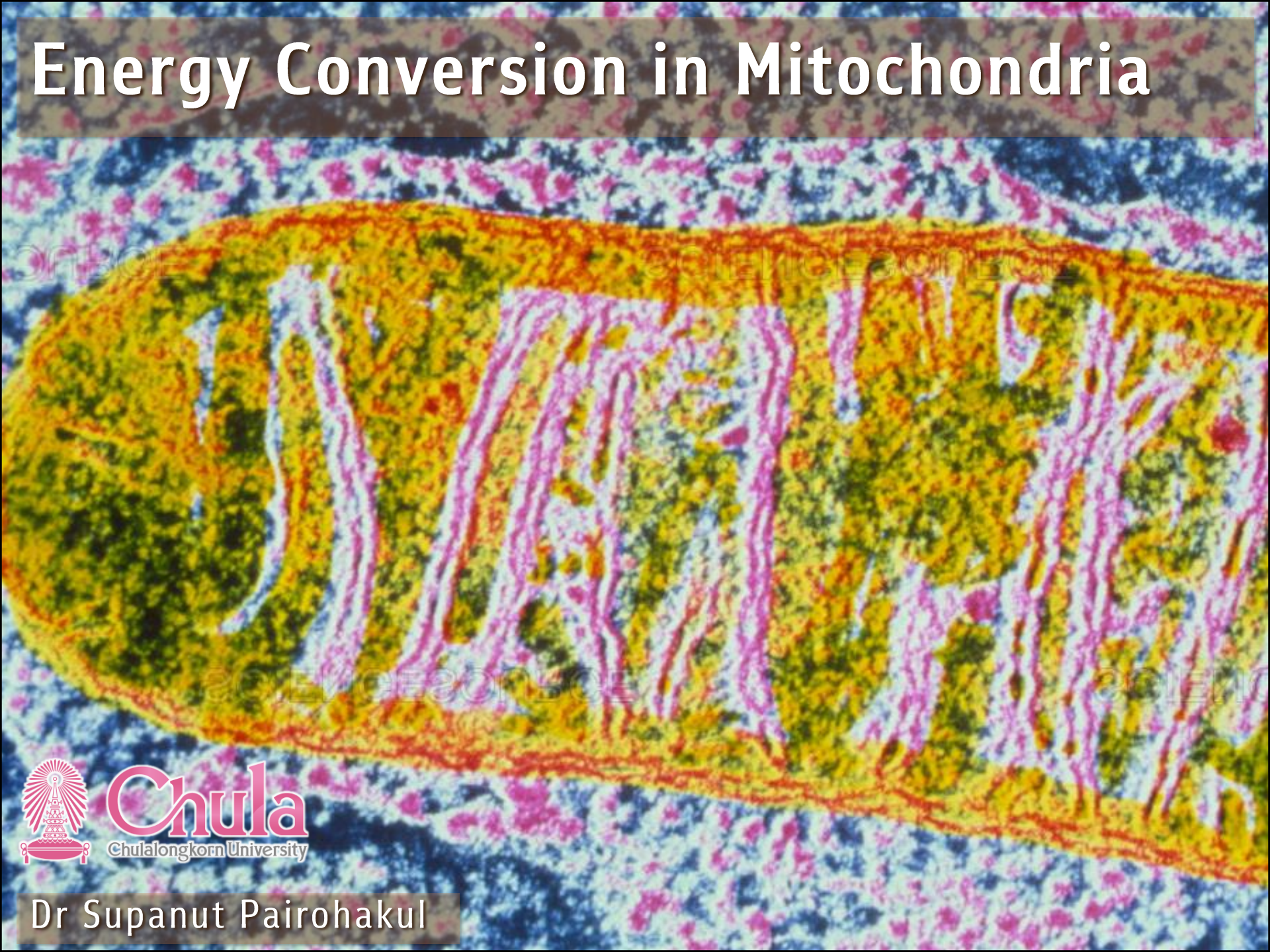
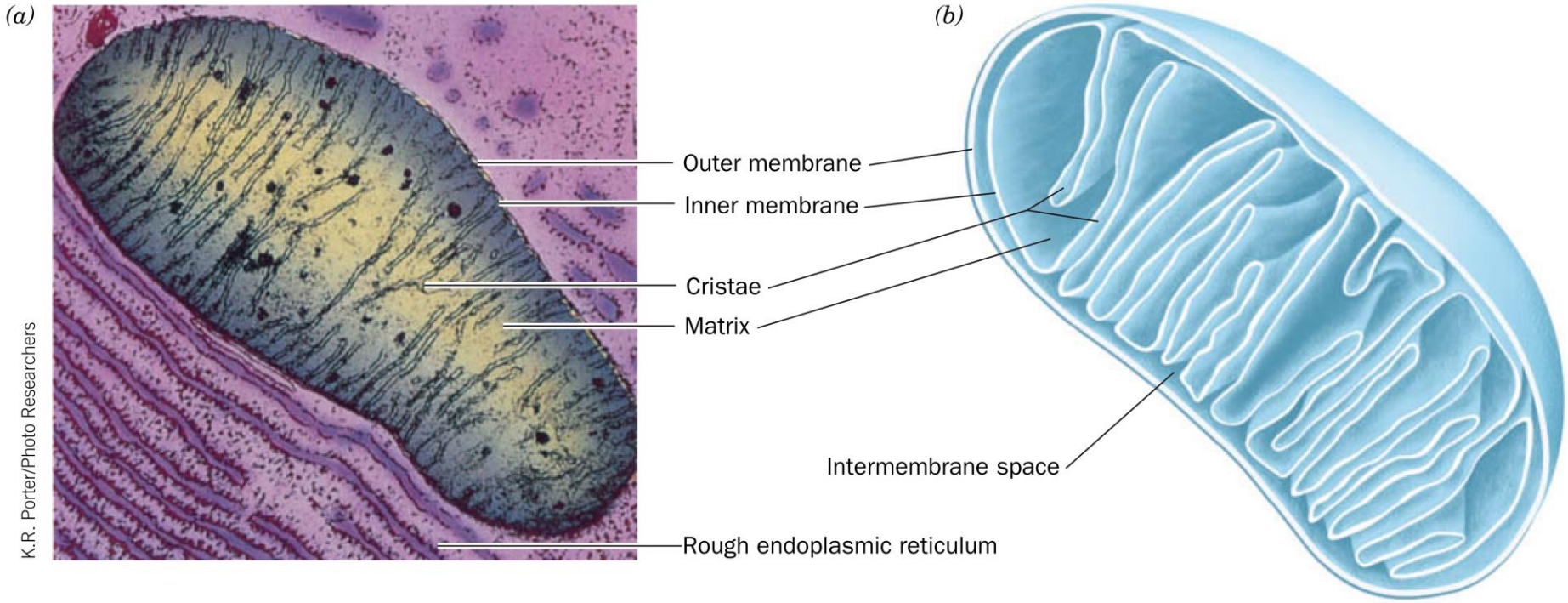
Energy Conversion in Mitochondria



