CHAPTER 14

Energy Conversion: Mitochondria and Chloroplasts

IN THIS CHAPTER

To maintain their high degree of organization in a universe that is constantly drifting toward chaos, cells have a constant need for a plentiful supply of ATP, as we have explained in Chapter 2. In eukaryotic cells, most of the ATP that powers life processes is produced by specialized, membrane-enclosed, *energy-converting organelles*. These are of two types. **Mitochondria**, which occur in virtually all cells of animals, plants, and fungi, burn food molecules to produce ATP by *oxidative phosphorylation*. **Chloroplasts**, which occur only in plants and green algae, harness solar energy to produce ATP by *photosynthesis*. In electron micrographs, the most striking features of both mitochondria and chloroplasts are their extensive internal membrane systems. These internal membranes contain sets of membrane protein complexes that work together to produce most of the cell's ATP. In bacteria, simpler versions of essentially the same protein complexes produce ATP, but they are located in the cell's plasma membrane (**Figure 14–1**).

Comparisons of DNA sequences indicate that the energy-converting organelles in present-day eukaryotes originated from prokaryotic cells that were endocytosed during the evolution of eukaryotes (discussed in Chapter 1). This explains why mitochondria and chloroplasts contain their own DNA, which still encodes a subset of their proteins. Over time, these organelles have lost most of their own genomes and become heavily dependent on proteins that are encoded by genes in the nucleus, synthesized in the cytosol, and then imported into the organelle. And the eukaryotic cells now rely on these organelles not only for the ATP they need for biosynthesis, solute transport, and movement, but also for many important biosynthetic reactions that occur inside each organelle.

The common evolutionary origin of the energy-converting machinery in mitochondria, chloroplasts, and prokaryotes (archaea and bacteria) is reflected in the fundamental mechanism that they share for harnessing energy. This is known as **chemiosmotic coupling**, signifying a link between the chemical bond-forming reactions that generate ATP ("chemi") and membrane transport processes

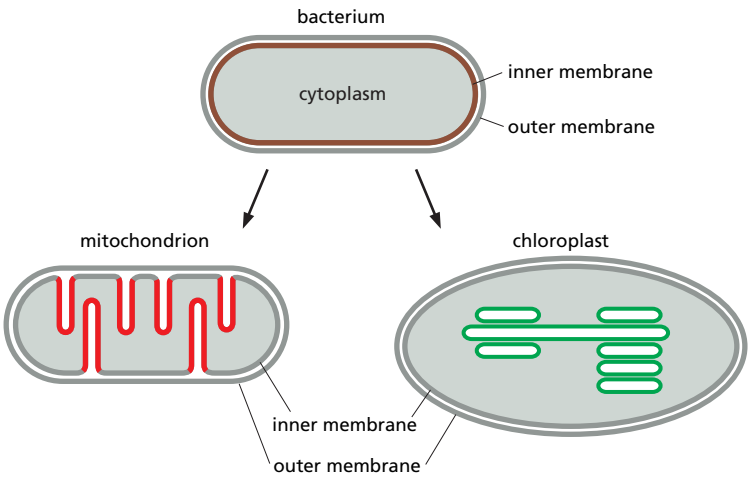


Figure 14–1 The membrane systems of bacteria, mitochondria, and chloroplasts are related. Mitochondria and chloroplasts are cell organelles that have originated from bacteria and have retained the bacterial energy-conversion mechanisms. Like their bacterial ancestors, mitochondria and chloroplasts have an outer and an inner membrane. Each of the membranes colored in this diagram contains energy-harvesting electron-transport chains. The deep invaginations of the mitochondrial inner membrane and the internal membrane system of the chloroplast harbor the machinery for cellular respiration and photosynthesis, respectively.

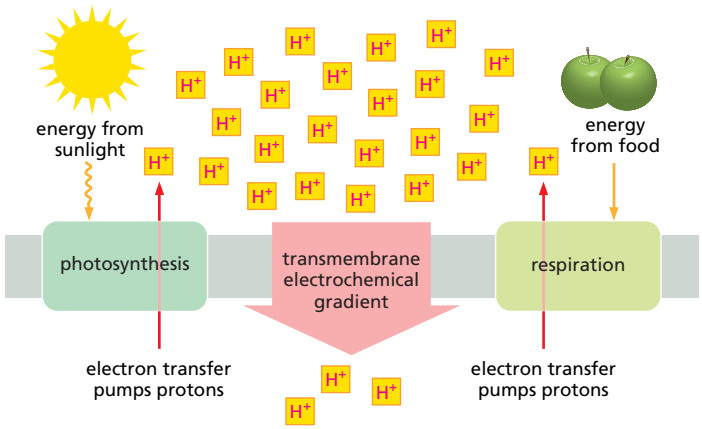


Figure 14–2 Stage 1 of chemiosmotic coupling. Energy from sunlight or the oxidation of food compounds is captured to generate an electrochemical proton gradient across a membrane. The electrochemical gradient serves as a versatile energy store that drives energy-requiring reactions in mitochondria, chloroplasts, and bacteria.

("osmotic"). The chemiosmotic process occurs in two linked stages, both of which are performed by protein complexes in a membrane.

Stage 1: High-energy electrons (derived from the oxidation of food molecules, from pigments excited by sunlight, or from other sources described later) are transferred along a series of electron-transport protein complexes that form an *electron-transport chain* embedded in a membrane. Each electron transfer releases a small amount of energy that is used to pump protons (H^+) and thereby generate a large *electrochemical gradient* across the membrane (**Figure 14–2**). As discussed in Chapter 11, such an electrochemical gradient provides a way of storing energy, and it can be harnessed to do useful work when ions flow back across the membrane.

Stage 2: The protons flow back down their electrochemical gradient through an elaborate membrane protein machine called *ATP synthase*, which catalyzes the production of ATP from ADP and inorganic phosphate (P_i). This ubiquitous enzyme works like a turbine in the membrane, driven by protons, to synthesize ATP (**Figure 14–3**). In this way, the energy derived from food or sunlight in stage 1 is converted into the chemical energy of a phosphate bond in ATP.

Electrons move through protein complexes in biological systems via tightly bound metal ions or other carriers that take up and release electrons easily, or by special small molecules that pick electrons up at one location and deliver them to another. For mitochondria, the first of these electron carriers is NAD^+, a water-soluble small molecule that takes up two electrons and one H^+ derived from food molecules (fats and carbohydrates) to become NADH. NADH transfers these electrons from the sites where the food molecules are degraded to the inner mitochondrial membrane. There, the electrons from the energy-rich NADH are passed from one membrane protein complex to the next, passing to a lower-energy compound at each step, until they reach a final complex in which they combine with molecular oxygen (O_2) to produce water. The energy released at each step as the electrons flow down this path from the energy-rich NADH to the low-energy water molecule drives H^+ pumps in the inner mitochondrial membrane, utilizing three different membrane protein complexes. Together, these complexes generate the proton-motive force harnessed by ATP synthase to produce the ATP that serves as the universal energy currency throughout the cell (see Chapter 2).

Figure 14–4 compares the electron-transport processes in mitochondria, which harness energy from food molecules, with those in chloroplasts, which harness energy from sunlight. The energy-conversion systems of mitochondria and chloroplasts can be described in similar terms, and we shall see later in the chapter that two of their key components are closely related. One of these is the ATP synthase, and the other is a proton pump (colored green in Figure 14–4).

Among the crucial constituents that are unique to photosynthetic organisms are the two *photosystems*. These use the green pigment chlorophyll to capture

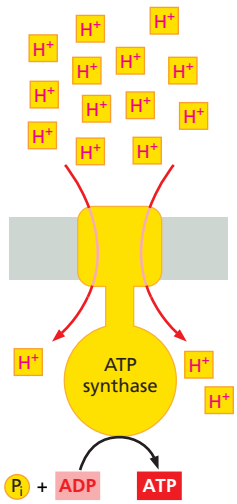


Figure 14–3 Stage 2 of chemiosmotic coupling. An ATP synthase *(yellow)* embedded in the lipid bilayer of a membrane harnesses the electrochemical proton gradient across the membrane, using it as a local energy store to drive ATP synthesis. The *red arrows* show the direction of proton movement through the ATP synthase.

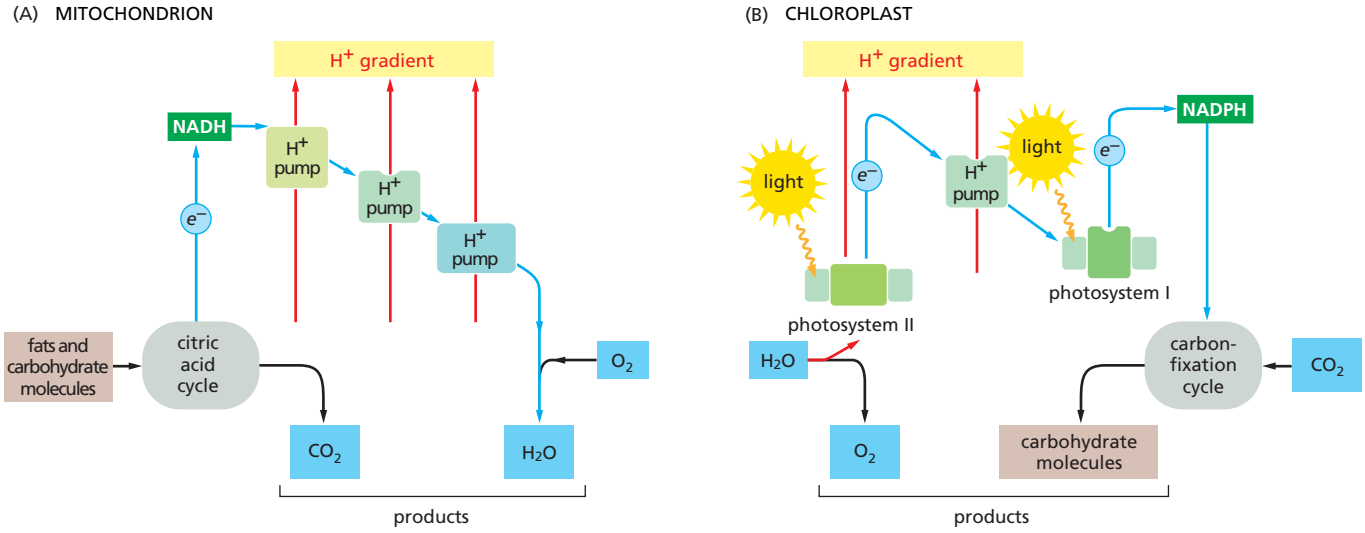


Figure 14–4 Electron-transport processes. (A) The mitochondrion converts energy from chemical fuels. (B) The chloroplast converts energy from sunlight. In both cases, electron flow is indicated by *blue arrows*. Each of the protein complexes *(green)* is embedded in a membrane. In the mitochondrion, fats and carbohydrates from food molecules are fed into the citric acid cycle and provide electrons to generate the energy-rich compound NADH from NAD^+. These electrons then flow down an energy gradient as they pass from one complex to the next in the electron-transport chain, until they combine with molecular O_2 in the last complex to produce water. The energy released at each stage is harnessed to pump H^+ across the membrane. In the chloroplast, by contrast, electrons are extracted from water through the action of light in the photosystem II complex and molecular O_2 is released. The electrons pass on to the next complex in the chain, which uses some of their energy to pump protons across the membrane, before passing to photosystem I, where sunlight generates high-energy electrons that combine with $NADP^+$ to produce NADPH. NADPH then enters the *carbon-fixation cycle* along with CO_2 to generate carbohydrates.

light energy and power the transfer of electrons, not unlike a photocell in a solar panel. The chloroplasts drive electron transfer in the direction opposite to that in mitochondria: electrons are taken from water to produce O_2, and these electrons are used (via NADPH, a molecule closely related to the NADH used in mitochondria) to synthesize carbohydrates from CO_2 and water. These carbohydrates then serve as the source for all other compounds a plant cell needs.

Thus, both mitochondria and chloroplasts make use of an electron-transfer chain to produce an H^+ gradient that powers reactions that are critical for the cell. However, chloroplasts generate O_2 and take up CO_2, whereas mitochondria consume O_2 and release CO_2 (see Figure 14–4).

THE MITOCHONDRION

Mitochondria occupy up to 20% of the cytoplasmic volume of a eukaryotic cell. Although they are often depicted as short, bacterium-like bodies with a diameter of 0.5–1 μm, they are in fact remarkably dynamic and plastic, moving about the cell, constantly changing shape, dividing, and fusing (**Movie 14.1**). Mitochondria are often associated with the microtubular cytoskeleton (**Figure 14–5**), which determines their orientation and distribution in different cell types. Thus, in highly polarized cells such as neurons, mitochondria can move long distances (up to a meter or more in the extended axons of neurons), being propelled along the tracks of the microtubular cytoskeleton. In other cells, mitochondria remain fixed at points of high energy demand; for example, in skeletal or cardiac muscle cells, they pack between myofibrils, and in sperm cells they wrap tightly around the flagellum (**Figure 14–6**).

Mitochondria also interact with other membrane systems in the cell, most notably the endoplasmic reticulum (ER). Contacts between mitochondria and ER define specialized domains thought to facilitate the exchange of lipids between the two membrane systems. These contacts also appear to induce mitochondrial

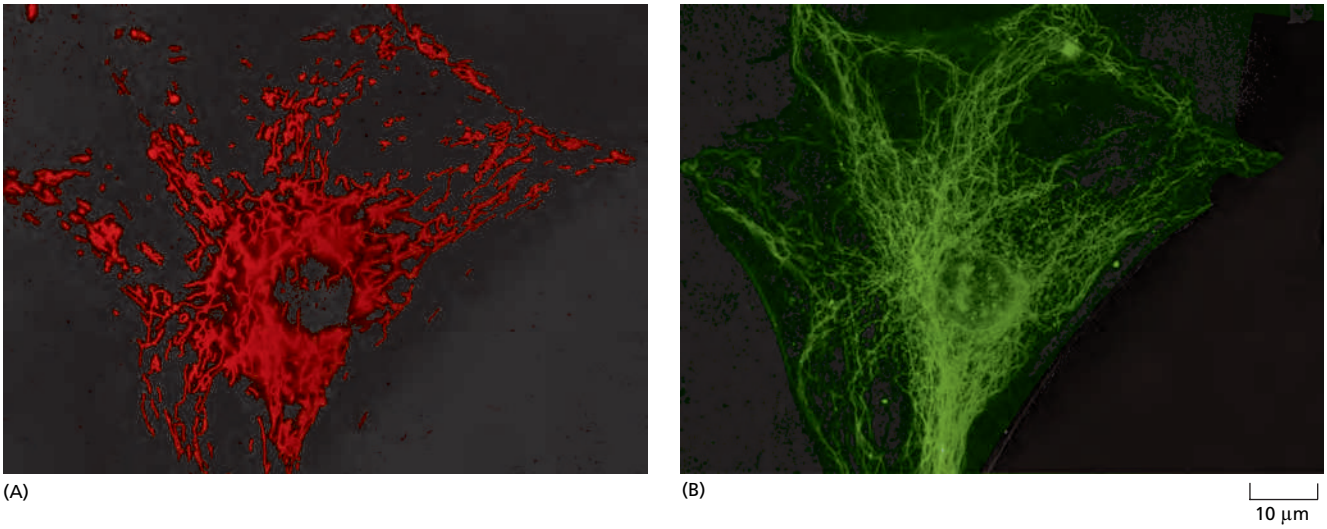


Figure 14–5 The relationship between mitochondria and microtubules. (A) A light micrograph of chains of elongated mitochondria in a living mammalian cell in culture. The cell was stained with a fluorescent dye (rhodamine 123) that specifically labels mitochondria in living cells. (B) An immunofluorescence micrograph of the same cell stained (after fixation) with fluorescent antibodies that bind to microtubules. Note that the mitochondria tend to be aligned along microtubules. (Courtesy of Lan Bo Chen.)

fission, which, as we discuss later, is involved in the distribution and partitioning of mitochondria within cells (Figure 14–7).

The acquisition of mitochondria was a prerequisite for the evolution of complex animals. Without mitochondria, present-day animal cells would have had to generate all of their ATP through anaerobic glycolysis. When glycolysis converts glucose to pyruvate, it releases only a small fraction of the total free energy that is potentially available from glucose oxidation (see Chapter 2). In mitochondria, the metabolism of sugars is complete: pyruvate is imported into the mitochondrion and ultimately oxidized by O_2 to CO_2 and H_2O, which allows 15 times more ATP to be made from a sugar than by glycolysis alone. As explained later, this became possible only when enough molecular oxygen accumulated in the Earth's atmosphere to allow organisms to take full advantage, via respiration, of the large amounts of energy potentially available from the oxidation of organic compounds.

Mitochondria are large enough to be seen in the light microscope, and they were first identified in the nineteenth century. Real progress in understanding their internal structure and function, however, depended on biochemical procedures developed in 1948 for isolating intact mitochondria, and on electron microscopy, which was first used to look at cells at about the same time.

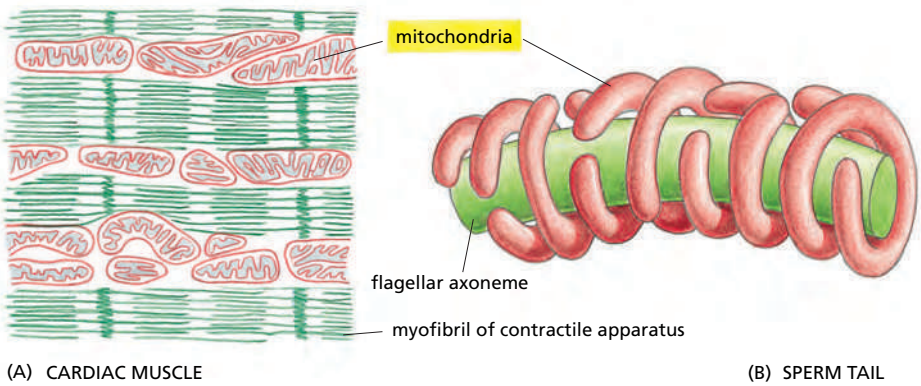


Figure 14–6 Localization of mitochondria near sites of high ATP demand in cardiac muscle and a sperm tail. (A) Cardiac muscle in the wall of the heart is the most heavily used muscle in the body, and its continual contractions require a reliable energy supply. It has limited built-in energy stores and has to depend on a steady supply of ATP from the copious mitochondria aligned close to the contractile myofibrils (see Figure 16–32). (B) During sperm development, microtubules wind helically around the flagellar axoneme, where they are thought to help localize the mitochondria in the tail to produce the structure shown.

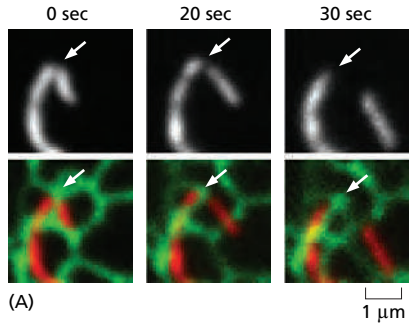
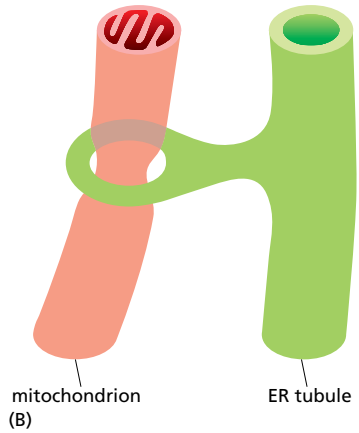


Figure 14–7 Interaction of mitochondria with the endoplasmic reticulum. (A) Fluorescence light microscopy shows that tubules of the ER *(green)* wrap around parts of the mitochondrial network *(red)* in mammalian cells. The mitochondria then divide at the contact sites. After contact is established, fission occurs within less than a minute, as indicated by time-lapse microscopy. (B) Schematic drawing of an ER tubule wrapped around part of the mitochondrial reticulum. It is thought that ER–mitochondrial contacts also mediate the exchange of lipids between the two membrane systems. (A, adapted from J.R. Friedman et al., *Science* 334:358–362, 2011.)

The Mitochondrion Has an Outer Membrane and an Inner Membrane



Like the bacteria from which they originated, mitochondria have an outer and an inner membrane. The two membranes have distinct functions and properties, and delineate separate compartments within the organelle. The inner membrane, which surrounds the internal **mitochondrial matrix** compartment (**Figure 14–8**), is highly folded to form invaginations known as **cristae** (the singular is crista), which contain in their membranes the proteins of the electron-transport chain. Where the inner membrane runs parallel to the outer membrane, between the cristae, it is known as the *inner boundary membrane*. The narrow (20–30 nm) gap between the inner boundary membrane and the outer membrane is known as the **intermembrane space**. The cristae are about 20 nm-wide membrane discs or tubules that protrude deeply into the matrix and enclose the *crista space*. The *crista membrane* is continuous with the inner boundary membrane, and where their membranes join, the membrane forms narrow membrane tubes or slits, known as *crista junctions*.

Like the bacterial outer membrane, the **outer mitochondrial membrane** is freely permeable to ions and to small molecules as large as 5000 daltons. This

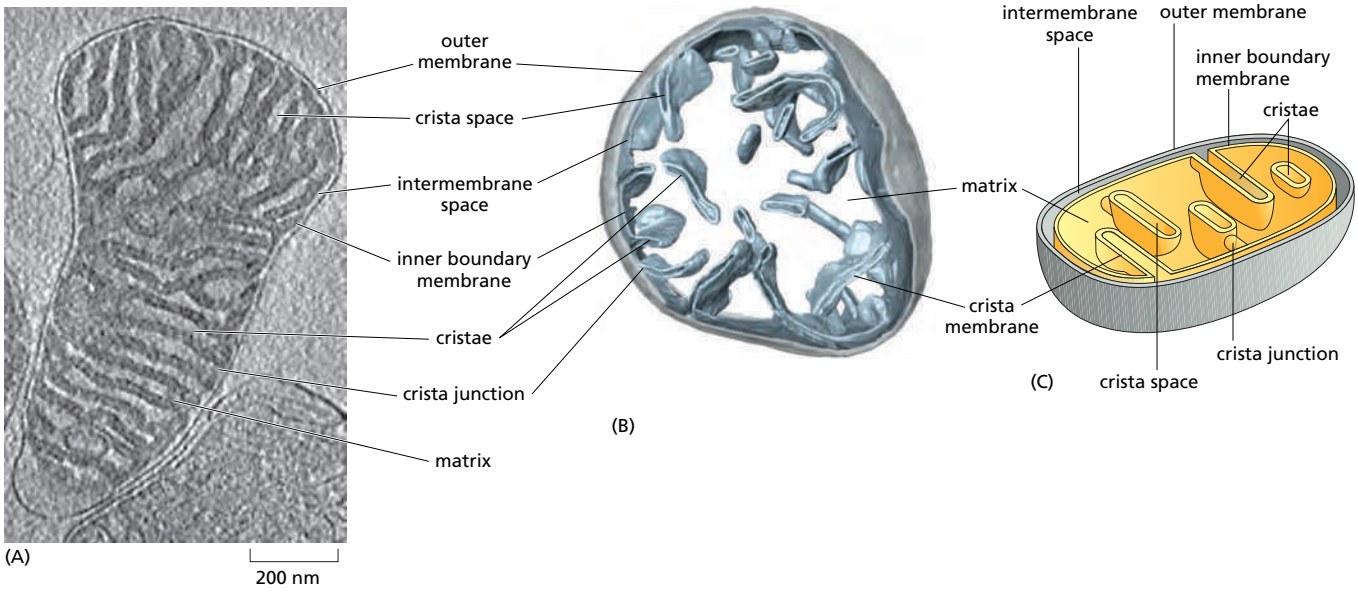


Figure 14–8 Structure of a mitochondrion. (A) Tomographic slice through a three-dimensional map of a mouse heart mitochondrion determined by electron-microscope tomography. The outer membrane envelops the inner boundary membrane. The inner membrane is highly folded into tubular or lamellar cristae, which crisscross the matrix. The dense matrix, which contains most of the mitochondrial protein, appears dark in the electron microscope, whereas the intermembrane space and the crista space appear light due to their lower protein content. The inner boundary membrane follows the outer membrane closely at a distance of ≈20 nm. The inner membrane turns sharply at the cristae junctions, where the cristae join the inner boundary membrane. (B) Tomographic surface-rendered portion of a yeast mitochondrion, showing how flattened cristae project into the matrix from the inner membrane (Movie 14.2). (C) Schematic drawing of a mitochondrion showing the outer membrane *(gray)*, and the inner membrane *(yellow)*. Note that the inner membrane is compartmentalized into the inner boundary membrane and the crista membrane. There are three distinct spaces: the inner membrane space, the crista space, and the matrix. (A, courtesy of Tobias Brandt; B, from K. Davies et al., *Proc. Natl Acad. Sci. USA* 109:13602–13607, 2012. With permission from the National Academy of Sciences.)

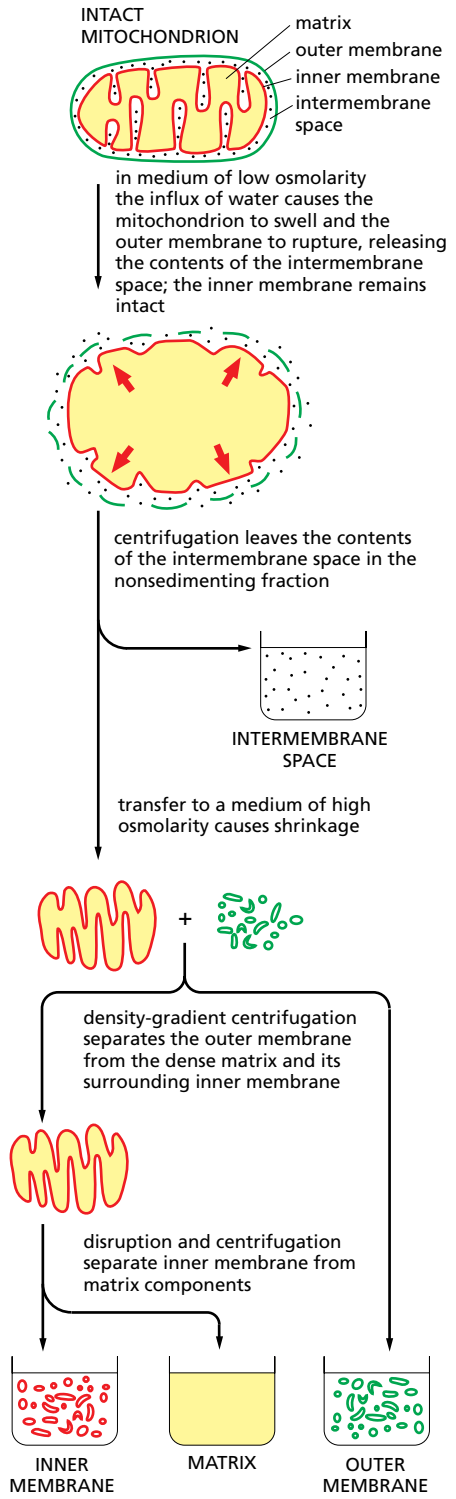


Figure 14–9 Biochemical fractionation of purified mitochondria into separate components. Large numbers of mitochondria are isolated from homogenized tissue by centrifugation and then suspended in a medium of low osmotic strength. In such a medium, water flows into mitochondria and greatly expands the matrix space *(yellow)*. While the cristae of the inner membrane unfold to accommodate the swelling, the outer membrane—which has no folds—breaks, releasing structures composed of the inner membrane surrounding the matrix. These techniques have made it possible to study the protein composition of the inner membrane (comprising a mixture of cristae, boundary membranes, and cristae junctions), the outer membrane, and the matrix.

is because it contains many porin molecules, a special class of β-barrel-type membrane protein that creates aqueous pores across the membrane (see Figure 10–23). As a consequence, the intermembrane space between the outer and inner membrane has the same pH and ionic composition as the cytoplasm, and there is no electrochemical gradient across the outer membrane.

If purified mitochondria are gently disrupted and then fractionated (**Figure 14–9**), the biochemical composition of membranes and mitochondrial compartments can be determined.

The Inner Membrane Cristae Contain the Machinery for Electron Transport and ATP Synthesis

Unlike the outer mitochondrial membrane, the **inner mitochondrial membrane** is a diffusion barrier to ions and small molecules, just like the bacterial inner membrane. However, selected ions, most notably protons and phosphate, as well as essential metabolites such as ATP and ADP, can pass through it by means of special transport proteins.

The inner mitochondrial membrane is highly differentiated into functionally distinct regions with different protein compositions. As discussed in Chapter 10, the lateral segregation of membrane regions with different protein and lipid compositions is a key feature of cells. In the inner mitochondrial membrane, the boundary membrane region is thought to contain the machinery for protein import, new membrane insertion, and assembly of the respiratory-chain complexes. The membranes of the cristae, which are continuous with the boundary membrane, contain the ATP synthase enzyme that produces most of the cell's ATP; they also contain the large protein complexes of the **respiratory chain**—the name given to the mitochondrion's electron-transport chain.

At the cristae junctions, where the membranes of the cristae join the boundary membrane, special protein complexes provide a diffusion barrier that segregates the membrane proteins in the two regions of the inner membrane; these complexes are also thought to anchor the cristae to the outer membrane, thus maintaining the highly folded topology of the inner membrane. Cristae membranes have one of the highest protein densities of all biological membranes, with a lipid content of 25% and a protein content of 75% by weight. The folding of the inner membrane into cristae greatly increases the membrane area available for oxidative phosphorylation. In highly active cardiac muscle cells, for example, the total area of cristae membranes can be up to 20 times larger than the area of the cell's plasma membrane. In total, the surface area of cristae membranes in each human body adds up to roughly the size of a football field.

The Citric Acid Cycle in the Matrix Produces NADH

Together with the cristae that project into it, the matrix is the major working part of the mitochondrion. Mitochondria can use both pyruvate and fatty acids as fuel. Pyruvate is derived from glucose and other sugars, whereas fatty acids are derived from fats. Both of these fuel molecules are transported across the inner mitochondrial membrane by specialized transport proteins, and they are then converted to the crucial metabolic intermediate *acetyl CoA* by enzymes located in the mitochondrial matrix (see Chapter 2).

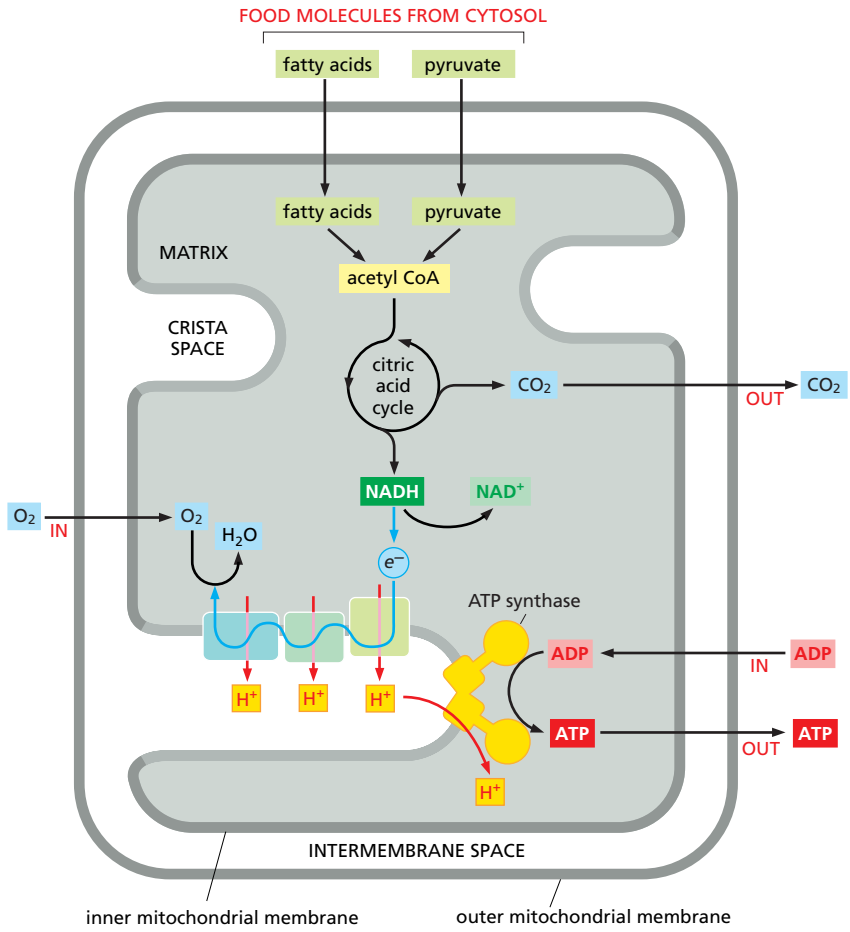


Figure 14–10 A summary of the energy-converting metabolism in mitochondria. Pyruvate and fatty acids enter the mitochondrion *(top of the figure)* and are broken down to acetyl CoA. The acetyl CoA is metabolized by the citric acid cycle, which reduces NAD^+ to NADH, which then passes its high-energy electrons to the first complex in the electron-transport chain. In the process of oxidative phosphorylation, these electrons pass along the electron-transport chain in the inner membrane cristae to oxygen (O_2). This electron transport generates a proton gradient, which drives the production of ATP by the ATP synthase (see Figure 14–3). Electrons from the oxidation of succinate, a reaction intermediate in the citric acid cycle (see Panel 2–9, pp. 106–107), take a separate path to enter this electron-transport chain (not shown, see p. 772).

The membranes that comprise the mitochondrial inner membrane—the inner boundary membrane and the crista membrane—contain different mixtures of proteins and they are therefore shaded differently in this diagram.

The acetyl groups in acetyl CoA are oxidized in the matrix via the *citric acid cycle*, also called the Krebs cycle (see Figure 2–57 and Movie 2.6). The oxidation of these carbon atoms in acetyl CoA produces CO_2, which diffuses out of the mitochondrion to be released to the environment as a waste product. More importantly, the citric acid cycle saves a great deal of the bond energy released by this oxidation in the form of electrons carried by NADH. This NADH transfers its electrons from the matrix to the electron-transport chain in the inner mitochondrial membrane, where—through the *chemiosmotic coupling* process described previously (see Figures 14–2 and 14–3)—the energy that was carried by NADH electrons is converted into phosphate-bond energy in ATP. **Figure 14–10** outlines this sequence of reactions schematically.

The matrix contains the genetic system of the mitochondrion, including the mitochondrial DNA and the ribosomes. The mitochondrial DNA (see section on genetic systems, p. 800) is organized into compact bodies—the nucleoids—by special scaffolding proteins that also function as transcription regulatory proteins. The large number of enzymes required for the maintenance of the mitochondrial genetic system, as well as for many other essential reactions to be outlined next, accounts for the very high protein concentration in the matrix; at more than 500 mg/mL, this concentration is close to that in a protein crystal.

