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 traced to their quest for energy. Oxygen did not appear in the atmosphere until more than a billion years after the first cells appeared on Earth. It is therefore thought that the earliest cells may have produced ATP by breaking down organic molecules that had been generated by geochemical processes. Such fermentation reactions, discussed in Chapter 13, can occur in the cytosol of present-day cells, when they use the energy derived from the partial oxidation of energy-rich food molecules to form ATP.

But very early in the history of life, a much more efficient mechanism for generating energy and synthesizing ATP appeared—one based on the transport of electrons along membranes. This mechanism is so central to the survival of life on Earth that we devote this entire chapter to it. Membrane-based electron transport first appeared in bacteria more than 3 billion years ago, and the progeny of these pioneering cells currently crowd every crevice of our planet's land and oceans in a wild menagerie of living forms. Perhaps most remarkably, remnants of these energy-generating electron-transport systems can be found in the bacterial descendants that labor within living eukaryotic cells: chloroplasts and mitochondria.

In this chapter, we consider the molecular mechanisms that enable electron-transport systems to generate the energy that cells need to survive. We begin with a brief overview of the general principles central to the generation of energy in all living things: the use of a membrane to harness the energy of moving electrons. We describe how such processes

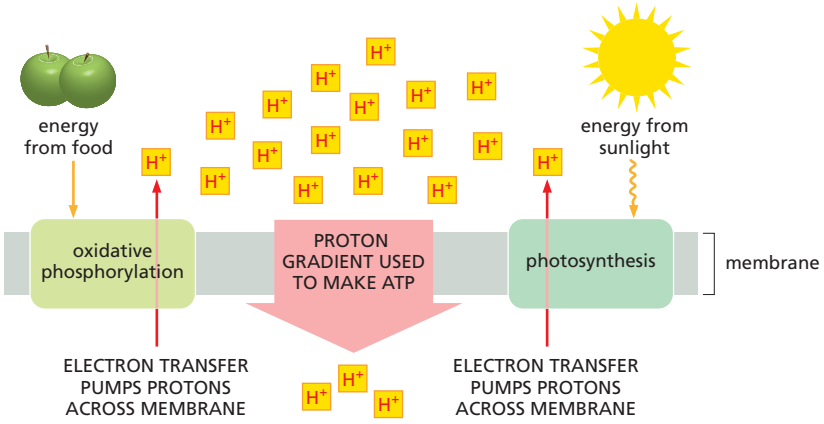


Figure 14–1 Membrane-based mechanisms use the energy provided by food or sunlight to generate ATP. In oxidative phosphorylation, which occurs in mitochondria, an electron-transport system uses energy derived from the oxidation of food to generate a proton (H^+) gradient across a membrane. In photosynthesis,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

operate in both mitochondria and chloroplasts, and we review the chemical principles that allow the transfer of electrons to release large amounts of energy. Finally, we trace the evolutionary pathways that most likely gave rise to these marvelous mechanisms.

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

The main chemical energy currency in cells is ATP (see Figure 3–31). Although small amounts of ATP are generated during glycolysis in the cell cytosol (discussed in Chapter 13), most of the ATP needed by cells is produced by *oxidative phosphorylation*. The generation of ATP by oxidative phosphorylation differs from the way ATP is produced during glycolysis, in that it requires a membrane-bound compartment. In eukaryotic cells, oxidative phosphorylation takes place in mitochondria, and it depends on an electron-transport process that drives the transport of protons (H^+) across the inner mitochondrial membrane. A related membrane-based process produces ATP during photosynthesis in plants, algae, and photosynthetic bacteria (**Figure 14–1**).

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

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

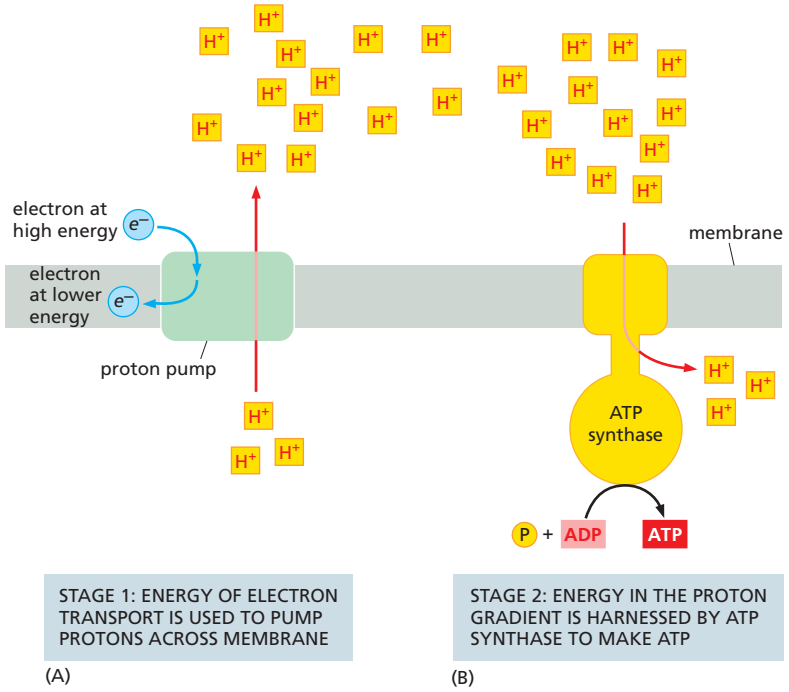


Figure 14–2 Membrane-based systems use the energy stored in an electrochemical proton gradient to synthesize ATP. The process occurs in two stages. (A) In the first stage, a proton pump harnesses the energy of electron transfer (described later in the chapter) to pump protons (H^+) across a membrane, creating a proton gradient. The *blue* arrow shows the direction of electron movement. These high-energy electrons can come from organic or inorganic molecules, or they can be produced by the action of light on special molecules such as chlorophyll. The protons are derived from water, which is ubiquitous in the aqueous environment of the cell. (B) The proton gradient produced in (A) serves as a versatile energy store that can be used to drive a variety of energy-requiring reactions in mitochondria, chloroplasts, and prokaryotes—most importantly, the synthesis of ATP by ATP synthase.

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

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

The membrane-based, chemiosmotic mechanism for making ATP arose very early in life's history, more than 3 billion years ago. The exact same type of ATP-generating processes occur in the plasma membrane of modern bacteria and archaea. The mechanism was so successful that its essential features have been retained in the long evolutionary journey from early prokaryotes to present-day cells.

The remarkable resemblance of the mechanism in prokaryotes and eukaryotes can be attributed in part to the fact that the organelles that produce ATP in eukaryotic cells—the chloroplasts and mitochondria—evolved from bacteria that were engulfed by ancestral cells more than a billion years ago (see Figures 1–19 and 1–21). As evidence of their bacterial ancestry, both chloroplasts and mitochondria reproduce in a manner

QUESTION 14–1

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

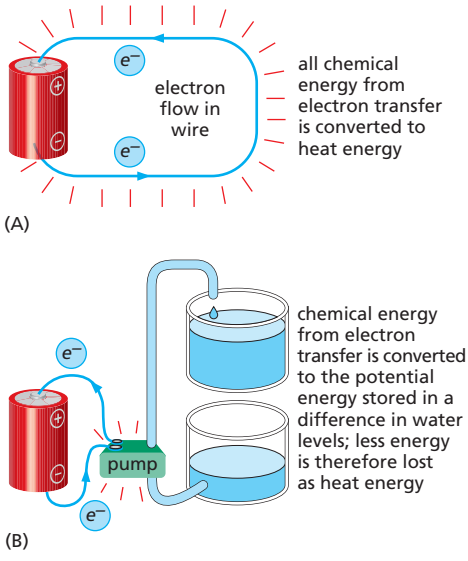
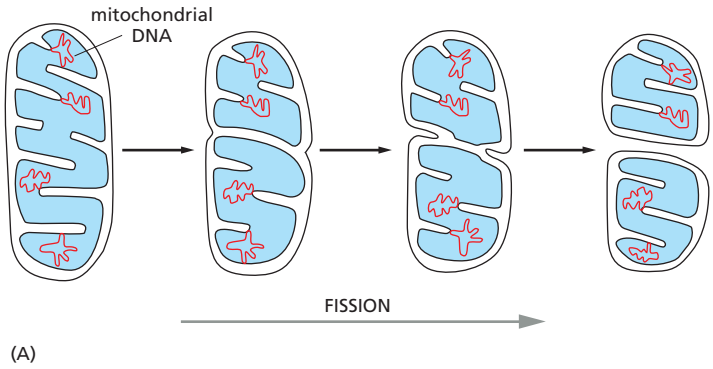


Figure 14–3 Batteries can use the energy of electron transfer to perform work. (A) If a battery's terminals are directly connected to each other, the energy released by electron transfer is all converted into heat. (B) If the battery is connected to a pump, much of the energy released by electron transfer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



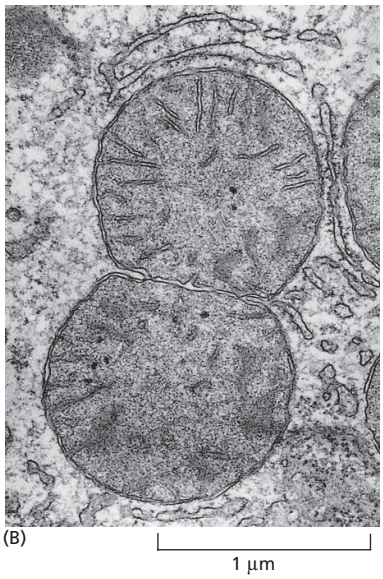


Figure 14–4 A mitochondrion can divide like a bacterium. (A) It undergoes a fission process that is conceptually similar to bacterial division. (B) An electron micrograph of a dividing mitochondrion in a liver cell. (B, courtesy of Daniel S. Friend, by permission of E.L. Bearer.)

similar to that of most prokaryotes (**Figure 14–4**). The organelles also harbor bacterial-like biosynthetic machinery for making RNA and proteins, and they possess DNA-based genomes (**Figure 14–5**). Not surprisingly, many chloroplast genes are strikingly similar to those of cyanobacteria—the photosynthetic bacteria from which these organelles were derived.

Although mitochondria and chloroplasts retain their own genomes, the bacteria from which they arose gave up many of the genes required for independent living as they developed their symbiotic relationships with eukaryotic animal and plant cells. Many of these jettisoned genes were not lost, however; they were relocated to the cell nucleus, where they continue to direct the production of proteins that mitochondria and chloroplasts import to carry out their specialized functions—including the generation of ATP.

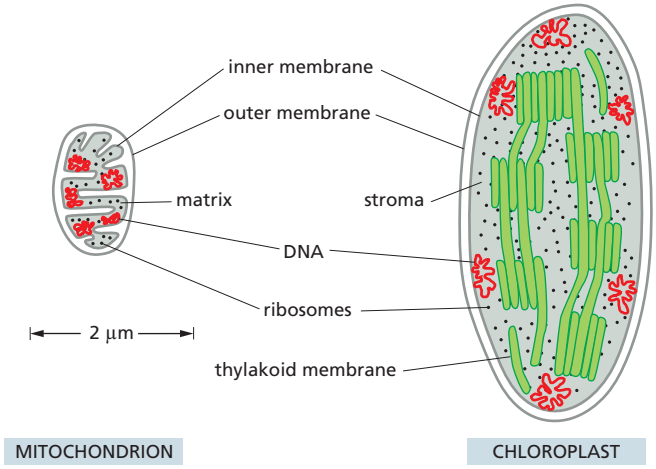


Figure 14–5 Mitochondria and chloroplasts share many of the features of their bacterial ancestors. Both organelles contain their own DNA-based genome and the machinery to replicate this DNA and to make RNA and protein. The inner compartments of these organelles—the mitochondrial matrix and the chloroplast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

MITOCHONDRIA AND OXIDATIVE PHOSPHORYLATION

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

The importance of mitochondria is further highlighted by the dire consequences of mitochondrial dysfunction. Defects in the proteins required for electron transport, for example, are responsible for an inherited disorder called *myoclonic epilepsy and ragged red fiber disease* (MERRF). Because muscle and nerve cells need large amounts of ATP to function normally, individuals with this condition typically experience muscle weakness, heart problems, epilepsy, and often dementia.

MERFF, like many of the disorders that affect mitochondrial function, stems from mutations that disable genes present in mitochondrial DNA (see Figure 14–5). Because mitochondria are passed down from mother to child (sperm mitochondria are lost after fertilization), such mutations are transmitted by the egg. To prevent the transmission of these life-threatening defects, reproductive biologists have developed methods for removing the nucleus from an egg that carries faulty mitochondria and transferring it to a donor egg that has healthy mitochondria. Although a baby boy produced using this form of *mitochondrial replacement therapy* was born in 2016, the approach remains controversial, in part because the effects of having genetic material from three "parents"—mother, father, and mitochondrial donor—are unknown.

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

Mitochondria Are Dynamic in Structure, Location, and Number

Isolated mitochondria are generally similar in appearance to their bacterial ancestors. Inside a cell, however, mitochondria are remarkably adaptable and can adjust their location, shape, and number to suit that particular cell's needs. In some cell types, mitochondria remain fixed in one location, where they supply ATP directly to a site of unusually high energy consumption. In a heart muscle cell, for example, mitochondria are located close to the contractile apparatus, whereas in a sperm they are wrapped tightly around the motile flagellum (**Figure 14–6**). In other cells, mitochondria fuse to form elongated, tubular networks, which are diffusely distributed through the cytoplasm (**Figure 14–7**). These networks are dynamic, continually breaking apart by fission (see Figure 14–4) and fusing again (**Movie 14.1** and **Movie 14.2**).

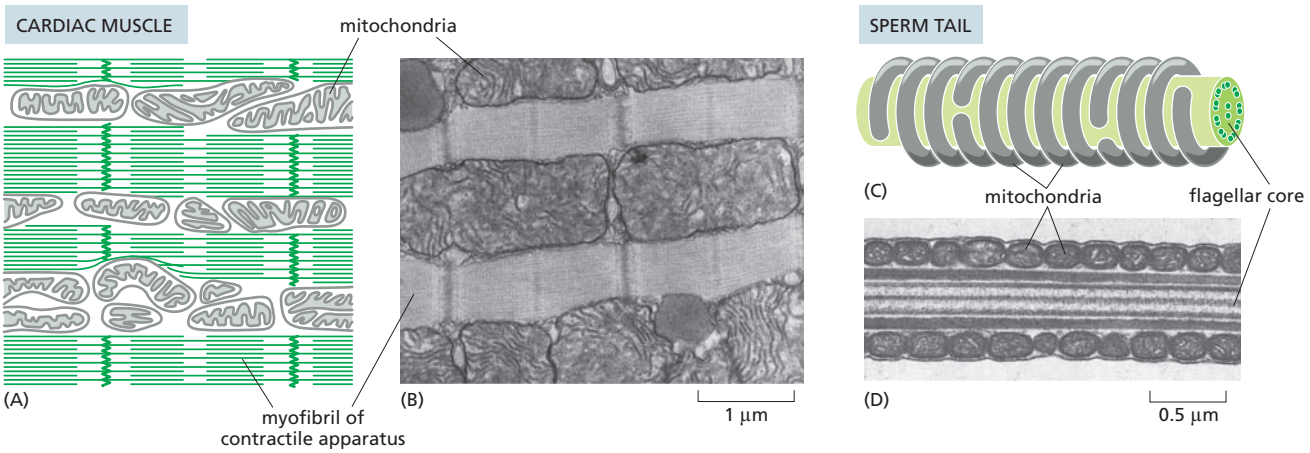


Figure 14–6 Mitochondria are located near sites of high ATP utilization. (A) In a cardiac muscle cell, mitochondria are located close to the contractile apparatus, where ATP hydrolysis provides the energy for contraction. The structure of the contractile apparatus is discussed in Chapter 17. (B) An electron micrograph of cardiac muscle shows a preponderance of mitochondria. (C) In a sperm, mitochondria are located in the tail, wrapped around a portion of the motile flagellum that requires ATP for its movement. The internal structure of the flagellar core is discussed in Chapter 17. (D) Micrograph showing a flagellum that has been thinly sliced to reveal the internal core structure as well as the surrounding mitochondria. (B, Keith Porter papers, Center for Biological Sciences Archives, University of Maryland, Baltimore Country; D, from W. Bloom and D.W. Fawcett, *A Textbook of Histology*, 10th ed. Philadelphia: W.B. Saunders Company, 1975. Reprinted with permission from the Estate of D.W. Fawcett.)

Mitochondria are present in large numbers—1000 to 2000 in a liver cell, for example. But their numbers vary depending on the cell type and can change with the energy needs of the cell. In skeletal muscle cells that have been repeatedly stimulated to contract, mitochondria can divide until their numbers increase five- to tenfold. Marathon runners, for example, may have twice the volume of mitochondria in their leg muscles than do individuals who are more sedentary.