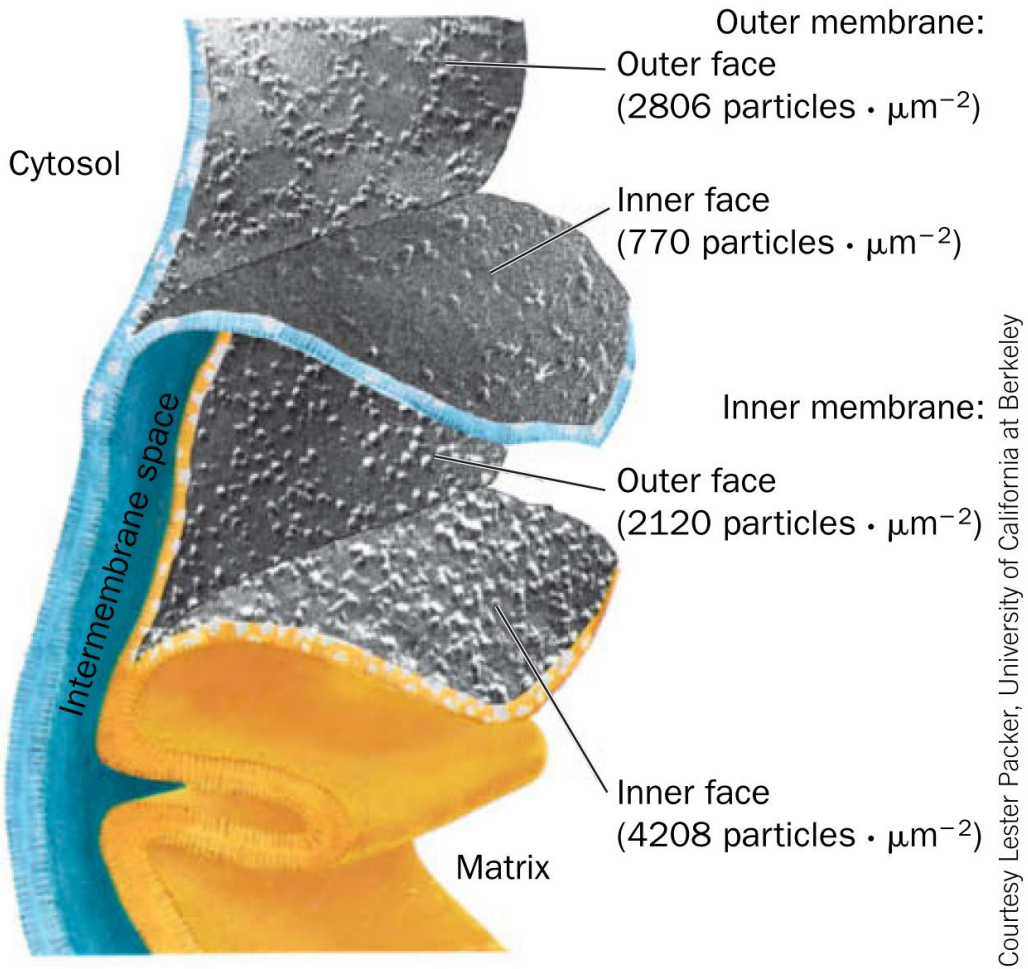
Chula
Chulalongkorn University

Dr Supanut Pairohakul

Mitochondria



Mitochondria

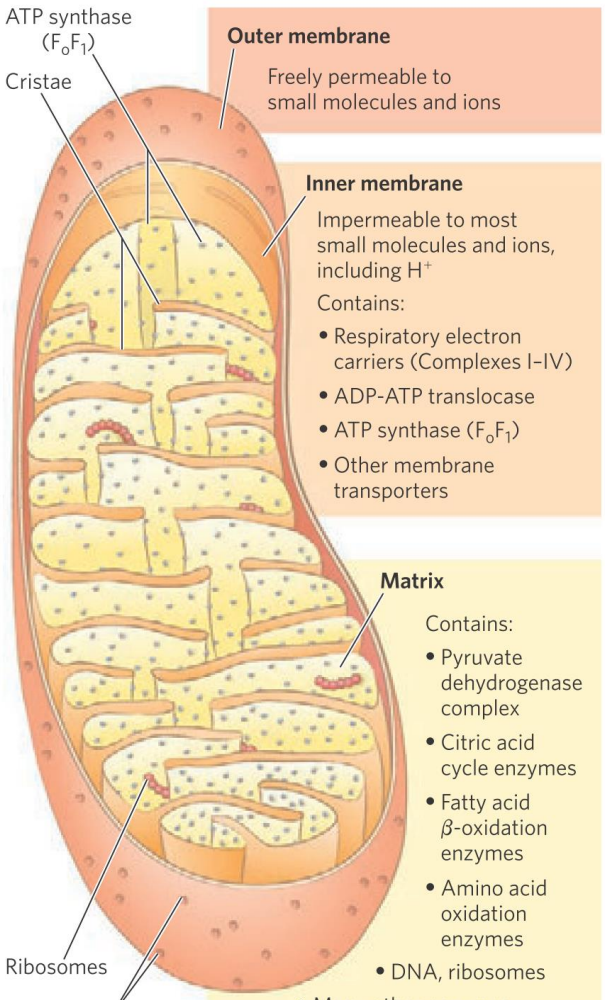


Courtesy Lester Packer, University of California at Berkeley

FIG. 18-4 Electron micrographs of the inner and outer mitochondrial membranes that have been split to expose the inner surfaces of their bilayer leaflets. Note that the inner membrane contains about twice the density of embedded particles as does the outer membrane. The particles are the portions of integral membrane proteins that were exposed when the bilayers were split.

Mitochondria

(a)



Outer membrane

Freely permeable to small molecules and ions

Inner membrane

Impermeable to most small molecules and ions, including H^+

Contains:

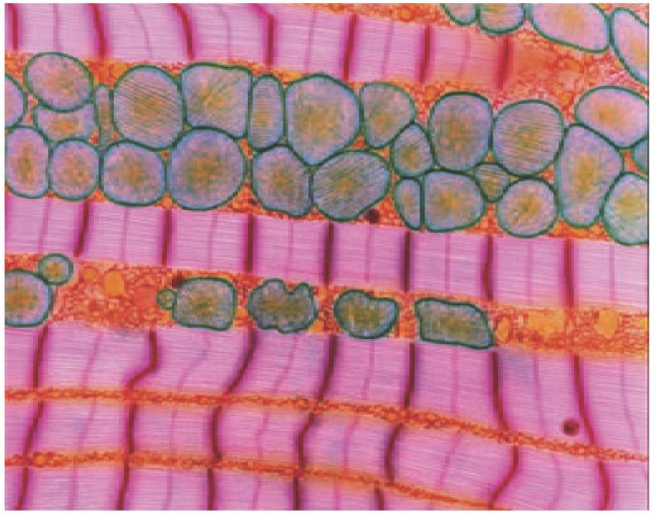
- Respiratory electron carriers (Complexes I–IV)
- ADP-ATP translocase
- ATP synthase (F_oF_1)
- Other membrane transporters

Matrix

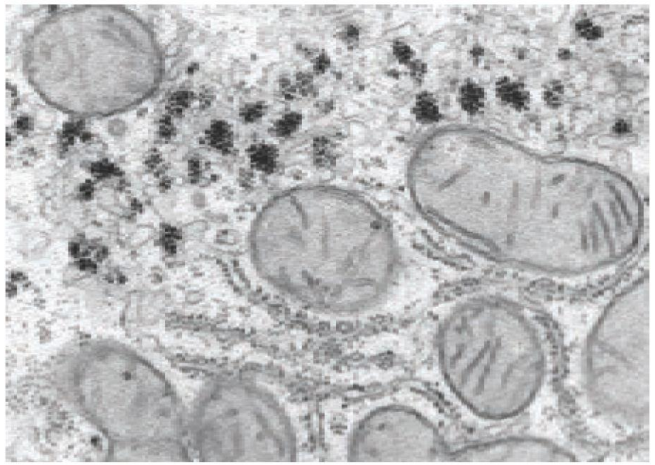
Contains:

- Pyruvate dehydrogenase complex
- Citric acid cycle enzymes
- Fatty acid β-oxidation enzymes
- Amino acid oxidation enzymes
- DNA, ribosomes
- Many other enzymes
- ATP, ADP, P_i, Mg^{2+}, Ca^{2+}, K^+
- Many soluble metabolic intermediates

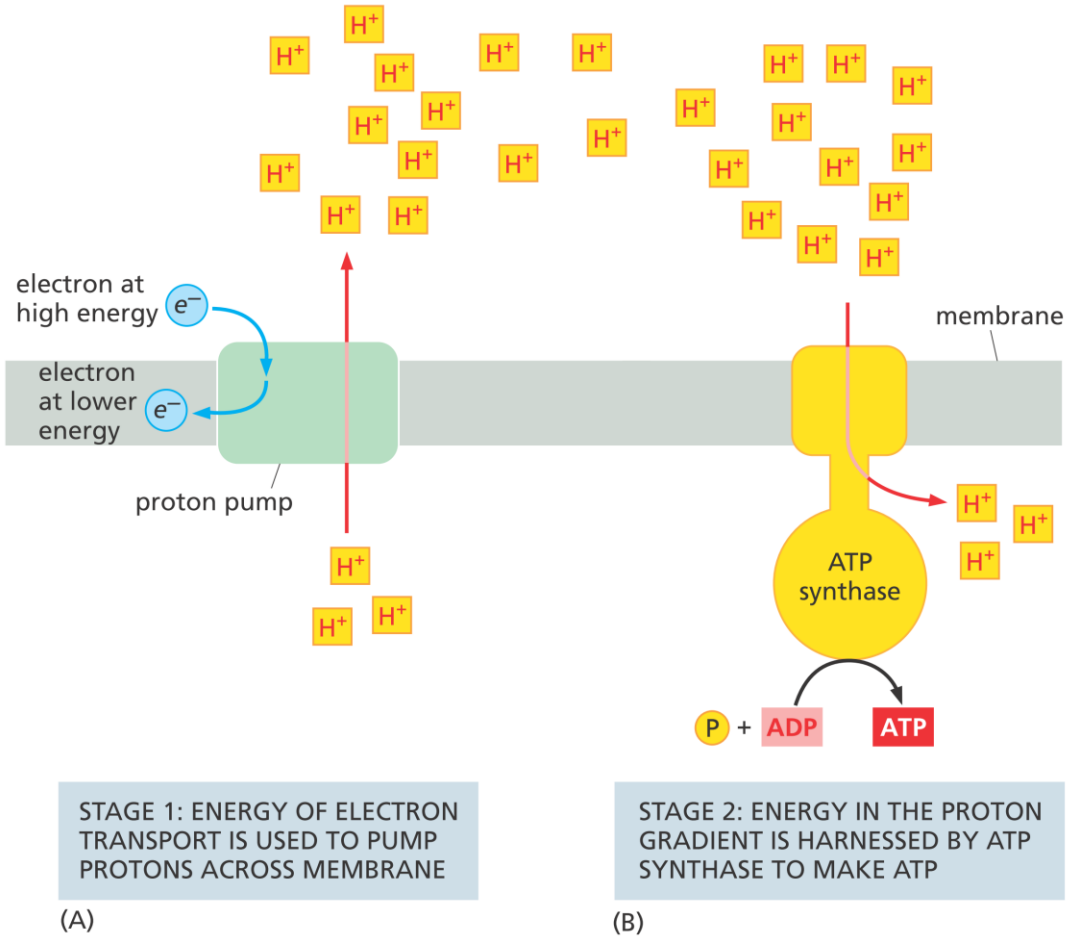
(b)