Mitochondria Have Many Essential Roles in Cellular Metabolism

Mitochondria not only generate most of the cell's ATP; they also provide many other essential resources for biosynthesis and cell growth. Before describing in detail the remarkable machinery of the respiratory chain, we diverge briefly to touch on some of these important roles.

Mitochondria are critical for buffering the redox potential in the cytosol. Cells need a constant supply of the electron acceptor NAD^+ for the central reaction in glycolysis that converts glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (see Figure 2–48). This NAD^+ is converted to NADH in the process, and the NAD^+ needs to be regenerated by transferring the high-energy NADH electrons somewhere. The NADH electrons will eventually be used to help drive oxidative phosphorylation inside the mitochondrion. But the inner mitochondrial membrane is impermeable to NADH. The electrons are therefore passed from the NADH to smaller molecules in the cytosol that can move through the inner mitochondrial membrane. Once in the matrix, these smaller molecules transfer their electrons to NAD^+ to form mitochondrial NADH, after which they are returned to the cytosol for recharging—creating a so-called *shuttle system* for the NADH electrons.

In addition to ATP, biosynthesis in the cytosol requires both a constant supply of reducing power in the form of NADPH and small carbon-rich molecules to serve as building blocks (discussed in Chapter 2). Descriptions of biosynthesis often state that the needed carbon skeletons come directly from the breakdown of sugars, whereas the NADPH is produced in the cytosol by a side pathway for the breakdown of sugars (the *pentose phosphate pathway*, an alternative to glycolysis). But under conditions where nutrients abound and plenty of ATP is available, mitochondria help to generate both the reducing power and the carbon-rich building blocks (the "carbon skeletons" in Panel 2–1, pp. 90–91) needed for cell growth. For this purpose, excess citrate produced in the mitochondrial matrix by the citric acid cycle (see Panel 2–9, pp. 106–107) is transported down its electrochemical gradient to the cytosol, where it is metabolized to produce essential components of the cell. Thus, for example, as part of a cell's response to growth signals, large amounts of acetyl CoA are produced in the cytosol from citrate exported from mitochondria, accelerating the production of the fatty acids and sterols that build new membranes (described in Chapter 10). Cancer cells are frequently mutated in ways that enhance this pathway, as part of their program of abnormal growth (see Figure 20–26).

The urea cycle is a central metabolic pathway in mammals that converts the ammonia (NH_4^+) produced by the breakdown of nitrogen-containing compounds (such as amino acids) to the urea excreted in urine. Two critical steps of the urea cycle are carried out in the mitochondria of liver cells, while the remaining steps occur in the cytosol. Mitochondria also play an essential part in the metabolic adaptation of cells to different nutritional conditions. For example, under conditions of starvation, proteins in our bodies are broken down to amino acids, and the amino acids are imported into mitochondria and oxidized to produce NADH for ATP production.

The biosynthesis of *heme groups*—which, as we shall see in the next section, play a central part in electron transfer—is another critical process that is shared between the mitochondrion and the cytoplasm. Iron–sulfur clusters, which are essential not only for electron transfer in the respiratory chain (see p. 766), but also for the maintenance and stability of the nuclear genome, are produced in mitochondria (and chloroplasts). Nuclear genome instability, a hallmark of cancer, can sometimes be linked to the decreased function of cellular proteins that contain iron–sulfur clusters.

Mitochondria also have a central role in membrane biosynthesis. Cardiolipin is a two-headed phospholipid (**Figure 14–11**) that is confined to the inner mitochondrial membrane, where it is also produced. But mitochondria are also a major source of phospholipids for the biogenesis of other cell membranes. Phosphatidylethanolamine, phosphatidylglycerol, and phosphatidic acid are synthesized in the mitochondrion, while phosphatidylinositol, phosphatidylcholine, and phosphatidylserine are primarily synthesized in the endoplasmic reticulum (ER). As described in Chapter 12, most of the cell's membranes are assembled in the ER. The exchange of lipids between the ER and mitochondria is thought to occur at special sites of close contact (see Figure 14–7) by an as-yet unknown mechanism.

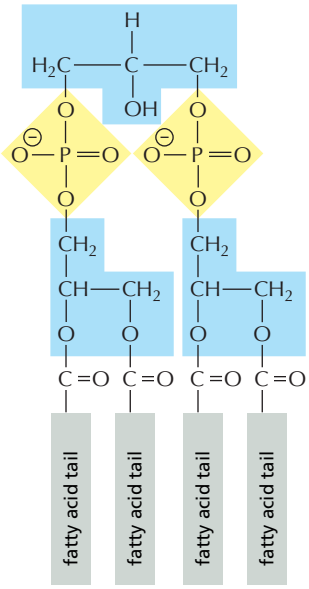


Figure 14–11 The structure of cardiolipin. Cardiolipin consists of two covalently linked phospholipid units, with a total of four rather than the usual two fatty acid chains (see Figure 10–3). Cardiolipin is only produced in the mitochondrial inner membrane, where it interacts closely with membrane proteins involved in oxidative phosphorylation and ATP transport. In cristae, its two juxtaposed phosphate groups may act as a local proton trap on the membrane surface.

Finally, mitochondria are important calcium buffers, taking up calcium from the ER and sarcoplasmic reticulum at special membrane junctions. Cellular calcium levels control muscle contraction (see Chapter 16) and alterations are implicated in neurodegeneration and apoptosis. Clearly, cells and organisms depend on mitochondria in many different ways.

We now return to the central function of the mitochondrion in respiratory ATP generation.

A Chemiosmotic Process Couples Oxidation Energy to ATP Production

Although the citric acid cycle that takes place in the mitochondrial matrix is considered to be part of aerobic metabolism, it does not itself use oxygen. Only the final step of oxidative metabolism consumes molecular oxygen (O_2) directly (see Figure 14–10). Nearly all the energy available from metabolizing carbohydrates, fats, and other foodstuffs in earlier stages is saved in the form of energy-rich compounds that feed electrons into the respiratory chain in the inner mitochondrial membrane. These electrons, most of which are carried by NADH, finally combine with O_2 at the end of the respiratory chain to form water. The energy released during the complex series of electron transfers from NADH to O_2 is harnessed in the inner membrane to generate an electrochemical gradient that drives the conversion of ADP + P_i to ATP. For this reason, the term **oxidative phosphorylation** is used to describe this final series of reactions (**Figure 14–12**).

The total amount of energy released by biological oxidation in the respiratory chain is equivalent to that released by the explosive combustion of hydrogen when it combines with oxygen in a single step to form water. But the combustion of hydrogen in a single-step chemical reaction, which has a strongly negative ΔG, releases this large amount of energy unproductively as heat. In the respiratory chain, the same energetically favorable reaction $H_2 + \frac{1}{2}\,O_2 \rightarrow H_2O$ is divided into small steps (**Figure 14–13**). This stepwise process allows the cell to store nearly half of the total energy that is released in a useful form. At each step, the electrons, which can be thought of as having been removed from a hydrogen molecule to

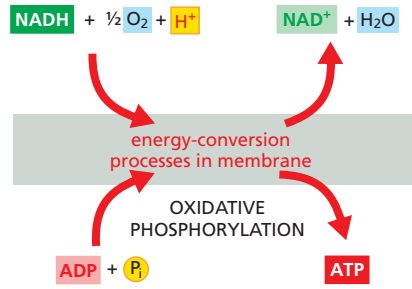


Figure 14–12 The major net energy conversion catalyzed by the mitochondrion. In the process of oxidative phosphorylation, the mitochondrial inner membrane serves as a device that changes one form of chemical-bond energy to another, converting a major part of the energy of NADH oxidation into phosphate-bond energy in ATP.

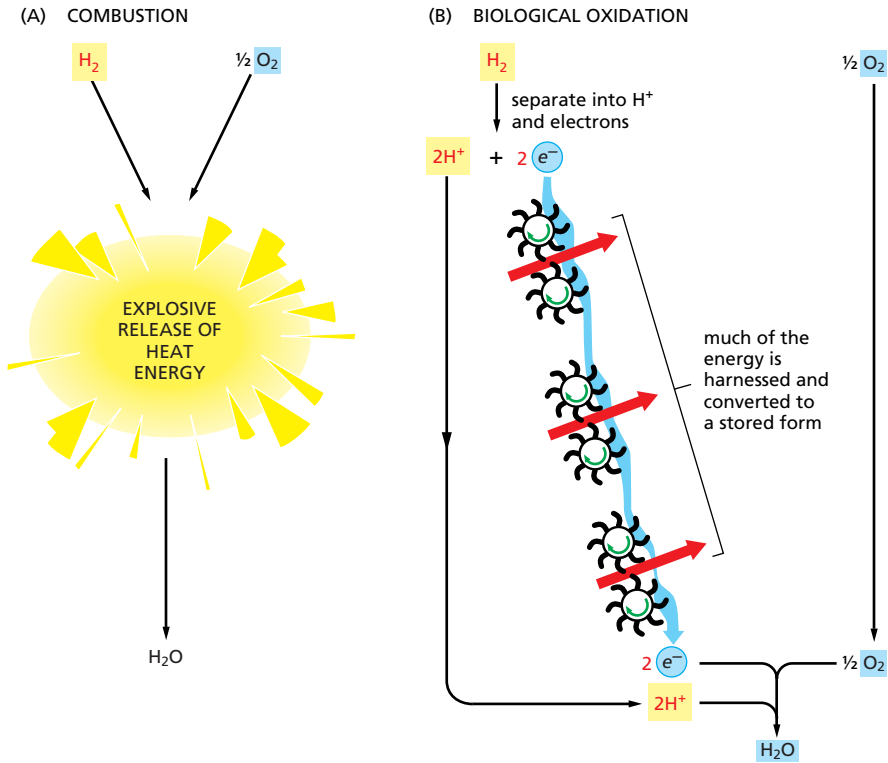


Figure 14–13 A comparison of biological oxidation with combustion. (A) If hydrogen were simply burned, nearly all of the energy would be released in the form of heat. (B) In biological oxidation reactions, about half of the released energy is stored in a form useful to the cell by means of the electron-transport chain (the respiratory chain) in the crista membrane of the mitochondrion. Only the rest of the energy is released as heat. In the cell, the protons and electrons shown here as being derived from H_2 are removed from hydrogen atoms that are covalently linked to NADH molecules.

produce two protons, pass through a series of electron carriers in the inner mitochondrial membrane. At each of three distinct steps along the way (marked by the three electron-transport complexes of the respiratory chain, see below), much of the energy is utilized for pumping protons across the membrane. At the end of the electron-transport chain, the electrons and protons recombine with molecular oxygen into water.

Water is a very low-energy molecule and is thus very stable; it can serve as an electron donor only when a large amount of energy from an external source is spent on splitting it into protons, electrons, and molecular oxygen. This is exactly what happens in oxygenic photosynthesis, where the external energy source is the sun, as we shall see later in the section on chloroplasts (p. 782).

The Energy Derived from Oxidation Is Stored as an Electrochemical Gradient

In mitochondria, the process of electron transport begins when two electrons and a proton are removed from NADH (to regenerate NAD^+). These electrons are passed to the first of about 20 different electron carriers in the respiratory chain. The electrons start at a large negative redox potential (see Panel 14–1, p. 765)—that is, at a high energy level—which gradually drops as they pass along the chain. The proteins involved are grouped into three large *respiratory enzyme complexes*, each composed of protein subunits that sit in the inner mitochondrial membrane. Each complex in the chain has a higher affinity for electrons than its predecessor, and electrons pass sequentially from one complex to the next until they are finally transferred to molecular oxygen, which has the highest electron affinity of all.

The net result is the pumping of H^+ out of the matrix across the inner membrane, driven by the energetically favorable flow of electrons. This transmembrane movement of H^+ has two major consequences:

1. It generates a pH gradient across the inner mitochondrial membrane, with a high pH in the matrix (close to 8) and a lower pH in the intermembrane space. Since ions and small molecules equilibrate freely across the outer mitochondrial membrane, the pH in the intermembrane space is the same as in the cytosol (generally around pH 7.4).
2. It generates a voltage gradient across the inner mitochondrial membrane, creating a *membrane potential* with the matrix side negative and the crista space side positive.

The pH gradient (ΔpH) reinforces the effect of the membrane potential (ΔV), because the latter acts to attract any positive ion into the matrix and to push any negative ion out. Together, ΔpH and ΔV make up the **electrochemical gradient**, which is measured in units of millivolts (mV). This gradient exerts a **proton-motive force**, which tends to drive H^+ back into the matrix (**Figure 14–14**).

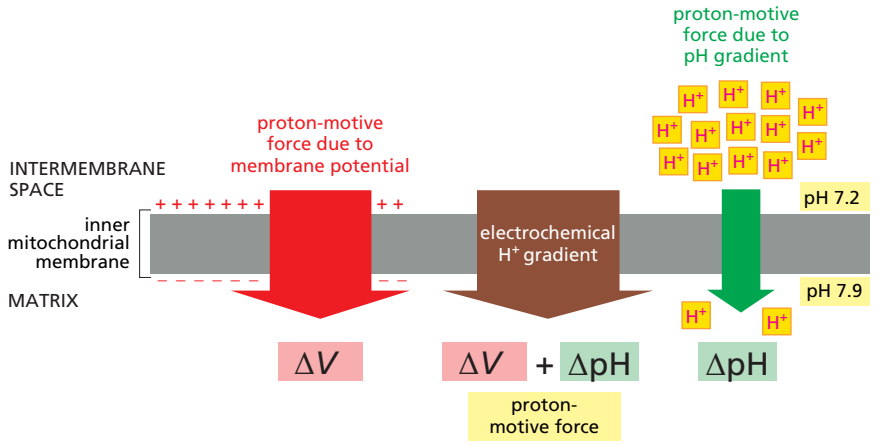


Figure 14–14 The electrochemical proton gradient across the inner mitochondrial membrane. This gradient is composed of a large force due to the membrane potential (ΔV) and a smaller force due to the H^+ concentration gradient—that is, the pH gradient (ΔpH). Both forces combine to generate the proton-motive force, which pulls H^+ back into the mitochondrial matrix. The exact relationship between these forces is expressed by the Nernst equation (see Panel 11–1, p. 616).

The electrochemical gradient across the inner membrane of a respiring mitochondrion is typically about 180 mV (inside negative), and it consists of a membrane potential of about 150 mV and a pH gradient of about 0.5 to 0.6 pH units (each ΔpH of 1 pH unit is equivalent to a membrane potential of about 60 mV). The electrochemical gradient drives not only ATP synthesis but also the transport of selected molecules across the inner mitochondrial membrane, including the import of selected proteins from the cytoplasm (discussed in Chapter 12).

Summary

The mitochondrion performs most cellular oxidations and produces the bulk of the animal cell's ATP. A mitochondrion has two separate membranes: the outer membrane and the inner membrane. The inner membrane surrounds the innermost space (the matrix) of the mitochondrion and forms the cristae, which project into the matrix. The matrix and the inner membrane cristae are the major working parts of the mitochondrion. The membranes that form cristae account for a major part of the membrane surface area in most cells, and they contain the mitochondrion's electron-transport chain (the respiratory chain).

The mitochondrial matrix contains a large variety of enzymes, including those that convert pyruvate and fatty acids to acetyl CoA and those that oxidize this acetyl CoA to CO_2 through the citric acid cycle. These oxidation reactions produce large amounts of NADH, whose high-energy electrons are passed to the respiratory chain. The respiratory chain then uses the energy derived from transporting electrons from NADH to molecular oxygen to pump H^+ out of the matrix. This produces a large electrochemical proton gradient across the inner mitochondrial membrane, composed of contributions from both a membrane potential and a pH difference. This electrochemical gradient exerts a force to drive H^+ back into the matrix. This proton-motive force is harnessed both to produce ATP and for the selective transport of metabolites across the inner mitochondrial membrane.

THE PROTON PUMPS OF THE ELECTRON-TRANSPORT CHAIN

Having considered in general terms how a mitochondrion uses electron transport to generate a proton-motive force, we now turn to the molecular mechanisms that underlie this membrane-based energy-conversion process. In describing the respiratory chain of mitochondria, we accomplish the larger purpose of explaining how an electron-transport process can pump protons across a membrane. As stated at the beginning of this chapter, mitochondria, chloroplasts, archaea, and bacteria use very similar chemiosmotic mechanisms. In fact, these mechanisms underlie the function of all living organisms—including anaerobes that derive all their energy from electron transfers between two inorganic molecules, as we shall see later.

We start with some of the basic principles on which all of these processes depend.

The Redox Potential Is a Measure of Electron Affinities

In chemical reactions, any electrons removed from one molecule are always passed to another, so that whenever one molecule is oxidized, another is reduced. As with any other chemical reaction, the tendency of such **redox reactions** to proceed spontaneously depends on the free-energy change (ΔG) for the electron transfer, which in turn depends on the relative affinities of the two molecules for electrons.

Because electron transfers provide most of the energy for life, it is worth taking the time to understand them. As discussed in Chapter 2, acids donate protons and bases accept them (see Panel 2–2, p. 93). Acids and bases 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, the acetate ion (CH_3COO^-), in the reaction:

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

In an exactly analogous way, pairs of compounds such as NADH and NAD^+ are called **redox pairs**, since NADH is converted to NAD^+ by the loss of electrons in the reaction:

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

NADH is a strong electron donor: because two of its electrons are engaged in a covalent bond which releases energy when broken, the free-energy change for passing these electrons to many other molecules is favorable. Energy is required to form this bond from NAD^+, two electrons, and a proton (the same amount of energy that was released when the bond was broken). Therefore NAD^+, the redox partner of NADH, is of necessity a weak electron acceptor.

We can measure the tendency to transfer electrons from any redox pair experimentally. All that is required is the formation of an electrical circuit linking a 1:1 (equimolar) mixture of the redox pair to a second redox pair that has been arbitrarily selected as a reference standard, so that we can measure the voltage difference between them (**Panel 14–1**). This voltage difference is defined as the **redox potential**; electrons move spontaneously from a redox pair like NADH/NAD^+ with a lower redox potential (a lower affinity for electrons) to a redox pair like O_2/H_2O with a higher redox potential (a higher affinity for electrons). Thus, NADH is a good molecule for donating electrons to the respiratory chain, while O_2 is well suited to act as the "sink" for electrons at the end of the chain. 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.

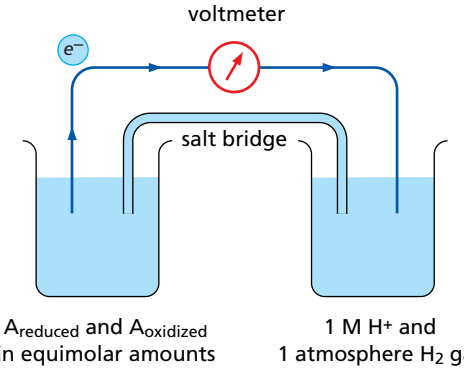
Electron Transfers Release Large Amounts of Energy

As just discussed, those pairs of compounds that have the most negative redox potentials have the weakest affinity for electrons and therefore are useful as carriers with a strong tendency to donate electrons. Conversely, those pairs that have the most positive redox potentials have the greatest affinity for electrons and therefore are useful as carriers with a strong tendency to accept electrons. A 1:1 mixture of NADH and NAD^+ has a redox potential of –320 mV, indicating that NADH has a strong tendency to donate electrons; a 1:1 mixture of H_2O and $\frac{1}{2}O_2$ has a redox potential of +820 mV, indicating that O_2 has a strong tendency to accept electrons. The difference in redox potential is 1140 mV, which means that the transfer of each electron from NADH to O_2 under these standard conditions is enormously favorable, since $\Delta G° = -109$ kJ/mole, and twice this amount of energy is gained for the two electrons transferred per NADH molecule (see Panel 14–1). If we compare this free-energy change with that for the formation of the phosphoanhydride bonds in ATP, where $\Delta G° = 30.6$ kJ/mole (see Figure 2–50), we see that, under standard conditions, the oxidation of one NADH molecule releases more than enough energy to synthesize seven molecules of ATP from ADP and P_i. (In the cell, the number of ATP molecules generated will be lower because the standard conditions are far from the physiological ones; in addition, small amounts of energy are inevitably dissipated as heat along the way.)

Transition Metal Ions and Quinones Accept and Release Electrons Readily

The electron-transport properties of the membrane protein complexes in the respiratory chain depend upon electron-carrying *cofactors*, most of which are *transition metals* such as Fe, Cu, Ni, and Mn, bound to proteins in the complexes. These metals have special properties that allow them to promote both enzyme catalysis and electron-transfer reactions. Most relevant here is the fact that their ions exist in several different oxidation states with closely spaced redox potentials, which enables them to accept or give up electrons readily; this property is

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

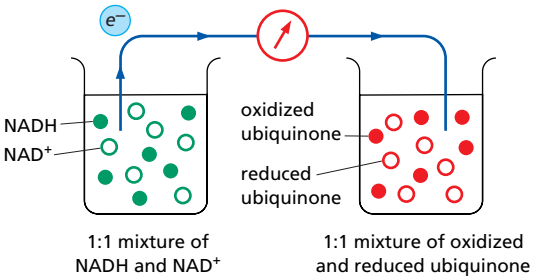
CALCULATION OF ΔG° FROM REDOX POTENTIALS

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:

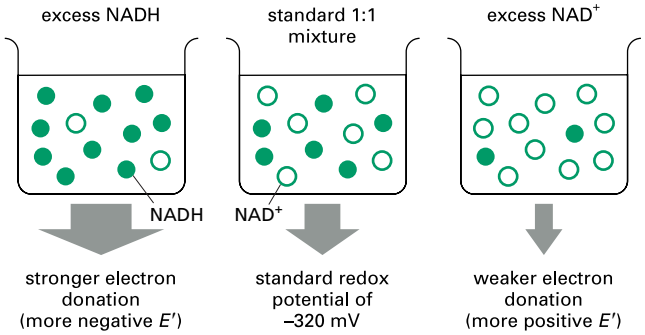
$$\Delta E'_0 = +30 - (-320) = +350 \text{ mV}$$

$$\Delta G^\circ = -n(0.096)\Delta E'_0 = -1(0.096)(350) = -34 \text{ kJ/mole}$$

The same 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 2 (see p. 60), the actual free-energy change for a reaction, ΔG, depends on the concentration of the reactants and generally will be different from the standard free-energy change, ΔG°. The standard redox potentials are for a 1:1 mixture of the redox pair. For example, the standard redox potential of –320 mV is for a 1:1 mixture of NADH and NAD^+. But when there is an excess of NADH over NAD^+, electron transfer from NADH to an electron acceptor becomes more favorable. This is reflected by a more negative redox potential and a more negative ΔG for electron transfer.



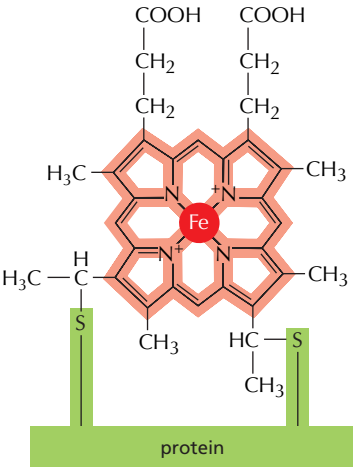


Figure 14–15 The structure of the heme group attached covalently to cytochrome *c*. The porphyrin ring of the heme is shown in *red*. There are six different cytochromes in the respiratory chain. Because the hemes in different cytochromes have slightly different structures and are kept in different local environments by their respective proteins, each has a different affinity for an electron, and a slightly different spectroscopic signature.

exploited by the membrane protein complexes in the respiratory chain to move electrons both within and between complexes.

Unlike the colorless atoms H, C, N, and O that constitute the bulk of biological molecules, transition metal ions are often brightly colored, which makes the proteins that contain them easy to study by spectroscopic methods using visible light. One family of such colored proteins, the **cytochromes**, contains a bound *heme group*, in which an iron atom is tightly held by four nitrogen atoms at the corners of a square in a *porphyrin ring* (**Figure 14–15**). Similar porphyrin rings are responsible both for the red color of blood and for the green color of leaves, binding an iron in hemoglobin or a magnesium in chlorophyll, respectively.

Iron–sulfur proteins contain a second major family of electron-transfer cofactors. In this case, either two or four iron atoms are bound to an equal number of sulfur atoms and to cysteine side chains, forming **iron–sulfur clusters** in the protein (**Figure 14–16**). Like the cytochrome hemes, these clusters carry one electron at a time.

The simplest of the electron-transfer cofactors in the respiratory chain—and the only one that is not always bound to a protein—is a quinone (called *ubiquinone*, or *coenzyme Q*). A **quinone** (**Q**) is a small hydrophobic molecule that is freely mobile in the lipid bilayer. This *electron carrier* can accept or donate either one or two electrons. Upon reduction (note that reduced quinones are called quinols), it picks up a proton from water along with each electron (**Figure 14–17**).

In the mitochondrial electron-transport chain, six different cytochrome hemes, eight iron–sulfur clusters, three copper atoms, a flavin mononucleotide (another electron-transfer cofactor), and ubiquinone work in a defined sequence to carry electrons from NADH to O_2. In total, this pathway involves more than 60 different polypeptides arranged in three large membrane protein complexes, each of which binds several of the above electron-carrying cofactors.

As we would expect, the electron-transfer cofactors have increasing affinities for electrons (higher redox potentials) as the electrons move along the respiratory chain. The redox potentials have been fine-tuned during evolution by the protein environment of each cofactor, which alters the cofactor's normal affinity for electrons. Because iron–sulfur clusters have a relatively low affinity for electrons, they predominate in the first half of the respiratory chain; in contrast, the heme cytochromes predominate further down the chain, where a higher electron affinity is required.

NADH Transfers Its Electrons to Oxygen Through Three Large Enzyme Complexes Embedded in the Inner Membrane

Membrane proteins are difficult to purify because they are insoluble in aqueous solutions, and they are easily disrupted by the detergents that are required to solubilize them. But by using mild nonionic detergents, such as octylglucoside or dodecyl maltoside (see Figure 10–28), they can be solubilized and purified in their native form, and even crystallized for structure determination. Each of the three different detergent-solubilized respiratory-chain complexes can be re-inserted

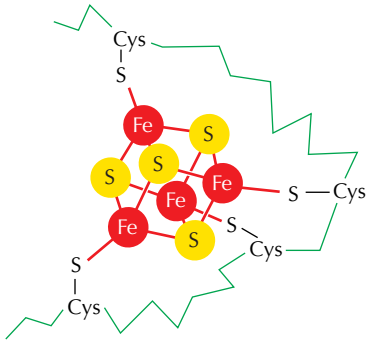


Figure 14–16 The structure of an iron–sulfur cluster. These dark brown clusters consist either of four iron and four sulfur atoms, as shown here, or of two irons and two sulfurs linked to cysteines in the polypeptide chain via covalent sulfur bridges, or to histidines. Although they contain several iron atoms, each iron–sulfur cluster can carry only one electron at a time. Nine different iron–sulfur clusters participate in electron transport in the respiratory chain.

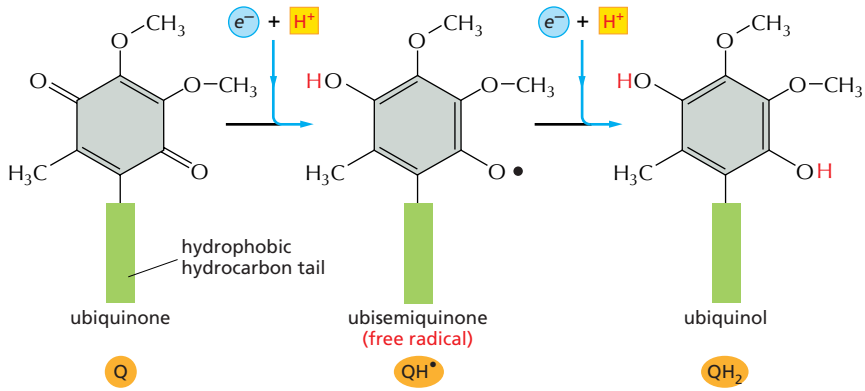


Figure 14–17 Quinone electron carriers. Ubiquinone in the lipid bilayer picks up one H^+ *(red)* from the aqueous environment for each electron *(blue)* it accepts, in two steps, from respiratory-chain complexes. The first step involves the acquisition of a proton and an electron and converts the ubiquinone into an unstable ubisemiquinone radical. In the second step, it becomes a fully reduced ubiquinone (called ubiquinol), which is freely mobile as an electron carrier in the lipid bilayer of the membrane. When the ubiquinol donates its electrons to the next complex in the chain, the two protons are released. The long hydrophobic tail *(green)* that confines ubiquinone to the membrane consists of 6–10 five-carbon isoprene units, depending on the organism. The corresponding electron carrier in the photosynthetic membranes of chloroplasts is plastoquinone, which has almost the same structure and works in the same way. For simplicity, we refer to both ubiquinone and plastoquinone in this chapter as quinone (abbreviated as Q).

into artificial lipid bilayer vesicles and shown to pump protons across the membrane as electrons pass through them.

In the mitochondrion, the three complexes are linked in series, serving as electron-transport-driven H^+ pumps that pump protons out of the matrix to acidify the crista space (Figure 14–18):

1. The **NADH dehydrogenase complex** (often referred to as *Complex I*) is the largest of these respiratory enzyme complexes. It accepts electrons from NADH and passes them through a flavin mononucleotide and eight iron–sulfur clusters to the lipid-soluble electron carrier ubiquinone. The reduced ubiquinol then transfers its electrons to cytochrome *c* reductase.
2. The **cytochrome *c* reductase** (also called the *cytochrome b-c_1 complex*) is a large membrane protein assembly that functions as a dimer. Each monomer contains three cytochrome hemes and an iron–sulfur cluster. The complex accepts electrons from ubiquinol and passes them on to the small, soluble protein cytochrome *c*, which is located in the crista space and carries electrons one at a time to cytochrome *c* oxidase.
3. The **cytochrome *c* oxidase complex** contains two cytochrome hemes and three copper atoms. The complex accepts electrons one at a time from cytochrome *c* and passes them to molecular oxygen. In total, four electrons and four protons are needed to convert one molecule of oxygen to water.

We have previously discussed how the redox potential reflects electron affinities. Figure 14–19 presents an outline of the redox potentials measured along the respiratory chain. These potentials change in three large steps, one across each proton-translocating respiratory complex. The change in redox potential between any two electron carriers is directly proportional to the free energy released when an electron transfers between them. Each complex acts as an energy-conversion device by harnessing some of this free-energy change to pump H^+ across the inner membrane, thereby creating an electrochemical proton gradient as electrons pass along the chain.

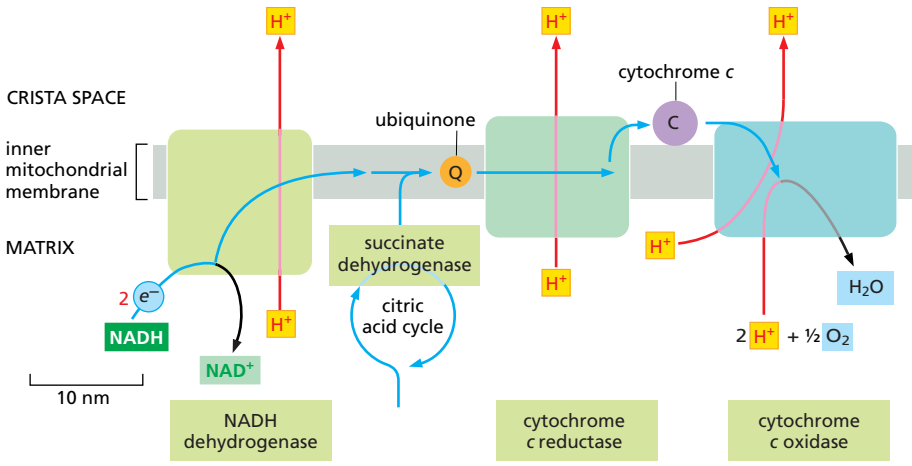


Figure 14–18 The path of electrons through the three respiratory-chain proton pumps. (Movie 14.3) The approximate size and shape of each complex is shown. During the transfer of electrons from NADH to oxygen *(blue arrows)*, ubiquinone and cytochrome *c* serve as mobile carriers that ferry electrons from one complex to the next. During the electron-transfer reactions, protons are pumped across the membrane by each of the respiratory enzyme complexes, as indicated *(red arrows)*.

For historical reasons, the three proton pumps in the respiratory chain are sometimes denoted as Complex I, Complex III, and Complex IV, according to the order in which electrons pass through them from NADH. Electrons from the oxidation of succinate by succinate dehydrogenase (designated as Complex II) are fed into the electron-transport chain in the form of reduced ubiquinone. Although embedded in the crista membrane, succinate dehydrogenase does not pump protons and thus does not contribute to the proton-motive force; it is therefore not considered to be an integral part of the respiratory chain.

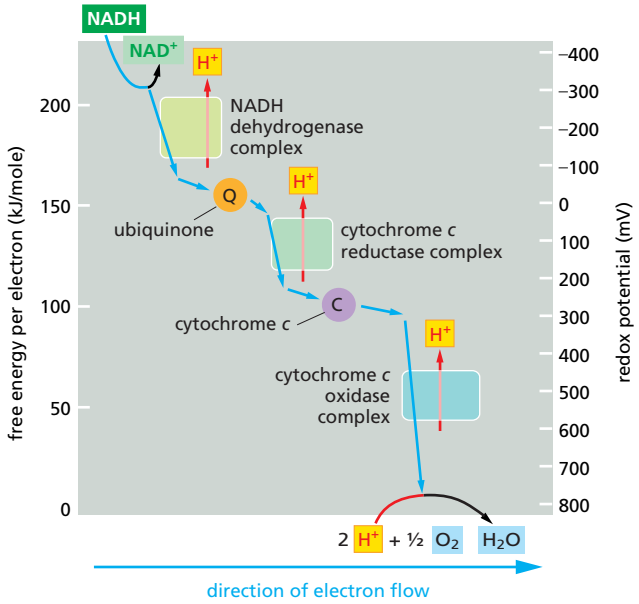


Figure 14–19 Redox potential changes along the mitochondrial electron-transport chain. The redox potential (designated E'_0) increases as electrons flow down the respiratory chain to oxygen. The standard free-energy change in kilojoules, $\Delta G°$, for the transfer of each of the two electrons donated by an NADH molecule can be obtained from the left-hand ordinate [$\Delta G° = -n(0.096)\ \Delta E'_0$, where n is the number of electrons transferred across a redox potential change of $\Delta E'_0$ mV]. Electrons flow through a respiratory enzyme complex by passing in sequence through the multiple electron carriers in each complex *(blue arrows)*. As indicated, part of the favorable free-energy change is harnessed by each enzyme complex to pump H^+ across the inner mitochondrial membrane *(red arrows)*. The NADH dehydrogenase pumps up to four H^+ per electron, the cytochrome *c* reductase complex pumps two, whereas the cytochrome *c* oxidase complex pumps one per electron.

