CHAPTER **THIRTEEN** 13

How Cells Obtain Energy From Food

THE BREAKDOWN AND UTILIZATION OF SUGARS AND FATS

REGULATION OF METABOLISM

As we discussed in Chapter 3, cells require a constant supply of energy to generate and maintain the biological order that allows them to grow, divide, and carry out their day-to-day activities. This energy comes from the chemical-bond energy in food molecules, which thereby serve as fuel for cells.

Perhaps the most important fuel molecules are the sugars. Plants make their own sugars from CO_2 by photosynthesis. Animals obtain sugars—and other organic molecules that can be chemically transformed into sugars—by eating plants and other organisms. Nevertheless, the process whereby all these sugars are broken down to generate energy is very similar in both animals and plants. In both cases, the organism's cells harvest useful energy from the chemical-bond energy locked in sugars as the sugar molecule is broken down and oxidized to carbon dioxide (CO_2) and water (H_2O)—a process called **cell respiration**. The energy released during these reactions is captured in the form of "high-energy" chemical bonds—covalent bonds that release large amounts of energy when hydrolyzed—in *activated carriers* such as ATP and NADH. These carriers in turn serve as portable sources of the chemical groups and electrons needed for biosynthesis (discussed in Chapter 3).

In this chapter, we trace the major steps in the breakdown of sugars and show how ATP, NADH, and other activated carriers are produced along the way. We concentrate on the breakdown of glucose because it generates most of the energy produced in the majority of animal cells. A very similar pathway operates in plants, fungi, and many bacteria. Other molecules, such as fatty acids and proteins, can also serve as energy sources if they are funneled through appropriate enzymatic pathways. We will

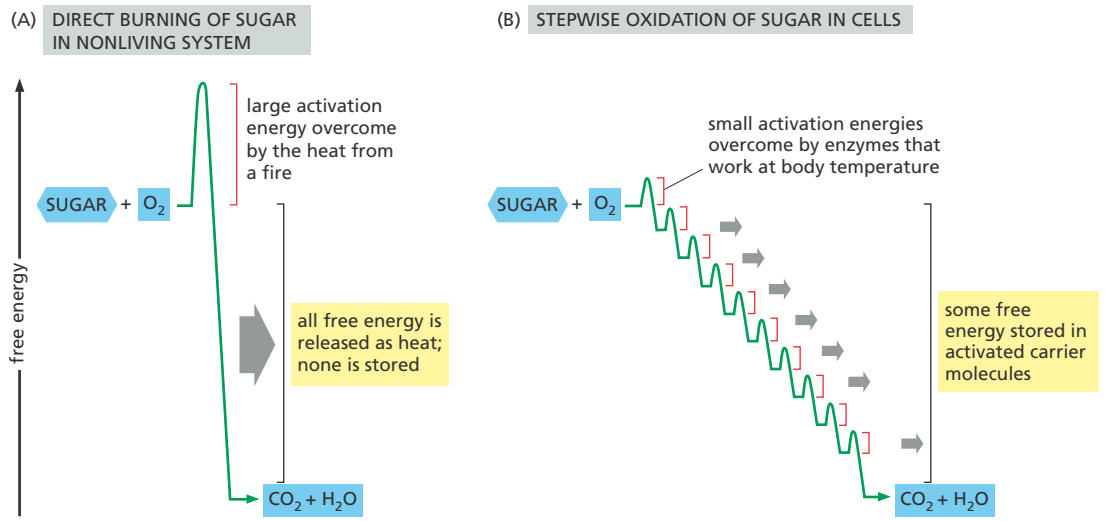


Figure 13–1 The controlled, stepwise oxidation of sugar in cells captures useful energy, unlike the simple burning of the same fuel molecule. (A) The direct burning of sugar in nonliving systems generates more energy than can be stored by any carrier molecule. This energy is thus released as heat. (B) In a cell, enzymes catalyze the breakdown of sugars via a series of small steps, in which a portion of the free energy released is captured by the formation of activated carriers—most often ATP and NADH. Each step is catalyzed by an enzyme that lowers the activation energy barrier that must be surmounted by the random collision of molecules at the temperature of cells (body temperature), so as to allow the reaction to occur. The total free energy released by the oxidative breakdown of glucose—686 kcal/mole (2880 kJ/mole)—is exactly the same in (A) and (B).

see how cells use many of the molecules generated from the breakdown of sugars and fats as starting points to make other organic molecules.

Finally, we examine how cells regulate their metabolism and how they store food molecules for their future metabolic needs. We will save our discussion of the elaborate mechanism cells use to produce the bulk of their ATP for Chapter 14.

THE BREAKDOWN AND UTILIZATION OF SUGARS AND FATS

If a fuel molecule such as glucose were oxidized to CO_2 and H_2O in a single step—by, for example, the direct application of fire—it would release an amount of energy many times larger than any carrier molecule could capture (**Figure 13–1A**). Instead, cells use enzymes to carry out the oxidation of sugars in a tightly controlled series of reactions. Thanks to the action of enzymes—which operate at temperatures typical of living things—cells degrade each glucose molecule step by step, paying out energy in small packets to activated carriers by means of coupled reactions (**Figure 13–1B**). In this way, much of the energy released by the breakdown of glucose is saved in the high-energy bonds of ATP and other activated carriers, which can then be made available to do useful work for the cell.

Animal cells make ATP in two ways. First, certain energetically favorable, enzyme-catalyzed reactions involved in the breakdown of foods are directly coupled to the energetically unfavorable reaction **ADP** + P_i → **ATP**. Thus the oxidation of food molecules can provide energy for the immediate production of ATP. Most ATP synthesis, however, requires an intermediary. In this second pathway to making ATP, the energy from other activated carriers is used to drive ATP production. This process, called *oxidative phosphorylation*, takes place on the inner mitochondrial membrane (**Figure 13–2**), and it is described in detail in Chapter 14. In this chapter, we focus on the first sequence of reactions by which food molecules are oxidized—both in the cytosol and in the mitochondrial matrix (see Figure 13–2). These reactions produce both ATP and the additional

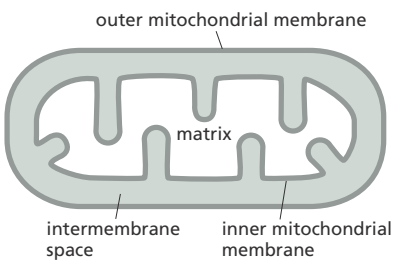


Figure 13–2 A mitochondrion has two membranes and a large internal space called the matrix. Most of the energy from food molecules is harvested in mitochondria—both in the matrix and in the inner mitochondrial membrane.

activated carriers that will subsequently help drive the production of much larger amounts of ATP by oxidative phosphorylation.

Food Molecules Are Broken Down in Three Stages

The proteins, fats, and polysaccharides that make up most of the food we eat must be broken down into smaller molecules before our cells can use them—either as a source of energy or as building blocks for making other organic molecules. This breakdown process—in which enzymes degrade complex organic molecules into simpler ones—is called **catabolism**. The process takes place in three stages, as illustrated in Figure 13–3.

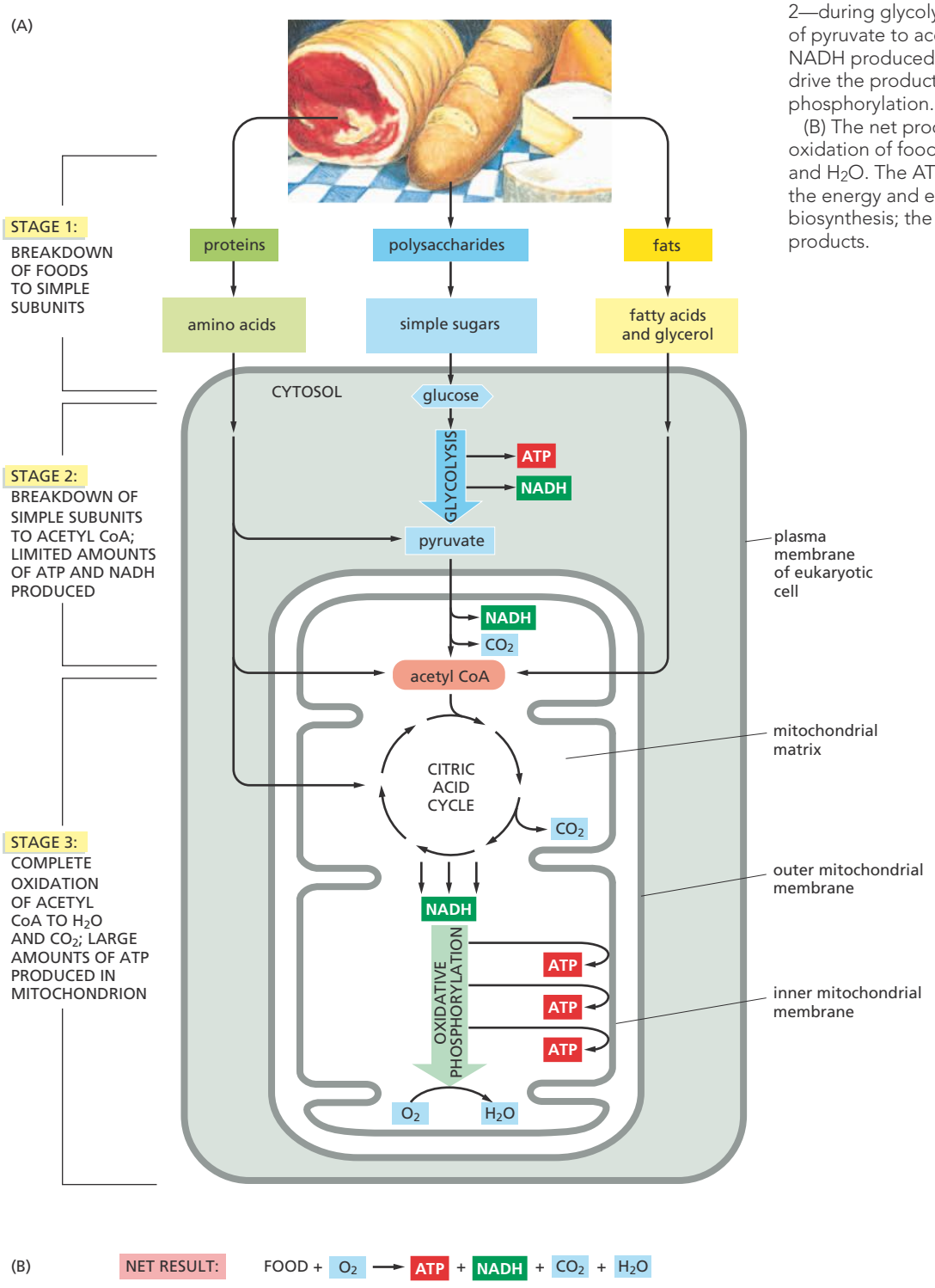


Figure 13–3 The breakdown of food molecules occurs in three stages. (A) Stage 1 mostly occurs outside cells in the mouth and the gut—although intracellular lysosomes can also digest large organic molecules. Stage 2 occurs mainly in the cytosol, except for the final step of conversion of pyruvate to acetyl groups on acetyl CoA, which occurs in the mitochondrial matrix. Stage 3 begins with the citric acid cycle in the mitochondrial matrix and concludes with oxidative phosphorylation on the mitochondrial inner membrane. The NADH generated in stage 2—during glycolysis and the conversion of pyruvate to acetyl CoA—adds to the NADH produced by the citric acid cycle to drive the production of ATP by oxidative phosphorylation.

(B) The net products of the complete oxidation of food include ATP, NADH, CO_2, and H_2O. The ATP and NADH provide the energy and electrons needed for biosynthesis; the CO_2 and H_2O are waste products.

In *stage 1* of catabolism, enzymes convert the large polymeric molecules in food into simpler monomeric subunits: proteins into amino acids, polysaccharides into sugars, and fats into fatty acids and glycerol. This stage—also called *digestion*—occurs either outside cells (in the intestine) or in specialized organelles within cells called lysosomes (discussed in Chapter 15). After digestion, the small organic molecules derived from food enter the cytosol of a cell, where their gradual oxidative breakdown begins.

In *stage 2* of catabolism, a chain of reactions called *glycolysis* splits each molecule of *glucose* into two smaller molecules of *pyruvate.* Sugars other than glucose can also be used, after first being converted into one of the intermediates in this sugar-splitting pathway. Glycolysis takes place in the cytosol and, in addition to producing pyruvate, it generates two types of activated carriers: ATP and NADH. The pyruvate is transported from the cytosol into the mitochondrion's large, internal compartment called the *matrix*. There, a giant enzyme complex converts each pyruvate molecule into CO_2 plus *acetyl CoA*, another of the activated carriers discussed in Chapter 3 (see Figure 3–36). In the same compartment, large amounts of acetyl CoA are also produced by the stepwise oxidative breakdown of fatty acids derived from fats (see Figure 13–3).

Stage 3 of catabolism takes place entirely in mitochondria. The acetyl group in acetyl CoA is transferred to an oxaloacetate molecule to form citrate, which enters a series of reactions called the *citric acid cycle.* In these reactions, the transferred acetyl group is oxidized to CO_2 with the production of large amounts of NADH. Finally, the high-energy electrons from NADH are passed along a series of enzymes within the mitochondrial inner membrane called an *electron-transport chain*, where the energy released by their transfer is used to drive oxidative phosphorylation—a process that produces ATP and consumes molecular oxygen (O_2 gas). It is in these final steps of catabolism that the majority of the energy released by oxidation is harnessed to produce most of the cell's ATP.

Through the production of ATP, the energy derived from the breakdown of sugars and fats is redistributed into packets of chemical energy in a form convenient for use in the cell. In total, nearly half of the energy that could, in theory, be derived from the breakdown of glucose or fatty acids to H_2O and CO_2 is captured and used to drive the energetically unfavorable reaction $ADP + P_i \rightarrow ATP$. By contrast, a modern combustion engine, such as a car engine, can convert no more than 20% of the available energy in its fuel into useful work. In both cases, the remaining energy is released as heat, which in animals helps to keep the body warm.

Roughly 10^9 molecules of ATP are in solution in a typical cell at any instant. In many cells, all of this ATP is turned over (that is, consumed and replaced) every 1–2 minutes. An average person at rest will hydrolyze his or her weight in ATP molecules every 24 hours.

Glycolysis Extracts Energy from the Splitting of Sugar

The central process in stage 2 of catabolism is the oxidative breakdown of **glucose** in the sequence of reactions known as **glycolysis**. Glycolysis produces ATP without the involvement of oxygen. It occurs in the cytosol of most cells, including many anaerobic microorganisms that thrive in the absence of oxygen. Glycolysis probably evolved early in the history of life on Earth, before photosynthetic organisms introduced oxygen into the atmosphere.

The term "glycolysis" comes from the Greek *glykys*, "sweet," and *lysis*, "splitting." It is an appropriate name, as glycolysis splits a molecule of glucose, which has six carbon atoms, to form two molecules of pyruvate, each of which contains three carbon atoms. The series of chemical

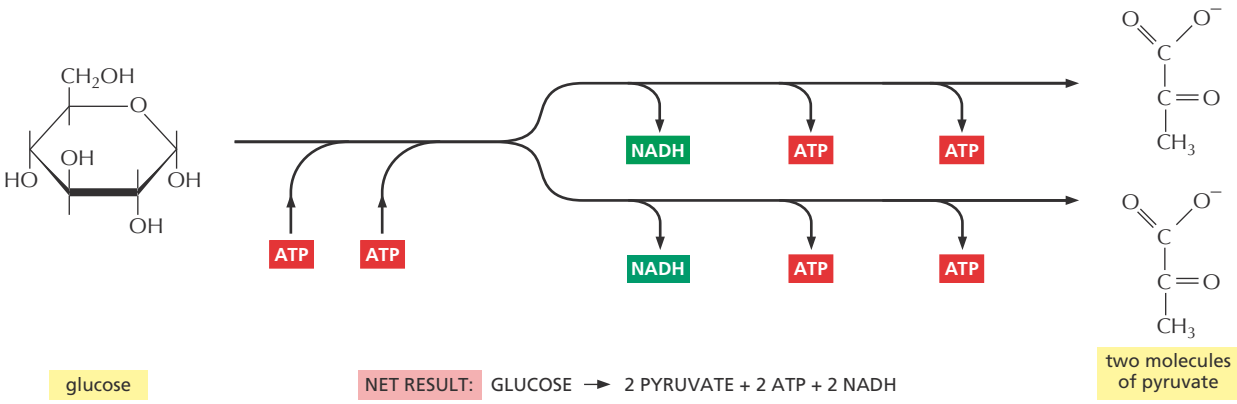


Figure 13–4 Glycolysis splits a molecule of glucose to form two molecules of pyruvate. The process requires an input of energy, in the form of ATP, at the start. This energy investment is later recouped by the production of two NADHs and four ATPs.

rearrangements that ultimately generate pyruvate release energy because the electrons in a molecule of pyruvate are, overall, at a lower energy state than those in a molecule of glucose. Nevertheless, for each molecule of glucose that enters glycolysis, two molecules of ATP are initially consumed to provide the energy needed to prepare the sugar to be split. This investment of energy is more than recouped in the later steps of glycolysis, when four molecules of ATP are produced. Energy is also captured in this "payoff phase" in the form of NADH. Thus, at the end of glycolysis, there is a net gain of two molecules of ATP and two molecules of NADH for each glucose molecule broken down (**Figure 13–4**).

Glycolysis Produces Both ATP and NADH

Piecing together the complete glycolytic pathway in the 1930s was a major triumph of biochemistry, as the pathway consists of a sequence of 10 separate reactions, each producing a different sugar intermediate and each catalyzed by a different enzyme. Like most enzymes, those that catalyze glycolysis all have names ending in *-ase*—like isomerase and dehydrogenase—which specify the type of reaction they catalyze (**Table 13–1**). The reactions of the glycolytic pathway are presented in outline in **Figure 13–5** and in detail in **Panel 13–1** (pp. 428–429).

TABLE 13–1 SOME TYPES OF ENZYMES INVOLVED IN GLYCOLYSIS

Enzyme type	General function	Role in glycolysis
Kinase	catalyzes the addition of a phosphate group to molecules	a kinase transfers a phosphate group from ATP to a substrate in steps 1 and 3; other kinases transfer a phosphate to ADP to form ATP in steps 7 and 10
Isomerase	catalyzes the rearrangement of bonds within a single molecule	isomerases in steps 2 and 5 prepare molecules for the chemical alterations to come
Dehydrogenase	catalyzes the oxidation of a molecule by removing a hydrogen atom plus an electron (a hydride ion, H^-)	the enzyme glyceraldehyde 3-phosphate dehydrogenase generates NADH in step 6
Mutase	catalyzes the shifting of a chemical group from one position to another within a molecule	the movement of a phosphate by phosphoglycerate mutase in step 8 helps prepare the substrate to transfer this group to ADP to make ATP in step 10

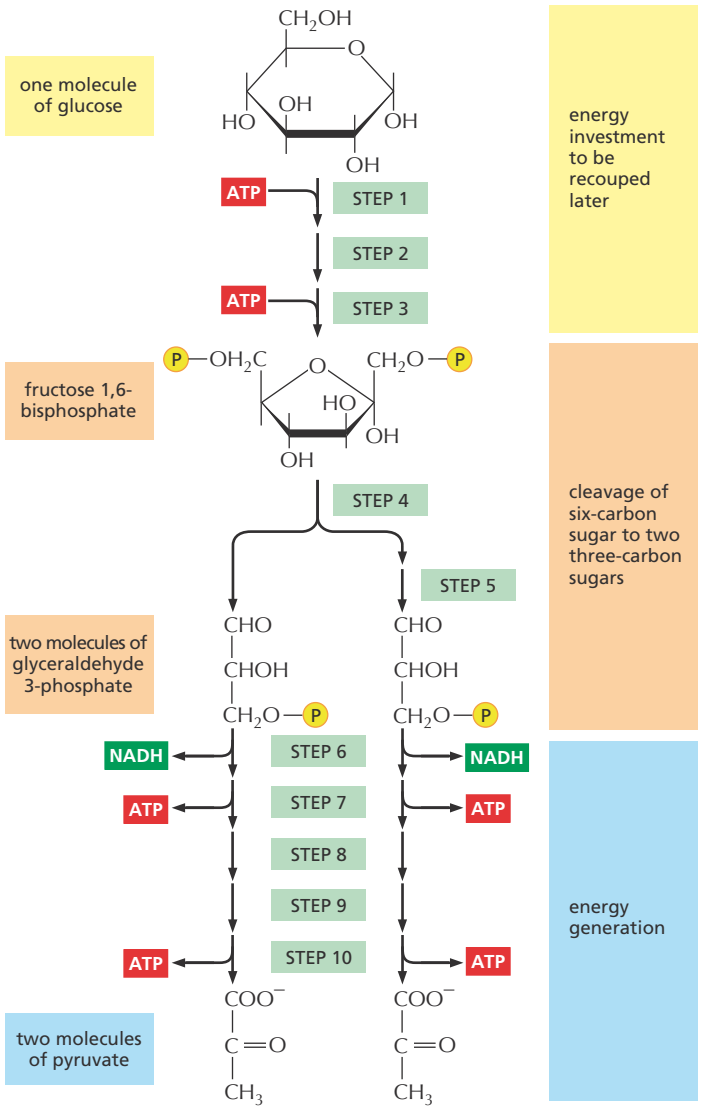


Figure 13–5 The stepwise breakdown of sugars begins with glycolysis. Each of the 10 steps of glycolysis is catalyzed by a different enzyme. Note that step 4 cleaves a six-carbon sugar into two three-carbon sugars, so that the number of molecules at every stage after this doubles. Note also that one of the products of step 4 needs to be modified (isomerized) in step 5 before it can proceed to step 6 (see Panel 13–1). As indicated, step 6 begins the energy-generation phase of glycolysis, which results in the net synthesis of ATP and NADH (see also Figure 13–4). Glycolysis is also sometimes referred to as the Embden–Meyerhof pathway, named for the chemists who first described it. All the steps of glycolysis are reviewed in **Movie 13.1**.

Much of the energy released by the breakdown of glucose is used to drive the synthesis of ATP molecules from ADP and P_i. This form of ATP synthesis, which takes place in steps 7 and 10 in glycolysis, is known as *substrate-level phosphorylation* because it occurs by the transfer of a phosphate group directly from a substrate molecule—one of the sugar intermediates—to ADP. By contrast, most phosphorylations in cells occur by the transfer of phosphate from ATP to a substrate molecule.

The remainder of the energy released during glycolysis is stored in the electrons in the **NADH** molecule produced in step 6 by an oxidation reaction. As discussed in Chapter 3, oxidation does not always involve oxygen; it occurs in any reaction in which electrons are lost from one atom and transferred to another. So, although no molecular oxygen is involved in glycolysis, oxidation does occur: in step 6, a hydrogen atom plus an electron is removed from the sugar intermediate, glyceraldehyde 3-phosphate, and transferred to **NAD^+**, producing NADH (see Panel 13–1, p. 428).

Over the course of glycolysis, two molecules of NADH are formed per molecule of glucose. In aerobic organisms, these NADH molecules donate their electrons to the electron-transport chain in the inner mitochondrial membrane, as described in detail in Chapter 14. Such electron transfers release energy as the electrons fall from a state of higher energy to a

lower one. The electrons that are passed along the electron-transport chain are ultimately passed on to O_2, forming water.

In giving up its electrons, NADH is converted back into NAD^+, which is then available to be used again for glycolysis. In the absence of oxygen, NAD^+ can be regenerated by an alternate type of energy-yielding reaction called a fermentation, as we discuss next.

Fermentations Can Produce ATP in the Absence of Oxygen

For most animal and plant cells, glycolysis is only a prelude to the third and final stage of the breakdown of food molecules, in which large amounts of ATP are generated in mitochondria by oxidative phosphorylation, a process that requires the consumption of oxygen. However, for many anaerobic microorganisms, which can grow and divide in the absence of oxygen, glycolysis is the principal source of ATP. The same is true for certain animal cells, such as skeletal muscle cells, which can continue to function at low levels of oxygen.

In these anaerobic conditions, the pyruvate and NADH made by glycolysis remain in the cytosol. The pyruvate is converted into products that are excreted from the cell: lactate in muscle cells, for example, or ethanol and CO_2 in the yeast cells used in brewing and breadmaking. The NADH gives up its electrons in the cytosol, and is converted back to the NAD^+ required to maintain the reactions of glycolysis (**Figure 13–6**). Such energy-yielding pathways that break down sugar in the absence of oxygen are called **fermentations**. Scientific studies of the commercially important fermentations carried out by yeasts laid the foundations for early biochemistry.

QUESTION 13–1

At first glance, the final steps in fermentation appear to be unnecessary: the generation of lactate or ethanol does not produce any additional energy for the cell. Explain why cells growing in the absence of oxygen could not simply discard pyruvate as a waste product. Which products derived from glucose would accumulate in cells unable to generate either lactate or ethanol by fermentation?

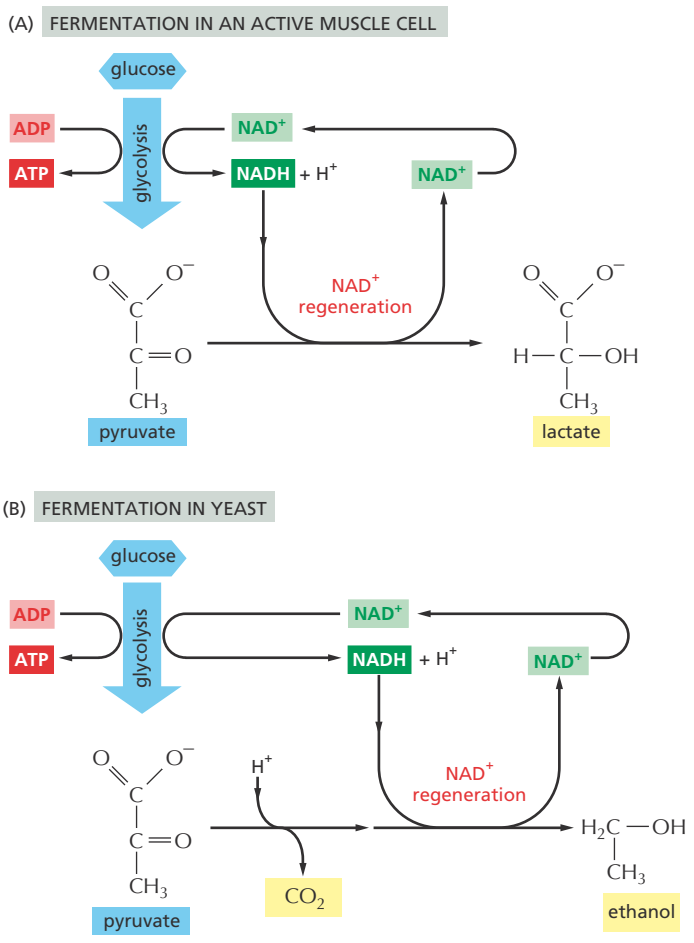


Figure 13–6 Pyruvate is broken down in the absence of oxygen by fermentation. (A) When inadequate oxygen is present, for example, in a muscle cell undergoing vigorous contraction, the pyruvate produced by glycolysis is converted to lactate in the cytosol. This reaction restores the NAD^+ consumed in step 6 of glycolysis, but the whole pathway yields much less energy overall than if the pyruvate were oxidized in mitochondria. (B) In microorganisms that can grow anaerobically, pyruvate is converted into carbon dioxide and ethanol. Again, this pathway regenerates NAD^+ from NADH, as required to enable glycolysis to continue. Both (A) and (B) are examples of fermentations. Note that in both cases, for each molecule of glucose that enters glycolysis, two molecules of pyruvate are generated (only a single pyruvate is shown here). Fermentation of these two pyruvates subsequently yields two molecules of lactate—or two molecules of CO_2 and ethanol—plus two molecules of NAD^+.

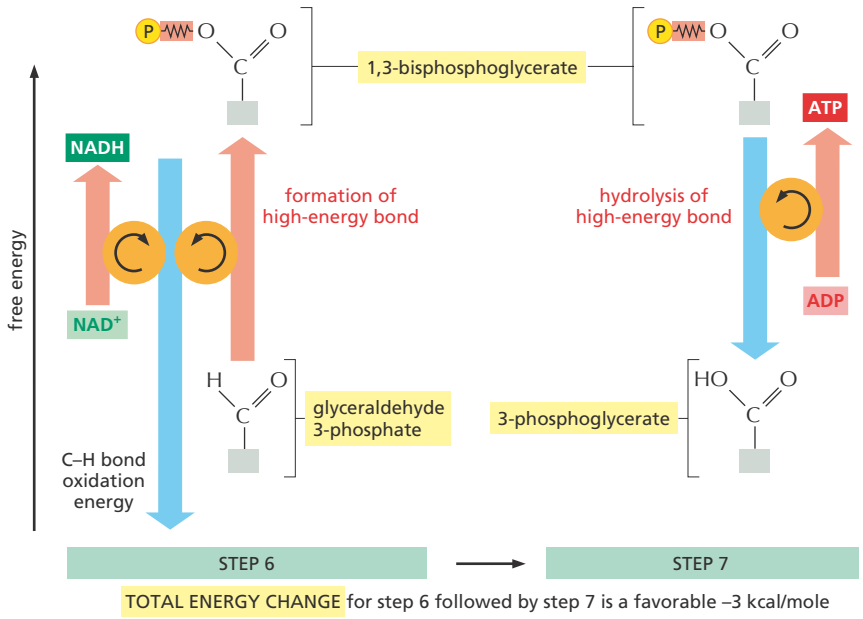


Figure 13–7 A pair of coupled reactions drives the energetically unfavorable formation of ATP in steps 6 and 7 of glycolysis. In this diagram, energetically favorable reactions are represented by *blue arrows*; energetically costly reactions by *red arrows*. In step 6, the energy released by the energetically favorable oxidation of a C–H bond in glyceraldehyde 3-phosphate (*blue arrow*) is large enough to drive two energetically costly reactions: the formation of both NADH and a high-energy phosphate bond in 1,3-bisphosphoglycerate (*red arrows*). The subsequent energetically favorable hydrolysis of that high-energy phosphate bond in step 7 then drives the formation of ATP.

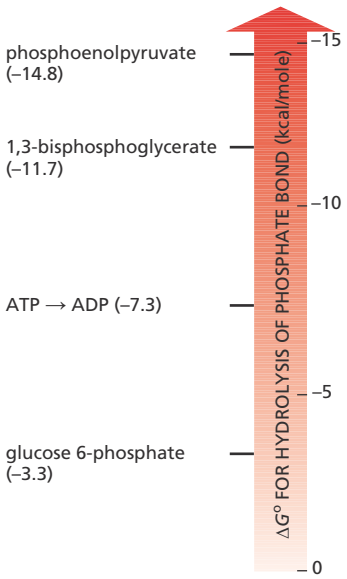


Figure 13–8 Differences in the energies of different phosphate bonds allow the formation of ATP by substrate-level phosphorylation. Examples of molecules containing different types of phosphate bonds are shown, along with the free-energy change for hydrolysis of those bonds in kcal/mole (1 kcal = 4.184 kJ). The transfer of a phosphate group from one molecule to another is energetically favorable if the standard free-energy change (ΔG°) for hydrolysis of the phosphate bond is more negative for the donor molecule than for the acceptor. (The hydrolysis reactions can be thought of as the transfer of the phosphate group to water.) Thus, a phosphate group is readily transferred from 1,3-bisphosphoglycerate to ADP to form ATP. Transfer reactions involving the phosphate groups in these molecules are detailed in Panel 13–1 (pp. 428–429).

Many bacteria and archaea can also generate ATP in the absence of oxygen by *anaerobic respiration*, a process that uses a molecule other than oxygen as a final electron acceptor. Anaerobic respiration differs from fermentation in that it involves an electron-transport chain embedded in a membrane—in this case, the plasma membrane of the microbe.

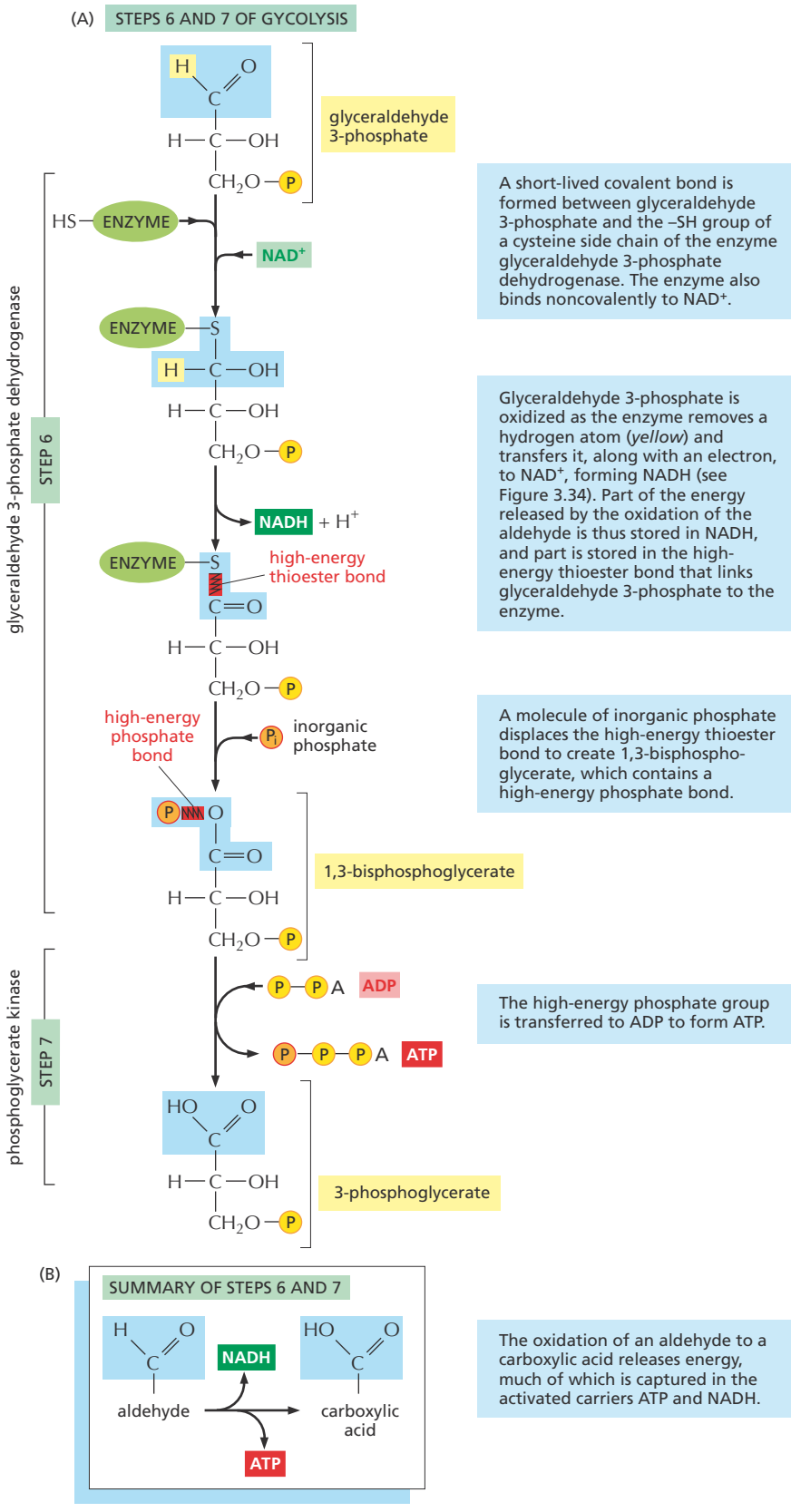
Glycolytic Enzymes Couple Oxidation to Energy Storage in Activated Carriers

The "paddle wheel" analogy in Chapter 3 explained how cells harvest useful energy from the oxidation of organic molecules by coupling an energetically unfavorable reaction to an energetically favorable one (see Figure 3–30). Here, we take a closer look at a key pair of glycolytic reactions that demonstrate how enzymes—the paddle wheel in our analogy—allow coupled reactions to facilitate the transfer of chemical energy to ATP and NADH.

The reactions in question—steps 6 and 7 in Panel 13–1—convert the three-carbon sugar intermediate glyceraldehyde 3-phosphate (an aldehyde) into 3-phosphoglycerate (a carboxylic acid). This conversion, which entails the oxidation of an aldehyde group to a carboxylic acid group, occurs in two steps. The overall reaction releases enough free energy to transfer two electrons from the aldehyde to NAD^+ to form NADH and to transfer a phosphate group to a molecule of ADP to form ATP. It also releases enough heat to the environment to make the overall reaction energetically favorable: the ΔG° for step 6 followed by step 7 is –3.0 kcal/mole (**Figure 13–7**).

The energy contained in any phosphate bond can be determined by measuring the standard free-energy change (ΔG°) when that bond is broken by hydrolysis. Molecules that contain phosphate bonds that have more energy than those found in ATP—including the high-energy 1,3-bisphosphoglycerate generated in step 6 of glycolysis—readily transfer their phosphate group to ADP to form ATP. **Figure 13–8** compares the high-energy phosphoanhydride bond in ATP with a few of the other phosphate bonds that are generated during glycolysis. As explained in Panel 13–1, we describe these bonds as "high energy" only in that their hydrolysis is particularly energetically favorable.

The reaction in step 6 is the only one in glycolysis that creates a high-energy phosphate linkage directly from inorganic phosphate—an example of the substrate-level phosphorylation mentioned earlier. How this high-energy linkage is generated in step 6—and then consumed in step 7 to produce ATP—is detailed in **Figure 13–9**.