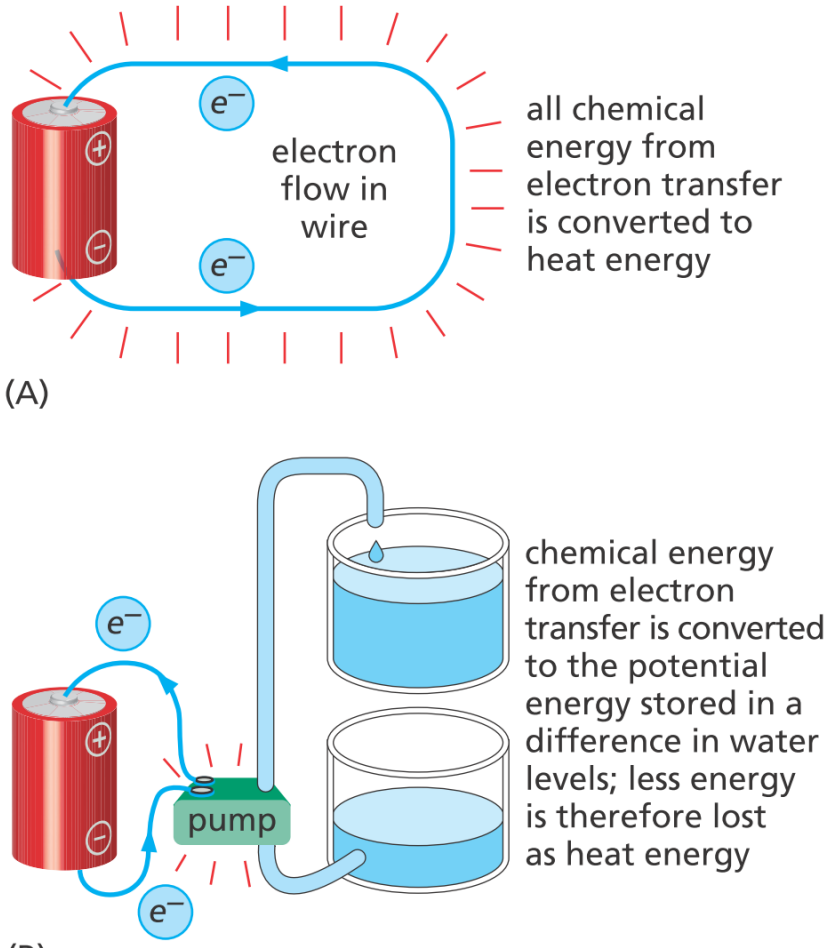
(c)



An Overview of Oxidative Phosphorylation

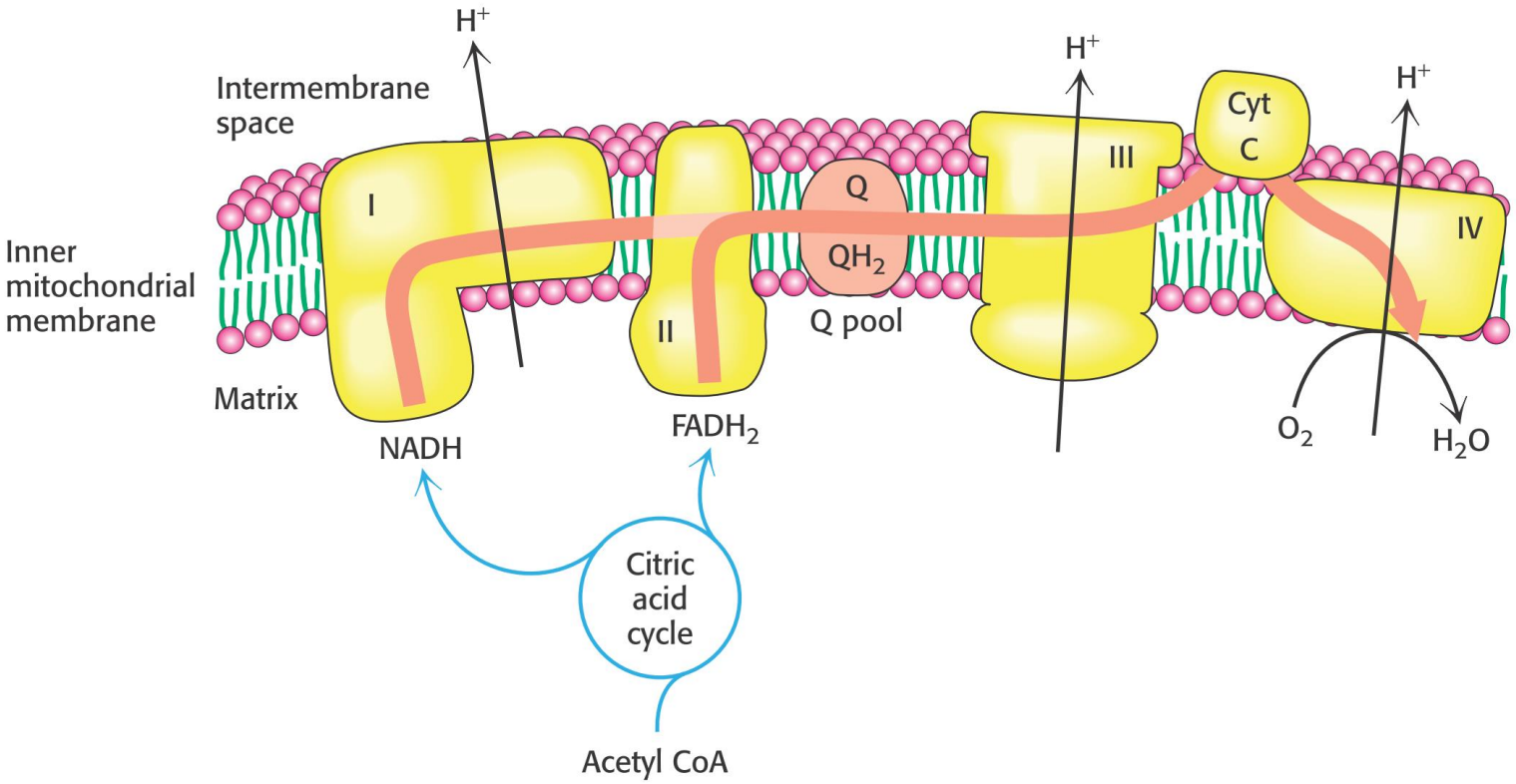


An Overview of Oxidative Phosphorylation



Electron Transport

- An Overview of Electron Transport



Electron Transport

- Reduction Potential (E_0')
  - Redox potential / oxidation-reduction potential
  - The measurement of the electromotive force generated by a sample half-cell connected to a standard reference half-cell
  - Electron flow from one half-cell to the other through the wire connecting the two cells

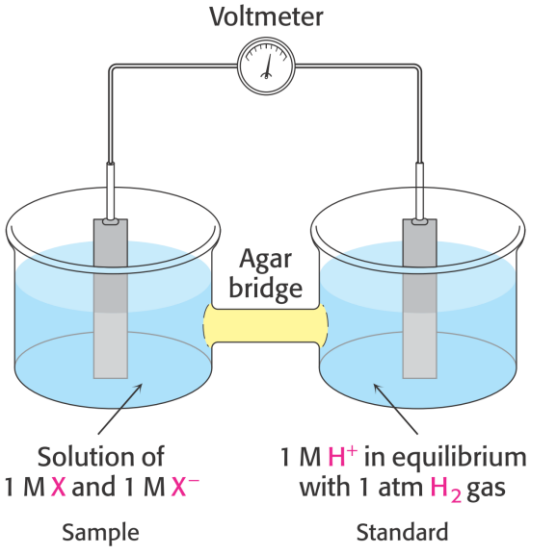


Figure 20.4 The measurement of redox potential. Apparatus for the measurement of the standard oxidation–reduction potential of a redox couple. Electrons flow through the wire connecting the cells, whereas ions flow through the agar bridge.

Electron Transport

Table 20.1 Standard reduction potentials of some reactions

Oxidant	Reductant	n	E_0' (V)
Succinate + CO_2	α-Ketoglutarate	2	−0.67
Acetate	Acetaldehyde	2	−0.60
Ferredoxin (oxidized)	Ferredoxin (reduced)	1	−0.43
2 H^+	H_2	2	−0.42
NAD^+	NADH + H^+	2	−0.32
$NADP^+$	NADPH + H^+	2	−0.32
Lipoate (oxidized)	Lipoate (reduced)	2	−0.29
Glutathione (oxidized)	Glutathione (reduced)	2	−0.23
FAD	$FADH_2$	2	−0.22
Acetaldehyde	Ethanol	2	−0.20
Pyruvate	Lactate	2	−0.19
2 H^+	H_2	2	0.00[1]
Cytochrome *b* (+3)	Cytochrome *b* (+2)	1	+0.07
Dehydroascorbate	Ascorbate	2	+0.08
Ubiquinone (oxidized)	Ubiquinone (reduced)	2	+0.10
Cytochrome *c* (+3)	Cytochrome *c* (+2)	1	+0.22
Fe (+3)	Fe (+2)	1	+0.77
½ O_2 + 2 H^+	H_2O	2	+0.82

Note: E_0' is the standard oxidation–reduction potential (pH 7, 25°C, except where noted), and n is the number of electrons transferred. E_0' refers to the partial reaction written as Oxidant + e^- ⟶ reductant

[1]Standard oxidation–reduction potential at pH = 0.

Electron Transport

- Reduction Potential (E_0')
  - The standard free-energy change $\Delta G°$ is related to the change in reduction potential ΔE_0' by

$$\Delta G^{0\prime} = -nF\Delta E_0'$$

  - How much energy is released by the reduction of O_2 with NADH?

