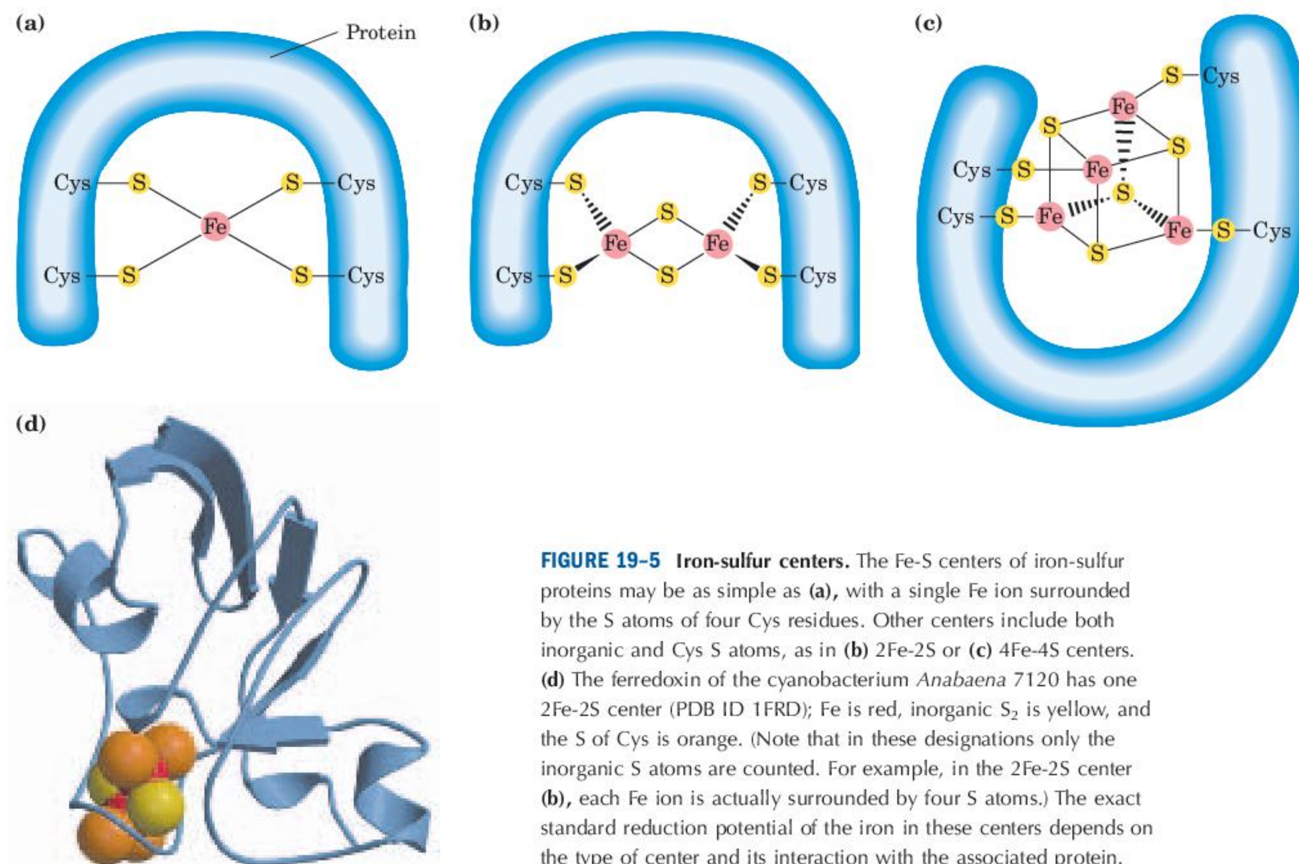


FIGURE 19–5 Iron-sulfur centers. The Fe-S centers of iron-sulfur proteins may be as simple as **(a)**, with a single Fe ion surrounded by the S atoms of four Cys residues. Other centers include both inorganic and Cys S atoms, as in **(b)** 2Fe-2S or **(c)** 4Fe-4S centers. **(d)** The ferredoxin of the cyanobacterium *Anabaena* 7120 has one 2Fe-2S center (PDB ID 1FRD); Fe is red, inorganic S_2 is yellow, and the S of Cys is orange. (Note that in these designations only the inorganic S atoms are counted. For example, in the 2Fe-2S center **(b)**, each Fe ion is actually surrounded by four S atoms.) The exact standard reduction potential of the iron in these centers depends on the type of center and its interaction with the associated protein.

