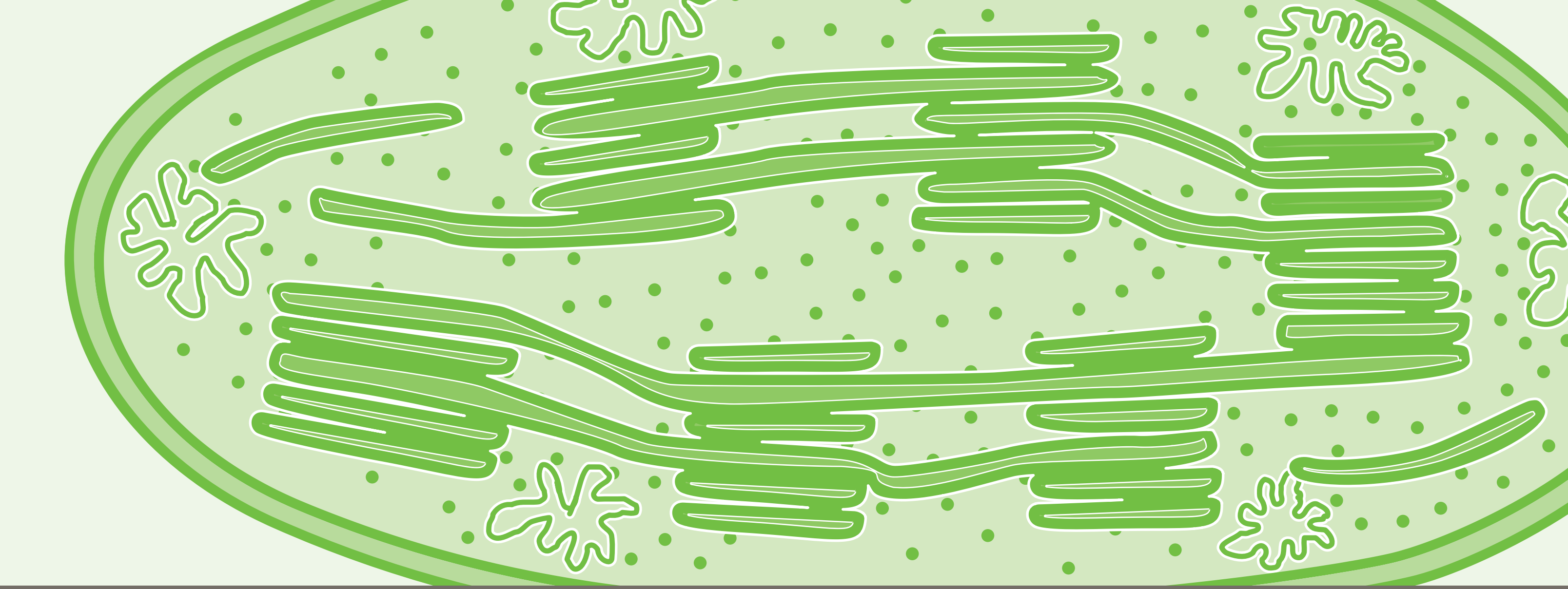


CHAPTER **FOURTEEN**

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. The earliest cells may have produced ATP by breaking down organic molecules, left by earlier geochemical processes, using some form of fermentation. Fermentation reactions occur in the cytosol of present-day cells. As discussed in Chapter 13, these reactions use the energy derived from the partial oxidation of energy-rich food molecules to form ATP, the chemical energy currency of cells.

But very early in the history of life, a much more efficient method for generating energy and synthesizing ATP appeared. This process is based on the transport of electrons along membranes. Billions of years later, it is so central to the survival of life on Earth that we devote this entire chapter to it. As we shall see, this membrane-based mechanism is used by cells to acquire energy from a wide variety of sources: for example, it is central to the conversion of light energy into chemical-bond energy in photosynthesis, and to the aerobic respiration that enables us to use oxygen to produce large amounts of ATP from food molecules. The mechanism we will describe first appeared in bacteria more than 3 billion years ago. The descendants of these pioneering cells crowd every corner and crevice of the land and the oceans with a wild menagerie of living forms, and they survive within eucaryotic cells in the form of chloroplasts and mitochondria.

Where we come from and how we are related to other living things are puzzles that have fascinated humans since the beginning of recorded time. The story that we can tell now, worked out through a long chain of scientific investigation, is one of the most dramatic and exciting histories ever told. And we are not yet done. Each year, further discoveries in cell biology enable us to add more details through molecular detective work of dramatically increasing power.

Absolutely central to life's progression was the ability to provide an abundant source of energy for cells. In this chapter, we discuss the remarkable mechanism that made this possible.

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

The main chemical energy currency in cells is ATP (see Figure 3–32). In eucaryotic cells, small amounts of ATP are generated during glycolysis in the cytosol, but most ATP is produced by oxidative phosphorylation in mitochondria (as outlined in Chapter 13). The mechanism by which the bulk of ATP is generated in the mitochondria differs from the way ATP is produced by glycolysis in that it involves a membrane: oxidative phosphorylation depends on electron transport within the mitochondrial membrane and the transport of ions across it. The same type of ATP-generating process occurs in the plasma membrane of bacteria. The membrane-based mechanism for making ATP arose very early in life's history and was so successful that its essential features have been retained in the long evolutionary journey from early procaryotes to modern cells. In photosynthetic bacteria, plants, and algae, a related membrane-based process produces ATP during photosynthesis.

The membrane-based process for making ATP consists of two linked stages; both are carried out by protein complexes in the membrane.

Stage 1. Electrons derived from the oxidation of food molecules (as discussed in Chapter 13) 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 (H^+), derived from the water that is ubiquitous in cells, across the membrane and thus generate an electrochemical proton gradient (Figure 14–1A). 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 gradient (as discussed in Chapter 12).

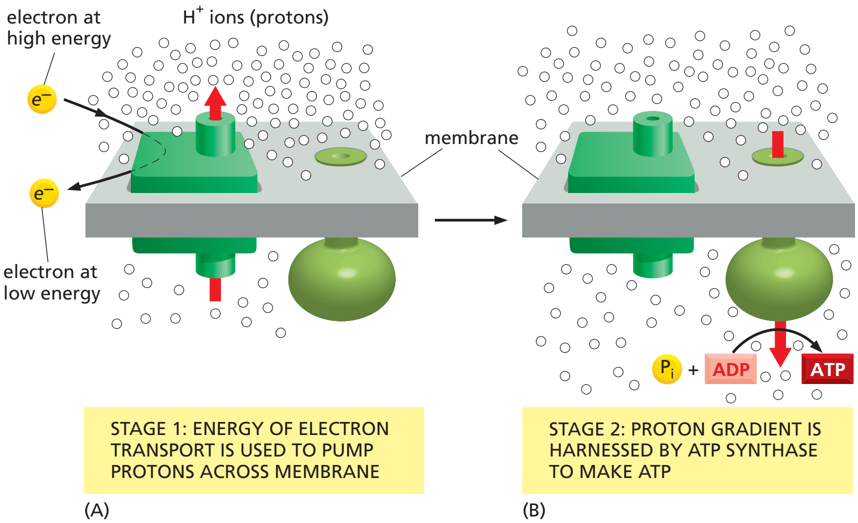


Figure 14–1 **Cells have evolved systems for harnessing the energy required for life.** (A) The essential requirements for this process are a membrane, in which are embedded a pump protein and an ATP synthase, and sources of high-energy electrons (e^-) and of protons (H^+). The pump harnesses the energy of electron transfer (details not shown here) to pump protons derived from water, creating a proton gradient across the membrane. The high-energy electrons can be provided by organic or inorganic molecules or they can be produced by the action of light on special molecules such as chlorophyll. (B) The 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 bacteria—including the synthesis of ATP by the ATP synthase. The *red arrow* shows the direction of proton movement at each stage.

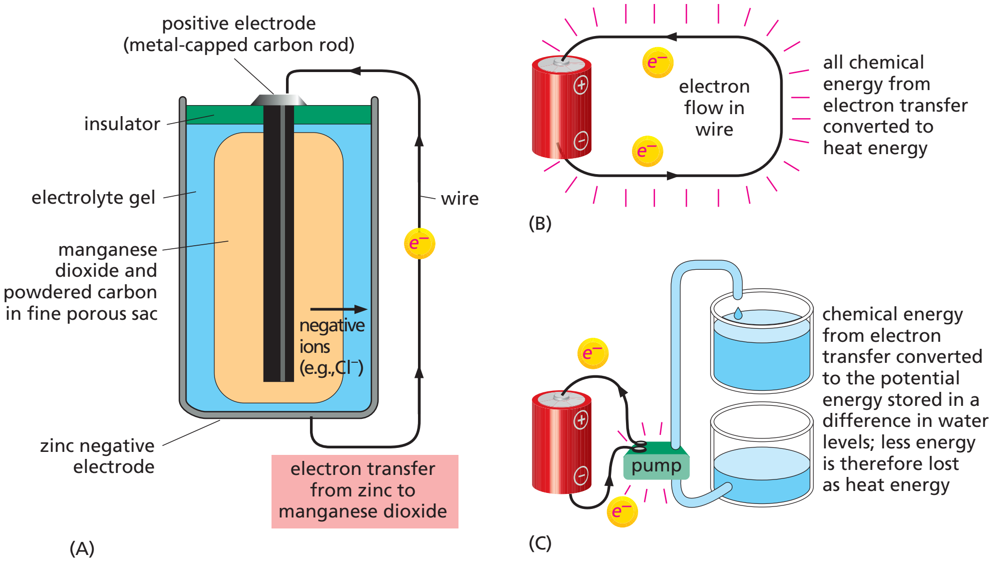


Figure 14–2 **Batteries are powered by chemical reactions based on electron transfers.** (A) When a standard flashlight battery is connected into a circuit, electrons flow from the metal container, which is made of zinc (Zn), to the manganese atom in manganese dioxide (MnO_2). Zn^{2+} and manganous oxide (MnO) are formed as products. (The carbon in the battery simply serves to conduct electrons.) (B) If the battery terminals are directly connected to each other, the energy released by electron transfer is all converted into heat. (C) 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 a pumping mechanism, as illustrated in Figure 14–1.

Stage 2. H^+ flows back down its 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 serves the role of a turbine, permitting the proton gradient to drive the production of ATP (Figure 14–1B).

The linkage of electron transport, proton pumping, and ATP synthesis was called the *chemiosmotic hypothesis* when it was first proposed in the 1960s, because of the link between the chemical bond-forming reactions that synthesize ATP ("chemi-") and the membrane transport processes ("osmotic," from the Greek *osmos*, "to push"). It is now known as **chemiosmotic coupling**. Chemiosmotic mechanisms allow cells to 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 14–2).

Chemiosmotic coupling first evolved in bacteria. Aerobic eucaryotic cells appear to have adopted the bacterial chemiosmotic mechanisms intact, first by engulfing aerobic bacteria to form mitochondria, and somewhat later—in the lineages leading to algae and plants—by engulfing cyanobacteria to form chloroplasts, as described in Chapter 1 (see Figures 1–19 and 1–21).

In this chapter we shall consider energy generation in both mitochondria and chloroplasts, emphasizing the common principles by which proton gradients are created and used in these organelles and in the plasma membranes of bacteria. We start by describing the structure and function of mitochondria, looking in detail at the events that occur in the mitochondrial membrane to create the proton gradient and generate ATP. We next consider photosynthesis in the chloroplasts of plant cells. Finally, we trace the evolutionary pathways that gave rise to these mechanisms of energy generation. By examining the lifestyles of a variety of single-celled organisms—including those that might resemble our early ancestors—we can begin to see the role that chemiosmotic coupling has played in the rise of complex eucaryotes and in the development of all life on Earth.

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.

MITOCHONDRIA AND OXIDATIVE PHOSPHORYLATION

Mitochondria are present in nearly all eucaryotic cells—in plants, animals, and most eucaryotic microorganisms—and most of a cell's ATP is produced in these organelles. Without them, present-day eucaryotes would be dependent on the relatively inefficient process of glycolysis for all of their ATP production, and it seems unlikely that complex multicellular organisms could have been supported in this way. When glucose is converted to pyruvate by glycolysis, only two molecules of ATP are produced per glucose molecule (less than 10% of the total free energy potentially available). By contrast, in mitochondria, the metabolism of sugars is completed, and the energy released is harnessed so efficiently that about 30 molecules of ATP are produced for each molecule of glucose oxidized.

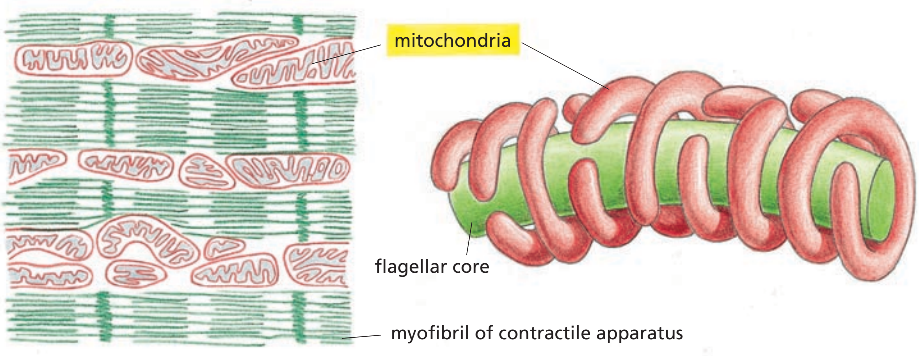
Defects in mitochondrial function can have serious repercussions for an organism. Consider, for example, an inherited disorder called *myoclonic epilepsy and ragged red fiber disease (MERRF)*. This disease, caused by a mutation in one of the mitochondrial transfer RNA (tRNA) genes, is characterized by a decrease in synthesis of the mitochondrial proteins required for electron transport and ATP production. As a result, patients with this disorder typically experience muscle weakness or heart problems (from effects on cardiac muscle) and epilepsy or dementia (from effects on nerve cells). Muscle and nerve cells suffer most when mitochondria are defective, because they need especially large amounts of ATP to function well.

The same metabolic reactions that occur in mitochondria also take place in aerobic bacteria, which do not possess these organelles; in these organisms the plasma membrane carries out the chemiosmotic coupling. Of course, a bacterial cell also has to carry out many other functions; the mitochondrion by contrast has become highly specialized for energy generation.

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

Mitochondria are generally similar in size and shape to bacteria, although these attributes can vary depending on the cell type. They contain their own DNA and RNA, and a complete transcription and translation system including ribosomes, which allows them to synthesize some of their own proteins. Time-lapse movies of living cells reveal mitochondria as remarkably mobile organelles, constantly changing shape and position. Present in large numbers—1000 to 2000 in a liver cell, for example—these organelles can form long, moving chains in association with the microtubules of the cytoskeleton (discussed in Chapter 17). In other cells, they remain fixed in one location to target ATP directly to a site of unusually high ATP 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 14–3). The number of mitochondria present in different cell types varies dramatically, and can change with the energy needs of the cell. In skeletal muscle cells, for example, the number of mitochondria may increase five- to tenfold, due to mitochondrial growth and division that occurs if the muscle has been repeatedly stimulated to contract.

An individual mitochondrion is bounded by two highly specialized membranes—one surrounding the other—that play a crucial part in its activities. The outer and inner mitochondrial membranes create two mito-



(A) CARDIAC MUSCLE CELL

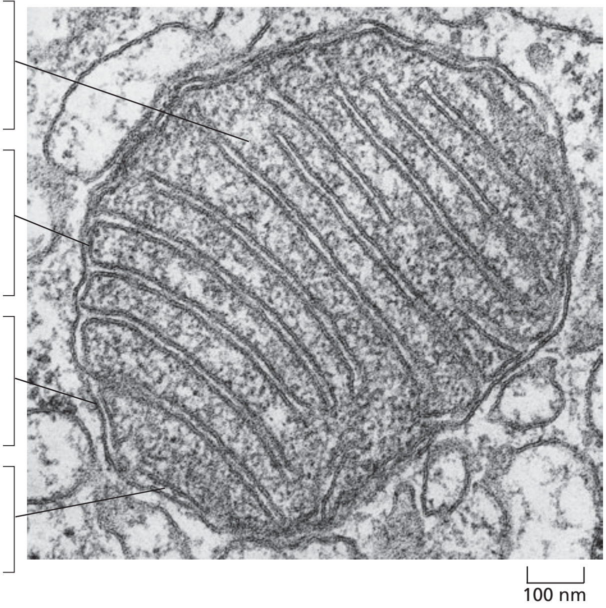
(B) SPERM TAIL

Figure 14–3 **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.

chondrial compartments: a large internal space called the **matrix** and the much narrower *intermembrane space* (Figure 14–4). If purified mitochondria are gently processed and fractionated into separate components by differential centrifugation (see Panel 4–4, pp. 164–165), the biochemical composition of each of the two membranes and of the spaces enclosed by them can be determined. Each contains a unique collection of proteins.

The *outer membrane* contains many molecules of a transport protein called porin, which, as described in Chapter 11, forms wide aqueous channels through the lipid bilayer. 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 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 membrane transport proteins. The mitochondrial matrix therefore contains only molecules that can be selectively transported into the matrix across the inner membrane, and its contents are highly specialized.

The inner mitochondrial membrane is the site of electron transport and proton pumping, and it contains the ATP synthase. Most of the proteins embedded in the inner mitochondrial membrane are components of the electron-transport chains required for oxidative phosphorylation. This



Matrix. This space contains a highly concentrated mixture of hundreds of enzymes, including those required for the oxidation of pyruvate and fatty acids and for the citric acid cycle.

Inner membrane. Folded into numerous cristae, the inner membrane contains proteins that carry out the oxidation reactions of the electron-transport chain and the ATP synthase that makes ATP in the matrix.

Outer membrane. Because it contains a large channel-forming protein (called porin), the outer membrane is permeable to all molecules of 5000 daltons or less.

Intermembrane space. This space contains several enzymes that use the ATP passing out of the matrix to phosphorylate other nucleotides.

Figure 14–4 **A mitochondrion is organized into four separate compartments.** Each compartment contains a unique set of proteins that enables it to perform its distinct functions. In liver mitochondria, an estimated 67% of the total mitochondrial protein is located in the matrix, 21% is located in the inner membrane, 6% in the outer membrane, and 6% in the intermembrane space. (Courtesy of Daniel S. Friend.)

membrane also contains a variety of transport proteins that allow the entry of selected small molecules, such as pyruvate and fatty acids, into the matrix.

The inner membrane is usually highly convoluted, forming a series of infoldings, known as *cristae*, that project into the matrix space to greatly increase the surface area of the inner membrane (see Figure 14–4). These folds provide a large surface on which ATP synthesis can take place; in a liver cell, for example, the inner mitochondrial membranes of all the mitochondria constitute about a third of the total membranes of the cell. And the number of cristae is three times greater in a mitochondrion of a cardiac muscle cell than in a mitochondrion of a liver cell.

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.

The Citric Acid Cycle Generates High-Energy Electrons

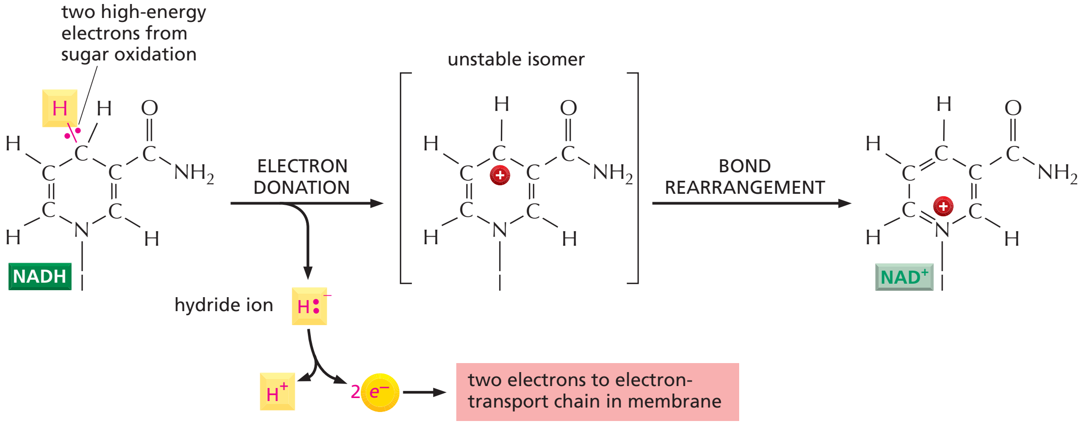
Mitochondria use both pyruvate and fatty acids as fuel, the pyruvate coming mainly from glucose and other sugars, and the fatty acids from fats. These fuel molecules are transported across the inner mitochondrial membrane and then converted to the crucial metabolic intermediate acetyl CoA by enzymes located in the mitochondrial matrix (see Figure 13–10). The acetyl groups in acetyl CoA are then oxidized in the matrix via the citric acid cycle (see Panel 13–2, pp. 442-443). The cycle converts the carbon atoms in acetyl CoA to CO_2, which is released from the cell as a waste product. In addition, the cycle generates high-energy electrons, carried by the activated carrier molecules NADH and $FADH_2$ (Figure 14–5).

Although the citric acid cycle is considered to be part of aerobic metabolism, it does not itself use molecular oxygen (O_2). Oxygen is directly consumed only in the final catabolic reactions that take place on the inner mitochondrial membrane, as we see next.