Electron Transport

- Electron Carriers

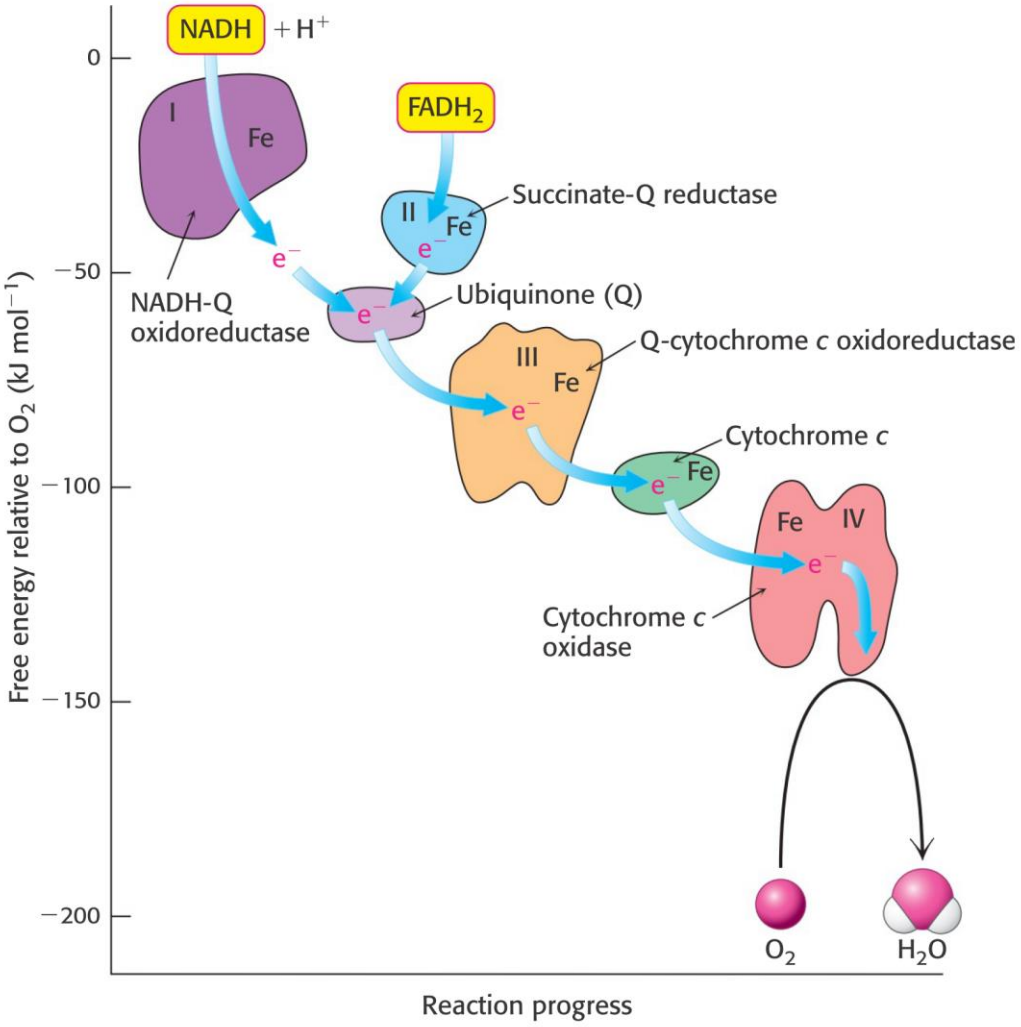


Figure 20.6 Components of the electron-transport chain. Electrons flow down an energy gradient from NADH to O_2. The flow is catalyzed by four protein complexes. Iron is a component of all of the complexes as well as cytochrome *c*. [Data from D. Sadava et al., *Life,* 8th ed. (Sinauer, 2008), p. 150.]

Electron Transport

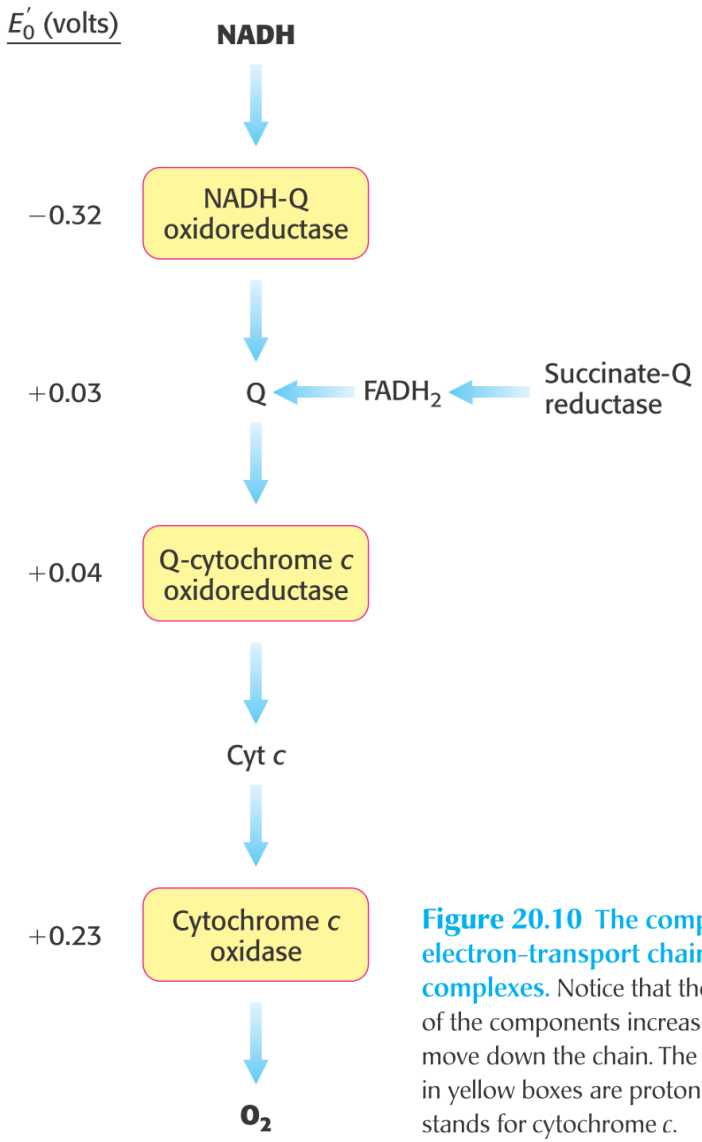


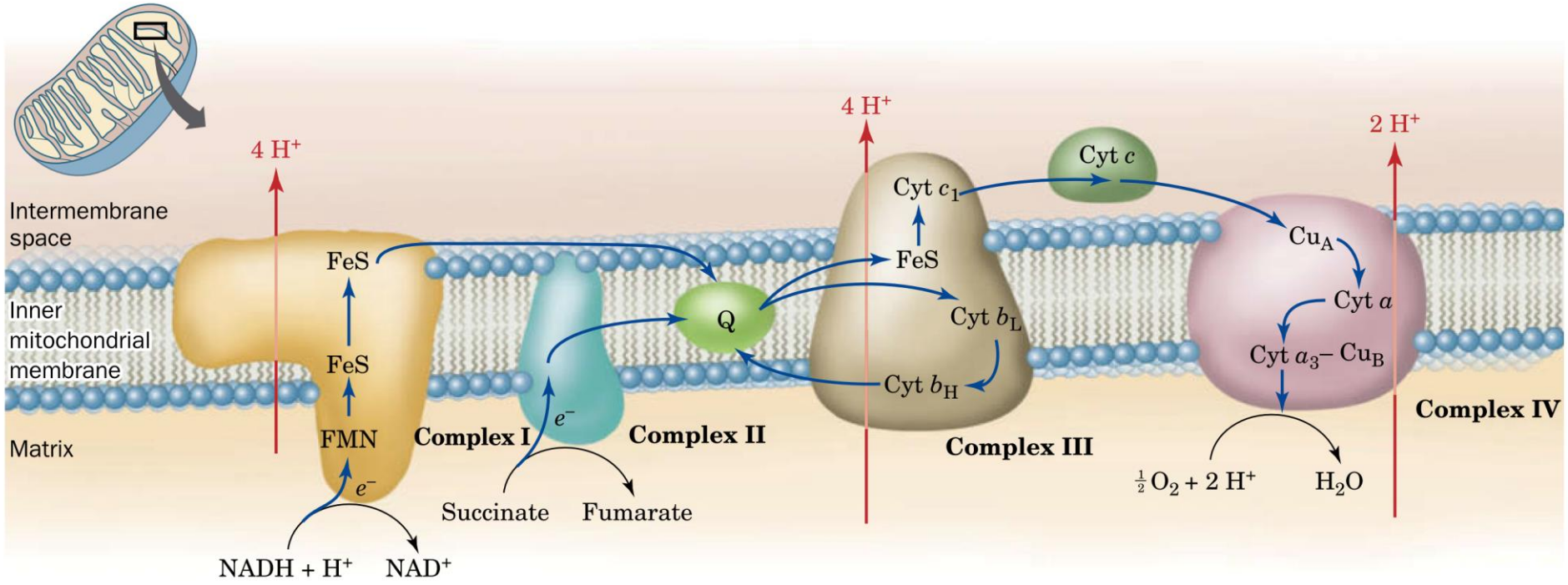
Figure 20.10 The components of the electron-transport chain are arranged in complexes. Notice that the electron affinity of the components increases as electrons move down the chain. The complexes shown in yellow boxes are proton pumps. Cyt *c* stands for cytochrome *c*.

TABLE 18-1 Reduction Potentials of Electron-Transport Chain Components in Resting Mitochondria

Component	$\mathscr{E}^{\circ\prime}$ (V)
NADH	−0.315
Complex I (NADH–CoQ oxidoreductase; ~1000 kD monomer, 44 unique subunits):	
FMN	−0.380
[2Fe–2S]N1a	−0.370
[2Fe–2S]N1b	−0.250
[4Fe–4S]N3, 4, 5, 6a, 6b, 7	−0.250
[4Fe–4S]N2	−0.150
Succinate	0.031
Complex II (succinate–CoQ oxidoreductase; ~420 kD trimer, 4 unique subunits):	
FAD	−0.040
[2Fe–2S]	−0.030
[4Fe–4S]	−0.245
[3Fe–4S]	−0.060
Heme b_{560}	−0.080
Coenzyme Q	0.045
Complex III (CoQ–cytochrome *c* oxidoreductase; ~450 kD dimer, 9−11 unique subunits):	
Heme b_H (b_{562})	0.030
Heme b_L (b_{566})	−0.030
[2Fe–2S]	0.280
Heme c_1	0.215
Cytochrome *c*	0.235
Complex IV (cytochrome *c* oxidase; ~410 kD dimer, 8–13 unique subunits):	
Heme *a*	0.210
Cu_A	0.245
Cu_B	0.340
Heme a_3	0.385
O_2	0.815

Source: Mainly Wilson, D.F., Erecinska, M., and Dutton, P.L., *Annu. Rev. Biophys. Bioeng.* **3,** 205 and 208 (1974); *and* Wilson, D.F., *in* Bittar, E.E. (Ed.), *Membrane Structure and Function,* Vol. 1, p. 160, Wiley (1980).

