roles in all forms of life. Furthermore, although the number of reactions in metabolism is large, the number of *kinds* of reactions is small and the mechanisms of these reactions are usually quite simple. Metabolic pathways are also regulated in common ways. The purpose of this chapter is to introduce some general principles and motifs of metabolism to provide a foundation for the more detailed studies to follow. These principles are:

1. Fuels are degraded and large molecules are constructed step by step in a series of linked reactions called *metabolic pathways*.
2. An energy currency common to all life forms, adenosine triphosphate (ATP), links energy-releasing pathways with energy-requiring pathways.
3. The oxidation of carbon fuels powers the formation of ATP.
4. Although there are many metabolic pathways, a limited number of types of reactions and particular intermediates are common to many pathways.
5. Metabolic pathways are highly regulated.

15.1 Metabolism Is Composed of Many Coupled, Interconnecting Reactions

Living organisms require a continual input of free energy for three major purposes: (1) the performance of mechanical work in muscle contraction and cellular movements, (2) the active transport of molecules and ions, and (3) the synthesis of macromolecules and other biomolecules from simple precursors. The free energy used in these processes, which maintain an organism in a state that is far from equilibrium, is derived from the environment. Photosynthetic organisms, or *phototrophs,* obtain this energy by trapping sunlight, whereas *chemotrophs,* which include animals, obtain energy through the oxidation of foodstuffs generated by phototrophs.

Metabolism consists of energy-yielding and energy-requiring reactions

Metabolism is essentially a linked series of chemical reactions that begins with a particular molecule and converts it into some other molecule or molecules in a carefully defined fashion (Figure 15.1). There are many such defined pathways in the cell (Figure 15.2), and we will examine a few of them in some detail later. These pathways are interdependent, and their activity is coordinated by exquisitely sensitive means of communication in which allosteric enzymes are predominant (Section 10.1). We considered the principles of this communication in Chapter 14.

We can divide metabolic pathways into two broad classes: (1) those that convert energy from fuels into biologically useful forms and (2) those that require inputs of energy to proceed. Although this division is often imprecise, it is nonetheless a useful distinction in an examination of metabolism. Those reactions that transform fuels into cellular energy are called *catabolic reactions* or, more generally, *catabolism*.

$$\text{Fuel (carbohydrates, fats)} \xrightarrow{\text{Catabolism}} CO_2 + H_2O + \text{useful energy}$$

Those reactions that require energy—such as the synthesis of glucose, fats, or DNA—are called *anabolic reactions* or *anabolism*. The useful forms of energy that are produced in catabolism are employed in anabolism to generate complex structures from simple ones, or energy-rich states from energy-poor ones.

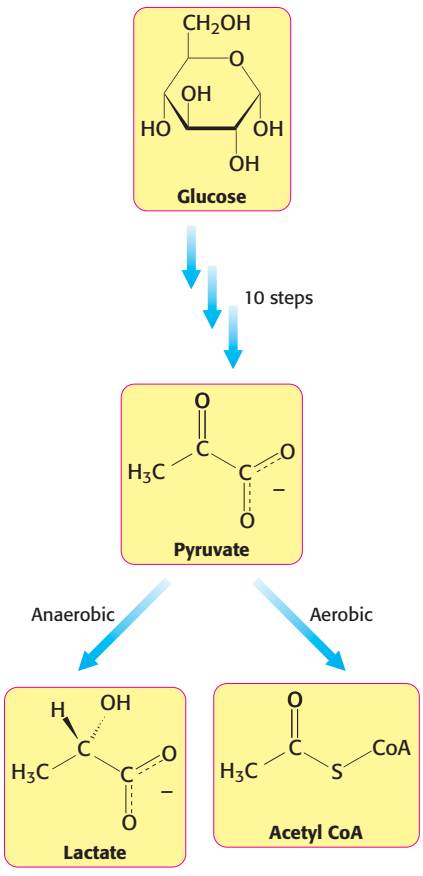


Figure 15.1 Glucose metabolism. Glucose is metabolized to pyruvate in 10 linked reactions. Under anaerobic conditions, pyruvate is metabolized to lactate and, under aerobic conditions, to acetyl CoA. The glucose-derived carbons of acetyl CoA are subsequently oxidized to CO_2.

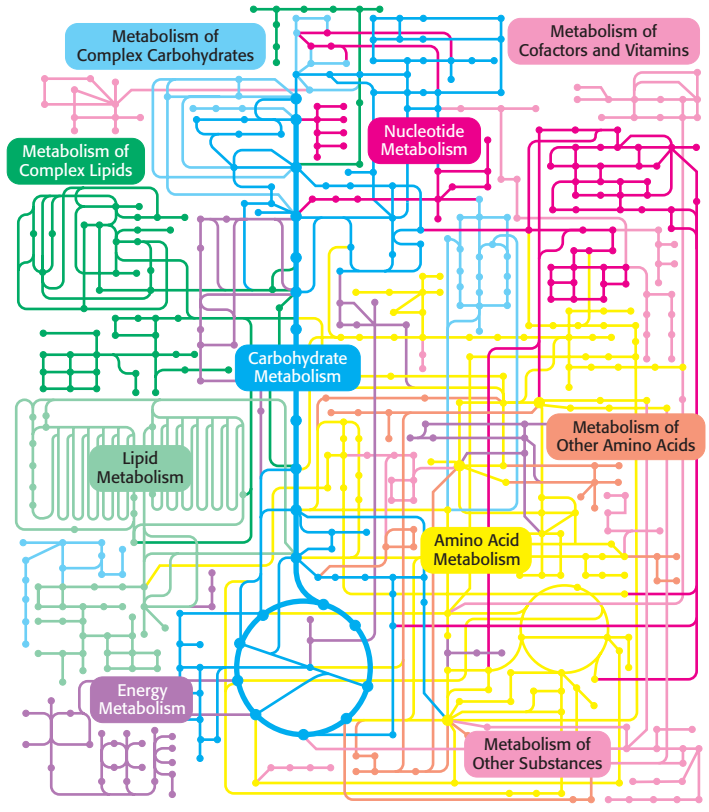


Figure 15.2 Metabolic pathways. [From the Kyoto Encyclopedia of Genes and Genomes (www.genome.ad.jp/kegg).]

$$\text{Useful energy} + \text{simple precursors} \xrightarrow{\text{Anabolism}} \text{complex molecules}$$

Some pathways can be either anabolic or catabolic, depending on the energy conditions in the cell. These pathways are referred to as *amphibolic pathways*.

An important general principle of metabolism is that *biosynthetic and degradative pathways are almost always distinct*. This separation is necessary for energetic reasons, as will be evident in subsequent chapters. It also facilitates the control of metabolism.

A thermodynamically unfavorable reaction can be driven by a favorable reaction

How are specific pathways constructed from individual reactions? A pathway must satisfy minimally two criteria: (1) the individual reactions must be *specific* and (2) the entire set of reactions that constitute the pathway must be *thermodynamically favored*. A reaction that is specific will yield only one particular product or set of products from its reactants. As discussed in Chapter 8, a function of enzymes is to provide this specificity. The thermodynamics of metabolism is most readily approached in relation to free energy, which was also discussed in Chapter 8. A reaction can occur spontaneously only if ΔG, the change in free energy, is negative. Recall that ΔG for the formation of products C and D from substrates A and B is given by

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

Thus, the ΔG of a reaction depends on the *nature* of the reactants and products (expressed by the $\Delta G^{\circ\prime}$ term, the standard free-energy change) and on their *concentrations* (expressed by the second term).

An important thermodynamic fact is that *the overall free-energy change for a chemically coupled series of reactions is equal to the sum of the free-energy changes of the individual steps*. Consider the following reactions:

$$\begin{array}{ll} A \rightleftharpoons B + C & \Delta G^{\circ\prime} = +21\ \text{kJ mol}^{-1}\ (+5\ \text{kcal mol}^{-1}) \\ B \rightleftharpoons D & \Delta G^{\circ\prime} = -34\ \text{kJ mol}^{-1}\ (-8\ \text{kcal mol}^{-1}) \\ \hline A \rightleftharpoons C + D & \Delta G^{\circ\prime} = -13\ \text{kJ mol}^{-1}\ (-3\ \text{kcal mol}^{-1}) \end{array}$$

Under standard conditions, A cannot be spontaneously converted into B and C, because $\Delta G^{\circ\prime}$ is positive. However, the conversion of B into D under standard conditions is thermodynamically feasible. Because free-energy changes are additive, the conversion of A into C and D has a $\Delta G^{\circ\prime}$ of -13 kJ mol^{-1} (-3 kcal mol^{-1}), which means that it can occur spontaneously under standard conditions. Thus, *a thermodynamically unfavorable reaction can be driven by a thermodynamically favorable reaction to which it is coupled*. In this example, the reactions are coupled by the shared chemical intermediate B. Thus, metabolic pathways are formed by the coupling of enzyme-catalyzed reactions such that the overall free energy of the pathway is negative.

15.2 ATP Is the Universal Currency of Free Energy in Biological Systems

Just as commerce is facilitated by the use of a common currency, the commerce of the cell—metabolism—is facilitated by the use of a common energy currency, *adenosine triphosphate* (ATP). Part of the free energy derived from the oxidation of foodstuffs and from light is transformed into this highly accessible molecule, which acts as the free-energy donor in most energy-requiring processes such as motion, active transport, and biosynthesis. Indeed, most of catabolism consists of reactions that extract energy from fuels such as carbohydrates and fats and convert it into ATP.

ATP hydrolysis is exergonic

ATP is a nucleotide consisting of adenine, a ribose, and a triphosphate unit (Figure 15.3). The active form of ATP is usually a complex of ATP with Mg^{2+} or Mn^{2+}. In considering the role of ATP as an energy carrier, we can focus on its triphosphate moiety. *ATP is an energy-rich molecule because its triphosphate unit contains two phosphoanhydride bonds*. A large amount of free energy is liberated when ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (P_i) or when ATP is hydrolyzed to adenosine monophosphate (AMP) and pyrophosphate (PP_i).

$$\begin{aligned} \text{ATP} + H_2O &\rightleftharpoons \text{ADP} + P_i \\ \Delta G^{\circ\prime} &= -30.5\ \text{kJ mol}^{-1}\ (-7.3\ \text{kcal mol}^{-1}) \end{aligned}$$

$$\begin{aligned} \text{ATP} + H_2O &\rightleftharpoons \text{AMP} + PP_i \\ \Delta G^{\circ\prime} &= -45.6\ \text{kJ mol}^{-1}\ (-10.9\ \text{kcal mol}^{-1}) \end{aligned}$$

The precise $\Delta G^{\circ\prime}$ for these reactions depends on the ionic strength of the medium and on the concentrations of Mg^{2+} and other metal ions. Under typical cellular concentrations, the actual ΔG for these hydrolyses is approximately -50 kJ mol^{-1} (-12 kcal mol^{-1}).

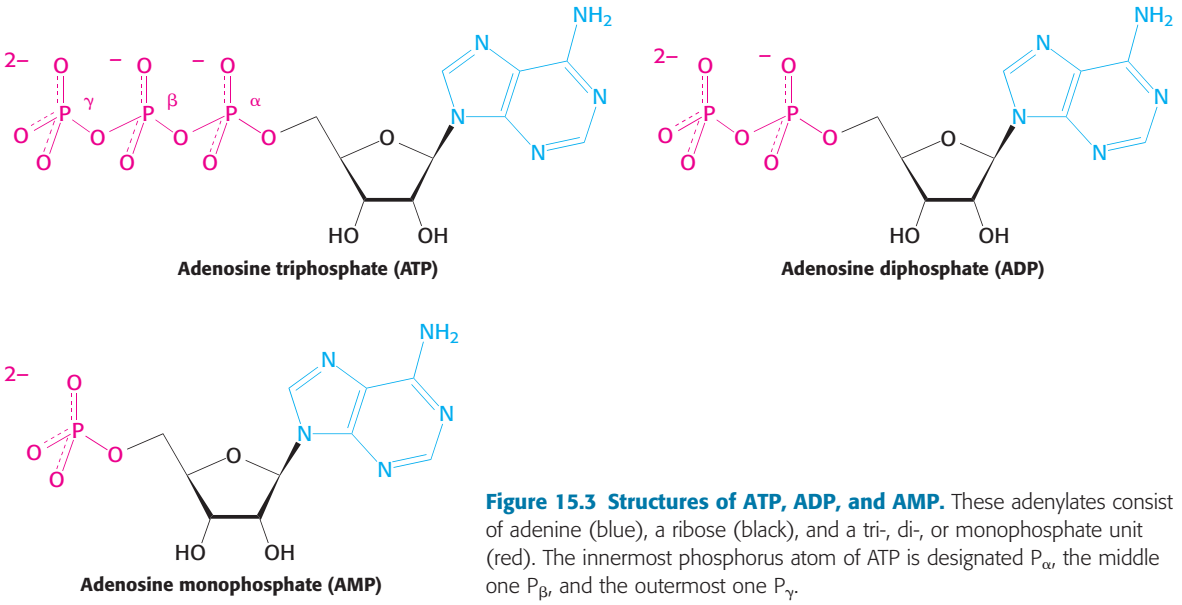


Figure 15.3 Structures of ATP, ADP, and AMP. These adenylates consist of adenine (blue), a ribose (black), and a tri-, di-, or monophosphate unit (red). The innermost phosphorus atom of ATP is designated P_α, the middle one P_β, and the outermost one P_γ.

The free energy liberated in the hydrolysis of ATP is harnessed to drive reactions that require an input of free energy, such as muscle contraction. In turn, ATP is formed from ADP and P_i when fuel molecules are oxidized in chemotrophs or when light is trapped by phototrophs. *This ATP–ADP cycle is the fundamental mode of energy exchange in biological systems.*

Some biosynthetic reactions are driven by the hydrolysis of nucleoside triphosphates that are analogous to ATP—namely, guanosine triphosphate (GTP), uridine triphosphate (UTP), and cytidine triphosphate (CTP). The diphosphate forms of these nucleotides are denoted by GDP, UDP, and CDP, and the monophosphate forms are denoted by GMP, UMP, and CMP. Enzymes catalyze the transfer of the terminal phosphoryl group from one nucleotide to another. The phosphorylation of nucleoside monophosphates is catalyzed by a family of *nucleoside monophosphate kinases,* as discussed in Section 9.4. The phosphorylation of nucleoside diphosphates is catalyzed by *nucleoside diphosphate kinase,* an enzyme with broad specificity.

$$\text{NMP} + \text{ATP} \xrightleftharpoons{\text{Nucleoside monophosphate kinase}} \text{NDP} + \text{ADP}$$

(NMP: Nucleoside monophosphate)

$$\text{NDP} + \text{ATP} \xrightleftharpoons{\text{Nucleoside diphosphate kinase}} \text{NTP} + \text{ADP}$$

(NDP: Nucleoside diphosphate)

It is intriguing to note that although all of the nucleotide triphosphates are energetically equivalent, ATP is nonetheless the primary cellular energy carrier. In addition, two important electron carriers, NAD^+ and FAD, are derivatives of ATP. *The role of ATP in energy metabolism is paramount.*

ATP hydrolysis drives metabolism by shifting the equilibrium of coupled reactions

An otherwise unfavorable reaction can be made possible by coupling to ATP hydrolysis. Consider a chemical reaction that is thermodynamically unfavorable without an input of free energy, a situation common to many biosynthetic reactions. Suppose that the standard free energy of

the conversion of compound A into compound B is +16.7 kJ mol^{-1} (+4.0 kcal mol^{-1}):

$$A \rightleftharpoons B \qquad \Delta G^{\circ\prime} = +16.7 \text{ kJ mol}^{-1} (+4 \text{ kcal mol}^{-1})$$

The equilibrium constant K'_{eq} of this reaction at 25°C is related to $\Delta G^{\circ\prime}$ (in units of kilojoules per mole) by

$$K'_{eq} = [B]_{eq}/[A]_{eq} = 10^{-\Delta G^{\circ\prime}/5.69} = 1.15 \times 10^{-3}$$

Thus, net conversion of A into B cannot take place when the molar ratio of B to A is equal to or greater than 1.15×10^{-3}. However, A can be converted into B under these conditions if the reaction is coupled to the hydrolysis of ATP. Under standard conditions, the $\Delta G^{\circ\prime}$ of hydrolysis is approximately −30.5 kJ mol^{-1} (−7.3 kcal mol^{-1}). The new overall reaction is

$$A + ATP + H_2O \rightleftharpoons B + ATP + P_i$$
$$\Delta G^{\circ\prime} = -13.8 \text{ kJ mol}^{-1} (-3.3 \text{ kcal mol}^{-1})$$

Its free-energy change of −13.8 kJ mol^{-1} (−3.3 kcal mol^{-1}) is the sum of the value of $\Delta G^{\circ\prime}$ for the conversion of A into B [+16.7 kJ mol^{-1} (+4.0 kcal mol^{-1})] and the value of $\Delta G^{\circ\prime}$ for the hydrolysis of ATP [−30.5 kJ mol^{-1} (−7.3 kcal mol^{-1})]. At pH 7, the equilibrium constant of this coupled reaction is

$$K'_{eq} = \frac{[B]_{eq}}{[A]_{eq}} \times \frac{[ADP]_{eq}[P_i]_{eq}}{[ATP]_{eq}} = 10^{13.8/5.69} = 2.67 \times 10^2$$

At equilibrium, the ratio of [B] to [A] is given by

$$\frac{[B]_{eq}}{[A]_{eq}} = K'_{eq}\frac{[ATP]_{eq}}{[ADP]_{eq}[P_i]_{eq}}$$

which means that the hydrolysis of ATP enables A to be converted into B until the [B]/[A] ratio reaches a value of 2.67×10^2. This equilibrium ratio is strikingly different from the value of 1.15×10^{-3} for the reaction A → B in the absence of ATP hydrolysis. In other words, coupling the hydrolysis of ATP with the conversion of A into B under standard conditions has changed the equilibrium ratio of B to A by a factor of about 10^5. If we were to use the ΔG of hydrolysis of ATP under cellular conditions [−50.2 kJ mol^{-1} (−12 kcal mol^{-1})] in our calculations instead of $\Delta G^{\circ\prime}$, the change in the equilibrium ratio would be even more dramatic, on the order of 10^8.

We see here the thermodynamic essence of ATP's action as an *energy-coupling agent*. Cells maintain a high level of ATP by using oxidizable substrates or light as sources of free energy for synthesizing the molecule. In the cell, the hydrolysis of an ATP molecule in a coupled reaction then changes the equilibrium ratio of products to reactants by a very large factor, of the order of 10^8. More generally, the hydrolysis of *n* ATP molecules changes the equilibrium ratio of a coupled reaction (or sequence of reactions) by a factor of 10^{8n}. For example, the hydrolysis of three ATP molecules in a coupled reaction changes the equilibrium ratio by a factor of 10^{24}. Thus, *a thermodynamically unfavorable reaction sequence can be converted into a favorable one by coupling it to the hydrolysis of a sufficient number of ATP molecules in a new reaction*. It should also be emphasized that A and B in the preceding coupled reaction may be interpreted very generally, not only as different chemical species. For example, A and B may represent activated and unactivated conformations of a protein that is activated by phosphorylation with ATP. Through such changes in protein conformation, molecular motors such as myosin, kinesin, and dynein convert the chemical energy of ATP into

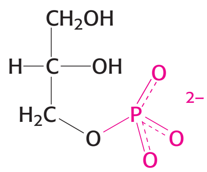
mechanical energy (Chapter 34). Indeed, this conversion is the basis of muscle contraction.

Alternatively, A and B may refer to the concentrations of an ion or molecule on the outside and inside of a cell, as in the active transport of a nutrient. The active transport of Na^+ and K^+ across membranes is driven by the phosphorylation of the sodium–potassium pump by ATP and its subsequent dephosphorylation (Section 13.2).

The high phosphoryl potential of ATP results from structural differences between ATP and its hydrolysis products

What makes ATP a particularly efficient phosphoryl-group donor? Let us compare the standard free energy of hydrolysis of ATP with that of a phosphate ester, such as glycerol 3-phosphate:

$$\text{ATP} + H_2O \rightleftharpoons \text{ADP} + P_i$$
$$\Delta G^{\circ\prime} = -30.5 \text{ kJ mol}^{-1} \ (-7.3 \text{ kcal mol}^{-1})$$
$$\text{Glycerol 3-phosphate} + H_2O \rightleftharpoons \text{glycerol} + P_i$$
$$\Delta G^{\circ\prime} = -9.2 \text{ kJ mol}^{-1} \ (-2.2 \text{ kcal mol}^{-1})$$



Glycerol 3-phosphate

The magnitude of $\Delta G^{\circ\prime}$ for the hydrolysis of glycerol 3-phosphate is much smaller than that of ATP, which means that ATP has a stronger tendency to transfer its terminal phosphoryl group to water than does glycerol 3-phosphate. In other words, ATP has a higher *phosphoryl-transfer potential (phosphoryl-group-transfer potential)* than does glycerol 3-phosphate.

The high phosphoryl-transfer potential of ATP can be explained by features of the ATP structure. Because $\Delta G^{\circ\prime}$ depends on the *difference* in free energies of the products and reactants, we need to examine the structures of both ATP and its hydrolysis products, ADP and P_i, to answer this question. Three factors are important: *resonance stabilization, electrostatic repulsion*, and *stabilization due to hydration*.

1. *Resonance Stabilization.* ADP and, particularly, P_i, have greater resonance stabilization than does ATP. Orthophosphate has a number of resonance forms of similar energy (Figure 15.4), whereas the γ phosphoryl group of ATP has a smaller number. Forms like that shown in Figure 15.5 are unfavorable because a positively charged oxygen atom is adjacent to a positively charged phosphorus atom, an electrostatically unfavorable juxtaposition.

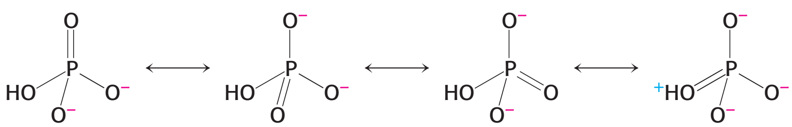


Figure 15.4 Resonance structures of orthophosphate.

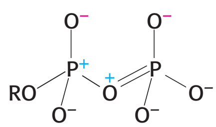


Figure 15.5 Improbable resonance structure. The structure contributes little to the terminal part of ATP, because two positive charges are placed adjacent to each other.

2. *Electrostatic Repulsion.* At pH 7, the triphosphate unit of ATP carries about four negative charges. These charges repel one another because they are in close proximity. The repulsion between them is reduced when ATP is hydrolyzed.

3. *Stabilization Due to Hydration.* More water can bind more effectively to ADP and P_i than can bind to the phosphoanhydride part of ATP, stabilizing the ADP and P_i by hydration.

ATP is often called a high-energy phosphate compound, and its phosphoanhydride bonds are referred to as high-energy bonds. Indeed, a

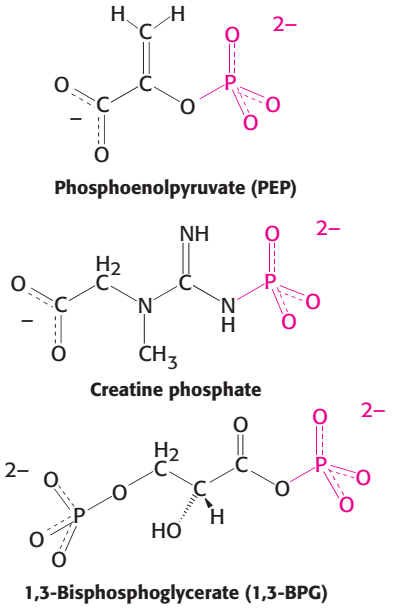


Figure 15.6 Compounds with high phosphoryl-transfer potential. These compounds have a higher phosphoryl-transfer potential than that of ATP and can be used to phosphorylate ADP to form ATP.

"squiggle" (~P) is often used to indicate such a bond. Nonetheless, there is nothing special about the bonds themselves. *They are high-energy bonds in the sense that much free energy is released when they are hydrolyzed*, for the reasons listed in factors 1 through 3.

Phosphoryl-transfer potential is an important form of cellular energy transformation

The standard free energies of hydrolysis provide a convenient means of comparing the phosphoryl-transfer potential of phosphorylated compounds. Such comparisons reveal that ATP is not the only compound with a high phosphoryl-transfer potential. In fact, some compounds in biological systems have a higher phosphoryl-transfer potential than that of ATP. These compounds include phosphoenolpyruvate (PEP), 1,3-bisphosphoglycerate (1,3-BPG), and creatine phosphate (Figure 15.6). Thus, PEP can transfer its phosphoryl group to ADP to form ATP. Indeed, this transfer is one of the ways in which ATP is generated in the breakdown of sugars (Chapter 16). It is significant that ATP has a phosphoryl-transfer potential that is intermediate among the biologically important phosphorylated molecules (Table 15.1). *This intermediate position enables ATP to function efficiently as a carrier of phosphoryl groups*.

The amount of ATP in muscle suffices to sustain contractile activity for less than a second. Creatine phosphate in vertebrate muscle serves as a reservoir of high-potential phosphoryl groups that can be readily transferred to ADP. Indeed, we use creatine phosphate to regenerate ATP from ADP every time that we exercise strenuously. This reaction is catalyzed by *creatine kinase*.

$$\text{Creatine phosphate} + \text{ADP} \overset{\text{Creatine kinase}}{\rightleftharpoons} \text{ATP} + \text{creatine}$$

At pH 7, the standard free energy of hydrolysis of creatine phosphate is -43.1 kJ mol^{-1} (-10.3 kcal mol^{-1}), compared with -30.5 kJ mol^{-1} (-7.3 kcal mol^{-1}) for ATP. Hence, the standard free-energy change in forming ATP from creatine phosphate is -12.6 kJ mol^{-1} (-3.0 kcal mol^{-1}), which corresponds to an equilibrium constant of 162.

$$K_{eq} = \frac{[\text{ATP}][\text{creatine}]}{[\text{ADP}][\text{creatine phosphate}]} = 10^{-\Delta G^{\circ\prime}/5.69} = 10^{12.6/5.69} = 162$$

In resting muscle, typical concentrations of these metabolites are [ATP] = 4 mM, [ADP] = 0.013 mM [creatine phosphate] = 25 mM, and [creatine] = 13 mM. Because of its abundance and high phosphoryl-transfer potential relative to that of ATP, creatine phosphate is a highly effective phosphoryl

Table 15.1 Standard free energies of hydrolysis of some phosphorylated compounds

Compound	kJ mol^{-1}	kcal mol^{-1}
Phosphoenolpyruvate	−61.9	−14.8
1,3-Bisphosphoglycerate	−49.4	−11.8
Creatine phosphate	−43.1	−10.3
ATP (to ADP)	−30.5	− 7.3
Glucose 1-phosphate	−20.9	− 5.0
Pyrophosphate	−19.3	− 4.6
Glucose 6-phosphate	−13.8	− 3.3
Glycerol 3-phosphate	− 9.2	− 2.2

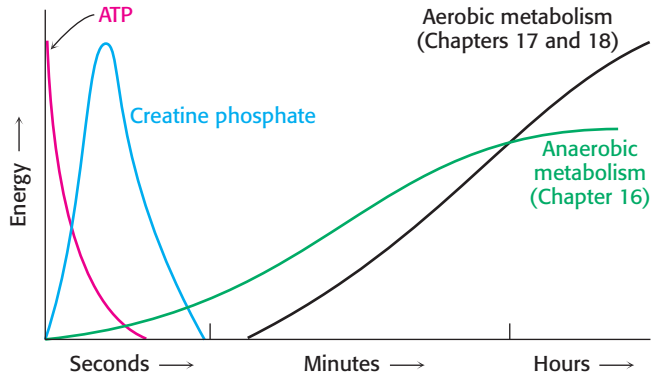


Figure 15.7 Sources of ATP during exercise. In the initial seconds, exercise is powered by existing high-phosphoryl-transfer compounds (ATP and creatine phosphate). Subsequently, the ATP must be regenerated by metabolic pathways.

buffer. Indeed, creatine phosphate is the major source of phosphoryl groups for ATP regeneration for a runner during the first 4 seconds of a 100-meter sprint. The fact that creatine phosphate can replenish ATP pools is the basis of the use of creatine as a dietary supplement by athletes in sports requiring short bursts of intense activity. After the creatine phosphate pool is depleted, ATP must be generated through metabolism (Figure 15.7).

15.3 The Oxidation of Carbon Fuels Is an Important Source of Cellular Energy

ATP serves as the principal *immediate donor of free energy* in biological systems rather than as a long-term storage form of free energy. In a typical cell, an ATP molecule is consumed within a minute of its formation. Although the total quantity of ATP in the body is limited to approximately 100 g, *the turnover of this small quantity of ATP is very high*. For example, a resting human being consumes about 40 kg of ATP in 24 hours. During strenuous exertion, the rate of utilization of ATP may be as high as 0.5 kg/minute. For a 2-hour run, 60 kg (132 pounds) of ATP is utilized. Clearly, having mechanisms for regenerating ATP is vital. Motion, active transport, signal amplification, and biosynthesis can take place only if ATP is continually regenerated from ADP (Figure 15.8). The generation of ATP is one of the primary roles of catabolism. The carbon in fuel molecules—such as glucose and fats—is oxidized to CO_2. The resulting electrons are captured and used to regenerate ATP from ADP and P_i.

In aerobic organisms, the ultimate electron acceptor in the oxidation of carbon is O_2 and the oxidation product is CO_2. Consequently, the more reduced a carbon is to begin with, the more free energy is released by its oxidation. Figure 15.9 shows the $\Delta G°'$ of oxidation for one-carbon compounds.

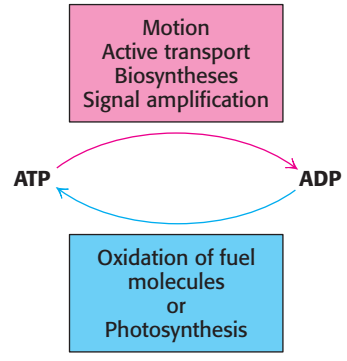
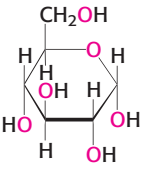


Figure 15.8 ATP–ADP cycle. This cycle is the fundamental mode of energy exchange in biological systems.

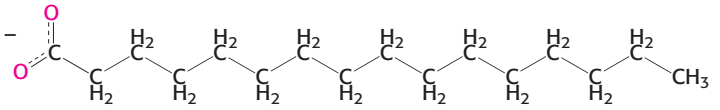
Most energy → Least energy

	Methane	Methanol	Formaldehyde	Formic acid	Carbon dioxide
$\Delta G°'_{oxidation}$ (kJ mol^{-1})	−820	−703	−523	−285	0
$\Delta G°'_{oxidation}$ (kcal mol^{-1})	−196	−168	−125	−68	0

Figure 15.9 Free energy of oxidation of single-carbon compounds.



Glucose

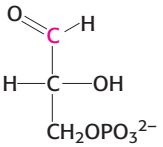


Fatty acid

Figure 15.10 Prominent fuels. Fats are a more efficient fuel source than carbohydrates such as glucose because the carbon in fats is more reduced.

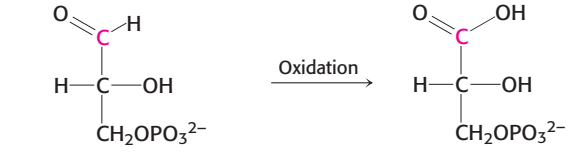
Although fuel molecules are more complex (Figure 15.10) than the single-carbon compounds depicted in Figure 15.9, when a fuel is oxidized the oxidation takes place one carbon at a time. The carbon-oxidation energy is used in some cases to create a compound with high phosphoryl-transfer potential and in other cases to create an ion gradient. In either case, the end point is the formation of ATP.

Compounds with high phosphoryl-transfer potential can couple carbon oxidation to ATP synthesis



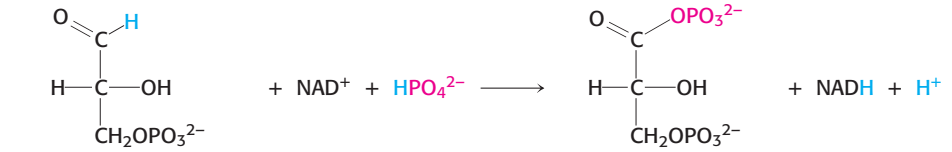
Glyceraldehyde 3-phosphate (GAP)

How is the energy released in the oxidation of a carbon compound converted into ATP? As an example, consider glyceraldehyde 3-phosphate (shown in the margin), which is a metabolite of glucose formed in the oxidation of that sugar. The C-1 carbon (shown in red) is at the aldehyde-oxidation level and is not in its most oxidized state. Oxidation of the aldehyde to an acid will release energy.



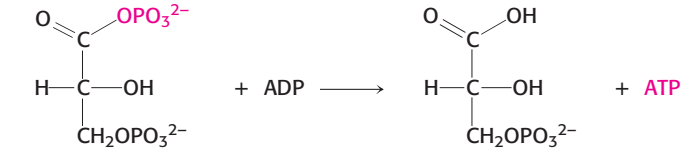
Glyceraldehyde 3-phosphate — 3-Phosphoglyceric acid

However, the oxidation does not take place directly. Instead, the carbon oxidation generates an acyl phosphate, 1,3-bisphosphoglycerate. The electrons released are captured by NAD^+, which we will consider shortly.



Glyceraldehyde 3-phosphate (GAP) — 1,3-Bisphosphoglycerate (1,3-BPG)

For reasons similar to those discussed for ATP, 1,3-bisphosphoglycerate has a high phosphoryl-transfer potential. Thus, the cleavage of 1,3-BPG can be coupled to the synthesis of ATP.



1,3-Bisphosphoglycerate — 3-Phosphoglyceric acid

The energy of oxidation is initially trapped as a high-phosphoryl-transfer-potential compound and then used to form ATP. The oxidation energy of a

carbon atom is transformed into phosphoryl-transfer potential, first as 1,3-bisphosphoglycerate and ultimately as ATP. We will consider these reactions in mechanistic detail in Chapter 16.

Ion gradients across membranes provide an important form of cellular energy that can be coupled to ATP synthesis

As described in Chapter 13, electrochemical potential is an effective means of storing free energy. Indeed, the electrochemical potential of *ion gradients across membranes*, produced by the oxidation of fuel molecules or by photosynthesis, ultimately powers the synthesis of most of the ATP in cells. In general, ion gradients are versatile means of coupling thermodynamically unfavorable reactions to favorable ones. Indeed, in animals, *proton gradients* generated by the oxidation of carbon fuels account for more than 90% of ATP generation (Figure 15.11). This process is called *oxidative phosphorylation* (Chapter 18). ATP hydrolysis can then be used to form ion gradients of different types and functions. The electrochemical potential of a Na^+ gradient, for example, can be tapped to pump Ca^{2+} out of cells or to transport nutrients such as sugars and amino acids into cells.

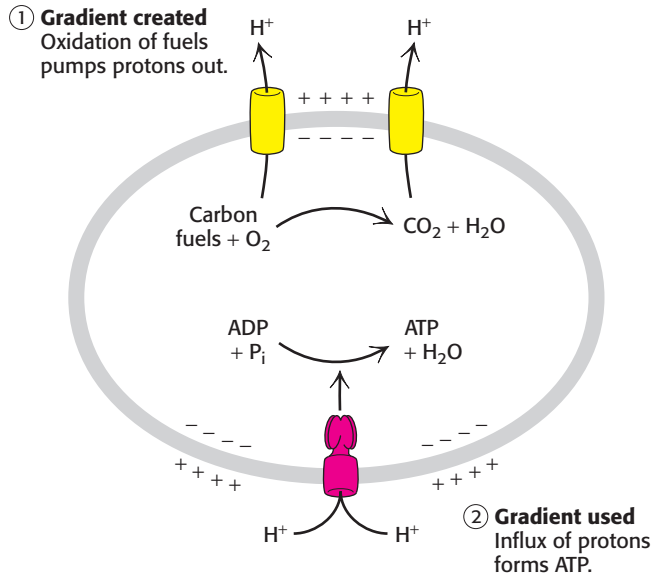


Figure 15.11 Proton gradients. The oxidation of fuels can power the formation of proton gradients by the action of specific proton pumps. These proton gradients can in turn drive the synthesis of ATP when the protons flow through an ATP-synthesizing enzyme.

Energy from foodstuffs is extracted in three stages

Let us take an overall view of the processes of energy conversion in higher organisms before considering them in detail in subsequent chapters. Hans Krebs described three stages in the generation of energy from the oxidation of foodstuffs (Figure 15.12).

In the first stage, large molecules in food are broken down into smaller units. This process is *digestion*. Proteins are hydrolyzed to their 20 different amino acids, polysaccharides are hydrolyzed to simple sugars such as glucose, and fats are hydrolyzed to glycerol and fatty acids. The degradation products are then absorbed by the cells of the intestine and distributed throughout the body. This stage is strictly a preparation stage; no useful energy is captured in this phase.

In the second stage, these numerous small molecules are degraded to a few simple units that play a central role in metabolism. In fact, most of them—sugars, fatty acids, glycerol, and several amino acids—are converted into the acetyl unit of acetyl CoA. Some ATP is generated in this stage, but the amount is small compared with that obtained in the third stage.

In the third stage, ATP is produced from the complete oxidation of the acetyl unit of acetyl CoA. The third stage consists of the citric acid cycle and oxidative phosphorylation, which are *the final common pathways in the oxidation of fuel molecules*. Acetyl CoA brings acetyl units into the citric acid cycle [also called the tricarboxylic acid (TCA) cycle or Krebs cycle], where they are completely oxidized to CO_2. Four pairs of electrons are transferred (three to NAD^+ and one to FAD) for each acetyl group that is oxidized. Then, a

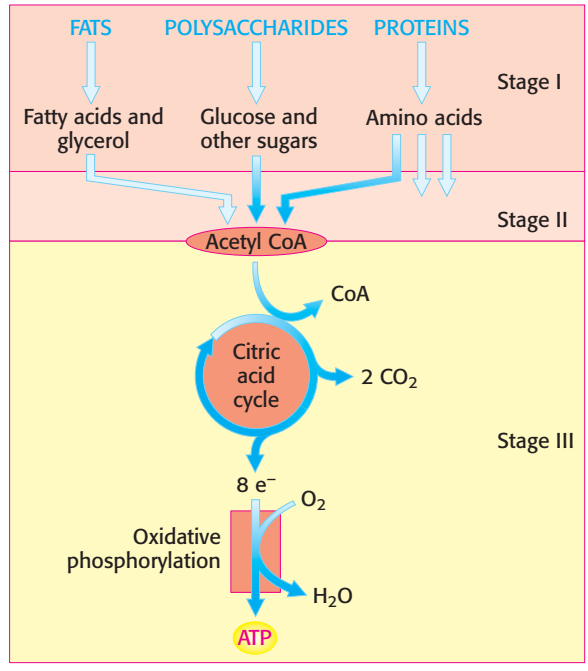


Figure 15.12 Stages of catabolism. The extraction of energy from fuels can be divided into three stages.

proton gradient is generated as electrons flow from the reduced forms of these carriers to O_2, and this gradient is used to synthesize ATP.

15.4 Metabolic Pathways Contain Many Recurring Motifs

At first glance, metabolism appears intimidating because of the sheer number of reactants and reactions. Nevertheless, there are unifying themes that make the comprehension of this complexity more manageable. These unifying themes include common metabolites, reactions, and regulatory schemes that stem from a common evolutionary heritage.

Activated carriers exemplify the modular design and economy of metabolism

We have seen that phosphoryl transfer can be used to drive otherwise endergonic reactions, alter the energy of conformation of a protein, or serve as a signal to alter the activity of a protein. The phosphoryl-group donor in all of these reactions is ATP. In other words, *ATP is an activated carrier of phosphoryl groups because phosphoryl transfer from ATP is an exergonic process*. The use of activated carriers is a recurring motif in biochemistry, and we will consider several such carriers here. Many such activated carriers function as coenzymes:

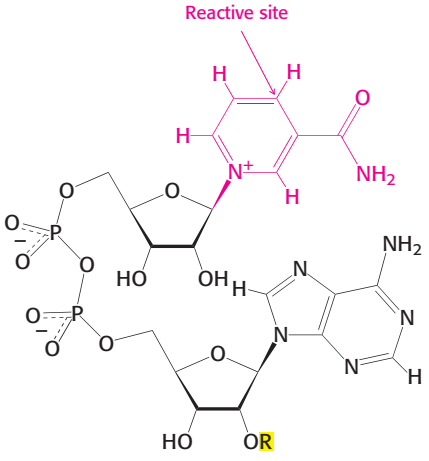
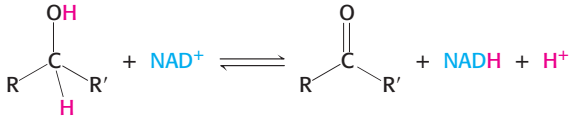


Figure 15.13 Structures of the oxidized forms of nicotinamide-derived electron carriers. Nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) are prominent carriers of high-energy electrons. In NAD^+, R = H; in $NADP^+$, R = PO_3^{2-}.

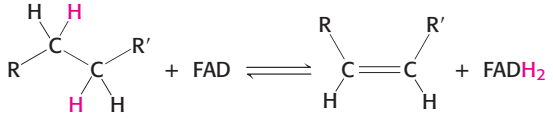
1. *Activated Carriers of Electrons for Fuel Oxidation*. In aerobic organisms, the ultimate electron acceptor in the oxidation of fuel molecules is O_2. However, electrons are not transferred directly to O_2. Instead, fuel molecules transfer electrons to special carriers, which are either *pyridine nucleotides* or *flavins*. The reduced forms of these carriers then transfer their high-potential electrons to O_2.

Nicotinamide adenine dinucleotide is a major electron carrier in the oxidation of fuel molecules (Figure 15.13). The reactive part of NAD^+ is its nicotinamide ring, a pyridine derivative synthesized from the vitamin niacin. *In the oxidation of a substrate, the nicotinamide ring of NAD^+ accepts a hydrogen ion and two electrons, which are equivalent to a hydride ion* ($H:^-$). The reduced form of this carrier is called *NADH*. In the oxidized form, the nitrogen atom carries a positive charge, as indicated by NAD^+. NAD^+ is the electron acceptor in many reactions of the type



In this dehydrogenation, one hydrogen atom of the substrate is directly transferred to NAD^+, whereas the other appears in the solvent as a proton. Both electrons lost by the substrate are transferred to the nicotinamide ring.

The other major electron carrier in the oxidation of fuel molecules is the coenzyme *flavin adenine dinucleotide* (Figure 15.14). The abbreviations for the oxidized and reduced forms of this carrier are FAD and $FADH_2$, respectively. FAD is the electron acceptor in reactions of the type



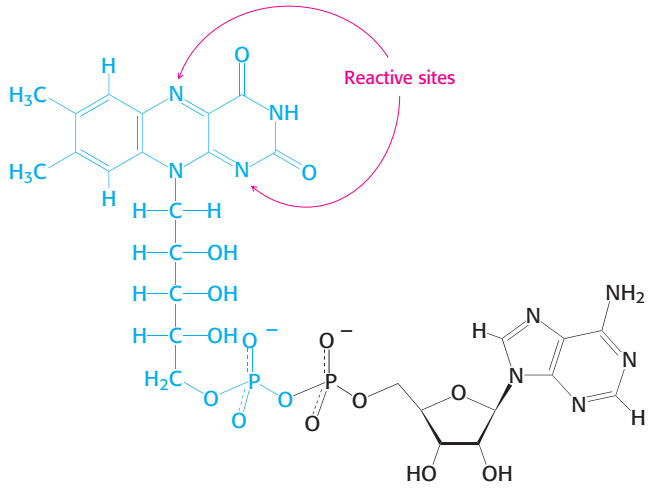


Figure 15.14 Structure of the oxidized form of flavin adenine dinucleotide (FAD). This electron carrier consists of a flavin mononucleotide (FMN) unit (shown in blue) and an AMP unit (shown in black).

The reactive part of FAD is its isoalloxazine ring, a derivative of the vitamin riboflavin (Figure 15.15). FAD, like NAD^+, can accept two electrons. In doing so, FAD, unlike NAD^+, takes up two protons. These carriers of high-potential electrons as well as flavin mononucleotide (FMN), an electron similar to FAD but lacking the adenine nucleotide, will be considered further in Chapter 18.

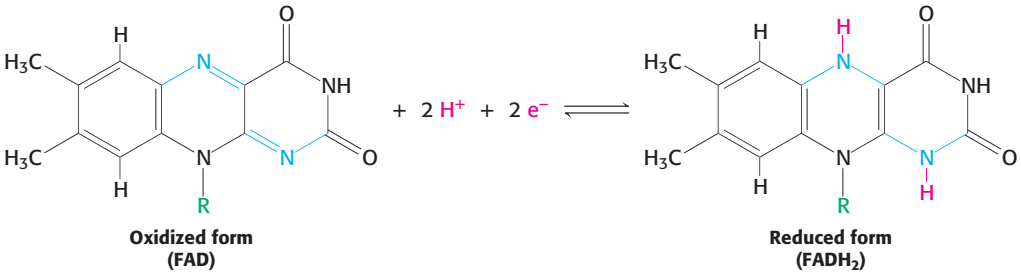
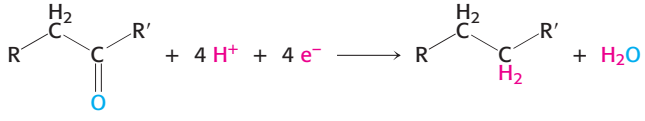


Figure 15.15 Structures of the reactive parts of FAD and $FADH_2$. The electrons and protons are carried by the isoalloxazine ring component of FAD and $FADH_2$.

2. *An Activated Carrier of Electrons for Reductive Biosynthesis*. High-potential electrons are required in most biosyntheses because the precursors are more oxidized than the products. Hence, reducing power is needed in addition to ATP. For example, in the biosynthesis of fatty acids, the keto group of an added two-carbon unit is reduced to a methylene group in several steps. This sequence of reactions requires an input of four electrons.



The electron donor in most reductive biosyntheses is NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate ($NADP^+$; see Figure 15.13). NADPH differs from NADH in that the 2′-hydroxyl group of its adenosine moiety is esterified with phosphate. NADPH carries electrons in the same way as NADH. However, *NADPH is used almost exclusively for reductive biosyntheses, whereas NADH is used primarily for the generation of ATP*. The extra phosphoryl group on NADPH is a tag that enables enzymes to distinguish between high-potential electrons to be used in anabolism and those to be used in catabolism.

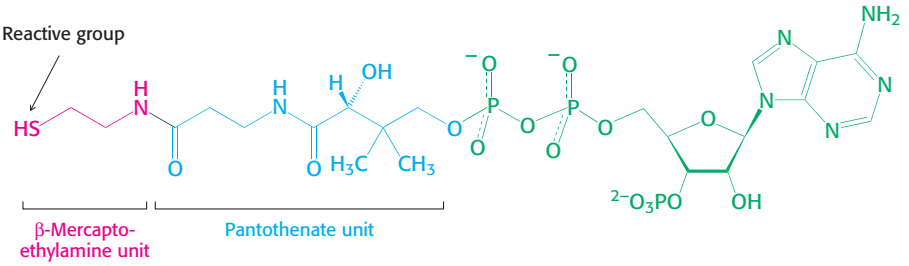
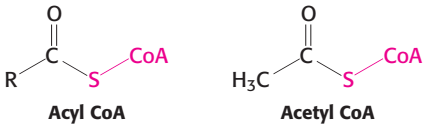
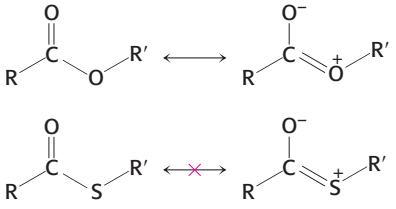


Figure 15.16 Structure of coenzyme A (CoA-SH).





Oxygen esters are stabilized by resonance structures not available to thioesters.

3. *An Activated Carrier of Two-Carbon Fragments*. Coenzyme A, another central molecule in metabolism, is a carrier of acyl groups derived from the vitamin pantothenate (Figure 15.16). Acyl groups are important constituents both in catabolism, as in the oxidation of fatty acids, and in anabolism, as in the synthesis of membrane lipids. The terminal sulfhydryl group in CoA is the reactive site. Acyl groups are linked to CoA by thioester bonds. The resulting derivative is called an *acyl CoA*. An acyl group often linked to CoA is the acetyl unit; this derivative is called *acetyl CoA*. The $\Delta G^{\circ\prime}$ for the hydrolysis of acetyl CoA has a large negative value:

$$\text{Acetyl CoA} + H_2O \rightleftharpoons \text{acetate} + \text{CoA} + H^+$$
$$\Delta G^{\circ\prime} = -31.4\ \text{kJ mol}^{-1}(-7.5\ \text{kcal mol}^{-1})$$

The hydrolysis of a thioester is thermodynamically more favorable than that of an oxygen ester because the electrons of the C═O bond cannot form resonance structures with the C—S bond that are as stable as those that they can form with the C—O bond. Consequently, *acetyl CoA has a high acetyl-group-transfer potential because transfer of the acetyl group is exergonic*. Acetyl CoA carries an activated acetyl group, just as ATP carries an activated phosphoryl group.

The use of activated carriers illustrates two key aspects of metabolism. First, NADH, NADPH, and $FADH_2$ react slowly with O_2 in the absence of a catalyst. Likewise, ATP and acetyl CoA are hydrolyzed slowly (in times of many hours or even days) in the absence of a catalyst. These molecules are kinetically quite stable in the face of a large thermodynamic driving force for reaction with O_2 (in regard to the electron carriers) and H_2O (for ATP and acetyl CoA). *The kinetic stability of these molecules in the absence of specific catalysts is essential for their biological function because it enables enzymes to control the flow of free energy and reducing power*.

Second, *most interchanges of activated groups in metabolism are accomplished by a rather small set of carriers* (Table 15.2). The existence of a recurring set

Table 15.2 Some activated carriers in metabolism

Carrier molecule in activated form	Group carried	Vitamin precursor
ATP	Phosphoryl	
NADH and NADPH	Electrons	Nicotinate (niacin)
$FADH_2$	Electrons	Riboflavin (vitamin B_2)
$FMNH_2$	Electrons	Riboflavin (vitamin B_2)
Coenzyme A	Acyl	Pantothenate
Lipoamide	Acyl	
Thiamine pyrophosphate	Aldehyde	Thiamine (vitamin B_1)
Biotin	CO_2	Biotin
Tetrahydrofolate	One-carbon units	Folate
S-Adenosylmethionine	Methyl	
Uridine diphosphate glucose	Glucose	
Cytidine diphosphate diacylglycerol	Phosphatidate	
Nucleoside triphosphates	Nucleotides	

Note: Many of the activated carriers are coenzymes that are derived from water-soluble vitamins.

Table 15.3 The B vitamins

Vitamin	Coenzyme	Typical reaction type	Consequences of deficiency
Thiamine (B_1)	Thiamine pyrophosphate	Aldehyde transfer	Beriberi (weight loss, heart problems, neurological dysfunction)
Riboflavin (B_2)	Flavin adenine dinucleotide (FAD)	Oxidation–reduction	Cheliosis and angular stomatitis (lesions of the mouth), dermatitis
Pyridoxine (B_6)	Pyridoxal phosphate	Group transfer to or from amino acids	Depression, confusion, convulsions
Nicotinic acid (niacin)	Nicotinamide adenine dinucleotide (NAD^+)	Oxidation–reduction	Pellagra (dermatitis, depression, diarrhea)
Pantothenic acid	Coenzyme A	Acyl-group transfer	Hypertension
Biotin	Biotin–lysine adducts (biocytin)	ATP-dependent carboxylation and carboxyl-group transfer	Rash about the eyebrows, muscle pain, fatigue (rare)
Folic acid	Tetrahydrofolate	Transfer of one-carbon components; thymine synthesis	Anemia, neural-tube defects in development
B_{12}	5′-Deoxyadenosyl cobalamin	Transfer of methyl groups; intramolecular rearrangements	Anemia, pernicious anemia, methylmalonic acidosis

of activated carriers in all organisms is one of the unifying motifs of biochemistry. Furthermore, it illustrates the modular design of metabolism. A small set of molecules carries out a very wide range of tasks. Metabolism is readily comprehended because of the economy and elegance of its underlying design.

Many activated carriers are derived from vitamins

Almost all the activated carriers that act as coenzymes are derived from *vitamins*. Vitamins are organic molecules that are needed in small amounts in the diets of some higher animals. Table 15.3 lists the vitamins that act as coenzymes and Figure 15.17 shows the structures of some. This series of vitamins is known as the vitamin B group. Note that, in all cases, the vitamin must be modified before it can serve its function. We have already touched on the roles of niacin, riboflavin, and pantothenate.We will see these three and the other B vitamins many times in our study of biochemistry.

Vitamins serve the same roles in nearly all forms of life, but higher animals lost the capacity to synthesize them in the course of evolution. For instance, whereas *E. coli* can thrive on glucose and organic salts,

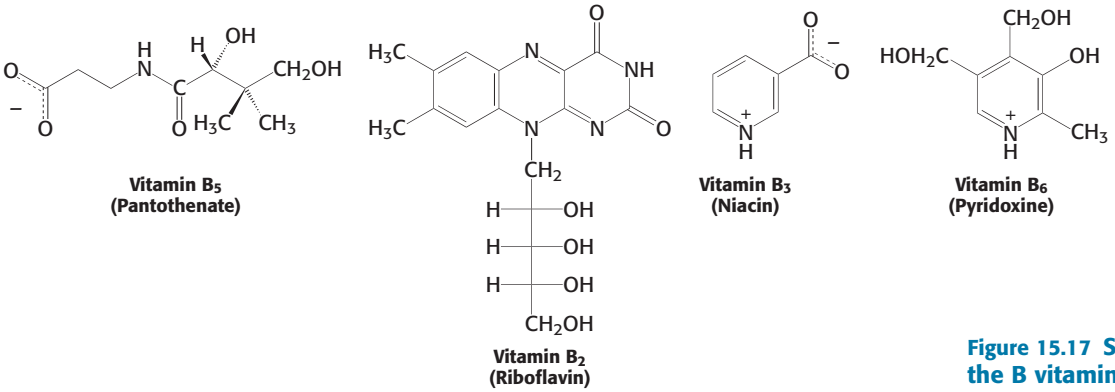


Figure 15.17 Structures of some of the B vitamins.

Table 15.4 Noncoenzyme vitamins

Vitamin	Function	Deficiency
A	Roles in vision, growth, reproduction	Night blindness, cornea damage, damage to respiratory and gastrointestinal tract
C (ascorbic acid)	Antioxidant	Scurvy (swollen and bleeding gums, subdermal hemorrhaging)
D	Regulation of calcium and phosphate metabolism	Rickets (children): skeletal deformities, impaired growth Osteomalacia (adults): soft, bending bones
E	Antioxidant	Inhibition of sperm production; lesions in muscles and nerves (rare)
K	Blood coagulation	Subdermal hemorrhaging

human beings require at least 12 vitamins in their diet. The biosynthetic pathways for vitamins can be complex; thus, it is biologically more efficient to ingest vitamins than to synthesize the enzymes required to construct them from simple molecules. This efficiency comes at the cost of dependence on other organisms for chemicals essential for life. Indeed, vitamin deficiency can generate diseases in all organisms requiring these molecules (see Tables 15.3 and 15.4).

Not all vitamins function as coenzymes. Vitamins designated by the letters A, C, D, E, and K (Figure 15.18 and Table 15.4) have a diverse array of functions. Vitamin A (retinol) is the precursor of retinal, the light-sensitive group in rhodopsin and other visual pigments (Section 32.3), and retinoic acid, an important signaling molecule. A deficiency of this vitamin leads to night blindness. In addition, young animals require vitamin A for growth. Vitamin C, or ascorbate, acts as an antioxidant. A deficiency in vitamin C can lead to scurvy, a disease due to malformed collagen and characterized by skin lesions and blood-vessel fragility (Section 27.6).A metabolite of vitamin D is a hormone that regulates the metabolism of calcium and phosphorus. A deficiency in vitamin D impairs bone formation in growing animals. Infertility in rats is a consequence of vitamin E (α-tocopherol) deficiency. This vitamin reacts with reactive oxygen species such as hydroxyl radicals and inactivates them before they can oxidize unsaturated membrane lipids, damaging cell structures. Vitamin K is required for normal blood clotting (Section 10.4).

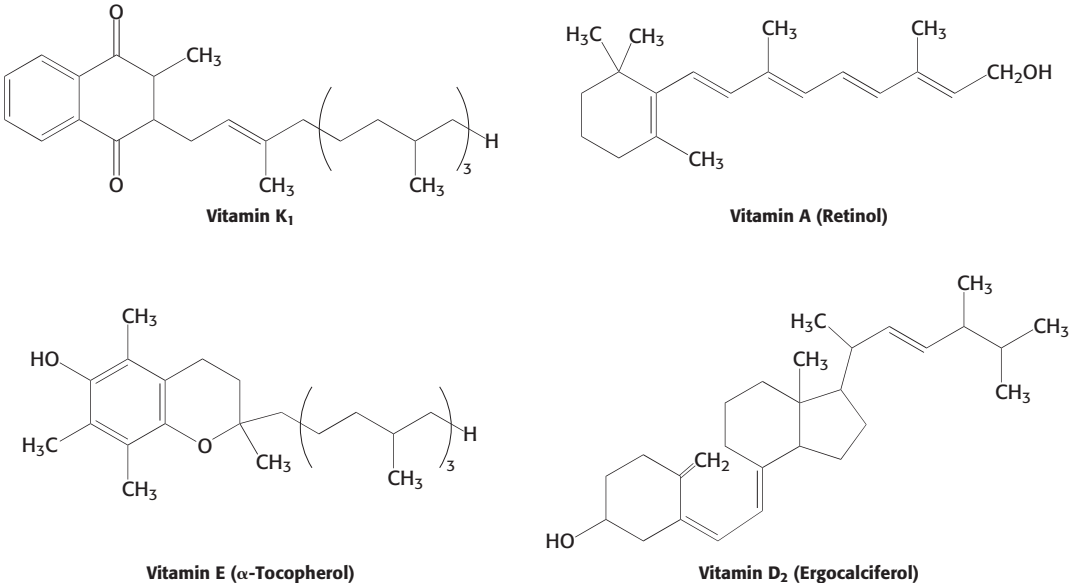


Figure 15.18 Structures of some vitamins that do not function as coenzymes.

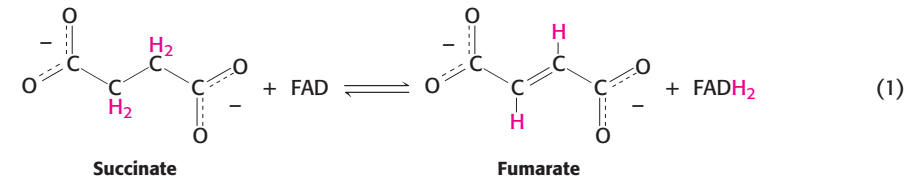
Table 15.5 Types of chemical reactions in metabolism

Type of reaction	Description
Oxidation–reduction	Electron transfer
Ligation requiring ATP cleavage	Formation of covalent bonds (i.e., carbon–carbon bonds)
Isomerization	Rearrangement of atoms to form isomers
Group transfer	Transfer of a functional group from one molecule to another
Hydrolytic	Cleavage of bonds by the addition of water
Addition or removal of functional groups	Addition of functional groups to double bonds or their removal to form double bonds

Key reactions are reiterated throughout metabolism

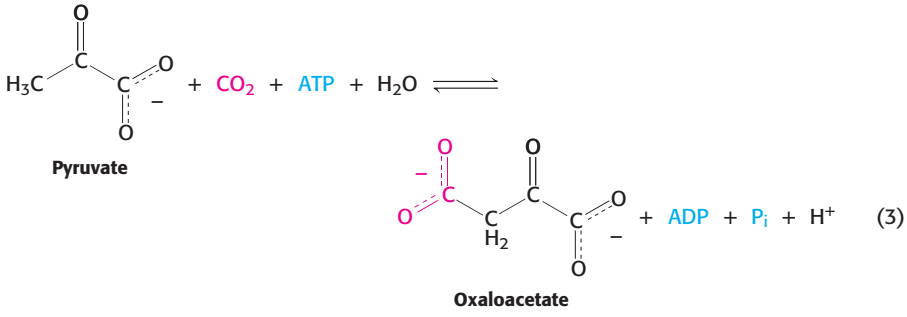
Just as there is an economy of design in the use of activated carriers, so is there an economy of design in biochemical reactions. The thousands of metabolic reactions, bewildering at first in their variety, can be subdivided into just six types (Table 15.5). Specific reactions of each type appear repeatedly, reducing the number of reactions that a student needs to learn.

1. *Oxidation–reduction reactions* are essential components of many pathways. Useful energy is often derived from the oxidation of carbon compounds. Consider the following two reactions:



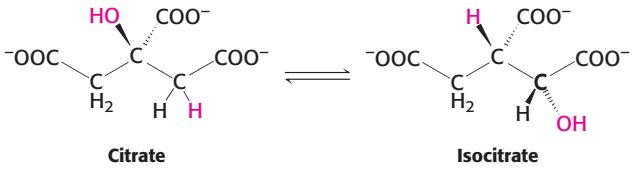
These two oxidation–reduction reactions are components of the citric acid cycle (Chapter 17), which completely oxidizes the activated two-carbon fragment of acetyl CoA to two molecules of CO_2. In reaction 1, $FADH_2$ carries the electrons, whereas, in reaction 2, electrons are carried by NADH.

2. *Ligation reactions* form bonds by using free energy from ATP cleavage. Reaction 3 illustrates the ATP-dependent formation of a carbon–carbon bond, necessary to combine smaller molecules to form larger ones. Oxaloacetate is formed from pyruvate and CO_2.



The oxaloacetate can be used in the citric acid cycle, or converted into glucose or amino acids such as aspartic acid.

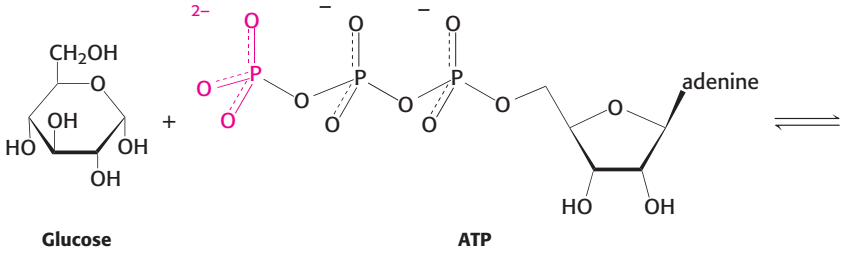
3. *Isomerization reactions* rearrange particular atoms within a molecule. Their role is often to prepare the molecule for subsequent reactions such as the oxidation–reduction reactions described in point 1.



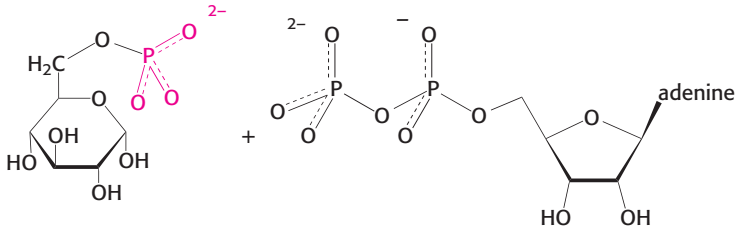
Citrate **Isocitrate** (4)

Reaction 4 is, again, a component of the citric acid cycle. This isomerization prepares the molecule for subsequent oxidation and decarboxylation by moving the hydroxyl group of citrate from a tertiary to a secondary position.

4. *Group-transfer reactions* play a variety of roles. Reaction 5 is representative of such a reaction. A phosphoryl group is transferred from the activated phosphoryl-group carrier, ATP, to glucose, the initial step in glycolysis, a key pathway for extracting energy from glucose (Chapter 16). This reaction traps glucose in the cell so that further catabolism can take place.



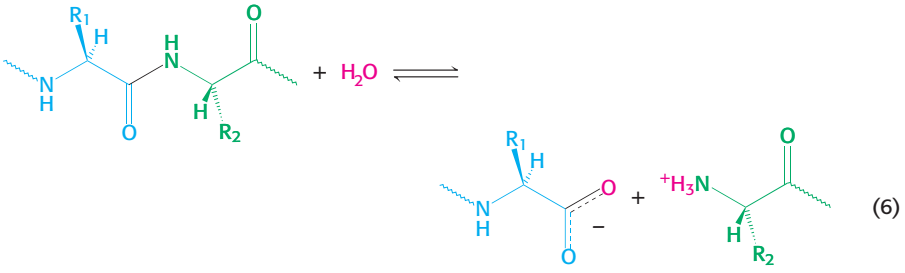
Glucose **ATP**



Glucose 6-phosphate (G-6P) **ADP** (5)

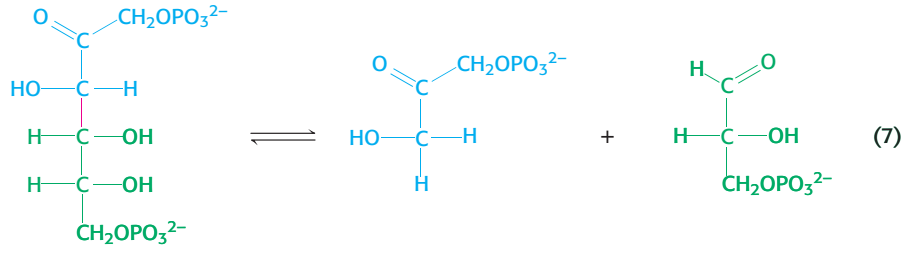
As stated earlier, group-transfer reactions are used to synthesize ATP. We also saw examples of their use in signaling pathways (Chapter 14).

5. *Hydrolytic reactions* cleave bonds by the addition of water. Hydrolysis is a common means employed to break down large molecules, either to facilitate further metabolism or to reuse some of the components for biosynthetic purposes. Proteins are digested by hydrolytic cleavage (Chapters 9 and 10). Reaction 6 illustrates the hydrolysis of a peptide to yield two smaller peptides.



(6)

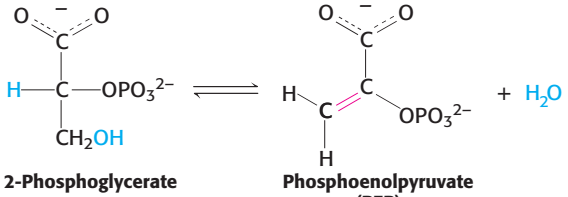
6. *Functional groups may be added to double bonds to form single bonds or removed from single bonds to form double bonds.* The enzymes that catalyze these types of reaction are classified as *lyases.* An important example, illustrated in reaction 7, is the conversion of the six-carbon molecule fructose 1,6-bisphosphate into two three-carbon fragments: dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.



Fructose 1,6-bisphosphate (F-1,6-BP) | **Dihydroxyacetone phosphate (DHAP)** | **Glyceraldehyde 3-phosphate (GAP)**

(7)

This reaction is a critical step in glycolysis (Chapter 16). Dehydrations to form double bonds, such as the formation of phosphoenolpyruvate (see Table 15.1) from 2-phosphoglycerate (reaction 8), are important reactions of this type.



2-Phosphoglycerate | **Phosphoenolpyruvate (PEP)**

(8)

The dehydration sets up the next step in the pathway, a group-transfer reaction that uses the high phosphoryl-transfer potential of the product PEP to form ATP from ADP.

These six fundamental reaction types are the basis of metabolism. Remember that all six types can proceed in either direction, depending on the standard free energy for the specific reaction and the concentrations of the reactants and products inside the cell. An effective way to learn is to look for commonalities in the diverse metabolic pathways that we will be examining. There is a chemical logic that, when exposed, renders the complexity of the chemistry of living systems more manageable and reveals its elegance.

Metabolic processes are regulated in three principal ways

It is evident that the complex network of metabolic reactions must be rigorously regulated. At the same time, metabolic control must be flexible, to

adjust metabolic activity to the constantly changing external environments of cells. Metabolism is regulated through control of (1) *the amounts of enzymes,* (2) *their catalytic activities,* and (3) *the accessibility of substrates*.

Controlling the amounts of enzymes. The amount of a particular enzyme depends on both its rate of synthesis and its rate of degradation. The level of many enzymes is adjusted primarily by a change in the *rate of transcription* of the genes encoding them (Chapters 29 and 31). In *E. coli,* for example, the presence of lactose induces within minutes a more than 50-fold increase in the rate of synthesis of β-galactosidase, an enzyme required for the breakdown of this disaccharide.

Controlling catalytic activity. The catalytic activity of enzymes is controlled in several ways. *Reversible allosteric control* is especially important. For example, the first reaction in many biosynthetic pathways is allosterically inhibited by the ultimate product of the pathway. The inhibition of aspartate transcarbamoylase by cytidine triphosphate (Section 10.1) is a well-understood example of *feedback inhibition*. This type of control can be almost instantaneous. Another recurring mechanism is *reversible covalent modification*. For example, glycogen phosphorylase, the enzyme catalyzing the breakdown of glycogen, a storage form of sugar, is activated by the phosphorylation of a particular serine residue when glucose is scarce (Section 21.1).

Hormones coordinate metabolic relations between different tissues, often by regulating the reversible modification of key enzymes. For instance, the hormone epinephrine triggers a signal-transduction cascade in muscle, resulting in the phosphorylation and activation of key enzymes and leading to the rapid degradation of glycogen to glucose, which is then used to supply ATP for muscle contraction. As described in Chapter 14, many hormones act through intracellular messengers, such as cyclic AMP and calcium ion, that coordinate the activities of many target proteins.

Many reactions in metabolism are controlled by the *energy status* of the cell. One index of the energy status is the *energy charge,* which is proportional to the mole fraction of ATP plus half the mole fraction of ADP, given that ATP contains two anhydride bonds, whereas ADP contains one. Hence, the energy charge is defined as

$$\text{Energy charge} = \frac{[\text{ATP}] + \frac{1}{2}[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$$

The energy charge can have a value ranging from 0 (all AMP) to 1 (all ATP). Daniel Atkinson showed that *ATP-generating (catabolic) pathways are inhibited by a high energy charge, whereas ATP-utilizing (anabolic) pathways are stimulated by a high energy charge*. In plots of the reaction rates of such pathways versus the energy charge, the curves are steep near an energy charge of 0.9, where they usually intersect (Figure 15.19). It is evident that

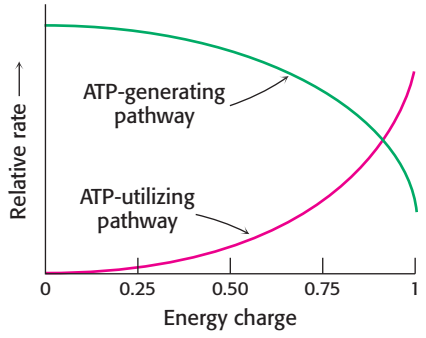


Figure 15.19 Energy charge regulates metabolism. High concentrations of ATP inhibit the relative rates of a typical ATP-generating (catabolic) pathway and stimulate the typical ATP-utilizing (anabolic) pathway.

the control of these pathways has evolved to maintain the energy charge within rather narrow limits. In other words, *the energy charge, like the pH of a cell, is buffered.* The energy charge of most cells ranges from 0.80 to 0.95. An alternative index of the energy status is the *phosphorylation potential*, which is defined as

$$\text{Phosphorylation potential} = \frac{[\text{ATP}]}{[\text{ADP}] + [\text{P}_\text{i}]}$$

The phosphorylation potential, in contrast with the energy charge, depends on the concentration of P_i and is directly related to the free-energy storage available from ATP.

Controlling the accessibility of substrates. In eukaryotes, metabolic regulation and flexibility are enhanced by compartmentalization. For example, fatty acid oxidation takes place in mitochondria, whereas fatty acid synthesis takes place in the cytoplasm. *Compartmentalization segregates opposed reactions.*

Controlling the *flux of substrates* is another means of regulating metabolism. Glucose breakdown can take place in many cells only if insulin is present to promote the entry of glucose into the cell. The transfer of substrates from one compartment of a cell to another (e.g., from the cytoplasm to mitochondria) can serve as a control point.

Aspects of metabolism may have evolved from an RNA world

How did the complex pathways that constitute metabolism evolve? The current thinking is that RNA was an early biomolecule and that, in an early RNA world, RNA served as catalysts and information-storage molecules.

Why do activated carriers such as ATP, NADH, $FADH_2$, and coenzyme A contain adenosine diphosphate units (Figure 15.20)? A possible explanation is that these molecules evolved from the early RNA catalysts. Non-RNA units such as the isoalloxazine ring may have been recruited to serve as efficient carriers of activated electrons and chemical units, a function not readily performed by RNA itself. We can picture the adenine ring of $FADH_2$ binding to a uracil unit in a niche of an RNA enzyme (ribozyme) by base-pairing, whereas the isoalloxazine ring protrudes and functions as an electron carrier. When the more versatile proteins replaced RNA as the major catalysts, the ribonucleotide coenzymes stayed essentially unchanged because they were already well suited to their metabolic roles. The nicotinamide unit of NADH, for example, can readily transfer electrons irrespective of whether the adenine unit interacts with a base in an RNA enzyme or with amino acid residues in a protein enzyme. With the advent of protein enzymes, these important cofactors evolved as free molecules without losing the adenosine diphosphate vestige of their RNA-world ancestry. That molecules and motifs of metabolism are common to all forms of life testifies to their common origin and to the retention of functioning modules through billions of years of evolution. Our understanding of metabolism, like that of other biological processes, is enriched by inquiry into how these beautifully integrated patterns of reactions came into being.

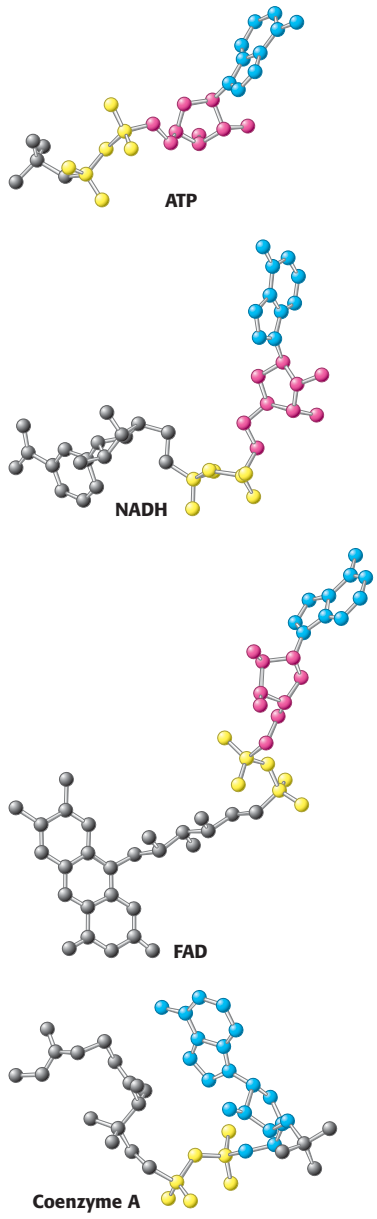


Figure 15.20 Adenosine diphosphate (ADP) is an ancient module in metabolism. This fundamental building block is present in key molecules such as ATP, NADH, FAD, and coenzyme A. The adenine unit is shown in blue, the ribose unit in red, and the diphosphate unit in yellow.

Summary

All cells transform energy. They extract energy from their environment and use this energy to convert simple molecules into cellular components.

15.1 Metabolism Is Composed of Many Coupled, Interconnecting Reactions

The process of energy transduction takes place through metabolism, a highly integrated network of chemical reactions. Metabolism can be subdivided into catabolism (reactions employed to extract energy from fuels) and anabolism (reactions that use this energy for biosynthesis). The most valuable thermodynamic concept for understanding bioenergetics is free energy. A reaction can occur spontaneously only if the change in free energy (ΔG) is negative. A thermodynamically unfavorable reaction can be driven by a thermodynamically favorable one, which is the hydrolysis of ATP in many cases.

15.2 ATP Is the Universal Currency of Free Energy in Biological Systems

The energy derived from catabolism is transformed into adenosine triphosphate. ATP hydrolysis is exergonic and the energy released can be used to power cellular processes, including motion, active transport, and biosynthesis. Under cellular conditions, the hydrolysis of ATP shifts the equilibrium of a coupled reaction by a factor of 10^8. ATP, the universal currency of energy in biological systems, is an energy-rich molecule because it contains two phosphoanhydride bonds.

15.3 The Oxidation of Carbon Fuels Is an Important Source of Cellular Energy

ATP formation is coupled to the oxidation of carbon fuels, either directly or through the formation of ion gradients. Photosynthetic organisms can use light to generate such gradients. ATP is consumed in muscle contraction and other motions of cells, in active transport, in signal-transduction processes, and in biosyntheses. The extraction of energy from foodstuffs by aerobic organisms comprises three stages. In the first stage, large molecules are broken down into smaller ones, such as amino acids, sugars, and fatty acids. In the second stage, these small molecules are degraded to a few simple units that have pervasive roles in metabolism. One of them is the acetyl unit of acetyl CoA, a carrier of activated acyl groups. The third stage of metabolism is the citric acid cycle and oxidative phosphorylation, in which ATP is generated as electrons flow to O_2, the ultimate electron acceptor, and fuels are completely oxidized to CO_2.

15.4 Metabolic Pathways Contain Many Recurring Motifs

Metabolism is characterized by common motifs. A small number of recurring activated carriers, such as ATP, NADH, and acetyl CoA, transfer activated groups in many metabolic pathways. NADPH, which carries two electrons at a high potential, provides reducing power in the biosynthesis of cell components from more-oxidized precursors. Many activated carriers are derived from vitamins, small organic molecules required in the diets of many higher organisms. Moreover, key reaction types are used repeatedly in metabolic pathways.

Metabolism is regulated in a variety of ways. The amounts of some critical enzymes are controlled by regulation of the rate of synthesis and degradation. In addition, the catalytic activities of many enzymes are regulated by allosteric interactions (as in feedback inhibition) and by covalent modification. The movement of many substrates into cells and subcellular compartments also is controlled. The energy charge, which depends on the relative amounts of ATP, ADP, and AMP, plays a role in metabolic regulation. A high energy charge inhibits ATP-generating (catabolic) pathways, whereas it stimulates ATP-utilizing (anabolic) pathways.

Key Terms

metabolism or intermediary metabolism (p. 427)
phototroph (p. 428)
chemotroph (p. 428)
catabolism (p. 428)
anabolism (p. 428)
amphibolic pathway (p. 429)
adenosine triphosphate (ATP) (p. 430)
phosphoryl-transfer potential (p. 433)
oxidative phosphorylation (p. 437)
activated carrier (p. 438)
vitamin (p. 441)
oxidation–reduction reaction (p. 443)
ligation reaction (p. 443)
isomerization reaction (p. 444)
group-transfer reaction (p. 444)
hydrolytic reaction (p. 444)
addition to or formation of double-bond reaction (p. 445)
lyase (p. 445)
energy charge (p. 446)
phosphorylation potential (p. 447)

Problems

1. *Complex patterns.* What is meant by *intermediary metabolism?*

2. *Opposites.* Differentiate between anabolism and catabolism.

3. *Why bother to eat?* What are the three primary uses for cellular energy?

4. *Match 'em.*

1. Cellular energy currency	a. NAD^+
2. Anabolic electron carrier	b. Coenzyme A
3. Phototroph	c. Precursor to coenzymes
4. Catabolic electron carrier reaction	d. Yields energy
5. Oxidation-reduction reaction	e. Requires energy
6. Activated carrier of two carbon fragments	f. ATP
7. Vitamin	g. Transfers electrons
8. Anabolism	h. $NADP^+$
9. Amphibolic reaction	i. Converts light energy to chemical energy
10. Catabolism	j. Used in anabolism and catabolism

5. *Energy to burn.* What factors account for the high-phosphoryl transfer potential of nucleoside triphosphates?

6. *Back in time.* Account for the fact that ATP, and not another nucleoside triphosphate, is the cellular energy currency.

7. *Currency Issues.* Why does it make good sense to have a single nucleotide, ATP, function as the cellular energy currency?

8. *Environmental conditions.* The standard free energy of hydrolysis for ATP is -30.5 kJ mol^{-1} (-7.3 kcal mol^{-1}).

$$ATP + H_2O \rightleftharpoons ADP + P_i$$

What conditions might be changed to alter the free energy of hydrolysis?

9. *Brute force?* Metabolic pathways frequently contain reactions with positive standard free energy values, yet the reactions still take place. How is this possible?

10. *Energy flow.* What is the direction of each of the following reactions when the reactants are initially present in equimolar amounts? Use the data given in Table 15.1.

(a) ATP + creatine $\rightleftharpoons$ creatine phosphate + ADP
(b) ATP + glycerol $\rightleftharpoons$ glycerol 3-phosphate + ADP
(c) ATP + pyruvate $\rightleftharpoons$ phosphoenolpyruvate + ADP
(d) ATP + glucose $\rightleftharpoons$ glucose 6-phosphate + ADP

11. *A proper inference.* What information do the $\Delta G^{\circ\prime}$ data given in Table 15.1 provide about the relative rates of hydrolysis of pyrophosphate and acetyl phosphate?

12. *A potent donor.* Consider the following reaction:

ATP + pyruvate $\rightleftharpoons$ phosphoenolpyruvate + ADP

(a) Calculate $\Delta G^{\circ\prime}$ and K'_{eq} at 25°C for this reaction by using the data given in Table 15.1.

(b) What is the equilibrium ratio of pyruvate to phosphoenolpyruvate if the ratio of ATP to ADP is 10?

13. *Isomeric equilibrium.* Calculate $\Delta G^{\circ\prime}$ for the isomerization of glucose 6-phosphate to glucose 1-phosphate. What is the equilibrium ratio of glucose 6-phosphate to glucose 1-phosphate at 25°C?

14. *Activated acetate.* The formation of acetyl CoA from acetate is an ATP-driven reaction:

Acetate + ATP + CoA $\rightleftharpoons$ acetyl CoA + AMP + PP_i

(a) Calculate $\Delta G^{\circ\prime}$ for this reaction by using data given in this chapter.

(b) The PP_i formed in the preceding reaction is rapidly hydrolyzed in vivo because of the ubiquity of inorganic pyrophosphatase. The $\Delta G^{\circ\prime}$ for the hydrolysis of PP_i is -19.2 kJ mol^{-1} (-4.6 kcal mol^{-1}). Calculate the $\Delta G^{\circ\prime}$ for the overall reaction, including pyrophosphate hydrolysis.

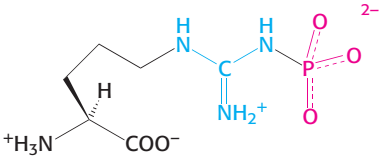
What effect does the hydrolysis of PP_i have on the formation of acetyl CoA?

15. *Acid strength*. The pK of an acid is a measure of its proton-group-transfer potential.

(a) Derive a relation between $\Delta G^{\circ\prime}$ and pK.

(b) What is the $\Delta G^{\circ\prime}$ for the ionization of acetic acid, which has a pK of 4.8?

16. *Raison d'être*. The muscles of some invertebrates are rich in arginine phosphate (phosphoarginine). Propose a function for this amino acid derivative.



Arginine phosphate

17. *Recurring motif*. What is the structural feature common to ATP, FAD, NAD^+, and CoA?

18. *Ergogenic help or hindrance?* Creatine is a popular, but untested, dietary supplement.

(a) What is the biochemical rationale for the use of creatine?

(b) What type of exercise would most benefit from creatine supplementation?

19. *Standard conditions versus real life 1*. The enzyme aldolase catalyzes the following reaction in the glycolytic pathway:

$$\text{Fructose 1, 6-bisphosphate} \overset{\text{Aldolase}}{\rightleftharpoons} \text{dihydroxyacetone phosphate} + \text{glyceraldehyde 3-phosphate}$$

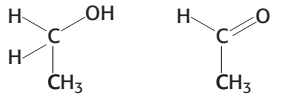
The $\Delta G^{\circ\prime}$ for the reaction is + 23.8 kJ mol^{-1} (+ 5.7 kcal mol^{-1}), whereas the ΔG in the cell is −1.3 kJ mol^{-1} (−0.3 kcal mol^{-1}). Calculate the ratio of reactants to products under equilibrium and intracellular conditions. Using your results, explain how the reaction can be endergonic under standard conditions and exergonic under intracellular conditions.

20. *Standard conditions versus real life 2*. On page 430, we showed that a reaction, A ⇌ B, with a $\Delta G' = +13$ kJ mol^{-1} (+ 4.0 kcal mol^{-1}) has an K_{eq} of 1.15×10^{-3}. The K_{eq} is increased to 2.67×10^{2} if the reaction is coupled to ATP hydrolysis under standard conditions. The ATP-generating system of cells maintains the [ATP]/[ADP][P_i] ratio at a high level, typically of the order of 500 M^{-1}. Calculate the ratio of B/A under cellular conditions.

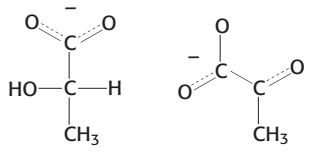
21. *Not all alike*. The concentrations of ATP, ADP, and P_i differ with cell type. Consequently, the release of free energy with the hydrolysis of ATP will vary with cell type. Using the following table, calculate the ΔG for the hydrolysis of ATP in liver, muscle, and brain cells. In which cell type is the free energy of ATP hydrolysis most negative?

	ATP (mM)	ADP (mM)	P_i (mM)
Liver	3.5	1.8	5.0
Muscle	8.0	0.9	8.0
Brain	2.6	0.7	2.7

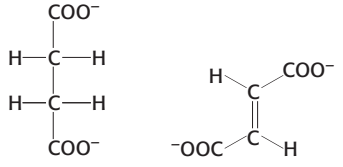
22. *Oxidation issues*. Examine the pairs of molecules and identify the more-reduced molecule in each pair.

(a) 

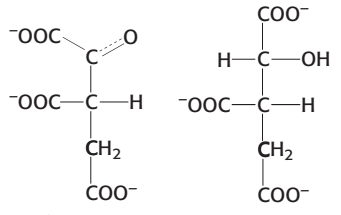
Ethanol **Acetaldehyde**

(b) 

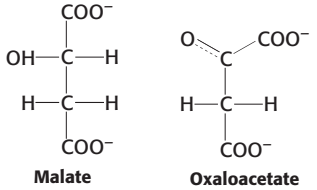
Lactate **Pyruvate**

(c) 

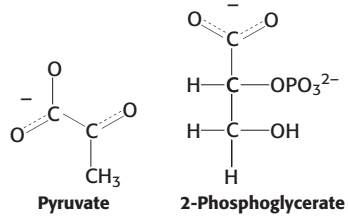
Succinate **Fumarate**

(d) 

Oxalosuccinate **Isocitrate**

(e) 

Malate **Oxaloacetate**

(f) 

Pyruvate **2-Phosphoglycerate**

23. *Running downhill*. Glycolysis is a series of 10 linked reactions that convert one molecule of glucose into two molecules of pyruvate with the concomitant synthesis of two molecules of ATP (Chapter 16). The $\Delta G^{\circ\prime}$ for this set of reactions is -35.6 kJ mol^{-1} (-8.5 kcal mol^{-1}), whereas the ΔG is -76.6 kJ mol^{-1} (-18.3 kcal mol^{-1}). Explain why the free-energy release is so much greater under intracellular conditions than under standard conditions.

24. *Breakdown products*. Digestion is the first stage in the extraction of energy from food, but no useful energy is acquired during this stage. Why is digestion considered a stage in energy extraction?

25. *High-energy electrons*. What are the activated electron carriers for catabolism? For anabolism?

26. *Less reverberation*. Thioesters, common in biochemistry, are more unstable (energy-rich) than oxygen esters. Explain why this is the case.

27. *Classifying reactions*. What are the six common types of reactions seen in biochemistry?

28. *Staying in control*. What are the three principal means of controlling metabolic reactions?

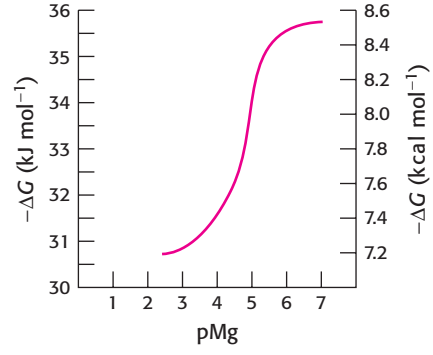
Chapter Integration Problems

29. *Kinetic vs. thermodynamic*. The reaction of NADH with oxygen to produce NAD^+ and H_2O is very exergonic, yet the reaction of NADH and oxygen takes place very slowly. Why does a thermodynamically favorable reaction not occur rapidly?

30. *Activated sulfate*. Fibrinogen contains tyrosine-*O*-sulfate. Propose an activated form of sulfate that could react in vivo with the aromatic hydroxyl group of a tyrosine residue in a protein to form tyrosine-*O*-sulfate.

Data Interpretation Problem

31. *Opposites attract*. The following graph shows how the ΔG for the hydrolysis of ATP varies as a function of the Mg^{2+} concentration (pMg = $-\log[Mg^{2+}]$).



(a) How does decreasing $[Mg^{2+}]$ affect the ΔG of hydrolysis for ATP?

(b) Explain this effect.

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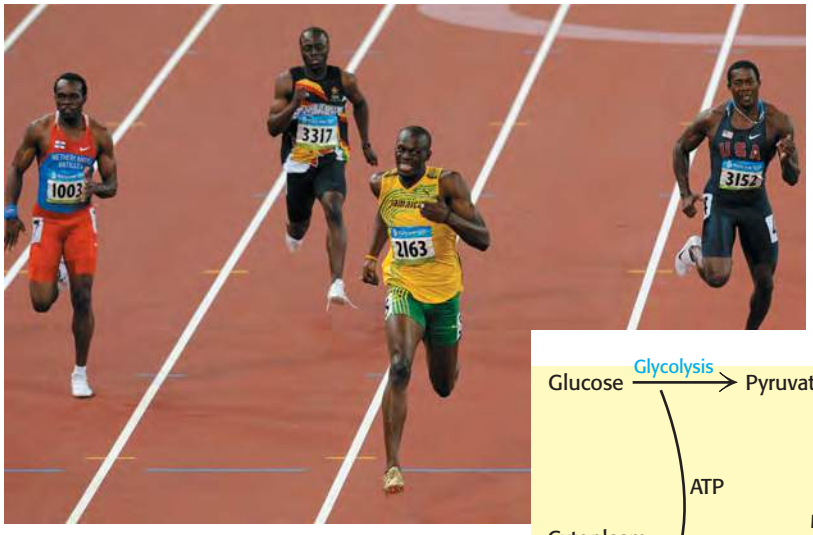
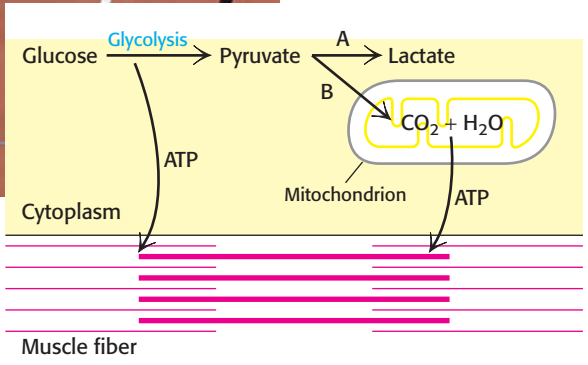
Glycolysis and Gluconeogenesis



Usain Bolt sprints through a world record in the 200-meter finals at the Olympics in Beijing in 2008. Glucose metabolism can generate the ATP to power muscle contraction. During a sprint, when the ATP needs outpace oxygen delivery, as would be the case for Bolt, glucose is metabolized to lactate. When oxygen delivery is adequate, glucose is metabolized more efficiently to carbon dioxide and water. [Reix-Liews/For Photo/Corbis.]



The first metabolic pathway that we encounter is *glycolysis,* an ancient pathway employed by a host of organisms. *Glycolysis is the sequence of reactions that metabolizes one molecule of glucose to two molecules of pyruvate with the concomitant net production of two molecules of ATP.* This process is anaerobic (i.e., it does not require O_2) because it evolved before substantial amounts of oxygen accumulated in the atmosphere. Pyruvate can be further processed anaerobically to lactate (*lactic acid fermentation*) or ethanol (*alcoholic fermentation*). Under aerobic conditions, pyruvate can be completely oxidized to CO_2, generating much more ATP, as will be described in Chapters 17 and 18. Figure 16.1 shows some possible fates of pyruvate produced by glycolysis.

Because glucose is such a precious fuel, metabolic products, such as pyruvate and lactate, are salvaged to synthesize glucose in the process of *gluconeogenesis.* Although glycolysis and gluconeogenesis have some enzymes in common, the two pathways are not simply the reverse of each other. In particular, the highly exergonic, irreversible steps of glycolysis are bypassed in gluconeogenesis. The two pathways are reciprocally regulated so that glycolysis and gluconeogenesis do not take place simultaneously in the same cell to a significant extent.

Our understanding of glucose metabolism, especially glycolysis, has a rich history. Indeed, the development of biochemistry and the delineation of

OUTLINE

Glycolysis

Derived from the Greek stem *glyk-,* "sweet," and the word *lysis,* "dissolution."

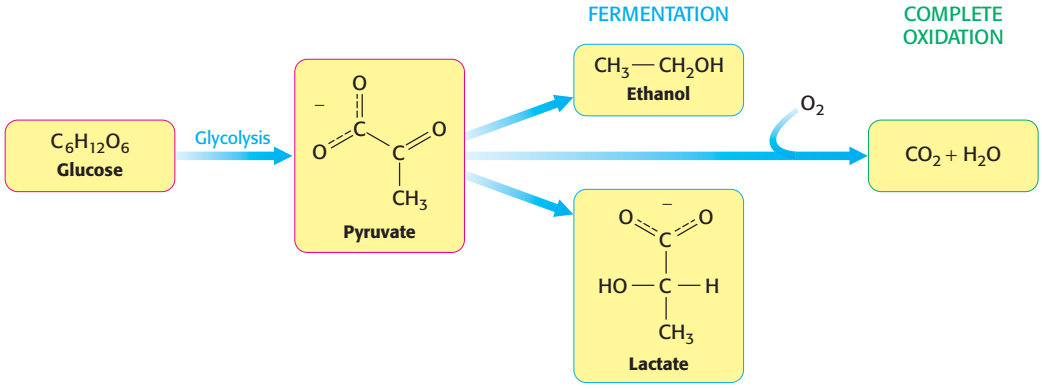


Figure 16.1 Some fates of glucose.

glycolysis went hand in hand. A key discovery was made by Hans Buchner and Eduard Buchner in 1897, quite by accident. The Buchners were interested in manufacturing cell-free extracts of yeast for possible therapeutic use. These extracts had to be preserved without the use of antiseptics such as phenol, and so they decided to try sucrose, a commonly used preservative in kitchen chemistry. They obtained a startling result: sucrose was rapidly fermented into alcohol by the yeast juice. The significance of this finding was immense. *The Buchners demonstrated for the first time that fermentation could take place outside living cells.* The accepted view of their day, asserted by Louis Pasteur in 1860, was that fermentation is inextricably tied to living cells. The chance discovery by the Buchners refuted this dogma and opened the door to modern biochemistry. The Buchners' discovery inspired the search for the biochemicals that catalyze the conversion of sucrose into alcohol. *The study of metabolism became the study of chemistry.*

Enzyme
A term coined by Friedrich Wilhelm Kühne in 1878 to designate catalytically active substances that had formerly been called ferments. Derived from the Greek words *en,* "in," and *zyme,* "leaven."

Studies of muscle extracts then showed that many of the reactions of lactic acid fermentation were the same as those of alcoholic fermentation. *This exciting discovery revealed an underlying unity in biochemistry.* The complete glycolytic pathway was elucidated by 1940, largely through the pioneering contributions of Gustav Embden, Otto Meyerhof, Carl Neuberg, Jacob Parnas, Otto Warburg, Gerty Cori, and Carl Cori. Glycolysis is also known as the *Embden–Meyerhof pathway.*

Glucose is generated from dietary carbohydrates

We typically consume in our diets a generous amount of starch and a smaller amount of glycogen. These complex carbohydrates must be converted into simpler carbohydrates for absorption by the intestine and transport in the blood. Starch and glycogen are digested primarily by the pancreatic enzyme *α-amylase* and to a lesser extent by salivary α-amylase. Amylase cleaves the α-1,4 bonds of starch and glycogen, but not the α-1,6 bonds. The products are the di- and trisaccharides maltose and maltotriose. The material not digestible because of the α-1,6 bonds is called the *limit dextrin.*

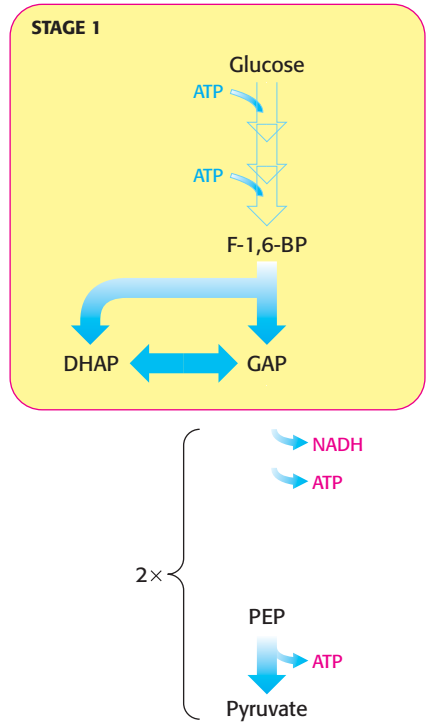
Maltase cleaves maltose into two glucose molecules, whereas *α-glucosidase* digests maltotriose and any other oligosaccharides that may have escaped digestion by the amylase. *α-Dextrinase* further digests the limit dextrin. Maltase and α-glucosidase are located on the surface of the intestinal cells, as is *sucrase,* an enzyme that degrades the sucrose contributed by vegetables to fructose and glucose. The enzyme *lactase* is responsible for degrading the milk sugar lactose into glucose and galactose. The monosaccharides are transported into the cells lining the intestine and then into the bloodstream.

Glucose is an important fuel for most organisms

Glucose is a common and important fuel. In mammals, glucose is the only fuel that the brain uses under nonstarvation conditions and the only fuel that red blood cells can use at all. Indeed, almost all organisms use glucose, and most that do process it in a similar fashion. Recall from Chapter 11 that there are many carbohydrates. Why is glucose instead of some other monosaccharide such a prominent fuel? We can speculate on the reasons. First, glucose is one of several monosaccharides formed from formaldehyde under prebiotic conditions, and so it may have been available as a fuel source for primitive biochemical systems. Second, glucose has a low tendency, relative to other monosaccharides, to nonenzymatically glycosylate proteins. In their open-chain forms, monosaccharides contain carbonyl groups that can react with the amino groups of proteins to form Schiff bases, which rearrange to form a more stable amino–ketone linkage. Such nonspecifically modified proteins often do not function effectively. Glucose has a strong tendency to exist in the ring conformation and, consequently, relatively little tendency to modify proteins. Recall that all the hydroxyl groups in the ring conformation of β-glucose are equatorial, contributing to the sugar's high relative stability (Section 11.1).

16.1 Glycolysis Is an Energy-Conversion Pathway in Many Organisms

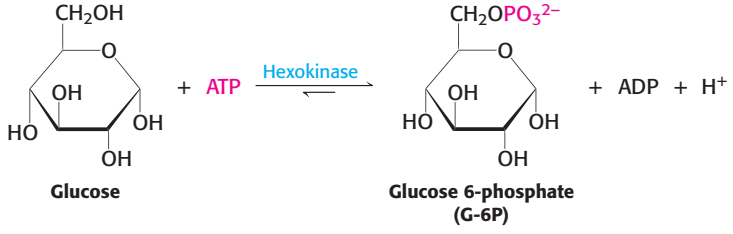
We now begin our consideration of the glycolytic pathway. This pathway is common to virtually all cells, both prokaryotic and eukaryotic. In eukaryotic cells, glycolysis takes place in the cytoplasm. This pathway can be thought of as comprising two stages (Figure 16.2). Stage 1 is the trapping and preparation phase. No ATP is generated in this stage. Stage 1 begins with the conversion of glucose into fructose 1,6-bisphosphate, which consists of three steps: a phosphorylation, an isomerization, and a second phosphorylation reaction. *The strategy of these initial steps in glycolysis is to trap the glucose in the cell and form a compound that can be readily cleaved into phosphorylated three-carbon units.* Stage 1 is completed with the cleavage of the fructose 1,6-bisphosphate into two three-carbon fragments. These resulting three-carbon units are readily interconvertible. In stage 2, ATP is harvested when the three-carbon fragments are oxidized to pyruvate.



First stage of glycolysis. The first stage of glycolysis begins with the phosphorylation of glucose by hexokinase and ends with the isomerization of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate.

Hexokinase traps glucose in the cell and begins glycolysis

Glucose enters cells through specific transport proteins (p. 477) and has one principal fate: *it is phosphorylated by ATP to form glucose 6-phosphate.* This step is notable for two reasons: (1) glucose 6-phosphate cannot pass through the membrane because it is not a substrate for the glucose transporters, and (2) the addition of the phosphoryl group acts to destabilize glucose, thus facilitating its further metabolism. The transfer of the phosphoryl group from ATP to the hydroxyl group on carbon 6 of glucose is catalyzed by *hexokinase.*



Glucose **Glucose 6-phosphate (G-6P)**

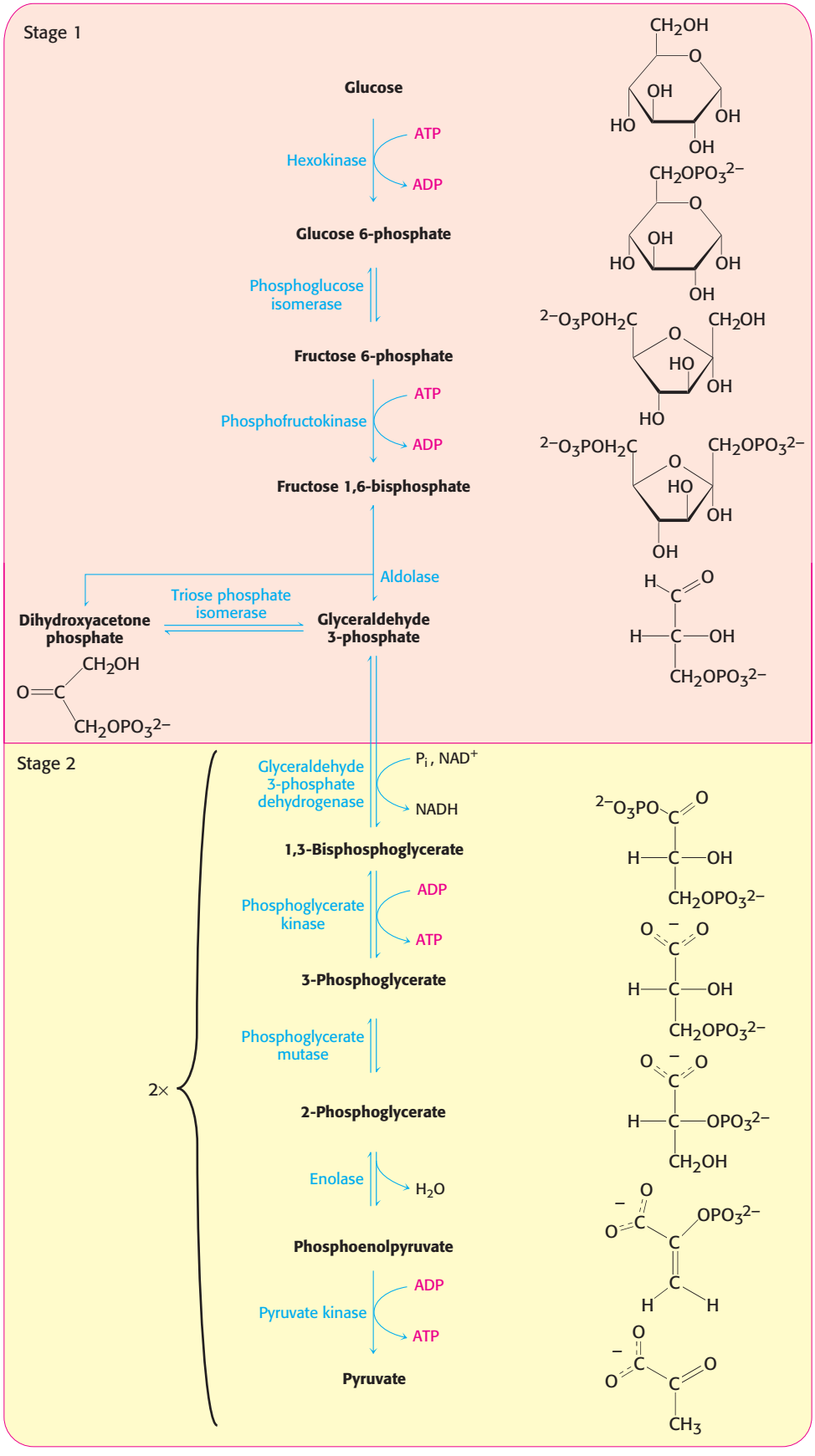


Figure 16.2 Stages of glycolysis. The glycolytic pathway can be divided into two stages: (1) glucose is trapped, destabilized, and cleaved into two interconvertible three-carbon molecules generated by cleavage of six-carbon fructose; and (2) ATP is generated.

Phosphoryl transfer is a fundamental reaction in biochemistry. *Kinases* are enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor. Hexokinase, then, catalyzes the transfer of a phosphoryl group from ATP to a variety of six-carbon sugars (*hexoses*), such as glucose and mannose. *Hexokinase, like adenylate kinase* (Section 9.4) *and all other kinases, requires* Mg^{2+} *(or another divalent metal ion such as* Mn^{2+}*) for activity.* The divalent metal ion forms a complex with ATP.

X-ray crystallographic studies of yeast hexokinase revealed that the binding of glucose induces a large conformational change in the enzyme. Hexokinase consists of two lobes, which move toward each other when glucose is bound (Figure 16.3). On glucose binding, one lobe rotates 12 degrees with respect to the other, resulting in movements of the polypeptide backbone of as much as 8 Å. The cleft between the lobes closes, and the bound glucose becomes surrounded by protein, except for the hydroxyl group of carbon 6, which will accept the phosphoryl group from ATP. The closing of the cleft in hexokinase is a striking example of the role *of induced fit* in enzyme action (Section 8.3).

The glucose-induced structural changes are significant in two respects. First, the environment around the glucose becomes more nonpolar, which favors reaction between the hydrophilic hydroxyl group of glucose and the terminal phosphoryl group of ATP. Second, the conformational changes enable the kinase to discriminate against H_2O as a substrate. The closing of the cleft keeps water molecules away from the active site. If hexokinase were rigid, a molecule of H_2O occupying the binding site for the $—CH_2OH$ of glucose could attack the γ phosphoryl group of ATP, forming ADP and P_i. In other words, a rigid kinase would likely also be an ATPase. It is interesting to note that other kinases taking part in glycolysis—phosphofructokinase, phosphoglycerate kinase, and pyruvate kinase—also contain clefts between lobes that close when substrate is bound, although the structures of these enzymes are different in other regards. *Substrate-induced cleft closing is a general feature of kinases.*

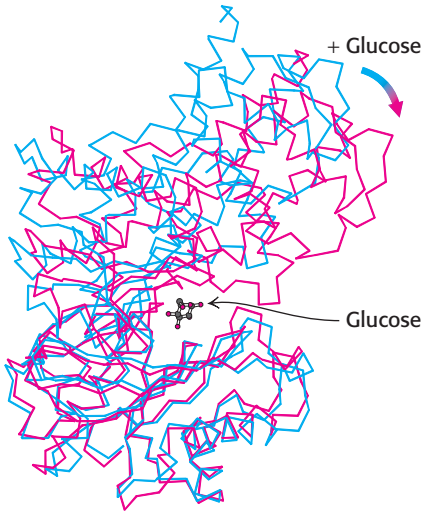
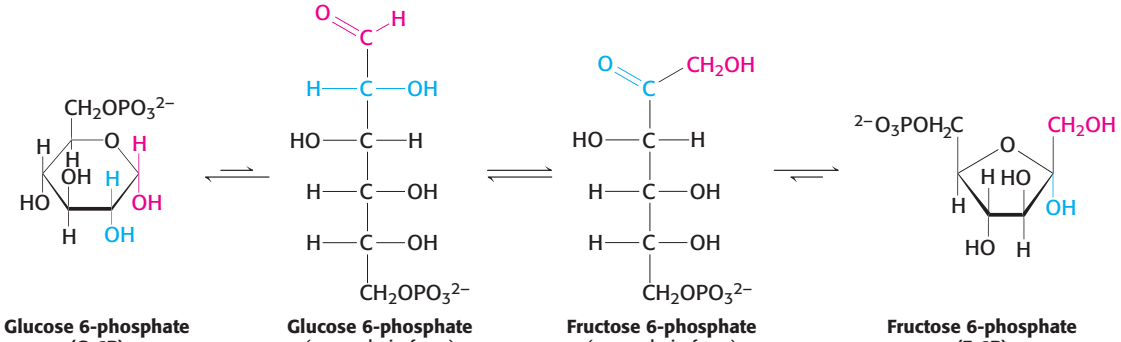


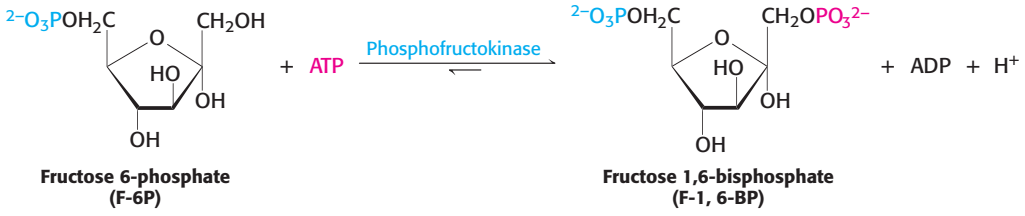
Figure 16.3 Induced fit in hexokinase. As shown in blue, the two lobes of hexokinase are separated in the absence of glucose. The conformation of hexokinase changes markedly on binding glucose, as shown in red. *Notice* that two lobes of the enzyme come together and surround the substrate, creating the necessary environment for catalysis. [Courtesy of Dr. Thomas Steitz.]

Fructose 1,6-bisphosphate is generated from glucose 6-phosphate

The next step in glycolysis is the *isomerization of glucose 6-phosphate to fructose 6-phosphate.* Recall that the open-chain form of glucose has an aldehyde group at carbon 1, whereas the open-chain form of fructose has a keto group at carbon 2. Thus, the isomerization of glucose 6-phosphate to fructose 6-phosphate is a *conversion of an aldose into a ketose.* The reaction catalyzed by *phosphoglucose isomerase* takes several steps because both glucose 6-phosphate and fructose 6-phosphate are present primarily in the cyclic forms. The enzyme must first open the six-membered ring of glucose 6-phosphate, catalyze the isomerization, and then promote the formation of the five-membered ring of fructose 6-phosphate.



A second phosphorylation reaction follows the isomerization step. *Fructose 6-phosphate is phosphorylated at the expense of ATP to fructose 1,6-bisphosphate* (F-l,6-BP). The prefix *bis-* in bisphosphate means that two separate monophosphoryl groups are present, whereas the prefix *di-* in diphosphate (as in adenosine diphosphate) means that two phosphoryl groups are present and are connected by an anhydride bond.

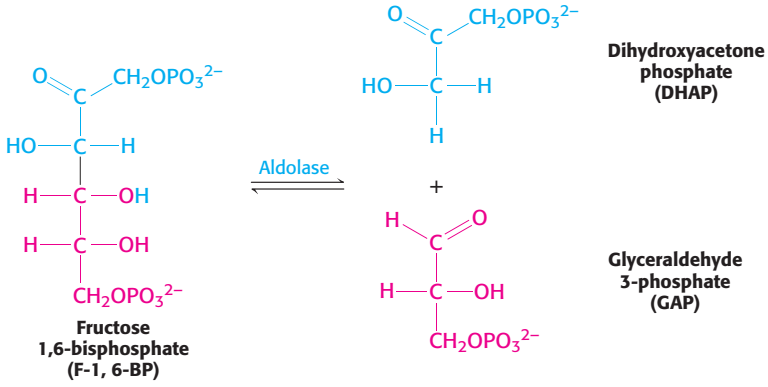


Fructose 6-phosphate (F-6P) — **Fructose 1,6-bisphosphate (F-1, 6-BP)**

This reaction is catalyzed by *phosphofructokinase* (PFK), an allosteric enzyme that sets the pace of glycolysis. As we will learn, this enzyme plays a central role in the metabolism of many molecules in all parts of the body.

The six-carbon sugar is cleaved into two three-carbon fragments

The newly formed fructose 1,6-bisphosphate is cleaved into *glyceraldehyde 3-phosphate* (GAP) and *dihydroxyacetone phosphate* (DHAP), completing stage 1 of glycolysis. The products of the remaining steps in glycolysis consist of three-carbon units rather than six-carbon units.



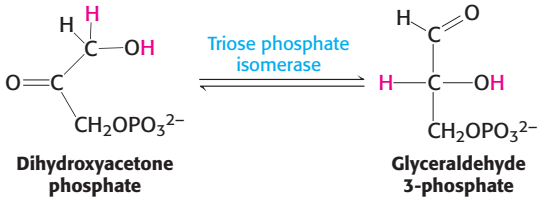
Fructose 1,6-bisphosphate (F-1, 6-BP)

Dihydroxyacetone phosphate (DHAP)

Glyceraldehyde 3-phosphate (GAP)

This reaction, which is readily reversible, is catalyzed by *aldolase*. This enzyme derives its name from the nature of the reverse reaction, an aldol condensation.

Glyceraldehyde 3-phosphate is on the direct pathway of glycolysis, whereas dihydroxyacetone phosphate is not. Unless a means exists to convert dihydroxyacetone phosphate into glyceraldehyde 3-phosphate, a three-carbon fragment useful for generating ATP will be lost. These compounds are isomers that can be readily interconverted: dihydroxyacetone phosphate is a ketose, whereas glyceraldehyde 3-phosphate is an aldose. The isomerization of these three-carbon phosphorylated sugars is catalyzed by *triose phosphate isomerase* (TPI, sometimes abbreviated TIM; Figure 16.4).



Dihydroxyacetone phosphate — **Glyceraldehyde 3-phosphate**

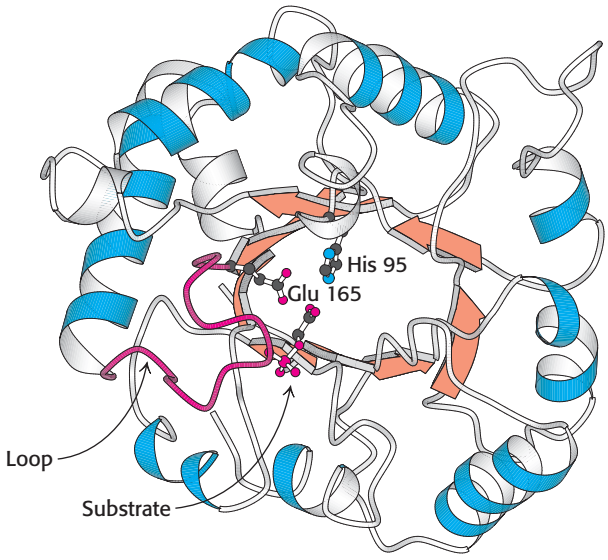


Figure 16.4 Structure of triose phosphate isomerase. This enzyme consists of a central core of eight parallel β strands (orange) surrounded by eight α helices (blue). This structural motif, called an αβ barrel, is also found in the glycolytic enzymes aldolase, enolase, and pyruvate kinase. *Notice* that histidine 95 and glutamate 165, essential components of the active site of triose phosphate isomerase, are located in the barrel. A loop (red) closes off the active site on substrate binding. [Drawn from 2YPI.pdb.]

This reaction is rapid and reversible. At equilibrium, 96% of the triose phosphate is dihydroxyacetone phosphate. However, the reaction proceeds readily from dihydroxyacetone phosphate to glyceraldehyde 3-phosphate because the subsequent reactions of glycolysis remove this product.

We now see the significance of the isomerization of glucose 6-phosphate to fructose 6-phosphate and its subsequent phosphorylation to form fructose 1,6-bisphosphate. Had the aldol cleavage taken place in the aldose glucose, a two-carbon and a four-carbon fragment would have resulted. Two different metabolic pathways, one to process the two-carbon fragment and one for the four-carbon fragment, would have been required to extract energy. Isomerization to the ketose fructose followed by aldol cleavage yields two phosphorylated interconvertable three-carbon fragments that will be oxidized in the later steps of glycolysis to capture energy in the form of ATP.

Mechanism: Triose phosphate isomerase salvages a three-carbon fragment

Much is known about the catalytic mechanism of triose phosphate isomerase. TPI catalyzes the transfer of a hydrogen atom from carbon 1 to carbon 2, an intramolecular oxidation–reduction. This isomerization of a ketose into an aldose proceeds through an *enediol intermediate* (Figure 16.5).

X-ray crystallographic and other studies showed that glutamate 165 plays the role of a general acid–base catalyst: it abstracts a proton (H^+) from carbon 1 and then donates it to carbon 2. However, the carboxylate group of glutamate 165 by itself is not basic enough to pull a proton away from a carbon atom adjacent to a carbonyl group. Histidine 95 assists catalysis by donating a proton to stabilize the negative charge that develops on the C-2 carbonyl group.

Two features of this enzyme are noteworthy. First, TPI displays great catalytic prowess. It accelerates isomerization by a factor of 10^{10} compared with the rate obtained with a simple base catalyst such as acetate ion. Indeed, the k_{cat}/K_M ratio for the isomerization of glyceraldehyde 3-phosphate is $2 \times 10^8\ M^{-1}\ s^{-1}$, which is close to the diffusion-controlled limit. In other words, catalysis takes place every time that enzyme and substrate meet. The diffusion-controlled encounter of substrate and enzyme is thus the rate-limiting step in catalysis. TPI is an example of a *kinetically perfect enzyme* (Section 8.4). Second, TPI suppresses an undesired side

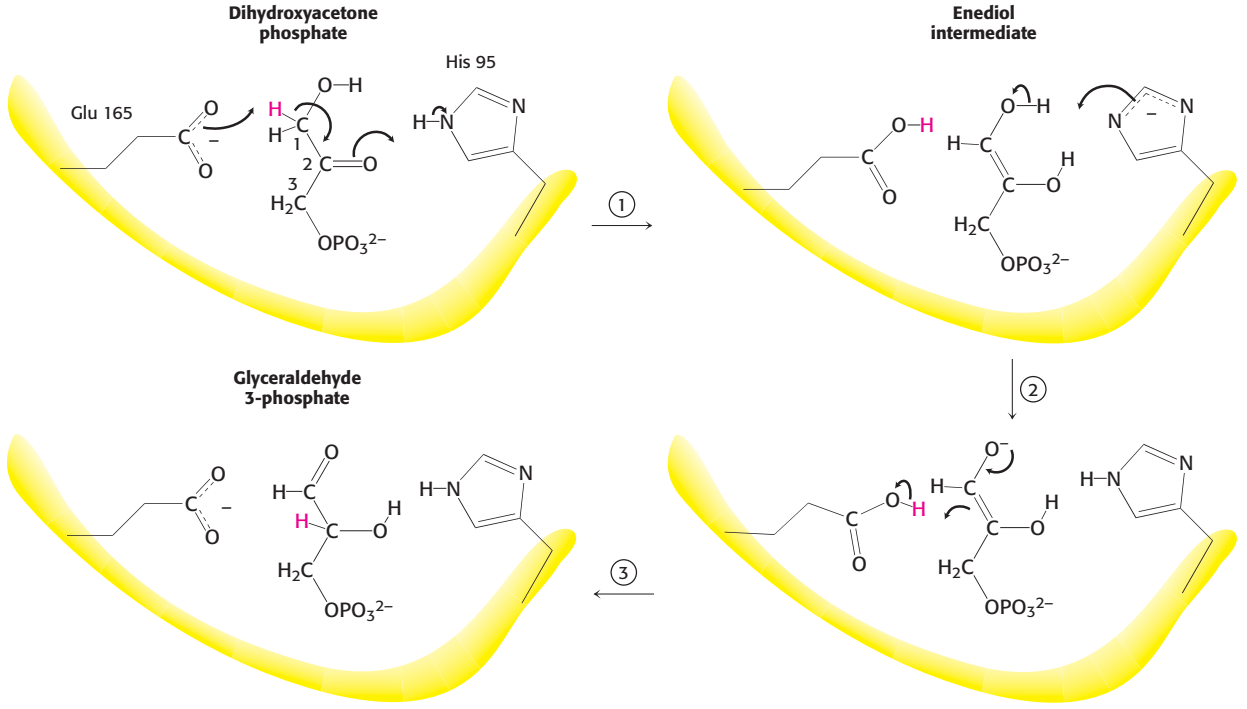
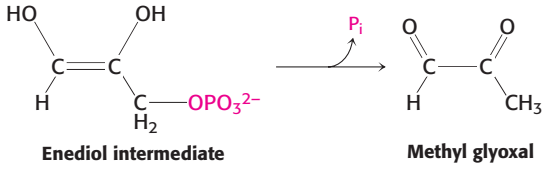


Figure 16.5 Catalytic mechanism of triose phosphate isomerase. (1) Glutamate 165 acts as a general base by abstracting a proton (H^+) from carbon 1. Histidine 95, acting as a general acid, donates a proton to the oxygen atom bonded to carbon 2, forming the enediol intermediate. (2) Glutamic acid, now acting as a general acid, donates a proton to C-2 while histidine removes a proton from the OH group of C-l. (3) The product is formed, and glutamate and histidine are returned to their ionized and neutral forms, respectively.

reaction, the decomposition of the enediol intermediate into methyl glyoxal and orthophosphate.



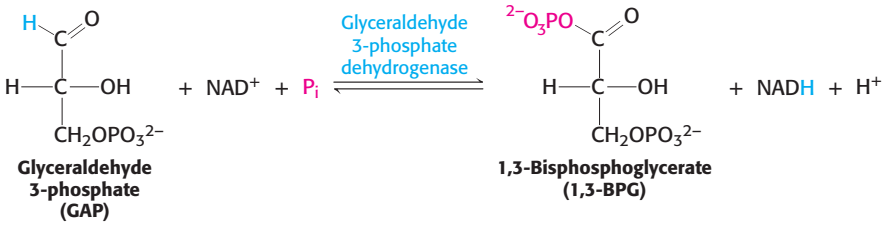
In solution, this physiologically useless reaction is 100 times as fast as isomerization. Moreover, methyl glyoxal is a highly reactive compound that can modify the structure and function of a variety of biomolecules, including proteins and DNA. The reaction of methyl glyoxal with a biomolecule is an example of deleterious reactions called advance glycation end products, discussed earlier (AGEs, Section 11.1). Hence, TPI must prevent the enediol from leaving the enzyme. This labile intermediate is trapped in the active site by the movement of a loop of 10 residues (see Figure 16.4). This loop serves as a lid on the active site, shutting it when the enediol is present and reopening it when isomerization is completed. *We see here a striking example of one means of preventing an undesirable alternative reaction: the active site is kept closed until the desirable reaction takes place.*

Thus, two molecules of glyceraldehyde 3-phosphate are formed from one molecule of fructose 1,6-bisphosphate by the sequential action of aldolase and triose phosphate isomerase. The economy of metabolism is evident in this reaction sequence. The isomerase funnels dihydroxyacetone phosphate into the main glycolytic pathway; a separate set of reactions is not needed.

The oxidation of an aldehyde to an acid powers the formation of a compound with high phosphoryl-transfer potential

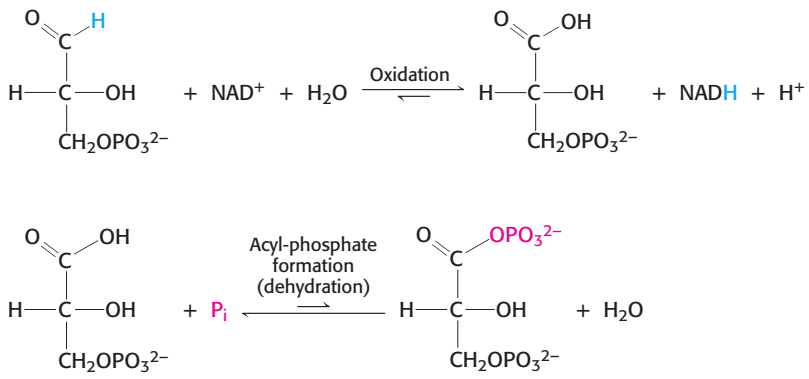
The preceding steps in glycolysis have transformed one molecule of glucose into two molecules of glyceraldehyde 3-phosphate, but no energy has yet

been extracted. On the contrary, thus far, two molecules of ATP have been invested. We come now to the second stage of glycolysis, a series of steps that harvest some of the energy contained in glyceraldehyde 3-phosphate as ATP. The initial reaction in this sequence is the *conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate* (1,3-BPG), a reaction catalyzed by *glyceraldehyde 3-phosphate dehydrogenase.*



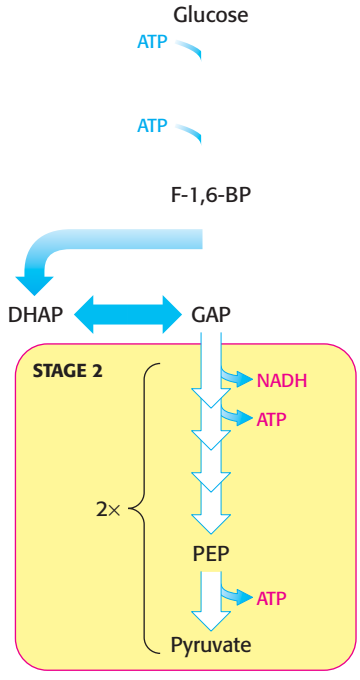
1,3-Bisphosphoglycerate is an acyl phosphate, which is a mixed anhydride of phosphoric acid and a carboxylic acid. Such compounds have a high phosphoryl-transfer potential; one of its phosphoryl groups is transferred to ADP in the next step in glycolysis.

The reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase can be viewed as the sum of two processes: the *oxidation* of the aldehyde to a carboxylic acid by NAD^+ and the *joining* of the carboxylic acid and orthophosphate to form the acyl-phosphate product.



The first reaction is thermodynamically quite favorable, with a standard free-energy change, $\Delta G^{\circ\prime}$, of approximately $-50\ kJ\ mol^{-1}$ ($-12\ kcal\ mol^{-1}$), whereas the second reaction is quite unfavorable, with a standard free-energy change of the same magnitude but the opposite sign. If these two reactions simply took place in succession, the second reaction would have a very large activation energy and thus not take place at a biologically significant rate. These two processes *must be coupled* so that the favorable aldehyde oxidation can be used to drive the formation of the acyl phosphate. How are these reactions coupled? *The key is an intermediate, formed as a result of the aldehyde oxidation, that is linked to the enzyme by a thioester bond.* Thioesters are high-energy compounds found in many biochemical pathways (Section 15.4). This intermediate reacts with orthophosphate to form the high-energy compound 1,3-bisphosphoglycerate.

The thioester intermediate is higher in free energy than the free carboxylic acid is. The favorable oxidation and unfavorable phosphorylation reactions are coupled by the thioester intermediate, which preserves much of the free energy released in the oxidation reaction. We see here the *use of a covalent enzyme-bound intermediate as a mechanism of energy coupling.* A free-energy profile of the glyceraldehyde 3-phosphate dehydrogenase reaction, compared



Second stage of glycolysis. The oxidation of three-carbon fragments yields ATP.

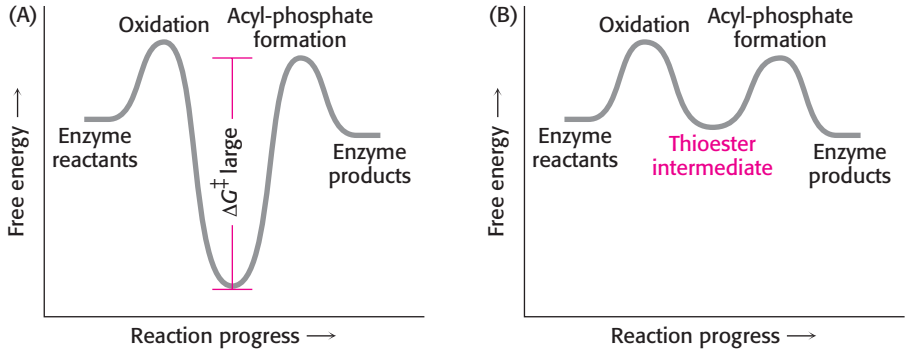


Figure 16.6 Free-energy profiles for glyceraldehyde oxidation followed by acyl-phosphate formation. (A) A hypothetical case with no coupling between the two processes. The second step must have a large activation barrier, making the reaction very slow. (B) The actual case with the two reactions coupled through a thioester intermediate.

with a hypothetical process in which the reaction proceeds without this intermediate, reveals how this intermediate allows a favorable process to drive an unfavorable one (Figure 16.6).

Mechanism: Phosphorylation is coupled to the oxidation of glyceraldehyde 3-phosphate by a thioester intermediate

The active site of glyceraldehyde 3-phosphate dehydrogenase includes a reactive cysteine residue, as well as NAD^+ and a crucial histidine (Figure 16.7). Let us consider in detail how these components cooperate in the reaction mechanism (Figure 16.8). In step 1, the aldehyde substrate reacts with the sulfhydryl group of cysteine 149 on the enzyme to form a hemithioacetal. Step 2 is the *transfer of a hydride ion to a molecule of NAD^+ that is tightly bound to the enzyme and is adjacent to the cysteine residue.* This reaction is favored by the deprotonation of the hemithioacetal by histidine 176. The products of this reaction are the reduced coenzyme NADH and a thioester intermediate. *This thioester intermediate has a free energy close to that of the reactants* (see Figure 16.6). In step 3, the NADH formed from the aldehyde oxidation leaves the enzyme and is replaced by a second molecule of NAD^+. This step is important because the positive charge on NAD^+ polarizes the thioester intermediate to facilitate the attack by orthophosphate. In step 4, orthophosphate attacks the thioester to form

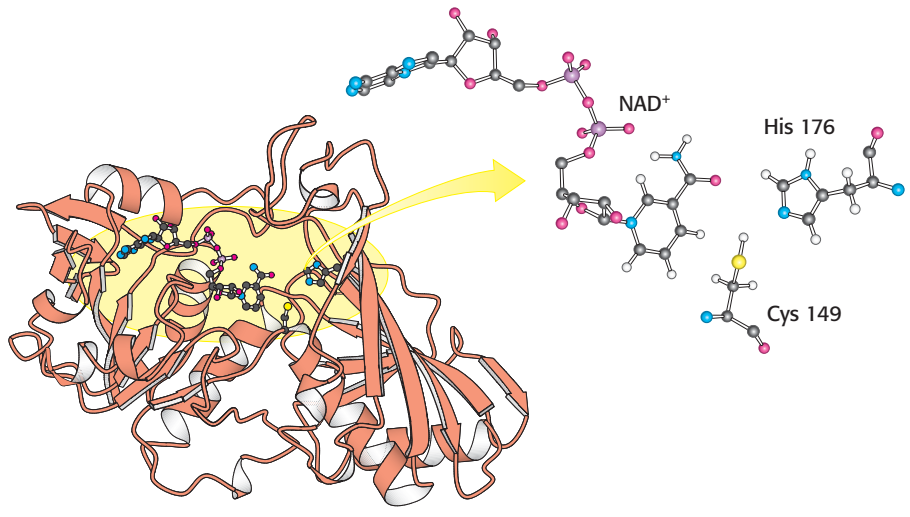


Figure 16.7 Structure of glyceraldehyde 3-phosphate dehydrogenase. *Notice* that the active site includes a cysteine residue and a histidine residue adjacent to a bound NAD^+ molecule. The sulfur atom of cysteine will link with the substrate to form a transitory thioester intermediate. [Drawn from 1GAD.pdb.]

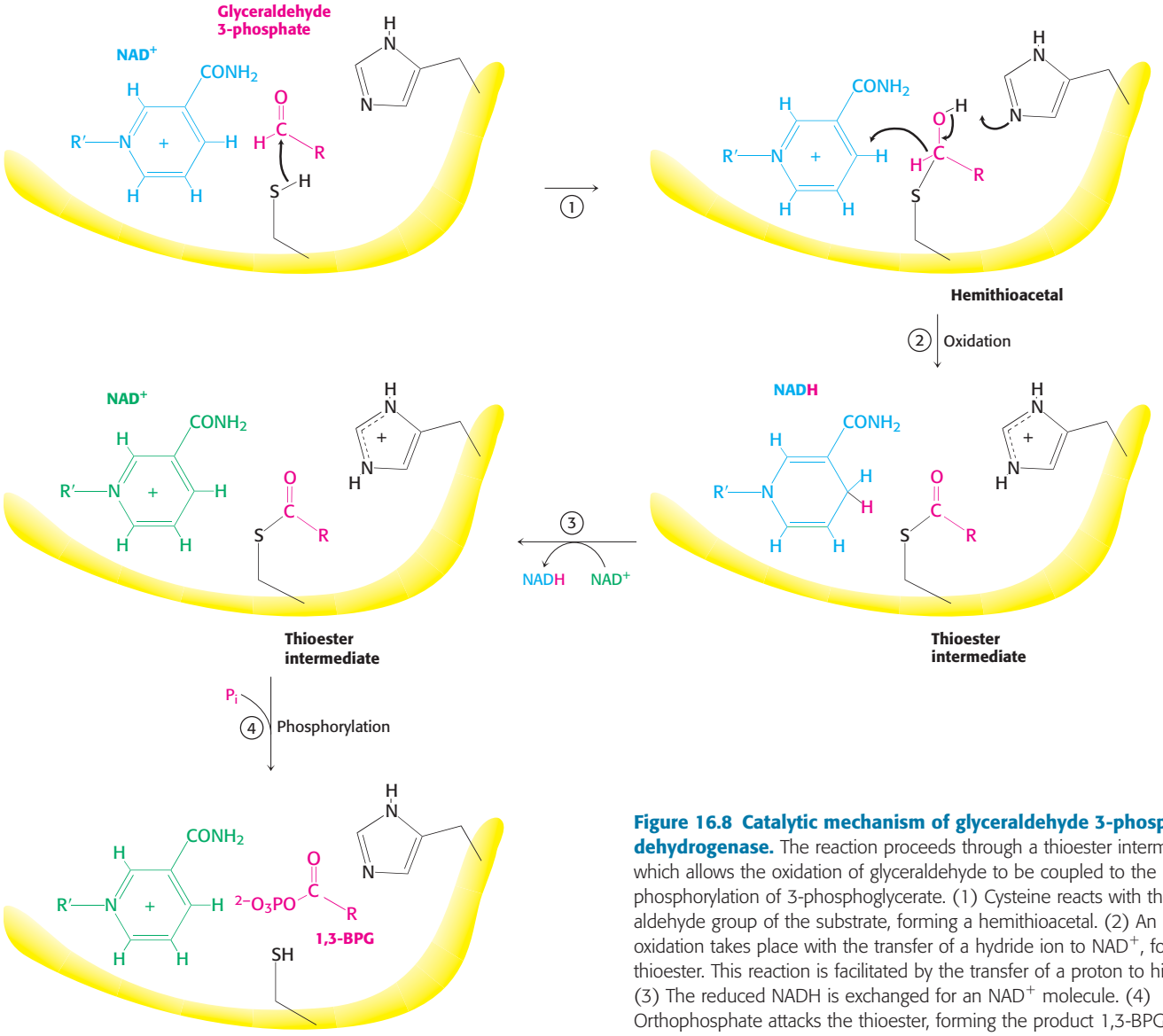
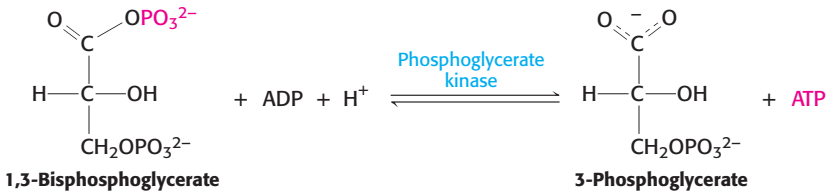


Figure 16.8 Catalytic mechanism of glyceraldehyde 3-phosphate dehydrogenase. The reaction proceeds through a thioester intermediate, which allows the oxidation of glyceraldehyde to be coupled to the phosphorylation of 3-phosphoglycerate. (1) Cysteine reacts with the aldehyde group of the substrate, forming a hemithioacetal. (2) An oxidation takes place with the transfer of a hydride ion to NAD^+, forming a thioester. This reaction is facilitated by the transfer of a proton to histidine. (3) The reduced NADH is exchanged for an NAD^+ molecule. (4) Orthophosphate attacks the thioester, forming the product 1,3-BPG.

1,3-BPG and free the cysteine residue. This example illustrates the essence of energy transformations and of metabolism itself: energy released by carbon oxidation is converted into high phosphoryl-transfer potential.

ATP is formed by phosphoryl transfer from 1,3-bisphosphoglycerate

1,3-Bisphosphoglycerate is an energy-rich molecule with a greater phosphoryl-transfer potential than that of ATP (Section 15.2). Thus, 1,3-BPG can be used to power the synthesis of ATP from ADP. *Phosphoglycerate kinase* catalyzes the transfer of the phosphoryl group from the acyl phosphate of 1,3-bisphosphoglycerate to ADP. ATP and 3-phosphoglycerate are the products.



The formation of ATP in this manner is referred to as *substrate-level phosphorylation* because the phosphate donor, 1,3-BPG, is a substrate with high phosphoryl-transfer potential. We will contrast this manner of ATP formation with the formation of ATP from ionic gradients in Chapters 18 and 19.

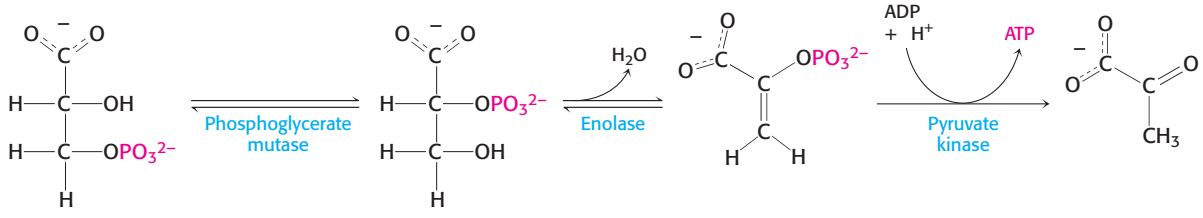
Thus, the outcomes of the reactions catalyzed by glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase are as follows:

1. Glyceraldehyde 3-phosphate, an aldehyde, is oxidized to 3-phosphoglycerate, a carboxylic acid.
2. NAD^+ is concomitantly reduced to NADH.
3. ATP is formed from P_i and ADP at the expense of carbon-oxidation energy.

In essence, the energy released during the oxidation of glyceraldehyde 3-phosphate to 3-phosphoglycerate is temporarily trapped as 1,3-bisphosphoglycerate. This energy powers the transfer of a phosphoryl group from 1,3-bisphosphoglycerate to ADP to yield ATP. Keep in mind that, because of the actions of aldolase and triose phosphate isomerase, two molecules of glyceraldehyde 3-phosphate were formed and hence two molecules of ATP were generated. These ATP molecules make up for the two molecules of ATP consumed in the first stage of glycolysis.

Additional ATP is generated with the formation of pyruvate

In the remaining steps of glycolysis, 3-phosphoglycerate is converted into pyruvate, and a second molecule of ATP is formed from ADP.



The first reaction is a rearrangement. The position of the phosphoryl group shifts in the *conversion of 3-phosphoglycerate into 2-phosphoglycerate,* a reaction catalyzed by *phosphoglycerate mutase.* In general, a *mutase* is an enzyme that catalyzes the intramolecular shift of a chemical group, such as a phosphoryl group. The phosphoglycerate mutase reaction has an interesting mechanism: the phosphoryl group is not simply moved from one carbon atom to another. This enzyme requires catalytic amounts of 2,3-bisphosphoglycerate (2,3-BPG) to maintain an active-site histidine residue in a phosphorylated form. This phosphoryl group is transferred to 3-phosphoglycerate to re-form 2,3-bisphosphoglycerate.

Enz-His-phosphate + 3-phosphoglycerate $\rightleftharpoons$
Enz-His + 2,3-bisphosphoglycerate

The mutase then functions as a phosphatase: it converts 2,3-bisphosphoglycerate into 2-phosphoglycerate. The mutase retains the phosphoryl group to regenerate the modified histidine.

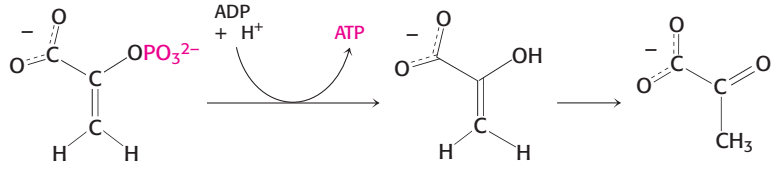
Enz-His + 2,3-bisphosphoglycerate $\rightleftharpoons$
Enz-His-phosphate + 2-phosphoglycerate

The sum of these reactions yields the mutase reaction:

$$\text{3-Phosphoglycerate} \rightleftharpoons \text{2-phosphoglycerate}$$

In the next reaction, the dehydration of 2-phosphoglycerate introduces a double bond, creating an *enol*. *Enolase* catalyzes this formation of the enol phosphate *phosphoenolpyruvate* (PEP). This dehydration markedly elevates the transfer potential of the phosphoryl group. An *enol phosphate* has a high phosphoryl-transfer potential, whereas the phosphate ester of an ordinary alcohol, such as 2-phosphoglycerate, has a low one. The $\Delta G^{\circ\prime}$ of the hydrolysis of a phosphate ester of an ordinary alcohol is -13 kJ mol^{-1} (-3 kcal mol^{-1}), whereas that of phosphoenolpyruvate is -62 kJ mol^{-1} (-15 kcal mol^{-1}).

Why does phosphoenolpyruvate have such a high phosphoryl-transfer potential? The phosphoryl group traps the molecule in its unstable enol form. When the phosphoryl group has been donated to ATP, the enol undergoes a conversion into the more stable ketone—namely, pyruvate.



Thus, *the high phosphoryl-transfer potential of phosphoenolpyruvate arises primarily from the large driving force of the subsequent enol–ketone conversion.* Hence, pyruvate is formed, and ATP is generated concomitantly. The virtually irreversible transfer of a phosphoryl group from phosphoenolpyruvate to ADP is catalyzed *by pyruvate kinase.* What is the energy source for the formation of phosphoenolpyruvate? The answer to this question becomes clear when we compare the structures of 2-phosphoglycerate and pyruvate. The formation of pyruvate from 2-phosphoglycerate is, in essence, an internal oxidation–reduction reaction; carbon 3 takes electrons from carbon 2 in the conversion of 2-phosphoglycerate into pyruvate. Compared with 2-phosphoglycerate, C-3 is more reduced in pyruvate, whereas C-2 is more oxidized. Once again, carbon oxidation powers the synthesis of a compound with high phosphoryl-transfer potential, phosphoenolpyruvate here and 1,3-bisphosphoglycerate earlier, which allows the synthesis of ATP.

Because the molecules of ATP used in forming fructose 1,6-bisphosphate have already been regenerated, the two molecules of ATP generated from phosphoenolpyruvate are "profit."

Two ATP molecules are formed in the conversion of glucose into pyruvate

The net reaction in the transformation of glucose into pyruvate is

$$\text{Glucose} + 2\,P_i + 2\,\text{ADP} + 2\,\text{NAD}^+ \rightarrow 2\,\text{pyruvate} + 2\,\text{ATP} + 2\,\text{NADH} + 2\,H^+ + 2\,H_2O$$

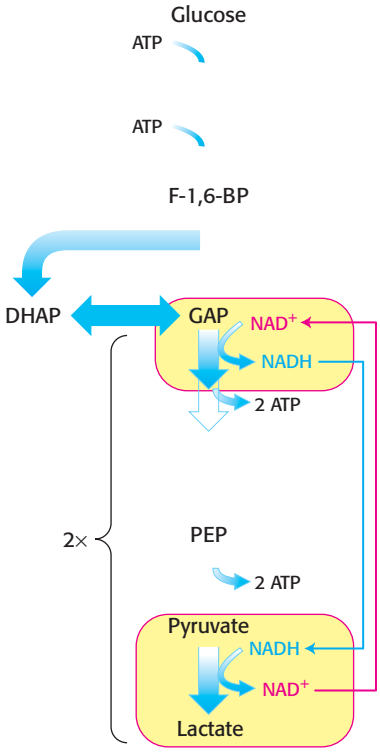
Thus, *two molecules of ATP are generated in the conversion of glucose into two molecules of pyruvate.* The reactions of glycolysis are summarized in Table 16.1.