QUESTION 13–2

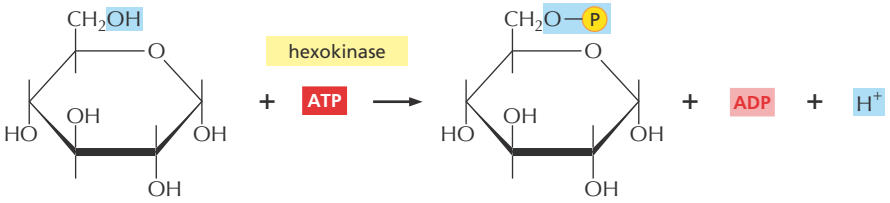
Arsenate (AsO_4^{3-}) is chemically very similar to phosphate (PO_4^{3-}) and is used as an alternative substrate by many phosphate-requiring enzymes. In contrast to phosphate, however, an anhydride bond between arsenate and carbon is very quickly hydrolyzed nonenzymatically in water. Knowing this, suggest why arsenate is a compound of choice for murderers but not for cells. Formulate your explanation in the context of Figure 13–7.

Figure 13–9 The oxidation of glyceraldehyde 3-phosphate is coupled to the formation of ATP and NADH in steps 6 and 7 of glycolysis. (A) In step 6, the enzyme glyceraldehyde 3-phosphate dehydrogenase couples the energetically favorable oxidation of an aldehyde to the energetically unfavorable formation of a high-energy phosphate bond. At the same time, it enables energy to be stored in NADH. The formation of the high-energy phosphate bond is driven by the oxidation reaction, and the enzyme thereby acts like the "paddle wheel" coupler in Figure 3–30B. In step 7, the newly formed high-energy phosphate bond in 1,3-bisphosphoglycerate is transferred to ADP, forming a molecule of ATP and leaving a free carboxylic acid group on the oxidized sugar. The part of the molecule that undergoes a change is shaded in *blue*; the rest of the molecule remains unchanged throughout all these reactions. (B) Summary of the overall chemical change produced by the reactions of steps 6 and 7.

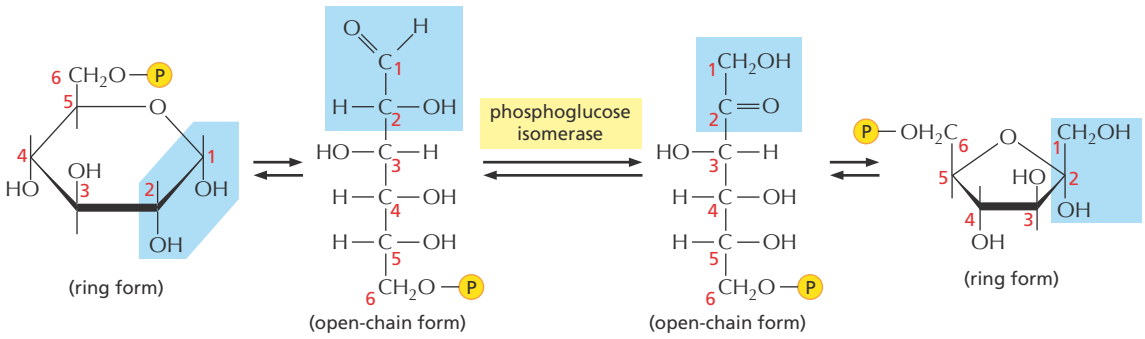
PANEL 13–1 DETAILS OF THE 10 STEPS OF GLYCOLYSIS

For each step, the part of the molecule that undergoes a change is shadowed in blue, and the name of the enzyme that catalyzes the reaction is in a yellow box. To watch a video of the reactions of glycolysis, see Movie 13.1.

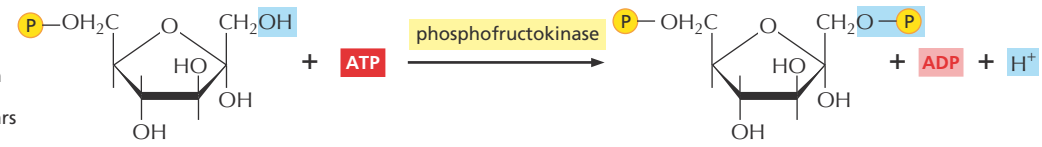
Step 1 Glucose is phosphorylated by ATP to form a sugar phosphate. The negative charge of the phosphate prevents passage of the sugar phosphate through the plasma membrane, trapping glucose inside the cell.



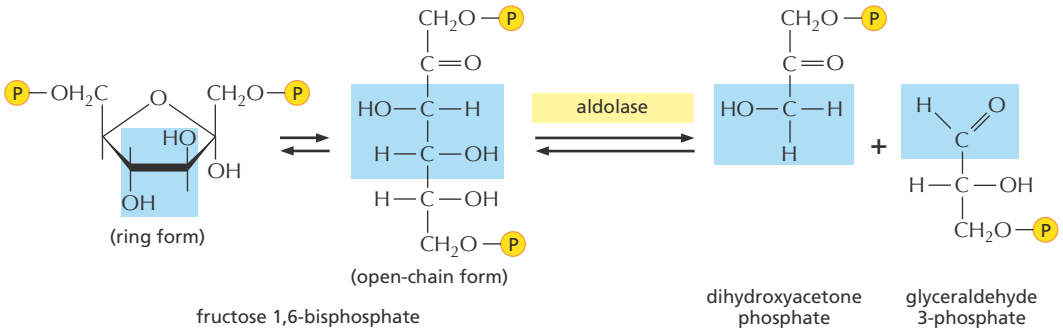
Step 2 A readily reversible rearrangement of the chemical structure (isomerization) moves the carbonyl oxygen from carbon 1 to carbon 2, forming a ketose from an aldose sugar. (See Panel 2–3, pp. 70–71.)



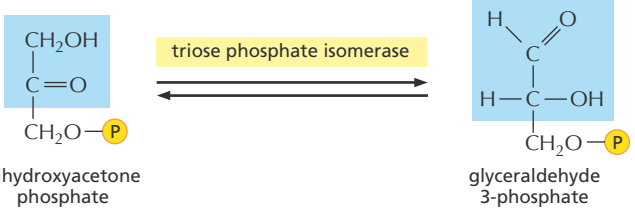
Step 3 The new hydroxyl group on carbon 1 is phosphorylated by ATP, in preparation for the formation of two three-carbon sugar phosphates. The entry of sugars into glycolysis is controlled at this step, through regulation of the enzyme *phosphofructokinase*.



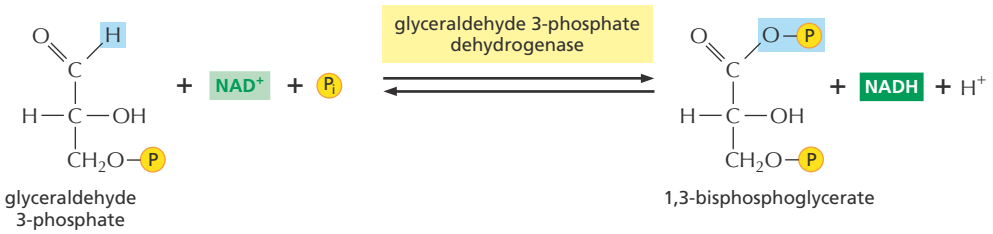
Step 4 The six-carbon sugar is cleaved to produce two three-carbon molecules. Only the glyceraldehyde 3-phosphate can proceed immediately through glycolysis.



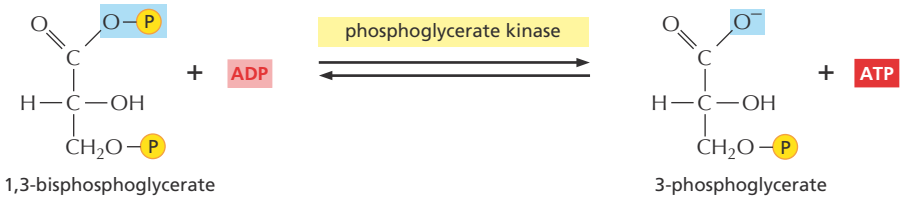
Step 5 The other product of step 4, dihydroxyacetone phosphate, is isomerized to form glyceraldehyde 3-phosphate.



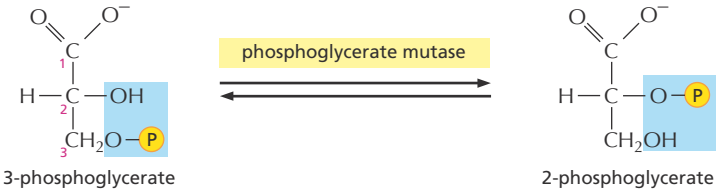
Step 6 The two molecules of glyceraldehyde 3-phosphate are oxidized. The energy-generation phase of glycolysis begins, as NADH and a new high-energy anhydride linkage to phosphate are formed (see Figure 13–5).



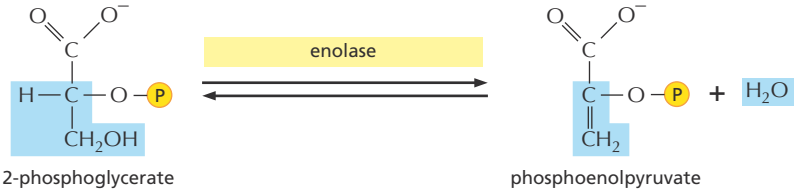
Step 7 The transfer to ADP of the high-energy phosphate group that was generated in step 6 forms ATP.



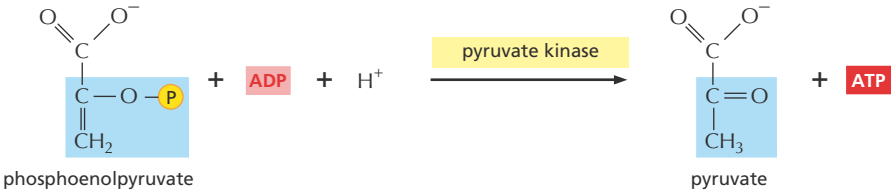
Step 8 The remaining phosphate ester linkage in 3-phosphoglycerate, which has a relatively low free energy of hydrolysis, is moved from carbon 3 to carbon 2 to form 2-phosphoglycerate.



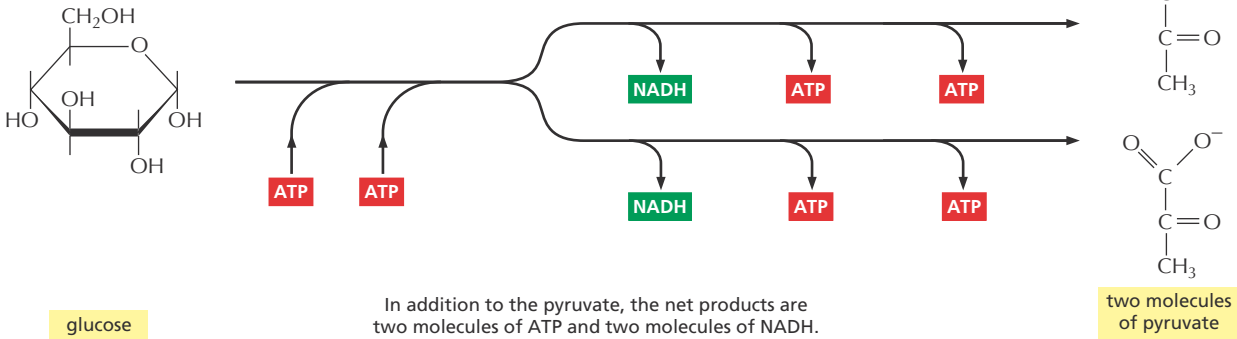
Step 9 The removal of water from 2-phosphoglycerate creates a high-energy enol phosphate linkage.



Step 10 The transfer to ADP of the high-energy phosphate group that was generated in step 9 forms ATP, completing glycolysis.



NET RESULT OF GLYCOLYSIS



In addition to the pyruvate, the net products are two molecules of ATP and two molecules of NADH.

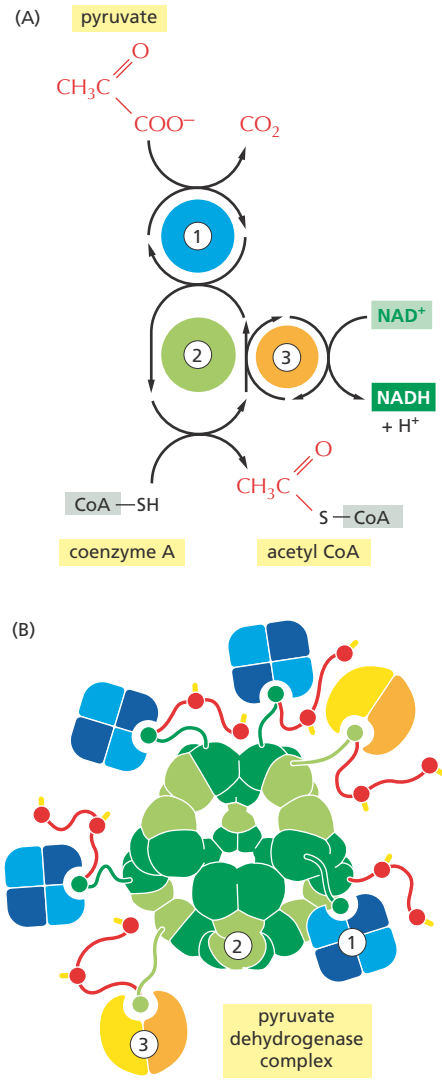


Figure 13–10 Pyruvate is converted into acetyl CoA and CO_2 by the pyruvate dehydrogenase complex in the mitochondrial matrix. (A) The pyruvate dehydrogenase complex, which contains multiple copies of three different enzymes—*pyruvate dehydrogenase* (1), *dihydrolipoyl transacetylase* (2), and *dihydrolipoyl dehydrogenase* (3)—converts pyruvate to acetyl CoA; NADH and CO_2 are also produced in this reaction. Pyruvate and its products are shown in *red lettering*. (B) In this large multienzyme complex, reaction intermediates are passed directly from one enzyme to another via flexible tethers. Only one-tenth of the subunits labeled 1 and 3, attached to the core formed by subunit 2, are shown here. To get a sense of scale, the pyruvate dehydrogenase complex is larger than a ribosome.

Several Organic Molecules Are Converted to Acetyl CoA in the Mitochondrial Matrix

In aerobic metabolism in eukaryotic cells, the **pyruvate** produced by glycolysis is actively pumped into the mitochondrial matrix (see Figure 13–3). There, it is rapidly decarboxylated by a giant complex of three enzymes, called the *pyruvate dehydrogenase complex.* The products of pyruvate decarboxylation are CO_2 (a waste product), NADH, and **acetyl CoA** (**Figure 13–10**).

In addition to sugar, which is broken down during glycolysis, **fat** is a major source of energy for most nonphotosynthetic organisms, including humans. Like the pyruvate derived from glycolysis, the fatty acids derived from fat are also converted into acetyl CoA in the mitochondrial matrix (see Figure 13–3). Fatty acids are first activated by covalent linkage to CoA and are then broken down completely by a cycle of reactions that trims two carbons at a time from their carboxyl end, generating one molecule of acetyl CoA for each turn of the cycle. Two activated carriers—NADH and another high-energy electron carrier, $FADH_2$—are also produced in this process (**Figure 13–11**).

In addition to pyruvate and fatty acids, some amino acids are transported from the cytosol into the mitochondrial matrix, where they are also converted into acetyl CoA or one of the other intermediates of the citric acid cycle (see Figure 13–3). Thus, in the eukaryotic cell, the mitochondrion is the center toward which all energy-yielding catabolic processes lead, whether they begin with sugars, fats, or proteins. In aerobic bacteria—which have no mitochondria—glycolysis and acetyl CoA production, as well as the citric acid cycle, take place in the cytosol.

Catabolism does not end with the production of acetyl CoA. In the process of converting food molecules to acetyl CoA, only a small part of their stored energy is extracted and converted into ATP, NADH, or $FADH_2$. Most of that energy is still locked up in acetyl CoA. The next stage in cell respiration is the citric acid cycle, in which the acetyl group in acetyl CoA is oxidized to CO_2 and H_2O in the mitochondrial matrix, as we now discuss.

The Citric Acid Cycle Generates NADH by Oxidizing Acetyl Groups to CO_2

The **citric acid cycle** accounts for about two-thirds of the total oxidation of carbon compounds in most cells, and its major end products are CO_2 and high-energy electrons in the form of NADH. The CO_2 is released as a waste product, while the high-energy electrons from NADH are passed to the electron-transport chain in the inner mitochondrial membrane. At the end of the chain, these electrons combine with O_2 to produce H_2O.

The citric acid cycle, which takes place in the mitochondrial matrix, does not itself use O_2. However, it requires O_2 to proceed because the

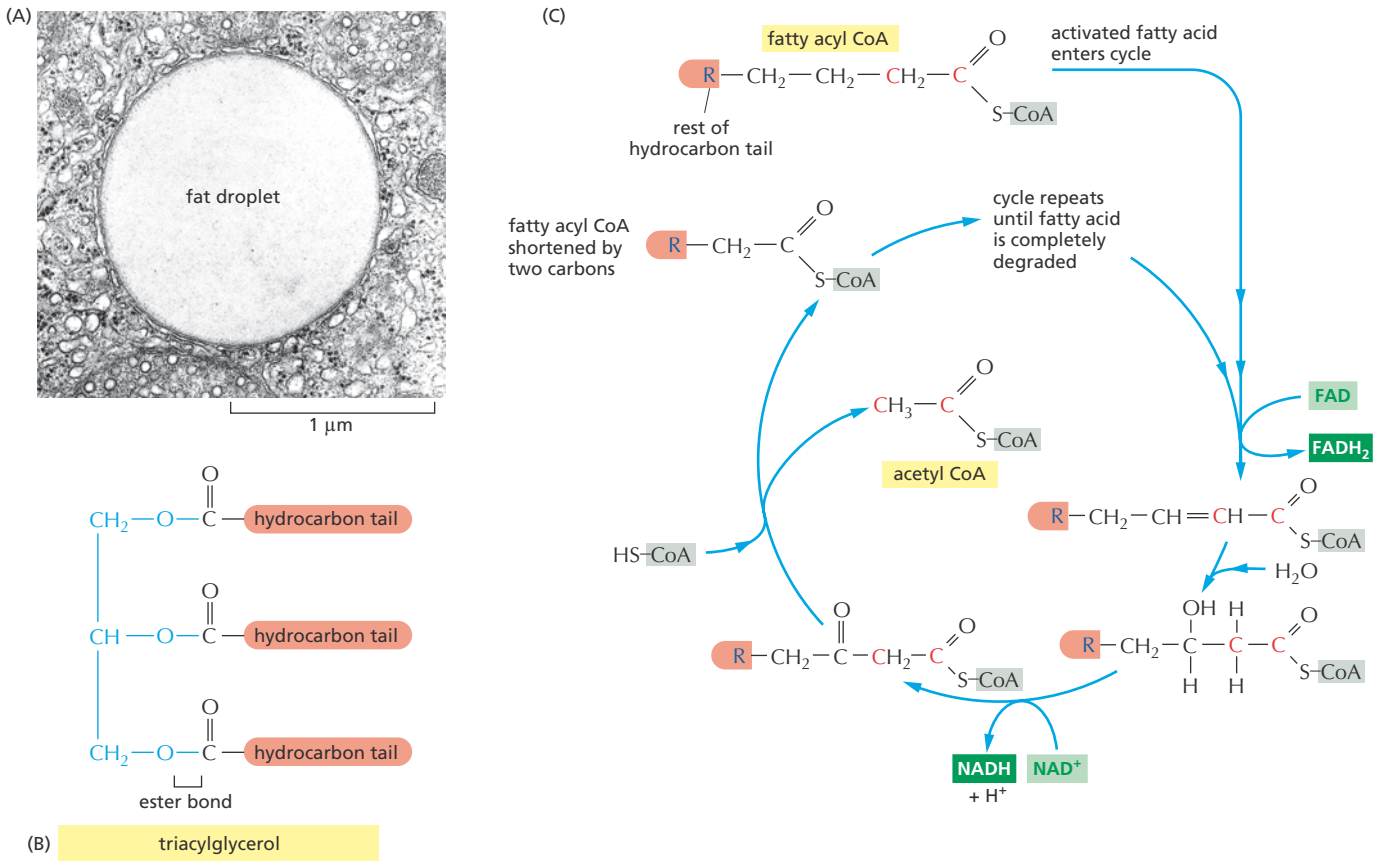


Figure 13–11 Fatty acids derived from fats are also converted to acetyl CoA in the mitochondrial matrix. (A) Fats are insoluble in water and spontaneously form large lipid droplets in specialized fat cells called adipocytes. This electron micrograph shows a lipid droplet in the cytoplasm of an adipocyte. (B) Fats are stored in the form of triacylglycerol. The glycerol portion, to which three fatty acid chains (shaded in *red*) are linked through ester bonds, is shown in *blue*. Enzymes called lipases can cleave the ester bonds that link the fatty acid chains to glycerol when fatty acids are needed for energy. (C) Fatty acids are first coupled to coenzyme A in a reaction requiring ATP (not shown). The activated fatty acid chains (fatty acyl CoA) are then oxidized in a cycle containing four enzymes. Each turn of the cycle shortens a fatty acyl CoA molecule by two carbons (*red*) and generates one molecule of acetyl CoA and one molecule each of NADH and $FADH_2$. (A, courtesy of Daniel S. Friend.)

electron-transport chain—which uses O_2 as its final acceptor—allows NADH to get rid of its electrons and thus regenerate the NAD^+ needed to keep the cycle going. Although living organisms have inhabited Earth for the past 3.5 billion years, the planet is thought to have developed an atmosphere containing O_2 gas only some 1 to 2 billion years ago (see Figure 14–45). Many of the energy-generating reactions of the citric acid cycle—also called the *tricarboxylic acid cycle* or the *Krebs cycle*—are therefore likely to be of relatively recent origin.

The citric acid cycle catalyzes the complete oxidation of the carbon atoms of the acetyl groups in acetyl CoA, converting them into CO_2. The acetyl group is not oxidized directly, however. Instead, it is transferred from acetyl CoA to a larger four-carbon molecule, oxaloacetate, to form the six-carbon tricarboxylic acid, citric acid, for which the subsequent cycle of reactions is named. The citric acid molecule (also called citrate) is then progressively oxidized, and the energy of this oxidation is harnessed to produce activated carriers in much the same manner as we described for glycolysis. The chain of eight reactions forms a cycle, because the oxaloacetate that began the process is regenerated at the end (**Figure 13–12**). The citric acid cycle is presented in detail in **Panel 13–2** (pp. 434–435), and the experiments that first revealed the cyclic nature of this series of oxidative reactions are described in **How We Know**, pp. 436–437.

QUESTION 13–3

Many catabolic and anabolic reactions are based on reactions that are similar but work in opposite directions, such as the hydrolysis and condensation reactions described in Figure 3–38. This is true for fatty acid breakdown and fatty acid synthesis. From what you know about the mechanism of fatty acid breakdown outlined in Figure 13–11, would you expect the fatty acids found in cells to most commonly have an even or an odd number of carbon atoms?

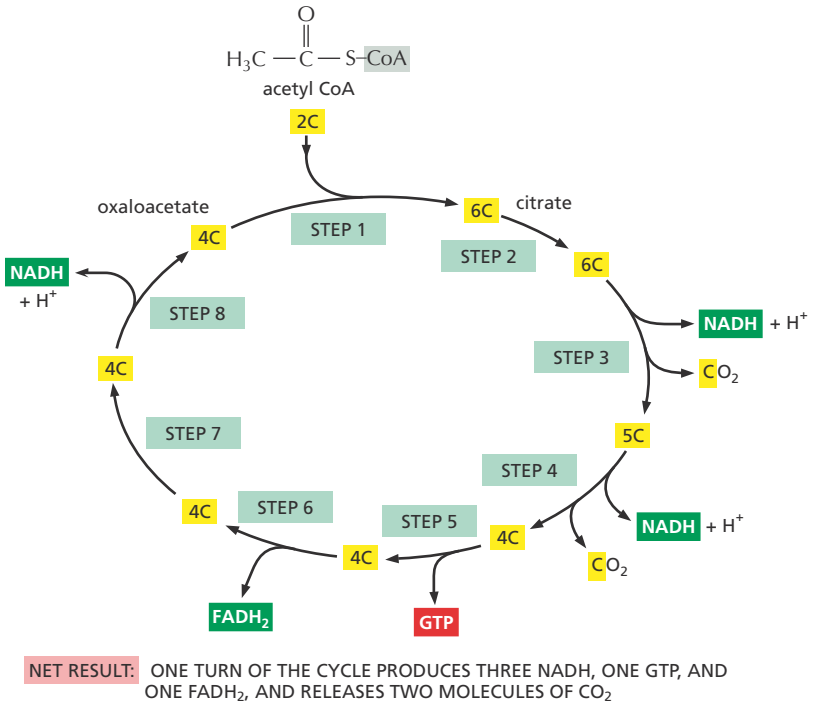


Figure 13–12 The citric acid cycle catalyzes the complete oxidation of acetyl groups derived from food. The cycle begins with the reaction of acetyl CoA (derived from pyruvate as shown in Figure 13–10) with oxaloacetate to produce citrate (citric acid). The number of carbon atoms in each intermediate is shaded in *yellow*. (See also Panel 13–2, pp. 434–435.) The steps of the citric acid cycle are reviewed in **Movie 13.2**.

Thus far, we have discussed only one of the three types of activated carriers that are produced by the citric acid cycle—NADH. In addition to three molecules of NADH, each turn of the cycle also produces one molecule of **$FADH_2$** (**reduced flavin adenine dinucleotide**) from FAD and one molecule of the ribonucleoside triphosphate **GTP** (**guanosine triphosphate**) from **GDP** (see Figure 13–12). The structures of these two activated carriers are illustrated in **Figure 13–13**. GTP is a close relative of ATP, and the transfer of its terminal phosphate group to ADP produces one ATP molecule in each cycle. Like NADH, $FADH_2$ is a carrier of high-energy electrons and hydrogen. As we discuss shortly, the energy stored in the readily transferred high-energy electrons of NADH and $FADH_2$ is

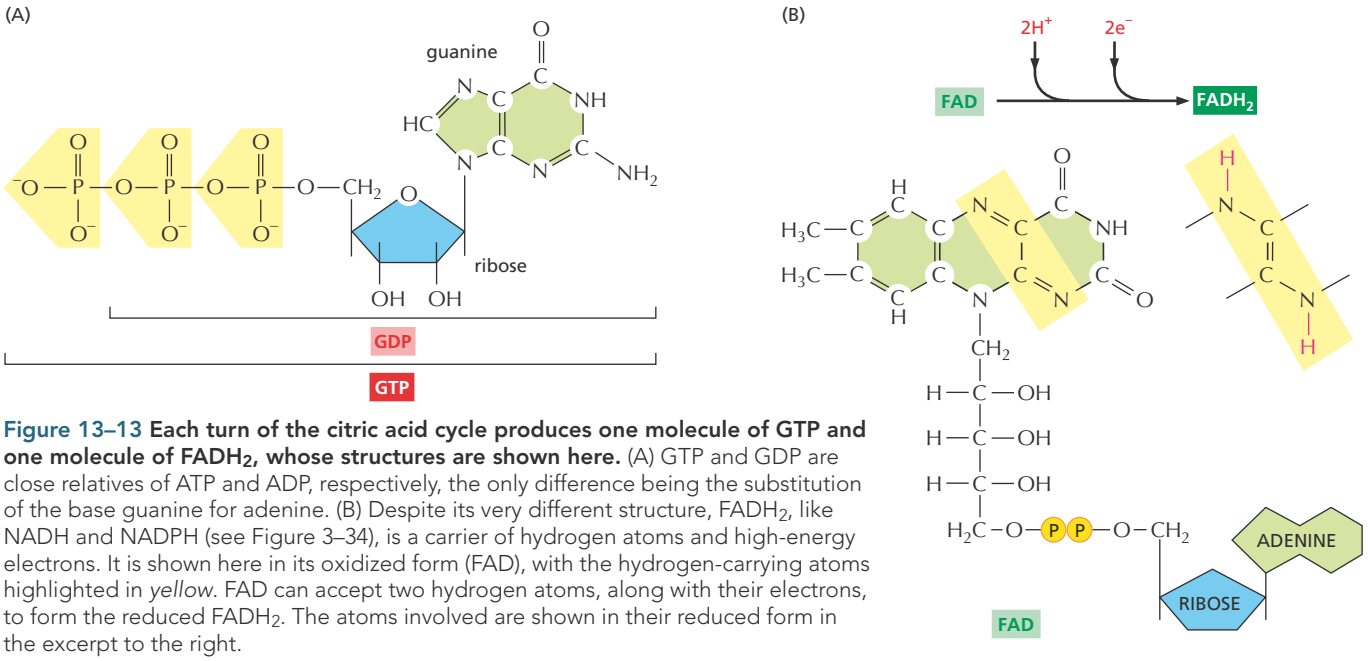


Figure 13–13 Each turn of the citric acid cycle produces one molecule of GTP and one molecule of $FADH_2$, whose structures are shown here. (A) GTP and GDP are close relatives of ATP and ADP, respectively, the only difference being the substitution of the base guanine for adenine. (B) Despite its very different structure, $FADH_2$, like NADH and NADPH (see Figure 3–34), is a carrier of hydrogen atoms and high-energy electrons. It is shown here in its oxidized form (FAD), with the hydrogen-carrying atoms highlighted in *yellow*. FAD can accept two hydrogen atoms, along with their electrons, to form the reduced $FADH_2$. The atoms involved are shown in their reduced form in the excerpt to the right.

subsequently used to produce ATP through oxidative phosphorylation on the inner mitochondrial membrane, the only step in the oxidative catabolism of foodstuffs that directly requires O_2 from the atmosphere.

A common misconception about the citric acid cycle is that the atmospheric O_2 required for the process to proceed is converted into the CO_2 that is released as a waste product. In fact, the oxygen atoms required to make CO_2 from the acetyl groups entering the citric acid cycle are supplied not by O_2 but by water. As illustrated in Panel 13–2, three molecules of water are split in each cycle, and the oxygen atoms of some of them are ultimately used to make CO_2. As we see shortly, the O_2 that we breathe is actually reduced to water by the electron-transport chain; it is not incorporated directly into the CO_2 we exhale.

Many Biosynthetic Pathways Begin with Glycolysis or the Citric Acid Cycle

Catabolic reactions, such as those of glycolysis and the citric acid cycle, produce both energy for the cell and the building blocks from which many other organic molecules are made. So far, we have emphasized energy production rather than the provision of starting materials for biosynthesis. But many of the intermediates formed in glycolysis and the citric acid cycle are siphoned off by such **anabolic pathways**, in which they are converted by series of enzyme-catalyzed reactions into amino acids, nucleotides, lipids, and other small organic molecules that the cell needs. Oxaloacetate and α-ketoglutarate from the citric acid cycle, for example, are transferred from the mitochondrial matrix back to the cytosol, where they serve as precursors for the production of many essential molecules, such as the amino acids aspartate and glutamate, respectively. An idea of the complexity of this process can be gathered from **Figure 13–14,** which illustrates some of the branches leading from the central catabolic reactions to biosyntheses.

QUESTION 13–4

Looking at the chemistry detailed in Panel 13–2 (pp. 434–435), why do you suppose it is useful to link the acetyl group first to another, larger carbon skeleton, oxaloacetate, before completely oxidizing both carbons to CO_2?

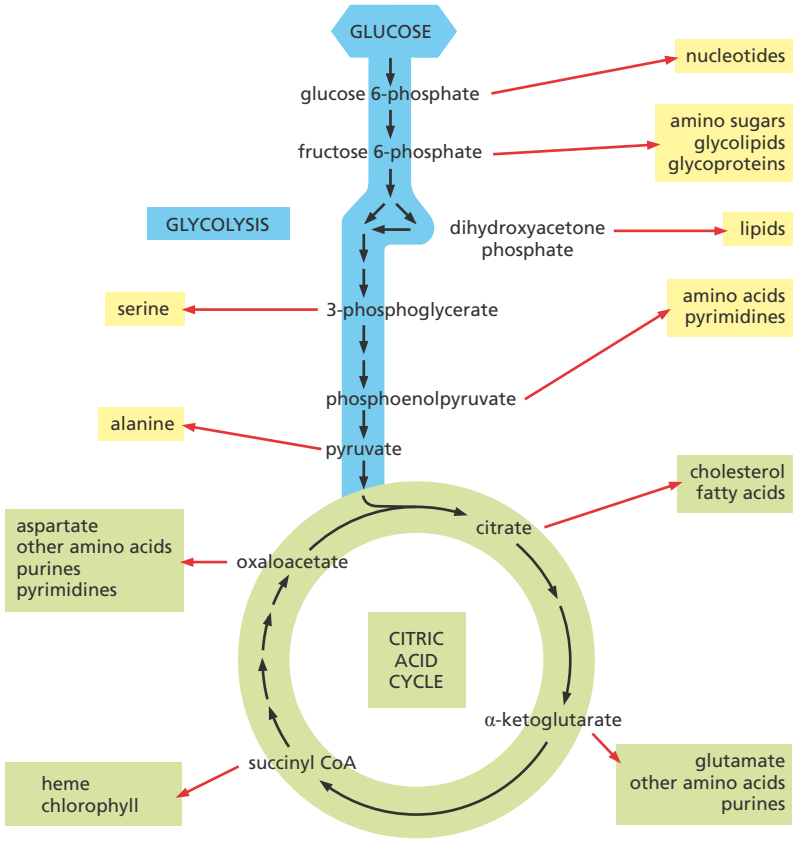
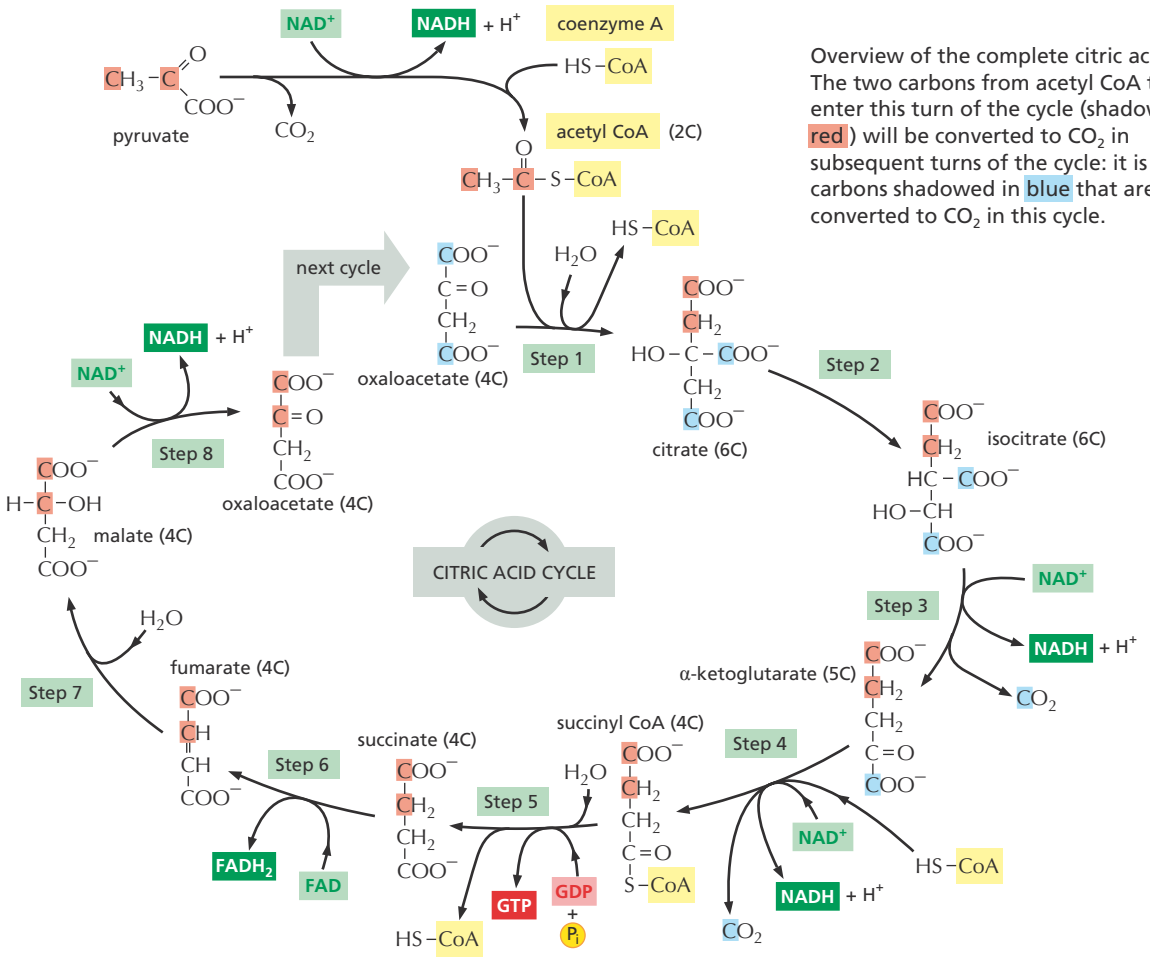


Figure 13–14 Glycolysis and the citric acid cycle provide the precursors needed for cells to synthesize many important organic molecules. The amino acids, nucleotides, lipids, sugars, and other molecules—shown here as products—in turn serve as the precursors for many of the cell's macromolecules. Each *black* arrow in this diagram denotes a single enzyme-catalyzed reaction; the *red* arrows generally represent pathways with many steps that are required to produce the indicated products.