A Chemiosmotic Process Converts the Energy From Activated Carrier Molecules into ATP

Nearly all the energy available from burning carbohydrates, fats, and other foodstuffs in the earlier stages of their oxidation is initially saved in the form of the activated carrier molecules generated during glycolysis and the citric acid cycle—NADH and $FADH_2$. These carrier molecules donate their high-energy electrons to the electron-transport chain in the mitochondrial membrane, and thus become oxidized to NAD^+ and FAD. The electrons are quickly passed along the chain to molecular oxygen (O_2) to form water (H_2O). The passage of the high-energy electrons along the electron-transport chain releases energy that is harnessed to pump protons across the inner mitochondrial membrane (Figure 14–6).

Figure 14–5 **NADH donates its electrons to the electron-transport chain.** In this drawing, the high-energy electrons are shown as two *red dots* on a *yellow* hydrogen atom. A hydride ion (a hydrogen atom with an extra electron) is removed from NADH and is converted into a proton and two high-energy electrons. Only the ring that carries the electrons in a high-energy linkage 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 13–12B.



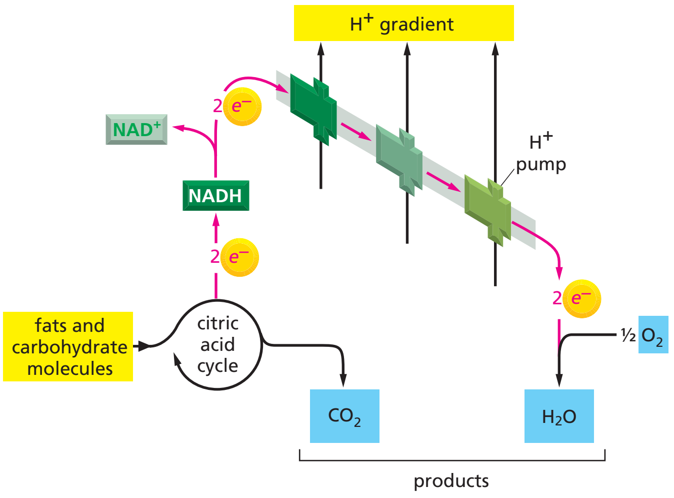


Figure 14–6 **Protons are pumped across the inner mitochondrial membrane.** Only stage 1 of chemiosmotic coupling is shown (see Figure 14–1). The path of electron flow is indicated by *red arrows*.

The resulting proton gradient in turn drives the synthesis of ATP. The full sequence of reactions is shown in Figure 14–7. The inner mitochondrial membrane thus serves as a device that converts the energy contained in the high-energy electrons of NADH into the high-energy phosphate bond of ATP (Figure 14–8). This chemiosmotic mechanism of 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.

Although chemiosmotic coupling escaped detection for many years, the vast majority of living organisms use this mechanism to generate ATP. The source of the electrons that power the proton pumping differs widely between different organisms and different processes. In aerobic respiration in mitochondria and aerobic bacteria, the electrons are ultimately derived from glucose or fatty acids. In photosynthesis, the required

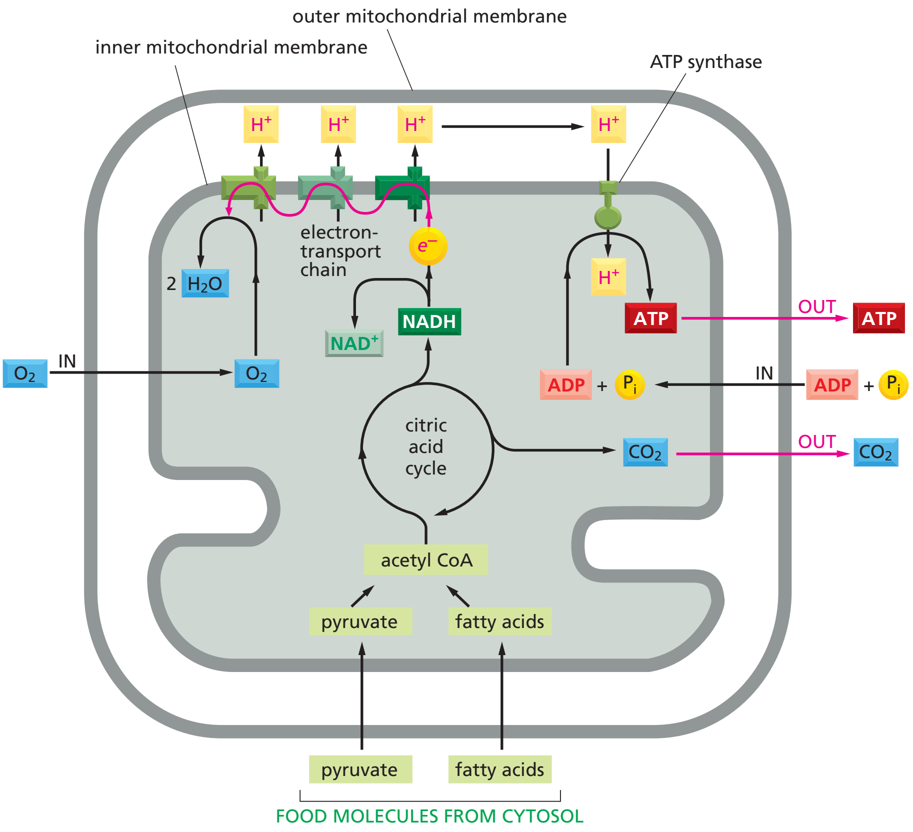


Figure 14–7 **High-energy electrons, generated during the citric acid cycle, power the production of ATP.** Pyruvate and fatty acids enter the mitochondrion (*bottom*), are converted to acetyl CoA, and are then metabolized by the citric acid cycle, which reduces NAD^+ to NADH (and FAD to $FADH_2$, not shown). In the process of oxidative phosphorylation, high-energy electrons from 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. In this diagram, the exact ratios of "reactants" and "products" have been omitted. For example, we will see shortly that it requires 4 electrons from 4 NADH molecules to convert O_2 to 2 H_2O molecules.

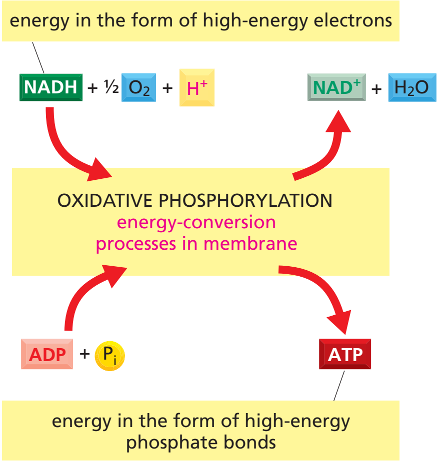


Figure 14–8 Mitochondria catalyze a major conversion of energy. In oxidative phosphorylation the energy released by the oxidation of NADH to NAD^+ is harnessed—through energy-conversion processes in the membrane—to drive the energy-requiring phosphorylation of ADP to form ATP. The net equation for this process, in which two electrons pass from NADH to oxygen, is $NADH + ½O_2 + H^+ \rightarrow NAD^+ + H_2O$.

electrons are derived from the action of light on the green pigment *chlorophyll*. And many bacteria use inorganic substances such as hydrogen, iron, and sulfur as the source of the high-energy electrons that they need to make ATP.

The Electron-Transport Chain Pumps Protons Across the Inner Mitochondrial Membrane

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, most of which are embedded in the lipid bilayer and function only in the intact membrane, making them difficult to study. However, the components of the electron-transport chain, like other membrane proteins, can be solubilized using nonionic detergents (see Figure 11–27), purified, and then reconstituted in operational form in small membrane vesicles. Such studies reveal that most of the proteins involved in the mitochondrial electron-transport chain are grouped into three large *respiratory enzyme complexes,* each containing multiple individual proteins. Each complex includes transmembrane proteins that hold the entire complex firmly in the inner mitochondrial membrane.

The three respiratory enzyme complexes, in the order that they receive electrons, are: (1) the *NADH dehydrogenase complex,* (2) the *cytochrome* b-c_1 *complex,* and (3) the *cytochrome oxidase complex.* Each contains metal ions and other chemical groups that form a pathway for the passage of electrons through the complex. The respiratory complexes are the sites of proton pumping, and each can be thought of as a protein machine that pumps protons across the membrane as electrons are transferred through it.

Electron transport begins when a hydride ion (H^-) is removed from NADH and is converted into a proton and two high-energy electrons: $H^- \rightarrow H^+ + 2e^-$, as shown previously in Figure 14–5. This reaction is catalyzed by the first of the respiratory enzyme complexes, the NADH dehydrogenase, which accepts electrons from NADH (Figure 14–9). 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. The transfer of electrons along the chain is energetically favorable: the electrons start out at very high energy and lose energy at each transfer step, eventually entering cytochrome oxidase where they combine with a molecule of O_2 to form water. This is the oxygen-requiring step of cellular respiration, and it consumes nearly all of the oxygen that we breathe.

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

Without a mechanism for harnessing the energy released by electron transfers, this energy would simply be liberated as heat. But cells utilize much of the energy of electron transfer by having the transfers take place within proteins that are capable of pumping protons. In this way, the energetically favorable flow of electrons along the electron-transport

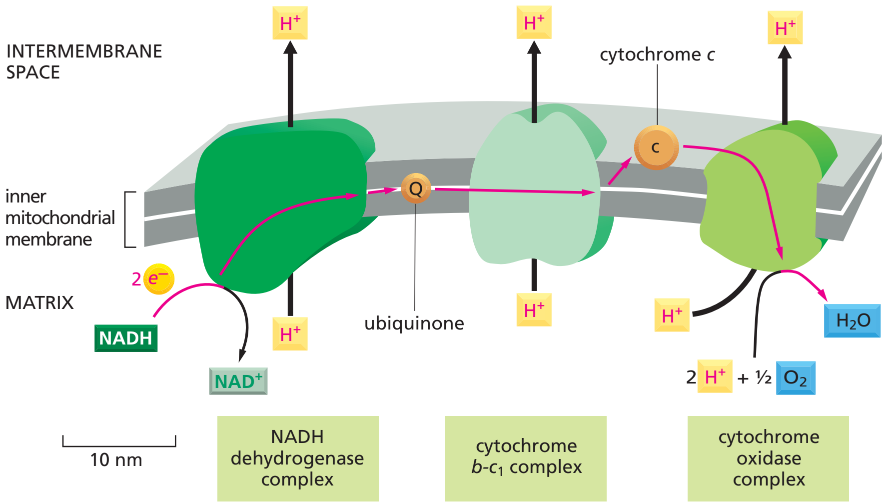


Figure 14–9 **Electrons are transferred through three respiratory enzyme complexes in the inner mitochondrial membrane.** The relative size and shape of each complex is indicated. During the transfer of electrons from NADH to oxygen (*red lines*), protons derived from water are pumped across the membrane from the matrix into the intermembrane space by each of the respiratory enzyme complexes (Movie 14.2). Ubiquinone (Q) and cytochrome *c* (c) serve as mobile carriers that ferry electrons from one complex to the next.

chain results in the pumping of protons across the membrane out of the mitochondrial matrix and into the space between the inner and outer mitochondrial membranes (see Figure 14–9).

Later in the chapter we review the detailed molecular mechanisms that couple electron transport to the movement of protons. For now, we focus on the consequences of this nifty biological maneuver. First, the active pumping of protons generates a gradient of H^+ concentration—a pH gradient—across the inner mitochondrial membrane, where the pH is about 0.5 unit higher in the matrix (around pH 7.5) than in the intermembrane space (which is close to 7, the same pH as the cytosol). Second, proton pumping generates a membrane potential across the inner mitochondrial membrane, with the inside (the matrix side) negative and the outside positive as a result of the net outflow of H^+.

As discussed in Chapter 12, the force driving the passive flow of an ion across a membrane is proportional to the electrochemical gradient for the ion across the membrane. This in turn depends on the voltage across the membrane, which is measured as the membrane potential, and on the concentration gradient of the ion (see Figure 12–7). Because protons are positively charged, they will move more readily across a membrane if the membrane has an excess of negative electrical charges on the other side. In the case of the inner mitochondrial membrane, the pH gradient and membrane potential work together to create a steep electrochemical proton gradient that makes it energetically very favorable for H^+ to flow back into the mitochondrial matrix. In the energy-producing membranes we discuss in this chapter, the membrane potential adds to the driving force pulling H^+ back across the membrane, which is called the *proton-motive force;* hence the membrane potential increases the amount of energy stored in the proton gradient (Figure 14–10).

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 will the electrochemical proton gradient change in response to DNP? (B) How will it change in response to nigericin?

The Electrochemical Proton Gradient Drives ATP Synthesis

As explained previously, the electrochemical proton gradient across the inner mitochondrial membrane is used to drive ATP synthesis. The device that makes this possible is a large enzyme called **ATP synthase**, which is also embedded in the inner mitochondrial membrane. ATP synthase creates a hydrophilic pathway across the inner mitochondrial membrane that allows protons to flow back across the membrane down their electrochemical gradient (Figure 14–11). As these ions thread their way through the enzyme, they are used to drive the energetically unfavora-

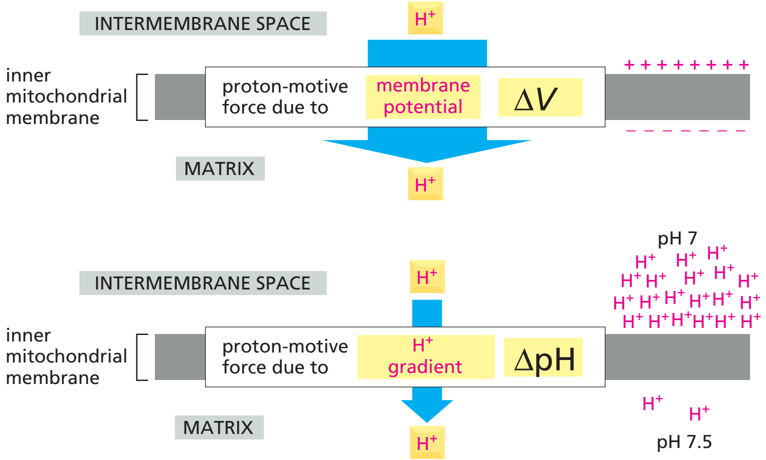


Figure 14–10 **The total electrochemical gradient of H^+ across the inner mitochondrial membrane consists of a large force due to the membrane potential (ΔV) and a smaller force due to the H^+ concentration gradient (ΔpH).** Both forces combine to produce the total proton-motive force that drives H^+ into the matrix space. The relationship between these forces is expressed by the Nernst equation (see Figure 12–29).

ble reaction between ADP and P_i that makes ATP (see Figure 2–24). The ATP synthase is of ancient origin; the same enzyme occurs in the mitochondria of animal cells, the chloroplasts of plants and algae, and in the plasma membrane of bacteria.

ATP synthase is a large, multisubunit protein (Figure 14–12). A large enzymatic portion, shaped like a lollipop head, projects into the matrix and carries out the phosphorylation reaction. This enzymatic structure is attached through a thinner multisubunit "stalk" to a transmembrane proton carrier. As protons pass through a narrow channel within the carrier, their movement causes the stalk to spin rapidly within the head, inducing the head to make ATP (Figure 14–12A). The synthase essentially acts as an energy-generating molecular motor, converting the energy of proton flow down a gradient into the mechanical energy of two sets of proteins rubbing against one another—rotating stalk proteins pushing against stationary head proteins. The movement of the stalk changes the conformation of subunits within the head. This mechanical deformation gets converted into chemical bond energy as the subunits produce ATP. This marvelous device can produce more than 100 molecules of ATP per second, and about three protons need to pass through the synthase to make each molecule of ATP.

The ATP synthase is a reversible coupling device. It can either harness the flow of protons down their electrochemical gradient to make ATP (its normal role in mitochondria and the plasma membrane of bacteria growing aerobically) or use the energy of ATP hydrolysis to pump protons across a membrane (Figure 14–13). In the latter mode, ATP synthase functions like the H^+ pumps described in Chapter 12. Whether the ATP synthase primarily makes or consumes ATP depends on the magnitude of the electrochemical proton gradient across the membrane in which it

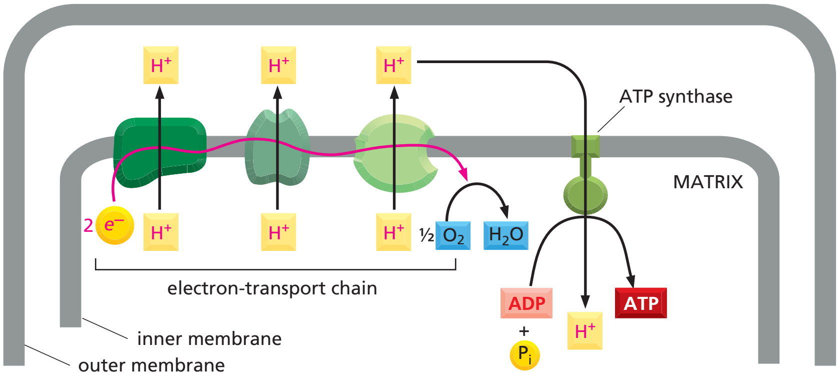


Figure 14–11 **The electrochemical proton gradient across the inner mitochondrial membrane allows ATP synthase to generate ATP.** As a high-energy electron is passed along the electron-transport chain, much of the energy released is used to drive the three respiratory enzyme complexes that pump H^+ out of the matrix space. The resulting electrochemical proton gradient across the inner membrane drives H^+ back through the ATP synthase, a transmembrane protein complex that uses the energy of the H^+ flow to synthesize ATP from ADP and P_i in the matrix.

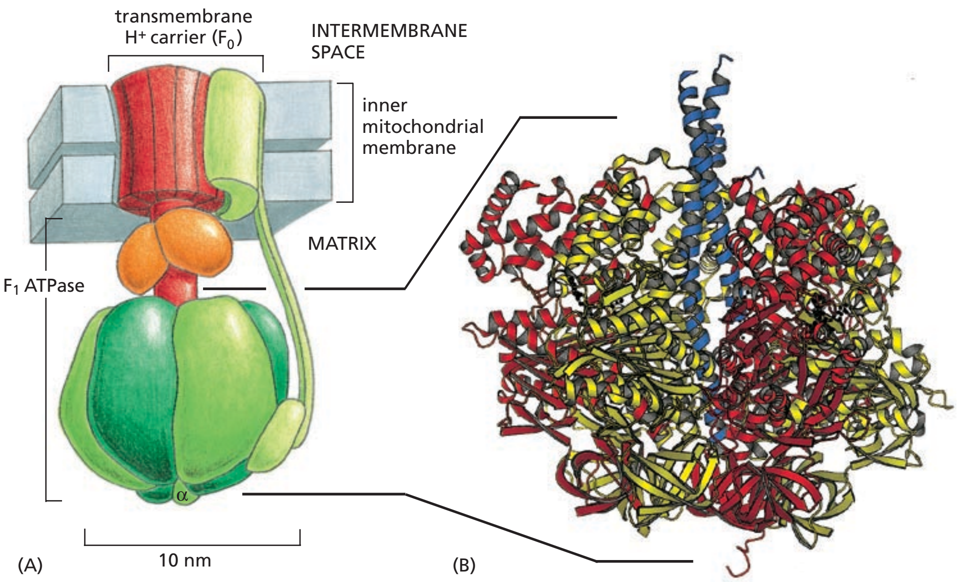
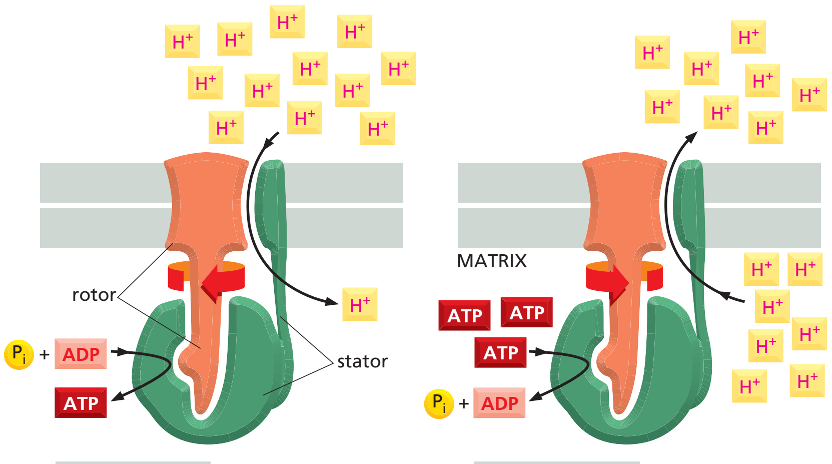


Figure 14–12 **ATP synthase is shaped like a lollipop.** (A) The enzyme is composed of a head portion, called the F_1 ATPase, and a transmembrane H^+ carrier, called F_0. Both F_1 and F_0 are formed from multiple subunits, as indicated. The F_0 portion, consisting of a rotor plus a stalk (*red*), rotates in the membrane driven by the proton gradient. The stator (*light green*) is formed from transmembrane a subunits, tied to other subunits that create an elongated arm. This arm fixes the stator to a ring of subunits that forms the stationary head of the ATPase. Its β subunits (*dark green*) generate the ATP. (B) The three-dimensional structure of the F_1 ATPase, as determined by X-ray crystallography. This part of the ATP synthase derives its name from its ability to carry out the reverse of the ATP synthesis reaction, namely, the hydrolysis of ATP to ADP and P_i, when detached from the transmembrane portion. (B, courtesy of John Walker, from J.P. Abrahams et al., *Nature* 370:621–628, 1994. With permission from Macmillan Publishers Ltd.)

sits. In many bacteria that can grow either aerobically or anaerobically, the direction in which the ATP synthase works is routinely reversed when the bacterium runs out of O_2. At this point, 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, as we see next.

