitochondria and the modern versions of the citric acid cycle and electron transport in eukaryotes.

The evolution of the citric acid cycle pathway involved several of the pathway evolution mechanisms discussed in Chapter 10. There is evidence for gene duplication, pathway extension, retro-evolution, pathway reversal, and enzyme theft.

Summary

1. The pyruvate dehydrogenase complex catalyzes the oxidation of pyruvate to form acetyl CoA and CO_2.

2. For each molecule of acetyl CoA oxidized via the citric acid cycle, two molecules of CO_2 are produced, three molecules of $NAD^{\oplus}$ are reduced to NADH, one molecule of Q is reduced to QH_2 and one molecule of GTP is generated from GDP + P_i (or ATP from ADP + P_i, depending on the species).

3. The eight enzyme-catalyzed reactions of the citric acid cycle can function as a multistep catalyst.

4. In eukaryotic cells, pyruvate must be imported into the mitochondria by a specific transporter before it can serve as a substrate for the pyruvate dehydrogenase reaction.

5. Oxidation of the reduced coenzymes generated by the citric acid cycle leads to the formation of about 10 ATP molecules per molecule of acetyl CoA entering the pathway, for a total of about 32 ATP molecules per complete oxidation of 1 molecule of glucose.

6. The oxidation of pyruvate is regulated at the steps catalyzed by the pyruvate dehydrogenase complex, isocitrate dehydrogenase, and the α-ketoglutarate dehydrogenase complex.

7. In addition to its role in oxidative catabolism, the citric acid cycle provides precursors for biosynthetic pathways. Anaplerotic reactions replenish cycle intermediates.

8. The glyoxylate cycle, a modification of the citric acid cycle, allows many organisms to use acetyl CoA to generate four-carbon intermediates for gluconeogenesis.

9. The citric acid cycle probably evolved from the more primitive forked pathway found in many modern species of bacteria.

Problems

1. (a) The citric acid cycle converts one molecule of citrate to one molecule of oxaloacetate, which is required for the cycle to continue. If other cycle intermediates are depleted by being used as precursors for amino acid biosynthesis, can a net synthesis of oxaloacetate occur from acetyl CoA via the enzymes of the citric acid cycle?

   (b) How can the cycle continue to function if insufficient oxaloacetate is present?

2. Fluoroacetate, a very toxic molecule that blocks the citric acid cycle, has been used as a rodent poison. It is converted enzymatically *in vivo* to fluoroacetyl CoA, which is then converted by the action of citrate synthase to 2R,3S-fluorocitrate, a potent competitive inhibitor of the next enzyme in the pathway. Predict the effect of fluoroacetate on the concentrations of the intermediates in the citric acid cycle. How can this blockage of the cycle be overcome?

3. Calculate the number of ATP molecules generated by the following net reactions of the citric acid cycle. Assume that all NADH and QH_2 are oxidized to yield ATP, pyruvate is converted to acetyl CoA, and the malate–aspartate shuttle is operating.

   (a) 1 Pyruvate $\longrightarrow$ 3 CO_2
   (b) Citrate $\longrightarrow$ Oxaloacetate + 2 CO_2

4. When one molecule of glucose is completely oxidized to six molecules of CO_2 under the conditions in Problem 3, what percentage of ATP is produced by substrate level phosphorylation?

5. The disease beriberi, which results from a dietary deficiency of vitamin B_1 (thiamine), is characterized by neurologic and cardiac symptoms, as well as increased levels of pyruvate and α-ketoglutarate in the blood. How does a deficiency of thiamine account for the increased levels of pyruvate and α-ketoglutarate

6. In three separate experiments, pyruvate labeled with ^{14}C at C-1, at C-2, or at C-3 is metabolized via the pyruvate dehydrogenase complex and the citric acid cycle. Which labeled pyruvate molecule is the first to yield $^{14}CO_2$? Which is the last to yield $^{14}CO_2$, and how many turns of the cycle are required to release all of the labeled carbon atoms as $^{14}CO_2$?

7. Patients in shock experience decreased delivery of O_2 to tissues, decreased activity of the pyruvate dehydrogenase complex, and increased anaerobic metabolism. Excess pyruvate is converted to lactate, which accumulates in tissues and in the blood, causing lactic acidosis.

   (a) Since O_2 is not a reactant or product of the citric acid cycle, why do low levels of O_2 decrease the activity of the pyruvate dehydrogenase complex?

   (b) To alleviate lactic acidosis, shock patients are sometimes given dichloroacetate, which inhibits pyruvate dehydrogenase kinase. How does this treatment affect the activity of the pyruvate dehydrogenase complex?

8. A deficiency of a citric acid cycle enzyme in both mitochondria and the cytosol of some tissues (e.g., blood lymphocytes) results in severe neurological abnormalities in newborns. The disease is characterized by excretion in the urine of abnormally large amounts of α-ketoglutarate, succinate, and fumarate. What enzyme deficiency would lead to these symptoms?

9. Acetyl CoA inhibits dihydrolipoamide acetyltransferase (E_2 of the pyruvate dehydrogenase complex) but activates the pyruvate dehydrogenase kinase component of the pyruvate dehydrogenase complex. How are these two different actions of acetyl CoA consistent with the overall regulation of the complex?

10. Pyruvate dehydrogenase complex deficiency is a disease that results in various metabolic and neurological effects. Pyruvate dehydrogenase complex deficiency can cause lactic acidosis in affected children. Other clinical symptoms include increased concentrations of pyruvate and alanine in the blood. Explain the increase in the levels of pyruvate, lactate, and alanine in individuals with pyruvate dehydrogenase complex deficiency.

11. In response to a signal for contraction and the resulting increased need for ATP in vertebrate muscle, Ca^{2+} is released into the cytosol from storage sites in the endoplasmic reticulum. How does the citric acid cycle respond to the influx of Ca^{2+} in satisfying the increased need for cellular ATP?

12. (a) The degradation of alanine yields pyruvate, and the degradation of leucine yields acetyl CoA. Can the degradation of these amino acids replenish the pool of citric acid cycle intermediates?

    (b) Fats (triacylglycerols) stored in adipose tissue are a significant source of energy in animals. Fatty acids are degraded to acetyl CoA, which activates pyruvate carboxylase. How does the activation of this enzyme help recover energy from fatty acids?

13. Amino acids resulting from the degradation of proteins can be further metabolized by conversion to intermediates of the citric acid cycle. If the degradation of a labeled protein leads to the following labeled amino acids, write the structure of the first intermediate of the citric acid cycle into which these amino acids would be converted and identify the labeled carbon in each case.

(a) Glutamate
(b) Alanine
(c) Aspartate
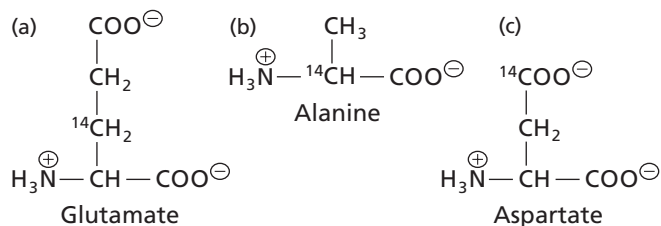
14. (a) How many molecules of ATP are eventually generated when two molecules of acetyl CoA are converted to four molecules of CO_2 via the citric acid cycle? (Assume NADH 2.5 ATP and QH_2 ~1.5ATP) How many molecules of ATP are generated when two molecules of acetyl CoA are converted to oxaloacetate in the glyoxylate cycle?

    (b) How do the yields of ATP relate to the primary functions of the two pathways?

15. The activities of PFK-2 and fructose 2,6-*bis*phosphatase are contained in a bifunctional protein that effects tight control over glycolysis and gluconeogenesis through the action of fructose 2,6-*bis*phosphate. Describe another protein that contains kinase and phosphatase activities in a single protein molecule. What pathways does it control?

Selected Readings

Pyruvate Dehydrogenase Complex

Harris, R. A., Bowker-Kinley, M. M., Huang, B., and Wu, P. (2002). Regulation of the activity of the pyruvate dehydrogenase complex. *Advances in Enzyme Regulation* 42:249–259.

Knoechel, T. R., Tucker, A. D., Robinson, C. M., Phillips, C., Taylor, W., Bungay, P. J., Kasten, S. A., Roche, T. E., and Brown, D. G. (2006). Regulatory roles of the N-terminal domain based on crystal structures of human pyruvate dehydrogenase kinase 2 containing physiological and synthetic ligands. *Biochem.* 45:402–415.

Maeng, C.-Y., Yazdi, M. A., Niu, X.-D., Lee, H. Y., and Reed, L. J. (1994). Expression, purification, and characterization of the dihydrolipoamide dehydrogenase-binding protein of the pyruvate dehydrogenase complex from *Saccharomyces cerevisiae*. *Biochem.* 33:13801–13807.

Mattevi, A., Obmolova, G., Schulze, E., Kalk, K. H., Westphal, A. H., de Kok, A., and Hol, W. G. J. (1992). Atomic structure of the cubic core of the pyruvate dehydrogenase multienzyme complex. *Science* 255:1544–1550.

Reed, L. J., and Hackert, M. L. (1990). Structure-function relationships in dihydrolipoamide acyltransferases. *J. Biol. Chem.* 265:8971–8974.

Citric Acid Cycle

Beinert, H., and Kennedy, M. C. (1989). Engineering of protein bound iron–sulfur clusters. *Eur. J. Biochem.* 186:5–15.

Gruer, M. J., Artymiuk, P. J., and Guest, J. R. (1997). The aconitase family: three structural variations on a comon theme. *Trends Biochem. Sci.* 22:3–6.

Hurley, J. H., Dean, A. M., Sohl, J. L., Koshland, D. E., Jr., and Stroud, R. M. (1990). Regulation of an enzyme by phosphorylation at the active site. *Science* 249:1012–1016.

Kay, J., and Weitzman, P. D. J., eds. (1987). *Krebs' Citric Acid Cycle—Half a Century and Still Turning* (London: The Biochemical Society).

Krebs, H. A., and Johnson, W. A. (1937). The role of citric acid in intermediate metabolism in animal tissues. *Enzymologia* 4:148–156.

McCormack, J. G., and Denton, R. M. (1988). The regulation of mitochondrial function in mammalian cells by Ca^{2+} ions. *Biochem. Soc. Trans.* 109:523–527.

Remington, S. J. (1992). Mechanisms of citrate synthase and related enzymes (triose phosphate isomerase and mandelate racemase). *Curr. Opin. Struct. Biol.* 2:730–735.

Williamson, J. R., and Cooper, R. H. (1980). Regulation of the citric acid cycle in mammalian systems. *FEBS Lett.* 117 (Suppl.):K73–K85.

Wolodko, W. T., Fraser, M. E., James, M. N. G., and Bridger, W. A. (1994). The crystal structure of succinyl-CoA synthetase from *Escherichia coli* at 2.5-Å resolution. *J. Biol. Chem.* 269:10883–10890.

Yankovskaya, V., Horsefield, R., Törnroth, S., Luna-Chavez, C., Miyoshi, H., Léger, C., Byrne, B., Cecchini, G. and Iwata, S. (2003). Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science* 299:700–704.

Glyoxylate Cycle

Beevers, H. (1980). The role of the glyoxylate cycle. In *The Biochemistry of Plants: A Comprehensive Treatise*, Vol. 4, P. K. Stumpf and E. E. Conn, eds. (New York: Academic Press), pp. 117–130.
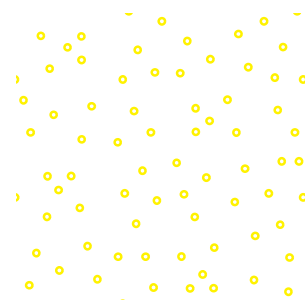
Electron Transport and ATP Synthesis

We now come to one of the most complicated metabolic pathways encountered in biochemistry—the membrane-associated electron transport system coupled to ATP synthesis. The role of this pathway is to convert reducing equivalents into ATP. We usually think of reducing equivalents as products of glycolysis and the citric acid cycle since the oxidation of glucose and acetyl CoA is coupled to the reduction of $NAD^{\oplus}$ and Q. In this chapter we learn that the subsequent reoxidation of NADH and QH_2 results in the passage of electrons through a membrane-associated electron transport system where the energy released can be saved through the phosphorylation of ADP to ATP. The electrons are eventually passed to a terminal electron acceptor. This terminal electron acceptor is usually molecular oxygen (O_2) and this is why the overall process is often called oxidative phosphorylation.

The combined pathway of electron transport and ATP synthesis involves numerous enzymes and coenzymes. It also depends absolutely on the presence of a membrane compartment since one of the key steps in coupling electron transport to ATP synthesis involves the creation of a pH gradient across a membrane. In eukaryotes the membrane is the inner mitochondrial membrane and in prokaryotes it is the plasma membrane.

We begin this chapter with an overview of the thermodynamics of a proton gradient and how it can drive ATP synthesis. We then describe the structure and function of the membrane-associated electron transport complexes and the ATP synthase complex. We conclude with a description of other terminal electron acceptors and a brief discussion of some enzymes involved in oxygen metabolism. Chapter 15 describes the similar membrane-associated electron transport and ATP synthesis pathway that operates during photosynthesis.

According to the chemiosmotic hypothesis of oxidative and photosynthetic phosphorylation proposed by Mitchell, the linkage between electron transport and phosphorylation occurs not because of hypothetical energy-rich chemical intermediates as in the orthodox view, but because oxidoreduction and adenosine triphosphate (ATP) hydrolysis are each separately associated with the net translocation of a certain number of electrons in one direction and the net translocation of the same number of hydrogen atoms in the opposite direction across a relatively ion-, acid-, and base-impermeable coupling membrane.

P. Mitchell, and J. Moyle, (1965)

Top: Sunflowers, cheetahs, and mushrooms all use the same mechanism to make ATP using a proton gradient.

14.1 Overview of Membrane-associated Electron Transport and ATP Synthesis

Membrane-associated electron transport requires several enzyme complexes embedded in a membrane. We will start by examining the pathway that occurs in mitochondria and later we will look at the common features of the prokaryotic and eukaryotic systems. The two processes of membrane-associated electron transport and ATP synthesis are coupled—neither process can occur without the other.

In the common pathway, electrons are passed from NADH to the terminal electron acceptor. There are many different terminal electron acceptors but we are mostly interested in the pathway found in eukaryotic mitochondria where molecular oxygen (O_2) is reduced to form water. As electrons pass along the electron transport chain from NADH to O_2 the energy they release is used to transfer protons from inside the mitochondrion to the intermembrane space between the double membranes. This proton gradient is used to drive ATP synthesis in a reaction catalyzed by ATP synthase (Figure 14.1). A very similar system operates in bacteria.

As mentioned above, the entire mitochondrial pathway is often called oxidative phosphorylation because, historically, the biochemical puzzle was to explain the linkage between oxygen uptake and ATP synthesis. You will also see frequent references to "respiration" and "respiratory electron transport." These terms also refer to the pathway that exploits oxygen as the terminal electron acceptor.

The coenzymes mentioned in this chapter are described in detail in Chapter 7: NAD⁺, Section 7.4; ubiquinone, Section 7.15; FMN and FAD, Section 7.5; iron–sulfur clusters, Section 7.1; and cytochromes, Section 7.17.

14.2 The Mitochondrion

Much of the aerobic oxidation of biomolecules in eukaryotes takes place in the mitochondrion. This organelle is the site of the citric acid cycle and fatty acid oxidation, both of which generate reduced coenzymes. The reduced coenzymes are oxidized by the electron transport complexes embedded in the mitochondrial membranes. The structure of a typical mitochondrion is shown in Figure 14.2.

The number of mitochondria in cells varies dramatically. Some unicellular algae contain only one mitochondrion whereas the cell of the protozoan *Chaos chaos* contains half a million mitochondria. A mammalian liver cell contains up to 5000 mitochondria. The number of mitochondria is related to the overall energy requirements of the cell. White muscle tissue, for example, relies on anaerobic glycolysis for its energy needs and it contains relatively few mitochondria. The rapidly contracting but swiftly exhausted jaw muscles of the alligator are an extreme example of white muscle. Alligators can snap their jaws with astonishing speed and force but cannot continue this motion beyond a

▼ Figure 14.1

Overview of membrane-associated electron transport and ATP synthesis in mitochondria. A proton concentration gradient is produced from reactions catalyzed by the electron transport chain. Protons are translocated across the inner mitochondrial membrane from the matrix to the intermembrane space as electrons from reduced substrates flow through the complexes. The free energy stored in the proton concentration gradient is utilized when protons flow back across the membrane via ATP synthase; their reentry is coupled to the conversion of ADP and P_i to ATP.

a)

(b)

Inner membrane

Outer membrane

Matrix

ntermembrane pace

Cristae

Matrix

Inner membrane

Outer membrane

Structure of the mitochondrion. The outer mitochondrial membrane is freely permeable to small molecules but the inner membrane is impermeable to polar and ionic substances. The inner membrane is highly folded and convoluted, forming structures called cristae. The protein complexes that catalyze the reactions of membrane-associated electron transport and ATP synthesis are located in the inner membrane. **(a)** Illustration. **(b)** Electron micrograph: longitudinal section from bat pancreas cell.

very few repetitions. By contrast, red muscle tissue has many mitochondria. The cells of the flight muscles of migratory birds are an example of red muscle cells. These muscles must sustain substantial and steady outputs of power and this power requires prodigious amounts of ATP.

Mitochondria vary greatly in size and shape among different species, in different tissues, and even within a cell. A typical mammalian mitochondrion has a diameter of 0.2 to 0.8 μm and a length of 0.5 to 1.5 μm—this is about the size and shape of an *E. coli* cell. (Recall from Chapter 1 that mitochondria are descendants of bacteria cells that entered into a symbiotic relationship with a primitive eukaryotic cell.)

Mitochondria are separated from the cytoplasm by a double membrane. The two membranes have markedly different properties. The outer mitochondrial membrane has few proteins. One of these proteins is the transmembrane protein porin (Section 9.11A) that forms channels allowing free diffusion of ions and water-soluble metabolites with molecular weights less than 10,000. In contrast, the inner mitochondrial membrane is very rich in protein with a protein-to-lipid ratio of about 4:1 by mass. This membrane is permeable to uncharged molecules such as water, O_2, and CO_2 but it is a barrier to protons and larger polar and ionic substances. These polar substances must be actively transported across the inner membrane using specific transport proteins such as pyruvate translocase (Section 13.4). The entry of anionic metabolites into the negatively charged interior of a mitochondrion is energetically unfavorable. Such metabolites are usually exchanged for other anions from the interior or are accompanied by protons flowing down the concentration gradient that is generated by the electron transport chain.

The inner membrane is often highly folded resulting in a greatly increased surface area. The folds are called cristae. The expansion and folding of the inner membrane also creates a greatly expanded intermembrane space (Figure 14.2a). Since the outer membrane is freely permeable to small molecules, the intermembrane space has about the same composition of ions and metabolites as the cytosol that surrounds the mitochondrion.

The contents of the matrix include the pyruvate dehydrogenase complex, the enzymes of the citric acid cycle (except for the succinate dehydrogenase complex, which is embedded in the inner membrane), and most of the enzymes that catalyze fatty acid oxidation. The protein concentration in the matrix is very high (approaching 500 mg ml^{-1}). Nevertheless, diffusion is only slightly less rapid than in the cytosol (Section 2.3b).

▲ **Alligator jaw muscles.** You're probably safe after this alligator has already snapped at you several times and missed. (If you trust your biochemistry textbook.)

▲ **Canada geese.** If you had more mitochondria in your muscle cells you might be able to fly to a warmer climate for the winter.

BOX 14.1 AN EXCEPTION TO EVERY RULE

One of the most fascinating things about biology is that there are very few universal rules. We can propose certain general principles that apply in most cases but there are almost always a few examples that don't fit. For example, we can say that eukaryotic cells contain mitochondria as a general rule but we know of some species that don't have mitochondria.

One of the "rules" that seemed valid was that all *animal* cells had mitochondria and they all require oxygen. Now there's even an exception to that rule. Some small microscopic animals of the phylum Loricifera live in deep ocean basins where there is no light and the nearly salt-saturated water is devoid of oxygen. They are incapable of aerobic oxidation and their cells have no mitochondria.

▶ *Spinoloricus* **sp., an anaerobic animal.**

▲ **Peter Mitchell (1920–1992).** Mitchell was awarded the Nobel Prize in Chemistry in 1978 "for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory." In 1963 Mitchell resigned from his position at Edinburgh University in Scotland and in 1965 he set up a private research institute with his long-time friend and collaborator, Jennifer Moyle. They continued to work on bioenergetics in a laboratory in Mitchell's home, Glynn House, in Cornwall (UK).

KEY CONCEPT

Chemiosmotic theory states that the energy from the oxidation–reduction reactions of electron transport is used to create a proton gradient across the membrane and the resulting protonmotive force is used in the synthesis of ATP.

The matrix also contains metabolites and inorganic ions and a pool of $NAD^{\oplus}$ and $NADP^{\oplus}$ that remains separate from the pyridine nucleotide coenzymes of the cytosol. Mitochondrial DNA and all of the enzymes required for DNA replication, transcription, and translation are located in the matrix. Mitochondrial DNA contains many of the genes that encode the electron transport proteins (see Figure 14.19).

14.3 The Chemiosmotic Theory and Protonmotive Force

Before considering the individual reactions of oxidative phosphorylation we will examine the nature of the energy stored in a proton concentration gradient. The **chemiosmotic theory** is the concept that a proton concentration gradient serves as the energy reservoir that drives ATP formation. The essential elements of this theory were originally formulated by Peter Mitchell in the early 1960s. At the time, the mechanism by which cells carry out oxidative phosphorylation was the subject of intensive research and much controversy. The pathway linking oxidation reactions to the phosphorylation of ADP was not known and many early attempts to identify a "high energy" phosphorylated metabolite that could transfer a phosphoryl group to ADP had ended in failure. Today, thanks to decades of work by many scientists, the formation and dissipation of ion gradients are acknowledged as a central motif in bioenergetics. Mitchell was awarded the Nobel Prize in Chemistry in 1978 for his contribution to our understanding of bioenergetics.

A. Historical Background: The Chemiosmotic Theory

By the time Mitchell proposed the chemiosmotic theory, much information had accumulated on the oxidation of substrates and the cyclic oxidation and reduction of mitochondrial electron carriers. In 1956 Britton Chance and Ronald Williams had shown that when intact isolated mitochondria are suspended in phosphate buffer they oxidize substrates and consume oxygen only when ADP is added to the suspension. In other words, the oxidation of a substrate must be *coupled* to the phosphorylation of ADP. Subsequent experiments showed that respiration proceeds rapidly until all the ADP has been phosphorylated (Figure 14.3a) and that the amount of O_2 consumed depends on the amount of ADP added.

Synthetic compounds called **uncouplers** stimulate the oxidation of substrates in the absence of ADP (Figure 14.3b). The phenomenon of uncoupling helped show how oxidation reactions are linked to ATP formation. In the presence of an uncoupler, oxygen uptake (respiration) proceeds until all the available oxygen is consumed. This rapid oxidation of substrates proceeds with little or no phosphorylation of ADP. In other words, these synthetic compounds uncouple oxidation from phosphorylation. There are many

different kinds of uncouplers and they have little in common chemically except that all are lipid-soluble weak acids. Both their protonated and conjugate base forms can cross the inner mitochondrial membrane—the anionic conjugate base retains lipid solubility because the negative charge is delocalized. The resonance structures of the uncoupler 2,4-dinitrophenol are shown in Figure 14.4.

The effect of uncouplers, and many other experiments, revealed that electron transport (oxygen uptake) and ATP synthesis were normally coupled but the underlying mechanism was unknown. Throughout the 1960s it was commonly believed that there must be several steps in the electron transport process where the Gibbs free energy change was sufficient to drive ATP synthesis. This form of coupling was thought to be analogous to substrate level phosphorylation.

Mitchell proposed that the action of mitochondrial enzyme complexes generates a proton concentration gradient across the inner mitochondrial membrane. He suggested that this gradient provides the energy for ADP phosphorylation via an *indirect* coupling to electron transport. Mitchell's ideas accounted for the effect of the lipid-soluble un-coupling agents—they bind protons in the cytosol, carry them through the inner membrane, and release them in the matrix, thereby dissipating the proton concentration gradient. The proton carriers uncouple electron transport (oxidation) from ATP synthesis because protons enter the matrix without passing through ATP synthase.

ATP synthase activity was first recognized in 1948 as ATPase activity in damaged mitochondria (i.e., damaged mitochondria catalyze hydrolysis of ATP to ADP and P_i). Most workers assumed that mitochondrial ATPase catalyzes the reverse reaction in un-damaged mitochondria and this assumption proved to be correct. Efraim Racker and his coworkers isolated and characterized this membrane-bound oligomeric ATPase in the 1960s. The proton driven reversibility of the ATPase reaction was demonstrated by observing the expulsion of protons on hydrolysis of ATP in mitochondria. Further support came from experiments with small membrane vesicles where the enzyme was incorporated into the membrane. When a suitable proton gradient was created across the vesicle membrane, ATP was synthesized from ADP and P_i (Section 14.9).

B. The Protonmotive Force

Protons are translocated into the intermembrane space by the membrane-associated electron transport complexes and they flow back into the matrix via ATP synthase. This circular flow forms a circuit that is similar to an electrical circuit. The energy of the proton concentration gradient, called the **protonmotive force**, is analogous to the electromotive force of electrochemistry (Section 10.9A). This analogy is illustrated in Figure 14.5.

Consider a reaction such as the reduction of molecular oxygen by the reducing agent XH_2 in an electrochemical cell.

$$XH_2 + \tfrac{1}{2}O_2 \rightleftharpoons X + H_2O \tag{14.1}$$

(a)

▲ **Figure 14.3**
Oxygen uptake and ATP synthesis in mitochondria. (a) In the presence of excess P_i and substrate, intact mitochondria consume oxygen rapidly only when ADP is added. Oxygen uptake ceases when all the ADP has been phosphorylated. **(b)** Adding the uncoupler 2,4-dinitrophenol allows oxidation of the substrate to proceed in the absence of phosphorylation of ADP. The arrows indicate the times at which additions were made to the solution of suspended mitochondria.

See Box 15.4 for a description of Racker's key experiment.

▲ **Figure 14.4**
Protonated and conjugate base forms of 2,4-dinitrophenol. The dinitrophenolate anion is resonance stabilized and its negative ionic charge is broadly distributed over the ring structure of the molecule. Because the negative charge is delocalized, both the acid and base forms of dinitrophenol are sufficiently hydrophobic to dissolve in the membrane.

(a)

(b)

▲ **Figure 14.5**
Electromotive and protonmotive force. (a) In an electrochemical cell, electrons pass from the reducing agent XH_2 to the oxidizing agent O_2 through a wire connecting the two electrodes. The measured electrical potential between cells is the electromotive force. **(b)** When the configuration is reversed (i.e., the external pathway for electrons is replaced by an aqueous pathway for protons), the potential is the protonmotive force. In mitochondria, protons are translocated across the inner membrane when electrons are transported within the membrane by the electron transport chain.

Electrons from XH_2 pass along a wire that connects the two electrodes where the oxidation and reduction half-reactions occur. Electrons flow from the electrode where XH_2 is oxidized

$$XH_2 \rightleftharpoons X + 2\,H^\oplus + 2\,e^\ominus \tag{14.2}$$
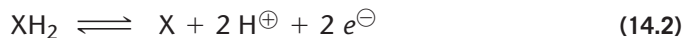
to the electrode where O_2 is reduced.

$$^1/_2O_2 + 2\,H^\oplus + 2\,e^\ominus \rightleftharpoons H_2O \tag{14.3}$$
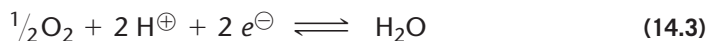
In the electrochemical cell, protons pass freely from one reaction cell to the other through the solvent in a salt bridge. Electrons move through an external wire because of a potential difference between the cells. This potential, measured in volts, is the electromotive force. The direction of electron flow and the extent of reduction of the oxidizing agent depend on the difference in free energy between XH_2 and O_2 that in turn depends on their respective reduction potentials.

In mitochondria, it is protons—not electrons—that flow through the external connection, an aqueous circuit connecting the membrane-associated electron transport chain and ATP synthase. This connection is analogous to the wire of the electrochemical reaction. The electrons still pass from the reducing agent XH_2 to the oxidizing agent O_2 but in this case it is through the membrane-associated electron transport chain. The free energy of these oxidation–reduction reactions is stored as the protonmotive force of the proton concentration gradient and is recovered in the phosphorylation of ADP.

Recall from Section 9.10 that the Gibbs free energy change for transport of a charged molecule is

$$\Delta G_{transport} = 2.303\,RT \log \frac{[A_{in}]}{[A_{out}]} + zF\Delta\Psi \tag{14.4}$$

where the first term is the Gibbs free energy due to the concentration gradient and the second term $\mathbb{1}zF\Delta\Psi\mathbb{2}$ is due to the charge difference across the membrane. For protons the charge per molecule is 1 ($z = 1.0$) and the overall Gibbs free energy change of the proton gradient is

$$\Delta G = 2.303\,RT \log \frac{[H^\oplus{}_{in}]}{[H^\oplus{}_{out}]} + F\Delta\Psi = 2.303\,RT\,\Delta pH + F\Delta\Psi \tag{14.5}$$

This equation can be used to calculate the protonmotive force generated by the proton gradient and the charge difference across the membrane. In liver mitochondria the membrane potential ($\Delta\Psi$) is -0.17 V (inside negative, Section 9.10A) and the pH difference is -0.5 ($\Delta pH = pH_{out} - pH_{in}$). The membrane potential is favorable for movement of protons into the mitochondrial matrix so the $F\Delta\Psi$ term will be negative because $\Delta\Psi$ is negative. The pH gradient is also favorable so the first term in Equation 14.5 must be negative. Thus, the equation for protonmotive force is

$$\Delta G_{in} = F\Delta\Psi + 2.303\,RT\,\Delta pH \tag{14.6}$$

Using the above values at 37° ($T = 310$ K) the available Gibbs free energy is

$$\begin{aligned} \Delta G &= [96485 \times -0.17] + [2.303 \times 8.315 \times 310 \times -0.5] \\ &= -16402\;J\;mol^{-1} - 2968\;J\;mol^{-1} = -19.4\;kJ\;mol^{-1} \end{aligned} \tag{14.7}$$

This means that the transport of a single mole of protons back across the membrane is associated with a free energy change of -19.4 kJ. That's a lot of energy for moving such a small ion!

The standard Gibbs free energy change for the synthesis of one molecule of ATP from ADP is 32 kJ mol^{-1} ($\Delta G°' = 32$ kJ mol^{-1}) but the actual Gibbs free energy change is about -48 kJ mol^{-1} (Section 10.6). At least three protons must be translocated in order to drive synthesis of one ATP molecule ($3 \times -19.4 = -58.2$ kJ mol^{-1}) .

Note that 85% ($-16.4/-19.4 = 85\%$) of the Gibbs free energy change is due to the charge gradient across the membrane and only 15% ($-3.0/-19.4 = 15\%$) is due to the proton concentration gradient. Keep in mind that the energy required to *create* the proton gradient is $+19.4$ kJ mol^{-1}.

14.4 Electron Transport

We now consider the individual reactions of the membrane-associated electron transport chain. Four oligomeric assemblies of proteins are found in the inner membrane of mitochondria or the plasma membrane of bacteria. These enzyme complexes have been isolated in their active forms by careful solubilization using detergents. Each complex catalyzes a separate portion of the energy transduction process. The numbers I through IV are assigned to these complexes. Complex V is ATP synthase.

A. Complexes I Through IV

The four enzyme complexes contain a wide variety of oxidation–reduction centers. These may be cofactors such as FAD, FMN, or ubiquinone (Q). Other centers include Fe–S clusters, heme-containing cytochromes, and copper proteins. Electron flow occurs via the sequential reduction and oxidation of these redox centers with flow proceeding from a reducing agent to an oxidizing agent. There are many reactions that involve electron transport processes in biochemistry. We have already seen several of these reactions in previous chapters—the flow of electrons in the pyruvate dehydrogenase complex is a good example (Section 13.1).

Electrons flow through the components of an electron transport chain in the direction of increasing reduction potential. The reduction potentials of each redox center fall between that of the strong reducing agent, NADH, and that of the terminal oxidizing agent, O$_2$. The mobile coenzymes ubiquinone (Q) and cytochrome c serve as links between different complexes of the electron transport chain. Q transfers electrons from complexes I or II to complex III. Cytochrome c transfers electrons from complex III to complex IV. Complex IV uses the electrons for the reduction of O$_2$ to water.

The order of the electron transport reactions is shown in Figure 14.6 against a scale of standard reduction potential on the left and a relative scale of Gibbs standard free energy change on the right. Recall from Section 10.9 that the standard reduction potential (in units of volts) is directly related to the standard Gibbs free energy change (in units of kJ mol^{-1}) by the formula

$$\Delta G°' = -n\text{F}\ \Delta E°' \tag{14.8}$$

As you can see from Figure 14.6, a substantial amount of energy is released during the electron transport process. Much of this energy is stored in the protonmotive force that drives ATP synthesis. It is this coupling of electron transport to the generation of a protonmotive force that distinguishes membrane-associated electron transport from other examples of electron transport.

The values shown in Figure 14.6 are strictly true only under standard conditions where the temperature is 25°C, the pH is 7.0, and the concentrations of reactants and products are equal (1M each). The relationship between actual reduction potentials (E) and standard ones ($E°'$) is similar to the relationship between actual and standard free energy (Section 1.4B),

$$E = E°' - \frac{RT}{n\text{F}} \ln \frac{[S_{red}]}{[S_{ox}]} = E°' - \frac{2.303RT}{n\text{F}} \log \frac{[S_{red}]}{[S_{ox}]} \tag{14.9}$$

The Gibbs Free Energy of Electron Transport

$$E°' = E°'_{acceptor} - E°'_{donor} \tag{10.26}$$

$$= E°'_{O_2} - E°'_{NADH}$$

$$= +0.82 - (-0.32) \text{ (Table 10.4)}$$

$$= 1.14 \text{ V}$$

$$\Delta G°' = -n\text{F}\Delta E°'$$

$$= -2(96485)(1.14)$$

$$= 220 \text{ kJ mol}^{-1}$$

Cofactors in electron transport

$$NADH \longrightarrow FMN \longrightarrow Fe\text{--}S \longrightarrow Q \longrightarrow \begin{bmatrix} Fe\text{--}S \\ Cyt\ b \end{bmatrix} \longrightarrow Cyt\ c_1 \longrightarrow Cyt\ c \longrightarrow Cyt\ a \longrightarrow Cyt\ a_3 \longrightarrow O_2$$

$$Succinate \longrightarrow FAD \longrightarrow Fe\text{--}S$$
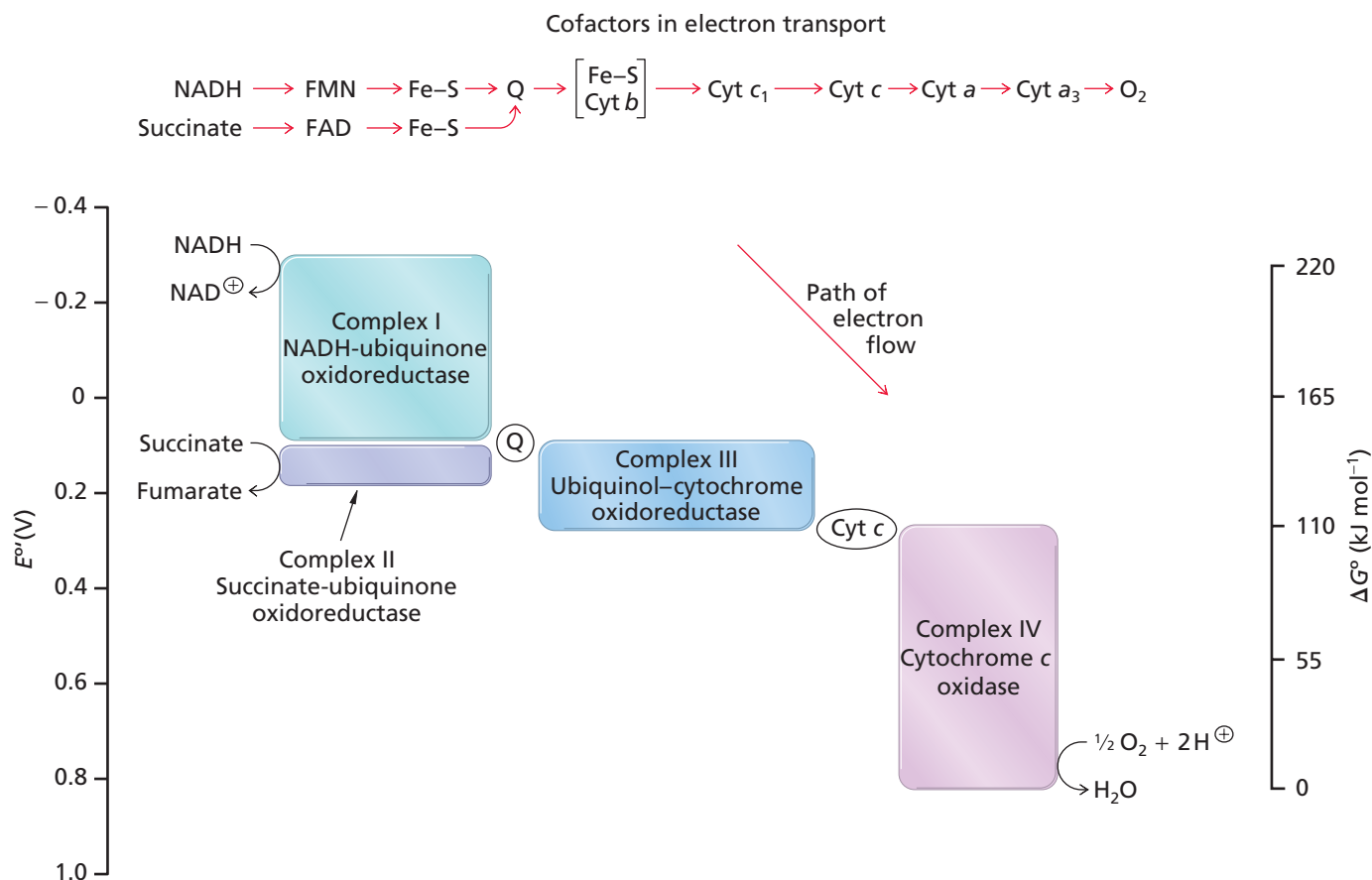
▲ **Figure 14.6**
Electron transport. Each of the four complexes of the electron transport chain, composed of several protein subunits and cofactors, undergoes cyclic reduction and oxidation. The complexes are linked by the mobile carriers ubiquinone (Q) and cytochrome c. The height of each complex indicates the $\Delta E^{\circ\prime}$ between its reducing agent (substrate) and its oxidizing agent (which becomes the reduced product). Standard reduction potentials are plotted with the lowest value at the top pf the graph (see Section 10.9B).

KEY CONCEPT

Aerobic organisms need oxygen because it serves as the terminal electron acceptor in membrane-associated electron transport.

where $[S_{red}]$ and $[S_{ox}]$ represent the actual concentrations of the two oxidation states of the electron carrier. Under standard conditions, the concentrations of reduced and oxidized carrier molecules are equal; thus, the ratio $[S_{red}]/[S_{ox}]$ is one, and the second term in Equation 14.9 is zero. In this case, the actual reduction potential is equal to the standard reduction potential (at 25°C and pH 7.0). In order for electron carriers to be efficiently reduced and reoxidized in a linear fashion, appreciable quantities of both the reduced and oxidized forms of the carriers must be present under steady state conditions. This is the situation found in mitochondria. We can therefore assume that for any given oxidation–reduction reaction in the electron transport complexes the concentrations of the two oxidation states of the electron carriers are fairly similar. Since physiological pH is close to 7 under most circumstances and since most electron transport processes operate at temperatures close to 25°C, we can safely assume that E is not much different from $E^{\circ\prime}$. From now on, our discussion refers only to $E^{\circ\prime}$ values.

The standard reduction potentials of the substrates and cofactors of the electron transport chain are listed in Table 14.1. Note that the values progress from negative to positive so that, in general, each substrate or intermediate is oxidized by a cofactor or substrate that has a more positive $E^{\circ\prime}$. In fact, one consideration in determining the actual sequence of the electron carriers was their reduction potentials.

The Gibbs standard free energy available from the reactions catalyzed by each complex is shown in Table 14.2. The overall free energy totals -220 kJ mol^{-1} as shown in Figure 14.6. Complexes I, III, and IV translocate protons across the membrane as electrons pass through the complex. Complex II, which is also the succinate dehydrogenase complex we examined as a component of the citric acid cycle, does not directly contribute to formation of the proton concentration gradient. Complex II transfers electrons from succinate to Q and thus represents a tributary of the respiratory chain.

B. Cofactors in Electron Transport

As shown at the top of Figure 14.6, the electrons that flow through complexes I through IV are actually transferred between coupled cofactors. Electrons enter the membrane-associated electron transport chain two at a time from the reduced substrates NADH and succinate. The flavin coenzymes FMN and FAD are reduced in complexes I and II, respectively. The reduced coenzymes FMNH$_2$ and FADH$_2$ donate one electron at a time and all subsequent steps in the electron transport chain proceed by single electron transfers. Iron–sulfur (Fe–S) clusters of both the [2 Fe–2 S] and [4 Fe–4 S] type are present in complexes I, II, and III. Each iron–sulfur cluster can accept or donate one electron as an iron atom undergoes reduction and oxidation between the ferric [Fe^{3+}, Fe(III)] and ferrous [Fe^{2+}, Fe(II)] states. Copper ions and cytochromes are also single electron oxidation–reduction agents.

Several different cytochromes are present in the mammalian mitochondrial enzyme complexes. These include cytochrome b_L, cytochrome b_H, cytochrome c_1, cytochrome a, and cytochrome a_3. Very similar cytochromes are found in other species. Cytochromes transfer electrons from a reducing agent to an oxidizing agent by cycling between the ferric and ferrous oxidation states of the iron atoms of their heme prosthetic groups (Section 7.17). Individual cytochromes have different reduction potentials because of differences in the structures of their apoproteins and sometimes their heme groups (Table 14.1). These differences allow heme groups to function as electron carriers at several points in the electron transport chain. Similarly, the reduction potentials of iron–sulfur clusters can vary widely depending on the local protein environment.

The membrane-associated electron transport complexes are functionally linked by the mobile electron carriers ubiquinone (Q) and cytochrome c. Q is a lipid-soluble molecule that can accept and donate two electrons, one at a time (Section 7.15). Q diffuses within the lipid bilayer accepting electrons from complexes I and II and passing them to complex III. The other mobile electron carrier is cytochrome c, a peripheral membrane protein associated with the outer face of the membrane. Cytochrome c carries electrons from complex III to complex IV. The structures and the oxidation–reduction reactions of each of the four electron transport complexes are examined in detail in the following sections.

Table 14.1 Standard reduction potentials of mitochondrial oxidation–reduction components

Substrate of Complex	$E^{\circ\prime}$ (V)
NADH	-0.32
Complex I	
FMN	-0.30
Fe–S clusters	-0.25 to -0.05
Succinate	$+0.03$
Complex II	
FAD	0.0
Fe–S clusters	-0.26 to 0.00
QH$_2$/Q	$+0.04$
$(\cdot Q^{\ominus}/Q$	$-0.16)$
$(QH_2/\cdot Q^{\ominus}$	$+0.28)$
Complex III	
Cytochrome b_1.	-0.01
Cytochrome b_H	$+0.03$
Fe–S cluster	$+0.28$
Cytochrome c_1	$+0.22$
Cytochrome c	$+0.22$
Complex IV	
Cytochrome a	$+0.21$
Cu$_A$	$+0.24$
Cytochrome a_3	$+0.39$
Cu$_B$	$+0.34$
O$_2$	$+0.82$

KEY CONCEPT

The transfer of electrons from NADH to O$_2$ releases enough energy to drive synthesis of many ATP molecules.

Table 14.2 Standard free energy released in the oxidation reaction catalyzed by each complex

Complex	$E^{\circ\prime}_{\text{reductant}}$ (V)	$E^{\circ\prime}_{\text{oxidant}}$ (V)	$\Delta E^{\circ\prime a}$ (V)	$\Delta G^{\circ\prime b}$ (kJ mol^{-1})
I (NADH/Q)	-0.32	-0.04	$+0.36$	-60
II (Succinate/Q)	$+0.03$	$+0.04$	$+0.01$	-2
III (QH$_2$/Cytochrome c)	$+0.04$	$+0.22$	$+0.18$	-35
IV (Cytochrome c/O$_2$)	$+0.22$	$+0.82$	$+0.59$	-116

$^a\Delta E^{\circ\prime}$ was calculated as the difference between $E^{\circ\prime}_{\text{reductant}}$ and $E^{\circ\prime}_{\text{oxidant}}$.
^{b}The **Gibbs** standard free energy was calculated using Equation **14.8** where $n = 2$ electrons.
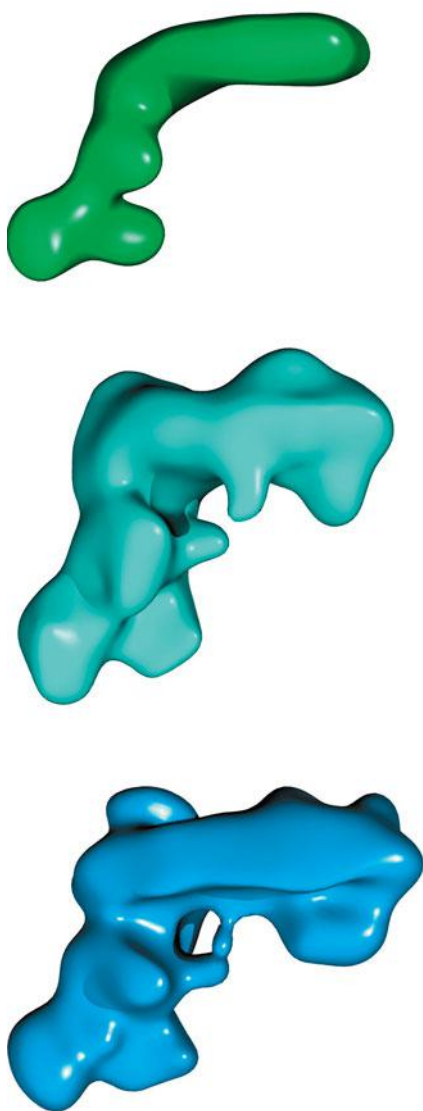
14.5 Complex I

Complex I catalyzes the transfer of two electrons from NADH to Q. The systematic name of this enzyme is NADH:ubiquinone oxidoreductase. It is a very complicated enzyme whose structure has not been completely solved. The prokaryotic versions contain 14 different polypeptide chains. The eukaryotic forms have 14 homologous subunits plus 20–32 additional subunits, depending on the species. The extra eukaryotic subunits probably stabilize the complex and prevent electron leakage.

The structure of the complex is L-shaped as seen in the electron microscope (Figure 14.7). The membrane-bound component consists of multiple subunits that span the membrane. This module contains a proton transporter activity. A larger component projects into the mitochondrial matrix, or the cytoplasm in bacteria (Figure 14.8). This arm contains a terminal NADH dehydrogenase activity and FMN. The connector module is composed of multiple subunits with 8 or 9 Fe–S clusters (Figure 14.9).

NADH molecules on the inside surface of the membrane donate electrons to complex I. The electrons are passed two at a time as a hydride ion ($H^{\ominus}$, two electrons and a proton). In the first step of electron transfer the hydride ion is transferred to FMN forming $FMNH_2$. $FMNH_2$ is then oxidized in two steps via a semiquinone intermediate. The two electrons are transferred one at a time to the next oxidizing agent, an iron–sulfur cluster.

$$FMN \xrightarrow{+H^{\oplus}, +H^{\ominus}} FMNH_2 \xrightarrow{-H^{\oplus}, -e^{\ominus}} FMNH\cdot \xrightarrow{-H^{\oplus}, -e^{\ominus}} FMN \qquad (14.10)$$
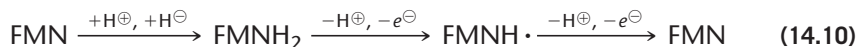
FMN is a transducer that converts two-electron transfer from NAD-linked dehydrogenases to one-electron transfer for the rest of the electron transport chain. In complex I the cofactor $FMNH_2$ transfers electrons to sequentially linked iron–sulfur clusters. There are at least eight Fe–S clusters positioned within the same arm of complex I that contains the NADH dehydrogenase activity. These Fe–S clusters provide a channel for electrons by directing them to the membrane-bound portion of the complex where ubiquinone (Q) accepts electrons one at a time passing through a semiquinone anion intermediate ($\cdot Q^{\ominus}$) before reaching its fully reduced state, ubiquinol (QH_2).

$$Q \xrightarrow{+e^{\ominus}} \cdot Q^{\ominus} \xrightarrow{+e^{\ominus}, +2\,H^{\oplus}} QH_2 \qquad (14.11)$$
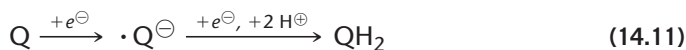
Q and QH_2 are lipid-soluble cofactors. They remain within the lipid bilayer and can diffuse freely in two dimensions. Note that the Q binding site of complex I is within the membrane. One of the reasons for the complicated electron transport chain within complex I is to carry electrons from an aqueous environment to a hydrophobic environment within the membrane.

As electrons move through complex I, two protons (one originating from the hydride ion of NADH and one from the interior) are transferred to FMN to form $FMNH_2$. These two protons or their equivalents are consumed in the reduction of Q to QH_2. Thus, two protons are taken up from the interior and transferred to QH_2. They are not released to the exterior in the complex I reactions. (QH_2 is subsequently reoxidized by complex III and the protons are then released to the exterior. This is part of the proton translocation activity of complex III described in Section 14.7.)

In complex I, four protons are directly translocated across the membrane for every pair of electrons that pass from NADH to QH_2. These do *not* include the protons required for ubiquinone reduction. The proton pump is probably an $H^{\oplus}/Na^{\oplus}$ antiporter

▲ **Figure 14.7**
Structure of complex I. The structures of complex I have been determined at low resolution by analyzing electron micrographic images. **(a)** Complex I from the bacterium *Aquifex aeolicus*. **(b)** Complex I from cow, *Bos taurus*. **(c)** Complex I from the yeast, *Yarrowia lipolytica*.

◄ **Figure 14.8**
Complex orientation. The electron transport complexes are embedded in the inner membrane. They can be drawn with the outside of the membrane at the top or at the bottom of the figure. Both views are seen in the scientific literature. We have chosen the orientation with the outside on top and the inside of the matrix on the bottom.
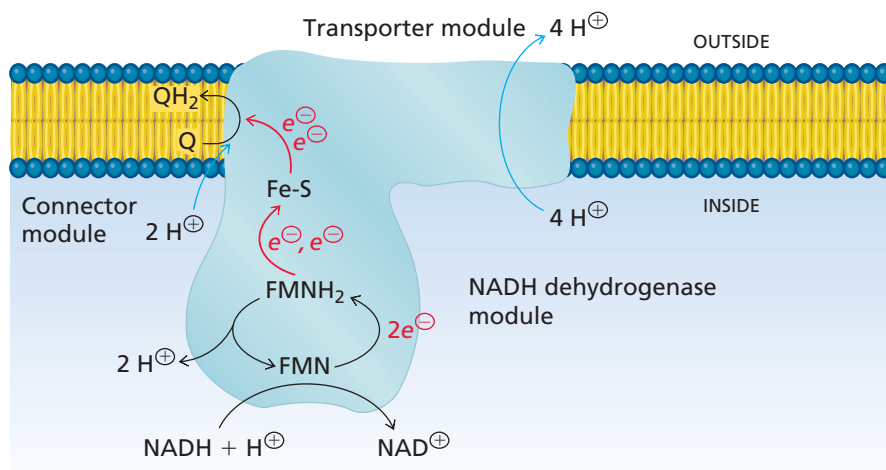
Electron transfer and proton flow in Complex I. Electrons are passed from NADH to Q via FMN and a series of Fe–S clusters. The reduction of Q to QH$_2$ requires two protons taken up from the inside compartment. In addition, four protons are translocated across the membrane for each pair of electrons transferred.

located in the membrane-bound module. The mechanism of proton translocation is not clear—it is likely coupled to conformational changes in the structure of complex I as electrons flow from the NADH dehydrogenase site to the ubiquinone binding site.

14.6 Complex II

Complex II is succinate:ubiquinone oxidoreductase, also called the succinate dehydrogenase complex. This is the same enzyme that we encountered in the previous chapter (Section 13.3#6). It catalyzes one of the reactions of the citric acid cycle. Complex II accepts electrons from succinate and, like complex I, catalyzes the reduction of Q to QH$_2$.

Complex II contains three identical multisubunit enzymes that associate to form a trimeric structure that is firmly embedded in the membrane (Figure 14.10). The overall shape resembles a mushroom with its head projecting into the interior of the membrane compartment. Each of the three succinate dehydrogenase enzymes has two subunits forming the head and one or two subunits (depending on the species) forming the membrane-bound stalk. One of the head subunits contains the substrate binding site and a covalently bound flavin adenine dinucleotide (FAD). The other head subunit contains three Fe–S clusters.

The head subunits from all species are closely related and share significant sequence similarity with other members of the succinate dehydrogenase family (e.g., fumarate reductase, Section 14.13). The membrane subunits, on the other hand, may be very different (and unrelated) in various species. In general, the membrane component has one or two subunits that consist exclusively of membrane-spanning α helices. Most of them have a bound heme b molecule and this subunit is often called cytochrome b. All of the membrane subunits have a Q binding site positioned near the interior surface of the membrane at the point where the head subunits are in contact with the membrane subunits.

The sequence of reactions for the transfer of two electrons from succinate to Q begins with the reduction of FAD by a hydride ion. This is followed by two single electron transfers from the reduced flavin to the series of three iron–sulfur clusters (Figure 14.11). (In those species with a cytochrome b anchor, the heme group is not part of the electron transfer pathway.)

Very little free energy is released in the reactions catalyzed by complex II (Table 14.2). This means that the complex cannot contribute directly to the proton concentration gradient across the membrane. Instead, it supplies electrons from the oxidation of succinate midway along the electron transport sequence. Q can accept electrons from complex I or II and donate them to complex III and then to the rest of the electron transport chain. Reactions in several other pathways also donate electrons to Q. We saw one of them, the reaction catalyzed by the glycerol 3-phosphate dehydrogenase complex, in Section 12.2C.
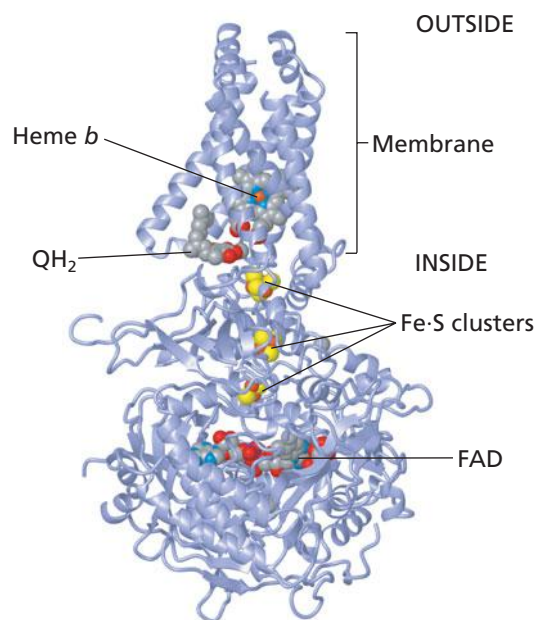
▲ Figure 14.10
Structure of the *E. coli* succinate dehydrogenase complex. A single copy of the enzyme showing the positions of FAD, the three Fe–S clusters, QH$_2$, and heme b. Complex II contains three copies of this multisubunit enzyme. [PDB 1NEK]

Electron transfer in complex II. A pair of electrons is passed from succinate to FAD as part of the citric acid cycle. Electrons are transferred one at a time from $FADH_2$ to three Fe–S clusters and then to Q. (Only one Fe–S cluster is shown in the figure.) Complex II does not directly contribute to the proton concentration gradient but serves as a tributary that supplies electrons (as QH_2) to the rest of the electron transport chain.
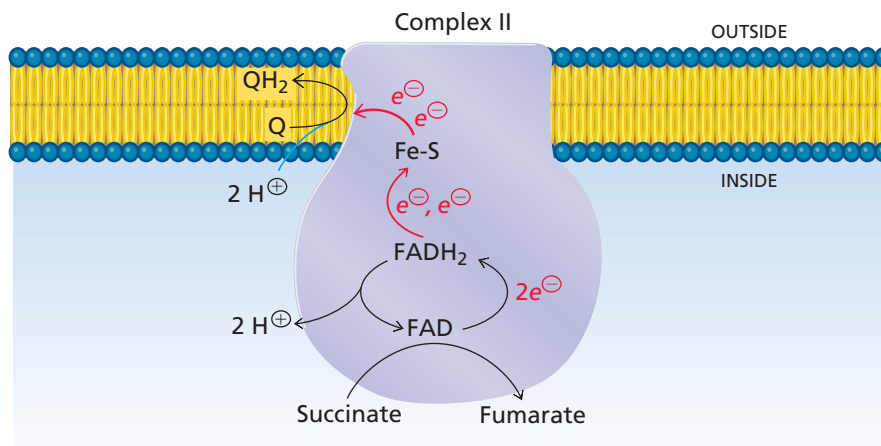
Complex III is, arguably, the most important enzyme in metabolism. Very similar complexes are present in chloroplasts where they participate in electron transport and proton translocation during photosynthesis.

14.7 Complex III

Complex III is ubiquinol:cytochrome c oxidoreductase, also called the cytochrome bc_1 complex. This enzyme catalyzes the oxidation of ubiquinol (QH_2) molecules in the membrane and the reduction of a mobile water-soluble cytochrome c molecule on the exterior surface. Electron transport through complex III is coupled to the transfer of $H^\oplus$ across the membrane by a process known as the Q cycle.

The structures of the cytochrome bc_1 complexes from many bacterial and eukaryotic species have been solved by X-ray crystallography. Complex III contains two copies of the enzyme and is firmly anchored to the membrane by a large number of α helices that span the lipid bilayer (Figure 14.12). The functional enzyme consists of three main subunits: cytochrome c_1, cytochrome b, and the Rieske iron sulfur protein (ISP) (Figure 14.13). (Note that the cytochrome c_1 subunit is a different protein than the mobile cytochrome c product of the reaction.) Other subunits are present on the inside surface but they do not play a direct role in the ubiquinol:cytochrome c oxidoreductase reaction. The mobile cytochrome c electron acceptor binds at the top of the complex—the part that is exposed to the exterior side of the membrane.

Complex III from cow (*Bos taurus*) mitochondria. The cytochrome bc_1 complex contains two copies of the enzyme ubiquinone: cytochrome c oxidoreductase. [PDB 1PP9]
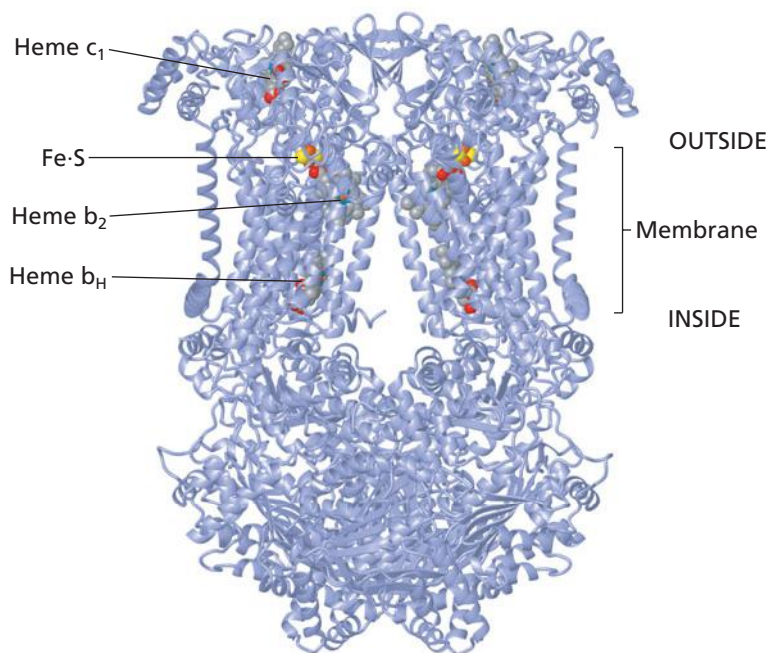
The path of electrons through the complex is shown in Figure 14.14. The reaction begins when QH_2 (from complex I or complex II) binds to the Q_0 site in the cytochrome b subunit. QH_2 is oxidized to the semiquinone and a single electron is passed to the adjacent Fe–S complex in the ISP subunit. From there, the electron transfers to the heme group in cytochrome c_1. This transfer is facilitated by movement of the head group of ISP. In the electron accepting position, the Fe–S cluster is adjacent to the Q_0 site and in the electron donating position the Fe–S cluster shifts to a position near the heme group in cytochrome c_1. Soluble cytochrome c is oxidized by transfer of an electron from the membrane-bound cytochrome c_1 subunit of complex III.

In this reaction, the terminal electron acceptor is cytochrome c (Section 7.17). This molecule serves as a mobile electron carrier transferring electrons to complex IV, the next component of the chain (Figure 14.15). The role of reduced cytochrome c is similar to that of QH_2, which carries electrons from complex I to complex III. The structures of cytochrome c electron carriers from all species are remarkably similar (Section 4.7B, Figure 4.21) and the amino acid sequences of the polypeptide chain are well conserved (Section 3.11, Figure 3.23).

The oxidation of QH_2 at the Q_0 site is a two-step process with a single electron transferred at each step. The path of electrons from the second step, oxidation of the semiquinone intermediate, follows a different route than the first electron. In this case, the electron is passed sequentially to two different b-type hemes within the membrane portion of the complex. The first heme group (b_L) has a lower reduction potential and the second heme (b_H) has a higher reduction potential (Table 14.1).

The b_H heme is part of the Q_1 site where a molecule of Q is reduced to QH_2 in a two-step reaction that involves a semiquinone intermediate. A single electron is transported from b_L (at the Q_0 site) to b_H (at the Q_1 site) to Q to produce the semiquinone.
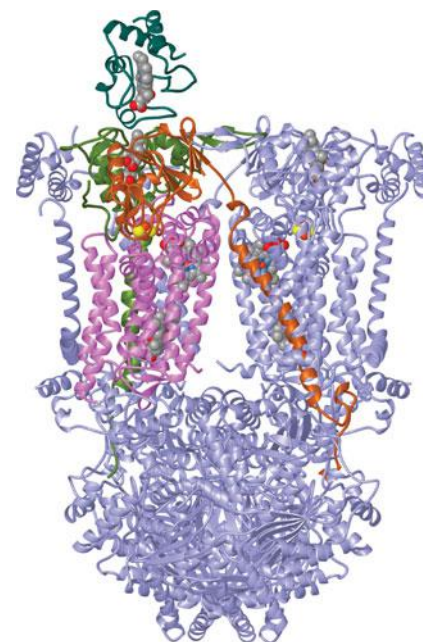
▲ **Figure 14.13**
Subunits of complex III. The three catalytic subunits of each dimer are Cytochrome c_1 (green), cytochrome b (blue) and the Rieske iron sulfur protein (ISP) (red). Cytochrome c (dark blue) binds to the Cytochrome c_1 subunit. [PDB 1PP9]
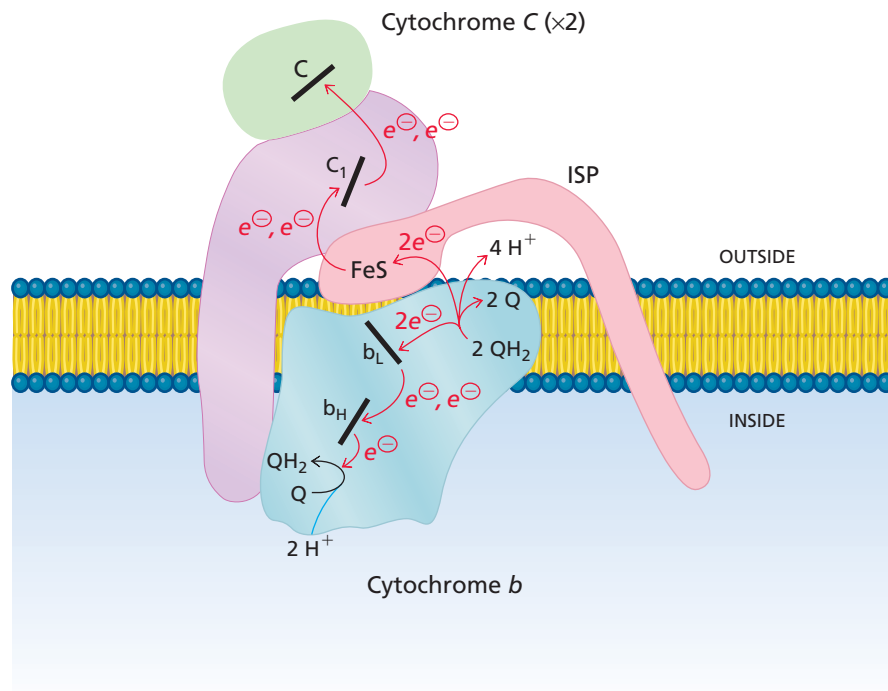
▲ **Figure 14.14**
Electron transfer and proton flow in complex III. Two pairs of electrons are passed separately from two molecules of QH_2 at the Q_0 site. Each pair of electrons is split so that individual electrons follow separate pathways. One electron is transferred to an Fe–S cluster then to cytochrome c_1 and finally to cytochrome c, the terminal electron carrier. The other electron from each oxidation of QH_2 is transferred to heme b_H (Q_1 site) and then to Q. A total of four protons are translocated across the membrane: two from the inside compartment and two from QH_2. (Only the left-hand half of the dimer is shown and the bottom subunits that project into the matrix are not shown.)
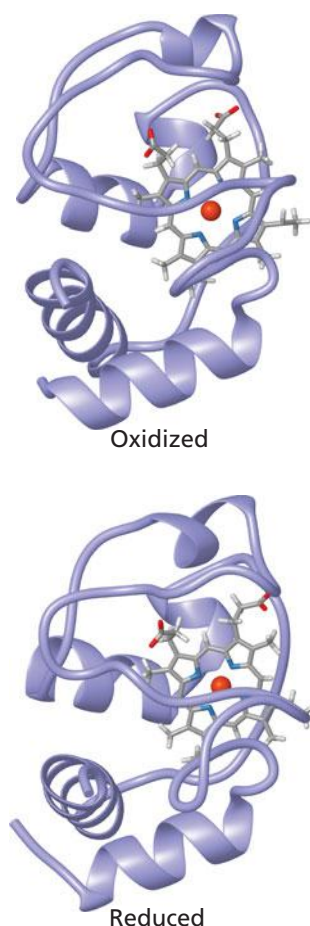
Oxidized

Reduced

▲ **Figure 14.15**
Cytochrome c. Oxidized (top) and reduced (bottom) forms of cytochrome c from horse (*Equus caballus*). The iron atom in the center of the heme group (orange) shifts from Fe^{3+} to Fe^{2+} as it gains an electron from complex III. This reduction is accompanied by small changes in the conformation of the protein. [PDB 1OCD (top) 1GIW (bottom)]

KEY CONCEPT

The net effect of the Q cycle is transfer of four protons to the exterior of the membrane for every two electrons transferred from QH_2 to cytochrome c.

Table 14.3

Q_o :	$2\ QH_2 + 2\ \text{cyt}\ c(Fe^{3+}) \longrightarrow 2\ Q + 2\ \text{cyt}\ c(Fe^{2+}) + 2\ e^{-} + 4\ H^{+}_{out}$
Q_i :	$Q + 2\ H^{+}_{in} + 2\ e^{-} \longrightarrow QH_2$
Sum:	$QH_2 + 2\ \text{cyt}\ c(Fe^{3+}) + 2\ H^{+}_{in} \longrightarrow Q + 2\ \text{cyt}\ c(Fe^{2+}) + 4\ H^{+}_{out}$
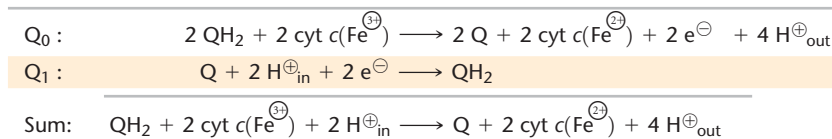
Then, a second electron is transferred to reduce the semiquinone to QH_2. The second electron is derived from the oxidation of a second molecule of QH_2 at the Q_o site. This second oxidation of QH_2 also results in the reduction of a second molecule of cytochrome c since the two electrons from the second QH_2 follow separate paths. The net result is that the oxidation of two molecules of QH_2 at the Q_o site produces two molecules of reduced cytochrome c and regenerates a molecule of QH_2 at the Q_i site. The two cycles of QH_2 oxidation are shown in Figure 14.16. The entire pathway is known as the Q cycle and it is one of the most important reactions in all of metabolism because it is the one most responsible for creating the protonmotive force.

Four protons are produced during the oxidation of two molecules of QH_2 at the Q_o site. These protons are released to the exterior of the membrane compartment and they contribute to the proton gradient that is formed during membrane-associated electron transport. The protons originate in the interior compartment. They may have been taken up in the reactions catalyzed by complex I or complex II or they may be derived from protons taken up on the inside of the membrane during reduction of Q at the Q_i site in complex III as shown in Figure 14.16.

The stoichiometry of the complete Q-cycle reaction is shown in Table 14.3. For every pair of electrons that pass through complex III from QH_2 to cytochrome c there are four protons translocated across the membrane. Two molecules of cytochrome c are reduced and these mobile carriers transport one electron each to complex IV. Note that there are actually two molecules of QH_2 oxidized (giving up four electrons) but two of these electrons are recycled to regenerate a molecule of QH_2.

The complete reaction catalyzed by ubiquinone:cytochrome c oxidoreductase (complex III) includes the Q cycle and proton translocation across the membrane. The complex III reaction is a fine example of the relationship between structure and function. While the stoichiometry of the Q cycle had been known for many years, the actual mechanism of the reaction only became apparent once the complete structure was solved in 1998.
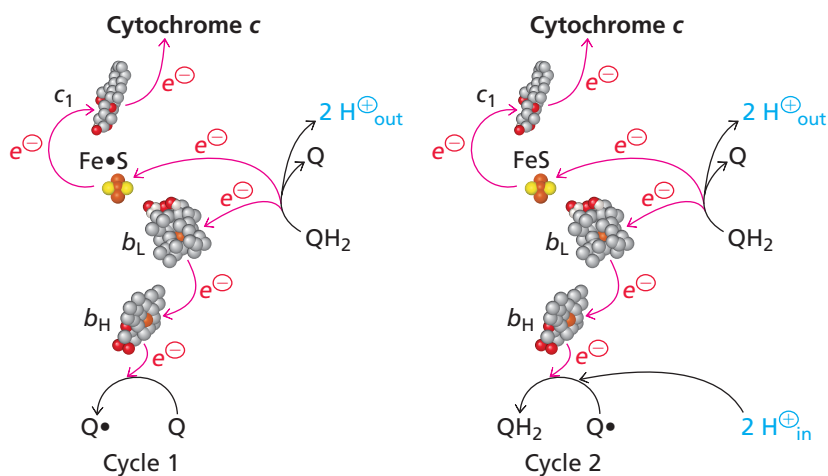
Figure 14.16 ▶
Q cycle. A molecule of QH_2 is oxidized in cycle 1 and a separate molecule is oxidized in cycle 2. Each cycle produces a molecule of reduced cytochrome c. The combination of cycle 1 and cycle 2 results in a two-stage reduction of Q to QH_2. Four protons are released on the exterior side of the membrane.

14.8 Complex IV

Complex IV is cytochrome c oxidase. This complex catalyzes the oxidation of the reduced cytochrome c molecules produced by complex III. The reaction includes a four-electron reduction of molecular oxygen (O_2) to water (2 H_2O) and translocation of four protons across the membrane.

Complex IV contains two functional units of cytochrome c oxidase. Each cytochrome c oxidase contains single copies of subunits I, II, and III (Figure 14.17). The bacterial enzymes contain only one additional subunit in each functional unit but the eukaryotic (mitochondrial) enzymes have up to ten additional subunits. Additional subunits in the eukaryotic complexes play a role in assembling complex IV and in stabilizing the structure.

The core structure of cytochrome c oxidase is formed from the three conserved subunits—I, II, and III. These polypeptides are encoded by mitochondrial genes in all eukaryotes. Subunit I is almost entirely embedded in the membrane. The bulk of this polypeptide consists of 12 transmembrane α helices. There are three redox centers buried within subunit I—two of them are a-type hemes (heme a and heme a_3) and the third is a copper ion (Cu_B). The copper atom is in close proximity to the iron atom of heme a_3 forming a binuclear center where the reduction of molecular oxygen takes place (Figure 14.18).

Subunit II has two transmembrane helices that anchor it to the membrane. Most of the polypeptide chain forms a β-barrel domain located on the exterior surface of the membrane. This domain contains a copper redox center (Cu_A) composed of two copper ions. These two copper atoms share electrons forming a mixed valence state. The external domain of subunit II is the site where cytochrome c binds to cytochrome c oxidase.

Subunit III has seven transmembrane helices and is completely embedded in the membrane. There are no redox centers in subunit III and it can be artificially removed without loss of catalytic activity. Its role *in vivo* is to stabilize subunits I and II and help protect the redox centers from inappropriate oxidation–reduction reactions.

Figure 14.19 shows the sequence of electron transfers in complex IV. Cytochrome c binds to subunit II and transfers an electron to the Cu_A site. The pair of copper ions at the Cu_A site can accept and donate one electron at a time—much like an Fe–S cluster. The complete oxidation of O_2 requires four electrons. Thus, four cytochrome c molecules have to bind and sequentially transfer a single electron each to the Cu_A redox center.
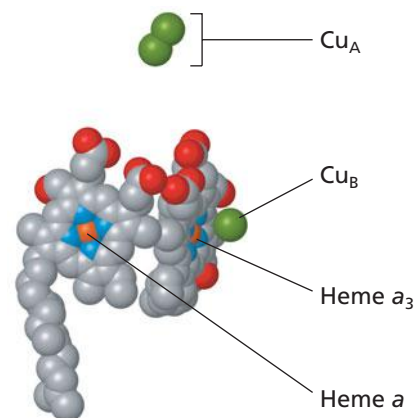
▲ **Figure 14.18**
Redox centers in cytochrome *c* oxidase. Organization of the heme and copper cofactors in one of the cytochrome *c* oxidase units. [PDB 1OCC]
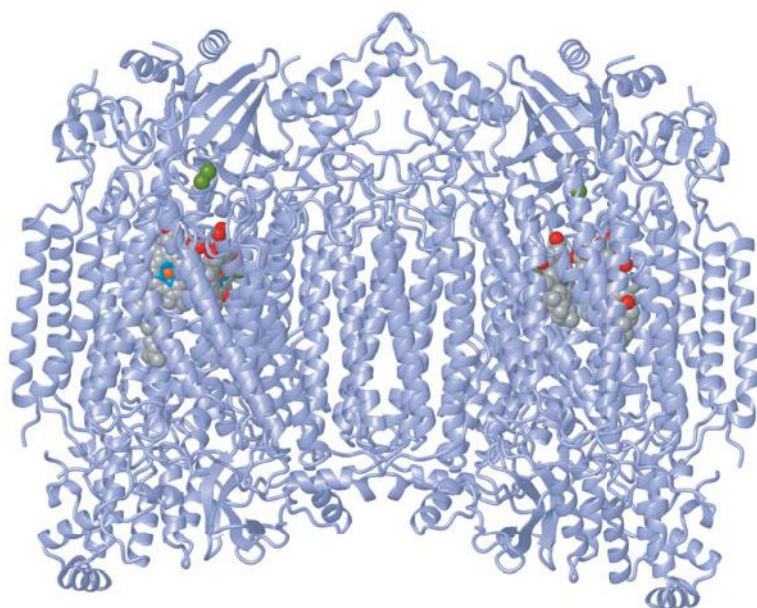
◄ **Figure 14.17**
Structure of cow (*Bos taurus*) complex IV from mitochondria. The complex consists of two functional units of cytochrome *c* oxidase. Each unit is composed of 13 subunits with multiple membrane-spanning α helices. [PDB 1OCC]
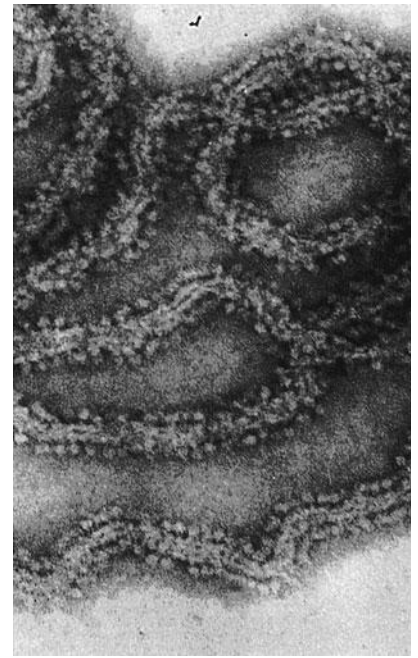
▲ Figure 14.20
Mitochondrial genome. Mitochondrial genomes are small, circular, double-stranded DNA molecules. They contain genes for ribosomal RNAs (12S rRNA, 16S rRNA) and tRNAs (labeled according to the amino acid they carry). The human mitochondrial genome, shown here, is only 16,589 bp in size and it encodes only a few of the subunits of the electron transport complexes. Genes for the subunits of complex I are colored green, a complex III subunit is purple, complex IV subunits are pink, and complex V subunits are yellow. The D-loop is a highly variable region required for DNA replication. Sequences of individual D-loop regions have been used to trace the evolution of modern humans providing early evidence that we all descend from a population in Africa.
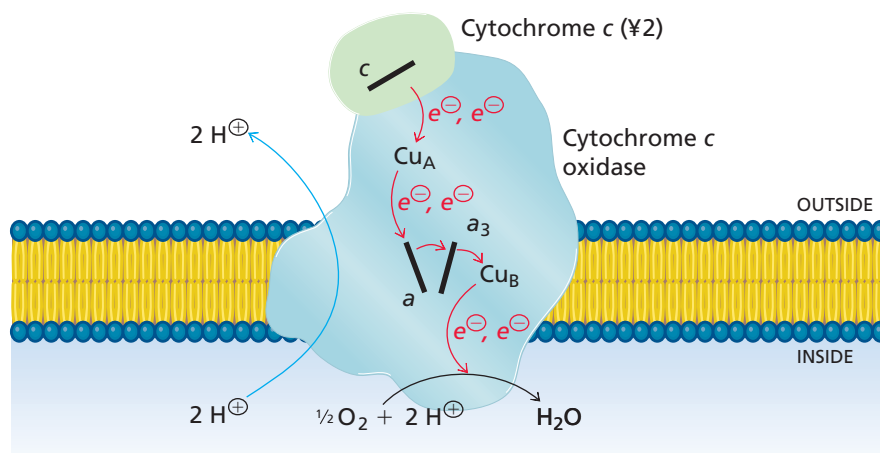
Electrons are transferred one at a time from the Cu_A site to the heme *a* prosthetic group in subunit I. From there they are transferred to the heme a_3–Cu_B binuclear center. The two heme groups (*a* and a_3) have identical structures but differ in their standard reduction potentials due to the local microenvironment formed by surrounding amino acid side chains in subunit I. Electrons can accumulate at the binuclear center as the heme iron alternates between Fe^{3+} and Fe^{2+} states and the copper atom shifts from Cu^{2+} to Cu^{+}. The detailed mechanism for reduction of molecular oxygen at the binuclear center is under active investigation in a number of laboratories. The first step involves the rapid splitting of molecular oxygen. One oxygen atom is bound to the iron atom of the a_3-heme group and the other is bound to the copper atom. Subsequent protonation and electron transfer results in the release of a water molecule from the copper site followed by release of a second water molecule from the iron ligand. The overall reaction requires the uptake of four protons from the inside surface of the membrane

$$O_2 + 4\,e^{\ominus} + 4\,H^{\oplus}_{in} \longrightarrow 2\,H_2O \qquad (14.12)$$
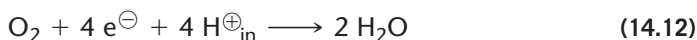
The site where oxygen is reduced is buried within the protein in the middle of the lipid bilayer of the membrane. Charged protons cannot access this site by passive diffusion—instead, the enzyme contains a channel leading from the inside of the membrane to the active site. This channel is filled with a single line of water molecules that rapidly exchange protons leading to the net movement of protons along this "water wire."

The reactions of cytochrome *c* oxidase are coupled to the transfer of protons across the membrane. One proton is translocated for each electron that passes from cytochrome *c* to the final product (H_2O). The protons move through a water-filled channel in complex IV and this movement is driven by conformational changes in the enzyme as oxygen is reduced. The stoichiometry of the complete reaction catalyzed by complex IV is

$$4\,\text{cyt}\,c^{2+} + O_2 + 8\,H^{\oplus}_{in} \longrightarrow 4\,\text{cyt}\,c^{3+} + 2\,H_2O + 4\,H^{\oplus}_{out} \qquad (14.13)$$

Complex IV contributes to the proton gradient that will drive ATP synthesis. *Two protons are translocated for each pair of electrons that pass through this complex.* Recall that complex I transfers four protons for each pair of electrons and complex III also translocates four protons for each electron pair. Thus, the membrane-associated electron transport system pumps ten protons across the membrane for every molecule of NADH that is oxidized.

The genes encoding the various subunits of the mitochondrial complexes may be in the nucleus or the mitochondria, depending on the species. The genes for cytochrome *c* oxidase subunits are always found in the mitochondrial genome (Figure 14.20).

14.9 Complex V: ATP Synthase

Complex V is ATP synthase. It catalyzes the synthesis of ATP from $ADP + P_i$ in a reaction that is driven by the proton gradient generated during membrane-associated electron transport. ATP synthase is a specific F-type ATPase called F_0F_1 ATPase—named after the reverse reaction. In spite of its name, F-type ATPase is responsible for *synthesizing* ATP—not hydrolyzing it. The enzyme is embedded in the membrane and has a characteristic knob-and-stalk structure that has been observed in electron micrographs for over half a century (Figure 14.21). The F_1 (knob) component contains the catalytic subunits—when released from membrane preparations it catalyzes the hydrolysis of ATP. For this reason, it has traditionally been referred to as F_1 ATPase. This part of the enzyme projects into the mitochondrial matrix in eukaryotes and into the cytoplasm in bacteria. (ATP synthase is also found in chloroplast membranes, as we will see in the next chapter.) The F_0 (stalk) component is embedded in the membrane. It has a proton channel that spans the membrane, and the passage of protons through this channel from the outside of the membrane to the inside is coupled to the formation of ATP by the F_1 component.

Recent cryo electron micrograph structures of ATP synthase have revealed details of its overall structure. These can be correlated with the X-ray crystallographic structures of the various components (Figure 14.22).

The subunit composition of the F_1 component (knob) is $\alpha_3\beta_3\gamma\delta\varepsilon$ and that of the F_0 membrane component is $a_1b_2c_{10-14}$. The c subunits of F_0 interact to form a cylindrical base within the membrane. The core of the F_1 (knob) structure is formed from three copies each of subunits α and β arranged as a cylindrical hexamer. The nucleotide binding sites lie in the clefts between adjacent α and β subunits. Thus, the binding sites are spaced 120° apart on the surface of the $\alpha_3\beta_3$ cylinder. The catalytic site of ATP synthesis is mostly associated with amino acid residues of the β subunit.

▲ **Figure 14.21**
Knobs and stalks. The internal mitochondrial membranes are studded with structures that look like knobs projecting into the mitochondrial matrix at the end of short membrane-embedded stalks.
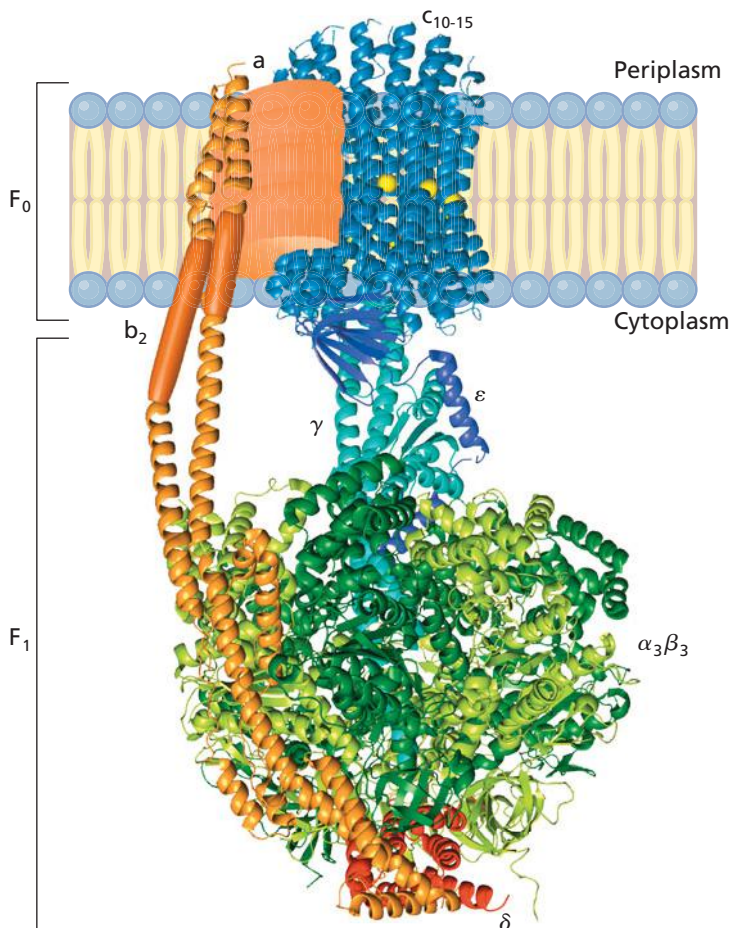
◄ **Figure 14.22**
ATP synthase structure. The F_1 component is on the inner face of the membrane. The F_0 component, which spans the membrane, forms a proton channel at the interface between the *a* and *c* subunits. The passage of protons through this channel causes the *c* subunit rotor (blue) to rotate relative to the stator of *a* and *b* subunits (orange). The torque of these rotations is transmitted to F_1 where it is used to drive ATP synthesis as the γ subunit (cyan) rotates within the head formed by α and β subunits (green). (The ε subunit is part of the stalk—it lies behind the γ subunit in this view.) (Modified from von Ballmoos et al., 2009.)

The $\alpha_3\beta_3$ oligomer of F_1 is connected to the transmembrane c subunits by a multi-subunit stalk made up of the γ and ε subunits. The c-ε-γ unit forms a "rotor" that spins within the membrane. Rotation of the γ subunit inside the $\alpha_3\beta_3$ hexamer alters the conformation of the β subunits, opening and closing the active sites. The a, b, and δ subunits form an arm that also attaches the F_0 component to the $\alpha_3\beta_3$ oligomer. This a-b-δ-$\alpha_3\beta_3$ unit is termed the "stator." Passage of protons through the channel at the interface between the a and c subunits causes the rotor assembly to spin in one direction relative to the stator. The entire structure is often called a molecular motor.

There are 10–14 c subunits in the membrane-associated c-ring at the base of the rotor. The number of subunits depends on the species—yeast and *E. coli* have a 10-subunit ring but plants and animals have up to 14 subunits. There is good evidence to indicate that the rotation of each c subunit past the stator is driven by translocation of a single proton. Rotation of the γ subunit within the F_1 component takes place in a stepwise, jerky manner where each step is 120° of rotation. As the c-ring rotates it twists the γ shaft until enough tension builds up to cause it to snap into the next position within the $\alpha_3\beta_3$ hexamer. If the c-ring has ten subunits then a complete rotation requires translocation of ten protons and results in the production of three ATP molecules but the exact stoichiometry is still being worked out. The results of many experiments indicate that, on average, three protons must be translocated for each ATP molecule synthesized and that's the value that we will use in the rest of this book. It suggests that only nine proton translocations are required for one complete rotation of the c-ring.

The mechanism of ATP synthesis from ADP and P_i has been the target of intensive research for several decades. In 1979 Paul Boyer proposed the *binding change mechanism* based on observations suggesting that the substrate and product binding properties of the active site could change as protons moved across the membrane. The $\alpha_3\beta_3$ oligomer of ATP synthase contains three catalytic sites. At any given time, each site can be in one of three different conformations: (1) open: newly synthesized ATP can be released and ADP + P_i can bind; (2) loose: bound ADP + P_i cannot be released; (3) tight: ATP is very tightly bound and condensation of ADP + P_i is favored. All three sites pass sequentially through these conformations as the γ subunit rotates within the knob. The rate of this reaction is comparable to that of many enzymes. The rotor turns at ten revolutions per second producing 30 ATP molecules per second. Typical turnover numbers (k_{cat}) are in the range of 100–1000 reactions per second.

The formation and release of ATP are believed to occur by the following steps, summarized in Figure 14.23:

1. One molecule of ADP and one molecule of P_i bind to an open site.

2. Rotation of the γ shaft causes each of the three catalytic sites to change conformation. The open conformation (containing the newly bound ADP and P_i) becomes a loose site. The loose site, already filled with ADP and P_i, becomes a tight site. The site containing ATP becomes an open site.

3. ATP is released from the open site and ADP and P_i condense to form ATP in the tight site.

V-ATPases have a similar structure. They use ATP hydrolysis to drive the import of protons into acidic vesicles (vacuoles). This is the reverse of the reaction catalyzed by ATP synthase.

▼ **Figure 14.23**
Binding change mechanism of ATP synthase. The different conformations of the three catalytic sites are indicated by different shapes. ADP and P_i bind to the yellow site in the open conformation. As the γ shaft rotates in the counterclockwise direction (viewed from the cytoplasmic/matrix end of the F_1 component), the yellow site is converted to a loose conformation where ADP and P_i are more firmly bound. Following the next step of the rotation the yellow site is converted to a tight conformation and ATP is synthesized. Meanwhile, the site that had bound ATP tightly has become an open site and a loose site containing other molecules of ADP and P_i has become a tight site. ATP is released from the open site and ATP is synthesized in the tight site.
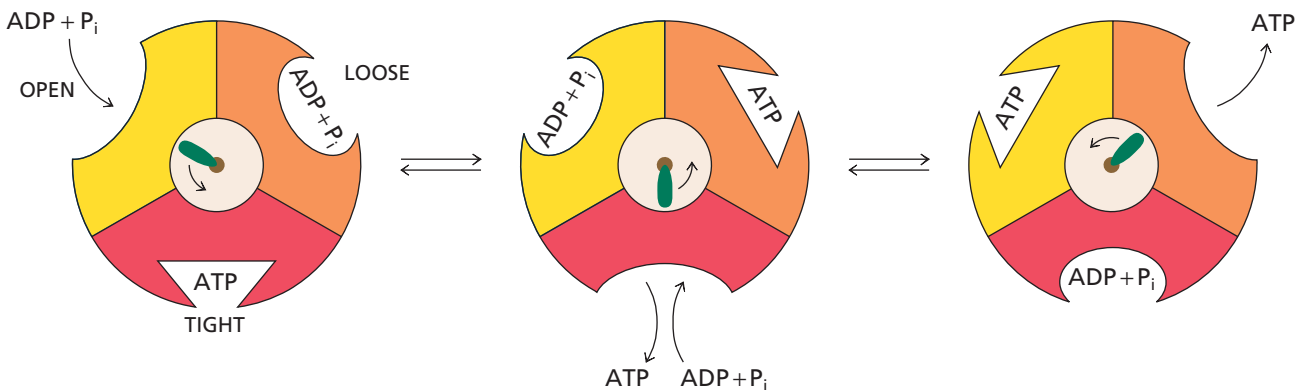
BOX 14.2 PROTON LEAKS AND HEAT PRODUCTION

Proton leaks appear to be a major consumer of free energy in mammals. In a resting adult mammal, about 90% of oxygen consumption takes place in the mitochondria and about 80% of this is coupled to ATP synthesis. Quantitative estimates indicate that the ATP produced by mitochondria is used for protein synthesis (almost 30% of the available ATP), for active transport of ions by Na$^\oplus$—K$^\oplus$ ATPase and Ca$^{2\oplus}$ ATPase (25% to 35%), for gluconeogenesis (up to 10%), and for other metabolic processes including heat generation. A significant amount of the energy from oxidation is not used for the synthesis of ATP. In resting mammals, at least 20% of the oxygen consumed by mitochondria is uncoupled by mitochondrial proton leakage. This leakage produces heat directly without apparent use.

The generation of heat in newborns and hibernating animals is a special example of deliberate uncoupling of proton translocation and ATP synthesis. This physiological uncoupling occurs in brown adipose tissue, whose brown color is due to its many mitochondria. Brown adipose tissue is found in abundance in newborn mammals and in species that hibernate. The free energy of NADH is not conserved as ATP but is lost as heat because oxidation is uncoupled from phosphorylation. The uncoupling is due to uncoupling protein 1 (UCP1, thermogenin) that forms a channel for the re-entry of protons into the mitochondrial matrix. When UCP1 is active the free energy released is dissipated as heat, raising the body temperature of the animal.

The strongest evidence that ATP synthase is a rotating motor has been obtained using the $\alpha_3\beta_3\gamma$ complex immobilized on a glass plate and modified by attachment of a fluorescent actin filament (Figure 14.24). Rotation of single molecules was observed by microscopy in the presence of ATP. In this experiment, the labeled γ subunit rotates inside the $\alpha_3\beta_3$ oligomer in response to ATP hydrolysis. This rotation is counterclockwise as depicted in Figure 14.24. Note that rotation driven by ATP hydrolysis is in the opposite direction to that observed when rotation is driven by the proton gradient and ATP is synthesized. The rotation of the γ shaft took place in 120° increments with one step for each ATP molecule hydrolyzed. Under ideal conditions, rates of more than 130 revolutions per second have been observed. This is the expected rotation rate based on the measured rate of ATP hydrolysis. It is much faster than the *in vivo* rate of rotation during ATP synthesis.
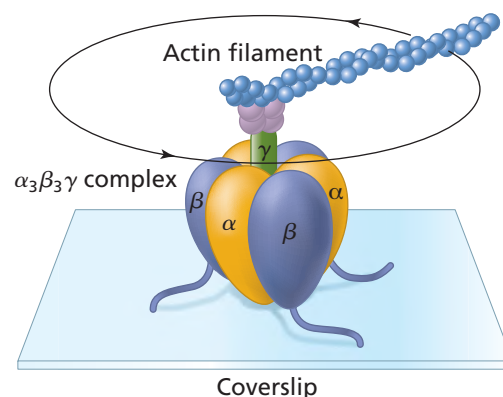
▲ **Figure 14.24**
Demonstration of the rotation of a single molecule of ATP synthase. $\alpha_3\beta_3$ complexes were bound to a glass coverslip and the γ subunit was attached to a long fluorescent protein arm. The arms on the molecules rotated when ATP was added. [Adapted from Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K., Jr. (1997). Direct observation of rotation of F$_1$-ATPase. *Nature* 386:299–302.]

14.10 Active Transport of ATP, ADP, and P$_i$ Across the Mitochondrial Membrane

A large fraction of the total ATP synthesized in eukaryotic cells is made in the mitochondria. These molecules must be exported since most of them are used in the cytoplasm. An active transporter is required to allow ADP to enter and ATP to leave mitochondria because the inner mitochondrial membrane is impermeable to charged substances. This transporter is called the adenine nucleotide translocase—it exchanges mitochondrial ATP and cytosolic ADP (Figure 14.25). Normally adenine nucleotides are complexed with Mg$^{2\oplus}$ but this is not the case when they are transported across the membrane. Exchange of ADP$^{3\ominus}$ and ATP$^{4\ominus}$ causes the loss of a net charge of –1 in the matrix. This type of exchange draws on the electrical part of the protonmotive force ($\Delta\Psi$) and some of the free energy of the proton concentration gradient is expended to drive this transport process.

The formation of ATP from ADP and P$_i$ in the mitochondrial matrix also requires a phosphate transporter to import P$_i$ from the cytosol. Phosphate (H$_2$PO$_4^-$) is transported into mitochondria in electroneutral symport with H$^\oplus$ (Figure 14.25). The phosphate carrier does not draw on the electrical component of the protonmotive force but does draw on the concentration difference, ΔpH. Thus, both transporters necessary for ATP formation use up some of the protonmotive force generated by proton translocation. The combined energy cost of transporting ATP out of the matrix and ADP and P$_i$ into it is approximately equivalent to the influx of one proton. Therefore, the synthesis of one molecule of cytoplasmic ATP by ATP synthase requires the

KEY CONCEPT

The chemical energy of the protonmotive force is converted to mechanical energy by causing the rotation of the ATP synthase rotor.

Active transport by ATPases is discussed in Section 9.11D.

net influx of four protons from the intermembrane space—one for transport and three that pass through the F$_0$ component of ATP synthase. Bacteria do not need to transport ATP or ADP across a membrane so the overall expense of ATP synthesis is less than that in eukaryotic cells.

14.11 The P/O Ratio

Before the chemiosmotic theory was proposed, many researchers were searching for a "high energy" intermediate capable of forming ATP by direct phosphoryl group transfer. They assumed that complexes I, III, and IV each contributed to ATP formation with one-to-one stoichiometry. We now know that energy transduction occurs by generating and consuming a proton concentration gradient. The yield of ATP need not be equivalent for each proton translocating electron transport complex nor must the yield of ATP per molecule of substrate oxidized be an integral number.

Many different membrane-associated electron transport complexes contribute simultaneously to the proton concentration gradient. This common energy reservoir is drawn on by many ATP synthase complexes. We saw in the preceding sections that the formation of one molecule of ATP from ADP and P$_i$ catalyzed by ATP synthase requires the inward passage of about three protons and one more proton is needed to transport P$_i$, ADP, and ATP across the inner membrane.

The first biochemists who studied these processes were primarily interested in the relationship between oxygen consumption (respiration) and ATP synthesis (phosphorylation). The P/O ratio is the ratio of molecules phosphorylated to atoms of oxygen reduced. It takes two electrons to reduce a single atom of oxygen (1/2 O$_2$) so we are interested in the number of protons translocated for each pair of electrons that pass through complexes I, III, and IV. Four protons are translocated by complex I, four by complex III, and two by complex IV. Thus, for each pair of electrons that pass through these complexes from NADH to O$_2$ a total of ten protons are moved across the membrane.

Since four protons are moved back across the membrane for each molecule of cytoplasmic ATP, the P/O ratio is 10 ÷ 4 = 2.5. The P/O ratio for succinate is only 6 ÷ 4 = 1.5 since electrons contributed by succinate oxidation do not pass through complex I. These calculated values are close to the P/O ratios that have been observed in experiments measuring the amount of O$_2$ reduced when a given amount of ADP is phosphorylated (Figure 14.3a). Recall that the overall energy available in the oxidation–reduction reactions is 220 kJ mol^{-1} (Section 14.4A) and this is more than enough for the synthesis of 2.5 molecules of ATP.

14.12 NADH Shuttle Mechanisms in Eukaryotes

NADH is produced by a variety of different reactions, especially the reactions catalyzed by glyceraldehyde-3-phosphate dehydrogenase during glycolysis and those of the citric acid cycle. NADH can be used directly in biosynthesis reactions such as amino acid synthesis and gluconeogenesis (where glceraldehyde-3-phosphate dehydrogenase operates in the reverse direction).

Excess NADH is used to produce ATP by the process that we have described in this chapter. In bacteria, the oxidation of NADH from all sources is readily accomplished since the membrane-associated electron transport system is embedded in the plasma membrane and the inside surface is exposed to the cytosol. In eukaryotic cells on the other hand, the only NADH molecules that have direct access to complex I are those found in the mitochondrial matrix. This is not a problem for reducing equivalents produced by the citric acid cycle since that pathway is localized to the mitochondria. However, the reducing equivalents produced by glycolysis in the cytosol must enter mitochondria in order to fuel ATP synthesis. Because neither NADH nor $NAD^{\oplus}$ can diffuse across the inner mitochondrial membrane, reducing equivalents must enter the mitochondrion by shuttle mechanisms. The glycerol phosphate shuttle and malate–aspartate shuttles are pathways by which a reduced coenzyme in the cytosol passes its reducing power to a mitochondrial molecule that then becomes a substrate for the electron transport chain.