First, the standard reduction potentials of the individual electron carriers have been determined experimentally (Table 19–2). We would expect the carriers to function in order of increasing reduction potential, because electrons tend to flow spontaneously from carriers of lower E'° to carriers of higher E'°. The order of carriers deduced by this method is NADH → Q → cytochrome b → cytochrome c_1 → cytochrome c → cytochrome a → cytochrome a_3 → O_2. Note, however, that the order of standard reduction potentials is not necessarily the same as the order of *actual* reduction potentials under cellular conditions, which depend on the concentration of reduced and oxidized forms (p. 510). A second method for determining the sequence

TABLE 19–2 Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

Redox reaction (half-reaction)	E'° *(V)*
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow$ NADH	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow$ NADPH	−0.324
NADH dehydrogenase (FMN) $+ 2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone $+ 2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome b (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome b (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome c (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome c (Fe^{2+})	0.254
Cytochrome a (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome a (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.8166

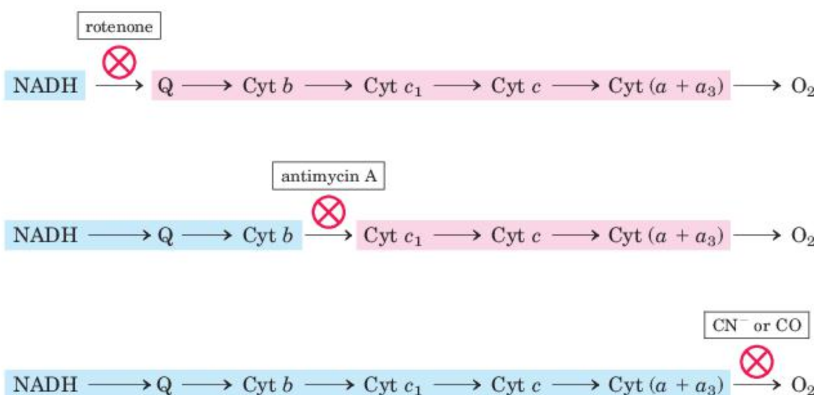


FIGURE 19–6 Method for determining the sequence of electron carriers. This method measures the effects of inhibitors of electron transfer on the oxidation state of each carrier. In the presence of an electron donor and O_2, each inhibitor causes a characteristic pattern of oxidized/reduced carriers: those before the block become reduced (blue), and those after the block become oxidized (pink).

of electron carriers involves reducing the entire chain of carriers experimentally by providing an electron source but no electron acceptor (no O_2). When O_2 is suddenly introduced into the system, the rate at which each electron carrier becomes oxidized (measured spectroscopically) reveals the order in which the carriers function. The carrier nearest O_2 (at the end of the chain) gives up its electrons first, the second carrier from the end is oxidized next, and so on. Such experiments have confirmed the sequence deduced from standard reduction potentials.

In a final confirmation, agents that inhibit the flow of electrons through the chain have been used in combination with measurements of the degree of oxidation of each carrier. In the presence of O_2 and an electron donor, carriers that function before the inhibited step become fully reduced, and those that function after this step are completely oxidized (Fig. 19–6). By using several inhibitors that block different steps in the chain, investigators have determined the entire sequence; it is the same as deduced in the first two approaches.

Electron Carriers Function in Multienzyme Complexes

The electron carriers of the respiratory chain are organized into membrane-embedded supramolecular complexes that can be physically separated. Gentle treatment of the inner mitochondrial membrane with detergents allows the resolution of four unique electron-carrier complexes, each capable of catalyzing electron transfer through a portion of the chain (Table 19–3; Fig. 19–7). Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II). Complex III carries electrons from reduced ubiquinone to cytochrome *c*, and Complex IV completes the sequence by transferring electrons from cytochrome *c* to O_2.

We now look in more detail at the structure and function of each complex of the mitochondrial respiratory chain.

Complex I: NADH to Ubiquinone Figure 19–8 illustrates the relationship between Complexes I and II and ubiquinone. **Complex I,** also called **NADH:ubiquinone oxidoreductase** or **NADH dehydrogenase,** is a large enzyme composed of 42 different polypeptide chains, including an FMN-containing flavoprotein and at least six iron-sulfur centers. High-resolution electron microscopy shows Complex I to be L-shaped, with one arm of the L in the membrane and the other extending into the matrix. As shown in Figure 19–9, Complex I catalyzes two simultaneous and obligately coupled processes: (1) the

TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

Enzyme complex/protein	*Mass (kDa)*	*Number of subunits**	*Prosthetic group(s)*
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome *c*†	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

*Numbers of subunits in the bacterial equivalents in parentheses.
†Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

