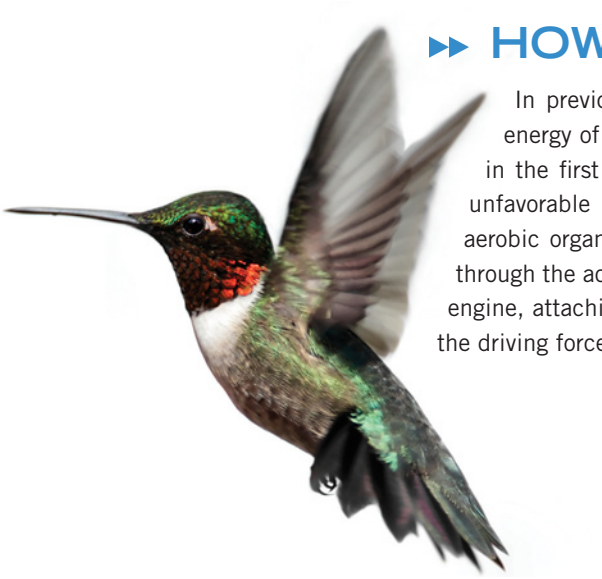
chapter 15

# OXIDATIVE PHOSPHORYLATION



[Steve Byland/iStockphoto]

## ▸▸ HOW does ATP synthase carry out an unfavorable reaction?

In previous chapters, we have seen numerous examples of cells using the free energy of the ATP reaction to carry out cellular work. But in order to produce ATP in the first place, cells must add a third phosphate to ADP, a thermodynamically unfavorable reaction. Glycolysis and the citric acid cycle provide some ATP, but in aerobic organisms, such as the hummingbird shown here, most of the ATP is made through the action of a mitochondrial ATP synthase. This enzyme functions like a rotary engine, attaching a phosphoryl group to ADP as it spins. In this chapter, we'll examine the driving force for this unusual molecular machine.

## THIS CHAPTER IN CONTEXT



### *Do You Remember?*

- **Living organisms obey the laws of thermodynamics (Section 1-3).**
- **Transporters obey the laws of thermodynamics, providing a way for solutes to move down their concentration gradients or using ATP to move substances against their gradients (Section 9-1).**
- **Coenzymes such as $NAD^+$ and ubiquinone collect electrons from compounds that become oxidized (Section 12-2).**
- **A reaction that breaks a phosphoanhydride bond in ATP occurs with a large change in free energy (Section 12-3).**

The oxidation of metabolic fuels such as glucose, fatty acids, and amino acids as well as the oxidation of acetyl carbons to $CO_2$ via the citric acid cycle yields the reduced cofactors NADH and ubiquinol ($QH_2$). These compounds are forms of energy currency (see Section 12-3) not because they are chemically special but because their reoxidation—ultimately by molecular oxygen in aerobic organisms—is an exergonic reaction. The free energy released thereby is harvested to synthesize ATP, a phenomenon called **oxidative phosphorylation.** In the scheme introduced in Figure 12-10, oxidative phosphorylation represents the final phase of the catabolism of metabolic fuels and the major source of the cell's ATP **(Fig. 15-1)**.

Oxidative phosphorylation differs from the conventional biochemical reactions we have focused on in the last two chapters. In particular, ATP synthesis is not directly coupled to a single discrete chemical reaction, such as a kinase-catalyzed reaction. Rather, *oxidative phosphorylation is a more indirect process in which free energy is converted to, or conserved as, a transmembrane gradient of protons that is then used to drive ATP synthesis.*

To understand oxidative phosphorylation, we must first consider how the reduced cofactors produced in other metabolic reactions are reoxidized by molecular oxygen. The flow of electrons from reduced compounds such as NADH and $QH_2$ to an oxidized compound such as $O_2$ is a thermodynamically favorable process. We will see that the free energy changes for electron-transfer reactions can be quantified by considering the reduction potentials of the chemical species involved. Next, we will track the movements of the electrons through a series of electron carriers that include small molecules as well as prosthetic groups of large integral membrane proteins. As electrons are shuttled from NADH and $QH_2$ to molecular oxygen, the membrane proteins translocate protons from one side of the membrane to the other. This process is the first step of chemiosmosis, the formation and dissipation of a transmembrane chemical gradient. Finally, we will examine the structure of ATP synthase, the enzyme complex that taps the free energy of the proton gradient to synthesize ATP from ADP + $P_i$.

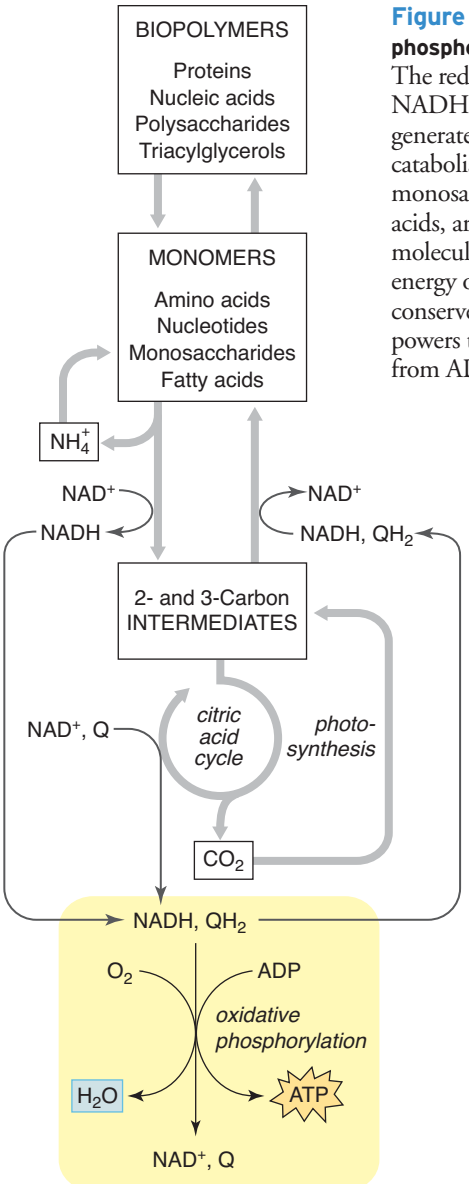



**Figure 15-1 Oxidative phosphorylation in context.** The reduced cofactors NADH and $QH_2$, which are generated in the oxidative catabolism of amino acids, monosaccharides, and fatty acids, are reoxidized by molecular oxygen. The free energy of this process is conserved in a manner that powers the synthesis of ATP from ADP + $P_i$.

# 15-1 The Thermodynamics of Oxidation-Reduction Reactions

Oxidation–reduction reactions (or redox reactions, introduced in Section 12-2) are similar to other chemical reactions in which a portion of a molecule—electrons in this case—is transferred. In any oxidation–reduction reaction, one reactant (called the **oxidizing agent** or **oxidant**) is reduced as it gains electrons. The other reactant (called the **reducing agent** or **reductant**) is oxidized as it gives up electrons:

$$A_{oxidized} + B_{reduced} \rightleftharpoons A_{reduced} + B_{oxidized}$$

For example, in the succinate dehydrogenase reaction (step 6 of the citric acid cycle; see Section 14-2), the two electrons of the reduced $FADH_2$ prosthetic group of the enzyme are transferred to ubiquinone (Q) so that $FADH_2$ is oxidized and ubiquinone is reduced:

$$\underset{(reduced)}{FADH_2} + \underset{(oxidized)}{Q} \rightleftharpoons \underset{(oxidized)}{FAD} + \underset{(reduced)}{QH_2}$$

**KEY CONCEPTS**

- The standard reduction potential indicates a substance's tendency to become reduced; the actual reduction potential depends on the concentrations of reactants.
- Electrons are transferred from a substance with a lower reduction potential to a substance with a higher reduction potential.
- The free energy change for an oxidation–reduction reaction depends on the change in reduction potential.

In this reaction, the two electrons are transferred as H atoms (an H atom consists of a proton and an electron, or $H^+$ and $e^-$). In oxidation–reduction reactions involving the cofactor $NAD^+$, the electron pair takes the form of a hydride ion ($H^-$, a proton with two electrons). In biological systems, electrons usually travel in pairs, although, as we will see, they may also be transferred one at a time. Note that the change in oxidation state of a reactant may be obvious, such as when $Fe^{3+}$ is reduced to $Fe^{2+}$, or it may require closer inspection of the molecule's structure, such as when succinate is oxidized to fumarate (Section 14-2).

## Reduction potential indicates a substance's tendency to accept electrons

The tendency of a substance to accept electrons (to become reduced) or to donate electrons (become oxidized) can be quantified. Although an oxidation–reduction reaction necessarily requires both an oxidant and a reductant, it is helpful to consider just one substance at a time, that is, a **half-reaction.** Using the example above, the half-reaction for ubiquinone (by convention, written as a reduction reaction) is

$$Q + 2H^+ + 2e^- \rightleftharpoons QH_2$$

(the reverse reaction would describe an oxidation half-reaction).

The affinity of a substance such as ubiquinone for electrons is its **standard reduction potential ($\mathcal{E}^{\circ\prime}$),** which has units of volts (note that the degree and prime symbols indicate a value under standard biochemical conditions where the pressure is 1 atm, the temperature is 25°C, the pH is 7.0, and all species are present at concentrations of 1 M). *The greater the value of $\mathcal{E}^{\circ\prime}$, the greater the tendency of the oxidized form of the substance to accept electrons and become reduced.* The standard reduction potentials of some biological substances are given in Table 15-1.

**TABLE 15-1** Standard Reduction Potentials of Some Biological Substances

| Half-Reaction | $\mathcal{E}^{\circ\prime}$ (V) |
|---|---|
| $\frac{1}{2}O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O$ | 0.815 |
| $SO_4^{2-} + 2H^+ + 2e^- \rightleftharpoons SO_3^{2-} + H_2O$ | 0.48 |
| $NO_3^- + 2H^+ + 2e^- \rightleftharpoons NO_2^- + H_2O$ | 0.42 |
| Cytochrome $a_3$ ($Fe^{3+}$) + $e^- \rightleftharpoons$ cytochrome $a_3$ ($Fe^{2+}$) | 0.385 |
| Cytochrome $a$ ($Fe^{3+}$) + $e^- \rightleftharpoons$ cytochrome $a$ ($Fe^{2+}$) | 0.29 |
| Cytochrome $c$ ($Fe^{3+}$) + $e^- \rightleftharpoons$ cytochrome $c$ ($Fe^{2+}$) | 0.235 |
| Cytochrome $c_1$ ($Fe^{3+}$) + $e^- \rightleftharpoons$ cytochrome $c_1$ ($Fe^{2+}$) | 0.22 |
| Cytochrome $b$ ($Fe^{3+}$) + $e^- \rightleftharpoons$ cytochrome $b$ ($Fe^{2+}$)(*mitochondrial*) | 0.077 |
| Ubiquinone + $2H^+ + 2e^- \rightleftharpoons$ ubiquinol | 0.045 |
| Fumarate$^-$ + $2H^+ + 2e^- \rightleftharpoons$ succinate$^-$ | 0.031 |
| FAD + $2H^+ + 2e^- \rightleftharpoons FADH_2$ (*in flavoproteins*) | ~0. |
| Oxaloacetate$^-$ + $2H^+ + 2e^- \rightleftharpoons$ malate$^-$ | −0.166 |
| Pyruvate$^-$ + $2H^+ + 2e^- \rightleftharpoons$ lactate$^-$ | −0.185 |
| Acetaldehyde + $2H^+ + 2e^- \rightleftharpoons$ ethanol | −0.197 |
| S + $2H^+ + 2e^- \rightleftharpoons H_2S$ | −0.23 |
| Lipoic acid + $2H^+ + 2e^- \rightleftharpoons$ dihydrolipoic acid | −0.29 |
| $NAD^+ + H^+ + 2e^- \rightleftharpoons$ NADH | −0.315 |
| $NADP^+ + H^+ + 2e^- \rightleftharpoons$ NADPH | −0.320 |
| Acetoacetate$^-$ + $2H^+ + 2e^- \rightleftharpoons$ 3-hydroxybutyrate$^-$ | −0.346 |
| Acetate$^-$ + $3H^+ + 2e^- \rightleftharpoons$ acetaldehyde + $H_2O$ | −0.581 |

*Source*: Mostly from Loach, P. A., in Fasman, G. D. (ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), Physical and Chemical Data, Vol. I, pp. 123–130, CRC Press (1976).

Like a $\Delta G$ value, *the actual reduction potential depends on the actual concentrations of the oxidized and reduced species.* The actual reduction potential ($\mathcal{E}$) is related to the standard reduction potential ($\mathcal{E}^{\circ\prime}$) by the **Nernst equation:**

$$\mathcal{E} = \mathcal{E}^{\circ\prime} - \frac{RT}{n\mathcal{F}} \ln \frac{[A_{reduced}]}{[A_{oxidized}]} \qquad \textbf{[15-1]}$$

$R$ (the gas constant) has a value of 8.3145 J · $K^{-1}$ · $mol^{-1}$, $T$ is the temperature in Kelvin, $n$ is the number of electrons transferred (one or two in most of the reactions we will encounter), and $\mathcal{F}$ is the **Faraday constant** (96,485 J · $V^{-1}$ · $mol^{-1}$; it is equivalent to the electrical charge of one mole of electrons). At 25°C (298 K), the Nernst equation reduces to

$$\mathcal{E} = \mathcal{E}^{\circ\prime} - \frac{0.026\ \text{V}}{n} \ln \frac{[A_{reduced}]}{[A_{oxidized}]} \qquad \textbf{[15-2]}$$

In fact, for many substances in biological systems, the concentrations of the oxidized and reduced species are similar, so the logarithmic term is small (recall that ln 1 = 0) and $\mathcal{E}$ is close to $\mathcal{E}^{\circ\prime}$ (Sample Calculation 15-1).

**SAMPLE CALCULATION 15-1**

**PROBLEM**

Calculate the reduction potential of fumarate ($\mathcal{E}^{\circ\prime}$ = 0.031 V) at 25°C when [fumarate] = 40 μM and [succinate] = 200 μM.

**SOLUTION**

Use Equation 15-2. Fumarate is the oxidized compound and succinate is the reduced compound.

$$\begin{aligned}\mathcal{E} &= \mathcal{E}^{\circ\prime} - \frac{0.026\ \text{V}}{n} \ln \frac{[A_{reduced}]}{[A_{oxidized}]} \\ &= 0.031\ \text{V} - \frac{0.026\ \text{V}}{2} \ln \frac{(2 \times 10^{-4})}{(4 \times 10^{-5})} \\ &= 0.031\ \text{V} - 0.021\ \text{V} = 0.010\ \text{V}\end{aligned}$$

**PRACTICE PROBLEMS**

1. Calculate the reduction potential of fumarate at 37°C when [fumarate] = 80 μM and [succinate] = 100 μM.
2. Calculate the standard reduction potential of substance A when $\mathcal{E}$ = 0.5 V at 25°C, $[A_{reduced}]$ = 5 μM, and $[A_{oxidized}]$ = 200 μM. Assume that $n$ = 2.

## The free energy change can be calculated from the change in reduction potential

Knowing the reduction potentials of different substances is useful for predicting the movement of electrons between the two substances. When the substances are together in solution or connected by wire in an electrical circuit, *electrons flow spontaneously from the substance with the lower reduction potential to the substance with the higher reduction potential.* For example, in a system containing $Q/QH_2$ and $NAD^+$/NADH, we can predict whether electrons will flow from $QH_2$ to $NAD^+$ or from NADH to Q. Using the standard reduction potentials given in Table 15-1, we note that $\mathcal{E}^{\circ\prime}$ for $NAD^+$ (−0.315 V) is lower than $\mathcal{E}^{\circ\prime}$ for ubiquinone (0.045 V). Therefore, NADH will tend to transfer its electrons to ubiquinone; that is, NADH will be oxidized and Q will be reduced.