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

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

An individual mitochondrion is bounded by two highly specialized membranes—one inside the other. These membranes, called the inner and outer mitochondrial membranes, create two mitochondrial

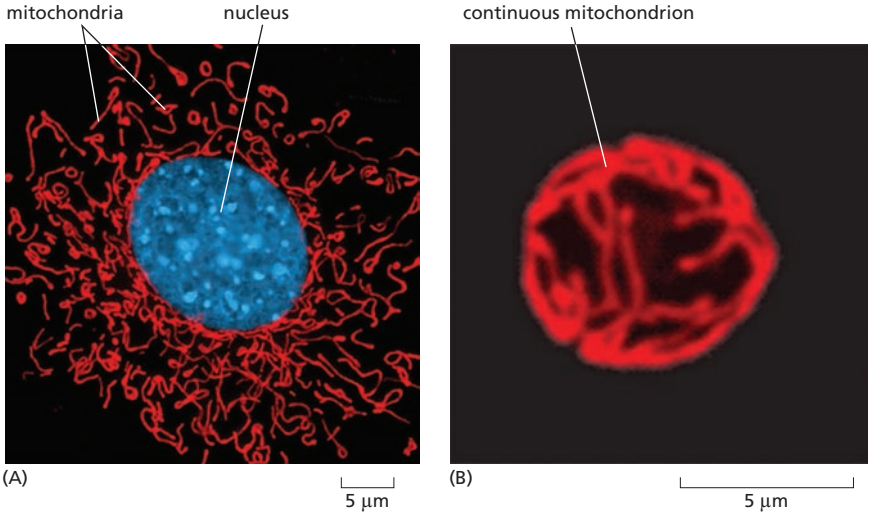


Figure 14–7 Mitochondria often form elongated, tubular networks, which can extend throughout the cytoplasm. (A) Mitochondria (*red*) are fluorescently labeled in this cultured mouse fibroblast. (B) In a yeast cell, the mitochondria (*red*) form a continuous network, tucked against the plasma membrane.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

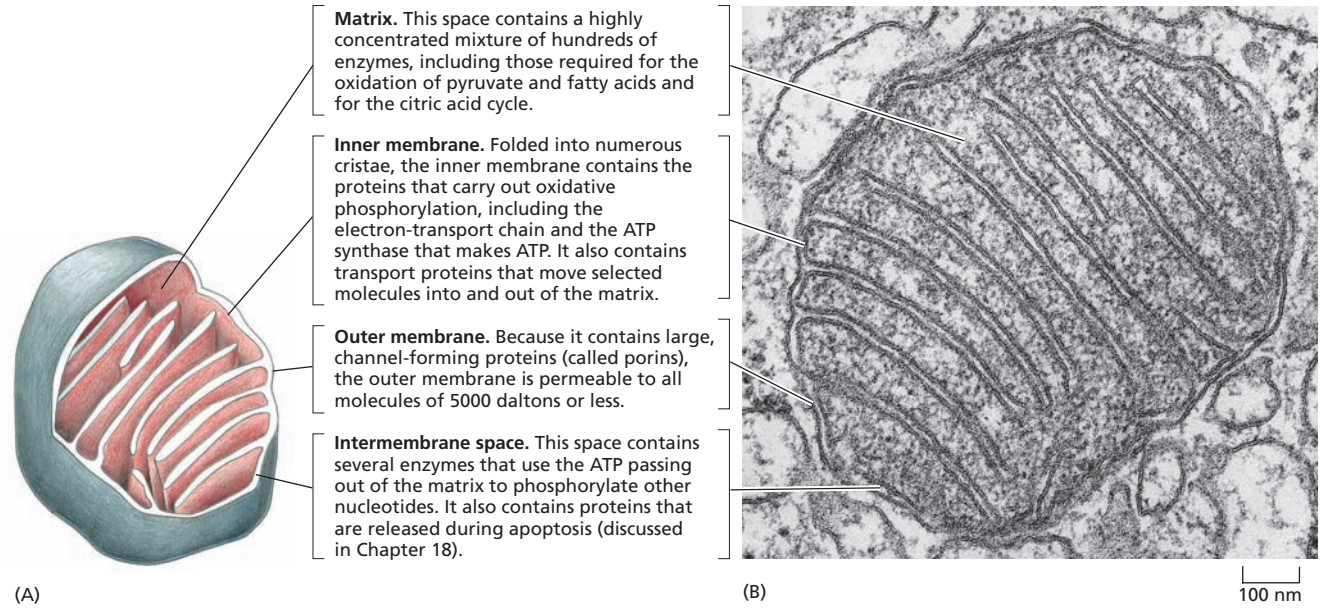


Figure 14–8 A mitochondrion is organized into four separate compartments. (A) A schematic drawing and (B) an electron micrograph of a mitochondrion. Each compartment contains a unique set of proteins, enabling it to perform its distinct functions. In liver mitochondria, an estimated 67% of the total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

compartments: a large internal space called the **matrix** and a much narrower *intermembrane space* (**Figure 14–8**). When purified mitochondria are gently fractionated into separate components and their contents analyzed (see Panel 4–3, pp. 164–165), each of the membranes, and the spaces they enclose, are found to contain a unique collection of proteins.

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

The inner mitochondrial membrane is the site of oxidative phosphorylation, and it is here that the proteins of the electron-transport chain and the ATP synthase required for ATP production are concentrated. This membrane is highly convoluted, forming a series of infoldings—known as *cristae*—that project into the matrix space (see Figure 14–8 and **Movie 14.3**). These folds greatly increase the surface area of the membrane. In a liver cell, the inner membranes of all the mitochondria make up about one-third of the total membranes of the cell.

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

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

QUESTION 14–2

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

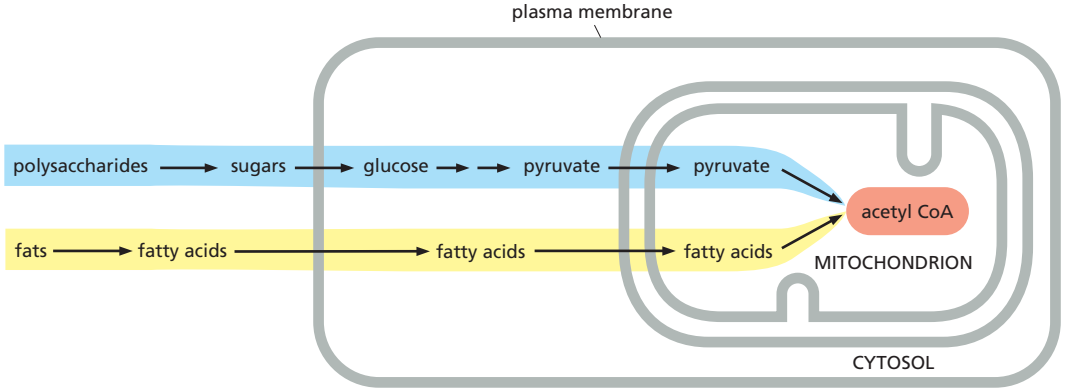


Figure 14–9 Acetyl CoA is produced in the mitochondria. In animal cells and other eukaryotes, pyruvate produced during glycolysis and fatty acids derived from the breakdown of fats enter the mitochondrion from the cytosol. Once inside the mitochondrial matrix, both of these food-derived molecules are converted to acetyl CoA and then oxidized to CO_2.

The citric acid cycle gets the fuel it needs to produce these activated carriers from food-derived molecules that make their way into mitochondria from the cytosol. Both the pyruvate produced by glycolysis and the fatty acids derived from the breakdown of fats (see Figure 13–3) can enter the mitochondrial intermembrane space through the porins in the outer mitochondrial membrane. These fuel molecules are then transported across the inner mitochondrial membrane into the matrix, where they are converted into the crucial metabolic intermediate, acetyl CoA (**Figure 14–9**). The acetyl groups in acetyl CoA are then oxidized to CO_2 via the citric acid cycle (see Figure 13–12). Some of the energy derived from this oxidation is saved in the form of high-energy electrons, held by the activated carriers NADH and $FADH_2$. These two activated carriers can then donate their electrons to the electron-transport chain in the inner mitochondrial membrane (**Figure 14–10**).

The Movement of Electrons Is Coupled to the Pumping of Protons

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

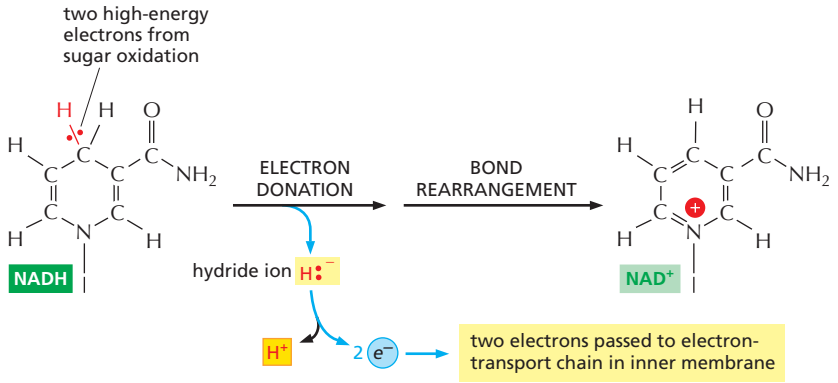


Figure 14–10 NADH donates its "high-energy" electrons to an electron-transport chain. A hydride ion (a hydrogen atom with two electrons, *red*) is removed from NADH and is converted into a proton and two electrons (*blue*). Only the part of NADH that carries these high-energy electrons is shown; for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

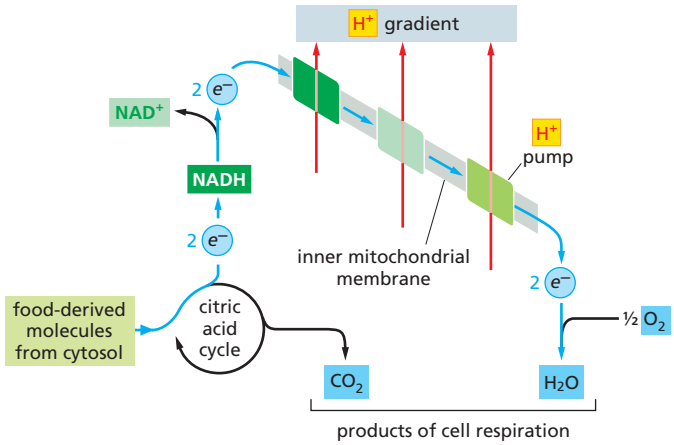


Figure 14–11 As electrons are transferred from activated carriers to oxygen, protons are pumped across the inner mitochondrial membrane. This is stage 1 of chemiosmotic coupling (see Figure 14–2). The path of electron flow is indicated by *blue* arrows. Only the pathway for NADH is shown here.

mechanism for ATP synthesis is called **oxidative phosphorylation** because it involves both the consumption of O_2 and the addition of a phosphate group to ADP to form ATP.

The source of the high-energy electrons that power the proton pumping differs widely between different organisms and different processes. During cell respiration—the energy-generating process that takes place in both mitochondria and aerobic bacteria—these electrons are ultimately derived from sugars or fats. In photosynthesis, the high-energy electrons come from the organic green pigment *chlorophyll*, which captures energy from sunlight. And many single-celled organisms (archaea and bacteria) use inorganic substances such as hydrogen, iron, and sulfur as the source of the high-energy electrons that they need to make ATP (see, for example, Figure 1–13).

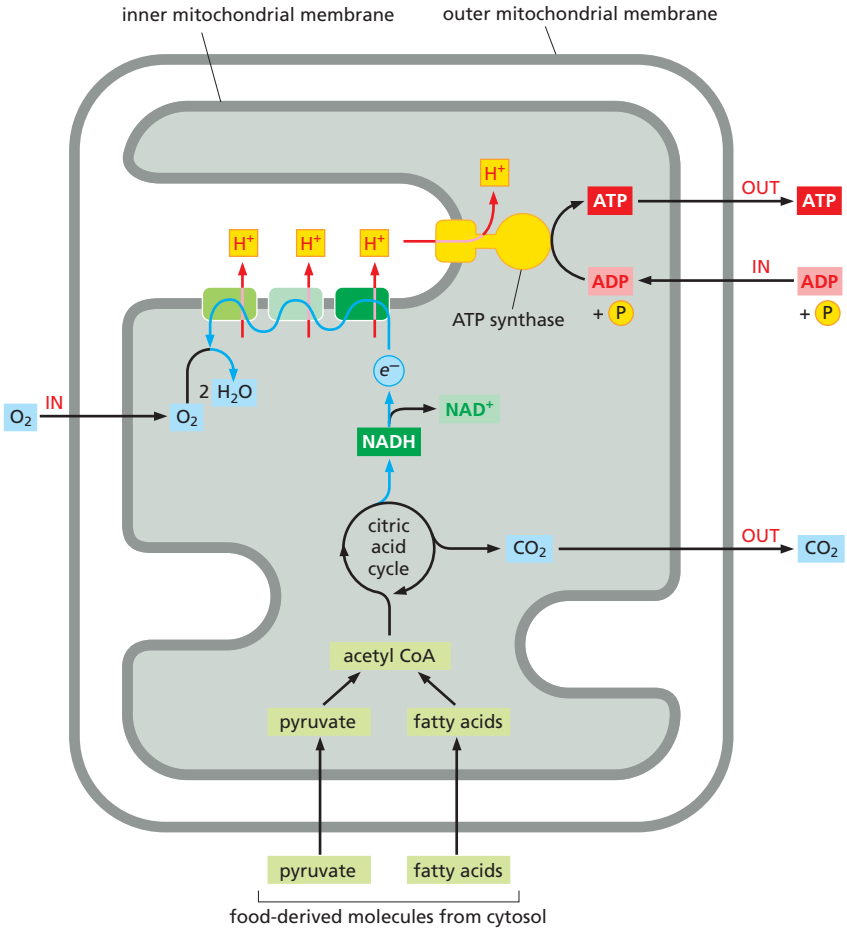


Figure 14–12 Activated carriers generated during the citric acid cycle power the production of ATP. Pyruvate and fatty acids enter the mitochondrial matrix (*bottom*), where they are converted to acetyl CoA. The acetyl CoA is then metabolized by the citric acid cycle, which produces NADH (and $FADH_2$,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

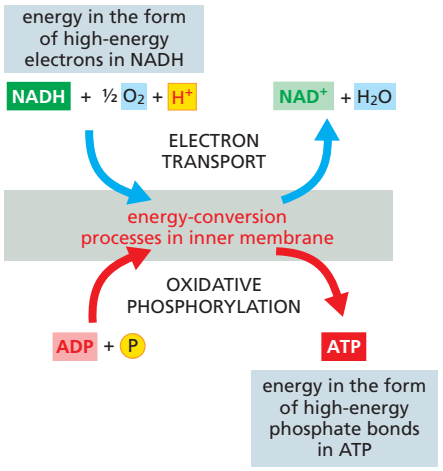


Figure 14–13 To produce ATP, mitochondria catalyze a major conversion of energy. In oxidative phosphorylation, the energy released by the oxidation of NADH to NAD^+ is harnessed—through energy-conversion processes in the inner mitochondrial membrane—to drive the energy-requiring phosphorylation of ADP to form ATP. The net equation for this process, in which four electrons pass from NADH to oxygen, is $2NADH + O_2 + 2H^+ \rightarrow 2NAD^+ + 2H_2O$. A smaller amount of ATP is similarly generated from energy released by the oxidation of $FADH_2$ to FAD (not shown).

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

Electrons Pass Through Three Large Enzyme Complexes in 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, grouped into three large **respiratory enzyme complexes**. These complexes each contain multiple individual proteins, including transmembrane proteins that anchor the complex firmly in the inner mitochondrial membrane.

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

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

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

Without a mechanism for harnessing the energy released by the energetically favorable transfer of electrons from NADH to O_2, this energy

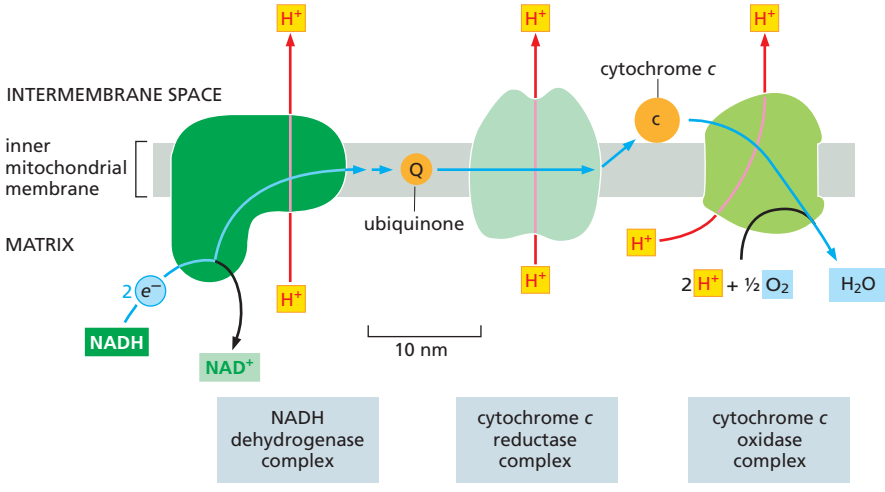


Figure 14–14 High-energy electrons are transferred through three respiratory enzyme complexes in the inner mitochondrial membrane. The relative size and shape of each complex are indicated, but the numerous individual protein components that form each complex are not. During the transfer of electrons from NADH to oxygen (*blue* lines), protons derived from water are pumped across the membrane from the matrix into the intermembrane space by each of the complexes (**Movie 14.4**). Ubiquinone (Q) and cytochrome *c* (c) serve as mobile carriers that ferry electrons from one complex to the next.

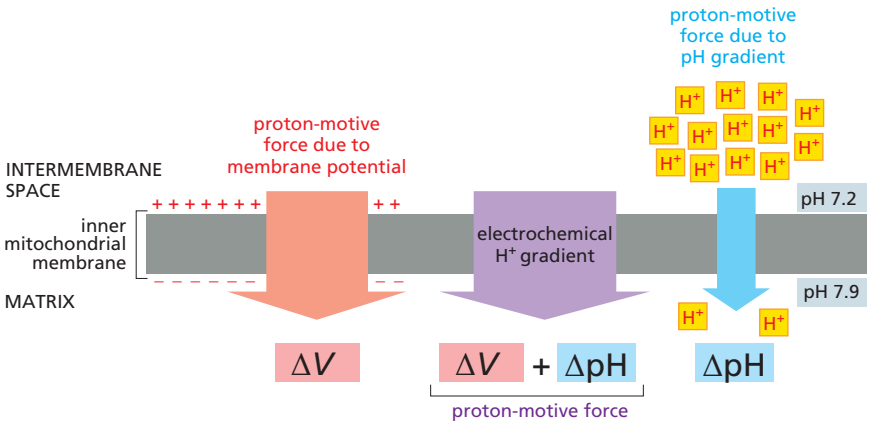


Figure 14–15 The electrochemical H^+ gradient across the inner mitochondrial membrane includes a large force due to the membrane potential (ΔV) and a smaller force due to the H^+ concentration gradient—that is, the pH gradient (ΔpH). The intermembrane space is slightly more acidic than the matrix, because the higher the concentration of protons, the more acidic the solution (see Panel 2–2, pp. 68–69). Both the membrane potential and the pH gradient combine to generate the proton-motive force, which pulls H^+ back into the mitochondrial matrix. The exact, mathematical relationship between these forces is expressed by the Nernst equation (see Figure 12–24).

would simply be liberated as heat. Cells are able to recover much of this energy because each of the respiratory enzyme complexes in the electron-transport chain uses it to pump protons across the inner mitochondrial membrane, from the matrix into the intermembrane space (see Figure 14–14). Later, we will outline the molecular mechanisms involved. For now, we focus on the consequences of this nifty maneuver. First, the pumping of protons generates an H^+ gradient—or pH gradient—across the inner membrane. As a result, the pH in the matrix (around 7.9) is about 0.7 unit higher than it is in the intermembrane space (which is 7.2, the same pH as the cytosol). Second, proton pumping generates a voltage gradient—or membrane potential—across the inner membrane; as H^+ flows outward, the matrix side of the membrane becomes negative and the side facing the intermembrane space becomes positive.