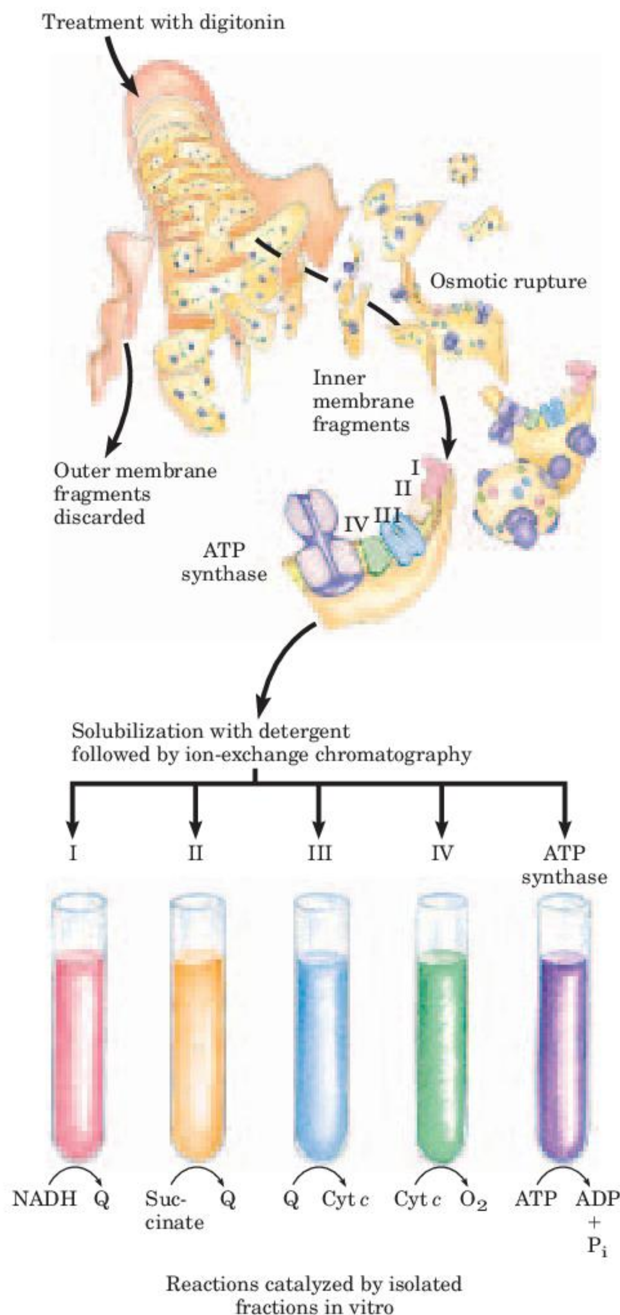


FIGURE 19–7 Separation of functional complexes of the respiratory chain. The outer mitochondrial membrane is first removed by treatment with the detergent digitonin. Fragments of inner membrane are then obtained by osmotic rupture of the mitochondria, and the fragments are gently dissolved in a second detergent. The resulting mixture of inner membrane proteins is resolved by ion-exchange chromatography into different complexes (I through IV) of the respiratory chain, each with its unique protein composition (see Table 19–3), and the enzyme ATP synthase (sometimes called Complex V). The isolated Complexes I through IV catalyze transfers between donors (NADH and succinate), intermediate carriers (Q and cytochrome c), and O_2, as shown. In vitro, isolated ATP synthase has only ATP-hydrolyzing (ATPase), not ATP-synthesizing, activity.

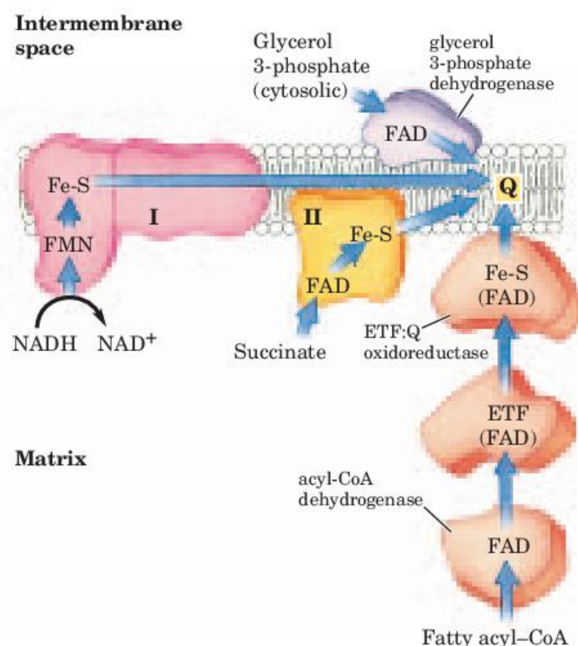
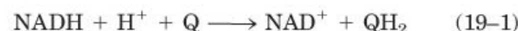


FIGURE 19–8 Path of electrons from NADH, succinate, fatty acyl–CoA, and glycerol 3-phosphate to ubiquinone. Electrons from NADH pass through a flavoprotein to a series of iron-sulfur proteins (in Complex I) and then to Q. Electrons from succinate pass through a flavoprotein and several Fe-S centers (in Complex II) on the way to Q. Glycerol 3-phosphate donates electrons to a flavoprotein (glycerol 3-phosphate dehydrogenase) on the outer face of the inner mitochondrial membrane, from which they pass to Q. Acyl-CoA dehydrogenase (the first enzyme of β oxidation) transfers electrons to electron-transferring flavoprotein (ETF), from which they pass to Q via ETF:ubiquinone oxidoreductase.