A complete oxidation–reduction reaction is just a combination of two half-reactions. For the NADH–ubiquinone reaction, the net reaction is the ubiquinone reduction half-reaction (the half-reaction as listed in Table 15-1) combined with the NADH oxidation half-reaction (the reverse of the half-reaction listed in Table 15-1).

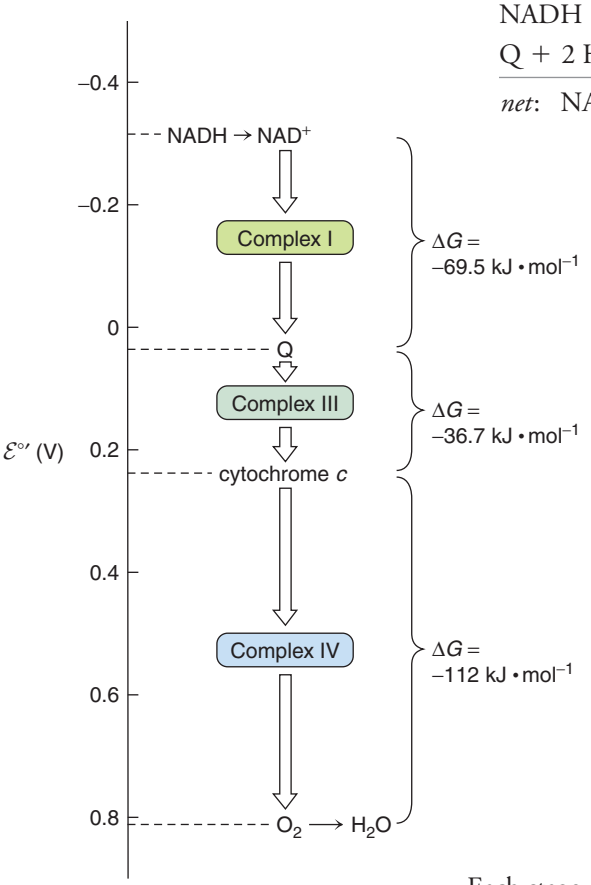



**Figure 15-2 Overview of mitochondrial electron transport.** The reduction potentials of the key electron carriers are indicated. The oxidation–reduction reactions mediated by Complexes I, III, and IV release free energy.

**? Determine the total free energy change for the oxidation of NADH by $O_2$.**

Note that because the $NAD^+$ half-reaction has been reversed to indicate oxidation, we have also reversed the sign of its $\mathcal{E}^{\circ\prime}$ value:

$$\text{NADH} \rightleftharpoons \text{NAD}^+ + \text{H}^+ + 2\,e^- \qquad \mathcal{E}^{\circ\prime} = +0.315\ \text{V}$$
$$\text{Q} + 2\,\text{H}^+ + 2\,e^- \rightleftharpoons \text{QH}_2 \qquad \mathcal{E}^{\circ\prime} = 0.045\ \text{V}$$
$$\textit{net:}\ \text{NADH} + \text{Q} + \text{H}^+ \rightleftharpoons \text{NAD}^+ + \text{QH}_2 \qquad \Delta\mathcal{E}^{\circ\prime} = +0.360\ \text{V}$$

When the two half-reactions are added, their reduction potentials are also added, yielding a $\Delta\mathcal{E}^{\circ\prime}$ value. Keep in mind that the reduction potential is a property of the half-reaction and is independent of the direction in which the reaction occurs. Reversing the sign of $\mathcal{E}^{\circ\prime}$, as shown above, is just a shortcut to simplify the task of calculating $\Delta\mathcal{E}^{\circ\prime}$. Another method for calculating $\Delta\mathcal{E}^{\circ\prime}$ uses the following equation:

$$\Delta\mathcal{E}^{\circ\prime} = \mathcal{E}^{\circ\prime}_{(e^- \text{ acceptor})} - \mathcal{E}^{\circ\prime}_{(e^- \text{ donor})} \qquad \textbf{[15-3]}$$

Not surprisingly, *the larger the difference in $\mathcal{E}$ values (the greater the $\Delta\mathcal{E}$ value), the greater the tendency of electrons to flow from one substance to the other, and the greater the change in free energy of the system.* $\Delta G$ is related to $\Delta\mathcal{E}$ as follows:

$$\Delta G^{\circ\prime} = -n\mathcal{F}\mathcal{E}^{\circ\prime} \text{ or } \Delta G = -n\mathcal{F}\Delta\mathcal{E} \qquad \textbf{[15-4]}$$

Accordingly, an oxidation–reduction reaction with a large positive $\Delta\mathcal{E}$ value has a large negative value of $\Delta G$ (see Sample Calculation 15-2). Depending on the relevant reduction potentials, an oxidation–reduction reaction can release considerable amounts of free energy. This is what happens in the mitochondria, where the reduced cofactors generated by the oxidation of metabolic fuels are reoxidized. The free energy released in this process powers ATP synthesis by oxidative phosphorylation. Figure 15-2 shows the major mitochondrial electron transport components arranged by their reduction potentials. Each stage of electron transfer, from NADH to $O_2$, the final electron acceptor, occurs with a negative change in free energy.

**CONCEPT REVIEW**

- Explain why an oxidation-reduction reaction must include both an oxidant and a reductant.
- When two reactants are mixed together, how can you predict which one will become reduced and which one will become oxidized?
- Explain how adding the $\mathcal{E}^{\circ\prime}$ values for two half-reactions yields a value of $\Delta\mathcal{E}^{\circ\prime}$ and $\Delta G^{\circ\prime}$ for an oxidation-reduction reaction.

## SAMPLE CALCULATION 15-2

**PROBLEM** Calculate the standard free energy change for the oxidation of malate by $NAD^+$. Is the reaction spontaneous under standard conditions?

**SOLUTION**

***Method 1***

Write the relevant half-reactions, reversing the malate half-reaction (so that it becomes an oxidation reaction) and reversing the sign of its $\mathcal{E}^{\circ\prime}$:

$$\text{malate} \longrightarrow \text{oxaloacetate} + 2\,\text{H}^+ + e^- \qquad \mathcal{E}^{\circ\prime} = +0.166\ \text{V}$$
$$\text{NAD}^+ + \text{H}^+ + 2e^- \longrightarrow \text{NADH} \qquad \mathcal{E}^{\circ\prime} = -0.315\ \text{V}$$
$$\textit{net:}\ \text{malate} + \text{NAD}^+ \longrightarrow \text{oxaloacetate} + \text{NADH} + \text{H}^+ \qquad \Delta\mathcal{E}^{\circ\prime} = -0.149\ \text{V}$$

***Method 2***

Identify the electron acceptor ($NAD^+$) and electron donor (malate). Substitute their standard reduction potentials into Equation 15-3:

$$\begin{aligned}\Delta\mathcal{E}^{\circ\prime} &= \mathcal{E}^{\circ\prime}_{(e^- \text{ acceptor})} - \mathcal{E}^{\circ\prime}_{(e^- \text{ donor})} \\ &= -0.315\ \text{V} - (-0.166\ \text{V}) \\ &= -0.149\ \text{V}\end{aligned}$$

***Both Methods***

The $\Delta\mathcal{E}^{\circ\prime}$ for the net reaction is $-0.149$ V. Use Equation 15-4 to calculate $\Delta G^{\circ\prime}$:

$$\begin{aligned}\Delta G^{\circ\prime} &= -n\mathcal{F}\Delta\mathcal{E}^{\circ\prime} \\ &= -(2)(96{,}485\ \text{J}\cdot\text{V}^{-1}\cdot\text{mol}^{-1})(-0.149\ \text{V}) \\ &= +28{,}750\ \text{J}\cdot\text{mol}^{-1} = +28.8\ \text{kJ}\cdot\text{mol}^{-1}\end{aligned}$$

The reaction has a positive value of $\Delta G^{\circ\prime}$ and so is not spontaneous. (*In vivo*, this endergonic reaction occurs as step 8 of the citric acid cycle and is coupled to step 1, which is exergonic.)

**PRACTICE PROBLEMS**

3. Calculate the standard free energy change for the oxidation of malate by ubiquinone. Is the reaction spontaneous under standard conditions?
4. In yeast, alcohol dehydrogenase reduces acetaldehyde to ethanol (Section 13-1). Calculate the free energy change for this reaction under standard conditions.
5. In cells, cytochrome *c* oxidizes cytochrome $c_1$. Calculate the change in free energy for the reverse reaction.

# 15-2 Mitochondrial Electron Transport

In aerobic organisms, the NADH and ubiquinol produced by glycolysis, the citric acid cycle, fatty acid oxidation, and other metabolic pathways is ultimately reoxidized by molecular oxygen, a process called **respiration.** The standard reduction potential of +0.815 V for the reduction of $O_2$ to $H_2O$ indicates that $O_2$ is a more effective oxidizing agent than any other biological compound (see Table 15-1). The oxidation of NADH by $O_2$, that is, the transfer of electrons from NADH directly to $O_2$, would release a large amount of free energy, but this reaction does not occur in a single step. Instead, *electrons are shuttled from NADH to $O_2$ in a multistep process that offers several opportunities to conserve the free energy of oxidation.* In eukaryotes, all the steps of oxidative phosphorylation are carried out by a series of membrane-bound protein complexes in the mitochondria (in prokaryotes, plasma membrane proteins perform similar functions). The following sections describe how electrons flow through this "respiratory chain" from reduced cofactors to oxygen.

**KEY CONCEPTS**

- The inner mitochondrial membrane encloses the matrix and includes specific transport proteins.
- Complex I transfers electrons from NADH to ubiquinone.
- The citric acid cycle, fatty acid oxidation, and other processes also generate mitochondrial ubiquinol.
- The Q cycle mediated by Complex III reduces cytochrome *c*.
- Complex IV uses electrons from cytochrome *c* to reduce $O_2$ to $H_2O$.

## Mitochondrial membranes define two compartments

In accordance with its origin as a bacterial symbiont, the **mitochondrion** (plural, *mitochondria*) has two membranes. The outer membrane, analogous to the outer membrane of some bacteria, is relatively porous due to the presence of porin-like proteins that permit the transmembrane diffusion of substances with masses up to about 10 kD (see Section 9-2 for an example of porin structure and function). The inner membrane has a convoluted architecture that encloses a space called the **mitochondrial matrix.** Because the inner mitochondrial membrane prevents the transmembrane movements of ions and small molecules (except via specific transport proteins), the composition of the matrix differs from that of the space between the inner and outer membranes. In fact, the ionic composition of the **intermembrane space** is considered to be equivalent to that of the cytosol due to the presence of the porins in the outer mitochondrial membrane (Fig. 15-3).

Mitochondria are customarily shown as kidney-shaped organelles with the inner mitochondrial membrane forming a system of baffles called **cristae** (Fig. 15-4a).

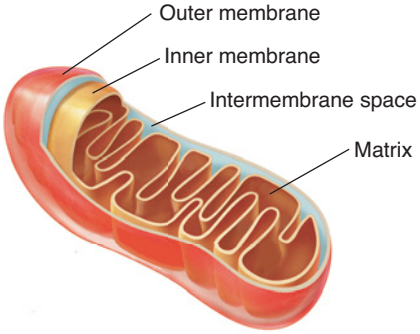
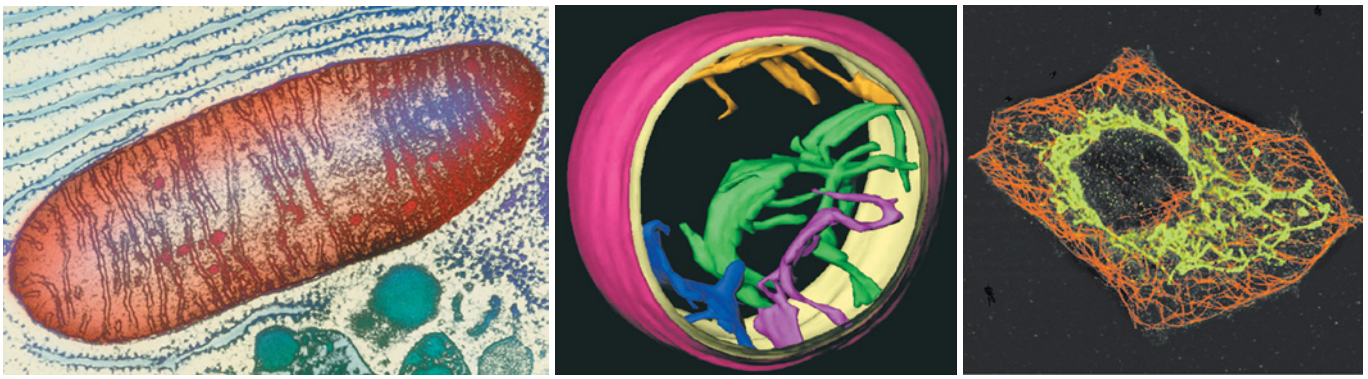



**Figure 15-3 Model of mitochondrial structure.** The relatively impermeable inner mitochondrial membrane encloses the protein-rich matrix. The intermembrane space has an ionic composition similar to that of the cytosol because the outer mitochondrial membrane is permeable to substances with masses of less than about 10 kD.



(a) (b) (c)

**Figure 15-4 Images of mitochondria.** (a) Conventional electron micrograph showing cristae as a system of planar baffles. [K. Porter/Photo Researchers.] (b) Three-dimensional reconstruction of a mitochondrion by electron tomography, showing irregular tubular cristae. [Courtesy Carmen Mannella, Wadsworth Center, Albany, New York.] (c) Electron micrograph of a mammalian fibroblast, showing a network of tubular mitochondria (labeled with a green fluorescent dye). The remainder of the cytosol is delineated by microtubules (labeled with a red fluorescent dye). [Courtesy Michael P. Yaffe. From *Science* 283, 1493–1497 (1991).]

**See Guided Exploration. Electron transport and oxidative phosphorylation overview.**

However, **electron tomography,** a technique for visualizing cellular structures in three dimensions by analyzing micrographs of sequential cell slices, reveals that mitochondria are highly variable structures. For example, the cristae may be irregular and bulbous rather than planar and may make several tubular connections with the rest of the inner mitochondrial membrane (Fig. 15-4b). Moreover, a cell may contain hundreds to thousands of discrete bacteria-shaped mitochondria, or a single tubular organelle may take the form of an extended network with many branches and interconnections (Fig. 15-4c). Individual mitochondria can move around the cell and undergo fusion (joining) and fission (separating).

Reflecting its ancient origin as a free-living organism, the mitochondrion has its own genome and protein-synthesizing machinery consisting of mitochondrially encoded rRNA and tRNA. The mitochondrial genome encodes 13 proteins, all of which are components of the respiratory chain complexes. This is only a small subset of the approximately 1500 proteins required for mitochondrial function; the other respiratory chain proteins, matrix enzymes, transporters, and so on are encoded by the cell's nuclear genome, synthesized in the cytosol, and imported into the mitochondria (across one or both membranes) by special mechanisms.

Much of the cell's NADH and $QH_2$ is generated by the citric acid cycle in the mitochondrial matrix. Fatty acid oxidation also takes place largely in the matrix and yields NADH and $QH_2$. These reduced cofactors transfer their electrons to the protein complexes of the respiratory electron transport chain, which are tightly associated with the inner mitochondrial membrane. However, NADH produced by glycolysis and other oxidative processes in the cytosol cannot directly reach the respiratory chain. There is no transport protein that can ferry NADH across the inner mitochondrial membrane. Instead, "reducing equivalents" are imported into the matrix by the chemical reactions of systems such as the malate–aspartate shuttle system **(Fig. 15-5)**.