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 charged molecules, such as pyruvate, ADP, and P_i, are pumped into the matrix from the cytosol, while others, such as ATP, must be moved in the opposite direction. Carrier proteins that bind these molecules can couple their transport to the energetically favorable flow of H^+ into the mitochondrial matrix. Pyruvate and inorganic phosphate (P_i), for example, are individually co-transported inward with H^+ as the latter moves down its electrochemical gradient, into the matrix.



(A) ATP SYNTHESIS

(B) ATP HYDROLYSIS

Figure 14–13 **ATP synthase is a reversible coupling device that can convert the energy of the electrochemical proton gradient into chemical-bond energy or vice versa.** The ATP synthase can either synthesize ATP by harnessing the H^+ gradient (A) or pump protons against their electrochemical 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 gradient. The action of ATP synthase is shown in Movie 14.3 and Movie 14.4.

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 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?

Other transporters take advantage of the fact that the electrochemical proton gradient generates a membrane potential, such that the matrix-side of the inner mitochondrial membrane is more negatively charged than the intermembrane space on the other side. An antiport carrier protein exploits this voltage gradient to expel ATP from—and import ADP to—the mitochondrial matrix. Because an ATP molecule has one more negative charge than ADP, swapping these nucleotides results in the movement of one negative charge out of the mitochondrion. This nucleotide exchange—which sends ATP to the cytosol—is thus driven by the charge difference across the inner mitochondrial membrane (Figure 14–14).

In eucaryotic cells, therefore, the electrochemical proton gradient is used to drive both the formation of ATP and the transport of certain metabolites across the inner mitochondrial membrane. In bacteria, the proton gradient across the bacterial plasma membrane serves all of these functions. But, in bacteria, this gradient is also an important source of directly usable energy: in motile bacteria, a flow of protons into the cell drives the rapid rotation of the bacterial flagellum, which propels the bacterium along (Movie 14.5).

Oxidative Phosphorylation Produces Most of the Cell's ATP

As we mentioned earlier, glycolysis on its own produces a net yield of two molecules of ATP for every molecule of glucose, whereas the complete oxidation of glucose—which includes glycolysis and oxidative phosphorylation—generates about 30 ATPs. In the case of glycolysis, it's obvious where those ATP molecules come from: two molecules of ATP are consumed early in the process and four molecules of ATP are produced toward the end (see Figure 13–3). But for oxidative phosphorylation, the accounting is less straightforward, because the ATPs are not produced directly, as they are in glycolysis. Instead, they are produced from the energy carried by NADH and $FADH_2$, which are generated during glycolysis and the citric acid cycle. These activated carrier molecules donate their electrons to the electron transport chain that lies in the inner mitochondrial membrane. These movement of these electrons along the respiratory chain fuels the formation of the proton gradient, which in turn powers the production of ATP.

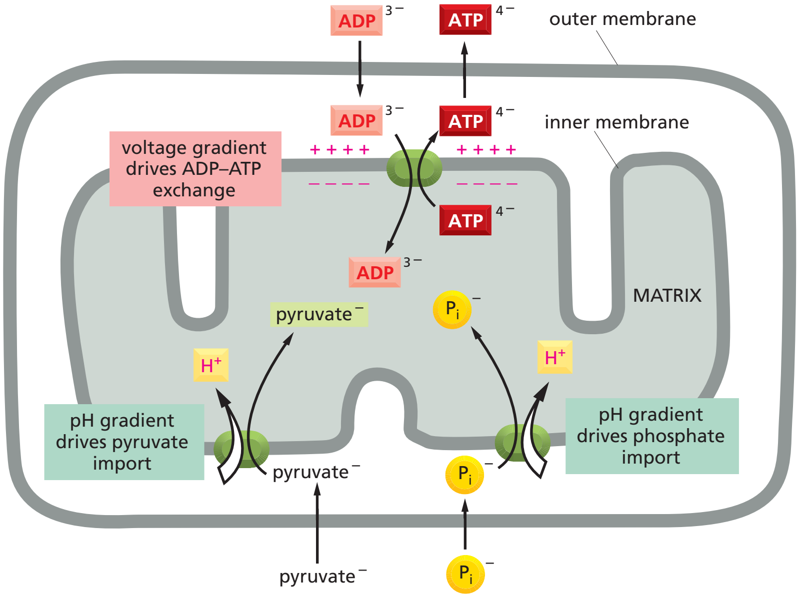


Figure 14–14 **The electrochemical proton gradient across the inner mitochondrial membrane is used to drive some coupled transport processes.** Pyruvate and inorganic phosphate (P_i) are moved into the matrix along with H^+ ions as they move down their electrochemical gradient. ADP is pumped in and ATP is pumped out by an antiport process (ADP–ATP exchange) that depends on a voltage gradient across the membrane (the membrane potential). The charge on each of the transported molecules is indicated for comparison with the membrane potential, which is negative inside as shown. The outer membrane is freely permeable to all of these compounds. The active transport of molecules across membranes by carrier proteins and the generation of a membrane potential are discussed in Chapter 12.

TABLE 14–1 PRODUCE 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 hydrogenase—thus requires energy.

How much ATP each carrier molecule ultimately produces depends on several factors, including where its electrons enter the respiratory chain. The NADH molecules produced during the citric acid cycle, which takes place inside the mitochondria, pass their electrons to NADH dehydrogenase—the first respiratory enzyme complex in the chain. These electrons then pass from one enzyme complex to the next, promoting the pumping of protons across the inner mitochondrial membrane at each step along the way. These NADH molecules provide energy for the net formation of about 2.5 molecules of ATP (see Question 14–5 and its answer).

The $FADH_2$ generated during the citric acid cycle, on the other hand, produces a net of only 1.5 molecules of ATP. That's because $FADH_2$ molecules bypass the NADH dehydrogenase complex and pass their electrons to the membrane-embedded mobile carrier ubiquinone (see Figure 14–9). These electrons enter further down the respiratory chain, and they therefore promote the pumping of fewer protons and generate less ATP. Table 14–1 provides a complete accounting of the ATP produced by the oxidation of each molecule of glucose.

The oxidation of fatty acids also produces large amounts of NADH and $FADH_2$, which in turn produce large amounts of ATP via oxidative phosphorylation (see Figures 13–9 and 13–10). Thus, the vast majority of the ATP produced in an animal cell is produced by chemiosmotic mechanisms on the mitochondrial membrane.

QUESTION 14–5

Calculate the number of 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.

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

As a result of the co-transport process discussed earlier, ADP molecules produced by ATP hydrolysis in the cytosol are rapidly drawn back into mitochondria for recharging, and the bulk of the ATP molecules formed in the mitochondrial matrix by oxidative phosphorylation are pumped into the cytosol where they are needed. A small amount of ATP is used within the mitochondrion itself to power the replication of its DNA, protein synthesis, and other energy-consuming reactions. All in all, a typical ATP molecule in the human body shuttles out of a mitochondrion and back into it (as ADP) for recharging more than once a minute, keeping the concentration of ATP in the cell about 10 times higher than that of ADP.

As discussed in Chapter 3, biosynthetic enzymes often drive energetically unfavorable reactions by coupling them to the energetically favorable hydrolysis of ATP (see Figure 3–33A). The ATP pool is thus used to drive cellular processes in much the same way that a battery can drive an electric engine. If the activity of the mitochondria were halted, ATP levels would fall 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 death in exactly this way.

MOLECULAR MECHANISMS OF ELECTRON-TRANSPORT AND PROTON PUMPING

We have already considered in general terms how a mitochondrion couples electron transport to the generation of ATP. We now examine in more detail the molecular mechanisms that underlie its membrane-based energy-conversion processes. In doing so, we will also be accomplishing a larger purpose. As emphasized at the beginning of this chapter, very similar energy-conversion devices are used by mitochondria, chloroplasts, and bacteria, and the basic principles that we shall discuss next therefore underlie the function of nearly all living things.

For many years, the reason that electron-transport chains were embedded in membranes eluded the biochemists who were struggling to understand them. The process of chemiosmotic coupling entails an interplay between chemical and electrical forces that is not easy to decipher at a molecular level. The puzzle was essentially solved as soon as the fundamental role of transmembrane proton gradients in energy generation was proposed in the early 1960s. However, the idea was so novel that it was not widely accepted until many years later, after additional supporting evidence had accumulated from experiments designed to test rigorously the chemiosmotic hypothesis (see How We Know, pp. 468–469).

Although investigators today are still unraveling the details of chemiosmotic coupling at the atomic level, the fundamentals are now clear. In this part of the chapter we shall look at some of the principles that underlie the electron-transport process and explain in detail how it can generate a proton gradient.

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 type of atom in living organisms and 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, flickering through the hydrogen-bonded network of water molecules by rapidly dissociating from one water molecule in order to associate with its neighbor. Thus, water, which is everywhere in cells, serves as a ready reservoir for donating and accepting protons.

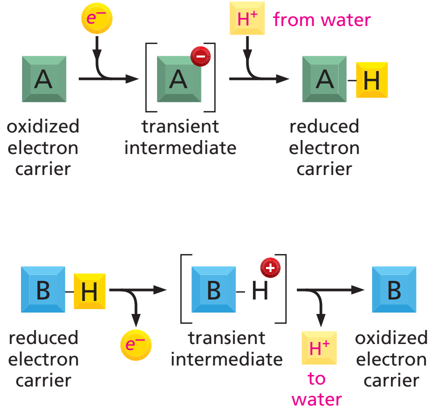
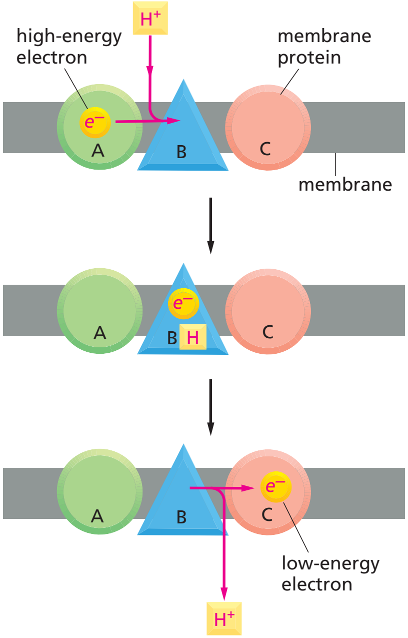


Figure 14–15 **The protons in water are highly mobile.** Electron transfer can cause the transfer of entire hydrogen atoms, because protons are readily accepted from or donated to water. In this example, A picks up an electron plus a proton when it is reduced, and B loses an electron plus a proton when it is oxidized.

Whenever a molecule is reduced by acquiring an electron, the electron (e^-) brings with it a negative charge. In many cases, this charge is rapidly 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 14–15). Similarly, when a molecule is oxidized, the hydrogen atom can be readily dissociated into its constituent electron and proton, allowing the electron to be transferred separately to a molecule that accepts electrons, while the proton is passed to the water. Therefore, in a membrane

Figure 14–16 **The orientation of the 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, electron carrier B picks up a proton (H^+) from one side of the membrane when it accepts an electron (e^-) from carrier A; it releases the proton to the other side of the membrane when it donates its electron to carrier C.



in which electrons are being passed along an electron-transport chain, it is a relatively simple matter, in principle, to pump protons from one side of the membrane to another. All that is required is that the electron carrier be arranged in the membrane in a way that causes it to pick up a proton from one side of the membrane when it accepts an electron, while releasing the proton on the other side of the membrane as the electron is passed on to the next carrier molecule in the chain (Figure 14–16).

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. 87–88). Electrons will pass spontaneously from molecules that have a relatively low affinity for their available electrons, and thus lose them easily, to molecules with a higher electron affinity. For example, NADH with its high-energy electrons has a low electron affinity, so that its electrons are readily passed to the NADH dehydrogenase. The electrical batteries of our common experience are based on similar electron transfers between two 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. 91–92.)

Because electron transfers provide most of the energy for living things, it is worth spending a little time to understand them. Many readers are already familiar with acids and bases, which donate and accept protons (see Panel 2–2, pp. 66–67). Acids and bases exist in conjugate acid–base pairs, where 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

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

In exactly 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: because its electrons are held in a high-energy linkage, the ΔG for passing its electrons to many other molecules is favorable. Conversely, it is difficult to form the high-energy linkage in NADH, so its partner, NAD^+, is of necessity a weak electron acceptor.

HOW WE KNOW:
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: electron-transport systems produce about 30 molecules of ATP for each molecule of glucose oxidized, compared with the two molecules of ATP generated by glycolysis alone. But it took another hundred years for researchers to determine that the process of chemiosmotic coupling—using proton pumping to power ATP synthesis—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. 430–431). It was assumed that the electron-transport chain in mitochondria would similarly generate some high-energy intermediate that could then donate its phosphate group directly to ADP. This model inspired a frustrating search for this mysterious intermediate that lasted for years. Investigators occasionally claimed to discover 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 a H^+ gradient formed during the transfer of electrons through the transport chain could be tapped to drive ATP synthesis.

Several lines of evidence offered support for such chemiosmotic coupling. First, mitochondria do generate a proton gradient across their inner membrane. But what does this gradient do? If the H^+ electrochemical gradient (also called the proton-motive force) is required to drive ATP synthesis, as the chemiosmotic hypothesis posits, then destruction of that gradient—or of the membrane itself—should inhibit energy generation. In fact researchers found this to be true. Physical disruption of the inner mitochondrial membrane halts ATP synthesis. Similarly, dissipation of the proton gradient by chemical "uncoupling" agents such as 2,4-dinitrophenol (DNP) also prevents ATP from being made. These 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 14–17). In this way they uncouple electron transport from ATP synthesis. As a result of this short-circuiting, the proton-motive force is dissipated completely and ATP can no longer be made.

Such uncoupling occurs naturally in some specialized fat cells. In these cells, called *brown fat cells*, most of the energy from oxidation is dissipated as heat rather than converted into ATP. The inner membranes of the large mitochondria in these cells contain a special transport protein that allows protons to move down their electrochemical gradient, circumventing ATP synthase.

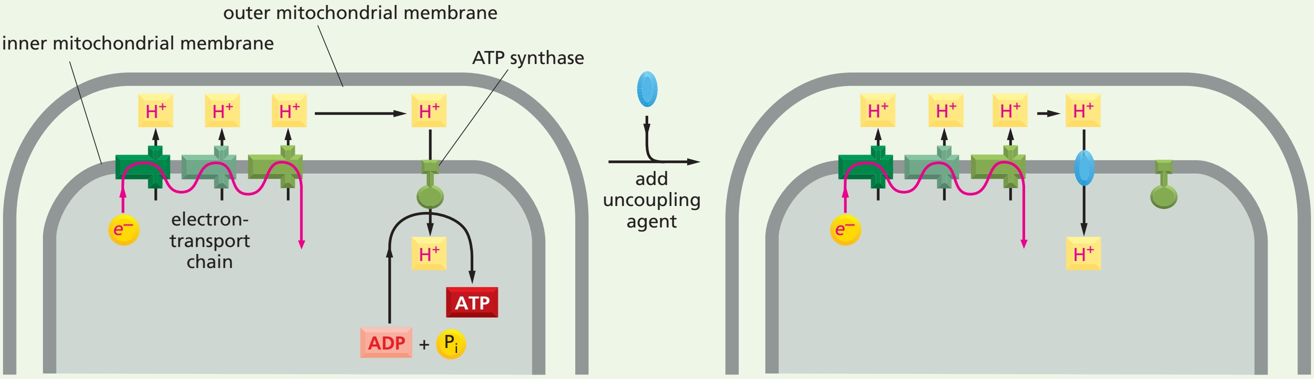
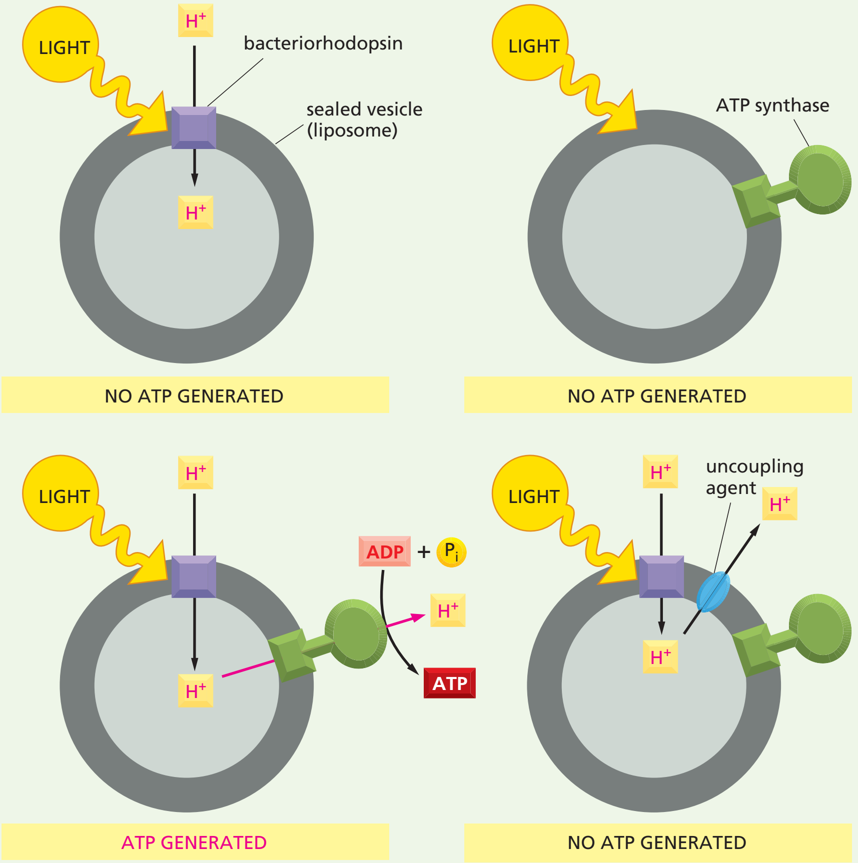


Figure 14–17 **Uncoupling agents are H^+ carriers that can insert into the mitochondrial inner membrane.** They render the membrane permeable to protons, allowing H^+ to flow into the mitochondrion without passing through ATP synthase. This short circuit effectively uncouples electron transport from ATP synthesis.

Figure 14–18 **Experiments with bacteriorhodopsin and an ATP synthase from cow-heart mitochondria provided strong evidence that proton gradients can power ATP production.** When bacteriorhodopsin is added to artificial vesicles, the protein generates a proton gradient in response to light. In artificial vesicles containing both bacteriorhodopsin and an ATP synthase, this proton gradient drives the formation of ATP. Uncoupling agents that abolish the gradient eliminate the ATP synthesis.



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 proton gradient across the mitochondrial membrane terminates ATP synthesis, then, conversely, generating an artificial proton gradient should stimulate the production of ATP. Again, this is exactly what happens. When a H^+ gradient is imposed artificially by lowering the pH on the cytoplasmic side of the mitochondrial membrane, ATP is synthesized, even in the absence of an oxidizable substrate.

How does this proton gradient drive ATP production? That's where the ATP synthase comes in. In 1974 Efraim Racker and Walther Stoeckenius demonstrated elegantly that the combination of an ATP synthase plus a proton gradient will produce ATP. They found that they could reconstitute a complete artificial energy-generating system by combining an ATPase from cow-heart mitochondria with a protein from the purple membrane of the procaryote, *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 11–28). Thus, this membrane protein generates a proton gradient when exposed to light.

When bacteriorhodopsin is reconstituted into artificial lipid vesicles, Racker and Stoeckenius showed that in the presence of light it pumps H^+ into the vesicles, generating a proton gradient. (For some reason the orientation of the protein is reversed in these membranes, so that H^+ ions are transported into the vesicles; in the bacterium protons are pumped out.) And when an ATPase purified from mitochondria is incorporated into these vesicles, the system catalyzes ATP synthesis in response to light. This ATP formation requires the H^+ gradient, as the researchers found that eliminating bacteriorhodopsin from the system or adding uncoupling agents abolished ATP synthesis (Figure 14–18).

Thus, although Mitchell's hypothesis initially met with considerable resistance—biochemists had hoped to discover a high-energy intermediate rather than having to settle for an elusive electrochemical force—the experimental evidence that eventually accumulated to support the importance of chemiosmotic coupling in cellular energy generation could not be ignored. Mitchell was awarded a Nobel Prize in 1978.

The tendency to transfer electrons from any redox pair can be measured experimentally. All that is required is to form an electrical circuit that links a 1:1 (equimolar) mixture of the redox pair to a second redox pair that has been arbitrarily selected as a reference standard, so that the voltage difference between them can be measured (Panel 14–1, p. 471). This voltage difference is defined as the **redox potential**; as defined, electrons will move spontaneously from a redox pair like $NADH/NAD^+$ with a low redox potential (a low affinity for electrons) to a redox pair like O_2/H_2O with a high redox potential (a high affinity for electrons). Thus NADH is a good molecule to donate electrons to the respiratory chain, while O_2 is well suited to act as the "sink" for electrons at the end of the pathway. As explained in Panel 14–1 (p. 471), 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 simply equal to $\Delta G°$ times a negative number that is a constant.

Electron Transfers Release Large Amounts of Energy

As just discussed, those pairs of compounds that have the most negative redox potential (E_0') have the weakest affinity for electrons and therefore the strongest tendency to donate electrons. Conversely, those pairs that have the most positive redox potential have the strongest affinity for electrons and therefore the strongest tendency to accept electrons. A 1:1 mixture of NADH and NAD^+ has a redox potential of –320 mV, indicating that NADH has a strong tendency to donate electrons; 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 tendency to accept electrons. 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 $\Delta G° = -26.2$ kcal/mole per electron or –52.4 kcal/mole for the two electrons transferred per NADH molecule (see Panel 14–1). If we compare this free-energy change with that for the formation of the phosphoanhydride bonds in ATP ($\Delta G° = +7.3$ kcal/mole; see Figure 13–7), we see that more than enough energy is released by the oxidization of one NADH molecule to synthesize several molecules of ATP from ADP and P_i.