exergonic transfer to ubiquinone of a hydride ion from NADH and a proton from the matrix, expressed by



$$\text{NADH} + \text{H}^+ + \text{Q} \longrightarrow \text{NAD}^+ + \text{QH}_2 \qquad (19\text{–}1)$$

and (2) the endergonic transfer of four protons from the matrix to the intermembrane space. Complex I is therefore a proton pump driven by the energy of electron transfer, and the reaction it catalyzes is **vectorial:** it moves protons in a specific direction from one location (the matrix, which becomes negatively charged with the departure of protons) to another (the intermembrane space, which becomes positively charged). To emphasize the vectorial nature of the process, the overall reaction is often written with subscripts that indicate the location of the protons: P for the positive side of the inner membrane (the intermembrane space), N for the negative side (the matrix):

$$\text{NADH} + 5\text{H}^+_\text{N} + \text{Q} \longrightarrow \text{NAD}^+ + \text{QH}_2 + 4\text{H}^+_\text{P} \quad (19\text{–}2)$$

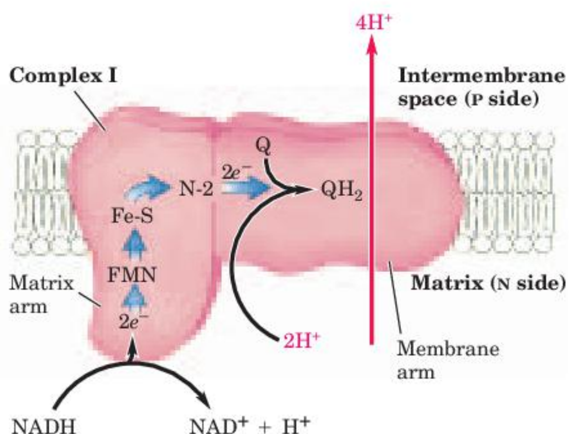


FIGURE 19–9 NADH:ubiquinone oxidoreductase (Complex I). Complex I catalyzes the transfer of a hydride ion from NADH to FMN, from which two electrons pass through a series of Fe-S centers to the iron-sulfur protein N-2 in the matrix arm of the complex. Electron transfer from N-2 to ubiquinone on the membrane arm forms QH_2, which diffuses into the lipid bilayer. This electron transfer also drives the expulsion from the matrix of four protons per pair of electrons. The detailed mechanism that couples electron and proton transfer in Complex I is not yet known, but probably involves a Q cycle similar to that in Complex III in which QH_2 participates twice per electron pair (see Fig. 19–12). Proton flux produces an electrochemical potential across the inner mitochondrial membrane (N side negative, P side positive), which conserves some of the energy released by the electron-transfer reactions. This electrochemical potential drives ATP synthesis.

Amytal (a barbiturate drug), rotenone (a plant product commonly used as an insecticide), and piericidin A (an antibiotic) inhibit electron flow from the Fe-S centers of Complex I to ubiquinone (Table 19–4) and therefore block the overall process of oxidative phosphorylation.

Ubiquinol (QH_2, the fully reduced form; Fig. 19–2) diffuses in the inner mitochondrial membrane from Complex I to Complex III, where it is oxidized to Q in a process that also involves the outward movement of H^+.

Complex II: Succinate to Ubiquinone We encountered **Complex II** in Chapter 16 as **succinate dehydrogenase,** the only membrane-bound enzyme in the citric acid cycle (p. 612). Although smaller and simpler than Complex I, it contains five prosthetic groups of two types and four different protein subunits (Fig. 19–10). Subunits C and D are integral membrane proteins, each with three transmembrane helices. They contain a heme group, heme *b,* and a binding site for ubiquinone, the final electron acceptor in the reaction catalyzed by Complex II. Subunits A and B extend into the matrix (or the cytosol of a bacterium); they contain three 2Fe-2S centers, bound FAD, and a binding site for the substrate, succinate. The path of electron transfer from the succinate-binding site to FAD, then through the Fe-S centers to the Q-binding site, is more than 40 Å long, but none of the individual electron-transfer distances exceeds about 11 Å—a reasonable distance for rapid electron transfer (Fig. 19–10).

TABLE 19–4 Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation

Type of interference	*Compound**	*Target/mode of action*
Inhibition of electron transfer	Cyanide	Inhibit cytochrome oxidase
	Carbon monoxide	
	Antimycin A	Blocks electron transfer from cytochrome *b* to cytochrome c_1
	Myxothiazol	Prevent electron transfer from Fe-S center to ubiquinone
	Rotenone	
	Amytal	
	Piericidin A	
	DCMU	Competes with Q_B for binding site in PSII
Inhibition of ATP synthase	Aurovertin	Inhibits F_1
	Oligomycin	Inhibit F_o and CF_o
	Venturicidin	
	DCCD	Blocks proton flow through F_o and CF_o
Uncoupling of phosphorylation from electron transfer	FCCP	Hydrophobic proton carriers
	DNP	
	Valinomycin	K^+ ionophore
	Thermogenin	In brown fat, forms proton-conducting pores in inner mitochondrial membrane
Inhibition of ATP-ADP exchange	Atractyloside	Inhibits adenine nucleotide translocase

*DCMU is 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCCD, dicyclohexylcarbodiimide; FCCP, cyanide-*p*-trifluoromethoxyphenylhydrazone; DNP, 2,4-dinitrophenol.