> **A simplified version of the malate–aspartate shuttle is described in Section 13.4.**

The glycerol phosphate shuttle (Figure 14.26) is prominent in insect flight muscles that sustain very high rates of ATP synthesis. It is also present to a lesser extent in most mammalian cells. Two glycerol 3-phosphate dehydrogenases are required—an $NAD^{\oplus}$-dependent cytosolic enzyme and a membrane-embedded dehydrogenase complex that contains an FAD prosthetic group and has a substrate binding site on the outer face of the inner mitochondrial membrane. In the cytosol, NADH reduces dihydroxyacetone phosphate in a reaction catalyzed by cytosolic glycerol 3-phosphate dehydrogenase.

$$\text{(14.14)}$$

Glycerol 3-phosphate is then converted back to dihydroxyacetone phosphate by the membrane dehydrogenase complex and two electrons are transferred to the FAD prosthetic group of the enzyme. $FADH_2$ transfers two electrons to the mobile electron carrier Q, that then carries the electrons to ubiquinol:cytochrome c oxidoreductase (complex III). The oxidation of cytosolic NADH equivalents by this pathway produces less energy (1.5 ATP per molecule of cytosolic NADH) than the oxidation of mitochondrial NADH because the reducing equivalents introduced by the shuttle bypass NADH:ubiquinone oxidoreductase (complex I).
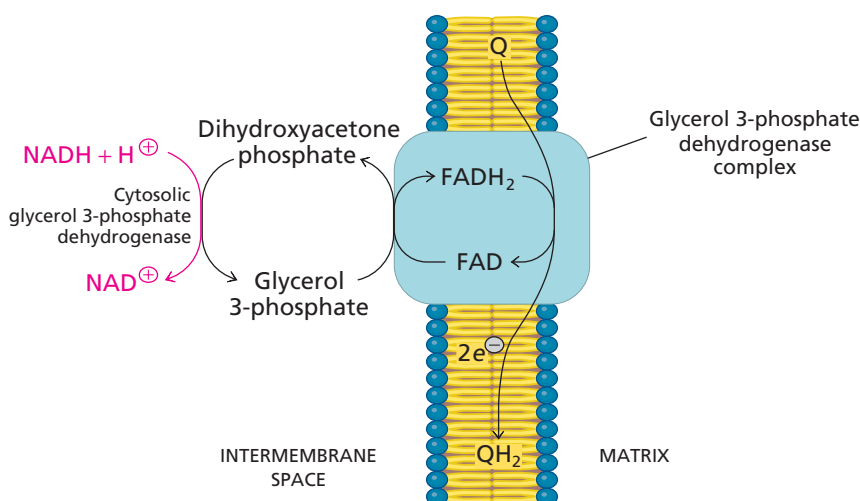
◄ **Figure 14.26**
Glycerol phosphate shuttle. Cytosolic NADH reduces dihydroxyacetone phosphate to glycerol 3-phosphate in a reaction catalyzed by cytosolic glycerol 3-phosphate dehydrogenase. The reverse reaction is catalyzed by an integral membrane flavoprotein that transfers electrons to ubiquinone.

Figure 14.27 ▶
Malate–aspartate shuttle. NADH in the cytosol reduces oxaloacetate to malate that is transported into the mitochondrial matrix. The reoxidation of malate generates NADH that can pass electrons to the electron transport chain. Completion of the shuttle cycle requires the activities of mitochondrial and cytosolic aspartate transaminase.

▲ **Another kind of shuttle.** This one required a great deal of energy.
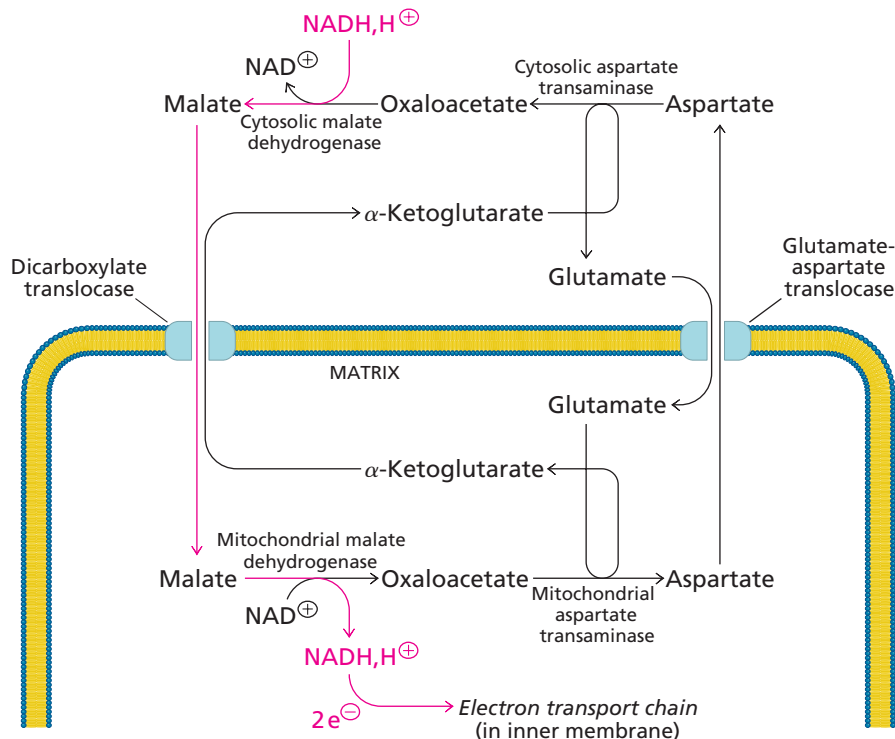
The malate–aspartate shuttle is more common. This shuttle requires cytosolic versions of malate dehydrogenase—the same enzyme used to convert cytosolic malate to oxaloacetate for gluconeogenesis. The reverse reaction is required for the malate–aspartate shuttle. The operation of the shuttle is diagrammed in Figure 14.27. NADH in the cytosol reduces oxaloacetate to malate in a reaction catalyzed by cytosolic malate dehydrogenase. Malate enters the mitochondrial matrix via the dicarboxylate translocase in electroneutral exchange for α-ketoglutarate. Inside the mitochondria, the citric acid cycle version of malate dehydrogenase catalyzes the reoxidation of malate to oxaloacetate with the reduction of mitochondrial $NAD^{\oplus}$ to NADH. NADH is then oxidized by complex I of the membrane-associated electron transport chain.

Continued operation of the shuttle requires the return of oxaloacetate to the cytosol but oxaloacetate cannot be directly transported across the inner mitochondrial membrane. Instead, oxaloacetate reacts with glutamate in a reversible reaction catalyzed by mitochondrial aspartate transaminase (Section 17.7C). This reaction transfers an amino group to oxaloacetate producing aspartate and α-ketoglutarate. Each molecule of α-ketoglutarate exits the mitochondrion via the dicarboxylate translocase in exchange for malate. Aspartate exits through the glutamate–aspartate translocase in exchange for glutamate. Once they are in the cytosol, aspartate and α-ketoglutarate become the substrates for a cytosolic form of aspartate transaminase that catalyzes the formation of glutamate and oxaloacetate. Glutamate re-enters the mitochondrion in antiport with aspartate and oxaloacetate reacts with another molecule of cytosolic NADH, repeating the cycle.

This complex shuttle system requires several enzymes that have distinctive cytoplasmic and mitochondrial versions (e.g., malate dehydrogenase). As a general rule, these enzymes are encoded by different, but related, genes that are descended from a common ancestor by an ancient gene duplication event. The compartmentalization of metabolic pathways in eukaryotic cells provides them with some advantages over bacterial cells but it requires mechanisms for moving metabolites across internal membranes. Part of the cost of compartmentalization is the duplication of enzymes that need to be present in several compartments. This partly explains why eukaryotic genomes contain so many families of related genes while bacterial genomes usually have only a single copy. One of the striking features of the human genome sequence is the presence of many gene families of this sort. Another major discovery is the presence

BOX 14.3 THE HIGH COST OF LIVING

The average active adult needs about 2400 kilocalories (10,080 kJ) per day. If all of this energy was translated to ATP equivalents, then it would correspond to the hydrolysis of 210 moles of ATP per day. (Assuming that the Gibbs free energy of hydrolysis is 48 kJ mol^{-1}.) This is approximately equal to 100 kg of ATP ($M_r = 507$).

All these ATP molecules have to be synthesized and by far the most common pathway is the synthesis of ATP driven by mitochondrial proton gradients. Actual calculated and measured values suggest that the average person makes

9×10^{20} molecules of ATP per second or 78×10^{24} molecules per day. This is 130 moles or 66 kg of ATP.

Thus, a significant percentage of our calorie intake is converted into a mitochondrial proton gradient in order to drive ATP synthesis. These calculations also tell us that ATP molecules turn over very rapidly since our bodies don't contain 66 kg of ATP.

Rich, P. (2003). The cost of living. *Nature* 421, 583.

of hundreds of genes involved in the translocation of molecules across membranes. The dicarboxylate translocase and glutamate–aspartate translocase described here (Figure 14.27) are examples of transport proteins.

14.13 Other Terminal Electron Acceptors and Donors

Up to this point we have only considered NADH and succinate as important sources of electrons in membrane-associated electron transport. These reduced compounds are mostly derived from catabolic oxidation–reduction reactions such as those in glycolysis and the citric acid cycle. You can imagine that the ultimate source of glucose is a biosynthesis pathway within a photosynthetic organism. The electrons in the chemical bonds of glucose were put there using light energy—the energy from sunlight is ultimately what powers ATP synthesis in mitochondria.

This is a reasonably accurate picture of energy flow in the modern biosphere but it doesn't explain how life survived before photosynthesis evolved. Not only did photosynthesis provide an abundant source of carbon compounds but it is also responsible for the increase in oxygen levels in the atmosphere. As we will see in the next chapter, photosynthesis also requires a membrane-associated electron transport system coupled to ATP synthesis. It's quite likely that respiratory electron transport, as described in this chapter, evolved first and the photosynthesis mechanism came later. There was probably life on this planet for several hundred million years before photosynthesis became commonplace.

What was the ultimate source of energy before sunlight? We have a pretty good idea of how metabolism worked in the beginning because there are still chemoautotrophic bacteria alive today. These species do not need organic molecules as carbon or energy sources and they do not capture energy from sunlight.

Chemoautotrophs derive their energy from oxidizing inorganic compounds such as H_2, $NH_4^{\oplus}$, $NO_2^{\ominus}$, H_2S, S, or Fe^{2+}. These inorganic molecules serve as a direct source of energetic electrons in membrane-associated electron transport. The terminal electron acceptors can be O_2, fumarate, or a wide variety of other molecules. As electrons pass through their electron transport chain a protonmotive force is generated and ATP is synthesized. An example of such a pathway is shown in Figure 14.28.

The electron donor is hydrogen in this example. A membrane-bound hydrogenase oxidizes hydrogen to protons. Such hydrogenases are common in a wide variety of bacteria species. Electrons pass through cytochrome complexes similar to those of respiratory electron transport. In most bacteria, the mobile quinone is not ubiquinone but a related molecule called menaquinone (Section 7.15). Fumarate reductase catalyzes the reduction of fumarate to succinate using reduced menaquinone (MQH_2) as the electron donor.

E. coli can use fumarate instead of oxygen as a terminal electron acceptor when it is growing under anaerobic conditions. Fumarate reductase is a multisubunit enzyme embedded in the plasma membrane. It is homologous to succinate dehydrogenase and the two enzymes catalyze a very similar reaction but in different directions. In *E. coli*,
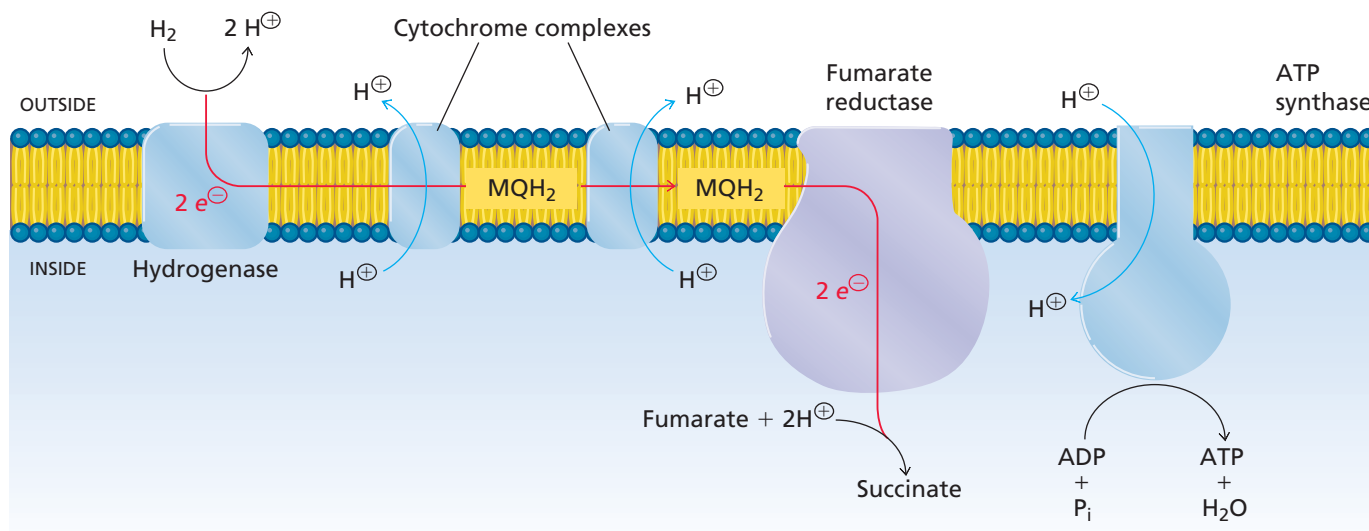
▲ Figure 14.28
One possible pathway for ATP synthesis in chemoautotrophic bacteria. Hydrogen is oxidized by a membrane-bound hydrogenase and electrons are passed through various membrane cytochrome complexes. Electron transfer is coupled to the translocation of protons across the membrane and the resulting protonmotive force is used to drive ATP synthesis. The terminal electron acceptor is fumarate. Fumarate is reduced to succinate by fumarate reductase.

these two enzymes are not expressed at the same time, and *in vivo* each catalyzes its reaction in only one direction (the direction related to the enzyme name). This is one of the few cases where bacterial genomes contain a family of related genes. Each gene encodes a slightly different version of the same enzyme.

In addition to oxygen and fumarate, nitrate and sulfate and many other inorganic molecules can serve as electron acceptors. There are many different combinations of electron donors, acceptors, and electron transport complexes in chemoautotrophic bacteria. The important point is that these bacteria extract energy from inorganic compounds in the absence of light and they may survive without oxygen.

Chemoautotrophic bacteria represent possible metabolic strategies that were present in very ancient organisms but there are still modern bacteria that grow and reproduce in the absence of sunlight and oxygen such as the extreme thermophiles described in Box 2.1 and species that live deep underground.

14.14 Superoxide Anions

One of the unfortunate consequences of oxygen metabolism is the production of reactive oxygen species such as the superoxide radical ($\cdot O_2^{\ominus}$), hydroxyl radical ($OH\cdot$), and hydrogen peroxide (H_2O_2). All of these species are highly toxic to cells. They are produced by flavoproteins, quinones, and iron–sulfur proteins. Almost all of the electron transport reactions produce small amounts of these reactive species, especially $\cdot O_2^{\ominus}$. If a superoxide radical is not rapidly removed by superoxide dismutase it will cause breakdown of proteins and nucleic acids.

We have already discussed superoxide dismutase as an example of an enzyme with a diffusion controlled mechanism (Section 6.4B). The overall reaction catalyzed by this enzyme is the dismutation of two superoxide anions to hydrogen peroxide. This reaction proceeds extremely rapidly.

$$2 \cdot O_2^{\ominus} + 2\,H^{\oplus} \longrightarrow H_2O_2 + O_2 \tag{14.15}$$
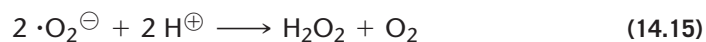
The rapidity of this process is typical of electron transfer reactions. In this case, a copper ion is the only electron transfer agent bound to the enzyme. The copper ion is reduced by superoxide anion ($\cdot O_2^{\ominus}$), and it then reduces another molecule of $\cdot O_2^{\ominus}$. The hydrogen peroxide formed can be converted to H_2O and O_2 by the action of catalase.

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2 \tag{14.16}$$
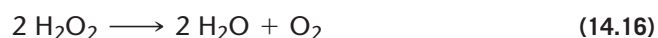
Some bacteria species are obligate anaerobes. They die in the presence of oxygen because they cannot deplete reactive oxygen species that arise as a by-product of oxidation–reduction reactions. These species do not have superoxide dismutase. All aerobic species have enzymes that scavenge reactive oxygen molecules.

Summary

1. The energy in reduced coenzymes is recovered as ATP through a membrane-associated electron transport system coupled to ATP synthesis.

2. Mitochondria are surrounded by a double membrane. The electron transport complexes and ATP synthase are embedded in the inner membrane. This inner membrane is highly folded.

3. The chemiosmotic theory explains how the energy of a proton gradient can be used to synthesize ATP. The free energy associated with the protonmotive force is mostly due to the charge difference across the membrane.

4. The electron transport complexes I through IV contain multiple polypeptides and cofactors. The electron carriers are arranged roughly in order of increasing reduction potential. The mobile carriers ubiquinone (Q) and cytochrome c link the oxidation–reduction reactions of the complexes.

5. The transfer of a pair of electrons from NADH to Q by complex I contributes four protons to the proton concentration gradient.

6. Complex II does not directly contribute to the proton concentration gradient but rather supplies electrons from succinate oxidation to the electron transport chain.

7. The transfer of a pair of electrons from QH_2 to cytochrome c by complex III is coupled to the transport of four protons by the Q cycle.

8. The transfer of a pair electrons from cytochrome c and the reduction of $1/2\ O_2$ to H_2O by complex IV contributes two protons to the gradient.

9. Protons move back across the membrane through complex V (ATP synthase). Proton flow drives ATP synthesis from ADP + P_i by conformational changes produced by the operation of a molecular motor.

10. The transport of ADP and P_i into and ATP out of the mitochondrial matrix consumes the equivalent of one proton.

11. The P/O ratio, the ATP yield per pair of electrons transferred by complexes I through IV, depends on the number of protons translocated. The oxidation of mitochondrial NADH generates 2.5 ATP; the oxidation of succinate generates 1.5 ATP.

12. Cytosolic NADH can contribute to oxidative phosphorylation when the reducing power is transferred to mitochondria by the action of shuttles.

13. Superoxide dismutase converts superoxide radicals to hydrogen peroxide. Hydrogen peroxide is removed by catalase.

Problems

1. In a typical marine bacterium the membrane potential across the inner membrane is –0.15 V. The protonmotive force is –21.2 kJ mol^{-1}. If the pH in the periplasmic space is 6.35, what is the pH in the cytoplasm if the cells are at 25°C?

2. The iron atoms of six different cytochromes in the respiratory electron transport chain participate in one-electron transfer reactions and cycle between the Fe(II) and the Fe(III) states. Explain why the reduction potentials of the cytochromes are not identical but range from -0.10 V to 0.39 V.

3. Functional electron transport systems can be reconstituted from purified respiratory electron transport chain components and membrane particles. For each of the following sets of components, determine the final electron acceptor. Assume O_2 is present.

   (a) NADH, Q, complexes I, III, and IV
   (b) NADH, Q, cytochrome c, complexes II and III
   (c) succinate, Q, cytochrome c, complexes II, III, and IV
   (d) succinate, Q, cytochrome c, complexes II and III

4. A gene has been identified in humans that appears to play a role in the efficiency with which calories are utilized, and anti-obesity drugs have been proposed to regulate the amount of the uncoupling protein-2 (UCP-2) produced by this gene. The UCP-2 protein is present in many human tissues and has been shown to be a proton translocator in mitochondrial membranes. Explain how increasing the presence of the UCP-2 protein might lead to weight loss in humans.

5. (a) When the widely prescribed painkiller Demerol (mepiridine) is added to a suspension of respiring mitochondria, the ratios NADH/NAD$^{\oplus}$ and Q/QH$_2$ increase. Which electron transport complex is inhibited by Demerol?

   (b) When the antibiotic myxothiazole is added to respiring mitochondria, the ratios cytochrome c_1(Fe$^{\oplus}$)/cytochrome c_1(Fe$^{\oplus}$) and cytochrome b_{566}(Fe$^{\oplus}$)/cytochrome b_L(Fe$^{\oplus}$) increase.

   Where does myxothiazole inhibit the electron transport chain?

6. (a) The toxicity of cyanide (CN$^{\ominus}$) results from its binding to the iron atoms of the cytochrome a,a_3 complex and subsequent inhibition of mitochondrial electron transport. How does this cyanide–iron complex prevent oxygen from accepting electrons from the electron transport chain?

   (b) Patients who have been exposed to cyanide can be given nitrites that convert the Fe$^{\oplus}$ iron in oxyhemoglobin to Fe$^{\oplus}$ (methemoglobin). Given the affinity of cyanide for Fe$^{\oplus}$, suggest how this nitrite treat mentmight function to decrease the effects of cyanide on the electron transport chain.

7. Acyl CoA dehydrogenase catalyzes the oxidation of fatty acids. Electrons from the oxidation reactions are transferred to FAD and enter the electron transport chain via Q. The reduction potential of the fatty acid in the dehydrogenase-catalyzed reaction is about –0.05 V. Calculate the free energy changes to show why FAD—not NAD$^{\oplus}$—is the preferred oxidizing agent.

8. For each of the following two-electron donors, state the number of protons translocated from the mitochondrion, the number of ATP molecules synthesized, and the P/O ratio. Assume that electrons pass eventually to O_2, NADH is generated in the mitochondrion, and the electron *transport and oxidative phosphorylation systems are fully* functional.

   (a) NADH
   (b) succinate
   (c) ascorbate/tetramethyl-p-phenylenediamine (donates two electrons to cytochrome c)

9. (a) Why is the outward transport of ATP favored over the outward transport of ADP by the adenine nucleotide transporter?

   (b) Does this ATP translocation have an energy cost to the cell?

10. Atractyloside is a toxic glycoside from a Mediterranean thistle that specifically inhibits the ADP/ATP carrier. Why does atractyloside cause electron transport to be inhibited as well?

11. (a) Calculate the protonmotive force across the inner mitochondrial membrane at 25°C when the electrical difference is −0.18 V (inside negative), the pH outside is 6.7, and the pH inside is 7.5.

    (b) What percentage of the energy is from the chemical (pH) gradient, and what percentage is from the charge gradient?

    (c) What is the total free energy available for the phosphorylation of ADP?

12. (a) Why does NADH generated in the cytosol and transported into the mitochondrion by the malate–aspartate shuttle produce fewer ATP molecules than NADH generated in the mitochondrion?

    (b) Calculate the number of *ATP equivalents produced* from the complete oxidation of one molecule of glucose to six molecules of CO_2 in the liver when the malate–aspartate shuttle is operating. Assume aerobic conditions and fully functional electron transport and oxidative phosphorylation systems.

Selected Readings

Mitochondria

Mentel, M., and Martin, W. (2010). Anaerobic animals from an ancient, anoxic ecological niche. *BMC Biology* 8:32–38.

Taylor, R. W., and Turnbull, D. M. (2005). Mitochondrial DNA mutations in human disease. *Nature Reviews: Genetics* 6:390–402.

Chemiosmotic Theory

Lane, N. (2006) Batteries not included. *Nature* 441:274–277.

Mitchell, P. (1979). Keilin's respiratory chain concept and its chemiosmotic consequences. *Science* 206:1148–1159.

Mitchell, P., and Moyle J. (1965). Stoichiometry of proton translocation through the respiratory chain and adenosine triphosphatase systems of rat liver mitochondria. *Nature* 208:147–151.

Schultz, B., and Chan, S. I. (2001). Structures and proton-pumping strategies of mitochondrial respiratory enzymes. *Annu. Rev. Biophys. Biomol. Struct.* 30:23–65.

Electron Transport Complexes

Berry, E. A., Guergova-Kuras, M., Huang, L., and Crofts, A. R. (2000). Structure and function of cytochrome *bc* complexes. *Annu. Rev. Biochem.* 69:1005–1075.

Brandt, U. (2006). Energy converting NADH: quinone oxidoreductase (complex I). *Annu. Rev. Biochem.* 75:69–92.

Cecchini, G. (2003). Function and structure of Complex II of the respiratory chain. *Annu. Rev. Biochem.* 72:77–100.

Clason, T., Ruiz, T., Schägger, H., Peng, G., Zickerman, V., Brandt, U., Michel, H., and Radermacher, M. (2010). The structure of eukaryotic and prokaryotic complex I. *J. Struct. Biol.* 169:81–88.

Clason, T., Ruiz, T., Schägger, H., Peng, G., Zickerman, V., Brandt, U., Michel, H., and Radermacher, M. (2010). The structure of eukaryotic and prokaryotic complex I. *J. Struct. Biol.* 169:81–88.

Crofts, A. R. (2004). The cytochrome bc_1 complex: function in the context of structure. *Annu. Rev. Physiol.* 66:689–733.

Hosler, J. P., Ferguson-Miller, S., and Mills, D. A. (2006). Energy transduction: proton transfer through the respiratory complexes. *Annu. Rev. Biochem.* 75:165–187.

Hunte, C., Palsdottir, H., and Trumpower, B. L. (2003). Protonmotive pathways and mechanisms in the cytochrome bc_1 complex. *FEBS Letters* 545:39–46.

Hunte, C., Zickerman, V., and Brandt, U. (2010). Functional modules and structural basis of conformational coupling in mitochondrial complex I. *Science* 329:448–457.

Richter, O.-M., and Ludwig, B. (2003). Cytochrome *c* oxidase—structure, function, and physiology of a redox-driven molecular machine. *Rev. Physiol. Biochem. Pharmacol.* 147:47–74.

ATP Synthase

Capaldi, R. A., and Aggler, R. (2002). Mechanism of the F_1F_0-type ATP synthase, a biological rotary motor. *Trends in Biochem. Sci.* 27:154–160.

Lau, W. C. Y., and Rubinstein, J. (2010). Structure of intact *Thermus thermophilus V-ATPase* by cryo-EM reveals organization of the membrane-bound V_o motor. *Proc. Natl. Acad. Sci. (USA)* 107:1367–1372.

Nishio, K., Iwamoto-Kihara, A., Yamamoto, A., Wada, Y., and Futai, M. (2002). Subunit rotation of ATP synthase: α or β subunit rotation relative to the *c* subunit ring. *Proc. Natl. Acad. Sci. (USA)* 99:13448–13452.

Oster, G., and Wang, H. (2003). Rotary protein motors. *Trends in Cell Biology* 13:114–121.

Other Electron Donors and Acceptors

Hederstedt, L. (1999). Respiration without O_2. *Science* 284:1941–1942.

Iverson, T. M., Luna-Chavez, C., Cecchini, G., and Rees, D. C. (1999). Structure of the *Escherichia coli* fumarate reductase respiratory complex. *Science* 284:1961–1966.

Peters, J. W., Lanzilotta, W. N., Lemon, B. J., and Seefeldt, L. C. (1998). X-ray crystal structure of the Fe-only hydrogenase (CpI) from *Clostridium pasteurianum* to 1.8 Ångstrom resolution. *Science* 282:1853–1858.

Tielens, A. G. M., Rotte, C., van Hellemond, J. J., and Martin, W. (2002). Mitochondria as we don't know them. *Trends in Biochem. Sci.* 27:564-572.

von Ballmoos, C., Cook, G. M., and Dimroth, P. (2008). Unique rotary ATP synthase and its biological diversity. *Annu. Rev. Biophys.* 37:43–64.

von Ballmoos, C., Wiedenmann, A., and Dimroth, P. (2009). Essentials for ATP synthesis by F_1F_0 ATP synthases. *Annu. Rev. Biochem.* 78:649–672.

Yankovskaya, V., Horsefield, R., Törnroth, S., Luna-Chavez, C., Miyoshi, H., Léger, C., Byrne, B., and Iwata, S. (2003). Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science* 299:700–704.
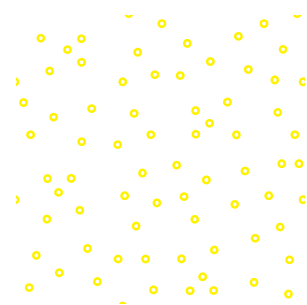
Photosynthesis

The most important part of photosynthesis is the conversion of light energy into chemical energy in the form of ATP. The basic principle behind this fundamental reaction is similar to that of membrane-associated electron transport covered in the previous chapter. In photosynthesis, light shines on a pigment molecule (e.g., chlorophyll) and an electron is excited to a higher energy level. As the electron falls back to its initial state it gives up energy and this energy is used to translocate protons across a membrane. This creates a proton gradient that is used to drive phosphorylation of ADP in a reaction catalyzed by ATP synthase. In some cases, reducing equivalents in the form of NADPH are synthesized directly when the excited electron is used to reduce $NADP^{\oplus}$. These reactions are called the **light reactions** since they are absolutely dependent on sunlight.

Photosynthetic species use their abundant supply of cheap ATP and NADPH to carry out all of the metabolic reactions that require energy. This includes synthesis of proteins, nucleic acids, carbohydrates, and lipids. This is why photosynthetic bacteria and algae are such successful organisms.

Most photosynthetic organisms have a special CO_2 fixing pathway called the *Calvin cycle*. Strictly speaking, the fixation of CO_2 does not require light and is not directly coupled to the light reactions. For this reason, these reactions are often called the **dark reactions** but this does not mean they take place in the dark. This pathway is closely related to the pentose–phosphate pathway described in Section 12.4.

The details of photosynthesis reactions are extremely important in understanding the biochemistry of all life on the planet. The ability to harvest light energy to synthesize macromolecules led to a rapid expansion of photosynthetic organisms. This, in turn, created opportunities for species that could secondarily exploit photosynthetic organisms as food sources. Animals, such as us, ultimately derive much of their energy by degrading molecules that were originally synthesized using the energy from sunlight. In addition, oxygen is a by-product of photosynthesis in plants and some bacteria. The buildup of oxygen in Earth's atmosphere led to its role as an electron acceptor in membrane-associated electron transport. With few exceptions, modern eukaryotes now absolutely depend on the supply of oxygen produced by photosynthesis in order to synthesize ATP in their mitochondria.

Why does this particular group of radiations, rather than some other, make the leaves grow and the flowers burst forth, cause the mating of fireflies and the spawning of palolo worms, and, when reflecting off the surface of the moon, excite the imagination of poets and lovers?

Helena Curtis and Sue Barnes
(1989). *Biology*, 5th ed.

Top: Sunlight on trillium in the woods. Solar energy captured by photosynthetic organisms ultimately sustains the activities of nearly all organisms on Earth.

▲ **Photosynthetic organisms.** Left: cyanobacteria. Middle: leaves of a flowering plant. Right: purple bacteria.

The major components of the photosynthesis reactions are large complexes of proteins, pigments, and cofactors embedded in a membrane. A complex containing the light-sensitive pigments is called a **photosystem**. Different species employ a variety of different strategies to utilize light energy in order to synthesize ATP and/or NADPH. We will first describe the structure and function of photosystems in bacteria and then move on to the more complex photosynthesis pathway in eukaryotes such as algae and plants. The eukaryotic photosynthesis complexes clearly evolved from the simple bacterial ones.

15.1 Light-Gathering Pigments

There are several kinds of light-gathering pigments. They have different structures, different properties, and different functions.

A. The Structures of Chlorophylls

Chlorophylls are the most important pigments in photosynthesis. The structures of the most common chlorophyll molecules are shown in Figure 15.1. Note that the tetrapyrrole ring of chlorophylls is similar to that of heme (Figure 7.38) except that the chlorophyll ring is reduced—it has one less double bond in the conjugated ring system between position 7 and 8 in ring IV. Chlorophylls contain a central chelated Mg^{2+} ion instead of the Fe^{2+} found in heme. Another distinguishing feature of chlorophylls is that they possess a long phytol side chain that contributes to their hydrophobicity.

There are many different types of chlorophylls. They differ mostly in the side chains labeled R_1, R_2, and R_3 in Figure 15.1. Chlorophyll a (Chl a) and chlorophyll b

Phytol side chain
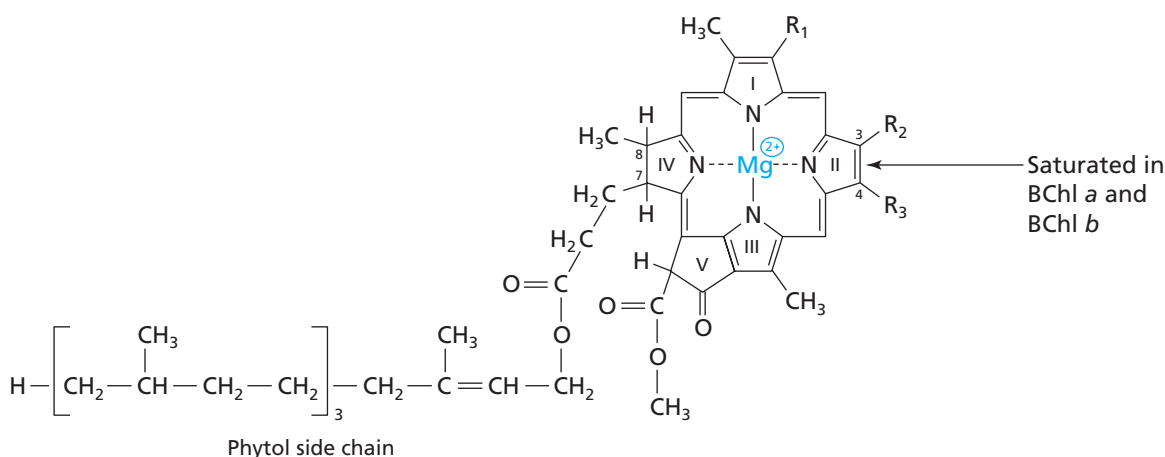
Chl species	R_1	R_2	R_3
Chl a	$-CH=CH_2$	$-CH_3$	$-CH_2-CH_3$
Chl b	$-CH=CH_2$	$-\overset{\overset{O}{\|\|}}{C}-H$	$-CH_2-CH_3$
BChl a	$-\overset{\overset{O}{\|\|}}{C}-CH_3$	$-CH_3$	$-CH_2-CH_3$
BChl b	$-\overset{\overset{O}{\|\|}}{C}-CH_3$	$-CH_3$	$-CH=CH-CH_3$

▲ **Figure 15.1**
Structures of chlorophyll and bacteriochlorophyll pigments. Differences in substituent groups indicated as R_1, R_2 and R_3 are shown in the table. In the bacteriochlorophylls, the double bond indicated in ring II is saturated. In some molecules of bacteriochlorophyll a, the phytol side chain has three additional double bonds. The hydrophobic phytol side chain and hydrophilic porphyrin ring give chlorophyll amphipathic characteristics. Chlorophyll (bound to proteins) is found in photosystems and in associated light-harvesting complexes.
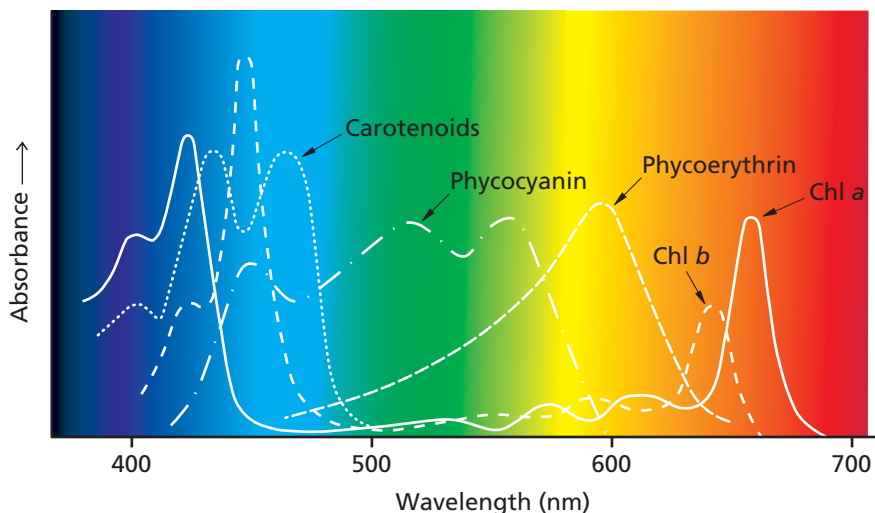
Absorption spectra of major photosynthetic pigments. Collectively, the pigments absorb radiant energy across the spectrum of visible light.

(Chl *b*) are found in a large number of species. Bacteriochlorophyll *a* (BChl *a*) and bacteriochlorophyll *b* (BChl *b*) are only found in photosynthetic bacteria. They differ from the other chlorophylls because they have one less double bond in ring II. Pheophytin (Ph) and bacteriopheophytin (BPh) are similar pigments where the Mg^{2+} in the central cavity is replaced by two covalently bound hydrogens.

Chlorophyll molecules are specifically oriented in the membrane by noncovalent binding to integral membrane proteins. The hydrophobic phytol side chain helps anchor chlorophyll in the membrane. The light-absorbing ability of chlorophyll is due to the tetrapyrrole ring with its network of conjugated double bonds. Chlorophylls absorb light in the violet-to-blue region (absorption maximum 400 to 500 nm) and the orange-to-red region (absorption maximum 650 to 700 nm) of the electromagnetic spectrum (Figure 15.2). This is why chlorophylls are green—that's the part of the spectrum that is reflected, not absorbed. The exact absorption maxima of chlorophylls depend on their structures; for example, Chl *a* differs from Chl *b*. The absorption maxima of particular chlorophyll molecules is also affected by their microenvironment within the pigment–protein complex.

B. Light Energy

A single quantum of light energy is called a **photon**. When a chlorophyll molecule absorbs a photon, an electron from a low energy orbital in the pigment is promoted to a higher energy molecular orbital. The energy of the absorbed photon must match the difference in energy between the ground state and higher energy orbitals—this is why chlorophyll absorbs only certain wavelengths of light. The excited "high energy" electron can be transferred to nearby oxidation–reduction centers in the same way that "high energy" electrons can be transferred from NADH to FMN in complex I during respiratory electron transport (Section 14.5). The main difference between photosynthesis and respiratory electron transport is the source of excited electrons. In respiratory electron transport the electrons are derived from chemical oxidation–reduction reactions that produce NADH and QH_2. In photosynthesis the electrons are directly promoted to a "high energy" state by absorption of a photon of light.

Chlorophyll molecules can exist in three different states. In the ground state (Chl or Chl^0), all electrons are at their normal stable level. In the excited state (Chl*) a photon of light has been absorbed. Following electron transfer, the chlorophyll molecule is in the oxidized state ($Chl^{\oplus}$) and must be regenerated by receiving an electron from an electron donor.

The energy of a photon of light can be calculated from the following equation

$$E = \frac{hc}{\lambda} \qquad \textbf{(15.1)}$$

where *h* is Planck's constant (6.63×10^{-34} J s), *c* is the velocity of light (3.00×10^8 m s^{-1}), and λ is the wavelength of light. It's often convenient to calculate the total energy of a

KEY CONCEPT

Chlorophyll molecules are oxidized (loss of an electron) when they absorb a photon of light.
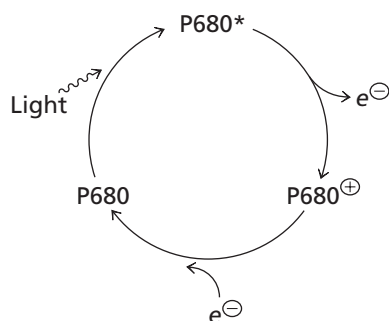
▲ **The states of chlorophyll.** Reduction, excitation, and oxidation of chlorophyll P680. P680* is the excited state following absorption of a photon of light. Loss of an electron produces the oxidized state, P680$^{\oplus}$. Gain of an electron from an outside source (such as the oxidation of water) yields the reduced P680 state.

> The Gibbs free energy change associated with the protonmotive force is calculated in Section 14.3B

"mole" of photons by multiplying E by 6.022×10^{23} (Avogadro's number). Thus, for light at a wavelength of 680 nm, the energy is 176 kJ mol^{-1}. This is similar to a standard Gibbs free energy change. It means that when a mole of chlorophyll molecules absorbs a mole of photons the excited electrons acquire an amount of energy equal to 176 kJ mol^{-1}. As they fall back to their ground state they give up this energy and some of it is captured and used to pump protons across the membrane or to synthesize NADPH.

C. The Special Pair and Antenna Chlorophylls

A typical photosystem contains dozens of chlorophyll molecules but only two special chlorophyll molecules actually give up electrons to begin the electron transfer chain. These two chlorophyll molecules are called the **special pair**. In most cases the special pair is identified simply as pigments (P) that absorb light at a specific wavelength. Thus, P680 is the special pair of chlorophyll molecules that absorbs light at 680 nm (red). Its three states are P680, P680*, and P680$^{\oplus}$. P680 is the ground state. P680* is the state following absorption of a photon of light when the chlorophyll macromolecules have an excited electron. P680$^{\oplus}$ is the electron-deficient (oxidized) state following transfer of an electron to another molecule. P680$^{\oplus}$ is reduced to P680 by transfer of an electron from an electron donor.

In addition to the special pair there are other specialized chlorophyll molecules that function as part of the electron transfer chain. They accept electrons from the special pair and transfer them to the next molecule on the pathway. Not all chlorophylls are directly involved in electron transfer. The remaining chlorophylls act as antenna molecules by capturing light energy and transferring it to the special pair. These antenna chlorophylls are much more numerous than the molecules in the electron transfer chain. The mode of excitation energy transfer between antenna chlorophylls is called **resonance energy transfer**. It does not involve the movement of electrons. You can think of excitation energy transfer as a transfer of vibrational energy between adjacent chlorophyll molecules in the densely packed antenna complex.

Figure 15.3 illustrates the transfer of excitation energy from antenna chlorophylls to the special pair in one of the photosystems. The figure shows only a few of the many antenna molecules surrounding the special pair. All chlorophyll molecules are held in

Figure 15.3 ▶
Transfer of light energy from antenna chlorophyll pigments to the special pair of chlorophyll molecules. Light can be captured by the antenna pigments (gray) and excitation energy is transferred between antenna chlorophylls until it reaches the special pair of chlorophyll molecules in the electron transfer pathway (green). The path of excitation energy transfer is shown in red. The special pair gives up an electron to the electron transfer pathway. The chlorophyll molecules are held in fixed positions because they are tightly bound to membrane proteins (not shown).
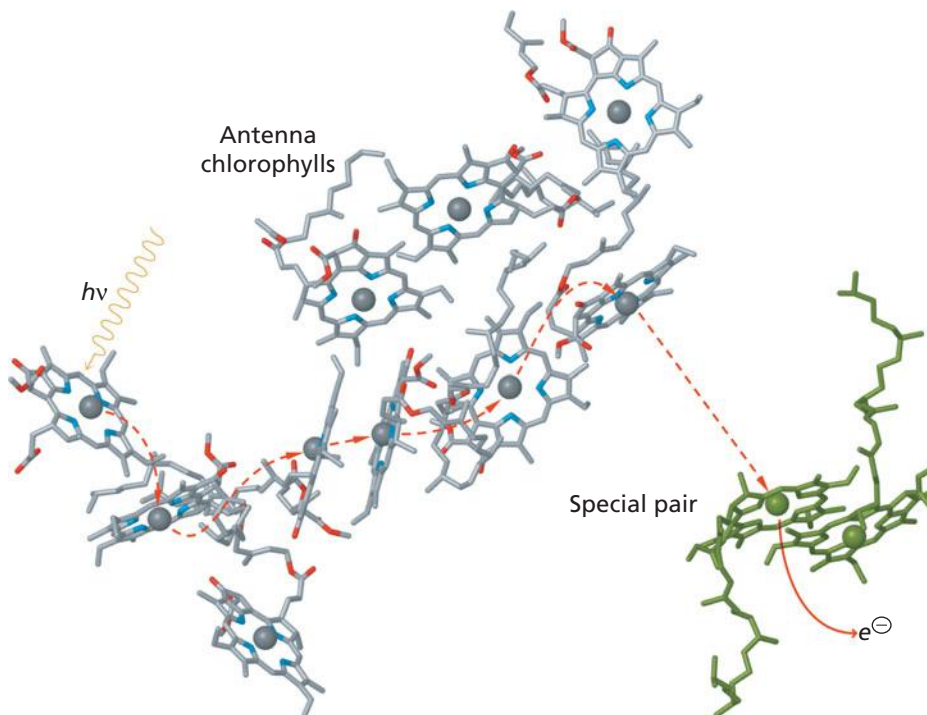
BOX 15.1 MENDEL'S SEED COLOR MUTANT

One of Gregor Mendel's original mutants affected the color of the peas in a pod. The normal color of mature seeds is yellow (I) and the recessive mutant confers a green color to the seeds (i). The mutation affects the "stay-green" (*sgr*) gene that encodes a chloroplast protein responsible for the degradation of chlorophyll as the seeds mature. When the protein is defective, chlorophyll is not broken down in the chloroplasts and the seeds stay green.

In normal wild-type plants (II) the seed are yellow and in the heterozygotes (Ii) the deficiency in the amount of chlorophyll degradation protein is not sufficient to affect chlorophyll breakdown. The seeds of the heterozygotes are also yellow. In homozygous mutant plants (ii) chlorophyll is not degraded and the seeds are green. Mendel determined that the wild-type trait (I) was dominant and the mutant trait (i) was recessive. Crosses between heterozygotes (Ii x Ii) gave the famous 3:1 ratio of yellow seeds to green seeds.

Some strains of food plants are homozygous for mutations in the genes that break down chlorophyll. These "cosmetic stay-greens," such as the one used by Mendel, produce seeds and fruit that are more attractive to consumers.

All the peas that we buy in supermarkets and farmer's markets have been genetically modified (by breeding) to be homozygous for the deficient *sgr* allele. That's why we never see the "normal" yellow peas.

▶ Normal mature peas turn yellow in color as they mature (bottom) but a mutation causes the seeds to retain their green color (top). The seed coat has been removed from the lower pair of each group in order to make the color difference more obvious.

fixed positions through interactions with the side chains of amino acids in the polypeptides of the photosystem. Excitation energy is efficiently transferred from any molecule that absorbs a photon because these molecules are so close to each other.

D. Accessory Pigments

Photosynthetic membranes contain several **accessory pigments** in addition to chlorophyll. The carotenoids include β-carotene (Figure 15.4) and related pigments such as xanthophylls. Xanthophylls have extra hydroxyl groups on the two rings. Note that the carotenoids, like chlorophyll, contain a series of conjugated double bonds allowing them to absorb light. Their absorption maxima lie in the blue region of the spectrum, which is why carotenoids appear red, yellow, or brown (Figure 15.2). The autumn colors of deciduous trees are due, in part, to carotenoids, as is the brown color of sea kelp (brown algae).

β-Carotene

Phycoerythrin

Ethyl group in phycocyanin

Unsaturated in phycocyanin
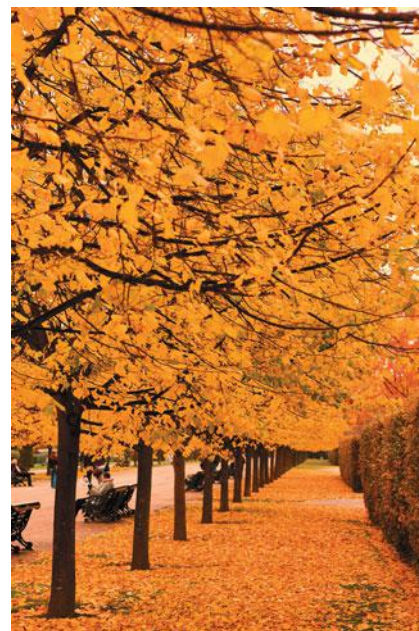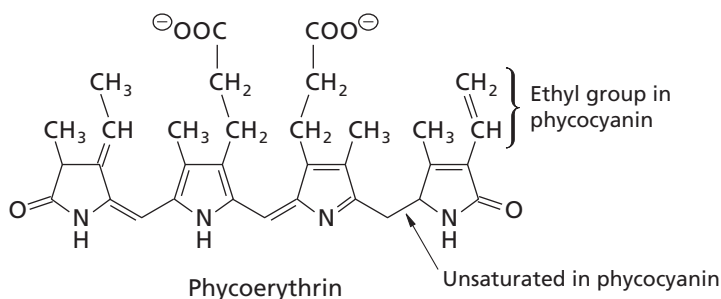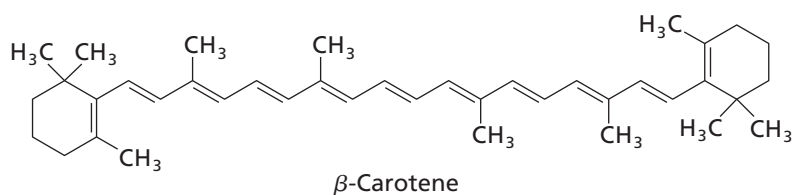
▲ The autumn colors of the leaves are due, in part, to the presence of accessory carotenoid pigments that become visible when chlorophyll molecules are degraded as the leaves die.

◀ Figure 15.4
Structures of some accessory pigments.
β-Carotene is a carotenoid, and phycoerythrin and phycocyanin are phycobilins. Phycobilins are covalently attached to proteins whereas carotenoids are bound noncovalently.

▲ **Red tide.** This red tide off the coast of Fujian, China, is due to the presence of red algae.
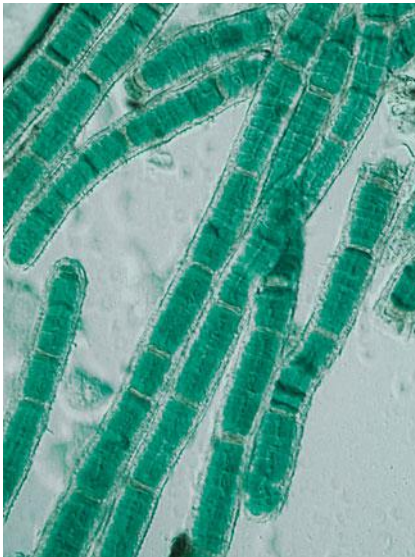
▲ **Scytonema—a blue-green cyanobacterium.**

The structure of the photosystem of the purple bacterium, *Rhodopseudomas viridis*, is shown in Figure 4.25f.

Carotenoids are closely associated with chlorophyll molecules in antenna complexes. They absorb light and transfer excitation energy to adjacent chlorophylls. In addition to serving as light-gathering pigments carotenoids also play a protective role in photosynthesis. They take up any electrons that are accidently released from antenna chlorophylls and return them to the oxidized chlorophyll molecule. This quenching process prevents the formation of reactive oxygen species such as the superoxide radical ($\cdot O_2^{\ominus}$). If allowed to form, these reactive oxygen species can be highly toxic to cells as described in Section 14.14.

Phycobilins, such as red phycoerythrin and blue phycocyanin (Figure 15.4), are found in some algae and cyanobacteria. They resemble a linear version of chlorophyll without the central magnesium ion. Like chlorophylls and carotenoids, these molecules contain a series of conjugated double bonds that allow them to absorb light. Like carotenoids, the absorption maxima of phycobilins complement those of chlorophylls and thus broaden the range of light energy that can be absorbed. In most cases, the phycobilins are found in special antenna complexes called phycobilisomes. Unlike other pigment molecules, the phycobilins are covalently attached to their supporting polypeptides. The bluish color of blue-green cyanobacteria and the red color of red algae are due to the presence of numerous phycobilisomes associated with their photosystems.

15.2 Bacterial Photosystems

We begin our discussion by describing simple bacterial systems. These simple systems evolved into more complicated structures in the cyanobacteria. The cyanobacterial version of photosynthesis was then adopted by algae and plants when a primitive cyanobacterium gave rise to chloroplasts.

Photosynthetic bacteria contain typical light-gathering photosystems. There are two basic types of photosystems that appear to have diverged from a common ancestor more than two billion years ago. Both types of photosystem contain a large number of antenna pigments surrounding a small reaction center located in the middle of the structure. The reaction center consists of a few chlorophyll molecules that include the special pair and others forming a short electron transfer chain.

Photosystem I (PSI) contains a **type I reaction center**. **Photosystem II (PSII)** contains a **type II reaction center**. Heliobacteria and green sulfur bacteria rely on photosystems with a type I reaction center whereas purple bacteria and green filamentous bacteria use photosystems with a type II reaction center. Cyanobacteria, the most abundant class of photosynthetic bacteria, utilize both photosystem I and photosystem II coupled in series. This coupled system resembles the one found in algae and plants.

A. Photosystem II

We begin by describing photosynthesis in purple bacteria and green filamentous bacteria. Most of these species of bacteria are strict anaerobes—they cannot survive in the presence of oxygen. Thus, they do not produce oxygen as a by-product of photosynthesis or consume it in respiratory electron transport. Purple bacteria and green filamentous bacteria have photosystems with a type II reaction center. These membrane complexes are often referred to as the bacterial reaction center (BRC) but this is misleading since bacteria also contain the other type of reaction center. We will refer to it here as photosystem II since it is evolutionarily related to photosystem II in cyanobacteria and eukaryotes.

The structure of the purple bacteria photosystem is shown in Figure 15.5. The pigment molecules of the internal type II reaction center form an electron transfer chain with two branches. The special pair of bacteriochlorophylls (P870) are positioned near the periplasmic (outside) surface of the membrane. Each branch contains a molecule of bacteriochlorophyll *a* and a bacteriopheophytin molecule (Figure 15.6). The right-hand branch terminates in a tightly bound quinone molecule while the equivalent position in the left-hand branch is occupied by a loosely bound quinone that can dissociate and diffuse within the lipid bilayer. Note in Figure 15.5 that the bound quinone is buried within the α helix barrel spanning the membrane while the equivalent site on the other side of the complex is open to the lipid bilayer.
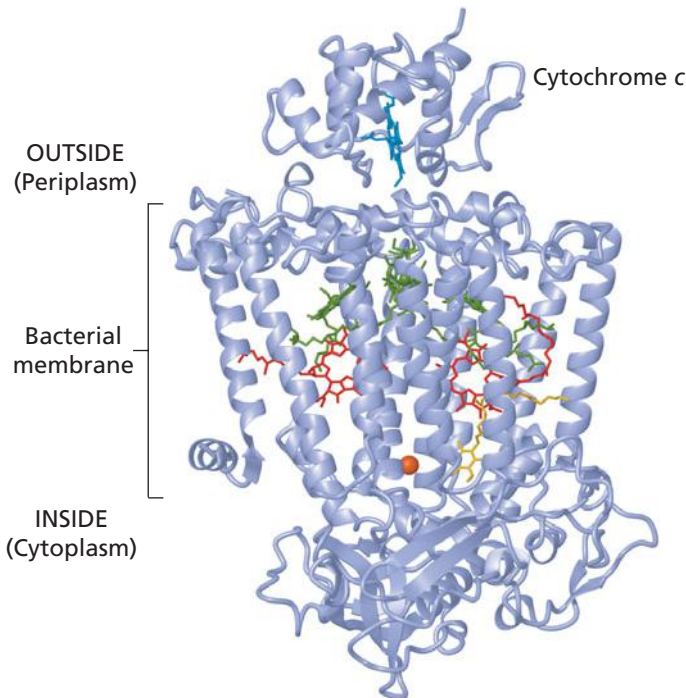
Photosystem II in the purple bacterium
Rhodobacter spaeroides. The core of the
structure consists of two homologous
membrane-spanning polypeptide subunits
(L and M). Each subunit has five transmem-
brane α helices. The electron transfer mole-
cules of the reaction center are sandwiched
between the core polypeptides. Cytochrome
c binds to PSII on the periplasmic side of
the membrane (top). An additional subunit
covers the core subunits on the cytoplasmic
surface (bottom). [PDB 1L9B]

Electron transfer begins with the release of an excited electron from P870 following
absorption of a photon of light or the transfer of excitation energy from antenna pig-
ments. (Antenna pigment molecules are not shown in Figure 15.6.) Electrons are then
transferred exclusively down the right-hand branch of the reaction center complex
resulting in the reduction of the bound quinone molecule. From there, electrons are
passed to the mobile quinone on the opposite side of the complex. This transfer is
mediated by a single bound iron atom on the central axis near the cytoplasmic side of
the membrane. The mobile quinone (Q) is reduced to QH_2 in a two-step process via the
sequential transfer of two electrons and the uptake of two $H^{\oplus}$ from the cytoplasm. Two
photons of light are absorbed for each molecule of QH_2 produced. Modern type II reac-
tion centers probably evolved from a more primitive system in which electrons were
transferred down both branches to produce QH_2 at both of the Q sites.

QH$_2$ diffuses within the lipid bilayer to the cytochrome bc_1 complex (complex III)
of the bacterial respiratory electron transport system. This is the same complex that we
described in the previous chapter (Section 14.7). The cytochrome bc_1 complex catalyzes
the oxidation of QH_2 and the reduction of cytochrome *c*—the enzyme is ubiquinol:
cytochrome *c* oxidoreductase. This reaction is coupled to the transfer of $H^{\oplus}$ from the
cytoplasm to the periplasmic space via the Q cycle. The resulting proton gradient drives
the synthesis of ATP by ATP synthase (Figure 15.7).

The P870$^{\oplus}$ special pair of chlorophyll molecules is reduced by the cytochrome *c*
(Fe$^{2\oplus}$) molecules produced by the cytochrome bc_1 complex. Cytochrome *c* diffuses
within the periplasmic space enclosed by the two membranes surrounding the bacterial
cell. The net effect is that electrons are shuffled from PSII to the cytochrome bc_1 com-
plex and back again. Note that the structure shown in Figure 15.5 includes a bound cy-
tochrome *c* molecule with its heme group positioned near the P870 special pair in order
to facilitate electron transfer.

The movement of electrons between complexes is mediated by the mobile cofactors
QH$_2$ and cytochrome *c* just as we saw in respiratory electron transport. The main differ-
ence between photosynthesis in purple bacteria and respiratory electron transport is
that photosynthesis is a cyclic process. There is no net gain or loss of electrons to other
reactions and consequently no outside source of electrons is needed. Cyclic electron
flow is a characteristic of many, but not all, photosynthesis reactions. The result of cou-
pling PSII and the cytochrome bc_1 complex is that absorption of light creates a proton
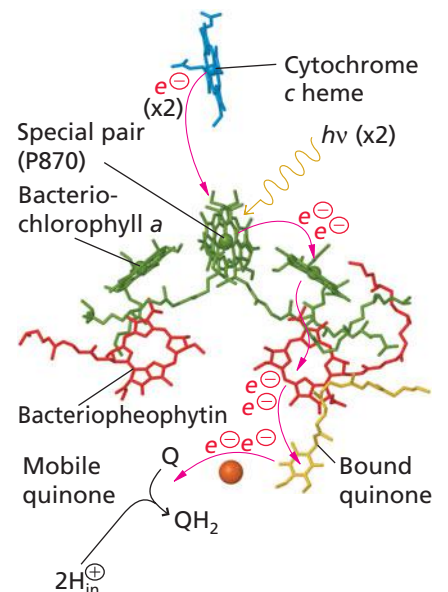
▲ **Figure 15.6**
**The type II reaction center contains the elec-
tron transfer chain.** The special pair (P870)
is located near the periplasmic surface close
to the heme group of cytochrome *c*. When
light is absorbed, electrons are transferred
one at a time from P870 to BChl *a* to BPh
to a bound quinone and from there to a
quinone located at a loosely bound site next
to a central iron atom (orange). Electrons
are restored to P870 from cytochrome *c*.

Figure 15.7 ▶
Photosynthesis in purple bacteria. Light is absorbed by the pigments of the PSII complex resulting in the transfer of electrons from P870 to QH_2 via the reaction center electron transfer chain. QH_2 diffuses to the cytochrome bc_1 complex where the electrons are transferred to cytochrome c. This reaction is coupled to the transfer of protons across the membrane. The proton gradient drives the synthesis of ATP. Reduced cytochrome c diffuses within the periplasmic space to PSII where it reduces $P870^+$. The Q-cycle reactions are shown in more detail in Figure 14.11.
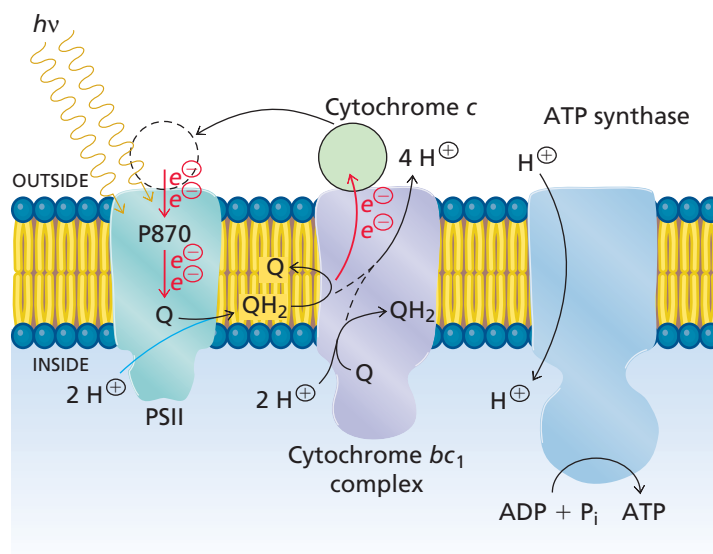
KEY CONCEPT

Bacteria with photosystem II use sunlight to produce a proton gradient that drives ATP synthesis.

KEY CONCEPT

Photosynthesis in purple bacteria is a cyclic process. It does not require an external source of electrons such as H_2O or H_eS.

gradient for ATP synthesis. The reactions are listed in Table 15.1. (The cytochrome bc_1 reactions are the same ones shown in Table 14.3.) Four protons are transferred across the membrane for every two photons of light that are absorbed. The ATP molecules produced as a result of this cycle are used by bacteria to synthesize proteins, nucleic acids, carbohydrates, and lipids. Thus, captured light energy is ultimately used in biosynthesis reactions.

We can calculate the energy of two "moles" of light at 870 nm using Equation 15.1. It works out to 274 kJ mol^{-1}. This light energy is used to pump four protons across the membrane. Pumping requires approximately 4×19.4 kJ $mol^{-1} = 77.6$ kJ mol^{-1} using our estimate from the previous chapter (Section 14.3). The result suggests that the production of chemical energy from light energy is not very efficient in purple bacteria (77.6/274 = 28%).

The basic principle of photosynthesis is the conversion of light energy (photons) to chemical energy (e.g. ATP). The pathway clearly evolved, in part, from the electron transport system we described in the previous chapter. Photosynthesis evolved several hundred million years after the main energy-producing pathway that uses complex III and ATP synthase. It's important to note that the ATP produced in bacterial photosynthesis is not restricted to the synthesis of carbohydrate and oxygen is not produced as part of the process.

Table 15.1 Photosystem II reactions

PSII:	2 P870 + 2 photons $\longrightarrow$ 2 $P870^{\oplus}$ + 2 $e^{\ominus}$
	Q + 2 $e^{\ominus}$ + 2 $H^{\oplus}_{in}$ $\longrightarrow$ QH_2
Cyt bc_1:	2 QH_2 + 2 cyt c (Fe^{3+}) $\longrightarrow$ 2 Q + 2 cyt c (Fe^{2+}) + 4 $H^{\oplus}_{out}$ + 2 $e^{\ominus}$
	Q + 2 $e^{\ominus}$ + 2 $H^{\oplus}_{in}$ $\longrightarrow$ QH_2
PSII:	2 cyt c (Fe^{2+}) + 2 $P870^{\oplus}$ $\longrightarrow$ 2 cyt c (Fe^{3+}) + 2 P870
Sum:	2 photons + 4 $H^{\oplus}_{in}$ $\longrightarrow$ 4 $H^{\oplus}_{out}$

B. Photosystem I

The structure of a typical photosystem I (PSI) complex is shown in Figure 15.8. The central part of the complex is formed by two homologous polypeptides with multiple membrane-spanning α helices. Each subunit of this dimer has two domains—an interior domain that binds the electron transfer chain pigments of the type I reaction center and a peripheral domain that binds antenna pigments. The reaction center protein domains in PSI subunits are related by structure and amino acid sequence to the core polypeptides in PSII. This is strong evidence for a common ancestor of type I and type II reaction centers.
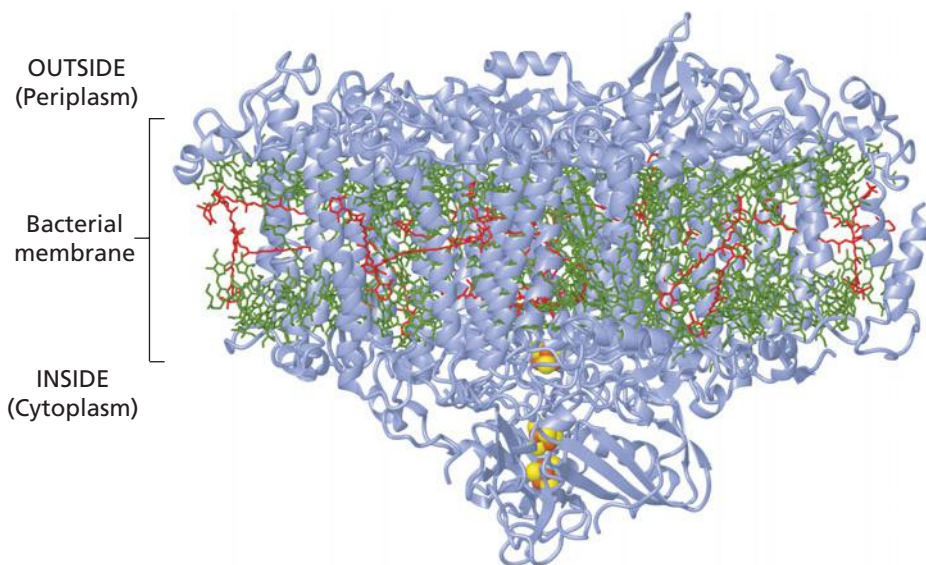
Structure of photosystem I (PSI). This version of PSI is from the cyanobacterium *Thermosynechococcus elongatus* (*Synechococcus elongatus*). The complex contains 96 chlorophylls (green), 22 carotenoids (red), and three iron–sulfur clusters (orange). There are 14 polypeptide subunits, most of which have membrane-spanning α helices. [PBD 1JBO]

The most obvious difference between PSI and PSII is the presence of a more complex antenna structure in PSI than in PSII. The PSI antenna complex is packed with chlorophyll and carotenoid pigment molecules. The example shown in Figure 15.8 is from cyanobacteria whose PSI complexes contain 96 chlorophylls and 22 carotenoids. Many of the light-gathering pigment molecules are tightly bound to additional membrane-spanning polypeptide subunits that surround the core subunits. The contrast between the structures shown in Figure 15.5 and Figure 15.8 is a bit misleading since there are simpler forms of PSI in some bacteria and more complex versions of PSII in other species (see below). Nevertheless, as a general rule, PSI is larger and more complicated than PSII.

The organization of the electron transfer chain molecules in PSI reveals striking parallels to that of PSII (Figure 15.9). In both cases, the reaction center contains two short branches of pigment molecules that terminate at bound quinones. The PSI pigment molecules are both chlorophylls and not one chlorophyll and one pheophytin as in PSII. The bound quinones in PSI are usually phylloquinones whereas in PSII they are related to ubiquinone (or menaquinone in bacteria). The phylloquinones in type I reaction centers are tightly bound to the complex and form part of the electron transfer chain. (Recall that one of the quinones in type II reaction centers is a mobile terminal electron acceptor.)

Electron transfer begins with a special pair of chlorophyll molecules located near the periplasmic surface of the membrane. This special pair is known as P700 since it absorbs light at a wavelength of 700 nm. The two chlorophyll molecules are not identical—the molecule closest to the A-branch is an epimer of chlorophyll *a* (bacteriochlorophyll *a* in bacteria). P700 is excited by absorbing a photon of light or by excitation energy transfer from antenna molecules. The excited electron is then transferred down one of the branches of the electron transfer chain to one of the bound phylloquinones. Electron transfer from P700 to phylloquinone takes about 20 picoseconds (10^{-12} s). This is extremely rapid compared to other electron transfer systems. In type II reaction centers, for example, the transfer from P680 to the bound quinone takes two or three times longer.

Electrons are subsequently transferred from bound phylloquinone to the three Fe–S clusters, F_X, F_A, and F_B. The terminal electron acceptor in PSI is ferredoxin (or flavodoxin) (Figure 7.36). Ferredoxin contains two [4Fe-4S] iron–sulfur clusters and reduction involves a $Fe^{3+} \rightarrow Fe^{2+}$ reduction with a standard reduction potential of −0.43 V (Table 10.5).

Reduced ferredoxin (Fd_{red}) becomes the substrate for an oxidation–reduction reaction catalyzed by an enzyme called ferredoxin:NADP$^{\oplus}$ oxidoreductase, more commonly known as ferredoxin:NADP$^{\oplus}$ reductase or FNR. The enzyme is a flavoprotein (containing FAD) and the reaction proceeds in three steps involving a typical semiquinone intermediate (Section 7.5). The product of the reaction is reducing equivalents in the form of NADPH. The coupled reactions involving PSI are shown in Table 15.2.

Note that the standard reduction potential of ferredoxin is considerably lower than that of NADP$^{\oplus}$, allowing for transfer of electrons from ferredoxin to NDAP$^{\oplus}$. The terminal electron acceptor is Q in photosystem II and its standard reduction potential is

Phylloquinone is also known as vitamin K (Section 7.14D, Figure 7.29).
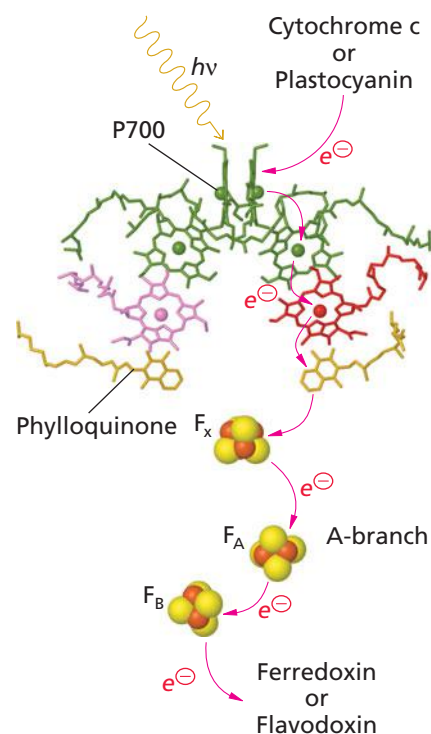
PSI electron transfer chain (type I reaction center). Electron transfer begins with the special pair of chlorophyll molecules (P700) and proceeds down one of the branches to phylloquinone. From there, electrons are transferred to the Fe–S clusters and eventually to ferredoxin. P700$^{\oplus}$ is reduced by cytochrome *c* or plastocyanin.
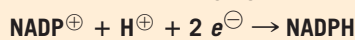
Table 15.2 The photosystem I reactions

PSI:	$2\ P700 + 2\ photons \longrightarrow 2\ P700^{\oplus} + 2\ e^{\ominus}$
	$2\ Fd_{ox} + 2\ e^{\ominus} + \longrightarrow 2\ Fd_{red}$
FNR:	$Fd_{red} + H^{\oplus} + FAD \rightleftharpoons Fd_{ox} + FADH{\cdot}$
	$Fd_{red} + H^{\oplus} + FADH{\cdot} \rightleftharpoons Fd_{ox} + FADH_2$
	$FADH_2 + NADP^{\oplus} \rightleftharpoons FAD + NADPH + H^{\oplus}$
Sum:	$2\ P700 + 2\ photons + NADP^{\oplus} + H^{\oplus} \longrightarrow 2\ P700^{\oplus} + NADPH$

KEY CONCEPT

Bacteria with photosystem I use sunlight to produce NADPH.
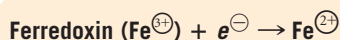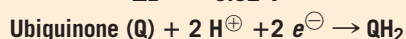
$$Ferredoxin\ (Fe^{\oplus}) + e^{\ominus} \rightarrow Fe^{2\oplus}$$
$$\Delta E = -0.43\ V$$
$$NADP^{\oplus} + H^{\oplus} + 2\ e^{\ominus} \rightarrow NADPH$$
$$\Delta E = -0.32\ V$$
$$Ubiquinone\ (Q) + 2\ H^{\oplus} + 2\ e^{\ominus} \rightarrow QH_2$$
$$\Delta E = +0.04\ V$$

too high to allow transfer of electrons to $NADP^{\oplus}$. This means that energy capture from sunlight is more efficient in PSII than in PSI.

The reactions in PSI do not create a cyclic pathway. The oxidized special pair in type I reaction centers ($P700^{\oplus}$) must be reduced by electrons from an outside source since the excited chlorophyll electrons were eventually transferred to NADPH. Some bacteria contain versions of PSI that bind cytochrome c on the outside surface of the membrane next to the special pair. In these bacteria $P700^{\oplus}$ is reduced by reduced cytochrome c in a manner similar to the reduction of the special pair in PSII. The source of electrons for reduced cytochrome c depends on the species. In green sulfur bacteria it is various reduced sulfur compounds such as H_2S and $S_2O_3^{2\ominus}$. The oxidation of these sulfur compounds is coupled to the transfer of electrons to cytochrome c by special enzymes that are found in these species (Figure 15.10). Green sulfur bacteria are photoautotrophs (Section 10.3) that grow in the absence of oxygen.

Noncyclic electron transfer is a characteristic feature of PSI but there can also be a cyclic process of electron transfer. Some electrons from PSI are occasionally passed from ferredoxin to a quinone—probably by ferredoxin:quinone oxidoreductase (ferredoxin: quinone reductase, FQR). Quinol (QH_2) interacts with the cytochrome bc_1 complex

▲ **Green sulfur bacteria.** Agar plate with streaks of *Chlorobium tepidum*.
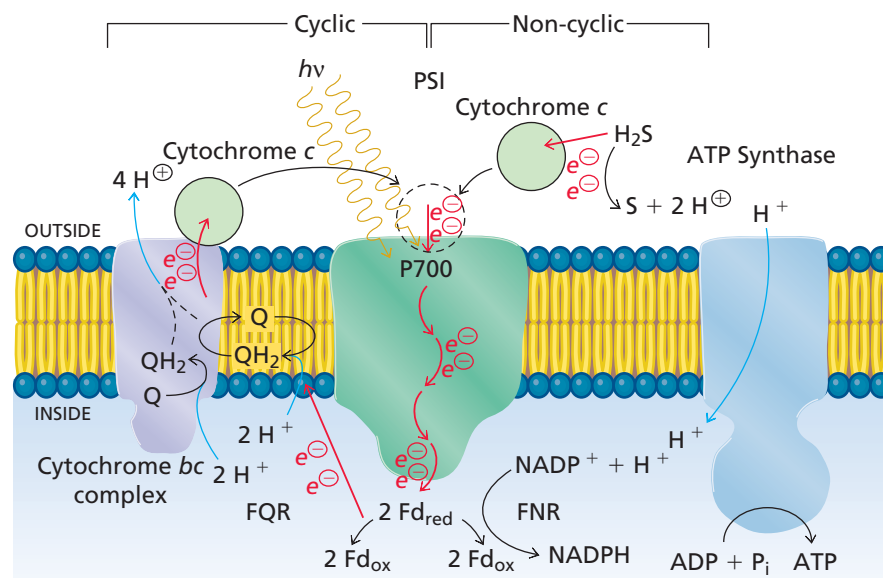
▲ **Figure 15.10**

Photosynthesis in green sulfur bacteria. Photoactivation of P700 leads to production of reduced ferredoxin on the cytoplasmic side of the membrane. Ferredoxin becomes the electron donor in a reaction catalyzed by ferredoxin:NADP$^{\oplus}$ reductase (FNR) resulting in production of NADPH in the cytoplasm. Ferredoxin can also reduce Q to QH_2 in a reaction catalyzed by ferredoxin:quinone reductase (FQR). QH_2 is oxidized by the cytochrome bc_1 complex, resulting in the transfer of electrons to reduced cytochrome c and the transfer of protons across the membrane. $P700^{\oplus}$ is normally reduced by cytochrome c on the periplasmic side of the membrane. In the noncyclic process, reduced cytochrome c is made in reactions that are coupled to the oxidation of sulfur compounds such as H_2S. The transfer of electrons is shown by red arrows.

transferring electrons via cytochrome bc_1 to cytochrome c and cytochrome c reduces $P700^{\oplus}$ (Figure 15.10). This cyclic process is very similar to the coupled reactions involving PSII. It allows for light-mediated synthesis of ATP because the passage of electrons through cytochrome bc_1 is associated with the translocation of protons across the membrane via the Q cycle. In most cases, the noncyclic process predominates and NADPH is produced; however, if NADPH cannot be efficiently used in biosynthesis reactions, electrons will be transferred through cytochrome bc_1 to produce ATP.

C. Coupled Photosystems and Cytochrome *bf*

Cyanobacterial membranes contain both PSI and PSII. The two photosystems are coupled in series to produce both NADPH and ATP in response to light. The photosynthetic reactions in cyanobacteria are illustrated in Figure 15.11. Light is absorbed by PSII leading to excitation of P680 and transfer of an electron to a mobile quinone called plastoquinone (PQ, Figure 7.33). Electrons are then transferred to a cytochrome *bf* complex similar to the cytochrome bc_1 complex in respiratory electron transport. Electron transport within the cytochrome *bf* complex is coupled to the movement of $H^{\oplus}$ across the membrane by a photosynthetic Q cycle. The coupling of PSII and a cytochrome *bf* complex is similar in principle to photosynthesis reactions in purple bacteria with one major difference—in purple bacteria electrons are returned to PSII by the terminal electron acceptor of the cytochrome bc_1 complex (cytochrome c) whereas in cyanobacteria electrons are passed on to PSI. The terminal electron acceptor of the unique cytochrome *bf* complex is either cytochrome c or a blue copper-containing protein called plastocyanin (PC). Reduced cytochrome c and reduced plastocyanin are mobile carriers that bind to the outside (periplasmic) surface of PSI and reduce $P700^{\oplus}$. (Most cyanobacteria and algae use cytochrome c while some cyanobacteria and all plants use plastocyanin, or a different cytochrome called cytochrome c_6, as the terminal electron acceptor of the cytochrome *bf* complex.)

The structure of the photosynthetic cytochrome *bf* complex has been solved by X-ray crystallography (Figure 15.12). It contains a cytochrome b with two cytochrome reaction centers whose role in the Q cycle is similar to that of cytochrome b in the cytochrome bc_1 complex (complex III) of respiratory electron transport. A Rieske iron–sulfur protein (ISP) transports electrons from one of the cytochrome b sites to cytochrome f and reduced cytochrome f passes electrons to plastocyanin. Cytochrome f (*f* stands for *feuille*, the French word for leaf) is a distinct protein unrelated to cytochrome c_1 of the respiratory cytochrome bc_1 complex but cytochrome b and ISP are homologues of the proteins found in complex III.

The cytochrome *bf* complex evolved from the original cytochrome bc_1 complex that was present in ancient cyanobacteria. The most important adaptation was the replacement of cytochrome c_1 of the bacterial *bc* complex with cytochrome f in the cyanobacterial complex. This change allowed for the transfer of electrons to the copper-containing plastocyanin via cytochrome f. (Recall that mobile cytochrome c, not plastocyanin, is the normal electron acceptor of the cytochrome bc_1 complex.)

> Reduced ferredoxin can be used directly in other pathways, notably in nitrogen fixation (Section 17.1)

KEY CONCEPT

Organisms with coupled photosystem I and photosystem II use sunlight to produce both NADPH *and* a proton gradient that drives ATP synthesis.

▼ **Figure 15.11**
Photosynthesis in cyanobacteria. Light (wavy arrows) is captured and used to drive the transport of electrons (obtained from water) from PSII through the cytochrome *bf* complex to PSI and ferredoxin. This process can generate NADPH and a proton concentration gradient that is used to drive phosphorylation of ADP. For each water molecule oxidized to 1/2 O_2 by the oxygen evolving complex (OEC), one molecule of $NADP^{\oplus}$ is reduced to NADPH. For simplicity, PSI, PSII, and cytochrome *bf* are shown close together in the plasma membrane but in most species they are located within internal membrane structures. Plastoquinone (PQ) is the mobile carrier between PSII and the cytochrome *bf* complex. In this example, plastocyanin (PC) is the mobile carrier between the cytochrome *bf* complex and PSI.
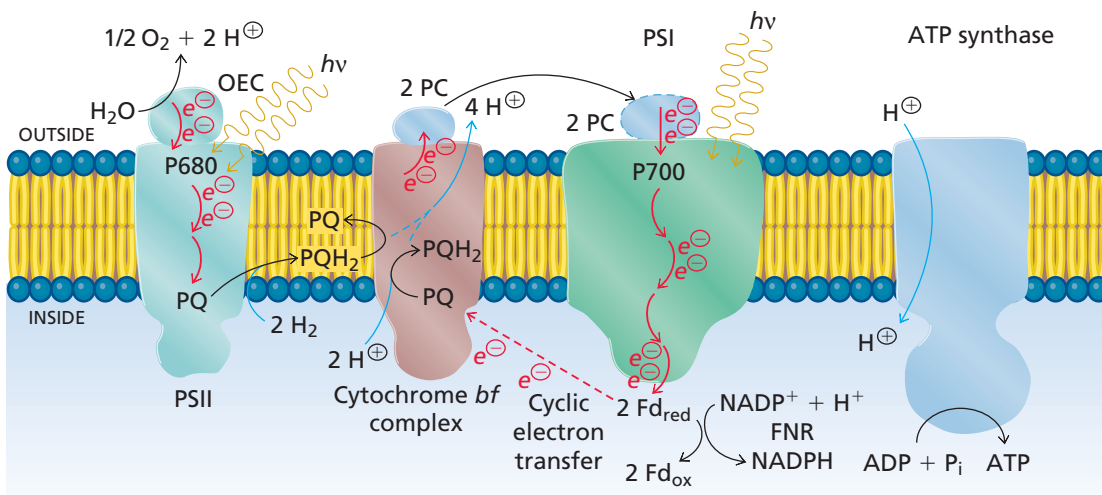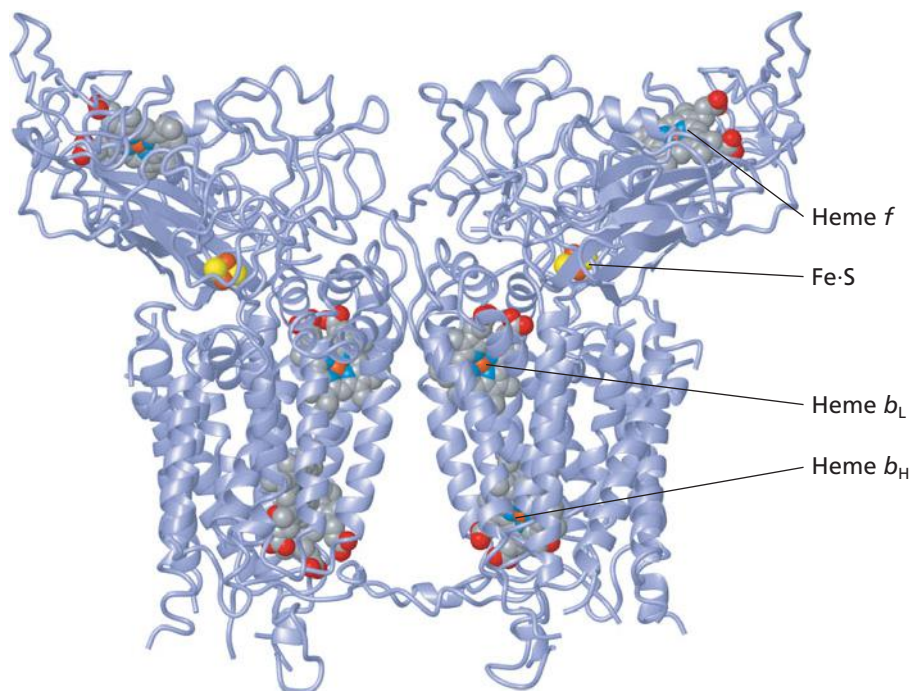
Heme *f*

Fe·S

Heme b_L

Heme b_H

KEY CONCEPT

The splitting of water to form molecular oxygen arose in order to supply electrons to photosystem II.

Plastocyanin binds specifically to PSI in cyanobacteria and transfers electrons to $P700^{\oplus}$. This allows for a unidirectional flow of electrons from PSII $\rightarrow PQH_2 \rightarrow$ cytochrome $bf \rightarrow PC \rightarrow PSI \rightarrow NADPH$.

Cyanobacteria do not contain cytochrome bc_1. Thus, cytochrome *bf* also plays a role in respiratory electron transport because it replaces the normal complex III. Reduced plastocyanin is the electron donor to the terminal oxidase (complex IV) possibly via an intermediate cytochrome *c* - like carrier. Plastoquinone is the mobile quinone electron carrier in both photosynthesis and respiratory electron transport.

Photoactivation of PSI results in synthesis of NADPH in a manner similar to that in green sulfur bacteria. As in green sulfur bacteria, some electrons are recycled but in this case it is through the cytochrome *bf* complex. Note that PSII, cytochrome *bf*, and PSI are coupled in series and the transfer of electrons to NADPH results in a deficiency of electrons at $P680^{\oplus}$ in PSII. The reduction of $P680^{\oplus}$ in cyanobacteria is accomplished by extracting electrons from water with the production of oxygen as a byproduct. The enzyme that splits water is called the oxygen evolving complex (OEC) and it is tightly bound to PSII on the outer surface of the membrane. The evolution of an oxygen evolving complex in primitive cyanobacteria was one of the most important biochemical events in the history of life.

The oxygen evolving complex (OEC) contains a cluster of $Mn^{2\oplus}$ ions, a $Ca^{2\oplus}$ ion, and a $Cl^{\ominus}$ ion. It catalyzes a complex reaction in which four electrons are extracted, one at a time, from two molecules of water. The reaction takes place on the outside of the PSII complex near the special pair of chlorophyll molecules (P680). The electrons from the splitting of water are transferred to $P680^{\oplus}$ (Figure 15.13). The exact mechanism of the water splitting reaction is being investigated in a number of laboratories. It is similar, in principle, to the reverse reaction catalyzed by complex IV of the respiratory electron transport chain (Section 14.8). Note that the oxygen evolving complex is located on the exterior surface of the membrane and the release of protons from water contributes to the formation of the proton gradient across the membrane.

As mentioned earlier, the similarities between PSI and PSII indicate that they evolved from a common ancestor. Over time, these two photosystems diverged in those species of photosynthetic bacteria that contain only one of the two types (e.g., purple bacteria, green sulfur bacteria). At some point, about 2.5 billion years ago, a primitive ancestor of cyanobacteria acquired both types of photosystem—probably by taking up a large part of the genome from an unrelated bacterial species. At first the two types of

Oxygen Evolving
Complex

photosystem must have worked in parallel but they began to function in series with the evolution of a photosynthetic cytochrome *bf* complex (from cytochrome bc_1) and an oxygen evolving complex. Later on, a species of cyanobacteria entered into a symbiotic relationship with a primitive eukaryotic cell and this led to the modern chloroplasts found in algae and plants.

The coupled photosystems are able to capture light energy and use it to produce both ATP (from the proton gradient) and reducing equivalents in the form of NADPH. Neither photosystem by itself can accomplish these two goals with the same efficiency.

The net result of this simplified linear pathway is the production of one molecule of NADPH and the transfer of four protons across the membrane for each *pair* of electrons excited by the absorption of light energy in each photosystem. The two separate excitation steps in PSI and PSII require a total of four photons of light energy. The splitting of water by the OEC contributes to the proton gradient and produces molecular oxygen. The individual reactions are summarized in Table 15.3.

D. Reduction Potentials and Gibbs Free Energy in Photosynthesis

The path of electron flow during photosynthesis can be depicted in a zigzag figure called the **Z-scheme** (Figure 15.14). The Z-scheme plots the reduction potentials of the photosynthetic electron transfer components in PSI, PSII, and cytochrome *bf*. It shows that the absorption of light energy converts P680 and P700—pigment molecules that are poor reducing agents—to excited molecules (P680* and P700*) that are good

Table 15.3 The photosynthesis reactions in species with both photosystems

PSII:	$2 \text{ P680} + 2 \text{ photons} \longrightarrow 2 \text{ P680}^{\oplus} + 2 \, e^{\ominus}$
	$\text{PQ} + 2 \, e^{\ominus} + 2 \text{ H}^{\oplus}_{in} \longrightarrow \text{PQH}_2$
OEC:	$\text{H}_2\text{O} \longrightarrow \frac{1}{2}\text{O}_2 + 2 \text{ H}^{\oplus}_{out} + 2 \, e^{\ominus}$
	$2 \text{ P680}^{\oplus} + 2 \, e^{\ominus} \longrightarrow 2 \text{ P680}$
Cyt *bf*:	$2 \text{ PQH}_2 + 2 \text{ plastocyanin (Cu}^{\oplus 2}) \longrightarrow 2 \text{ PQ} + 2 \text{ plastocyanin (Cu}^{\oplus}) + 4 \text{ H}^{\oplus}_{out} + 2 \, e^{\ominus}$
	$\text{PQ} + 2 \text{ H}^{\oplus}_{in} + 2 \, e^{\ominus} \longrightarrow \text{PQH}_2$
PSI:	$2 \text{ P700} + 2 \text{ photons} \longrightarrow 2 \text{ P700}^{\oplus} + 2 \, e^{\ominus}$
	$2 \text{ Fd}_{ox} + 2 \, e^{\ominus} \longrightarrow 2 \text{ Fd}_{red}$
	$2 \text{ plastocyanin (Cu}^{\oplus}) + 2 \text{ P700}^{\oplus} \longrightarrow 2 \text{ plastocyanin (Cu}^{2+}) + 2 \text{ P700}$
FNR:	$2 \text{ Fd}_{red} + \text{H}^{\oplus} + \text{NADP}^{\oplus} \rightleftharpoons 2 \text{ Fd}_{ox} + \text{NADPH}$
Sum:	$\text{H}_2\text{O} + 4 \text{ photons} + 4 \text{ H}^{\oplus}_{in} + \text{NADP}^{\oplus} + \text{H}^{\oplus} \longrightarrow \frac{1}{2}\text{O}_2 + 6 \text{ H}^{\oplus}_{out} + \text{NADPH}$

reducing agents. (Recall that a reducing agent is one that gives up electrons to reduce another molecule. The reducing agent is oxidized in such reactions.) The oxidized forms of the pigment molecules are P680$^\oplus$ and P700$^\oplus$. Energy is recovered when P680* and P700* are oxidized and electrons are passed to cytochrome *bf* and NADPH.

The standard reduction potentials of many of these components are listed in Table 10.5. The difference between any two reduction potentials can be converted to a standard Gibbs free energy change as we saw in Chapter 10. Looking at Figure 15.14 we can see that the absorption of a photon by either P680 or P700 lowers the standard reduction potential by about 1.85 V. In these examples, a difference of 1.85 V corresponds to a standard Gibbs free energy change of about 180 kJ mol^{-1} ($\Delta G^{\circ\prime} = 180$ kJ mol^{-1}). This value is almost identical to the calculated energy of a "mole" of photons at a wavelength of 680 nm (176 kJ mol^{-1}, Section 15.1). What this means is that the energy of sunlight is very efficiently converted to a change in reduction potential.

There are many similarities between electron transfer in photosynthesis and the membrane-associated electron transport chain that we saw in the last chapter. In both cases electrons pass through a cytochrome complex that transports H$^\oplus$ across a membrane. The resulting proton gradient is expended when ATP is synthesized by ATP synthase.

The structure and orientation of cytochrome bc_1 (complex III) and cytochrome *bf* are similar. Both complexes release protons into the space between the inner and outer membranes. The orientation of ATP synthase is also identical—the "head" of the structure is located in the cytoplasm of bacterial cells or the inside compartment of mitochondria. In the next section we'll see that the orientation of ATP synthase in chloroplasts is topologically similar.

KEY CONCEPT

The energy from a photon of light is used to excite an electron in the special pair of chlorophyll molecules. The excited state has a much lower reduction potential making it easy to give up an electron to an oxidation reaction.
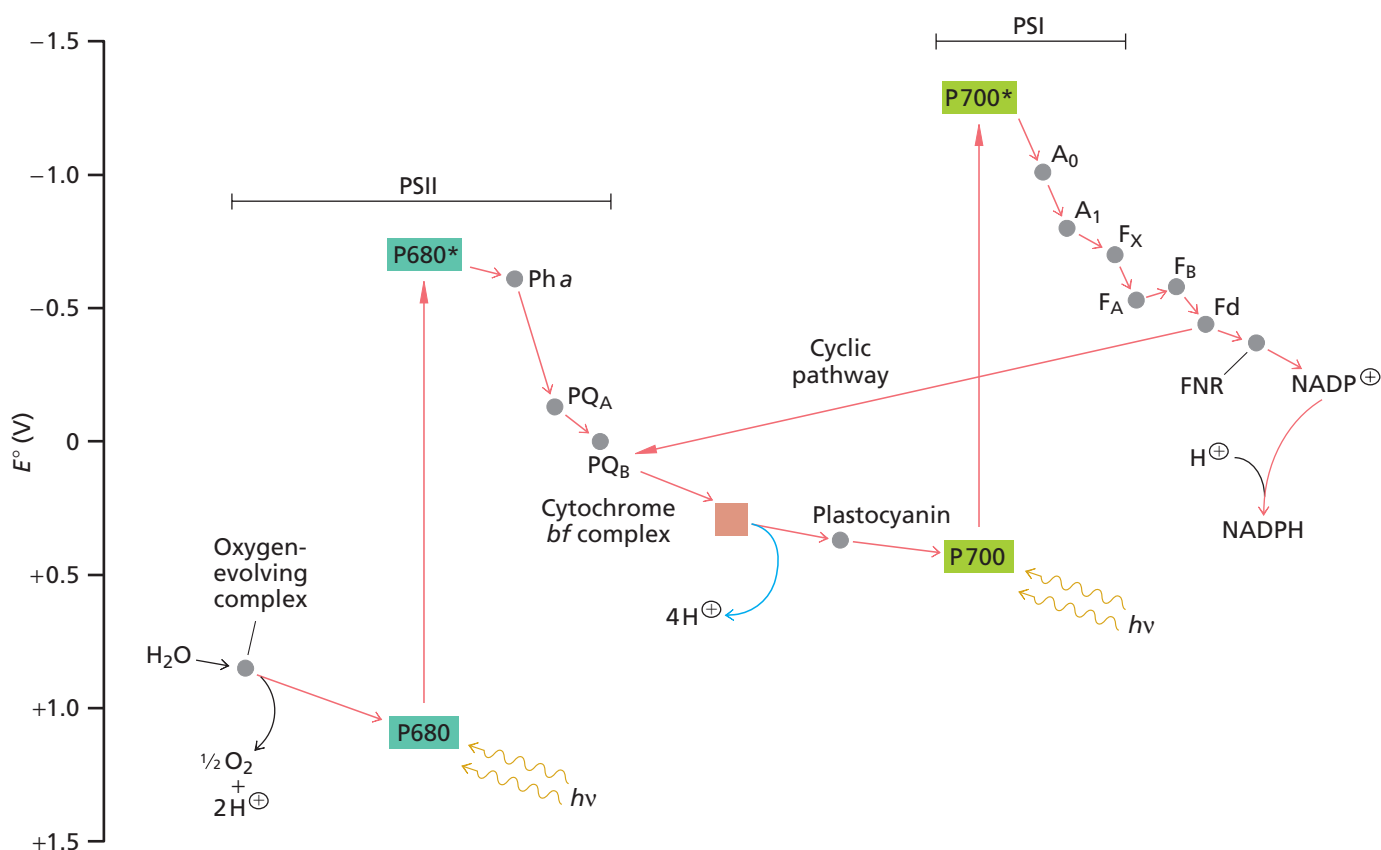
▲ Figure 15.14

Z-scheme, showing reduction potentials and electron flow during photosynthesis in cyanobacteria. Light energy is absorbed by the special pair pigments, P680 and P700. This converts these molecules into strong reducing agents as shown by the huge drop in standard reduction potential. The values shown are approximate because the reduction potentials of the carriers vary with experimental conditions. The pathway shows the stoichiometry when a *pair* of electrons is transferred from H$_2$O to NADPH. Abbreviations: Ph *a*, pheophytin *a*, electron acceptor of P680; PQ$_A$, bound plastoquinone; PQ$_B$, mobile plastoquinone; A$_0$, chlorophyll *a*, the primary electron acceptor of P700; A$_1$, phylloquinone; F$_x$, F$_B$, and F$_A$, iron–sulfur clusters; Fd, ferredoxin; FNR, ferredoxin:NADP$^+$ reductase.

The main difference between photosynthesis and respiratory electron transport is the source of electrons and the terminal electron acceptors. In mitochondria, for example, "high energy" electrons are supplied by reducing equivalents such as NADH ($E°' = -0.32$ V) and accepted by O_2 ($E°' = +0.82$ V) to produce water. In the coupled photosynthesis pathway the flow of electrons is reversed—water ($E°' = +0.82$ V) is the electron donor and $NADP^{\oplus}$ ($E°' = -0.32$ V) is the electron acceptor. This "reversal" of electron flow is thermodynamically unfavorable unless it is coupled to other reactions with a larger Gibbs free energy change. Those other reactions are, of course, the excitation of PSI and PSII by sunlight.

In order to extract electrons from water the cell needs to generate a powerful oxidizing agent with a reduction potential greater than that of the $H_2O \rightarrow 1/2\ O_2 + 2\ H^{\oplus} + 2\ e^{\ominus}$ reaction. This strong oxidizing agent is the P680 special pair after it has given up an electron. The half reaction is $P680^{\oplus} + e^{\ominus} \rightarrow P680°$ ($E°' = +1.1$ V). Note that this standard reduction potential is higher than that of water so that electrons can flow "down" from water to $P680^{\oplus}$ as shown in Figure 15.14. $P680^{\oplus}$ is the most powerful oxidizing agent in biochemical reactions. It is much more potent than $P870^{\oplus}$ in purple bacteria even though purple bacteria have a similar type II reaction center.

Similarly, P700* is a strong reducing agent with a lower reduction potential than $NADP^{\oplus}$. In this case, the absorption of a photon of light by PSI creates an energetic electron that can be passed "down" to $NADP^{\oplus}$ to create reducing equivalents in the form of NADPH. Thus, the "reversal" of electron flow in photosynthesis, compared to respiratory electron transport, is achieved by the special light-absorbing properties of chlorophyll molecules in the two photosystems.

E. Photosynthesis Takes Place Within Internal Membranes

All four of the photosynthesis complexes (PSI, PSII, cytochrome *bf,* and ATP synthase) are embedded in membranes. Most cyanobacteria contain a complex internal network of membranes where these complexes are concentrated (Figure 15.15). The internal membranes are called **thylakoid membranes**. They form by invagination of the inner plasma membrane creating structures that are similar to the mitochondrial cristae. As the membrane folds inward it encloses a space called the **lumen** where protons accumulate during photosynthesis. The thylakoid lumen may remain connected to the periplasmic space or it may form an internal compartment if a membrane loop (or bubble) pinches off from the plasma membrane.
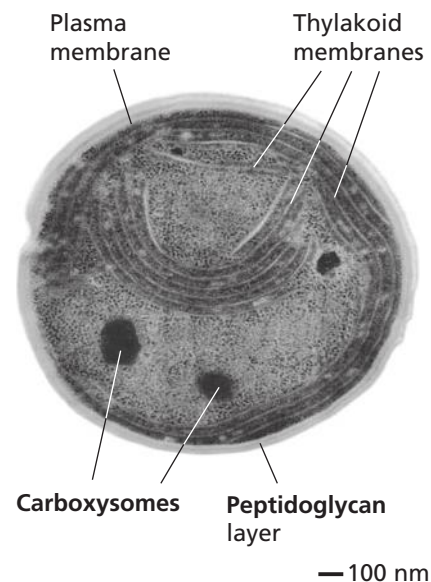
▲ **Figure 15.15**
Internal structure of the cyanobacterium *Synechocystis* **PCC 6803.** (Carboxysomes are described in Section 15.6A.)