Living systems could certainly have evolved enzymes that would allow NADH to donate electrons directly to O_2 to make water in the reaction

$$2H^+ + 2e^- + \tfrac{1}{2}O_2 \rightarrow H_2O$$

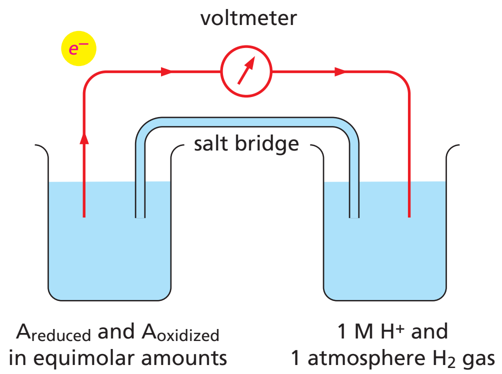
But because of the huge free-energy drop, this reaction would proceed with almost explosive force and nearly all of the energy would be released as heat. Instead, the energetically favorable reaction $2H^+ + 2e^- + \frac{1}{2}O_2 \rightarrow H_2O$ is made to occur in many small steps enabling nearly half of the released energy to be stored, rather than being lost to the environment as heat.

Metals Tightly Bound to Proteins Form Versatile Electron Carriers

Within each of the three respiratory enzyme complexes, electrons move mainly between metal atoms that are tightly bound to the proteins, traveling by skipping from one metal ion to another one with a greater affinity for electrons. In contrast, electrons are carried between the different respiratory complexes by molecules that diffuse along the lipid bilayer, picking up electrons from one complex and delivering them to another in an orderly sequence. In both the respiratory and photosynthetic electron-transport chains, one of these carriers is a **quinone**, a small hydrophobic molecule that dissolves in the lipid bilayer; in the mitochondrial respi-

PANEL 14–1 Redox potentials

HOW REDOX POTENTIALS ARE MEASURED



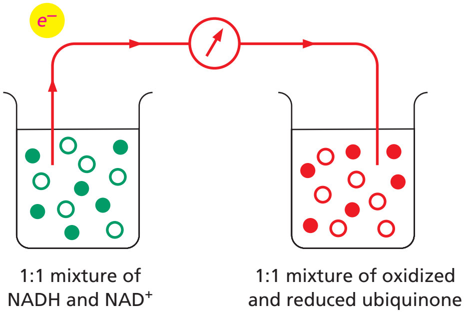
One beaker *(left)* contains substance A with an equimolar mixture of the reduced ($A_{reduced}$) and oxidized ($A_{oxidized}$) members of its redox pair. The other beaker contains the hydrogen reference standard ($2H^+ + 2e^- \rightleftharpoons H_2$), whose redox potential is arbitrarily assigned as zero by international agreement. (A salt bridge formed from a concentrated KCl solution allows K^+ and Cl^- to move between the beakers and neutralize the charges when electrons flow between the beakers.) The metal wire *(red)* 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 $A_{oxidized}$, this redox pair is said to have a postive redox potential.

By convention, the redox potential for a redox pair is designated as *E*. Since biological reactions occur at pH 7, biologists define the standard state as $A_{reduced}$ = $A_{oxidized}$ and $H^+ = 10^{-7}$ M and use it to determine the standard redox potential E_0'.

examples of redox reactions	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

$$\Delta E_0' = E_0'(\text{acceptor}) - E_0'(\text{donor})$$
$$\Delta E_0' = +30 - (-320) = +350$$



$\Delta G^\circ = -n(0.023)\,\Delta E_0'$ where *n* is the number of electrons transferred across a redox potential change of $\Delta E_0'$ millivolts (mV)

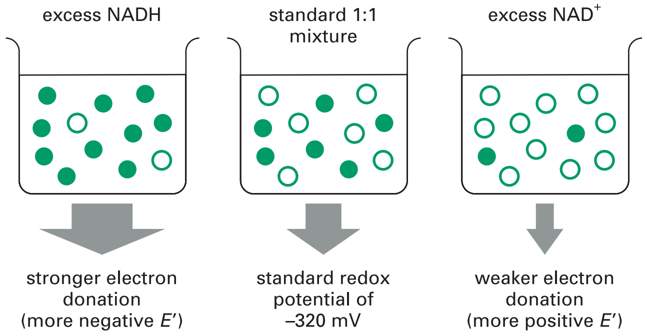
Example: The transfer of one electron from NADH to ubiquinone has a favorable ΔG° of –8.0 kcal/mole, calculated as follows:

$$\Delta G^\circ = -n(0.023)\Delta E_0' = -1(0.023)(350) = -8.0 \text{ kcal/mole}$$

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. 92), 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.



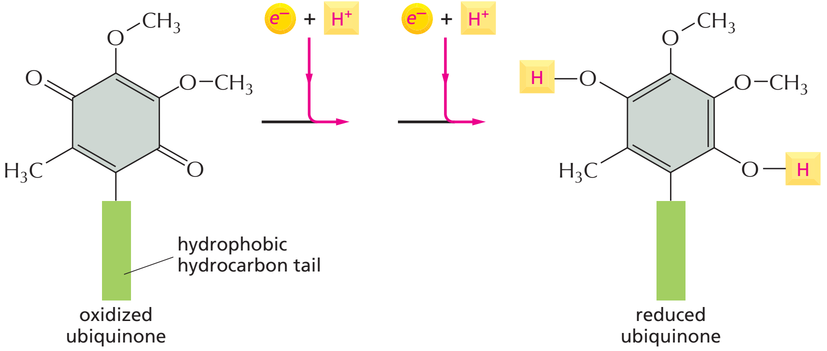


Figure 14–19 **Quinones carry electrons within the lipid bilayer.** The quinone in the mitochondrial electron-transport chain is called ubiquinone. It picks up one H^+ from the aqueous environment for every electron it accepts, and it can carry two electrons as part of a hydrogen atom (*yellow*). When reduced ubiquinone donates its electrons to the next carrier in the chain, the protons are released. The long hydrophobic tail confines ubiquinone to the membrane and consists of 6–10 five-carbon isoprene units, the number depending on the organism.

ratory chain, the quinone is called *ubiquinone*. Quinones are the only electron carriers in electron-transport chains that can function without being tightly bound to a protein.

Ubiquinone picks up electrons from the NADH dehydrogenase complex and delivers them to the cytochrome *b-c*$_1$ complex (see Figure 14–9). Ubiquinone can pick up or donate either one or two electrons, and it picks up one H^+ from the surroundings with each electron that it carries (Figure 14–19). Its redox potential of +30 mV places ubiquinone about one-quarter of the way down the chain from NADH in terms of energy loss (Figure 14–20). Ubiquinone can also receive electrons directly from the $FADH_2$ generated by the citric acid cycle or by fatty acid oxidation. Because these electrons bypass NADH hydrogenase—which is one of the proton pumps in the electron transport chain—they cause less proton pumping than do the two electrons transported from NADH.

The rest of the electron carriers in the electron-transport chain are either small molecules or metal-containing groups that are all tightly bound to proteins. To get from NADH to ubiquinone, for example, the electrons are passed inside the NADH dehydrogenase complex between a flavin group (see Figure 13–12 for structure) bound to one of the proteins and a set of **iron–sulfur centers** of increasing redox potentials. The final iron–sulfur center in the dehydrogenase donates its electrons to ubiquinone.

Iron–sulfur centers have relatively low affinities for electrons and thus are prominant in the early part of the electron-transport chain. Later, in

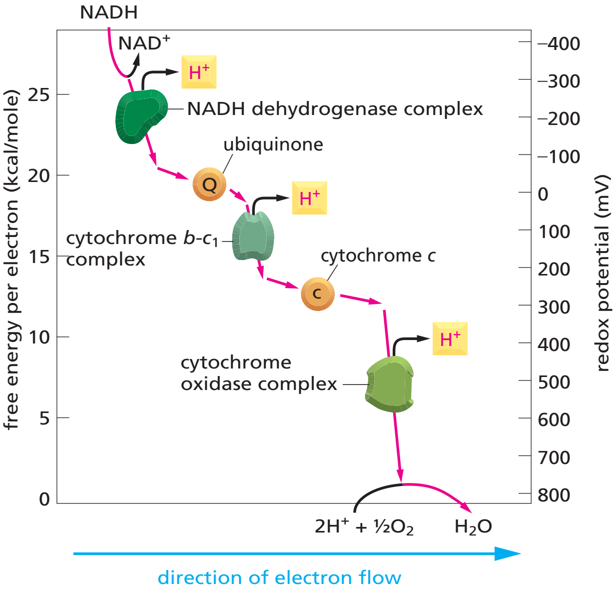


Figure 14–20 **Redox potential increases along the mitochondrial electron-transport chain.** The big increases in redox potential occur across each of the three respiratory enzyme complexes, as required for each of them to pump protons. To convert free energy values to kJ/mole, recall that 1 kilocalorie is equal to about 4.2 kilojoules.

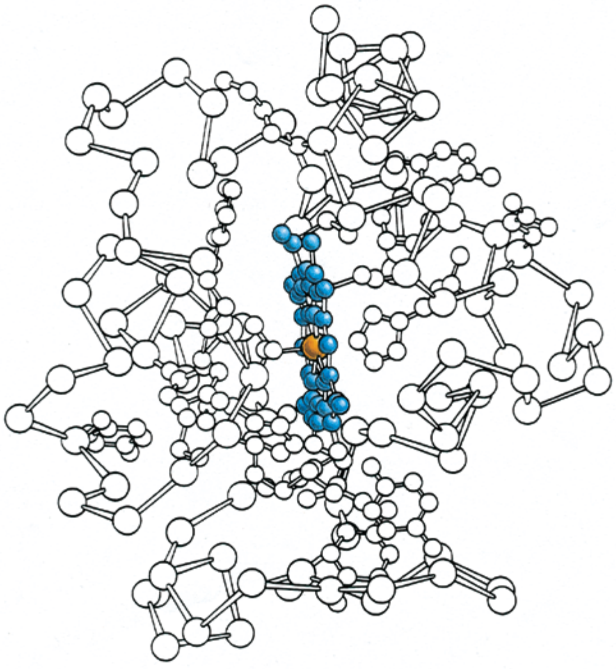


Figure 14–21 **Cytochrome *c* is an electron carrier in the electron-transport chain.** This small protein contains just over 100 amino acids and is held loosely on the outer face of the inner membrane by ionic interactions (see Figure 14–9). The iron atom (*orange*) in the bound heme (*blue*) can carry a single electron. The structure of the heme group in hemoglobin, which reversibly binds O_2 rather than an electron, was shown in Figure 4–33.

the pathway from ubiquinone to O_2, iron atoms in heme groups that are tightly bound to cytochrome proteins are commonly used as electron carriers, as in the cytochrome *b-c*$_1$ and cytochrome oxidase complexes. The **cytochromes** constitute a family of colored proteins (hence their name, from the Greek *chroma*, "color"); each contains one or more heme groups whose iron atom changes from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state whenever it accepts an electron. As one would expect, the various cytochromes increase in redox potential as one progresses down the mitochondrial electron-transport chain towards O_2. The structure of *cytochrome* c, a small protein that shuttles electrons between the cytochrome *b-c*$_1$ complex and the cytochrome oxidase complex, is shown in Figure 14–21: its redox potential is +230 mV.

At the very end of the respiratory chain, just before O_2, the electron carriers are those in the cytochrome oxidase complex. The carriers here are either iron atoms in heme groups or copper atoms that are tightly bound to the complex in specific ways that give them a high redox potential.

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.

Cytochrome Oxidase Catalyzes the Reduction of Molecular Oxygen

Cytochrome oxidase is a protein complex that receives electrons from cytochrome *c*, thus oxidizing it (hence the name cytochrome oxidase). It then donates these electrons to O_2. In brief, four electrons from 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 couple with O_2, four other protons are pumped across the membrane during electron transfer, further increasing the electrochemical proton gradient.

Of course, for proton pumping to occur, it must be coupled in some way to energetically favorable reactions. In the case of cytochrome oxidase, the energy comes from the transfer of a series of four electrons to an O_2 molecule that is bound tightly to the protein; these electron transfers drive allosteric changes in the conformation of the protein that move protons out of the mitochondrial matrix. At its active site, where O_2 is bound, cytochrome oxidase contains a complex of a heme iron atom juxtaposed with a tightly bound copper atom (Figure 14–22). It is here that nearly all of the oxygen we breathe is used, serving as the final repository for the electrons that NADH donated at the start of the electron-transport chain.

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. One of the roles of cytochrome oxidase is to hold on tightly to an oxygen molecule until all four electrons needed to convert it to two H_2O molecules are in hand, thereby preventing a random attack on cellular macromolecules by superoxide radicals—damage that has been postulated to be a cause of human aging.

The invention of cytochrome oxidase was crucial to the evolution of cells that could use O_2 as an electron acceptor, and it is estimated to account for 90% of the total uptake of O_2 in most cells. This protein complex is therefore essential for all aerobic life. The poisons cyanide and azide are extremely toxic because they bind tightly to the cell's cytochrome oxidase complexes to stop electron transport, thereby greatly reducing ATP production.

The Mechanism of H^+ Pumping Can Be Studied in Atomic Detail

The detailed mechanism by which electron transport is coupled to H^+ pumping is different in each of the three different respiratory enzyme complexes. For example, ubiquinone has a central role in proton pumping through the cytochrome *b*-c_1 complex, as the quinone picks up an H^+ from the aqueous medium along with each electron it carries and liberates it when it releases the electron (see Figure 14–19). Because ubi-

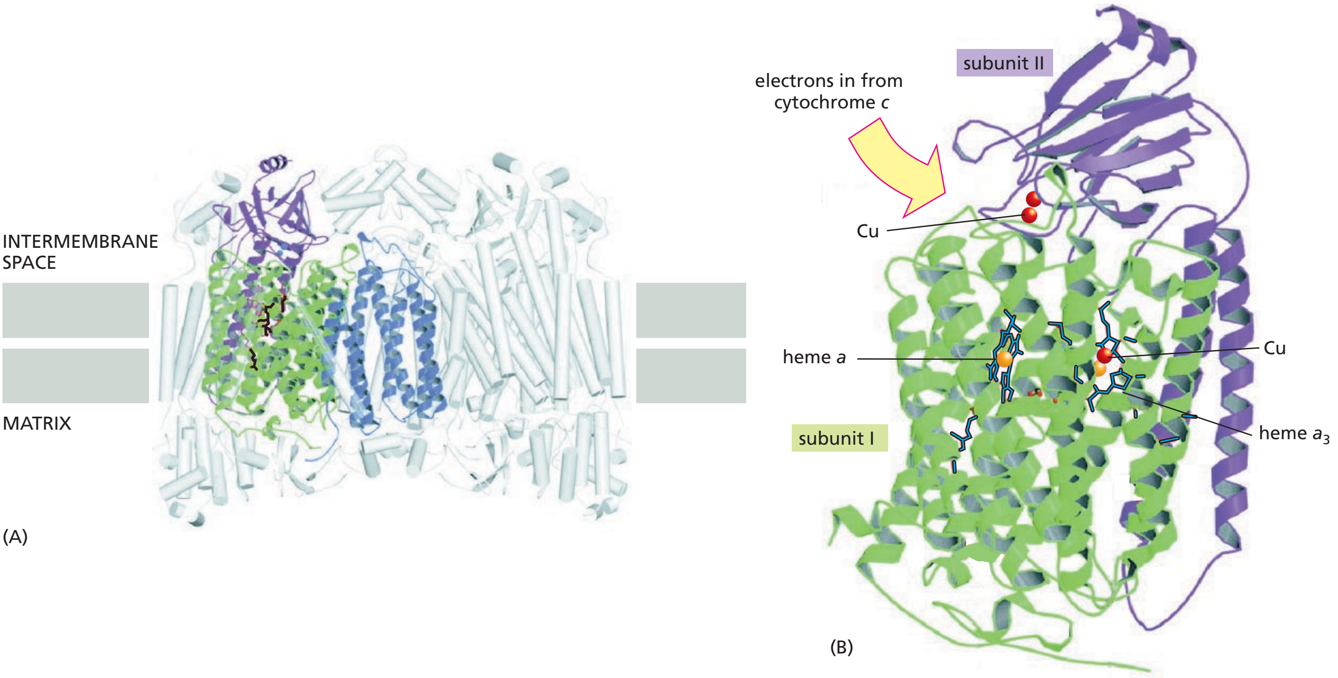


Figure 14–22 **Cytochrome oxidase is a finely tuned protein machine.** The protein is a dimer formed from a monomer with 13 different protein subunits. The three colored subunits that form the functional core of the complex are encoded by the mitochondrial genome. As electrons pass through this protein on the way to its bound O_2 molecule, they cause the protein to pump protons across the membrane. (A) The entire protein is shown, positioned in the inner mitochondrial membrane. (B) The electron carriers are located in subunits I and II, as indicated.

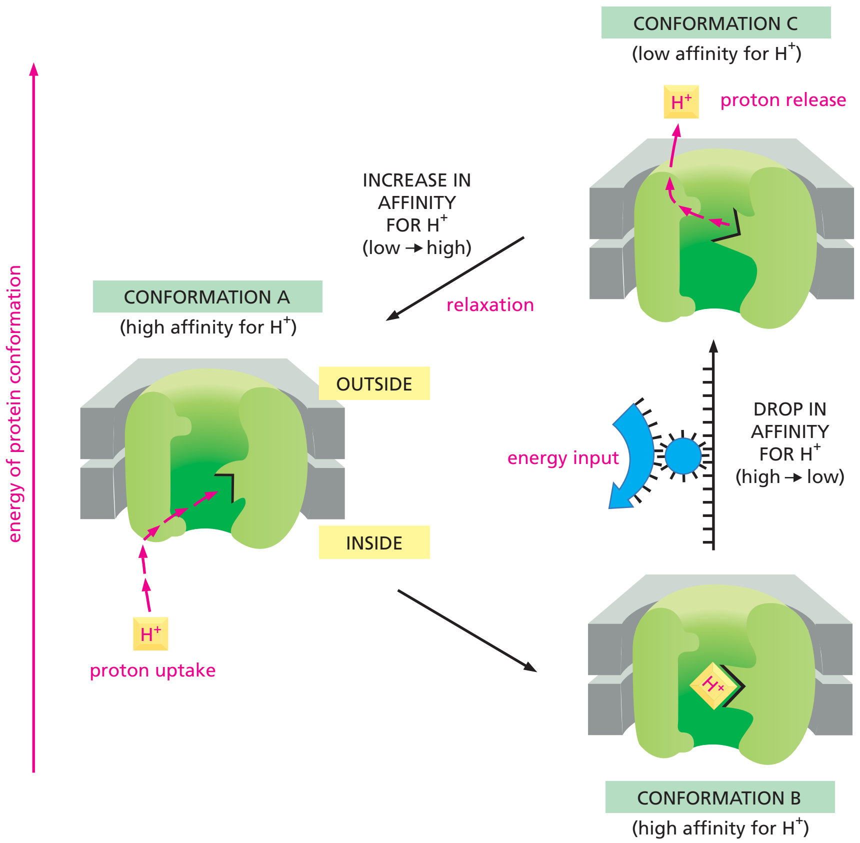


Figure 14–23 **H^+ pumping can be caused by a conformational change in a protein pump driven by an energetically favorable reaction.** This mechanism for H^+ pumping by a transmembrane protein is thought to be used by NADH dehydrogenase and cytochrome oxidase, and by many other proton pumps. The protein is driven through a cycle of three conformations: A, B, and C. As indicated by their vertical spacing, these protein conformations have different energies. In conformations A and B, the protein has a high affinity for H^+, causing it to pick up an H^+ on the inside of the membrane. In conformation C, the protein has a low affinity for H^+, causing it to release an H^+ on the outside of the membrane. The transition from conformation B to conformation C that releases the H^+ is energetically unfavorable, and it occurs only because it is driven by being allosterically coupled to an energetically favorable reaction occurring elsewhere on the protein (*blue* arrow). For cytochrome oxidase and NADH dehydrogenase, this reaction is electron transport. The other two conformational changes, A → B and C → A, lead to states of lower energy, and they proceed spontaneously. Because the overall cycle A → B → C → A releases free energy, H^+ is pumped from inside the mitochondrial matrix out to the intermembrane space (Movie 14.6). For other proton pumps, such as the Ca^{2+} pump in muscle cells, the energy required for the conformational change B → C is provided by ATP hydrolysis (see Figure 12–15). For bacteriorhodopsin, this energy comes from sunlight (see Figure 11–28).

quinone is freely mobile in the lipid bilayer, it can accept electrons near the inside surface of the membrane and donate them to the cytochrome b-c_1 complex near the outside surface. Thus ubiquinone transfers one H^+ across the bilayer for every electron it transports. However, two protons are pumped per electron in the cytochrome b-c_1 complex, and there is good evidence for a so-called *Q-cycle,* in which ubiquinone is recycled through the protein complex in a complicated, but ordered way that makes this two-for-one transfer possible.

Allosteric changes in protein conformations can also pump H^+. For both the NADH dehydrogenase complex and the cytochrome oxidase complex, electron transport drives sequential allosteric changes in the protein that cause it to pump H^+ across the mitochondrial inner membrane. A general mechanism for this type of H^+ pumping is presented in Figure 14–23.

Respiration Is Amazingly Efficient

The free-energy changes for burning fats and carbohydrates directly to CO_2 and H_2O can be compared with the total amount of energy generated and stored in the phosphate bonds of ATP during the corresponding biological oxidations. When this is done, one finds that the efficiency with which oxidation energy is converted into ATP bond energy is often greater than 40%. This is considerably better than the efficiency of most nonbiological energy-conversion devices. If cells worked only with the efficiency of an electric motor or a gasoline engine (10–20%), an organism would have to eat voraciously in order to maintain itself. Moreover, because wasted energy is liberated as heat, large organisms (including ourselves) would need more efficient mechanisms than they presently have for giving up heat to the environment.