The energy released in the anaerobic conversion of glucose into two molecules of pyruvate is about -96 kJ mol^{-1} (-23 kcal mol^{-1}). We shall see

Table 16.1 Reactions of glycolysis

Step	Reaction
1	Glucose + ATP ⟶ glucose 6-phosphate + ADP + H^+
2	Glucose 6-phosphate ⇌ fructose 6-phosphate
3	Fructose 6-phosphate + ATP ⟶ fructose 1,6-bisphosphate + ADP + H^+
4	Fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate
5	Dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate
6	Glyceraldehyde 3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + NADH + H^+
7	1,3-Bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP
8	3-Phosphoglycerate ⇌ 2-phosphoglycerate
9	2-Phosphoglycerate ⇌ phosphoenolpyruvate + H_2O
10	Phosphoenolpyruvate + ADP + H^+ ⟶ pyruvate + ATP

Note: ΔG, the actual free-energy change, has been calculated from $\Delta G°'$ and known concentrations of reactants under typical physiological conditions. Glycolysis can proceed only if the ΔG values of all reactions are negative. The smalls positive ΔG values of three of the above reactions indicate that the concentrations of metabolites in vivo in cells undergoing glycolysis are not precisely known.



Regeneration of NAD^+.

in Chapters 17 and 18 that much more energy can be released from glucose in the presence of oxygen.

NAD^+ is regenerated from the metabolism of pyruvate

The conversion of glucose into two molecules of pyruvate has resulted in the net synthesis of ATP. However, an energy-converting pathway that stops at pyruvate will not proceed for long, because redox balance has not been maintained. As we have seen, the activity of glyceraldehyde 3-phosphate dehydrogenase, in addition to generating a compound with high phosphoryl-transfer potential, of necessity leads to the reduction of NAD^+ to NADH. In the cell, there are limited amounts of NAD^+, which is derived from the vitamin niacin, a dietary requirement for human beings. Consequently, NAD^+ must be regenerated for glycolysis to proceed. Thus, the final process in the pathway is the regeneration of NAD^+ through the metabolism of pyruvate.

The sequence of reactions from glucose to pyruvate is similar in most organisms and most types of cells. In contrast, the fate of pyruvate is variable. Three reactions of pyruvate are of primary importance: conversion into ethanol, lactate, or carbon dioxide (Figure 16.9). The first two reactions are fermentations that take place in the absence of oxygen. A *fermentation* is an ATP-generating process in which organic compounds act both as the donor and as the acceptor of electrons. In the presence of oxygen, the most common situation in multicellular organisms and in many unicellular ones, pyruvate is metabolized to carbon dioxide and water through the citric acid cycle and the electron-transport chain with oxygen serving as the final electron acceptor. We now take a closer look at these three possible fates of pyruvate.

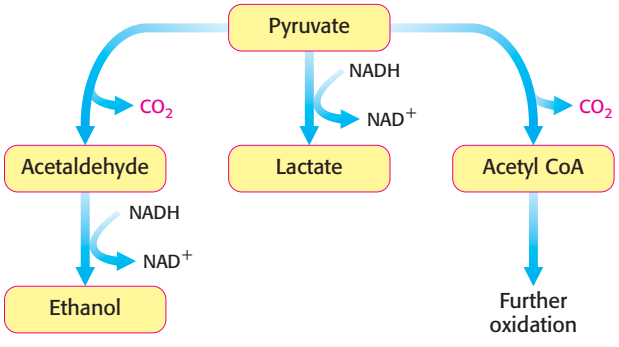
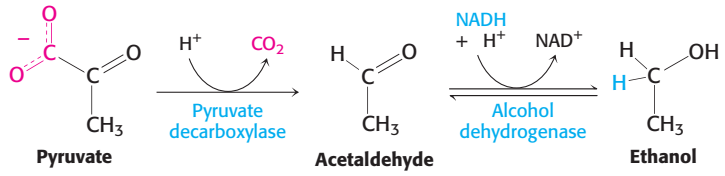


Figure 16.9 Diverse fates of pyruvate. Ethanol and lactate can be formed by reactions that include NADH. Alternatively, a two-carbon unit from pyruvate can be coupled to coenzyme A (Chapter 17) to form acetyl CoA.

1. *Ethanol* is formed from pyruvate in yeast and several other microorganisms. The first step is the decarboxylation of pyruvate. This reaction is catalyzed by *pyruvate decarboxylase,* which requires the coenzyme thiamine pyrophosphate. This coenzyme, derived from the vitamin thiamine (B_1), also participates in reactions catalyzed by other enzymes (Section 17.1). The second step is the reduction of

Enzyme	Reaction type	$\Delta G^{\circ\prime}$ in kJ mol^{-1} (kcal mol^{-1})	ΔG in kJ mol^{-1} (kcal mol^{-1})
Hexokinase	Phosphoryl transfer	−16.7 (−4.0)	−33.5 (−8.0)
Phosphoglucose isomerase	Isomerization	+1.7 (+0.4)	−2.5 (−0.6)
Phosphofructokinase	Phosphoryl transfer	−14.2 (−3.4)	−22.2 (−5.3)
Aldolase	Aldol cleavage	+23.8 (+5.7)	−1.3 (−0.3)
Triose phosphate isomerase	Isomerization	+7.5 (+1.8)	+2.5 (+0.6)
Glyceraldehyde 3-phosphate dehydrogenase	Phosphorylation coupled to oxidation	+6.3 (+1.5)	−1.7 (−0.4)
Phosphoglycerate kinase	Phosphoryl transfer	−18.8 (−4.5)	+1.3 (+0.3)
Phosphoglycerate mutase	Phosphoryl shift	+4.6 (+1.1)	+0.8 (+0.2)
Enolase	Dehydration	+1.7 (+0.4)	−3.3 (−0.8)
Pyruvate kinase	Phosphoryl transfer	−31.4 (−7.5)	−16.7 (−4.0)

acetaldehyde to ethanol by NADH, in a reaction catalyzed by *alcohol dehydrogenase.* This process regenerates NAD^+.



The active site of alcohol dehydrogenase contains a zinc ion that is coordinated to the sulfur atoms of two cysteine residues and a nitrogen atom of histidine (Figure 16.10). This zinc ion polarizes the carbonyl group of the substrate to favor the transfer of a hydride from NADH.

The conversion of glucose into ethanol is an example *of alcoholic fermentation.* The net result of this anaerobic process is

$$\text{Glucose} + 2\,P_i + 2\,\text{ADP} + 2\,H^+ \rightarrow 2\,\text{ethanol} + 2\,CO_2 + 2\,\text{ATP} + 2\,H_2O$$

Note that NAD^+ and NADH do not appear in this equation, even though they are crucial for the overall process. NADH generated by the oxidation of glyceraldehyde 3-phosphate is consumed in the reduction of acetaldehyde to ethanol. Thus, *there is no net oxidation–reduction in the conversion of glucose into ethanol* (Figure 16.11). The ethanol formed in alcoholic fermentation provides a key ingredient for brewing and winemaking.

2. *Lactate* is formed from pyruvate in a variety of microorganisms in a process called *lactic acid fermentation.* The reaction also takes place in the cells of higher organisms when the amount of oxygen is limiting, as in

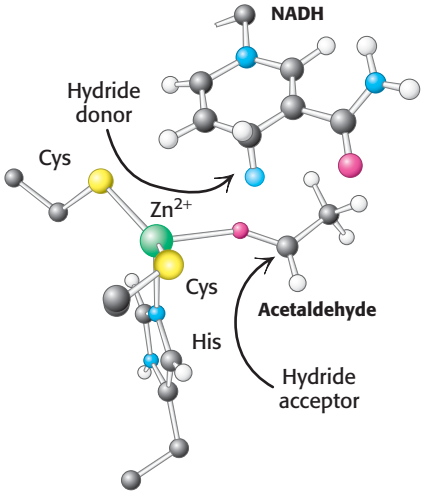


Figure 16.10 Active site of alcohol dehydrogenase. The active site contains a zinc ion bound to two cysteine residues and one histidine residue. *Notice* that the zinc ion binds the acetaldehyde substrate through its oxygen atom, polarizing the substrate so that it more easily accepts a hydride from NADH. Only the nicotinamide ring of NADH is shown.

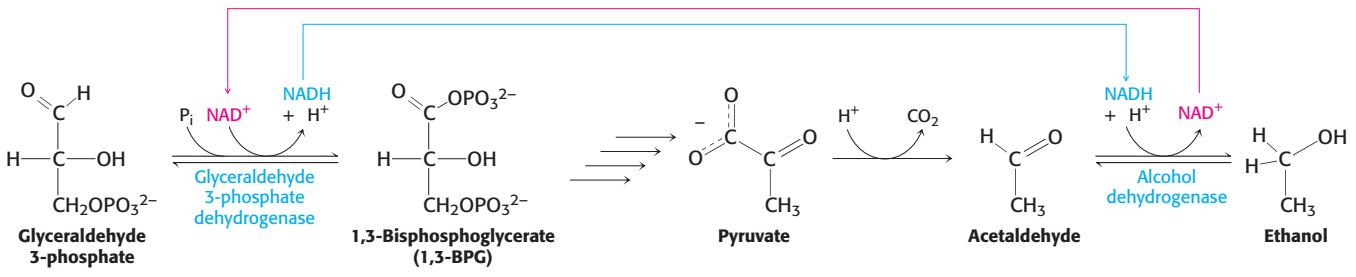
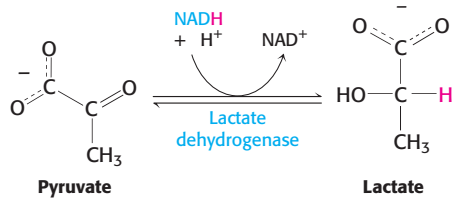


Figure 16.11 Maintaining redox balance. The NADH produced by the glyceraldehyde 3-phosphate dehydrogenase reaction must be reoxidized to NAD^+ for the glycolytic pathway to continue. In alcoholic fermentation, alcohol dehydrogenase oxidizes NADH and generates ethanol. In lactic acid fermentation (not shown), lactate dehydrogenase oxidizes NADH while generating lactic acid.

muscle cells during intense activity. The reduction of pyruvate by NADH to form lactate is catalyzed by *lactate dehydrogenase.*



The overall reaction in the conversion of glucose into lactate is

$$\text{Glucose} + 2\ P_i + 2\ \text{ADP} \rightarrow 2\ \text{lactate} + 2\ \text{ATP} + 2\ H_2O$$

As in alcoholic fermentation, there is no net oxidation–reduction. The NADH formed in the oxidation of glyceraldehyde 3-phosphate is consumed in the reduction of pyruvate. *The regeneration of NAD^+ in the reduction of pyruvate to lactate or ethanol sustains the continued process of glycolysis under anaerobic conditions.*

3. Only a fraction of the energy of glucose is released in its anaerobic conversion into ethanol or lactate. Much more energy can be extracted aerobically by means of the citric acid cycle and the electron-transport chain. The entry point to this oxidative pathway is *acetyl coenzyme A* (acetyl CoA), which is formed inside mitochondria by the oxidative decarboxylation of pyruvate.

$$\text{Pyruvate} + NAD^+ + \text{CoA} \rightarrow \text{acetyl CoA} + CO_2 + \text{NADH} + \text{H}$$

This reaction, which is catalyzed by the pyruvate dehydrogenase complex, will be considered in detail in Chapter 17. The NAD^+ required for this reaction and for the oxidation of glyceraldehyde 3-phosphate is regenerated when NADH ultimately transfers its electrons to O_2 through the electron-transport chain in mitochondria.

Fermentations provide usable energy in the absence of oxygen

Fermentations yield only a fraction of the energy available from the complete combustion of glucose. Why is a relatively inefficient metabolic pathway so extensively used? The fundamental reason is that oxygen is not required. The ability to survive without oxygen affords a host of living accommodations such as soils, deep water, and skin pores. Some organisms, called *obligate anaerobes,* cannot survive in the presence of O_2, a highly reactive compound. The bacterium *Clostridium perfringens,* the cause of gangrene, is an example of an obligate anaerobe. Other pathogenic obligate anaerobes are listed in Table 16.2. Skeletal muscles in most animals can function anaerobically for short periods. For example, when animals perform bursts of intense exercise, their ATP needs rise faster than the ability of the body

Table 16.2 Examples of pathogenic obligate anaerobes

Bacterium	Result of infection
Clostridium tetani	Tetanus (lockjaw)
Clostridium botulinum	Botulism (an especially severe type of food poisoning)
Clostridium perfringens	Gas gangrene (gas is produced as an end point of the fermentation, distorting and destroying the tissue)
Bartonella hensela	Cat scratch fever (flu-like symptoms)
Bacteroides fragilis	Abdominal, pelvic, pulmonary, and blood infections

to provide oxygen to the muscle. The muscle functions anaerobically until fatigue sets in, which is caused, in part, by lactate buildup.

Although we have considered only lactic acid and alcoholic fermentation, microorganisms are capable of generating a wide array of molecules as end points of fermentation (Table 16.3). Indeed, many food products, including sour cream, yogurt, various cheeses, beer, wine, and sauerkraut, result from fermentation.

Table 16.3 Starting and ending points of various fermentations

Glucose	→	lactate
Lactate	→	acetate
Glucose	→	ethanol
Ethanol	→	acetate
Arginine	→	carbon dioxide
Pyrimidines	→	carbon dioxide
Purines	→	formate
Ethylene glycol	→	acetate
Threonine	→	propionate
Leucine	→	2-alkylacetate
Phenylalanine	→	propionate

Note: The products of some fermentations are the substrates for others.

The binding site for NAD^+ is similar in many dehydrogenases

The three dehydrogenases—glyceraldehyde 3-phosphate dehydrogenase, alcohol dehydrogenase, and lactate dehydrogenase—have quite different three-dimensional structures. However, their NAD^+-binding domains are strikingly similar (Figure 16.12). This nucleotide-binding region is made up of four α helices and a sheet of six parallel β strands. Moreover, in all cases, the bound NAD^+ displays nearly the same conformation. This common structural domain was one of the first recurring structural domains to be discovered. It is often called a *Rossmann fold* after Michael Rossmann, who first recognized it. This fold likely represents a primordial dinucleotide-binding domain that recurs in the dehydrogenases of glycolysis and other enzymes because of their descent from a common ancestor.

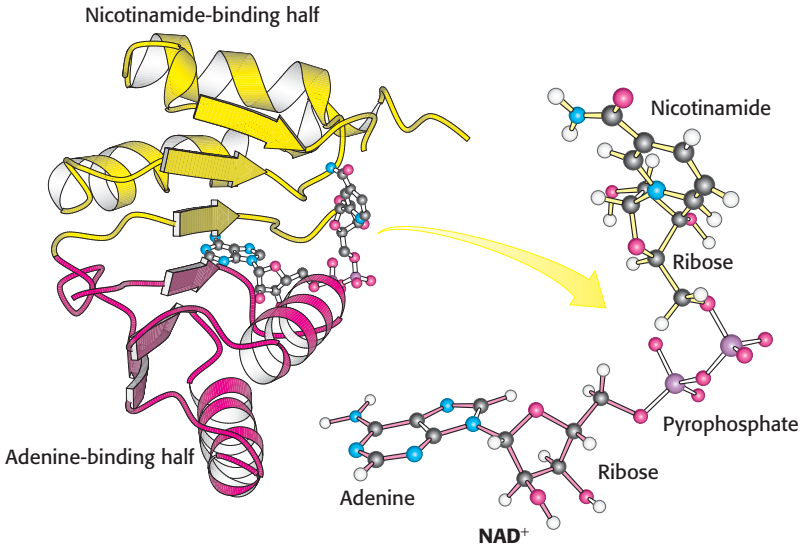


Figure 16.12 NAD^+-binding region in dehydrogenases. *Notice* that the nicotinamide-binding half (yellow) is structurally similar to the adenine-binding half (red). The two halves together form a structural motif called a Rossmann fold. The NAD^+ molecule binds in an extended conformation. [Drawn from 3LDH.pdb.]

Fructose and galactose are converted into glycolytic intermediates

Although glucose is the most widely used monosaccharide, others also are important fuels. Let us consider how two abundant sugars—fructose and galactose—can be funneled into the glycolytic pathway (Figure 16.13). There are no catabolic pathways for metabolizing fructose or galactose, and so the strategy is to convert these sugars into a metabolite of glucose.

Fructose can take one of two pathways to enter the glycolytic pathway. Much of the ingested fructose is metabolized by the liver, using *the fructose 1-phosphate pathway* (Figure 16.14). The first step is the phosphorylation of *fructose* to *fructose 1-phosphate* by *fructokinase.* Fructose 1-phosphate is then split into *glyceraldehyde* and *dihydroxyacetone phosphate,* an

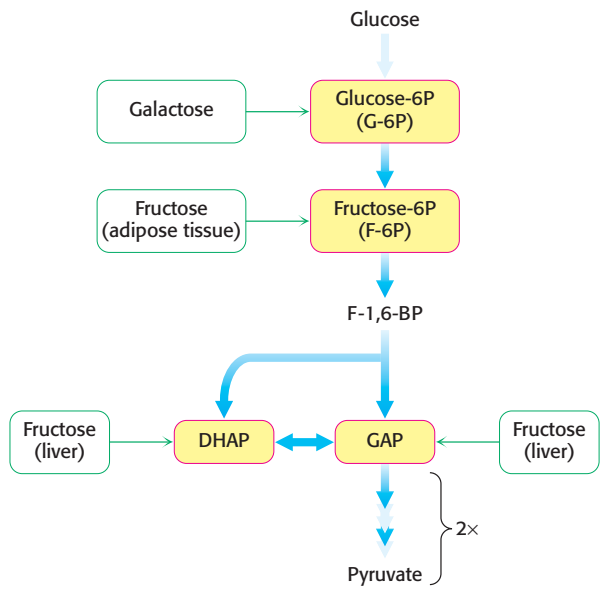


Figure 16.13 Entry points in glycolysis for galactose and fructose.

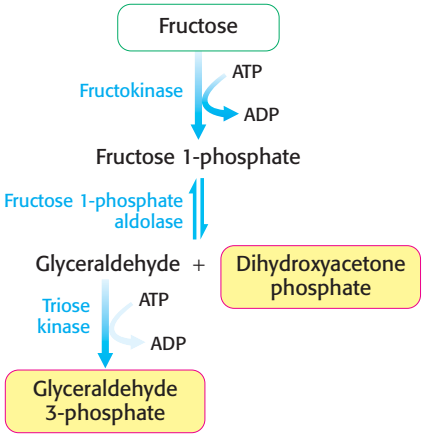
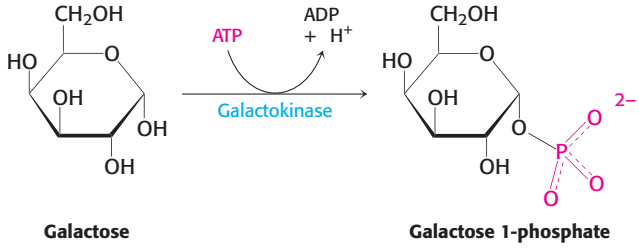


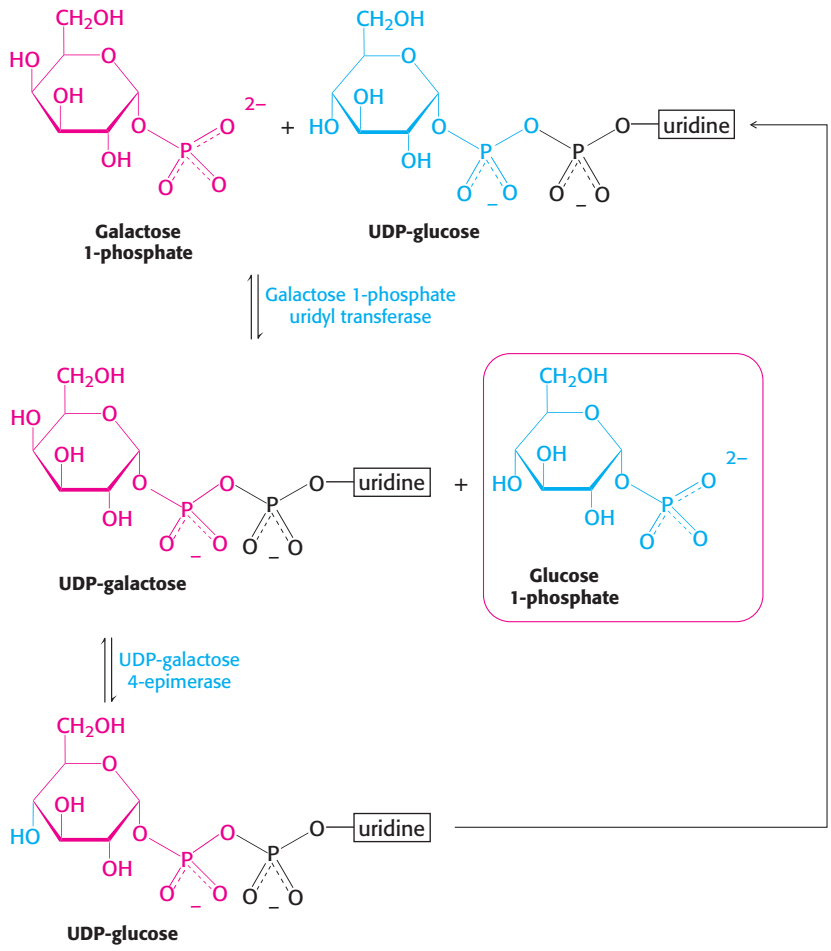
Figure 16.14 Fructose metabolism.
Fructose enters the glycolytic pathway in the liver through the fructose 1-phosphate pathway.

intermediate in glycolysis. This aldol cleavage is catalyzed by a specific *fructose 1-phosphate aldolase.* Glyceraldehyde is then phosphorylated to *glyceraldehyde 3-phosphate,* a glycolytic intermediate, by *triose kinase.* In other tissues, *fructose can be phosphorylated to fructose 6-phosphate by hexokinase.*

Galactose is converted into *glucose 6-phosphate* in four steps. The first reaction in the *galactose–glucose interconversion pathway* is the phosphorylation of galactose to galactose 1-phosphate by *galactokinase.*



Galactose 1-phosphate then acquires a uridyl group from uridine diphosphate glucose (UDP-glucose), an activated intermediate in the synthesis of carbohydrates (Section 21.4).



The products of this reaction, which is catalyzed by *galactose 1-phosphate uridyl transferase,* are UDP-galactose and glucose 1-phosphate. The galactose moiety of UDP-galactose is then epimerized to glucose. The configuration of the hydroxyl group at carbon 4 is inverted by *UDP-galactose 4-epimerase.*

The sum of the reactions catalyzed by galactokinase, the transferase, and the epimerase is

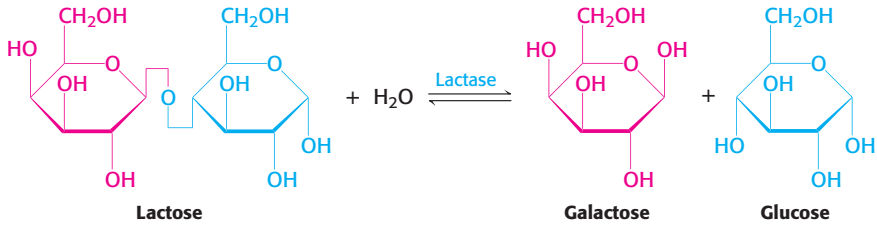
$$\text{Galactose} + \text{ATP} \rightarrow \text{glucose 1-phosphate} + \text{ADP} + \text{H}^+$$

Note that UDP-glucose is not consumed in the conversion of galactose into glucose, because it is regenerated from UDP-galactose by the epimerase. This reaction is reversible, and the product of the reverse direction also is important. *The conversion of UDP-glucose into UDP-galactose is essential for the synthesis of galactosyl residues in complex polysaccharides and glycoproteins if the amount of galactose in the diet is inadequate to meet these needs.*

Finally, glucose 1-phosphate, formed from galactose, is isomerized to glucose 6-phosphate by *phosphoglucomutase.* We shall return to this reaction when we consider the synthesis and degradation of glycogen, which proceeds through glucose 1-phosphate, in Chapter 21.

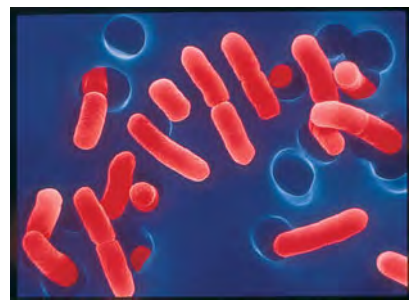
Many adults are intolerant of milk because they are deficient in lactase

Many adults are unable to metabolize the milk sugar lactose and experience gastrointestinal disturbances if they drink milk. *Lactose intolerance,* or hypolactasia, is most commonly caused by a deficiency of the enzyme lactase, which cleaves lactose into glucose and galactose.



"Deficiency" is not quite the appropriate term, because a decrease in lactase is normal in the course of development in all mammals. As children are weaned and milk becomes less prominent in their diets, lactase activity normally declines to about 5% to 10% of the level at birth. This decrease is not as pronounced with some groups of people, most notably Northern Europeans, and people from these groups can continue to ingest milk without gastrointestinal difficulties. With the appearance of milk-producing domesticated animals, an adult with active lactase would have a selective advantage in being able to consume calories from the readily available milk. Because dairy farming originated only about 10,000 years ago, the evolutionary selective pressure on lactase persistence must have been substantial, attesting to the biochemical value of being able to use milk as an energy source into adulthood.

What happens to the lactose in the intestine of a lactase-deficient person? The lactose is a good energy source for microorganisms in the colon, and they ferment it to lactic acid while generating methane (CH_4) and hydrogen gas (H_2). The gas produced creates the uncomfortable feeling of gut distension and the annoying problem of flatulence. The lactate produced by the microorganisms is osmotically active and draws water into the intestine, as does any undigested lactose, resulting in diarrhea. If severe enough, the gas and diarrhea hinder the absorption of other nutrients such as fats and proteins. The simplest treatment is to avoid the consumption of products containing much lactose. Alternatively, the enzyme lactase can be ingested with milk products.



Scanning electron micrograph of *Lactobacillus.* The anaerobic bacterium *Lactobacillus* is shown here (artificially colored) at a magnification of 22.245×. As suggested by its name, this genus of bacteria ferments glucose into lactic acid and is widely used in the food industry. *Lactobacillus* is also a component of the normal human bacterial flora of the urogenital tract where, because of its ability to generate an acidic environment, it prevents the growth of harmful bacteria. [Dr. Dennis Kunkel/ PhotoTake.]

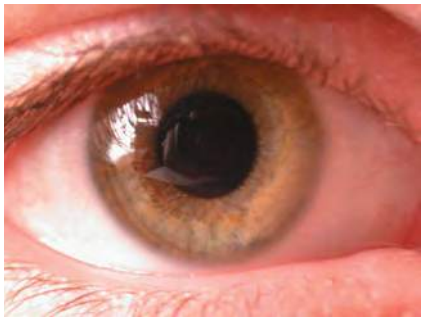
Galactose is highly toxic if the transferase is missing

Less common than lactose intolerance are disorders that interfere with the metabolism of galactose. The disruption of galactose metabolism is referred to as *galactosemia*. The most common form, called classic galactosemia, is an inherited deficiency in galactose 1-phosphate uridyl transferase activity. Afflicted infants fail to thrive. They vomit or have diarrhea after consuming milk, and enlargement of the liver and jaundice are common, sometimes progressing to cirrhosis. Cataracts will form, and lethargy and retarded mental development also are common. The blood-galactose level is markedly elevated, and galactose is found in the urine. The absence of the transferase in red blood cells is a definitive diagnostic criterion.

The most common treatment is to remove galactose (and lactose) from the diet. An enigma of galactosemia is that, although elimination of galactose from the diet prevents liver disease and cataract development, the majority of patients still suffer from central nervous system malfunction, most commonly a delayed acquisition of language skills. Female patients also display ovarian failure.

Cataract formation is better understood. A cataract is the clouding of the normally clear lens of the eye (Figure 16.15). If the transferase is not active in the lens of the eye, the presence of aldose reductase causes the accumulating galactose to be reduced to galactitol.

(A)



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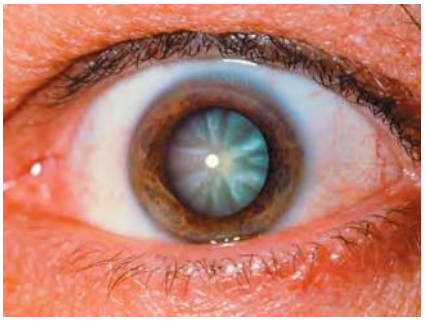
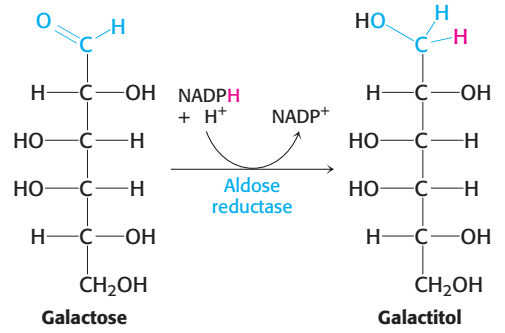


Figure 16.15 Cataracts are evident as the clouding of the lens. (A) A healthy eye. (B) An eye with a cataract. [(A) © Imageafter; (B) SPL/Photo Researchers.]



Galactitol is osmotically active, and water will diffuse into the lens, instigating the formation of cataracts. In fact, there is a high incidence of cataract formation with age in populations that consume substantial amounts of milk into adulthood.

16.2 The Glycolytic Pathway Is Tightly Controlled

The glycolytic pathway has a dual role: it degrades glucose to generate ATP and it provides building blocks for synthetic reactions, such as the formation of fatty acids. The rate of conversion of glucose into pyruvate is regulated to meet these two major cellular needs. *In metabolic pathways, enzymes catalyzing essentially irreversible reactions are potential sites of control.* In glycolysis, the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase are virtually irreversible; hence, these enzymes would be expected to have regulatory as well as catalytic roles. In fact, each of them serves as a control site. These enzymes become more active or less so in response to the reversible binding of allosteric effectors or covalent modification. In addition, the amounts of these important enzymes are varied by the regulation of transcription to meet changing metabolic needs. The time required for reversible allosteric control, regulation by phosphorylation, and transcriptional control is measured typically in milliseconds, seconds,

and hours, respectively. We will consider the control of glycolysis in two different tissues—skeletal muscle and liver.

Glycolysis in muscle is regulated to meet the need for ATP

Glycolysis in skeletal muscle provides ATP primarily to power contraction. Consequently, the primary control of muscle glycolysis is the energy charge of the cell—the ratio of ATP to AMP. Let us examine how each of the key regulatory enzymes responds to changes in the amounts of ATP and AMP present in the cell.

Phosphofructokinase. *Phosphofructokinase is the most important control site in the mammalian glycolytic pathway* (Figure 16.16). High levels of ATP allosterically inhibit the enzyme (a 340-kd tetramer). ATP binds to a specific regulatory site that is distinct from the catalytic site. The binding of ATP lowers the enzyme's affinity for fructose 6-phosphate. Thus, a high concentration of ATP converts the hyperbolic binding curve of fructose 6-phosphate into a sigmoidal one (Figure 16.17). AMP reverses the inhibitory action of ATP, and so *the activity of the enzyme increases when the ATP/AMP ratio is lowered.* In other words, *glycolysis is stimulated as the energy charge falls.* A decrease in pH also inhibits phosphofructokinase activity by augmenting the inhibitory effect of ATP. The pH might fall when muscle is functioning anaerobically, producing excessive quantities of lactic acid. The inhibitory effect protects the muscle from damage that would result from the accumulation of too much acid.

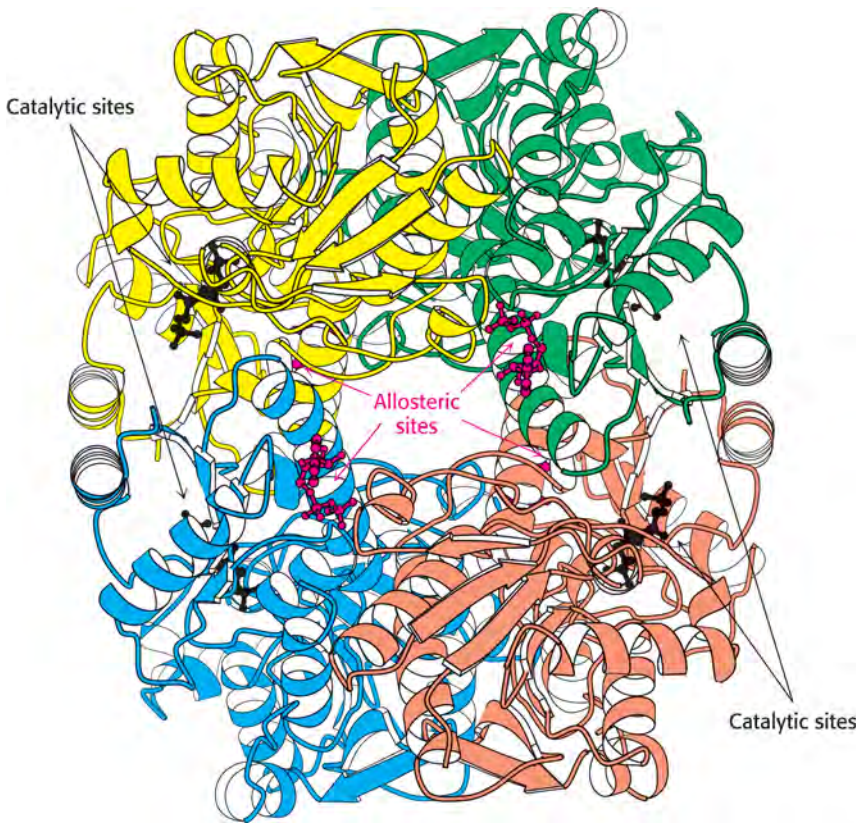


Figure 16.16 Structure of phosphofructokinase. The structure of phosphofructokinase from *E. coli* comprises a tetramer of four identical subunits. *Notice* the separation of the catalytic and allosteric sites. Each subunit of the human liver enzyme consists of two domains that are similar to the *E. coli* enzyme. [Drawn from 1PFK.pdb.]

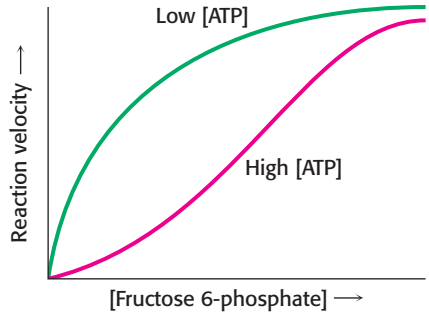


Figure 16.17 Allosteric regulation of phosphofructokinase. A high level of ATP inhibits the enzyme by decreasing its affinity for fructose 6-phosphate. AMP diminishes and citrate enhances the inhibitory effect of ATP.

Why is AMP and not ADP the positive regulator of phosphofructokinase? When ATP is being utilized rapidly, the enzyme *adenylate kinase* (Section 9.4) can form ATP from ADP by the following reaction:

$$\text{ADP} + \text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$$

Thus, some ATP is salvaged from ADP, and AMP becomes the signal for the low-energy state. Moreover, the use of AMP as an allosteric regulator provides an especially sensitive control. We can understand why by considering, first, that the total adenylate pool ([ATP], [ADP], [AMP]) in a cell is constant over the short term and, second, that the concentration of ATP is greater than that of ADP and the concentration of ADP is, in turn, greater than that of AMP. Consequently, small-percentage changes in [ATP] result in larger-percentage changes in the concentrations of the other adenylate nucleotides. This magnification of small changes in [ATP] to larger changes in [AMP] leads to tighter control by increasing the range of sensitivity of phosphofructokinase.

Hexokinase. Phosphofructokinase is the most prominent regulatory enzyme in glycolysis, but it is not the only one. Hexokinase, the enzyme catalyzing the first step of glycolysis, is inhibited by its product, glucose 6-phosphate. High concentrations of this molecule signal that the cell no longer requires glucose for energy or for the synthesis of glycogen, a storage form of glucose (Chapter 21), and the glucose will be left in the blood. A rise in glucose 6-phosphate concentration is a means by which phosphofructokinase communicates with hexokinase. When phosphofructokinase is inactive, the concentration of fructose 6-phosphate rises. In turn, the level of glucose 6-phosphate rises because it is in equilibrium with fructose 6-phosphate. Hence, *the inhibition of phosphofructokinase leads to the inhibition of hexokinase.*

Why is phosphofructokinase rather than hexokinase the pacemaker of glycolysis? The reason becomes evident on noting that glucose 6-phosphate is not solely a glycolytic intermediate. In muscle, glucose 6-phosphate can also be converted into glycogen. The first irreversible reaction unique to the glycolytic pathway, the *committed step* (Section 10.1), is the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate. Thus, it is highly appropriate for phosphofructokinase to be the primary control site in glycolysis. In general, *the enzyme catalyzing the committed step in a metabolic sequence is the most important control element in the pathway.*

Pyruvate kinase. Pyruvate kinase, the enzyme catalyzing the third irreversible step in glycolysis, controls the outflow from this pathway. This final step yields ATP and pyruvate, a central metabolic intermediate that can be oxidized further or used as a building block. ATP allosterically inhibits pyruvate kinase to slow glycolysis when the energy charge is high. Finally, alanine (synthesized in one step from pyruvate, Section 23.3) also allosterically inhibits pyruvate kinase—in this case, to signal that building blocks are abundant. When the pace of glycolysis increases, fructose 1,6-bisphosphate, the product of the preceding irreversible step in glycolysis, activates the kinase to enable it to keep pace with the oncoming high flux of intermediates. A summary of the regulation of glycolysis in resting and active muscle is shown in Figure 16.18.

The regulation of glycolysis in the liver illustrates the biochemical versatility of the liver

The liver has more-diverse biochemical functions than muscle. Significantly, the liver maintains blood-glucose levels: it stores glucose as glycogen when

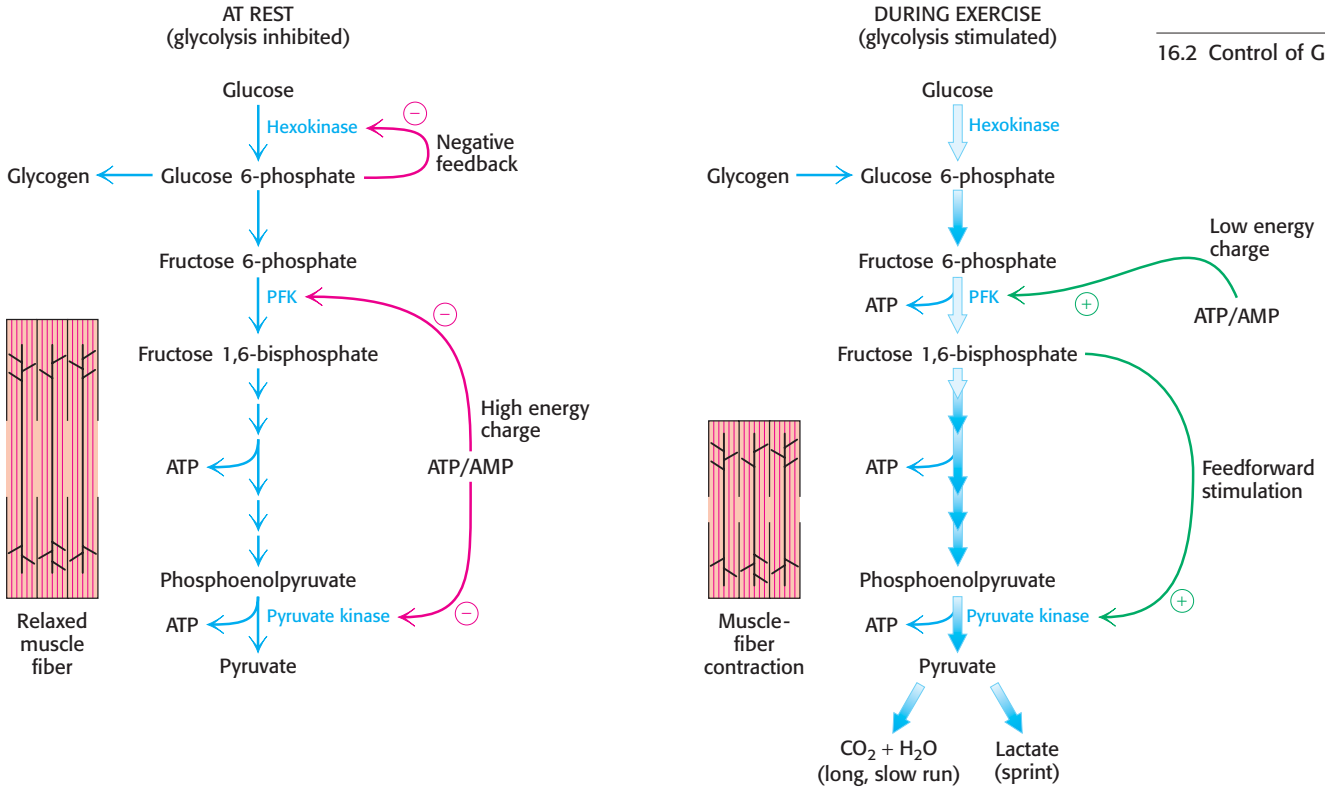


Figure 16.18 Regulation of glycolysis in muscle. At rest (left), glycolysis is not very active (thin arrows). The high concentration of ATP inhibits phosphofructokinase (PFK), pyruvate kinase, and hexokinase. Glucose 6-phosphate is converted into glycogen (Chapter 21). During exercise (right), the decrease in the ATP/AMP ratio resulting from muscle contraction activates phosphofructokinase and hence glycolysis. The flux down the pathway is increased, as represented by the thick arrows.

glucose is plentiful, and it releases glucose when supplies are low. It also uses glucose to generate reducing power for biosynthesis (Section 20.3) as well as to synthesize a host of biochemicals. So, although the liver has many of the regulatory features of muscle glycolysis, the regulation of glycolysis in the liver is more complex.

Phosphofructokinase. Regulation with respect to ATP is the same in the liver as in muscle. Low pH is not a metabolic signal for the liver enzyme, because lactate is not normally produced in the liver. Indeed, as we will see, lactate is converted into glucose in the liver.

Glycolysis also furnishes carbon skeletons for biosyntheses, and so a signal indicating whether building blocks are abundant or scarce should also regulate phosphofructokinase. In the liver, *phosphofructokinase is inhibited by citrate,* an early intermediate in the citric acid cycle (Chapter 17). A high level of citrate in the cytoplasm means that biosynthetic precursors are abundant, and so there is no need to degrade additional glucose for this purpose. Citrate inhibits phosphofructokinase by enhancing the inhibitory effect of ATP.

One means by which glycolysis in the liver responds to changes in blood glucose is through the signal molecule *fructose 2,6-bisphosphate* (F-2,6-BP), a potent activator of phosphofructokinase (Figure 16.19). In the liver, the concentration of fructose 6-phosphate rises when blood-glucose concentration is high, and the abundance of fructose 6-phosphate accelerates the synthesis of F-2,6-BP (Figure 16.20). Hence, *an abundance of fructose 6-phosphate leads to a higher concentration of F-2,6-BP.* The binding of

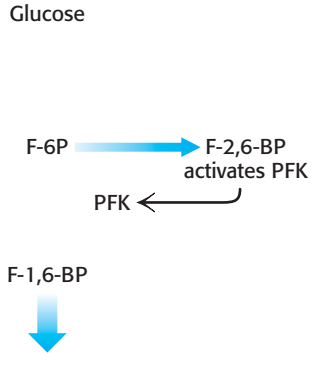


Figure 16.19 Regulation of phosphofructokinase by fructose 2,6-bisphosphate. In high concentrations, fructose 6-phosphate (F-6P) activates the enzyme phosphofructokinase (PFK) through an intermediary, fructose 2,6-bisphosphate (F-2,6-BP).

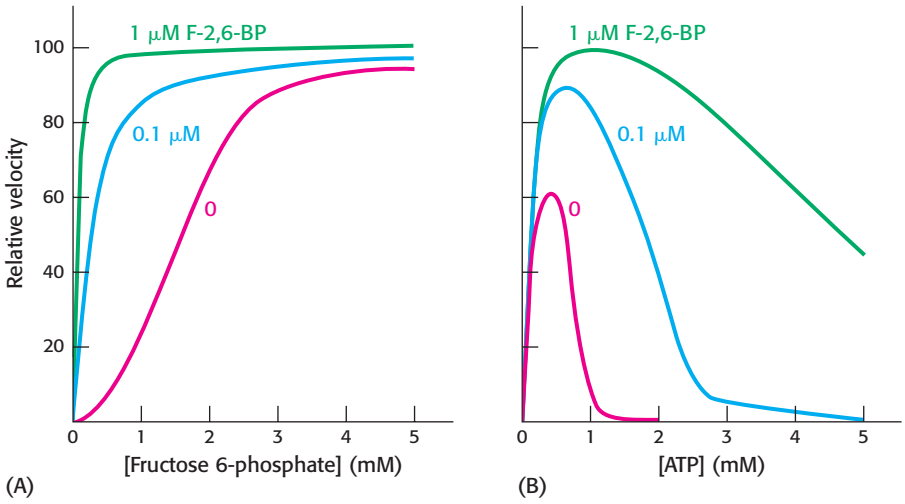
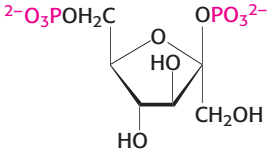


Figure 16.20 Activation of phosphofructokinase by fructose 2,6-bisphosphate. (A) The sigmoidal dependence of velocity on substrate concentration becomes hyperbolic in the presence of 1 μM fructose 2,6-bisphosphate. (B) ATP, acting as a substrate, initially stimulates the reaction. As the concentration of ATP increases, it acts as an allosteric inhibitor. The inhibitory effect of ATP is reversed by fructose 2,6-bisphosphate. [After E. Van Schaftingen, M. F. Jett, L. Hue, and H. G. Hers. *Proc. Natl. Acad. Sci. U. S. A.* 78:3483–3486, 1981.]



Fructose 2,6-bisphosphate (F-2,6-BP)

fructose 2,6-bisphosphate increases the affinity of phosphofructokinase for fructose 6-phosphate and diminishes the inhibitory effect of ATP. Glycolysis is thus accelerated when glucose is abundant. Such a process is called *feed-forward stimulation.* We will turn to the synthesis and degradation of this important regulatory molecule after we have considered gluconeogenesis.

Hexokinase. The hexokinase reaction in the liver is controlled as in the muscle. However, the liver, in keeping with its role as monitor of blood-glucose levels, possesses another specialized isozyme of hexokinase, called *glucokinase,* which is not inhibited by glucose 6-phosphate. Glucokinase phosphorylates glucose only when glucose is abundant because the affinity of glucokinase for glucose is about 50-fold lower than that of hexokinase. The role of glucokinase is to provide glucose 6-phosphate for the synthesis of glycogen and for the formation of fatty acids (Section 22.1). The low affinity of glucokinase for glucose in the liver gives the brain and muscles first call on glucose when its supply is limited, and it ensures that glucose will not be wasted when it is abundant. Glucokinase is also present in the β cells of the pancreas, where the increased formation of glucose 6-phosphate by glucokinase when blood-glucose levels are elevated leads to the secretion of the hormone insulin. Insulin signals the need to remove glucose from the blood for storage as glycogen or conversion into fat.

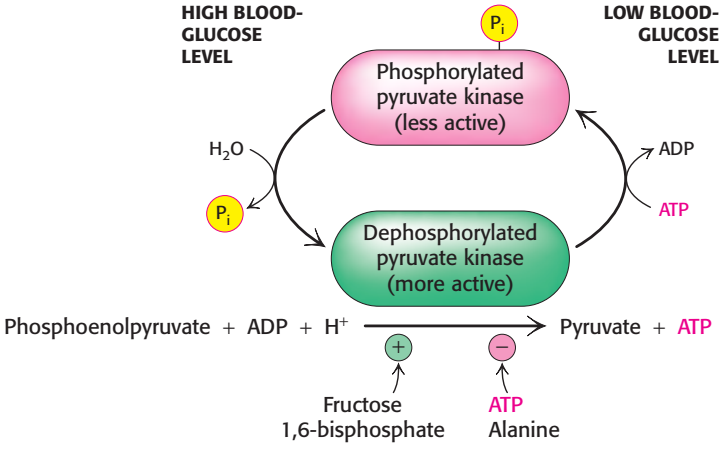


Figure 16.21 Control of the catalytic activity of pyruvate kinase. Pyruvate kinase is regulated by allosteric effectors and covalent modification.

Pyruvate kinase. Several isozymic forms of pyruvate kinase (a tetramer of 57-kd subunits) encoded by different genes are present in mammals: the L type predominates in the liver, and the M type in muscle and the brain. The L and M forms of pyruvate kinase have many properties in common. Indeed, the liver enzyme behaves much like the muscle enzyme with regard to allosteric regulation. However, the isozymic forms differ in their susceptibility to covalent modification. The catalytic properties of the L form—but not of the M form—are also controlled by reversible phosphorylation (Figure 16.21). When the blood-glucose level is low, the glucagon-triggered cyclic AMP cascade (p. 487) leads to the phosphorylation of pyruvate kinase, which diminishes its activity. This hormone-triggered phosphorylation prevents

Table 16.4 Family of glucose transporters

Name	Tissue location	K_M	Comments
GLUT1	All mammalian tissues	1 mM	Basal glucose uptake
GLUT2	Liver and pancreatic β cells	15–20 mM	In the pancreas, plays a role in the regulation of insulin In the liver, removes excess glucose from the blood
GLUT3	All mammalian tissues	1 mM	Basal glucose uptake
GLUT4	Muscle and fat cells	5 mM	Amount in muscle plasma membrane increases with endurance training
GLUT5	Small intestine	—	Primarily a fructose transporter

the liver from consuming glucose when it is more urgently needed by the brain and muscle. We see here a clear-cut example of how isoenzymes contribute to the metabolic diversity of different organs. We will return to the control of glycolysis after considering gluconeogenesis.

A family of transporters enables glucose to enter and leave animal cells

Several glucose transporters mediate the thermodynamically downhill movement of glucose across the plasma membranes of animal cells. Each member of this protein family, named GLUT1 to GLUT5, consists of a single polypeptide chain about 500 residues long (Table 16.4). Each glucose transporter has a 12-transmembrane-helix structure similar to that of lactose permease (Section 13.3).

The members of this family have distinctive roles:

1. GLUT1 and GLUT3, present in nearly all mammalian cells, are responsible for basal glucose uptake. Their K_M value for glucose is about 1 mM, significantly less than the normal serum-glucose level, which typically ranges from 4 mM to 8 mM. Hence, GLUT1 and GLUT3 continually transport glucose into cells at an essentially constant rate.

2. GLUT2, present in liver and pancreatic β cells, is distinctive in having a very high K_M value for glucose (15–20 mM). Hence, glucose enters these tissues at a biologically significant rate only when there is much glucose in the blood. The pancreas can sense the glucose level and accordingly adjust the rate of insulin secretion. The high K_M value of GLUT2 also ensures that glucose rapidly enters liver cells only in times of plenty.

3. GLUT4, which has a K_M value of 5 mM, transports glucose into muscle and fat cells. The number of GLUT4 transporters in the plasma membrane increases rapidly in the presence of insulin, which signals the fed state. Hence, insulin promotes the uptake of glucose by muscle and fat. Endurance exercise training increases the amount of this transporter present in muscle membranes.

4. GLUT5, present in the small intestine, functions primarily as a fructose transporter.

This family of transporters vividly illustrates how isoforms of a single protein can significantly shape the metabolic character of cells and contribute to their diversity and functional specialization. The transporters are members of a superfamily of transporters called the major facilitator (MF) superfamily. Members of this family transport sugars in organisms as diverse as *E. coli*, *Trypanosoma brucei* (a parasitic protozoan that causes sleeping sickness), and human beings. An elegant solution to the problem of

fuel transport evolved early and has been tailored to meet the needs of different organisms and even different tissues.

Cancer and exercise training affect glycolysis in a similar fashion

Tumors have been known for decades to display enhanced rates of glucose uptake and glycolysis. Indeed, rapidly growing tumor cells will metabolize glucose to lactate even in the presence of oxygen, a process called *aerobic glycolysis* or the "Warburg effect," after Otto Warburg, the biochemist who first noted this characteristic of cancer cells in the 1920s. In fact, tumors with a high glucose uptake are particularly aggressive, and the cancer is likely to have a poor prognosis. A nonmetabolizable glucose analog, 2-^{18}F-2-D-deoxyglucose, detectable by a combination of positron emission tomography (PET) and computer-aided tomography (CAT), easily visualizes tumors (Figure 16.22).

What selective advantage does aerobic glycolysis offer the tumor over the energetically more efficient oxidative phosphorylation? Research is being actively pursued to answer the question, but we can speculate on the benefits. First, aerobic glycolysis generates lactic acid that is then secreted. Acidification of the tumor environment has been shown to facilitate tumor invasion and inhibit the immune system from attacking the tumor. Second, the increased uptake of glucose and formation of glucose 6-phosphate provides substrates for another metabolic pathway, the pentose phosphate

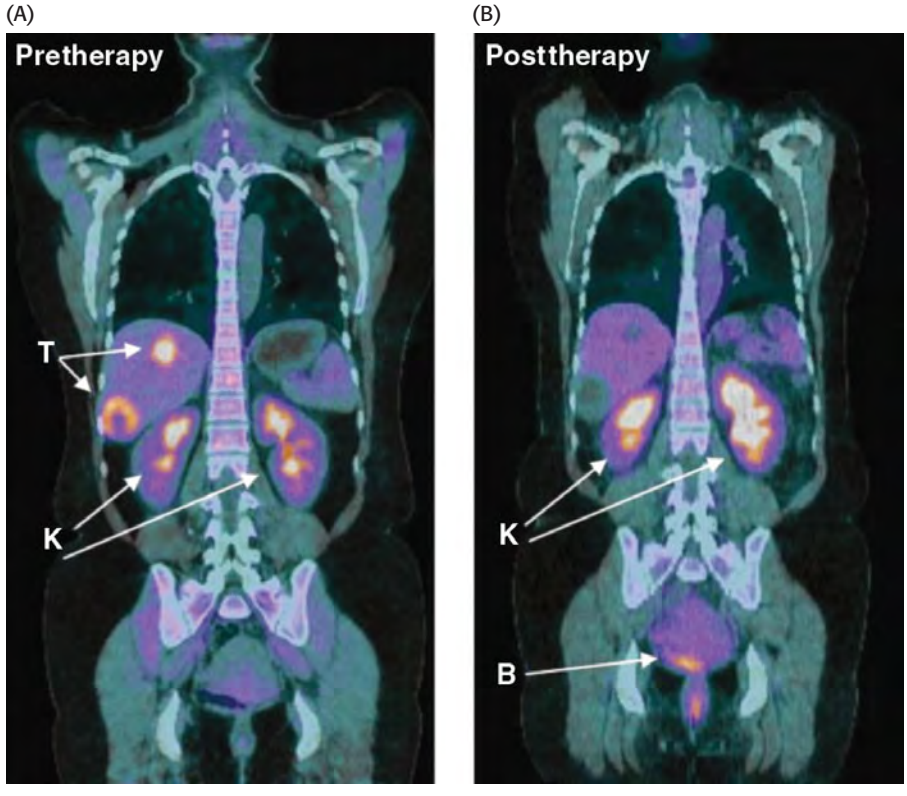


Figure 16.22 Tumors can be visualized with 2-^{18}F-2-D-deoxyglucose (FDG) and positron emission tomography. (A) A nonmetabolizable glucose analog infused into a patient and detected by a combination of positron emission and computer-aided tomography reveals the presence of a malignant tumor (T). (B) After 4 weeks of treatment with a tyrosine kinase inhibitor (Section 14.5), the tumor shows no uptake of FDG, indicating decreased metabolism. Excess FDG, which is excreted in the urine, also visualizes the kidney (K) and bladder (B). [Images courtesy of A. D. Van den Abbeele, Dana-Farber Cancer Institute, Boston.]

pathway (Chapter 20), that generates biosynthetic reducing power. Moreover, the pentose phosphate pathway, in cooperation with glycolysis, produces precursors for biomolecules necessary for growth, such as nucleotides. Finally, cancer cells grow more rapidly than the blood vessels that nourish them; thus, as solid tumors grow, the oxygen concentration in their environment falls. In other words, they begin to experience *hypoxia,* a deficiency of oxygen. The use of aerobic glycolysis reduces the dependence of cell growth on oxygen.

Hypoxia itself enhances tumor growth by activating a transcription factor, *hypoxia-inducible transcription factor* (HIF-1). HIF-1 increases the expression of most glycolytic enzymes and the glucose transporters GLUT1 and GLUT3 (Table 16.5). These adaptations by the cancer cells enable a tumor to survive until blood vessels can grow. HIF-1 also increases the expression of signal molecules, such as vascular endothelial growth factor (VEGF), that facilitate the growth of blood vessels that will provide nutrients to the cells (Figure 16.23). Without new blood vessels, a tumor would cease to grow and either die or remain harmlessly small. Efforts are underway to develop drugs that inhibit the growth of blood vessels in tumors.

What biochemical alterations facilitate the switch to aerobic glycolysis? Again, the answers are not complete, but changes in gene expression of isozymic forms of two glycolytic enzymes may be crucial. Tumor cells express an isozyme of hexokinase that binds to mitochondria. There, the enzyme has ready access to any ATP generated by oxidative phosphorylation and is no longer susceptible to feedback inhibition by its product, glucose 6-phosphate. An embryonic isozyme of pyruvate kinase also is expressed; it facilitates uses of glycolytic intermediates for biosynthetic reactions and is sensitive to growth-factor regulation.

Interestingly, anaerobic exercise training activates HIF-1, producing the same effects as those seen in the tumor—enhanced ability to generate ATP anaerobically and a stimulation of blood-vessel growth. These biochemical effects account for the improved athletic performance that results from training and demonstrate how behavior can affect biochemistry.

Table 16.5 Proteins in glucose metabolism encoded by genes regulated by hypoxia-inducible factor

GLUT1
GLUT3
Hexokinase
Phosphofructokinase
Aldolase
Glyceraldehyde 3-phosphate dehydrogenase
Phosphoglycerate kinase
Enolase
Pyruvate kinase

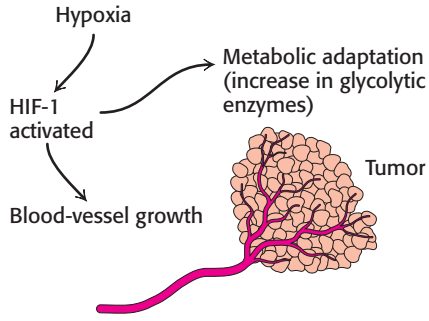


Figure 16.23 Alteration of gene expression in tumors owing to hypoxia. The hypoxic conditions inside a tumor mass lead to the activation of the hypoxia-inducible transcription factor (HIF-1), which induces metabolic adaptation (an increase in glycolytic enzymes) and activates angiogenic factors that stimulate the growth of new blood vessels. [After C. V. Dang and G. L. Semenza. *Trends Biochem. Sci.* 24:68–72, 1999.]

16.3 Glucose Can Be Synthesized from Noncarbohydrate Precursors

We now turn to the *synthesis of glucose from noncarbohydrate precursors,* a process called *gluconeogenesis.* Maintaining levels of glucose is important because the brain depends on glucose as its primary fuel and red blood cells use glucose as their only fuel. The daily glucose requirement of the brain in a typical adult human being is about 120 g, which accounts for most of the 160 g of glucose needed daily by the whole body. The amount of glucose present in body fluids is about 20 g, and that readily available from glycogen is approximately 190 g. Thus, the direct glucose reserves are sufficient to meet glucose needs for about a day. Gluconeogenesis is especially important during a longer period of fasting or starvation (Section 27.5).

The gluconeogenic pathway converts pyruvate into glucose. Noncarbohydrate precursors of glucose are first converted into pyruvate or enter the pathway at later intermediates such as oxaloacetate and dihydroxyacetone phosphate (Figure 16.24). The major noncarbohydrate precursors are *lactate, amino acids,* and *glycerol.* Lactate is formed by active skeletal muscle when the rate of glycolysis exceeds the rate of oxidative metabolism. Lactate is readily converted into pyruvate by the action of lactate dehydrogenase (p. 468). Amino acids are derived from proteins in the diet and,

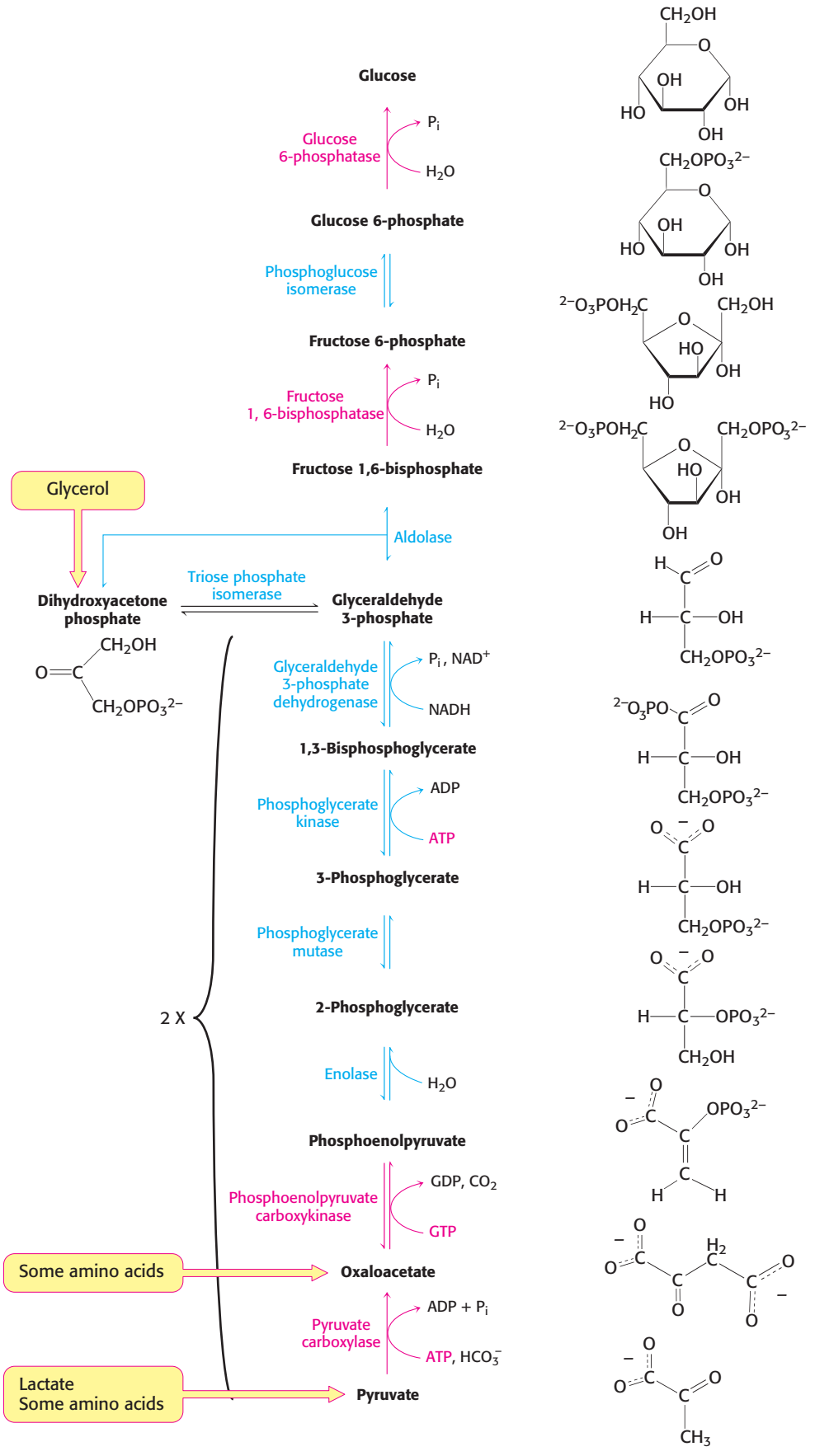
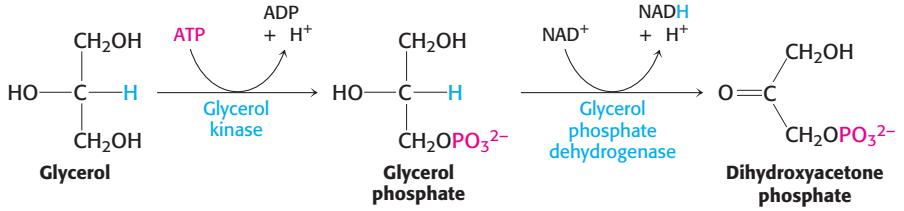


Figure 16.24 Pathway of gluconeogenesis. The distinctive reactions and enzymes of this pathway are shown in red. The other reactions are common to glycolysis. The enzymes for gluconeogenesis are located in the cytoplasm, except for pyruvate carboxylase (in the mitochondria) and glucose 6-phosphatase (membrane bound in the endoplasmic reticulum). The entry points for lactate, glycerol, and amino acids are shown.

during starvation, from the breakdown of proteins in skeletal muscle (Section 23.1). The hydrolysis of triacylglycerols (Section 22.2) in fat cells yields glycerol and fatty acids. Glycerol is a precursor of glucose, but animals cannot convert fatty acids into glucose, for reasons that will be given later. Glycerol may enter either the gluconeogenic or the glycolytic pathway at dihydroxyacetone phosphate.



The major site of gluconeogenesis is the *liver,* with a small amount also taking place in the *kidney.* Little gluconeogenesis takes place in the brain, skeletal muscle, or heart muscle. Rather, *gluconeogenesis in the liver and kidney helps to maintain the glucose level in the blood so that the brain and muscle can extract sufficient glucose from it to meet their metabolic demands.*

Gluconeogenesis is not a reversal of glycolysis

In glycolysis, glucose is converted into pyruvate; in gluconeogenesis, pyruvate is converted into glucose. However, *gluconeogenesis is not a reversal of glycolysis.* Several reactions must differ because the equilibrium of glycolysis lies far on the side of pyruvate formation. The actual ΔG for the formation of pyruvate from glucose is about -84 kJ mol^{-1} (-20 kcal mol^{-1}) under typical cellular conditions. Most of the decrease in free energy in glycolysis takes place in the three essentially irreversible steps catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{glucose 6-phosphate} + \text{ADP}$$
$$\Delta G = -33 \text{ kJ mol}^{-1} (-8.0 \text{ kcal mol}^{-1})$$

$$\text{Fructose 6-phosphate} + \text{ATP} \xrightarrow{\text{Phosphofructokinase}} \text{fructose 1,6-bisphosphate} + \text{ADP}$$
$$\Delta G = -22 \text{ kJ mol}^{-1} (-5.3 \text{ kcal mol}^{-1})$$

$$\text{Phosphoenolpyruvate} + \text{ADP} \xrightarrow{\text{Pyruvate kinase}} \text{pyruvate} + \text{ATP}$$
$$\Delta G = -17 \text{ kJ mol}^{-1} (-4.0 \text{ kcal mol}^{-1})$$

In gluconeogenesis, the following new steps bypass these virtually irreversible reactions of glycolysis:

1. *Phosphoenolpyruvate is formed from pyruvate by way of oxaloacetate* through the action of pyruvate carboxylase and phosphoenolpyruvate carboxykinase.

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \xrightarrow{\text{Pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i + 2\,H^+$$

$$\text{Oxaloacetate} + \text{GTP} \xrightarrow{\text{Phosphoenolpyruvate carboxykinase}} \text{phosphoenolpyruvate} + \text{GDP} + CO_2$$

2. *Fructose 6-phosphate is formed from fructose 1,6-bisphosphate by hydrolysis of the phosphate ester at carbon 1.* Fructose 1,6-bisphosphatase catalyzes this exergonic hydrolysis.

$$\text{Fructose 1,6-bisphosphate} + H_2O \longrightarrow \text{fructose 6-phosphate} + P_i$$

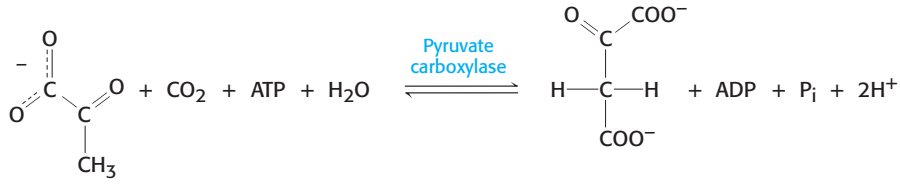
3. *Glucose is formed by the hydrolysis of glucose 6-phosphate* in a reaction catalyzed by glucose 6-phosphatase.

$$\text{Glucose 6-phosphate} + H_2O \longrightarrow \text{glucose} + P_i$$

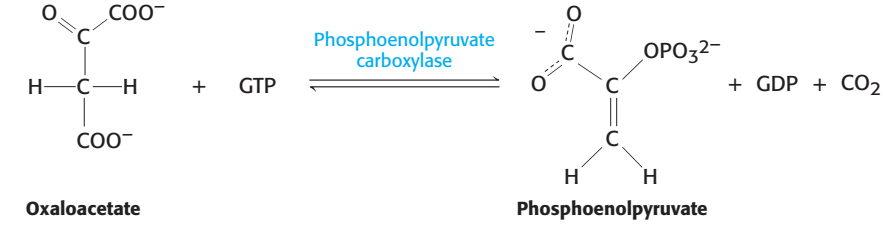
We will examine each of these steps in turn.

The conversion of pyruvate into phosphoenolpyruvate begins with the formation of oxaloacetate

The first step in gluconeogenesis is the carboxylation of pyruvate to form oxaloacetate at the expense of a molecule of ATP.



Then, oxaloacetate is decarboxylated and phosphorylated to yield phosphoenolpyruvate, at the expense of the high phosphoryl-transfer potential of GTP.



The first of these reactions takes place inside the mitochondria.

The first reaction is catalyzed by *pyruvate carboxylase* and the second by *phosphoenolpyruvate carboxykinase* (PEPCK). The sum of these reactions is

$$\text{Pyruvate} + \text{ATP} + \text{GTP} + H_2O \rightleftharpoons \text{phosphoenolpyruvate} + \text{ADP} + \text{GDP} + P_i + 2\,H^+$$

Pyruvate carboxylase is of special interest because of its structural, catalytic, and allosteric properties. The N-terminal 300 to 350 amino acids form an *ATP-grasp domain* (Figure 16.25), which is an ATP-activating domain found in many enzymes, to be considered in more detail when we examine nucleotide biosynthesis (Chapter 25). The C-terminal 80 amino acids constitute a biotin-binding domain (Figure 16.26) that we will see again in fatty acid synthesis (Section 22.4). *Biotin* is a covalently attached prosthetic

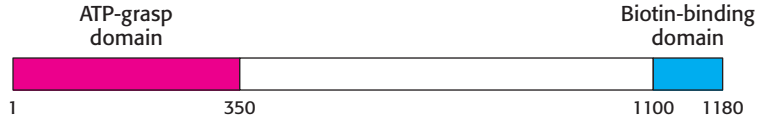


Figure 16.25 Domain structure of pyruvate carboxylase. The ATP-grasp domain activates HCO_3^- and transfers CO_2 to the biotin-binding domain. From there, the CO_2 is transferred to pyruvate generated in the central domain.

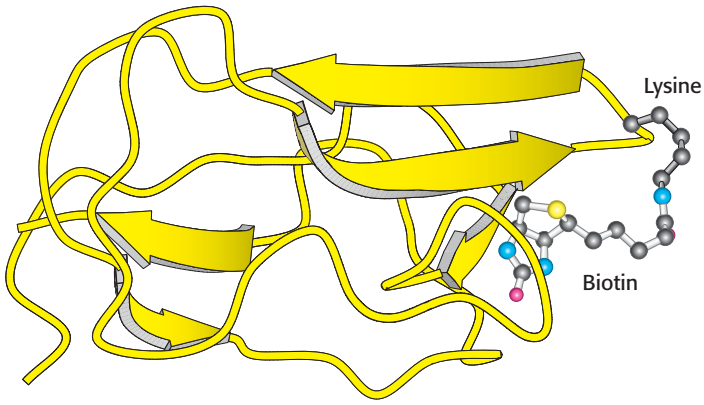


Figure 16.26 Biotin-binding domain of pyruvate carboxylase. This likely structure is based on the structure of the homologous domain of the enzyme acetyl CoA carboxylase (Section 22.4). *Notice* that the biotin is on a flexible tether, allowing it to move between the ATP-bicarbonate site and the pyruvate site. [Drawn from 1BDO.pdb.]

group, which serves as a *carrier of activated* CO_2. The carboxylate group of biotin is linked to the ε-amino group of a specific lysine residue by an amide bond (Figure 16.27). Note that biotin is attached to pyruvate carboxylase by a *long, flexible chain*.

The carboxylation of pyruvate takes place in three stages:

$$HCO_3^- + ATP \rightleftharpoons HOCO_2\text{-}PO_3^{2-} + ADP$$
$$\text{Biotin–enzyme} + HOCO_2\text{-}PO_3^{2-} \rightleftharpoons CO_2 - \text{biotin–enzyme} + P_i$$
$$CO_2 - \text{biotin–enzyme} + \text{pyruvate} \rightleftharpoons \text{biotin–enzyme} + \text{oxaloacetate}$$

Recall that, in aqueous solutions, CO_2 exists primarily as HCO_3^- with the aid of carbonic anhydrase (Section 9.2). HCO_3^- is activated to carboxyphosphate. This activated CO_2 is subsequently bonded to the N-1 atom of the biotin ring to form the carboxybiotin–enzyme intermediate (see Figure 16.27). The CO_2 attached to biotin is quite activated. The $\Delta G^{\circ\prime}$ for its cleavage

$$CO_2\text{–biotin–enzyme} + H^+ \rightarrow CO_2 + \text{biotin–enzyme}$$

is -20 kJ mol^{-1} (-4.7 kcal mol^{-1}). This negative $\Delta G^{\circ\prime}$ indicates that carboxybiotin is able to transfer CO_2 to acceptors without the input of additional free energy.

The activated carboxyl group is then transferred from carboxybiotin to pyruvate to form oxaloacetate. The long, flexible link between biotin and the enzyme enables this prosthetic group to rotate from one active site of the enzyme (the ATP-bicarbonate site) to the other (the pyruvate site).

The first partial reaction of pyruvate carboxylase, the formation of carboxybiotin, depends on the presence of acetyl CoA. *Biotin is not carboxylated unless acetyl CoA is bound to the enzyme.* Acetyl CoA has no effect on the second partial reaction. The allosteric activation of pyruvate carboxylase by acetyl CoA is an important physiological control mechanism that will be discussed in Section 17.4.

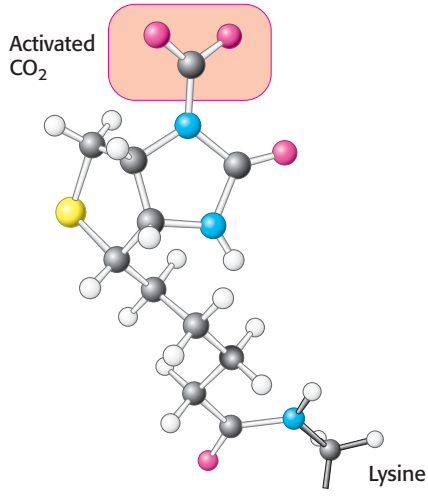


Figure 16.27 Structure of carboxybiotin.

Oxaloacetate is shuttled into the cytoplasm and converted into phosphoenolpyruvate

Pyruvate carboxylase is a mitochondrial enzyme, whereas the other enzymes of gluconeogenesis are present primarily in the cytoplasm. Oxaloacetate, the product of the pyruvate carboxylase reaction, must thus be transported to the cytoplasm to complete the pathway. Oxaloacetate is transported from a mitochondrion in the form of malate: oxaloacetate is reduced to malate inside the mitochondrion by an NADH-linked malate dehydrogenase. After malate has been transported across the mitochondrial membrane, it is reoxidized to oxaloacetate by an NAD^+-linked malate dehydrogenase in the

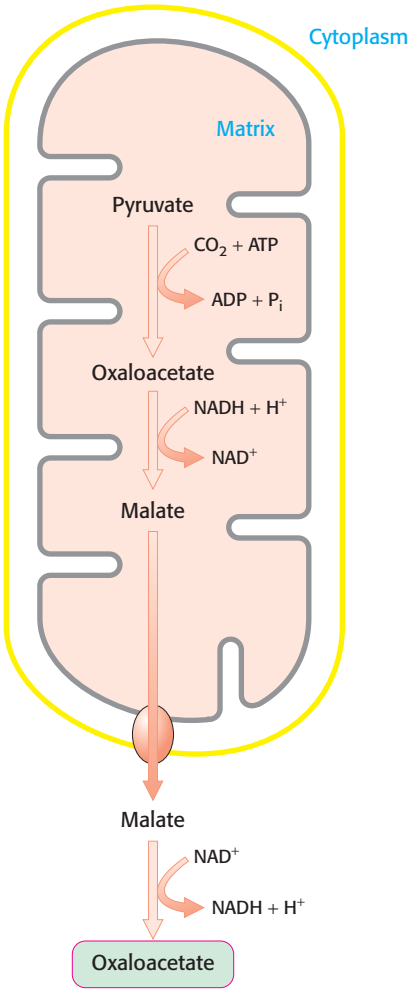


Figure 16.28 Compartmental cooperation. Oxaloacetate used in the cytoplasm for gluconeogenesis is formed in the mitochondrial matrix by the carboxylation of pyruvate. Oxaloacetate leaves the mitochondrion by a specific transport system (not shown) in the form of malate, which is reoxidized to oxaloacetate in the cytoplasm.

cytoplasm (Figure 16.28). The formation of oxaloacetate from malate also provides NADH for use in subsequent steps in gluconeogenesis. Finally, oxaloacetate is simultaneously *decarboxylated* and *phosphorylated* by phosphoenolpyruvate carboxykinase to generate phosphoenolpyruvate. The phosphoryl donor is GTP. The CO_2 that was added to pyruvate by pyruvate carboxylase comes off in this step.

Why is a carboxylation and a decarboxylation required to form phosphoenolpyruvate from pyruvate? Recall that, in glycolysis, the presence of a phosphoryl group traps the unstable enol isomer of pyruvate as phosphoenolpyruvate (p. 465). However, the addition of a phosphoryl group to pyruvate is a highly unfavorable reaction: the $\Delta G^{\circ\prime}$ of the reverse of the glycolytic reaction catalyzed by pyruvate kinase is $+31$ kJ mol^{-1} ($+7.5$ kcal mol^{-1}). In gluconeogenesis, the use of the carboxylation and decarboxylation steps results in a much more favorable $\Delta G^{\circ\prime}$. The formation of phosphoenolpyruvate from pyruvate in the gluconeogenic pathway has a $\Delta G^{\circ\prime}$ of $+0.8$ kJ mol^{-1} ($+0.2$ kcal mol^{-1}). A molecule of ATP is used to power the addition of a molecule of CO_2 to pyruvate in the carboxylation step. That CO_2 is then removed to power the formation of phosphoenolpyruvate in the decarboxylation step. *Decarboxylations often drive reactions that are otherwise highly endergonic.* This metabolic motif is used in the citric acid cycle (Chapter 17), the pentose phosphate pathway (Chapter 20), and fatty acid synthesis (Section 22.4).

The conversion of fructose 1,6-bisphosphate into fructose 6-phosphate and orthophosphate is an irreversible step

On formation, phosphoenolpyruvate is metabolized by the enzymes of glycolysis but in the reverse direction. These reactions are near equilibrium under intracellular conditions; so, when conditions favor gluconeogenesis, the reverse reactions will take place until the next irreversible step is reached. This step is the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate and P_i.

$$\text{Fructose 1,6-bisphosphate} + H_2O \xrightarrow{\text{Fructose 1,6-bisphosphatase}} \text{fructose 6-phosphate} + P_i$$

The enzyme responsible for this step is fructose 1,6-bisphosphatase. Like its glycolytic counterpart, it is an allosteric enzyme that participates in the regulation of gluconeogenesis. We will return to its regulatory properties later in the chapter.

The generation of free glucose is an important control point

The fructose 6-phosphate generated by fructose 1,6-bisphosphatase is readily converted into glucose 6-phosphate. In most tissues, gluconeogenesis ends here. Free glucose is not generated; rather, the glucose 6-phosphate is processed in some other fashion, notably to form glycogen. One advantage to ending gluconeogenesis at glucose 6-phosphate is that, unlike free glucose, the molecule is not transported out of the cell. To keep glucose inside the cell, the generation of free glucose is controlled in two ways. First, the enzyme responsible for the conversion of glucose 6-phosphate into glucose, *glucose 6-phosphatase,* is regulated. Second, the enzyme is present only in tissues whose metabolic duty is to maintain blood-glucose homeostasis—tissues that release glucose into the blood. These tissues are the liver and to a lesser extent the kidney.

This final step in the generation of glucose does not take place in the cytoplasm. Rather, glucose 6-phosphate is transported into the lumen of the endoplasmic reticulum, where it is hydrolyzed to glucose by glucose

6-phosphatase, which is bound to the membrane (Figure 16.29). An associated Ca^{2+}-binding stabilizing protein is essential for phosphatase activity. Glucose and P_i are then shuttled back to the cytoplasm by a pair of transporters. The glucose transporter in the endoplasmic reticulum membrane is like those found in the plasma membrane. It is striking that five proteins are needed to transform cytoplasmic glucose 6-phosphate into glucose.

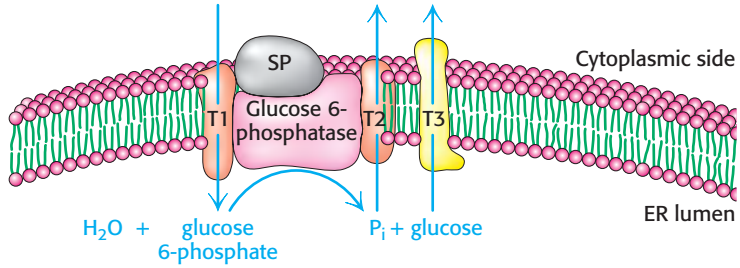


Figure 16.29 Generation of glucose from glucose 6-phosphate. Several endoplasmic reticulum (ER) proteins play a role in the generation of glucose from glucose 6-phosphate. T1 transports glucose 6-phosphate into the lumen of the ER, whereas T2 and T3 transport P_i and glucose, respectively, back into the cytoplasm. Glucose 6-phosphatase is stabilized by a Ca^{2+}-binding protein (SP). [After A. Buchell and I. D. Waddel. *Biochem. Biophys. Acta* 1092:129–137, 1991.]

Six high-transfer-potential phosphoryl groups are spent in synthesizing glucose from pyruvate

The formation of glucose from pyruvate is energetically unfavorable unless it is coupled to reactions that are favorable. Compare the stoichiometry of gluconeogenesis with that of the reverse of glycolysis.

The stoichiometry of gluconeogenesis is

$$2\,\text{Pyruvate} + 4\,\text{ATP} + 2\,\text{GTP} + 2\,\text{NADH} + 6\,H_2O \rightarrow \text{glucose} + 4\,\text{ADP} + 2\,\text{GDP} + 6\,P_i + 2\,NAD^+ + 2\,H^+$$
$$\Delta G^{\circ\prime} = -48\ \text{kJ mol}^{-1}(-11\ \text{kcal mol}^{-1})$$

In contrast, the stoichiometry for the reversal of glycolysis is

$$2\,\text{Pyruvate} + 2\,\text{ATP} + \text{NADH} + 2\,H_2O \rightarrow \text{glucose} + 2\,\text{ADP} + 2\,P_i + 2\,NAD^+ + 2\,H^+$$
$$\Delta G^{\circ\prime} = +84\ \text{kJ mol}^{-1}(+20\ \text{kcal mol}^{-1})$$

Note that *six* nucleoside triphosphate molecules are hydrolyzed to synthesize glucose from pyruvate in gluconeogenesis, whereas only *two* molecules of ATP are generated in glycolysis in the conversion of glucose into pyruvate. Thus, the extra cost of gluconeogenesis is four high-phosphoryl-transfer-potential molecules for each molecule of glucose synthesized from pyruvate. The four additional molecules having high phosphoryl-transfer potential are needed to turn an energetically unfavorable process (the reversal of glycolysis) into a favorable one (gluconeogenesis). Here we have a clear example of the coupling of reactions: NTP hydrolysis is used to power an energetically unfavorable reaction. The reactions of gluconeogenesis are summarized in Table 16.6.

Table 16.6 Reactions of gluconeogenesis

Step	Reaction
1	Pyruvate + CO_2 + ATP + H_2O ⟶ oxaloacetate + ADP + P_i + $2H^+$
2	Oxaloacetate + GTP ⇌ phosphoenolpyruvate + GDP + CO_2
3	Phosphoenolpyruvate + H_2O ⇌ 2-phosphoglycerate
4	2-Phosphoglycerate ⇌ 3-phosphoglycerate
5	3-Phosphoglycerate + ATP ⇌ 1,3-bisphosphoglycerate + ADP
6	1,3-Bisphosphoglycerate + NADH + H^+ ⇌ glyceraldehyde 3-phosphate + NAD^+ + P_i
7	Glyceraldehyde 3-phosphate ⇌ dihydroxyacetone phosphate
8	Glyceraldehyde 3-phosphate + dihydroxyacetone phosphate ⇌ fructose 1,6-bisphosphate
9	Fructose 1,6-bisphosphate + H_2O ⟶ fructose 6-phosphate + P_i
10	Fructose 6-phosphate ⇌ glucose 6-phosphate
11	Glucose 6-phosphate + H_2O ⟶ + glucose + P_i

16.4 Gluconeogenesis and Glycolysis Are Reciprocally Regulated

Gluconeogenesis and glycolysis are coordinated so that, within a cell, one pathway is relatively inactive while the other is highly active. If both sets of reactions were highly active at the same time, the net result would be the hydrolysis of four nucleoside triphosphates (two ATP molecules plus two GTP molecules) per reaction cycle. Both glycolysis and gluconeogenesis are highly exergonic under cellular conditions, and so there is no thermodynamic barrier to such simultaneous activity. However, the *amounts* and *activities* of the distinctive enzymes of each pathway are controlled so that both pathways are not highly active at the same time. The rate of glycolysis is also determined by the concentration of glucose, and the rate of gluconeogenesis by the concentrations of lactate and other precursors of glucose. The basic premise of the reciprocal regulation is that, when energy is needed, glycolysis will predominate. When there is a surplus of energy, gluconeogenesis will take over.

Energy charge determines whether glycolysis or gluconeogenesis will be most active

The first important regulation site is the interconversion of fructose 6-phosphate and fructose 1,6-bisphosphate (Figure 16.30). Consider first a situation in which energy is needed. In this case, the concentration of AMP is high. Under this condition, AMP stimulates phosphofructokinase but inhibits fructose 1,6-bisphosphatase. Thus, glycolysis is turned on and gluconeogenesis is inhibited. Conversely, high levels of ATP and citrate indicate that the energy charge is high and that biosynthetic intermediates

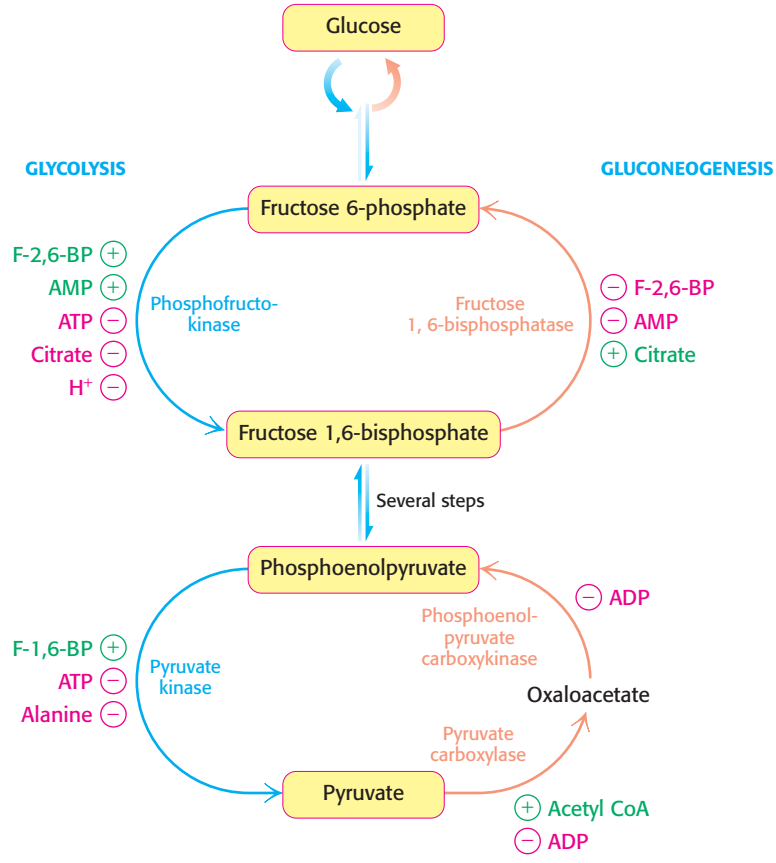


Figure 16.30 Reciprocal regulation of gluconeogenesis and glycolysis in the liver. The level of fructose 2,6-bisphosphate is high in the fed state and low in starvation. Another important control is the inhibition of pyruvate kinase by phosphorylation during starvation.

are abundant. ATP and citrate inhibit phosphofructokinase, whereas citrate activates fructose 1,6-bisphosphatase. Under these conditions, glycolysis is nearly switched off and gluconeogenesis is promoted. Why does citrate take part in this regulatory scheme? As we will see in Chapter 17, citrate reports on the status of the citric acid cycle, the primary pathway for oxidizing fuels in the presence of oxygen. High levels of citrate indicate an energy-rich situation and the presence of precursors for biosynthesis.

Glycolysis and gluconeogenesis are also reciprocally regulated at the interconversion of phosphoenolpyruvate and pyruvate in the liver. The glycolytic enzyme pyruvate kinase is inhibited by allosteric effectors ATP and alanine, which signal that the energy charge is high and that building blocks are abundant. Conversely, pyruvate carboxylase, which catalyzes the first step in gluconeogenesis from pyruvate, is inhibited by ADP. Likewise, ADP inhibits phosphoenolpyruvate carboxykinase. Pyruvate carboxylase is activated by acetyl CoA, which, like citrate, indicates that the citric acid cycle is producing energy and biosynthetic intermediates (Chapter 17). Hence, gluconeogenesis is favored when the cell is rich in biosynthetic precursors and ATP.

The balance between glycolysis and gluconeogenesis in the liver is sensitive to blood-glucose concentration

In the liver, rates of glycolysis and gluconeogenesis are adjusted to maintain blood-glucose levels. *The signal molecule fructose 2,6-bisphosphate strongly stimulates phosphofructokinase* (PFK) *and inhibits fructose 1,6-bisphosphatase* (p. 475). When blood glucose is low, fructose 2,6-bisphosphate loses a phosphoryl group to form fructose 6-phosphate, which no longer binds to PFK. How is the concentration of fructose 2,6-bisphosphate controlled to rise and fall with blood-glucose levels? Two enzymes regulate the concentration of this molecule: one phosphorylates fructose 6-phosphate and the other dephosphorylates fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate is formed in a reaction catalyzed by *phosphofructokinase 2* (PFK2), a different enzyme from phosphofructokinase. Fructose 6-phosphate is formed through the hydrolysis of fructose 2,6-bisphosphate by a specific phosphatase, *fructose bisphosphatase 2* (FBPase2). The striking finding is that *both PFK2 and FBPase2 are present in a single* 55-*kd polypeptide chain* (Figure 16.31). This *bifunctional enzyme* contains an N-terminal *regulatory domain,* followed by a *kinase domain* and a *phosphatase domain.* PFK2 resembles adenylate kinase in having a P-loop NTPase domain (Section 9.4), whereas FBPase2 resembles phosphoglycerate mutase (p. 464). Recall that the mutase is essentially a phosphatase. In the bifunctional enzyme, the phosphatase activity evolved to become specific for F-2,6-BP. The bifunctional enzyme itself probably arose by the fusion of genes encoding the kinase and phosphatase domains.

What controls whether PFK2 or FBPase2 dominates the bifunctional enzyme's activities in the liver? The activities of PFK2 and FBPase2 are reciprocally controlled by *phosphorylation of a single serine residue.* When glucose is scarce, such as during a night's fast, a rise in the blood level of the hormone glucagon triggers a cyclic AMP signal cascade (Section 14.1), leading to the phosphorylation of this bifunctional enzyme by protein kinase A (Figure 16.32). This covalent modification activates FBPase2 and inhibits PFK2, lowering the level of F-2,6-BP. Gluconeogenesis

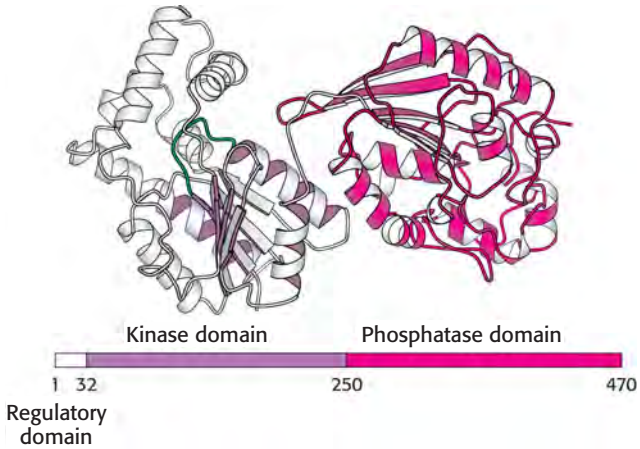


Figure 16.31 Domain structure of the bifunctional enzyme phosphofructokinase 2. The kinase domain (purple) is fused to the phosphatase domain (red). The kinase domain is a P-loop NTP hydrolase domain, as indicated by the purple shading (Section 9.4). The bar represents the amino acid sequence of the enzyme. [Drawn from 1BIF.pdb.]

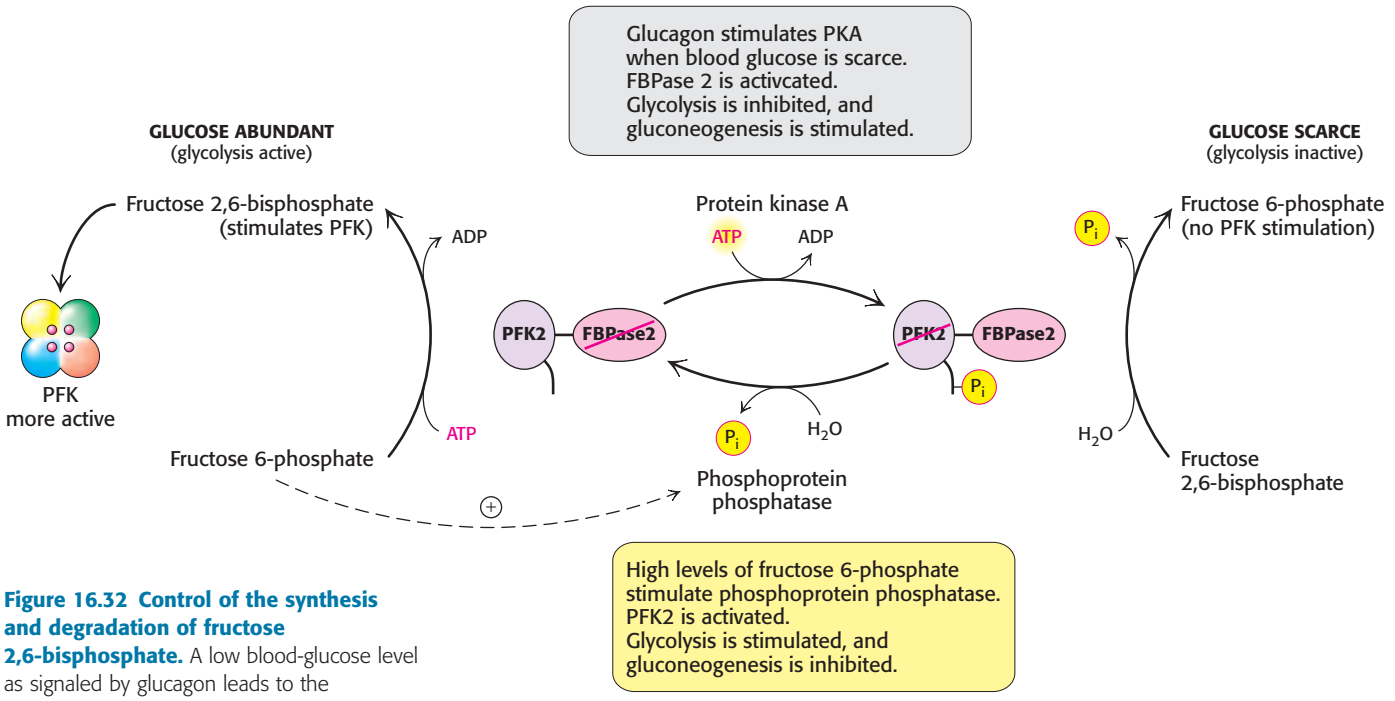


Figure 16.32 Control of the synthesis and degradation of fructose 2,6-bisphosphate. A low blood-glucose level as signaled by glucagon leads to the phosphorylation of the bifunctional enzyme and hence to a lower level of fructose 2,6-bisphosphate, slowing glycolysis. High levels of fructose 6-phosphate accelerate the formation of fructose 2,6-bisphosphate by facilitating the dephosphorylation of the bifunctional enzyme.

predominates. Glucose formed by the liver under these conditions is essential for the viability of the brain. Glucagon stimulation of protein kinase A also inactivates pyruvate kinase in the liver (p. 476).

Conversely, when blood-glucose levels are high, such as after a meal, gluconeogenesis is not needed. Insulin is secreted and initiates a signal pathway that activates a protein phosphatase, which removes the phosphoryl group from the bifunctional enzyme. This covalent modification activates PFK2 and inhibits FBPase2. The resulting rise in the level of F-2,6-BP accelerates glycolysis. The coordinated control of glycolysis and gluconeogenesis is facilitated by the location of the kinase and phosphatase domains on the same polypeptide chain as the regulatory domain.

The hormones insulin and glucagon also regulate the amounts of essential enzymes. These hormones alter gene expression primarily by changing the rate of transcription. Insulin levels rise subsequent to eating, when there is plenty of glucose for glycolysis. To encourage glycolysis, insulin stimulates the expression of phosphofructokinase, pyruvate kinase, and the bifunctional enzyme that makes and degrades F-2,6-BP. Glucagon rises during fasting, when gluconeogenesis is needed to replace scarce glucose. To encourage gluconeogenesis, glucagon inhibits the expression of the three regulated glycolytic enzymes and stimulates instead the production of two key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase and fructose 1,6-bisphosphatase. Transcriptional control in eukaryotes is much slower than allosteric control, taking hours or days instead of seconds to minutes. The richness and complexity of hormonal control are graphically displayed by the promoter of the phosphoenolpyruvate carboxykinase gene, which contains regulatory sequences that respond to insulin, glucagon (through the cAMP response elements), glucocorticoids, and thyroid hormone (Figure 16.33).

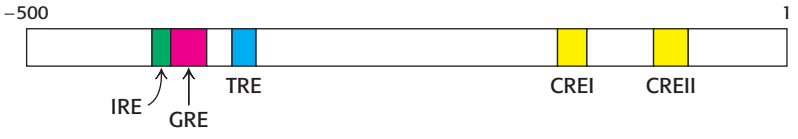


Figure 16.33 The promoter of the phosphoenolpyruvate carboxykinase gene. This promoter is approximately 500 bp in length and contains regulatory sequences (response elements) that mediate the action of several hormones. Abbreviations: IRE, insulin response element; GRE, glucocorticoid response element; TRE, thyroid hormone response element; CREI and CREII, cAMP response elements. [After M. M. McGrane, J. S. Jun, Y. M. Patel, and R. W. Hanson. *Trends Biochem. Sci.* 17:40–44, 1992.]

Substrate cycles amplify metabolic signals and produce heat

A pair of reactions such as the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate and its hydrolysis back to fructose 6-phosphate is called a *substrate cycle.* As already mentioned, both reactions are not simultaneously fully active in most cells, because of reciprocal allosteric controls. However, isotope-labeling studies have shown that some fructose 6-phosphate is phosphorylated to fructose 1,6-bisphosphate even during gluconeogenesis. There also is a limited degree of cycling in other pairs of opposed irreversible reactions. This cycling was regarded as an imperfection in metabolic control, and so substrate cycles have sometimes been called *futile cycles.* Indeed, there are pathological conditions, such as malignant hyperthermia, in which control is lost and both pathways proceed rapidly. One result is the rapid, uncontrolled hydrolysis of ATP, which generates heat.

Despite such extraordinary circumstances, substrate cycles now seem likely to be biologically important. One possibility is that *substrate cycles amplify metabolic signals.* Suppose that the rate of conversion of A into B is 100 and of B into A is 90, giving an initial net flux of 10. Assume that an allosteric effector increases the A → B rate by 20% to 120 and reciprocally decreases the B → A rate by 20% to 72. The new net flux is 48, and so a 20% change in the rates of the opposing reactions has led to a 380% increase in the net flux. In the example shown in Figure 16.34, this amplification is made possible by the rapid hydrolysis of ATP. The flux down the glycolytic pathway has been suggested to increase as much as 1000-fold at the initiation of intense exercise. Because the allosteric activation of enzymes alone seems unlikely to explain this increased flux, the existence of substrate cycles may partly account for the rapid rise in the rate of glycolysis.

The other potential biological role of substrate cycles is the generation of heat produced by the hydrolysis of ATP. In European bumblebees, cycling is used for both signal amplification and heat generation. Phosphofructokinase and fructose 1,6-bisphosphatase in a bee's flight muscle are simultaneously active. The cycling augments other means of thermogenesis, such as shivering, and amplifies the flux down the glycolytic pathway in preparation for the transition from rest to flight.

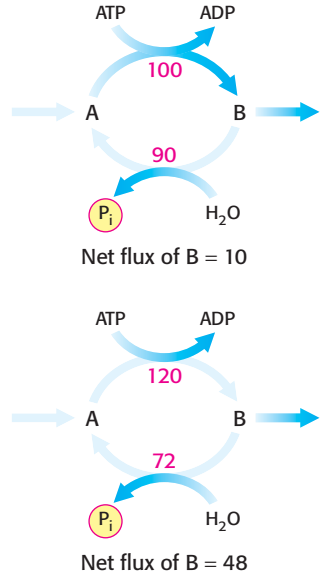


Figure 16.34 Substrate cycle. This ATP-driven cycle operates at two different rates. A small change in the rates of the two opposing reactions results in a large change in the *net* flux of product B.

Lactate and alanine formed by contracting muscle are used by other organs

Lactate produced by active skeletal muscle and erythrocytes is a source of energy for other organs. Erythrocytes lack mitochondria and can never oxidize glucose completely. In contracting skeletal muscle during vigorous exercise, the rate at which glycolysis produces pyruvate exceeds the rate at which the citric acid cycle oxidizes it. In these cells, lactate dehydrogenase reduces excess pyruvate to lactate to restore redox balance (p. 466). However, lactate is a dead end in metabolism. It must be converted back into pyruvate before it can be metabolized. Both pyruvate and lactate diffuse out of these cells through carriers into the blood. *In contracting skeletal muscle, the formation and release of lactate lets the muscle generate ATP in the absence of oxygen and shifts the burden of metabolizing lactate from muscle to other organs.* The pyruvate and lactate in the bloodstream have two fates. In one fate, the plasma membranes of some cells, particularly cells in cardiac muscle, contain carriers that make the cells highly permeable to lactate and pyruvate. These molecules diffuse from the blood into such permeable cells. Once inside these well-oxygenated cells, lactate can be reverted back to pyruvate and metabolized through the citric acid cycle and oxidative phosphorylation to generate ATP. The use of lactate in place of glucose by these

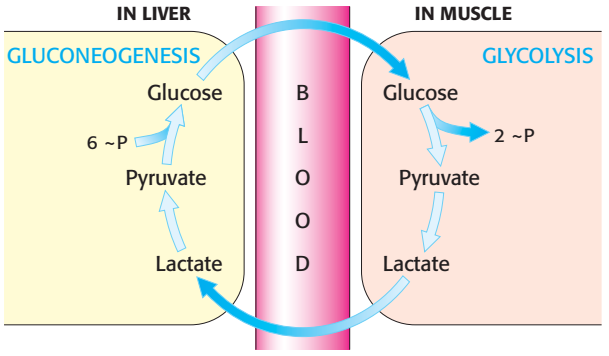


Figure 16.35 The Cori cycle. Lactate formed by active muscle is converted into glucose by the liver. This cycle shifts part of the metabolic burden of active muscle to the liver.

cells makes more circulating glucose available to the active muscle cells. In the other fate, excess lactate enters the liver and is converted first into pyruvate and then into glucose by the gluconeogenic pathway. *Contracting skeletal muscle supplies lactate to the liver, which uses it to synthesize and release glucose. Thus, the liver restores the level of glucose necessary for active muscle cells, which derive ATP from the glycolytic conversion of glucose into lactate. These reactions constitute the Cori cycle* (Figure 16.35).

Studies have shown that alanine, like lactate, is a major precursor of glucose in the liver. The alanine is generated in muscle when the carbon skeletons of some amino acids are used as fuels. The nitrogens from these amino acids are transferred to pyruvate to form alanine; the reverse reaction takes place in the liver. This process also helps maintain nitrogen balance. The interplay between glycolysis and gluconeogenesis is summarized in Figure 16.36, which shows how these pathways help meet the energy needs of different cell types.

Isozymic forms of lactate dehydrogenase in different tissues catalyze the interconversions of pyruvate and lactate (Section 10.2). Lactate dehydrogenase is a tetramer of two kinds of 35-kd subunits encoded by similar genes: the H type predominates in the heart, and the homologous M type in skeletal muscle and the liver. These subunits associate to form five types of tetramers: H_4, H_3M_1, H_2M_2, H_1M_3, and M_4. The H_4 isozyme (type 1) has higher affinity for substrates than that of the M_4 isozyme (type 5) and, unlike M_4, is allosterically inhibited by high levels of pyruvate. The other isozymes have intermediate properties, depending on

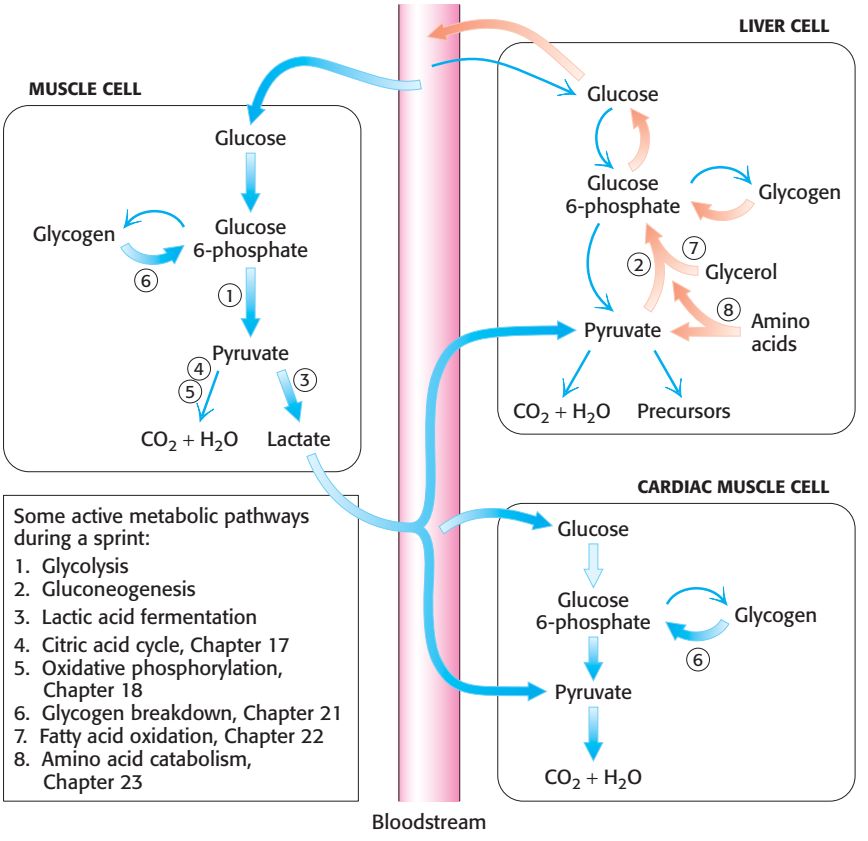


Figure 16.36 PATHWAY INTEGRATION: Cooperation between glycolysis and gluconeogenesis during a sprint. Glycolysis and gluconeogenesis are coordinated, in a tissue-specific fashion, to ensure that the energy needs of all cells are met. Consider a sprinter. In skeletal leg muscle, glucose will be metabolized aerobically to CO_2 and H_2O or, more likely (thick arrows) during a sprint, anaerobically to lactate. In cardiac muscle, the lactate can be converted into pyruvate and used as a fuel, along with glucose, to power the heartbeats to keep the sprinter's blood flowing. Gluconeogenesis, a primary function of the liver, will be taking place rapidly (thick arrows) to ensure that enough glucose is present in the blood for skeletal and cardiac muscle, as well as for other tissues. Glycogen, glycerol, and amino acids are other sources of energy that we will learn about in later chapters.

the ratio of the two kinds of chains. The H_4 isozyme oxidizes lactate to pyruvate, which is then used as a fuel by the heart through aerobic metabolism. Indeed, heart muscle never functions anaerobically. In contrast, M_4 is optimized to operate in the reverse direction, to convert pyruvate into lactate to allow glycolysis to proceed under anaerobic conditions. We see here an example of how gene duplication and divergence generate a series of homologous enzymes that foster metabolic cooperation between organs.

Glycolysis and gluconeogenesis are evolutionarily intertwined

The metabolism of glucose has ancient origins. Organisms living in the early biosphere depended on the anaerobic generation of energy until significant amounts of oxygen began to accumulate 2 billion years ago. Glycolytic enzymes were most likely derived independently rather than by gene duplication, because glycolytic enzymes with similar properties do not have similar amino acid sequences. Although there are four kinases and two isomerases in the pathway, both sequence and structural comparisons do not suggest that these sets of enzymes are related to one another by divergent evolution. The common dinucleotide-binding domain found in the dehydrogenases (see Figure 16.12) and the αβ barrels are the only major recurring elements.

We can speculate on the relationship between glycolysis and gluconeogenesis if we think of glycolysis as consisting of two segments: the metabolism of hexoses (the upper segment) and the metabolism of trioses (the lower segment). The enzymes of the upper segment are different in some species and are missing entirely in some archaea, whereas enzymes of the lower segment are quite conserved. In fact, four enzymes of the lower segment are present in all species. *This lower part of the pathway is common to glycolysis and gluconeogenesis.* This common part of the two pathways may be the oldest part, constituting the core to which the other steps were added. The upper part would have varied according to the sugars that were available to evolving organisms in particular niches. Interestingly, this core part of carbohydrate metabolism can generate triose precursors for ribose sugars, a component of RNA and a critical requirement for the RNA world. Thus, we are left with the unanswered question, Was the original core pathway used for energy conversion or biosynthesis?

Summary

16.1 Glycolysis Is an Energy-Conversion Pathway in Many Organisms

Glycolysis is the set of reactions that converts glucose into pyruvate. The 10 reactions of glycolysis take place in the cytoplasm. In the first stage, glucose is converted into fructose 1,6-bisphosphate by a phosphorylation, an isomerization, and a second phosphorylation reaction. Fructose 1,6-bisphosphate is then cleaved by aldolase into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, which are readily interconvertible. Two molecules of ATP are consumed per molecule of glucose in these reactions, which are the prelude to the net synthesis of ATP. In the second stage, ATP is generated. Glyceraldehyde 3-phosphate is oxidized and phosphorylated to form 1,3-bisphosphoglycerate, an acyl phosphate with a high phosphoryl-transfer potential. This molecule transfers a phosphoryl group to ADP to form ATP and 3-phosphoglycerate. A phosphoryl shift and a dehydration form phosphoenolpyruvate, a second intermediate with a high phosphoryl-transfer potential. Another molecule of ATP is generated as phosphoenolpyruvate is converted into pyruvate. There is a net gain of two

molecules of ATP in the formation of two molecules of pyruvate from one molecule of glucose.

The electron acceptor in the oxidation of glyceraldehyde 3-phosphate is NAD^+, which must be regenerated for glycolysis to continue. In aerobic organisms, the NADH formed in glycolysis transfers its electrons to O_2 through the electron-transport chain, which thereby regenerates NAD^+. Under anaerobic conditions and in some microorganisms, NAD^+ is regenerated by the reduction of pyruvate to lactate. In other microorganisms, NAD^+ is regenerated by the reduction of pyruvate to ethanol. These two processes are examples of fermentations.

16.2 The Glycolytic Pathway Is Tightly Controlled

The glycolytic pathway has a dual role: it degrades glucose to generate ATP, and it provides building blocks for the synthesis of cellular components. The rate of conversion of glucose into pyruvate is regulated to meet these two major cellular needs. Under physiological conditions, the reactions of glycolysis are readily reversible except for those catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase. Phosphofructokinase, the most important control element in glycolysis, is inhibited by high levels of ATP and citrate, and it is activated by AMP and fructose 2,6-bisphosphate. In the liver, this bisphosphate signals that glucose is abundant. Hence, phosphofructokinase is active when either energy or building blocks are needed. Hexokinase is inhibited by glucose 6-phosphate, which accumulates when phosphofructokinase is inactive. ATP and alanine allosterically inhibit pyruvate kinase, the other control site, and fructose 1,6-bisphosphate activates the enzyme. Consequently, pyruvate kinase is maximally active when the energy charge is low and glycolytic intermediates accumulate.

16.3 Glucose Can Be Synthesized from Noncarbohydrate Precursors

Gluconeogenesis is the synthesis of glucose from noncarbohydrate sources, such as lactate, amino acids, and glycerol. Several of the reactions that convert pyruvate into glucose are common to glycolysis. Gluconeogenesis, however, requires four new reactions to bypass the essential irreversibility of three reactions in glycolysis. In two of the new reactions, pyruvate is carboxylated in mitochondria to oxaloacetate, which in turn is decarboxylated and phosphorylated in the cytoplasm to phosphoenolpyruvate. Two molecules having high phosphoryl-transfer potential are consumed in these reactions, which are catalyzed by pyruvate carboxylase and phosphoenolpyruvate carboxykinase. Pyruvate carboxylase contains a biotin prosthetic group. The other distinctive reactions of gluconeogenesis are the hydrolyses of fructose 1,6-bisphosphate and glucose 6-phosphate, which are catalyzed by specific phosphatases. The major raw materials for gluconeogenesis by the liver are lactate and alanine produced from pyruvate by active skeletal muscle. The formation of lactate during intense muscular activity buys time and shifts part of the metabolic burden from muscle to the liver.

16.4 Gluconeogenesis and Glycolysis Are Reciprocally Regulated

Gluconeogenesis and glycolysis are reciprocally regulated so that one pathway is relatively inactive while the other is highly active. Phosphofructokinase and fructose 1,6-bisphosphatase are key control points. Fructose 2,6-bisphosphate, an intracellular signal molecule present

at higher levels when glucose is abundant, activates glycolysis and inhibits gluconeogenesis by regulating these enzymes. Pyruvate kinase and pyruvate carboxylase are regulated by other effectors so that both are not maximally active at the same time. Allosteric regulation and reversible phosphorylation, which are rapid, are complemented by transcriptional control, which takes place in hours or days.

Key Terms

glycolysis (p. 453)
lactic acid fermentation (p. 453)
alcoholic fermentation (p. 453)
gluconeogenesis (p. 453)
α-amylase (p. 454)
hexokinase (p. 455)
kinase (p. 457)
phosphofructokinase (PFK) (p. 458)
thioester intermediate (p. 462)
substrate-level phosphorylation (p. 464)
phosphoglycerate mutase (p. 464)
enol phosphate (p. 465)
pyruvate kinase (p. 465)
fermentation (p. 466)
obligate anaerobe (p. 468)
Rossmann fold (p. 469)
committed step (p. 474)
feedforward stimulation (p. 476)
aerobic glycolysis (p. 478)
pyruvate carboxylase (p. 482)
biotin (p. 482)
glucose 6-phosphatase (p. 484)
bifunctional enzyme (p. 487)
substrate cycle (p. 489)
Cori cycle (p. 490)

Problems

1. *Gross versus net*. The gross yield of ATP from the metabolism of glucose to two molecules of pyruvate is four molecules of ATP. However, the net yield is only two molecules of ATP. Why are the gross and net values different?

2. *Who takes? Who gives?* Lactic acid fermentation and alcoholic fermentation are oxidation–reduction reactions. Identify the ultimate electron donor and electron acceptor.

3. *ATP yield.* Each of the following molecules is processed by glycolysis to lactate. How much ATP is generated from each molecule?

(a) Glucose 6-phosphate
(b) Dihydroxyacetone phosphate
(c) Glyceraldehyde 3-phosphate
(d) Fructose
(e) Sucrose

4. *Enzyme redundancy?* Why is it advantageous for the liver to have both hexokinase and glucokinase to phosphorylate glucose?

5. *Corporate sponsors*. Some of the early research on glycolysis was supported by the brewing industry. Why would the brewing industry be interested in glycolysis?

6. *Recommended daily allowance*. The recommended daily allowance for the vitamin niacin is 15 mg per day. How would glycolysis be affected by niacin deficiency?

7. *Who's on first?* Although both hexokinase and phosphofructokinase catalyze irreversible steps in glycolysis and the hexokinase-catalyzed step is first, phosphofructokinase is nonetheless the pacemaker of glycolysis. What does this information tell you about the fate of the glucose 6-phosphate formed by hexokinase?

8. *The tortoise and the hare*. Why is the regulation of phosphofructokinase by energy charge not as important in the liver as it is in muscle?

9. *Running in reverse*. Why can't the reactions of the glycolytic pathway simply be run in reverse to synthesize glucose?

10. *Road blocks*. What reactions of glycolysis are not readily reversible under intracellular conditions?

11. *No pickling*. Why is it in the muscle's best interest to export lactic acid into the blood during intense exercise?

12. *Après vous*. Why is it physiologically advantageous for the pancreas to use GLUT2, with a high K_M, as the transporter that allows glucose entry into β cells?

13. *Bypass*. In the liver, fructose can be converted into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate without passing through the phosphofructokinase-regulated reaction. Show the reactions that make this conversion possible. Why might ingesting high levels of fructose have deleterious physiological effects?

14. *Trouble ahead*. Suppose that a microorganism that was an obligate anaerobe suffered a mutation that resulted in the loss of triose phosphate isomerase activity. How would this loss affect the ATP yield of fermentation? Could such an organism survive?

15. *Kitchen chemistry.* Sucrose is commonly used to preserve fruits. Why is glucose not suitable for preserving foods?

16. *Tracing carbon atoms 1.* Glucose labeled with ^{14}C at C-1 is incubated with the glycolytic enzymes and necessary cofactors.

(a) What is the distribution of ^{14}C in the pyruvate that is formed? (Assume that the interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate is very rapid compared with the subsequent step.)
(b) If the specific activity of the glucose substrate is 10 mCi $mmol^{-1}$ (millicuries per mole, a measure of radioactivity per mole), what is the specific activity of the pyruvate that is formed?

17. *Lactic acid fermentation.* (a) Write a balanced equation for the conversion of glucose into lactate. (b) Calculate the standard free-energy change of this reaction by using the data given in Table 16.1 and the fact that $\Delta G^{\circ\prime}$ is -25 kJ mol^{-1} (-6 kcal mol^{-1}) for the following reaction:

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{lactate} + \text{NAD}^+$$

What is the free-energy change (ΔG, not $\Delta G^{\circ\prime}$) of this reaction when the concentrations of reactants are: glucose, 5 mM; lactate, 0.05 mM; ATP, 2 mM; ADP, 0.2 mM; and Pi, 1 mM?

18. *High potential.* What is the equilibrium ratio of phosphoenolpyruvate to pyruvate under standard conditions when [ATP]/[ADP] = 10?

19. *Hexose–triose equilibrium.* What are the equilibrium concentrations of fructose 1,6-bisphosphate, dihydroxyacetone phosphate, and glyceraldehyde 3-phosphate when 1 mM fructose 1,6-bisphosphate is incubated with aldolase under standard conditions?

20. *Double labeling.* 3-Phosphoglycerate labeled uniformly with ^{14}C is incubated with 1,3-BPG labeled with ^{32}P at C-1. What is the radioisotope distribution of the 2,3-BPG that is formed on addition of BPG mutase?

21. *An informative analog.* Xylose has the same structure as that of glucose except that it has a hydrogen atom at G-5 in place of a hydroxymethyl group. The rate of ATP hydrolysis by hexokinase is markedly enhanced by the addition of xylose. Why?

22. *Distinctive sugars.* The intravenous infusion of fructose into healthy volunteers leads to a two- to fivefold increase in the level of lactate in the blood, a far greater increase than that observed after the infusion of the same amount of glucose.

(a) Why is glycolysis more rapid after the infusion of fructose?
(b) Fructose has been used in place of glucose for intravenous feeding. Why is this use of fructose unwise?

23. *It is not hard to meet expenses. They are everywhere.* What energetic barrier prevents glycolysis from simply running in reverse to synthesis glucose? What is the energetic cost to overcome this barrier?

24. *Waste not, want not.* Why is the conversion of lactic acid from the blood into glucose in the liver in an organism's best interest?

25. *Road blocks bypassed.* How are the irreversible reactions of glycolysis bypassed in gluconeogenesis?

26. *Pointlessness averted.* What are the regulatory means that prevent high levels of activity in glycolysis and gluconeogenesis simultaneously?

27. *Different needs.* Liver is primarily a gluconeogenic tissue, whereas muscle is primarily glycolytic. Why does this division of labor make good physiological sense?

28. *Metabolic mutants.* What would be the effect on an organism's ability to use glucose as an energy source if a mutation inactivated glucose 6-phosphatase in the liver?

29. *Never let me go.* Why does the lack of glucose 6-phosphatase activity in the brain and muscle make good physiological sense?

30. *Counting high-energy compounds 1.* How many NTP molecules are required for the synthesis of one molecule of glucose from two molecules of pyruvate? How many NADH molecules?

31. *Counting high-energy compounds 2.* How many NTP molecules are required to synthesize glucose from each of the following compounds?

(a) Glucose 6-phosphate
(b) Fructose 1,6-bisphosphate
(c) Two molecules of oxaloacetate
(d) Two molecules of dihydroxyacetone phosphate

32. *Lending a hand.* How might enzymes that remove amino groups from alanine and aspartate contribute to gluconeogenesis?

33. *More metabolic mutants.* Predict the effect of each of the following mutations on the pace of glycolysis in liver cells:

(a) Loss of the allosteric site for ATP in phosphofructokinase
(b) Loss of the binding site for citrate in phosphofructokinase
(c) Loss of the phosphatase domain of the bifunctional enzyme that controls the level of fructose 2,6-bisphosphate
(d) Loss of the binding site for fructose 1,6-bisphosphate in pyruvate kinase

34. *Yet another metabolic mutant.* What are the likely consequences of a genetic disorder rendering fructose 1,6-bisphosphatase in the liver less sensitive to regulation by fructose 2,6-bisphosphate?

35. *Biotin snatcher.* Avidin, a 70-kd protein in egg white, has very high affinity for biotin. In fact, it is a highly

specific inhibitor of biotin enzymes. Which of the following conversions would be blocked by the addition of avidin to a cell homogenate?

(a) Glucose → pyruvate
(b) Pyruvate → glucose
(c) Oxaloacetate → glucose
(d) Malate → oxaloacetate
(e) Pyruvate → oxaloacetate
(f) Glyceraldehyde 3-phosphate → fructose 1,6-bisphosphate

36. *Tracing carbon atoms 2.* If cells synthesizing glucose from lactate are exposed to CO_2 labeled with ^{14}C, what will be the distribution of label in the newly synthesized glucose?

37. *Arsenate poisoning.* Arsenate (AsO_4^{3-}) closely resembles P_i in structure and reactivity. In the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase, arsenate can replace phosphate in attacking the energy-rich thioester intermediate. The product of this reaction, 1-arseno-3-phosphoglycerate, is unstable. It and other acyl arsenates are rapidly and spontaneously hydrolyzed. What is the effect of arsenate on energy generation in a cell?

38. *Reduce, reuse, recycle.* In the conversion of glucose into two molecules of lactate, the NADH generated earlier in the pathway is oxidized to NAD^+. Why is it not to the cell's advantage to simply make more NAD^+ so that the regeneration would not be necessary? After all, the cell would save much energy because it would no longer need to synthesize lactic acid dehydrogenase.

39. *Adenylate kinase again.* Adenylate kinase, an enzyme considered in great detail in Chapter 9, is responsible for interconverting the adenylate nucleotide pool:

$$ADP + ADP \rightleftharpoons ATP + AMP$$

The equilibrium constant for this reaction is close to 1, inasmuch as the number of phosphoanhydride bonds is the same on each side of the equation. Using the equation for the equilibrium constant for this reaction, show why changes in [AMP] are a more effective indicator of the adenylate pool than [ATP].

40. *Working at cross-purposes?* Gluconeogenesis takes place during intense exercise, which seems counterintuitive. Why would an organism synthesize glucose and at the same time use glucose to generate energy?

41. *Powering pathways.* Compare the stoichiometries of glycolysis and gluconeogenesis. Recall that the input of one ATP equivalent changes the equilibrium constant of a reaction by a factor of about 10^8 (Section 15.2). By what factor do the additional high-phosphoryl-transfer compounds alter the equilibrium constant of gluconeogenesis?

Mechanism Problem

42. *Argument by analogy.* Propose a mechanism for the conversion of glucose 6-phosphate into fructose 6-phosphate by phosphoglucose isomerase based on the mechanism of triose phosphate isomerase.

Chapter Integration Problems

43. *Not just for energy.* People with galactosemia display central nervous system abnormalities even if galactose is eliminated from the diet. The precise reason for it is not known. Suggest a plausible explanation.

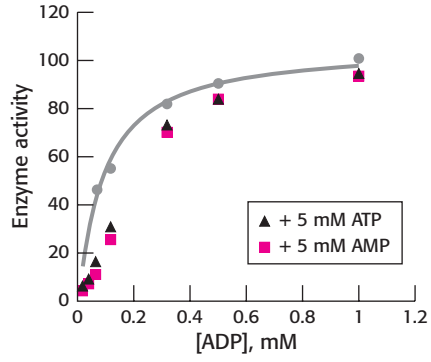
44. *State function.* Fructose 2,6-bisphosphate is a potent stimulator of phosphofructokinase. Explain how fructose 2,6-bisphosphate might function in the concerted model for allosteric enzymes.

Data Interpretation Problems

45. *Now, that's unusual.* Phosphofructokinase has recently been isolated from the hyperthermophilic archaeon *Pyrococcus furiosus.* It was subjected to standard biochemical analysis to determine basic catalytic parameters. The processes under study were of the form

$$\text{Fructose 6-phosphate} + (x - P_i) \rightarrow \text{fructose 1,6-bisphosphate} + (x)$$

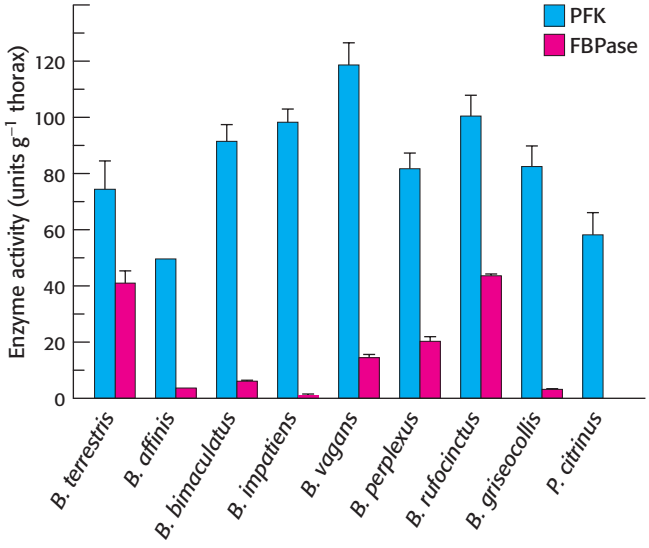
The assay measured the increase in fructose 1,6-bisphosphate. Selected results are shown in the adjoining graph.



[Data from J. E. Tuininga et al. *J. Biol. Chem.* 274:21023–21028, 1999.]

(a) How does the *P. furiosus* phosphofructokinase differ from the phosphofructokinase considered in this chapter?
(b) What effects do AMP and ATP have on the reaction with ADP?

46. *Cool bees.* In principle, a futile cycle that includes phosphofructokinase and fructose 2,6-bisphosphatase could be used to generate heat. The heat could be used to warm tissues. For instance, certain bumblebees have been reported



[After J. F. Staples, E. L. Koen, and T. M. Laverty, *J. Exp. Biol.* 207:749–754, 2004, p. 751.]

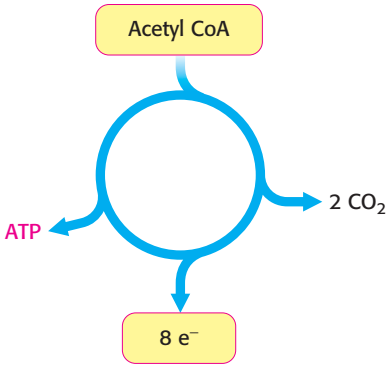
to use such a futile cycle to warm their flight muscles on cool mornings.

Scientists undertook a series of experiments to determine if a number of species of bumblebee use this futile cycle. Their approach was to measure the activity of PFK and F-1,6-BPase in flight muscle.

(a) What was the rationale for comparing the activities of these two enzymes?
(b) The data at the left show the activites of both enzymes for a variety of bumblebee species (genera *Bombus* and *Psithyrus*). Do these results support the notion that bumblebees use futile cycles to generate heat? Explain.
(c) In which species might futile cycling take place? Explain your reasoning.
(d) Do these results prove that futile cycling does not participate in heat generation?

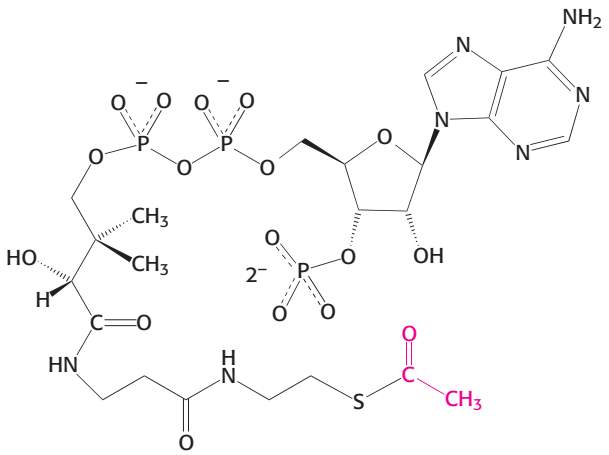
CHAPTER 17

The Citric Acid Cycle



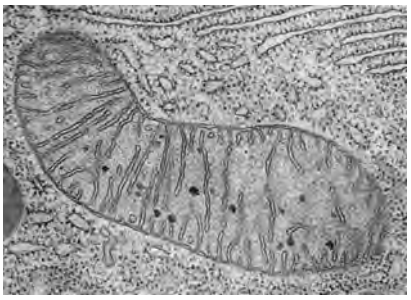
Roundabouts, or traffic circles, function as hubs to facilitate traffic flow. The citric acid cycle is the biochemical hub of the cell, oxidizing carbon fuels, usually in the form of acetyl CoA, as well as serving as a source of precursors for biosynthesis. [(Left) Lynn Saville/Getty Images.]

The metabolism of glucose to pyruvate in glycolysis, an anaerobic process, harvests but a fraction of the ATP available from glucose. Most of the ATP generated in metabolism is provided by the *aerobic* processing of glucose. This process starts with the complete oxidation of glucose derivatives to carbon dioxide. This oxidation takes place in a series of reactions called the *citric acid cycle,* also known as the *tricarboxylic acid* (TCA) *cycle* or the *Krebs cycle.* The citric acid cycle is the *final common pathway for the oxidation of fuel molecules*—carbohydrates, fatty acids, and amino acids. Most fuel molecules enter the cycle as *acetyl coenzyme A*.



Acetyl coenzyme A (Acetyl CoA)

OUTLINE



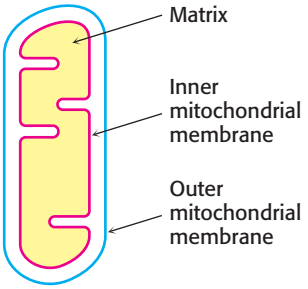


Figure 17.1 Mitochondrion. The double membrane of the mitochondrion is evident in this electron micrograph. The numerous invaginations of the inner mitochondrial membrane are called cristae. The oxidative decarboxylation of pyruvate and the sequence of reactions in the citric acid cycle take place within the matrix. [(Left) Omikron/Photo Researchers.]

Under aerobic conditions, the pyruvate generated from glucose is oxidatively decarboxylated to form acetyl CoA. In eukaryotes, the reactions of the citric acid cycle take place inside mitochondria (Figure 17.1), in contrast with those of glycolysis, which take place in the cytoplasm.

The citric acid cycle harvests high-energy electrons

The citric acid cycle is the central metabolic hub of the cell. It is the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group or a component of the citric acid cycle. The cycle is also an important source of precursors for the building blocks of many other molecules such as amino acids, nucleotide bases, and porphyrin (the organic component of heme). The citric acid cycle component, oxaloacetate, is also an important precursor to glucose (Section 16.3).

What is the function of the citric acid cycle in transforming fuel molecules into ATP? Recall that fuel molecules are carbon compounds that are capable of being oxidized—that is, of losing electrons (Chapter 15). The citric acid cycle includes a series of oxidation–reduction reactions that result in the oxidation of an acetyl group to two molecules of carbon dioxide. This oxidation generates high-energy electrons that will be used to power the synthesis of ATP. *The function of the citric acid cycle is the harvesting of high-energy electrons from carbon fuels.*

The overall pattern of the citric acid cycle is shown in Figure 17.2. A four-carbon compound (oxaloacetate) condenses with a two-carbon acetyl unit to yield a six-carbon tricarboxylic acid. The six-carbon compound releases CO_2 twice in two successive oxidative decarboxylations that yield high-energy electrons. A four-carbon compound remains. This four-carbon compound is further processed to regenerate oxaloacetate, which can initiate another round of the cycle. Two carbon atoms enter the cycle as an acetyl unit and two carbon atoms leave the cycle in the form of two molecules of CO_2.

Note that the citric acid cycle itself neither generates a large amount of ATP nor includes oxygen as a reactant (Figure 17.3). Instead, the citric acid cycle removes electrons from acetyl CoA and uses these electrons to form NADH and $FADH_2$. Three hydride ions (hence, six electrons) are transferred to three molecules of nicotinamide adenine dinucleotide (NAD^+), and one pair of hydrogen atoms (hence, two electrons) is transferred to one molecule of flavin adenine dinucleotide (FAD). These electron carriers yield nine molecules of ATP when they are oxidized by O_2 in *oxidative phosphorylation* (Chapter 18). Electrons released in the reoxidation of NADH and $FADH_2$ flow through a series of membrane proteins (referred to as the *electron-transport chain*) to generate a proton gradient across the membrane. These protons then flow through ATP synthase to generate ATP from ADP and inorganic phosphate.

The citric acid cycle, in conjunction with oxidative phosphorylation, provides the vast preponderance of energy used by aerobic cells—in human

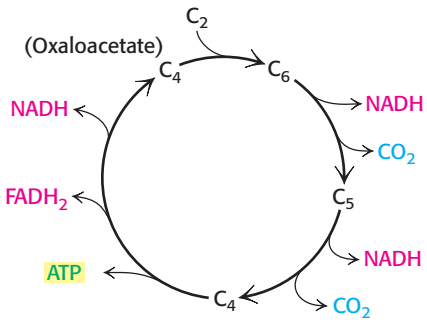


Figure 17.2 Overview of the citric acid cycle. The citric acid cycle oxidizes two-carbon units, producing two molecules of CO_2, one molecule of ATP, and high-energy electrons in the form of NADH and $FADH_2$.

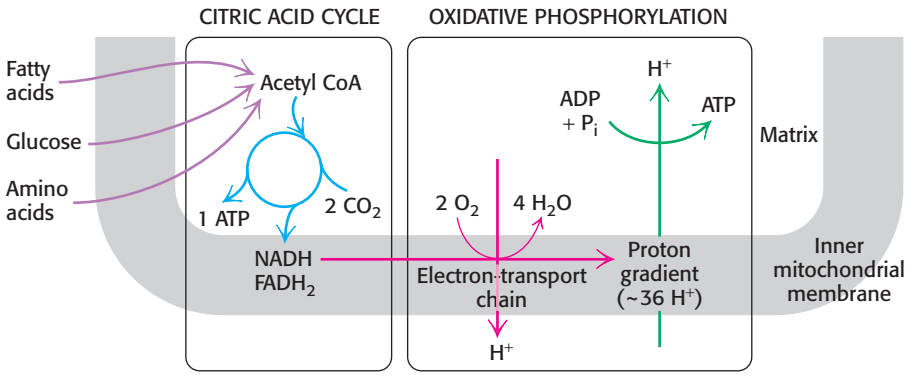


Figure 17.3 Cellular respiration. The citric acid cycle constitutes the first stage in cellular respiration, the removal of high-energy electrons from carbon fuels in the form of NADH and $FADH_2$ (left). These electrons reduce O_2 to generate a proton gradient (red pathway), which is used to synthesize ATP (green pathway). The reduction of O_2 and the synthesis of ATP constitute oxidative phosphorylation.

beings, greater than 90%. It is highly efficient because the oxidation of a limited number of citric acid cycle molecules can generate large amounts of NADH and $FADH_2$. Note in Figure 17.2 that the four-carbon molecule, oxaloacetate, that initiates the first step in the citric acid cycle is regenerated at the end of one passage through the cycle. Thus, one molecule of oxaloacetate is capable of participating in the oxidation of many acetyl molecules.

17.1 Pyruvate Dehydrogenase Links Glycolysis to the Citric Acid Cycle

Carbohydrates, most notably glucose, are processed by glycolysis into pyruvate (Chapter 16). Under anaerobic conditions, the pyruvate is converted into lactate or ethanol, depending on the organism. Under aerobic conditions, the pyruvate is transported into mitochondria by a specific carrier protein embedded in the mitochondrial membrane. In the mitochondrial matrix, pyruvate is oxidatively decarboxylated by the *pyruvate dehydrogenase complex* to form acetyl CoA.

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

This irreversible reaction is the link between glycolysis and the citric acid cycle (Figure 17.4). Note that the pyruvate dehydrogenase complex produces CO_2 and captures high-transfer-potential electrons in the form of NADH. Thus, the pyruvate dehydrogenase reaction has many of the key features of the reactions of the citric acid cycle itself.

The pyruvate dehydrogenase complex is a large, highly integrated complex of three distinct enzymes (Table 17.1). Pyruvate dehydrogenase complex is a member of a family of homologous complexes that include the citric acid cycle enzyme α-ketoglutarate dehydrogenase complex (p. 507). These complexes are giant, larger than ribosomes, with molecular masses ranging from 4 million to 10 million daltons (Figure 17.5). As we will see,

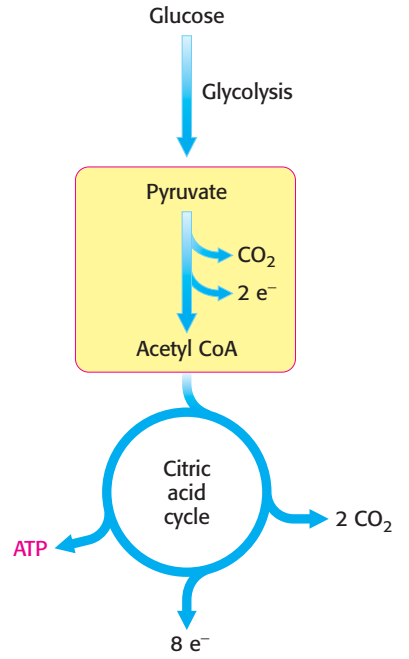


Figure 17.4 The link between glycolysis and the citric acid cycle. Pyruvate produced by glycolysis is converted into acetyl CoA, the fuel of the citric acid cycle.

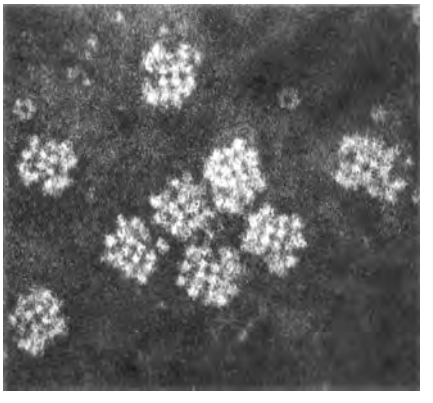


Figure 17.5 Electron micrograph of the pyruvate dehydrogenase complex from *E. coli*. [Courtesy of Dr. Lester Reed.]

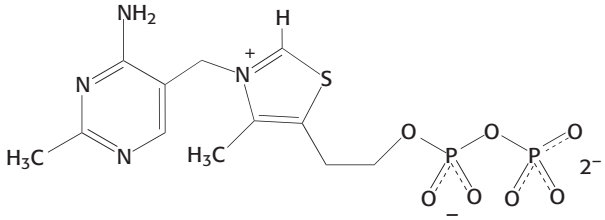
Table 17.1 Pyruvate dehydrogenase complex of *E. coli*

Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	24	Lipoamide	Transfer of acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	12	FAD	Regeneration of the oxidized form of lipoamide

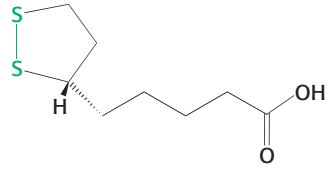
their elaborate structures allow groups to travel from one active site to another, connected by tethers to the core of the structure.

Mechanism: The synthesis of acetyl coenzyme A from pyruvate requires three enzymes and five coenzymes

The mechanism of the pyruvate dehydrogenase reaction is wonderfully complex, more so than is suggested by its simple stoichiometry. The reaction requires the participation of the three enzymes of the pyruvate dehydrogenase complex and five coenzymes. The coenzymes *thiamine pyrophosphate* (TPP), *lipoic acid*, and *FAD* serve as catalytic cofactors, and CoA and NAD^+ are stoichiometric cofactors, cofactors that function as substrates.

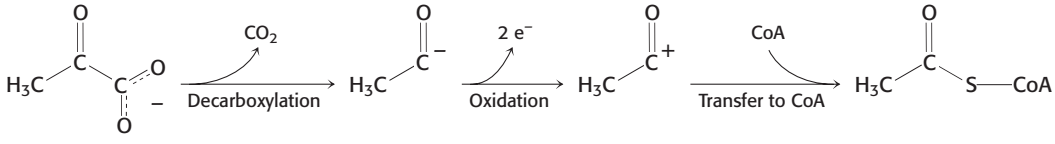


Thiamine pyrophosphate (TPP)



Lipoic acid

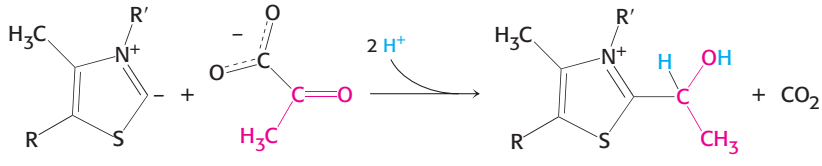
The conversion of pyruvate into acetyl CoA consists of three steps: decarboxylation, oxidation, and transfer of the resultant acetyl group to CoA.



Pyruvate **Acetyl CoA**

These steps must be coupled to preserve the free energy derived from the decarboxylation step to drive the formation of NADH and acetyl CoA.

1. *Decarboxylation*. Pyruvate combines with TPP and is then decarboxylated to yield hydroxyethyl-TPP (Figure 17.6).



Carbanion of TPP **Pyruvate** **Hydroxyethyl-TPP**

This reaction is catalyzed by the *pyruvate dehydrogenase component* (E_1) of the multienzyme complex. TPP is the prosthetic group of the pyruvate dehydrogenase component.

2. *Oxidation*. The hydroxyethyl group attached to TPP is *oxidized* to form an acetyl group while being simultaneously transferred to lipoamide, a derivative of lipoic acid that is linked to the side chain of a lysine residue by an amide linkage. Note that this transfer results in the formation of an energy-rich thioester bond.