## BOX 15.2 OXYGEN "POLLUTION" OF EARTH'S ATMOSPHERE

Photosynthetic bacteria probably evolved three billion years ago but the earliest fossil evidence of oxygen producing cyanobacteria dates only from 2.1 billion years ago—claims of much earlier fossils have recently been discredited. The geological record strongly indicates that bacteria began "polluting" the atmosphere with oxygen about 2.4–2.7 billion years ago. This likely corresponds to the evolution of the oxygen evolving complex in PSII and it predates the earliest cyanobacteria fossils.

At that time, oxygen levels rose to about 25% of the present level and they remained at that level for more than a billion years except for a brief drop around 1.9 billion years ago. The cause of this decline isn't known. Primitive plants—probably lichens and mosses—invaded land about 700 million years ago and this led to a steep rise in oxygen levels that eventually reached the present-day concentration of 21%.

Oxygen was highly toxic to most of the species that were around 2 billion years ago but gradually new species arose that could not only tolerate the "pollutant" but used it in respiratory electron transport.
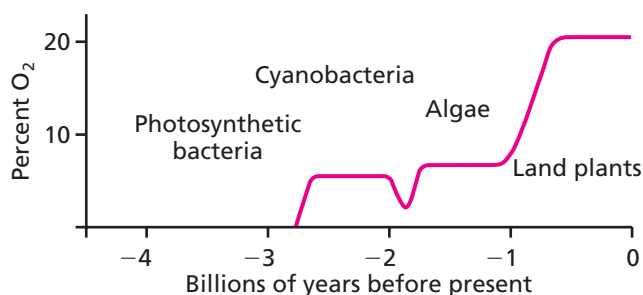
▲ **Oxygen levels in Earth's atmosphere.**
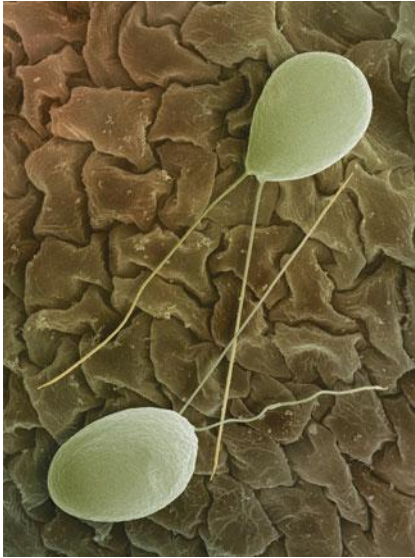
▲ ***Chlamydomonas sp.*** Chlamydomonas species are green algae that are closely related to plants. They contain a single large chloroplast. "Chlamy" is a model organism that is easily grown in the laboratory.
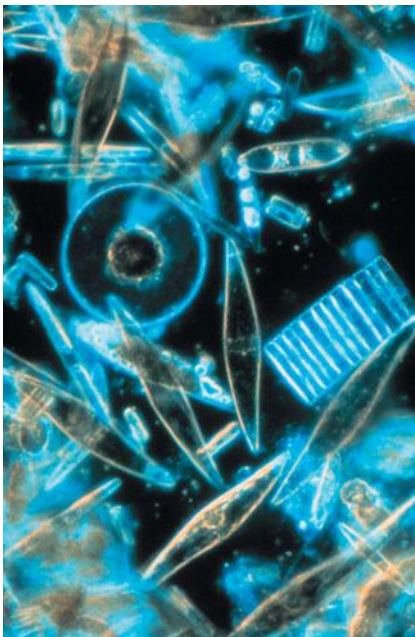
▲ **Diatoms.** About 30% of the oxygen in our atmosphere comes from marine photosynthetic organisms.

The internal membrane network presents a much greater surface area for membrane proteins. As a result, cyanobacteria contain a much higher concentration of photosynthesis complexes compared to other species of photosynthetic bacteria. This means that cyanobacteria are very efficient at capturing light energy and converting it to chemical energy. This, in turn, has led to their evolutionary success and the formation of an oxygen enriched atmosphere.

15.3 Plant Photosynthesis

Up to this point we have been describing bacterial photosynthesis but many eukaryotic species are capable of photosynthesis. The photosynthesizing eukaryotes we are most familiar with are flowering plants and other terrestrial species such as mosses and ferns. In addition to these obvious examples, there are many simpler species such as algae and diatoms.

In all photosynthesizing eukaryotes the light-gathering photosystems are localized to a specific cellular organelle called the chloroplast. Thus, unlike bacterial metabolism, photosynthesis and respiratory electron transport are not integrated since they take place in different compartments (chloroplasts and mitochondria). Chloroplasts evolved from a species of cyanobacteria that entered into a symbiotic relationship with a primitive eukaryotic cell over 1 billion years ago. Modern chloroplasts still retain a reduced form of the original bacterial genome. This DNA contains many of the genes for the proteins of the photosystems and genes for some of the enzymes involved in CO_2 fixation. The transcription of these genes and the translation of their mRNAs resemble the prokaryotic mechanisms described in Chapters 21 and 22. This prokaryotic flavor of gene expression reflects the evolutionary origin of chloroplasts.

In the modern world, a large percentage (~70%) of total atmospheric oxygen is produced by photosynthesis in land plants, especially in tropical rain forests. The remaining oxygen is produced by small marine organisms, mostly bacteria, diatoms, and algae. Almost all of the food for animals comes directly or indirectly from plants and the synthesis of these food molecules relies on the energy of sunlight.

A. Chloroplasts

The chloroplast is enclosed by a double membrane (Figure 15.16). As in mitochondria, the outer membrane is exposed to the cytoplasm and the inner membrane forms highly folded internal structures. During photosynthesis protons are translocated from the interior of the chloroplast, called the **stroma**, to the compartments between the membranes.

The interior membrane is called the thylakoid membrane. Recall that cyanobacteria possess a similar thylakoid membrane (Figure 15.15). In the chloroplast this membrane forms an extensive network of sheets within the organelle. As the chloroplast develops, projections grow out from these sheets to form flattened disk-like structures. These disk-like structures stack on top of one another like a pile of coins to form **grana** (singular, granum). A typical chloroplast contains dozens of grana, or stacked disks of thylakoid membranes. The grana in mature chloroplasts are connected to each other by thin sheets of thylakoid membrane called stroma thylakoids. These stroma thylakoid membranes are exposed to the stroma on both surfaces whereas grana thylakoid membranes within a stack are in close contact with the membranes immediately above and below them.

The three-dimensional organization of the thylakoid membrane is shown in Figure 15.17. Each disk in the stack is connected to the stroma thylakoids by short bridges. The interior of each disk is called the lumen and it is the same compartment as the region between the two membranes of the stroma thylakoid. All thylakoid membranes are likely derived from the inner chloroplast membrane. This means that the lumen is topologically equivalent to the space between the inner and outer membranes of the chloroplast although in some cases the direct connection may be lost. The thylakoid membranes contain PSI, PSII, cytochrome *bf*, and ATP synthase complexes as in cyanobacteria. In mitochondria, protons accumulate in the compartment between the inner and outer membranes (Section 14.3); similarly, in chloroplasts, protons are translocated into the thylakoid lumen and the space between the two membranes of

(a)

(b)
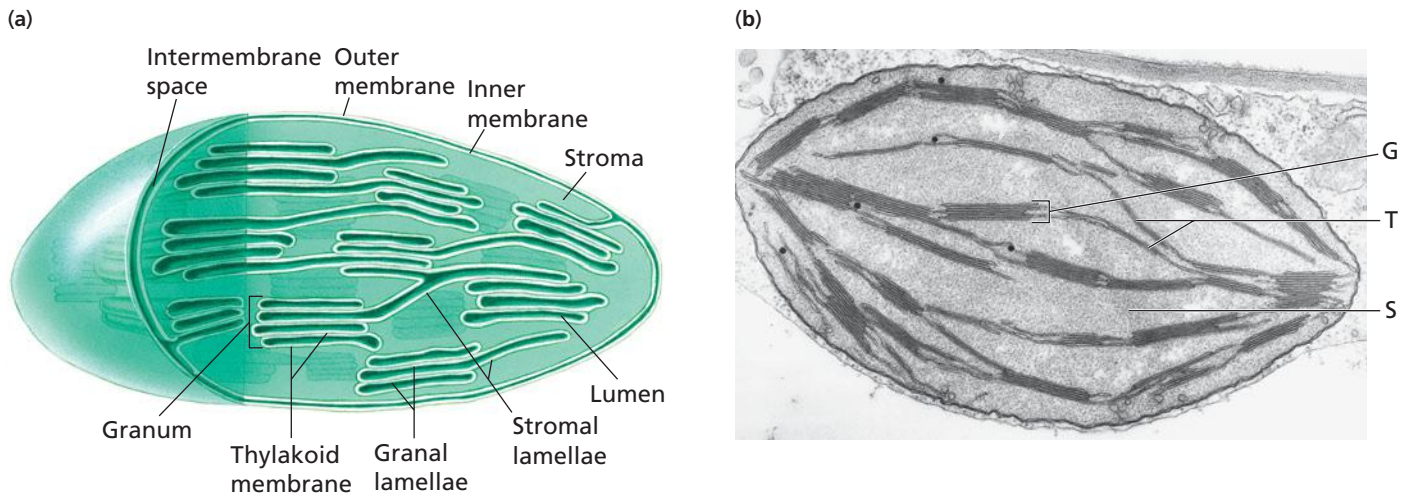
▲ **Figure 15.16**
Structure of the chloroplast. (a) Illustration. **(b)** Electron micrograph: cross-section of a chloroplast from a spinach leaf. Shown are grana (G), the thylakoid membrane (T), and the stroma (S).

the stroma thylakoids. It's important to keep in mind that the chloroplast stroma is equivalent to the cytoplasm in bacteria and the matrix in mitochondria.

B. Plant Photosystems

The photosynthesis complexes in eukaryotic chloroplasts evolved from the complexes present in primitive cyanobacteria. Chloroplast PSI is structurally and functionally similar to its bacterial ancestor—the only significant structural difference is that eukaryotic PSI contains chlorophyll molecules instead of bacteriochlorophyll in the electron transfer chain of the reaction center. The eukaryotic version oxidizes plastocyanin (or cytochrome *c*) and reduces ferredoxin (or flavodoxin). Eukaryotic PSI associates with a light-harvesting complex called LHCI that resembles the complex found in some bacteria.

Chloroplast PSII is also similar to the one in cyanobacteria. Plant chloroplasts contain a light-harvesting complex called LHCII that associates with PSII in the chloroplast membrane. LHCII is a large structure containing 140 chlorophylls and 40 carotenoids and it completely surrounds PSII. As a result, photon capture in plants is more efficient than in bacteria. Cyanobacteria and chloroplasts contain similar cytochrome *bf* complexes.

The ATP synthase in chloroplasts is related to the cyanobacterial ATP synthase, as expected. The protein components differ from the mitochondrial version described in the previous chapter. This is not surprising since the mitochondrial ATP synthase evolved from the proteobacterial ancestor of bacteria and proteobacteria are distantly related to cyanobacteria. Species such as algae, diatoms, and plants that contain both mitochondria and chloroplasts have distinctive versions of ATP synthase in each organelle.

The chloroplast ATP synthase is a CF_0F_1 ATPase where the "C" stands for chloroplast. The overall molecular structure is very similar to that of mitochondria even though the various subunits of the two enzymes are encoded by different genes. As in mitochondria, the membrane component of the chloroplast ATP synthase consists of a multimeric ring and a rod that projects into a hexameric head structure. The ring rotates as protons move across the membrane and ATP is synthesized from ADP + P_i by a binding change mechanism as described in Section 14.9. The "knob" projects into the chloroplast stroma (Figure 15.18).

C. Organization of Chloroplast Photosystems

Figure 15.19 illustrates the locations of the membrane-spanning photosynthetic components within the chloroplast thylakoid membrane. PSI is located predominantly in the stroma thylakoid and is therefore exposed to the chloroplast stroma. PSII is located
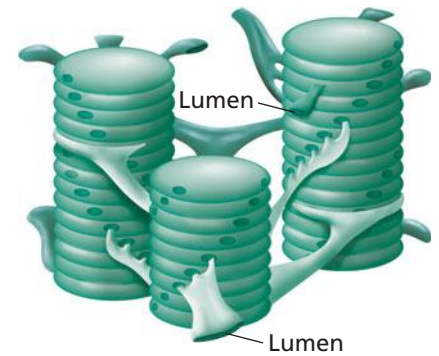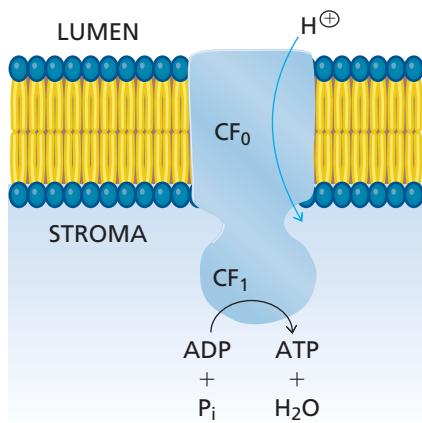

▲ **Figure 15.17**
Organization of stacked disks in a granum and their connection to the stroma thylakoids.

Adapted from Staehlin, L. A. (2003) Chloroplast structure: from chlorophyll granules to supramolecular architecture of thylakoid membranes. *Photosynthesis Research* 76:185–196.

The locations of various photosynthetic components in the stroma and grana thylakoid membranes are shown in Figure 15.19.

KEY CONCEPT

Photosynthetic bacteria and chloroplasts make use of internal thylakoid membranes to increase the number of photosystem complexes.

▲ **Figure 15.18**
Chloroplast ATP synthase.

predominantly in the grana thylakoid membrane, away from the stroma. The oxygen-evolving complex is associated with PSII on the luminal side of the thylakoid membrane. The cytochrome *bf* complex spans the thylakoid membrane and is found in both the stroma and grana thylakoid membranes. ATP synthase is found exclusively in the stroma thylakoids with the CF_1 component, the site of ATP synthesis, projecting into the stroma.

The membranes of the top and bottom surfaces of each disk in a granum are in contact with each other forming a double-membrane structure. This region is densely packed with the light-absorbing PSII complexes and their associated LHCII complexes. Light passes through the plasma membrane of the plant cell, through the cytoplasm, and across the outer membrane of the chloroplast. When light reaches the grana, the photons are efficiently absorbed by the pigment molecules in the membrane.

Excited electrons are transferred within PSII to PQ forming PQH_2. The protons for this reaction are taken up from the stroma. The PSII reaction center is replenished with electrons from the oxidation of water taking place in the lumen. PQH_2 diffuses within the membrane to the cytochrome *bf* complex where it is oxidized to PQ. The protons released in the Q cycle enter the lumen. Electrons are passed to plastocyanin that diffuses freely in the lumen to reach PSI. PSI absorbs light leading to the transfer of electrons from reduced plastocyanin to ferredoxin. Ferredoxin is formed in the stroma. It can participate in the reduction of $NADP^{\oplus}$ to NADPH in the stroma or serve as an electron donor to cytochrome *bf* complexes in the stroma thylakoid membrane (cyclic electron transport, Section 15.2B).

Note that PSII is not directly exposed to the stroma but is exposed to the thylakoid lumen. The lumen is topologically equivalent to the outside of the bacterial membrane as shown in Figure 15.11. PSI projects into the stroma compartment since it produces ferredoxin that accumulates within chloroplasts. The stroma is topologically equivalent to the bacterial cytoplasm (inside the cell). The distribution of cytochrome *bf* complexes is explained by the fact they can receive electrons from both PSII and PSI. Supercomplexes of PSII and cytochrome *bf* in the grana participate in linear electron transfer from water to plastocyanin. In the stroma thylakoids there are complexes of PSI, cytochrome *bf*, and ferredoxin:quinone oxidoreductase (FQR) that are involved in cyclic electron flow.

The proton gradient is used to generate ATP. As protons are translocated from the lumen compartment to the stroma, ATP is synthesized from ADP and P_i in the stroma. Both ATP and NADPH accumulate in the stroma where they can be used in biosynthesis reactions. In plants, but not other photosynthetic species, a high percentage of ATP and NADPH molecules are used in the fixation of CO_2 and the synthesis of carbohydrates.

Figure 15.19 ►

Distribution of membrane-spanning photosynthetic components between stroma and granal thylakoids. PSI is found predominantly in stroma thylakoids. PSII is found predominantly in grana thylakoids. The cytochrome *bf* complex in found in both stroma and grana thylakoid membranes. ATP synthase is localized exclusively to stroma thylakoids.
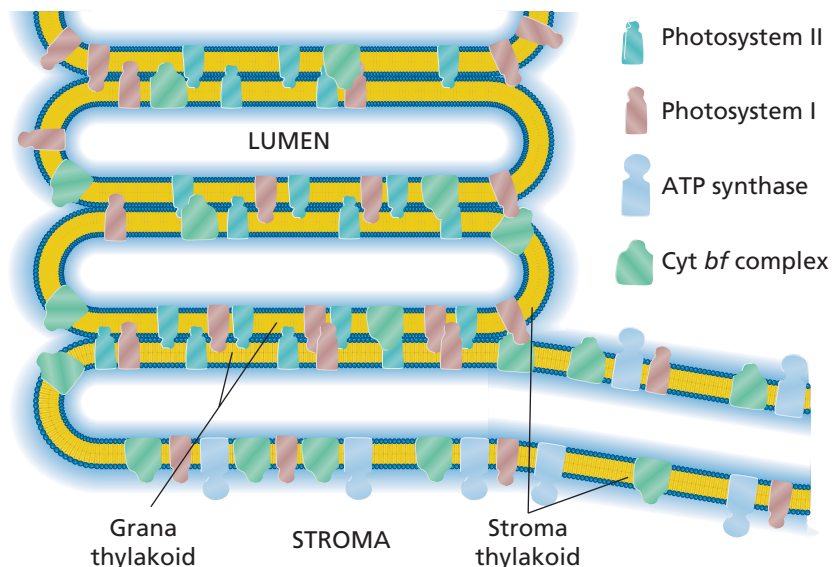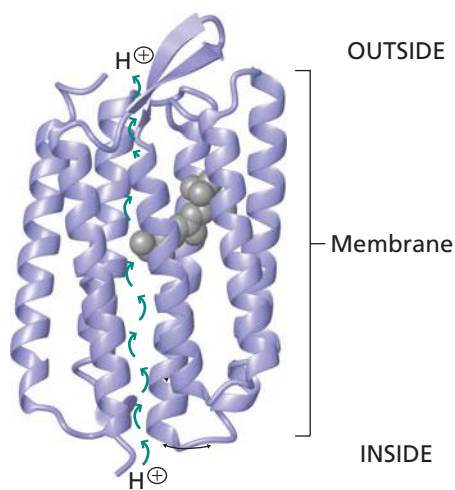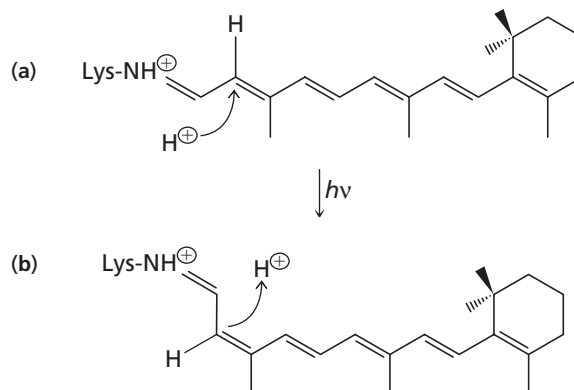
BOX 15.3 BACTERIORHODOPSIN

Bacteriorhodopsin is a membrane protein found in a few specialized species of archaebacteria such as *Halobacterium salinarium*. The protein has seven membrane-spanning α helices that form a channel in the membrane. (See ribbon structure below.) A single retinal molecule is covalently bound to a lysine side chain in the middle of the channel. The normal configuration of the retinal is all-*trans* but when it absorbs a photon of light it converts to the 13-*cis* configuration. (See structure below.) The light-induced change in configuration is coupled to deprotonation and reprotonation of the retinol molecule.

When light is absorbed, the shift in configuration to 13-*cis* retinal releases a proton that then passes up the channel to be released on the outside of the membrane. This proton is replaced by a proton that is taken up from the cytosol and the retinol configuration shifts back to the all-*trans* form. For every photon of light that is absorbed by bacteriorhodopsin a single proton is translocated across the membrane.

Bacteriorhodopsin creates a light-induced proton gradient and this proton gradient drives ATP synthesis by ATP synthase.

▲ **Two configurations of retinal-lysine in bacteriorhodopsin. (a)** All-*trans* retinal. **(b)** 13-*cis* retinal. The configuration shifts from the all-*trans* form to the 13-*cis* form when a photon of light is absorbed.

The coupling of bacteriorhodopsin and ATP synthase can be directly demonstrated by artificially synthesizing lipid vesicles containing both complexes. In the orientation shown below, the vesicles will synthesize ATP from ADP + P$_i$ when they are illuminated. This experiment, first carried out by Efraim Racker and his colleagues in 1974, was one of the first confirmations of the chemiosmotic theory (Section 14.3).
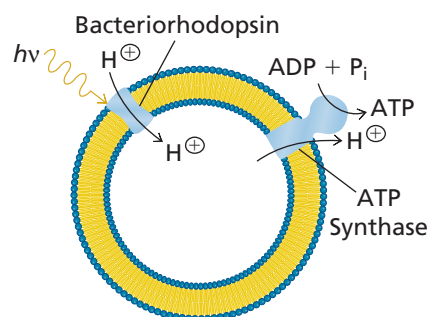
▲ **Bacteriorhodopsin creates a proton gradient that drives ATP synthesis.** Artificial lipid vesicles containing bacteriorhodopsin and ATP synthase were created with the orientation shown. When these vesicles were illuminated, bacteriorhodopsin pumped protons into the vesicle and the resulting proton gradient activated ATP synthase.

▲ **Bacteriorhodopsin.**

15.4 Fixation of CO$_2$: The Calvin Cycle

In photosynthetic species there is a special pathway for the reductive conversion of atmospheric CO$_2$ to carbohydrates. The reactions are powered by the ATP and NADPH formed during the light reactions of photosynthesis. The fixation of CO$_2$ and the synthesis of carbohydrates occurs in the cytoplasm of bacteria and in the chloroplast stroma. This biosynthesis pathway is a cycle of enzyme-catalyzed reactions with three major stages: (1) the carboxylation of a five-carbon sugar molecule, (2) the reductive synthesis of carbohydrate for use in other pathways, and (3) the regeneration of the molecule that accepts CO$_2$. This pathway of carbon assimilation has several names, such

as *the reductive pentose phosphate cycle*, *the C$_3$ pathway* (the first intermediate is a three-carbon molecule), and *the Calvin cycle*. (Workers in Melvin Calvin's laboratory discovered the carbon-fixing pathway using $^{14}CO_2$ tracer experiments in algae.) We refer to the pathway as the **Calvin cycle**.

The fixation of CO_2 and the synthesis of carbohydrates are often described as "photosynthesis." In this textbook we refer to photosynthesis and the Calvin cycle as two separate pathways.

A. The Calvin Cycle

The Calvin cycle is outlined in Figure 15.20. The first stage is the carboxylation of ribulose 1,5-*bis*phosphate, a reaction catalyzed by the enzyme ribulose 1,5-*bis*phosphate carboxylase–oxygenase, better known as Rubisco. The second stage is a reduction stage where 3-phosphoglycerate is converted to glyceraldehyde 3-phosphate. Most of the glyceraldehyde 3-phosphate is converted to ribulose 1,5-*bis*phosphate in the third (regeneration) stage. Some of the glyceraldehyde 3-phosphate produced in the Calvin cycle is used in carbohydrate synthesis pathways. Glyceraldehye 3-phosphate is the main product of the Calvin cycle.

Figure 15.21 on page 464 shows all reactions of the Calvin cycle. The pathway begins with steps for assimilating *three* molecules of carbon dioxide because the smallest carbon intermediate in the Calvin cycle is a C$_3$ molecule. Therefore, three CO_2 molecules must be fixed before one C$_3$ unit (glyceraldehyde 3-phosphate) can be removed from the cycle without diminishing the metabolic pools.

B. Rubisco: Ribulose 1,5-*bis*phosphate Carboxylase–oxygenase

Rubisco (ribulose 1,5-*bis*phosphate carboxylase–oxygenase) is the key enzyme of the Calvin cycle. It catalyzes the fixation of atmospheric CO_2 into carbon compounds. This reaction involves the carboxylation of the five-carbon sugar, ribulose 1,5-*bis*phosphate, by CO_2. This leads to the eventual release of two three-carbon molecules of 3-phosphoglycerate. The reaction mechanism of Rubisco is shown in Figure 15.22.

Rubisco makes up about 50% of the soluble protein in plant leaves, making it one of the most abundant enzymes on Earth. Interestingly, its status as an abundant enzyme is due partly to the fact that it is not very efficient—the low turnover number of ~3 s^{-1} means that large amounts of the enzyme are required to support CO_2 fixation!

The Rubisco of plants, algae, and cyanobacteria is composed of eight large (L) subunits and eight small (S) subunits (Figure 15.23). There are eight active sites located in the eight large subunits. Four additional small subunits are located at each end of the core formed by the large subunits. The Rubisco molecules in other photosynthetic bacteria have only the large subunits containing the active sites. For example, in the purple bacterium *Rhodospirillum rubrum*, Rubisco consists of a simple dimer of large subunits.
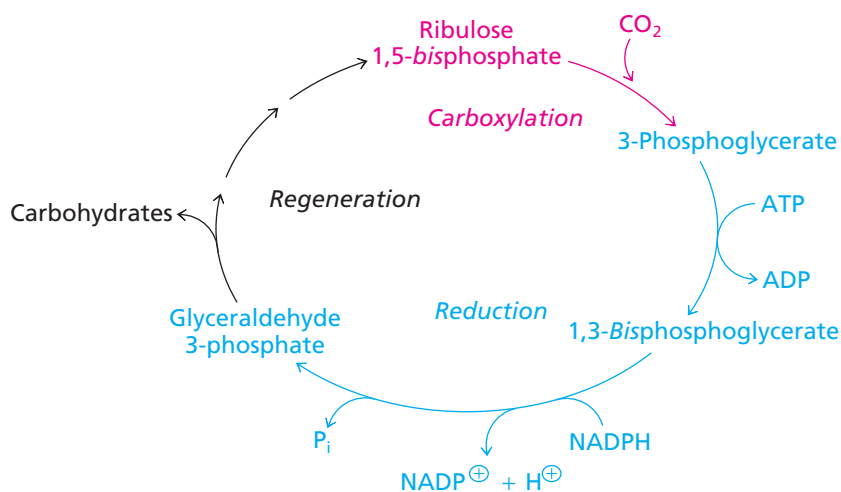

▲ **Melvin Calvin (1911–1997).** Calvin won the Nobel Prize in Chemistry in 1961 for his work on carbon dioxide assimilation in plants.

lbl.gov/Science-Articles/Research-Review/Magazine/1997/story12.html]

KEY CONCEPT

The Calvin cycle utilizes the products of photosynthesis, ATP and NADPH, to fix CO_2 into carbohydrates.
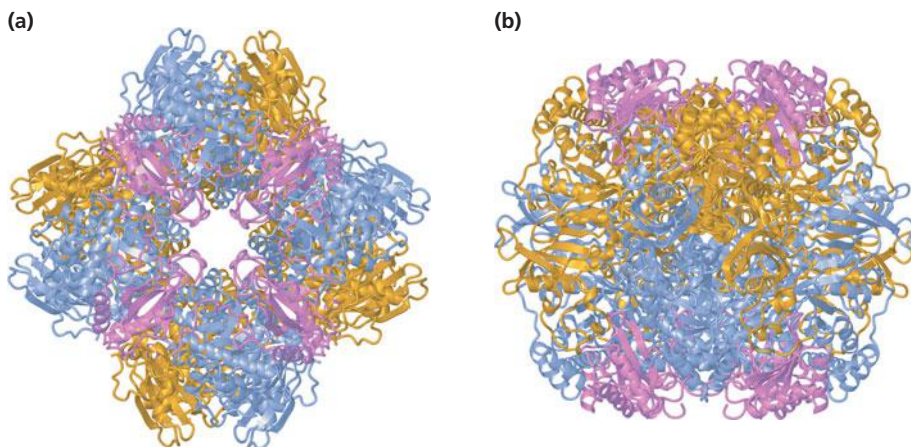

Figure 15.20 ▶
Summary of the Calvin cycle. The cycle has three stages: carboxylation of ribulose 1,5-*bis*phosphate, reduction of 3-phosphoglycerate to glyceraldehyde 3-phosphate, and regeneration of ribulose 1,5-*bis*phosphate.
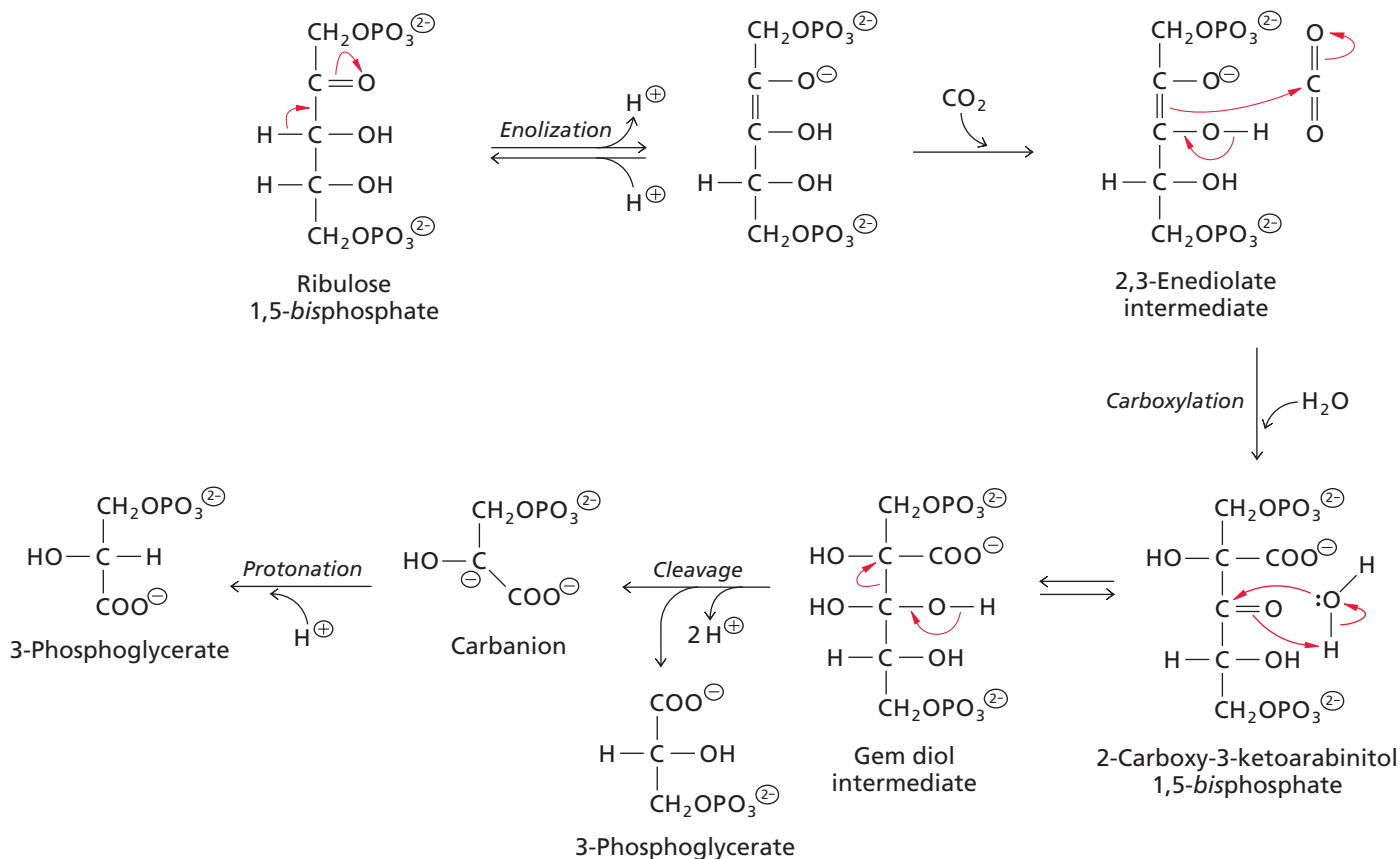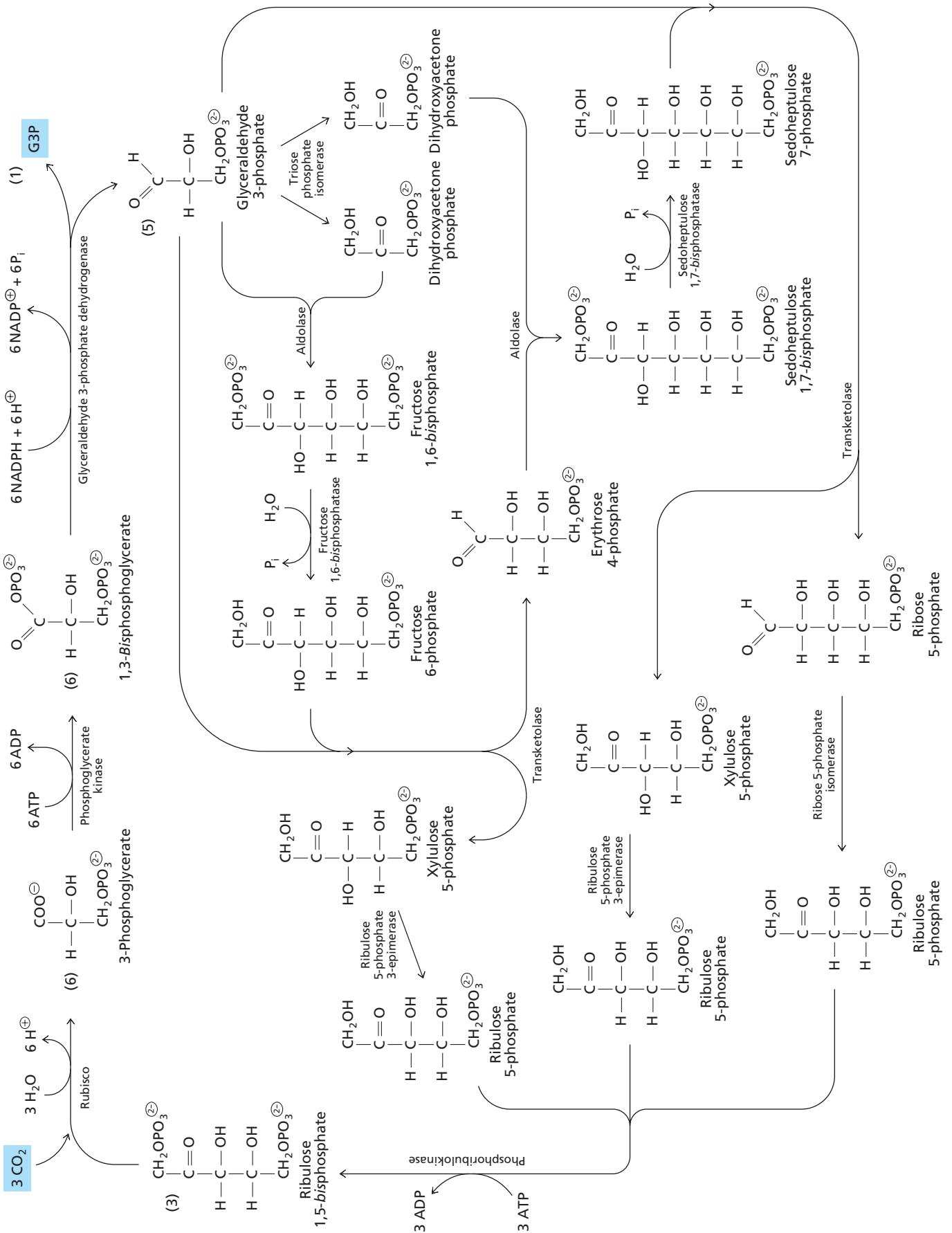
▲ **Figure 15.22**

Mechanism of Rubisco-catalyzed carboxylation of ribulose 1,5-*bis*phosphate to form two molecules of 3-phosphoglycerate. A proton is abstracted from C-3 of ribulose 1,5-*bis*phosphate to create a 2,3-enediolate intermediate. The nucleophilic enediolate attacks CO_2, producing 2-carboxy-3-ketoarabinitol 1,5-*bis*phosphate, which is hydrated to an unstable gem diol intermediate. The C-2–C-3 bond of the intermediate is immediately cleaved, generating a carbanion and one molecule of 3-phosphoglycerate. Stereospecific protonation of the carbanion yields a second molecule of 3-phosphoglycerate. This step completes the carbon fixation stage of the RPP cycle—two molecules of 3-phosphoglycerate are formed from CO_2 and the five-carbon sugar ribulose 1,5-*bis*phosphate.

The purple bacterium version of Rubisco has a much lower affinity for CO_2 than the more complex multisubunit enzymes in other species but it catalyzes the same reaction. In a spectacular demonstration of this functional similarity, tobacco plants were genetically engineered by replacing the normal plant gene with the one from the purple bacterium *Rhodospirillum rubrum*. The modified plants contained only the dimeric bacterial form of the enzyme but they grew normally and reproduced as long as they were kept in an atmosphere of high CO_2 concentration.

(a)   (b)

◄ **Figure 15.23**

The quaternary structure (L_8S_8) of ribulose 1,5-*bis*phosphate carboxylase–oxygenase (Rubisco). (a) Top and **(b)** side views of the enzyme from spinach (*Spinacia oleracea*). Large subunits are shown alternately yellow and blue; small subunits are purple. [PDB 1RCX].

▲ **Figure 15.21**
Calvin cycle. The concentrations of Calvin cycle intermediates are maintained when one molecule of glyceraldehyde 3-phosphate (G3P) exits the cycle after three molecules of CO_2 are fixed.

Rubisco cycles between an active form (in the light) and an inactive form (in the dark). It must be activated to catalyze the fixation of CO_2. In the light, Rubisco activity increases in response to the higher, more basic pH that develops in the stroma (or bacterial cytoplasm) during proton translocation. Under alkaline conditions an activating molecule of CO_2, which is not the substrate CO_2 molecule, reacts reversibly with the side chain of a lysine residue of Rubisco to form a carbamate adduct. Mg^{2+} binds to and stabilizes this CO_2–lysine adduct. The enzyme must be carbamylated in order to carry out CO_2 fixation; however, the carbamate adduct readily dissociates, making the enzyme inactive. Carbamylation is normally inhibited because Rubico is usually in an inactive conformation. During the day, a light-activated ATP-dependent enzyme called Rubisco activase binds to Rubisco and facilitates carbamylation by inducing a conformational change. Under these conditions Rubisco is active.

When the sun goes down Rubisco activase is no longer effective in activating Rubisco and CO_2 fixation stops. This regulation makes sense since photosynthesis is not active at night and ATP + NADPH are not produced in chloroplasts during the night. These cofactors are required for the Calvin cycle so the Calvin cycle is not active at night as a result of the regulation of Rubisco activity. Inhibition of Rubisco in the dark prevents the inefficient accumulation of 3-phosphoglycerate and the wasteful oxygenation reaction described in the next section.

In plants, an additional level of inhibition is mediated by 2-carboxyarabinitol 1-phosphate (Figure 15.24). This compound is an analog of the unstable gem diol intermediate of the carboxylation reaction. It is synthesized only at night and it binds to, and inhibits, any residual carbamylated Rubisco, thus ensuring that the Calvin cycle is shut down. Some plants synthesize sufficient amounts of the inhibitor to keep Rubisco completely inactive in the dark.

C. Oxygenation of Ribulose 1,5-*bis*phosphate

As its complete name indicates, ribulose 1,5-*bis*phosphate carboxylase–oxygenase catalyzes not only carboxylation but also the oxygenation of ribulose 1,5-*bis*phosphate. The two reactions are competitive since CO_2 and O_2 compete for the same active sites on Rubisco. The oxygenation reaction produces one molecule of 3-phosphoglycerate and one molecule of 2-phosphoglycolate (Figure 15.25). Oxygenation consumes significant amounts of ribulose 1,5-*bis*phosphate *in vivo*. Under normal growth conditions, the rate of carboxylation is only about three to four times the rate of oxygenation.

The 3-phosphoglycerate formed from the oxygenation of ribulose 1,5-*bis*phosphate enters the Calvin cycle. The other product of the oxygenation reaction follows a different pathway. Two molecules of 2-phosphoglycolate (C_2) are metabolized in peroxisomes and the mitochondria by an oxidative pathway (via glyoxylate and the amino acids glycine and serine) to one molecule of CO_2 and one molecule of 3-phosphoglycerate (C_3), which also enters the Calvin cycle. This oxidative pathway consumes NADH and ATP. The light-dependent uptake of O_2 catalyzed by Rubisco and followed by the release of CO_2 during the metabolism of 2-phosphoglycolate is called *photorespiration*. Like carboxylation, photorespiration is normally inhibited in darkness when Rubisco is inactive. The appreciable release of fixed CO_2 and the consumption of
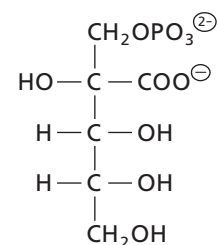
▲ **Figure 15.24**
2-Carboxyarabinitol 1-phosphate.

KEY CONCEPT

Some enzymes cannot distinguish between very similar substrates.
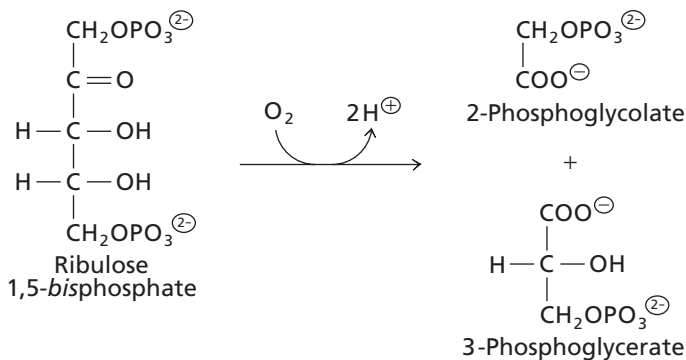
◄ **Figure 15.25**
Oxygenation of ribulose 1,5-*bis*phosphate catalyzed by Rubisco.

BOX 15.4 BUILDING A BETTER RUBISCO

Many labs are attempting to genetically modify plants in order to enhance the carboxylation reaction and suppress the oxygenation reaction. If successful, these attempts to make a better Rubisco could greatly increase food production.

The "perfect" enzyme would have very low oxygenase activity and very efficient carboxylase activity. The kinetic parameters of the oxygenase activity of Rubisco enzymes from several species are listed in the accompanying table. The low catalytic efficiency of the enzyme is indicated by the k_{cat}/K_m values.

Kinetic parameters of Rubisco carboxylase activity in various species

Species	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (M^{-1}s^{-1})
Tobacco	3.4	10.7	3.2×10^5
Red algae	2.6	9.3	2.8×10^5
Purple bacteria	7.3	89	8.2×10^4
"Perfect" enzyme	1070	10.7	10^8

Data from Andrews, J. T., and Whitney, S. M. (2003). Manipulating ribulose *bis*phosphate carboxylase/oxygenase in the chloroplasts of higher plants. *Arch. Biochem. Biophys.* 414: 159–169.

These values should be compared to those in Table 5.2. It seems likely that the carboxylase efficiency can be improved 1000-fold by modifying the amino acid side chains in the active site.

The difficult part of the genetic modification is choosing the appropriate amino acid changes. The choice is informed by a detailed knowledge of the structures of several Rubisco enzymes from different species and by examination of the contacts between amino acid side chains and substrate molecules. Models of the presumed transition states are also important. Additional key residues can be identified by comparing the conservation of amino acid sequences in enzymes from a wide variety of species
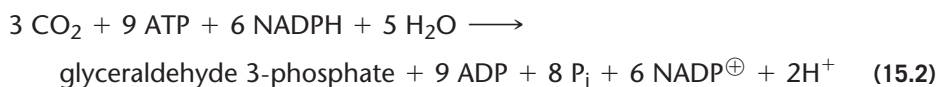
The underlying strategy assumes that evolution has not yet selected for the most well-designed enzyme. This assumption seems reasonable since there are many examples of ongoing evolution in biochemistry. However, several billion years of evolution have not resulted in a better Rubisco and neither have several decades of human effort. It may not be possible to build a better Rubisco.

▲ **Figure 15.26**
Outline of the regeneration stage of the Calvin cycle.

energy as a result of oxygenation—with no apparent benefit to the organism—arise from the lack of absolute substrate specificity of Rubisco. This is a serious problem in agriculture because photorespiration limits crop yields.
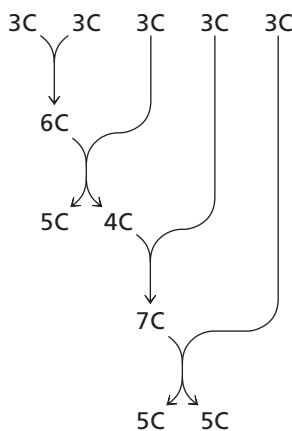
D. Calvin Cycle: Reduction and Regeneration Stages

The reduction stage of the Calvin cycle begins with the ATP-dependent conversion of 3-phosphoglycerate to 1,3-*bis*phosphoglycerate in a reaction catalyzed by phosphoglycerate kinase. Next, 1,3-*bis*phosphoglycerate is reduced by NADPH (not NADH, as in gluconeogenesis, Section 11.2#6) in a reaction catalyzed by a glyceraldehyde 3-phosphate dehydrogenase isozyme. As in gluconeogenesis, some of the glyceraldehyde 3-phosphate is rearranged to its isomer, dihydroxyacetone phosphate, by triose phosphate isomerase. For every six glyceraldehyde 3-phosphate molecules produced by this pathway, one is removed from the cycle to be used in carbohydrate synthesis and the five others are used in the regeneration stage.

In the regeneration stage, glyceraldehyde 3-phosphate is diverted into three different branches of the pathway and is interconverted between three-carbon (3C), four-carbon (4C), five-carbon (5C), six-carbon (6C), and seven-carbon (7C) phosphorylated sugars (Figure 15.21). The pathway is schematically outlined in Figure 15.26. Two of the reactions, those catalyzed by aldolase and fructose 1,6-*bis*phosphatase, are familiar because they are part of the gluconeogenesis pathway (Section 12.1). Many of the other reactions are part of the normal pentose phosphate pathway (Section 12.4) including two tranketolase reactions. The net result of the Calvin cycle reactions is

$$3 \text{ CO}_2 + 9 \text{ ATP} + 6 \text{ NADPH} + 5 \text{ H}_2\text{O} \longrightarrow$$

$$\text{glyceraldehyde 3-phosphate} + 9 \text{ ADP} + 8 \text{ P}_i + 6 \text{ NADP}^{\oplus} + 2\text{H}^+ \quad \textbf{(15.2)}$$

Both ATP and NADPH are required for CO$_2$ fixation by the Calvin cycle. These are the major products of the light reactions of photosynthesis. The fact that the requirement for ATP exceeds that of NADPH is one reason why cyclic electron flow from PSI to cytochrome *bf* is important in photosynthesis. Cyclic electron flow results in increased production of ATP relative to NADPH.
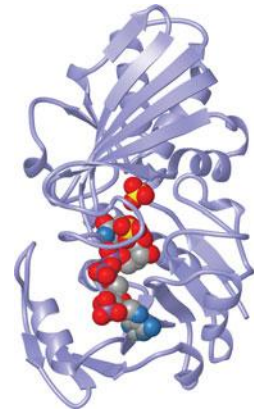
It's interesting to compare the cost of synthesizing carbohydrates from CO_2 and the energy yield from degrading it via glycolysis and the citric acid cycle. We can use Reaction 15.2 to estimate the cost of synthesizing acetyl CoA—the substrate for the citric acid cycle. Recall that the pathway from glyceraldehyde 3-phosphate to acetyl CoA is coupled to the synthesis of two molecules of NADH and two molecules of ATP (Section 11.2). If we subtract these from the cost of making glyceraldehyde 3-phosphate then the total cost of synthesizing acetyl CoA from CO_2 is 7 ATP + 4 NAD(P)H. This can be expressed as 17 ATP equivalents since each NADH is equivalent to 2.5 ATP (Section 14.11). The net gain from complete oxidation of acetyl CoA by the citric acid cycle is 10 ATP equivalents (Section 13.4). The biosynthesis pathway is more expensive than the energy gained from catabolism. In this case, the "efficiency" of acetyl CoA oxidation is only about 60% (10/17 = 59%) but this value is misleading since it's actually the biosynthesis pathway (costing 17 ATP equivalents) that is complex and inefficient.

We can estimate the cost of synthesizing glucose because it is simply the cost of making two molecules of glyceraldehyde 3-phosphate. It's equivalent to 18 molecules of ATP and 12 molecules of NADPH or 48 ATP equivalents. Recall that the net gain of energy from the complete oxidation of glucose via glycolysis and the citric acid cycle is 32 ATP equivalents (Section 13.4). In this case, catabolism recovers two-thirds of amount of the ATP equivalents used in the biosynthesis pathway.


▲ **Glyceraldehyde 3-phosphate dehydrogenase.** This NADPH-dependent enzyme from spinach (*Spinacia oleracea*) crystallizes as a tetramer. Only a single subunit is shown here. NADPH is bound in the active site of the enzyme. [PDB 2PKQ]

15.5 Sucrose and Starch Metabolism in Plants

Glyceraldehyde 3-phosphate (G3P) is the main product of carbon fixation in most photosynthetic species. G3P is subsequently converted to glucose by the gluconeogenesis pathway. Newly synthesized hexoses can be used immediately as substrates in a number of biosynthesis pathways or they can be stored as polysaccharides for use later on. In bacteria, most algae, and some plants, the storage polysaccharide is glycogen, just as in animals. The storage polysaccharide in vascular plants is usually starch.

Starch is synthesized in chloroplasts from glucose 6-phosphate, the primary product of gluconeogenesis (Section 12.1D). In the first step, glucose 6-phosphate is converted to glucose 1-phosphate in a reaction catalyzed by phosphoglucomutase (Figure 15.27). This is the same enzyme we encountered in the glycogen synthesis pathway (Section 12.5A). The second step is the activation of glucose by synthesis of ADP–glucose. This reaction is catalyzed by ADP–glucose pyrophosphorylase. The metabolic strategy is similar to that of glycogen biosynthesis except that the key intermediate in glycogen

KEY CONCEPT

The energy recovered in catabolic pathways is usually about two-thirds of the energy used in biosynthesis.

The structures of starch and glycogen are described in Section 8.6A.

The nucleotide sugar ADP–glucose is also required for synthesis of glycogen by some bacteria (Section 12.5A).
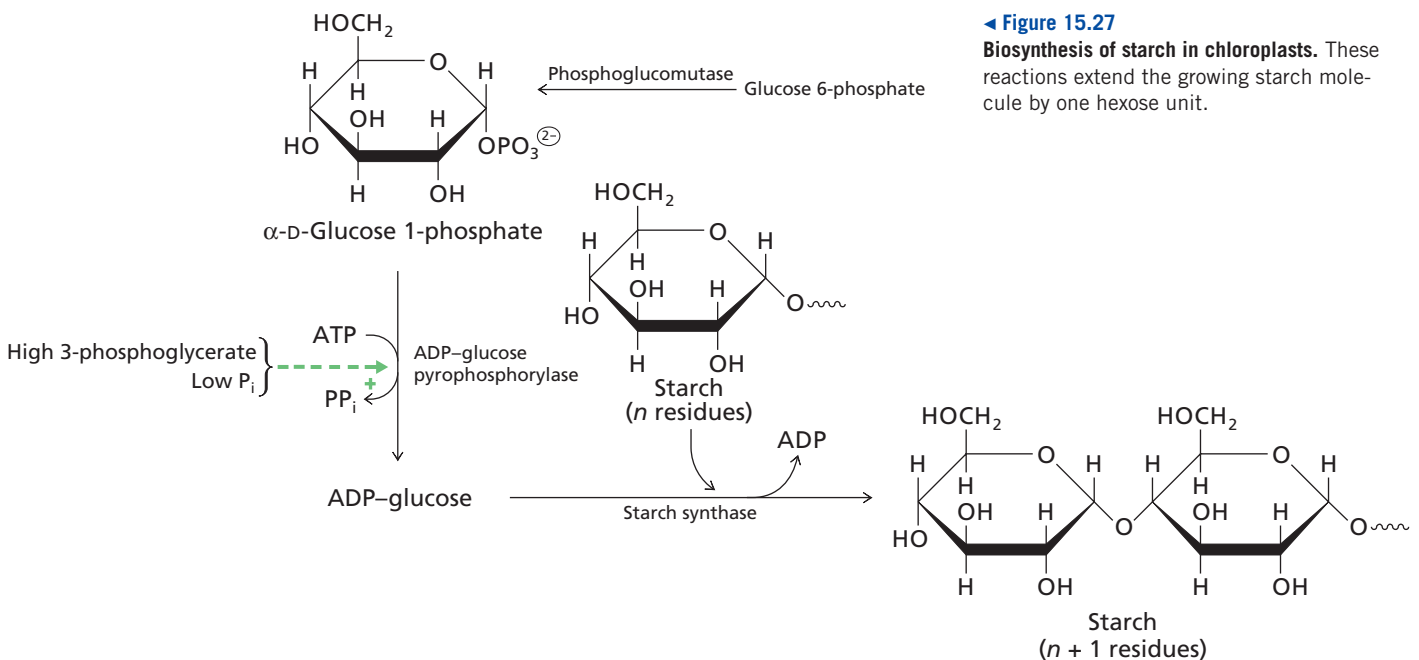
◄ **Figure 15.27**
Biosynthesis of starch in chloroplasts. These reactions extend the growing starch molecule by one hexose unit.

▲ **Maple syrup.** The sucrose-rich sap of maple trees is collected and concentrated to produce maple syrup.

synthesis is UDP–glucose. The polymerization reaction in starch biosynthesis is carried out by starch synthase. This pathway consumes one molecule of ATP and releases one molecule of pyrophosphate for each residue that is added to the growing polysaccharide chain. ATP is supplied by the reactions of photosynthesis.

Starch is synthesized in daylight when photosynthesis is active and ATP molecules accumulate within the chloroplast. During the night starch becomes a source of carbon and energy for the plant. The starch molecule is cleaved by the action of starch phosphorylase to generate glucose 1-phosphate that is converted to triose phosphates by glycolysis. The triose phosphates are exported from the chloroplast to the cytoplasm. Alternatively, starch can be hydrolyzed by the action of amylases to dextrins and eventually to maltose and then glucose. Glucose formed via this route is phosphorylated by the action of hexokinase and enters the glycolytic pathway.

Sucrose is a mobile form of carbohydrate in plants. It is synthesized in the cytoplasm of cells that contain chloroplasts (e.g., leaf cells) and exported to the plant vascular system where it is taken up by non-photosynthetic cells (e.g., root cells). Thus, sucrose is functionally equivalent to glucose, the mobile form of carbohydrate in those animals that possess a circulatory system (Section 12.5).

The pathway for sucrose synthesis is shown in Figure 15.28. Four molecules of triose phosphate produce one molecule of sucrose. The triose phosphates follow the gluconeogenesis pathway, condensing to form fructose 1,6-*bis*phosphate that is hydrolyzed to yield fructose 6-phosphate. Fructose 6-phosphate isomerizes to glucose 6-phosphate that is diverted from the gluconeogenesis pathway and converted to α-D-glucose 1-phosphate. Glucose 1-phosphate reacts with UTP to form UDP–glucose and this activated glucose molecule donates its glucosyl group to a molecule of fructose 6-phosphate,
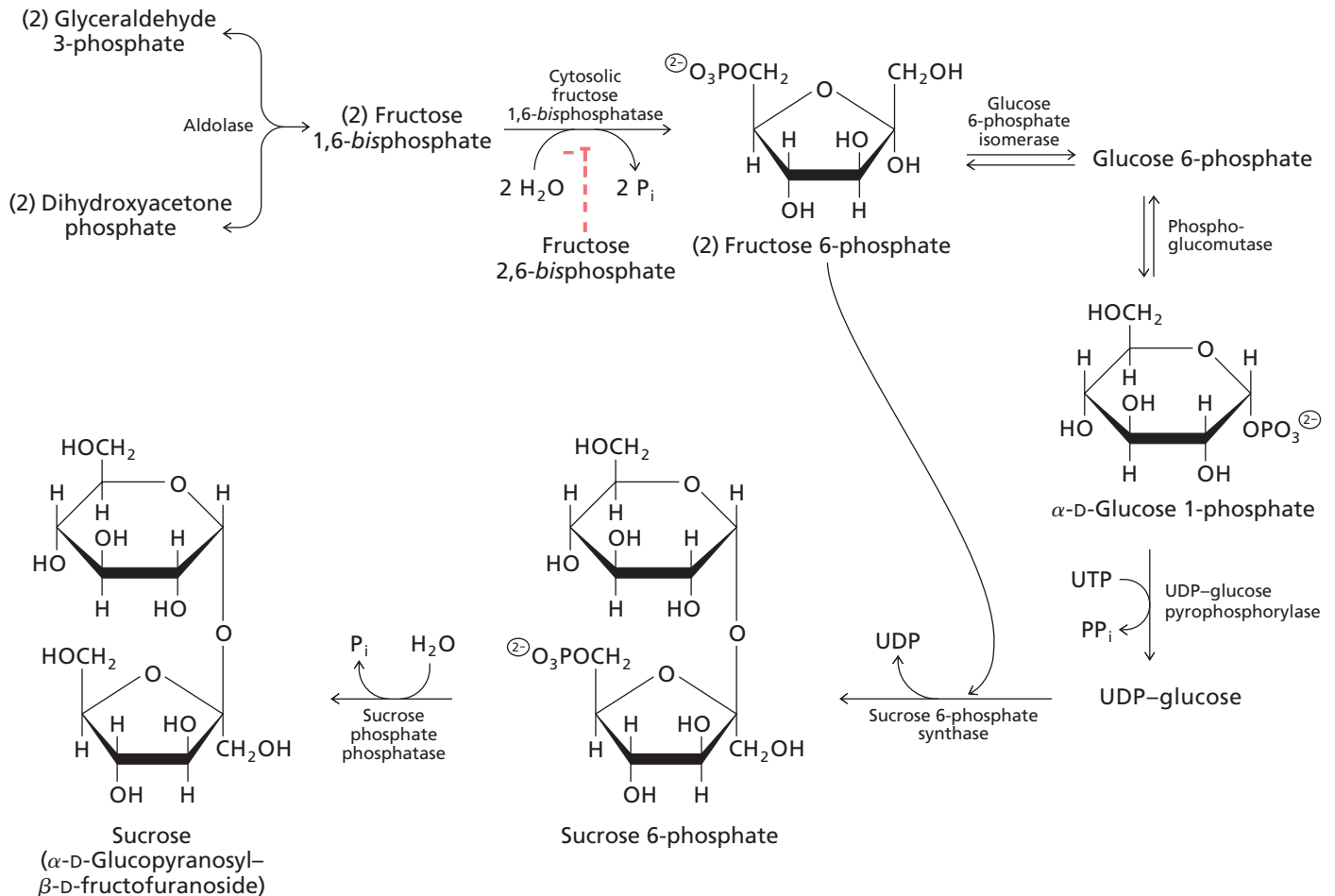
▲ **Figure 15.28**
Biosynthesis of sucrose from glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in the cytosol. Four molecules of triose phosphate (4 C_3) are converted to one molecule of sucrose (C_{12}).

BOX 15.5 GREGOR MENDEL'S WRINKLED PEAS

One of the genetic traits that Gregor Mendel studied was round (R) vs. wrinkled (r) peas. The wrinkled pea phenotype is caused by a defect in the gene for starch branching enzyme. Starch synthesis is partially blocked in the absence of this enzyme and the developing peas have a higher concentration of sucrose. This causes them to absorb more water than the normal peas and they swell to a larger size. When the seeds begin to dry out the mutant peas lose more water and their outer surface takes on a wrinkled appearance.

The mutation is caused by insertion of a transposon into the gene. It is a *recessive* loss-of-function mutation because a single copy of the normal wild-type allele in heterozygotes can produce enough starch branching enzyme to produce starch granules.

▲ **Round and wrinkled peas in a pod.**

to form sucrose 6-phosphate. The final step is the hydrolysis of sucrose 6-phosphate to form sucrose.

Inorganic phosphate (P_i) is produced in the sucrose synthesis pathway by the reactions catalyzed by fructose 1,6-*bis*phosphatase and sucrose phosphate phosphatase. Pyrophosphate (PP_i) is produced in the reaction catalyzed by UDP–glucose pyrophosphorylase. The pathway consumes one ATP equivalent (as UTP). Sucrose synthesis and glycogen synthesis require an activated glucose molecule in the form of UDP–glucose whereas starch biosynthesis uses ADP–glucose.

The first metabolically irreversible step in the sucrose biosynthesis pathway is the hydrolysis of fructose 1,6-*bis*phosphate to yield fructose 6-phosphate and P_i. The activity of fructose 1,6-*bis*phosphatase is inhibited by the allosteric modulator fructose 2,6-*bis*phosphate (Figure 12.9)—a molecule we encountered in our examinations of glycolysis and gluconeogenesis. In plants, the level of fructose 2,6-*bis*phosphate is regulated by several metabolites that reflect the suitability of conditions for sucrose synthesis.

Sucrose is taken up by non-photosynthetic cells where it is degraded by sucrase (invertase) to glucose and fructose that supply energy via glycolysis and the citric acid cycle (Section 11.6A). These hexoses can also be converted to starch in those tissues that store carbohydrate for future use. In root cells, for example, sucrose is converted to hexose monomers and these sugars are taken up by specialized organelles called **amyloplasts**. Amyloplasts are modified chloroplasts that lack the photosynthesis complexes but retain the enzymes for starch synthesis. In some plants, such as potatos, turnips, and carrots, the root cells can store huge reservoirs of starch.
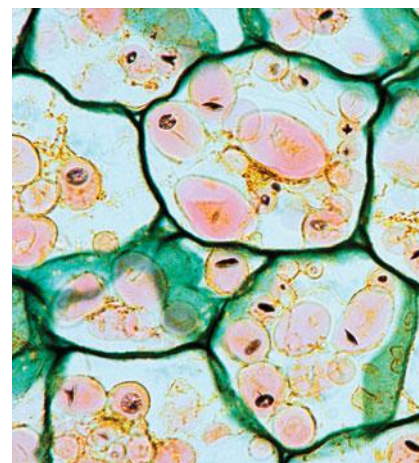
▲ **Amyloplasts in potato cells.**

15.6 Additional Carbon Fixation Pathways

As mentioned earlier, one of the most important problems with carbon fixation is the inefficiency of Rubisco, especially the oxygenation reaction that greatly limits crop yields (Section 15.4C) . Different species have evolved a variety of ways of overcoming this problem.
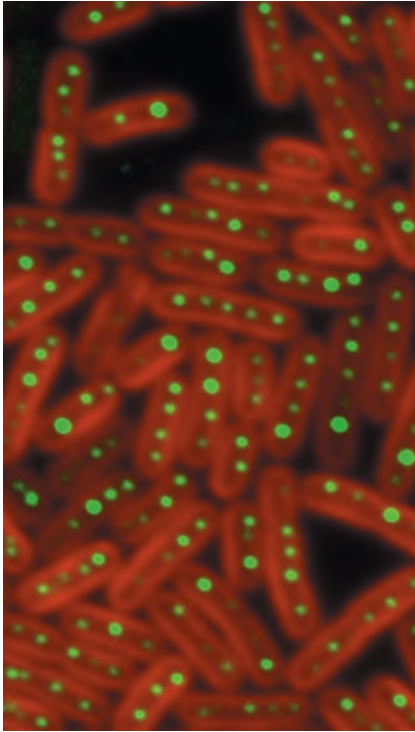
A. Compartmentalization in Bacteria

Bacteria avoid the problems of photorespiration by confining Rubisco to specialized compartments called carboxysomes. Carboxysomes are surrounded by a protein coat that is impermeable to oxygen. Rubisco is localized to carboxysomes and so is the enzyme carbonic anhydrase that converts bicarbonate ($HCO_3^{\ominus}$) to CO_2 (see Section 2.10 and Figure 7.1). The advantage of compartmentalization is that Rubisco is supplied with an abundant source of CO_2 while protecting it against O_2, thus avoiding the inefficiencies of photorespiration.

B. The C$_4$ Pathway

Several plant species avoid wasteful photorespiration by means of secondary pathways for carbon fixation. The net effect of these secondary pathways is to increase the local

▲ **Potatoes are an excellent source of starch.**
French fries are served in Québec with gravy and cheese curds. The dish is called poutine.

concentration of CO_2 relative to O_2 in those cells where Rubisco is active. One of these pathways is called the C_4 pathway because it involves four-carbon intermediates. C_4 plants tend to grow at high temperatures and high light intensities. They include such economically important species as maize (corn), sorghum, and sugarcane, and many of the most troublesome weeds. The avoidance of photorespiration by tropical plants is essential because the ratio of oxygenation to carboxylation by Rubisco increases with temperature.

The C_4 pathway concentrates CO_2 and delivers it to cells in the interior of the leaf where the Calvin cycle is active. The initial product of carbon fixation is a four-carbon acid (C_4) rather than a three-carbon acid as in the Calvin cycle. The C_4 pathway occurs in two different cell types within the leaf. First, CO_2 is hydrated to bicarbonate that reacts with the C_3 compound phosphoenolpyruvate to form a C_4 acid in mesophyll cells (near the leaf exterior). This reaction is catalyzed by an isozyme of phosphoenolpyruvate (PEP) carboxylase (Section 13.6). Next, the C_4 acid is transported to bundle sheath cells in the interior of the leaf where it is decarboxylated. Because they are not directly exposed to the atmosphere, the bundle sheath cells have a much lower O_2 concentration than mesophyll cells. The released CO_2 is fixed by the action of Rubisco and incorporated into the Calvin cycle. Phosphoenolpyruvate is regenerated from the remaining C_3 product. Figure 15.29 outlines the sequence of C_4 pathway reactions.
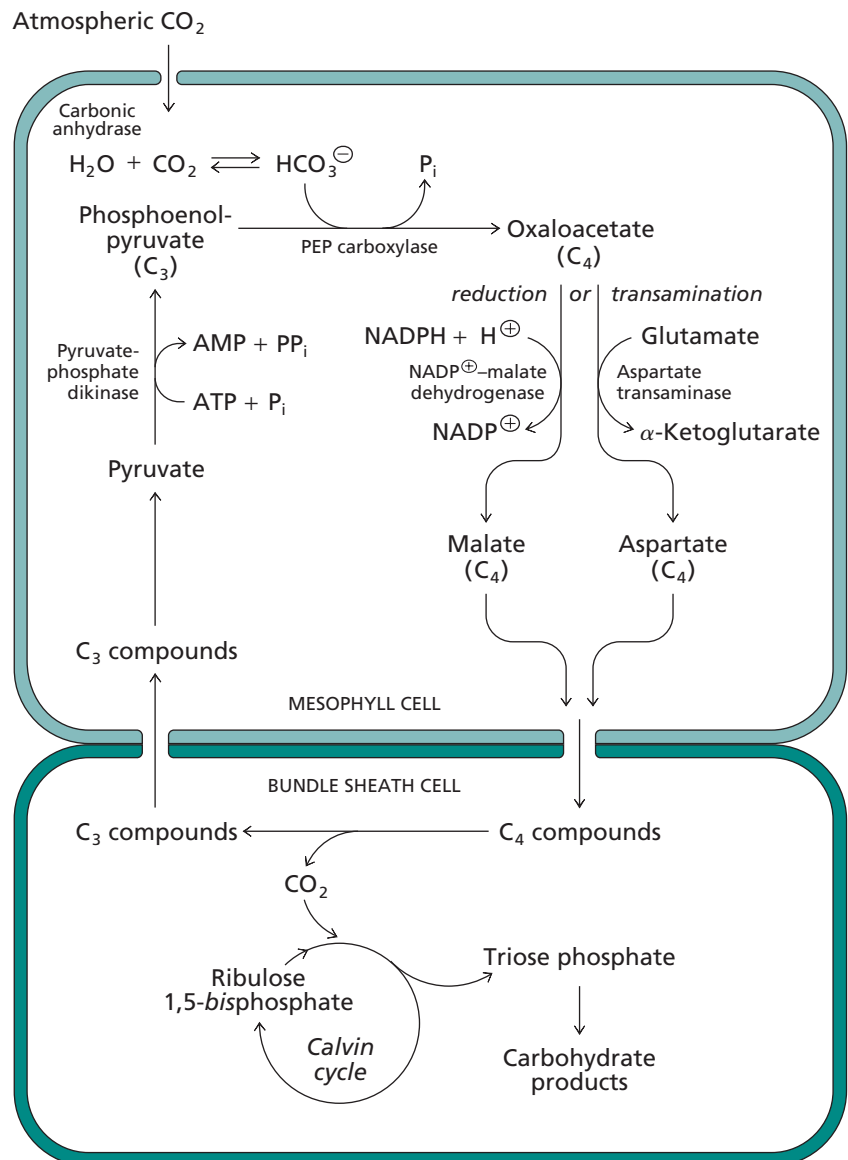

▲ **Carboxysomes.**
Cyanobacteria (*Synechococcus elongatus*) cells are stained with a fluorescent dye showing thylakoid membranes (red) and carboxysomes (green).

Figure 15.29 ▶
C$_4$ pathway. CO_2 is hydrated to bicarbonate (HCO_3^-) in the mesophyll cytosol. Bicarbonate reacts with phosphoenolpyruvate in a carboxylation reaction catalyzed by phosphoenolpyruvate (PEP) carboxylase, a cytosolic enzyme that has no oxygenase activity. Depending on the species, the oxaloacetate produced is either reduced or transaminated to form a four-carbon carboxylic acid or amino acid, which is transported to an adjacent bundle sheath cell and decarboxylated. The released CO_2 is fixed by the Rubisco reaction and enters the RPP cycle. The remaining three-carbon compound is converted back to the CO_2 acceptor, phosphoenolpyruvate.

The cell walls of internal bundle sheath cells are impermeable to gases. The decarboxylation of C_4 acids in these cells greatly increases the CO_2 concentration and creates a high ratio of CO_2 to O_2. The oxygenase activity of Rubisco is minimized because there is an insignificant amount of Rubisco in mesophyll cells and the ratio of CO_2 to O_2 is extremely high in bundle sheath cells. As a result, C_4 plants have essentially no photorespiration activity. Although there is an extra energy cost to form phosphoenolpyruvate for C_4 carbon assimilation, the absence of photorespiration gives C_4 plants a significant advantage over C_3 plants.

C. Crassulacean Acid Metabolism (CAM)

Succulent plants, such as many species of cactus, grow primarily in arid environments where water loss can be a serious problem. A large amount of water can be lost from the leaf tissues during carbon fixation since the cells must be exposed to atmospheric CO_2 and water can evaporate from the surface. These plants minimize water loss during photosynthesis by assimilating carbon at night. The pathway is called Crassulacean acid metabolism because it was first discovered in the family Crassulaceae.

The surface of the leaf in terrestrial vascular plants is often covered with an impermeable waxy coating and CO_2 passes through structures called stomata to reach photosynthetic cells. **Stomata** are formed by two adjacent cells on the surface of the leaf. These guard cells define the entrance to a cavity lined with cells containing chloroplasts. The aperture between the guard cells changes in response to ion fluxes and the resulting osmotic uptake of water. The flux of ions across the guard cells is regulated by conditions that affect CO_2 fixation such as temperature and the availability of water. In the heat of the day, CAM plants keep their stomata closed to minimize water loss. At night, mesophyll cells take up CO_2 through open stomata. Water loss through the stomata is much lower at cooler nighttime temperatures than during the day. CO_2 is fixed by the PEP carboxylase reaction, and the oxaloacetate formed is reduced to malate (Figure 15.30).

Malate is stored in a large central vacuole in order to maintain a nearly neutral pH in the cytosol since the cellular concentration of this acid can reach 0.2 M by the end of the night. The vacuoles of CAM plants generally occupy more than 90% of the total volume of the cell. Malate is released from the vacuole and decarboxylated during the day when ATP and NADPH are formed by photosynthesis. Thus, the large pool of malate accumulated at night supplies CO_2 for carbon assimilation during the day. Leaf stomata are tightly closed when malate is decarboxylated so that neither water nor CO_2 can escape from the leaf and the level of cellular CO_2 can be much higher than the level of atmospheric CO_2. As in C_4 plants, the higher internal CO_2 concentration greatly reduces photorespiration.

In CAM plants the phosphoenolpyruvate required for malate formation is derived from starch via glycolysis. The phosphoenolpyruvate formed by malate decarboxylation (either directly by PEP carboxykinase or via malic enzyme and pyruvate phosphate dikinase) is converted to starch via gluconeogenesis and stored in the chloroplast.

CAM is analogous to C_4 metabolism in that the C_4 acid formed by the action of PEP carboxylase is subsequently decarboxylated to supply CO_2 to the Calvin cycle. In the C_4 pathway the carboxylation and decarboxylation phases of the cycle are spatially separated in distinct cell types whereas in CAM they are temporally separated in day and night cycles.

An important regulatory feature of the CAM pathway is the inhibition of PEP carboxylase by malate and low pH. PEP carboxylase is effectively inhibited during the day when the cytosolic concentration of malate is high and pH is low. This inhibition prevents futile cycling of CO_2 and malate by PEP carboxylase and avoids competition between PEP carboxylase and Rubisco for CO_2.


▲ **Field of Dreams.** These baseball players were probably studying the biochemistry of carbon fixation in the corn field.

▲ **Cactus is a CAM plant.**

Figure 15.30 ▶
Crassulacean acid metabolism (CAM). At night, CO_2 is taken up, and PEP carboxylase and $NAD^{\oplus}$–malate dehydrogenase catalyze the formation of malate. The phosphoenolpyruvate required for malate synthesis is derived from starch. The next day, when NADPH and ATP are formed by the light reactions, the decarboxylation of malate increases the cellular concentration of CO_2 that can be fixed by the Calvin cycle. The decarboxylation of malate occurs by either of two pathways, depending on the species, and yields phosphoenolpyruvate, which is subsequently converted to starch through gluconeogenesis.
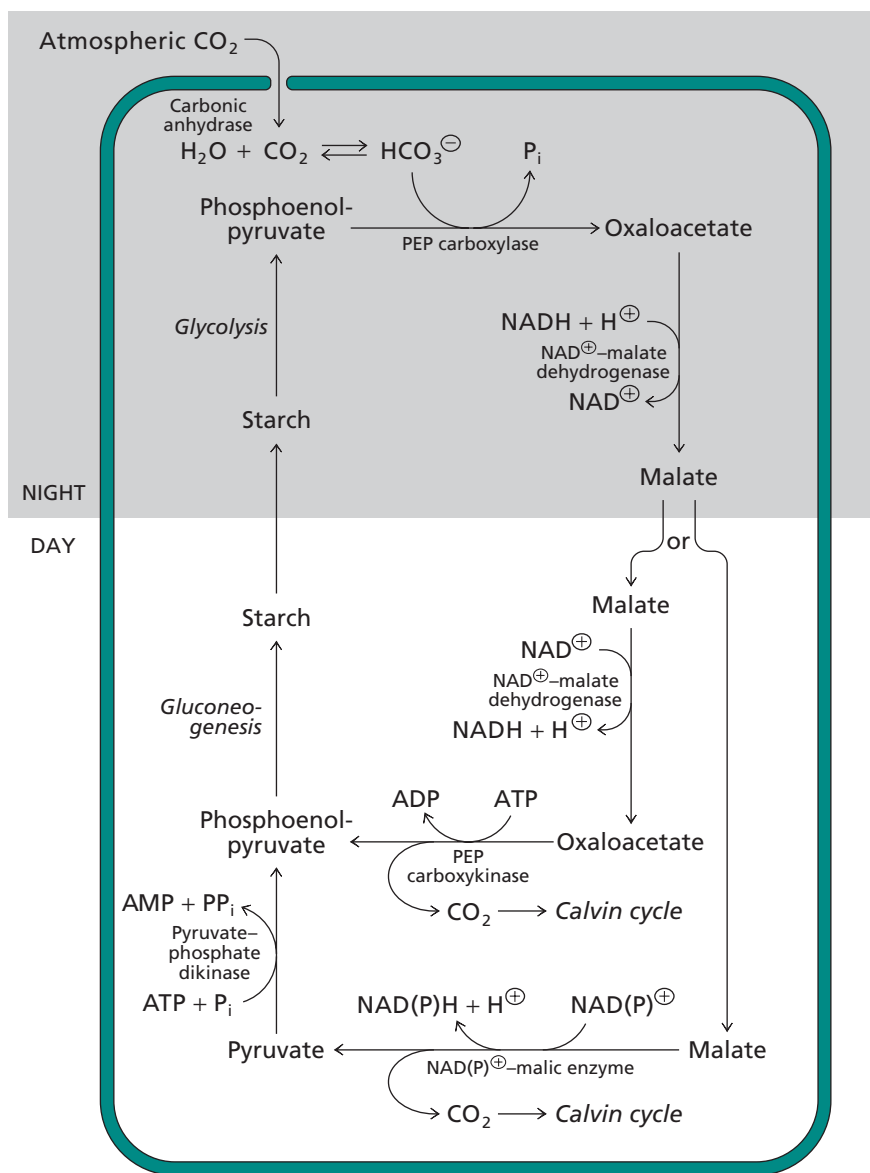
Summary

1. Chlorophyll is the major light-gathering pigment in photosynthesis. When chlorophyll molecules absorb a photon of light, an electron is promoted to a higher-energy molecular orbital. This electron can be transferred to an electron transfer chain giving rise to an electron-deficient chlorophyll molecule.

2. Accessory pigments transfer energy to the special pair of chlorophyll molecules by resonance energy transfer.

3. Photosystem II (PSII) complexes contain a type II reaction center. Electrons are transferred from the special pair of chlorophyll molecules to a short electron transfer chain consisting of a chlorophyll, a pheophytin, a bound quinone, and a mobile quinone.

4. In some bacteria QH_2 molecules from PSII bind to the cytochrome bc_1 complex. Electrons are transferred to cytochrome c and this process is coupled to the transfer of protons across the membrane via the Q cycle. Cytochrome c then binds to PSII and transfers electrons back to the electron-deficient special pair in a cyclic

process of electron transfer. The resulting proton gradient drives ATP synthesis.

5. Photosystem I (PSI) complexes contain a type I reaction center. The electron transfer chain consists of two chlorophylls, a phylloquinone, three [Fe–S] clusters, and ferredoxin (or flavodoxin).

6. Reduced ferredoxin is the substrate for ferredoxin: $NADP^{\oplus}$ reductase (FNR), and NADPH is the product of photosystem I photosynthesis in a noncyclic electron transfer. In some cases, electrons are passed from ferredoxin to the cytochrome bc_1 complex and back to PSI via cytochrome c in a cyclic process of electron transfer.

7. Cyanobacteria, and chloroplasts, contain coupled photosystems consisting of PSI, PSII, and cytochrome bf—a photosynthetic version of cytochrome bc_1. When PSII absorbs a photon of light, electrons are transferred from PSII to cytochrome bf and plastocyanin. Plastocyanin resupplies electrons to PSI. When PSI absorbs a photon of light, excited electrons are used to synthesize NADPH.

In coupled photosystems, PSII is associated with an oxygen evolving complex (OEC) that catalyzes the oxidation of water to O_2 and supplies electrons to the PSII special pair.

8. The Z-scheme depicts electron flow during photosynthesis in terms of the change in reduction potentials of the various components of the electron transfer chains.

9. Photosynthesis complexes are concentrated in thylakoid membranes in cyanobacteria. Chloroplasts contain a complex internal membrane system of thylakoid membranes.

10. The Calvin cycle is responsible for fixing CO_2 into carbohydrates. The key enzyme is ribulose 1,5-*bis*phosphate carboxylase–oxygenase (Rubisco). Rubisco is an inefficient enzyme that catalyzes carboxylation of ribulose 1,5-*bis*phosphate. It also catalyzes an oxygenation reaction.

11. Sucrose and starch are the main products of photosynthetic carbohydrate synthesis in plants.

12. Additional carbon-fixation pathways in some plants serve to increase the concentration of CO_2 at the site of the Calvin cycle reactions.

Problems

1. In plants the transport of a single pair of electrons from P680 to NADPH is coupled to the accumulation of six protons in the lumen. This will result in production of 1.5 molecules of ATP (Section 14.11). Assuming that NADPH ≈ 2.5 ATP, this means that in photosynthesis transport of a pair of electrons through the complexes produces 1.5 + 2.5 = 4 ATP equivalents. Why is this process so much more efficient than respiratory electron transport?

2. The dragonfish is a deepwater species that flashes a red bioluminescent light to illuminate its prey. Although the visual pigments normally present in the retina of fish are not sensitive enough to pick up the red light, the dragonfish retina contains other pigments, derived from chlorophyll, that absorb at 667 nm. Suggest how these chlorophyll pigments might act as a photosensitizer to aid the dragonfish to detect prey using its own red light beacon, which other fish cannot see.

3. (a) Ribulose 1,5-*bis*phosphate carboxylase–oxygenase (Rubisco) has been called the "enzyme that feeds the world." Explain the basis for this statement.
   (b) Rubisco has also been accused of being the world's most incompetent enzyme and the most inefficient enzyme in primary metabolism. Explain the basis for this statement.

4. You frequently see photosynthesis plus the Calvin cycle described as

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

Write a similar equation for the reactions in purple bacteria and in green sulfur bacteria.

5. (a) Some photosynthesis bacteria use H_2S as a hydrogen donor and produce elemental sulfur, whereas others use ethanol and produce acetaldehyde. Write the net reactions for photosynthesis for these bacteria.
   (b) Why is no oxygen produced by these bacteria?
   (c) Write a general equation for the photosynthetic fixation of CO_2 to carbohydrate using H_2A as the hydrogen donor.

6. Can a suspension of chloroplasts in the dark synthesize glucose from CO_2 and H_2O? If not, what must be added for glucose synthesis to occur? Assume that all the components of the Calvin cycle are present.

7. (a) How many photons are absorbed for every O_2 molecule produced in photosynthesis?
   (b) How many photons must be absorbed to generate enough NADPH reducing power for the synthesis of one molecule of a triose phosphate?

8. The herbicide 3-(3, 4-dichlorophenyl)-1,1-dimethylurea (DCMU) blocks photosynthetic electron transport from PSII to the cytochrome *bf* complex.
   (a) When DCMU is added to isolated chloroplasts, will both O_2 evolution and photophosphorylation cease?
   (b) If an external electron acceptor that reoxidizes P680* is added, how will this affect O_2 production and photophosphorylation?

9. (a) The luminal pH of chloroplasts suspended in a solution of pH 4.0 reaches pH 4.0 within a few minutes. Explain why there is a burst of ATP synthesis when the pH of the external solution is quickly raised to 8.0 and ADP and P_i are added.
   (b) If ample ADP and P_i are present, why does ATP synthesis cease after a few seconds?

10. Cyclic electron transport may occur simultaneously with noncyclic electron transport under certain conditions in chloroplasts. Is any ATP, O_2, or NADPH produced by cyclic electron transport?

11. A plant has been genetically engineered to contain a smaller percentage than normal of unsaturated lipids in the thylakoid membranes of the chloroplasts. This genetically changed plant has an improved tolerance to higher temperatures and also shows improved rates of photosynthesis and growth at 40°C. What major components of the photosynthesis system might be affected by changing the lipid composition of the thylakoid membranes?

12. A compound was added to isolated spinach chloroplasts and the effect on photosynthetic photophosphorylation, proton uptake, and noncyclic electron transport determined. Addition of the compound resulted in an inhibition of photosynthetic photophosphorylation (ATP synthesis), inhibition of proton uptake, and an enhancement in noncylic electron transport. Suggest a mechanism for the compound.

13. How many molecules of ATP (or ATP equivalents) and NADPH are required to synthesize (a) one molecule of glucose via photosynthetic CO_2 fixation in plants and (b) one glucose residue incorporated into starch?

14. After one complete turn of the Calvin cycle, where will the labeled carbon atoms from $^{14}CO_2$ appear in (a) glyceraldehyde 3-phosphate (b) fructose 6-phosphate, and (c) erythrose 4-phosphate?

15. (a) How many additional ATP equivalents are required to synthesize glucose from CO_2 in C_4 plants than are required in C_3 plants?
   (b) Explain why C_4 plants fix CO_2 much more efficiently than C_3 plants despite the extra ATP needed.

16. Explain how the following changes in metabolic conditions alter the Calvin cycle: (a) an increase in stromal pH, and (b) a decrease in stromal concentration of Mg^{2+}.

Selected Readings

Pigments

Armstead, I., Donnison, I., Aubry, S., Harper, J., Hörtensteiner, S., James, C., Mani, J., Moffet, M., Ougham, H., Roberts, L., Thomas, A., Weeden, N., Thomas, H., and King, I. (2007). Cross-species identification of Mendel's I locus. *Science* 315:73.

Sato Y., Morita R., Nishimura M., Yamaguchi H., and Kusaba M. (2007). Mendel's green cotyledon gene encodes a positive regulator of the chlorophyll-degrading pathway. *Proc. Natl. Acad. Sci. (USA)* 104:14169–14174.

Photosynthetic Electron Transport

Allen, J. F. (2004). Cytochrome b_6f: structure for signalling and vectorial metabolism. *Trends in Plant Sci.* 9:130–137.

Allen, J. P., and Williams, J. C. (2010). The evolutionary pathway from anoxygenic to oxygenic photosynthesis examined by comparison of the properties of photosystem II and bacterial reaction centers. *Photosynth. Res.* Published online May 7, 2010: Doi 10.1007/s11120-010-9552-x

Amunts, A., Toporik, H., Borovikova, A. B., and Nelson, N. (2010). Structure determination and improved model of plant photosystem I. *J. Biol. Chem*. 285:3478–3486.

Barber, J., Nield, J., Morris, E. P., and Hankamer, B. (1999). Subunit positioning in photosystem II revisited. *Trends Biochem. Sci.* 24:43–45.

Cramer, W. A., Zhang, H., Yan, J., Kurisu, G., and Smith, J. L. (2004). Evolution of photosynthesis: time-independent structure of the cytochrome b_6f complex. *Biochem.* 43:5921–5929.

Cramer, W. A., Zhang, H., Yan, J., Kurisu, G., and Smith, J. L. (2006). Transmembrane traffic in the cytochrome b_6f complex. *Annu. Rev. Biochem.* 75:769–790.

Ferreira, K. N., Iverson, T. M., Maghlaoui, K., Barber, J., and Iwata, S. (2004). Architecture of the photosynthetic oxygen-evolving center. *Science* 303:1831–1838.

Golbeck, J. H. (1992). Structure and function of photosystem I. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:293–324.

Kühlbrandt, W., Wang, D. N., and Fujiyoshi, Y. (1994). Atomic model of plant light-harvesting complex by electron crystallography. *Nature* 367: 614–621.

Leslie, M. (2009). On the origin of photosynthesis. *Science* 323:1286–1287.

Müller, M. G., Slavov, C., Luthra, R., Redding, K. E., and Holzwarth, A. R. (2010). Independent initiation of primary electron transfer in the two branches of the photosystem I reaction center. *Proc. Natl. Acad. Sci. (USA)* 107:4123–4128.

Nugent, J. H. A. (1996). Oxygenic photosynthesis. Electron transfer in photosystem I and photosystem II. *Eur. J. Biochem*. 237:519–531.

Rhee, K.-H., Morris, E. P., Barber, J., and Kühlbrandt, W. (1998). Three-dimensional structure of the plant photosystem II reaction centre at 8 Å resolution. *Nature* 396:283–286.

Staehlin, L. A., and Arntzen, C. J., eds. (1986). *Photosynthesis III: Photosynthetic Membranes and Light Harvesting Systems*. Vol. 19 of *Encyclopedia of Plant Physiology* (New York: Springer-Verlag).

Photophosphorylation

Bennett, J. (1991). Phosphorylation in green plant chloroplasts. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:281–311.

Photosynthetic Carbon Metabolism

Andrews, T. J. and Whitney, S. M. (2003). Manipulating ribulose *bis*phosphate carboxylase/oxygenase in the chloroplasts of higher plants. *Arch. Biochem. Biophys.* 414:159–169.

Bassham, J. A., and Calvin, M. (1957). *The Path of Carbon in Photosynthesis* (Englewood Cliffs, NJ: Prentice Hall).

Edwards, G. E., and Walker, D. (1983). C_3 C_4: *Mechanisms and Cellular and Environmental Regulation of Photosynthesis* (Berkeley: University of California Press).

Hartman, F. C., and Harpel, M. R. (1994). Structure, function, regulation, and assembly of D-ribulose-1,5-*bis*phosphate carboxylase/oxygenase. *Annu. Rev. Biochem.* 63:197–234.

Savage, D. F., Afonso, B., Chen, A. H., and Silver, P. A. (2010). Spatially ordered dynamics of the bacterial carbon fixation machinery. *Science* 327:1258–1261.

Schnarrenberger, C., and Martin, W. (1997). The Calvin cycle—a historical perspective. *Photosynthetica* 33:331–345. An outline of the advances made since the 1950s.
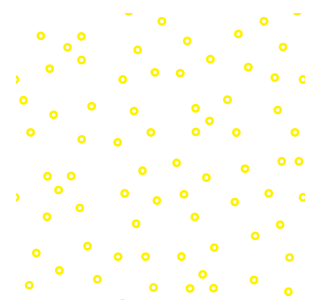
Lipid Metabolism

The synthesis of lipids is an essential part of cellular metabolism since lipids are crucial components of cell membranes. In this chapter we describe the pathways for synthesis of the major lipids that were described in Chapter 9. The most important of these pathways is fatty acid synthesis since fatty acids are required in triacylglycerols. Other important biosynthesis pathways include cholesterol synthesis, eicosanoid synthesis, and the synthesis of sphingolipids.

Lipids can also be degraded as a normal part of cellular metabolism. The most important catabolic pathway is that of fatty acid oxidation (β-oxidation). In this pathway, long-chain fatty acids are broken down to acetyl CoA. The opposing pathways of fatty acid biosynthesis and fatty acid oxidation provide another example of how cells handle energy production and utilization in a manner that's compatible with the fundamentals of thermodynamics.

The catabolic pathways of lipid metabolism are part of basic fuel metabolism in animals. Triacylglycerols and glycogen are the two major forms of stored energy. Glycogen can supply ATP for muscle contraction for only a fraction of an hour. Sustained intense work, such as the migration of birds or the effort of marathon runners, is fueled by the metabolism of triacylglycerols. Triacylglycerols are anhydrous and their fatty acids are more reduced than amino acids or monosaccharides—this makes them very efficient at storing energy for use later on (Section 9.3). Triacylglycerols are oxidized when the energy demand increases. In most cases, fat is only used when other energy sources, such as glucose, are unavailable.

We will begin by examining the fundamental pathways of lipid metabolism—the ones that are present in all living species. Where necessary, we'll point out the differences between the bacterial and the eukaryotic pathways. These differences are minor. We then go on to describe the absorption and utilization of dietary lipids in mammals, including the hormonal regulation of lipid metabolism.
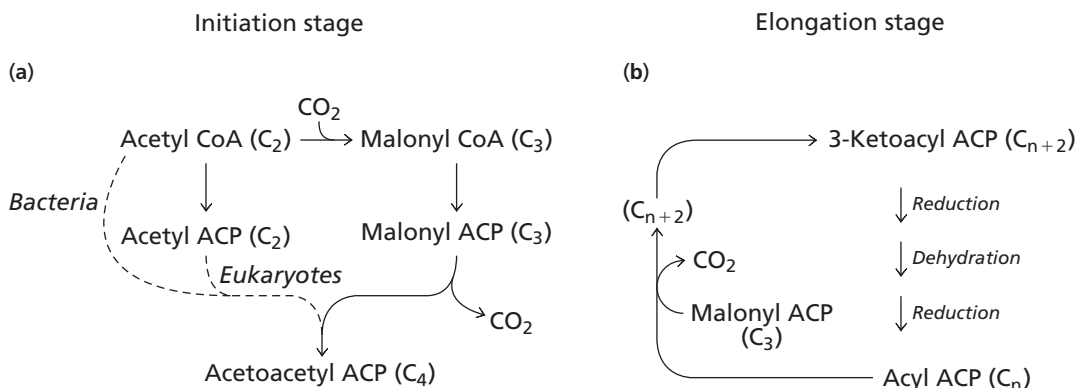
16.1 Fatty Acid Synthesis

Fatty acids are synthesized by the repetitive addition of two-carbon units to the growing end of a hydrocarbon chain. The growing chain is covalently attached to acyl carrier

Derangements of this complicated mechanism of formation and metabolism of lipids are in many cases responsible for the genesis of some of our most important diseases, especially in the cardiovascular field. A detailed knowledge of the mechanisms of lipid metabolism is necessary to deal with these medical problems in a rational manner.

—S. Bergström, presentation speech on awarding the 1964 Nobel Prize in Physiology or Medicine to Konrad Bloch and Feodor Lynen

Top: Whereas the polar bear lives off its stored fat for much of the year, the bird uses its fat stores for long flights.

Figure 16.1 ▶
Outline of fatty acid synthesis.

Malonate
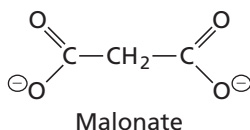
protein (ACP), a protein coenzyme (Section 7.6). The linkage is a thioester as in acetyl CoA. An overview of fatty acid synthesis is shown in Figure 16.1.

The first steps in the fatty acid synthesis pathway are the production of acetyl ACP and malonyl ACP from acetyl CoA. (Malonic acid, or malonate, is the name of the standard C_3 dicarboxylic acid.) The initiation step involves a condensation of acetyl and malonyl groups to give a four-carbon precursor and CO_2. This precursor serves as the primer for fatty acid synthesis. In the elongation stage, the acyl group attached to ACP (acyl ACP) is extended by two-carbon units donated by malonyl ACP. The product of the initial condensation (3-ketoacyl ACP) is modified by two reduction reactions and a dehydration reaction to produce a longer acyl ACP. Acyl ACP then serves as the substrate for additional condensation reactions.

Fatty acid synthesis takes place in the cytosol of all species. In adult mammals it occurs largely in liver cells and adipocytes. Some fatty acid synthesis takes place in specialized cells such as mammary glands during lactation.

A. Synthesis of Malonyl ACP and Acetyl ACP

The regulation of fatty acid metabolism is described in Section 16.9.

Malonyl ACP is the substrate for fatty acid biosynthesis. It is synthesized in two steps, the first of which is the carboxylation of acetyl CoA in the cytosol to form malonyl CoA (Figure 16.2). The carboxylation reaction is catalyzed by the biotin-dependent enzyme acetyl-CoA carboxylase using a mechanism similar to the reaction catalyzed by pyruvate carboxylase (Figure 7.20). The ATP-dependent activation of $HCO_3^\ominus$ forms carboxybiotin. This reaction is followed by the transfer of activated CO_2 to acetyl CoA, forming malonyl CoA. These reactions are catalyzed in eukaryotes by a bifunctional enzyme and the biotin moiety is on a flexible arm that moves between the two active sites. The bacterial version of acetyl-CoA carboxylase is a multisubunit enzyme complex containing biotin carboxylase, biotin carboxylase carrier protein, and a heterodimeric transcarboxylase. In all species, acetyl-CoA carboxylase is the key regulatory enzyme of fatty acid synthesis and the carboxylation reaction is metabolically irreversible.

The second step in the synthesis of malonyl ACP is the transfer of the malonyl moiety from coenzyme A to ACP. This reaction is catalyzed by malonyl CoA:ACP transacylase (Figure 16.3). A similar enzyme called acetyl CoA:ACP transacylase converts acetyl CoA to the acetyl ACP. In most species these are separate enzymes with specificity for malonyl CoA or acetyl CoA but in mammals the two activities are combined in a bifunctional enzyme, malonyl–acetyl transferase (MAT) that's part of a larger complex (see below).

Figure 16.2 ▶
Carboxylation of acetyl CoA to malonyl CoA, catalyzed by acetyl-CoA carboxylase.
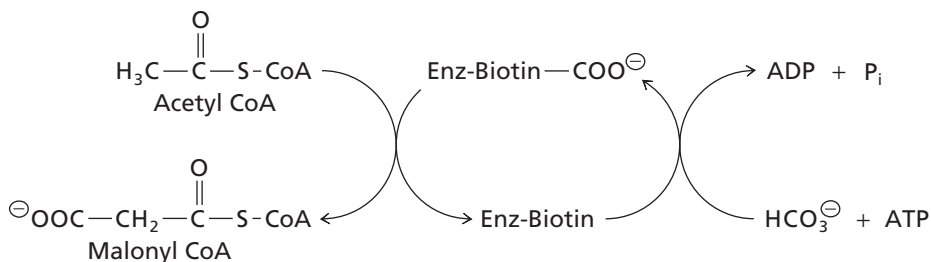
B. The Initiation Reaction of Fatty Acid Synthesis

The synthesis of long-chain fatty acids begins with the formation of a four-carbon unit attached to ACP. This molecule, called acetoacetyl ACP, is formed by condensation of a two-carbon substrate (acetyl CoA or acetyl ACP) and a three-carbon substrate (malonyl ACP) with the loss of CO_2. The reaction is catalyzed by 3-ketoacyl ACP synthase (KAS).

There are several versions of KAS in bacterial cells. One form of the enzyme (KAS III) is used in the initiation reaction and other versions (KAS I, KAS II) are used in subsequent elongation reactions. Bacterial KAS III uses acetyl CoA for the initial condensation reaction with malonyl ACP (Figure 16.4).

A two-carbon unit from acetyl CoA is transferred to the enzyme where it is covalently bound via a thioester linkage. The enzyme then catalyzes the transfer of this two-carbon unit to the end of malonyl ACP creating a four-carbon intermediate and releasing CO_2. Eukaryotic versions of 3-ketoacyl ACP synthase carry out the same reaction except that they use acetyl ACP as the initial substrate instead of acetyl CoA.

Recall that synthesis of malonyl CoA involves ATP-dependent carboxylation of acetyl CoA (Figure 16.2). This strategy of first carboxylating and then decarboxylating a compound results in a favorable free energy change for the process at the expense of ATP consumed in the carboxylation step. A similar strategy is seen in mammalian gluconeogenesis where pyruvate (C_3) is first carboxylated to form oxaloacetate (C_4) and then oxaloacetate is decarboxylated to form the C_3 molecule phosphoenolpyruvate (Section 12.1).

C. The Elongation Reactions of Fatty Acid Synthesis

Acetoacetyl ACP contains the smallest version of a 3-ketoacyl moiety. The "3-keto-" in the name of this molecule refers to the presence of a keto group at the C-3 position. In the older terminology this carbon atom was the β-carbon and the product was called a β-ketoacyl moiety. The condensation enzyme is also called β-ketoacyl ACP synthase.

In order to prepare for subsequent condensation reactions, this oxidized 3-ketoacyl moiety has to be reduced by the transfer of electrons (and protons) to the C-3 position. Three separate reactions are required,

▲ **Figure 16.3**
Synthesis of malonyl ACP from malonyl CoA and acetyl ACP from acetyl CoA.