Note that NADH is not the only source of electrons for the respiratory chain. The flavin $FADH_2$, which is generated by fatty acid oxidation (see Figure 2–56) and by succinate dehydrogenase in the citric acid cycle (see Figure 2–57), also contributes. Its two electrons are passed directly to ubiquinone, bypassing NADH dehydrogenase.

X-ray crystallography has elucidated the structure of each of the three respiratory-chain complexes in great detail, and we next examine each of them in turn to see how they work.

The NADH Dehydrogenase Complex Contains Separate Modules for Electron Transport and Proton Pumping

The NADH dehydrogenase complex is a massive assembly of membrane and nonmembrane proteins that receives electrons from NADH and passes them to ubiquinone. In animal mitochondria, it consists of more than 40 different protein subunits, with a molecular mass of nearly a million daltons. The x-ray structures of the NADH dehydrogenase complex from fungi and bacteria show that it is L-shaped, with both a hydrophobic membrane arm and a hydrophilic arm that projects into the mitochondrial matrix (**Figure 14–20**).

Electron transfer and proton pumping are physically separated in the NADH dehydrogenase complex, with electron transfer occurring in the matrix arm and proton pumping in the membrane arm. The NADH docks near the tip of the matrix arm, where it transfers its electrons via a bound flavin mononucleotide to a string of iron–sulfur clusters that runs down the arm, acting like a wire to carry electrons to a protein-bound molecule of ubiquinone. Electron transfer to the quinone is thought to trigger proton translocation in a set of proton pumps in the membrane arm, and for this to happen the two processes must be energetically and mechanically linked. A mechanical link is thought to be provided by a 6-nm long, amphipathic α helix that runs parallel to the membrane surface on the matrix side of the membrane arm. This helix may act like the connecting rod in a steam engine to generate a mechanical, energy-transducing power stroke that links the quinone-binding site to the proton-translocating modules in the membrane (see Figure 14–20).

The reduction of each quinone by the transfer of two electrons can cause four protons to be pumped out of the matrix into the crista space. In this way, NADH dehydrogenase generates roughly half of the total proton-motive force in mitochondria.

Cytochrome *c* Reductase Takes Up and Releases Protons on the Opposite Side of the Crista Membrane, Thereby Pumping Protons

As described previously, when a quinone molecule (Q) accepts its two electrons, it also takes up two protons to form a quinol (QH_2; see Figure 14–17). In

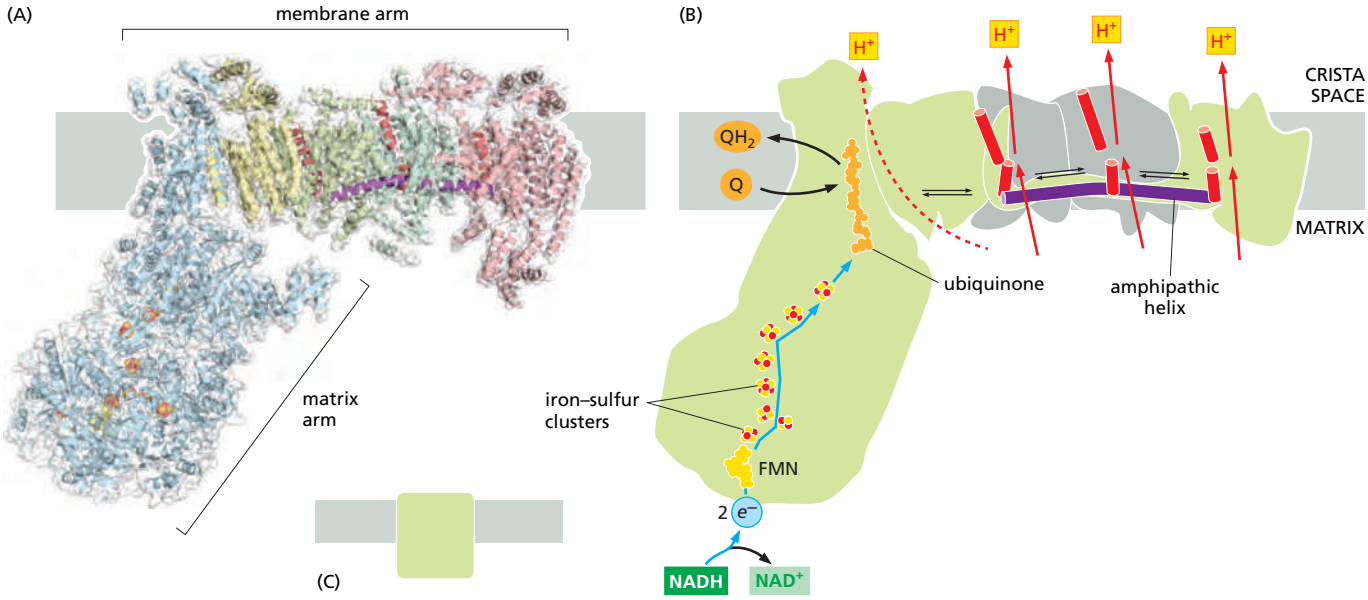


Figure 14–20 The structure of NADH dehydrogenase. (A) The model of the mitochondrial complex shown here is based on the x-ray structure of the smaller bacterial complex, which works in the same way. The matrix arm of NADH dehydrogenase (also known as Complex I) contains eight iron–sulfur (FeS) clusters that appear to participate in electron transport. The membrane contains more than 70 transmembrane helices, forming three distinct proton-pumping modules, while the matrix arm contains the electron-transport cofactors. (B) NADH donates two electrons, via a bound flavin mononucleotide (FMN; *yellow*), to a chain of seven iron–sulfur clusters *(red and yellow spheres)*. From the terminal iron–sulfur cluster, the electrons pass to ubiquinone *(orange)*. Electron transfer results in conformational changes *(black arrows)* that are thought to be transmitted to a long amphipathic α helix *(purple)* on the matrix side of the membrane arm, which pulls on discontinuous transmembrane helices *(red)* in three membrane subunits, each of which resembles an antiporter (see Chapter 11). This movement is thought to change the conformation of charged residues in the three proton channels, resulting in the translocation of three protons out of the matrix. A fourth proton may be translocated at the interface of the two arms *(dotted line)*. (C) This shows the symbol for NADH dehydrogenase used throughout this chapter. (Adapted from R.G. Efremov, R. Baradaran and L.A. Sazanov, *Nature* 465:441–445, 2010. PDB code: 3M9S.)

the respiratory chain, ubiquinol tranfers electrons from NADH dehydrogenase to cytochrome *c* reductase. Because the protons in this QH_2 molecule are taken up from the matrix and released on the opposite side of the crista membrane, two protons are transferred from the matrix into the crista space per pair of electrons transferred (Figure 14–21). This vectorial transfer of protons supplements the electrochemical proton gradient that is created by the NADH dehydrogenase proton pumping just discussed.

Cytochrome *c* reductase is a large assembly of membrane protein subunits. Three subunits form a catalytic core that passes electrons from ubiquinol to cytochrome *c*, with a structure that has been highly conserved from bacterial ancestors (Figure 14–22). It pumps protons by a vectorial transfer of protons that involves a binding site for a second molecule of ubiquinone; the elaborate

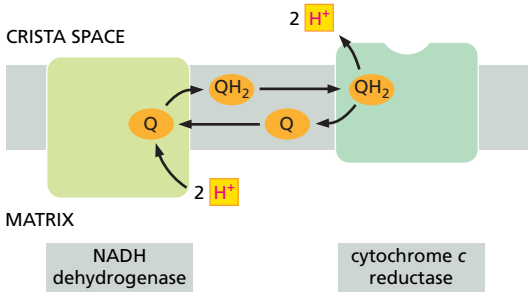


Figure 14–21 How a directional release and uptake of protons by a quinone pumps protons across a membrane. Two protons are picked up on the matrix side of the inner mitochondrial membrane when the reaction $Q + 2e^- + 2H^+ \rightarrow QH_2$ is catalyzed by the NADH dehydrogenase complex. This molecule of ubiquinol (QH_2) diffuses rapidly in the plane of the membrane, becoming bound to the crista side of cytochrome *c* reductase. When its oxidation by cytochrome *c* reductase generates two protons and two electrons (see Figure 14–17), the two protons are released into the crista space. The flow of electrons is not shown in this diagram.

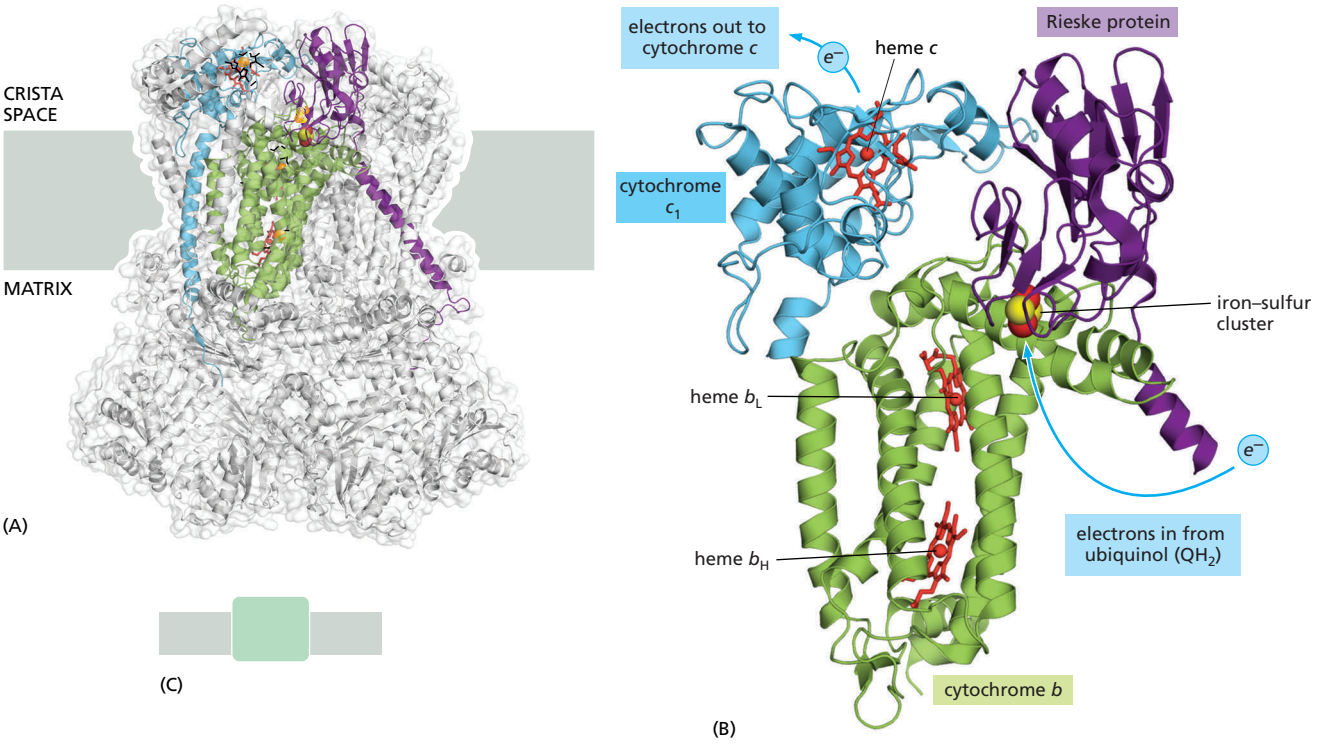


Figure 14–22 The structure of cytochrome *c* reductase. Cytochrome *c* reductase (also known as the cytochrome *b*-c_1 complex) is a dimer of two identical 240,000-dalton halves, each composed of 11 different protein molecules in mammals. (A) A structure graphic of the entire dimer, showing in color the three proteins that form the functional core of the enzyme complex: cytochrome *b (green)* and cytochrome c_1 *(blue)* are colored in one half, and the Rieske protein *(purple)* containing an Fe_2S_2 iron–sulfur cluster *(red and yellow)* is colored in the other. These three protein subunits interact across the two halves. (B) Transfer of electrons through cytochrome *c* reductase to the small, soluble carrier protein cytochrome *c*. Electrons entering from ubiquinol near the matrix side of the membrane are captured by the iron–sulfur cluster of the Rieske protein, which moves its iron–sulfur group back and forth to transfer these electrons to heme *c (red)*. Heme *c* then transfers them to the carrier molecule cytochrome *c*.

As detailed in Figure 14–23, only one of the two electrons from each ubiquinol is transferred through this path. To increase proton pumping, the second ubiquinol electron is passed to a molecule of ubiquinone bound to cytochrome *c* reductase on the opposite side of the membrane—near the matrix. (C) This shows the symbol for cytochrome *c* reductase used throughout this chapter. (PDB code: 1EZV.)

redox loop mechanism used is called the *Q cycle* because while one of the electrons received from each QH_2 molecule is transferred from ubiquinone through the complex to the carrier protein cytochrome *c*, the other electron is recycled back into the quinone pool. Through the mechanism illustrated in **Figure 14–23**, the Q cycle increases the total amount of redox energy that can be stored in the electrochemical proton gradient. As a result, two protons are pumped across the crista membrane for every electron that is transferred from NADH dehydrogenase to cytochrome *c*.

The Cytochrome *c* Oxidase Complex Pumps Protons and Reduces O_2 Using a Catalytic Iron–Copper Center

The final link in the mitochondrial electron-transport chain is cytochrome *c* oxidase. The cytochrome *c* oxidase complex accepts electrons from the soluble electron carrier cytochrome *c*, and it uses yet a different, third mechanism to pump protons across the inner mitochondrial membrane. The structure of the mammalian complex is illustrated in **Figure 14–24**. The atomic-resolution structures, combined with studies of the effect of mutations introduced into the enzyme by genetic engineering of the yeast and bacterial proteins, have revealed the detailed mechanisms of this electron-driven proton pump.

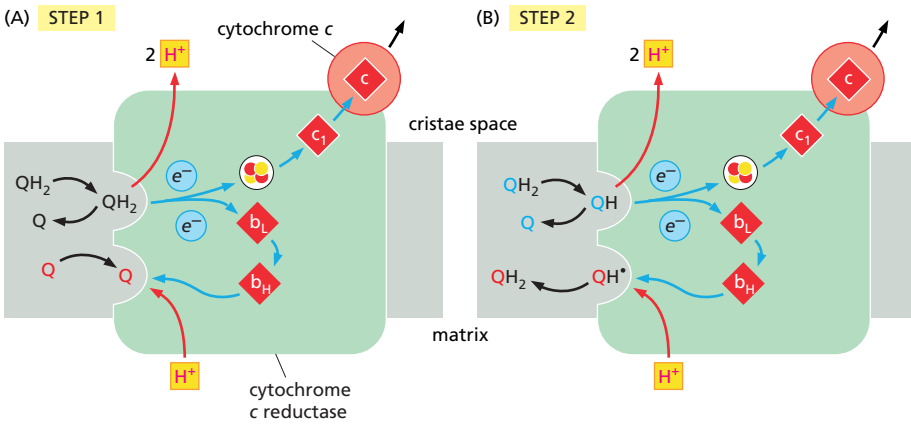


Figure 14–23 The two-step mechanism of the cytochrome *c* reductase Q-cycle. (A) In step 1, ubiquinol reduced by NADH dehydrogenase docks to the cytochrome *c* reductase complex. Oxidation of the quinol produces two protons and two electrons. The protons are released into the cristae space. One electron passes via an iron-sulfur cluster to heme c_1, and then to the soluble electron carrier protein cytochrome *c* on the membrane surface. The second electron passes via hemes b_L and b_H to a ubiquinone (*red* Q) bound at a separate site near the matrix side of the protein. Uptake of a proton from the matrix produces an ubisemiquinone radical (see Figure 14–17), which remains bound to this site (*red* $QH^{\bullet}$ in B).

(B) In step 2, a second ubiquinol (*blue* QH_2) docks and releases two protons and two electrons, as described for step 1. One electron is passed to a second cytochrome *c*, whereas the other electron is accepted by the ubisemiquinone. The ubisemiquinone takes up a proton from the matrix and is released into the lipid bilayer as fully reduced ubiquinol (*red* QH_2).

On balance, the oxidation of one ubiquinol in the Q cycle pumps two protons through the membrane by a directional release and uptake of protons (see Figure 14–21), while releasing another two into the cristae space. In addition, in each of the two steps (A) and (B), one electron is transferred to a cytochrome *c* carrier (Movie 14.4).

Because oxygen has a high affinity for electrons, it can release a large amount of free energy when it is reduced to form water. Thus, the evolution of cellular respiration, in which O_2 is converted to water, enabled organisms to harness much more energy than can be derived from anaerobic metabolism. As we discuss later, the availability of the large amount of energy released by the reduction of molecular oxygen to form water is thought to have been essential to the emergence of multicellular life: this would explain why all large organisms respire. The ability of biological systems to use O_2 in this way, however, requires sophisticated chemistry. Once a molecule of O_2 has picked up one electron, it forms a superoxide radical anion ($O_2^{\bullet -}$) that is dangerously reactive and rapidly takes up an additional three electrons wherever it can get them, with destructive effects on its immediate environment. We can tolerate oxygen in the air we breathe only because the uptake of the first electron by the O_2 molecule is slow, allowing cells to use enzymes to control electron uptake by oxygen. Thus, cytochrome *c* oxidase holds

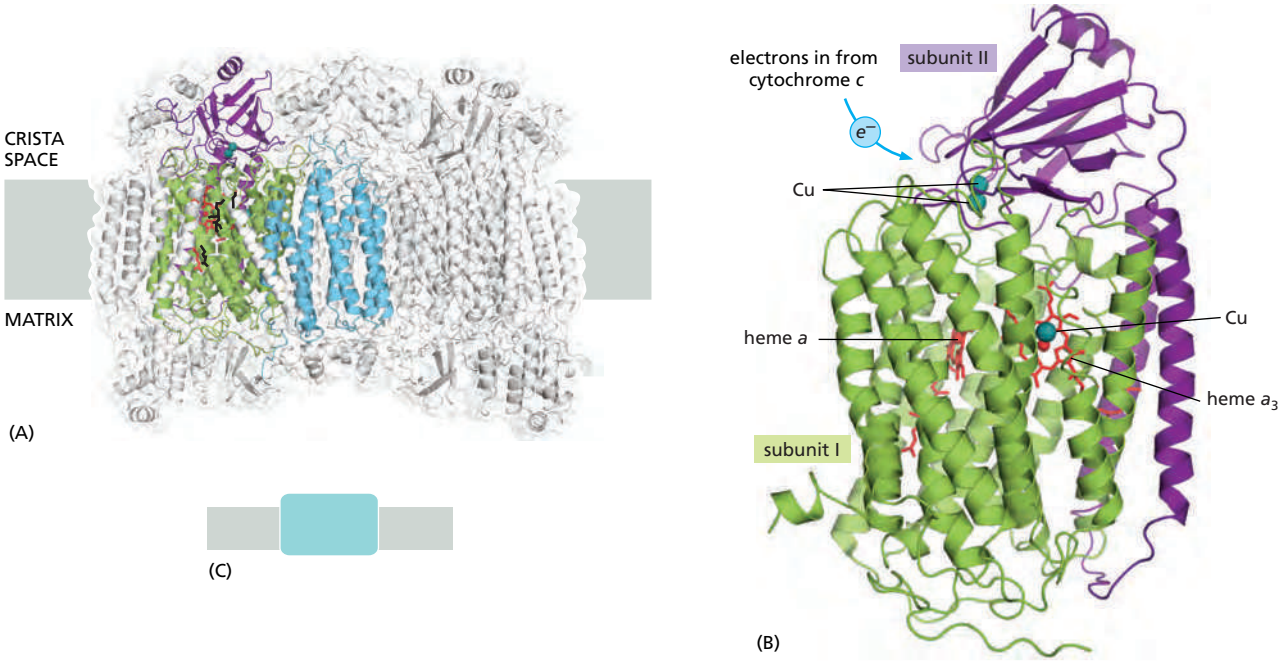


Figure 14–24 The structure of cytochrome *c* oxidase. The final complex in the mitochondrial electron-transfer chain consists of 13 different protein subunits, with a total mass of 204,000 daltons. (A) The entire dimeric complex is shown, positioned in the crista membrane. The highly conserved subunits I *(green)*, II *(purple)*, and III *(blue)* are encoded by the mitochondrial genome, and they form the functional core of the enzyme. (B) The functional core of the complex. Electrons pass through this structure from cytochrome *c* via bound copper ions *(blue spheres)* and hemes *(red)* to an O_2 molecule bound between heme a_3 and a copper ion. The four protons needed to reduce O_2 to water are taken up from the matrix; see also Figure 14–25. (C) This shows the symbol for cytochrome *c* oxidase used throughout this chapter. (PDB code: 2OCC.)

on to oxygen at a special bimetallic center, where it remains clamped between a heme-linked iron atom and a copper ion until it has picked up a total of four electrons. Only then are the two oxygen atoms of the oxygen molecule safely released as two molecules of water (**Figure 14–25**).

The cytochrome *c* oxidase reaction accounts for about 90% of the total oxygen uptake in most cells. This protein complex is therefore crucial for all aerobic life. Cyanide and azide are extremely toxic because they bind to the heme iron atoms in cytochrome *c* oxidase much more tightly than does oxygen, thereby greatly reducing ATP production.

The Respiratory Chain Forms a Supercomplex in the Crista Membrane

By using cryoelectron microscopy to examine proteins that have been very gently isolated, it can be shown that the three protein complexes that form the respiratory chain assemble into an even larger *supercomplex* in the crista membrane. As illustrated in **Figure 14–26**, this structure is thought to help the mobile electron carriers ubiquinone (in the crista membrane) and cytochrome *c* (in the crista space) transfer electrons with high efficiency. The formation of the supercomplex depends on the presence of the mitochondrial lipid cardiolipin (see Figure 14–11), which presumably works like a hydrophobic glue that holds the components together.

In addition to the three proton pumps in the supercomplex just discussed, one of the enzymes in the citric acid cycle, *succinate dehydrogenase*, is embedded in the mitochondrial crista membrane. In the course of oxidizing succinate to fumarate in the matrix, this enzyme complex captures electrons in the form of a tightly bound $FADH_2$ molecule (see Panel 2–9, pp. 106–107) and passes them to

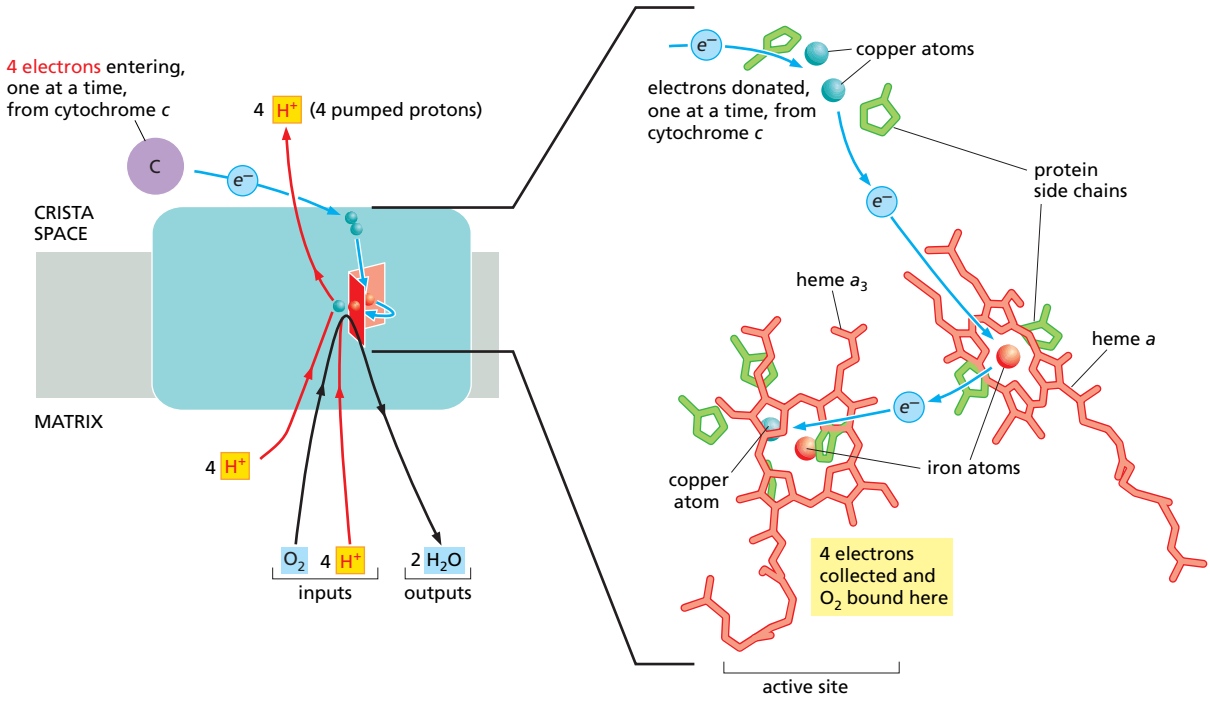


Figure 14–25 The reaction of O_2 with electrons in cytochrome *c* oxidase. Electrons from cytochrome *c* pass through the complex via bound copper ions (*blue spheres*) and hemes *(red)* to an O_2 molecule bound between heme a_3 and a copper ion. Iron ions are shown as *red spheres*. The iron atom in heme *a* serves as an electron queuing point where electrons are held so that they can be released to an O_2 molecule (not shown) that is held at the bimetallic center active site, which is formed by the central iron of the other heme (heme a_3) and a closely apposed copper atom. The four protons needed to reduce O_2 to water are removed from the matrix. For each O_2 molecule that undergoes the reaction $4e^- + 4H^+ + O_2 \rightarrow 2H_2O$, another four protons are pumped out of the matrix by mechanisms that are driven by allosteric changes in protein conformation (see Figure 14–28).

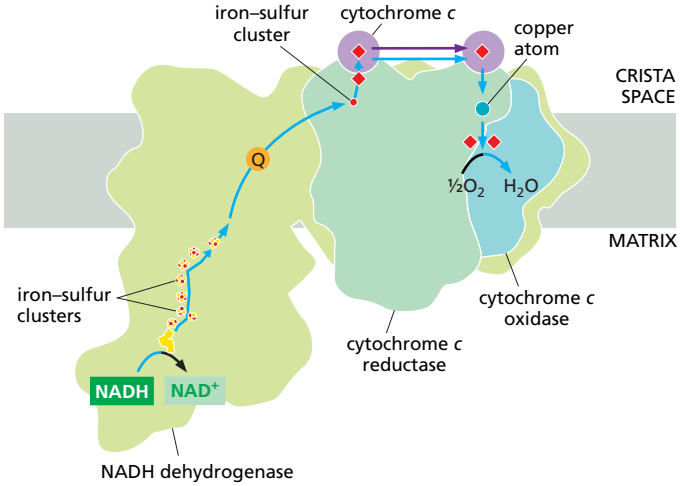


Figure 14–26 The respiratory-chain supercomplex from bovine heart mitochondria. The three proton-pumping complexes of the mitochondrial respiratory chain of mammalian mitochondria assemble into large supercomplexes in the crista membrane. Supercomplexes can be isolated by mild detergent treatment of mitochondria, and their structure has been deciphered by single-particle cryoelectron microscopy. The bovine heart supercomplex has a total mass of 1.7 megadaltons. Shown is a schematic of such a complex that consists of NADH dehydrogenase, cytochrome *c* reductase, and cytochrome *c* oxidase, as indicated. The facing quinol-binding sites of NADH dehydrogenase and cytochrome *c* reductase, plus the short distance between the cytochrome *c*-binding sites in cytochrome *c* reductase and cytochrome *c* oxidase, facilitate fast, efficient electron transfer. Cofactors active in electron transport are marked as a *yellow dot* (flavin mononucleotide), *red and yellow dots* (iron–sulfur clusters), Q (quinone), *red squares* (hemes), and a *blue dot* (copper atom). Only cofactors participating in the linear flow of electrons from NADH to water are shown. *Blue arrows* indicate the path of the electrons through the supercomplex. (Adapted from T. Athoff et al., *EMBO J.* 30:4652–4664, 2011.)

a molecule of ubiquinone. The reduced ubiquinol then passes its two electrons to the respiratory chain via cytochrome *c* reductase (see Figure 14–18). Succinate dehydrogenase is not a proton pump, and it does not contribute directly to the electrochemical potential utilized for ATP production in mitochondria. Thus, it is not considered to be an integral part of the respiratory chain.

Protons Can Move Rapidly Through Proteins Along Predefined Pathways

The protons in water are highly mobile: by rapidly dissociating from one water molecule and associating with its neighbor, they can rapidly flit through a hydrogen-bonded network of water molecules (see Figure 2–5). But how can a proton move through the hydrophobic interior of a protein embedded in the lipid bilayer? Proton-translocating proteins contain so-called *proton wires*, which are rows of polar or ionic side chains, or water molecules spaced at short distances, so that the protons can jump from one to the next (**Figure 14–27**). Along such predefined pathways, protons move up to 40 times faster than through bulk water. The three-dimensional structure of cytochrome *c* oxidase indicates two different proton-uptake pathways. This confirmed earlier mutagenesis studies, which had shown that replacing the side chains of particular aspartate or arginine residues, whose side chains can bind and release protons, made the cytochrome *c* oxidase less efficient as a proton pump.

But how can electron transport cause allosteric changes in protein conformations that pump protons? From the most basic point of view, if electron transport drives sequential allosteric changes in protein conformation that alter the redox state of the components, these conformational changes can be connected to protein wires that allow the protein to pump H^+ across the crista membrane. This type of H^+ pumping requires at least three distinct conformations for the pump protein, as schematically illustrated in **Figure 14–28.**

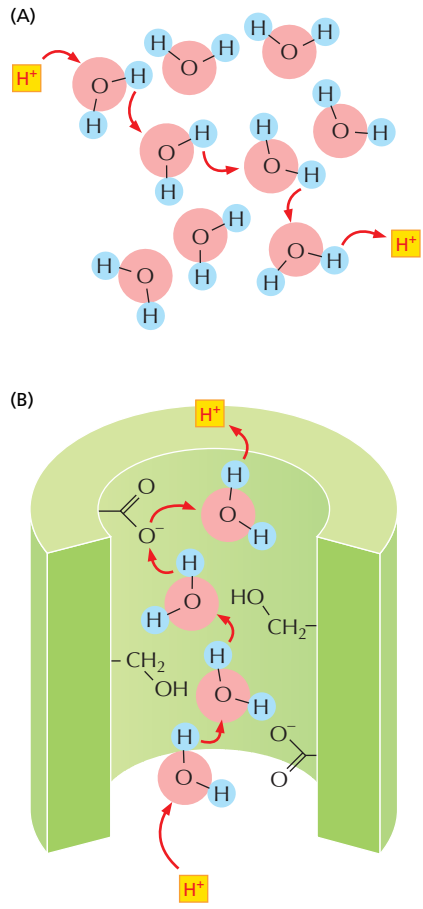


Figure 14–27 Proton movement through water and proteins. (A) Protons move rapidly through water, hopping from one H_2O molecule to the next by the continuous formation and dissociation of hydronium ions, H_3O^+ (see Chapter 2). In this diagram, proton jumps are indicated by *red arrows*. (B) Protons can move even more rapidly through a protein along "proton wires." These are predefined proton paths consisting of suitably spaced amino acid side chains that accept and release protons easily (Asp, Glu) or carry a waterlike hydroxyl group (Ser, Thr), along with water molecules trapped in the protein interior.

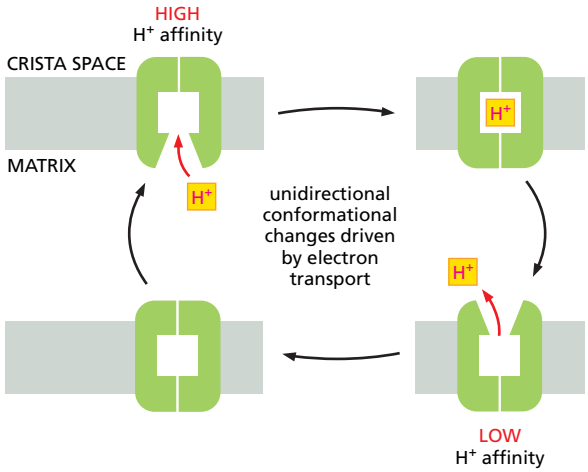


Figure 14–28 A general model for H^+ pumping coupled to electron transport. This mechanism for H^+ pumping by a transmembrane protein is thought to be used by NADH dehydrogenase and cytochrome *c* oxidase, and by many other proton pumps. The protein is driven through a cycle of three conformations. In one of these conformations, the protein has a high affinity for H^+, causing it to pick up an H^+ on the inside of the membrane. In another conformation, the protein has a low affinity for H^+, causing it to release an H^+ on the outside of the membrane. As indicated, the transitions from one conformation to another occur only in one direction, because they are being driven by being allosterically coupled to the energetically favorable process of electron transport (discussed in Chapter 11).

Summary

The respiratory chain embedded in the inner mitochondrial membrane contains three respiratory enzyme complexes, through which electrons pass on their way from NADH to O_2. In these complexes, electrons are transferred along a series of protein-bound electron carriers, including hemes and iron–sulfur clusters. The energy released as the electrons move to lower and lower energy levels is used to pump protons by different mechanisms in the three respiratory enzyme complexes, each coupling lateral electron transport to vectorial proton transport across the membrane. Electrons are shuttled between enzyme complexes by the mobile electron carriers ubiquinone and cytochrome c *to complete the electron-transport chain. The path of electron flow is NADH → NADH dehydrogenase complex → ubiquinone → cytochrome* c *reductase → cytochrome* c *→ cytochrome* c *oxidase complex → molecular oxygen (O_2).*

ATP PRODUCTION IN MITOCHONDRIA

As we have just discussed, the three proton pumps of the respiratory chain each contribute to the formation of an electrochemical proton gradient across the inner mitochondrial membrane. This gradient drives ATP synthesis by ATP synthase, a large membrane-bound protein complex that performs the extraordinary feat of converting the energy contained in this electrochemical gradient into biologically useful, chemical-bond energy in the form of ATP (see Figure 14–10). Protons flow down their electrochemical gradient through the membrane part of this proton turbine, thereby driving the synthesis of ATP from ADP and P_i in the extramembranous part of the complex. As discussed in Chapter 2, the formation of ATP from ADP and inorganic phosphate is highly unfavorable energetically. As we shall see, ATP synthase can produce ATP only because of allosteric shape changes in this protein complex that directly couple ATP synthesis to the energetically favorable flow of protons across its membrane.