Mitochondria also need a mechanism to export ATP and to import ADP and $P_i$, since most of the cell's ATP is generated in the matrix by oxidative phosphorylation and is consumed in the cytosol. A transport protein called the adenine nucleotide translocase exports ATP and imports ADP, binding one or the other and changing its conformation to release the bound nucleotide on the other side of the membrane **(Fig. 15-6a)**. Inorganic phosphate, a substrate for oxidative phosphorylation, is imported from the cytosol in symport with $H^+$ (Fig. 15-6b).

The protein complexes that carry out electron transport and ATP synthesis are oriented in the inner mitochondrial membrane so that they can bind the NADH, ADP, and $P_i$ present in the matrix. Electron microscopy studies strongly suggest that

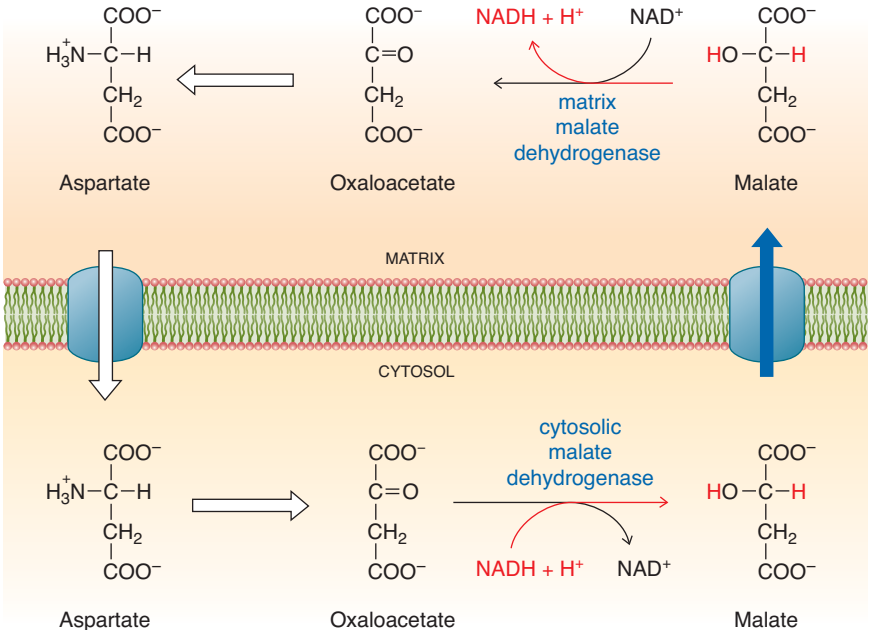



**Figure 15-5 The malate-aspartate shuttle system.** Cytosolic oxaloacetate is reduced to malate for transport into mitochondria. Malate is then reoxidized in the matrix. The net result is the transfer of "reducing equivalents" from the cytosol to the matrix. Mitochondrial oxaloacetate can be exported back to the cytosol after being converted to aspartate by an aminotransferase.

the complexes associate with each other, which would increase the efficiency of electron transfer between them.

## Complex I transfers electrons from NADH to ubiquinone

The path electrons travel through the respiratory chain begins with Complex I, also called NADH:ubiquinone oxidoreductase or NADH dehydrogenase. This enzyme catalyzes the transfer of a pair of electrons from NADH to ubiquinone:

$$NADH + H^+ + Q \rightleftharpoons NAD^+ + QH_2$$

Complex I is the largest of the electron transport proteins in the mitochondrial respiratory chain, with 45 different subunits and a total mass of about 980 kD in mammals. The crystal structure of the smaller (550-kD) bacterial Complex I reveals an L-shaped protein with numerous transmembrane helices and a peripheral arm (Fig. 15-7). Electron transport takes place in the peripheral arm, which includes several prosthetic groups that undergo reduction as they receive electrons and become oxidized as they give up their electrons to the next group. All these groups, or **redox centers,** appear to have reduction potentials approximately between the reduction potentials of $NAD^+$ ($\mathcal{E}^{\circ\prime} = -0.315$ V) and ubiquinone ($\mathcal{E}^{\circ\prime} = +0.045$ V). This allows them to form a chain where the electrons travel a path of increasing reduction potential. The redox centers do not need to be in intimate contact with each

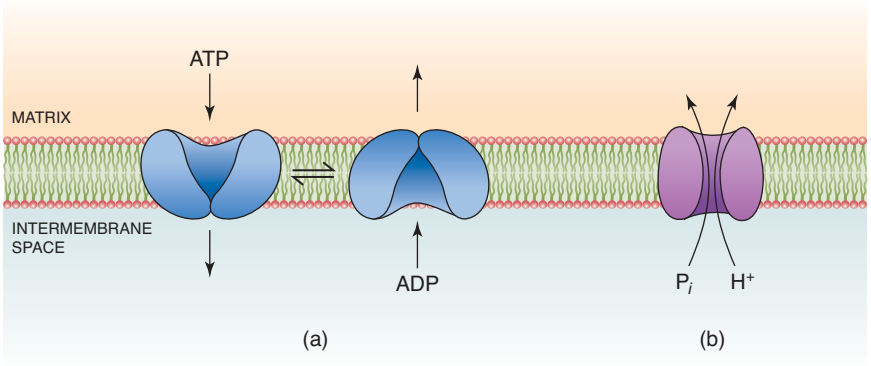



**Figure 15-6 Mitochondrial transport systems.** (a) The adenine nucleotide translocase binds either ATP or ADP and changes its conformation to release the nucleotide on the opposite side of the inner mitochondrial membrane. This transporter can therefore export ATP and import ADP. (b) A $P_i$–$H^+$ symport protein permits the simultaneous movement of inorganic phosphate and a proton into the mitochondrial matrix.

**? Does the activity of either of these transporters contribute to the mitochondrial membrane potential?**

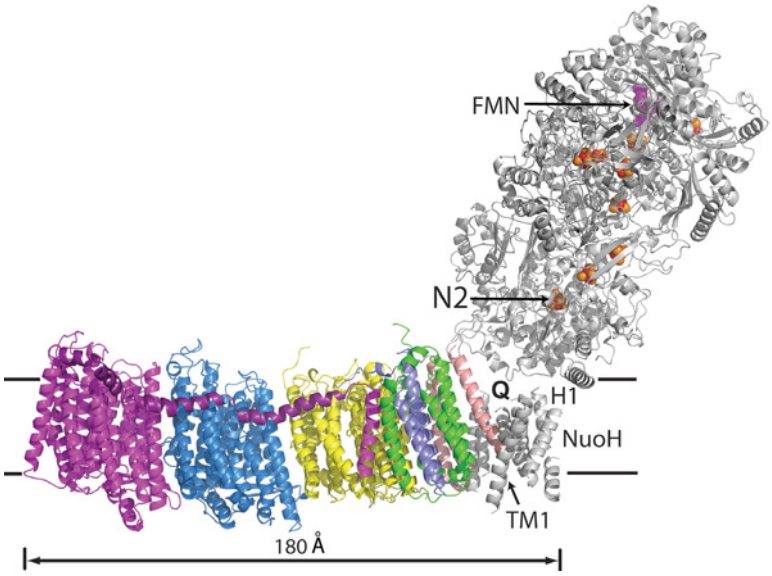



**Figure 15-7 Structure of bacterial Complex I.** This image is a composite of the membrane arm from *E. coli* (colored portion) and the peripheral arm from *Thermus thermophilus* (gray portion). Redox centers are shown in space-filling form, including FMN and nine iron–sulfur clusters. The ubiquinone-binding site is marked by a Q. Horizontal lines represent the boundaries of the lipid bilayer. The cytosol (equivalent to the mitochondrial matrix) is at the top. [Courtesy of Leonid Sazanov, Medical Research Council, Cambridge, U.K.]

other, as they would be if the transferred group were a larger chemical entity. An electron can move between redox centers up to 14 Å apart by "tunneling" through the covalent bonds of the protein.

The two electrons donated by NADH are first picked up by flavin mononucleotide (FMN; Fig. 15-8). This noncovalently bound prosthetic group, which is similar to FAD, then transfers the electrons, one at a time, to a second type of redox center, an iron–sulfur (Fe–S) cluster. Complex I bears nine of these prosthetic groups, which contain equal numbers of iron and sulfide ions (Fig. 15-9). Unlike the electron carriers we have introduced so far, Fe–S clusters are one-electron carriers. They have an oxidation state of either +3 (oxidized) or +2 (reduced), regardless of the number of Fe atoms in the cluster (each cluster is a conjugated structure that functions as a single unit). Electrons travel between several Fe–S clusters before reaching ubiquinone. Like FMN, ubiquinone is a two-electron carrier (see Section 12-2), but it accepts one electron at a time from an Fe–S donor. Iron–sulfur clusters may be among the most ancient of electron carriers, dating from a time when the earth's abundant iron and sulfur were major players in prebiotic chemical reactions.

*As electrons are transferred from NADH to ubiquinone, Complex I transfers four protons from the matrix to the intermembrane space.* Comparisons with other transport proteins and detailed analysis of the crystal structure indicate the presence of

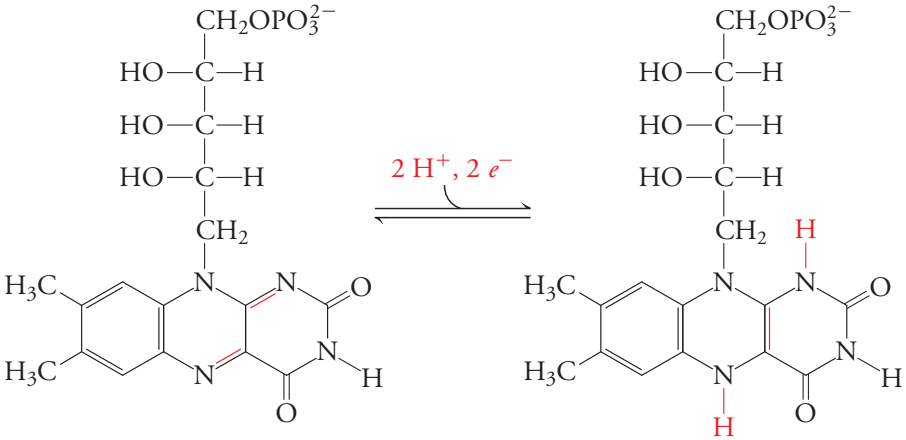



**Figure 15-8 Flavin mononucleotide (FMN).** This prosthetic group resembles flavin adenine dinucleotide (FAD; see Fig. 3-3c) but lacks the AMP group of FAD. The transfer of two electrons and two protons to FMN yields $FMNH_2$.

four proton-translocating "channels" in the membrane-embedded arm of Complex I. When redox groups in the peripheral arm are transiently reduced and reoxidized, the protein undergoes conformational changes that are transmitted from the peripheral arm to the membrane arm in part by a horizontally oriented helix that lies within the membrane portion of the complex (see Fig. 15-7). These conformational changes do not open passageways, as occurs in $Na^+$ and $K^+$ transporters (Section 9-2). Instead, each proton passes from one side of the membrane to the other via a **proton wire,** a series of hydrogen-bonded protein groups plus water molecules that form a chain through which a proton can be rapidly relayed. (Recall from Fig. 2-14 that protons readily jump between water molecules.) Note that in this relay mechanism, the protons taken up from the matrix are not the same ones that are released into the intermembrane space. The reactions of Complex I are summarized in **Figure 15-10**.

## Other oxidation reactions contribute to the ubiquinol pool

The reduced quinone product of the Complex I reaction joins a pool of quinones that are soluble in the inner mitochondrial membrane by virtue of their long hydrophobic isoprenoid tails (see Section 12-2). *The pool of reduced quinones is augmented by the activity of other oxidation–reduction reactions.* One of these is catalyzed by succinate dehydrogenase, which carries out step 6 of the citric acid cycle (see Section 14-2).

$$\text{succinate} + \text{Q} \rightleftharpoons \text{fumarate} + \text{QH}_2$$

Succinate dehydrogenase is the only one of the citric acid cycle enzymes that is not soluble in the mitochondrial matrix; it is embedded in the inner membrane. Like the other respiratory complexes, it contains several redox centers, including an FAD group. Succinate dehydrogenase is also called Complex II of the mitochondrial respiratory chain. However, it is more like a tributary because it does not undertake proton translocation and therefore does not directly contribute the free energy of its oxidation–reduction reaction toward ATP synthesis. Nevertheless, it does feed reducing equivalents as ubiquinol into the electron transport chain **(Fig. 15-11a)**.

A major source of ubiquinol is fatty acid oxidation, another energy-generating catabolic pathway that takes place in the mitochondrial matrix. A membrane-bound fatty acyl-CoA dehydrogenase catalyzes the oxidation of a C—C bond in a fatty acid attached to coenzyme A. The electrons removed in this dehydrogenation reaction are transferred to ubiquinone (Fig. 15-11b). As we will see in Section 17-1, the complete oxidation of a fatty acid also produces NADH that is reoxidized by the mitochondrial electron transport chain, starting with Complex I.

Electrons from cytosolic NADH can also enter the mitochondrial ubiquinol pool through the actions of a cytosolic and a mitochondrial glycerol-3-phosphate dehydrogenase (Fig. 15-11c). This system, which shuttles electrons from NADH to ubiquinol, bypasses Complex I.

## Complex III transfers electrons from ubiquinol to cytochrome *c*

Ubiquinol is reoxidized by Complex III, an integral membrane protein with 11 subunits in each of its two monomeric units. Complex III, also called ubiquinol:cytochrome *c* oxidoreductase or cytochrome $bc_1$, transfers electrons to the peripheral membrane protein cytochrome *c*. **Cytochromes** are proteins with heme prosthetic groups. The name *cytochrome* literally means "cell color"; cytochromes are largely responsible for the purplish-brown color of mitochondria. Cytochromes are commonly named with a letter (*a*, *b*, or *c*) indicating the exact

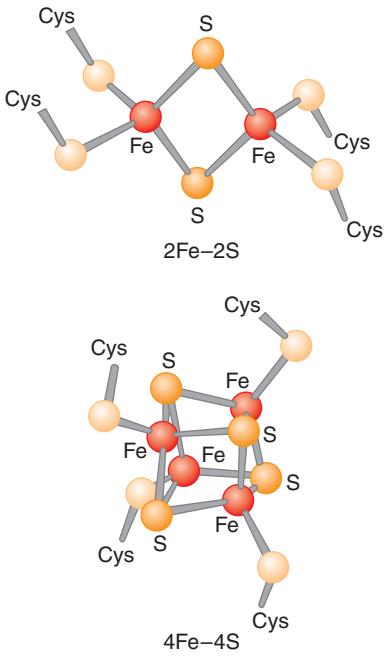



**Figure 15-9 Iron–sulfur clusters.** Although some Fe–S clusters contain up to eight Fe atoms, the most common are the 2Fe–2S and 4Fe–4S clusters. In all cases, the iron–sulfur clusters are coordinated by the S atoms of Cys side chains. These prosthetic groups undergo one-electron redox reactions.

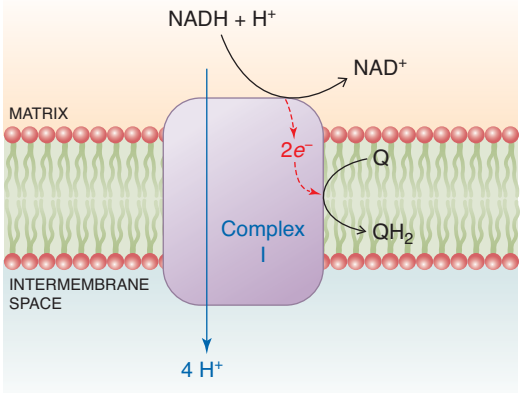



**Figure 15-10 Complex I function.** As two electrons from the water-soluble NADH are transferred to the lipid-soluble ubiquinone, four protons are translocated from the matrix into the intermembrane space.