Electron Transport



Electron Transport

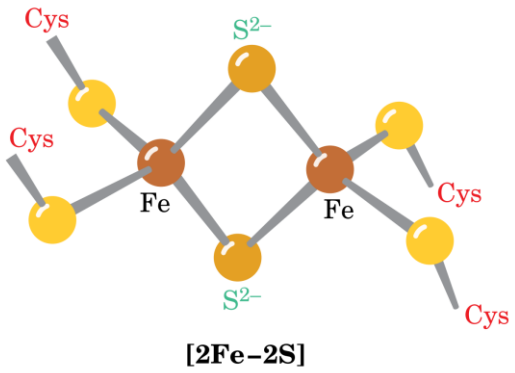
Table 10-3 Properties of the Mitochondrial Respiratory Complexes

Respiratory Complex		Number of Polypeptides*	Cofactors	Electron Flow		Protons Translocated (per electron pair)
Number	Name			Accepted from	Passed to	
I	NADH–coenzyme Q oxidoreductase (NADH dehydrogenase)	43 (7)	1 FMN 6–9 Fe-S centers	NADH	Coenzyme Q	4
II	Succinate–coenzyme Q oxidoreductase (succinate dehydrogenase)	4 (0)	1 FAD 3 Fe-S centers	Succinate (via enzyme-bound FAD)	Coenzyme Q	0
III	Coenzyme Q–cytochrome *c* oxidoreductase (cytochrome b/c_1 complex)	11 (1)	2 cytochrome *b* 1 cytochrome c_1 1 Fe-S center	Coenzyme Q	Cytochrome *c*	4**
IV	Cytochrome *c* oxidase	13 (3)	1 cytochrome *a* 1 cytochrome a_3 2 Cu centers (as Fe-Cu centers with cytochrome a_3)	Cytochrome *c*	Oxygen (O_2)	2

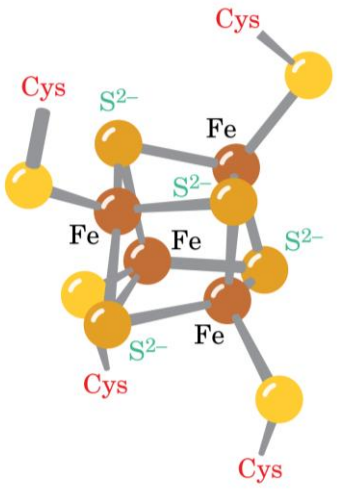
*The number of polypeptides encoded by the mitochondrial genome is indicated in parentheses for each complex.
**The value for complex III includes two protons translocated by coenzyme Q.

Electron Transport

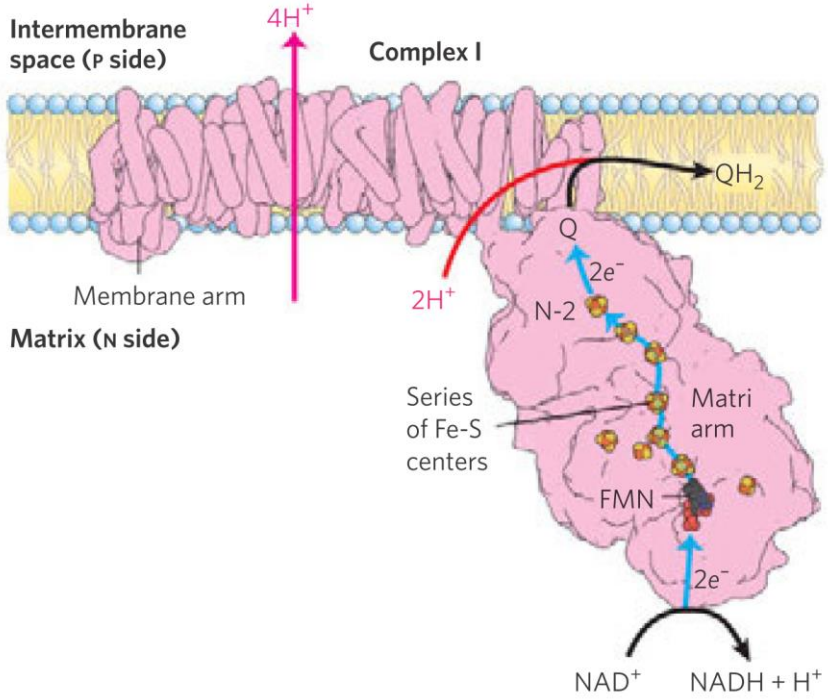
- Complex I (NADH-coenzyme Q oxidoreductase)
  - The largest protein complex in the inner membrane (L-shaped)
  - Passes electrons from NADH to CoQ
  - Contains one molecule of FMN and varied numbers of iron-sulfur clusters (e.g. [2Fe-2S] and 4Fe-4S)



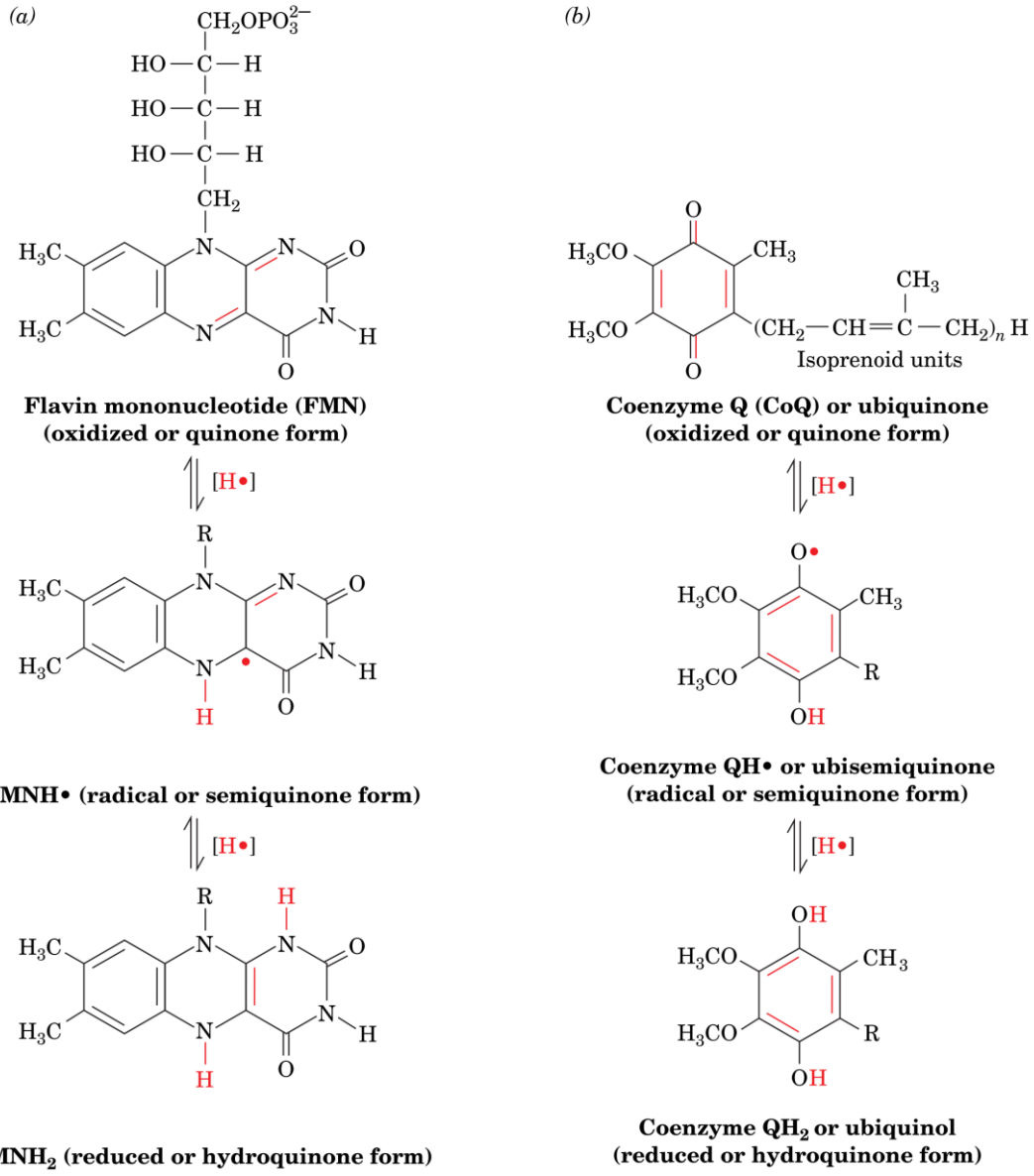
[2Fe–2S]



[4Fe–4S]