The Large Negative Value of ΔG for ATP Hydrolysis Makes ATP Useful to the Cell

An average person turns over roughly 50 kg of ATP per day. In athletes running a marathon, this figure can go up to several hundred kilograms. The ATP produced in mitochondria is derived from the energy available in the intermediates NADH, $FADH_2$, and GTP. These three energy-rich compounds are produced both by the oxidation of glucose (**Table 14–1**A), and by the oxidation of fats (Table 14–1B; see also Figure 2–56).

Glycolysis alone can produce only two molecules of ATP for every molecule of glucose that is metabolized, and this is the total energy yield for the fermentation processes that occur in the absence of O_2 (discussed in Chapter 2). In oxidative

TABLE 14–1 Product Yields from the Oxidation of Sugars and Fats

A. Net products from oxidation of one molecule of glucose
In cytosol (glycolysis) 1 glucose ⟶ 2 pyruvate + 2 NADH + 2 ATP In mitochondrion (pyruvate dehydrogenase and citric acid cycle) 2 pyruvate ⟶ 2 acetyl CoA + 2 NADH 2 acetyl CoA ⟶ 6 NADH + 2 $FADH_2$ + 2 GTP
Net result in mitochondrion 2 pyruvate ⟶ 8 NADH + 2 $FADH_2$ + 2 GTP
B. Net products from oxidation of one molecule of palmitoyl CoA (activated form of palmitate, a fatty acid)
In mitochondrion (fatty acid oxidation and citric acid cycle) 1 palmitoyl CoA ⟶ 8 acetyl CoA + 7 NADH + 7 $FADH_2$ 8 acetyl CoA ⟶ 24 NADH + 8 $FADH_2$ + 8 GTP
Net result in mitochondrion 1 palmitoyl CoA ⟶ 31 NADH + 15 $FADH_2$ + 8 GTP

phosphorylation, each pair of electrons donated by the NADH produced in mitochondria can provide energy for the formation of about 2.5 molecules of ATP. Oxidative phosphorylation also produces 1.5 ATP molecules per electron pair from the $FADH_2$ produced by succinate dehydrogenase in the mitochondrial matrix, and from the NADH molecules produced by glycolysis in the cytosol. From the product yields of glycolysis and the citric acid cycle, we can calculate that the complete oxidation of one molecule of glucose—starting with glycolysis and ending with oxidative phosphorylation—gives a net yield of about 30 molecules of ATP. Nearly all this ATP is produced by the mitochondrial ATP synthase.

In Chapter 2, we introduced the concept of free energy (G). The free-energy change for a reaction, ΔG, determines whether that reaction will occur in a cell. We showed on pp. 60–63 that the ΔG for a given reaction can be written as the sum of two parts: the first, called the standard free-energy change, $\Delta G°$, depends only on the intrinsic characters of the reacting molecules; the second depends only on their concentrations. For the simple reaction A ⟶ B,

$$\Delta G = \Delta G° + RT \ln \frac{[B]}{[A]}$$

where [A] and [B] denote the concentrations of A and B, and ln is the natural logarithm. $\Delta G°$ is the standard reference value, which can be seen to be equal to the value of ΔG when the molar concentrations of A and B are equal (since ln 1 = 0).

In Chapter 2, we discussed how the large, favorable free-energy change (large negative ΔG) for ATP hydrolysis is used, through coupled reactions, to drive many other chemical reactions in the cell that would otherwise not occur (see pp. 65–66). The ATP hydrolysis reaction produces two products, ADP and P_i; it is therefore of the type A ⟶ B + C, where, as demonstrated in **Figure 14–29**,

$$\Delta G = \Delta G° + RT \ln \frac{[B][C]}{[A]}$$

When ATP is hydrolyzed to ADP and P_i under the conditions that normally exist in a cell, the free-energy change is roughly –46 to –54 kJ/mole (–11 to –13 kcal/mole). This extremely favorable ΔG depends on maintaining a high concentration of ATP compared with the concentrations of ADP and P_i. When ATP, ADP, and P_i are all present at the same concentration of 1 mole/liter (so-called standard conditions), the ΔG for ATP hydrolysis drops to the standard free-energy change ($\Delta G°$), which is only –30.5 kJ/mole (–7.3 kcal/mole). At much lower concentrations of ATP relative to ADP and P_i, ΔG becomes zero. At this point, the rate at

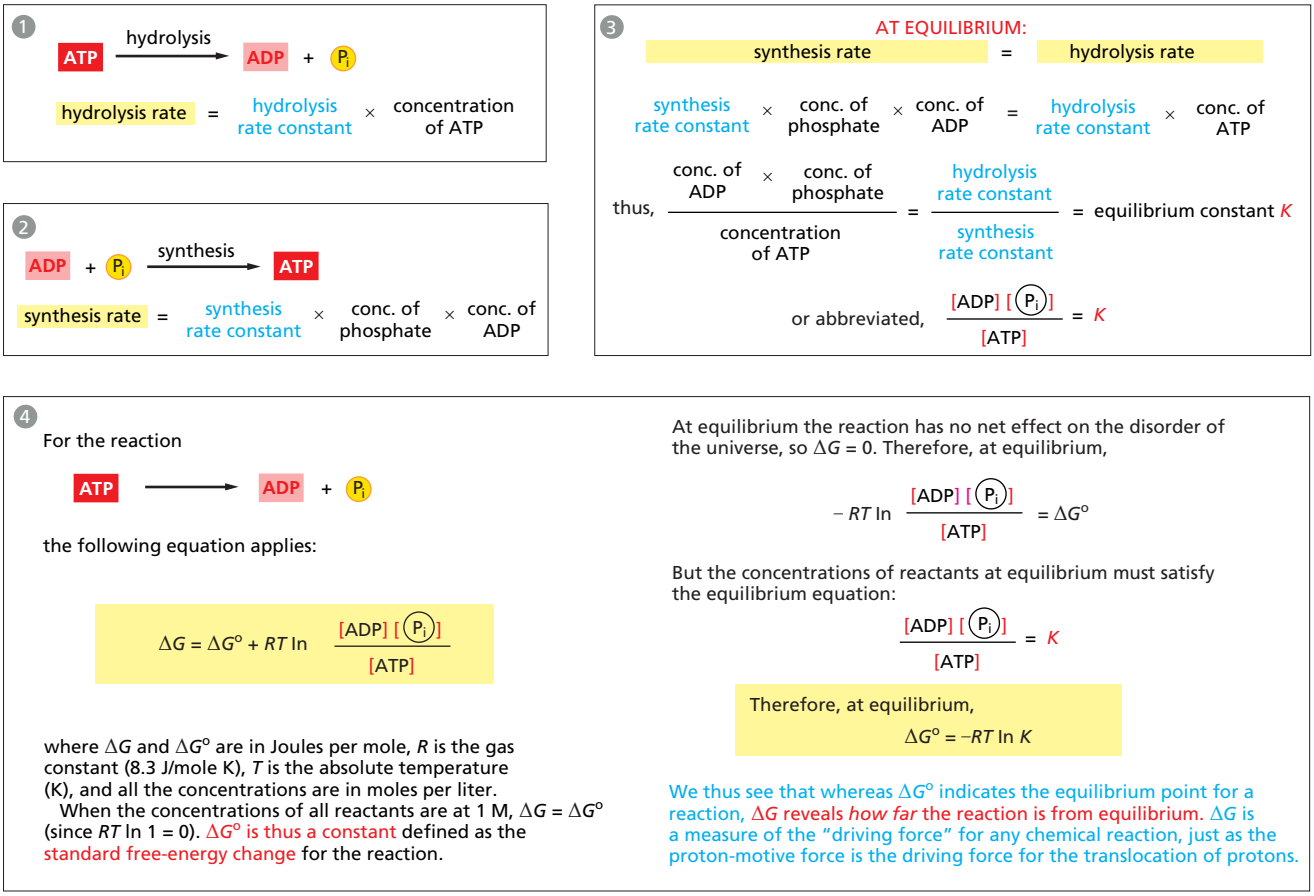


Figure 14–29 The basic relationship between free-energy changes and equilibrium in the ATP hydrolysis reaction. The rate constants in boxes 1 and 2 are determined from experiments in which product accumulation is measured as a function of time (conc., concentration). The equilibrium constant shown here, *K*, is in units of moles per liter. (See Panel 2–7, pp. 102–103, for a discussion of free energy and see Figure 3–44 for a discussion of the equilibrium constant.)

which ADP and P_i will join to form ATP will be equal to the rate at which ATP hydrolyzes to form ADP and P_i. In other words, when $\Delta G = 0$, the reaction is at *equilibrium* (see Figure 14–29).

It is ΔG, not $\Delta G°$, that indicates how far a reaction is from equilibrium and determines whether it can drive other reactions. Because the efficient conversion of ADP to ATP in mitochondria maintains such a high concentration of ATP relative to ADP and P_i, the ATP hydrolysis reaction in cells is kept very far from equilibrium and ΔG is correspondingly very negative. Without this large disequilibrium, ATP hydrolysis could not be used to drive the reactions of the cell. At low ATP concentrations, many biosynthetic reactions would run backward and the cell would die.

The ATP Synthase Is a Nanomachine that Produces ATP by Rotary Catalysis

The **ATP synthase** is a finely tuned nanomachine composed of 23 or more separate protein subunits, with a total mass of about 600,000 daltons. The ATP synthase can work both in the forward direction, producing ATP from ADP and phosphate in response to an electrochemical gradient, or in reverse, generating an electrochemical gradient by ATP hydrolysis. To distinguish it from other enzymes that hydrolyze ATP, it is also called an F_1F_o ATP synthase or F-type ATPase.

Resembling a turbine, ATP synthase is composed of both a rotor and a stator (**Figure 14–30**). To prevent the catalytic head from rotating, a stalk at the periphery of the complex (the stator stalk) connects the head to stator subunits embedded in the membrane. A second stalk in the center of the assembly (the rotor stalk) is connected to the rotor ring in the membrane that turns as protons flow through it, driven by the electrochemical gradient across the membrane. As a result, proton

flow makes the rotor stalk rotate inside the stationary head, where the catalytic sites that assemble ATP from ADP and P_i are located. Three α and three β subunits of similar structure alternate to form the head. Each of the three β subunits has a catalytic nucleotide-binding site at the α/β interface. These catalytic sites are all in different conformations, depending on their interaction with the rotor stalk. This stalk acts like a camshaft, the device that opens and closes the valves in a combustion engine. As it rotates within the head, the stalk changes the conformations of the β subunits sequentially. One of the possible conformations of the catalytic sites has high affinity for ADP and P_i, and as the rotor stalk pushes the binding site into a different conformation, these two substrates are driven to form ATP. In this way, the mechanical force exerted by the central rotor stalk is directly converted into the chemical energy of the ATP phosphate bond.

Serving as a proton-driven turbine, the ATP synthase is driven by H^+ flow into the matrix to spin at about 8000 revolutions per minute, generating three molecules of ATP per turn. In this way, each ATP synthase can produce roughly 400 molecules of ATP per second.

Proton-driven Turbines Are of Ancient Origin

The membrane-embedded rotors of ATP synthases consist of a ring of identical *c* subunits (**Figure 14–31**). Each *c* subunit is a hairpin of two membrane-spanning α helices that contain a proton-binding site defined by a glutamate or aspartate in the middle of the lipid bilayer. The *a* subunit, which is part of the stator (see Figure 14–30), makes two narrow channels at the interface between the rotor and stator, each spanning half of the membrane and converging on the proton-binding site at the middle of the rotor subunit. Protons flow through the two half-channels down their electrochemical gradient from the crista space back into the matrix. A negatively charged side chain in the binding site accepts a proton arriving from the crista space through the first half-channel, as it rotates past the *a* subunit. The bound proton then rides round in the ring for a full cycle, whereupon it is thought to be displaced by a positively charged arginine in the *a* subunit, and escapes

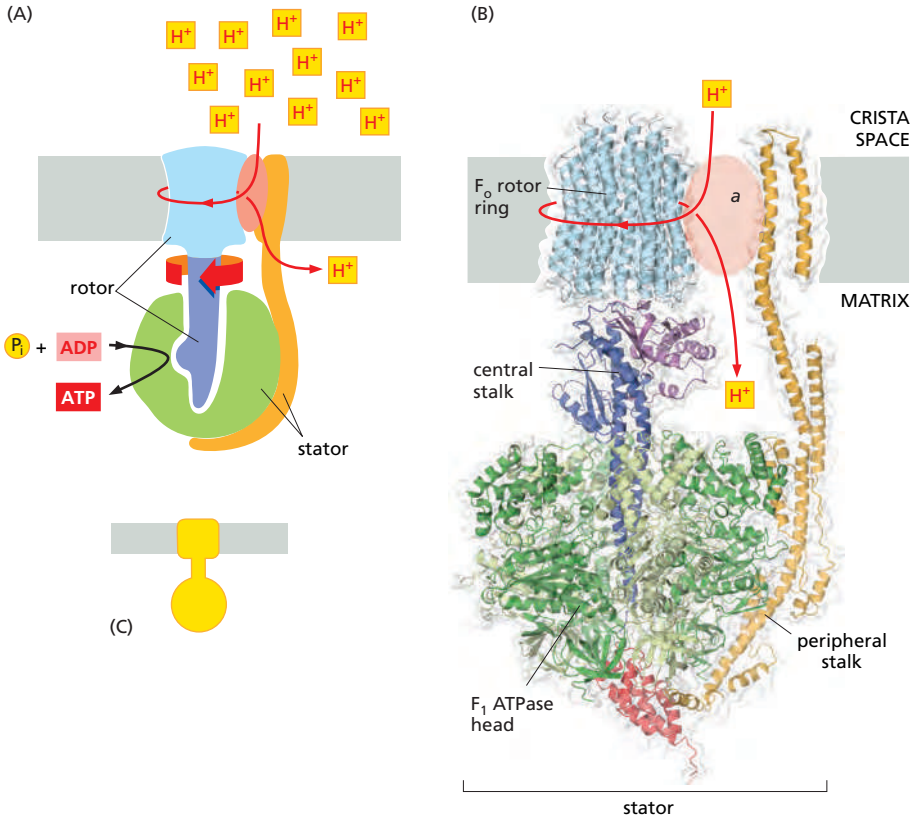


Figure 14–30 ATP synthase. The three-dimensional structure of the F_1F_o ATP synthase, determined by x-ray crystallography. Also known as an F-type ATPase, it consists of an F_o part (from "oligomycin-sensitive factor") in the membrane and the large, catalytic F_1 head in the matrix. Under mild dissociation conditions, this complex separates into its F_1 and F_o components, which can be isolated and studied individually. (A) Diagram of the enzyme complex showing how its globular head portion *(green)* is kept stationary as proton-flow across the membrane drives a rotor *(blue)* that turns inside it. (B) In bovine heart mitochondria, the F_o rotor ring in the membrane *(light blue)* has eight *c* subunits. It is attached to the γ subunit of the central stalk *(dark blue)* by the ε subunit *(purple)*. The catalytic F_1 head consists of a ring of three α and three β subunits *(light and dark green)*, and it directly converts mechanical energy into chemical-bond energy in ATP, as described in the text. The elongated peripheral stalk of the stator *(orange)* is connected to the F_1 head by the small δ subunit *(red)* at one end, and to the *a* subunit in the membrane *(pink oval)* at the other. Together with the *c* subunits of the ring rotating past it, the *a* subunit creates a path for protons through the membrane. (C) The symbol for ATP synthase used throughout this book.

The closely related ATP synthases of mitochondria, chloroplasts, and bacteria synthesize ATP by harnessing the proton-motive force across a membrane. This powers the rotation of the rotor against the stator in a counterclockwise direction, as seen from the F_1 head. The same enzyme complex can also pump protons against their electrochemical gradient by hydrolyzing ATP, which then drives the clockwise rotation of the rotor. The direction of operation depends on the net free-energy change (ΔG) for the coupled processes of H^+ translocation across the membrane and the synthesis of ATP from ADP and P_i (**Movie 14.5** and **Movie 14.6**).

Measurement of the torque that the ATP synthase can produce by ATP hydrolysis reveals that the ATP synthase is 60 times more powerful than a diesel engine of equal dimensions. (B, courtesy of K. Davies. PDB codes: 2WPD, 2CLY, 2WSS, 2BO5.)

through the second half-channel into the matrix. Thus proton flow causes the rotor ring to spin against the stator like a proton-driven turbine.

The mitochondrial ATP synthase is of ancient origin: essentially the same enzyme occurs in plant chloroplasts and in the plasma membrane of bacteria or archaea. The main difference between them is the number of *c* subunits in the rotor ring. In mammalian mitochondria, the ring has 8 subunits. In yeast mitochondria, the number is 10; in bacteria and archaea, it ranges from 11 to 13; in plant chloroplasts, there are 14; and the rings of some cyanobacteria contain 15 *c* subunits.

The *c* subunits in the rotor ring can be thought of as cogs in the gears of a bicycle. A high gear, with a small number of cogs, is advantageous when the supply of protons is limited, as in mitochondria, but a low gear, with a large number of cogs in the wheel, is preferable when the proton gradient is high. This is the case in chloroplasts and cyanobacteria, where protons produced through the action of sunlight are plentiful. Because each rotation produces three molecules of ATP in the head, the synthesis of one ATP requires around three protons in mitochondria but up to five in photosynthetic organisms. It is the number of *c* subunits in the ring that defines how many protons need to pass through this marvelous device to make each molecule of ATP, and thereby how high a ratio of ATP to ADP can be maintained by the ATP synthase.

In principle, ATP synthase can also run in reverse as an ATP-powered proton pump that converts the energy of ATP back into a proton gradient across the membrane. In many bacteria, the rotor of the ATP synthase in the plasma membrane changes direction routinely, from ATP synthesis mode in aerobic respiration, to ATP hydrolysis mode in anaerobic metabolism. In this latter case, ATP hydrolysis serves to maintain the proton gradient across the plasma membrane, which is used to power many other essential cell functions including nutrient transport and the rotation of bacterial flagella. The V-type ATPases that acidify certain cellular organelles are architecturally similar to the F-type ATP synthases, but they normally function in reverse (see Figure 13–37).

Mitochondrial Cristae Help to Make ATP Synthesis Efficient

In the electron microscope, the mitochondrial ATP synthase complexes can be seen to project like lollipops on the matrix side of cristae membranes. Recent studies by cryoelectron microscopy and tomography have shown that this large complex is not distributed randomly in the membrane, but forms long rows of dimers along the cristae ridges (**Figure 14–32**). The dimer rows induce or stabilize these regions of high membrane curvature, which are otherwise energetically unfavorable. Indeed, the formation of ATP synthase dimers and their assembly into rows are required for cristae formation and have far-reaching consequences for cellular fitness. By contrast with bacterial or chloroplast ATP synthases, which do not form dimers, the mitochondrial complex contains additional subunits, located mostly near the membrane end of the stator stalk. Several of these subunits are found to be dimer-specific. If these subunits are mutated in yeast, the ATP synthase in the membrane remains monomeric, the mitochondria have no cristae, cellular respiration drops by half, and the cells grow more slowly.

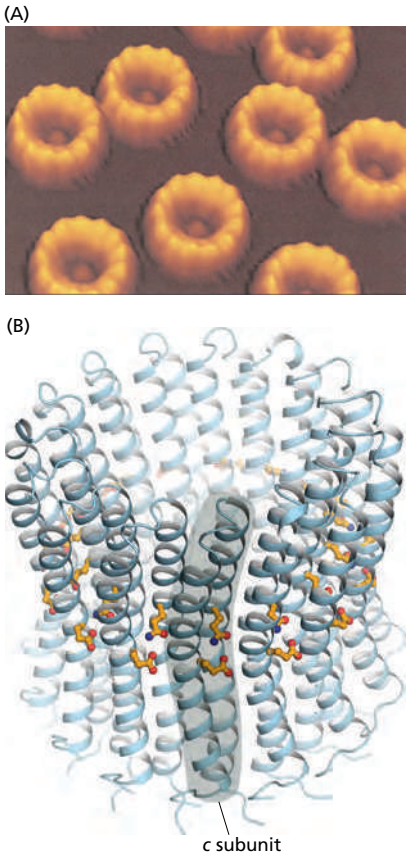


Figure 14–31 F_o ATP synthase rotor rings. (A) Atomic force microscopy image of ATP synthase rotors from the cyanobacterium *Synechococcus elongatus* in a lipid bilayer. Whereas 8 *c* subunits form the rotor in Figure 14–30, there are 13 *c* subunits in this ring. (B) The x-ray structure of the F_o ring of the ATP synthase from *Spirulina platensis*, another cyanobacterium, shows that this rotor has 15 *c* subunits. In all ATP synthases, the *c* subunits are hairpins of two membrane-spanning α helices (one subunit is highlighted in *gray*). The helices are highly hydrophobic, except for two glutamine and glutamate side chains *(yellow)* that create proton-binding sites in the membrane. (A, courtesy of Thomas Meier and Denys Pogoryelov; B, PDB code: 2WIE.)

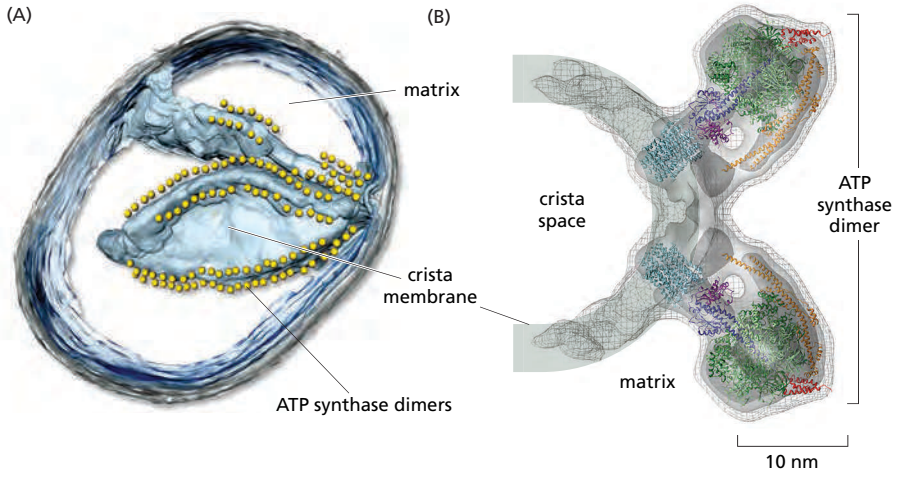


Figure 14–32 Dimers of mitochondrial ATP synthase in cristae membranes. (A) A three-dimensional map of a small mitochondrion obtained by electron microscope tomography shows that ATP synthases form long paired rows along cristae ridges. The outer membrane is *gray*, the inner membrane and cristae membranes have been colored *light blue*. Each head of an ATP synthase is indicated by a *yellow sphere*. (B) A three-dimensional map of a mitochondrial ATP synthase dimer in the crista membrane obtained by subtomogram averaging, with fitted x-ray structures (Movie 14.7). (A, from K. Davies et al., *Proc. Natl Acad. Sci. USA* 108:14121–14126, 2011. With permission from the National Academy of Sciences; B, from K. Davies et al., *Proc. Natl Acad. Sci. USA* 109:13602–13607, 2012. With permission from the National Academy of Sciences.)

Electron tomography suggests that the proton pumps of the respiratory chain are located in the membrane regions at either side of the dimer rows. Protons pumped into the crista space by these respiratory-chain complexes are thought to diffuse very rapidly along the membrane surface, with the ATP synthase rows creating a proton "sink" at the cristae tips (Figure 14–33). *In vitro* studies suggest that the ATP synthase needs a proton gradient of about 2 pH units to produce ATP at the rate required by the cell, irrespective of the membrane potential. The H^+ gradient across the inner mitochondrial membrane is only 0.5 to 0.6 pH units. The cristae thus seem to work as proton traps that enable the ATP synthase to make efficient use of the protons pumped out of the mitochondrial matrix. As we shall see in the next section, this elaborate arrangement of membrane protein complexes is absent in chloroplasts, where the H^+ gradient is much higher.

Special Transport Proteins Exchange ATP and ADP Through the Inner Membrane

Like all biological membranes, the inner mitochondrial membrane contains numerous specific *transport proteins* that allow particular substances to pass through. One of the most abundant of these is the *ADP/ATP carrier protein* (Figure 14–34). This carrier shuttles the ATP produced in the matrix through the inner membrane to the intermembrane space, from where it diffuses through the outer mitochondrial membrane to the cytosol. In exchange, ADP passes from the cytosol into the matrix for recycling into ATP. ATP^{4-} has one more negative charge than ADP^{3-}, and the exchange of ATP and ADP is driven by the electrochemical gradient across the inner membrane, so that the more negatively charged ATP is pushed out of the matrix, and the less negatively charged ADP is pulled in. The ADP/ATP carrier is but one member of a *mitochondrial carrier family*: the inner

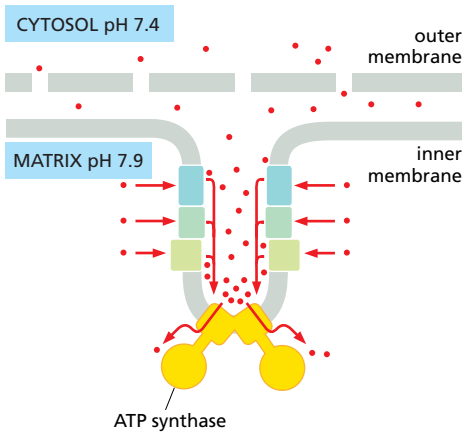


Figure 14–33 ATP synthase dimers at cristae ridges and ATP production. At the crista ridges, the ATP synthases *(yellow)* form a sink for protons *(red)*. The proton pumps of the electron-transport chain *(green)* are located in the membrane regions on either side of the crista. As illustrated, protons tend to diffuse along the membrane from their source to the proton sink created by the ATP synthase. This allows efficient ATP production despite the small H^+ gradient between the cytosol and matrix. *Red arrows* show the direction of the proton flow.

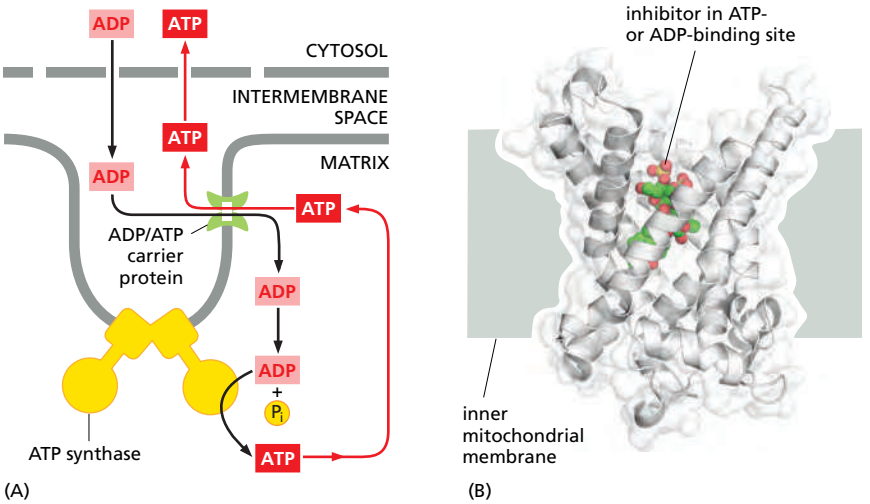


Figure 14–34 The ADP/ATP carrier protein. (A) The ADP/ATP carrier protein is a small membrane protein that carries the ATP produced on the matrix side of the inner membrane to the intermembrane space, and the ADP that is needed for ATP synthesis into the matrix. (B) In the ADP/ATP carrier, six transmembrane α helices define a cavity that binds either ADP or ATP. In this x-ray structure, the substrate is replaced by a tightly bound inhibitor instead *(colored)*. When ADP binds from outside the inner membrane, it triggers a conformational change and is released into the matrix. In exchange, a molecule of ATP quickly binds to the matrix side of the carrier and is transported to the intermembrane space. From there the ATP diffuses through the outer mitochondrial membrane to the cytoplasm, where it powers the energy-requiring processes in the cell. (B, PDB code: 1OKC.)

mitochondrial membrane contains about 20 related carrier proteins exchanging various other metabolites, including the phosphate that is required along with ADP for ATP synthesis.

In some specialized fat cells, mitochondrial respiration is uncoupled from ATP synthesis by the *uncoupling protein*, another member of the mitochondrial carrier family. In these cells, known as brown fat cells, most of the energy of oxidation is dissipated as heat rather than being converted into ATP. In the inner membranes of the large mitochondria in these cells, the uncoupling protein allows protons to move down their electrochemical gradient without passing through ATP synthase. This process is switched on when heat generation is required, causing the cells to oxidize their fat stores at a rapid rate and produce heat rather than ATP. Tissues containing brown fat serve as "heating pads," helping to revive hibernating animals and to protect newborn human babies from the cold.

Chemiosmotic Mechanisms First Arose in Bacteria

Bacteria use enormously diverse energy sources. Some, like animal cells, are aerobic; they synthesize ATP from sugars they oxidize to CO_2 and H_2O by glycolysis, the citric acid cycle, and a respiratory chain in their plasma membrane that is similar to the one in the inner mitochondrial membrane. Others are strict anaerobes, deriving their energy either from glycolysis alone (by fermentation, see Figure 2–47) or from an electron-transport chain that employs a molecule other than oxygen as the final electron acceptor. The alternative electron acceptor can be a nitrogen compound (nitrate or nitrite), a sulfur compound (sulfate or sulfite), or a carbon compound (fumarate or carbonate), for example. A series of electron carriers in the plasma membrane that are comparable to those in mitochondrial respiratory chains transfers the electrons to these acceptors.

Despite this diversity, the plasma membrane of the vast majority of bacteria contains an ATP synthase that is very similar to the one in mitochondria. In bacteria that use an electron-transport chain to harvest energy, the electron-transport chain pumps H^+ out of the cell and thereby establishes a proton-motive force across the plasma membrane that drives the ATP synthase to make ATP. In other

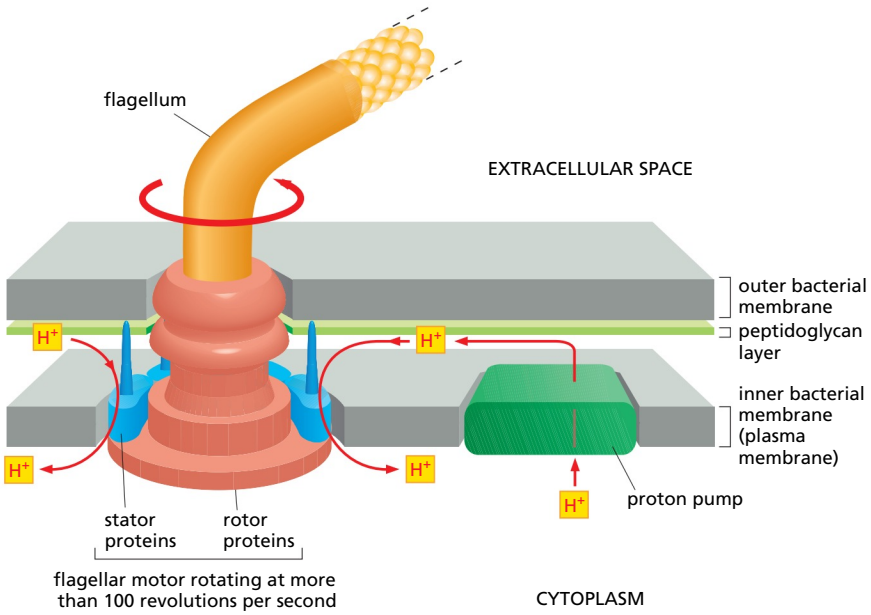


Figure 14–35 The rotation of the bacterial flagellum driven by H^+ flow. The flagellum is attached to a series of protein rings *(pink)*, which are embedded in the outer and inner membranes and rotate with the flagellum. The rotation is driven by a flow of protons through an outer ring of proteins (the stator) by mechanisms that may resemble those used by the ATP synthase. However, the flow of protons in the flagellar motor is always toward the cytosol, both during clockwise and counterclockwise rotation, whereas in ATP synthase this flow reverses with the direction of rotation (Movie 14.8).

bacteria, the ATP synthase works in reverse, using the ATP produced by glycolysis to pump H^+ and establish a proton gradient across the plasma membrane.

Bacteria, including the strict anaerobes, maintain a proton gradient across their plasma membrane that is harnessed to drive many other processes. It can be used to drive a flagellar motor, for example (Figure 14–35). This gradient is harnessed to pump Na^+ out of the bacterium via a Na^+–H^+ antiporter that takes the place of the Na^+-K^+ pump of eukaryotic cells. The gradient is also used for the active inward transport of nutrients, such as most amino acids and many sugars: each nutrient is dragged into the cell along with one or more protons through a specific symporter (Figure 14–36; see also Chapter 11). In animal cells, by contrast, most inward transport across the plasma membrane is driven by the Na^+ gradient (high Na^+ outside, low Na^+ inside) that is established by the Na^+-K^+ pump (see Figure 11–15).

Some unusual bacteria have adapted to live in a very alkaline environment and yet must maintain their cytoplasm at a physiological pH. For these cells, any attempt to generate an electrochemical H^+ gradient would be opposed by a large H^+ concentration gradient in the wrong direction (H^+ higher inside than outside). Presumably for this reason, some of these bacteria substitute Na^+ for H^+ in all of their chemiosmotic mechanisms. The respiratory chain pumps Na^+ out of the cell, the transport systems and flagellar motor are driven by an inward flux of Na^+, and a Na^+-driven ATP synthase synthesizes ATP. The existence of such bacteria demonstrates a critical point: the principle of chemiosmosis is more fundamental than the proton-motive force on which it is normally based.

As we discuss next, an ATP synthase coupled to chemiosmotic processes is also a central feature of plants, where it plays critical roles in both mitochondria and chloroplasts.