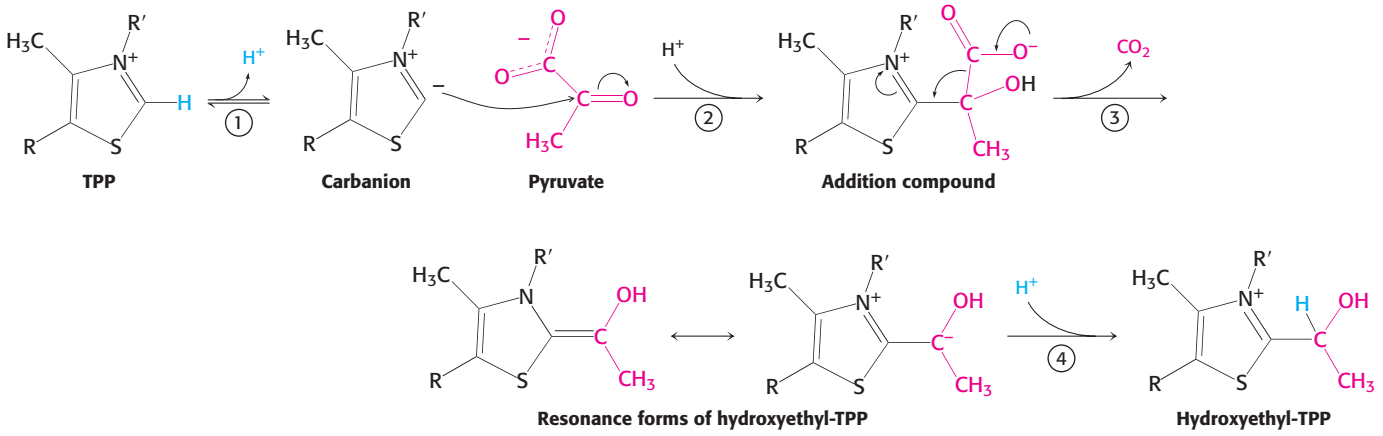
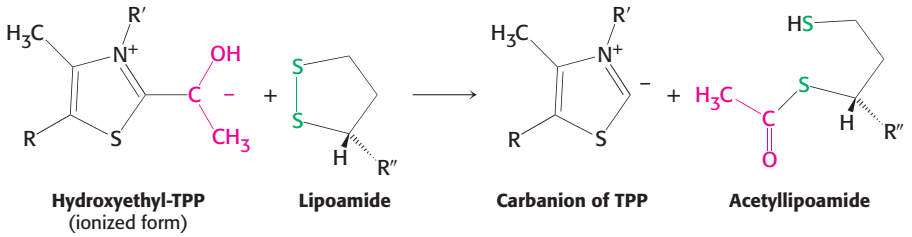
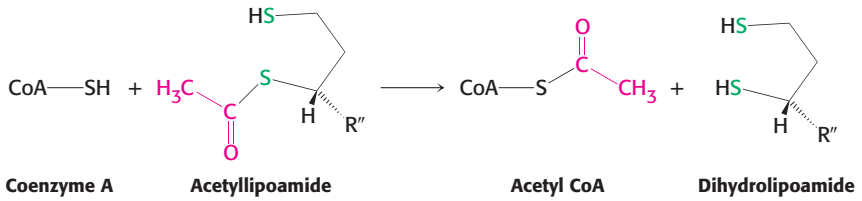


Figure 17.6 Mechanism of the E_1 decarboxylation reaction. E_1 is the pyruvate dehydrogenase component of the pyruvate dehydrogenase complex. A key feature of the prosthetic group, TPP, is that the carbon atom between the nitrogen and sulfur atoms in the thiazole ring is much more acidic than most =CH– groups, with a pK_a value near 10. (1) This carbon center ionizes to form a *carbanion*. (2) The carbanion readily adds to the carbonyl group of pyruvate. (3) This addition is followed by the decarboxylation of pyruvate. The positively charged ring of TPP acts as an electron sink that stabilizes the negative charge that is transferred to the ring as part of the decarboxylation. (4) Protonation yields hydroxyethyl-TPP.



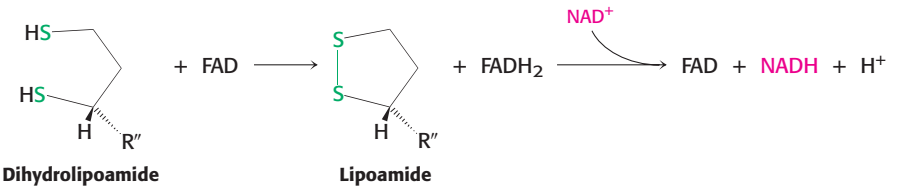
The oxidant in this reaction is the disulfide group of lipoamide, which is reduced to its disulfhydryl form. This reaction, also catalyzed by the pyruvate dehydrogenase component E_1, yields *acetyllipoamide*.

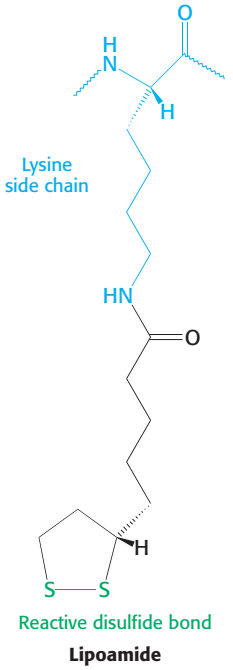
3. *Formation of Acetyl CoA. The acetyl group is transferred from acetyllipoamide to CoA to form acetyl CoA.*



Dihydrolipoyl transacetylase (E_2) catalyzes this reaction. The energy-rich thioester bond is preserved as the acetyl group is transferred to CoA. Recall that CoA serves as a carrier of many activated acyl groups, of which acetyl is the simplest (Section 15.3). Acetyl CoA, the fuel for the citric acid cycle, has now been generated from pyruvate.

The pyruvate dehydrogenase complex cannot complete another catalytic cycle until the dihydrolipoamide is oxidized to lipoamide. In a fourth step, *the oxidized form of lipoamide is regenerated by dihydrolipoyl dehydrogenase* (E_3). Two electrons are transferred to an FAD prosthetic group of the enzyme and then to NAD^+.





This electron transfer from FAD to NAD^+ is unusual because the common role for FAD is to receive electrons from NADH. The electron-transfer potential of FAD is increased by its chemical environment within the enzyme, enabling it to transfer electrons to NAD^+. Proteins tightly associated with FAD or flavin mononucleotide (FMN) are called *flavoproteins.*

Flexible linkages allow lipoamide to move between different active sites

The structures of all of the component enzymes of the pyruvate dehydrogenase complex are known, albeit from different complexes and species. Thus, it is now possible to construct an atomic model of the complex to understand its activity (Figure 17.7).

The core of the complex is formed by the transacetylase component E_2. Transacetylase consists of eight catalytic trimers assembled to form a hollow cube. Each of the three subunits forming a trimer has three major domains (Figure 17.8). At the amino terminus is a small domain that contains a bound flexible lipoamide cofactor attached to a lysine residue. This domain is homologous to biotin-binding domains such as that of pyruvate carboxylase (see Figure 16.26). The lipoamide domain is followed by a small domain that interacts with E_3 within the complex. A larger transacetylase domain completes an E_2 subunit. E_1 is an $\alpha_2\beta_2$ tetramer, and E_3 is an $\alpha\beta$ dimer. Multiples copies of E_1 and E_3 surround the E_2 core. How do the three distinct active sites work in concert (Figure 17.9)? The key is the long, flexible lipoamide arm of the E_2 subunit, which carries substrate from active site to active site.

1. Pyruvate is decarboxylated at the active site of E_1, forming the hydroxyethyl-TPP intermediate, and CO_2 leaves as the first product. This active site lies deep within the E_1 complex, connected to the enzyme surface by a 20-Å-long hydrophobic channel.

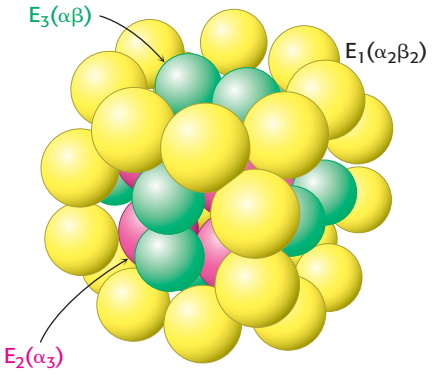


Figure 17.7 Schematic representation of the pyruvate dehydrogenase complex. The transacetylase core (E_2) is shown in red, the pyruvate dehydrogenase component (E_1) in yellow, and the dihydrolipoyl dehydrogenase (E_3) in green.

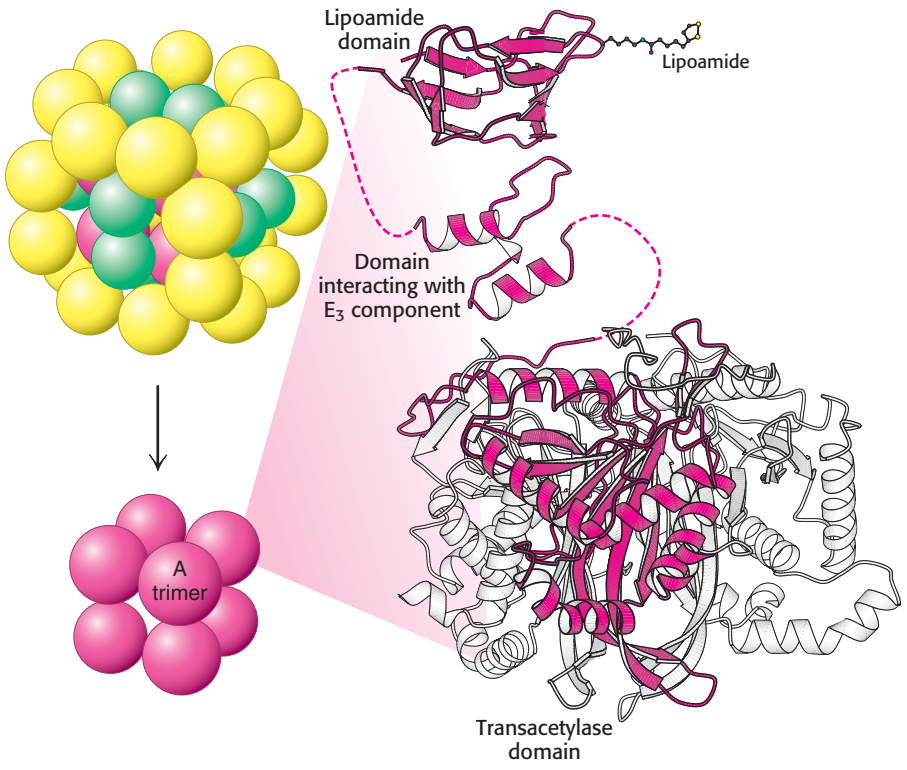


Figure 17.8 Structure of the transacetylase (E_2) core. Each red ball represents a trimer of three E_2 subunits. *Notice* that each subunit consists of three domains: a lipoamide-binding domain, a small domain for interaction with E_3, and a large transacetylase catalytic domain. The transacetylase domain has three identical subunits, with one depicted in red and the others in white in the ribbon representation.

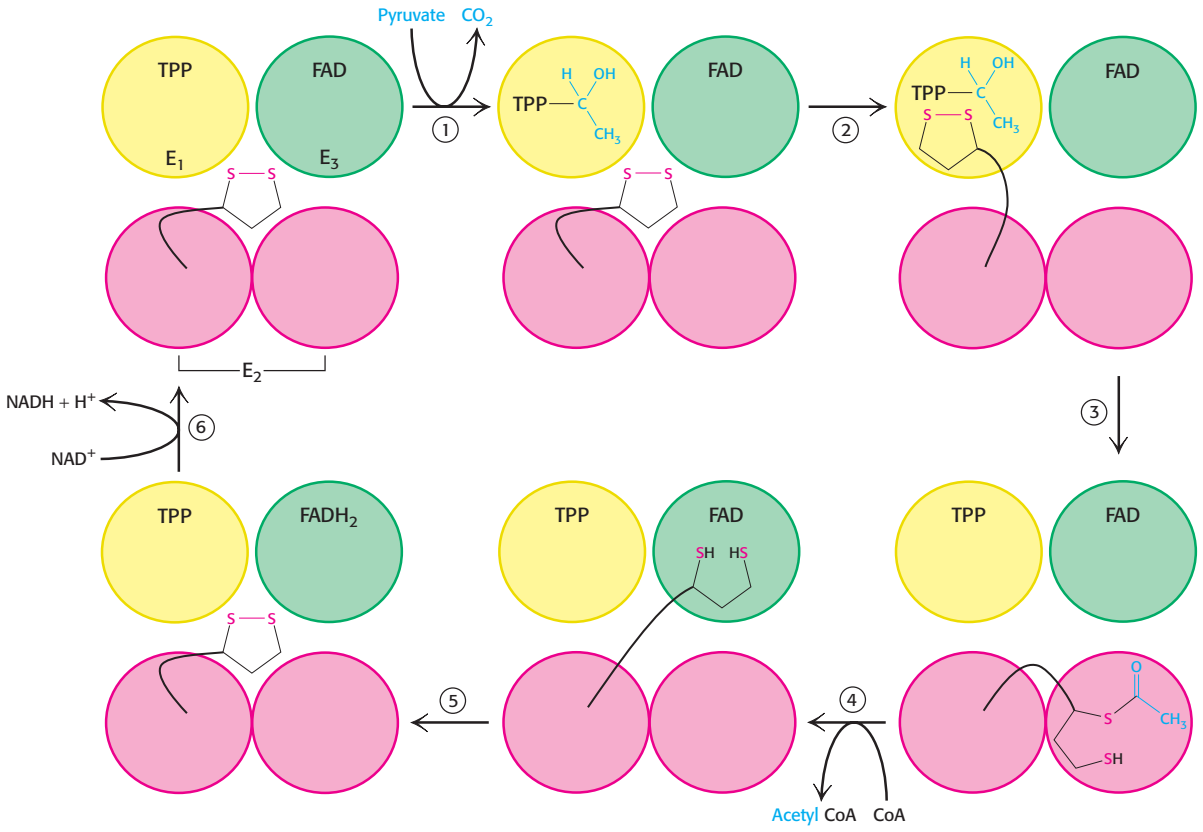


Figure 17.9 Reactions of the pyruvate dehydrogenase complex. At the top (left), the enzyme (represented by a yellow, a green, and two red spheres) is unmodified and ready for a catalytic cycle. (1) Pyruvate is decarboxylated to form hydroxyethyl-TPP. (2) The lipoamide arm of E_2 moves into the active site of E_1. (3) E_1 catalyzes the transfer of the two-carbon group to the lipoamide group to form the acetyl–lipoamide complex. (4) E_2 catalyzes the transfer of the acetyl moiety to CoA to form the product acetyl CoA. The dihydrolipoamide arm then swings to the active site of E_3. E_3 catalyzes (5) the oxidation of the dihydrolipoamide acid and (6) the transfer of the protons and electrons to NAD^+ to complete the reaction cycle.

2. E_2 inserts the lipoamide arm of the lipoamide domain into the deep channel in E_1 leading to the active site.

3. E_1 catalyzes the transfer of the acetyl group to the lipoamide. The acetylated arm then leaves E_1 and enters the E_2 cube to visit the active site of E_2, located deep in the cube at the subunit interface.

4. The acetyl moiety is then transferred to CoA, and the second product, acetyl CoA, leaves the cube. The reduced lipoamide arm then swings to the active site of the E_3 flavoprotein.

5. At the E_3 active site, the lipoamide is oxidized by coenzyme FAD. The reactivated lipoamide is ready to begin another reaction cycle.

6. The final product, NADH, is produced with the reoxidation of $FADH_2$ to FAD.

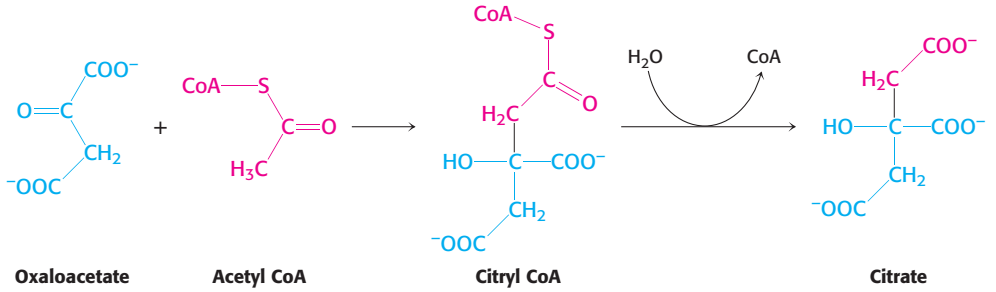
The structural integration of three kinds of enzymes and the long, flexible lipoamide arm make the coordinated catalysis of a complex reaction possible. The proximity of one enzyme to another *increases the overall reaction rate and minimizes side reactions.* All the intermediates in the oxidative decarboxylation of pyruvate remain bound to the complex throughout the reaction sequence and are readily transferred as the flexible arm of E_2 calls on each active site in turn.

17.2 The Citric Acid Cycle Oxidizes Two-Carbon Units

The conversion of pyruvate into acetyl CoA by the pyruvate dehydrogenase complex is the link between glycolysis and cellular respiration because *acetyl CoA is the fuel for the citric acid cycle*. Indeed, all fuels are ultimately metabolized to acetyl CoA or components of the citric acid cycle.

Citrate synthase forms citrate from oxaloacetate and acetyl coenzyme A

The citric acid cycle begins with the condensation of a four-carbon unit, oxaloacetate, and a two-carbon unit, the acetyl group of acetyl CoA. Oxaloacetate reacts with acetyl CoA and H_2O to yield citrate and CoA.



Oxaloacetate **Acetyl CoA** **Citryl CoA** **Citrate**

Synthase
An enzyme catalyzing a synthetic reaction in which two units are joined usually without the direct participation of ATP (or another nucleoside triphosphate).

This reaction, which is an aldol condensation followed by a hydrolysis, is catalyzed by *citrate synthase.* Oxaloacetate first condenses with acetyl CoA to form *citryl CoA,* a molecule that is energy rich because it contains the thioester bond that originated in acetyl CoA. The hydrolysis of citryl CoA thioester to citrate and CoA drives the overall reaction far in the direction of the synthesis of citrate. In essence, the hydrolysis of the thioester powers the synthesis of a new molecule from two precursors.

Mechanism: The mechanism of citrate synthase prevents undesirable reactions

Because the condensation of acetyl CoA and oxaloacetate initiates the citric acid cycle, it is very important that side reactions, notably the hydrolysis of acetyl CoA to acetate and CoA, be minimized. Let us briefly consider how the citrate synthase prevents the wasteful hydrolysis of acetyl CoA.

Mammalian citrate synthase is a dimer of identical 49-kd subunits. Each active site is located in a cleft between the large and the small domains of a subunit, adjacent to the subunit interface. X-ray crystallographic studies of citrate synthase and its complexes with several substrates and inhibitors revealed that the enzyme undergoes large conformational changes in the course of catalysis. Citrate synthase exhibits sequential, ordered kinetics: oxaloacetate binds first, followed by acetyl CoA. The reason for the ordered binding is that *oxaloacetate induces a major structural rearrangement leading to the creation of a binding site for acetyl CoA.* The binding of oxaloacetate converts the open form of the enzyme into a closed form (Figure 17.10). In each subunit, the small domain rotates 19 degrees relative to the large domain. *Movements as large as 15 Å are produced by the rotation of α helices elicited by quite small shifts of side chains around bound oxaloacetate.* These structural changes create a binding site for acetyl CoA. This conformational transition is reminiscent of the cleft closure in hexokinase induced by the binding of glucose (Section 16.1).

Citrate synthase catalyzes the condensation reaction by bringing the substrates into close proximity, orienting them, and polarizing certain bonds (Figure 17.11). The donation and removal of protons transforms acetyl CoA into an *enol intermediate*. The enol attacks oxaloacetate to form a carbon–carbon double bond linking acetyl CoA and oxaloacetate. The newly formed citryl CoA induces additional structural changes in the enzyme, causing the active site to become completely enclosed. The enzyme cleaves the citryl CoA thioester by hydrolysis. CoA leaves the enzyme, followed by citrate, and the enzyme returns to the initial open conformation.

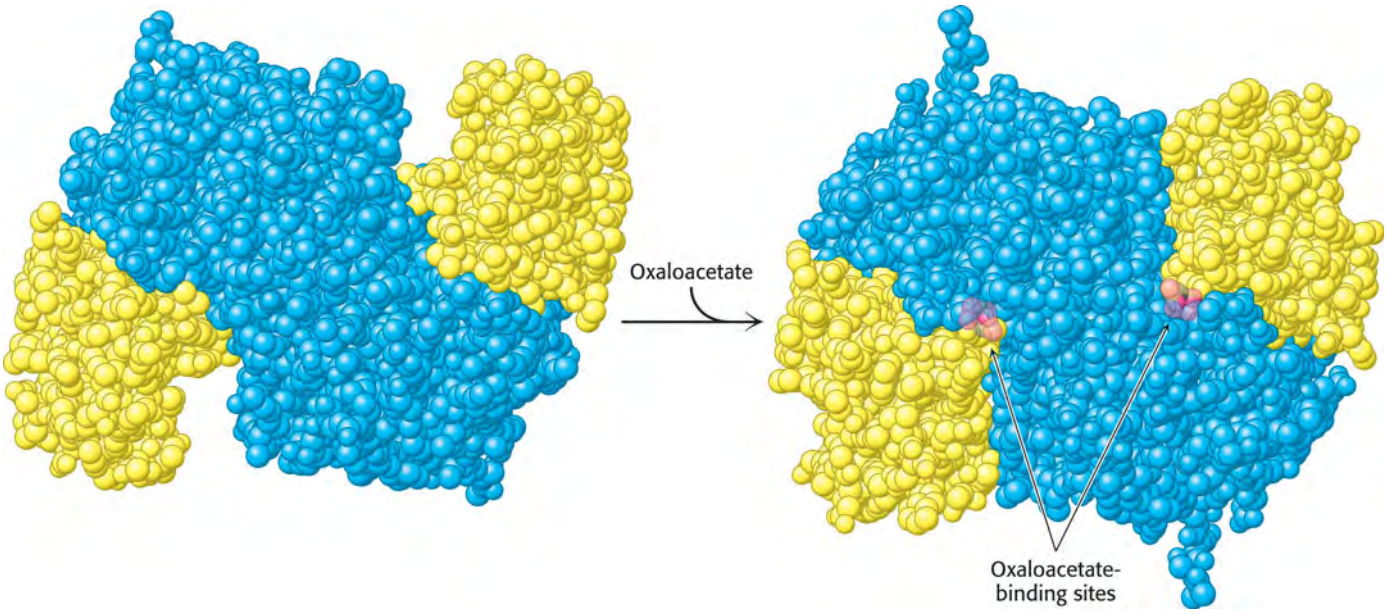


Figure 17.10 Conformational changes in citrate synthase on binding oxaloacetate. The small domain of each subunit of the homodimer is shown in yellow; the large domains are shown in blue. (Left) Open form of enzyme alone. (Right) Closed form of the liganded enzyme. [Drawn from 5CSC.pdb and 4CTS.pdb.]

We can now understand how the wasteful hydrolysis of acetyl CoA is prevented. Citrate synthase is well suited to hydrolyze *citryl* CoA but not *acetyl* CoA. How is this discrimination accomplished? First, acetyl CoA does not bind to the enzyme until oxaloacetate is bound and ready for condensation. Second, the catalytic residues crucial for the hydrolysis of the thioester linkage are not appropriately positioned *until citryl CoA is formed.* As with hexokinase and triose phosphate isomerase (Section 16.1), *induced fit prevents an undesirable side reaction.*

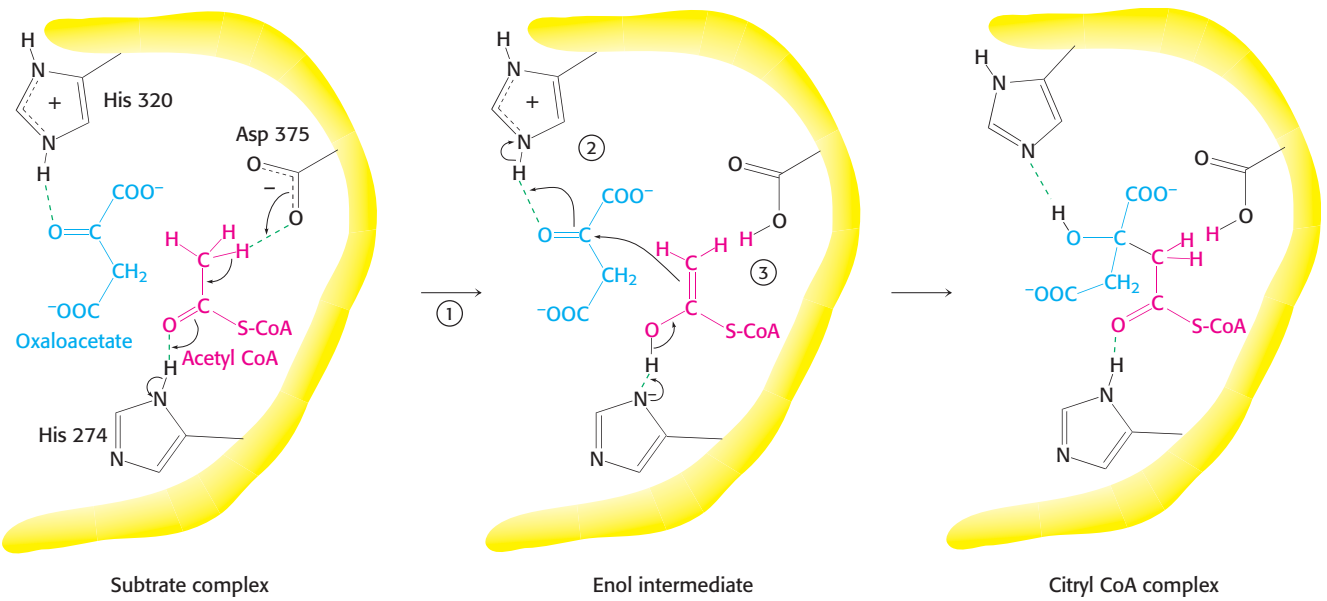
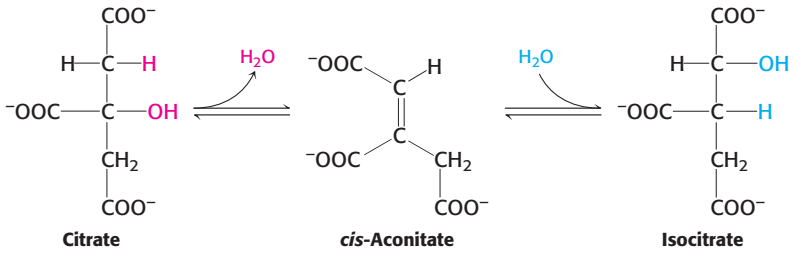


Figure 17.11 Mechanism of synthesis of citryl CoA by citrate synthase. (1) In the substrate complex (left), His 274 donates a proton to the carbonyl oxygen of acetyl CoA to promote the removal of a methyl proton by Asp 375 to form the enol intermediate (center). (2) Oxaloacetate is activated by the transfer of a proton from His 320 to its carbonyl carbon atom. (3) Simultaneously, the enol of acetyl CoA attacks the carbonyl carbon of oxaloacetate to form a carbon–carbon bond linking acetyl CoA and oxaloacetate. His 274 is reprotonated. Citryl CoA is formed. His 274 participates again as a proton donor to hydrolyze the thioester (not shown), yielding citrate and CoA.

Citrate is isomerized into isocitrate

The hydroxyl group is not properly located in the citrate molecule for the oxidative decarboxylations that follow. Thus, citrate is isomerized into isocitrate to enable the six-carbon unit to undergo oxidative decarboxylation. The isomerization of citrate is accomplished by a *dehydration* step followed by a *hydration* step. The result is an interchange of an H and an OH. The enzyme catalyzing both steps is called *aconitase* because cis-*aconitate* is an intermediate.



Aconitase is an *iron–sulfur protein*, or *nonheme-iron protein*, in that it contains iron that is not bonded to heme. Rather, its four iron atoms are complexed to four inorganic sulfides and three cysteine sulfur atoms, leaving one iron atom available to bind citrate through one of its COO^- groups and an OH group (Figure 17.12). This Fe-S cluster participates in dehydrating and rehydrating the bound substrate.

Isocitrate is oxidized and decarboxylated to alpha-ketoglutarate

We come now to the first of four oxidation–reduction reactions in the citric acid cycle. The oxidative decarboxylation of isocitrate is catalyzed by *isocitrate dehydrogenase*.

$$\text{Isocitrate} + NAD^+ \longrightarrow \alpha\text{-ketoglutarate} + CO_2 + NADH$$

The intermediate in this reaction is oxalosuccinate, an unstable β-ketoacid. While bound to the enzyme, it loses CO_2 to form α-ketoglutarate.

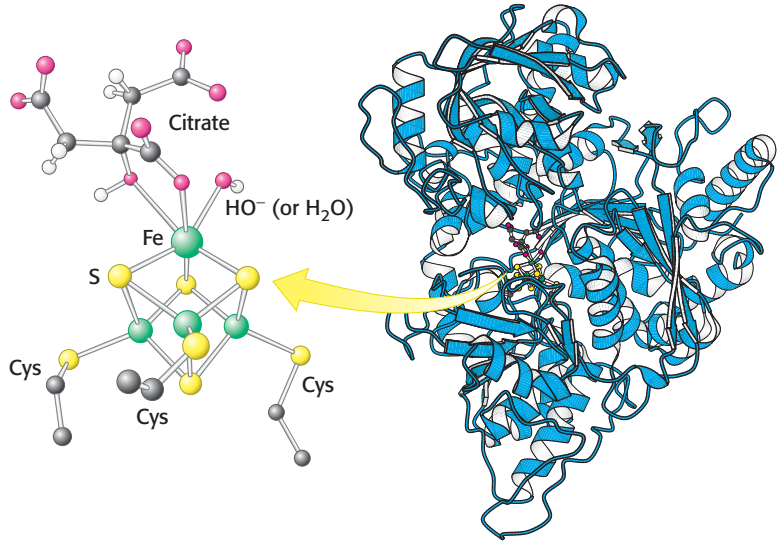
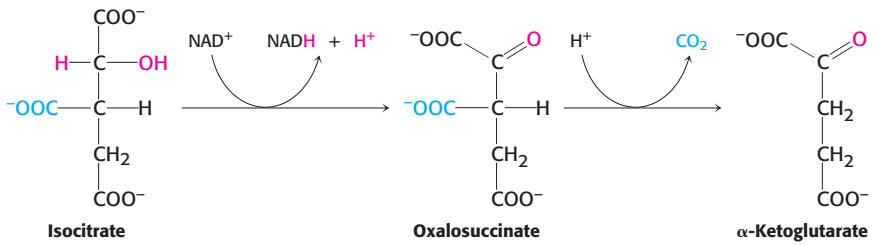


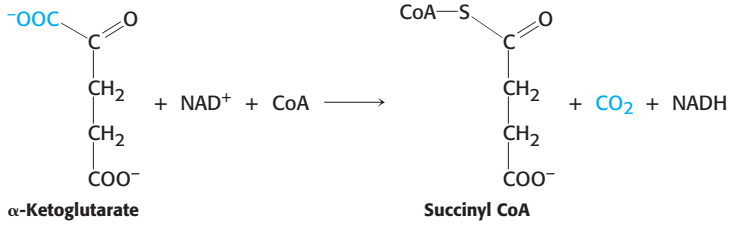
Figure 17.12 Binding of citrate to the iron–sulfur complex of aconitase. A 4Fe-4S iron–sulfur cluster is a component of the active site of aconitase. *Notice* that one of the iron atoms of the cluster binds to a COO^- group and an OH group of citrate. [Drawn from 1C96.pdb.]



The rate of formation of α-ketoglutarate is important in determining the overall rate of the cycle, as will be discussed on page 514. This oxidation generates the first high-transfer-potential electron carrier, NADH, in the cycle.

Succinyl coenzyme A is formed by the oxidative decarboxylation of alpha-ketoglutarate

The conversion of isocitrate into α-ketoglutarate is followed by a second oxidative decarboxylation reaction, the formation of succinyl CoA from α-ketoglutarate.



This reaction is catalyzed by the *α-ketoglutarate dehydrogenase complex*, an organized assembly of three kinds of enzymes that is homologous to the pyruvate dehydrogenase complex. In fact, the oxidative decarboxylation of α-ketoglutarate closely resembles that of pyruvate, also an α-ketoacid.

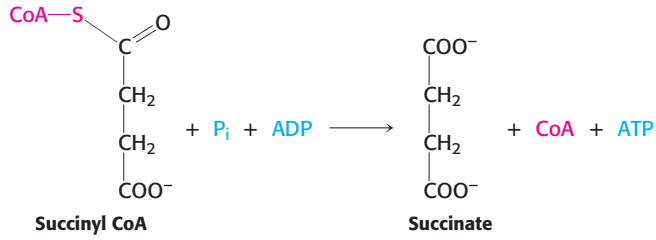
$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \xrightarrow{\text{Pyruvate dehydrogenase complex}} \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

$$\alpha\text{-Ketoglutarate} + \text{CoA} + \text{NAD}^+ \xrightarrow{\alpha\text{-Ketoglutarate dehydrogenase complex}} \text{succinyl CoA} + \text{CO}_2 + \text{NADH}$$

Both reactions include the decarboxylation of an α-ketoacid and the subsequent formation of a thioester linkage with CoA that has a high transfer potential. The reaction mechanisms are entirely analogous (p. 500).

A compound with high phosphoryl-transfer potential is generated from succinyl coenzyme A

Succinyl CoA is an energy-rich thioester compound. The $\Delta G^{\circ\prime}$ for the hydrolysis of succinyl CoA is about –33.5 kJ mol^{-1} (–8.0 kcal mol^{-1}), which is comparable to that of ATP (–30.5 kJ mol^{-1}, or –7.3 kcal mol^{-1}). In the citrate synthase reaction, the cleavage of the thioester bond powers the synthesis of the six-carbon citrate from the four-carbon oxaloacetate and the two-carbon fragment. *The cleavage of the thioester bond of succinyl CoA is coupled to the phosphorylation of a purine nucleoside diphosphate, usually ADP.* This reaction, which is readily reversible, is catalyzed by *succinyl CoA synthetase* (succinate thiokinase).



This reaction is the only step in the citric acid cycle that directly yields a compound with high phosphoryl-transfer potential. In mammals, there are two isozymic forms of the enzyme, one specific for ADP and one for GDP. In tissues that perform large amounts of cellular respiration, such as skeletal and heart muscle, the ADP-requiring isozyme predominates. In tissues that perform many anabolic reactions, such as the liver, the GDP-requiring enzyme is common. The GDP-requiring enzyme is believed to work in reverse of the direction observed in the TCA cycle; that is, GTP is used to power the synthesis of succinyl CoA, which is a precursor for heme synthesis. The *E. coli* enzyme uses either GDP or ADP as the phosphoryl-group acceptor.

Note that the enzyme *nucleoside diphosphokinase,* which catalyzes the following reaction,

$$\text{GTP} + \text{ADP} \rightleftharpoons \text{GDP} + \text{ATP}$$

allows the γ phosphoryl group to be readily transferred from GTP to form ATP, thereby allowing the adjustment of the concentration of GTP or ATP to meet the cell's need.

Mechanism: Succinyl coenzyme A synthetase transforms types of biochemical energy

The mechanism of this reaction is a clear example of an energy transformation: energy inherent in the thioester molecule is transformed into phosphoryl-group-transfer potential (Figure 17.13). The first step is the displacement of coenzyme A by orthophosphate, which generates another

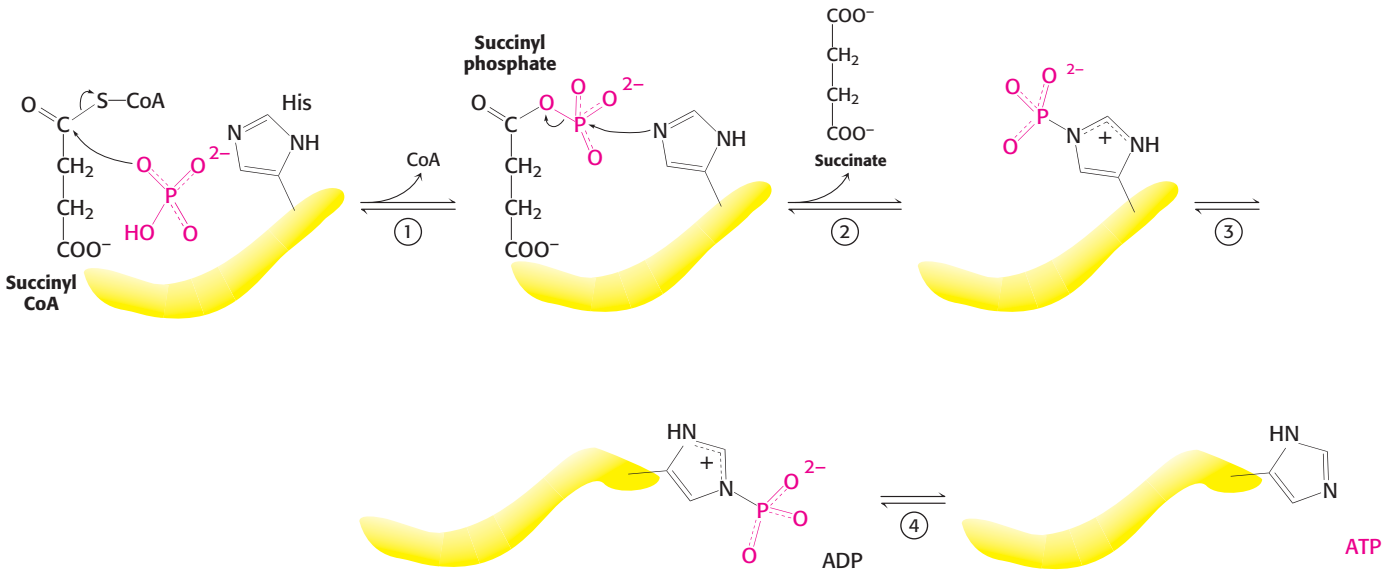


Figure 17.13 Reaction mechanism of succinyl CoA synthetase. The reaction proceeds through a phosphorylated enzyme intermediate. (1) Orthophosphate displaces coenzyme A, which generates another energy-rich compound, succinyl phosphate. (2) A histidine residue removes the phosphoryl group with the concomitant generation of succinate and phosphohistidine. (3) The phosphohistidine residue then swings over to a bound nucleoside diphosphate, and (4) the phosphoryl group is transferred to form the nucleoside triphosphate.

energy-rich compound, succinyl phosphate. A histidine residue plays a key role as a moving arm that detaches the phosphoryl group, then swings over to a bound nucleoside diphosphate and transfers the group to form the nucleoside triphosphate. The participation of high-energy compounds in all the steps is attested to by the fact that the reaction is readily reversible: $\Delta G^{\circ\prime} = -3.4$ kJ mol^{-1} (-0.8 kcal mol^{-1}). The formation of ATP at the expense of succinyl CoA is an example of substrate-level phosphorylation.

Succinyl CoA synthetase is an $\alpha_2\beta_2$ heterodimer; the functional unit is one $\alpha\beta$ pair. The enzyme mechanism shows that a phosphoryl group is transferred first to succinyl CoA bound in the α subunit and then to a nucleoside diphosphate bound in the β subunit. Examination of the three-dimensional structure of succinyl CoA synthetase reveals that each subunit comprises two domains (Figure 17.14). The amino-terminal domains of the two subunits have different structures, each characteristic of its role in the mechanism. The amino-terminal domain of the α subunit forms a Rossmann fold (Section 16.1), which binds the ADP substrate of succinyl CoA synthetase. The amino-terminal domain of the β subunit is an ATP-grasp domain, found in many enzymes, which here binds and activates ADP. Succinyl CoA synthetase has evolved by adopting these domains and harnessing them to capture the energy associated with succinyl CoA cleavage, which is used to drive the generation of a nucleoside triphosphate.

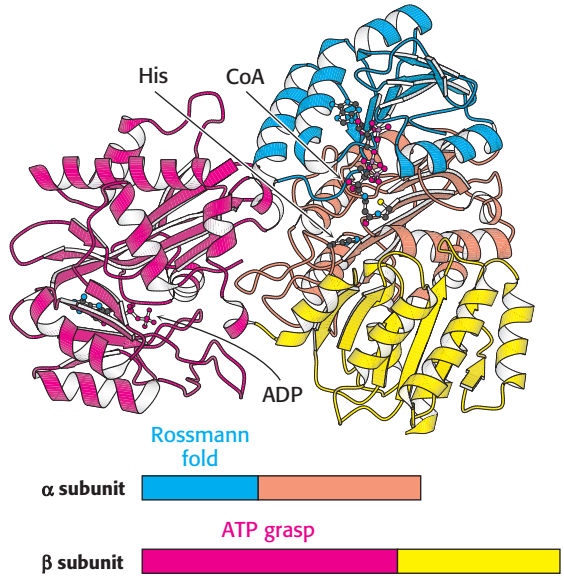
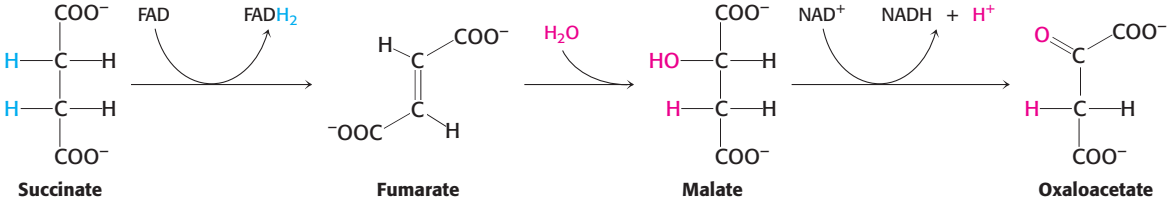


Figure 17.14 Structure of succinyl CoA synthetase. The enzyme is composed of two subunits. The α subunit contains a Rossmann fold that binds the ADP component of CoA, and the β subunit contains a nucleotide-activating region called the ATP-grasp domain. The ATP-grasp domain is shown here binding a molecule of ADP. *Notice* that the histidine residue is between the CoA and the ADP. This histidine residue picks up the phosphoryl group from near the CoA and swings over to transfer it to the nucleotide bound in the ATP-grasp domain. [Drawn from 1CGI.pdb.]

Oxaloacetate is regenerated by the oxidation of succinate

Reactions of four-carbon compounds constitute the final stage of the citric acid cycle: the regeneration of oxaloacetate.



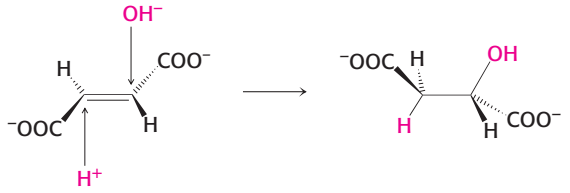
The reactions constitute a metabolic motif that we will see again in fatty acid synthesis and degradation as well as in the degradation of some amino acids. A methylene group (CH_2) is converted into a carbonyl group (C=O) in three steps: an oxidation, a hydration, and a second oxidation reaction. Oxaloacetate is thereby regenerated for another round of the cycle, and more energy is extracted in the form of $FADH_2$ and NADH.

Succinate is oxidized to fumarate by *succinate dehydrogenase*. The hydrogen acceptor is FAD rather than NAD^+, which is used in the other three oxidation reactions in the cycle. FAD is the hydrogen acceptor in this reaction because the free-energy change is insufficient to reduce NAD^+. FAD is nearly always the electron acceptor in oxidations that remove two hydrogen *atoms* from a substrate. In succinate dehydrogenase, the isoalloxazine ring of FAD is covalently attached to a histidine side chain of the enzyme (denoted E-FAD).

$$\text{E-FAD} + \text{succinate} \rightleftharpoons \text{E-FADH}_2 + \text{fumarate}$$

Succinate dehydrogenase, like aconitase, is an iron–sulfur protein. Indeed, succinate dehydrogenase contains three different kinds of iron–sulfur clusters: 2Fe-2S (two iron atoms bonded to two inorganic sulfides), 3Fe-4S, and 4Fe-4S. Succinate dehydrogenase—which consists of a 70-kd and a 27-kd subunit—differs from other enzymes in the citric acid cycle in being embedded in the inner mitochondrial membrane. In fact, *succinate dehydrogenase is directly associated with the electron-transport chain, the link between the citric acid cycle and ATP formation.* $FADH_2$ produced by the oxidation of succinate does not dissociate from the enzyme, in contrast with NADH produced in other oxidation–reduction reactions. Rather, two electrons are transferred from $FADH_2$ directly to iron–sulfur clusters of the enzyme, which in turn passes the electrons to coenzyme Q (CoQ). Coenzyme Q, an important member of the electron-transport chain, passes electrons to the ultimate acceptor, molecular oxygen, as we shall see in Chapter 18.

The next step is the hydration of fumarate to form L-malate. *Fumarase* catalyzes a stereospecific trans addition of H^+ and OH^-. The OH^- group adds to only one side of the double bond of fumarate; hence, only the L isomer of malate is formed.



Fumarate **L-Malate**

Finally, malate is oxidized to form oxaloacetate. This reaction is catalyzed by *malate dehydrogenase,* and NAD^+ is again the hydrogen acceptor.

$$\text{Malate} + NAD^+ \rightleftharpoons \text{oxaloacetate} + NADH + H^+$$

The standard free energy for this reaction, unlike that for the other steps in the citric acid cycle, is significantly positive ($\Delta G^{\circ\prime} = +29.7$ kJ mol^{-1}, or $+7.1$ kcal mol^{-1}). The oxidation of malate is driven by the use of the products—oxaloacetate by citrate synthase and NADH by the electron-transport chain.

The citric acid cycle produces high-transfer-potential electrons, ATP, and CO_2

The net reaction of the citric acid cycle is

$$\text{Acetyl CoA} + 3\,NAD^+ + FAD + ADP + P_i + 2\,H_2O \longrightarrow 2\,CO_2 + 3\,NADH + FADH_2 + ATP + 2\,H^+ + CoA$$

Let us recapitulate the reactions that give this stoichiometry (Figure 17.15 and Table 17.2):

1. Two carbon atoms enter the cycle in the condensation of an acetyl unit (from acetyl CoA) with oxaloacetate. Two carbon atoms leave the cycle in the form of CO_2 in the successive decarboxylations catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.

2. Four pairs of hydrogen atoms leave the cycle in four oxidation reactions. Two NAD^+ molecules are reduced in the oxidative decarboxylations of isocitrate and α-ketoglutarate, one FAD molecule is reduced in the oxidation

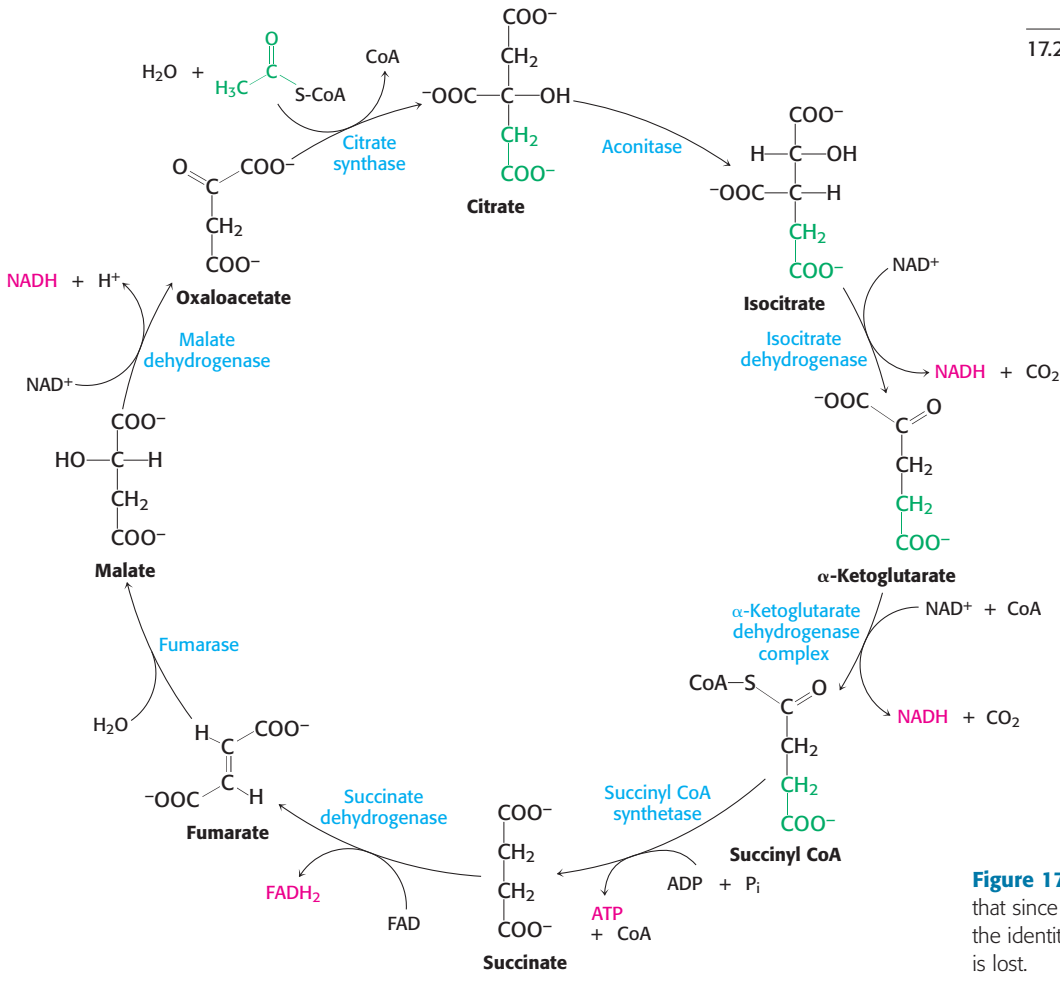


Figure 17.15 The citric acid cycle. *Notice* that since succinate is a symmetric molecule, the identity of the carbons from the acetyl unit is lost.

Table 17.2 Citric acid cycle

Step	Reaction	Enzyme	Prosthetic group	Type*	$\Delta G^{\circ\prime}$ kJ mol^{-1}	$\Delta G^{\circ\prime}$ kcal mol^{-1}
1	Acetyl CoA + oxaloacetate + $H_2O \rightarrow$ citrate + CoA + H^+	Citrate synthase		a	–31.4	–7.5
2a	Citrate $\rightleftharpoons$ *cis*-aconitate + H_2O	Aconitase	Fe-S	b	+8.4	+2.0
2b	*cis*-Aconitate + $H_2O \rightleftharpoons$ isocitrate	Aconitase	Fe-S	c	–2.1	–0.5
3	Isocitrate + $NAD^+ \rightleftharpoons$ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase		d + e	–8.4	–2.0
4	α-Ketoglutarate + NAD^+ + CoA $\rightleftharpoons$ succinyl CoA + CO_2 + NADH	α-Ketoglutarate dehydrogenase complex	Lipoic acid, FAD, TPP	d + e	–30.1	–7.2
5	Succinyl CoA + P_i + ADP $\rightleftharpoons$ succinate + ATP + CoA	Succinyl CoA synthetase		f	–3.3	–0.8
6	Succinate + FAD (enzyme-bound) $\rightleftharpoons$ fumarate + $FADH_2$(enzyme-bound)	Succinate dehydrogenase	FAD, Fe-S	e	0	0
7	Fumarate + $H_2O \rightleftharpoons$ L-malate	Fumarase		c	–3.8	–0.9
8	L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+	Malate dehydrogenase		e	+29.7	+7.1

*Reaction type: (a) condensation; (b) dehydration; (c) hydration; (d) decarboxylation; (e) oxidation; (f) substrate-level phosphorylation.

of succinate, and one NAD^+ molecule is reduced in the oxidation of malate. Recall also that one NAD^+ molecule is reduced in the oxidative decarboxylation of pyruvate to form acetyl CoA.

3. One compound with high phosphoryl-transfer potential, usually ATP, is generated from the cleavage of the thioester linkage in succinyl CoA.

4. Two water molecules are consumed: one in the synthesis of citrate by the hydrolysis of citryl CoA and the other in the hydration of fumarate.

Isotope-labeling studies revealed that the two carbon atoms that enter each cycle are not the ones that leave. The two carbon atoms that enter the cycle as the acetyl group are retained during the initial two decarboxylation reactions (see Figure 17.15) and then remain incorporated in the four-carbon acids of the cycle. Note that succinate is a symmetric molecule. Consequently, the two carbon atoms that enter the cycle can occupy any of the carbon positions in the subsequent metabolism of the four-carbon acids. The two carbons that enter the cycle as the acetyl group will be released as CO_2 in *subsequent* trips through the cycle. To understand why citrate is not processed as a symmetric molecule, see Problems 27 and 28.

Evidence is accumulating that the enzymes of the citric acid cycle are physically associated with one another. The close arrangement of enzymes enhances the efficiency of the citric acid cycle because a reaction product can pass directly from one active site to the next through connecting channels, a process called *substrate channeling*. The word *metabolon* has been suggested as the name for such multienzyme complexes.

As will be considered in Chapter 18, the electron-transport chain oxidizes the NADH and $FADH_2$ formed in the citric acid cycle. The transfer of electrons from these carriers to O_2, the ultimate electron acceptor, leads to the generation of a proton gradient across the inner mitochondrial membrane. This proton-motive force then powers the generation of ATP; the net stoichiometry is about 2.5 ATP per NADH, and 1.5 ATP per $FADH_2$. Consequently, nine high-transfer-potential phosphoryl groups are generated when the electron-transport chain oxidizes 3 NADH molecules and 1 $FADH_2$ molecule, and one high-transfer-potential phosphoryl group is directly formed in one round of the citric acid cycle. Thus, one acetyl unit generates approximately 10 molecules of ATP. In dramatic contrast, the anaerobic glycolysis of 1 glucose molecule generates only 2 molecules of ATP (and 2 molecules of lactate).

Recall that molecular oxygen does not participate directly in the citric acid cycle. However, the cycle operates only under aerobic conditions because NAD^+ and FAD can be regenerated in the mitochondrion only by the transfer of electrons to molecular oxygen. *Glycolysis has both an aerobic and an anaerobic mode, whereas the citric acid cycle is strictly aerobic.* Glycolysis can proceed under anaerobic conditions because NAD^+ is regenerated in the conversion of pyruvate into lactate or ethanol.

17.3 Entry to the Citric Acid Cycle and Metabolism Through It Are Controlled

The citric acid cycle is the final common pathway for the aerobic oxidation of fuel molecules. Moreover, as we will see shortly (Section 17.4) and repeatedly elsewhere in our study of biochemistry, the cycle is an important source of building blocks for a host of important biomolecules. As befits its role as the metabolic hub of the cell, entry into the cycle and the rate of the cycle itself are controlled at several stages.

The pyruvate dehydrogenase complex is regulated allosterically and by reversible phosphorylation

As stated earlier, glucose can be formed from pyruvate (Section 16.3). *However, the formation of acetyl CoA from pyruvate is an irreversible step in animals and thus they are unable to convert acetyl CoA back into glucose.* The oxidative decarboxylation of pyruvate to acetyl CoA commits the carbon atoms of glucose to one of two principal fates: oxidation to CO_2 by the citric acid cycle, with the concomitant generation of energy, or incorporation into lipid (Figure 17.16). As expected of an enzyme at a critical branch point in metabolism, the activity of the pyruvate dehydrogenase complex is stringently controlled. High concentrations of reaction products inhibit the reaction: acetyl CoA inhibits the transacetylase component (E_2) by binding directly, whereas NADH inhibits the dihydrolipoyl dehydrogenase (E_3). High concentrations of NADH and acetyl CoA inform the enzyme that the energy needs of the cell have been met or that fatty acids are being degraded to produce acetyl CoA and NADH. In either case, there is no need to metabolize pyruvate to acetyl CoA. This inhibition has the effect of sparing glucose, because most pyruvate is derived from glucose by glycolysis (Section 16.1).

The key means of regulation of the complex in eukaryotes is covalent modification (Figure 17.17). *Phosphorylation of the pyruvate dehydrogenase component (E_1) by pyruvate dehydrogenase kinase I* (PDK) *switches off the activity of the complex. Deactivation is reversed by the pyruvate dehydrogenase phosphatase* (PDP). The kinase is associated with the transacetylase component (E_2), again highlighting the structural and mechanistic importance of this core. Both the kinase and the phosphatase are regulated. To see how this regulation works in biological conditions, consider muscle that is becoming active after a period of rest (Figure 17.18). At rest, the muscle will not have significant energy demands. Consequently, the $NADH/NAD^+$, acetyl CoA/CoA, and ATP/ADP ratios will be high. These high ratios promote phosphorylation and, hence, deactivation of the pyruvate dehydrogenase complex. In other words, high concentrations of immediate (acetyl CoA and NADH) and ultimate (ATP) products inhibit the activity. Thus, *pyruvate dehydrogenase is switched off when the energy charge is high.*

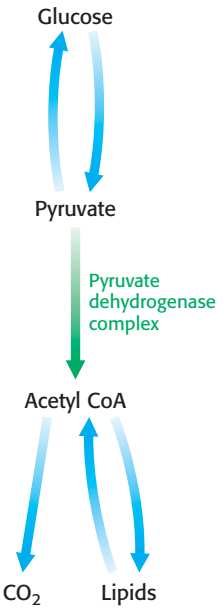


Figure 17.16 From glucose to acetyl CoA. The synthesis of acetyl CoA by the pyruvate dehydrogenase complex is a key irreversible step in the metabolism of glucose.

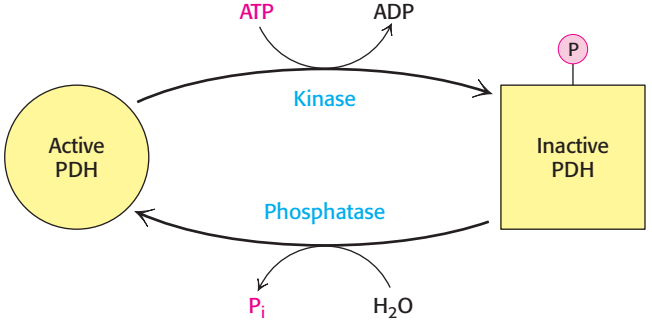


Figure 17.17 Regulation of the pyruvate dehydrogenase complex. A specific kinase phosphorylates and inactivates pyruvate dehydrogenase (PDH), and a phosphatase activates the dehydrogenase by removing the phosphoryl group. The kinase and the phosphatase also are highly regulated enzymes.

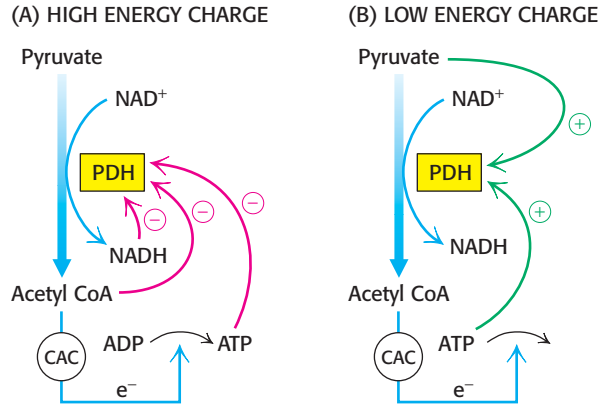


Figure 17.18 Response of the pyruvate dehydrogenase complex to the energy charge. The pyruvate dehydrogenase complex is regulated to respond to the energy charge of the cell. (A) The complex is inhibited by its immediate products, NADH and acetyl CoA, as well as by the ultimate product of cellular respiration, ATP. (B) The complex is activated by pyruvate and ADP, which inhibit the kinase that phosphorylates PDH.

As exercise begins, the concentrations of ADP and pyruvate will increase as muscle contraction consumes ATP and glucose is converted into pyruvate to meet the energy demands. Both ADP and pyruvate activate the dehydrogenase by inhibiting the kinase. Moreover, the phosphatase is stimulated by Ca^{2+}, the same signal that initiates muscle contraction. A rise in the cytoplasmic Ca^{2+} level (Section 35.2) elevates the mitochondrial Ca^{2+} level. The rise in mitochondrial Ca^{2+} activates the phosphatase, enhancing pyruvate dehydrogenase activity.

In some tissues, the phosphatase is regulated by hormones. In liver, epinephrine binds to the α-adrenergic receptor to initiate the phosphatidylinositol pathway (Section 14.1), causing an increase in Ca^{2+} concentration that activates the phosphatase. In tissues capable of fatty acid synthesis, such as the liver and adipose tissue, insulin, the hormone that signifies the fed state, stimulates the phosphatase, increasing the conversion of pyruvate into acetyl CoA. Acetyl CoA is the precursor for fatty acid synthesis (Section 22.4). In these tissues, the pyruvate dehydrogenase complex is activated to funnel glucose to pyruvate and then to acetyl CoA and ultimately to fatty acids.

In people with a phosphatase deficiency, pyruvate dehydrogenase is always phosphorylated and thus inactive. Consequently, glucose is processed to lactate rather than acetyl CoA. This condition results in unremitting lactic acidosis—high blood levels of lactic acid. In such an acidic environment, many tissues malfunction, most notably the central nervous system.

The citric acid cycle is controlled at several points

The rate of the citric acid cycle is precisely adjusted to meet an animal cell's needs for ATP (Figure 17.19). The primary control points are the allosteric enzymes isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, the first two enzymes in the cycle to generate high-energy electrons.

The first control site is isocitrate dehydrogenase. The enzyme is allosterically stimulated by ADP, which enhances the enzyme's affinity for substrates. The binding of isocitrate, NAD^+, Mg^{2+}, and ADP is mutually cooperative. In contrast, ATP is inhibitory. The reaction product NADH also inhibits isocitrate dehydrogenase by directly displacing NAD^+. It is important to note that several steps in the cycle require NAD^+ or FAD, which are abundant only when the energy charge is low.

A second control site in the citric acid cycle is α-ketoglutarate dehydrogenase. Some aspects of this enzyme's control are like those of the pyruvate dehydrogenase complex, as might be expected from the homology of the two enzymes. α-Ketoglutarate dehydrogenase is inhibited by succinyl CoA and NADH, the products of the reaction that it catalyzes. In addition, α-ketoglutarate dehydrogenase is inhibited by a high energy charge. Thus, *the rate of the cycle is reduced when the cell has a high level of ATP.*

The use of isocitrate dehydrogenase and α-ketoglutarate dehydrogenase as control points integrates the citric acid cycle with other pathways and highlights the central role of the citric acid cycle in metabolism. For instance, the inhibition of isocitrate dehydrogenase leads to a buildup of citrate, because the interconversion of isocitrate and citrate is readily reversible under intracellular conditions. Citrate can be transported to the cytoplasm, where it signals phosphofructokinase to halt glycolysis (Section 16.2) and where it can serve as a source of acetyl CoA for fatty acid synthesis (Section 22.4). The α-ketoglutarate that accumulates when α-ketoglutarate

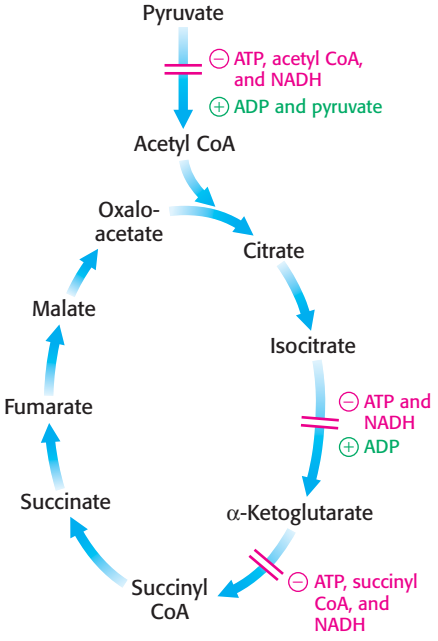


Figure 17.19 Control of the citric acid cycle. The citric acid cycle is regulated primarily by the concentration of ATP and NADH. The key control points are the enzymes isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.

dehydrogenase is inhibited can be used as a precursor for several amino acids and the purine bases (Chapter 23 and Chapter 25).

In many bacteria, the funneling of two-carbon fragments into the cycle also is controlled. *The synthesis of citrate from oxaloacetate and acetyl CoA carbon units is an important control point in these organisms.* ATP is an allosteric inhibitor of citrate synthase. The effect of ATP is to increase the value of K_M for acetyl CoA. Thus, as the level of ATP increases, less of this enzyme is saturated with acetyl CoA and so less citrate is formed.

Defects in the citric acid cycle contribute to the development of cancer

Three enzymes crucial to cellular respiration are known to contribute to the development of cancer: succinate dehydrogenase, fumarase, and pyruvate dehydrogenase kinase. Mutations that alter the activity of all three of these enzymes enhance aerobic glycolysis. In aerobic glycolysis, cancer cells preferentially metabolize glucose to lactate even in the presence of oxygen. Defects in all of these enzymes share a common biochemical link: the transcription factor *hypoxia inducible factor 1* (HIF-1).

Normally, HIF-1 up-regulates the enzymes and transporters that enhance glycolysis only when oxygen concentration falls, a condition called hypoxia. Under normal conditions, HIF-1 is hydroxylated by prolyl hydroxylase 2 and is subsequently destroyed by the proteasome, a large complex of proteolytic enzymes (Chapter 23). The degradation of HIF-1 prevents the stimulation of glycolysis. Prolyl hydroxylase 2 requires α-ketoglutarate, ascorbate, and oxygen for activity. Thus, when oxygen concentration falls, the prolyl hydroxylase 2 is inactive, HIF-1 is not hydroxylated and not degraded, and the synthesis of proteins required for glycolysis is stimulated. As a result, the rate of glycolysis is increased.

Defects in the enzymes of the citric acid cycle can significantly affect the regulation of prolyl hydroxylase 2. When either succinate dehydrogenase or fumarase is defective, succinate and fumarate accumulate in the mitochondria and spill over into the cytoplasm. Both succinate and fumarate are competitive inhibitors of prolyl hydroxylase 2. The inhibition of prolyl hydroxylase 2 results in the stabilization of HIF-1, since HIF-1 is no longer hydroxylated. Lactate, the end product of glycolysis, also appears to inhibit prolyl hydroxylase 2 by interfering with the action of ascorbate. In addition to increasing the amount of the proteins required for glycolysis, HIF-1 also stimulates the production of pyruvate dehydrogenase kinase (PDK). The kinase inhibits the pyruvate dehydrogenase complex, preventing the conversion of pyruvate into acetyl CoA. The pyruvate remains in the cytoplasm, further increasing the rate of aerobic glycolysis. Moreover, mutations in PDK that lead to enhanced activity contribute to increased aerobic glycolysis and the subsequent development of cancer. By enhancing glycolysis and increasing the concentration of lactate, the mutations in PDK result in the inhibition of hydroxylase and the stabilization of HIF-1.

These observations linking citric acid cycle enzymes to cancer suggest that cancer is also a metabolic disease, not simply a disease of mutant growth factors and cell cycle control proteins. The realization that there is a metabolic component to cancer opens the door to new thinking about the control of cancer. Indeed, preliminary experiments suggest that if cancer cells undergoing aerobic glycolysis are forced by pharmacological manipulation to use oxidative phosphorylation, the cancer cells lose their malignant properties. It is also interesting to note that the citric acid cycle, which has been studied for decades, still has secrets to be revealed by future biochemists.

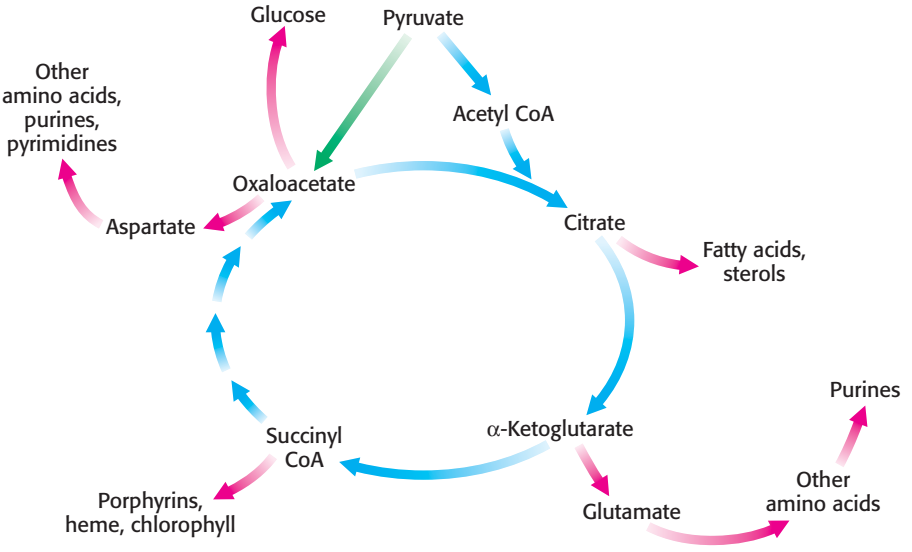


Figure 17.20 Biosynthetic roles of the citric acid cycle. Intermediates are drawn off for biosyntheses (shown by red arrows) when the energy needs of the cell are met. Intermediates are replenished by the formation of oxaloacetate from pyruvate.

17.4 The Citric Acid Cycle Is a Source of Biosynthetic Precursors

Thus far, discussion has focused on the citric acid cycle as the *major degradative pathway for the generation of ATP.* As a major metabolic hub of the cell, the citric acid cycle also *provides intermediates for biosyntheses* (Figure 17.20). For example, most of the carbon atoms in porphyrins come from *succinyl CoA.* Many of the amino acids are derived from *α-ketoglutarate* and *oxaloacetate.* These biosynthetic processes will be considered in subsequent chapters.

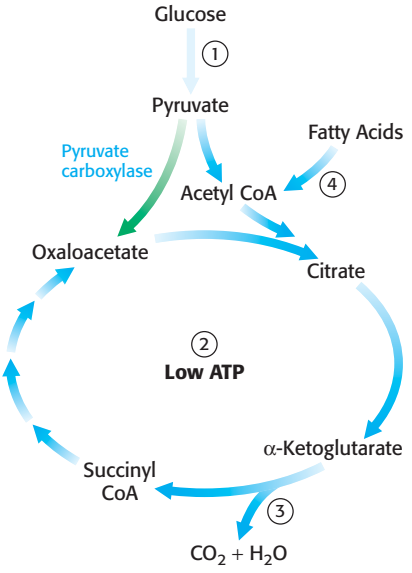
The citric acid cycle must be capable of being rapidly replenished

The important point now is that *citric acid cycle intermediates must be replenished if any are drawn off for biosyntheses.* Suppose that much oxaloacetate is converted into amino acids for protein synthesis and, subsequently, the energy needs of the cell rise. The citric acid cycle will operate to a reduced extent unless new oxaloacetate is formed, because acetyl CoA cannot enter the cycle unless it condenses with oxaloacetate. Even though oxaloacetate is recycled, a minimal level must be maintained to allow the cycle to function.

How is oxaloacetate replenished? Mammals lack the enzymes for the net conversion of acetyl CoA into oxaloacetate or any other citric acid cycle intermediate. Rather, oxaloacetate is formed by the carboxylation of pyruvate, in a reaction catalyzed by the biotin-dependent enzyme *pyruvate carboxylase* (Figure 17.21).

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \rightarrow \text{oxaloacetate} + \text{ADP} + P_i + 2\,H^+$$

Recall that this enzyme plays a crucial role in gluconeogenesis (Section 16.3). It is active only in the presence of acetyl CoA, which signifies the need for more oxaloacetate. If the energy charge is high, oxaloacetate is converted into glucose. If the energy charge is low, oxaloacetate replenishes the citric acid cycle. The synthesis of oxaloacetate by the carboxylation of pyruvate is an example of an *anaplerotic reaction* (*anaplerotic* is of Greek origin, meaning to "fill up"), a reaction that leads to the net synthesis, or



Active pathways

1. Glycolysis, Ch. 16
2. Citric acid cycle, Ch. 17
3. Oxidative phosphorylation, Ch. 18
4. Fatty acid oxidation, Ch. 22

Figure 17.21 PATHWAY INTEGRATION: Pathways active during exercise after a night's rest. The rate of the citric acid cycle increases during exercise, requiring the replenishment of oxaloacetate and acetyl CoA. Oxaloacetate is replenished by its formation from pyruvate. Acetyl CoA may be produced from the metabolism of both pyruvate and fatty acids.

replenishment, of pathway components. Note that because the citric acid cycle is a cycle, it can be replenished by the generation of any of the intermediates.

The disruption of pyruvate metabolism is the cause of beriberi and poisoning by mercury and arsenic

Beriberi, a neurologic and cardiovascular disorder, is caused by a dietary deficiency of thiamine (also called *vitamin* B_1). The disease has been and continues to be a serious health problem in the Far East because rice, the major food, has a rather low content of thiamine. This deficiency is partly ameliorated if the whole rice grain is soaked in water before milling; some of the thiamine in the husk then leaches into the rice kernel. The problem is exacerbated if the rice is polished (that is, converted from brown to white rice), because only the outer layer contains significant amounts of thiamine. Beriberi is also occasionally seen in alcoholics who are severely malnourished and thus thiamine deficient. The disease is characterized by neurologic and cardiac symptoms. Damage to the peripheral nervous system is expressed as pain in the limbs, weakness of the musculature, and distorted skin sensation. The heart may be enlarged and the cardiac output inadequate.

Beriberi

A vitamin-deficiency disease first described in 1630 by Jacob Bonitus, a Dutch physician working in Java:

"A certain very troublesome affliction, which attacks men, is called by the inhabitants Beriberi (which means sheep). I believe those, whom this same disease attacks, with their knees shaking and the legs raised up, walk like sheep. It is a kind of paralysis, or rather Tremor: for it penetrates the motion and sensation of the hands and feet indeed sometimes of the whole body."

Which biochemical processes might be affected by a deficiency of thiamine? Thiamine is the precursor of the cofactor thiamine pyrophosphate. *This cofactor is the prosthetic group of three important enzymes: pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and transketolase.* Transketolase functions in the pentose phosphate pathway, which will be considered in Chapter 20. *The common feature of enzymatic reactions utilizing TPP is the transfer of an activated aldehyde unit.* In beriberi, the levels of pyruvate and α-ketoglutarate in the blood are higher than normal. The increase in the level of pyruvate in the blood is especially pronounced after the ingestion of glucose. A related finding is that the activities of the pyruvate and α-ketoglutarate dehydrogenase complexes in vivo are abnormally low. The low transketolase activity of red blood cells in beriberi is an easily measured and reliable diagnostic indicator of the disease.

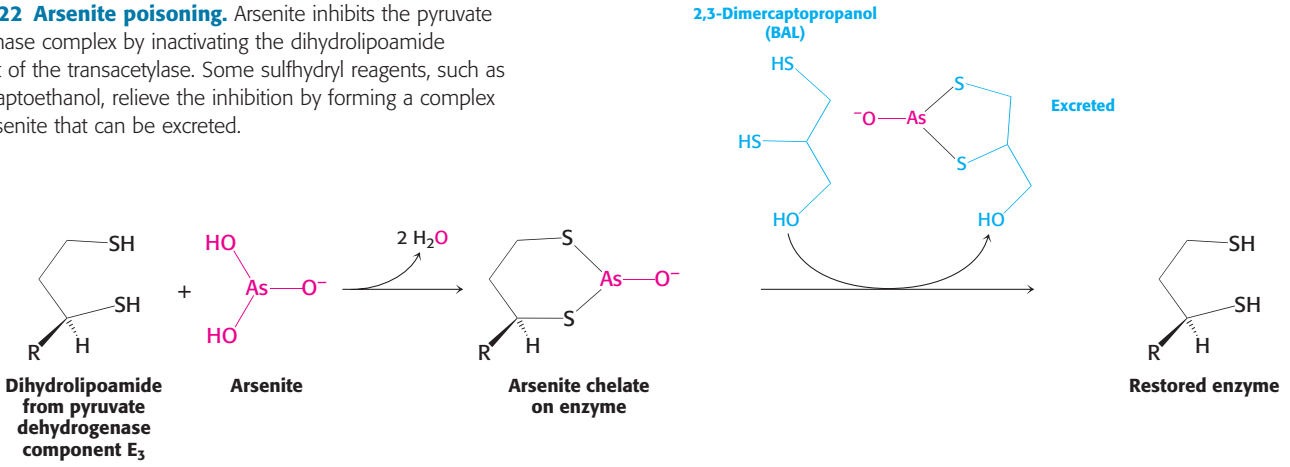
Why does TPP deficiency lead primarily to neurological disorders? The nervous system relies essentially on glucose as its only fuel. The product of glycolysis, pyruvate, can enter the citric acid cycle only through the pyruvate dehydrogenase complex. With that enzyme deactivated, the nervous system has no source of fuel. In contrast, most other tissues can use fats as a source of fuel for the citric acid cycle.

Symptoms similar to those of beriberi appear in organisms exposed to mercury or arsenite (AsO_3^{3-}). Both materials have a high affinity for neighboring sulfhydryls, such as those in the reduced dihydrolipoyl groups of the E_3 component of the pyruvate dehydrogenase complex (Figure 17.22). The binding of mercury or arsenite to the dihydrolipoyl groups inhibits the complex and leads to central nervous system pathologies. The proverbial phrase "mad as a hatter" refers to the strange behavior of poisoned hat makers who used mercury nitrate to soften and shape animal furs. This form of mercury is absorbed through the skin. Similar symptoms afflicted the early photographers, who used vaporized mercury to create daguerreotypes.

[The Granger Collection.]

Treatment for these poisons is the administration of sulfhydryl reagents with adjacent sulfhydryl groups to compete with the dihydrolipoyl residues for binding with the metal ion. The reagent–metal complex is then excreted in the urine. Indeed, 2,3-dimercaptopropanol (see Figure 17.22) was developed after World War I as an antidote to lewisite, an arsenic-based chemical weapon. This compound was initially called BAL, for British anti-lewisite.

Figure 17.22 Arsenite poisoning. Arsenite inhibits the pyruvate dehydrogenase complex by inactivating the dihydrolipoamide component of the transacetylase. Some sulfhydryl reagents, such as 2,3-dimercaptoethanol, relieve the inhibition by forming a complex with the arsenite that can be excreted.



The manuscript proposing the citric acid cycle was submitted for publication to *Nature* but was rejected in June 1937. That same year it was published in *Enzymologia*. Dr. Krebs proudly displayed the rejection letter throughout his career as encouragement for young scientists.

"The editor of NATURE presents his compliments to Dr. H. A. Krebs and regrets that as he has already sufficient letters to fill the correspondence columns of NATURE for seven or eight weeks, it is undesirable to accept further letters at the present time on account of the time delay which must occur in their publication.

If Dr. Krebs does not mind much delay the editor is prepared to keep the letter until the congestion is relieved in the hope of making use of it.

He returns it now, in case Dr. Krebs prefers to submit it for early publication to another periodical."

The citric acid cycle may have evolved from preexisting pathways

How did the citric acid cycle come into being? Although definitive answers are elusive, informed speculation is possible. We can perhaps begin to comprehend how evolution might work at the level of biochemical pathways.

The citric acid cycle was most likely assembled from preexisting reaction pathways. As noted earlier, many of the intermediates formed in the citric acid cycle are used in metabolic pathways for amino acids and porphyrins. Thus, compounds such as pyruvate, α-ketoglutarate, and oxaloacetate were likely present early in evolution for biosynthetic purposes. The oxidative decarboxylation of these α-ketoacids is quite favorable thermodynamically and can be used to drive the synthesis of both acyl CoA derivatives and NADH. These reactions almost certainly formed the core of processes that preceded the citric acid cycle evolutionarily. Interestingly, α-ketoglutarate can be directly converted into oxaloacetate by transamination of the respective amino acids by aspartate aminotransferase, another key biosynthetic enzyme. Thus, cycles comprising smaller numbers of intermediates used for a variety of biochemical purposes could have existed before the present form evolved.

17.5 The Glyoxylate Cycle Enables Plants and Bacteria to Grow on Acetate

Acetyl CoA that enters the citric acid cycle has but one fate: oxidation to CO_2 and H_2O. Most organisms thus cannot convert acetyl CoA into glucose, because although oxaloacetate, a key precursor to glucose, is formed in the citric acid cycle, the two decarboxylations that take place before the regeneration of oxaloacetate preclude the *net* conversion of acetyl CoA into glucose.

In plants and in some microorganisms, there is a metabolic pathway that allows the conversion of acetyl CoA generated from fats stores into glucose. This reaction sequence, called the *glyoxylate cycle,* is similar to the citric acid cycle but bypasses the two decarboxylation steps of the cycle. Another important difference is that two molecules of acetyl CoA enter per turn of the glyoxylate cycle, compared with one in the citric acid cycle.

The glyoxylate cycle (Figure 17.23), like the citric acid cycle, begins with the condensation of acetyl CoA and oxaloacetate to form citrate, which is then isomerized to isocitrate. Instead of being decarboxylated, as in the citric acid cycle, isocitrate is cleaved by *isocitrate lyase* into succinate and

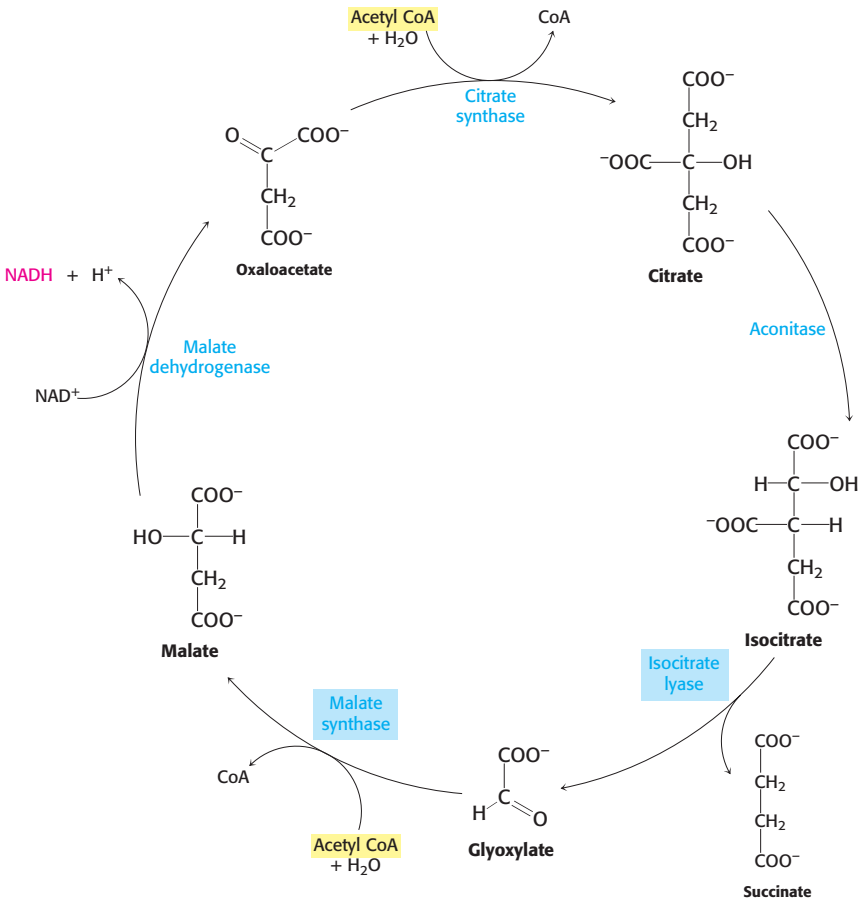


Figure 17.23 The glyoxylate pathway. The glyoxylate cycle allows plants and some microorganisms to grow on acetate because the cycle bypasses the decarboxylation steps of the citric acid cycle. The reactions of this cycle are the same as those of the citric acid cycle except for the ones catalyzed by isocitrate lyase and malate synthase, which are boxed in blue.

glyoxylate. The ensuing steps regenerate oxaloacetate from glyoxylate. First, acetyl CoA condenses with glyoxylate to form malate in a reaction catalyzed by *malate synthase,* and then malate is oxidized to oxaloacetate, as in the citric acid cycle. The sum of these reactions is

$$2\ \text{Acetyl CoA} + \text{NAD}^+ + 2\ \text{H}_2\text{O} \longrightarrow \text{succinate} + 2\ \text{CoASH} + \text{NADH} + 2\ \text{H}^+$$

In plants, these reactions take place in organelles called *glyoxysomes.* This cycle is especially prominent in oil-rich seeds, such as those from sunflowers, cucumbers, and castor beans. Succinate, released midcycle, can be converted into carbohydrates by a combination of the citric acid cycle and gluconeogenesis. The carbohydrates power seedling growth until the cell can begin photosynthesis. Thus, organisms with the glyoxylate cycle gain a metabolic versatility because they can use acetyl CoA as a precursor of glucose and other biomolecules.

Summary

The citric acid cycle is the final common pathway for the oxidation of fuel molecules. It also serves as a source of building blocks for biosyntheses.

17.1 Pyruvate Dehydrogenase Links Glycolysis to the Citric Acid Cycle

Most fuel molecules enter the cycle as acetyl CoA. The link between glycolysis and the citric acid cycle is the oxidative decarboxylation of pyruvate to form acetyl CoA. In eukaryotes, this reaction and those of

the cycle take place inside mitochondria, in contrast with glycolysis, which takes place in the cytoplasm.

17.2 The Citric Acid Cycle Oxidizes Two-Carbon Units

The cycle starts with the condensation of oxaloacetate (C_4) and acetyl CoA (C_2) to give citrate (C_6), which is isomerized to isocitrate (C_6). Oxidative decarboxylation of this intermediate gives α-ketoglutarate (C_5). The second molecule of carbon dioxide comes off in the next reaction, in which α-ketoglutarate is oxidatively decarboxylated to succinyl CoA (C_4). The thioester bond of succinyl CoA is cleaved by orthophosphate to yield succinate, and an ATP is concomitantly generated. Succinate is oxidized to fumarate (C_4), which is then hydrated to form malate (C_4). Finally, malate is oxidized to regenerate oxaloacetate (C_4). Thus, two carbon atoms from acetyl CoA enter the cycle, and two carbon atoms leave the cycle as CO_2 in the successive decarboxylations catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. In the four oxidation–reduction reactions in the cycle, three pairs of electrons are transferred to NAD^+ and one pair to FAD. These reduced electron carriers are subsequently oxidized by the electron-transport chain to generate approximately 9 molecules of ATP. In addition, 1 molecule of a compound having a high phosphoryl-transfer potential is directly formed in the citric acid cycle. Hence, a total of 10 molecules of compounds having high phosphoryl-transfer potential are generated for each two-carbon fragment that is completely oxidized to H_2O and CO_2.

17.3 Entry to the Citric Acid Cycle and Metabolism Through It Are Controlled

The citric acid cycle operates only under aerobic conditions because it requires a supply of NAD^+ and FAD. The irreversible formation of acetyl CoA from pyruvate is an important regulatory point for the entry of glucose-derived pyruvate into the citric acid cycle. The activity of the pyruvate dehydrogenase complex is stringently controlled by reversible phosphorylation. The electron acceptors are regenerated when NADH and $FADH_2$ transfer their electrons to O_2 through the electron-transport chain, with the concomitant production of ATP. Consequently, the rate of the citric acid cycle depends on the need for ATP. In eukaryotes, the regulation of two enzymes in the cycle also is important for control. A high energy charge diminishes the activities of isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. These mechanisms complement each other in reducing the rate of formation of acetyl CoA when the energy charge of the cell is high and when biosynthetic intermediates are abundant.

17.4 The Citric Acid Cycle Is a Source of Biosynthetic Precursors

When the cell has adequate energy available, the citric acid cycle can also provide a source of building blocks for a host of important biomolecules, such as nucleotide bases, proteins, and heme groups. This use depletes the cycle of intermediates. When the cycle again needs to metabolize fuel, anaplerotic reactions replenish the cycle intermediates.

17.5 The Glyoxylate Cycle Enables Plants and Bacteria to Grow on Acetate

The glyoxylate cycle enhances the metabolic versatility of many plants and bacteria. This cycle, which uses some of the reactions of the citric acid cycle, enables these organisms to subsist on acetate because it bypasses the two decarboxylation steps of the citric acid cycle.

Key Terms

citric acid (tricarboxylic acid, TCA; Krebs) cycle (p. 497)
acetyl CoA (p. 497)
oxidative phosphorylation (p. 498)
pyruvate dehydrogenase complex (p. 499)
flavoprotein (p. 502)
citrate synthase (p. 504)
iron–sulfur (nonheme iron) protein (p. 506)
isocitrate dehydrogenase (p. 506)
α-ketoglutarate dehydrogenase (p. 507)
metabolon (p. 512)
anaplerotic reaction (p. 516)
beriberi (p. 517)
glyoxylate cycle (p. 518)
isocitrate lyase (p. 518)
malate synthase (p. 519)
glyoxysome (p. 519)

Problems

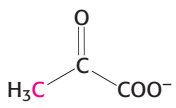
1. *Naming names.* What are the five enzymes (including regulatory enzymes) that constitute the pyruvate dehydrogenase complex? Which reactions do they catalyze?

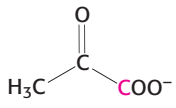
2. *Coenzymes.* What coenzymes are required by the pyruvate dehydrogenase complex? What are their roles?

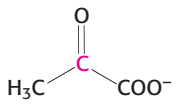
3. *More coenzymes.* Distinguish between catalytic coenzymes and stoichiometric coenzymes in the pyruvate dehydrogenase complex.

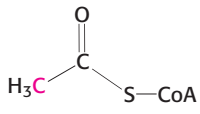
4. *Joined at the hip.* List some of the advantages of organizing the enzymes that catalyze the formation of acetyl CoA from pyruvate into a single large complex.

5. *Flow of carbon atoms.* What is the fate of the radioactive label when each of the following compounds is added to a cell extract containing the enzymes and cofactors of the glycolytic pathway, the citric acid cycle, and the pyruvate dehydrogenase complex? (The ^{14}C label is printed in red.)

(a) 

(b) 

(c) 

(d) 

(e) Glucose 6-phosphate labeled at C-1.

6. $C_2 + C_2 \rightarrow C_4$.

(a) Which enzymes are required to get *net synthesis* of oxaloacetate from acetyl CoA?

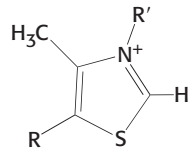
(b) Write a balanced equation for the net synthesis.

(c) Do mammalian cells contain the requisite enzymes?

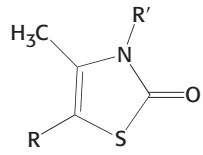
7. *Driving force.* What is the $\Delta G^{\circ\prime}$ for the complete oxidation of the acetyl unit of acetyl CoA by the citric acid cycle?

8. *Acting catalytically.* The citric acid cycle itself, which is composed of enzymatically catalyzed steps, can be thought of essentially as a supramolecular enzyme. Explain.

9. *A potent inhibitor.* Thiamine thiazolone pyrophosphate binds to pyruvate dehydrogenase about 20,000 times as strongly as does thiamine pyrophosphate, and it competitively inhibits the enzyme. Why?



TPP



Thiazolone analog of TPP

10. *Lactic acidosis.* Patients in shock often suffer from lactic acidosis owing to a deficiency of O_2. Why does a lack of O_2 lead to lactic acid accumulation? One treatment for shock is to administer dichloroacetate, which inhibits the kinase associated with the pyruvate dehydrogenase complex. What is the biochemical rationale for this treatment?

11. *Energy rich.* What are the thioesters in the reaction catalyzed by PDH complex?

12. *Alternative fates.* Compare the regulation of the pyruvate dehydrogenase complex in muscle and in liver.

13. *Mutations.* (a) Predict the effect of a mutation that enhances the activity of the kinase associated with the PDH complex. (b) Predict the effect of a mutation that reduces the activity of the phosphatase associated with the PDH complex.

14. *Flaking paint, green wallpaper.* Clare Boothe Luce, ambassador to Italy in the 1950s (and Connecticut congressperson, playwright, editor of *Vanity Fair,* and the wife of Henry Luce, founder of *Time* magazine and *Sports Illustrated*) became ill when she was staying at the ambassadorial residence in Rome. The paint on the dining room ceiling, an arsenic-based paint, was flaking; the wallpaper of her bedroom in the ambassadorial residence was colored a mellow green owing to the presence of cupric arsenite in the pigment. Suggest a possible cause of Ambassador Luce's illness.

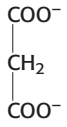
15. *A hoax, perhaps?* The citric acid cycle is part of aerobic respiration, but no O_2 is required for the cycle. Explain this paradox.

16. *Coupling reactions.* The oxidation of malate by NAD^+ to form oxaloacetate is a highly endergonic reaction under standard conditions [$\Delta G^{\circ\prime} = 29$ kJ mol^{-1} (7 kcal mol^{-1})]. The reaction proceeds readily under physiological conditions.

(a) Why?
(b) Assuming an $[NAD^+]/[NADH]$ ratio of 8 and a pH of 7, what is the lowest [malate]/[oxaloacetate] ratio at which oxaloacetate can be formed from malate?

17. *Synthesizing α-ketoglutarate.* It is possible, with the use of the reactions and enzymes considered in this chapter, to convert pyruvate into α-ketoglutarate without depleting any of the citric acid cycle components. Write a balanced reaction scheme for this conversion, showing cofactors and identifying the required enzymes.

18. *Seven o'clock roadblock.* Malonate is a competitive inhibitor of succinate dehydrogenase. How will the concentrations of citric acid cycle intermediates change immediately after the addition of malonate? Why is malonate not a substrate for succinate dehydrogenase?



Malonate

19. *No signal, no activity.* Why is acetyl CoA an especially appropriate activator for pyruvate carboxylase?

20. *Power differentials.* As we will see in the next chapter, when NADH reacts with oxygen 2.5 ATP are generated. When $FADH_2$ reduces oxygen only 1.5 ATP are generated. Why then does succinate dehydrogenase produce $FADH_2$ and not NADH when succinate is reduced to fumarate?

21. *Back to Orgo.* Before any oxidation can occur in the citric acid cycle, citrate must be isomerized into isocitrate. Why is this the case?

22. *A nod is as good as a wink to a blind horse.* Explain why a GTP molecule, or another nucleoside triphosphate, is energetically equivalent to an ATP molecule in metabolism.

23. *One from two.* The synthesis of citrate from acetyl CoA and oxaloacetate is a biosynthetic reaction. What is the energy source that drives formation of citrate?

Chapter Integration Problems

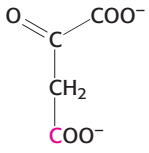
24. *Fats into glucose?* Fats are usually metabolized into acetyl CoA and then further processed through the citric acid cycle. In Chapter 16, we saw that glucose can be synthesized from oxaloacetate, a citric acid cycle intermediate. Why, then, after a long bout of exercise depletes our carbohydrate stores, do we need to replenish those stores by eating carbohydrates? Why do we not simply replace them by converting fats into carbohydrates?

25. *Alternative fuels.* As we will see (Chapter 22), fatty acid breakdown generates a large amount of acetyl CoA. What will be the effect of fatty acid breakdown on pyruvate dehydrogenase complex activity? On glycolysis?

Mechanism Problems

26. *Theme and variation.* Propose a reaction mechanism for the condensation of acetyl CoA and glyoxylate in the glyoxylate cycle of plants and bacteria.

27. *Symmetry problems.* In experiments carried out in 1941 to investigate the citric acid cycle, oxaloacetate labeled with ^{14}C in the carboxyl carbon atom farthest from the keto group was introduced to an active preparation of mitochondria.



Oxaloacetate

Analysis of the α-ketoglutarate formed showed that none of the radioactive label had been lost. Decarboxylation of α-ketoglutarate then yielded succinate devoid of radioactivity. All the label was in the released CO_2. Why were the early investigators of the citric acid cycle surprised that *all* the label emerged in the CO_2?

28. *Symmetric molecules reacting asymmetrically.* The interpretation of the experiments described in Problem 27 was that citrate (or any other symmetric compound) cannot be an intermediate in the formation of α-ketoglutarate, because of the asymmetric fate of the label. This view seemed compelling until Alexander Ogston incisively pointed out in 1948 that "it is possible that *an asymmetric enzyme which attacks a symmetrical compound can distinguish between its identical groups* [italics added]." For simplicity, consider a molecule in which two hydrogen atoms, a group X, and a different group Y are bonded to a tetrahedral carbon atom as a model for citrate. Explain how a symmetric molecule can react with an enzyme in an asymmetric way.

Data Interpretation Problem

29. *A little goes a long way.* As will become clearer in Chapter 18, the activity of the citric acid cycle can be monitored by measuring the amount of O_2 consumed. The greater the rate of O_2 consumption, the faster the rate of the cycle. Hans Krebs used this assay to investigate the cycle in 1937. He used as his experimental system minced pigeon-breast muscle, which is rich in mitochondria. In one set of experiments, Krebs measured the O_2 consumption in the presence of carbohydrate only and in the presence of

carbohydrate and citrate. The results are shown in the following table.

Effect of citrate on oxygen consumption by minced pigeon-breast muscle

	Micromoles of oxygen consumed	
Time (min)	Carbohydrate only	Carbohydrate plus 3 μmol of citrate
10	26	28
60	43	62
90	46	77
150	49	85

(a) How much O_2 would be absorbed if the added citrate were completely oxidized to H_2O and CO_2?

(b) On the basis of your answer to part *a*, what do the results given in the table suggest?

30. *Arsenite poisoning.* The effect of arsenite on the experimental system of Problem 29 was then examined. Experimental data (not presented here) showed that the amount of citrate present did not change in the course of the experiment in the absence of arsenite. However, if arsenite was added to the system, different results were obtained, as shown in the following table.

Disappearance of citric acid in pigeon-breast muscle in the presence of arsenite

Micromoles of citrate added	Micromoles of citrate found after 40 minutes	Micromoles of citrate used
22	00.6	21
44	20.0	24
90	56.0	34

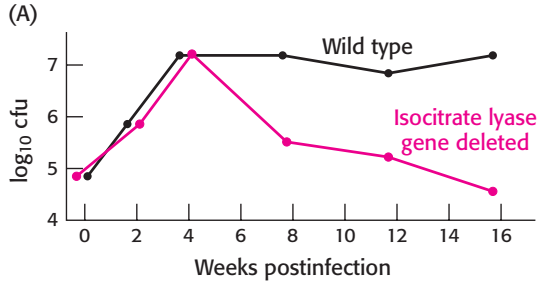
(a) What is the effect of arsenite on the disappearance of citrate?

(b) How is the action of arsenite altered by the addition of more citrate?

(c) What do these data suggest about the site of action of arsenite?

31. *Isocitrate lyase and tuberculosis.* The bacterium *Mycobacterium tuberculosis,* the cause of tuberculosis, can invade the lungs and persist in a latent state for years. During this time, the bacteria reside in granulomas—nodular scars containing bacteria and host-cell debris in the center and surrounded by immune cells. The granulomas are lipid-rich, oxygen-poor environments. How these bacteria manage to persist is something of a mystery. The results of recent research suggest that the glyoxylate cycle is required for the persistence. The following data show the amount of bacteria [presented as colony-forming units (cfu)] in mice lungs in the weeks after an infection.

In graph A, the black circles represent the results for wild-type bacteria and the red circles represent the results for bacteria from which the gene for isocitrate lyase was deleted.



(a) What is the effect of the absence of isocitrate lyase?

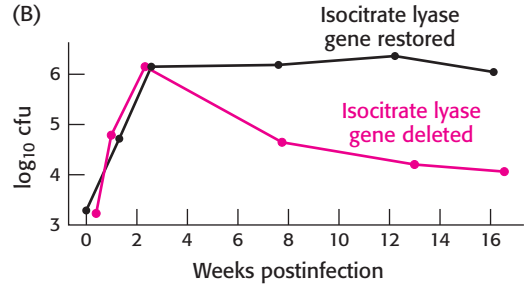
The techniques described in Chapter 5 were used to reinsert the gene encoding isocitrate lyase into bacteria from which it had previously been deleted.

In graph B, black circles represent bacteria into which the gene was reinserted and red circles represent bacteria in which the gene was still missing.

(b) Do these results support those obtained in part *a*?

(c) What is the purpose of the experiment in part *b*?

(d) Why do these bacteria perish in the absence of the glyoxylate cycle?



[Data after McKinney et al., *Nature* 406(2000):735–738.]

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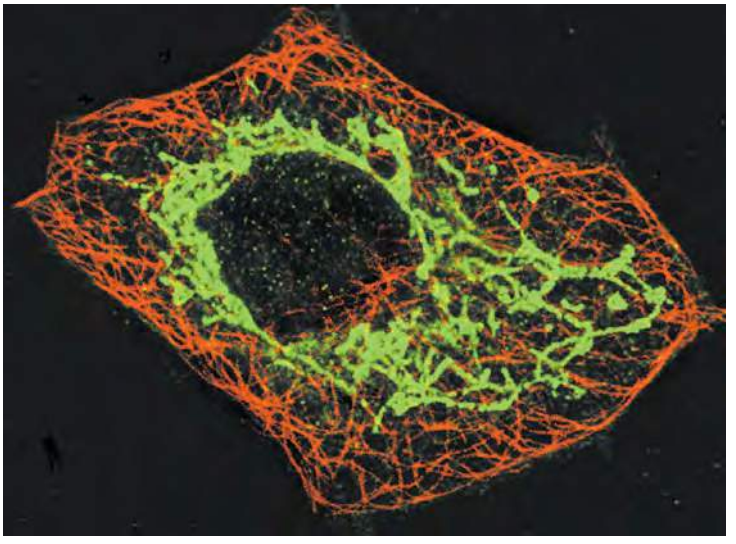
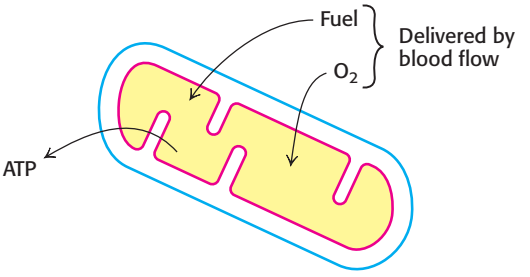
CHAPTER 18

Oxidative Phosphorylation





Mitochondria, stained green, form a network inside a fibroblast cell (left). Mitochondria oxidize carbon fuels to form cellular energy in the form of ATP. [(Left) Courtesy of Michael P. Yaffee, Department of Biology, University of California at San Diego.]

The amount of ATP that human beings require to go about their lives is staggering. A sedentary male of 70 kg (154 lbs) requires about 8400 kJ (2000 kcal) for a day's worth of activity. To provide this much energy requires 83 kg of ATP. However, human beings possess only about 250 g of ATP at any given moment. The disparity between the amount of ATP that we have and the amount that we require is compensated by recycling ADP back to ATP. Each ATP molecule is recycled approximately 300 times per day. This recycling takes place primarily through *oxidative phosphorylation*.

We begin our study of oxidative phosphorylation by examining the oxidation–reduction reactions that allow the flow of electrons from NADH and $FADH_2$ to oxygen. The electron flow takes place in four large protein complexes that are embedded in the inner mitochondrial membrane, together called the *respiratory chain* or the *electron-transport chain*.

$$NADH + \tfrac{1}{2}O_2 + H^+ \longrightarrow H_2O + NAD^+$$
$$\Delta G^{\circ\prime} = -220.1 \text{ kJ mol}^{-1} (-52.6 \text{ kcal mol}^{-1})$$

The overall reaction is exergonic. Importantly, three of the complexes of the electron-transport chain use the energy released by the electron flow to pump protons from the mitochondrial matrix into the cytoplasm. In essence, energy is transformed. The resulting unequal distribution of protons generates a pH gradient and a transmembrane electrical potential that creates a *proton-motive force*. ATP is synthesized when protons flow back to the mitochondrial matrix through an enzyme complex.

$$ADP + P_i + H^+ \longrightarrow ATP + H_2O$$
$$\Delta G^{\circ\prime} = +30.5 \text{ kJ mol}^{-1} (+7.3 \text{ kcal mol}^{-1})$$

OUTLINE

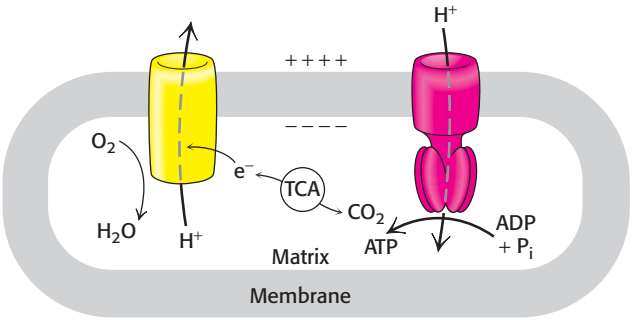


Figure 18.1 Overview of oxidative phosphorylation. Oxidation and ATP synthesis are coupled by transmembrane proton fluxes. Electrons flow from NADH and $FADH_2$ through four protein complexes to reduce oxygen to water. Three of the complexes pump protons from the mitochondrial matrix to the exterior of the mitochondria. The protons return to the matrix by flowing through another protein complex, ATP synthase, powering the synthesis of ATP.

Respiration

An ATP-generating process in which an inorganic compound (such as molecular oxygen) serves as the ultimate electron acceptor. The electron donor can be either an organic compound or an inorganic one.

Thus, *the oxidation of fuels and the phosphorylation of ADP are coupled by a proton gradient across the inner mitochondrial membrane* (Figure 18.1).

Collectively, the generation of high-transfer-potential electrons by the citric acid cycle, their flow through the respiratory chain, and the accompanying synthesis of ATP is called *respiration* or *cellular respiration*.

18.1 Eukaryotic Oxidative Phosphorylation Takes Place in Mitochondria

Recall that the biochemical purpose of the citric acid cycle, which takes place in mitochondria, is to generate high-energy electrons. It is fitting, therefore, that oxidative phosphorylation, which will convert the energy of these electrons into ATP, also takes place in mitochondria. Mitochondria are oval-shaped organelles, typically about 2 μm in length and 0.5 μm in diameter, about the size of a bacterium. Eugene Kennedy and Albert Lehninger discovered more than a half-century ago that *mitochondria contain the respiratory assembly, the enzymes of the citric acid cycle, and the enzymes of fatty acid oxidation.*

Mitochondria are bounded by a double membrane

Electron microscopic studies by George Palade and Fritjof Sjöstrand revealed that mitochondria have two membrane systems: an *outer membrane* and an extensive, highly folded *inner membrane.* The inner membrane is folded into a series of internal ridges called *cristae.* Hence, there are two compartments in mitochondria: (1) the *intermembrane space* between the outer and the inner membranes and (2) the *matrix,* which is bounded by the inner membrane (Figure 18.2). The mitochondrial matrix is the site of most of the reactions of the citric acid cycle and fatty acid oxidation. In contrast, oxidative phosphorylation takes place in the inner mitochondrial membrane. The increase in surface area of the inner mitochondrial membrane provided by the cristae creates more sites for oxidative phosphorylation than would be the case with a simple, unfolded membrane. Humans contain an estimated 14,000 m^2 of inner mitochondrial membrane, which is the approximate equivalent of three football fields in the United States.

The outer membrane is quite permeable to most small molecules and ions because it contains many copies of *mitochondrial porin,* a 30- to 35-kd pore-forming protein also known as VDAC, for *v*oltage-*d*ependent *a*nion *c*hannel. VDAC, the most prevalent protein in the outer mitochondrial membrane, plays a role in the regulated flux of metabolites—usually anionic species such as phosphate, chloride, organic anions, and the adenine nucleotides—across the outer membrane. In contrast, the inner membrane is impermeable to nearly all ions and polar molecules. A large family of

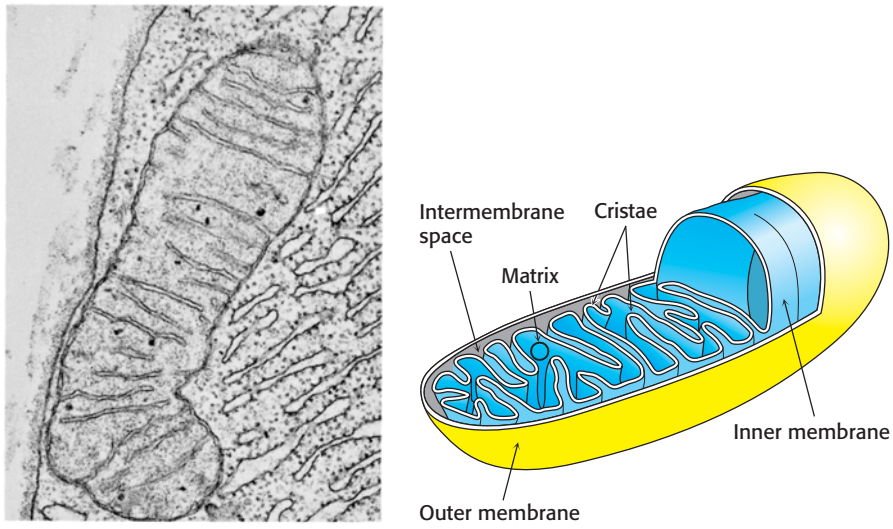


Figure 18.2 Electron micrograph (A) and diagram (B) of a mitochondrion. [(A) Courtesy of George Palade. (B) From Wolfe, *Biology of the Cell,* 2e, © 1981 Brooks/Cole, a part of Cengage Learning, Inc. Reproduced by permission www. cengage.com/permission 3.]

transporters shuttles metabolites such as ATP, pyruvate, and citrate across the inner mitochondrial membrane. The two faces of this membrane will be referred to as the *matrix side* and the *cytoplasmic side* (the latter because it is freely accessible to most small molecules in the cytoplasm). They are also called the *N* and *P* sides, respectively, because the membrane potential is negative on the matrix side and positive on the cytoplasmic side.

In prokaryotes, the electron-driven proton pumps and ATP-synthesizing complex are located in the cytoplasmic membrane, the inner of two membranes. The outer membrane of bacteria, like that of mitochondria, is permeable to most small metabolites because of the presence of porins.

Mitochondria are the result of an endosymbiotic event

Mitochondria are semiautonomous organelles that live in an endosymbiotic relation with the host cell. These organelles contain their own DNA, which encodes a variety of different proteins and RNAs. Mitochondrial DNA is usually portrayed as being circular, but recent research suggests that the mitochondrial DNA of many organisms may be linear. The genomes of mitochondria range broadly in size across species. The mitochondrial genome of the protist *Plasmodium falciparum* consists of fewer than 6000 base pairs (bp), whereas those of some land plants comprise more than 200,000 bp (Figure 18.3). Human mitochondrial DNA comprises 16,569 bp and encodes 13 respiratory-chain proteins as well as the small and large ribosomal RNAs and enough tRNAs to translate all codons. However, mitochondria also contain many proteins encoded by nuclear DNA. Cells that contain mitochondria depend on these organelles for oxidative phosphorylation, and the mitochondria in turn depend on the cell for their very existence. How did this intimate symbiotic relation come to exist?

An *endosymbiotic event* is thought to have occurred whereby a free-living organism capable of oxidative phosphorylation was engulfed by another cell. The double-membrane, circular DNA (with exceptions) and the mitochondrial-specific transcription and translation machinery all point to this conclusion. Thanks to the rapid accumulation of sequence data for mitochondrial and bacterial genomes, speculation on the origin of the "original" mitochondrion with some authority is now possible. The most mitochondrial-like bacterial genome is that of *Rickettsia prowazekii,* the cause of louse-borne typhus. The genome for this organism is more than

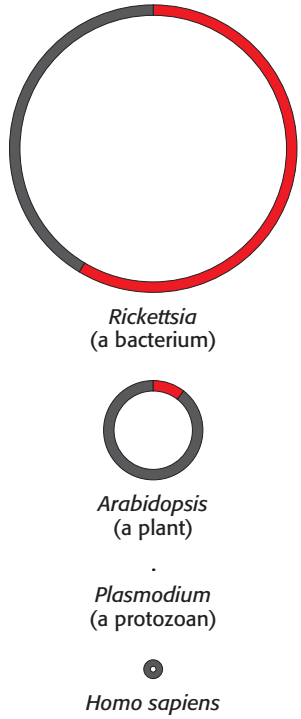


Figure 18.3 Sizes of mitochondrial genomes. The sizes of three mitochondrial genomes compared with the genome of *Rickettsia,* a relative of the presumed ancestor of all mitochondria. For genomes of more than 60 kbp, the DNA coding region for genes with known function is shown in red.

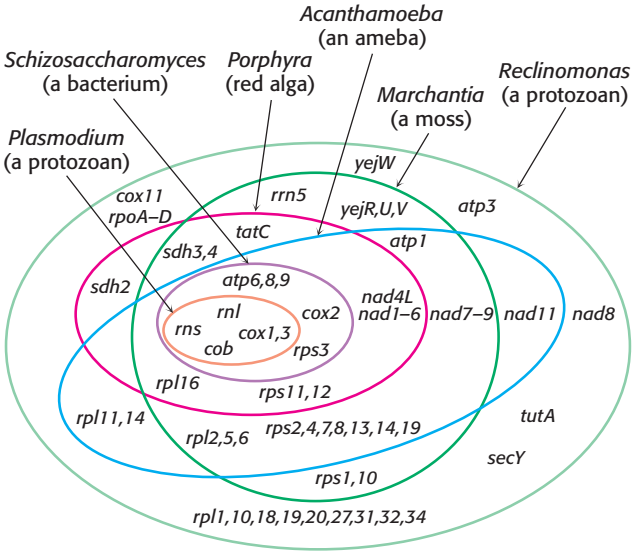


Figure 18.4 Overlapping gene complements of mitochondria. The genes present within each oval are those present within the organism represented by the oval. Only rRNA- and protein-coding genes are shown. The genome of *Reclinomonas* contains all the protein-coding genes found in all the sequenced mitochondrial genomes. [After M. W. Gray, G. Burger, and B. F. Lang. *Science* 283:1476–1481, 1999.]

1 million base pairs in size and contains 834 protein-encoding genes. Sequence data suggest that all extant mitochondria are derived from an ancestor of *R. prowazekii* as the result of a single endosymbiotic event.

The evidence that modern mitochondria result from a single event comes from examination of the most bacteria-like mitochondrial genome, that of the protozoan *Reclinomonas americana*. Its genome contains 97 genes, of which 62 specify proteins. The genes encoding these proteins include all of the protein-coding genes found in all of the sequenced mitochondrial genomes (Figure 18.4). Yet, this genome encodes less than 2% of the protein-coding genes in the bacterium *E. coli*. In other words, a small fraction of bacterial genes—2%—are found in all examined mitochondria. How is it possible that all mitochondria have the same 2% of the bacterial genome? It seems unlikely that mitochondrial genomes resulting from several endosymbiotic events could have been independently reduced to the same set of genes found in *R. americana*. Thus, the simplest explanation is that the endosymbiotic event took place just once and all existing mitochondria are descendants of that ancestor.

Note that transient engulfment of prokaryotic cells by larger cells is not uncommon in the microbial world. In regard to mitochondria, such a transient relation became permanent as the bacterial cell lost DNA, making it incapable of independent living, and the host cell became dependent on the ATP generated by its tenant.

18.2 Oxidative Phosphorylation Depends on Electron Transfer

In Chapter 17, the primary function of the citric acid cycle was identified as the generation of NADH and $FADH_2$ by the oxidation of acetyl CoA. In oxidative phosphorylation, electrons from NADH and $FADH_2$ are used to reduce molecular oxygen to water. The highly exergonic reduction of molecular oxygen by NADH and $FADH_2$ is accomplished by a number of electron-transfer reactions, which take place in a set of membrane proteins known as the *electron-transport chain*.

The electron-transfer potential of an electron is measured as redox potential

In oxidative phosphorylation, the *electron-transfer potential* of NADH or $FADH_2$ is converted into the *phosphoryl-transfer potential* of ATP. We need quantitative expressions for these forms of free energy. The measure of phosphoryl-transfer potential is already familiar to us: it is given by $\Delta G^{\circ\prime}$ for the hydrolysis of the activated phosphoryl compound. The corresponding expression for the electron-transfer potential is E'_0, the *reduction potential* (also called the *redox potential* or *oxidation–reduction potential*).

The reduction potential is an electrochemical concept. Consider a substance that can exist in an oxidized form X and a reduced form X^-. Such a pair is called a *redox couple* and is designated $X : X^-$. The reduction potential of this couple can be determined by measuring the electromotive force generated by an apparatus called a *sample half-cell* connected to a *standard reference half-cell* (Figure 18.5). The sample half-cell consists of an electrode

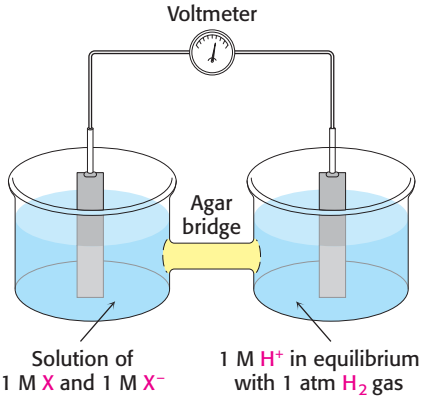


Figure 18.5 Measurement of redox potential. Apparatus for the measurement of the standard oxidation–reduction potential of a redox couple. Electrons, but not X or X^-, can flow through the agar bridge.

immersed in a solution of 1 M oxidant (X) and 1 M reductant (X^-). The standard reference half-cell consists of an electrode immersed in a 1 M H^+ solution that is in equilibrium with H_2 gas at 1 atmosphere (1 atm) of pressure. The electrodes are connected to a voltmeter, and an agar bridge allows ions to move from one half-cell to the other, establishing electrical continuity between the half-cells. Electrons then flow from one half-cell to the other through the wire connecting the two half-cells to the voltmeter. If the reaction proceeds in the direction

$$X^- + H^+ \rightarrow X + {}^1/_2\, H_2$$

the reactions in the half-cells (referred to as *half-reactions* or *couples*) must be

$$X^- \rightarrow X + e^- \qquad H^+ + e^- \rightarrow {}^1/_2\, H_2$$

Thus, electrons flow from the sample half-cell to the standard reference half-cell, and the sample-cell electrode is taken to be negative with respect to the standard-cell electrode. *The reduction potential of the $X : X^-$ couple is the observed voltage at the start of the experiment* (when X, X^-, and H^+ are 1 M with 1 atm of H_2). *The reduction potential of the $H^+ : H_2$ couple is defined to be 0 volts.* In oxidation–reduction reactions, the donor of electrons, in this case X, is called the reductant or reducing agent, whereas the acceptor of electrons, H^+ here, is called the oxidant.

The meaning of the reduction potential is now evident. A negative reduction potential means that the oxidized form of a substance has lower affinity for electrons than does H_2, as in the preceding example. A positive reduction potential means that the oxidized form of a substance has higher affinity for electrons than does H_2. These comparisons refer to standard conditions—namely, 1 M oxidant, 1 M reductant, 1 M H^+, and 1 atm H_2. Thus, *a strong reducing agent (such as NADH) is poised to donate electrons and has a negative reduction potential, whereas a strong oxidizing agent (such as O_2) is ready to accept electrons and has a positive reduction potential.*

The reduction potentials of many biologically important redox couples are known (Table 18.1). Table 18.1 is like those presented in chemistry

Table 18.1 Standard reduction potentials of some reactions

Oxidant	Reductant	n	E'_0 (V)
Succinate + CO_2	α-Ketoglutarate	2	−0.67
Acetate	Acetaldehyde	2	−0.60
Ferredoxin (oxidized)	Ferredoxin (reduced)	1	−0.43
2 H^+	H_2	2	−0.42
NAD^+	NADH + H^+	2	−0.32
$NADP^+$	NADPH + H^+	2	−0.32
Lipoate (oxidized)	Lipoate (reduced)	2	−0.29
Glutathione (oxidized)	Glutathione (reduced)	2	−0.23
FAD	$FADH_2$	2	−0.22
Acetaldehyde	Ethanol	2	−0.20
Pyruvate	Lactate	2	−0.19
Fumarate	Succinate	2	+0.03
Cytochrome *b* (+3)	Cytochrome *b* (+2)	1	+0.07
Dehydroascorbate	Ascorbate	2	+0.08
Ubiquinone (oxidized)	Ubiquinone (reduced)	2	+0.10
Cytochrome *c* (+3)	Cytochrome *c* (+2)	1	+0.22
Fe (+3)	Fe (+2)	1	+0.77
½ O_2 + 2 H^+	H_2O	2	+0.82

Note: E'_0 is the standard oxidation–reduction potential (pH 7, 25°C) and n is the number of electrons transferred. E'_0 refers to the partial reaction written as Oxidant + $e^- \rightarrow$ reductant

textbooks, except that a hydrogen ion concentration of 10^{-7} M (pH 7) instead of 1 M (pH 0) is the standard state adopted by biochemists. This difference is denoted by the prime in E'_0. Recall that the prime in $\Delta G^{\circ\prime}$ denotes a standard free-energy change at pH 7.

The standard free-energy change $\Delta G^{\circ\prime}$ is related to the change in reduction potential $\Delta E'_0$ by

$$\Delta G^{\circ\prime} = -nF\Delta E'_0$$

in which n is the number of electrons transferred, F is a proportionality constant called the *faraday* [96.48 kJ mol^{-1} V^{-1} (23.06 kcal mol^{-1} V^{-1})], $\Delta E'_0$ is in volts, and $\Delta G^{\circ\prime}$ is in kilojoules or kilocalories per mole.

The free-energy change of an oxidation–reduction reaction can be readily calculated from the reduction potentials of the reactants. For example, consider the reduction of pyruvate by NADH, catalyzed by lactate dehydrogenase. Recall that this reaction maintains redox balance in lactic acid fermentation (see Figure 16.11).

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{lactate} + \text{NAD}^+ \qquad \text{(A)}$$

The reduction potential of the NAD^+ : NADH couple, or half-reaction, is -0.32 V, whereas that of the pyruvate : lactate couple is -0.19 V. By convention, reduction potentials (as in Table 18.1) refer to partial reactions written as reductions: oxidant + e^- $\longrightarrow$ reductant. Hence,

$$\text{Pyruvate} + 2\,\text{H}^+ + 2\,\text{e}^- \longrightarrow \text{lactate} \qquad E'_0 = -0.19\,\text{V} \qquad \text{(B)}$$
$$\text{NAD}^+ + \text{H}^+ + 2\,\text{e}^- \longrightarrow \text{NADH} \qquad E'_0 = -0.32\,\text{V} \qquad \text{(C)}$$

To obtain reaction A from reactions B and C, we need to reverse the direction of reaction C so that NADH appears on the left side of the arrow. In doing so, the sign of E'_0 must be changed.

$$\text{Pyruvate} + 2\,\text{H}^+ + 2\,\text{e}^- \longrightarrow \text{lactate} \qquad E'_0 = -0.19\,\text{V} \qquad \text{(B)}$$
$$\text{NADH} \longrightarrow \text{NAD}^+ + \text{H}^+ + 2\,\text{e}^- \qquad E'_0 = +0.32\,\text{V} \qquad \text{(D)}$$

For reaction B, the free energy can be calculated with $n = 2$.

$$\begin{aligned}\Delta G^{\circ\prime} &= -2 \times 96.48\ \text{kJ mol}^{-1}\ \text{V}^{-1} \times -0.19\,\text{V} \\ &= +36.7\ \text{kJ mol}^{-1}\ (+8.8\ \text{kcal mol}^{-1})\end{aligned}$$

Likewise, for reaction D,

$$\begin{aligned}\Delta G^{\circ\prime} &= -2 \times 96.48\ \text{kJ mol}^{-1}\ \text{V}^{-1} \times +0.32\,\text{V} \\ &= -61.8\ \text{kJ mol}^{-1}\ (-14.8\ \text{kcal mol}^{-1})\end{aligned}$$

Thus, the free energy for reaction A is given by

$$\begin{aligned}\Delta G^{\circ\prime} &= \Delta G^{\circ\prime}\ (\text{for reaction B}) + \Delta G^{\circ\prime}\ (\text{for reaction D}) \\ &= +36.7\ \text{kJ mol}^{-1} + (-61.8\ \text{kJ mol}^{-1}) \\ &= -25.1\ \text{kJ mol}^{-1}\ (-6.0\ \text{kcal mol}^{-1})\end{aligned}$$

A 1.14-volt potential difference between NADH and molecular oxygen drives electron transport through the chain and favors the formation of a proton gradient

The driving force of oxidative phosphorylation is the electron-transfer potential of NADH or $FADH_2$ relative to that of O_2. How much energy is released by the reduction of O_2 with NADH? Let us calculate $\Delta G^{\circ\prime}$ for this reaction. The pertinent half-reactions are

$$\tfrac{1}{2}\,O_2 + 2\,H^+ + 2\,e^- \longrightarrow H_2O \qquad E'_0 = +0.82\,V \qquad (A)$$
$$NAD^+ + H^+ + 2\,e^- \longrightarrow NADH \qquad E'_0 = -0.32\,V \qquad (B)$$

The combination of the two half-reactions, as it proceeds in the electron-transport chain, yields

$$\tfrac{1}{2}\,O_2 + NADH + H^+ \longrightarrow H_2O + NAD^+ \qquad (C)$$

The standard free energy for this reaction is then given by

$$\begin{aligned}\Delta G^{\circ\prime} &= (-2 \times 96.48\ \text{kJ mol}^{-1}\ \text{V}^{-1} \times +0.82\,\text{V}) - \\ &\quad (-2 \times 96.48\ \text{kJ mol}^{-1}\ \text{V}^{-1} \times 0.32\,\text{V}) \\ &= -158.2\ \text{kJ mol}^{-1} - 61.9\ \text{kJ mol}^{-1} \\ &= -220.1\ \text{kJ mol}^{-1}\ (-52.6\ \text{kcal mol}^{-1})\end{aligned}$$

This release of free energy is substantial. Recall that $\Delta G^{\circ\prime}$ for the hydrolysis of ATP is -30.5 kJ mol^{-1} (-7.3 kcal mol^{-1}). The released energy is initially used to generate a proton gradient that is then used for the synthesis of ATP and the transport of metabolites across the mitochondrial membrane.

How can the energy associated with a proton gradient be quantified? Recall that the free-energy change for a species moving from one side of a membrane where it is at concentration c_1 to the other side where it is at a concentration c_2 is given by

$$\Delta G = RT \ln (c_2/c_1) + ZF\Delta V$$

in which Z is the electrical charge of the transported species and ΔV is the potential in volts across the membrane (Section 13.1). Under typical conditions for the inner mitochondrial membrane, the pH outside is 1.4 units lower than inside [corresponding to $\ln(c_2/c_1)$ of 1.4] and the membrane potential is 0.14 V, the outside being positive. Because $Z = +1$ for protons, the free-energy change is $(8.32 \times 10^{-3}\ \text{kJ mol}^{-1}\ \text{K}^{-1} \times 310\ \text{K} \times 1.4) + (+1 \times 96.48\ \text{kJ mol}^{-1}\ \text{V}^{-1} \times 0.14\,\text{V}) = 21.8\ \text{kJ mol}^{-1}$ (5.2 kcal mol^{-1}). Thus, each proton that is transported out of the matrix to the cytoplasmic side corresponds to 21.8 kJ mol^{-1} of free energy.

18.3 The Respiratory Chain Consists of Four Complexes: Three Proton Pumps and a Physical Link to the Citric Acid Cycle

Electrons are transferred from NADH to O_2 through a chain of three large protein complexes called *NADH-Q oxidoreductase, Q-cytochrome* c *oxidoreductase,* and *cytochrome* c *oxidase* (Figure 18.6 and Table 18.2). *Electron flow within these transmembrane complexes leads to the transport of protons across the inner mitochondrial membrane.* A fourth large protein complex, called *succinate-Q reductase,* contains the succinate dehydrogenase that generates $FADH_2$ in the citric acid cycle. Electrons from this $FADH_2$ enter the electron-transport chain at Q-cytochrome oxidoreductase. Succinate-Q reductase, in contrast with the other complexes, does not pump protons. NADH-Q oxidoreductase, succinate-Q reductase, Q-cytochrome *c* oxidoreductase, and cytochrome *c* oxidase are also called *Complex I, II, III,* and *IV,* respectively. Complexes I, II, and III appear to be associated in a supramolecular complex termed the *respirasome*. As we have seen before, such supramolecular complexes facilitate the rapid transfer of substrate and prevent the release of reaction intermediates.

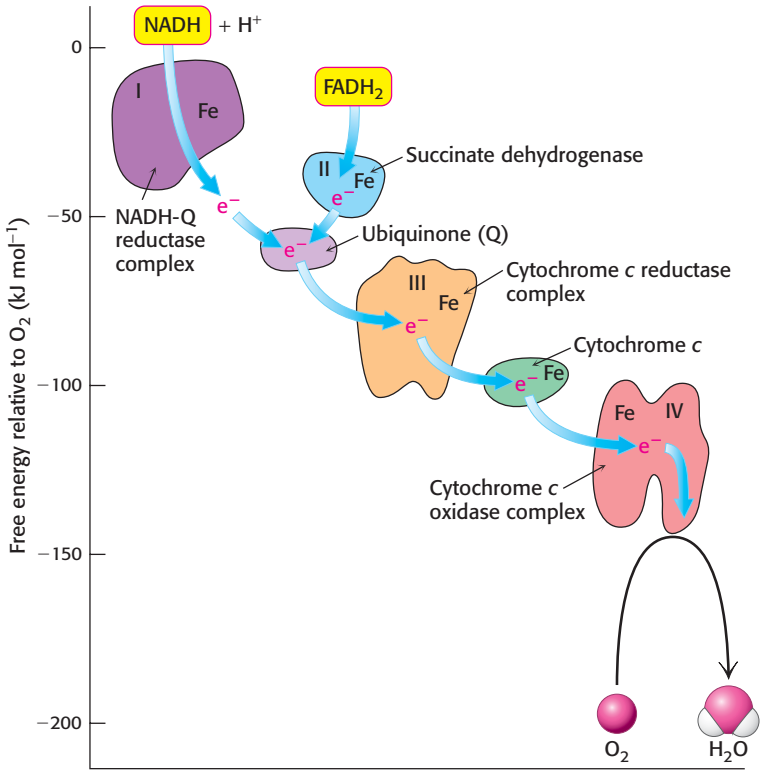


Figure 18.6 Components of the electron-transport chain. Electrons flow down an energy gradient from NADH to O_2. The flow is catalyzed by four protein complexes. Iron is a component of Complexes I, III, IV and cytochrome *c*. [After D. Sadava et al., *Life,* 8th ed. (Sinauer, 2008), p. 150.]

Two special electron carriers ferry the electrons from one complex to the next. The first is *coenzyme Q* (Q), also known as *ubiquinone* because it is a *ubi*quitous *quinone* in biological systems. Ubiquinone is a hydrophobic quinone that diffuses rapidly within the inner mitochondrial membrane. Electrons are carried from NADH-Q oxidoreductase to Q-cytochrome *c* oxidoreductase, the second complex of the chain, by the reduced form of Q. Electrons from the $FADH_2$ generated by the citric acid cycle are transferred first to ubiquinone and then to the Q-cytochrome *c* oxidoreductase complex.

Coenzyme Q is a quinone derivative with a long tail consisting of five-carbon isoprene units that account for its hydrophobic nature. The number of isoprene units in the tail depends on the species. The most common mammalian form contains 10 isoprene units (coenzyme Q_{10}). For simplicity,

Table 18.2 Components of the mitochondrial electron-transport chain

Enzyme complex	Mass (kd)	Subunits	Prosthetic group	Oxidant or reductant		
				Matrix side	Membrane core	Cytoplasmic side
NADH-Q oxidoreductase	>900	46	FMN Fe-S	NADH	Q	
Succinate-Q reductase	140	4	FAD Fe-S	Succinate	Q	
Q-cytochrome *c* oxidoreductase	250	11	Heme b_H Heme b_L Heme c_1 Fe-S		Q	Cytochrome *c*
Cytochrome *c* oxidase	160	13	Heme *a* Heme a_3 Cu_A and Cu_B			Cytochrome *c*

Sources: J. W. DePierre and L. Ernster. *Annu. Rev. Biochem.* 46:215, 1977; Y. Hatefi. *Annu Rev. Biochem.* 54:1015, 1985; and J. E. Walker. *Q. Rev. Biophys.* 25:253, 1992.

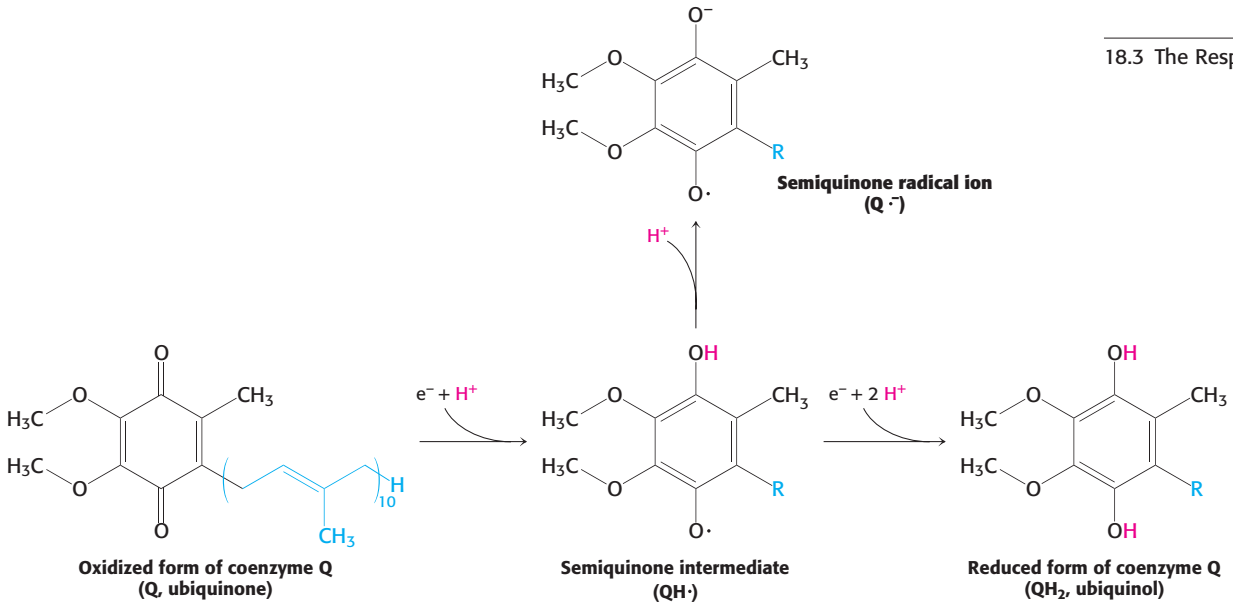


Figure 18.7 Oxidation states of quinones. The reduction of ubiquinone (Q) to ubiquinol (QH_2) proceeds through a semiquinone intermediate (QH·).

the subscript will be omitted from this abbreviation because all varieties function in an identical manner. Quinones can exist in three oxidation states. In the fully oxidized state (Q), coenzyme Q has two keto groups (Figure 18.7). The addition of one electron and one proton results in the semiquinone form (QH·). The semiquinone can lose a proton to form a semiquinone radical anion ($Q\cdot^-$). The addition of a second electron and proton to the semiquinone generates ubiquinol (QH_2), the fully reduced form of coenzyme Q, which holds its protons more tightly. Thus, *for quinones, electron-transfer reactions are coupled to proton binding and release,* a property that is key to transmembrane proton transport. Because ubiquinone is soluble in the membrane, a pool of Q and QH_2—the *Q pool*—is thought to exist in the inner mitochondrial membrane.

In contrast with Q, the second special electron carrier is a protein. Cytochrome *c,* a small soluble protein, shuttles electrons from Q-cytochrome *c* oxidoreductase to cytochrome *c* oxidase, the final component in the chain and the one that catalyzes the reduction of O_2.

The high-potential electrons of NADH enter the respiratory chain at NADH-Q oxidoreductase

The electrons of NADH enter the chain at *NADH-Q oxidoreductase* (also called *Complex I* and *NADH dehydrogenase*), an enormous enzyme (>900 kd) consisting of approximately 46 polypeptide chains. This proton pump, like that of the other two in the respiratory chain, is encoded by genes residing in both the mitochondria and the nucleus. NADH-Q oxidoreductase is L-shaped, with a horizontal arm lying in the membrane and a vertical arm that projects into the matrix.

The reaction catalyzed by this enzyme appears to be

$$NADH + Q + 5\,H^+_{matrix} \rightarrow NAD^+ + QH_2 + 4\,H^+_{cytoplasm}$$

The initial step is the binding of NADH and the transfer of its two high-potential electrons to the *flavin mononucleotide* (FMN) prosthetic group of this complex to give the reduced form, $FMNH_2$ (Figure 18.8). The electron acceptor of FMN, the isoalloxazine ring, is identical with that of FAD.

Electrons are then transferred from $FMNH_2$ to a series of *iron–sulfur clusters,* the second type of prosthetic group in NADH-Q oxidoreductase.

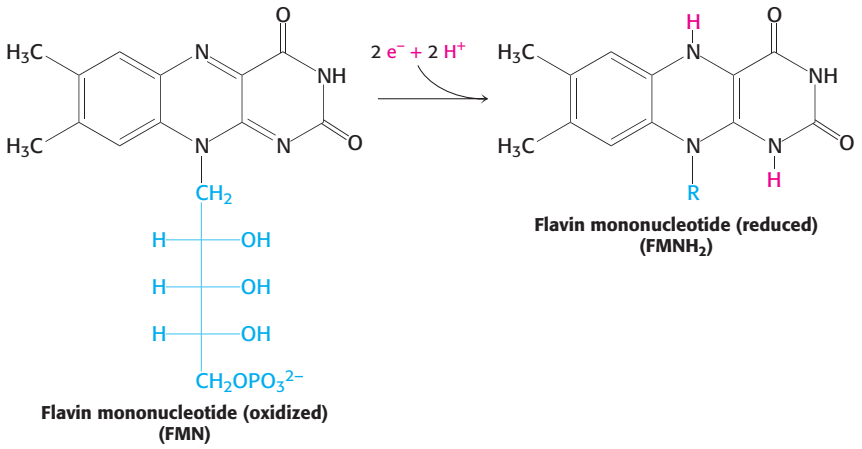


Figure 18.8 Oxidation states of flavins.

Fe-S clusters in *iron–sulfur proteins* (also called *nonheme iron proteins*) play a critical role in a wide range of reduction reactions in biological systems. Several types of Fe-S clusters are known (Figure 18.9). In the simplest kind, a single iron ion is tetrahedrally coordinated to the sulfhydryl groups of four cysteine residues of the protein. A second kind, denoted by 2Fe-2S, contains two iron ions, two inorganic sulfides, and usually four cysteine residues. A third type, designated 4Fe-4S, contains four iron ions, four inorganic sulfides, and four cysteine residues. NADH-Q oxidoreductase contains both 2Fe-2S and 4Fe-4S clusters. Iron ions in these Fe-S complexes cycle between Fe^{2+} (reduced) and Fe^{3+} (oxidized) states. Unlike quinones and flavins, iron–sulfur clusters generally undergo oxidation–reduction reactions without releasing or binding protons.

All of the redox reactions take place in the extramembranous part of NADH-Q oxidoreductase. Although the details of electron transfer through this complex remain the subject of ongoing investigation, NADH clearly binds to a site in the extramembranous domain. NADH transfers its two electrons to FMN. These electrons flow through a series of Fe-S centers and then to coenzyme Q. *The flow of two electrons from NADH to coenzyme Q through NADH-Q oxidoreductase leads to the pumping of four hydrogen ions out of the matrix of the mitochondrion. In accepting two electrons, Q takes up two protons from the matrix as it is reduced to* QH_2 (Figure 18.10). The QH_2 leaves the enzyme for the hydrophobic interior of the membrane.

It is important to note that the citric acid cycle is not the only source of mitochondrial NADH. As we will see in Chapter 22, fatty acid degradation,

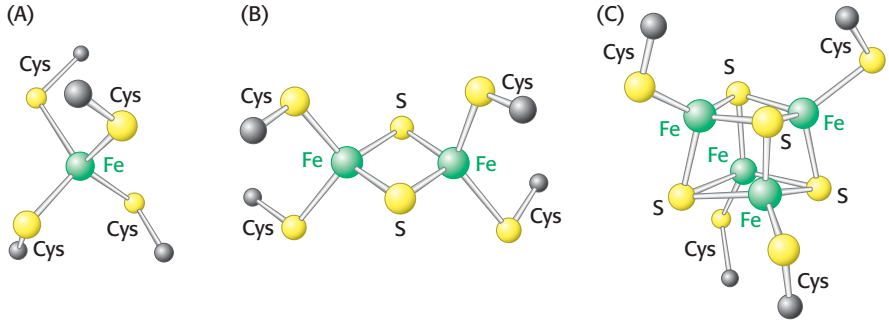


Figure 18.9 Iron–sulfur clusters. (A) A single iron ion bound by four cysteine residues. (B) 2Fe-2S cluster with iron ions bridged by sulfide ions. (C) 4Fe-4S cluster. Each of these clusters can undergo oxidation–reduction reactions.

which also takes place in mitochondria, is another crucial source of NADH for the electron-transport chain. Moreover, cytoplasmically generated NADH can be transported into mitochondria for use by the electron-transport chain (Section 18.5).

Ubiquinol is the entry point for electrons from $FADH_2$ of flavoproteins

$FADH_2$ enters the electron-transport chain at the second protein complex of the chain. Recall that $FADH_2$ is formed in the citric acid cycle, in the oxidation of succinate to fumarate by succinate dehydrogenase (Section 17.2). Succinate dehydrogenase, a citric acid cycle enzyme, is part of the *succinate-Q reductase complex (Complex II),* an integral membrane protein of the inner mitochondrial membrane. $FADH_2$ does not leave the complex. Rather, its electrons are transferred to Fe-S centers and then finally to Q to form QH_2, which then is ready to transfer electrons further down the electron-transport chain. The succinate-Q reductase complex, in contrast with NADH-Q oxidoreductase, does not pump protons from one side of the membrane to the other. Consequently, less ATP is formed from the oxidation of $FADH_2$ than from NADH.

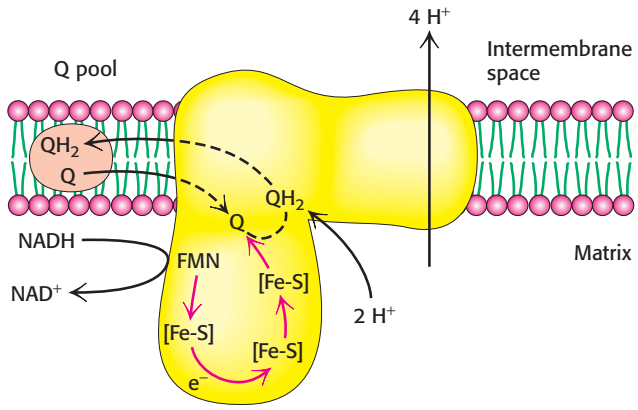


Figure 18.10 Coupled electron–proton transfer reactions through NADH-Q oxidoreductase. Electrons flow in Complex I from NADH through FMN and a series of iron–sulfur clusters to ubiquinone (Q). The electron flow (red arrows) results in the pumping of four protons and the uptake of two protons from the mitochondrial matrix. [Based on U. Brandt et al. *FEBS Letters* 545:9–17, 2003, Fig. 2.]

Two other enzymes that we will encounter later, *glycerol phosphate dehydrogenase* (p. 551) and *fatty acyl CoA dehydrogenase* (Section 22.2), likewise transfer their high-potential electrons from $FADH_2$ to Q to form ubiquinol (QH_2), the reduced state of ubiquinone. These enzymes oxidize glycerol and fats, respectively, providing electrons for oxidative phosphorylation. These enzymes also do not pump protons.