(16.1)

The ketone is reduced to the corresponding alcohol in the first reduction. The second step is the removal of water by a dehydratase producing a C=C double bond. Finally, a
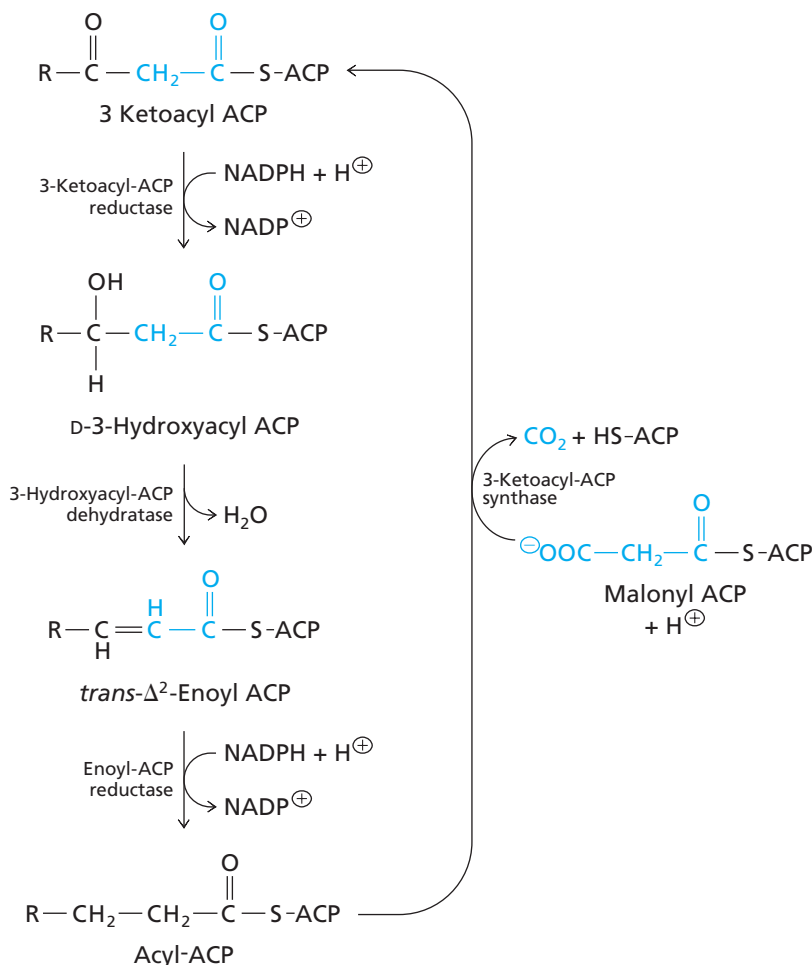
◄ **Figure 16.4**
Synthesis of acetoacetyl ACP in bacteria.

Figure 16.5 ▶
The elongation stage of fatty acid synthesis.
R represents —CH$_3$ in acetoacetyl ACP or
[—CH$_2$—CH$_2$]$_n$—CH$_3$ in other 3-ketoacyl
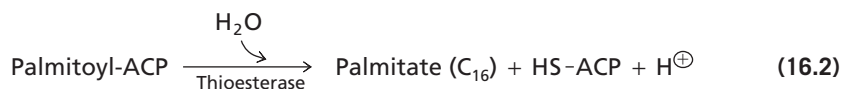ACP molecules.

KEY CONCEPT

**Malonyl ACP, formed from acetyl CoA, is
the precursor for all fatty acid synthesis.**

second reduction adds hydrogens to create the reduced acyl group. This is a common oxidation–reduction strategy in biochemical pathways. We have seen an example of the reverse reactions in the citric acid cycle where succinate is oxidized to oxaloacetate (Figure 13.5).

The specific reactions of the elongation cycle are shown in Figure 16.5. The first reduction is catalyzed by 3-ketoacyl ACP reductase (KR). The full name of the dehydratase enzyme is 3-hydroxyacyl ACP dehydratase (DH). The second reduction step is catalyzed by enoyl ACP reductase (ER). Note that during synthesis the D isomer of the β-hydroxy intermediate is formed in an NADPH-dependent reaction. We will see in Section 16.7 that the L isomer is formed during the degradation of fatty acids.

The final product of the reduction, dehydration, and reduction steps is an acyl ACP that is two carbons longer. This acyl ACP becomes the substrate for the elongation forms of 3-ketoacyl ACP synthase (KAS I and KAS II). All species use malonyl ACP as the carbon donor in the condensation reaction. The elongation reactions are repeated many times resulting in longer and longer fatty acid chains.

The end products of saturated fatty acid synthesis are 16- and 18-carbon fatty acids. Larger chain lengths cannot be accommodated in the binding site of the condensing enzyme. The completed fatty acid is released from ACP by the action of a thioesterase (TE) that catalyzes a cleavage reaction regenerating HS–ACP. For example, palmitoyl ACP is a substrate for a thioesterase that catalyzes formation of palmitate and HS–ACP.

$$\text{Palmitoyl-ACP} \xrightarrow[\text{Thioesterase}]{\text{H}_2\text{O}} \text{Palmitate (C}_{16}) + \text{HS-ACP} + \text{H}^\oplus \qquad \textbf{(16.2)}$$

The overall stoichiometry of palmitate synthesis from acetyl CoA and malonyl CoA is

$$\text{Acetyl CoA} + 7\,\text{Malonyl CoA} + 14\,\text{NADPH} + 20\,\text{H}^{\oplus} \longrightarrow$$

(16.3)

$$\text{Palmitate} + 7\,\text{CO}_2 + 14\,\text{NADP}^{\oplus} + 8\,\text{HS–CoA} + 6\,\text{H}_2\text{O}$$
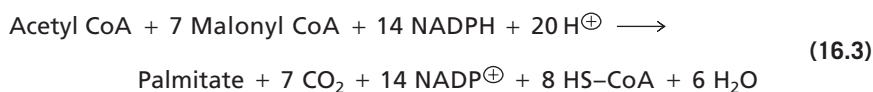
In bacteria, each reaction in fatty acid synthesis is catalyzed by a discrete monofunctional enzyme. This type of pathway is known as a type II fatty acid synthesis system (FAS II). In fungi and animals, the various enzymatic activities are localized to individual domains in a large multifunctional enzyme and the complex is described as a type I fatty acid synthesis system (FAS I).

The large mammalian polypeptide is about 2500 amino acid residues in length ($M_r = 270$ kDa). Fatty acid synthase is a dimer where the two monomers are tightly bound, creating an enzyme with two sites where the fatty acids are synthesized on either side of the dimer axis (Figure 16.6). The bottom part of the enzyme in Figure 16.6 contains the condensing activities of malonyl/acetyl transferase (MAT) and 3-ketoacyl ACP synthase (KAS) that are responsible for adding a new two-carbon unit to the growing chain. These enzymes attach the fatty acid to a bound ACP phosphopantetheine prosthetic group (ACP) that is positioned on a flexible loop. The ACP-bound fatty acid visits the active sites of the modifying activities: 3-ketoacyl ACP reductase (KR), 3-hydroxyacyl ACP dehydratase (DH), and enoyl ACP reductase (ER). The fatty acid chain is eventually released by a thioesterase (TE) activity.

The structures of the ACP domain and the TE domain are not resolved in the crystal structure because they are tethered to the main part of the enzyme by a short stretch of residues that are intrinsically disordered (Section 4.7D). These flexible domains must be free to move during the reaction.

▲ **Figure 16.6**
Mammalian fatty acid synthase. The structure of the pig (*Sus scrofa*) enzyme is shown. It is a large dimer consisting of the following enzyme activities: malonyl/acetyl transferase (MAT), 3-ketoacyl ACP synthase (KAS), 3-ketoacyl ACP reductase (KR), 3-hydroxyacyl ACP dehydratase (DH), enoyl ACP reductase (ER), and thioesterase (TE). The fatty acid chain is attached to a bound ACP cofactor (ACP). The structures of the ACP and TE domains are not resolved because they are bound to a flexible tether. [PDB 2VZ9]
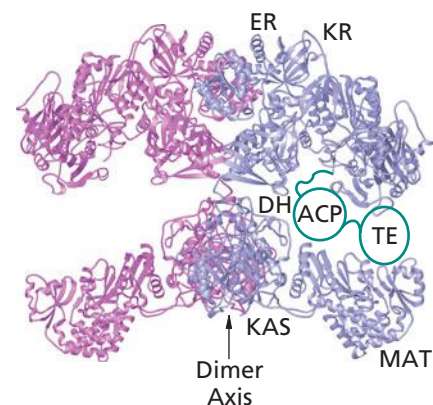
D. Activation of Fatty Acids

The thioesterase reaction (Reaction 16.2) results in release of free fatty acids but subsequent modifications of these fatty acids require an activation step where they are converted to thioesters of coenzyme A in an ATP-dependent reaction catalyzed by acyl-CoA synthetase (Figure 16.7). The pyrophosphate released in this reaction is hydrolyzed to two molecules of phosphate by the action of pyrophosphatase. As a result, two phosphoanhydride bonds, or two ATP equivalents, are consumed to form the CoA thioesters of fatty acids. Bacteria generally have a single acyl-CoA synthetase but in mammals there are at least four different acyl-CoA synthetase isoforms. Each of the distinct enzymes is specific for a particular fatty acid chain length: short ($<C_6$), medium (C_6 to C_{12}), long ($>C_{12}$), or very long ($>C_{16}$). The mechanism of the activation reaction is the same as that for the synthesis of acetyl CoA from acetate and CoA (Figure 10.13). Activation of fatty acids is required for their incorporation into membrane lipids (Section 16.2).

▲ **Figure 16.7**
Activation of fatty acids.

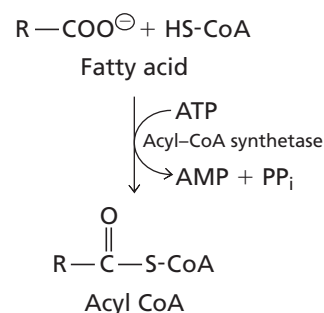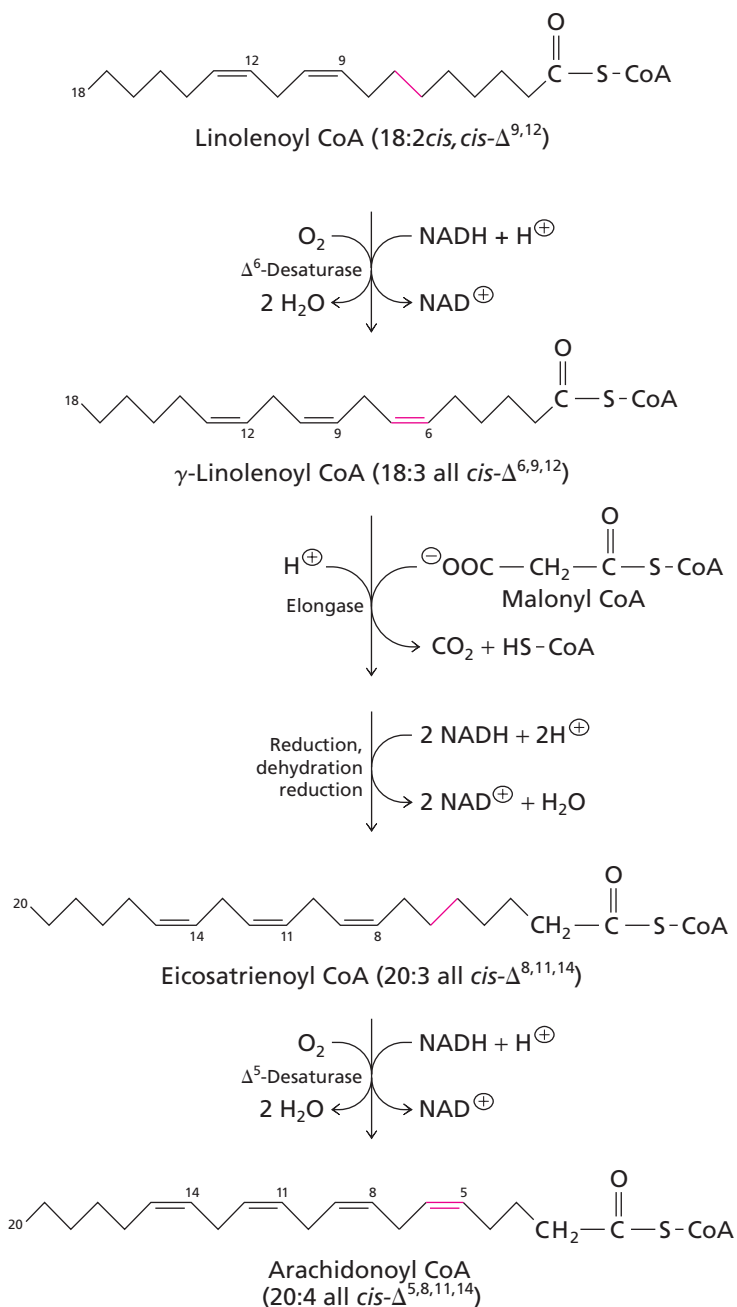## E. Fatty Acid Extension and Desaturation

The fatty acid synthase pathway cannot make fatty acids that are longer than 16 or 18 carbons (C_{16} or C_{18}). Longer fatty acids are made by extending palmitoyl CoA or stearoyl CoA in separate extension reactions. The enzymes that catalyze such extensions are known as elongases and they use malonyl CoA (not malonyl ACP) as the source of the two-carbon extension unit. An example of an elongase reaction is shown below in step 2 of Figure 16.8. Long chain fatty acids such as C_{20} and C_{22} fatty acids are common but C_{24} and C_{26} fatty acids are rare.

Unsaturated fatty acids are synthesized in both bacteria and eukaryotes but the pathways are quite different. In type II fatty acid synthesis systems (bacteria) a double bond is added to the growing chain when it reaches a length of ten carbon atoms. The reaction is catalyzed by specific enzymes that recognize the C_{10} intermediate. For example, 3-hydroxydecanoyl–ACP dehydratase specifically introduces a double bond at the 2 position just as in the normal dehydratase reaction during fatty acid synthesis (Figure 16.5). However, the specific C_{10} dehydratase creates a *cis*-2-decanoyl ACP and not the *trans* configuration that serves as a substrate for enoyl ACP reductase.

Linolenoyl CoA (18:2*cis, cis*-$\Delta^{9,12}$)

Δ^6-Desaturase

γ-Linolenoyl CoA (18:3 all *cis*-$\Delta^{6,9,12}$)

Malonyl CoA

Elongase

Reduction,
dehydration
reduction

Eicosatrienoyl CoA (20:3 all *cis*-$\Delta^{8,11,14}$)

Δ^5-Desaturase
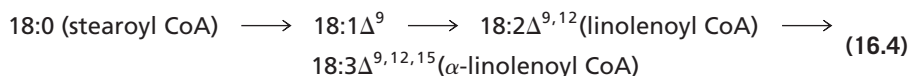
Arachidonoyl CoA
(20:4 all *cis*-$\Delta^{5,8,11,14}$)

Subsequent elongation of this unsaturated fatty acid proceeds by the normal fatty acid synthase pathway except that a specific 3-ketoacyl–ACP synthase enzyme recognizes the unsaturated fatty acid in the condensation reaction. The final products will be 16:1 Δ^8 and 18:1 Δ^{10} unsaturated fatty acids. These products can be further modified to create polyunsaturated fatty acids (PUFAs) in bacteria. The chains can be extended by elongase enzymes and additional double bonds are introduced by a class of enzymes called desaturases. Bacteria contain a huge variety of PUFAs that serve to increase the fluidity of membranes when species encounter low temperatures (Section 9.9). For example, many species of marine bacteria synthesize 20:5 and 22:6 PUFAs. Up to 25% of the membrane fatty acids are large polyunsaturated fatty acids in these species.

The introduction of a double bond during synthesis of fatty acids is not possible in eukaryotes since they employ a type I fatty acid synthase. This fatty acid synthase contains a single 3-ketoacyl–ACP synthase (KAS) activity that is part of a large multifunctional protein. The eukaryotic KAS active site does not recognize unsaturated fatty acid intermediates and could not extend them if they were created at the C_{10} step as in bacteria.

Consequently, eukaryotes synthesize unsaturated fatty acids entirely by using desaturases that act on the completed fatty acid derivatives palmitoyl CoA and stearoyl CoA.

Most eukaryotic cells contain various desaturases that catalyze the formation of double bonds as far as 15 carbons away from the carboxyl end of a fatty acid. For example, palmitoyl CoA is oxidized to its 16:1 Δ^9 analog that can be hydrolyzed to form the common fatty acid palmitoleate. Polyunsaturated fatty acids are synthesized by the sequential action of different, highly specific desaturases. In most cases, the double bonds are spaced at 3-carbon intervals as in synthesis of α-linolenate in plants.

$$18\text{:}0 \text{ (stearoyl CoA)} \longrightarrow 18\text{:}1\Delta^9 \longrightarrow 18\text{:}2\Delta^{9,12} \text{(linolenoyl CoA)} \longrightarrow$$
$$18\text{:}3\Delta^{9,12,15} (\alpha\text{-linolenoyl CoA}) \qquad \textbf{(16.4)}$$

Mammalian cells do not contain a desaturase that acts beyond the C-9 position and they are not able to synthesize linoleate or α-linolenate. However, PUFAs with double bonds at the 12 position are absolutely essential for survival since they are precursors for synthesis of important eicosanoids such as prostaglandins. Because they lack a Δ^{12} desaturase, mammals must obtain linoleate from the diet. This is an essential fatty acid in the human diet. Deficiencies of α-lineolate are rare since most food contains adequate quantities. Plants, for example, are rich sources of PUFAs. Nevertheless, the composition of many "vitamin" supplements will include linoleic acid.

Mammals can convert dietary linoleate (activated to linolenoyl CoA) to arachidonoyl CoA (20:4) by a series of desaturation and elongation reactions (Figure 16.8). (Arachidonate derived from phospholipids is a precursor of eicosanoids, Section 16.3.) This pathway illustrates typical examples of elongase and desaturase activity in the synthesis of complex PUFAs. The intermediate γ-linolenoyl CoA (18:3) in the arachidonate pathway can undergo elongation and desaturation to produce C_{20} and C_{22} polyunsaturated fatty acids. Note that the double bonds of polyunsaturated fatty acids are not conjugated but are interrupted by a methylene group. Thus, a Δ^9 double bond, for example, directs insertion of the next double bond to the Δ^6 position or the Δ^{12} position.

▲ **Linoleate.** Linoleate is an essential component of the human diet.

In addition to the essential fatty acids, mammalian diets must supply a number of essential vitamins (Chapter 7) and essential amino acids (Chapter 17).

In the older biochemistry literature triacylglycerols were called triglycerides (Section 9.3).

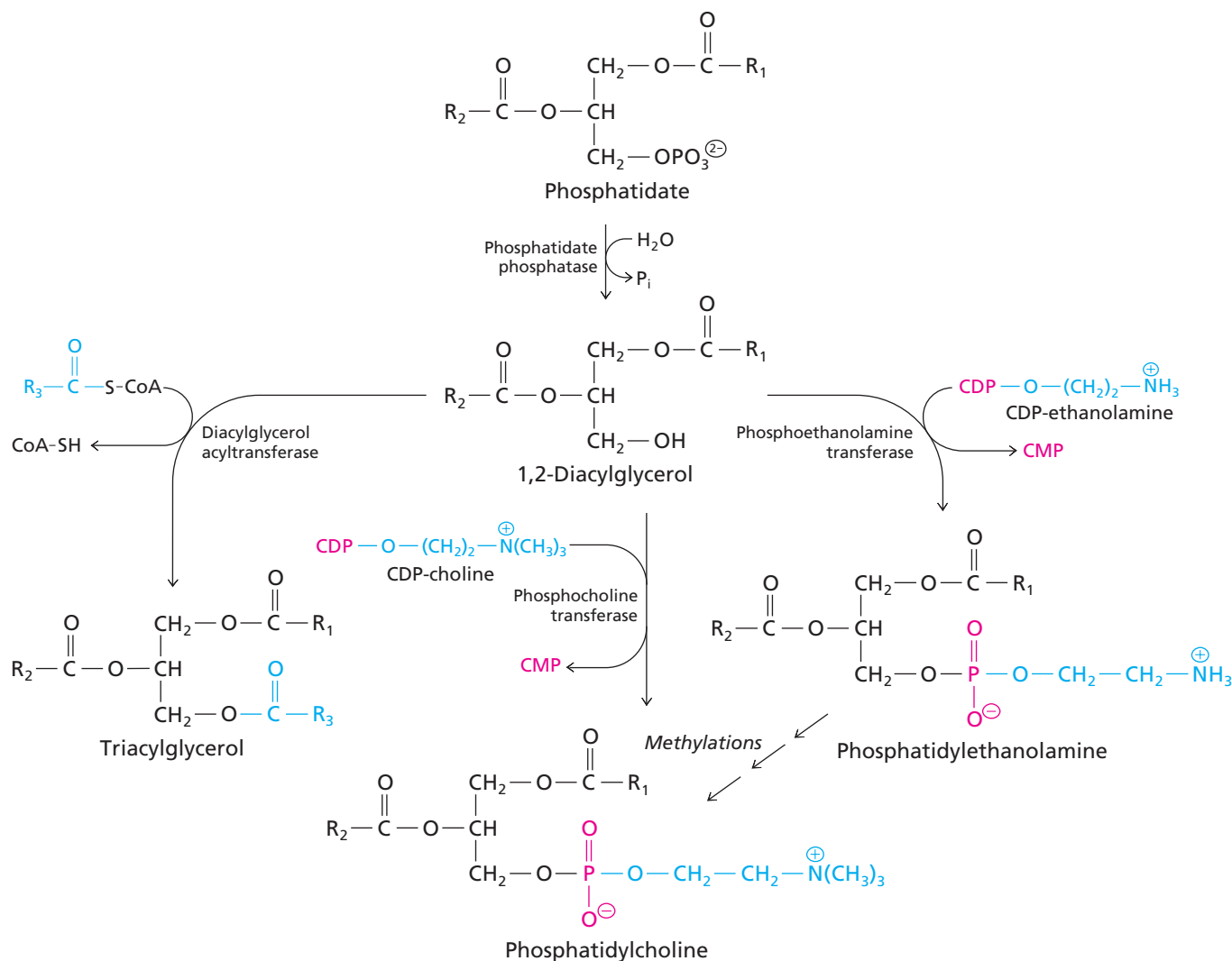## 16.2 Synthesis of Triacylglycerols and Glycerophospholipids

Most fatty acids are found in esterified forms as triacylglycerols or glycerophospholipids (Sections 9.3 and 9.4). Phosphatidate is an intermediate in the synthesis of triacylglycerols and glycerophospholipids. It is formed by transferring the acyl groups from fatty acid CoA molecules to the C-1 and C-2 positions of glycerol 3-phosphate (Figure 16.9). Glycerol 3-phosphate is synthesized from dihydroxyacetone phosphate in a reduction reaction catalyzed by glycerol 3-phosphate dehydrogenase. We encountered this enzyme when we discussed NADH shuttle mechanisms in Chapter 14 (Section 14.12).

The lipid synthesis reactions are catalyzed by two separate acyltransferases that use fatty acyl CoA molecules as the acyl group donors. The first acyltransferase is glycerol-3-phosphate acyltransferase. It catalyzes esterification at C-1 of glycerol 3-phosphate to form 1-acylglycerol 3-phosphate (lysophosphatidate) and it exhibits a preference for saturated fatty acyl chains. The second acyltransferase is 1-acylglycerol-3-phosphate acyltransferase and it catalyzes esterification at C-2 of 1-acylglycerol 3-phosphate. This enzyme

Glycerol 3-phosphate

1-Acylglycerol 3-phosphate
(Lysophosphatidate)
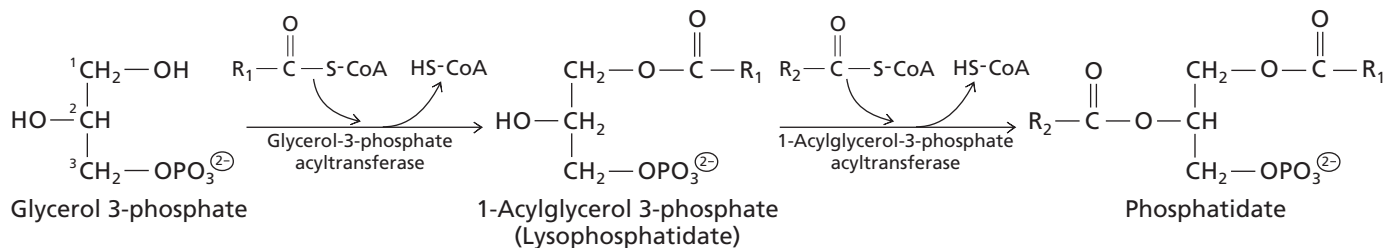
Phosphatidate

▲ **Figure 16.9**
Formation of phosphatidate. Glycerol 3-phosphate acyltransferase catalyzes esterification at C-1 of glycerol 3-phosphate. It has a preference for saturated acyl chains. 1-Acylglycerol-3-phosphate acyltransferase catalyzes esterification at C-2 and has a preference for unsaturated acyl chains.

▲ **Figure 16.10**
Synthesis of triacylglycerols and neutral phospholipids. The formation of triacylglycerols, phosphatidylcholine, and phosphatidylethanolamine proceeds via a diacylglycerol intermediate. A cytosine-nucleotide derivative donates the polar head groups of the phospholipids. Three enzymatic methylation reactions, in which *S*-adenosylmethionine is the methyl-group donor, convert phosphatidylethanolamine to phosphatidylcholine.

prefers unsaturated chains. The product of the two reactions is a phosphatidate, one of a family of molecules whose specific properties depend on the attached acyl groups.

The formation of triacylglycerols and neutral phospholipids from phosphatidate begins with a dephosphorylation catalyzed by phosphatidate phosphatase (Figure 16.10). The product of this reaction is a 1,2-diacylglycerol that can be directly acylated to form a triacylglycerol. Alternatively, 1,2-diacylglycerol can react with a nucleotide–alcohol derivative, such as CDP–choline or CDP–ethanolamine (Section 7.3), to form phosphatidylcholine or phosphatidylethanolamine, respectively. These derivatives are formed from CTP by the general reaction

$$\text{CTP + Alcohol phosphate} \longrightarrow \text{CDP–alcohol + PP}_i \qquad \textbf{(16.5)}$$

Phosphatidylcholine can also be synthesized by methylation of phosphatidylethanolamine by *S*-adenosylmethionine (Section 7.3).

Phosphatidate is also the precursor of acidic phospholipids. In this pathway, phosphatidate is first activated by reacting with CTP to form CDP–diacylglycerol with the release of pyrophosphate (Figure 16.11). In some bacteria, the displacement of CMP by serine produces phosphatidylserine. In both prokaryotes and eukaryotes, displacement of CMP by inositol produces phosphatidylinositol. Phosphatidylinositol can be converted to phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-*bis*phosphate

Figure 16.11 ▶
Synthesis of acidic phospholipids.
Phosphatidate accepts a cytidylyl group
from CTP to form CDP–diacylglycerol. CMP
is then displaced by an alcohol group of
serine or inositol to form phosphatidylserine
or phosphatidylinositol, respectively.
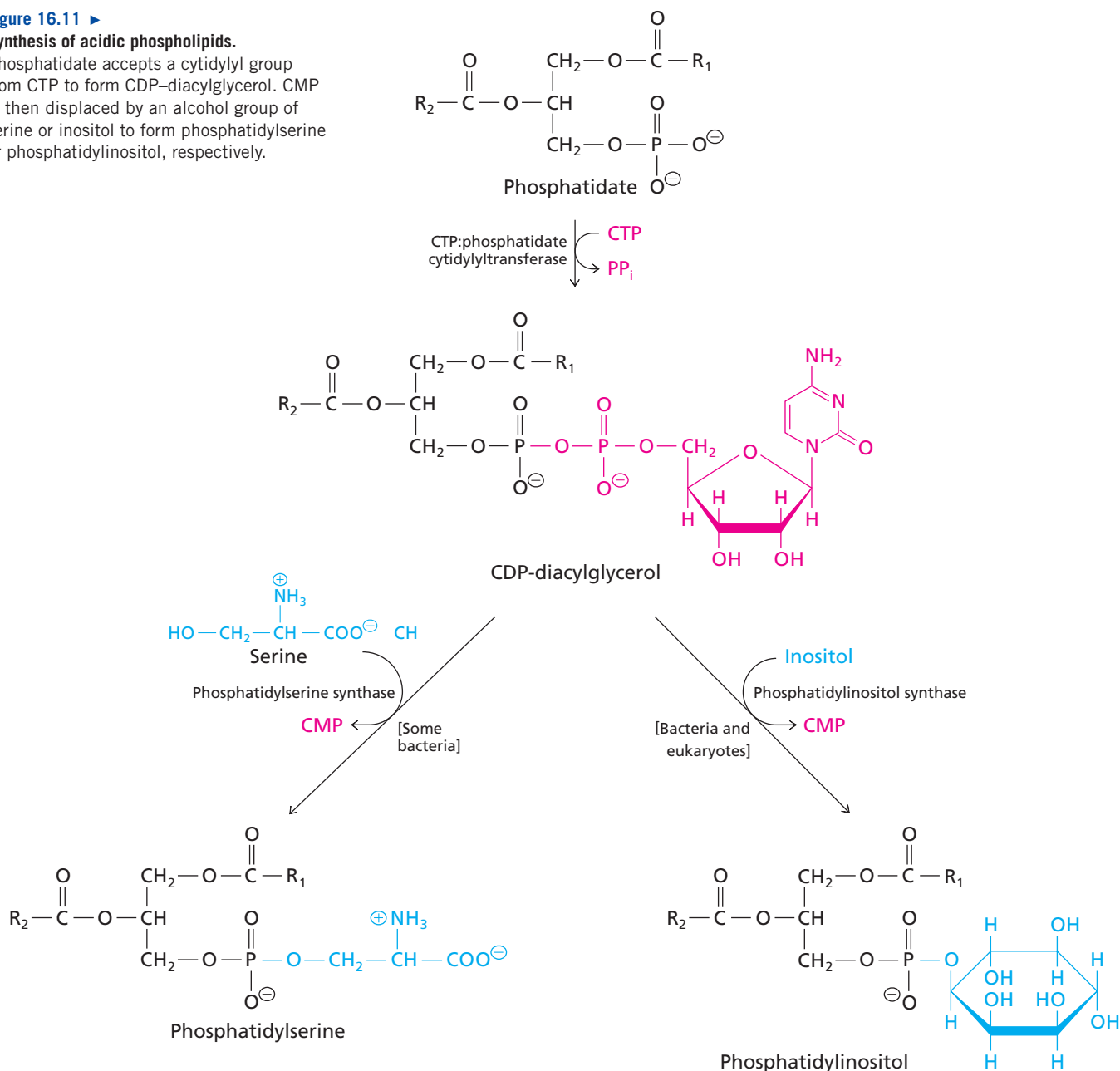
(PIP$_2$) through successive ATP-dependent phosphorylation reactions. Recall that PIP$_2$ is
the precursor of the second messengers inositol 1,4,5-*tris*phosphate (IP$_3$) and diacylglyc-
erol (Section 9.11D).

Most eukaryotes use a different pathway for the synthesis of phosphatidylserine. It
is formed from phosphatidylethanolamine via the reversible displacement of ethanolamine
by serine, catalyzed by phosphatidylethanolamine:serine transferase (Figure 16.12).
Phosphatidylserine can be converted back to phosphatidylethanolamine in a decar-
boxylation reaction catalyzed by phosphatidylserine decarboxylase.

16.3 Synthesis of Eicosanoids

There are two general classes of eicosanoids: prostaglandins + thromboxanes, and
leukotrienes. Arachidonate (20:4 $\Delta^{5,8,11,14}$) is the precursor of many eicosanoids. Recall
that arachidonate is synthesized from linoleoyl CoA (18:2 $\Delta^{9,12}$) in a pathway that re-
quires a Δ^6 desaturase, an elongase, and a Δ^5 desaturase as shown in Figure 16.8.

Prostaglandins are synthesized by the cyclization of arachidonate in a reaction cat-
alyzed by a bifunctional enzyme called prostaglandin endoperoxide H synthase (PGHS).

Figure 16.12 ▶
Interconversions of phosphatidylethanolamine and phosphatidylserine.

Phosphatidylethanolamine

Phosphatidylserine
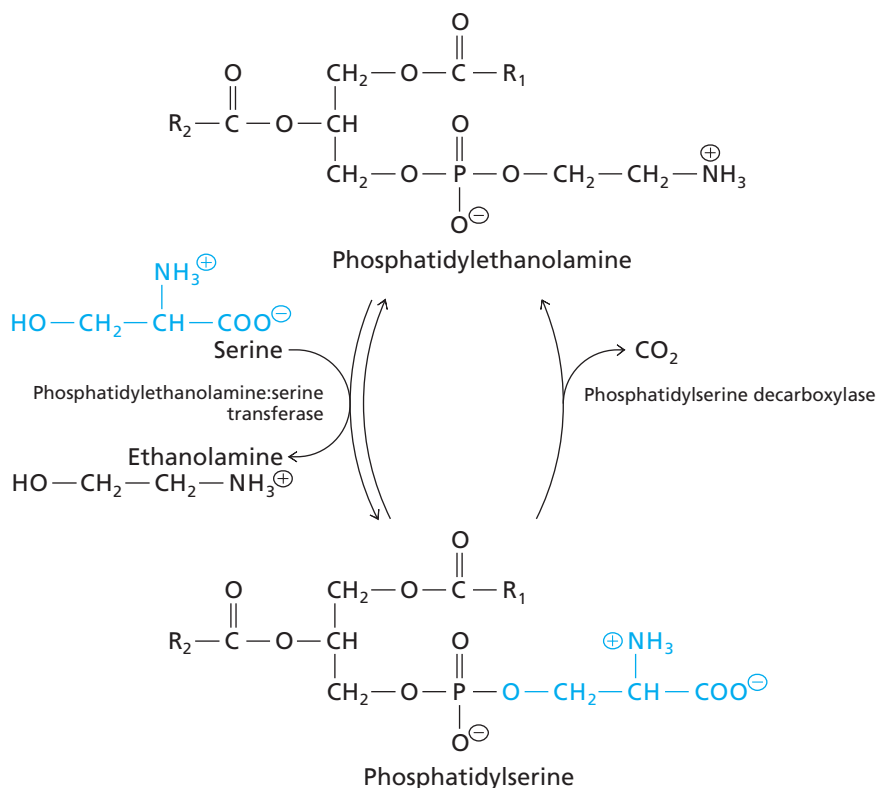
The enzyme is bound to the inner surface of the endoplasmic reticulum through a cluster of hydrophobic α helices that penetrate one of the lipid bilayers (Figure 16.13). The cyclooxygenase (COX) activity of the enzyme catalyzes the formation of a hydroperoxide (prostaglandin G_2). The PGHS enzyme contains a second active site for a hydroperoxidase activity that rapidly converts the unstable hydroperoxide to prostaglandin H_2 (Figure 16.14). This product is converted to various short-lived regulatory molecules including prostacyclin, prostaglandins, and thromboxane A_2. Unlike hormones, which are

---

BOX 16.1 *sn*-GLYCEROL 3-PHOSPHATE

One of the precursors for synthesis of triacylglycerols is glycerol 3-phosphate shown as a Fischer projection in Figure 16.9. This molecule could also be accurately drawn upside down as glycerol 1-phosphate. This changes the stereochemical naming convention from L to D. Similarly, D-glycerol 3-phosphate and L-glycerol 1-phosphate are different names for the same molecule.

Having different names for the same molecule could lead to confusion since the glycerol phosphate precursor is a prochiral molecule meaning that modified lipids will have different stereochemical names depending on whether you start with L-glycerol 3-phosphate or D-glycerol 1-phosphate. In order to avoid this, a new convention is introduced to number the carbon atoms. In a Fischer projection where the hydroxyl group on C-2 is on the left, the "top" carbon atom becomes C-1 and the "bottom" one is C-3. Thus, L-glycerol 3-phosphate becomes *sn*-glycerol 3-phosphate where "*sn*" stands for *Stereochemical numbering*.
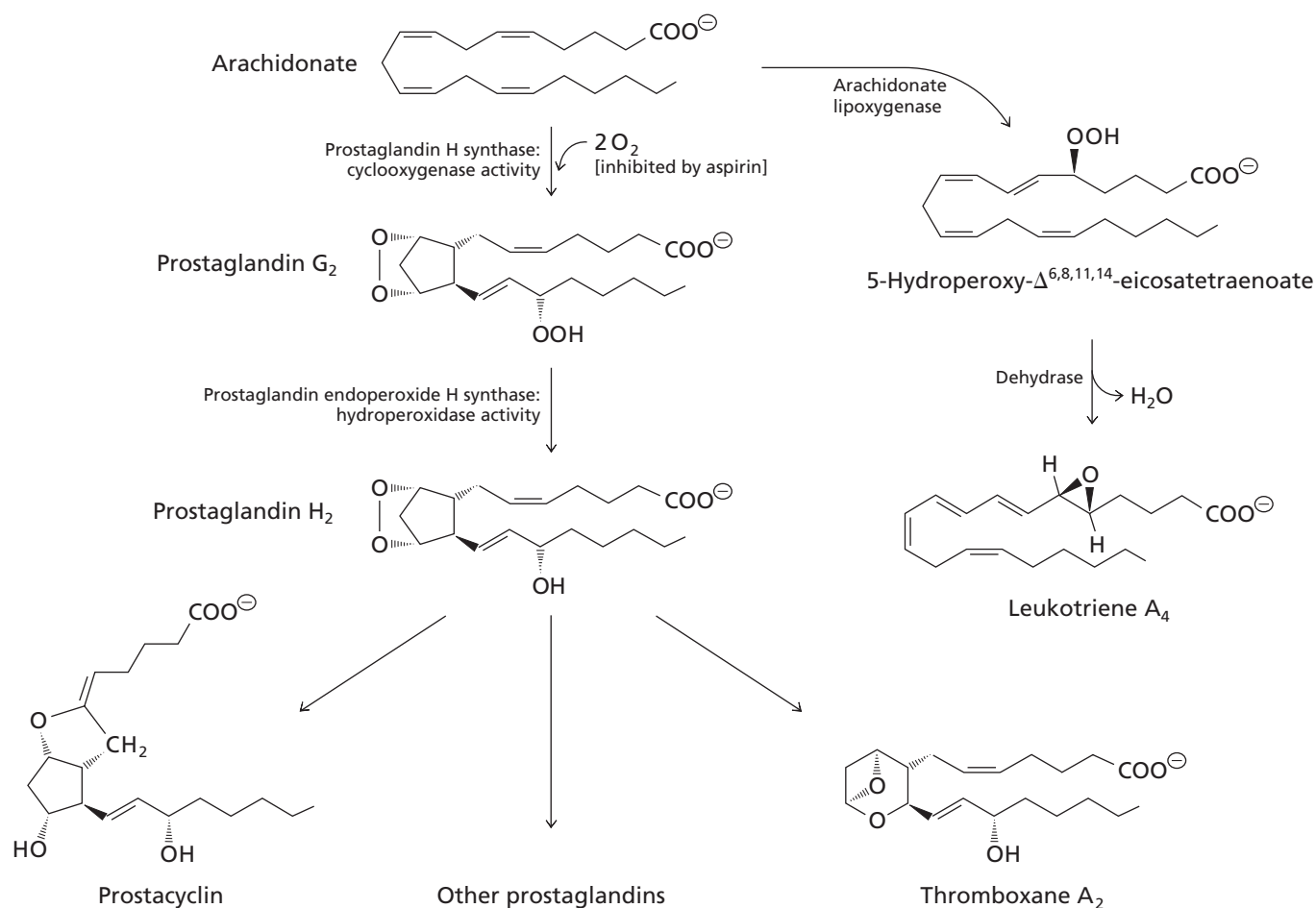
The accurate name for the triglyceride precursor is *sn*-glycerol 3-phosphate in most cases. In archaebacteria the precursor is *sn*-glycerol 1-phosphate (Box 9.5).

L-Glycerol 3-phosphate
sn-Glycerol 3-phosphate

D-Glycerol 1-phosphate

D-Glycerol 3-phosphate

L-Glycerol 1-phosphate
sn-Glycerol 1-phosphate

▲ **Figure 16.14**
Major pathways for the formation of eicosanoids. The prostaglandin H synthase (PGHS) pathway leads to prostaglandin H_2 that can be converted to prostacyclin, thromboxane A_2 and a variety of prostaglandins. The lipoxygenase pathway shown produces leukotriene A_4 a precursor of some other leukotrienes. The cyclooxygenase activity of PGHS is inhibited by aspirin.

▲ **The bark of willow trees is a natural source of salicylates.**

produced by glands and travel in the blood to their sites of action, eicosanoids typically act in the immediate neighborhood of the cell in which they are produced. For example, thromboxane A_2 is produced by blood platelets and it leads to platelet aggregation and blood clots and constriction of the smooth muscles in arterial walls causing localized changes in blood flow. The uterus produces contraction-triggering prostaglandins during labor. Eicosanoids also mediate pain sensitivity, inflammation, and swelling.

Recall that linoleate must be supplied in the human diet, usually from plants, in order to support the synthesis of arachidonate and eicosanoids. One of the reasons why linoleate is essential is because it's required for synthesis of prostaglandins and prostaglandins are necessary for survival.

Aspirin blocks production of some eicosanoids and thus relieves the symptoms of pain and reduces fever. The active ingredient of aspirin, acetylsalicylic acid, irreversibly inhibits COX activity by transferring an acetyl group to an active-site serine residue of the bifunctional enzyme. By blocking the activity of COX, aspirin prevents the formation of a variety of eicosanoids that are synthesized after the COX reaction. Aspirin was first developed as a marketable drug in 1897 but other salicylates have long been used in the treatment of pain. The ancient Greeks, for example, used the bark of willow trees for pain relief. Willow bark is a natural source of salicylates.

The second class of eicosanoids are the products of reactions catalyzed by lipoxygenases. In Figure 16.14, arachidonate lipoxygenase is shown catalyzing the first step in the pathway leading to leukotriene A_4. (The term *triene* refers to the presence of three conjugated double bonds.) Further reactions produce other leukotrienes, such as the compounds once called the "slow-reacting substances of anaphylaxis" (allergic response) that are responsible for the occasionally fatal effects of exposure to antigens.


BOX 16.2 THE SEARCH FOR A REPLACEMENT FOR ASPIRIN

Most natural salicylates have serious side effects. They cause inflamation of the mouth, throat, and stomach and they taste horrible. Aspirin avoids most of these side effects, which is why it became such a popular drug when it was first introduced. However, aspirin can cause dizziness, ringing in the ears, and bleeding or ulcers of the stomach lining. There are two different forms of PGHS (also called COX after their cyclooxygenase activity). COX-1 is a constitutive enzyme that regulates secretion of mucin in the stomach, thus protecting the gastric wall. COX-2 is an inducible enzyme that promotes inflammation, pain, and fever. Aspirin inhibits both isozymes.

There are many other nonsteroidal anti-inflammatory drugs (NSAIDS) that inhibit COX activity. Aspirin is the only one that inhibits by covalent modification of the enzyme. The others act by competing with arachidonate for binding to the COX active site. Ibuprofen (Advil®), for example, binds rapidly, but weakly, to the active site and its inhibition is readily reversed when drug levels drop. Acetaminophen (Tylenol®) is an effective inhibitor of COX activity in intact cells.

Physicians would like to have a drug that selectively inhibits COX-2 and not COX-1. Such a compound would not cause stomach irritation. A number of specific COX-2 inhibitors have been synthesized and many are currently available for patients. These drugs, while expensive, are important for patients with arthritis who must take pain killers on a regular basis. In some cases, the new NSAIDS have been associated with increased risk of cardiovascular disease and they have been taken off the market (e.g., Vioxx®). X-ray crystallographic studies of COX-2 and its interaction with these inhibitors has aided the search for even better replacements for aspirin.
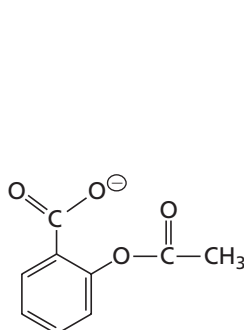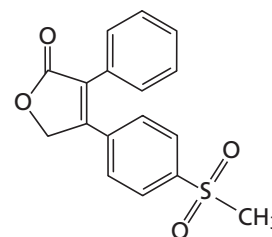

Aspirin
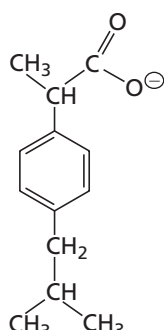
Ibuprofen
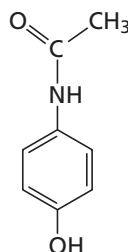
Acetaminophen

Rofecoxib (Vioxx®)
(COX-2 specific NSAID)

16.4 Synthesis of Ether Lipids

Ether lipids have an ether linkage in place of one of the usual ester linkages (Section 9.4). The pathway for the formation of ether lipids in mammals begins with dihydroxyacetone phosphate (Figure 16.15). First, an acyl group from fatty acyl CoA is esterified to the oxygen atom at C-1 of dihydroxyacetone phosphate producing 1-acyldihydroxyacetone phosphate. Next, a fatty alcohol displaces the fatty acid to produce 1-alkyldihydroxyacetone phosphate. The keto group of this compound is then reduced by NADPH to form 1-alkylglycero-3-phosphate. This reduction is followed by esterification at C-2 of the glycerol residue to produce 1-alkyl-2-acylglycero-3-phosphate. The subsequent reactions—dephosphorylation and addition of a polar head group (either choline or ethanolamine)—are the same as those shown earlier in Figure 16.10. Plasmalogens, which contain a vinyl ether linkage at C-1 of the glycerol backbone (Figure 9.9), are formed from alkyl ethers by oxidation of the alkyl ether linkage. This reaction is catalyzed by an oxidase that requires NADH and O_2. The oxidase is similar to the acyl-CoA desaturases (Figure 16.8) that introduce double bonds into fatty acids.

In eukaryotes, ether lipids are not as common as the glycerophospholipids containing ester linkages although some species and some tissues have membranes that are enriched in plasmalogens. Ether lipids are more common in bacteria, especially in archaebacteria where the majority of membrane lipids are ether lipids (Box 9.5).

Figure 16.15 ▼
Synthesis of ether lipids. Plasmalogens are synthesized from ether lipids by the formation of a double bond at the position marked with a red arrow.
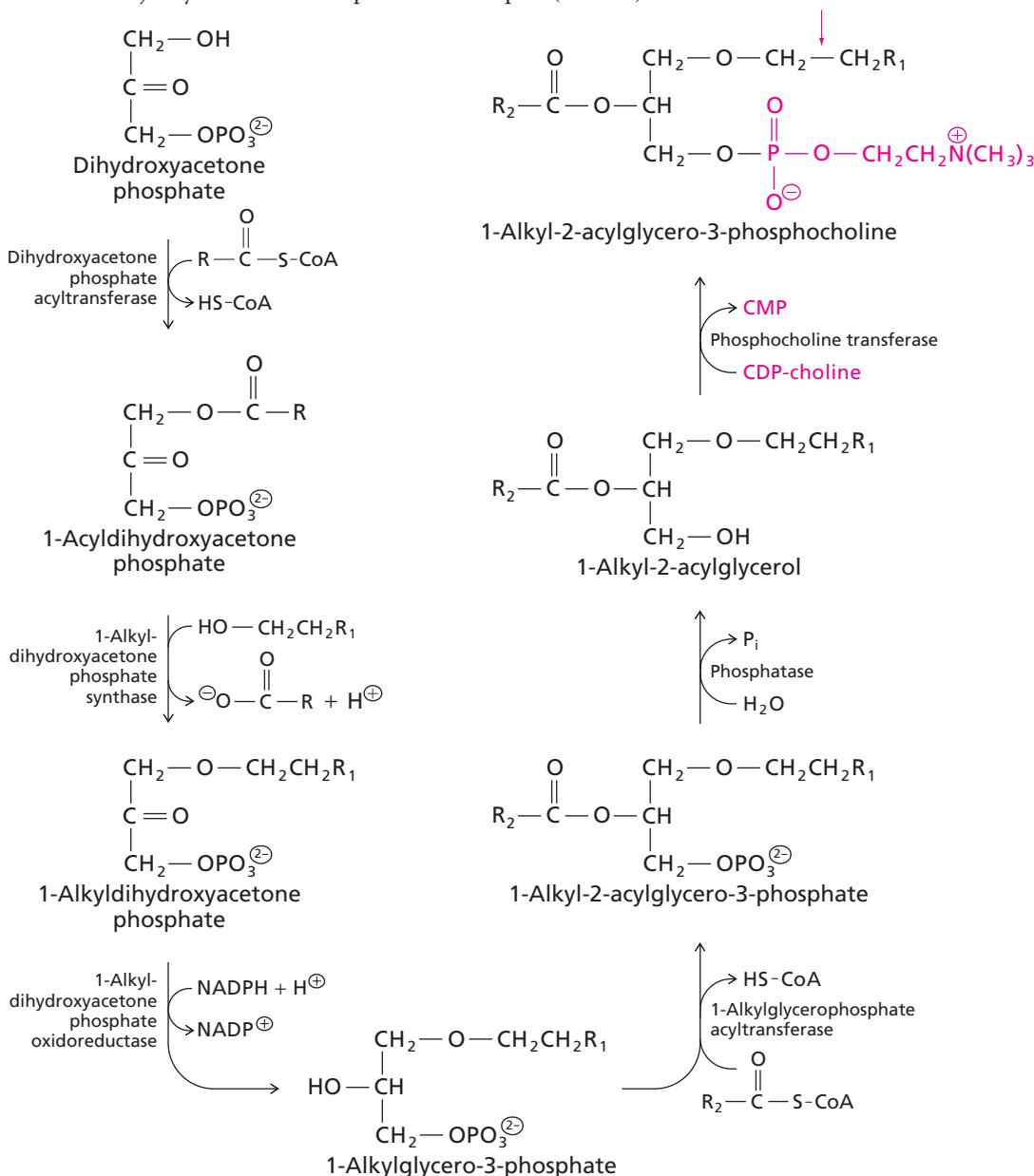
16.5 Synthesis of Sphingolipids

Sphingolipids are membrane lipids that have sphingosine (a C_{18} unsaturated amino alcohol) as their structural backbone (Figure 9.10). In the first step of sphingolipid biosynthesis, serine (a C_3 unit) condenses with palmitoyl CoA, producing 3-ketosphinganine and CO_2 (Figure 16.16). Reduction of 3-ketosphinganine by NADPH produces sphinganine. Next, a fatty acyl group is transferred from acyl CoA to the amino group of sphinganine in an *N*-acylation reaction. The product of this reaction is dihydroceramide, or ceramide without the characteristic double bond between C-4 and C-5 of a typical sphingosine. This double bond is introduced in a reaction catalyzed by dihydroceramide Δ^4-desaturase, an enzyme that is similar to other desaturases that we have encountered. The final product is ceramide (*N*-acylsphingosine).

Ceramide is the source of all the other sphingolipids. It can react with phosphatidylcholine to form sphingomyelin or with a UDP–sugar to form a cerebroside. Complex sugar–lipid conjugates, gangliosides, can be formed by reaction of a cerebroside with additional UDP-sugars and CMP-*N*-acetylneuraminic acid (Figure 9.12). Gangliosides are found in the outer leaflet of the plasma membrane, as are most glycolipids.

16.6 Synthesis of Cholesterol

The steroid cholesterol is an important component of many membranes (Section 9.8) and a precursor of steroid hormones and bile salts in mammals. All the carbon atoms in cholesterol come from acetyl CoA, a fact that emerged from early radioisotopic labeling experiments. Squalene, a C_{30} linear hydrocarbon, is an intermediate in the biosynthesis of the 27-carbon cholesterol molecule. Squalene is formed from 5-carbon units related to isoprene. The stages in the cholesterol biosynthesis pathway are

$$\text{Acetate (C}_2) \longrightarrow \text{Isoprenoid (C}_5) \longrightarrow \text{Squalene (C}_{30}) \longrightarrow \text{Cholesterol (C}_{27}) \quad \textbf{(16.6)}$$

A. Stage 1: Acetyl CoA to Isopentenyl Diphosphate

> **Mitochondrial isozymes of acetoacetyl-CoA thiolase and HMG-CoA synthase are involved in the synthesis of ketone bodies (Section 16.11).**

The first step in cholesterol synthesis is sequential condensation of three molecules of acetyl CoA. These condensation steps are catalyzed by acetoacetyl-CoA thiolase and HMG-CoA synthase. The product, HMG CoA, is then reduced to mevalonate in a reaction catalyzed by HMG-CoA reductase (Figure 16.17). This is the first committed step in cholesterol synthesis. Mevalonate is converted to the C_5 compound isopentenyl diphosphate by two phosphorylations followed by decarboxylation. The conversion of three molecules of acetyl CoA to isopentenyl diphosphate requires energy in the form of three ATP and two NADPH. In addition to its role in cholesterol synthesis, isopentenyl diphosphate is an important donor of isoprenyl units for many other biosynthesis reactions.

Many species of bacteria have a completely different, mevalonate-independent pathway for synthesis of isopentyl diphosphate. The initial precursors in this pathway are glyceraldehyde 3-phosphate + pyruvate and not acetyl CoA. The mevalonate-independent pathway is more ancient than the mevalonate-dependent pathway shown here.

B. Stage 2: Isopentenyl Diphosphate to Squalene

> **KEY CONCEPT**
>
> **Isopentenyl diphosphate is the precursor for synthesis of all isoprenoids.**

Isopentenyl diphosphate is converted to dimethylallyl diphosphate by a specific isomerase called isopentenyl diphosphate isomerase (IDI). The two isomers are then joined in a head-to-tail condensation reaction catalyzed by prenyl transferase (Figure 16.18). The products of this reaction are a C_{10} molecule (geranyl diphosphate) and pyrophosphate. A second condensation reaction, also catalyzed by prenyl transferease, produces the important C_{15} intermediate, farnesyl diphosphate. The condensation of isoprenyl units produces a characteristic branched hydrocarbon with regularly spaced double bonds at the branch position. These isoprene units (Figure 9.13) are present in a number of important cofactors.
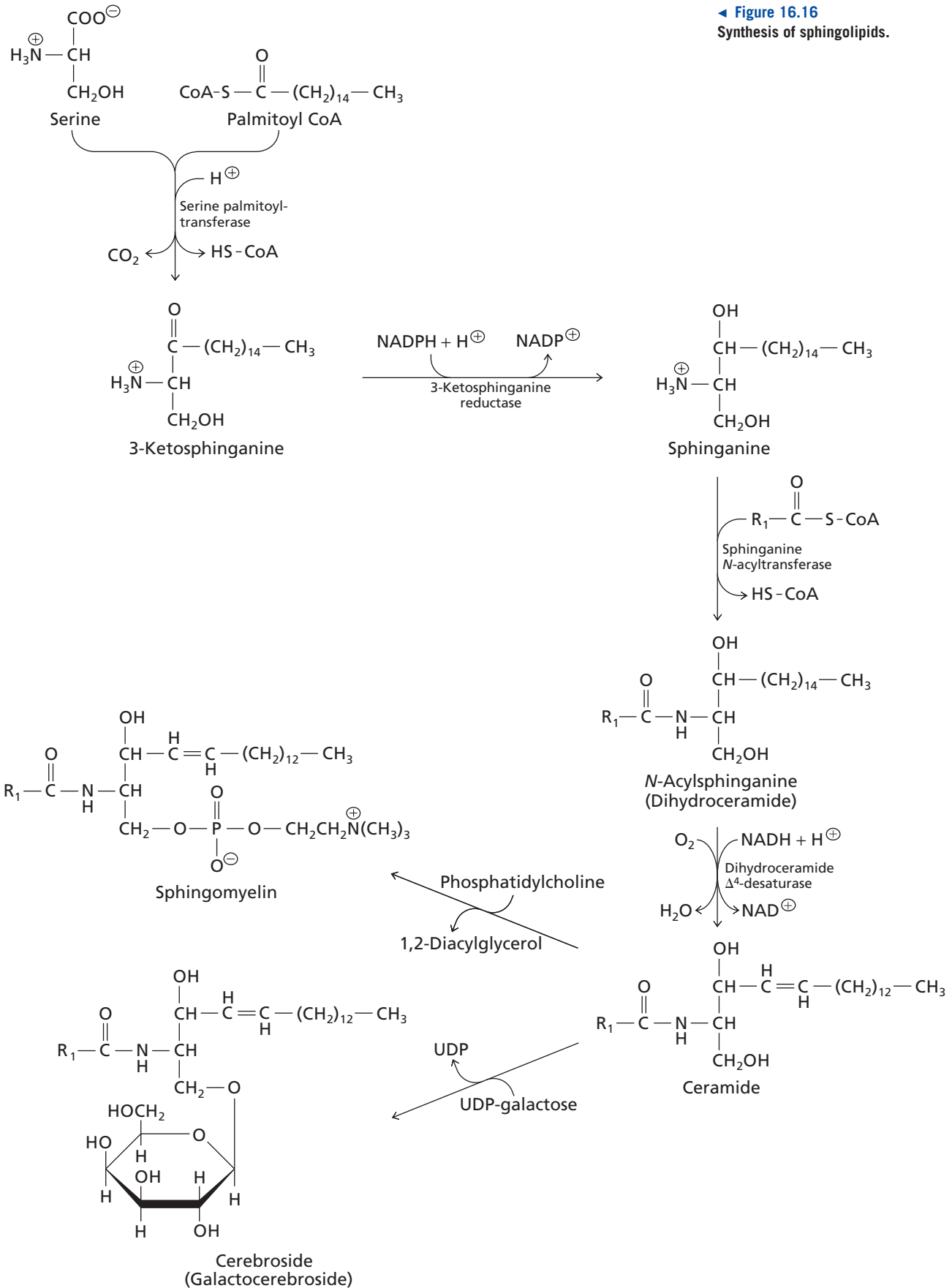
Two molecules of farnesyl diphosphate are joined in a head-to-head condensation reaction to form the C_{30} molecule squalene. Pyrophosphate, whose hydrolysis drives reaction equilibria toward completion, is produced in three steps in the squalene synthesis pathway. Note that all double bonds in squalene are *trans*.
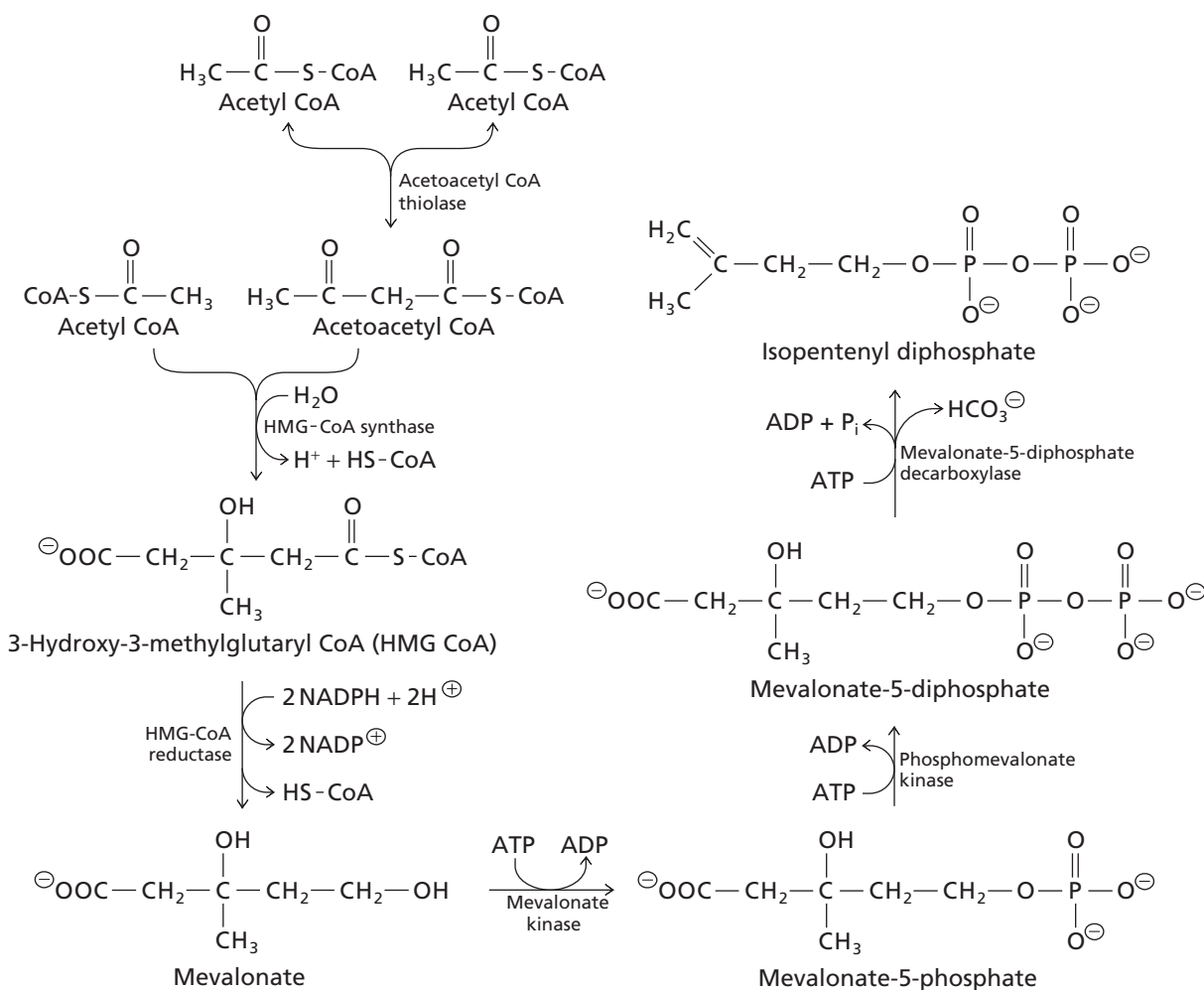
Serine

Palmitoyl CoA

H⁺

Serine palmitoyl-transferase

CO_2 HS–CoA

3-Ketosphinganine

$NADPH + H^{\oplus}$ $NADP^{\oplus}$

3-Ketosphinganine reductase

Sphinganine

Sphinganine N-acyltransferase

HS–CoA

N-Acylsphinganine
(Dihydroceramide)

O_2 $NADH + H^{\oplus}$

Dihydroceramide Δ⁴-desaturase

H_2O $NAD^{\oplus}$

Ceramide

Phosphatidylcholine

1,2-Diacylglycerol

Sphingomyelin

UDP

UDP-galactose

Cerebroside
(Galactocerebroside)

▲ **Figure 16.17**
Stage I of cholesterol synthesis: formation of isopentenyl diphosphate. The condensation of three acetyl CoA molecules leads to HMG CoA, which is reduced to mevalonate. Mevalonate is then converted to the five-carbon molecule isopentenyl diphosphate via two phosphorylations and one decarboxylation.

C. Stage 3: Squalene to Cholesterol

The steps between squalene and the first fully cyclized intermediate, lanosterol, include the addition of a hydroxyl group followed by a concerted series of cyclizations to form the four-ring steroid nucleus (Figure 16.19). Lanosterol accumulates in appreciable quantities in cells that are actively synthesizing cholesterol. The conversion of lanosterol to cholesterol occurs via two pathways, both involving many steps.

D. Other Products of Isoprenoid Metabolism

A multitude of isoprenoids are synthesized from cholesterol or its precursors. Isopentenyl diphosphate, the C_5 precursor of squalene, is the precursor of a large number of other products, such as quinones; the lipid vitamins A, E, and K; carotenoids; terpenes; the side chains of some cytochrome heme groups; and the phytol side chain of chlorophyll (Figure 16.20). Many of these isoprenoids are made in bacteria, which do not synthesize
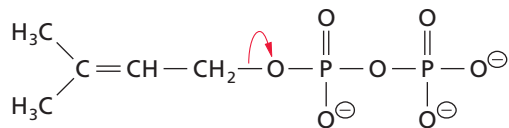
◀ **Konrad Bloch (1912–2000)** (top) and **Feodor Lynen (1911–1979)** (bottom) received the Nobel Prize in Physiology or Medicine in 1964 "for their discoveries concerning the mechanism and regulation of the cholesterol and fatty acid metabolism".
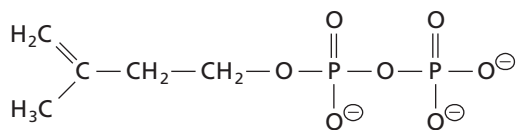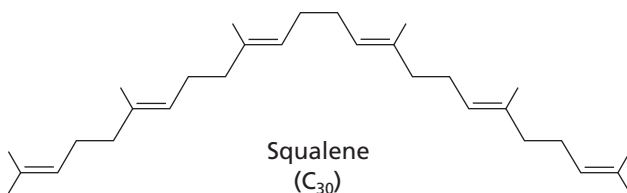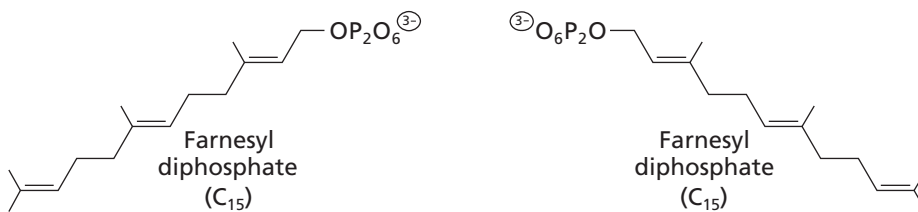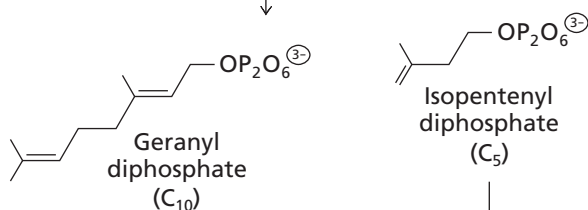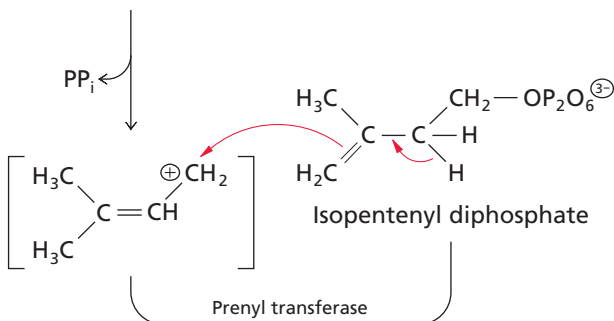
Condensation reactions in the second stage of cholesterol synthesis.

▲ **Figure 16.19**
Final stage of cholesterol synthesis: squalene to cholesterol. The conversion of lanosterol to cholesterol requires up to 20 steps.

BOX 16.3 REGULATING CHOLESTEROL LEVELS

The HMG-CoA reductase reaction appears to be the principal site for the regulation of cholesterol synthesis. HMG-CoA reductase has three regulatory mechanisms—covalent modification, repression of transcription, and control of degradation. Short-term control is effected by covalent modification: HMG-CoA reductase is an interconvertible enzyme that is inactivated by phosphorylation. This phosphorylation is catalyzed by an unusual AMP-activated protein kinase that can also catalyze the phosphorylation and concomitant inactivation of acetyl-CoA carboxylase (Section 16.9). The action of the kinase appears to decrease the ATP-consuming synthesis of both cholesterol and fatty acids when AMP levels rise. The amount of HMG-CoA reductase in cells is also closely regulated. Cholesterol (endogenous cholesterol delivered by plasma lipoproteins or dietary cholesterol delivered by chylomicrons) can repress transcription of the gene that encodes

HMG-CoA reductase. In addition, high levels of cholesterol and its derivatives increase the rate of degradation of HMG-CoA reductase, possibly by increasing the rate of transport of the membrane-bound enzyme to the site of its degradation.

Lowering of serum cholesterol levels decreases the risk of coronary heart disease. A number of drugs called statins are potent competitive inhibitors of HMG-CoA reductase. Statins are often used as part of the treatment of hypercholesterolemia because they can effectively lower blood cholesterol levels. Another useful approach is to bind bile salts in the intestine to resin particles, to prevent their reabsorption. More cholesterol must then be converted to bile salts. Inhibition of HMG-CoA reductase may not be the most desirable method for controlling cholesterol levels because mevalonate is needed for the synthesis of important molecules such as ubiquinone.

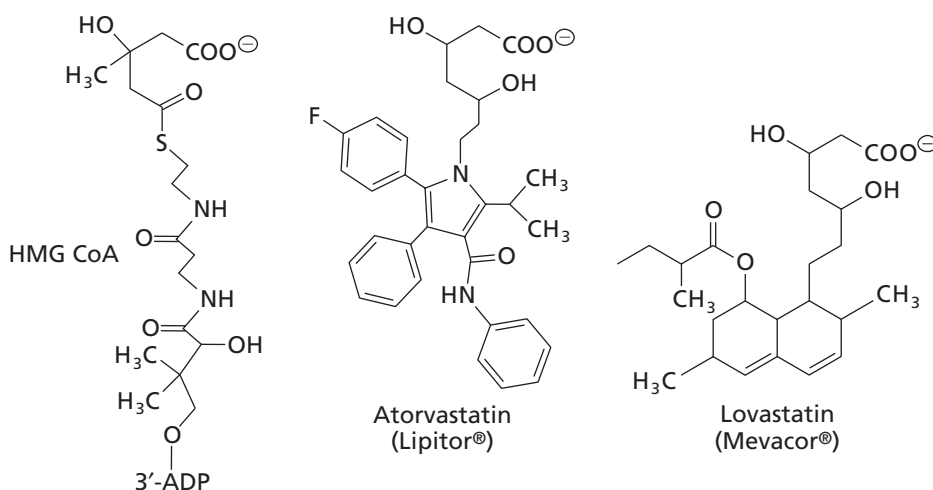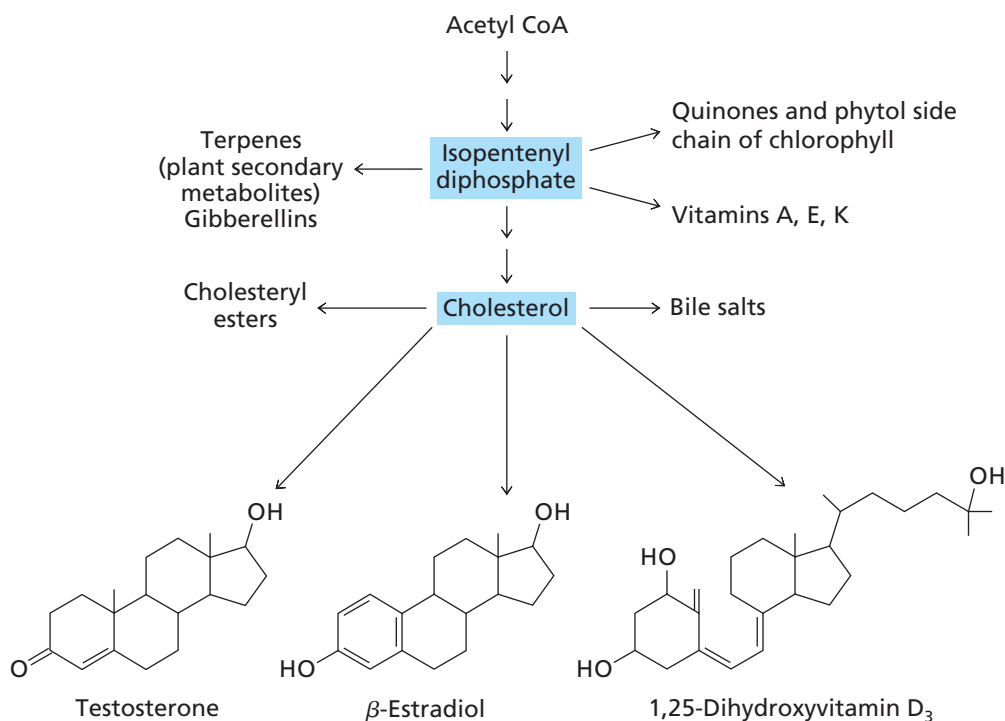▶ **Structure of HMG-CoA and two common statins.**

HMG CoA

3'-ADP

Atorvastatin
(Lipitor®)

Lovastatin
(Mevacor®)

Figure 16.20 ▶
Other products of isopentenyl diphosphate and cholesterol metabolism.

Acetyl CoA

Terpenes (plant secondary metabolites) Gibberellins

Isopentenyl diphosphate

Quinones and phytol side chain of chlorophyll

Vitamins A, E, K

Cholesteryl esters

Cholesterol

Bile salts

Testosterone

β-Estradiol

1,25-Dihydroxyvitamin D₃

cholesterol. The two pathways for the biosynthesis of isopentyl diphosphate (Section 16.6A) are much more ancient than the more recent cholesterol biosynthesis pathway.

Cholesterol is the precursor of bile salts, which facilitate intestinal absorption of lipids; vitamin D that stimulates Ca^{2+} uptake from the intestine; steroid hormones such as testosterone and β-estradiol that control sex characteristics; and steroids that control salt balance. The principal product of steroid synthesis in mammals is cholesterol itself, which modulates membrane fluidity and is an essential component of the plasma membrane of animal cells.

16.7 Fatty Acid Oxidation

Fatty acids, released from triacylglycerols (Section 16.9), are oxidized by a pathway that degrades them by removing two-carbon units at each step. The two-carbon fragments are transferred to coenzyme A to form acetyl CoA, and the remainder of the fatty acid re-enters the oxidative pathway. This degradative process is called the β-oxidation pathway because the β-carbon atom (C-3) of the fatty acid is oxidized. Fatty acid oxidation is divided into two stages: activation of fatty acids and degradation to two-carbon fragments (as acetyl CoA). The NADH and ubiquinol (QH_2) produced by the oxidation of fatty acids can be oxidized by the respiratory electron transport chain, and the acetyl CoA can enter the citric acid cycle.

Acetyl CoA can be completely oxidized by the citric acid cycle to yield energy (in the form of ATP) that can be used in other biochemical pathways. The carbon atoms from fatty acids can also be used as substrates for amino acid synthesis since several of the intermediates in the citric acid cycle are diverted to amino acid biosynthesis pathways (Section 13.6). In those organisms that possess a glyoxylate pathway (Section 13.7), acetyl CoA from fatty acid oxidation can be used to synthesize glucose via the gluconeogenesis pathway.

The oxidation of fatty acids occurs as part of the normal turnover of membrane lipids. Thus, bacteria, protists, fungi, plants, and animals all have a β-oxidation pathway. In addition to its role in normal cellular metabolism, fatty acid oxidation is a major component of fuel metabolism in animals. A significant percentage of dietary food consists of membrane lipids and fat and this rich course of energy is exploited by oxidizing fatty acids. In this section we describe the basic biochemical pathways of fatty acid oxidation. In the following sections we will discuss the role of fatty acid oxidation in mammalian fuel metabolism.

A. Activation of Fatty Acids

The activation of fatty acids for oxidation is catalyzed by acyl-CoA synthetase (Figure 16.7). This is the same activation step that is required for the synthesis of polyunsaturated fatty acids and complex lipids.

B. The Reactions of β-Oxidation

In eukaryotes, β-oxidation takes place in mitochondria and in specialized organelles called peroxisomes. In bacteria, the reactions take place in the cytosol. Four steps are required to produce acetyl CoA from fatty acyl CoA: oxidation, hydration, further oxidation, and thiolysis (Figure 16.21). We focus first on the oxidation of a saturated fatty acid with an even number of carbon atoms.

In the first oxidation step, acyl-CoA dehydrogenase catalyzes the formation of a double bond between the C-2 and C-3 atoms of the acyl group forming *trans* 2-enoyl CoA. There are several separate acyl-CoA dehydrogenase isozymes, each with a different chain length preference: short, medium, long, or very long.

When the double bond is formed, electrons from fatty acyl CoA are transferred to the FAD prosthetic group of acyl-CoA dehydrogenase and then to another FAD prosthetic group bound to a mobile, water-soluble, protein coenzyme called electron
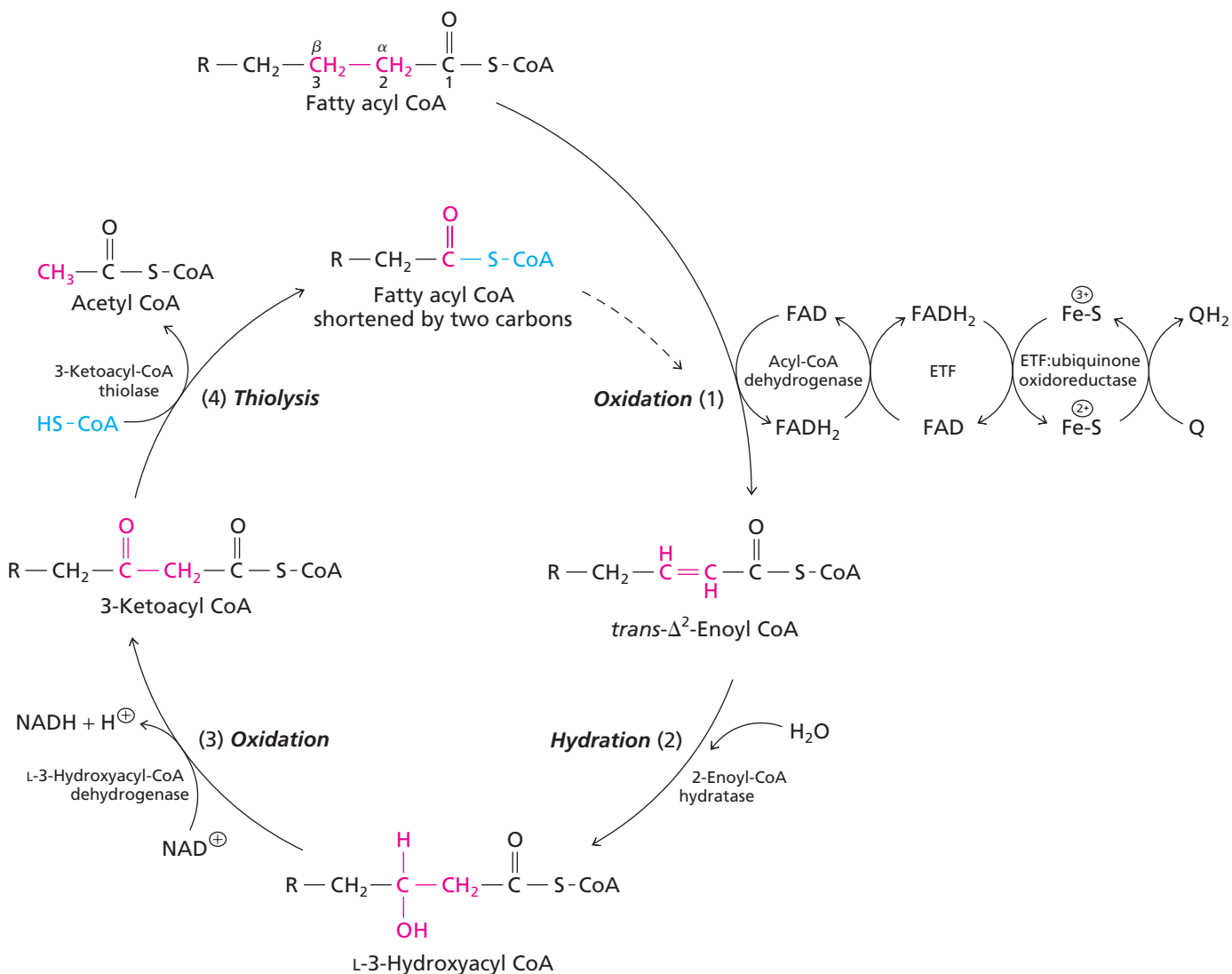
KEY CONCEPT

β-Oxidation is an ancient and ubiquitous pathway for degradation of fatty acids.
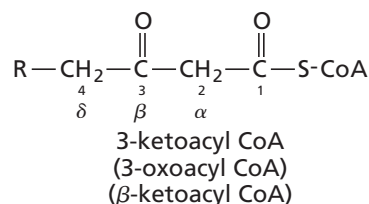
▲ **3-ketoacyl CoA, 3-oxoacyl CoA, β-ketoacyl CoA**

▲ **Bear bile.** In Vietnam bears are kept in captivity—often under deplorable conditions—and bile is extracted from their stomachs on a regular basis. Bear bile is thought to be an effective remedy for fever and poor eyesight.

▲ **Figure 16.21**

β-oxidation of saturated fatty acids. One round of β-oxidation consists of four enzyme-catalyzed reactions. Each round generates one molecule each of QH_2, NADH, acetyl CoA, and a fatty acyl CoA molecule two carbon atoms shorter than the molecule that entered the round. (ETF is the electron-transferring flavoprotein, a water-soluble protein coenzyme.)
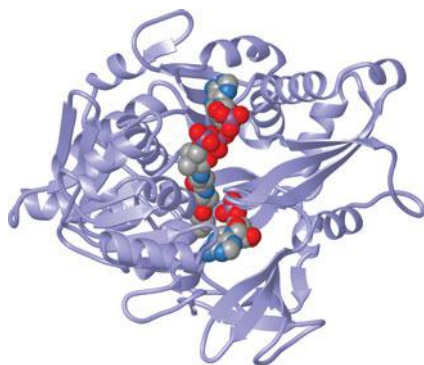

▲ **Human medium chain acyl-CoA synthetase.** The products of the reaction, AMP and acyl CoA, are bound in the active site. The enzyme is a dimer but only one subunit is shown. [PDB 3EQ6]

transferring flavoprotein (ETF, Figure 16.22). (ETF also accepts electrons from several other flavoproteins that are not involved in fatty acid metabolism.) Electrons are then passed to Q in a reaction catalyzed by ETF:ubiquinone oxidoreductase. This enzyme is embedded in the membrane and QH_2 from fatty acid oxidation enters the pool of QH_2 that can be oxidized by the membrane-associated electron transport system.

The second step is a *hydration* reaction. Water is added to the unsaturated *trans* 2-enoyl CoA produced in the first step to form the L isomer of 3-hydroxyacyl CoA. The enzyme is 2-enoyl-CoA hydratase.

The third step is a second *oxidation* catalyzed by L-3-hydroxyacyl-CoA dehydrogenase. This production of 3-ketoacyl CoA from 3-hydroxyacyl CoA is an $NAD^{\oplus}$-dependent reaction. The resulting reducing equivalents (NADH) can be used directly in biosynthesis pathways or they can be oxidized by the membrane-associated electron transport system.

Finally, in Step 4, the nucleophilic sulfhydryl group of HS–CoA attacks the carbonyl carbon of 3-ketoacyl CoA in a reaction catalyzed by 3-ketoacyl-CoA thiolase. This enzyme, also called thiolase II, is related to the acetoacyl-CoA thiolase (thiolase I) that we encountered
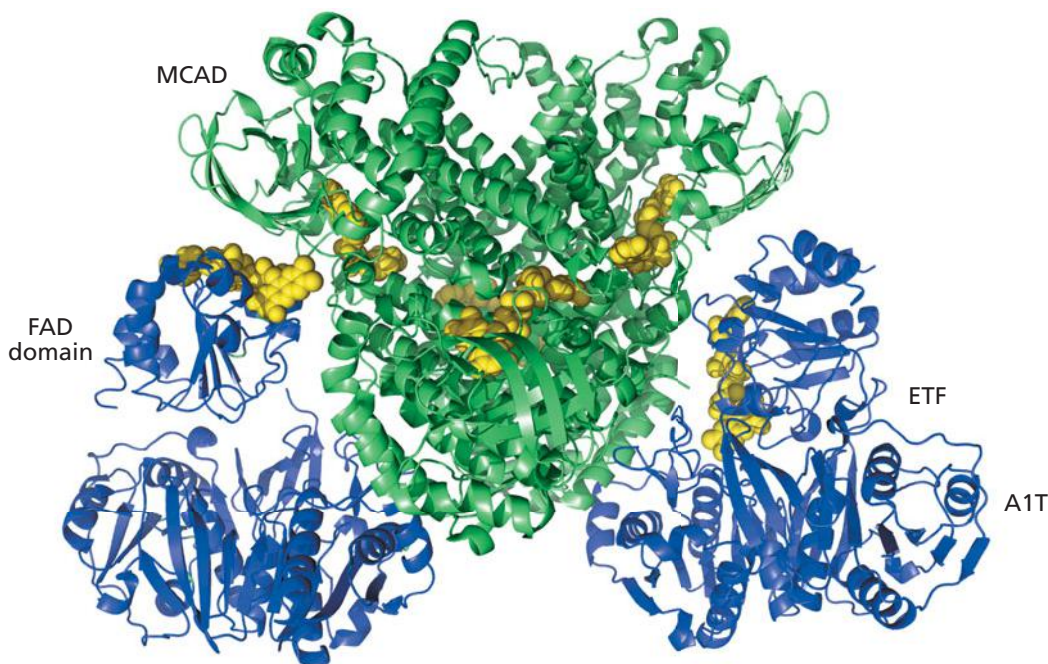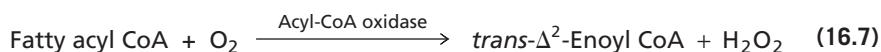
▲ **Figure 16.22**
Model of the medium chain acyl-CoA dehydrogenase (MCAD) bound to ETF. The MCAD subunits are colored green and the ETF subunits are colored blue. Bound FADs are represented as space-filling molecules (yellow). The model is based on the structure in PDB entry 2A1T containing a mutant protein that blocks movement of the FAD domain of ETF. The left-hand side of the dimer shows the probable position of the FAD domain during transfer of electrons from MCAD to ETF and the right-hand side shows the position of the FAD domain in free, unbound ETF. The flexibility of the FAD domain as it shifts from one position to another is responsible for its lack of resolution in the wild-type ETF: MCAD crystal structure. (Toogood et al., 2004; Toogood et al., 2005)

in the isopentenyl diphosphate pathway (Section 16.6A). Acetoacetyl-CoA thiolase is specific for acetoacetyl CoA, while 3-ketoacyl-CoA thiolase acts on long chain fatty acid derivatives. The release of acetyl CoA leaves a fatty acyl CoA molecule shortened by two carbons. This acyl CoA molecule is a substrate for another round of the four reactions and the metabolic spiral continues until the entire molecule has been converted to acetyl CoA.

As the fatty acyl chain becomes shorter, the first step is catalyzed by acyl-CoA dehydrogenase isozymes with preferences for shorter chains. Interestingly, the first three reactions of fatty acid oxidation are chemically parallel to three steps of the citric acid cycle. In these reactions, an ethylene group ($-CH_2CH_2-$, as in succinate) is oxidized to a two-carbon unit containing a carbonyl group ($-COCH_2-$, as in oxaloacetate). The steps are the reverse of the reactions in the fatty acid synthesis pathway (Section 16.1C).

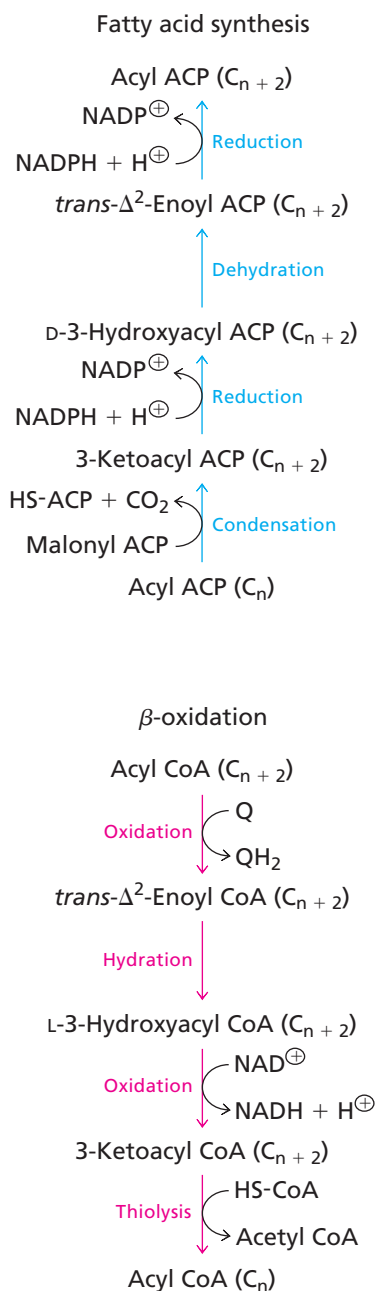In eukaryotes, fatty acid oxidation also occurs in peroxisomes. In fact, peroxisomes are the only site of fatty acid β-oxidation in most eukaryotes (but not mammals). In peroxisomes, the initial oxidation step is catalyzed by acyl-CoA oxidase—an enzyme that is homologous to the acyl-CoA dehydrogenase that catalyzes the first oxidation in mitochondria. The peroxisomal enzyme transfers electrons to O_2 to form hydrogen peroxide (H_2O_2).

$$\text{Fatty acyl CoA } + \text{ O}_2 \xrightarrow{\text{Acyl-CoA oxidase}} trans\text{-}\Delta^2\text{-Enoyl CoA } + \text{ H}_2\text{O}_2 \qquad \textbf{(16.7)}$$

In bacterial and mitochondrial β-oxidation the product of the first oxidation step is QH_2 that can be used in the respiratory electron transport chain. This results in synthesis of ATP—each QH_2 molecule is equivalent to 1.5 molecules of ATP (Section 14.11). There is no membrane-associated electron transport system in peroxisomes and this is why a different type of oxidation–reduction takes place in peroxisomes. It also means that fewer ATP equivalents are produced during peroxisomal β-oxidation. In mammals, where both mitochondrial and peroxisomal pathways exist, the peroxisomal β-oxidation pathway degrades very long chain fatty acids, branched fatty acids, long chain dicarboxylic acids, and possibly
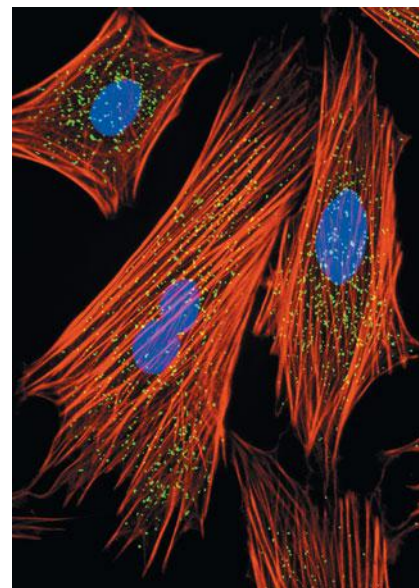
▲ **Peroxisomes**. Indian Muntjac (*Muntiacus muntjak*) fibroblast cells were stained with green reagent to show peroxisomes. Actin fibers are stained red and nuclear DNA is purple. The small peroxisomes are scattered throughout the cytoplasm. [http://www.microscopyu.com/staticgallery/fluorescence/muntjac.html]

Fatty acid synthesis

Acyl ACP (C_{n+2})

NADP$^\oplus$ ↖

NADPH + H$^\oplus$ ⤴ Reduction

trans-Δ^2-Enoyl ACP (C_{n+2})

↑ Dehydration

D-3-Hydroxyacyl ACP (C_{n+2})

NADP$^\oplus$ ↖

NADPH + H$^\oplus$ ⤴ Reduction

3-Ketoacyl ACP (C_{n+2})

HS-ACP + CO_2 ↖

Malonyl ACP ⤴ Condensation

Acyl ACP (C_n)

β-oxidation

Acyl CoA (C_{n+2})

Oxidation │ Q
          └→ QH_2

trans-Δ^2-Enoyl CoA (C_{n+2})

↓ Hydration

L-3-Hydroxyacyl CoA (C_{n+2})

Oxidation │ NAD$^\oplus$
          └→ NADH + H$^\oplus$

3-Ketoacyl CoA (C_{n+2})

Thiolysis │ HS-CoA
          └→ Acetyl CoA

Acyl CoA (C_n)

▲ **Figure 16.23**
Fatty acid synthesis and β-oxidation.

trans unsaturated fatty acids producing smaller, more polar compounds that can be excreted. Most of the common fatty acids are degraded in mitochondria.

C. Fatty Acid Synthesis and β-Oxidation

Fatty acid synthesis involves carbon-carbon bond formation (condensation) followed by reduction, dehydration, and reduction steps in preparation for the next condensation reaction. The reverse reactions—oxidation, hydration, oxidation, and carbon-carbon bond cleavage—are part of the degradation pathway of β-oxidation. We compare the two pathways in Figure 16.23.

The active thioesters in fatty acid oxidation are CoA derivatives whereas the intermediates in fatty acid synthesis are bound as thioesters to acyl carrier protein (ACP). In both cases, the acyl groups are attached to phosphopantetheine. Synthesis and degradation both proceed in two-carbon steps. However, oxidation results in a two-carbon product, acetyl CoA, whereas synthesis requires a three-carbon substrate, malonyl ACP that transfers a two-carbon unit to the growing chain releasing CO_2. Reducing power for synthesis is supplied by NADPH, whereas oxidation depends on NAD$^\oplus$ and ubiquinone (via the electron-transferring flavoprotein). Finally, the intermediate in fatty acid synthesis is D-3-hydroxyacyl-ACP whereas the L isomer (L-3-hydroxyacyl-CoA) is produced during β-oxidation.

The biosynthesis and catabolic pathways are catalyzed by a completely different set of enzymes and the intermediates form separate pools due to the fact that they are bound to different cofactors (CoA and ACP). In eukaryotic cells the two opposing pathways are physically separated. The biosynthesis enzymes are found in the cytosol and the β-oxidation enzymes are confined to mitochondria and peroxisomes.

D. Transport of Fatty Acyl CoA into Mitochondria

Long-chain fatty acyl CoA formed in the cytosol cannot diffuse across the inner mitochondrial membrane into the mitochondrial matrix where the reactions of β-oxidation occur in mammals. A transport system, called the carnitine shuttle system, actively transports fatty acids into mitochondria (Figure 16.24). In the cytosol, the acyl group of
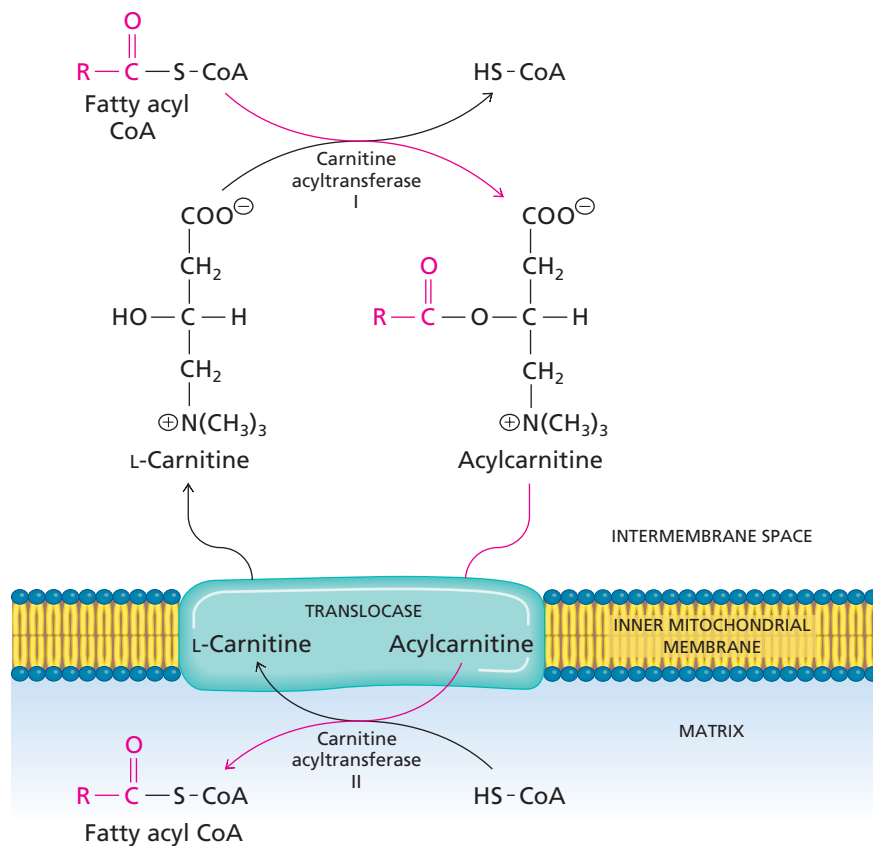
Figure 16.24 ►
Carnitine shuttle system for transporting fatty acyl CoA into the mitochondrial matrix. The path of the acyl group is traced in red.

BOX 16.4 A TRIFUNCTIONAL ENZYME FOR β-OXIDATION

Many species contain a trifunctional enzyme for β-oxidation. The 2-enoyl-CoA hydratase (ECH) and L-3-hydroxyacyl-CoA dehydrogenase (HACD) activities are located on a single polypeptide chain (α subunit). The 3-ketoacyl-CoA thiolase (KACT) activity is localized to the β subunit and the two subunits combine to form a protein with $\alpha_2\beta_2$ quaternary structure.

The structure of a bacterial enzyme is shown in the figure. During β-oxidation the product of the first reaction, *trans*-2-enoyl CoA, binds to the ECH site of the trifunctional enzyme. The substrate then undergoes the next three reactions within the cavity formed by the ECH, HACD, and KACT active sites in each half of the dimer. The two intermediates in the pathway are not released during these reactions because they are bound by their CoA termini. This is an example of metabolic channeling by a multienzyme complex.
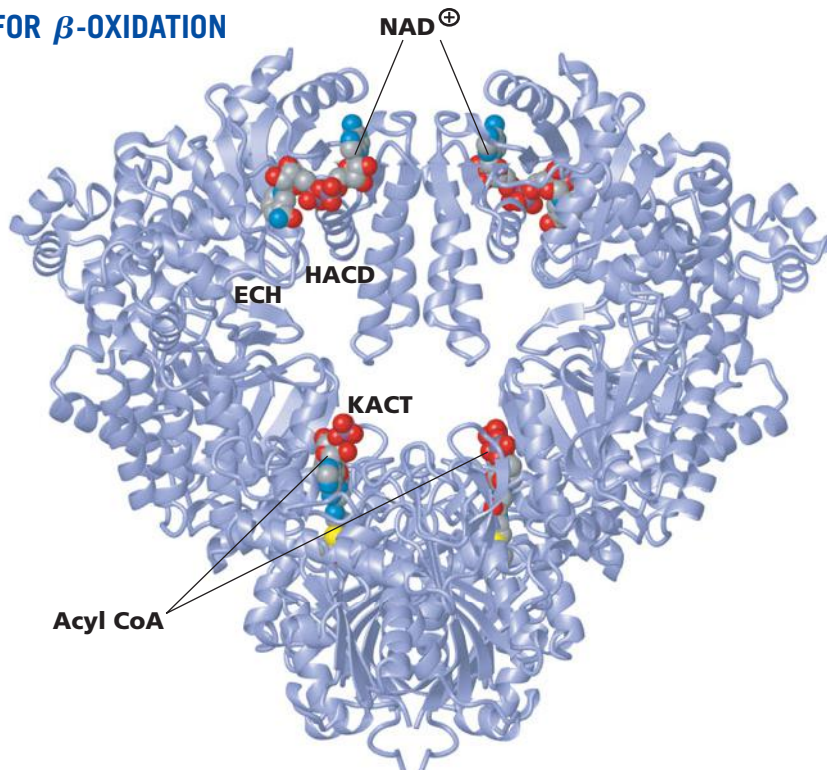
▲ **Structure of the fatty acid β-oxidation multienzyme complex from the bacterium *Pseudomonas fragi*.** In this structure a molecule of acyl CoA is bound at each of the KACT sites. [PDB 1WDK]

fatty acyl CoA is transferred to the hydroxyl group of carnitine to form acylcarnitine in a reaction catalyzed by carnitine acyltransferase I, also called carnitine palmitoyltransferase I (CPTI). The enzyme is associated with the outer membrane of the mitochondria.

This reaction is a key site for regulation of the oxidation of intracellular fatty acids. The acyl ester acylcarnitine is a "high energy" molecule with a free energy of hydrolysis similar to that of a thiol ester. Acylcarnitine then enters the mitochondrial matrix in exchange for free carnitine via the carnitine:acylcarnitine translocase. In the mitochondrial matrix, the isozyme carnitine acyltransferase II catalyzes the reverse of the reaction catalyzed by carnitine acyltransferase I. The effect of the carnitine shuttle system is to remove fatty acyl CoA from the cytosol and regenerate fatty acyl CoA in the mitochondrial matrix.

The carnitine shuttle system is not used in most eukaryotes since fatty acid oxidation takes place in the peroxisomes. Fatty acids are transported into peroxisomes by a different mechanisms of course, no transport mechanism is required in prokaryotes since all these reactions take place in the cytoplasm.

E. ATP Generation from Fatty Acid Oxidation

The complete oxidation of fatty acids supplies more energy than the oxidation of an equivalent amount of glucose. As is the case in glycolysis, the energy yield of fatty acid oxidation can be estimated from the total theoretical yield of ATP (Section 13.5). As an example, let's consider the balanced equation for the complete oxidation of one molecule of stearate (C_{18}) by eight cycles of β-oxidation. Stearate is converted to stearoyl CoA at a cost of two ATP equivalents and the oxidation of steroyl CoA yields acetyl CoA and the reduced coenzymes QH_2 and NADH.

$$\text{Stearoyl CoA} + 8\ \text{HS–CoA} + 8\ Q + 8\ \text{NAD}^{\oplus} \longrightarrow$$
$$9\ \text{Acetyl CoA} + 8\ QH_2 + 8\ \text{NADH} + 8\ H^{\oplus} \tag{16.8}$$

KEY CONCEPT

Unlike the pathways for gluconeogenesis and glycolysis, the pathways for the synthesis and degradation of fatty acids are completely different.