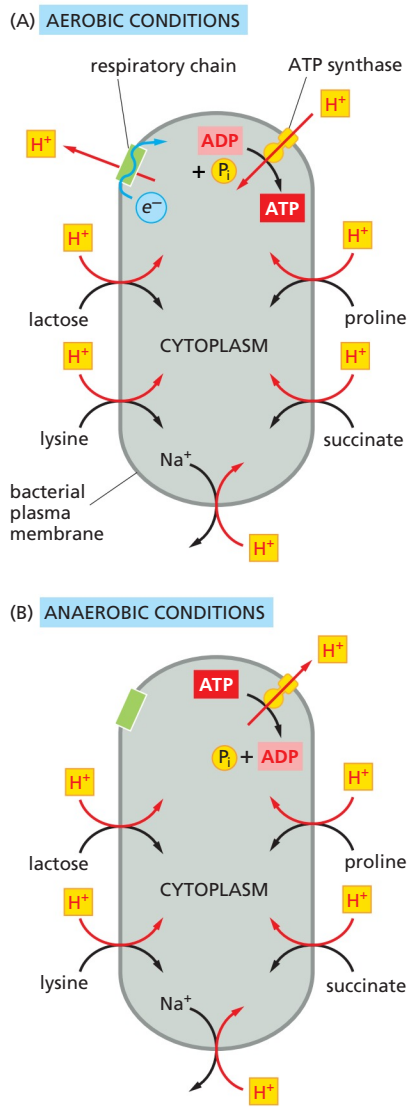


Figure 14–36 The importance of H^+-driven transport in bacteria. A proton-motive force generated across the plasma membrane pumps nutrients into the cell and expels Na^+. (A) In an aerobic bacterium, a respiratory chain fed by the oxidation of substrates produces an electrochemical proton gradient across the plasma membrane. This gradient is then harnessed to make ATP, as well as to transport nutrients (proline, succinate, lactose, and lysine) into the cell and to pump Na^+ out of the cell. (B) When the same bacterium grows under anaerobic conditions, it derives its ATP from glycolysis. As indicated, the ATP synthase in the plasma membrane then hydrolyzes some of this ATP to establish an electrochemical proton gradient that drives the same transport processes that depend on respiratory chain proton-pumping in (A).

Summary

The large amount of free energy released when H^+ flows back into the matrix from the cristae provides the basis for ATP production on the matrix side of mitochondrial cristae membranes by a remarkable protein machine—the ATP synthase. The ATP synthase functions like a miniature turbine, and it is a reversible device that can couple proton flow to either ATP synthesis or ATP hydrolysis. The transmembrane electrochemical gradient that drives ATP production in mitochondria also drives the active transport of selected metabolites across the inner mitochondrial membrane, including an efficient ADP/ATP exchange between the mitochondrion and the cytosol that keeps the cell's ATP pool highly charged. The resulting high cellular concentration of ATP makes the free-energy change for ATP hydrolysis extremely favorable, allowing this hydrolysis reaction to drive a very large number of energy-requiring processes throughout the cell. The universal presence of ATP synthase in bacteria, mitochondria, and chloroplasts testifies to the central importance of chemiosmotic mechanisms in cells.

CHLOROPLASTS AND PHOTOSYNTHESIS

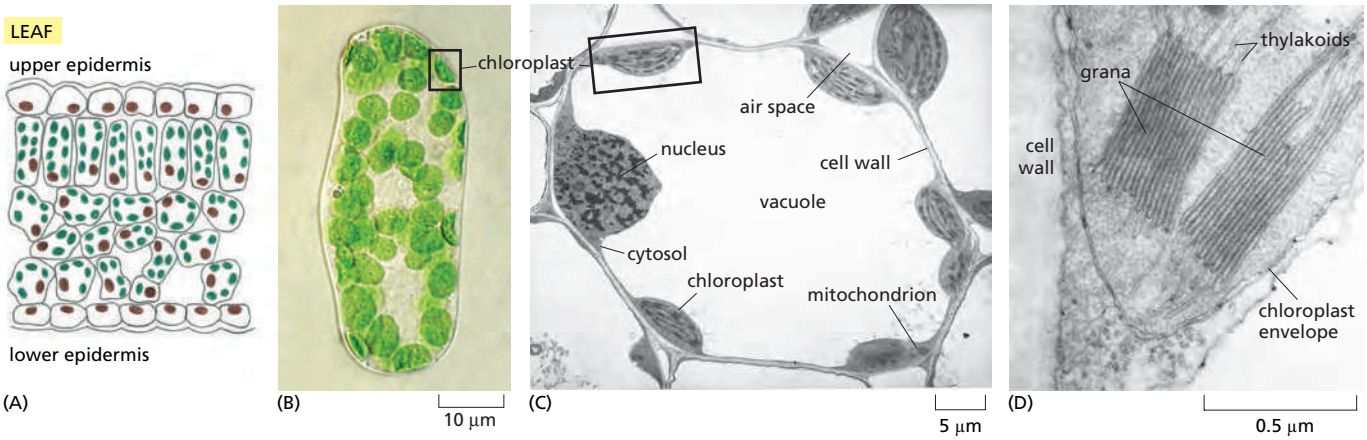
All animals and most microorganisms rely on the continual uptake of large amounts of organic compounds from their environment. These compounds provide both the carbon-rich building blocks for biosynthesis and the metabolic energy for life. It is likely that the first organisms on the primitive Earth had access to an abundance of organic compounds produced by geochemical processes, but it is clear that these were used up billions of years ago. Since that time, virtually all of the organic materials required by living cells have been produced by *photosynthetic organisms*, including plants and photosynthetic bacteria. The core machinery that drives all photosynthesis appears to have evolved more than 3 billion years ago in the ancestors of present-day bacteria; today it provides the only major solar energy storage mechanism on Earth.

The most advanced photosynthetic bacteria are the cyanobacteria, which have minimal nutrient requirements. They use electrons from water and the energy of sunlight to convert atmospheric CO_2 into organic compounds—a process called *carbon fixation*. In the course of the overall reaction $n H_2O + n CO_2 \rightarrow$ (light) $(CH_2O)_n + n O_2$, they also liberate into the atmosphere the molecular oxygen that then powers oxidative phosphorylation. In this way, it is thought that the evolution of cyanobacteria from more primitive photosynthetic bacteria eventually made possible the development of the many different aerobic life-forms that populate the Earth today.

Chloroplasts Resemble Mitochondria But Have a Separate Thylakoid Compartment

Plants (including algae) developed much later than cyanobacteria, and their photosynthesis occurs in a specialized intracellular organelle—the **chloroplast** (**Figure 14–37**). Chloroplasts use chemiosmotic mechanisms to carry out their energy interconversions in much the same way that mitochondria do. Although much larger than mitochondria, they are organized on the same principles. They have a highly permeable outer membrane; a much less permeable inner membrane, in which membrane transport proteins are embedded; and a narrow intermembrane space in between. Together, these two membranes form the chloroplast envelope (Figure 14–37D). The inner chloroplast membrane surrounds a large space called the **stroma**, which is analogous to the mitochondrial matrix. The stroma contains many metabolic enzymes and, as for the mitochondrial matrix, it is the place where ATP is made by the head of an ATP synthase. Like the mitochondrion, the chloroplast has its own genome and genetic system. The stroma therefore also contains a special set of ribosomes, RNAs, and the chloroplast DNA.

An important difference between the organization of mitochondria and chloroplasts is highlighted in **Figure 14–38**. The inner membrane of the chloroplast is



not folded into cristae and does not contain electron-transport chains. Instead, the electron-transport chains, photosynthetic light-capturing systems, and ATP synthase are all contained in the **thylakoid membrane**, a separate, distinct membrane that forms a set of flattened, disc-like sacs, the *thylakoids*. The thylakoid membrane is highly folded into numerous local stacks of flattened vesicles called *grana*, interconnected by nonstacked thylakoids. The lumen of each thylakoid is connected with the lumen of other thylakoids, thereby defining a third internal compartment called the *thylakoid space*. This space represents a separate compartment in each chloroplast that is not connected to either the intermembrane space or the stroma.

Figure 14–37 Chloroplasts in the cell. (A) Schematic cross section through the leaf of a green plant. (B) Light microscopy of a plant leaf cell—here, a mesophyll cell from *Zinnia elegans*—shows chloroplasts as bright green bodies, measuring several micrometers across, in the transparent cell interior. (C) The electron micrograph of a thin, stained section through a wheat leaf cell shows a thin rim of cytoplasm—containing chloroplasts, the nucleus, and mitochondria—surrounding a large, water-filled vacuole. (D) At higher magnification, electron microscopy reveals the chloroplast envelope membrane and the thylakoid membrane within the chloroplast that is highly folded into grana stacks (Movie 14.9). (B, courtesy of John Innes Foundation; C and D, courtesy of K. Plaskitt.)

Chloroplasts Capture Energy from Sunlight and Use It to Fix Carbon

We can group the reactions that occur during photosynthesis in chloroplasts into two broad categories:

1. The **photosynthetic electron-transfer reactions** (also called the "light reactions") occur in two large protein complexes, called *reaction centers*, embedded in the thylakoid membrane. A photon (a quantum of light) knocks an electron out of the green pigment molecule *chlorophyll* in the first reaction center, creating a positively charged chlorophyll ion. This electron then moves along an electron-transport chain and through a second reaction center in much the same way that an electron moves along the respiratory chain in mitochondria. During this electron-transport process, H^+ is pumped across the thylakoid membrane, and the resulting

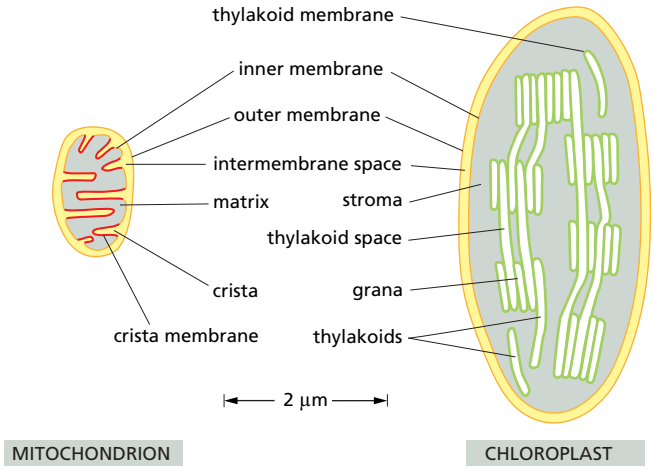


Figure 14–38 A mitochondrion and chloroplast compared. Chloroplasts are generally larger than mitochondria. In addition to an outer and inner envelope membrane, they contain the thylakoid membrane with its internal thylakoid space. The chloroplast thylakoid membrane, which is the site of solar energy conversion in plants and algae, corresponds to the mitochondrial cristae, which are the sites of energy conversion by cellular respiration. Unlike the crista membrane, which is continuous with the inner mitochondrial membrane at cristae junctions, the thylakoid membrane is not connected to the inner chloroplast membrane at any point.

electrochemical proton gradient drives the synthesis of ATP in the stroma. As the final step in this series of reactions, electrons are loaded (together with H^+) onto $NADP^+$, converting it to the energy-rich NADPH molecule. Because the positively charged chlorophyll in the first reaction center quickly regains its electrons from water (H_2O), O_2 gas is produced as a by-product. All of these reactions are confined to the chloroplast.

2. The **carbon-fixation reactions** do not require sunlight. Here the ATP and NADPH generated by the light reactions serve as the source of energy and reducing power, respectively, to drive the conversion of CO_2 to carbohydrate. These carbon-fixation reactions begin in the chloroplast stroma, where they generate the three-carbon sugar *glyceraldehyde 3-phosphate*. This simple sugar is exported to the cytosol, where it is used to produce sucrose and many other organic metabolites in the leaves of the plant. The sucrose is then exported to meet the metabolic needs of the nonphotosynthetic plant tissues, serving as a source of both carbon skeletons and energy for growth.

Thus, the formation of ATP, NADPH, and O_2 (which requires light energy directly) and the conversion of CO_2 to carbohydrate (which requires light energy only indirectly) are separate processes (**Figure 14–39**). However, 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 chloroplast enzymes required for carbon fixation, for example, are inactive in the dark and reactivated by light-stimulated electron-transport processes.

Carbon Fixation Uses ATP and NADPH to Convert CO_2 into Sugars

We have seen earlier in this chapter how animal cells produce ATP by using the large amount of free energy released when carbohydrates are oxidized to CO_2 and H_2O. The reverse reaction, in which plants make carbohydrate from CO_2 and H_2O, takes place in the chloroplast stroma. The large amounts of ATP and NADPH produced by the photosynthetic electron-transfer reactions are required to drive this energetically unfavorable reaction.

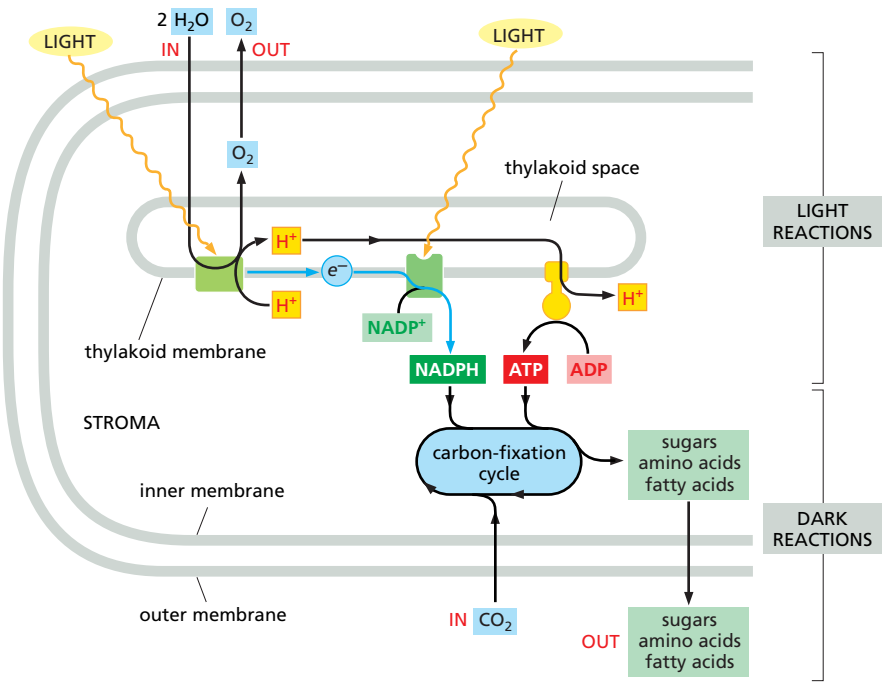


Figure 14–39 A summary of the energy-converting metabolism in chloroplasts. Chloroplasts require only water and carbon dioxide as inputs for their light-driven photosynthesis reactions, and they produce the nutrients for most other organisms on the planet. Each oxidation of two water molecules by a photochemical reaction center in the thylakoid membrane produces one molecule of oxygen, which is released into the atmosphere. At the same time, protons are concentrated in the thylakoid space. These protons create a large electrochemical gradient across the thylakoid membrane, which is utilized by the chloroplast ATP synthase to produce ATP from ADP and phosphate. The electrons withdrawn from water are transferred to a second type of photochemical reaction center to produce NADPH from $NADP^+$. As indicated, the NADPH and ATP are fed into the *carbon-fixation cycle* to reduce carbon dioxide, thereby producing the precursors for sugars, amino acids, and fatty acids. The CO_2 that is taken up from the atmosphere here is the source of the carbon atoms for most organic molecules on Earth.

In a plant cell, a variety of metabolites produced in the chloroplast are exported to the cytoplasm for biosyntheses. Some of the sugar produced is stored in the form of starch granules in the chloroplast, but the rest is transported throughout the plant as sucrose or converted to starch in special storage tissues. These storage tissues serve as a major food source for animals.

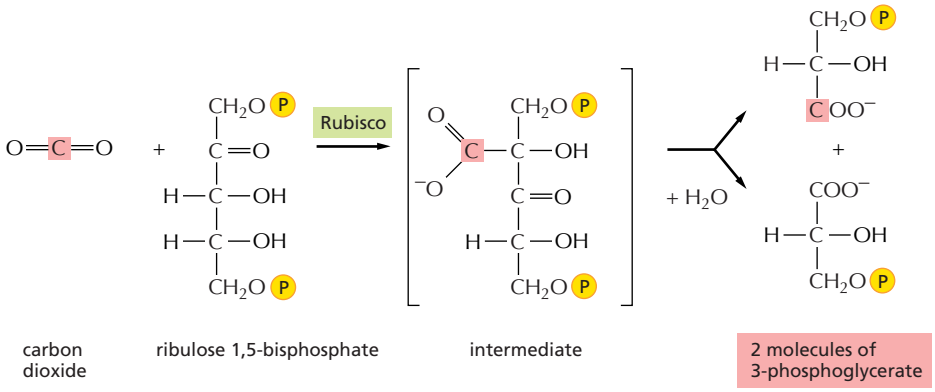


Figure 14–40 The initial reaction in carbon fixation. This carboxylation reaction allows one molecule each of carbon dioxide and water to be incorporated into organic carbon molecules. It is catalyzed in the chloroplast stroma by the abundant enzyme ribulose bisphosphate carboxylase, or Rubisco. As indicated, the product is two molecules of 3-phosphoglycerate.

Figure 14–40 illustrates the central reaction of **carbon fixation**, in which an atom of inorganic carbon is converted to organic carbon: CO_2 from the atmosphere combines with the five-carbon compound ribulose 1,5-bisphosphate plus water to yield two molecules of the three-carbon compound 3-phosphoglycerate. This carboxylation reaction is catalyzed in the chloroplast stroma by a large enzyme called *ribulose bisphosphate carboxylase*, or *Rubisco* for short. Because the reaction is so slow (each Rubisco molecule turns over only about 3 molecules of substrate per second, compared to 1000 molecules per second for a typical enzyme), an unusually large number of enzyme molecules are needed. Rubisco often constitutes more than 50% of the chloroplast protein mass, and it is thought to be the most abundant protein on Earth. In a global context, Rubisco also keeps the amount of the greenhouse gas CO_2 in the atmosphere at a low level.

Although the production of carbohydrates from CO_2 and H_2O is energetically unfavorable, the fixation of CO_2 catalyzed by Rubisco is an energetically favorable reaction. Carbon fixation is energetically favorable because a continuous supply of the energy-rich ribulose 1,5-bisphosphate is fed into the process. This compound is consumed by the addition of CO_2, and it must be replenished. The energy and reducing power needed to regenerate ribulose 1,5-bisphosphate come from the ATP and NADPH produced by the photosynthetic light reactions.

The elaborate series of reactions in which CO_2 combines with ribulose 1,5-bisphosphate to produce a simple sugar—a portion of which is used to regenerate ribulose 1,5-bisphosphate—forms a cycle, called the *carbon-fixation cycle*, or the Calvin cycle (**Figure 14–41**). This cycle was one of the first metabolic pathways to be worked out by applying radioisotopes as tracers in biochemistry. As indicated, each turn of the cycle converts six molecules of 3-phosphoglycerate to three molecules of ribulose 1,5-bisphosphate plus one molecule of glyceraldehyde 3-phosphate. *Glyceraldehyde 3-phosphate*, the three-carbon sugar produced by the cycle, then provides the starting material for the synthesis of many other sugars and all of the other organic molecules that form the plant.

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

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

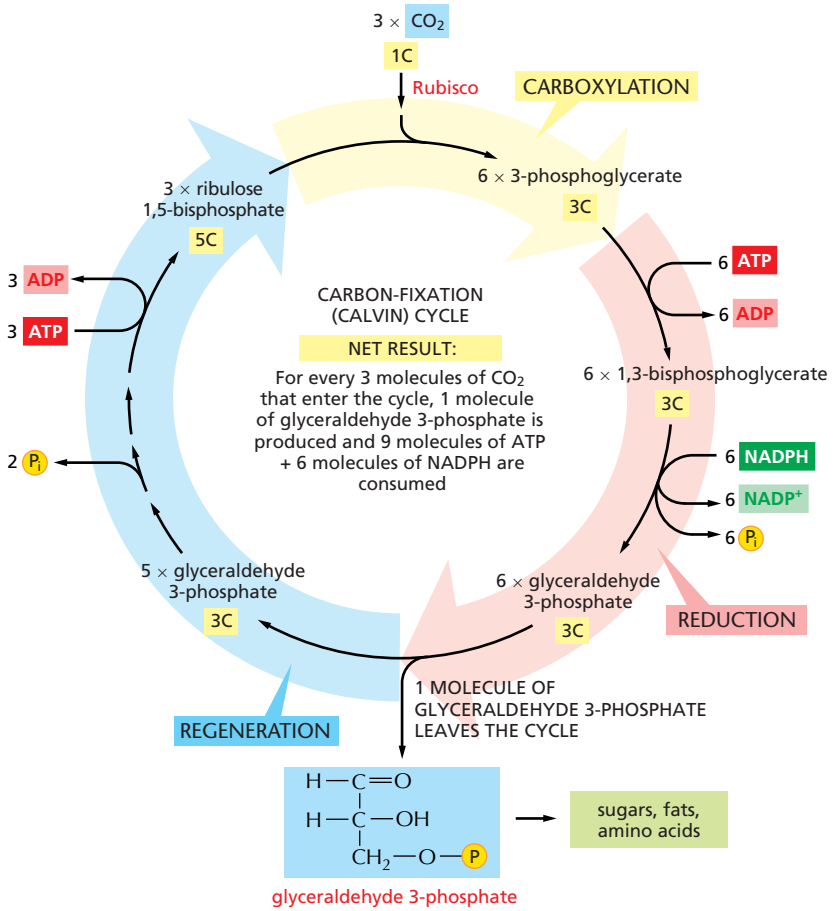


Figure 14–41 The carbon-fixation cycle. This central metabolic pathway allows organic molecules to be produced from CO_2 and H_2O. In the first stage of the cycle (carboxylation), CO_2 is added to ribulose 1,5-bisphosphate, as shown in Figure 14–40. In the second stage (reduction), ATP and NADPH are consumed to produce glyceraldehyde 3-phosphate molecules. In the final stage (regeneration), some of the glyceraldehyde 3-phosphate produced is used to regenerate ribulose 1,5-bisphosphate. Other glyceraldehyde 3-phosphate molecules are either converted to starch and fat in the chloroplast stroma, or transported out of the chloroplast into the cytosol. The number of carbon atoms in each type of molecule is indicated in *yellow*. There are many intermediates between glyceraldehyde 3-phosphate and ribulose 5-phosphate, but they have been omitted here for clarity. The entry of water into the cycle is also not shown (but see Figure 14–40).

glycolytic pathway (see Figure 2–46), where it is converted to pyruvate. Both that pyruvate and the fatty acids can enter the plant cell mitochondria and be fed into the citric acid cycle, ultimately leading to the production of large amounts of ATP by oxidative phosphorylation (**Figure 14–42**). Plants use this ATP in the same way that animal cells and other nonphotosynthetic organisms do to power a variety of metabolic reactions.

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 to provide carbohydrate to the rest of the plant.

The Thylakoid Membranes of Chloroplasts Contain the Protein Complexes Required for Photosynthesis and ATP Generation

We next need to explain how the large amounts of ATP and NADPH required for carbon fixation are generated in the chloroplast. Chloroplasts are much larger and less dynamic than mitochondria, but they make use of chemiosmotic energy conversion in much the same way. As we saw in Figure 14–38, chloroplasts and mitochondria are organized on the same principles, although the chloroplast contains a separate thylakoid membrane system in which its chemiosmotic mechanisms occur. The thylakoid membranes contain two large membrane protein complexes, called *photosystems*, which endow plants and other photosynthetic organisms with the ability to capture and convert solar energy for their own use. Two other protein complexes in the thylakoid membrane that work together with the photosystems in photophosphorylation—the generation of ATP with sunlight—have mitochondrial equivalents. These are the heme-containing cytochrome

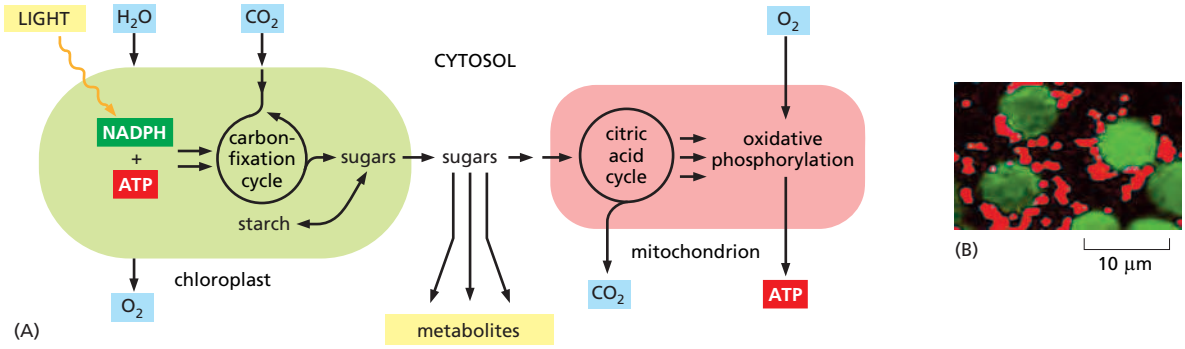


Figure 14–42 How chloroplasts and mitochondria collaborate to supply cells with both metabolites and ATP. (A)The inner chloroplast membrane is impermeable to the ATP and NADPH that are produced in the stroma during the light reactions of photosynthesis. These molecules are therefore funneled into the carbon-fixation cycle, where they are used to make sugars. The resulting sugars and their metabolites are either stored within the chloroplast—in the form of starch or fat—or exported to the rest of the plant cell. There, they can enter the energy-generating pathway that ends in ATP synthesis linked to oxidative phosphorylation inside the mitochondrion. Unlike the chloroplast, mitochondrial membranes contain a specific transporter that makes them permeable to ATP (see Figure 14–34). Note that the O_2 released to the atmosphere by photosynthesis in chloroplasts is used for oxidative phosphorylation in mitochondria; similarly, the CO_2 released by the citric acid cycle in mitochondria is used for carbon fixation in chloroplasts. (B) In a leaf, mitochondria *(red)* tend to cluster close to the chloroplasts *(green)*, as seen in this light micrograph. (B, courtesy of Olivier Grandjean.)

b_6-f complex, which both functionally and structurally resembles cytochrome c reductase in the respiratory chain; and the chloroplast ATP synthase, which closely resembles the mitochondrial ATP synthase and works in the same way.

Chlorophyll–Protein Complexes Can Transfer Either Excitation Energy or Electrons

The photosystems in the thylakoid membrane are multiprotein assemblies of a complexity comparable to that of the protein complexes in the mitochondrial electron-transport chain. They contain large numbers of specifically bound chlorophyll molecules, in addition to cofactors that will be familiar from our discussion of mitochondria (heme, iron–sulfur clusters, and quinones). **Chlorophyll**, the green pigment of photosynthetic organisms, has a long hydrophobic tail that makes it behave like a lipid, plus a porphyrin ring that has a central Mg atom and an extensive system of delocalized electrons in conjugated double bonds (**Figure 14–43**). When a chlorophyll molecule absorbs a quantum of sunlight (a photon), the energy of the photon causes one of these electrons to move from a low-energy molecular orbital to another orbital of higher energy.

The excited electron in a chlorophyll molecule tends to return quickly to its ground state, which can occur in one of three ways:

1. By converting the extra energy into heat (molecular motion) or to some combination of heat and light of a longer wavelength (fluorescence); this is what usually happens when light is absorbed by an isolated chlorophyll molecule in solution.
2. By transferring the energy—but not the electron—directly to a neighboring chlorophyll molecule by a process called *resonance energy transfer*.
3. By transferring the excited electron with its negative charge to another nearby molecule, an *electron acceptor*, after which the positively charged chlorophyll returns to its original state by taking up an electron from some other molecule, an *electron donor.*

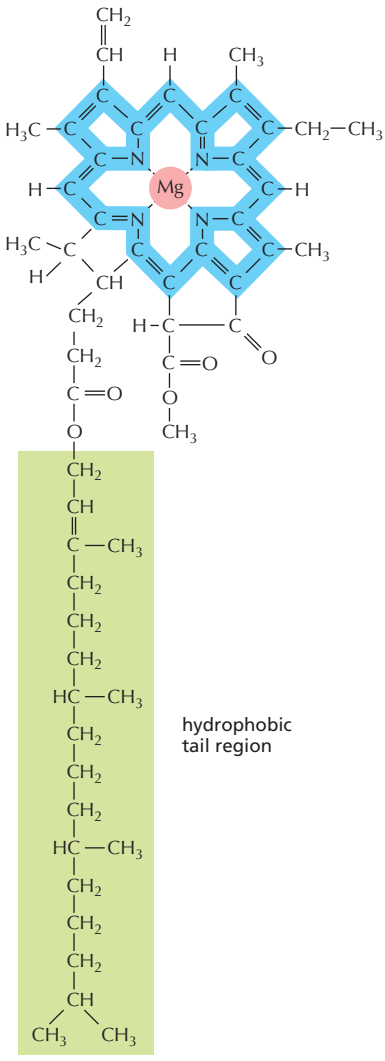


Figure 14–43 The structure of chlorophyll. A magnesium atom is held in a porphyrin ring, which is related to the porphyrin ring that binds iron in heme (see Figure 14–15). Electrons are delocalized over the bonds shaded in *blue*.

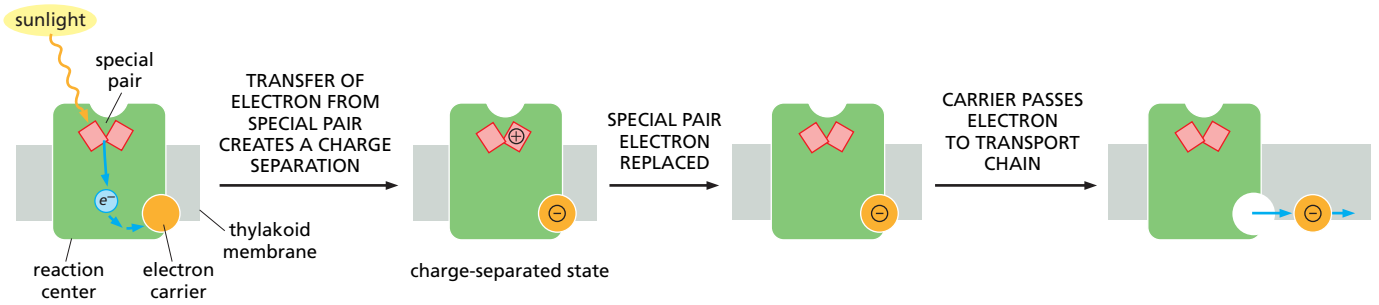


Figure 14–44 A general scheme for the charge-separation step in a photosynthetic reaction center. In a reaction center, light energy is harnessed to generate electrons that are held at a high energy level by mobile electron carriers in a membrane. Light energy is thereby converted to chemical energy. The process starts when a photon absorbed by the special pair of chlorophylls in the reaction center knocks an electron out of one of the chlorophylls. The electron is taken up by a mobile electron carrier *(orange)* bound at the opposite membrane surface. A set of intermediary carriers embedded in the reaction center provide the path from the special pair to this carrier (not shown). The physical distance between the positively charged chlorophyll ion and the negatively charged electron carrier stabilizes the charge-separated state for a short time, during which the chlorophyll ion, a strong oxidant, withdraws an electron from a suitable compound (for example, from water, an event we will discuss in detail shortly). The electron carrier then diffuses away from the reaction center as a strong electron donor that will transfer its electron to an electron-transport chain.

The latter two mechanisms occur when chlorophylls are attached to proteins in a *chlorophyll–protein complex*. The protein coordinates the central Mg atom in the chlorophyll porphyrin, most often through a histidine side chain located in the hydrophobic interior of a membrane, causing each of the chlorophylls in a protein complex to be held at exactly defined distances and orientations. The flow of excitation energy or electrons then depends on both the precise spatial arrangement and the local protein environment of the protein-bound chlorophylls.

When excited by a photon, most protein-bound chlorophylls simply transmit the absorbed energy to another nearby chlorophyll by the process of resonance energy transfer. However, in a few specially positioned chlorophylls, the energy difference between the ground state and the excited state is just right for the photon to trigger a light-induced chemical reaction. The special state of such chlorophyll molecules derives from their close interaction with a second chlorophyll molecule in the same chlorophyll–protein complex. Together, these two chlorophylls form a *special pair*.

The photosynthetic electron transfer process starts when a photon of suitable energy ionizes a chlorophyll molecule in such a special pair, dissociating it into an electron and a positively charged chlorophyll ion. The energized electron is passed rapidly to a quinone in the same protein complex, preventing its unproductive reassociation with the chlorophyll ion. This light-induced transfer of an electron from a chlorophyll to a mobile electron carrier is the central **charge-separation** step in photosynthesis, in which a chlorophyll becomes positively charged and an electron carrier becomes negatively charged (**Figure 14–44**). The chlorophyll ion is a very strong oxidant that is able to withdraw an electron from a low-energy substrate; in the first step of oxygenic photosynthesis, this low-energy substrate is water.

Upon transfer to a mobile carrier in the electron-transport chain, the electron is stabilized as part of a strong electron donor and made available for subsequent reactions. These subsequent reactions require more time to complete, and they result in light-generated energy-rich compounds.

A Photosystem Consists of an Antenna Complex and a Reaction Center

There are two distinct types of chlorophyll–protein complexes in the photosynthetic membrane. One type, called a *photochemical reaction center*, contains the special pair of chlorophylls just described. The other type engages exclusively in light absorption and resonance energy transfer and is called an *antenna complex*. Together, the two types of complex make up a **photosystem** (**Figure 14–45**).

The role of the **antenna complex** in the photosystem is to collect the energy of a sufficient number of photons for photosynthesis. Without it, the process would be slow and inefficient, as each reaction-center chlorophyll would absorb only about one light quantum per second, even in broad daylight, whereas hundreds per second are needed for effective photosynthesis. When light excites a chlorophyll molecule in the antenna complex, the energy passes rapidly from one protein-bound chlorophyll to another by resonance energy transfer until it reaches the special pair in the reaction center. The antenna complex is also known as a

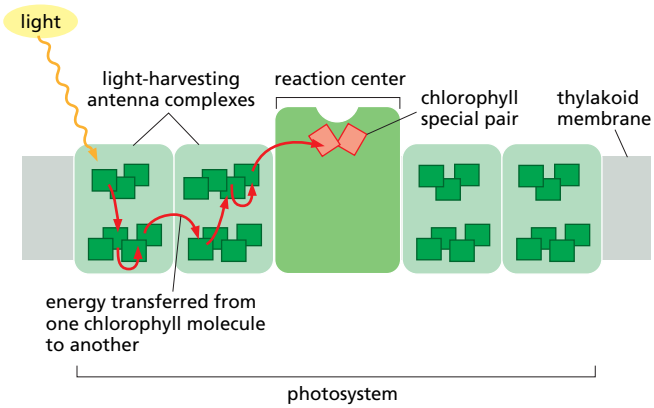


Figure 14–45 A photosystem. Each photosystem consists of a reaction center plus a number of light-harvesting antenna complexes. The solar energy for photosynthesis is collected by the antenna complexes, which account for most of the chlorophyll in a plant cell. The energy hops randomly by resonance energy transfer *(red arrows)* from one chlorophyll molecule to another, until it reaches the reaction center complex, where it ionizes a chlorophyll in the special pair. The chlorophyll special pair holds its electrons at a lower energy than the chlorophyll in the antenna complexes, causing the energy transferred to it from the antenna complex to become trapped there. Note that it is only energy that moves from one chlorophyll molecule to another in the antenna complex, not electrons (Movie 14.10).

light-harvesting complex, or LHC. In addition to many chlorophyll molecules, an LHC contains orange carotenoid pigments. The carotenoids collect light of a different wavelength from that absorbed by chlorophylls, helping to make the antenna complex more efficient. They also have an important protective role in preventing the formation of harmful oxygen radicals in the photosynthetic membrane.