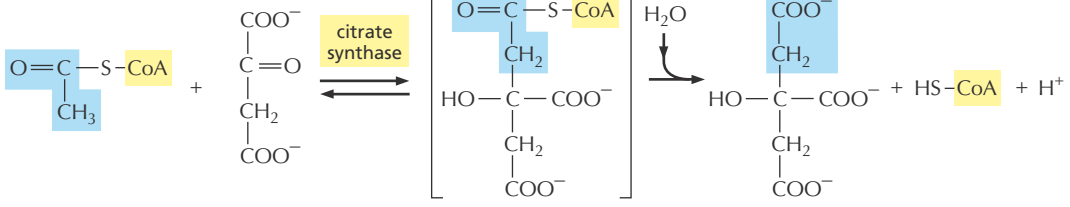
PANEL 13–2 THE COMPLETE CITRIC ACID CYCLE



Overview of the complete citric acid cycle. The two carbons from acetyl CoA that enter this turn of the cycle (shadowed in red) will be converted to CO_2 in subsequent turns of the cycle: it is the two carbons shadowed in blue that are converted to CO_2 in this cycle.

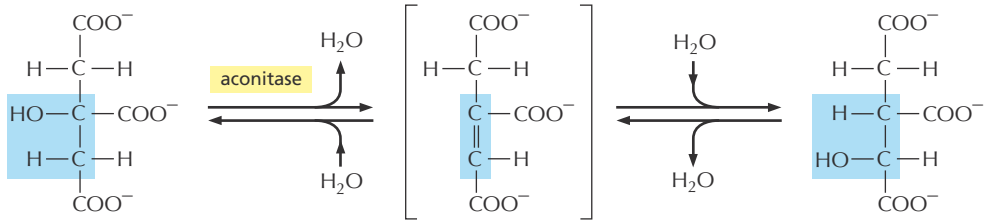
Details of these eight steps are shown below. In this part of the panel, for each step, the part of the molecule that undergoes a change is shadowed in blue, and the name of the enzyme that catalyzes the reaction is in a yellow box.
To watch a video of the reactions of the citric acid cycle, see **Movie 13.2**.

Step 1 After the enzyme removes a proton from the CH_3 group on acetyl CoA, the negatively charged CH_2^- forms a bond to a carbonyl carbon of oxaloacetate. The subsequent loss by hydrolysis of the coenzyme A (HS–CoA) drives the reaction strongly forward.



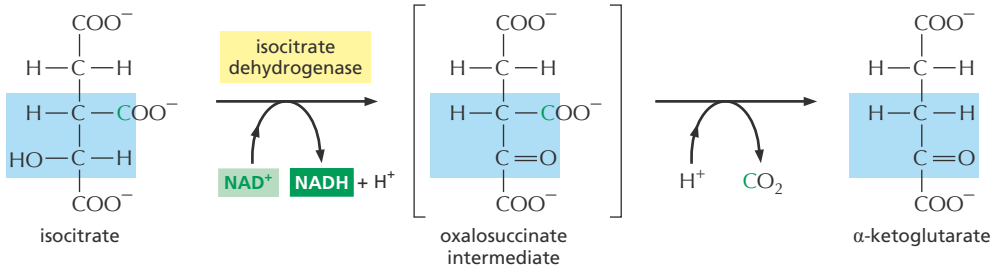
acetyl CoA · oxaloacetate · S-citryl-CoA intermediate · citrate

Step 2 An isomerization reaction, in which water is first removed and then added back, moves the hydroxyl group from one carbon atom to its neighbor.

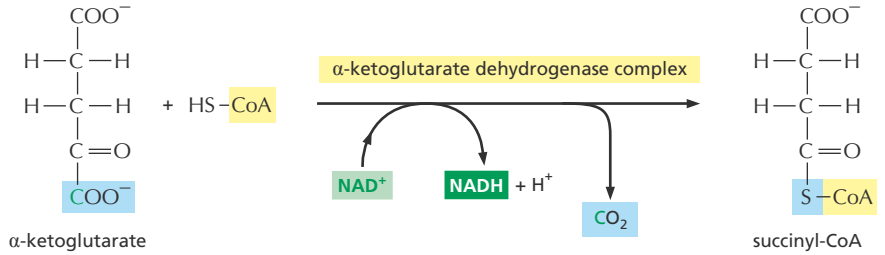


citrate · *cis*-aconitate intermediate · isocitrate

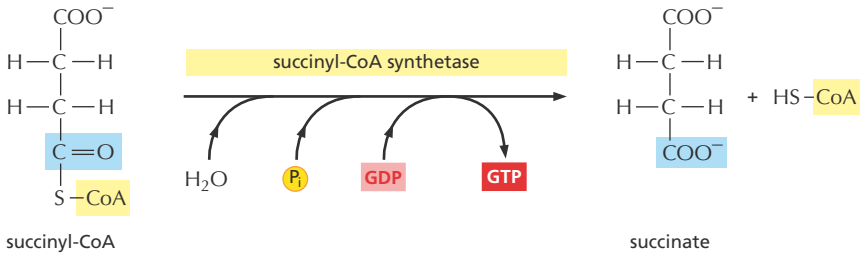
Step 3 In the first of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group. The immediate product is unstable, losing CO_2 while still bound to the enzyme.



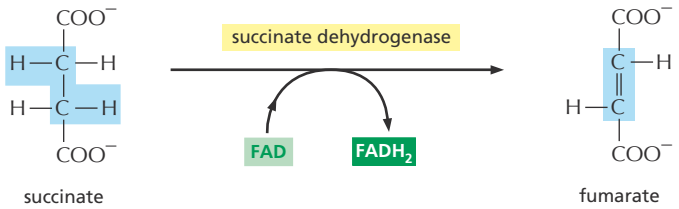
Step 4 The *α-ketoglutarate dehydrogenase complex* closely resembles the large enzyme complex that converts pyruvate to acetyl CoA, the *pyruvate dehydrogenase* complex in Figure 13–10. It likewise catalyzes an oxidation that produces NADH, CO_2, and a high-energy thioester bond to coenzyme A (CoA).



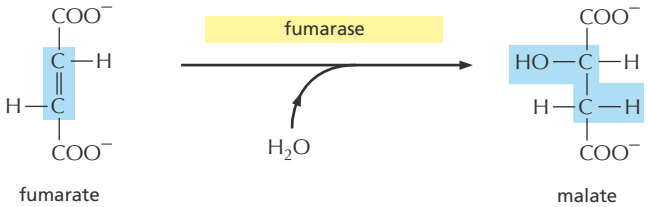
Step 5 A phosphate molecule from solution displaces the CoA, forming a high-energy phosphate linkage to succinate. This phosphate is then passed to GDP to form GTP. (In bacteria and plants, ATP is formed instead.)



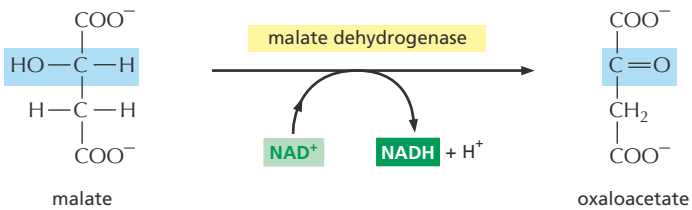
Step 6 In the third oxidation step in the cycle, FAD accepts two hydrogen atoms from succinate.



Step 7 The addition of water to fumarate places a hydroxyl group next to a carbonyl carbon.



Step 8 In the last of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group, regenerating the oxaloacetate needed for step 1.



UNRAVELING THE CITRIC ACID CYCLE

"I have often been asked how the work on the citric acid cycle arose and developed," stated biochemist Hans Krebs in a lecture and review article in which he described his Nobel Prize-winning discovery of the cycle of reactions that lies at the center of cell metabolism. Did the concept stem from a sudden inspiration, a revelatory vision? "It was nothing of the kind," answered Krebs. Instead, his realization that these reactions occur in a cycle—rather than a set of linear pathways, as in glycolysis—arose from a "very slow evolutionary process" that occurred over a five-year period, during which Krebs coupled insight and reasoning to careful experimentation to discover one of the central pathways that underlies energy metabolism.

Minced tissues, curious catalysis

By the early 1930s, Krebs and other investigators had discovered that a select set of small organic molecules are oxidized extraordinarily rapidly in various types of tissue preparations—slices of kidney or liver, or suspensions of minced pigeon muscle. Because these reactions were seen to depend on the presence of oxygen, the researchers surmised that this set of molecules might include intermediates that are important in *cell respiration*—the consumption of O_2 and production of CO_2 that occurs when tissues break down foodstuffs.

Using the minced-tissue preparations, Krebs and others made the following observations. First, in the presence of oxygen, certain organic acids—citrate, succinate, fumarate, and malate—were readily oxidized to CO_2. These reactions depended on a continuous supply of oxygen.

Second, the oxidation of these acids occurred in two linear, sequential pathways:

citrate → α-ketoglutarate → succinate

and

succinate → fumarate → malate → oxaloacetate

Third, the addition of small amounts of several of these compounds to the minced-muscle suspensions stimulated an unusually large uptake of O_2—far greater than that needed to oxidize only the added molecules. To explain this surprising observation, Albert Szent-Györgyi (the Nobel laureate who worked out the second pathway above) suggested that a single molecule of each compound must somehow act catalytically to stimulate the oxidation of many molecules of some endogenous substance in the muscle.

At this point, most of the reactions central to the citric acid cycle were known. What was not yet clear—and caused great confusion, even to future Nobel laureates—was how these apparently linear reactions could drive such a catalytic consumption of oxygen, where each molecule of metabolite fuels the oxidation of many more molecules. To simplify the discussion of how Krebs ultimately solved this puzzle—by linking these linear reactions together into a circle—we will now refer to the molecules involved by a sequence of letters, A through H (**Figure 13–15**).

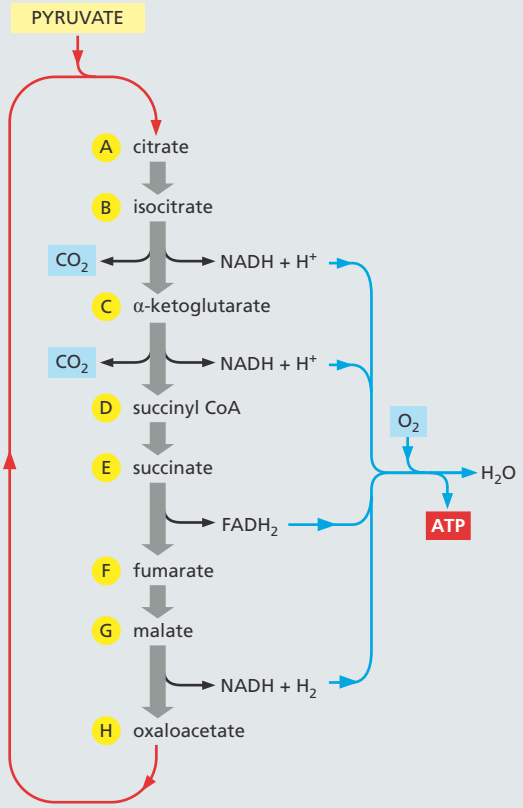


Figure 13–15 In this simplified representation of the citric acid cycle, O_2 is consumed and CO_2 is liberated as the molecular intermediates become oxidized. Krebs and others did not initially realize that these oxidation reactions occur in a cycle, as shown here.

A poison suggests a cycle

Many of the clues that Krebs used to work out the citric acid cycle came from experiments using malonate—a poisonous compound that specifically inhibits the enzyme succinate dehydrogenase, which converts E to F. Malonate closely resembles succinate (E) in its structure (**Figure 13–16**), and it serves as a competitive inhibitor of

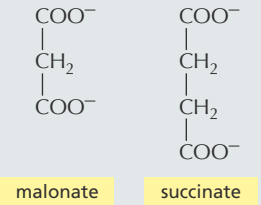


Figure 13–16 The structure of malonate closely resembles that of succinate.

the enzyme. Because the addition of malonate poisons cell respiration in tissues, Krebs concluded that succinate dehydrogenase (and the entire pathway linked to it) must play a critical role in the respiration process.

Krebs then discovered that when A, B, or C was added to malonate-poisoned tissue suspensions, E accumulated (**Figure 13–17A**). This observation reinforced the importance of succinate dehydrogenase for successful cell respiration. However, he found that E also accumulated when F, G, or H was added to malonate-poisoned muscle (**Figure 13–17B**). The latter result suggested that an additional set of reactions must exist that can convert F, G, and H molecules into E, since E was previously shown to be a precursor for F, G, and H, rather than a product of their reactions.

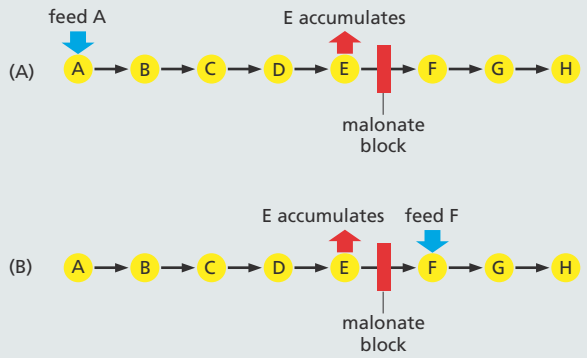


Figure 13–17 Poisoning muscle preparations with malonate provided clues to the cyclic nature of these oxidative reactions. (A) Adding A (or B or C—not shown) to malonate-poisoned muscle causes an accumulation of E. (B) Addition of F (or G or H—not shown) to a malonate-poisoned preparation also causes an accumulation of E, suggesting that enzymatic reactions can convert these molecules into E. The discovery that citrate (A) can be formed from oxaloacetate (H) and pyruvate allowed Krebs to join these two reaction pathways into a complete circle.

At about this time, Krebs also determined that when muscle suspensions were incubated with pyruvate and oxaloacetate, citrate formed: pyruvate + H → A.

This observation led Krebs to postulate that when oxygen is present, pyruvate and H condense to form A, converting the previously delineated string of linear reactions into a cyclic sequence (see Figure 13–15).

Explaining the mysterious stimulatory effects

The cycle of reactions that Krebs proposed clearly explained how the addition of small amounts of any of the intermediates A through H could cause the large increase in the uptake of O_2 that had been observed. Pyruvate is abundant in minced tissues, being readily produced by glycolysis (see Figure 13–4), using glucose derived from stored glycogen. Its oxidation requires a functioning citric acid cycle, in which each turn of the cycle results in the oxidation of one molecule of pyruvate. If the intermediates A through H are in small enough supply, the rate at which the entire cycle turns will be restricted. Adding a supply of any one of these intermediates will then have a dramatic effect on the rate at which the entire cycle operates. Thus, it is easy to see how a large number of pyruvate molecules can be oxidized, and a great deal of oxygen consumed, for every molecule of a citric acid cycle intermediate that is added (**Figure 13–18**).

Krebs went on to demonstrate that all of the individual enzymatic reactions in his postulated cycle took place in tissue preparations. Furthermore, they occured at rates high enough to account for the rate of pyruvate and oxygen consumption in these tissues. Krebs therefore concluded that this series of reactions is the major, if not the sole, pathway for the oxidation of pyruvate—at least in muscle. By fitting together pieces of information like a jigsaw puzzle, he arrived at a coherent picture of the intricate metabolic processes that underlie the oxidation—and took home a share of the 1953 Nobel Prize in Physiology or Medicine.

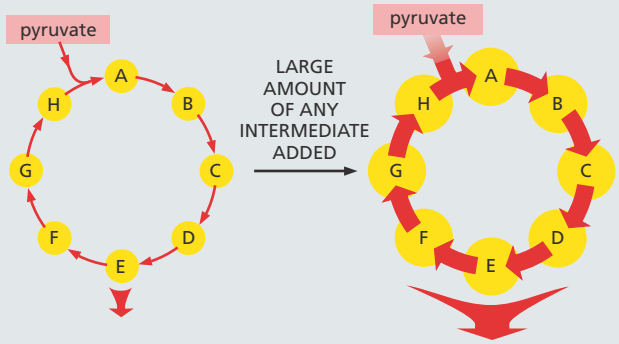


Figure 13–18 Replenishing the supply of any single intermediate has a dramatic effect on the rate at which the entire citric acid cycle operates. When the concentrations of intermediates are limiting, the cycle turns slowly and little pyruvate is used. O_2 uptake is low because only small amounts of NADH and $FADH_2$ are produced to feed oxidative phosphorylation (see Figure 13–19). But when a large amount of any one intermediate is added, the cycle turns rapidly; more of all the intermediates is made, and O_2 uptake is high.

Electron Transport Drives the Synthesis of the Majority of the ATP in Most Cells

We now return briefly to the final stage in the oxidation of food molecules: **oxidative phosphorylation**. It is in this stage that the chemical energy captured by the activated carriers produced during glycolysis and the citric acid cycle is used to generate ATP. During oxidative phosphorylation, NADH and $FADH_2$ transfer their high-energy electrons to the **electron-transport chain**—a series of electron carriers embedded in the inner mitochondrial membrane in eukaryotic cells (and in the plasma membrane of aerobic bacteria). As the electrons pass through the series of electron acceptor and donor molecules that form the chain, they fall to successively lower energy states. At specific sites in the chain, the energy released is used to drive H^+ (protons) across the inner membrane, from the mitochondrial matrix to the intermembrane space (see Figure 13–2). This movement generates a proton gradient across the inner membrane, which serves as a source of energy (like a battery) that can be tapped to drive a variety of energy-requiring reactions (discussed in Chapter 12). The most prominent of these reactions is the phosphorylation of ADP to generate ATP on the matrix side of the inner membrane (**Figure 13–19**).

At the end of the transport chain, the electrons are added to molecules of O_2 that have diffused into the mitochondrion, and the resulting reduced oxygen molecules immediately combine with protons (H^+) from the surrounding solution to produce water (see Figure 13–19). The electrons have now reached their lowest energy level, with all the available energy extracted from the food molecule being oxidized. In total, the complete oxidation of a molecule of glucose to H_2O and CO_2 can produce about 30 molecules of ATP. In contrast, only two molecules of ATP are produced per molecule of glucose by glycolysis alone.

Oxidative phosphorylation occurs in both eukaryotic cells and in aerobic bacteria. It represents a remarkable evolutionary achievement, and the ability to extract energy from food with such great efficiency has shaped the entire character of life on Earth. In the next chapter, we describe the mechanisms behind this game-changing molecular process and discuss how it likely arose.

QUESTION 13–5

What, if anything, is wrong with the following statement: "The oxygen consumed during the oxidation of glucose in animal cells is returned as part of CO_2 to the atmosphere." How could you support your answer experimentally?

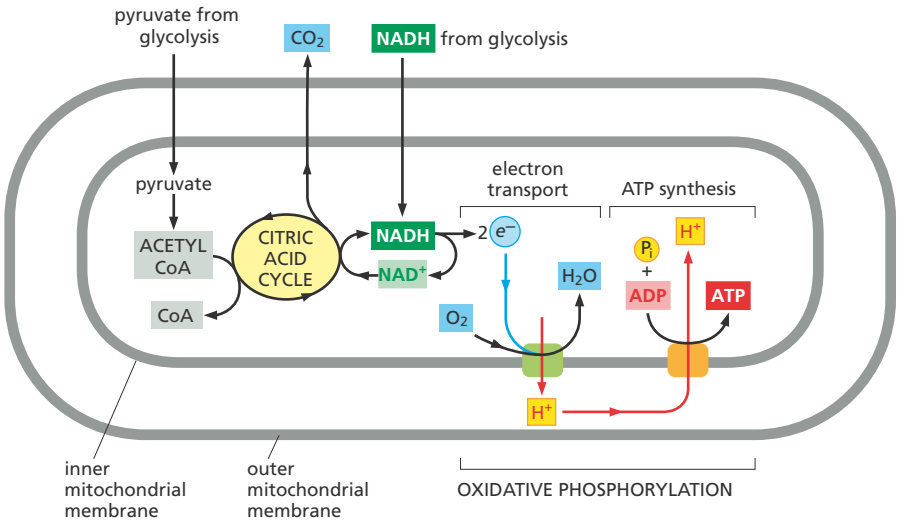


Figure 13–19 Oxidative phosphorylation completes the catabolism of food molecules and generates the bulk of the ATP made by the cell. Electron-bearing activated carriers produced by the citric acid cycle and glycolysis donate their high-energy electrons to an electron-transport chain in the inner mitochondrial membrane (or in the plasma membrane of aerobic bacteria). This electron transfer pumps protons across the inner membrane (*red arrows*). The resulting proton gradient is then used to drive the synthesis of ATP through the process of oxidative phosphorylation.

REGULATION OF METABOLISM

A cell is an intricate chemical machine, and our discussion of metabolism—with a focus on glycolysis and the citric acid cycle—has considered only a tiny fraction of the many enzymatic reactions that can take place in a cell at any time (**Figure 13–20**). For all these pathways to work together smoothly, as is required to allow the cell to survive and to respond to its environment, the choice of which pathway each metabolite will follow must be carefully regulated at every branch point.

Many sets of reactions need to be coordinated and controlled. For example, to maintain order within their cells, all organisms need to replenish their ATP pools continuously through the oxidation of sugars or fats. Yet animals have only periodic access to food, and plants need to survive without sunlight overnight, when they are unable to produce sugar through photosynthesis. Animals and plants have evolved several ways to cope with this problem. One is to synthesize food reserves in times of plenty that can be later consumed when other energy sources are scarce. Thus, depending on conditions, a cell must decide whether to route key metabolites into anabolic or catabolic pathways—in other words, whether to use them to build other molecules or burn them to provide

QUESTION 13–6

A cyclic reaction pathway requires that the starting material be regenerated and available at the end of each cycle. If compounds of the citric acid cycle are siphoned off as building blocks to make other organic molecules via a variety of metabolic reactions, why does the citric acid cycle not quickly grind to a halt?

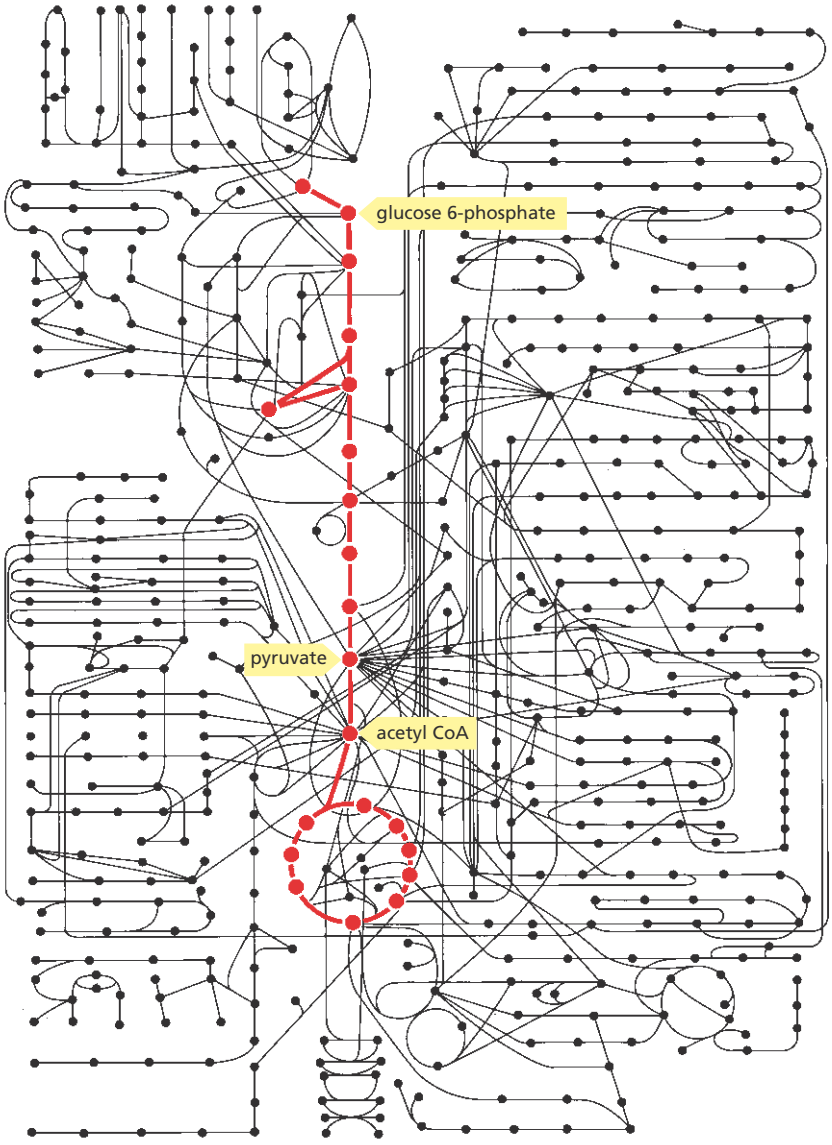


Figure 13–20 Glycolysis and the citric acid cycle constitute a small fraction of the reactions that occur in a cell. In this diagram, the filled circles represent molecules in various metabolic pathways, and the lines that connect them represent the enzymatic reactions that convert one metabolite to another. The reactions of glycolysis and the citric acid cycle are shown in *red*. Many other reactions either lead into these two central catabolic pathways—delivering small organic molecules to be oxidized for energy—or lead outward to the anabolic pathways that supply carbon compounds for biosynthesis.

immediate energy. In this section, we discuss how a cell regulates its intricate web of interconnected metabolic pathways to best serve both its immediate and long-term needs.

Catabolic and Anabolic Reactions Are Organized and Regulated

All the reactions shown in Figure 13–20 occur in a cell that is less than 0.1 mm in diameter, and each step requires a different enzyme. To add to the complexity, the same substrate is often a part of many different pathways. Pyruvate, for example, is a substrate for half a dozen or more different enzymes, each of which modifies it chemically in a different way. We have already seen that the pyruvate dehydrogenase complex converts pyruvate to acetyl CoA, and that, during fermentation, lactate dehydrogenase converts it to lactate. A third enzyme converts pyruvate to oxaloacetate, a fourth to the amino acid alanine, and so on. All these pathways compete for pyruvate molecules, and similar competitions for thousands of other small molecules go on at the same time.

To balance the activities of these interrelated reactions—and to allow organisms to adapt swiftly to changes in food availability or energy expenditure—an elaborate network of *control mechanisms* regulates and coordinates the activity of the enzymes that catalyze the myriad metabolic reactions that go on in a cell. As we discuss in Chapter 4, the activity of enzymes can be controlled by covalent modification—such as the addition or removal of a phosphate group (see Figure 4–41)—and by the binding of small regulatory molecules, often a metabolite (see pp. 150–151). Such regulation can either enhance the activity of the enzyme or inhibit it. As we see next, both types of regulation—positive and negative—control the activity of key enzymes involved in the breakdown and synthesis of glucose.

Feedback Regulation Allows Cells to Switch from Glucose Breakdown to Glucose Synthesis

Animals need an ample supply of glucose. Active muscles need glucose to power their contraction, and brain cells depend almost completely on glucose for energy. During periods of fasting or intense physical exercise, the body's glucose reserves get used up faster than they can be replenished from food. One way to increase available glucose is to synthesize it from pyruvate by a process called **gluconeogenesis**.

Gluconeogenesis is, in many ways, a reversal of glycolysis: it builds glucose from pyruvate, whereas glycolysis does the opposite. Indeed, gluconeogenesis makes use of many of the same enzymes as glycolysis; it simply runs them in reverse. For example, the isomerase that converts glucose 6-phosphate to fructose 6-phosphate in step 2 of glycolysis (see Panel 13–1, pp. 428–429) will readily catalyze the reverse reaction. There are, however, three steps in glycolysis that so strongly favor the direction of glucose breakdown that they are effectively irreversible. To get around these one-way steps, gluconeogenesis uses a special set of enzymes to catalyze a set of bypass reactions. In step 3 of glycolysis, for example, the enzyme phosphofructokinase catalyzes the phosphorylation of fructose 6-phosphate to produce the intermediate fructose 1, 6-bisphosphate. In gluconeogenesis, the enzyme fructose 1,6-bisphosphatase removes a phosphate from this intermediate to produce fructose 6-phosphate (**Figure 13–21**).

How does a cell decide whether to synthesize glucose or to degrade it? Part of the decision centers on the reactions shown in Figure 13–21. The activity of the enzyme phosphofructokinase is allosterically regulated by

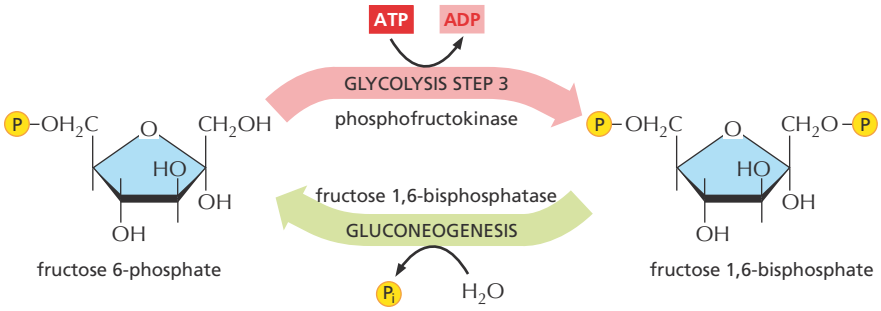


Figure 13–21 Gluconeogenesis uses specific enzymes to bypass those steps in glycolysis that are essentially irreversible. The enzyme phosphofructokinase catalyzes the phosphorylation of fructose 6-phosphate to form fructose 1, 6-bisphosphate in step 3 of glycolysis. This reaction is so energetically favorable that the enzyme will not work in reverse. To produce fructose 6-phosphate in gluconeogenesis, the enzyme fructose 1,6-bisphosphatase removes the phosphate from fructose 1,6-bisphosphate. Coordinated feedback regulation of these two enzymes helps control the flow of metabolites toward glucose synthesis or glucose breakdown.

the binding of a variety of metabolites, which provide both positive and negative *feedback regulation*. The enzyme is activated by byproducts of ATP hydrolysis—including ADP, AMP, and inorganic phosphate—and it is inhibited by ATP. Thus, when ATP is depleted and its metabolic byproducts accumulate, phosphofructokinase is turned on and glycolysis proceeds to generate ATP; when ATP is abundant, the enzyme is turned off and glycolysis shuts down. The enzyme that catalyzes the reverse reaction, fructose 1, 6–bisphosphatase (see Figure 13–21), is regulated by the same molecules but in the opposite direction. Thus this enzyme is activated when phosphofructokinase is turned off, allowing gluconeogenesis to proceed. Many such coordinated regulatory mechanisms enable a cell to respond rapidly to changing conditions and to adjust its metabolism accordingly.

Some of the biosynthetic bypass reactions required for gluconeogenesis are energetically costly. Production of a single molecule of glucose by gluconeogenesis consumes four molecules of ATP and two molecules of GTP. Thus a cell must tightly regulate the balance between glycolysis and gluconeogenesis. If both processes were to proceed simultaneously, they would shuttle metabolites back and forth in a futile cycle that would consume large amounts of energy and generate heat for no purpose.

Cells Store Food Molecules in Special Reservoirs to Prepare for Periods of Need

As we have seen, gluconeogenesis is a costly process, requiring substantial amounts of energy from the hydrolysis of ATP and GTP. During periods when food is scarce, this expensive way of producing glucose is suppressed if alternatives are available. Thus fasting cells can mobilize glucose that has been stored in the form of **glycogen**, a branched polymer of glucose (**Figure 13–22A** and see Panel 2–3, pp. 70–71). This large polysaccharide is stored as small granules in the cytoplasm of many animal cells, but mainly in liver and muscle cells (**Figure 13–22B**). The synthesis and degradation of glycogen occur by separate metabolic pathways, which can be rapidly and coordinately regulated according to need. When more ATP is needed than can be generated from food molecules taken in from the bloodstream, cells break down glycogen in a reaction that is catalyzed by the enzyme *glycogen phosphorylase*. This enzyme produces *glucose 1-phosphate*, which is then converted to the glucose 6-phosphate that feeds into the glycolytic pathway (**Figure 13–22C**).

The glycogen synthetic and degradative pathways are coordinated by feedback regulation. Enzymes in each pathway are allosterically regulated by glucose 6-phosphate, but in opposite directions: *glycogen synthetase* in the synthetic pathway is activated by glucose 6-phosphate, whereas glycogen phosphorylase, which breaks down glycogen (see Figure 13–22C), is inhibited by glucose 6-phosphate, as well as by ATP. This regulation

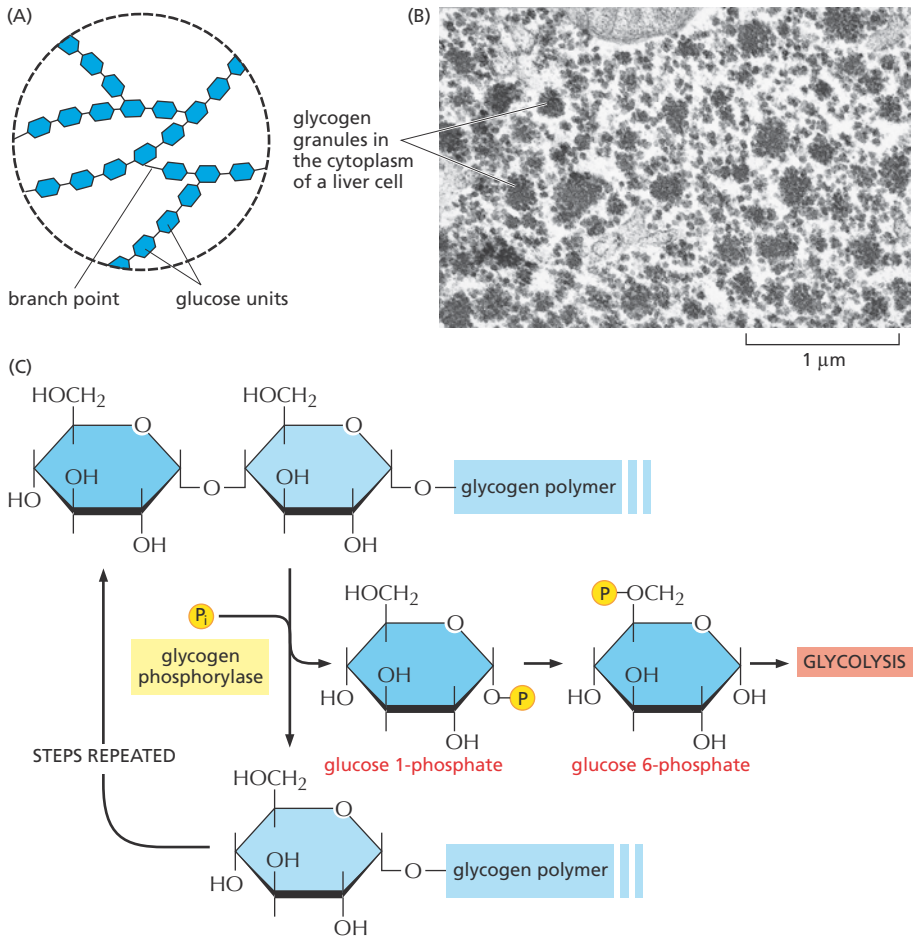


Figure 13–22 Animal cells store glucose in the form of glycogen to provide energy in times of need. (A) The structure of glycogen (starch in plants is a very similar branched polymer of glucose but has many fewer branch points). (B) An electron micrograph showing glycogen granules in the cytoplasm of a liver cell; each granule contains both glycogen and the enzymes required for glycogen synthesis and breakdown. (C) The enzyme glycogen phosphorylase breaks down glycogen when cells need more glucose. (B, courtesy of Robert Fletterick and Daniel S. Friend.)

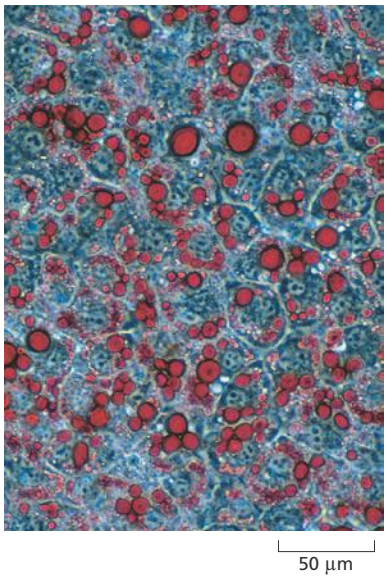


Figure 13–23 Fats are stored in the form of fat droplets in animal cells. The fat droplets (stained *red*) shown here are in the cytoplasm of developing adipocytes. (Courtesy of Peter Tontonoz and Ronald M. Evans.)

helps to prevent glycogen breakdown when ATP is plentiful and to favor glycogen synthesis when glucose 6-phosphate concentration is high. The balance between glycogen synthesis and breakdown is further regulated by intracellular signaling pathways that are controlled by the hormones insulin, adrenaline, and glucagon (see Table 16–1, p. 529 and Figure 16–25, p. 546).

Quantitatively, fat is a far more important storage material than glycogen, in part because the oxidation of a gram of fat releases about twice as much energy as the oxidation of a gram of glycogen. Moreover, glycogen binds a great deal of water, producing a sixfold difference in the actual mass of glycogen required to store the same amount of energy as fat. An average adult human stores enough glycogen for only about a day of normal activity, but enough fat to last nearly a month. If our main fuel reserves had to be carried as glycogen instead of fat, body weight would need to be increased by an average of about 60 pounds (nearly 30 kilograms).