As discussed in Chapter 12, the force that drives the passive flow of an ion across a membrane is proportional to the ion's *electrochemical gradient*. The strength of that electrochemical gradient depends both on the voltage across the membrane, as measured by the membrane potential, and on the ion's concentration gradient (see Figure 12–5). Because protons are positively charged, they will more readily cross a membrane if there is an excess of negative charge on the other side. In the case of the inner mitochondrial membrane, the pH gradient and membrane potential work together to create a steep electrochemical proton gradient that makes it energetically very favorable for H^+ to flow back into the mitochondrial matrix. The membrane potential contributes significantly to this *proton-motive force*, which pulls H^+ back across the membrane; the greater the membrane potential, the more energy is stored in the proton gradient (**Figure 14–15**).

QUESTION 14–3

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

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

If protons in the intermembrane space were simply allowed to flow back into the mitochondrial matrix, the energy stored in the electrochemical proton gradient would be lost as heat. Such a seemingly wasteful process allows hibernating bears to stay warm, as we discuss further in How We Know (pp. 476–477). In most cells, however, the electrochemical proton gradient across the inner mitochondrial membrane is used to drive the synthesis of ATP from ADP and P_i (see Figure 2–27). The device that makes this possible is **ATP synthase**, a large, multisubunit protein embedded in the inner mitochondrial membrane.

ATP synthase is of ancient origin; the same enzyme generates ATP in the mitochondria of animal cells, the chloroplasts of plants and algae, and

the plasma membrane of bacteria and archaea. The part of the protein that catalyzes the phosphorylation of ADP is shaped like a lollipop head that projects into the mitochondrial matrix; it is penetrated by a central stalk that is attached to a transmembrane H^+ carrier (**Figure 14–16**). The passage of protons through the carrier causes the carrier and its stalk to spin rapidly, like a tiny motor. As the stalk rotates, it rubs against proteins in the enzyme's stationary head, altering their conformation and causing them to produce ATP. In this way, a mechanical deformation gets converted into the chemical-bond energy of ATP (**Movie 14.5**). This fine-tuned sequence of interactions allows ATP synthase to produce more than 100 molecules of ATP per second—3 molecules of ATP per revolution.

ATP synthase can also operate in reverse—using the energy of ATP hydrolysis to pump protons "uphill," against their electrochemical gradient (**Figure 14–17**). In this mode, ATP synthase functions like the H^+ pumps described in Chapter 12. Whether ATP synthase primarily makes ATP—or consumes it to pump protons—depends on the magnitude of the electrochemical proton gradient across the membrane in which the enzyme is embedded. In many bacteria that can grow either aerobically or anaerobically, the direction in which the ATP synthase works is routinely reversed when the bacterium runs out of O_2. Under these conditions, the ATP synthase uses some of the ATP generated inside the cell by glycolysis to pump protons out of the cell, creating the proton gradient that the bacterial cell needs to import its essential nutrients by coupled transport. A proton gradient is similarly used to drive the transport of small molecules in and out of the mitochondrial matrix, as we discuss next.

The Electrochemical Proton Gradient Also Drives Transport Across the Inner Mitochondrial Membrane

The synthesis of ATP is not the only process driven by the electrochemical proton gradient in mitochondria. Many small, charged molecules, such as pyruvate, ADP, and inorganic phosphate (P_i), are imported into the

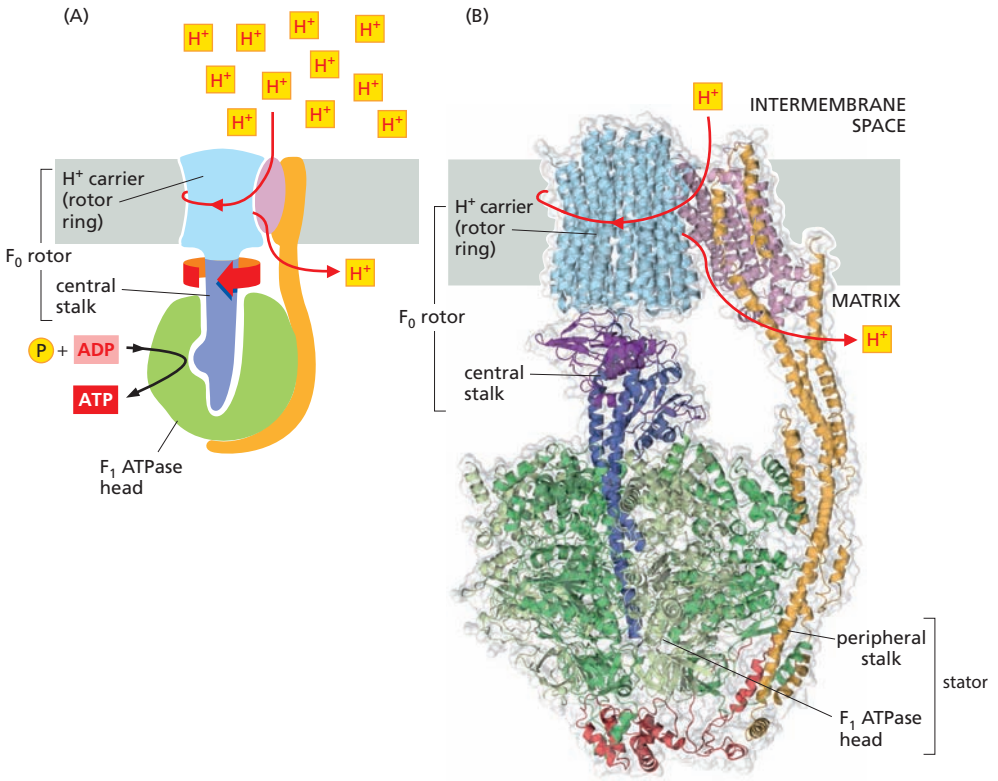


Figure 14–16 ATP synthase acts like a motor to convert the energy of protons flowing down their electrochemical gradient to chemical-bond energy in ATP. (A) The multisubunit protein is composed of a stationary head, called the F_1 ATPase, and a rotating portion called F_0. Both F_1 and F_0 are formed from multiple subunits. Driven by the electrochemical proton gradient, the F_0 part of the protein—which consists of the transmembrane H^+ carrier (*blue*) plus a central stalk (*dark purple*)—spins rapidly within the stationary head of the F_1 ATPase (*green*), causing it to generate ATP from ADP and P_i. The stationary head is secured to the inner membrane by an elongated protein "arm" called the peripheral stalk (*orange*). The F_1 ATPase is so named because it can carry out the reverse reaction—the hydrolysis of ATP to ADP and P_i—when detached from the F_0 portion of the complex. (B) The three-dimensional structure of ATP synthase, as determined by x-ray crystallography. The peripheral stalk is anchored to the membrane with the help of an additional subunit (*light purple*). At its other end, this stalk is attached to the F_1 ATPase head via a small *red* subunit. **Movie 14.6** shows how the ATP synthase proteins are organized into mitochondrial cristae.

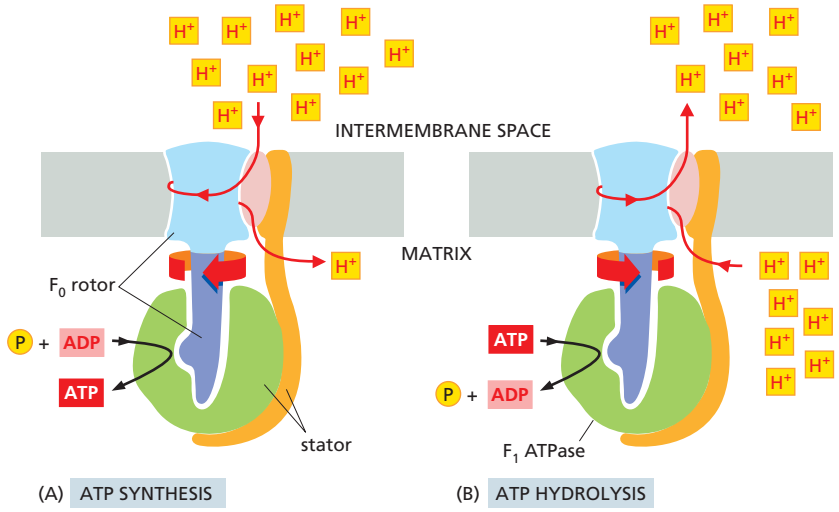


Figure 14–17 ATP synthase is a reversible coupling device. The protein can either (A) synthesize ATP by harnessing the electrochemical H^+ gradient or (B) pump protons against this gradient by hydrolyzing ATP. The direction of operation (and of rotation) at any given instant depends on the net free-energy change (ΔG, discussed in Chapter 3) for the coupled processes of H^+ translocation across the membrane and the synthesis of ATP from ADP and P_i. For example, if the electrochemical proton gradient falls below a certain level, the ΔG for H^+ transport into the matrix will no longer be large enough to drive ATP production; instead, ATP will be hydrolyzed by the ATP synthase to rebuild the proton gradient. A tribute to the activity of this all-important protein complex is shown in Movie 14.7.

mitochondrial matrix from the cytosol, while others, such as ATP, must be transported in the opposite direction. Carrier proteins that bind some of these molecules couple their transport to the energetically favorable flow of H^+ into the matrix (see the "coupled transporters" in Figure 12–15). Pyruvate and P_i, for example, are each co-transported inward, along with protons, as the protons move down their electrochemical gradient into the matrix.

Other transporters take advantage of the membrane potential generated by the electrochemical proton gradient, which makes the matrix side of the inner mitochondrial membrane more negatively charged than the side that faces the intermembrane space (see Figure 14–15). A special antiport carrier protein exploits this voltage gradient to export ATP from the mitochondrial matrix and to bring ADP in. This exchange allows the ATP synthesized in the mitochondrion to be exported rapidly, which is important for energizing the rest of the cell (Figure 14–18).

The electrochemical proton gradient is also required for the translocation of proteins across the inner mitochondrial membrane and into the matrix. As mentioned earlier, although mitochondria retain their own genome—and synthesize some of their own proteins—most of the proteins required for mitochondrial function are made in the cytosol and must be actively imported into the organelle. We discuss this transport process—which requires energy supplied by the electrochemical proton gradient as well as ATP hydrolysis—in Chapter 15.

In eukaryotic cells, therefore, the electrochemical proton gradient is used to drive both the generation of ATP and the transport of selected metabolites and proteins across the inner mitochondrial membrane. In bacteria, the proton gradient across the plasma membrane is similarly used to drive ATP synthesis and metabolite transport. But it also serves as an important source of directly usable energy: in motile bacteria, for instance, the flow of protons into the cell drives the rapid rotation of the bacterial flagellum, which propels the bacterium along (Movie 14.8).

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

As a result of the nucleotide exchange shown in Figure 14–18, ADP molecules—produced by hydrolysis of ATP in the cytosol—are rapidly drawn back into mitochondria for recharging, while the bulk of the ATP molecules

QUESTION 14–4

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

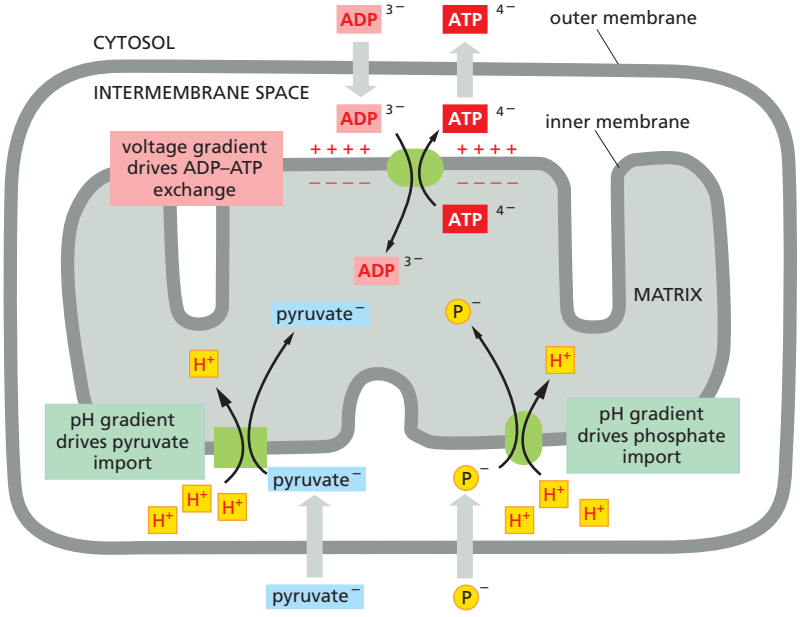


Figure 14–18 The electrochemical proton gradient across the inner mitochondrial membrane is used to drive some coupled transport processes. The charge on each of the transported molecules is indicated for comparison with the membrane potential, which is negative inside, as shown. Pyruvate and inorganic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

produced in mitochondria are exported into the cytosol, where they are most needed. (A small amount of ATP is used within the mitochondrion itself to power DNA replication, protein synthesis and translocation, and other energy-consuming reactions that occur there.) With all of this back-and-forth, a typical ATP molecule in a human cell will shuttle out of a mitochondrion, then back in as ADP, more than once every minute.

As discussed in Chapter 3, most biosynthetic enzymes drive energetically unfavorable reactions by coupling them to the energetically favorable hydrolysis of ATP (see Figure 3–32). The pool of ATP in a cell is thus used to drive a huge variety of cell processes in much the same way that a battery is used to drive an electric engine. To serve as a readily available energy source, the concentration of ATP in the cytosol must be kept about 10 times higher than that of ADP. When the activity of mitochondria is halted, ATP levels fall dramatically and the cell's battery runs down. Eventually, energetically unfavorable reactions can no longer take place and the cell dies. The poison cyanide, which blocks electron transport in the inner mitochondrial membrane, causes cell death in exactly this way.

Cell Respiration Is Amazingly Efficient

The oxidation of sugars to produce ATP may seem unnecessarily complex. Surely the process could be accomplished more directly—perhaps by eliminating the citric acid cycle or some of the steps in the respiratory chain. Such simplification would certainly make the chemistry easier to learn—but it would not be as helpful for the cell. As discussed in Chapter 13, the oxidative pathways that allow cells to extract energy from food in a usable form involve many intermediates, each differing only slightly from its predecessor. In this way, the huge amount of energy locked up in food molecules can be parceled out into small packets that can be captured in activated carriers such as NADH and $FADH_2$ (see Figure 13–1).

Much of the energy carried by NADH and $FADH_2$ is ultimately converted into the bond energy of ATP. How much ATP each of these activated carriers can produce depends on several factors, including where its electrons enter the respiratory chain. The NADH molecules produced in the mitochondrial matrix during the citric acid cycle pass their high-energy

electrons to the NADH dehydrogenase complex—the first complex in the chain. As the electrons pass from one enzyme complex to the next, they promote the pumping of protons across the inner mitochondrial membrane. In this way, each NADH molecule provides enough net energy to generate about 2.5 molecules of ATP (see Question 14–5 and its answer).

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

Although the biological oxidation of glucose to CO_2 and H_2O consists of many interdependent steps, the overall process—known as **cell respiration**—is remarkably efficient. Almost 50% of the total energy that could be released by burning sugars or fats is captured and stored in the phosphate bonds of ATP during cell respiration. That might not seem impressive, but it is considerably better than most nonbiological energy-conversion devices. Electric motors and gasoline engines operate at about 10–20% efficiency. If cells operated at this efficiency, an organism would have to eat voraciously just to maintain itself. Moreover, because the wasted energy is liberated as heat, large organisms (including humans) would need far better mechanisms for cooling themselves. It is hard to imagine how animals could have evolved without the elaborate yet economical mechanisms that allow cells to extract a maximum amount of energy from food.

TABLE 14–1 PRODUCT YIELDS FROM GLUCOSE OXIDATION

Process	Direct Product	Final ATP Yield per Glucose
Glycolysis	2 NADH (cytosolic)	3*
	2 ATP	2
Pyruvate oxidation to acetyl CoA (two per glucose)	2 NADH (mitochondrial matrix)	5
Complete oxidation of the acetyl group of acetyl CoA (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 can pass electrons to NADH dehydrogenase—thus requires energy.

MOLECULAR MECHANISMS OF ELECTRON TRANSPORT AND PROTON PUMPING

For many years, biochemists struggled to understand why electron-transport chains had to be embedded in membranes to function in ATP production. The puzzle was essentially solved in the 1960s, when it was discovered that transmembrane proton gradients drive the process. The concept of chemiosmotic coupling was so novel, however, that it was not widely accepted until more than a decade later, when experiments with artificial energy-generating systems put the power of proton gradients to the test, as described in **How We Know** (pp. 476–477).

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

Protons Are Readily Moved by the Transfer of Electrons

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

QUESTION 14–5

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

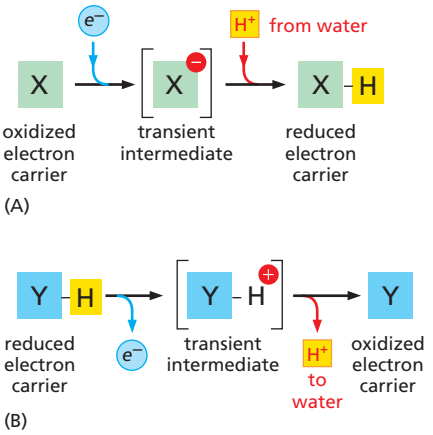


Figure 14–19 Electron transfers can cause the movement of entire hydrogen atoms, because protons are readily accepted from or donated to water. In these examples, (A) an oxidized electron carrier molecule, X, picks up an electron plus a proton when it is reduced, and (B) a reduced electron carrier molecule, Y, loses an electron plus a proton when it is oxidized.

and acceptance of protons. These nomadic protons often accompany the electrons that are transferred during oxidation and reduction. An isolated electron (e^-) bears a negative charge. But when a molecule is reduced by acquiring an electron, in many cases, this charge is immediately neutralized by the addition of a proton from water. Thus the net effect of the reduction is to transfer an entire hydrogen atom, $H^+ + e^-$ (**Figure 14–19A**).

Similarly, when a molecule is oxidized, it often loses an electron belonging to one of its hydrogen atoms: in most instances, when this electron is transferred to an electron carrier, the proton that is left behind is passed on to water (**Figure 14–19B**). Therefore, in a membrane in which electrons are being passed along an electron-transport chain, it is a relatively simple matter, in principle, to move protons from one side of the membrane to the other. All that is required is that the electron carrier be oriented in the membrane in such a way that it accepts an electron—along with a proton from water—on one side of the membrane, and then releases a proton on the other side of the membrane when it passes an electron on to the next electron carrier molecule in the chain (**Figure 14–20**).

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 the next. Each of these electron transfers 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). These reactions are necessarily coupled: 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 electron affinities of the participating molecules. Electrons will pass spontaneously from molecules that have a relatively low affinity for some of their electrons, and thus lose them easily, to molecules that have a higher affinity for electrons. For example, NADH has a low electron affinity, so that its electrons are readily passed to the NADH dehydrogenase complex (see Figure 14–14). The batteries that power our electronic gadgets are based on similar electron transfers between chemical substances with different electron affinities.