Electrons flow from ubiquinol to cytochrome *c* through Q-cytochrome *c* oxidoreductase

What is the fate of ubiquinol generated by Complexes I and II? The electrons from QH_2 are passed on to cytochrome *c* by the second of the three proton pumps in the respiratory chain, *Q-cytochrome* c *oxidoreductase* (also known as *Complex III* and as *cytochrome reductase*). The function of Q-cytochrome *c* oxidoreductase is to catalyze the transfer of electrons from QH_2 to oxidized *cytochrome* c (Cyt *c*), a water-soluble protein, and concomitantly pump protons out of the mitochondrial matrix. The flow of a pair of electrons through this complex leads to the effective net transport of 2 H^+ to the cytoplasmic side, half the yield obtained with NADH-Q reductase because of a smaller thermodynamic driving force.

$$QH_2 + 2\ \text{Cyt}\ c_{ox} + 2\ H^+_{matrix} \rightarrow Q + 2\ \text{Cyt}\ c_{red} + 4\ H^+_{cytoplasm}$$

Q-cytochrome *c* oxidoreductase itself contains two types of cytochromes, named *b* and c_1 (Figure 18.11). *A cytochrome is an electron-transferring protein that contains a heme prosthetic group.* The iron ion of a cytochrome alternates between a reduced ferrous (+2) state and an oxidized ferric (+3) state during electron transport. The two cytochrome subunits of Q-cytochrome *c* oxidoreductase contain a total of three hemes: two hemes within cytochrome *b*, termed heme b_L (L for low affinity) and heme b_H (H for high affinity), and one heme within cytochrome c_1. The heme prosthetic group in cytochromes *b*, c_1, and *c* is iron-protoporphyrin IX, the same heme present in myoglobin and hemoglobin (Section 7.1). These identical hemes have different electron affinities because they are in different

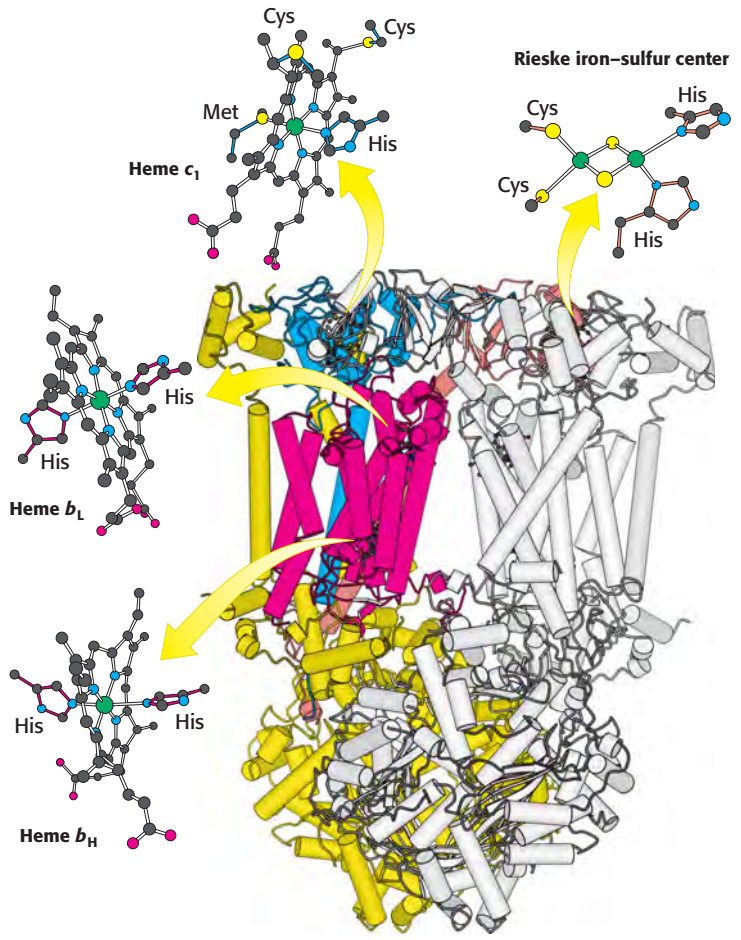


Figure 18.11 Structure of Q-cytochrome *c* oxidoreductase (cytochrome bc_1). This enzyme is a homodimer with 11 distinct polypeptide chains. *Notice* that the major prosthetic groups, three hemes and a 2Fe-2S cluster, are located either near the cytoplasmic edge of the complex bordering the intermembrane space (top) or in the region embedded in the membrane (α helices represented by tubes). They are well positioned to mediate the electron-transfer reactions between quinones in the membrane and cytochrome *c* in the intermembrane space. [Drawn from 1BCC.pdb.]

polypeptide environments. For example, heme b_L, which is located in a cluster of helices near the cytoplasmic face of the membrane, has lower affinity for an electron than does heme b_H, which is near the matrix side. Q-cytochrome *c* oxidoreductase is also known as cytochrome bc_1 after its cytochrome groups.

In addition to the hemes, the enzyme contains an iron–sulfur protein with a 2Fe-2S center. This center, termed the *Rieske center,* is unusual in that one of the iron ions is coordinated by two histidine residues rather than two cysteine residues. This coordination stabilizes the center in its reduced form, raising its reduction potential so that it can readily accept electrons from QH_2.

The Q cycle funnels electrons from a two-electron carrier to a one-electron carrier and pumps protons

QH_2 passes two electrons to Q-cytochrome *c* oxidoreductase, but the acceptor of electrons in this complex, cytochrome *c*, can accept only one electron. How does the switch from the two-electron carrier ubiquinol to the one-electron carrier cytochrome *c* take place? The mechanism for the coupling of electron transfer from Q to cytochrome *c* to transmembrane proton transport is known as the *Q cycle* (Figure 18.12). Two QH_2 molecules bind to the complex consecutively, each giving up two electrons and two H^+. *These protons are released to the cytoplasmic side of the membrane.* The first QH_2 to exit the Q pool binds to the first Q binding site (Q_o), and its two electrons travel through the complex to different destinations. One electron flows, first, to the Rieske 2Fe-2S cluster; then, to cytochrome c_1; and, finally, to a

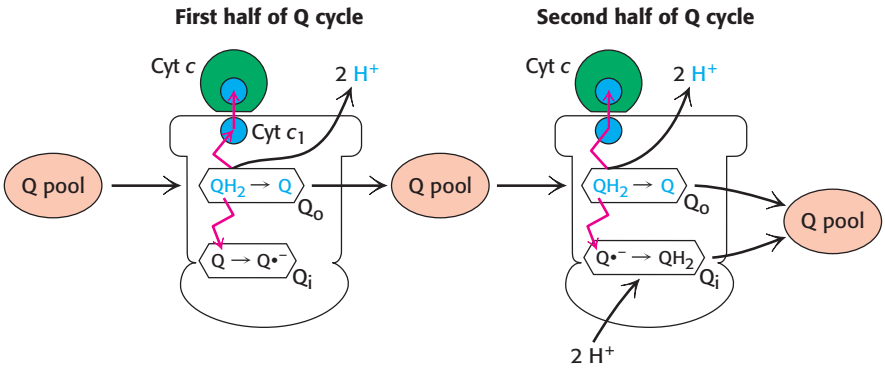


Figure 18.12 Q cycle. The Q cycle takes place in Complex III, which is represented in outline form. In the first half of the cycle, two electrons of a bound QH_2 are transferred, one to cytochrome *c* and the other to a bound Q in a second binding site to form the semiquinone radical anion $Q\cdot^-$. The newly formed Q dissociates and enters the Q pool. In the second half of the cycle, a second QH_2 also gives up its electrons to complex II, one to a second molecule of cytochrome *c* and the other to reduce $Q\cdot^-$ to QH_2. This second electron transfer results in the uptake of two protons from the matrix. The path of electron transfer is shown in red.

molecule of oxidized cytochrome *c*, converting it into its reduced form. The reduced cytochrome *c* molecule is free to diffuse away from the enzyme to continue down the respiratory chain.

The second electron passes through two heme groups of cytochrome *b* to an oxidized ubiquinone in a second Q binding site (Q_i). The Q in the second binding site is reduced to a semiquinone radical anion ($Q\cdot^-$) by the electron from the first QH_2. The now fully oxidized Q leaves the first Q site, free to reenter the Q pool.

A second molecule of QH_2 binds to the Q_o site of Q-cytochrome *c* oxidoreductase and reacts in the same way as the first. One of the electrons is transferred to cytochrome *c*. The second electron passes through the two heme groups of cytochrome *b* to partly reduced ubiquinone bound in the Q_i binding site. On the addition of the electron from the second QH_2 molecule, this quinone radical anion takes up two protons from the matrix side to form QH_2. *The removal of these two protons from the matrix contributes to the formation of the proton gradient.* In sum, four protons are released on the cytoplasmic side, and two protons are removed from the mitochondrial matrix.

$$2\,QH_2 + Q + 2\,\text{Cyt}\,c_{ox} + 2\,H^+_{matrix} \longrightarrow 2\,Q + QH_2 + 2\,\text{Cyt}\,c_{red} + 4\,H^+_{cytoplasm}$$

In one Q cycle, two QH_2 molecules are oxidized to form two Q molecules, and then one Q molecule is reduced to QH_2. The problem of how to efficiently funnel electrons from a two-electron carrier (QH_2) to a one-electron carrier (cytochrome *c*) is solved by the Q cycle. The cytochrome *b* component of the reductase is in essence a recycling device that enables both electrons of QH_2 to be used effectively.

Cytochrome *c* oxidase catalyzes the reduction of molecular oxygen to water

The last of the three proton-pumping assemblies of the respiratory chain is *cytochrome* c *oxidase (Complex IV)*. Cytochrome *c* oxidase catalyzes the transfer of electrons from the reduced form of cytochrome *c* to molecular oxygen, the final acceptor.

$$4\,\text{Cyt}\,c_{red} + 8\,H^+_{matrix} + \longrightarrow 4\,\text{Cyt}\,c_{ox} + 2\,H_2O + 4\,H^+_{cytoplasm}$$

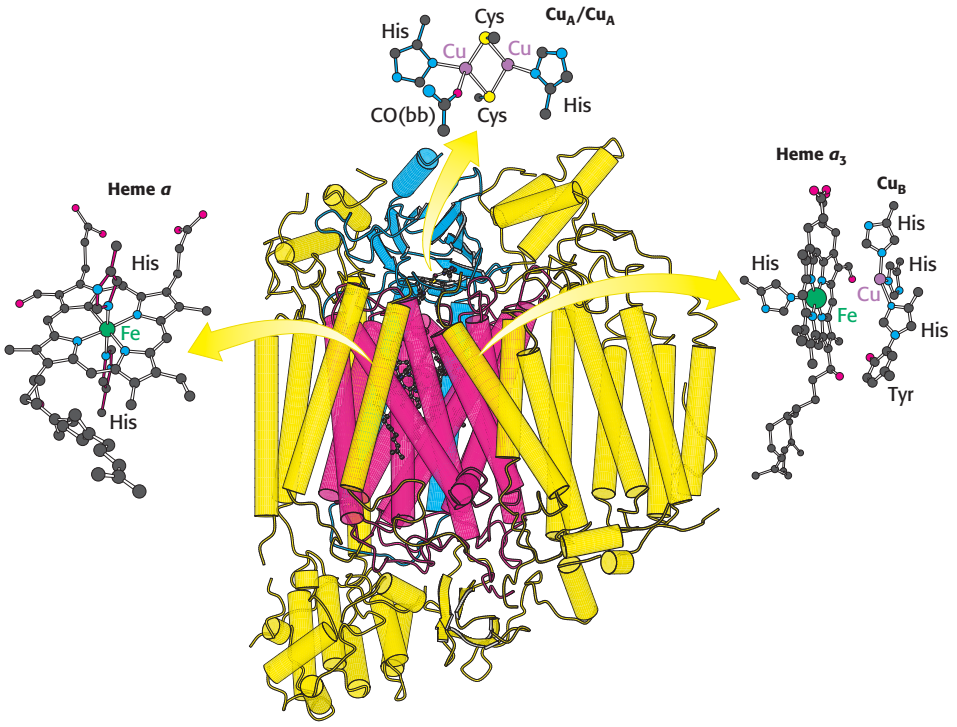
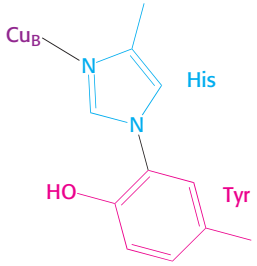


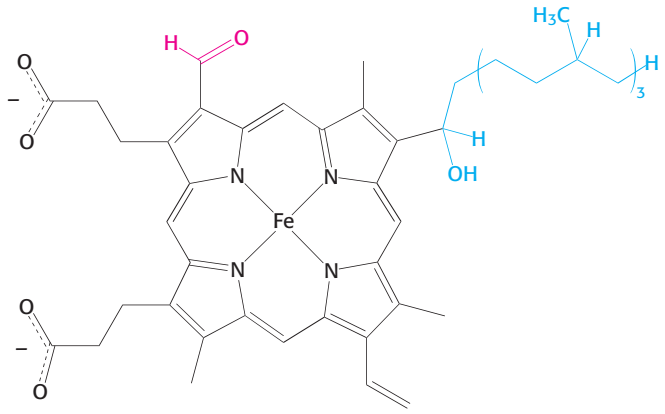
Figure 18.13 Structure of cytochrome *c* oxidase. This enzyme consists of 13 polypeptide chains. *Notice* that most of the complex, as well as two major prosthetic groups (heme *a* and heme a_3–Cu_B) are embedded in the membrane (α helices represented by vertical tubes). Heme a_3–Cu_B is the site of the reduction of oxygen to water. The Cu_A/Cu_A prosthetic group is positioned near the intermembrane space to better accept electrons from cytochrome *c*. CO(bb) is a carbonyl group of the peptide backbone. [Drawn from 20CC.pdb.]

The requirement of oxygen for this reaction is what makes "aerobic" organisms aerobic. To obtain oxygen for this reaction is the reason that human beings must breath. Four electrons are funneled to O_2 to completely reduce it to H_2O, and, concomitantly, protons are pumped from the matrix to the cytoplasmic side of the inner mitochondrial membrane. This reaction is quite thermodynamically favorable. From the reduction potentials in Table 18.1, the standard free-energy change for this reaction is calculated to be $\Delta G^{\circ\prime} = -231.8$ kJ mol^{-1} (-55.4 kcal mol^{-1}). As much of this free energy as possible must be captured in the form of a proton gradient for subsequent use in ATP synthesis.

Bovine cytochrome *c* oxidase is reasonably well understood at the structural level (Figure 18.13). It consists of 13 subunits, 3 of which are encoded by the mitochondrion's own genome. Cytochrome *c* oxidase contains two *heme A* groups and three *copper ions*, arranged as two copper centers, designated A and B. One center, Cu_A/Cu_A, contains two copper ions linked by two bridging cysteine residues. This center initially accepts electrons from reduced cytochrome *c*. The remaining copper ion, Cu_B, is coordinated by three histidine residues, one of which is modified by covalent linkage to a tyrosine residue. The copper centers alternate between the reduced Cu^+ (cuprous) form and the oxidized Cu^{2+} (cupric) form as they accept and donate electrons.



There are two heme A molecules, called *heme* a and *heme* a_3, in cytochrome *c* oxidase. Heme A differs from the heme in cytochrome *c* and c_1 in three ways: (1) a formyl group replaces a methyl group, (2) a C_{17} hydrocarbon chain replaces one of the vinyl groups, and (3) the heme is not covalently attached to the protein.



Heme A

Heme *a* and heme a_3 have distinct redox potentials because they are located in different environments within cytochrome *c* oxidase. An electron flows from cytochrome *c* to Cu_A/Cu_A, to heme *a* to heme a_3 to Cu_B, and finally to O_2. Heme a_3 and Cu_B are directly adjacent. Together, *heme* a_3 and Cu_B form the active center at which O_2 is reduced to H_2O.

Four molecules of cytochrome *c* bind consecutively to the enzyme and transfer an electron to reduce one molecule of O_2 to H_2O (Figure 18.14).

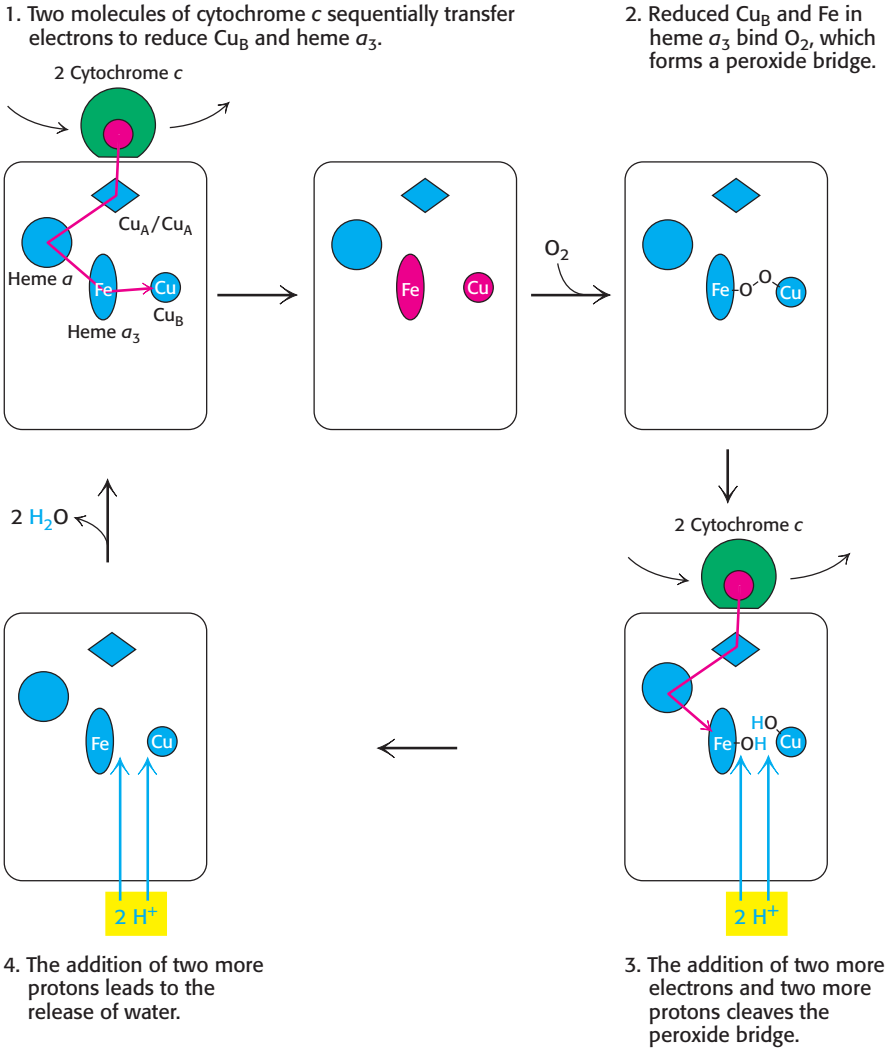


Figure 18.14 Cytochrome *c* oxidase mechanism. The cycle begins and ends with all prosthetic groups in their oxidized forms (shown in blue). Reduced forms are in red. Four cytochrome *c* molecules donate four electrons, which, in allowing the binding and cleavage of an O_2 molecule, also makes possible the import of four H^+ from the matrix to form two molecules of H_2O, which are released from the enzyme to regenerate the initial state.

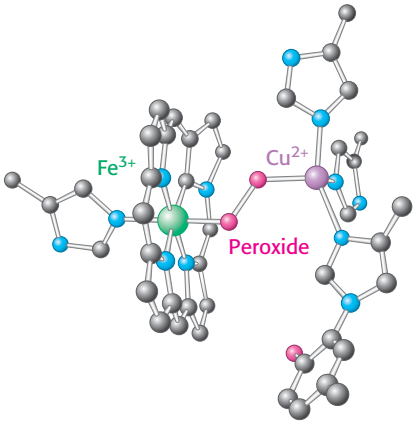


Figure 18.15 Peroxide bridge. The oxygen bound to heme a_3 is reduced to peroxide by the presence of Cu_B.

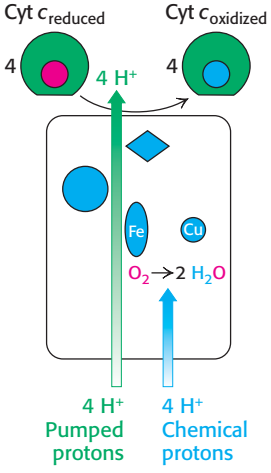


Figure 18.16 Proton transport by cytochrome *c* oxidase. Four protons are taken up from the matrix side to reduce one molecule of O_2 to two molecules of H_2O. These protons are called "chemical protons" because they participate in a clearly defined reaction with O_2. Four additional "pumped" protons are transported out of the matrix and released on the cytoplasmic side in the course of the reaction. The pumped protons double the efficiency of free-energy storage in the form of a proton gradient for this final step in the electron-transport chain.

1. Electrons from two molecules of reduced cytochrome *c* flow down an electron-transfer pathway within cytochrome *c* oxidase, one stopping at Cu_B and the other at heme a_3. With both centers in the reduced state, they together can now bind an oxygen molecule.

2. As molecular oxygen binds, it abstracts an electron from each of the nearby ions in the active center to form a peroxide (O_2^{2-}) bridge between them (Figure 18.15).

3. Two more molecules of cytochrome *c* bind and release electrons that travel to the active center. The addition of an electron as well as H^+ to each oxygen atom reduces the two ion–oxygen groups to Cu_B^{2+}—OH and Fe^{3+}—OH.

4. Reaction with two more H^+ ions allows the release of two molecules of H_2O and resets the enzyme to its initial, fully oxidized form.

$$4\ \text{Cyt}\ c_{red} + 4\ H^+_{matrix} + O_2 \rightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

The four protons in this reaction come exclusively from the matrix. Thus, the consumption of these four protons contributes directly to the proton gradient. Recall that each proton contributes 21.8 kJ mol^{-1} (5.2 kcal mol^{-1}) to the free energy associated with the proton gradient; so these four protons contribute 87.2 kJ mol^{-1} (20.8 kcal mol^{-1}), an amount substantially less than the free energy available from the reduction of oxygen to water. What is the fate of this missing energy? Remarkably, *cytochrome* c *oxidase uses this energy to pump four additional protons from the matrix to the cytoplasmic side of the membrane in the course of each reaction cycle for a total of eight protons removed from the matrix* (Figure 18.16). The details of how these protons are transported through the protein is still under study. However, two effects contribute to the mechanism. First, charge neutrality tends to be maintained in the interior of proteins. Thus, the addition of an electron to a site inside a protein tends to favor the binding of H^+ to a nearby site. Second, conformational changes take place, particularly around the heme a_3–Cu_B center, in the course of the reaction cycle. Presumably, in one conformation, protons may enter the protein exclusively from the matrix side, whereas, in another, they may exit exclusively to the cytoplasmic side. Thus, the overall process catalyzed by cytochrome *c* oxidase is

$$4\ \text{Cyt}\ c_{red} + 8\ H^+_{matrix} + O_2 \rightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O + 4\ H^+_{cytoplasm}$$

Figure 18.17 summarizes the flow of electrons from NADH and $FADH_2$ through the respiratory chain. This series of exergonic reactions is coupled to the pumping of protons from the matrix. As we will see shortly, the energy inherent in the proton gradient will be used to synthesize ATP.

Toxic derivatives of molecular oxygen such as superoxide radical are scavenged by protective enzymes

As discussed earlier, molecular oxygen is an ideal terminal electron acceptor, because its high affinity for electrons provides a large thermodynamic driving force. However, danger lurks in the reduction of O_2. The transfer of four electrons leads to safe products (two molecules of H_2O), but partial reduction generates hazardous compounds. In particular, *the transfer of a single electron to O_2 forms superoxide anion, whereas the transfer of two electrons yields peroxide.*

$$O_2 \xrightarrow{e^-} O_2^{\cdot -} \xrightarrow{e^-} O_2^{2-}$$

Superoxide ion — Peroxide

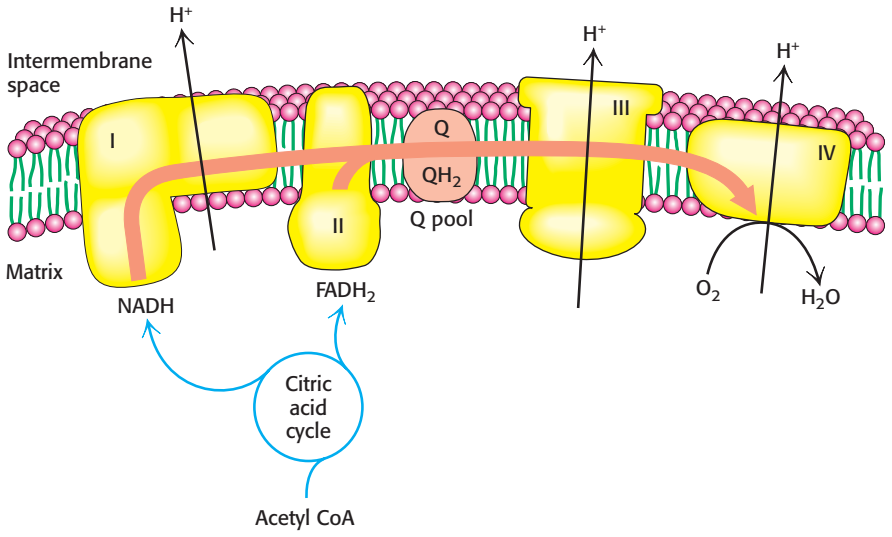


Figure 18.17 The electron-transport chain. High-energy electrons in the form of NADH and $FADH_2$ are generated by the citric acid cycle. These electrons flow through the respiratory chain, which powers proton pumping and results in the reduction of O_2.

Both compounds are potentially destructive. The strategy for the safe reduction of O_2 is clear: *the catalyst does not release partly reduced intermediates.* Cytochrome *c* oxidase meets this crucial criterion by holding O_2 tightly between Fe and Cu ions.

Although cytochrome *c* oxidase and other proteins that reduce O_2 are remarkably successful in not releasing intermediates, small amounts of superoxide anion and hydrogen peroxide are unavoidably formed. Superoxide, hydrogen peroxide, and species that can be generated from them such as OH· are collectively referred to as *reactive oxygen species* or *ROS*. Oxidative damage caused by ROS has been implicated in the aging process as well as in a growing list of diseases (Table 18.3).

What are the cellular defense strategies against oxidative damage by ROS? Chief among them is the enzyme *superoxide dismutase.* This enzyme scavenges superoxide radicals by catalyzing the conversion of two of these radicals into hydrogen peroxide and molecular oxygen.

Dismutation
A reaction in which a single reactant is converted into two different products.

$$O_2^{\cdot-} + 2H^+ \xrightleftharpoons{\text{Superoxide dismutase}} O_2 + H_2O_2$$

Eukaryotes contain two forms of this enzyme, a manganese-containing version located in mitochondria and a copper- and zinc-dependent cytoplasmic

Table 18.3 Pathological and other conditions that may entail free-radical injury

Atherogenesis
Emphysema; bronchitis
Parkinson disease
Duchenne muscular dystrophy
Cervical cancer
Alcoholic liver disease
Diabetes
Acute renal failure
Down syndrome
Retrolental fibroplasia (conversion of the retina into a fibrous mass in premature infants)
Cerebrovascular disorders
Ischemia; reperfusion injury

Source: After D. B. Marks, A. D. Marks, and C. M. Smith, *Basic Medical Biochemistry: A Clinical Approach* (Williams & Wilkins, 1996), p. 331.

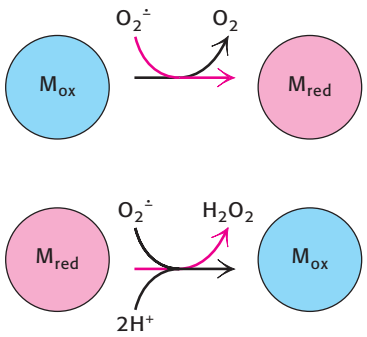


Figure 18.18 Superoxide dismutase mechanism. The oxidized form of superoxide dismutase (M_{ox}) reacts with one superoxide ion to form O_2 and generate the reduced form of the enzyme (M_{red}). The reduced form then reacts with a second superoxide and two protons to form hydrogen peroxide and regenerate the oxidized form of the enzyme.

form. These enzymes perform the dismutation reaction by a similar mechanism (Figure 18.18). The oxidized form of the enzyme is reduced by superoxide to form oxygen. The reduced form of the enzyme, formed in this reaction, then reacts with a second superoxide ion to form peroxide, which takes up two protons along the reaction path to yield hydrogen peroxide.

The hydrogen peroxide formed by superoxide dismutase and by other processes is scavenged by *catalase*, a ubiquitous heme protein that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen.

$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} O_2 + 2\,H_2O$$

Superoxide dismutase and catalase are remarkably efficient, performing their reactions at or near the diffusion-limited rate (Section 8.4). Glutathione peroxidase also plays a role in scavenging H_2O_2 (Section 20.5). Other cellular defenses against oxidative damage include the antioxidant vitamins, vitamins E and C. Because it is lipophilic, vitamin E is especially useful in protecting membranes from lipid peroxidation.

A long-term benefit of exercise may be to increase the amount of superoxide dismutase in the cell. The elevated aerobic metabolism during exercise causes more ROS to be generated. In response, the cell synthesizes more protective enzymes. The net effect is one of protection, because the increase in superoxide dismutase more effectively protects the cell during periods of rest.

Despite the fact that reactive oxygen species are known hazards, recent evidence suggests that, under certain circumstances, the controlled generation of these molecules may be important components of signal-transduction pathways. For instance, growth factors have been shown to increase ROS levels as part of their signaling pathway, and ROS regulate channels and transcription factors. The dual roles of ROS is a excellent example of the wondrous complexity of biochemistry of living systems: even potentially harmful substances can be harnessed to play useful roles.

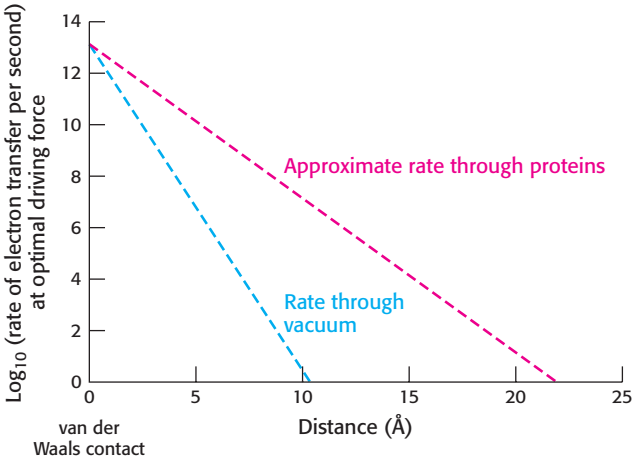


Figure 18.19 Distance dependence of electron-transfer rate. The rate of electron transfer decreases as the electron donor and the electron acceptor move apart. In a vacuum, the rate decreases by a factor of 10 for every increase of 0.8 Å. In proteins, the rate decreases more gradually, by a factor of 10 for every increase of 1.7 Å. This rate is only approximate because variations in the structure of the intervening protein medium can affect the rate.

Electrons can be transferred between groups that are not in contact

How are electrons transferred between electron-carrying groups of the respiratory chain? This question is intriguing because these groups are frequently buried in the interior of a protein in fixed positions and are therefore not directly in contact with one another. Electrons can move through space, even through a vacuum. However, the rate of electron transfer through space falls off rapidly as the electron donor and electron acceptor move apart from each other, decreasing by a factor of 10 for each increase in separation of 0.8 Å. The protein environment provides more-efficient pathways for electron conduction: typically, the rate of electron transfer decreases by a factor of 10 every 1.7 Å (Figure 18.19). For groups in contact, electron-transfer reactions can be quite fast, with rates of approximately 10^{13} s^{-1}. Within proteins in the electron-transport chain, electron-carrying groups are typically separated by 15 Å beyond their van der Waals contact distance. For such separations, we expect electron-transfer rates of approximately 10^4 s^{-1} (i.e., electron transfer in less than 1 ms), assuming that all other factors

are optimal. Without the mediation of the protein, an electron transfer over this distance would take approximately 1 day.

The case is more complicated when electrons must be transferred between two distinct proteins, such as when cytochrome *c* accepts electrons from Complex III or passes them on to Complex IV. A series of hydrophobic interactions bring the heme groups of cytochrome *c* and c_1 to within 4.5 Å of each other, with the iron atoms separated by 17.4 Å. This distance could allow cytochrome *c* reduction at a rate of $8.3 \times 10^6\ s^{-1}$.

The conformation of cytochrome *c* has remained essentially constant for more than a billion years

Cytochrome *c* is present in all organisms having mitochondrial respiratory chains: plants, animals, and eukaryotic microorganisms. This electron carrier evolved more than 1.5 billion years ago, before the divergence of plants and animals. Its function has been conserved throughout this period, as evidenced by the fact that *the cytochrome* c *of any eukaryotic species reacts in vitro with the cytochrome* c *oxidase of any other species tested thus far.* For example, wheat-germ cytochrome *c* reacts with human cytochrome *c* oxidase. Additionally, some prokaryotic cytochromes, such as cytochrome c_2 from the photosynthetic bacterium *Rhodospirillum rubrum* and cytochrome c_{550} from the denitrifying bacterium *Paracoccus denitrificans*, closely resemble cytochrome *c* from tuna-heart mitochondria (Figure 18.20). This evidence attests to an efficient evolutionary solution to electron transfer bestowed by the structural and functional characteristics of cytochrome *c*.

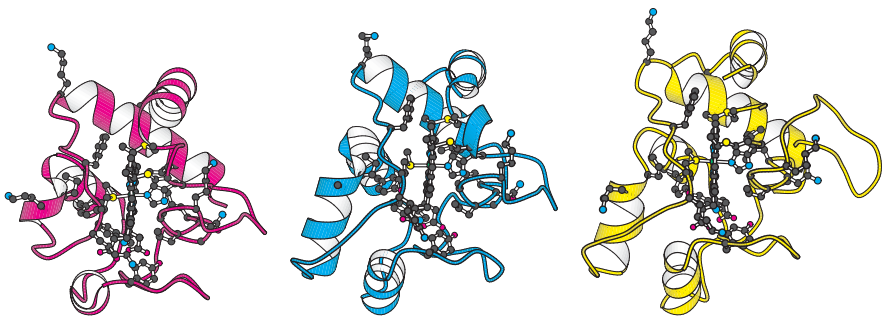


Figure 18.20 Conservation of the three-dimensional structure of cytochrome c. The side chains are shown for the 21 conserved amino acids and the heme. [Drawn from 3CYT.pdb, 3C2C.pdb, and 1SSC.pdb.]

The resemblance among cytochrome *c* molecules extends to the level of amino acid sequence. Because of the molecule's small size and ubiquity, the amino acid sequences of cytochrome *c* from more than 80 widely ranging eukaryotic species have been determined by direct protein sequencing by Emil Smith, Emanuel Margoliash, and others. The striking finding is that *21 of 104 residues have been invariant for more than one and a half billion years of evolution.* A phylogenetic tree, constructed from the amino acid sequences of cytochrome *c*, reveals the evolutionary relationships between many animal species (Figure 18.21).

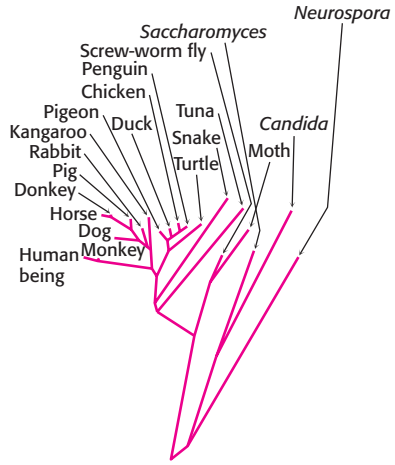


Figure 18.21 Evolutionary tree constructed from sequences of cytochrome c. Branch lengths are proportional to the number of amino acid changes that are believed to have occurred. This drawing is an adaptation of the work of Walter M. Fitch and Emanuel Margoliash.

18.4 A Proton Gradient Powers the Synthesis of ATP

Thus far, we have considered the flow of electrons from NADH to O_2, an exergonic process.

$$NADH + \tfrac{1}{2}O_2 + H^+ \rightleftharpoons H_2O + NAD^+$$
$$\Delta G^{\circ\prime} = -220.1\ kJ\ mol^{-1}\ (-52.6\ kcal\ mol^{-1})$$

Next, we consider how this process is coupled to the synthesis of ATP, an endergonic process.

$$ADP + P_i + H^+ \rightleftharpoons ATP + H_2O$$
$$\Delta G^{\circ\prime} = +30.5 \text{ kJ mol}^{-1} (+7.3 \text{ kcal mol}^{-1})$$

A molecular assembly in the inner mitochondrial membrane carries out the synthesis of ATP. This enzyme complex was originally called the *mitochondrial ATPase* or *F_1F_0 ATPase* because it was discovered through its catalysis of the reverse reaction, the hydrolysis of ATP. *ATP synthase,* its preferred name, emphasizes its actual role in the mitochondrion. It is also called *Complex V*.

How is the oxidation of NADH coupled to the phosphorylation of ADP? Electron transfer was first suggested to lead to the formation of a covalent high-energy intermediate that serves as a compound having a high phosphoryl-transfer potential, analogous to the generation of ATP by the formation of 1,3-bisphosphoglycerate in glycolysis (Section 16.1). An alternative proposal was that electron transfer aids the formation of an activated protein conformation, which then drives ATP synthesis. The search for such intermediates for several decades proved fruitless.

In 1961, Peter Mitchell suggested a radically different mechanism, *the chemiosmotic hypothesis*. He proposed that electron transport and ATP synthesis are coupled by *a proton gradient across the inner mitochondrial membrane*. In his model, the transfer of electrons through the respiratory chain leads to the pumping of protons from the matrix to the cytoplasmic side of the inner mitochondrial membrane. The H^+ concentration becomes lower in the matrix, and an electric field with the matrix side negative is generated (Figure 18.22). Protons then flow back into the matrix to equalize the distribution. Mitchell's idea was that this flow of protons drives the synthesis of ATP by ATP synthase. The energy-rich unequal distribution of protons is called the *proton-motive force*. The proton-motive force can be thought of as being composed of two components: a chemical gradient and a charge gradient. The chemical gradient for protons can be represented as a pH gradient. The charge gradient is created by the positive charge on the unequally distributed protons forming the chemical gradient. Mitchell proposed that both components power the synthesis of ATP.

Proton-motive force (Δp) =
chemical gradient (ΔpH) + charge gradient ($\Delta\psi$)

Some have argued that, along with the elucidation of the structure of DNA, the discovery that ATP synthesis is powered by a proton gradient is one of the two major advances in biology in the twentieth century. Mitchell's initial postulation of the chemiosmotic theory was not warmly received by all. Efraim Racker, one of the early investigators of ATP synthase, recalls that some thought of Mitchell as a court jester, whose work was of no consequence. Peter Mitchell was awarded the Nobel Prize in chemistry in 1978 for his contributions to understanding oxidative phosphorylation.

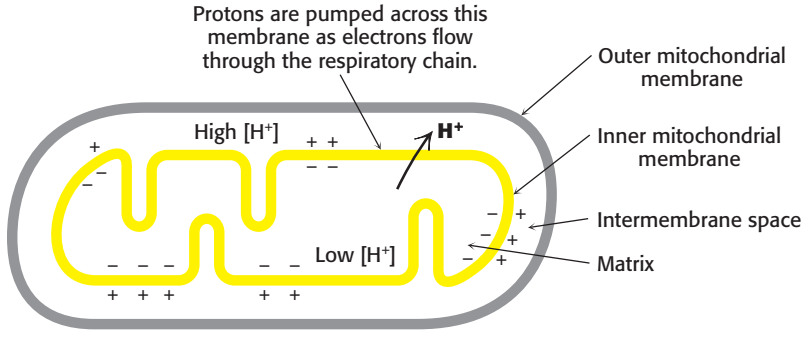


Figure 18.22 Chemiosmotic hypothesis. Electron transfer through the respiratory chain leads to the pumping of protons from the matrix to the cytoplasmic side of the inner mitochondrial membrane. The pH gradient and membrane potential constitute a proton-motive force that is used to drive ATP synthesis.

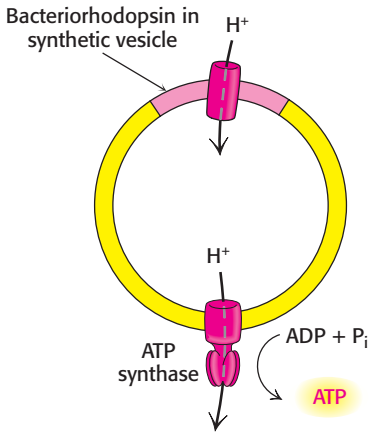


Figure 18.23 Testing the chemiosmotic hypothesis. ATP is synthesized when reconstituted membrane vesicles containing bacteriorhodopsin (a light-driven proton pump) and ATP synthase are illuminated. The orientation of ATP synthase in this reconstituted membrane is the reverse of that in the mitochondrion.

Mitchell's highly innovative hypothesis that oxidation and phosphorylation are coupled by a proton gradient is now supported by a wealth of evidence. Indeed, electron transport does generate a proton gradient across the inner mitochondrial membrane. The pH outside is 1.4 units lower than inside, and the membrane potential is 0.14 V, the outside being positive. As calculated on page 531, this membrane potential corresponds to a free energy of 21.8 kJ (5.2 kcal) per mole of protons.

An artificial system was created to elegantly demonstrate the basic principle of the chemiosmotic hypothesis. The role of the respiratory chain was played by bacteriorhodopsin, a membrane protein from halobacteria that pumps proteins when illuminated. Synthetic vesicles containing bacteriorhodopsin and mitochondrial ATP synthase purified from beef heart were created (Figure 18.23). When the vesicles were exposed to light, ATP was formed. This key experiment clearly showed that *the respiratory chain and ATP synthase are biochemically separate systems, linked only by a proton-motive force.*

ATP synthase is composed of a proton-conducting unit and a catalytic unit

Two parts of the puzzle of how NADH oxidation is coupled to ATP synthesis are now evident: (1) electron transport generates a proton-motive force; (2) ATP synthesis by ATP synthase can be powered by a proton-motive force. How is the proton-motive force converted into the high phosphoryl-transfer potential of ATP?

Biochemical, electron microscopic, and crystallographic studies of ATP synthase have revealed many details of its structure (Figure 18.24). It is a large, complex enzyme that looks like a ball on a stick. Much of the "stick" part, called the F_0 subunit, is embedded in the inner mitochondrial membrane. The 85-Å-diameter ball, called the F_1 subunit, protrudes into the mitochondrial matrix. The F_1 subunit contains the catalytic activity of the synthase. In fact, isolated F_1 subunits display ATPase activity.

The F_1 subunit consists of five types of polypeptide chains (α_3, β_3, γ, δ, and ε) with the indicated stoichiometry. The α and β subunits, which make up the bulk of the F_1, are arranged alternately in a hexameric ring; they are homologous to one another and are members of the P-loop NTPase family (Section 9.4). Both bind nucleotides but only the β subunits participate directly in catalysis. Beginning just below the α and β subunits is a central stalk consisting of the γ and ε proteins. The γ subunit includes a long helical coiled coil that extends into the center of the $\alpha_3\beta_3$ hexamer. *The γ subunit breaks the symmetry of the $\alpha_3\beta_3$ hexamer: each of the β subunits is distinct by*

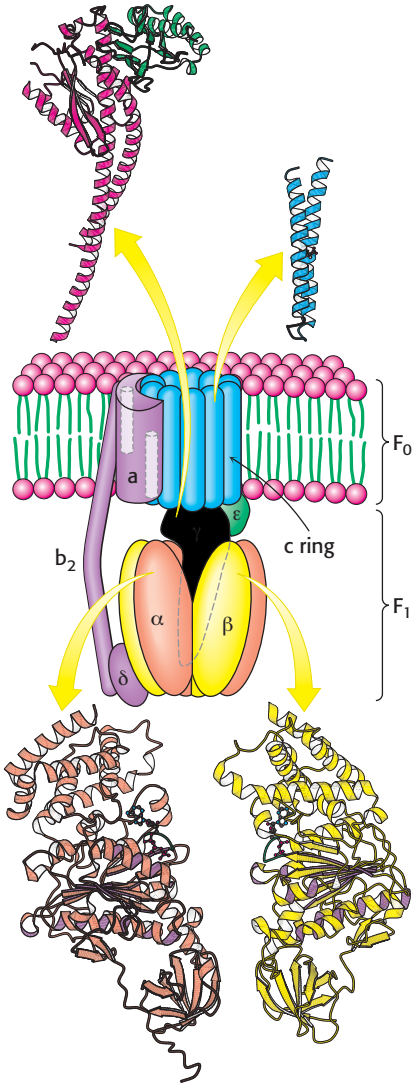


Figure 18.24 Structure of ATP synthase. A schematic structure is shown along with representations of the components for which structures have been determined to high resolution. The P-loop NTPase domains of the α and β subunits are indicated by purple shading. *Notice* that part of the enzyme complex is embedded in the inner mitochondrial membrane, whereas the remainder resides in the matrix. [Drawn from 1E79.pdb and 1COV.pdb.]

virtue of its interaction with a different face of γ. Distinguishing the three β subunits is crucial for understanding the mechanism of ATP synthesis.

The F_0 subunit is a hydrophobic segment that spans the inner mitochondrial membrane. *F_0 contains the proton channel of the complex.* This channel consists of a ring comprising from 10 to 14 **c** subunits that are embedded in the membrane. A single **a** subunit binds to the outside of the ring. The F_0 and F_1 subunits are connected in two ways: by the central γε stalk and by an exterior column. The exterior column consists of one **a** subunit, two **b** subunits, and the δ subunit.

Proton flow through ATP synthase leads to the release of tightly bound ATP: The binding-change mechanism

ATP synthase catalyzes the formation of ATP from ADP and orthophosphate.

$$ADP^{3-} + HPO_4^{2-} + H^+ \rightleftharpoons ATP^{4-} + H_2O$$

The actual substrates are ADP and ATP complexed with Mg^{2+}, as in all known phosphoryl-transfer reactions with these nucleotides. A terminal oxygen atom of ADP attacks the phosphorus atom of P_i to form a pentacovalent intermediate, which then dissociates into ATP and H_2O (Figure 18.25).

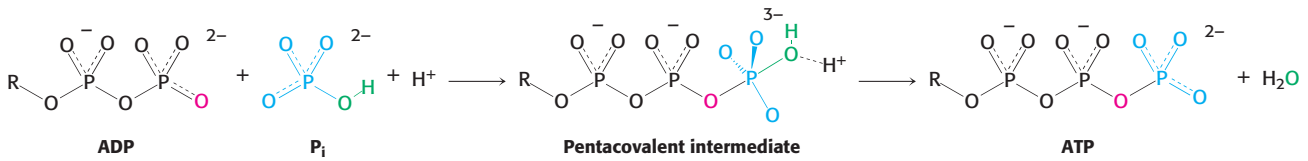


Figure 18.25 ATP-synthesis mechanism. One of the oxygen atoms of ADP attacks the phosphorus atom of P_i to form a pentacovalent intermediate, which then forms ATP and releases a molecule of H_2O.

How does the flow of protons drive the synthesis of ATP? Isotopic-exchange experiments unexpectedly revealed that *enzyme-bound ATP forms readily in the absence of a proton-motive force.* When ADP and P_i were added to ATP synthase in $H_2{}^{18}O$, ^{18}O became incorporated into P_i through the synthesis of ATP and its subsequent hydrolysis (Figure 18.26). The rate of incorporation of ^{18}O into P_i showed that about equal amounts of bound ATP and ADP are in equilibrium at the catalytic site, even in the absence of a proton gradient. However, ATP does not leave the catalytic site unless protons flow through the enzyme. Thus, *the role of the proton gradient is not to form ATP but to release it from the synthase.*

The fact that three β subunits are components of the F_1 moiety of the ATPase means that there are three active sites on the enzyme, each performing one of three different functions at any instant. The proton-motive force causes the three active sites to sequentially change functions as pro-

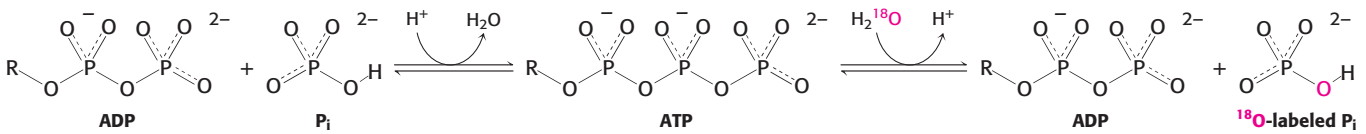


Figure 18.26 ATP forms without a proton-motive force but is not released. The results of isotopic-exchange experiments indicate that enzyme-bound ATP is formed from ADP and P_i in the absence of a proton-motive force.

tons flow through the membrane-embedded component of the enzyme. Indeed, we can think of the enzyme as consisting of a moving part and a stationary part: (1) the moving unit, or *rotor,* consists of the **c** ring and the γε stalk and (2) the stationary unit, or *stator,* is composed of the remainder of the molecule.

How do the three active sites of ATP synthase respond to the flow of protons? A number of experimental observations suggested a *binding-change mechanism* for proton-driven ATP synthesis. This proposal states that a β subunit can perform each of three sequential steps in the synthesis of ATP by changing conformation. These steps are (1) ADP and P_i binding, (2) ATP synthesis, and (3) ATP release. As already noted, interactions with the γ subunit make the three β subunits unequivalent (Figure 18.27). At any given moment, one β subunit will be in the L, or loose, conformation. This conformation binds ADP and P_i. A second subunit will be in the T, or tight, conformation. This conformation binds ATP with great avidity, so much so that it will convert bound ADP and P_i into ATP. Both the T and L conformations are sufficiently constrained that they cannot release bound nucleotides. The final subunit will be in the O, or open, form. This form has a more open conformation and can bind or release adenine nucleotides.

The rotation of the γ subunit drives the interconversion of these three forms (Figure 18.28). ADP and P_i bound in the subunit in the T form are transiently combining to form ATP. Suppose that the γ subunit is rotated by 120 degrees in a counterclockwise direction (as viewed from the top). This rotation converts the T-form site into an O-form site with the nucleotide bound as ATP. Concomitantly, the L-form site is converted into a T-form site, enabling the transformation of an additional ADP and P_i into ATP. The ATP in the O-form site can now depart from the enzyme to be replaced by ADP and P_i. An additional 120-degree rotation converts this O-form site into an L-form site, trapping these substrates. Each subunit progresses from the T to the O to the L form with no two subunits ever present in the same conformational form. This mechanism suggests that ATP can be synthesized and released by driving the rotation of the γ subunit in the appropriate direction.

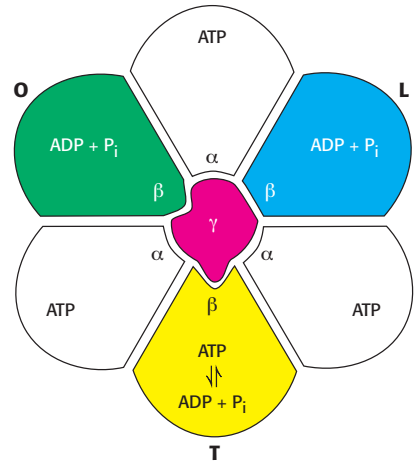


Figure 18.27 ATP synthase nucleotide-binding sites are not equivalent. The γ subunit passes through the center of the $\alpha_3\beta_3$ hexamer and makes the nucleotide-binding sites in the β subunits distinct from one another. *Notice* that each α subunit contains bound ATP, but these nucleotides do not participate in any reactions. The β subunits are colored to distinguish them from one another.

Progressive alteration of the forms of the three active sites of ATP synthase

Subunit 1	L → T → O → L → T → O……
Subunit 2	O → L → T → O → L → T……
Subunit 3	T → O → L → T → O → L……

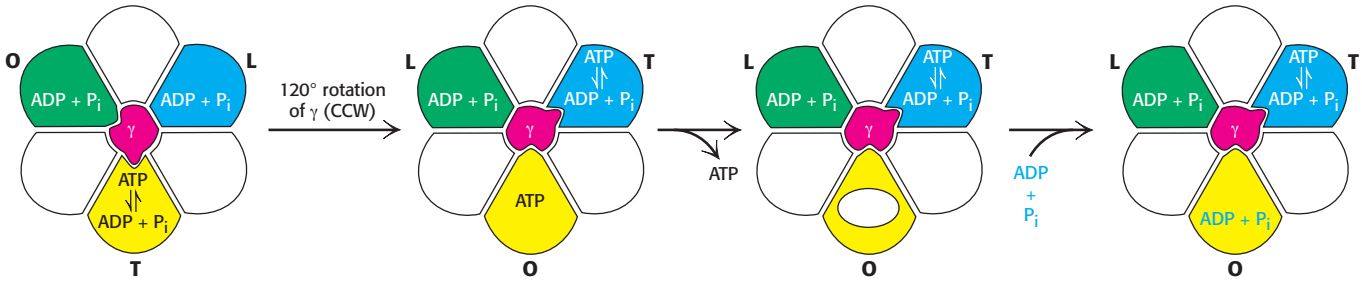


Figure 18.28 Binding-change mechanism for ATP synthase. The rotation of the γ subunit interconverts the three β subunits. The subunit in the T (tight) form interconverts ADP and P_i and ATP but does not allow ATP be released. When the γ subunit is rotated by 120 degrees in a counterclockwise (CCW) direction, the T-form subunit is converted into the O form, allowing ATP release. ADP and P_i can then bind to the O-form subunit. An additional 120-degree rotation (not shown) traps these substrates in an L-form subunit.

Rotational catalysis is the world's smallest molecular motor

Is it possible to observe the proposed rotation directly? Elegant experiments, using single-molecule techniques (Section 8.6), have demonstrated the rotation through the use of a simple experimental system consisting solely of cloned $\alpha_3\beta_3\gamma$ subunits (Figure 18.29). The β subunits were engineered to contain amino-terminal polyhistidine tags, which have a high affinity for nickel ions (Section 3.1). This property of the tags allowed the $\alpha_3\beta_3$ assembly to be immobilized on a glass surface that had been coated with nickel ions. The γ subunit was linked to a fluorescently labeled actin

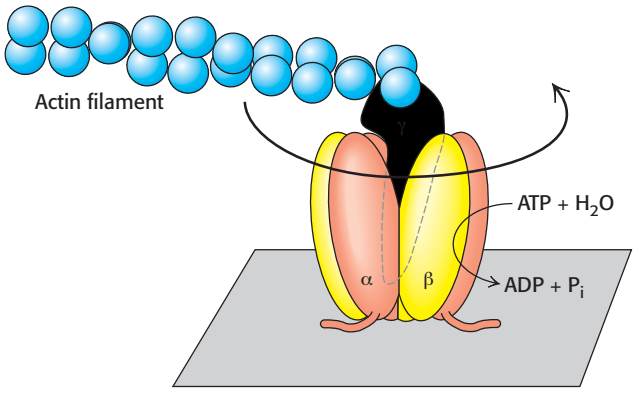


Figure 18.29 Direct observation of ATP-driven rotation in ATP synthase. The $\alpha_3\beta_3$ hexamer of ATP synthase is fixed to a surface, with the γ subunit projecting upward and linked to a fluorescently labeled actin filament. The addition and subsequent hydrolysis of ATP result in the counterclockwise rotation of the γ subunit, which can be directly seen under a fluorescence microscope.

filament to provide a long segment that could be observed under a fluorescence microscope. Remarkably, the addition of ATP caused the actin filament to rotate unidirectionally in a counterclockwise direction. *The γ subunit was rotating, driven by the hydrolysis of ATP.* Thus, the catalytic activity of an individual molecule could be observed. The counterclockwise rotation is consistent with the predicted mechanism for hydrolysis because the molecule was viewed from below relative to the view shown in Figure 18.29.

More-detailed analysis in the presence of lower concentrations of ATP revealed that the γ subunit rotates in 120-degree increments. Each increment corresponds to the hydrolysis of a single ATP molecule. In addition, from the results obtained by varying the length of the actin filament and measuring the rate of rotation, the enzyme appears to operate near 100% efficiency; that is, essentially all of the energy released by ATP hydrolysis is converted into rotational motion.

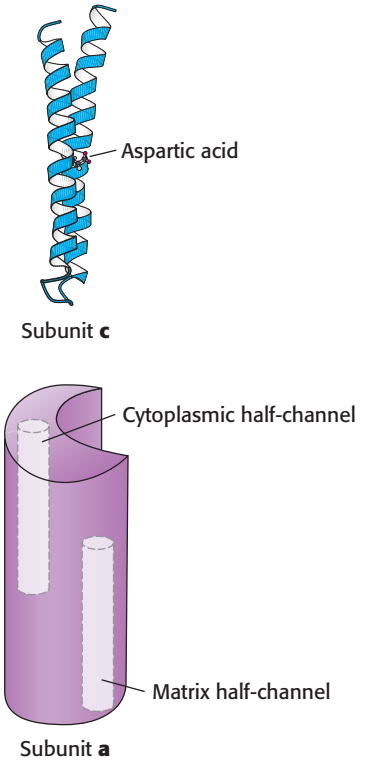


Figure 18.30 Components of the proton-conducting unit of ATP synthase. The **c** subunit consists of two α helices that span the membrane. An aspartic acid residue in one of the helices lies on the center of the membrane. The structure of the **a** subunit has not yet been directly observed, but it appears to include two half-channels that allow protons to enter and pass partway but not completely through the membrane.

Proton flow around the c ring powers ATP synthesis

The direct observation of rotary motion of the γ subunit is strong evidence for the rotational mechanism for ATP synthesis. The last remaining question is: How does proton flow through F_0 drive the rotation of the γ subunit? Howard Berg and George Oster proposed an elegant mechanism that provides a clear answer to this question. The mechanism depends on the structures of the **a** and **c** subunits of F_0 (Figure 18.30). The stationary **a** subunit directly abuts the membrane-spanning ring formed by 10 to 14 **c** subunits. Although the structure of the **a** subunit has not yet been experimentally determined, a variety of evidence is consistent with a structure that includes two hydrophilic half-channels that do not span the membrane (see Figure 18.30). Thus, protons can pass into either of these channels, but they cannot move completely across the membrane. The **a** subunit is positioned such that each half-channel directly interacts with one **c** subunit.

The structure of the **c** subunit was determined both by NMR methods and by x-ray crystallography. Each polypeptide chain forms a pair of α helices that span the membrane. An aspartic acid residue (Asp 61) is found in the middle of one of the helices. The key to proton movement across the membrane is that, in a proton-rich environment, such as the cytoplasmic side of the mitochondrial membrane, a proton will enter a channel and bind the aspartate residue (Figure 18.31). The **c** subunit with the bound proton then rotates through the membrane until the aspartic acid is in a proton-poor environment of the other half-channel, where the proton is released. *The movement of protons through the half-channels from the high proton concentration of the cytoplasm to the low proton concentration of the matrix*

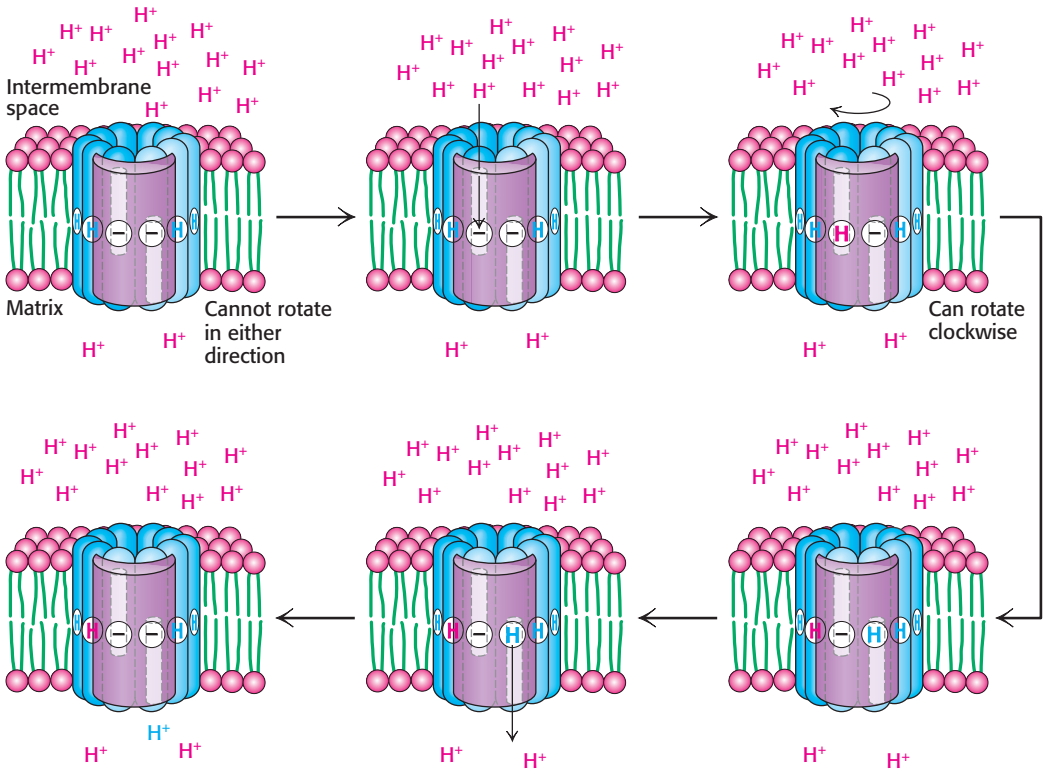


Figure 18.31 Proton motion across the membrane drives rotation of the c ring. A proton enters from the intermembrane space into the cytoplasmic half-channel to neutralize the charge on an aspartate residue in a **c** subunit. With this charge neutralized, the **c** ring can rotate clockwise by one **c** subunit, moving an aspartic acid residue out of the membrane into the matrix half-channel. This proton can move into the matrix, resetting the system to its initial state.

powers the rotation of the **c** *ring.* Its rotation is favored by the ability of the newly protonated (neutralized) aspartic acid residue to occupy the hydrophobic environment of the membrane. Thus, the **c** subunit with the newly protonated aspartic acid moves from contact with the cytoplasmic half-channel into the membrane, and the other **c** subunits move in unison. The **a** unit remains stationary as the **c** ring rotates. Each proton that enters the cytoplasmic half-channel of the **a** unit moves through the membrane by riding around on the rotating **c** ring to exit through the matrix half-channel into the proton-poor environment of the matrix (Figure 18.32).

How does the rotation of the **c** ring lead to the synthesis of ATP? The **c** ring is tightly linked to the γ and ε subunits. Thus, as the **c** ring turns, the γ and ε subunits are turned inside the $\alpha_3\beta_3$ hexamer unit of F_1. The rotation of the γ subunit in turn promotes the synthesis of ATP through the binding-change mechanism. The exterior column formed by the two **b** chains and the δ subunit prevents the $\alpha_3\beta_3$ hexamer from rotating. Recall that the number of **c** subunits in the **c** ring appears to range between 10 and 14. This number is significant because it determines the number of protons that must be transported to generate a molecule of ATP. Each 360-degree rotation of the γ subunit leads to the synthesis and release of three molecules of ATP. Thus, if there are 10 **c** subunits in the ring (as was observed in a crystal structure of yeast mitochondrial ATP synthase), each ATP generated requires the transport of $10/3 = 3.33$ protons. For simplicity, we will assume that three protons must flow into the matrix for each ATP formed, but we must keep in mind that the true value may differ. As we will see, the

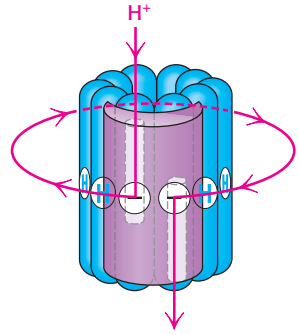


Figure 18.32 Proton path through the membrane. Each proton enters the cytoplasmic half-channel, follows a complete rotation of the **c** ring, and exits through the other half-channel into the matrix.

A little goes a long way

Despite the various molecular machinations and the vast numbers of ATPs synthesized and protons pumped, a resting human being requires surprisingly little power. Approximately 116 watts, the energy output of a typical light bulb, provides enough energy to sustain a resting person.

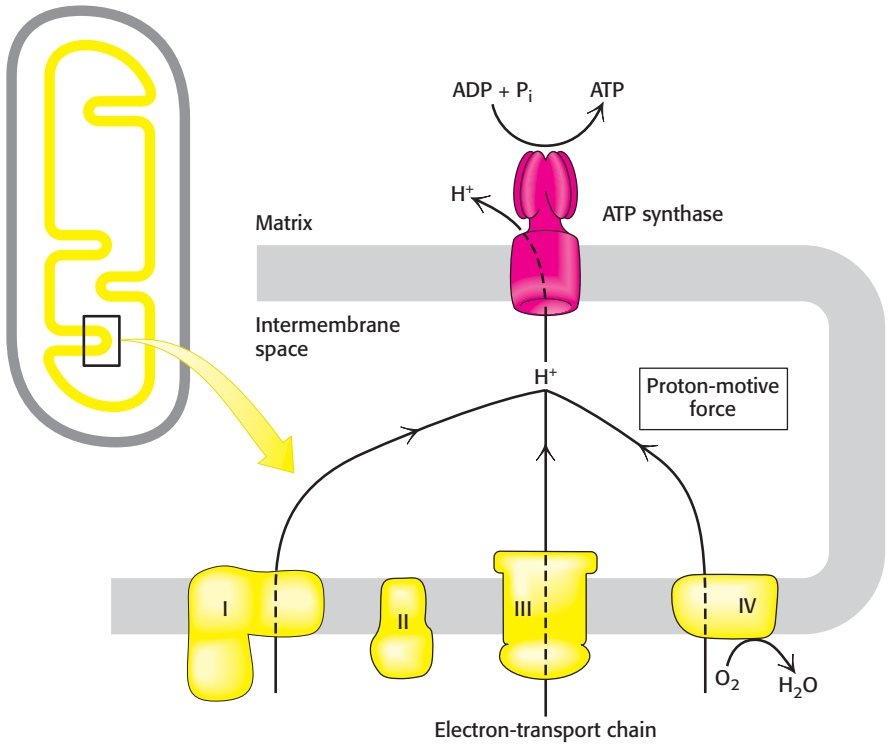


Figure 18.33 Overview of oxidative phosphorylation. The electron-transport chain generates a proton gradient, which is used to synthesize ATP.

electrons from NADH pump enough protons to generate 2.5 molecules of ATP, whereas those from $FADH_2$ yield 1.5 molecules of ATP.

Let us return for a moment to the example with which we began this chapter. If a resting human being requires 85 kg of ATP per day for bodily functions, then 3.3×10^{25} protons must flow through the ATP synthase per day, or 3.3×10^{21} protons per second. Figure 18.33 summarizes the process of oxidative phosphorylation.

ATP synthase and G proteins have several common features

The α and β subunits of ATP synthase are members of the P-loop NTPase family of proteins. In Chapter 14, we learned that the signaling properties of other members of this family, the G proteins, depend on their ability to bind nucleoside triphosphates and diphosphates with great tenacity. They do not exchange nucleotides unless they are stimulated to do so by interaction with other proteins. The binding-change mechanism of ATP synthase is a variation on this theme. The P-loop regions of the β subunits will bind either ADP or ATP (or release ATP), depending on which of three different faces of the γ subunit they interact with. The conformational changes take place in an orderly way, driven by the rotation of the γ subunit.

18.5 Many Shuttles Allow Movement Across Mitochondrial Membranes

The inner mitochondrial membrane must be impermeable to most molecules, yet much exchange has to take place between the cytoplasm and the mitochondria. This exchange is mediated by an array of membrane-spanning transporter proteins (Section 13.4).

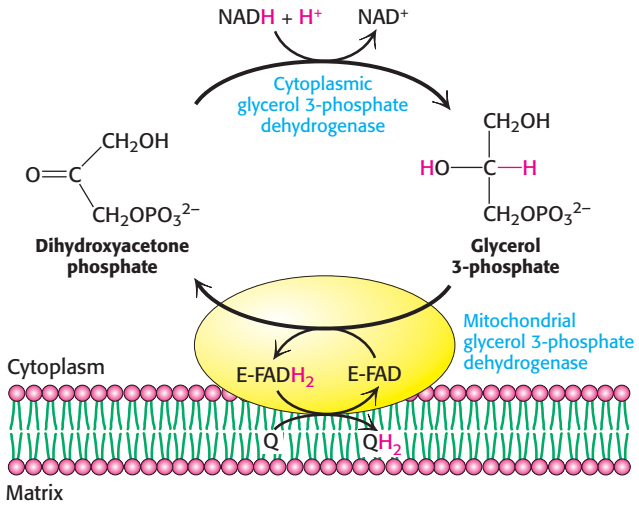
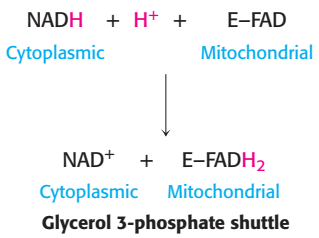


Figure 18.34 Glycerol 3-phosphate shuttle. Electrons from NADH can enter the mitochondrial electron-transport chain by being used to reduce dihydroxyacetone phosphate to glycerol 3-phosphate. Glycerol 3-phosphate is reoxidized by electron transfer to an FAD prosthetic group in a membrane-bound glycerol 3-phosphate dehydrogenase. Subsequent electron transfer to Q to form QH_2 allows these electrons to enter the electron-transport chain.

Electrons from cytoplasmic NADH enter mitochondria by shuttles

One function of the respiratory chain is to regenerate NAD^+ for use in glycolysis. How is cytoplasmic NADH reoxidized to NAD^+ under aerobic conditions? NADH cannot simply pass into mitochondria for oxidation by the respiratory chain, because the inner mitochondrial membrane is impermeable to NADH and NAD^+. The solution is that *electrons from NADH,* rather than NADH itself, are carried across the mitochondrial membrane. One of several means of introducing electrons from NADH into the electron-transport chain is the *glycerol 3-phosphate shuttle* (Figure 18.34). The first step in this shuttle is the transfer of a pair of electrons from NADH to dihydroxyacetone phosphate, a glycolytic intermediate, to form glycerol 3-phosphate. This reaction is catalyzed by a glycerol 3-phosphate dehydrogenase in the cytoplasm. Glycerol 3-phosphate is reoxidized to dihydroxyacetone phosphate on the outer surface of the inner mitochondrial membrane by a membrane-bound isozyme of glycerol 3-phosphate dehydrogenase. An electron pair from glycerol 3-phosphate is transferred to an FAD prosthetic group in this enzyme to form $FADH_2$. This reaction also regenerates dihydroxyacetone phosphate.

The reduced flavin transfers its electrons to the electron carrier Q, which then enters the respiratory chain as QH_2. *When cytoplasmic NADH transported by the glycerol 3-phosphate shuttle is oxidized by the respiratory chain, 1.5 rather than 2.5 molecules of ATP are formed.* The yield is lower because FAD rather than NAD^+ is the electron acceptor in mitochondrial glycerol 3-phosphate dehydrogenase. The use of FAD enables electrons from cytoplasmic NADH to be transported into mitochondria against an NADH concentration gradient. The price of this transport is one molecule of ATP per two electrons. This glycerol 3-phosphate shuttle is especially prominent in muscle and enables it to sustain a very high rate of oxidative phosphorylation. Indeed, some insects lack lactate dehydrogenase and are completely dependent on the glycerol 3-phosphate shuttle for the regeneration of cytoplasmic NAD^+.



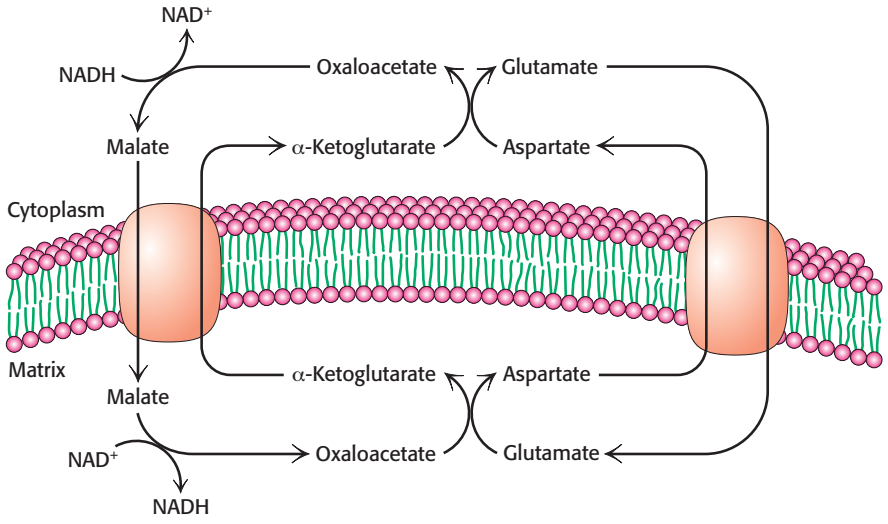
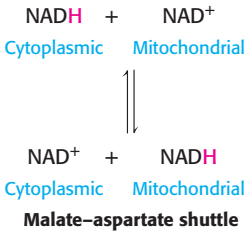


Figure 18.35 Malate–aspartate shuttle.



In the heart and liver, electrons from cytoplasmic NADH are brought into mitochondria by the *malate–aspartate shuttle,* which is mediated by two membrane carriers and four enzymes (Figure 18.35). Electrons are transferred from NADH in the cytoplasm to oxaloacetate, forming malate, which traverses the inner mitochondrial membrane in exchange for α-ketoglutarate and is then reoxidized by NAD^+ in the matrix to form NADH in a reaction catalyzed by the citric acid cycle enzyme malate dehydrogenase. The resulting oxaloacetate does not readily cross the inner mitochondrial membrane and so a transamination reaction (Section 23.3) is needed to form aspartate, which can be transported to the cytoplasmic side in exchange for glutamate. Glutamate donates an amino group to oxaloacetate, forming aspartate and α-ketoglutarate. In the cytoplasm, aspartate is then deaminated to form oxaloacetate and the cycle is restarted.

The entry of ADP into mitochondria is coupled to the exit of ATP by ATP-ADP translocase

The major function of oxidative phosphorylation is to generate ATP from ADP. ATP and ADP do not diffuse freely across the inner mitochondrial membrane. How are these highly charged molecules moved across the inner membrane into the cytoplasm? A specific transport protein, *ATP-ADP translocase,* enables these molecules to transverse this permeability barrier. Most important, the flows of ATP and ADP are coupled. *ADP enters the mitochondrial matrix only if ATP exits, and vice versa.* This process is carried out by the translocase, an antiporter:

$$ADP^{3-}_{cytoplasm} + ATP^{4-}_{matrix} \longrightarrow ADP^{3-}_{matrix} + ATP^{4-}_{cytoplasm}$$

ATP-ADP translocase is highly abundant, constituting about 15% of the protein in the inner mitochondrial membrane. The abundance is a manifestation of the fact that human beings exchange the equivalent of their weight in ATP each day. The 30-kd translocase contains a single nucleotide-binding site that alternately faces the matrix and the cytoplasmic sides of the membrane (Figure 18.36). ATP and ADP bind to the translocase without Mg^{2+}, and ATP has one more negative charge than that of ADP. Thus, in an actively respiring mitochondrion with a positive membrane potential, ATP transport out of the mitochondrial matrix and ADP transport into the matrix are favored. This ATP–ADP exchange is

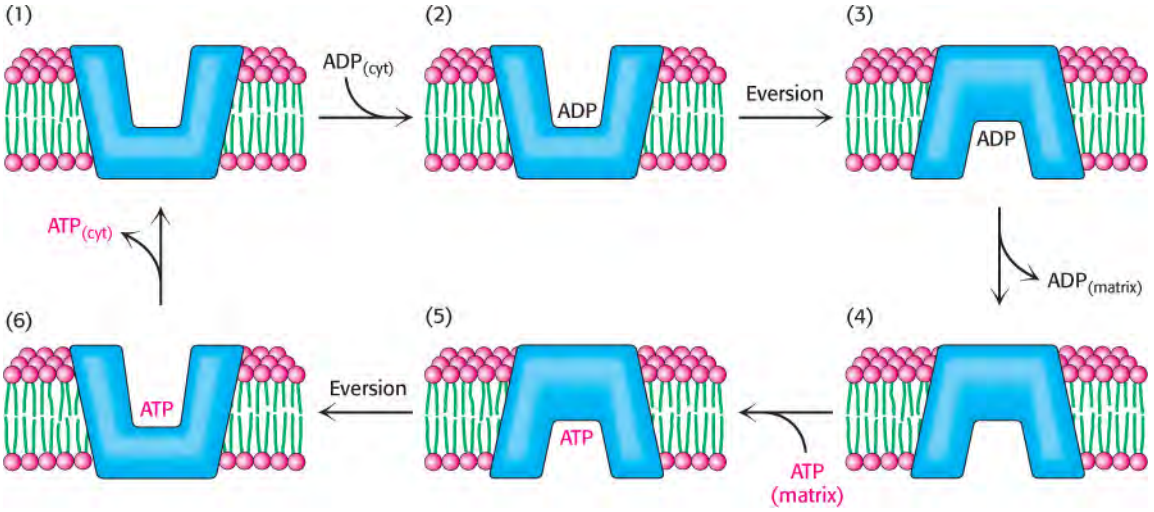


Figure 18.36 Mechanism of mitochondrial ATP-ADP translocase. The translocase catalyzes the coupled entry of ADP into the matrix and the exit of ATP from it. The binding of ADP (1) from the cytoplasm favors eversion of the transporter (2) to release ADP into the matrix (3). Subsequent binding of ATP from the matrix to the everted form (4) favors eversion back to the original conformation (5), releasing ATP into the cytoplasm (6).

energetically expensive; about a quarter of the energy yield from electron transfer by the respiratory chain is consumed to regenerate the membrane potential that is tapped by this exchange process. The inhibition of this process leads to the subsequent inhibition of cellular respiration as well (p. 558).

Mitochondrial transporters for metabolites have a common tripartite structure

Examination of the amino acid sequence of the ATP-ADP translocase revealed that this protein consists of three tandem repeats of a 100-amino-acid module, each of which appears to have two transmembrane segments. This tripartite structure has recently been confirmed by the determination of the three-dimensional structure of this transporter (Figure 18.37). The

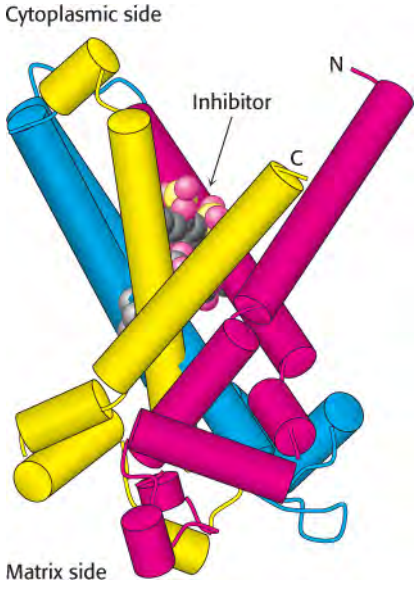


Figure 18.37 Structure of mitochondrial transporters. The structure of the ATP-ADP translocase is shown. *Notice* that this structure comprises three similar units (shown in red, blue, and yellow) that come together to form a binding site, here occupied by an inhibitor of this transporter. Other members of the mitochondrial transporter family adopt similar tripartite structures. [Drawn from 10KC.pdb.]

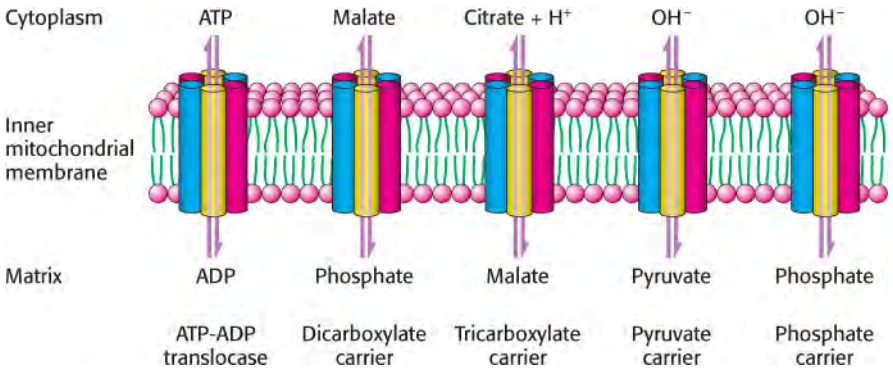


Figure 18.38 Mitochondrial transporters. Transporters (also called carriers) are transmembrane proteins that carry specific ions and charged metabolites across the inner mitochondrial membrane.

transmembrane helices form a tepeelike structure with the nucleotide-binding site (marked by a bound inhibitor) lying in the center. Each of the three repeats adopts a similar structure.

ATP-ADP translocase is but one of many mitochondrial transporters for ions and charged metabolites (Figure 18.38). The *phosphate carrier,* which works in concert with ATP-ADP translocase, mediates the electroneutral exchange of $H_2PO_4^-$ for OH^-. The combined action of these two transporters leads to the exchange of cytoplasmic ADP and P_i for matrix ATP at the cost of the influx of one H^+ (owing to the transport of one OH^- out of the matrix). These two transporters, which provide ATP synthase with its substrates, are associated with the synthase to form a large complex called the *ATP synthasome.*

Other homologous carriers also are present in the inner mitochondrial membrane. The dicarboxylate carrier enables malate, succinate, and fumarate to be exported from the mitochondrial matrix in exchange for P_i. The tricarboxylate carrier exchanges citrate and H^+ for malate. Pyruvate in the cytoplasm enters the mitochondrial membrane in exchange for OH^- by means of the pyruvate carrier. In all, more than 40 such carriers are encoded in the human genome.

18.6 The Regulation of Cellular Respiration Is Governed Primarily by the Need for ATP

Because ATP is the end product of cellular respiration, the ATP needs of the cell are the ultimate determinant of the rate of respiratory pathways and their components.

The complete oxidation of glucose yields about 30 molecules of ATP

We can now estimate how many molecules of ATP are formed when glucose is completely oxidized to CO_2. The number of ATP (or GTP) molecules formed in glycolysis and the citric acid cycle is unequivocally known because it is determined by the stoichiometries of chemical reactions. In contrast, the ATP yield of oxidative phosphorylation is less certain because the stoichiometries of proton pumping, ATP synthesis, and metabolite-transport processes need not be integer numbers or even have fixed values. As stated earlier, the best current estimates for the number of protons pumped out of the matrix by NADH-Q oxidoreductase, Q-cytochrome *c* oxidoreductase, and cytochrome *c* oxidase per electron pair are four, two, and four, respectively. The synthesis of a molecule of ATP is driven by the flow of about three protons through ATP synthase. An additional proton is

Table 18.4 ATP yield from the complete oxidation of glucose

Reaction sequence	ATP yield per glucose molecule
Glycolysis: Conversion of glucose into pyruvate (in the cytoplasm)	
Phosphorylation of glucose	−1
Phosphorylation of fructose 6-phosphate	−1
Dephosphorylation of 2 molecules of 1,3-BPG	+2
Dephosphorylation of 2 molecules of phosphoenolpyruvate	+2
2 molecules of NADH are formed in the oxidation of 2 molecules of glyceraldehyde 3-phosphate	
Conversion of pyruvate into acetyl CoA (inside mitochondria)	
2 molecules of NADH are formed	
Citric acid cycle (inside mitochondria)	
2 molecules of adenosine triphosphate are formed from 2 molecules of succinyl CoA	+2
6 molecules of NADH are formed in the oxidation of 2 molecules each of isocitrate, α-ketoglutarate, and malate	
2 molecules of $FADH_2$ are formed in the oxidation of 2 molecules of succinate	
Oxidative phosphorylation (inside mitochondria)	
2 molecules of NADH formed in glycolysis; each yields 1.5 molecules of ATP (assuming transport of NADH by the glycerol 3-phosphate shuttle)	+3
2 molecules of NADH formed in the oxidative decarboxylation of pyruvate; each yields 2.5 molecules of ATP	+5
2 molecules of $FADH_2$ formed in the citric acid cycle; each yields 1.5 molecules of ATP	+3
6 molecules of NADH formed in the citric acid cycle; each yields 2.5 molecules of ATP	+15
Net Yield per Molecule of Glucose	+30

Source: The ATP yield of oxidative phosphorylation is based on values given in P. C. Hinkle, M. A. Kumar, A. Resetar, and D. L. Harris. *Biochemistry* 30:3576, 1991.
Note: The current value of 30 molecules of ATP per molecule of glucose supersedes the earlier value of 36 molecules of ATP. The stoichiometries of proton pumping, ATP synthesis, and metabolite transport should be regarded as estimates. About 2 more molecules of ATP are formed per molecule of glucose oxidized when the malate–aspartate shuttle rather than the glycerol 3-phosphate shuttle is used.

consumed in transporting ATP from the matrix to the cytoplasm. Hence, about 2.5 molecules of cytoplasmic ATP are generated as a result of the flow of a pair of electrons from NADH to O_2. For electrons that enter at the level of Q-cytochrome *c* oxidoreductase, such as those from the oxidation of succinate or cytoplasmic NADH, the yield is about 1.5 molecules of ATP per electron pair. Hence, as tallied in Table 18.4, *about 30 molecules of ATP are formed when glucose is completely oxidized to CO_2;* this value supersedes the traditional estimate of 36 molecules of ATP. Most of the ATP, 26 of 30 molecules formed, is generated by oxidative phosphorylation. Recall that the anaerobic metabolism of glucose yields only 2 molecules of ATP. The efficiency of cellular respiration is manifested in the fact that one of the effects of endurance exercise, a practice that calls for much ATP for an extended period of time, is to increase the number of mitochondria and blood vessels in muscle and thus increase the extent of ATP generation by oxidative phosphorylation.

The rate of oxidative phosphorylation is determined by the need for ATP

How is the rate of the electron-transport chain controlled? Under most physiological conditions, electron transport is tightly coupled to phosphorylation. *Electrons do not usually flow through the electron-transport chain to O_2 unless ADP is simultaneously phosphorylated to ATP.* When ADP concentration rises, as would be the case in active muscle, the rate of oxidative

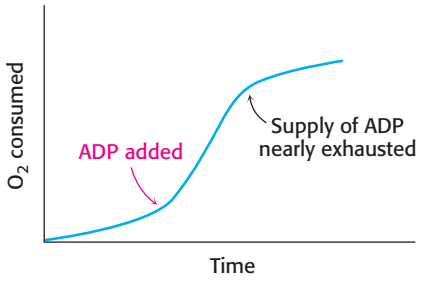


Figure 18.39 Respiratory control. Electrons are transferred to O_2 only if ADP is concomitantly phosphorylated to ATP.

phosphorylation increases to meet the ATP needs of the muscle. The regulation of the rate of oxidative phosphorylation by the ADP level is called *respiratory control* or *acceptor control*. Experiments on isolated mitochondria demonstrate the importance of ADP level (Figure 18.39). The rate of oxygen consumption by mitochondria increases markedly when ADP is added and then returns to its initial value when the added ADP has been converted into ATP.

The level of ADP likewise affects the rate of the citric acid cycle. At low concentrations of ADP, as in a resting muscle, NADH and $FADH_2$ are not consumed by the electron-transport chain. The citric acid cycle slows because there is less NAD^+ and FAD to feed the cycle. As the ADP level rises and oxidative phosphorylation speeds up, NADH and $FADH_2$ are oxidized, and the citric acid cycle becomes more active. *Electrons do not flow from fuel molecules to O_2 unless ATP needs to be synthesized.* We see here another example of the regulatory significance of the energy charge (Figure 18.40).

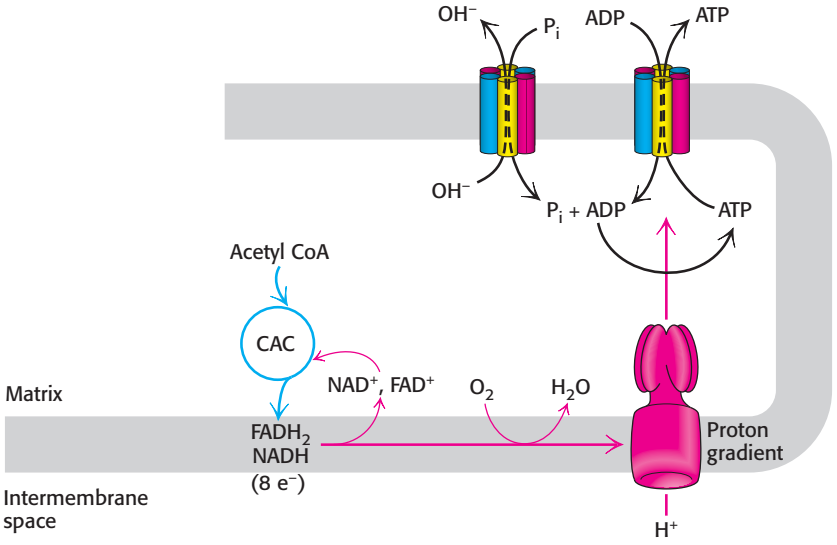


Figure 18.40 Energy charge regulates the use of fuels. The synthesis of ATP from ADP and P_i controls the flow of electrons from NADH and $FADH_2$ to oxygen. The availability of NAD^+ and FAD in turn control the rate of the citric acid cycle (CAC).

Regulated uncoupling leads to the generation of heat

Some organisms possess the ability to uncouple oxidative phosphorylation from ATP synthesis to generate heat. Such uncoupling is a means to maintain body temperature in hibernating animals, in some newborn animals (including human beings), and in many adult mammals, especially those adapted to cold. The skunk cabbage uses an analogous mechanism to heat its floral spikes in early spring, increasing the evaporation of odoriferous molecules that attract insects to fertilize its flowers. In animals, the uncoupling is in *brown adipose tissue* (BAT), which is specialized tissue for the process of *nonshivering thermogenesis*. In contrast, *white adipose tissue* (WAT), which constitutes the bulk of adipose tissue, plays no role in thermogenesis but serves as an energy source and an endocrine gland (Chapters 26 and 27).

Brown adipose tissue is very rich in mitochondria, often called *brown fat mitochondria*. The tissue appears brown from the combination of the greenish-colored cytochromes in the numerous mitochondria and the red hemoglobin present in the extensive blood supply, which helps to carry the heat through the body. The inner mitochondrial membrane of these

mitochondria contains a large amount of *uncoupling protein* (UCP-1), or *thermogenin*, a dimer of 33-kd subunits that resembles ATP-ADP translocase. UCP-1 forms a pathway for the flow of protons from the cytoplasm to the matrix. In essence, *UCP-1 generates heat by short-circuiting the mitochondrial proton battery.* The energy of the proton gradient, normally captured as ATP, is released as heat as the protons flow through UCP-1 to the mitochondrial matrix. This dissipative proton pathway is activated when the core body temperature begins to fall. In response to a temperature drop, the release of hormones leads to the liberation of free fatty acids from triacylglycerols that in turn activate thermogenin (Figure 18.41).

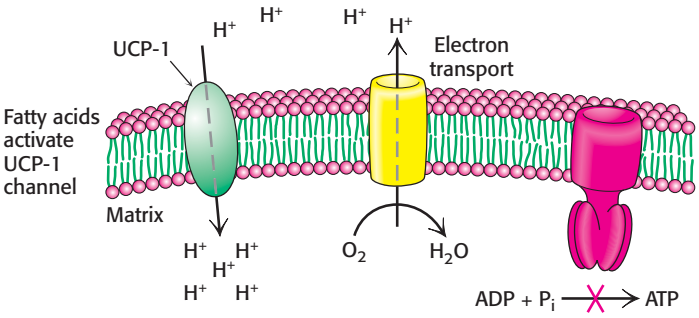


Figure 18.41 Action of an uncoupling protein. Uncoupling protein (UCP-1) generates heat by permitting the influx of protons into the mitochondria without the synthesis of ATP.

We can witness the effects of a lack of nonshivering thermogenesis by examining pig behavior. Pigs are unusual mammals in that they have large litters and are the only ungulates (hoofed animals) that build nests for birth. These behavioral characteristics appear to be the result of a biochemical deficiency. Pigs lack UCP-1 and, hence, brown fat. Piglets must rely on other means of thermogenesis, such as nesting, large litter size, and shivering.

Until recently, adult humans were believed to lack brown fat tissue. However, new studies have established that adults, women especially, have brown adipose tissue in the neck and upper chest regions that is activated by cold (Figure 18.42). Obesity leads to a decrease in brown adipose tissue.

In addition to UCP-1, two other uncoupling proteins have been identified. UCP-2, which is 56% identical in sequence with UCP-1, is found in a wide variety of tissues. UCP-3 (57% identical with UCP-1 and 73% identical with UCP-2) is localized to skeletal muscle and brown fat. This family of uncoupling proteins, especially UCP-2 and UCP-3, may play a role in energy homeostasis. In fact, the genes for UCP-2 and UCP-3 map to regions of the human and mouse chromosomes that have been linked to obesity, supporting the notion that they function as a means of regulating body weight.

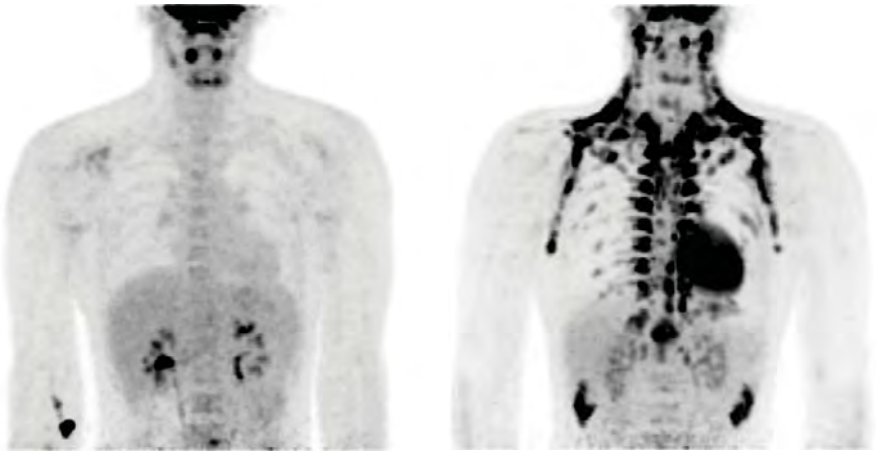


Figure 18.42 Brown adipose tissue is revealed on exposure to cold. The results of PET–CT scanning show the uptake and distribution of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) in adipose tissue. The patterns of ^{18}F-FDG uptake in the same subject are dramatically different under thermoneutral conditions (left) and after exposure to cold (right). [Courtesy of Wouter van Marken Lichtenbelt. Copyright 2009 Massachusetts Medical Society. All rights reserved.]

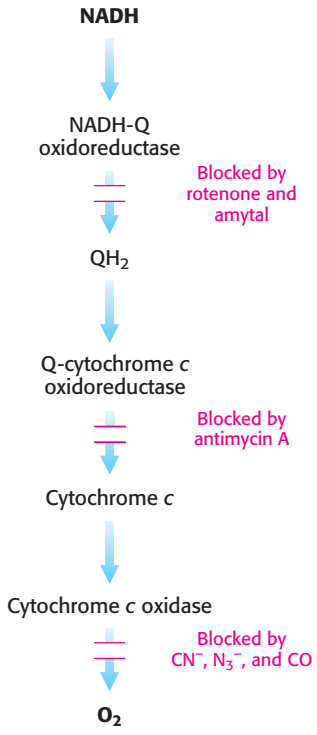
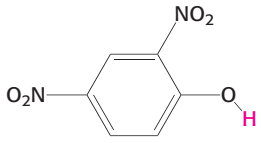


Figure 18.43 Sites of action of some inhibitors of electron transport.



2,4-Dinitrophenol (DNP)

Oxidative phosphorylation can be inhibited at many stages

Many potent and lethal poisons exert their effect by inhibiting oxidative phosphorylation at one of a number of different locations (Figure 18.43).

1. *Inhibition of the electron-transport chain. Rotenone,* which is used as a fish and insect poison, and *amytal,* a barbiturate sedative, block electron transfer in NADH-Q oxidoreductase and thereby prevent the utilization of NADH as a substrate. Rotenone, as an electron-transport-chain inhibitor, may play a role, along with genetic susceptibility, in the development of Parkinson disease. In the presence of rotenone and amytal, electron flow resulting from the oxidation of succinate is unimpaired, because these electrons enter through QH_2, beyond the block. *Antimycin A* interferes with electron flow from cytochrome b_H in Q-cytochrome *c* oxidoreductase. Furthermore, electron flow in cytochrome *c* oxidase can be blocked by *cyanide* (CN^-), *azide* (N_3^-), and *carbon monoxide* (CO). Cyanide and azide react with the ferric form of heme a_3, whereas carbon monoxide inhibits the ferrous form. Inhibition of the electron-transport chain also inhibits ATP synthesis because the proton-motive force can no longer be generated.

2. *Inhibition of ATP synthase.* Oligomycin, an antibiotic used as an antifungal agent, and dicyclohexylcarbodiimide (DCC) prevent the influx of protons through ATP synthase. If actively respiring mitochondria are exposed to an inhibitor of ATP synthase, the electron-transport chain ceases to operate. This observation clearly illustrates that electron transport and ATP synthesis are normally tightly coupled.

3. *Uncoupling electron transport from ATP synthesis.* The tight coupling of electron transport and phosphorylation in mitochondria can be uncoupled by 2,4-dinitrophenol (DNP) and certain other acidic aromatic compounds. These substances carry protons across the inner mitochondrial membrane, down their concentration gradient. In the presence of these uncouplers, electron transport from NADH to O_2 proceeds in a normal fashion, but ATP is not formed by mitochondrial ATP synthase, because the proton-motive force across the inner mitochondrial membrane is continuously dissipated. This loss of respiratory control leads to increased oxygen consumption and oxidation of NADH. Indeed, in the accidental ingestion of uncouplers, large amounts of metabolic fuels are consumed, but no energy is captured as ATP. Rather, energy is released as heat. DNP is the active ingredient in some herbicides and fungicides. Remarkably, some people consume DNP as a weight-loss drug, despite the fact that the FDA banned its use in 1938. There are also reports that Soviet soldiers were given DNP to keep them warm during the long Russian winters. Chemical uncouplers are nonphysiological, unregulated counterparts of uncoupling proteins.

4. *Inhibition of ATP export.* ATP-ADP translocase is specifically inhibited by very low concentrations of *atractyloside* (a plant glycoside) or *bongkrekic acid* (an antibiotic from a mold). Atractyloside binds to the translocase when its nucleotide site faces the cytoplasm, whereas bongkrekic acid binds when this site faces the mitochondrial matrix. Oxidative phosphorylation stops soon after either inhibitor is added, showing that ATP-ADP translocase is essential for maintaining adequate amounts of ADP to accept the energy associated with the proton-motive force.

Mitochondrial diseases are being discovered

The number of diseases that can be attributed to mitochondrial mutations is steadily growing in step with our growing understanding

of the biochemistry and genetics of mitochondria. The prevalence of mitochondrial diseases is estimated to be from 10 to 15 per 100,000 people, roughly equivalent to the prevalence of the muscular dystrophies. The first mitochondrial disease to be understood was *Leber hereditary optic neuropathy* (LHON), a form of blindness that strikes in midlife as a result of mutations in Complex I. Some of these mutations impair NADH utilization, whereas others block electron transfer to Q. Mutations in Complex I are the most frequent cause of mitochondrial diseases. The accumulation of mutations in mitochondrial genes in a span of several decades may contribute to aging, degenerative disorders, and cancer.

A human egg harbors several hundred thousand molecules of mitochondrial DNA, whereas a sperm contributes only a few hundred and thus has little effect on the mitochondrial genotype. Because the maternally inherited mitochondria are present in large numbers and not all of the mitochondria may be affected, the pathologies of mitochondrial mutants can be quite complex. Even within a single family carrying an identical mutation, chance fluctuations in the percentage of mitochondria with the mutation lead to large variations in the nature and severity of the symptoms of the pathological condition as well as the time of onset. As the percentage of defective mitochondria increases, energy-generating capacity diminishes until, at some threshold, the cell can no longer function properly. Defects in cellular respiration are doubly dangerous. Not only does energy transduction decrease, but also the likelihood that reactive oxygen species will be generated increases. Organs that are highly dependent on oxidative phosphorylation, such as the nervous system and the heart, are most vulnerable to mutations in mitochondrial DNA.

Mitochondria play a key role in apoptosis

In the course of development or in cases of significant cell damage, individual cells within multicellular organisms undergo *programmed cell death,* or *apoptosis.* Mitochondria act as control centers regulating this process. Although the details have not yet been established, the outer membrane of damaged mitochondria becomes highly permeable, a process referred to as *mitochondrial outer membrane permeabilization* (MOMP). This permeabilization is instigated by a family of proteins (Bcl family) that were initially discovered because of their role in cancer. One of the most potent activators of apoptosis, cytochrome *c,* exits the mitochondria and interacts with apoptotic peptidase-activating factor 1 (APAF-1), which leads to the formation of the *apoptosome*. The apoptosome recruits and activates a proteolytic enzyme called *caspase 9,* a member of the cysteine protease family (Section 9.1), that in turn activates a cascade of other caspases. Each caspase type destroys a particular target, such as the proteins that maintain cell structure. Another target is a protein that inhibits an enzyme that destroys DNA (an enzyme called caspase-activated DNAse or CAD), freeing CAD to cleave the genetic material. This cascade of proteolytic enzymes has been called "death by a thousand tiny cuts."

Power transmission by proton gradients is a central motif of bioenergetics

The main concept presented in this chapter is that mitochondrial electron transfer and ATP synthesis are linked by a transmembrane proton gradient. ATP synthesis in bacteria and chloroplasts also is driven by proton gradients. In fact, proton gradients power a variety of energy-requiring processes such as the active transport of calcium ions by mitochondria, the

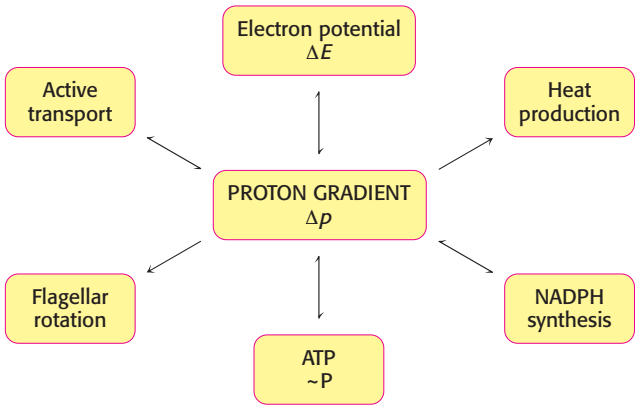


Figure 18.44 The proton gradient is an interconvertible form of free energy.

entry of some amino acids and sugars into bacteria, the rotation of bacterial flagella, and the transfer of electrons from $NADP^+$ to NADPH. Proton gradients can also be used to generate heat, as in hibernation. It is evident that *proton gradients are a central interconvertible currency of free energy in biological systems* (Figure 18.44). Mitchell noted that the proton-motive force is a marvelously simple and effective store of free energy because it requires only a thin, closed lipid membrane between two aqueous phases.

Summary

18.1 Eukaryotic Oxidative Phosphorylation Takes Place in Mitochondria

Mitochondria generate most of the ATP required by aerobic cells through a joint endeavor of the reactions of the citric acid cycle, which take place in the mitochondrial matrix, and oxidative phosphorylation, which takes place in the inner mitochondrial membrane. Mitochondria are descendants of a free-living bacterium that established a symbiotic relation with another cell.

18.2 Oxidative Phosphorylation Depends on Electron Transfer

In oxidative phosphorylation, the synthesis of ATP is coupled to the flow of electrons from NADH or $FADH_2$ to O_2 by a proton gradient across the inner mitochondrial membrane. Electron flow through three asymmetrically oriented transmembrane complexes results in the pumping of protons out of the mitochondrial matrix and the generation of a membrane potential. ATP is synthesized when protons flow back to the matrix through a channel in an ATP-synthesizing complex, called ATP synthase (also known as F_0F_1-ATPase). Oxidative phosphorylation exemplifies a fundamental theme of bioenergetics: the transmission of free energy by proton gradients.

18.3 The Respiratory Chain Consists of Four Complexes: Three Proton Pumps and a Physical Link to the Citric Acid Cycle

The electron carriers in the respiratory assembly of the inner mitochondrial membrane are quinones, flavins, iron–sulfur complexes, heme groups of cytochromes, and copper ions. Electrons from NADH are transferred to the FMN prosthetic group of NADH-Q oxidoreductase (Complex I), the first of four complexes. This oxidoreductase also contains Fe-S centers. The electrons emerge in QH_2, the reduced form of ubiquinone (Q). The citric acid cycle enzyme succinate dehydrogenase is a component of the succinate-Q reductase complex (Complex II), which donates electrons from $FADH_2$ to Q to form QH_2.This highly mobile hydrophobic carrier transfers its electrons to Q-cytochrome *c* oxidoreductase (Complex III), a complex that contains cytochromes *b* and c_1 and an Fe-S center. This complex reduces cytochrome *c*, a water-soluble peripheral membrane protein. Cytochrome *c*, like Q, is a mobile carrier of electrons, which it then transfers to cytochrome *c* oxidase (Complex IV). This complex contains cytochromes *a* and a_3 and three copper ions. A heme iron ion and a copper ion in this oxidase transfer electrons to O_2, the ultimate acceptor, to form H_2O.

18.4 A Proton Gradient Powers the Synthesis of ATP

The flow of electrons through Complexes I, III, and IV leads to the transfer of protons from the matrix side to the cytoplasmic side of the inner mitochondrial membrane. A proton-motive force consisting of a pH gradient (matrix side basic) and a membrane potential (matrix side negative) is generated. The flow of protons back to the matrix side through ATP synthase drives ATP synthesis. The enzyme complex is a molecular motor made of two operational units: a rotating component and a stationary component. The rotation of the γ subunit induces structural changes in the β subunit that result in the synthesis and release of ATP from the enzyme. Proton influx provides the force for the rotation.

The flow of two electrons through NADH-Q oxidoreductase, Q-cytochrome *c* oxidoreductase, and cytochrome *c* oxidase generates a gradient sufficient to synthesize 1, 0.5, and 1 molecule of ATP, respectively. Hence, 2.5 molecules of ATP are formed per molecule of NADH oxidized in the mitochondrial matrix, whereas only 1.5 molecules of ATP are made per molecule of $FADH_2$ oxidized, because its electrons enter the chain at QH_2, after the first proton-pumping site.

18.5 Many Shuttles Allow Movement Across Mitochondrial Membranes

Mitochondria employ a host of transporters, or carriers, to move molecules across the inner mitochondrial membrane. The electrons of cytoplasmic NADH are transferred into mitochondria by the glycerol phosphate shuttle to form $FADH_2$ from FAD or by the malate–aspartate shuttle to form mitochondrial NADH. The entry of ADP into the mitochondrial matrix is coupled to the exit of ATP by ATP-ADP translocase, a transporter driven by membrane potential.

18.6 The Regulation of Oxidative Phosphorylation Is Governed Primarily by the Need for ATP

About 30 molecules of ATP are generated when a molecule of glucose is completely oxidized to CO_2 and H_2O. Electron transport is normally tightly coupled to phosphorylation. NADH and $FADH_2$ are oxidized only if ADP is simultaneously phosphorylated to ATP, a form of regulation called acceptor or respiratory control. Proteins have been identified that uncouple electron transport and ATP synthesis for the generation of heat. Uncouplers such as DNP also can disrupt this coupling; they dissipate the proton gradient by carrying protons across the inner mitochondrial membrane.

Key Terms

oxidative phosphorylation (p. 525)
proton-motive force (p. 525)
cellular respiration (p. 526)
electron-transport chain (p. 528)
reduction (redox, oxidation–reduction, E'_0) potential (p. 528)
coenzyme Q (Q, ubiquinone) (p. 532)
Q pool (p. 533)
NADH-Q oxidoreductase (Complex I) (p. 533)
flavin mononucleotide (FMN) (p. 533)
iron–sulfur (nonheme iron) protein (p. 534)
succinate-Q reductase (Complex II) (p. 535)
Q-cytochrome *c* oxidoreductase (Complex III) (p. 535)
cytochrome *c* (Cyt *c*) (p. 535)
Rieske center (p. 536)
Q cycle (p. 536)
cytochrome *c* oxidase (Complex IV) (p. 537)
superoxide dismutase (p. 541)
catalase (p. 542)
ATP synthase (Complex V, F_1F_0 ATPase) (p. 544)
glycerol 3-phosphate shuttle (p. 551)
malate–aspartate shuttle (p. 552)
ATP-ADP translocase (adenine nucleotide translocase, ANT) (p. 552)
respiratory (acceptor) control (p. 556)
uncoupling protein (UCP) (p. 557)
programmed cell death (apoptosis) (p. 559)
mitochondrial outer membrane permeabilization (MOMP) (p. 559)
apoptosome (p. 559)
caspase (p. 559)

Problems

1. *Breathe or ferment?* Compare fermentation and respiration with respect to electron donors and electron acceptors.

2. *Reference states.* The standard oxidation–reduction potential for the reduction of O_2 to H_2O is given as 0.82 V in Table 18.1. However, the value given in textbooks of chemistry is 1.23 V. Account for this difference.

3. *Less energetic electrons.* Why are electrons carried by $FADH_2$ not as energy rich as those carried by NADH? What is the consequence of this difference?

4. *Now prove it.* Calculate the energy released by the reduction of O_2 with $FADH_2$.

5. *Thermodynamic constraint.* Compare the $\Delta G^{\circ\prime}$ values for the oxidation of succinate by NAD^+ and by FAD. Use the data given in Table 18.1 to find the E'_0 of the NAD^+ $^-$NADH and fumarate-succinate couples, and assume that E'_0 for the FAD–$FADH_2$ redox couple is nearly 0.05 V. Why is FAD rather than NAD^+ the electron acceptor in the reaction catalyzed by succinate dehydrogenase?

6. *The benefactor and beneficiary.* Identify the oxidant and the reductant in the following reaction.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{lactate} + \text{NAD}^+$$

7. *Six of one, half dozen of the other.* How is the redox potential ($\Delta E'_0$) related to the free-energy change of a reaction ($\Delta G^{\circ\prime}$)?

8. *Location, location, location.* Iron is a component of many of the electron carriers of the electron-transport chain. How can it participate in a series of coupled redox reactions if the E'_0 value is +0.77 V, as seen in Table 18.1?

9. *Line up.* Place the following components of the electron-transport chain in their proper order: (a) cytochrome *c;* (b) Q-cytochrome *c* oxidoreductase; (c) NADH-Q reductase; (d) cytochrome *c* oxidase; (e) ubiquinone.

10. *Match 'em.*

(a) Complex I___	1. Q-cytochrome c oxidoreductase
(b) Complex II___	2. Coenzyme Q
(c) Complex III___	3. Succinate-Q reductase
(d) Complex IV___	4. NADH-Q oxidoreductase
(e) Ubiquinone___	5. Cytochrome *c* oxidase

11. *Structural considerations.* Explain why coenzyme Q is an effective mobile electron carrier in the electron-transport chain.

12. *Inhibitors.* Rotenone inhibits electron flow through NADH-Q oxidoreductase. Antimycin A blocks electron flow between cytochromes *b* and c_1. Cyanide blocks electron flow through cytochrome oxidase to O_2. Predict the relative oxidation–reduction state of each of the following respiratory-chain components in mitochondria that are treated with each of the inhibitors: NAD^+; NADH-Q oxidoreductase; coenzyme Q; cytochrome c_1; cytochrome *c;* cytochrome *a*.

13. *Rumored to be a favorite of Elvis.* Amytal is a barbiturate sedative that inhibits electron flow through Complex I. How would the addition of amytal to actively respiring mitochondria affect the relative oxidation–reduction states of the components of the electron-transport chain and the citric acid cycle?

14. *Efficiency.* What is the advantage of having Complexes I, III, and IV associated with one another in the form of a respirasome?

15. *ROS, not ROUS.* What are the reactive oxygen species and why are they especially dangerous to cells?

16. *Reclaim resources.* Humans have only about 250 g of ATP, but even a couch potato needs about 83 kg of ATP to open the bag of chips and use the remote. How is this discrepancy between requirements and resources reconciled?

17. *Energy harvest.* What is the yield of ATP when each of the following substrates is completely oxidized to CO_2 by a mammalian cell homogenate? Assume that glycolysis, the citric acid cycle, and oxidative phosphorylation are fully active.

(a) Pyruvate	(d) Phosphoenolpyruvate
(b) Lactate	(e) Galactose
(c) Fructose 1,6-bisphosphate	(f) Dihydroxyacetone phosphate

18. *Potent poisons.* What is the effect of each of the following inhibitors on electron transport and ATP formation by the respiratory chain?

(a) Azide	(d) DNP
(b) Atractyloside	(e) Carbon monoxide
(c) Rotenone	(f) Antimycin A

19. *A question of coupling.* What is the mechanistic basis for the observation that the inhibitors of ATP synthase also lead to an inhibition of the electron-transport chain?

20. *A Brownian ratchet wrench.* What causes the c subunits of ATP synthase to rotate? What determines the direction of rotation?

21. *Alternative routes.* The most common metabolic sign of mitochondrial disorders is lactic acidosis. Why?

22. *Connections.* How does the inhibition of ATP-ADP translocase affect the citric acid cycle? Glycolysis?

23. *O_2 consumption.* Oxidative phosphorylation in mitochondria is often monitored by measuring oxygen con-

sumption. When oxidative phosphorylation is proceeding rapidly, the mitochondria will rapidly consume oxygen. If there is little oxidative phosphorylation, only small amounts of oxygen will be used. You are given a suspension of isolated mitochondria and directed to add the following compounds in the order from *a* to *h*. With the addition of each compound, all of the previously added compounds remain present. Predict the effect of each addition on oxygen consumption by the isolated mitochondria.

(a) Glucose
(b) ADP + P_i
(c) Citrate
(d) Oligomycin
(e) Succinate
(f) Dinitrophenol
(g) Rotenone
(h) Cyanide

24. *P : O ratios.* The number of molecules of inorganic phosphate incorporated into organic form per atom of oxygen consumed, termed the *P : O ratio,* was frequently used as an index of oxidative phosphorylation.

(a) What is the relation of the P : O ratio to the ratio of the number of protons translocated per electron pair ($H^+/2\ e^-$) and the ratio of the number of protons needed to synthesize ATP and transport it to the cytoplasm (P/H^+)?

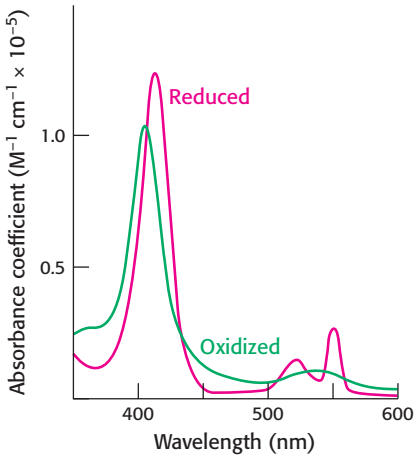
(b) What are the P : O ratios for electrons donated by matrix NADH and by succinate?

25. *Cyanide antidote.* The immediate administration of nitrite is a highly effective treatment for cyanide poisoning. What is the basis for the action of this antidote? (Hint: Nitrite oxidizes ferrohemoglobin to ferrihemoglobin.)

26. *Runaway mitochondria 1.* Suppose that the mitochondria of a patient oxidize NADH irrespective of whether ADP is present. The P : O ratio for oxidative phosphorylation by these mitochondria is less than normal. Predict the likely symptoms of this disorder.

27. *Recycling device.* The cytochrome *b* component of Q-cytochrome *c* oxidoreductase enables both electrons of QH_2 to be effectively utilized in generating a proton-motive force. Cite another recycling device in metabolism that brings a potentially dead end reaction product back into the mainstream.

28. *Crossover point.* The precise site of action of a respiratory-chain inhibitor can be revealed by the *crossover technique*. Britton Chance devised elegant spectroscopic methods for determining the proportions of the oxidized and reduced forms of each carrier. This determination is feasible because the forms have distinctive absorption spectra, as illustrated in the adjoining graph for cytochrome *c*. You are given a new inhibitor and find that its addition to respiring mitochondria causes the carriers between NADH and QH_2 to become more reduced and those between cytochrome *c* and O_2 to become more oxidized. Where does your inhibitor act?



29. *Runaway mitochondria 2.* Years ago, uncouplers were suggested to make wonderful diet drugs. Explain why this idea was proposed and why it was rejected. Why might the producers of antiperspirants be supportive of the idea?

30. *Everything is connected.* If actively respiring mitochondria are exposed to an inhibitor of ATP-ADP translocase, the electron-transport chain ceases to operate. Why?

31. *Identifying the inhibition.* You are asked to determine whether a chemical is an electron-transport-chain inhibitor or an inhibitor of ATP synthase. Design an experiment to make this determination.

32. *To each according to its needs.* It has been noted that the mitochondria of muscle cells often have more cristae than the mitochondria of liver cells. Provide an explanation for this observation.

33. *Opposites attract.* An arginine residue (Arg 210) in the **a** subunit of ATP synthase is near the aspartate residue (Asp 61) in the matrix-side proton channel. How might Arg 210 assist proton flow?

34. *Variable **c** subunits.* Recall that the number of **c** subunits in the **c** ring appears to range between 10 and 14. This number is significant because it determines the number of protons that must be transported to generate a molecule of ATP. Each 360-degree rotation of the γ subunit leads to the synthesis and release of three molecules of ATP. Thus, if there are 10 **c** subunits in the ring (as was observed in a crystal structure of yeast mitochondrial ATP synthase), each ATP generated requires the transport of $10/3 = 3.33$ protons. How many protons are required to form ATP if the ring has 12 **c** subunits? 14?

35. *Counterintuitive.* Under some conditions, mitochondrial ATP synthase has been observed to actually run in reverse. How would that situation affect the proton-motive force?

36. *Etiology? What does that mean?* What does the fact that rotenone appears to increase the susceptibility to Parkinson disease indicate about the etiology of Parkinson disease?

37. *Exaggerating the difference.* Why must ATP-ADP translocase (also called adenine nucleotide translocase or ANT) use Mg^{2+}-free forms of ATP and ADP?

38. *Respiratory control.* The rate of oxygen consumption by mitochondria increases markedly when ADP is added and then returns to its initial value when the added ADP has been converted into ATP (see Figure 18.39). Why does the rate decrease?

39. *Same, but different.* Why is the electroneutral exchange of $H_2PO_4^-$ for OH^- indistinguishable from the electroneutral symport of $H_2PO_4^-$ and H^+?

40. *Multiple uses.* Give an example of the use of the protonmotive force in ways other than for the synthesis of ATP?

Chapter Integration Problems

41. *Just obeying the laws.* Why do isolated F_1 subunits of ATP synthase catalyze ATP hydrolysis?

42. *The right location.* Some cytoplasmic kinases, enzymes that phosphorylate substrates at the expense of ATP, bind to voltage-dependent anion channels. What might the advantage of this binding be?

43. *No exchange.* Mice that completely lack ATP-ADP translocase (ANT^-/ANT^-) can be made by using the knockout technique. Remarkably, these mice are viable but have the following pathological conditions: (1) high serum levels of lactate, alanine, and succinate; (2) little electron transport; and (3) a six- to eightfold increase in the level of mitochondrial H_2O_2 compared with that in normal mice. Provide a possible biochemical explanation for each of these conditions.

44. *Maybe you shouldn't take your vitamins.* Exercise is known to increase insulin sensitivity and to ameliorate type 2 diabetes (Chapter 27). Recent research suggests that taking antioxidant vitamins might mitigate the beneficial effects of exercise with respect to ROS protection.

(a) What are the antioxidant vitamins?
(b) How does exercise protect against ROS?
(c) Explain why vitamins might counteract the effects of exercise.

Data Interpretation Problem

45. *Mitochondrial disease.* A mutation in a mitochondrial gene encoding a component of ATP synthase has been identified. People who have this mutation suffer from muscle weakness, ataxia, and retinitis pigmentosa. A tissue biopsy was performed on each of three patients having this mutation, and submitochondrial particles were isolated that were capable of succinate-sustained ATP synthesis. First, the activity of the ATP synthase was measured on the addition of succinate and the following results were obtained.

	ATP synthase activity (nmol of ATP formed min^{-1} mg^{-1})
Controls	3.0
Patient 1	0.25
Patient 2	0.11
Patient 3	0.17

(a) What was the purpose of the addition of succinate?
(b) What is the effect of the mutation on succinate-coupled ATP synthesis?

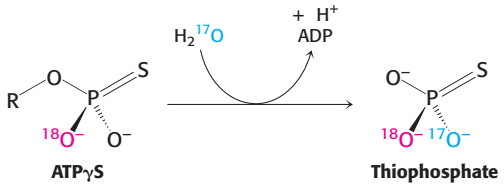
Next, the ATPase activity of the enzyme was measured by incubating the submitochondrial particles with ATP in the absence of succinate.

	ATP hydrolysis (nmol of ATP hydrolyzed min^{-1} mg^{-1})
Controls	33
Patient 1	30
Patient 2	25
Patient 3	31

(c) Why was succinate omitted from the reaction?
(d) What is the effect of the mutation on ATP hydrolysis?
(e) What do these results, in conjunction with those obtained in the first experiment, tell you about the nature of the mutation?

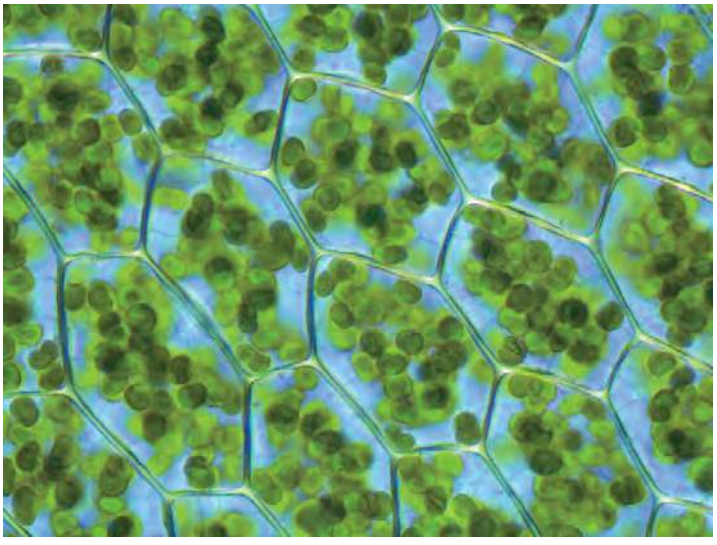
Mechanism Problem

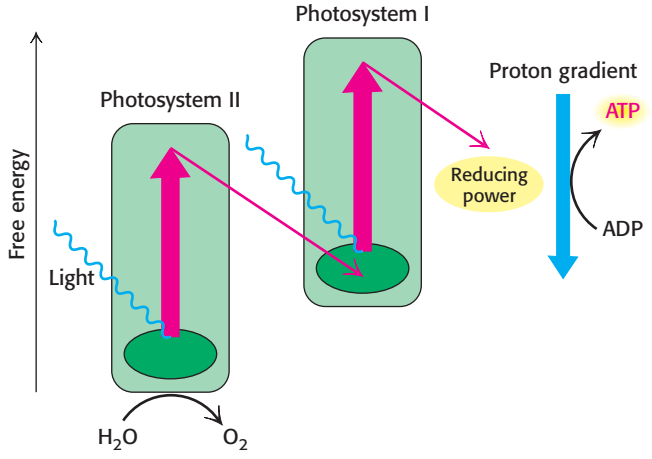
46. *Chiral clue.* ATPγS, a slowly hydrolyzed analog of ATP, can be used to probe the mechanism of phosphoryl-transfer reactions. Chiral ATPγS has been synthesized containing ^{18}O in a specific γ position and ordinary ^{16}O elsewhere in the molecule. The hydrolysis of this chiral molecule by ATP synthase in ^{17}O-enriched water yields inorganic [^{16}O,^{17}O,^{18}O]thiophosphate having the following absolute configuration. In contrast, the hydrolysis of this chiral ATPγS by a calcium-pumping ATPase from muscle gives thiophosphate of the opposite configuration. What is the simplest interpretation of these data?



CHAPTER 19

The Light Reactions of Photosynthesis





Chloroplasts (left) convert light energy into chemical energy. High-energy electrons in chloroplasts are transported through two photosystems (right). In this transit, which culminates in the generation of reducing power, ATP is synthesized in a manner analogous to mitochondrial ATP synthesis. In contrast with mitochondrial electron transport, however, electrons in chloroplasts are energized by light. [(Left) Created by Kristian Peters/GNU Free Documentation Licencse.]

On our planet are organisms capable of collecting the electromagnetic energy of the visible spectrum and converting it into chemical energy. Green plants are the most obvious of these organisms, though 60% of this conversion is carried out by algae and bacteria. This transformation is perhaps the most important of all of the energy transformations that we will see in our study of biochemistry; without it, life on our planet as we know it simply could not exist.

The process of converting electromagnetic radiation into chemical energy is called *photosynthesis,* which uses light energy to convert carbon dioxide and water into carbohydrates and oxygen.

$$CO_2 + H_2O \xrightarrow{\text{Light}} (CH_2O) + O_2$$

In this equation, CH_2O represents carbohydrate, primarily sucrose and starch. These carbohydrates provide not only the energy to run the biological world, but also the carbon molecules to make a wide array of biomolecules. Photosynthetic organisms are called *autotrophs* (literally, "self-feeders") because they can synthesize chemical fuels such as glucose from carbon dioxide and water by using sunlight as an energy source and then recover some of this energy from the synthesized glucose through the glycolytic

OUTLINE

pathway and aerobic metabolism. Organisms that obtain energy from chemical fuels only are called *heterotrophs,* which ultimately depend on autotrophs for their fuel.

We can think of photosynthesis as comprising two parts: the light reactions and the dark reactions. In the *light reactions,* light energy is transformed into two forms of biochemical energy with which we are already familiar: reducing power and ATP. The products of the light reactions are then used in the dark reactions to drive the reduction of CO_2 and its conversion into glucose and other sugars. The dark reactions are also called the *Calvin cycle* or *light-independent reactions* and will be discussed in Chapter 20.

Photosynthesis converts light energy into chemical energy

The light reactions of photosynthesis closely resemble the events of oxidative phosphorylation. In Chapters 17 and 18, we learned that cellular respiration is the oxidation of glucose to CO_2 with the reduction of O_2 to water, a process that generates ATP. In photosynthesis, this process must be reversed—reducing CO_2 and oxidizing H_2O to synthesize glucose.

$$\text{Energy} + 6\,H_2O + 6\,CO_2 \xrightarrow{\text{Photosynthesis}} C_6H_{12}O_6 + 6\,O_2$$

$$C_6H_{12}O_6 + 6\,O_2 \xrightarrow{\text{Cellular respiration}} 6\,O_2 + 6\,H_2O + \text{energy}$$

Although the processes of respiration and photosynthesis are chemically opposite each other, the biochemical principles governing the two processes are nearly identical. The key to both processes is the generation of high-energy electrons. The citric acid cycle oxidizes carbon fuels to CO_2 to generate high-energy electrons. The flow of these high-energy electrons down an electron-transport chain generates a proton-motive force. This proton-motive force is then transduced by ATP synthase to form ATP. To synthesize glucose from CO_2, high-energy electrons are required for two purposes: (1) to provide reducing power in the form of NADPH to reduce CO_2 and (2) to generate ATP to power the reduction. How can high-energy electrons be generated without using a chemical fuel? *Photosynthesis uses energy from light to boost electrons from a low-energy state to a high-energy state.* In the high-energy, unstable state, nearby molecules can abscond with the excited electrons. These electrons are used to produce reducing power, and they are used indirectly through an electron-transport chain and a proton-motive force across a membrane, which subsequently drives the synthesis of ATP. The reactions that are powered by sunlight are called the *light reactions* (Figure 19.1).

Photosynthetic yield

"If a year's yield of photosynthesis were amassed in the form of sugar cane, it would form a heap over two miles high and with a base 43 square miles."

—G. E. Fogge

If all of this sugar cane were converted into sugar cubes (0.5 inch, or 1.27 cm, on a side) and stacked end to end, the sugar cubes would extend 1.6×10^{10} miles (2.6×10^{10} kilometers) or to the planet Pluto.

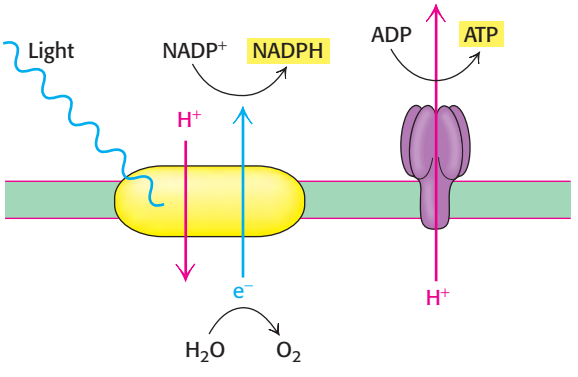


Figure 19.1 The light reactions of photosynthesis. Light is absorbed and the energy is used to drive electrons from water to generate NADPH and to drive protons across a membrane. These protons return through ATP synthase to make ATP.

Photosynthesis in green plants is mediated by two kinds of light reactions. Photosystem I generates reducing power in the form of NADPH but, in the process, becomes electron deficient. Photosystem II oxidizes water and transfers the electrons to replenish the electrons lost by photosystem I. A side product of these reactions is O_2. Electron flow from photosystem II to photosystem I generates the transmembrane proton gradient, augmented by the protons released by the oxidation of water, that drives the synthesis of ATP. In keeping with the similarity of their principles of operation, both processes take place in double-membrane organelles: mitochondria for cellular respiration and chloroplasts for photosynthesis.

Photosynthetic catastrophe

If photosynthesis were to cease, all higher forms of life would be extinct in about 25 years. A milder version of such a catastrophe ended the Cretaceous period 65.1 million years ago when a large asteroid struck the Yucatan Peninsula of Mexico. Enough dust was sent into the atmosphere that photosynthetic capacity was greatly diminished, which apparently led to the disappearance of the dinosaurs and allowed the mammals to rise to prominence.

19.1 Photosynthesis Takes Place in Chloroplasts

Photosynthesis, the means of converting light into chemical energy, takes place in organelles called *chloroplasts,* typically 5 μm long. Like a mitochondrion, a chloroplast has an outer membrane and an inner membrane, with an intervening intermembrane space (Figure 19.2). The inner membrane surrounds a space called the *stroma,* which is the site of the dark reactions of photosynthesis (Section 20.1). In the stroma are membranous structures called *thylakoids,* which are flattened sacs, or discs. The thylakoid sacs are stacked to form a *granum*. Different grana are linked by regions of thylakoid membrane called *stroma lamellae* (Figure 19.3). The thylakoid membranes separate the thylakoid space from the stroma space. Thus, chloroplasts have three different membranes (*outer, inner*, and *thylakoid membranes*) and three separate spaces (*intermembrane, stroma,* and *thylakoid spaces*). In developing chloroplasts, thylakoids arise from budding of the inner membrane, and so they are analogous to the mitochondrial cristae. Like the mitochondrial cristae, they are the site of coupled oxidation–reduction reactions of the light reactions that generate the proton-motive force.

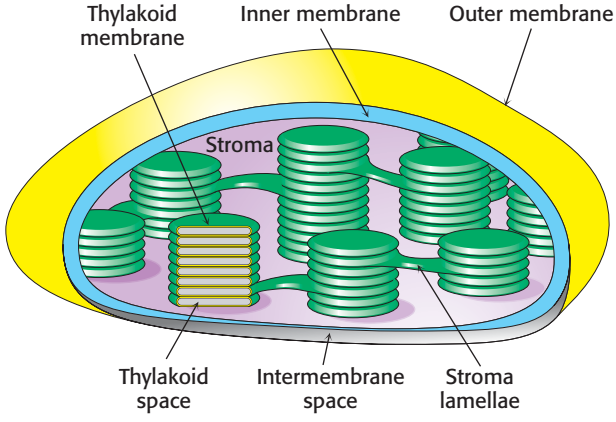


Figure 19.2 Diagram of a chloroplast.

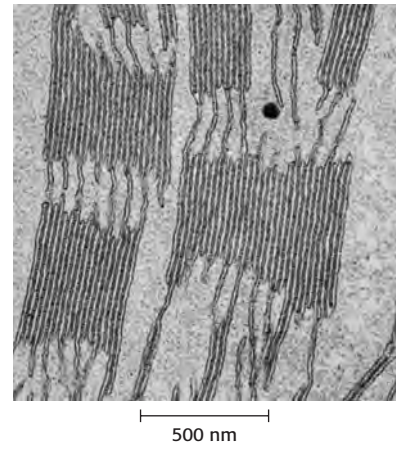


Figure 19.3 Electron micrograph of a chloroplast from a spinach leaf. The thylakoid membranes pack together to form grana. [Courtesy of Dr. Kenneth Miller.]

The primary events of photosynthesis take place in thylakoid membranes

The thylakoid membranes contain the energy-transforming machinery: light-harvesting proteins, reaction centers, electron-transport chains, and ATP synthase. These membranes contain nearly equal amounts of lipids and proteins. The lipid composition is highly distinctive: about 40% of the total lipids are *galactolipids* and 4% are *sulfolipids*, whereas only 10% are phospholipids. The thylakoid membrane and the inner membrane, like

the inner mitochondrial membrane, are impermeable to most molecules and ions. The outer membrane of a chloroplast, like that of a mitochondrion, is highly permeable to small molecules and ions. The stroma contains the soluble enzymes that utilize the NADPH and ATP synthesized by the thylakoids to convert CO_2 into sugar. Plant-leaf cells contain between 1 and 100 chloroplasts, depending on the species, cell type, and growth conditions.

Chloroplasts arose from an endosymbiotic event

Chloroplasts contain their own DNA and the machinery for replicating and expressing it. However, chloroplasts are not autonomous: they also contain many proteins encoded by nuclear DNA. How did the intriguing relation between the cell and its chloroplasts develop? We now believe that, in a manner analogous to the evolution of mitochondria (Section 18.1), chloroplasts are the result of endosymbiotic events in which a photosynthetic microorganism, most likely an ancestor of a cyanobacterium (Figure 19.4), was engulfed by a eukaryotic host. Evidence suggests that chloroplasts in higher plants and green algae are derived from a single endosymbiotic event, whereas those in red and brown algae are derived from at least one additional event.

The chloroplast genome is smaller than that of a cyanobacterium, but the two genomes have key features in common. Both are circular and have a single start site for DNA replication, and the genes of both are arranged in operons—sequences of functionally related genes under common control (Chapter 31). In the course of evolution, many of the genes of the chloroplast ancestor were transferred to the plant cell's nucleus or, in some cases, lost entirely, thus establishing a fully dependent relation.

Figure 19.4 Cyanobacteria. A colony of the photosynthetic filamentous cyanobacterium *Anabaena* shown at 450× magnification. Ancestors of these bacteria are thought to have evolved into present-day chloroplasts. [James W. Richardson/Visuals Unlimited.]

19.2 Light Absorption by Chlorophyll Induces Electron Transfer

The trapping of light energy is the key to photosynthesis. The first event is the absorption of light by a photoreceptor molecule. The principal photoreceptor in the chloroplasts of most green plants is the pigment molecule *chlorophyll* a, a substituted tetrapyrrole (Figure 19.5). The four nitrogen

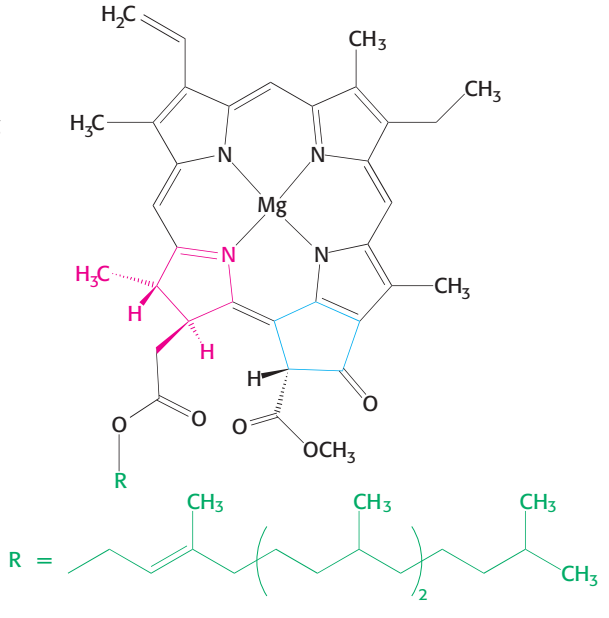


Figure 19.5 Chlorophyll. Like heme, chlorophyll *a* is a cyclic tetrapyrrole. One of the pyrrole rings (shown in red) is reduced, and an additional five-carbon ring (shown in blue) is fused to another pyrrole ring. A phytol chain (shown in green) is connected by an ester linkage. Magnesium ion binds at the center of the structure.

atoms of the pyrroles are coordinated to a magnesium ion. Unlike a porphyrin such as heme, chlorophyll has a reduced pyrrole ring and an additional 5-carbon ring fused to one of the pyrrole rings. Another distinctive feature of chlorophyll is the presence of *phytol*, a highly hydrophobic 20-carbon alcohol, esterified to an acid side chain.

Chlorophylls are very effective photoreceptors because they contain networks of conjugated double bonds—alternating single and double bonds. Such compounds are called conjugated *polyenes*. In polyenes, the electrons are not localized to a particular atomic nucleus and consequently can more readily absorb light energy. Chlorophylls have very strong absorption bands in the visible region of the spectrum, where the solar output reaching Earth is maximal (Figure 19.6). Chlorophyll *a*'s peak molar extinction coefficient (ε), a measure of a compound's ability to absorb light, is higher than 10^5 M^{-1} cm^{-1}, among the highest observed for organic compounds.

What happens when light is absorbed by a pigment molecule such as chlorophyll? The energy from the light excites an electron from its ground energy level to an excited energy level (Figure 19.7). This high-energy electron can have one of two fates. For most compounds that absorb light, the electron simply returns to the ground state and the absorbed energy is converted into heat. However, if a suitable electron acceptor is nearby, the excited electron can move from the initial molecule to the acceptor (Figure 19.8). A positive charge forms on the initial molecule, owing to the loss of an electron, and a negative charge forms on the acceptor, owing to the gain of an electron. Hence, this process is referred to as *photoinduced charge separation*.

In chloroplasts, the sites at which the charge separation takes place within each photosystem is called the *reaction center*. The photosynthetic apparatus is arranged to maximize photoinduced charge separation and minimize an unproductive return of the electron to its ground state. The electron, extracted from its initial site by the absorption of light, now has reducing power: it can reduce other molecules to store the energy originally obtained from light in chemical forms.

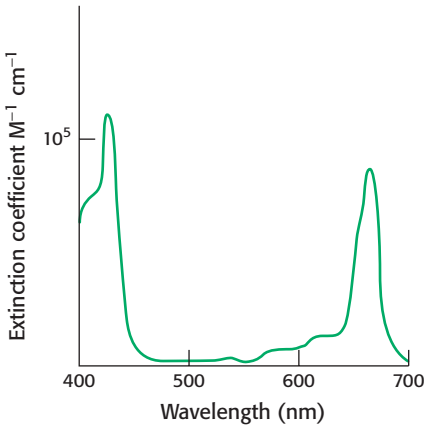


Figure 19.6 Light absorption by chlorophyll *a*. Chlorophyll *a* absorbs visible light efficiently as judged by the extinction coefficient near 10^5 M^{-1} cm^{-1}.

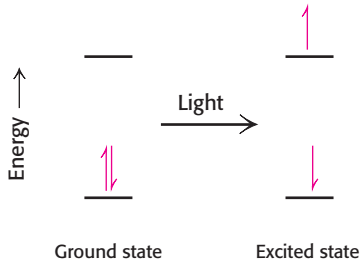


Figure 19.7 Light absorption. The absorption of light leads to the excitation of an electron from its ground state to a higher energy level.

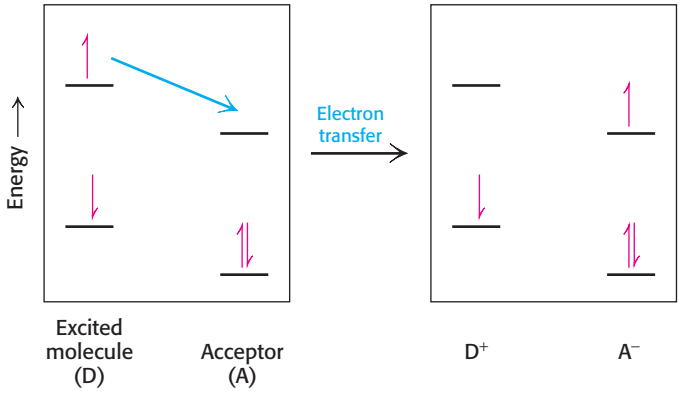


Figure 19.8 Photoinduced charge separation. If a suitable electron acceptor is nearby, an electron that has been moved to a high energy level by light absorption can move from the excited molecule to the acceptor.

A special pair of chlorophylls initiate charge separation

Photosynthetic bacteria such as *Rhodopseudomonas viridis* contain a photosynthetic reaction center that has been revealed at atomic resolution. The

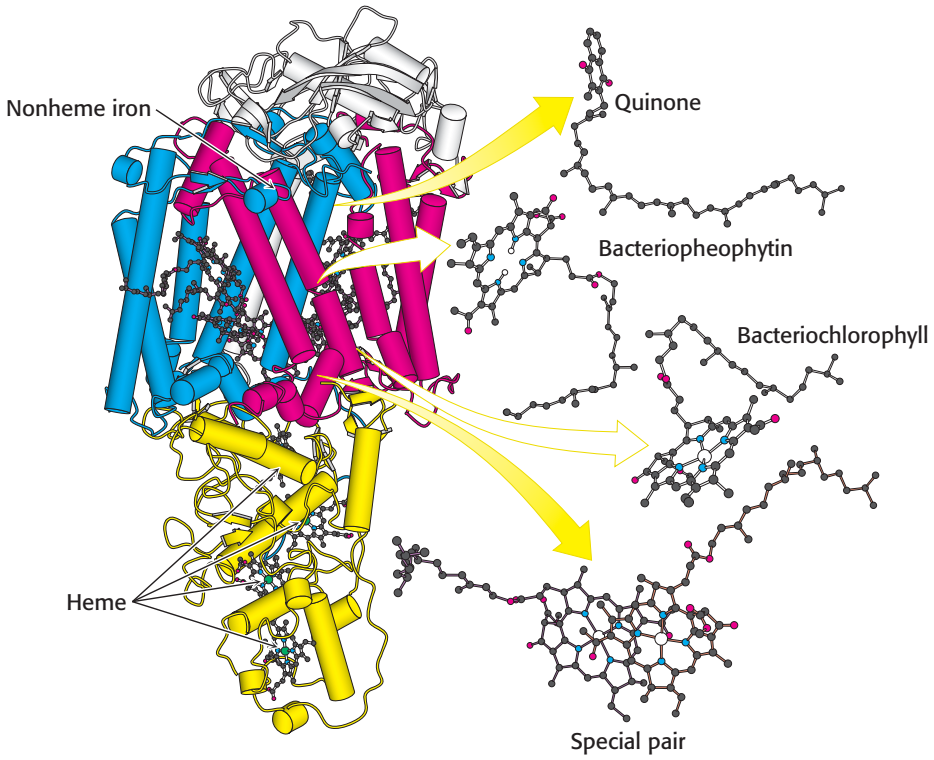
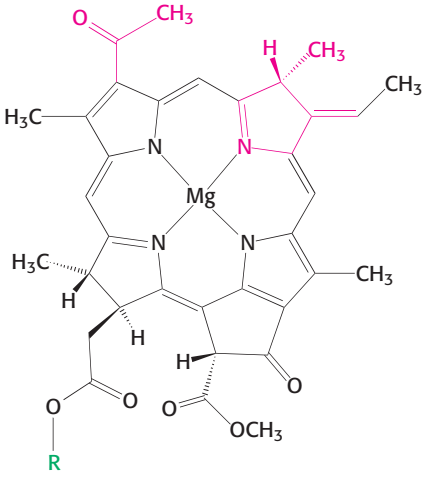
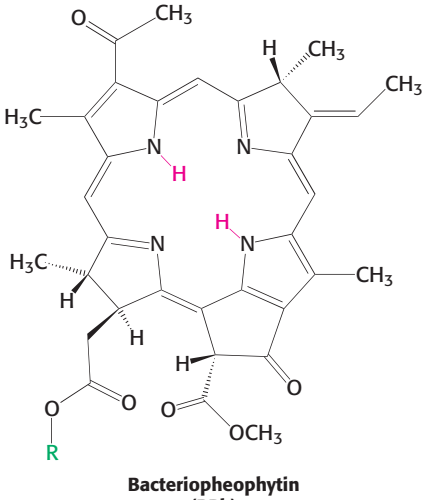


Figure 19.9 Bacterial photosynthetic reaction center. The core of the reaction center from *Rhodopseudomonas viridis* consists of two similar chains: L (red) and M (blue). An H chain (white) and a cytochrome subunit (yellow) complete the structure. *Notice* that the L and M subunits are composed largely of α helices that span the membrane. *Also notice* that a chain of electron-carrying prosthetic groups, beginning with a special pair of bacteriochlorophylls and ending at a bound quinone, runs through the structure from bottom to top in this view. [Drawn from 1PRC.pdb.]



Bacteriochlorophyll *b* (BChl-*b*)



Bacteriopheophytin (BPh)

bacterial reaction center consists of four polypeptides: L (31 kd), M (36 kd), and H (28 kd) subunits and C, a *c*-type cytochrome with four *c*-type hemes (Figure 19.9). *Sequence comparisons and low-resolution structural studies have revealed that the bacterial reaction center is homologous to the more complex plant systems*. Thus, many of our observations of the bacterial system will apply to plant systems as well.

The L and M subunits form the structural and functional core of the bacterial photosynthetic reaction center (see Figure 19.9). Each of these homologous subunits contains five transmembrane helices, in contrast with the H subunit, which has just one. The H subunit lies on the cytoplasmic side of the cell membrane, and the cytochrome subunit lies on the exterior face of the cell membrane, called the periplasmic side because it faces the periplasm, the space between the cell membrane and the cell wall. Four bacteriochlorophyll *b* (BChl-*b*) molecules, two bacteriopheophytin *b* (BPh) molecules, two quinones (Q_A and Q_B), and a ferrous ion are associated with the L and M subunits.

Bacteriochlorophylls are photoreceptors similar to chlorophylls, except for the reduction of an additional pyrrole ring and other minor differences that shift their absorption maxima to the near infrared, to wavelengths as long as 1000 nm. *Bacteriopheophytin* is the term for a bacteriochlorophyll that has two protons instead of a magnesium ion at its center.