Most of our fat is stored as droplets of water-insoluble triacylglycerols in specialized fat cells called *adipocytes* (**Figure 13–23** and see Figure 13–11 A and B). In response to hormonal signals, fatty acids can be released from these depots into the bloodstream for other cells to use as required. Such a need arises after a period of not eating. Even a normal overnight fast results in the mobilization of fat: in the morning, most of the acetyl CoA that enters the citric acid cycle is derived from fatty acids rather than from glucose. After a meal, however, most of the acetyl CoA entering the citric acid cycle comes from glucose derived from food, and any excess

Figure 13–24 Some plant seeds serve as important foods for humans. Corn, nuts, and peas all contain rich stores of starch and fats, which provide the plant embryo in the seed with energy and building blocks for biosynthesis. (Courtesy of the John Innes Foundation.)

glucose is used to make glycogen or fat. (Although animal cells can readily convert sugars to fats, they cannot convert fatty acids to sugars.)

The food reserves in both animals and plants form a vital part of the human diet. Plants convert some of the sugars they make through photosynthesis during daylight into fats and into **starch**, a branched polymer of glucose very similar to animal glycogen. The fats in plants are triacylglycerols, as they are in animals, and they differ only in the types of fatty acids that predominate (see Figures 2–19 and 2–20).

The embryo inside a plant seed must live on stored food reserves for a long time, until the seed germinates to produce a plant with leaves that can harvest the energy in sunlight. The embryo uses these food stores as sources of energy and of small molecules to build cell walls and to synthesize many other biological molecules as it develops. For this reason, plant seeds often contain especially large amounts of fats and starch—which make them a major food source for animals, including ourselves (**Figure 13–24**). Germinating seeds convert the stored fat and starch into glucose as needed.

In plant cells, fats and starch are both stored in chloroplasts—specialized organelles that carry out photosynthesis (**Figure 13–25**). These energy-rich molecules serve as food reservoirs that are mobilized by the cell to produce ATP in mitochondria during periods of darkness. In the next chapter, we take a closer look at chloroplasts and mitochondria, and review the elaborate mechanisms by which they harvest energy from sunlight and from food.

QUESTION 13–7

After looking at the structures of sugars and fatty acids (discussed in Chapter 2), give an intuitive explanation as to why oxidation of a sugar yields only about half as much energy as the oxidation of an equivalent dry weight of a fatty acid.

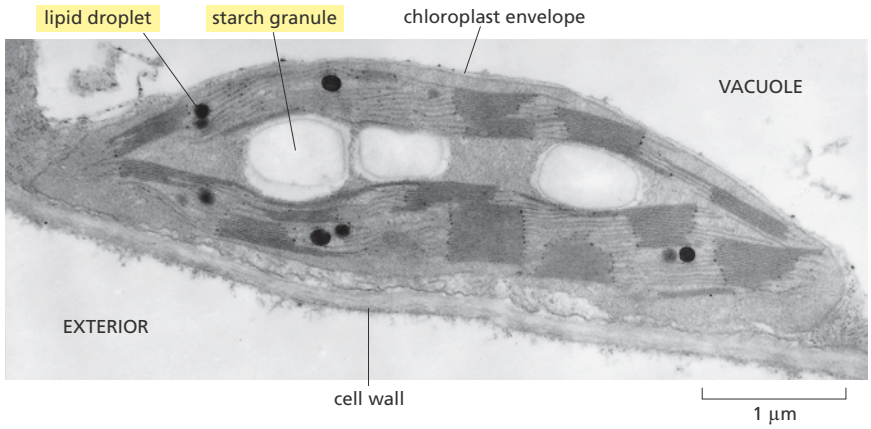


Figure 13–25 Plant cells store both starch and fats in their chloroplasts. An electron micrograph of a single chloroplast in a plant cell shows the starch granules and lipid droplets (fats) that have been synthesized in the organelle. (Courtesy of K. Plaskitt.)

ESSENTIAL CONCEPTS

- Food molecules are broken down in successive steps, in which energy is captured in the form of activated carriers such as ATP and NADH.
- In plants and animals, these catabolic reactions occur in different cell compartments: glycolysis in the cytosol, the citric acid cycle in the mitochondrial matrix, and oxidative phosphorylation on the inner mitochondrial membrane.
- During glycolysis, the six-carbon sugar glucose is split to form two molecules of the three-carbon sugar pyruvate, producing small amounts of ATP and NADH.
- In the presence of oxygen, eukaryotic cells convert pyruvate into acetyl CoA plus CO_2 in the mitochondrial matrix. The citric acid cycle then converts the acetyl group in acetyl CoA to CO_2 and H_2O, capturing much of the energy released as high-energy electrons in the activated carriers NADH and $FADH_2$.
- Fatty acids produced from the digestion of fats are also imported into mitochondria and converted to acetyl CoA molecules, which are then further oxidized through the citric acid cycle.
- In the mitochondrial matrix, NADH and $FADH_2$ pass their high-energy electrons to an electron-transport chain in the inner mitochondrial membrane, where a series of electron transfers is used to drive the formation of ATP. Most of the energy captured during the breakdown of food molecules is harvested during this process of oxidative phosphorylation (described in detail in Chapter 14).
- Many intermediates of glycolysis and the citric acid cycle are starting points for the anabolic pathways that lead to the synthesis of proteins, nucleic acids, and the many other organic molecules of the cell.
- The thousands of different reactions carried out simultaneously by a cell are regulated and coordinated by positive and negative feedback, enabling the cell to adapt to changing conditions; for example, such feedback allows a cell to switch from glucose breakdown to glucose synthesis when food is scarce.
- Cells store food molecules in special reserves. Glucose subunits are stored as glycogen in animal cells and as starch in plant cells; both animal and plant cells store fatty acids as fats. The food reserves stored by plants are major sources of food for animals, including humans.

KEY TERMS

acetyl CoA
ADP, ATP
anabolic pathways
catabolism
cell respiration
citric acid cycle
electron-transport chain
FAD, $FADH_2$
fat
fermentation
GDP, GTP
gluconeogenesis
glucose
glycogen
glycolysis
NAD^+, NADH
oxidative phosphorylation
pyruvate
starch

QUESTIONS

QUESTION 13–8

The oxidation of sugar molecules by the cell takes place according to the general reaction $C_6H_{12}O_6$ (glucose) + $6O_2 \rightarrow 6CO_2 + 6H_2O$ + energy. Which of the following statements are correct? Explain your answers.

A. All of the energy produced is in the form of heat.

B. None of the produced energy is in the form of heat.

C. The energy is produced by a process that involves the oxidation of carbon atoms.

D. The reaction supplies the cell with essential water.

E. In cells, the reaction takes place in more than one step.

F. Many steps in the oxidation of sugar molecules involve reaction with oxygen gas.

G. Some organisms carry out the reverse reaction.

H. Some cells that grow in the absence of O_2 produce CO_2.

QUESTION 13–9

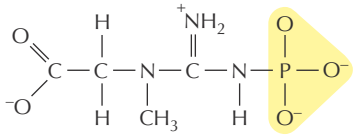
An exceedingly sensitive instrument (yet to be devised) shows that one of the carbon atoms in Charles Darwin's last breath is resident in your bloodstream, where it forms part of a hemoglobin molecule. Suggest how this carbon atom might have traveled from Darwin to you, and list some of the molecules it could have entered en route.

QUESTION 13–10

Yeast cells can grow both in the presence of O_2 (aerobically) and in its absence (anaerobically). Under which of the two conditions could you expect the cells to grow better? Explain your answer.

QUESTION 13–11

During movement, muscle cells require large amounts of ATP to fuel their contractile apparatus. These cells contain high levels of creatine phosphate (**Figure Q13–11**), which has a standard free-energy change ($\Delta G°$) for hydrolysis of its phosphate bond of –10.3 kcal/mole. Why is this a useful compound to store energy? Justify your answer with the information shown in Figure 13–8.



creatine phosphate

Figure Q13–11

QUESTION 13–12

Identical pathways that make up the complicated sequence of reactions of glycolysis, shown in Panel 13–1 (pp. 428–429), are found in most living cells, from bacteria to humans. One could envision, however, countless alternative chemical reaction mechanisms that would allow the oxidation of sugar molecules and that could, in principle, have evolved to take the place of glycolysis. Discuss this fact in the context of evolution.

QUESTION 13–13

An animal cell, roughly cubical in shape with side length of 10 μm, uses 10^9 ATP molecules every minute. Assume that the cell replaces this ATP by the oxidation of glucose according to the overall reaction $6O_2 + C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O$ and that complete oxidation of each glucose molecule produces 30 ATP molecules. How much oxygen does the cell consume every minute? How long will it take before the cell has used up an amount of oxygen gas equal to its own volume? (Recall that one mole of a gas has a volume of 22.4 liter.)

QUESTION 13–14

Under the conditions existing in the cell, the free energies of the first few reactions in glycolysis (in Panel 13–1, pp. 428–429) are:

step 1 ΔG = –8.0 kcal/mole

step 2 ΔG = –0.6 kcal/mole

step 3 ΔG = –5.3 kcal/mole

step 4 ΔG = –0.3 kcal/mole

Are these reactions energetically favorable? Using these values, draw to scale an energy diagram (A) for the overall reaction and (B) for the pathway composed of the four individual reactions.

QUESTION 13–15

The chemistry of most metabolic reactions was deciphered by synthesizing metabolites containing atoms that are different isotopes from those occurring naturally. The products of reactions starting with isotopically labeled metabolites can be analyzed to determine precisely which atoms in the products are derived from which atoms in the starting material. The methods of detection exploit, for example, the fact that different isotopes have different masses that can be distinguished using biophysical techniques such as mass spectrometry. Moreover, some isotopes are radioactive and can therefore be readily recognized with electronic counters or photographic film that becomes exposed by radiation.

A. Assume that pyruvate containing radioactive ^{14}C in its carboxyl group is added to a cell extract that can support oxidative phosphorylation. Which of the molecules produced should contain the vast majority of the ^{14}C that was added?

B. Assume that oxaloacetate containing radioactive ^{14}C in its keto group (refer to Panel 13–2, pp. 434–435) is added to the extract. Where should the ^{14}C atom be located after precisely one turn of the cycle?

QUESTION 13–16

In cells that can grow both aerobically and anaerobically, fermentation is inhibited in the presence of O_2. Suggest a reason for this observation.

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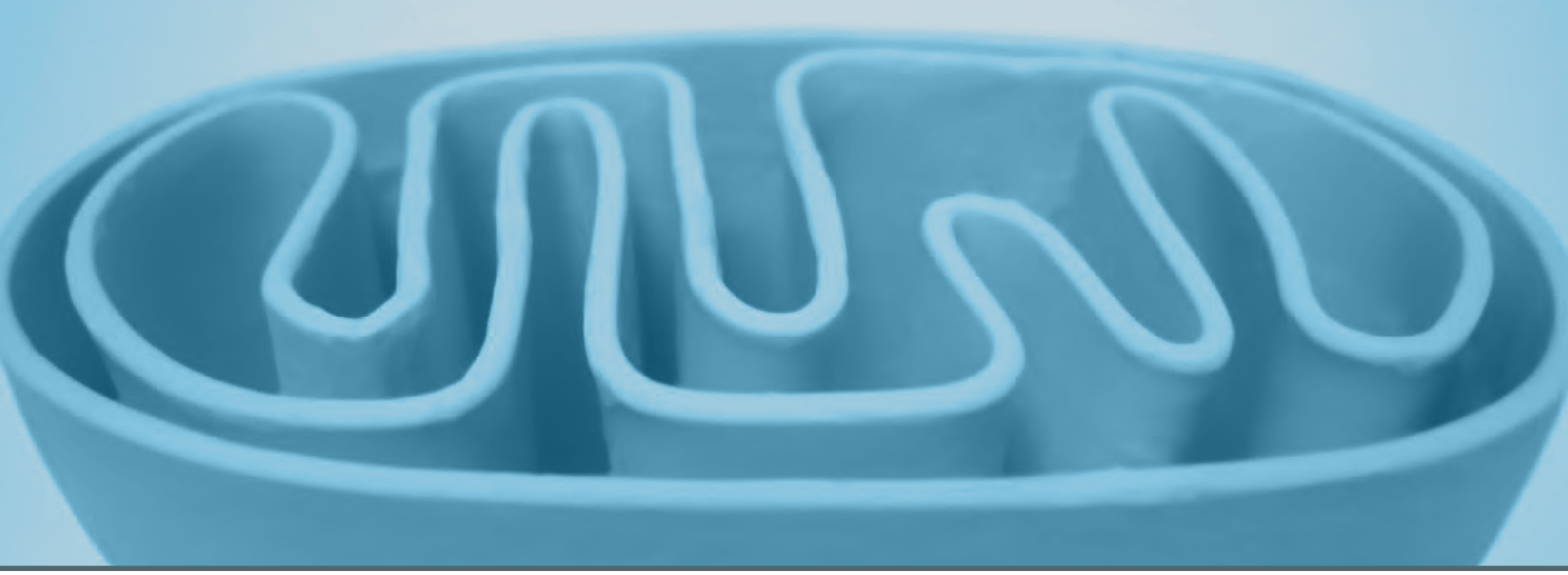


CHAPTER **FOURTEEN** 14

Energy Generation in Mitochondria and Chloroplasts

The fundamental need to generate energy efficiently has had a profound influence on the history of life on Earth. Much of the structure, function, and evolution of cells and organisms can be related to their need for energy. With no oxygen in the atmosphere, it is thought that the earliest cells may have produced ATP by breaking down organic molecules that had been generated by geochemical processes. Such fermentation reactions, discussed in Chapter 13, occur in the cytosol of present-day cells, where they use the energy derived from the partial oxidation of energy-rich food molecules to form ATP.

But early in the history of life, a much more efficient mechanism for generating energy and synthesizing ATP appeared—one based on the transport of electrons along membranes. Billions of years later, this mechanism is so central to the survival of life on Earth that we devote this entire chapter to it. As we will see, membrane-based electron-transport mechanisms are used by cells to extract energy from a wide variety of sources. These mechanisms are central to both the conversion of light energy into chemical-bond energy in photosynthesis and to the generation of large amounts of ATP from food molecules during **cell respiration**. Although membrane-based electron transport first appeared in bacteria more than 3 billion years ago, the descendants of these pioneering cells now crowd every corner and crevice of our planet's land and oceans with a wild menagerie of living forms. Perhaps even more remarkably, remnants of these bacteria survive within every living eukaryotic cell in the form of chloroplasts and mitochondria.

In this chapter, we consider the molecular mechanisms by which electron transport enables cells to generate the energy they need to survive.

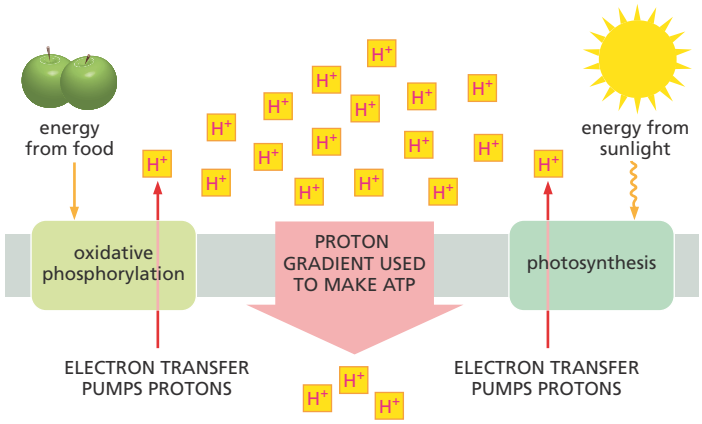


Figure 14–1 Membrane-based mechanisms use the energy provided by food or sunlight to generate ATP. In oxidative phosphorylation, which occurs in mitochondria, an electron-transport system uses energy derived from the oxidation of food to generate a proton (H^+) gradient across a membrane. In photosynthesis, which occurs in chloroplasts, an electron-transport system uses energy derived from the sun to generate a proton gradient across a membrane. In both cases, this proton gradient is then used to drive ATP synthesis.

We describe how such systems operate in both mitochondria and chloroplasts, and we review the chemical principles that allow the transfer of electrons to release large amounts of energy. Finally, we trace the evolutionary pathways that gave rise to these mechanisms.

But first, we take a brief look at the general principles central to the generation of energy in all living things: the use of a membrane to harness the energy of moving electrons.

Cells Obtain Most of Their Energy by a Membrane-based Mechanism

The main chemical energy currency in cells is ATP (see Figure 3–32). Small amounts of ATP are generated during glycolysis in the cytosol of all cells (discussed in Chapter 13). But for the majority of cells, most of their ATP is produced by *oxidative phosphorylation*. The generation of ATP by oxidative phosphorylation differs from the way ATP is produced during glycolysis, in that it requires a membrane. In eukaryotic cells, oxidative phosphorylation takes place in mitochondria, and it depends on an electron-transport process that drives the transport of protons (H^+) across the inner mitochondrial membrane. A related membrane-based process produces ATP during photosynthesis in plants, algae, and photosynthetic bacteria (**Figure 14–1**).

This membrane-based process for making ATP consists of two linked stages: one sets up an electrochemical proton gradient, the other uses that gradient to generate ATP. Both stages are carried out by special protein complexes in the membrane.

1. In Stage 1, high-energy electrons derived from the oxidation of food molecules (discussed in Chapter 13), from sunlight, or from other sources (discussed later) are transferred along a series of electron carriers—called an **electron-transport chain**—embedded in the membrane. These electron transfers release energy that is used to pump protons, derived from the water that is ubiquitous in cells, across the membrane and thus generate an electrochemical proton gradient (**Figure 14–2A**). An ion gradient across a membrane is a form of stored energy that can be harnessed to do useful work when the ions are allowed to flow back across the membrane down their electrochemical gradient (discussed in Chapter 12).
2. In Stage 2 of oxidative phosphorylation, protons flow back down their electrochemical gradient through a protein complex called *ATP synthase*, which catalyzes the energy-requiring synthesis of ATP from ADP and inorganic phosphate (P_i). This ubiquitous enzyme functions like a turbine, permitting the proton gradient to drive the production of ATP (**Figure 14–2B**).

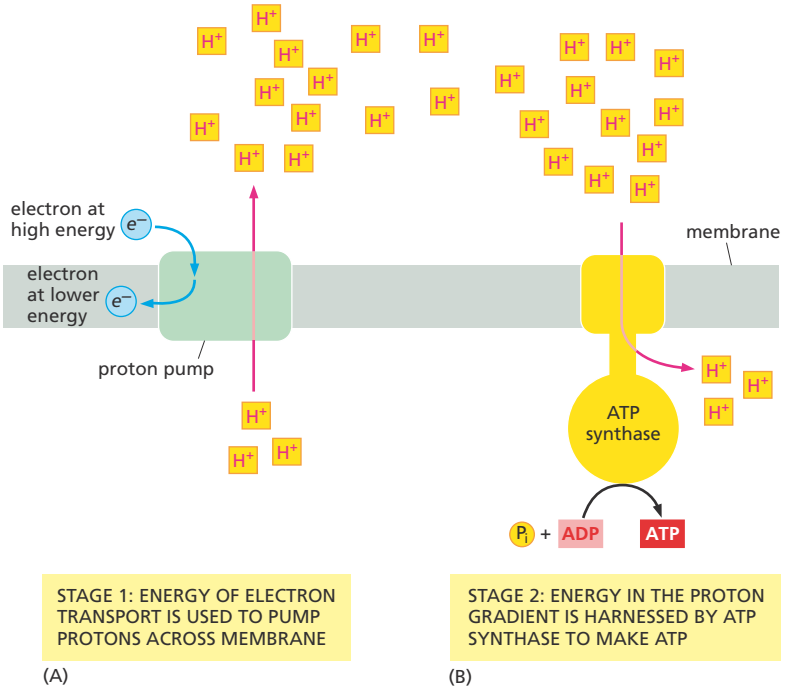


Figure 14–2 Membrane-based systems use the energy stored in an electrochemical proton gradient to synthesize ATP. The process occurs in two stages. (A) In the first stage, a proton pump harnesses the energy of electron transfer (details not shown here) to pump protons (H^+) derived from water, creating a proton gradient across the membrane. The *blue arrow* shows the direction of electron movement. These high-energy electrons can come from organic or inorganic molecules, or they can be produced by the action of light on special molecules such as chlorophyll. (B) The proton gradient produced in (A) serves as a versatile energy store. It is used to drive a variety of energy-requiring reactions in mitochondria, chloroplasts, and prokaryotes—including the synthesis of ATP by an ATP synthase.

When it was first proposed in 1961, this mechanism for generating energy was called the *chemiosmotic hypothesis*, because it linked the chemical bond-forming reactions that synthesize ATP ("chemi-") with the membrane transport processes that pump protons ("osmotic," from the Greek *osmos*, "to push"). Thanks to this chemiosmotic mechanism, now known as **chemiosmotic coupling**, cells can harness the energy of electron transfers in much the same way that the energy stored in a battery can be harnessed to do useful work (**Figure 14–3**).

Chemiosmotic Coupling is an Ancient Process, Preserved in Present-Day Cells

The membrane-based, chemiosmotic mechanism for making ATP arose very early in life's history. The exact same type of ATP-generating processes occur in the plasma membrane of modern bacteria and archaea. Apparently, the mechanism was so successful that its essential features have been retained in the long evolutionary journey from early prokaryotes to present-day cells.

This remarkable resemblance can be attributed in part to the fact that the organelles that produce ATP in eukaryotic cells—the chloroplasts and mitochondria—evolved from bacteria that were engulfed by ancestral cells more than a billion years ago (see Figures 1–18 and 1–20). As evidence of their bacterial ancestry, both chloroplasts and mitochondria reproduce in a manner similar to that of most prokaryotes (**Figure 14–4**).

QUESTION 14–1

Dinitrophenol (DNP) is a small molecule that renders membranes permeable to protons. In the 1940s, small amounts of this highly toxic compound were given to patients to induce weight loss. DNP was effective in melting away the pounds, especially promoting the loss of fat reserves. Can you explain how it might cause such loss? As an unpleasant side reaction, however, patients had an elevated temperature and sweated profusely during the treatment. Provide an explanation for these symptoms.

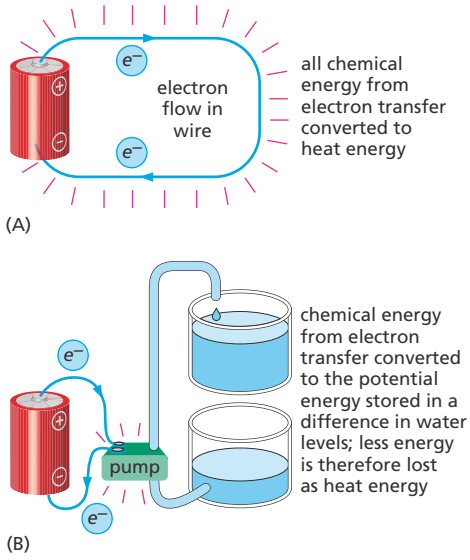
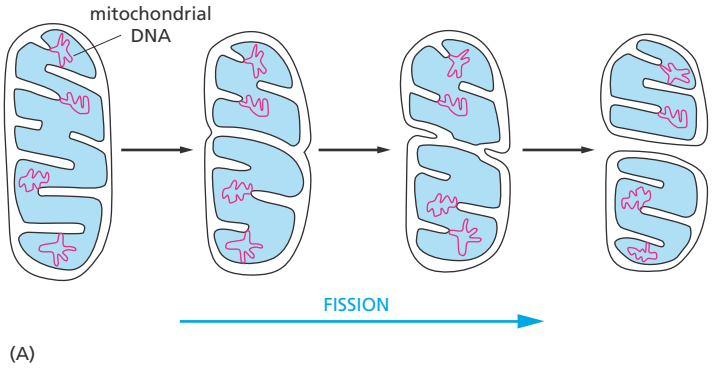


Figure 14–3 Batteries can use the energy of electron transfer to perform work. (A) If a battery's terminals are directly connected to each other, the energy released by electron transfer is all converted into heat. (B) If the battery is connected to a pump, much of the energy released by electron transfers can be harnessed to do work instead (in this case, to pump water). Cells can similarly harness the energy of electron transfer to do work—for example, pumping H^+ (see Figure 14–2A).



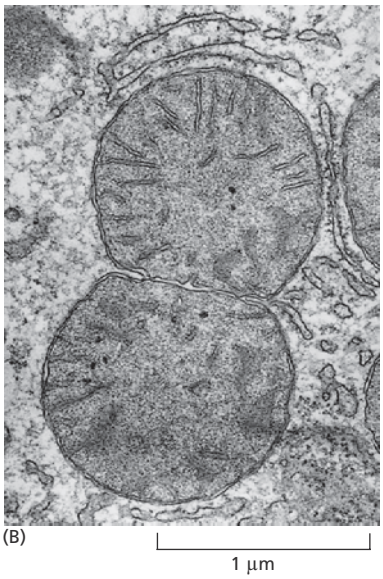


Figure 14–4 A mitochondrion can divide like a bacterium. (A) It undergoes a fission process that is conceptually similar to bacterial division. (B) An electron micrograph of a dividing mitochondrion in a liver cell. (B, courtesy of Daniel S. Friend.)

They also harbor bacterial-like biosynthetic machinery for making RNA and proteins, and they retain their own genomes (**Figure 14–5**). Many chloroplast genes are strikingly similar to those of cyanobacteria—the photosynthetic bacteria from which chloroplasts are thought to have been derived.

Although mitochondria and chloroplasts still contain DNA, the bacteria that gave rise to these organelles gave up many of the genes required for independent living as they developed the symbiotic relationships that led to the evolution of eukaryotic animal and plant cells. These jettisoned genes were not lost, however; many moved to the cell nucleus, where they continue to direct the production of proteins that mitochondria and chloroplasts import to carry out their specialized functions—including the generation of ATP, a process we discuss in detail throughout the remainder of the chapter.

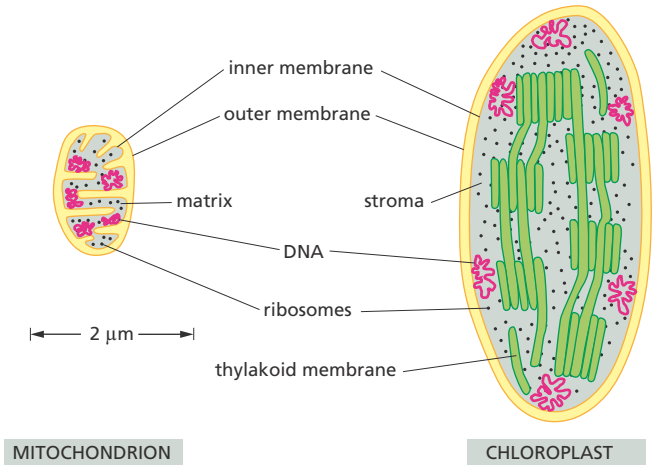


Figure 14–5 Mitochondria and chloroplasts share many of the features of their bacterial ancestors. Both organelles contain their own DNA-based genome and the machinery to copy this DNA and to make RNA and protein. The inner compartments of these organelles—the mitochondrial matrix and the chloroplast stroma—contain the DNA (*red*) and a special set of ribosomes. Membranes in both organelles—the mitochondrial inner membrane and the chloroplast thylakoid membrane—contain the protein complexes involved in ATP production.

MITOCHONDRIA AND OXIDATIVE PHOSPHORYLATION

Mitochondria are present in nearly all eukaryotic cells, where they produce the bulk of the cell's ATP. Without mitochondria, eukaryotes would have to rely on the relatively inefficient process of glycolysis for all of their ATP production. When glucose is converted to pyruvate by glycolysis in the cytosol, the net result is that only two molecules of ATP are produced per glucose molecule, which is less than 10% of the total free energy potentially available from oxidizing the sugar. By contrast, about 30 molecules of ATP are produced when mitochondria are recruited to complete the oxidation of glucose that begins in glycolysis. Had ancestral cells not established the relationship with the bacteria that gave rise to modern mitochondria, it seems unlikely that complex multicellular organisms could have evolved.

The importance of mitochondria is further highlighted by the dire consequences of mitochondrial dysfunction. For example, patients with an inherited disorder called *myoclonic epilepsy and ragged red fiber disease* (*MERRF*) are deficient in multiple proteins required for electron transport. As a result, they typically experience muscle weakness, heart problems, epilepsy, and often dementia. Muscle and nerve cells are especially sensitive to mitochondrial defects, because they need so much ATP to function normally.

In this section, we review the structure and function of mitochondria. We outline how this organelle makes use of an electron-transport chain, embedded in its inner membrane, to generate the proton gradient needed to drive the synthesis of ATP. And we consider the overall efficiency with which this membrane-based system converts the energy stored in food molecules into the energy contained in the phosphate bonds of ATP.

Mitochondria Can Change Their Shape, Location, and Number to Suit a Cell's Needs

Isolated mitochondria are generally similar in size and shape to their bacterial ancestors. Although they are no longer capable of living independently, mitochondria are remarkably adaptable and can adjust their location, shape, and number to suit the needs of the cell. In some cells, mitochondria remain fixed in one location, where they supply ATP directly to a site of unusually high energy consumption. In a heart muscle cell, for example, mitochondria are located close to the contractile apparatus, whereas in a sperm they are wrapped tightly around the motile flagellum (**Figure 14–6**). In other cells, mitochondria fuse to form elongated, dynamic tubular networks, which are diffusely distributed through

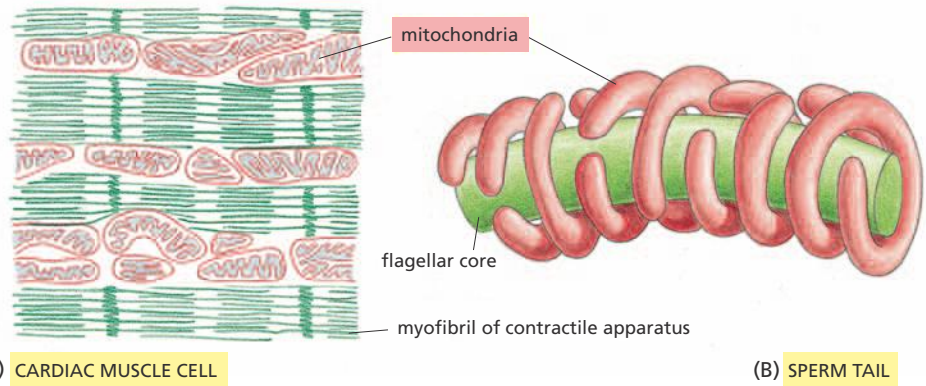


Figure 14–6 Some mitochondria are located near sites of high ATP utilization. (A) In a cardiac muscle cell, mitochondria are located close to the contractile apparatus, in which ATP hydrolysis provides the energy for contraction. (B) In a sperm, mitochondria are located in the tail, wrapped around a portion of the motile flagellum that requires ATP for its movement.

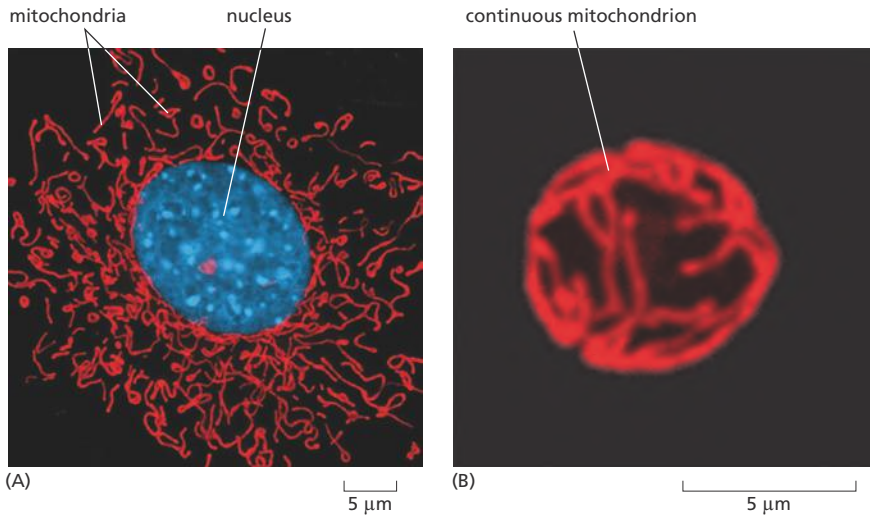


Figure 14–7 Mitochondria often fuse to form elongated tubular networks, which can extend throughout the cytoplasm. (A) Mitochondria (*red*) are fluorescently labeled in this cultured mouse fibroblast. (B) In a yeast cell, the mitochondria (*red*) form a continuous network, tucked against the plasma membrane. (A, courtesy of Michael W. Davidson, Carl Zeiss Microscopy Online Campus; B, from J. Nunnari et al., *Mol. Biol. Cell*. 8:1233–1242, 1997. With permission by The American Society for Cell Biology.)

the cytoplasm (**Figure 14–7**). These networks are dynamic, continually breaking apart by fission (see Figure 14–4) and fusing again.

Mitochondria are present in large numbers—1000 to 2000 in a liver cell, for example. But their numbers vary depending on the cell type and can change with the energy needs of the cell. In skeletal muscle cells, for example, mitochondria can divide until their numbers increase five- to tenfold if the muscle has been repeatedly stimulated to contract.

Regardless of their varied appearance, location, and number, however, all mitochondria have the same basic internal structure—a design that supports the efficient production of ATP, as we see next.

A Mitochondrion Contains an Outer Membrane, an Inner Membrane, and Two Internal Compartments

An individual mitochondrion is bounded by two highly specialized membranes—one surrounding the other. These membranes, called the outer and inner mitochondrial membranes, create two mitochondrial compartments: a large internal space called the **matrix** and a much narrower *intermembrane space* (**Figure 14–8**). When purified mitochondria are gently fractionated into separate components and their contents analyzed (see Panel 4–3, pp. 164–165), each of the membranes, and the spaces they enclose, are found to contain a unique collection of proteins.

The *outer membrane* contains many molecules of a transport protein called *porin*, which, forms wide aqueous channels through the lipid bilayer (described in Chapter 11). As a result, the outer membrane is like a sieve that is permeable to all molecules of 5000 daltons or less, including small proteins. This makes the intermembrane space chemically equivalent to the cytosol with respect to the small molecules and inorganic ions it contains. In contrast, the *inner membrane*, like other membranes in the cell, is impermeable to the passage of ions and most small molecules, except where a path is provided by specific membrane transport proteins. The mitochondrial matrix therefore contains only molecules that are selectively transported into the matrix across the inner membrane, and so its contents are highly specialized.

The inner mitochondrial membrane is the site of oxidative phosphorylation, and it contains the proteins of the electron-transport chain, the proton pumps, and the ATP synthase required for ATP production. It also contains a variety of transport proteins that allow the entry of selected small molecules—such as pyruvate and fatty acids that will be oxidized by the mitochondrion—into the matrix.

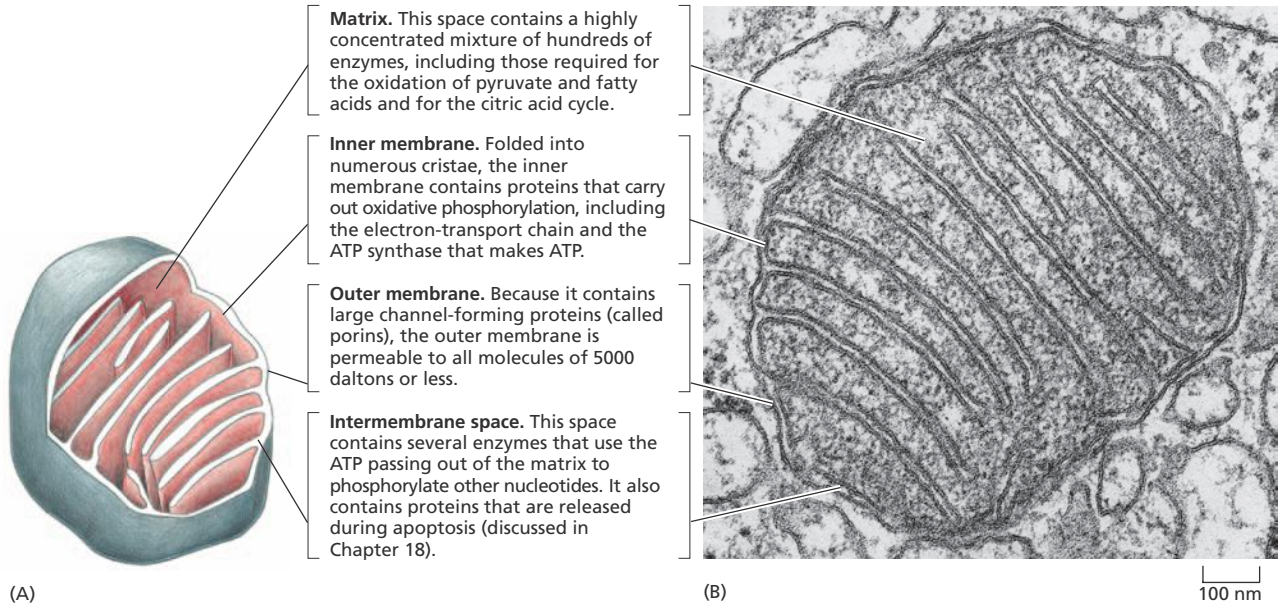


Figure 14–8 A mitochondrion is organized into four separate compartments. (A) A schematic drawing and (B) an electron micrograph of a mitochondrion. Each compartment contains a unique set of proteins, enabling it to perform its distinct functions. In liver mitochondria, an estimated 67% of the total mitochondrial protein is located in the matrix, 21% in the inner membrane, 6% in the outer membrane, and 6% in the intermembrane space. (B, courtesy of Daniel S. Friend.)