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

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

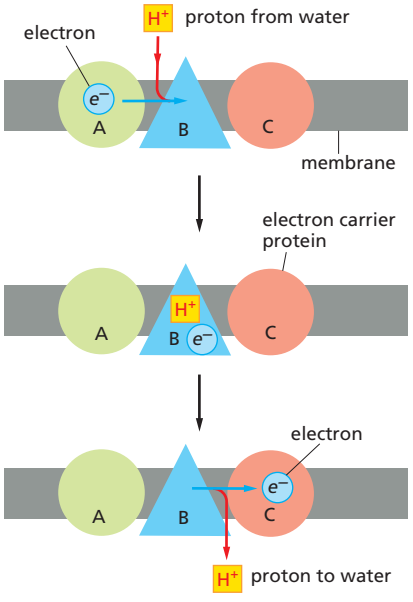


Figure 14–20 The orientation of a membrane-embedded electron carrier allows electron transfer to drive proton pumping. As an electron passes along an electron-transport chain, it can bind and release a proton at each step. In this schematic diagram, the electron carrier, protein B, picks up a proton (H^+) from one side of the membrane when it accepts an electron (e^-) from protein A; protein B releases the proton to the other side of the membrane when it donates its electron to the electron carrier, protein C. In this example, the transfer of a single electron thereby pumps the equivalent of one proton across a membrane.

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

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

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

The tendency for a redox pair such as NADH/NAD^+ to donate or accept electrons can be determined experimentally by measuring its **redox potential** (**Panel 14–1**, p. 472). The lower the redox potential, the lower the molecules' affinity for electrons—and the more likely they are to act as electron donors. Redox potentials are expressed in units of volts, as for a standard battery.

Electrons will move spontaneously from a redox pair with a low redox potential (or low affinity for electrons), such as NADH/NAD^+, to a redox pair with a high redox potential (or high affinity for electrons), such as O_2/H_2O. Thus, NADH is an excellent molecule to donate electrons to the respiratory chain, while O_2 is well suited to act as an electron "sink" at the end of the pathway. As explained in Panel 14–1, the difference in redox potential, $\Delta E'_0$, is a direct measure of the standard free-energy change ($\Delta G°$) for the transfer of an electron from one molecule to another.

Electron Transfers Release Large Amounts of Energy

The amount of energy that can be released by an electron transfer can be determined by comparing the redox potentials of the molecules involved. Again, let's look at the transfer of electrons from NADH and to O_2. As shown in Panel 14–1, a 1:1 mixture of NADH and NAD^+ has a redox potential of –320 mV, indicating that NADH has a weak affinity for electrons—and a strong tendency to donate them; a 1:1 mixture of H_2O and $\frac{1}{2}O_2$ has a redox potential of +820 mV, indicating that O_2 has a strong affinity for electrons—and a strong tendency to accept them. The difference in redox potential between these two pairs is 1.14 volts (1140 mV), which means that the transfer of each electron from NADH to O_2 under these standard conditions is enormously favorable: the $\Delta G°$ for that electron transfer is –109.6 kJ/mole per electron—or –219.2 kJ/mole for the two electrons that are donated from each NADH molecule (see Panel 14–1). If we compare this free-energy change with that needed for the formation of the terminal phosphoanhydride bond of ATP in cells (about 54 kJ/mole), we see that enough energy is released by the oxidization of one NADH molecule to synthesize several molecules of ATP.

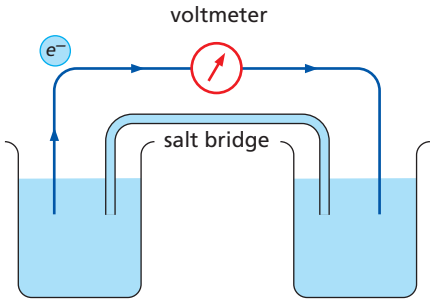
Living systems could have evolved enzymes that would allow NADH to donate electrons directly to O_2 to make water. But because of the huge drop in free energy, this reaction would proceed with almost explosive force and nearly all of the energy would be released as heat. Instead, as we have seen, the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

Metals Tightly Bound to Proteins Form Versatile Electron Carriers

Each of the three respiratory enzyme complexes includes metal atoms that are tightly bound to the proteins. Once an electron has been donated

PANEL 14–1 REDOX POTENTIALS

HOW REDOX POTENTIALS ARE MEASURED



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

THE STANDARD REDOX POTENTIAL, E_0'

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

examples of redox reactions	standard redox potential E_0'
$NADH \rightleftharpoons NAD^+ + H^+ + 2e^-$	–320 mV
reduced ubiquinone $\rightleftharpoons$ oxidized ubiquinone $+ 2H^+ + 2e^-$	+30 mV
reduced cytochrome *c* $\rightleftharpoons$ oxidized cytochrome *c* $+ e^-$	+230 mV
$H_2O \rightleftharpoons \frac{1}{2}O_2 + 2H^+ + 2e^-$	+820 mV

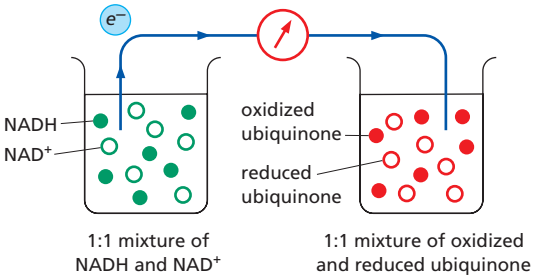
CALCULATION OF ΔG° FROM REDOX POTENTIALS

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

$\Delta G^\circ = -n(0.096)\,\Delta E_0'$, where n is the number of electrons transferred across a redox potential change of $\Delta E_0'$ millivolts (mV), and

$\Delta E_0' = E_0'(\text{acceptor}) - E_0'(\text{donor})$

EXAMPLE:



For the transfer of one electron from NADH to ubiquinone:

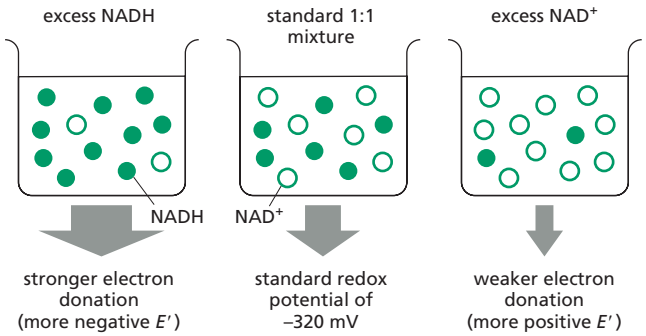
$$\Delta E_0' = +30 - (-320) = +350 \text{ mV}$$

$\Delta G^\circ = -n(0.096)\Delta E_0' = -1(0.096)(350) = -34$ kJ/mole

A similar calculation reveals that the transfer of one electron from ubiquinone to oxygen has an even more favorable ΔG° of –76 kJ/mole. The ΔG° value for the transfer of one electron from NADH to oxygen is the sum of these two values, –110 kJ/mole.

EFFECT OF CONCENTRATION CHANGES

As explained in Chapter 3 (see p. 93), the actual free-energy change for a reaction, ΔG, depends on the concentrations 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.



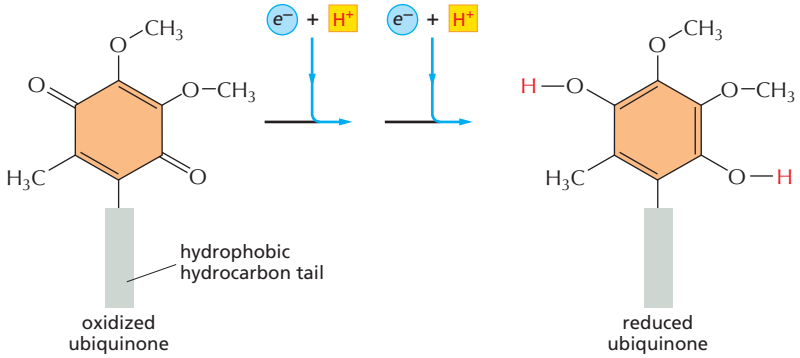


Figure 14–21 Quinones carry electrons within the lipid bilayer. The quinone in the mitochondrial electron-transport chain is called ubiquinone. It picks up one H^+ from the aqueous environment for every electron it accepts, and it can carry two electrons as part of its hydrogen atoms (*red*). When this reduced ubiquinone donates its electrons to the next carrier in the chain, the protons are released. Its long, hydrophobic hydrocarbon tail confines ubiquinone to the inner mitochondrial membrane.

to a respiratory complex, it can move within the complex by skipping from one embedded metal ion to another ion with an even greater affinity for electrons.

When electrons pass from one respiratory complex to the next, in contrast, they are ferried by electron carriers that can diffuse freely within or along the lipid bilayer. These mobile molecules pick up electrons from one complex and deliver them to the next in line. In the mitochondrial respiratory chain, for example, a small, hydrophobic molecule called ubiquinone picks up electrons from the NADH dehydrogenase complex and delivers them to the cytochrome *c* reductase complex (see Figure 14–14). A related **quinone** functions similarly during electron transport in photosynthesis. A ubiquinone molecule can accept or donate either one or two electrons, and it picks up one H^+ from water with each electron that it carries (**Figure 14–21**). Its redox potential of +30 mV places ubiquinone between the NADH dehydrogenase complex and the cytochrome *c* reductase complex in terms of its tendency to gain or lose electrons—which explains why ubiquinone receives electrons from the former and donates them to the latter (**Figure 14–22**). Ubiquinone also serves as the entry point for electrons donated by the $FADH_2$ that is generated both during the citric acid cycle and from fatty acid oxidation (see Figures 13–11 and 13–12).

The redox potentials of different metal complexes influence where they are used along the electron-transport chain. **Iron–sulfur centers** have relatively low affinities for electrons and thus are prominent in the electron carriers that operate in the early part of the chain. An iron–sulfur center

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 reasons why this is necessary.

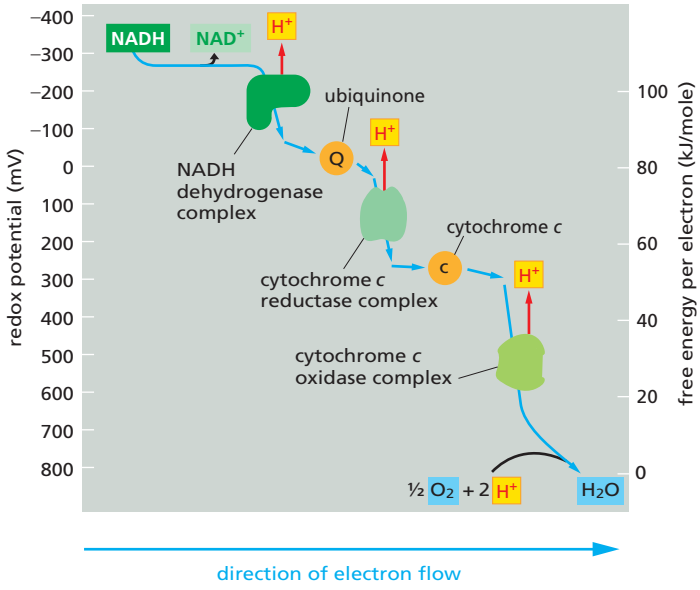


Figure 14–22 Redox potential increases along the mitochondrial electron-transport chain. The biggest increases in redox potential occur across each of the three respiratory enzyme complexes, which allows each of them to pump protons.

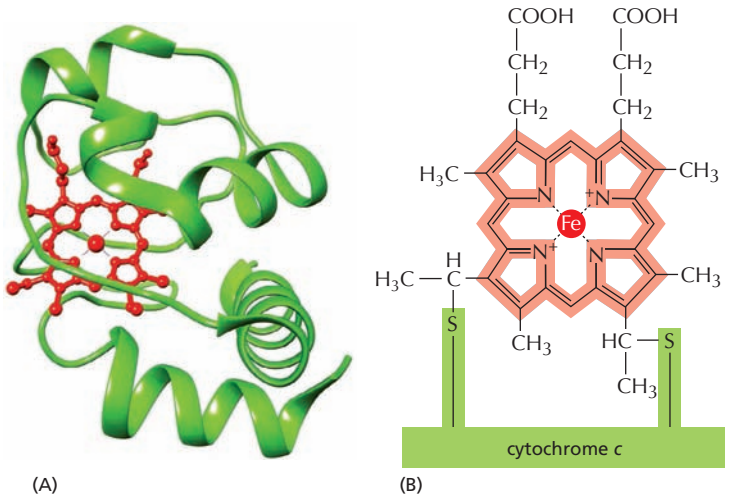


Figure 14–23 The iron in a heme group can serve as an electron acceptor. (A) Ribbon structure showing the position of the heme group (*red*) associated with cytochrome *c* (*green*). (B) The porphyrin ring of the heme group (*light red*) is attached covalently to side chains in the protein. The heme groups of different cytochromes have different electron affinities because they differ slightly in structure and are held in different local environments within each protein.

in the NADH dehydrogenase complex, for example, passes electrons to ubiquinone. Later in the pathway, iron atoms that are held in the heme groups bound to cytochrome proteins are commonly used as electron carriers (**Figure 14–23**). These heme groups give **cytochromes,** such as the cytochrome *c* reductase and cytochrome *c* oxidase complexes, their color ("cytochrome" from the Greek *chroma*, "color"). Like other electron carriers, the cytochrome proteins increase in redox potential the further down the mitochondrial electron-transport chain they are located. For example, cytochrome *c*, a small protein that accepts electrons from the cytochrome *c* reductase complex and transfers them to the cytochrome *c* oxidase complex, has a redox potential of +230 mV—a value about midway between those of the cytochromes with which it interacts (see Figure 14–22).

Cytochrome *c* Oxidase Catalyzes the Reduction of Molecular Oxygen

Cytochrome *c* oxidase, the final electron carrier in the respiratory chain, has the highest redox potential of all. This protein complex removes electrons from cytochrome *c*, thereby oxidizing it—hence the name "cytochrome *c* oxidase." The exceptionally high electron affinity stems in part from a special oxygen-binding site within cytochrome *c* oxidase that contains a heme group plus a copper atom (**Figure 14–24**). It is here that nearly all the oxygen we breathe is consumed, when the electrons that had been donated by NADH at the start of the electron-transport chain are handed off to O_2 to produce H_2O.

In total, four electrons donated by cytochrome *c* and four protons extracted from the aqueous environment are added to each O_2 molecule in the reaction $4e^- + 4H^+ + O_2 \rightarrow 2H_2O$. In addition to the protons that combine with O_2, four other protons are pumped across the membrane during the transfer of the four electrons from cytochrome *c* to O_2. This pumping occurs because the transfer of electrons drives allosteric changes in the conformation of cytochrome *c* oxidase that cause protons to be ejected from the mitochondrial matrix (**Figure 14–25**).

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.

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.

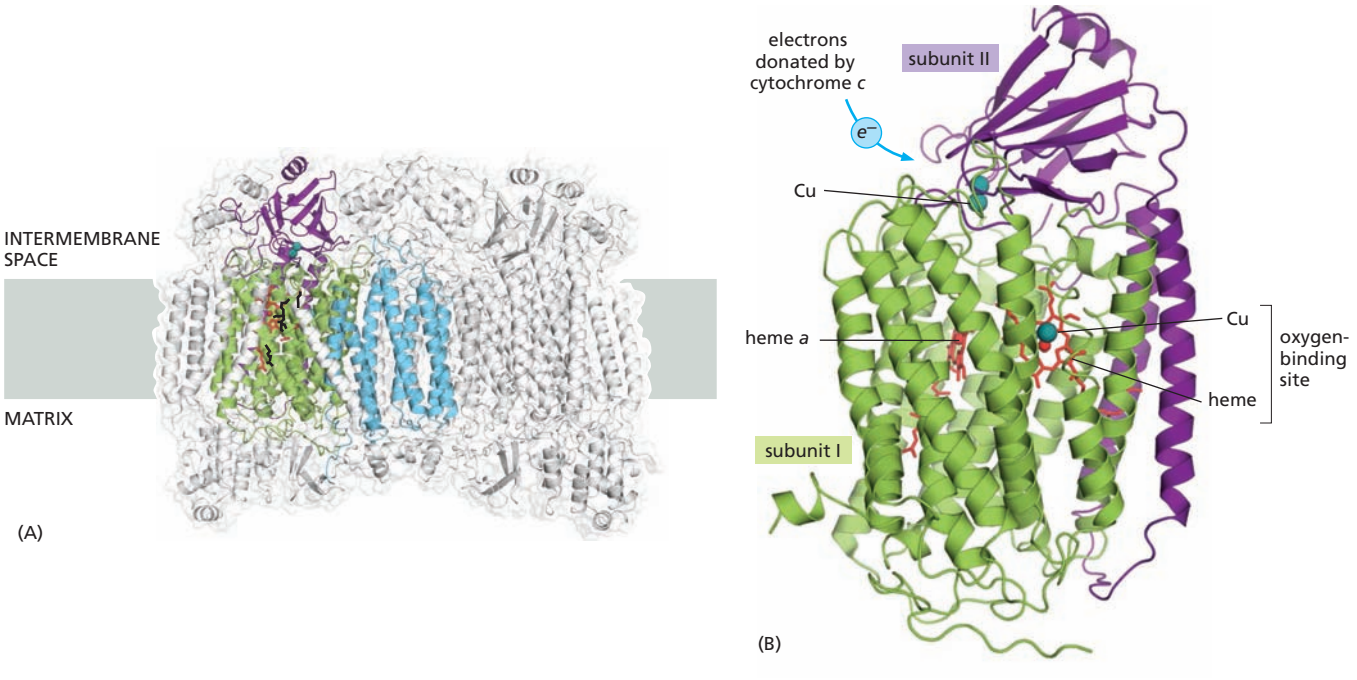


Figure 14–24 Cytochrome *c* oxidase is a finely tuned protein machine. The protein is a dimer formed from a monomer with 13 different protein subunits. (A) The entire protein is shown positioned in the inner mitochondrial membrane. The three colored subunits that form the functional core of the complex are encoded by the mitochondrial genome; the remaining subunits are encoded by the nuclear genome. (B) As electrons pass through this protein on the way to its bound O_2 molecule, they cause the protein to pump protons across the membrane. As indicated, a heme and a copper atom (Cu) form the site where a tightly bound O_2 molecule will receive four electrons to produce H_2O. Only two of the 13 subunits are shown.

The active site of cytochrome *c* oxidase therefore holds on tightly to an oxygen molecule until it receives all four of the electrons needed to convert it to two molecules of H_2O. This retention is critical, because it prevents superoxide radicals from attacking macromolecules throughout the cell—a type of damage that has been postulated to contribute to human aging.