Electron Transport



Electron Transport

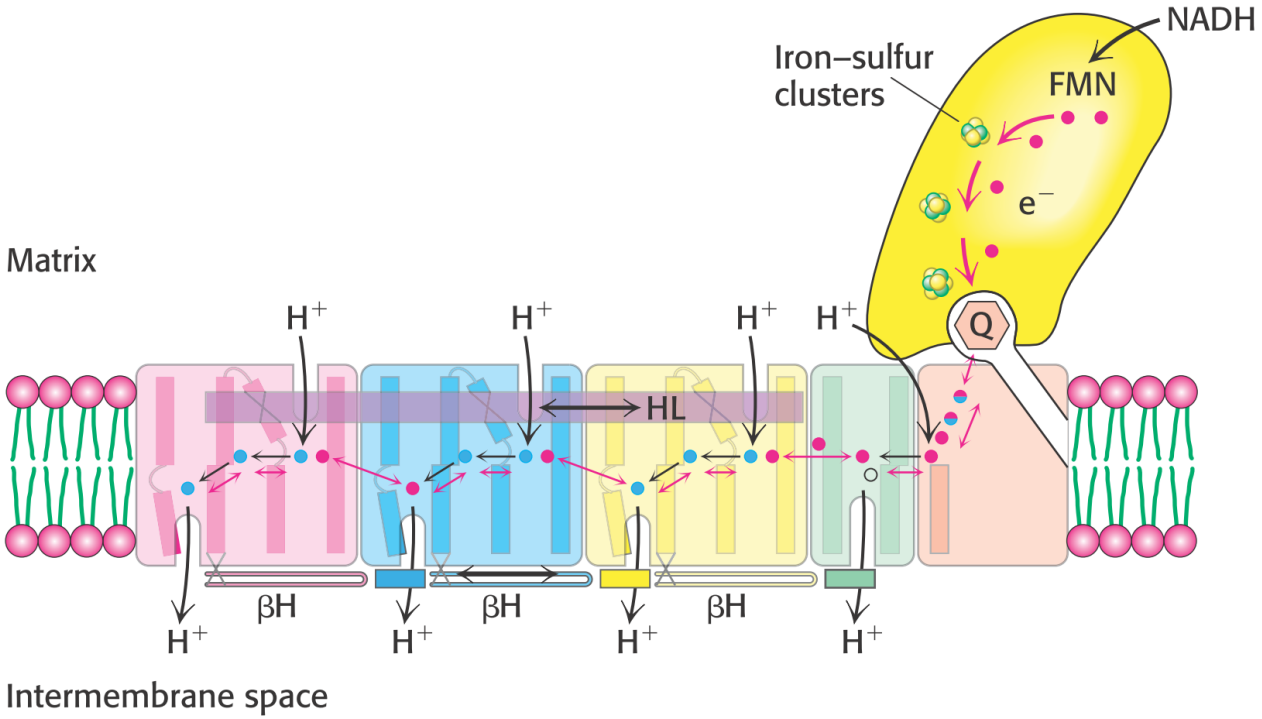


Figure 20.11 Coupled electron–proton transfer reactions through NADH-Q oxidoreductase. Electrons flow in Complex I from NADH through FMN and a series of iron–sulfur clusters to ubiquinone (Q), forming Q^{2-}. The charges on Q^{2-} are electrostatically transmitted to hydrophilic amino acid residues (shown as red and blue balls) that power the movement of HL and βH components. This movement changes the conformation of the transmembrane helices and results in the transport of four protons out of the mitochondrial matrix. [Information from R. Baradaran et al., *Nature* 494:443–448, 2013.]

Electron Transport

- Complex II (Succinate-coenzyme Q oxidoreductase)
  - Contains the citric acid cycle enzyme succinate dehydrogenase
  - Passes electrons from succinate to CoQ
  - [4Fe-4S] → [3Fe-4S] → [2Fe-2S] → cytochrome b_{560}

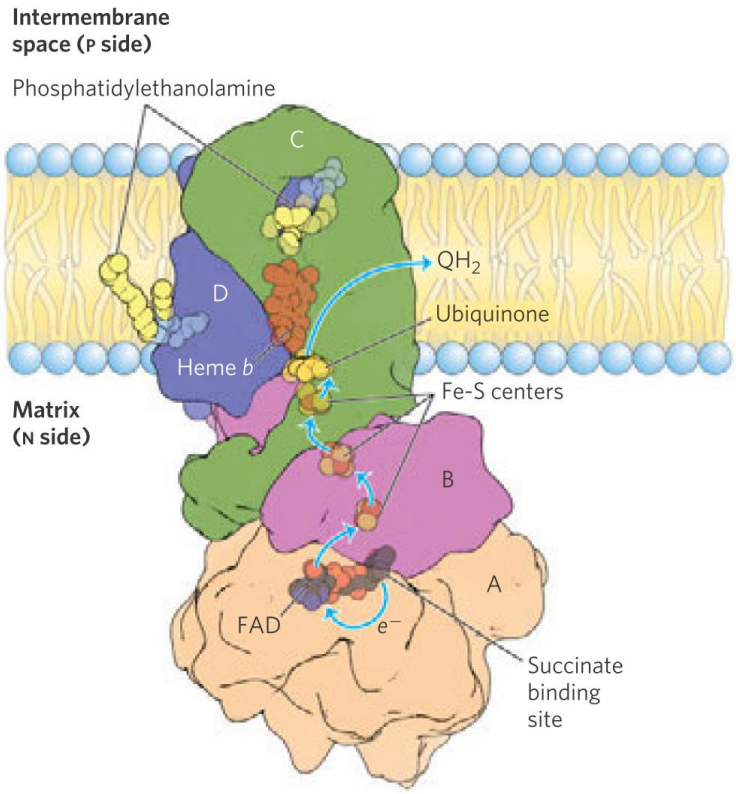
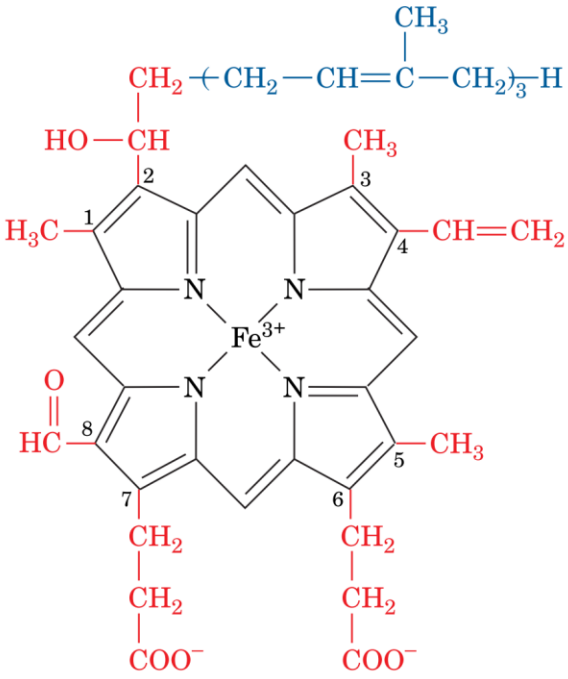
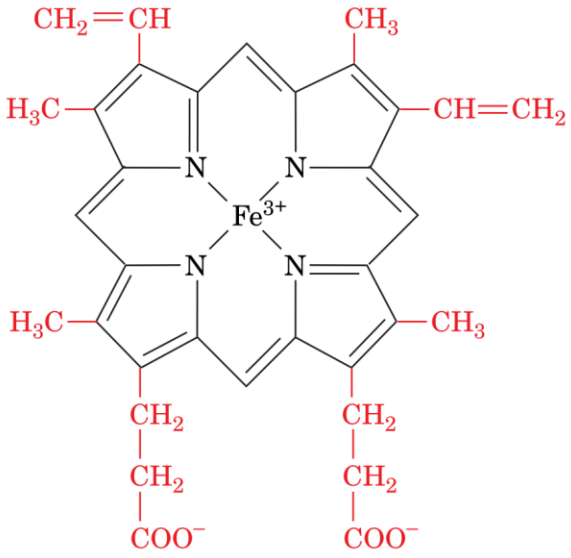


FIGURE 19–10 Structure of Complex II (succinate dehydrogenase). (PDB ID 1ZOY) This complex (shown here is the porcine heart enzyme) has two transmembrane subunits, C and D; the cytoplasmic extensions contain subunits A and B. Just behind the FAD in subunit A is the binding site for succinate. Subunit B has three Fe-S centers, ubiquinone is bound to subunit B, and heme *b* is sandwiched between subunits C and D. Two phosphatidylethanolamine molecules are so tightly bound to subunit D that they show up in the crystal structure. 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.

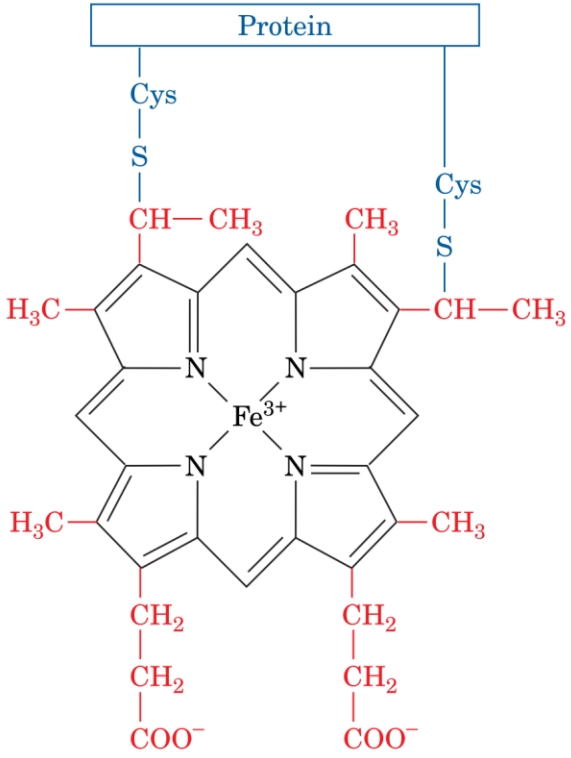
Electron Transport



Heme *a*



Heme *b*
(iron–protoporphyrin IX)