In Section 16.7D we compare the cost of fatty acid synthesis to the energy recovered in β-oxidation.

We can calculate the theoretical yield of 9 molecules of acetyl CoA by assuming that they enter the citric acid cycle where they are completely oxidized to CO_2. These reactions produce 10 ATP equivalents for each molecule of acetyl CoA. The net yield from oxidation of stearate is 120 ATP equivalents.

Eight cycles of β-oxidation yield

8 QH$_2$	$\approx$	12 ATP
8 NADH	$\approx$	20 ATP
9 molecules of acetyl CoA	$\approx$	90 ATP
activation of stearate	$\approx$	-2 ATP
Total	$=$	120 ATP

By comparison, the oxidation of glucose to CO_2 and water yields approximately 32 ATP molecules. Since stearate has 18 carbons and glucose has only six carbons, we normalize the yield of ATP from glucose by comparing the oxidation of three molecules of glucose: $3 \times 32 = 96$ ATP. This theoretical ATP yield is only 80% of the value for stearate. Fatty acids provide more energy per carbon atom than carbohydrates because carbohydrates are already partially oxidized. Furthermore, because fatty acid moieties are hydrophobic, they can be stored in large quantities as triacylglycerols without large amounts of bound water, as are found with carbohydrates. Anhydrous storage allows far more energy to be stored per gram.

We can also calculate the cost of synthesizing stearate in order to compare it to the energy recovered during β-oxidation. For this calculation we need to know the cost of synthesizing acetyl CoA from CO_2. This value (17 ATP equivalents) is obtained from the reactions of CO_2 fixation in plants (Section 15.4C).

8 acetyl CoA $\rightarrow$ 8 malonyl ACP		$\approx$	8 ATP
8 synthesis steps	16 NADPH	$\approx$	40 ATP
9 acetyl CoA	9×17	$\approx$	153 ATP
Total		$=$	201 ATP

The energy recovered in the degradation of stearate is about 60% (120/201) of the total theoretical energy required for its synthesis. This is a typical example of biochemical efficiency.
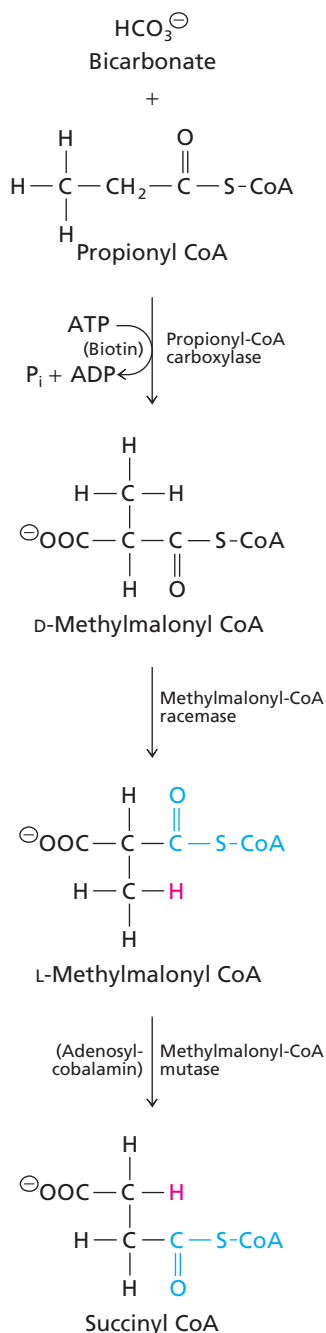
F. β-Oxidation of Odd-Chain and Unsaturated Fatty Acids

Most fatty acids have an even number of carbon atoms. Odd-chain fatty acids are synthesized by bacteria and by some other organisms. Odd-chain fatty acids are oxidized by the same sequence of reactions as even-chain fatty acids except that the product of the final thiolytic cleavage is propionyl CoA (CoA with a C_3 acyl group) rather than acetyl CoA (CoA with a C_2 acyl group). In mammals, propionyl CoA can be converted to succinyl CoA in a three step pathway (Figure 16.25).

The first reaction is catalyzed by propionyl-CoA carboxylase, a biotin-dependent enzyme that incorporates bicarbonate into propionyl CoA to produce D-methylmalonyl CoA. Methylmalonyl-CoA racemase catalyzes the conversion of D-methylmalonyl CoA to its L isomer. Finally, methylmalonyl-CoA mutase catalyzes the formation of succinyl CoA.

Methylmalonyl-CoA mutase is one of the few enzymes that require adenosylcobalamin as a cofactor. We learned in Section 7.12 that adenosylcobalamin-dependent enzymes catalyze intramolecular rearrangements in which a hydrogen atom and a substituent on an adjacent carbon atom exchange places. In the reaction catalyzed by methylmalonyl-CoA mutase, the $-C(O)-S$-CoA group exchanges with a hydrogen atom of a methyl group (Figure 7.28).

The succinyl CoA molecule formed by the action of methylmalonyl-CoA mutase is metabolized to oxaloacetate. Since oxaloacetate is a substrate for gluconeogenesis, the

▲ **Figure 16.25**
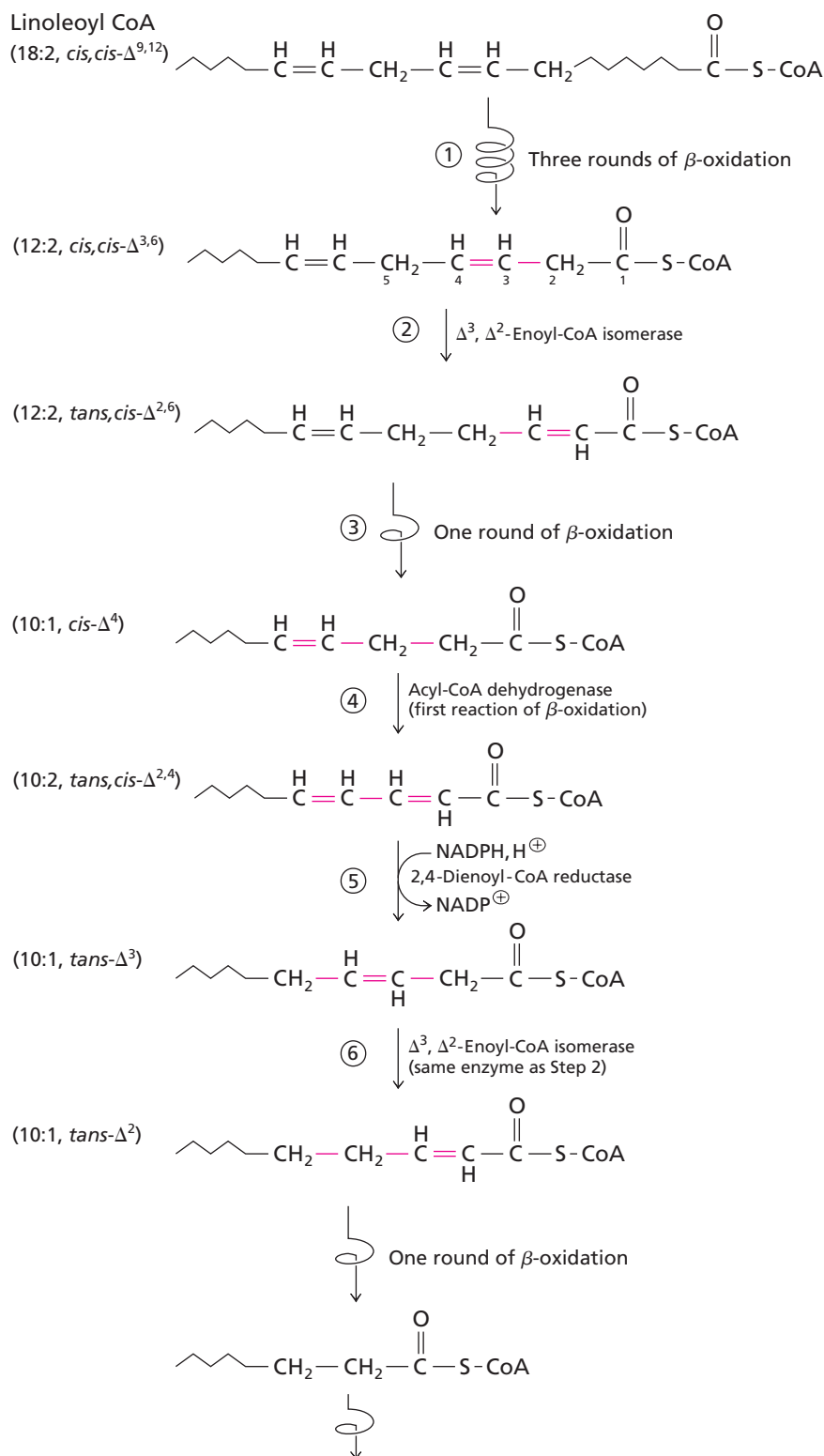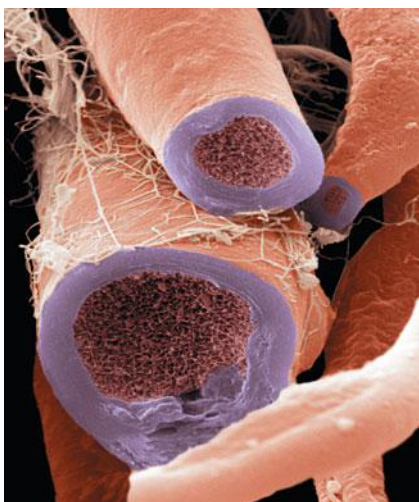**Conversion of propionyl CoA to succinyl CoA.**

Linoleoyl CoA
(18:2, cis,cis-$\Delta^{9,12}$)

① Three rounds of β-oxidation

(12:2, cis,cis-$\Delta^{3,6}$)

② Δ^3, Δ^2-Enoyl-CoA isomerase

(12:2, tans,cis-$\Delta^{2,6}$)

③ One round of β-oxidation

(10:1, cis-Δ^4)

④ Acyl-CoA dehydrogenase
(first reaction of β-oxidation)

(10:2, tans,cis-$\Delta^{2,4}$)

⑤ NADPH, H$^{\oplus}$
2,4-Dienoyl-CoA reductase
NADP$^{\oplus}$

(10:1, tans-Δ^3)

⑥ Δ^3, Δ^2-Enoyl-CoA isomerase
(same enzyme as Step 2)

(10:1, tans-Δ^2)

One round of β-oxidation

propionyl group derived from the β-oxidation of an odd-chain fatty acid can be con-
verted to glucose.

The oxidation of unsaturated fatty acids requires two enzymes in addition to those
usually needed for the oxidation of saturated fatty acids. The oxidation of the Coenzyme A
derivative of linoleate (18:2 cis,cis $\Delta^{9,12}$-octadecadienoate) illustrates the modified path-
way (Figure 16.26).
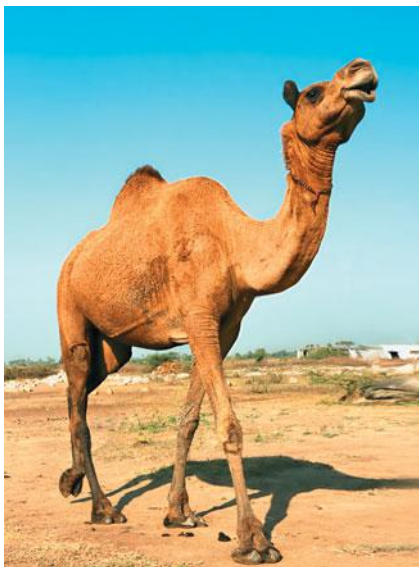
▲ **The camel's hump stores fat for energy production when food is scarce.** The hump of the camel contains fat that is used to supply energy. It does not store water. The ability of camels to go for long periods of time without water is due to completely different adaptations having nothing to do with fat metabolism. The camel shown here is the Arabian camel or dromedary, *Camelus dromedarius*.

Like all polyunsaturated fatty acids linoleoyl CoA has both odd-numbered and even-numbered double bonds (its double bonds are separated by a methylene group). Unsaturated fatty acids are normal substrates for the enzymes of the β-oxidation pathway until an odd-numbered double bond of the shortened fatty acid chain interferes with catalysis. In this example, three rounds of β-oxidation convert linoleoyl CoA to the C_{12} molecule 12:2 *cis,cis*-$\Delta^{3,6}$-dienoyl CoA (step 1). This molecule has a *cis*-3,4 double bond rather than the usual *trans*-2,3 double bond that would be produced during β-oxidation of saturated fatty acids. The *cis*-3,4 intermediate is not a substrate for 2-enoyl-CoA hydratase since the normal β-oxidation enzyme is specific for *trans* acyl CoAs and, in addition, the double bond is in the wrong position for hydration.

The inappropriate double bond is rearranged from Δ^3 to Δ^2 to produce the C_{12} molecule 12:2 *trans,cis*-$\Delta^{2,6}$-dienoyl CoA in a reaction catalyzed by Δ^3,Δ^2-enoyl-CoA isomerase (step 2). This product can re-enter the β-oxidation pathway and another round of β-oxidation can be completed resulting in the C_{10} molecule 10:1 *cis*-Δ^4-enoyl CoA (step 3). The first enzyme of the β-oxidation pathway, acyl-CoA dehydrogenase, acts on this compound, producing the C_{10} molecule 10:2 *trans,cis*-$\Delta^{2,4}$-dienoyl CoA. This resonance-stabilized diene resists hydration. 2,4-Dienoyl-CoA reductase catalyzes the NADPH-dependent reduction of the diene (step 5) to produce a C_{10} molecule with a single double bond (10:1 *trans*-Δ^3-enoyl CoA). This product (like the substrate in step 2) is acted on by Δ^3,Δ^2-enoyl-CoA isomerase to produce a compound that continues through the β-oxidation pathway. Note that the isomerase can convert both *cis*-Δ^3 and *trans*-Δ^3 double bonds to the *trans*-Δ^2 intermediate.

The oxidation of a monounsaturated fatty acid with a *cis* double bond at an odd-numbered carbon (e.g., oleate) requires the activity of the isomerase but not the reductase, in addition to the enzymes of β-oxidation. Oleoyl (18:1 *cis*-Δ^3) CoA undergoes three cycles of β-oxidation, forming three molecules of acetyl CoA and the CoA ester of the (12:1 *cis*-Δ^3) acid. Δ^3,Δ^2-Enoyl-CoA isomerase then catalyzes conversion of the 12-carbon enoyl CoA to a 12-carbon *trans*-Δ^2 enoyl CoA, which can undergo β-oxidation.

16.8 Eukaryotic Lipids Are Made at a Variety of Sites

Eukaryotic cells are highly compartmentalized. The compartments can have quite different functions, and their surrounding membranes can have quite distinct phospholipid and fatty acyl constituents. Most lipid biosynthesis in eukaryotic cells occurs in the endoplasmic reticulum. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine, for example, are all synthesized in the ER. The biosynthesis enzymes are membrane bound with their active sites oriented toward the cytosol so that they have access to the water-soluble cytosolic compounds. The major phospholipids are incorporated into the ER membrane. From there they are transported to other membranes in the cell in vesicles that travel between the endoplasmic reticulum and Golgi apparatus and between the Golgi apparatus and various membrane target sites. Soluble transport proteins also participate in carrying phospholipids and cholesterol to other membranes.

Although the endoplasmic reticulum is the principal site of lipid metabolism in the cell, there are also lipid-metabolizing enzymes at other locations. For instance, membrane lipids can be tailored to give the lipid profile characteristic of individual cellular organelles. In the plasma membrane, acyltransferase activities catalyze the acylation of lysophospholipids. Mitochondria have the enzyme phosphatidylserine decarboxylase that catalyzes the conversion of phosphatidylserine to phosphatidylethanolamine. Mitochondria also contain the enzymes responsible for the synthesis of diphosphatidylglycerol (cardiolipin, Table 9.2), a molecule found uniquely in the inner membrane of the mitochondrion. Lysosomes possess various hydrolases that degrade phospholipids and sphingolipids. Peroxisomes possess enzymes involved in the early stages of ether

▲ **Myelin sheath.** These nerve fibers are coated with several layers of myelin membranes (colored purple) forming a protective sheath around the axons. Plasmalogens are important components of myelin membranes. The symptoms of multiple sclerosis (MS) are caused by degradation of myelin in the brain and spinal cord leading to loss of motor control.

lipid synthesis. Defects in peroxisomal formation can lead to poor plasmalogen synthesis, with potentially fatal consequences.

The tissues of the central nervous system are especially prone to damage. In those tissues plasmalogens constitute a substantial portion of the lipids of the myelin sheath. Often, different subcellular locations have a different set of enzymes (isozymes) responsible for the biosynthesis of different, segregated pools of lipids, with each pool having its own biological function.

16.9 Lipid Metabolism is Regulated by Hormones in Mammals

Fatty acids are no longer oxidized in mitochondria when the energy supply is sufficient to meet the immediate needs of an organism. Instead, they are transported to adipose tissue where they are stored for future use when energy is needed (e.g., lack of food). This aspect of lipid metabolism is similar to the strategy in carbohydrate metabolism where excess glucose is stored in specialized cells as glycogen (animals) or starch (plants).

The mobilization and storage of lipids requires communication between different tissues. Hormones that circulate in the blood are ideally suited to act as signals between cells. Lipid metabolism must be coordinated with carbohydrate metabolism, so it's not surprising that the same hormones also affect the synthesis, degradation, and storage of carbohydrates.

Glucagon, epinephrine, and insulin are the principal hormonal regulators of fatty acid metabolism. Glucagon and epinephrine are present in high concentrations in the fasted state and insulin is present in high concentrations in the fed state. The concentration of circulating glucose must be maintained within fairly narrow limits at all times. In the fasted state, carbohydrate stores become depleted and synthesis of carbohydrates must occur to maintain the level of glucose in the blood. To further relieve pressure on the limited supply of glucose, fatty acids are mobilized to serve as fuel, and many tissues undergo regulatory transitions that decrease their use of carbohydrates and increase their use of fatty acids. The opposite occurs in the fed state when carbohydrates are used as fuel and precursors for fatty acid synthesis.

The key regulatory enzyme for fatty acid synthesis is acetyl-CoA carboxylase. High insulin levels after a meal inhibit the hydrolysis of stored triacylglycerols and stimulate the formation of malonyl CoA by acetyl-CoA carboxylase. Malonyl CoA allosterically inhibits carnitine acyltransferase I. As a result, fatty acids remain in the cytosol rather than being transported into mitochondria for oxidation. Regulation of fatty acid synthesis and degradation is reciprocal, with increased metabolism by one pathway balanced by decreased activity in the opposing pathway. In animals this regulation is achieved by hormones that indirectly affect the activities of the enzymes.

Triacylglycerols are delivered to adipose tissue in the form of lipoproteins that circulate in blood plasma (Section 16.10B). When they arrive at adipose tissue the triglycerols are hydrolyzed to release fatty acids and glycerol that are then taken up by adipocytes. Hydrolysis is catalyzed by lipoprotein lipase (LPL), an extracellular enzyme bound to endothelial cells of the capillaries of adipose tissue. Following entry into adipocytes, the fatty acids are re-esterified for storage as triacylglycerols.

Subsequent mobilization, or release, of fatty acids from adipocytes depends on metabolic needs. A hormone-sensitive lipase in adipocytes catalyzes the hydrolysis of triacylglycerols to free fatty acids and monoacylglycerols. Although hormone-sensitive lipase can also catalyze the conversion of monoacylglycerols to glycerol and free fatty acids, a more specific and more active monoacylglycerol lipase probably accounts for most of this catalytic activity.

The hydrolysis of triacylglycerols is inhibited in the fed state by high concentrations of insulin. When carbohydrate stores are depleted and insulin concentrations are low, an increased concentration of epinephrine stimulates triacylglycerol hydrolysis. Epinephrine binds to the β-adrenergic receptors of adipocytes leading to activation of the
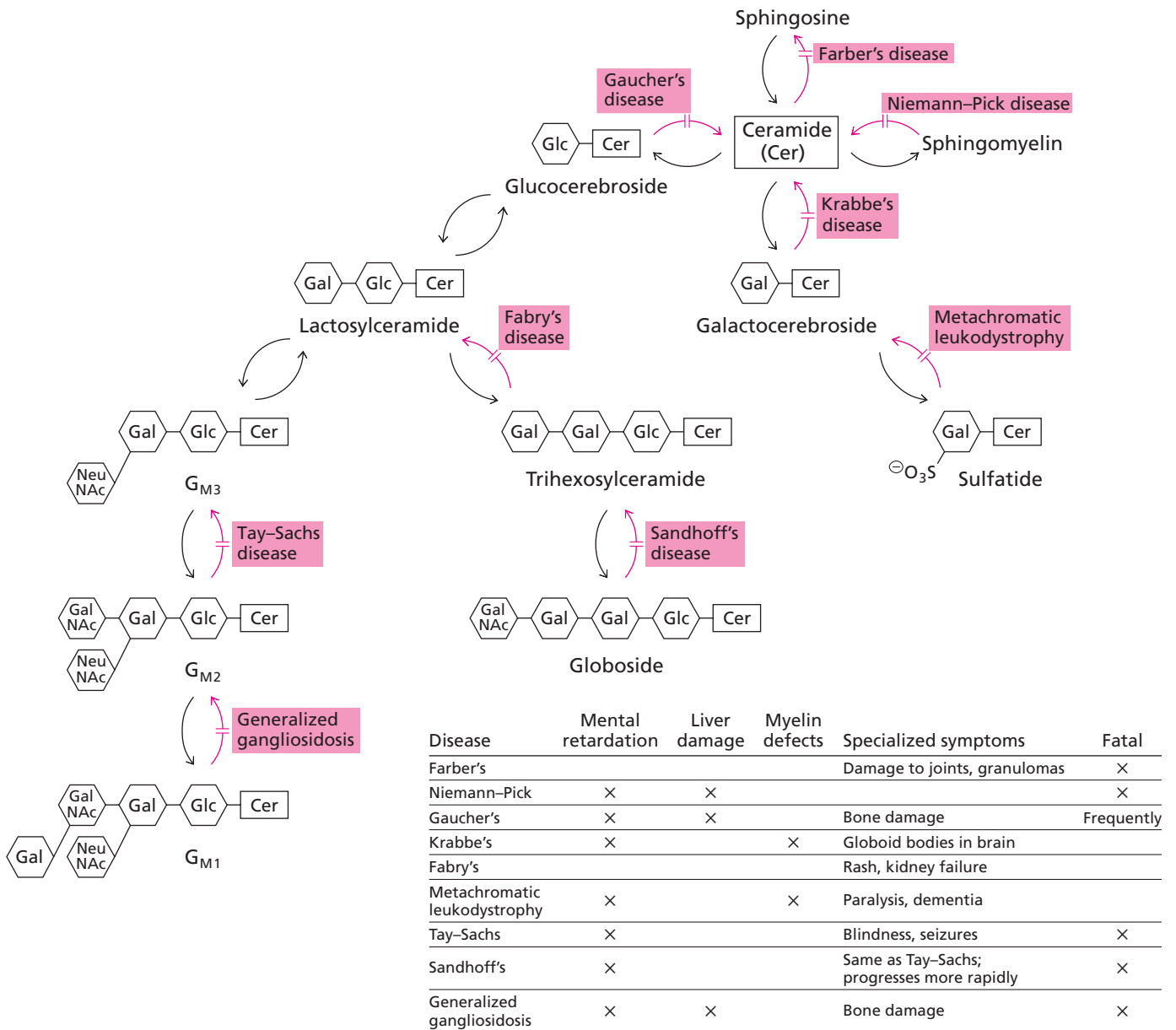
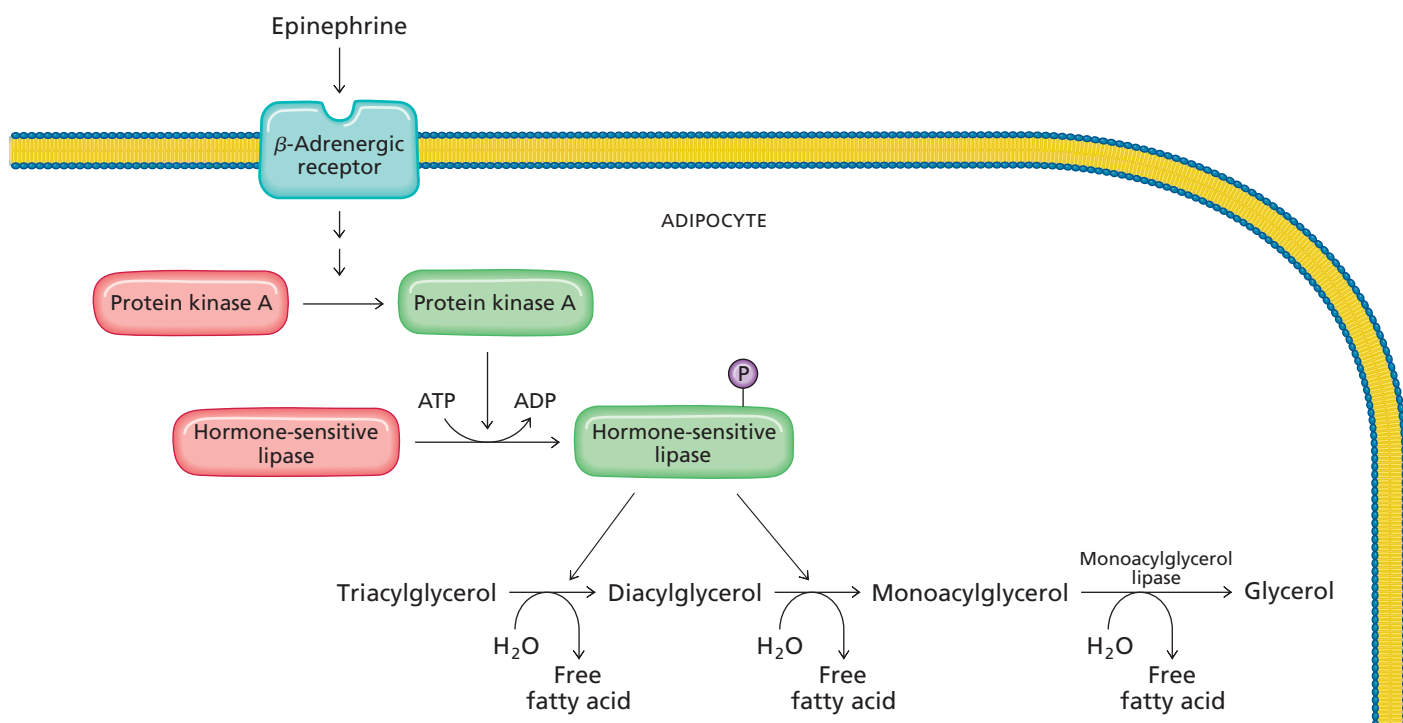**Hormone signaling pathways are described in Section 9.12.**

BOX 16.5 LYSOSOMAL STORAGE DISEASES

There are no metabolic diseases associated with defects in the sphingolipid biosynthesis pathways. It is likely that mutations in the genes for biosynthesis enzymes are lethal since sphingolipids are essential membrane components. In contrast, defects in the sphingolipid degradation pathway can have serious clinical consequences. Sphingolipid catabolism is largely carried out in the lysosomes of cells. Lysosomes contain a variety of glycosidases that catalyze the stepwise hydrolytic removal of sugars from the oligosaccharide chains of sphingolipids. There are certain inborn errors of metabolism in which a genetic defect leads to a deficiency in a particular degradative lysosomal enzyme resulting in lysosomal storage diseases. The accumulation of nondegradable lipid by-products can cause lysosomes to swell leading to cellular and ultimately tissue enlargement. This is particularly deleterious in central nervous tissue that has little room for expansion. Swollen lysosomes accumulate in the cell bodies of nerve cells and lead to neuronal death, possibly by leakage of lysosomal enzymes into the cell. As a result, blindness, mental retardation, and death can occur. In Tay–Sachs disease, for instance, there is a deficiency in hexosaminidase A, which catalyzes the removal of N-acetylgalactosamine from the oligosaccharide chain of gangliosides. If removal of this sugar does not occur, the disassembly of gangliosides is blocked, leading to a buildup of the nondegradable by-product, ganglioside G_{M2}. (The complete structure of ganglioside G_{M2} is shown in Figure 9.12.)

Schematic pathways for the formation and degradation of a variety of sphingolipids are shown in the accompanying figure. A number of defects in sphingolipid metabolism, whose clinical manifestations are termed *sphingolipidoses*, are identified there.

Disease	Mental retardation	Liver damage	Myelin defects	Specialized symptoms	Fatal
Farber's				Damage to joints, granulomas	×
Niemann–Pick	×	×			×
Gaucher's	×	×		Bone damage	Frequently
Krabbe's	×		×	Globoid bodies in brain	
Fabry's				Rash, kidney failure	
Metachromatic leukodystrophy	×		×	Paralysis, dementia	
Tay–Sachs	×			Blindness, seizures	×
Sandhoff's	×			Same as Tay–Sachs; progresses more rapidly	×
Generalized gangliosidosis	×	×		Bone damage	×

▲ **Figure 16.27**
Triacylglycerol degradation in adipocytes. Epinephrine initiates the activation of protein kinase A, which catalyzes the phosphorylation and activation of hormone-sensitive lipase. The lipase catalyzes the hydrolysis of triacylglycerols to monoacylglycerols and free fatty acids. The hydrolysis of monoacylglycerols is catalyzed by monoacylglycerol lipase.
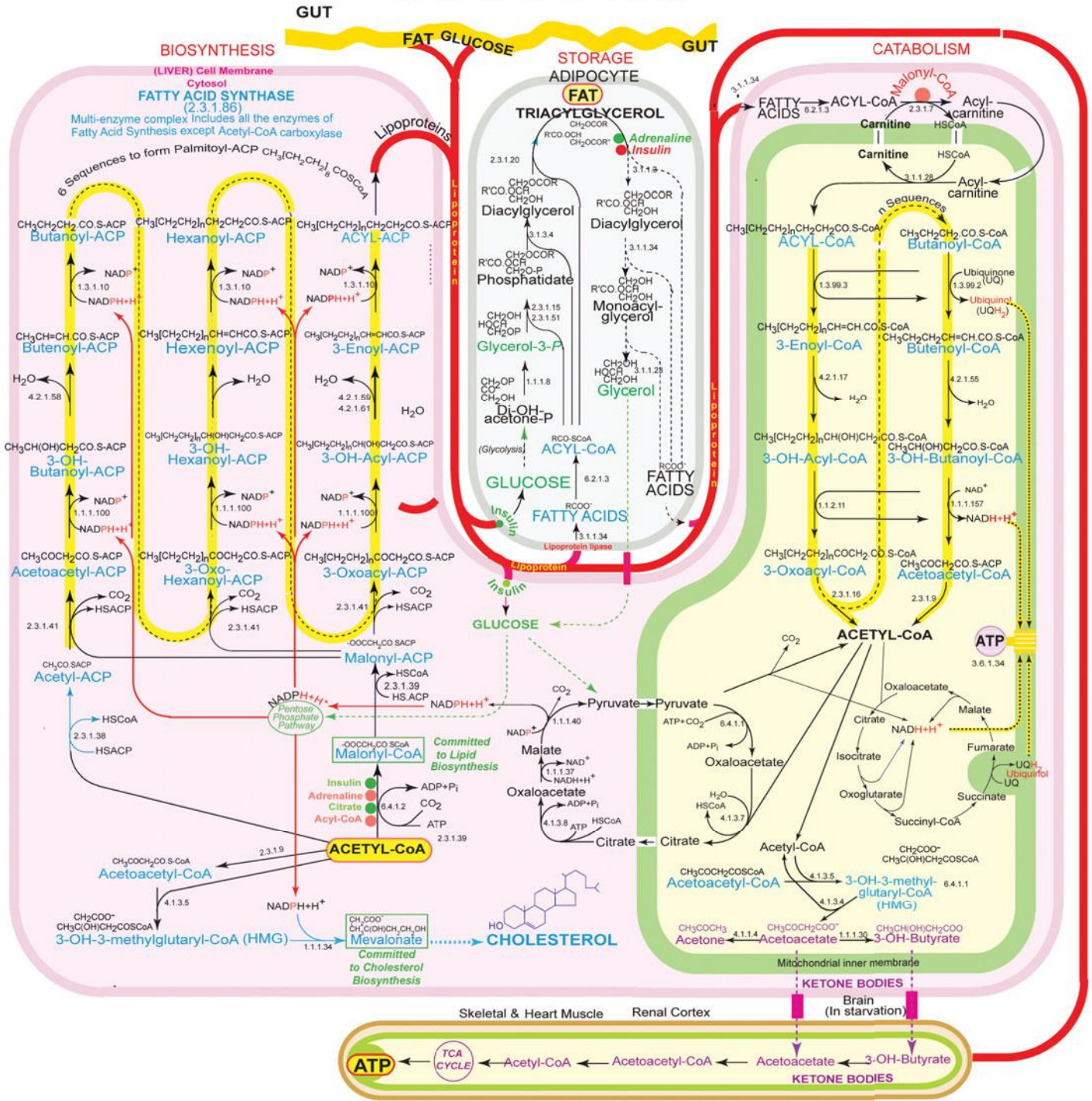
cAMP-dependent protein kinase A. Protein kinase A catalyzes the phosphorylation and activation of hormone-sensitive lipase (Figure 16.27).

Glycerol and free fatty acids diffuse through the adipocyte plasma membrane and enter the bloodstream. Glycerol is metabolized by the liver, where most of it is converted to glucose via gluconeogenesis. Free fatty acids are poorly soluble in aqueous solution and travel through blood bound to serum albumin (Section 16.9C). Fatty acids are carried to tissues such as heart, skeletal muscle, and liver, where they are oxidized in mitochondria to release energy. Fatty acids are a major source of energy during the fasting state (e.g., while we sleep).

At the same time, an increase in glucagon levels inactivates acetyl-CoA carboxylase, the enzyme that catalyzes the synthesis of malonyl CoA in the liver. The result is increased transport of fatty acids into mitochondria and greater flux through the β-oxidation pathway. The high concentrations of acetyl CoA and NADH that are produced by fatty acid oxidation decrease glucose and pyruvate oxidation by inhibiting the pyruvate dehydrogenase complex. Thus, not only are fatty acid oxidation and storage reciprocally regulated but fatty acid metabolism is also regulated so that storage is favored in times of plenty (such as immediately after feeding) and fatty acid oxidation proceeds when glucose must be spared.

Citrate—a precursor of cytosolic acetyl CoA—activates acetyl-CoA carboxylase *in vitro*, but the physiological relevance of this activation has not been fully established. Acetyl-CoA carboxylase is inhibited by fatty acyl CoA. The ability of fatty acid derivatives to regulate acetyl-CoA carboxylase is physiologically appropriate; an increased concentration of fatty acids causes a decrease in the rate of the first committed step of fatty acid synthesis. Acetyl CoA-carboxylase activity is also under hormonal control. Glucagon stimulates phosphorylation and concomitant inactivation of the enzyme in the liver, and epinephrine stimulates its inactivation by phosphorylation in adipocytes. Several protein kinases can catalyze phosphorylation and thus inhibition of acetyl-CoA carboxylase. The action of AMP activated protein kinase inactivates both fatty acid synthesis (by inhibiting the acetyl-CoA carboxylase step) and steroid synthesis in the presence of a high AMP/ATP ratio.

LIPID METABOLISM

GUT

FAT GLUCOSE

GUT

BIOSYNTHESIS

CATABOLISM

STORAGE

(LIVER) Cell Membrane
Cytosol

FATTY ACID SYNTHASE
(2.3.1.86)
Multi-enzyme complex Includes all the enzymes of
Fatty Acid Synthesis except Acetyl-CoA carboxylase

6 Sequences to form Palmitoyl-ACP $CH_3[CH_2CH_2]_8$COSCoA

Lipoproteins

ADIPOCYTE

FAT

TRIACYLGLYCEROL

Adrenaline
Insulin

$CH_3CH_2CH_2.CO.S-ACP$
Butanoyl-ACP

$CH_3[CH_2CH_2]_nCH_2CH_2CO.S-ACP$
Hexanoyl-ACP

ACYL-ACP

$3.1.1.34$

FATTY
ACIDS

ACYL-CoA $6.2.1.3$

Malonyl-CoA

Acyl-
carnitine
$2.3.1.7$

HSCoA

Carnitine

Carnitine

HSCoA

NADP$^+$
$1.3.1.10$
NADPH+H$^+$

NADP$^+$
$1.3.1.10$
NADPH+H$^+$

NADP$^+$
$1.3.1.10$
NADPH+H$^+$

$3.1.1.28$

Acyl-
carnitine

$CH_3[CH_2CH_2]_nCH_2CH_2CO.S-CoA$
ACYL-CoA

n Sequences

$CH_3CH_2CH_2.CO.S-CoA$
Butanoyl-CoA

$CH_3CH=CH.CO.S-ACP$
Butenoyl-ACP

$CH_3[CH_2CH_2]_nCH=CHCO.S-ACP$
Hexenoyl-ACP

$CH_3[CH_2CH_2]_nCH=CHCO.S-ACP$
3-Enoyl-ACP

$1.3.99.3$

Ubiquinone (UQ)
$1.3.99.2$
Ubiquinol (UQH$_2$)

H_2O
$4.2.1.58$

H_2O

H_2O
$4.2.1.59$
$4.2.1.61$

H_2O

$CH_3[CH_2CH_2]_nCH=CH.CO.S-CoA$
3-Enoyl-CoA

$CH_3CH_2CH_2.CH=CH.CO.S-CoA$
Butenoyl-CoA

$4.2.1.17$
H_2O

$4.2.1.55$
H_2O

$CH_3CH(OH)CH_2CO.S-ACP$
3-OH-Butanoyl-ACP

$CH_3[CH_2CH_2]_nCH(OH)CH_2CO.S-ACP$
3-OH-Hexanoyl-ACP

$CH_3[CH_2CH_2]_nCH(OH)CH_2CO.S-ACP$
3-OH-Acyl-ACP

$CH_3[CH_2CH_2]_nCH(OH)CH_2CO.S-CoA$
3-OH-Acyl-CoA

$CH_3CH(OH)CH_2CO.S-CoA$
3-OH-Butanoyl-CoA

NADP$^+$
$1.1.1.100$
NADPH+H$^+$

NADP$^+$
$1.1.1.100$
NADPH+H$^+$

NADP$^+$
$1.1.1.100$
NADPH+H$^+$

$1.1.2.11$

NAD$^+$
$1.1.1.157$
NADH+H$^+$

$CH_3COCH_2CO.S-ACP$
Acetoacetyl-ACP

$CH_3[CH_2CH_2]_nCOCH_2CO.S-ACP$
3-Oxo-Hexanoyl-ACP

$CH_3[CH_2CH_2]_nCOCH_2CO.S-ACP$
3-Oxoacyl-ACP

$CH_3[CH_2CH_2]_nCOCH_2.CO.S-CoA$
3-Oxoacyl-CoA

$CH_3COCH_2CO.S-ACP$
Acetoacetyl-CoA

ATP
$3.6.1.34$

CO_2
HSACP

CO_2
HSACP

$2.3.1.41$

CO_2
HSACP

$2.3.1.16$

$2.3.1.9$

$2.3.1.41$

$2.3.1.41$

$CH_2CO.SACP$
Acetyl-ACP

$-OOCCH_2.CO.SACP$
Malonyl-ACP

HSCoA
$2.3.1.39$
HS.ACP

NADPH+H$^+$
Pentose
Phosphate
Pathway

NADPH+H$^+$

CO_2

Pyruvate Pyruvate

ACETYL-CoA

CO_2

Oxaloacetate

Malate

HSCoA

$2.3.1.38$

HSACP

$-OOCCH_2.CO.SCoA$
Malonyl-CoA

Committed to Lipid Biosynthesis

NADP$^+$
$1.1.1.40$

$ATP+CO_2$
$6.4.1.1$

$ADP+Pi$

Citrate

Isocitrate

NADH+H$^+$

Malate

Fumarate

UQH$_2$
Ubiquinol
UQ

Insulin
Adrenaline
Citrate
Acyl-CoA

ADP+Pi
$6.4.1.2$
CO_2
ATP

$2.3.1.39$

Malate
NAD$^+$
$1.1.1.37$
NADH+H$^+$

Oxaloacetate

H_2O
HSCoA
$4.1.3.7$

Oxaloacetate

Oxoglutarate

Succinate

Succinyl-CoA

$2.3.1.9$

$CH_3COCH_2CO.S-CoA$
Acetoacetyl-CoA

ACETYL-CoA

ADP+Pi
$4.1.3.8$ ATP HSCoA

Citrate Citrate

Acetyl-CoA

$CH_3COCH_2COSCoA$
Acetoacetyl-CoA

CH_2COO^-
$CH_3C(OH)CH_2COSCoA$
3-OH-3-methyl-glutaryl-CoA (HMG)

$6.4.1.1$

NADPH+H$^+$

$4.1.3.5$

CH_2COO^-
$CH_3C(OH)CH_2COSCoA$
3-OH-3-methylglutaryl-CoA (HMG)

CH_2COO^-
$CH_2C(OH)CH_3.CH_2OH$
Mevalonate

Committed to Cholesterol Biosynthesis

HO **CHOLESTEROL**

$1.1.1.34$

$4.1.3.5$
$4.1.3.4$

CH_3COCH_3
Acetone

$4.1.1.4$

$CH_3COCH_2COO^-$
Acetoacetate

$1.1.1.30$

$CH_3CH(OH)CH_2COO^-$
3-OH-Butyrate

Mitochondrial inner membrane

KETONE BODIES

Brain
(In starvation)

Skeletal & Heart Muscle   Renal Cortex

ATP   TCA CYCLE   Acetyl-CoA ← Acetoacetyl-CoA ← Acetoacetate ← 3-OH-Butyrate

KETONE BODIES

GLUCOSE

Insulin

Lipoprotein lipase

FATTY ACIDS

GLUCOSE

Diacylglycerol

Phosphatidate

Glycerol-3-P

Di-OH-acetone-P

(Glycolysis)

GLUCOSE

Diacylglycerol

Monoacyl-glycerol

Glycerol

ACYL-CoA

FATTY ACIDS

ANABOLISM $8CH_3CO.SCoA + 14NADPH + 14H^+ + 7ATP \longrightarrow CH_3[CH_2CH_2]_7CO.SCoA + 14NADP^+ + 7HSCoA + 7ADP + 7P_i$
Acetyl-CoA   Palmitoyl-CoA

CATABOLISM $CH_3[CH_2CH_2]_7CO.SCoA + 7Ubiquinone + 7H_2O + 7NAD^+ + 7HSCoA \longrightarrow 8CH_3CO.SCoA + 7\ Ubiquinol + 7NADH + 7H^+$
Palmitoyl-CoA   Acetyl-CoA

COMPLETE (AEROBIC) OXIDATION OF PALMITOYL CoA

$CH_3[CH_2CH_2]_7CO.SCoA + 23O_2 + \sim (106\ ADP + 106P_i) \longrightarrow 16CO_2 + 119\ H_2O + HSCoA + \sim106ATP$

This is a fascinating equation which explains how some animals, such as camels and polar bears can survive in the most adverse environments
They can use fat, not only as the sole source of energy, but also of water. The killer whale cannot utilise sea-water but creates its own from fat

ENZYMES

1.1.1.8	Glycerol-3-P-dehydrogenase	1.3.1.10	Enoyl-[ACP]-reductase	2.3.1.51	1-Acylglycerol-3-P O-acyl
1.1.1.34	HMG-CoA reductase	1.3.99.2	Butyryl-CoA dehydrogenase		transferase
1.1.1.35	3-OH-acyl-CoA dehydrogenase	1.3.99.3	Acyl-CoA dehydrogenase	3.1.1.3	Triacylglycerol lipase
1.1.1.37	Malatedehydrogenase	2.3.1.7	Carnitine-O-acyltransferase	3.1.1.23	Acylglycerol lipase
1.1.1.40	Malatedehydrogenase	2.3.1.9	Acetyl-CoA-C-acetyl transferase	3.1.1.28	Acylcarnitine hydrolase
	(oxaloacetate	2.3.1.15	Glycerol-3-P O-acyl transferase	3.1.1.34	Lipoprotein lipase
1.1.1.100	3-Oxoacyl-[ACP]	2.3.1.16	Acetyl-CoA C-acyltransferase	3.1.3.4	Phosphatidate phosphatase
1.1.1.157	3-OH-butyryl-CoA	2.3.1.20	DiacylglycerolO-acyltransferase	4.1.1.4	Acetoacetate decarboxylase
1.1.1.211	Long-chain 3-OH-acyl-CoA	2.3.1.38	[ACP] S-acyl transferase	4.1.1.9	Malonyl-CoA decarboxylase
1.2.4.1	Pyruvatedehydrogenase	2.3.1.39	[ACP] S-malonyl transferase	4.1.3.4	OH-Methylglutaryl-CoA lyase
				4.1.3.5	OH-Methylglutaryl-CoA synthase
				4.1.3.7	Citrate synthase
				4.1.3.8	ATP Citrate lyase
				4.2.1.17	Enoyl-CoA hydratase
				4.2.1.55	3-OH-Butyryl-CoA dehydratase
				4.2.1.58	Crotonyl-[ACP] hydratase
				4.2.1.59	3-OH-octanoyl-[ACP] dehydratase
				4.2.1.61	3-OH-Palmitoyl-[ACP] dehydratase
				6.2.1.3	Long-chain-fatty.-acid-CoA ligase
				6.4.1.1	Pyruvate carboxylase
				6.4.1.2	Acetyl-CoA carboxylase

504

16.10 Absorption and Mobilization of Fuel Lipids

The fatty acids and glycerol that mammals use as metabolic fuels are obtained from triacylglycerols in the diet and from adipocytes. The fats stored in adipocytes include fats synthesized from the catabolism of carbohydrates and amino acids. Free fatty acids occur only in trace amounts in cells—this is fortunate because, as anions, they are detergents and at high concentrations could disrupt cell membranes. We begin our study of lipid metabolism by examining the dietary uptake, transport, and mobilization of fatty acids in mammals.

A. Absorption of Dietary Lipids

Most lipids in the diets of mammals are triacylglycerols with smaller amounts of phospholipids and cholesterol. The digestion of dietary lipids occurs mainly in the small intestine, where suspended fat particles are coated with bile salts (Figure 16.28). Bile salts are amphipathic cholesterol derivatives synthesized in the liver, collected in the gallbladder, and secreted into the lumen of the intestine. Micelles of bile salts solubilize fatty acids and monoacylglycerols so that they can diffuse to and be absorbed by the cells of the intestinal wall. Lipids are transported through the body as complexes of lipid and protein known as lipoproteins.

Triacylglycerols are broken down in the small intestine by the action of lipases. These enzymes are synthesized as zymogens in the pancreas and secreted into the small intestine where they are activated. Pancreatic lipase catalyzes hydrolysis of the primary esters (at C-1 and C-3) of triacylglycerols releasing fatty acids and generating monoacylglycerols (Figure 16.29). A small protein called colipase helps bind the water-soluble lipase to the lipid substrates. Colipase also activates lipase by holding it in a conformation with an open active site. The fatty acids derived from dietary triacylglycerols are primarily long chain molecules.

Most of these bile salts recirculate through the lower parts of the small intestine, the hepatic portal blood, and then the liver. Bile salts circulate through the liver and intestine several times during the digestion of a single meal. Fatty acids are converted to fatty acyl CoA molecules within the intestinal cells. Three of these molecules can combine with glycerol, or two with a monoacylglycerol, to form a triacylglycerol. As described below, these water-insoluble triacylglycerols combine with cholesterol and specific proteins to form chylomicrons for transport to other tissues.

The fate of dietary phospholipids is similar to that of triacylglycerols. Pancreatic phospholipases secreted into the intestine catalyze the hydrolysis of phospholipids (Figure 9.8), which aggregate in micelles. The major phospholipase in the pancreatic secretion is phospholipase A_2, which catalyzes hydrolysis of the ester bond at C-2 of a glycerophospholipid to form a lysophosphoglyceride and a fatty acid (Figure 16.30). A model of phospholipase A_2 with a lipid substrate is shown in Figure 16.31. Lysophosphoglycerides are absorbed by the intestine and re-esterified to glycerophospholipids in intestinal cells.

Lysophosphoglycerides are normally present in cells only at low concentrations. High concentrations can disrupt cellular membranes by acting as detergents. This occurs, for example, when snake venom phospholipase A_2 acts on phospholipids in red blood cells, causing lysis of erythrocyte membranes. This is probably what killed Cleopatra.

Unlike other types of dietary lipids, most dietary cholesterol is unesterified. Dietary cholesteryl esters are hydrolyzed in the lumen of the intestine by the action of an esterase. Free cholesterol, which is insoluble in water, is solubilized by bile-salt micelles for absorption. Most cholesterol reacts with acyl CoA to form cholesteryl esters (Figure 9.16) in the intestinal cells.

B. Lipoproteins

Triacylglycerols, cholesterol, and cholesteryl esters cannot be transported in blood or lymph as free molecules because they are insoluble in water. Instead, these lipids assemble with phospholipids and amphipathic lipid-binding proteins to form spherical

◀ **IUMBM–Nicholson metabolic chart for lipid metabolism in mammals.**
Designed by Donald Nicholson ©2002 IUBMB.
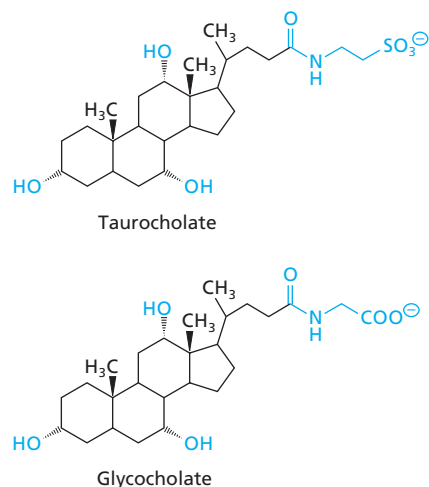
▲ **Figure 16.28**
Bile salts. The cholesterol derivatives taurocholate and glycocholate are the most abundant bile salts in humans. Bile salts are amphipathic: the hydrophilic parts are shown in blue, and the hydrophobic parts are shown in black.
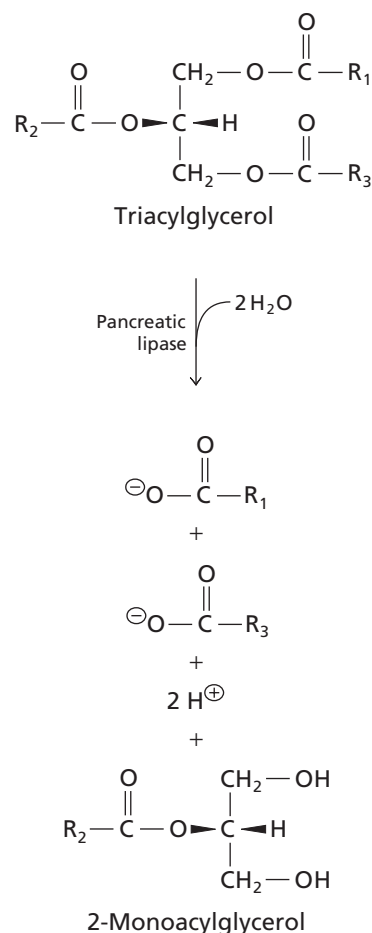
▲ **Figure 16.29**
Action of pancreatic lipase. Removal of the C-1 and C-3 acyl chains produces free fatty acids and a 2-monoacylglycerol. The intermediates, 1,2- and 2,3-diacylglycerol, are not shown.

Figure 16.30 ▶
Action of phospholipase A$_2$. X represents a polar head group. R$_1$ and R$_2$ are long hydrophobic chains, making up much of the phospholipid molecule.

Glycerophospholipid   Phospholipase A$_2$ Lysophosphoglyceride
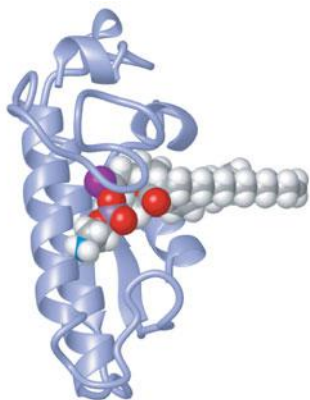
▲ **Figure 16.31**
Structure of phospholipase A$_2$ from cobra venom. Phospholipase A$_2$ catalyzes the hydrolysis of phospholipids at lipid–water interfaces. The model shows how a phospholipid substrate (dimyristoyl phosphatidylethanolamine, space-filling model) can fit into the active site of the water-soluble enzyme. A calcium ion (purple) in the active site probably helps bind the anionic head group. About half of the hydrophobic portion of the lipid would be buried in the lipid aggregate. Mammalian phospholipases are structurally similar to the venom enzyme. [PDB 1POB].
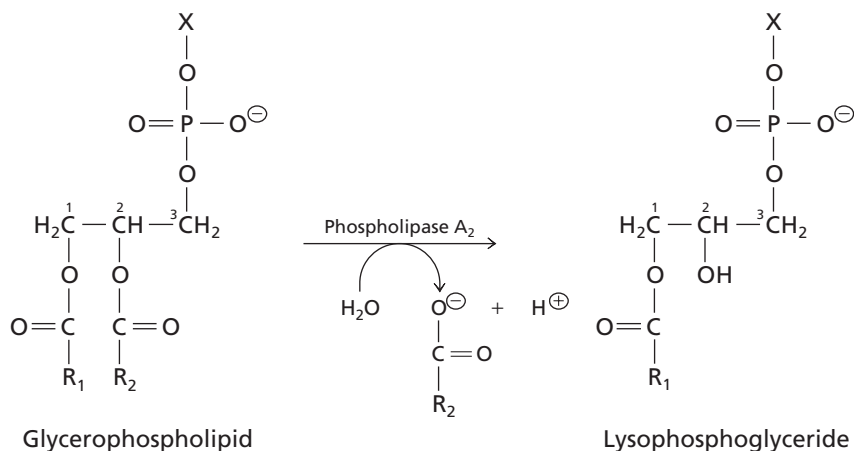
macromolecular particles known as lipoproteins. A lipoprotein has a hydrophobic core containing triacylglycerols and cholesteryl esters and a hydrophilic surface consisting of a layer of amphipathic molecules such as cholesterol, phospholipids, and proteins (Figure 16.32).

The largest lipoproteins are chylomicrons that deliver triacylglycerols and cholesterol from the intestine via the lymph and blood to tissues such as muscle (for oxidation) and adipose tissue (for storage) (Figure 16.33). Chylomicrons are present in blood only after a meal. The cholesterol-rich remnants of chylomicrons—having lost most of their triacylglycerol—deliver cholesterol to the liver. Liver cells are responsible for synthesizing most of the newly synthesized cholesterol that enters the bloodstream but almost all cell types make cholesterol for internal use. Lipoproteins deliver both dietary and liver-derived cholesterol to the rest of the body's cells. Cholesterol biosynthesis is regulated by hormones and by the levels of cholesterol in the blood.

Blood plasma contains several other types of lipoproteins. They are classified according to their relative densities and types of lipid (Table 16.1). Since proteins are more dense than lipids, the greater the protein content of a lipoprotein, the greater its density. Very low density lipoproteins (VLDLs) consist of approximately 98% lipid and only 2% protein. VLDLs are formed in the liver and carry lipids synthesized in the liver, or not needed by the liver, to other tissues such as adipose tissue. Lipases within capillaries of muscle and adipose tissue degrade VLDLs and chylomicrons. When VLDLs give up triacylglycerols to tissue cells their lipid content decreases and their remnants

BOX 16.6 EXTRA VIRGIN OLIVE OIL

Olive oil contains mostly triacylglycerols. If it has been produced by crushing olives with no additional chemical treatment, then it is called *virgin* olive oil according to the International Olive Oil Council (IOOC).

The quality of olive oil is often determined by the presence of free fatty acids that form when triacylglycerols break down during production. Virgin olive oil should have less than 2% free fatty acids (acidity) and *extra virgin* olive oil has less than 0.8% free fatty acids (acidity).

▶ **Extra virgin olive oil.** Extra virgin olive oil has less than 0.8% free fatty acids. http://www.examiner.com/fountain-of-youth-in-atlanta/extra-virgin-olive-oil-benefits

are degraded to intermediate density lipoproteins (IDLs). Of the IDLs formed during the breakdown of VLDLs, some are taken up by the liver and others are degraded to low density lipoproteins (LDLs). LDLs are enriched in cholesterol and cholesteryl esters and deliver these lipids to peripheral tissues. High density lipoproteins (HDLs) are formed as protein-rich particles in blood plasma. They pick up cholesterol from peripheral tissues, chylomicrons, and VLDL remnants and convert it into cholesterol esters. HDLs transport cholesterol and cholesteryl esters back to the liver. Cholesteryl esters from HDLs can be picked up by IDLs, which become LDLs.

Large lipoprotein particles contain a number of different lipid binding proteins. These are often called apolipoproteins—the "apo-" prefix usually refers to polypeptides that bind to a tightly associated cofactor as described in Chapter 7. Two of these apolipoproteins are large, hydrophobic, monomeric proteins. ApoB-100 (M_r 513,000) is firmly bound to the outer layer of VLDLs, IDLs, and LDLs. The smaller apolipoproteins of VLDLs and IDLs are weakly bound and most dissociate during lipoprotein degradation, leaving apoB-100 as the major protein component of LDLs. ApoB-48 (M_r 241,000), which is present only in chylomicrons, is identical in primary structure to the N-terminal 48% of apoB-100.

ApoB-100 and apoB-48 form much of the amphipathic crust or shell over the hydrophobic lipoprotein core of their respective lipoproteins. ApoB-100 is the protein that attaches LDL to its cell surface receptor; apoB-48 lacks this property. The other apolipoproteins are smaller than apoB-48. They have a variety of functions, including modulating the activity of certain enzymes involved in lipid mobilization and interacting with cell surface receptors.

Cholesterol, an essential component of eukaryotic cell membranes, is delivered to peripheral tissues by LDLs. The lipoprotein particles bind to the LDL receptor on the cell surface. A complex between LDL and its receptor enters the cell by endocytosis and fuses with a lysosome. Lysosomal lipases and proteases degrade the LDL releasing cholesterol that is then incorporated into cell membranes or stored as cholesteryl esters. An abundance of intracellular cholesterol suppresses synthesis of HMG-CoA reductase, a key enzyme in the biosynthesis of cholesterol and it also inhibits synthesis of the LDL receptor. Individuals lacking LDL receptors suffer from familial hypercholesterolemia, a disease in which cholesterol accumulates in the blood and is deposited in the skin and in arteries. Such patients die of heart disease at an early age.

HDLs remove cholesterol from plasma and from cells of nonhepatic tissues returning it to the liver. They bind to a receptor called SR-B1 at the liver surface and transfer cholesterol and cholesterol esters into liver cells. The lipid depleted HDL particles return to the plasma. In the liver, the cholesterol can be converted to bile salts that are secreted into the gallbladder.

The buildup of lipid deposits in the arteries (atherosclerosis) is associated with increased risk of coronary heart disease that can lead to a heart attack. High levels of LDL ("bad" cholesterol) *increase* the chance of developing atherosclerosis. High levels of
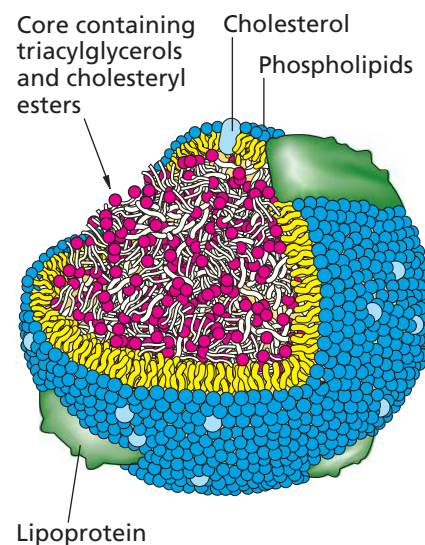
▲ **Figure 16.32**
Structure of a lipoprotein. A core of neutral lipids, including triacylglycerols and cholesteryl esters, is coated with phospholipids in which apolipoproteins and cholesterol are embedded.

▲ Chylomicrons.

BOX 16.7 LIPOPROTEIN LIPASE AND CORONARY HEART DISEASE

Lipoprotein lipase (Section 16.9) is the enzyme that releases fatty acid from the triacylglcerols in lipoproteins. It plays an important role in clearing triacylglycerols from the blood plasma. High concentrations of triacylglycerols are associated with coronary heart disease.

The human population contains several variants (mutations) of the lipoprotein lipase (LPL) gene. Some of these are associated with decreased LPL activity. One example is the D9N variant where an asparagine residue substitutes for the normal aspartate residue at position 9. Individuals who carry this variant are more likely to suffer from coronary heart disease due to the buildup of triacyglycerol-containing lipoproteins in the blood plasma.

In the S447X variant a normal serine codon is mutated to a stop codon (X) at position 447. The result is a truncated protein that is shorter than the normal protein. About 17% of the population carries at least one copy of this variant gene and 1% of the population is homozygous for this variant. The S447X enzyme is more active than the wild-type enzyme and this results in lower triacylglycerol levels in plasma. Males (but not females) who carry this variant are less likely to suffer heart attacks. This is an example of a beneficial allele that has arisen in the human population.

[Online Mendelian Inheritance in Man (OMIM) MIM=609708]

Figure 16.33 ▶
Summary of lipoprotein metabolism.
Chylomicrons formed in intestinal cells carry dietary triacylglycerols to peripheral tissues, including muscle and adipose tissue. Chylomicron remnants deliver cholesteryl esters to the liver. VLDLs assemble in the liver and carry endogenous lipids to peripheral tissues. When VLDLs are degraded (via IDLs), they pick up cholesterol and cholesteryl esters from HDLs and become LDLs, which carry cholesterol to nonhepatic tissues. HDLs deliver cholesterol from peripheral tissues to the liver.
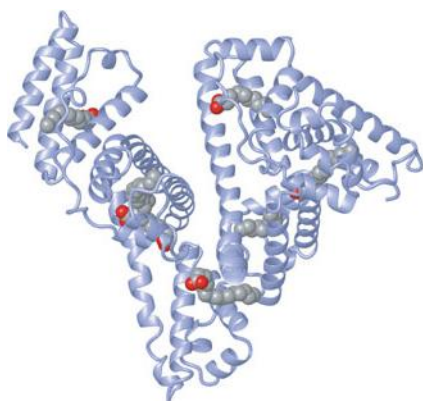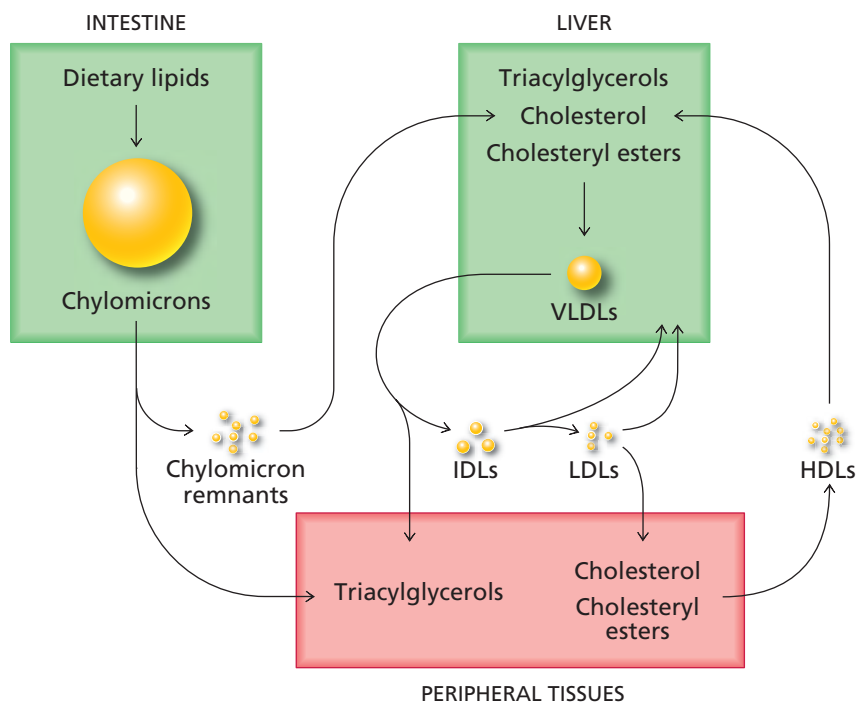
▲ Figure 16.34
Human serum albumin. Seven bound molecules of palmitate are shown. [PDB 1E7H]

HDL ("good" cholesterol), on the other hand, are correlated with a *decrease* in the risk of having a heart attack. Statins (Box 16.4) block synthesis of cholesterol in the liver and lower LDL levels.

C. Serum Albumin

In addition to complex lipids such as cholesterol and triacylglycerols, free fatty acids are also transported in blood plasma. Fatty acids bind to serum albumin, an abundant plasma protein. This protein, especially the bovine version (bovine serum albumin, BSA) has been intensely studied for over 40 years. Recently, the structure of human serum albumin (HSA) in association with free fatty acids of various chain lengths (Figure 16.34) has been solved by X-ray crystallography.

HSA belongs to the all-α category of tertiary structures (Section 4.7, Figure 4.24a). There are seven distinct binding sites for palmitic acid (16:0) and other medium and long chain fatty acids. In most cases, the carboxylate end of the fatty acids interacts with the side chains of basic amino acid residues and the methylene tails fit into hydrophobic pockets that can accommodate chains of 10–18 carbons. HSA also binds many important drugs that are only sparingly soluble in water.

16.11 Ketone Bodies Are Fuel Molecules

Most acetyl CoA produced in the liver from fatty acid oxidation is routed to the citric acid cycle but some of it can follow an alternate pathway. During periods of fasting, glycolysis is decreased and the gluconeogenic pathway is active. Under these conditions the

Table 16.1 Lipoproteins in human plasma

	Chylomicrons	**VLDLs**	**IDLs**	**LDLs**	**HDLs**
Molecular weight $\times 10^{-6}$	>400	10–80	5–10	2.3	0.18–0.36
Density (g cm^{-3})	<0.95	0.95–1.006	1.006–1.019	1.019–1.063	1.063–1.210
Chemical composition (%)					
Protein	2	10	18	25	33
Triacylglycerol	85	50	31	10	8
Cholesterol	4	22	29	45	30
Phospholipid	9	18	22	20	29

pool of oxaloacetate molecules becomes temporarily depleted. The amount of acetyl CoA from enhanced β-oxidation exceeds the capacity of the citric acid cycle (recall that oxaloacetate reacts with acetyl CoA in the first step of the citric acid cycle). The excess acetyl CoA is used to form ketone bodies—β-hydroxybutyrate, acetoacetate, and acetone. As indicated by their structures (Figure 16.35), not all ketone bodies are ketones. The only quantitatively significant ketone bodies are β-hydroxybutyrate and acetoacetate; small amounts of acetone are produced by the nonenzymatic decarboxylation of acetoacetate, a β-keto acid.

β-Hydroxybutyrate and acetoacetate are fuel molecules. They have less potential metabolic energy than the fatty acids from which they are derived but they make up for this deficiency by serving as "water-soluble lipids" that can be more readily transported in the blood plasma. During starvation, ketone bodies are produced in large amounts becoming substitutes for glucose as the principal fuel for brain cells. Ketone bodies are also metabolized in skeletal muscle and in the intestine during starvation.

A. Ketone Bodies Are Synthesized in the Liver

In mammals, ketone bodies are synthesized in the liver and exported for use by other tissues. The pathway for ketone body synthesis is shown in Figure 16.36. First, two molecules of acetyl CoA condense to form acetoacetyl CoA and HS-CoA in a reaction catalyzed by acetoacetyl-CoA thiolase. Subsequently, a third molecule of acetyl CoA is added to acetoacetyl CoA to form 3-hydroxy-3-methylglutaryl CoA (HMG CoA) in a reaction catalyzed by HMG-CoA synthase. These steps are identical to the first two steps in the isopentenyl diphosphate biosynthesis pathway (Figure 16.17). The synthesis of
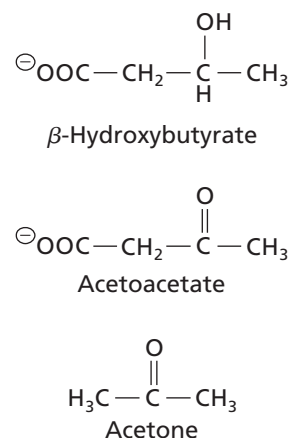
▲ **Figure 16.35**
Ketone bodies.
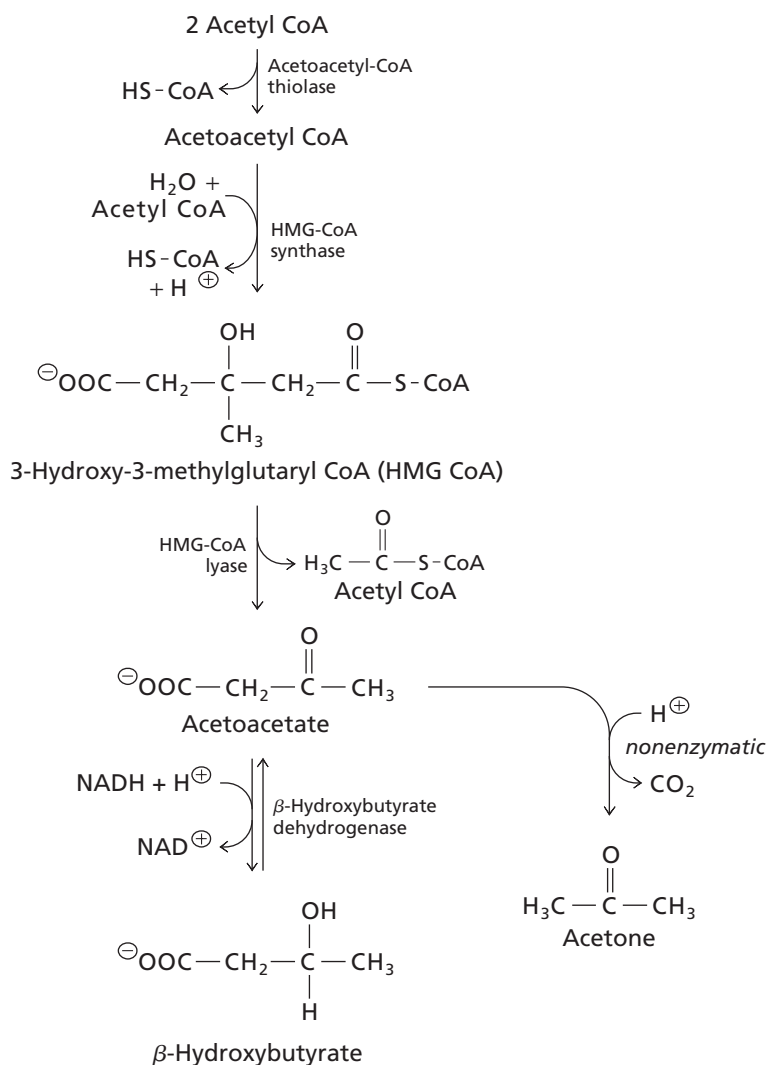
◄ **Figure 16.36**
Biosynthesis of β-hydroxybutyrate, acetoacetate, and acetone.
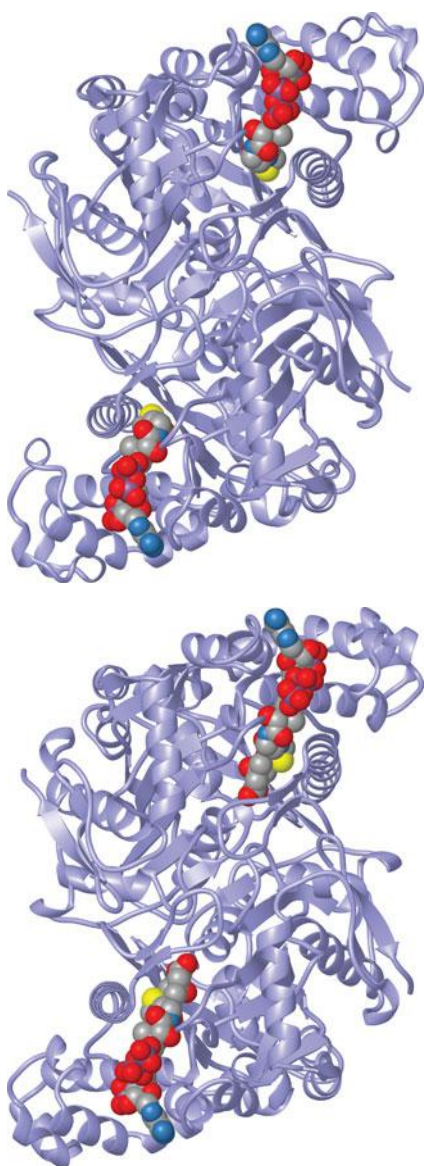
▲ **HMG-CoA synthase.** The human (*Homo sapiens*) isozymes are shown with bound HMG CoA. The cytosolic enzyme (top: PDB 2P8U) and the mitochondrial version (bottom: PDB 2WYA) are very similar.

> **Changes in carbohydrate metabolism during starvation are described in Section 13.10.**

ketone bodies takes place in mitochondria but the synthesis of isopentenyl diphosphate (and cholesterol) takes place in the cytosol. Mammals have distinct isozymes of acetoacetyl-CoA thiolase and HMG-CoA synthase in the mitochondria and the cytosol. HMG-CoA synthase is only present in the mitochondria of liver cells and not in the mitochondria of any other cell types.

In the next step, HMG-CoA lyase catalyzes the cleavage of HMG CoA producing acetoacetate and acetyl CoA. HMG-CoA lyase is not present in the cytosol, which is why cytosolic HMG CoA is used exclusively in isopentenyl diphosphate synthesis and no ketone bodies are produced in the cytosol. NADH-dependent reduction of acetoacetate produces β-hydroxybutyrate in a reaction catalyzed by β-hydroxybutyrate dehydrogenase. Both acetoacetate and β-hydroxybutyrate can be transported across the inner mitochondrial membrane and the plasma membrane of liver cells. They enter the blood to be used as fuel by other cells of the body. Small amounts of acetoacetate are nonenzymatically decarboxylated to acetone in the bloodstream.

The main control point for ketogenesis is the mitochondrial isozyme of HMG-CoA synthase provided that fatty acyl CoA and acetyl CoA are available in the mitochondria. Succinyl CoA specifically inhibits this enzyme by covalent modification through succinylation. This is a short-term inactivation since reactivation occurs frequently by spontaneous desuccinylation. Glucagon lowers the amount of succinyl CoA in mitochondria, stimulating ketogenesis. Long-term regulation occurs by modification of gene expression. Starvation increases the level of HMG-CoA synthase (and its mRNA); refeeding or insulin produces a decrease in both activity and mRNA.

B. Ketone Bodies Are Oxidized in Mitochondria

In cells that use them as an energy source, β-hydroxybutyrate and acetoacetate enter mitochondria where they are converted to acetyl CoA that is oxidized by the citric acid cycle. β-Hydroxybutyrate is converted to acetoacetate in a reaction catalyzed by an isozyme of β-hydroxybutyrate dehydrogenase that is distinct from the liver enzyme. Acetoacetate reacts with succinyl CoA to form acetoacetyl CoA in a reaction catalyzed by succinyl-CoA transferase (also called succinyl-CoA:3-ketoacid-CoA transferase; Figure 16.37). Ketone bodies are broken down only in nonhepatic tissues because this transferase is present in all tissues except liver. The succinyl-CoA transferase reaction siphons some succinyl CoA from the citric acid cycle. Energy that would normally be captured as GTP in the substrate-level phosphorylation catalyzed by succinyl-CoA synthetase (Section 13.3#5) is used instead to activate acetoacetate to its CoA ester. Thiolase then catalyzes the conversion of acetoacetyl CoA to two molecules of acetyl CoA that can be oxidized by the citric acid cycle.
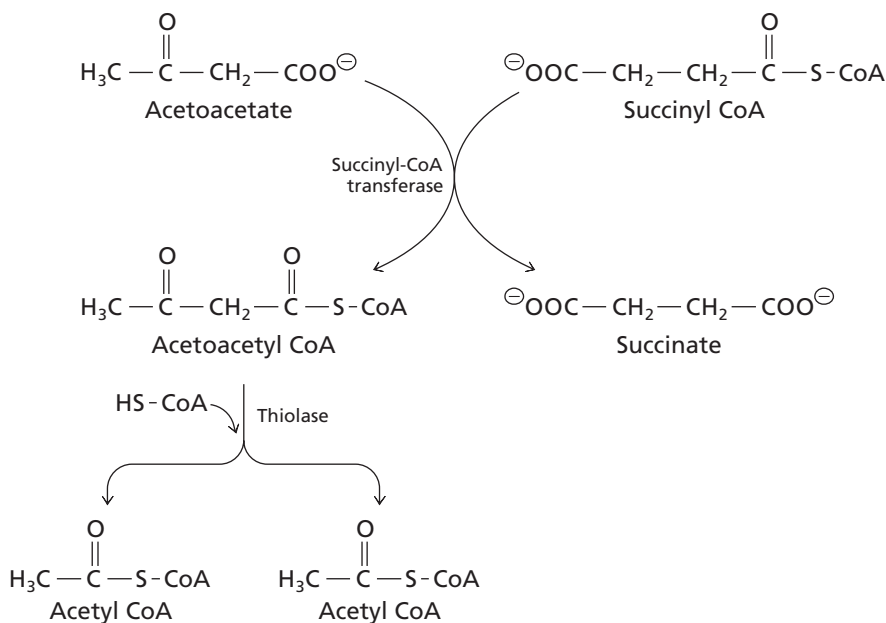
Figure 16.37 ▶
Conversion of acetoacetate to acetyl CoA.

BOX 16.8 LIPID METABOLISM IN DIABETES

The breakdown of fats occurs because lipolysis is not inhibited by insulin, and other hormones trigger the release of fatty acids from adipocytes. The large amounts of fatty acids available to the liver lead to excess acetyl CoA that is diverted to form ketone bodies. In Type 2 diabetes (Section 12.7), the accumulation of glucose in the blood is caused mainly by poor uptake of glucose by peripheral tissues. Because obesity strongly predisposes a person to developing Type 2 diabetes, much research is focusing on the role of lipids in decreased insulin sensitivity. It appears that elevated free fatty acids in the blood may interfere with insulin signaling for glucose uptake into tissues.

Individuals suffering from untreated Type 1 diabetes produce large amounts of ketone bodies—more than the peripheral tissues can use. The smell of acetone can be discerned on the breath of diabetics. In fact, the levels of acetoacetic acid and β-hydroxybutyric acid in the blood can be so high that the pH of the serum can be lowered—a life-threatening condition called diabetic ketoacidosis. Type 1 diabetes must be treated with repeated injections of insulin and restricted glucose intake.

Although acute complications are rare in Type 2 diabetes, hyperglycemia can lead to tissue damage, particularly in the eye and the cardiovascular and renal systems. Dietary modifications are often sufficient to control Type 2 diabetes. In addition, oral drugs can increase insulin secretion and potentiate its action at peripheral tissues.

A novel approach for the treatment of Type 2 diabetes may be inhibition of the tyrosine phosphatase PTP-1B. PTP-1B inactivates the insulin receptor by catalyzing the removal of phosphate added to the receptor when insulin binds to it. After insulin injection, mice lacking PTP-1B have increased phosphorylation of insulin receptors in liver and muscle and enhanced sensitivity to insulin. These mice also maintain normal levels of blood glucose after a meal. A surprising observation was that mice lacking PTP-1B could eat a high fat diet yet be resistant to weight gain. PTP-1B may therefore also be a target for the treatment of obesity.

Summary

1. The pathway for fatty acid synthesis begins with synthesis of malonyl CoA in a reaction catalyzed by acetyl CoA-carboxylase. Malonyl CoA is converted to malonyl ACP and one molecule of malonyl ACP condenses with acetyl CoA (or acetyl ACP) to form acetoacetyl ACP.

2. The formation of long-chain fatty acids from a 3-ketoacyl ACP precursor occurs in four stages: reduction, dehydration, further reduction, and condensation. These four stages repeat to form a long-chain fatty acid. Fatty acids with more than 18 carbons and unsaturated fatty acids are produced by additional reactions.

3. Triacylglycerols and glycerophospholipids are derived from phosphatidate. The synthesis of triacylglycerols and neutral phospholipids proceeds via a 1,2-diacylglycerol intermediate. Acidic phospholipids are synthesized via a CDP–diacylglycerol intermediate.

4. Many eicosanoids are derived from arachidonate. The cyclooxygenase pathway leads to prostacyclin, prostaglandins, and thromboxane A_2. The products of the lipoxygenase pathway include leukotrienes.

5. Sphingolipids are synthesized from serine and palmitoyl CoA. Reduction, acylation, and oxidation produce ceramide, which can be modified by adding a polar head group and sugar residues.

6. Cholesterol is synthesized from acetyl CoA in a pathway leading to mevalonate and isopentenyl diphosphate. Both cholesterol and isopentenyl diphosphate are precursors of many other compounds.

7. Fatty acids are degraded to acetyl CoA by β-oxidation, the sequential removal of two-carbon fragments. Fatty acids are first activated by esterification to CoA and fatty acyl CoA is oxidized by a repeated series of four enzyme-catalyzed steps: oxidation, hydration, further oxidation, and thiolysis. Fatty acids yield more ATP per gram than glucose.

8. β-Oxidation of odd-chain fatty acids produces acetyl CoA and one molecule of propionyl CoA. The oxidation of most unsaturated fatty acids requires two enzymes, an isomerase and a reductase, in addition to those required for the oxidation of saturated fatty acids.

9. Fatty acid oxidation in animals is regulated by hormones according to the energy needs of the organism.

10. Dietary fat is hydrolyzed in the intestine to fatty acids and monoacylglycerols, which are absorbed. Lipoproteins transport lipids in the blood. In adipocytes, fatty acids are esterified for storage as triacylglycerols. Fatty acids are mobilized by the action of hormone-sensitive lipase.

11. The ketone bodies β-hydroxybutyrate and acetoacetate are water-soluble fuel molecules produced in the liver by the condensation of acetyl-CoA molecules.

Problems

1. (a) Familial hypercholesterolemia is a human genetic disorder in which LDL receptors are defective, leading to very high blood cholesterol levels and severe atherosclerosis at an early age. Explain why this disease results in high blood cholesterol levels.

   (b) Do high blood cholesterol levels affect cellular cholesterol synthesis in individuals with this disease?

   (c) Individuals with Tangier's disease lack the cellular protein ABCl, which is required for cholesterol uptake by HDL. How will this disease affect cholesterol transport?

2. Individuals with abnormally low levels of carnitine in their muscles suffer from muscular weakness during moderate exercise. In addition, their muscles have significantly increased levels of triacylglycerols.

   (a) Explain these two effects.
   (b) Can these individuals metabolize muscle glycogen aerobically?

3. How many ATP equivalents are generated by the complete oxidation of (a) laurate (dodecanoate) and (b) palmitoleate (cis-Δ^9-hexadecenoate)? Assume that the citric acid cycle is functioning.

4. Tetrahydrolipstatin (Orlistat) is a drug treatment for obesity. It is an inhibitor of pancreatic lipase. Suggest a rationale for use of tetrahydrolipstatin to treat obesity.

5. In addition to the enzymes of β-oxidation, what enzymes are necessary to degrade the following fatty acids to acetyl CoA or acetyl CoA and succinyl CoA?

   (a) oleate (cis $CH_3(CH_2)_7\,CH{=}CH(CH_2)_7\,COO^{\ominus}$)
   (b) arachidonate
       (all cis $CH_3(CH_2)_4\,(CH{=}CHCH_2)_4\,(CH_2)_2COO^{\ominus}$)
   (c) cis $CH_3(CH_2)_9CH{=}CH(CH_2)_4\,COO^{\ominus}$

6. Animals cannot carry out a net conversion of even chain fatty acid carbons to glucose. On the other hand, some of the carbons in odd-chain fatty acids can be gluconeogenic precursors to glucose. Explain.

7. Where is the labeled carbon found when the following molecules are added to a liver homogenate carrying out palmitate synthesis?

   (a) $H^{14}CO_3^{\ominus}$
   (b)
$$H_3{}^{14}C{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}S{-}CoA$$

8. Triclosan (2,4,4-trichloro-2-hydroxydiphenyl ether) is an effective antimicrobial agent that is used in a wide range of consumer products including soaps, toothpaste, toys, and cutting boards. Triclosan is effective against a broad spectrum of bacteria and mycobacteria and is an inhibitor of type II FAS enoyl acyl carrier protein reductase.

   (a) What reaction is catalyzed by enoyl acyl carrier protein reductase?
   (b) Why is enoyl acyl carrier protein reductase an appropriate target for antimicrobials?
   (c) Suggest a reason why a compound may selectively inhibit fatty acid synthesis in bacteria and not in humans.

9. It has been proposed that malonyl CoA may be one of the signals sent to the brain to decrease the appetite response. When mice are given a derivative of cerulenin (a fungal epoxide) named C75, their appetite is suppressed and they rapidly lose weight. Cerulenin and its derivatives have been shown to be potent inhibitors of fatty acid synthase (FAS). Suggest how C75 might act as a potential weight reduction drug.

10. (a) Draw a general pathway for converting carbohydrates to fatty acids in a liver cell, and indicate which processes occur in the cytosol and which occur in motochondria.
    (b) About half the reducing equivalents necessary for fatty acid synthesis are generated by glycolysis. Explain how these reducing equivalents can be used for fatty acid synthesis.

11. (a) Acetyl CoA carboxylase (ACC), a key regulator for fatty acid synthesis, exists in two different interconvertible forms:

(1) an active filamentous polymer (dephosphorylated), and (2) an inactive protomeric form (phosphorylated). Citrate and palmitoyl CoA can regulate fatty acid synthesis by preferentially binding tightly to and stabilizing different forms of ACC. Explain how each of these regulator functions by interacting with ACC.

Filamentous polymer (active) $\rightleftharpoons$ Protomer (inactive)

   (b) What role do glucagon and epinephrine play in regulating fatty acid synthesis?

12. Obesity is a serious health problem worldwide due in part to increased food intake and reduced physical activity. Obesity is associated with a variety of human disease including Type 2 diabetes and cardiovascular diseases. Selective and specific inhibitors of acetyl-CoA carboxylase have been proposed as potential anti-obesity drugs.

    (a) What effect would an inhibitor of acetyl-CoA carboxylase have on fatty acid synthesis and fatty acid oxidation?
    (b) One such inhibitor of acetyl-CoA carboxylase is CABI (structure below). What structural feature of CABI makes it a potential acetyl-CoA carboxylase inhibitor? (Levert, K. L., Waldrop, G. L., Stephens, J. M. (2002). *J. Biol. Chem.* A biotin analog inhibits acetyl CoA carboxylase activity and adipogenesis. 277:16347–16350.)

CABI
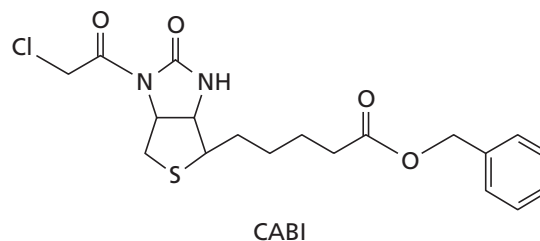
13. Write the equation for the conversion of eight acetyl CoA molecules to palmitate.

14. (a) In response to tissue damage in such injuries as heart attacks and rheumatoid arthritis, inflammatory cells (e.g., monocytes and neutrophils) invade the injured tissue and promote the synthesis of arachadonic acid. Explain the reason for this response.
    (b) The biosynthesis of eicosanoids is affected by nonsteroidal drugs such as aspirin and ibuprofen and by steroidal drugs such as hydrocortisone and prednisone (which inhibit a specific phospholipase). Why do steroidal drugs inhibit the biosynthesis of both prostaglandins and leukotrienes, whereas aspirin-like drugs inhibit the biosynthesis of only prostaglandins?

15. Draw the correct structures of the following complex lipids.

    (a) Phosphatidyl glycerol.
    (b) Ethanolamine plasmalogen (1-alkyl-2-glycero-3-phosphoethanolamine).
    (c) Glucocerebroside (1-β-D-glucoceramide).

16. Excess dietary fat can be converted to cholesterol in the liver. When palmitate labeled with ^{14}C at every odd-numbered carbon is added to a liver homogenate, where does the label appear in mevalonate?

17. The therapeutic anti-inflammatory effects of aspirin arise from its inhibition of the enzyme cyclooxygenase-2 (COX-2)—involved in the synthesis of prostaglandins, mediators of inflammation, pain, and fever. Aspirin irreversibly inhibits COX-2 by covalently

transferring an acetyl group to a serine residue at the enzyme active site. However, the undesirable side effect of stomach irritation arises from the irreversible inhibition of the related intestinal enzyme cyclooxygenase-1 (COX-1) by aspirin. COX-1 is involved in the synthesis of prostaglandins that regulate secretion of gastric mucin, which protects the stomach from acid. The aspirin analog APHS was synthesized and shown to be 60 times more selective as an inhibitor of COX-2 than of COX-1, suggesting that it could be an anti-inflammatory drug with far less gastrointestinal side effects. Draw the structure of the inactivated COX-2 enzyme-inhibitor complex with APHS. Since aspirin and structural analogs act at the active site of COX enzymes, will they exhibit competitive inhibition patterns?

APHS
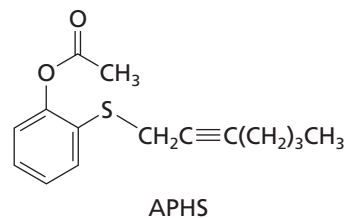
Selected Readings

General

Nicholson, D. E. (2001). IUBMB–Nicholson metabolic pathways charts. *Biochem. Mol. Bio. Educ.* 29:42–44.

Vance, J. E., and Vance, D. E., eds. (2008). *Biochemistry of Lipids, Lipoproteins, and Membranes* (Amsterdam: Elsevier Science).

Lipid Synthesis

Athenstaedt, K., and Daum, G. (1999). Phosphatidic acid, a key intermediate in lipid metabolism. *Eur. J. Biochem.* 266:1–16.

Frye, L. L., and Leonard, D. A. (1999). Lanosterol analogs: dual-action inhibitors of cholesterol biosynthesis. *Crit. Rev. Biochem. Mol. Biol.* 34:123–124.

Kent, C. (1995). Eukaryotic phospholipid synthesis. *Annu. Rev. Biochem.* 64:315–343.

Leibundgut, M., Maier, T., Jenni, S., and Ban, N. (2008). The multienzyme architecture of eukaryotic fatty acid synthases. *Curr. Opin. Struct. Biol.* 18:714–726.

Simmons, D. L., Botting, R. M., and Hla, T. (2004). Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacol. Rev.* 56:387–437.

Sommerville, C., and Browse, J. (1996). Dissecting desaturation: plants prove advantageous. *Trends in Cell Biol.* 6:148–153.

Wallis, J. G., Watts, J. L., and Browse, J. (2002). Polyunsaturated fatty acid synthesis: what will they think of next? *Trends Biochem. Sci.* 27:467–473.

White, S. W. Zheng, J., Zhang, Y-M., and Rock, C. O. (2005). The structural biology of type II fatty acid biosynthesis. *Ann. Rev. Biochem.* 74:791–831.

Lipid Catabolism

Bartlett, K., and Eaton, S. (2004). Mitochondrial β-oxidation. *Eur. J. Biochem.* 271:462–469.

Candlish, J. (1981). Metabolic water and the camel's hump—a textbook survey. *Biochem Ed.* 9:96–97.

Ishikawa, M., Tsuchiya, D., Oyama, T., Tsunaka, D., and Morikawa, K. (2004). Structural basis for channelling mechanism of a fatty acid β-oxidation multienzyme complex. *EMBO J.* 23:2745–2754.

Kim, J-J., and Battaile, K. P. (2002). Burning fat: the structural basis of fatty acid β-oxidation. *Curr. Opin. Struct. Biol.* 12:721–728.

Toogood. H. S., van Thiel, A., Basran, J., Sutcliffe, M. J., Scrutton, N. S., and Leys, D. (2004). Extensive domain motion and electron transfer in the human electron transferring flavoprotein medium chain acyl-CoA dehydrogenase complex. *J. Biol. Chem.* 279: 32904–32912.

Toogood, H. S., van Thiel, A., Scrutton, N. S., and Leys, D. (2005). Stabilization of non-productive conformations underpins rapid electron transfer to electron-transferring flavoprotein. *J. Biol. Chem.* 280:30361–30366.

Wanders, R. J. A., and Waterman, H. R. (2006). Biochemistry of mammalian peroxisomes revisited. *Ann. Rev. Biochem.* 75:295–332.

Lipoproteins

Bhattacharya, A. A., Grüne, T., and Curry, S. (2000). Crystallographic analysis reveals common modes of binding of medium- and long-chain fatty acids to human serum albumin. *J. Mol. Biol.* 303:721–732.

Fidge, N. H. (1999). High density lipoprotein receptors, binding proteins, and ligands. *J. Lipid Res.* 40:187–201.

Gagné, S. E., Larson, M. G., Pimstone, S. N., Schaefer, E. J., Kastelein, J. J. P., Wilson, P. W. F., Ordovas, J. M., and Hayden, M. R. (1999). A common truncation variant of lipoprotein lipase (S447X) confers protection against coronary heart disease: the Framingham Offspring Study. *Clin. Genet.* 55:450–454.

Kreiger, M. (1998). The "best" of cholesterols, the "worst" of cholesterols: a tale of two receptors. *Proc. Natl. Acad. Sci. USA* 95:4077–4080.
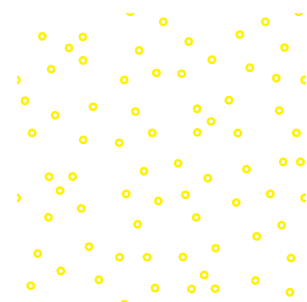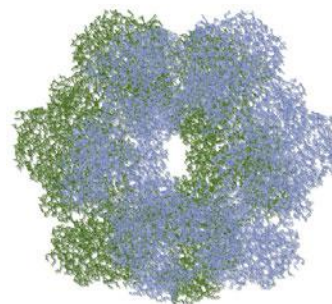
Amino Acid Metabolism

Authors writing a chapter on amino acid metabolism have a nearly impossible task. Any description will be incomplete since there are 20 different amino acids and many intermediates in the biosynthesis and degradation pathways. Furthermore, alternate pathways are used by different tissues, organelles, and organisms. Fortunately, metabolic highlights can show the biological rationale of how amino acids are formed and degraded without getting into excessive detail. Here we describe a number of these highlights in order to illustrate the principles and concepts of amino acid metabolism.

The metabolism of amino acids includes hundreds of enzymatic interconversions of small molecules. Many of these reactions involve nitrogen atoms. Some of the intermediates appear in the metabolic pathways described in preceding chapters but many are described here for the first time. Although amino acids from the degradation of proteins can be a source of energy, we are more concerned with their biosynthesis. Life is compromised if all the amino acids are not available at the same time for protein synthesis. We can consider the metabolism of the 20 common amino acids from two points of view: the origins and fates of their nitrogen atoms and the origins and fates of their carbon skeletons.

The abilities of organisms to synthesize amino acids differ widely. A few organisms can assimilate N_2 and simple carbon compounds into amino acids—in other words, they are totally self-supporting for amino acid synthesis. Other species can synthesize the carbon chains of amino acids but require nitrogen in the form of ammonia. We begin this chapter with an overview of the principles of nitrogen metabolism.

Some species cannot synthesize the carbon skeletons of every amino acid. Mammals, for example, can make only about half of the amino acids they require; the rest—called **essential amino acids**—must be obtained from the diet. **Nonessential amino acids** are those that mammals can synthesize in sufficient quantity, provided they receive adequate total dietary protein.

The routes for disposal of the nitrogen-containing waste products of amino acid metabolism also vary among species. For example, excess nitrogen is excreted by aquatic animals as ammonia, by birds and most reptiles as uric acid, and by many other terrestrial

We live now in the "Age of Bacteria." Our planet has always been in the "Age of Bacteria," ever since the first fossils—bacteria, of course—were entombed in rocks more than 3 and a half billion years ago. On any possible, reasonable, or fair criterion, bacteria are—and always have been—the dominant forms of life on Earth.

Stephen Jay Gould, (1996),
Full House, p. 176

Top: Glutamine synthetase from the bacterium *Salmonella typhimurium*. Twelve identical subunits are arranged with hexagonal symmetry. [PDB 2GLS].

vertebrates as urea. We will end the chapter with a description of the urea cycle, a pathway for elimination of nitrogen in mammals.

17.1 The Nitrogen Cycle and Nitrogen Fixation

The nitrogen needed for amino acids (and for the heterocyclic bases of nucleotides; Chapter 18) comes from two major sources—nitrogen gas in the atmosphere and nitrate ($NO_3^{\ominus}$) in soil and water. Atmospheric N_2, which constitutes about 80% of the atmosphere, is the ultimate source of biological nitrogen. This molecule can be metabolized, or fixed, by only a few species of bacteria. N_2 and $NO_3^{\ominus}$ must be reduced to ammonia in order to be used in metabolism. The ammonia produced is incorporated into amino acids via glutamate, glutamine, and carbamoyl phosphate.

N_2 is chemically unreactive because of the great strength of the $N \equiv N$ triple bond. Some bacteria have a very specific, sophisticated enzyme, called nitrogenase, that can catalyze the reduction of N_2 to ammonia in a process called **nitrogen fixation**. Ammonia is essential for life and bacteria are the only organisms capable of producing it from atmospheric nitrogen. Half of all biological nitrogen fixation is performed by various species of cyanobacteria in the ocean. The other half comes from soil bacteria.

There are two additional nitrogen-converting processes in addition to biological nitrogen fixation. During lightning storms, high-voltage discharges catalyze the oxidation of N_2 to nitrate and nitrite ($NO_2^{\ominus}$). Nitrogen is converted to ammonia for use in plant fertilizers by an energetically expensive industrial process that requires high temperature and pressure as well as special catalysts to drive the reduction of N_2 by H_2. The availability of biologically useful nitrogen is often a limiting factor for plant growth, and the application of nitrogenous fertilizers is important for obtaining high crop yields. Humans are now responsible for a substantial fraction of the total nitrogen fixation on the planet. Although only a small percentage of the nitrogen undergoing metabolism comes *directly* from nitrogen fixation, this process is the only way that organisms can use the huge pool of atmospheric N_2.

The overall scheme for the interconversion of the major nitrogen-containing compounds is shown in Figure 17.1. The flow of nitrogen from N_2 to nitrogen oxides, ammonia, and nitrogenous biomolecules and then back to N_2 is called the **nitrogen cycle**. Most of the nitrogen shuttles between ammonia and nitrate. Ammonia from decayed organisms is oxidized by soil bacteria to nitrate. This formation of nitrate is called nitrification. Some anaerobic bacteria can reduce nitrate or nitrite to N_2 (denitrification).

▲ **Blooms of *Trichodesmium*.** *Trichodesmium* is one of the main nitrogen-fixing species of cyanobacteria. This large bloom of bacteria formed giant streaks in the ocean off the coast of Australia. The photograph was taken from the space shuttle. The average concentration of nitrogen-fixing bacteria in the ocean is about one million cells per liter.

KEY CONCEPT

Nitrogen is the most abundant gas in the atmosphere but only a few species of bacteria are capable of nitrogen fixation.

▲ **Lightning.** Lightning causes the conversion of nitrogen gas to nitrates. It is an important source of usable nitrogen for living organisms. This photograph was taken in 1908.

▲ **Figure 17.1**
Nitrogen cycle. A few free-living or symbiotic microorganisms can convert N_2 directly to ammonia. Ammonia is incorporated into biomolecules such as amino acids and proteins that subsequently are degraded, re-forming ammonia. Many soil bacteria and plants can carry out the reduction of nitrate to ammonia via nitrite. Several bacteria convert ammonia to nitrite. Others oxidize nitrite to nitrate and some can reduce nitrate to N_2.

▲ **Figure 17.2**
Nodules on alfalfa roots. Symbiotic bacteria of the genus *Rhizobium* reside in these nodules where they reduce atmospheric nitrogen to ammonia.