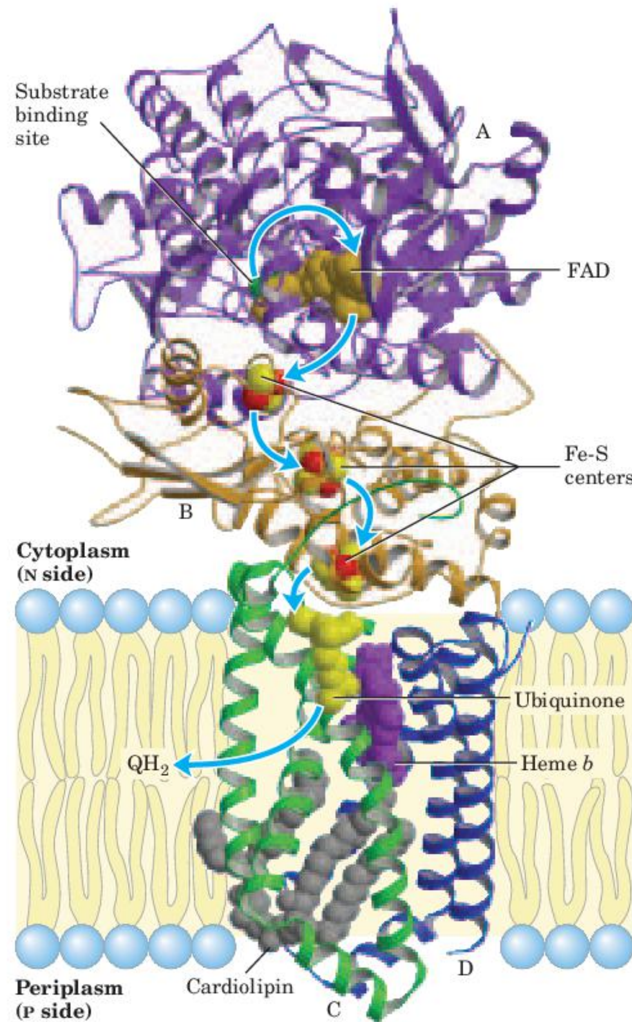
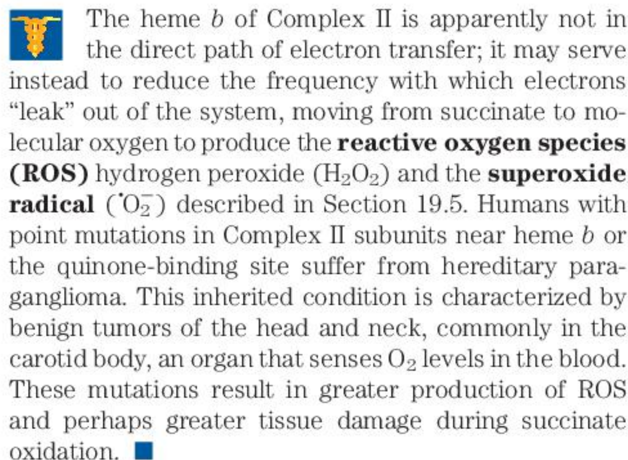


FIGURE 19–10 Structure of Complex II (succinate dehydrogenase) of *E. coli* (PDB ID 1NEK). The enzyme has two transmembrane subunits, C (green) and D (blue); the cytoplasmic extensions contain subunits B (orange) and A (purple). Just behind the FAD in subunit A (gold) is the binding site for succinate (occupied in this crystal structure by the inhibitor oxaloacetate, green). Subunit B has three sets of Fe-S centers (yellow and red); ubiquinone (yellow) is bound to subunit C; and heme *b* (purple) is sandwiched between subunits C and D. A cardiolipin molecule is so tightly bound to subunit C that it shows up in the crystal structure (gray spacefilling). Electrons move (blue arrows) from succinate to FAD, then through the three Fe-S centers to ubiquinone. The heme *b* is not on the main path of electron transfer but protects against the formation of reactive oxygen species (ROS) by electrons that go astray.



The heme *b* of Complex II is apparently not in the direct path of electron transfer; it may serve instead to reduce the frequency with which electrons "leak" out of the system, moving from succinate to molecular oxygen to produce the **reactive oxygen species (ROS)** hydrogen peroxide (H_2O_2) and the **superoxide radical** ($\cdot O_2^-$) described in Section 19.5. Humans with point mutations in Complex II subunits near heme *b* or the quinone-binding site suffer from hereditary paraganglioma. This inherited condition is characterized by benign tumors of the head and neck, commonly in the carotid body, an organ that senses O_2 levels in the blood. These mutations result in greater production of ROS and perhaps greater tissue damage during succinate oxidation. ■