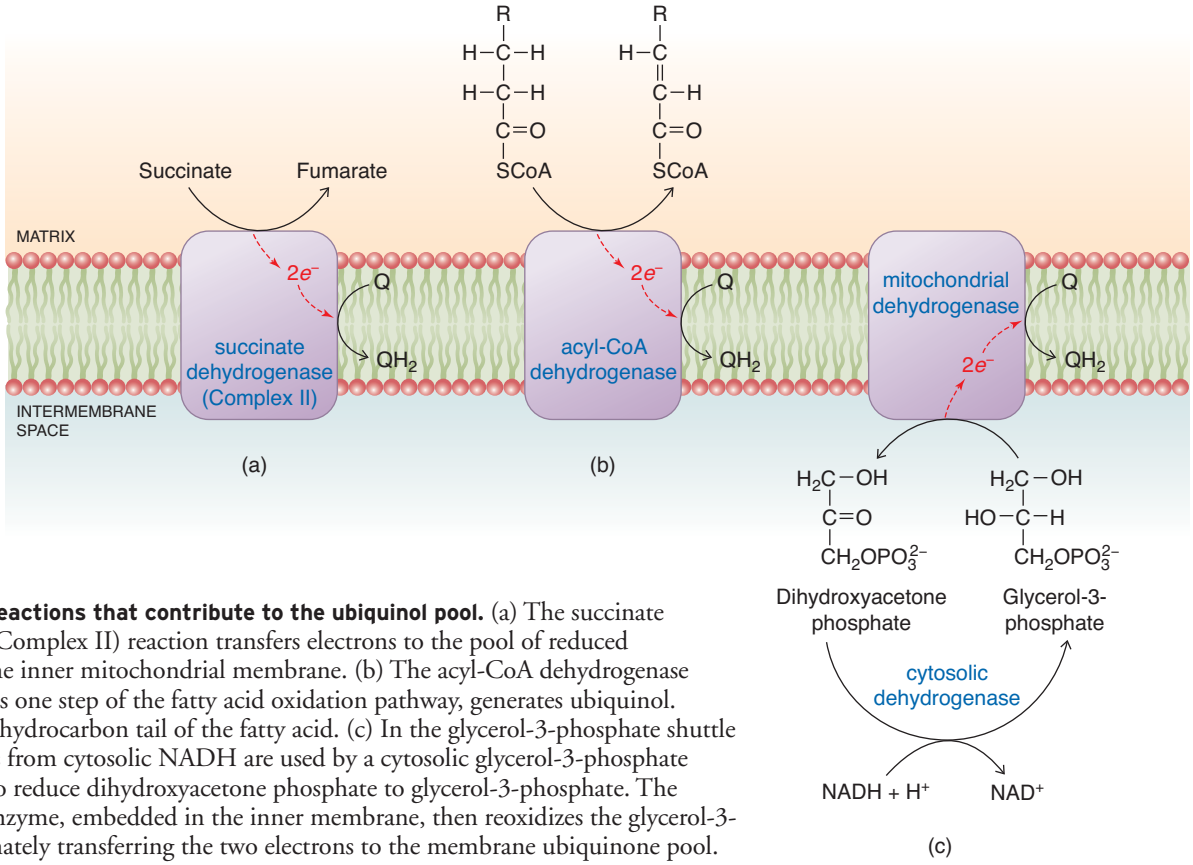



**Figure 15-11 Reactions that contribute to the ubiquinol pool.** (a) The succinate dehydrogenase (Complex II) reaction transfers electrons to the pool of reduced ubiquinone in the inner mitochondrial membrane. (b) The acyl-CoA dehydrogenase reaction, which is one step of the fatty acid oxidation pathway, generates ubiquinol. R represents the hydrocarbon tail of the fatty acid. (c) In the glycerol-3-phosphate shuttle system, electrons from cytosolic NADH are used by a cytosolic glycerol-3-phosphate dehydrogenase to reduce dihydroxyacetone phosphate to glycerol-3-phosphate. The mitochondrial enzyme, embedded in the inner membrane, then reoxidizes the glycerol-3-phosphate, ultimately transferring the two electrons to the membrane ubiquinone pool.

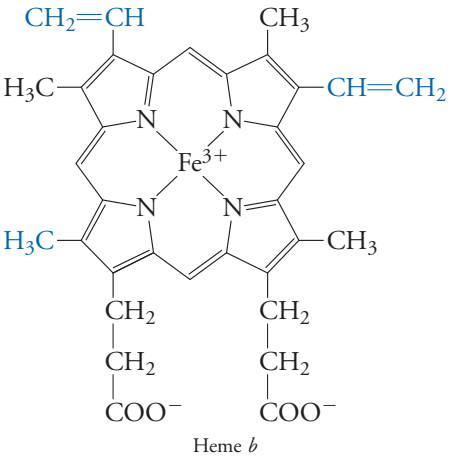



**Figure 15-12 The heme group of a *b* cytochrome.** The planar porphyrin ring surrounds a central Fe atom, shown here in its oxidized ($Fe^{3+}$) state. The heme substituent groups that are colored blue differ in the *a* and *c* cytochromes (the heme group of hemoglobin and myoglobin has the *b* structure; see Section 5-1).

structure of the porphyrin ring of their heme group (Fig. 15-12). The structure of the heme group and the surrounding protein microenvironment influence the protein's absorption spectrum. They also determine the reduction potentials of cytochromes, which range from about −0.080 V to about +0.385 V.

Unlike the heme prosthetic groups of hemoglobin and myoglobin, the heme groups of cytochromes undergo reversible one-electron reduction, with the central Fe atom cycling between the $Fe^{3+}$ (oxidized) and $Fe^{2+}$ (reduced) states. Consequently, the net reaction for Complex III, in which two electrons are transferred, includes two cytochrome *c* proteins:

$$QH_2 + 2 \text{ cytochrome } c\ (Fe^{3+}) \rightleftharpoons Q + 2 \text{ cytochrome } c\ (Fe^{2+}) + 2\ H^+$$

Complex III itself contains two cytochromes (cytochrome *b* and cytochrome $c_1$) that are integral membrane proteins. These two proteins, along with an iron–sulfur protein (also called the Rieske protein), form the functional core of Complex III (these same three subunits are the only ones that have homologs in the corresponding bacterial respiratory complex). Altogether, each monomer of Complex III is anchored in the membrane by 14 transmembrane α helices (Fig. 15-13).

The flow of electrons through Complex III is complicated, in part because the two electrons donated by ubiquinol must split up in order to travel through a series of one-electron carriers that includes the 2Fe–2S cluster of the iron–sulfur protein, cytochrome $c_1$, and cytochrome *b* (which actually contains two heme groups with slightly different reduction potentials). Except for the 2Fe–2S cluster, all the redox centers are arranged in such a way that electrons can tunnel from one to another. The iron–sulfur protein must change its conformation by rotating and moving about 22 Å in order to pick up and deliver an electron. Further complicating the picture is the fact that each monomeric unit of Complex III has two active sites where quinone cofactors undergo reduction and oxidation.

The circuitous route of electrons from ubiquinol to cytochrome *c* is described by the two-round **Q cycle,** diagrammed in Figure 15-14. *The net result of the Q cycle is*

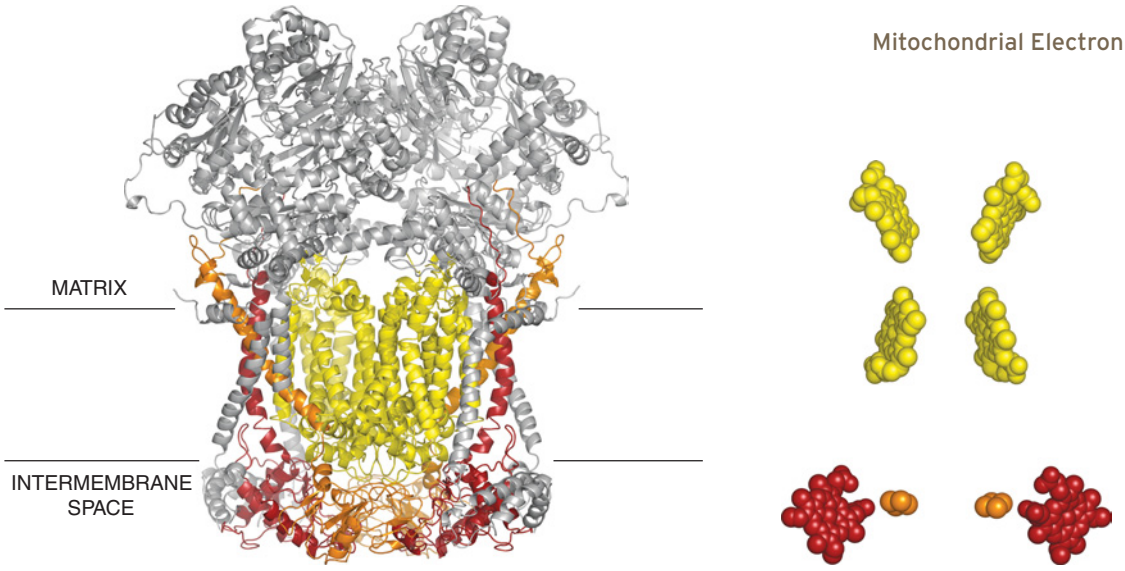
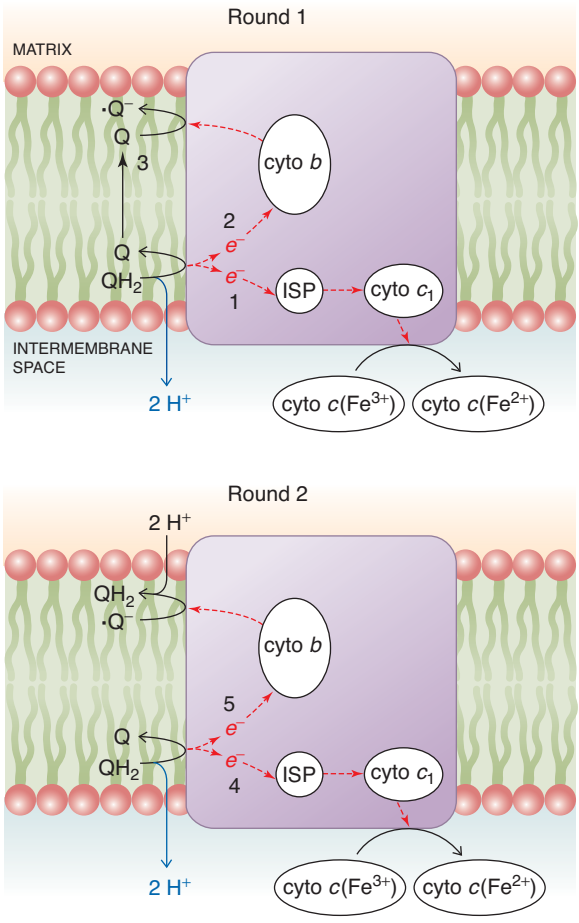



**Figure 15-13 Structure of mammalian Complex III.** (a) Backbone model. Eight transmembrane helices in each monomer of the dimeric complex are contributed by cytochrome *b* (yellow). The iron–sulfur protein (orange) and cytochrome $c_1$ (red) project into the intermembrane space. The approximate position of the membrane is indicated. (b) Arrangement of prosthetic groups. The two heme groups of each cytochrome *b* (yellow) and the heme group of cytochrome $c_1$ (red), along with the iron–sulfur clusters (orange), provide a pathway for electrons between ubiquinol (in the membrane) and cytochrome *c* (in the intermembrane space). [Structure (pdb 1BE3) determined by S. Iwata, J. W. Lee, K. Okada, J. K. Lee, M. Iwata, S. Ramaswamy, and B. K. Jap.] **See Interactive Exercise. Complex III.**




1. *In the first round, $QH_2$ donates one electron to the iron–sulfur protein (ISP). The electron then travels to cytochrome $c_1$ and then to cytochrome c.*
2. *$QH_2$ donates its other electron to cytochrome b. The two protons from $QH_2$ are released into the intermembrane space.*
3. *The oxidized ubiquinone diffuses to another quinone-binding site, where it accepts the electron from cytochrome b, becoming a half-reduced semiquinone ($\cdot Q^-$).*
4. *In the second round, a second $QH_2$ surrenders its two electrons to Complex III and its two protons to the intermembrane space. One electron goes to reduce cytochrome c.*
5. *The other electron goes to cytochrome b and then to the waiting semiquinone produced in the first part of the cycle. This step regenerates $QH_2$, using protons from the matrix.*

**Figure 15-14 The Q cycle.**

**? Write an equation for round 1 and for round 2.**

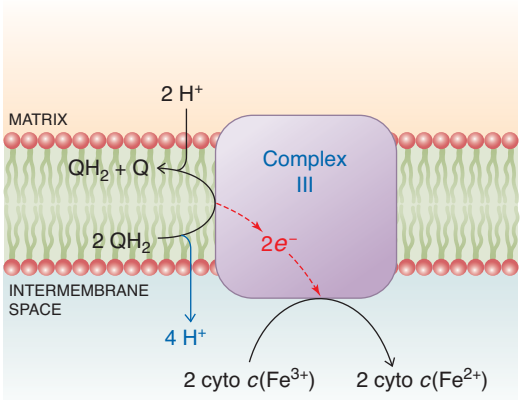



**Figure 15-15 Complex III function.** For every two electrons that pass from ubiquinol to cytochrome *c*, four protons are translocated to the intermembrane space.

**? How does the proton-translocating mechanism of Complex III differ from the one in Complex I?**

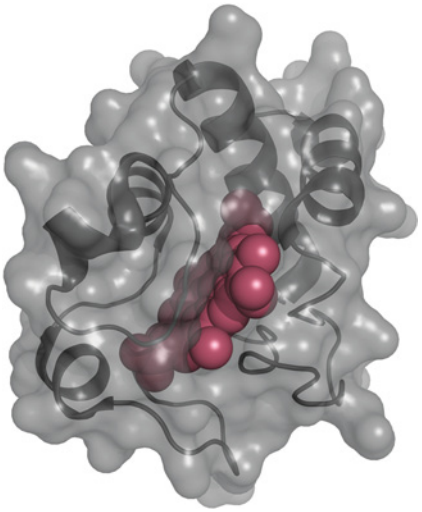


**Figure 15-16 Cytochrome *c*.** The protein is shown as a gray transparent surface over its ribbon backbone. The heme group (pink) lies in a deep pocket. Cytochrome *c* transfers one electron at a time from Complex III to Complex IV. [Structure of tuna cytochrome *c* (pdb 5CYT) determined by T. Takano.]

*that two electrons from $QH_2$ reduce two molecules of cytochrome c. In addition, four protons are translocated to the intermembrane space, two from $QH_2$ in the first round of the Q cycle and two from $QH_2$ in the second round.* This proton movement contributes to the transmembrane proton gradient. The reactions of Complex III are summarized in Figure 15-15.

## Complex IV oxidizes cytochrome c and reduces $O_2$

Just as ubiquinone ferries electrons from Complex I and other enzymes to Complex III, cytochrome *c* ferries electrons between Complexes III and IV. Unlike ubiquinone and the other proteins of the respiratory chain, cytochrome *c* is soluble in the intermembrane space (Fig. 15-16). Because this small peripheral membrane protein is central to the metabolism of many organisms, analysis of its sequence played a large role in elucidating evolutionary relationships.

Complex IV, also called cytochrome *c* oxidase, is the last enzyme to deal with the electrons derived from the oxidation of metabolic fuels. Four electrons delivered by cytochrome *c* are consumed in the reduction of molecular oxygen to water:

$$4 \text{ cytochrome } c\,(Fe^{2+}) + O_2 + 4\,H^+ \rightarrow 4 \text{ cytochrome } c\,(Fe^{3+}) + 2\,H_2O$$

The redox centers of mammalian Complex IV include heme groups and copper ions situated among the 13 subunits in each half of the dimeric complex (Fig. 15-17).