Students sometimes wonder why the chemical interconversions in cells follow such complex pathways. The oxidation of sugars to CO_2 plus H_2O could certainly be accomplished more directly, eliminating the citric acid

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.

cycle and many of the steps in the respiratory chain. This would make respiration easier for students to learn, but it would be a disaster for the cell. Oxidation produces huge amounts of free energy, which can be utilized efficiently only in small bits. Biological oxidative pathways involve many intermediates, each differing only slightly from its predecessor. The energy released is thereby parceled out into small packets that can be efficiently converted to high-energy bonds in useful molecules, such as ATP and NADH, by means of coupled reactions (see Figure 13–1).

Having seen how chemiosmotic coupling is used to generate ATP in mitochondria, we now look at how it harnesses light energy for the generation of ATP in chloroplasts.

CHLOROPLASTS AND PHOTOSYNTHESIS

Virtually all of the organic material required by present-day living cells is produced by **photosynthesis**—the series of light-driven reactions that creates organic molecules from atmospheric carbon dioxide (CO_2). Plants, algae, and the most advanced photosynthetic bacteria, such as the cyanobacteria, use electrons from water and the energy of sunlight to convert atmospheric CO_2 into organic compounds. In the course of splitting water they liberate into the atmosphere vast quantities of O_2 gas. This oxygen is in turn required for cellular respiration—not only in animals but also in plants and many bacteria. Thus the activity of early photosynthetic bacteria, which filled the atmosphere with oxygen, enabled the evolution of life forms that use aerobic metabolism to make their ATP (Figure 14–24).

In plants, photosynthesis is carried out in a specialized intracellular organelle—the **chloroplast**, which contains light-capturing pigments such as the green pigment chlorophyll. All green parts of a plant contain chloroplasts, but for most plants the leaves are the major sites of photosynthesis. Chloroplasts perform photosynthesis during the daylight hours. The process produces ATP and NADPH, which in turn are used to convert CO_2 into sugar inside the chloroplast. Thus we begin our discussion of photosynthesis by describing the structure of this specialized organelle.

Figure 14–24 **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 lay down successive layers of material. (B) Cross section of a modern stromatolite, showing its layered structure. (C) Cross section through a fossil stromatolite in a rock 3.5 billion years old. Note the layered structure similar to that in (B). Fossil stromatolites are thought to have been formed by photosynthetic bacteria very similar to modern cyanobacteria. The activities of bacteria like these, which liberate O_2 gas as a waste product of photosynthesis, would have slowly changed Earth's atmosphere. (A, courtesy of Sally Birch, Oxford Scientific Films; B and C, courtesy of S.M. Awramik, University of California/Biological Photo Service.)

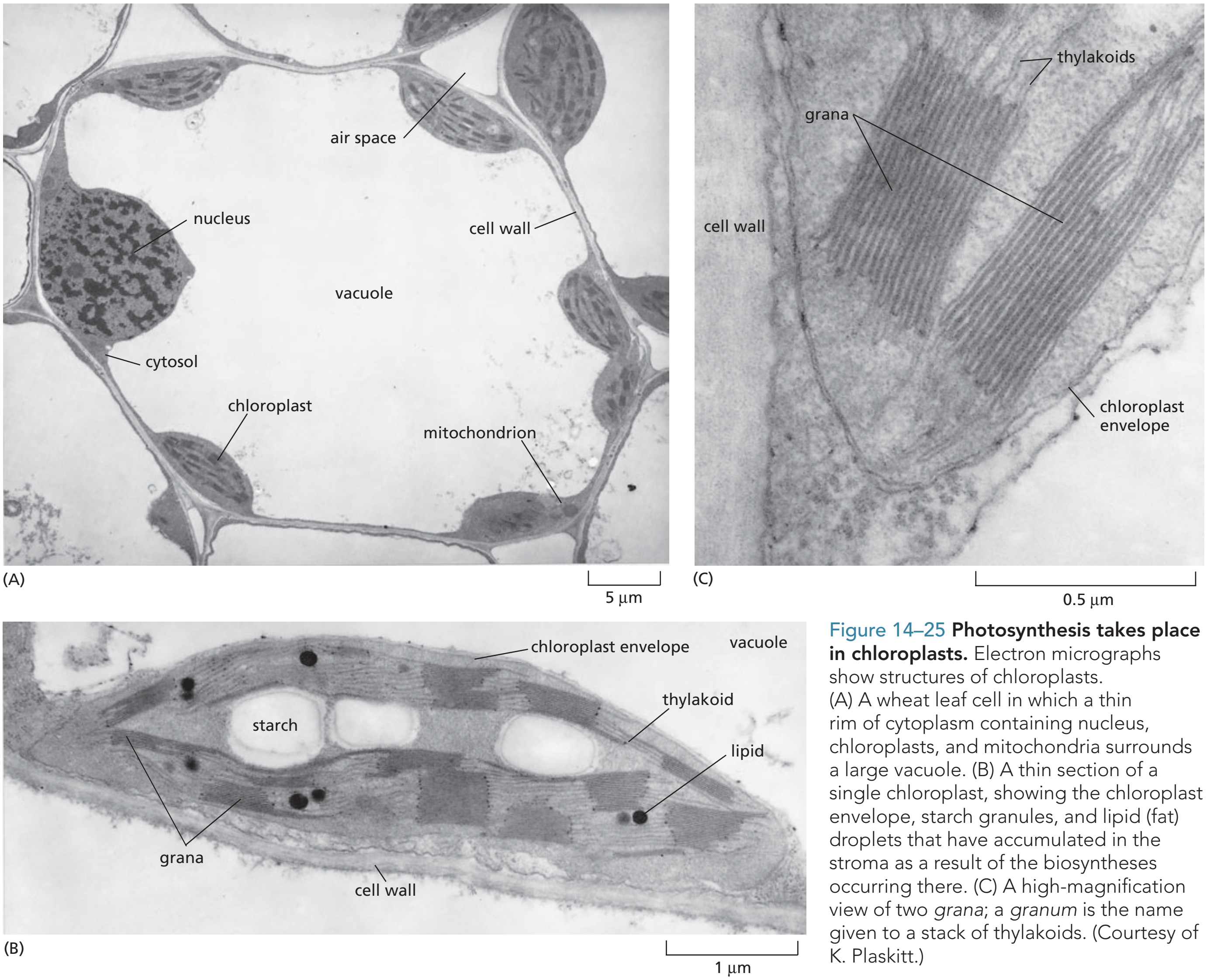


Figure 14–25 Photosynthesis takes place in chloroplasts. Electron micrographs show structures of chloroplasts. (A) A wheat leaf cell in which a thin rim of cytoplasm containing nucleus, chloroplasts, and mitochondria surrounds a large vacuole. (B) A thin section of a single chloroplast, showing the chloroplast envelope, starch granules, and lipid (fat) droplets that have accumulated in the stroma as a result of the biosyntheses occurring there. (C) A high-magnification view of two *grana*; a *granum* is the name given to a stack of thylakoids. (Courtesy of K. Plaskitt.)

Chloroplasts Resemble Mitochondria but Have an Extra Compartment

Chloroplasts carry out their energy interconversions by means of proton gradients in much the same way that mitochondria do. Although chloroplasts are larger (Figure 14–25A), they are organized on the same principles as mitochondria. Chloroplasts have a highly permeable outer membrane and a much less permeable inner membrane, in which membrane transport proteins are embedded. Together these membranes—and the narrow, intermembrane space that separates them—form the chloroplast envelope (Figure 14–25B). The inner membrane surrounds a large space called the **stroma**, which is analogous to the mitochondrial matrix and contains many metabolic enzymes.

There is, however, an important difference between the organization of mitochondria and that of chloroplasts. The inner membrane of the chloroplast does not contain the electron-transport chains. Instead, the light-capturing systems, the electron-transport chains, and ATP synthase are all contained in the *thylakoid membrane*, a third membrane that forms a set of flattened disclike sacs, called the *thylakoids* (Figure 14–25C). These are arranged in stacks, and the space inside each thylakoid is thought to be connected with that of other thylakoids, thereby defining a continuous third internal compartment that is separated from the stroma by the

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?

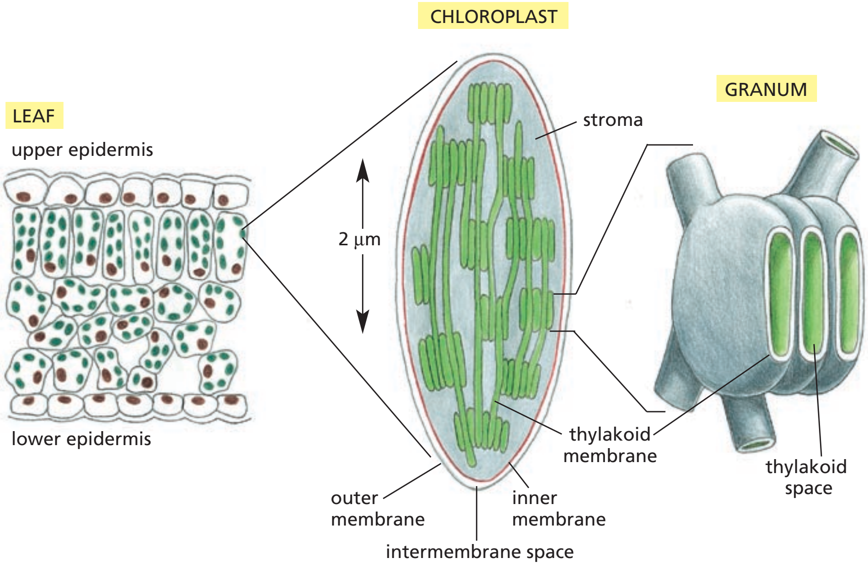


Figure 14–26 **A chloroplast contains a third internal compartment.** This photosynthetic organelle contains three distinct membranes (the outer membrane, the inner membrane, and the thylakoid membrane) that define three separate internal compartments (the intermembrane space, the stroma, and the thylakoid space). The thylakoid membrane contains all of the energy-generating systems of the chloroplast, including its light-capturing chlorophyll. In electron micrographs this membrane appears to be broken up into separate units that enclose individual flattened vesicles (see Figure 14–25C), but these are probably joined into a single, highly folded membrane in each chloroplast. As indicated, the individual thylakoids are interconnected, and they tend to stack to form grana.

thylakoid membrane (Figure 14–26). The structural similarities and differences between mitochondria and chloroplasts are illustrated in Figure 14–27.

Chloroplasts Capture Energy from Sunlight and Use It to Fix Carbon

As we saw in Chapter 3, the overall equation that summarizes the net result of photosynthesis can be written as follows:

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

Although the equation is quite simple, the reactions that allow the process to occur are fairly elaborate. Generally speaking, however, the many reactions that make up photosynthesis in plants take place in two stages (see Figure 3–8):

1. In the first stage, which is dependent on light, energy from sunlight is captured and stored transiently in the high-energy bonds of ATP and the activated carrier molecule NADPH. These energy-producing *photosynthetic electron-transfer reactions*, also called the 'light reactions,' occur entirely within the thylakoid membrane of the chloroplast. In this series of reactions, energy derived from sunlight energizes an electron in the green organic pigment **chlorophyll**, enabling the electron to move along an electron-transport chain in the thylakoid membrane in much the same way that an electron moves along the respiratory chain in mitochondria. The

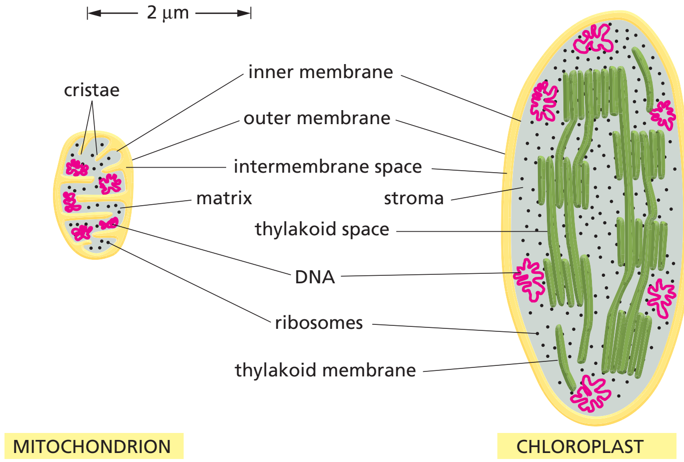


Figure 14–27 **Compared with mitochondria, chloroplasts are larger and have an extra compartment.** A chloroplast contains, in addition to an inner and an outer membrane, a thylakoid membrane enclosing a thylakoid space. The thylakoid membrane contains the light-capturing systems, the electron-transport chains, and ATP synthase. Unlike the chloroplast inner membrane, the mitochondrial inner membrane is folded into cristae in order to increase its surface area. As we discuss later in the chapter, both organelles contain their own genome and genetic system. The stroma, therefore, like the mitochondrial matrix, also contains a special set of ribosomes, RNA, and DNA (*red*).

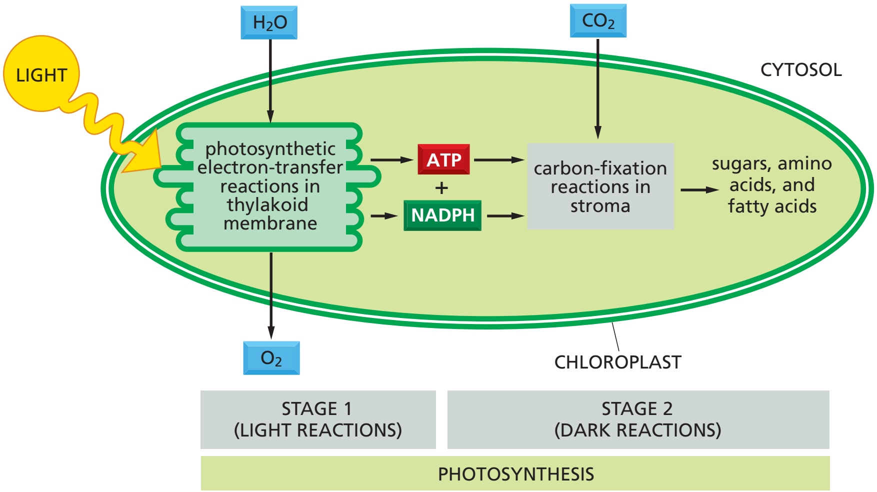


Figure 14–28 **Both stages of photosynthesis depend on the chloroplast.** Water is oxidized and oxygen is released in the photosynthetic electron-transfer reactions that produce ATP and NADPH (stage 1), while carbon dioxide is assimilated (fixed) to produce sugars and a variety of other organic molecules in the carbon-fixation reactions (stage 2). Stage 2 begins in the chloroplast stroma (as shown) and continues in the cytosol.

electron that chlorophyll donates to the electron-transport chain is ultimately replaced by an electron extracted from water. This electron shuffle splits a molecule of water (H_2O), producing O_2 as a by-product. During the electron-transport process, H^+ is pumped across the thylakoid membrane, and the resulting electrochemical proton gradient drives the synthesis of ATP in the stroma. As the final step in this series of reactions, high-energy electrons are loaded (together with H^+) onto $NADP^+$, converting it to NADPH (Figure 14–28).

2. In the second, light-independent stage of photosynthesis, the ATP and the NADPH produced by the photosynthetic electron-transfer reactions serve as the source of energy and reducing power, respectively, to drive the manufacture of sugars from CO_2 (see Figure 14–28. These carbon-fixation reactions, also called the ‘dark reactions,’ begin in the chloroplast stroma and continue in the plant cell cytosol. They produce sucrose and many other organic molecules in the leaves of the plant. The sucrose is exported to other tissues as a source of both organic molecules and energy for growth.

Thus the formation of ATP, NADPH, and O_2 (which requires light energy directly) and the conversion of CO_2 to carbohydrate (which requires light energy only indirectly) are separate processes, although elaborate feedback mechanisms interconnect the two sets of reactions. Several of the chloroplast enzymes required for carbon fixation, for example, are inactivated in the dark and reactivated by light-stimulated electron-transport processes.

Sunlight is Absorbed by Chlorophyll Molecules

Visible light is a form of electromagnetic radiation composed of many different wavelengths, ranging from violet (wavelength 400 nm) to deep red (700 nm). But when we consider events at the level of a single molecule—such as the absorption of light by a molecule of chlorophyll—we have to picture light as being composed of discrete packets of energy called *photons*. Light of different colors is distinguished by photons of different energy, with longer wavelengths corresponding to lower energies. Thus photons of red light have a lower energy than photons of green light.

When sunlight is absorbed by a molecule of chlorophyll, electrons in the molecule interact with photons of light and are raised to a higher energy level. The electrons in the extensive network of alternating single and double bonds in the chlorophyll molecule (Figure 14–29) absorb red light most strongly, which is why chlorophyll looks green to us.

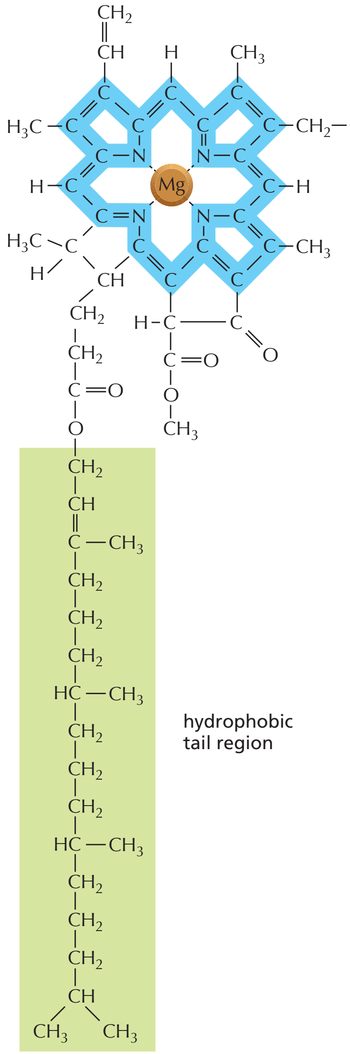


Figure 14–29 **Chlorophyll is a green pigment that absorbs energy from photons of light.** A magnesium atom (*orange*) is held in the center of a porphyrin ring, which is structurally similar to the porphyrin ring that binds iron in heme. Light is absorbed by electrons within the bond network shown in *blue*, while the long hydrophobic tail (*green*) helps to hold the chlorophyll in the thylakoid membrane.

Excited Chlorophyll Molecules Funnel Energy into a Reaction Center

An isolated molecule of chlorophyll is incapable of converting the light it absorbs to a form of energy useful to living systems. It can accomplish this feat only when it is associated with the appropriate proteins and embedded in a membrane. In plant thylakoid membranes and in the membranes of photosynthetic bacteria, the light-absorbing chlorophylls are held in large multiprotein complexes called **photosystems**. Each photosystem consists of an **antenna complex** that captures light energy and a **reaction center** that enables this light energy to be converted into chemical energy. The antenna portion of a photosystem consists of hundreds of chlorophyll molecules that capture light energy in the form of excited (high-energy) electrons. These chlorophylls are arranged so that the energy of an excited electron can be passed from one molecule to another, until finally the energy is funneled into two chlorophyll molecules called the *special pair* (Figure 14–30). These two chlorophyll molecules are located in the reaction center, a protein complex that sits adjacent to the antenna complex in the membrane. There the energy is trapped and used to energize one electron in the special pair of chlorophyll molecules.

The reaction center is a transmembrane complex of proteins and organic pigments that lies at the heart of photosynthesis. It is thought to have first evolved more than 3 billion years ago in primitive photosynthetic bacteria. Detailed structural and functional studies have revealed how it functions at an atomic level of detail (Movie 14.7). The reaction center acts as an irreversible trap for an excited electron, because the special pair of chlorophylls are poised to pass the high-energy electron to a precisely positioned neighboring molecule in the same protein complex. By moving the energized electron rapidly away from the chlorophylls, a process known as *charge separation*, the reaction center transfers this high-energy electron to an environment where it is much more stable.

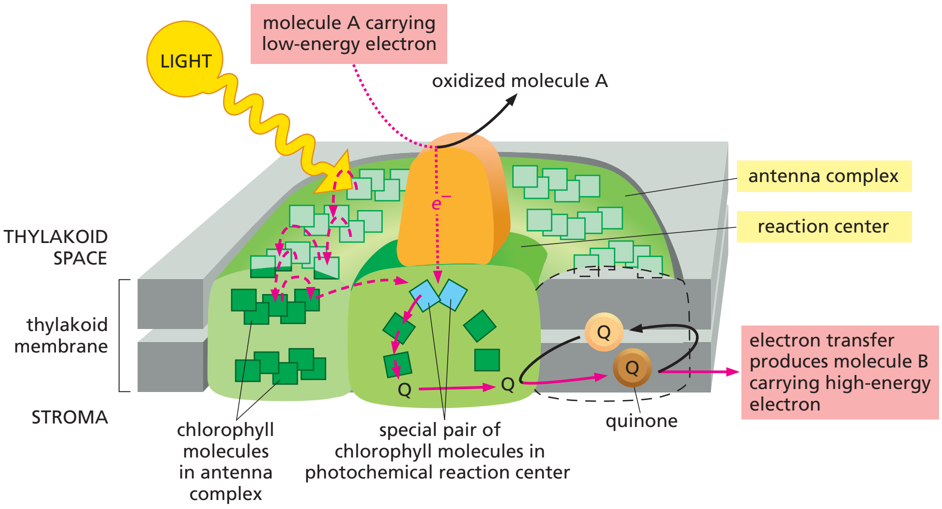


Figure 14–30 **A photosystem contains a reaction center and an antenna.** The antenna collects the energy of electrons that have been excited by light and funnels this energy (energy transfers shown by *red* dashed arrows) to a special pair of chlorophyll molecules in the reaction center. This produces a high-energy electron in the special pair that can be passed rapidly (electron transfers shown as *red* solid arrows) to the electron-transport chain in the thylakoid membrane, via the quinone (Q). In addition to chlorophyll, the antenna contains additional accessory pigments (not shown) that help to capture light energy of different wavelengths. A protein in the reaction center (*orange*) collects the low-energy electrons needed to return the system to its original unexcited state (electron transfers shown as *red* dotted arrows), as we shall see in Figure 14–31.