Other substrates for mitochondrial dehydrogenases pass electrons into the respiratory chain at the level of ubiquinone, but not through Complex II. The first step in the β oxidation of fatty acyl–CoA, catalyzed by the flavoprotein **acyl-CoA dehydrogenase** (see Fig. 17–8), involves transfer of electrons from the substrate to the FAD of the dehydrogenase, then to electron-transferring flavoprotein (ETF), which in turn passes its electrons to **ETF : ubiquinone oxidoreductase** (Fig. 19–8). This enzyme transfers electrons into the respiratory chain by reducing ubiquinone. Glycerol 3-phosphate, formed either from glycerol released by triacylglycerol breakdown or by the reduction of dihydroxyacetone phosphate from glycolysis, is oxidized by **glycerol 3-phosphate dehydrogenase** (see Fig. 17–4). This enzyme is a flavoprotein located on the outer face of the inner mitochondrial membrane, and like succinate dehydrogenase and acyl-CoA dehydrogenase it channels electrons into the respiratory chain by reducing ubiquinone (Fig. 19–8). The important role of glycerol 3-phosphate dehydrogenase in shuttling reducing equivalents from cytosolic NADH into the mitochondrial matrix is described in Section 19.2 (see Fig. 19–28). The effect of each of these electron-transferring enzymes is to contribute to the pool of reduced ubiquinone. QH_2 from all these reactions is reoxidized by Complex III.

Complex III: Ubiquinone to Cytochrome c The next respiratory complex, **Complex III,** also called **cytochrome *bc*$_1$ complex** or **ubiquinone:cytochrome *c* oxidoreductase,** couples the transfer of electrons from ubiquinol (QH_2) to cytochrome *c* with the vectorial transport of protons from the matrix to the intermembrane space. The determination of the complete structure of this huge complex (Fig. 19–11) and of Complex IV (below) by x-ray crystallography, achieved between 1995 and 1998, were landmarks in the study of mitochondrial electron transfer, providing the structural framework to integrate the many biochemical observations on the functions of the respiratory complexes.

Based on the structure of Complex III and detailed biochemical studies of the redox reactions, a reasonable model has been proposed for the passage of electrons

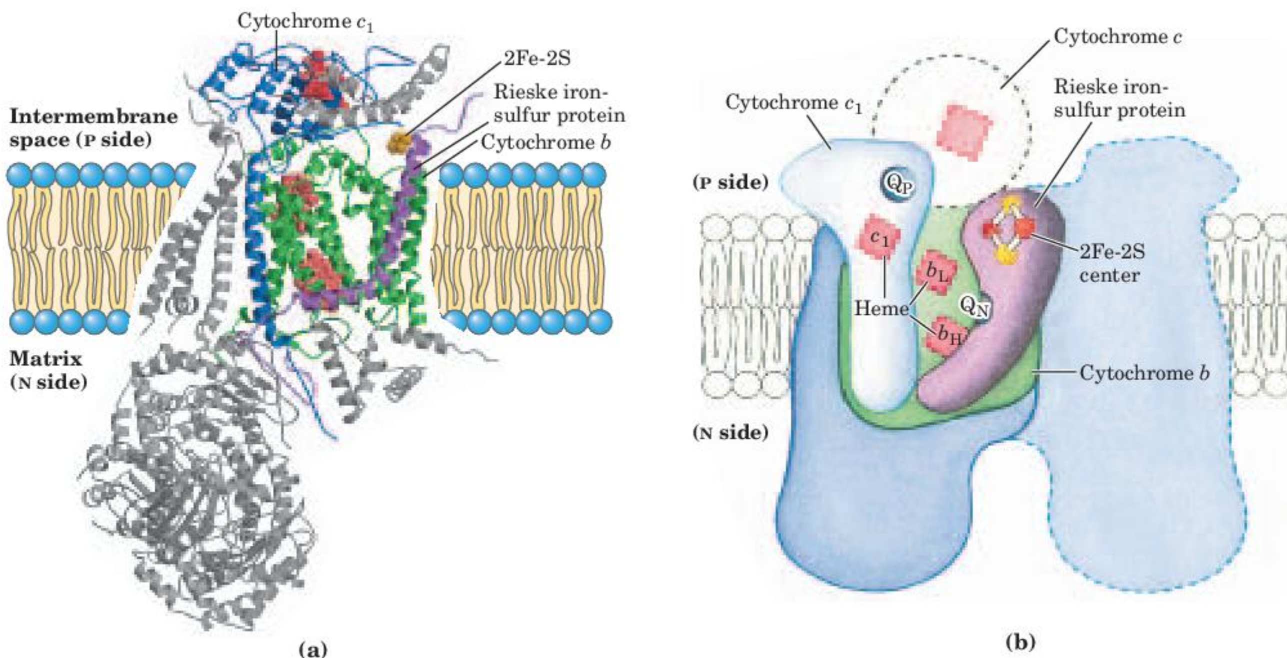
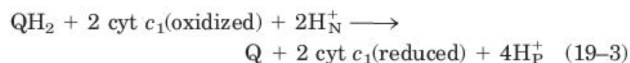