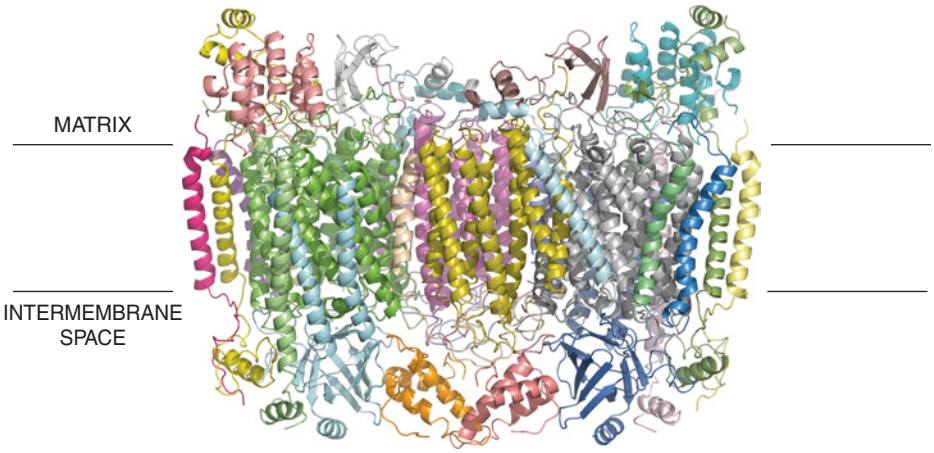



**Figure 15-17 Structure of cytochrome *c* oxidase.** The 13 subunits in each monomeric half of the mammalian complex comprise 28 transmembrane α helices. [Structure (pdb 2OCC) determined by T. Tsukihara and M. Yao.] **See Interactive Exercise. Cytochrome *c* oxidase.**

Each electron travels from cytochrome *c* to the $Cu_A$ redox center, which has two copper ions, and then to a heme *a* group. From there it travels to a binuclear center consisting of the iron atom of heme $a_3$ and a copper ion ($Cu_B$). The four-electron reduction of $O_2$ occurs at the Fe–Cu binuclear center. Note that the chemical reduction of $O_2$ to $H_2O$ consumes four protons from the mitochondrial matrix. One possible sequence of reaction intermediates is shown in **Figure 15-18**. The incomplete reduction of $O_2$ to $H_2O$ is believed to generate free radicals that can damage mitochondria (Box 15-A).

Cytochrome *c* oxidase also relays four additional protons from the matrix to the intermembrane space (two protons for every pair of electrons). The protein complex appears to harbor two proton wires. One delivers $H^+$ ions from the matrix to the oxygen-reducing active site. The other one spans the 50-Å distance between the matrix and intermembrane faces of the protein. Protons are relayed through the proton wires when the protein changes its conformation in response to changes in its oxidation

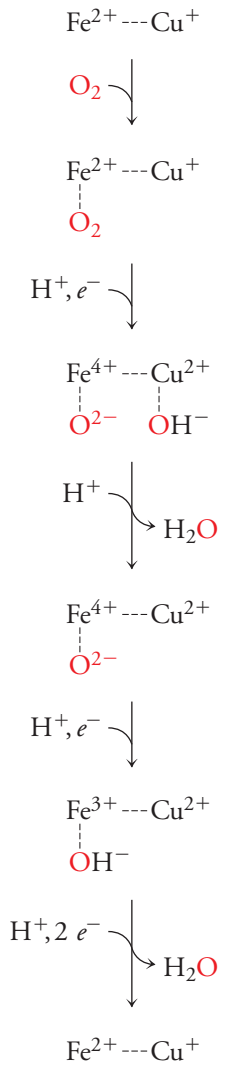



**Figure 15-18 A proposed model for the cytochrome *c* oxidase reaction.** Although the exact sequence of proton and electron transfers is not known, the reaction intermediates shown here are inferred from spectroscopic and other evidence. An enzyme tyrosine radical (not shown) plays a role in electron transfer.

**BOX 15-A BIOCHEMISTRY NOTE**

## Free Radicals and Aging

The oxidizing power of molecular oxygen allows aerobic metabolism—a far more efficient strategy than anaerobic metabolism—but comes at a cost. Partial reduction of $O_2$ by Complex IV, or possibly via side reactions carried out in Complexes I and III, can produce the superoxide free radical, $\cdot O_2^-$.

$$O_2 + e^- \rightarrow \cdot O_2^-$$

A **free radical** is an atom or molecule with a single unpaired electron and is highly reactive as it seeks another electron to form a pair. Such reactivity means that a free radical, although extremely short-lived (the half-life of $\cdot O_2^-$ is $1 \times 10^{-6}$ seconds), can chemically alter nearby molecules. Presumably, the most damage is felt by mitochondria, whose proteins, lipids, and DNA are all susceptible to oxidation as superoxide steals an electron.

As the damage accumulates, the mitochondria become less efficient and eventually nonfunctional, at which point the cell self-destructs. According to the free radical theory of aging, oxidative damage mediated by $\cdot O_2^-$ and other free radicals is responsible for the degeneration of tissues that occurs with aging. Oxidative damage has also been implicated in the pathogenesis of disorders such as Parkinson's disease and Alzheimer's disease (see Box 4-C).

Several lines of evidence support the link between free radicals and aging. First, the tissues of some individuals with progeria, a form of accelerated aging, appear to produce higher than normal levels of oxygen free radicals. Second, cells of all kinds are equipped with antioxidant mechanisms, suggesting that these components perform an essential function. For example, the enzyme superoxide dismutase converts superoxide to a less toxic product, peroxide:

$$2 \cdot O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2$$

Other cellular components, such as ascorbate (see Box 5-D) and α-tocopherol (see Box 8-B) may protect cells from oxidative damage by scavenging free radicals. Finally, animal experiments suggest that caloric restriction, which extends life spans, generates fewer free radicals by decreasing the availability of fuel molecules that undergo oxidative metabolism. Unfortunately, studies in humans have not yielded conclusive evidence that consuming particular antioxidants or decreasing fuel consumption diminishes the degeneration that normally accompanies aging.

**Question:** Free radicals have been identified as hormone-like signaling molecules in both animals and plants. How might this information alter the free radical theory of aging?

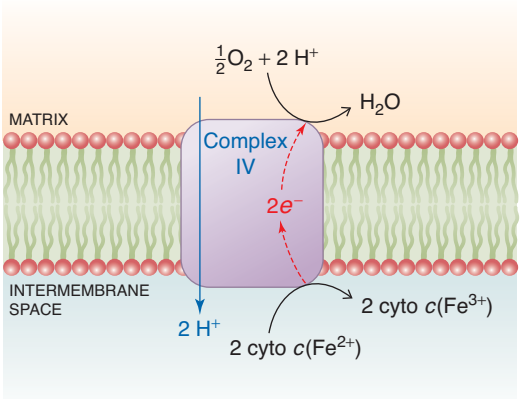



**Figure 15-19 Complex IV function.** For every two electrons donated by cytochrome *c*, two protons are translocated to the intermembrane space. Two protons from the matrix are also consumed in the reaction $\frac{1}{2}\,O_2 \rightarrow H_2O$ (the full reduction of $O_2$ requires four electrons).

state. *The production of water and the proton relays both deplete the matrix proton concentration and thereby contribute to the formation of a proton gradient across the inner mitochondrial membrane* (Fig. 15-19).

**CONCEPT REVIEW**

- Describe the compartments of a mitochondrion.
- What transport proteins occur in the inner mitochondrial membrane?
- List the different types of redox centers in the respiratory electron transport chain. Which are one-electron carriers and which are two-electron carriers?
- How are electrons delivered to Complexes I, III, and IV?
- Why is oxygen the final electron acceptor of the respiratory chain?
- What is the function of a proton wire?

# 15-3 Chemiosmosis

**KEY CONCEPTS**

- The formation of a transmembrane proton gradient during electron transport provides the free energy to synthesize ATP.
- Both concentration and charge contribute to the free energy of the proton gradient.

The electrons collected from metabolic fuels during their oxidation are now fully disposed of in the reduction of $O_2$ to $H_2O$. However, their free energy has been conserved. How much free energy is potentially available? Using the $\Delta G$ values calculated from the standard reduction potentials of the substrates and products of Complexes I, III, and IV (presented graphically in Fig. 15-2), we can see that each of the three respiratory complexes theoretically releases enough free energy to drive the endergonic phosphorylation of ADP to form ATP ($\Delta G^{\circ\prime} = +30.5\ \text{kJ} \cdot \text{mol}^{-1}$).

| | |
|---|---|
| Complex I: NADH $\rightarrow$ $QH_2$ | $\Delta G^{\circ\prime} = -69.5\ \text{kJ} \cdot \text{mol}^{-1}$ |
| Complex III: $QH_2$ $\rightarrow$ cytochrome *c* | $\Delta G^{\circ\prime} = -36.7\ \text{kJ} \cdot \text{mol}^{-1}$ |
| Complex IV: cytochrome *c* $\rightarrow$ $O_2$ | $\Delta G^{\circ\prime} = -112.0\ \text{kJ} \cdot \text{mol}^{-1}$ |
| NADH $\rightarrow$ $O_2$ | $\Delta G^{\circ\prime} = -218.2\ \text{kJ} \cdot \text{mol}^{-1}$ |

## Chemiosmosis links electron transport and oxidative phosphorylation

Until the 1960s, the connection between respiratory electron transport (measured as $O_2$ consumption) and ATP synthesis was a mystery. Credit for discovering the connection belongs primarily to Peter Mitchell, who was inspired by his work on mitochondrial phosphate transport and recognized the importance of compartmentation in biological systems. Mitchell's **chemiosmotic theory** proposed that the proton-translocating activity of the electron transport complexes in the inner mitochondrial membrane generates a proton gradient across the membrane. The protons cannot diffuse back into the matrix because the membrane is impermeable to ions. *The imbalance of protons represents a source of free energy, also called a* ***protonmotive force,*** *that can drive the activity of an ATP synthase.*

We now know that for each pair of electrons that flow through Complexes I, III, and IV, 10 protons are translocated from the matrix to the intermembrane space (which is ionically equivalent to the cytosol). In bacteria, electron transport complexes in the plasma membrane translocate protons from the cytosol to the cell exterior. Mitchell's theory of chemiosmosis actually explains more than just aerobic respiration. It also applies to systems where the energy from sunlight is used to generate a transmembrane proton gradient (this aspect of photosynthesis is described in Section 16-2).

## The proton gradient is an electrochemical gradient

When the mitochondrial complexes translocate protons across the inner mitochondrial membrane, the concentration of $H^+$ outside increases and the concentration of $H^+$ inside decreases **(Fig. 15-20)**. *This imbalance of protons, a nonequilibrium state, has an associated free energy (the force that would restore the system to equilibrium).* The free energy of the proton gradient has two components, reflecting the difference in the concentration of the chemical species and the difference in electrical charge of the positively charged protons (for this reason, the mitochondrial proton gradient is referred to as an electrochemical gradient rather than a simple concentration gradient). The free energy change for generating the chemical imbalance of protons is

$$\Delta G = RT \ln \frac{[H^+_{out}]}{[H^+_{in}]} \qquad \textbf{[15-5]}$$

The pH ($-\log [H^+]$) of the intermembrane space (*out*) is typically about 0.75 units less than the pH of the matrix (*in*).

The free energy change for generating the electrical imbalance of protons is

$$\Delta G = Z\mathcal{F}\Delta\psi \qquad \textbf{[15-6]}$$

where $Z$ is the ion's charge (+1 in this case) and $\Delta\psi$ is the membrane potential caused by the imbalance in positive charges (see Section 9-1). For mitochondria, $\Delta\psi$ is positive, usually 150 to 200 mV. This value indicates that the intermembrane space or cytosol is more positive than the matrix (recall from Section 9-1 that for a whole cell, the cytosol is more negative than the extracellular space and $\Delta\psi$ is negative).

Combining the chemical and electrical effects gives an overall free energy change for transporting protons from the matrix (*in*) to the intermembrane space (*out*):

$$\Delta G = RT \ln \frac{[H^+_{out}]}{[H^+_{in}]} + Z\mathcal{F}\Delta\psi \qquad \textbf{[15-7]}$$

Typically, the free energy change for translocating one proton out of the matrix is about $+20$ kJ $\cdot$ mol$^{-1}$ (see Sample Calculation 15-3 for a detailed application of Equation 15-7). This is a thermodynamically costly event. Passage of the proton back *into* the matrix, following its electrochemical gradient, would have a free energy change of about $-20$ kJ $\cdot$ mol$^{-1}$. This event is thermodynamically favorable, but it does not provide enough free energy to drive the synthesis of ATP. However, the 10 protons translocated for each pair of electrons transferred from NADH to $O_2$ have an associated proton motive force of over 200 kJ $\cdot$ mol$^{-1}$, enough to drive the phosphorylation of several molecules of ADP.

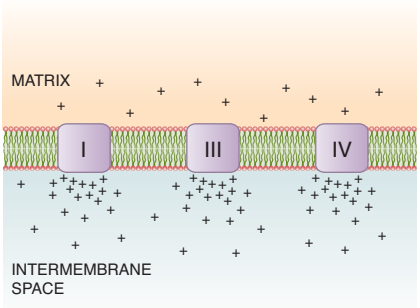



**Figure 15-20 Generation of a proton gradient.** During the oxidation–reduction reactions catalyzed by mitochondrial Complexes I, III, and IV, protons (represented by positive charges) are translocated out of the matrix into the intermembrane space. This creates an imbalance in both proton concentration and electrical charge.

### SAMPLE CALCULATION 15-3

**PROBLEM**

Calculate the free energy change for translocating a proton out of the mitochondrial matrix, where $pH_{matrix} = 7.8$, $pH_{cytosol} = 7.15$, $\Delta\psi = 170$ mV, and $T = 25°C$.

**SOLUTION**

Since $pH = -\log [H^+]$ (Equation 2-4), the logarithmic term of Equation 15-7 can be rewritten. Equation 15-7 then becomes

$$\Delta G = 2.303\, RT\,(pH_{in} - pH_{out}) + Z\mathcal{F}\Delta\psi$$

(*continued on next page*)

Substituting known values gives

$$\Delta G = 2.303\ (8.3145\ \text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1})(298\ \text{K})(7.8 - 7.15) + (1)(96{,}485\ \text{J} \cdot \text{V}^{-1} \cdot \text{mol}^{-1})(0.170\ \text{V})$$
$$= 3700\ \text{J} \cdot \text{mol}^{-1} + 16{,}400\ \text{J} \cdot \text{mol}^{-1}$$
$$= +20.1\ \text{kJ} \cdot \text{mol}^{-1}$$

**PRACTICE PROBLEMS**

6. Calculate the free energy change for transporting a proton out of the mitochondrial matrix, where $pH_{matrix} = 7.6$, $pH_{cytosol} = 7.35$, $\Delta\psi = 170$ mV, and $T = 37°C$.
7. What size pH gradient (the difference between $pH_{matrix}$ and $pH_{cytosol}$) would correspond to a free energy change of 30.5 kJ · $mol^{-1}$? Assume that $\Delta\psi = 170$ mV and $T = 25°C$.

**CONCEPT REVIEW**

- What is the source of the protons that form the gradient across the inner mitochondrial membrane?
- Why does the free energy of the proton gradient have a chemical and an electrical component?

# 15-4 ATP Synthase

**KEY CONCEPTS**

- Proton translocation drives the rotation of a portion of ATP synthase.
- Rotation-induced conformational changes allow ATP synthase to bind ADP and $P_i$, to phosphorylate ADP, and to release ATP.
- Because ATP synthesis is indirectly linked to electron transport, the P:O ratio is not a whole number.
- The supply of reduced cofactors determines the rate of oxidative phosphorylation.