The inner membrane is highly convoluted, forming a series of infoldings—known as *cristae*—that project into the matrix space (see Figure 14–8 and **Movie 14.1**). These folds greatly increase the surface area of the membrane. In a liver cell, for example, the inner membranes of all the mitochondria make up about one-third of the total membranes of the cell. And the number of cristae in a mitochondrion of a cardiac muscle cell is three times greater than that in a mitochondrion from a liver cell.

The Citric Acid Cycle Generates the High-Energy Electrons Required for ATP Production

The generation of ATP is powered by the flow of electrons that are derived from the burning of carbohydrates, fats, and other foodstuffs during glycolysis and the citric acid cycle (discussed in Chapter 13). These high-energy electrons are provided by activated carriers generated during these two stages of catabolism, with the majority being churned out by the citric acid cycle that operates in the mitochondrial matrix.

The citric acid cycle gets the fuel it needs to produce these activated carriers from food-derived molecules that make their way into mitochondria from the cytosol. Both the pyruvate produced by glycolysis, which takes place in the cytosol, and the fatty acids derived from the breakdown of fats (see Figure 13–3) can enter the mitochondrial intermembrane space through the porins in the outer mitochondrial membrane. These fuel molecules are then transported across the inner mitochondrial membrane into the matrix, where they are converted into the crucial metabolic intermediate, acetyl CoA (**Figure 14–9**). The acetyl groups in acetyl CoA are

QUESTION 14–2

Electron micrographs show that mitochondria in heart muscle have a much higher density of cristae than mitochondria in skin cells. Suggest an explanation for this observation.

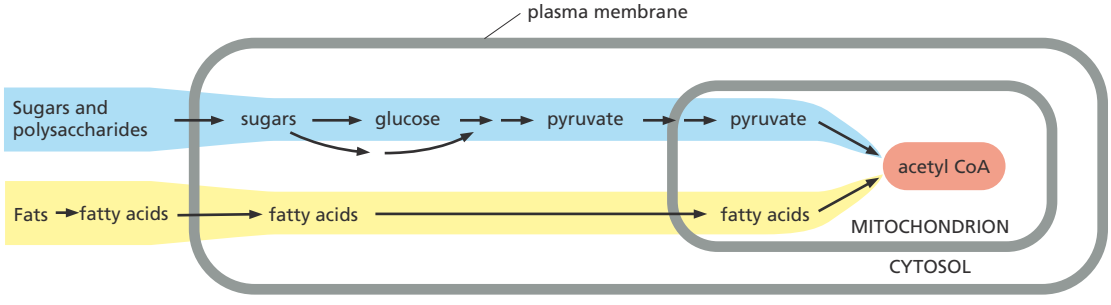


Figure 14–9 In eukaryotic cells, acetyl CoA is produced in the mitochondria from molecules derived from sugars and fats. Most of the cell's oxidation reactions occur in these organelles, and most of its ATP is made here.

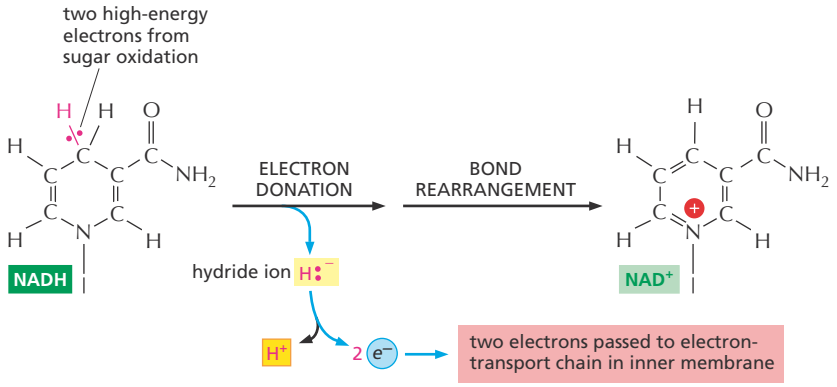


Figure 14–10 NADH donates its high-energy electrons to the electron-transport chain. In this drawing, the electrons being transferred are shown as two *red dots* on a *red* hydrogen atom. A hydride ion (a hydrogen atom with an extra electron) is removed from NADH and is converted into a proton and two electrons. Only the part of NADH that carries these high-energy electrons is shown; for the complete structure and the conversion of NAD^+ back to NADH, see the structure of the closely related NADPH in Figure 3–34. Electrons are also carried in a similar way by $FADH_2$, whose structure is shown in Figure 13–13B.

then oxidized to CO_2 via the citric acid cycle (see Figure 13–12). Some of the energy derived from this oxidation is saved in the form of high-energy electrons, held by the activated carriers NADH and $FADH_2$. These activated carriers can then donate their high-energy electrons to the electron-transport chain in the inner mitochondrial membrane (**Figure 14–10**).

The Movement of Electrons is Coupled to the Pumping of Protons

The chemiosmotic generation of energy begins when the activated carriers NADH and $FADH_2$ donate their high-energy electrons to the electron-transport chain in the inner mitochondrial membrane, becoming oxidized to NAD^+ and FAD in the process (see Figure 14–10). The electrons are quickly passed along the chain to molecular oxygen (O_2) to form water (H_2O). The stepwise movement of these high-energy electrons through the components of the electron-transport chain releases energy that can then be used to pump protons across the inner membrane (**Figure 14–11**). The resulting proton gradient, in turn, is used to drive the synthesis of ATP. The full sequence of reactions is shown in **Figure 14–12**. The inner mitochondrial membrane thus serves as a device that converts the energy contained in the high-energy electrons of NADH (and $FADH_2$) into the phosphate bond of ATP molecules (**Figure 14–13**). This chemiosmotic mechanism for ATP synthesis is called **oxidative phosphorylation**, because it involves both the consumption of O_2 and the addition of a phosphate group to ADP to form ATP.

The source of the high-energy electrons that power the proton pumping differs widely between different organisms and different processes. In cell respiration—which takes place in both mitochondria and aerobic bacteria—the high-energy electrons are ultimately derived from sugars

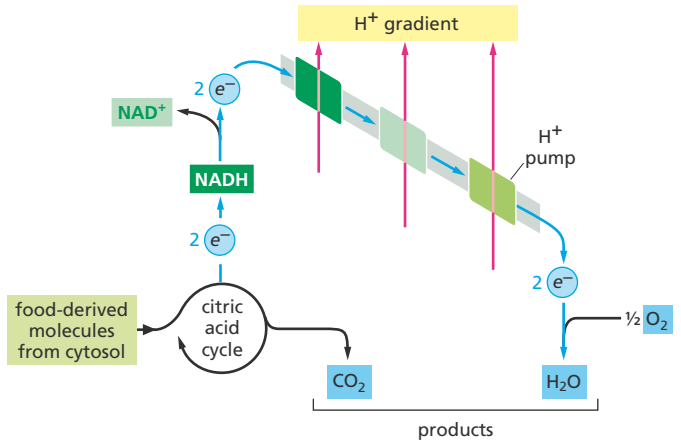


Figure 14–11 As electrons are transferred from activated carriers to oxygen, protons are pumped across the inner mitochondrial membrane. This is stage 1 of chemiosmotic coupling (see Figure 14–2). The path of electron flow is indicated by *blue arrows*.

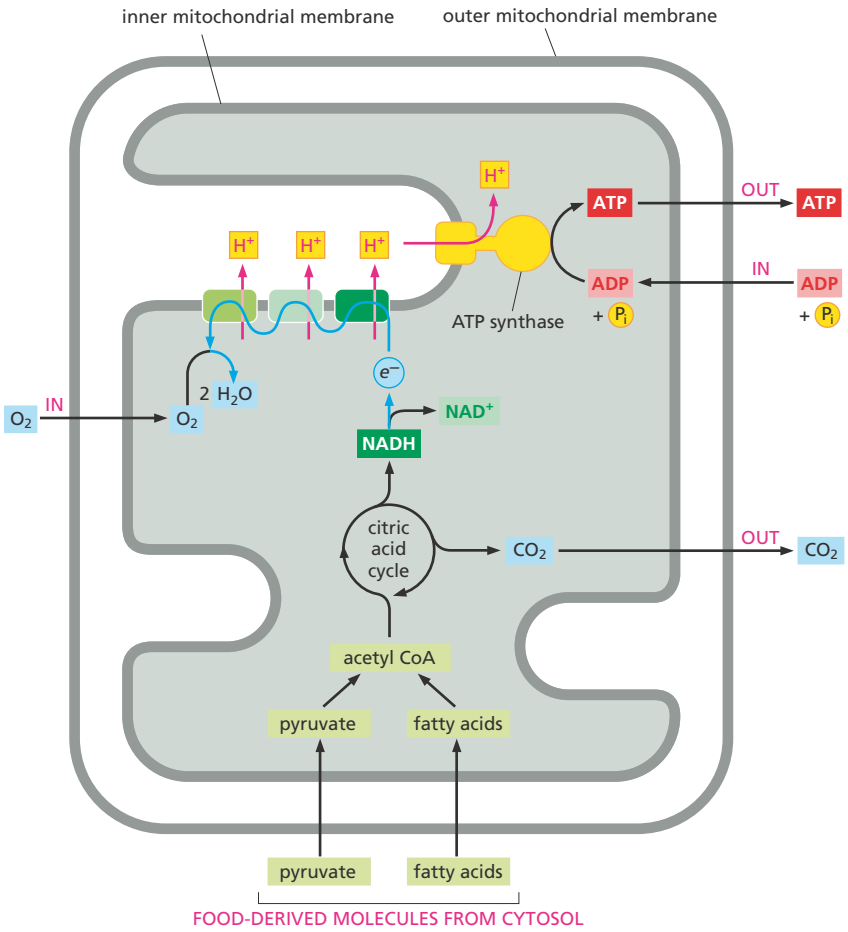


Figure 14–12 Activated carriers generated during the citric acid cycle power the production of ATP. Pyruvate and fatty acids enter the mitochondrial matrix (*bottom*), where they are converted to acetyl CoA. The acetyl CoA is then metabolized by the citric acid cycle, which produces NADH (and $FADH_2$, not shown). During oxidative phosphorylation, high-energy electrons donated by NADH (and $FADH_2$) are then passed along the electron-transport chain in the inner membrane to oxygen (O_2); this electron transport generates a proton gradient across the inner membrane, which is used to drive the production of ATP by ATP synthase. The exact ratios of "reactants" and "products" are not indicated in this diagram: for example, we will see shortly that it requires four electrons from four NADH molecules to convert O_2 to two H_2O molecules.

or fats. In photosynthesis, the high-energy electrons come from the organic green pigment *chlorophyll*, which captures energy from sunlight. And many single-celled organisms (archaea and bacteria) use inorganic substances such as hydrogen, iron, and sulfur as the source of the high-energy electrons that they need to make ATP (see, for example, Figure 1–12).

Regardless of the electron source, the vast majority of living organisms use a chemiosmotic mechanism to generate ATP. In the following sections, we describe in detail how this process occurs.

Protons Are Pumped Across the Inner Mitochondrial Membrane by Proteins in the Electron-Transport Chain

The electron-transport chain—or *respiratory chain*—that carries out oxidative phosphorylation is present in many copies in the inner mitochondrial membrane. Each chain contains over 40 proteins, grouped into three large **respiratory enzyme complexes**. These complexes each contain multiple individual proteins, including transmembrane proteins that anchor the complex firmly in the inner mitochondrial membrane.

The three respiratory enzyme complexes, in the order in which they receive electrons, are: (1) *NADH dehydrogenase complex*, (2) *cytochrome* c *reductase complex*, and (3) *cytochrome* c *oxidase complex* (**Figure 14–14**). Each complex contains metal ions and other chemical groups that act as stepping stones to facilitate the passage of electrons. The movement of electrons through these respiratory complexes is accompanied by the pumping of protons from the mitochondrial matrix to the intermembrane space. Thus each complex can be thought of as a proton pump.

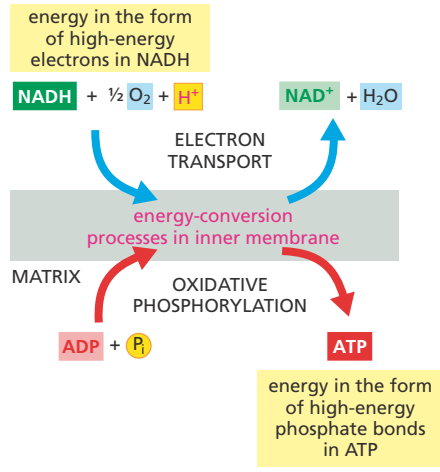


Figure 14–13 Mitochondria catalyze a major conversion of energy. In oxidative phosphorylation, the energy released by the oxidation of NADH to NAD^+ is harnessed—through energy-conversion processes in the inner mitochondrial membrane—to drive the energy-requiring phosphorylation of ADP to form ATP. The net equation for this process, in which four electrons pass from NADH to oxygen, is $2NADH + O_2 + 2H^+ \rightarrow 2NAD^+ + 2H_2O$.

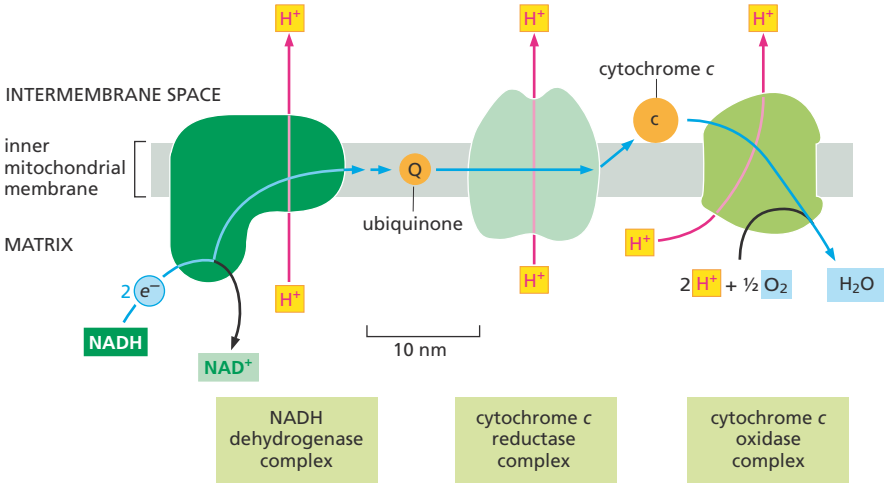


Figure 14–14 High-energy electrons are transferred through three respiratory enzyme complexes in the inner mitochondrial membrane. The relative size and shape of each complex are indicated, although the numerous individual protein components that form each complex are not. During the transfer of high-energy electrons from NADH to oxygen (*blue lines*), protons derived from water are pumped across the membrane from the matrix into the intermembrane space by each of the complexes (Movie 14.2). Ubiquinone (Q) and cytochrome *c* (c) serve as mobile carriers that ferry electrons from one complex to the next.

The first respiratory complex in the chain, NADH dehydrogenase, accepts electrons from NADH. These electrons are extracted from NADH in the form of a hydride ion (H^-), which is then converted into a proton and two high-energy electrons. That reaction, $H^- \rightarrow H^+ + 2e^-$ (see Figure 14–10), is catalyzed by the NADH dehydrogenase complex. The electrons are then passed along the chain to each of the other enzyme complexes in turn, using mobile electron carriers to ferry electrons between complexes (see Figure 14–14). This transfer of electrons is energetically favorable: the electrons are passed from electron carriers with weaker electron affinity to those with stronger electron affinity, until they combine with a molecule of O_2 to form water. This final reaction is the only oxygen-requiring step in cell respiration, and it consumes nearly all of the oxygen that we breathe.

Proton Pumping Produces a Steep Electrochemical Proton Gradient Across the Inner Mitochondrial Membrane

Without a mechanism for harnessing the energy released by the energetically favorable transfer of electrons from NADH to O_2, this energy would simply be liberated as heat. Cells are able to recover much of this energy because the three respiratory enzyme complexes in the electron-transport chain use it to pump protons across the inner mitochondrial membrane, from the matrix into the intermembrane space (see Figure 14–14). Later, we will outline the molecular mechanisms involved. For now, we focus on the consequences of this nifty maneuver. First, the pumping of protons generates a H^+ gradient—or pH gradient—across the inner membrane. As a result, the pH in the matrix (around 7.9) is about 0.7 unit higher than it is in the intermembrane space (which is 7.2, the same pH as the cytosol). Second, proton pumping generates a voltage gradient—or membrane potential—across the inner membrane; as H^+ flows outward, the matrix side of the membrane becomes negative and the side facing the intermembrane space becomes positive.

As discussed in Chapter 12, the force that drives the passive flow of an ion across a membrane is proportional to the ion's *electrochemical gradient*. The strength of that electrochemical gradient depends both on the voltage across the membrane, as measured by the membrane potential, and on the ion's concentration gradient (see Figure 12–5). Because protons are positively charged, they will more readily cross a membrane if there is an excess of negative charge on the other side. In the case of the inner mitochondrial membrane, the pH gradient and membrane potential work

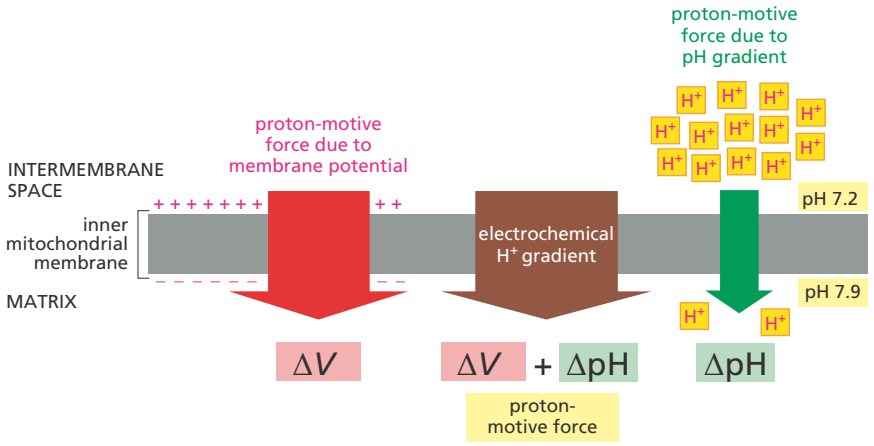


Figure 14–15 The electrochemical H^+ gradient across the inner mitochondrial membrane includes a large force due to the membrane potential (ΔV) and a smaller force due to the H^+ concentration gradient—that is, the pH gradient (ΔpH). Both forces combine to generate the proton-motive force, which pulls H^+ back into the mitochondrial matrix. The exact, mathematical relationship between these forces is expressed by the Nernst equation (see Figure 12–23).

QUESTION 14–3

When the drug dinitrophenol (DNP) is added to mitochondria, the inner membrane becomes permeable to protons (H^+). In contrast, when the drug nigericin is added to mitochondria, the inner membrane becomes permeable to K^+. (A) How does the electrochemical proton gradient change in response to DNP? (B) How does it change in response to nigericin?

together to create a steep electrochemical proton gradient that makes it energetically very favorable for H^+ to flow back into the mitochondrial matrix. The membrane potential contributes significantly to this *proton-motive force*, which pulls H^+ back across the membrane; the greater the membrane potential, the more energy is stored in the proton gradient (**Figure 14–15**).

ATP Synthase Uses the Energy Stored in the Electrochemical Proton Gradient to Produce ATP

If protons in the intermembrane space were allowed simply to flow back into the mitochondrial matrix, the energy stored in the electrochemical proton gradient would be lost as heat. Such a seemingly wasteful process allows hibernating bears to stay warm, as we discuss further in How We Know (pp. 462–463). In most cells, however, the electrochemical proton gradient across the inner mitochondrial membrane is used to drive the synthesis of ATP from ADP and P_i (see Figure 2–25). The device that makes this possible is **ATP synthase**, a large, multisubunit protein embedded in the inner mitochondrial membrane.

ATP synthase is of ancient origin; the same enzyme generates ATP in the mitochondria of animal cells, the chloroplasts of plants and algae, and the plasma membrane of bacteria. The part of the protein that catalyzes the phosphorylation of ADP is shaped like a lollipop head and projects into the mitochondrial matrix; it is attached by a central stalk to a transmembrane H^+ carrier (**Figure 14–16**). The passage of protons through the carrier causes the carrier and its stalk to spin rapidly, like a tiny motor. As the stalk rotates, it rubs against proteins in the stationary head, altering their conformation and prompting them to produce ATP. In this way, a mechanical deformation gets converted into the chemical-bond energy of ATP (**Movie 14.3**). This fine-tuned sequence of interactions allows ATP synthase to produce more than 100 molecules of ATP per second—3 molecules of ATP per revolution.

ATP synthase can also operate in reverse—using the energy of ATP hydrolysis to pump protons "uphill," against their electrochemical gradient across the membrane (**Figure 14–17**). In this mode, ATP synthase functions like the H^+ pumps described in Chapter 12. Whether ATP synthase primarily makes ATP—or consumes it to pump protons—depends on the magnitude of the electrochemical proton gradient across the membrane in which the enzyme is embedded. In many bacteria that can grow either aerobically or anaerobically, the direction in which the ATP

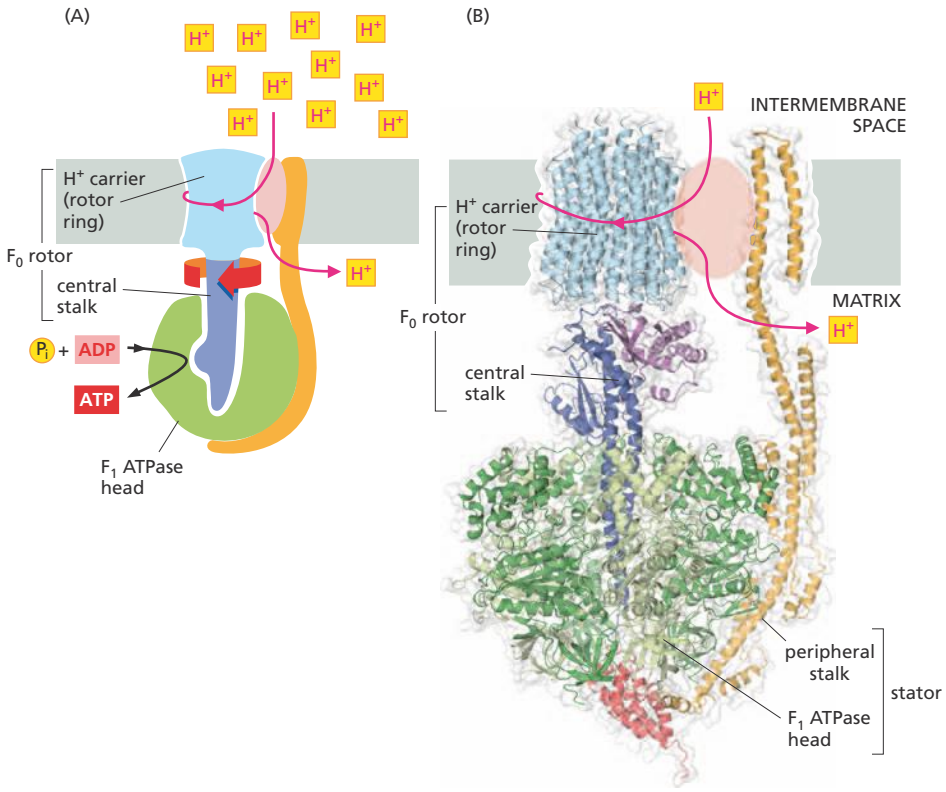


Figure 14–16 ATP synthase acts like a motor to convert the energy of protons flowing down their electrochemical gradient to chemical-bond energy in ATP. (A) The multisubunit protein is composed of a stationary head, called the F_1 ATPase, and a rotating portion called F_0. Both F_1 and F_0 are formed from multiple subunits. Driven by the electrochemical proton gradient, the F_0 part of the protein—which consists of the transmembrane H^+ carrier (*blue*) plus a central stalk (*purple*)—spins rapidly within the stationary head of the F_1 ATPase (*green*), causing it to generate ATP from ADP and P_i. The stationary head is secured to the inner membrane by an elongated protein "arm" called the peripheral stalk (*orange*). The F_1 ATPase is so named because it can carry out the reverse reaction—the hydrolysis of ATP to ADP and P_i—when detached from the F_0 portion of the complex.

(B) The three-dimensional structure of ATP synthase, as determined by X-ray crystallography. The peripheral stalk is fixed to the membrane with the help of the subunit denoted by the *pink* oval, which is the only part of the complex still lacking structural details. At its other end, this stalk is tied to the F_1 ATPase head via the small *red* subunit. (B, courtesy of K. Davies.)

synthase works is routinely reversed when the bacterium runs out of O_2. Under these conditions, the ATP synthase uses some of the ATP generated inside the cell by glycolysis to pump protons out of the cell, creating the proton gradient that the bacterial cell needs to import its essential nutrients by coupled transport. A similar mechanism is used to drive the transport of small molecules in and out of the mitochondrial matrix, as we discuss next.

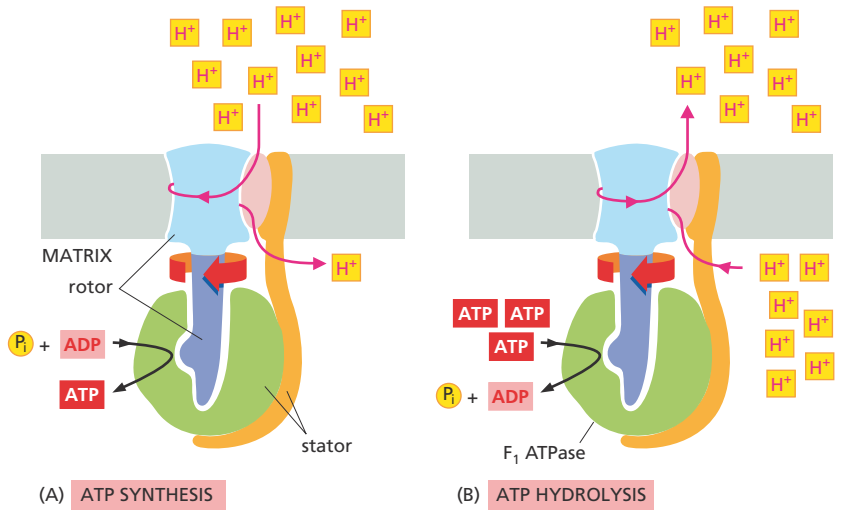


Figure 14–17 ATP synthase is a reversible coupling device. It can either synthesize ATP by harnessing the electrochemical H^+ gradient (A) or pump protons against this gradient by hydrolyzing ATP (B). The direction of operation at any given instant depends on the net free-energy change (ΔG, discussed in Chapter 3) for the coupled processes of H^+ translocation across the membrane and the synthesis of ATP from ADP and P_i. For example, if the electrochemical proton gradient falls below a certain level, the ΔG for H^+ transport into the matrix will no longer be large enough to drive ATP production; instead, ATP will be hydrolyzed by the ATP synthase to rebuild the proton gradient. A tribute to the activity of ATP synthase is shown in Movie 14.4.

Coupled Transport Across the Inner Mitochondrial Membrane Is Also Driven by the Electrochemical Proton Gradient

The synthesis of ATP is not the only process driven by the electrochemical proton gradient in mitochondria. Many small, charged molecules, such as pyruvate, ADP, and inorganic phosphate (P_i), are imported into the mitochondrial matrix from the cytosol, while others, such as ATP, must be transported in the opposite direction. Carrier proteins that bind these molecules can couple their transport to the energetically favorable flow of H^+ into the matrix (see the "coupled transporters" in Figure 12–14). Pyruvate and P_i, for example, are each co-transported inward along with protons, as the protons move down their electrochemical gradient into the matrix.

Other transporters take advantage of the membrane potential generated by the electrochemical proton gradient, which makes the matrix side of the inner mitochondrial membrane more negatively charged than the side that faces the intermembrane space. An antiport carrier protein exploits this voltage gradient to export ATP from the mitochondrial matrix and to bring ADP in. This exchange allows the ATP synthesized in the mitochondrion to be exported rapidly (**Figure 14–18**).

In eukaryotic cells, therefore, the electrochemical proton gradient is used to drive both the formation of ATP and the transport of selected metabolites across the inner mitochondrial membrane. In bacteria, the proton gradient across the plasma membrane is similarly used to drive ATP synthesis and metabolite transport. But it also serves as an important source of directly usable energy: in motile bacteria, for instance, the flow of protons into the cell drives the rapid rotation of the bacterial flagellum, which propels the bacterium along (**Movie 14.5**).

The Rapid Conversion of ADP to ATP in Mitochondria Maintains a High ATP/ADP Ratio in Cells

As a result of the nucleotide exchange shown in Figure 14–18, ADP molecules—produced by hydrolysis of ATP in the cytosol—are rapidly drawn back into mitochondria for recharging, while the bulk of the ATP molecules produced in mitochondria are exported into the cytosol, where they are most needed. (A small amount of ATP is used within mitochondria

QUESTION 14–4

The remarkable properties that allow ATP synthase to run in either direction allow the interconversion of energy stored in the H^+ gradient and energy stored in ATP to proceed in either direction. (A) If ATP synthase making ATP can be likened to a water-driven turbine producing electricity, what would be an appropriate analogy when it works in the opposite direction? (B) Under what conditions would one expect the ATP synthase to stall, running neither forward nor backward? (C) What determines the direction in which the ATP synthase operates?

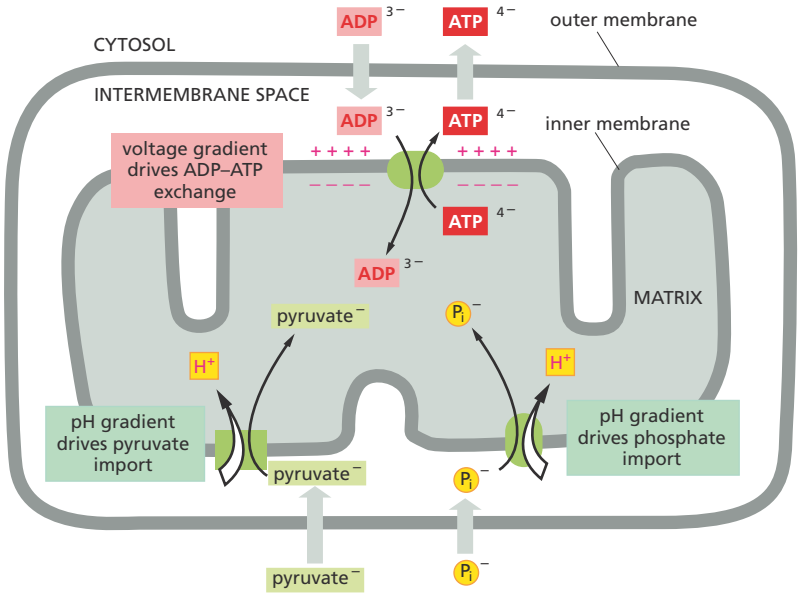


Figure 14–18 The electrochemical proton gradient across the inner mitochondrial membrane is used to drive some coupled transport processes. The charge on each of the transported molecules is indicated for comparison with the membrane potential, which is negative inside, as shown. Pyruvate and inorganic phosphate (P_i) are moved into the matrix along with protons, as the protons move down their electrochemical gradient. Both are negatively charged, so their movement is opposed by the negative membrane potential; however, the H^+ concentration gradient—the pH gradient—is harnessed in a way that nevertheless drives their inward transport. ADP is pumped into the matrix and ATP is pumped out by an antiport process that uses the voltage gradient across the membrane to drive this exchange. The outer mitochondrial membrane is freely permeable to all of these compounds due to the presence of porins in the membrane (not shown). The active transport of molecules across membranes by carrier proteins and the generation of a membrane potential are discussed in Chapter 12.

themselves to power DNA replication, protein synthesis, and other energy-consuming reactions that occur there.) With all of this back-and-forth, a typical ATP molecule in a human cell will shuttle out of a mitochondrion and back in (as ADP) more than once every minute.

As discussed in Chapter 3, most biosynthetic enzymes drive energetically unfavorable reactions by coupling them to the energetically favorable hydrolysis of ATP (see Figure 3–33A). The pool of ATP in a cell is thus used to drive a huge variety of cell processes in much the same way that a battery is used to drive an electric engine. To be useful in this way, the concentration of ATP in the cytosol must be kept about 10 times higher than that of ADP. If the activity of mitochondria were halted, ATP levels would fall dramatically and the cell's battery would run down. Eventually, energetically unfavorable reactions could no longer take place and the cell would die. The poison cyanide, which blocks electron transport in the inner mitochondrial membrane, causes cell death in exactly this way.

Cell Respiration Is Amazingly Efficient

The oxidation of sugars to produce ATP may seem unnecessarily complex. Surely the process could be accomplished more directly—perhaps by eliminating the citric acid cycle or some of the steps in the respiratory chain. Such simplification would certainly make the chemistry easier for students to learn—but it would be bad news for the cell. As discussed in Chapter 13, the oxidative pathways that allow cells to extract energy from food efficiently and in a usable form involve many intermediates, each differing only slightly from its predecessor. In this way, the huge amounts of energy locked up in food molecules can be parceled out into small packets that can be captured in activated carriers, such as NADH and $FADH_2$ (see Figure 13–1).

Much of the energy carried by NADH and $FADH_2$ is ultimately converted into the bond energy of ATP. How much ATP each of these activated carriers can produce depends on several factors, including where its electrons enter the respiratory chain. The NADH molecules produced in the mitochondrial matrix during the citric acid cycle pass their high-energy electrons to the NADH dehydrogenase complex—the first complex in the chain. As the electrons pass from one enzyme complex to the next, they promote the pumping of protons across the inner mitochondrial membrane at each step along the way. In this way, each NADH molecule provides enough net energy to generate about 2.5 molecules of ATP (see Question 14–5 and its answer).

$FADH_2$ molecules, on the other hand, bypass the NADH dehydrogenase complex and pass their electrons to the membrane-embedded mobile carrier ubiquinone (see Figure 14–14). Because these electrons enter further down the respiratory chain than those donated by NADH, they promote the pumping of fewer protons: each molecule of $FADH_2$ thus produces only 1.5 molecules of ATP. **Table 14–1** provides a full accounting of the ATP produced by the complete oxidation of glucose.

Although the biological oxidation of glucose to CO_2 and H_2O consists of many interdependent steps, the overall process is remarkably efficient. Almost 50% of the total energy that could be released by burning sugars or fats is captured and stored in the phosphate bonds of ATP during cell respiration. That might not seem impressive, but it is considerably better than most nonbiological energy-conversion devices. Electric motors and gasoline engines operate at about 10–20% efficiency. If cells operated at this efficiency, an organism would have to eat voraciously just to maintain itself. Moreover, because the wasted energy is liberated as heat, large organisms (including ourselves) would need far better mechanisms

TABLE 14–1 PRODUCT YIELDS FROM GLUCOSE OXIDATION

Process	Direct product	Final ATP yield per molecule of glucose
Glycolysis	2 NADH (cytosolic)	3*
	2 ATP	2
Pyruvate oxidation to acetyl CoA (two per glucose)	2 NADH (mitochondrial matrix)	5
Complete acetyl CoA oxidation (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 $FADH_2$	3
	2 GTP	2
	TOTAL	30

*NADH produced in the cytosol yields fewer ATP molecules than NADH produced in the mitochondrial matrix because the mitochondrial inner membrane is impermeable to NADH. Transporting NADH into the mitochondrial matrix—where it encounters NADH dehydrogenase—thus requires energy.

for cooling themselves. It is hard to imagine how animals could have evolved without the elaborate yet economical mechanisms that allow cells to extract a maximum amount of energy from food.

MOLECULAR MECHANISMS OF ELECTRON TRANSPORT AND PROTON PUMPING

For many years, biochemists struggled to understand why electron-transport chains had to be embedded in membranes to function in ATP production. The puzzle was essentially solved in the 1960s, when it was discovered that transmembrane proton gradients drive the process. The concept of chemiosmotic coupling was so novel, however, that it was not widely accepted until many years later, when additional experiments with artificial energy-generating systems put the power of proton gradients to the test (see **How We Know**, pp. 462–463).

Although investigators are still unraveling some of the details of chemiosmotic coupling at the atomic level, the fundamentals are now clear. In this section, we examine the basic principles that drive the movement of electrons, and we explain in molecular detail how electron transport can generate a proton gradient. Because very similar mechanisms are used by mitochondria, chloroplasts, and prokaryotes, these principles apply to nearly all living things.

Protons Are Readily Moved by the Transfer of Electrons

Although protons resemble other positive ions such as Na^+ and K^+ in the way they move across membranes, in some respects they are unique. Hydrogen atoms are by far the most abundant atom in living organisms: they are plentiful not only in all carbon-containing biological molecules but also in the water molecules that surround them. The protons in water are highly mobile: by rapidly dissociating from one water molecule and associating with its neighbor, they can rapidly flit through a hydrogen-bonded network of water molecules (see Figure 2–15B). Thus water, which is everywhere in cells, serves as a ready reservoir for donating and accepting protons.

QUESTION 14–5

Calculate the number of usable ATP molecules produced per pair of electrons transferred from NADH to oxygen, if (i) five protons are pumped across the inner mitochondrial membrane for each electron passed through the three respiratory enzyme complexes, (ii) three protons must pass through the ATP synthase for each ATP molecule that it produces from ADP and inorganic phosphate inside the mitochondrion, and (iii) one proton is used to produce the voltage gradient needed to transport each ATP molecule out of the mitochondrion to the cytosol where it is used.