FIGURE 19–11 Cytochrome bc_1 complex (Complex III). The complex is a dimer of identical monomers, each with 11 different subunits. **(a)** Structure of a monomer. The functional core is three subunits: cytochrome b (green) with its two hemes (b_H and b_L, light red); the Rieske iron-sulfur protein (purple) with its 2Fe-2S centers (yellow); and cytochrome c_1 (blue) with its heme (red) (PDB ID 1BGY). **(b)** The dimeric functional unit. Cytochrome c_1 and the Rieske iron-sulfur protein project from the P surface and can interact with cytochrome c (not part of the functional complex) in the intermembrane space. The complex has two distinct binding sites for ubiquinone, Q_N and Q_P, which correspond to the sites of inhibition by two drugs that block oxidative phosphorylation. Antimycin A, which blocks electron flow from heme b_H to Q, binds at Q_N, close to heme b_H on the N (matrix) side of the membrane. Myxothiazol, which prevents electron flow from QH_2 to the Rieske iron-sulfur protein, binds at Q_P, near the 2Fe-2S center and heme b_L on the P side. The dimeric structure is essential to the function of Complex III. The interface between monomers forms two pockets, each containing a Q_P site from one monomer and a Q_N site from the other. The ubiquinone intermediates move within these sheltered pockets.

Complex III crystallizes in two distinct conformations (not shown). In one, the Rieske Fe-S center is close to its electron acceptor, the heme of cytochrome c_1, but relatively distant from cytochrome b and the QH_2-binding site at which the Rieske Fe-S center receives electrons. In the other, the Fe-S center has moved away from cytochrome c_1 and toward cytochrome b. The Rieske protein is thought to oscillate between these two conformations as it is reduced, then oxidized.

and protons through the complex. The net equation for the redox reactions of this **Q cycle** (Fig. 19–12) is



$$QH_2 + 2\ \text{cyt}\ c_1(\text{oxidized}) + 2H^+_N \longrightarrow Q + 2\ \text{cyt}\ c_1(\text{reduced}) + 4H^+_P \quad (19\text{–}3)$$

The Q cycle accommodates the switch between the two-electron carrier ubiquinone and the one-electron carriers—cytochromes b_{562}, b_{566}, c_1, and c—and explains the measured stoichiometry of four protons translocated per pair of electrons passing through the Complex III to cytochrome c. Although the path of electrons through this segment of the respiratory chain is complicated, the net effect of the transfer is simple: QH_2 is oxidized to Q and two molecules of cytochrome c are reduced.

Cytochrome c (see Fig. 4–18) is a soluble protein of the intermembrane space. After its single heme accepts an electron from Complex III, cytochrome c moves to Complex IV to donate the electron to a binuclear copper center.

Complex IV: Cytochrome c to O_2 In the final step of the respiratory chain, **Complex IV,** also called **cytochrome oxidase,** carries electrons from cytochrome c to molecular oxygen, reducing it to H_2O. Complex IV is a large enzyme (13 subunits; M_r 204,000) of the inner mitochondrial membrane. Bacteria contain a form that is much simpler, with only three or four subunits, but still capable of catalyzing both electron transfer and proton pumping. Comparison of the mitochondrial and bacterial complexes suggests that three subunits are critical to the function (Fig. 19–13).

Mitochondrial subunit II contains two Cu ions complexed with the —SH groups of two Cys residues in a binuclear center (Cu_A; Fig. 19–13b) that resembles the 2Fe-2S centers of iron-sulfur proteins. Subunit I contains two heme groups, designated a and a_3, and another copper ion (Cu_B). Heme a_3 and Cu_B form a second binuclear center that accepts electrons from heme a and transfers them to O_2 bound to heme a_3.