**See Guided Exploration. ATP synthase.**

The protein that taps the electrochemical proton gradient to phosphorylate ADP is known as the F-ATP synthase (or Complex V). One part of the protein, called $F_0$, functions as a transmembrane channel that permits $H^+$ to flow back into the matrix, following its gradient. The $F_1$ component catalyzes the reaction ADP + $P_i \rightarrow$ ATP + $H_2O$ (Fig. 15-21). This section describes the structures of the two components of ATP synthase and shows how their activities are linked so that exergonic $H^+$ transport can be coupled to endergonic ATP synthesis.

## ATP synthase rotates as it translocates protons

Not surprisingly, the overall structure of ATP synthase is conserved among different species. The $F_1$ component consists of three α and three β subunits surrounding a central shaft. The membrane-embedded portion of ATP synthase includes an *a* subunit, two *b* subunits that extend upward to interact with the $F_1$ component, and a ring of *c* subunits (Fig. 15-22). The exact number of *c* subunits varies with the source; bovine mitochondrial ATP synthase, for example, has 8 *c* subunits, while some bacterial enzymes have 15 *c* subunits.

In all species, proton transport through ATP synthase involves the rotation of the *c* ring past the stationary *a* subunit. The carboxylate side chain of a highly conserved Asp or Glu residue on each *c* subunit serves as a proton binding site (Fig. 15-23). When properly positioned at the *a* subunit, a *c* subunit can take up a proton from the intermembrane space. A slight rotation of the *c* ring brings another *c* subunit into position so that it can release its bound proton into the matrix. The favorable thermodynamics of proton translocation force the *c* ring to keep moving in one direction. Experiments show that depending on the relative concentrations of protons on the two sides of the membrane, the *c* ring can actually spin in either direction. Related proteins, known as P- and V-ATPases, in fact function as

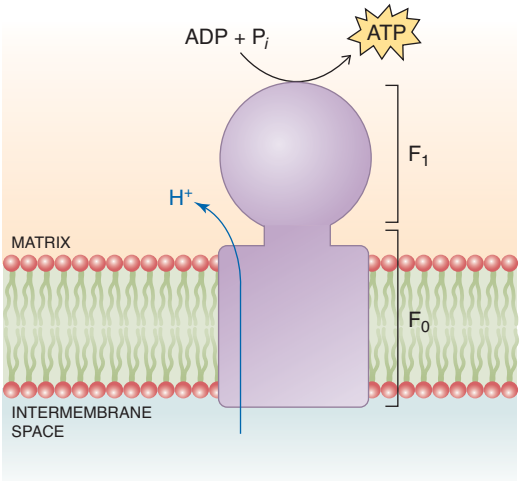



**Figure 15-21 ATP synthase function.** As protons flow through the $F_0$ component from the intermembrane space to the matrix, the $F_1$ component catalyzes the synthesis of ATP from ADP + $P_i$.

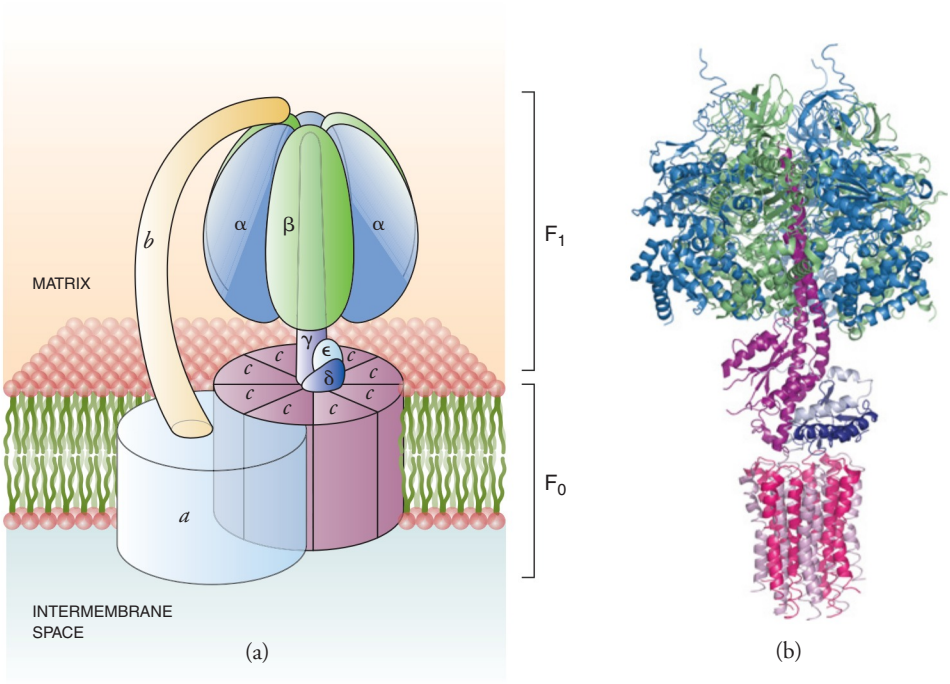



**Figure 15-22 Structure of ATP synthase.** (a) Model of the mammalian enzyme with individual subunits labeled. A spherical structure consisting of three α and three β subunits is connected via a central stalk (subunits γ, δ, and ε) to the membrane-embedded *c* ring. The *a* subunit is closely associated with the *c* ring, and a peripheral stalk containing several subunits (including *b*) links subunit *a* to the catalytic domain. (b) X-Ray structure of bovine ATP synthase at 3.5-Å resolution. Some subunits are not visible in the crystal structure. [Structure (pdb 2XND) determined by I. N. Watt, M. G. Montgomery, M. J. Runswick, A. G. W. Leslie, and J. E. Walker.]

active transporters that use the free energy of the ATP hydrolysis reaction to drive ion movement across the membrane.

Attached to the *c* ring and rotating along with it are the γ, δ, and ε subunits (Fig. 15-22). The δ and ε subunits are relatively small, but the γ subunit consists of two long α helices arranged as a bent coiled coil that protrudes into the center of the globular $F_1$ structure. The three α and three β subunits of $F_1$ have similar tertiary structures and are arranged like the sections of an orange around the γ subunit **(Fig. 15-24)**. Although all six subunits can bind adenine nucleotides, only the β subunits have catalytic activity (nucleotide binding to the α subunits may play a regulatory role).

A close examination of the $F_1$ assembly reveals that the γ subunit interacts asymmetrically with the three pairs of αβ units. In fact, each αβ unit has a slightly different conformation, and model-building indicates that for steric reasons, the three units cannot simultaneously adopt the same conformation. *The three αβ pairs change their conformations as the γ subunit rotates* (it is like a shaft driven by the *c* ring

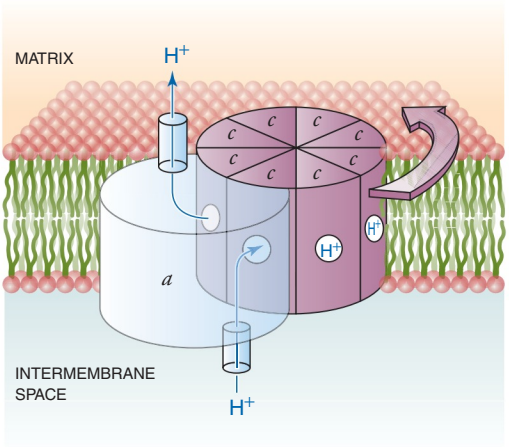



**Figure 15-23 Mechanism of proton transport by ATP synthase.** When a *c* subunit (purple) binds a proton from one side of the membrane, it moves away from the *a* subunit (blue). Because the *c* subunits form a ring, rotation brings another *c* subunit toward the *a* subunit, where it releases its bound proton to the opposite side of the membrane. In mammalian ATP synthase (shown here), one complete rotation of the *c* ring corresponds to the translocation of eight protons.

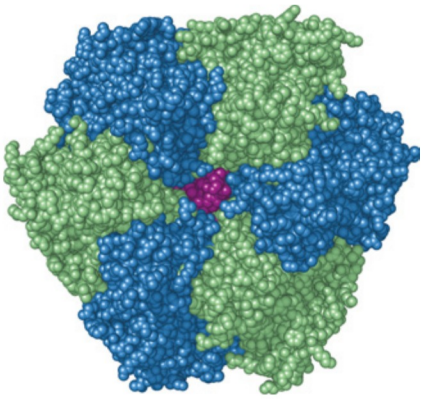


**Figure 15-24 Structure of the $F_1$ component of ATP synthase.** The alternating α (blue) and β (green) subunits form a hexamer around the end of the γ shaft (purple). This view is looking from the matrix down onto the top of the ATP synthase structure shown in Figure 15-22b. [Structure (pdb 1E79) determined by C. Gibbons, M. G. Montgomery, A. G. W. Leslie, and J. E. Walker.]

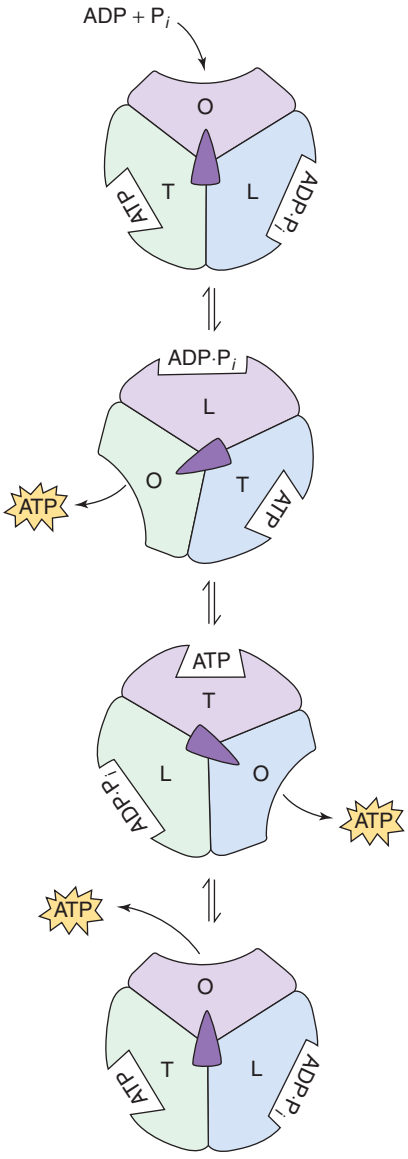



**Figure 15-25 The binding change mechanism.** The diagram shows the catalytic (β) subunits of the $F_1$ component of ATP synthase from the same perspective as in Fig. 15-24. Each of the three β subunits adopts a different conformation: open (O), loose (L), or tight (T). The substrates ADP and $P_i$ bind to a loose site, ATP is synthesized when the site becomes tight, and ATP is released when the subunit becomes open. The conformational shifts are triggered by the 120° rotation of the γ subunit, arbitrarily represented by the purple shape. Because each of the three catalytic sites cycles through the three conformational states, ATP is released from one of the three β subunits with each 120° rotation of the γ subunit. ⊕ **See Animated Figure. The binding change mechanism of ATP synthesis.**

"rotor"). The αβ hexamer itself does not rotate, since it is held in place by the peripheral arm that is anchored to the *a* subunit (see Fig. 15-22a).

For an ATP synthase containing 8 *c* subunits, the transmembrane movement of each proton could potentially turn the γ shaft by 45° (360° ÷ 8). However, videomicroscopy experiments indicate that the γ subunit rotates in steps of 120°, interacting successively with each of the three αβ pairs in one full rotation of 360°. Electrostatic interactions between the γ and β subunits apparently act as a catch that holds the γ subunit in place while translocation of two to three protons builds up strain. Translocation of the next proton causes the γ subunit to suddenly snap into position at the next β subunit, a movement of 120°. This mechanism accounts for the variation in the number of *c* subunits in ATP synthases from different sources. The *c* ring spins in small increments (24° to 45°, depending on the number of *c* subunits), but the γ subunit makes just three large shifts of 120°.

▶▶ **HOW does ATP synthase carry out an unfavorable reaction?**

## The binding change mechanism explains how ATP is made

At the start of the chapter, we pointed out that ATP synthase catalyzes a highly endergonic reaction ($\Delta G^{\circ\prime} = +30.5$ kJ · mol$^{-1}$) in order to produce the bulk of a cell's ATP supply. This enzyme operates in an unusual fashion, using mechanical energy (rotation) to form a chemical bond (the attachment of a phosphoryl group to ADP). In other words, the enzyme converts mechanical energy to the chemical energy of ATP. The interaction between the γ subunit and the αβ hexamer explains this energy transduction.

According to the **binding change mechanism** described by Paul Boyer, *rotation-driven conformational changes alter the affinity of each catalytic β subunit for an adenine nucleotide.* At any moment, each catalytic site has a different conformation (and binding affinity), referred to as the open, loose, or tight state. ATP synthesis occurs as follows (Fig. 15-25):

1. The substrates ADP and $P_i$ bind to a β subunit in the loose state.
2. The substrates are converted to ATP as rotation of the γ subunit causes the β subunit to shift to the tight conformation.
3. The product ATP is released after the next rotation, when the β subunit shifts to the open conformation.

Because the three β subunits of ATP synthase act cooperatively, they all change their conformations simultaneously as the γ subunit turns. A full rotation of 360° is required to restore the enzyme to its initial state, but each rotation of 120° results in the release of ATP from one of the three active sites.

Experiments with the isolated $F_1$ component of ATP synthase show that in the absence of $F_0$, $F_1$ functions as an ATPase, hydrolyzing ATP to ADP + $P_i$ (a thermodynamically favorable reaction). In the intact ATP synthase, dissipation of the proton gradient is tightly coupled to ATP synthesis with near 100% efficiency. Consequently, *in the absence of a proton gradient, no ATP is synthesized because there is no free energy to drive the rotation of the γ subunit.* Agents that dissipate the proton gradient can therefore "uncouple" ATP synthesis from electron transport, the source of the proton gradient (Box 15-B).

## The P:O ratio describes the stoichiometry of oxidative phosphorylation

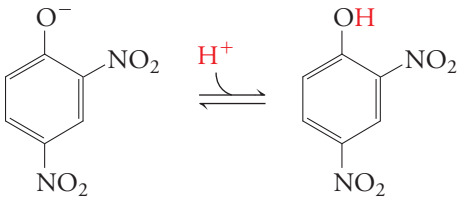
Since the γ shaft of ATP synthase is attached to the *c*-subunit rotor, 3 ATP molecules are synthesized for every complete *c*-ring rotation. However, the number

**BOX 15-B BIOCHEMISTRY NOTE**

## Uncoupling Agents Prevent ATP Synthesis

When the metabolic need for ATP is low, the oxidation of reduced cofactors proceeds until the transmembrane proton gradient builds up enough to halt further electron transport. When the protons reenter the matrix via the $F_0$ component of ATP synthase, electron transport resumes. However, if the protons leak back into the matrix by a route other than ATP synthase, then electron transport will continue without any ATP being synthesized. ATP synthesis is said to be "uncoupled" from electron transport, and the agent that allows the proton gradient to dissipate in this way is called an **uncoupler.**

One well-known uncoupler is the dinitrophenolate ion, which can take up a proton (its p$K$ is near neutral):




Dinitrophenolate Dinitrophenol

In its neutral state, dinitrophenol can diffuse through a lipid bilayer and release the proton on the other side. Dinitrophenol can therefore dissipate a proton gradient by providing a way for protons to cross the membrane.

Dinitrophenol, of course, is not usually present in mitochondria; however, physiological uncoupling does occur. Dissipating a proton gradient prevents ATP synthesis, but it allows oxidative metabolism to continue at a high rate. The by-product of this metabolic activity is heat.

Uncoupling for **thermogenesis** (heat production) occurs in specialized adipose tissue known as brown fat (its dark color is due to the relatively high concentration of cytochrome-containing mitochondria; ordinary adipose tissue is lighter). The inner membrane of the mitochondria in brown fat contains a transmembrane proton channel called a UCP (uncoupling protein). Protons translocated to the intermembrane space during respiration can reenter the mitochondrial matrix via the uncoupling protein, bypassing ATP synthase. The free energy of respiration is therefore given off as heat rather than used to synthesize ATP. Brown fat is abundant in hibernating mammals and newborn humans, and the activity of the UCP is under the control of hormones that also mobilize the stored fatty acids to be oxidized in the brown fat mitochondria.