The Thylakoid Membrane Contains Two Different Photosystems Working in Series

The excitation energy collected by the antenna complex is delivered to the special pair in the **photochemical reaction center**. The reaction center is a transmembrane chlorophyll–protein complex that lies at the heart of photosynthesis. It harbors the special pair of chlorophyll molecules, which acts as an irreversible trap for excitation energy (see Figure 14–45).

Chloroplasts contain two functionally different although structurally related photosystems, each of which feeds electrons generated by the action of sunlight into an electron-transfer chain. In the chloroplast thylakoid membrane, *photosystem I* is confined to the unstacked stroma thylakoids, while the stacked grana thylakoids contain *photosystem II*. The two photosystems were named in order of their discovery, not of their actions in the photosynthetic pathway, and electrons are first activated in photosystem II before being transferred to photosystem I (**Figure 14–46**). The path of the electron through the two photosystems can be described as a Z-like trajectory and is known as the *Z scheme*. In the Z scheme, the reaction center of *photosystem II* first withdraws an electron from water. The electron passes via an electron-transport chain (composed of the electron carrier plastoquinone, the cytochrome b_6-f complex, and the protein plastocyanin) to *photosystem I*, which propels the electron across the membrane in a second light-driven charge-separation reaction that leads to NADPH production.

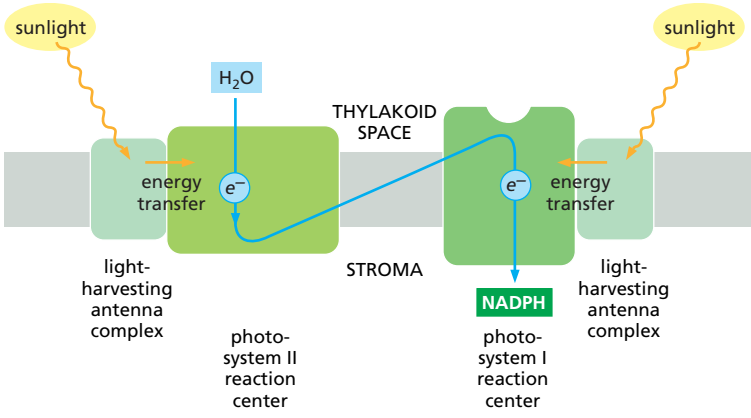


Figure 14–46 The Z scheme for photosynthesis. The thylakoids of plants and cyanobacteria contain two different photosystems, known as photosystem I and photosystem II, which work in series. Each of the photosystem I and II reaction centers receives excitation energy from its own set of tightly associated antenna complexes, known as LHC-I and LHC-II, by resonance energy transfer. Note that, for historical reasons, the two photosystems were named opposite to the order in which they act, with photosystem II passing its electrons to photosystem I.

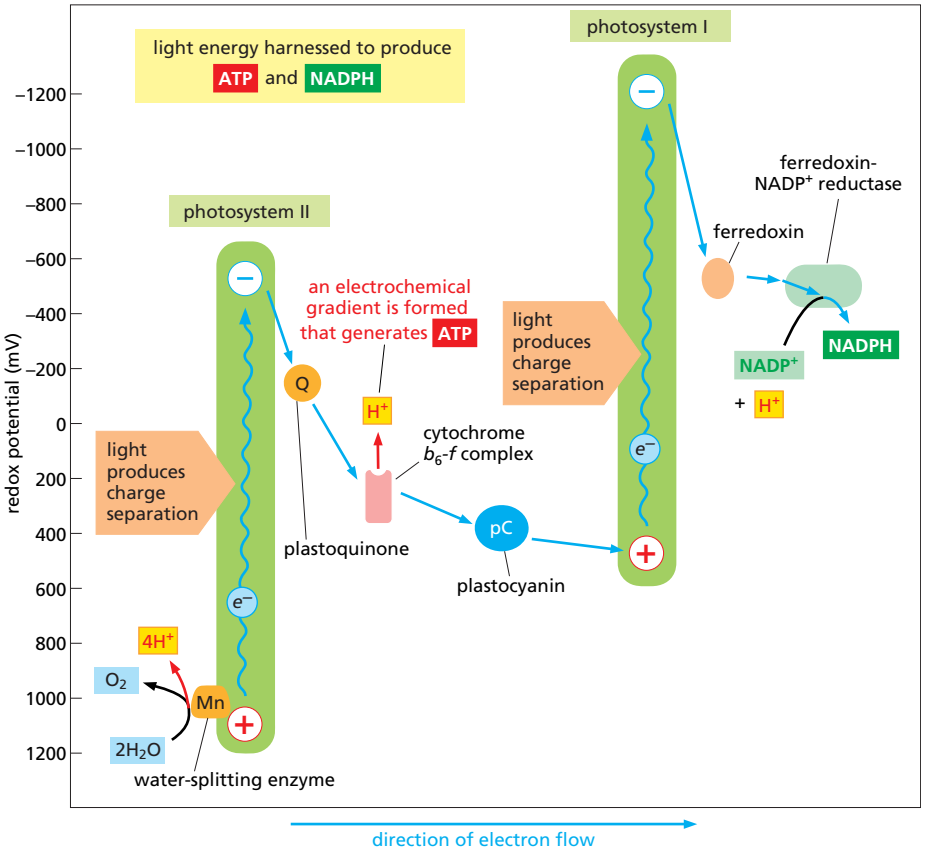


Figure 14–47 Changes in redox potential during photosynthesis. The redox potential for each molecule is indicated by its position along the vertical axis. Photosystem II passes electrons derived from water to photosystem I, which in turn passes them to $NADP^+$ through ferredoxin-$NADP^+$ reductase. The net electron flow through the two photosystems is from water to $NADP^+$, and it produces NADPH as well as an electrochemical proton gradient. This proton gradient is used by the ATP synthase to produce ATP. Details in this figure will be explained in the subsequent text.

The Z scheme is necessary to bridge the very large energy gap between water and NADPH (**Figure 14–47**). A single quantum of visible light does not contain enough energy both to withdraw electrons from water, which holds on to its electrons very tightly (redox potential +820 mV) and therefore is a very poor electron donor, and to force them on to $NADP^+$, which is a very poor electron acceptor (redox potential –320 mV). The Z scheme first evolved in cyanobacteria to enable them to use water as a universally available electron source. Other, simpler photosynthetic bacteria have only one photosystem. As we shall see, they cannot use water as an electron source and must rely on other, more energy-rich substrates instead, from which electrons are more readily withdrawn. The ability to extract electrons from water (and thereby to produce molecular oxygen) was acquired by plants when their ancestors took up the endosymbiotic cyanobacteria that later evolved into chloroplasts (see Figure 1–31).

Photosystem II Uses a Manganese Cluster to Withdraw Electrons From Water

In biology, only photosystem II is able to withdraw electrons from water and to generate molecular oxygen as a waste product. This remarkable specialization of photosystem II is conferred by the unique properties of one of the two chlorophyll molecules of its special pair and by a *manganese cluster* linked to the protein. These chlorophyll molecules and the manganese cluster form the catalytic core of the photosystem II reaction center, whose mechanism is outlined in **Figure 14–48**.

Water is an inexhaustible source of electrons, but it is also extremely stable; therefore a large amount of energy is required to make it part with its electrons. The only compound in living organisms that is able to achieve this feat after its ionization by light, is the chlorophyll special pair called P_{680} (P_{680}/P_{680}^+ redox potential = +1270 mV). The reaction $2H_2O + 4$ photons $\rightarrow 4H^+ + 4e^- + O_2$ is catalyzed by its adjacent manganese cluster. The intermediates remain firmly attached to the manganese cluster until two water molecules have been fully oxidized to O_2, thus

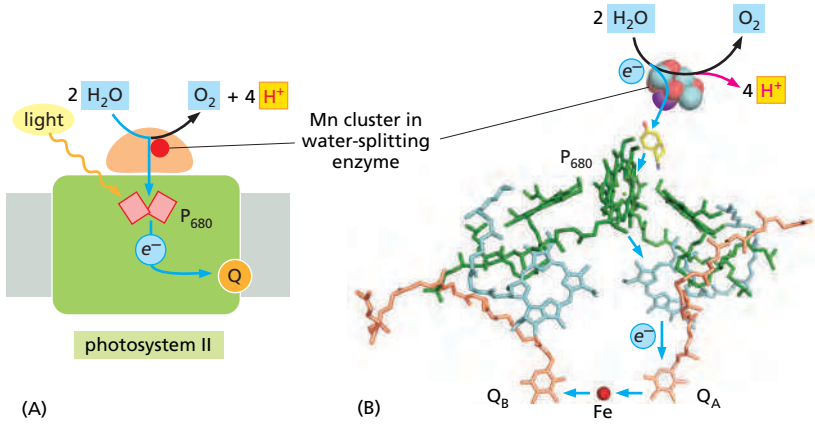


Figure 14–48 The conversion of light energy to chemical energy in the photosystem II complex. (A) Schematic diagram of the photosystem II reaction center, whose special pair of chlorophyll molecules is designated as P_{680} based on the wavelength of its absorbance maximum (680 nm). (B) Cofactors and pigments at the core of the reaction center. Shown are the manganese (Mn) cluster, the tyrosine side chain that links it to the P_{680} special pair, four chlorophylls *(green)*, two pheophytins *(light blue)*, two plastoquinones *(pink),* and an iron atom *(red)*. The path of electrons is shown by *blue arrows*. In the manganese cluster, four manganese atoms *(light blue)*, one calcium atom *(purple)*, and five oxygen atoms *(red)* work together to catalyze the oxidation of water. The water-splitting reaction occurs in four successive steps, each requiring the energy of one photon. Each photon turns a P_{680} reaction-center chlorophyll into a positively charged chlorophyll ion. Through an ionized tyrosine side chain *(yellow)*, this chlorophyll ion pulls an electron away from a water molecule bound at the manganese cluster. In this way, a total of four electrons are withdrawn from two water molecules to generate molecular oxygen, which is released into the atmosphere.

Each electron that is energized by light passes from the special pair along an electron-transfer chain inside the complex, along the indicated path to the permanently bound plastoquinone Q_A and then to plastoquinone Q_B as electron acceptors. Once Q_B has picked up two electrons (plus two protons; see Figure 14–17), it dissociates from its binding site in the complex and enters the lipid bilayer as a mobile electron carrier, being immediately replaced by a new, nonreduced molecule of plastoquinone. Note that the chlorophylls and pheophytins form two symmetrical branches of a potential electron-transport chain. Only one branch is active, thus ensuring that the plastoquinones become fully reduced in minimum time.

ensuring that no dangerous oxygen radicals are released as the reaction proceeds. The protons released by the two water molecules are discharged to the thylakoid space, contributing to the proton gradient across the thylakoid membrane (pH lower in the thylakoid space than in the stroma). The unique protein environment that endows life with this all-important ability to oxidize water has remained essentially unchanged throughout billions of years of evolution (**Figure 14–49**).

All of the oxygen in the Earth's atmosphere has been generated in this way. Although the exact details of the water-oxidation reaction in photosystem II are still not fully understood, scientists are trying to construct an artificial system that mimics the process. If successful, this might provide a virtually endless supply of clean energy, helping to solve the world's energy crisis.

The Cytochrome b_6-f Complex Connects Photosystem II to Photosystem I

Following the path shown previously in Figure 14–48, the electrons extracted from water by photosystem II are transferred to plastoquinol, a strong electron donor similar to ubiquinol in mitochondria. This quinol, which can diffuse rapidly in the lipid bilayer of the thylakoid membrane, transfers its electrons to the *cytochrome b_6-f complex,* whose structure is homologous to the cytochrome *c* reductase in mitochondria. The cytochrome b_6-f complex pumps H^+ into the thylakoid space using the same Q cycle that is utilized in mitochondria (see Figure 14–21), thereby adding to the proton gradient across the thylakoid membrane.

The cytochrome b_6-f complex forms the connecting link between photosystems II and I in the chloroplast electron-transport chain. It passes its electrons

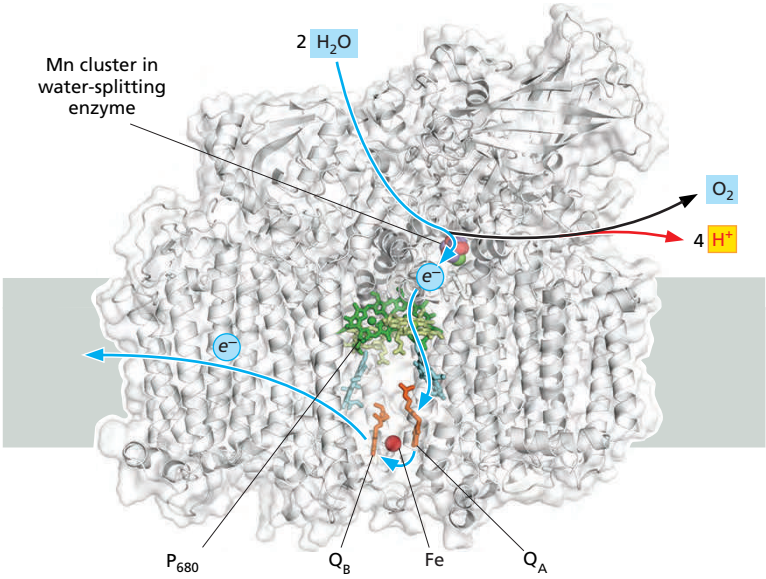


Figure 14–49 The structure of the complete photosystem II complex. This photosystem contains at least 16 protein subunits, along with 36 chlorophylls, two pheophytins, two hemes, and a number of protective carotenoids *(colored).* Most of these pigments and cofactors are deeply buried, tightly complexed to protein *(gray)*. The path of electrons is indicated by the *blue arrows*, and is explained in Figure 14–48B. The photosystem II complex presented here is the cyanobacterial complex, which is simpler and more stable than the plant complex, which works in the same way. (PDB code: 3ARC.)

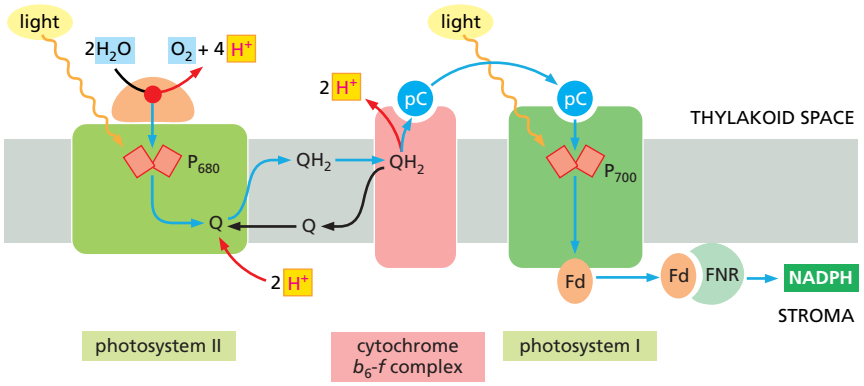


Figure 14–50 Electron flow through the cytochrome b_6-f complex to NADPH. The cytochrome b_6-f complex is the functional equivalent of cytochrome c reductase (the cytochrome b-c_1 complex) in mitochondria (see Figure 14–22). Like its mitochondrial homolog, the b_6-f complex receives its electrons from a quinone and engages in a complicated Q cycle that pumps two protons across the membrane (details not shown). It hands its electrons, one at a time, to plastocyanin (pC). Plastocyanin diffuses along the membrane surface to photosystem I and transfers the electrons via ferredoxin (Fd) to the ferredoxin-NADP$^+$ reductase (FNR), where they are utilized to produce NADPH. P_{700} is a special pair of chlorophylls that absorbs light of wavelength 700 nm.

one at a time to the mobile electron carrier plastocyanin (a small copper-containing protein that takes the place of the cytochrome c in mitochondria), which will transfer them to photosystem I (**Figure 14–50**). As we discuss next, photosystem I then harnesses a second photon of light to further energize the electrons that it receives.

Photosystem I Carries Out the Second Charge-Separation Step in the Z Scheme

Photosystem I receives electrons from plastocyanin in the thylakoid space and transfers them, via a second charge-separation reaction, to the small protein ferredoxin on the opposite membrane surface (**Figure 14–51**). Then, in a final step, ferredoxin feeds its electrons to a membrane-associated enzyme complex, the *ferredoxin-NADP$^+$ reductase*, which uses the electrons to produce NADPH from NADP$^+$ (see Figure 14–50).

The redox potential of the NADP$^+$/NADPH pair (–320 mV) is already very low, and reduction of NADP$^+$ therefore requires a compound with an even lower redox potential. This turns out to be a chlorophyll molecule near the stromal membrane surface of photosystem I that has a redox potential of –1000 mV (chlorophyll A_0), making it the strongest known electron donor in biology. The reduced NADPH is released into the chloroplast stroma, where it is used for biosynthesis of glyceraldehyde 3-phosphate, amino acid precursors, and fatty acids, much of it to be exported to the cytoplasm.

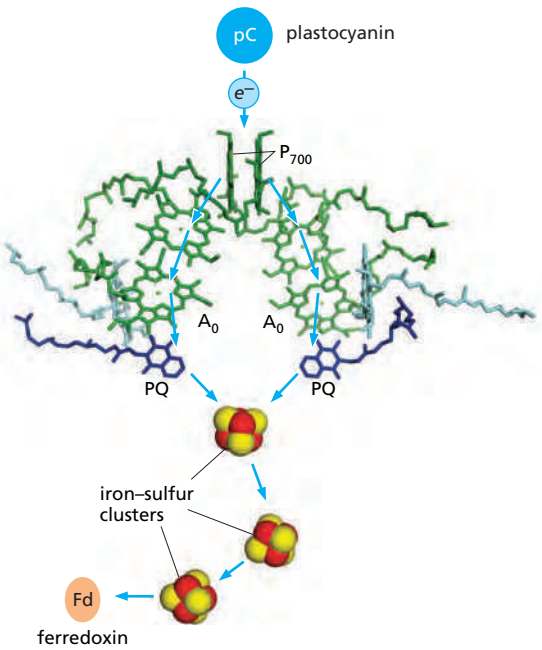
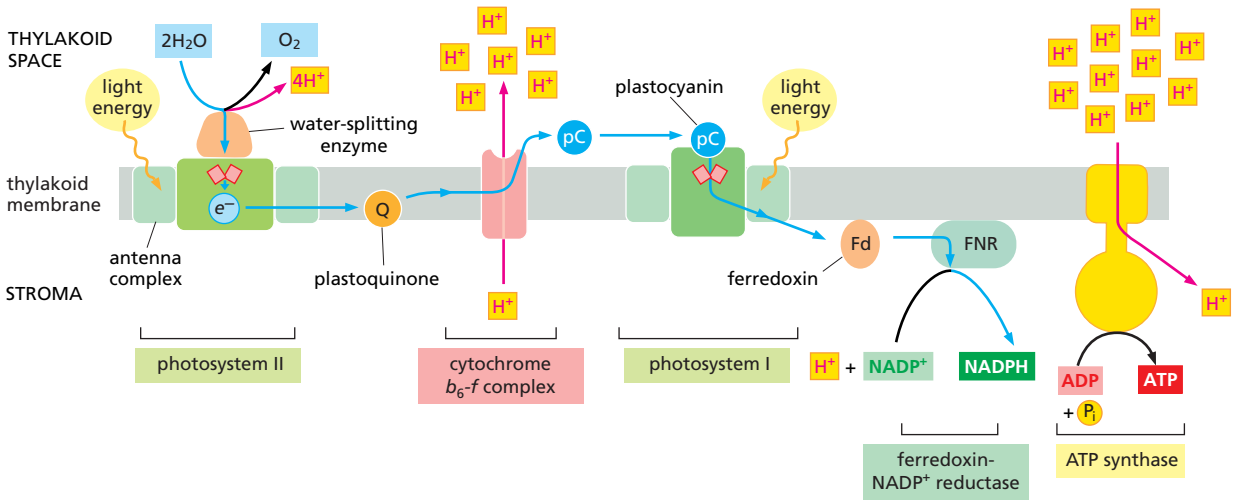


Figure 14–51 Structure and function of photosystem I. At the heart of the photosystem I complex assembly is the electron-transfer chain shown. At one end is a special pair of chlorophylls called P_{700} (because it absorbs light of 700 nm wavelength), receiving electrons from plastocyanin (pC). At the other end are the A_0 chlorophylls, which hand the electrons on to ferredoxin via two plastoquinones (PQ; *purple*) and three iron–sulfur clusters. Even though the roles of photosystems I and II in photosynthesis are very different, their central electron-transfer chains are structurally similar, indicating a common evolutionary origin (see Figure 14–53). Note that in photosystem I both branches of the electron-transfer chain are active, unlike in photosystem II (see Figure 14–48). (PDB code: 3LW5.)



The Chloroplast ATP Synthase Uses the Proton Gradient Generated by the Photosynthetic Light Reactions to Produce ATP

The sequence of events that results in light-driven production of ATP and NADPH in chloroplasts and cyanobacteria is summarized in **Figure 14–52**. Starting with the withdrawal of electrons from water, the light-driven charge-separation steps in photosystems II and I enable the energetically unfavorable (uphill) flow of electrons from water to NADPH (see Figure 14–47). Three small mobile electron carriers—plastoquinone, plastocyanin, and ferredoxin—participate in this process. Together with the electron-driven proton pump of the cytochrome b_6-f complex, the photosystems generate a large proton gradient across the thylakoid membrane. The ATP synthase molecules embedded in the thylakoid membranes then harness this proton gradient to produce large amounts of ATP in the chloroplast stroma, mimicking the synthesis of ATP in the mitochondrial matrix.

The linear Z scheme for photosynthesis thus far discussed can switch to a circular mode of electron flow through photosystem I and the b_6-f complex. Here, the reduced ferredoxin diffuses back to the b_6-f complex to reduce plastoquinone, instead of passing its electrons to the ferredoxin-$NADP^+$ reductase enzyme complex. This, in effect, turns photosystem I into a light-driven proton pump, thereby increasing the proton gradient and thus the amount of ATP made by the ATP synthase. An elaborate set of regulatory mechanisms control this switch, which enables the chloroplast to generate either more NADPH (linear mode) or more ATP (circular mode), depending on the metabolic needs of the cell.

All Photosynthetic Reaction Centers Have Evolved From a Common Ancestor

Evidence for the prokaryotic origins of mitochondria and chloroplasts abounds in their genetic systems, as we will see in the next section. But strong and direct evidence for the evolutionary origins of chloroplasts can also be found in the molecular structures of photosynthetic reaction centers revealed in recent years by crystallography. The positions of the chlorophylls in the special pair and the two branches of the electron-transfer chain are basically the same in photosystem I, photosystem II, and the photochemical reaction centers of photosynthetic bacteria (**Movie 14.11**). As a result, one can conclude that they all have evolved from a common ancestor. Evidently, the molecular architecture of the photosynthetic reaction center originated only once and has remained essentially unchanged during evolution. By contrast, the less critical antenna systems have evolved in several different ways and are correspondingly diverse in present-day photosynthetic organisms (**Figure 14–53**).

Figure 14–52 Summary of electron and proton movements during photosynthesis in the thylakoid membrane. Electrons are withdrawn, through the action of light energy, from a water molecule that is held by the manganese cluster in photosystem II. The electrons pass on to plastoquinone, which delivers them to the cytochrome b_6-f complex that resembles the cytochrome c reductase of mitochondria and the b-c complex of bacteria. They are then carried to photosystem I by the soluble electron carrier plastocyanin, the functional equivalent of cytochrome c in mitochondria. From photosystem I they are transferred to ferredoxin-$NADP^+$ reductase (FNR) by the soluble carrier ferredoxin (Fd; a small protein containing an iron–sulfur center). Protons are pumped into the thylakoid space by the cytochrome b_6-f complex, in the same way that protons are pumped into mitochondrial cristae by cytochrome c reductase (see Figure 14–21). In addition, the H^+ released into the thylakoid space by water oxidation, and the H^+ consumed during NADPH formation in the stroma, contribute to the generation of the electrochemical H^+ gradient across the thylakoid membrane. As illustrated, this gradient drives ATP synthesis by an ATP synthase that sits in the same membrane (see Figure 14–47).

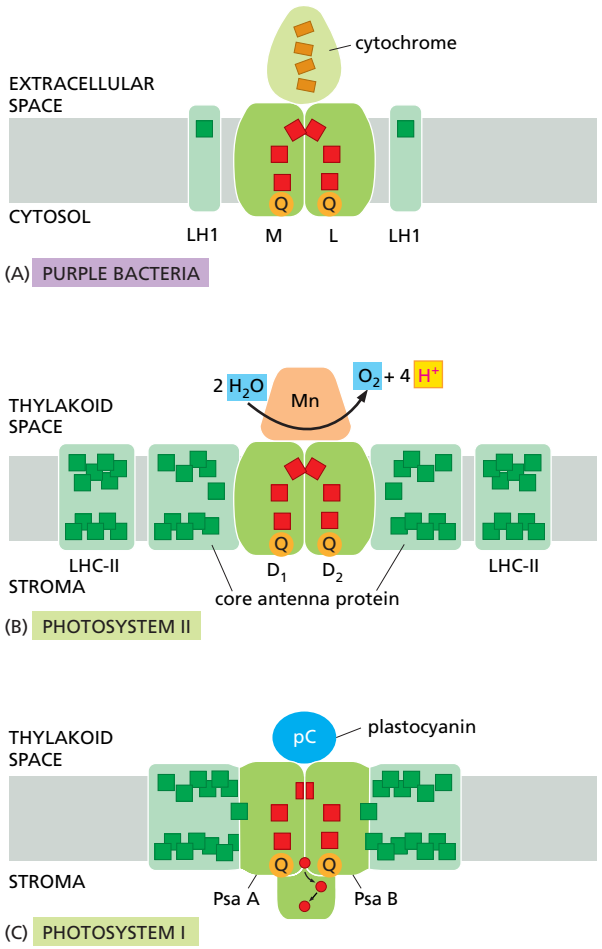


Figure 14–53 Evolution of photosynthetic reaction centers. Pigments involved in light-harvesting are colored *green*; those involved in the central photochemical events are colored *red*. (A) The primitive photochemical reaction center of purple bacteria contains two related protein subunits, L and M, that bind the pigments involved in the central process of photosynthesis, including a special pair of chlorophyll molecules. Electrons are fed into the excited chlorophylls by a cytochrome. LH1 is a bacterial antenna complex. (B) Photosystem II contains the D_1 and D_2 proteins, which are homologous to the L and M subunits in (A). The excited P_{680} chlorophyll in the special pair withdraws electrons from water held by the manganese cluster. LHC-II is the light-harvesting complex that feeds energy into the core antenna proteins. (C) Photosystem I contains the Psa A and Psa B proteins, each of which is equivalent to a fusion of the D_1 or D_2 protein to a core antenna protein of photosystem II. The loosely bound plastocyanin (pC) feeds electrons into the excited chlorophyll pair. As indicated, in photosystem I, electrons are passed from a bound quinone (Q) through a series of three iron–sulfur centers *(red circles)*. (Modified from K. Rhee, E. Morris, J. Barber and W. Kühlbrandt, *Nature* 396:283–286, 1998; and W. Kühlbrandt, *Nature* 411:896–899, 2001. With permission from Macmillan Publishers Ltd.)

The Proton-Motive Force for ATP Production in Mitochondria and Chloroplasts Is Essentially the Same

The proton gradient across the thylakoid membrane depends both on the proton-pumping activity of the cytochrome b_6-f complex and on the photosynthetic activity of the two photosystems, which in turn depends on light intensity. In chloroplasts exposed to light, H^+ is pumped out of the stroma (pH around 8, similar to the mitochondrial matrix) into the thylakoid space (pH 5–6), creating a gradient of 2–3 pH units across the thylakoid membrane, representing a proton-motive force of about 180 mV. This is very similar to the proton-motive force in respiring mitochondria. However, a membrane potential across the inner mitochondrial membrane makes the largest contribution to the proton-motive force that drives the mitochondrial ATP synthase to make ATP, whereas a H^+ gradient predominates for chloroplasts.

In contrast to mitochondrial ATP synthase, which forms long rows of dimers along the cristae ridges, the chloroplast ATP synthase is monomeric and located in flat membrane regions (**Figure 14–54**). Evidently, the H^+ gradient across the thylakoid membrane is high enough for ATP synthesis without the need for the elaborate arrangement of ATP synthase seen in mitochondria.

Chemiosmotic Mechanisms Evolved in Stages

The first living cells on Earth may have consumed geochemically produced organic molecules and generated their 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

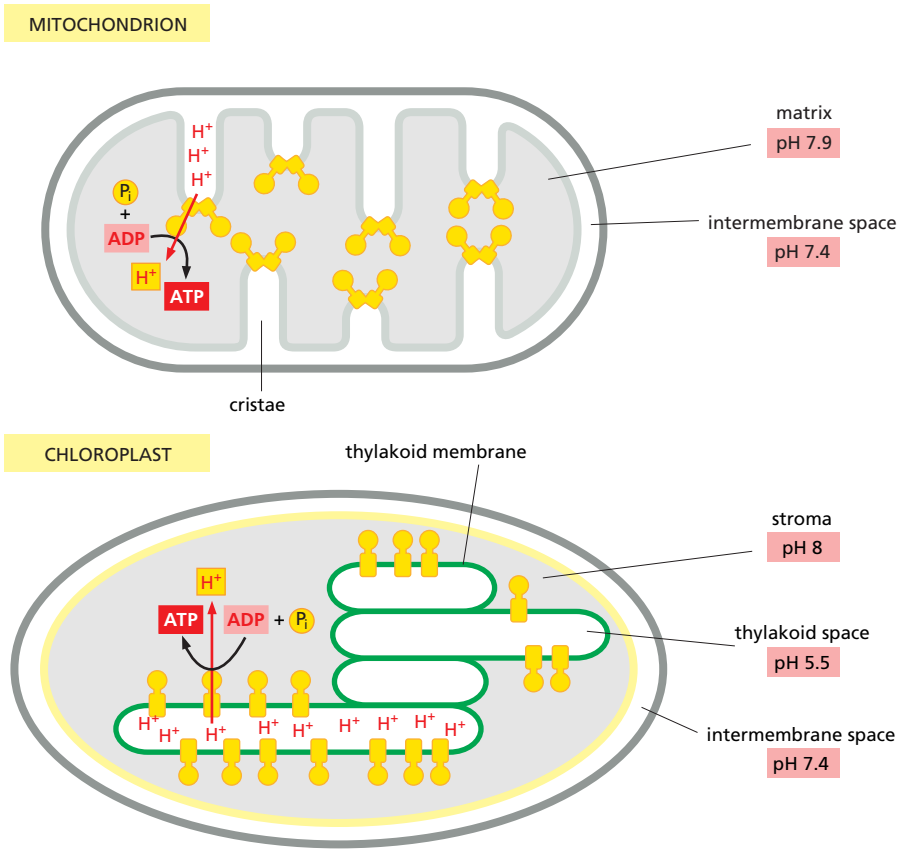


Figure 14–54 A comparison of H^+ concentrations and the arrangement of ATP synthase in mitochondria and chloroplasts. In both organelles, the pH in the intermembrane space is 7.4, as in the cytoplasm. The pH of the mitochondrial matrix and the pH of the chloroplast stroma are both about 8 *(light gray)*. The pH in the thylakoid space is around 5.5, depending on photosynthetic activity. This results in a high proton-motive force across the thylakoid membrane, consisting largely of the H^+ gradient (a high permeability of this membrane to Mg^{2+} and Cl^- ions allows the flow of these ions to dissipate most of the membrane potential).

In contrast to chloroplasts, the H^+ gradient across the inner mitochondrial membrane is insufficient for ATP production, and mitochondria need a membrane potential to bring the proton-motive force to the same level as in chloroplasts. The arrangement of the mitochondrial ATP synthase in rows of dimers along the cristae ridges (see Figure 14–32) next to the respiratory-chain proton pumps may help the flow of protons along the membrane surface toward the ATP synthase, as the availability of protons is limiting for ATP production. In the chloroplast, the ATP synthase is distributed randomly in thylakoid membranes.

environment (see Figure 2–47). Perhaps such acids lowered the pH of the environment, favoring the survival of cells that evolved transmembrane proteins that could pump H^+ out of the cytosol, thereby preventing the cell from becoming too acidic (stage 1 in Figure 14–55). One of these pumps may have used the energy available from ATP hydrolysis to eject H^+ from the cell; such a proton pump could have been the ancestor of present-day ATP synthases.

As the Earth's supply of geochemically produced nutrients began to dwindle, organisms that could find a way to pump H^+ without consuming ATP would have been at an advantage: they could save the small amounts of ATP they derived from the fermentation of increasingly scarce foodstuffs to fuel other important activities. This need to conserve resources might have led to the evolution of electron-transport proteins that allowed cells to use the movement of electrons between molecules of different redox potentials as a source of energy for pumping H^+ across the plasma membrane (stage 2 in Figure 14–55). Some of these cells might have used the nonfermentable organic acids that neighboring cells had excreted as waste to provide the electrons needed to feed this electron-transport system. Some present-day bacteria grow on formic acid, for example, using the small amount of redox energy derived from the transfer of electrons from formic acid to fumarate to pump H^+.

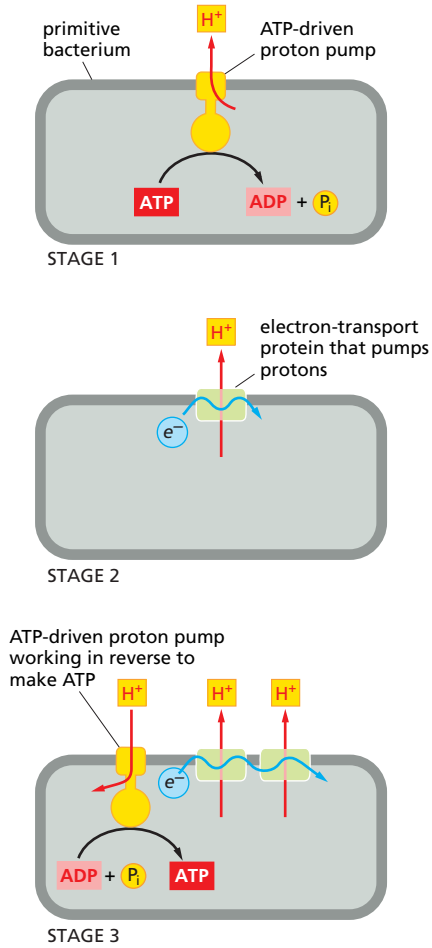


Figure 14–55 How ATP synthesis by chemiosmosis might have evolved in stages. The first stage could have involved the evolution of an ATPase that pumped protons out of the cell using the energy of ATP hydrolysis. Stage 2 could have involved the evolution of a different proton pump, driven by an electron-transport chain. Stage 3 would then have linked these two systems together to generate a primitive ATP synthase that used the protons pumped by the electron-transport chain to synthesize ATP. An early bacterium with this final system would have had a selective advantage over bacteria with neither of the systems or only one.