Heme *c*

Electron Transport

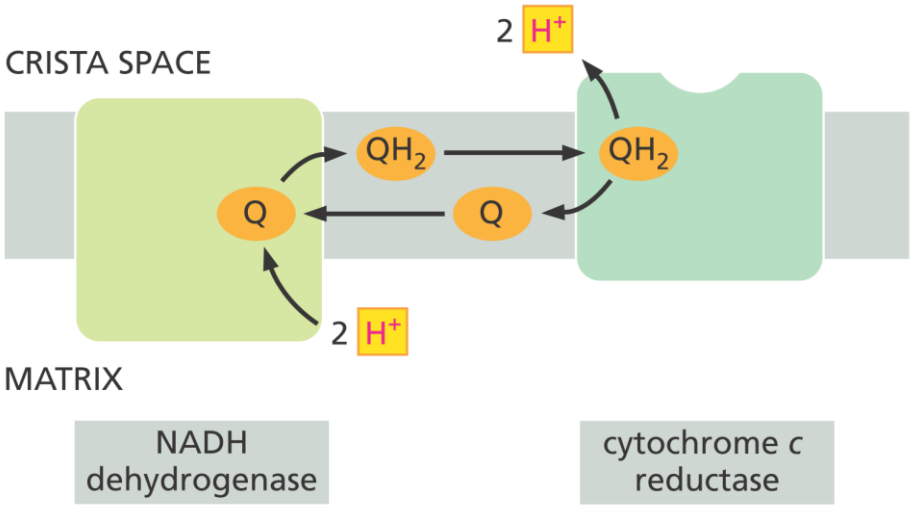


Figure 14–21 How a directional release and uptake of protons by a quinone pumps protons across a membrane. Two protons are picked up on the matrix side of the inner mitochondrial membrane when the reaction $Q + 2e^- + 2H^+ \rightarrow QH_2$ is catalyzed by the NADH dehydrogenase complex. This molecule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

Electron Transport

- Complex III (Coenzyme Q-cytochrome c oxidoreductase)
  - Passes electrons from reduced CoQ to cytochrome c
  - It contains two b-type cytochromes, one cytochrome c_1, and one [2Fe-s2S] cluster

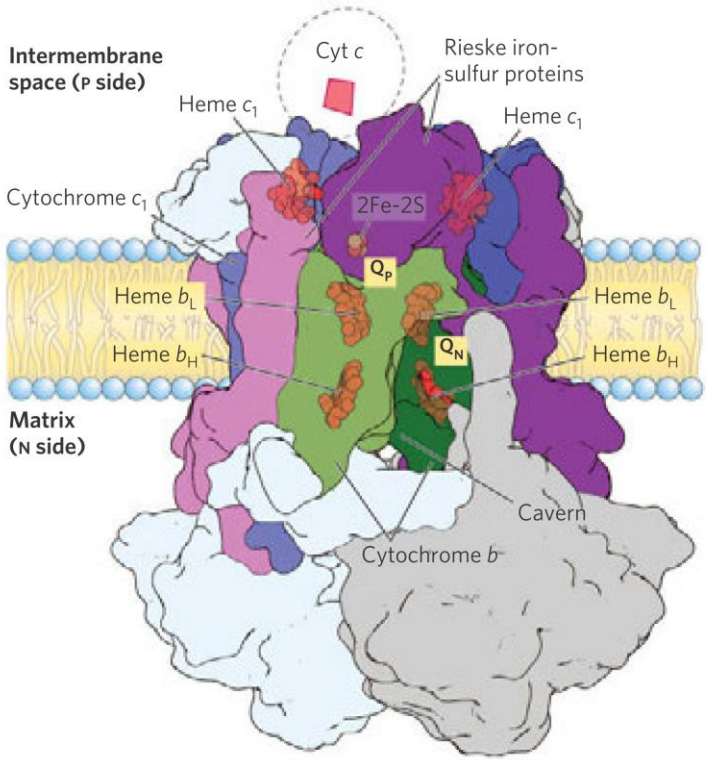
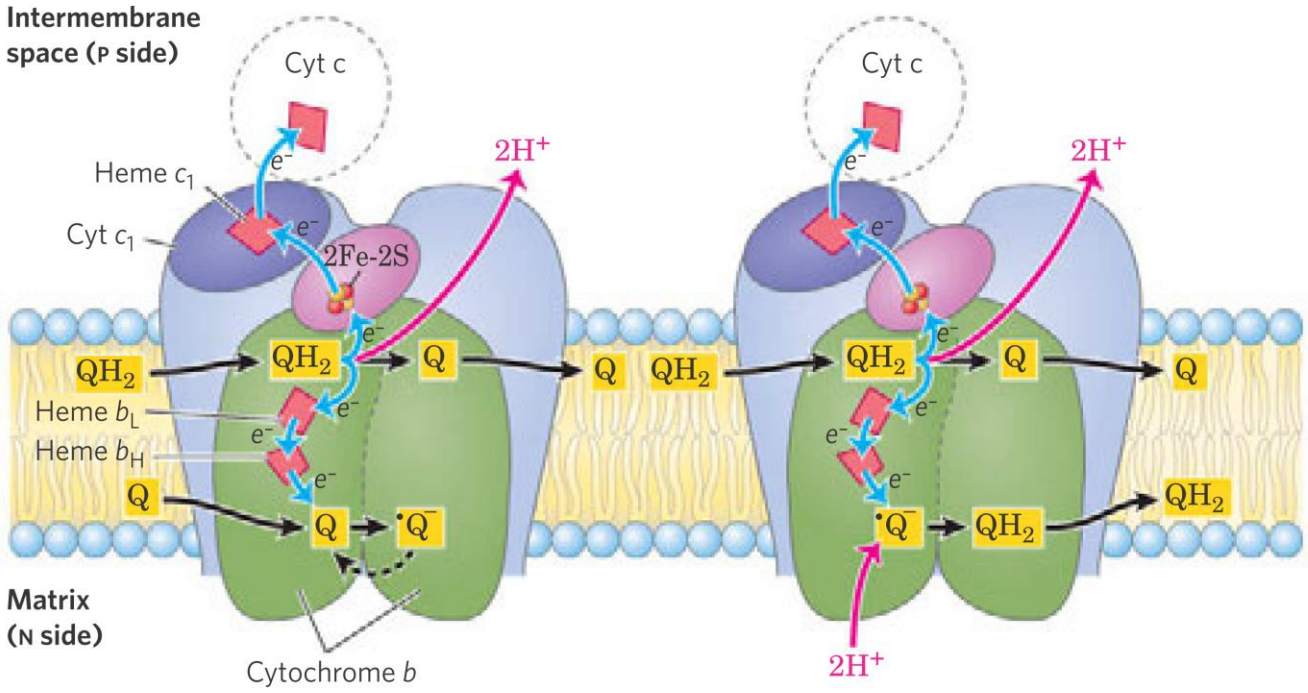


FIGURE 19–11 Cytochrome bc_1 complex (Complex III). (PDB ID 1BGY) The complex is a dimer of identical monomers, each with 11 different subunits. The functional core of each monomer is three subunits: cytochrome *b* (green) with its two hemes (b_H and b_L), the Rieske iron-sulfur protein (purple) with its 2Fe-2S centers, and cytochrome c_1 (blue) with its heme. This cartoon view of the complex shows how 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 caverns, each containing a Q_P site from one monomer and a Q_N site from the other. The ubiquinone intermediates move within these sheltered caverns.

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 first reduced, then oxidized.

Electron Transport



$$QH_2 + Q + \text{cyt } c_1\,(\text{oxidized}) \longrightarrow Q + {}^{\bullet}Q^- + 2H_P^+ + \text{cyt } c_1\,(\text{reduced})$$

$$QH_2 + {}^{\bullet}Q^- + 2H_N^+ + \text{cyt } c_1\,(\text{oxidized}) \longrightarrow Q + 2H_P^+ + QH_2 + \text{cyt } c_1\,(\text{reduced})$$

$$\text{Net equation: } QH_2 + 2 \text{ cyt } c_1\,(\text{oxidized}) + 2H_N^+ \longrightarrow Q + 2 \text{ cyt } c_1\,(\text{reduced}) + 4H_P^+$$

FIGURE 19–12 The Q cycle, shown in two stages. The path of electrons through Complex III is shown by blue arrows. The movement of various forms of ubiquinone is shown with black arrows. In the first stage (left), Q on the N side is reduced to the semiquinone radical, which moves back into position to accept another electron. In the second stage (right), the semiquinone radical is converted to QH_2. Meanwhile, on the P side of the membrane, two molecules of QH_2 are oxidized to Q, releasing two protons per Q molecule (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 consumes two protons per Q, which are taken up from the matrix (N side). Reduced cyt c_1 passes electrons one at a time to cyt *c*, which dissociates and carries electrons to Complex IV.

Electron Transport

- Complex IV (Cytochrome c oxidase)
  - Catalyzes the one-electron oxidations of four consecutive reduced cytochrome c molecules and the contaminant four-electron reduction of one O_2 molecule:

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

  - Contains 4 redox centers: cytochrome a, cytochrome a_3, CuB center and CuA center