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

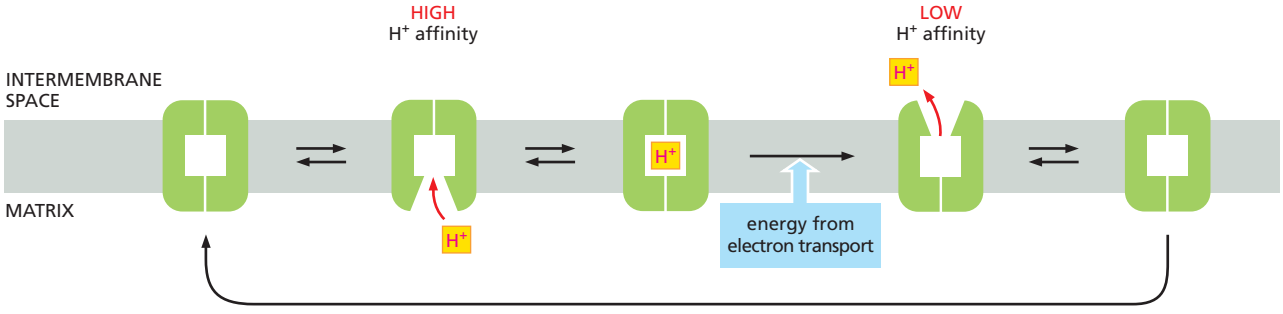


Figure 14–25 Proton pumping is coupled to electron transport. This type of mechanism is thought to be used by the NADH dehydrogenase complex and by cytochrome *c* oxidase, as well as by many other proton pumps. The protein is driven through a cycle of three conformations. In one of these conformations, the protein has a high affinity for H^+, causing it to pick up an H^+ on the matrix side of the membrane. In another conformation, the protein has a low affinity for H^+, causing it to release an H^+ on the other side of the membrane. As indicated, the cycle goes only in one direction—releasing the proton into the intermembrane space—because one of the steps is driven by allosteric change in conformation driven by the energetically favorable transport of electrons.

HOW CHEMIOSMOTIC COUPLING DRIVES ATP SYNTHESIS

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

Imaginary intermediates

In the 1950s, many researchers believed that the oxidative phosphorylation that takes place in mitochondria generated 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. 436–437). It was assumed that the electron-transport chain in mitochondria would similarly generate some phosphorylated intermediate that could then donate its phosphate group directly to ADP. This assumption inspired a long and frustrating search for this mysterious high-energy intermediate. Investigators occasionally claimed to have discovered the missing intermediate, but the compounds turned out to be either unrelated to electron transport or, as one researcher put it in a review of the history of bioenergetics, "products of high-energy imagination."

Harnessing the force

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

Several lines of evidence offered support for Mitchell's proposed mechanism. First, it was known that mitochondria do generate an electrochemical proton gradient across their inner membrane. But what does this gradient—also called the proton-motive force—actually do? If the gradient is required to drive ATP synthesis, as the chemiosmotic hypothesis posits, then either disrupting the inner membrane or eliminating the proton gradient across it should inhibit ATP production. In fact, researchers found both these predictions to be true. Physical disruption of the inner mitochondrial membrane halts ATP synthesis in that organelle. Similarly, dissipation of the proton gradient by a chemical "uncoupling" agent such as 2,4-dinitrophenol (DNP) also inhibits mitochondrial ATP production. Such gradient-busting chemicals carry H^+ across the inner mitochondrial membrane, forming a shuttle system for the movement of H^+ that bypasses the ATP synthase that generates ATP (**Figure 14–26**). In this way, compounds such as DNP uncouple electron transport from ATP synthesis. As a result of this short-circuiting, the proton-motive force is dissipated completely, and the organelle can no longer make ATP.

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

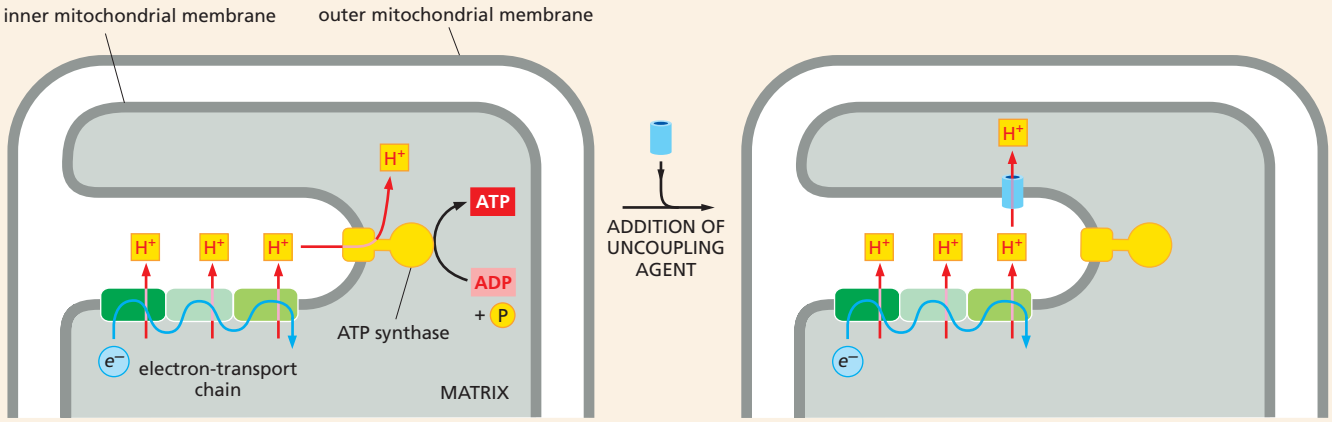


Figure 14–26 Uncoupling agents are H^+ carriers that can insert into the inner mitochondrial membrane. They render the membrane permeable to protons, allowing H^+ to flow into the mitochondrial matrix without passing through ATP synthase. This short-circuit effectively uncouples electron transport from ATP synthesis.

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

Artificial ATP generation

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

How does the electrochemical proton gradient drive ATP production? This is where the ATP synthase comes in. In 1974, Efraim Racker and Walther Stoeckenius demonstrated that they could assemble an artificial ATP-generating system by combining an ATP synthase isolated from the mitochondria of cow heart muscle with a proton pump purified from the purple membrane of the archaean *Halobacterium halobium*. As discussed in Chapter 11, the plasma membrane of this prokaryote is packed with bacteriorhodopsin, a protein that pumps H^+ out of the cell in response to sunlight (see Figure 11–28).

When bacteriorhodopsin alone was reconstituted into artificial lipid vesicles (liposomes), Racker and Stoeckenius showed that, in the presence of light, the protein pumps H^+ into the vesicles, generating a proton gradient. (The orientation of the protein is reversed in these membranes, so that protons are transported into the vesicles; in the organism, protons are pumped out.) When the bovine ATP synthase was then incorporated into these vesicles, much to the amazement of many biochemists, the system catalyzed the synthesis of ATP from ADP and inorganic phosphate in response to light. This ATP formation showed an absolute dependence on an intact proton gradient, as either eliminating bacteriorhodopsin from the system or adding uncoupling agents such as DNP abolished ATP synthesis (**Figure 14–27**).

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

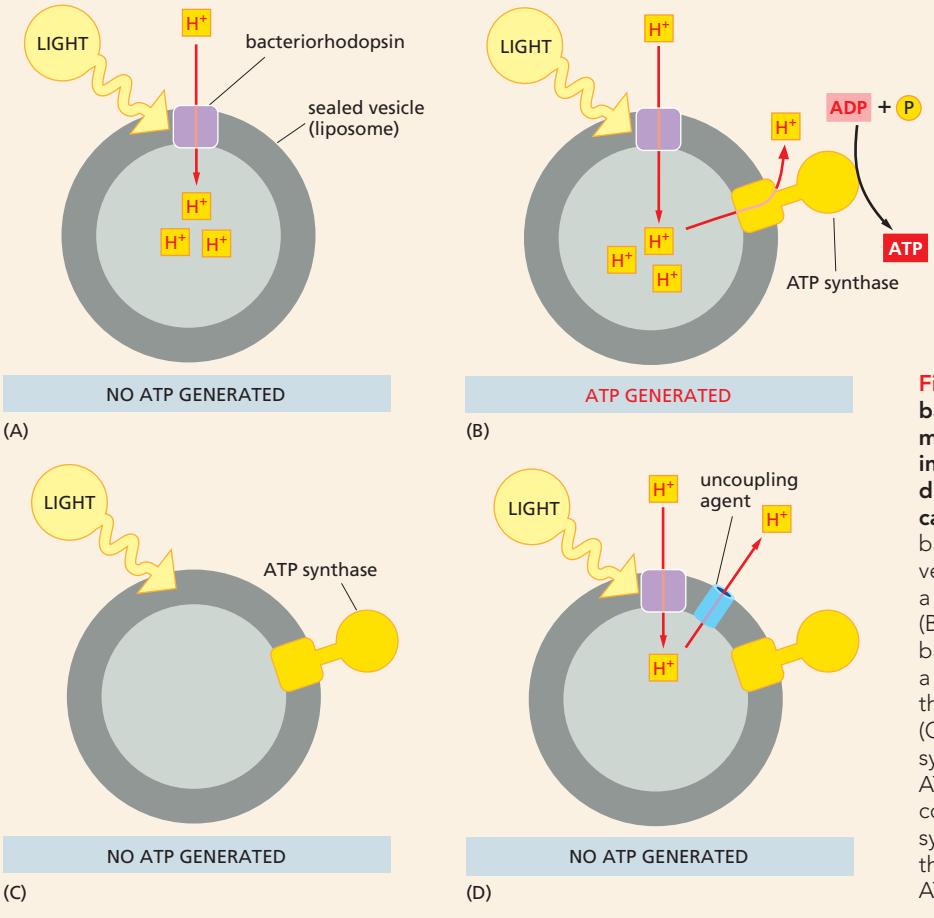


Figure 14–27 Experiments in which bacteriorhodopsin and bovine mitochondrial ATP synthase were introduced into liposomes provided direct evidence that proton gradients can power ATP production. (A) When bacteriorhodopsin is added to artificial lipid vesicles (liposomes), the protein generates a proton gradient in response to light. (B) In artificial vesicles containing both bacteriorhodopsin and an ATP synthase, a light-generated proton gradient drives the formation of ATP from ADP and P_i. (C) Artificial vesicles containing only ATP synthase do not on their own produce ATP in response to light. (D) In vesicles containing both bacteriorhodopsin and ATP synthase, uncoupling agents that abolish the proton gradient eliminate light-induced ATP synthesis.

CHLOROPLASTS AND PHOTOSYNTHESIS

Virtually all the organic material in present-day cells is produced by **photosynthesis**—the series of light-driven reactions that creates organic molecules from atmospheric carbon dioxide (CO_2). Plants, algae, and photosynthetic bacteria such as cyanobacteria use electrons from water and the energy of sunlight to perform this chemical feat. In the process, water molecules are split, releasing vast quantities of O_2 gas into the atmosphere. This oxygen in turn supports oxidative phosphorylation—not only in animals but also in plants and aerobic bacteria. As we discuss in detail at the end of the chapter, it was the activity of photosynthetic bacteria that eventually filled the atmosphere with oxygen, enabling the subsequent evolution of the myriad life-forms that today use aerobic metabolism to make their ATP (**Figure 14–28**).

For most plants, photosynthesis occurs mainly in the leaves. There, specialized intracellular organelles called **chloroplasts** capture light energy and use it to produce ATP and NADPH. These activated carriers are used to convert CO_2 into organic molecules that serve as the precursors for sugars—a process called *carbon fixation*.

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

Chloroplasts Resemble Mitochondria but Have an Extra Compartment—the Thylakoid

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

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 molecular machinery needed to produce energy. Instead, the light-capturing systems, electron-transport chain, and ATP synthase that convert light energy into ATP during

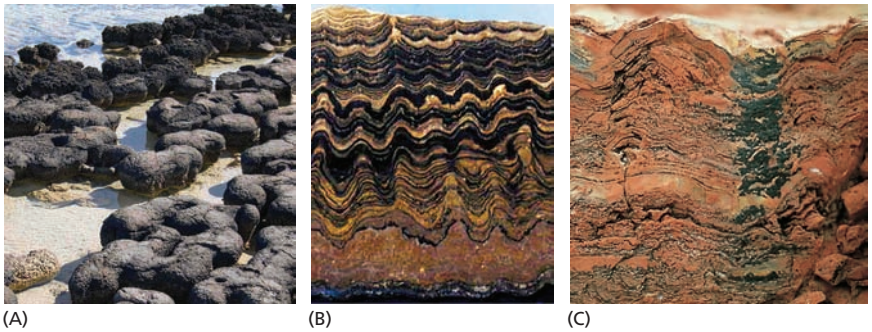
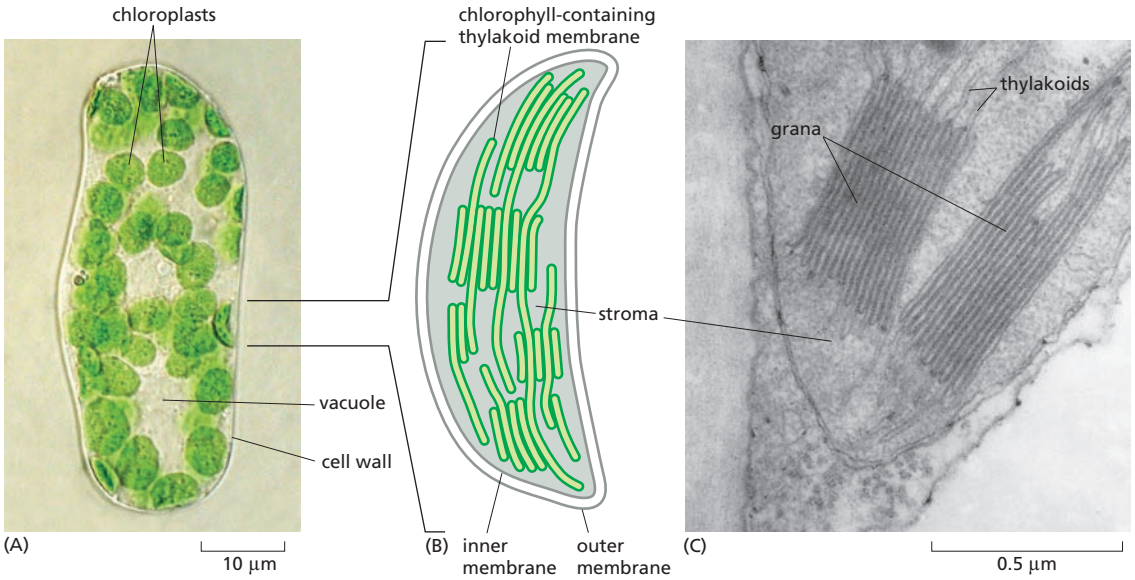

(A) (B) (C)

Figure 14–28 Microorganisms that carry out oxygen-producing photosynthesis changed Earth's atmosphere. (A) Living stromatolites from a lagoon in Western Australia. These structures are formed in specialized environments by large colonies of oxygen-producing photosynthetic cyanobacteria, which form mats that trap sand or minerals in thin layers. (B) Cross section of a modern stromatolite, showing its stratification. (C) A similar, layered structure can be seen in a fossilized stromatolite. These ancient accretions, some more than 3.5 billion years old, contain the remnants of the photosynthetic bacteria whose O_2-liberating activities ultimately transformed the Earth's atmosphere. (A, courtesy of Cambridge Carbonates Ltd.; B, courtesy of Roger Perkins, Virtual Fossil Museum, https://creativecommons.org/licenses/by-nc/4.0/; C, courtesy of S.M. Awramik, University of California/Biological Photo Service.)



photosynthesis are all contained in the *thylakoid membrane*. This third membrane is folded to form a set of flattened, disclike sacs, called the **thylakoids**, which are arranged in stacks called grana (**Figure 14–29**). The interior of each thylakoid is thought to be connected with that of other thylakoids, creating the *thylakoid space*—a compartment that is separate from the chloroplast stroma.

Photosynthesis Generates—and Then Consumes—ATP and NADPH

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

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

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

1. *Stage 1* of photosynthesis resembles the oxidative phosphorylation that takes place on the mitochondrial inner membrane. In this stage, an electron-transport chain in the thylakoid membrane harnesses the energy of electron transport to pump protons into the thylakoid space; the resulting proton gradient then drives the synthesis of ATP by ATP synthase. What makes photosynthesis very different is that the high-energy electrons donated to the *photosynthetic electron-transport chain* come from a molecule of **chlorophyll** that has absorbed energy from sunlight. Thus the energy-producing reactions of stage 1 are sometimes called the **light reactions** (**Figure 14–30**). Another major difference between photosynthesis and oxidative phosphorylation is where the high-energy electrons ultimately wind up: those that make their way down the photosynthetic electron-transport chain in chloroplasts are donated not to O_2 but to $NADP^+$, to produce NADPH.

Figure 14–29 Chloroplasts, like mitochondria, are composed of a set of specialized membranes and compartments. (A) Light micrograph showing chloroplasts (*green*) in the cell of a flowering plant. (B) Drawing of a single chloroplast showing the organelle's three sets of membranes, including the thylakoid membrane (*dark green*), which contains the light-capturing and ATP-generating systems. (C) A high-magnification view of an electron micrograph shows the thylakoids arranged in stacks called *grana*; a single thylakoid stack is called a *granum* (**Movie 14.9**). (A, courtesy of Preeti Dahiya; C, courtesy of K. Plaskitt.)

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?

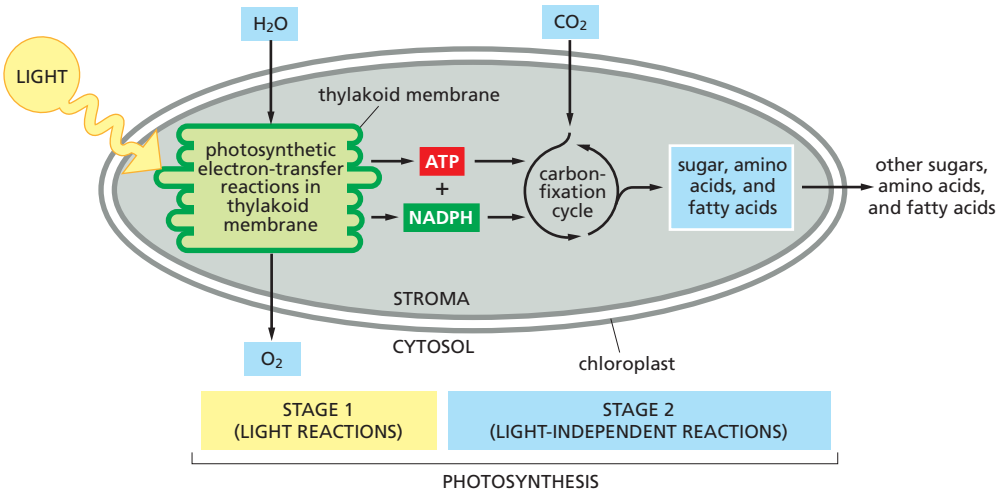


Figure 14–30 Both stages of photosynthesis depend on the chloroplast. In stage 1, a series of photosynthetic electron-transfer reactions produce ATP and NADPH; in the process, electrons are extracted from water and oxygen is released as a by-product, as we discuss shortly. In stage 2, carbon dioxide is assimilated (fixed) to produce sugars and a variety of other organic molecules. Stage 1 occurs in the thylakoid membrane, whereas stage 2 begins in the chloroplast stroma (as shown) and continues in the cytosol.