Eventually, some bacteria would have developed H^+-pumping electron-transport systems that were so efficient that they could harvest more redox energy than they needed to maintain their internal pH. Such cells would probably have generated large electrochemical proton gradients, which they could then use to produce ATP. Protons could leak back into the cell through the ATP-driven H^+ pumps, essentially running them in reverse so that they synthesized ATP (stage 3 in Figure 14–55). Because such cells would require much less of the dwindling supply of fermentable nutrients, they would have proliferated at the expense of their neighbors.

By Providing an Inexhaustible Source of Reducing Power, Photosynthetic Bacteria Overcame a Major Evolutionary Obstacle

The gradual depletion of nutrients from the environment on the early Earth meant that organisms had to find some alternative source of carbon to make the sugars that serve as the precursors for so many other cell components. Although the CO_2 in the atmosphere provides an abundant potential carbon source, to convert it into an organic molecule such as a carbohydrate requires reducing the fixed CO_2 with a strong electron donor, such as NADPH, which can generate (CH_2O) units from CO_2 (see Figure 14–41). Early in cellular evolution, strong reducing agents (electron donors) are thought to have been plentiful. But once an ancestor of ATP synthase began to generate most of the ATP, it would have become imperative for cells to evolve a new way of generating strong reducing agents.

A major evolutionary breakthrough in energy metabolism came with the development of photochemical reaction centers that could use the energy of sunlight to produce molecules such as NADPH. It is thought that this occurred early in the process of cellular evolution in the ancestors of the green sulfur bacteria. Present-day green sulfur bacteria use light energy to transfer hydrogen atoms (as an electron plus a proton) from H_2S to NADPH, thereby producing the strong reducing power required for carbon fixation. Because the redox potential of H_2S is much lower than that of H_2O (–230 mV for H_2S compared with +820 mV for H_2O), one quantum of light absorbed by the single photosystem in these bacteria is sufficient to generate NADPH via a relatively simple photosynthetic electron-transport chain.

The Photosynthetic Electron-Transport Chains of Cyanobacteria Produced Atmospheric Oxygen and Permitted New Life-Forms

The next evolutionary step, which is thought to have occurred with the development of the cyanobacteria perhaps 3 billion years ago, was the evolution of organisms capable of using water as the electron source for CO_2 reduction. This entailed the evolution of a water-splitting enzyme and also required the addition of a second photosystem, acting in series with the first, to bridge the large gap in redox potential between H_2O and NADPH. The biological consequences of this evolutionary step were far-reaching. For the first time, there would have been organisms that could survive on water, CO_2, and sunlight (plus a few trace elements). These cells would have been able to spread and evolve in ways denied to the earlier photosynthetic bacteria, which needed H_2S or organic acids as a source of electrons. Consequently, large amounts of biologically synthesized, reduced organic materials accumulated and oxygen entered the atmosphere for the first time.

Oxygen is highly toxic because the oxidation of biological molecules alters their structure and properties indiscriminately and irreversibly. Most anaerobic bacteria, for example, are rapidly killed when exposed to air. Thus, organisms on the primitive Earth would have had to evolve protective mechanisms against the rising O_2 levels in the environment. Late evolutionary arrivals, such as ourselves, have numerous detoxifying mechanisms that protect our cells from the ill effects of oxygen. Even so, an accumulation of oxidative damage to our macromolecules has been postulated to contribute to human aging, as we discuss in the next section.

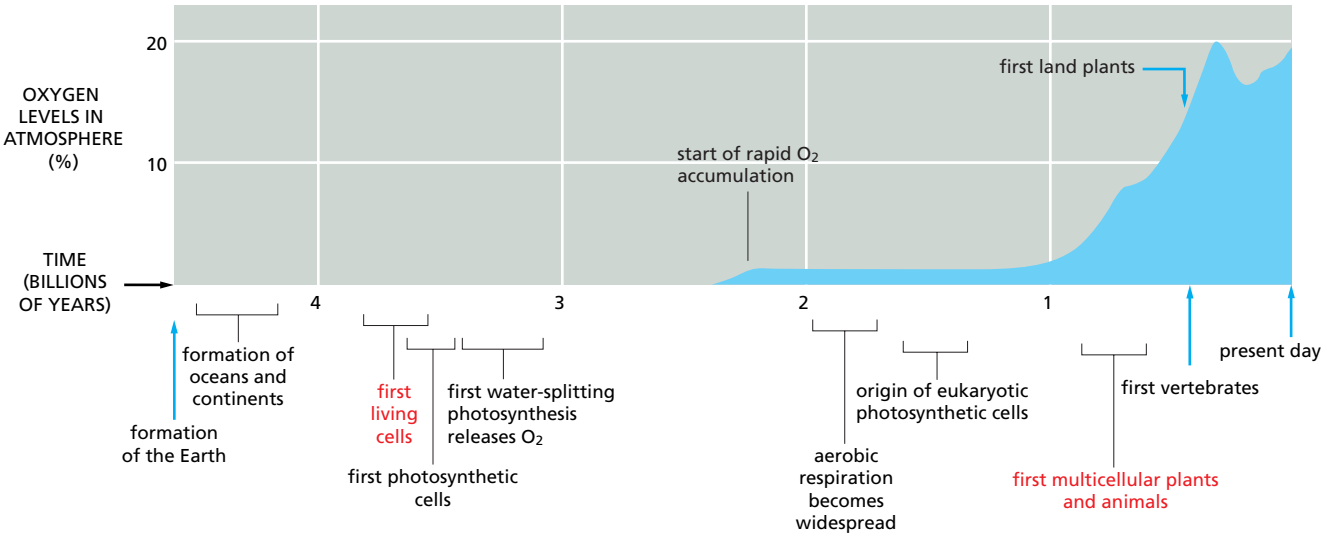


Figure 14–56 Major events during the evolution of living organisms on Earth. With the evolution of the membrane-based process of photosynthesis, organisms were able to make their own organic molecules from CO_2 gas. The delay of more than 10^9 years between the appearance of bacteria that split water and released O_2 during photosynthesis and the accumulation of high levels of O_2 in the atmosphere is thought to be due to the initial reaction of the oxygen with the abundant ferrous iron (Fe^{2+}) that was dissolved in the early oceans. Only when the ferrous iron was used up would oxygen have started to accumulate in the atmosphere. In response to the rising oxygen levels, nonphotosynthetic oxygen-consuming organisms evolved, and the concentration of oxygen in the atmosphere equilibrated at its present-day level.

The increase in atmospheric O_2 was very slow at first and would have allowed a gradual evolution of protective devices. For example, the early seas contained large amounts of iron in its reduced, ferrous state (Fe^{2+}), and nearly all the O_2 produced by early photosynthetic bacteria would have been used up in oxidizing Fe^{2+} to ferric Fe^{3+}. This conversion caused the precipitation of huge amounts of stable oxides, and the extensive banded iron formations in sedimentary rocks, beginning about 2.7 billion years ago, help to date the spread of the cyanobacteria. By about 2 billion years ago, the supply of Fe^{2+} was exhausted, and the deposition of further iron precipitates ceased. Geological evidence reveals how O_2 levels in the atmosphere have changed over billions of years, approximating current levels only about 0.5 billion years ago (**Figure 14–56**).

The availability of O_2 enabled the rise of bacteria that developed an aerobic metabolism to make their ATP. These organisms could harness the large amount of energy released by breaking down carbohydrates and other reduced organic molecules all the way to CO_2 and H_2O, as explained when we discussed mitochondria. Components of preexisting electron-transport complexes were modified to produce a cytochrome oxidase, so that the electrons obtained from organic or inorganic substrates could be transported to O_2 as the terminal electron acceptor. Some present-day purple photosynthetic bacteria can switch between photosynthesis and respiration depending on the availability of light and O_2, with only relatively minor reorganizations of their electron-transport chains.

In **Figure 14–57**, we relate these postulated evolutionary pathways to different types of bacteria. By necessity, evolution is always conservative, taking parts of the old and building on them to create something new. Thus, parts of the electron-transport chains that were derived to service anaerobic bacteria 3–4 billion years ago survive, in altered form, in the mitochondria and chloroplasts of today's higher eukaryotes. A good example is the overall similarity in structure and function between the cytochrome *c* reductase that pumps H^+ in the central segment of the mitochondrial respiratory chain and the analogous cytochrome *b-f* complex in the electron-transport chains of both bacteria and chloroplasts, revealing their common evolutionary origin (**Figure 14–58**).

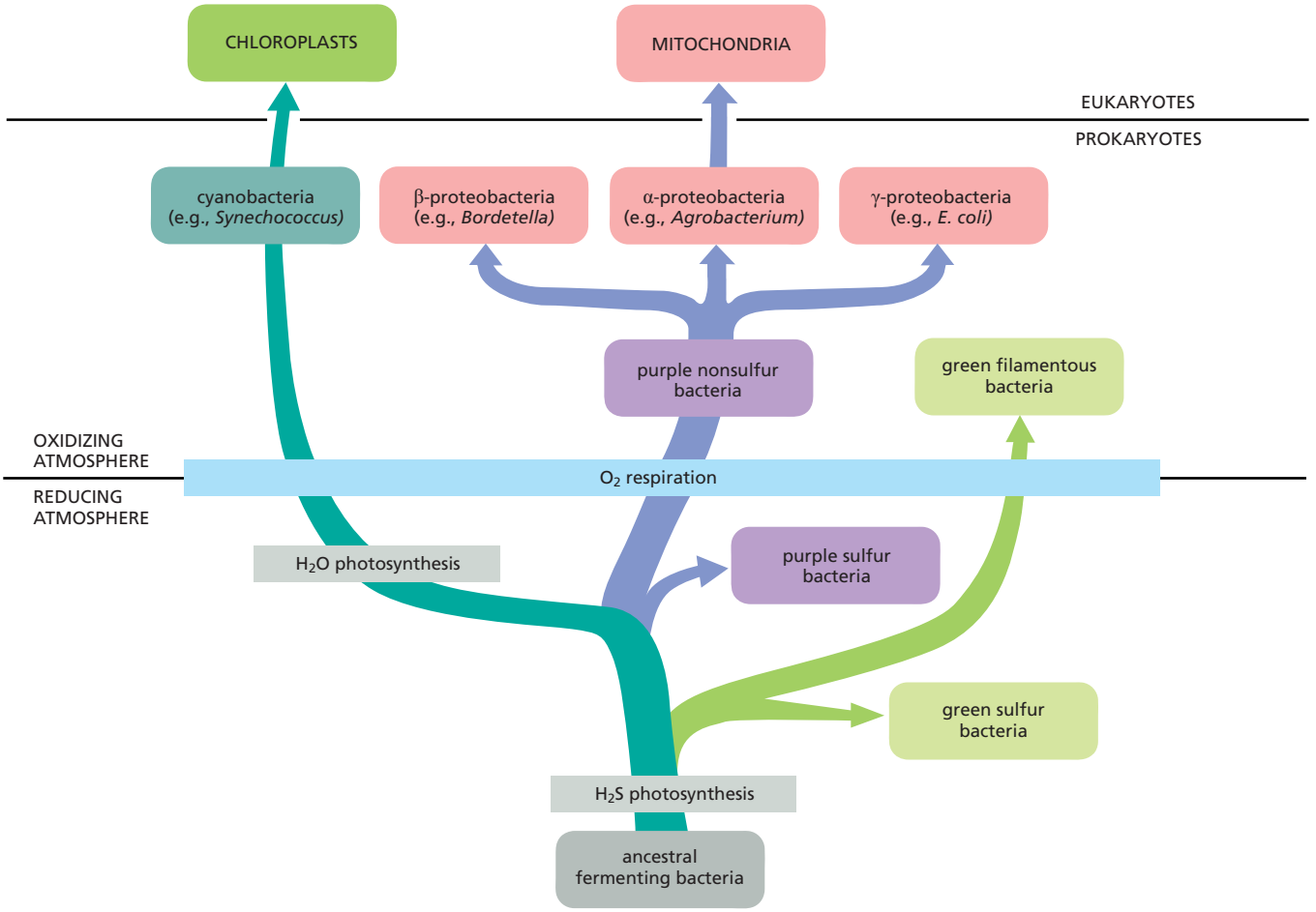


Figure 14–57 Evolutionary scheme showing the postulated origins of mitochondria and chloroplasts and their bacterial ancestors. The consumption of oxygen by respiration is thought to have first developed about 2 billion years ago. Nucleotide-sequence analyses suggest that an endosymbiotic oxygen-evolving cyanobacterium *(cyan)* gave rise to chloroplasts *(dark green)*, while mitochondria arose from an α-proteobacterium. The nearest relatives of mitochondria *(pink)* are members of three closely related groups of α-proteobacteria—the rhizobacteria, agrobacteria, and rickettsias—known to form intimate associations with present-day eukaryotic cells. Proteobacteria are *pink*, purple photosynthetic bacteria are *purple*, and other photosynthetic bacteria are *light green*.

Summary

Chloroplasts and photosynthetic bacteria have the unique ability to harness the energy of sunlight to produce energy-rich compounds. This is achieved by the photosystems, in which chlorophyll molecules attached to proteins are excited when hit by a photon. Photosystems are composed of an antenna complex that collects solar energy and a photochemical reaction center, in which the collected energy is funneled to a chlorophyll molecule held in a special position, enabling it to withdraw electrons from an electron donor. Chloroplasts and cyanobacteria contain two distinct photosystems. The two photosystems are normally linked in series in the Z scheme, and they transfer electrons from water to $NADP^+$ to form NADPH, generating a transmembrane electrochemical potential. One of the two photosystems—photosystem II—can split water by removing electrons from this ubiquitous, low-energy compound. All the molecular oxygen (O_2) in our atmosphere is a by-product of the water-splitting reaction in this photosystem. The three-dimensional structures of photosystems I and II are strikingly similar to the photosystems of purple photosynthetic bacteria, demonstrating a remarkable degree of conservation over billions of years of evolution.

The two photosystems and the cytochrome b_6-f *complex reside in the thylakoid membrane, a separate membrane system in the central stroma compartment of the*

chloroplast that is differentiated into stacked grana and unstacked stroma thylakoids. Electron-transport processes in the thylakoid membrane cause protons to be released into the thylakoid space. The backflow of protons through the chloroplast ATP synthase then generates ATP. This ATP is used in conjunction with the NADPH produced by photosynthesis to drive a large number of biosynthetic reactions in the chloroplast stroma, including the carbon-fixation cycle, which generates large amounts of carbohydrates from CO_2.

In the early evolution of life, cyanobacteria overcame a major obstacle in devising a way to use solar energy to split water and fix carbon dioxide. Cyanobacteria produced both abundant organic nutrients and molecular oxygen, enabling the rise of a multitude of aerobic life-forms. The chloroplasts in plants have evolved from a cyanobacterium that was endocytosed long ago by an aerobic eukaryotic host organism.

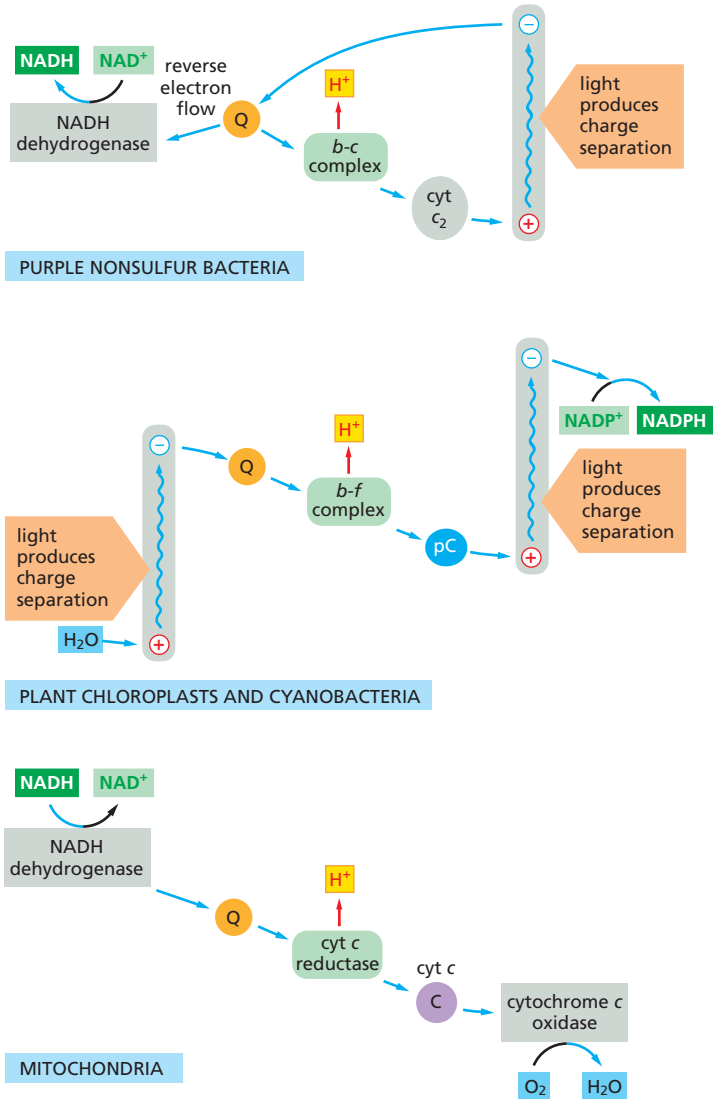


Figure 14–58 A comparison of three electron-transport chains discussed in this chapter. Bacteria, chloroplasts, and mitochondria all contain a membrane-bound enzyme complex that resembles the cytochrome *c* reductase of mitochondria. These complexes all accept electrons from a quinone carrier (Q) and pump H^+ across their respective membranes. Moreover, in reconstituted *in vitro* systems, the different complexes can substitute for one another, and the structures of their protein components reveal that they are evolutionarily related. Note that the purple nonsulfur bacteria use a cyclic flow of electrons to produce a large electrochemical proton gradient that drives a *reverse electron flow* through NADH dehydrogenase to produce NADH from $NAD^+ + H^+ + e^-$.

THE GENETIC SYSTEMS OF MITOCHONDRIA AND CHLOROPLASTS

As we discussed in Chapter 1, mitochondria and chloroplasts are thought to have evolved from endosymbiotic bacteria (see Figures 1–29 and 1–31). Both types of organelles still contain their own genomes (**Figure 14–59**). As we will discuss shortly, they also retain their own biosynthetic machinery for making RNA and organellar proteins.

Like bacteria, mitochondria and chloroplasts proliferate by growth and division of an existing organelle. In actively dividing cells, each type of organelle must double in mass in each cell generation and then be distributed into each daughter cell. In addition, nondividing cells must replenish organelles that are degraded as part of the continual process of organelle turnover, or produce additional organelles as the need arises. Organelle growth and proliferation are therefore carefully controlled. The process is complicated because mitochondrial and chloroplast proteins are encoded in two places: the nuclear genome and the separate genomes harbored in the organelles themselves. The biogenesis of mitochondria and chloroplasts thus requires contributions from two separate genetic systems, which must be closely coordinated.

Most organellar proteins are encoded by the nuclear DNA. The organelle imports these proteins from the cytosol, after they have been synthesized on cytosolic ribosomes, through the mitochondrial protein translocases of the outer and inner mitochondrial membrane—TOM and TIM. In Chapter 12, we discussed how this happens. Here, we describe the organelle genomes and genetic systems, and consider the consequences of separate organelle genomes for the cell and the organism as a whole.

The Genetic Systems of Mitochondria and Chloroplasts Resemble Those of Prokaryotes

As discussed in Chapter 12, it is thought that eukaryotic cells originated through a symbiotic relationship between an archaeon and an aerobic bacterium (a proteobacterium). The two organisms are postulated to have merged to form the ancestor of all nucleated cells, with the archeaon providing the nucleus and the proteobacterium serving as a respiring, ATP-producing endosymbiont—one that would eventually evolve into the mitochondrion (see Figure 12–3). This most likely occurred roughly 1.6 billion years ago, when oxygen had entered the atmosphere in substantial amounts (see Figure 14–56). The chloroplast was derived later, after the plant and animal lineages diverged, through endocytosis of an oxygen-producing cyanobacterium.

This *endosymbiont hypothesis* of organelle development receives strong support from the observation that the genetic systems of mitochondria and chloroplasts are similar to those of present-day bacteria. For example, chloroplast ribosomes are very similar to bacterial ribosomes, both in their structure and in their sensitivity to various antibiotics (such as chloramphenicol, streptomycin, erythromycin, and tetracyclin). In addition, protein synthesis in chloroplasts starts with *N*-formylmethionine, as in bacteria, and not with methionine as in the cytosol of eukaryotic cells. Although mitochondrial genetic systems are much less similar to those of present-day bacteria than are the genetic systems of chloroplasts, their ribosomes are also sensitive to antibacterial antibiotics, and protein synthesis in mitochondria also starts with *N*-formylmethionine.

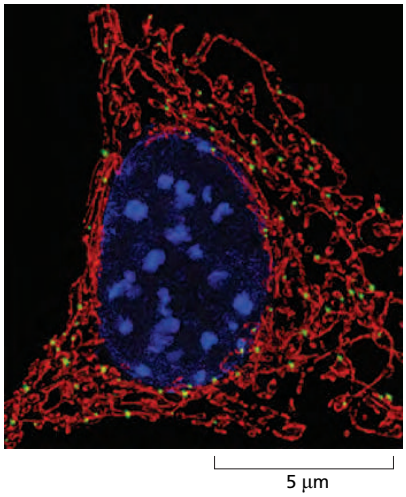


Figure 14–59 Staining of nuclear and mitochondrial DNA. In this confocal micrograph of a human fibroblast, the nuclear DNA is stained with the dye DAPI *(blue)* and mitochondrial DNA is visualized with fluorescent antibodies that bind DNA *(green)*. The mitochondria are stained with fluorescent antibodies that recognize a specialized protein translocase specific to the outer mitochondrial membrane *(red)*. Numerous copies of the mitochondrial genome are distributed in distinct nucleoids throughout the mitochondria that snake through the cytoplasm. (From C. Kukat et al., *Proc. Natl Acad. Sci. USA* 108:13534–13539, 2011. With permission from the National Academy of Sciences.)

The processes of organelle DNA transcription, protein synthesis, and DNA replication take place where the genome is located: in the matrix of mitochondria or the stroma of chloroplasts. Although the enzymes that mediate these genetic processes are unique to the organelle, and resemble those of bacteria (or even of bacterial viruses) rather than their eukaryotic analogs, the nuclear genome encodes the vast majority of these enzymes. Indeed, most present-day mitochondrial and chloroplast proteins are encoded by genes that reside in the cell nucleus.

Over Time, Mitochondria and Chloroplasts Have Exported Most of Their Genes to the Nucleus by Gene Transfer

The nature of the organelle genes located in the nucleus of the cell demonstrates that an extensive transfer of genes from organelle to nuclear DNA has occurred in the course of eukaryotic evolution. Such successful *gene transfer* is expected to be rare, because any gene moved from the organelle needs to adapt to both nuclear transcription and cytoplasmic translation requirements. In addition, the protein needs to acquire a signal sequence that directs it to the correct organelle after its synthesis in the cytosol. By comparing the genes in the mitochondria from different organisms, we can infer that some of the gene transfers to the nucleus occurred relatively recently. The smallest and presumably most highly evolved mitochondrial genomes, for example, encode only a few hydrophobic inner-membrane proteins of the electron-transport chain, plus ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs). Other mitochondrial genomes that have remained more complex tend to contain this same subset of genes along with others (**Figure 14–60**). The most complex mitochondrial genomes include genes that encode components of the mitochondrial genetic system, such as RNA polymerase subunits and ribosomal proteins; these same genes are found in the cell nucleus in yeast and all animal cells.

The proteins that are encoded by genes in the organellar DNA are synthesized on ribosomes within the organelle, using organelle-produced messenger RNA (mRNA) to specify their amino acid sequence (**Figure 14–61**). The protein traffic between the cytosol and these organelles seems to be unidirectional: proteins are normally not exported from mitochondria or chloroplasts to the cytosol. An important exception occurs when a cell is about to undergo apoptosis. As will be

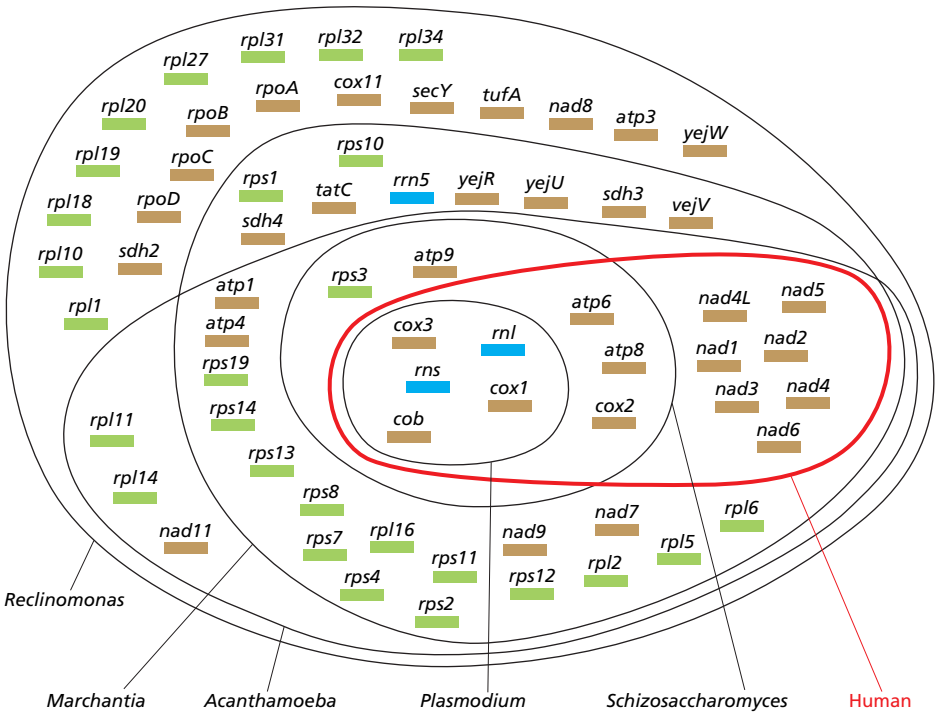


Figure 14–60 Comparison of mitochondrial genomes. Less complex mitochondrial genomes encode subsets of the proteins and ribosomal RNAs that are encoded by larger mitochondrial genomes. In this comparison, there are only five genes that are shared by the six mitochondrial genomes; these encode ribosomal RNAs (*rns* and *rnl*), cytochrome *b* (*cob*), and two cytochrome oxidase subunits (*cox1* and *cox3*). *Blue* indicates ribosomal RNAs; *green*, ribosomal proteins; and *brown*, components of the respiratory chain and other proteins. (Adapted from M.W. Gray, G. Burger and B.F. Lang, *Science* 283:1476–1481, 1999.)

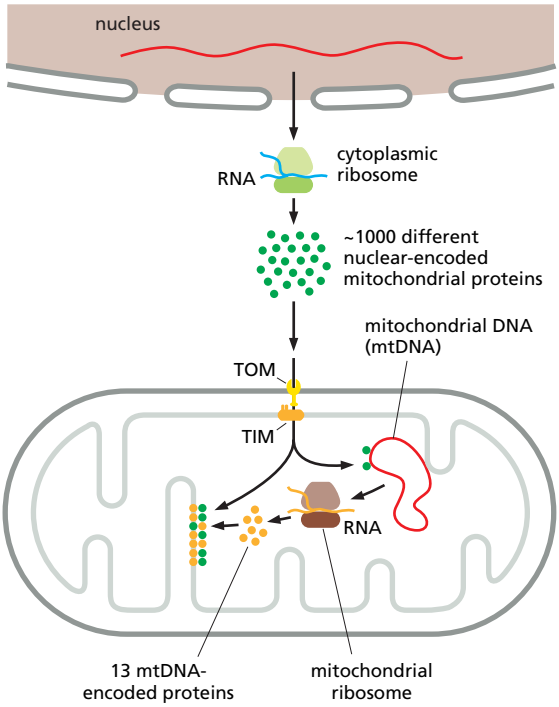


Figure 14–61 Biogenesis of the respiratory-chain proteins in human mitochondria. Most of the protein components of the mitochondrial respiratory chain are encoded by nuclear DNA, with only a small number encoded by mitochondrial DNA (mtDNA). Transcription of mtDNA produces 13 mRNAs, all of which encode subunits of the oxidative phosphorylation system, and the 24 RNAs (22 transfer RNAs and 2 ribosomal RNAs) needed for translation of these mRNAs on the mitochondrial ribosomes *(brown)*.

The mRNAs produced by transcription of nuclear genes are translated on cytoplasmic ribosomes *(green)*, which are distinct from the mitochondrial ribosomes. The nuclear-encoded mitochondrial proteins *(dark green)* are imported into mitochondria through two protein translocases called TOM and TIM, and constitute the vast majority of the approximately 1000 different protein species present in mammalian mitochondria. The nuclear-encoded mitochondrial proteins in humans include the majority of the oxidative phosphorylation system subunits, all proteins needed for expression and maintenance of mtDNA, and all proteins of the mitochondrial ribosomes.

The mtDNA-encoded subunits *(orange)* assemble together with the nuclear subunits to form a functional oxidative phosphorylation system. (Adapted from N.G. Larsson, *Annu. Rev. Biochem.* 79:683–706, 2010.)

discussed in detail in Chapter 18, during apoptosis the mitochondrion releases proteins (most notably cytochrome *c*) from the crista space through its outer mitochondrial membrane, as part of an elaborate signaling pathway that is triggered to cause cells to undergo programmed cell death.

The Fission and Fusion of Mitochondria Are Topologically Complex Processes

In mammalian cells, mitochondrial DNA makes up less than 1% of the total cellular DNA. In other cells, however, such as the leaves of higher plants or the very large egg cells of amphibians, a much larger fraction of the cellular DNA may be present in mitochondria or chloroplasts (**Table 14–2**), and a large fraction of the total RNA and protein synthesis takes place in the organelles.

Mitochondria and chloroplasts are large enough to be visible by light microscopy in living cells. For example, mitochondria can be visualized by expressing in cells a genetically engineered fusion of a mitochondrial protein linked to green

TABLE 14–2 Relative Amounts of Organelle DNA in Some Cells and Tissues

Organism	Tissue or cell type	DNA molecules per organelle	Organelles per cell	Organelle DNA as percentage of total cellular DNA
Mitochondrial DNA				
Rat	Liver	5–10	1000	1
Yeast*	Vegetative	2–50	1–50	15
Frog	Egg	5–10	10^7	99
Chloroplast DNA				
Chlamydomonas	Vegetative	80	1	7
Maize	Leaves	0–300**	20–40	0–15**

*The large variation in the number and size of mitochondria per cell in yeasts is due to mitochondrial fusion and fission.**In maize, the amount of chloroplast DNA drops precipitously in mature leaves, after cell division ceases: the chloroplast DNA is degraded and stable mRNAs persist to provide for protein synthesis.

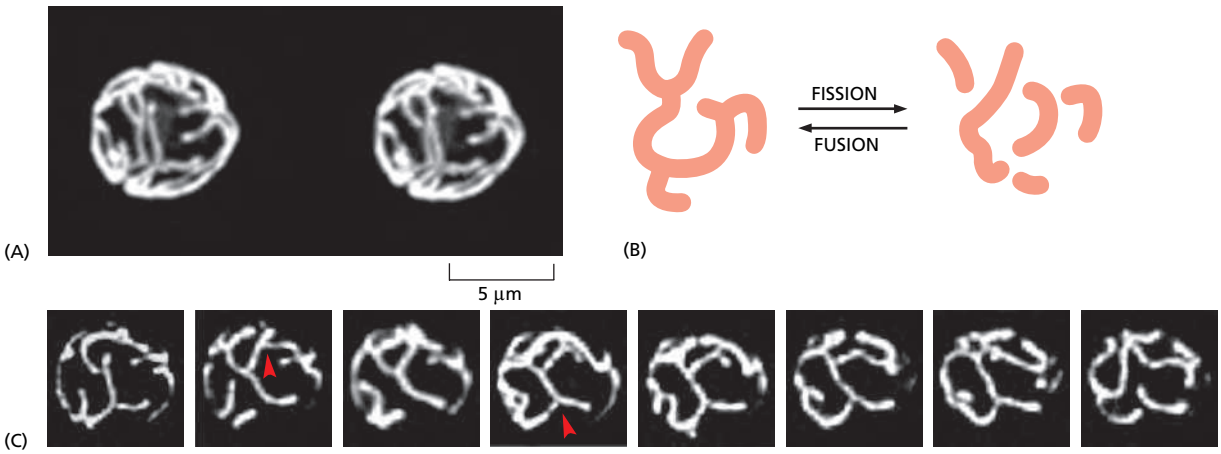


Figure 14–62 The mitochondrial reticulum is dynamic. (A) In yeast cells, mitochondria form a continuous reticulum on the cytoplasmic side of the plasma membrane (stereo pair). (B) A balance between fission and fusion determines the arrangement of the mitochondria in different cells. (C) Time-lapse fluorescent microscopy shows the dynamic behavior of the mitochondrial network in a yeast cell. In addition to shape changes, fission and fusion constantly remodel the network *(red arrows)*. These pictures were taken at 3-minute intervals. (A and C, from J. Nunnari et al., *Mol. Biol. Cell* 8:1233–1242, 1997. With permission from the American Society for Cell Biology.)

fluorescent protein (GFP), or cells can be incubated with a fluorescent dye that is specifically taken up by mitochondria because of their membrane potential. Such images demonstrate that the mitochondria in living cells are dynamic—frequently dividing by fission, fusing, and changing shape (**Figure 14–62** and **Movie 14.12**). The fission of mitochondria may be necessary so that small parts of the network can pinch off and reach remote regions of the cell—for example in the thin, extended axon and dendrites of a neuron.

The fission and fusion of mitochondria are topologically complex processes that must ensure the integrity of the separate mitochondrial compartments defined by the inner and outer membranes. These processes control the number and shape of mitochondria, which can vary dramatically in different cell types, ranging from multiple spherical or wormlike organelles to a highly branched, net-shaped single organelle called a *reticulum*. Each depends on its own special set of proteins. The mitochondrial fission machine works by assembling dynamin-related GTPases (discussed in Chapter 13) into helical oligomers that cause local constrictions in tubular mitochondria. GTP hydrolysis then generates the mechanical force that severs the inner and outer mitochondrial membranes in one step (**Figure 14–63**). Mitochondrial fusion requires two separate machineries, one each for the outer and the inner membrane (**Figure 14–64**). In addition to GTP hydrolysis for force generation, both mechanisms also depend on the mitochondrial proton-motive force for reasons that are still unknown.