The reaction begins with light absorption by a pair of BChl-*b* molecules that lie near the periplasmic side of the membrane in the L-M dimer. The

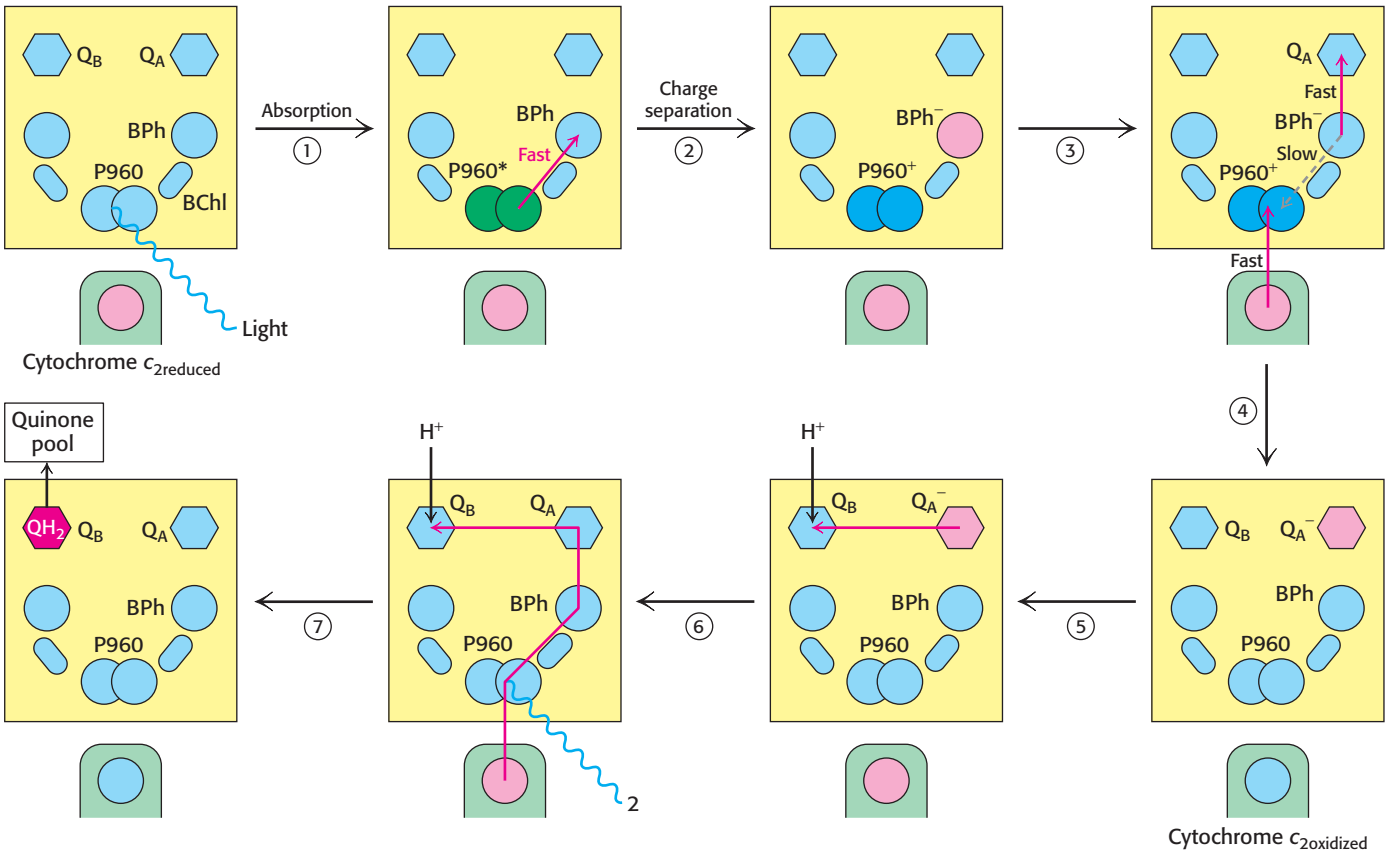


Figure 19.10 Electron chain in the photosynthetic bacterial reaction center. The absorption of light by the special pair (P960) results in the rapid transfer of an electron from this site to a bacteriopheophytin (BPh), creating a photoinduced charge separation (steps 1 and 2). (The asterisk on P960 stands for excited state.) The possible return of the electron from the pheophytin to the oxidized special pair is suppressed by the "hole" in the special pair being refilled with an electron from the cytochrome subunit and the electron from the pheophytin being transferred to a quinone (Q_A) that is farther away from the special pair (steps 3 and 4). Q_A passes the electron to Q_B. The reduction of a quinone (Q_B) on the cytoplasmic side of the membrane results in the uptake of two protons from the cytoplasm (steps 5 and 6). The reduced quinone can move into the quinone pool in the membrane (step 7).

pair of BChl-*b* molecules is called the *special pair* because of its fundamental role in photosynthesis. The special pair absorbs light maximally at 960 nm, and, for this reason, is often called *P960* (P stands for pigment). After absorbing light, the excited special pair ejects an electron, which is transferred through another BChl-*b* to a bacteriopheophytin (Figure 19.10, steps 1 and 2). This initial charge separation yields a positive charge on the special pair ($P960^+$) and a negative charge on BPh. The electron ejection and transfer take place in less than 10 picoseconds (10^{-11} s).

A nearby electron acceptor, a tightly bound quinone (Q_A), quickly grabs the electron away from BPh^- before the electron has a chance to fall back to the P960 special pair. From Q_A, the electron moves to a more loosely associated quinone, Q_B. The absorption of a second photon and the movement of a second electron from the special pair through the bacteriopheophytin to the quinones completes the two-electron reduction of Q_B from Q to QH_2. Because the Q_B-binding site lies near the cytoplasmic side of the membrane, *two protons are taken up from the cytoplasm, contributing to the development of a proton gradient across the cell membrane* (Figure 19.10, steps 5, 6, and 7).

In their high-energy states, $P960^+$ and BPh^- could undergo charge recombination; that is, the electron on BPh^- could move back to neutralize the positive charge on the special pair. Its return to the special pair would waste a valuable high-energy electron and simply convert the absorbed light energy into heat. How is charge recombination prevented? Two factors in the structure of the reaction center work together to suppress charge recombination nearly completely (Figure 19.10, steps 3 and 4). First, the next electron acceptor (Q_A) is less than 10 Å away from BPh^-, and so the electron is rapidly transferred farther away from the special pair. Second, one of

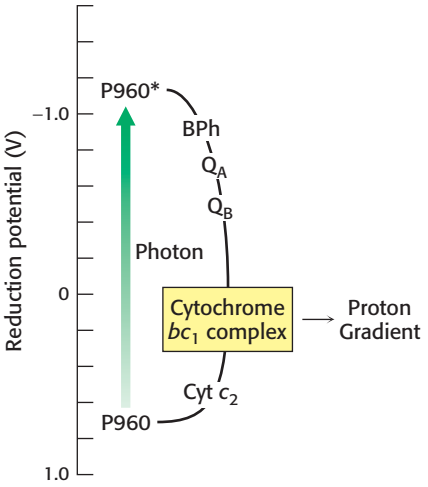


Figure 19.11 Cyclic electron flow in the bacterial reaction center. Excited electrons from the P960 reaction center flow through bacteriopheophytin (BPh), a pair of quinone molecules (Q_A and Q_B), cytochrome bc_1 complex, and finally through cytochrome c_2 to the reaction center. The cytochrome bc_1 complex pumps protons as a result of electron flow, which powers the formation of ATP.

the hemes of the cytochrome subunit is less than 10 Å away from the special pair, and so the positive charge on P960 is neutralized by the transfer of an electron from the reduced cytochrome.

Cyclic electron flow reduces the cytochrome of the reaction center

The cytochrome subunit of the reaction center must regain an electron to complete the cycle. It does so by taking back two electrons from reduced quinone (QH_2). QH_2 first enters the Q pool in the membrane where it is reoxidized to Q by complex bc_1, which is homologous to complex III of the respiratory electron-transport chain. Complex bc_1 transfers the electrons from QH_2 to cytochrome c_2, a water-soluble protein in the periplasm, and in the process pumps protons into the periplasmic space. The electrons now on cytochrome c_2 flow to the cytochrome subunit of the reaction center. The flow of electrons is thus cyclic (Figure 19.11). The proton gradient generated in the course of this cycle drives the generation of ATP through the action of ATP synthase.

19.3 Two Photosystems Generate a Proton Gradient and NADPH in Oxygenic Photosynthesis

Photosynthesis is more complicated in green plants than in photosynthetic bacteria. In green plants, photosynthesis depends on the interplay of two kinds of membrane-bound, light-sensitive complexes—*photosystem I* (PS I) and *photosystem II* (PS II), as shown in Figure 19.12. There are similarities in photosynthesis between green plants and photosynthetic bacteria. Both require light to energize reaction centers consisting of special pairs, called P680 for photosystem I and P700 for photosystem II, and both transfer electrons by using electron-transport chains. However, in plants, electron flow is not cyclic but progresses from photosystem II to photosystem I under most circumstances.

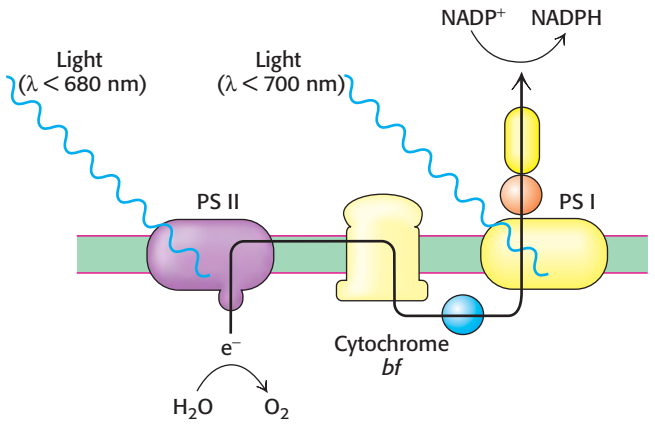


Figure 19.12 Two photosystems. The absorption of photons by two distinct photosystems (PS I and PS II) is required for complete electron flow from water to $NADP^+$.

Photosystem I, which responds to light with wavelengths shorter than 700 nm, uses light-derived high-energy electrons to create biosynthetic reducing power in the form of NADPH, a versatile reagent for driving biosynthetic processes. The electrons for creating one molecule of NADPH are taken from two molecules of water by photosystem II, which responds to wavelengths shorter than 680 nm. A molecule of O_2 is generated as a side product of the actions of photosystem II. The electrons travel from photosystem II to photosystem I through cytochrome *bf*, a membrane-bound complex homologous to Complex III in oxidative phosphorylation. Cytochrome *bf* generates a proton gradient across the thylakoid membrane that drives the formation of ATP. Thus, the two photosystems cooperate to produce NADPH and ATP.

Photosystem II transfers electrons from water to plastoquinone and generates a proton gradient

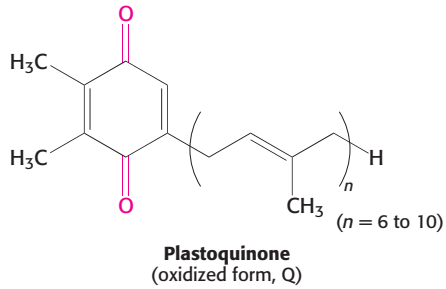
Photosystem II, an enormous transmembrane assembly of more than 20 subunits, catalyzes the light-driven transfer of electrons from water to plastoquinone. This electron acceptor closely resembles ubiquinone, a component of the mitochondrial electron-transport chain. Plastoquinone cycles

between an oxidized form (Q) and a reduced form (QH_2, plastoquinol). The overall reaction catalyzed by photosystem II is

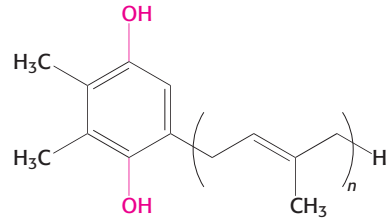
$$2\,Q + 2\,H_2O \xrightarrow{\text{Light}} O_2 + 2\,QH_2$$

The electrons in QH_2 are at a higher redox potential than those in H_2O. Recall that, in oxidative phosphorylation, electrons flow from ubiquinol to an acceptor, O_2, which is at a *lower* potential. Photosystem II drives the reaction in a thermodynamically uphill direction by using the free energy of light.

This reaction is similar to one catalyzed by the bacterial system in that a quinone is converted from its oxidized into its reduced form. Photosystem II is reasonably similar to the bacterial reaction center (Figure 19.13). The core of the photosystem is formed by D1 and D2, a pair of similar 32-kd subunits that span the thylakoid membrane. These subunits are homologous to the L and M chains of the bacterial reaction center. Unlike the bacterial system, photosystem II contains a large number of additional subunits that bind more than 30 chlorophyll molecules altogether and increase the efficiency with which light energy is absorbed and transferred to the reaction center (Section 19.5).



Plastoquinone
(oxidized form, Q)



Plastoquinol
(reduced form, QH_2)

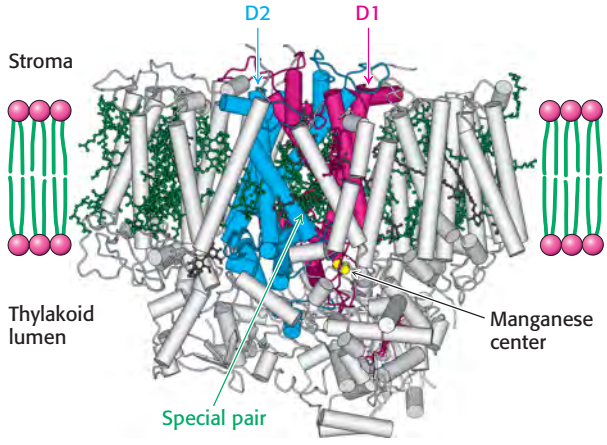


Figure 19.13 The structure of photosystem II. The D1 and D2 subunits are shown in red and blue, respectively, and the numerous bound chlorophyll molecules are shown in green. *Notice* that the special pair and the manganese center (the site of oxygen evolution) lie toward the thylakoid-lumen side of the membrane. [Drawn from 1S5L.pdb.]

The photochemistry of photosystem II begins with excitation of a special pair of chlorophyll molecules that are bound by the D1 and D2 subunits (Figure 19.14). Because the chlorophyll *a* molecules of the special pair absorb light at 680 nm, the special pair is often called *P680*. On excitation, P680 rapidly transfers an electron to a nearby pheophytin. From there, the electron is transferred first to a tightly bound plastoquinone at site Q_A and then to a mobile plastoquinone at site Q_B. This electron flow is entirely analogous to that in the bacterial system. With the arrival of a second electron and the uptake of two protons, the mobile plastoquinone is reduced to QH_2. At this point, the energy of two photons has been safely and efficiently stored in the reducing potential of QH_2.

The major difference between the bacterial system and photosystem II is the source of the electrons that are used to neutralize the positive charge formed on the special pair. *$P680^+$, a very strong oxidant, extracts electrons from*

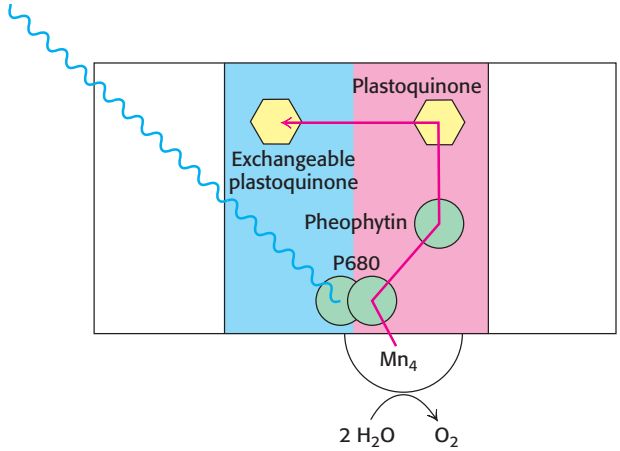


Figure 19.14 Electron flow through photosystem II. Light absorption induces electron transfer from P680 down an electron-transfer pathway to an exchangeable plastoquinone. The positive charge on P680 is neutralized by electron flow from water molecules bound at the manganese center.

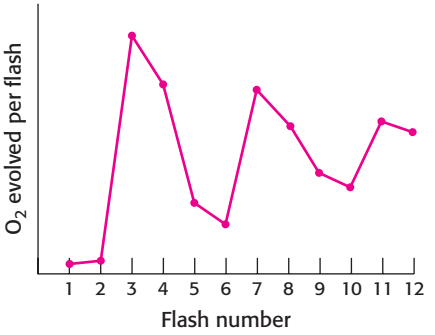
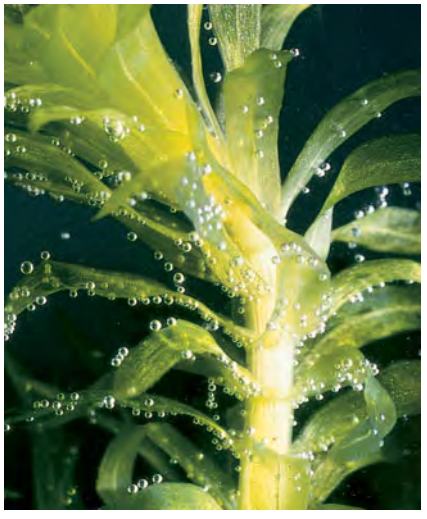


Figure 19.15 Four photons are required to generate one oxygen molecule. When dark-adapted chloroplasts are exposed to a brief flash of light, one electron passes through photosystem II. Monitoring the O_2 released after each flash reveals that four flashes are required to generate each O_2 molecule. The peaks in O_2 release are after the 3rd, 7th, and 11th flashes because the dark-adapted chloroplasts start in the S_1 state—that is, the one-electron reduced state.



Evolution of oxygen is evident by the generation of bubbles in the aquatic plant *Elodea*. [Colin Milkins/Oxford Scientific Films/Photolibrary.]

water molecules bound at the manganese center. The structure of this center includes a calcium ion and four manganese ions. Manganese was apparently evolutionarily selected for this role because of its ability to exist in multiple oxidation states (Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{5+}) and to form strong bonds with oxygen-containing species. The *manganese center,* in its reduced form, oxidizes two molecules of water to form a single molecule of oxygen. Each time the absorbance of a photon kicks an electron out of P680, the positively charged special pair extracts an electron from the manganese center (Figure 19.15). However, the electrons do not come directly from the manganese ions. A tyrosine residue (often designated Z) of subunit D1 in photosystem II is the immediate electron donor, forming a tyrosine radical. The tyrosine radical removes electrons from the manganese ions, which in turn remove electrons from H_2O to generate O_2 and H^+. Four photons must be absorbed to extract four electrons from a water molecule (Figure 19.16). The four electrons harvested from water are used to reduce two molecules of Q to QH_2.

Photosystem II spans the thylakoid membrane such that the site of quinone reduction is on the side of the stroma, whereas the manganese center, hence the site of water oxidation, lies in the thylakoid lumen. Thus, the two protons that are taken up with the reduction of Q to QH_2 come from the stroma, and the four protons that are liberated in the course of water oxidation are released into the lumen. This distribution of protons gener-

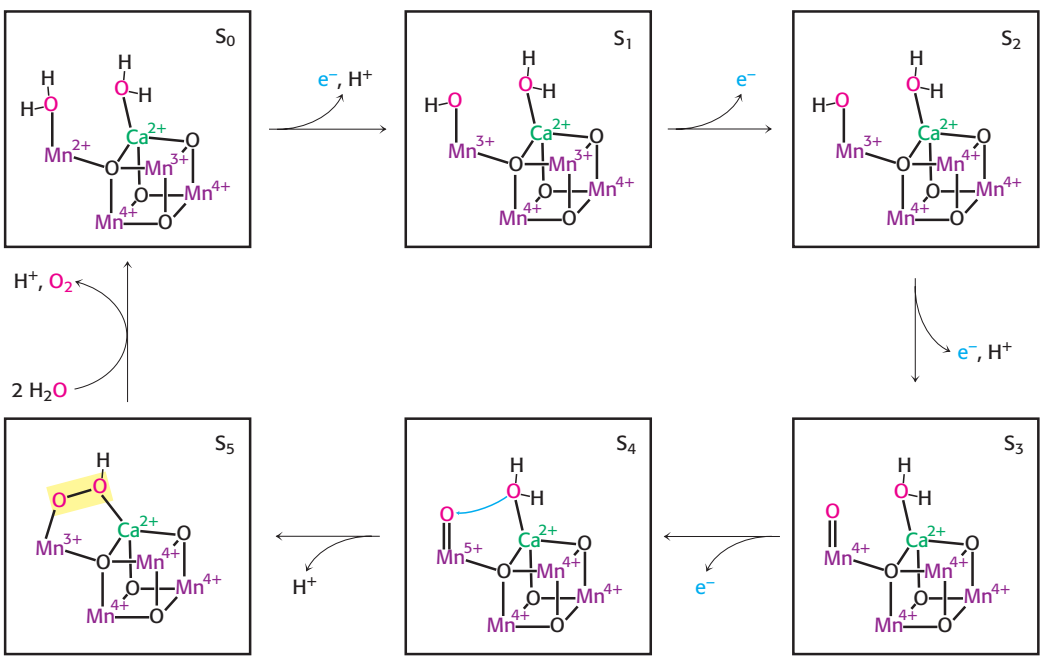


Figure 19.16 A plausible scheme for oxygen evolution from the manganese center. The deduced core structure of the manganese center including four manganese ions and one calcium ion is shown, although many additional ligands are omitted for clarity. The center is oxidized, one electron at a time, until two bound H_2O molecules are linked to form a molecule of O_2, which is then released from the center. A tyrosine residue (not shown) participates in the coupled proton–electron transfer steps. The structures are designated S_0 to S_4 to indicate the number of electrons that have been removed.

ates a proton gradient across the thylakoid membrane characterized by an excess of protons in the thylakoid lumen compared with the stroma (Figure 19.17).

Cytochrome *bf* links photosystem II to photosystem I

Electrons flow from photosystem II to photosystem I through the *cytochrome* bf complex. This complex catalyzes the transfer of electrons from plastoquinol (QH_2) to plastocyanin (Pc), a small, soluble copper protein in the thylakoid lumen.

$$QH_2 + 2\,Pc(Cu^{2+}) \rightarrow Q + 2\,Pc(Cu^{+}) + 2\,H^{+}_{\text{thylakoid lumen}}$$

The two protons from plastoquinol are released into the thylakoid lumen. This reaction is reminiscent of that catalyzed by Complex III in oxidative phosphorylation, and most components of the *cytochrome* bf complex are homologous to those of Complex III. The cytochrome *bf* complex includes four subunits: a 23-kd cytochrome with two *b*-type hemes, a 20-kd Rieske-type Fe-S protein, a 33-kd cytochrome *f* with a *c*-type cytochrome, and a 17-kd chain.

This complex catalyzes the reaction by proceeding through the Q cycle (see Figure 18.12). In the first half of the Q cycle, plastoquinol (QH_2) is oxidized to plastoquinone (Q), one electron at a time. The electrons from plastoquinol flow through the Fe-S protein to convert oxidized plastocyanin (Pc) into its reduced form.

In the second half of the Q cycle, cytochrome *bf* reduces a molecule of plastoquinone from the Q pool to plastoquinol, taking up two protons from one side of the membrane, and then reoxidizes plastoquinol to release these protons on the other side. The enzyme is oriented so that protons are released into the thylakoid lumen and taken up from the stroma, contributing further to the proton gradient across the thylakoid membrane (Figure 19.18).

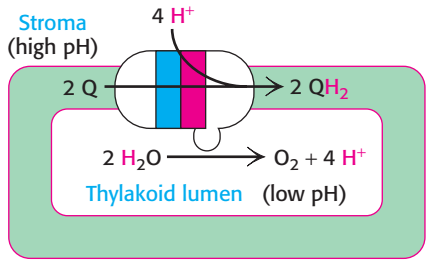


Figure 19.17 Proton-gradient direction. Photosystem II releases protons into the thylakoid lumen and takes them up from the stroma. The result is a pH gradient across the thylakoid membrane with an excess of protons (low pH) inside.

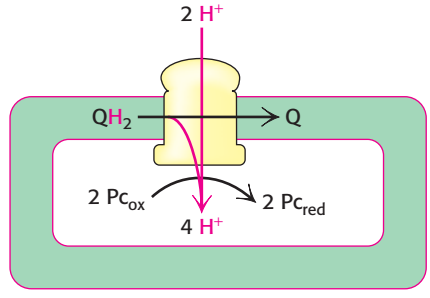


Figure 19.18 Cytochrome *bf* contribution to proton gradient. The cytochrome *bf* complex oxidizes QH_2 to Q through the Q cycle. Four protons are released into the thylakoid lumen in each cycle.

Photosystem I uses light energy to generate reduced ferredoxin, a powerful reductant

The final stage of the light reactions is catalyzed by photosystem I, a transmembrane complex consisting of about 14 polypeptide chains and multiple associated proteins and cofactors (Figure 19.19). The core of this system is a pair of similar subunits, psaA (83 kd) and psaB (82 kd). These subunits are quite a bit larger than the core subunits of photosystem II and the bacterial

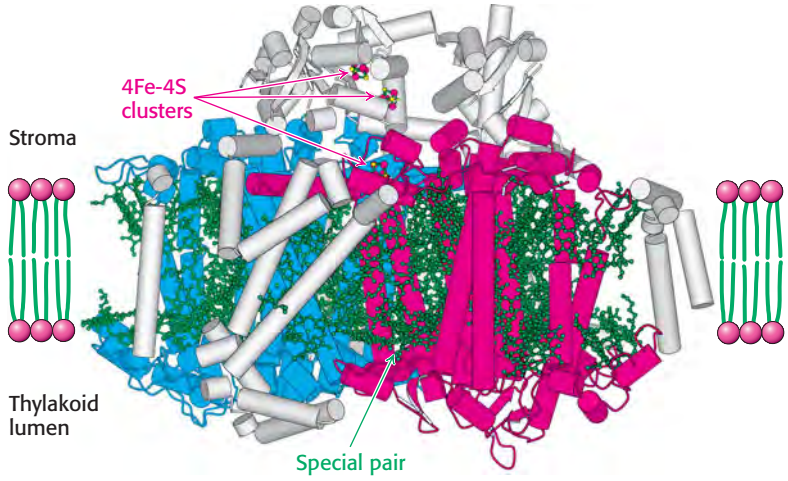


Figure 19.19 The structure of photosystem I. The psaA and psaB subunits are shown in red and blue, respectively. *Notice* the numerous bound chlorophyll molecules, shown in green, including the special pair, as well as the iron–sulfur clusters that facilitate electron transfer from the stroma. [Drawn from 1JB0.pdb.]

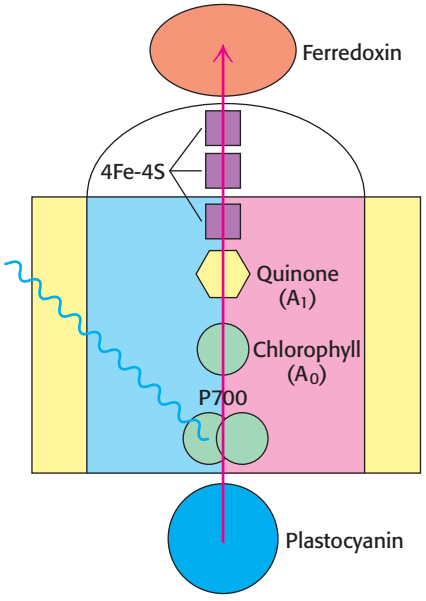


Figure 19.20 Electron flow through photosystem I to ferredoxin. Light absorption induces electron transfer from P700 down an electron-transfer pathway that includes a chlorophyll molecule, a quinone molecule, and three 4Fe-4S clusters to reach ferredoxin. The positive charge left on P700 is neutralized by electron transfer from reduced plastocyanin.

reaction center. Nonetheless, they appear to be homologous; the terminal 40% of each subunit is similar to a corresponding subunit of photosystem II. A special pair of chlorophyll *a* molecules lie at the center of the structure and absorb light maximally at 700 nm. This center, called *P700*, initiates photo-induced charge separation (Figure 19.20). The electron travels from P700 down a pathway through chlorophyll at site A_0 and quinone at site A_1 to a set of 4Fe-4S clusters. The next step is the transfer of the electron to ferredoxin (Fd), a soluble protein containing a 2Fe-2S cluster coordinated to four cysteine residues (Figure 19.21). Ferredoxin transfers electrons to $NADP^+$. Meanwhile, $P700^+$ captures an electron from reduced plastocyanin to return to P700 so that P700 can be excited again. Thus, the overall reaction catalyzed by photosystem I is a simple one-electron oxidation–reduction reaction.

$$Pc(Cu^+) + Fd_{ox} \xrightarrow{\text{Light}} Pc(Cu^{2+}) + Fd_{red}$$

Given that the reduction potentials for plastocyanin and ferredoxin are +0.37 V and –0.45 V, respectively, the standard free energy for this reaction is +79.1 kJ mol^{-1} (+18.9 kcal mol^{-1}). This uphill reaction is driven by the absorption of a 700-nm photon, which has an energy of 171 kJ mol^{-1} (40.9 kcal mol^{-1}).

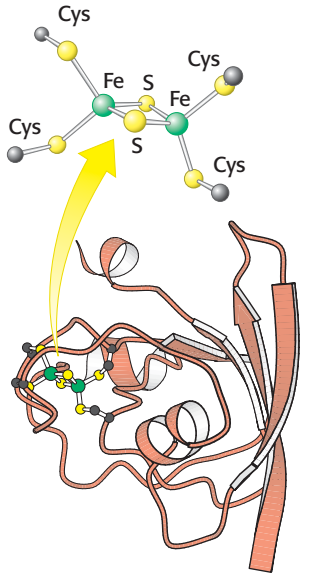


Figure 19.21 Structure of ferredoxin. In plants, ferredoxin contains a 2Fe-2S cluster. This protein accepts electrons from photosystem I and carries them to ferredoxin–NADP reductase. [Drawn from 1FXA.pdb.]

Ferredoxin–$NADP^+$ reductase converts $NADP^+$ into NADPH

Although reduced ferredoxin is a strong reductant, it is not useful for driving many reactions, in part because ferredoxin carries only one available electron. In contrast, NADPH, a two-electron reductant, is a widely used electron donor in biosynthetic processes, including the reactions of the Calvin cycle (Chapter 20). How is reduced ferredoxin used to drive the reduction of $NADP^+$ to NADPH? This reaction is catalyzed by *ferredoxin–$NADP^+$ reductase,* a flavoprotein with an FAD prosthetic group (Figure 19.22A). The bound FAD moiety accepts two electrons and two protons from two molecules of reduced ferredoxin to form $FADH_2$ (Figure 19.22B). The enzyme then transfers a hydride ion (H^-) to $NADP^+$ to form NADPH. This reaction takes place on the stromal side of the membrane. Hence, the uptake of a proton in the reduction of $NADP^+$

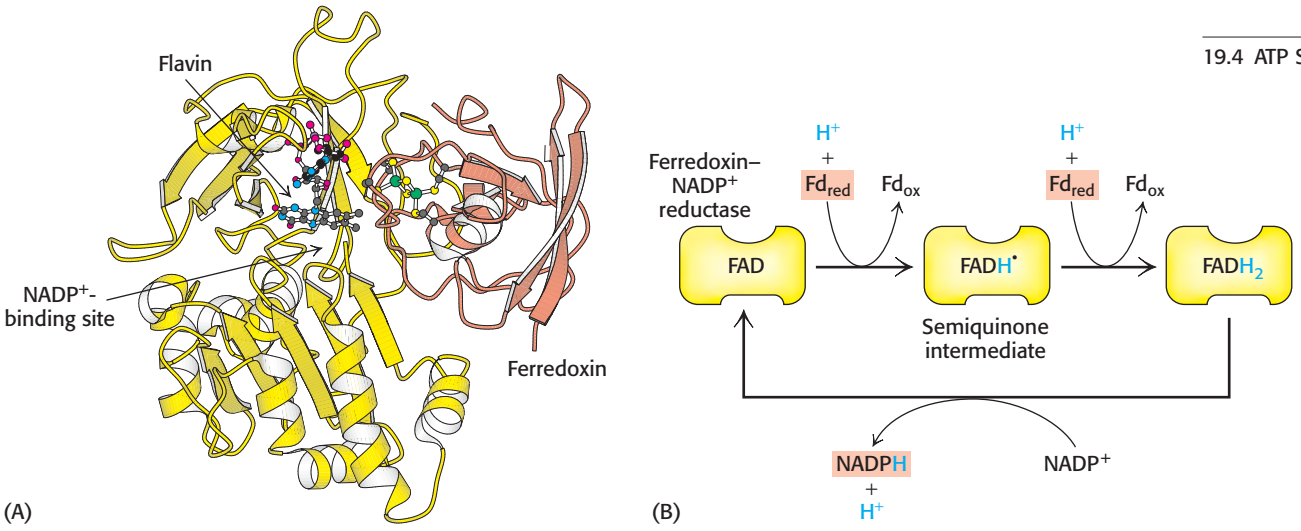


Figure 19.22 Structure and function of ferredoxin–NADP$^+$ reductase. (A) Structure of ferredoxin–NADP$^+$ reductase. This enzyme accepts electrons, one at a time, from ferredoxin (shown in orange). (B) Ferredoxin–NADP$^+$ reductase first accepts two electrons and two protons from two molecules of reduced ferredoxin (Fd) to form $FADH_2$, which then transfers two electrons and a proton to NADP$^+$ to form NADPH. [Drawn from 1EWY.pdb.]

further contributes to the generation of the proton gradient across the thylakoid membrane.

The cooperation between photosystem I and photosystem II creates a flow of electrons from H_2O to NADP$^+$. The pathway of electron flow is called the *Z scheme of photosynthesis* because the redox diagram from P680 to P700* looks like the letter Z (Figure 19.23).

19.4 A Proton Gradient Across the Thylakoid Membrane Drives ATP Synthesis

In 1966, André Jagendorf showed that chloroplasts synthesize ATP in the dark when an artificial pH gradient is imposed across the thylakoid membrane. To create this transient pH gradient, he soaked chloroplasts in a pH 4 buffer for several hours and then rapidly mixed them with a pH 8 buffer

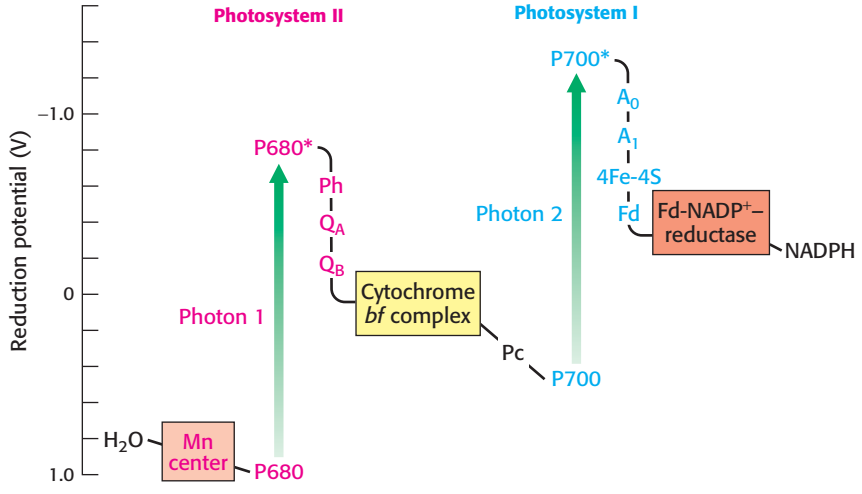


Figure 19.23 Pathway of electron flow from H_2O to NADP$^+$ in photosynthesis. This endergonic reaction is made possible by the absorption of light by photosystem II (P680) and photosystem I (P700). Abbreviations: Ph, pheophytin; Q_A and Q_B, plastoquinone-binding proteins; Pc, plastocyanin; A_0 and A_1, acceptors of electrons from P700*; Fd, ferredoxin; Mn, manganese.

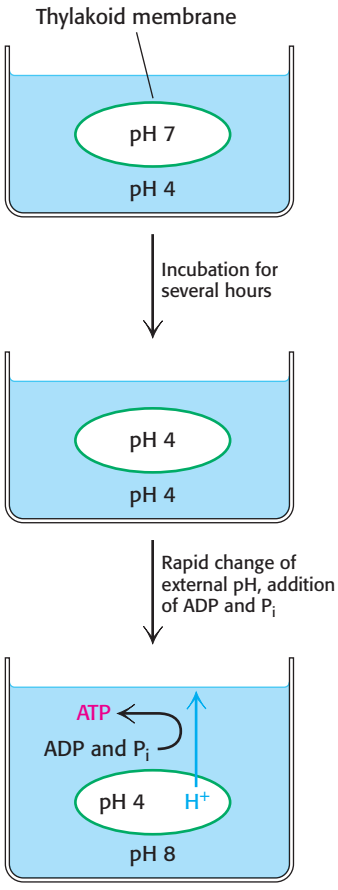


Figure 19.24 Jagendorf's demonstration. Chloroplasts synthesize ATP after the imposition of a pH gradient.

containing ADP and P_i. The pH of the stroma suddenly increased to 8, whereas the pH of the thylakoid space remained at 4. *A burst of ATP synthesis then accompanied the disappearance of the pH gradient across the thylakoid membrane* (Figure 19.24). This incisive experiment was one of the first to unequivocally support the hypothesis put forth by Peter Mitchell that ATP synthesis is driven by proton-motive force.

The principles of ATP synthesis in chloroplasts are nearly identical with those in mitochondria. *ATP formation is driven by a proton-motive force in both photophosphorylation and oxidative phosphorylation.* We have seen how light induces electron transfer through photosystems II and I and the cytochrome *bf* complex. At various stages in this process, protons are released into the thylakoid lumen or taken up from the stroma, generating a proton gradient. The gradient is maintained because the thylakoid membrane is essentially impermeable to protons. *The thylakoid space becomes markedly acidic, with the pH approaching 4. The light-induced transmembrane proton gradient is about 3.5 pH units.* As discussed in Section 18.4, energy inherent in the proton gradient, called the *proton-motive force* (Δp), is described as the sum of two components: a charge gradient and a chemical gradient. In chloroplasts, nearly all of Δp arises from the pH gradient, whereas, in mitochondria, the contribution from the membrane potential is larger. The reason for this difference is that the thylakoid membrane is quite permeable to Cl^- and Mg^{2+}. The light-induced transfer of H^+ into the thylakoid space is accompanied by the transfer of either Cl^- in the same direction or Mg^{2+} ($1\ Mg^{2+}$ per $2\ H^+$) in the opposite direction. Consequently, electrical neutrality is maintained and no membrane potential is generated. The influx of Mg^{2+} into the stroma plays a role in the regulation of the Calvin Cycle (Section 20.2). A pH gradient of 3.5 units across the thylakoid membrane corresponds to a proton-motive force of 0.20 V or a ΔG of –20.0 kJ mol^{-1} (-4.8 kcal mol^{-1}).

The ATP synthase of chloroplasts closely resembles those of mitochondria and prokaryotes

The proton-motive force generated by the light reactions is converted into ATP by the *ATP synthase* of chloroplasts, also called the *CF_1–CF_0 complex* (*C* stands for chloroplast and *F* for factor). CF_1–CF_0 ATP synthase closely resembles the F_1–F_0 complex of mitochondria (Section 18.4). CF_0 conducts protons across the thylakoid membrane, whereas CF_1 catalyzes the formation of ATP from ADP and P_i.

CF_0 is embedded in the thylakoid membrane. It consists of four different polypeptide chains known as I (17 kd), II (16.5 kd), III (8 kd), and IV (27 kd) having an estimated stoichiometry of 1 : 2 : 12 : 1. Subunits I and II have sequence similarity to subunit **b** of the mitochondrial F_0 subunit, III corresponds to subunit **c** of the mitochondrial complex, and subunit IV is similar in sequence to subunit **a.** CF_1, the site of ATP synthesis, has a subunit composition $\alpha_3\beta_3\gamma\delta_\varepsilon$. The β subunits contain the catalytic sites, similarly to the F_1 subunit of mitochondrial ATP synthase. Remarkably, the β subunits of ATP synthase in corn chloroplasts are more than 60% identical in amino acid sequence with those of human ATP synthase, despite the passage of approximately 1 billion years since the separation of the plant and animal kingdoms.

Note that the membrane orientation of CF_1–CF_0 is reversed compared with that of the mitochondrial ATP synthase (Figure 19.25). However, the functional orientation of the two synthases is identical: protons flow from the lumen through the enzyme to the stroma or matrix where ATP is synthesized. Because CF_1 is on the stromal surface of the thylakoid

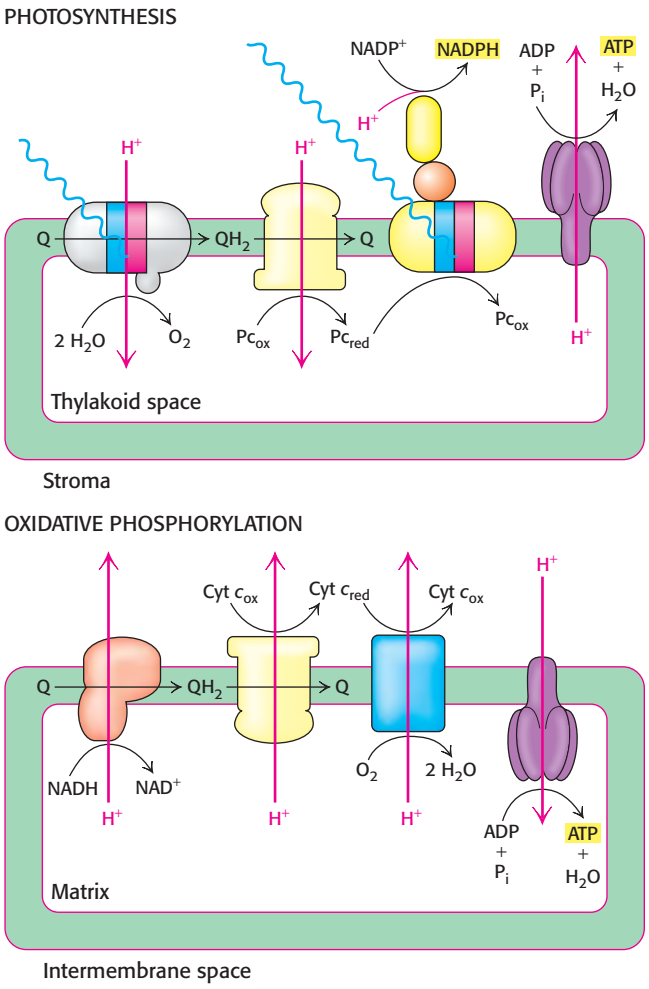


Figure 19.25 Comparison of photosynthesis and oxidative phosphorylation. The light-induced electron transfer in photosynthesis drives protons into the thylakoid lumen. The excess protons flow out of the lumen through ATP synthase to generate ATP in the stroma. In oxidative phosphorylation, electron flow down the electron-transport chain pumps protons out of the mitochondrial matrix. Excess protons from the intermembrane space flow into the matrix through ATP synthase to generate ATP in the matrix.

membrane, the newly synthesized ATP is released directly into the stromal space. Likewise, NADPH formed by photosystem I is released into the stromal space. Thus, *ATP and NADPH, the products of the light reactions of photosynthesis, are appropriately positioned for the subsequent dark reactions, in which CO_2 is converted into carbohydrate.*

Cyclic electron flow through photosystem I leads to the production of ATP instead of NADPH

On occasion, when the ratio of NADPH to $NADP^+$ is very high as might be the case if there was another source of electrons to form NADPH (Section 20.3), $NADP^+$ may be unavailable to accept electrons from reduced ferredoxin. In this case, electrons arising from P700, the reaction center of photosystem I, may take an alternative pathway that does not end at NADPH. The electron in reduced ferredoxin is transferred to the cytochrome *bf* complex rather than to $NADP^+$. This electron then flows back through the cytochrome *bf* complex to reduce plastocyanin, which can then be reoxidized by $P700^+$ to complete a cycle. The net outcome of this cyclic flow of electrons is the pumping of protons by the cytochrome *bf*

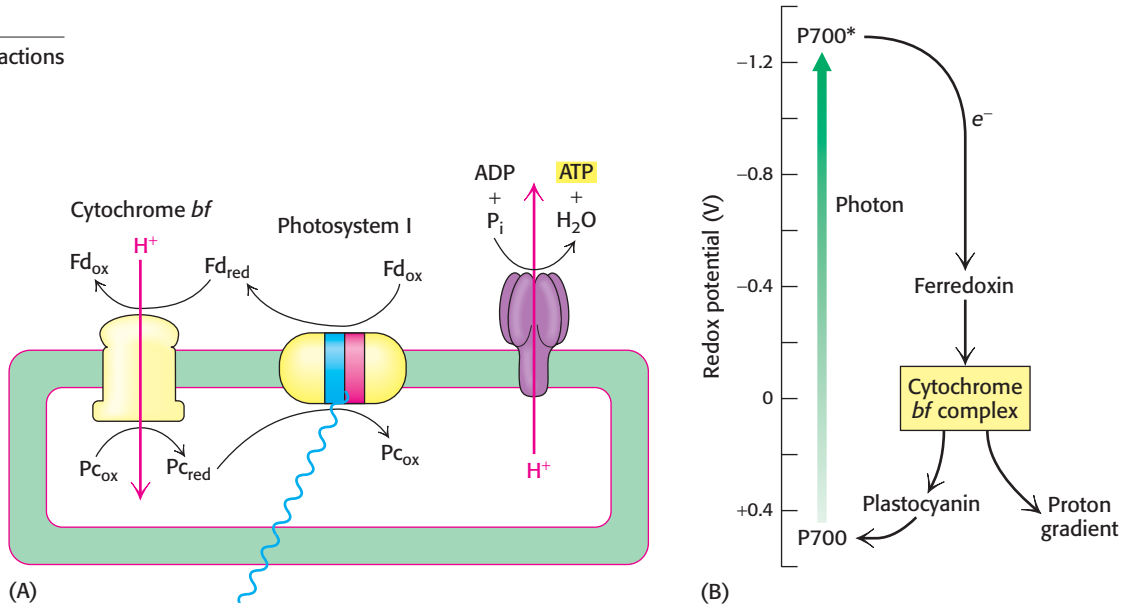


Figure 19.26 Cyclic photophosphorylation. (A) In this pathway, electrons from reduced ferredoxin are transferred to cytochrome *bf* rather than to ferredoxin–$NADP^+$ reductase. The flow of electrons through cytochrome *bf* pumps protons into the thylakoid lumen. These protons flow through ATP synthase to generate ATP. Neither NADPH nor O_2 is generated by this pathway. (B) A scheme showing the energetic basis for cyclic photophosphorylation. Abbreviations: Fd, ferredoxin; Pc, plastocyanin.

complex. The resulting proton gradient then drives the synthesis of ATP. In this process, called *cyclic photophosphorylation, ATP is generated without the concomitant formation of NADPH* (Figure 19.26). Photosystem II does not participate in cyclic photophosphorylation, and so O_2 is not formed from H_2O.

The absorption of eight photons yields one O_2, two NADPH, and three ATP molecules

We can now estimate the overall stoichiometry for the light reactions. The absorption of four photons by photosystem II generates one molecule of O_2 and releases 4 protons into the thylakoid lumen. The two molecules of plastoquinol are oxidized by the Q cycle of the cytochrome *bf* complex to release 8 protons into the lumen. Finally, the electrons from four molecules of reduced plastocyanin are driven to ferredoxin by the absorption of four additional photons. The four molecules of reduced ferredoxin generate two molecules of NADPH. Thus, the overall reaction is

$$2\,H_2O + 2\,NADP^+ + 10\,H^+_{stroma} \rightarrow O_2 + 2\,NADPH + 12\,H^+_{lumen}$$

The 12 protons released in the lumen can then flow through ATP synthase. Given that there are apparently 12 subunit III components in CF_0, we expect that 12 protons must pass through CF_0 to complete one full rotation of CF_1. A single rotation generates three molecules of ATP. Given the ratio of 3 ATP for 12 protons, the overall reaction is

$$2\,H_2O + 2\,NADP^+ + 10\,H^+_{stroma} \longrightarrow O_2 + 2\,NADPH + 12\,H^+_{lumen}$$
$$3\,ADP^{3-} + 3\,P_i^{2-} + 3\,H^+ + 12\,H^+_{lumen} \longrightarrow 3\,ATP^{4-} + 3\,H_2O + 12\,H^+_{stroma}$$
$$2\,NADP^+ + 3\,ADP^{3-} + 3\,P_i^{2-} + H^+ \longrightarrow O_2 + 2\,NADPH + 3\,ATP^{4-} + H_2O$$

Thus, eight photons are required to yield three molecules of ATP (2.7 photons/ATP).

Cyclic photophosphorylation is a somewhat more productive way to synthesize ATP. The absorption of four photons by photosystem I leads to the release of 8 protons into the lumen by the cytochrome *bf* system. These protons flow through ATP synthase to yield two molecules of ATP. Thus, each two absorbed photons yield one molecule of ATP. No NADPH is produced.

19.5 Accessory Pigments Funnel Energy into Reaction Centers

A light-harvesting system that relied only on the chlorophyll *a* molecules of the special pair would be rather inefficient for two reasons. First, chlorophyll *a* molecules absorb light only at specific wavelengths (see Figure 19.6). A large gap is present in the middle of the visible region between approximately 450 and 650 nm. This gap falls right at the peak of the solar spectrum, and so failure to collect this light would constitute a considerable lost opportunity. Second, even on a cloudless day, many photons that can be absorbed by chlorophyll *a* pass through the chloroplast without being absorbed, because the density of chlorophyll *a* molecules in a reaction center is not very great. Accessory pigments, both additional chlorophylls and other classes of molecules, are closely associated with reaction centers. *These pigments absorb light and funnel the energy to the reaction center for conversion into chemical forms*. Accessory pigments prevent the reaction center from sitting idle.

Resonance energy transfer allows energy to move from the site of initial absorbance to the reaction center

How is energy funneled from an associated pigment to a reaction center? The absorption of a photon does not always lead to electron excitation and transfer. More commonly, excitation energy is transferred from one molecule to a nearby molecule through electromagnetic interactions through space (Figure 19.27). The rate of this process, called *resonance energy transfer,* depends strongly on the distance between the energy-donor and the energy-acceptor molecules; an increase in the distance between the donor and the acceptor by a factor of two typically results in a decrease in the energy-transfer rate by a factor of $2^6 = 64$. For reasons of conservation of energy, energy transfer must be from a donor in the excited state to an acceptor of equal or lower energy. *The excited state of the special pair of*

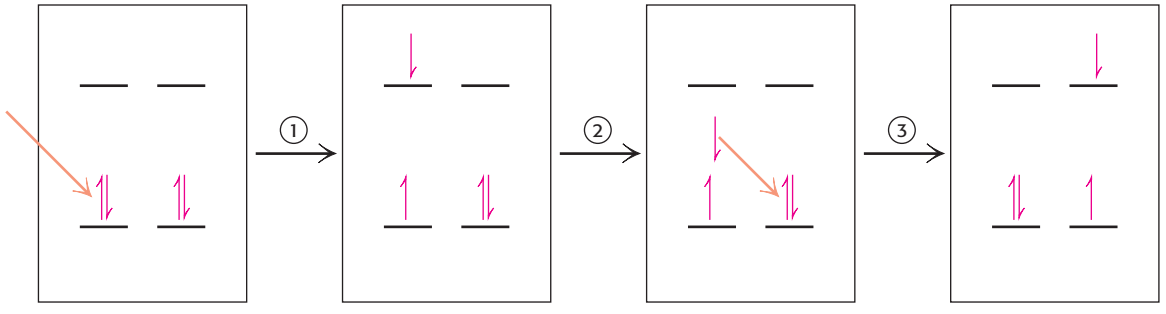


Figure 19.27 Resonance energy transfer. (1) An electron can accept energy from electron magnetic radiation of appropriate wavelength and jump to a higher energy state. (2) When the excited electron falls back to its lower energy state, the absorbed energy is released. (3) The released energy can be absorbed by an electron in a nearby molecule, and this electron jumps to a high energy state.

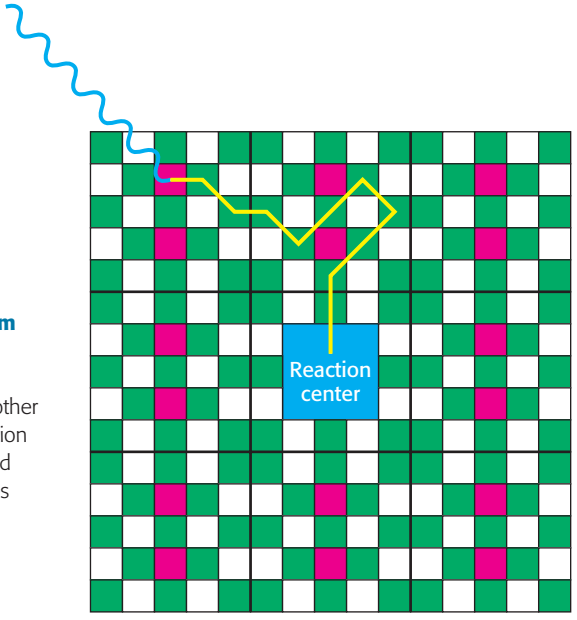
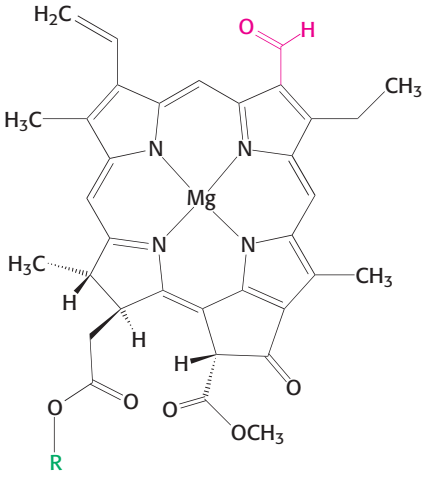


Figure 19.28 Energy transfer from accessory pigments to reaction centers. Light energy absorbed by accessory chlorophyll molecules or other pigments can be transferred to reaction centers, where it drives photoinduced charge separation. The green squares represent accessory chlorophyll molecules and the red squares represent carotenoid molecules; the white squares designate protein.



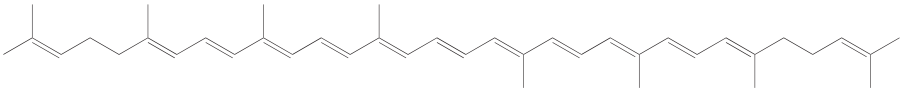
Chlorophyll *b*

chlorophyll molecules is lower in energy than that of single chlorophyll molecules, allowing reaction centers to trap the energy transferred from other molecules (Figure 19.28).

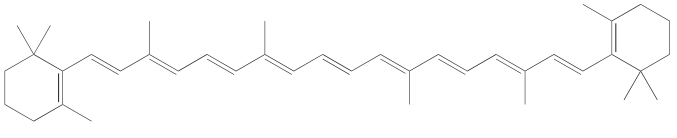
Light-harvesting complexes contain additional chlorophylls and carotinoids

Chlorophyll b and *carotenoids* are important light-harvesting molecules that funnel energy to the reaction center. Chlorophyll *b* differs from chlorophyll *a* in having a formyl group in place of a methyl group. This small difference shifts its two major absorption peaks toward the center of the visible region. In particular, chlorophyll *b* efficiently absorbs light with wavelengths between 450 and 500 nm (Figure 19.29).

Carotenoids are extended polyenes that absorb light between 400 and 500 nm. The carotenoids are responsible for most of the yellow and red colors of fruits and flowers, and they provide the brilliance of fall, when the chlorophyll molecules degrade, revealing the carotenoids.



Lycopene



β-Carotene

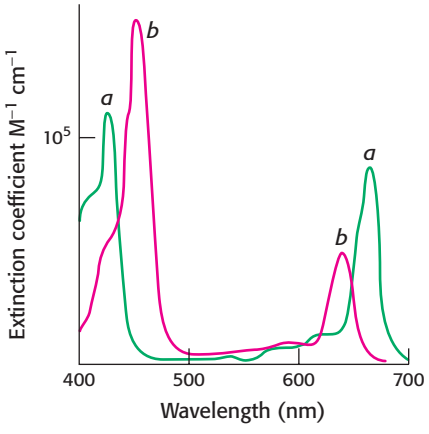


Figure 19.29 Absorption spectra of chlorophylls *a* and *b*.

In addition to their role in transferring energy to reaction centers, the carotenoids serve a safeguarding function. Carotenoids suppress damaging photochemical reactions, particularly those including oxygen that can be induced by bright sunlight. This protection may be especially important in the fall when the primary pigment chlorophyll is being degraded and thus not able to absorb light energy. Plants lacking carotenoids are quickly killed on exposure to light and oxygen.

The accessory pigments are arranged in numerous *light-harvesting complexes* that completely surround the reaction center. The 26-kd subunit of light-harvesting complex II (LHC-II) is the most abundant membrane protein in chloroplasts. This subunit binds seven chlorophyll *a* molecules, six chlorophyll *b* molecules, and two carotenoid molecules. Similar light-harvesting assemblies exist in photosynthetic bacteria (Figure 19.30).

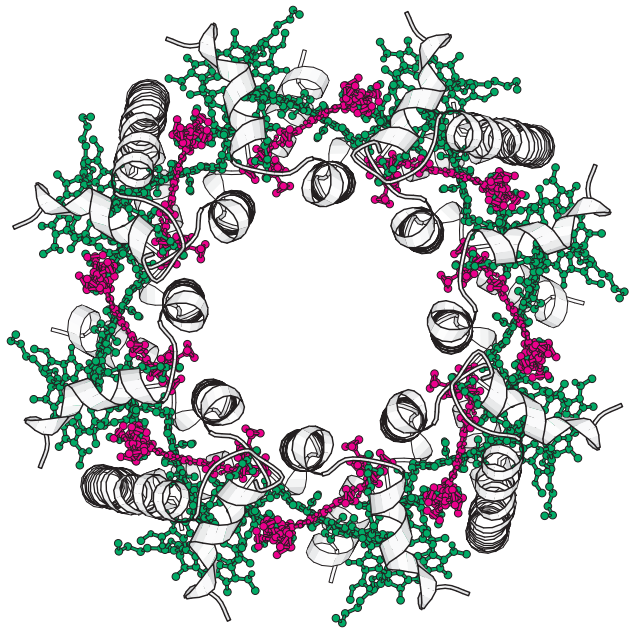


Figure 19.30 Structure of a bacterial light-harvesting complex. Eight polypeptides, each of which binds three chlorophyll molecules (green) and a carotenoid molecule (red), surround a central cavity that contains the reaction center (not shown). *Notice* the high concentration of accessory pigments that surround the reaction center. [Drawn from 1LGH.pdb.]

The components of photosynthesis are highly organized

The complexity of photosynthesis, seen already in the elaborate interplay of complex components, extends even to the placement of the components in the thylakoid membranes. *Thylakoid membranes of most plants are differentiated into stacked* (appressed) and *unstacked* (nonappressed) *regions* (see Figures 19.2 and 19.3). Stacking increases the amount of thylakoid membrane in a given chloroplast volume. Both regions surround a common internal thylakoid space, but only unstacked regions make direct contact with the chloroplast stroma. Stacked and unstacked regions differ in the nature of their photosynthetic assemblies (Figure 19.31). Photosystem I and ATP synthase are located almost exclusively in unstacked regions, whereas photosystem II is present mostly in stacked regions. The cytochrome *bf* complex is found in both regions. Indeed, this complex rapidly moves back and forth between the stacked and the unstacked regions. Plastoquinone and plastocyanin are the mobile carriers of electrons between assemblies located in different regions of the thylakoid membrane. A common internal thylakoid space enables protons liberated by photosystem II in stacked membranes to be utilized by ATP synthase molecules that are located far away in unstacked membranes.

What is the functional significance of this lateral differentiation of the thylakoid membrane system? The positioning of photosystem I in the unstacked membranes also gives it direct access to the stroma for the reduction of $NADP^+$. ATP synthase, too, is located in the unstacked region to provide space for its large CF_1 globule and to give access to ADP. In contrast, the tight quarters of the appressed region pose no problem for

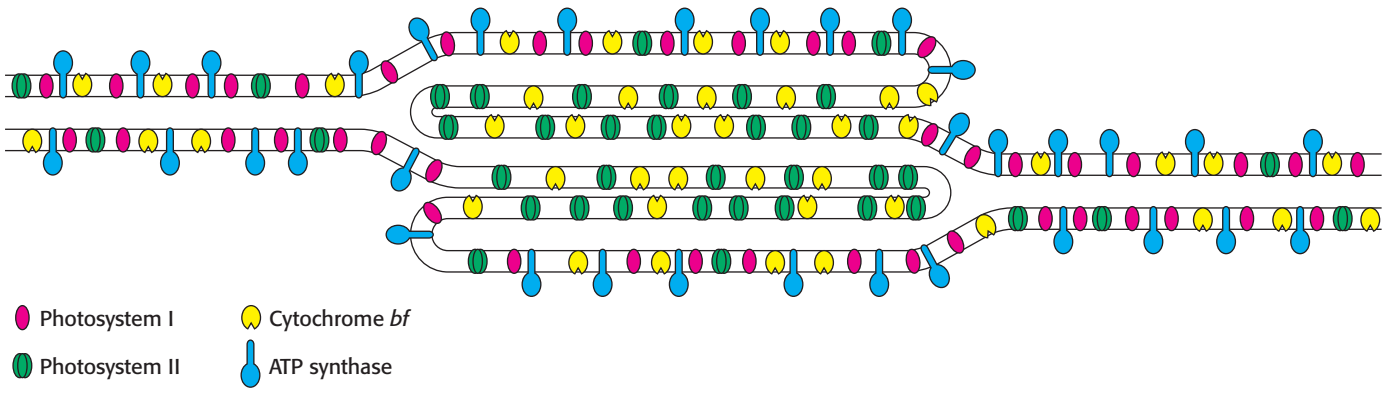
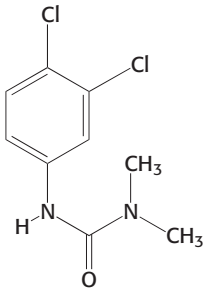


Figure 19.31 Location of photosynthesis components. Photosynthetic assemblies are differentially distributed in the stacked (appressed) and unstacked (nonappressed) regions of thylakoid membranes. [After a drawing kindly provided by Dr. Jan M. Anderson and Dr. Bertil Andersson.]

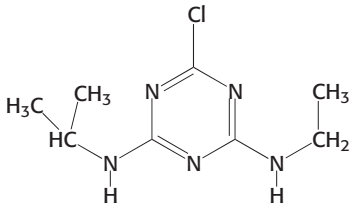
photosystem II, which interacts with a small polar electron donor (H_2O) and a highly lipid soluble electron carrier (plastoquinone).

Many herbicides inhibit the light reactions of photosynthesis

Many commercial herbicides kill weeds by interfering with the action of photosystem II or photosystem I. Inhibitors of photosystem II block electron flow, whereas inhibitors of photosystem I divert electrons from the terminal part of this photosystem. Photosystem II inhibitors include urea derivatives such as *diuron* and triazine derivatives such as *atrazine*. These chemicals bind to the Q_B site of the D1 subunit of photosystem II and block the formation of plastoquinol (QH_2).



Diuron



Atrazine

Paraquat (1,1′-dimethyl-4-4′-bipyridinium) is an inhibitor of photosystem I. Paraquat, a dication, can accept electrons from photosystem I to become a radical. This radical reacts with O_2 to produce reactive oxygen species such as superoxide (O_2^-) and hydroxyl radical (OH•). Such reactive oxygen species react with double bonds in membrane lipids, damaging the membrane.

19.6 The Ability to Convert Light into Chemical Energy Is Ancient

The ability to convert light energy into chemical energy is a tremendous evolutionary advantage. Geological evidence suggests that oxygenic photosynthesis became important approximately 2 billion years ago. Anoxygenic photosynthetic systems arose much earlier in the 3.5-billion-year history of life on Earth (Table 19.1). The photosynthetic system of the nonsulfur purple bacterium *Rhodopseudomonas viridis* has most features common to oxygenic photosynthetic systems and clearly predates them. Green sulfur bacteria such as *Chlorobium thiosulfatophilum* carry out a reaction that also seems to have appeared before oxygenic photosynthesis and is even more similar to oxygenic photosynthesis than *R. viridis* is. Reduced sulfur species such as H_2S are electron donors in the overall photosynthetic reaction:

$$CO_2 + 2\,H_2S \xrightarrow{\text{Light}} (CH_2O) + 2\,S + H_2O$$

Nonetheless, photosynthesis did not evolve immediately at the origin of life. No photosynthetic organisms have been discovered in the domain of Archaea, implying that photosynthesis evolved in the domain of Bacteria after Archaea and Bacteria diverged from a common ancestor. All domains of life do have electron-transport chains in common, however. As we have seen, components such as the ubiquinone–cytochrome *c* oxidoreductase and cytochrome *bf* family are present in both respiratory and photosynthetic electron-transport chains. These components were the foundations on which light-energy-capturing systems evolved.

Table 19.1 Major groups of photosynthetic prokaryotes

Bacteria	Photosynthetic electron donor	O_2 use
Green sulfur	H_2, H_2S, S	Anoxygenic
Green nonsulfur	Variety of amino acids and organic acids	Anoxygenic
Purple sulfur	H_2, H_2S, S	Anoxygenic
Purple nonsulfur	Usually organic molecules	Anoxygenic
Cyanobacteria	H_2O	Oxygenic

Summary

19.1 Photosynthesis Takes Place in Chloroplasts

The proteins that participate in the light reactions of photosynthesis are located in the thylakoid membranes of chloroplasts. The light reactions result in (1) the creation of reducing power for the production of NADPH, (2) the generation of a transmembrane proton gradient for the formation of ATP, and (3) the production of O_2.

19.2 Light Absorption by Chlorophyll Induces Electron Transfer

Chlorophyll molecules—tetrapyrroles with a central magnesium ion—absorb light quite efficiently because they are polyenes. An electron excited to a high-energy state by the absorption of a photon can move to nearby electron acceptors. In photosynthesis, an excited electron leaves a pair of associated chlorophyll molecules known as the special pair. The functional core of photosynthesis, a reaction center, from a photosynthetic bacterium has been studied in great detail. In this system, the electron moves from the special pair (containing bacteriochlorophyll) to a bacteriopheophytin (a bacteriochlorophyll lacking the central magnesium ion) to quinones. The reduction of quinones leads to the generation of a proton gradient, which drives ATP synthesis in a manner analogous to that of oxidative phosphorylation.

19.3 Two Photosystems Generate a Proton Gradient and NADPH in Oxygenic Photosynthesis

Photosynthesis in green plants is mediated by two linked photosystems. In photosystem II, the excitation of a special pair of chlorophyll molecules called P680 leads to electron transfer to plastoquinone in a manner analogous to that of the bacterial reaction center. The electrons are replenished by the extraction of electrons from a water molecule at a center containing four manganese ions. One molecule of O_2 is generated at this center for each four electrons transferred. The plastoquinol produced at photosystem II is reoxidized by the cytochrome *bf* complex, which transfers the electrons to plastocyanin, a soluble copper protein. From plastocyanin, the electrons enter photosystem I. In photosystem I, the excitation of special pair P700 releases electrons that flow to ferredoxin, a powerful reductant. Ferredoxin–$NADP^+$ reductase, a flavoprotein located on the stromal side of the membrane, then catalyzes the formation of NADPH. A proton gradient is generated as electrons pass through photosystem II, through the cytochrome *bf* complex, and through ferredoxin–$NADP^+$ reductase.

19.4 A Proton Gradient Across the Thylakoid Membrane Drives ATP Synthesis

The proton gradient across the thylakoid membrane creates a protonmotive force, used by ATP synthase to form ATP. The ATP synthase of chloroplasts (also called CF_0–CF_1) closely resembles the ATP-synthesizing assemblies of bacteria and mitochondria (F_0–F_1). If the NADPH:$NADP^+$ ratio is high, electrons transferred to ferredoxin by photosystem I can reenter the cytochrome *bf* complex. This process, called cyclic photophosphorylation, leads to the generation of a proton gradient by the cytochrome *bf* complex without the formation of NADPH or O_2.

19.5 Accessory Pigments Funnel Energy into Reaction Centers

Light-harvesting complexes that surround the reaction centers contain additional molecules of chlorophyll *a*, as well as carotenoids and chlorophyll *b* molecules, which absorb light in the center of the visible

spectrum. These accessory pigments increase the efficiency of light capture by absorbing light and transferring the energy to reaction centers through resonance energy transfer.

19.6 The Ability to Convert Light into Chemical Energy Is Ancient

The photosystems have structural features in common that suggest a common evolutionary origin. Similarities in organization and molecular structure to those of oxidative phosphorylation suggest that the photosynthetic apparatus evolved from an early energy-transduction system.

Key Terms

light reactions (p. 566)
chloroplast (p. 567)
stroma (p. 567)
thylakoid (p. 567)
granum (p. 567)
chlorophyll *a* (p. 568)
photoinduced charge separation (p. 569)
reaction center (p. 569)
special pair (p. 571)
P960 (p. 571)
photosystem I (PS I) (p. 572)
photosystem II (PS II) (p. 572)
P680 (p. 573)
manganese center (p. 574)
cytochrome *bf* (p. 575)
P700 (p. 576)
Z scheme of photosynthesis (p. 577)
proton-motive force (p. 578)
ATP synthase (CF_1–CF_0 complex) (p. 578)
cyclic photophosphorylation (p. 580)
carotenoid (p. 582)
light-harvesting complex (p. 583)

Problems

1. *Complementary powers.* Photosystem I produces a powerful reductant, whereas photosystem II produces a powerful oxidant. Identify the reductant and oxidant and describe their roles.

2. *If a little is good.* What is the advantage of having an extensive set of thylakoid membranes in the chloroplasts?

3. *Cooperation.* Explain how light-harvesting complexes enhance the efficiency of photosynthesis.

4. *One thing leads to another.* What is the ultimate electron acceptor in photosynthesis? The ultimate electron donor? What powers the electron flow between the donor and the acceptor?

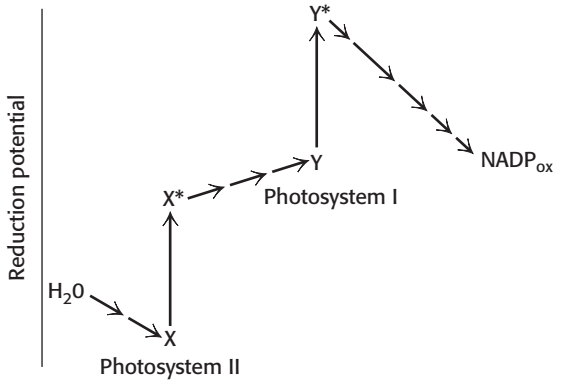
5. *Neutralization compensation.* In chloroplasts, a greater pH gradient across the thylakoid membrane is required to power the synthesis of ATP than is required across the mitochondrial inner membrane. Explain this difference.

6. *Environmentally appropriate.* Chlorophyll is a hydrophobic molecule. Why is this property crucial for the function of chlorophyll?

7. *Proton origins.* What are the various sources of protons that contribute to the generation of a proton gradient in chloroplasts?

8. *Efficiency matters.* What fraction of the energy of 700-nm light absorbed by photosystem I is trapped as high-energy electrons?

9. *That's not right.* Explain the defect or defects in the hypothetical scheme for the light reactions of photosynthesis depicted here.



10. *Electron transfer.* Calculate the $\Delta E'_0$ and $\Delta G^{\circ\prime}$ for the reduction of $NADP^+$ by ferredoxin. Use data given in Table 18.1.

11. *To boldly go.* (a) It can be argued that, if life were to exist elsewhere in the universe, it would require some process like photosynthesis. Why is this argument reasonable? (b) If the *Enterprise* were to land on a distant plant and find no measurable oxygen in the atmosphere, could the crew conclude that photosynthesis is not taking place?

12. *Weed killer 1.* Dichlorophenyldimethylurea (DCMU), a herbicide, interferes with photophosphorylation and O_2 evolution. However, it does not block O_2 evolution in the presence of an artificial electron acceptor such as ferricyanide. Propose a site for the inhibitory action of DCMU.

13. *Weed killer 2.* Predict the effect of the herbicide dichlorophenyldimethylurea (DCMU) on a plant's ability to perform cyclic photophosphorylation.

14. *Infrared harvest.* Consider the relation between the energy of a photon and its wavelength.

(a) Some bacteria are able to harvest 1000-nm light. What is the energy (in kilojoules or kilocalories) of a mole (also called an einstein) of 1000-nm photons?

(b) What is the maximum increase in redox potential that can be induced by a 1000-nm photon?

(c) What is the minimum number of 1000-nm photons needed to form ATP from ADP and P_i? Assume a ΔG of 50 kJ mol^{-1} (12 kcal mol^{-1}) for the phosphorylation reaction.

15. *Missing acceptors.* Suppose that a bacterial reaction center containing only the special pair and the quinones has been prepared. Given the separation of 22 Å between the special pair and the closest quinone, estimate the rate of electron transfer between the excited special pair and this quinone.

16. *Close approach.* Suppose that energy transfer between two chlorophyll *a* molecules separated by 10 Å takes place in 10 picoseconds. Suppose that this distance is increased to 20 Å with all other factors remaining the same. How long would energy transfer take?

Chapter Integration Problems

17. *Functional equivalents.* What structural feature of mitochondria corresponds to the thylakoid membranes?

18. *Compare and contrast.* Compare and contrast oxidative phosphorylation and photosynthesis.

19. *Energy accounts.* On page 580, the balance sheet for the cost of the photosynthetically powered synthesis of glucose is presented. Eighteen molecules of ATP are required. Yet, when glucose undergoes combustion in cellular respiration, 30 molecules of ATP are produced. Account for the difference.

20. *Looking for a place to rest.* Albert Szent-Györgyi, Nobel Prize–winning biochemist, once said something to the effect: Life is nothing more than an electron looking for a place to rest. Explain how this pithy statement applies to photosynthesis and cellular respiration.

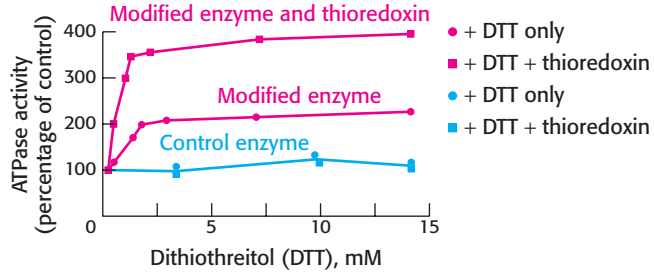
Mechanism Problem

21. *Hill reaction.* In 1939, Robert Hill discovered that chloroplasts evolve O_2 when they are illuminated in the presence of an artificial electron acceptor such as ferricyanide $[Fe^{3+}(CN)_6]^{3-}$. Ferricyanide is reduced to ferrocyanide $[Fe^{2+}(CN)_6]^{4-}$ in this process. No NADPH or reduced plastocyanin is produced. Propose a mechanism for the Hill reaction.

Data Interpretation and Chapter Integration Problem

22. *The same, but different.* The $\alpha_3\beta_3\gamma$ complex of mitochondrial or chloroplast ATP synthase will function as an ATPase in vitro. The chloroplast enzyme (both synthase and ATPase activity) is sensitive to redox control, whereas the mitochondrial enzyme is not. To determine where the enzymes differ, a segment of the mitochondrial γ subunit was removed and replaced with the equivalent segment from the chloroplast γ subunit. The ATPase activity of the modified enzyme was then measured as a function of redox conditions.

(a) What is the redox regulator of the ATP synthase in vivo? The adjoining graph shows the ATPase activity of modified and control enzymes under various redox conditions.



[Data from O. Bald et al. *J. Biol. Chem.* 275:12757–12762, 2000.]

(b) What is the effect of increasing the reducing power of the reaction mixture for the control and the modified enzymes?

(c) What is the effect of the addition of thioredoxin? How do these results differ from those in the presence of DTT alone? Suggest a possible explanation for the difference.

(d) Did the researchers succeed in identifying the region of the γ subunit responsible for redox regulation?

(e) What is the biological rationale of regulation by high concentrations of reducing agents?

(f) What amino acids in the γ subunit are most likely affected by the reducing conditions?

(g) What experiments might confirm your answer to part *e*?

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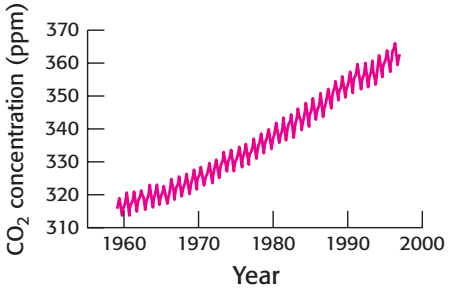
CHAPTER 20

The Calvin Cycle and the Pentose Phosphate Pathway



Atmospheric carbon dioxide measurements at Mauna Loa, Hawaii. These measurements show annual cycles resulting from seasonal variation in carbon dioxide fixation by the Calvin cycle in terrestrial plants. Much of this fixation takes place in rain forests, which account for approximately 50% of terrestrial fixation. [Dennis Potokar/Photo Researchers.]

Photosynthesis proceeds in two parts: the light reactions and the dark reactions. The light reactions, discussed in Chapter 19, transform light energy into ATP and biosynthetic reducing power, NADPH. The dark reactions use the ATP and NADPH produced by the light reactions to reduce carbon atoms from their fully oxidized state as carbon dioxide to a more reduced state as a hexose. Carbon dioxide is thereby trapped in a form that is useful for many processes and most especially as a fuel. Together, *the light reactions and dark reactions of photosynthesis cooperate to transform light energy into carbon fuel*. The dark reactions are also called the *Calvin cycle,* after Melvin Calvin, the biochemist who elucidated the pathway. The components of the Calvin cycle are called the dark reactions because, in contrast with the light reactions, these reactions do not directly depend on the presence of light.

The second half of this chapter examines a pathway common to all organisms, known variously as the pentose phosphate pathway, the hexose monophosphate pathway, the phosphogluconate pathway, or the pentose shunt. The pathway provides a means by which glucose can be oxidized to generate NADPH, *the currency of readily available reducing power in cells*. The phosphoryl group on the 2′-hydroxyl group of one of the ribose units of NADPH distinguishes NADPH from NADH. *There is a fundamental distinction between NADPH and NADH in biochemistry: NADH is oxidized*

OUTLINE

by the respiratory chain to generate ATP, whereas NADPH serves as a reductant in biosynthetic processes. The pentose phosphate pathway can also be used for the catabolism of pentose sugars from the diet, the synthesis of pentose sugars for nucleotide biosynthesis, and the catabolism and synthesis of less common four- and seven-carbon sugars. The pentose phosphate pathway and the Calvin cycle have in common several enzymes and intermediates that attest to an evolutionary kinship. Like glycolysis and gluconeogenesis, these pathways are mirror images of each other: the Calvin cycle uses NADPH to reduce carbon dioxide to generate hexoses, whereas the pentose phosphate pathway breaks down glucose into carbon dioxide to generate NADPH.

20.1 The Calvin Cycle Synthesizes Hexoses from Carbon Dioxide and Water

As stated in Chapter 16, glucose can be formed from noncarbohydrate precursors, such as lactate and amino acids, by gluconeogenesis. The energy powering gluconeogenesis ultimately comes from previous catabolism of carbon fuels. In contrast, photosynthetic organisms can use the Calvin cycle to synthesize glucose from carbon dioxide gas and water, by using sunlight as an energy source. The Calvin cycle introduces into life all of the carbon atoms that will be used as fuel and as the carbon backbones of biomolecules. Photosynthetic organisms are called *autotrophs* (literally, "self-feeders") because they can convert sunlight into chemical energy, which they subsequently use to power their biosynthetic processes. Organisms that obtain energy from chemical fuels only are called *heterotrophs*, and such organisms ultimately depend on autotrophs for their fuel.

The Calvin cycle comprises three stages (Figure 20.1):

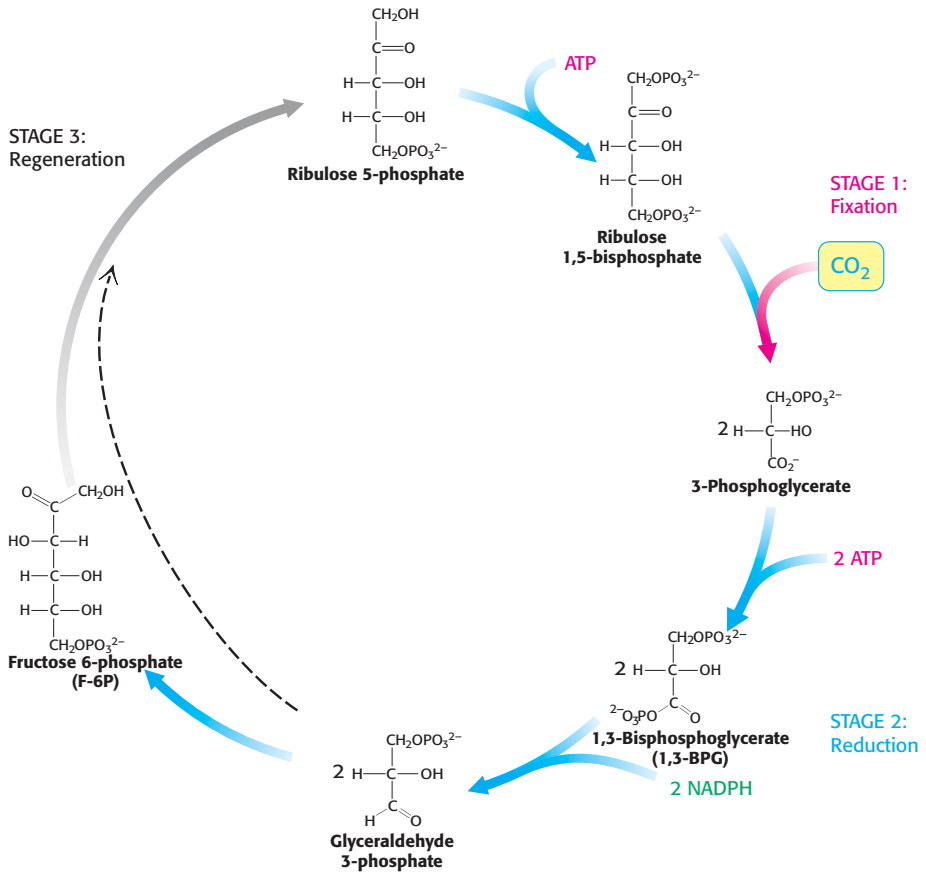


Figure 20.1 Calvin cycle. The Calvin cycle consists of three stages. Stage 1 is the fixation of carbon by the carboxylation of ribulose 1,5-bisphosphate. Stage 2 is the reduction of the fixed carbon to begin the synthesis of hexose. Stage 3 is the regeneration of the starting compound, ribulose 1,5-bisphosphate.

1. The fixation of CO_2 by ribulose 1,5-bisphosphate to form two molecules of 3-phosphoglycerate;

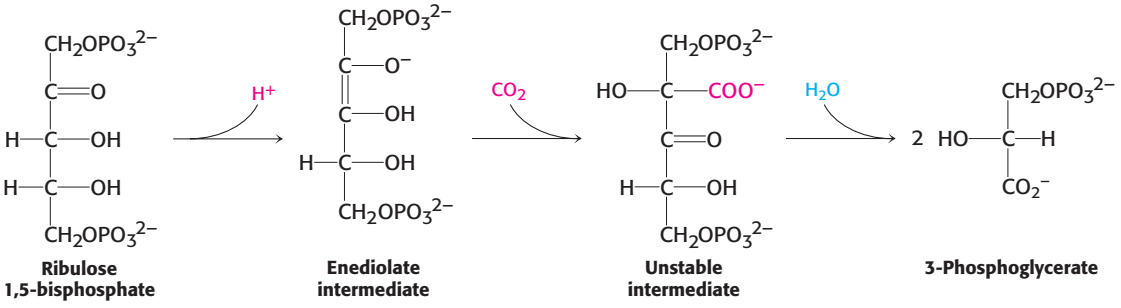
2. The reduction of 3-phosphoglycerate to form hexose sugars; and

3. The regeneration of ribulose 1,5-bisphosphate so that more CO_2 can be fixed.

This set of reactions takes place in the stroma of chloroplasts, the photosynthetic organelles.

Carbon dioxide reacts with ribulose 1,5-bisphosphate to form two molecules of 3-phosphoglycerate

The first step in the Calvin cycle is the fixation of CO_2. This begins with the conversion of ribulose 1,5-bisphosphate into a highly reactive enediol intermediate. The CO_2 molecule condenses with the enediol intermediate to form an unstable six-carbon compound, which is rapidly hydrolyzed to two molecules of 3-phosphoglycerate.



This highly exergonic reaction [$\Delta G^{\circ\prime} = -51.9$ kJ mol^{-1} (-12.4 kcal mol^{-1})] is catalyzed by *ribulose 1,5-bisphosphate carboxylase/oxygenase* (usually called *rubisco*), an enzyme located on the stromal surface of the thylakoid membranes of chloroplasts. This important reaction is the rate-limiting step in hexose synthesis. Rubisco in chloroplasts consists of eight large (L, 55-kd) subunits and eight small (S, 13-kd) ones (Figure 20.2). Each L chain contains a catalytic site and a regulatory site. The S chains enhance the catalytic activity of the L chains. This enzyme is abundant in chloroplasts, accounting for approximately 30% of the total leaf protein in some plants. In fact, rubisco is the most abundant enzyme and probably the most abundant protein in the biosphere. Large amounts are present because rubisco is a slow enzyme; its maximal catalytic rate is only 3 s^{-1}.

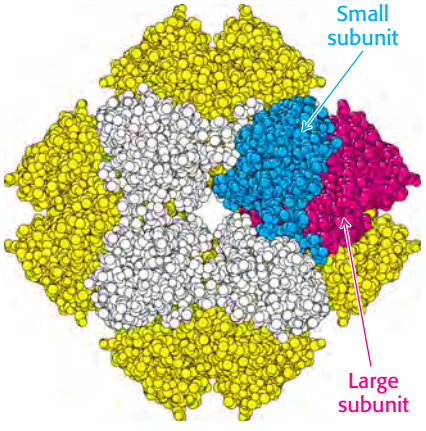
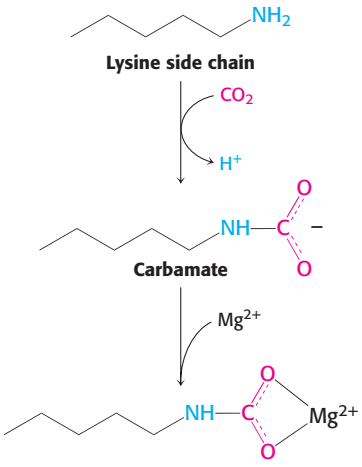


Figure 20.2 Structure of rubisco. The enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) comprises eight large subunits (one shown in red and the others in yellow) and eight small subunits (one shown in blue and the others in white). The active sites lie in the large subunits. [Drawn from 1RXO.pdb.]



Rubisco activity depends on magnesium and carbamate

Rubisco requires a bound divalent metal ion for activity, usually magnesium ion. Like the zinc ion in the active site of carbonic anhydrase (Section 9.2), this metal ion serves to activate a bound substrate molecule by stabilizing a negative charge. Interestingly, a CO_2 molecule other than the substrate is required to complete the assembly of the Mg^{2+}-binding site in rubisco. This CO_2 molecule adds to the uncharged ε-amino group of lysine 201 to form a *carbamate*. This negatively charged adduct then binds the Mg^{2+} ion. The formation of the carbamate is facilitated by the enzyme *rubisco activase*, although it will also form spontaneously at a lower rate.

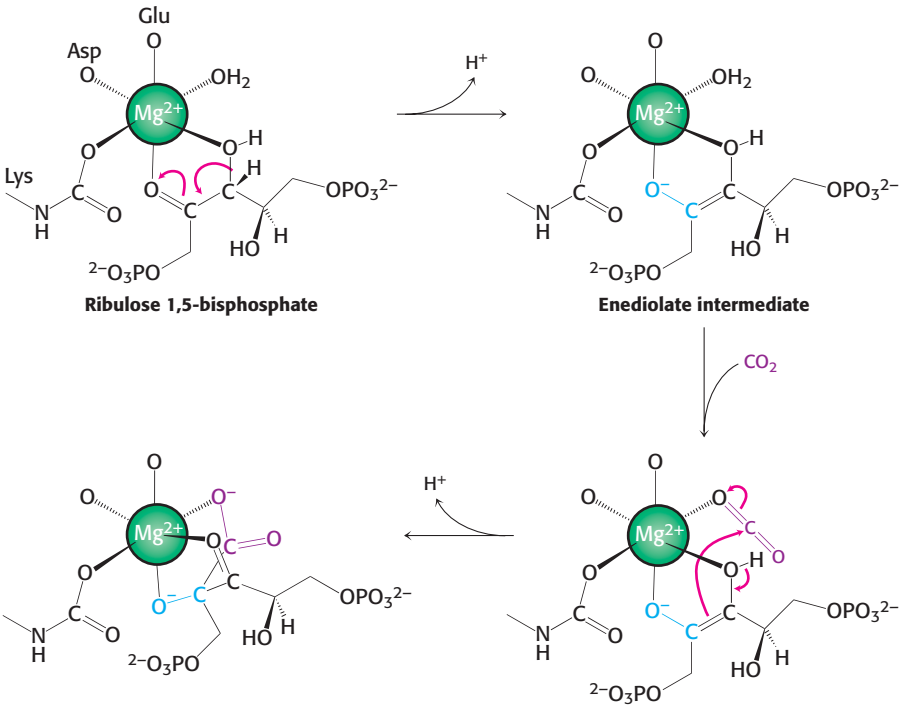


Figure 20.3 Role of the magnesium ion in the rubisco mechanism. Ribulose 1,5-bisphosphate binds to a magnesium ion that is linked to rubisco through a glutamate residue, an aspartate residue, and the lysine carbamate. The coordinated ribulose 1,5-bisphosphate gives up a proton to form a reactive enediolate species that reacts with CO_2 to form a new carbon–carbon bond.

The metal center plays a key role in binding ribulose 1,5-bisphosphate and activating it so that it will react with CO_2 (Figure 20.3). Ribulose 1,5-bisphosphate binds to Mg^{2+} through its keto group and an adjacent hydroxyl group. This complex is readily deprotonated to form an enediolate intermediate. This reactive species, analogous to the zinc–hydroxide species in carbonic anhydrase, couples with CO_2, forming the new carbon–carbon bond. The resulting product is coordinated to the Mg^{2+} ion through three groups, including the newly formed carboxylate. A molecule of H_2O is then added to this β-ketoacid to form an intermediate that cleaves to form two molecules of 3-phosphoglycerate (Figure 20.4).

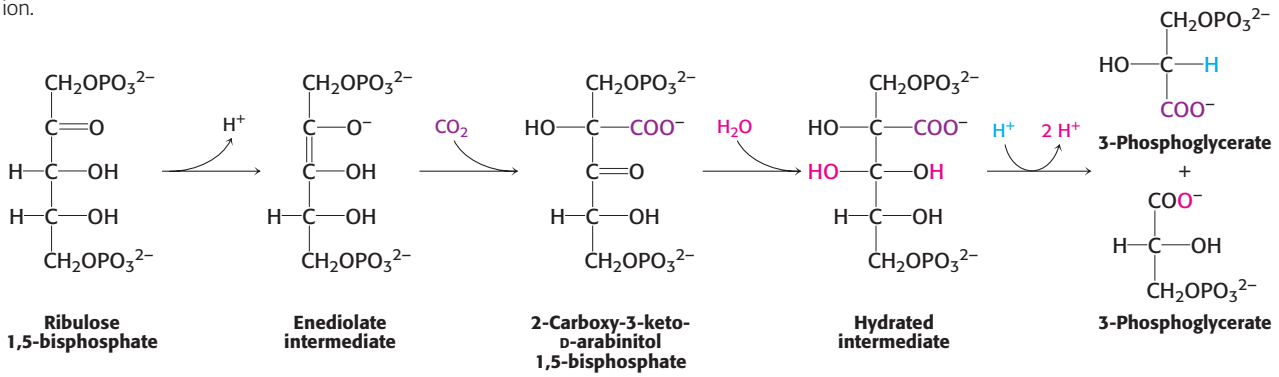


Figure 20.4 Formation of 3-phosphoglycerate. The overall pathway for the conversion of ribulose 1,5 bisphosphate and CO_2 into two molecules of 3-phosphoglycerate. Although the free species are shown, these steps take place on the magnesium ion.

Rubisco also catalyzes a wasteful oxygenase reaction: Catalytic imperfection

The reactive intermediate generated on the Mg^{2+} ion sometimes reacts with O_2 instead of CO_2. Thus, rubisco also catalyzes a deleterious oxygenase reaction. The products of this reaction are *phosphoglycolate* and *3-phosphoglycerate* (Figure 20.5). The rate of the carboxylase reaction is four times that of the oxygenase reaction under normal atmospheric conditions at 25°C; the stromal concentration of CO_2 is then 10 μM and that of O_2 is 250 μM. The oxygenase reaction, like the carboxylase reaction, requires that lysine 201 be in the carbamate form. Because this carbamate forms only in the presence of CO_2, rubisco is prevented from catalyzing the oxygenase reaction exclusively when CO_2 is absent.

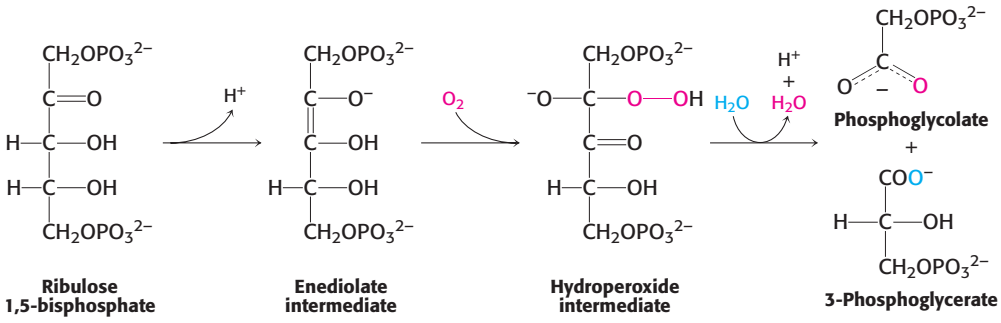


Figure 20.5 A wasteful side reaction. The reactive enediolate intermediate on rubisco also reacts with molecular oxygen to form a hydroperoxide intermediate, which then proceeds to form one molecule of 3-phosphoglycerate and one molecule of phosphoglycolate.

Phosphoglycolate is not a versatile metabolite. A salvage pathway recovers part of its carbon skeleton (Figure 20.6). A specific phosphatase converts phosphoglycolate into *glycolate,* which enters *peroxisomes* (also called *microbodies;* Figure 20.7). Glycolate is then oxidized to *glyoxylate*

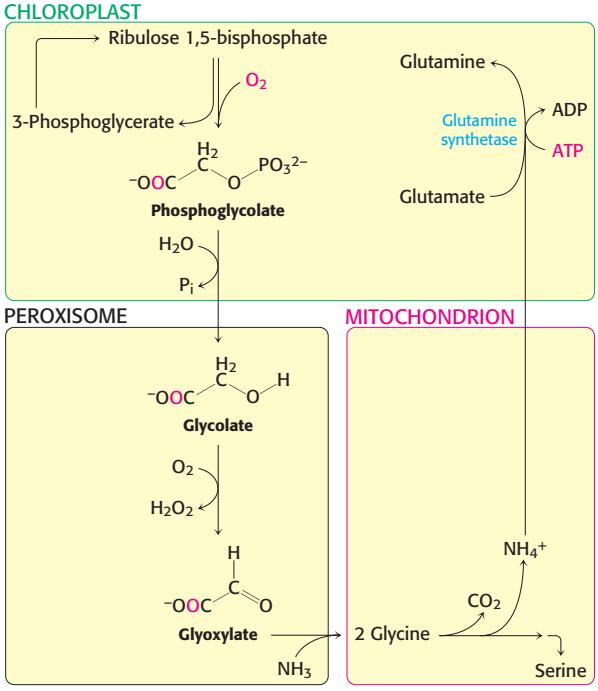


Figure 20.6 Photorespiratory reactions. Phosphoglycolate is formed as a product of the oxygenase reaction in chloroplasts. After dephosphorylation, glycolate is transported into peroxisomes where it is converted into glyoxylate and then glycine. In mitochondria, two glycines are converted into serine, after losing a carbon as CO_2 and ammonium ion. The ammonium ion is salvaged in chloroplasts.

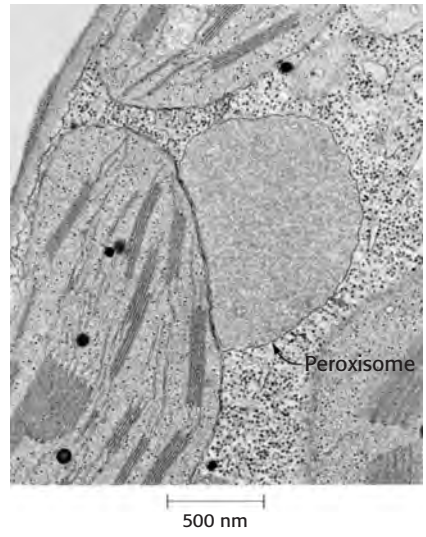


Figure 20.7 Electron micrograph of a peroxisome nestled between two chloroplasts. [Courtesy of Dr. Sue Ellen Frederick.]

by glycolate oxidase, an enzyme with a flavin mononucleotide prosthetic group. The H_2O_2 produced in this reaction is cleaved by catalase to H_2O and O_2. Transamination of glyoxylate then yields *glycine*. Two glycine molecules can unite to form serine, a potential precursor of glucose, with the release of CO_2 and ammonium ion (NH_4^+). The ammonium ion, used in the synthesis of nitrogen-containing compounds, is salvaged by a glutamine synthetase reaction (see Figure 20.6 and Section 23.3).

This salvage pathway serves to recycle three of the four carbon atoms of two molecules of glycolate. However, one carbon atom is lost as CO_2. This process is called *photorespiration* because O_2 is consumed and CO_2 is released. Photorespiration is wasteful because organic carbon is converted into CO_2 without the production of ATP, NADPH, or another energy-rich metabolite. Indeed, photorespiration accounts for the loss of up to 25% of the carbon fixed. Evolutionary processes have presumably enhanced the preference of rubisco for carboxylation. For instance, the rubisco of higher plants is eightfold as specific for carboxylation as that of photosynthetic bacteria.

Much research has been undertaken to generate recombinant forms of rubisco that display reduced oxygenase activity, but all such attempts have failed. This raises the question, what is the biochemical basis of this inefficiency? Structural studies show that when the reactive enediol intermediate is formed, loops close over the active site to protect the enediol. A channel to the environment is maintained to allow access by CO_2. However, like CO_2, O_2 is a linear molecule that also fits the channel. In essence, the problem lies not with the enzyme but in the unremarkable structure of CO_2. CO_2 lacks any chemical features that would allow discrimination between it and other gases such as O_2, and thus the oxygenase activity is an inevitable failing of the enzyme. Another possibility exists, however. The oxygenase activity may not be an imperfection of the enzyme, but rather an imperfection in our understanding. Perhaps the oxygenase activity performs a biochemically important role that we have not yet discovered.

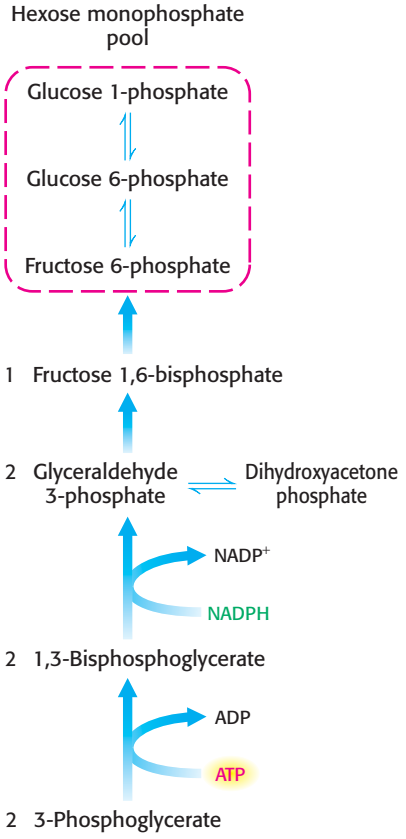
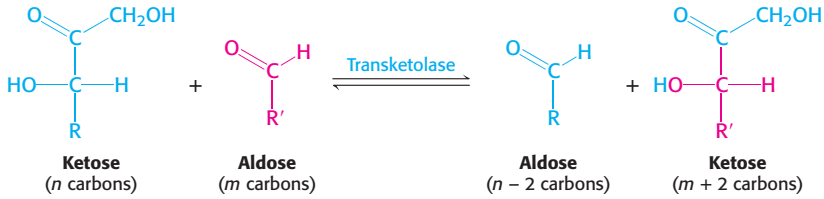


Figure 20.8 Hexose phosphate formation. 3-Phosphoglycerate is converted into fructose 6-phosphate in a pathway parallel to that of gluconeogenesis.

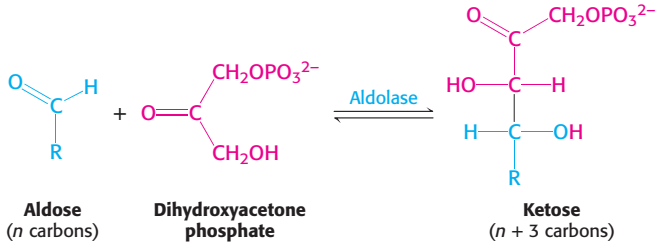
Hexose phosphates are made from phosphoglycerate, and ribulose 1,5-bisphosphate is regenerated

The 3-phosphoglycerate product of rubisco is next converted into fructose 6-phosphate, which readily isomerizes to glucose 1-phosphate and glucose 6-phosphate. The mixture of the three phosphorylated hexoses is called the *hexose monophosphate pool*. The steps in this conversion (Figure 20.8) are like those of the gluconeogenic pathway (see Figure 16.24), except that glyceraldehyde 3-phosphate dehydrogenase in chloroplasts, which generates glyceraldehyde 3-phosphate (GAP), is specific for NADPH rather than NADH. These reactions and that catalyzed by rubisco bring CO_2 to the level of a hexose, converting CO_2 into a chemical fuel at the expense of NADPH and ATP generated from the light reactions.

The third phase of the Calvin cycle is the regeneration of ribulose 1,5-bisphosphate, the acceptor of CO_2 in the first step. The problem is to construct a five-carbon sugar from six-carbon and three-carbon sugars. A transketolase and an aldolase play the major role in the rearrangement of the carbon atoms. The *transketolase,* which we will see again in the pentose phosphate pathway, requires the coenzyme thiamine pyrophosphate (TPP) to transfer a two-carbon unit ($CO—CH_2OH$) from a ketose to an aldose.



Aldolase, which we have already encountered in glycolysis (Section 16.1), catalyzes an aldol condensation between dihydroxyacetone phosphate (DHAP) and an aldehyde. This enzyme is highly specific for dihydroxyacetone phosphate, but it accepts a wide variety of aldehydes.



With these enzymes, the construction of the five-carbon sugar proceeds as shown in Figure 20.9.

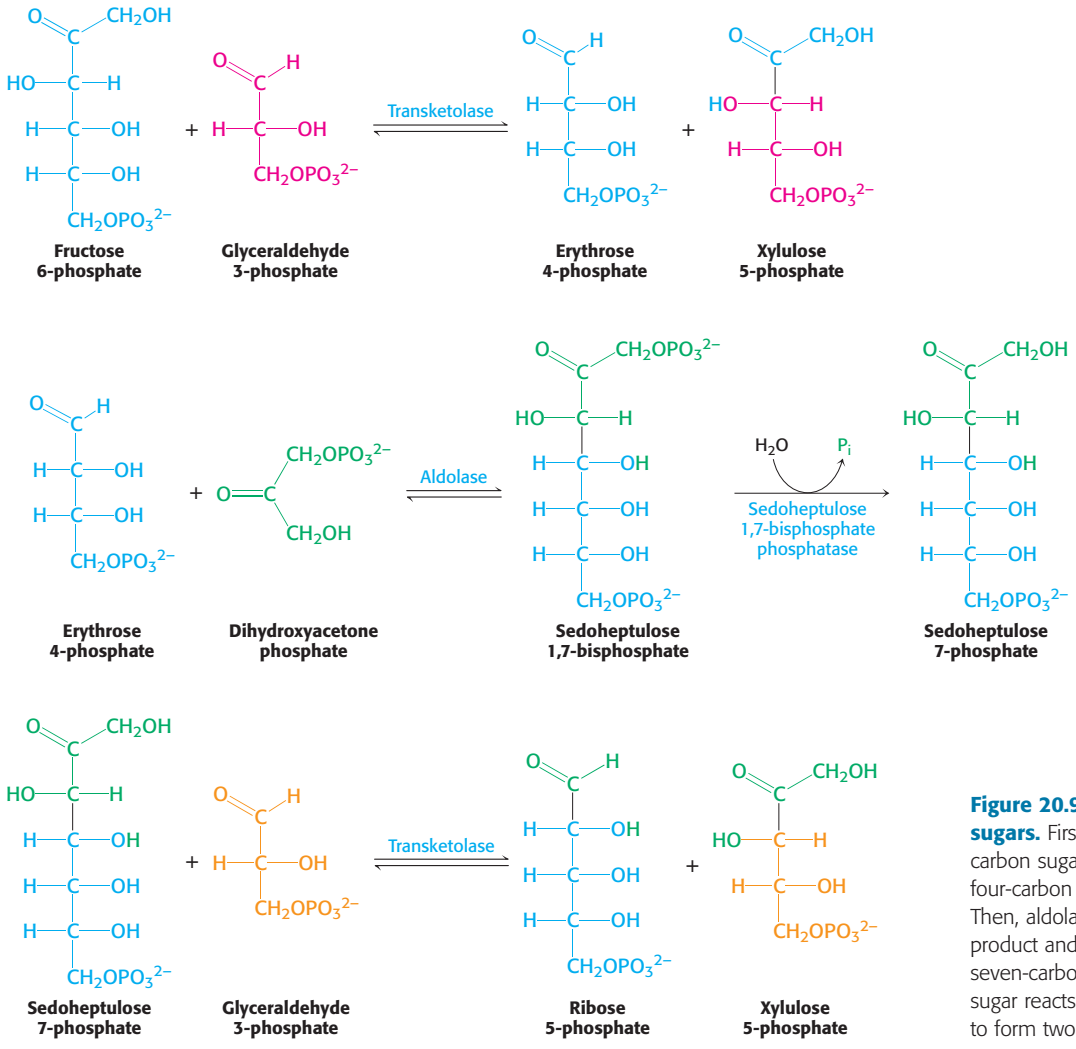


Figure 20.9 Formation of five-carbon sugars. First, transketolase converts a six-carbon sugar and a three-carbon sugar into a four-carbon sugar and a five-carbon sugar. Then, aldolase combines the four-carbon product and a three-carbon sugar to form a seven-carbon sugar. Finally, this seven-carbon sugar reacts with another three-carbon sugar to form two additional five-carbon sugars.

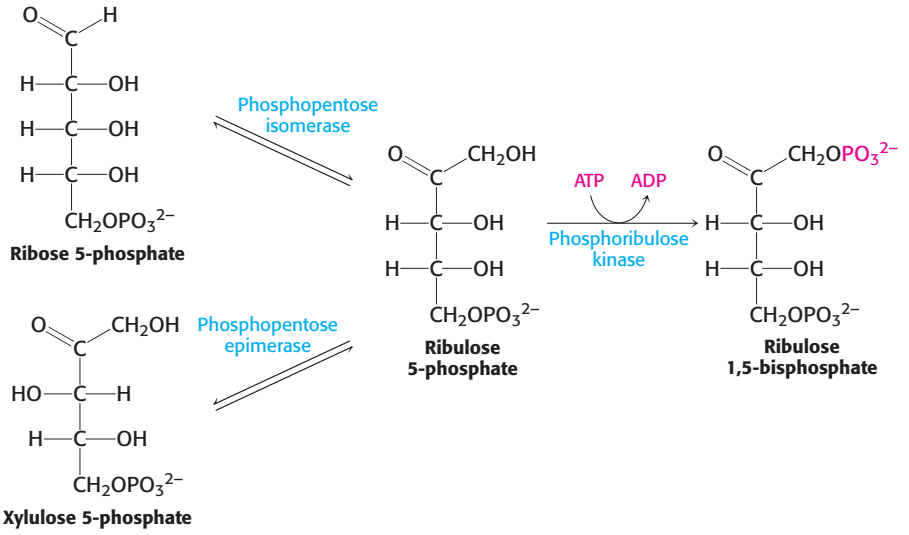


Figure 20.10 Regeneration of ribulose 1,5-bisphosphate. Both ribose 5-phosphate and xylulose 5-phosphate are converted into ribulose 5-phosphate, which is then phosphorylated to complete the regeneration of ribulose 1,5-bisphosphate.

Finally, ribose 5-phosphate is converted into ribulose 5-phosphate by *phosphopentose isomerase,* whereas xylulose 5-phosphate is converted into ribulose 5-phosphate by *phosphopentose epimerase*. Ribulose 5-phosphate is converted into ribulose 1,5-bisphosphate through the action of *phosphoribulose kinase* (Figure 20.10). The sum of these reactions is

Fructose 6-phosphate + 2 glyceraldehyde 3-phosphate
+ dihydroxyacetone phosphate + 3 ATP ⟶
3 ribulose 1,5-bisphosphate + 3 ADP

This series of reactions completes the Calvin cycle (Figure 20.11). Figure 20.11 presents the required reactions with the proper stoichiometry

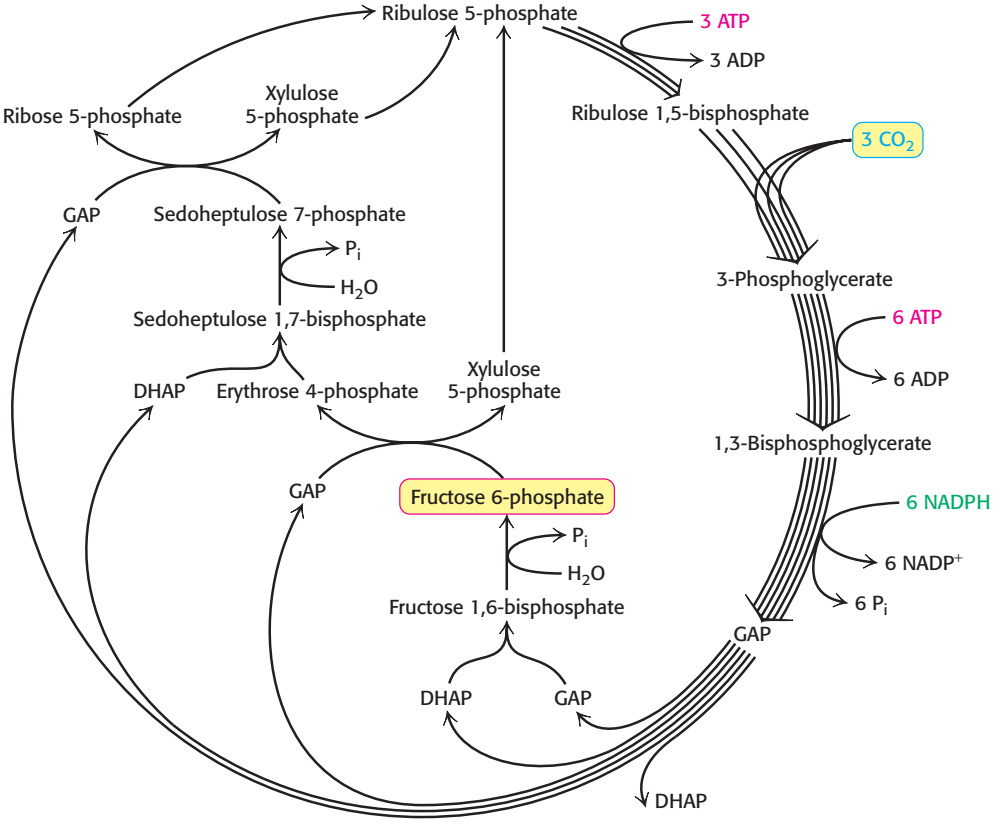


Figure 20.11 Calvin cycle. The diagram shows the reactions necessary with the correct stoichiometry to convert three molecules of CO_2 into one molecule of dihydroxyacetone phosphate (DHAP). The cycle is not as simple as presented in Figure 20.1; rather, it entails many reactions that lead ultimately to the synthesis of glucose and the regeneration of ribulose 1,5-bisphosphate. [After J. R. Bowyer and R. C. Leegood. "Photosynthesis," in *Plant Biochemistry,* P. M. Dey and J. B. Harborne, Eds. (Academic Press, 1997), p. 85.]

to convert three molecules of CO_2 into one molecule of dihydroxyacetone phosphate (DHAP). However, two molecules of DHAP are required for the synthesis of a member of the hexose monophosphate pool. Consequently, the cycle as presented must take place twice to yield a hexose monophosphate. The outcome of the Calvin cycle is the generation of a hexose and the regeneration of the starting compound, ribulose 1,5-bisphosphate. In essence, ribulose 1,5-bisphosphate acts catalytically, similarly to oxaloacetate in the citric acid cycle.

Three ATP and two NADPH molecules are used to bring carbon dioxide to the level of a hexose

What is the energy expenditure for synthesizing a hexose? Six rounds of the Calvin cycle are required, because one carbon atom is reduced in each round. Twelve molecules of ATP are expended in phosphorylating 12 molecules of 3-phosphoglycerate to 1,3-bisphosphoglycerate, and 12 molecules of NADPH are consumed in reducing 12 molecules of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate. An additional six molecules of ATP are spent in regenerating ribulose 1,5-bisphosphate.

We can now write a balanced equation for the net reaction of the Calvin cycle:

$$6\,CO_2 + 18\,ATP + 12\,NADPH + 12\,H_2O \longrightarrow C_6H_{12}O_6 + 18\,ADP + 18\,P_i + 12\,NADP^+ + 6\,H^+$$

Thus, three molecules of ATP and two molecules of NADPH are consumed in incorporating a single CO_2 molecule into a hexose such as glucose or fructose.

Starch and sucrose are the major carbohydrate stores in plants

What are the fates of the members of the hexose monophosphate pool? These molecules are used in a variety of ways, but there are two primary roles. Plants contain two major storage forms of sugar: *starch* and *sucrose*. Starch, like its animal counterpart glycogen, is a polymer of glucose residues, but it is less branched than glycogen because it contains a smaller proportion of α-1,6-glycosidic linkages (Section 11.2). Another difference is that ADP-glucose, not UDP-glucose, is the activated precursor. Starch is synthesized and stored in chloroplasts.

In contrast, sucrose (common table sugar), a disaccharide, is synthesized in the cytoplasm. Plants lack the ability to transport hexose phosphates across the chloroplast membrane, but they are able to transport *triose* phosphates from chloroplasts to the cytoplasm. Triose phosphate intermediates such as glyceraldehyde 3-phosphate cross into the cytoplasm in exchange for phosphate through the action of an abundant phosphate translocator. Fructose 6-phosphate formed from triose phosphates joins the glucose unit of UDP-glucose to form sucrose 6-phosphate (Figure 20.12). The hydrolysis of the phosphate ester yields sucrose, a readily transportable and mobilizable sugar that is stored in many plant cells, as in sugar beets and sugar cane.

20.2 The Activity of the Calvin Cycle Depends on Environmental Conditions

How do the light reactions communicate with the dark reactions to regulate this crucial process of fixing CO_2 into biomolecules? *The principal means of regulation is alteration of the stromal environment by the light reactions*. The light reactions lead to an increase in pH and in the stromal concentrations of

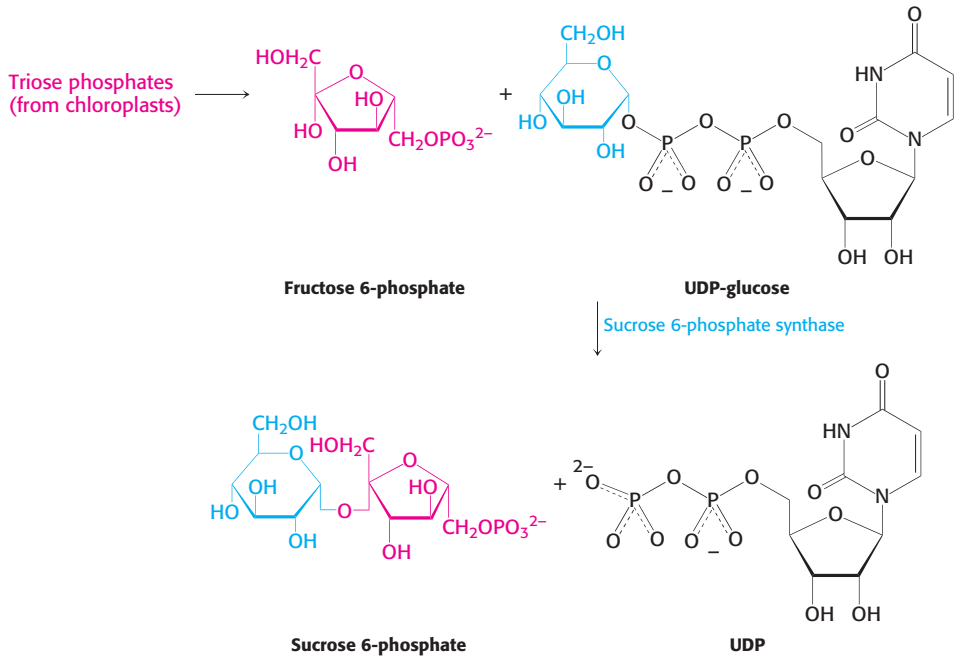


Figure 20.12 Synthesis of sucrose. Sucrose 6-phosphate is formed by the reaction between fructose 6-phosphate and the activated intermediate uridine diphosphate glucose (UDP-glucose).

Mg^{2+}, NADPH, and reduced ferredoxin—all of which contribute to the activation of certain Calvin-cycle enzymes (Figure 20.13).

Rubisco is activated by light-driven changes in proton and magnesium ion concentrations

As stated earlier, the rate-limiting step in the Calvin cycle is the carboxylation of ribulose 1,5-bisphosphate to form two molecules of 3-phosphoglycerate. *The activity of rubisco increases markedly on illumination because light facilitates the carbamate formation necessary to enzyme activity*. In the stroma, the pH increases from 7 to 8, and the level of Mg^{2+} rises. Both effects are consequences of the light-driven pumping of protons into the thylakoid space. Mg^{2+} ions from the thylakoid space are released into the stroma to compensate for the influx of protons. Carbamate formation is favored at alkaline pH. CO_2 adds to a deprotonated from of lysine 201 of rubisco, and Mg^{2+} ion binds to the carbamate to generate the active form of the enzyme. Thus, light leads to the generation of regulatory signals as well as ATP and NADPH.

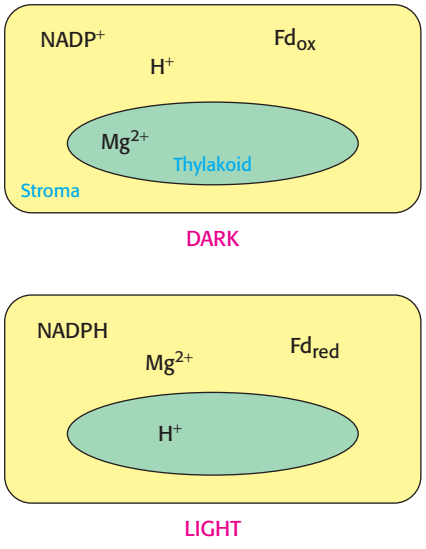


Figure 20.13 Light regulation of the Calvin cycle. The light reactions of photosynthesis transfer electrons out of the thylakoid lumen into the stroma and transfer protons from the stroma into the thylakoid lumen. As a consequence of these processes, the concentrations of NADPH, reduced ferredoxin (Fd), and Mg^{2+} in the stroma are higher in the light than in the dark, whereas the concentration of H^+ is lower in the dark. Each of these concentration changes helps couple the Calvin cycle reactions to the light reactions.

Thioredoxin plays a key role in regulating the Calvin cycle

Light-driven reactions lead to electron transfer from water to ferredoxin and, eventually, to NADPH. The presence of reduced ferredoxin and

Table 20.1 Enzymes regulated by thioredoxin

Enzyme	Pathway
Rubisco	Carbon fixation in the Calvin cycle
Fructose 1,6-bisphosphatase	Gluconeogenesis
Glyceraldehyde 3-phosphate dehydrogenase	Calvin cycle, gluconeogenesis, glycolysis
Sedoheptulose 1,7-bisphosphatase	Calvin cycle
Glucose 6-phosphate dehydrogenase	Pentose phosphate pathway
Phenylalanine ammonia lyase	Lignin synthesis
Phosphoribulose kinase	Calvin cycle
$NADP^+$-malate dehydrogenase	C_4 pathway

NADPH are good signals that conditions are right for biosynthesis. One way in which this information is conveyed to biosynthetic enzymes is by *thioredoxin*, a 12-kd protein containing neighboring cysteine residues that cycle between a reduced sulfhydryl and an oxidized disulfide form (Figure 20.14). The reduced form of thioredoxin activates many biosynthetic enzymes by reducing disulfide bridges that control their activity and inhibits several degradative enzymes by the same means (Table 20.1). In chloroplasts, oxidized thioredoxin is reduced by ferredoxin in a reaction catalyzed by *ferredoxin–thioredoxin reductase*. This enzyme contains a 4Fe-4S cluster that couples two one-electron oxidations of reduced ferredoxin to the two-electron reduction of thioredoxin. Thus, *the activities of the light and dark reactions of photosynthesis are coordinated through electron transfer from reduced ferredoxin to thioredoxin and then to component enzymes containing regulatory disulfide bonds* (Figure 20.15). We shall return to thioredoxin when we consider the reduction of ribonucleotides (Section 25.3).

NADPH is a signal molecule that activates two biosynthetic enzymes, phosphoribulose kinase and glyceraldehyde 3-phosphate dehydrogenase. In the dark, these enzymes are inhibited by association with an 8.5-kd protein called CP12. NADPH disrupts this association, leading to the release of the active enzymes.

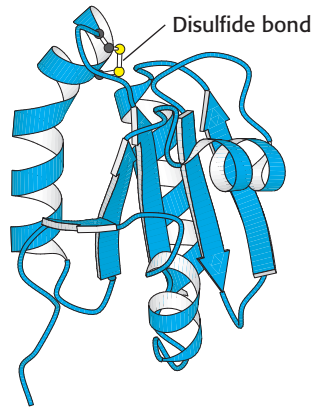


Figure 20.14 Thioredoxin. The oxidized form of thioredoxin contains a disulfide bond. When thioredoxin is reduced by reduced ferredoxin, the disulfide bond is converted into two free sulfhydryl groups. Reduced thioredoxin can cleave disulfide bonds in enzymes, activating certain Calvin cycle enzymes and inactivating some degradative enzymes. [Drawn from 1F9M.pdb.]

The C_4 pathway of tropical plants accelerates photosynthesis by concentrating carbon dioxide

The oxygenase activity of rubisco presents a biochemical challenge to tropical plants because the oxygenase activity increases more rapidly with temperature than does the carboxylase activity. How, then, do plants, such as sugar cane, that grow in hot climates prevent very high rates of wasteful photorespiration? Their solution to this problem is to achieve a high local concentration of CO_2 at the site of the Calvin cycle in their photosynthetic cells. The essence of this process, which was elucidated by Marshall Davidson Hatch and C. Roger Slack, is that *four-carbon (C_4) compounds such as oxaloacetate and malate carry CO_2 from mesophyll cells, which are in contact with air, to bundle-sheath cells, which are the major sites of photosynthesis* (Figure 20.16). The decarboxylation of the four-carbon compound in a bundle-sheath cell maintains a high concentration of CO_2 at the site of the Calvin cycle. The three-carbon product returns to the mesophyll cell for another round of carboxylation.

The *C_4 pathway* for the transport of CO_2 starts in a mesophyll cell with the condensation of CO_2 and phosphoenolpyruvate to form *oxaloacetate* in a reaction catalyzed by *phosphoenolpyruvate carboxylase*. In some species, oxaloacetate is converted into *malate* by an $NADP^+$-linked malate dehydrogenase. Malate enters the bundle-sheath cell and is oxidatively decarboxylated within the chloroplasts by an $NADP^+$-linked malate dehydrogenase. The released CO_2 enters the Calvin cycle in the usual way by condensing with ribulose 1,5-bisphosphate. Pyruvate formed in this decarboxylation reaction returns to the mesophyll cell. Finally, phosphoenolpyruvate is formed from pyruvate by *pyruvate-P_i dikinase*.

The net reaction of this C_4 pathway is

$$CO_2 \text{ (in mesophyll cell)} + ATP + 2\,H_2O \longrightarrow CO_2 \text{ (in bundle-sheath cell)} + AMP + 2\,P_i + 2\,H^+$$

Thus, *the energetic equivalent of two ATP molecules is consumed in transporting CO_2 to the chloroplasts of the bundle-sheath cells*. In essence, this process is active transport: the pumping of CO_2 into the bundle-sheath cell is driven by the hydrolysis of one molecule of ATP to one molecule of AMP

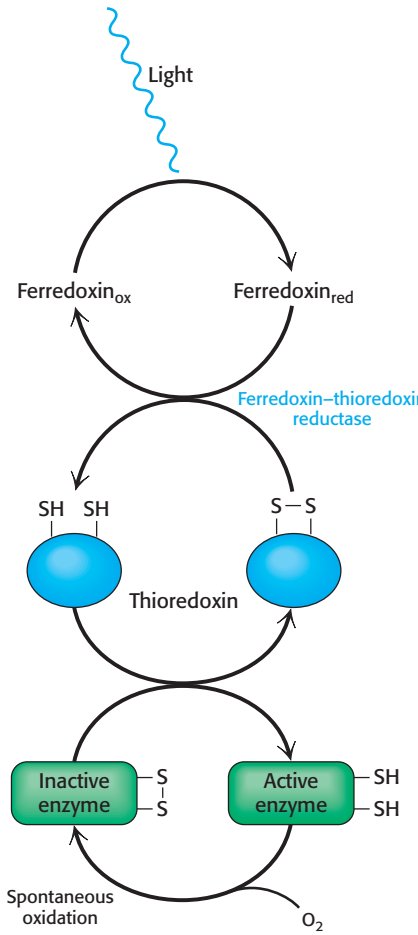


Figure 20.15 Enzyme activation by thioredoxin. Reduced thioredoxin activates certain Calvin cycle enzymes by cleaving regulatory disulfide bonds.

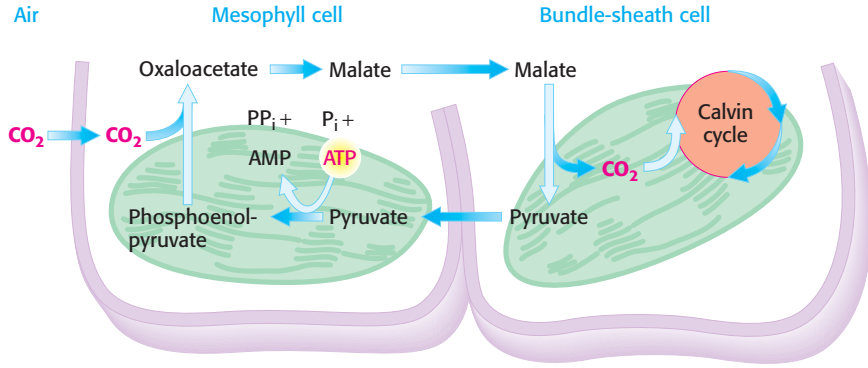


Figure 20.16 C_4 pathway. Carbon dioxide is concentrated in bundle-sheath cells by the expenditure of ATP in mesophyll cells.

and two molecules of orthophosphate. The CO_2 concentration can be 20-fold as great in the bundle-sheath cells as in the mesophyll cells.

When the C_4 pathway and the Calvin cycle operate together, the net reaction is

$$6\,CO_2 + 30\,ATP + 12\,NADPH + 24\,H_2O \longrightarrow C_6H_{12}O_6 + 30\,ADP + 30\,P_i + 12\,NADP^+ + 18\,H^+$$

Note that 30 molecules of ATP are consumed per hexose molecule formed when the C_4 pathway delivers CO_2 to the Calvin cycle, in contrast with 18 molecules of ATP per hexose molecule in the absence of the C_4 pathway. The high concentration of CO_2 in the bundle-sheath cells of C_4 plants, which is due to the expenditure of the additional 12 molecules of ATP, is critical for their rapid photosynthetic rate, because CO_2 is limiting when light is abundant. A high CO_2 concentration also minimizes the energy loss caused by photorespiration.

Tropical plants with a C_4 pathway do little photorespiration because the high concentration of CO_2 in their bundle-sheath cells accelerates the carboxylase reaction relative to the oxygenase reaction. This effect is especially important at higher temperatures. The geographic distribution of plants having this pathway (C_4 plants) and those lacking it (C_3 plants) can now be understood in molecular terms. *C_4 plants* have the advantage in a hot environment and under high illumination, which accounts for their prevalence in the tropics. *C_3 plants,* which consume only 18 molecules of ATP per hexose molecule formed in the absence of photorespiration (compared with 30 molecules of ATP for C_4 plants), are more efficient at temperatures lower than about 28°C, and so they predominate in temperate environments.

Rubisco is present in bacteria, eukaryotes, and even archaea, though other photosynthetic components have not been found in archaea. Thus, rubisco emerged early in evolution, when the atmosphere was rich in CO_2 and almost devoid of O_2. The enzyme was not originally selected to operate in an environment like the present one, which is almost devoid of CO_2 and rich in O_2. Photorespiration became significant about 600 million years ago, when the CO_2 concentration fell to present levels. The C_4 pathway is thought to have evolved in response no more than 30 million years ago and possibly as recently as 7 million years ago. It is interesting that none of the enzymes are unique to C_4 plants, suggesting that this pathway made use of already existing enzymes.

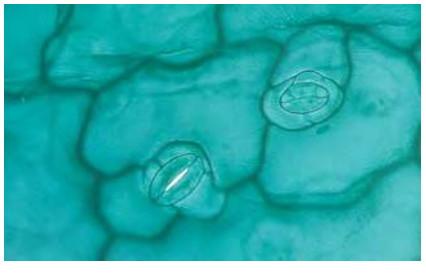


Figure 20.17 Electron micrograph of an open stoma and a closed stoma. [Herb Charles Ohlmeyer/Fran Heyl Associates.]

Crassulacean acid metabolism permits growth in arid ecosystems

Many plants growing in hot, dry climates keep the stomata of their leaves closed in the heat of the day to prevent water loss (Figure 20.17). As a con-

sequence, CO_2 cannot be absorbed during the daylight hours, when it is needed for glucose synthesis. Rather, CO_2 enters the leaf when the stomata open at the cooler temperatures of night. To store the CO_2 until it can be used during the day, such plants make use of an adaptation called *crassulacean acid metabolism* (CAM), named after the genus *Crassulacea* (the succulents). Carbon dioxide is fixed by the C_4 pathway into malate, which is stored in vacuoles. During the day, malate is decarboxylated and the CO_2 becomes available to the Calvin cycle. In contrast with C_4 plants, CAM plants separate CO_2 accumulation from CO_2 utilization temporally rather than spatially.

20.3 The Pentose Phosphate Pathway Generates NADPH and Synthesizes Five-Carbon Sugars

Photosynthetic organisms can use the light reactions for generation of some NADPH. Another pathway, present in all organisms, meets the NADPH needs of nonphotosynthetic organisms and of the nonphotosynthetic tissues in plants. The *pentose phosphate pathway* is a crucial source of NADPH to use in reductive biosynthesis (Table 20.2) as well as for protection against oxidative stress. This pathway consists of two phases: (1) the oxidative generation of NADPH and (2) the nonoxidative interconversion of sugars (Figure 20.18). In the oxidative phase, NADPH is generated when glucose 6-phosphate is oxidized to ribulose 5-phosphate, which is subsequently converted into ribose 5-phosphate. Ribose 5-phosphate and its derivatives are components of RNA and DNA, as well as of ATP, NADH, FAD, and coenzyme A.

Table 20.2 Pathways requiring NADPH

Synthesis
Fatty acid biosynthesis
Cholesterol biosynthesis
Neurotransmitter biosynthesis
Nucleotide biosynthesis
Detoxification
Reduction of oxidized glutathione
Cytochrome P450 monooxygenases

$$\text{Glucose 6-phosphate} + 2\,NADP^+ + H_2O \longrightarrow \text{ribulose 5-phosphate} + 2\,NADPH + 2\,H^+ + CO_2$$

In the nonoxidative phase, the pathway catalyzes the interconversion of three-, four-, five-, six-, and seven-carbon sugars in a series of nonoxidative reactions. Excess five-carbon sugars may be converted into intermediates of the glycolytic pathway. All these reactions take place in the cytoplasm. These interconversions rely on the same reactions that lead to the regeneration of ribulose 1,5-bisphosphate in the Calvin cycle.

Two molecules of NADPH are generated in the conversion of glucose 6-phosphate into ribulose 5-phosphate

The oxidative phase of the pentose phosphate pathway starts with the dehydrogenation of glucose 6-phosphate at carbon 1, a reaction catalyzed by *glucose 6-phosphate dehydrogenase* (Figure 20.19). This enzyme is highly specific for $NADP^+$; the K_M for NAD^+ is about a thousand times as great as that for $NADP^+$. The product is *6-phosphoglucono-δ-lactone,* which is an intramolecular ester between the C-1 carboxyl group and the C-5 hydroxyl group. The next step is the hydrolysis of 6-phosphoglucono-δ-lactone by a specific *lactonase* to give *6-phosphogluconate*. This six-carbon sugar is then oxidatively decarboxylated by *6-phosphogluconate dehydrogenase* to yield *ribulose 5-phosphate*. $NADP^+$ is again the electron acceptor.

The pentose phosphate pathway and glycolysis are linked by transketolase and transaldolase

The preceding reactions yield two molecules of NADPH and one molecule of ribulose 5-phosphate for each molecule of glucose 6-phosphate oxidized.

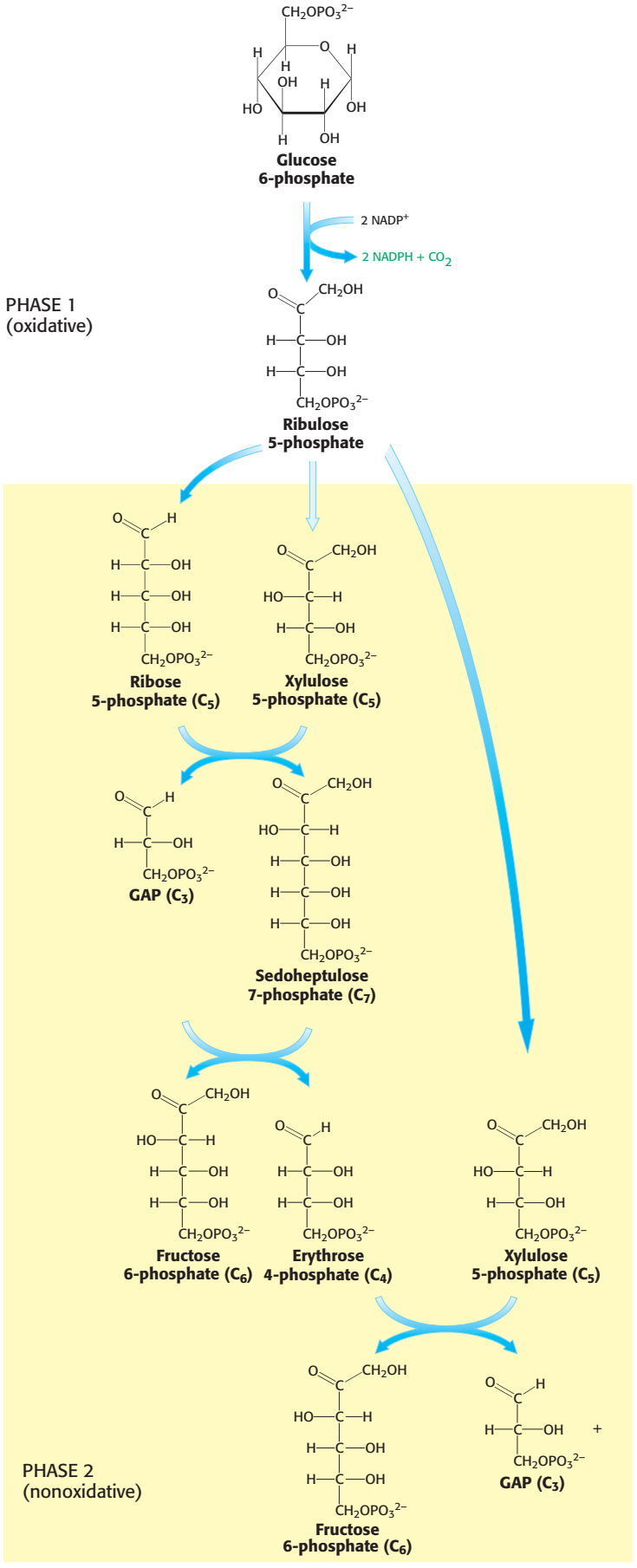
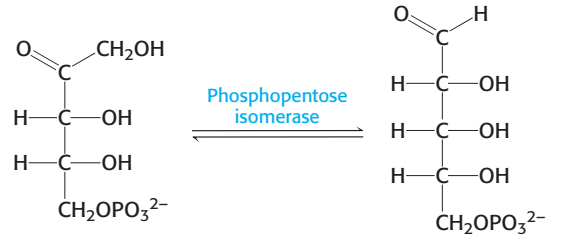


Figure 20.18 Pentose phosphate pathway. The pathway consists of (1) an oxidative phase that generates NADPH and (2) a nonoxidative phase that interconverts phosphorylated sugars.

The ribulose 5-phosphate is subsequently isomerized to ribose 5-phosphate by phosphopentose isomerase.



Ribulose 5-phosphate **Ribose 5-phosphate**

Although ribose 5-phosphate is a precursor to many biomolecules, many cells need NADPH for reductive biosyntheses much more than they need ribose 5-phosphate for incorporation into nucleotides and nucleic acids. For instance, adipose tissue, the liver, and mammary glands require large amounts of NADPH for fatty acid synthesis (Chapter 22). In these cases, ribose 5-phosphate is converted into the glycolytic intermediates glyceraldehyde 3-phosphate and fructose 6-phosphate by *transketolase* and *transaldolase. These enzymes create a reversible link between the pentose phosphate pathway and glycolysis by catalyzing these three successive reactions.*

$$C_5 + C_5 \xrightleftharpoons{\text{Transketolase}} C_3 + C_7$$

$$C_3 + C_7 \xrightleftharpoons{\text{Transketolase}} C_6 + C_4$$

$$C_4 + C_5 \xrightleftharpoons{\text{Transketolase}} C_6 + C_3$$

The net result of these reactions is the *formation of two hexoses and one triose from three pentoses:*

$$3\ C_5 \rightleftharpoons 2\ C_6 + C_3$$

The first of the three reactions linking the pentose phosphate pathway and glycolysis is the formation of *glyceraldehyde 3-phosphate* and *sedoheptulose 7-phosphate* from two pentoses.

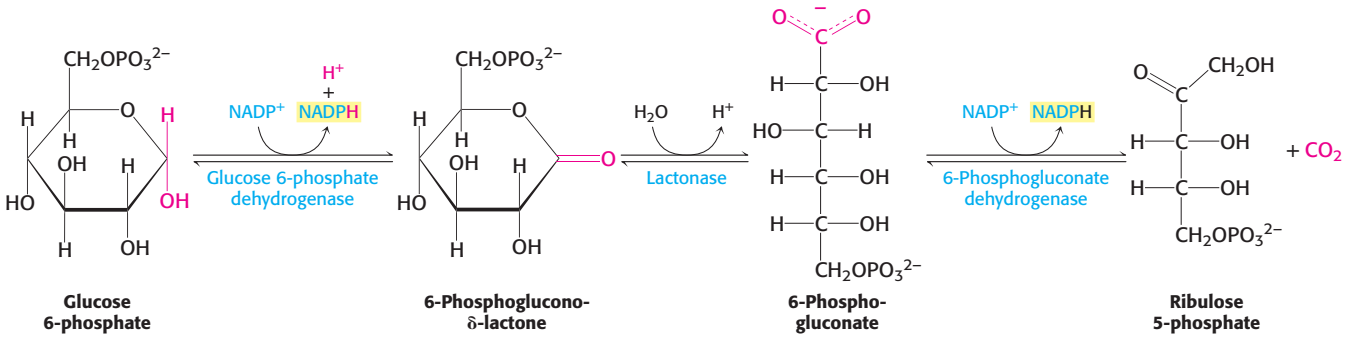
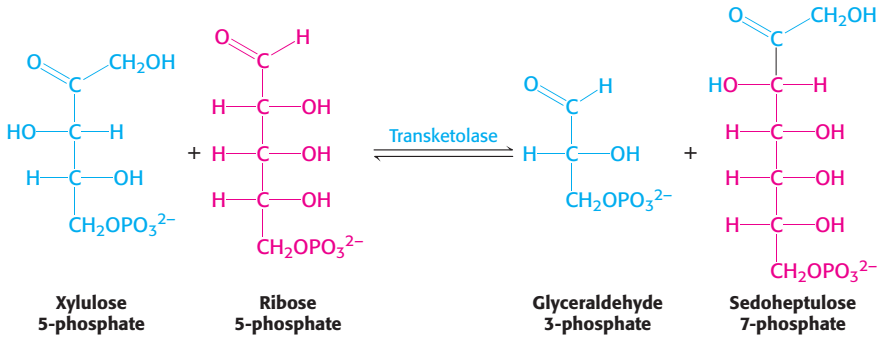
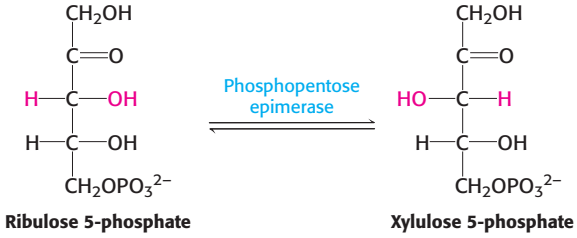


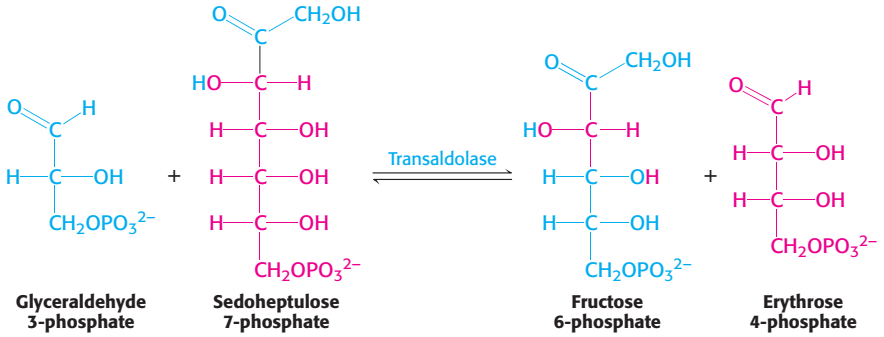
Figure 20.19 Oxidative phase of the pentose phosphate pathway. Glucose 6-phosphate is oxidized to 6-phosphoglucono-δ-lactone to generate one molecule of NADPH. The lactone product is hydrolyzed to 6-phosphogluconate, which is oxidatively decarboxylated to ribulose 5-phosphate with the generation of a second molecule of NADPH.



The donor of the two-carbon unit in this reaction is xylulose 5-phosphate, an epimer of ribulose 5-phosphate. A ketose is a substrate of transketolase only if its hydroxyl group at C-3 has the configuration of xylulose rather than ribulose. Ribulose 5-phosphate is converted into the appropriate epimer for the transketolase reaction by *phosphopentose epimerase* in the reverse reaction of that which takes place in the Calvin cycle.

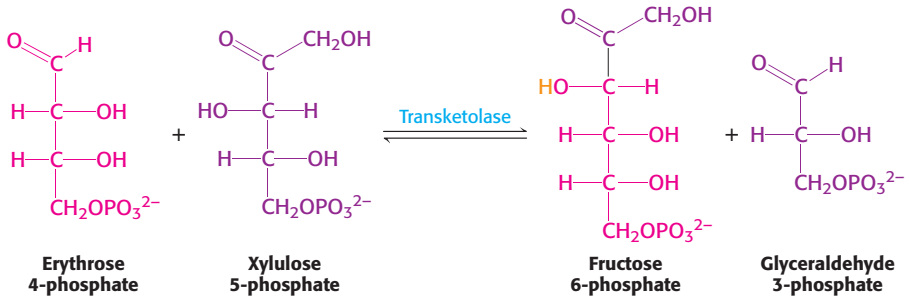


Glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate generated by the transketolase then react to form *fructose 6-phosphate* and *erythrose 4-phosphate*.