2. In *stage 2* of photosynthesis, the ATP and the NADPH produced by the photosynthetic electron-transfer reactions of stage 1 are used to drive the manufacture of sugars from CO_2 (see Figure 14–30). These *carbon-fixation reactions,* which do not directly require sunlight, begin in the chloroplast stroma. There they generate a three-carbon sugar called *glyceraldehyde 3-phosphate*. This simple sugar is exported to the cytosol, where it is used to produce a large number of organic molecules in the leaves of the plant, including the disaccharide sucrose, which is exported from the leaves to nourish the rest of the plant.

Although the formation of ATP and NADPH during stage 1, and the conversion of CO_2 to carbohydrate during stage 2, are mediated by two separate sets of reactions, they are linked by elaborate feedback mechanisms that allow a plant to manufacture sugars only when it is appropriate to do so. Several of the enzymes required for carbon fixation, for example, are inactivated in the dark and reactivated by light-stimulated electron transport.

Chlorophyll Molecules Absorb the Energy of Sunlight

Visible light is a form of electromagnetic radiation composed of many wavelengths, ranging from violet (wavelength 400 nm) to deep red (700 nm). Most chlorophylls absorb light best in the blue and red wavelengths, and they absorb green light poorly (**Figure 14–31**). Plants look green to us because the green light that is not absorbed is reflected back to our eyes.

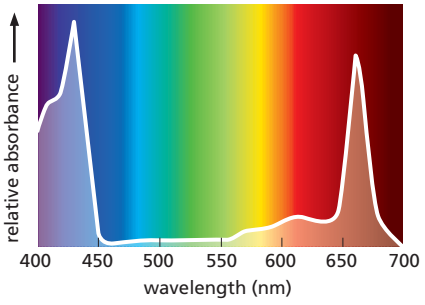


Figure 14–31 Chlorophylls absorb light of blue and red wavelengths. As shown in this absorption spectrum, one form of chlorophyll preferentially absorbs light around wavelengths of 430 nm (blue) and 660 nm (red). Green light, in contrast, is absorbed poorly by this pigment. Other chlorophylls can absorb light of slightly different wavelengths.

Chlorophyll's ability to harness energy derived from sunlight stems from its unique structure. The electrons in a chlorophyll molecule are distributed in a decentralized cloud around the molecule's light-absorbing porphyrin ring (**Figure 14–32**). When light of an appropriate wavelength hits a molecule of chlorophyll, it excites electrons within this diffuse network. This high-energy state is unstable, and an excited chlorophyll molecule will rapidly release this excess energy and return to its more stable, unexcited state.

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

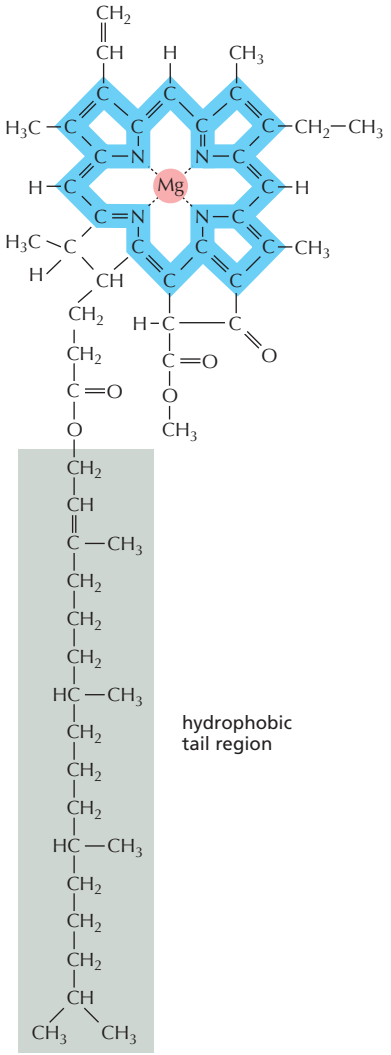


Figure 14–32 Chlorophyll's structure allows it to absorb energy from light. Each chlorophyll molecule contains a porphyrin ring with a magnesium atom (*pink*) at its center. This porphyrin ring is structurally similar to the one that binds iron in heme (see Figure 14–25). Light is absorbed by electrons within the bond network shown in *blue*, while the long, hydrophobic tail (*gray*) helps hold the chlorophyll in the thylakoid membrane.

Excited Chlorophyll Molecules Funnel Energy into a Reaction Center

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

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

The chlorophyll special pair is not located in an antenna complex. Instead, it is part of the **reaction center**—a transmembrane complex of proteins and pigments that is thought to have first evolved more than 3 billion years ago in primitive photosynthetic bacteria (**Movie 14.10**). Within the reaction center, the special pair is positioned directly next to a set of electron carriers that are poised to accept a high-energy electron from the excited chlorophyll special pair (**Figure 14–33**). This electron transfer converts the light energy that entered the special pair into the chemical energy of a transferable electron—a transformation that lies at the heart of photosynthesis.

As soon as a high-energy electron is passed from chlorophyll to an electron carrier, the chlorophyll special pair becomes positively charged, and the electron carrier that accepts the electron becomes negatively charged. The rapid movement of this electron along a set of intermediary electron carriers within the reaction center then creates a *charge separation* that sets in motion the flow of high-energy electrons from the reaction center to the electron-transport chain (**Figure 14–34**).

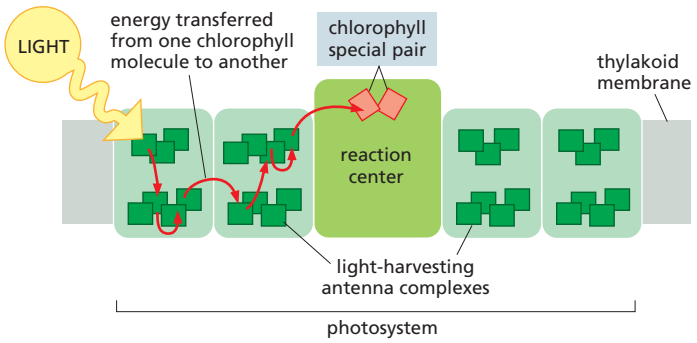


Figure 14–33 A photosystem consists of a reaction center surrounded by chlorophyll-containing antenna complexes. Once light energy has been captured by a chlorophyll molecule in an antenna complex, it will pass randomly from one chlorophyll molecule to another (*red* lines), until it gets trapped by a chlorophyll dimer called the *special pair*, located in the reaction center. The chlorophyll special pair holds its electrons at a somewhat lower energy than the antenna chlorophylls, so the energy transferred to it from the antenna gets trapped there. Note that in the antenna complex, it is energy that moves from one chlorophyll molecule to another, not electrons.

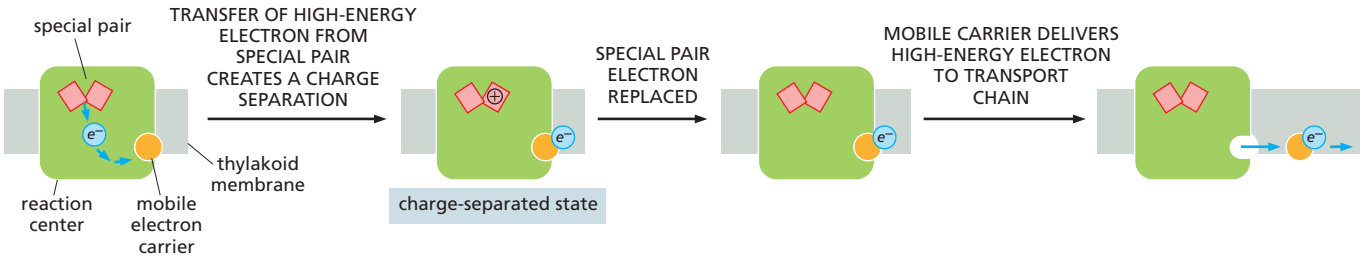


Figure 14–34 In a reaction center, a high-energy electron is transferred from the chlorophyll special pair to a carrier that becomes part of an electron-transport chain. Not shown is the set of intermediary carriers, embedded within the reaction center, that provides a rapid path (*blue* arrows) from the special pair to a mobile electron carrier (*orange*). As illustrated, the transfer of the high-energy electron from the excited chlorophyll special pair leaves behind a positive charge that creates a charge-separated state, thereby converting light energy to chemical energy. Once the electron in the special pair has been replaced (an event we will discuss in detail shortly), the mobile carrier diffuses away from the reaction center, transferring the high-energy electron to the transport chain.

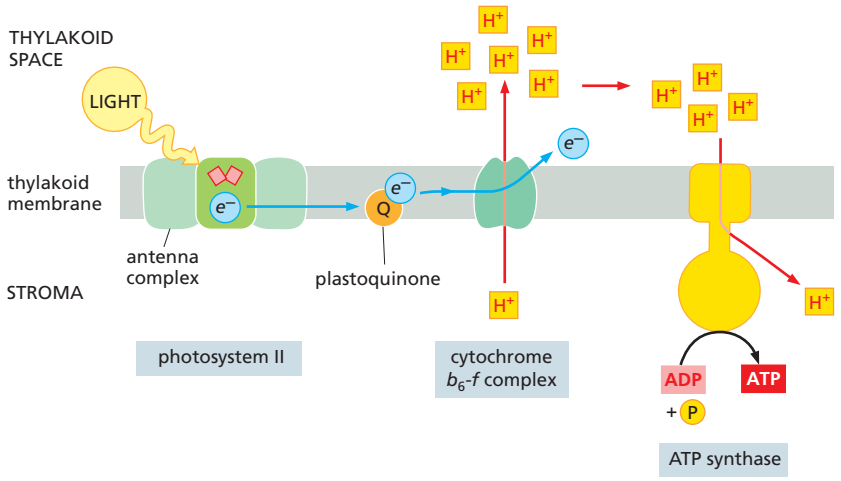
A Pair of Photosystems Cooperate to Generate both ATP and NADPH

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

When the first photosystem (which, paradoxically, is called photosystem II for historical reasons) absorbs light energy, its reaction center passes electrons to a mobile electron carrier called *plastoquinone*, which is part of the photosynthetic electron-transport chain. This carrier transfers the high-energy electrons to a proton pump, which—like the proton pumps in the mitochondrial inner membrane—uses the movement of electrons to generate an electrochemical proton gradient. The electrochemical proton gradient then drives the production of ATP by an ATP synthase located in the thylakoid membrane (**Figure 14–35**).

At the same time, a second, nearby photosystem—called photosystem I—has been also busy capturing the energy from sunlight. The reaction center of this photosystem passes its high-energy electrons to a different mobile electron carrier, called *ferredoxin*, which brings them to an enzyme that uses the electrons to reduce $NADP^+$ to NADPH (**Figure 14–36**). It is the combined action of these two photosystems that produces both the ATP (photosystem II) and the NADPH (photosystem I) required for carbon fixation in stage 2 of photosynthesis (see Figure 14–30).

Figure 14–35 Photosystem II feeds electrons to a photosynthetic proton pump, leading to the generation of ATP by ATP synthase. When light energy is captured by photosystem II, a high-energy electron is transferred to a mobile electron carrier called plastoquinone (Q), which closely resembles the ubiquinone of mitochondria. This carrier transfers its electrons to a proton pump called the cytochrome b_6-f complex, which resembles the cytochrome c reductase complex of mitochondria and is the sole site of active proton pumping in the chloroplast electron-transport chain. As in mitochondria, an ATP synthase embedded in the membrane then uses the energy of the electrochemical proton gradient to produce ATP.



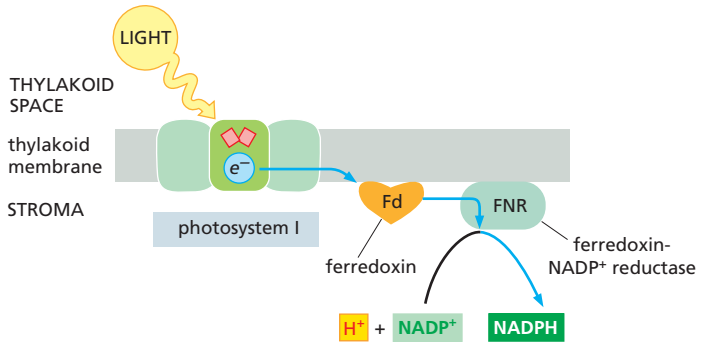


Figure 14–36 Photosystem I transfers high-energy electrons to an enzyme that produces NADPH. When light energy is captured by photosystem I, a high-energy electron is passed to a mobile electron carrier called ferredoxin (Fd), a small protein that contains an iron–sulfur center. Ferredoxin carries its electrons to ferredoxin-$NADP^+$ reductase (FNR), the final protein in the electron-transport chain that catalyzes the production of NADPH.

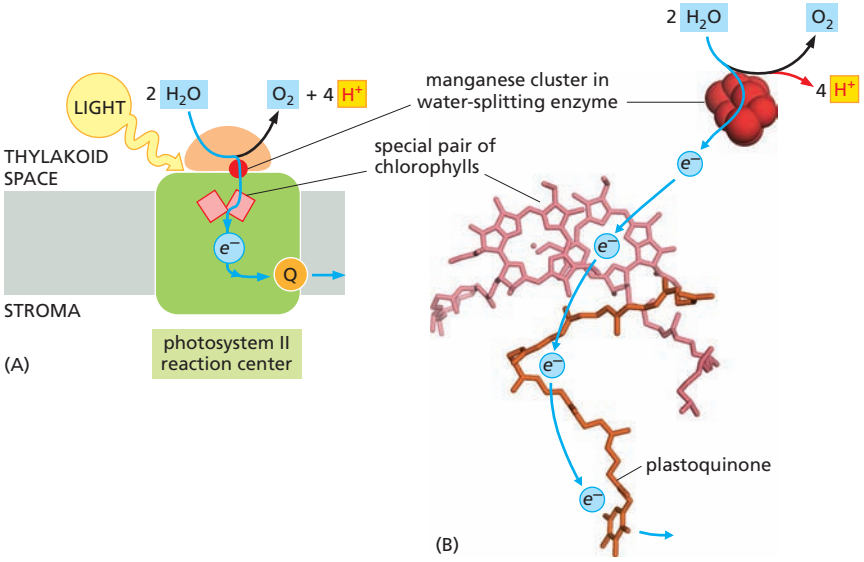
Oxygen Is Generated by a Water-Splitting Complex Associated with Photosystem II

The scheme that we have thus far described for photosynthesis has ignored a major chemical conundrum. When a mobile electron carrier removes an electron from a reaction center (whether in photosystem I or photosystem II), it leaves behind a positively charged chlorophyll special pair (see Figure 14–34). To reset the system and allow photosynthesis to proceed, this missing electron must be replaced.

For photosystem II, the missing electron is replaced by a special manganese-containing protein complex that removes the electrons from water. The cluster of manganese atoms in this *water-splitting enzyme* holds onto two water molecules from which electrons are extracted one at a time. Once four electrons have been removed from these two water molecules—and used to replace the electrons lost by four excited chlorophyll special pairs—O_2 is released (**Figure 14–37**). It is by this means that all of the O_2 in our atmosphere—all of the O_2 we breathe—is produced. Life on Earth would be a very different affair without the water-splitting enzyme of photosystem II.

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 biosynthesis?



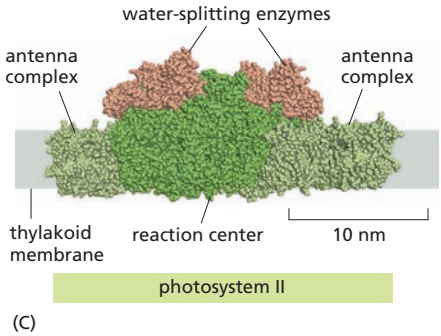


Figure 14–37 The reaction center of photosystem II includes a water-splitting enzyme that catalyzes the extraction of electrons from water. (A) Schematic diagram showing the flow of electrons through the reaction center of photosystem II. When light energy excites the chlorophyll special pair, an electron is passed to the mobile electron carrier plastoquinone (Q). An electron is then returned to the special pair by a water-splitting enzyme that extracts electrons from water. The manganese (Mn) cluster that participates in the electron extraction is shown as a *red spot*. Once four electrons have been withdrawn from two water molecules, O_2 is released into the atmosphere. (B) The structure and position of some of the electron carriers involved. (C) Structure of a membrane-embedded photosystem II (PSII) complex, including a reaction center and several light-harvesting antenna complexes. This structure, obtained from spinach, was determined by cryoelectron microscopy (see Panel 4–6, pp. 168–169). Note that this complex exists as a dimer in the membrane, and thus contains two copies of the water-splitting enzyme.

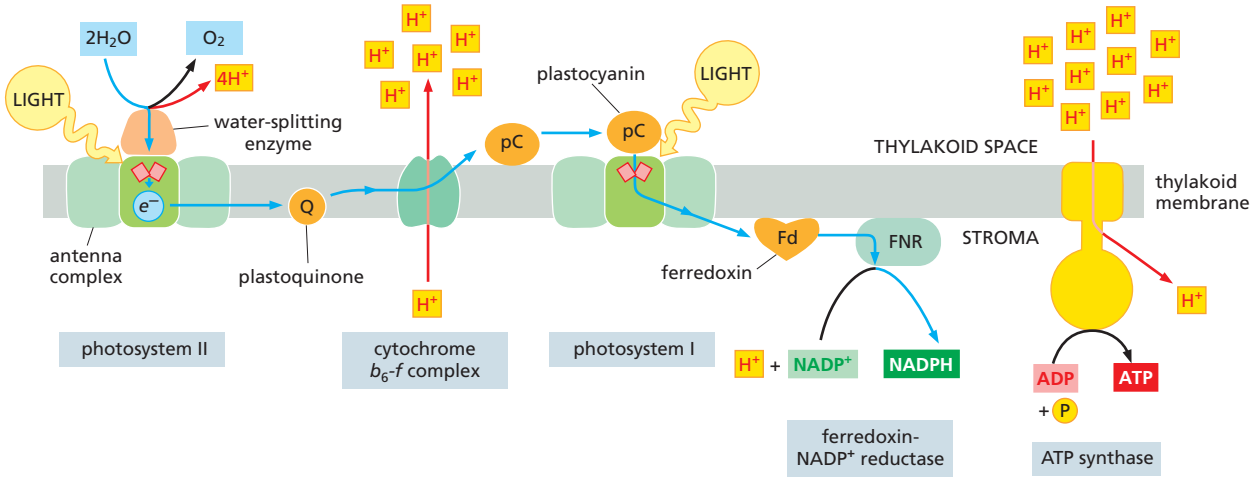


Figure 14–38 The serial movement of electrons through two photosystems powers the production of both ATP and NADPH. Electrons are supplied to photosystem II by a water-splitting enzyme that extracts four electrons from two molecules of water, producing O_2 as a by-product. Their energy is raised by the absorption of light to power the pumping of protons by the cytochrome b_6-f complex. Electrons that pass through this complex are then donated to a copper-containing protein, the mobile electron carrier plastocyanin (pC), which ferries them to the reaction center of photosystem I. After a second energy boost from light, these electrons are used to generate NADPH. An overview of these reactions is shown in **Movie 14.11**.