Most green plants and some microorganisms contain nitrate reductase and nitrite reductase, enzymes that together catalyze the reduction of nitrogen oxides to ammonia.

$$NO_3^{\ominus} \xrightarrow[\text{Nitrate}]{2\,e^{\ominus},\,2\,H^{\oplus} \quad H_2O} NO_2^{\ominus} \xrightarrow[\text{Nitrite}]{6\,e^{\ominus},\,7\,H^{\oplus} \quad 2\,H_2O} NH_3 \quad \text{Ammonia}} \tag{17.1}$$
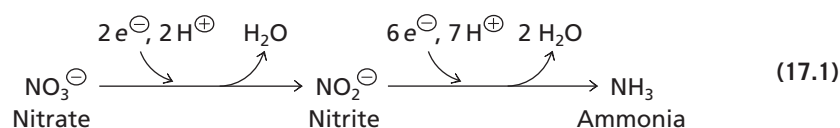
This ammonia is used by plants, which supply amino acids to animals. Reduced ferredoxin (formed in the light reactions of photosynthesis, Section 15.2B) is the source of the reducing power in plants and photosynthetic bacteria.

Let's examine the enzymatic reduction of N_2. Most nitrogen fixation in the biosphere is carried out by bacteria that synthesize the enzyme nitrogenase. This multisubunit protein catalyzes the conversion of each molecule of N_2 to two molecules of NH_3. Nitrogenase is present in various species of *Rhizobium* and *Bradyrhizobium* that live symbiotically in root nodules of many leguminous plants, including soybeans, peas, alfalfa, and clover (Figure 17.2). N_2 is also fixed by free-living soil bacteria such as *Agrobacteria, Azotobacter, Klebsiella*, and *Clostridium* and by cyanobacteria (mostly *Trichodesmuim spp.*) found in the ocean. Most plants require a supply of fixed nitrogen from sources such as decayed animal and plant tissue, nitrogen compounds excreted by bacteria, and fertilizers. Vertebrates obtain fixed nitrogen by ingesting plant and animal matter.

Nitrogenase is a protein complex that consists of two different polypeptide subunits forming an $\alpha_2\beta_2$ dimer of dimers (Figure 17.3). The two halves of the complex contain an [8 Fe–7 S] iron–sulfur cluster called the P-cluster. It is near the outer surface of the protein. The reactive center is a complex cluster of molybdenum, iron, and homocitrate [$MoFe_7S_9$-homocitrate]. A single $\alpha\beta$ dimer is called the iron–molybdenum (MoFe) protein.

Electrons are donated to the P-cluster by a mobile iron (Fe) protein containing a [4 Fe–4 S] cluster. Fe protein, a homodimer, binds to the ends of MoFe protein near the P-cluster and a single electron is passed from Fe protein to MoFe protein. The reduction of iron in Fe protein is coupled to oxidation of ferredoxin or flavodoxin and each of these reduction reactions requires hydrolysis of two bound ATP molecules. Electrons are passed from Fe protein to the P-cluster to the FeMo-cluster. A total of six electrons are required for conversion of N_2 to $2NH_3$ and these must be passed one at a time from Fe protein as it binds and then dissociates from MoFe protein. An obligatory reduction of 2 $H^{\oplus}$ to H_2 accompanies the reduction of N_2. The overall stoichiometry is

$$N_2 + 8\,H^{\oplus} + 8\,e^{\ominus} + 16\,ATP \longrightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i \tag{17.2}$$

Figure 17.3 ▶

Structure of *Azotobacter vinelandii* nitrogenase. The Fe protein subunits are colored red and orange and the α and β subunits of each half of the MoFe protein are colored blue/green and purple/pink. This structure with bound Fe protein is stabilized by bound transition-state ATP analogs ADP-AlF$_4$ at the ATP binding sites. [PDB 1N2C]

Fe Protein   MoFe Protein   MoFe Protein   Fe Protein

[4 Fe–4 S] cluster

P-cluster

MoFe$_7$S$_9$N-homocitrate reactive center
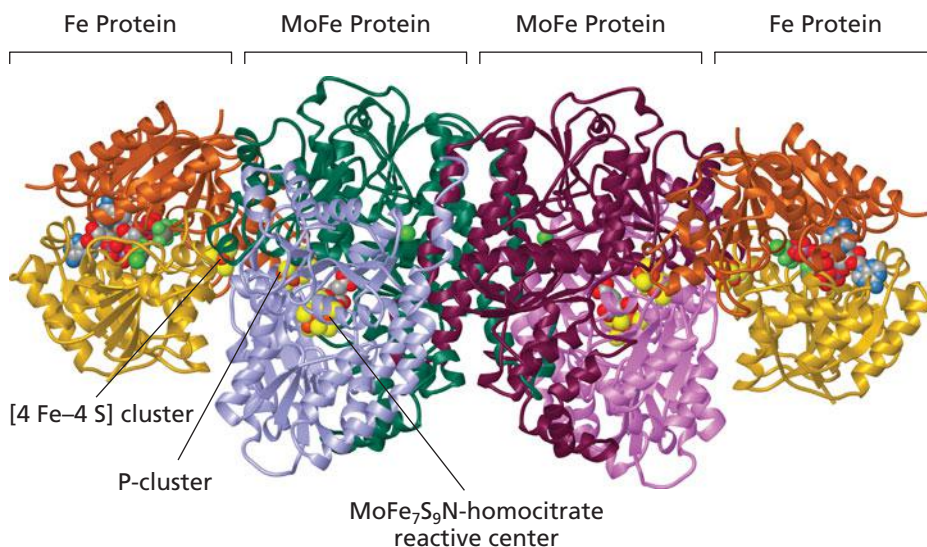
This is a very expensive reaction in terms of ATP equivalents. It is also a very slow reaction in biochemical terms with a turnover number of only five ammonia atoms produced per second. The slowness of the reaction is due to the fact that eight reduced Fe proteins have to bind and dissociate from the MoFe protein during the conversion of nitrogen to ammonia.

Nitrogenases must be protected from oxygen because the various oxidation–reduction centers are highly susceptible to inactivation by O_2. Strict anaerobes carry out nitrogen fixation in the absence of O_2. Within the root nodules of leguminous plants, the protein leghemoglobin (a homolog of vertebrate myoglobin; Section 4.12) binds O_2 and thereby keeps its concentration sufficiently low in the immediate environment of the nitrogen-fixing enzymes of *Rhizobium*. Nitrogen fixation in cyanobacteria is carried out in specialized cells (heterocysts) whose thick membranes inhibit entry of O_2 (Figure 10.8). In order to obtain the reducing power and ATP required for this process, symbiotic nitrogen-fixing microorganisms rely on nutrients obtained through photosynthesis carried out by the plants with which they are associated.

The actual reduction of nitrogen takes place at the iron–molybdenum–homocitrate cluster in the MoFe protein. This cluster is remarkably complex. It consists of a cage of Fe and S atoms surrounding a central N atom. A single Mo atom is bound to one edge of the Fe—S cage. It is chelated to a single molecule of homocitrate to form a $MoFe_7S_9N$·homocitrate cluster (Figure 17.4).

The detailed reaction mechanism of nitrogenase is unknown in spite of many years of intense study. It is likely that each of the three $N{\equiv}N$ bonds is broken sequentially, giving rise to the intermediates diimine and hydrazine.

$$
N{\equiv}N \xrightarrow{2\,e^{\ominus},\ 2H^{\oplus}} \underset{\text{Diimine}}{H{-}N{=}N{-}H} \xrightarrow{2\,e^{\ominus},\ 2H^{\oplus}} \underset{\text{Hydrazine}}{H_2N{-}NH_2} \xrightarrow{2\,e^{\ominus},\ 2H^{\oplus}} NH_3 + NH_3
$$

(17.3)
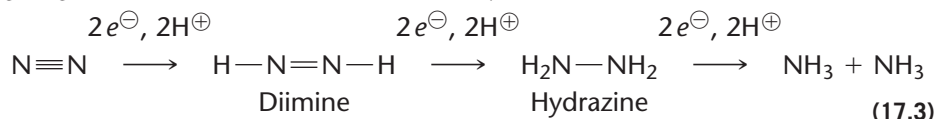
The reduction of $2\,H^{\oplus}$ to H_2, an essential coupled reaction, consumes the extra pair of electrons from ferredoxin as shown in Reaction 17.2.
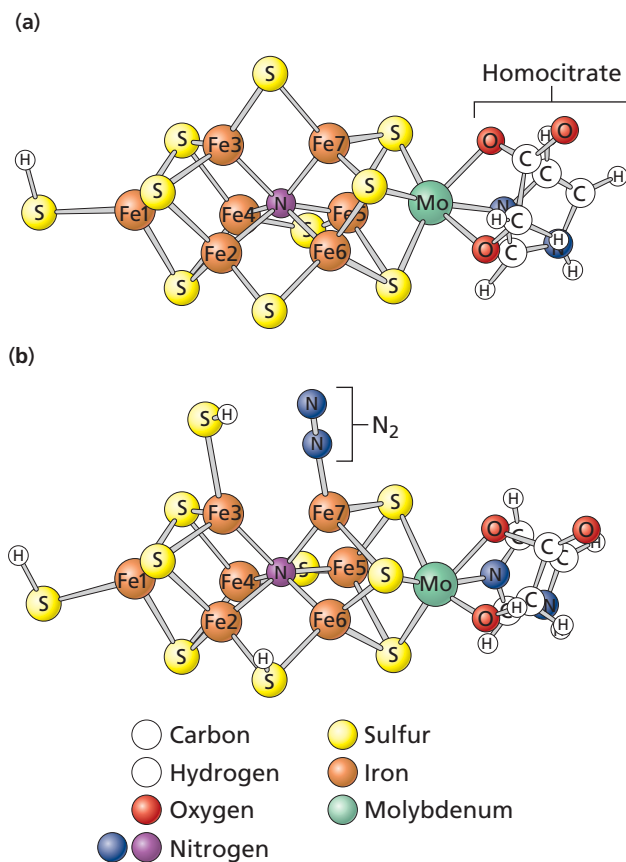
◄ **Figure 17.4**
Structure of the $MoFe_7S_9N$ · homocitrate reactive center in *Azotobacter vinelandii*. (a) Resting state. **(b)** One possible structure with bound N_2. [PDB 2MIN]

(a)

(b)

○ Carbon   ○ Sulfur
○ Hydrogen   ● Iron
● Oxygen   ● Molybdenum
● ● Nitrogen
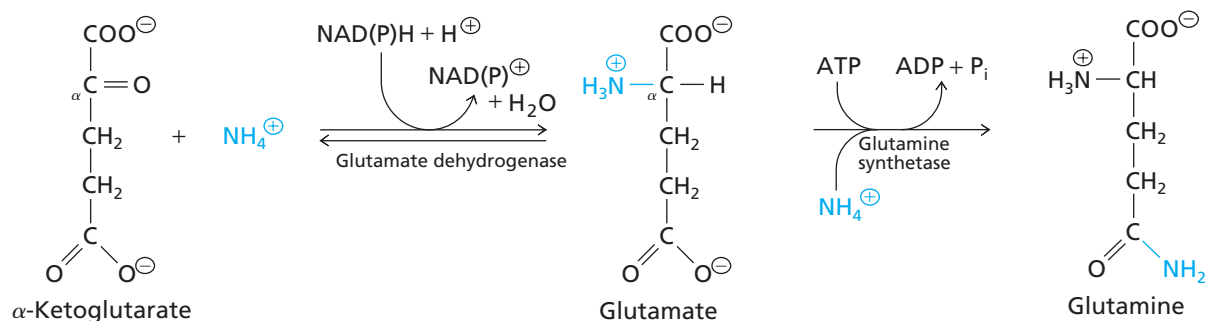
▲ **Figure 17.5**
Incorporation of ammonia into glutamate and glutamine.

17.2 Assimilation of Ammonia

Ammonia is assimilated into a large number of low molecular weight metabolites, often via the amino acids glutamate and glutamine. At physiological pH the main ionic form of ammonia is the ammonium ion, $NH_4^{\oplus}$ ($pK_a = 9.2$). However, unprotonated ammonia (NH_3) is the reactive species in the catalytic centers of many enzymes.

A. Ammonia Is Incorporated into Glutamate and Glutamine

The reductive amination of α-ketoglutarate to glutamate by glutamate dehydrogenase is one highly efficient route for the incorporation of ammonia into the central pathways of amino acid metabolism (Figure 17.5). The glutamate dehydrogenases of some species or tissues are specific for NADH while others are specific for NADPH. Still others can use either cofactor.

> Synthetases are members of the Ligase class of enzymes. They require ATP as a cosubstrate. Synthases are members of the Transferase or Lyase class of enzymes. They do not use ATP as a cofactor. (Section 5.1, Section 13.3#1).

The glutamate dehydrogenase reaction can play different physiological roles depending on substrate and coenzyme availability and enzyme specificity. In *Escherichia coli*, for example, the enzyme generates glutamate when $NH_4^{\oplus}$ is present at high concentrations. In the mold *Neurospora crassa* an NADPH-dependent enzyme is used for the reductive amination of α-ketoglutarate to glutamate and the reverse reaction is catalyzed by an $NAD^{\oplus}$-dependent enzyme. Glutamate dehydrogenase is located in mitochondria in mammals and plants and it catalyzes a near equilibrium reaction with net flux usually from glutamate to α-ketoglutarate. The primary role of glutamate dehydrogenase in mammals is the degradation of amino acids and the release of $NH_4^{\oplus}$. Mammals probably assimilate very little nitrogen as free ammonia because they get most of their nitrogen from amino acids and nucleotides in the diet.

Another reaction critical to the assimilation of ammonia in many organisms is the formation of glutamine from glutamate and ammonia. This reaction is catalyzed by glutamine synthetase (Figure 17.5). Glutamine is a nitrogen donor in many biosynthetic reactions; for example, the amide nitrogen of glutamine is the direct precursor of several of the nitrogen atoms of the purine and pyrimidine ring systems of nucleotides (Sections 18.1 and 18.3). In mammals, glutamine carries nitrogen and carbon between tissues in order to avoid high levels of toxic $NH_4^{\oplus}$ in the bloodstream.

The amide nitrogen of glutamine can be transferred to α-ketoglutarate to produce two molecules of glutamate in a reductive amination reaction catalyzed by glutamate synthase (Figure 17.6). Like glutamate dehydrogenase, glutamate synthase requires a reduced pyridine nucleotide to reductively aminate α-ketoglutarate. Unlike the dehydrogenase, the synthase uses glutamine as the source of nitrogen. Animals do not have glutamate synthase.
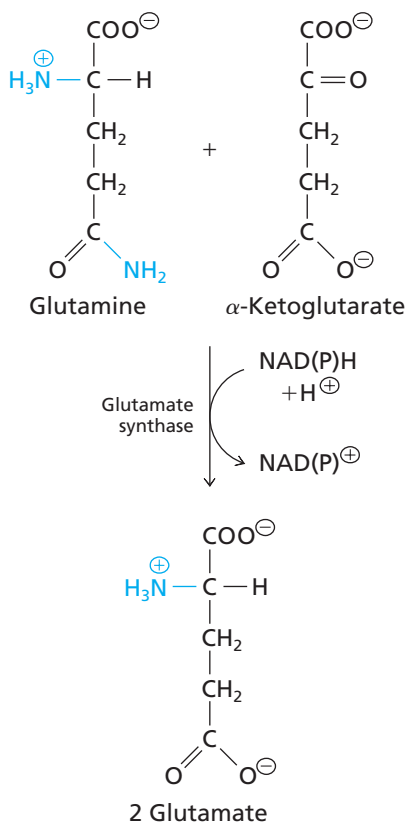
▲ **Figure 17.6**
Glutamate synthase catalyzes the reductive amination of α-ketoglutarate.

B. Transamination Reactions

The amino group of glutamate can be transferred to many α-keto acids in reactions catalyzed by enzymes known as transaminases or aminotransferases. The general transamination reaction is shown in Figure 17.7.
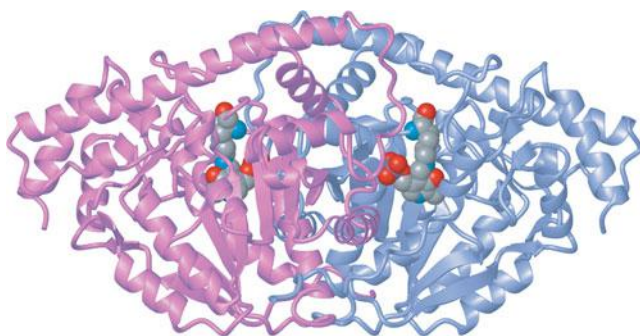
▲ **Pig (*Sus scrofa*) cytosolic aspartate transaminase.** The enzyme is a dimer of identical subunits (individual monomers are shown in purple and blue). A molecule of the coenzyme pyridoxal phosphate is shown (space-filling model) in each active site. [PDB 1AJR]

The amino group of glutamate is transferred to various α-keto acids generating the corresponding α-amino acids during amino acid synthesis. Most of the common amino acids can be formed by transamination. In amino acid catabolism, amino groups are transferred from various amino acids to α-ketoglutarate or oxaloacetate generating glutamate or aspartate.

All known transaminases require the coenzyme pyridoxal phosphate (Section 7.8). The chemical mechanism of the initial half-reaction of transamination was shown in Figure 7.18. The complete transamination requires two coupled half-reactions, with enzyme-bound pyridoxamine phosphate (PMP) transiently carrying the amino group being transferred.

The transaminases catalyze near-equilibrium reactions. The direction in which the reactions proceed *in vivo* (flux) depends on the supply of substrates and the removal of products. For example, in cells with an excess of α-amino nitrogen groups the amino groups can be transferred via one or a series of transamination reactions to α-ketoglutarate to yield glutamate that can undergo oxidative deamination catalyzed by glutamate dehydrogenase. Transamination occurs in the opposite direction when amino acids are being actively formed and the amino groups are donated by glutamate.

An important alternative to the glutamate dehydrogenase reaction in bacteria uses coupled reactions catalyzed by glutamine synthetase and glutamate synthase for the assimilation of ammonia into glutamate, especially when the concentration of ammonia is low. Figure 17.8 shows how the combined actions of glutamine synthetase and glutamate synthase can lead to the incorporation of ammonia into a variety of amino acids. After formation, glutamate undergoes transamination with α-keto acids to form the corresponding amino acids. The conversion of α-ketoglutarate to glutamate can occur via the glutamine synthetase–glutamate synthase pathway at the low concentrations of $NH_4^{\oplus}$ present in most bacterial cells because the K_m of glutamine synthetase for NH_3 is much lower than the K_m of glutamate dehydrogenase for $NH_4^{\oplus}$.
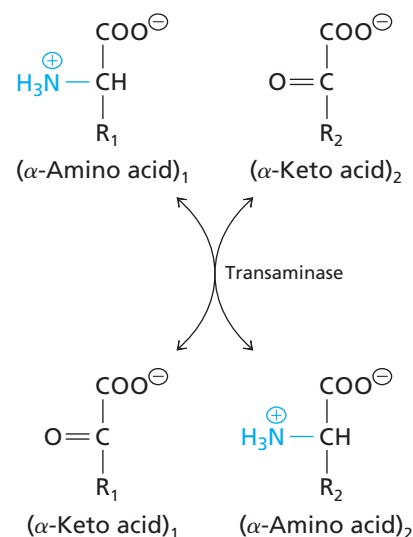
▲ **Figure 17.7**
Transfer of an amino group from an α-amino acid to an α-keto acid, catalyzed by a transaminase. In biosynthetic reactions $(\alpha\text{-amino acid})_1$ is often glutamate, with its carbon skeleton producing α-ketoglutarate [= $(\alpha\text{-keto acid})_1$]. $(\alpha\text{-keto acid})_2$ represents the precursor of a newly formed acid, $(\alpha\text{-amino acid})_2$.
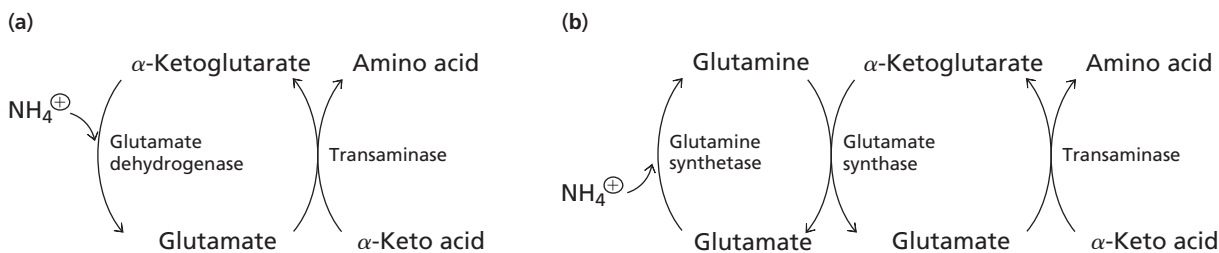
▲ **Figure 17.8**
Assimilation of ammonia into amino acids. (a) The glutamate dehydrogenase pathway. **(b)** Combined action of glutamine synthetase and glutamate synthase under conditions of low $NH_4^{\oplus}$ concentration.

17.3 Synthesis of Amino Acids

We now turn our attention to the origins of the carbon skeletons of amino acids. Figure 17.9 shows how the biosynthesis pathways leading to the 20 common amino acids are related to other metabolic pathways. Note that 11 of the 20 common amino acids are synthesized from intermediates in the citric acid cycle. The others require simple precursors that we have encountered in previous chapters.

A. Aspartate and Asparagine

Oxaloacetate is the amino group acceptor in a transamination reaction that produces aspartate (Figure 17.10). The enzyme that catalyzes this reaction is aspartate transaminase (L-aspartate:2-oxoglutarate aminotransferase). Asparagine is synthesized in most species by an ATP-dependent transfer of the amide nitrogen of glutamine to aspartate in a reaction catalyzed by asparagine synthetase. In some bacteria, asparagine synthetase catalyzes the formation of asparagine from aspartate using ammonia instead of glutamine as the source of the amide group. This reaction is similar to the reaction catalyzed by glutamine synthetase.

Some asparagine synthetases can use either ammonia or glutamine as the substrate. These enzymes use $NH_4^{\oplus}$ at the primary reaction site but they have a second site that catalyzes hydrolysis of glutamine and release of $NH_4^{\oplus}$. The $NH_4^{\oplus}$ intermediate diffuses through a tunnel in the protein that connects the two active sites. This example of molecular channeling ensures that the hydrolysis of glutamine is tightly coupled to the formation of asparagine and it prevents the accumulation of $NH_4^{\oplus}$ in the cell. There are many examples of molecular tunnels that facilitate the channeling of $NH_4^{\oplus}$ (see Box 18.2).

B. Lysine, Methionine, and Threonine

Aspartate is the precursor for synthesis of lysine, methionine, and threonine (Figure 17.11). The first step in the pathway is the phosphorylation of aspartate in a reaction catalyzed by aspartate kinase. In the second step, aspartyl phosphate is converted to aspartate β-semialdehyde. This second reaction is catalyzed by aspartate semialdehyde dehydrogenase. These two enzymes are present in bacteria, protists, fungi, and plants but they are missing in animals. Consequently, animals are not able to synthesize lysine, methionine, and threonine (see Box 17.3).

The first two reactions leading to aspartate β-semialdehyde are common to the formation of all three amino acids. In the branch leading to lysine, pyruvate is the source of carbon atoms added to the skeleton of aspartate β-semialdehyde and glutamate is the

▼ **Figure 17.9**
Biosynthesis of amino acids, showing the connections to glycolysis/gluconeogenesis and the citric acid cycle.
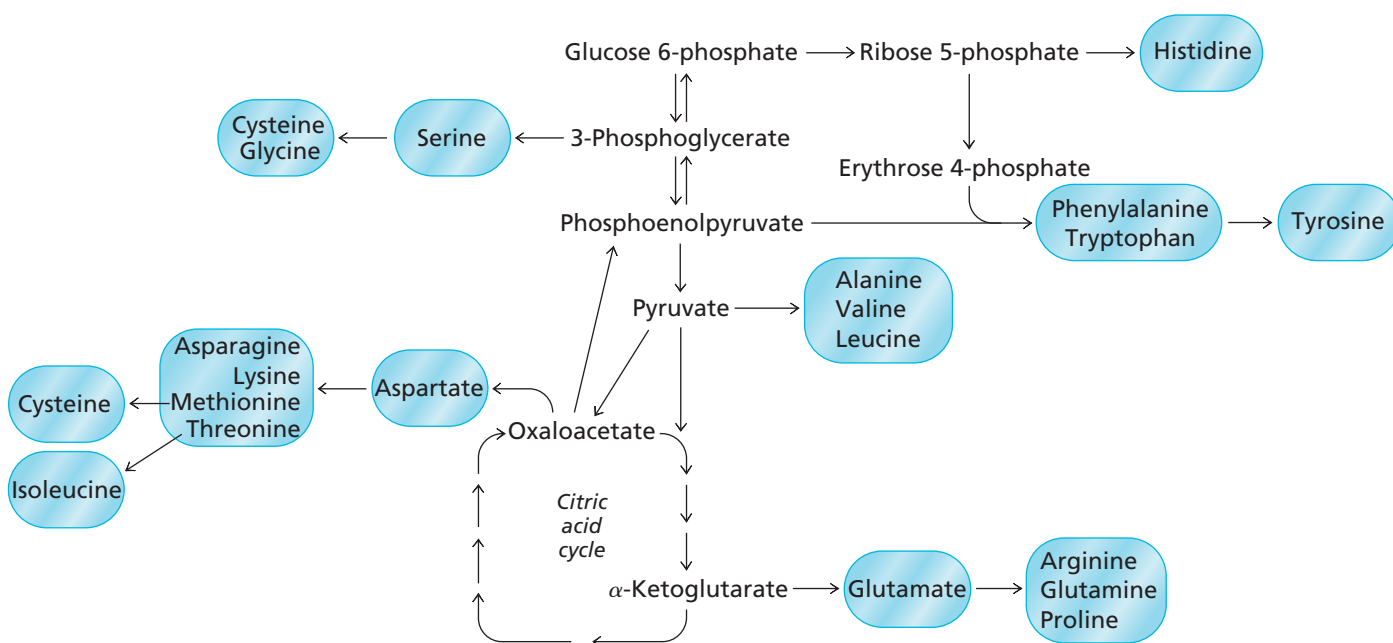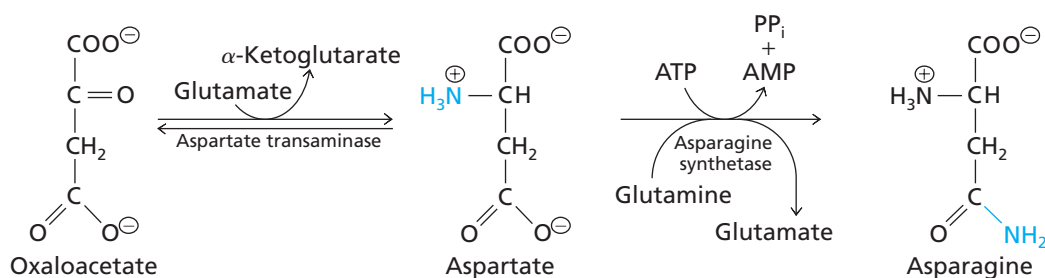
Synthesis of aspartate and asparagine.

(a)

(b)

▲ **Asparaginases. (a)** *Escherichia coli* [PDB 1NNS] **(b)** *Erwinia chrysanthemi* [PDB 1O7J]

source of the ε-amino group. Lysine is produced by an entirely different route in yeast and some algae.

Homoserine is formed from aspartate β-semialdehyde. It is a branch point for the formation of threonine and methionine. Threonine is derived from homoserine in two steps, one of which requires PLP. In the methionine pathway homoserine is converted to homocysteine in three steps. The sulfur atom of homocysteine then accepts a methyl group derived from 5-methyltetrahydrofolate forming methionine. The enzyme that catalyzes this reaction is homocysteine methyltransferase, one of the few enzymes that requires cobalamin (Section 7.12). Homocysteine methyltransferase is found in mammals but its activity is low and the supply of homocysteine is limited. Therefore, methionine remains an essential amino acid in mammals due primarily to the absence of the first two enzymes in the pathway.

C. Alanine, Valine, Leucine, and Isoleucine

Pyruvate is the amino group acceptor in the synthesis of alanine by a transamination reaction (Figure 17.12). Pyruvate is also a precursor in the synthesis of the branched chain amino acids valine, leucine, and isoleucine. The first step in the branched chain pathway is the synthesis of α-ketobutyrate from threonine.

Pyruvate combines with α-ketobutyrate in a series of three reactions leading to the branched chain intermediate α-keto-β-methylvalerate. This intermediate is converted to isoleucine in a transamination reaction. Note the similarity between the structures of α-ketobutyrate and pyruvate. The same enzymes that catalyze the synthesis of α-keto-β-methylvalerate also catalyze the synthesis of α-ketoisovalerate by combining two molecules of pyruvate instead of one molecule of pyruvate and one molecule of α-ketobutyrate. α-Ketoisovalerate is converted directly to valine by valine transaminase—the same enzyme catalyzes the synthesis of isoleucine from α-keto-β-methylvalerate (Figure 17.13). These pathways illustrate an important point, namely that some enzymes recognize several different but similar substrates. At some point in the future the eukaryotic genes for these enzymes might be duplicated and each of the two copies would evolve to become specific for either the isoleucine or valine pathways. If this happened, it would be an example of pathway evolution by gene duplication and divergence (Section 10.2D). We see
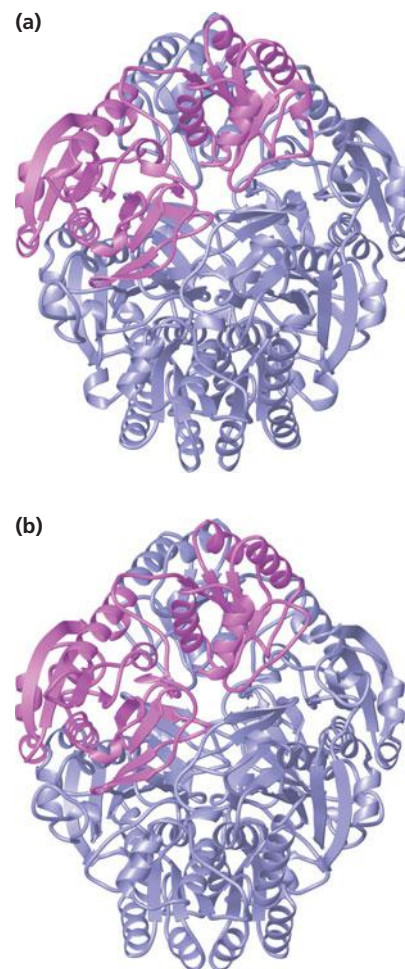
---

BOX 17.1 CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA CAN BE TREATED WITH ASPARAGINASE

Acute lymphoblastic leukemia (ALL) is caused by the proliferation of malignant T-cell lymphoblasts due, in most cases, to a mutation caused by mistakes in genetic recombination during the activation of T-cell receptor genes. Malignant lymphoblasts have reduced levels of asparagine synthetase and are unable to synthesize enough asparagine to support their rapid growth and proliferation. Unlike normal cells, they must obtain asparagine from the blood plasma.

This cancer can be successfully treated with injections of asparaginase, an *E. coli* enzyme that breaks down asparagine in the plasma (Section 17.6A). The malignant cells die in the absence of an available source of asparagine. Treatment with

asparaginase alone causes remission in 50% of all cases of childhood acute lymphoblastic leukemia and the success rate is even higher when the enzyme treatment is combined with other chemotherapy. The primary cause of resistance to the treatment is due to increased expression of asparagine synthetase in the cancer cells.

Patients often develop antibodies to the *E. coli* enzyme during treatment. Switching to the homologous enzyme from *Erwinia chrysanthemi* is often effective because the amino acid side chains on the surface of the two proteins are different. Antibodies directed against one enzyme usually don't recognize the other.
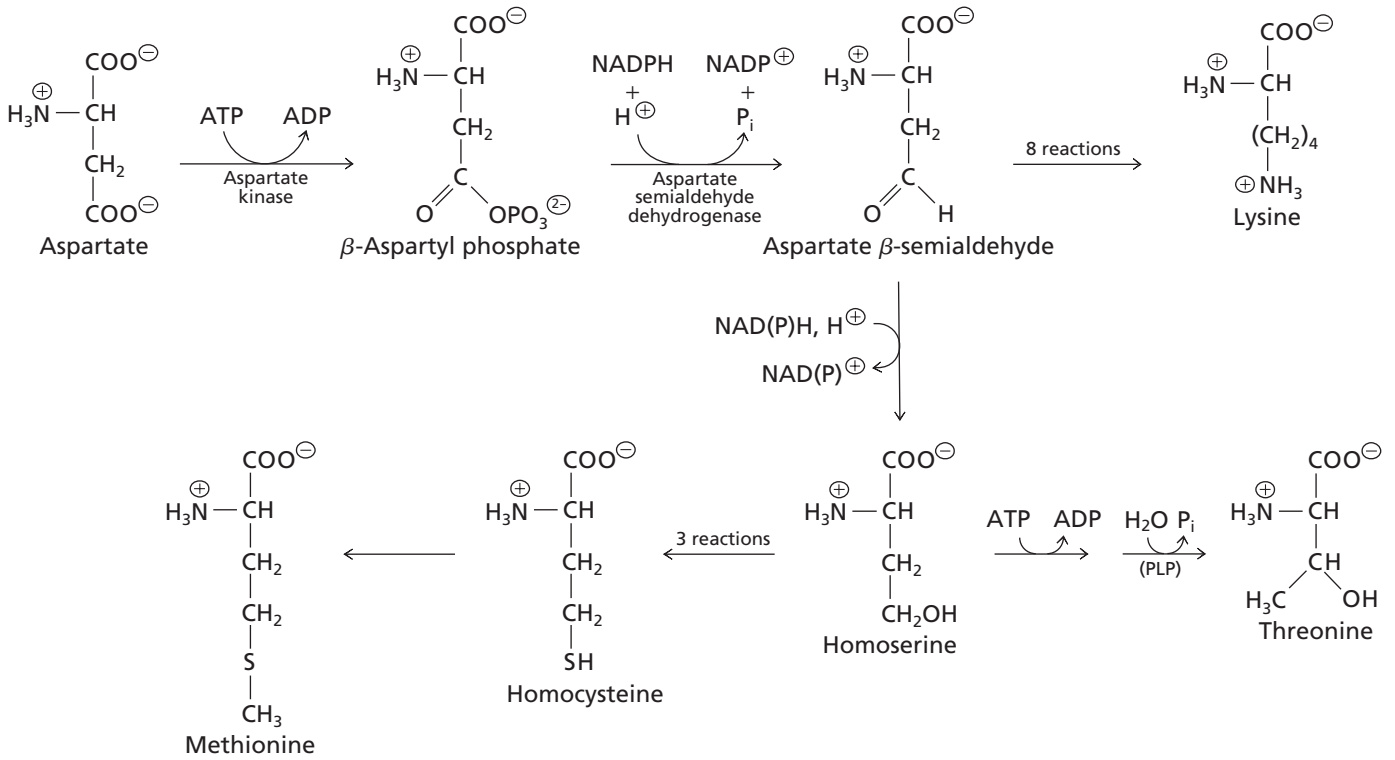
▲ Figure 17.11
Biosynthesis of lysine, threonine, and methionine from aspartate.
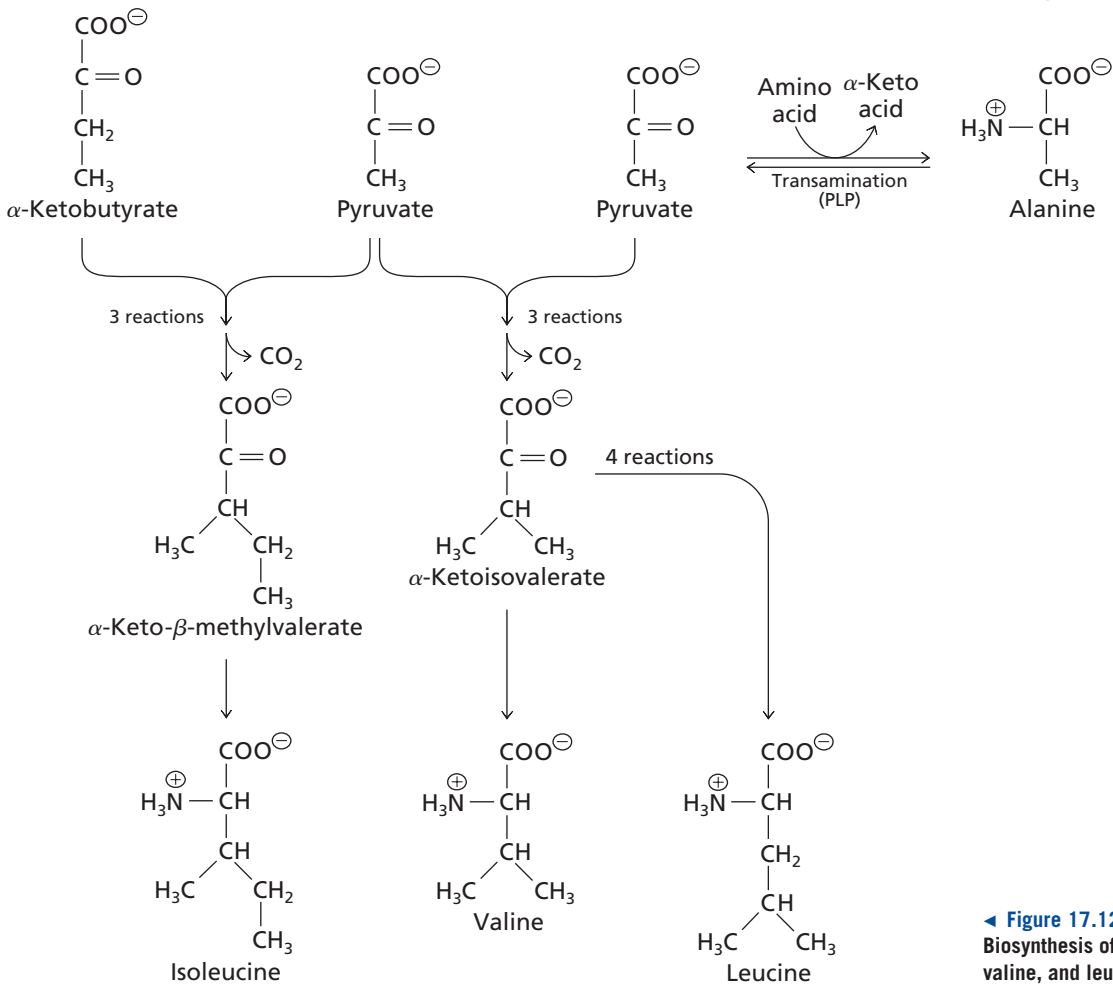
◄ Figure 17.12
Biosynthesis of alanine, isoleucine, valine, and leucine.

many examples of pathway evolution by gene duplication involving enzymes of amino acid metabolism (see below). The basic requirement is that in the early stages the same enzyme can catalyze two similar reactions and that is what we see in the isoleucine and valine synthesis pathways.

The carbon skeleton of α-ketoisovalerate is lengthened by one methylene group to form leucine in a pathway that branches from the valine biosynthetic pathway. Two of the enzymes in this pathway are homologous to aconitase and isocitrate dehydrogenase in the citric acid cycle lending support to the idea that citric acid cycle enzymes evolved from preexisting enzymes required for amino acid biosynthesis (Section 13.8).

D. Glutamate, Glutamine, Arginine, and Proline

We have seen how glutamate and glutamine are formed from the citric acid cycle intermediate α-ketoglutarate (Section 17.2B). The carbon atoms of proline and arginine also come from α-ketoglutarate, via glutamate. Proline is synthesized from glutamate by a four-step pathway in which the 5-carboxylate group of glutamate is reduced to an aldehyde. The glutamate 5-semialdehyde intermediate undergoes nonenzymatic cyclization to a Schiff base, 5-carboxylate, that is reduced by a pyridine nucleotide coenzyme to produce proline (Figure 17.14).

The pathway to arginine is similar in most species except that the α-amino group of glutamate is acetylated before the aldehyde is formed. This step prevents the cyclization that occurs in the synthesis of proline. The N-acetylglutamate 5-semialdehyde intermediate is then converted to N-acetyl ornithine and ornithine. In mammals, glutamate 5-semialdehyde is transaminated to ornithine and ornithine is converted to arginine by the reactions of the urea cycle (Section 17.7).

E. Serine, Glycine, and Cysteine

Three amino acids—serine, glycine, and cysteine—are derived from the glycolytic/gluconeogenic intermediate 3-phosphoglycerate. Serine is synthesized from 3-phosphoglycerate in three steps (Figure 17.15). First, the secondary hydroxyl substituent of
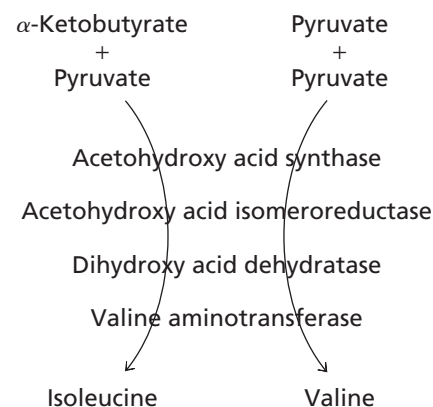
▲ **Figure 17.13**
The isoleucine and valine synthesis pathways share four enzymes.

Figure 17.14 ▶
Conversion of glutamate to proline and arginine.
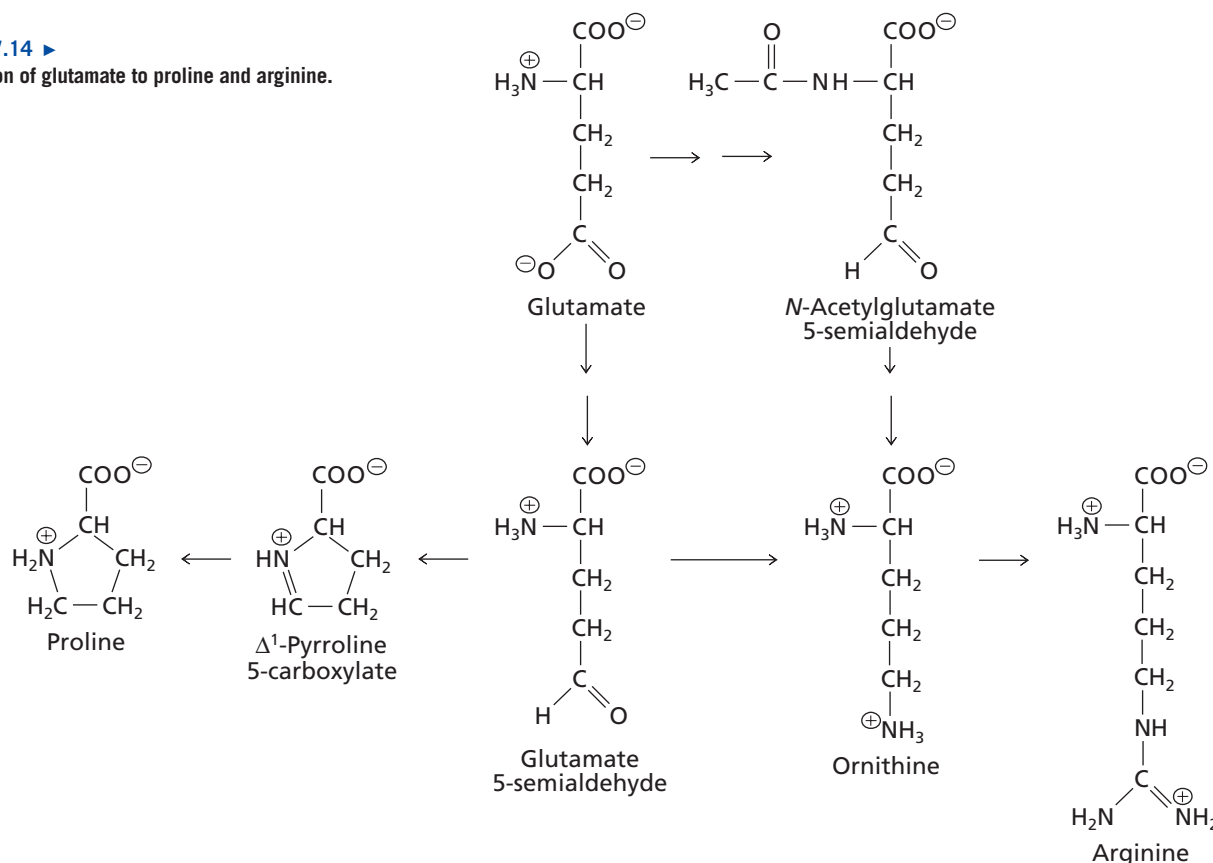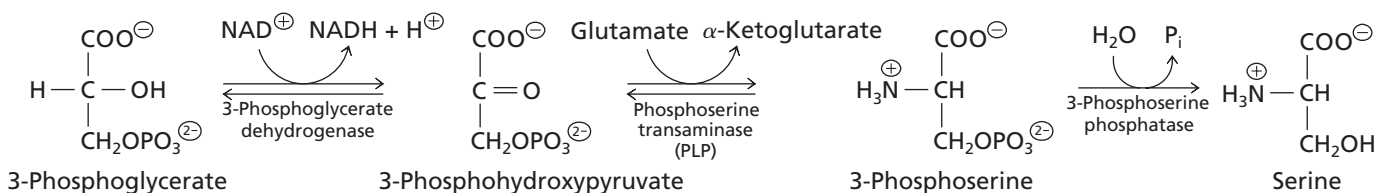
▲ **Figure 17.15**
Biosynthesis of serine.

3-phosphoglycerate is oxidized to a keto group, forming 3-phosphohydroxypyruvate. This compound undergoes transamination with glutamate to form 3-phosphoserine and α-ketoglutarate. Finally, 3-phosphoserine is hydrolyzed to give serine and P_i.

Serine is a major source of glycine via a reversible reaction catalyzed by serine hydroxymethyltransferase (Figure 17.16). In plant mitochondria and bacteria, the flux through this reaction is toward serine providing a route to serine that differs from that in Figure 17.15. The serine hydroxymethyltransferase reaction requires two cofactors: the prosthetic group PLP and the cosubstrate tetrahydrofolate.

The biosynthesis of cysteine from serine occurs in two steps (Figure 17.17). First, an acetyl group from acetyl CoA is transferred to the β-hydroxyl substituent of serine, forming O-acetylserine. Next, sulfide ($S^{\ominus}$) displaces the acetate group, and cysteine is formed.

Animals do not have the normal cysteine biosynthesis pathway shown in Figure 17.17. Nevertheless, cysteine can still be synthesized in animals as a by-product of methionine degradation (Section 17.6F). Serine condenses with homocysteine, an intermediate in the degradation of methionine. The product of the condensation reaction, crystathionine, is cleaved to α-ketobutyrate and cysteine (Figure 17.18).

F. Phenylalanine, Tyrosine, and Tryptophan

The key to elucidation of the pathway for aromatic amino acid synthesis was the observation that some bacteria with single-gene mutations require as many as five compounds for growth: phenylalanine, tyrosine, tryptophan, p-hydroxybenzoate, and p-aminobenzoate. These compounds all contain an aromatic ring. The inability of these mutants to grow without these compounds is reversed when shikimate is provided indicating that shikimate is an intermediate in the biosynthesis of all these aromatic compounds.

Chorismate, a derivative of shikimate, is a key branch-point intermediate in aromatic amino acid synthesis. The pathway to shikimate and chorismate (Figure 17.19) begins with condensation of phosphoenolpyruvate and erythrose 4-phosphate to form a seven-carbon sugar derivative and P_i. Three additional steps, including cyclization, are required to produce shikimate. The pathway from shikimate to chorismate involves phosphorylation of shikimate, addition of a three-carbon group from phosphoenolpyruvate, and dephosphorylation. Pathways from chorismate lead to phenylalanine, tyrosine, and tryptophan. Animals do not have the enzymes of the chorismate pathway. They cannot synthesize chorismate and, consequently, cannot synthesize any of the aromatic amino acids.

A branched pathway leads from chorismate to phenylalanine or tyrosine (Figure 17.20). In phenylalanine synthesis in *E. coli*, a bifunctional chorismate mutase–prephenate dehydratase catalyzes the rearrangement of chorismate to produce prephenate, a highly reactive compound. Next, the enzyme catalyzes the elimination of a hydroxide ion and CO_2

▼ **Figure 17.16**
Biosynthesis of glycine.
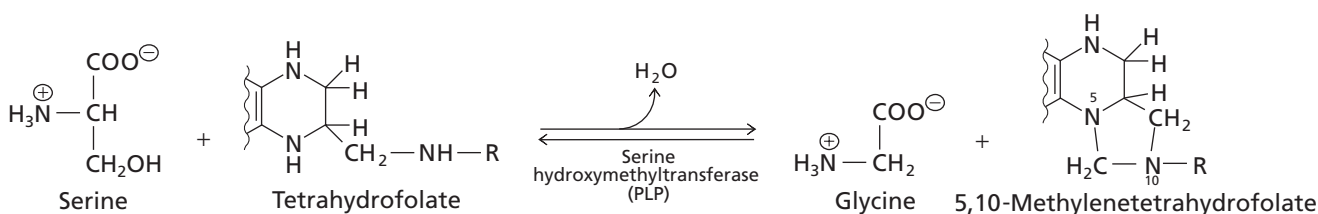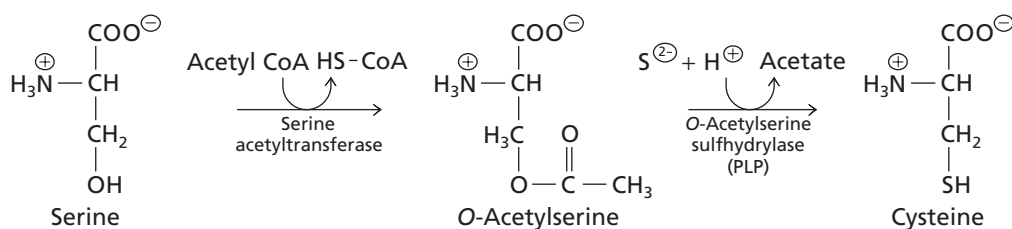
from prephenate to form the fully aromatic product phenylpyruvate that is then transaminated to phenylalanine.

A similar bifunctional chorismate mutase–prephenate dehydrogenase catalyzes the formation of prephenate and then 4-hydroxyphenylpyruvate in the tyrosine branch. The intermediate undergoes transamination to form tyrosine. Several bacteria and some plants follow the same pathways from chorismate to phenylalanine and tyrosine as *E. coli* although their chorismate mutase and prephenate dehydratase or prephenate dehydrogenase activities are on separate polypeptide chains. Some other bacteria use alternate pathways in which prephenate is first transaminated and then decarboxylated.

The biosynthesis of tryptophan from chorismate requires five enzymes. In the first step, the amide nitrogen of glutamine is transferred to chorismate. Subsequent elimination of the hydroxyl group and the adjacent pyruvate moiety of chorismate produces the aromatic compound anthranilate (Figure 17.21). Anthranilate accepts a phosphoribosyl moiety from PRPP. Rearrangement of the ribose, decarboxylation, and ring closure generate indole glycerol phosphate.

The final two reactions of tryptophan biosynthesis are catalyzed by tryptophan synthase (Figure 17.22). In some organisms, the two independent catalytic domains of tryptophan synthase are contained on a single polypeptide chain but in some species the enzyme contains two types of subunits in an $\alpha_2\beta_2$ tetramer. The α subunit, or domain, catalyzes the cleavage of indole glycerol phosphate to glyceraldehyde 3-phosphate and indole. The β subunit, or domain, catalyzes the condensation of indole and serine in a reaction that requires PLP as a cofactor. The indole produced in the reaction catalyzed by the α subunit of $\alpha_2\beta_2$ tetramers is channeled (i.e., transferred directly) to the active site of the β subunit. When the three-dimensional structure of tryptophan synthase from *Salmonella typhimurium* (an organism whose tryptophan synthase has the $\alpha_2\beta_2$ oligomeric structure) was determined by X-ray crystallography, a tunnel joining the α and β active sites was discovered. The diameter of the tunnel matches the molecular dimensions of indole, so passage of indole through the tunnel would explain why
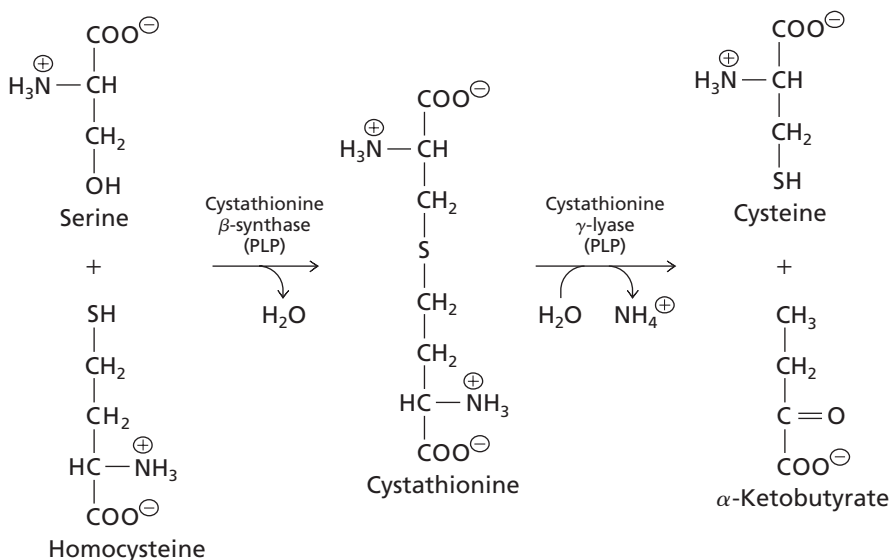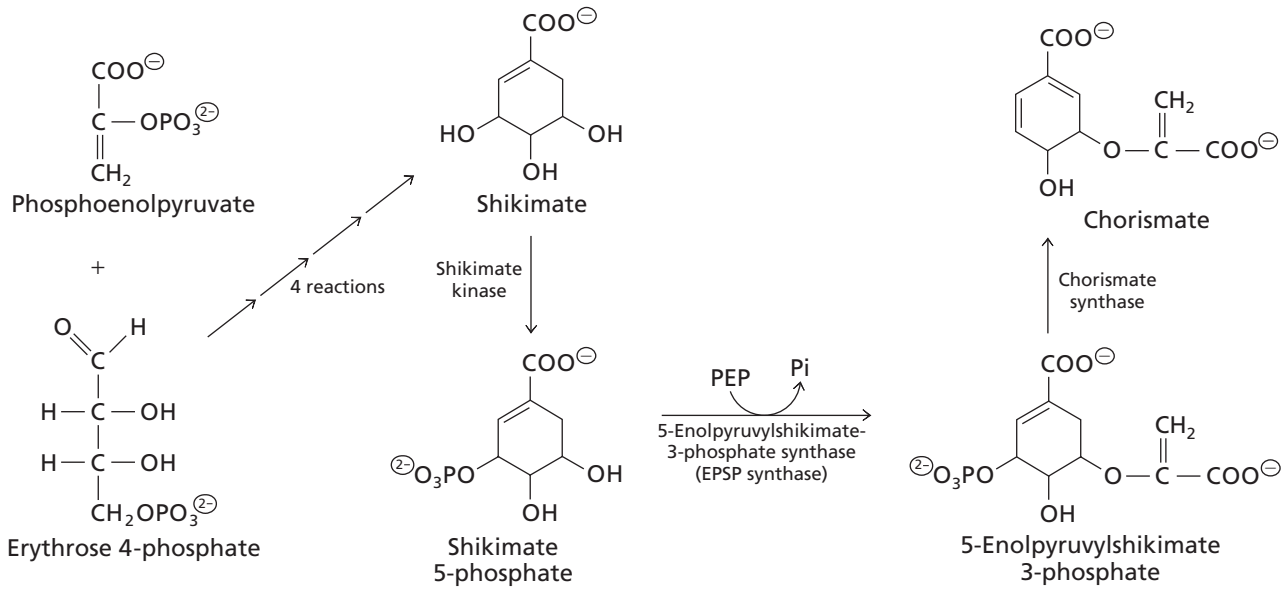
▲ **Figure 17.19**
Synthesis of shikimate and chorismate.

Figure 17.20 ►
Biosynthesis of tryptophan, phenylalanine, and tyrosine from chorismate in *E. coli*.
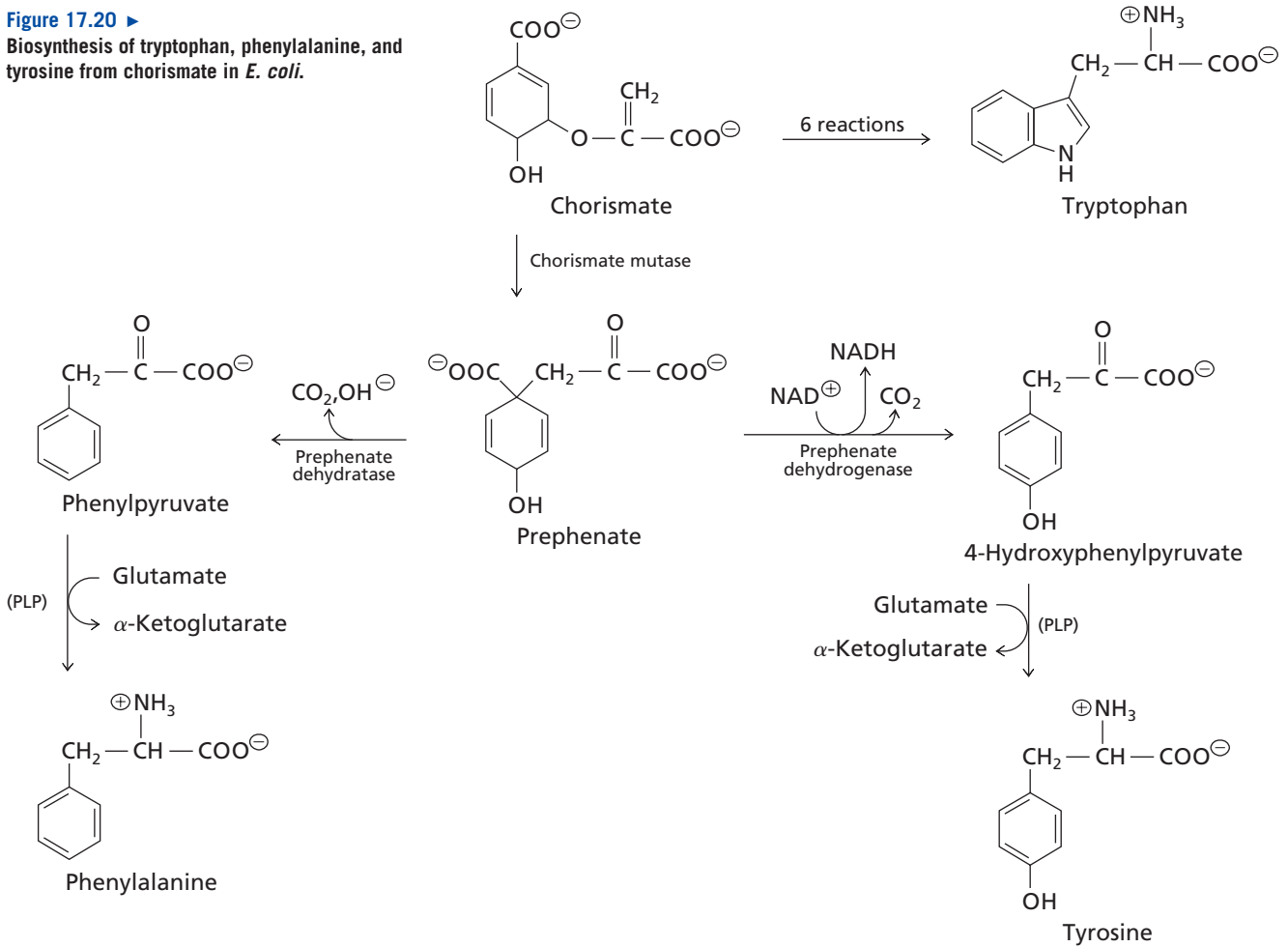
indole does not diffuse away. This was one of the earliest examples of metabolic channeling (Section 5.10). Up until quite recently there were only a few other examples and the phenomenon was thought to be rare. The huge increase in structural and genomic studies has revealed many more examples—including half a dozen in this chapter alone.

G. Histidine

The ten-step pathway for the biosynthesis of histidine in bacteria begins with a condensation between the six-membered ring of ATP and a ribose derivative, phosphoribosyl pyrophosphate (PRPP) (Figure 17.23). The six-membered ring of the adenine moiety is then cleaved and glutamine donates a nitrogen atom that is incorporated via cyclization
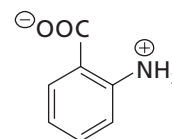
▲ **Figure 17.21**
Anthranilate.
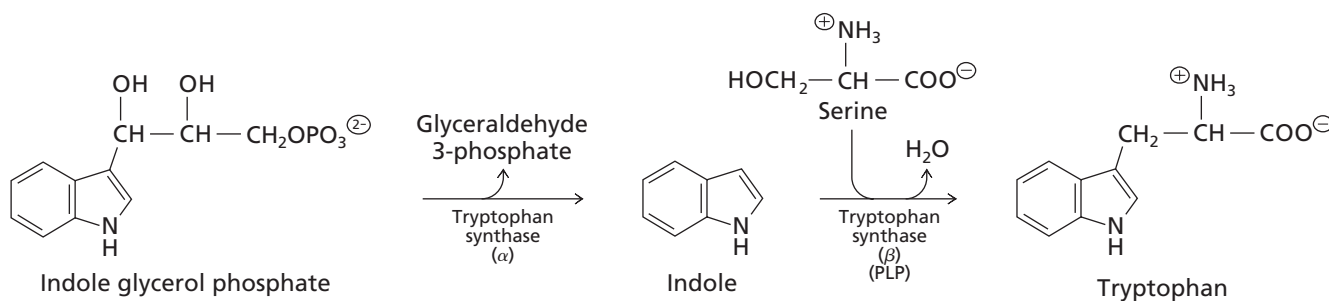
▲ **Figure 17.22**
Reactions catalyzed by tryptophan synthase.

▼ **Figure 17.23**
Synthesis of histidine from phosphoribosyl pyrophosphate (PRPP) and ATP.
Histidine is derived from PRPP (5 C atoms), the purine ring of ATP
(1 N and 1 C), glutamine (1 N), and glutamate (1 N).
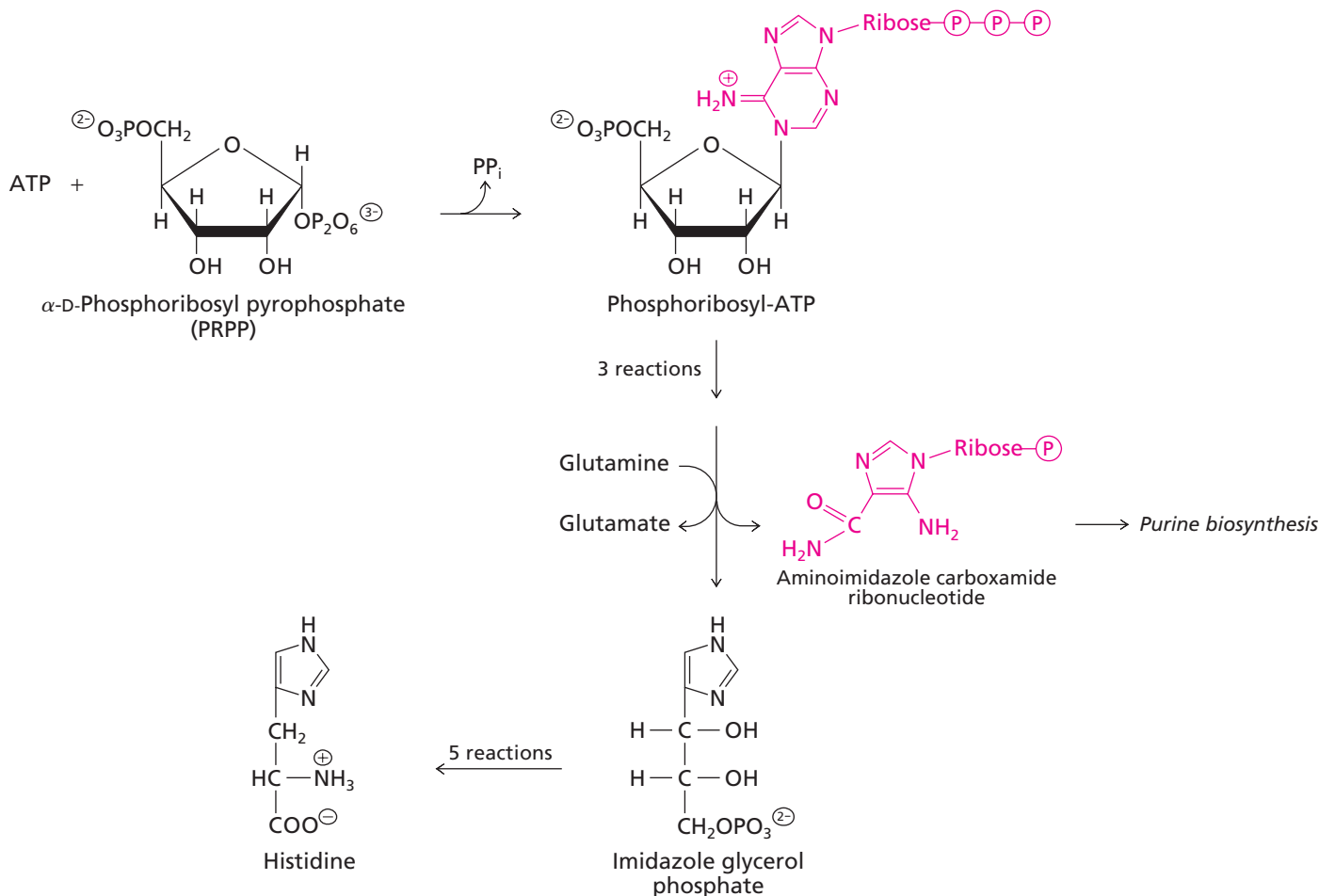
BOX 17.2 GENETICALLY MODIFIED FOOD

The chorismate pathway is an effective target for herbicides since compounds that specifically block this pathway in plants will have no effect on animals. One of the most effective general herbicides is glyphosate. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) by acting as a competitive inhibitor of PEP binding (Section 5.7A).

Glyphosate is the active ingredient in Roundup®, a herbicide that kills all plants. It is used to remove weeds from driveways and stone pathways. Although it is cheap and effective as a weed killer, glyphosate cannot be used to spray actively growing food crops since it indiscriminately kills all plants, including the crop!

$$^{\ominus}O_3P—CH_2—NH—CH_2—COO^{\ominus}$$
Glyphosate
(*N*-(phosphonomethyl) glycine)
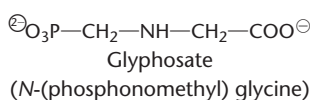
Resistant versions of EPSP synthase have been identified in many species of bacteria. The enzyme from strain CP4 of *Agrobacterium sp.* has been genetically modified to remain fully active in the presence of high concentrations of glyphosate. The gene for this bacterial CP4-EPSP synthase was patented and then introduced into soybeans creating a genetically modified plant that is resistant to glyphosate. The new strain

of soybeans is marketed by Monsanto as Roundup Ready® soybeans. Farmers who grow crops of Roundup Ready® soybeans are able to spray them with Roundup® (also sold by Monsanto) to kill weeds. The economic advantages to farmers are significant. Most of the soybeans currently grown in North America are genetically modified.

Other Roundup Ready® crop plants are now available. Versions of corn, cotton, and canola are widely used.

▲ *E. coli* 5-enolpyruvylshikimate-3-phosphate synthase with a molecule of glyphosate bound to the active site. [PDB 2AAY]

KEY CONCEPT

Metabolic channeling evolves to improve kinetic efficiency.

into the imidazole ring of the product, imidazole glycerol phosphate. Most of the carbon and nitrogen atoms of ATP are released as aminoimidazole carboxamide ribonucleotide, an intermediate in purine biosynthesis (Section 18.1). This metabolite then can be recycled into ATP. Imidazole glycerol phosphate undergoes dehydration, transamination by glutamate, hydrolytic removal of its phosphate, and oxidation from the level of a primary alcohol to that of a carboxylic acid in two sequential $NAD^{\oplus}$-dependent steps forming histidine.
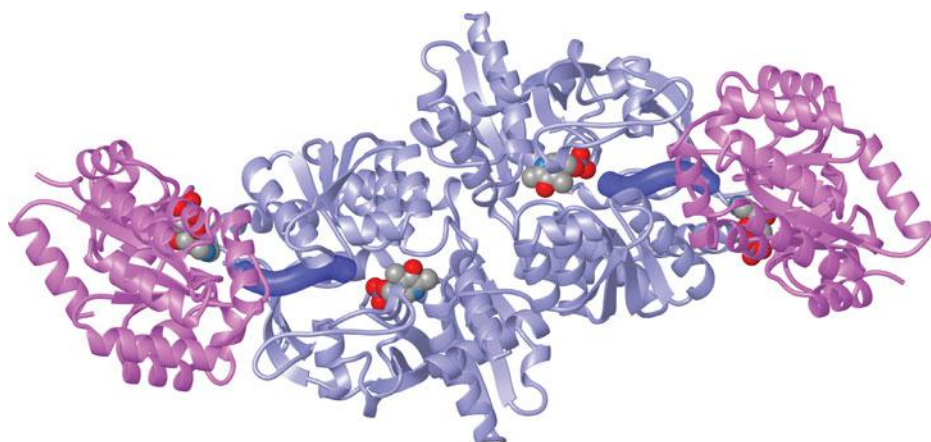
▲ **Tryptophan synthase from *Salmonella typhimurium*.** The substrate indole glycerol phosphate is shown as a space-filling molecule bound to the α subunits. The cofactor PLP is bound to the β subunits. The enzyme contains a channel leading from the indole glycerol phosphate binding site to the PLP reaction site. [PDB 1QOQ]

BOX 17.3 ESSENTIAL AND NONESSENTIAL AMINO ACIDS IN ANIMALS

Humans and other animals do not possess the enzymes required for the synthesis of all amino acids. Those that cannot be synthesized are, therefore, essential components of the human diet. As a general rule, the pathways that have been lost are the ones with the most steps. A crude measure of the complexity of a pathway is the number of moles of ATP (or its equivalent) required in a pathway.

The table shows the correlation between the expense of a particular pathway and whether an amino acid is essential. The amino acids are grouped according to their common precursors as described in the previous sections. Note that lysine, methionine, and threonine are derived from a common precursor (Section 17.3B). All three amino acids are essential because animals cannot synthesize the precursor. Valine, leucine, and isoleucine are essential because animals lack the key enzymes that all three biosynthesis pathways share (Section 17.3C).

Energy requirements for biosynthesis of amino acids

Amino acids	Moles of ATP required per mole of amino acid produced[a]	
	Nonessential	Essential
Aspartate	21	
Asparagine	22–24	
Lysine		50 or 51
Methionine		44
Threonine		31
Alanine	20	
Valine		39
Leucine		47
Isoleucine		55
Glutamate	30	
Glutamine	31	
Arginine	44[b]	
Proline	39	
Serine	18	
Glycine	12	
Cysteine	19[c]	
Phenylalanine		65
Tyrosine	62[d]	
Tryptophan		78
Histidine		42

[a]Moles of ATP required includes ATP used for synthesis of precursors and conversion of precursors to products.

[b]Essential in some mammals.

[c]Cysteine can be synthesized from homocysteine and homocysteine is a degradation product of methionine. The biosynthesis of cysteine depends on an adequate supply of methionine in the diet.

[d]Tyrosine can be synthesized from the essential amino acid phenylalanine.

17.4 Amino Acids as Metabolic Precursors

The primary role of amino acids is to serve as substrates for protein synthesis. In this role, newly synthesized amino acids are activated by covalent attachment to tRNA and the pool of aminoacyl–tRNAs is used as the substrate for polypeptide synthesis by the protein synthesis machinery. We devote an entire chapter to this fundamentally important biosynthesis pathway (Chapter 22).

Some amino acids are essential precursors in other biosynthesis pathways. The list is long and it's impossible to mention every pathway. Some important regulatory amines were described in Section 3.3 (histamine, GABA, epinephrine, thyroxine). The important role of methionine in the synthesis of *S*-adenosylmethionine will be described in Section 17.6F.

A. Products Derived from Glutamate, Glutamine, and Aspartate

We've already seen that glutamate and glutamine are important players in nitrogen assimilation. In addition, glutamate and aspartate are amino group donors in many transamination reactions. We will see that glutamate and aspartate are required in the urea cycle. Glutamine and aspartate are also required as precursors in both purine biosynthesis (Section 18.1) and pyrimidine biosynthesis (Section 18.3). Recall that synthesis of biologically active tertahydrofolate involves addition of up to six glutamate residues to the tetrahydrofolate moiety (Section 7.10).

B. Products Derived from Serine and Glycine

Serine and glycine are metabolic precursors of many other compounds (Figure 17.24). The role of serine in lipid biosynthesis has already been described in the previous chapter.

Phenylalanine
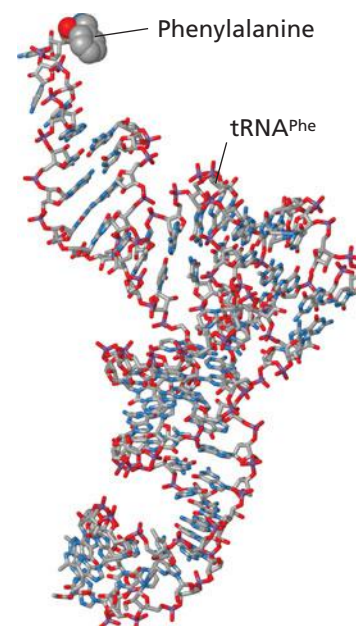
tRNA^Phe

▲ **Phenylanyl–tRNA^Phe.** Most newly synthesized amino acids are rapidly attached to their corresponding tRNAs and used in protein synthesis. [PDB 1TTT]
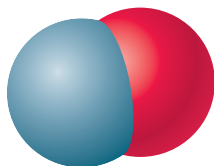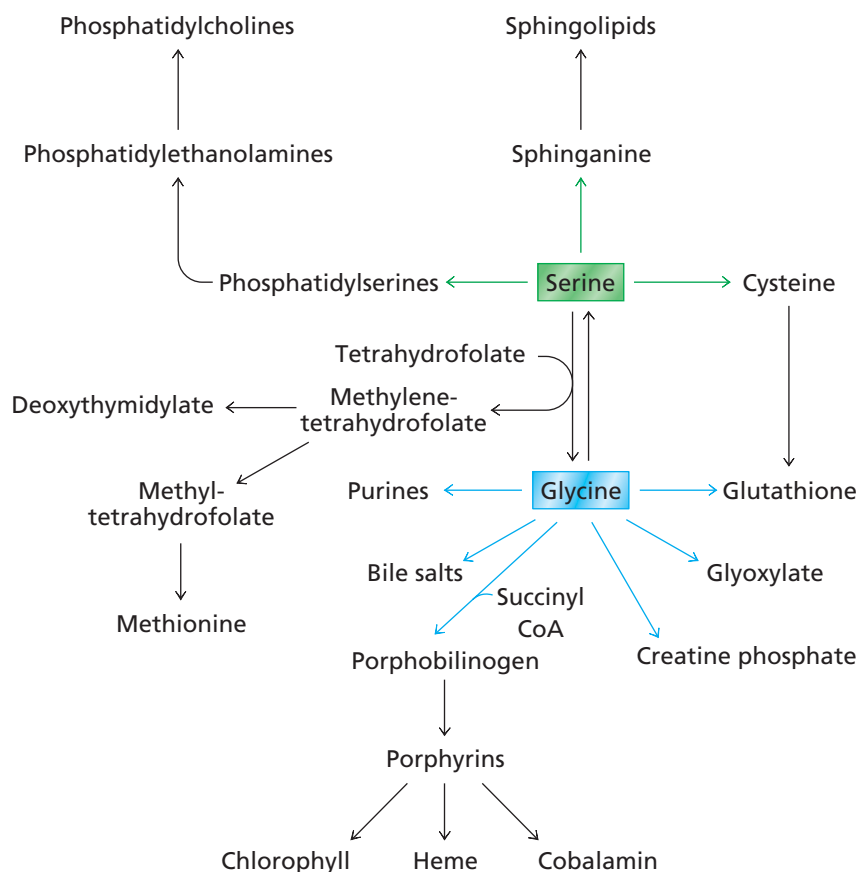
Glycine and succinyl CoA are the main precursors in the porphyrin pathway leading to heme and chlorophyll. Glycine is also required in purine biosynthesis (Section 18.1).

The conversion of serine to glycine is coupled to the synthesis of methylene tetrahydrofolate. Tetrahydrofolate derivatives are important in many reactions that catalyze transfer of one-carbon units (Section 17.10). One of the most important of these reactions is the synthesis of deoxythymidylate (Figure 18.15).

C. Synthesis of Nitric Oxide from Arginine

One of the more interesting examples of amino acids as metabolic precursors is the role of arginine as substrate for synthesis of nitric oxide, an unstable gaseous derivative of nitrogen with an odd number of electrons ($\cdot N {=} O$). Although it is a reactive free radical and potentially toxic, nitric oxide is physiologically important—so important, in fact, that it was named the 1992 "Molecule of the Year" by the journal *Science*. As a gas, NO can diffuse rapidly into cells. It exists *in vivo* for only a few seconds because nitric oxide in aqueous solution reacts rapidly with oxygen and water to form nitrates and nitrites.

An enzyme found in mammals, nitric oxide synthase, catalyzes the formation of nitric oxide and citrulline from arginine (Figure 17.25). The reaction requires the cofactors NADPH, FMN, FAD, a cytochrome P450, and tetrahydrobiopterin (Section 7.10). The mechanism of action of tetrahydrobiopterin in this reaction has not yet been elucidated but it appears to be a reducing agent needed for the hydroxylation of arginine. Nitric oxide synthase is present in two forms, a constitutive (i.e., constantly synthesized) calcium-dependent form in brain and endothelial cells and an inducible (i.e., variably synthesized) calcium-independent form in macrophages (a type of white blood cell).

Nitric oxide is a messenger molecule that binds to a soluble guanylyl cyclase and stimulates the formation of cyclic GMP (Section 9.12B). It has several functions; for example, when macrophages are stimulated, they synthesize nitric oxide. The short-lived nitric oxide free radical is one of the weapons used by macrophages to kill bacteria and tumor cells. Nitric oxide may interact with superoxide anions ($\cdot O_2^{\ominus}$) to form more toxic reactants that account for the cell-killing activity.

▲ **Nitric oxide**

In 1998 Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad were awarded the Nobel Prize in Physiology or Medicine "for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system."
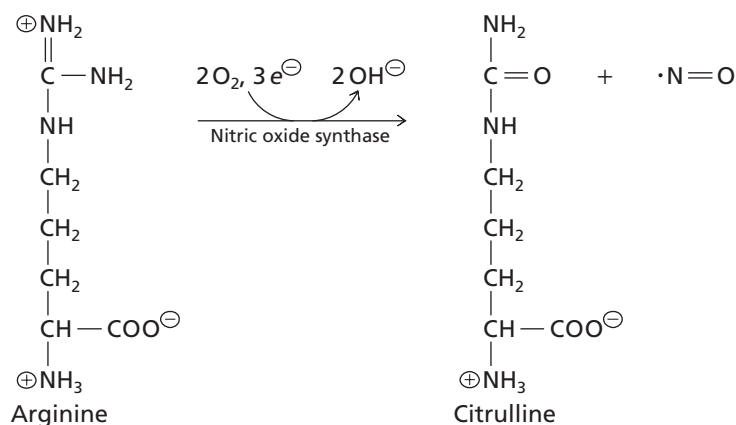
Conversion of arginine to nitric oxide and citrulline. NADPH is the source of the three electrons.

Nitric oxide synthase is also present in the cells that line blood vessels. Under certain conditions, nitric oxide is produced and diffuses to the smooth muscle cells of the vessels, causing them to relax and lower blood pressure. Hypertension and heart failure involve impaired relaxation of blood vessels. Nitroglycerin, used to dilate coronary arteries in the treatment of angina pectoris, exerts its effect by virtue of its metabolic conversion to nitric oxide.

Nitric oxide also functions as a neurotransmitter in brain tissue. Abnormally high amounts of nitric oxide formed during a stroke appear to kill some neurons in the same way macrophages kill bacteria. Administering an inhibitor of nitric oxide synthase to an animal produces some protection from stroke damage. One role of nitric oxide as a neurotransmitter is to stimulate erection of the penis. Sildenafil, the active ingredient in Viagra, is a drug used to alleviate erectile dysfunction. Sildenafil is a phosphodiesterase inhibitor that blocks the hydrolysis of cyclic GMP and therefore prolongs the stimulatory effect of nitric oxide. Tadalafil (Cialis) and vardenafil (Levitra) inhibit the same enzyme.

▲ **Sildenafil.** Sildenafil is the active ingredient in Viagra®.

D. Synthesis of Lignin from Phenylalanine

Lignin (Figure 17.26) is a series of complex polymers synthesized from phenylalanine. It is a major component of wood in flowering plants and may be the second most abundant biopolymer on the planet (after cellulose). Lignin cannot be broken down during digestion so in spite of the fact that animals ingest huge amounts of lignin it is metabolically inert. The only species that can break it down are various fungi that degrade fallen trees in the forest.

E. Melanin Is Made from Tyrosine

Melanin is a dark pigment found in bacteria, fungi, and animals. In humans it is responsible for skin color and hair color. Melanin is also the main component of the ink released by a frightened octopus.

The structure of melanin (eumelanin) is complex but the precursors are well known and the enzymes required in the pathway have been identified in a number of species. The first steps involve the conversion of L-tyrosine to L-DOPA and L-dopaquinone (Figure 17.27).

17.5 Protein Turnover

One might assume that only growing or reproducing cells would require new protein molecules (and therefore a supply of amino acids) but this is not the case. Proteins are continually synthesized and degraded in all cells, a process called turnover. Individual proteins turn over at different rates. Their half-lives can vary from a few minutes to several weeks but the half-life of a given protein in different organs and species is generally similar. Rapid protein turnover ensures that some regulatory proteins are degraded so that the cell can respond to constantly changing conditions. Such proteins have evolved to be relatively unstable.

▲ **Octopus ink is mostly melanin.**

Figure 17.26 ▶
Lignin. This is one of many possible structures of plant lignin.
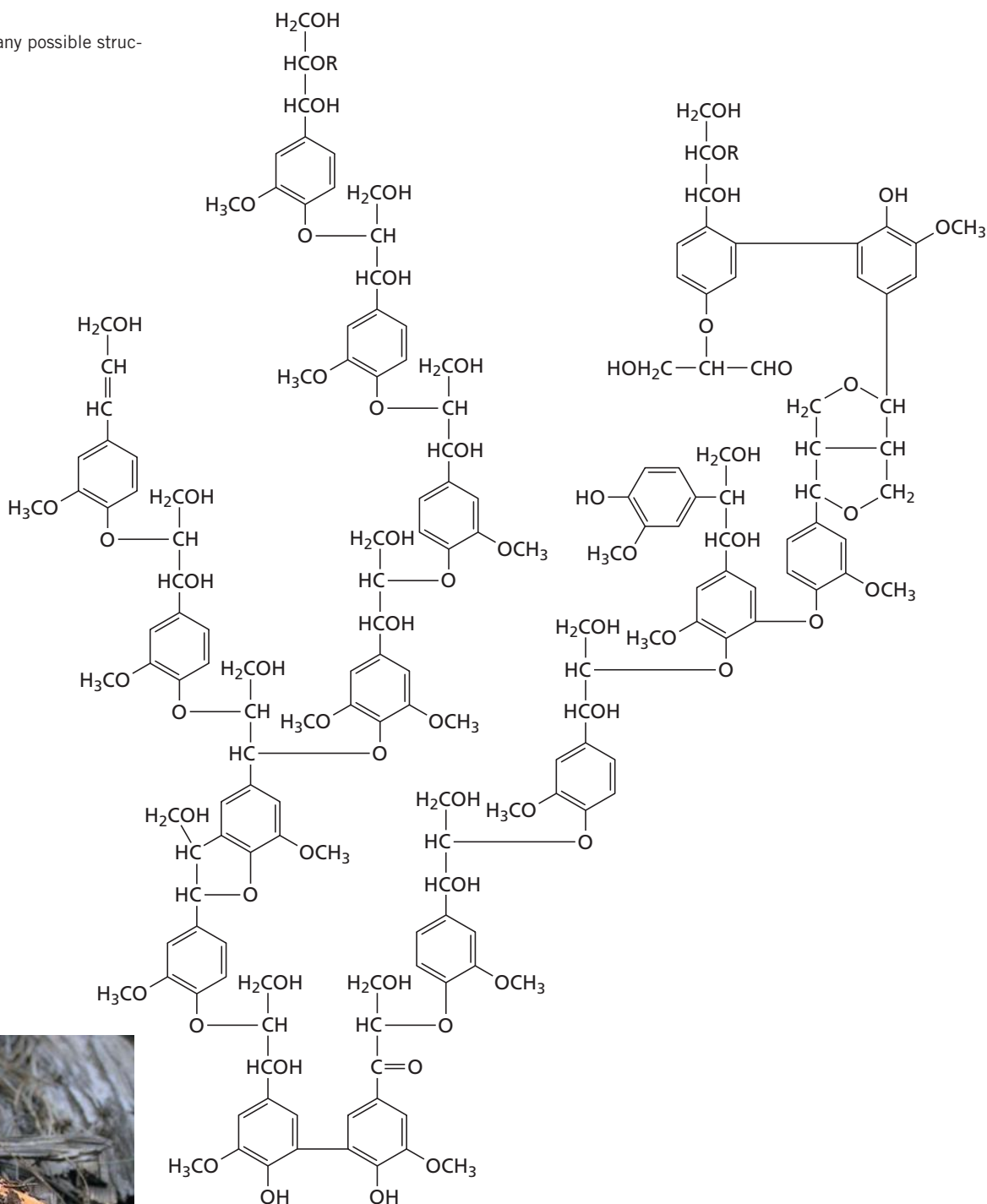
▲ **Rotting wood.** This mushroom is growing on rotting wood in a deciduous forest. Fungi are the only organisms that produce enzymes for breaking down lignin.

The rate of hydrolysis of a protein can be inversely related to the stability of its tertiary structure. Misfolded and unfolded proteins are quickly degraded (Section 4.10).

Some proteins are degraded to amino acids through lysosomal hydrolysis (in eukaryotic cells). Vesicles containing material to be destroyed fuse with lysosomes, and various lysosomal proteases hydrolyze the engulfed proteins. The lysosomal enzymes have broad substrate specificities so all the trapped proteins are extensively degraded.

Some proteins have very short half-lives because they are specifically targeted for degradation. Abnormal (mutated) proteins are also selectively hydrolyzed. The pathway for the selective hydrolysis of these proteins in eukaryotic cells requires the protein ubiquitin. Side-chain amino groups of lysine residues in the target protein are covalently linked to the C-terminus of ubiquitin in a complex pathway that involves
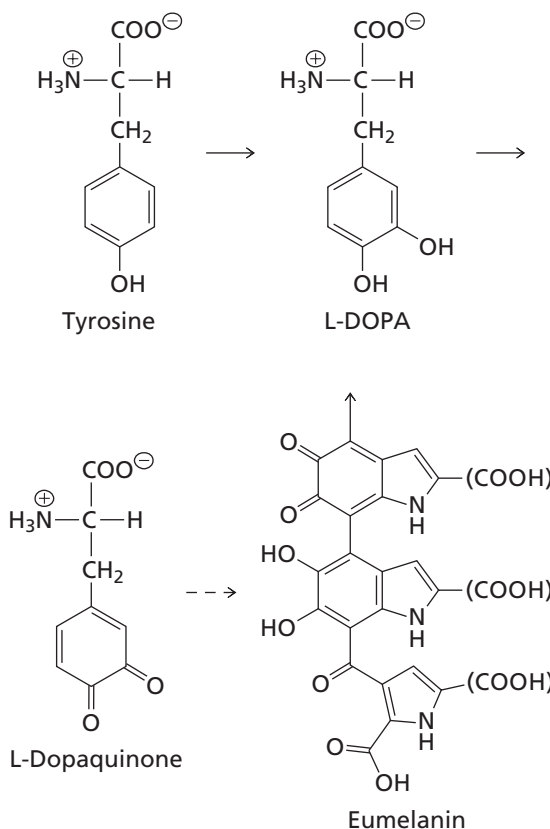
Synthesis of eumelanin from tyrosine and L-DOPA.
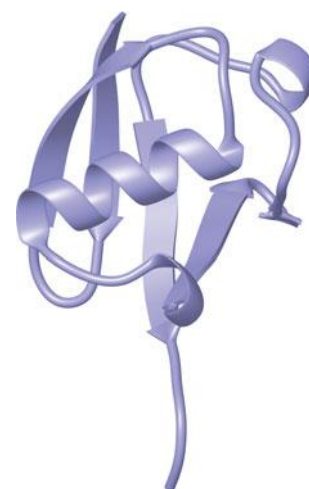
▲ **Ubiquitin (*Homo sapiens*).** Ubiquitin is a small, highly conserved, eukaryotic protein used as a marker that targets proteins for degradation. [PDB 1UBI]

ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin-protein ligase (E3). This pathway is coupled to ATP hydrolysis—one ATP molecule is hydroylzed for every ubiquitin molecule attached to the target protein. The ubiquitinated protein is hydrolyzed to peptides by the action of a large multiprotein complex called the proteasome (or proteosome) (Figure 17.28). This process occurs in both the cytosol and the nucleus. Other proteases catalyze hydrolysis of the resulting peptides. ATP is required to assemble the proteasome and to hydrolyze the ubiquitinated protein. Before this pathway was discovered there was no explanation for the surprising observation that the degradation of many proteins requires ATP. (Recall from Section 2.6 that hydrolysis of a peptide bond is a thermodynamically favorable reaction.)

Aaron Ciechanover (1947–), Avram Hershko (1937–), and Irwin Rose (1926–) won the 2004 Nobel Prize in Chemistry "for the discovery of ubiquitin-mediated protein degradation."
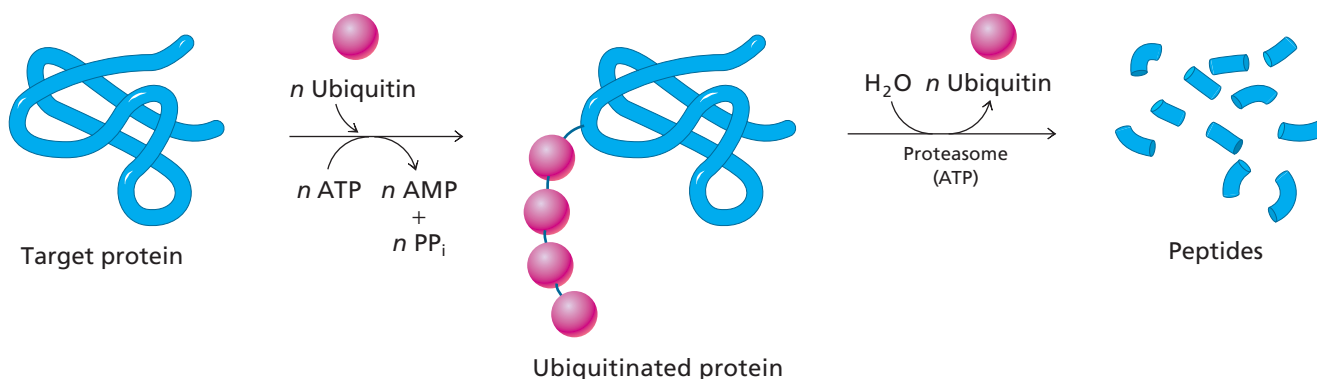
Ubiquitination and hydrolysis of a protein. Ubiquitination enzymes catalyze the attachment of numerous molecules of ubiquitin to the protein targeted for degradation. The proteasome catalyzes ATP-dependent hydrolysis of the substituted protein, releasing peptides and ubiquitin.

BOX 17.4 APOPTOSIS—PROGRAMMED CELL DEATH

Apoptosis (often pronounced with the second *p* silent) is a series of morphological changes in a cell that leads to its death. The changes include a decrease in cell volume, damage to the plasma membrane, swelling of mitochondria, and fragmentation of chromatin. Surplus and harmful cells are removed principally by the action of proteases.

Some cells die normally during development or in the regulation of antibody production. Others die as a result of diseases or from faulty apoptosis (as in some neurodegenerative diseases). As a result of apoptosis, vesicles containing cellular contents form and are engulfed by neighboring cells. Some of the protein contents of the vesicles can be saved and reused by the other cells.

All eukaryotes have a similar set of endogenous enzymes responsible for cell death. These enzymes (first described as being involved in apoptosis in 1993) include about a dozen proteases called caspases—meaning cysteine-containing hydrolases that act on the carboxyl side of aspartate residues.
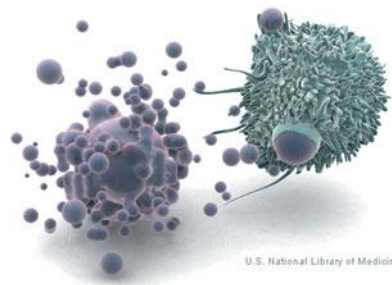
▲ **Apoptosis.** The drawing depicts vesicles from a dead apoptotic cell (purple) being taken up by a white blood cell (green). [Courtesy of the United States National Library of Medicine.]
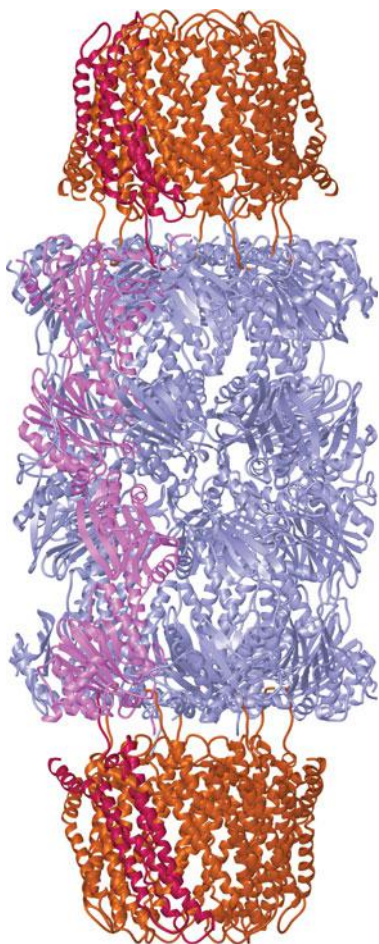
▲ **Proteasome from yeast (*Saccharomyces cerevisiae*). (a)** Side view. The complete proteasome consists of two seven-member rings of β subunits (blue) with their active protease sites on the interior of the cylinder. The outer two rings have seven α subunits (purple). **(b)** Top view. Ubiquinated proteins enter the cylinder through a pore at the top or bottom of the structure. [PDB 1FNT]

17.6 Amino Acid Catabolism

Amino acids obtained from the degradation of endogenous proteins or from the diet can be used for the biosynthesis of new proteins. Amino acids not needed for the synthesis of proteins are catabolized in order to make use of their nitrogen and their carbon skeletons. The first step in amino acid degradation is often removal of the α-amino group. Next, the carbon chains are altered in specific ways for entry into the central pathways of carbon metabolism. We first consider the metabolic fates of the various carbon skeletons. In the next section we examine the metabolism of the ammonia arising from amino acid degradation. These catabolic pathways are present in all species but they are especially important in animals since amino acids are a significant part of fuel metabolism.

Removal of the α-amino group of an amino acid occurs in several ways. The amino acid usually undergoes transamination with α-ketoglutarate to form an α-keto acid and glutamate. The glutamate is oxidized to α-ketoglutarate and ammonia by the action of mitochondrial glutamate dehydrogenase. The net effect of these two reactions is the release of α-amino groups as ammonia and the formation of NADH and α-keto acids. This is the reverse of the pathway shown in Figure 17.8A.

$$\text{Amino acid} + \alpha\text{-Ketoglutarate} \rightleftharpoons \alpha\text{-Keto acid} + \text{Glutamate}$$

$$\text{Glutamate} + NAD^{\oplus} + H_2O \rightleftharpoons \alpha\text{-Ketoglutarate} + NADH + H^{\oplus} + NH_4^{\oplus}$$

$$\text{Sum: Amino acid} + NAD^{\oplus} + H_2O \rightleftharpoons \alpha\text{-Keto acid} + NADH + H^{\oplus} + NH_4^{\oplus} \quad \textbf{(17.4)}$$
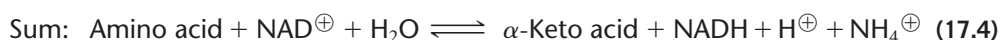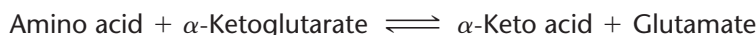
The amide groups of glutamine and asparagine are hydrolyzed by specific enzymes—glutaminase and asparaginase, respectively—to produce ammonia and the corresponding dicarboxylic amino acids glutamate and aspartate. Ammonia from amides and amino groups that is not used in biosynthesis reactions is excreted.

Once the amino groups have been removed, the carbon chains of the 20 amino acids can be degraded. Some are degraded to one of four citric acid cycle intermediates while others are degraded to pyruvate, and still others to acetyl CoA or acetoacetate (Figure 17.29). Each amino acid follows its own route to one or more of these seven compounds.

While all these products can be oxidized to CO_2 and H_2O they can also have other metabolic fates. Amino acids that are degraded to pyruvate or citric acid cycle intermediates are called glucogenic because they can directly supply the pathway of gluconeogenesis. Those that form acetyl CoA or acetoacetate can contribute to the formation of fatty acids or ketone bodies and are called ketogenic. Some amino acids are both glucogenic
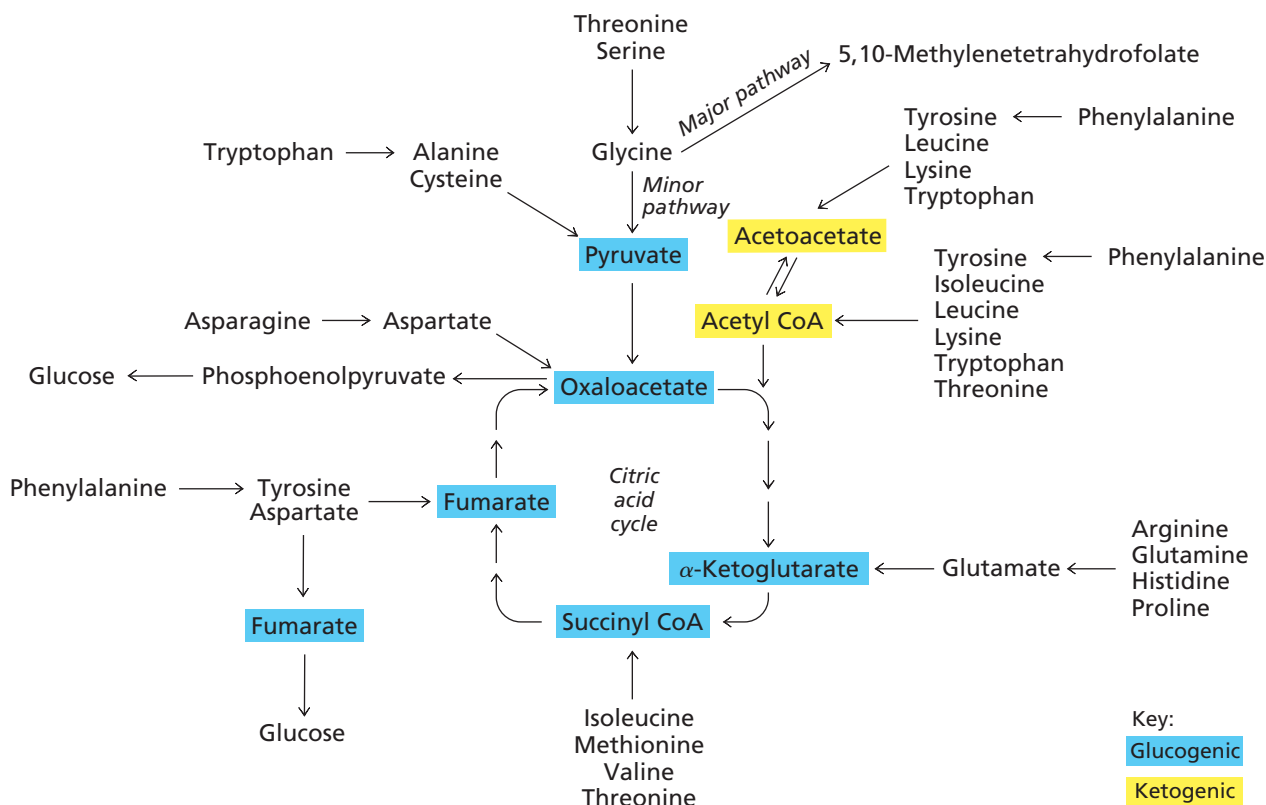
▲ **Figure 17.29**
Degradation of amino acids. The carbon skeletons of amino acids are converted to pyruvate, acetoacetate, acetyl CoA, or citric acid cycle intermediates.

and ketogenic because different parts of their carbon chains form different products. The distinction between glucogenic and ketogenic products is important in animals since amino acids are significant fuel metabolites in the diet. Animals do not possess a direct pathway leading from acetyl CoA to glucose and the production of excess acetyl CoA stimulates formation of ketone bodies (Section 16.11). The distinction between glucogenic and ketogenic products is less important in bacteria, protists, fungi, and plants since they can convert acetyl CoA to oxaloacetate via the glyoxylate pathway (Section 13.7). In these organisms, acetyl CoA is glucogenic.