**Question:** Why would increasing the activity of UCP promote weight loss?

of protons translocated per ATP depends on the number of *c* subunits. For mammalian ATP synthase, which has 8 *c* subunits, the stoichiometry is 8 $H^+$ per 3 ATP, or 2.7 $H^+$ per ATP. Such nonintegral values would be difficult to reconcile with most biochemical reactions, but they are consistent with the chemiosmotic theory: *Chemical energy (from the respiratory oxidation–reduction reactions) is transduced to a proton motive force, then to the mechanical movement of a rotary engine (the c ring and its attached γ shaft), and finally back to chemical energy in the form of ATP.*

The relationship between respiration (the activity of the electron transport complexes) and ATP synthesis is traditionally expressed as a **P:O ratio,** that is, the number of phosphorylations of ADP relative to the number of oxygen atoms reduced. For example, the oxidation of NADH by $O_2$ (carried out by the sequential activities of Complexes I, III, and IV) translocates 10 protons into the intermembrane space. The movement of these 10 protons back into the matrix via the $F_0$ component

would theoretically drive the synthesis of about 3.7 ATP since 1 ATP can be made for every 2.7 protons translocated, at least in mammalian mitochondria:

$$\frac{1\ \text{ATP}}{2.7\ \text{H}^+} \times 10\ \text{H}^+ = 3.7$$

Thus, the P:O ratio would be about 3.7 (3.7 ATP per $\frac{1}{2}O_2$ reduced). For an electron pair originating as $QH_2$, only 6 protons would be translocated (by the activities of Complexes III and IV), and the P:O ratio would be approximately 2.2:

$$\frac{1\ \text{ATP}}{2.7\ \text{H}^+} \times 6\ \text{H}^+ = 2.2$$

*In vivo*, the P:O ratios are actually a bit lower than the theoretical values, because some of the protons translocated during electron transport do leak across the membrane or are consumed in other processes, such as the transport of $P_i$ into the mitochondrial matrix (see Fig. 15-6). Consequently, experimentally determined P:O ratios are closer to 2.5 when NADH is the source of electrons and 1.5 for ubiquinol. These values are the basis for our tally of the ATP yield for the complete oxidation of glucose by glycolysis and the citric acid cycle (see Section 14-2).

## The rate of oxidative phosphorylation depends on the rate of fuel catabolism

In most metabolic pathways, control is exerted at highly exergonic (irreversible) steps. In oxidative phosphorylation, this step would be the reaction catalyzed by cytochrome *c* oxidase (Complex IV; see Fig. 15-2). However, there are no known effectors of cytochrome *c* oxidase activity. Apparently, *the close coupling between generation of the proton gradient and ATP synthesis allows oxidative phosphorylation to be regulated primarily by the availability of reduced cofactors (NADH and $QH_2$) produced by other metabolic processes.*

Less important regulatory mechanisms may involve the availability of the substrates ADP and $P_i$ (which depend on the activity of their respective transport proteins). Experiments with ATP synthase show that when ADP and $P_i$ are absent, the β subunits cannot undergo the conformational changes required by the binding change mechanism. The γ subunit therefore remains immobile, and no protons are translocated through the *c* ring. This tight coupling between ATP synthesis and proton translocation prevents the waste of the free energy of the proton gradient.

There is also evidence that mitochondria contain a regulatory protein that binds to ATP synthase to inhibit its rate of ATP hydrolysis. The inhibitor is sensitive to pH, so it does not bind to ATP synthase when the matrix pH is high (as it is when electron transport is occurring). However, if the matrix pH drops as a result of a momentary disruption of the proton gradient, the inhibitor binds to ATP synthase. This regulatory mechanism prevents ATP synthase from operating in reverse as an ATPase.

**CONCEPT REVIEW**

- How does ATP synthase dissipate the proton gradient? How is this activity related to the activity of the ATP-synthesizing catalytic sites?
- Describe how the three conformational states of the β subunits of ATP synthase are involved in ATP synthesis.
- How does the binding change mechanism account for ATP hydrolysis by ATP synthase?
- Why does the number of protons translocated per ATP synthesized vary among species?
- What is a P:O ratio and why is it nonintegral?
- Explain why the availability of reduced substrates is the primary mechanism for regulating oxidative phosphorylation.

## SUMMARY

### 15-1 The Thermodynamics of Oxidation-Reduction Reactions

- The electron affinity of a substance participating in an oxidation–reduction reaction, which involves the transfer of electrons, is indicated by its reduction potential, $\mathcal{E}^{\circ\prime}$.
- The difference in reduction potential between species undergoing oxidation and reduction is related to the free energy change for the reaction.

### 15-2 Mitochondrial Electron Transport

- Oxidation of reduced cofactors generated by metabolic reactions takes place in the mitochondrion. Shuttle systems and transport proteins allow the transmembrane movement of reducing equivalents, ATP, ADP, and $P_i$.
- The electron transport chain consists of a series of integral membrane protein complexes that contain multiple redox groups, including iron–sulfur clusters, flavins, cytochromes, and copper ions, and that are linked by mobile electron carriers. Starting from NADH, electrons travel a path of increasing reduction potential through Complex I, ubiquinone, Complex III, cytochrome *c*, and then to Complex IV, where $O_2$ is reduced to $H_2O$.
- As electrons are transferred, protons are translocated to the intermembrane space via proton wires in Complexes I and IV and by the action of the Q cycle associated with Complex III.

### 15-3 Chemiosmosis

- The chemiosmotic theory describes how proton translocation during mitochondrial electron transport generates an electrochemical gradient whose free energy drives ATP synthesis.

### 15-4 ATP Synthase

- The energy of the proton gradient is tapped as protons spontaneously flow through ATP synthase. Proton transport allows rotation of a ring of integral membrane *c* subunits. The linked $\gamma$ subunit thereby rotates, triggering conformational changes in the $F_1$ portion of ATP synthase.
- According to the binding change mechanism, the three functional units of the $F_1$ portion cycle through three conformational states to sequentially bind ADP and $P_i$, convert the substrates to ATP, and release ATP.
- The P:O ratio quantifies the link between electron transport and oxidative phosphorylation in terms of the ATP synthesized and the $O_2$ reduced. Because these processes are coupled, the rate of oxidative phosphorylation is controlled primarily by the availability of reduced cofactors.

## GLOSSARY TERMS

oxidative phosphorylation
oxidizing agent (oxidant)
reducing agent (reductant)
half-reaction
$\mathcal{E}^{\circ\prime}$
$\mathcal{E}$
Nernst equation
$\mathcal{F}$
respiration
mitochondrion
mitochondrial matrix
intermembrane space
cristae
electron tomography
redox center
proton wire
cytochrome
Q cycle
free radical
chemiosmotic theory
protonmotive force
$\mathcal{Z}$
binding change mechanism
P:O ratio
uncoupler
thermogenesis

## PROBLEMS

### 15-1 The Thermodynamics of Oxidation-Reduction Reactions

**1.** Calculate the standard free energy change for the reduction of pyruvate by NADH. Consult Table 15-1 for the relevant half-reactions. Is this reaction spontaneous under standard conditions?

**2.** Calculate the standard free energy change for the reduction of oxygen by cytochrome $a_3$. Consult Table 15-1 for the relevant half-reactions. Is this reaction spontaneous under standard conditions?

**3.** In one of the final steps of the pyruvate dehydrogenase reaction (see Section 14-1), E3 reoxidizes the lipoamide group of E2, then $NAD^+$ reoxidizes E3. Calculate $\Delta G^{\circ\prime}$ for the electron transfer from dihydrolipoic acid to $NAD^+$.

**4.** Each electron from cytochrome *c* is donated to a $Cu_A$ redox center in Complex IV. The $\mathcal{E}^{\circ\prime}$ value for the $Cu_A$ redox center is 0.245 V. Calculate $\Delta G^{\circ\prime}$ for this electron transfer.

**5.** Acetaldehyde may be oxidized to acetate. Would $NAD^+$ be an effective oxidizing agent? Explain.

**6.** Acetoacetate may be reduced to 3-hydroxybutyrate. What serves as a better reducing agent, NADH or $FADH_2$? Explain.

**7.** For every two $QH_2$ that enter the Q cycle, one is regenerated and the other passes its two electrons to two cytochrome $c_1$ centers. The overall equation is

$$QH_2 + 2\ \text{cyt}\ c_1\ (Fe^{3+}) + 2\ H^+ \rightarrow Q + 2\ \text{cyt}\ c_1\ (Fe^{2+}) + 4\ H^+$$

Calculate the free energy change associated with the Q cycle.

**8.** Why is succinate oxidized by FAD instead of by $NAD^+$?

**9.** **(a)** What is the $\Delta\mathcal{E}$ value for the oxidation of ubiquinol by cytochrome *c* when the ratio of $QH_2/Q$ is 10 and the ratio of cyt *c* ($Fe^{3+}$)/cyt *c* ($Fe^{2+}$) is 5?
**(b)** Calculate the $\Delta G$ for the reaction in part (a).

**10.** An iron–sulfur protein in Complex III donates an electron to cytochrome $c_1$. The reduction half-reactions and $\mathcal{E}^{\circ\prime}$ values are shown below. Write the balanced equation for the reaction and calculate the standard free energy change. How can you account for the fact that this reaction occurs spontaneously in the cell?

$$\text{FeS } (ox) + e^- \rightarrow \text{FeS } (red) \qquad \mathcal{E}^{\circ\prime} = 0.280 \text{ V}$$

$$\text{cyt } c_1 \ (Fe^{3+}) + e^- \rightarrow \text{cyt } c_1 \ (Fe^{2+}) \qquad \mathcal{E}^{\circ\prime} = 0.215 \text{ V}$$

**11.** Calculate the overall efficiency of oxidative phosphorylation, assuming standard conditions, by comparing the free energy potentially available from the oxidation of NADH by $O_2$ and the free energy required to synthesize 2.5 ATP from 2.5 ADP.

**12.** Using the percent efficiency calculated in Problem 11, calculate the number of ATP generated by **(a)** Complex I (where NADH is oxidized by ubiquinone), **(b)** Complex III (where ubiquinol is oxidized by cytochrome *c*), and **(c)** Complex IV (where cytochrome *c* is oxidized by molecular oxygen).

**13.** Calculate $\Delta\mathcal{E}^{\circ\prime}$ and $\Delta G^{\circ\prime}$ for the succinate dehydrogenase (Complex II) reaction.

**14.** Refer to the solution for Problem 13. Does this reaction provide sufficient free energy to drive ATP synthesis under standard conditions? Explain.

## 15-2 Mitochondrial Electron Transport

**15.** The sequence of events in electron transport was elucidated in part by the use of inhibitors that block electron transfer at specific points along the chain. For example, adding rotenone (a plant toxin) or amytal (a barbiturate) blocks electron transport in Complex I; antimycin A (an antibiotic) blocks electron transport in Complex III; and cyanide ($CN^-$) blocks electron transport in Complex IV by binding to the $Fe^{2+}$ in the Fe–Cu binuclear center.
**(a)** What happens to oxygen consumption when these inhibitors are added to a suspension of respiring mitochondria?
**(b)** What is the redox state of the electron carriers in the electron transport chain when each of the inhibitors is added separately to the mitochondrial suspension?

**16.** What is the effect of added succinate to rotenone-blocked, amytal-blocked, or cyanide-blocked mitochondria (see Problem 15)? In other words, can succinate help "bypass" the block? Explain.

**17.** The compound tetramethyl-*p*-phenylenediamine (TMPD) donates a pair of electrons directly to Complex IV. What is the P:O ratio of this compound?

**18.** Ascorbate (vitamin C) can donate a pair of electrons to cytochrome *c*. What is the P:O ratio for ascorbate?

**19.** Can tetramethyl-*p*-phenylenediamine (see Problem 17) act as a bypass for the rotenone-blocked, amytal-blocked, or cyanide-blocked mitochondria described in Problem 15? Can ascorbate (see Problem 18) act as a bypass? Explain.

**20.** When the antifungal agent myxothiazol is added to a suspension of respiring mitochondria, the $QH_2/Q$ ratio increases. Where in the electron transport chain does myxothiazol inhibit electron transfer?

**21.** If cyanide poisoning (see Problem 15) is diagnosed immediately, it can be treated by administering nitrites that can oxidize the $Fe^{2+}$ in hemoglobin to $Fe^{3+}$. Why is this treatment effective?

**22.** The effect of the drug fluoxetine (Prozac) on isolated rat brain mitochondria was examined by measuring the rate of electron transport (units not given) in the presence of various combinations of substrates and inhibitors (see Problems 15–17).
**(a)** How do pyruvate and malate serve as substrates for electron transport?
**(b)** What is the effect of fluoxetine on electron transport? Explain.
**(c)** Fluoxetine can also inhibit ATP synthase. Why might long-term use of fluoxetine be a concern?

**Rate of Electron Transport**

| [Fluoxetine], (mM) | Pyruvate + malate | Succinate + rotenone | Ascorbate + TMPD |
|---|---|---|---|
| 0 | 163 ± 15.1 | 145 ± 14.2 | 184 ± 22.2 |
| 0.15 | 77 ± 7.3 | 131 ± 13.5 | 116 ± 13.9 |

**23.** Complex I, succinate dehydrogenase, acyl-CoA dehydrogenase, and glycerol-3-phosphate dehydrogenase (see Fig. 15-11) are all flavoproteins; that is, they contain an FMN or FAD prosthetic group. Explain the function of the flavin group in these enzymes.

**24.** What side chains would you expect to find as part of a proton wire in a proton-translocating membrane protein?

**25.** Ubiquinone is not anchored in the mitochondrial membrane but is free to diffuse laterally throughout the membrane among the electron transport chain components. What aspects of its structure account for this behavior?

**26.** Explain why the ubiquinone-binding site of Complex I (Fig. 15-7) is located at the end of the peripheral arm closest to the membrane.

**27.** Cytochrome *c* is easily dissociated from isolated mitochondrial membrane preparations, but the isolation of cytochrome $c_1$ requires the use of strong detergents. Explain why.

**28.** Release of cytochrome *c* from the mitochondrion to the cytosol is one of the signals that induces apoptosis, a form of programmed cell death. What structural features of cytochrome *c* allow it to play this role?

**29.** In coastal marine environments, high concentrations of nutrients from terrestrial runoff may lead to algal blooms. When the nutrients are depleted, the algae die and sink and are degraded by other microorganisms. The algal die-off may be followed by a sharp drop in oxygen in the depths, which can kill fish and bottom-dwelling invertebrates. Why do these "dead zones" form?

**30.** Chromium is most toxic and highly soluble in its oxidized Cr(VI) state but is less toxic and less soluble in its more reduced Cr(III) state. Efforts to detoxify Cr-contaminated groundwater have involved injecting chemical reducing agents underground. Another approach is bioremediation, which involves injecting molasses or cooking oil into the contaminated groundwater. Explain how these substances would promote the reduction of Cr(VI) to Cr(III).

**31.** At one time, it was believed that myoglobin functioned simply as an oxygen-storage protein. New evidence suggests that myoglobin plays a much more active role in the muscle cell. The phrase *myoglobin-facilitated oxygen diffusion* describes myoglobin's role in transporting oxygen from the muscle cell sarcolemma to

the mitochondrial membrane surface. Mice in which the myoglobin gene was knocked out had higher tissue capillary density, elevated red blood cell counts, and increased coronary blood flow. Explain the reasons for these compensatory mechanisms in the knockout mice.