This synthesis of a four-carbon sugar and a six-carbon sugar is catalyzed by *transaldolase*.

In the third reaction, transketolase catalyzes the synthesis of *fructose 6-phosphate* and *glyceraldehyde 3-phosphate* from erythrose 4-phosphate and xylulose 5-phosphate.



The sum of these reactions is

2 Xylulose 5-phosphate + ribose 5-phosphate $\rightleftharpoons$
2 fructose 6-phosphate + glyceraldehyde 3-phosphate

Xylulose 5-phosphate can be formed from ribose 5-phosphate by the sequential action of phosphopentose isomerase and phosphopentose epimerase, and so the net reaction starting from ribose 5-phosphate is

3 Ribose 5-phosphate $\rightleftharpoons$
2 fructose 6-phosphate + glyceraldehyde 3-phosphate

Thus, *excess ribose 5-phosphate formed by the pentose phosphate pathway can be completely converted into glycolytic intermediates*. Moreover, any ribose ingested in the diet can be processed into glycolytic intermediates by this pathway. It is evident that the carbon skeletons of sugars can be extensively rearranged to meet physiological needs (Table 20.3).

Mechanism: Transketolase and transaldolase stabilize carbanionic intermediates by different mechanisms

The reactions catalyzed by transketolase and transaldolase are distinct, yet similar in many ways. One difference is that transketolase transfers a two-carbon unit, whereas transaldolase transfers a three-carbon unit. Each of these units is transiently attached to the enzyme in the course of the reaction.

Table 20.3 Pentose phosphate pathway

Reaction	Enzyme
Oxidative phase	
Glucose 6-phosphate + $NADP^+$ $\longrightarrow$ 6-phosphoglucono-δ-lactone + NADPH + H^+	Glucose 6-phosphate dehydrogenase
6-Phosphoglucono-δ-lactone + H_2O $\longrightarrow$ 6-phosphogluconate + H^+	Lactonase
6-Phosphogluconate + $NADP^+$ $\longrightarrow$ ribulose 5-phosphate + CO_2 + NADPH + H^+	6-Phosphogluconate dehydrogenase
Nonoxidative Phase	
Ribulose 5-phosphate $\rightleftharpoons$ ribose 5-phosphate	Phosphopentose isomerase
Ribulose 5-phosphate $\rightleftharpoons$ xylulose 5-phosphate	Phosphopentose epimerase
Xylulose 5-phosphate + ribose 5-phosphate $\rightleftharpoons$ sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate	Transketolase
Sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate $\rightleftharpoons$ fructose 6-phosphate + erythrose 4-phosphate	Transaldolase
Xylulose 5-phosphate + erythrose 4-phosphate $\rightleftharpoons$ fructose 6-phosphate + glyceraldehyde 3-phosphate	Transketolase

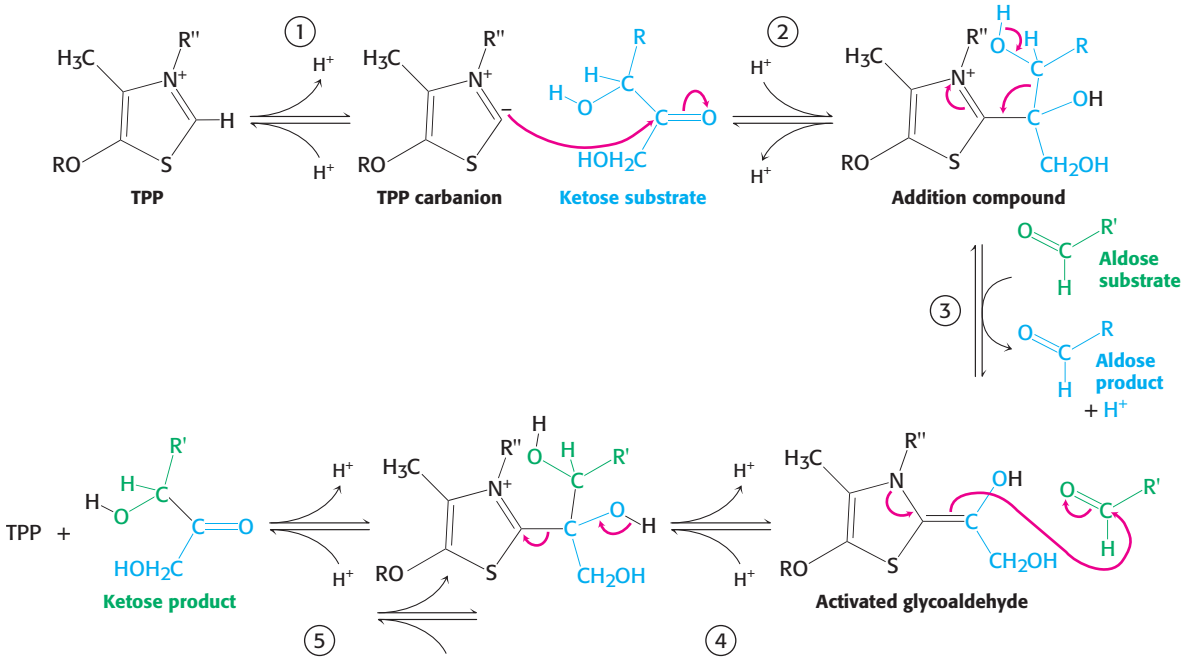


Figure 20.20 Transketolase mechanism. (1) Thiamine pyrophosphate (TPP) ionizes to form a carbanion. (2) The carbanion of TPP attacks the ketose substrate. (3) Cleavage of a carbon–carbon bond frees the aldose product and leaves a two-carbon fragment joined to TPP. (4) This activated glycoaldehyde intermediate attacks the aldose substrate to form a new carbon–carbon bond. (5) The ketose product is released, freeing the TPP for the next reaction cycle.

Transketolase reaction. Transketolase contains a tightly bound thiamine pyrophosphate as its prosthetic group. The enzyme transfers a two-carbon glycoaldehyde from a ketose donor to an aldose acceptor. The site of the addition of the two-carbon unit is the thiazole ring of TPP. Transketolase is homologous to the E_1 subunit of the pyruvate dehydrogenase complex (Section 17.1) and the reaction mechanism is similar (Figure 20.20).

The C-2 carbon atom of bound TPP readily ionizes to give a *carbanion*. The negatively charged carbon atom of this reactive intermediate attacks the carbonyl group of the ketose substrate. The resulting addition compound releases the aldose product to yield an *activated glycoaldehyde unit*. The positively charged nitrogen atom in the thiazole ring acts as an *electron sink* in the development of this activated intermediate. The carbonyl group of a suitable aldose acceptor then condenses with the activated glycoaldehyde unit to form a new ketose, which is released from the enzyme.

Transaldolase reaction. Transaldolase transfers a three-carbon *dihydroxyacetone* unit from a ketose donor to an aldose acceptor. Transaldolase, in contrast with transketolase, does not contain a prosthetic group. Rather, *a Schiff base is formed between the carbonyl group of the ketose substrate and the ε-amino group of a lysine residue at the active site of the enzyme* (Figure 20.21). This kind of covalent enzyme–substrate intermediate is like that formed in fructose 1,6-bisphosphate aldolase in the glycolytic pathway (Section 16.1) and, indeed, the enzymes are homologous. The Schiff base becomes protonated, the bond between C-3 and C-4 is split, and an aldose is released. The negative charge on the Schiff-base carbanion moiety is stabilized by resonance. The positively charged nitrogen atom of the protonated Schiff base acts as an electron sink. The Schiff-base adduct is stable until a suitable aldose becomes bound. The dihydroxyacetone moiety then reacts with the carbonyl group of the aldose. The ketose product is released by

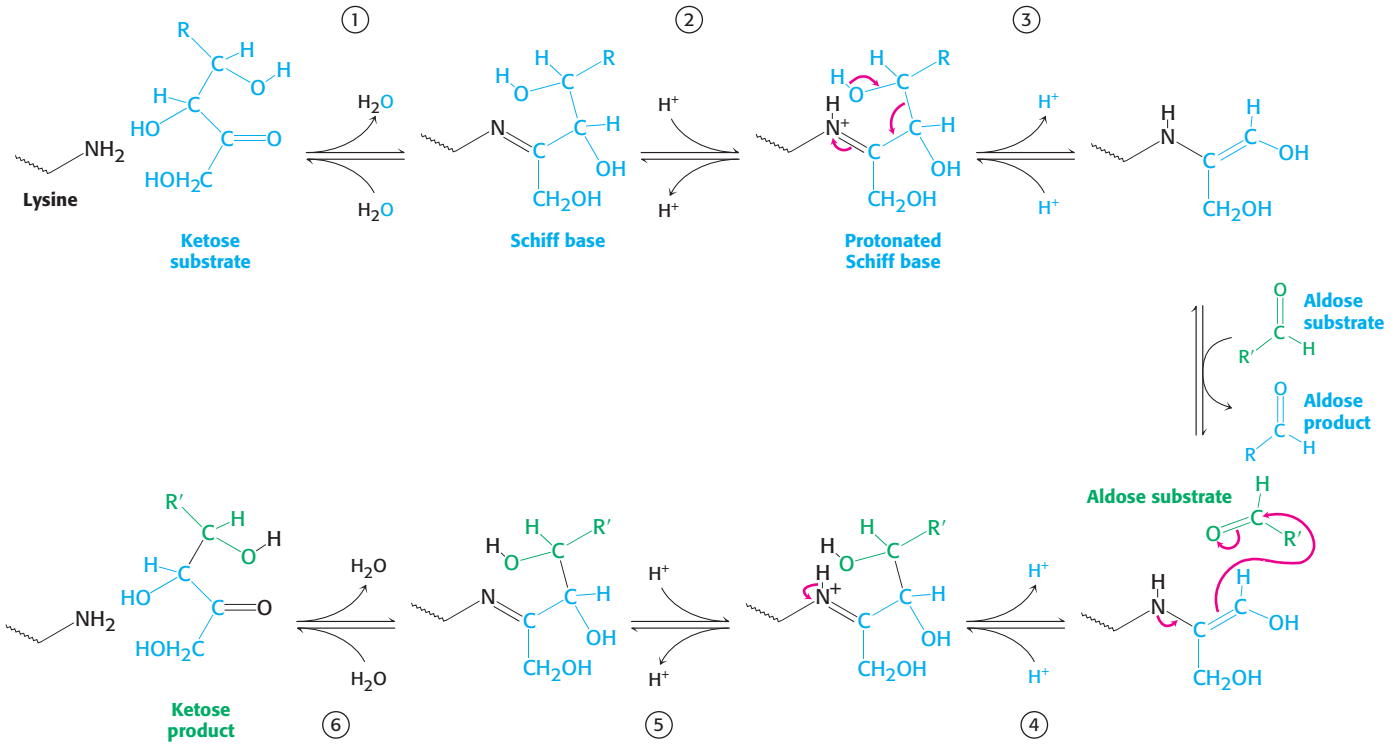


Figure 20.21 Transaldolase mechanism. (1) The reaction begins with the formation of a Schiff base between a lysine residue in transaldolase and the ketose substrate. Protonation of the Schiff base (2) leads to the release of the aldose product (3), leaving a three-carbon fragment attached to the lysine residue. (4) This intermediate adds to the aldose substrate, with a concomitant protonation to form a new carbon–carbon bond. Subsequent deprotonation (5) and hydrolysis of the Schiff base (6) release the ketose product from the lysine side chain, completing the reaction cycle.

hydrolysis of the Schiff base. The nitrogen atom of the protonated Schiff base plays the same role in transaldolase as the thiazole-ring nitrogen atom does in transketolase. In each enzyme, a group within an intermediate reacts like a carbanion in attacking a carbonyl group to form a new carbon–carbon bond. In each case, the charge on the carbanion is stabilized by resonance (Figure 20.22).

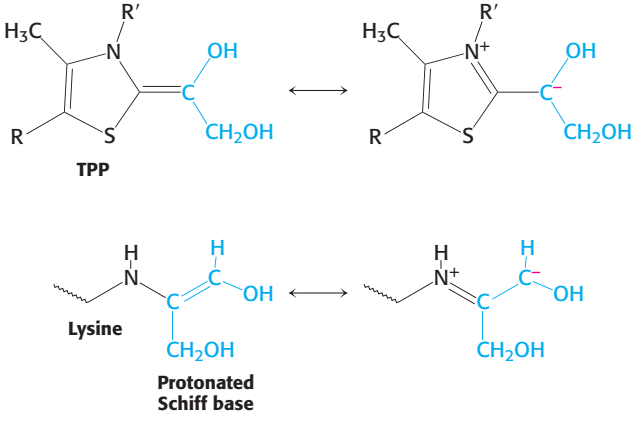


Figure 20.22 Carbanion intermediates. For transketolase and transaldolase, a carbanion intermediate is stabilized by resonance. In transketolase, TPP stabilizes this intermediate; in transaldolase, a protonated Schiff base plays this role.

20.4 The Metabolism of Glucose 6-phosphate by the Pentose Phosphate Pathway Is Coordinated with Glycolysis

Glucose 6-phosphate is metabolized by both the glycolytic pathway (Chapter 16) and the pentose phosphate pathway. How is the processing of this important metabolite partitioned between these two metabolic routes? The cytoplasmic concentration of $NADP^+$ plays a key role in determining the fate of glucose 6-phosphate.

The rate of the pentose phosphate pathway is controlled by the level of $NADP^+$

The first reaction in the oxidative branch of the pentose phosphate pathway, the dehydrogenation of glucose 6-phosphate, is essentially irreversible. In fact, this reaction is rate limiting under physiological conditions and serves as the control site. The most important regulatory factor is the level of $NADP^+$. Low levels of $NADP^+$ reduce the dehydrogenation of glucose 6-phosphate because it is needed as the electron acceptor. The effect of low levels of $NADP^+$ is intensified by the fact that NADPH competes with $NADP^+$ in binding to the enzyme. The ratio of $NADP^+$ to NADPH

in the cytoplasm of a liver cell from a well-fed rat is about 0.014, several orders of magnitude lower than the ratio of NAD^+ to NADH, which is 700 under the same conditions. The marked effect of the $NADP^+$ level on the rate of the oxidative phase ensures that NADPH is not generated unless the supply needed for reductive biosyntheses is low. The nonoxidative phase of the pentose phosphate pathway is controlled primarily by the availability of substrates.

The flow of glucose 6-phosphate depends on the need for NADPH, ribose 5-phosphate, and ATP

We can grasp the intricate interplay between glycolysis and the pentose phosphate pathway by examining the metabolism of glucose 6-phosphate in four different metabolic situations (Figure 20.23).

Mode 1. *Much more ribose 5-phosphate than NADPH is required.* For example, rapidly dividing cells need ribose 5-phosphate for the synthesis of nucleotide precursors of DNA. Most of the glucose 6-phosphate is converted into fructose 6-phosphate and glyceraldehyde 3-phosphate by the glycolytic pathway. Transaldolase and transketolase then convert two molecules of fructose 6-phosphate and one molecule of glyceraldehyde

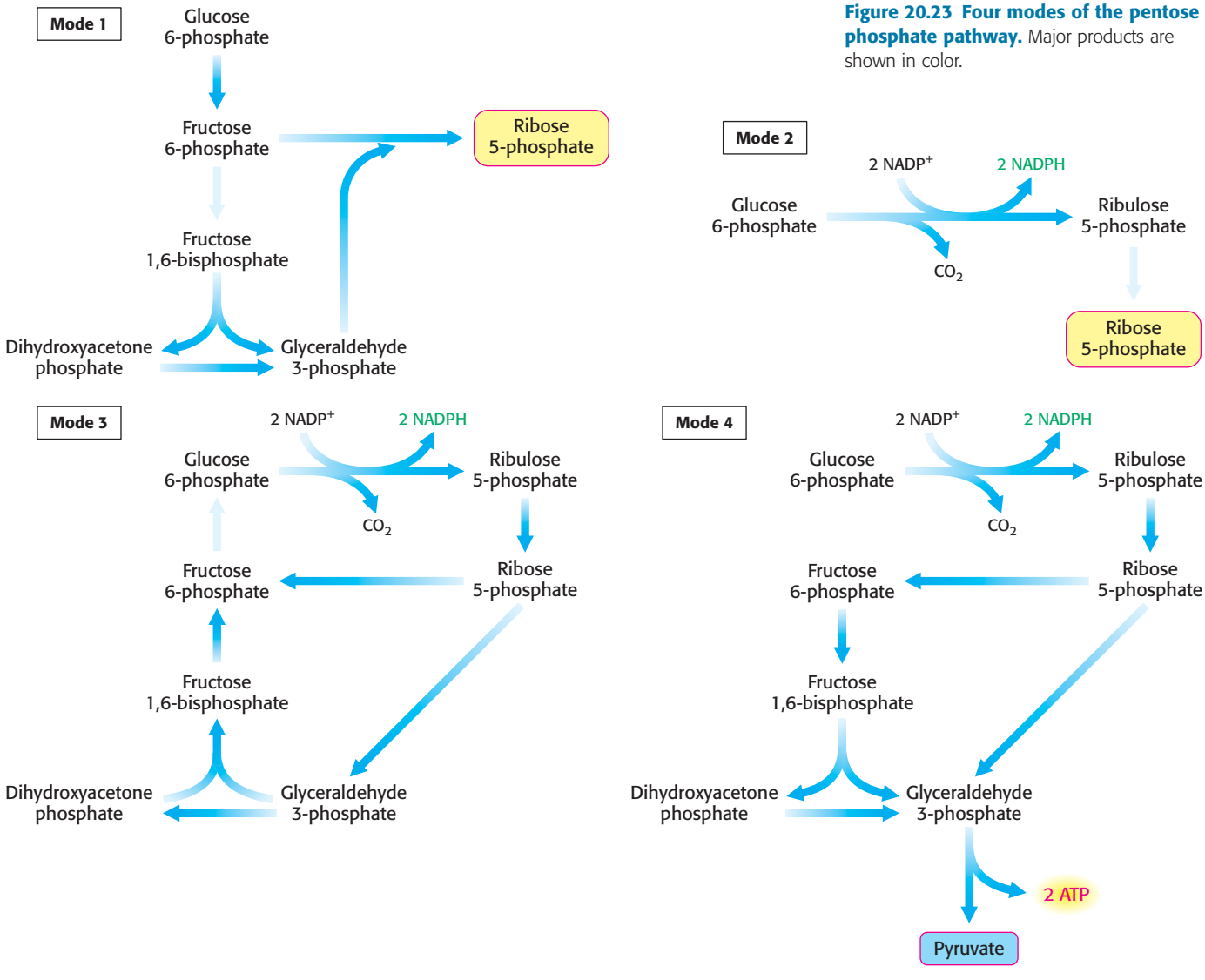


Figure 20.23 Four modes of the pentose phosphate pathway. Major products are shown in color.

Table 20.4 Tissues with active pentose phosphate pathways

Tissue	Function
Adrenal gland	Steroid synthesis
Liver	Fatty acid and cholesterol synthesis
Testes	Steroid synthesis
Adipose tissue	Fatty acid synthesis
Ovary	Steroid synthesis
Mammary gland	Fatty acid synthesis
Red blood cells	Maintenance of reduced glutathione

3-phosphate into three molecules of ribose 5-phosphate by a reversal of the reactions described earlier. The stoichiometry of mode 1 is

$$5 \text{ Glucose 6-phosphate} + \text{ATP} \longrightarrow 6 \text{ ribose 5-phosphate} + \text{ADP} + 2\,H^+$$

Mode 2. *The needs for NADPH and for ribose 5-phosphate are balanced.* The predominant reaction under these conditions is the formation of two molecules of NADPH and one molecule of ribose 5-phosphate from one molecule of glucose 6-phosphate in the oxidative phase of the pentose phosphate pathway. The stoichiometry of mode 2 is

$$\text{Glucose 6-phosphate} + 2\,NADP^+ + H_2O \longrightarrow \text{ribose 5-phosphate} + 2\,NADPH + 2\,H^+ + CO_2$$

Mode 3. *Much more NADPH than ribose 5-phosphate is required.* For example, adipose tissue requires a high level of NADPH for the synthesis of fatty acids (Table 20.4). In this case, glucose 6-phosphate is completely oxidized to CO_2. Three groups of reactions are active in this situation. First, the oxidative phase of the pentose phosphate pathway forms two molecules of NADPH and one molecule of ribose 5-phosphate. Then, ribose 5-phosphate is converted into fructose 6-phosphate and glyceraldehyde 3-phosphate by transketolase and transaldolase. Finally, glucose 6-phosphate is resynthesized from fructose 6-phosphate and glyceraldehyde 3-phosphate by the gluconeogenic pathway. The stoichiometries of these three sets of reactions are

$$6 \text{ Glucose 6-phosphate} + 12\,NADP^+ + 6\,H_2O \longrightarrow 6 \text{ Ribose 5-phosphate} + 12\,NADPH + 12\,H^+ + 6CO_2$$

$$6 \text{ Ribose 5-phosphate} \longrightarrow 4 \text{ Fructose 6-phosphate} + 2 \text{ glyceraldehyde 3-phosphate}$$

$$4 \text{ Fructose 6-phosphate} + 2 \text{ glyceraldehyde 3-phosphate} + H_2O \longrightarrow 5\text{-Glucose 6-phosphate} + P_i$$

The sum of the mode 3 reactions is

$$\text{Glucose 6-phosphate} + 12\,NADP^+ + 7\,H_2O \longrightarrow 6\,CO_2 + 12\,NADPH + 12\,H^+ + P_i$$

Thus, *the equivalent of glucose 6-phosphate can be completely oxidized to CO_2 with the concomitant generation of NADPH.* In essence, ribose 5-phosphate produced by the pentose phosphate pathway is recycled into glucose 6-phosphate by transketolase, transaldolase, and some of the enzymes of the gluconeogenic pathway.

Mode 4. *Both NADPH and ATP are required.* Alternatively, ribose 5-phosphate formed by the oxidative phase of the pentose phosphate

pathway can be converted into pyruvate. Fructose 6-phosphate and glyceraldehyde 3-phosphate derived from ribose 5-phosphate enter the glycolytic pathway rather than reverting to glucose 6-phosphate. In this mode, *ATP and NADPH are concomitantly generated, and five of the six carbons of glucose 6-phosphate emerge in pyruvate.*

$$3\ \text{Glucose 6-phosphate} + 6\ NADP^+ + 5\ NAD^+ + 5\ P_i + 8\ ADP \longrightarrow 5\ \text{pyruvate} + 3\ CO_2 + 6\ NADPH + 5\ NADH + 8\ ATP + 2\ H_2O + 8\ H^+$$

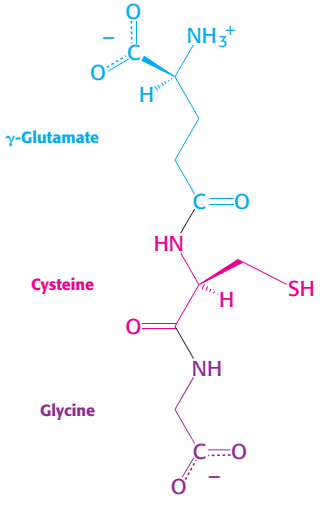
Pyruvate formed by these reactions can be oxidized to generate more ATP or it can be used as a building block in a variety of biosyntheses.

Through the looking-glass: The Calvin cycle and the pentose phosphate pathway are mirror images

The complexities of the Calvin cycle and the pentose phosphate pathway are easier to comprehend if we consider them as functional mirror images of each other. The Calvin cycle begins with the fixation of CO_2 and proceeds to use NADPH in the synthesis of glucose. The pentose phosphate pathway begins with the oxidation of a glucose-derived carbon atom to CO_2 and concomitantly generates NADPH. The regeneration phase of the Calvin cycle converts C_6 and C_3 molecules back into the starting material—the C_5 molecule ribulose 1,5-bisphosphate. The pentose phosphate pathway converts a C_5 molecule, ribose 5-phosphate, into C_6 and C_3 intermediates of the glycolytic pathway. Not surprisingly, in photosynthetic organisms, many enzymes are common to the two pathways. We see the economy of evolution: the use of identical enzymes for similar reactions with different ends.

20.5 Glucose 6-phosphate Dehydrogenase Plays a Key Role in Protection Against Reactive Oxygen Species

The NADPH generated by the pentose phosphate pathway plays a vital role in protecting the cells from reactive oxygen species (ROS). Reactive oxygen species generated in oxidative metabolism inflict damage on all classes of macromolecules and can ultimately lead to cell death. Indeed, ROS are implicated in a number of human diseases (see Table 18.3). Reduced *glutathione* (GSH), a tripeptide with a free sulfhydryl group, combats oxidative stress by reducing ROS to harmless forms. Its task accomplished, the glutathione is now in the oxidized form (GSSG) and must be reduced to regenerate GSH. The reducing power is supplied by the NADPH generated by glucose 6-phosphate dehydrogenase in the pentose phosphate pathway. Indeed, cells with reduced levels of glucose 6-phosphate dehydrogenase are especially sensitive to oxidative stress. This stress is most acute in red blood cells because they lack mitochondria and have no alternative means of generating reducing power.



Glutathione (reduced)
(γ-Glutamylcysteinylglycine)

Glucose 6-phosphate dehydrogenase deficiency causes a drug-induced hemolytic anemia

The importance of the pentose phosphate pathway is highlighted by some people's anomalous responses to certain drugs. For instance, pamaquine, an antimalarial drug introduced in 1926, was associated with the appearance of severe and mysterious ailments. Most patients tolerated the drug well, but a few developed severe symptoms within a few days after therapy was started. The urine turned black, jaundice developed, and the

hemoglobin content of the blood dropped sharply. In some cases, massive destruction of red blood cells caused death.

This drug-induced *hemolytic anemia* was shown 30 years later to be caused by a *deficiency of glucose 6-phosphate dehydrogenase,* the enzyme catalyzing the first step in the oxidative branch of the pentose phosphate pathway. The result is a dearth of NADPH in all cells, but this deficiency is most acute in red blood cells. This defect, which is inherited on the X chromosome, is the most common disease that results from an enzyme malfunction, affecting hundreds of millions of people. The major role of NADPH in red cells is to reduce the disulfide form of glutathione to the sulfhydryl form. The enzyme that catalyzes the regeneration of reduced glutathione is *glutathione reductase*.

Vicia faba. The Mediterranean plant *Vicia faba* is a source of fava beans that contain the purine glycoside vicine. [Inga Spence/ Visuals Unlimited.]

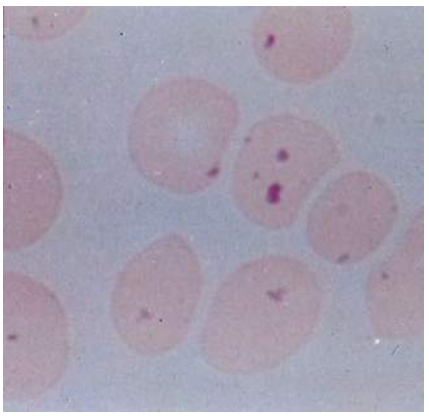
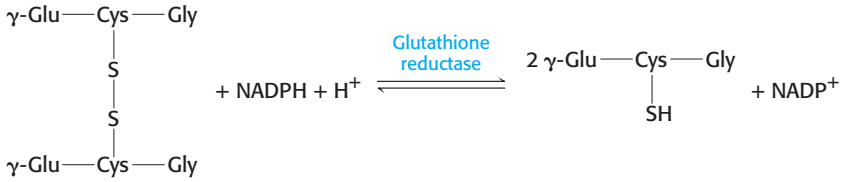


Figure 20.24 Red blood cells with Heinz bodies. The light micrograph shows red blood cells obtained from a person deficient in glucose 6-phosphate dehydrogenase. The dark particles, called Heinz bodies, inside the cells are clumps of denatured hemoglobin that adhere to the plasma membrane and stain with basic dyes. Red blood cells in such people are highly susceptible to oxidative damage. [Courtesy of Dr. Stanley Schrier.]



Oxidized glutathione (GSSG) **Reduced glutathione (GSH)**

Red blood cells with a lowered level of reduced glutathione are more susceptible to hemolysis.

Pamaquine sensitivity is not simply a historical oddity about malaria treatment many decades ago. Pamaquine is purine glycoside of fava beans, a bean that is still consumed today in countries surrounding the Mediterranean. People deficient in glucose 6-phosphate dehydrogenase suffer hemolysis from eating fava beans or inhaling the pollen of the fava flowers, a response called favism. How can we explain pamaquine-induced hemolysis biochemically? Pamaquine is an oxidative agent that leads to the generation of peroxides, reactive oxygen species that can damage membranes as well as other biomolecules. Peroxides are normally eliminated by the enzyme *glutathione peroxidase,* which uses reduced glutathione as a reducing agent.

$$2\,GSH + ROOH \xrightarrow{\text{Glutathione peroxidase}} GSSG + H_2O + ROH$$

In the absence of glucose 6-phosphate dehydrogenase, peroxides continue to damage membranes because no NADPH is being produced to restore reduced glutathione.

Reduced glutathione is also essential for maintaining the normal structure of red blood cells by maintaining the structure of hemoglobin. The reduced form of glutathione serves as a sulfhydryl buffer that keeps the residues of hemoglobin in the reduced sulfhydryl form. Without adequate levels of reduced glutathione, the hemoglobin sulfhydryl groups can no longer be maintained in the reduced form. Hemoglobin molecules then cross-link with one another to form aggregates called *Heinz bodies* on cell membranes (Figure 20.24). Membranes damaged by Heinz bodies and reactive oxygen species become deformed, and the cell is likely to undergo lysis. Thus, the answer to our question is that glucose 6-phosphate dehydrogenase is required to maintain reduced glutathione levels to protect against oxidative stress. In the absence of oxidative stress, however, the deficiency is quite benign. The sensitivity to pamaquine of people having this dehydrogenase deficiency also clearly demonstrates that *atypical reactions to drugs may have a genetic basis.*

A deficiency of glucose 6-phosphate dehydrogenase confers an evolutionary advantage in some circumstances

The incidence of the most common form of glucose 6-phosphate dehydrogenase deficiency, characterized by a 10-fold reduction in enzymatic activity in red blood cells, is 11% among Americans of African heritage. This high frequency suggests that the deficiency may be advantageous under certain environmental conditions. Indeed, *glucose 6-phosphate dehydrogenase deficiency protects against falciparum malaria*. The parasites causing this disease require reduced glutathione and the products of the pentose phosphate pathway for optimal growth. Thus, glucose 6-phosphate dehydrogenase deficiency is a mechanism of protection against malaria, which accounts for its high frequency in malaria-infested regions of the world. We see here once again the interplay of heredity and environment in the production of disease.

Summary

20.1 The Calvin Cycle Synthesizes Hexoses from Carbon Dioxide and Water

ATP and NADPH formed in the light reactions of photosynthesis are used to convert CO_2 into hexoses and other organic compounds. The dark phase of photosynthesis, called the Calvin cycle, starts with the reaction of CO_2 and ribulose 1,5-bisphosphate to form two molecules of 3-phosphoglycerate. The steps in the conversion of 3-phosphoglycerate into fructose 6-phosphate and glucose 6-phosphate are like those of gluconeogenesis, except that glyceraldehyde 3-phosphate dehydrogenase in chloroplasts is specific for NADPH rather than NADH. Ribulose 1,5-bisphosphate is regenerated from fructose 6-phosphate, glyceraldehyde 3-phosphate, and dihydroxyacetone phosphate by a complex series of reactions. Several of the steps in the regeneration of ribulose 1,5-bisphosphate are like those of the pentose phosphate pathway. Three molecules of ATP and two molecules of NADPH are consumed for each molecule of CO_2 converted into a hexose. Starch in chloroplasts and sucrose in the cytoplasm are the major carbohydrate stores in plants.

Rubisco also catalyzes a competing oxygenase reaction, which produces phosphoglycolate and 3-phosphoglycerate. The recycling of phosphoglycolate leads to the release of CO_2 and further consumption of O_2 in a process called photorespiration.

20.2 The Activity of the Calvin Cycle Depends on Environmental Conditions

Reduced thioredoxin formed by the light-driven transfer of electrons from ferredoxin activates enzymes of the Calvin cycle by reducing disulfide bridges. The light-induced increase in pH and Mg^{2+} level of the stroma is important in stimulating the carboxylation of ribulose 1,5-bisphosphate by rubisco. Photorespiration is minimized in tropical plants, which have an accessory pathway—the C_4 pathway—for concentrating CO_2 at the site of the Calvin cycle. This pathway enables tropical plants to take advantage of high levels of light and minimize the oxygenation of ribulose 1,5-bisphosphate. Plants in arid ecosystems employ crassulacean acid metabolism to prevent dehydration. In CAM plants, the C_4 pathway is active during the night, when the plant exchanges gases with the air. During the day, gas exchange is eliminated and CO_2 is generated from malate stored in vacuoles.

20.3 The Pentose Phosphate Pathway Generates NADPH and Synthesizes Five-Carbon Sugars

Whereas the Calvin cycle is present only in photosynthetic organisms, the pentose phosphate pathway is present in all organisms. The pentose phosphate pathway generates NADPH and ribulose 5-phosphate in the cytoplasm, which is subsequently isomerized to ribose 5-phosphate. NADPH is used in reductive biosyntheses, whereas ribose 5-phosphate is used in the synthesis of RNA, DNA, and nucleotide coenzymes. The pentose phosphate pathway starts with the dehydrogenation of glucose 6-phosphate to form a lactone, which is hydrolyzed to give 6-phosphogluconate and then oxidatively decarboxylated to yield ribulose 5-phosphate. $NADP^+$ is the electron acceptor in both of these oxidations. The last step is the isomerization of ribulose 5-phosphate (a ketose) to ribose 5-phosphate (an aldose). A different mode of the pathway is active when cells need much more NADPH than ribose 5-phosphate. Under these conditions, ribose 5-phosphate is converted into glyceraldehyde 3-phosphate and fructose 6-phosphate by *transketolase* and *transaldolase*. These two enzymes create a reversible link between the pentose phosphate pathway and gluconeogenesis. Xylulose 5-phosphate, sedoheptulose 7-phosphate, and erythrose 4-phosphate are intermediates in these interconversions. In this way, 12 molecules of NADPH can be generated for each molecule of glucose 6-phosphate that is completely oxidized to CO_2.

20.4 The Metabolism of Glucose 6-phosphate by the Pentose Phosphate Pathway Is Coordinated with Glycolysis

Only the nonoxidative branch of the pathway is significantly active when much more ribose 5-phosphate than NADPH needs to be synthesized. Under these conditions, fructose 6-phosphate and glyceraldehyde 3-phosphate (formed by the glycolytic pathway) are converted into ribose 5-phosphate without the formation of NADPH. Alternatively, ribose 5-phosphate formed by the oxidative branch can be converted into pyruvate through fructose 6-phosphate and glyceraldehyde 3-phosphate. In this mode, ATP and NADPH are generated, and five of the six carbons of glucose 6-phosphate emerge in pyruvate. The interplay of the glycolytic and pentose phosphate pathways enables the levels of NADPH, ATP, and building blocks such as ribose 5-phosphate and pyruvate to be continuously adjusted to meet cellular needs.

20.5 Glucose 6-phosphate Dehydrogenase Plays a Key Role in Protection Against Reactive Oxygen Species

NADPH generated by glucose 6-phosphate dehydrogenase maintains the appropriate levels of reduced glutathione required to combat oxidative stress and maintain the proper reducing environment in the cell. Cells with diminished glucose 6-phosphate dehydrogenase activity are especially sensitive to oxidative stress.

Key Terms

Calvin cycle (dark reactions) (p. 589)
autotroph (p. 590)
heterotroph (p. 590)
rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) (p. 591)
peroxisome (microbody) (p. 593)
photorespiration (p. 594)
hexose monophosphate pool (p. 594)
transketolase (p. 594)
aldolase (p. 595)
starch (p. 597)
sucrose (p. 597)
thioredoxin (p. 599)
C_4 pathway (p. 600)
C_4 plant (p. 600)

C_3 plant (p. 600)
crassulacean acid metabolism (CAM) (p. 601)
pentose phosphate pathway (p. 601)
glucose 6-phosphate dehydrogenase (p. 601)
glutathione (p. 609)

Problems

1. *A vital cycle*. Why is the Calvin cycle crucial to the functioning of all life forms?

2. *Compare and contrast*. Identify the similarities and differences between the Krebs cycle and the Calvin cycle.

3. *Labeling experiments*. When Melvin Calvin performed his initial experiments on carbon fixation, he exposed algae to radioactive carbon dioxide. After 5 seconds, only a single organic compound contained radioactivity but, after 60 seconds, many compounds had incorporated radioactivity. (a) What compound initially contained the radioactivity? (b) What compounds contained radioactivity after 60 seconds?

4. *Three-part harmony*. The Calvin cycle can be thought of as occurring in three parts or stages. Describe the stages.

5. *Not always to the swiftest*. Suggest a reason why rubisco might be the most abundant enzyme in the world.

6. *A requirement*. In an atmosphere devoid of CO_2 but rich in O_2, the oxygenase activity of rubisco disappears. Why?

7. *Reduce locally*. Glyceraldehyde 3-phosphate dehydrogenase in chloroplasts uses NADPH to participate in the synthesis of glucose. In gluconeogenesis in the cytoplasm, the isozyme of the dehydrogenase uses NADH. Why is it advantageous for the chloroplast enzyme to use NADPH?

8. *Total eclipse*. An illuminated suspension of *Chlorella* is actively carrying out photosynthesis. Suppose that the light is suddenly switched off. How would the levels of 3-phosphoglycerate and ribulose 1,5-bisphosphate change in the next minute?

9. *CO_2 deprivation*. An illuminated suspension of *Chlorella* is actively carrying out photosynthesis in the presence of 1% CO_2. The concentration of CO_2 is abruptly reduced to 0.003%. What effect would this reduction have on the levels of 3-phosphoglycerate and ribulose 1,5-bisphosphate in the next minute?

10. *Salvage operation*. Write a balanced equation for the transamination of glyoxylate to yield glycine.

11. *Dog days of August*. Before the days of pampered lawns, most homeowners practiced horticultural Darwinism. A result was that the lush lawns of early summer would often convert into robust cultures of crabgrass in the dog days of August. Provide a possible biochemical explanation for this transition.

12. *Is it hot in here, or is it just me?* Why is the C_4 pathway valuable for tropical plants?

13. *No free lunch*. Explain why maintaining a high concentration of CO_2 in the bundle-sheath cells of C_4 plants is an example of active transport. How much ATP is required per CO_2 to maintain a high concentration of CO_2 in the bundle-sheath cells of C_4 plants?

14. *Breathing pictures?* What is photorespiration, what is its cause, and why is it believed to be wasteful?

15. *Global warming*. C_3 plants are most common in higher latitudes and become less common at latitudes near the equator. The reverse is true of C_4 plants. How might global warming affect this distribution?

16. *Communication*. What are the light-dependent changes in the stroma that regulate the Calvin cycle?

17. *Linked in*. Describe how the pentose phosphate pathway and glycolysis are linked by transaldolase and transketolase.

18. *Tracing glucose*. Glucose labeled with ^{14}C at C-6 is added to a solution containing the enzymes and cofactors of the oxidative phase of the pentose phosphate pathway. What is the fate of the radioactive label?

19. *Recurring decarboxylations*. Which reaction in the citric acid cycle is most analogous to the oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate? What kind of enzyme-bound intermediate is formed in both reactions?

20. *Synthetic stoichiometries*. What is the stoichiometry of the synthesis of (a) ribose 5-phosphate from glucose 6-phosphate without the concomitant generation of NADPH? (b) NADPH from glucose 6-phosphate without the concomitant formation of pentose sugars?

21. *Offal or awful?* Liver and other organ meats contain large quantities of nucleic acids. In the course of digestion, RNA is hydrolyzed to ribose, among other chemicals. Explain how ribose can be used as a fuel.

22. *A required ATP*. The metabolism of glucose 6-phosphate into ribose 5-phosphate by the joint efforts of the pentose phosphate pathway and glycolysis can be summarized by the following equation.

$$5 \text{ glucose} + 6\text{-phosphate} + \text{ATP} \longrightarrow 6 \text{ ribose 5-phosphate} + \text{ADP}$$

Which reaction requires the ATP?

23. *No respiration.* Glucose is normally completely oxidized to CO_2 in the mitochondria. In what circumstance can glucose be complete oxidized to CO_2 in the cytoplasm?

24. *Watch your diet, doctor.* The noted psychiatrist Hannibal Lecter once remarked to FBI Agent Clarice Starling that he enjoyed liver with some fava beans and a nice Chianti. Why might this diet be dangerous for some people?

25. *No redundancy.* Why do deficiencies in glucose 6-phosphate dehydrogenase frequently present as anemia?

26. *Damage control.* What is the role of glutathione in protection against damage by reactive oxygen species? Why is the pentose phosphate pathway crucial to this protection?

27. *Reductive power.* What ratio of NADPH to $NADP^+$ is required to sustain [GSH] = 10 mM and [GSSG] = 1 mM? Use the redox potentials given in Table 18.1.

Mechanism Problems

28. *An alternative approach.* The mechanisms of some aldolases do not include Schiff-base intermediates. Instead, these enzymes require bound metal ions. Propose such a mechanism for the conversion of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate into fructose 1,6-bisphosphate.

29. *A recurring intermediate.* Phosphopentose isomerase interconverts the aldose ribose 5-phosphate and the ketose ribulose 5-phosphate. Propose a mechanism.

Chapter Integration Problems

30. *Catching carbons.* Radioactive-labeling experiments can yield estimates of how much glucose 6-phosphate is metabolized by the pentose phosphate pathway and how much is metabolized by the combined action of glycolysis and the citric acid cycle. Suppose that you have samples of two different tissues as well as two radioactively labeled glucose samples, one with glucose labeled with ^{14}C at C-1 and the other with glucose labeled with ^{14}C at C-6. Design an experiment that would enable you to determine the relative activity of the aerobic metabolism of glucose compared with metabolism by the pentose phosphate pathway.

31. *Photosynthetic efficiency.* Use the following information to estimate the efficiency of photosynthesis.

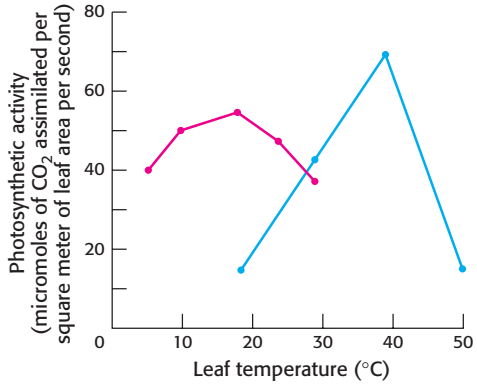
The $\Delta G^{\circ\prime}$ for the reduction of CO_2 to the level of hexose is $+477$ kJ mol^{-1} ($+114$ kcal mol^{-1}).
A mole of 600-nm photons has an energy content of 199 kJ (47.6 kcal).

Assume that the proton gradient generated in producing the required NADPH is sufficient to drive the synthesis of the required ATP.

32. *A violation of the First Law?* The complete combustion of glucose to CO_2 and H_2O yields 30 ATP, as shown in Table 18.4. However, the synthesis of glucose requires only 18 ATP. How is it possible that glucose synthesis from CO_2 and H_2O requires only 18 ATP but combustion to CO_2 and H_2O yields 30 ATP? Is it a violation of the First Law of Thermodynamics or perhaps a miracle?

Data Interpretation Problem

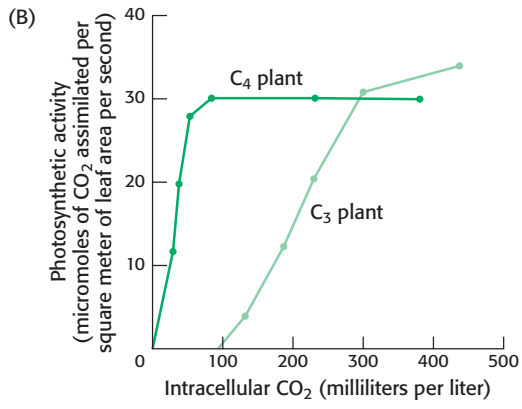
33. *Deciding between 3 and 4.* Graph A shows the photosynthetic activity of two species of plant, one a C_4 plant and the other a C_3 plant, as a function of leaf temperature.



(a) Which data were most likely generated by the C_4 plant and which by the C_3 plant? Explain.

(b) Suggest some possible explanations for why the photosynthetic activity falls at higher temperatures.

Graph B illustrates how the photosynthetic activity of C_3 and C_4 plants varies with CO_2 concentration when temperature (30°C) and light intensity (high) are constant.

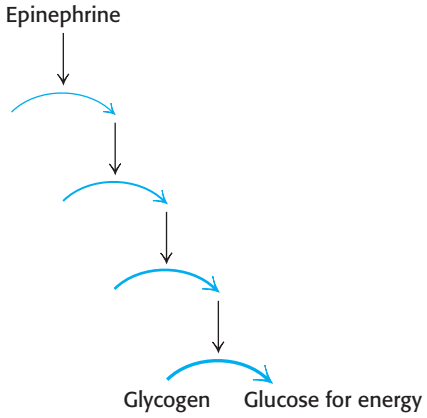


(c) Why can C_4 plants thrive at CO_2 concentrations that do not support the growth of C_3 plants?

(d) Suggest a plausible explanation why C_3 plants continue to increase photosynthetic activity at higher CO_2 concentrations, whereas C_4 plants reach a plateau.

CHAPTER 21

Glycogen Metabolism



Signaling cascades lead to the mobilization of glycogen to produce glucose, an energy source for runners. [(Left) Steve Krull/Alamy.]

Glucose is an important fuel and, as we will see, a key precursor for the biosynthesis of many molecules. However, glucose cannot be stored, because high concentrations of glucose disrupt the osmotic balance of the cell, which would cause cell damage or death. How can adequate stores of glucose be maintained without damaging the cell? The solution to this problem is to store glucose as a nonosmotically active polymer called glycogen.

Glycogen is a *readily mobilized storage form of glucose.* It is a very large, branched polymer of glucose residues that can be broken down to yield glucose molecules when energy is needed (Figure 21.1). A glycogen molecule has approximately 12 layers of glucose molecules and can be as large as 40 nm. Most of the glucose residues in glycogen are linked by α-1,4-glycosidic bonds (Figure 21.2). Branches at about every tenth residue are created by α-1,6-glycosidic bonds. Recall that α-glycosidic linkages form open helical polymers, whereas β linkages produce nearly straight strands that form structural fibrils, as in cellulose (see Figure 11.14).

Glycogen is not as reduced as fatty acids are and consequently not as energy rich. Why isn't all excess fuel stored as fatty acids rather than as glycogen? The controlled release of glucose from glycogen maintains blood-glucose levels between meals. The circulating blood keeps the brain supplied

OUTLINE

Figure 21.1 Glycogen. At the core of the glycogen molecule is the protein glycogenin (p. 628). The nonreducing ends of the glycogen molecule form the surface of the glycogen granule. Degradation takes place at this surface. [After R. Melendez et al. *Biophys. J.* 77:1327–1332, 1999.]

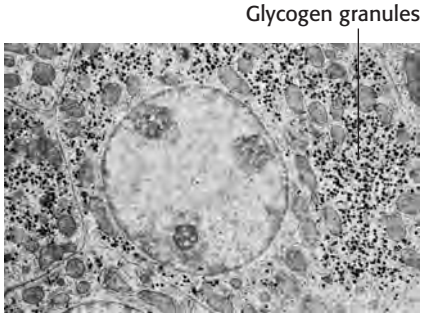


Figure 21.3 Electron micrograph of a liver cell. The dense particles in the cytoplasm are glycogen granules. [Courtesy of Dr. George Palade.]

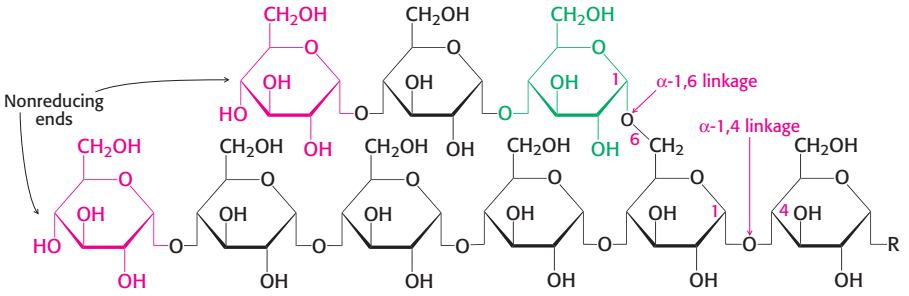


Figure 21.2 Glycogen structure. In this structure of two outer branches of a glycogen molecule, the residues at the nonreducing ends are shown in red and the residue that starts a branch is shown in green. The rest of the glycogen molecule is represented by R.

with glucose, which is virtually the only fuel used by the brain, except during prolonged starvation. Moreover, the readily mobilized glucose from glycogen is a good source of energy for sudden, strenuous activity. Unlike fatty acids, the released glucose can provide energy in the absence of oxygen and can thus supply energy for anaerobic activity.

Although most tissues have some glycogen, the two major sites of glycogen storage are the liver and skeletal muscle. The concentration of glycogen is higher in the liver than in muscle (10% versus 2% by weight), but more glycogen is stored in skeletal muscle overall because of muscle's much greater mass. Glycogen is present in the cytoplasm, with the molecule appearing as granules (Figure 21.3). In the liver, glycogen synthesis and degradation are regulated to maintain blood-glucose levels as required to meet the needs of the organism as a whole. In contrast, in muscle, these processes are regulated to meet the energy needs of the muscle itself.

Glycogen metabolism is the regulated release and storage of glucose

Glycogen degradation and synthesis are simple biochemical processes. Glycogen degradation consists of three steps: (1) the release of glucose 1-phosphate from glycogen, (2) the remodeling of the glycogen substrate to permit further degradation, and (3) the conversion of glucose 1-phosphate into glucose 6-phosphate for further metabolism. The glucose 6-phosphate

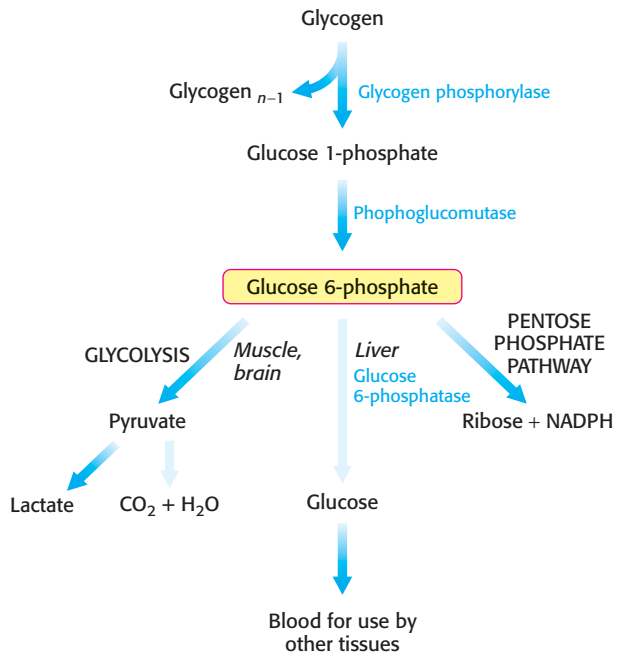


Figure 21.4 Fates of glucose 6-phosphate. Glucose 6-phosphate derived from glycogen can (1) be used as a fuel for anaerobic or aerobic metabolism as in, for instance, muscle; (2) be converted into free glucose in the liver and subsequently released into the blood; (3) be processed by the pentose phosphate pathway to generate NADPH or ribose in a variety of tissues.

derived from the breakdown of glycogen has three possible fates (Figure 21.4): (1) it is the initial substrate for glycolysis, (2) it can be converted into free glucose for release into the bloodstream, and (3) it can be processed by the pentose phosphate pathway to yield NADPH and ribose derivatives. The conversion of glycogen into free glucose takes place mainly in the liver.

Glycogen synthesis, which takes place when glucose is abundant, requires an activated form of glucose, uridine diphosphate glucose (UDP-glucose), formed by the reaction of UTP and glucose 1-phosphate. As is the case for glycogen degradation, the glycogen molecule must be remodeled for continued synthesis.

The regulation of glycogen degradation and synthesis is complex. Several enzymes taking part in glycogen metabolism allosterically respond to metabolites that signal the energy needs of the cell. *Through these allosteric responses, enzyme activity is adjusted to meet the needs of the cell.* In addition, hormones may initiate signal cascades that lead to the reversible phosphorylation of enzymes, which alters their catalytic rates. *Regulation by hormones adjusts glycogen metabolism to meet the needs of the entire organism.*

21.1 Glycogen Breakdown Requires the Interplay of Several Enzymes

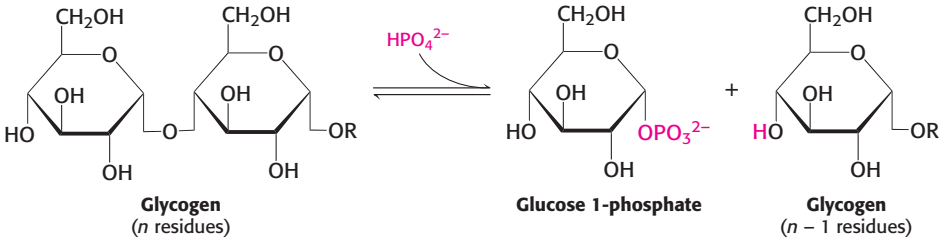
The efficient breakdown of glycogen to provide glucose 6-phosphate for further metabolism requires four enzyme activities: one to degrade glycogen, two to remodel glycogen so that it can be a substrate for degradation, and one to convert the product of glycogen breakdown into a form suitable for further metabolism. We will examine each of these activities in turn.

Phosphorylase catalyzes the phosphorolytic cleavage of glycogen to release glucose 1-phosphate

Glycogen phosphorylase, the key enzyme in glycogen breakdown, cleaves its substrate by the addition of orthophosphate (P_i) to yield *glucose 1-phosphate.* The cleavage of a bond by the addition of orthophosphate is referred to as *phosphorolysis*.

$$\underset{(n \text{ residues})}{\text{Glycogen}} + P_i \rightleftharpoons \text{glucose 1-phophase} + \underset{(n-1 \text{ residues})}{\text{glycogen}}$$

Phosphorylase catalyzes the sequential removal of glucosyl residues from the nonreducing ends of the glycogen molecule (the ends with a free OH group on carbon 4). Orthophosphate splits the glycosidic linkage between C-1 of the terminal residue and C-4 of the adjacent one. Specifically, it cleaves the bond between the C-1 carbon atom and the glycosidic oxygen atom, and the α configuration at C-1 is retained.



Glucose 1-phosphate released from glycogen can be readily converted into glucose 6-phosphate, an important metabolic intermediate, by the enzyme phosphoglucomutase.

The reaction catalyzed by phosphorylase is readily reversible in vitro. At pH 6.8, the equilibrium ratio of orthophosphate to glucose 1-phosphate is 3.6. The value of $\Delta G^{\circ\prime}$ for this reaction is small because a glycosidic bond is replaced by a phosphoryl ester bond that has a nearly equal transfer potential. However, phosphorolysis proceeds far in the direction of glycogen breakdown in vivo because the $[P_i]/[\text{glucose 1-phosphate}]$ ratio is usually greater than 100, substantially favoring phosphorolysis. We see here an example of how the cell can alter the free-energy change to favor a reaction's occurrence by altering the ratio of substrate and product.

The phosphorolytic cleavage of glycogen is energetically advantageous because the released sugar is already phosphorylated. In contrast, a hydrolytic cleavage would yield glucose, which would then have to be phosphorylated at the expense of a molecule of ATP to enter the glycolytic pathway. An additional advantage of phosphorolytic cleavage for muscle cells is that no transporters exist for glucose 1-phosphate, which is negatively charged under physiological conditions, and so it cannot be transported out of the cell.

Mechanism: Pyridoxal phosphate participates in the phosphorolytic cleavage of glycogen

The special challenge faced by phosphorylase is to cleave glycogen phosphorolytically rather than hydrolytically to save the ATP required to phosphorylate free glucose. Thus, water must be excluded from the active site. Phosphorylase is a dimer of two identical 97-kd subunits. Each subunit is compactly folded into an *amino-terminal domain* (480 residues) containing a *glycogen-binding site* and a *carboxyl-terminal domain* (360 residues; Figure 21.5). The catalytic site in each subunit is located in a deep crevice formed by residues from both domains. What is the mechanistic basis of the phosphorolytic cleavage of glycogen?

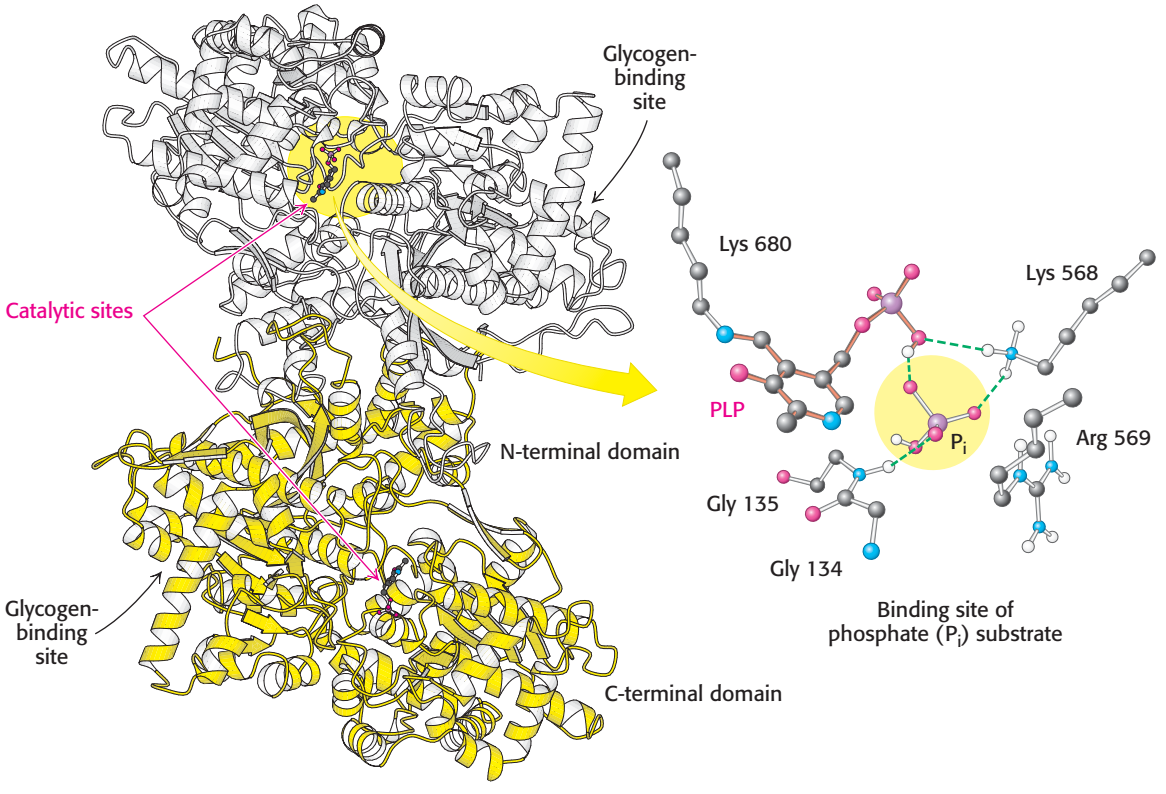


Figure 21.5 Structure of glycogen phosphorylase. This enzyme forms a homodimer: one subunit is shown in white and the other in yellow. Each catalytic site includes a pyridoxal phosphate (PLP) group, linked to lysine 680 of the enzyme. The binding site for the phosphate (P_i) substrate is shown. *Notice* that the catalytic site lies between the C-terminal domain and the glycogen-binding site. A narrow crevice, which binds four or five glucose units of glycogen, connects the two sites. The separation of the sites allows the catalytic site to phosphorolyze several glucose units before the enzyme must rebind the glycogen substrate. [Drawn from 1NOI.pdb.]

Several clues suggest a mechanism by which phosphorylase achieves the exclusion of water. First, both the glycogen substrate and the glucose 1-phosphate product have an α configuration at C-1. A direct attack by phosphate on C-1 of a sugar would invert the configuration at this carbon atom because the reaction would proceed through a pentacovalent transition state. The fact that the glucose 1-phosphate formed has an α rather than a β configuration suggests that an even number of steps (most simply, two) is required. The most likely explanation for these results is that a *carbonium ion intermediate* is formed from the glucose residue.

A second clue to the catalytic mechanism of phosphorylase is its requirement for the coenzyme *pyridoxal phosphate* (PLP), a derivative of pyridoxine (vitamin B_6, Section 15.4). The aldehyde group of this coenzyme forms a Schiff-base linkage with a specific lysine side chain of the enzyme (Figure 21.6). Structural studies indicate that the reacting orthophosphate group takes a position between the 5′-phosphate group of PLP and the glycogen substrate (Figure 21.7). *The 5′-phosphate group of PLP acts in tandem with orthophosphate by serving as a proton donor and then as a proton acceptor (i.e., as a general acid–base catalyst).* Orthophosphate (in the HPO_4^{2-} form) donates a proton to the oxygen atom attached to carbon 4 of the departing glycogen chain and simultaneously acquires a proton from PLP. The carbocation (carbonium ion) intermediate formed in this step is then attacked by orthophosphate to form α-glucose 1-phosphate, with the concomitant return of a hydrogen atom to pyridoxal phosphate. The special role of pyridoxal phosphate in the reaction is necessary because water is excluded from the active site.

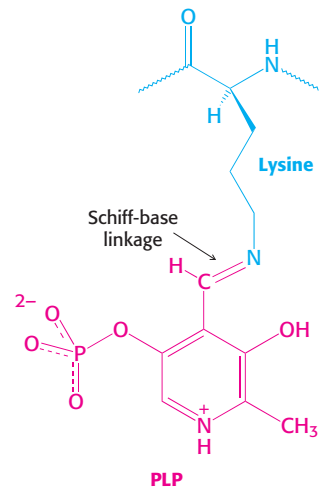


Figure 21.6 PLP–Schiff-base linkage. A pyridoxal phosphate (PLP) group (red) forms a Schiff base with a lysine residue (blue) at the active site of phosphorylase.

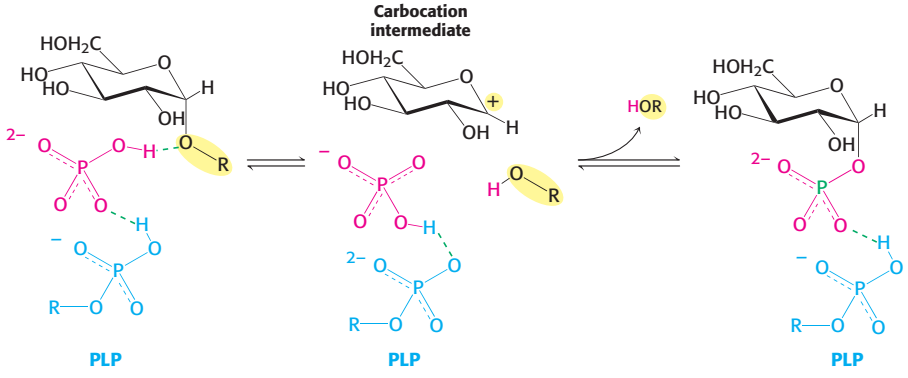


Figure 21.7 Phosphorylase mechanism. A bound HPO_4^{2-} group (red) favors the cleavage of the glycosidic bond by donating a proton to the departing glucose (black). This reaction results in the formation of a carbocation and is favored by the transfer of a proton from the protonated phosphate group of the bound pyridoxal phosphate (PLP) group (blue). The carbocation and the orthophosphate combine to form glucose 1-phosphate.

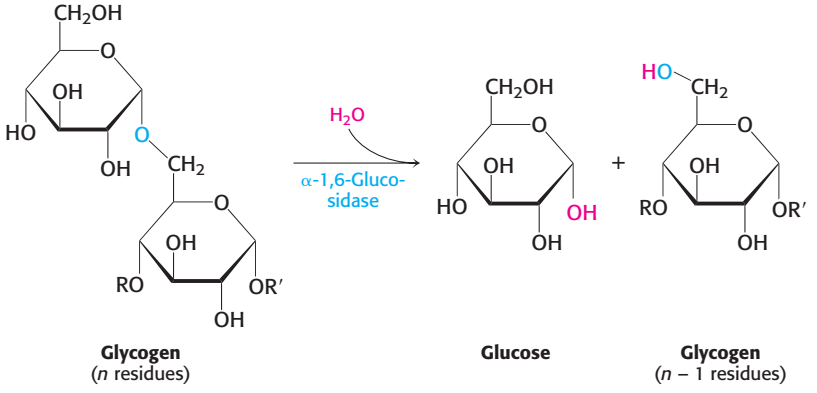
The glycogen-binding site is 30 Å away from the catalytic site (see Figure 21.5), but it is connected to the catalytic site by a narrow crevice able to accommodate four or five glucose units. The large separation between the binding site and the catalytic site enables the enzyme to phosphorolyze many residues without having to dissociate and reassociate after each catalytic cycle. An enzyme that can catalyze many reactions without having to dissociate and reassociate after each catalytic step is said to be *processive*—a property of enzymes that synthesize and degrade large polymers. We will see such enzymes again when we consider DNA and RNA synthesis.

A debranching enzyme also is needed for the breakdown of glycogen

Glycogen phosphorylase acting alone degrades glycogen to a limited extent. The enzyme can break α-1,4-glycosidic bonds on glycogen branches but soon encounters an obstacle. The α-1,6-glycosidic bonds at the branch points are not susceptible to cleavage by phosphorylase. Indeed, phosphorylase stops cleaving α-1,4 linkages when it reaches a terminal residue four

residues away from a branch point. Because about 1 in 10 residues is branched, cleavage by the phosphorylase alone would come to a halt after the release of six glucose molecules per branch.

How can the remainder of the glycogen molecule be mobilized for use as a fuel? Two additional enzymes, a *transferase* and *α-1,6-glucosidase,* remodel the glycogen for continued degradation by the phosphorylase (Figure 21.8). *The transferase shifts a block of three glucosyl residues from one outer branch to another.* This transfer exposes a single glucose residue joined by an α-1,6-glycosidic linkage. α-1,6-Glucosidase, also known as the debranching enzyme, hydrolyzes the α-1,6-glycosidic bond.



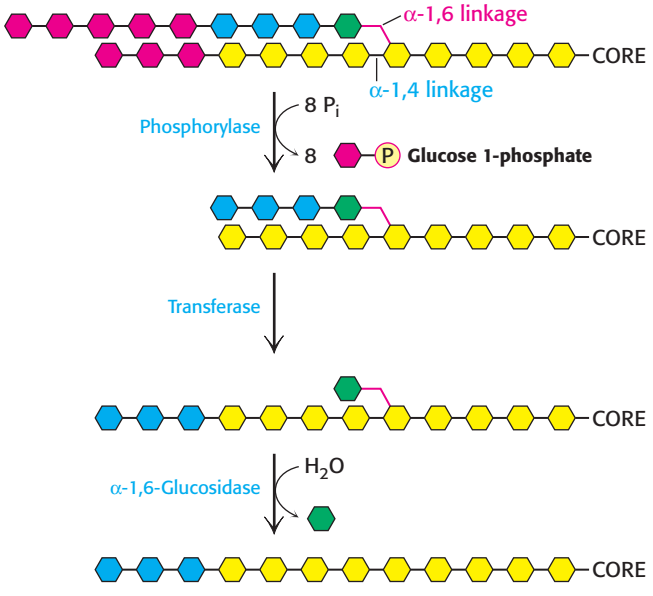


Figure 21.8 Glycogen remodeling. First, α-1,4-glycosidic bonds on each branch are cleaved by phosphorylase, leaving four residues along each branch. The transferase shifts a block of three glucosyl residues from one outer branch to the other. In this reaction, the α-1,4-glycosidic link between the blue and the green residues is broken and a new α-1,4 link between the blue and the yellow residues is formed. The green residue is then removed by α-1,6-glucosidase, leaving a linear chain with all α-1,4 linkages, suitable for further cleavage by phosphorylase.

A free glucose molecule is released and then phosphorylated by the glycolytic enzyme hexokinase. Thus, the transferase and α-1,6-glucosidase convert the branched structure into a linear one, which paves the way for further cleavage by phosphorylase. In eukaryotes, the transferase and the α-1,6-glucosidase activities are present in a single 160-kd polypeptide chain, providing yet another example of a bifunctional enzyme (see Figure 16.29).

Phosphoglucomutase converts glucose 1-phosphate into glucose 6-phosphate

Glucose 1-phosphate formed in the phosphorolytic cleavage of glycogen must be converted into glucose 6-phosphate to enter the metabolic mainstream. This shift of a phosphoryl group is catalyzed by *phosphoglucomutase.* Recall that this enzyme is also used in galactose metabolism (Section 16.1). To effect this shift, the enzyme exchanges a phosphoryl group with the substrate (Figure 21.9). The catalytic site of an active mutase molecule contains a phosphorylated serine residue. The phosphoryl group is transferred from the serine residue to the C-6 hydroxyl group of glucose 1-phosphate to form glucose 1,6-bisphosphate. The C-1 phosphoryl group of this intermediate is then shuttled to the same serine residue, resulting in the formation of glucose 6-phosphate and the regeneration of the phosphoenzyme.

These reactions are like those of *phosphoglycerate mutase,* a glycolytic enzyme (Section 16.1). The role of glucose 1,6-bisphosphate in the interconversion of the phosphoglucoses is like that of 2,3-bisphosphoglycerate (2,3-BPG) in the interconversion of 2-phosphoglycerate and 3-phosphoglycerate in glycolysis. A phosphoenzyme intermediate participates in both reactions.

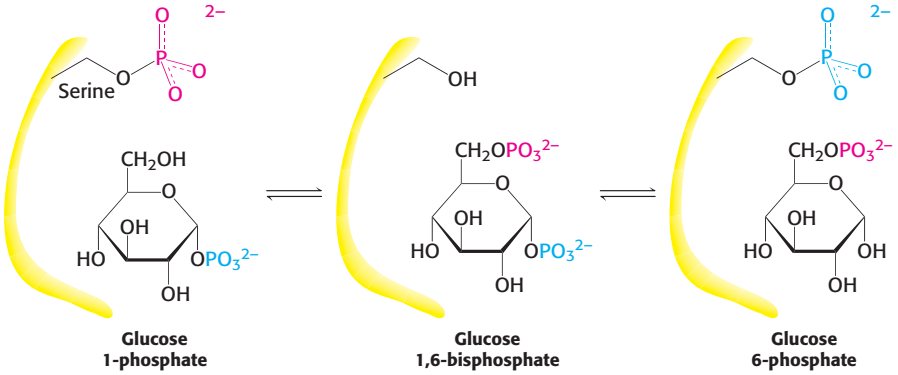


Figure 21.9 Reaction catalyzed by phosphoglucomutase. A phosphoryl group is transferred from the enzyme to the substrate, and a different phosphoryl group is transferred back to restore the enzyme to its initial state.

The liver contains glucose 6-phosphatase, a hydrolytic enzyme absent from muscle

A major function of the liver is to maintain a nearly constant level of glucose in the blood. The liver releases glucose into the blood during muscular activity and between meals. The released glucose is taken up primarily by the brain and skeletal muscle. In contrast with unmodified glucose, however, the phosphorylated glucose produced by glycogen breakdown is not transported out of cells. The liver contains a hydrolytic enzyme, *glucose 6-phosphatase* that enables glucose to leave that organ. This enzyme cleaves the phosphoryl group to form free glucose and orthophosphate. This glucose 6-phosphatase is the same enzyme that releases free glucose at the conclusion of gluconeogenesis. It is located on the lumenal side of the smooth endoplasmic reticulum membrane. Recall that glucose 6-phosphate is transported into the endoplasmic reticulum; glucose and orthophosphate formed by hydrolysis are then shuttled back into the cytoplasm (Section 16.1).

$$\text{Glucose 6-phosphate} + H_2O \longrightarrow \text{glucose} + P_i$$

Glucose 6-phosphatase is absent from most other tissues. Muscle tissues retain glucose 6-phosphate for the generation of ATP. In contrast, glucose is not a major fuel for the liver.

21.2 Phosphorylase Is Regulated by Allosteric Interactions and Reversible Phosphorylation

Glycogen metabolism is precisely controlled by multiple interlocking mechanisms. The focus of this control is the enzyme glycogen phosphorylase. *Phosphorylase is regulated by several allosteric effectors that signal the energy state of the cell as well as by reversible phosphorylation, which is responsive to hormones such as insulin, epinephrine, and glucagon.* We will examine the differences in the control of glycogen metabolism in two tissues: skeletal muscle and liver. These differences are due to the fact that *the muscle uses glucose to produce energy for itself, whereas the liver maintains glucose homeostasis of the organism as a whole.*

Muscle phosphorylase is regulated by the intracellular energy charge

The dimeric skeletal-muscle phosphorylase exists in two interconvertible forms: a *usually active* phosphorylase *a* and a *usually inactive* phosphorylase *b* (Figure 21.10). Each of these two forms exists in equilibrium between an active relaxed (R) state and a much less active tense (T) state, but the equilibrium for phosphorylase *a* favors the active R state, whereas the equilibrium for phosphorylase *b* favors the less-active T state (Figure 21.11). The

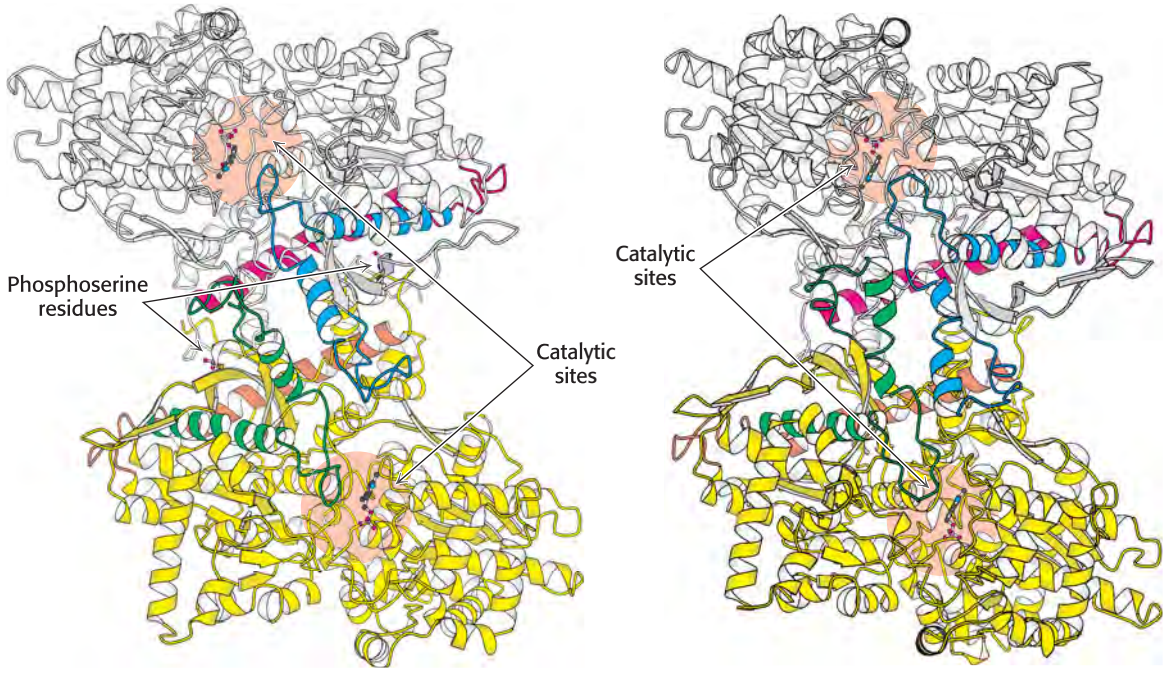


Figure 21.10 Structures of phosphorylase *a* and phosphorylase *b*. Phosphorylase *a* is phosphorylated on serine 14 of each subunit. This modification favors the structure of the more-active R state. One subunit is shown in white, with helices and loops important for regulation shown in blue and red. The other subunit is shown in yellow, with the regulatory structures shown in orange and green. Phosphorylase *b* is not phosphorylated and exists predominantly in the T state. *Notice* that the catalytic sites are partly occluded in the T state. [Drawn from 1GPA.pdb and 1NOJ.pdb.]

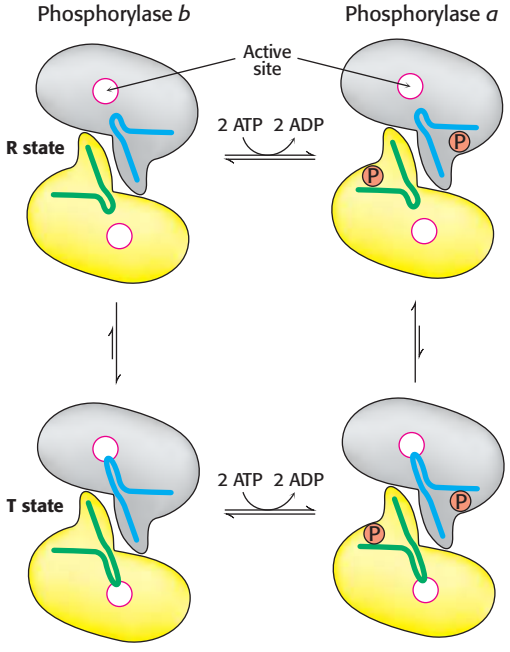


Figure 21.11 Phosphorylase regulation. Both phosphorylase *b* and phosphorylase *a* exist as equilibria between an active R state and a less-active T state. Phosphorylase *b* is usually inactive because the equilibrium favors the T state. Phosphorylase *a* is usually active because the equilibrium favors the R state. Regulatory structures are shown in blue and green.

default state of muscle phosphorylase is the *b* form, owing to the fact that, for muscle, phosphorylase needs to be active during muscle contraction. Muscle phosphorylase *b* is activated by the presence of high concentrations of AMP, which binds to a nucleotide-binding site and stabilizes the conformation of phosphorylase *b* in the active R state (Figure 21.12). Thus, when a muscle contracts and ATP is converted into AMP, the phosphorylase is signaled to degrade glycogen. ATP acts as a negative allosteric effector by competing with AMP. Thus, *the transition of phosphorylase* b *between the active R state and the less-active T state is controlled by the energy charge of the muscle cell.* Glucose 6-phosphate also binds to and stabilizes the less-

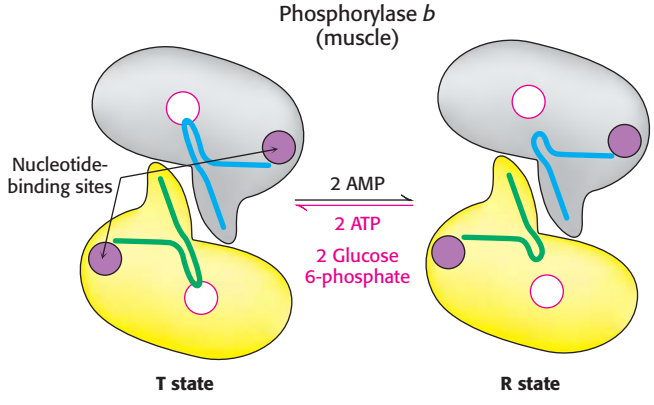


Figure 21.12 Allosteric regulation of muscle phosphorylase. A low energy charge, represented by high concentrations of AMP, favors the transition to the R state.

active state of phosphorylase *b*, an example of feedback inhibition. Under most physiological conditions, *phosphorylase* b *is inactive because of the inhibitory effects of ATP and glucose 6-phosphate.* In contrast, *phosphorylase* a *is fully active,* regardless of the levels of AMP, ATP, and glucose 6-phosphate. In resting muscle, nearly all the enzyme is in the inactive *b* form.

Phosphorylase *b* is converted into phosphorylase *a* by the phosphorylation of a single serine residue (serine 14) in each subunit. This conversion is initiated by hormones. Fear or the excitement of exercise will cause levels of the hormone epinephrine to increase. The increase in hormone levels and the electrical stimulation of muscle result in phosphorylation of the enzyme to the phosphorylase *a* form. The regulatory enzyme *phosphorylase kinase* catalyzes this covalent modification.

Comparison of the structures of phosphorylase *a* in the R state and phosphorylase *b* in the T state reveals that subtle structural changes at the subunit interfaces are transmitted to the active sites (see Figure 21.10). The transition from the T state (the prevalent state of phosphorylase *b*) to the R state (the prevalent state of phosphorylase *a*) entails a 10-degree rotation around the twofold axis of the dimer. Most importantly, this transition is associated with structural changes in α helices that move a loop out of the active site of each subunit. Thus, the T state is less active because the catalytic site is partly blocked. In the R state, the catalytic site is more accessible and a binding site for orthophosphate is well organized.

Liver phosphorylase produces glucose for use by other tissues

The role of glycogen degradation in the liver is to form glucose for *export to other tissues* when the blood-glucose level is low. Consequently, we can think of the default state of liver phosphorylase as being the *a* form: glucose is to be generated unless the enzyme is signaled otherwise. The liver phosphorylase *a* form thus exhibits the most responsive R ↔ T transition (Figure 21.13). The binding of glucose shifts the *a* form from the active R state to the less-active T state. In essence, the enzyme reverts to the low-activity T state only when it detects the presence of sufficient glucose. If glucose is present in the diet, there is no need to degrade glycogen. As we will see later, the presence of glucose also facilitates the *a*-to-*b* transition.

The regulation of liver phosphorylase differs from that of muscle phosphorylase. In muscle, the default state is the *b* form: there is no need to generate glucose unless energy is required. As discussed previously, AMP shifts the muscle *b* form from the T to the R state. Unlike the enzyme in muscle, the liver phosphorylase is insensitive to regulation by AMP because the liver does not undergo the dramatic changes in energy charge seen in a contracting muscle. We see here a clear example of the use of isozymic forms of the same enzyme to establish the tissue-specific biochemical properties of muscle and the liver. In human beings, liver phosphorylase and muscle phosphorylase are approximately 90% identical in amino acid sequence, yet the 10% difference results in subtle but important shifts in the stability of various forms of the enzyme.

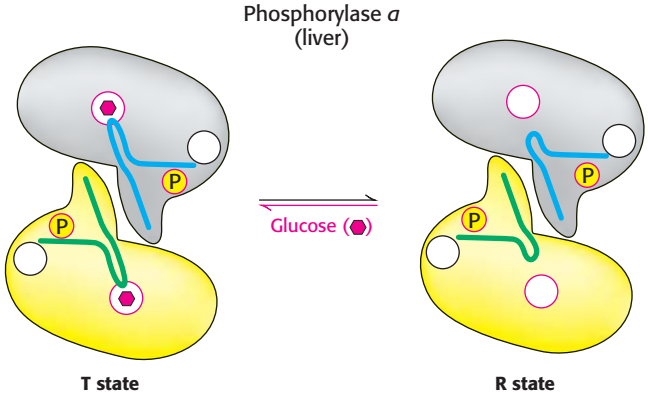


Figure 21.13 Allosteric regulation of liver phosphorylase. The binding of glucose to phosphorylase *a* shifts the equilibrium to the T state and inactivates the enzyme. Thus, glycogen is not mobilized when glucose is already abundant.

Phosphorylase kinase is activated by phosphorylation and calcium ions

Phosphorylase kinase activates phosphorylase *b* by attaching a phosphoryl group. The subunit composition of phosphorylase kinase in skeletal muscle

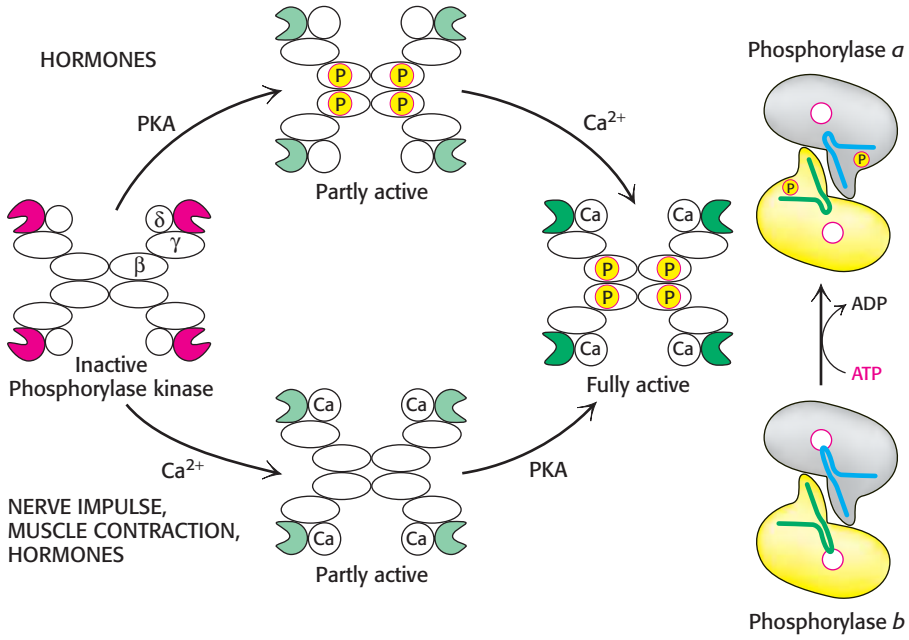


Figure 21.14 Activation of phosphorylase kinase. Phosphorylase kinase, an $(\alpha\beta\gamma\delta)_4$ assembly, is activated by hormones that lead to the phosphorylation of the β subunit and by Ca^{2+} binding to the δ subunit. Both types of stimulation are required for maximal enzyme activity. When active, the enzyme converts phosphorylase *b* into phosphorylase *a*.

is $(\alpha\beta\gamma\delta)_4$, and the mass of this very large protein is 1200 kd. The catalytic activity resides in the γ subunit, whereas the other subunits serve regulatory functions. This kinase is under dual control: it is activated both by phosphorylation by phosphorylase kinase A (PKA) and by increases in Ca^{2+} levels (Figure 21.14). Like its own substrate, phosphorylase kinase is activated by phosphorylation: the kinase is converted from *a low-activity form into a high-activity one by phosphorylation of its β subunit.* The activation of phosphorylase kinase is one step in a signal-transduction cascade initiated by hormones.

Phosphorylase kinase can also be partly activated by Ca^{2+} levels of the order of 1 μM. Its δ subunit is *calmodulin,* a calcium sensor that stimulates many enzymes in eukaryotes. This mode of activation of the kinase is especially noteworthy in muscle, where contraction is triggered by the release of Ca^{2+} from the sarcoplasmic reticulum. Phosphorylase kinase attains maximal activity only after both phosphorylation of the β subunit and activation of the δ subunit by Ca^{2+} binding.

21.3 Epinephrine and Glucagon Signal the Need for Glycogen Breakdown

Protein kinase A activates phosphorylase kinase, which in turn activates glycogen phosphorylase. What activates protein kinase A? What is the signal that ultimately triggers an increase in glycogen breakdown?

G proteins transmit the signal for the initiation of glycogen breakdown

Several hormones greatly affect glycogen metabolism. Glucagon and epinephrine trigger the breakdown of glycogen. Muscular activity or its anticipation leads to the release of *epinephrine (adrenaline),* a catecholamine derived from tyrosine, from the adrenal medulla. Epinephrine markedly stimulates glycogen breakdown in muscle and, to a lesser extent, in the liver. The liver is more responsive to *glucagon,* a polypeptide hormone secreted by the α cells of the pancreas when the blood-sugar level is low. Physiologically, glucagon signifies the starved state (Figure 21.15).

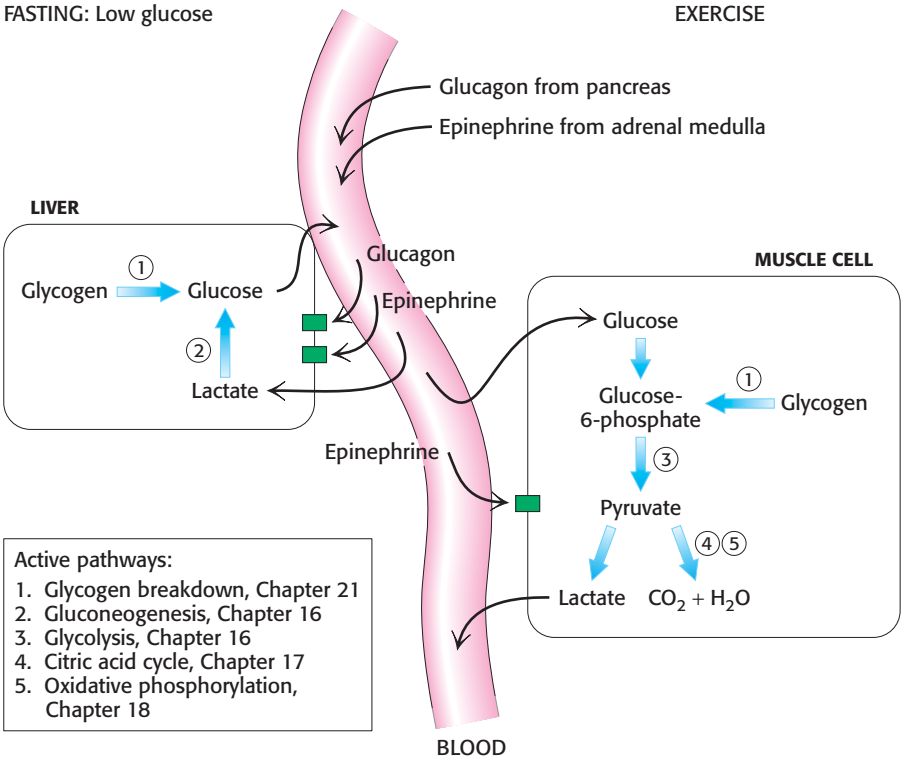


Figure 21.15 PATHWAY INTEGRATION: Hormonal control of glycogen breakdown. Glucagon stimulates liver-glycogen breakdown when blood glucose is low. Epinephrine enhances glycogen breakdown in muscle and the liver to provide fuel for muscle contraction.

How do hormones trigger the breakdown of glycogen? They initiate a cyclic AMP signal-transduction cascade, already discussed in Section 16.1 (Figure 21.16).

1. The signal molecules epinephrine and glucagon bind to specific seven-transmembrane (7TM) receptors in the plasma membranes of target cells (Section 14.1). Epinephrine binds to the β-adrenergic receptor in muscle,

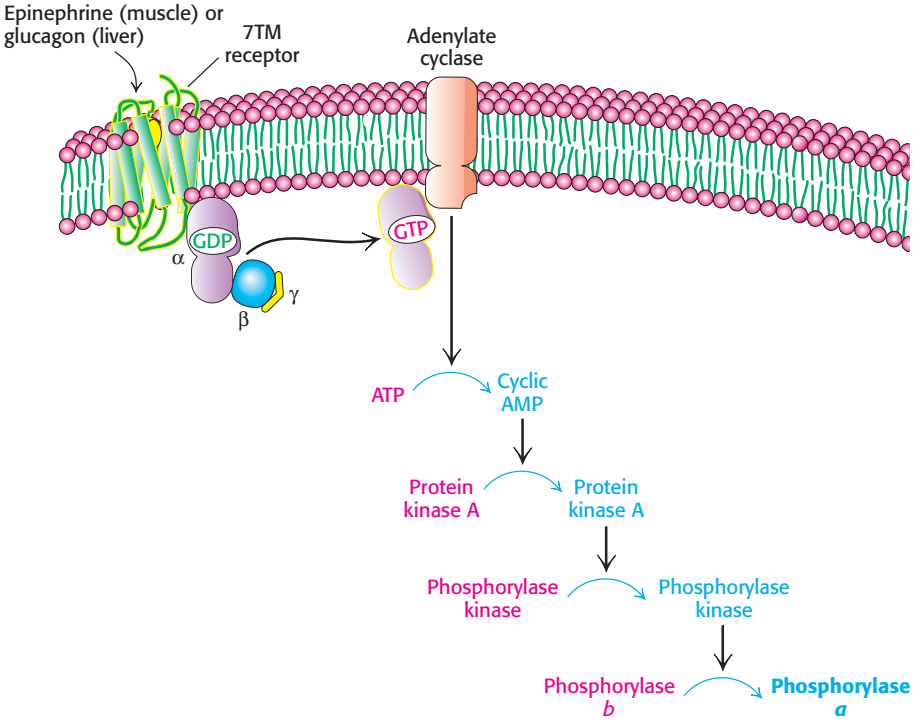
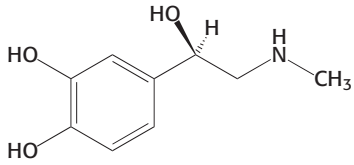


Figure 21.16 Regulatory cascade for glycogen breakdown. Glycogen degradation is stimulated by hormone binding to 7TM receptors. Hormone binding initiates a G-protein-dependent signal-transduction pathway that results in the phosphorylation and activation of glycogen phosphorylase.

whereas glucagon binds to the glucagon receptor in the liver. These binding events activate the G_s protein. *A specific external signal has been transmitted into the cell through structural changes,* first in the receptor and then in the G protein.



Epinephrine

$^{+}H_3N$–His–Ser–Glu–Gly–Thr–Phe–Thr–Ser–Asp–Tyr–
–Ser–Lys–Tyr–Leu–Asp–Ser–Arg–Arg–Ala–Gln–
–Asp–Phe–Val–Gln–Trp–Leu–Met–Asn–Thr–COO^{-}

Glucagon

2. The GTP-bound subunit of G_s activates the transmembrane protein adenylate cyclase. This enzyme catalyzes the formation of the second messenger cyclic AMP from ATP.

3. The elevated cytoplasmic level of cyclic AMP activates *protein kinase A* (Section 10.3). The binding of cyclic AMP to inhibitory regulatory subunits triggers their dissociation from the catalytic subunits. The free catalytic subunits are now active.

4. Protein kinase A phosphorylates phosphorylase kinase first on β subunit and then on the α subunit, which subsequently activates glycogen phosphorylase.

The cyclic AMP cascade highly amplifies the effects of hormones. The binding of a small number of hormone molecules to cell-surface receptors leads to the release of a very large number of sugar units. Indeed, much of the stored glycogen would be mobilized within seconds were it not for a counterregulatory system.

The signal-transduction processes in the liver are more complex than those in muscle. Epinephrine can also elicit glycogen degradation in the liver. However, in addition to binding to the β-adrenergic receptor, it binds to the 7TM α-adrenergic receptor, which then initiates the *phosphoinositide cascade* (Section 14.2) that induces the release of Ca^{2+} from endoplasmic reticulum stores. Recall that the δ subunit of phosphorylase kinase is the Ca^{2+} sensor calmodulin. The binding of Ca^{2+} to calmodulin leads to a partial activation of phosphorylase kinase. Stimulation by both glucagon and epinephrine leads to maximal mobilization of liver glycogen.

Glycogen breakdown must be rapidly turned off when necessary

There must be a way to shut down the high-gain system of glycogen breakdown quickly to prevent the wasteful depletion of glycogen after energy needs have been met. When glucose needs have been satisfied, phosphorylase kinase and glycogen phosphorylase are dephosphorylated and inactivated. Simultaneously, glycogen synthesis is activated.

The signal-transduction pathway leading to the activation of glycogen phosphorylase is shut down automatically when the initiating hormone is no longer present. The inherent GTPase activity of the G protein converts the bound GTP into inactive GDP, and phosphodiesterases always present in the cell convert cyclic AMP into AMP. Protein phosphatase 1 (PP1) removes the phosphoryl groups from phosphorylase kinase, thereby inactivating the enzyme. Finally, protein phosphatase 1 also removes the phosphoryl group from glycogen phosphorylase, converting the enzyme into the usually inactive *b* form.

The regulation of glycogen phosphorylase became more sophisticated as the enzyme evolved

Analyses of the primary structures of glycogen phosphorylase from human beings, rats, *Dictyostelium* (slime mold), yeast, potatoes, and *E. coli* have enabled inferences to be made about the evolution of this important enzyme. The 16 residues that come into contact with glucose at the active site are identical in nearly all the enzymes. There is more variation but still substantial conservation of the 15 residues at the pyridoxal phosphate-binding site. Likewise, the glycogen-binding site is well conserved in all the enzymes. The high degree of similarity among these three sites shows that the catalytic mechanism has been maintained throughout evolution.

Differences arise, however, when we compare the regulatory sites. The simplest type of regulation would be feedback inhibition by glucose 6-phosphate. Indeed, the glucose 6-phosphate regulatory site is highly conserved among most of the phosphorylases. The crucial amino acid residues that participate in regulation by phosphorylation and nucleotide binding are well conserved only in the mammalian enzymes. Thus, this level of regulation was a later evolutionary acquisition.

21.4 Glycogen Is Synthesized and Degraded by Different Pathways

As with glycolysis and gluconeogenesis, biosynthetic and degradative pathways rarely operate by precisely the same reactions in the forward and reverse directions. Glycogen metabolism provided the first known example of this important principle. *Separate pathways afford much greater flexibility, both in energetics and in control.*

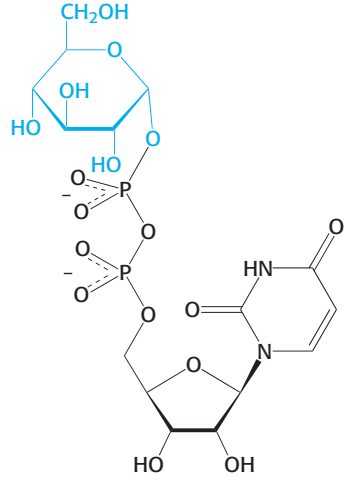
In 1957, Luis Leloir and his coworkers showed that glycogen is synthesized by a pathway that utilizes *uridine diphosphate glucose* (UDP-glucose) rather than glucose 1-phosphate as the activated glucose donor.

$$\text{Synthesis: Glycogen}_n + \text{UDP-glucose} \longrightarrow \text{glycogen}_{n+1} + \text{UDP}$$
$$\text{Degradation: Glycogen}_{n+1} + P_i \longrightarrow \text{glycogen}_n + \text{glucose 1-phosphate}$$

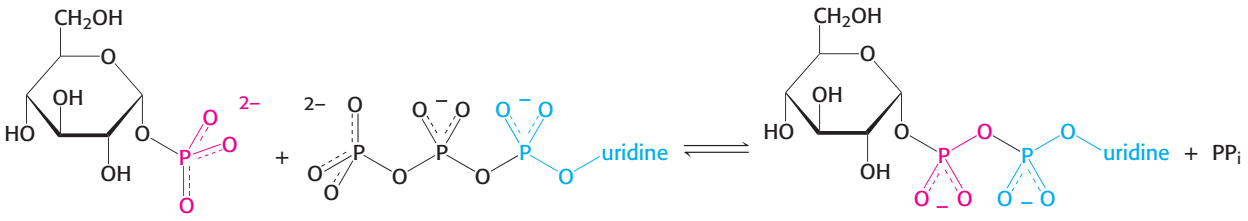
UDP-glucose is an activated form of glucose

UDP-glucose, the glucose donor in the biosynthesis of glycogen, is an *activated form of glucose,* just as ATP and acetyl CoA are activated forms of orthophosphate and acetate, respectively. The C-1 carbon atom of the glucosyl unit of UDP-glucose is activated because its hydroxyl group is esterified to the diphosphate moiety of UDP.

UDP-glucose is synthesized from glucose 1-phosphate and uridine triphosphate (UTP) in a reaction catalyzed by *UDP-glucose pyrophosphorylase.* This reaction liberates the outer two phosphoryl residues of UTP as pyrophosphate.



Uridine diphosphate glucose (UDP-glucose)



Glucose 1-phosphate **UTP** **UDP-glucose**

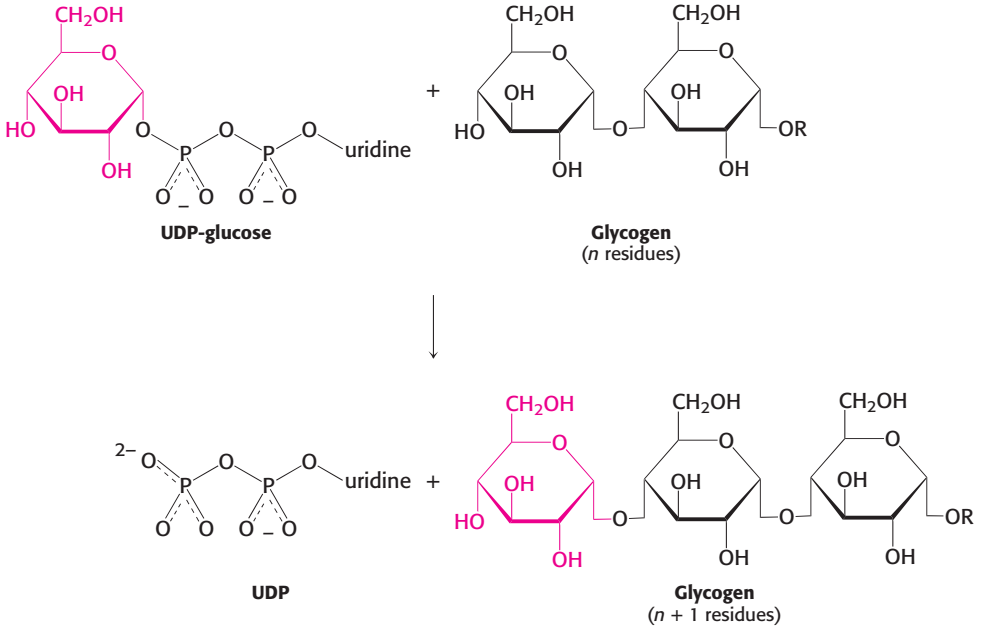
This reaction is readily reversible. However, pyrophosphate is rapidly hydrolyzed in vivo to orthophosphate by an inorganic pyrophosphatase. The essentially irreversible hydrolysis of pyrophosphate drives the synthesis of UDP-glucose.

$$\begin{array}{r} \text{Glucose 1-phosphate} + \text{UTP} \rightleftharpoons \text{UDP-glucose} + \text{PP}_i \\ \text{PP}_i + \text{H}_2\text{O} \longrightarrow 2\,\text{P}_i \\ \hline \text{Glucose 1-phosphate} + \text{UTP} + \text{H}_2\text{O} \longrightarrow \text{UDP-glucose} + 2\,\text{P}_i \end{array}$$

The synthesis of UDP-glucose exemplifies another recurring theme in biochemistry: *many biosynthetic reactions are driven by the hydrolysis of pyrophosphate.*

Glycogen synthase catalyzes the transfer of glucose from UDP-glucose to a growing chain

New glucosyl units are added to the nonreducing terminal residues of glycogen. The activated glucosyl unit of UDP-glucose is transferred to the hydroxyl group at C-4 of a terminal residue to form an α-1,4-glycosidic linkage. UDP is displaced by the terminal hydroxyl group of the growing glycogen molecule. This reaction is catalyzed by *glycogen synthase, the key regulatory enzyme in glycogen synthesis.*



Glycogen synthase can add glucosyl residues only to a polysaccharide chain already containing more than four residues. Thus, glycogen synthesis requires a *primer*. This priming function is carried out by *glycogenin,* a glycosyltransferase (see Figure 11.25) composed of two identical 37-kd subunits. Each subunit of glycogenin catalyzes the addition of eight glucosyl units to the other subunit. These glucosyl units form short α-1,4-glucose polymers, which are covalently attached to the phenolic hydroxyl group of a specific tyrosine residue in each glycogenin subunit. UDP-glucose is the donor in this autoglycosylation. At this point, glycogen synthase takes over to extend the glycogen molecule. Thus, every glycogen molecule has a glycogenin molecule at its core (see Figure 21.1).

Despite no detectable sequence similarity, structural studies have revealed that glycogen synthase is homologous to glycogen phosphorylase. The binding site for UDP-glucose in glycogen synthase corresponds in position to the pyridoxal phosphate in glycogen phosphorylase.

A branching enzyme forms α-1,6 linkages

Glycogen synthase catalyzes only the synthesis of α-1,4 linkages. Another enzyme is required to form the α-1,6 linkages that make glycogen a branched polymer. Branching takes place after a number of glucosyl residues are joined in α-1,4 linkages by glycogen synthase (Figure 21.17). A branch is created by the breaking of an α-1,4 link and the formation of an α-1,6 link: this reaction is different from debranching. A block of residues, typically 7 in number, is transferred to a more interior site. The *branching enzyme* that catalyzes this reaction requires that the block of 7 or so residues must include the nonreducing terminus, and must come from a chain at least 11 residues long. In addition, the new branch point must be at least 4 residues away from a preexisting one.

Branching is important because it increases the solubility of glycogen. Furthermore, branching creates a large number of terminal residues, the sites of action of glycogen phosphorylase and synthase (Figure 21.18). Thus, *branching increases the rate of glycogen synthesis and degradation.*

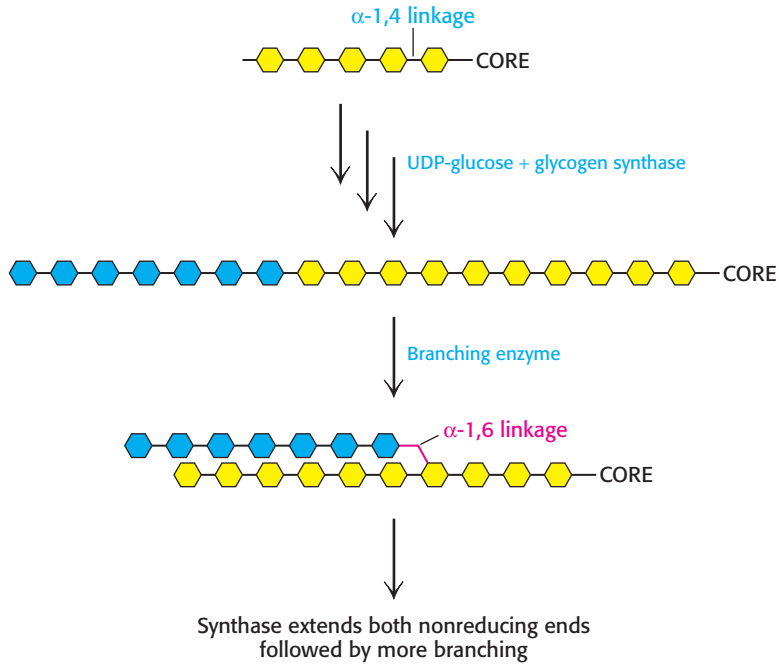


Figure 21.17 Branching reaction. The branching enzyme removes an oligosaccharide of approximately seven residues from the nonreducing end and creates an internal α-1,6 linkage.

Glycogen branching requires a single transferase activity. Glycogen debranching requires two enzyme activities: a transferase and an α-1,6 glucosidase. Sequence analysis suggests that the two transferases and, perhaps, the α-1,6 glucosidase are members of the same enzyme family, termed the *α-amylase family.* An enzyme of this family catalyzes a reaction by forming a covalent intermediate attached to a conserved aspartate residue. Thus, the branching enzyme appears to transfer a chain of glucose molecules from an α-1,4 linkage to an aspartate residue on the enzyme and then from this site to a more interior location on the glycogen molecule to form an α-1,6 linkage.

Glycogen synthase is the key regulatory enzyme in glycogen synthesis

The activity of glycogen synthase, like that of phosphorylase, is regulated by covalent modification. Glycogen synthase is phosphorylated at multiple sites by several protein kinases, notably protein kinase A and *glycogen synthase kinase* (GSK). The resulting alteration of the charges in the protein lead to its inactivation. *Phosphorylation has opposite effects on the enzymatic activities of glycogen synthase and phosphorylase.* Phosphorylation converts the active *a* form of the synthase into a usually inactive *b* form. The phosphorylated *b* form is active only if a high level of the allosteric activator glucose 6-phosphate is present, whereas the *a* form is active whether or not glucose 6-phosphate is present.

Glycogen is an efficient storage form of glucose

What is the cost of converting glucose 6-phosphate into glycogen and back into glucose 6-phosphate? The pertinent reactions have already been described, except for reaction 5, which is the regeneration of UTP. ATP phosphorylates UDP in a reaction catalyzed by *nucleoside diphosphokinase.*

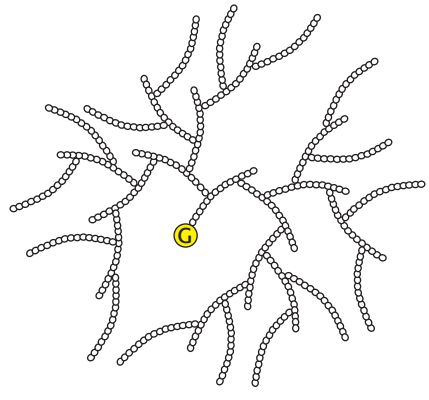


Figure 21.18 Cross section of a glycogen molecule. The component labeled G is glycogenin.

$$\text{Glucose 6-phosphate} \longrightarrow \text{glucose 1-phosphate} \quad (1)$$

$$\text{Glucose 1-phosphate} + \text{UTP} \longrightarrow \text{UDP-glucose} + PP_i \quad (2)$$

$$PP_i + H_2O \longrightarrow 2\,P_i \quad (3)$$

$$\text{UDP-glucose} + \text{glycogen}_n \longrightarrow \text{glycogen}_{n+1} + \text{UDP} \quad (4)$$

$$\text{UDP} + \text{ATP} \longrightarrow \text{UTP} + \text{ADP} \quad (5)$$

$$\text{Sum: Glucose 6-phosphate} + \text{ATP} + \text{glycogen}_n + H_2O \longrightarrow \text{glycogen}_{n+1} + \text{ADP} + 2\,P_i$$

Thus, 1 molecule of ATP is hydrolyzed to incorporate glucose 6-phosphate into glycogen. The energy yield from the breakdown of glycogen is highly efficient. About 90% of the residues are phosphorolytically cleaved to glucose 1-phosphate, which is converted at no cost into glucose 6-phosphate. The other 10% are branch residues, which are hydrolytically cleaved. One molecule of ATP is then used to phosphorylate each of these glucose molecules to glucose 6-phosphate. The complete oxidation of glucose 6-phosphate yields about 31 molecules of ATP, and storage consumes slightly more than 1 molecule of ATP per molecule of glucose 6-phosphate; so *the overall efficiency of storage is nearly 97%.*

21.5 Glycogen Breakdown and Synthesis Are Reciprocally Regulated

An important control mechanism prevents glycogen from being synthesized at the same time as it is being broken down. *The same glucagon- and epinephrine-triggered cAMP cascades that initiate glycogen breakdown in the liver and muscle, respectively, also shut off glycogen synthesis. Glucagon and*

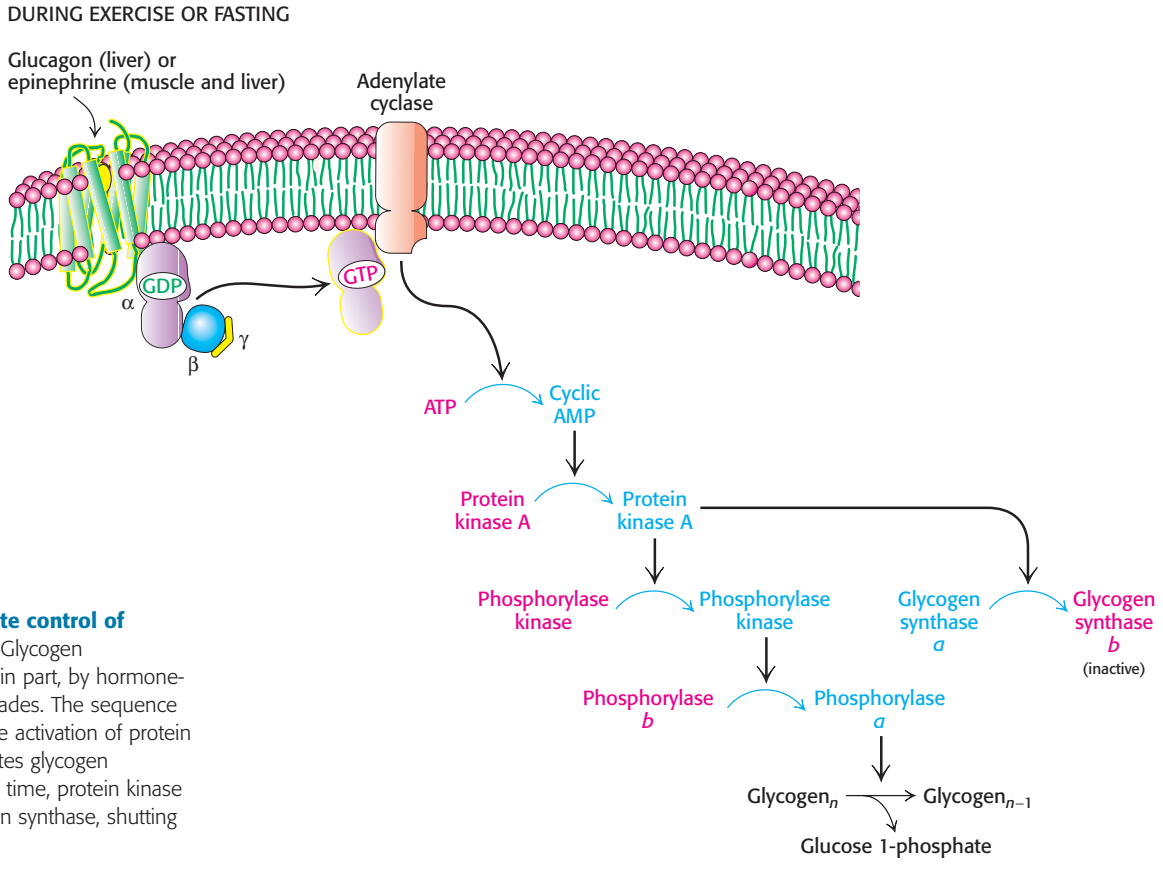


Figure 21.19 Coordinate control of glycogen metabolism. Glycogen metabolism is regulated, in part, by hormone-triggered cyclic AMP cascades. The sequence of reactions leading to the activation of protein kinase A ultimately activates glycogen degradation. At the same time, protein kinase A also inactivates glycogen synthase, shutting down glycogen synthesis.

epinephrine control both glycogen breakdown and glycogen synthesis through protein kinase A (Figure 21.19). Recall that protein kinase A adds a phosphoryl group to phosphorylase kinase, activating that enzyme and initiating glycogen breakdown. Likewise, protein kinase A adds a phosphoryl group to glycogen synthase, but this phosphorylation leads to a *decrease* in enzymatic activity. Other kinases, such as glycogen synthase kinase, help to inactivate the synthase. In this way, glycogen breakdown and synthesis are reciprocally regulated. How is the enzymatic activity reversed so that glycogen breakdown halts and glycogen synthesis begins?

Protein phosphatase 1 reverses the regulatory effects of kinases on glycogen metabolism

After a bout of exercise, muscle must shift from a glycogen-degrading mode to one of glycogen replenishment. A first step in this metabolic task is to shut down the phosphorylated proteins that stimulate glycogen breakdown. This task is accomplished by *protein phosphatases* that catalyze the hydrolysis of phosphorylated serine and threonine residues in proteins. *Protein phosphatase 1 plays key roles in regulating glycogen metabolism* (Figure 21.20). PP1 inactivates phosphorylase *a* and phosphorylase kinase by dephosphorylating them. PP1 decreases the rate of glycogen breakdown; it reverses the effects of the phosphorylation cascade. Moreover, *PP1 also removes phosphoryl groups from glycogen synthase* b *to convert it into the much more active glycogen synthase* a *form.* Here, PP1 also accelerates glycogen synthesis. PP1 is yet another molecular device for coordinating carbohydrate storage.

The catalytic subunit of PP1 is a 37-kd single-domain protein. This subunit is usually bound to one of a family of regulatory subunits with masses of approximately 120 kd; in skeletal muscle and heart, the most prevalent regulatory subunit is called G_M, whereas, in the liver, the most

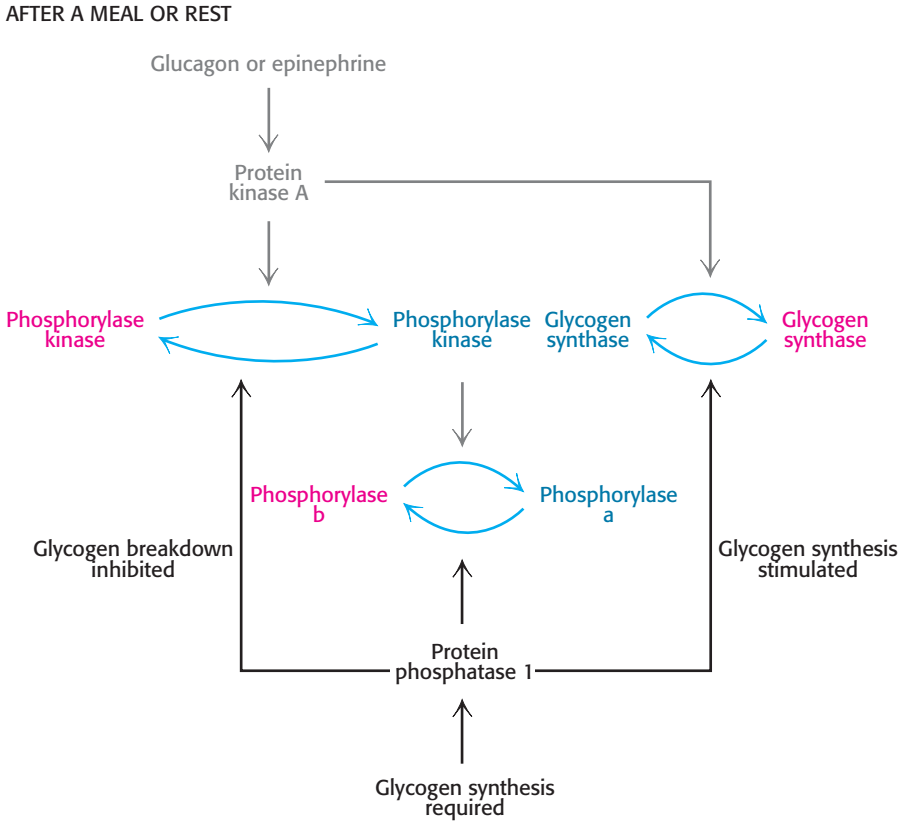


Figure 21.20 Regulation of glycogen synthesis by protein phosphatase 1. Protein phosphatase 1 stimulates glycogen synthesis while inhibiting glycogen breakdown.

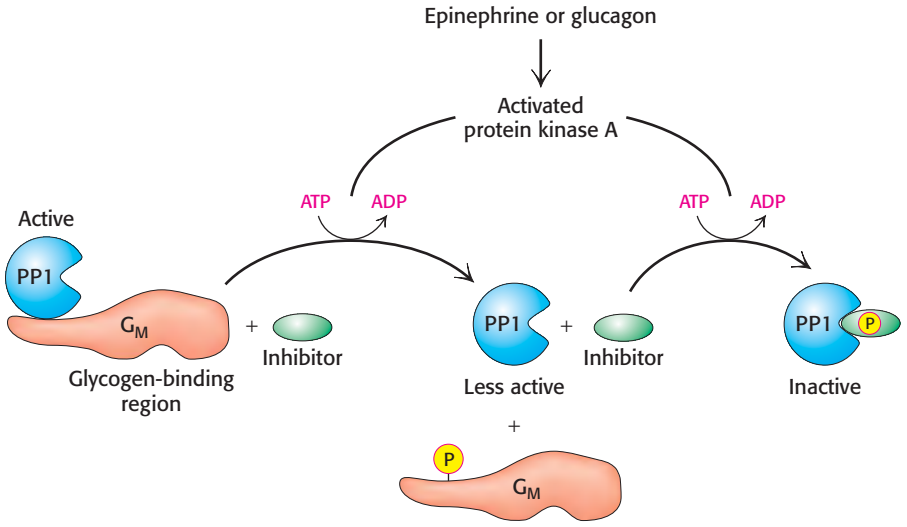


Figure 21.21 Regulation of protein phosphatase 1 (PP1) in muscle takes place in two steps. Phosphorylation of G_M by protein kinase A dissociates the catalytic subunit from its substrates in the glycogen particle. Phosphorylation of the inhibitor subunit by protein kinase A inactivates the catalytic unit of PP1.

prevalent subunit is G_L. These regulatory subunits have modular structures with domains that participate in interactions with glycogen, with the catalytic subunit, and with target enzymes. Thus, *these regulatory subunits act as scaffolds, bringing together the phosphatase and its substrates in the context of a glycogen particle.*

The phosphatase activity of PP1 must be reduced when glycogen degradation is called for (Figure 21.21). In such cases, epinephrine or glucagon has activated the cAMP cascade and protein kinase A is active. Protein kinase A reduces the activity of PP1 by two mechanisms. First, in muscle, G_M is phosphorylated in the domain responsible for binding the catalytic subunit. The catalytic subunit is released from glycogen and from its substrates and dephosphoryation is greatly reduced. Second, almost all tissues contain small proteins that, when phosphorylated, bind to the catalytic subunit of PP1 and inhibit it. Thus, when glycogen degradation is switched on by cAMP, the accompanying phosphorylation of these inhibitors keeps phosphorylase in its active *a* form and glycogen synthase in its inactive *b* form.

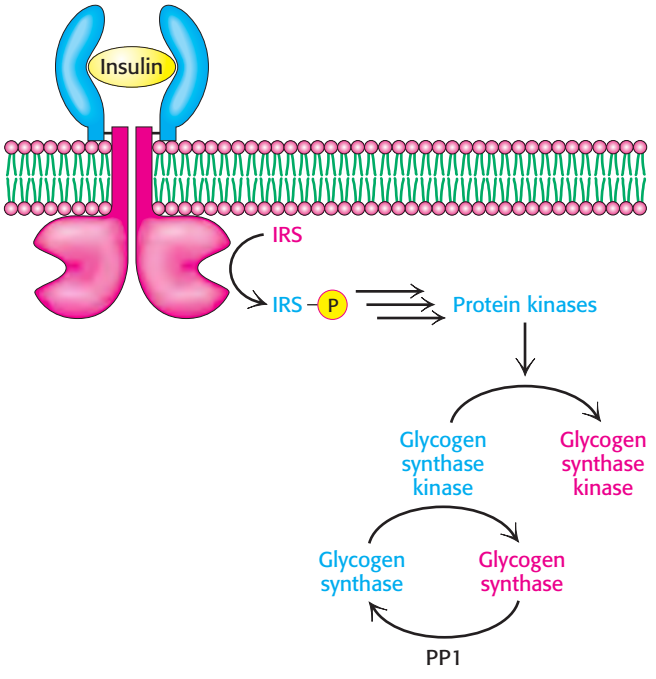


Figure 21.22 Insulin inactivates glycogen synthase kinase. Insulin triggers a cascade that leads to the phosphorylation and inactivation of glycogen synthase kinase and prevents the phosphorylation of glycogen synthase. Protein phosphatase 1 (PP1) removes the phosphates from glycogen synthase, thereby activating the enzyme and allowing glycogen synthesis. IRS, insulin-receptor substrate.

Insulin stimulates glycogen synthesis by inactivating glycogen synthase kinase

After exercise, people often consume carbohydrate-rich foods to restock their glycogen stores. How is glycogen synthesis stimulated? When blood-glucose levels are high, *insulin stimulates the synthesis of glycogen by inactivating glycogen synthase kinase,* the enzyme that maintains glycogen synthase in its phosphorylated, inactive state (Figure 21.22). The first step in the action of insulin is its binding to a receptor tyrosine kinase in the plasma membrane (Section 14.2). The binding of insulin activates the tyrosine kinase activity of the receptor so that it phosphorylates insulin-receptor substrates (IRSs). These phosphorylated proteins trigger signal-transduction pathways that eventually lead to the activation of protein kinases that phosphorylate and inactivate glycogen synthase kinase. The inactive kinase can no longer maintain

glycogen synthase in its phosphorylated, inactive state. Protein phosphatase 1 dephosphorylates glycogen synthase, activating it, and restoring glycogen reserves. Recall that insulin also generates an increase in the amount of glucose in the cell by increasing the number of glucose transports in the membrane. The net effect of insulin is thus the replenishment of glycogen stores.

Glycogen metabolism in the liver regulates the blood-glucose level

After a meal rich in carbohydrates, blood-glucose levels rise, and glycogen synthesis is stepped up in the liver. Although insulin is the primary signal for glycogen synthesis, another is the concentration of glucose in the blood, which normally ranges from about 80 to 120 mg per 100 ml (4.4–6.7 mM). The liver senses the concentration of glucose in the blood and takes up or releases glucose accordingly. The amount of liver phosphorylase *a* decreases rapidly when glucose is infused (Figure 21.23). After a lag period, the amount of glycogen synthase *a* increases, which results in glycogen synthesis. In fact, *phosphorylase* a *is the glucose sensor in liver cells.* Phosphorylase *a* and PP1 are localized to the glycogen particle by interactions with the G_L subunit of PP1. The binding of glucose to phosphorylase *a* shifts its allosteric equilibrium from the active R form to the inactive T form. This conformational change *renders the phosphoryl group on serine 14 a substrate for protein phosphatase 1.* PP1 binds tightly to phosphorylase *a* only when the phosphorylase is in the R state but is inactive when bound. When glucose induces the transition to the T form, PP1 and the phosphorylase dissociate from each other and the glycogen particle, and PP1 becomes active. Recall that the R ↔ T transition of muscle phosphorylase *a* is unaffected by glucose and is thus unaffected by the rise in blood-glucose levels (Section 21.2). Efforts are underway to develop drugs that disrupt the interaction of liver phosphorylase with the G_L subunit as a treatment for type 2 diabetes (Section 27.2). Type 2 diabetes is characterized by excess blood glucose. Hence, disrupting the association of phosphorylase with the G_L would render it a substrate for PP1, and glucose release into the blood would be inhibited.

How does glucose activate glycogen synthase? The conversion of *a* into *b* is accompanied by the *release of PP1, which is then free to activate glycogen synthase* and *dephosphorylate glycogen phosphorylase* (Figure 21.24). The

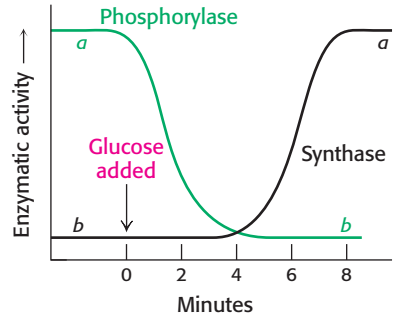


Figure 21.23 Blood glucose regulates liver-glycogen metabolism. The infusion of glucose into the bloodstream leads to the inactivation of phosphorylase, followed by the activation of glycogen synthase, in the liver. [After W. Stalmans, H. De Wulf, L. Hue, and H.-G. Hers. *Eur. J. Biochem.* 41:117–134, 1974.]

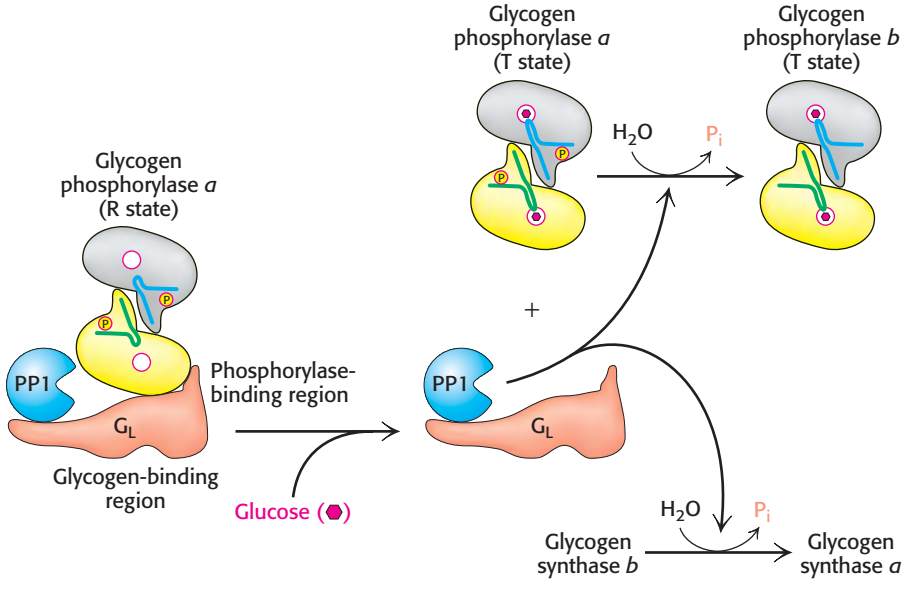


Figure 21.24 Glucose regulation of liver-glycogen metabolism. Glucose binds to and inhibits glycogen phosphorylase *a* in the liver, facilitating the formation of the T state of phosphorylase *a*. The T state of phosphorylase *a* does not bind protein phosphate 1 (PP1), leading to the dissociation and activation of PP1 from glycogen phosphorylase *a*. The free PP1 dephosphorylates glycogen phosphorylase *a* and glycogen synthase *b*, leading to the inactivation of glycogen breakdown and the activation of glycogen synthesis.

removal of the phosphoryl group of inactive glycogen synthase *b* converts it into the active *a* form. Initially, there are about 10 phosphorylase *a* molecules per molecule of phosphatase. Hence, *the activity of glycogen synthase begins to increase only after most of phosphorylase* a *is converted into* b. The lag between the decrease in glycogen degradation and the increase in glycogen synthesis prevents the two pathways from operating simultaneously. This remarkable glucose-sensing system depends on three key elements: (1) communication between the allosteric site for glucose and the serine phosphate, (2) the use of PP1 to inactivate phosphorylase and activate glycogen synthase, and (3) the binding of the phosphatase to phosphorylase *a* to prevent the premature activation of glycogen synthase.

A biochemical understanding of glycogen-storage diseases is possible

Edgar von Gierke described the first glycogen-storage disease in 1929. A patient with this disease has a huge abdomen caused by a massive enlargement of the liver. There is a pronounced hypoglycemia between meals. Furthermore, the blood-glucose level does not rise on administration of epinephrine and glucagon. An infant with this glycogen-storage disease may have convulsions because of the low blood-glucose level.

The enzymatic defect in von Gierke disease was elucidated in 1952 by Carl and Gerty Cori. They found that *glucose 6-phosphatase is missing from the liver of a patient with this disease.* This finding was the first demonstration of an inherited deficiency of a liver enzyme. The glycogen in the liver is normal in structure but is present in abnormally large amounts. The absence of glucose 6-phosphatase in the liver causes hypoglycemia because glucose cannot be formed from glucose 6-phosphate. This phosphorylated sugar does not leave the liver, because it cannot cross the plasma membrane. The presence of excess glucose 6-phosphate triggers an increase in glycolysis in the liver, leading to a high level of lactate and pyruvate in the blood. Patients who have von Gierke disease also have an increased dependence on fat metabolism. This disease can also be produced by a mutation in the gene

Table 21.1 Glycogen-storage diseases

Type	Defective enzyme	Organ affected	Glycogen in the affected organ	Clinical features
I Von Gierke	Glucose 6-phosphatase or transport system	Liver and kidney	Increased amount; normal structure.	Massive enlargement of the liver. Failure to thrive. Severe hypoglycemia, ketosis, hyperuricemia, hyperlipemia.
II Pompe	α-1,4-Glucosidase (lysosomal)	All organs	Massive increase in amount; normal structure.	Cardiorespiratory failure causes death, usually before age 2.
III Cori	Amylo-1,6-glucosidase (debranching enzyme)	Muscle and liver	Increased amount; short outer branches.	Like type I, but milder course.
IV Andersen	Branching enzyme (α-1,4 ⟶ α-1,6)	Liver and spleen	Normal amount; very long outer branches.	Progressive cirrhosis of the liver. Liver failure causes death, usually before age 2.
V McArdle	Phosphorylase	Muscle	Moderately increased amount; normal structure.	Limited ability to perform strenuous exercise because of painful muscle cramps. Otherwise patient is normal and well developed.
VI Hers	Phosphorylase	Liver	Increased amount.	Like type l, but milder course.
VII	Phosphofructokinase	Muscle	Increased amount; normal structure.	Like type V.
VIII	Phosphorylase kinase	Liver	Increased amount; normal structure.	Mild liver enlargement. Mild hypoglycemia.

Note: Types I through VII are inherited as autosomal recessives. Type VIII is sex linked.

that encodes the *glucose 6-phosphate transporter.* Recall that glucose 6-phosphate must be transported into the lumen of the endoplasmic reticulum to be hydrolyzed by phosphatase. Mutations in the other three essential proteins of this system can likewise lead to von Gierke disease.

Seven other glycogen-storage diseases have been characterized (Table 21.1). In Pompe disease (type II), lysosomes become engorged with glycogen because they lack α-1,4-glucosidase, a hydrolytic enzyme confined to these organelles (Figure 21.25). Carl and Gerty Cori also elucidated the biochemical defect in another glycogen-storage disease (type III), which cannot be distinguished from von Gierke disease (type I) by physical examination alone. In type III disease, the structure of liver and muscle glycogen is abnormal and the amount is markedly increased. Most striking, the outer branches of the glycogen are very short. *Patients having this type lack the debranching enzyme (α-1,6-glucosidase),* and so only the outermost branches of glycogen can be effectively utilized. Thus, only a small fraction of this abnormal glycogen is functionally active as an accessible store of glucose.

A defect in glycogen metabolism confined to muscle is found in McArdle disease (type V). *Muscle phosphorylase activity is absent,* and a patient's capacity to perform strenuous exercise is limited because of painful muscle cramps. The patient is otherwise normal and well developed. Thus, effective utilization of muscle glycogen is not essential for life. Phosphorus-31 nuclear magnetic resonance studies of these patients have been very informative. The pH of skeletal-muscle cells of normal people drops during strenuous exercise because of the production of lactate. In contrast, the muscle cells of patients with McArdle disease become more alkaline during exercise because of the breakdown of creatine phosphate (Section 15.2). Lactate does not accumulate in these patients, because the glycolytic rate of their muscle is much lower than normal; their glycogen cannot be mobilized. NMR studies have also shown that the painful cramps in this disease are correlated with high levels of ADP (Figure 21.26). NMR spectroscopy is a valuable, noninvasive technique for assessing dietary and exercise therapy for this disease.

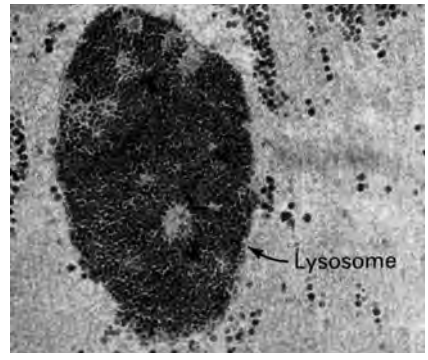


Figure 21.25 Glycogen-engorged lysosome. This electron micrograph shows skeletal muscle from an infant with type II glycogen-storage disease (Pompe disease). The lysosomes are filled with glycogen because of a deficiency in α-1,4-glucosidase, a hydrolytic enzyme confined to lysosomes. The amount of glycogen in the cytoplasm is normal. [From H.-G. Hers and F. Van Hoof, Eds., *Lysosomes and Storage Diseases* (Academic Press, 1973), p. 205.]

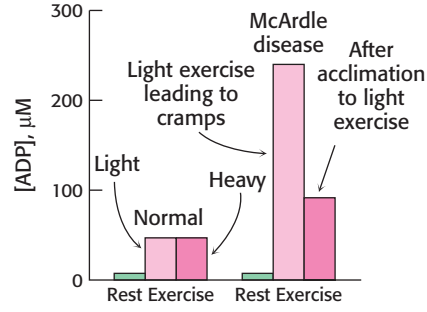


Figure 21.26 NMR study of human arm muscle. The level of ADP during exercise increases much more in a patient with McArdle glycogen-storage disease (type V) than in normal controls. [After G. K. Radda. *Biochem. Soc. Trans.* 14:517–525, 1986.]

Summary

Glycogen, a readily mobilized fuel store, is a branched polymer of glucose residues. Most of the glucose units in glycogen are linked by α-1,4-glycosidic bonds. At about every tenth residue, a branch is created by an α-1,6-glycosidic bond. Glycogen is present in large amounts in muscle cells and in liver cells, where it is stored in the cytoplasm in the form of hydrated granules.

21.1 Glycogen Breakdown Requires the Interplay of Several Enzymes

Most of the glycogen molecule is degraded to glucose 1-phosphate by the action of glycogen phosphorylase, the key enzyme in glycogen breakdown. The glycosidic linkage between C-1 of a terminal residue and C-4 of the adjacent one is split by orthophosphate to give glucose 1-phosphate, which can be reversibly converted into glucose 6-phosphate. Branch points are degraded by the concerted action of an oligosaccharide transferase and an α-1,6-glucosidase.

21.2 Phosphorylase Is Regulated by Allosteric Interactions and Reversible Phosphorylation

Phosphorylase *b,* which is usually inactive, is converted into active phosphorylase *a* by the phosphorylation of a single serine residue in each subunit. This reaction is catalyzed by phosphorylase kinase. The

b form in muscle can also be activated by the binding of AMP, an effect counteracted by ATP and glucose 6-phosphate. The *a* form in the liver is inhibited by glucose. The AMP-binding sites and phosphorylation sites are located at the subunit interface. In muscle, phosphorylase is activated to generate glucose for use inside the cell as a fuel for contractile activity. In contrast, liver phosphorylase is activated to liberate glucose for export to other organs, such as skeletal muscle and the brain.

21.3 Epinephrine and Glucagon Signal the Need for Glycogen Breakdown

Epinephrine and glucagon stimulate glycogen breakdown through specific 7TM receptors. Muscle is the primary target of epinephrine, whereas the liver is responsive to glucagon. Both signal molecules initiate a kinase cascade that leads to the activation of glycogen phosphorylase.

21.4 Glycogen Is Synthesized and Degraded by Different Pathways

The pathway for glycogen synthesis differs from that for glycogen breakdown. UDP-glucose, the activated intermediate in glycogen synthesis, is formed from glucose 1-phosphate and UTP. Glycogen synthase catalyzes the transfer of glucose from UDP-glucose to the C-4 hydroxyl group of a terminal residue in the growing glycogen molecule. Synthesis is primed by glycogenin, an autoglycosylating protein that contains a covalently attached oligosaccharide unit on a specific tyrosine residue. A branching enzyme converts some of the α-1,4 linkages into α-1,6 linkages to increase the number of ends so that glycogen can be made and degraded more rapidly.

21.5 Glycogen Breakdown and Synthesis Are Reciprocally Regulated

Glycogen synthesis and degradation are coordinated by several amplifying reaction cascades. Epinephrine and glucagon stimulate glycogen breakdown and inhibit its synthesis by increasing the cytoplasmic level of cyclic AMP, which activates protein kinase A. Protein kinase A activates glycogen breakdown by attaching a phosphate to phosphorylase kinase and inhibits glycogen synthesis by phosphorylating glycogen synthase.

The glycogen-mobilizing actions of protein kinase A are reversed by protein phosphatase 1, which is regulated by several hormones. Epinephrine inhibits this phosphatase by blocking its attachment to glycogen molecules and by turning on an inhibitor. Insulin, in contrast, triggers a cascade that phosphorylates and inactivates glycogen synthase kinase, one of the enzymes that inhibits glycogen synthase. Hence, glycogen synthesis is decreased by epinephrine and increased by insulin. Glycogen synthase and phosphorylase are also regulated by noncovalent allosteric interactions. In fact, phosphorylase is a key part of the glucose-sensing system of liver cells. Glycogen metabolism exemplifies the power and precision of reversible phosphorylation in regulating biological processes.

Key Terms

glycogen phosphorylase (p. 617)
phosphorolysis (p. 617)
pyridoxal phosphate (PLP) (p. 619)
phosphorylase kinase (p. 623)
calmodulin (p. 624)
epinephrine (adrenaline) (p. 624)
glucagon (p. 624)
protein kinase A (PKA) (p. 626)
uridine diphosphate glucose (UDP-glucose) (p. 627)
glycogen synthase (p. 628)
glycogenin (p. 628)
protein phosphatase 1 (PP1) (p. 631)
insulin (p. 632)

Problems

1. *Choice is good.* Glycogen is not as reduced as fatty acids are and consequently not as energy rich. Why do animals store any energy as glycogen? Why not convert all excess fuel into fatty acids?

2. *If a little is good, a lot is better.* α-Amylose is an unbranched glucose polymer. Why would this polymer not be as effective a storage form of glucose as glycogen?

3. *Telltale products.* A sample of glycogen from a patient with liver disease is incubated with orthophosphate, phosphorylase, the transferase, and the debranching enzyme (α-1,6-glucosidase). The ratio of glucose 1-phosphate to glucose formed in this mixture is 100. What is the most likely enzymatic deficiency in this patient?

4. *Dare to be different.* Compare the allosteric regulation of phosphorylase in the liver and in muscle, and explain the significance of the difference.

5. *A thumb on the balance.* The reaction catalyzed by phosphorylase is readily reversible in vitro. At pH 6.8, the equilibrium ratio of orthophosphate to glucose 1-phosphate is 3.6. The value of $\Delta G^{\circ\prime}$ for this reaction is small because a glycosidic bond is replaced by a phosphoryl ester bond that has a nearly equal transfer potential. However, phosphorolysis proceeds far in the direction of glycogen breakdown in vivo. Suggest one means by which the reaction can be made irreversible in vivo.

6. *Excessive storage.* Suggest an explanation for the fact that the amount of glycogen in type I glycogen-storage disease (von Gierke disease) is increased.

7. *Recouping an essential phosphoryl.* The phosphoryl group on phosphoglucomutase is slowly lost by hydrolysis. Propose a mechanism that utilizes a known catalytic intermediate for restoring this essential phosphoryl group. How might this phosphoryl donor be formed?

8. *Not all absences are equal.* Hers disease results from an absence of liver glycogen phosphorylase and may result in serious illness. In McArdle disease, muscle glycogen phosphorylase is absent. Although exercise is difficult for patients suffering from McArdle disease, the disease is rarely life threatening. Account for the different manifestations of the absence of glycogen phosphorylase in the two tissues. What does the existence of these two different diseases indicate about the genetic nature of the phosphorylase?

9. *Hydrophobia.* Why is water excluded from the active site of phosphorylase? Predict the effect of a mutation that allows water molecules to enter.

10. *Removing all traces.* In human liver extracts, the catalytic activity of glycogenin was detectable only after treatment with α-amylase (p. 629). Why was α-amylase necessary to reveal the glycogenin activity?

11. *Two in one.* A single polypeptide chain houses the transferase and debranching enzyme. Cite a potential advantage of this arrangement.

12. *How did they do that?* A strain of mice has been developed that lack the enzyme phosphorylase kinase. Yet, after strenuous exercise, the glycogen stores of a mouse of this strain are depleted. Explain how this depletion is possible.

13. *An appropriate inhibitor.* What is the rationale for the inhibition of muscle glycogen phosphorylase by glucose 6-phosphate when glucose 1-phosphate is the product of the phosphorylase reaction?

14. *Passing along the information.* Outline the signal-transduction cascade for glycogen degradation in muscle.

15. *Slammin' on the breaks.* There must be a way to shut down glycogen breakdown quickly to prevent the wasteful depletion of glycogen after energy needs have been met. What mechanisms are employed to turn off glycogen breakdown?

16. *Diametrically opposed.* Phosphorylation has opposite effects on glycogen synthesis and breakdown. What is the advantage of its having opposing effects?

17. *Feeling depleted.* Glycogen depletion resulting from intense, extensive exercise can lead to exhaustion and the inability to continue exercising. Some people also experience dizziness, an inability to concentrate, and a loss of muscle control. Account for these symptoms.

18. *Everyone had a job to do.* What accounts for the fact that liver phosphorylase is a glucose sensor, whereas muscle phosphorylase is not?

19. *If you insist.* Why does activation of the phosphorylated *b* form of glycogen synthase by high concentrations of glucose 6-phosphate make good biochemical sense?

20. *An ATP saved is an ATP earned.* The complete oxidation of glucose 6-phosphate derived from free glucose yields 30 molecules ATP, whereas the complete oxidation of glucose 6-phosphate derived from glycogen yields 31 molecules of ATP. Account for this difference.

21. *Dual roles.* Phosphoglucomutase is crucial for glycogen breakdown as well as for glycogen synthesis. Explain the role of this enzyme in each of the two processes.

22. *Working at cross-purposes.* Write a balanced equation showing the effect of simultaneous activation of glycogen phosphorylase and glycogen synthase. Include the reactions catalyzed by phosphoglucomutase and UDP-glucose pyrophosphorylase.

23. *Achieving immortality*. Glycogen synthase requires a primer. A primer was formerly thought to be provided when the existing glycogen granules are divided between the daughter cells produced by cell division. In other words, parts of the original glycogen molecule were simply passed from generation to generation. Would this strategy have been successful in passing glycogen stores from generation to generation? How are new glycogen molecules now known to be synthesized?

24. *Synthesis signal*. How does insulin stimulate glycogen synthesis?

Mechanism Problem

25. *Family resemblance*. Propose mechanisms for the two enzymes catalyzing steps in glycogen debranching on the basis of their potential membership in the α-amylase family.