HOW CHEMIOSMOTIC COUPLING DRIVES ATP SYNTHESIS

In 1861, Louis Pasteur discovered that yeast cells grow and divide more vigorously when air is present—the first demonstration that aerobic metabolism is more efficient than anaerobic metabolism. His observations make sense now that we know that oxidative phosphorylation is a much more efficient means of generating ATP than is glycolysis, producing about 30 molecules of ATP for each molecule of glucose oxidized, compared with the 2 ATPs generated by glycolysis alone. But it took another hundred years for researchers to determine that it is the process of chemiosmotic coupling—using proton pumping to power ATP synthesis—that allows cells to generate energy with such efficiency.

Imaginary intermediates

In the 1950s, many researchers believed that the oxidative phosphorylation that takes place in mitochondria generates ATP via a mechanism similar to that used in glycolysis. During glycolysis, ATP is produced when a molecule of ADP receives a phosphate group directly from a "high-energy" intermediate. Such substrate-level phosphorylation occurs in steps 7 and 10 of glycolysis, where the high-energy phosphate groups from 1,3-bisphosphoglycerate and phosphoenolpyruvate, respectively, are transferred to ADP to form ATP (see Panel 13–1, pp. 428–429). It was assumed that the electron-transport chain in mitochondria would similarly generate some phosphorylated intermediate that could then donate its phosphate group directly to ADP. This model inspired a long and frustrating search for this mysterious high-energy intermediate. Investigators occasionally claimed to have discovered the missing intermediate, but the compounds turned out to be either unrelated to electron transport or, as one researcher put it in a review of the history of bioenergetics, "products of high-energy imagination."

Harnessing the force

It wasn't until 1961 that Peter Mitchell suggested that the "high-energy intermediate" his colleagues were seeking was, in fact, the electrochemical proton gradient generated by the electron-transport system. His proposal, dubbed the chemiosmotic hypothesis, stated that the energy of an electrochemical proton gradient formed during the transfer of electrons through the electron-transport chain could be tapped to drive ATP synthesis.

Several lines of evidence offered support for Mitchell's proposed mechanism. First, mitochondria do generate an electrochemical proton gradient across their inner membrane. But what does this gradient—also called the proton-motive force—actually do? If the gradient is required to drive ATP synthesis, as the chemiosmotic hypothesis posits, then either disrupting the inner membrane or eliminating the proton gradient across it should inhibit ATP production. In fact, researchers found both these predictions to be true. Physical disruption of the inner mitochondrial membrane halts ATP synthesis in that organelle. Similarly, dissipation of the proton gradient by a chemical "uncoupling" agent such as 2,4-dinitrophenol (DNP) also inhibits mitochondrial ATP production. Such gradient-busting chemicals carry H^+ across the inner mitochondrial membrane, forming a shuttle system for the movement of H^+ that bypasses the ATP synthase (**Figure 14–19**). In this way, compounds such as DNP uncouple electron transport from ATP synthesis. As a result of this short-circuiting, the proton-motive force is dissipated completely, and the organelle can no longer make ATP.

Such uncoupling occurs naturally in some specialized fat cells. In these cells, called *brown fat cells*, most of the energy from the oxidation of fat is dissipated as heat rather than converted into ATP. The inner membranes

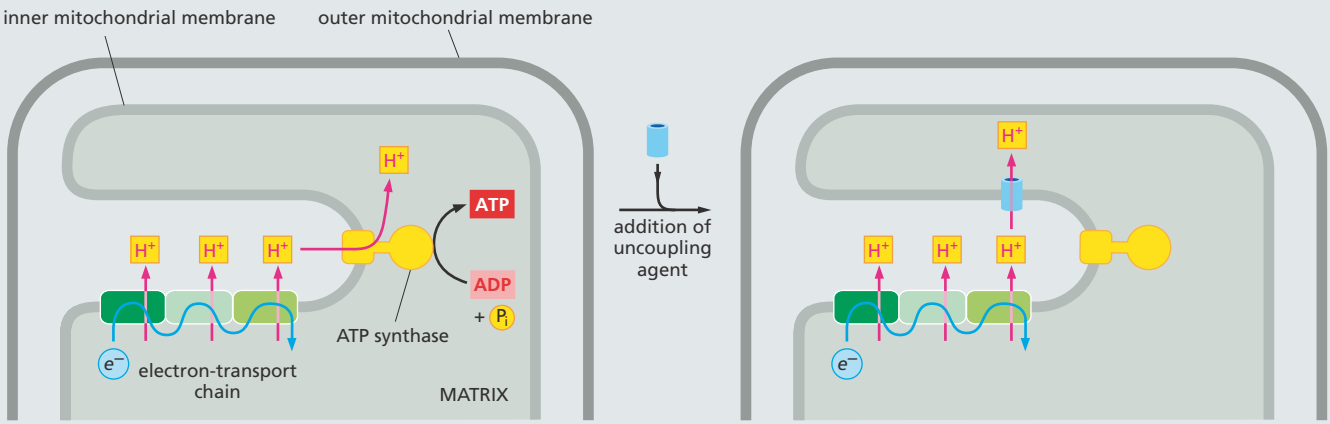


Figure 14–19 Uncoupling agents are H^+ carriers that can insert into the inner mitochondrial membrane. They render the membrane permeable to protons, allowing H^+ to flow into the mitochondrial matrix without passing through ATP synthase. This short circuit effectively uncouples electron transport from ATP synthesis.

of the large mitochondria in these cells contain a carrier protein that allows protons to move down their electrochemical gradient, circumventing ATP synthase. As a result, the cells oxidize their fat stores at a rapid rate and produce more heat than ATP. Tissues containing brown fat serve as biological heating pads, helping to revive hibernating animals and to protect sensitive areas of newborn human babies (such as the backs of their necks) from the cold.

Artificial ATP generation

If disrupting the electrochemical proton gradient across the mitochondrial inner membrane terminates ATP synthesis, then, conversely, generating an artificial proton gradient should stimulate ATP synthesis. Again, this is exactly what happens. When a proton gradient is imposed artificially by lowering the pH on the outside of the mitochondrial inner membrane, out pours ATP.

How does the electrochemical proton gradient drive ATP production? This is where the ATP synthase comes in. In 1974, Efraim Racker and Walther Stoeckenius demonstrated that they could reconstitute a complete artificial ATP-generating system by combining an ATP synthase isolated from the mitochondria of cow heart muscle with a proton pump purified from the purple membrane of the prokaryote *Halobacterium halobium*. As discussed in Chapter 11, the plasma membrane of this archaean is packed with bacteriorhodopsin, a protein that pumps H^+ out of the cell in response to sunlight (see Figure 11–27).

When bacteriorhodopsin was reconstituted into artificial lipid vesicles (liposomes), Racker and Stoeckenius showed that, in the presence of light, the bacterial protein pumps H^+ into the vesicles, generating a proton gradient. (The orientation of the protein is reversed in these membranes, so that protons are transported into the vesicles; in the bacterium, protons are pumped out.) When the bovine ATP synthase was then incorporated into these vesicles, much to the amazement of many biochemists, the system could catalyze the synthesis of ATP from ADP and inorganic phosphate in response to light. This ATP formation showed an absolute dependence on an intact proton gradient, as either eliminating bacteriorhodopsin from the system or adding uncoupling agents such as DNP abolished ATP synthesis (**Figure 14–20**).

This remarkable experiment demonstrated without a doubt that a proton gradient could stimulate ATP synthase to make ATP. Thus, although biochemists had initially hoped to discover a high-energy intermediate involved in oxidative phosphorylation, the experimental evidence eventually convinced them that their search was in vain and that the chemiosmotic hypothesis was correct. Mitchell was awarded a Nobel Prize in 1978.

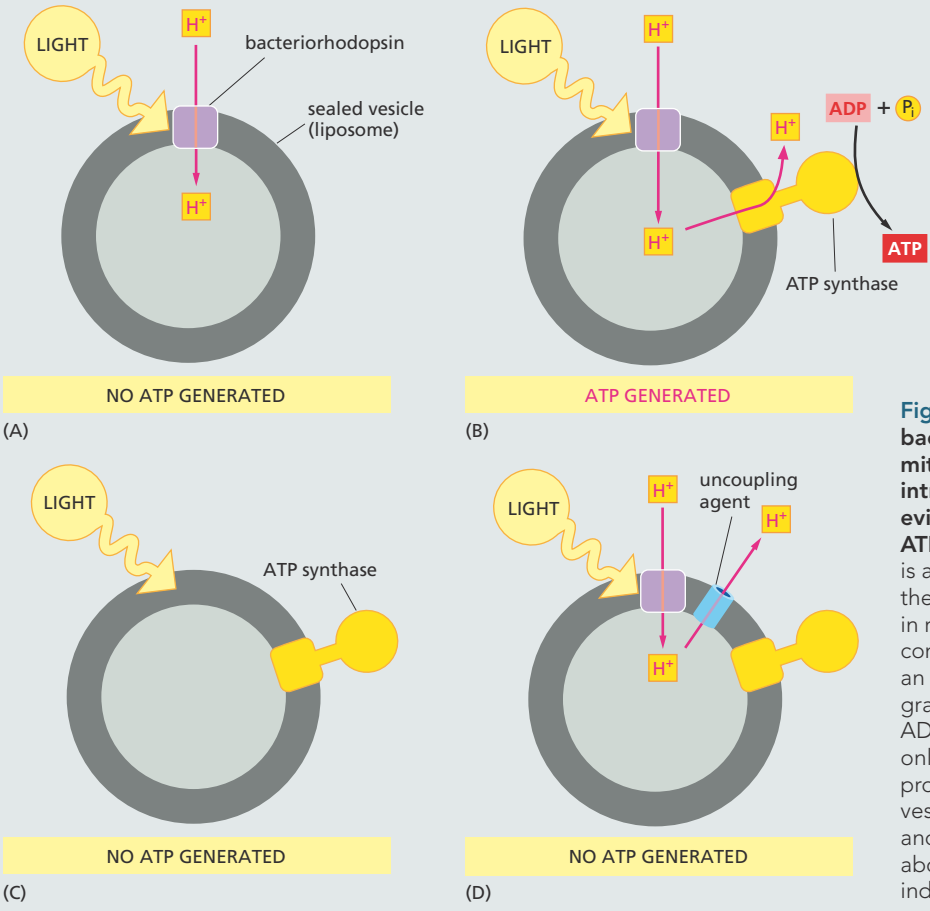


Figure 14–20 Experiments in which bacteriorhodopsin and bovine mitochondrial ATP synthase were introduced into liposomes provided direct evidence that proton gradients can power ATP production. (A) When bacteriorhodopsin is added to artificial lipid vesicles (liposomes), the protein generates a proton gradient in response to light. (B) In artificial vesicles containing both bacteriorhodopsin and an ATP synthase, a light-generated proton gradient drives the formation of ATP from ADP and P_i. (C) Artificial vesicles containing only ATP synthase do not on their own produce ATP in response to light. (D) In vesicles containing both bacteriorhodopsin and ATP synthase, uncoupling agents that abolish the proton gradient eliminate light-induced ATP synthesis.

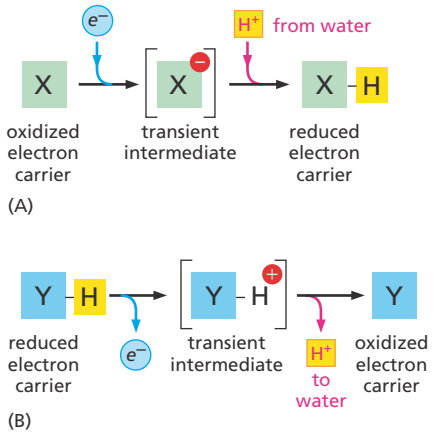


Figure 14–21 Electron transfers can cause the movement of entire hydrogen atoms, because protons are readily accepted from or donated to water. In these examples, an oxidized electron carrier molecule, X, picks up an electron plus a proton when it is reduced (A), and a reduced electron carrier molecule, Y, loses an electron plus a proton when it is oxidized (B).

These protons often accompany the electrons that are transferred during oxidation and reduction. When a molecule is reduced by acquiring an electron (e^-), the electron brings with it a negative charge; in many cases, this charge is immediately neutralized by the addition of a proton from water, so that the net effect of the reduction is to transfer an entire hydrogen atom, $H^+ + e^-$ (**Figure 14–21A**). Similarly, when a molecule is oxidized, it often loses an electron from one of its hydrogen atoms: in most instances, the electron is transferred to an electron carrier, and the proton is passed on to water (**Figure 14–21B**). Therefore, in a membrane in which electrons are being passed along an electron-transport chain, it is a relatively simple matter, in principle, to move protons from one side of the membrane to the other. All that is required is that the electron carrier be oriented in the membrane in such a way that it accepts an electron—along with a proton from water—on one side of the membrane, and then releases that proton on the other side of the membrane when the electron is passed on to the next electron carrier molecule in the chain (**Figure 14–22**).

The Redox Potential Is a Measure of Electron Affinities

The proteins of the respiratory chain guide the electrons so that they move sequentially from one enzyme complex to another—with no short circuits that skip a complex. Each electron transfer is an oxidation–reduction reaction: as described in Chapter 3, the molecule or atom donating the electron becomes oxidized, while the receiving molecule or atom becomes reduced (see pp. 89–90). Electrons will pass spontaneously from molecules that have a relatively low affinity for their outer-shell electrons, and thus lose them easily, to molecules that have a higher affinity for electrons. For example, NADH has a low electron affinity, so that its electrons are readily passed to the NADH dehydrogenase complex (see Figure 14–14). The batteries we use to power our electronic gadgets are based on similar electron transfers between chemical substances with different electron affinities.

In biochemical reactions, any electrons removed from one molecule are always passed to another, so that whenever one molecule is oxidized, another is reduced. Like any other chemical reaction, the tendency of such oxidation–reduction reactions, or **redox reactions**, to proceed spontaneously depends on the free-energy change (ΔG) for the electron transfer, which in turn depends on the relative affinities of the two molecules for electrons. (The role of free energy in chemical reactions is discussed in Chapter 3, pp. 90–100.)

Because electron transfers provide most of the energy in living things, it is worth taking time to understand them. Molecules that donate protons are known as acids; those that accept protons are called bases (see Panel 2–2, pp. 68–69). These molecules exist in conjugate acid–base pairs, in which the acid is readily converted into the base by the loss of a proton. For example, acetic acid (CH_3COOH) is converted into its conjugate base (CH_3COO^-) in the reaction

$$CH_3COOH \rightleftharpoons CH_3COO^- + H^+$$

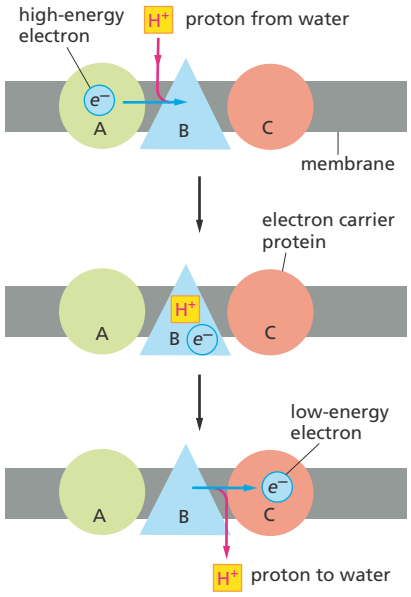


Figure 14–22 The orientation of a membrane-embedded electron carrier allows electron transfer to drive proton pumping. As an electron passes along an electron-transport chain, it can bind and release a proton at each step. In this schematic diagram, the electron carrier, protein B, picks up a proton (H^+) from one side of the membrane when it accepts an electron (e^-) from protein A; protein B releases the proton to the other side of the membrane when it donates its electron to the electron carrier, protein C.

In the same way, pairs of compounds such as NADH and NAD^+ are called **redox pairs**, because NADH is converted to NAD^+ by the loss of electrons in the reaction

$$NADH \rightleftharpoons NAD^+ + H^+ + 2e^-$$

NADH is a strong electron donor. Its electrons can be said to be held at high-energy because the ΔG for passing them to many other molecules is favorable. Conversely, it is difficult to produce the high-energy electrons in NADH, so its partner, NAD^+, is of necessity a weak electron acceptor.

The tendency for a redox pair such as NADH/NAD^+ to donate or accept electrons can be determined experimentally by measuring its **redox potential** (**Panel 14–1**, p. 466). Electrons will move spontaneously from a redox pair with a low redox potential (or low affinity for electrons), such as NADH/NAD^+, to a redox pair with a high redox potential (or high affinity for electrons), such as O_2/H_2O. Thus, NADH is an excellent molecule to donate electrons to the respiratory chain, while O_2 is well suited to act as an electron "sink" at the end of the pathway. As explained in Panel 14–1, the difference in redox potential, $\Delta E_0'$, is a direct measure of the standard free-energy change ($\Delta G°$) for the transfer of an electron from one molecule to another. In fact, $\Delta E_0'$ is equal to $\Delta G°$ times a negative number that is a constant.

Electron Transfers Release Large Amounts of Energy

The amount of energy that can be released by an electron transfer can be determined by comparing the redox potentials of the molecules involved. Again, let's look at the transfer of electrons from NADH and to O_2. As shown in Panel 14–1, a 1:1 mixture of NADH and NAD^+ has a redox potential of –320 mV, indicating that NADH has a weak affinity for electrons—and a strong tendency to donate them; a 1:1 mixture of H_2O and $\frac{1}{2}O_2$ has a redox potential of +820 mV, indicating that O_2 has a strong affinity for electrons—and a strong tendency to accept them. The difference in redox potential between these two pairs is 1.14 volts (1140 mV), which means that the transfer of each electron from NADH to O_2 under these standard conditions is enormously favorable: the $\Delta G°$ for that electron transfer is –26.2 kcal/mole per electron—or –52.4 kcal/mole for the two electrons that are donated from each NADH molecule (see Panel 14–1). If we compare this free-energy change with that needed for the formation of the phosphoanhydride bonds in ATP in cells (about 13 kcal/mole), we see that enough energy is released by the oxidization of one NADH molecule to synthesize a couple of molecules of ATP.

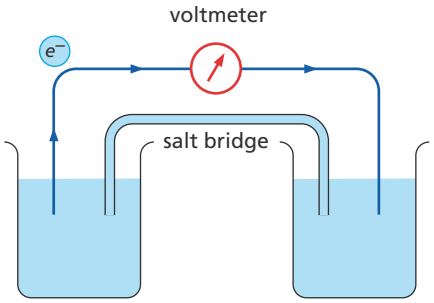
Living systems could have evolved enzymes that would allow NADH to donate electrons directly to O_2 to make water. But because of the huge free-energy drop, this reaction would proceed with almost explosive force and nearly all of the energy would be released as heat. Instead, as we have seen, the transfer of electrons from NADH to O_2 is made in many small steps along the electron-transport chain, enabling nearly half of the released energy to be stored in the proton gradient across the inner membrane rather than getting lost to the environment as heat.

Metals Tightly Bound to Proteins Form Versatile Electron Carriers

Each of the three respiratory enzyme complexes includes metal atoms that are tightly bound to the proteins. Once an electron has been donated to a respiratory complex, it moves within the complex by skipping from one embedded metal ion to another with a greater affinity for electrons.

PANEL 14–1 REDOX POTENTIALS

HOW REDOX POTENTIALS ARE MEASURED



One beaker (*left*) contains substance A with an equimolar mixture of the reduced ($A_{reduced}$) and oxidized ($A_{oxidized}$) members of its redox pair. The other beaker contains the hydrogen reference standard ($2H^+ + 2e^- \rightleftharpoons H_2$), whose redox potential is arbitrarily assigned as zero by international agreement. (A salt bridge formed from a concentrated KCl solution allows K^+ and Cl^- to move between the beakers; as required to neutralize the charges when electrons flow between the beakers.) The metal wire (*dark blue*) provides a resistance-free path for electrons, and a voltmeter then measures the redox potential of substance A. If electrons flow from $A_{reduced}$ to H^+, as indicated here, the redox pair formed by substance A is said to have a negative redox potential. If they instead flow from H_2 to $A_{oxidized}$, the redox pair is said to have a positive redox potential.

THE STANDARD REDOX POTENTIAL, E'_0

The standard redox potential for a redox pair, defined as E_0, is measured for a standard state where all of the reactants are at a concentration of 1 M, including H^+. Since biological reactions occur at pH 7, biologists instead define the standard state as $A_{reduced} = A_{oxidized}$ and $H^+ = 10^{-7}$ M. This standard redox potential is designated by the symbol E'_0, in place of E_0.

examples of redox reactions	standard redox potential E'_0
$NADH \rightleftharpoons NAD^+ + H^+ + 2e^-$	–320 mV
reduced ubiquinone $\rightleftharpoons$ oxidized ubiquinone $+ 2H^+ + 2e^-$	+30 mV
reduced cytochrome *c* $\rightleftharpoons$ oxidized cytochrome *c* $+ e^-$	+230 mV
$H_2O \rightleftharpoons \frac{1}{2}O_2 + 2H^+ + 2e^-$	+820 mV

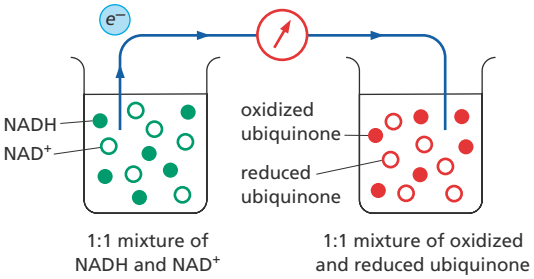
CALCULATION OF ΔG° FROM REDOX POTENTIALS

To determine the energy change for an electron transfer, the ΔG° of the reaction (kcal/mole) is calculated as follows:

$\Delta G^\circ = -n(0.023)\,\Delta E'_0$, where n is the number of electrons transferred across a redox potential change of $\Delta E'_0$ millivolts (mV), and

$\Delta E'_0 = E'_0(\text{acceptor}) - E'_0(\text{donor})$

EXAMPLE:



For the transfer of one electron from NADH to ubiquinone:

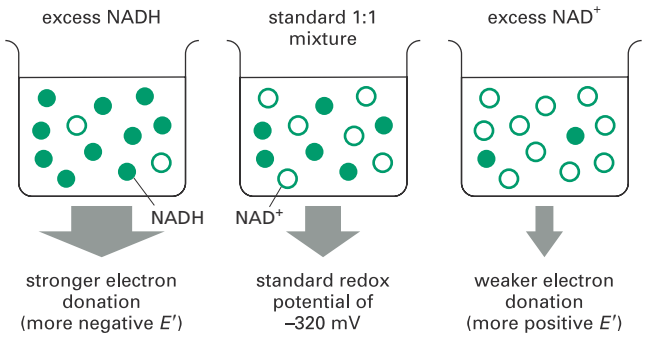
$$\Delta E'_0 = +30 - (-320) = +350 \text{ mV}$$

$$\Delta G^\circ = -n(0.023)\Delta E'_0 = -1(0.023)(350) = -8.0 \text{ kcal/mole}$$

The same calculation reveals that the transfer of one electron from ubiquinone to oxygen has an even more favorable ΔG° of –18.2 kcal/mole. The ΔG° value for the transfer of one electron from NADH to oxygen is the sum of these two values, –26.2 kcal/mole.

EFFECT OF CONCENTRATION CHANGES

As explained in Chapter 3 (see p. 94), the actual free-energy change for a reaction, ΔG, depends on the concentration of the reactants and generally will be different from the standard free-energy change, ΔG°. The standard redox potentials are for a 1:1 mixture of the redox pair. For example, the standard redox potential of –320 mV is for a 1:1 mixture of NADH and NAD^+. But when there is an excess of NADH over NAD^+, electron transfer from NADH to an electron acceptor becomes more favorable. This is reflected by a more negative redox potential and a more negative ΔG for electron transfer.



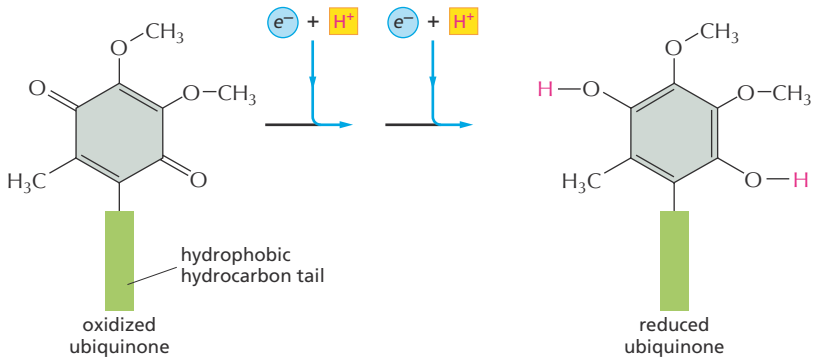


Figure 14–23 Quinones carry electrons within the lipid bilayer. The quinone in the mitochondrial electron-transport chain is called ubiquinone. It picks up one H^+ from the aqueous environment for every electron it accepts, and it can carry two electrons as part of its hydrogen atoms (*red*). When this reduced ubiquinone donates its electrons to the next carrier in the chain, the protons are released. Its long, hydrophobic hydrocarbon tail confines ubiquinone to the inner mitochondrial membrane.

When passing from one respiratory complex to the next, in contrast, the electrons are ferried by electron carriers that diffuse freely within the lipid bilayer. These mobile molecules pick up electrons from one complex and deliver them to the next in line. In the mitochondrial respiratory chain, for example, a small, hydrophobic molecule called ubiquinone picks up electrons from the NADH dehydrogenase complex and delivers them to the cytochrome *c* reductase complex (see Figure 14–14). A related **quinone** functions similarly during electron transport in photosynthesis. Ubiquinone can accept or donate either one or two electrons, and it picks up one H^+ from water with each electron that it carries (**Figure 14–23**). Its redox potential of +30 mV places ubiquinone between the NADH dehydrogenase complex and the cytochrome *c* reductase complex in terms of its tendency to gain or lose electrons—which explains why ubiquinone receives electrons from the former and donates them to the latter (**Figure 14–24**). Ubiquinone also serves as the entry point for electrons donated by the $FADH_2$ that is generated during the citric acid cycle and from fatty acid oxidation (see Figures 13–11 and 13–12).

The redox potentials of different metal complexes influence where they are used along the electron-transport chain. **Iron–sulfur centers** have relatively low affinities for electrons and thus are prominent in the electron carriers that operate in the early part of the chain. An iron–sulfur center in the NADH dehydrogenase complex, for example, passes electrons to ubiquinone. Later in the pathway, iron atoms held in the heme groups bound to cytochrome proteins are commonly used as electron carriers (**Figure 14–25**). These heme groups give **cytochromes**, such as

QUESTION 14–6

At many steps in the electron-transport chain, Fe ions are used as part of heme or FeS clusters to bind the electrons in transit. Why do these functional groups that carry out the chemistry of electron transfer need to be bound to proteins? Provide several different reasons why this is necessary.

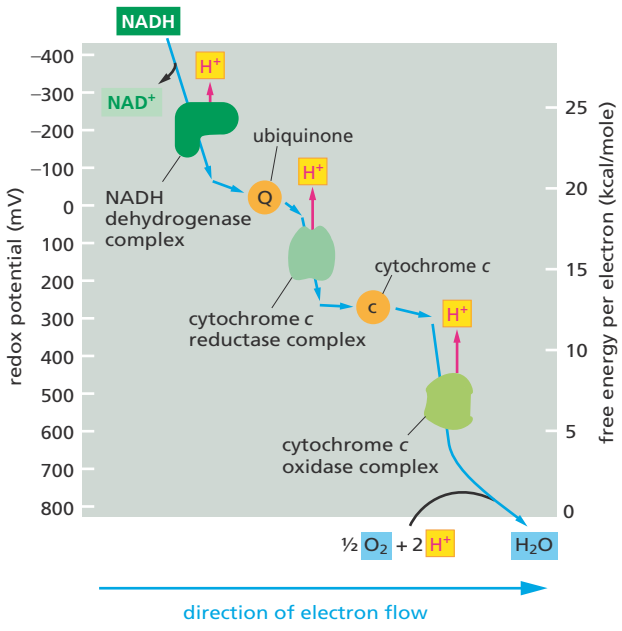


Figure 14–24 Redox potential increases along the mitochondrial electron-transport chain. The big increases in redox potential occur across each of the three respiratory enzyme complexes, as required for each of them to pump protons. To convert free-energy values to kJ/mole, recall that 1 kilocalorie is equal to about 4.2 kilojoules.

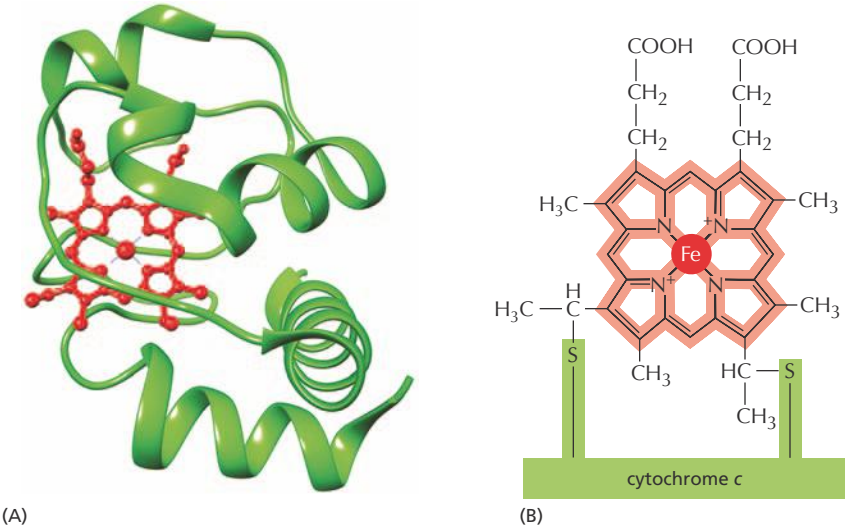


Figure 14–25 The iron in a heme group can serve as an electron acceptor. (A) Ribbon structure shows the position of the heme group (*red*) associated with cytochrome *c* (*green*). (B) The porphyrin ring of the heme group (*light red*) is attached covalently to side chains in the protein. The heme groups of different cytochromes have different electron affinities because they differ slightly in structure and are held in different local environments within each protein.

the cytochrome *c* reductase and cytochrome *c* oxidase complexes, their color ("cytochrome" from the Greek *chroma*, "color"). Like other electron carriers, the cytochrome proteins increase in redox potential the further down the mitochondrial electron-transport chain they are located. For example, *cytochrome* c, a small protein that accepts electrons from the cytochrome *c* reductase complex and transfers them to the cytochrome *c* oxidase complex, has a redox potential of +230 mV—a value about midway between those of the cytochromes with which it interacts (see Figure 14–24).

Cytochrome *c* Oxidase Catalyzes the Reduction of Molecular Oxygen

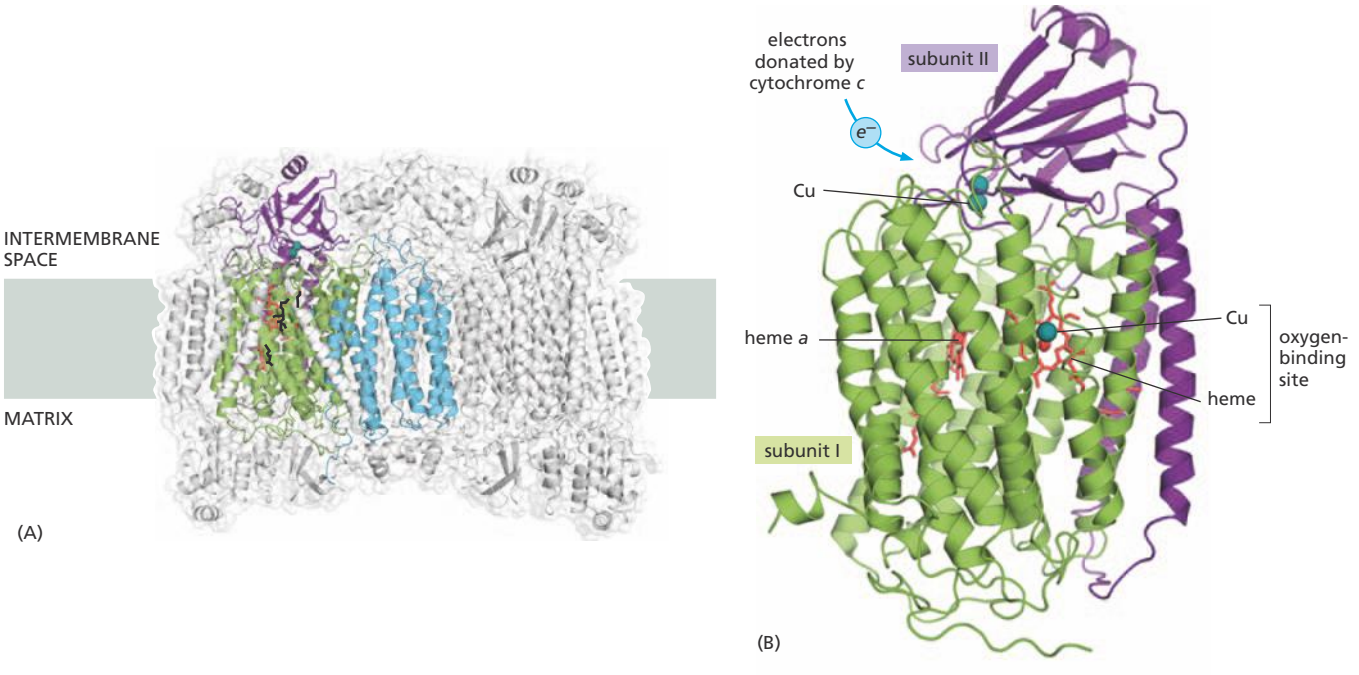
Cytochrome *c* oxidase, the final electron carrier in the respiratory chain, has the highest redox potential of all. This protein complex removes electrons from cytochrome *c*, thereby oxidizing it—hence the name "cytochrome *c* oxidase." These electrons are then handed off to O_2 to produce H_2O. In total, four electrons donated by cytochrome *c* and four protons from the aqueous environment are added to each O_2 molecule in the reaction $4e^- + 4H^+ + O_2 \rightarrow 2H_2O$.

In addition to the protons that combine with O_2, four other protons are pumped across the membrane during the transfer of four electrons from cytochrome *c* to O_2. This transfer of electrons drives allosteric changes in the conformation of the protein that move protons out of the mitochondrial matrix. A special oxygen-binding site within this protein complex—which contains both a heme group plus a copper atom—serves as the final repository for all of the electrons donated by NADH at the start of the electron-transport chain (**Figure 14–26**). It is here that nearly all the oxygen we breathe is consumed.

Oxygen is useful as an electron sink because of its very high affinity for electrons. However, once O_2 picks up one electron, it forms the superoxide radical O_2^-; this radical is dangerously reactive and will avidly take up another three electrons wherever it can find them, a tendency that can cause serious damage to nearby DNA, proteins, and lipid membranes. The active site of cytochrome *c* oxidase holds on tightly to an oxygen molecule until it receives all four of the electrons needed to convert it to two molecules of H_2O. This retention helps prevent superoxide radicals from attacking macromolecules throughout the cell—damage that has been postulated to contribute to human aging.

QUESTION 14–7

Two different diffusible electron carriers, ubiquinone and cytochrome *c*, shuttle electrons between the three protein complexes of the electron-transport chain. Could the same diffusible carrier, in principle, be used for both steps? Explain your answer.



The evolution of cytochrome *c* oxidase was crucial to the formation of cells that could use O_2 as an electron acceptor, and this protein complex is therefore essential for all aerobic life. Poisons such as cyanide are extremely toxic because they bind tightly to cytochrome *c* oxidase complexes, thereby halting electron transport and the production of ATP.

Figure 14–26 Cytochrome *c* oxidase is a finely tuned protein machine. The protein is a dimer formed from a monomer with 13 different protein subunits. (A) The entire protein is shown positioned in the inner mitochondrial membrane. The three colored subunits that form the functional core of the complex are encoded by the mitochondrial genome; the remaining subunits are encoded by the nuclear genome. (B) As electrons pass through this protein on the way to its bound O_2 molecule, they cause the protein to pump protons across the membrane. As indicated, a heme and copper atom (Cu) form the site where a tightly bound O_2 molecule is reduced to H_2O.

CHLOROPLASTS AND PHOTOSYNTHESIS

Virtually all the organic material in present-day cells is produced by **photosynthesis**—the series of light-driven reactions that creates organic molecules from atmospheric carbon dioxide (CO_2). Plants, algae, and photosynthetic bacteria such as cyanobacteria use electrons from water and the energy of sunlight to convert atmospheric CO_2 into organic compounds. In the course of these reactions, water molecules are split, releasing vast quantities of O_2 gas into the atmosphere. This oxygen in turn supports oxidative phosphorylation—not only in animals but also in plants and aerobic bacteria. Thus the activity of early photosynthetic bacteria, which filled the atmosphere with oxygen, enabled the evolution of the myriad life-forms that use aerobic metabolism to make their ATP (**Figure 14–27**).

In plants, photosynthesis is carried out in a specialized intracellular organelle—the **chloroplast**, which contains light-capturing pigments such as the green pigment *chlorophyll*. For most plants, the leaves are the major sites of photosynthesis. Photosynthesis occurs only during the daylight hours, producing ATP and NADPH. These activated carriers can then be used, at any time of day, to convert CO_2 into sugar inside the chloroplast—a process called *carbon fixation*.