The "waiting for four electrons" maneuver executed by the water-splitting enzyme ensures that no partly oxidized water molecules are released as dangerous, highly reactive chemicals. As we discussed earlier, that same trick is used by the cytochrome *c* oxidase that catalyzes the reverse reaction—the transfer of electrons to O_2 to produce water—during oxidative phosphorylation (see Figure 14–24).

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

We have seen that photosystem II replaces electrons lost by its chlorophyll special pair with electrons extracted from water. But where does photosystem I get the electrons it needs to reset its special pair? These electrons come from photosystem II: the two photosystems work in series, such that the chlorophyll special pair in photosystem I serves as the final electron acceptor for the electron-transport chain that carries electrons from photosystem II. The overall flow of electrons through this linked system is shown in **Figure 14–38**. In sum, electrons removed from water by photosystem II are passed, through a proton pump (the cytochrome b_6-f complex), to a mobile electron carrier called plastocyanin. Plastocyanin then carries these electrons to photosystem I, to replace the electrons lost by its excited chlorophyll special pair. When light is again absorbed by this photosystem, the electrons will be boosted to the very high energy level needed to reduce $NADP^+$ to NADPH.

Having these two photosystems operating in series effectively couples their two electron-energizing steps. This extra boost of energy—provided by the light harvested by both photosystems—allows an electron to be transferred from water, which normally holds onto its electrons very tightly (redox potential = +820 mV), to NADPH, which normally holds onto its electrons loosely (redox potential = –320 mV). In addition to powering this chemistry, there is enough energy left over to enable the electron-transport chain that links the two photosystems to pump H^+ across the thylakoid membrane, so that the ATP synthase embedded in this membrane can also harness light-derived energy to produce ATP (**Figure 14–39**).

Carbon Fixation Uses ATP and NADPH to Convert CO_2 into Sugars

The light reactions of photosynthesis generate ATP and NADPH in the chloroplast stroma, as we have just seen. But the inner membrane of the chloroplast is impermeable to both of these compounds, which means

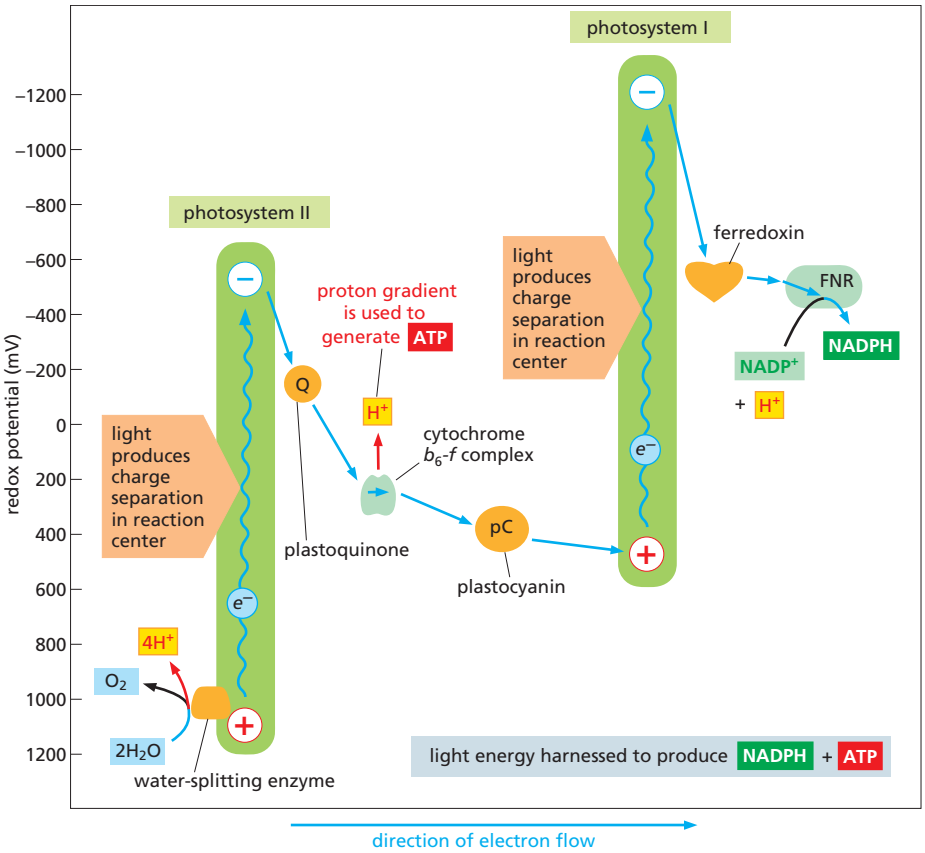


Figure 14–39 The combined actions of photosystems I and II boost electrons to the energy level needed to produce both ATP and NADPH. The redox potential for each molecule is indicated by its position on the vertical axis. Electron transfers are shown with non-wavy *blue* arrows. Photosystem II passes electrons from its excited chlorophyll special pair to an electron-transport chain in the thylakoid membrane that leads to photosystem I (see Figure 14–38). The net electron flow through these two photosystems linked in series is from water to $NADP^+$, to form NADPH.

that they cannot be exported directly to the cytosol. To provide energy and reducing power for the rest of the cell, the ATP and NADPH are instead used within the chloroplast stroma to produce a simple three-carbon sugar that can be exported to the cytosol by specific carrier proteins in the chloroplast inner membrane. This production of sugar from CO_2 and water, which occurs during stage 2 of photosynthesis, is called **carbon fixation**.

In the central reaction of photosynthetic carbon fixation, CO_2 from the atmosphere is attached to a five-carbon sugar derivative, ribulose 1,5-bisphosphate, to yield two molecules of the three-carbon compound *3-phosphoglycerate*. This carbon-fixing reaction, which was discovered in 1948, is catalyzed in the chloroplast stroma by a large enzyme called ribulose bisphosphate carboxylase or *Rubisco* (**Figure 14–40**). Rubisco works much more slowly than most other enzymes: it processes about three molecules of substrate per second—compared with 1000 molecules per second for a typical enzyme. To compensate for this sluggish behavior,

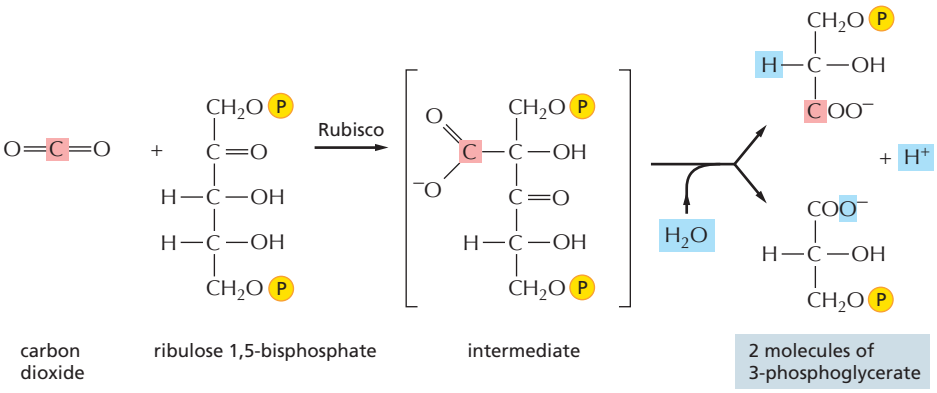


Figure 14–40 Carbon fixation is catalyzed by the enzyme ribulose bisphosphate carboxylase, also called Rubisco. In this reaction, which takes place in the chloroplast stroma, a covalent bond is formed between carbon dioxide and an energy-rich molecule of ribulose 1,5-bisphosphate. This union generates a chemical intermediate that then reacts with water (highlighted in *blue*) to generate two molecules of 3-phosphoglycerate.

plants maintain a surplus of Rubisco to ensure the efficient production of sugars. The enzyme generally represents more than 50% of the total chloroplast protein, and it is widely claimed to be the most abundant protein on Earth.

Although the production of carbohydrates from CO_2 and H_2O is extremely energetically unfavorable, the fixation of CO_2 catalyzed by Rubisco is actually an energetically favorable reaction. That's because a continuous supply of energy-rich ribulose 1,5-bisphosphate is fed into the reaction. As this compound is consumed—by the addition of CO_2 (see Figure 14–40)—it must be replenished. The energy and reducing power needed to regenerate ribulose 1,5-bisphosphate come from the ATP and NADPH produced by the photosynthetic light reactions.

The elaborate series of reactions in which CO_2 combines with ribulose 1,5-bisphosphate to produce a simple three-carbon sugar—a portion of which is used to regenerate the ribulose 1,5-bisphosphate that's consumed—forms a cycle, called the *carbon-fixation cycle*, or the Calvin cycle (**Figure 14–41**). For every three molecules of CO_2 that enter the cycle, one molecule of glyceraldehyde 3-phosphate is ultimately produced, at the

QUESTION 14–10

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

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

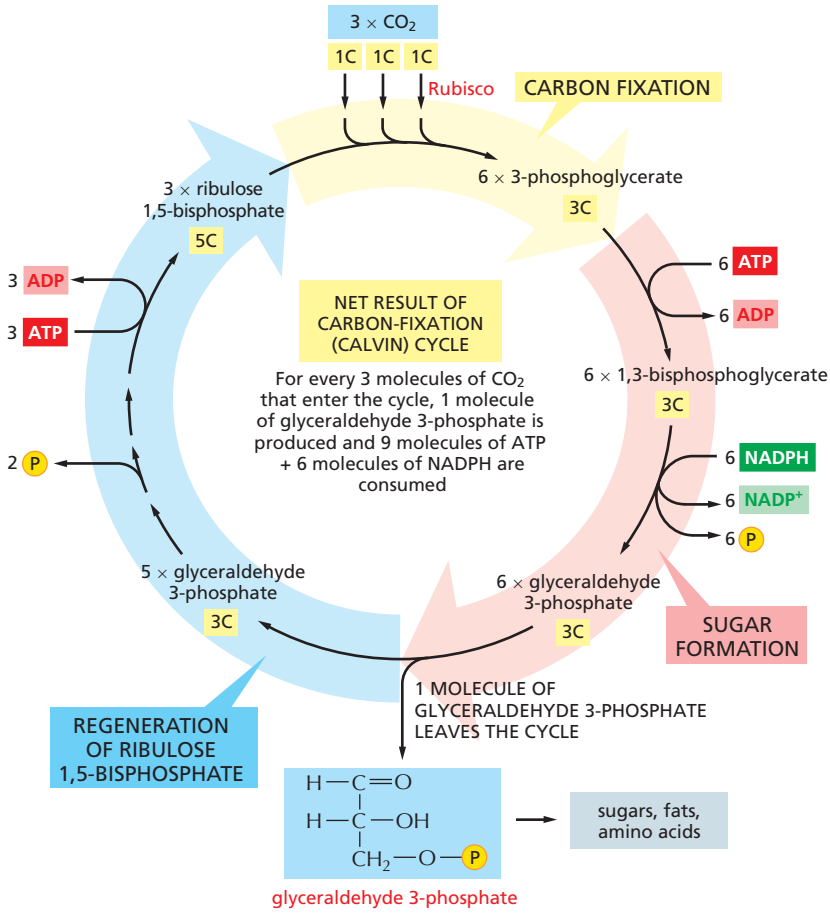


Figure 14–41 The carbon-fixation cycle consumes ATP and NADPH to form glyceraldehyde 3-phosphate from CO_2 and H_2O. In the first stage of the cycle (highlighted in *yellow*), CO_2 is added to ribulose 1,5-bisphosphate (as shown in Figure 14–40). In the second stage (highlighted in *red*), ATP and NADPH are consumed to convert 3-phosphoglycerate to glyceraldehyde 3-phosphate. In the final stage (highlighted in *blue*), most of the glyceraldehyde 3-phosphate produced is used to regenerate ribulose 1,5-bisphosphate; the rest is transported out of the chloroplast stroma into the cytosol. The number of carbon atoms in each type of molecule is indicated in *yellow*. There are many intermediates between glyceraldehyde 3-phosphate and ribulose 1,5-bisphosphate, but they have been omitted here for clarity. The entry of water into the cycle is also not shown.

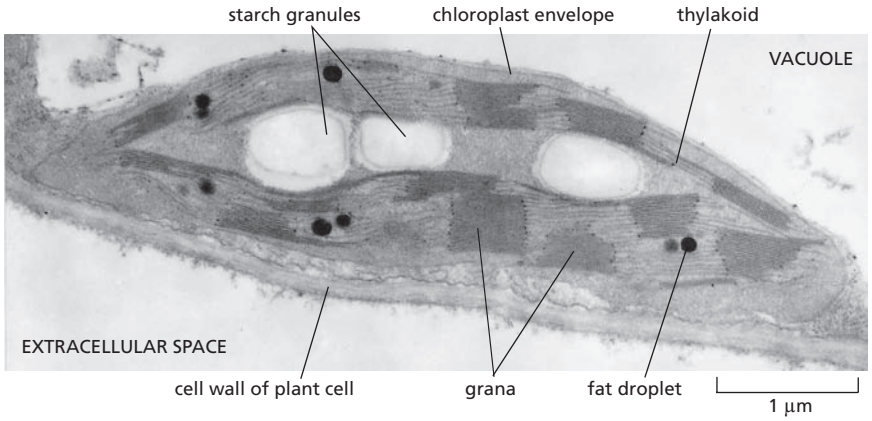


Figure 14–42 Chloroplasts often contain large stores of carbohydrates and fatty acids. An electron micrograph of a thin section of a single chloroplast shows the chloroplast envelope and the starch granules and fat droplets that have accumulated in the stroma as a result of the biosynthetic processes that occur there. (Courtesy of K. Plaskitt.)

expense of nine molecules of ATP and six molecules of NADPH, which are consumed in the process. *Glyceraldehyde 3-phosphate*, the three-carbon sugar that is the final product of the cycle, provides the starting material for the synthesis of the many other sugars and other organic molecules that the plant needs.

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

The glyceraldehyde 3-phosphate generated by carbon fixation in the chloroplast stroma can be used in a number of ways, depending on the needs of the plant. During periods of excess photosynthetic activity, much of the sugar is retained in the chloroplast stroma and converted to *starch*. Like glycogen in animal cells, starch is a large polymer of glucose that serves as a carbohydrate reserve, and it is stored as large granules in the chloroplast stroma. Starch forms an important part of the diet of all animals that eat plants. Other glyceraldehyde 3-phosphate molecules are converted to fat in the stroma. This material, which accumulates as fat droplets, likewise serves as an energy reserve (**Figure 14–42**).

At night, this stored starch and fat can be broken down to sugars and fatty acids, which are exported to the cytosol to help support the metabolic needs of the plant. Some of the exported sugar enters the glycolytic pathway (see Figure 13–5), where it is converted to pyruvate. Most of that pyruvate, along with the fatty acids, enters the plant cell mitochondria and is fed into the citric acid cycle, ultimately leading to the production of ATP by oxidative phosphorylation (**Figure 14–43**). Plants use this ATP to power a huge variety of metabolic reactions, just as animal cells and other nonphotosynthetic organisms do.

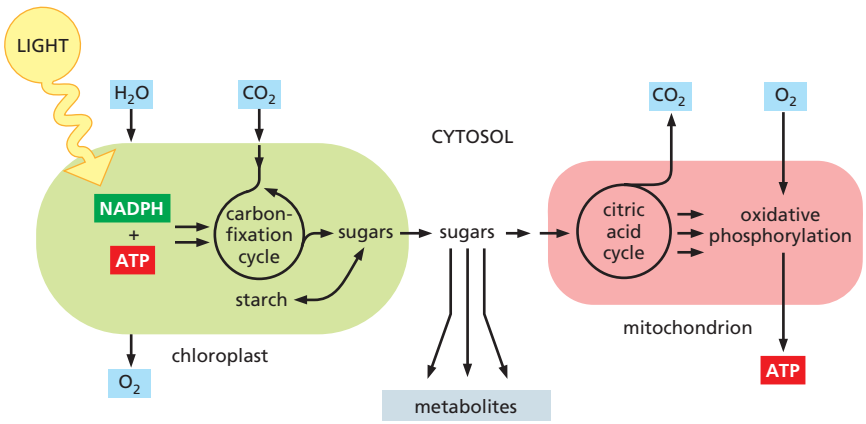


Figure 14–43 In plants, the chloroplasts and mitochondria collaborate to supply cells with metabolites and ATP. The chloroplast's inner membrane is impermeable to the ATP and NADPH that are produced in the stroma during the light reactions of photosynthesis. These molecules are funneled into the carbon-fixation cycle, where they are used to make sugars. The resulting sugars and their metabolites are either stored within the chloroplast—in the form of starch or fat—or exported to the rest of the plant cell. There, they can enter the energy-generating pathway that ends in ATP synthesis in the mitochondria. Unlike those chloroplasts, mitochondrial membranes are permeable to ATP, as indicated. Note that some of the O_2 released to the atmosphere by photosynthesis in chloroplasts is used for oxidative phosphorylation in mitochondria; similarly, some of the CO_2 released by the citric acid cycle in mitochondria is used for carbon fixation in chloroplasts.

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

THE EVOLUTION OF ENERGY-GENERATING SYSTEMS

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

Oxidative Phosphorylation Evolved in Stages

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

A buildup of such 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, preventing the cell interior from becoming too acidic. Some of these pumps may have used the energy available from ATP hydrolysis to eject H^+ from the cell (stage 1 in **Figure 14–44**). Such a proton pump could have been the ancestor of present-day ATP synthases. Other pumps, like those in modern respiratory chain complexes, eventually evolved to use the movement of electrons between molecules of different redox potentials as a source of energy for pumping H^+ across the plasma membrane (stage 2 in Figure 14–44). Indeed, some present-day bacteria that grow on formic acid use the small amount of redox energy derived from the transfer of electrons from formic acid to fumarate to pump H^+.