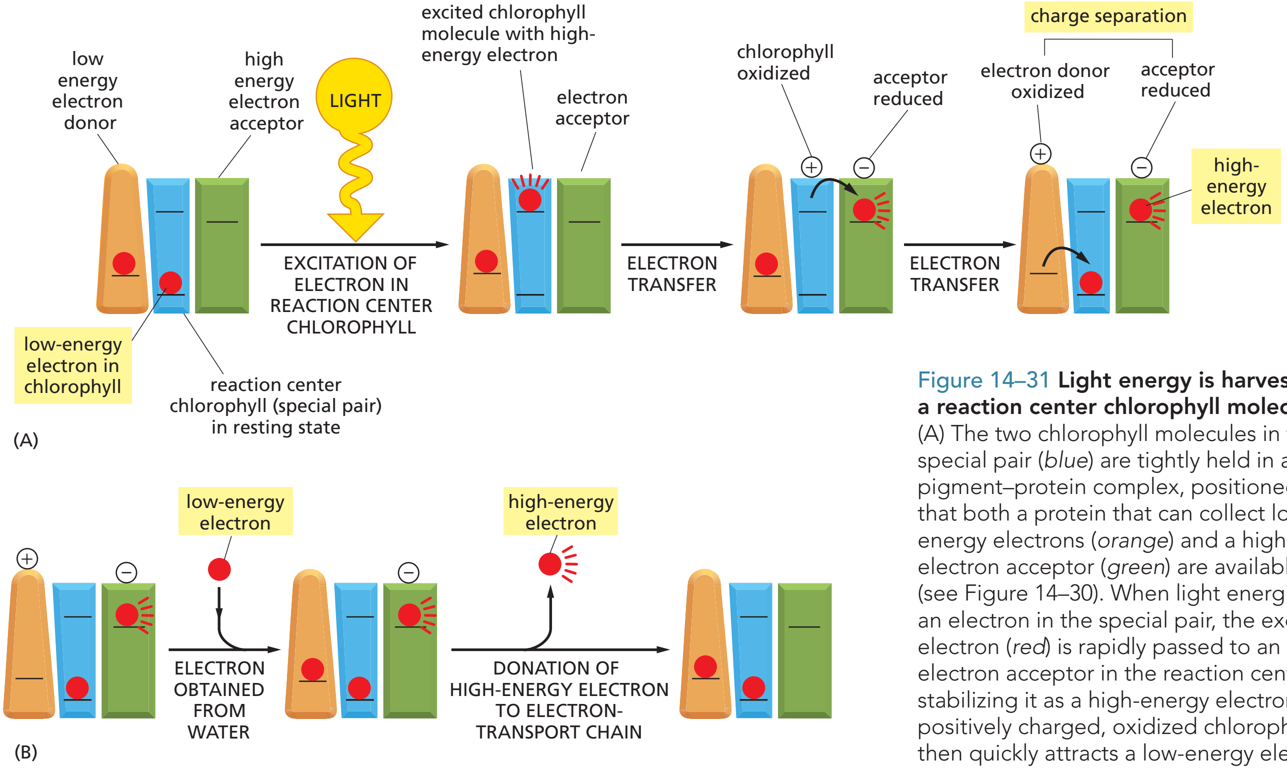


Figure 14–31 **Light energy is harvested by a reaction center chlorophyll molecule.** (A) The two chlorophyll molecules in the special pair (*blue*) are tightly held in a pigment–protein complex, positioned so that both a protein that can collect low-energy electrons (*orange*) and a high-energy electron acceptor (*green*) are available (see Figure 14–30). When light energizes an electron in the special pair, the excited electron (*red*) is rapidly passed to an electron acceptor in the reaction center, stabilizing it as a high-energy electron. The positively charged, oxidized chlorophyll then quickly attracts a low-energy electron from the electron donor, thereby returning to a resting state. These reactions, which create a charge separation between the electron donor and the electron acceptor, require less than 10^{-6} second to complete. (B) In the final stage of the process, which follows the steps in (A), the entire reaction center is restored to its original state by both extracting a new, low-energy electron (from water in this case) and passing its high-energy electron on to the electron-transport chain. Thus, in the overall reaction, low-energy electrons obtained from water are being consumed and high-energy electrons are being produced in the plant thylakoid membrane.

When the chlorophyll molecule in the reaction center loses an electron, it becomes positively charged; it then rapidly regains an electron from an adjacent electron donor to return to its unexcited, uncharged state (Figure 14–31A). Then, in slower reactions, the electron donor has its missing electron replaced with an electron removed from water. The high-energy electron that was generated by the excited chlorophyll is then transferred to the electron-transport chain. This transfer leaves the reaction center ready to receive the next high-energy electron excited by sunlight (Figure 14–31B).

Light Energy Drives the Synthesis of Both ATP and NADPH

In mitochondria, the electron-transport chain functions solely to generate ATP. But in the chloroplast, and in free-living photosynthetic organisms like cyanobacteria, electron transport has an additional role: it also produces the activated carrier molecule NADPH (see Figure 3–34). NADPH is needed because photosynthesis is ultimately a biosynthetic process. To build organic molecules from CO_2, a cell requires a huge input of energy, in the form of ATP, and a very large amount of reducing power, in the form of NADPH. To produce this NADPH from $NADP^+$, the cell uses energy captured from sunlight to convert the low-energy electrons in water to the high-energy electrons in NADPH.

To produce both ATP and NADPH, plant cells and cyanobacteria use two photons of light: ATP is made after the first photon is absorbed, NADPH after the second. These photons are absorbed by two different photosystems that operate in series. Working together, these photosystems impart to an electron a high enough energy to produce NADPH. Along the way, a proton gradient is generated, allowing ATP to be made.

In outline, the process works as follows: the first photon of light is absorbed by one photosystem (which is paradoxically called photosystem II for historical reasons). As we have seen, that photon is used to produce a high-energy electron that is handed off to an electron-transport chain (see Figure 14–31). While traveling down the electron-transport chain, the electron drives an H^+ pump in the thylakoid membrane and creates a proton gradient in the manner described previously for oxidative phosphorylation. An ATP synthase in the thylakoid membrane then uses this proton gradient to drive the synthesis of ATP on the stromal side of the membrane (Figure 14–32).

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?

In the meantime, the electron-transport chain delivers the electron generated by photosystem II to the second photosystem in the pathway (called photosystem I). There the electron fills the positively charged 'hole' that was left in the reaction center of photosystem I when it absorbed the second photon of light. Because photosystem I starts at a higher energy level than photosystem II, it is able to boost electrons to the very high energy level needed to make NADPH from $NADP^+$ (see Figure 14–32). The redox potentials of the components along this electron-transport chain are shown in Figure 14–33.

In the overall process described thus far, we have seen that an electron removed from a chlorophyll molecule at the reaction center of photosystem II travels all the way through the electron-transport chain in the thylakoid membrane until it winds up being donated to NADPH. This initial electron must be replaced to return the system to its unexcited state. The replacement electron comes from a low-energy electron donor, which, in plants and many photosynthetic bacteria, is water (see Figure 14–31B). The reaction center of photosystem II includes a water-splitting enzyme that holds the oxygen atoms of two water molecules bound to a cluster of manganese atoms in the protein (Figure 14–34; see also Figure 14–32). This enzyme removes electrons one at a time from the water to fill the holes created by light in the chlorophyll molecules of the reaction center. When four electrons have been removed from two water molecules (which requires four photons of light), O_2 is released. It is this critical process, occurring over billions of years, that has generated all of the O_2 in the Earth's atmosphere.

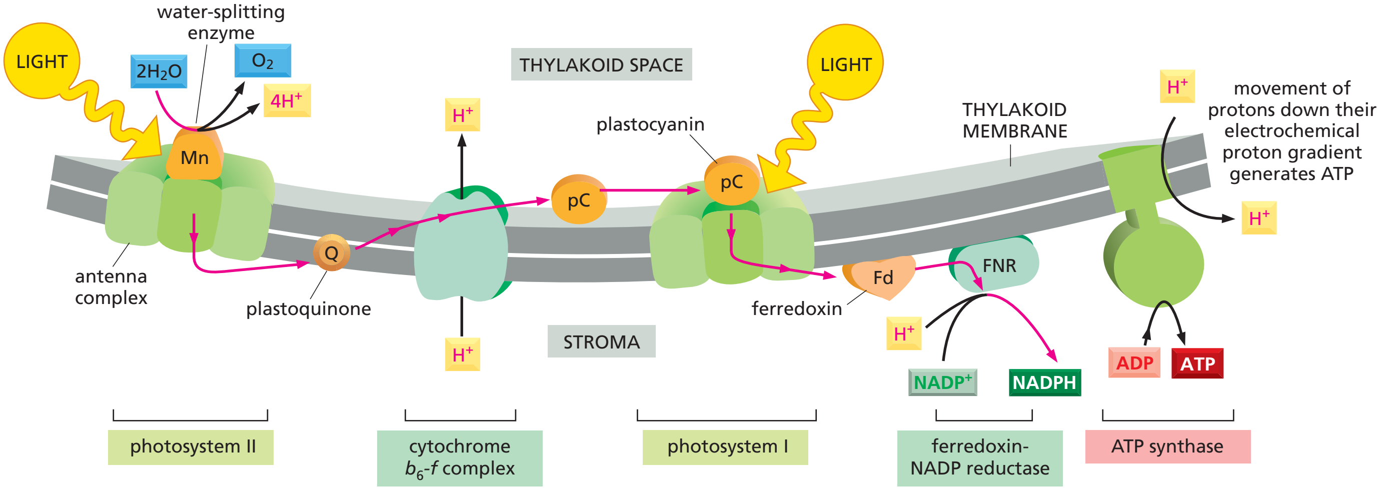


Figure 14–32 **During photosynthesis electrons travel down an electron transport chain in the thylakoid membrane.** Light energy is harvested by the antenna complexes in each of the two membrane-embedded photosystems and funneled to a special pair of chlorophyll molecules in the reaction center. As shown in Figure 14–30, an excited, high-energy electron is thereby created in the special pair, which is transferred through a series of electron acceptors inside the reaction center before being passed to the electron-transport chain in the chloroplast membrane. The mobile electron carriers in the chloroplast electron-transport chain are plastoquinone (Q, which closely resembles the ubiquinone of mitochondria), plastocyanin (pC, a small, copper-containing protein), and ferredoxin (Fd, a small protein containing an iron–sulfur center). The cytochrome b_6-*f* complex resembles the cytochrome *b*-c_1 complex of mitochondria, and it is the sole site of active H^+ pumping in the chloroplast electron-transport chain. The H^+ released by the splitting of water by photosystem II and the H^+ taken up during NADPH formation by the protein ferredoxin NADP-reductase, the last protein in the electron transport chain, also contribute to generating the electrochemical proton gradient. As indicated, the proton gradient drives an ATP synthase located in the same membrane to generate ATP. An overview of the photosynthetic reactions is shown in Movie 14.8.

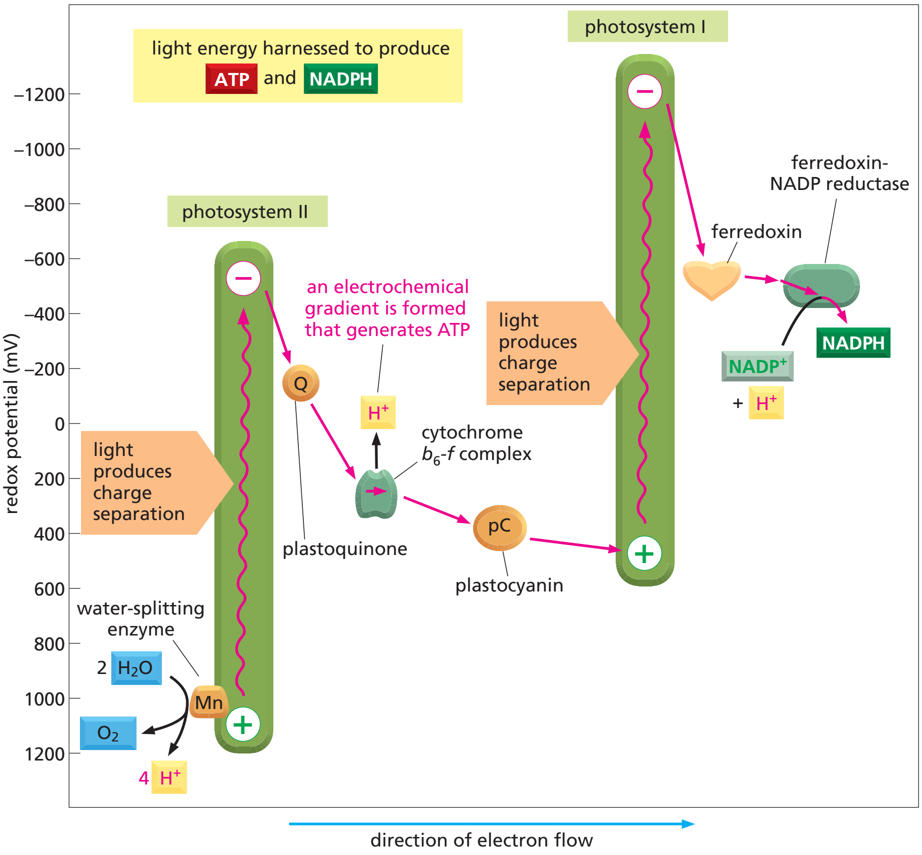


Figure 14–33 The coupling of photosystems I and II boosts electrons to the energy level needed to produce NADPH. The redox potential for each molecule is indicated by its position on the vertical axis. Photosystem II passes electrons from its excited chlorophyll special pair through an electron-transport chain in the thylakoid membrane that leads to photosystem I. The net electron flow through the two photosystems linked in series is from water to $NADP^+$, and it produces NADPH as well as ATP. The ATP is synthesized by an ATP synthase (not shown here) that harnesses the electrochemical proton gradient produced by electron transport.

Chloroplasts Can Adjust their ATP Production

In addition to carrying out the photosynthetic process outlined so far, chloroplasts can also generate ATP without making NADPH. To produce this extra ATP, chloroplasts can switch photosystem I into a cyclic mode so that it produces ATP instead of NADPH. In this process, called **cyclic photophosphorylation**, the high-energy electrons produced by light activation of photosystem I are transferred back to the cytochrome b_6-f complex rather than being passed on to $NADP^+$. From the b_6-f complex, the electrons are handed back to photosystem I at low energy (Figure 14–35). The net result, aside from the conversion of some light energy to heat, is that H^+ is pumped across the thylakoid membrane by the b_6-f complex as electrons pass through it. This cycle increases the electrochemical proton gradient that drives the synthesis of ATP. Cells adjust the relative amounts of cyclic photophosphorylation (which involves only photosystem I) and the standard, noncyclic form of phosphorylation

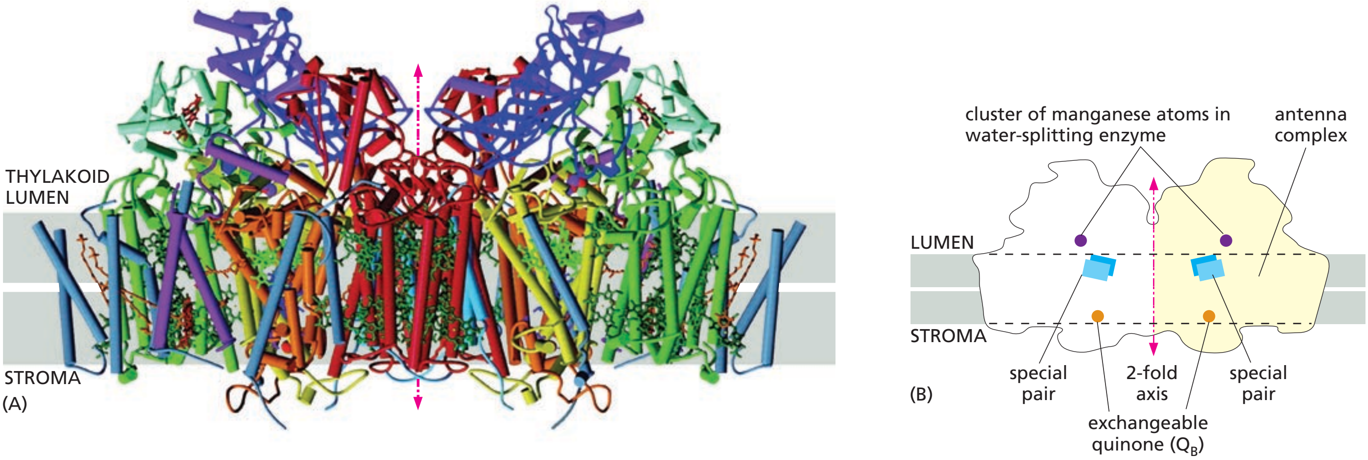
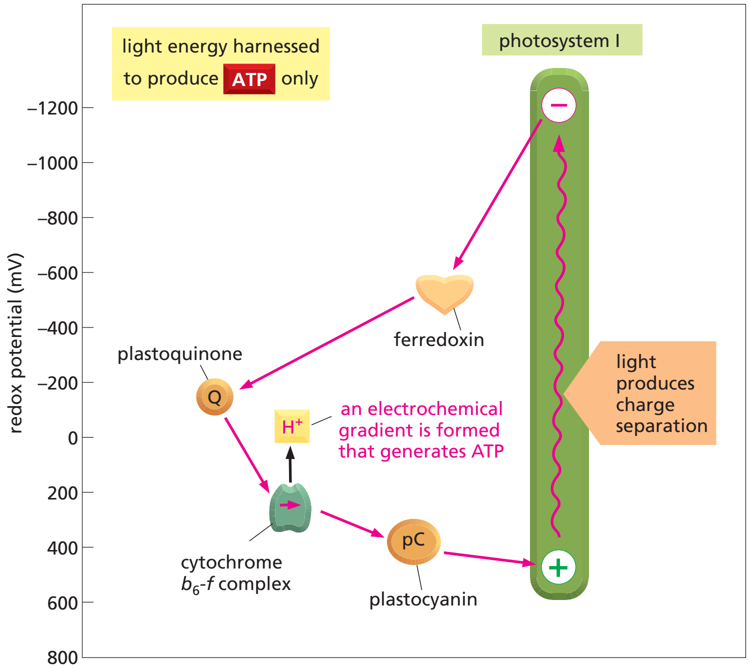


Figure 14–34 The complete three-dimensional structures of photosystems I and II are known. This diagram shows the structure of photosystem II, which is a dimeric complex that contains more than 20 proteins and several dozen chlorophylls. (Adapted from K. N. Ferreira et al., *Science* 303:1831–1838, 2004. With permission from the AAAS.)

Figure 14–35 Chloroplasts can also produce ATP by cyclic photophosphorylation. This pathway allows ATP to be made without producing either NADPH or O_2. When NADPH accumulates, the cell tends to favor this cyclic scheme.



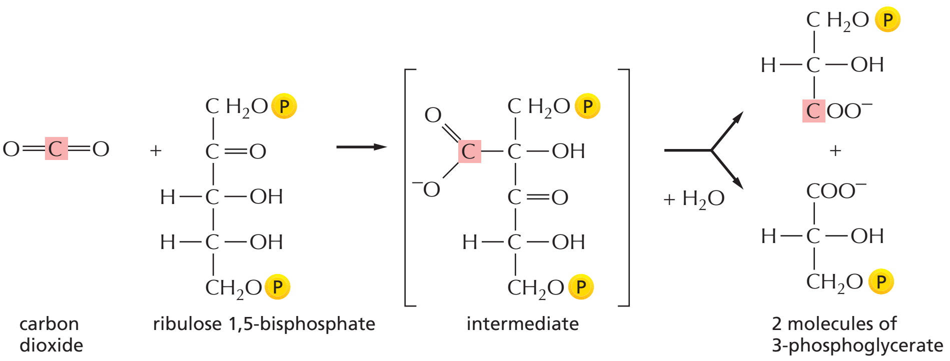
(which involves both photosystems I and II) depending on their relative need for reducing power (in the form of NADPH) and high-energy phosphate bonds (in the form of ATP).

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. 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 reducing power and energy for the rest of the cell, ATP and NADPH are instead used within the chloroplast stroma to produce sugars that can then be directly exported. This sugar production, which occurs during the dark reactions of photosynthesis, is called **carbon fixation**.

The central reaction of photosynthetic carbon fixation, in which an atom of inorganic carbon (as CO_2) is converted to organic carbon, is illustrated in Figure 14–36. CO_2 from the atmosphere combines with the five-carbon sugar derivative *ribulose 1,5-bisphosphate* plus water to give 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* (also called *ribulose bisphosphate carboxylase/oxygenase* or *Rubisco*). Because this enzyme works extremely sluggishly compared with most other enzymes (processing about three molecules of substrate per second

Figure 14–36 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. As shown, the product is two molecules of 3-phosphoglycerate.



compared with 1000 molecules per second for a typical enzyme), very large numbers of enzyme molecules are needed by the plant. Ribulose bisphosphate carboxylase often represents more than 50% of the total chloroplast protein, and it is widely claimed to be the most abundant protein on Earth.