Given the chloroplast's central role in photosynthesis, we begin this section by describing the structure of this highly specialized organelle. We then provide an overview of photosynthesis, followed by a detailed accounting of the mechanism by which chloroplasts harvest energy from sunlight to produce huge amounts of ATP and NADPH. We next describe how plants use these two activated carriers to synthesize the sugars and other food molecules that sustain them—and the many organisms that eat plants.

Figure 14–27 Microorganisms that carry out oxygen-producing photosynthesis changed Earth's atmosphere. (A) Living stromatolites from a lagoon in Western Australia. These structures are formed in specialized environments by large colonies of oxygen-producing photosynthetic cyanobacteria, which form mats that trap sand or minerals in thin layers. (B) Cross section of a modern stromatolite, showing its stratification. A similar structure is seen in fossilized stromatolites (not shown). These ancient accretions, some more than 3.5 billion years old, contain the remnants of the photosynthetic bacteria whose O_2-liberating activities transformed the Earth's atmosphere. (A, courtesy of Cambridge Carbonates Ltd.; B, courtesy of Roger Perkins, Virtual Fossil Museum.)

Chloroplasts Resemble Mitochondria but Have an Extra Compartment—the Thylakoid

Chloroplasts are larger than mitochondria, but both are organized along structurally similar principles. Chloroplasts have a highly permeable outer membrane and a much less permeable inner membrane, in which various membrane transport proteins are embedded. Together, these two membranes—and the narrow, intermembrane space that separates them—form the chloroplast envelope. The inner membrane surrounds a large space called the **stroma**, which is analogous to the mitochondrial matrix and contains many metabolic enzymes (see Figure 14–5).

There is, however, one important difference between the organization of mitochondria and that of chloroplasts. The inner membrane of the chloroplast does not contain the photosynthetic machinery. Instead, the light-capturing systems, electron-transport chain, and ATP synthase that produce ATP during photosynthesis are all contained in the *thylakoid membrane*. This third membrane is folded to form a set of flattened, disclike sacs, called the **thylakoids**, which are arranged in stacks called *grana* (**Figure 14–28**). The space inside each thylakoid is thought to be connected with that of other thylakoids, creating a third internal compartment, the *thylakoid space*, which is separate from the stroma.

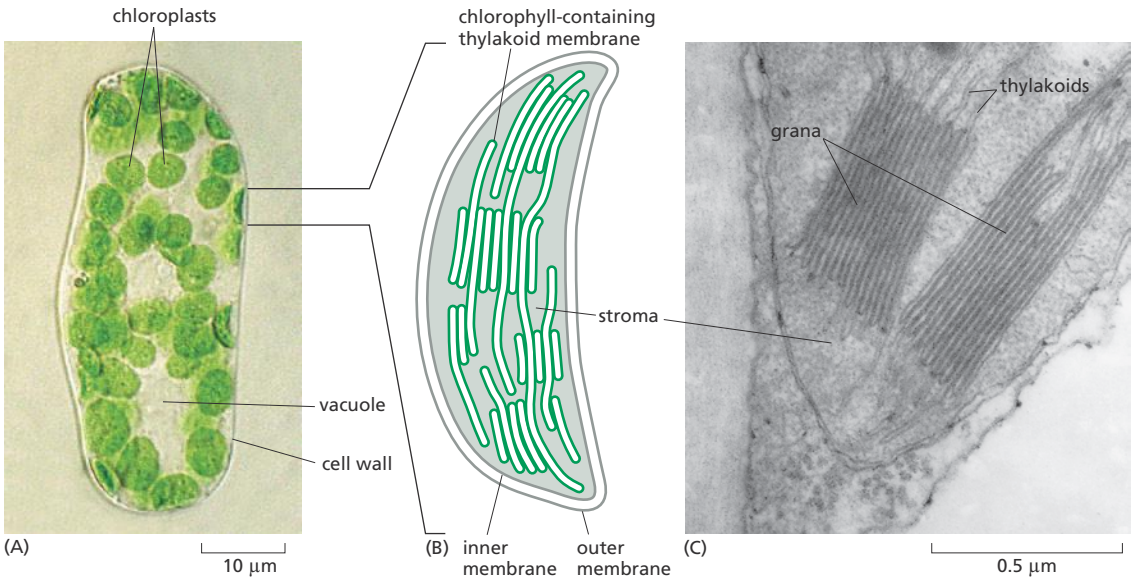


Figure 14–28 Chloroplasts, like mitochondria, are composed of a set of specialized membranes and compartments. (A) Light micrograph shows chloroplasts (*green*) in the cell of a flowering plant. (B) Drawing of a single chloroplast shows the organelle's three sets of membranes, including the thylakoid membrane, which contains the light-capturing and ATP-generating systems. (C) A high-magnification view of an electron micrograph shows the thylakoids arranged in stacks called *grana*; a single thylakoid stack is called a *granum*. (A, courtesy of Preeti Dahiya; C, courtesy of K. Plaskitt.)

Photosynthesis Generates—Then Consumes—ATP and NADPH

The chemistry carried out by photosynthesis can be summarized in one simple equation:

$$\text{light energy} + CO_2 + H_2O \rightarrow \text{sugars} + O_2 + \text{heat energy}$$

On its surface, the equation accurately represents the process by which light energy drives the production of sugars from CO_2. But this superficial accounting leaves out two of the most important players in photosynthesis: the activated carriers ATP and NADPH. In the first stage of photosynthesis, the energy from sunlight is used to produce ATP and NADPH; in the second stage, these activated carriers are consumed to fuel the synthesis of sugars.

1. *Stage 1* of photosynthesis is, in large part, equivalent to the oxidative phosphorylation that takes place on the mitochondrial inner membrane. In this stage, an electron-transport chain in the thylakoid membrane harnesses the energy of electron transport to pump protons into the thylakoid space; the resulting proton gradient then drives the synthesis of ATP by ATP synthase. What makes photosynthesis different is that the high-energy electrons donated to the *photosynthetic electron-transport chain* come from a molecule of **chlorophyll** that has absorbed energy from sunlight. Thus the energy-producing reactions of stage 1 are sometimes called the **light reactions** (**Figure 14–29**). The other major difference between photosynthesis and oxidative phosphorylation is where the high-energy electrons ultimately wind up: those that make their way down the photosynthetic electron-transport chain in chloroplasts are donated not to O_2, but to $NADP^+$, to produce NADPH.
2. In *Stage 2* of photosynthesis, the ATP and the NADPH produced by the photosynthetic electron-transfer reactions of stage 1 are used to drive the manufacture of sugars from CO_2 (see Figure 14–29). These *carbon-fixation reactions* can occur in the absence of sunlight and are thus also called the **dark reactions**. They begin in the chloroplast stroma, where they generate a three-carbon sugar called *glyceraldehyde 3-phosphate*. This simple sugar is exported to the cytosol, where it is used to produce sucrose and a large number of other organic molecules in the leaves of the plant.

Although the formation of ATP and NADPH during stage 1, and the conversion of CO_2 to carbohydrate during stage 2, are mediated by two separate sets of reactions, they are linked by elaborate feedback mechanisms that

QUESTION 14–8

Chloroplasts have a third internal compartment, the thylakoid space, bounded by the thylakoid membrane. This membrane contains the photosystems, reaction centers, electron-transport chain, and ATP synthase. In contrast, mitochondria use their inner membrane for electron transport and ATP synthesis. In both organelles, protons are pumped out of the largest internal compartment (the matrix in mitochondria and the stroma in chloroplasts). The thylakoid space is completely sealed off from the rest of the cell. Why does this arrangement allow a larger H^+ gradient in chloroplasts than can be achieved for mitochondria?

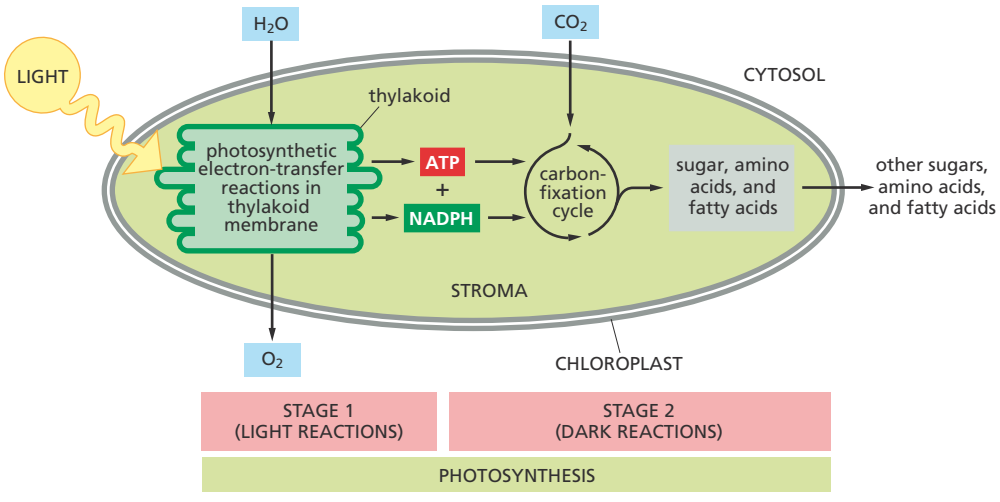


Figure 14–29 Both stages of photosynthesis depend on the chloroplast. In stage 1, a series of photosynthetic electron-transfer reactions produce ATP and NADPH; in the process, electrons are extracted from water and oxygen is released as a by-product, as we discuss shortly. In stage 2, carbon dioxide is assimilated (fixed) to produce sugars and a variety of other organic molecules. Stage 1 occurs in the thylakoid membrane, whereas stage 2 begins in the chloroplast stroma (as shown) and continues in the cytosol.

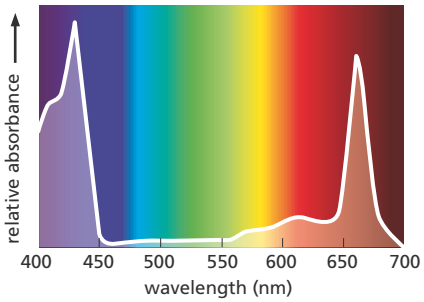
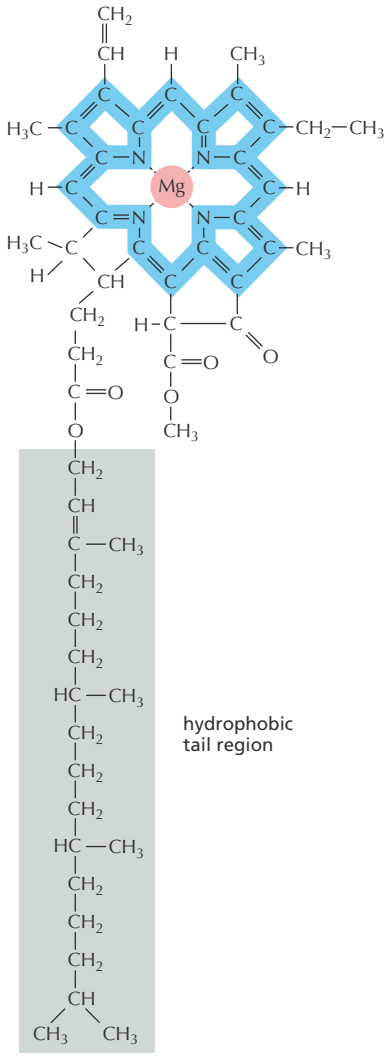


Figure 14–30 Chlorophylls absorb light of blue and red wavelengths. As shown in this absorption spectrum, one form of chlorophyll preferentially absorbs light around wavelengths of 430 nm (*blue*) and 660 nm (*red*). Green light, in contrast, is absorbed poorly by this pigment. Other chlorophylls can absorb light of slightly different wavelengths.



allow a plant to manufacture sugars only when it is appropriate to do so. Several of the enzymes required for carbon fixation, for example, are inactivated in the dark and reactivated by light-stimulated electron transport.

Chlorophyll Molecules Absorb the Energy of Sunlight

Visible light is a form of electromagnetic radiation composed of many wavelengths, ranging from violet (wavelength 400 nm) to deep red (700 nm). Most chlorophylls best absorb light in the blue and red wavelengths (**Figure 14–30**). Because these pigments absorb green light poorly, plants look green to us: the green light is reflected back to our eyes.

Chlorophyll's ability to harness energy derived from sunlight stems from its unique structure. The electrons in a chlorophyll molecule are distributed in a decentralized cloud around the molecule's light-absorbing porphyrin ring (**Figure 14–31**). When light of an appropriate wavelength hits a molecule of chlorophyll, it excites electrons in this diffuse network, perturbing the way the electrons are distributed. This perturbed high-energy state is unstable, and an excited chlorophyll molecule will seek to get rid of this excess energy so it can return to its more stable, unexcited state.

A molecule of chlorophyll, on its own in solution, would simply release its absorbed energy in the form of light or heat—accomplishing nothing useful. However, chlorophyll molecules in a chloroplast are able to convert light energy into a form of energy useful to the cell because they are associated with a special set of photosynthetic proteins in the thylakoid membrane, as we see next.

Excited Chlorophyll Molecules Funnel Energy into a Reaction Center

In the thylakoid membrane of plants and the plasma membrane of photosynthetic bacteria, chlorophyll molecules are held in large multiprotein complexes called **photosystems**. Each photosystem consists of a set of *antenna complexes*, which capture light energy, and a *reaction center*, which converts that light energy into chemical energy.

In each **antenna complex**, hundreds of chlorophyll molecules are arranged so that the light energy captured by one chlorophyll molecule can be transferred to a neighboring chlorophyll molecule in the network. In this way, energy jumps randomly from one chlorophyll molecule to the next—either within the same antenna or in an adjacent antenna. At some point, this wandering energy will encounter a chlorophyll dimer called the *special pair*, which holds its electrons at a lower energy than do the other chlorophyll molecules. Thus when energy is accepted by this special pair, it becomes effectively trapped there.

The chlorophyll special pair is not located in an antenna complex. Instead, it is part of the **reaction center**—a transmembrane complex of proteins and pigments that is thought to have first evolved more than 3 billion years ago in primitive photosynthetic bacteria (**Movie 14.6**). Within the reaction center, the special pair is positioned directly next to a set of electron carriers that are poised to accept a high-energy electron

Figure 14–31 Chlorophyll's structure allows it to absorb energy from light. Each chlorophyll molecule contains a porphyrin ring with a magnesium atom (*pink*) at its center. This porphyrin ring is structurally similar to the one that binds iron in heme (see Figure 14–25). Light is absorbed by electrons within the bond network shown in *blue*, while the long, hydrophobic tail (*gray*) helps hold the chlorophyll in the thylakoid membrane.

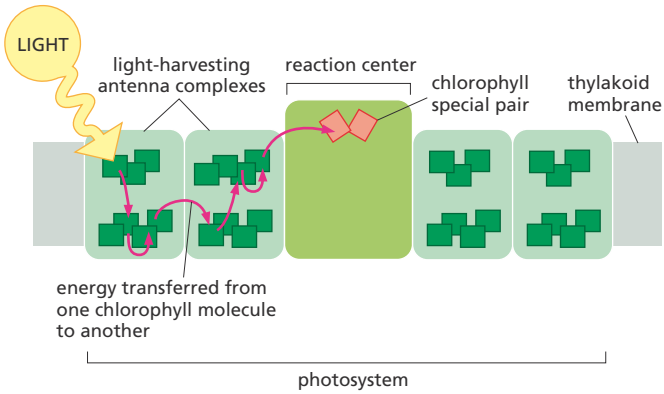


Figure 14–32 A photosystem consists of a reaction center surrounded by chlorophyll-containing antenna complexes. Once light energy has been captured by a chlorophyll molecule in an antenna complex, it will pass randomly from one chlorophyll molecule to another (*red lines*), until it gets trapped by a chlorophyll dimer called the *special pair*, located in the reaction center. The chlorophyll special pair holds its electrons at a lower energy than the antenna chlorophylls, so the energy transferred to it from the antenna gets trapped there. Note that in the antenna complex only energy moves from one chlorophyll molecule to another, not electrons.

from the excited chlorophyll special pair (**Figure 14–32**). This electron transfer lies at the heart of photosynthesis, because it converts the light energy that came into the special pair into chemical energy in the form of a transferable electron. As soon as the high-energy electron is handed off, the chlorophyll special pair becomes positively charged, and the electron carrier that accepts the electron becomes negatively charged. The rapid movement of this electron along a set of electron carriers in the reaction center then creates a *charge separation* that sets in motion the flow of electrons from the reaction center to an electron-transport chain (**Figure 14–33**).

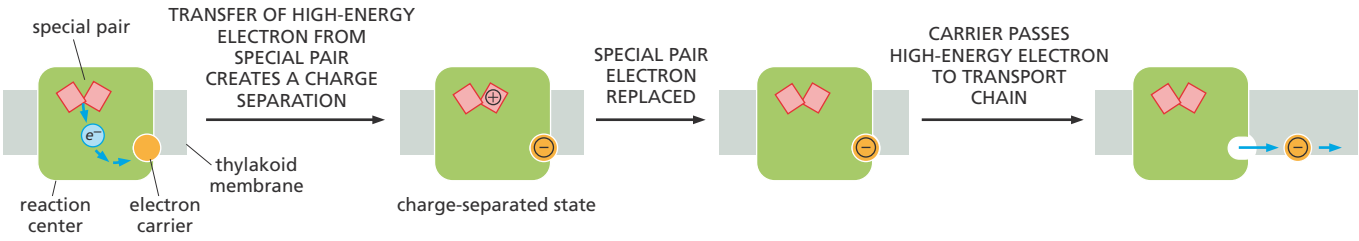
A Pair of Photosystems Cooperate to Generate Both ATP and NADPH

Photosynthesis is ultimately a biosynthetic process, and to build organic molecules from CO_2, a plant cell requires a huge input of energy, in the form of ATP, and a very large amount of reducing power, in the form of the activated carrier NADPH (see Figure 3–34). To generate both ATP and NADPH, plant cells—and free-living photosynthetic organisms such as cyanobacteria—use a pair of photosystems that are similar in structure, but that do different things with the high-energy electrons that leave their reaction center chlorophylls.

When the first photosystem (which, paradoxically, is called photosystem II for historical reasons) absorbs light energy, its reaction center passes electrons to a mobile electron carrier called *plastoquinone*, which is part of the photosynthetic electron-transport chain. This carrier transfers the high-energy electrons to a proton pump, which—like the proton pumps in the mitochondrial inner membrane—uses the movement of electrons to generate an electrochemical proton gradient. The electrochemical proton gradient then drives the production of ATP by an ATP synthase located in the thylakoid membrane (**Figure 14–34**).

At the same time, a second nearby photosystem—called photosystem I—has been also busy capturing the energy from sunlight. The reaction center of this photosystem passes its high-energy electrons to a different mobile electron carrier, which brings them to an enzyme that uses them to reduce $NADP^+$ to NADPH (**Figure 14–35**). The combined action of these

Figure 14–33 In a reaction center, a high-energy electron is transferred from the special pair to a carrier that becomes part of an electron-transport chain. Not shown is a set of intermediary carriers embedded in the reaction center that provide the path from the special pair to this carrier (*orange*). As illustrated, the transfer of the high-energy electron from the excited chlorophyll special pair leaves behind a positive charge that creates a charge-separated state, thereby converting light energy to chemical energy. Once the electron in the special pair has been replaced (an event we will discuss in detail shortly), the carrier diffuses away from the reaction center, transferring the high-energy electron to the transport chain.



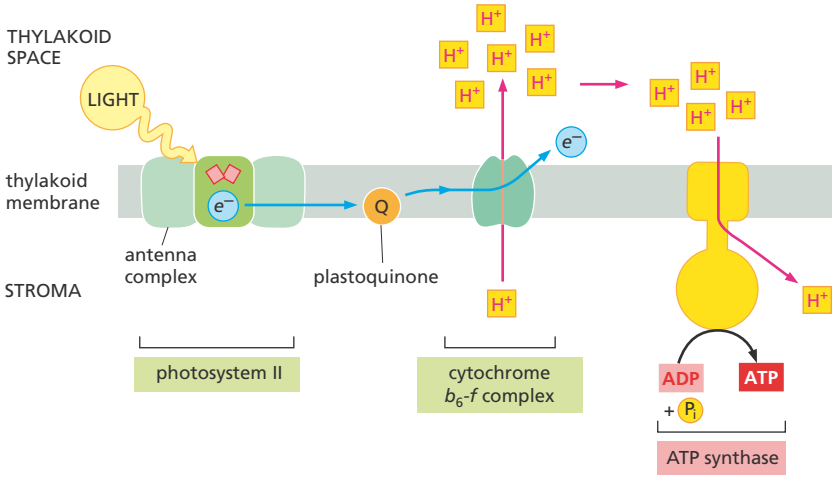


Figure 14–34 Photosystem II feeds electrons to a photosynthetic proton pump, leading to ATP synthesis by ATP synthase. When light energy is captured by photosystem II, a high-energy electron is transferred to a mobile electron carrier called plastoquinone (Q), which closely resembles the ubiquinone of mitochondria. This carrier transfers its electrons to a proton pump called the cytochrome b_6-f complex, which resembles the cytochrome *c* reductase complex of mitochondria and is the sole site of active proton pumping in the chloroplast electron-transport chain. As in mitochondria, an ATP synthase embedded in the membrane then uses the energy of the electrochemical proton gradient to produce ATP.

two photosystems thus produces both the ATP (photosystem II) and the NADPH (photosystem I) that will be used in stage 2 of photosynthesis (see Figure 14–29).

QUESTION 14–9

Both NADPH and the related carrier molecule NADH are strong electron donors. Why might plant cells have evolved to rely on NADPH, rather than NADH, to provide the reducing power for photosynthesis?

Oxygen Is Generated by a Water-Splitting Complex Associated with Photosystem II

The scheme that we have thus far described for photosynthesis has ignored a major chemical conundrum. When a mobile electron carrier removes an electron from a reaction center (whether in photosystem I or photosystem II), it leaves behind a positively charged chlorophyll special pair (see Figure 14–33). To reset the system and allow photosynthesis to proceed, this missing electron must be replaced.

For photosystem II, the missing electron is replaced by a special protein complex that removes the electrons from water. This *water-splitting enzyme* contains a cluster of manganese atoms that holds onto two water molecules from which electrons are extracted one at a time. Once four electrons have been removed from these two water molecules—and used to replace the electrons lost by four excited chlorophyll special pairs—O_2 is released (**Figure 14–36**).

This "waiting for four electrons" maneuver ensures that no partly oxidized water molecules are released as dangerous highly reactive chemicals. The same trick is used by the cytochrome *c* oxidase that catalyzes the reverse reaction—the transfer of electrons to O_2 to produce water—during oxidative phosphorylation (see Figure 14–26).

It is astounding to realize that essentially all of the oxygen in the Earth's atmosphere has been produced by the water-splitting enzyme of photosystem II.

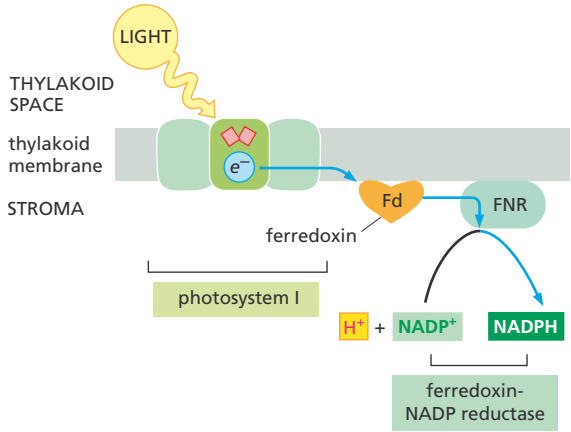


Figure 14–35 Photosystem I transfers high-energy electrons to an enzyme that produces NADPH. When light energy is captured by photosystem I, a high-energy electron is passed to a mobile electron carrier called ferredoxin (Fd), a small protein that contains an iron–sulfur center. Ferredoxin carries its electrons to ferredoxin-NADP reductase (FNR), the final protein in the electron-transport chain that generates NADPH.

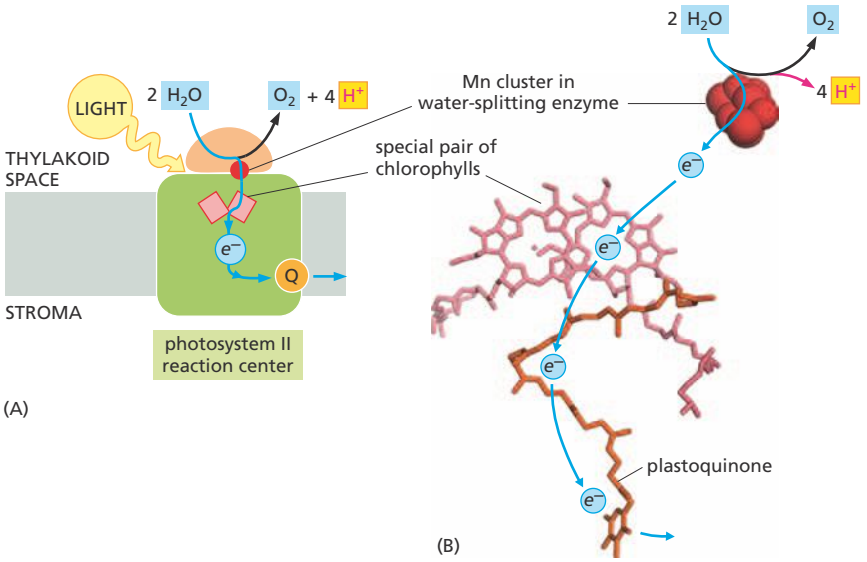


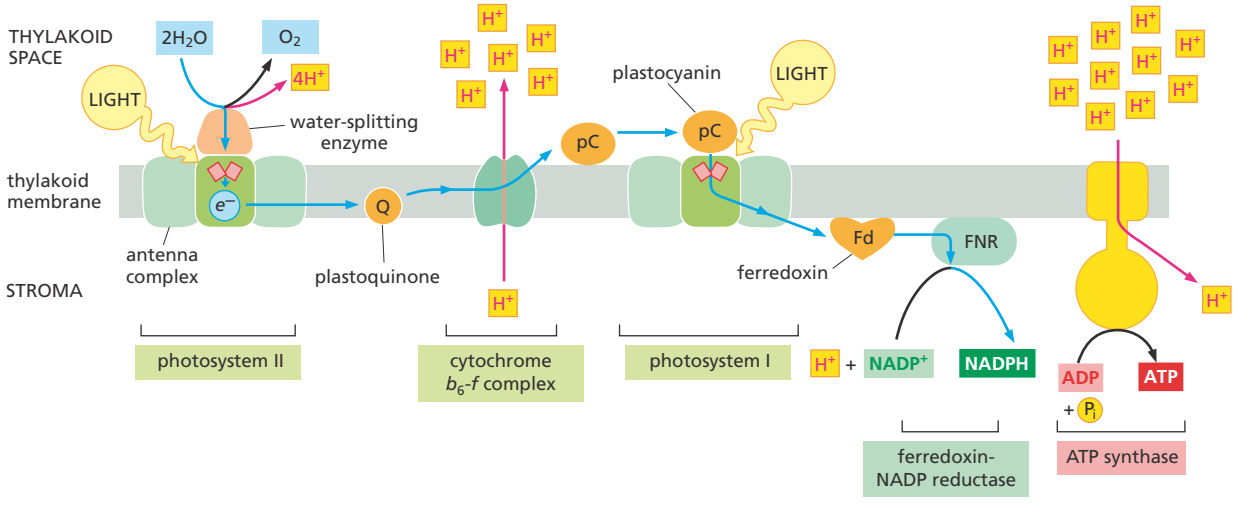
Figure 14–36 The reaction center of photosystem II includes an enzyme that catalyzes the extraction of electrons from water. (A) Schematic diagram shows the flow of electrons through the reaction center of photosystem II. When light energy excites the chlorophyll special pair, an electron is passed to the mobile electron carrier plastoquinone (Q). An electron is then returned to the special pair by a water-splitting enzyme that extracts electrons from water. The Mn cluster that participates in the electron extraction is shown as a *red spot*. Once four electrons have been withdrawn from two water molecules, O_2 is released into the atmosphere. (B) The structure and position of some of the electron carriers involved.

The Special Pair in Photosystem I Receives its Electrons from Photosystem II

We have seen that photosystem II receives electrons from water. But where does photosystem I get the electrons it needs to reset its special pair? It gets them from photosystem II: the chlorophyll special pair in photosystem I serves as the final electron acceptor for the electron-transport chain that carries electrons from photosystem II. The overall flow of electrons is shown in **Figure 14–37**. Electrons removed from water by photosystem II are passed, through a proton pump (the cytochrome b_6-f complex), to a mobile electron carrier called plastocyanin. Plastocyanin then carries these electrons to photosystem I, to replace the electrons lost by its excited chlorophyll special pair. When light is again absorbed by this photosystem, this electron will be boosted to the very high-energy level needed to reduce $NADP^+$ to NADPH.

Having these two photosystems operating in tandem effectively couples their two electron-energizing steps. This extra boost of energy—provided by the light harvested by both photosystems—allows an electron to be moved from water, which normally holds onto its electrons very tightly (redox potential = +820 mV), to NADPH, which normally holds onto its electrons loosely (redox potential = –320 mV). There is even enough energy left over to enable the electron-transport chain that links the two photosystems to pump H^+ across the thylakoid membrane, so that ATP

Figure 14–37 The movement of electrons along the photosynthetic electron-transport chain powers the production of both ATP and NADPH. Electrons are supplied to photosystem II by a water-splitting complex that extracts four electrons from two molecules of water, producing O_2 as a by-product. After their energy is raised by the absorption of light, these electrons power the pumping of protons by the cytochrome b_6-f complex. Electrons that pass through this complex are then donated to a copper-containing protein, the mobile electron carrier plastocyanin (pC), which ferries them to the reaction center of photosystem I. After an additional energy boost from light, these electrons are used to generate NADPH. An overview of these reactions is shown in Movie 14.7.



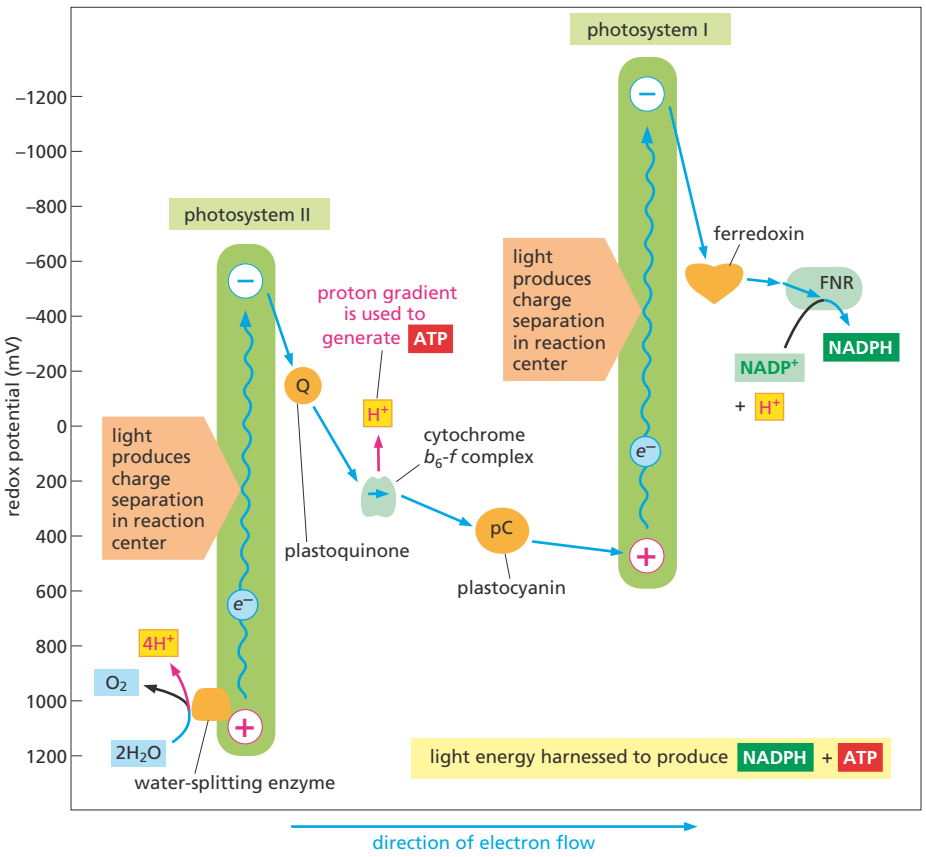


Figure 14–38 The combined actions of photosystems I and II boost electrons to the energy level needed to produce both ATP and NADPH. The redox potential for each molecule is indicated by its position on the vertical axis. Electron transfers are shown with non-wavy *blue arrows*. Photosystem II passes electrons from its excited chlorophyll special pair to an electron-transport chain in the thylakoid membrane that leads to photosystem I (see Figure 14–37). The net electron flow through the two photosystems linked in series is from water to $NADP^+$, to form NADPH.

synthase can harness some of the light-derived energy for ATP production (**Figure 14–38**).

Carbon Fixation Uses ATP and NADPH to Convert CO_2 into Sugars

The light reactions of photosynthesis generate ATP and NADPH in the chloroplast stroma, as we have just seen. But the inner membrane of the chloroplast is impermeable to both of these compounds, which means that they cannot be exported directly to the cytosol. To provide energy and reducing power for the rest of the cell, the ATP and NADPH are instead used within the chloroplast stroma to produce sugars, which can be exported by specific carrier proteins in the chloroplast inner membrane. This production of sugar from CO_2 and water, which occurs during the dark reactions (stage 2) of photosynthesis, is called **carbon fixation**.

In the central reaction of photosynthetic carbon fixation, CO_2 from the atmosphere is attached to a five-carbon sugar derivative, ribulose 1,5-bisphosphate, to yield two molecules of the three-carbon compound *3-phosphoglycerate*. This carbon-fixing reaction, which was discovered in 1948, is catalyzed in the chloroplast stroma by a large enzyme called ribulose bisphosphate carboxylase or *Rubisco* (**Figure 14–39**). Rubisco works much more slowly than most other enzymes: it processes about three molecules of substrate per second—compared with 1000 molecules per second for a typical enzyme. To compensate for this sluggish behavior, plants maintain a surplus of Rubisco to ensure the efficient production of sugars. The enzyme often represents more than 50% of the total chloroplast protein, and it is widely claimed to be the most abundant protein on Earth.

Although the production of carbohydrates from CO_2 and H_2O is energetically unfavorable, the fixation of CO_2 catalyzed by Rubisco is an

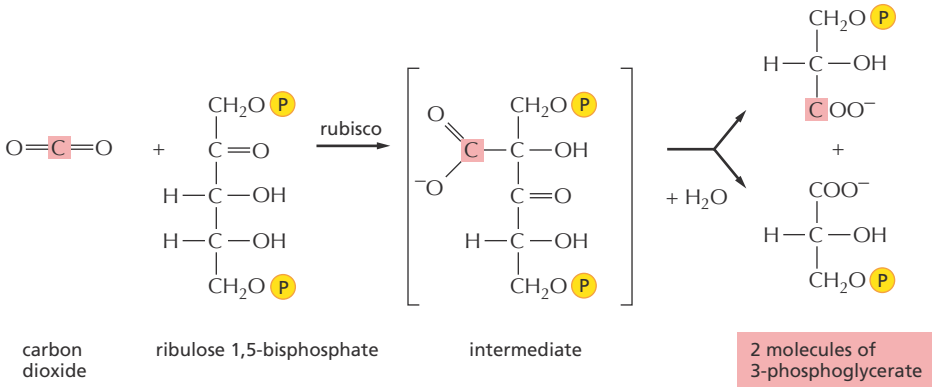


Figure 14–39 Carbon fixation involves the formation of a covalent bond that attaches carbon dioxide to ribulose 1,5-bisphosphate. The reaction is catalyzed in the chloroplast stroma by the abundant enzyme ribulose bisphosphate carboxylase, or Rubisco. As shown, the product is two molecules of 3-phosphoglycerate.

energetically favorable reaction. Carbon fixation is energetically favorable because a continuous supply of the energy-rich ribulose 1,5-bisphosphate is fed into it. As this compound is consumed—by the addition of CO_2 (see Figure 14–39)—it must be replenished. The energy and reducing power needed to regenerate ribulose 1,5-bisphosphate come from the ATP and NADPH produced by the photosynthetic light reactions.

The elaborate series of reactions in which CO_2 combines with ribulose 1,5-bisphosphate to produce a simple sugar—a portion of which is used to regenerate ribulose 1,5-bisphosphate—forms a cycle, called the *carbon-fixation cycle*, or the Calvin cycle (**Figure 14–40**). For every three

QUESTION 14–10

A. How do cells in plant roots survive, since they contain no chloroplasts and are not exposed to light?

B. Unlike mitochondria, chloroplasts do not have a transporter that allows them to export ATP to the cytosol. How, then, do plant cells obtain the ATP that they need to carry out energy-requiring metabolic reactions in the cytosol?