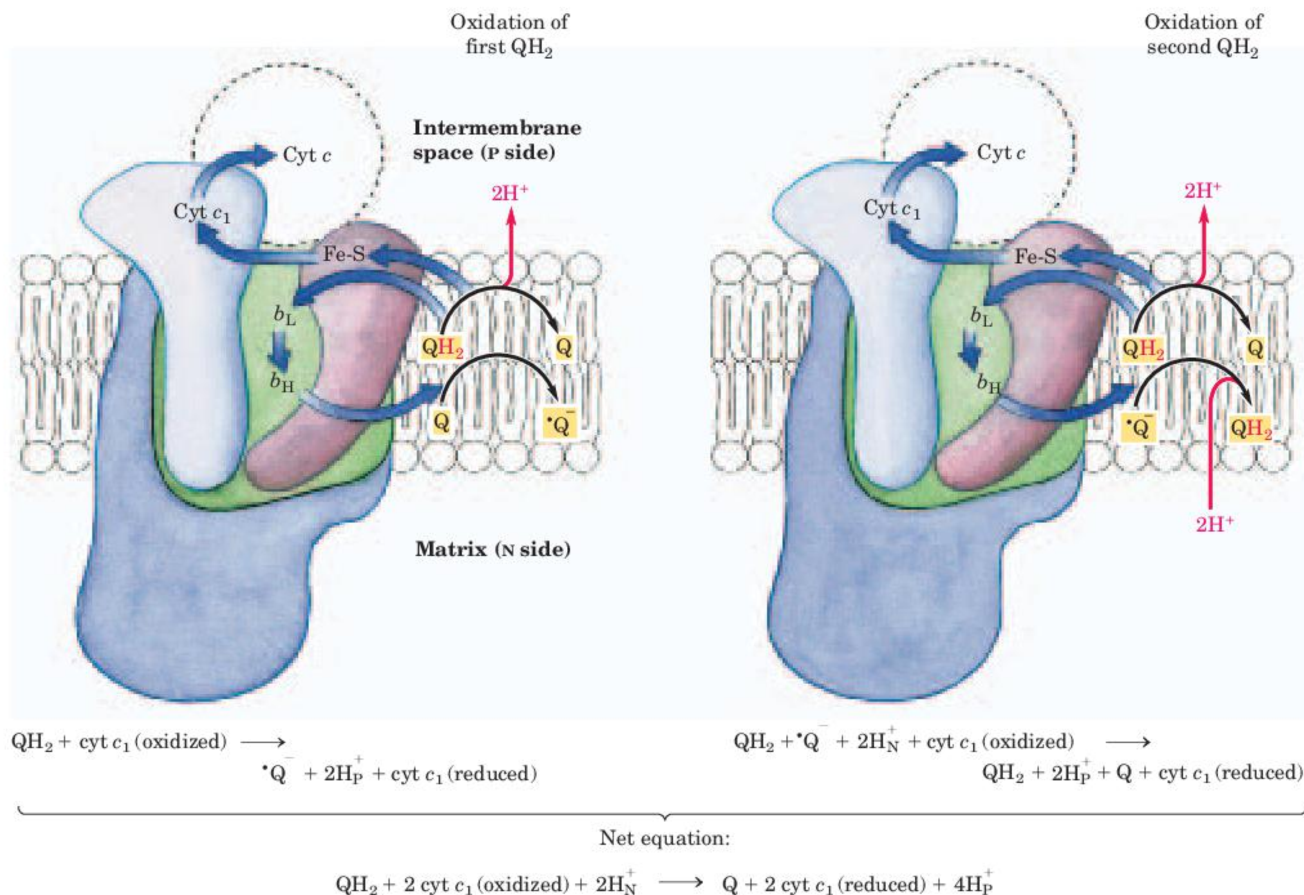
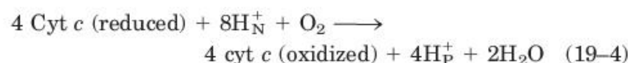


FIGURE 19–12 The Q cycle. The path of electrons through Complex III is shown by blue arrows. On the P side of the membrane, two molecules of QH_2 are oxidized to Q near the P side, releasing two protons per Q (four protons in all) into the intermembrane space. Each QH_2 donates one electron (via the Rieske Fe-S center) to cytochrome c_1, and one electron (via cytochrome *b*) to a molecule of Q near the N side, reducing it in two steps to QH_2. This reduction also uses two protons per Q, which are taken up from the matrix.

Electron transfer through Complex IV is from cytochrome *c* to the Cu_A center, to heme *a*, to the heme a_3–Cu_B center, and finally to O_2 (Fig. 19–14). For every four electrons passing through this complex, the enzyme consumes four "substrate" H^+ from the matrix (N side) in converting O_2 to $2H_2O$. It also uses the energy of this redox reaction to pump one proton outward into the intermembrane space (P side) for each electron that passes through, adding to the electrochemical potential produced by redox-driven proton transport through Complexes I and III. The overall reaction catalyzed by Complex IV is



$$4\ \text{Cyt}\ c\ (\text{reduced}) + 8H^+_N + O_2 \longrightarrow 4\ \text{cyt}\ c\ (\text{oxidized}) + 4H^+_P + 2H_2O \qquad (19\text{–}4)$$

This four-electron reduction of O_2 involves redox centers that carry only one electron at a time, and it must occur without the release of incompletely reduced intermediates such as hydrogen peroxide or hydroxyl free radicals—very reactive species that would damage cellular components. The intermediates remain tightly bound to the complex until completely converted to water.

The Energy of Electron Transfer Is Efficiently Conserved in a Proton Gradient

The transfer of two electrons from NADH through the respiratory chain to molecular oxygen can be written as

$$\text{NADH} + H^+ + \tfrac{1}{2}O_2 \longrightarrow \text{NAD}^+ + H_2O \qquad (19\text{–}5)$$

This net reaction is highly exergonic. For the redox pair NAD^+/NADH, E'° is -0.320 V, and for the pair O_2/H_2O, E'° is 0.816 V. The $\Delta E'^\circ$ for this reaction is therefore 1.14 V, and the standard free-energy change (see Eqn 13–6, p. 510) is

$$\begin{aligned}\Delta G'^\circ &= -n\mathcal{F}\Delta E'^\circ \qquad (19\text{–}6)\\ &= -2(96.5\ \text{kJ/V}\cdot\text{mol})(1.14\ \text{V})\\ &= -220\ \text{kJ/mol (of NADH)}\end{aligned}$$

This *standard* free-energy change is based on the assumption of equal concentrations (1 M) of NADH and