When these H^+-pumping electron-transport systems became efficient enough, cells could harvest more redox energy than they needed to

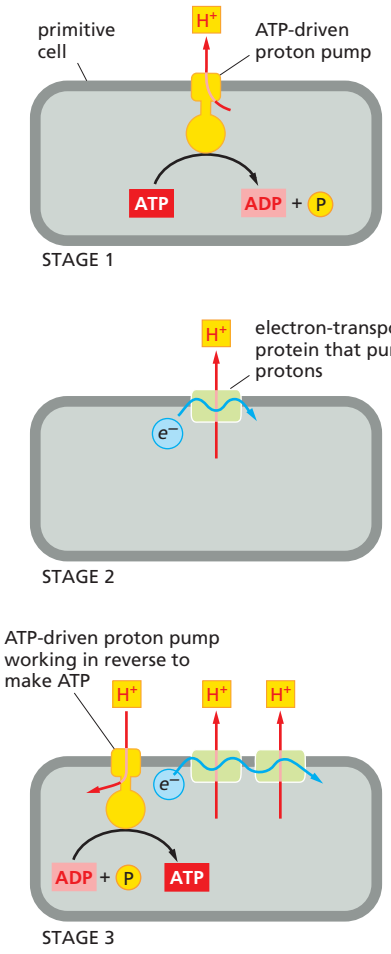


Figure 14–44 Chemiosmotic processes most likely evolved in stages. The first stage might have involved the evolution of an ATPase that pumped protons out of the cell using the energy of ATP hydrolysis. Stage 2 could have involved the evolution of a different proton pump, driven by an electron-transport chain. Stage 3 could then link these two systems together to generate an ATP synthase that uses the protons pumped by the electron-transport chain to synthesize ATP. An early cell with this final system would have had a large selective advantage over cells with neither of the systems or only one.

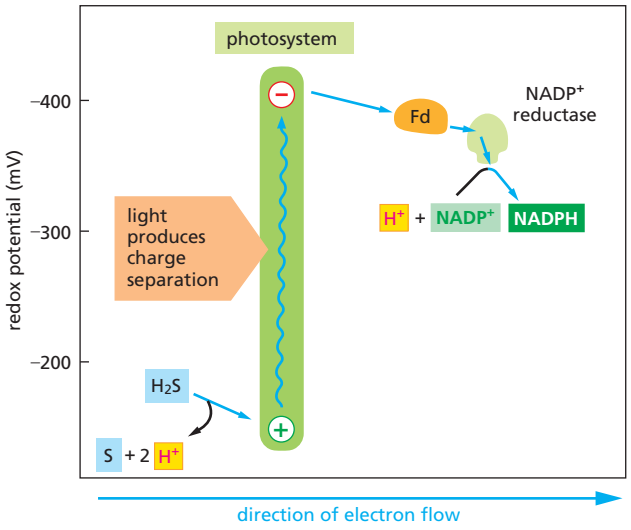


Figure 14–45 Photosynthesis in green sulfur bacteria uses hydrogen sulfide (H_2S) as an electron donor rather than water. Electrons are easier to extract from H_2S than from H_2O, because H_2S has a much higher redox potential (compare with Figure 14–39). Therefore, only one photosystem is needed to produce NADPH, and elemental sulfur is formed as a by-product instead of O_2. The photosystem in green sulfur bacteria resembles photosystem I in plants and cyanobacteria. These photosystems all use a series of iron–sulfur centers as the electron carriers that eventually donate their high-energy electrons to ferredoxin (Fd). A bacterium of this type is *Chlorobium tepidum*, which can thrive at high temperatures and low light intensities in hot springs.

maintain their internal pH. These cells could then generate large electrochemical proton gradients, which they could couple to the production of ATP (stage 3 in Figure 14–44). Because such cells would require much less of the dwindling supply of fermentable nutrients, they would have proliferated at the expense of their neighbors.

Photosynthetic Bacteria Made Even Fewer Demands on Their Environment

The major evolutionary breakthrough in energy metabolism, however, was almost certainly the formation of photochemical reaction centers that could use the energy of sunlight to produce molecules such as NADPH. It is thought that this development occurred early in the process of evolution—more than 3 billion years ago, in the ancestors of green sulfur bacteria. Present-day green sulfur bacteria use light energy to transfer hydrogen atoms (as an electron plus a proton) from H_2S to NADPH, thereby creating the strong reducing power required for carbon fixation (**Figure 14–45**).

The next step is thought to have involved the evolution of organisms capable of using water instead of H_2S as the electron source for photosynthesis. This entailed the evolution of a water-splitting enzyme and the addition of a second photosystem, acting in conjunction with the first, to bridge the enormous gap in redox potential between H_2O and NADPH (see Figure 14–39).

The biological consequences of this evolutionary step were far-reaching. For the first time, there were organisms that made only minimal chemical demands on their environment. These cells—including the first cyanobacteria (see Figure 14–28)—could spread and evolve in ways denied to the earlier photosynthetic bacteria, which needed H_2S, organic acids, or other sources of electrons. Consequently, large amounts of fermentable organic materials—produced by these cells and their ancestors—began to accumulate. Moreover, O_2 began to enter the atmosphere in large amounts (**Figure 14–46**).

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

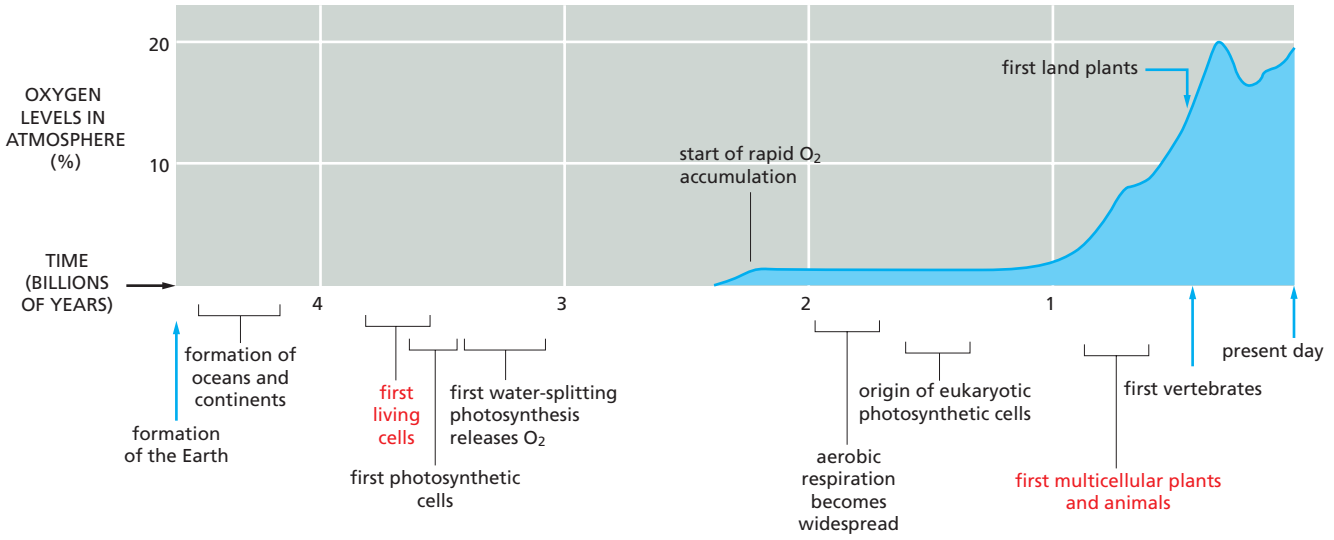


Figure 14–46 Oxygen entered Earth's atmosphere billions of years ago. With the evolution of photosynthesis in prokaryotes more than 3 billion years ago, organisms would have no longer depended on preformed organic chemicals: they could make their own organic molecules from CO_2. Note that there was a delay of about a billion years between the appearance of photosynthetic bacteria that split water and released O_2 and the accumulation of high levels of O_2 in the atmosphere. This delay is thought to have been due to the initial reaction of the O_2 with abundant ferrous iron (Fe^{2+}) dissolved in the early oceans. Only when the iron was used up could large amounts of O_2 begin to accumulate in the atmosphere. In response to rising amounts of O_2 in the atmosphere, nonphotosynthetic, aerobic organisms appeared, and the concentration of O_2 in the atmosphere eventually leveled out.

As organic materials accumulated as a by-product of photosynthesis, some photosynthetic bacteria—including the ancestors of the bacterium *Escherichia coli*—lost their ability to survive on light energy alone and came to rely entirely on cell respiration. Mitochondria arose when a pre-eukaryotic cell engulfed such an aerobic bacterium (see Figure 1–19). Plants arose somewhat later, when a descendant of this early aerobic eukaryote captured a photosynthetic bacterium, which became the precursor of chloroplasts (see Figure 1–21). Once eukaryotes had acquired the bacterial symbionts that became mitochondria and chloroplasts, they could then embark on the spectacular pathway of evolution that eventually led to complex multicellular organisms, including ourselves.

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

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

One of the archaea that live in this environment today is *Methanococcus jannaschii*. Originally isolated from a hydrothermal vent more than a mile beneath the ocean surface, the organism grows in the complete absence of light and gaseous oxygen, using as nutrients the inorganic gases—hydrogen (H_2), CO_2, and nitrogen (N_2)—that bubble up from the vent (**Figure 14–47**). Its mode of existence gives us a hint of how early cells might have used electron transport to derive energy and to extract carbon molecules from inorganic materials that were freely available on the hot early Earth.

Methanococcus relies on N_2 gas as its source of nitrogen for making organic molecules such as amino acids. The organism reduces N_2 to ammonia (NH_3) by the addition of hydrogen, a process called **nitrogen fixation**. Nitrogen fixation requires a large amount of energy, as does the carbon-fixation process that converts CO_2 and H_2O into sugars. Much

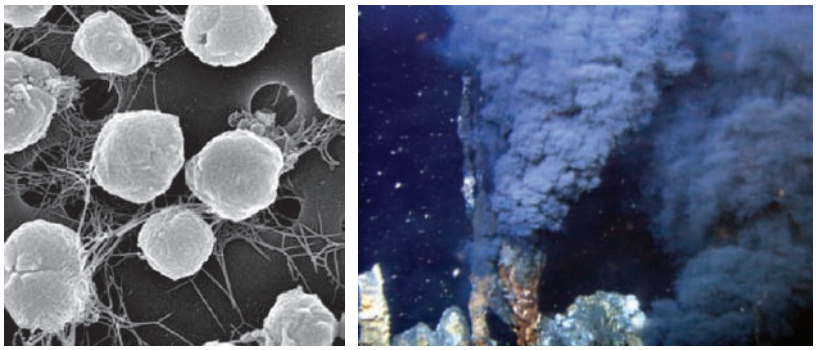


Figure 14–47 *Methanococcus* represents life-forms that might have existed early in Earth's history. (A) Scanning electron micrograph showing individual *Methanococcus* cells. These deep-sea archaea use the hydrogen gas (H_2) that bubbles from deep-sea vents (B) as the source of reducing power to generate energy via chemiosmotic coupling. (A, from C.B. Park & D.S. Clark, *Appl. Environ. Microbiol.* 68:1458–1463, 2002. With permission from the American Society for Microbiology; B, National Oceanic and Atmospheric Administration's Pacific Marine Environmental Laboratory Vents Program.)

of the energy that *Methanococcus* requires for both processes is derived from the transfer of electrons from H_2 to CO_2, with the release of large amounts of methane (CH_4) as a waste product (thus producing natural gas and giving the organism its name). Part of this electron transfer occurs in the plasma membrane and results in the pumping of protons (H^+) across it. The resulting electrochemical proton gradient drives an ATP synthase in the same membrane to make ATP.

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

ESSENTIAL CONCEPTS

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

- Electron-transport chains associated with photosystems transfer electrons from water to $NADP^+$ to form NADPH, which produces O_2 as a by-product.
- The photosynthetic electron-transport chains in chloroplasts also generate a proton gradient across the thylakoid membrane, which is used by an ATP synthase embedded in that membrane to generate ATP.
- The ATP and the NADPH made by photosynthesis are used within the chloroplast stroma to drive the carbon-fixation cycle, which produces carbohydrate from CO_2 and water.
- Carbohydrate is exported from the stroma to the plant cell cytosol; there it provides the starting material used for the synthesis of many other organic molecules and for the production of the materials used by plant cell mitochondria to produce ATP.
- Both mitochondria and chloroplasts are thought to have evolved from bacteria that were endocytosed by other cells. Each retains its own genome and divides by processes that resemble bacterial cell division.
- Chemiosmotic coupling mechanisms are of ancient origin. Modern microorganisms that live in environments similar to those thought to have been present on the early Earth also use chemiosmotic coupling to produce ATP.

KEY TERMS

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

QUESTIONS

QUESTION 14–11

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

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

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

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

QUESTION 14–12

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

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

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

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

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

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

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

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

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

QUESTION 14–13

A single proton moving down its electrochemical gradient into the mitochondrial matrix space liberates 19.2 kJ/mole of free energy (ΔG). How many protons have to flow across the inner mitochondrial membrane to synthesize one molecule of ATP if the ΔG for ATP synthesis under intracellular conditions is between 46 and 54 kJ/mole? (ΔG is discussed in Chapter 3, pp. 88–98.) 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 answers. "If no O_2 is available, all components of the mitochondrial electron-transport chain will accumulate in their *reduced/oxidized* form. If O_2 is suddenly added again, the electron carriers in cytochrome *c* oxidase will become *reduced/oxidized before/after* those in NADH dehydrogenase."

QUESTION 14–15

Assume that the conversion of oxidized ubiquinone to reduced ubiquinone by NADH dehydrogenase occurs on the matrix side of the inner mitochondrial membrane and that its oxidation by cytochrome *c* reductase occurs on the intermembrane-space side of the membrane (see Figures 14–14 and 14–21). What are the consequences of this arrangement for the generation of the H^+ gradient across the membrane?

QUESTION 14–16

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

QUESTION 14–17

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

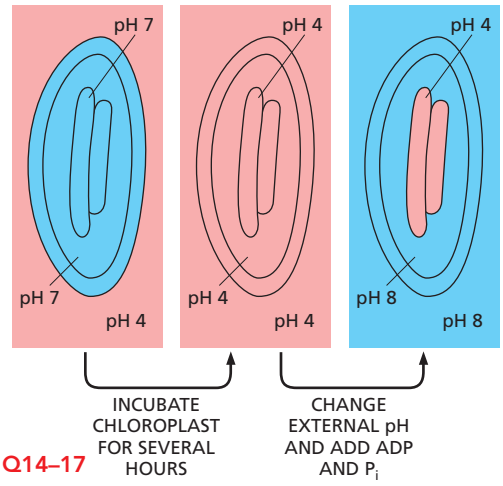


Figure Q14–17

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

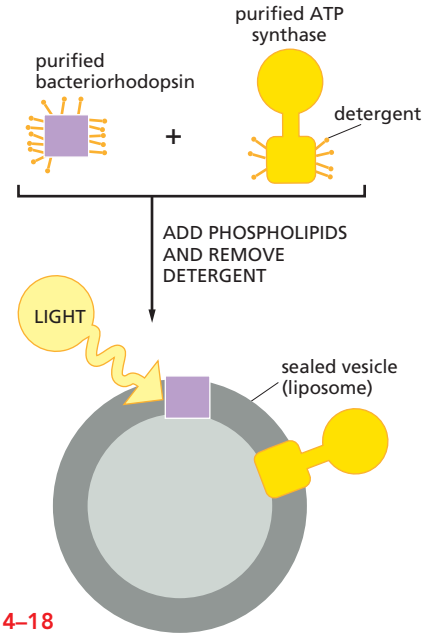


Figure Q14–18

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

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

and

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

The redox potential of $FADH_2$, however, is only –220 mV. Referring to Panel 14–1 (p. 472) and Figure 14–22, 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?

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 reconstituted membrane-bound system. Assuming that the electrons must follow the path specified in Figure 14–14, in which experiments would you expect a net transfer of electrons to cytochrome *c*? Discuss why electron transfer does not occur in the other experiments.

A. reduced ubiquinone and oxidized cytochrome *c*

B. oxidized ubiquinone and oxidized cytochrome *c*

C. reduced ubiquinone and reduced cytochrome *c*

D. oxidized ubiquinone and reduced cytochrome *c*

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

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

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

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

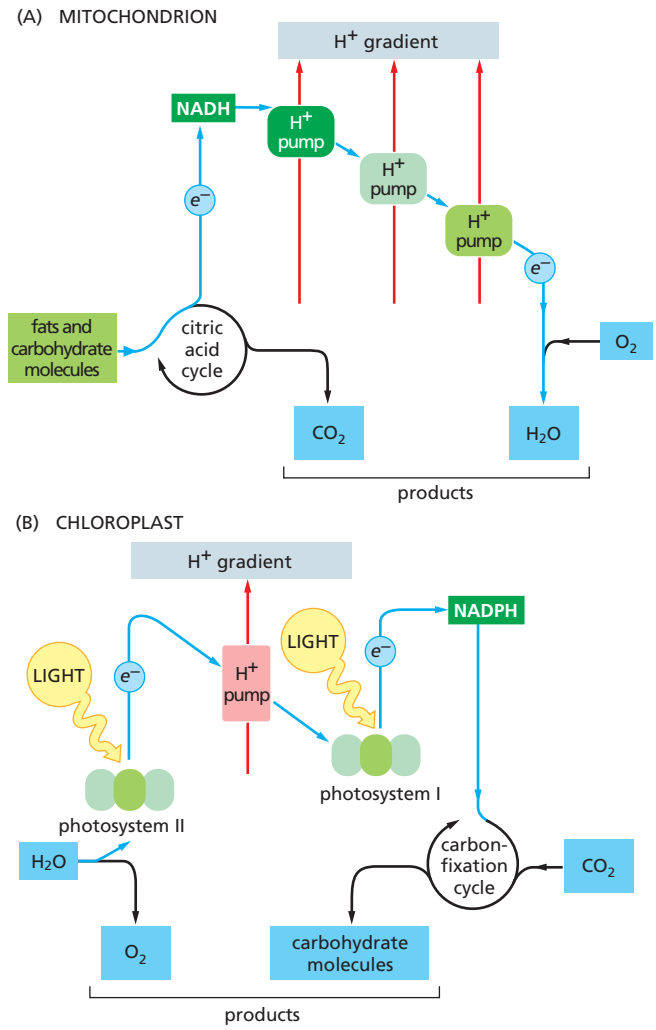


Figure Q14–21