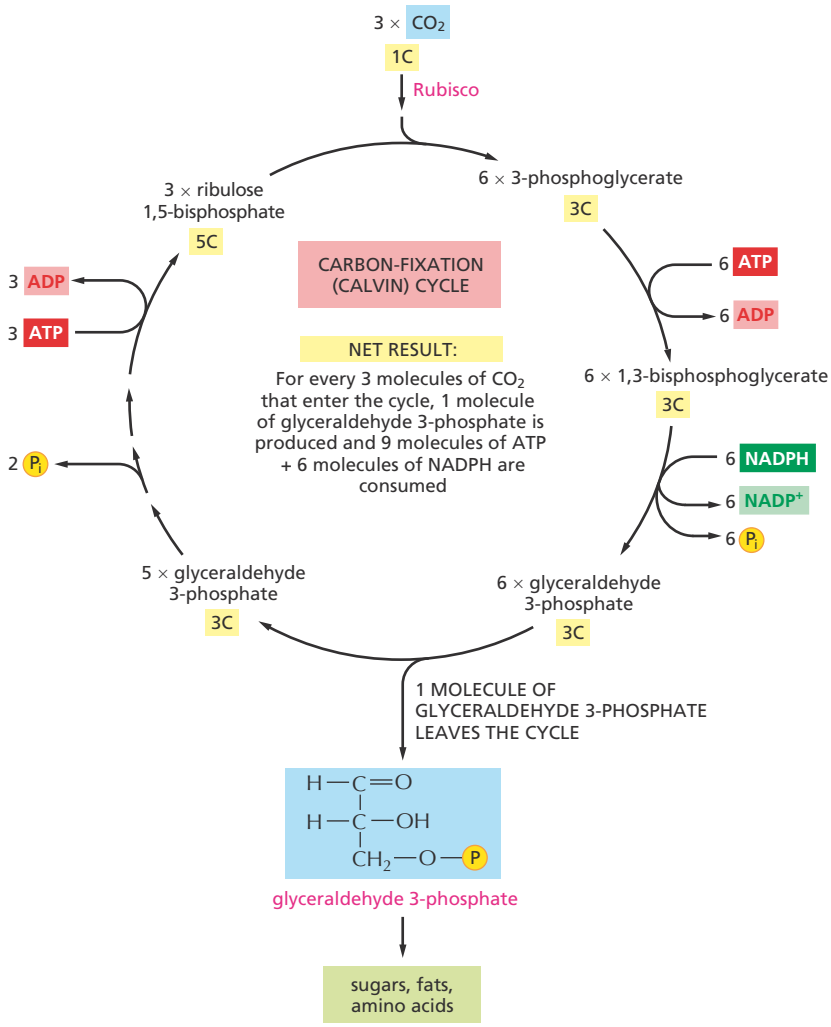


Figure 14–40 The carbon-fixation cycle consumes ATP and NADPH to form glyceraldehyde 3-phosphate from CO_2 and H_2O. In the first stage of the cycle, CO_2 is added to ribulose 1,5-bisphosphate (as shown in Figure 14–39). In the second stage, ATP and NADPH are consumed to produce glyceraldehyde 3-phosphate. In the final stage, some of the glyceraldehyde 3-phosphate produced is used to regenerate ribulose 1,5-bisphosphate; the rest is transported out of the chloroplast stroma into the cytosol. The number of carbon atoms in each type of molecule is indicated in *yellow*. There are many intermediates between glyceraldehyde 3-phosphate and ribulose 5-phosphate, but they have been omitted here for clarity. The entry of water into the cycle is also not shown.

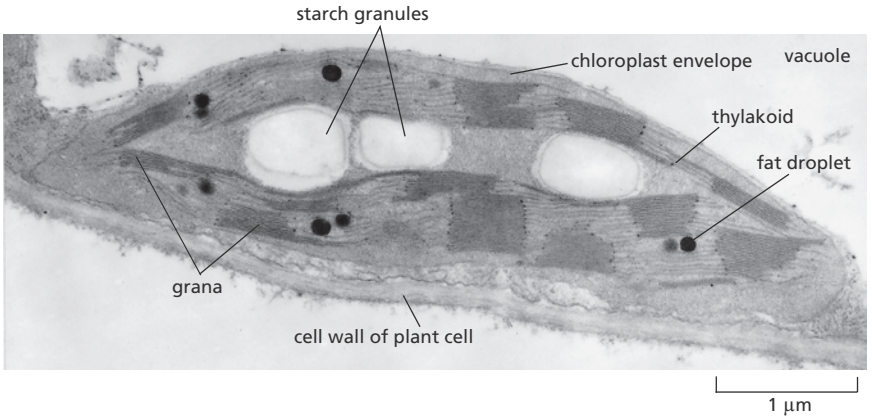


Figure 14–41 Chloroplasts often contain large stores of carbohydrates and fatty acids. A thin section of a single chloroplast shows the chloroplast envelope, starch granules, and fat droplets that have accumulated in the stroma as a result of the biosynthetic processes that occur there.

molecules of CO_2 that enter the cycle, one molecule of glyceraldehyde 3-phosphate is produced, and nine molecules of ATP and six molecules of NADPH are consumed. *Glyceraldehyde 3-phosphate*, the three-carbon sugar that is the final product of the cycle, then provides the starting material for the synthesis of many other sugars and other organic molecules.

Sugars Generated by Carbon Fixation Can Be Stored As Starch or Consumed to Produce ATP

The glyceraldehyde 3-phosphate generated by carbon fixation in the chloroplast stroma can be used in a number of ways, depending on the needs of the plant. During periods of excess photosynthetic activity, much of it is retained in the chloroplast stroma and converted to *starch*. Like glycogen in animal cells, starch is a large polymer of glucose that serves as a carbohydrate reserve, and it is stored as large granules in the chloroplast stroma. Starch forms an important part of the diet of all animals that eat plants. Other glyceraldehyde 3-phosphate molecules are converted to fat in the stroma. This material, which accumulates as fat droplets, likewise serves as an energy reserve (**Figure 14–41**).

At night, this stored starch and fat can be broken down to sugars and fatty acids, which are exported to the cytosol to help support the metabolic needs of the plant. Some of the exported sugar enters the glycolytic pathway (see Figure 13–5), where it is converted to pyruvate. That pyruvate, along with the fatty acids, can enter the plant cell mitochondria and be fed into the citric acid cycle, ultimately leading to the production of ATP by oxidative phosphorylation (**Figure 14–42**). Plants use this ATP in the same way that animal cells and other nonphotosynthetic organisms do to power a variety of metabolic reactions.

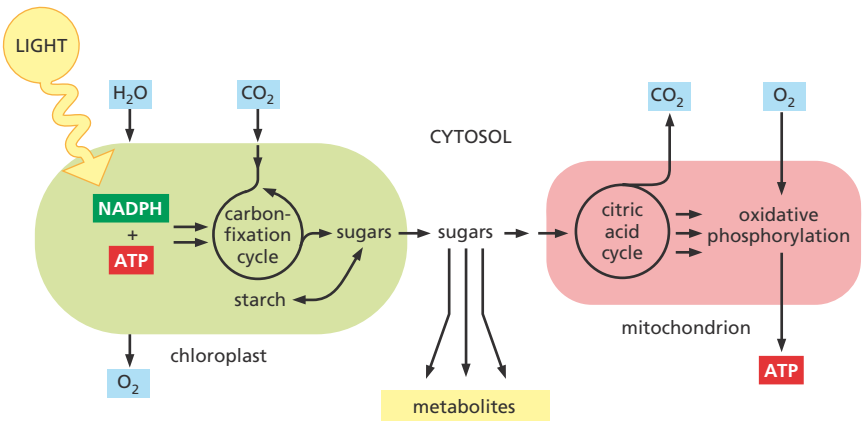


Figure 14–42 In plants, the chloroplasts and mitochondria collaborate to supply cells with metabolites and ATP. The chloroplast's inner membrane is impermeable to the ATP and NADPH that are produced in the stroma during the light reactions of photosynthesis. These molecules are therefore funneled into the carbon-fixation cycle, where they are used to make sugars. The resulting sugars and their metabolites are either stored within the chloroplast—in the form of starch or fat—or exported to the rest of the plant cell. There, they can enter the energy-generating pathway that ends in ATP synthesis in the mitochondria. Mitochondrial membranes are permeable to ATP, as indicated. Note that the O_2 released to the atmosphere by photosynthesis in chloroplasts is used for oxidative phosphorylation in mitochondria; similarly, the CO_2 released by the citric acid cycle in mitochondria is used for carbon fixation in chloroplasts.

The glyceraldehyde 3-phosphate exported from chloroplasts into the cytosol can also be converted into many other metabolites, including the disaccharide *sucrose*. Sucrose is the major form in which sugar is transported between the cells of a plant: just as glucose is transported in the blood of animals, so sucrose is exported from the leaves via the vascular bundle to provide carbohydrate to the rest of the plant.

THE EVOLUTION OF ENERGY-GENERATING SYSTEMS

The ability to sequence the genomes of microorganisms that are difficult, if not impossible, to grow in culture has made it possible to identify a huge variety of previously mysterious life-forms. Some of these unicellular organisms thrive in the most inhospitable habitats on the planet, including sulfurous hot springs and hydrothermal vents that lie deep on the ocean floor. In these remarkable microbes, we are finding clues to life's history. Like fingerprints left at the scene of a crime, the proteins and small molecules these organisms produce provide evidence that allows us to trace the history of ancient biological events, including those that gave rise to the ATP-generating systems present in the mitochondria and chloroplasts of modern eukaryotic cells. We therefore end this chapter with a brief review of what has been learned about the origins of present-day energy-harvesting systems, which have played such a critical part in fueling the evolution of life on Earth.

Oxidative Phosphorylation Evolved in Stages

As we mentioned earlier, the first living cells on Earth—both prokaryotes and primitive eukaryotes—may have consumed geochemically produced organic molecules and generated ATP by fermentation. Because oxygen was not yet present in the atmosphere, such anaerobic fermentation reactions would have dumped organic acids—such as lactic or formic acids, for example—into the environment (see Figure 13–6A).

Perhaps such acids lowered the pH of the environment, favoring the survival of cells that evolved transmembrane proteins that could pump H^+ out of the cytosol, thereby preventing the cell from becoming too acidic (stage 1 in **Figure 14–43**). One of these pumps may have used the energy available from ATP hydrolysis to eject H^+ from the cell; such a proton pump could have been the ancestor of present-day ATP synthases.

As the Earth's supply of geochemically produced nutrients began to dwindle, organisms that could find a way to pump H^+ without consuming ATP would have been at an advantage: they could save the small amounts of ATP they derived from the fermentation of increasingly scarce foodstuffs to fuel other important activities. This need to conserve resources might have led to the evolution of electron-transport proteins that allowed cells to use the movement of electrons between molecules of different redox potentials as a source of energy for pumping H^+ across the plasma membrane (stage 2 in Figure 14–43). Some of these cells might have used the nonfermentable organic acids that neighboring cells had excreted as waste to provide the electrons needed to feed this electron-transport system. Some present-day bacteria grow on formic acid, for example, using the small amount of redox energy derived from the transfer of electrons from formic acid to fumarate to pump H^+.

Eventually, some bacteria would have developed H^+-pumping electron-transport systems that were so efficient that they could harvest more redox energy than they needed to maintain their internal pH. Such cells would probably have generated large electrochemical proton gradients,

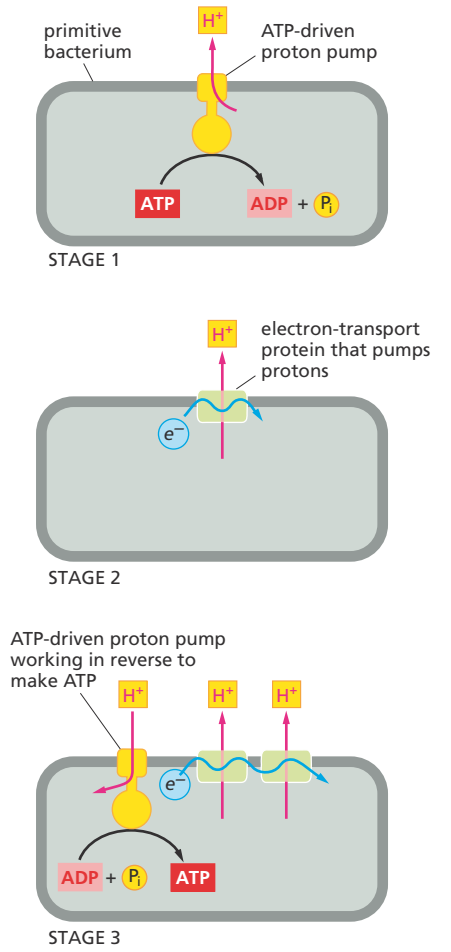


Figure 14–43 Oxidative phosphorylation might have evolved in stages. The first stage could have involved the evolution of an ATPase that pumped protons out of the cell using the energy of ATP hydrolysis. Stage 2 could have involved the evolution of a different proton pump, driven by an electron-transport chain. Stage 3 would then have linked these two systems together to generate an ATP synthase that uses the protons pumped by the electron-transport chain to synthesize ATP. A bacterium with this final system would have had a selective advantage over bacteria with neither of the systems or only one.

which they could then use to produce ATP. Protons could leak back into the cell through the ATP-driven H^+ pumps, essentially running them in reverse so that they synthesized ATP (stage 3 in Figure 14–43). Because such cells would require much less of the dwindling supply of fermentable nutrients, they would have proliferated at the expense of their neighbors.

Photosynthetic Bacteria Made Even Fewer Demands on Their Environment

The major evolutionary breakthrough in energy metabolism, however, was almost certainly the formation of photochemical reaction centers that could use the energy of sunlight to produce molecules such as NADH. It is thought that this development occurred early in the process of evolution—more than 3 billion years ago, in the ancestors of green sulfur bacteria. Present-day green sulfur bacteria use light energy to transfer hydrogen atoms (as an electron plus a proton) from H_2S to NADPH, thereby creating the strong reducing power required for carbon fixation (**Figure 14–44**).

The next step is thought to have involved the evolution of organisms capable of using water instead of H_2S as the electron source for photosynthesis. This entailed the evolution of a water-splitting enzyme and the addition of a second photosystem, acting in conjunction with the first, to bridge the enormous gap in redox potential between H_2O and NADPH (see Figure 14–38). The biological consequences of this evolutionary step were far-reaching. For the first time, there were organisms that made only minimal chemical demands on their environment. These cells—including the first cyanobacteria (see Figure 14–27)—could spread and evolve in ways denied to the earlier photosynthetic bacteria, which needed H_2S, organic acids, or other sources as a source of electrons. Consequently, large amounts of fermentable organic materials—produced by these cells and their ancestors—began to accumulate. Moreover, O_2 entered the atmosphere in large amounts (**Figure 14–45**).

The availability of O_2 made possible the development of bacteria that relied on aerobic metabolism to make their ATP. As explained previously, these organisms could harness the large amount of energy released when they break down carbohydrates and other reduced organic molecules all the way to CO_2 and H_2O.

As organic materials accumulated as a by-product of photosynthesis, some photosynthetic bacteria—including the ancestors of the bacterium

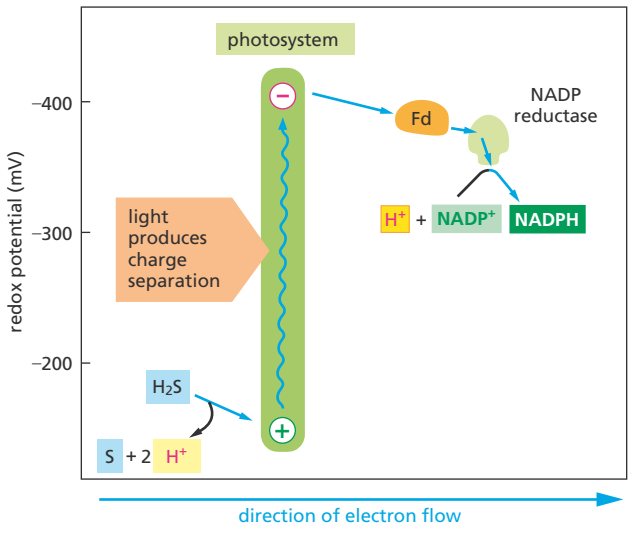


Figure 14–44 Photosynthesis in green sulfur bacteria uses hydrogen sulfide (H_2S) as an electron donor rather than water. Electrons are easier to extract from H_2S than from H_2O, because H_2S has a much higher redox potential (compare with Figure 14–38). Therefore, only one photosystem is needed to produce NADPH, and elemental sulfur is formed as a by-product instead of O_2.The photosystem in green sulfur bacteria resembles photosystem I in plants and cyanobacteria, in that they all use a series of iron–sulfur centers as the electron carriers that eventually donate their high-energy electrons to ferredoxin (Fd). A bacterium of this type is *Chlorobium tepidum*, which can thrive at high temperatures and low light intensities in hot springs.

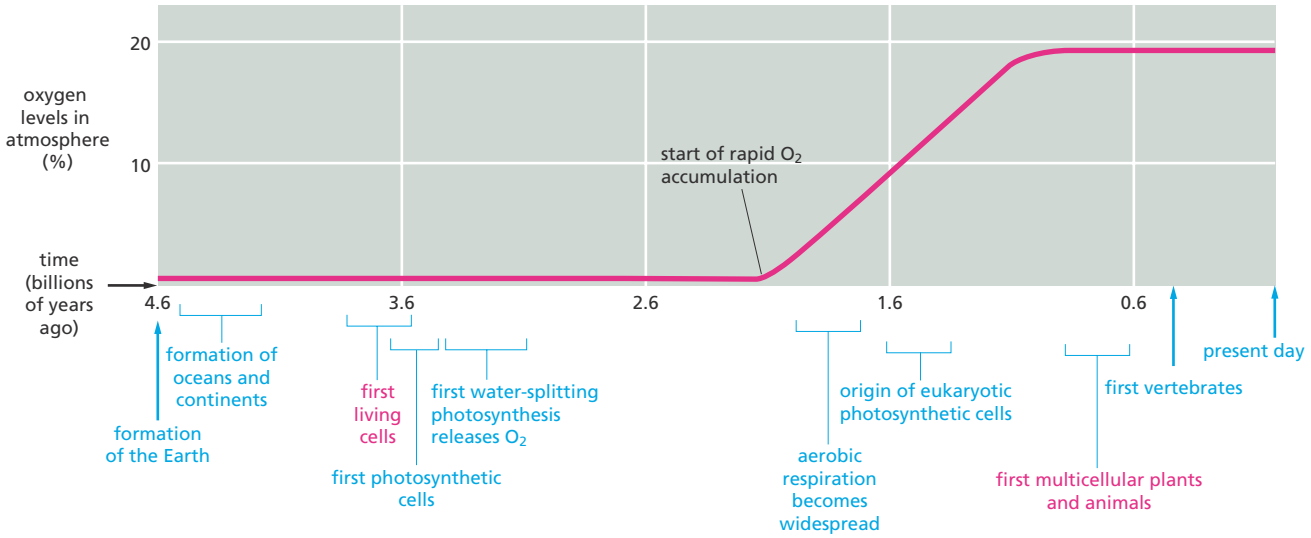


Figure 14–45 Oxygen entered Earth's atmosphere billions of years ago. With the evolution of photosynthesis in prokaryotes more than 3 billion years ago, organisms would have no longer depended on preformed organic chemicals. They could have then made their own organic molecules from CO_2. The delay of more than a billion years between the appearance of bacteria that split water and released O_2 during photosynthesis and the accumulation of high levels of O_2 in the atmosphere is thought to have been due to the initial reaction of the O_2 with abundant ferrous iron (Fe^{2+}) dissolved in the early oceans. Only when the iron was used up would large amounts of O_2 have started to accumulate in the atmosphere. In response to the rising amount of O_2 in the atmosphere, nonphotosynthetic, aerobic organisms appeared, and the concentration of O_2 in the atmosphere eventually leveled out.

E. coli—lost their ability to survive on light energy alone and came to rely entirely on cell respiration. Mitochondria probably arose when a pre-eukaryotic cell engulfed such an aerobic bacterium (see Figure 1–18). Plants arose somewhat later, when a descendant of this early aerobic eukaryote captured a photosynthetic bacterium, which became the precursor of chloroplasts (see Figure 1–20). Once eukaryotes had acquired the bacterial symbionts that became mitochondria and chloroplasts, they could then embark on the spectacular pathway of evolution that eventually led to complex multicellular organisms.

The Lifestyle of *Methanococcus* Suggests That Chemiosmotic Coupling Is an Ancient Process

The conditions today that most resemble those under which cells are thought to have lived 3.5–3.8 billion years ago may be those near deep-ocean hydrothermal vents. These vents represent places where the Earth's molten mantle is breaking through the overlying crust, expanding the width of the ocean floor. Indeed, the modern organisms that appear to be most closely related to the hypothetical cells from which all life evolved live at 75°C to 95°C, temperatures approaching that of boiling water. This ability to thrive at such extreme temperatures suggests that life's common ancestor—the cell that gave rise to bacteria, archaea, and eukaryotes—lived under very hot, anaerobic conditions.

One of the archaea that live in this environment today is *Methanococcus jannaschii*. Originally isolated from a hydrothermal vent more than a mile beneath the ocean surface, the organism grows in the complete absence of light and gaseous oxygen, using as nutrients the inorganic gases—hydrogen (H_2), CO_2, and nitrogen (N_2)—that bubble up from the vent (**Figure 14–46**). Its mode of existence gives us a hint of how early cells might have used electron transport to derive energy and to extract carbon molecules from inorganic materials that were freely available on the hot early Earth.

Methanococcus relies on N_2 gas as its source of nitrogen for making organic molecules such as amino acids. The organism reduces N_2 to ammonia (NH_3) by the addition of hydrogen, a process called **nitrogen fixation**. Nitrogen fixation requires a large amount of energy, as does the carbon-fixation process that converts CO_2 and H_2O into sugars. Much of the energy that *Methanococcus* requires for both processes is derived from the transfer of electrons from H_2 to CO_2, with the release of large amounts of methane (CH_4) as a waste product (thus producing natural gas

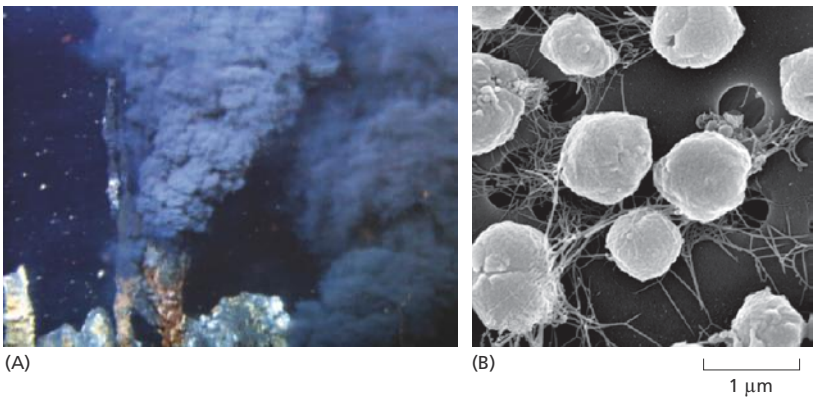


Figure 14–46 *Methanococcus* represents life-forms that might have existed early in Earth's history. (A) This deep-sea archaean lives in hydrothermal vents, such as the one shown, where temperatures reach near that of boiling water. (B) Scanning electron micrograph shows individual *Methanococcus* cells. These organisms use the hydrogen gas (H_2) that bubbles from deep-sea vents as the source of reducing power to generate energy via chemiosmotic coupling. (A, courtesy of the National Oceanic and Atmospheric Administration's Pacific Marine Environmental Laboratory Vents Program; B, courtesy of Chan B. Park.)

and giving the organism its name). Part of this electron transfer occurs in the plasma membrane and results in the pumping of protons (H^+) across it. The resulting electrochemical proton gradient drives an ATP synthase in the same membrane to make ATP.

The fact that such chemiosmotic coupling exists in an organism like *Methanococcus* suggests that the storage of energy in a proton gradient derived from electron transport is an extremely ancient process. Thus, chemiosmotic coupling may have fueled the evolution of nearly all life-forms on Earth.

ESSENTIAL CONCEPTS

- **Mitochondria, chloroplasts, and many prokaryotes generate energy by a membrane-based mechanism known as chemiosmotic coupling, which involves using an electrochemical proton gradient to drive the synthesis of ATP.**
- **Mitochondria produce most of an animal cell's ATP, using energy derived from oxidation of sugars and fatty acids.**
- **Mitochondria have an inner and an outer membrane. The inner membrane encloses the mitochondrial matrix, where the citric acid cycle produces large amounts of NADH and $FADH_2$ from the oxidation of acetyl CoA.**
- **In the inner mitochondrial membrane, high-energy electrons donated by NADH and $FADH_2$ pass along an electron-transport chain and eventually combine with molecular oxygen (O_2) to form water.**
- **Much of the energy released by electron transfers along the electron-transport chain is harnessed to pump protons (H^+) out of the matrix, creating an electrochemical proton gradient. The proton pumping is carried out by three large respiratory enzyme complexes embedded in the inner membrane.**
- **The electrochemical proton gradient across the inner mitochondrial membrane is harnessed to make ATP when protons move back into the matrix through an ATP synthase located in the inner membrane.**
- **The electrochemical proton gradient also drives the active transport of selected metabolites into and out of the mitochondrial matrix.**
- **In photosynthesis in chloroplasts and photosynthetic bacteria, the energy of sunlight is captured by chlorophyll molecules embedded in large protein complexes known as photosystems; in plants, these photosystems are located in the thylakoid membranes of chloroplasts in leaf cells.**
- **Electron-transport chains associated with photosystems transfer high-energy electrons from water to $NADP^+$ to form NADPH, which produces O_2 as a by-product.**

- The photosynthetic electron-transport chains in chloroplasts also generate a proton gradient across the thylakoid membrane, which is used by an ATP synthase embedded in the membrane to generate ATP.
- The ATP and the NADPH made by photosynthesis are used within the chloroplast stroma to drive the carbon-fixation cycle, which produces carbohydrate from CO_2 and water.
- Carbohydrate is exported from the stroma to the cell cytosol, where it provides the starting material for the synthesis of other organic molecules.
- Both mitochondria and chloroplasts are thought to have evolved from bacteria that were endocytosed by other cells. Each retains its own genome and divides by processes that resemble a bacterial cell division.
- Chemiosmotic coupling mechanisms are of ancient origin. Modern microorganisms that live in environments similar to those thought to have been present on the early Earth also use chemiosmotic coupling to produce ATP.

KEY TERMS

antenna complex
ATP synthase
carbon fixation
cell respiration
chemiosmotic coupling
chlorophyll
chloroplast
cytochrome
cytochrome *c* oxidase
dark reactions
electron-transport chain
iron–sulfur center
light reactions
matrix
mitochondrion
nitrogen fixation
oxidative phosphorylation
photosynthesis
photosystem
quinone
reaction center
redox pair
redox potential
redox reaction
respiratory enzyme complex
stroma
thylakoids

QUESTIONS

QUESTION 14–11

Which of the following statements are correct? Explain your answers.

A. After an electron has been removed by light, the affinity for electrons of the positively charged chlorophyll in the reaction center of the first photosystem (photosystem II) is even greater than the electron affinity of O_2.

B. Photosynthesis is the light-driven transfer of an electron from chlorophyll to a second molecule that normally has a much lower affinity for electrons.

C. Because it requires the removal of four electrons to release one O_2 molecule from two H_2O molecules, the water-splitting enzyme in photosystem II has to keep the reaction intermediates tightly bound so as to prevent partly reduced, and therefore hazardous, superoxide radicals from escaping.

QUESTION 14–12

Which of the following statements are correct? Explain your answers.

A. Many, but not all, electron-transfer reactions involve metal ions.

B. The electron-transport chain generates an electrical potential across the membrane because it moves electrons from the intermembrane space into the matrix.

C. The electrochemical proton gradient consists of two components: a pH difference and an electrical potential.

D. Ubiquinone and cytochrome *c* are both diffusible electron carriers.

E. Plants have chloroplasts and therefore can live without mitochondria.

F. Both chlorophyll and heme contain an extensive system of double bonds that allows them to absorb visible light.

G. The role of chlorophyll in photosynthesis is equivalent to that of heme in mitochondrial electron transport.

H. Most of the dry weight of a tree comes from the minerals that are taken up by the roots.

QUESTION 14–13

A single proton moving down its electrochemical gradient into the mitochondrial matrix space liberates 4.6 kcal/mole of free energy (ΔG). How many protons have to flow across the inner mitochondrial membrane to synthesize one molecule of ATP if the ΔG for ATP synthesis under intracellular conditions is between 11 and 13 kcal/mole? (ΔG is discussed in Chapter 3, pp. 90–100.) Why is a range given for this latter value, and not a precise number? Under which conditions would the lower value apply?

QUESTION 14–14

In the following statement, choose the correct one of the alternatives in italics and justify your answer. "If no O_2 is available, all components of the mitochondrial electron-transport chain will accumulate in their *reduced/oxidized* form. If O_2 is suddenly added again, the electron carriers in cytochrome *c* oxidase will become *reduced/oxidized before/after* those in NADH dehydrogenase."

QUESTION 14–15

Assume that the conversion of oxidized ubiquinone to reduced ubiquinone by NADH dehydrogenase occurs on the matrix side of the inner mitochondrial membrane and that its oxidation by cytochrome *c* reductase occurs on the intermembrane space side of the membrane (see Figures 14–14 and 14–23). What are the consequences of this arrangement for the generation of the H^+ gradient across the membrane?

QUESTION 14–16

If a voltage is applied to two platinum wires (electrodes) immersed in water, then water molecules become split into H_2 and O_2 gas. At the negative electrode, electrons are donated and H_2 gas is released; at the positive electrode, electrons are accepted and O_2 gas is produced. When photosynthetic bacteria and plant cells split water, they produce O_2, but no H_2. Why?

QUESTION 14–17

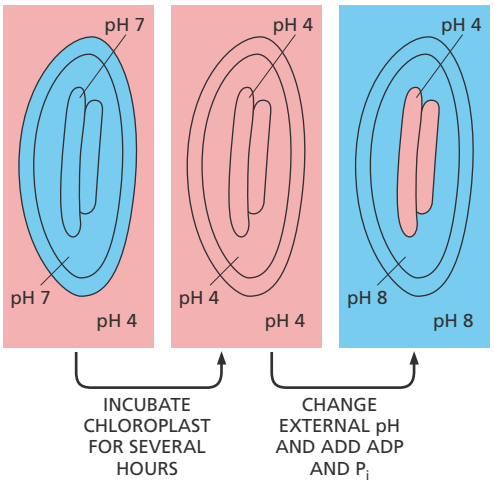
In an insightful experiment performed in the 1960s, chloroplasts were first soaked in an acidic solution at pH 4, so that the stroma and thylakoid space became acidified (**Figure Q14–17**). They were then transferred to a basic solution (pH 8). This quickly increased the pH of the stroma to 8, while the thylakoid space temporarily remained at pH 4. A burst of ATP synthesis was observed, and the pH difference between the thylakoid and the stroma then disappeared.

A. Explain why these conditions lead to ATP synthesis.

B. Is light needed for the experiment to work?

C. What would happen if the solutions were switched so that the first incubation is in the pH 8 solution and the second one in the pH 4 solution?

Figure Q14–17



D. Does the experiment support or question the chemiosmotic model?

Explain your answers.

QUESTION 14–18

As your first experiment in the laboratory, your adviser asks you to reconstitute purified bacteriorhodopsin, a light-driven H^+ pump from the plasma membrane of photosynthetic bacteria, and purified ATP synthase from ox-heart mitochondria together into the same membrane vesicles—as shown in **Figure Q14–18**. You are then asked to add ADP and P_i to the external medium and shine light into the suspension of vesicles.

A. What do you observe?

B. What do you observe if not all the detergent is removed and the vesicle membrane therefore remains leaky to ions?

C. You tell a friend over dinner about your new experiments, and he questions the validity of an approach that utilizes components from so widely divergent, unrelated organisms: "Why would anybody want to mix vanilla pudding with brake fluid?" Defend your approach against his critique.

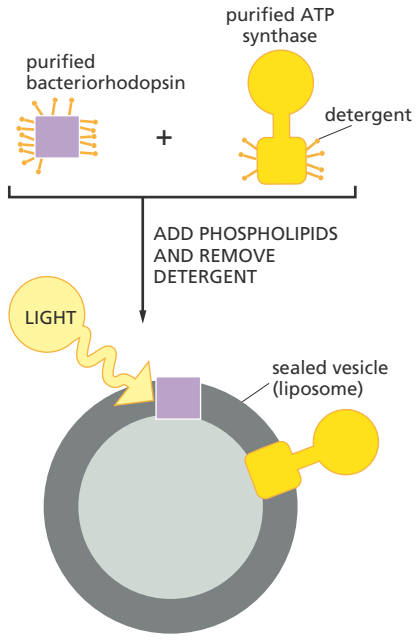


Figure Q14–18

QUESTION 14–19

$FADH_2$ is produced in the citric acid cycle by a membrane-embedded enzyme complex, called succinate dehydrogenase, that contains bound FAD and carries out the reactions

$$\text{succinate} + \text{FAD} \longrightarrow \text{fumarate} + FADH_2$$

and

$$FADH_2 \longrightarrow \text{FAD} + 2H^+ + 2e^-$$

The redox potential of $FADH_2$, however, is only –220 mV. Referring to Panel 14–1 (p. 466) and Figure 14–24, suggest a plausible mechanism by which its electrons could be fed into the electron-transport chain. Draw a diagram to illustrate your proposed mechanism.

QUESTION 14–20

Some bacteria have become specialized to live in an environment of high pH (pH ~10). Do you suppose that these bacteria use a proton gradient across their plasma membrane to produce their ATP? (Hint: all cells must maintain their cytoplasm at a pH close to neutrality.)

QUESTION 14–21

Figure Q14–21 summarizes the circuitry used by mitochondria and chloroplasts to interconvert different forms of energy. Is it accurate to say

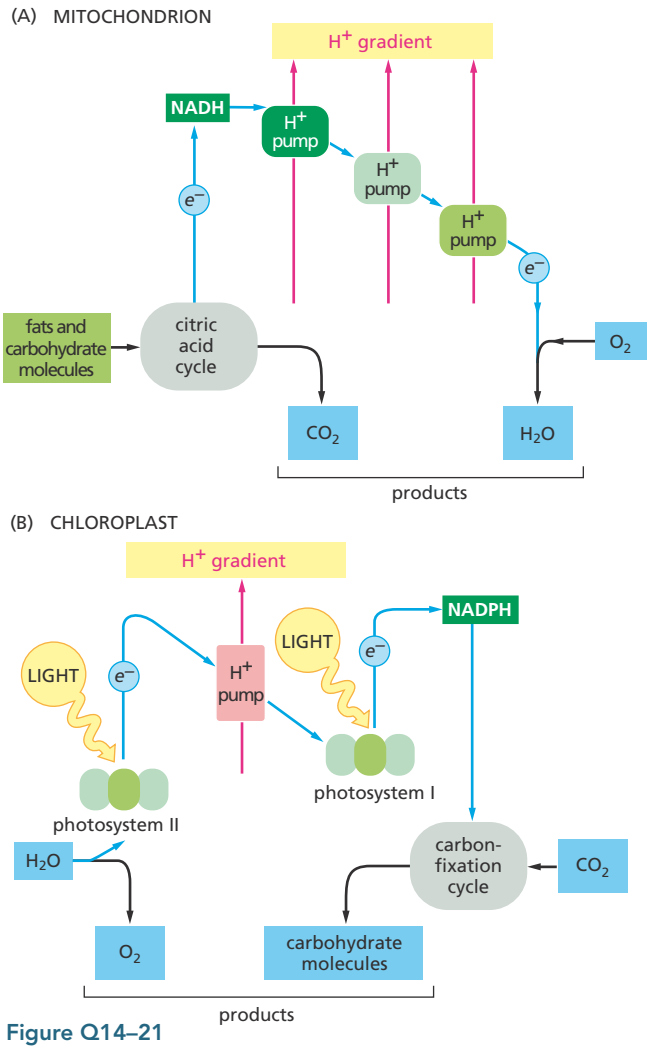


Figure Q14–21

A. that the products of chloroplasts are the substrates for mitochondria?

B. that the activation of electrons by the photosystems enables chloroplasts to drive electron transfer from H_2O to carbohydrate, which is the opposite direction of electron transfer in the mitochondrion?

C. that the citric acid cycle is the reverse of the normal carbon-fixation cycle?

QUESTION 14–22

A manuscript has been submitted for publication to a prestigious scientific journal. In the paper, the authors describe an experiment in which they have succeeded in trapping an individual ATP synthase molecule and then mechanically rotating its head by applying a force to it. The authors show that upon rotating the head of the ATP synthase, ATP is produced, in the absence of an H^+ gradient. What might this mean about the mechanism whereby ATP synthase functions? Should this manuscript be considered for publication in one of the best journals?

QUESTION 14–23

You mix the following components in a solution. Assuming that the electrons must follow the path specified in Figure 14–14, in which experiments would you expect a net transfer of electrons to cytochrome *c*? Discuss why electron transfer does not occur in the other experiments.

A. reduced ubiquinone and oxidized cytochrome *c*

B. oxidized ubiquinone and oxidized cytochrome *c*

C. reduced ubiquinone and reduced cytochrome *c*

D. oxidized ubiquinone and reduced cytochrome *c*

E. reduced ubiquinone, oxidized cytochrome *c*, and cytochrome *c* reductase complex

F. oxidized ubiquinone, oxidized cytochrome *c*, and cytochrome *c* reductase complex

G. reduced ubiquinone, reduced cytochrome *c*, and cytochrome *c* reductase complex

H. oxidized ubiquinone, reduced cytochrome *c*, and cytochrome *c* reductase complex

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