In this section, we examine the pathways of amino acid degradation beginning with the simplest routes. Our aim is to show how the carbon atoms of each amino acid reach "glucogenic" metabolites (pyruvate and citric acid cycle intermediates) or "ketogenic" metabolites (acetyl CoA and acetoacetate). The ultimate fates of these metabolites depend on the species and are covered in earlier chapters.

A. Alanine, Asparagine, Aspartate, Glutamate, and Glutamine

Alanine, aspartate, and glutamate are synthesized by reversible transamination reactions (Sections 17.3A,C,D). The breakdown of these three amino acids involves their re-entry into the pathways from which their carbon skeletons arose. Alanine gives rise to pyruvate, aspartate to oxaloacetate, and glutamate to α-ketoglutarate by reversal of the original transamination reactions. All three amino acids are glucogenic since aspartate and glutamate are converted to citric acid cycle intermediates and alanine is converted to pyruvate.

The degradation of both glutamine and asparagine begins with their hydrolysis to glutamate and aspartate, respectively. Thus, glutamine and asparagine are both glucogenic. The hydrolysis reactions are catalyzed by specific enzymes—asparaginase (Box 17.1) and glutaminase.

B. Arginine, Histidine, and Proline

The pathways for the degradation of arginine, histidine, and proline converge on glutamate (Figure 17.30). In the case of arginine and proline, the degradation pathways resemble the biosynthesis pathways. Arginine degradation commences with the reaction catalyzed by arginase. The ornithine produced is transaminated to glutamate 5-semialdehyde, which is oxidized to form glutamate.
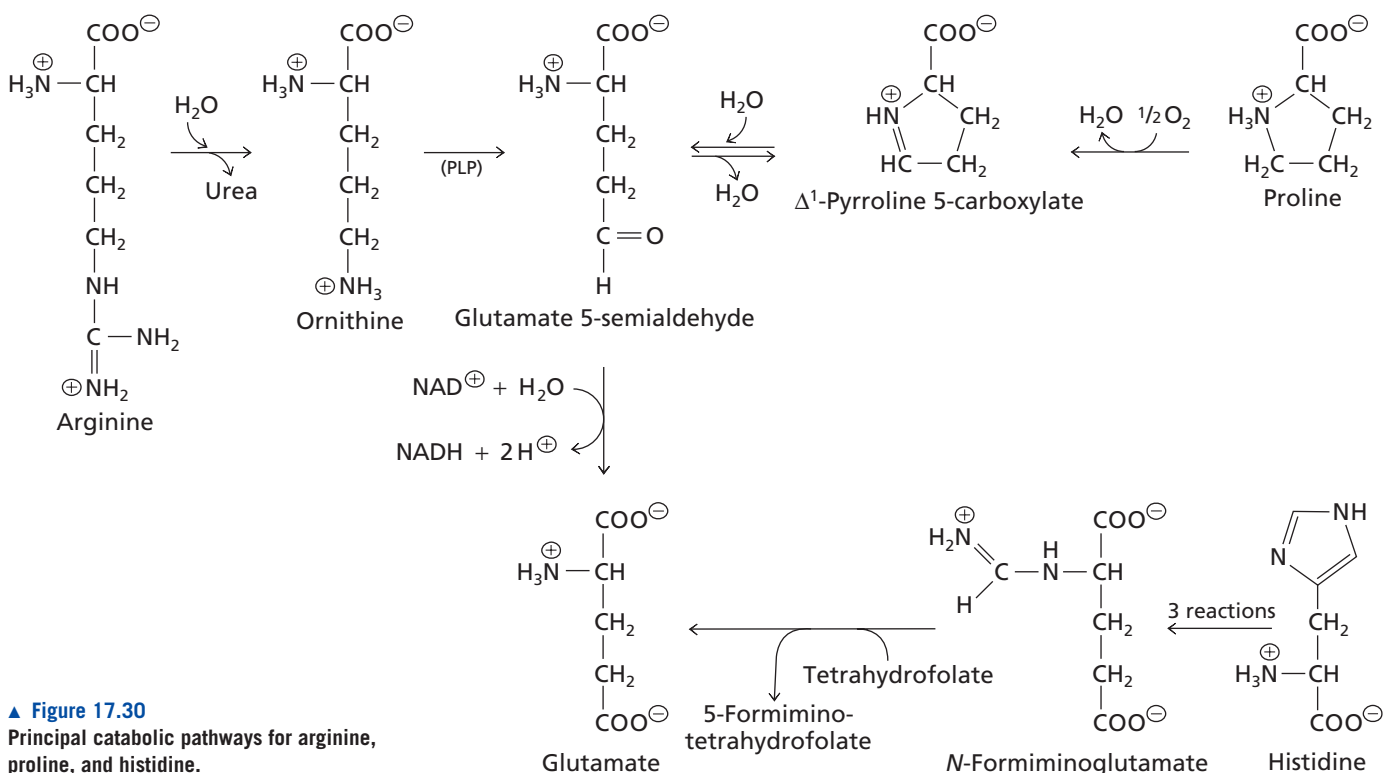
▲ **Figure 17.30**
Principal catabolic pathways for arginine, proline, and histidine.
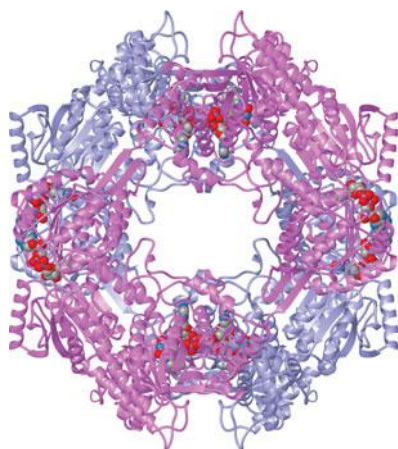
▲ **Proline utilization A flavoprotein.** This enzyme from *Bradyrhizobium japonicum* combines the first two enzymes in the proline degradation pathway into a large complex consisting of six subunits of bifunctional proteins. The two identical subunits of one core dimer are colored blue and purple and the entire structure consists of three such dimers arranged in a circle. The bound FAD and NAD$^{\oplus}$ coenzymes are shown as space-filling models. This enzyme presumably confers a selective advantage over species containing two separate enzymes so why hasn't it evolved in eukaryotes? [PDB 3HAZ]

Proline is converted to glutamate in three steps. The first step is an oxidation reaction catalyzed by the FAD-containing enzyme proline dehydrogenase. The electron acceptor is sometimes molecular oxygen although other acceptors can be used. The product of the first reaction is Δ^1-pyrroline 5-carboxylate (P5C) that exists in equilibrium with the open-chain form, glutamate 5-semialdehyde. Glutamate 5-semialdehyde is converted to glutamate by the action of NAD$^{\oplus}$-dependant P5C dehydrogenase. Note that the conversion of Δ^1-pyrroline 5-carboxylate to glutamate 5-semialdehyde is spontaneous as in the proline synthesis pathway (Section 17.3D).

The first two enzymes in this pathway are separate enzymes in all eukaryotes and most bacteria but in some species of bacteria the two genes for these enzymes have fused to create a bifunctional hexameric protein that catalyzes both reactions. This is kinetically advantageous since the intermediates (Δ^1-pyrroline 5-carboxylate and glutamate 5-semialdehyde) do not dissociate from the complex before being converted to glutamate.

The major pathway for histidine degradation also produces glutamate. Histidine undergoes nonoxidative deamination, hydration, and ring opening to form *N*-formiminoglutamate. The formimino moiety ($-CH{=}NH_2^{\oplus}$) is then transferred to tetrahydrofolate, forming 5-formiminotetrahydrofolate and glutamate. 5-Formiminotetrahydrofolate is then enzymatically deaminated to form 5,10-methenyltetrahydrofolate. The one-carbon (methenyl) group of this tetrahydrofolate derivative can be used in pathways such as pyrimidine synthesis (Section 18.6).

C. Glycine and Serine

There are two pathways for the breakdown of serine (Figure 17.31). A small amount of serine is converted directly to pyruvate by the action of serine dehydratase, a PLP-dependent enzyme. Most serine, however, is converted to glycine by the action of serine hydroxymethyltransferease. This is the same reaction that results in synthesis of glycine in the biosynthesis pathway (Figure 17.16) and it is a reaction that produces 5,10-methylene tetrahydrofolate (5,10-methylene THF).

Some glycine can be converted to serine by the reverse reaction of serine hydroxylmethyltransferase and the glycine carbon atoms can end up in pyruvate when the serine molecules are deaminated. However, the major pathway for degradation of glycine in all species is conversion to $NH_4^{\oplus}$ and $HCO_3^{\ominus}$ by the glycine cleavage system.

Catalysis by the glycine cleavage system requires an enzyme complex containing four nonidentical subunits. PLP, lipoamide, and FAD are prosthetic groups, and $NAD^{\oplus}$ and tetrahydrofolate (THF) are cosubstrates. Initially, glycine is decarboxylated and the $-CH_2-NH_3^{\oplus}$ group is transferred to lipoamide. Then, $NH_4^{\oplus}$ is released, and the remaining one-carbon group is transferred to tetrahydrofolate to form 5,10-methylenetetrahydrofolate (5,10-methylene THF). Reduced lipoamide is oxidized by FAD and $FADH_2$ reduces the mobile carrier $NAD^{\oplus}$.

As shown in Figure 17.32 the glycine cleavage system is another example of a lipoamide swinging arm mechanism similar in principle to that of pyruvate dehydrogenase (Section 13.1). Although glycine breakdown is reversible *in vitro*, the glycine cleavage system catalyzes an irreversible reaction in cells. The irreversibility of the reaction sequence is due in part to the K_m values for the products ammonia and methylenetetrahydrofolate that are far greater than the concentrations of these compounds *in vivo*.

D. Threonine

There are several routes for the degradation of threonine. In the major pathway, threonine is oxidized to 2-amino-3-ketobutyrate in a reaction catalyzed by threonine dehydrogenase (Figure 17.33). 2-Amino-3-ketobutyrate can undergo thiolysis to form acetyl CoA and glycine. Another route for threonine catabolism is cleavage to acetaldehyde and glycine by the action of threonine aldolase. Threonine aldolase is actually a minor activity of serine hydroxymethyltransferase in many tissues and organisms. Acetaldehyde can be oxidized to acetate by the action of acetaldehyde dehydrogenase and acetate can be converted to acetyl CoA by acetyl-CoA synthetase.

A third route for threonine catabolism in mammals is deamination to α-ketobutyrate. This reaction is catalyzed by serine dehydratase, the same enzyme that catalyzes the conversion of serine to pyruvate. This reaction produces α-ketobutyrate for synthesis of isoleucine in most species (Section 17.3C). α-Ketobutyrate can be converted to propionyl CoA in the degradative pathway and propionyl CoA is a precursor of the citric acid cycle intermediate succinyl CoA (Section 16.7 F). Threonine can thus produce either succinyl CoA or glycine + acetyl CoA depending on the pathway by which it is degraded.

E. The Branched Chain Amino Acids

Leucine, valine, and isoleucine are degraded by related pathways (Figure 17.34). The same three enzymes catalyze the first three steps in all pathways. The first step, transamination, is catalyzed by branched chain amino acid transaminase.

The second step in the catabolism of branched chain amino acids is catalyzed by branched chain α-keto acid dehydrogenase. In this reaction, the branched chain α-keto acids undergo oxidative decarboxylation to form branched chain acyl CoA molecules one carbon atom shorter than the precursor α-keto acids. Branched chain α-keto acid dehydrogenase is a multienzyme complex containing lipoamide and thiamine pyrophosphate (TPP) and requires $NAD^{\oplus}$ and coenzyme A. Its catalytic mechanism is similar to
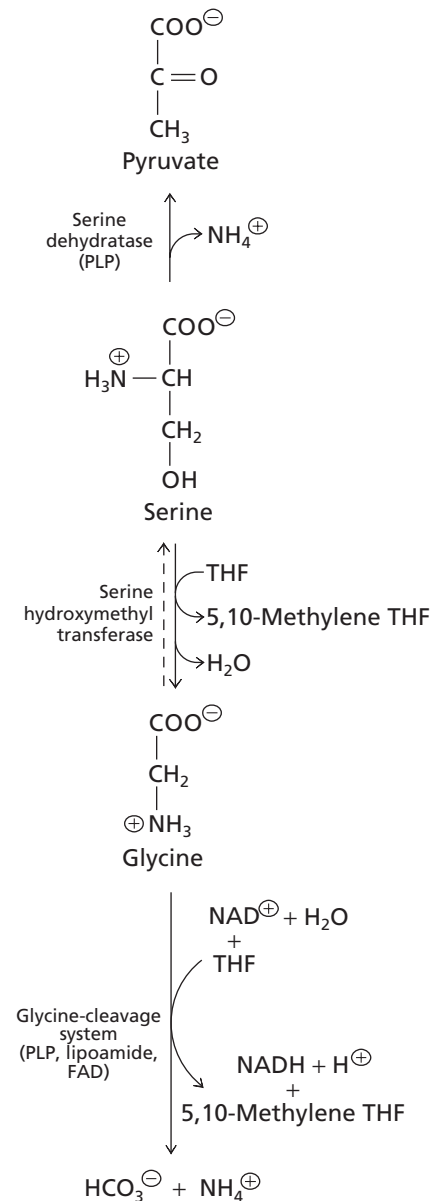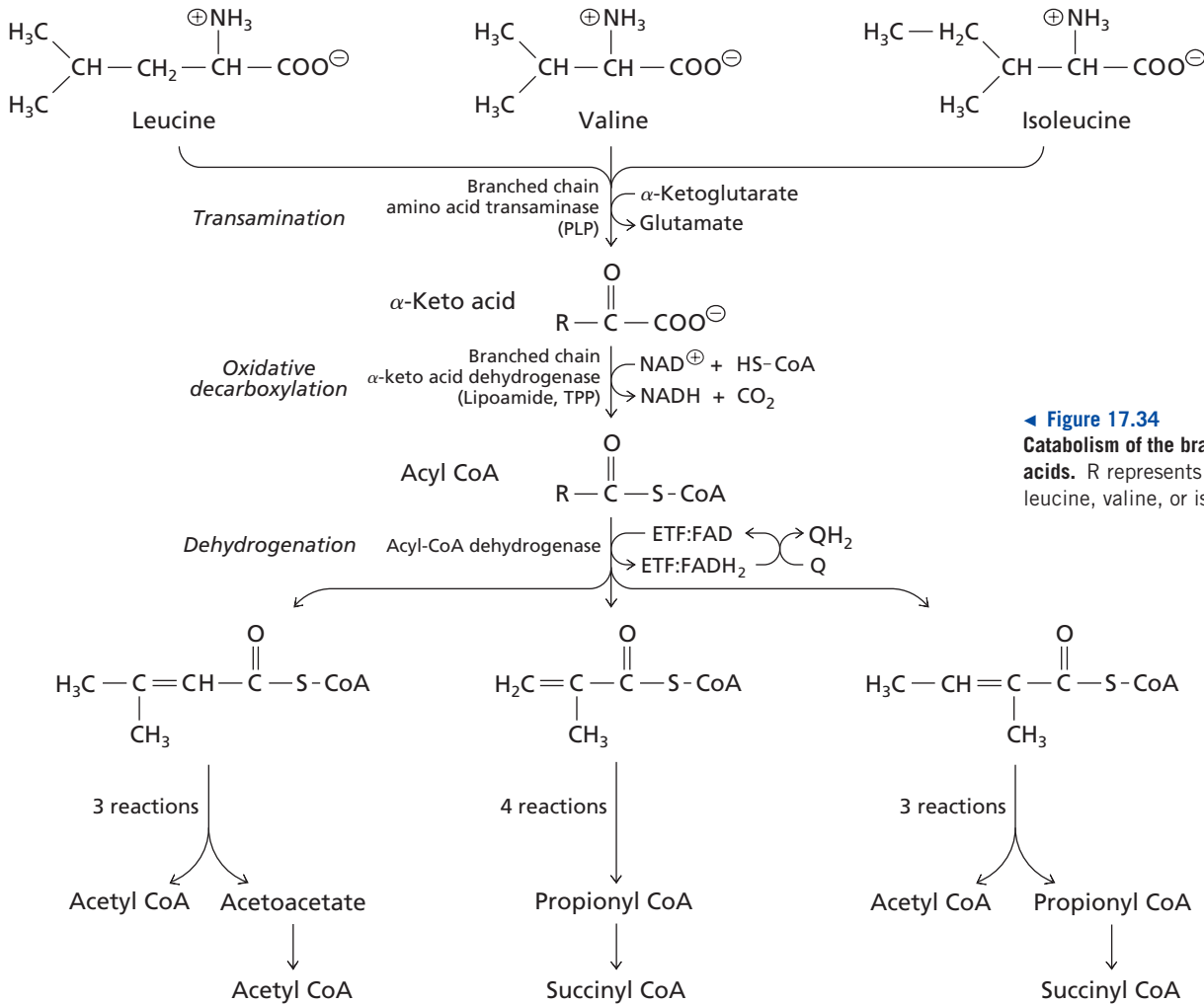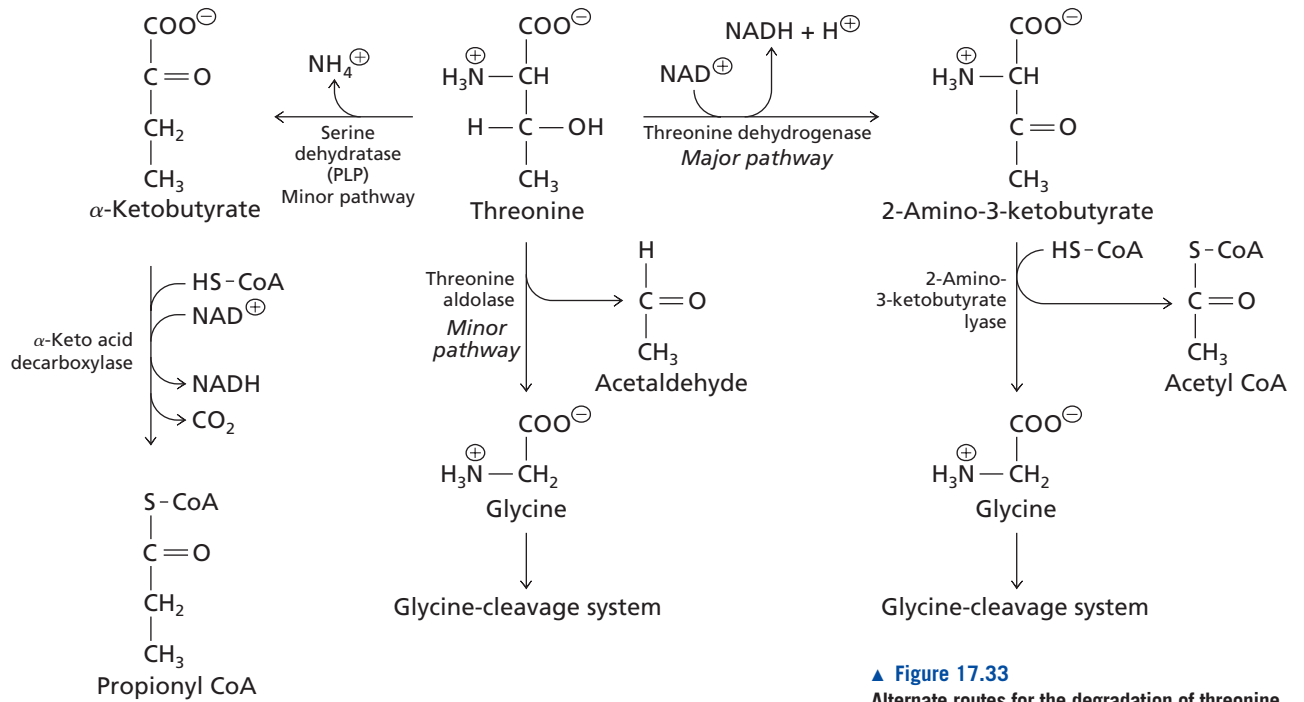

▲ Figure 17.31
Catabolism of serine and glycine.
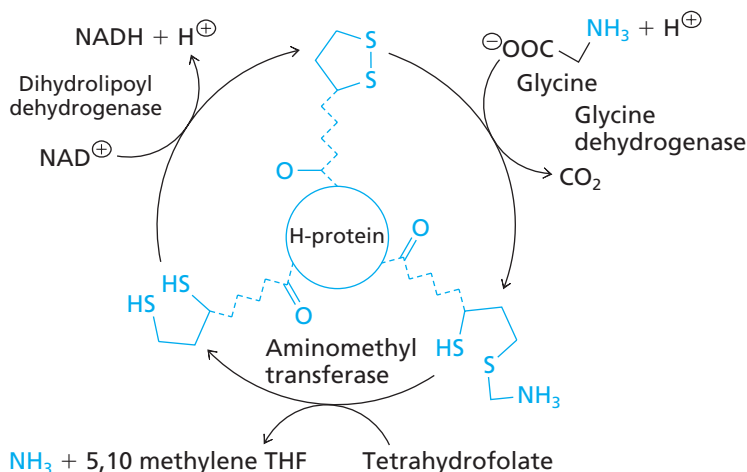
The pathway from propionyl CoA to succinyl CoA is shown in detail in Figure 16.22.

◄ Figure 17.32
Glycine cleavage system. A lipoamide swinging arm is attached to the core structural component (H-protein). The swinging arm visits the active sites of the three enzymes of the pathway.

α-Ketobutyrate

Threonine

2-Amino-3-ketobutyrate

NH₄⁺

Serine dehydratase (PLP) Minor pathway

NAD⁺ → NADH + H⁺

Threonine dehydrogenase *Major pathway*

HS–CoA → S–CoA

2-Amino-3-ketobutyrate lyase

α-Keto acid decarboxylase

HS–CoA
NAD⁺
NADH
CO₂

Propionyl CoA

Threonine aldolase *Minor pathway*

Acetaldehyde

Glycine

Glycine-cleavage system

Acetyl CoA

Glycine

Glycine-cleavage system

▲ **Figure 17.33**
Alternate routes for the degradation of threonine.

Leucine

Valine

Isoleucine

Transamination

Branched chain amino acid transaminase (PLP)

α-Ketoglutarate → Glutamate

α-Keto acid

R—C—COO⁻

Oxidative decarboxylation

Branched chain α-keto acid dehydrogenase (Lipoamide, TPP)

NAD⁺ + HS–CoA → NADH + CO₂

◄ **Figure 17.34**
Catabolism of the branched chain amino acids. R represents the side chain of leucine, valine, or isoleucine.

Acyl CoA

R—C—S-CoA

Dehydrogenation Acyl-CoA dehydrogenase

ETF:FAD → QH₂
ETF:FADH₂ → Q

H₃C—C=CH—C—S-CoA
|
CH₃

H₂C=C—C—S-CoA
|
CH₃

H₃C—CH=C—C—S-CoA
|
CH₃

3 reactions

4 reactions

3 reactions

Acetyl CoA   Acetoacetate

Propionyl CoA

Acetyl CoA   Propionyl CoA

Acetyl CoA

Succinyl CoA

Succinyl CoA

that of the pyruvate dehydrogenase complex (Section 13.1) and the α-ketoglutarate dehydrogenase complex (Section 13.3#4), and it contains the same dihydrolipoamide dehydrogenase (E_3) subunits as those found in the other two dehydrogenase complexes.

Branched chain acyl CoA molecules are oxidized by an FAD-containing acyl-CoA dehydrogenase in a reaction analogous to the first step in fatty acyl CoA oxidation (Figure 16.19). The electrons removed in this oxidation step are transferred via the electron transferring flavoprotein (ETF) to ubiquinone (Q).

At this point, the steps in the catabolism of branched chain amino acids diverge. All the carbons of leucine are ultimately converted to acetyl CoA, so leucine is purely ketogenic. Valine is ultimately converted to propionyl CoA. As in the degradation of threonine, propionyl CoA is converted to succinyl CoA that enters the citric acid cycle. Valine is glucogenic. The isoleucine degradation pathway leads to both propionyl CoA and acetyl CoA. Isoleucine is therefore both glucogenic (via succinyl CoA formed from propionyl CoA) and ketogenic (via acetyl CoA). Thus, although the initial steps in the degradation of the three branched chain amino acids are similar, their carbon skeletons have different fates—at least in animals.

> **Remember that the distinction between ketogenic and glucogenic pathways is only relevant in animals because all other species can convert acetyl CoA to glucose.**

F. Methionine

One major role of methionine is conversion to the activated methyl donor *S*-adenosylmethionine (Section 7.3). Transfer of the methyl group from *S*-adenosylmethionine to a methyl acceptor leaves *S*-adenosylhomocysteine that is degraded by hydrolysis to homocysteine and adenosine (Figure 17.35). Homocysteine can either be methylated by
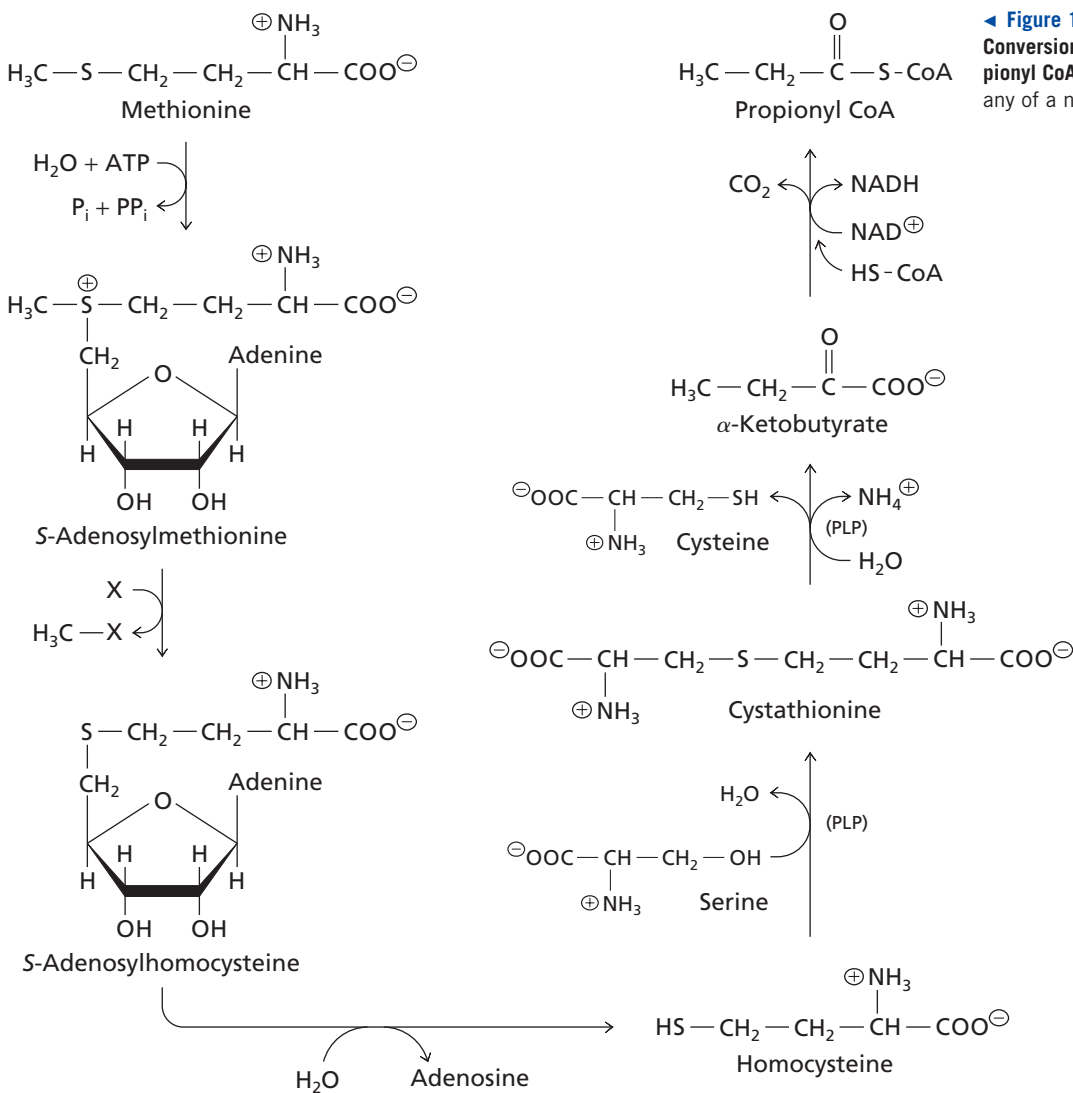
◄ **Figure 17.35**
Conversion of methionine to cysteine and propionyl CoA. X in the second step represents any of a number of methyl-group acceptors.

COO$^\ominus$
|
H$_3$N$^\oplus$ — CH
|
CH$_2$
|
SH

Cysteine

↓ O$_2$
↘ H$^\oplus$

COO$^\ominus$
|
H$_3$N$^\oplus$ — CH
|
CH$_2$
|
SO$_2^\ominus$

Cysteinesulfinate

↓ α-Keto-
　glutarate
(PLP)
↘ Glutamate

COO$^\ominus$
|
C = O
|
CH$_2$
|
SO$_2^\ominus$

β-Sulfinylpyruvate

Nonenzymatic
desulfurylation
↓ H$^\oplus$
↘ SO$_2$

COO$^\ominus$
|
C = O
|
CH$_3$
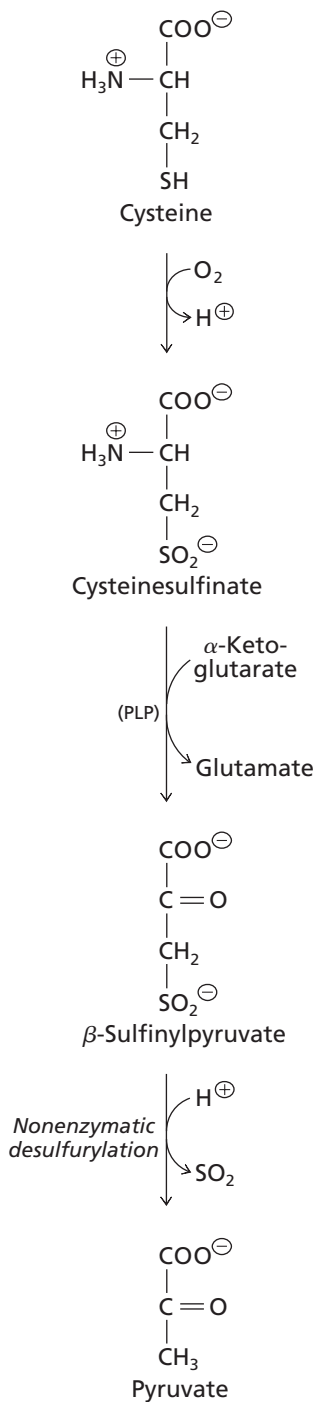
Pyruvate

▲ **Figure 17.36**
Conversion of cysteine to pyruvate.

BOX 17.5　PHENYLKETONURIA IS A DEFECT IN TYROSINE FORMATION

One of the most common disorders of amino acid metabolism is phenylketonuria (PKU). The disease is caused by a mutation in the gene that encodes phenylalanine hydroxylase (*PAH* gene on chromosome 12q: OMIN MIN=261600). Affected individuals are unable to convert dietary phenylalanine to tyrosine so the blood of children with this disease contains very high levels of phenylalanine and low levels of tyrosine. Instead of being converted to tyrosine, phenylalanine is metabolized to phenylpyruvate in the reverse of the transamination reaction shown in Figure 17.20. (Transamination of phenylalanine does not occur in unaffected individuals because the K_m of the transaminase for phenylalanine is much higher than the normal concentration of phenylalanine.) Elevated levels of phenylpyruvate and its derivatives inhibit brain development.

Newborns are routinely screened for PKU by testing for elevated levels of phenylpyruvate in the urine or of phenylalanine in the blood during the first days after birth. Phenylalanine hydroxylase-deficient individuals often develop normally if the dietary intake of phenylalanine is strictly limited during the first decade of life. Some women with PKU must restrict their dietary intake of phenylalanine during pregnancy to ensure proper fetal development. Elevated levels of phenylalanine are also observed in individuals with deficiencies in dihydropteridine reductase or 4a-carbinolamine dehydratase or defects in the biosynthesis of tetrahydrobiopterin because each of these disorders results in impairment of the hydroxylation of phenylalanine.

Control of diet can successfully treat PKU but the restrictions exclude many natural, protein-rich foods such as meat, fish, milk, bread, and cake. The food of this strict diet is not appetizing. Tests have been performed by feeding PKU victims an enzyme that catalyzes degradation of phenylalanine to ammonia and a nontoxic carbon product. This enzyme does not fully replace dietary restriction of phenylalanine but it may increase a patient's tolerance for protein-containing foods.

▲ Newborn infants are tested for phenylketonuria by analyzing blood drawn from the heel of the foot.

5-methyltetrahydrofolate to form methionine or it can react with serine to form cystathionine that can be cleaved to cysteine and α-ketobutyrate. We encountered this series of reactions earlier as part of a pathway for the formation of cysteine (Figure 17.18). By this pathway, mammals can form cysteine using a sulfur atom from the essential amino acid methionine. α-Ketobutyrate is converted to propionyl CoA by the action of an α-keto acid dehydrogenase. Propionyl CoA can be further metabolized to succinyl CoA, so methionine is glucogenic.

G.　Cysteine

The major route of cysteine catabolism is a three-step pathway leading to pyruvate (Figure 17.36). Therefore, cysteine is glucogenic. Cysteine is first oxidized to cysteinesulfinate that loses its amino group by transamination to form β-sulfinylpyruvate. Nonenzymatic desulfurylation produces pyruvate.
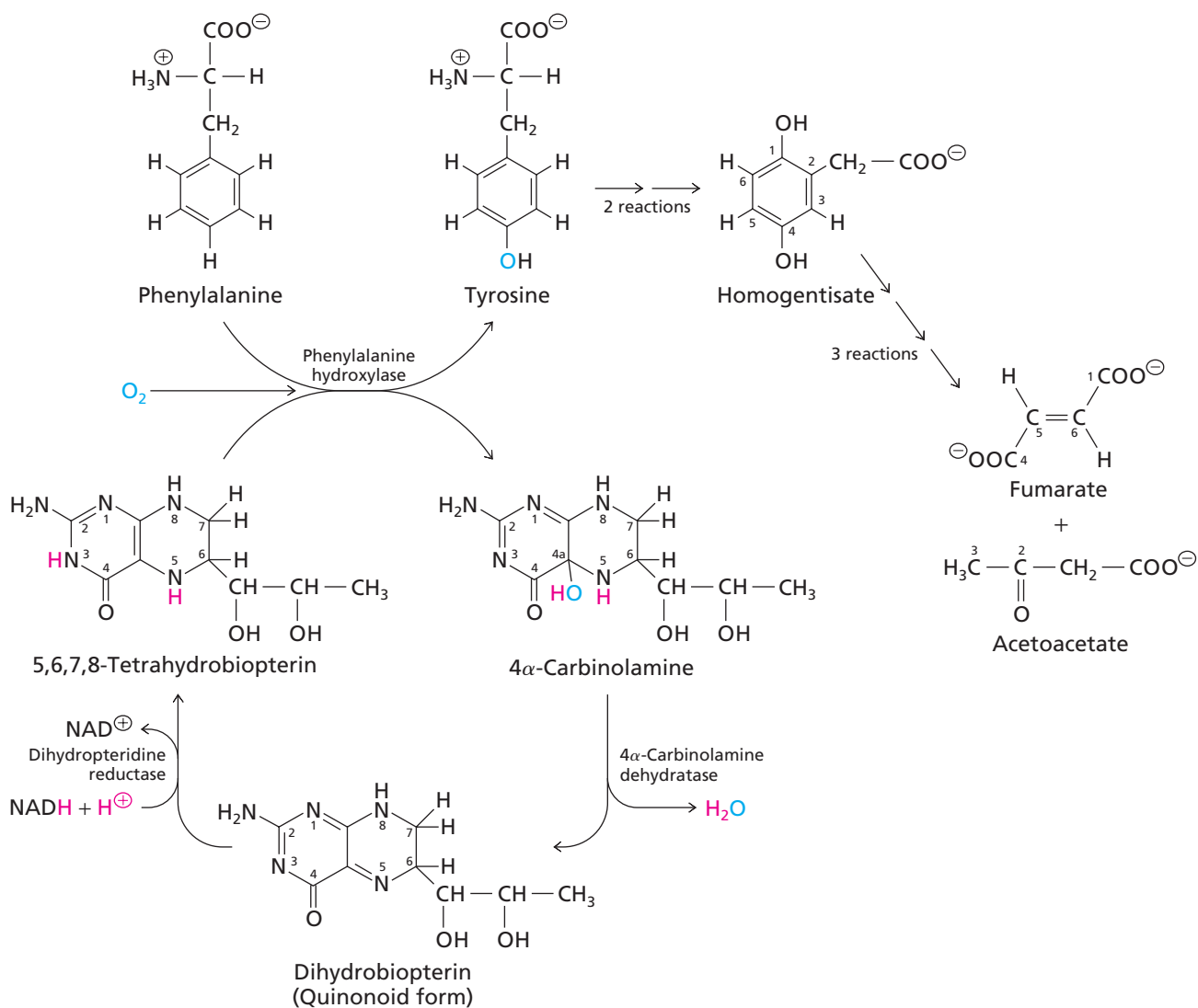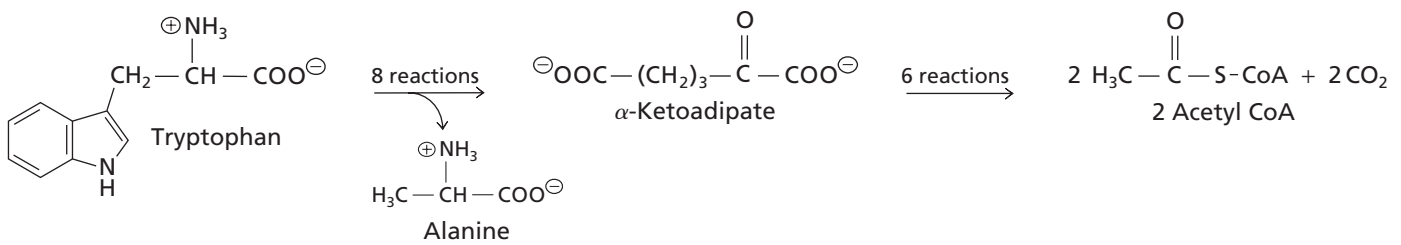
▲ **Figure 17.37**
Conversion of phenylalanine and tyrosine to fumarate and acetoacetate. The tetrahydrobiopterin cofactor is regenerated via dehydration and NADH-dependent reduction.

H. Phenylalanine, Tryptophan, and Tyrosine

The aromatic amino acids share a common pattern of catabolism. In general, the pathways begin with oxidation, followed by removal of nitrogen by transamination or hydrolysis and then ring opening coupled with oxidation.

The conversion of phenylalanine to tyrosine, catalyzed by phenylalanine hydroxylase, is an important step in the catabolism of phenylalanine (Figure 17.37). It also serves as a source of tyrosine in animals since they lack the normal chorismate pathway for tyrosine synthesis. The phenylalanine hydroxylase reaction requires molecular oxygen and the reducing agent tetrahydrobiopterin. One oxygen atom from O_2 is incorporated into tyrosine and the other is converted to water.

Tetrahydrobiopterin is regenerated in two steps. 4α-Carbinolamine dehydratase catalyzes the dehydration of the first oxidized product and prevents its isomerization to an inactive form in which the side chain is on C-7, not C-6. Dihydropteridine reductase catalyzes the reduction of the resulting quinonoid dihydrobiopterin to 5,6,7,8-tetrahydrobiopterin in a reaction that requires NADH. Tetrahydrobiopterin is also a reducing agent in the biosynthesis of nitric oxide from arginine (Section 17.4C).

▲ **Figure 17.38**
Conversion of tryptophan to alanine and acetyl CoA.

The catabolism of tyrosine begins with the removal of its α-amino group in a transamination reaction with α-ketoglutarate. Subsequent oxidation steps lead to ring opening and eventually to the final products, fumarate and acetoacetate. This fumarate is cytosolic and is converted to glucose. Acetoacetate is a ketone body. Thus, tyrosine is both glucogenic and ketogenic.

The indole ring system of tryptophan has a more complex degradation pathway that includes two ring-opening reactions. The major route of tryptophan catabolism in the liver and many microorganisms leads to α-ketoadipate and ultimately to acetyl CoA (Figure 17.38). Alanine, produced early in tryptophan catabolism, is transaminated to pyruvate. Thus, the catabolism of tryptophan is both ketogenic and glucogenic.

I. Lysine

The main pathway for the degradation of lysine generates the intermediate saccharopine, the product of the condensation of α-ketoglutarate with lysine (Figure 17.39). Sequential oxidation reactions produce α-aminoadipate that loses its amino group by transamination with α-ketoglutarate to become α-ketoadipate. α-Ketoadipate is subsequently converted to acetyl CoA by the same steps that occur in the degradation of tryptophan. Like leucine, lysine is ketogenic (these two are the only common amino acids that are purely ketogenic).
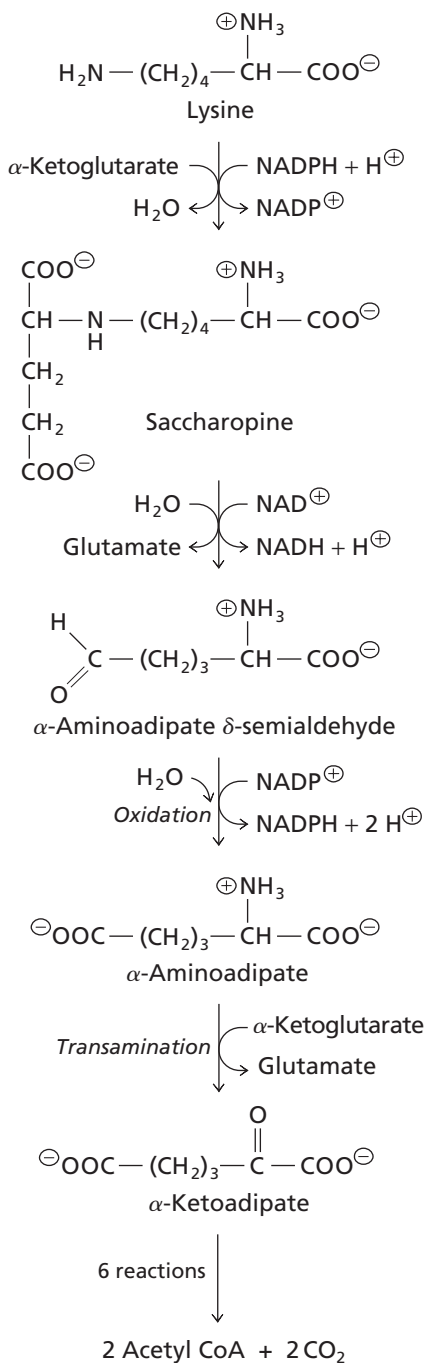
17.7 The Urea Cycle Converts Ammonia into Urea

High concentrations of ammonia are toxic to cells. Different organisms have evolved different strategies for eliminating waste ammonia. The nature of the excretory product depends on the availability of water. In many aquatic organisms, ammonia diffuses directly across the cell membranes and is diluted by the surrounding water. This route is inefficient in large terrestrial multicellular organisms and the buildup of ammonia inside internal cells must be avoided.

Most terrestrial vertebrates convert waste ammonia to urea, a less toxic product (Figure 17.40). Urea is an uncharged and highly water-soluble compound produced in the liver and carried in the blood to the kidneys where it is excreted as the major solute of urine. (Urea was first described around 1720 as the essential salt of urine. The name "urea" is derived from "urine.") Birds and many terrestrial reptiles convert surplus ammonia to uric acid, a relatively insoluble compound that precipitates from aqueous solution to form a semisolid slurry. Uric acid is also a product of the degradation of purine nucleotides by birds, some reptiles, and primates.

The synthesis of urea occurs almost exclusively in the liver. Urea is the product of a set of reactions called the urea cycle—a pathway discovered by Hans Krebs and Kurt Henseleit in 1932 several years before Krebs discovered the citric acid cycle. Several observations led to the identification of the urea cycle; for example, slices of rat liver can bring about the net conversion of ammonia to urea. Synthesis of urea by these preparations is markedly stimulated when the amino acid ornithine is added and the amount of urea synthesized greatly exceeds the amount of ornithine that is added, suggesting that ornithine acts catalytically. Finally, it was known that high levels of the enzyme arginase occur in the livers of all organisms that synthesize urea.
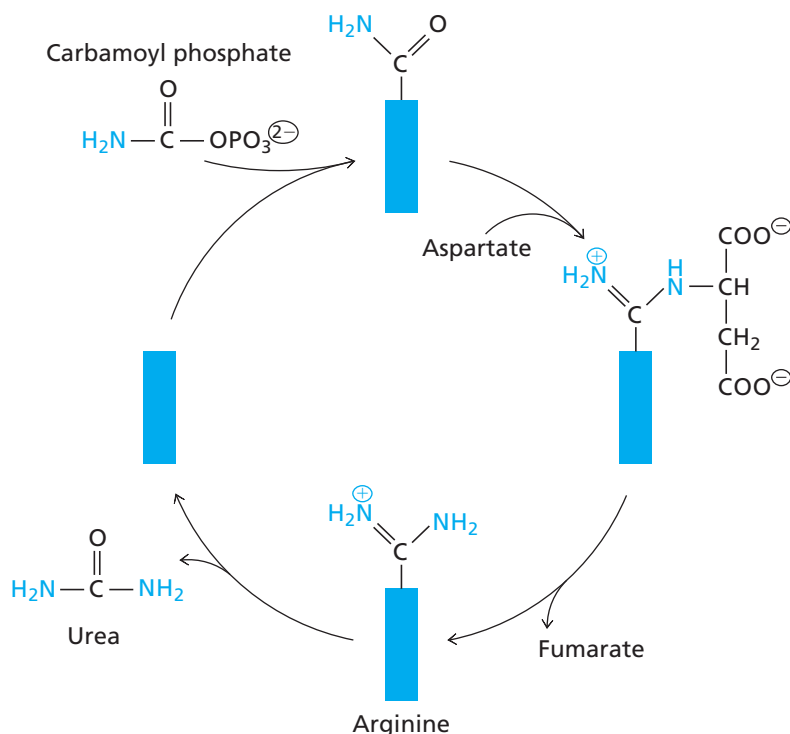

▲ **Figure 17.39**
Conversion of lysine to acetyl CoA.

Further degradation of uric acid is described in Section 18.8.
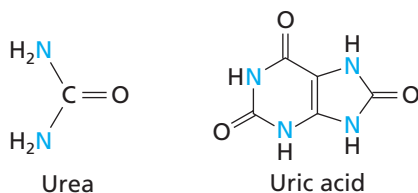
◀ **Figure 17.40**
Urea and uric acid.

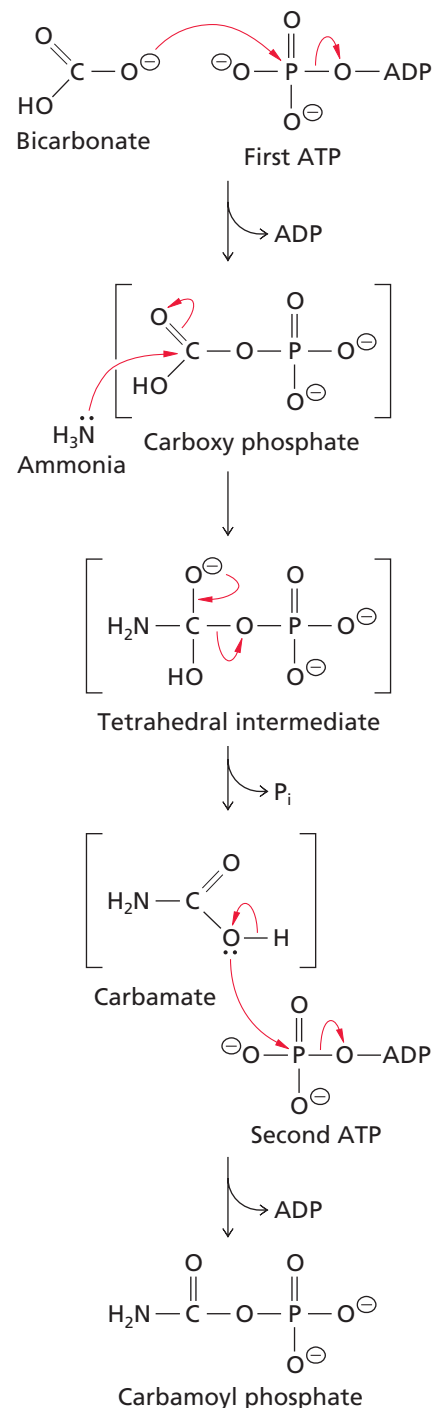## A. Synthesis of Carbamoyl Phosphate

The ammonia released by oxidative deamination of glutamate reacts with bicarbonate to form carbamoyl phosphate. This reaction requires two molecules of ATP and is catalyzed by carbamoyl phosphate synthetase (Figure 17.41). This enzyme is present in all species since carbamoyl phosphate is an essential precursor in pyrimidine biosynthesis and it's also required in the synthesis of arginine in species that don't have a urea cycle. Mammals have two versions of this enzyme. The cytosolic version is called carbamoyl phosphate synthetase II and it uses glutamine rather than ammonia as the nitrogen donor. This is the enzyme used in pyrimidine synthesis (Section 18.3). The bacterial enzymes also use glutamine. The second mammalian version, carbamoyl phosphate I, is the one involved in the urea cycle. It is one of the most abundant enzymes in liver mitochondria accounting for as much as 20% of the protein of the mitochondrial matrix. The nitrogen atom of carbamoyl phosphate is incorporated into urea via the urea cycle.

B. The Reactions of the Urea Cycle

The first nitrogen atom of urea is contributed by carbamoyl phosphate and the second is derived from aspartate. The synthesis of urea takes place while the intermediates are covalently bound to an ornithine skeleton. Ornithine is regenerated when urea is released and it re-enters the urea cycle. Thus, ornithine acts catalytically in the synthesis of urea (Figure 17.42). The carbon, nitrogen, and oxygen atoms of ornithine are not

▲ **Figure 17.42**
The urea cycle. The blue rectangular box represents ornithine.

▲ **Figure 17.41**
Synthesis of carbamoyl phosphate catalyzed by carbamoyl phosphate synthetase I. The reaction involves two phosphoryl-group transfers. First, nucleophilic attack by bicarbonate on ATP produces carboxy phosphate and ADP. Next, ammonia reacts with carboxy phosphate, forming a tetrahedral intermediate. Elimination of a phosphate group produces carbamate. A second phosphoryl-group transfer from another ATP forms carbamoyl phosphate and ADP. Structures in brackets remain enzyme bound during the reaction.

BOX 17.6 DISEASES OF AMINO ACID METABOLISM

Hundreds of human metabolic diseases involving single-gene defects (often termed inborn errors of metabolism) have been discovered. Many are due to defects in the breakdown of amino acids. We have already discussed phenylketonuria, the defect in tyrosine formation from phenylalanine (Box 17.5). A few more examples are mentioned here. Defects in some pathways are severe and even life-threatening; defects in other pathways can result in less severe symptoms. The results indicate that some amino acid degradation pathways are almost dispensable whereas others are essential for survival following birth.

Alkaptonuria

The first metabolic disease to be characterized as a genetic defect was alkaptonuria, a rare disease in which one of the intermediates in the catabolism of phenylalanine and tyrosine (homogentisate) accumulates (Figure 17.37). A deficiency of homogentisate dioxygenase, the enzyme that catalyzes oxidative cleavage of this intermediate, prevents further metabolism of this catabolite. The gene is *HGD* on chromosome 3 (OMIM MIM=203500).

Solutions of homogentisate turn dark on standing because this compound is converted to a pigment. Alkaptonuria was recognized by observing the darkening of urine. Individuals with alkaptonuria are prone to develop arthritis, but it is not known how the metabolic defect produces this complication; possibly it is from the deposit of pigments in bones and connective tissues.

Cystinuria

If there is a defect in kidney transport of cysteine and the basic amino acids, then cysteine accumulates in blood and oxidizes to cystine producing a condition called cystinuria. Cystine has a low solubility and forms calculi. Patients suffering from cystinuria drink large amounts of water to dissolve these stones or are given compounds that react with cystine to form soluble derivatives. (See OMIM MIM=220100.)

Gyrate Atrophy

A defect in ornithine transaminase activity causes the metabolic disease gyrate atrophy of the choroid and retina of the eyes. The affected gene is *OAT* on chromosome 10 (OMIM MIM=258870). Gyrate atrophy leads to tunnel vision and later to blindness. The progress of this disorder can be slowed by restricting the dietary intake of arginine or by the administration of pyridoxine.

Maple Syrup Urine Disease

Patients suffering from maple syrup urine disease excrete urine that smells like maple syrup. The disease is caused by a genetic defect at the second step in catabolism of branched chain amino acids—the step catalyzed by the branched chain α-keto acid dehydrogenase complex. Those afflicted with this disease have short lives unless they follow a diet very low in branched chain amino acids. (OMIM MIM=248600)

Nonketotic Hyperglycinemia (Glycine Encephalopathy)

Defects in the enzyme complex that catalyzes glycine cleavage lead to the accumulation of large amounts of glycine in body fluids. This is the main biochemical symptom of a disease called nonketotic hyperglycinemia. Most individuals with this disorder have severe mental deficiencies and die in infancy. The severity of the disease indicates the crucial importance of the glycine cleavage system. (OMIM MIM=605899)

---

exchanged in the urea cycle. Its role as a catalyst is more obvious than the role of oxaloacetate in the citric acid cycle (Section 13.3) but the principle is the same.

The actual urea cycle reactions are more complex than the simple scheme shown in Figure 17.42. This is because the first reaction occurs in the mitochondrial matrix and the other three occur in the cytosol (Figure 17.43). Two transport proteins connecting the mitochondrial matrix and the cytosol are required: the citrulline–ornithine exchanger and the glutamate-aspartate translocase.

KEY CONCEPT

All species need to eliminate ammonia produced by degradation reactions. Some can excrete it directly while others have to convert it to less toxic compounds that are subsequently excreted.

1. The cycle begins when carbamoyl phosphate reacts in the mitochondrion with ornithine to form citrulline in a reaction catalyzed by ornithine transcarbamoylase. This step incorporates the nitrogen atom originating from ammonia into citrulline; citrulline thus contains half the nitrogen destined for urea. Citrulline is then transported out of the mitochondrion in exchange for cytosolic ornithine.

2. The second nitrogen atom destined for urea comes from aspartate and is incorporated when citrulline condenses with aspartate to form argininosuccinate in the cytosol. This ATP-dependent reaction is catalyzed by argininosuccinate synthetase. Most aspartate in cells originates in mitochondria although aspartate is sometimes generated in the cytosol. Mitochondrial aspartate enters the cytosol in exchange for cytosolic glutamate. (This translocase reaction is part of the malate–aspartate shuttle we described in Section 14.12.)

3. Argininosuccinate is cleaved nonhydrolytically to form arginine plus fumarate in an elimination reaction catalyzed by argininosuccinate lyase. Arginine is the immediate
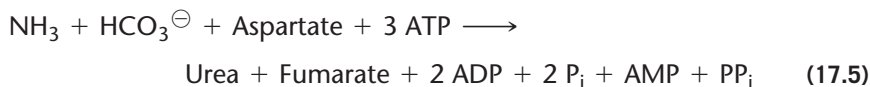
Figure 17.43 ▶
Urea cycle.
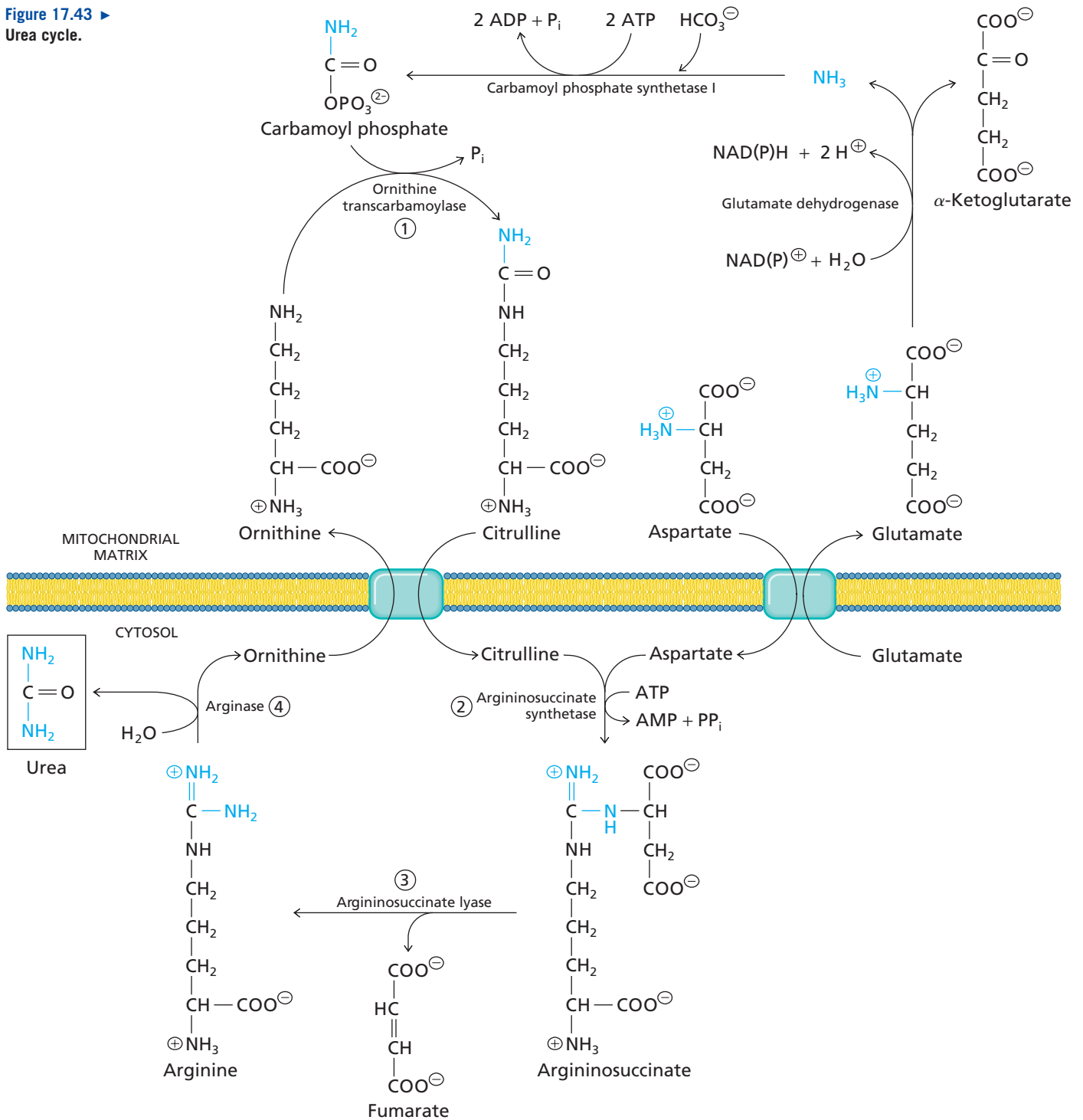
precursor of urea. (Together, the second and third steps of the urea cycle exemplify a strategy for donating the amino group of aspartate. We will encounter this strategy twice more in the next chapter as part of purine biosynthesis. The key processes are a nucleoside triphosphate–dependent condensation, followed by the elimination of fumarate.)

4. Finally, the guanidinium group of arginine is hydrolytically cleaved to form ornithine and urea in a reaction catalyzed by arginase. Arginase has a pair of Mn^{2+} ions in its active site and this binuclear manganese cluster binds a molecule of water forming a nucleophilic hydroxide ion that attacks the guanidinium carbon atom of arginine. The ornithine generated by the action of arginase is transported

into the mitochondrion where it reacts with carbamoyl phosphate to support continued operation of the urea cycle.

The overall reaction for urea synthesis is

$$NH_3 + HCO_3^{\ominus} + \text{Aspartate} + 3 \text{ ATP} \longrightarrow$$
$$\text{Urea} + \text{Fumarate} + 2 \text{ ADP} + 2 \text{ P}_i + \text{AMP} + \text{PP}_i \quad \textbf{(17.5)}$$

The two nitrogen atoms of urea are derived from ammonia and aspartate. The carbon atom of urea comes from bicarbonate. Four equivalents of ATP are consumed per molecule of urea synthesized. Three molecules of ATP are converted to two ADP and one AMP during the formation of one molecule of urea and the hydrolysis of inorganic pyrophosphate accounts for cleavage of the fourth phosphoanhydride bond.

The carbon skeleton of fumarate is converted to glucose and CO_2. Cytosolic fumarate does not enter the citric acid cycle (which occurs in mitochondria) but instead is hydrated to malate by the action of a cytosolic fumarase. Malate is oxidized to oxaloacetate by the action of malate dehydrogenase and oxaloacetate enters the pathway of gluconeogenesis. This fate is shared by the fumarate produced during tyrosine degradation (Section 17.6H).

C. Ancillary Reactions of the Urea Cycle

The reactions of the urea cycle convert equal amounts of nitrogen from ammonia and from aspartate into urea. Many amino acids can function as amino-group donors via transamination reactions with α-ketoglutarate to form glutamate. Glutamate can undergo either transamination with oxaloacetate to form aspartate or deamination to form ammonia. Both glutamate dehydrogenase and aspartate transaminase are abundant in liver mitochondria and catalyze near-equilibrium reactions. The concentrations of ammonia and aspartate must be approximately equal for efficient synthesis of urea and elimination of nitrogen.

Consider the theoretical case of a relative surplus of ammonia (Figure 17.44a). In this situation, the near-equilibrium reaction catalyzed by glutamate dehydrogenase would proceed in the direction of glutamate formation. Elevated concentrations of glutamate would then result in increased flux to aspartate through aspartate transaminase, also a near-equilibrium step. In contrast, when excess aspartate is present the net flux in the reactions catalyzed by glutamate dehydrogenase and aspartate transaminase would occur in the opposite direction to provide ammonia for urea formation (Figure 17.44b).

Figure 17.44 ▶
Balancing the supply of nitrogen for the urea cycle. Two theoretical situations are described: **(a)** NH_3 in extreme excess and **(b)** aspartate in extreme excess. Flux through the near-equilibrium reactions catalyzed by glutamate dehydrogenase and aspartate transaminase reverses, depending on the relative amounts of ammonia and amino acids.
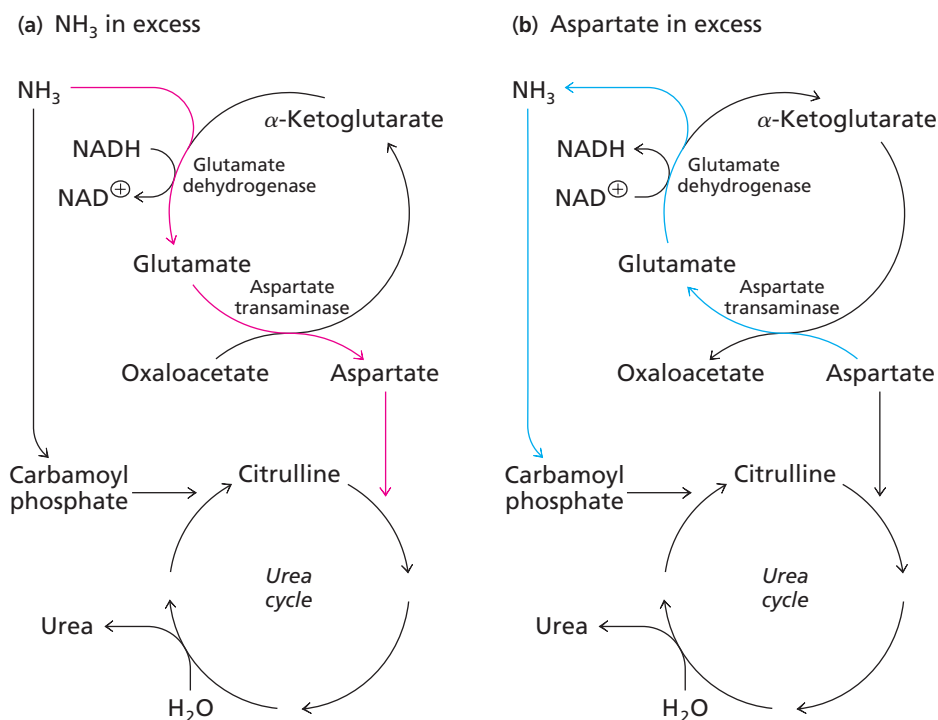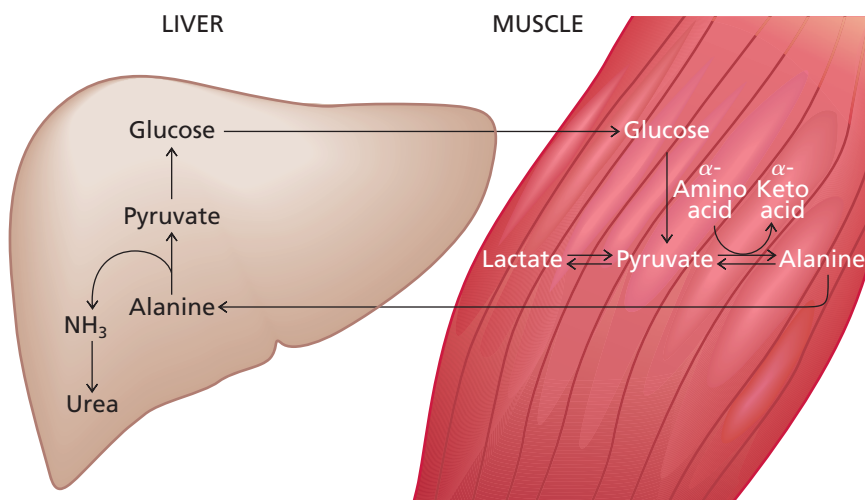
Some amino acids are deaminated in muscle, not in the liver. Glycolysis—a major source of energy in muscle—produces pyruvate. The transfer of amino groups from α-amino acids to pyruvate generates large amounts of alanine. Alanine travels through the bloodstream to the liver where it is deaminated back to pyruvate. The amino group is used for urea synthesis and the pyruvate is converted to glucose by gluconeogenesis. Recall that neither of these pathways operates in muscle. Glucose can return to the muscle tissue. Alternatively, pyruvate can be converted to oxaloacetate that becomes the carbon chain of aspartate—the metabolite that donates one of the nitrogen atoms of urea. The exchange of glucose and alanine between muscle and liver is called the glucose–alanine cycle (Figure 17.45) and it provides an indirect means for muscle to eliminate nitrogen and replenish its energy supply.

17.8 Renal Glutamine Metabolism Produces Bicarbonate

The body often produces acids as metabolic end products. The resulting anions are eliminated in the urine and the protons remain in the body. One example is β-hydroxybutyric acid, a ketone body that is produced in massive amounts during uncontrolled diabetes mellitus. Another example is sulfuric acid produced during catabolism of the sulfur-containing amino acids cysteine and methionine. These acid metabolites dissociate to give protons and the corresponding anion, β-hydroxybutyrate or sulfate ($SO_4^{\ominus}$). The blood has an effective buffer system for the protons—they react with bicarbonate to produce CO_2 that is eliminated by the lungs and H_2O (Figure 17.46). While this system effectively neutralizes the excess hydrogen ions it does so at the cost of depleting blood bicarbonate. Bicarbonate is replenished by glutamine catabolism in the kidneys.

In the kidneys, the two nitrogen atoms of glutamine are removed by the sequential action of glutaminase and of glutamate dehydrogenase to produce α-ketoglutarate$^{\ominus}$ and $2\ NH_4^{\oplus}$.

$$\text{Glutamine} \longrightarrow \longrightarrow \alpha\text{-Ketoglutarate}^{\circled{2}} + 2\ NH_4^{\oplus} \qquad \textbf{(17.6)}$$

Two molecules of the divalent anion α-ketoglutarate can be converted to one molecule of neutral glucose and four molecules of bicarbonate. The α-ketoglutarate is converted to glucose by oxidation to oxaloacetate, leading to gluconeogenesis. The overall process (ignoring ATP involvement) is

$$2\ C_5H_{10}N_2O_3 + 3\ O_2 + 6\ H_2O \longrightarrow$$
Glutamine

$$C_6H_{12}O_6 + 4\ HCO_3^{\ominus} + 4\ NH_4^{\oplus} \qquad \textbf{(17.7)}$$
Glucose
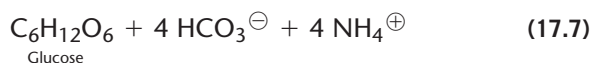
The $NH_4^{\oplus}$ is excreted in the urine and the $HCO_3^{\ominus}$ is added to the venous blood, replacing the bicarbonate lost in buffering metabolic acids. The excreted $NH_4^{\oplus}$ is accompanied in the urine by the anion (e.g., β-hydroxybutyrate or sulfate) of the original acid metabolite.
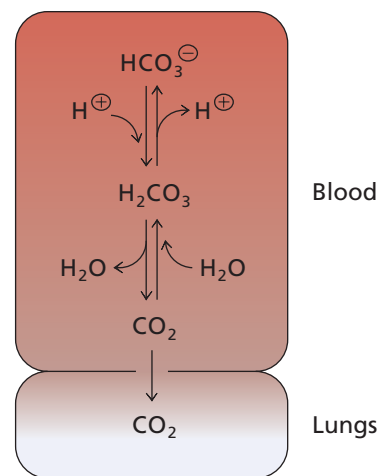
▲ **Figure 17.46**
**$H^{\oplus}$ buffering in blood.** The $H^{\oplus}$ buffer system leads to bicarbonate loss.

Summary

1. Nitrogen is fixed in only a few species of bacteria by the nitrogenase-catalyzed reduction of atmospheric N_2 to ammonia. Plants and microorganisms can reduce nitrate and nitrite to ammonia.

2. Ammonia is assimilated into metabolites by the reductive amination of α-ketoglutarate to glutamate, catalyzed by glutamate dehydrogenase. Glutamine, a nitrogen donor in many biosynthetic reactions, is formed from glutamate and ammonia by the action of glutamine synthetase.

3. The amino group of glutamate can be transferred to an α-keto acid in a reversible transamination reaction to form α-ketoglutarate and the corresponding α-amino acid.

4. Pathways for the biosynthesis of the carbon skeletons of amino acids begin with simple metabolic precursors such as pyruvate and citric acid cycle intermediates.

5. In addition to their role in protein synthesis, amino acids serve as precursors in a number of other metabolic pathways.

6. Protein molecules in all living cells are continually synthesized and degraded.

7. Amino acids obtained from protein degradation or directly from food can be catabolized. Catabolism often begins with deamination, followed by modification of the remaining carbon chains for entry into the central pathways of carbon metabolism.

8. The pathways for the degradation of amino acids lead to pyruvate, acetyl CoA, or intermediates of the citric acid cycle. Amino acids that are degraded to citric acid cycle intermediates are glucogenic. Those that form acetyl CoA are ketogenic.

9. Most nitrogen in mammals is excreted as urea that is formed by the urea cycle in the liver. The carbon atom of urea is derived from bicarbonate. One amino group is derived from ammonia and the other from aspartate.

10. The metabolism of glutamine in the kidneys produces the bicarbonate needed to neutralize acids produced in the body.

Problems

1. The heterocysts of cyanobacteria contain high concentrations of nitrogenase. These cells have retained photosystem I (PSI) but they do not contain photosystem II (PSII). Why?

2. Write the net equation for converting one molecule of α-ketoglutarate into one molecule of glutamine by assimilating two molecules of ammonia in the following coupled reactions: (a) glutamate dehydrogenase and glutamine synthetase and (b) glutamine synthetase and glutamate synthase. Compare the energy requirements of the two pathways and account for any difference.

3. When ^{15}N-labeled aspartate is fed to animals the ^{15}N label quickly appears in many amino acids. Explain this observation.

4. (a) Three of the 20 common amino acids are synthesized by simple transamination of carbohydrate metabolites. Write the equations for these three transamination reactions.

   (b) One amino acid can be synthesized by reductive amination of a carbohydrate metabolite. Write the equation for this reaction.

5. Animals rely on plants or microorganisms for the incorporation of sulfur into amino acids and their derivatives. However, methionine is an essential amino acid in animals while cysteine is not. If the donor of a sulfur atom in the conversion of homoserine to homocysteine by plants is cysteine, outline the overall path by which sulfur is incorporated into cysteine and methionine in plants and into cysteine in animals.

6. Serine is a source of one-carbon fragments for certain biosynthetic pathways.

   (a) Write the equations that show how two carbon atoms from serine are made available for biosynthesis.

   (b) Assuming that the precursor of serine is produced by glycolysis, which carbon atoms of glucose are the ultimate precursors of these one-carbon fragments?

7. Indicate where the label appears in the product for each of the following precursor–product pairs:

   (a) 3-$[^{14}C]$-Oxaloacetate $\longrightarrow$ Threonine

   (b) 3-$[^{14}C]$-Phosphoglycerate $\longrightarrow$ Tryptophan

   (c) 3-$[^{14}C]$-Glutamate $\longrightarrow$ Proline

   (d)

Chorismate $\longrightarrow$ Phenylalanine
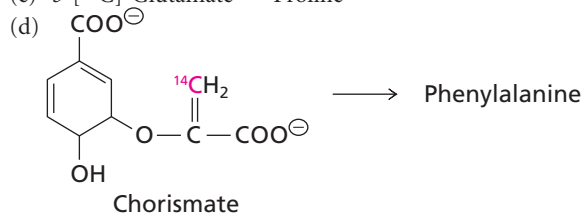
8. (a) PPT (phosphinothricin) is a herbicide that is relatively safe for animals because it is not transported from the blood into the brain and it is rapidly cleared by animal kidneys. PPT effectively inhibits an enzyme in plant amino acid metabolism because it is an analog of the amino acid substrate. What amino acid does PPT resemble?

PPT
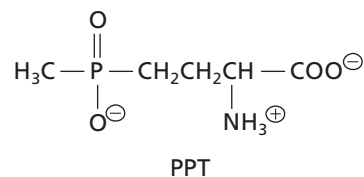
   (b) The herbicide aminotriazole inhibits imidazole glycerol phosphate dehydrogenase. What amino acid pathway is inhibited in plants?

Aminotriazole
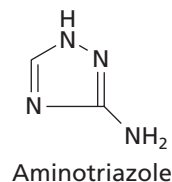
9. Children with phenylketonuria should not consume the artificial sweetener aspartame (Figure 3.10). Why?

10. (a) A deficiency of α-keto acid dehydrogenase is the most common enzyme abnormality in branched chain amino acid catabolism. Individuals with this disease excrete branched chain α-keto acids. Write the structures of the α-keto acids that would result during the catabolism of leucine, valine, and isoleucine when this enzyme is defective.

(b) A disorder of amino acid catabolism results in the accumulation and excretion of saccharopine. What amino acid pathway is involved and what enzyme is defective?

(c) Citrullinemia is characterized by accumulation of citrulline in the blood and its excretion in the urine. What metabolic pathway is involved and what enzyme is deficient for this disease?

11. Which amino acids yield the following α-keto acids by transamination?

(a)
$$^{\ominus}OOC - \overset{\overset{O}{\|}}{C} - CH_3$$

(b)
$$^{\ominus}OOC - \overset{\overset{O}{\|}}{C} - CH_2 - COO^{\ominus}$$

(c)
$$^{\ominus}OOC - CH$$

(d)
$$^{\ominus}OOC - \overset{\overset{O}{\|}}{C} - CH_2 - SH$$

12. Animal muscles use two mechanisms to eliminate excess nitrogen generated during the deamination of amino acids. What are the two pathways and why are they necessary?

13. Thiocitrulline and *S*-methylthiocitrulline prevent experimentally induced blood vessel dilation, reduced blood pressure, and shock in animals. What enzyme that produces a gaseous blood vessel dilating messenger is being inhibited? Suggest why these two molecules might act as inhibitors of this enzyme.

14. Why are there so few metabolic diseases associated with defects in amino acid biosynthesis?

15. Pathways for the biosynthesis of the 21st, 22nd, and 23rd amino acids (Section 3.3) are not described in this chapter. Why not? What are the immediate precursors of three additional amino acids?

16. The cost of making amino acids, in ATP equivalents, can be calculated using values for the cost of making each of the precursors plus the cost of each reaction in the amino acid biosynthesis pathway. Assuming that the cost of making glyceraldehyde-3-phosphate is 24 ATP equivalents (Section 15.4C), calculate the cost of making serine (Figure 17.15) and alanine (Figure 17.12). How do your values compare to those in Box 17.3?

Selected Readings

Nitrogen Cycle

Dixon, R., and Kahn, D. (2004). Genetic regulation of biological nitrogen fixation. *Nat. Rev. Microbiol.* 2:621–631.

Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., Montoya, J. P., and Zehr, J. P. (2010). Unicellular cyanobacterial distributions broaden the oceanic N_2 fixation domain. *Science* 327:1512–1524.

Montoya, J. P., Holl, C. M., Zehr, J. P., Hansen, A., Villareal, T. A., and Capone, D. G. (2004). High rates of N_2 fixation in the oligotrophic Pacific ocean. *Nature* 430:1027–1031.

Schimpl, J., Petrilli, H. M., and Blöchl, P. E. (2003). Nitrogen binding to the FeMo-cofactor of nitrogenase. *J. Am. Chem. Soc.* 125:15772–15778.

Seefeldt, L. C., Hoffman, B. M., and Dean, D. R. (2009). Mechanism of Mo-dependent nitrogenase. *Annu. Rev. Biochem.* 78:701–722.

Amino Acid Metabolism

Fitzpatrick, P. F. (1999). Tetrahydropterin-dependent amino acid hydroxylases. *Annu. Rev. Biochem.* 68:355–381.

Häussinger, D. (1998). Hepatic glutamine transport and metabolism. *Adv. Enzymol. Relat. Areas Mol. Biol.* 72:43–86.

Huang. X., Holden, H. M., and Raushel, F. M. (2001). Channeling of substrates and intermediates in enzyme-catalyzed reactions. *Annu. Rev. Biochem.* 70:149–180.

Katagiri, M., and Nakamura, M. (2003). Reappraisal of the 20th-century version of amino acid metabolism. *Biochem. Biophys, Res, Comm.* 312:205–208.

Levy, H. L. (1999). Phenylketonuria: old disease, new approach to treatment. *Proc. Natl. Acad. Sci. USA* 96:1811–1813.

Perham, R. N. (2000). Swinging arms and swinging domains in multifunctional enzymes: catalytic machines for multistep reactions. *Annu. Rev. Biochem.* 69:961–1004.

Purich, D. L. (1998). Advances in the enzymology of glutamine synthesis. *Adv. Enzymol. Relat. Areas Mol. Biol.* 72:9–42.

Raushel, F. M., Thoden, J. B., and Holden, H. M. (2003). Enzymes with molecular tunnels. *Acc. Chem. Res.* 36:539–548.

Richards, N. G. and Kilberg, M. S. (2006). Asparagine synthetase chemotherapy. *Annu. Rev. Biochem.* 75:629–654.

Scapin, G., and Blanchard, J. S. (1998). Enzymology of bacterial lysine biosynthesis. *Adv. Enzymol. Relat. Areas Mol. Biol.* 72:279–324.

Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds. (1995). *The Metabolic Basis of Inherited Disease*, Vols. 1, 2, and 3. (New York: McGraw-Hill).

Srivastava, D., Schuermann, J. P., White, T. A., Krishnan, N., Sanyal, N., Hura, G. L., Tan, A., Henzl, M. T., Becker, D. F., and Tanner, J. J. (2010). Crystal structure of the bifunctional proline utilization A flavoenzyme from *Bradyrhizobium japonicum. Proc. Natl. Acad. Sci. USA* 107:2878–2883.

Wu, G., and Morris, S. M., Jr. (1998). Arginine metabolism: nitric oxide and beyond. *Biochem. J.* 336:1–17.

Zalkin, H., and Smith, J. L. (1998). Enzymes utilizing glutamine as an amide donor. *Adv. Enzymol. Relat. Areas Mol. Biol.* 72:87–144.
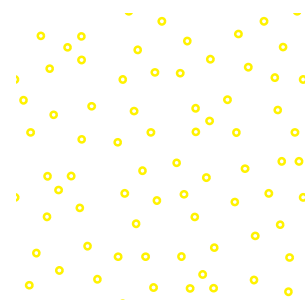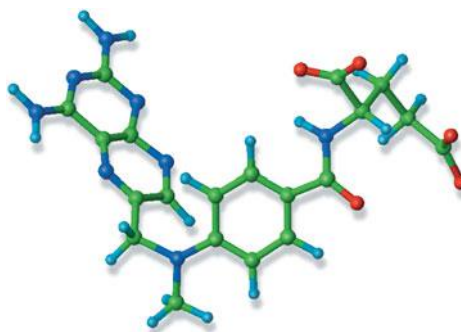
Nucleotide Metabolism

W e have encountered nucleotides and their constituents throughout this book. Nucleotides are probably best known as the building blocks of DNA and RNA; however, as we have seen, they are involved in almost all the activities of the cell either alone or in combination with other molecules. Some nucleotides (such as ATP) function as cosubstrates, and others (such as cyclic AMP and GTP) are regulatory compounds.

One of the components of every nucleotide is a purine or pyrimidine base. The other components are a five-carbon sugar—ribose or deoxyribose—and one or more phosphoryl groups. The standard bases (adenine, guanine, cytosine, thymine, uracil) are almost always found as constituents of nucleotides and polynucleotides. All organisms and cells can synthesize purine and pyrimidine nucleotides because these molecules are essential for information flow. In non-dividing cells, nucleotide biosynthesis is almost exclusively devoted to the production of ribonucleotides for RNA synthesis and various nucleotide cofactors. Deoxyribonucleotides are required for DNA replication in dividing cells and consequently, deoxynucleotide synthesis is closely linked to cell division. Its study is particularly important in modern medicine since synthetic agents that inhibit deoxyribonucleotide synthesis are useful as therapeutic agents against cancer.

We begin this chapter with a description of the biosynthesis of purine and pyrimidine nucleotides. Next, we present the conversion of purine and pyrimidine ribonucleotides to their 2′-deoxy forms, the forms incorporated into DNA. We then discuss how purines and pyrimidines obtained from the breakdown of nucleic acids or extracellularly from food can be incorporated directly into nucleotides—a process called salvage. The salvage pathways conserve energy by recycling the products of nucleic acid turnover. Finally, we examine the biological degradation of nucleotides. The breakdown of purines leads to the formation of potentially toxic compounds that are excreted, whereas the breakdown of pyrimidines leads to readily metabolized products.

[Sven] Furberg, reasoning with marked brilliance and luck from data that were meagre but included his own x-ray studies, got right the absolute three-dimensional configuration of the individual nucleotide . . . "Furberg's nucleotide . . . was absolutely essential to us." Crick told me.

Horace Freeland Judson (1996), *The Eighth Day of Creation*, p. 94.

18.1 Synthesis of Purine Nucleotides

The identification of the enzymes and intermediates in the pathway for the synthesis of the two purine nucleotides, adenosine 5′-monophosphate (AMP) and guanosine

---

Top: Methotrexate, one of the most commonly used anticancer drugs. Methotrexate is an analog of folate that inhibits the reaction cycle generating deoxythymidylate for DNA synthesis.

5′-monophosphate (GMP), began with studies of nitrogen metabolism in birds. The major end product of nitrogen metabolism in birds and some reptiles is uric acid (Figure 18.1) rather than urea, as in mammals. Researchers in the 1950s discovered that uric acid and nucleic acid purines arise from the same precursors and reaction sequence. Homogenates of pigeon liver—a tissue in which purines are actively synthesized—were a convenient source of enzymes for studying the steps in purine biosynthesis. The pathway in avian liver has since been found in many other organisms.

When isotopically labeled compounds such $^{13}CO_2$, $H^{13}COO^-$ (formate), and $^+H_3N-CH_2-^{13}COO^-$ (glycine) were administered to pigeons and rats the result was excretion of labeled uric acid. This uric acid was isolated and chemically degraded to identify the positions of the labeled carbon and nitrogen atoms. The carbon from carbon dioxide was incorporated into C-6, and the carbon from formate into C-2 and C-8 of purines. Ultimately, the sources of the ring atoms were shown to be N-1, aspartate; C-2 and C-8, formate via 10-formyltetrahydrofolate (Section 7.9); N-3 and N-9, amide groups from glutamine; C-4, C-5, and N-7, glycine; and C-6, carbon dioxide. These findings are summarized in Figure 18.2.

The purine ring structure is not synthesized as a free base but as a substituent of ribose 5-phosphate. The ribose 5-phosphate for purine biosynthesis comes from 5-phosphoribosyl 1-pyrophosphate (PRPP) also known as 5-phosphoribosyl 1-diphosphate. PRPP is synthesized from ribose 5-phosphate and ATP in a reaction catalyzed by ribose-phosphate diphosphokinase (Figure 18.3); PRPP then donates ribose 5-phosphate to serve as the foundation on which the purine structure is built. PRPP is also a precursor for the biosynthesis of pyrimidine nucleotides, although in that pathway it reacts with a preformed pyrimidine to form a nucleotide. PRPP is also used in the nucleotide salvage pathways and in the biosynthesis of histidine (Figure 17.23).

The initial product of the purine nucleotide biosynthetic pathway is inosine 5′-monophosphate (IMP, or inosinate) (Figure 18.4), a nucleotide containing hypoxanthine (6-oxopurine) as its base. The ten-step pathway for the *de novo* synthesis of IMP was discovered in the 1950s by the research groups of John M. Buchanan and G. Robert Greenberg. The painstaking isolation and structural characterization of the intermediates took about ten years.

The pathway to IMP is shown in Figure 18.5. It begins with displacement of the pyrophosphoryl group of PRPP by the amide nitrogen of glutamine in a reaction catalyzed by glutamine–PRPP amidotransferase. Note that the configuration of the anomeric carbon is inverted from α to β in this nucleophilic displacement—the β configuration persists in completed purine nucleotides. The amino group of the product, phosphoribosylamine, is then acylated by glycine to form glycinamide ribonucleotide. The mechanism of this reaction, in which an enzyme-bound glycyl phosphate is formed, resembles that of glutamine synthetase that has γ-glutamyl phosphate as an intermediate (Reaction 10.17).

Step 3 consists of transfer of a formyl group from 10-formyltetrahydrofolate to the amino group destined to become N-7 of IMP. In step 4, an amide is converted to an amidine ($RHN-C=NH$) in an ATP-dependent reaction in which glutamine is the nitrogen donor. Step 5 is a ring-closure reaction that requires ATP and produces an imidazole derivative. CO_2 is incorporated in step 6 by attachment to the carbon that becomes C-5 of IMP. This carboxylation is unusual because it does not require biotin. Bicarbonate is first attached, in an ATP-dependent step, to the amino group that becomes N-3 of IMP. The carboxylated intermediate then undergoes a rearrangement in which the carboxylate group is transferred to the carbon atom that becomes C-5 of the purine ring (Figure 18.6). These steps are catalyzed by two separate proteins in *Escherichia coli* but in eukaryotes they are catalyzed by a multifunctional enzyme. Vertebrate versions of this enzyme transfer the carboxylate group directly to the final position in carboxyaminoimidazole ribonucleotide (CAIR). The vertebrate enzymes are much more efficient. There doesn't seem to be any reason why the enzymes in other species have to undergo a two-step reaction.

The amino group of aspartate is incorporated into the growing purine ring system in steps 7 and 8. First, aspartate condenses with the newly added carboxylate group to form an amide, specifically a succinylocarboxamide. Then adenylosuccinate lyase catalyzes a nonhydrolytic cleavage reaction that releases fumarate. This two-step process
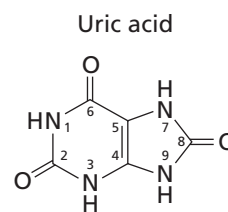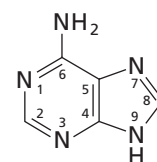
Uric acid
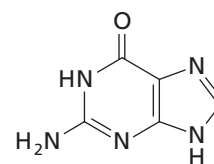

▲ **Figure 18.1**
Uric acid.
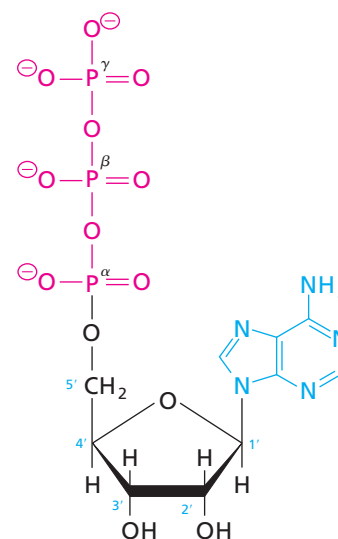
▲ **Structure of adenosine triphosphate (ATP).** The nitrogenous base adenine (blue) is attached to ribose (black). Three phosphoryl groups (red) are bound to the ribose at the 5′ position.

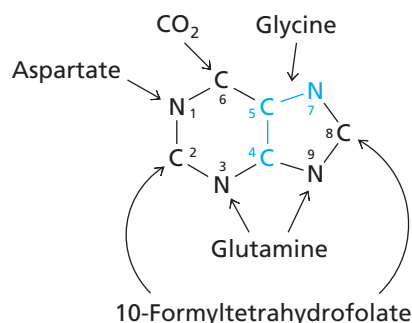**Ribose 5-phosphate is produced by the pentose phosphate pathway (Section 12.4).**
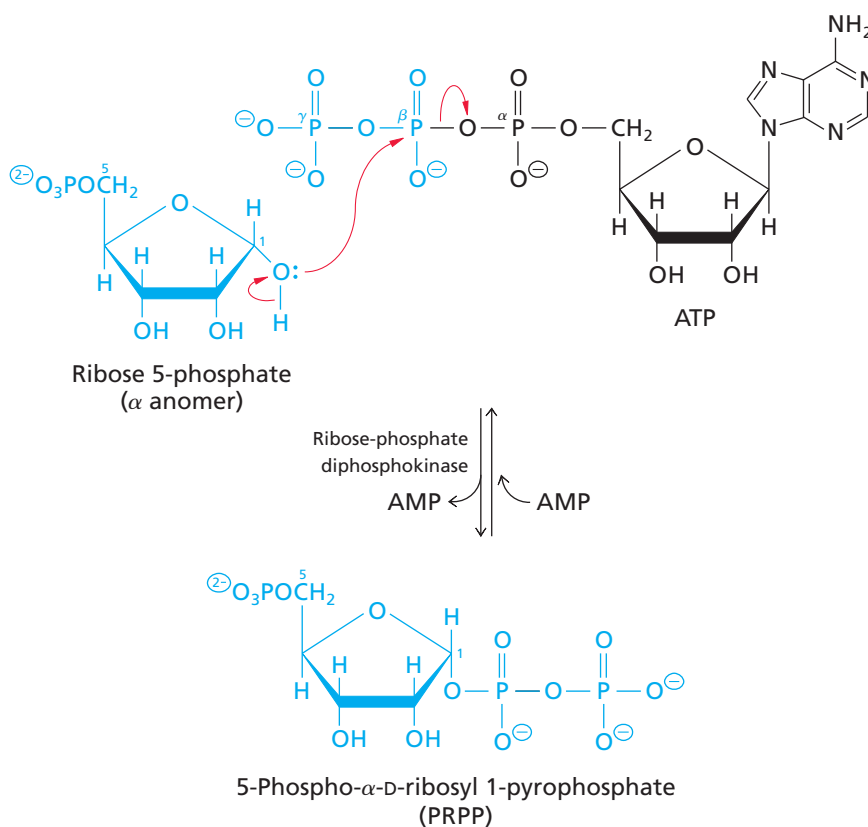
THE MAJOR PURINES

Adenine
(6-Aminopurine)

Guanine
(2-Amino-6-oxopurine)

▲ **Adenine, Guanine**

▲ **Figure 18.2**
Sources of the ring atoms in purines synthesized *de novo*.

Ribose 5-phosphate
(α anomer)

Ribose-phosphate
diphosphokinase
AMP ⇌ AMP

5-Phospho-α-D-ribosyl 1-pyrophosphate
(PRPP)

▲ **Figure 18.3**
Synthesis of 5-phosphoribosyl 1-pyrophosphate (PRPP) from ribose 5-phosphate and ATP. Ribose-phosphate diphosphokinase catalyzes the transfer of a pyrophosphoryl group from ATP to the 1-hydroxyl oxygen of ribose 5-phosphate.


▲ **G. Robert Greenberg (1918–2005).**
Greenberg's research group worked out many of the reactions of the purine biosynthesis pathway.

results in the transfer of an amino group containing the nitrogen destined to become N-1 of IMP. The two steps are similar to steps 2 and 3 of the urea cycle (Figure 17.43) except that in this case ATP is cleaved to ADP + P_i rather than to AMP + PP_i.

In step 9, which resembles step 3, the cosubstrate 10-formyltetrahydrofolate donates a formyl group ($-CH=O$) to the nucleophilic amino group of aminoimidazole carboxamide ribonucleotide. The amide nitrogen of the final intermediate then condenses with the formyl group in a ring closure that completes the purine ring system of IMP.

The synthesis of IMP consumes considerable energy. ATP is converted to AMP during the synthesis of PRPP and steps 2, 4, 5, 6, and 7 are driven by the conversion of ATP to ADP. Additional ATP is required for the synthesis of glutamine from glutamate and ammonia (Figure 17.4).
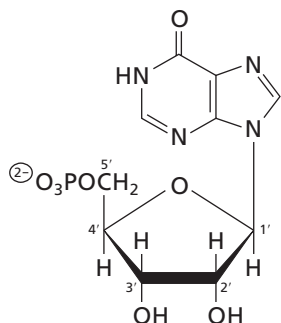
▲ **Figure 18.4**
Inosine 5′-monophosphate (IMP, or inosinate). IMP is converted to other purine nucleotides. Much of the IMP is degraded to uric acid in birds and primates.

BOX 18.1 COMMON NAMES OF THE BASES

Adenine	from the Greek *adenas*, "gland": first isolated from pancreatic glands (1885)
Cytosine	derived from *cyto-* from the Greek word for "receptacle," referring to cells (1894)
Guanine	originally isolated from "guano" or bird excrement (1850)
Uracil	origin uncertain, possibly from "urea" (1890)
Thymine	first isolated from thymus glands (1894)
Xanthine	from the Greek word for "yellow" (1857)
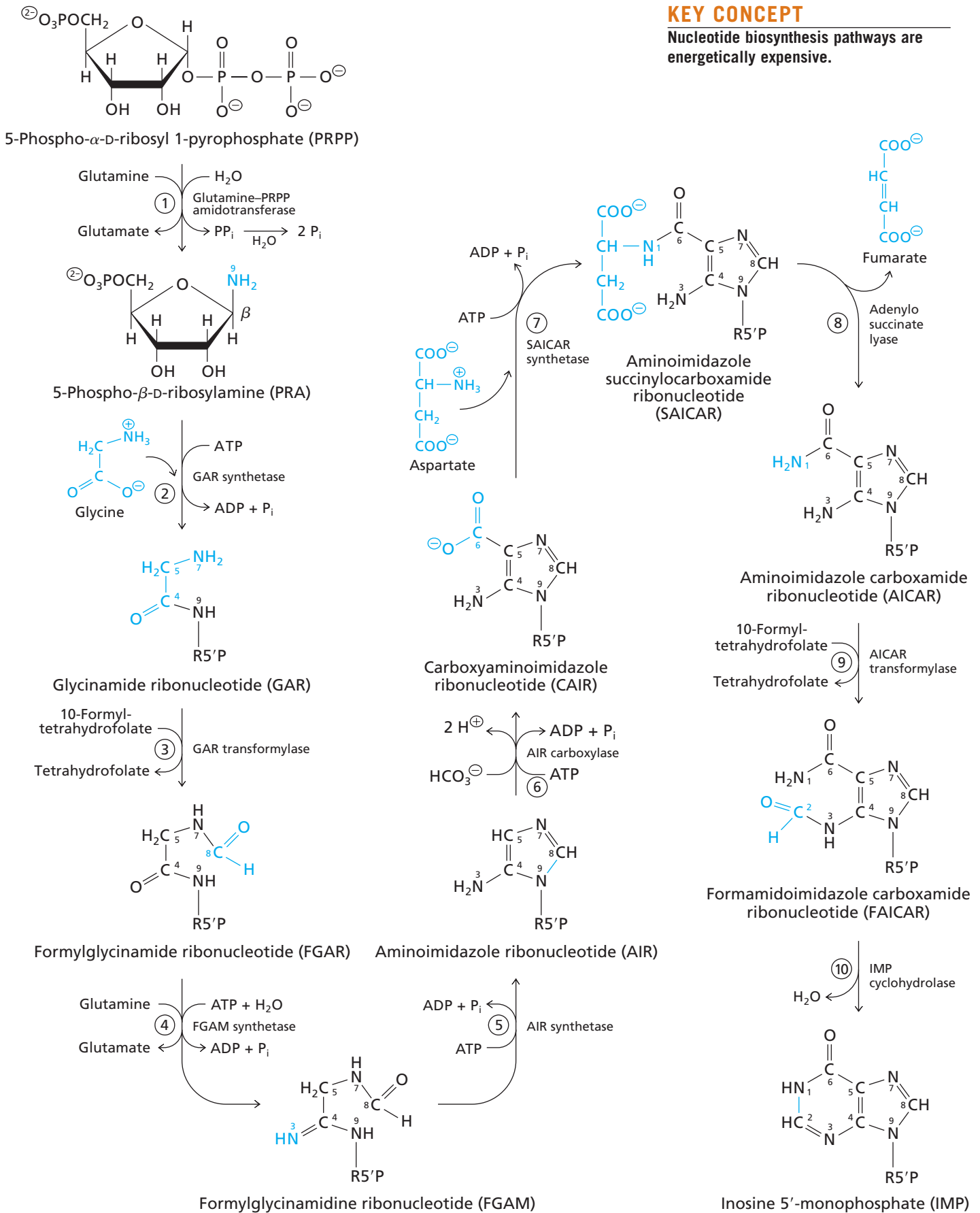
▲ **Figure 18.5**

Ten-step pathway for the _de novo_ synthesis of IMP. R5′P stands for ribose 5′-phosphate. The atoms are numbered according to their positions in the completed purine ring structure.