When carbohydrates are broken down and oxidized to CO_2 and H_2O by cells, a large amount of free energy is released. Clearly, the reverse overall reaction—in which CO_2 and H_2O combine to make carbohydrate during photosynthesis—must be energetically very unfavorable. For this process to occur, it must be coupled to an energetically favorable reaction that drives it. The reaction in which CO_2 is fixed by Rubisco is in fact energetically favorable, but only because it receives a continuous supply of the energy-rich compound ribulose 1,5-bisphosphate, to which each molecule of CO_2 is added (see Figure 14–36). The energy and reducing power required for the elaborate metabolic pathway by which this compound is regenerated is provided by the ATP and NADPH produced by the photosynthetic light reactions.

The series of reactions that allows cells to incorporate CO_2 into sugars forms a cycle that begins and ends with ribulose 1,5-bisphosphate (Figure 14–37). For every three molecules of CO_2 that enter the cycle, one new molecule of *glyceraldehyde 3-phosphate* is produced—the three-carbon sugar that is the net product—and three molecules of ATP and two molecules of NADPH are consumed. Glyceraldehyde 3-phosphate then provides the starting material for the synthesis of many other sugars and organic molecules. The *carbon-fixation cycle* (or Calvin cycle) was worked out in the 1940s and 50s in one of the first successful applications of radioisotopes as tracers in biochemistry.

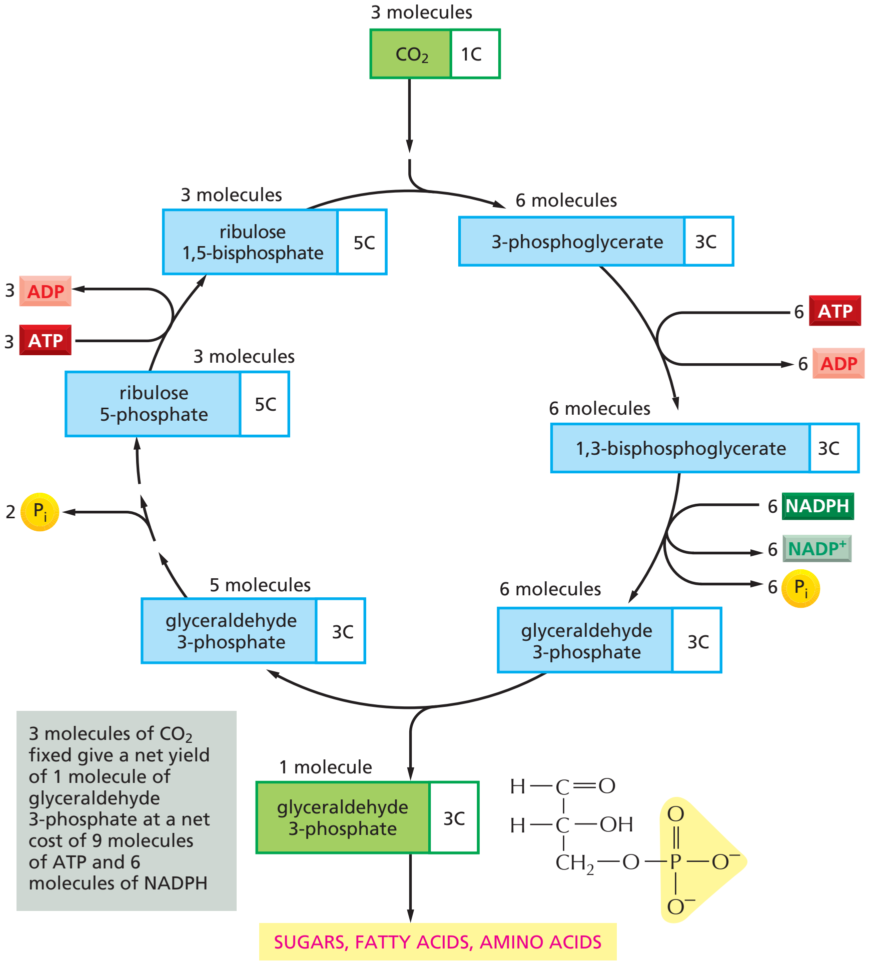


Figure 14–37 The carbon-fixation cycle forms organic molecules from CO_2 and H_2O. The cycle begins with the reaction shown in Figure 14–36, and it produces glyceraldehyde 3-phosphate. The number of carbon atoms in each type of molecule is indicated in the *white box*. There are many intermediates between 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.

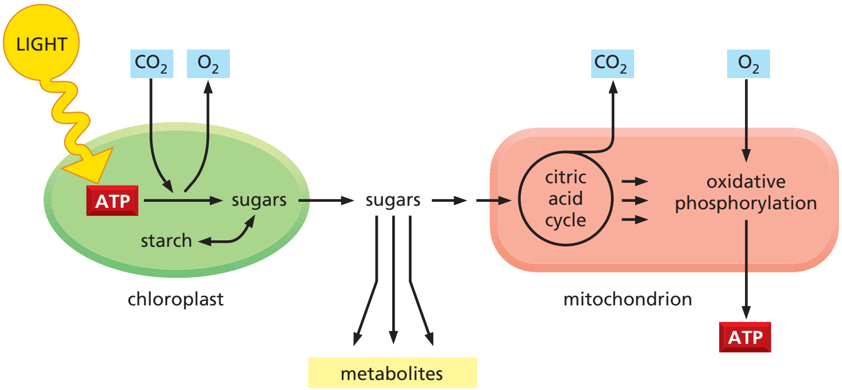


Figure 14–38 **In plants, the chloroplasts and mitochondria collaborate to supply cells with metabolites and ATP.** The chloroplast's inner membrane is impermeable to the ATP and NADPH that are produced during the light reactions of photosynthesis. These molecules are therefore funneled into the carbon-fixation cycle, where they are used to make sugars. The resulting sugars are either stored within the chloroplast—in the form of starch—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.

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 can be used in a number of different ways, depending on the needs of the plant. During periods of excess photosynthetic activity, glyceraldehyde 3-phosphate is retained in the chloroplast, where it is mainly converted to *starch* in the stroma (Figure 14–38). Like glycogen in animal cells, starch is a large polymer of glucose that serves as a carbohydrate reserve. Starch is stored as large grains in the chloroplast stroma (see Figure 14–25B), and at night, it is broken down to sugars to help support the metabolic needs of the plant. Starch forms an important part of the diet of all animals that eat plants.

But the chloroplast is not merely a storage depot. Much of the glyceraldehyde 3-phosphate produced in chloroplasts is moved out of the chloroplast into the cytosol. Some of it enters the glycolytic pathway (see Figure 13–5), where it is converted to pyruvate; this pyruvate then enters the citric acid cycle in the plant cell mitochondria and leads to the production of ATP by oxidative phosphorylation (see Figure 14–38). This is the ATP the plant uses for its general metabolism, and it is synthesized in mitochondria in the same way as in animal cells and other nonphotosynthetic organisms.

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

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?

THE ORIGINS OF CHLOROPLASTS AND MITOCHONDRIA

It is now widely accepted that chloroplasts and mitochondria most likely evolved from bacteria that were engulfed by ancestral eucaryotic cells more than a billion years ago (see Figures 1–19 and 1–21). As a relic of this evolutionary past, both types of organelles contain their own genomes, as well as their own biosynthetic machinery for making RNA and organelle proteins. The way that mitochondria and chloroplasts reproduce—through the growth and division of preexisting organelles—provides additional evidence of their bacterial ancestry (Figure 14–39).

The growth and proliferation of mitochondria and chloroplasts is complicated by the fact that their component proteins are encoded by two separate genetic systems—one in the organelle and one in the cell nucleus.

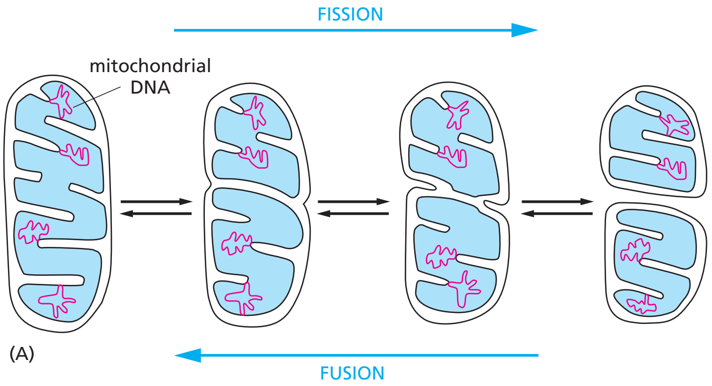


Figure 14–39 **A mitochondrion divides like a bacterium.** (A) Both mitochondrial fission and mitochondrial fusion are observed to occur. The fission process is conceptually similar to bacterial division processes. (B) An electron micrograph of a dividing mitochondrion in a liver cell. (B, Courtesy of Daniel S. Friend.)

In the case of the mitochondrion, most of the original bacterial genes have become transposed to the cell nucleus, leaving only relatively few genes inside the organelle itself. Animal mitochondria in fact contain a uniquely simple genetic system: the human mitochondrial genome, for example, contains only 16,569 nucleotide pairs of DNA encoding 37 genes. The vast majority of mitochondrial proteins—including those needed to make the mitochondrion's RNA polymerase and ribosomal proteins, and all of the enzymes of its citric acid cycle—are instead produced from nuclear genes, and these proteins must therefore be imported into the mitochondria from the cytosol, where they are made (discussed in Chapter 15).

Like the mitochondrion, the chloroplast contains many of its own genes, as well as a complete transcription and translation system for producing proteins from these genes. Chloroplast genomes are considerably larger than mitochondrial genomes: in higher plants, for example, the chloroplast genome contains about 120 genes in 120,000 nucleotide pairs. These genes are strikingly similar to the genes of cyanobacteria, the photosynthetic bacteria from which chloroplasts are thought to have been derived. Even so, many chloroplast proteins are now encoded by nuclear genes and must be imported from the cytosol.

The same techniques that have allowed us to analyze the genomes of mitochondria and chloroplasts have also permitted us to identify and explore the molecular biology of many microorganisms on the Earth. Some of these organisms thrive in the most inhospitable habitats on the planet. These include sulfurous hot springs or hydrothermal vents deep on the ocean floor. In these seemingly odd, modern microbes, we can readily find clues to life's history—in the form of the many molecules from which they are made. Like the fingerprints left at the scene of a crime, these molecules provide powerful evidence that allows us to trace the history of ancient events, permitting speculations on the origin of the ATP-generating systems that are found in today's mitochondria and chloroplasts. We therefore end this chapter with a discussion of the evolution of the energy-harvesting systems that we have discussed in detail previously in this chapter.

Oxidative Phosphorylation Might Have Given Ancient Bacteria an Evolutionary Advantage

As we mentioned earlier, the first living cells on Earth—both procaryotes and primitive eucaryotes—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 13–4A).

Such an excretion of organic acids would have lowered the pH of the environment, favoring the survival of cells that evolved transmembrane proteins that could pump H^+ out of the cytosol, keeping the cell from becoming too acidified (stage 1 in Figure 14–40). One of these pumps may have used the energy available from ATP hydrolysis to eject H^+ from the cell; such a protein pump could have been the ancestor of the present-day ATP synthase.

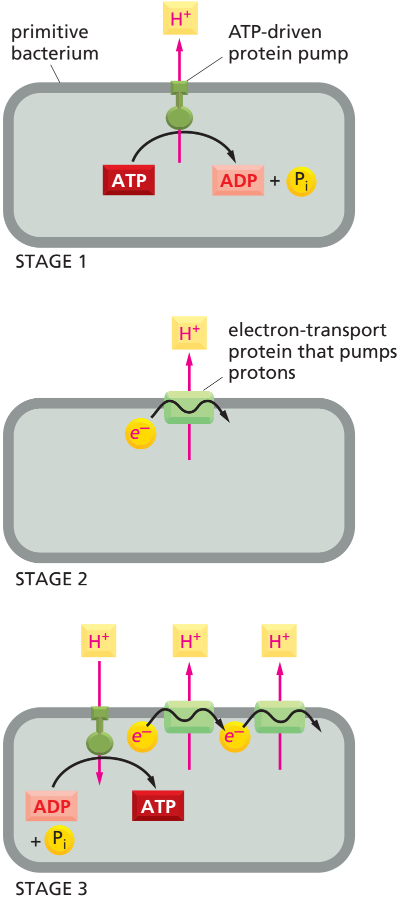


Figure 14–40 **Oxidative phosphorylation might have evolved in stages.** The first stage could have involved evolution of an ATPase that pumped protons out of the cell using the energy of ATP hydrolysis; stage 2 could have involved evolution of a different proton-pump driven by an electron-transport chain; stage 3 would have been the linking of 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 only one of the systems or none.

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. Selective pressures such as the scarcity of nutrients might therefore have led to the evolution of electron-transport proteins; these proteins allowed cells to use the movement of electrons between molecules of different redox potentials as an energy source for transporting H^+ across the plasma membrane (stage 2 in Figure 14–40). 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 the 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. These cells most likely generated large electrochemical proton gradients, 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 14–40). Because such cells required 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 the 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 14–41).

The next step, which is thought to have occurred with the rise of cyanobacteria (see Figure 14–24), was the evolution of organisms capable of using water as the electron source for photosynthesis. This entailed the evolution of a water-splitting enzyme and the addition of a second photosystem, acting in tandem with the first, to bridge the enormous gap in redox potential between H_2O and NADPH (see Figure 14–33). The biological consequences of this evolutionary step were far-reaching. For the first time, there were organisms that made only very minimal chemical demands on their environment. These cells could spread and evolve in ways denied to the earlier photosynthetic bacteria, which needed H_2S or organic acids as a source of electrons. Consequently, large amounts

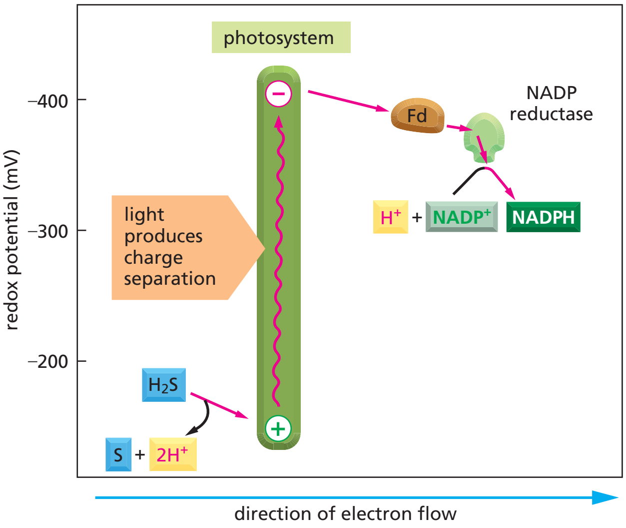


Figure 14–41 **Photosynthesis in green sulfur bacteria uses hydrogen sulfide (H_2S) as an electron donor rather than water.** Electrons are easier to extract from H_2S than from H_2O, because H_2S has a much higher redox potential (see Figure 14–33). Therefore, only one photosystem is needed to produce NADPH, and elemental sulfur is formed as a byproduct 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 acceptors 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.

of biologically synthesized, fermentable organic materials accumulated. Moreover, O_2 entered the atmosphere in large amounts (Figure 14–42).

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 by breaking 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 *E. coli*—lost their ability to survive on light energy alone and came to rely entirely on cellular respiration. Mitochondria probably arose when a primitive eucaryotic cell engulfed such a respiration-dependent bacterium. And plants arose somewhat later, when a descendant of this early aerobic eucaryote captured a photosynthetic bacterium, which became the precursor of the chloroplast. Once eucaryotes had acquired the bacterial

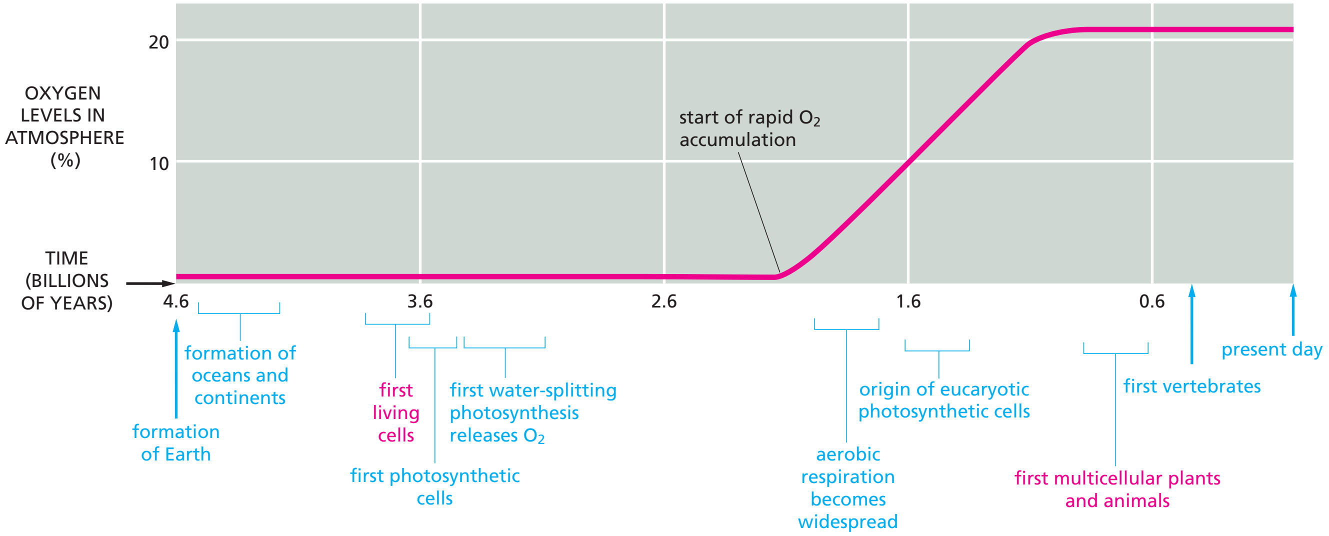


Figure 14–42 **Life on Earth has evolved over billions of years.** With the evolution of the membrane-based process of photosynthesis more than 3 billion years ago, organisms were no longer dependent on preformed organic chemicals. They could now make their own organic molecules from CO_2 gas. The delay 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 be due to the initial reaction of the oxygen with abundant ferrous iron (Fe^{2+}) dissolved in the early oceans. Only when the iron was used up would oxygen have started to accumulate in the atmosphere. In response to the rising amount of oxygen in the atmosphere, nonphotosynthetic oxygen-using organisms appeared, and the concentration of oxygen in the atmosphere eventually leveled out.

symbionts that became mitochondria and chloroplasts, they could then embark on the amazing 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 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 high temperatures (75°C to 95°C, close to the temperature 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 eucaryotes—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 entirely on inorganic nutrients in the complete absence of light and gaseous oxygen, utilizing as nutrients hydrogen gas (H_2), CO_2, and nitrogen gas (N_2) that bubble up from the vent. Its mode of existence gives us a hint of how early cells might have used electron transport to derive their energy and their 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 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 the bacterium needs to convert CO_2 into sugars. Much of the energy required 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 and giving the organism its name; Figure 14–43). Part of this electron transfer occurs in the 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 as primitive as *Methanococcus* suggests that the storage of energy derived from electron transport in an H^+ gradient is an extremely ancient process. Thus, chemiosmotic coupling is likely to have fueled the evolution of nearly all life forms on Earth.

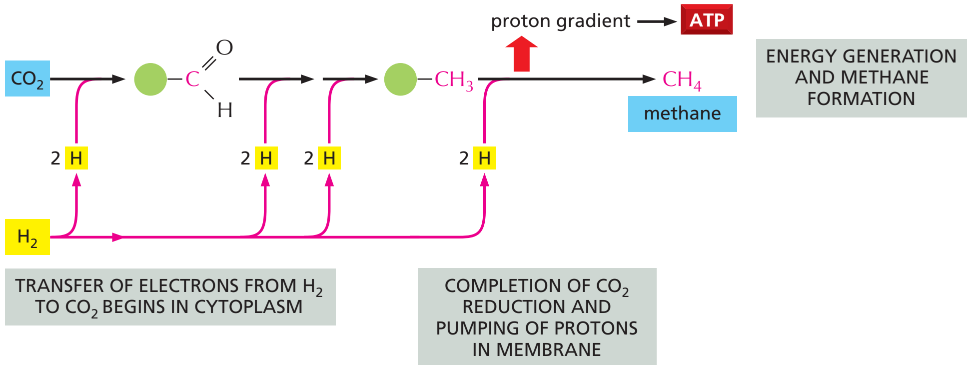


Figure 14–43 ***Methanococcus* uses chemiosmotic coupling to generate energy.** This deep-sea archean uses hydrogen gas (H_2) as the source of reducing power for energy generation. The initial reduction steps take place via enzyme-catalyzed reactions in the cytoplasm. In contrast, the final reduction step involves a membrane-based electron transfer that generates a proton gradient that drives ATP synthesis, while producing methane as a waste product. The *green* circles represent special coenzymes to which the metabolic intermediates are bound.