**32.** The myoglobin and cytochrome *c* oxidase content were determined in several animals, as shown in the table. What is the relationship between the two proteins? Explain.

| | Myoglobin content, mmol · kg$^{-1}$ | Cytochrome *c* oxidase activity |
|---|---|---|
| Hare | 0.1 | 900 |
| Sheep | 0.19 | 950 |
| Ox | 0.31 | 1200 |
| Horse | 0.38 | 1800 |

**33.** It has recently been found that myoglobin distribution is not confined to muscle cells. Tumor cells, which generally exist in hypoxic (low-oxygen) conditions because of limited blood flow, have been found to express myoglobin. How does this adaptation increase the chances of tumor cell survival?

**34.** Cancer cells, even when sufficient oxygen is available, produce large amounts of lactate. It has been observed that the concentration of fructose-2,6-bisphosphate is much higher in cancer cells than in normal cells. Why would this result in anaerobic metabolism being favored, even when oxygen is available?

**35.** A group of elderly patients who did not exercise regularly were asked to participate in a 12-week exercise program. Data collected from the patients are shown in the table. What was the result of the exercise intervention and why did this occur?

| | Prior to exercise intervention | Post 12-week exercise intervention |
|---|---|---|
| Total mitochondrial DNA (copies per diploid genome) | 1300 | 1900 |
| Complex II activity | 0.13 | 0.20 |
| Complex I–IV activity | 0.51 | 1.00 |

**36.** Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that causes muscle paralysis and eventually death. Researchers measured the activity of the electron transport chain complexes in various regions of the nervous system in patients with ALS. In a certain region of the spinal cord, Complex I showed decreased activity but not decreased concentration. How does this contribute to progression of the disease?

## 15-3 Chemiosmosis

**37.** What is the free energy change for generating the electrical imbalance of protons in neuroblastoma cells, where $\Delta\psi$ is 81 mV?

**38.** Calculate the free energy change for translocating a proton out of the mitochondrial matrix, where $pH_{matrix} = 7.6$, $pH_{cytosol} = 7.2$, $\Delta\psi = 200$ mV, and $T = 37°C$.

**39.** Several key experimental observations were important in the development of the chemiosmotic theory. Explain how each observation listed below is consistent with the chemiosmotic theory as described by Peter Mitchell.

**(a)** The pH of the intermembrane space is lower than the pH of the mitochondrial matrix.

**(b)** Oxidative phosphorylation does not occur in mitochondrial preparations to which detergents have been added.

**(c)** Lipid-soluble compounds such as DNP (see Box 15-B) inhibit oxidative phosphorylation while allowing electron transport to continue.

**40.** Mitchell's original chemiosmotic hypothesis relies on the impermeability of the inner mitochondrial membrane to ions other than $H^+$, such as $Na^+$ and $Cl^-$.

**(a)** Why was this thought to be important?

**(b)** Could ATP still be synthesized if the membrane were permeable to other ions?

**41.** Nigericin is an antibiotic that integrates into membranes and functions as a $K^+/H^+$ antiporter. Another antibiotic, valinomycin, is similar, but it allows the passage of $K^+$ ions. When both antibiotics are added simultaneously to suspensions of respiring mitochondria, the electrochemical gradient completely collapses.

**(a)** Draw a diagram of a mitochondrion in which nigericin and valinomycin have integrated into the inner mitochondrial membrane, in a manner that is consistent with the experimental results.

**(b)** Explain why the electrochemical gradient dissipates. What happens to ATP synthesis?

**42.** How does transport of inorganic phosphate from the intermembrane space to the mitochondrial matrix affect the pH difference across the inner mitochondrial membrane?

## 15-4 ATP Synthase

**43.** How much ATP can be obtained by the cell from the complete oxidation of one mole of glucose? Compare this value with the amount of ATP obtained when glucose is anaerobically converted to lactate or ethanol.

**44.** The glycerol-3-phosphate shuttle can transport NADH generated in the cytosol into the mitochondrial matrix (see Figure 15-11c). In this shuttle, the protons and electrons are donated to FAD, which is reduced to $FADH_2$. These protons and electrons are subsequently donated to coenzyme Q in the electron transport chain. How much ATP is generated per mole of glucose when the glycerol-3-phosphate shuttle is used?

**45.** The complete oxidation of glucose yields $-2850$ kJ · mol$^{-1}$ of free energy. Incomplete oxidation by conversion to lactate yields $-196$ kJ · mol$^{-1}$ and by alcoholic fermentation yields $-235$ kJ · mol$^{-1}$. Calculate the overall efficiencies of glucose oxidation by these three processes.

**46.** Do organisms that can completely oxidize glucose have an advantage over organisms that cannot? (*Hint:* See Problem 45).

**47.** A culture of yeast grown under anaerobic conditions is exposed to oxygen, resulting in a dramatic decrease in glucose consumption by the cells. This phenomenon is referred to as the Pasteur effect.

**(a)** Explain the Pasteur effect.

**(b)** The [NADH]/[$NAD^+$] and [ATP]/[ADP] ratios also change when an anaerobic culture is exposed to oxygen. Explain how these ratios change and what effect this has on glycolysis and the citric acid cycle in the yeast.

**48.** Experiments in the late 1970s attributed the Pasteur effect (see Problem 47) to the stimulation of hexokinase and phosphofructokinase under anaerobic conditions. Upon exposure to oxygen, the stimulation of these enzymes ceases. Why are these enzymes more active in the absence of oxygen?

**49.** Consider the adenine nucleotide translocase and the $P_i$–$H^+$ symport protein that import ADP and $P_i$, the substrates for oxidative phosphorylation, into the mitochondrion (see Fig. 15-6).

**(a)** How does the activity of the adenine nucleotide translocase affect the electrochemical gradient across the mitochondrial membrane?

**(b)** How does the activity of the $P_i$–$H^+$ symport protein affect the gradient?

**(c)** What can you conclude about the thermodynamic force that drives the two transport systems?

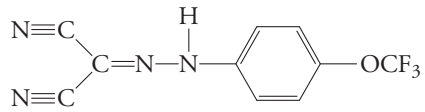
**50.** The compounds atractyloside and bongkrekic acid both bind tightly to and inhibit the ATP/ADP translocase. What is the effect of these compounds on ATP synthesis? On electron transport?

**51.** Dicyclohexylcarbodiimide (DCCD) is a reagent that reacts with Asp or Glu residues. Explain why the reaction of DCCD with just one *c* subunit completely blocks both the ATP-synthesizing and ATP-hydrolyzing activity of ATP synthase.

**52.** Oligomycin is an antibiotic that blocks proton transfer through the $F_0$ proton channel of ATP synthase. What is the effect on **(a)** ATP synthesis, **(b)** electron transport, and **(c)** oxygen consumption when oligomycin is added to a suspension of respiring mitochondria? **(d)** What changes occur when dinitrophenol is then added to the suspension?

**53.** The compound DNP was introduced as a "diet pill" in the 1920s. Its use was discontinued because the side effects were fatal in some cases. What was the rationale for believing that DNP would be an effective diet aid?

**54.** The compound carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone (FCCP) is an uncoupler similar to DNP. Describe how FCCP acts as an uncoupler.



FCCP

**55.** In the 1950s, experiments with isolated mitochondria showed that organic compounds are oxidized and $O_2$ is consumed only when ADP is included in the preparation. When the ADP supply runs out, oxygen consumption halts. Explain these results.

**56.** A patient seeks treatment because her metabolic rate is twice normal and her temperature is elevated. A biopsy reveals that her muscle mitochondria are structurally unusual and not subject to normal respiratory controls. Electron transport takes place regardless of the concentration of ADP.

**(a)** What is the P:O ratio (compared to normal) of NADH that enters the electron transport chain in the mitochondria of this patient?

**(b)** Why are the patient's metabolic rate and temperature elevated?

**(c)** Will this patient be able to carry out strenuous exercise?

**57.** In experimental systems, the $F_0$ component of ATP synthase can be reconstituted into a membrane. $F_0$ can then act as a proton channel that is blocked when the $F_1$ component is added to the system. What molecule must be added to the system in order to restore the proton-translocating activity of $F_0$? Explain.

**58.** Calculate the ratio of protons translocated to ATP synthesized for yeast ATP synthase, which has 10 *c* subunits, and for spinach chloroplast ATP synthase, which has 14 *c* subunits.

**59.** A bacterial ATP synthase has 10 *c* subunits, and a chloroplast ATP synthase has 14 *c* subunits. Would you expect the bacterium or the chloroplast to have a higher P:O ratio?

**60.** Experiments indicate that the *c* ring of ATP synthase spins at a rate of 6000 rpm. How many ATP molecules are generated each second?

**61.** Mutations that impair ATP synthase function are rare. Laboratory studies indicate that adding α-ketoglutarate boosts ATP production in ATP synthase–deficient cells, but only when aspartate is also added to the cells. Explain.

**62.** In yeast, pyruvate may be converted to ethanol in a two-step pathway catalyzed by pyruvate decarboxylase and alcohol dehydrogenase (see Section 13-1). Pyruvate may also be converted to acetyl-CoA by pyruvate dehydrogenase. Yeast mutants in which the pyruvate decarboxylase gene is missing (*pdc*–) are useful for studying the regulation of the pyruvate dehydrogenase enzyme. When wild-type yeast were pulsed with glucose, glycolytic flux increased dramatically and the rate of respiration increased. But when the same experiments were performed with *pdc*– mutants, only a small increase in glycolysis was observed, and pyruvate was the main product excreted by the yeast cells. Explain these results.

**63.** During anaerobic fermentation in yeast, the majority of the available glucose is oxidized via the glycolytic pathway and the rest enters the pentose phosphate pathway to generate NADPH and ribose. This occurs during aerobic respiration as well, except that the percentage of glucose entering the pentose phosphate pathway is much greater in aerobic respiration than during anaerobic fermentation. Explain why.

**64.** UCP1 is an uncoupling protein in brown fat (see Box 15-B). Experiments using UCP1-knockout mice (animals missing the gene for UCP1) resulted in the discovery of a second uncoupling protein named UCP2.

**(a)** Oxygen consumption increased over twofold when a $\beta_3$ adrenergic agonist that stimulates UCP1 was injected into normal mice. This was not observed when the agonist was injected into the knockout mice. Explain these results.

**(b)** The UCP1-knockout mice were essentially normal except for increased lipid deposition in their adipose tissue. Explain why.

**(c)** In one experiment, normal mice and UCP1-knockout mice were placed in a cold (5°C) room overnight. The normal mice were able to maintain their body temperature at 37°C even after 24 hours in the cold. But the body temperatures of the cold-exposed knockout mice decreased 10°C or more. Explain.

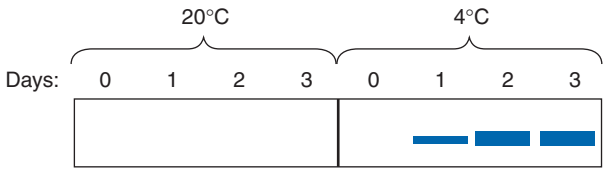
**(d)** UCP1-knockout mice did not become obese when fed a high-fat diet. Propose a hypothesis that explains this observation.

**65.** The Eastern skunk cabbage can maintain its temperature 15–35°C higher than ambient temperature during the months of February and March, when ambient temperatures range from −15 to +15°C. Thermogenesis in the skunk cabbage is critical to the survival of the plant since the spadix (a flower component) is not frost-resistant. An uncoupling protein was found to be responsible for the observed thermogenesis.

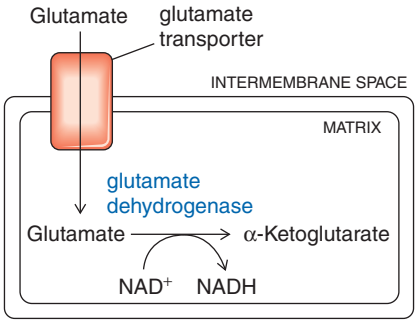
**(a)** The spadix relies on the skunk cabbage's massive root system, which stores appreciable quantities of starch. Why is a large quantity of starch required for the skunk cabbage to carry out sustained thermogenesis for weeks rather than hours?

**(b)** Oxygen consumption by the skunk cabbage increases as the temperature decreases, nearly doubling with every 10°C drop in ambient temperature. Oxygen consumption was observed to decrease during the day, when temperatures were close to 30°C, and increase at night. What is the biochemical explanation for these observations?

**66.** The gene that codes for the uncoupling protein (see Problem 65) in potatoes was recently isolated. The results of a Northern blot analysis (which detects mRNA) are shown below. What is your interpretation of these results? How does the mRNA level affect thermogenesis in the potato?




**67.** Glutamate can be used as an artificial substrate for mitochondrial respiration, as shown in the diagram below. When ceramide is added to a mitochondrial suspension respiring in the presence of glutamate, respiration decreases, leading scientists to hypothesize that ceramide might regulate mitochondrial function *in vivo*.




**(a)** How does glutamate act as a substrate for mitochondrial respiration?
**(b)** Ceramide-induced inhibition of respiration could be due to several different factors. List several possibilities.
**(c)** Mitochondria treated with ceramide were exposed to an uncoupler, but the respiration rate did not increase. What site(s) of inhibition can be ruled out?
**(d)** In another experiment, mitochondria were subjected to a freeze–thaw cycle that rendered the inner mitochondrial membrane permeable to NADH. NADH could then be added to a mitochondrial suspension as a substrate for electron transport. When NADH was used as a substrate, ceramide decreased the respiration rate to the same extent as when glutamate was the substrate. What site(s) of inhibition can be ruled out?

**68.** When cells cannot carry out oxidative phosphorylation, they can synthesize ATP through substrate-level phosphorylation.
**(a)** Which enzymes of glycolysis and the citric acid cycle catalyze substrate-level phosphorylation?
**(b)** The $O_2$ that we breathe in is not directly converted to the $CO_2$ that we breathe out. Write a balanced equation for the complete combustion of glucose and oxygen.

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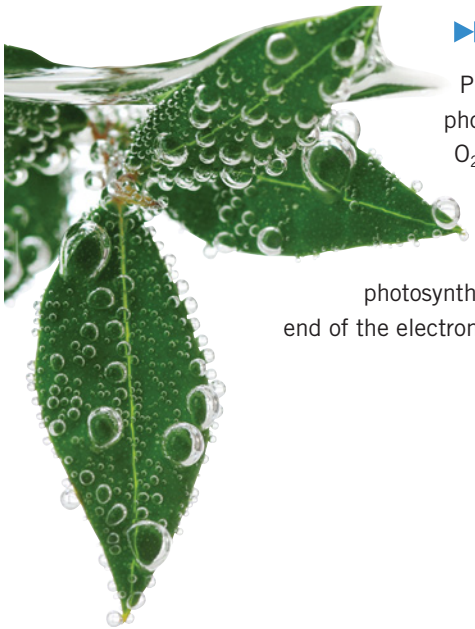
chapter 16

# PHOTOSYNTHESIS



[Elena Elisseeva/Alamy Limited]

## ▸▸ WHY do plants produce $O_2$?

Plants take up water and carbon dioxide and produce sugar during photosynthesis. A by-product of this process is molecular oxygen—$O_2$ gas—visible as tiny bubbles emanating from a plant placed under water. In the context of cellular respiration, $O_2$ is a vital reactant, providing the oxidizing power that is ultimately responsible for generating most of the cell's ATP. In this chapter, we'll explore the photosynthetic machinery and see why $O_2$, rather than being a key player at the end of the electron transport chain, is produced early in the photosynthetic process.

## THIS CHAPTER IN CONTEXT



### *Do You Remember?*

- **Glucose polymers include the fuel-storage polysaccharides starch and glycogen and the structural polysaccharide cellulose (Section 11-2).**
- **Coenzymes such as $NAD^+$ and ubiquinone collect electrons from compounds that become oxidized (Section 12-2).**
- **Electrons are transferred from a substance with a lower reduction potential to a substance with a higher reduction potential (Section 15-1).**
- **The formation of a transmembrane proton gradient during electron transport provides the free energy to synthesize ATP (Section 15-3).**