Vertebrates

Aminoimidazole
ribonucleotide
(AIR)

$HCO_3^{\ominus}$ $2H^{\oplus}$

ATP ADP
+
P_i

N-Carboxyaminoimidazole
ribonucleotide

Carboxyaminoimidazole
ribonucleotide
(CAIR)
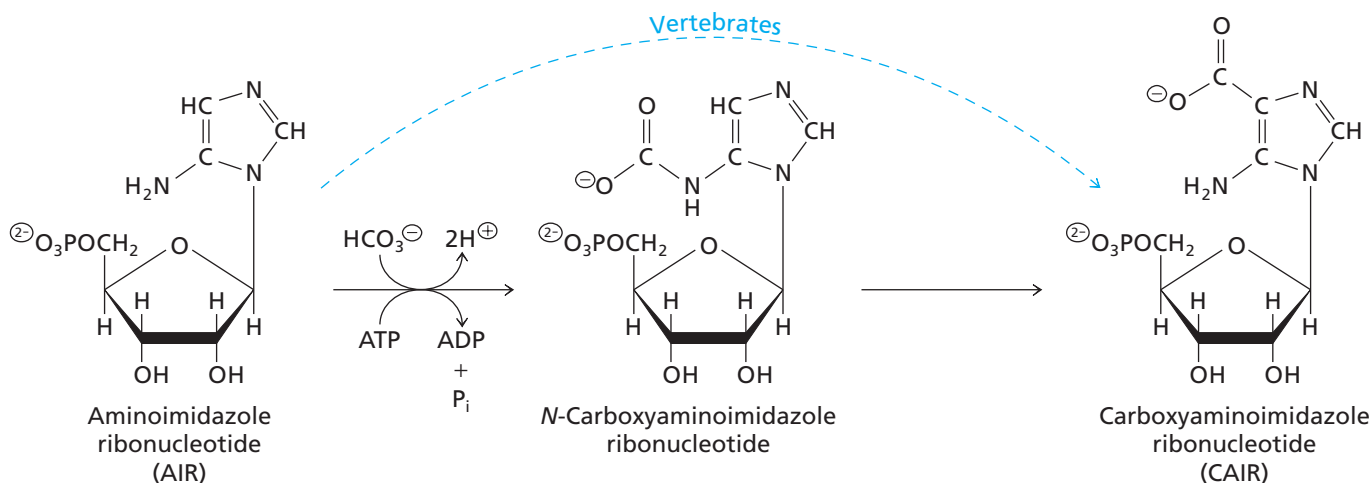
▲ **Figure 18.6**
***N*-Carboxyaminoimidazole ribonucleotide is sometimes an intermediate in the conversion of AIR to CAIR.**

▲ **John M. ("Jack") Buchanan (1917–2007).**
Buchanan's group discovered many of the purine biosynthesis pathway reactions. He and Greenberg were friendly competitors sharing many of their research results.

18.2 Other Purine Nucleotides Are Synthesized from IMP

IMP can be converted to AMP or GMP (Figure 18.7). Two enzymatic reactions are required for each of these conversions. AMP and GMP can then be phosphorylated to their di- and triphosphates by the actions of specific nucleotide kinases (adenylate kinase and guanylate kinase, respectively) and the broadly specific nucleoside diphosphate kinase (Section 10.6).

The two steps that convert IMP to AMP closely resemble steps 7 and 8 in the biosynthesis of IMP. First, the amino group of aspartate condenses with the keto group of IMP in a reaction catalyzed by GTP-dependent adenylosuccinate synthetase. Next, the elimination of fumarate from adenylosuccinate is catalyzed by adenylosuccinate lyase, the same enzyme that catalyzes step 8 of the *de novo* pathway to IMP.

The first step in the conversion of IMP to GMP is the oxidation of C-2 catalyzed by $NAD^{\oplus}$-dependent IMP dehydrogenase. This reaction proceeds by the addition of a molecule of water to the double bond between C-2 and N-3 followed by oxidation of the hydrate. The product of the oxidation is xanthosine monophosphate (XMP). Next, the amide nitrogen of glutamine replaces the oxygen at C-2 of XMP in an ATP-dependent reaction catalyzed by GMP synthetase. The use of GTP as a cosubstrate in the synthesis of AMP from IMP, and of ATP in the synthesis of GMP from IMP, helps balance the formation of the two products.

Purine nucleotide synthesis is regulated in cells by feedback inhibition. Several enzymes that catalyze steps in the biosynthesis of purine nucleotides exhibit allosteric behavior *in vitro*. Ribose-phosphate diphosphokinase is inhibited by several purine ribonucleotides but only at concentrations higher than those usually found in the cell. PRPP is a donor of ribose 5-phosphate in more than a dozen reactions so we would not expect PRPP synthesis to be regulated exclusively by the concentrations of purine nucleotides. The enzyme that catalyzes the first committed step in the pathway of purine nucleotide synthesis, glutamine–PRPP amidotransferase (step 1 in Figure 18.5), is allosterically inhibited by 5′-ribonucleotide end products (IMP, AMP, and GMP) at intracellular concentrations. This step appears to be the principal site of regulation of this pathway.

The paths leading from IMP to AMP and from IMP to GMP also appear to be regulated by feedback inhibition. Adenylosuccinate synthetase is inhibited *in vitro* by AMP, the product of this two-step branch. Both XMP and GMP inhibit IMP dehydrogenase. The pattern of feedback inhibition in the synthesis of AMP and GMP is shown in Figure 18.8. Note that the end products inhibit two of the initial common steps as well as steps leading from IMP at the branch point.
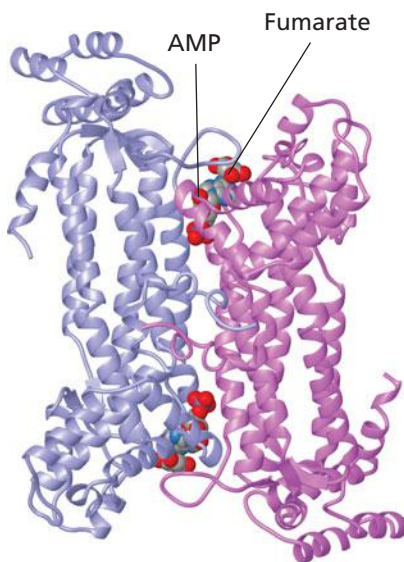
18.3 Synthesis of Pyrimidine Nucleotides

Uridine 5′-monophosphate is the precursor of other pyrimidine nucleotides. The pathway for the biosynthesis of UMP is simpler than the purine pathway and consumes fewer ATP molecules. The pyrimidine ring atoms come from bicarbonate that contributes C-2; the amide group of glutamine (N-3); and aspartate that contributes the remaining atoms (Figure 18.9). C-2 and N-3 are incorporated after formation of the intermediate carbamoyl phosphate.

PRPP is required for the biosynthesis of pyrimidine nucleotides but the sugar-phosphate from PRPP is donated after the ring has formed rather than entering the
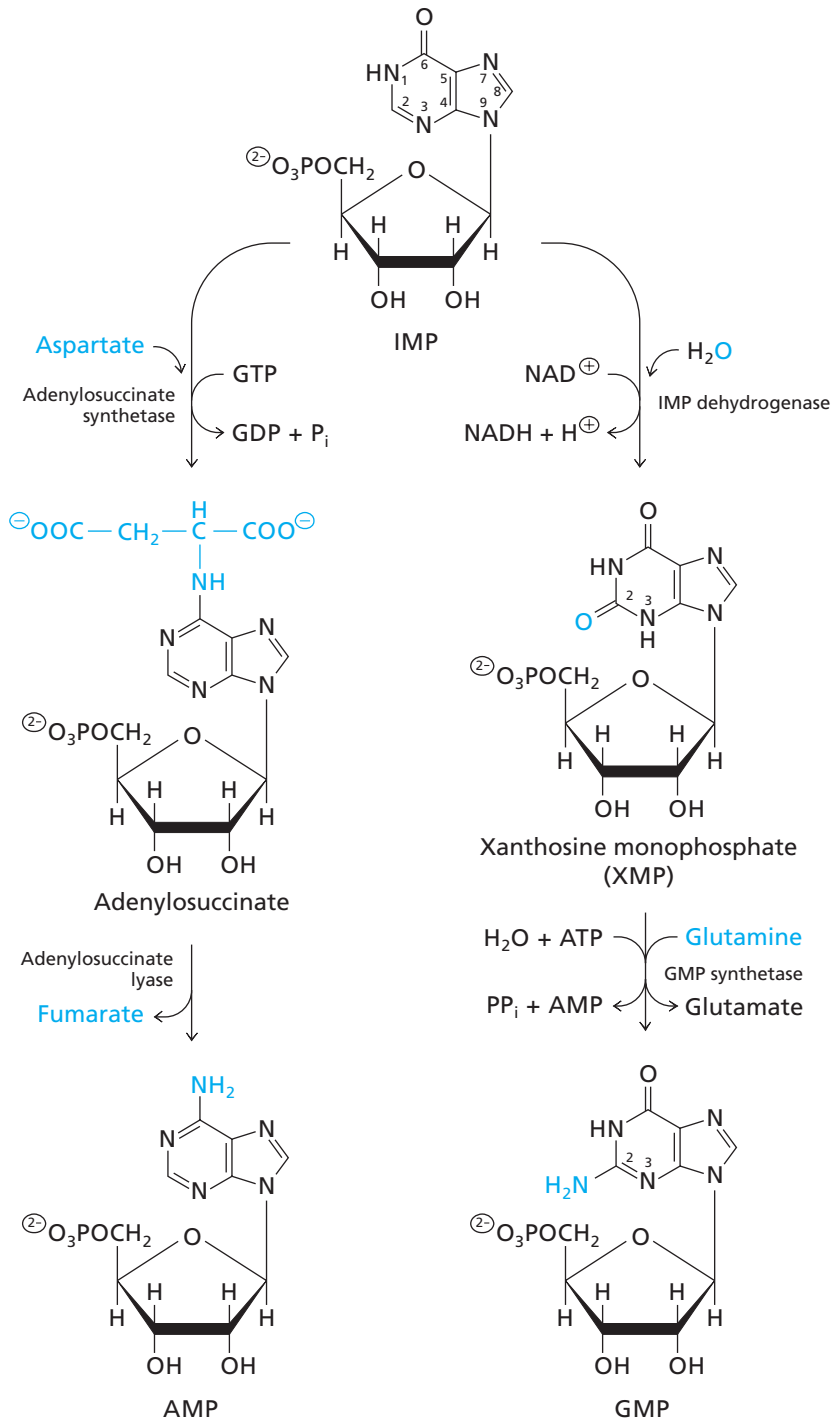
▲ **Figure 18.7**
Pathways for the conversion of IMP to AMP and to GMP.

Feedback inhibition in purine nucleotide biosynthesis.

Ribose 5-phosphate

Ribose-phosphate diphosphokinase

PRPP

Glutamine–PRPP amidotransferase

5-Phosphoribosylamine

(Steps 2–10)

IMP

Adenylosuccinate synthetase

IMP dehydrogenase

Adenylosuccinate

XMP

Adenylosuccinate lyase

GMP synthetase

AMP

GMP

▲ **Adenylosuccinate lyase from *E. coli*.** The enzyme is a homodimer. One subunit is colored blue and the other is purple. This is a mutant enzyme (H171N) showing the two products, AMP and fumarate, bound at the active sites. Adenylosuccinate lyase catalyzes similar steps in the IMP synthesis pathway and in the conversion of IMP to AMP. [PDB 2PTQ]
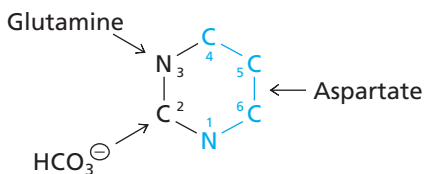
▲ **Figure 18.9**
Sources of the ring atoms in pyrimidines. The immediate precursor of C-2 and N-3 is carbamoyl phosphate.
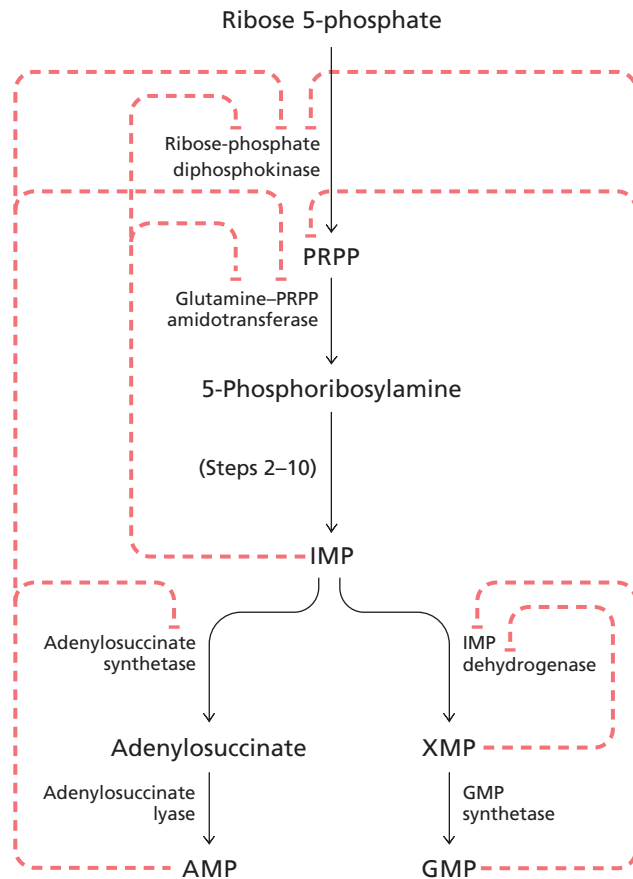
pathway in the first step. A compound with a completed pyrimidine ring—orotate (6-carboxyuracil)—reacts with PRPP to form a pyrimidine ribonucleotide in the fifth step of the six-step pathway.

A. The Pathway for Pyrimidine Synthesis

The six-step pathway for pyrimidine synthesis is shown in Figure 18.10. The first two steps generate a noncyclic intermediate that contains all the atoms destined for the pyrimidine ring. This intermediate, carbamoyl aspartate, is enzymatically cyclized. The product is dihydroorotate and it is subsequently oxidized to orotate. Orotate is then converted to the ribonucleotide orotidine 5′-monophosphate (OMP, or orotidylate) that undergoes decarboxylation to form UMP (uridylate). This pyrimidine nucleotide is the precursor not only of all other pyrimidine ribonucleotides but also of the pyrimidine deoxyribonucleotides. The enzymes required for pyrimidine synthesis are organized and regulated differently in prokaryotes and eukaryotes.

The first step in the pathway of pyrimidine biosynthesis is the formation of carbamoyl phosphate from bicarbonate plus the amide nitrogen of glutamine and ATP. This reaction is catalyzed by carbamoyl phosphate synthetase (or by carbamoyl phosphate synthetase II activity in mammalian cells). It requires two molecules of ATP—one to drive formation of the C—N bond and the other to donate a phosphoryl group. This enzyme is not the same carbamoyl phosphate synthetase that is used in the urea cycle. That enzyme, carbamoyl phophate synthetase I, assimilates free ammonia whereas this enzyme (carbamoyl phosphate synthetase II in animals) transfers an amino group from glutamine.

The activated carbamoyl group of carbamoyl phosphate is transferred to aspartate to form carbamoyl aspartate in the second step of UMP biosynthesis. This reaction is catalyzed by a famous enzyme, aspartate transcarbamoylase (ATCase). The mechanism involves the nucleophilic attack of the aspartate nitrogen on the carbonyl group of carbamoyl phosphate.

Dihydroorotase catalyzes the third step of UMP biosynthesis—the reversible closure of the pyrimidine ring (Figure 18.10). The product, dihydroorotate, is then oxidized by the
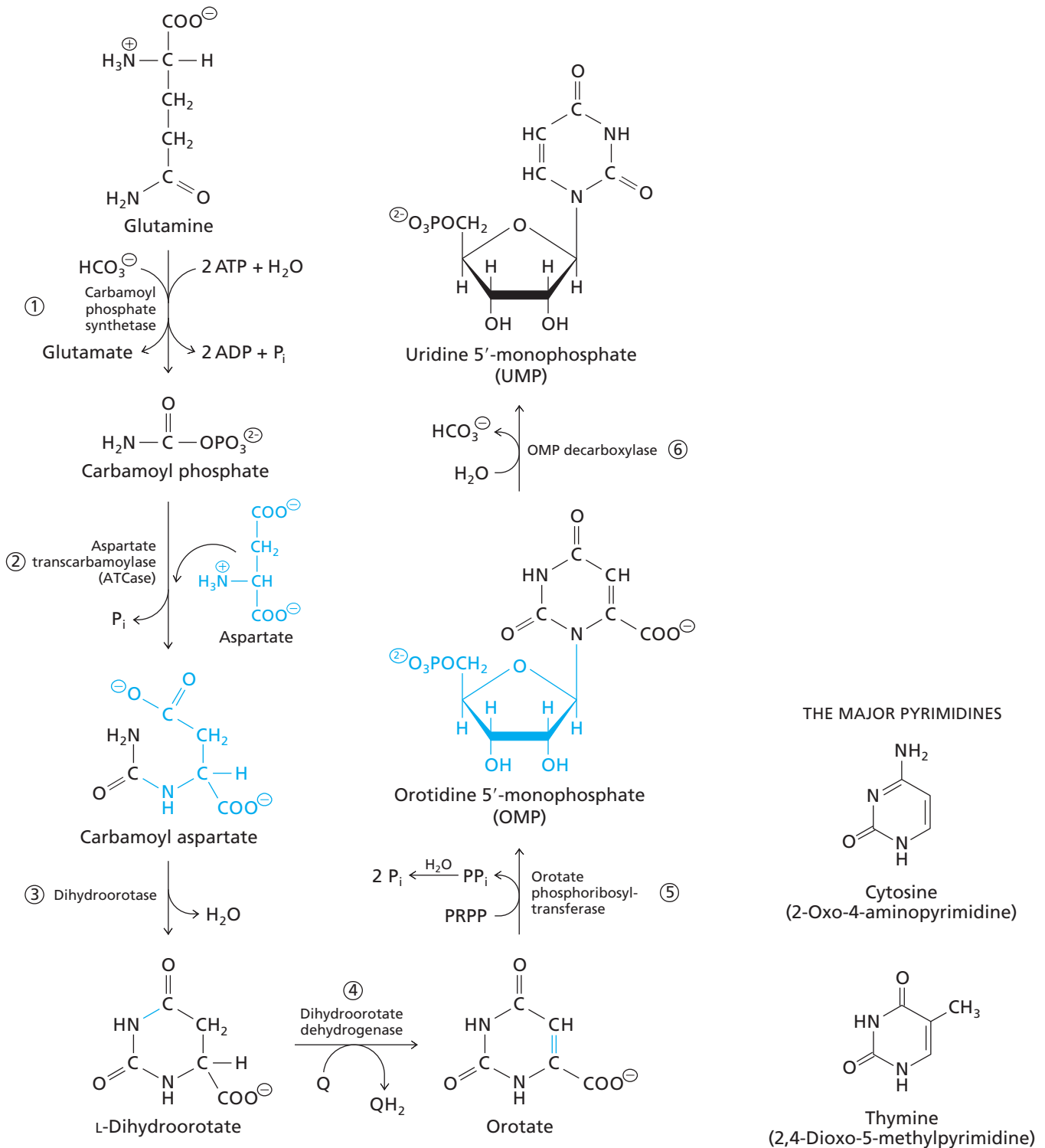
▲ **Figure 18.10**

Six-step pathway for the synthesis of UMP in prokaryotes. In eukaryotes, steps 1 through 3 are catalyzed by a multifunctional protein called dihydroorotate synthase, and steps 5 and 6 are catalyzed by a bifunctional enzyme, UMP synthase.

action of dihydroorotate dehydrogenase to form orotate. In eukaryotes, dihydroorotate is produced in the cytosol by steps 1 through 3. It then passes through the outer mitochondrial membrane prior to being oxidized to orotate by the action of dihydroorotate dehydrogenase. This enzyme is associated with the inner mitochondrial membrane. Its substrate binding site is located on the outer surface. The enzyme is an iron-containing

▲ **Cytosine, Thymine, Uracil**

flavoprotein that catalyzes the transfer of electrons to ubiquinone (Q) forming ubiquinol (QH_2). Electrons from QH_2 are then transferred to O_2 via the electron transport chain.

Once formed, orotate displaces the pyrophosphate group of PRPP, producing OMP in a reaction catalyzed by orotate phosphoribosyltransferase. The subsequent hydrolysis of pyrophosphate makes this reaction essentially irreversible.

Finally, OMP is decarboxylated to form UMP in a reaction catalyzed by OMP decarboxylase. In eukaryotes, orotate produced in the mitochondria moves to the cytosol where it is converted to UMP. A bifunctional enzyme known as UMP synthase catalyzes both the reaction of orotate with PRPP to form OMP and the rapid decarboxylation of OMP to UMP.

In mammals, the intermediates formed in steps 1 and 2 (carbamoyl phosphate and carbamoyl aspartate) and OMP (from step 5) are not normally released to solvent but remain bound to enzyme complexes and are channeled from one catalytic center to the next. Several multifunctional proteins, each catalyzing several steps, also occur in the pathway of purine nucleotide biosynthesis in some organisms.

> **Orotidine 5′-phosphate decarboxylase (OMP decarboxylase) is one of the most efficient enzymes known (Table 5.2).**

BOX 18.2 HOW SOME ENZYMES TRANSFER AMMONIA FROM GLUTAMINE

Several enzymes that use glutamine as an amide donor have a molecular tunnel running through the interior of the protein. This is an example of metabolite channeling (Section 5.11). Carbamoyl phosphate synthetase from *E. coli* is the most fully studied of these enzymes. It catalyzes the synthesis of carbamoyl phosphate from bicarbonate and glutamine:

$$\text{Glutamine} + \text{HCO}_3^{\ominus} + 2\ \text{ATP} + \text{H}_2\text{O}$$

↓ Carbamoyl phosphate synthetase II

$$\text{H}_2\text{N} - \overset{\overset{\displaystyle O}{\|}}{\text{C}} - \text{OPO}_3^{2-} + \text{Glutamate} + 2\ \text{ADP} + \text{P}_i$$
Carbamoyl phosphate
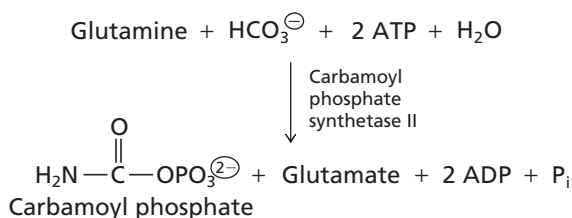
Carbamoyl phosphate formed in this reaction is used in the synthesis of pyrimidine nucleotides. (A different carbamoyl phosphate synthetase that uses ammonia rather than glutamine as its substrate is discussed in Section 17.7A.)

Carbamoyl phosphate synthetase of *E. coli* is a heterodimer with one small subunit and one large subunit (see figure). The synthesis of carbamoyl phosphate from glutamine proceeds via three intermediates, each formed at a different active site. ATP reacts at two of these sites. The three sites are connected by a tunnel that runs

from the glutamine-binding site, where a molecule of ammonia is released from glutamine, to the second ATP-binding site, where ammonia is carboxylated, and finally to the third site where carbamoyl phosphate is formed. Ammonia that is released from glutamine at the active site in the small subunit does not equilibrate with solvent but proceeds down the tunnel and undergoes the reactions that eventually produce carbamoyl phosphate. Several of the intermediates in the overall reaction are quite unstable and would be degraded by water if they were not protected by being in a tunnel.
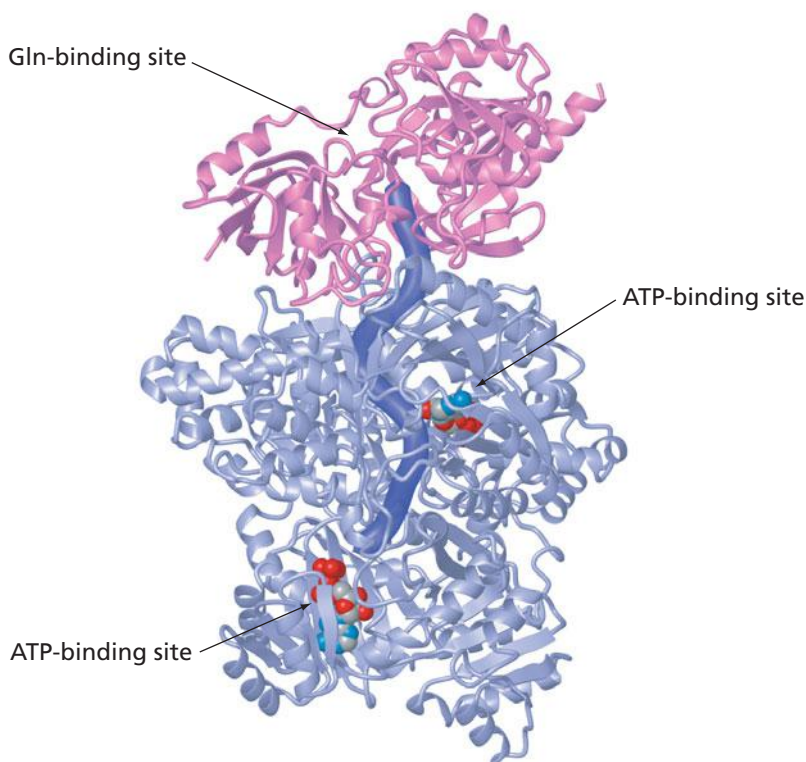
▶ **Carbamoyl phosphate synthetase from *E. coli*.** The small subunit (*N*-terminal domain, purple) contains the active site for glutamine hydrolysis releasing NH_3. The large subunit is shown in blue. NH_3 is converted to the unstable intermediate carbamate ($H_2N-COOH$) at its upper ATP-binding site. Carbamate is then phosphorylated at the *C*-terminal (lower) ATP-binding site. A molecule of ADP is bound in each ATP-binding site. The molecular tunnel connecting the three active sites is shown by the thick blue wire. [PDP 1A9X]

B. Regulation of Pyrimidine Synthesis

Regulation of pyrimidine biosynthesis also differs between prokaryotes and eukaryotes. Although the six enzymatic steps leading to UMP are the same in all species, the enzymes are organized differently in different organisms. In *E. coli*, each of the six reactions is catalyzed by a separate enzyme. In eukaryotes, a multifunctional protein in the cytosol known as dihydroorotate synthase contains separate catalytic sites (carbamoyl phosphate synthetase II, ATCase, and dihydroorotase) for the first three steps of the pathway.

In addition to being an intermediate in pyrimidine synthesis, carbamoyl phosphate is a metabolite in the pathway for the biosynthesis of arginine via citrulline (Figure 17.43). The same carbamoyl phosphate synthetase in prokaryotes is also used in both pyrimidine and arginine biosynthetic pathways. This enzyme is allosterically inhibited by pyrimidine ribonucleotides such as UMP, the product of the pyrimidine biosynthetic pathway. It is activated by L-ornithine, a precursor of citrulline, and by purine nucleotides, the substrates (along with pyrimidine nucleotides) for the synthesis of nucleic acids. Eukaryotic carbamoyl phosphate synthetase II is also allosterically regulated. PRPP and IMP activate the enzyme and several pyrimidine nucleotides inhibit it.

The next enzyme of the pathway is aspartate transcarbamoylase (ATCase). ATCase from *E. coli* is the most thoroughly studied allosteric enzyme. ATCase catalyzes the first committed step of pyrimidine biosynthesis since carbamoyl phosphate can enter pathways leading either to pyrimidines or to arginine in bacteria. This enzyme is inhibited by pyrimidine nucleotides and activated *in vitro* by ATP. ATCase in *E. coli* is only partially inhibited (50% to 70%) by the most potent inhibitor, CTP, but inhibition can be almost total when both CTP and UTP are present. UTP alone does not inhibit the enzyme. The allosteric controls—inhibition by pyrimidine nucleotides and activation by the purine nucleotide ATP—provide a means for carbamoyl phosphate synthetase and ATCase to balance the pyrimidine nucleotide and purine nucleotide pools in *E. coli*. The ratio of the concentrations of the two types of allosteric modulators determines the activity level of ATCase.

E. coli ATCase has a complex structure with binding sites for substrates and allosteric modulators on separate subunits. The enzyme contains six catalytic subunits arranged as two trimers and six regulatory subunits arranged as three dimers (Figure 18.11). Each subunit of a catalytic trimer is connected to a subunit of the other catalytic trimer through a regulatory dimer. When one molecule of aspartate binds, in the presence of carbamoyl phosphate, all six catalytic subunits change to a conformation having increased catalytic activity.

Eukaryotic ATCase is not feedback-inhibited. Regulation by feedback inhibition is not necessary because the pyrimidine pathway can be controlled by regulating the enzyme preceding ATCase, carbamoyl phosphate synthetase II. The substrate of ATCase in eukaryotes is not a branch-point metabolite—the synthesis of carbamoyl phosphate and citrulline for the urea cycle occurs in mitochondria, and the synthesis of carbamoyl phosphate for pyrimidines occurs in the cytosol. The pools of carbamoyl phosphate are separate.

18.4 CTP Is Synthesized from UMP

UMP is converted to CTP in three steps. Uridylate kinase (UMP kinase) catalyzes the transfer of the γ-phosphoryl group of ATP to UMP to generate UDP, and then nucleoside diphosphate kinase catalyzes the transfer of the γ-phosphoryl group of a second ATP molecule to UDP to form UTP. Two molecules of ATP are converted to two molecules of ADP during the synthesis of UTP from UMP.

$$\text{UMP} \xrightarrow{\text{ATP} \quad \text{ADP}} \text{UDP} \xrightarrow{\text{ATP} \quad \text{ADP}} \text{UTP} \qquad \textbf{(18.1)}$$
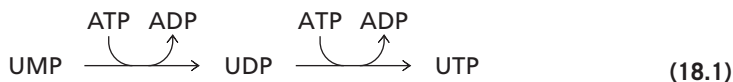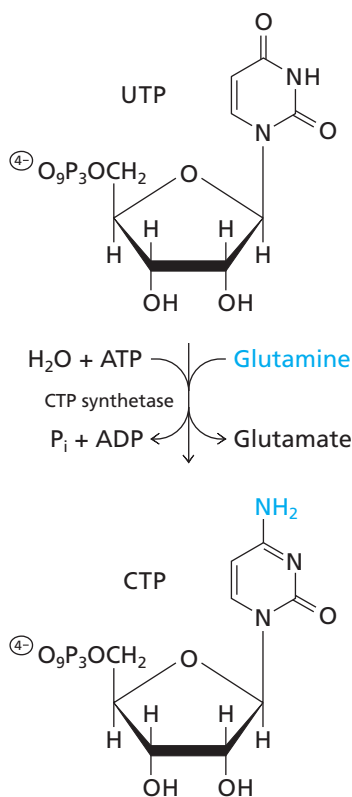
CTP synthetase then catalyzes the ATP-dependent transfer of the amide nitrogen from glutamine to C-4 of UTP forming CTP (Figure 18.12). This reaction is chemically analogous to step 4 of purine biosynthesis (Figure 18.5) and to GMP synthesis from XMP catalyzed by GMP synthetase (Figure 18.7).
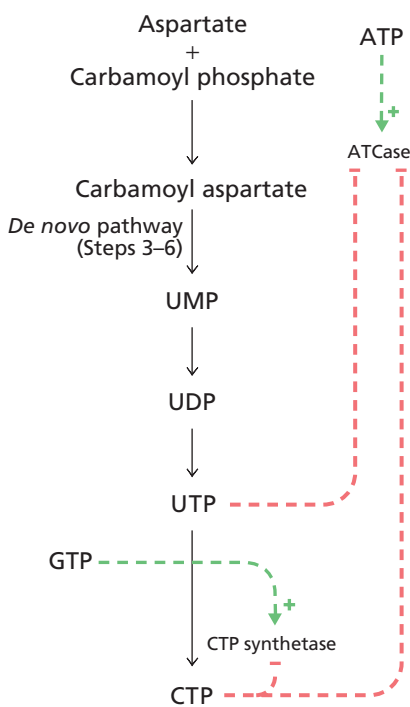
CTP synthetase is allosterically inhibited by its product, CTP, and in *E. coli* it is allosterically activated by GTP (Figure 18.13). The regulation of ATCase and CTP synthetase balances the concentrations of endogenous pyrimidine nucleotides. Elevated levels of CTP block further synthesis of CTP by inhibiting CTP synthetase. Under these
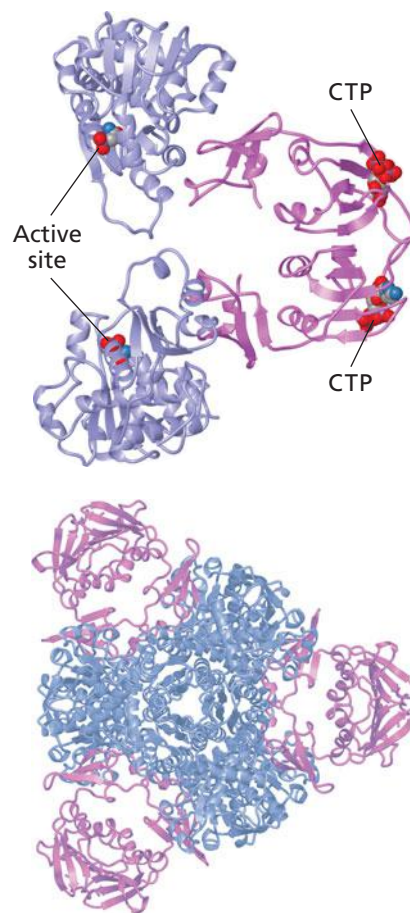
▲ Figure 18.11
ATCase from *Escherichia coli.* The top structure has two regulatory subunits (purple) with a bound CTP. The two catalytic subunits (blue) have a bound substrate analog that identifies the active site. Note the large distance between the allosteric site where CTP binds and the active site of the enzyme. Three of these units are bound together to produce a large hexameric ring (below) and two of these hexameric rings stack together to create the complete 12-subunit enzyme. [PDB 2FZC (top) 9ATC (bottom)].

UTP

$^{(4-)}O_9P_3OCH_2$

H₂O + ATP ——→ Glutamine

CTP synthetase

P_i + ADP ←——→ Glutamate

NH₂

CTP

$^{(4-)}O_9P_3OCH_2$

▲ **Figure 18.12**
Conversion of UTP to CTP.

Aspartate
+
Carbamoyl phosphate

ATP

Carbamoyl aspartate

De novo pathway
(Steps 3–6)

ATCase

UMP

UDP

UTP

GTP

CTP synthetase

CTP

▲ **Figure 18.13**
Regulation of pyrimidine nucleotide synthesis in *E. coli.* Allosteric regulation of ATCase and CTP synthetase by both purine and pyrimidine nucleotides helps balance nucleotide synthesis.

conditions, UMP synthesis will be slowed but not stopped since CTP only partially inhibits ATCase. UMP can still be used in RNA synthesis and as a precursor to dTTP (Section 18.6). ATCase is completely inhibited when the concentrations of both UTP and CTP are elevated. Elevated concentrations of the purine nucleotides ATP and GTP increase the rates of synthesis of the pyrimidine nucleotides and this helps balance the supplies of purine and pyrimidine nucleotides.

18.5 Reduction of Ribonucleotides to Deoxyribonucleotides

The 2′-deoxyribonucleoside triphosphates are synthesized by the enzymatic reduction of ribonucleotides. This reduction occurs at the nucleoside diphosphate level in most organisms. Peter Reichard and his colleagues showed that all four ribonucleoside diphosphates—ADP, GDP, CDP, and UDP—are substrates of a single, closely regulated, ribonucleoside diphosphate reductase. In some microorganisms, including species of *Lactobacillus, Clostridium*, and *Rhizobium*, ribonucleoside triphosphates are the substrates for reduction by a cobalamin-dependent reductase. Both types of enzymes are called ribonucleotide reductase (class I and class II, respectively), although the more precise names are ribonucleoside diphosphate reductase and ribonucleoside triphosphate reductase.

NADPH provides the reducing power for the synthesis of deoxyribonucleoside diphosphates in class I enzymes. A disulfide bond at the active site of ribonucleotide reductase is reduced to two thiol groups that reduce C-2′ of the ribose moiety of the nucleotide substrate by a complex free-radical mechanism. As shown in Figure 18.14, electrons are transferred from NADPH to ribonucleotide reductase via the flavoprotein thioredoxin reductase and the dithiol protein coenzyme thioredoxin (Figure 7.35). Thioredoxin reductase of prokaryotes and yeast has a dithiol/disulfide (cysteine pair) group in the active site. In mammalian thioredoxin reductase, the oxidation–reduction center differs by having one residue of cysteine and one of selenocysteine. Once formed, dADP, dGDP, and dCDP are phosphorylated to the triphosphate level by the action of nucleoside diphosphate kinases. dUDP, as we will see in the next section, is converted to dTMP via dUMP. A third version of ribonucleotide reductase (class III) uses *S*-adenosylmethionine as a cofactor.

Ribonucleotide reductase has a complicated mechanism of allosteric regulation that supplies a balanced pool of the deoxynucleotides required for DNA synthesis. Both the substrate specificity and the catalytic rate of ribonucleotide reductase are regulated in eukaryotic cells by the reversible binding of nucleotide metabolites. The allosteric modulators—ATP, dATP, dTTP, and dGTP—act by binding to ribonucleotide reductase at either of two regulatory sites. One allosteric site, called the *activity site*, controls the activity of the catalytic site. A second allosteric site, called the *specificity site*, controls the substrate specificity of the catalytic site (Figure 18.15). The binding of ATP to the activity site forms an activated enzyme whereas the binding of dATP to the activity site inhibits all enzymatic activity. When ATP is bound to the activity site and either ATP or dATP is bound to the specificity site, the reductase becomes pyrimidine specific, catalyzing the reduction of CDP and UDP. The binding of dTTP to the specificity site activates the reduction of GDP, and the binding of dGTP activates the reduction of ADP. The allosteric regulation of ribonucleotide reductase, summarized in Table 18.1, controls enzyme activity and ensures a balanced selection of deoxyribonucleotides for DNA synthesis.

18.6 Methylation of dUMP Produces dTMP

Deoxythymidylate (dTMP) is formed from UMP in four steps. UMP is phosphorylated to UDP that is reduced to dUDP and dUDP is dephosphorylated to dUMP. dUMP is then methylated to dTMP.

$$UMP \longrightarrow UDP \longrightarrow dUDP \longrightarrow dUMP \longrightarrow dTMP \qquad (18.2)$$
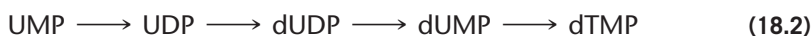
The conversion of dUDP to dUMP can occur by two routes. dUDP can react with ADP in the presence of a nucleoside monophosphate kinase to form dUMP and ATP.

$$dUDP + ADP \Longleftrightarrow dUMP + ATP \qquad (18.3)$$

Ribonucleoside
diphosphate

Deoxyribonucleoside
diphosphate
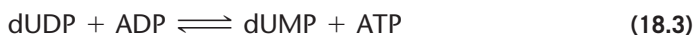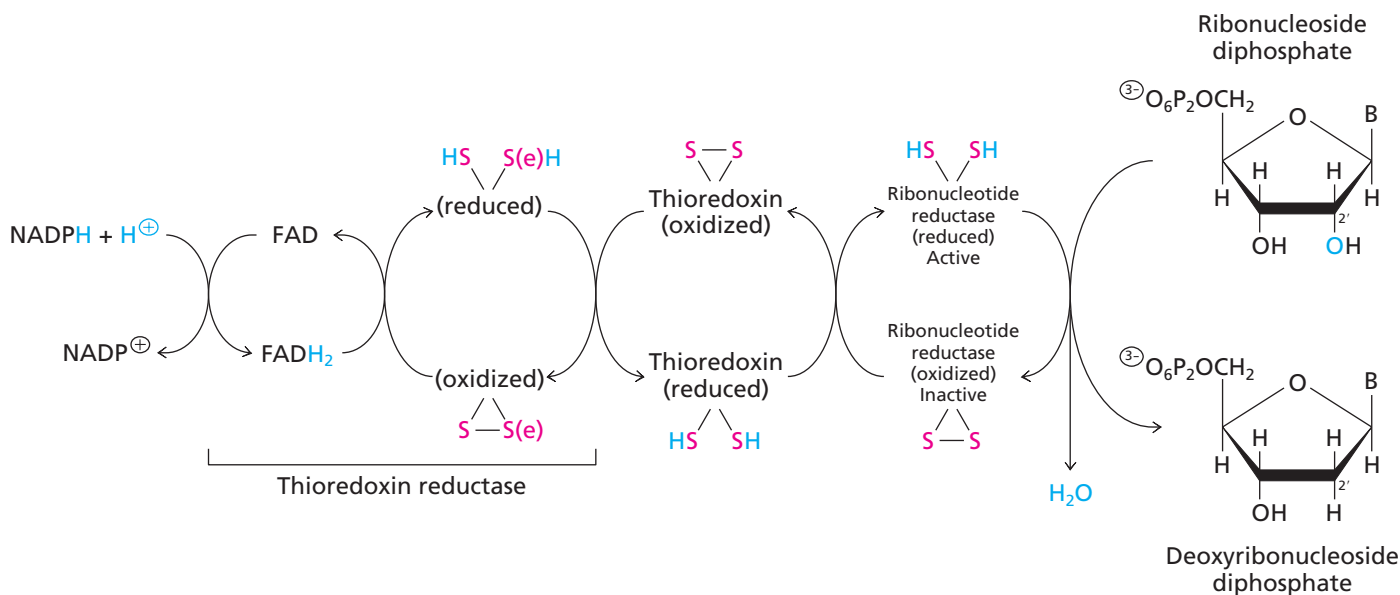
▲ **Figure 18.14**
Reduction of ribonucleoside diphosphates. Three proteins are involved: the NADPH-dependent flavoprotein thioredoxin reductase, thioredoxin, and ribonucleotide reductase. B represents a purine or pyrimidine base. S(e) represents either sulfur or selenium.

dUDP can also be phosphorylated to dUTP at the expense of ATP through the action of nucleoside diphosphate kinases. dUTP is then rapidly hydrolyzed to dUMP + PP$_i$ by the action of deoxyuridine triphosphate diphosphohydrolase (dUTPase).

$$dUDP + ATP \xrightarrow{\quad ADP \quad} dUTP \xrightarrow{\quad H_2O \quad} dUMP + PP_i \qquad (18.4)$$
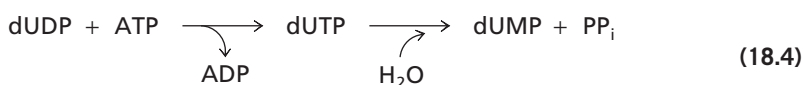
The rapid hydrolysis of dUTP prevents it from being accidentally incorporated into DNA in place of dTTP.

dCMP can also be a source of dUMP via hydrolysis catalyzed by dCMP deaminase.

$$dCMP + H_2O \longrightarrow dUMP + HN_4^{\oplus} \qquad (18.5)$$
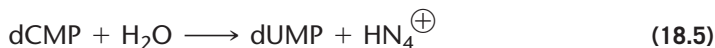
The conversion of dUMP to dTMP is catalyzed by the enzyme known as thymidylate synthase. (Because thymine occurs almost exclusively in DNA, the trivial names thymidine and thymidylate are commonly used instead of deoxythymidine and deoxythymidylate.) 5,10-Methylenetetrahydrofolate is the donor of the one-carbon group in this reaction (Figure 18.16). The carbon-bound methyl group (C—CH$_3$) in dTMP is more reduced than the nitrogen-bridged methylene group (N—CH$_2$—N) in 5,10-methylenetetrahydrofolate, whose oxidation state is equivalent to that of a nitrogen-bound hydroxymethyl group (N—CH$_2$OH) or formaldehyde. Thus, not only is methylenetetrahydrofolate a coenzyme donating a one-carbon unit but it is also the reducing agent for the reaction, furnishing a hydride ion and being oxidized to 7,8-dihydrofolate in the process. This is the only known reaction in which the transfer of a one-carbon unit from a tetrahydrofolate derivative results in its oxidation at N-5 and C-6 to produce dihydrofolate.

> **The structure of selenocysteine, the 22nd amino acid, is shown in Section 3.3.**
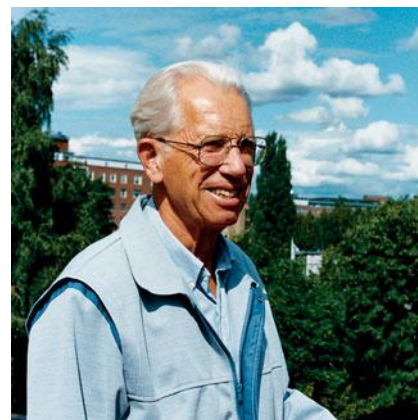

▲ **Peter Reichard (1925–)**.
Reichard worked for many years at the Karolinska Institute in Sweden. In addition to working on ribonucleotide reductase, he was an active member of the Nobel Committee that selects candidates to receive the Nobel Prize.

Table 18.1 Allosteric regulation of eukaryotic ribonucleotide reductase

| Ligand bound to activity site	Ligand bound to specificity site	Activity of catalytic site
dATP		Enzyme inactive
ATP	ATP or dATP	Specific for CDP or UDP
ATP	dTTP	Specific for GDP
ATP	dGTP	Specific for ADP
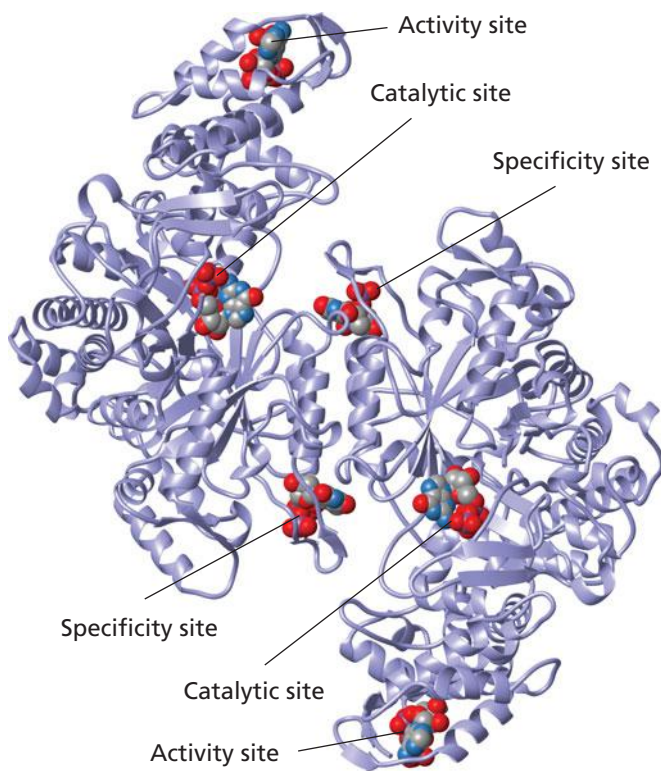
BOX 18.3 FREE RADICALS IN THE REDUCTION OF RIBONUCLEOTIDES

The ribonucleotide reductase reaction is an unusual reaction because it proceeds by a free radical mechanism. The first clue to the free radical nature of the reaction was the observation that the reductase from *E. coli* could be isolated with a tyrosine residue in the free radical form. This was the first free radical protein to be discovered. The role of the tyrosine radical is to convert the thiol group of an active-site cysteine residue to a thiyl radical. (In the *Lactobacillus* enzyme, cobalamin serves to convert the active-site thiol to a radical.)

The proposed mechanism is shown in the accompanying figure. The active site of the reductase has three cysteine residues—one forms the free radical and the other two are an oxidation–reduction group. The thiyl radical removes a hydrogen atom from the C-3′ position of the ribonucleotide forming a substrate radical. This substrate radical is first dehydrated (losing the C-2′—OH) and then reduced by the cysteine reduction pair. A hydrogen atom is returned to C-3′, regenerating the thiyl radical.
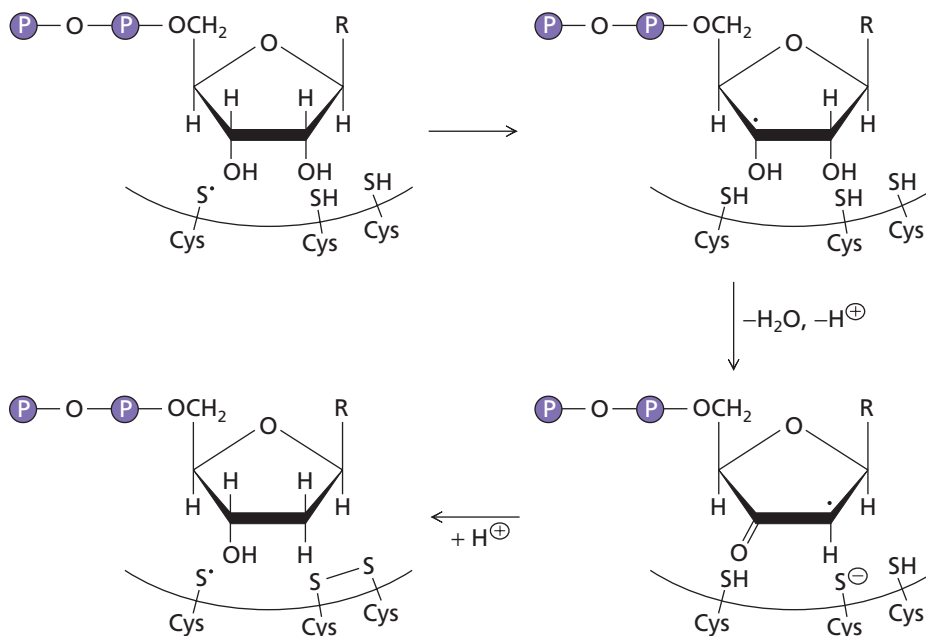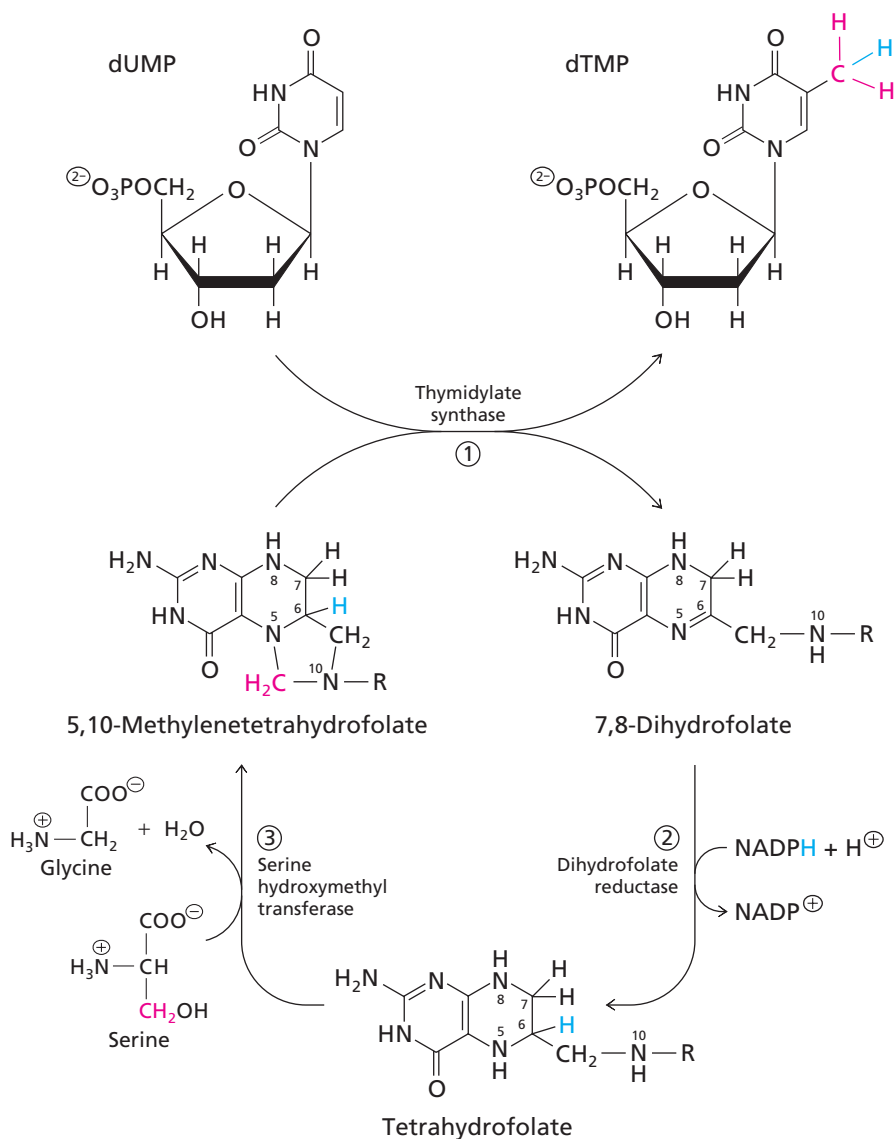
Dihydrofolate must be converted to tetrahydrofolate before the coenzyme can accept another one-carbon unit for further transfer reactions. The 5,6 double bond of dihydrofolate is reduced by NADPH in a reaction catalyzed by dihydrofolate reductase. Serine hydroxymethyltransferase (Figure 17.16) then catalyzes the transfer of the β-CH_2OH group of serine to tetrahydrofolate to regenerate 5,10-methylenetetrahydrofolate.

Thymidylate synthase and dihydrofolate reductase are distinct polypeptides in most organisms but in protozoa the two enzyme activities are contained on the same polypeptide chain. The dihydrofolate product of the first reaction is channeled from the thymidylate synthase active site to the dihydrofolate reductase active site. Charge–charge interactions between a positively charged region on the surface of the bifunctional enzyme and the negatively charged dihydrofolate (recall that it contains several γ-glutamate residues; Section 7.11) steer the product to the next active site.

dTMP can also be synthesized via the salvage of thymidine (deoxythymidine), catalyzed by ATP-dependent thymidine kinase.

$$\text{Deoxythymidine (Thymidine)} \xrightarrow[\text{Thymidine kinase}]{\text{ATP \quad ADP}} \text{dTMP} \qquad (18.6)$$
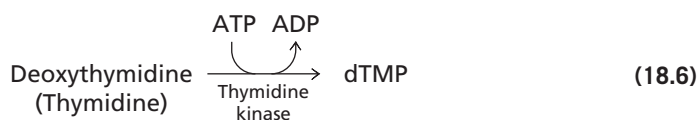
BOX 18.4 CANCER DRUGS INHIBIT dTTP SYNTHESIS

Since dTMP is an essential precursor of DNA, any agent that lowers dTMP levels drastically affects cell division. Thymidylate synthase and dihydrofolate reductase have been major targets for anticancer drugs because rapidly dividing cells are particularly dependent on the activities of these enzymes. The inhibition of either or both of these enzymes blocks the synthesis of dTMP and therefore the synthesis of DNA.

5-Fluorouracil, methotrexate, and Tomudex are effective in combating some types of cancer. 5-Fluorouracil is converted to its deoxyribonucleotide, 5-fluorodeoxyuridylate,

which binds tightly to thymidylate synthase inhibiting the enzyme and bringing the three-reaction cycle shown in Figure 18.16 to a halt. Methotrexate, an analog of folate, is a potent, relatively specific inhibitor of dihydrofolate reductase that catalyzes step 2 of the cycle shown in Figure 18.16. The resulting decrease in tetrahydrofolate levels greatly diminishes the formation of dTMP since dTMP synthesis depends on adequate concentrations of methylenetetrahydrofolate. Tomudex is a folate-based inhibitor of human thymidylate synthase that has been approved for the treatment of cancer.

5-Fluorouracil   Methotrexate

Tomudex (ZD1694)
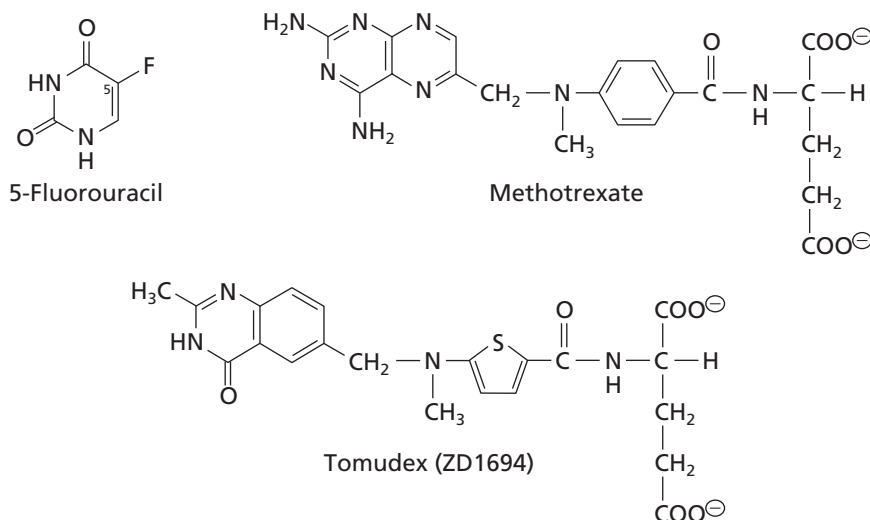
▲ 5-Fluorouracil, methotrexate, and Tomudex are drugs designed to inhibit thymidylate synthase and block the growth of rapidly dividing cells.

▲ Structure of human dihydrofolate reductase with the substrate analog methotrexate (red) and the cosubstrate NADPH (gold) bound in the active site. [PDB 1DLS]

Radioactive thymidine is often used as a highly specific tracer for monitoring intracellular synthesis of DNA because it enters cells easily and its principal metabolic fate is conversion to thymidylate and incorporation into DNA.

18.7 Modified Nucleotides

DNA and RNA contain a number of modified nucleotides. The ones present in transfer RNA are well known (Section 21.8B) but the modified nucleotides in DNA are just as important. Some of the more common modified bases in DNA are shown in Figure 18.17. Most of them are only found in a few species or in bacteriophage while others are more widespread.

We will encounter N^6-methyladenine in the next chapter when we discuss restriction endonucleases. 5-Methylcytosine is a common modified base in mammalian DNA because it plays a role in chromatin assembly and the regulation of transcription. About 3% of all deoxycytidylate residues in mammalian DNA are modified to 5-methylcytidine. The methylation occurs after DNA is synthesized and the modified residues are at CG sequences. All of these modified nucleotides are made *in situ* by enzymes that act on one of the four common nucleotides in the DNA molecule.

18.8 Salvage of Purines and Pyrimidines

▲ Salvage pathways are a form of biochemical recycling.

Nucleic acids are degraded to mononucleotides, nucleosides, and eventually, heterocyclic bases during normal cell metabolism (Figure 18.18). The catabolic reactions are catalyzed by

ribonucleases, deoxyribonucleases, and a variety of nucleotidases, nonspecific phosphatases, and nucleosidases or nucleoside phosphorylases. Some of the purine and pyrimidine bases formed in this way are further degraded (e.g., purines are converted to uric acid and other excretory products) but a considerable fraction is normally salvaged by direct conversion back to 5′-mononucleotides. PRPP is the donor of the 5-phosphoribosyl moiety for salvage reactions. The degradation pathways are part of fuel metabolism in animals. Purines and pyrimidines formed during digestion are more likely to be degraded while those formed inside the cell are usually salvaged. The recycling of intact bases conserves cellular energy.

5-Methylcytosine   5-Hydroxymethylcytosine   5-Hydroxymethyluracil

N^4-Methylcytosine N^6-Methyladenine   2-Aminoadenine

▲ **Figure 18.17**
Modified bases in DNA.

The degradation of purine nucleotides to their respective purines and their salvage through reaction with PRPP are outlined in Figure 18.19. Adenine phosphoribosyltransferase catalyzes the reaction of adenine with PRPP to form AMP and PP_i. The hydrolysis of PP_i, catalyzed by pyrophosphatase, renders the reaction metabolically irreversible. Hypoxanthine–guanine phosphoribosyltransferase catalyzes similar reactions—the conversion of hypoxanthine to IMP and of guanine to GMP with formation of PP_i.

Pyrimidines are salvaged by the action of orotate phosphoribosyltransferase, which catalyzes step 5 of the biosynthesis pathway (Figure 18.10). This enzyme can also catalyze the conversion of pyrimidines other than orotate to the corresponding pyrimidine nucleotides.

Nucleotides and their constituents are interconverted by many reactions, some of which we have seen already. The actions of phosphatases, nucleotidases, and nucleosidases or nucleoside phosphorylases can release bases from nucleotides. Reactions catalyzed by phosphoribosyltransferases or nucleoside phosphorylases can salvage the bases and nucleosides by converting them to the nucleotide level. Bases that are not salvaged can be catabolized. The interconversions of purine nucleotides and their constituents are summarized in Figure 18.20, and the interconversions of pyrimidine nucleotides and their constituents are summarized in Figure 18.21.

18.9 Purine Catabolism

Most free purine and pyrimidine molecules are salvaged but some are catabolized. Birds, some reptiles, and primates (including humans) convert purine nucleotides to uric acid or urate, which is then excreted. In birds and reptiles, amino acid catabolism also leads to uric acid; in mammals, surplus nitrogen from amino acid catabolism is disposed of in the form of urea. Birds and reptiles cannot further catabolize uric acid (urate) but many organisms degrade urate to other products.
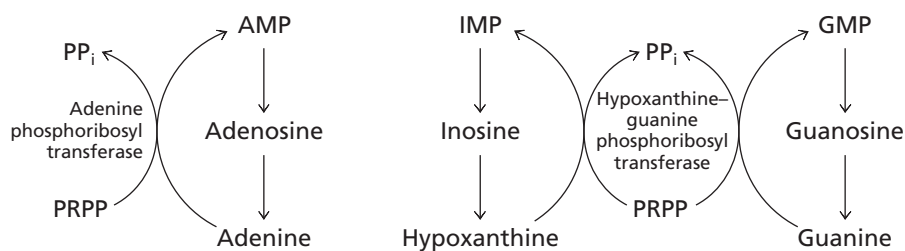
As shown in Figure 18.20, AMP can be broken down to hypoxanthine and GMP is broken down to guanine. The hydrolytic removal of phosphate from AMP and GMP produces adenosine and guanosine, respectively. Adenosine can be deaminated to inosine by the action of adenosine deaminase. Alternatively, AMP can be deaminated to
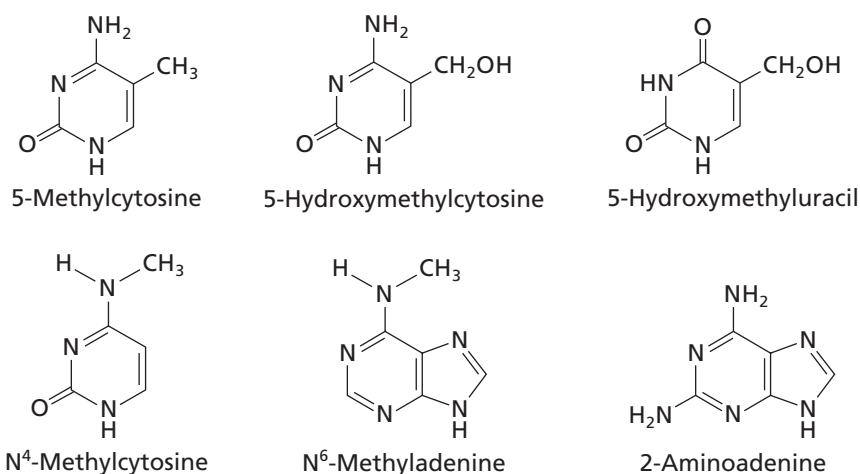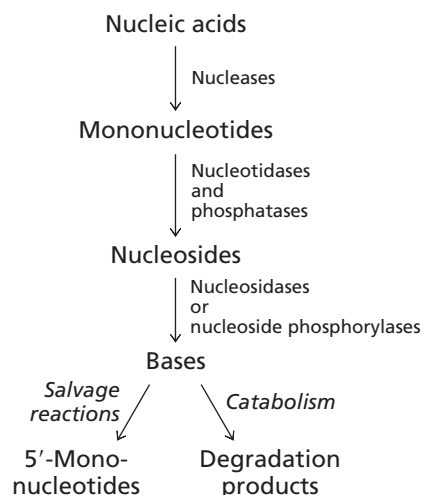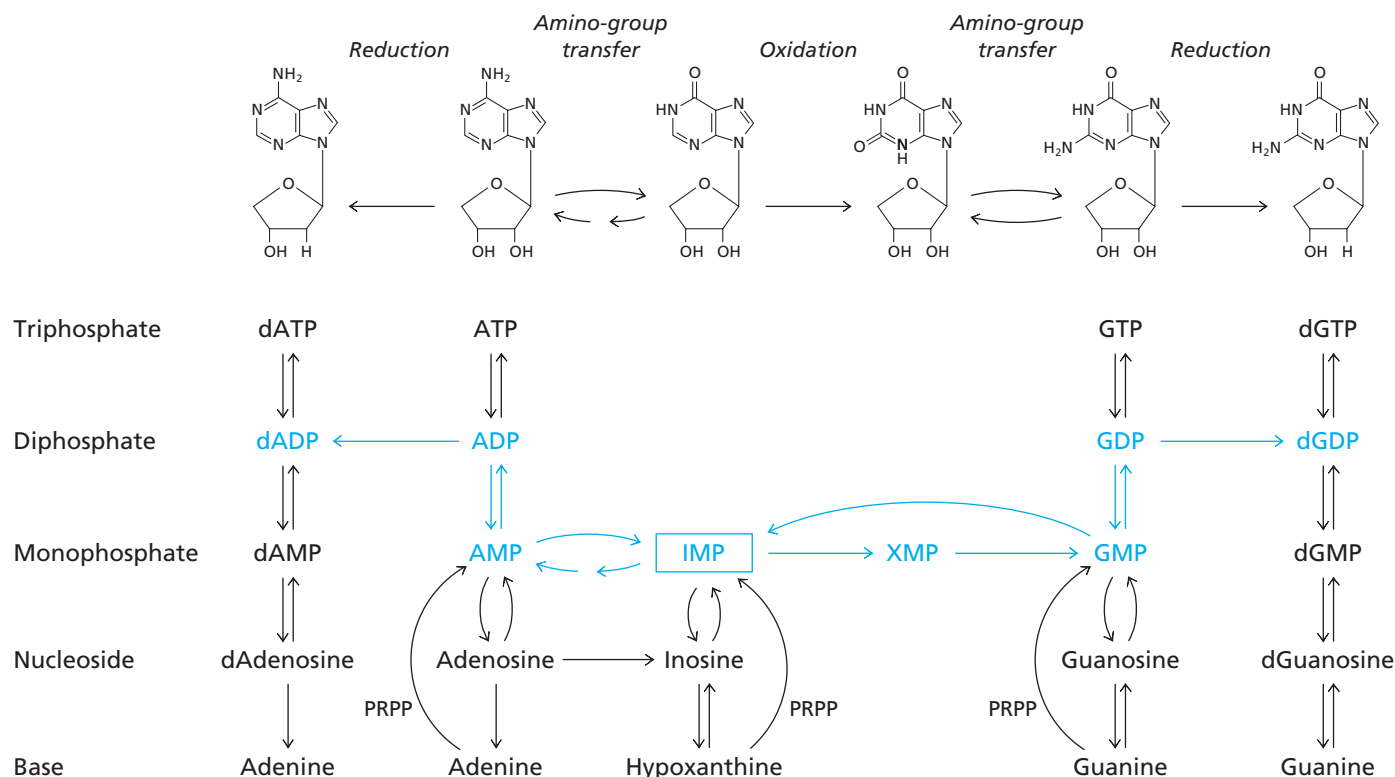

▲ **Figure 18.18**
**Breakdown of nucleic acids.**

◄ **Figure 18.19**
Degradation and salvage of purines.

▲ **Figure 18.20**

Interconversions of purine nucleotides and their constituents. IMP, the first nucleotide product of the *de novo* biosynthetic pathway, is readily converted to AMP and GMP, their di- and triphosphates, and the deoxy counterparts of these nucleotides. 5′-Phosphate groups are not shown in the abbreviated structures. [Adapted from Traut, T. W. (1988). Enzymes of nucleotide metabolism: the significance of subunit size and polymer size for biological function and regulatory properties. *Crit. Rev. Biochem.* 23:121–169.]

IMP by the action of AMP deaminase and then IMP can be hydrolyzed to inosine. The phosphorolysis of inosine produces hypoxanthine and the phosphorolysis of guanosine produces guanine. Both these reactions (as well as the phosphorolysis of several deoxynucleosides) are catalyzed by purine–nucleoside phosphorylase and produce α-D-ribose 1-phosphate (or deoxyribose 1-phosphate) and the free purine base.

$$\text{(Deoxy)Nucleoside} + P_i \rightleftharpoons \text{Base} + \text{(Deoxy)-}\alpha\text{-D-Ribose 1-phosphate} \quad \textbf{(18.7)}$$
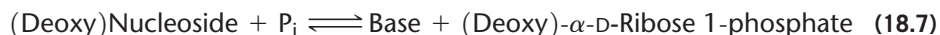
Adenosine is not a substrate of mammalian purine-nucleoside phosphorylase.

Hypoxanthine formed from inosine is oxidized to xanthine, and xanthine is oxidized to urate (Figure 18.22). Either xanthine oxidase or xanthine dehydrogenase can catalyze both reactions. Electrons are transferred to O_2 to form hydrogen peroxide (H_2O_2) in the reactions catalyzed by xanthine oxidase. (The H_2O_2 is converted to H_2O and O_2 by the action of catalase.) Xanthine oxidase is an extracellular enzyme in mammals and it appears to be an altered form of the intracellular enzyme xanthine dehydrogenase that generates the same products as xanthine oxidase but transfers electrons to $NAD^\oplus$ to form NADH. These two enzyme activities occur widely in nature and exhibit broad substrate specificity. Their active sites contain complex electron-transfer systems that include an iron–sulfur cluster, a pterin coenzyme with bound molybdenum, and FAD.

In most cells, guanine is deaminated to xanthine in a reaction catalyzed by guanase (Figure 18.22). Animals that lack guanase excrete guanine. For example, pigs excrete guanine but metabolize adenine derivatives further to allantoin, the major end product of the catabolism of purines in most mammals.

Urate can be further oxidized in most organisms. Up until recently it was thought that urate oxidase converted urate directly to allantoin but it is now known that the pathway is more complex. The conversion of urate to the stereospecific product (S)-allantoin

See Section 6.5D for a description of the adenosine deaminase mechanism.
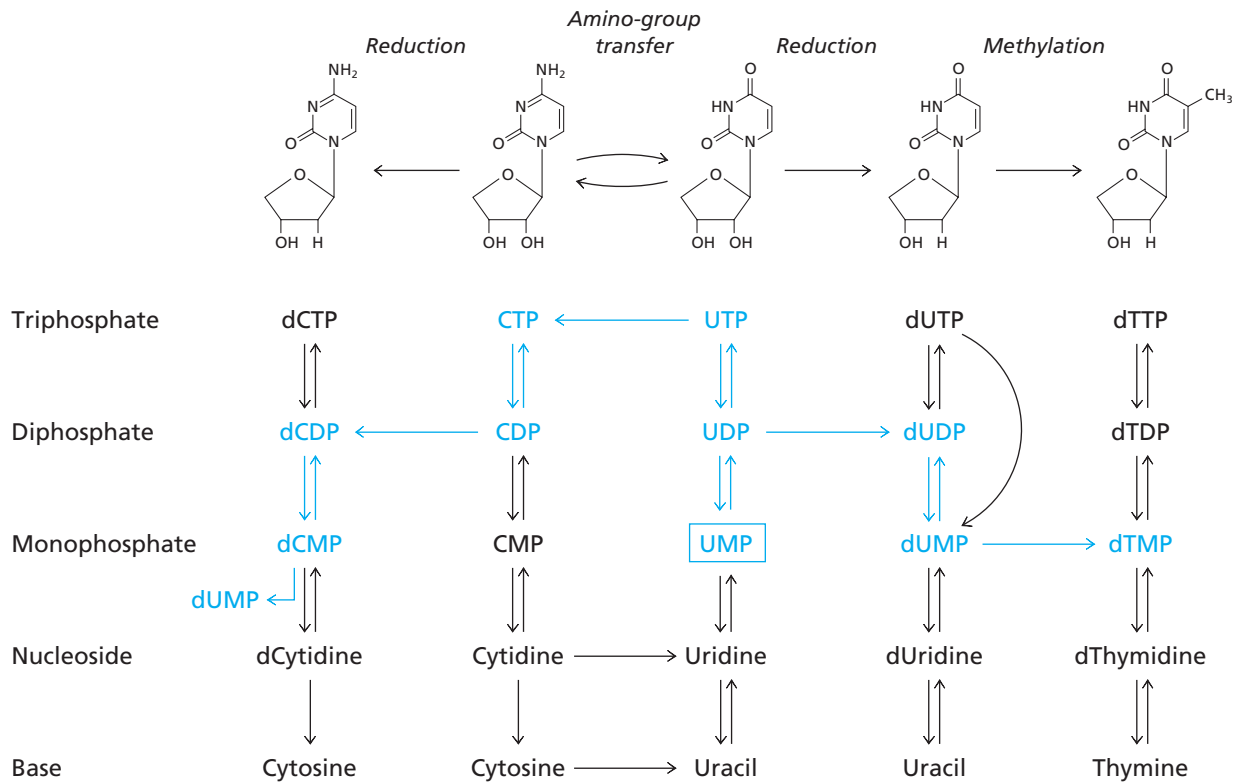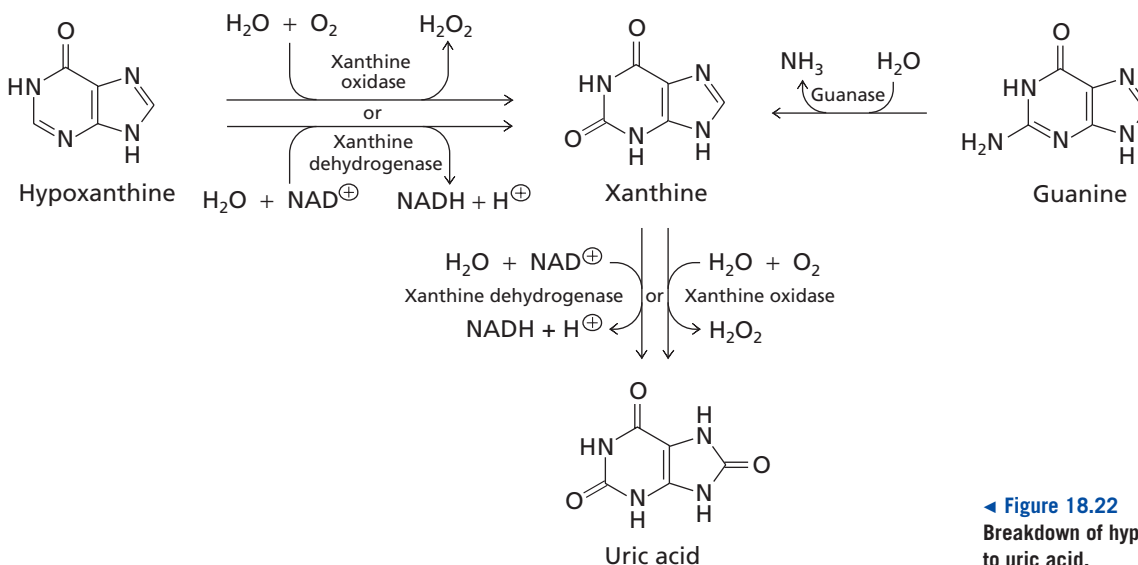
▲ **Figure 18.21**
Interconversions of pyrimidine nucleotides and their constituents. UMP formed by the *de novo* pathway can be converted to cytidine and thymidine phosphates, as well as to other uridine derivatives. 5′-Phosphate groups are not shown in the abbreviated structures. [Adapted from Traut, T. W. (1988). Enzymes of nucleotide metabolism: the significance of subunit size and polymer size for biological function and regulatory properties. *Crit. Rev. Biochem.* 23:121–169.]

requires urate oxidase plus two additional enzymes as shown in Figure 18.23. Peroxide (H_2O_2) and CO_2 are released in this series of reactions. Allantoin is the major end product of purine degradation in most mammals (though not in humans, for whom the end product is urate). It is also excreted by turtles, some insects, and also gastropods.

The enzyme allantoinase catalyzes hydrolytic opening of the imidazole ring of allantoin to produce allantoate, the conjugate base of allantoic acid. Some bony fishes (teleosts) possess allantoinase activity and excrete allantoate as the end product of purine degradation.

◄ **Figure 18.22**
Breakdown of hypoxanthine and guanine to uric acid.
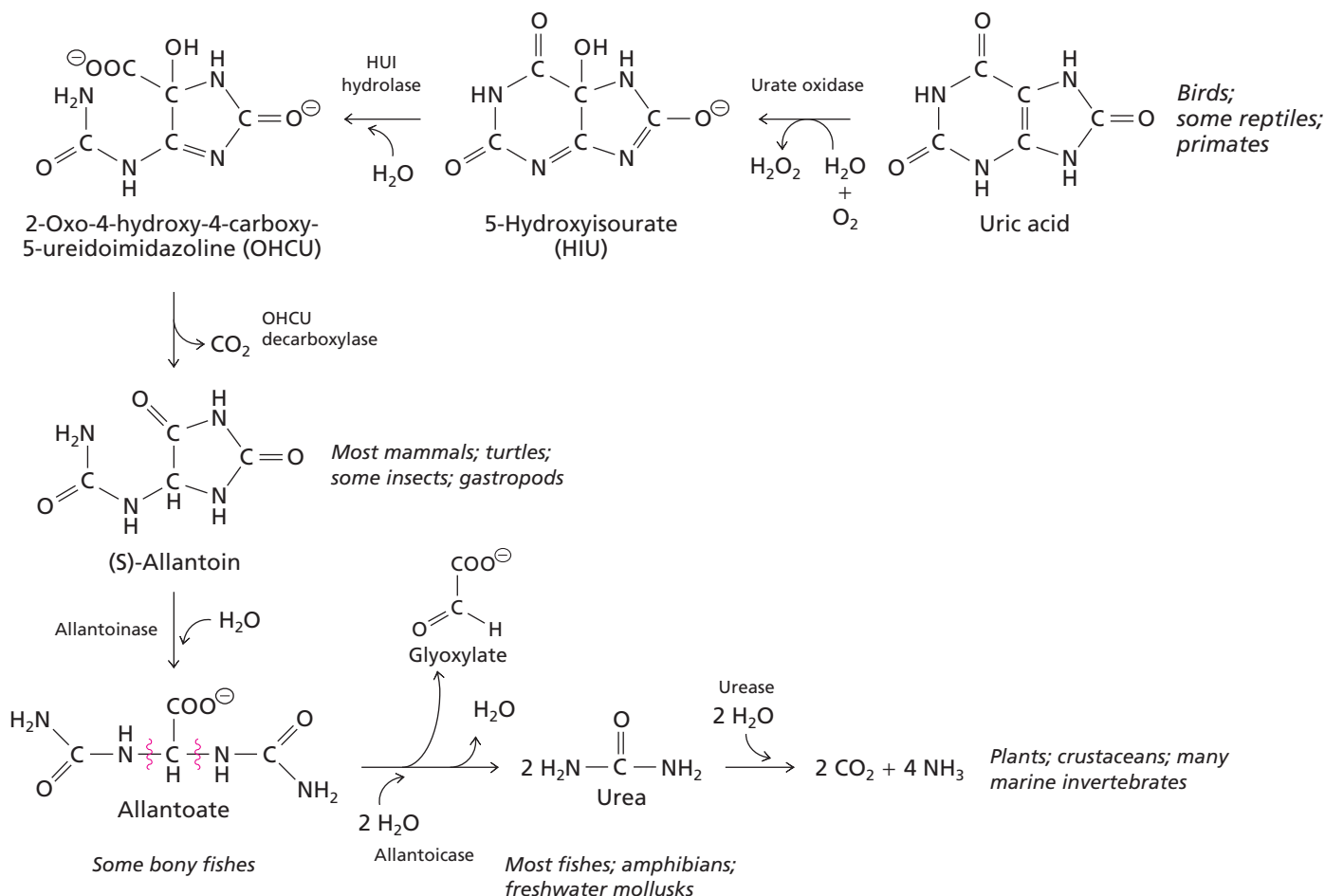
▲ **Figure 18.23**
Catabolism of uric acid through oxidation and hydrolysis. To the right of each compound are listed the organisms for which it is an excretory product.

▲ When they were alive, these snails could convert urate to allantoin. Humans can't do that.

Most fishes, amphibians, and freshwater mollusks can further degrade allantoate. These species contain allantoicase that catalyzes the hydrolysis of allantoate to one molecule of glyoxylate and two molecules of urea. Urea is the nitrogenous end product of purine catabolism in these organisms.

Finally, several organisms—including plants, crustaceans, and many marine invertebrates—can hydrolyze urea in a reaction catalyzed by urease. Carbon dioxide and ammonia are the products of this reaction. Urease is found only in the cells of organisms in which the hydrolysis of urea does not lead to ammonia toxicity. For example, in plants, ammonia generated from urea is rapidly assimilated by the action of glutamine synthetase. In marine animals, ammonia is produced in surface organs such as gills and is flushed away before it can accumulate to toxic levels. Most terrestrial organisms would be poisoned by the final nitrogen-containing product, ammonia. The enzymes that catalyze urate catabolism have been lost through evolution by organisms that excrete urate.

18.10 Pyrimidine Catabolism

The catabolism of pyrimidine nucleotides begins with hydrolysis to the corresponding nucleosides and P_i, catalyzed by 5′-nucleotidase (Figure 18.24). Initial hydrolysis to cytidine can be followed by deamination to uridine in a reaction catalyzed by cytidine deaminase.

BOX 18.5 LESCH–NYHAN SYNDROME AND GOUT

Defects in purine metabolism can have devastating effects. In 1964 Michael Lesch and William Nyhan described a severe metabolic disease characterized by slow mental development, palsylike spasticity, and a bizarre tendency toward self-mutilation. Individuals afflicted with this disease, called Lesch–Nyhan syndrome, rarely survive past childhood. Prominent biochemical features of the disease are the excretion of up to six times the normal amount of uric acid and a greatly increased rate of purine biosynthesis.

The disease is caused by a hereditary deficiency of the activity of the enzyme hypoxanthine–guanine phosphoribosyltransferase (Section 18.8). The deficiency is usually seen in males because the mutation is recessive and the gene for this enzyme is on the X chromosome. Lesch–Nyhan patients usually have less than 1% of the normal activity of the enzyme and most show a complete absence of activity. In the absence of hypoxanthine–guanine phosphoribosyltransferase, hypoxanthine and guanine are degraded to uric acid instead of being converted to IMP and GMP, respectively. The PRPP normally used for the salvage of hypoxanthine and guanine contributes to the synthesis of excessive amounts of IMP and the surplus IMP is degraded to uric acid. It is not known how this single enzyme defect causes the various behavioral symptoms. The catastrophic effects of the deficiency indicate that in some cells the purine salvage pathway in humans is not just an energy-saving addendum to the central pathways of purine nucleotide metabolism.

Gout is a disease caused by the overproduction or inadequate excretion of uric acid. Sodium urate is relatively insoluble and when its concentration in blood is elevated, it can crystallize (sometimes along with uric acid) in soft tissues, especially the kidney, and in toes and joints. Gout has several causes including a deficiency of hypoxanthine–guanine phosphoribosyltransferase activity resulting in less salvage of purines and more catabolic production of uric acid. The difference between gout and Lesch–Nyhan syndrome is due to the fact that gout patients retain up to 10% enzyme activity. Gout can also be caused by defective regulation of purine biosynthesis.
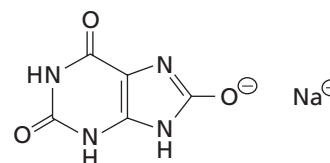
Sodium urate

Gout can be treated by giving patients allopurinol, a synthetic C-7, N-8 positional isomer of hypoxanthine. Allopurinol is converted in cells to oxypurinol, a powerful inhibitor of xanthine oxidase. Administration of allopurinol prevents the formation of abnormally high levels of uric acid. Hypoxanthine and xanthine are more soluble than sodium urate and uric acid and they are excreted when not reused by salvage reactions.

Hypoxanthine   Allopurinol   Oxypurinol
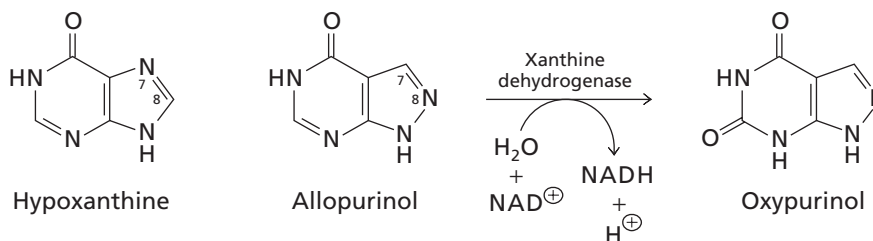
▲ **Allopurinol and oxypurinol.** Xanthine dehydrogenase catalyzes the oxidation of allopurinol, an isomer of hypoxanthine. The product, oxypurinol, binds tightly to xanthine dehydrogenase, inhibiting the enzyme.

The glycosidic bonds of uridine and thymidine are then cleaved by phosphorolysis in reactions catalyzed by uridine phosphorylase and thymidine phosphorylase, respectively. Deoxyuridine can also undergo phosphorolysis catalyzed by uridine phosphorylase. The products of these phosphorolysis reactions are α-D-ribose 1-phosphate or deoxyribose 1-phosphate, thymine, and uracil.

The catabolism of pyrimidines ends with intermediates of central metabolism, so no distinctive excretory products are formed. The breakdown of both uracil and thymine involves several steps (Figure 18.24). First, the pyrimidine ring is reduced to a 5,6-dihydropyrimidine in a reaction catalyzed by dihydrouracil dehydrogenase. The reduced ring
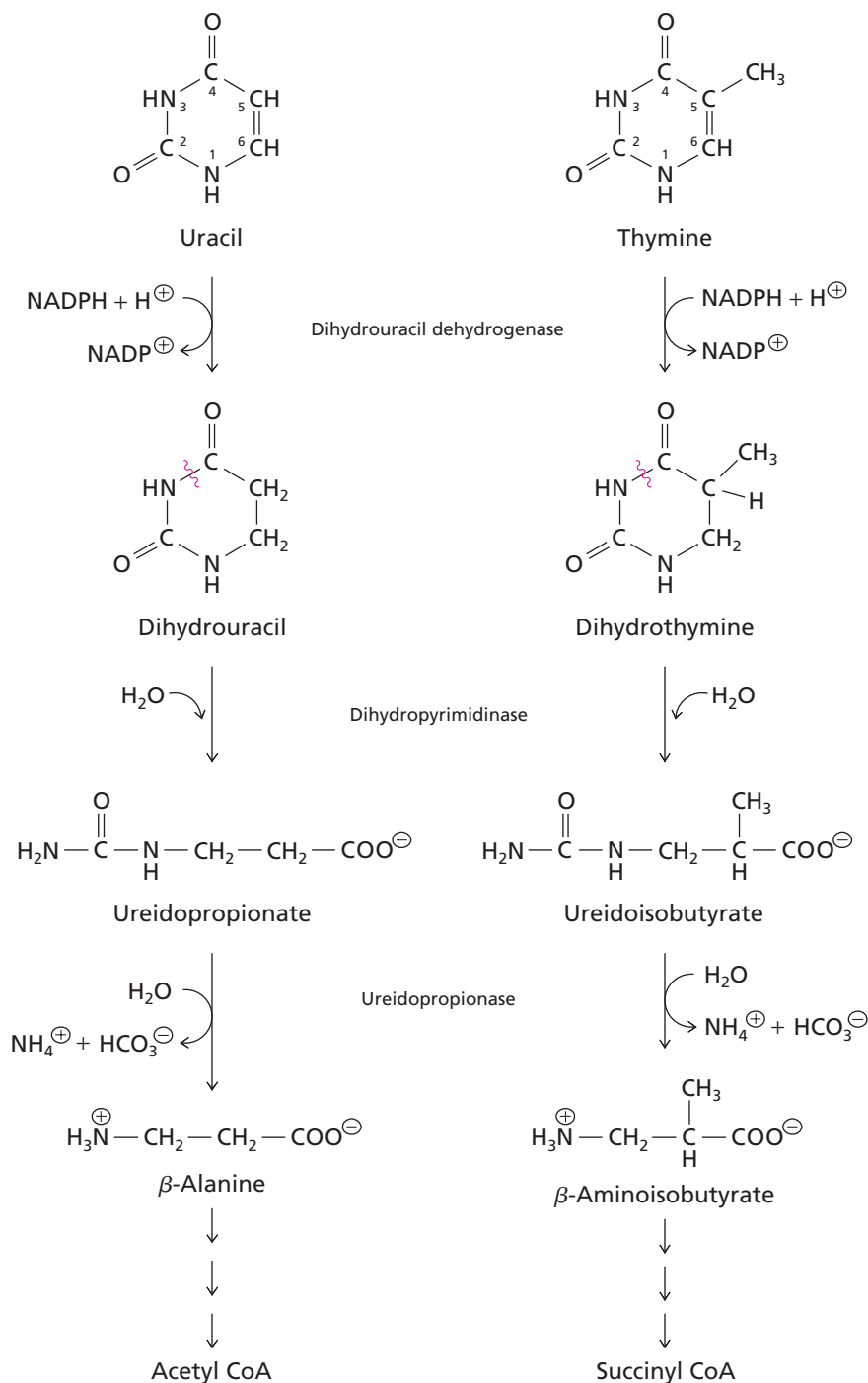
is then opened by hydrolytic cleavage of the N-3—C-4 bond in a reaction catalyzed by dihydropyrimidinase. The resulting carbamoyl-β-amino acid derivative (ureidopropionate or ureidoisobutyrate) is further hydrolyzed to $NH_4^\oplus$, $HCO_3^\ominus$, and a β-amino acid. β-Alanine (from uracil) and β-aminoisobutyrate (from thymine) can then be converted to acetyl CoA and succinyl CoA, respectively, which can enter the citric acid cycle and be converted to other compounds. In bacteria, β-alanine can also be used in the synthesis of pantothenate, a constituent of coenzyme A.

Summary

1. The synthesis of purine nucleotides is a ten-step pathway that leads to IMP (inosinate). The purine is assembled on a foundation of ribose 5-phosphate donated by 5-phosphoribosyl 1-pyrophosphate (PRPP).

2. IMP can be converted to AMP or GMP.

3. In the six-step synthesis of the pyrimidine nucleotide UMP, PRPP enters the pathway after completion of the ring structure.

4. CTP is formed by the amination of UTP.

5. Deoxyribonucleotides are synthesized by the reduction of ribonucleotides at C-2′ in a reaction catalyzed by ribonucleotide reductase.

6. Thymidylate (dTMP) is formed from deoxyuridylate (dUMP) by a methylation reaction in which 5,10-methylenetetrahydrofolate donates both a one-carbon group and a hydride ion. 7,8-Dihydrofolate, the other product of this methylation, is recycled by NADPH-dependent reduction to the active coenzyme tetrahydrofolate.

7. PRPP reacts with pyrimidines and purines in salvage reactions to yield nucleoside monophosphates. Nucleotides and their constituents are interconverted by a variety of enzymes.

8. Nitrogen from amino acids and purine nucleotides is excreted as uric acid in birds and some reptiles. Primates degrade purines to uric acid (urate). Most other organisms further catabolize urate to allantoin, allantoate, urea, or ammonia.

9. Pyrimidines are catabolized to ammonia, bicarbonate, and either acetyl CoA (from cytosine or uracil) or succinyl CoA (from thymine).

Problems

1. Indicate where the label appears in the product for each of the following precursor–product pairs:

   (a) ^{15}N-aspartate $\rightarrow$ AMP
   (b) 2-[^{14}C]-glycine $\rightarrow$ AMP
   (c) δ-[^{15}N]-glutamine $\rightarrow$ GMP
   (d) 2-[^{14}C]-aspartate $\rightarrow$ UMP
   (e) H^{14}CO$_3^{\ominus}$ $\rightarrow$ UMP

2. How many ATP equivalents are needed to synthesize one molecule of IMP, starting from ribose 5-phosphate? Assume that all necessary precursors in the pathway are present.

3. The incorporation of one-carbon units in the *de novo* pathways of purines and pyrimidines requires tetrahydrofolate (THF) derivatives as donors. List the reactions requiring THF derivatives, indicate the THF donor, and indicate which carbon of the purine or pyrimidine is derived from THF.

4. The glutamine analog acivicin, a potential anticancer agent, slows the rapid growth of cells by inhibiting nucleotide biosynthesis.

   (a) Show how acivicin structurally resembles glutamine.
   (b) What intermediate accumulates in the purine biosynthetic pathway when acivicin is present?
   (c) What enzyme is inhibited in the pyrimidine biosynthetic pathway when acivicin is present?

   COO$^{\ominus}$
   |
   H$_3\overset{\oplus}{N}$ — C — H
   |
   CH
   O ▲ CH$_2$
   | |
   N = C
           Cl
   Acivicin

5. A hypothetical bacterium synthesizes UMP by a pathway analogous to the pathway in *E. coli*, except that β-alanine is used instead of aspartate.

   H$_3\overset{\oplus}{N}$ — CH$_2$ — CH$_2$ — COO$^{\ominus}$
   β-Alanine

   (a) Why would this pathway be shorter than the pathway in *E. coli*?
   (b) When β-alanine uniformly labeled with ^{14}C is used, where would the label appear in UMP?

6. (a) The enzyme dCMP deaminase can provide a major route from cytidine to uridine nucleotides. What is the product of the action of dCMP deaminase on dCMP?

   (b) This allosteric enzyme is subject to inhibition by dTTP and activation by dCTP. Explain why this is reasonable in terms of the overall cellular needs of nucleoside triphosphates.

7. In eukaryotes, how many ATP equivalents are needed to synthesize one molecule of UMP from HCO$_3^{\ominus}$, aspartate, glutamine, and ribose 5-phosphate? (Ignore any ATP that might be produced by oxidizing the QH$_2$ generated in the pathway.)

8. Severe combined immunodeficiency syndrome (SCIDS) is characterized by the lack of an immune response to infectious diseases. One form of SCIDS is caused by a deficiency of adenosine deaminase (ADA), an enzyme that catalyzes the deamination of adenosine and deoxyadenosine to produce inosine and deoxyinosine, respectively. The enzyme deficiency increases dATP levels but decreases the levels of other deoxynucleotides, thereby inhibiting DNA replication and cell division in certain rapidly dividing cells. Explain how an adenosine deaminase deficiency affects the levels of deoxynucleotides. (The first effective gene therapy in humans was carried out by transforming a patient's T-cells with a normal ADA gene.)

9. One cause of gout is a deficiency in hypoxanthine–guanine phosphoribosyltransferase activity (Box 18.4). Another cause is due to an *increase* in PRPP synthetase activity. If PRPP is a positive effector of glutamine–PRPP amidotransferase in humans, how does this affect purine synthesis?

10. Identify the nucleotides involved in the following pathways:

    (a) the nucleoside triphosphate required as a substrate in the synthesis of NAD
    (b) the nucleoside triphosphate required in the synthesis of FMN
    (c) the nucleoside triphosphate that serves as a substrate in the synthesis of coenzyme A
    (d) the substrate for G proteins
    (e) the nucleotide used in the synthesis of glycogen from glucose 6-phosphate
    (f) the cofactor required in the reaction catalyzed by mammalian succinyl-CoA synthetase
    (g) the cosubstrate required for the synthesis of phosphatidylserine from phosphatidate
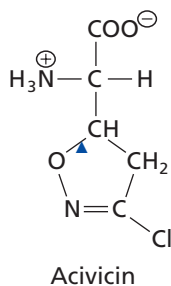
(h) the nucleotide required for activation of galactose in cerebro-side biosynthesis

(i) the nucleotide substrate used in histidine biosynthesis

(j) the common precursor of AMP and GMP

(k) the precursor of hypoxanthine

11. The catabolism of fats and carbohydrates provides considerable metabolic energy in the form of ATP. Does the degradation of purines and pyrimidines provide a significant source of energy in eukaryotic cells?

12. PPRP synthetase uses α-D-ribose 5-phosphate as a substrate. How is the α isomer formed inside the cell?

13. The systematic names of the common bases are given in Sections 18.1 and 18.2. What are the systematic names of xanthine, hypoxanthine, and orotate?

14. The sequential action of adenylosuccinate synthetase and adenylosuccinate lyase results in the transfer of an amino group from aspartate and the release of fumarate. Identify two other pairs of enzymes that accomplish the same goal.

Selected Readings

Purine Metabolism

Honzatko, R. B., Stayton, M. M., and Fromm, H. J. (1999). Adenylosuccinate synthetase: recent developments. *Adv. Enzymol. Relat. Areas Mol. Biol.* 73:57–102.

Cendron, L., Berni, R., Folli, C., Ramazzina, I., Percudani, R., and Zanotti, G. (2007). The structure of 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazo-line decarboxylase provides insights into the mechanism of uric acid degradation. *J Biol. Chem.* 282:18182–18189.

Kresge, N., Simoni, R. D., and Hill, R. L. (2006). Biosynthesis of purines: the work of John M. Buchanan. *J. Biol. Chem.* 281:e35–e36.

Ramazzina, I., Folli, C., Secchi, A., Berni, R., and Percudani, R. (2006). Completing the uric acid degradation pathway through phylogenetic comparison of whole genomes. *Nat Chem Biol.* 2:144–148.

Tipton, P. A. (2006). Urate to allantoin, specifically (S)-allantoin. *Nat. Chem. Biol.* 2:124–125.

Tsai. M., Koo, J., Yip, P., Colman, R. F., Segall, M. L., Howell, P. L. (2007). Substrate and product complexes of *Escherichia coli* adenylosuccinate lyase provide new insights into the enzymatic mechanism. *J. Mol. Biol.* 370:541–554.

Zhang, R.-G., Evans, G., Rotella, F. J., Westbrook, E. M., Beno, D., Huberman, E., Joachimiak, A., and

Collart, F. R. (1999). Characteristics and crystal structure of bacterial inosine-5′-monophosphate dehydrogenase. *Biochem.* 38:4691–4700.

Pyrimidine Metabolism

Blakley, R. L. (1995). Eukaryotic dihydrofolate reductase. *Adv. Enzymol. Relat. Areas Mol. Biol.* 70:23–102.

Carreras, C. W., and Santi, D. V. (1995). The catalytic mechanism and structure of thymidylate synthase. *Annu. Rev. Biochem.* 64:721–762.

Chan, R. S., Sakash, J. B., Macol, C. P., West, J. M., Tsuruta, H., and Kantrowitz, E. R. (2002). The role of intersubunit interactions for the stabilization of the T state of *Escherichia coli* aspartate transcarbamoylase. *J. Biol. Chem.* 277:49755–49760.

Lipscomb, W. N. (1994). Aspartate transcarbamoylase from *Escherichia coli*: activity and regulation. *Adv. Enzymol. Relat. Areas Mol. Biol.* 68:67–151.

Raushel, F. M., Thoden, J. B., and Holden, H. M. (1999). The amidotransferase family of enzymes: molecular machines for the production and delivery of ammonia. *Biochem.* 38:7891–7899.

Stroud, R. M. (1994). An electrostatic highway. *Struct. Biol.* 1:131–134.

Ribonucleotide Reduction

Eriksson, M., Uhlin, U., Ramaswamy, S., Ekberg, M., Regnström, K., Sjöberg, B. M., and Eklund, H.

(1997). Binding of allosteric effectors to ribonucleotide reductase protein R1: reduction of active-site cysteines promotes substrate binding. *Structure* 5:1077–1092.

Gorlatov, S. N., and Stadtman, T. C. (1998). Human thioredoxin reductase from HeLa cells: selective alkylation of selenocysteine in the protein inhibits enzyme activity and reduction with NADPH influences affinity to heparin. *Proc. Natl. Acad. Sci. USA.* 95:8520–8525.

Jordan, A., and Reichard, P. (1998). Ribonucleotide reductases. *Annu. Rev. Biochem.* 67:71–98.

Kresge, N., Simoni, R. D., and Hill, R. L. (2006). Peter Reichard and the reduction of ribonucleosides. *J. Biol. Chem.* 281:e13–e15.

Nordland, P. and Reichard, P. (2006). Ribonucleotide reductases. *Annu. Rev. Biochem.* 75:681–706.

Sjöberg, B.M. (2010). A never-ending story. *Science* 329:1475–1476.

Stubbe, J. (1998). Ribonucleotide reductases in the twenty-first century. *Proc. Natl. Acad. Sci. USA.* 95:2723–2724.

Uppsten, M., Färnegårdh, M., Domkin, V., and Uhlin, U. (2006). The first holocomplex structure of ribonucleotide reductase gives new insight into its mechanism of action. *J. Mol. Biol.* 359:365–377.