Animal Mitochondria Contain the Simplest Genetic Systems Known

Comparisons of DNA sequences in different organisms reveal that, in vertebrates (including ourselves), the mutation rate during evolution has been roughly 100 times greater in the mitochondrial genome than in the nuclear genome. This difference is likely to be due to lower fidelity of mitochondrial DNA replication,

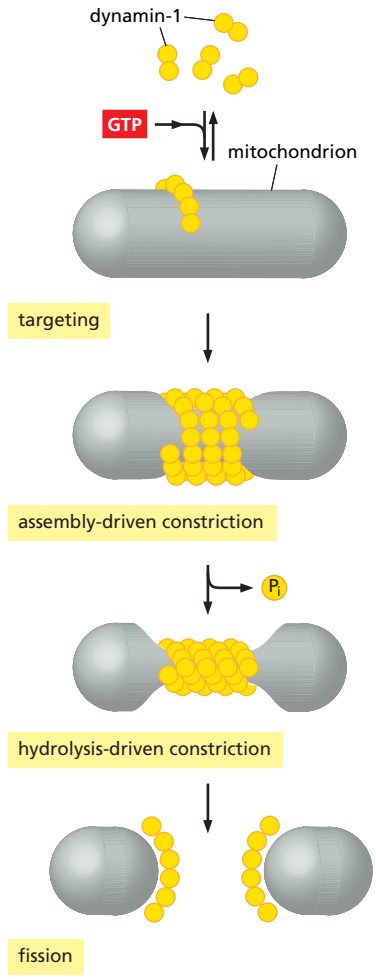


Figure 14–63 A model for mitochondrial division. Dynamin-1 *(yellow)* exists as dimers in the cytosol, which form larger oligomeric structures in a process that requires GTP hydrolysis. Dynamin assemblies interact with the outer mitochondrial membrane through special adaptor proteins, forming a spiral of GTP-dynamin around the mitochondrion that causes a constriction. A concerted GTP-hydrolysis event in the dynamin subunits is then thought to produce the conformational changes that result in fission. (Adapted from S. Hoppins, L. Lackner and J. Nunnari, *Annu. Rev. Biochem*. 76:751–780, 2007.)

inefficient DNA repair, or both, given that the mechanisms that perform these processes in the organelle are relatively simple compared with those in the nucleus. As discussed in Chapter 4, the relatively high rate of evolution of animal mitochondrial genes makes a comparison of mitochondrial DNA sequences especially useful for estimating the dates of relatively recent evolutionary events, such as the steps in primate evolution.

There are 13 protein-encoding genes in human mitochondrial DNA (**Figure 14–65**). These code for hydrophobic components of the respiratory-chain complexes and of ATP synthase. In contrast, roughly 1000 mitochondrial proteins are encoded in the nucleus, produced on cytosolic ribosomes, and imported by the protein import machinery in the outer and inner membrane (discussed in Chapter 12). It has been suggested that the cytosolic production of hydrophobic membrane proteins and their import into the organelle may present a problem to the cell, and that this is the reason why their genes have remained in the mitochondrion. However, some of the most hydrophobic mitochondrial proteins, such as the *c* subunit of the ATP synthase rotor ring, are imported from the cytosol in some species (though they are mitochondrially encoded in others). And the parasites *Plasmodium falciparum* and *Leishmania tarentolae*, which spend most of their life cycles inside cells of their host organisms, have retained only two or three mitochondrially encoded proteins.

The size range of mitochondrial DNAs is similar to that of viral DNAs. The mitochondrial DNA in *Plasmodium falciparum* (the human malaria parasite) has less than 6000 nucleotide pairs, whereas the mitochondrial DNAs of some land plants contain more than 300,000 nucleotide pairs (**Figure 14–66**). In animals, the mitochondrial genome is a simple DNA circle of about 16,600 nucleotide pairs (less than 0.001% of the nuclear genome), and it is nearly the same size in organisms as different from us as *Drosophila* and sea urchins.

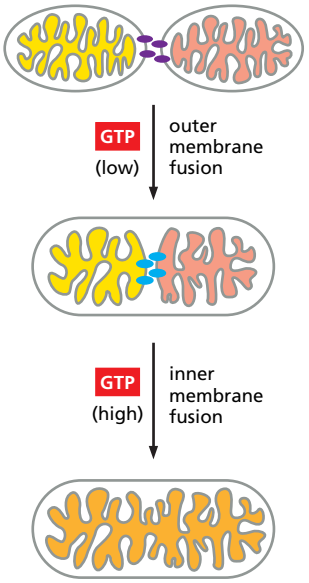


Figure 14–64 A model for mitochondrial fusion. The fusions of the outer and inner mitochondrial membranes are coordinated sequential events, each of which requires a separate set of protein factors. Outer membrane fusion is brought about by an outer-membrane GTPase *(purple)*, which forms an oligomeric complex that includes subunits anchored in the two membranes to be fused. Fusion of outer membranes requires GTP and an H^+ gradient across the inner membrane. For fusion of the inner membrane, a dynamin-related protein forms an oligomeric tethering complex *(blue)* that includes subunits anchored in the two inner membranes to be fused. Fusion of the inner membranes requires GTP and the electrical component of the potential across the inner membrane. (Adapted from S. Hoppins, L. Lackner and J. Nunnari, *Annu. Rev. Biochem.* 76:751–780, 2007.)

Mitochondria Have a Relaxed Codon Usage and Can Have a Variant Genetic Code

The human mitochondrial genome has several surprising features that distinguish it from nuclear, chloroplast, and bacterial genomes:

1. *Dense gene packing.* Unlike other genomes, the human mitochondrial genome seems to contain almost no noncoding DNA: nearly every nucleotide seems to be part of a coding sequence, either for a protein or for one of the rRNAs or tRNAs. Since these coding sequences run directly into each other, there is very little room left for regulatory DNA sequences.
2. *Relaxed codon usage.* Whereas 30 or more tRNAs specify amino acids in the cytosol and in chloroplasts, only 22 tRNAs are required for mitochondrial protein synthesis. The normal codon–anticodon pairing rules are relaxed in mitochondria, so that many tRNA molecules recognize any one of the four nucleotides in the third (wobble) position. Such "2 out of 3" pairing

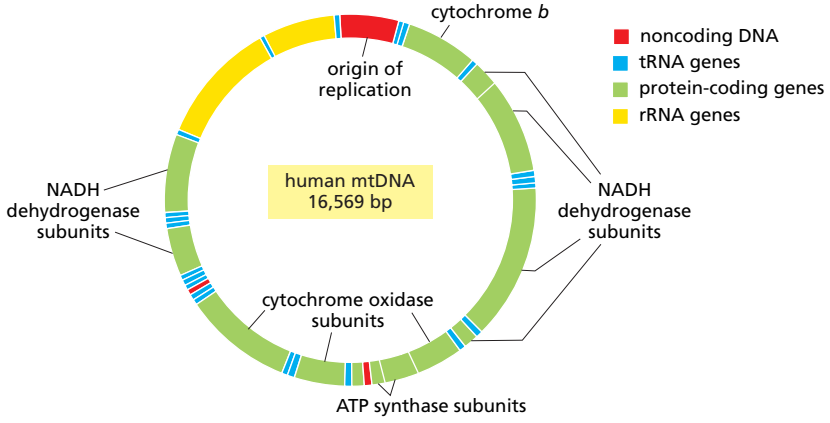


Figure 14–65 The organization of the human mitochondrial genome. The human mitochondrial genome of ≈16,600 nucleotide pairs contains 2 rRNA genes, 22 tRNA genes, and 13 protein-coding sequences. There are two transcriptional promoters, one for each strand of the mitochondrial DNA (mtDNA). The DNAs of many other animal mitochondrial genomes have been completely sequenced. Most of these animal mitochondrial DNAs encode precisely the same genes as humans, with the gene order being identical for animals ranging from fish to mammals.

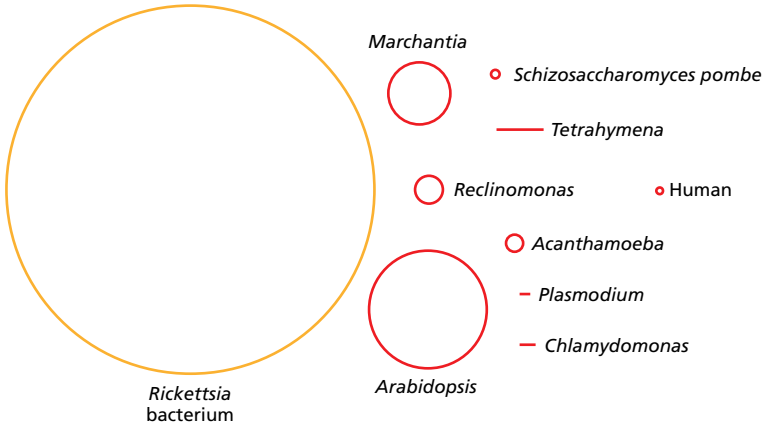


Figure 14–66 Comparison of various sizes of mitochondrial genomes with the genome of bacterial ancestors. The complete DNA sequences for thousands of mitochondrial genomes have been determined. The lengths of a few of these mitochondrial DNAs are shown to scale—as circles for those genomes thought to be circular and lines for linear genomes. The largest circle represents the genome of *Rickettsia prowazekii*, a small pathogenic bacterium whose genome most closely resembles that of mitochondria. The size of mitochondrial genomes does not correlate well with the number of proteins encoded in them: while human mitochondrial DNA encodes 13 proteins, the 22-fold larger mitochondrial DNA of *Arabidopsis thaliana* encodes only 32 proteins—that is, about 2.5-fold as many as human mitochondrial DNA. The extra DNA that is found in *Arabidopsis*, *Marchantia*, and other plant mitochondria may be "junk DNA"—that is, noncoding DNA with no apparent function. The mitochondrial DNA of the protozoan *Reclinomonas americana* has 98 genes. (Adapted from M.W. Gray, G. Burger and B.F. Lang, *Science* 283:1476–1481, 1999.)

allows one tRNA to pair with any one of four codons and permits protein synthesis with fewer tRNA molecules.

3. *Variant genetic code.* Perhaps most surprising, comparisons of mitochondrial gene sequences and the amino acid sequences of the corresponding proteins indicate that the genetic code is different: 4 of the 64 codons have different "meanings" from those of the same codons in other genomes (**Table 14–3**).

The close similarity of the genetic code in all organisms provides strong evidence that they all have evolved from a common ancestor. How, then, do we explain the differences in the genetic code in many mitochondria? A hint comes from the finding that the mitochondrial genetic code in different organisms is not the same. In the mitochondrion with the largest number of genes in Figure 14–60, that of the protozoan *Reclinomonas*, the genetic code is unchanged from the standard genetic code of the cell nucleus. Yet UGA, which is a stop codon elsewhere, is read as tryptophan in the mitochondria of mammals, fungi, and invertebrates. Similarly, the codon AGG normally codes for arginine, but it codes for *stop* in the mitochondria of mammals and codes for serine in the mitochondria of *Drosophila* (see Table 14–3). Such variation suggests that a random drift can occur in the genetic code in mitochondria. Presumably, the unusually small number of proteins encoded by the mitochondrial genome makes an occasional change in the meaning of a rare codon tolerable, whereas such a change in a larger genome would alter the function of many proteins and thereby destroy the cell.

Interestingly, in many species, one or two tRNAs for mitochondrial protein synthesis are encoded in the nucleus. Some parasites, for example trypanosomes, have not retained any tRNA genes in their mitochondrial DNA. Instead, the required tRNAs are all produced in the cytosol and are thought to be imported into the mitochondrion by special tRNA translocases that are distinct from the mitochondrial protein import system.

TABLE 14–3 Some Differences Between the "Universal" Code and Mitochondrial Genetic Codes*

Codon	"Universal" code	Mitochondrial codes			
		Mammals	Invertebrates	Yeasts	Plants
UGA	STOP	*Trp*	*Trp*	*Trp*	STOP
AUA	Ile	*Met*	*Met*	*Met*	Ile
CUA	Leu	Leu	Leu	*Thr*	Leu
AGA AGG	Arg	*STOP*	*Ser*	Arg	Arg

**Red* italics indicate that the code differs from the "Universal" code.

Chloroplasts and Bacteria Share Many Striking Similarities

The chloroplast genomes of land plants range in size from 70,000 to 200,000 nucleotide pairs. More than 300 chloroplast genomes have now been sequenced. Many are surprisingly similar, even in distantly related plants (such as tobacco and liverwort), and even those of green algae are closely related (**Figure 14–67**). Chloroplast genes are involved in three main processes: transcription, translation, and photosynthesis. Plant chloroplast genomes typically encode 80–90 proteins and around 45 RNAs, including 37 or more tRNAs. As in mitochondria, most of the organelle-encoded proteins are part of larger protein complexes that also contain one or more subunits encoded in the nucleus and imported from the cytosol.

The genomes of chloroplasts and bacteria have striking similarities. Basic regulatory sequences, such as transcription promoters and terminators, are virtually identical. The amino acid sequences of the proteins encoded in chloroplasts are clearly recognizable as bacterial, and several clusters of genes with related functions (such as those encoding ribosomal proteins) are organized in the same way in the genomes of chloroplasts, the bacterium *E. coli*, and cyanobacteria.

The mechanisms by which chloroplasts and bacteria divide are also similar. Both utilize *FtsZ* proteins, which are self-assembling GTPases related to tubulins (see Chapter 16). Bacterial FtsZ is a soluble protein that assembles into a dynamic ring of membrane-attached protofilaments beneath the plasma membrane in the middle of the dividing cell. The FtsZ ring acts as a scaffold for recruitment of other cell-division proteins and generates a contractile force that results in membrane constriction and eventually in cell division. Presumably, chloroplasts divide in very much the same way. Although both employ membrane-interacting GTPases, the mechanisms by which mitochondria and chloroplasts divide are fundamentally different. The machinery for chloroplast division acts from the inside, as in bacteria, while the dynamin-like GTPases divide mitochondria from the outside (see Figure 14–63). The chloroplasts have remained closer to their bacterial origins than have mitochondria, since the eukaryotic mechanisms of membrane constriction and vesicle formation have been adapted for mitochondrial fission.

The RNA editing and RNA processing that is prevalent in chloroplasts owes everything to their eukaryotic hosts. This RNA processing includes the generation of transcript 5′ and 3′ termini and the cleavage of polycistronic transcripts. In addition, an RNA editing process converts specific C residues to U and can change the amino acid specified by the edited codon. These and other RNA-based

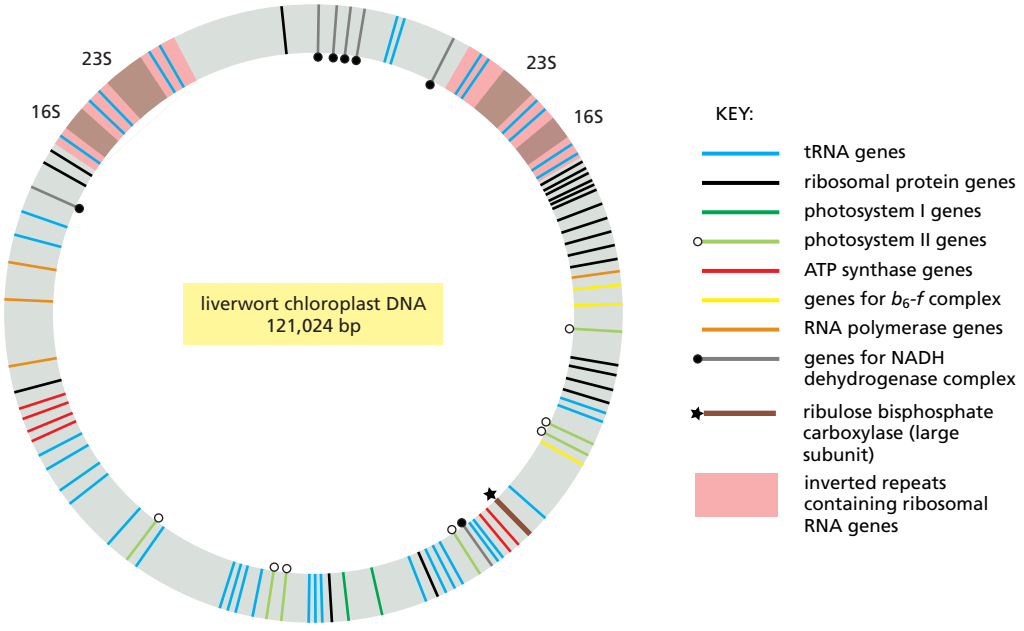


Figure 14–67 The organization of the liverwort chloroplast genome. The chloroplast genome organization is similar in all higher plants, although the size varies from species to species—depending on how much of the DNA surrounding the genes encoding the chloroplast's 16S and 23S ribosomal RNAs is present in two copies.

processes are catalyzed by protein families that are not found in prokaryotes. One can ask why the expression of so few chloroplast genes needs to be so complex. One explanation is that the expression of chloroplast and nuclear genes must be closely coordinated. More generally, the bacterial concept of the operon as a co-regulated set of genes in a single transcription unit has been largely abandoned in chloroplasts. Polycistronic transcripts are cleaved into smaller fragments, which then require splicing or RNA editing to become functional.

Organelle Genes Are Maternally Inherited in Animals and Plants

In *Saccharomyces cerevisiae* (baker's yeast), when two haploid cells mate, they are equal in size and contribute equal amounts of mitochondrial DNA to the diploid zygote. Mitochondrial inheritance in yeasts is therefore *biparental*: both parents contribute equally to the mitochondrial gene pool of the progeny. However, during the course of the subsequent asexual, vegetative growth, the mitochondria become distributed more or less randomly to daughter cells. After a few generations, the mitochondria of any given cell contain only the DNA from one or the other parent cell, because only a small sample of the mitochondrial DNA passes from the mother cell to the bud of the daughter cell. This process is known as *mitotic segregation*, and it gives rise to a distinct form of inheritance that is called non-Mendelian, or *cytoplasmic inheritance*, in contrast to the Mendelian inheritance of nuclear genes.

The inheritance of mitochondria in animals and plants is quite different. In these organisms, the egg cell contributes much more cytoplasm to the zygote than does the male gamete (sperm in animals, pollen in plants). For example, a typical human oocyte contains about 100,000 copies of maternal mitochondrial DNA, whereas a sperm cell contains only a few. In addition, an active process ensures that the sperm mitochondria do not compete with those in the egg. As sperm mature, the DNA is degraded in their mitochondria. Sperm mitochondria are also specifically recognized then eliminated from the fertilized egg cell by autophagy in very much the same way that damaged mitochondria are removed (by ubiquitylation followed by delivery to lysosomes, as discussed in Chapter 13). Because of these two processes, the mitochondrial inheritance in both animals and plants is *uniparental*. More precisely, the mitochondrial DNA passes from one generation to the next by **maternal inheritance**.

In about two-thirds of higher plants, the chloroplast precursors from the male parent (contained in pollen grains) fail to enter the zygote, so that chloroplast as well as mitochondrial DNA is maternally inherited. In other plants, the chloroplast precursors from the pollen grains enter the zygote, making chloroplast inheritance biparental. In such plants, defective chloroplasts are a cause of variegation: a mixture of normal and defective chloroplasts in a zygote may sort out by mitotic segregation during plant growth and development, thereby producing alternating green and white patches in leaves. Leaf cells in the green patches contain normal chloroplasts, while those in the white patches contain defective chloroplasts (**Figure 14–68**).

Figure 14–68 A variegated leaf. In the white patches, the plant cells have inherited a defective chloroplast. (Courtesy of John Innes Foundation.)

Mutations in Mitochondrial DNA Can Cause Severe Inherited Diseases

In humans, as we have explained, all the mitochondrial DNA in a fertilized egg cell is inherited from the mother. Some mothers carry a mixed population of both mutant and normal mitochondrial genomes. Their daughters and sons will inherit this mixture of normal and mutant mitochondrial DNAs and be healthy unless the process of mitotic segregation results in a majority of defective mitochondria in a particular tissue. Muscle and the nervous system are most at risk. Because they need particularly large amounts of ATP, muscle and nerve cells are particularly dependent on fully functional mitochondria.

Numerous diseases in humans are caused by mutations in mitochondrial DNA. These diseases are recognized by their passage from affected mothers to

both their daughters and their sons, with the daughters but not the sons producing children with the disease. As expected from the random nature of mitotic segregation, the symptoms of these diseases vary greatly between different family members—including not only the severity and age of onset, but also which tissue is affected. There are also mitochondrial diseases that are caused by mutations in nuclear-encoded mitochondrial proteins; these diseases are inherited in the regular, Mendelian fashion.

The Accumulation of Mitochondrial DNA Mutations Is a Contributor to Aging

Mitochondria are marvels of efficiency in energy conversion, and they supply the cells of our body with a readily available source of energy in the form of ATP. But in highly developed, long-lived animals such as ourselves, the cells in our body age and eventually die. A factor in this inevitable process is the accumulation of deletions and point mutations in mitochondrial DNA. Oxidative damage to the cell by *reactive oxygen species* (*ROS*) such as H_2O_2, superoxide, or hydroxyl radicals also increases with age. The mitochondrial respiratory chain is the main source of ROS in animal cells, and animals in which mitochondrial superoxide dismutase—the main ROS scavenger—has been knocked out, die prematurely.

The less complex DNA replication and repair systems in mitochondria mean that accidents are corrected less efficiently. This results in a 100-fold higher occurrence of deletions and point mutations than in nuclear DNA. Mathematical modeling suggests that most of these mutations and lesions are acquired in childhood or early adult life, and then proliferate by *clonal expansion* in later life. Due to mitotic segregation, some cells will accumulate higher levels of faulty mitochondrial DNA than others. Above some threshold, serious deficiencies in respiratory-chain function will develop, producing cells that are *senescent*. In many organs of the human body, senescent cells with high levels of mitochondrial DNA damage are intermingled with normal cells, resulting in a mosaic of cells with and without respiratory-chain deficiency.

The main role of mitochondrial fusion in cellular physiology is most likely to ensure an even distribution of mitochondrial DNA throughout the mitochondrial reticulum, and to prevent the accumulation of damaged DNA in one part of the network. When the fusion machinery is defective, DNA is lost from a subset of the mitochondria in the cell. Loss of mitochondrial DNA leads to a loss of respiratory-chain function, and it can cause disease.

All of the considerations just discussed have suggested to some scientists that changes in our mitochondria are major contributors to human aging. However, there are many other processes that tend to go wrong as cells and tissues age, as one might expect given the incredible complexity of human cell biology. Despite intensive research, the issue remains unresolved.

Why Do Mitochondria and Chloroplasts Maintain a Costly Separate System for DNA Transcription and Translation?

Why do mitochondria and chloroplasts require their own separate genetic systems, when other organelles that share the same cytoplasm, such as peroxisomes and lysosomes, do not? The question is not trivial, because maintaining a separate genetic system is costly: more than 90 proteins—including many ribosomal proteins, aminoacyl-tRNA synthetases, DNA polymerase, RNA polymerase, and RNA-processing and RNA-modifying enzymes—must be encoded by nuclear genes specifically for this purpose. Moreover, as we have seen, the mitochondrial genetic system entails the risk of aging and disease.

A possible reason for maintaining this costly and potentially hazardous arrangement is the highly hydrophobic nature of the nonribosomal proteins encoded by organelle genes. This may make their production in and import from the cytoplasm simply too difficult and energy-consuming. It is also possible that the evolution (and eventual elimination) of the organellar genetic systems is still

ongoing, but for now there is no alternative for the cell than to maintain separate genetic systems for its nuclear, mitochondrial, and chloroplast genes.

Summary

Mitochondria are organelles that allow eukaryotes to carry out oxidative phosphorylation, while chloroplasts are organelles that allow plants to carry out photosynthesis. Presumably as a result of their prokaryotic origins, each organelle maintains and reproduces itself in a highly coordinated process that requires the contribution of two separate genetic systems—one in the organelle and the other in the cell nucleus. The vast majority of the proteins in these organelles are encoded by nuclear DNA, synthesized in the cytosol, and then imported individually into the organelle. Other organelle proteins, as well as organelle ribosomal and transfer RNAs, are encoded by the organelle DNA; these are synthesized in the organelle itself.

The ribosomes of chloroplasts closely resemble bacterial ribosomes, while the origin of mitochondrial ribosomes is more difficult to trace. Extensive protein similarities, however, suggest that both organelles originated when a primitive eukaryotic cell entered into a stable endosymbiotic relationship with a bacterium. Although some of the genes of these former bacteria still function to make organelle proteins and RNA, most of them have been transferred into the nuclear genome, where they encode bacteria-like enzymes that are synthesized on cytosolic ribosomes and then imported into the organelle. The mitochondrial DNA replication and DNA repair processes are substantially less effective than the corresponding processes in the cell nucleus. Damage therefore accumulates in the genome of mitochondria over time; this damage may be a substantial contributor to the aging of cells and organisms, and it can cause serious diseases.

WHAT WE DON'T KNOW

- What structures are needed to form the barriers that separate and maintain the differentiated membrane domains in a single continuous membrane—as for the cristae and inner boundary membrane in mitochondria?
- How does a eukaryotic cell regulate the many functions of mitochondria, including ATP production?
- What are the origins and evolutionary history of photosynthetic complexes? Are there undiscovered types of photosynthesis present on Earth to help answer this question?
- Why is the mutation rate so much higher in mitochondria than in the nucleus (and chloroplasts)? Could this high rate have been useful to the cell?
- What mechanisms and pathways have been used during evolution to transfer genes from the mitochondrion to the nucleus?

PROBLEMS

Which statements are true? Explain why or why not.

14–1 The three respiratory enzyme complexes in the mitochondrial inner membrane tend to associate with each other in ways that facilitate the correct transfer of electrons between appropriate complexes.

14–2 The number of *c* subunits in the rotor ring of ATP synthase defines how many protons need to pass through the turbine to make each molecule of ATP.

14–3 Mutations that are inherited according to Mendelian rules affect nuclear genes; mutations whose inheritance violates Mendelian rules are likely to affect organelle genes.

Discuss the following problems.

14–4 In the 1860s, Louis Pasteur noticed that when he added O_2 to a culture of yeast growing anaerobically on glucose, the rate of glucose consumption declined dramatically. Explain the basis for this result, which is known as the Pasteur effect.

14–5 Heart muscle gets most of the ATP needed to power its continual contractions through oxidative phosphorylation. When oxidizing glucose to CO_2, heart muscle consumes O_2 at a rate of 10 μmol/min per g of tissue, in order to replace the ATP used in contraction and give a steady-state ATP concentration of 5 μmol/g of tissue. At this rate, how many seconds would it take the heart to consume an amount of ATP equal to its steady-state levels? (Complete oxidation of one molecule of glucose to CO_2 yields 30 ATP, 26 of which are derived by oxidative phosphorylation using the 12 pairs of electrons captured in the electron carriers NADH and $FADH_2$.)

14–6 Both H^+ and Ca^{2+} are ions that move through the cytosol. Why is the movement of H^+ ions so much faster than that of Ca^{2+} ions? How do you suppose the speed of these two ions would be affected by freezing the solution? Would you expect them to move faster or slower? Explain your answer.

14–7 If isolated mitochondria are incubated with a source of electrons such as succinate, but without oxygen, electrons enter the respiratory chain, reducing each of the electron carriers almost completely. When oxygen is then introduced, the carriers become oxidized at different rates (**Figure Q14–1**). How does this result allow you to order

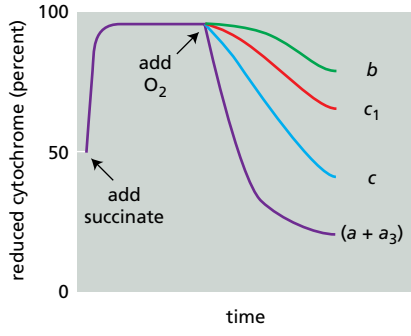


Figure Q14–1 Rapid spectrophotometric analysis of the rates of oxidation of electron carriers in the respiratory chain (Problem 14–7). Cytochromes *a* and a_3 cannot be distinguished and thus are listed as cytochrome ($a + a_3$).

the electron carriers in the respiratory chain? What is their order?

14–8 Normally, the flow of electrons to O_2 is tightly linked to the production of ATP via the electrochemical gradient. If ATP synthase is inhibited, for example, electrons do not flow down the electron-transport chain and respiration ceases. Since the 1940s, several substances—such as 2,4-dinitrophenol—have been known to uncouple electron flow from ATP synthesis. Dinitrophenol was once prescribed as a diet drug to aid in weight loss. How would an uncoupler of oxidative phosphorylation promote weight loss? Why do you suppose dinitrophenol is no longer prescribed?

14–9 In actively respiring liver mitochondria, the pH in the matrix is about half a pH unit higher than it is in the cytosol. Assuming that the cytosol is at pH 7 and the matrix is a sphere with a diameter of 1 μm [$V = (4/3)\pi r^3$], calculate the total number of protons in the matrix of a respiring liver mitochondrion. If the matrix began at pH 7 (equal to that in the cytosol), how many protons would have to be pumped out to establish a matrix pH of 7.5 (a difference of 0.5 pH units)?

14–10 ATP synthase is the world's smallest rotary motor. Passage of H^+ ions through the membrane-embedded portion of ATP synthase (the F_o component) causes rotation of the single, central, axle-like γ subunit inside the head group. The tripartite head is composed of the three αβ dimers, the β subunit of which is responsible for synthesis of ATP. The rotation of the γ subunit induces conformational changes in the αβ dimers that allow ADP and Pi to be converted into ATP. A variety of indirect evidence had suggested rotary catalysis by ATP synthase, but seeing is believing.

To demonstrate rotary motion, a modified form of the $\alpha_3\beta_3\gamma$ complex was used. The β subunits were modified so they could be firmly anchored to a solid support and the γ subunit was modified (on the end that normally inserts into the F_o component in the inner membrane) so that a fluorescently tagged, readily visible filament of actin could be attached (**Figure Q14–2**A). This arrangement allows rotations of the γ subunit to be visualized as revolutions of the long actin filament. In these experiments, ATP synthase was studied in the reverse of its normal mechanism by allowing it to hydrolyze ATP. At low ATP concentrations, the actin filament was observed to revolve in steps of 120° and then pause for variable lengths of time, as shown in Figure Q14–2B.

A. Why does the actin filament revolve in steps with pauses in between? What does this rotation correspond to in terms of the structure of the $\alpha_3\beta_3\gamma$ complex?

B. In its normal mode of operation inside the cell, how many ATP molecules do you suppose would be synthesized for each complete 360° rotation of the γ subunit? Explain your answer.

14–11 How much energy is available in visible light? How much energy does sunlight deliver to Earth? How efficient

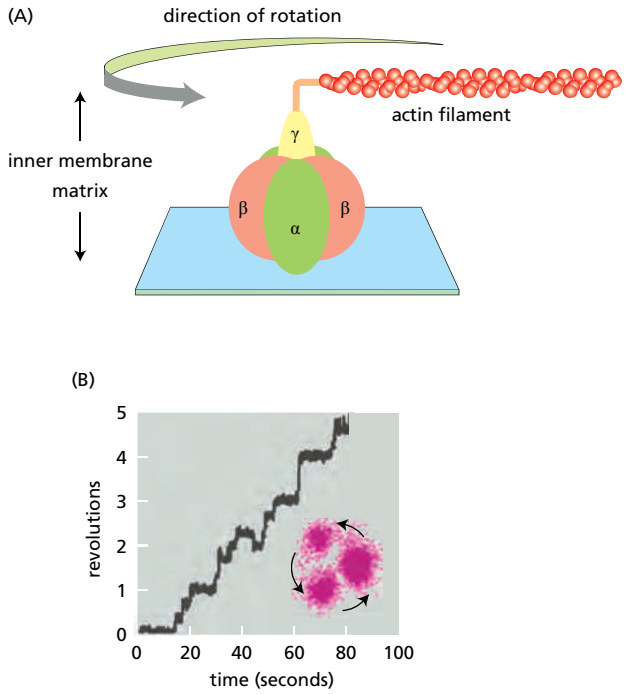


Figure Q14–2 Experimental set-up for observing rotation of the γ subunit of ATP synthase (Problem 14–10). (A) The immobilized $\alpha_3\beta_3\gamma$ complex. The β subunits are anchored to a solid support and a fluorescent actin filament is attached to the γ subunit. (B) Stepwise revolution of the actin filament. The indicated trace is a typical example from one experiment. The inset shows the positions in the revolution at which the actin filament pauses. (B, from R. Yasuda et al., *Cell* 93:1117–1124, 1998. With permission from Elsevier.)

are plants at converting light energy into chemical energy? The answers to these questions provide an important backdrop to the subject of photosynthesis.

Each quantum or photon of light has energy $h\nu$, where h is Planck's constant (6.6×10^{-37} kJ sec/photon) and ν is the frequency in sec^{-1}. The frequency of light is equal to c/λ, where c is the speed of light (3.0×10^{17} nm/sec) and λ is the wavelength in nm. Thus, the energy (E) of a photon is

$$E = h\nu = hc/\lambda$$

A. Calculate the energy of a mole of photons (6×10^{23} photons/mole) at 400 nm (violet light), at 680 nm (red light), and at 800 nm (near-infrared light).

B. Bright sunlight strikes Earth at the rate of about 1.3 kJ/sec per square meter. Assuming for the sake of calculation that sunlight consists of monochromatic light of wavelength 680 nm, how many seconds would it take for a mole of photons to strike a square meter?

C. Assuming that it takes eight photons to fix one molecule of CO_2 as carbohydrate under optimal conditions (8–10 photons is the currently accepted value), calculate how long it would take a tomato plant with a leaf area of 1 square meter to make a mole of glucose from CO_2. Assume that photons strike the leaf at the rate calculated above and, furthermore, that all the photons are absorbed and used to fix CO_2.

D. If it takes 468 kJ/mole to fix a mole of CO_2 into carbohydrate, what is the efficiency of conversion of light energy into chemical energy after photon capture? Assume again that eight photons of red light (680 nm) are required to fix one molecule of CO_2.

14–12 In chloroplasts, protons are pumped out of the stroma across the thylakoid membrane, whereas in mitochondria, they are pumped out of the matrix across the crista membrane. Explain how this arrangement allows chloroplasts to generate a larger proton gradient across the thylakoid membrane than mitochondria can generate across the inner membrane.

14–13 Examine the variegated leaf shown in **Figure Q14–3**. Yellow patches surrounded by green are common, but there are no green patches surrounded by yellow. Propose an explanation for this phenomenon.

Figure Q14–3 A variegated leaf of *Aucuba japonica* with green and yellow patches (Problem 14–13).

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