Chapter Integration Problems

26. *Carbohydrate conversion*. Write a balanced equation for the formation of glycogen from galactose.

27. *Working together*. What enzymes are required for the liver to release glucose into the blood when an organism is asleep and fasting?

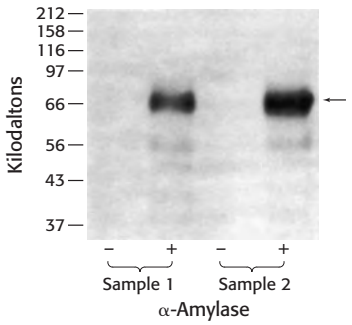
28. *A shattering experience*. Crystals of phosphorylase *a* grown in the presence of glucose shatter when a substrate such as glucose 1-phosphate is added. Why?

29. *I know I've seen that face before*. UDP-glucose is the activated form of glucose used in glycogen synthesis. However, we have previously met other similar activated forms of carbohydrate in our consideration of metabolism. Where else have we seen UDP-carbohydrate?

30. *Same symptoms, different cause*. Suggest another mutation in glucose metabolism that causes symptoms similar to those of von Gierke disease.

Data Interpretation Problems

31. *Glycogen isolation 1*. The liver is a major storage site for glycogen. Purified from two samples of human liver, glycogen was either treated or not treated with α-amylase and subsequently analyzed by SDS-PAGE and western blotting with the use of antibodies to glycogenin. The results are presented in the adjoining illustration.



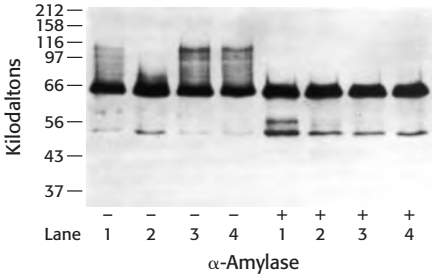
Glycogen isolation 1. [Courtesy of Dr. Peter J. Roach, Indiana University School of Medicine.]

(a) Why are no proteins visible in the lanes without amylase treatment?

(b) What is the effect of treating the samples with α-amylase? Explain the results.

(c) List other proteins that you might expect to be associated with glycogen. Why are other proteins not visible?

32. *Glycogen isolation 2*. The gene for glycogenin was transfected into a cell line that normally stores only small amounts of glycogen. The cells were then manipulated according to the following protocol, and glycogen was isolated and analyzed by SDS-PAGE and western blotting by using an antibody to glycogenin with and without α-amylase treatment. The results are presented in the adjoining illustration.



Glycogen isolation 2. [Courtesy of Dr. Peter J. Roach, Indiana University School of Medicine.]

The protocol: Cells cultured in growth medium and 25 mM glucose (lane 1) were switched to medium containing no glucose for 24 hours (lane 2). Glucose-starved cells were refed with medium containing 25 mM glucose for 1 hour (lane 3) or 3 hours (lane 4). Samples (12 μg of protein) were either treated or not treated with α-amylase, as indicated, before being loaded on the gel.

(a) Why did the western analysis produce a "smear"—that is, the high-molecular-weight staining in lane 1(−)?

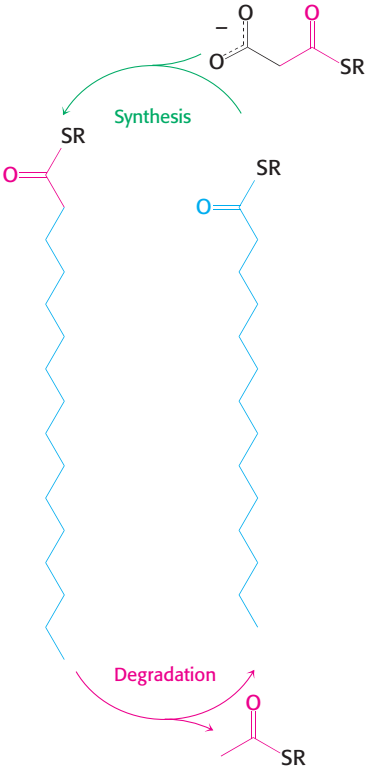
(b) What is the significance of the decrease in high-molecular-weight staining in lane 2(−)?

(c) What is the significance of the difference between lanes 2(−) and 3(−)?

(d) Suggest a plausible reason why there is essentially no difference between lanes 3(−) and 4(−)?

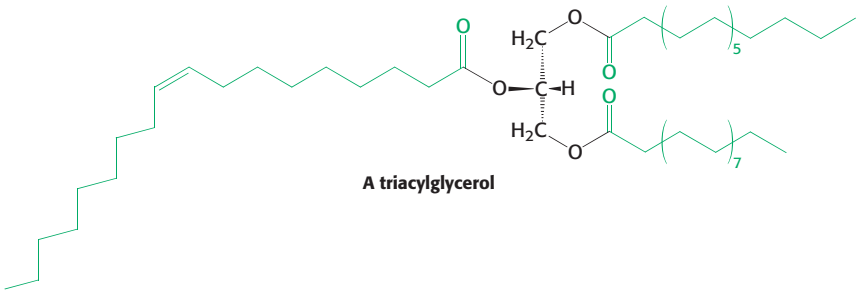
(e) Why are the bands at 66 kd the same in the lanes treated with amylase, despite the fact that the cells were treated differently?

Fatty Acid Metabolism



Fats provide efficient means for storing energy for later use. (Right) The processes of fatty acid synthesis (preparation for energy storage) and fatty acid degradation (preparation for energy use) are, in many ways, the reverse of each other. (Above) Studies of mice are revealing the interplay between these pathways and the biochemical bases of appetite and weight control. [Photograph © Jackson/Visuals Unlimited.]

We turn now from the metabolism of carbohydrates to that of fatty acids. A fatty acid contains a long hydrocarbon chain and a terminal carboxylate group. Fatty acids have four major physiological roles. First, *fatty acids are fuel molecules.* They are stored as *triacylglycerols* (also called *neutral fats* or *triglycerides*), which are uncharged esters of fatty acids with glycerol. Triacylglycerols are stored in adipose tissue, composed of cells called adipocytes (Figure 22.1). Fatty acids mobilized from triacylglycerols are oxidized to meet the energy needs of a cell or organism. During rest or moderate exercise, such as walking, fatty acids are our primary source of energy. Second, *fatty acids are building blocks of phospholipids and glycolipids.* These amphipathic molecules are important components of biological membranes, as discussed in Chapter 12. Third, many proteins are modified by the *covalent attachment of fatty acids, which targets the proteins to membrane locations.* Fourth, *fatty acid derivatives serve as hormones and intracellular messengers.* In this chapter, we focus on the degradation and synthesis of fatty acids.



A triacylglycerol

OUTLINE

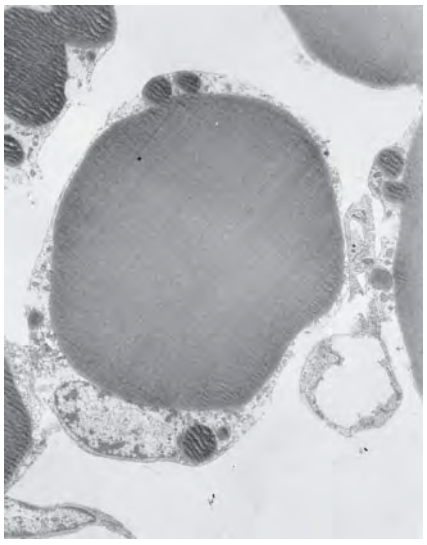


Figure 22.1 Electron micrograph of an adipocyte. A small band of cytoplasm surrounds the large deposit of triacylglycerols. [Biophoto Associates/Photo Researchers.]

Fatty acid degradation and synthesis mirror each other in their chemical reactions

Fatty acid degradation and synthesis consist of four steps that are the reverse of each other in their basic chemistry. Degradation is an oxidative process that converts a fatty acid into a set of activated acetyl units (acetyl CoA) that can be processed by the citric acid cycle (Figure 22.2). An activated fatty acid is oxidized to introduce a double bond; the double bond is hydrated to introduce a hydroxyl group; the alcohol is oxidized to a ketone; and, finally, the fatty acid is cleaved by coenzyme A to yield acetyl CoA and a fatty acid chain two carbons shorter. If the fatty acid has an even number of carbon atoms and is saturated, the process is simply repeated until the fatty acid is completely converted into acetyl CoA units.

Fatty acid synthesis is essentially the reverse of this process. The process starts with the individual units to be assembled—in this case with an activated acyl group (most simply, an acetyl unit) and a malonyl unit (see Figure 22.2). The malonyl unit condenses with the acetyl unit to form a four-carbon fragment. To produce the required hydrocarbon chain, the carbonyl group is reduced to a methylene group in three steps: a reduction,

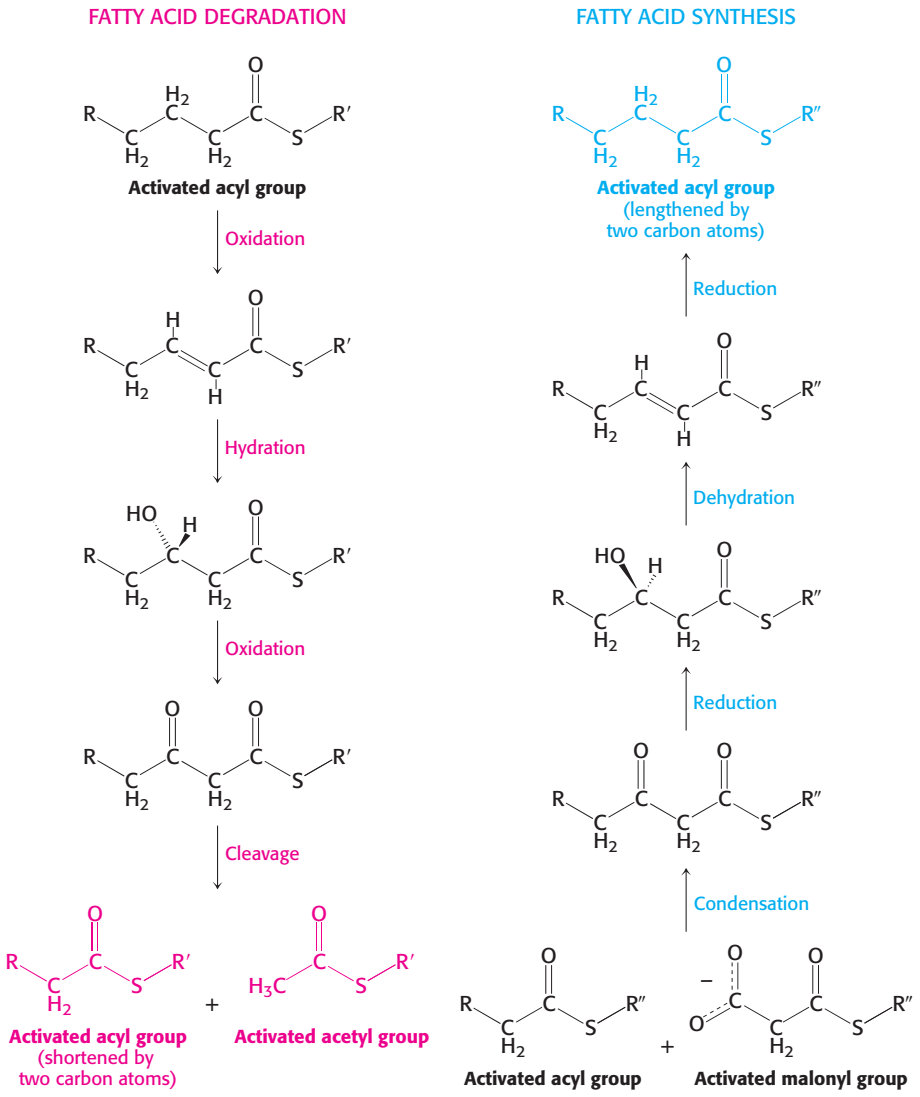


Figure 22.2 Steps in fatty acid degradation and synthesis. The two processes are in many ways mirror images of each other.

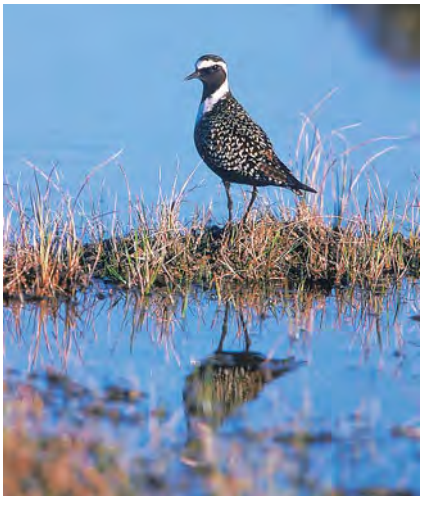
a dehydration, and another reduction, exactly the opposite of degradation. The product of the reduction is butyryl CoA. Another activated malonyl group condenses with the butyryl unit, and the process is repeated until a C_{16} or shorter fatty acid is synthesized.

22.1 Triacylglycerols Are Highly Concentrated Energy Stores

Triacylglycerols are highly concentrated stores of metabolic energy because they are *reduced* and *anhydrous*. The yield from the complete oxidation of fatty acids is about 38 kJ g^{-1} (9 kcal g^{-1}), in contrast with about 17 kJ g^{-1} (4 kcal g^{-1}) for carbohydrates and proteins. The basis of this large difference in caloric yield is that fatty acids are much more reduced than carbohydrates or proteins. Furthermore, triacylglycerols are nonpolar, and so they are stored in a nearly anhydrous form, whereas much more polar carbohydrates are more highly hydrated. In fact, 1 g of dry glycogen binds about 2 g of water. Consequently, *a gram of nearly anhydrous fat stores 6.75 times as much energy as a gram of hydrated glycogen,* which is likely the reason that triacylglycerols rather than glycogen were selected in evolution as the major energy reservoir. Consider a typical 70-kg man, who has fuel reserves of 420,000 kJ (100,000 kcal) in triacylglycerols, 100,000 kJ (24,000 kcal) in protein (mostly in muscle), 2500 kJ (600 kcal) in glycogen, and 170 kJ (40 kcal) in glucose. Triacylglycerols constitute about 11 kg of his total body weight. If this amount of energy were stored in glycogen, his total body weight would be 64 kg greater. The glycogen and glucose stores provide enough energy to sustain physiological function for about 24 hours, whereas the triacylglycerol stores allow survival for several weeks.

In mammals, the major site of triacylglycerol accumulation is the cytoplasm of *adipose cells (fat cells)*. This fuel-rich tissue is located throughout the body, notably under the skin (subcutaneous fat) and surrounding the internal organs (visceral fat). Droplets of triacylglycerol coalesce to form a large globule, called a lipid droplet, which may occupy most of the cell volume (see Figure 22.1). The lipid droplet is surrounded by a monolayer of phospholipids and proteins required for triacylglycerol metabolism. Adipose cells are specialized for the synthesis and storage of triacylglycerols and for their mobilization into fuel molecules that are transported to other tissues by the blood. Muscle also stores triacylglycerols for its own energy needs. Indeed, triacylglycerols are evident as the "marbling" of expensive cuts of beef.

The utility of triacylglycerols as an energy source is dramatically illustrated by the abilities of migratory birds, which can fly great distances without eating after having stored energy as triacylglycerols. Examples are the American golden plover and the ruby-throated hummingbird. The golden plover flies from Alaska to the southern tip of South America; a large segment of the flight (3800 km, or 2400 miles) is over open ocean, where the birds cannot feed. The ruby-throated hummingbird can fly nonstop across the Gulf of Mexico. Fatty acids provide the energy source for both these prodigious feats.



Triacylglycerols fuel the long migration flights of the American golden plover (*Pluvialis dominica*). [Gerard Fuehrer/ Visuals Unlimited.]

Dietary lipids are digested by pancreatic lipases

Most lipids are ingested in the form of triacylglycerols and must be degraded to fatty acids for absorption across the intestinal epithelium. Intestinal enzymes called *lipases,* secreted by the pancreas, degrade triacylglycerols to free fatty acids and monoacylglycerol (Figure 22.3). Lipids present a special problem because, unlike carbohydrates and proteins, these molecules are

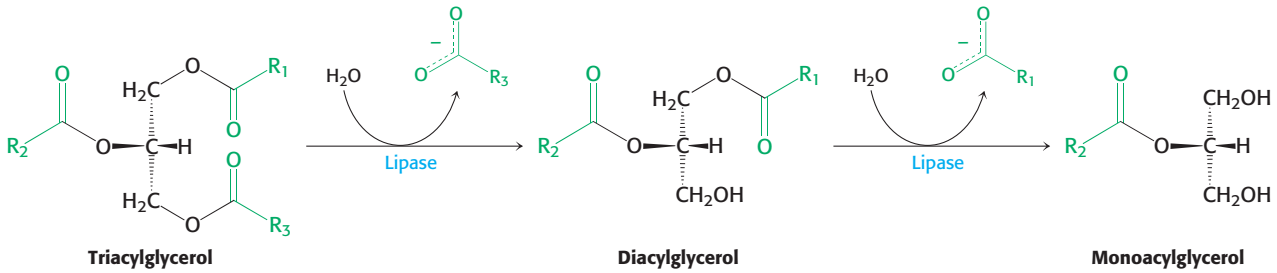


Figure 22.3 Action of pancreatic lipases. Lipases secreted by the pancreas convert triacylglycerols into fatty acids and monoacylglycerol for absorption into the intestine.

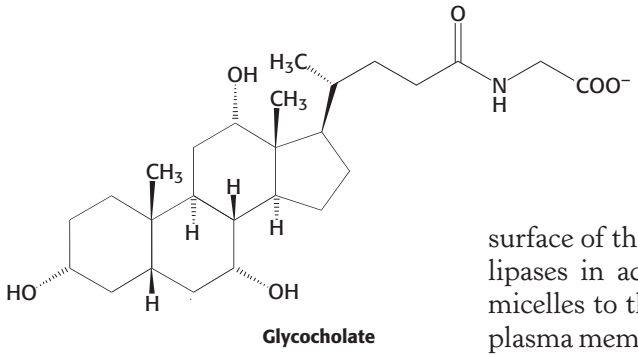


Figure 22.4 Glycocholate. Bile salts, such as glycocholate, facilitate lipid digestion in the intestine.

not soluble in water. How are they made accessible to the lipases, which are in aqueous solution? The solution is to wrap lipids in a soluble container. Triacylglycerols in the intestinal lumen are incorporated into micelles composed of *bile salts* (Figure 22.4), amphipathic molecules synthesized from cholesterol in the liver and secreted from the gall bladder. The ester bond of each lipid is oriented toward the surface of the micelle, rendering the bond more susceptible to digestion by lipases in aqueous solution. The final digestion products are carried in micelles to the intestinal epithelium where they are transported across the plasma membrane (Figure 22.5). If the production of bile salts is inadequate due to liver disease, large amounts of fats (as much as 30 g day^{-1}) are excreted in the feces. This condition is referred to as *steatorrhea*, after stearic acid, a common fatty acid.

Dietary lipids are transported in chylomicrons

In the intestinal mucosal cells, the triacylglycerols are resynthesized from fatty acids and monoacylglycerols and then packaged into lipoprotein transport particles called *chylomicrons*, stable particles approximately 2000 Å (200 nm) in diameter (see Figure 22.5). These particles are composed mainly of triacylglycerols, with apoliprotein B-48 (apo B-48) as the main protein component. Protein constituents of lipoprotein particles are called *apolipoproteins*. Chylomicrons also transport fat-soluble vitamins and cholesterol.

The chylomicrons are released into the lymph system and then into the blood. These particles bind to membrane-bound lipases, primarily at adipose tissue and muscle, where the triacylglycerols are once again degraded into free fatty acids and monoacylglycerol for transport into the tissue. The triacylglycerols are then resynthesized inside the cell and stored. In the muscle, they can be oxidized to provide energy.

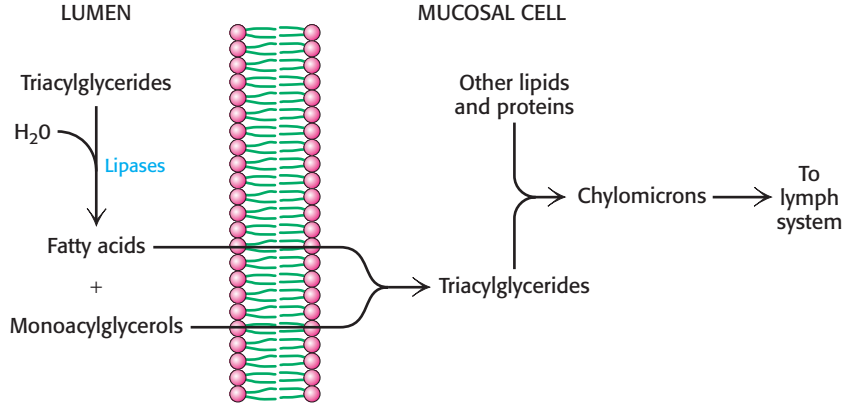


Figure 22.5 Chylomicron formation. Free fatty acids and monoacylglycerols are absorbed by intestinal epithelial cells. Triacylglycerols are resynthesized and packaged with other lipids and apolipoprotein B-48 to form chylomicrons, which are then released into the lymph system.

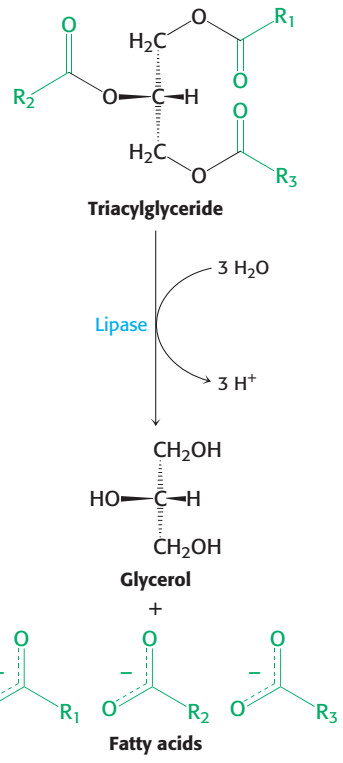
22.2 The Use of Fatty Acids As Fuel Requires Three Stages of Processing

Tissues throughout the body gain access to the lipid energy reserves stored in adipose tissue through three stages of processing. First, the lipids must be mobilized. In this process, triacylglycerols are degraded to fatty acids and glycerol, which are released from the adipose tissue and transported to the energy-requiring tissues. Second, at these tissues, the fatty acids must be activated and transported into mitochondria for degradation. Third, the fatty acids are broken down in a step-by-step fashion into acetyl CoA, which is then processed in the citric acid cycle.

Triacylglycerols are hydrolyzed by hormone-stimulated lipases

Consider someone who has just awakened from a night's sleep and begins a bout of exercise. Glycogen stores will be low, but lipids are readily available. How are these lipid stores mobilized?

Before fats can be used as fuels, the triacylglycerol storage form must be hydrolyzed to yield isolated fatty acids. This reaction is catalyzed by a hormonally controlled lipase. Under the physiological conditions facing an early-morning runner, glucagon and epinephrine will be present. In adipose tissue, these hormones trigger 7 TM receptors that activate adenylate cyclase (Section 14.1). The increased level of cyclic AMP then stimulates protein kinase A, which phosphorylates two key proteins: *perilipin*, a fat-droplet-associated protein, and hormone-sensitive lipase (Figure 22.6). The phosphorylation of perilipin has two crucial effects. First, it restructures the fat droplet so that the triacylglycerols are more accessible to the mobililzation. Second, the phosphorylation of perilipin triggers the release of a coactivator for the adipose triglyceride lipase (ATGL). ATGL initiates the mobilization of triacylglycerols by releasing a fatty acid from triacylglycerol, forming diacylglycerol. Diacylglycerol is converted into a free fatty acid and monoacylglycerol by the hormone-sensitive lipase. Finally, a monoacylglycerol lipase completes the mobilization of fatty acids with the production of a free fatty acid and glycerol. Thus, *epinephrine and glucagon induce lipolysis*. Although their role in muscle is not as firmly established, these hormones probably also regulate the use of triacylglycerol stores in that tissue.



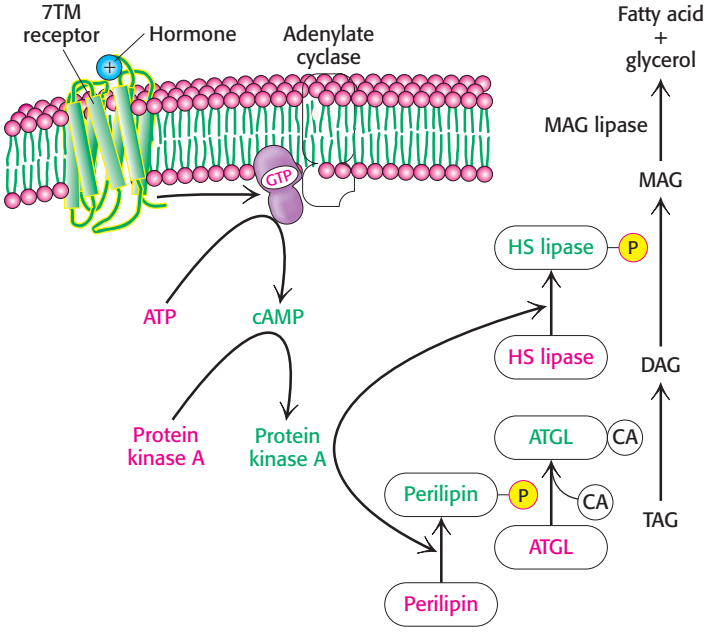
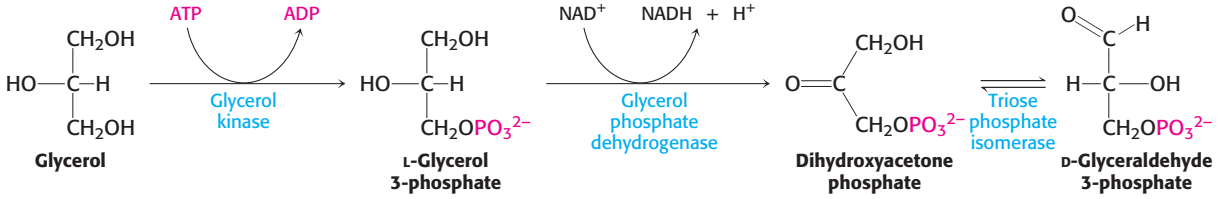


Figure 22.6 Mobilization of triacylglycerols. Triacylglycerols in adipose tissue are converted into free fatty acids in response to hormonal signals. The phosphorylation of perilipin restructures the lipid droplet and releases the coactivator of ATGL. The activation of ATGL by binding with its coactivator initiates the mobilization. Hormone-sensitive lipase releases a fatty acid from diacylglycerol. Monoacylglycerol lipase completes the mobilization process. Abbreviations: 7TM, seven transmembrane receptor; ATGL, adipose triglyceride lipase; CA, coactivator; HS lipase, hormone-sensitive lipase; MAG lipase, monoacylglycerol lipase; DAG, diacylglycerol; TAG, triacylglycerol.

The released fatty acids are not soluble in blood plasma, and so the blood protein albumin binds the fatty acids and serves as a carrier. By these means, free fatty acids are made accessible as a fuel in other tissues. At the tissues, fatty acid transport protein facilitates the transit of the fatty acids across the plasma membrane.

Glycerol formed by lipolysis is absorbed by the liver and phosphorylated. It is then oxidized to dihydroxyacetone phosphate, which is isomerized to glyceraldehyde 3-phosphate. This molecule is an intermediate in both the glycolytic and the gluconeogenic pathways.



Hence, glycerol can be converted into pyruvate or glucose in the liver, which contains the appropriate enzymes (Figure 22.7). The reverse process can take place by the reduction of dihydroxyacetone phosphate to glycerol 3-phosphate. Hydrolysis by a phosphatase then gives glycerol. Thus, glycerol and glycolytic intermediates are readily interconvertible.

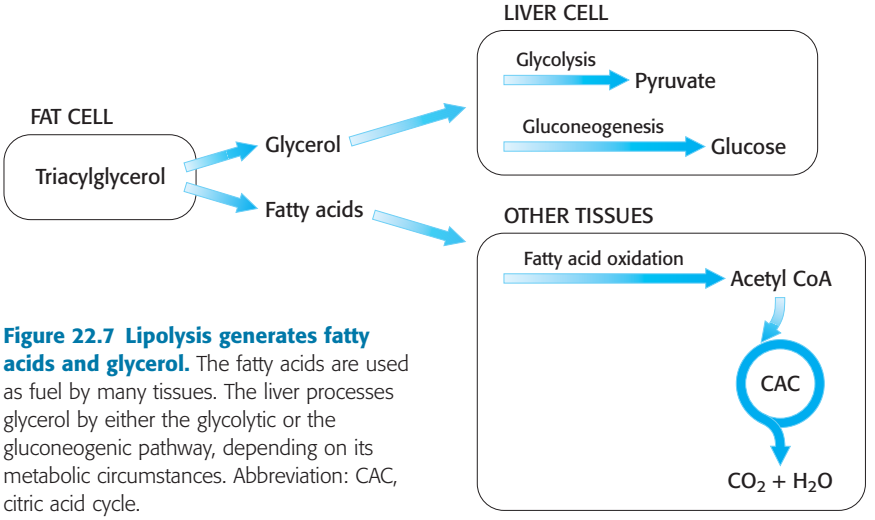
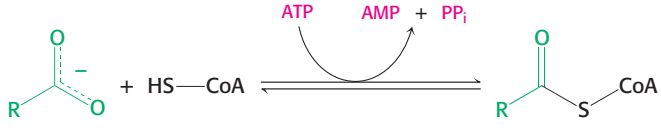
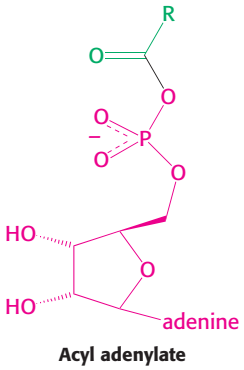


Figure 22.7 Lipolysis generates fatty acids and glycerol. The fatty acids are used as fuel by many tissues. The liver processes glycerol by either the glycolytic or the gluconeogenic pathway, depending on its metabolic circumstances. Abbreviation: CAC, citric acid cycle.

Fatty acids are linked to coenzyme A before they are oxidized

Eugene Kennedy and Albert Lehninger showed in 1949 that fatty acids are oxidized in mitochondria. Subsequent work demonstrated that they are first activated through the formation of a thioester linkage to coenzyme A before they enter the mitochondrial matrix. Adenosine triphosphate drives the formation of the thioester linkage between the carboxyl group of a fatty acid and the sulfhydryl group of coenzyme A. This activation reaction takes place on the outer mitochondrial membrane, where it is catalyzed by *acyl CoA synthetase* (also called *fatty acid thiokinase*).

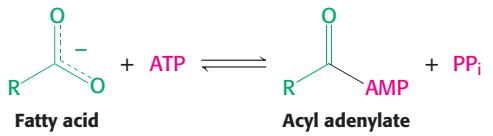




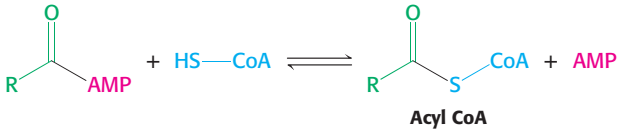
Acyl adenylate

Paul Berg showed that acyl CoA synthetase accomplishes the activation of a fatty acid in two steps. First, the fatty acid reacts with ATP to form an

acyl adenylate. In this mixed anhydride, the carboxyl group of a fatty acid is bonded to the phosphoryl group of AMP. The other two phosphoryl groups of the ATP substrate are released as pyrophosphate. In the second step, the sulfhydryl group of coenzyme A attacks the acyl adenylate, which is tightly bound to the enzyme, to form acyl CoA and AMP.



(1)



(2)

These partial reactions are freely reversible. In fact, the equilibrium constant for the sum of these reactions is close to 1. One high-transfer-potential compound is cleaved (between PP_i and AMP) and one high-transfer-potential compound is formed (the thioester acyl CoA). How is the overall reaction driven forward? The answer is that pyrophosphate is rapidly hydrolyzed by a pyrophosphatase. The complete reaction is

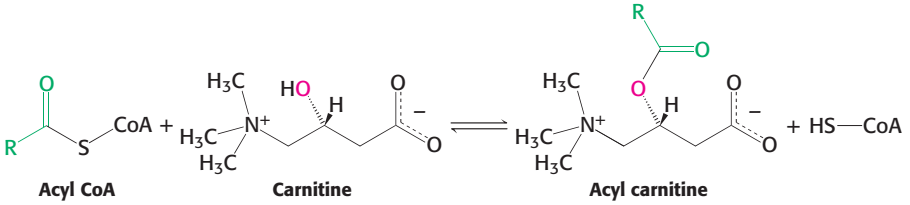
$$RCOO^- + CoA + ATP + H_2O \longrightarrow RCO\text{-}CoA + AMP + 2\ P_i + 2\ H^+$$

This reaction is quite favorable because the equivalent of two molecules of ATP is hydrolyzed, whereas only one high-transfer-potential compound is formed. We see here another example of a recurring theme in biochemistry: *many biosynthetic reactions are made irreversible by the hydrolysis of inorganic pyrophosphate.*

Another motif recurs in this activation reaction. The enzyme-bound acyl adenylate intermediate is not unique to the synthesis of acyl CoA. *Acyl adenylates are frequently formed when carboxyl groups are activated in biochemical reactions.* Amino acids are activated for protein synthesis by a similar mechanism (Section 30.2), although the enzymes that catalyze this process are not homologous to acyl CoA synthetase. Thus, *activation by adenylation recurs in part because of convergent evolution.*

Carnitine carries long-chain activated fatty acids into the mitochondrial matrix

Fatty acids are activated on the outer mitochondrial membrane, whereas they are oxidized in the mitochondrial matrix. A special transport mechanism is needed to carry activated long-chain fatty acids across the inner mitochondrial membrane. These fatty acids must be conjugated to *carnitine,* a zwitterionic alcohol. The acyl group is transferred from the sulfur atom of coenzyme A to the hydroxyl group of carnitine to form *acyl carnitine*. This reaction is catalyzed by *carnitine acyltransferase I,* also called *carnitine palmitoyl transferase I* (CPTI), which is bound to the outer mitochondrial membrane.



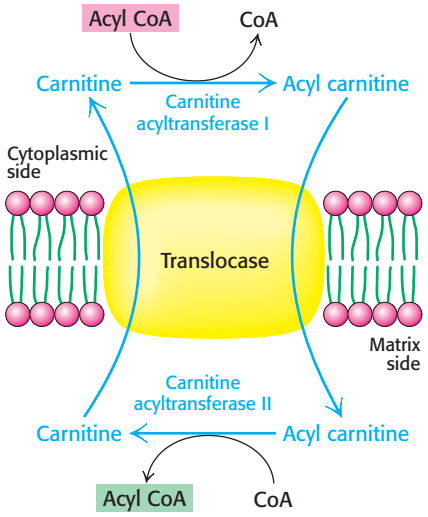
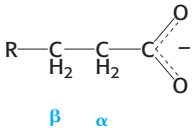


Figure 22.8 Acyl carnitine translocase. The entry of acyl carnitine into the mitochondrial matrix is mediated by a translocase. Carnitine returns to the cytoplasmic side of the inner mitochondrial membrane in exchange for acyl carnitine.



Acyl carnitine is then shuttled across the inner mitochondrial membrane by a translocase (Figure 22.8). The acyl group is transferred back to coenzyme A on the matrix side of the membrane. This reaction, which is catalyzed by *carnitine acyltransferase II (carnitine palmitoyl transferase II),* is simply the reverse of the reaction that takes place in the cytoplasm. The reaction is thermodynamically feasible because of the zwitterionic nature of carnitine. The *O*-acyl link in carnitine has a high group-transfer potential, apparently because, being zwitterions, carnitine and its esters are solvated differently from most other alcohols and their esters. Finally, the translocase returns carnitine to the cytoplasmic side in exchange for an incoming acyl carnitine.

A number of diseases have been traced to a deficiency of carnitine, the transferase, or the translocase. The symptoms of carnitine deficiency range from mild muscle cramping to severe weakness and even death. Muscle, kidney, and heart are the tissues primarily impaired. Muscle weakness during prolonged exercise is a symptom of a deficiency of carnitine acyltransferases because muscle relies on fatty acids as a long-term source of energy. Medium-chain (C_8–C_{10}) fatty acids are oxidized normally in these patients because these fatty acids do not require carnitine to enter the mitochondria. These diseases illustrate that *the impaired flow of a metabolite from one compartment of a cell to another can lead to a pathological condition.*

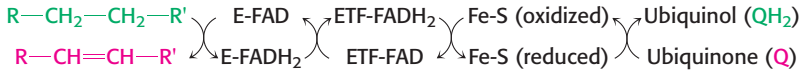
Acetyl CoA, NADH, and $FADH_2$ are generated in each round of fatty acid oxidation

A saturated acyl CoA is degraded by a recurring sequence of four reactions: oxidation by flavin adenine dinucleotide (FAD), hydration, oxidation by NAD^+, and thiolysis by coenzyme A (Figure 22.9). The fatty acid chain is shortened by two carbon atoms as a result of these reactions, and $FADH_2$, NADH, and acetyl CoA are generated. Because oxidation takes place at the β carbon atom, this series of reactions is called the *β-oxidation pathway.*

The first reaction in each round of degradation is the *oxidation* of acyl CoA by an *acyl CoA dehydrogenase* to give an enoyl CoA with a trans double bond between C-2 and C-3.

$$\text{Acyl CoA} + \text{E-FAD} \longrightarrow \textit{trans}\text{-}\Delta^2\text{-enoyl CoA} + \text{E-FADH}_2$$

As in the dehydrogenation of succinate in the citric acid cycle, FAD rather than NAD^+ is the electron acceptor because the ΔG for this reaction is insufficient to drive the reduction of NAD^+. Electrons from the $FADH_2$ prosthetic group of the reduced acyl CoA dehydrogenase are transferred to a second flavoprotein called *electron-transferring flavoprotein* (ETF). In turn, ETF donates electrons to *ETF:ubiquinone reductase,* an iron–sulfur protein. Ubiquinone is thereby reduced to ubiquinol, which delivers its high-potential electrons to the second proton-pumping site of the respiratory chain (Section 18.3). Consequently, 1.5 molecules of ATP are generated per molecule of $FADH_2$ formed in this dehydrogenation step, as in the oxidation of succinate to fumarate.



The next step is the *hydration* of the double bond between C-2 and C-3 by *enoyl CoA hydratase.*

$$\textit{trans}\text{-}\Delta^2\text{-Enoyl CoA} + H_2O \longrightarrow \text{L-3-hydroxyacyl CoA}$$

Table 22.1 Principal reactions in fatty acid oxidation

Step	Reaction	Enzyme
1	Fatty acid + CoA + ATP $\rightleftharpoons$ acyl CoA + AMP + PP_i	Acyl CoA synthetase (also called fatty acid thiokinase and fatty acid:CoA ligase)*
2	Carnitine + acyl CoA $\rightleftharpoons$ acyl carnitine + CoA	Carnitine acyltransferase (also called carnitine palmitoyl transferase)
3	Acyl CoA + E-FAD $\longrightarrow$ *trans*-Δ^2-enoyl CoA + E-$FADH_2$	Acyl CoA dehydrogenases (several isozymes having different chain-length specificity)
4	*trans*-Δ^2-Enoyl CoA + H_2O $\rightleftharpoons$ L-3-hydroxyacyl CoA	Enoyl CoA hydratase (also called crotonase or 3-hydroxyacyl CoA hydrolyase)
5	L-3-Hydroxyacyl CoA + NAD^+ $\rightleftharpoons$ 3-ketoacyl CoA + NADH + H^+	L-3-Hydroxyacyl CoA dehydrogenase
6	3-Ketoacyl CoA + CoA $\rightleftharpoons$ acetyl CoA + acyl CoA (shortened by C_2)	β-Ketothiolase (also called thiolase)

*An AMP-forming ligase.

The hydration of enoyl CoA is stereospecific. Only the L isomer of 3-hydroxyacyl CoA is formed when the trans-Δ^2 double bond is hydrated. The enzyme also hydrates a cis-Δ^2 double bond, but the product then is the D isomer. We shall return to this point shortly in considering how unsaturated fatty acids are oxidized.

The hydration of enoyl CoA is a prelude to the second *oxidation* reaction, which converts the hydroxyl group at C-3 into a keto group and generates NADH. This oxidation is catalyzed by *L-3-hydroxyacyl CoA dehydrogenase*, which is specific for the L isomer of the hydroxyacyl substrate.

$$\text{L-3-Hydroxyacyl CoA} + NAD^+ \rightleftharpoons \text{3-ketoacyl CoA} + NADH + H^+$$

The preceding reactions have oxidized the methylene group at C-3 to a keto group. The final step is the *cleavage* of 3-ketoacyl CoA by the thiol group of a second molecule of coenzyme A, which yields acetyl CoA and an acyl CoA shortened by two carbon atoms. This thiolytic cleavage is catalyzed by *β-ketothiolase*.

$$\underset{(n \text{ carbons})}{\text{3-Ketoacyl CoA}} + \text{HS-CoA} \rightleftharpoons \text{acetyl CoA} + \underset{(n-2 \text{ carbons})}{\text{acyl CoA}}$$

Table 22.1 summarizes the reactions in fatty acid oxidation.

The shortened acyl CoA then undergoes another cycle of oxidation, starting with the reaction catalyzed by acyl CoA dehydrogenase (Figure 22.10). Fatty acid chains containing from 12 to 18 carbon atoms are oxidized by the long-chain acyl CoA dehydrogenase. The medium-chain acyl CoA dehydrogenase oxidizes fatty acid chains having from 14 to 4 carbons, whereas the short-chain acyl CoA dehydrogenase acts only on 4- and 6-carbon fatty acid chains. In contrast, β-ketothiolase, hydroxyacyl dehydrogenase, and enoyl CoA hydratase act on fatty acid molecules of almost any length.

The complete oxidation of palmitate yields 106 molecules of ATP

We can now calculate the energy yield derived from the oxidation of a fatty acid. In each reaction cycle, an acyl CoA is shortened by two carbon atoms, and one molecule each of $FADH_2$, NADH, and acetyl CoA are formed.

$$C_n\text{-acyl CoA} + FAD + NAD^+ + H_2O + CoA \longrightarrow C_{n-2}\text{-acyl CoA} + FADH_2 + NADH + \text{acetyl CoA} + H^+$$

The degradation of palmitoyl CoA (C_{16}-acyl CoA) requires seven reaction cycles. In the seventh cycle, the C_4-ketoacyl CoA is thiolyzed to two molecules of acetyl CoA. Hence, the stoichiometry of the oxidation of palmitoyl CoA is

$$\text{Palmitoyl CoA} + 7\,FAD + 7\,NAD^+ + 7\,CoA + 7\,H_2O \longrightarrow 8\,\text{acetyl CoA} + 7\,FADH_2 + 7\,NADH + 7\,H^+$$

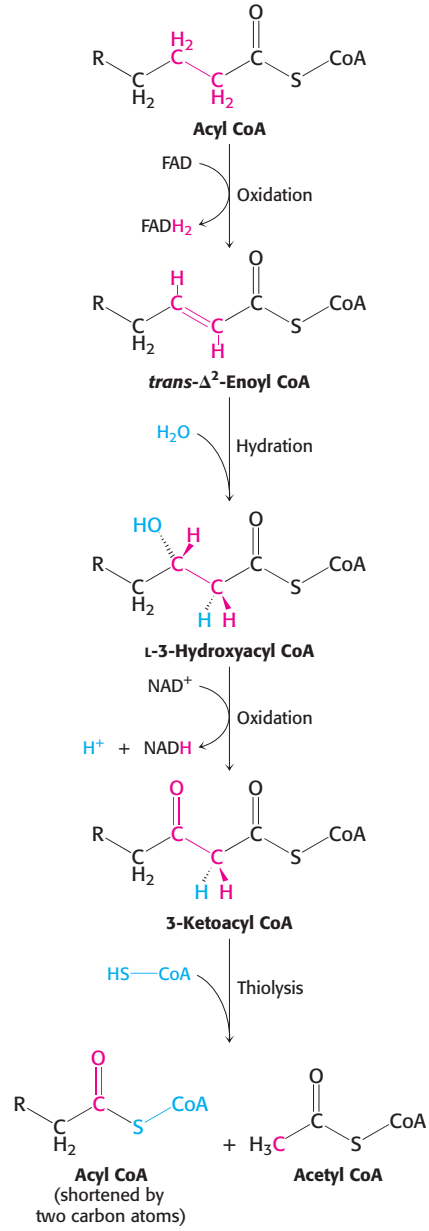


Figure 22.9 Reaction sequence for the degradation of fatty acids. Fatty acids are degraded by the repetition of a four-reaction sequence consisting of oxidation, hydration, oxidation, and thiolysis.

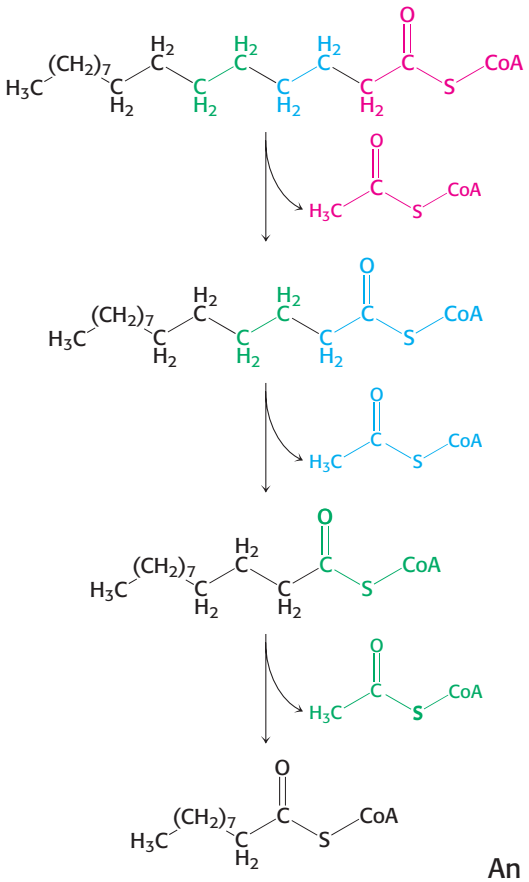


Figure 22.10 First three rounds in the degradation of palmitate. Two-carbon units are sequentially removed from the carboxyl end of the fatty acid.

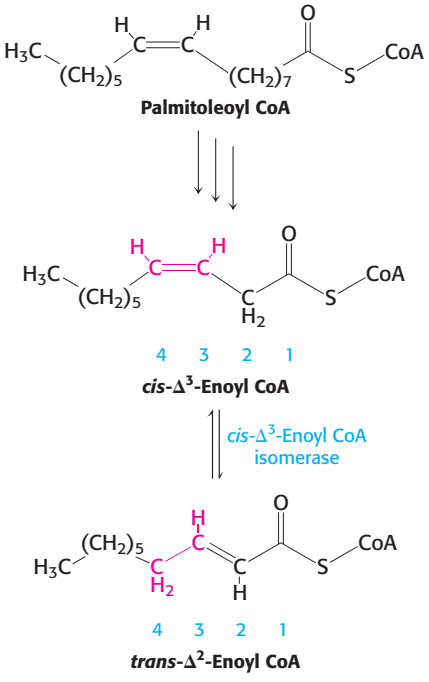


Figure 22.11 The degradation of a monounsaturated fatty acid. *Cis*-Δ^3-Enoyl CoA isomerase allows continued β-oxidation of fatty acids with a single double bond.

Approximately 2.5 molecules of ATP are generated when the respiratory chain oxidizes each of these NADH molecules, whereas 1.5 molecules of ATP are formed for each $FADH_2$ because their electrons enter the chain at the level of ubiquinol. Recall that the oxidation of acetyl CoA by the citric acid cycle yields 10 molecules of ATP. Hence, the number of ATP molecules formed in the oxidation of palmitoyl CoA is 10.5 from the seven $FADH_2$, 17.5 from the seven NADH, and 80 from the eight acetyl CoA molecules, which gives a total of 108. The equivalent of 2 molecules of ATP is consumed in the activation of palmitate, in which ATP is split into AMP and two molecules of orthophosphate. Thus, *the complete oxidation of a molecule of palmitate yields 106 molecules of ATP.*

22.3 Unsaturated and Odd-Chain Fatty Acids Require Additional Steps for Degradation

The β-oxidation pathway accomplishes the complete degradation of saturated fatty acids having an even number of carbon atoms. Most fatty acids have such structures because of their mode of synthesis (to be addressed later in this chapter). However, not all fatty acids are so simple. The oxidation of fatty acids containing double bonds requires additional steps, as does the oxidation of fatty acids containing an odd number of carbon atoms.

An isomerase and a reductase are required for the oxidation of unsaturated fatty acids

The oxidation of unsaturated fatty acids presents some difficulties, yet many such fatty acids are available in the diet. Most of the reactions are the same as those for saturated fatty acids. In fact, only two additional enzymes—an isomerase and a reductase—are needed to degrade a wide range of unsaturated fatty acids.

Consider the oxidation of palmitoleate (Figure 22.11) This C_{16} unsaturated fatty acid, which has one double bond between C-9 and C-10, is activated and transported across the inner mitochondrial membrane in the same way as saturated fatty acids are. Palmitoleoyl CoA then undergoes three cycles of degradation, which are carried out by the same enzymes as those in the oxidation of saturated fatty acids. However, the *cis*-Δ^3-enoyl CoA formed in the third round is not a substrate for acyl CoA dehydrogenase. The presence of a double bond between C-3 and C-4 prevents the formation of another double bond between C-2 and C-3. This impasse is resolved by a new reaction that shifts the position and configuration of the cis-Δ^3 double bond. *cis*-Δ^3-*Enoyl CoA isomerase converts this double bond into a trans*-Δ^2 *double bond.* The double bond is now between C-2 and C-3. The subsequent reactions are those of the saturated fatty acid oxidation pathway, in which the *trans*-Δ^2-enoyl CoA is a regular substrate.

Human beings require polyunsaturated fatty acids, which have multiple double bonds, as important precursors for signal molecules, but excess polyunsaturated fatty acids are degraded by β oxidation. However, another problem arises with the oxidation of polyunsaturated fatty acids. Consider linoleate, a C_{18} polyunsaturated fatty acid with cis-Δ^9 and cis-Δ^{12} double bonds (Figure 22.12). The cis-Δ^3 double bond (between carbons 3 and 4) formed after three rounds of β-oxidation is converted into a trans-Δ^2 double bond (between carbons 2 and 3) by the aforementioned isomerase. The acyl CoA produced by another round of β-oxidation contains a cis-Δ^4 (between

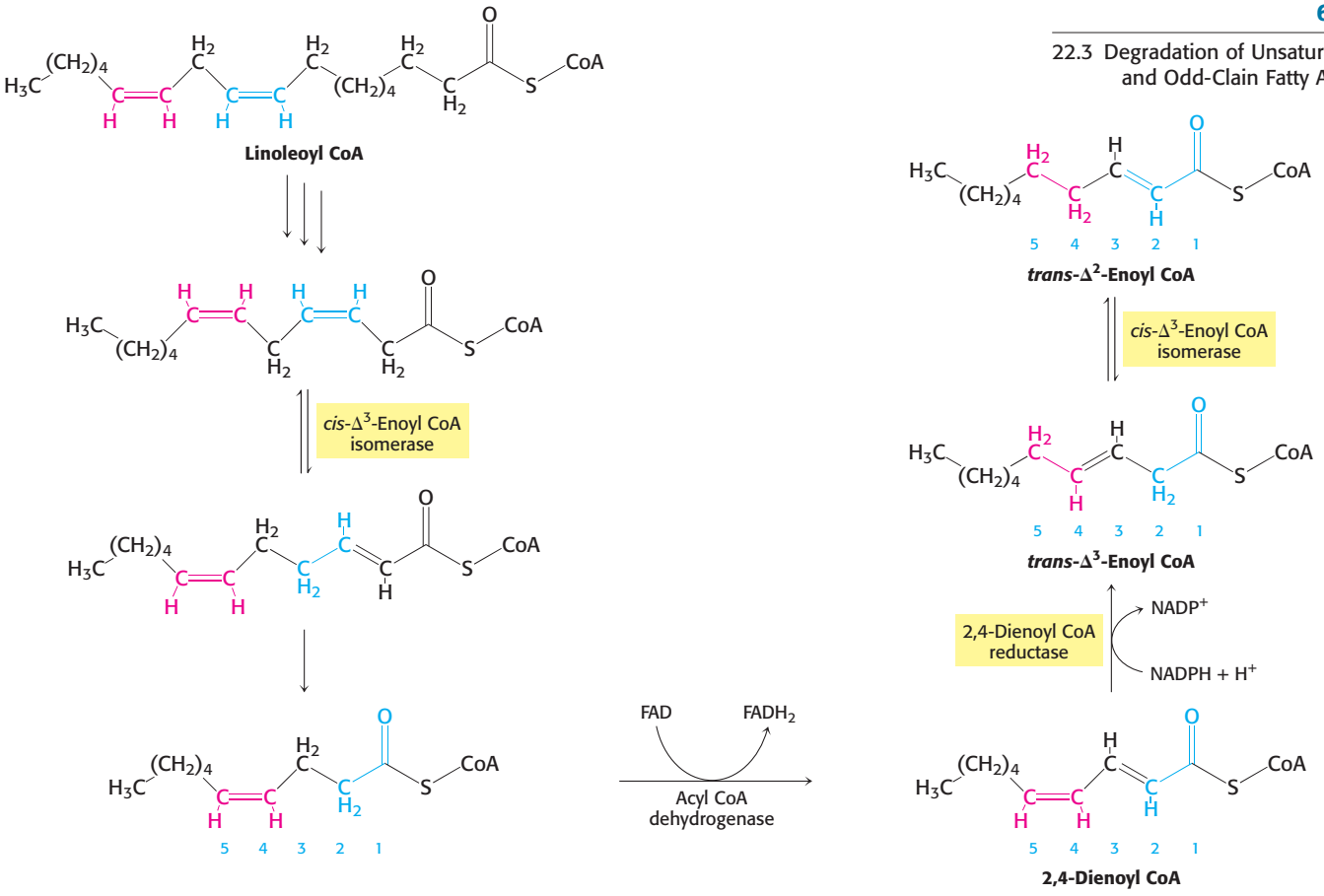
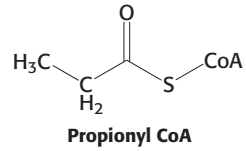


Figure 22.12 Oxidation of linoleoyl CoA. The complete oxidation of the diunsaturated fatty acid linoleate is facilitated by the activity of enoyl CoA isomerase and 2,4-dienoyl CoA reductase.

carbons 4 and 5) double bond. Dehydrogenation of this species by acyl CoA dehydrogenase yields a *2,4-dienoyl intermediate* (double bond between carbons 2 and 3 and carbons 4 and 5), which is not a substrate for the next enzyme in the β-oxidation pathway. This impasse is circumvented by *2,4-dienoyl CoA reductase,* an enzyme that uses NADPH to reduce the 2,4-dienoyl intermediate to *trans*-Δ^3-enoyl CoA. *cis*-Δ^3-Enoyl CoA isomerase then converts *trans*-Δ^3-enoyl CoA into the trans-Δ^2 form, a customary intermediate in the β-oxidation pathway. These catalytic strategies are elegant and economical. Only two extra enzymes are needed for the oxidation of *any* polyunsaturated fatty acid. *Odd-numbered double bonds are handled by the isomerase, and even-numbered ones by the reductase and the isomerase.*

Odd-chain fatty acids yield propionyl CoA in the final thiolysis step

Fatty acids having an odd number of carbon atoms are minor species. They are oxidized in the same way as fatty acids having an even number, except that propionyl CoA and acetyl CoA, rather than two molecules of acetyl CoA, are produced in the final round of degradation. The activated three-carbon unit in propionyl CoA enters the citric acid cycle after it has been converted into succinyl CoA.



The pathway from propionyl CoA to succinyl CoA is especially interesting because it entails a rearrangement that requires *vitamin* B_{12} (also known as *cobalamin*). Propionyl CoA is carboxylated at the expense of the hydrolysis of a molecule of ATP to yield the D isomer of methylmalonyl CoA (Figure 22.13). This carboxylation reaction is catalyzed by *propionyl*

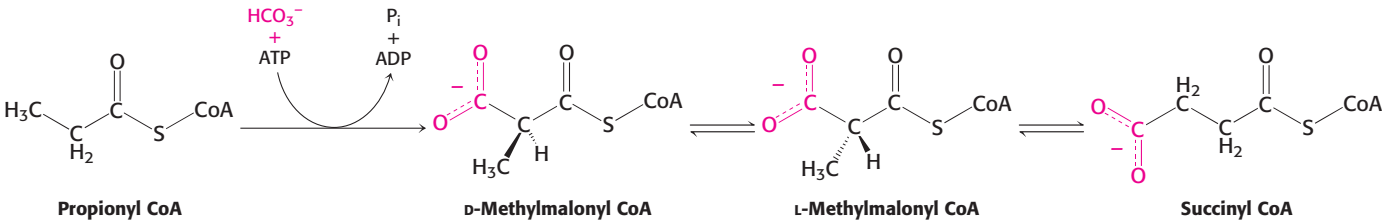


Figure 22.13 Conversion of propionyl CoA into succinyl CoA. Propionyl CoA, generated from fatty acids with an odd number of carbons as well as some amino acids, is converted into the citric acid cycle intermediate succinyl CoA.

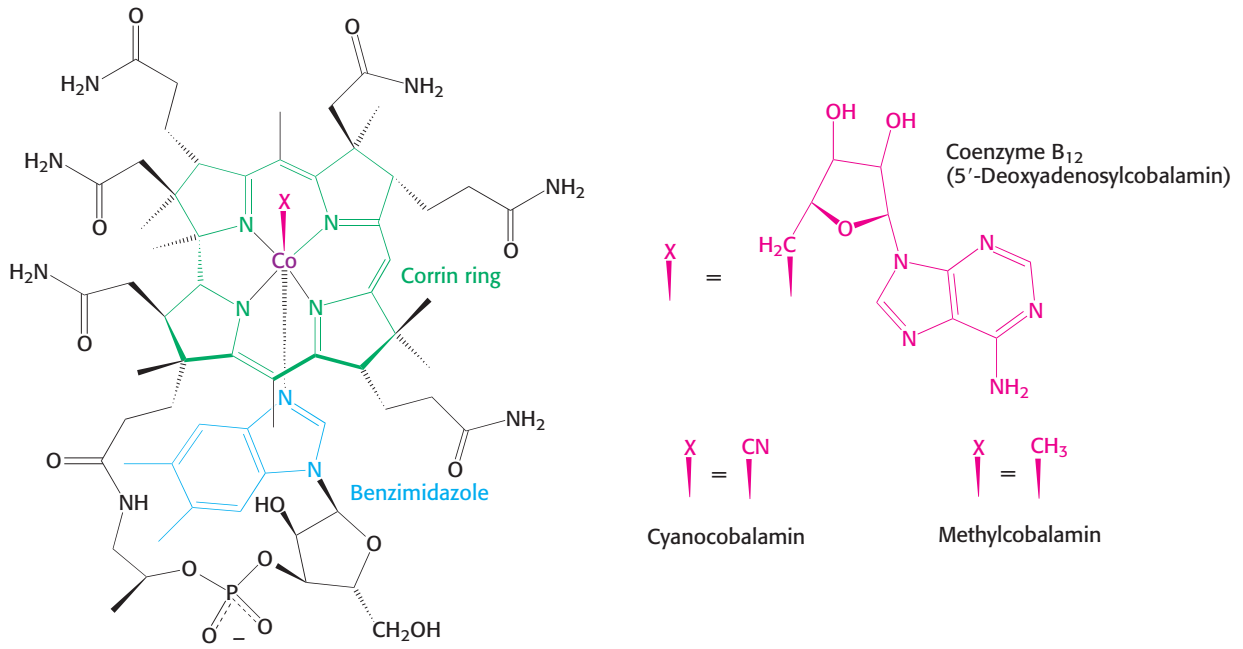
CoA carboxylase, a biotin enzyme that has a catalytic mechanism like that of the homologous enzyme pyruvate carboxylase. The D isomer of methylmalonyl CoA is racemized to the L isomer, the substrate for a mutase that converts it into *succinyl CoA* by an *intramolecular rearrangement*. The —CO—S—CoA group migrates from C-2 to a methyl group in exchange for a hydrogen atom. This very unusual isomerization is catalyzed by *methylmalonyl CoA mutase,* which contains a derivative of cobalamin as its coenzyme.

Vitamin B_{12} contains a corrin ring and a cobalt atom

Cobalamin enzymes, which are present in most organisms, catalyze three types of reactions: (1) *intramolecular rearrangements;* (2) *methylations,* as in the synthesis of methionine; and (3) the *reduction of ribonucleotides to deoxyribonucleotides* (Section 25.3). In mammals, only two reactions are known to require coenzyme B_{12}. The conversion of L-methylmalonyl CoA into succinyl CoA is one, and the formation of methionine by the methylation of homocysteine is the other. The latter reaction is especially important because methionine is required for the generation of coenzymes that participate in the synthesis of purines and thymine, which are needed for nucleic acid synthesis.

The core of cobalamin consists of a *corrin ring with a central cobalt atom* (Figure 22.14). The corrin ring, like a porphyrin, has *four pyrrole units.* Two of them are directly bonded to each other, whereas the others are joined by methine bridges, as in porphyrins. The corrin ring is more reduced than that of porphyrins and the substituents are different. A cobalt atom is bonded to the four pyrrole nitrogens. The fifth substituent linked to the cobalt atom is

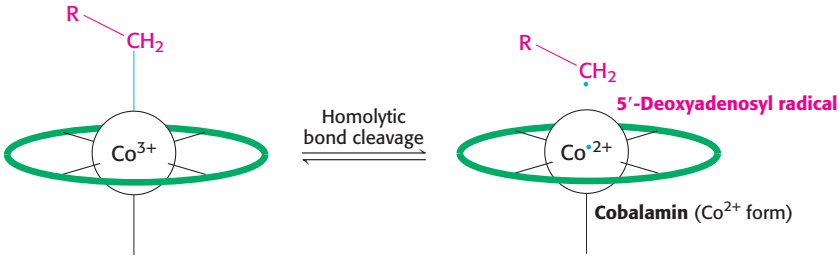
Figure 22.14 Structure of coenzyme B_{12}. Coenzyme B_{12} is a class of molecules that vary, depending on the component designated X in the left-hand structure. 5′-Deoxyadenosylcobalamin is the form of the coenzyme in methylmalonyl mutase. Substitution of cyano and methyl groups for X creates cyanocobalamin and methylcobalamin, respectively.



a derivative of *dimethylbenzimidazole* that contains ribose 3-phosphate and aminoisopropanol. One of the nitrogen atoms of dimethylbenzimidazole is linked to the cobalt atom. In coenzyme B_{12}, *the sixth substituent* linked to the cobalt atom is a *5′-deoxyadenosyl unit*. This position can also be occupied by a cyano group, a methyl group, or other ligands. In all of these compounds, the cobalt is in the +3 oxidation state.

Mechanism: Methylmalonyl CoA mutase catalyzes a rearrangement to form succinyl CoA

The rearrangement reactions catalyzed by coenzyme B_{12} are exchanges of two groups attached to adjacent carbon atoms of the substrate (Figure 22.15). A hydrogen atom migrates from one carbon atom to the next, and an R group (such as the —CO—S—CoA group of methylmalonyl CoA) concomitantly moves in the reverse direction. The first step in these intramolecular rearrangements is the cleavage of the carbon–cobalt bond of 5′-deoxyadenosylcobalamin to generate the Co^{2+} form of the coenzyme and a 5′-deoxyadenosyl radical, $—CH_2•$ (Figure 22.16). In this *homolytic cleavage reaction*, one electron of the Co–C bond stays with Co (reducing it from the +3 to the +2 oxidation state), whereas the other electron stays with the carbon atom, generating a free radical. In contrast, nearly all other cleavage reactions in biological systems are *heterolytic:* an electron *pair* is transferred to one of the two atoms that were bonded together.



What is the role of this very unusual $—CH_2•$ radical? This highly reactive species abstracts a *hydrogen atom* from the substrate to form 5′-deoxyadenosine and a substrate radical (Figure 22.17). This substrate radical spontaneously rearranges: the carbonyl CoA group migrates to the position formerly occupied by H on the neighboring carbon atom to produce a different radical. This product radical abstracts a hydrogen atom from the methyl group of 5′-deoxyadenosine to complete the rearrangement and return the deoxyadenosyl unit to the radical form. *The role of coenzyme B_{12} in such intramolecular migrations is to serve as a source of free radicals for the abstraction of hydrogen atoms.*

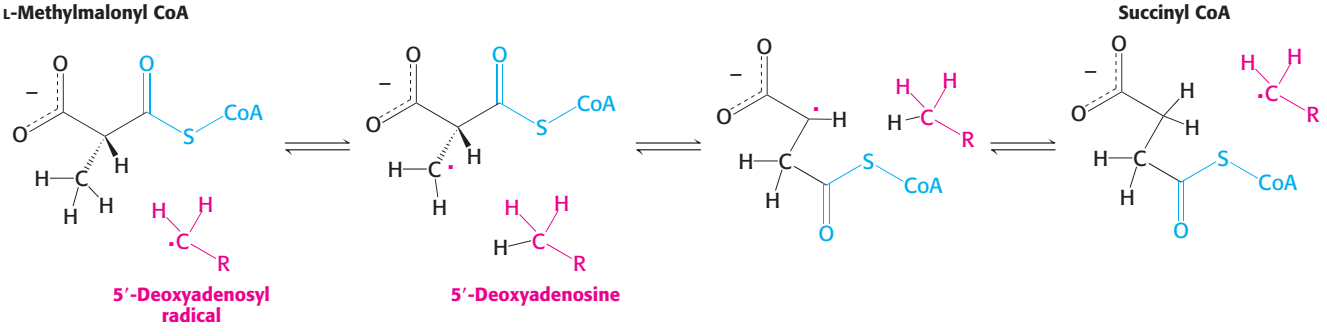


Figure 22.15 Rearrangement reaction catalyzed by cobalamin enzymes. The R group can be an amino group, a hydroxyl group, or a substituted carbon.

Figure 22.16 Formation of a 5′-deoxyadenosyl radical. The methylmalonyl CoA mutase reaction begins with the homolytic cleavage of the bond joining Co^{3+} of coenzyme B_{12} to a carbon atom of the ribose of the adenosine moiety of the enzyme. The cleavage generates a 5′-deoxyadenosyl radical and leads to the reduction of Co^{3+} to Co^{2+}. The letter R represents the 5′-deoxyadenosyl component of the coenzyme, and the green oval represents the remainder of the coenzyme.

Figure 22.17 Formation of succinyl CoA by a rearrangement reaction. A free radical abstracts a hydrogen atom in the rearrangement of methylmalonyl CoA to succinyl CoA.

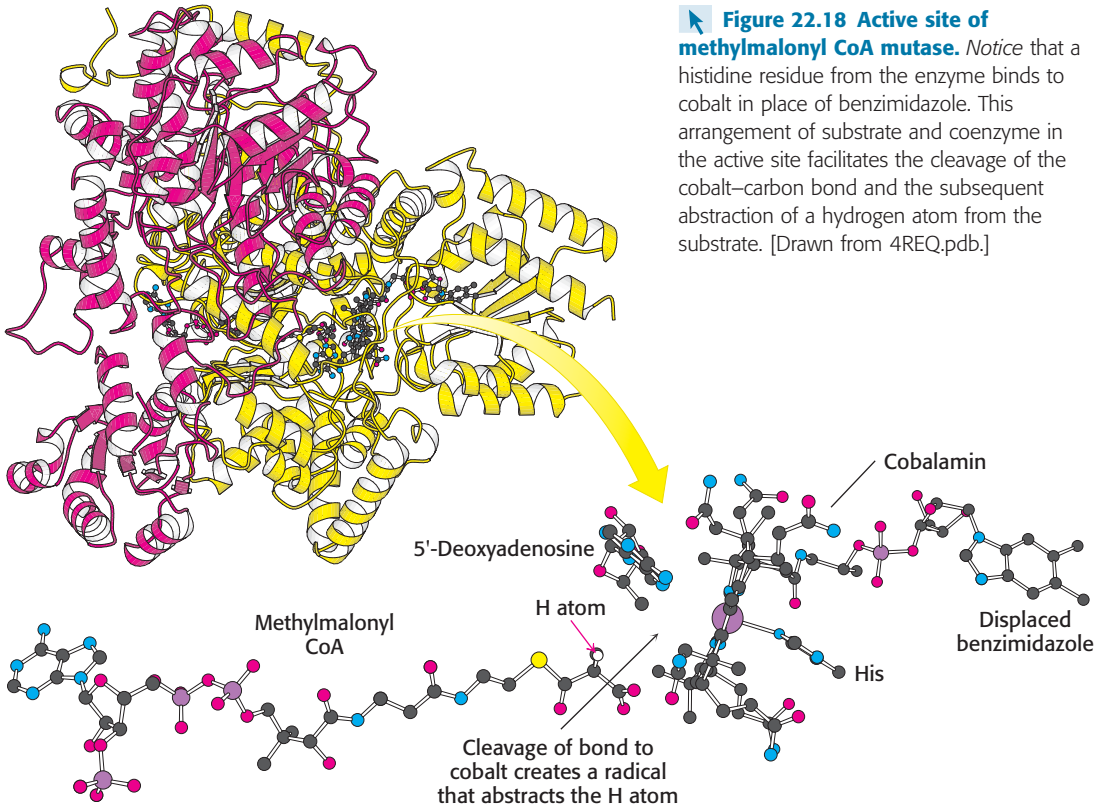


Figure 22.18 Active site of methylmalonyl CoA mutase. *Notice* that a histidine residue from the enzyme binds to cobalt in place of benzimidazole. This arrangement of substrate and coenzyme in the active site facilitates the cleavage of the cobalt–carbon bond and the subsequent abstraction of a hydrogen atom from the substrate. [Drawn from 4REQ.pdb.]

An essential property of coenzyme B_{12} is the weakness of its cobalt–carbon bond, which is readily cleaved to generate a radical. To facilitate the cleavage of this bond, enzymes such as methylmalonyl CoA mutase displace the benzimidazole group from the cobalamin and bind to the cobalt atom through a histidine residue (Figure 22.18). The steric crowding around the cobalt–carbon bond within the corrin ring system contributes to the bond weakness.

Fatty acids are also oxidized in peroxisomes

Although most fatty acid oxidation takes place in mitochondria, some oxidation of fatty acids can take place in cellular organelles called *peroxisomes* (Figure 22.19). These organelles are small membrane-bounded compartments that are present in the cells of most eukaryotes. Fatty acid oxidation in these organelles, which halts at octanoyl CoA, may serve to shorten long chains to make them better substrates of β oxidation in mitochondria. Peroxisomal oxidation differs from β oxidation in the initial dehydrogenation reaction (Figure 22.20). In peroxisomes, acyl CoA dehydrogenase, a flavoprotein, transfers electrons from the substrate to $FADH_2$ and then to O_2 to yield H_2O_2 instead of capturing high-energy electrons as $FADH_2$ for use in the electron-transport chain, as in mitochondrial β oxidation. Peroxisomes contain high concentrations of the enzyme catalase to degrade H_2O_2 into water and O_2. Subsequent steps are identical with those of their mitochondrial counterparts, although they are carried out by different isoforms of the enzymes.

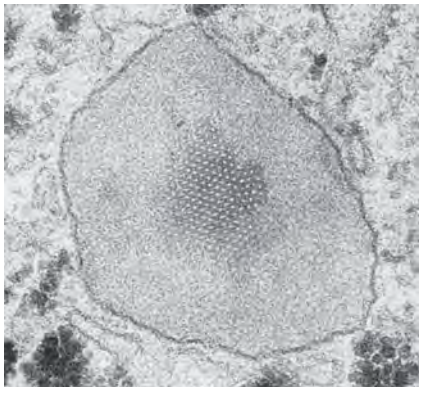


Figure 22.19 Electron micrograph of a peroxisome in a liver cell. A crystal of urate oxidase is present inside the organelle, which is bounded by a single bilayer membrane. The dark granular structures outside the peroxisome are glycogen particles. [Courtesy of Dr. George Palade.]

Peroxisomes do not function in patients with Zellweger syndrome. Liver, kidney, and muscle abnormalities usually lead to death by age six. The syndrome is caused by a defect in the import of enzymes into the peroxisomes. Here we see a pathological condition resulting from an inappropriate cellular distribution of enzymes.

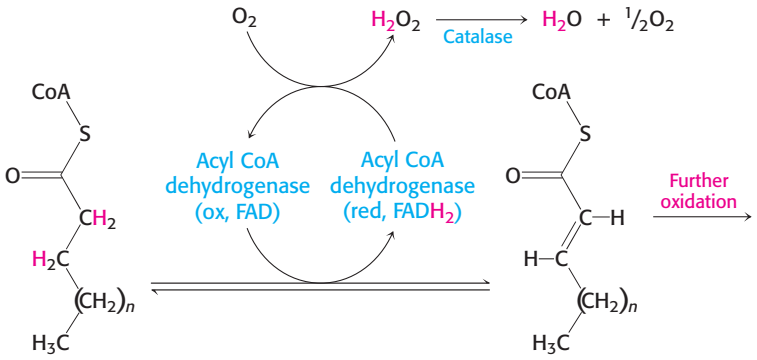


Figure 22.20 Initiation of peroxisomal fatty acid degradation. The first dehydration in the degradation of fatty acids in peroxisomes requires a flavoprotein dehydrogenase that transfers electrons from its $FADH_2$ moiety to O_2 to yield H_2O_2.

Ketone bodies are formed from acetyl CoA when fat breakdown predominates

The acetyl CoA formed in fatty acid oxidation enters the citric acid cycle only if fat and carbohydrate degradation are appropriately balanced. Acetyl CoA must combine with oxaloacetate to gain entry to the citric acid cycle. The availability of oxaloacetate, however, depends on an adequate supply of carbohydrate. Recall that oxaloacetate is normally formed from pyruvate, the product of glucose degradation in glycolysis. If carbohydrate is unavailable or improperly utilized, the concentration of oxaloacetate is lowered and acetyl CoA cannot enter the citric acid cycle. This dependency is the molecular basis of the adage that *fats burn in the flame of carbohydrates.*

In fasting or diabetes, oxaloacetate is consumed to form glucose by the gluconeogenic pathway (Section 16.3) and hence is unavailable for condensation with acetyl CoA. Under these conditions, acetyl CoA is diverted to the formation of acetoacetate and D-3-hydroxybutyrate. Acetoacetate, D-3-hydroxybutyrate, and acetone are often referred to as *ketone bodies.* Abnormally high levels of ketone bodies are present in the blood of untreated diabetics.

Acetoacetate is formed from acetyl CoA in three steps (Figure 22.21). Two molecules of acetyl CoA condense to form acetoacetyl CoA. This reaction, which is catalyzed by thiolase, is the reverse of the thiolysis step in the oxidation of fatty acids. Acetoacetyl CoA then reacts with acetyl CoA and water to give 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) and CoA.

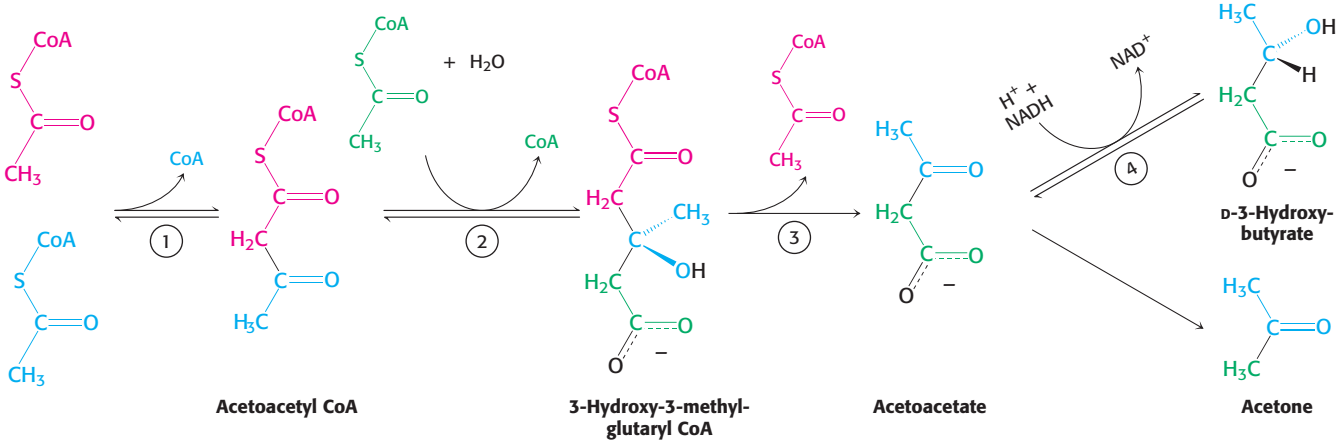


Figure 22.21 Formation of ketone bodies. The ketone bodies—acetoacetate, D-3-hydroxybutyrate, and acetone from acetyl CoA—are formed primarily in the liver. Enzymes catalyzing these reactions are (1) 3-ketothiolase, (2) hydroxymethylglutaryl CoA synthase, (3) hydroxymethylglutaryl CoA cleavage enzyme, and (4) D-3-hydroxybutyrate dehydrogenase. Acetoacetate spontaneously decarboxylates to form acetone.

This condensation resembles the one catalyzed by citrate synthase (Section 17.2). This reaction, which has a favorable equilibrium owing to the hydrolysis of a thioester linkage, compensates for the unfavorable equilibrium in the formation of acetoacetyl CoA. 3-Hydroxy-3-methylglutaryl CoA is then cleaved to acetyl CoA and acetoacetate. The sum of these reactions is

$$2\ \text{Acetyl CoA} + H_2O \longrightarrow \text{acetoacetate} + 2\ \text{CoA} + H^+$$

D-3-Hydroxybutyrate is formed by the reduction of acetoacetate in the mitochondrial matrix by D-3-hydroxybutyrate dehydrogenase. The ratio of hydroxybutyrate to acetoacetate depends on the $NADH/NAD^+$ ratio inside mitochondria.

Because it is a β-ketoacid, acetoacetate also undergoes a slow, spontaneous decarboxylation to acetone. The odor of acetone may be detected in the breath of a person who has a high level of acetoacetate in the blood.

Ketone bodies are a major fuel in some tissues

The major site of the production of acetoacetate and 3-hydroxybutyrate is the liver. These substances diffuse from the liver mitochondria into the blood and are transported to other tissues such as heart and kidney (Figure 22.22). Acetoacetate and 3-hydroxybutyrate are normal fuels of respiration and are quantitatively important as sources of energy. Indeed, heart muscle and the renal cortex use acetoacetate in preference to glucose. In contrast, glucose is the major fuel for the brain and red blood cells in

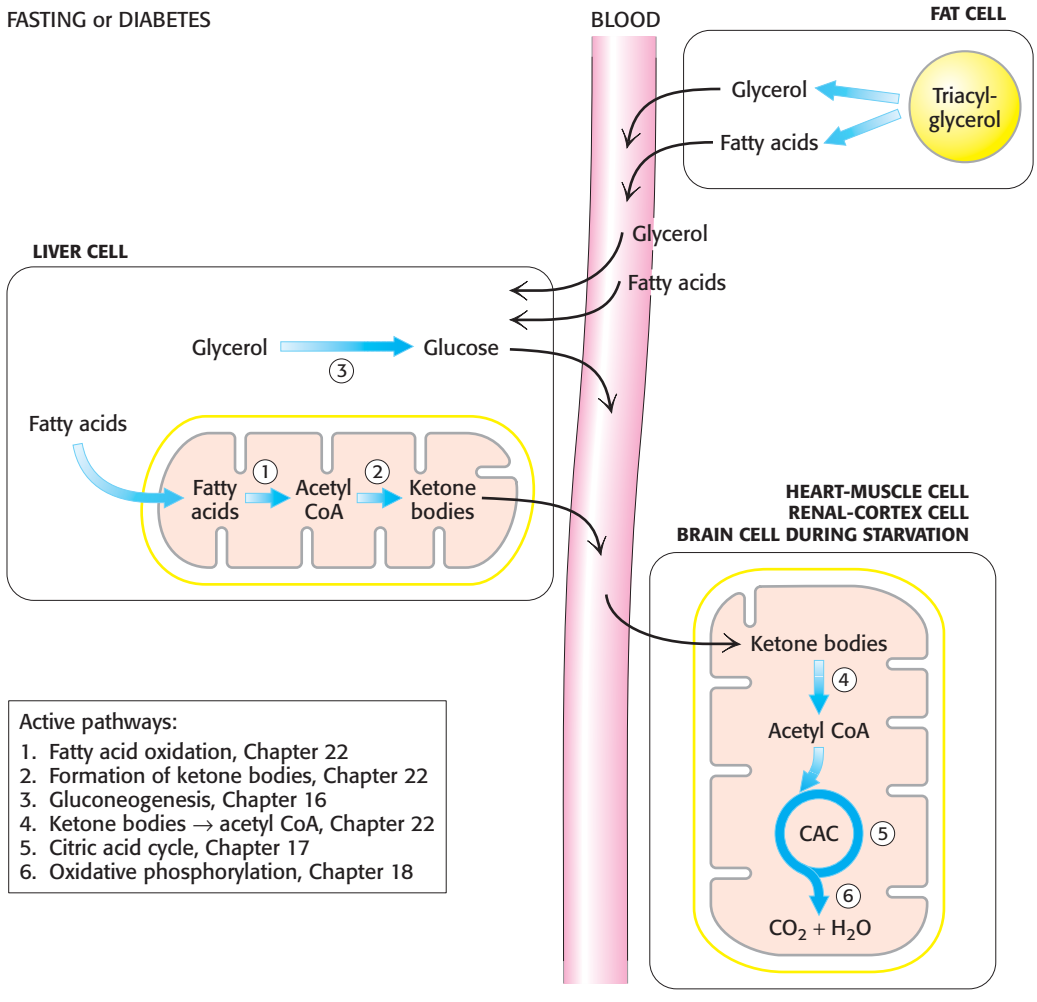
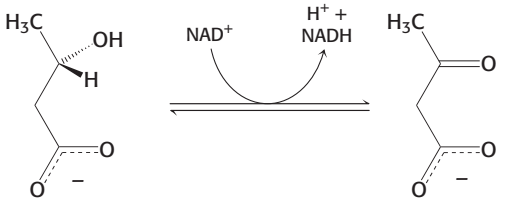


Figure 22.22 PATHWAY INTEGRATION: Liver supplies ketone bodies to the peripheral tissues. During fasting or in untreated diabetics, the liver converts fatty acids into ketone bodies, which are a fuel source for a number of tissues. Ketone-body production is especially important during starvation, when ketone bodies are the predominant fuel.

well-nourished people on a balanced diet. However, the brain adapts to the utilization of acetoacetate during starvation and diabetes. In prolonged starvation, 75% of the fuel needs of the brain are met by ketone bodies.

Acetoacetate is converted into acetyl CoA in two steps. First, acetoacetate is activated by the transfer of CoA from succinyl CoA in a reaction catalyzed by a specific CoA transferase. Second, acetoacetyl CoA is cleaved by thiolase to yield two molecules of acetyl CoA, which can then enter the citric acid cycle (Figure 22.23). The liver has acetoacetate available to supply to other organs because it lacks this particular CoA transferase. 3-Hydroxybutyrate requires an additional step to yield acetyl CoA. It is first oxidized to produce acetoacetate, which is processed as heretofore described, and NADH for use in oxidative phosphorylation.



Ketone bodies can be regarded as a water-soluble, transportable form of acetyl units. Fatty acids are released by adipose tissue and converted into acetyl units by the liver, which then exports them as acetoacetate. As might be expected, acetoacetate also has a regulatory role. *High levels of acetoacetate in the blood signify an abundance of acetyl units and lead to a decrease in the rate of lipolysis in adipose tissue.*

High blood levels of ketone bodies, the result of certain pathological conditions, can be life threatening. The most common of these conditions is diabetic ketosis in patients with insulin-dependent diabetes mellitus. These patients are unable to produce insulin. As stated earlier, this hormone, normally released after meals, signals tissues to take up glucose. In addition, it curtails fatty acid mobilization by adipose tissue. The absence of insulin has two major biochemical consequences (Figure 22.24). First, the liver cannot absorb glucose and consequently cannot provide oxaloacetate to process fatty acid-derived acetyl CoA. Second, adipose cells continue to

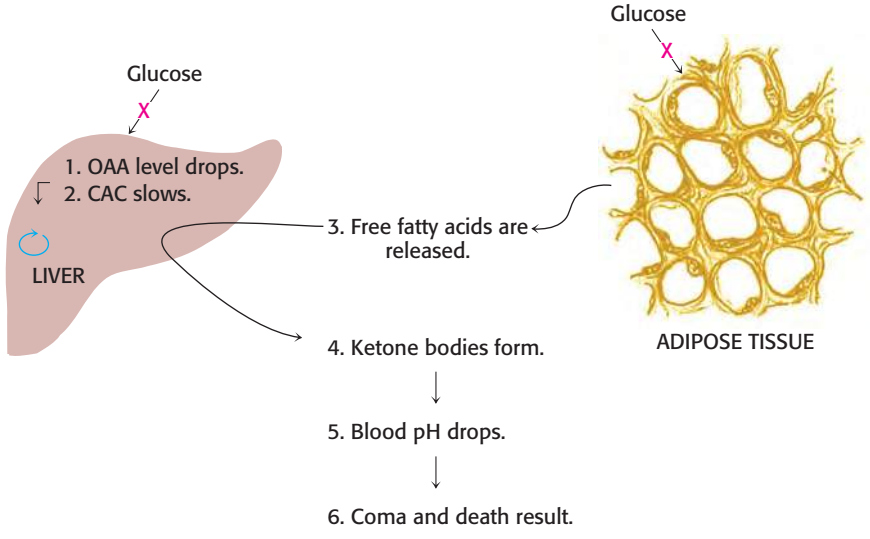


Figure 22.24 Diabetic ketosis results when insulin is absent. In the absence of insulin, fats are released from adipose tissue, and glucose cannot be absorbed by the liver or adipose tissue. The liver degrades the fatty acids by β oxidation but cannot process the acetyl CoA, because of a lack of glucose-derived oxaloacetate (OAA). Excess ketone bodies are formed and released into the blood.

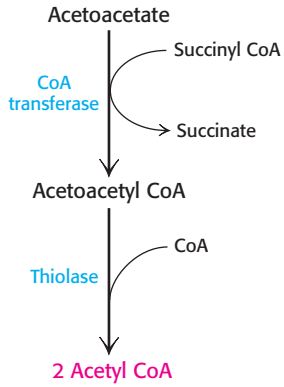


Figure 22.23 Utilization of acetoacetate as a fuel. Acetoacetate can be converted into two molecules of acetyl CoA, which then enter the citric acid cycle.

release fatty acids into the bloodstream, which are taken up by the liver and converted into ketone bodies. The liver thus produces large amounts of ketone bodies, which are moderately strong acids. The result is severe acidosis. The decrease in pH impairs tissue function, most importantly in the central nervous system.

Interestingly, diets that promote ketone-body formation, called ketogenic diets, are frequently used as a therapeutic option for children with drug-resistant epilepsy. Ketogenic diets are rich in fats and low in carbohydrates, with adequate amounts of protein. In essence, the body is forced into starvation mode, where fats and ketone bodies become the main fuel source (Section 27.5). How such diets reduce the seizures suffered by the children is currently unknown.

Animals cannot convert fatty acids into glucose

A typical human being has far greater fat stores than glycogen stores. However, glycogen is necessary to fuel very active muscle, as well as the brain, which normally uses only glucose as a fuel. When glycogen stores are low, why can't the body make use of fat stores and convert fatty acids into glucose? Because *animals are unable to effect the net synthesis of glucose from fatty acids.* Specifically, acetyl CoA cannot be converted into pyruvate or oxaloacetate in animals. Recall that the reaction that generates acetyl CoA from pyruvate is irreversible (Section 17.1). The two carbon atoms of the acetyl group of acetyl CoA enter the citric acid cycle, but two carbon atoms leave the cycle in the decarboxylations catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. Consequently, oxaloacetate is regenerated, but it is not formed de novo when the acetyl unit of acetyl CoA is oxidized by the citric acid cycle. In essence, two carbon atoms enter the cycle as an acetyl group, but two carbons leave the cycle as CO_2 before oxaloacetate is generated. Consequently, no net synthesis of oxaloacetate is possible. In contrast, plants have two additional enzymes enabling them to convert the carbon atoms of acetyl CoA into oxaloacetate (Section 17.5).

22.4 Fatty Acids Are Synthesized by Fatty Acid Synthase

Fatty acids are synthesized by a complex of enzymes that together are called *fatty acid synthase.* Because eating a typical Western diet meets our physiological needs for fats and lipids, adult human beings have little need for de novo fatty acid synthesis. However, many tissues, such as liver and adipose tissue, are capable of synthesizing fatty acids, and this synthesis is required under certain physiological conditions. For instance, fatty acid synthesis is necessary during embryonic development and during lactation in mammary glands. Inappropriate fatty acid synthesis in the liver of alcoholics contributes to liver failure.

Acetyl CoA, the end product of fatty acid degradation, is the precursor for virtually all fatty acids. The biochemical challenge is to link the two carbon units together and reduce the carbons to produce palmitate, a C_{16} fatty acid. Palmitate then serves as a precursor for the variety of other fatty acids.

Fatty acids are synthesized and degraded by different pathways

Although fatty acid synthesis is the reversal of the degradative pathway in regard to basic chemical reactions, the synthetic and degradative pathways are different mechanistically, again exemplifying the principle that *synthetic and degradative pathways are almost always distinct.* Some important differences between the pathways are as follows:

1. Synthesis takes place in the *cytoplasm,* in contrast with degradation, which takes place primarily in the mitochondrial matrix.

2. Intermediates in fatty acid synthesis are covalently linked to the sulfhydryl groups of an *acyl carrier protein* (ACP), whereas intermediates in fatty acid breakdown are covalently attached to the sulfhydryl group of coenzyme A.

3. The enzymes of fatty acid synthesis in higher organisms are joined in a *single polypeptide chain* called *fatty acid synthase.* In contrast, the degradative enzymes do not seem to be associated.

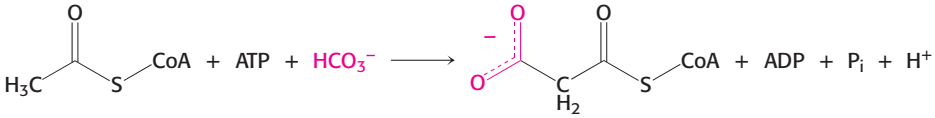
4. The growing fatty acid chain is elongated by the *sequential addition of two-carbon units* derived from acetyl CoA. The activated donor of two-carbon units in the elongation step is *malonyl ACP.* The elongation reaction is driven by the release of CO_2.

5. The reductant in fatty acid synthesis is *NADPH,* whereas the oxidants in fatty acid degradation are NAD^+ and *FAD.*

6. Elongation by the fatty acid synthase complex stops on the formation of *palmitate* (C_{16}). Further elongation and the insertion of double bonds are carried out by other enzyme systems.

The formation of malonyl CoA is the committed step in fatty acid synthesis

Fatty acid synthesis starts with the carboxylation of acetyl CoA to *malonyl CoA.* This irreversible reaction is the committed step in fatty acid synthesis.



Acetyl CoA **Malonyl CoA**

The synthesis of malonyl CoA is catalyzed by *acetyl CoA carboxylase,* which contains a biotin prosthetic group. The carboxyl group of biotin is covalently attached to the ε amino group of a lysine residue, as in pyruvate carboxylase (see Figure 16.24) and propionyl CoA carboxylase (p. 650). As with these other enzymes, a carboxybiotin intermediate is formed at the expense of the hydrolysis of a molecule of ATP. The activated CO_2 group in this intermediate is then transferred to acetyl CoA to form malonyl CoA.

$$\text{Biotin-enzyme} + \text{ATP} + HCO_3^- \rightleftharpoons CO_2\text{-biotin-enzyme} + \text{ADP} + P_i$$

$$CO_2\text{-biotin-enzyme} + \text{acetyl CoA} \longrightarrow \text{malonyl CoA} + \text{biotin-enzyme}$$

Acetyl CoA carbozylase is also the essential regulatory enzyme for fatty acid metabolism (Section 22.5).

Intermediates in fatty acid synthesis are attached to an acyl carrier protein

The intermediates in fatty acid synthesis are linked to an acyl carrier protein. Specifically, they are linked to the sulfhydryl terminus of a phosphopantetheine group. In the degradation of fatty acids, this unit is present as part of coenzyme A, whereas, in their synthesis, it is attached to a serine

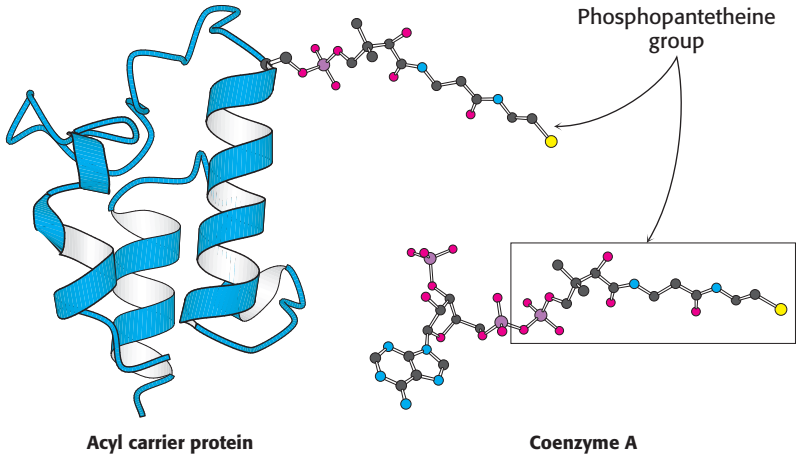


Figure 22.25 Phosphopantetheine. Both acyl carrier protein and coenzyme A include phosphopantetheine as their reactive units.

residue of the acyl carrier protein (Figure 22.25). Thus, ACP, a single polypeptide chain of 77 residues, can be regarded as a giant prosthetic group, a "macro CoA."

Fatty acid synthesis consists of a series of condensation, reduction, dehydration, and reduction reactions

The enzyme system that catalyzes the synthesis of saturated long-chain fatty acids from acetyl CoA, malonyl CoA, and NADPH is called the fatty acid synthase. The synthase is actually a complex of distinct enzymes. The fatty acid synthase complex in bacteria is readily dissociated into individual enzymes when the cells are broken apart. The availability of these isolated enzymes has helped biochemists elucidate the steps in fatty acid synthesis (Table 22.2). In fact, the reactions leading to fatty acid synthesis in higher organisms are very much like those of bacteria.

The elongation phase of fatty acid synthesis starts with the formation of acetyl ACP and malonyl ACP. *Acetyl transacylase* and *malonyl transacylase* catalyze these reactions.

$$\text{Acetyl CoA} + \text{ACP} \rightleftharpoons \text{acetyl ACP} + \text{CoA}$$

$$\text{Malonyl CoA} + \text{ACP} \rightleftharpoons \text{malonyl ACP} + \text{CoA}$$

Malonyl transacylase is highly specific, whereas acetyl transacylase can transfer acyl groups other than the acetyl unit, though at a much slower rate. The synthesis of fatty acids with an odd number of carbon atoms starts with propionyl ACP, which is formed from propionyl CoA by acetyl transacylase.

Acetyl ACP and malonyl ACP react to form acetoacetyl ACP (Figure 22.26). The *β-ketoacyl synthase*, also called the condensing enzyme, catalyzes this condensation reaction.

$$\text{Acetyl ACP} + \text{malonyl ACP} \longrightarrow \text{acetoacetyl ACP} + \text{ACP} + CO_2$$

Table 22.2 Principal reactions in fatty acid synthesis in bacteria

Step	Reaction	Enzyme
1	Acetyl CoA + HCO_3^- + ATP ⟶ malonyl CoA + ADP + P_i + H^+	Acetyl CoA carboxylase
2	Acetyl CoA + ACP ⇌ acetyl ACP + CoA	Acetyl transacylase
3	Malonyl CoA + ACP ⇌ malonyl ACP + CoA	Malonyl transacylase
4	Acetyl ACP + malonyl ACP ⟶ acetoacetyl ACP + ACP + CO_2	β-Ketoacyl synthase
5	Acetoacetyl ACP + NADPH + H^+ ⇌ D-3-hydroxybutyryl ACP + $NADP^+$	β-Ketoacyl reductase
6	D-3-Hydroxybutyryl ACP ⇌ crotonyl ACP + H_2O	3-Hydroxyacyl dehydratase
7	Crotonyl ACP + NADPH + H^+ ⟶ butyryl ACP + $NADP^+$	Enoyl reductase

In the condensation reaction, a four-carbon unit is formed from a two-carbon unit and a three-carbon unit, and CO_2 is released. Why is the four-carbon unit not formed from two 2-carbon units—say, two molecules of acetyl ACP? The answer is that the equilibrium for the synthesis of acetoacetyl ACP from two molecules of acetyl ACP is highly unfavorable. In contrast, *the equilibrium is favorable if malonyl ACP is a reactant because its decarboxylation contributes a substantial decrease in free energy.* In effect, ATP drives the condensation reaction, though ATP does not directly participate in the condensation reaction. Instead, ATP is used to carboxylate acetyl CoA to malonyl CoA. The free energy thus stored in malonyl CoA is released in the decarboxylation accompanying the formation of acetoacetyl ACP. Although HCO_3^- is required for fatty acid synthesis, its carbon atom does not appear in the product. Rather, *all the carbon atoms of fatty acids containing an even number of carbon atoms are derived from acetyl CoA.*

The next three steps in fatty acid synthesis reduce the keto group at C-3 to a methylene group (see Figure 22.26). First, acetoacetyl ACP is reduced to D-3-hydroxybutyryl ACP by β-ketoacyl reductase. This reaction differs from the corresponding one in fatty acid degradation in two respects: (1) the D rather than the L isomer is formed; and (2) NADPH is the reducing agent, whereas NAD^+ is the oxidizing agent in β oxidation. This difference exemplifies the general principle that *NADPH is consumed in biosynthetic reactions, whereas NADH is generated in energy-yielding reactions.* Then D-3-hydroxybutyryl ACP is *dehydrated* to form crotonyl ACP, which is a *trans*-Δ^2-enoyl ACP by 3-hydroxyacyl dehydratase. The final step in the cycle *reduces* crotonyl ACP to butyryl ACP. NADPH is again the reductant, whereas FAD is the oxidant in the corresponding reaction in β oxidation. The bacterial enzyme that catalyzes this step, *enoyl reductase,* can be inhibited by *triclosan,* a broad-spectrum antibacterial agent that is added to a variety of products such as toothpaste, soaps, and skin creams. These last three reactions—a reduction, a dehydration, and a second reduction—convert acetoacetyl ACP into butyryl ACP, which completes the first elongation cycle.

In the second round of fatty acid synthesis, butyryl ACP condenses with malonyl ACP to form a C_6-β-ketoacyl ACP. This reaction is like the one in the first round, in which acetyl ACP condenses with malonyl ACP to form a C_4-β-ketoacyl ACP. Reduction, dehydration, and a second reduction convert the C_6-β-ketoacyl ACP into a C_6-acyl ACP, which is ready for a third round of elongation. The elongation cycles continue until C_{16}-acyl ACP is formed. This intermediate is a good substrate for a thioesterase that hydrolyzes C_{16}-acyl ACP to yield palmitate and ACP. *The thioesterase acts as a ruler to determine fatty acid chain length.* The synthesis of longer-chain fatty acids is discussed in Section 22.6.

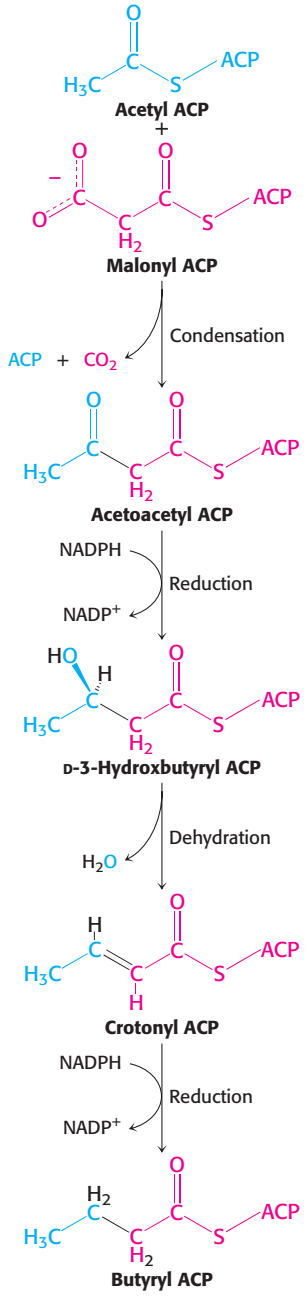


Figure 22.26 The steps of fatty acid synthesis. Fatty acid synthesis begins with the condensation of malonyl ACP and acetyl ACP to form acetoacetyl ACP. Acetoacetyl ACP is then reduced, dehydrated, and reduced again to form butyryl ACP. Another cycle begins with the condensation of butyryl ACP and malonyl ACP. The sequence of reactions is repeated until the final product palmitate is formed.

Fatty acids are synthesized by a multifunctional enzyme complex in animals

Although the basic biochemical reactions in fatty acid synthesis are very similar in *E. coli* and eukaryotes, the structure of the synthase varies considerably. The component enzymes of animal fatty acid synthases, in contrast with those of *E. coli* and plants, are linked in a large polypeptide chain.

The structure of a large part of the mammalian fatty acid synthase has recently been determined, with the acyl carrier protein and thioesterase remaining to be resolved. The enzyme is a dimer of identical 270-kd subunits. Each chain contains all of the active sites required for activity, as well as an acyl carrier protein tethered to the complex (Figure 22.27A). Despite the fact that each chain possesses all of the enzymes required for fatty acid synthesis, the monomers are not active. A dimer is required.

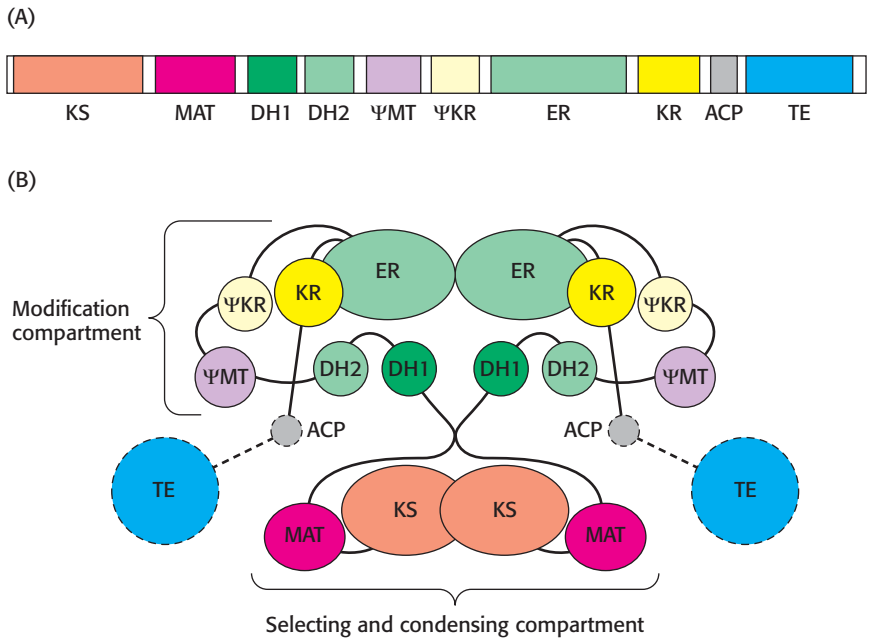


Figure 22.27 The structure of the mammalian fatty acid synthase. (A) The arrangement of the catalytic activities present in a single polypeptide chain. (B) A cartoon of the dimer based on an x-ray crystallographic result. The Ψ-MT and Ψ-KR are inactive domains similar to methyl transferase and ketoreductase sequences. Although there are two domains for DH, only one is active. The inactive domains are presented in faded colors. Dotted lines outline domains for which the structure has not yet been determined. Abbreviations: KS, ketosynthase; MAT, malonyl-acetyl transferase; DH, dehydratase; Ψ-MT, methyl transferase (inactive); Ψ-KR, ketoreductase (inactive); ER, enoyl reductase; KR, ketoreductase; ACP, acyl carrier protein; TE, thioesterase.

The two component chains interact such that the enzyme activities are partitioned into two distinct compartments (Figure 22.27B). The selecting and condensing compartment binds the acetyl and malonyl substrates and condenses them to form the growing chain. Interestingly, the mammalian fatty acid synthase has one active site, malonyl-acetyl transacylase, that adds both acetyl CoA and malonyl CoA. In contrast, most other fatty acid synthases have two separate enzyme activities, one for acetyl CoA and one for malonyl CoA. The modification compartment is responsible for the reduction and dehydration activities that result in the saturated fatty acid product.

Let us consider one catalytic cycle of the fatty acid synthase complex (Figure 22.28). An elongation cycle begins when methyl-acetyl transferase (MAT) moves an acetyl unit from coenzyme A to the acyl carrier protein (ACP). β-Keto synthase (β-KS) accepts the acetyl unit, which forms a thioester with a cysteine residue at the β-KS active site. The vacant ACP is reloaded by MAT, this time with a malonyl moiety. Malonyl ACP visits the active site of β-KS where the condensation of the two 2-carbon fragments takes place on the ACP with the concomitant release of CO_2. The selecting and condensing process concludes with the β-ketoacyl product attached to the ACP.

The loaded ACP then sequentially visits the active sites of the modification compartment of the enzyme, where the β-keto group of the substrate is reduced to —OH, dehydrated, and finally reduced to yield the saturated acyl product, still attached to the ACP. With the completion of the modification process, the reduced product is transferred to the β-KS while the ACP accepts another malonyl unit. Condensation takes place and is followed by another modification cycle. The process is repeated until the thioesterase releases the final C_{16} palmitic acid product.

Many eukaryotic multienzyme complexes are multifunctional proteins in which different enzymes are linked covalently. An advantage of this arrangement is that the synthetic activity of different enzymes is coordinated. In addition, intermediates can be efficiently handed from one active site to another without leaving the assembly. Furthermore, a complex of covalently joined enzymes is more stable than one formed by noncovalent attractions. Each of the component enzymes is recognizably homologous to

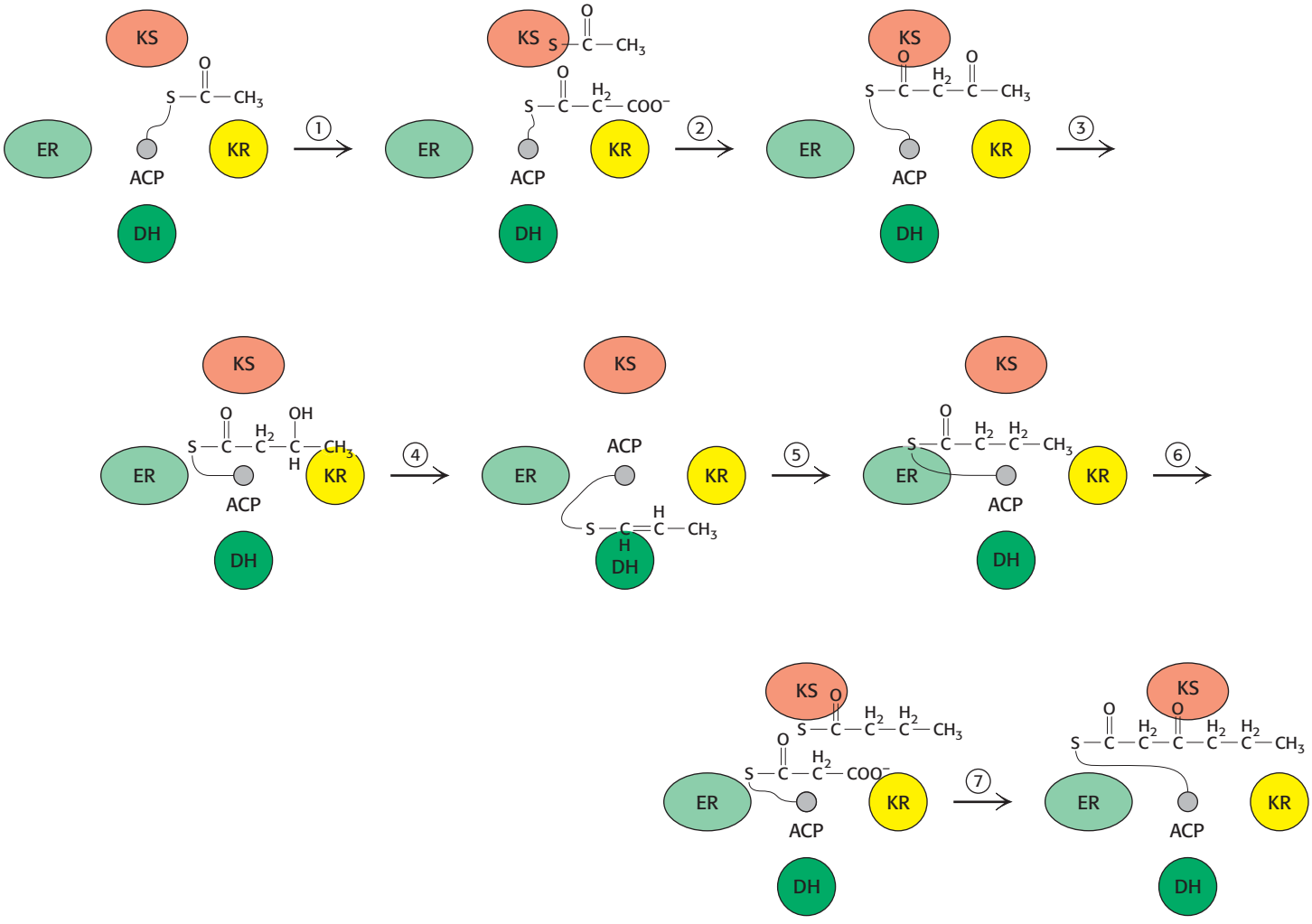


Figure 22.28 A catalytic cycle of mammalian fatty acid synthase. The cycle begins when MAT (not shown) attaches an acetyl unit to ACP. (1) ACP delivers the acetyl unit to KS, and MAT then attaches a malonyl unit to ACP. (2) ACP visits KS again, which condenses the acetyl and malonyl units to form the β-ketoacyl product, attached to the ACP. (3) ACP delivers the β-ketoacyl product to the KR enzyme, which reduces the keto group to an alcohol. (4) The β-hydroxyl product then visits the DH, which introduces a double bond with the loss of water. (5) The enoyl product is delivered to the ER enzyme, where the double bond is reduced. (6) ACP hands the reduced product to KS and is recharged with malonyl CoA by MAT. (7) KS condenses the two molecules on ACP, which is now ready to begin another cycle. See Figure 22.27 for abbreviations.

its bacterial counterpart. Multifunctional enzymes such as fatty acid synthase seem likely to have arisen in eukaryotic evolution by fusion of the individual genes of evolutionary ancestors.

The synthesis of palmitate requires 8 molecules of acetyl CoA, 14 molecules of NADPH, and 7 molecules of ATP

The stoichiometry of the synthesis of palmitate is

$$\text{Acetyl CoA} + 7\ \text{malonyl CoA} + 14\ \text{NADPH} + 20\ \text{H}^+ \longrightarrow \text{palmitate} + 7\ \text{CO}_2 + 14\ \text{NADP}^+ + 8\ \text{CoA} + 6\ \text{H}_2\text{O}$$

The equation for the synthesis of the malonyl CoA used in the preceding reaction is

$$7\ \text{Acetyl CoA} + 7\ \text{CO}_2 + 7\ \text{ATP} \longrightarrow 7\ \text{malonyl CoA} + 7\ \text{ADP} + 7\ \text{P}_i + 14\ \text{H}^+$$

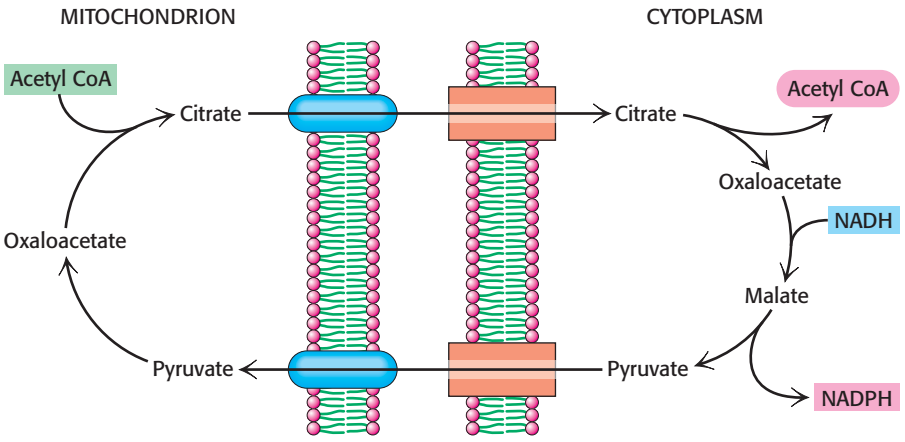


Figure 22.29 Transfer of acetyl CoA to the cytoplasm. Acetyl CoA is transferred from mitochondria to the cytoplasm, and the reducing potential of NADH is concomitantly converted into that of NADPH by this series of reactions.

Hence, the overall stoichiometry for the synthesis of palmitate is

$$8\ \text{Acetyl CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 6\ \text{H}^+ \longrightarrow \text{palmitate} + 14\ \text{NADP}^+ + 8\ \text{CoA} + 6\ \text{H}_2\text{O} + 7\ \text{ADP} + 7\ \text{P}_i$$

Citrate carries acetyl groups from mitochondria to the cytoplasm for fatty acid synthesis

Fatty acids are synthesized in the cytoplasm, whereas acetyl CoA is formed from pyruvate in mitochondria. Hence, acetyl CoA must be transferred from mitochondria to the cytoplasm for fatty acid synthesis. Mitochondria, however, are not readily permeable to acetyl CoA. Recall that carnitine carries only long-chain fatty acids. *The barrier to acetyl CoA is bypassed by citrate, which carries acetyl groups across the inner mitochondrial membrane.* Citrate is formed in the mitochondrial matrix by the condensation of acetyl CoA with oxaloacetate (Figure 22.29). When present at high levels, citrate is transported to the cytoplasm, where it is cleaved by *ATP-citrate lyase.*

Lyases
Enzymes catalyzing the cleavage of C—C, C—O, or C—N bonds by elimination. A double bond is formed in these reactions.

$$\text{Citrate} + \text{ATP} + \text{CoA} + \text{H}_2\text{O} \longrightarrow \text{acetyl CoA} + \text{ADP} + \text{P}_i + \text{oxaloacetate}$$

Thus, acetyl CoA and oxaloacetate are transferred from mitochondria to the cytoplasm at the expense of the hydrolysis of a molecule of ATP.

Several sources supply NADPH for fatty acid synthesis

Oxaloacetate formed in the transfer of acetyl groups to the cytoplasm must now be returned to the mitochondria. The inner mitochondrial membrane is impermeable to oxaloacetate. Hence, a series of bypass reactions are needed. Most importantly, these reactions generate much of the NADPH needed for fatty acid synthesis. First, oxaloacetate is reduced to malate by NADH. This reaction is catalyzed by a *malate dehydrogenase* in the cytoplasm.

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{malate} + \text{NAD}^+$$

Second, malate is oxidatively decarboxylated by an *$NADP^+$-linked malate enzyme* (also called *malic enzyme*).

$$\text{Malate} + \text{NADP}^+ \longrightarrow \text{pyruvate} + \text{CO}_2 + \text{NADPH}$$

The pyruvate formed in this reaction readily enters mitochondria, where it is carboxylated to oxaloacetate by pyruvate carboxylase.

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \longrightarrow \text{oxaloacetate} + \text{ADP} + P_i + 2\,H^+$$

The sum of these three reactions is

$$NADP^+ + \text{NADH} + \text{ATP} + H_2O \longrightarrow \text{NADPH} + NAD^+ + \text{ADP} + P_i + H^+$$

Thus, *one molecule of NADPH is generated for each molecule of acetyl CoA that is transferred from mitochondria to the cytoplasm.* Hence, eight molecules of NADPH are formed when eight molecules of acetyl CoA are transferred to the cytoplasm for the synthesis of palmitate. *The additional six molecules of NADPH required for this process come from the pentose phosphate pathway* (Section 20.3).

The accumulation of the precursors for fatty acid synthesis is a wonderful example of the coordinated use of multiple pathways. The citric acid cycle, transport of oxaloacetate from the mitochondria, and pentose phosphate pathway provide the carbon atoms and reducing power, whereas glycolysis and oxidative phosphorylation provide the ATP to meet the needs for fatty acid synthesis (Figure 22.30).

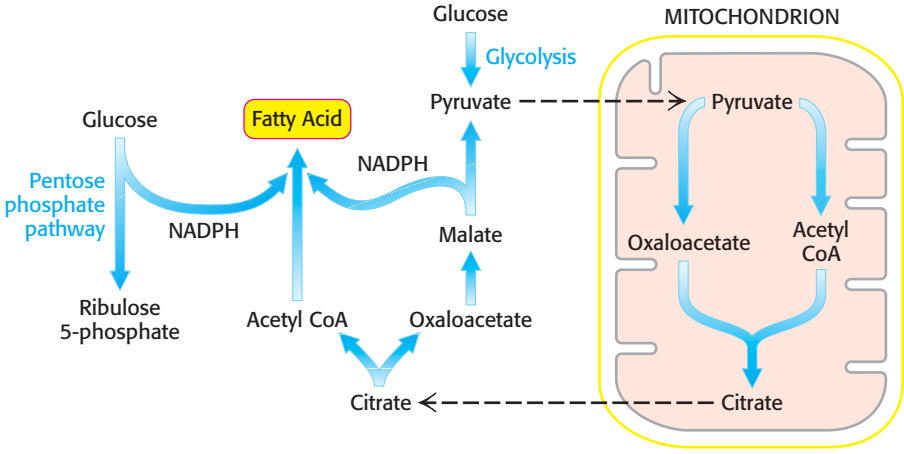


Figure 22.30 PATHWAY INTEGRATION: Fatty acid synthesis. Fatty acid synthesis requires the cooperation of various metabolic pathways located in different cellular compartments.

Fatty acid synthase inhibitors may be useful drugs

Fatty acid synthase is overexpressed in most human cancers and its expression is correlated with tumor malignancy. The fatty acids are not stored as an energy source, but rather are used as precursors for the synthesis of phospholipids, which are then incorporated into membranes in the rapidly growing cancer cells. Researchers intrigued by this observation have tested inhibitors of fatty acid synthase on mice to see if the inhibitors slow tumor growth. These inhibitors do indeed slow tumor growth, apparently by inducing apoptosis. However, another startling observation was made: *mice treated with inhibitors of the condensing enzyme showed remarkable weight loss* because they ate less. Thus, fatty acid synthase inhibitors are exciting candidates both as antitumor and as antiobesity drugs.

22.5 The Elongation and Unsaturation of Fatty Acids Are Accomplished by Accessory Enzyme Systems

The major product of the fatty acid synthase is palmitate. In eukaryotes, longer fatty acids are formed by elongation reactions catalyzed by enzymes

on the cytoplasmic face of the *endoplasmic reticulum membrane.* These reactions add two-carbon units sequentially to the carboxyl ends of both saturated and unsaturated fatty acyl CoA substrates. Malonyl CoA is the two-carbon donor in the elongation of fatty acyl CoAs. Again, condensation is driven by the decarboxylation of malonyl CoA.

Membrane-bound enzymes generate unsaturated fatty acids

Endoplasmic reticulum systems also introduce double bonds into long-chain acyl CoAs. For example, in the conversion of stearoyl CoA into oleoyl CoA, a cis-Δ^9 double bond is inserted by an oxidase that employs *molecular oxygen* and *NADH* (or *NADPH*).

$$\text{Stearoyl CoA} + \text{NADH} + \text{H}^+ + \text{O}_2 \longrightarrow \text{oleoyl CoA} + \text{NAD}^+ + 2\,\text{H}_2\text{O}$$

This reaction is catalyzed by a complex of three membrane-bound proteins: *NADH-cytochrome* b_5 *reductase, cytochrome* b_5, and a *desaturase* (Figure 22.31). First, electrons are transferred from NADH to the FAD moiety of NADH-cytochrome b_5 reductase. The heme iron atom of cytochrome b_5 is then reduced to the Fe^{2+} state. The nonheme iron atom of the desaturase is subsequently converted into the Fe^{2+} state, which enables it to interact with O_2 and the saturated fatty acyl CoA substrate. A double bond is formed and two molecules of H_2O are released. Two electrons come from NADH and two from the single bond of the fatty acyl substrate.

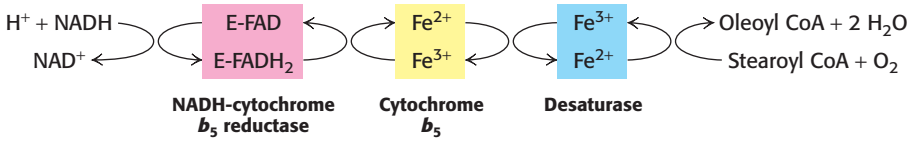


Figure 22.31 Electron-transport chain in the desaturation of fatty acids.

A variety of unsaturated fatty acids can be formed from oleate by a combination of elongation and desaturation reactions. For example, oleate can be elongated to a 20:1 cis-Δ^{11} fatty acid. Alternatively, a second double bond can be inserted to yield an 18:2 cis-Δ^6, Δ^9 fatty acid. Similarly, palmitate (16:0) can be oxidized to palmitoleate (16:1 cis-Δ^9), which can then be elongated to *cis*-vaccenate (18:1 cis-Δ^{11}).

Unsaturated fatty acids in mammals are derived from either palmitoleate (16:1), oleate (18:1), linoleate (18:2), or linolenate (18:3). The number of carbon atoms from the ω end of a derived unsaturated fatty acid to the nearest double bond identifies its precursor.

Precursor	Formula
Linolenate (ω-3)	$CH_3—(CH_2)_2═CH—R$
Linoleate (ω-6)	$CH_3—(CH_2)_5═CH—R$
Palmitoleate (ω-7)	$CH_3—(CH_2)_6═CH—R$
Oleate (ω-9)	$CH_3—(CH_2)_8═CH—R$

Mammals lack the enzymes to introduce double bonds at carbon atoms beyond C-9 in the fatty acid chain. Hence, mammals cannot synthesize linoleate (18:2 cis-Δ^9,Δ^{12}) and linolenate (18:3 cis-Δ^9,Δ^{12},Δ^{15}). Linoleate and linolenate are the two essential fatty acids. The term essential means that they must be supplied in the diet because they are required by an organism and cannot be synthesized by the organism itself. Linoleate and linolenate furnished by the diet are the starting points for the synthesis of a variety of other unsaturated fatty acids.

Eicosanoid hormones are derived from polyunsaturated fatty acids

Arachidonate, a 20:4 fatty acid derived from linoleate, is the major precursor of several classes of signal molecules: prostaglandins, prostacyclins, thromboxanes, and leukotrienes (Figure 22.32).

A prostaglandin is a 20-carbon fatty acid containing a 5-carbon ring (Figure 22.33). This basic compound is modified by reductases and isomerases to yield nine major classes of prostaglandins, designated PGA through PGI; a subscript denotes the number of carbon–carbon double bonds outside the ring. Prostaglandins with two double bonds, such as PGE_2, are derived from arachidonate; the other two double bonds of this precursor are lost in forming a 5-membered ring. *Prostacyclin* and *thromboxanes* are related compounds that arise from a nascent prostaglandin. They are generated by *prostacyclin synthase* and *thromboxane synthase*, respectively. Alternatively, arachidonate can be converted into *leukotrienes* by the action of *lipoxygenase*. Leukotrienes, first found in leukocytes, contain three conjugated double bonds—hence, the name. Prostaglandins, prostacyclin, thromboxanes, and leukotrienes are called *eicosanoids* (from the Greek *eikosi*, "twenty") because they contain 20 carbon atoms.

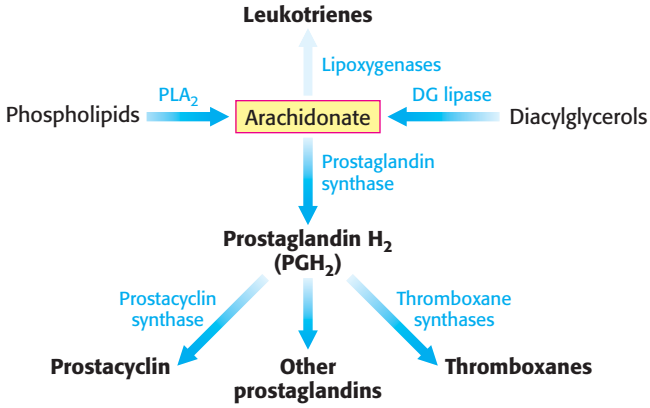
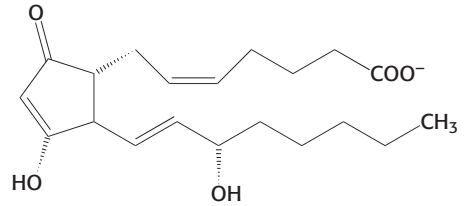


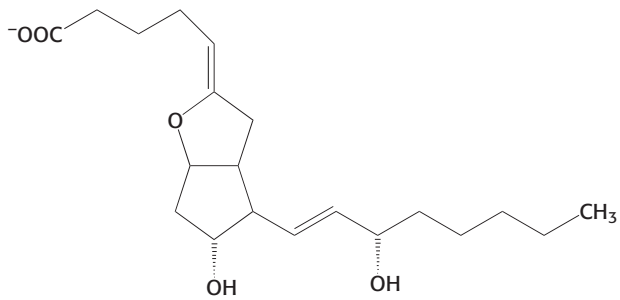
Figure 22.32 Arachidonate is the major precursor of eicosanoid hormones. Prostaglandin synthase catalyzes the first step in a pathway leading to prostaglandins, prostacyclins, and thromboxanes. Lipoxygenase catalyzes the initial step in a pathway leading to leukotrienes.

Prostaglandins and other eicosanoids are *local hormones* because they are short-lived. They alter the activities both of the cells in which they are synthesized and of adjoining cells by binding to 7TM receptors. Their effects may vary from one cell type to another, in contrast with the more-uniform actions of global hormones such as insulin and glucagon. Prostaglandins stimulate inflammation, regulate blood flow to particular organs, control ion transport across membranes, modulate synaptic transmission, and induce sleep.

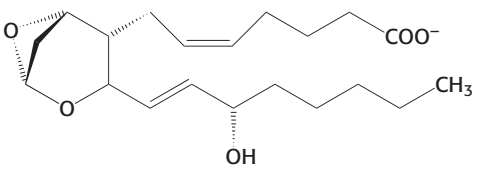
Recall that aspirin blocks access to the active site of the enzyme that converts arachidonate into prostaglandin H_2 (Section 12.3). Because arachidonate is the precursor of other prostaglandins, prostacyclin, and thromboxanes, blocking this step interferes with many signaling pathways. Aspirin's ability to obstruct these pathways accounts for its wide-ranging effects on inflammation, fever, pain, and blood clotting.



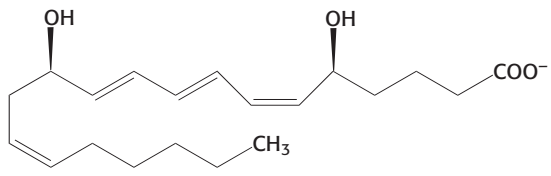
Prostaglandin E_2



Prostacyclin (PGI_2)



Thromboxane A_2 (TXA_2)



Leukotriene B_4

Figure 22.33 Structures of several eicosanoids.

22.6 Acetyl CoA Carboxylase Plays a Key Role in Controlling Fatty Acid Metabolism

Fatty acid metabolism is stringently controlled so that synthesis and degradation are highly responsive to physiological needs. Fatty acid synthesis is maximal when carbohydrates and energy are plentiful and when fatty acids are scarce. *Acetyl CoA carboxylase plays an essential role in regulating fatty acid synthesis and degradation.* Recall that this enzyme catalyzes the committed step in fatty acid synthesis: the production of malonyl CoA (the activated two-carbon donor). This important enzyme is subject to both local and hormonal regulation. We will examine each of these levels of regulation in turn.

Acetyl CoA carboxylase is regulated by conditions in the cell

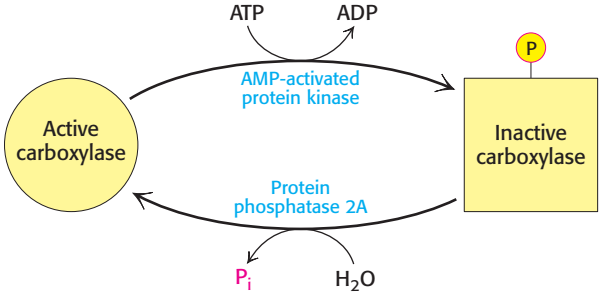


Figure 22.34 Control of acetyl CoA carboxylase. Acetyl CoA carboxylase is inhibited by phosphorylation.

Acetyl CoA carboxylase responds to changes in its immediate environment. *Acetyl CoA carboxylase is switched off by phosphorylation* and activated by dephosphorylation (Figure 22.34). *AMP-dependent protein kinase* (AMPK) converts the carboxylase into an inactive form by modifying three serine residues. AMPK is essentially a fuel gauge; it is activated by AMP and inhibited by ATP. Thus, the carboxylase is inactivated when the energy charge is low. Fats are not synthesized when energy is required.

The carboxylase is also allosterically stimulated by citrate. Citrate acts in an unusual manner on inactive acetyl CoA carboxylase, which exists as isolated inactive dimers. Citrate facilitates the polymerization of the inactive dimers into active filaments (Figure 22.35). Citrate-induced polymerization can partly reverse the inhibition produced by phosphorylation (Figure 22.36). The level of citrate is high when both acetyl CoA and ATP are abundant, signifying that raw materials and energy are available for fatty acid synthesis. The stimulatory effect of citrate on the carboxylase is counteracted by *palmitoyl CoA,* which is abundant when there is an excess of fatty acids. Palmitoyl CoA causes the filaments to disassemble into the inactive subunits. Palmitoyl CoA also inhibits the translocase that transports citrate from mitochondria to the cytoplasm, as well as glucose 6-phosphate dehydrogenase, which generates NADPH in the pentose phosphate pathway.

Acetyl CoA carboxylase also plays a role in the regulation of fatty acid degradation. Malonyl CoA, the product of the carboxylase reaction, is present at a high level when fuel molecules are abundant. *Malonyl CoA inhibits carnitine acyltransferase I, preventing the entry of fatty acyl CoAs into the mitochondrial matrix in times of plenty.* Malonyl CoA is an especially effective inhibitor of carnitine acyltransferase I in heart and muscle, tissues that have little fatty acid synthesis capacity of their own. In these tissues, acetyl CoA carboxylase may be a purely regulatory enzyme.

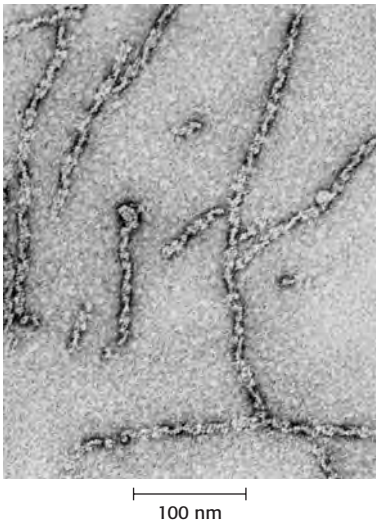


Figure 22.35 Filaments of acetyl CoA carboxylase. The electron micrograph shows the enzymatically active filamentous form of acetyl CoA carboxylase from chicken liver. The inactive form is a dimer of 265-kd subunits. [Courtesy of Dr. M. Daniel Lane.]

Acetyl CoA carboxylase is regulated by a variety of hormones

Acetyl CoA carboxylase is controlled by the hormones glucagon, epinephrine, and insulin, which denote the overall energy status of the organism. *Insulin stimulates fatty acid synthesis by activating the carboxylase, whereas glucagon and epinephrine have the reverse effect.*

Regulation by glucagon and epinephrine. Consider, again, a person who has just awakened from a night's sleep and begins a bout of exercise. As mentioned, glycogen stores will be low, but lipids are readily available for mobilization.

As stated earlier, the hormones glucagon and epinephrine, present under conditions of fasting and exercise, will stimulate the release of fatty acids from triacylglycerols in fat cells, which will be released into the blood, and probably from muscle cells, where they will be used immediately as fuel. These same hormones will inhibit fatty acid synthesis by inhibiting acetyl CoA carboxylase. Although the exact mechanism by which these hormones exert their effects is not known, the net result is to augment the inhibition by the AMP-dependent kinase. This result makes sound physiological sense: when the energy level of the cell is low, as signified by a high concentration of AMP, and the energy level of the organism is low, as signaled by glucagon, fats should not be synthesized. Epinephrine, which signals the need for immediate energy, enhances this effect. Hence, *these catabolic hormones switch off fatty acid synthesis by keeping the carboxylase in the inactive phosphorylated state.*

Regulation by insulin. Now consider the situation after the exercise has ended and the runner has had a meal. In this case, the hormone insulin inhibits the mobilization of fatty acids and stimulates their accumulation as triacylglycerols by muscle and adipose tissue. Insulin also stimulates fatty acid synthesis by activating acetyl CoA carboxylase. Insulin stimulates the carboxylase by stimulating the activity of a protein phosphatase that dephosphorylates and activates acetyl CoA carboxylase. Thus, the signal molecules glucagon, epinephrine, and insulin act in concert on triacylglycerol metabolism and acetyl CoA carboxylase to carefully regulate the utilization and storage of fatty acids.

Response to diet. *Long-term control is mediated by changes in the rates of synthesis and degradation of the enzymes participating in fatty acid synthesis.* Animals that have fasted and are then fed high-carbohydrate, low-fat diets show marked increases in their amounts of acetyl CoA carboxylase and fatty acid synthase within a few days. This type of regulation is known as *adaptive control*. This regulation, which is mediated both by insulin and glucose, is at the level of gene transcription.

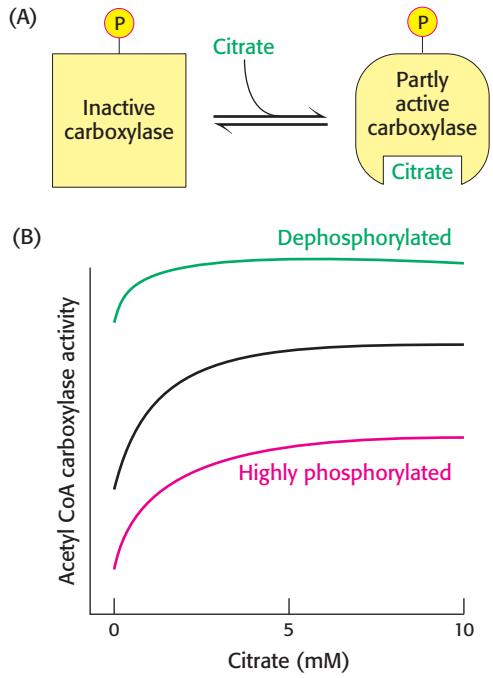


Figure 22.36 Dependence of the catalytic activity of acetyl CoA carboxylase on the concentration of citrate. (A) Citrate can partly activate the phosphorylated carboxylase. (B) The dephosphorylated form of the carboxylase is highly active even when citrate is absent. Citrate partly overcomes the inhibition produced by phosphorylation. [After G. M. Mabrouk, I. M. Helmy, K. G. Thampy, and S. J. Wakil. *J. Biol. Chem.* 265:6330–6338, 1990.]

Summary

22.1 Triacylglycerols Are Highly Concentrated Energy Stores

Fatty acids are physiologically important as (1) fuel molecules, (2) components of phospholipids and glycolipids, (3) hydrophobic modifiers of proteins, and (4) hormones and intracellular messengers. They are stored in adipose tissue as triacylglycerols (neutral fat).

22.2 The Use of Fatty Acids As Fuel Requires Three Stages of Processing

Triacylglycerols can be mobilized by the hydrolytic action of lipases that are under hormonal control. Glucagon and epinephrine stimulate triacylglycerol breakdown by activating the lipase. Insulin, in contrast, inhibits lipolysis. Fatty acids are activated to acyl CoAs, transported across the inner mitochondrial membrane by carnitine, and degraded in the mitochondrial matrix by a recurring sequence of four reactions: oxidation by FAD, hydration, oxidation by NAD^+, and thiolysis by coenzyme A. The $FADH_2$ and NADH formed in the oxidation steps transfer their electrons to O_2 by means of the respiratory chain, whereas the acetyl CoA formed in the thiolysis step normally enters the citric acid cycle by condensing with oxaloacetate. Mammals are unable to convert fatty acids into glucose, because they lack a pathway for the net production of oxaloacetate, pyruvate, or other gluconeogenic intermediates from acetyl CoA.

22.3 Unsaturated and Odd-Chain Fatty Acids Require Additional Steps for Degradation

Fatty acids that contain double bonds or odd numbers of carbon atoms require ancillary steps to be degraded. An isomerase and a reductase are required for the oxidation of unsaturated fatty acids, whereas propionyl CoA derived from chains with odd numbers of carbon atoms requires a vitamin B_{12}-dependent enzyme to be converted into succinyl CoA.

22.4 Fatty Acids Are Synthesized by Fatty Acid Synthase

Fatty acids are synthesized in the cytoplasm by a different pathway from that of β oxidation. Fatty acid synthase is the enzyme complex responsible for fatty acid synthase. Synthesis starts with the carboxylation of acetyl CoA to malonyl CoA, the committed step. This ATP-driven reaction is catalyzed by acetyl CoA carboxylase, a biotin enzyme. The intermediates in fatty acid synthesis are linked to an acyl carrier protein. Acetyl ACP is formed from acetyl CoA, and malonyl ACP is formed from malonyl CoA. Acetyl ACP and malonyl ACP condense to form acetoacetyl ACP, a reaction driven by the release of CO_2 from the activated malonyl unit. A reduction, a dehydration, and a second reduction follow. NADPH is the reductant in these steps. The butyryl ACP formed in this way is ready for a second round of elongation, starting with the addition of a two-carbon unit from malonyl ACP. Seven rounds of elongation yield palmitoyl ACP, which is hydrolyzed to palmitate. In higher organisms, the enzymes catalyzing fatty acid synthesis are covalently linked in a multifunctional enzyme complex. A reaction cycle based on the formation and cleavage of citrate carries acetyl groups from mitochondria to the cytoplasm. NADPH needed for synthesis is generated in the transfer of reducing equivalents from mitochondria by the combined action of cytoplasmic malate dehydrogenase and malic enzyme and by the pentose phosphate pathway.

22.5 The Elongation and Unsaturation of Fatty Acids Are Accomplished by Accessory Enzyme Systems

Fatty acids are elongated and desaturated by enzyme systems in the endoplasmic reticulum membrane. Desaturation requires NADH and O_2 and is carried out by a complex consisting of a flavoprotein, a cytochrome, and a nonheme iron protein. Mammals lack the enzymes to introduce double bonds distal to C-9, and so they require linoleate and linolenate in their diets.

Arachidonate, an essential precursor of prostaglandins and other signal molecules, is derived from linoleate. This 20:4 polyunsaturated fatty acid is the precursor of several classes of signal molecules—prostaglandins, prostacyclins, thromboxanes, and leukotrienes—that act as messengers and local hormones because of their transience. They are called eicosanoids because they contain 20 carbon atoms. Aspirin (acetylsalicylate), an anti-inflammatory and antithrombotic drug, irreversibly blocks the synthesis of these eicosanoids.

22.6 Acetyl CoA Carboxylase Plays a Key Role in Controlling Fatty Acid Metabolism

Fatty acid synthesis and degradation are reciprocally regulated so that both are not simultaneously active. Acetyl CoA carboxylase, the essential control site, is phosphorylated and inactivated by AMP-dependent kinase. The phosphorylation is reversed by a protein phosphatase. Citrate, which signals an abundance of building blocks and energy,

partly reverses the inhibition by phosphorylation. Carboxylase activity is stimulated by insulin and inhibited by glucagon and epinephrine. In times of plenty, fatty acyl CoAs do not enter the mitochondrial matrix, because malonyl CoA inhibits carnitine acyltransferase I.

Key Terms

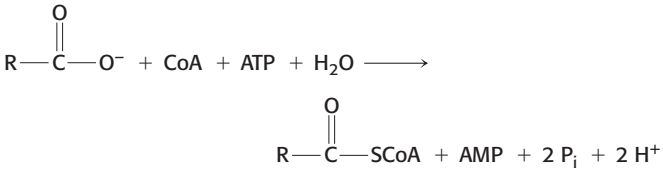
triacylglycerol (neutral fat, triglyceride) (p. 639)
acyl adenylate (p. 645)
carnitine (p. 645)
β-oxidation pathway (p. 646)
vitamin B_{12} (cobalamin) (p. 649)
peroxisome (p. 652)
ketone body (p. 653)
acyl carrier protein (ACP) (p. 657)
fatty acid synthase (p. 657)
malonyl CoA (p. 657)
acetyl CoA carboxylase (p. 657)
arachidonate (p. 664)
prostaglandin (p. 665)
eicosanoid (p. 665)
AMP-dependent protein kinase (AMPK) (p. 666)

Problems

1. *After lipolysis.* Write a balanced equation for the conversion of glycerol into pyruvate. Which enzymes are required in addition to those of the glycolytic pathway?

2. *Forms of energy.* The partial reactions leading to the synthesis of acyl CoA (equations 1 and 2, p. 645) are freely reversible. The equilibrium constant for the sum of these reactions is close to 1, meaning that the energy levels of the reactants and products are about equal, even though a molecule of ATP has been hydrolyzed. Explain why these reactions are readily reversible.

3. *Activation fee.* The reaction for the activation of fatty acids before degradation is



This reaction is quite favorable because the equivalent of two molecules of ATP is hydrolyzed. Explain why, from a biochemical bookkeeping point of view, the equivalent of two molecules of ATP is used despite the fact that the left side of the equation has only one molecule of ATP.

4. *Proper sequence.* Place the following list of reactions or relevant locations in the β oxidation of fatty acids in the proper order.

(a) Reaction with carnitine
(b) Fatty acid in the cytoplasm
(c) Activation of fatty acid by joining to CoA
(d) Hydration
(e) NAD^+-linked oxidation
(f) Thiolysis
(g) Acyl CoA in mitochondrion
(h) FAD-linked oxidation.

5. *Remembrance of reactions past.* We have encountered reactions similar to the oxidation, hydration, and oxidation reactions of fatty acid degradation earlier in our study of biochemistry. What other pathway employs this set of reactions?

6. *A phantom acetyl CoA?* In the equation for fatty acid degradation shown here, only seven molecules of CoA are required to yield eight molecules of acetyl CoA. How is this difference possible?

$$\text{Palmitoyl CoA} + 7\ \text{FAD} + 7\ \text{NAD}^+ + 7\ \text{CoASH} + 7\ \text{H}_2\text{O} \longrightarrow 8\ \text{Acetyl CoA} + 7\ \text{FADH}_2 + 7\ \text{NADH} + 7\ \text{H}^+$$

7. *Comparing yields.* Compare the ATP yields from palmitic acid and palmitoleic acid.

8. *Counting ATPs 1.* What is the ATP yield for the complete oxidation of C_{17} (heptadecanoic) fatty acid? Assume that the propionyl CoA ultimately yields oxaloacetate in the citric acid cycle.

9. *Sweet temptation.* Stearic acid is a C_{18} fatty acid component of chocolate. Suppose you had a depressing day and decided to settle matters by gorging on chocolate. How much ATP would you derive from the complete oxidation of stearic acid to CO_2?

10. *The best storage form.* Compare the ATP yield from the complete oxidation of glucose, a six-carbon carbohydrate, and hexanoic acid, a six-carbon fatty acid. Hexanoic acid is also called caprioic acid and is responsible for the "aroma" of goats. Why are fats better fuels than carbohydrates?

11. *From fatty acid to ketone body.* Write a balanced equation for the conversion of stearate into acetoacetate.

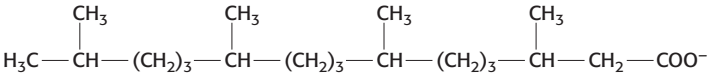
12. *Generous, but not to a fault.* Liver is the primary site of ketone-body synthesis. However, ketone bodies are not used by the liver but are released for other tissues to use. The liver does gain energy in the process of synthesizing and releasing ketone bodies. Calculate the number of molecules of ATP generated by the liver in the conversion of palmitate, a C_{16} fatty acid, into acetoacetate.

13. *Counting ATPs 2.* How much energy is attained with the complete oxidation of the ketone body D-3-hydroxybutyrate?

14. *Another view.* Why might someone argue that the answer to Problem 13 is wrong?