ESSENTIAL CONCEPTS

- Mitochondria, chloroplasts, and many bacteria produce ATP by a membrane-based mechanism known as chemiosmotic coupling.
- 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, a compartment which contains many enzymes, including those of the citric acid cycle. These enzymes produce 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—the respiratory chain—eventually combining with molecular oxygen (O_2) in an energetically favorable reaction.
- Much of the energy released by electron transfers along the respiratory chain is harnessed to pump H^+ out of the matrix, thereby creating a transmembrane proton (H^+) gradient. The proton pumping is carried out by three large respiratory enzyme complexes embedded in the membrane.
- The resulting electrochemical proton gradient across the inner mitochondrial membrane is harnessed to make ATP when H^+ ions flow back into the matrix through ATP synthase, an enzyme located in the inner mitochondrial membrane.
- The proton gradient also drives the active transport of metabolites into and out of the mitochondrion.
- In photosynthesis in chloroplasts and photosynthetic bacteria, high-energy electrons are generated when sunlight is absorbed by chlorophyll; this energy is captured by protein complexes known as photosystems, which in plant cells are located in the thylakoid membranes of chloroplasts.
- Electron-transport chains associated with photosystems transfer electrons from water to $NADP^+$ to form NADPH. O_2 is generated as a by-product.
- The electron-transport chains in chloroplasts also generate a proton gradient across the thylakoid membrane. As in mitochondria, this electrochemical proton gradient 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 to drive the carbon-fixation cycle in the chloroplast stroma, thereby producing carbohydrate from CO_2.
- Carbohydrate is exported to the cell cytosol, where it is metabolized to provide organic carbon, ATP (mostly via mitochondria), and reducing power for the rest of the cell.
- Both mitochondria and chloroplasts are thought to have evolved from bacteria that were endocytosed by primitive eucaryotic cells. Each retains its own genome and divides by processes that resemble a bacterial cell division.
- Chemiosmotic coupling mechanisms are widespread and 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
chemiosmotic coupling
chlorophyll
chloroplast
cyclic photophosphorylation
cytochrome
electron-transport chain
iron–sulfur center
matrix
mitochondrion
nitrogen fixation
oxidative phosphorylation
photosynthesis
photosystem
quinone
reaction center
redox pair
redox potential
redox reaction
stroma

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 with a much lower affinity for electrons.

C. Because it requires the absorption of four photons to release one O_2 molecule from two H_2O molecules, the water-splitting enzyme 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. 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. 91–98.) 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 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 b-c_1 occurs on the intermembrane space side of the membrane (see Figures 14–9 and 14–19). 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

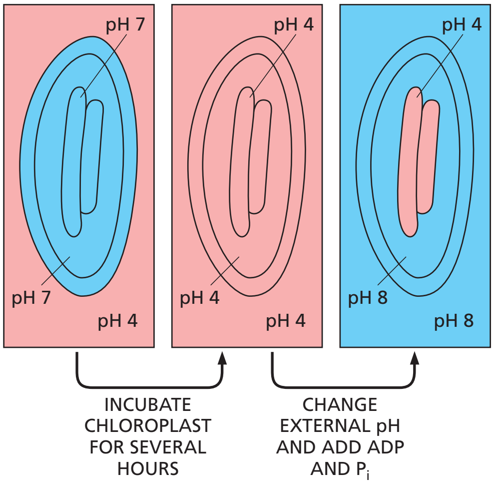


Figure Q14–17

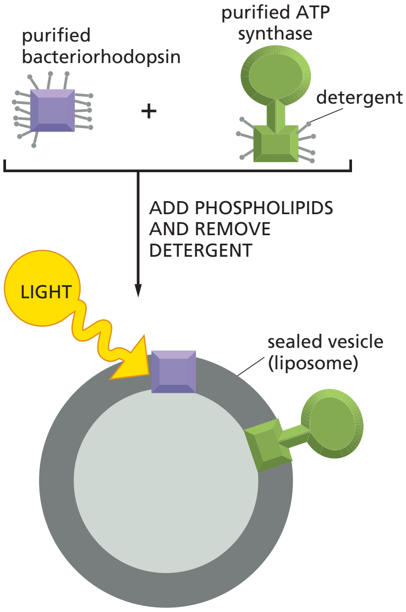


Figure Q14–18

(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?

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.

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

$$\text{succinate} + \text{FAD} \rightarrow \text{fumarate} + \text{FADH}_2$$

and

$$\text{FADH}_2 \rightarrow \text{FAD} + 2\text{H}^+ + 2e^-$$

The redox potential of $FADH_2$, however, is only –220 mV. Referring to Panel 14–1 (p.) and Figure 14–20, 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:

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?

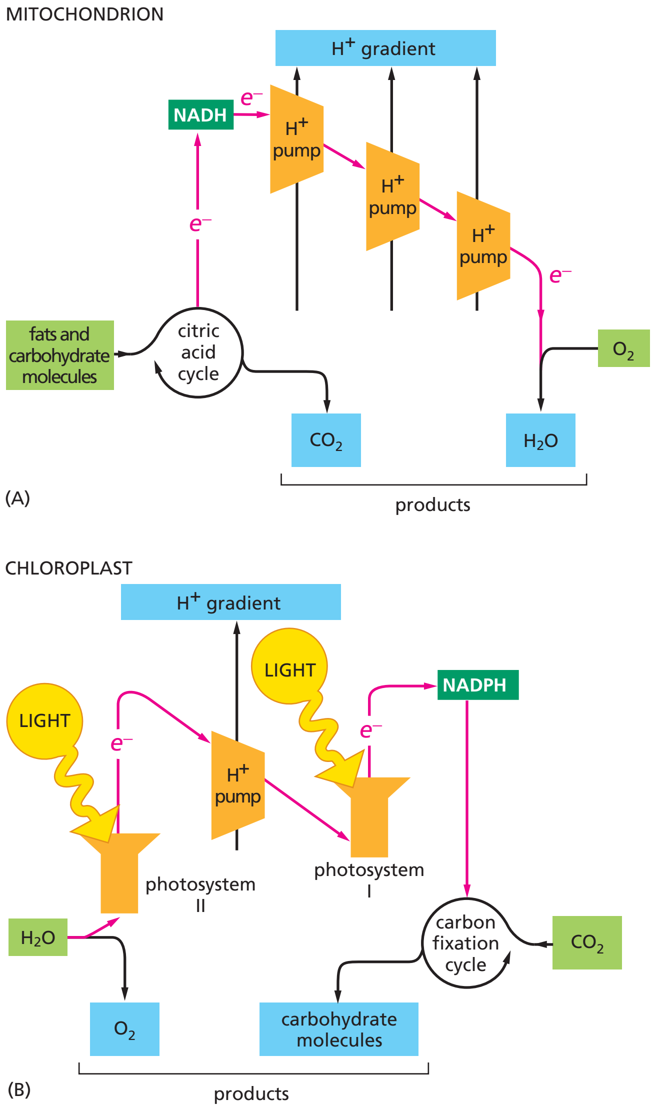


Figure Q14–21

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 14–9, 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 *b*-c_1 complex

F. oxidized ubiquinone, oxidized cytochrome *c*, and cytochrome *b*-c_1 complex

G. reduced ubiquinone, reduced cytochrome *c*, and cytochrome *b*-c_1 complex

H. oxidized ubiquinone, reduced cytochrome *c*, and cytochrome *b*-c_1 complex

ANSWER 14–1

By making membranes permeable to protons, DNP collapses—or at very small concentrations diminishes—the proton gradient across the inner mitochondrial membrane. Cells continue to oxidize food molecules to feed high-energy electrons into the electron-transport chain, but H^+ ions pumped across the membrane flow back into the mitochondria in a futile cycle. As a result, the energy of the electrons cannot be tapped to drive ATP synthesis, and instead is released as heat. Patients who have been given small doses of DNP lose weight because their fat reserves are used more rapidly to feed the electron-transport chain, and the whole process simply "wastes" energy.

A similar mechanism of heat production is used by a specialized tissue composed of brown fat cells, which is abundant in newborn humans and in hibernating animals. These cells are packed with mitochondria that leak part of their H^+ gradient futilely back across the membrane for the sole purpose of warming up the organism. These cells are brown because they are packed with mitochondria, which contain high concentrations of pigmented proteins, such as cytochromes.

ANSWER 14–2

The inner mitochondrial membrane is the site of oxidative phosphorylation, and it produces most of the cell's ATP. Cristae are portions of the mitochondrial inner membrane that are folded inward. Mitochondria that have a higher density of cristae have a larger area of inner membrane and therefore a greater capacity to carry out oxidative phosphorylation. Heart muscle expends a lot of energy during its continuous contractions, whereas skin cells have a lesser energy demand. An increased density of cristae therefore increases the ATP-production capacity of the heart muscle cell. This is a remarkable example of how cells adjust the abundance of their individual components according to need.

ANSWER 14–3

A. The DNP collapses the electrochemical proton gradient completely. H^+ ions that are pumped to one side of the membrane flow back freely, and therefore no energy can be stored across the membrane.

B. An electrochemical gradient is made up of two components: a concentration gradient and an electrical potential. If the membrane is made permeable to K^+ with nigericin, K^+ will be driven into the matrix by the electrical potential of the inner membrane (negative inside, positive outside). The influx of positively charged K^+ will abolish the membrane's electrical potential. In contrast, the concentration component of the H^+ gradient (the pH difference) is unaffected by nigericin. Therefore, only part of the driving force that makes it energetically favorable for H^+ ions to flow back into the matrix is lost.

ANSWER 14–4

A. Such a turbine running in reverse is an electrically driven water pump, which is analogous to what the ATP synthase becomes when it uses the energy of ATP hydrolysis to pump protons against their electrochemical gradient across the inner mitochondrial membrane.

B. The ATP synthase should stall when the energy that it can draw from the proton gradient is just equal to the ΔG required to make ATP; at this equilibrium point there will be neither net ATP synthesis nor net ATP consumption.

C. As the cell uses up ATP, the ATP/ADP ratio in the matrix falls below the equilibrium point just described, and ATP synthase uses the energy stored in the proton gradient to synthesize ATP in order to restore the original ATP/ADP ratio. Conversely, when the electrochemical proton gradient drops below that at the equilibrium point, ATP synthase uses ATP in the matrix to restore this gradient.

ANSWER 14–5

An electron pair causes 10 H^+ to be pumped across the membrane when passing from NADH to O_2 through the three respiratory complexes. Four H^+ are needed to make each ATP: three for synthesis from ADP and one for ATP export to the cytosol. Therefore, 2.5 ATP molecules are synthesized from each NADH molecule.

ANSWER 14–6

One can describe four essential roles for the proteins in the process. First, the chemical environment provided by a protein's amino acid side chains sets the redox potential of each Fe ion such that electrons can be passed in a defined order from one component to the next, giving up their energy in small steps and becoming more firmly bound as they proceed. Second, the proteins position the Fe ions so that the electrons can move efficiently between them. Third, the proteins prevent electrons from skipping an intermediate step; thus, as we have learned for other enzymes (discussed in Chapter 4), they channel the electron flow along a defined path. Fourth, the proteins couple the movement of the electrons down their energy ladder to the pumping of protons across the membrane, thereby harnessing the energy that is released and storing it in a proton gradient that is then used for ATP production.

ANSWER 14–7

It would not be productive to use the same carrier in two steps. If ubiquinone, for example, could transfer electrons directly to the cytochrome oxidase, the cytochrome *b*-c_1 complex would often be skipped when electrons are collected from NADH dehydrogenase. Given the large difference in redox potential between ubiquinone and cytochrome oxidase, a large amount of energy would be released as heat and thus be wasted. Electron transfer directly between NADH dehydrogenase and cytochrome *c* would similarly allow the cytochrome *b*-c_1 complex to be bypassed.

ANSWER 14–8

Protons pumped across the inner mitochondrial membrane into the intermembrane space equilibrate with the cytosol, which functions as a huge H^+ sink. Both the mitochondrial matrix and the cytosol support many metabolic reactions that require a pH around neutrality. The H^+ concentration difference, ΔpH, that can be achieved between mitochondrial matrix and cytosol is therefore relatively small (less than one pH unit). Much of the energy stored in the mitochondrial electrochemical proton gradient is instead due to the electrical potential of the membrane (see Figure 14–10).

In contrast, chloroplasts have a smaller, dedicated compartment into which H^+ ions are pumped. Much higher concentration differences can be achieved (up to a thousandfold, or 3 pH units), and much of the energy stored in the thylakoid H^+ gradient is due to the H^+ concentration difference between the thylakoid space and the stroma.

ANSWER 14–9

NADH and NADPH differ by the presence of a single phosphate group. That phosphate gives NADPH a slightly different shape from NADH, which allows these molecules to be recognized by different enzymes, and thus to deliver their electrons to different destinations. Such a division of labor is useful because NADPH tends to be involved in biosynthetic reactions, where high-energy electrons are used to produce energy-rich biological molecules. NADH, on the other hand, is involved in reactions that oxidize energy-rich food molecules to produce ATP. Inside the cell the ratio of NAD^+ to NADH is kept high, whereas the ratio of $NADP^+$ to NADPH is kept low. This provides plenty of NAD^+ to act as an oxidizing agent and plenty of NADPH to act as a reducing agent—as required for their special roles in catabolism and anabolism, respectively.

ANSWER 14–10

A. Photosynthesis produces sugars, most importantly sucrose, that are transported from the photosynthetic cells through the sap to root cells. There, the sugars are oxidized by glycolysis in the root cell cytoplasm and by oxidative phosphorylation in the root cell mitochondria to produce ATP, as well as being used as the building blocks for many other metabolites.

B. Mitochondria are required even during daylight hours in chloroplast-containing cells to supply the cell with ATP derived by oxidative phosphorylation. Glyceraldehyde 3-phosphate made by photosynthesis in chloroplasts moves to the cytosol and is eventually used as a source of energy to drive ATP production in mitochondria.

ANSWER 14–11

All statements are correct.

A. This is a necessary condition. If it were not true, electrons could not be removed from water and the reaction that splits water molecules ($H_2O \rightarrow 2H^+ + ½O_2 + 2e^-$) would not occur.

B. This transfer allows the energy of the photon to be harnessed as energy that can be utilized in chemical conversions.

C. It can be argued that this is one of the most important obstacles that had to be overcome during the evolution of photosynthesis: partially reduced oxygen molecules, such as the superoxide radical O_2^-, are dangerously reactive and will attack and destroy almost any biologically active molecule. These intermediates, therefore, have to remain tightly bound to the metals in the active site of the enzyme until all four electrons have been removed from two water molecules. This requires the sequential capture of four photons by the same reaction center.

ANSWER 14–12

A. True. NAD^+ and quinones are examples of compounds that do not have metal ions but can participate in electron transfer.

B. False. The potential is due to protons (H^+) that are pumped across the membrane from the matrix to the intermembrane space. Electrons remain bound to electron carriers in the inner mitochondrial membrane.

C. True. Both components add to the driving force that makes it energetically favorable for H^+ to flow back into the matrix.

D. True. Both move rapidly in the plane of the membrane.

E. False. Not only do plants need mitochondria to make ATP in cells that do not have chloroplasts, such as root cells, but mitochondria make most of the cytosolic ATP in all plant cells.

F. True. Chlorophyll's physiological function requires it to absorb light; heme just happens to be a colored compound from which blood derives its red color.

G. False. Chlorophyll absorbs light and transfers energy in the form of an energized electron, whereas the iron in heme is a simple electron carrier.

H. False. Most of the dry weight of a tree comes from carbon derived from the CO_2 that has been fixed during photosynthesis.

ANSWER 14–13

It takes three protons. The precise value of the ΔG for ATP synthesis depends on the concentrations of ATP, ADP, and P_i (as described in Chapter 3). The higher the ratio of the concentration of ATP to ADP, the more energy it takes to make additional ATP. The lower value of 11 kcal/mole therefore applies to conditions where cells have expended a lot of energy and have therefore decreased the normal ATP/ADP ratio.

ANSWER 14–14

If no O_2 is available, all components of the mitochondrial electron-transport chain will accumulate in their *reduced* form. This is the case because electrons derived from NADH enter the chain but cannot be transferred to O_2. The electron-transport chain therefore stalls with all of its components in the reduced form. If O_2 is suddenly added again, the electron carriers in cytochrome oxidase will become *oxidized before* those in NADH dehydrogenase. This is true because, after O_2 addition, cytochrome oxidase will donate its electrons directly to O_2, thereby becoming oxidized. A wave of increasing oxidation then passes backward with time from cytochrome oxidase through the components of the electron-transport chain, as each component regains the opportunity to pass on its electrons to downstream components.

ANSWER 14–15

As oxidized ubiquinone becomes reduced, it picks up two electrons but also two protons from water (Figure 14–19). Upon oxidation, these protons are released. If reduction occurs on one side of the membrane and oxidation at the other side, a proton is pumped across the membrane for each electron transported. Electron transport by ubiquinone thereby contributes directly to the generation of the H^+ gradient.

ANSWER 14–16

Photosynthetic bacteria and plant cells use the electrons derived in the reaction $2H_2O \rightarrow 4e^- + 4H^+ + O_2$ to reduce $NADP^+$ to NADPH, which is then used to produce useful metabolites. If the electrons were used instead to produce H_2 in addition to O_2, the cells would lose any benefit they derive from carrying out the reaction, because the electrons could not take part in metabolically useful reactions.

ANSWER 14–17

A. The switch in solutions creates a pH gradient across the thylakoid membrane. The flow of H^+ ions down its electrochemical potential drives ATP synthase, which converts ADP to ATP.

B. No light is needed, because the H^+ gradient is established artificially without a need for the light-driven electron-transport chain.

C. Nothing. The H^+ gradient would be in the wrong direction; ATP synthase would not work.

D. The experiment provided early supporting evidence for the chemiosmotic model by showing that an H^+ gradient alone is sufficient to drive ATP synthesis.

ANSWER 14–18

A. When the vesicles are exposed to light, H^+ ions (derived from H_2O) pumped into the vesicles by the bacteriorhodopsin flow back out through the ATP synthase, causing ATP to be made in the solution surrounding the vesicles in response to light.

B. If the vesicles are leaky, no H^+ gradient can form and thus ATP synthase cannot work.

C. Using components from widely divergent organisms can be a very powerful experimental tool. Because the two proteins come from such different sources, it is very unlikely that they form a direct functional interaction. The experiment therefore strongly suggests that electron transport and ATP synthesis are separate events. This approach is therefore a valid one.

ANSWER 14–19

The redox potential of $FADH_2$ is too low to transfer electrons to the NADH dehydrogenase complex, but high enough to transfer electrons to ubiquinone (Figure 14–20). Therefore, electrons from $FADH_2$ can enter the electron-transport chain only at this step (Figure A14–19). Because the NADH dehydrogenase complex is bypassed, fewer H^+ ions are pumped across the membrane and less ATP is made. This example shows the versatility of the electron-transport chain. The ability to use vastly different sources of electrons from the environment to feed electron transport is thought to have been an essential feature in the early evolution of life.

ANSWER 14–20

If these bacteria used a proton gradient to make their ATP in a fashion analogous to other bacteria (that is, fewer protons inside than outside), they would need to raise their cytoplasmic pH even higher than that of their environment (pH 10). Cells with a cytoplasmic pH greater than 10 would not be viable. These bacteria, therefore, must use gradients of ions other than H^+, such as Na^+ gradients, in the chemiosmotic coupling between electron transport and an ATP synthase.

ANSWER 14–21

Statements A and B are accurate. Statement C is incorrect, because the chemical reactions that are carried out in each cycle are completely different, even though the net effect is the same as that expected for simple reversal.

ANSWER 14–22

This experiment would suggest a two-step model for ATP synthase function. According to this model, the flow of protons through the base of the synthase drives rotation of the head, which in turn causes ATP synthesis. In their experiment, the authors have succeeded in uncoupling these two steps. If rotating the head mechanically is sufficient to produce ATP in the absence of any applied proton gradient, the ATP synthase is a protein machine that indeed functions like a "molecular turbine." This would be a very exciting experiment, indeed, because it would directly demonstrate the relationship between mechanical movement and enzymatic activity. There is no doubt that it should be published and that it would become a "classic."

ANSWER 14–23

Only under condition (E) is electron transfer observed, with cytochrome *c* becoming reduced. A portion of the electron-transport chain has been reconstituted in this mixture, so that electrons can flow in the energetically favored direction from reduced ubiquinone to the cytochrome *b*-c_1 complex to cytochrome *c*. Although energetically favorable, the transfer in (A) cannot occur spontaneously in the absence of the cytochrome *b*-c_1 complex to catalyze this reaction. No electron flow occurs in the other experiments, whether the cytochrome *b*-c_1 complex is present or not: in experiments (B) and (F) both ubiquinone and cytochrome *c*

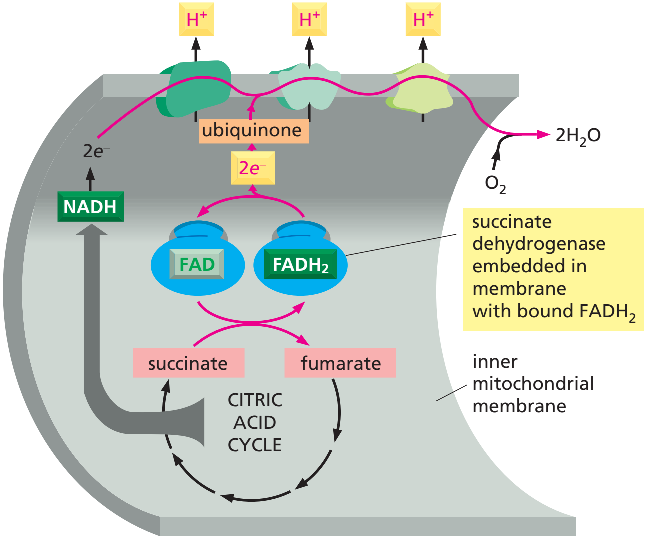


Figure A14–19

are oxidized; in experiments (C) and (G) both are reduced; and in experiments (D) and (H) electron flow is energetically disfavored because reduced cytochrome c has a lower free energy than oxidized ubiquinone.