15. *An accurate adage.* An old biochemistry adage is that *fats burn in the flame of carbohydrates.* What is the molecular basis of this adage?

16. *Refsum disease.* Phytanic acid is a branched-chain fatty acid component of chlorophyll and is a significant component of milk. In susceptible people, phytanic acid can accumulate, leading to neurological problems. This syndrome is called Refsum disease or phytanic acid storage disease.



Phytanic acid

(a) Why does phytanic acid accumulate?

(b) What enzyme activity would you invent to prevent its accumulation?

17. *A hot diet.* Tritium is a radioactive isotope of hydrogen and can be readily detected. A fully tritiated, six-carbon saturated fatty acid is administered to a rat, and a muscle biopsy of the rat is taken by concerned, sensitive, and discrete technical assistants. These assistants carefully isolate all of the acetyl CoA generated from the β oxidation of the radioactive fatty acid and remove the CoA to form acetate. What will be the overall tritium-to-carbon ratio of the isolated acetate?

18. *Finding triacylglycerols in all the wrong places.* Insulin-dependent diabetes is often accompanied by high levels of triacylglycerols in the blood. Suggest a biochemical explanation.

19. *Counterpoint.* Compare and contrast fatty acid oxidation and synthesis with respect to

(a) site of the process.

(b) acyl carrier.

(c) reductants and oxidants.

(d) stereochemistry of the intermediates.

(e) direction of synthesis or degradation.

(f) organization of the enzyme system.

20. *A supple synthesis.* Myristate, a saturated C_{14} fatty acid, is used as an emollient for cosmetics and topical medicinal preparations. Write a balanced equation for the synthesis of myristate.

21. *The cost of cleanliness.* Lauric acid is a 12-carbon fatty acid with no double bonds. The sodium salt of lauric acid (sodium laurate) is a common detergent used in a variety of products, including laundry detergent, shampoo, and toothpaste. How many molecules of ATP and NADPH are required to synthesize lauric acid?

22. *Proper organization.* Arrange the following steps in fatty acid synthesis in their proper order.

(a) Dehydration

(b) Condensation

(c) Release of a C_{16} fatty acid

(d) Reduction of a carbonyl group

(e) Formation of malonyl ACP

23. *No access to assets.* What would be the effect on fatty acid synthesis of a mutation in ATP-citrate lyase that reduces the enzyme's activity? Explain.

24. *The truth and nothing but.* True or False. If false, explain.

(a) Biotin is required for fatty acid synthase activity.

(b) The condensation reaction in fatty acid synthesis is powered by the decarboxylation of malonyl CoA.

(c) Fatty acid synthesis does not depend on ATP.

(d) Palmitate is the end product of fatty acid synthase.

(e) All of the enzyme activities required for fatty acid synthesis in mammals are contained in a single polypeptide chain.

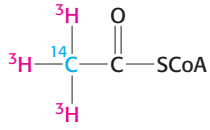
(f) Fatty acid synthase in mammals is active as a monomer.

(g) The fatty acid arachidonate is a precursor for signal molecules.

(h) Acetyl CoA carboxylase is inhibited by citrate.

25. *Odd fat out.* Suggest how fatty acids with odd numbers of carbons are synthesized.

26. *Labels.* Suppose that you had an in vitro fatty acid-synthesizing system that had all of the enzymes and cofactors required for fatty acid synthesis except for acetyl CoA. To this system, you added acetyl CoA that contained radioactive hydrogen (^3H, tritium) and carbon 14 (^{14}C) as shown here.



The ratio of $^3H/^{14}C$ is 3. What would the $^3H/^{14}C$ ratio be after the synthesis of palmitic acid (C_{16}) with the use of the radioactive acetyl CoA?

27. *A tight embrace.* Avidin, a glycoprotein found in eggs, has a high affinity for biotin. Avidin can bind biotin and

prevent its use by the body. How might a diet rich in raw eggs affect fatty acid synthesis? What will be the effect on fatty acid synthesis of a diet rich in cooked eggs? Explain.

28. *Alpha or omega?* Only one acetyl CoA molecule is used directly in fatty acid synthesis. Identify the carbon atoms in palmitic acid that were donated by acetyl CoA.

29. *Now you see it, now you don't.* Although HCO_3^- is required for fatty acid synthesis, its carbon atom does not appear in the product. Explain how this omission is possible.

30. *It is all about communication.* Why is citrate an appropriate inhibitor of phosphofructokinase?

31. *Tracing carbon atoms.* Consider a cell extract that actively synthesizes palmitate. Suppose that a fatty acid synthase in this preparation forms one molecule of palmitate in about 5 minutes. A large amount of malonyl CoA labeled with ^{14}C in each carbon atom of its malonyl unit is suddenly added to this system, and fatty acid synthesis is stopped a minute later by altering the pH. The fatty acids are analyzed for radioactivity. Which carbon atom of the palmitate formed by this system is more radioactive—C-1 or C-14?

32. *An unaccepting mutant.* The serine residues in acetyl CoA carboxylase that are the target of the AMP-dependent protein kinase are mutated to alanine. What is a likely consequence of this mutation?

33. *Sources.* For each of the following unsaturated fatty acids, indicate whether the biosynthetic precursor in animals is palmitoleate, oleate, linoleate, or linolenate.

(a) 18:1 cis-Δ^{11}
(b) 18:3 cis-Δ^6, Δ^9, Δ^{12}
(c) 20:2 cis-Δ^{11}, Δ^{14}
(d) 20:3 cis-Δ^5, Δ^8, Δ^{11}
(e) 22:1 cis-Δ^{13}
(f) 22:6 cis-Δ^4, Δ^7, Δ^{10}, Δ^{13}, Δ^{16}, Δ^{19}

34. *Driven by decarboxylation.* What is the role of decarboxylation in fatty acid synthesis? Name another key reaction in a metabolic pathway that employs this mechanistic motif.

35. *Kinase surfeit.* Suppose that a promoter mutation leads to the overproduction of protein kinase A in adipose cells. How might fatty acid metabolism be altered by this mutation?

36. *Blocked assets.* The presence of a fuel molecule in the cytoplasm does not ensure that the fuel molecule can be effectively used. Give two examples of how impaired transport of metabolites between compartments leads to disease.

37. *Elegant inversion.* Peroxisomes have an alternative pathway for oxidizing polyunsaturated fatty acids. They contain a hydratase that converts D-3-hydroxyacyl CoA into *trans*-Δ^2-enoyl CoA. How can this enzyme be used to oxidize CoAs containing a cis double bond at an even-numbered carbon atom (e.g., the cis-Δ^{12} double bond of linoleate)?

38. *Covalent catastrophe.* What is a potential disadvantage of having many catalytic sites together on one very long polypeptide chain?

39. *Missing acyl CoA dehydrogenases.* A number of genetic deficiencies in acyl CoA dehydrogenases have been described. This deficiency presents early in life after a period of fasting. Symptoms include vomiting, lethargy, and sometimes coma. Not only are blood levels of glucose low (hypoglycemia), but starvation-induced ketosis is absent. Provide a biochemical explanation for these last two observations.

40. *Effects of clofibrate.* High blood levels of triacylglycerides are associated with heart attacks and strokes. Clofibrate, a drug that increases the activity of peroxisomes, is sometimes used to treat patients with such a condition. What is the biochemical basis for this treatment?

41. *A different kind of enzyme.* Figure 22.36 shows the response of acetyl CoA carboxylase to varying amounts of citrate. Explain this effect in light of the allosteric effects that citrate has on the enzyme. Predict the effects of increasing concentrations of palmitoyl CoA.

Mechanism Problems

42. *Variation on a theme.* Thiolase is homologous in structure to the condensing enzyme. On the basis of this observation, propose a mechanism for the cleavage of 3-ketoacyl CoA by CoA.

43. *Two plus three to make four.* Propose a reaction mechanism for the condensation of an acetyl unit with a malonyl unit to form an acetoacetyl unit in fatty acid synthesis.

Chapter Integration Problems

44. *Ill-advised diet.* Suppose that, for some bizarre reason, you decided to exist on a diet of whale and seal blubber, exclusively.

(a) How would lack of carbohydrates affect your ability to utilize fats?

(b) What would your breath smell like?

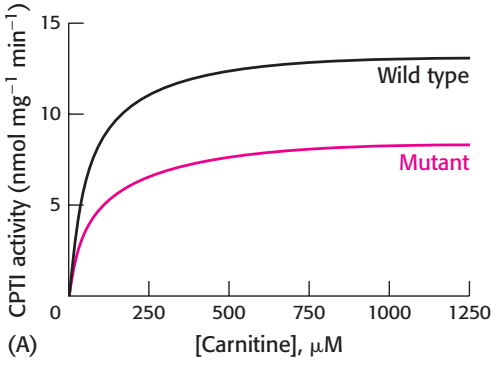
(c) One of your best friends, after trying unsuccessfully to convince you to abandon this diet, makes you promise to consume a healthy dose of odd-chain fatty acids. Does your friend have your best interests at heart? Explain.

45. *Fats to glycogen.* An animal is fed stearic acid that is radioactively labeled with [^{14}C]carbon. A liver biopsy reveals the presence of ^{14}C-labeled glycogen. How is this finding possible in light of the fact that animals cannot convert fats into carbohydrates?

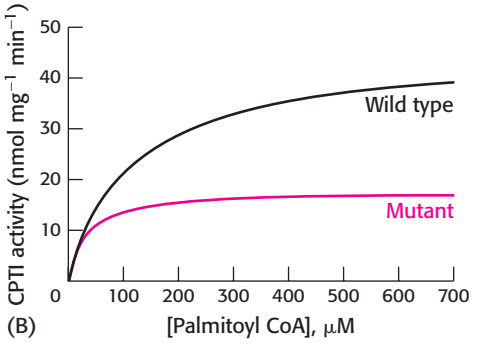
Data Interpretation Problem

46. *Mutant enzyme.* Carnitine palmitoyl transferase I (CPTI) catalyzes the conversion of long-chain acyl CoA into acyl carnitine, a prerequisite for transport into mitochondria and subsequent degradation. A mutant enzyme was constructed with a single amino acid change at position 3 of glutamic acid for alanine. Graphs A through C show data from studies performed to identify the effect of the mutation [data from J. Shi, H. Zhu, D. N. Arvidson, and G. J. Woldegiorgis. *J. Biol. Chem.* 274:9421–9426, 1999].

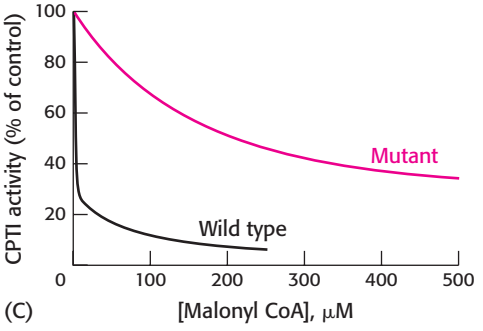
(a) What is the effect of the mutation on enzyme activity when the concentration of carnitine is varied (Graph A)? What are the K_M and V_{max} values for the wild-type and mutant enzymes?



(b) What is the effect when the experiment is repeated with varying concentrations of palmitoyl CoA (Graph B)? What are the K_M and V_{max} values for the wild-type and mutant enzymes?



(c) Graph C shows the inhibitory effect of malonyl CoA on the wild-type and mutant enzymes. Which enzyme is more sensitive to malonyl CoA inhibition?



(d) Suppose that the concentration of palmitoyl CoA = 100 μM, that of carnitine = 100 μM, and that of malonyl CoA = 5 10 μM. Under these conditions, what is the most prominent effect of the mutation on the properties of the enzyme?

(e) What can you conclude about the role of glutamate 3 in carnitine acyltransferase I function?

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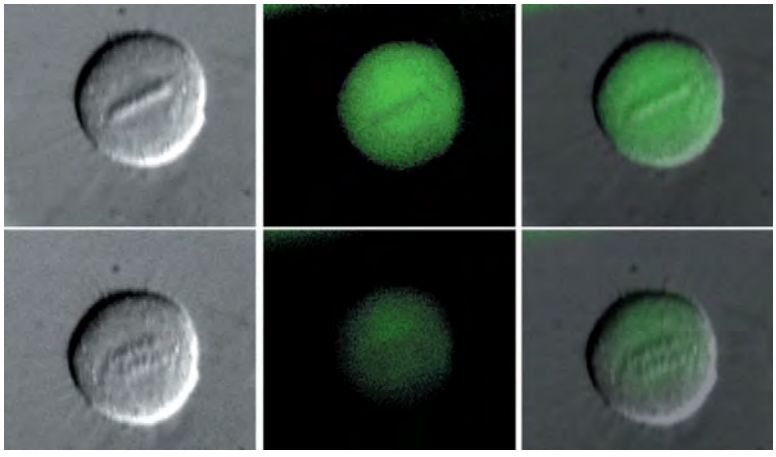
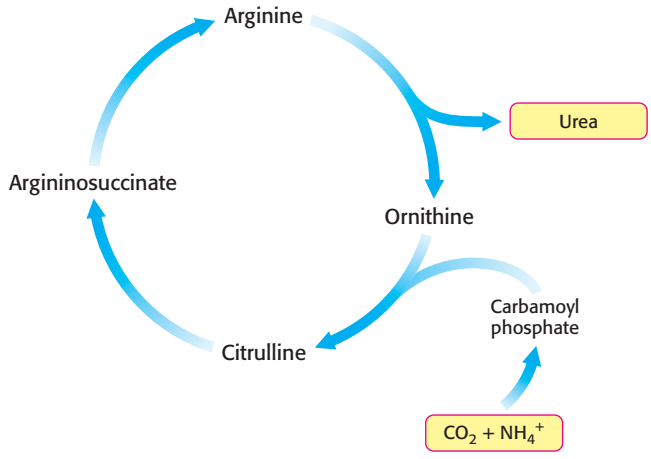
CHAPTER 23

Protein Turnover and Amino Acid Catabolism





Degradation of cyclin B. This important protein in cell-cycle regulation is visible as the green areas in the images above (the protein was fused with green fluorescent protein). Cyclin B is prominent during metaphase (top) but is degraded in anaphase (bottom) to prevent the premature initiation of another cell cycle. A large protease complex called the proteasome digests the protein into peptides, which are then degraded into amino acids. These amino acids are either reused or further processed so that the carbon skeletons can be used as fuel or building blocks. The released amino group is converted into urea for excretion by the urea cycle. [(Left) Courtesy of Dr. Jonathan Pines, University of Cambridge, Wellcome/CRC Institute of Cancer and Developmental Biology.]

The digestion of dietary proteins in the intestine and the degradation of proteins within the cell provide a steady supply of amino acids to the cell. Many cellular proteins are constantly degraded and resynthesized in response to changing metabolic demands. Others are misfolded or become damaged and they, too, must be degraded. Unneeded or damaged proteins are marked for destruction by the covalent attachment of chains of a small protein called *ubiquitin* and then degraded by a large, ATP-dependent complex called the *proteasome. The primary use of amino acids provided through degradation or digestion is as building blocks for the synthesis of proteins and other nitrogenous compounds such as nucleotide bases.*

Amino acids in excess of those needed for biosynthesis can neither be stored, in contrast with fatty acids and glucose, nor excreted. Rather, surplus amino acids are used as metabolic fuel. *The α-amino group is removed, and the resulting carbon skeleton is converted into a major metabolic intermediate.* Most of the amino groups harvested from surplus amino acids are converted into urea through the *urea cycle,* and their carbon skeletons are transformed into acetyl CoA, acetoacetyl CoA, pyruvate, or one of the intermediates of the citric acid cycle. The carbon skeletons are converted into glucose, glycogen, and fats.

OUTLINE

Several coenzymes play key roles in amino acid degradation; foremost among them is *pyridoxal phosphate*. This coenzyme forms Schiff-base intermediates, which are a type of aldimine, that allow α-amino groups to be shuttled between amino acids and ketoacids. We will consider several genetic errors of amino acid degradation that lead to brain damage and mental retardation unless remedial action is initiated soon after birth. *Phenylketonuria*, which is caused by a block in the conversion of phenylalanine into tyrosine, is readily diagnosed and can be treated by removing phenylalanine from the diet. The study of amino acid metabolism is especially rewarding because it is rich in connections between basic biochemistry and clinical medicine.

23.1 Proteins Are Degraded to Amino Acids

Dietary protein is a vital source of amino acids. Especially important dietary proteins are those containing the essential amino acids—amino acids that cannot be synthesized and must be acquired in the diet (Table 23.1). Proteins ingested in the diet are digested into amino acids or small peptides that can be absorbed by the intestine and transported in the blood. Another crucial source of amino acids is the degradation of cellular proteins.

Table 23.1 Essential amino acids in human beings

Histidine
Isoleucine
Leucine
Lysine
Methionine
Phenylalanine
Threonine
Tryptophan
Valine

The digestion of dietary proteins begins in the stomach and is completed in the intestine

Protein digestion begins in the stomach, where the acidic environment favors the denaturation of proteins into random coils. Denatured proteins are more accessible as substrates for proteolysis than are native proteins. The primary proteolytic enzyme of the stomach is *pepsin*, a nonspecific protease that, remarkably, is maximally active at pH 2. Thus, pepsin can function in the highly acidic environment of the stomach that disables other proteins.

Protein degradation continues in the lumen of the intestine. The pancreas secretes a variety of proteolytic enzymes into the intestinal lumen as inactive zymogens that are then converted into active enzymes (Sections 9.1 and 10.4). The battery of enzymes displays a wide array of specificity, and so the substrates are degraded into free amino acids as well as di- and tripeptides. Digestion is further enhanced by proteolytic enzymes, such as aminopeptidase N, that are located in the plasma membrane of the intestinal cells. Aminopeptidases digest proteins from the amino-terminal end. Single amino acids, as well as di- and tripeptides, are transported into the intestinal cells from the lumen and subsequently released into the blood for absorption by other tissues (Figure 23.1).

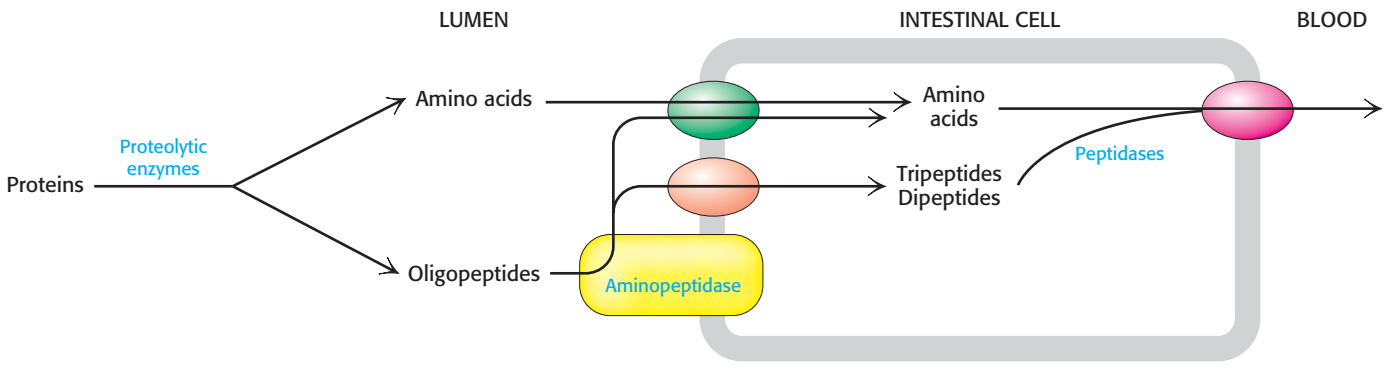


Figure 23.1 Digestion and absorption of proteins. Protein digestion is primarily a result of the activity of enzymes secreted by the pancreas. Aminopeptidases associated with the intestinal epithelium further digest proteins. The amino acids and di- and tripeptides are absorbed into the intestinal cells by specific transporters. Free amino acids are then released into the blood for use by other tissues.

Cellular proteins are degraded at different rates

Protein turnover—the degradation and resynthesis of proteins—takes place constantly in cells. Although some proteins are very stable, many proteins are short lived, particularly those that participate in metabolic regulation. These proteins can be quickly degraded to activate or shut down a signaling pathway. In addition, cells must eliminate damaged proteins. A significant proportion of newly synthesized protein molecules are defective because of errors in translation or misfolding. Even proteins that are normal when first synthesized may undergo oxidative damage or be altered in other ways with the passage of time. These proteins must be removed before they accumulate and aggregate. Indeed, a number of pathological conditions, such as certain forms of Parkinson disease and Huntington disease, are associated with protein aggregation.

The half-lives of proteins range over several orders of magnitude. Ornithine decarboxylase, at approximately 11 minutes, has one of the shortest half-lives of any mammalian protein. This enzyme participates in the synthesis of polyamines, which are cellular cations essential for growth and differentiation. The life of hemoglobin, on the other hand, is limited only by the life of the red blood cell, and the lens protein, crystallin, by the life of the organism.

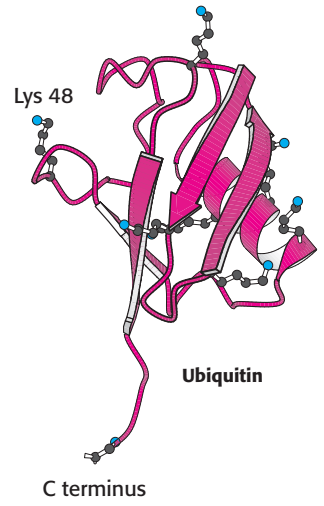


Figure 23.2 Structure of ubiquitin. *Notice* that ubiquitin has an extended carboxyl terminus, which is activated and linked to proteins targeted for destruction. Lysine residues, including lysine 48, the major site for linking additional ubiquitin molecules, are shown as ball-and-stick models. [Drawn from 1UBI.pdb.]

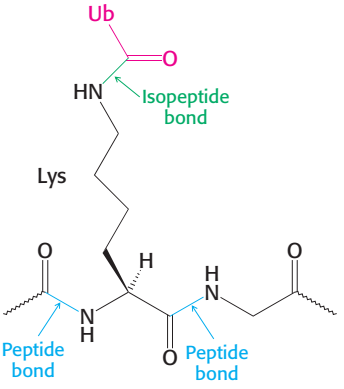
23.2 Protein Turnover Is Tightly Regulated

How can a cell distinguish proteins that should be degraded? *Ubiquitin* (Ub), a small (8.5-kd) protein present in all eukaryotic cells, is a tag that marks proteins for destruction (Figure 23.2). Ubiquitin is the cellular equivalent of the "black spot" of Robert Louis Stevenson's *Treasure Island:* the signal for death.

Ubiquitin tags proteins for destruction

Ubiquitin is highly conserved in eukaryotes: yeast and human ubiquitin differ at only 3 of 76 residues. The carboxyl-terminal glycine residue of ubiquitin becomes covalently attached to the ε-amino groups of several lysine residues on a protein destined to be degraded. The energy for the formation of these *isopeptide bonds* (*iso* because ε- rather than α-amino groups are targeted) comes from ATP hydrolysis.

Three enzymes participate in the attachment of ubiquitin to a protein (Figure 23.3): ubiquitin-activating enzyme, or E1; ubiquitin-conjugating enzyme, or E2; and ubiquitin–protein ligase, or E3. First, the C-terminal



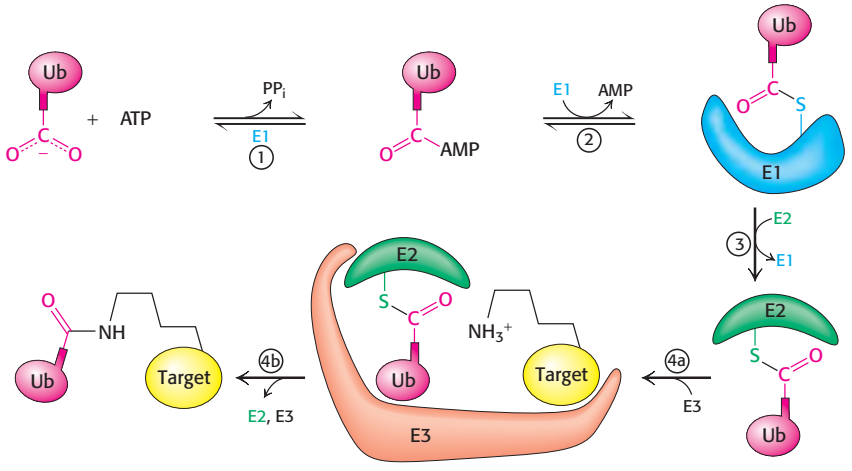


Figure 23.3 Ubiquitin conjugation. The ubiquitin-activating enzyme E1 adenylates ubiquitin (Ub) (1) and transfers the ubiquitin to one of its own cysteine residues (2). Ubiquitin is then transferred to a cysteine residue in the ubiquitin-conjugating enzyme E2 by the E2 enzyme. (3). Finally, the ubiquitin–protein ligase E3 transfers the ubiquitin to a lysine residue on the target protein (4a and 4b).

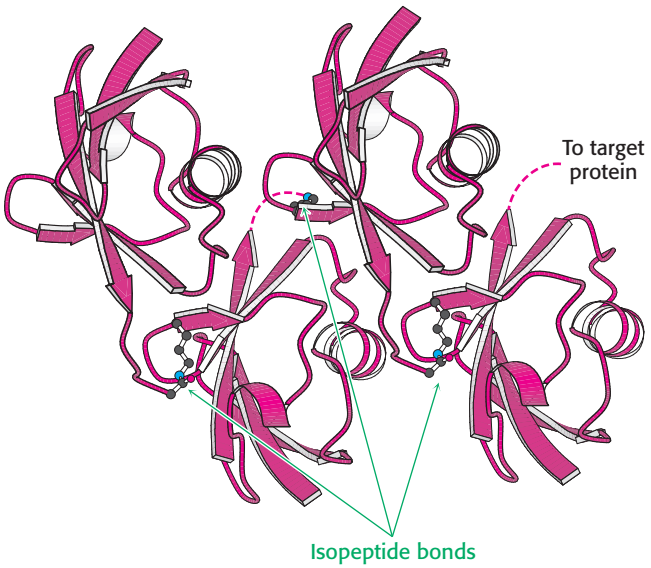


Figure 23.4 Structure of tetraubiquitin. Four ubiquitin molecules are linked by isopeptide bonds. *Notice* that each isopeptide bond is formed by the linkage of the carboxylate group at the end of the extended C terminus with the ε-amino group of a lysine residue. Dashed lines indicate the positions of the extended C-termini that were not observed in the crystal structure. This unit is the primary signal for degradation when linked to a target protein. [Drawn from 1TBE.pdb.]

carboxylate group of ubiquitin becomes linked to a sulfhydryl group of E1 by a thioester bond. This ATP-driven reaction is reminiscent of fatty acid activation (Section 22.2). In this reaction, ATP is linked to the C-terminal carboxylate of ubiquitin with the release of pyrophosphate, and the ubiquitin is transferred to a sulfhydryl group of a key cysteine residue in E1. The activated ubiquitin is then shuttled to a sulfhydryl group of E2, a reaction catalyzed by E2 itself. Finally, E3 catalyzes the transfer of ubiquitin from E2 to an ε-amino group on the target protein.

A chain of four or more ubiquitin molecules is especially effective in signaling the need for degradation (Figure 23.4). The ubiquitination reaction is processive: E3 remains bound to the target proteins and generates a chain of ubiquitin molecules by linking the ε-amino group of lysine residue 48 of one ubiquitin molecule to the terminal carboxylate of another.

What determines whether a protein becomes ubiquitinated? A specific sequence of amino acids, termed a *degron*, indicates that a protein should be degraded. One such signal turns out to be unexpectedly simple. *The half-life of a cytoplasmic protein is determined to a large extent by its amino-terminal residue* (Table 23.2). This dependency is referred to as the *N-terminal rule* or the *N-terminal degron*. A yeast protein with methionine at its N terminus typically has a half-life of more than 20 hours, whereas one with arginine at this position has a half-life of about 2 minutes. A highly destabilizing N-terminal residue such as arginine or leucine favors rapid ubiquitination, whereas a stabilizing residue such as methionine or proline does not. Other degrons thought to identify proteins for degradation include *cyclin destruction boxes,* which are amino acid sequences that mark cell-cycle proteins for destruction, and *PEST sequences*, which contain the amino acid sequence proline (P, single-letter abbreviation), glutamic acid (E), serine (S), and threonine (T).

E3 enzymes are the readers of N-terminal residues. Although most eukaryotes have only one or a small number of distinct E1 enzymes, all eukaryotes have many distinct E2 and E3 enzymes. Moreover, there appears to be only a single family of evolutionarily related E2 proteins but three distinct families of E3 proteins, all together consisting of hundreds of members. Indeed, the E3 family is one of the largest gene families in human beings. The diversity of target proteins that must be tagged for destruction requires a large number of E3 proteins as readers.

Table 23.2 Dependence of the half-lives of cytoplasmic yeast proteins on the identity of their amino-terminal residues

Highly stabilizing residues ($t_{1/2}$ > 20 hours)			
Ala	Cys	Gly	Met
Pro	Ser	Thr	Val
Intrinsically destabilizing residues ($t_{1/2}$ = 2 to 30 minutes)			
Arg	His	Ile	Leu
Lys	Phe	Trp	Tyr
Destabilizing residues after chemical modification ($t_{1/2}$ = 3 to 30 minutes)			
Asn	Asp	Gln	Glu

Source: J. W. Tobias, T. E. Schrader, G. Rocap, and A. Varshavsky. *Science* 254(1991):1374–1377.

Three examples demonstrate the importance of E3 proteins to normal cell function. Proteins that are not broken down owing to a defective E3 may accumulate to create a disease of protein aggregation such as juvenile or early-onset Parkinson disease. A defect in another member of the E3 family causes Angelman syndrome, a severe neurological disorder characterized by mental retardation, absence of speech, uncoordinated movement, and hyperactivity. Conversely, uncontrolled protein turnover also can create dangerous pathological conditions. For example, human papilloma virus (HPV) encodes a protein that activates a specific E3 enzyme. The enzyme ubiquitinates the tumor suppressor p53 and other proteins that control DNA repair, which are then destroyed. The activation of this E3 enzyme is observed in more than 90% of cervical carcinomas. Thus, the inappropriate marking of key regulatory proteins for destruction can trigger further events, leading to tumor formation.

It is important to note that the role of ubiquitin is much broader than merely marking proteins for destruction. Although we have focused on protein degradation, ubiquitination also regulates proteins involved in DNA repair, chromatin remodeling, and protein kinase activation, among other biochemical processes.

The proteasome digests the ubiquitin-tagged proteins

If ubiquitin is the mark of death, what is the executioner? *A large protease complex called the proteasome or the 26S proteasome digests the ubiquitinated proteins*. This ATP-driven multisubunit protease spares ubiquitin, which is then recycled. The 26S proteasome is a complex of two components: a 20S catalytic unit and a 19S regulatory unit.

The 20S unit is constructed from 14 copies each of two homologous subunits (α and β) and has a mass of 700 kd (Figure 23.5). The subunits are arranged in four rings of seven subunits that stack to form a structure resembling a barrel. The outer two rings of the barrel are made up of α subunits and the inner two rings of β subunits. The 20S catalytic core is a sealed barrel. *Access to its interior is controlled by a 19S regulatory unit*, itself a 700-kd complex made up of 20 subunits. Two such 19S complexes bind to the 20S proteasome core, one at each end, to form the complete 26S proteasome (Figure 23.6). The 19S regulatory unit has three functions. First, the 19S unit binds specifically to polyubiquitin chains, thereby ensuring that only ubiquitinated proteins are degraded. Second, an isopeptidase in the 19S unit cleaves off intact ubiquitin molecules from the proteins so that they can be reused. Finally, the doomed protein is unfolded and directed into the catalytic core. Key components of the 19S complex are six ATPases of a type called the AAA class (*A*TPase *a*ssociated with various cellular *a*ctivities). ATP hydrolysis assists the 19S complex to unfold the substrate and induce conformational changes in the 20S catalytic core so that the substrate can be passed into the center of the complex.

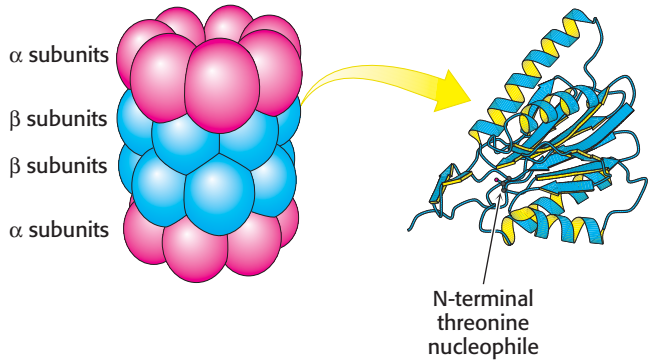


Figure 23.5 20S proteasome. The 20S proteasome comprises 28 homologous subunits (*α*, red; *β*, blue), arranged in four rings of 7 subunits each. Some of the β subunits (right) include protease active sites at their amino termini. [Subunit drawn from 1RYP.pdb.]

The proteolytic active sites are sequestered in the interior of the barrel to protect potential substrates until they are directed into the barrel. There are three types of active sites in the β subunits, each with a different specificity, but all employ an N-terminal threonine. The hydroxyl group of the threonine residue is converted into a nucleophile that attacks the carbonyl groups of peptide bonds to form acyl-enzyme intermediates. Substrates are degraded in a processive manner without the release of degradation intermediates, until the substrate is reduced to peptides ranging in length from seven to nine residues. These peptide products are released from the proteasome and further degraded by other cellular proteases to yield individual amino acids. Thus, the ubiquitination pathway and the proteasome cooperate to degrade unwanted proteins. Figure 23.7 presents an overview of the fates of amino acids following proteasomal digestion.

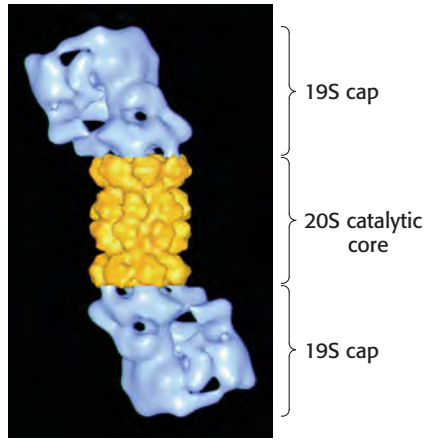


Figure 23.6 26S proteasome. A 19S cap is attached to each end of the 20S catalytic unit. [From W. Baumeister, J. Walz, F. Zuhl, and E. Seemuller. *Cell* 92(1998):367–380; courtesy of Dr. Wolfgang Baumeister.]

The ubiquitin pathway and the proteasome have prokaryotic counterparts

Both the ubiquitin pathway and the proteasome appear to be present in all eukaryotes. Homologs of the proteasome are also found in some prokaryotes. The proteasomes of some archaea are quite similar in overall structure to their eukaryotic counterparts and similarly have 28 subunits (Figure 23.8). In the archaeal proteasome, however, all α outer-ring subunits and all β inner-ring subunits are identical; in eukaryotes, each α or β subunit

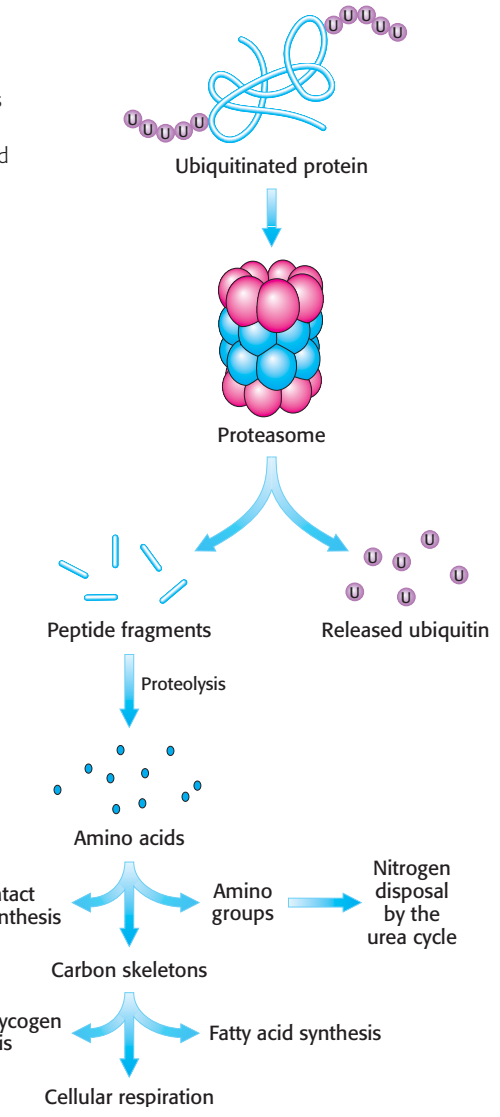
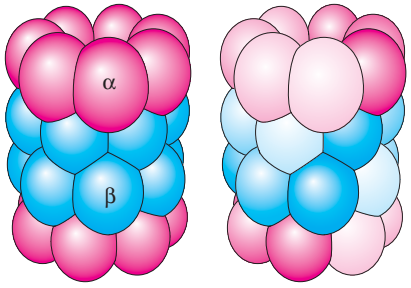
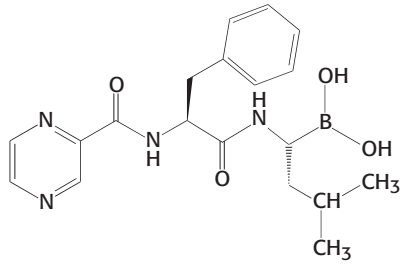


Figure 23.7 The proteasome and other proteases generate free amino acids. Ubiquitinated proteins are processed to peptide fragments from which the ubiquitin is subsequently removed and recycled. The peptide fragments are further digested to yield free amino acids, which can be used for biosynthetic reactions, most notably protein synthesis. Alternatively, the amino group can be removed and processed to urea (p. 685) and the carbon skeleton can be used to synthesize carbohydrate or fats or used directly as a fuel for cellular respiration.



Archaeal proteasome **Eukaryotic proteasome**

Figure 23.8 Proteasome evolution. The archaeal proteasome consists of 14 identical α subunits and 14 identical β subunits. In the eukaryotic proteasome, gene duplication and specialization has led to 7 distinct subunits of each type. The overall architecture of the proteasome is conserved.



Bortezomib
(a dipeptidyl boronic acid)

is one of seven different isoforms. This specialization provides distinct substrate specificity.

Although ubiquitin has not been found in prokaryotes, ubiquitin's molecular ancestors were recently identified in prokaryotes. Remarkably, these proteins take part not in protein modification but in biosynthesis of the coenzyme thiamine. A key enzyme in thiamine biosynthesis is ThiF, which activates the protein ThiS as an acyl adenylate and then adds a sulfide ion derived from cysteine (Figure 23.9). ThiF is homologous to human E1, which includes two tandem regions of 160 amino acids that are 28% identical in amino acid sequence with a region of ThiF from *E. coli*. The evolutionary relationships between these two pathways were cemented by the determination of the three-dimensional structure of ThiS, which revealed a structure very similar to that of ubiquitin, despite being only 14% identical in amino acid sequence (Figure 23.10). Thus, a eukaryotic system for protein modification evolved from a preexisting prokaryotic pathway for coenzyme biosynthesis.

Protein degradation can be used to regulate biological function

Table 23.3 lists a number of physiological processes that are controlled at least in part by protein degradation through the ubiquitin–

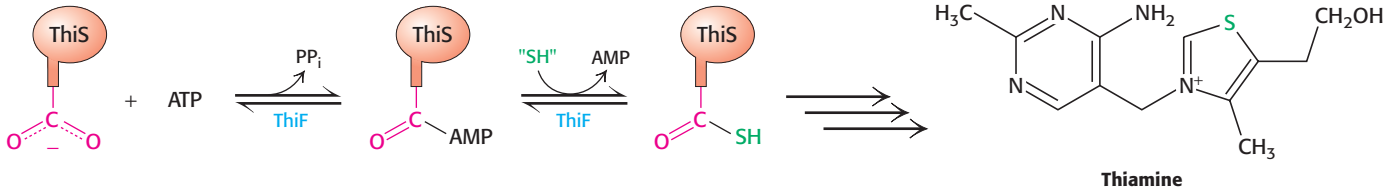


Figure 23.9 Biosynthesis of thiamine. The biosynthesis of thiamine begins with the addition of sulfide to the carboxyl terminus of the protein ThiS. This protein is activated by adenylation and conjugated in a manner analogous to the first steps in the ubiquitin pathway.

proteasome pathway. In each case, the proteins being degraded are regulatory proteins. Consider, for example, control of the inflammatory response. A transcription factor called *NF-κB* (NF for nuclear factor) initiates the expression of a number of the genes that take part in this response. This factor is itself activated by the degradation of an attached inhibitory protein, *I-κB* (I for inhibitor). In response to inflammatory signals that bind to membrane-bound receptors, I-κB is phosphorylated at two serine residues, creating an E3 binding site. The binding of E3 leads to the ubiquitination and degradation of I-κB, unleashing NF-κB. The liberated transcription factor migrates from the cytoplasm to the nucleus to stimulate the transcription of the target genes. The NF-κB–I-κB system illustrates the interplay of several key regulatory motifs: receptor-mediated signal transduction, phosphorylation, compartmentalization, controlled and specific degradation, and selective gene expression. The importance of the ubiquitin–proteasome system for the regulation of gene expression is highlighted by the recent approval of bortezomib (Velcade), a potent inhibitor of the proteasome, as a therapy for multiple myeloma. Bortezomib is a dipeptidyl boronic acid inhibitor of the proteasome.

Table 23.3 Processes regulated by protein degradation

Gene transcription
Cell-cycle progression
Organ formation
Circadian rhythms
Inflammatory response
Tumor suppression
Cholesterol metabolism
Antigen processing

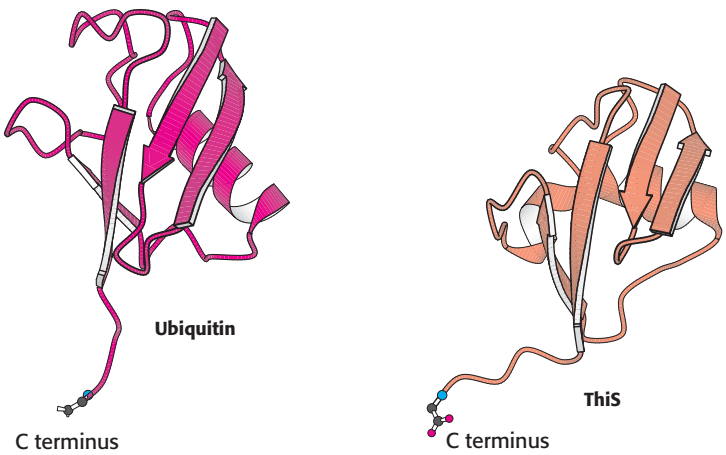
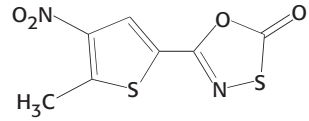


Figure 23.10 Structures of ThiS and ubiquitin compared. *Notice* that ThiS is structurally similar to ubiquitin despite only 14% sequence identity. This observation suggests that a prokaryotic protein such as ThiS evolved into ubiquitin. [Drawn from 1UBI.pdb and 1FOZ.pdb.]

The evolutionary studies of proteasomes described above have also yielded potential clinical benefits. The bacterial pathogen *Mycobacterium tuberculosis*, the cause of tuberculosis, harbors a proteasome that is very similar to the human counterpart. Nevertheless, recent work has shown that it is possible to exploit the differences between the human and the bacterial proteasomes to develop specific inhibitors of the *M. tuberculosis* complex. Oxathiazol-2-one compounds such as HT1171 are suicide inhibitors of the proteolytic activity of the *M. tuberculosis* proteasome, but have no effect on the proteasomes of the human host. This is especially exciting because these drugs kill the nonreplicating form of *M. tuberculosis*, and thus may not require the prolonged treatment required with conventional drugs, thereby reducing the likelihood of drug resistance due to interruption of the treatment regime.



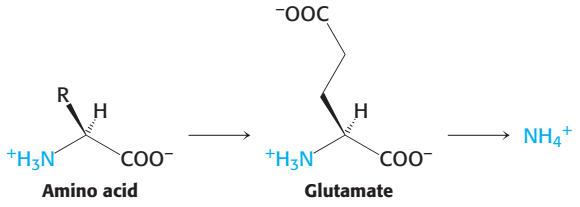
HT1171
[5-(2-methyl-3-nitrothiophen-2-yl)-1,3,4-oxathiazol-2-one]

23.3 The First Step in Amino Acid Degradation Is the Removal of Nitrogen

What is the fate of amino acids released on protein digestion or turnover? The first call is for use as building blocks for biosynthetic reactions. However, any not needed as building blocks are degraded to compounds able to enter the metabolic mainstream. The amino group is first removed, and then the remaining carbon skeleton is metabolized to glucose, one of several citric acid cycle intermediates, or to acetyl CoA. The major site of amino acid degradation in mammals is the liver, although muscles readily degrade the branched-chain amino acids (Leu, Ile, and Val). The fate of the α-amino group will be considered first, followed by that of the carbon skeleton (Section 23.5).

Alpha-amino groups are converted into ammonium ions by the oxidative deamination of glutamate

The α-amino group of many amino acids is transferred to α-ketoglutarate to form *glutamate,* which is then oxidatively deaminated to yield ammonium ion (NH_4^+).



Aminotransferases catalyze the transfer of an α-amino group from an α-amino acid to an α-ketoacid. These enzymes, also called *transaminases,* generally funnel α-amino groups from a variety of amino acids to α-ketoglutarate for conversion into NH_4^+.

Aspartate aminotransferase, one of the most important of these enzymes, catalyzes the transfer of the amino group of aspartate to α-ketoglutarate.

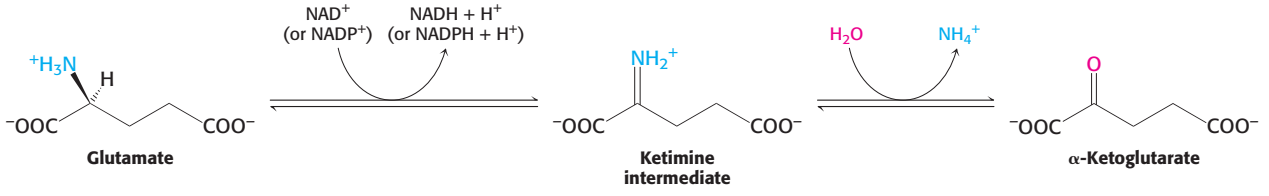
$$\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

Alanine aminotransferase catalyzes the transfer of the amino group of alanine to α-ketoglutarate.

$$\text{Alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}$$

These transamination reactions are reversible and can thus be used to synthesize amino acids from α-ketoacids, as we shall see in Chapter 24.

The nitrogen atom in glutamate is converted into free ammonium ion by oxidative deamination. This reaction is catalyzed by *glutamate dehydrogenase.* This enzyme is unusual in being able to utilize either NAD^+ or $NADP^+$, at least in some species. The reaction proceeds by dehydrogenation of the C—N bond, followed by hydrolysis of the resulting aldimine.

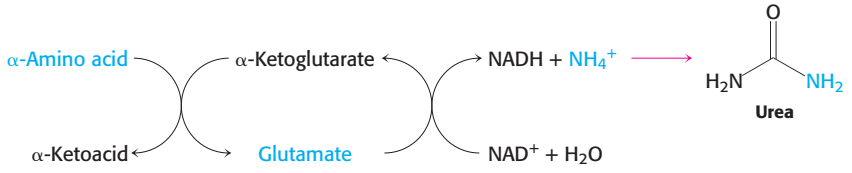


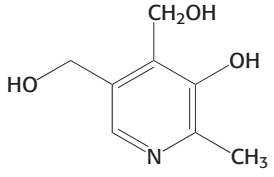
This reaction equilibrium constant is close to 1 in the liver, so the direction of the reaction is determined by the concentrations of reactants and products. Normally, the reaction is driven forward by the rapid removal of ammonium ion. In mammals but not in other organisms, glutamate is allosterically inhibited by GTP and palmitoyl CoA, and stimulated by ADP and leucine. Glutamate dehydrogenase, essentially a liver-specific enzyme, is located in mitochondria, as are some of the other enzymes required for the production of urea. This compartmentalization sequesters free ammonium ion, which is toxic.

The sum of the reactions catalyzed by aminotransferases and glutamate dehydrogenase is

$$\alpha\text{-Amino acid} + \underset{(\text{or NADP}^+)}{\text{NAD}^+} + \text{H}_2\text{O} \rightleftharpoons \alpha\text{-ketoacid} + \text{NH}_4^+ + \underset{(\text{or NADPH})}{\text{NADH}} + \text{H}^+$$

In most terrestrial vertebrates, NH_4^+ is converted into urea, which is excreted.

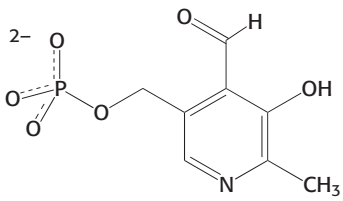




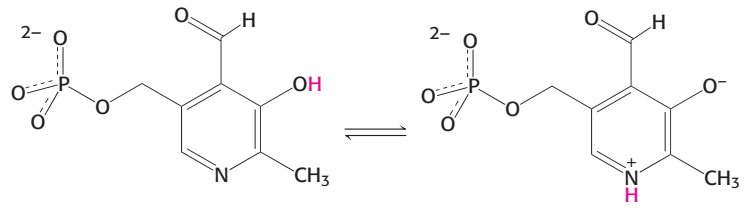
Pyridoxine (Vitamin B_6)

Mechanism: Pyridoxal phosphate forms Schiff-base intermediates in aminotransferases

All aminotransferases contain the prosthetic group *pyridoxal phosphate* (PLP), which is derived from *pyridoxine (vitamin B_6)*. Pyridoxal phosphate includes a pyridine ring that is slightly basic to which is attached an OH group that is slightly acidic. Thus, pyridoxal phosphate derivatives can form a stable tautomeric form in which the pyridine nitrogen atom is protonated and, hence, positively charged, while the OH group loses a proton and hence is negatively charged, forming a phenolate.

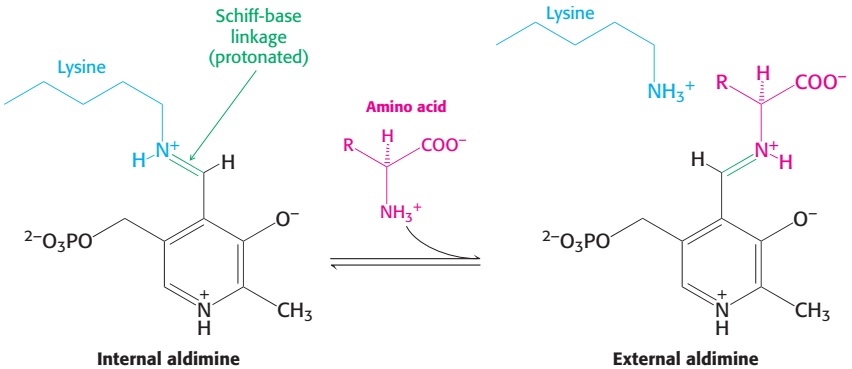


Pyridoxal phosphate (PLP)



PLP (protonated) **PLP (phenolate)**

The most important functional group on PLP is the aldehyde. This group forms covalent Schiff-base intermediates with amino acid substrates. Indeed, even in the absence of substrate, the aldehyde group of PLP usually forms a Schiff-base linkage with the ε-amino group of a specific lysine residue at the enzyme's active site. A new Schiff-base linkage is formed on addition of an amino acid substrate.



Internal aldimine **External aldimine**

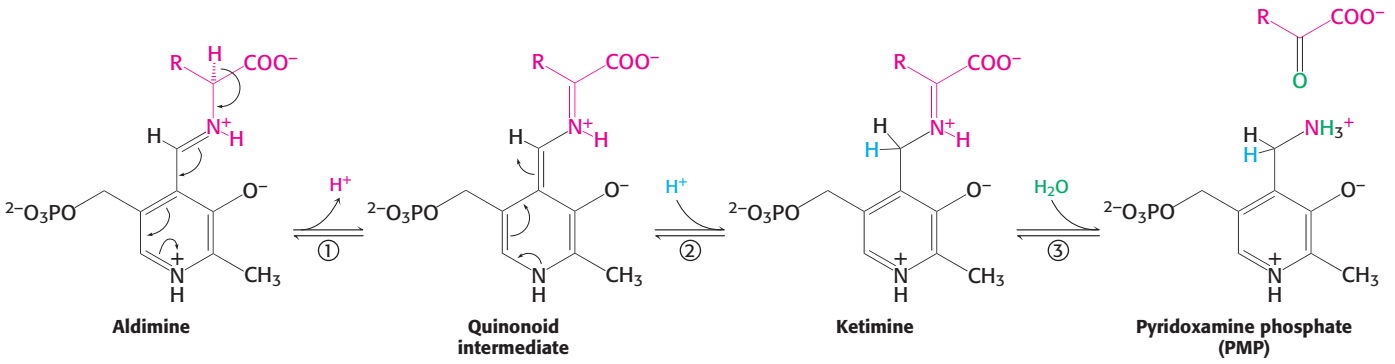
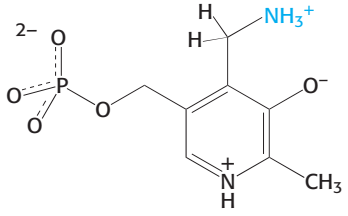


Figure 23.11 Transamination mechanism.
(1) The external aldimine loses a proton to form a quinonoid intermediate. (2) Reprotonation of this intermediate at the aldehyde carbon atom yields a ketimine. (3) This intermediate is hydrolyzed to generate the α-ketoacid product and pyridoxamine phosphate.



The α-amino group of the amino acid substrate displaces the ε-amino group of the active-site lysine residue. In other words, an *internal* aldimine becomes an *external* aldimine. The amino acid–PLP Schiff base that is formed remains tightly bound to the enzyme by multiple noncovalent interactions. The Schiff-base linkage often accepts a proton at the N, with the positive charge stabilized by interaction with the negatively charged phenolate group of PLP.

The Schiff base between the amino acid substrate and PLP, the external *aldimine,* loses a proton from the α-carbon atom of the amino acid to form a *quinonoid* intermediate (Figure 23.11). Reprotonation of this intermediate at the aldehyde carbon atom yields a *ketimine.* The ketimine is then hydrolyzed to an α-ketoacid and *pyridoxamine phosphate* (PMP). These steps constitute half of the transamination reaction.

$$\text{Amino acid}_1 + \text{E-PLP} \rightleftharpoons \alpha\text{-ketoacid}_1 + \text{E-PMP}$$

The second half takes place by the reverse of the preceding pathway. A second α-ketoacid reacts with the enzyme–pyridoxamine phosphate complex (E-PMP) to yield a second amino acid and regenerate the enzyme–pyridoxal phosphate complex (E-PLP).

$$\alpha\text{-Ketoacid}_2 + \text{E-PMP} \rightleftharpoons \text{amino acid}_2 + \text{E-PLP}$$

The sum of these partial reactions is

$$\text{Amino acid}_1 + \alpha\text{-ketoacid}_2 \rightleftharpoons \text{amino acid}_2 + \alpha\text{-ketoacid}_1$$

Aspartate aminotransferase is an archetypal pyridoxal-dependent transaminase

The mitochondrial enzyme aspartate aminotransferase provides an especially well studied example of PLP as a coenzyme for transamination reactions (Figure 23.12). X-ray crystallographic studies provided detailed views of how PLP and substrates are bound and confirmed much of the proposed catalytic mechanism. Each of the identical 45-kd subunits of this dimer consists of a large domain and a small one. PLP is bound to the large domain, in a pocket near the subunit interface. In the absence of substrate, the aldehyde group of PLP is in a Schiff-base linkage with lysine 258, as expected. Adjacent to the coenzyme's binding site is a conserved arginine residue that interacts with the α-carboxylate group of the amino acid substrate, helping to orient the substrate appropriately in the active site. A base is necessary to remove a proton from the α-carbon group of the amino acid and to transfer it to the aldehyde carbon atom of PLP (see Figure 23.11, steps 1 and 2). The lysine amino group that was initially in Schiff-base linkage with PLP appears to serve as the proton donor and acceptor.

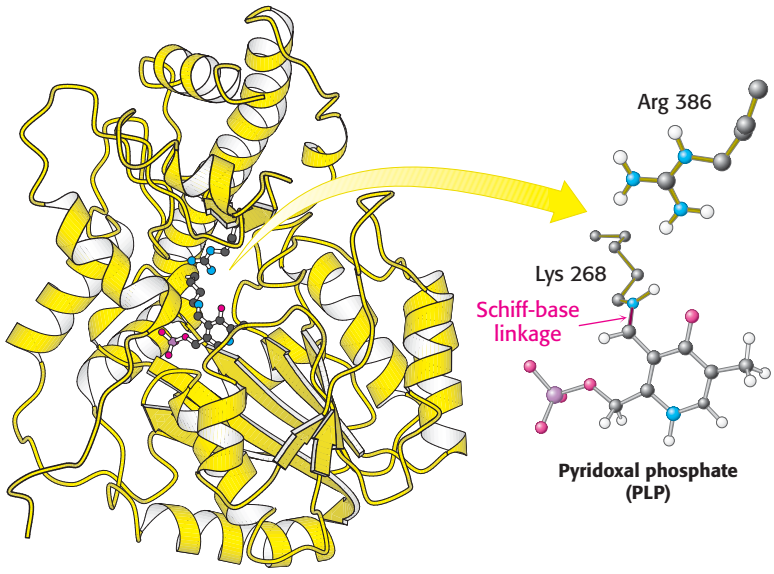


Figure 23.12 Aspartate aminotransferase. The active site of this prototypical PLP-dependent enzyme includes pyridoxal phosphate attached to the enzyme by a Schiff-base linkage with lysine 258. An arginine residue in the active site helps orient substrates by binding to their α-carboxylate groups. Only one of the enzyme's two subunits is shown. [Drawn from 1AAW.pdb.]

Pyridoxal phosphate enzymes catalyze a wide array of reactions

Transamination is just one of a wide range of amino acid transformations that are catalyzed by PLP enzymes. The other reactions catalyzed by PLP enzymes at the α-carbon atom of amino acids are decarboxylations, deaminations, racemizations, and aldol cleavages (Figure 23.13). In addition, PLP enzymes catalyze elimination and replacement reactions at the β-carbon atom (e.g., tryptophan synthetase in the synthesis of tryptophan) and the γ-carbon atom (e.g., cystathionine β-synthase in the synthesis of cysteine) of amino acid substrates. Three common features of PLP catalysis underlie these diverse reactions.

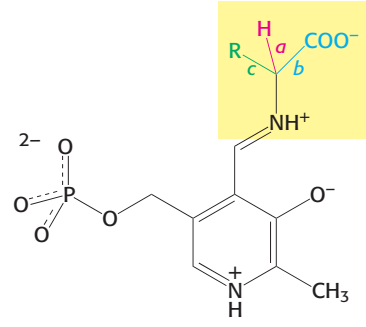


Figure 23.13 Bond cleavage by PLP enzymes. Pyridoxal phosphate enzymes labilize one of three bonds at the α-carbon atom of an amino acid substrate. For example, bond *a* is labilized by aminotransferases, bond *b* by decarboxylases, and bond *c* by aldolases (such as threonine aldolases). PLP enzymes also catalyze reactions at the β- and γ-carbon atoms of amino acids.

1. A Schiff base is formed by the amino acid substrate (the amine component) and PLP (the carbonyl component).

2. The protonated form of PLP acts as an *electron sink* to stabilize catalytic intermediates that are negatively charged. Electrons from these intermediates are attracted to the positive charge on the ring nitrogen atom. In other words, PLP is an *electrophilic catalyst*.

3. The product Schiff base is cleaved at the completion of the reaction.

How does an enzyme selectively break a particular one of three bonds at the α-carbon atom of an amino acid substrate? An important principle is that *the bond being broken must be perpendicular to the π orbitals of the electron sink* (Figure 23.14). An aminotransferase, for example, binds the amino acid substrate so that the C_α—H bond is perpendicular to the PLP ring (Figure 23.15). In serine hydroxymethyltransferase, the enzyme that converts serine into glycine, the N—C_α bond is rotated so that the C_α—C_β bond is most nearly perpendicular to the plane of the PLP ring, favoring its

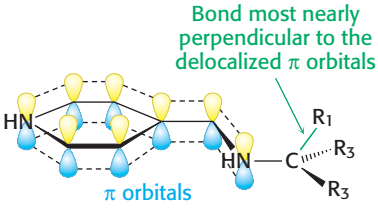


Figure 23.14 Stereoelectronic effects. The orientation about the NH—C_α bond determines the most favored reaction catalyzed by a pyridoxal phosphate enzyme. The bond that is most nearly perpendicular to the plane of delocalized π orbitals (represented by dashed lines) of the pyridoxal phosphate electron sink is most easily cleaved.

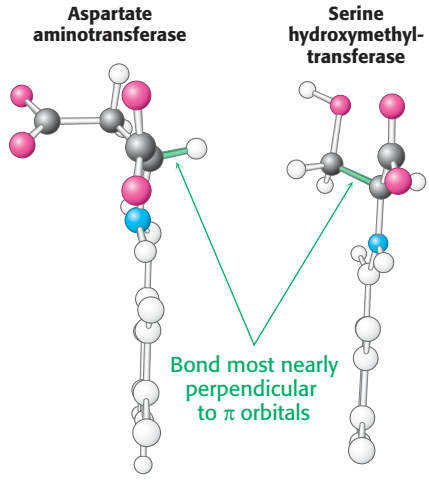


Figure 23.15 Reaction choice. In aspartate aminotransferase, the C_α—H bond is most nearly perpendicular to the π-orbital system and is cleaved. In serine hydroxymethyltransferase, a small rotation about the N—C_α bond places the C_α—C_β bond perpendicular to the π system, favoring its cleavage.

cleavage. This means of choosing one of several possible catalytic outcomes is called *stereoelectronic control*.

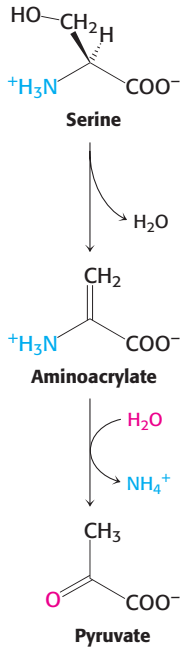
Many of the PLP enzymes that catalyze amino acid transformations, such as serine hydroxymethyltransferase, have a similar structure and are clearly related by divergent evolution. Others, such as tryptophan synthetase, have quite different overall structures. Nonetheless, the active sites of these enzymes are remarkably similar to that of aspartate aminotransferase, revealing the effects of convergent evolution.

Serine and threonine can be directly deaminated

The α-amino groups of serine and threonine can be directly converted into NH_4^+ without first being transferred to α-ketoglutarate. These direct deaminations are catalyzed by *serine dehydratase* and *threonine dehydratase*, in which PLP is the prosthetic group.

$$\text{Serine} \longrightarrow \text{pyruvate} + NH_4^+$$
$$\text{Threonine} \longrightarrow \alpha\text{-ketobutyrate} + NH_4^+$$

These enzymes are called *dehydratases* because *dehydration precedes deamination*. Serine loses a hydrogen ion from its α-carbon atom and a hydroxide ion group from its β-carbon atom to yield aminoacrylate. This unstable compound reacts with H_2O to give pyruvate and NH_4^+. Thus, the presence of a hydroxyl group attached to the β-carbon atom in each of these amino acids permits the direct deamination.



Peripheral tissues transport nitrogen to the liver

Although most amino acid degradation takes place in the liver, other tissues can degrade amino acids. For instance, muscle uses branched-chain amino acids as a source of fuel during prolonged exercise and fasting. How is the nitrogen processed in these other tissues? As in the liver, the first step is the removal of the nitrogen from the amino acid. However, muscle lacks the enzymes of the urea cycle, and so the nitrogen must be released in a nontoxic form that can be absorbed by the liver and converted into urea.

Nitrogen is transported from muscle to the liver in two principal transport forms. Glutamate is formed by transamination reactions, but the nitrogen is then transferred to pyruvate to form alanine, which is released into the blood (Figure 23.16). The liver takes up the alanine and converts it back into pyruvate by transamination. The pyruvate can be used for gluconeo-

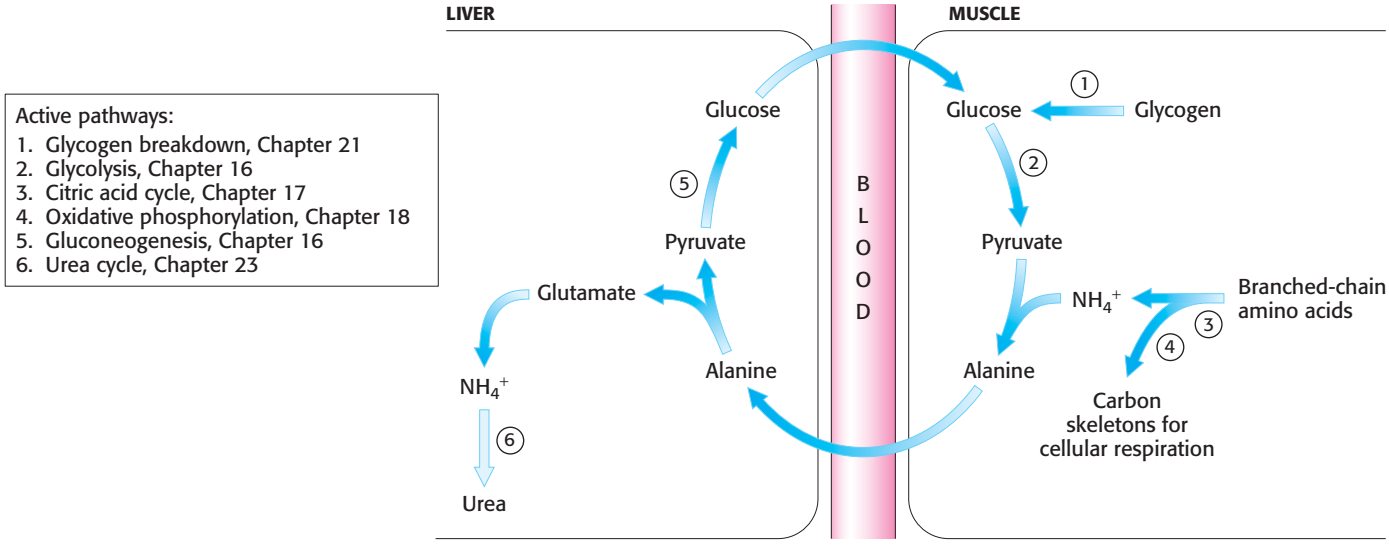


Figure 23.16 PATHWAY INTEGRATION: The glucose–alanine cycle. During prolonged exercise and fasting, muscle uses branched-chain amino acids as fuel. The nitrogen removed is transferred (through glutamate) to alanine, which is released into the bloodstream. In the liver, alanine is taken up and converted into pyruvate for the subsequent synthesis of glucose.

genesis and the amino group eventually appears as urea. This transport is referred to as the *glucose–alanine cycle.* It is reminiscent of the Cori cycle discussed earlier (see Figure 16.33). However, in contrast with the Cori cycle, pyruvate is not reduced to lactate by NADH, and thus more high-energy electrons are available for oxidative phosphorylation.

Nitrogen can also be transported as glutamine. Glutamine synthetase catalyzes the synthesis of glutamine from glutamate and NH_4^+ in an ATP-dependent reaction:

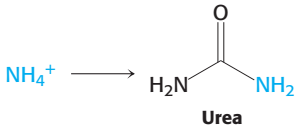
$$NH_4^+ + \text{glutamate} + ATP \xrightarrow{\text{Glutamine synthetase}} \text{glutamine} + ADP + P_i$$

The nitrogens of glutamine can be converted into urea in the liver.

23.4 Ammonium Ion Is Converted into Urea in Most Terrestrial Vertebrates

Some of the NH_4^+ formed in the breakdown of amino acids is consumed in the biosynthesis of nitrogen compounds. In most terrestrial vertebrates, the excess NH_4^+ is converted into *urea* and then excreted. Such organisms are referred to as *ureotelic.*

In terrestrial vertebrates, urea is synthesized by the *urea cycle* (Figure 23.17). The urea cycle, proposed by Hans Krebs and Kurt Henseleit in 1932, was the first cyclic metabolic pathway to be discovered. One of the nitrogen atoms of urea is transferred from an amino acid, aspartate. The other nitrogen atom is derived directly from free NH_4^+, and the carbon atom comes from HCO_3^- (derived by the hydration of CO_2; see Section 9.2).



The urea cycle begins with the formation of carbamoyl phosphate

The urea cycle begins with the coupling of free NH_3 with HCO_3^- to form carbamoyl phosphate. Carbamoyl phosphate

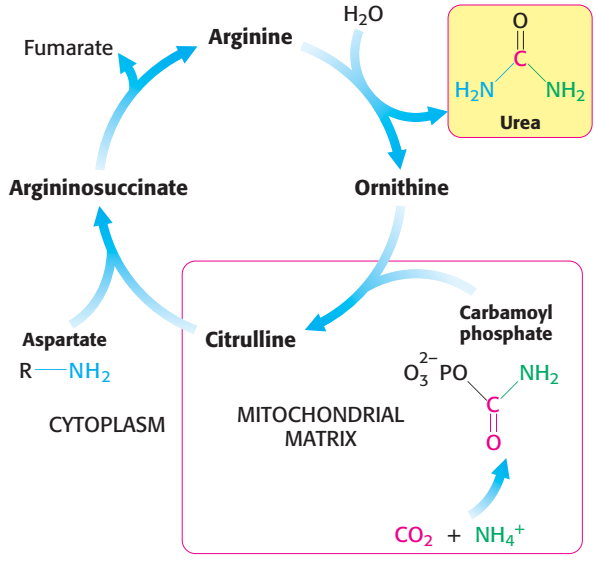
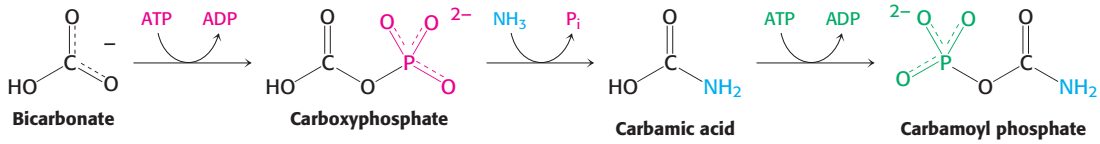


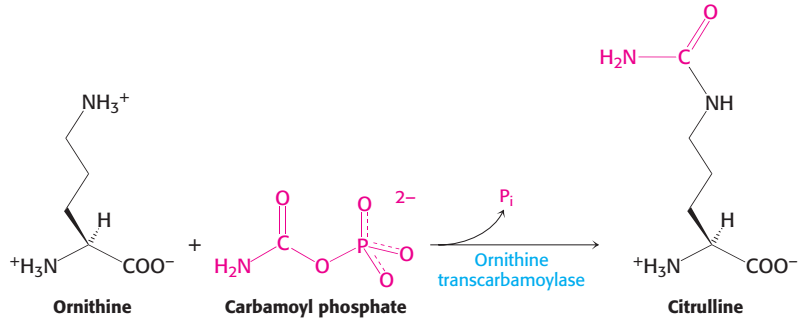
Figure 23.17 The urea cycle.

is a simple molecule, but its synthesis is complex. *Carbamoyl phosphate synthetase* catalyzes the required three steps.



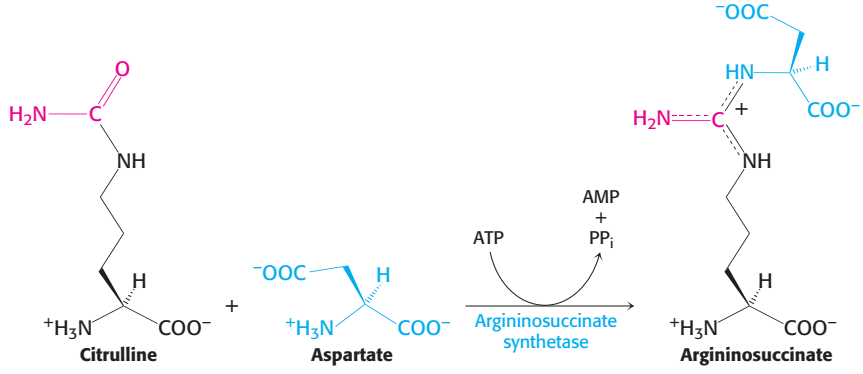
Note that NH_3, because it is a strong base, normally exists as NH_4^+ in aqueous solution. However, carbamoyl phosphate synthetase uses only NH_3 as a substrate. The reaction begins with the phosphorylation of HCO_3^- to form carboxyphosphate, which then reacts with NH_3 to form carbamic acid. Finally, a second molecule of ATP phosphorylates carbamic acid to form carbamoyl phosphate. The structure and mechanism of the enzyme that catalyzes these reactions will be presented in Chapter 25. The consumption of two molecules of ATP makes this synthesis of carbamoyl phosphate essentially irreversible. The mammalian enzyme requires N-*acetylglutamate* for activity, as will be described shortly.

The carbamoyl group of carbamoyl phosphate has a high transfer potential because of its anhydride bond. The carbamoyl group is transferred to *ornithine* to form *citrulline,* in a reaction catalyzed by *ornithine transcarbamoylase.*

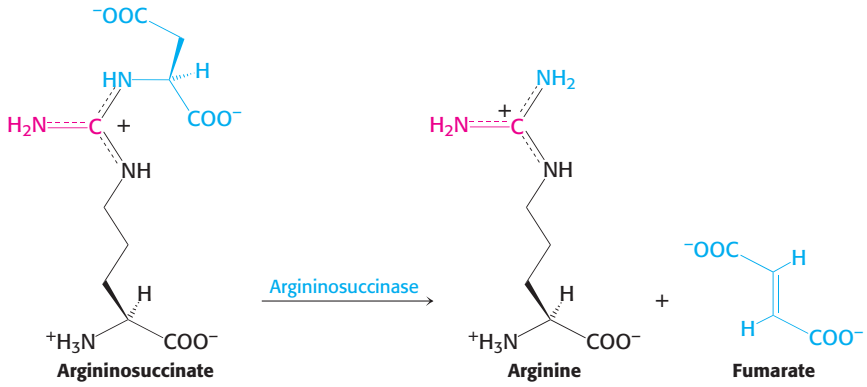


Ornithine and citrulline are amino acids, but they are not used as building blocks of proteins. The formation of NH_4^+ by glutamate dehydrogenase, its incorporation into carbamoyl phosphate as NH_3, and the subsequent synthesis of citrulline take place in the mitochondrial matrix. In contrast, the next three reactions of the urea cycle, which lead to the formation of urea, take place in the cytoplasm.

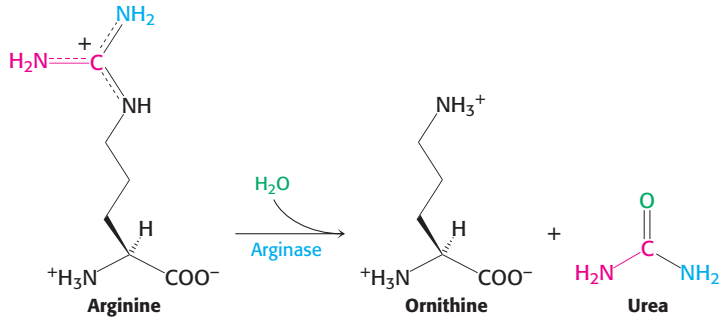
Citrulline is transported to the cytoplasm, where it condenses with aspartate, the donor of the second amino group of urea. This synthesis of *argininosuccinate,* catalyzed by *argininosuccinate synthetase,* is driven by the cleavage of ATP into AMP and pyrophosphate and by the subsequent hydrolysis of pyrophosphate.



Argininosuccinase cleaves argininosuccinate into *arginine* and *fumarate*. Thus, the carbon skeleton of aspartate is preserved in the form of fumarate.



Finally, arginine is hydrolyzed to generate urea and ornithine in a reaction catalyzed by *arginase*. Ornithine is then transported back into the mitochondrion to begin another cycle. The urea is excreted. Indeed, human beings excrete about 10 kg (22 pounds) of urea per year.



In ancient Rome, urine was a valuable commodity. Vessels were placed on street corners for passersby to urinate into. Bacteria would degrade the urea, releasing ammonium ion, which would act as a bleach to brighten togas.

The urea cycle is linked to gluconeogenesis

The stoichiometry of urea synthesis is

$$CO_2 + NH_4^+ + 3\,ATP + aspartate + 2\,H_2O \longrightarrow urea + 2\,ADP + P_i + AMP + PP_i + fumarate$$

Pyrophosphate is rapidly hydrolyzed, and so the equivalent of four molecules of ATP are consumed in these reactions to synthesize one molecule of urea. The synthesis of fumarate by the urea cycle is important because it is a precursor for glucose synthesis (Figure 23.18). Fumarate is hydrated to malate, which is in turn oxidized to oxaloacetate. Oxaloacetate can be converted into glucose by gluconeogenesis or transaminated to aspartate.

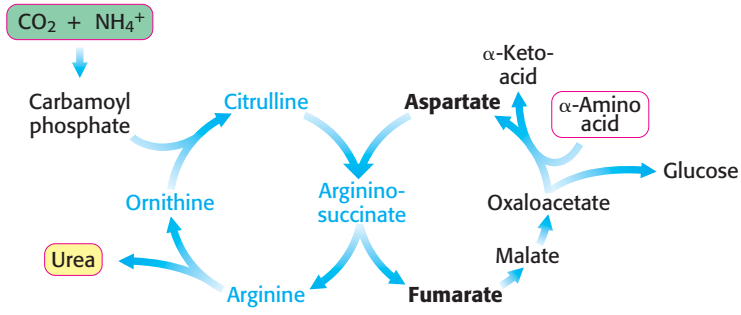


Figure 23.18 Metabolic integration of nitrogen metabolism. The urea cycle, gluconeogenesis, and the transamination of oxaloacetate are linked by fumarate and aspartate.

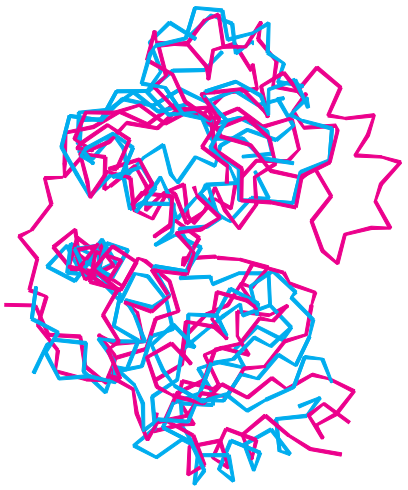
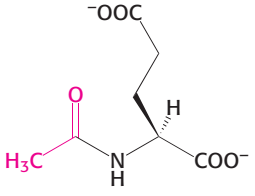


Figure 23.19 Homologous enzymes. The structure of the catalytic subunit of ornithine transcarbamoylase (blue) is quite similar to that of the catalytic subunit of aspartate transcarbamoylase (red), indicating that these two enzymes are homologs. [Drawn from 1RKM.pdb and 1RAI.pdb.]

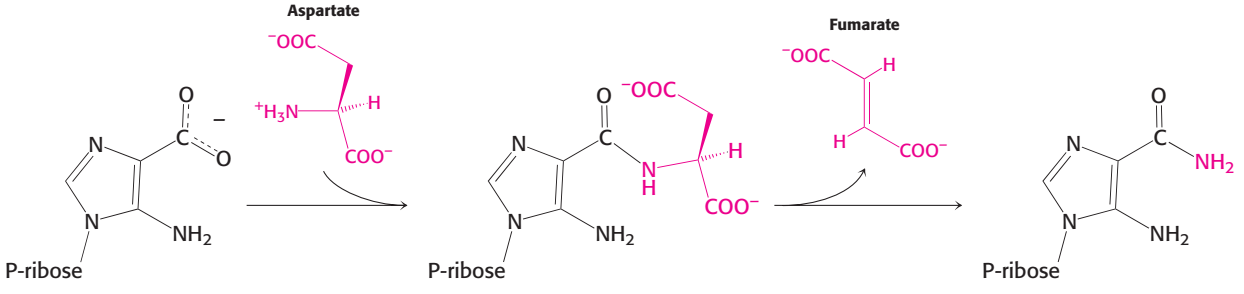


***N*-Acetylglutamate**

Urea-cycle enzymes are evolutionarily related to enzymes in other metabolic pathways

Carbamoyl phosphate synthetase generates carbamoyl phosphate for both the urea cycle and the first step in pyrimidine biosynthesis (Section 25.1). In mammals, two distinct isozymes of the enzyme are present. The carbamoyl phosphate synthetase used in pyrimidine biosynthesis differs in two important ways from its urea-cycle counterpart. First, this enzyme utilizes glutamine as a nitrogen source rather than NH_3. The side-chain amide of glutamine is hydrolyzed within one domain of the enzyme, and the ammonia generated moves through a tunnel in the enzyme to a second active site, where it reacts with carboxyphosphate. Second, this enzyme is part of a large polypeptide called CAD that comprises three distinct enzymes: *c*arbamoyl phosphate synthetase, *a*spartate transcarbamoylase, and *d*ihydroorotase. All three enzymes catalyze steps in pyrimidine biosynthesis (Section 25.1). Interestingly, the domain in which glutamine hydrolysis takes place is largely preserved in the urea-cycle enzyme, although that domain is catalytically inactive. This site binds *N*-acetylglutamate, an allosteric activator of the enzyme. *N*-Acetylglutamate is synthesized whenever the rate of amino acid catabolism increases and, consequently, signals that the ammonium ion generated in the catabolism of the free amino acids must be disposed of. *A catalytic site in one isozyme has been adapted to act as an allosteric site in another isozyme having a different physiological role.*

Can we find homologs for the other enzymes in the urea cycle? Ornithine transcarbamoylase is homologous to aspartate transcarbamoylase, which catalyzes the first step in pyrimidine biosynthesis, and the structures of their catalytic subunits are quite similar (Figure 23.19). Thus, two consecutive steps in the pyrimidine biosynthetic pathway were adapted for urea synthesis. The next step in the urea cycle is the addition of aspartate to citrulline to form argininosuccinate, and the subsequent step is the removal of fumarate. These two steps together accomplish the net addition of an amino group to citrulline to form arginine. Remarkably, these steps are analogous to two consecutive steps in the purine biosynthetic pathway (Section 25.2).



The enzymes that catalyze these steps are homologous to argininosuccinate synthetase and argininosuccinase, respectively. Thus, four of the five enzymes in the urea cycle were adapted from enzymes taking part in nucleotide biosynthesis. The remaining enzyme, arginase, appears to be an ancient enzyme found in all domains of life.

Inherited defects of the urea cycle cause hyperammonemia and can lead to brain damage

The synthesis of urea in the liver is the major route for the removal of NH_4^+. A blockage of carbamoyl phosphate synthesis or of any of the four steps of the urea cycle has devastating consequences because there is no

alternative pathway for the synthesis of urea. *All defects in the urea cycle lead to an elevated level of NH_4^+ in the blood (hyperammonemia).* Some of these genetic defects become evident a day or two after birth, when the afflicted infant becomes lethargic and vomits periodically. Coma and irreversible brain damage may soon follow. Why are high levels of NH_4^+ toxic? The answer to this question is not yet known. Recent work, however, suggests that NH_4^+ may inappropriately activate a sodium-potassium-chloride cotransporter. This activation disrupts the osmotic balance of the nerve cell, causing swelling that damages the cell and results in neurological disorders.

Ingenious strategies for coping with deficiencies in urea synthesis have been devised on the basis of a thorough understanding of the underlying biochemistry. Consider, for example, *argininosuccinase deficiency.* This defect can be partly bypassed by *providing a surplus of arginine in the diet and restricting the total protein intake.* In the liver, arginine is split into urea and ornithine, which then reacts with carbamoyl phosphate to form citrulline (Figure 23.20). This urea-cycle intermediate condenses with aspartate to yield argininosuccinate, which is then excreted. Note that two nitrogen atoms—one from carbamoyl phosphate and the other from aspartate—are eliminated from the body per molecule of arginine provided in the diet. In essence, *argininosuccinate substitutes for urea in carrying nitrogen out of the body.*

The treatment of *carbamoyl phosphate synthetase deficiency* or *ornithine transcarbamoylase deficiency* illustrates a different strategy for circumventing a metabolic block. Citrulline and argininosuccinate cannot be used to dispose of nitrogen atoms because their formation is impaired. Under these conditions, excess nitrogen accumulates in glycine and glutamine. The challenge then is to rid the body of the nitrogen accumulating in these two amino acids. That goal is accomplished by supplementing a protein-restricted diet with *large amounts of benzoate and phenylacetate.* Benzoate is activated to benzoyl CoA, which reacts with glycine to form hippurate (Figure 23.21). Likewise, phenylacetate is activated to phenylacetyl CoA, which reacts with glutamine to form phenylacetylglutamine. These conjugates substitute for urea in the disposal of nitrogen. Thus, *latent biochemical pathways can be activated to partly bypass a genetic defect.*

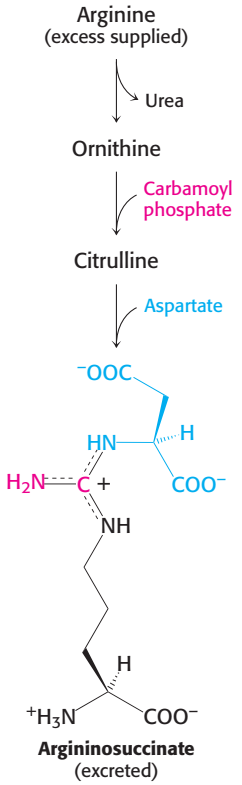


Figure 23.20 Treatment of argininosuccinase deficiency. Argininosuccinase deficiency can be managed by supplementing the diet with arginine. Nitrogen is excreted in the form of argininosuccinate.

Urea is not the only means of disposing of excess nitrogen

As stated earlier, most terrestrial vertebrates are ureotelic; they excrete excess nitrogen as urea. However, urea is not the only excretable form of

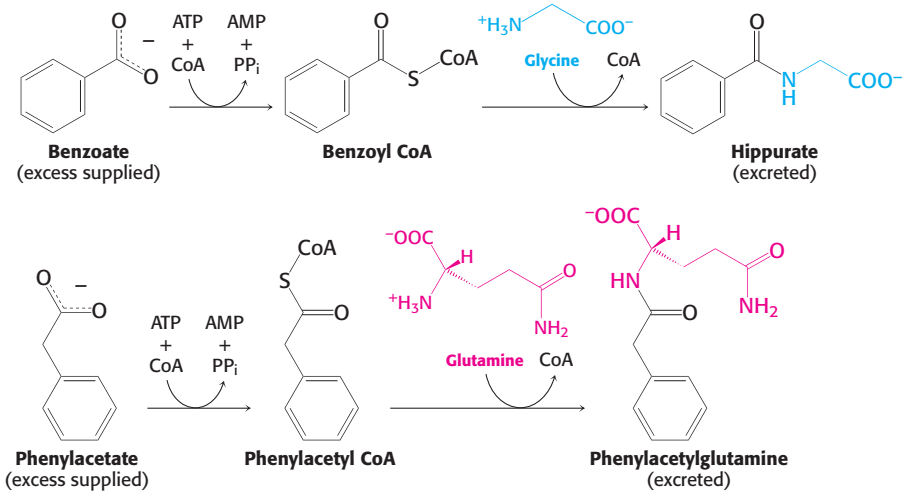


Figure 23.21 Treatment of carbamoyl phosphate synthetase and ornithine transcarbamoylase deficiencies. Both deficiencies can be treated by supplementing the diet with benzoate and phenylacetate. Nitrogen is excreted in the form of hippurate and phenylacetylglutamine.

nitrogen. *Ammoniotelic organisms, such as aquatic vertebrates and invertebrates, release nitrogen as* NH_4^+ and rely on the aqueous environment to dilute this toxic substance. Interestingly, lungfish, which are normally ammoniotelic, become ureotelic in time of drought, when they live out of the water.

Both ureotelic and ammoniotelic organisms depend on sufficient water, to varying degrees, for nitrogen excretion. *In contrast, uricotelic organisms, such as birds and reptiles, secrete nitrogen as the purine uric acid.* Uric acid is secreted as an almost solid slurry requiring little water. The secretion of uric acid also has the advantage of removing four atoms of nitrogen per molecule. The pathway for nitrogen excretion developed in the course of evolution clearly depends on the habitat of the organism.

23.5 Carbon Atoms of Degraded Amino Acids Emerge As Major Metabolic Intermediates

We now turn to the fates of the carbon skeletons of amino acids after the removal of the α-amino group. *The strategy of amino acid degradation is to transform the carbon skeletons into major metabolic intermediates that can be converted into glucose or oxidized by the citric acid cycle.* The conversion pathways range from extremely simple to quite complex. The carbon skeletons of the diverse set of 20 fundamental amino acids are funneled into only seven molecules: *pyruvate, acetyl CoA, acetoacetyl CoA, α-ketoglutarate, succinyl CoA, fumarate,* and *oxaloacetate.* We see here an example of the remarkable economy of metabolic conversions.

Amino acids that are degraded to acetyl CoA or acetoacetyl CoA are termed *ketogenic* amino acids because they can give rise to ketone bodies or fatty acids. Amino acids that are degraded to pyruvate, α-ketoglutarate, succinyl CoA, fumarate, or oxaloacetate are termed *glucogenic* amino acids. The net synthesis of glucose from these amino acids is feasible because these citric acid cycle intermediates and pyruvate can be converted into phosphoenolpyruvate and then into glucose (Section 16.3). Recall that mammals lack a pathway for the net synthesis of glucose from acetyl CoA or acetoacetyl CoA.

Of the basic set of 20 amino acids, only leucine and lysine are solely ketogenic (Figure 23.22). Isoleucine, phenylalanine, tryptophan, and

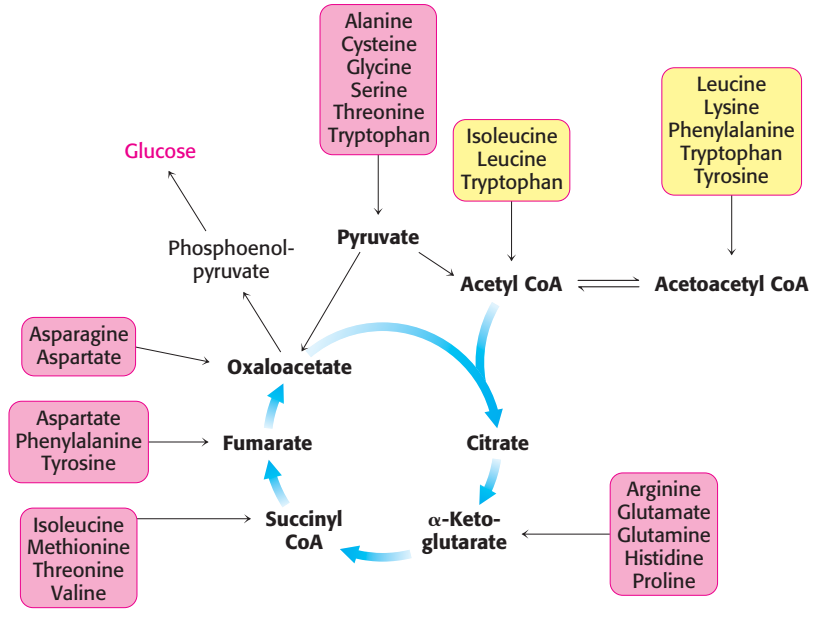


Figure 23.22 Fates of the carbon skeletons of amino acids. Glucogenic amino acids are shaded red, and ketogenic amino acids are shaded yellow. Several amino acids are both glucogenic and ketogenic.

tyrosine are both ketogenic and glucogenic. Some of their carbon atoms emerge in acetyl CoA or acetoacetyl CoA, whereas others appear in potential precursors of glucose. The other 14 amino acids are classed as solely glucogenic. This classification is not universally accepted, because different quantitative criteria are applied. Whether an amino acid is regarded as being glucogenic, ketogenic, or both depends partly on the eye of the beholder, although the majority of amino acid carbons clearly end up in glucose or glycogen. We will identify the degradation pathways by the entry point into metabolism.

Pyruvate is an entry point into metabolism for a number of amino acids

Pyruvate is the entry point of the three-carbon amino acids—alanine, serine, and cysteine—into the metabolic mainstream (Figure 23.23). The transamination of alanine directly yields pyruvate.

$$\text{Alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}$$

As mentioned earlier in the chapter, glutamate is then oxidatively deaminated, yielding NH_4^+ and regenerating α-ketoglutarate. The sum of these reactions is

$$\text{Alanine} + \text{NAD(P)}^+ + H_2O \longrightarrow \text{pyruvate} + NH_4^+ + \text{NAD(P)H} + H^+$$

Another simple reaction in the degradation of amino acids is the *deamination of serine to pyruvate* by *serine dehydratase* (p. 684).

$$\text{Serine} \longrightarrow \text{pyruvate} + NH_4^+$$

Cysteine can be converted into pyruvate by several pathways, with its sulfur atom emerging in H_2S, SCN^-, or SO_3^{2-}.

The carbon atoms of three other amino acids can be converted into pyruvate. *Glycine* can be converted into serine by the enzymatic addition of a hydroxymethyl group or it can be cleaved to give CO_2, NH_4^+, and an activated one-carbon unit. *Threonine* can give rise to pyruvate through the intermediate 2-amino-3-ketobutyrate. Three carbon atoms of *tryptophan* can emerge in alanine, which can be converted into pyruvate.

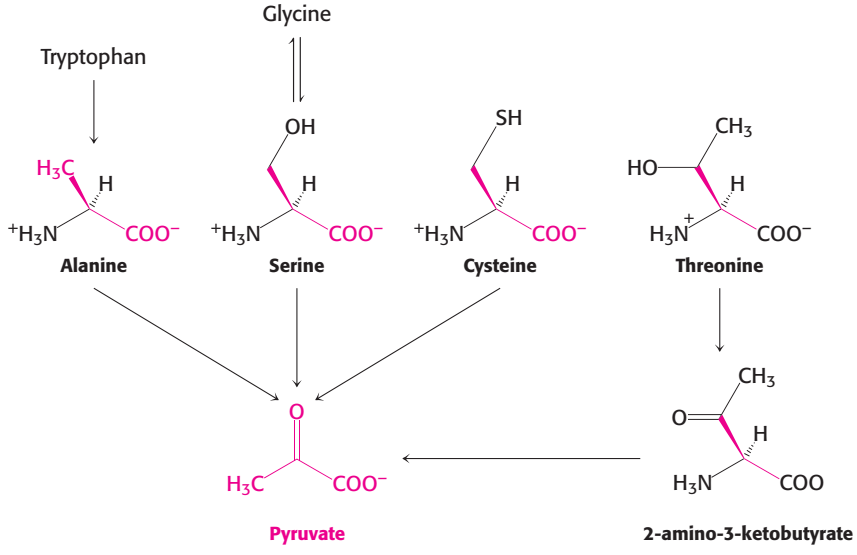


Figure 23.23 Pyruvate formation from amino acids. Pyruvate is the point of entry for alanine, serine, cysteine, glycine, threonine, and tryptophan.

Oxaloacetate is an entry point into metabolism for aspartate and asparagine

Aspartate and asparagine are converted into oxaloacetate, a citric acid cycle intermediate. *Aspartate,* a four-carbon amino acid, is directly *transaminated to oxaloacetate.*

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

Asparagine is hydrolyzed by *asparaginase* to NH_4^+ and aspartate, which is then transaminated.

Recall that aspartate can also be converted into *fumarate* by the urea cycle (see Figure 23.18). Fumarate is a point of entry for half the carbon atoms of tyrosine and phenylalanine, as will be discussed shortly.

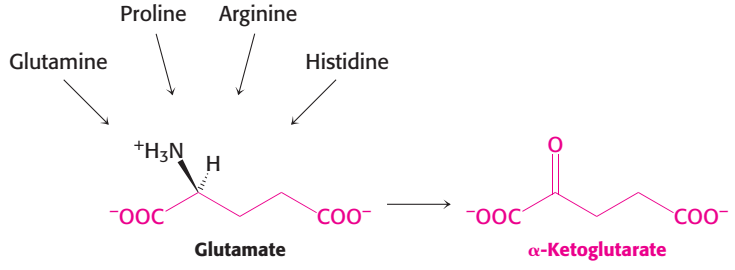


Figure 23.24 α-Ketoglutarate formation from amino acids. α-Ketoglutarate is the point of entry of several five-carbon amino acids that are first converted into glutamate.

Alpha-ketoglutarate is an entry point into metabolism for five-carbon amino acids

The carbon skeletons of several five-carbon amino acids enter the citric acid cycle at *α-ketoglutarate.* These amino acids are first converted into *glutamate,* which is then oxidatively deaminated by glutamate dehydrogenase to yield α-ketoglutarate (Figure 23.24).

Histidine is converted into 4-imidazolone 5-propionate (Figure 23.25). The amide bond in the ring of this intermediate is hydrolyzed to the *N*-formimino derivative of glutamate, which is then converted into glutamate by the transfer of its formimino group to tetrahydrofolate, a carrier of activated one-carbon units (see Figure 24.9).

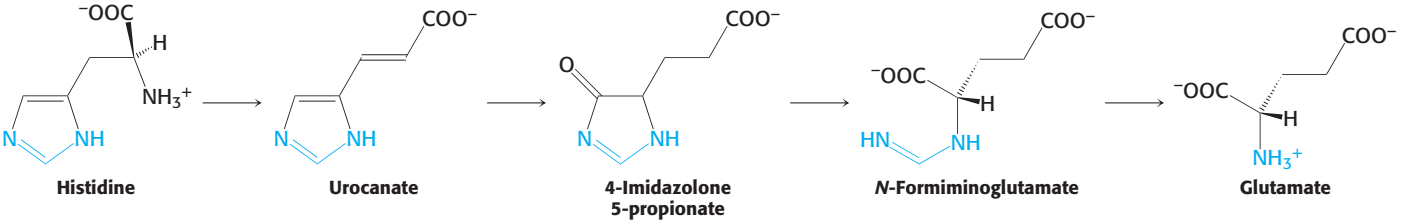


Figure 23.25 Histidine degradation. Conversion of histidine into glutamate.

Glutamine is hydrolyzed to glutamate and NH_4^+ by *glutaminase. Proline* and *arginine* are each converted into glutamate γ-semialdehyde, which is then oxidized to glutamate (Figure 23.26).

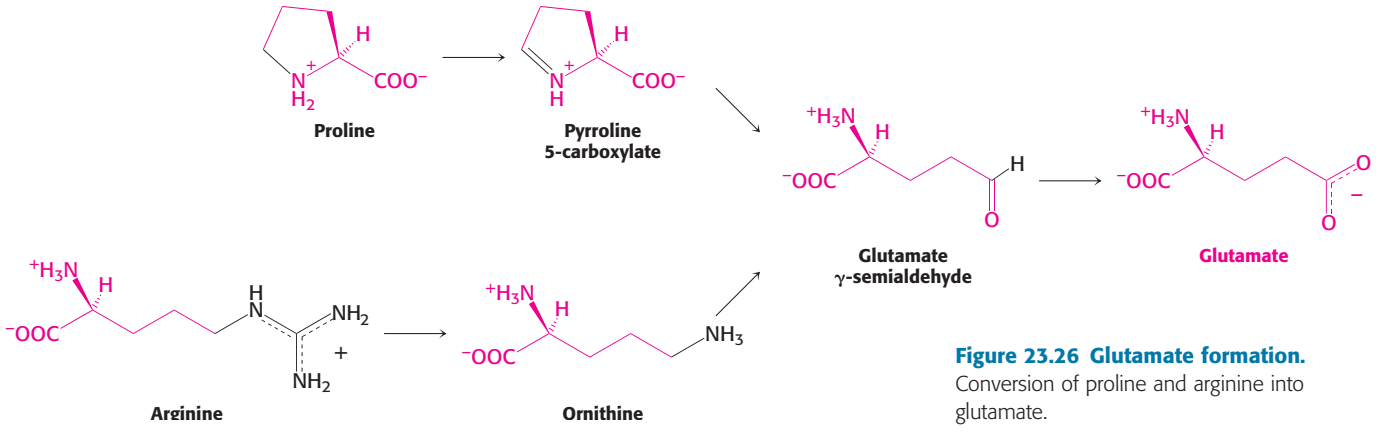


Figure 23.26 Glutamate formation. Conversion of proline and arginine into glutamate.

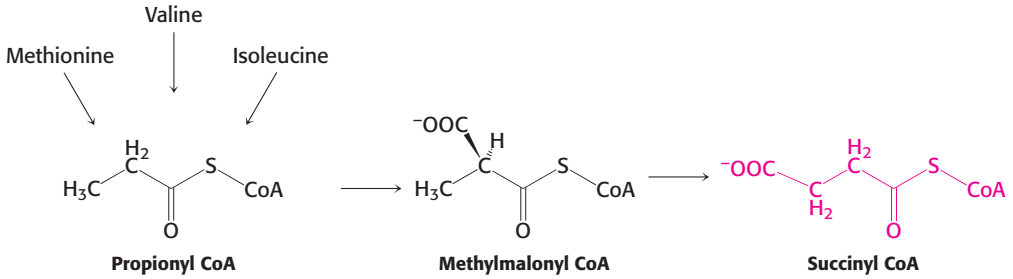


Figure 23.27 Succinyl CoA formation. Conversion of methionine, isoleucine, and valine into succinyl CoA.

Succinyl coenzyme A is a point of entry for several nonpolar amino acids

Succinyl CoA is a point of entry for some of the carbon atoms of methionine, isoleucine, and valine. Propionyl CoA and then methylmalonyl CoA are intermediates in the breakdown of these three nonpolar amino acids (Figure 23.27). The mechanism for the interconversion of propionyl CoA and methylmalonyl CoA was presented in Section 22.3. This pathway from propionyl CoA to succinyl CoA is also used in the oxidation of fatty acids that have an odd number of carbon atoms (Section 22.3).

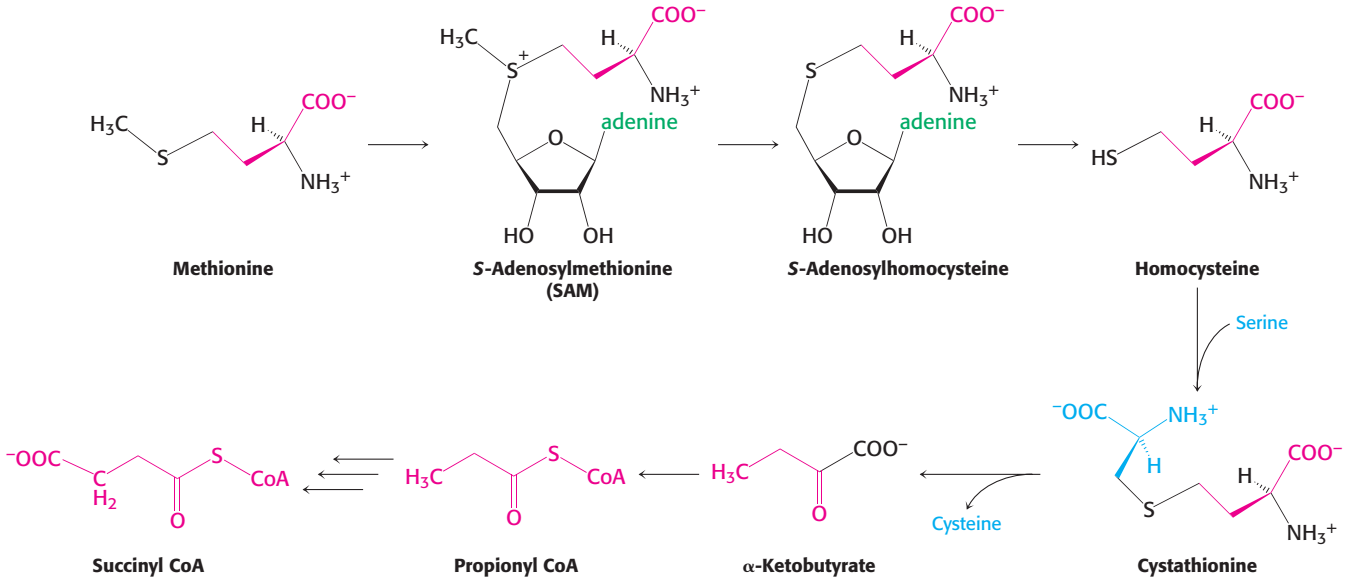
Methionine degradation requires the formation of a key methyl donor, *S*-adenosylmethionine

Methionine is converted into succinyl CoA in nine steps (Figure 23.28). The first step is the adenylation of methionine to form S-*adenosylmethionine* (SAM), a common methyl donor in the cell (Section 24.2). Loss of the methyl and adenosyl groups yields homocysteine, which is eventually processed to *α-ketobutyrate*. This α-ketoacid is oxidatively decarboxylated by the α-ketoacid dehydrogenase complex to *propionyl CoA*, which is processed to *succinyl CoA*, as described in Section 22.3.

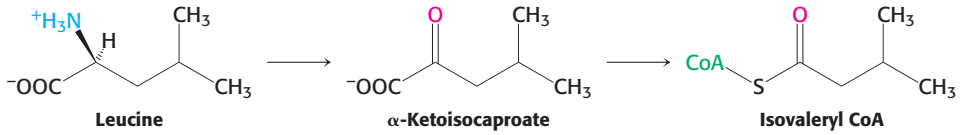
The branched-chain amino acids yield acetyl CoA, acetoacetate, or propionyl CoA

The branched-chain amino acids are degraded by reactions that we have already encountered in the citric acid cycle and fatty acid oxidation. Leucine is transaminated to the corresponding α-ketoacid, *α-ketoisocaproate*. This

Figure 23.28 Methionine metabolism. The pathway for the conversion of methionine into succinyl CoA. *S*-Adenosylmethionine, formed along this pathway, is an important molecule for transferring methyl groups.

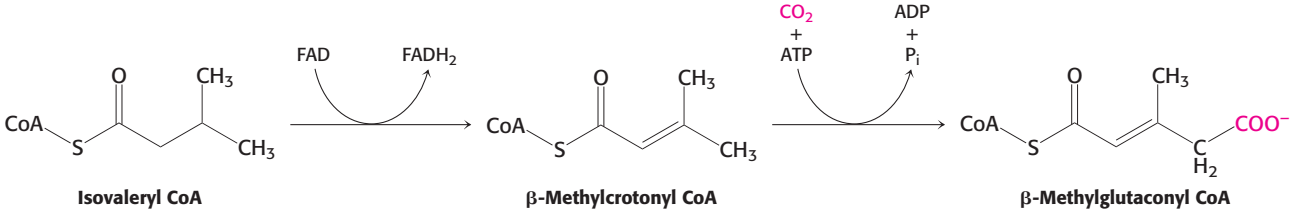


α-ketoacid is *oxidatively decarboxylated to isovaleryl CoA* by the *branched-chain α-ketoacid dehydrogenase complex*.

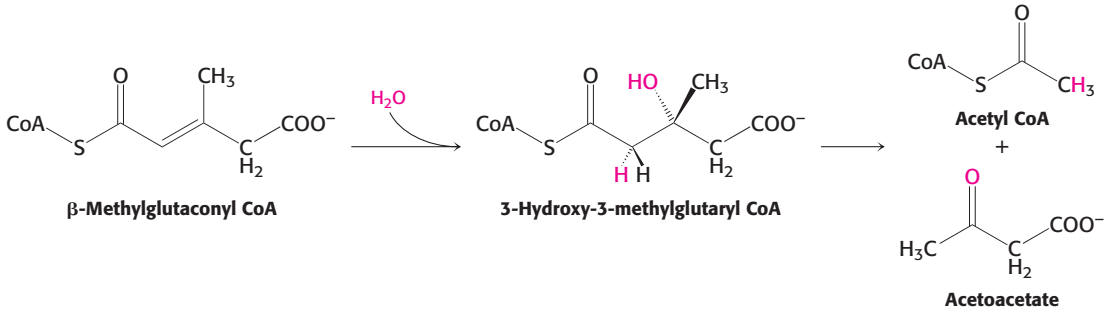


The α-ketoacids of valine and isoleucine, the other two branched-chain aliphatic amino acids, also are substrates (as is α-ketobutyrate derived from methionine). The oxidative decarboxylation of these α-ketoacids is analogous to that of pyruvate to acetyl CoA and of α-ketoglutarate to succinyl CoA. The branched-chain α-ketoacid dehydrogenase, a multienzyme complex, is a homolog of pyruvate dehydrogenase (Section 17.1) and α-ketoglutarate dehydrogenase (Section 17.2). Indeed, the E3 components of these enzymes, which regenerate the oxidized form of lipoamide, are identical.

The isovaleryl CoA derived from leucine is *dehydrogenated* to yield *β-methylcrotonyl CoA*. This oxidation is catalyzed by *isovaleryl CoA dehydrogenase*. The hydrogen acceptor is FAD, as in the analogous reaction in fatty acid oxidation that is catalyzed by acyl CoA dehydrogenase. *β-Methylglutaconyl CoA* is then formed by the *carboxylation* of β-methylcrotonyl CoA at the expense of the hydrolysis of a molecule of ATP. As might be expected, the carboxylation mechanism of β-methylcrotonyl CoA carboxylase is similar to that of pyruvate carboxylase and acetyl CoA carboxylase.



β-Methylglutaconyl CoA is then *hydrated* to form *3-hydroxy-3-methylglutaryl CoA*, which is cleaved into *acetyl CoA* and *acetoacetate*. This reaction has already been discussed in regard to the formation of ketone bodies from fatty acids (Section 22.3).



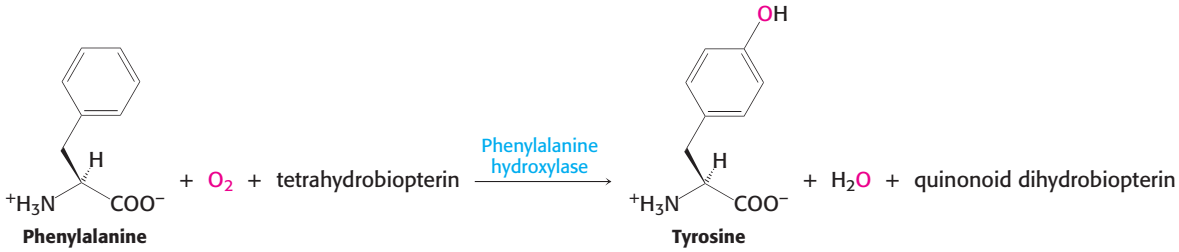
The degradative pathways of valine and isoleucine resemble that of leucine. After transamination and oxidative decarboxylation to yield a CoA derivative, the subsequent reactions are like those of fatty acid oxidation. Isoleucine yields acetyl CoA and propionyl CoA, whereas valine yields CO_2 and propionyl CoA. The degradation of leucine, valine, and isoleucine

validate a point made earlier (Chapter 15): the number of reactions in metabolism is large, but the number of *kinds* of reactions is relatively small. The degradation of leucine, valine, and isoleucine provides a striking illustration of the underlying simplicity and elegance of metabolism.

Oxygenases are required for the degradation of aromatic amino acids

The degradation of the aromatic amino acids yields the common intermediates acetoacetate, fumarate, and pyruvate. The degradation pathway is not as straightforward as that of the amino acids previously discussed. For the aromatic amino acids, *molecular oxygen is used to break an aromatic ring.*

The degradation of phenylalanine begins with its hydroxylation to tyrosine, a reaction catalyzed by *phenylalanine hydroxylase.* This enzyme is called a *monooxygenase* (or *mixed-function oxygenase*) because *one atom of* O_2 appears in the product and the other in H_2O.



The reductant here is *tetrahydrobiopterin,* an electron carrier that has not been previously discussed and is derived from the cofactor *biopterin*. Because biopterin is synthesized in the body, it is not a vitamin. Tetrahydrobiopterin is initially formed by the reduction of dihydrobiopterin by NADPH in a reaction catalyzed by *dihydrofolate reductase* (Figure 23.29). The quinonoid form of dihydrobiopterin is produced in the hydroxylation of phenylalanine. It is reduced back to tetrahydrobiopterin by NADPH in a reaction catalyzed by *dihydropteridine reductase.* The sum of the reactions catalyzed by phenylalanine hydroxylase and dihydropteridine reductase is

$$\text{Phenylalanine} + O_2 + \text{NADPH} + H^+ \longrightarrow \text{tyrosine} + \text{NADP}^+ + H_2O$$

Note that these reactions can also be used to synthesize tyrosine from phenylalanine.

The next step in the degradation of phenylalanine and tyrosine is the transamination of tyrosine to p-*hydroxyphenylpyruvate* (Figure 23.30). This α-ketoacid then reacts with O_2 to form *homogentisate.* The enzyme catalyzing

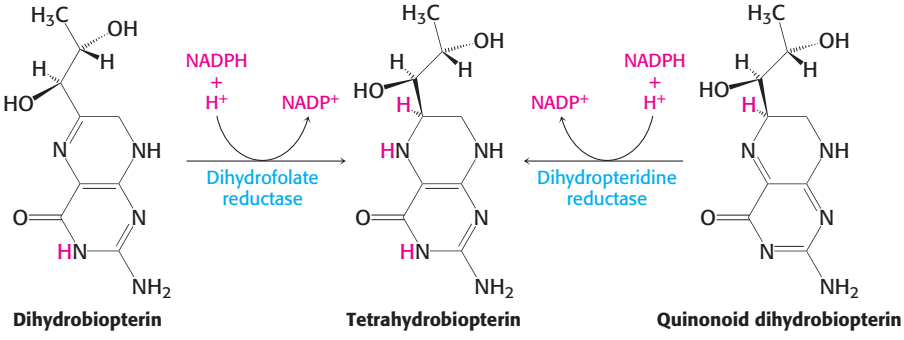


Figure 23.29 Formation of tetrahydrobiopterin, an important electron carrier.
Tetrahydrobiopterin can be formed by the reduction of either of two forms of dihydrobiopterin.

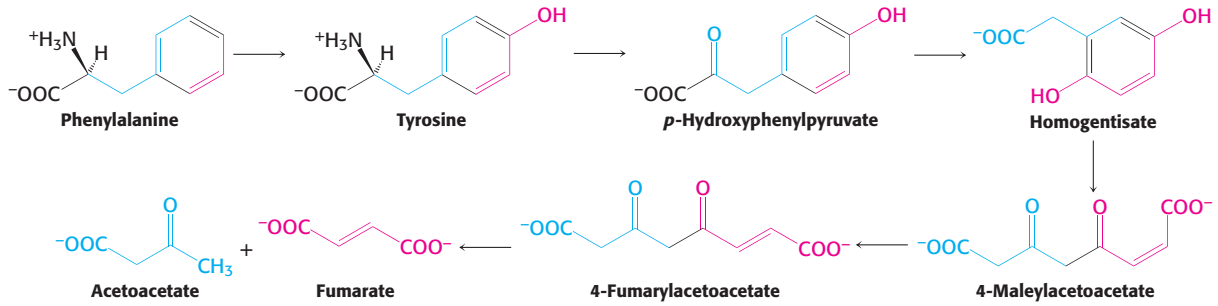


Figure 23.30 Phenylalanine and tyrosine degradation. The pathway for the conversion of phenylalanine into acetoacetate and fumarate.

this complex reaction, p-*hydroxyphenylpyruvate hydroxylase,* is called a *dioxygenase* because *both atoms of* O_2 become incorporated into the product, one on the ring and one in the carboxyl group. The aromatic ring of homogentisate is then cleaved by O_2, which yields 4-maleylacetoacetate. This reaction is catalyzed by *homogentisate oxidase,* another dioxygenase. 4-Maleylacetoacetate is then isomerized to *4-fumarylacetoacetate* by an enzyme that uses glutathione as a cofactor. Finally, 4-fumarylacetoacetate is hydrolyzed to *fumarate* and *acetoacetate.*

Tryptophan degradation requires several oxygenases (Figure 23.31). Tryptophan 2,3-dioxygenase cleaves the pyrrole ring, and kynureinine 3-monooxygenase hydroxylates the remaining benzene ring, a reaction similar to the hydroxylation of phenylalanine to form tyrosine. Alanine is removed and the 3-hydroxyanthranilate is cleaved by another dioxygenase and subsequently processed to acetoacetyl CoA. *Nearly all cleavages of aromatic rings in biological systems are catalyzed by dioxygenases,* a class of enzymes discovered by Osamu Hayaishi. The active sites of these enzymes contain iron that is not part of heme or an iron–sulfur cluster.

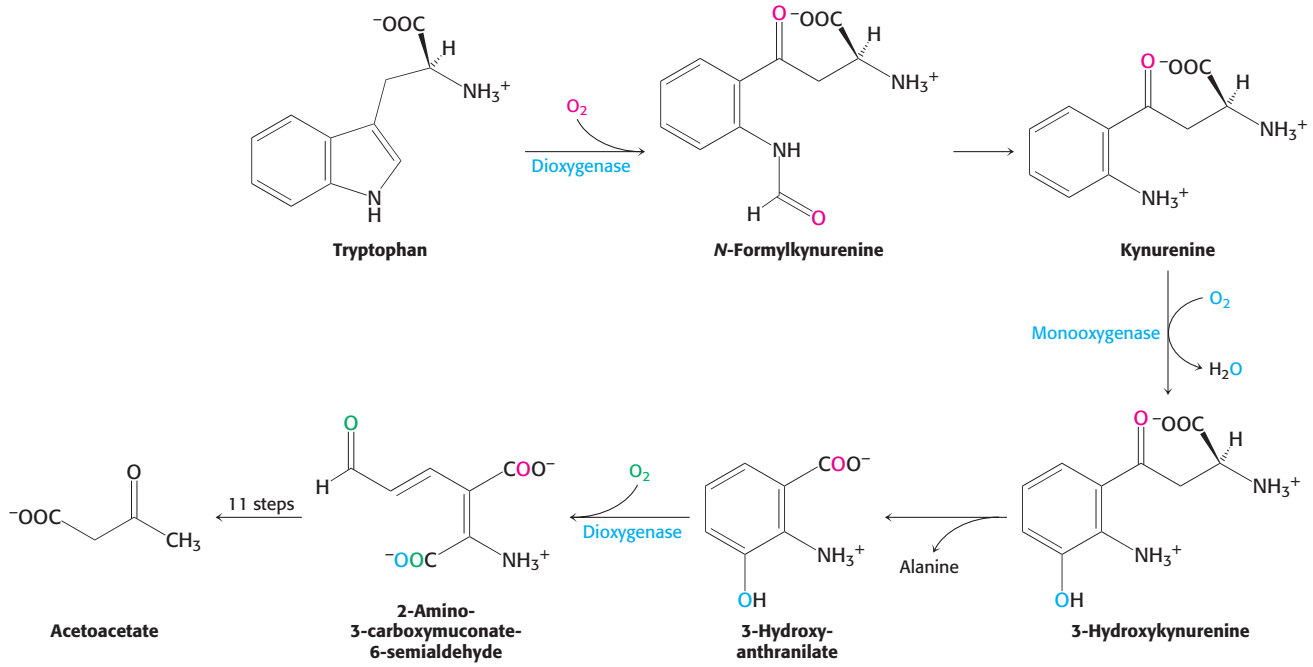
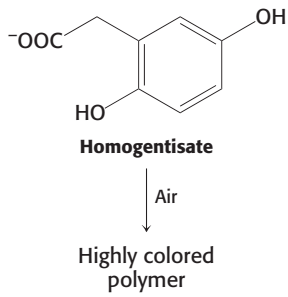


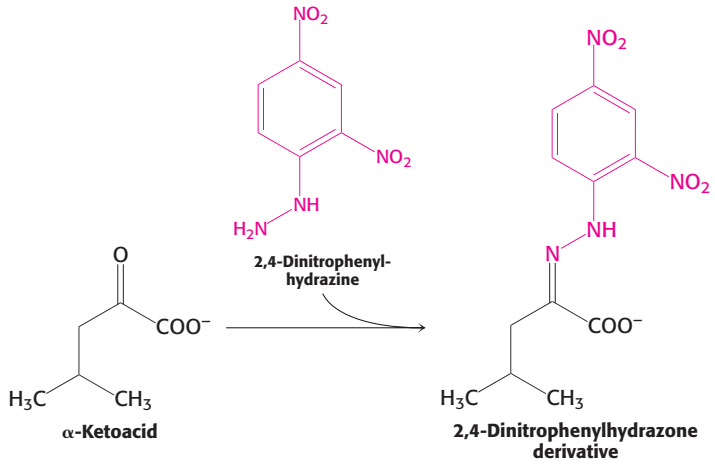
Figure 23.31 Tryptophan degradation. The pathway for the conversion of tryptophan into alanine and acetoacetate.

23.6 Inborn Errors of Metabolism Can Disrupt Amino Acid Degradation

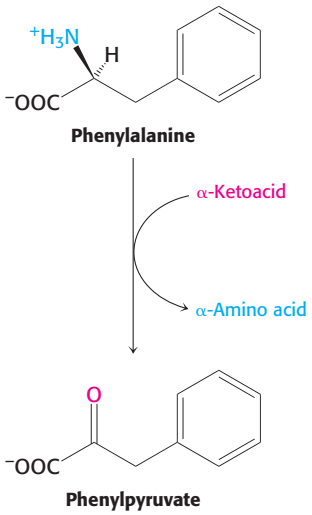
Errors in amino acid metabolism provided some of the first examples of biochemical defects linked to pathological conditions. For instance, *alcaptonuria* is an inherited metabolic disorder caused by the absence of homogentisate oxidase. In 1902, Archibald Garrod showed that alcaptonuria is transmitted as a single recessive Mendelian trait. Furthermore, he recognized that homogentisate is a normal intermediate in the degradation of phenylalanine and tyrosine (see Figure 23.30) and that it accumulates in alcaptonuria because its degradation is blocked. He concluded that "the splitting of the benzene ring in normal metabolism is the work of a special enzyme, that in congenital alcaptonuria this enzyme is wanting." Homogentisate accumulates and is excreted in the urine, which turns dark on standing as homogentisate is oxidized and polymerized to a melanin-like substance.



Although alcaptonuria is a relatively harmless condition, such is not the case with other errors in amino acid metabolism. In *maple syrup urine disease,* the oxidative decarboxylation of α-ketoacids derived from valine, isoleucine, and leucine is blocked because the branched-chain dehydrogenase is missing or defective. Hence, the levels of these α-ketoacids and the branched-chain amino acids that give rise to them are markedly elevated in both blood and urine. The urine of patients has the odor of maple syrup—hence the name of the disease (also called *branched-chain ketoaciduria*). Maple syrup urine disease usually leads to mental and physical retardation unless the patient is placed on a diet low in valine, isoleucine, and leucine early in life. The disease can be readily detected in newborns by screening urine samples with 2,4-dinitrophenylhydrazine, which reacts with α-ketoacids to form 2,4-dinitrophenylhydrazone derivatives. A definitive diagnosis can be made by mass spectrometry.



Phenylketonuria is perhaps the best known of the diseases of amino acid metabolism. Phenylketonuria is caused by an *absence or deficiency of phenylalanine hydroxylase* or, more rarely, of its tetrahydrobiopterin cofactor. *Phenylalanine accumulates in all body fluids because it cannot be converted into tyrosine.* Normally, three-quarters of phenylalanine molecules are converted into tyrosine, and the other quarter become incorporated into proteins. Because the major outflow pathway is blocked in phenylketonuria, the blood level of phenylalanine is typically at least 20-fold as high as in normal people. Minor fates of phenylalanine in normal people, such as the



formation of phenylpyruvate, become major fates in phenylketonurics. Indeed, the initial description of phenylketonuria in 1934 was made by observing the reaction of phenylpyruvate in the urine of phenylketonurics with $FeCl_3$, which turns the urine olive green.

Almost all untreated phenylketonurics are severely mentally retarded. In fact, about 1% of patients in mental institutions have phenylketonuria. The brain weight of these people is below normal, myelination of their nerves is defective, and their reflexes are hyperactive. The life expectancy of untreated phenylketonurics is drastically shortened. Half die by age 20 and three-quarters by age 30. *The biochemical basis of their mental retardation is an enigma.*

Phenylketonurics appear normal at birth but are severely defective by age 1 if untreated. The therapy for phenylketonuria is a *low-phenylalanine diet*, supplemented with tyrosine because tyrosine is normally synthesized from phenylalanine. The aim is to provide just enough phenylalanine to meet the needs for growth and replacement. Proteins that have a low content of phenylalanine, such as casein from milk, are hydrolyzed and phenylalanine is removed by adsorption. A low-phenylalanine diet must be started very soon after birth to prevent irreversible brain damage. In one study, the average IQ of phenylketonurics treated within a few weeks after birth was 93; a control group treated starting at age 1 had an average IQ of 53.

Early diagnosis of phenylketonuria is essential and has been accomplished by mass screening programs. The phenylalanine level in the blood is the preferred diagnostic criterion because it is more sensitive and reliable than the $FeCl_3$ test. Prenatal diagnosis of phenylketonuria with DNA probes has become feasible because the gene has been cloned and the exact locations of many mutations have been discovered in the protein. Interestingly, whereas some mutations lower the activity of the enzyme, others decrease the enzyme concentration instead. These latter mutations lead to degradation of the enzyme, at least in part by the ubiquitin–proteasome pathway (Section 23.2).

The incidence of phenylketonuria is about 1 in 20,000 newborns. The disease is inherited as an *autosomal recessive*. Heterozygotes, who make up about 1.5% of a typical population, appear normal. Carriers of the phenylketonuria gene have a reduced level of phenylalanine hydroxylase, as indicated by an increased level of phenylalanine in the blood. However, this criterion is not absolute, because the blood levels of phenylalanine in carriers and normal people overlap to some extent. The measurement of the kinetics of the disappearance of intravenously administered phenylalanine is a more definitive test for the carrier state. It should be noted that a high blood level of phenylalanine in a pregnant woman can result in abnormal development of the fetus. This is a striking example of maternal–fetal relationships at the molecular level. Table 23.4 lists some other diseases of amino acid metabolism.

Table 23.4 Inborn errors of amino acid metabolism

Disease	Enzyme deficiency	Symptoms
Citrullinema	Arginosuccinate lyase	Lethargy, seizures, reduced muscle tension
Tyrosinemia	Various enzymes of tyrosine degradation	Weakness, self-mutilation, liver damage, mental retardation
Albinism	Tyrosinase	Absence of pigmentation
Homocystinuria	Cystathionine β-synthase	Scoliosis, muscle weakness, mental retardation, thin blond hair
Hyperlysinemia	α-Aminoadipic semialdehyde dehydrogenase	Seizures, mental retardation, lack of muscle tone, ataxia

Summary

23.1 Proteins Are Degraded to Amino Acids

Dietary protein is digested in the intestine, producing amino acids that are transported throughout the body. Cellular proteins are degraded at widely variable rates, ranging from minutes to the life of the organism.

23.2 Protein Turnover Is Tightly Regulated

The turnover of cellular proteins is a regulated process requiring complex enzyme systems. Proteins to be degraded are conjugated with ubiquitin, a small conserved protein, in a reaction driven by ATP hydrolysis. The ubiquitin-conjugating system is composed of three distinct enzymes. A large, barrel-shaped complex called the proteasome digests the ubiquitinated proteins. The proteasome also requires ATP hydrolysis to function. The resulting amino acids provide a source of precursors for protein, nucleotide bases, and other nitrogenous compounds.

23.3 The First Step in Amino Acid Degradation Is the Removal of Nitrogen

Surplus amino acids are used as building blocks and as metabolic fuel. The first step in their degradation is the removal of their α-amino groups by transamination to α-ketoacids. Pyridoxal phosphate is the coenzyme in all aminotransferases and in many other enzymes catalyzing amino acid transformations. The α-amino group funnels into α-ketoglutarate to form glutamate, which is then oxidatively deaminated by glutamate dehydrogenase to give NH_4^+ and α-ketoglutarate. NAD^+ or $NADP^+$ is the electron acceptor in this reaction.

23.4 Ammonium Ion Is Converted into Urea in Most Terrestrial Vertebrates

The first step in the synthesis of urea is the formation of carbamoyl phosphate, which is synthesized from HCO_3^-, NH_3, and two molecules of ATP by carbamoyl phosphate synthetase. Ornithine is then carbamoylated to citrulline by ornithine transcarbamoylase. These two reactions take place in mitochondria. Citrulline leaves the mitochondrion and condenses with aspartate to form argininosuccinate, which is cleaved into arginine and fumarate. The other nitrogen atom of urea comes from aspartate. Urea is formed by the hydrolysis of arginine, which also regenerates ornithine.

23.5 Carbon Atoms of Degraded Amino Acids Emerge as Major Metabolic Intermediates

The carbon atoms of degraded amino acids are converted into pyruvate, acetyl CoA, acetoacetate, or an intermediate of the citric acid cycle. Most amino acids are solely glucogenic, two are solely ketogenic, and a few are both ketogenic and glucogenic. Alanine, serine, cysteine, glycine, threonine, and tryptophan are degraded to pyruvate. Asparagine and aspartate are converted into oxaloacetate. α-Ketoglutarate is the point of entry for glutamate and four amino acids (glutamine, histidine, proline, and arginine) that can be converted into glutamate. Succinyl CoA is the point of entry for some of the carbon atoms of three amino acids (methionine, isoleucine, and valine) that are degraded through the intermediate methylmalonyl CoA. Leucine is degraded to acetoacetate and acetyl CoA. The breakdown of valine and isoleucine is like that of leucine. Their α-ketoacid derivatives are oxidatively decarboxylated by the branched-chain α-ketoacid dehydrogenase.

The rings of aromatic amino acids are degraded by oxygenases. Phenylalanine hydroxylase, a monooxygenase, uses tetrahydrobiop-

terin as the reductant. One of the oxygen atoms of O_2 emerges in tyrosine and the other in water. Subsequent steps in the degradation of these aromatic amino acids are catalyzed by dioxygenases, which catalyze the insertion of both atoms of O_2 into organic products. Four of the carbon atoms of phenylalanine and tyrosine are converted into fumarate, and four emerge in acetoacetate.

23.6 Inborn Errors of Metabolism Can Disrupt Amino Acid Degradation

Errors in amino acid metabolism were sources of some of the first insights into the correlation between pathology and biochemistry. Phenylketonuria is the best known of the many hereditary errors of amino acid metabolism. This condition results from the accumulation of high levels of phenylalanine in the body fluids. By unknown mechanisms, this accumulation leads to mental retardation unless the afflicted are placed on low-phenylalanine diets immediately after birth.

Key Terms

ubiquitin (p. 675)
degron (p. 676)
proteasome (p. 677)
aminotransferase (transaminase) (p. 680)
glutamate dehydrogenase (p. 680)
pyridoxal phosphate (PLP) (p. 681)
pyridoxamine phosphate (PMP) (p. 682)
glucose–alanine cycle (p. 685)
urea cycle (p. 685)
carbamoyl phosphate synthetase (p. 686)
N-acetylglutamate (p. 686)
ketogenic amino acid (p. 690)
glucogenic amino acid (p. 690)
biopterin (p. 695)
phenylketonuria (p. 697)

Problems

1. *Getting exposure.* Proteins are denatured by acid in the stomach. This denaturation makes them better substrates for proteolysis. Explain why this is the case.

2. *Targeting for destruction.* What are the steps required to attach ubiquitin to a target protein?

3. *Not the dating service.* Match the description on the right with the term on the left.

a. Pepsin
b. N-terminal rule
c. Ubiquitin
d. PEST sequence
e. Threonine nucleophiles
f. ATP-dependent protein unfolding
g. Proteasome
h. Ubiquitin-activating enzyme
i. Ubiquitin-conjugating enzyme
j. Ubiquitin-ligase

1. Requires an adenylate intermediate
2. Marks a protein for destruction
3. 19S regulatory subunit
4. Determines half-life of a protein
5. 20S core
6. Substrate for ligase
7. Stomach proteolytic enzyme
8. Recognizes protein to be degraded
9. Protein degrading machine
10. Pro-Glu-Ser-Thr

4. *Wasted energy?* Protein hydrolysis is an exergonic process, yet the 26S proteasome is dependent on ATP hydrolysis for activity.

(a) Although the exact function of the ATPase activity is not known, suggest some likely functions.

(b) Small peptides can be hydrolyzed without the expenditure of ATP. How does this information concur with your answer to part *a*?

5. *Keto counterparts.* Name the α-ketoacid that is formed by the transamination of each of the following amino acids:

(a) Alanine
(b) Aspartate
(c) Glutamate
(d) Leucine
(e) Phenylalanine
(f) Tyrosine

6. *A versatile building block.* (a) Write a balanced equation for the conversion of aspartate into glucose through the intermediate oxaloacetate. Which coenzymes participate in this transformation? (b) Write a balanced equation for the conversion of aspartate into oxaloacetate through the intermediate fumarate.

7. *The benefits of specialization.* The archaeal proteasome contains 14 identical active β subunits, whereas the eukaryotic proteasome has 7 distinct β subunits. What are the

potential benefits of having several distinct active subunits?

8. *Propose a structure.* The 19S subunit of the proteasome contains six subunits that are members of the AAA ATPase family. Other members of this large family are associated into homohexamers with sixfold symmetry. Propose a structure for the AAA ATPases within the 19S proteasome. How might you test and refine your prediction?

9. *Effective electron sinks.* Pyridoxal phosphate stabilizes carbanionic intermediates by serving as an electron sink. Which other prosthetic group catalyzes reactions in this way?

10. *Cooperation.* How do aminotransferases and glutamate dehydrogenase cooperate in the metabolism of the amino group of amino acids?

11. *Taking away the nitrogen.* What amino acids yield citric acid cycle components and glycolysis intermediates when deaminated?

12. *One reaction only.* What amino acids can be deaminated directly?

13. *Useful products.* What are the common features of the breakdown products of the carbon skeletons of amino acids?

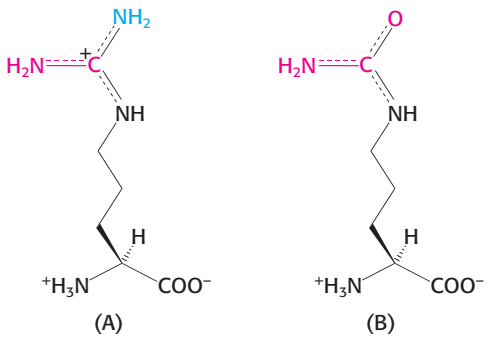
14. *Helping hand.* Propose a role for the positively charged guanidinium nitrogen atom in the cleavage of argininosuccinate into arginine and fumarate.

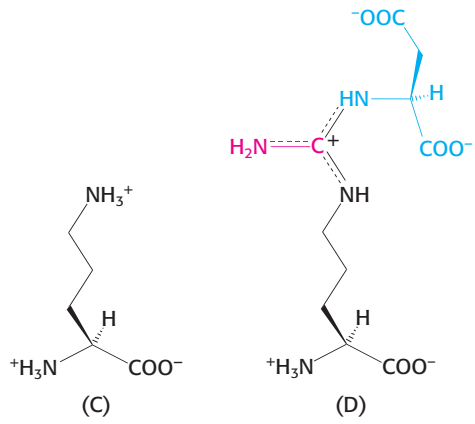
15. *Nitrogen sources.* What are the immediate biochemical sources for the two nitrogen atoms in urea?

16. *Counterparts.* Match the biochemical on the right with the property on the left.

a. Formed from NH_4^+	1. Aspartate
b. Hydrolyzed to yield urea	2. Urea
c. A second source of nitrogen	3. Ornithine
d. Reacts with aspartate	4. Carbamoyl phosphate
e. Cleavage yields fumarate	5. Arginine
f. Accepts the first nitrogen	6. Citrulline
g. Final product	7. Arginosuccinate

17. *Line up.* Identify structures A–D, and place them in the order that they appear in the urea cycle.

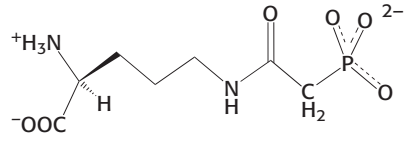




18. *Completing the cycle.* Four high-transfer-potential phosphoryl groups are consumed in the synthesis of urea according to the stoichiometry given on page 687. In this reaction, aspartate is converted into fumarate. Suppose that fumarate is converted into oxaloacetate. What is the resulting stoichiometry of urea synthesis? How many high-transfer-potential phosphoryl groups are spent?

19. *A good bet.* A friend bets you a bazillion dollars that you can't prove that the urea cycle is linked to the citric acid cycle and other metabolic pathways. Can you collect?

20. *Inhibitor design.* Compound A has been synthesized as a potential inhibitor of a urea-cycle enzyme. Which enzyme do you think compound A might inhibit?



Compound A

21. *Ammonia toxicity.* Glutamate is an important neurotransmitter whose levels must be carefully regulated in the brain. Explain how a high concentration of ammonia might disrupt this regulation. How might a high concentration of ammonia alter the citric acid cycle?

22. *A precise diagnosis.* The urine of an infant gives a positive reaction with 2,4-dinitrophenylhydrazine. Mass spectrometry shows abnormally high blood levels of pyruvate, α-ketoglutarate, and the α-ketoacids of valine, isoleucine, and leucine. Identify a likely molecular defect and propose a definitive test of your diagnosis.

23. *Therapeutic design.* How would you treat an infant who is deficient in argininosuccinate synthetase? Which molecules would carry nitrogen out of the body?

24. *Damaged liver.* As we will see later (Chapter 27), liver damage (cirrhosis) often results in ammonia poisoning. Explain why this is the case.

25. *Argininosuccinic aciduria.* Argininosuccinic aciduria is a condition that results when the urea-cycle enzyme argininosuccinase is deficient. Argininosuccinate is present in the blood and urine. Suggest how this condition might be treated while still removing nitrogen from the body.

26. *Sweet hazard.* Why should phenylketonurics avoid using aspartame, an artificial sweetener? (Hint: Aspartame is L-aspartyl-L-phenylalanine methyl ester.)

27. *Déjà vu. N*-Acetylglutamate is required as a cofactor in the synthesis of carbamoyl phosphate. How might *N*-acetylglutamate be synthesized from glutamate?

28. *Negative nitrogen balance.* A deficiency of even one amino acid results in a negative nitrogen balance. In this state, more protein is degraded than is synthesized, and so more nitrogen is excreted than is ingested. Why would protein be degraded if one amino acid were missing?

29. *Precursors.* Differentiate between ketogenic amino acids and glucogenic amino acids.

30. *Closely related.* Pyruvate dehydrogenase complex and α-ketoglutarate dehydrogenase complex are huge enzymes consisting of three discrete enzymatic activities. Which amino acids require a related enzyme complex, and what is the name of the enzyme?

31. *Supply lines.* The carbon skeletons of the 20 common amino acids can be degraded into a limited number of end products. What are the end products and in what metabolic pathway are they commonly found?

Mechanism Problems

32. *Serine dehydratase.* Write out a complete mechanism for the conversion of serine into aminoacrylate catalyzed by serine dehydratase.

33. *Serine racemase.* The nervous system contains a substantial amount of D-serine, which is generated from L-serine by serine racemase, a PLP-dependent enzyme. Propose a mechanism for this reaction. What is the equilibrium constant for the reaction L-serine $\rightleftharpoons$ D-serine?

Chapter Integration Problems

34. *Double duty.* Degradation signals are commonly located in protein regions that also facilitate protein–protein interactions. Explain why this coexistence of two functions in the same domain might be useful.

35. *Fuel choice.* Within a few days after a fast begins, nitrogen excretion accelerates to a higher-than-normal level. After a few weeks, the rate of nitrogen excretion falls to a lower level and continues at this low rate. However, after the fat stores have been depleted, nitrogen excretion rises to a high level.

(a) What events trigger the initial surge of nitrogen excretion?

(b) Why does nitrogen excretion fall after several weeks of fasting?

(c) Explain the increase in nitrogen excretion when the lipid stores have been depleted.

36. *Isoleucine degradation.* Isoleucine is degraded to acetyl CoA and succinyl CoA. Suggest a plausible reaction sequence, based on reactions discussed in the text, for this degradation pathway.

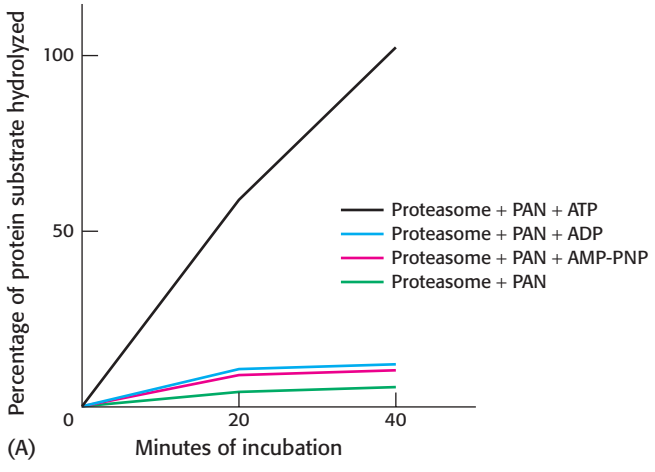
37. *Many roles.* Pyridoxal phosphate is an important coenzyme in transamination reactions. We have seen this coenzyme before, in glycogen metabolism. Which enzyme in glycogen metabolism requires pyridoxal phosphate and what role does the coenzyme play in this enzyme?

38. *Enough cycles to have a race.* The glucose–alanine cycle is reminiscent of the Cori cycle, but the glucose–alanine cycle can be said to be more energy efficient. Explain why this is so.

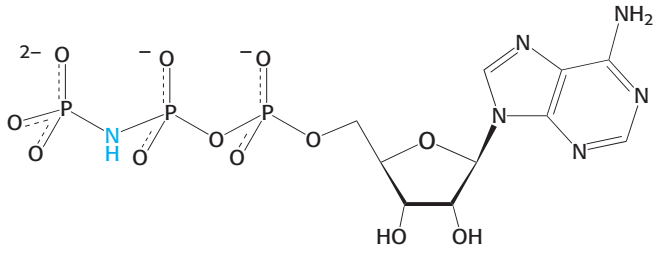
Data Interpretation Problem

39. *Another helping hand.* In eukaryotes, the 20S proteasome component in conjunction with the 19S component degrades ubiquitinated proteins with the hydrolysis of a molecule of ATP. Archaea lack ubiquitin and the 26S proteasome but do contain a 20S proteasome. Some archaea also contain an ATPase that is homologous to the ATPases of the eukaryotic 19S component. This archaeal ATPase activity was isolated as a 650-kd complex (called PAN) from the archaeon *Thermoplasma*, and experiments were performed to determine if PAN could enhance the activity of the 20S proteasome from *Thermoplasma* as well as other 20S proteasomes.

Protein degradation was measured as a function of time and in the presence of various combinations of components. Graph A shows the results.



(A)



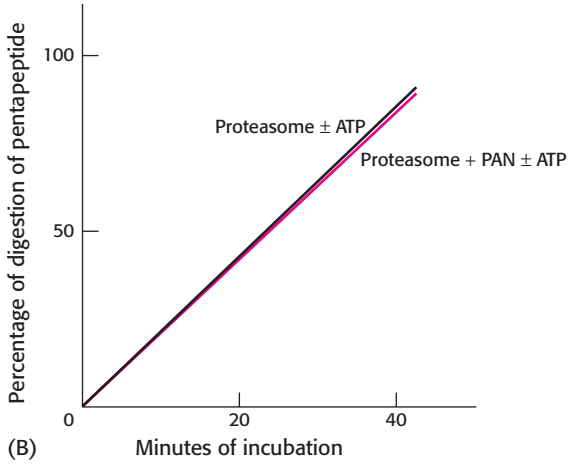
AMP-PNP

(a) What is the effect of PAN on archaeal proteasome activity in the absence of nucleotides?

(b) What is the nucleotide requirement for protein digestion?

(c) What evidence suggests that ATP hydrolysis, and not just the presence of ATP, is required for digestion?

A similar experiment was performed with a small peptide as a substrate for the proteasome instead of a protein. The results obtained are shown in graph B.



(d) How do the requirements for peptide digestion differ from those of protein digestion?

(e) Suggest some reasons for the difference.

The ability of PAN from the archaeon *Thermoplasma* to support protein degradation by the 20S proteasomes from the archaeon *Methanosarcina* and rabbit muscle was then examined.

Percentage of digestion of protein substrate (20S proteasome source)

Additions	Thermoplasma	Methanosarcina	Rabbit muscle
None	11	10	10
PAN	8	8	8
PAN + ATP	100	40	30
PAN + ADP	12	9	10

[Data from P. Zwickl, D. Ng, K. M. Woo, H.-P. Klenk, and A. L. Goldberg. An archaebacterial ATPase, homologous to ATPase in the eukaryotic 26S proteasome, activates protein breakdown by 20S proteasomes. *J. Biol. Chem.* 274(1999): 26008–26014.]

(f) Can the *Thermoplasma* PAN augment protein digestion by the proteasomes from other organisms?

(g) What is the significance of the stimulation of rabbit muscle proteasome by *Thermoplasma* PAN?

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