Electron Transport

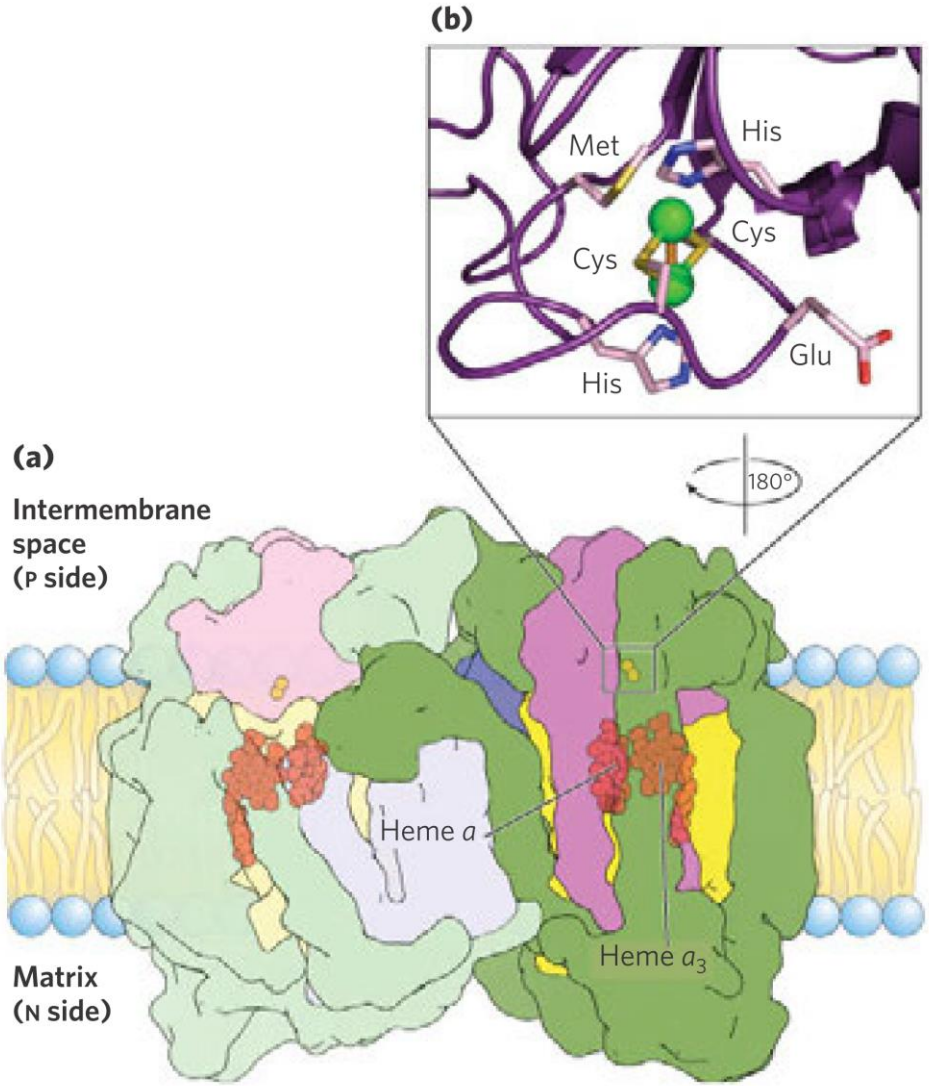


FIGURE 19–13 Structure of cytochrome oxidase (Complex IV). This complex from bovine mitochondria has 13 subunits, but only four core proteins are shown here (PDB ID 1OCC). **(a)** Complex IV, with four subunits in each of two identical units of a dimer. Subunit I (yellow) has two heme groups, *a* and a_3, near a single copper ion, Cu_B (not visible here). Heme a_3 and Cu_B form a binuclear Fe-Cu center. Subunit II (purple) contains two Cu ions complexed with the —SH groups of two Cys residues in a binuclear center, Cu_A, that resembles the 2Fe-2S centers of iron-sulfur proteins. This binuclear center and the cytochrome *c*-binding site are located in a domain of subunit II that protrudes from the P side of the inner membrane (into the intermembrane space). Subunit III (blue) is essential for rapid proton movement through subunit II. The role of subunit IV (green) is not yet known. **(b)** The binuclear center of Cu_A. The Cu ions (green spheres) share electrons equally. When the center is reduced, the ions have the formal charges $Cu^{1+}Cu^{1+}$; when oxidized, $Cu^{1.5+}Cu^{1.5+}$. Six amino acid residues are ligands around the Cu ions: two His, two Cys, Glu, and Met.

Electron Transport

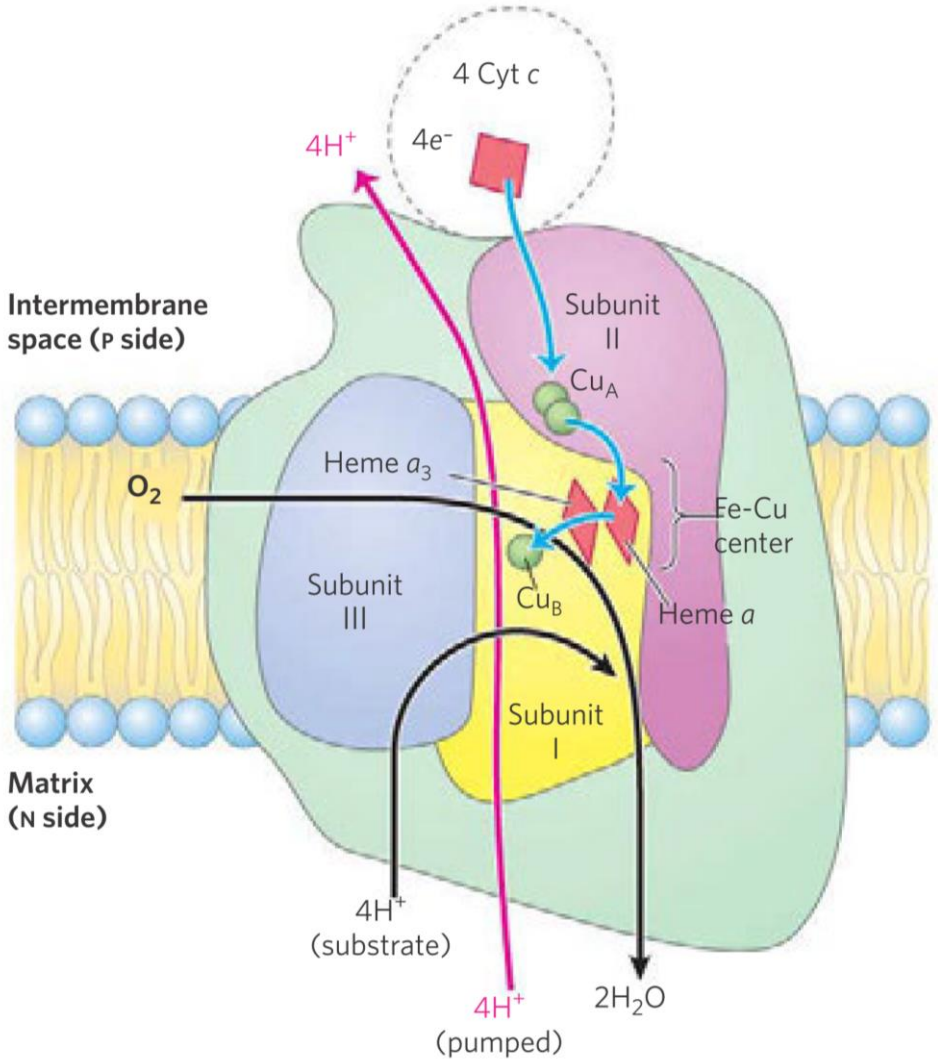
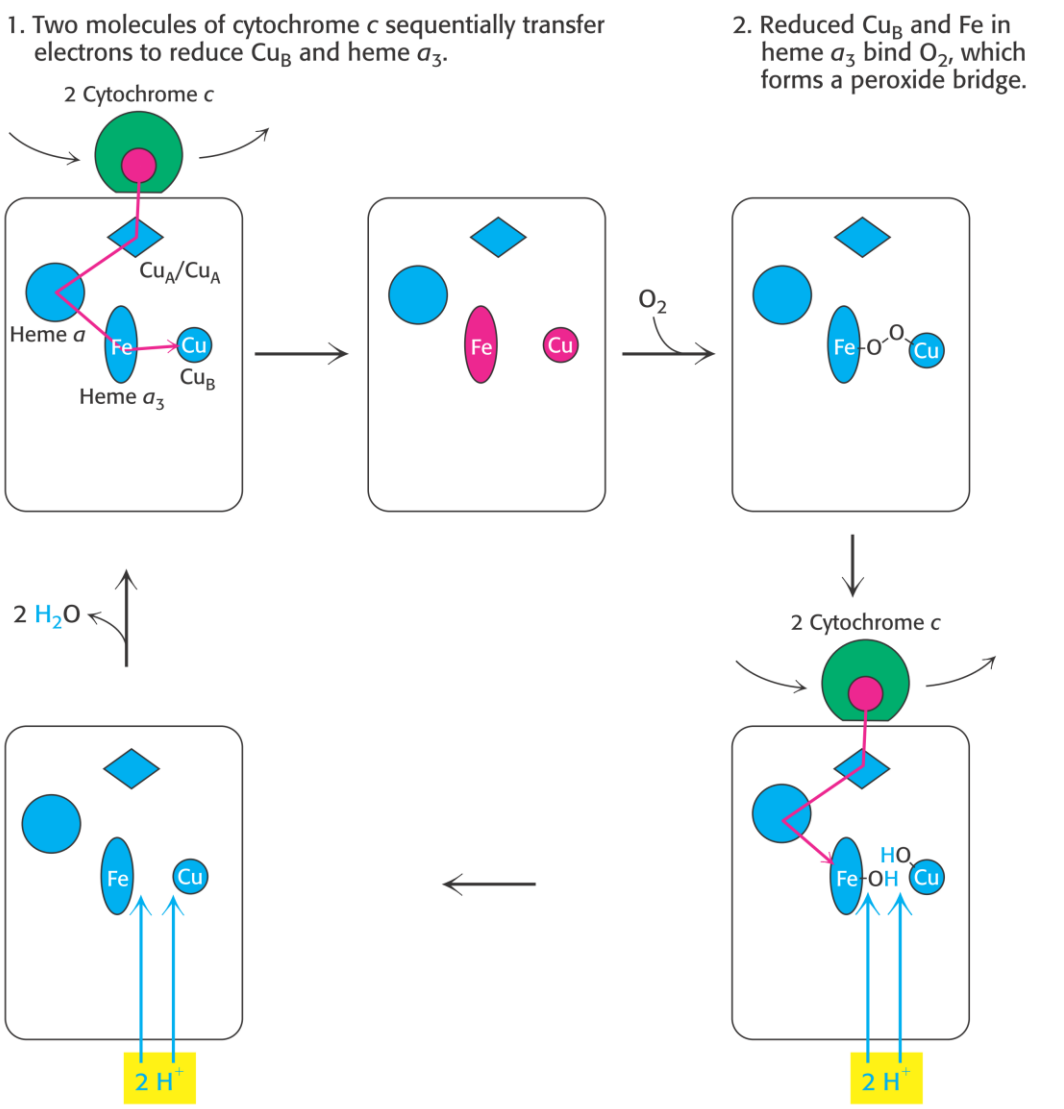


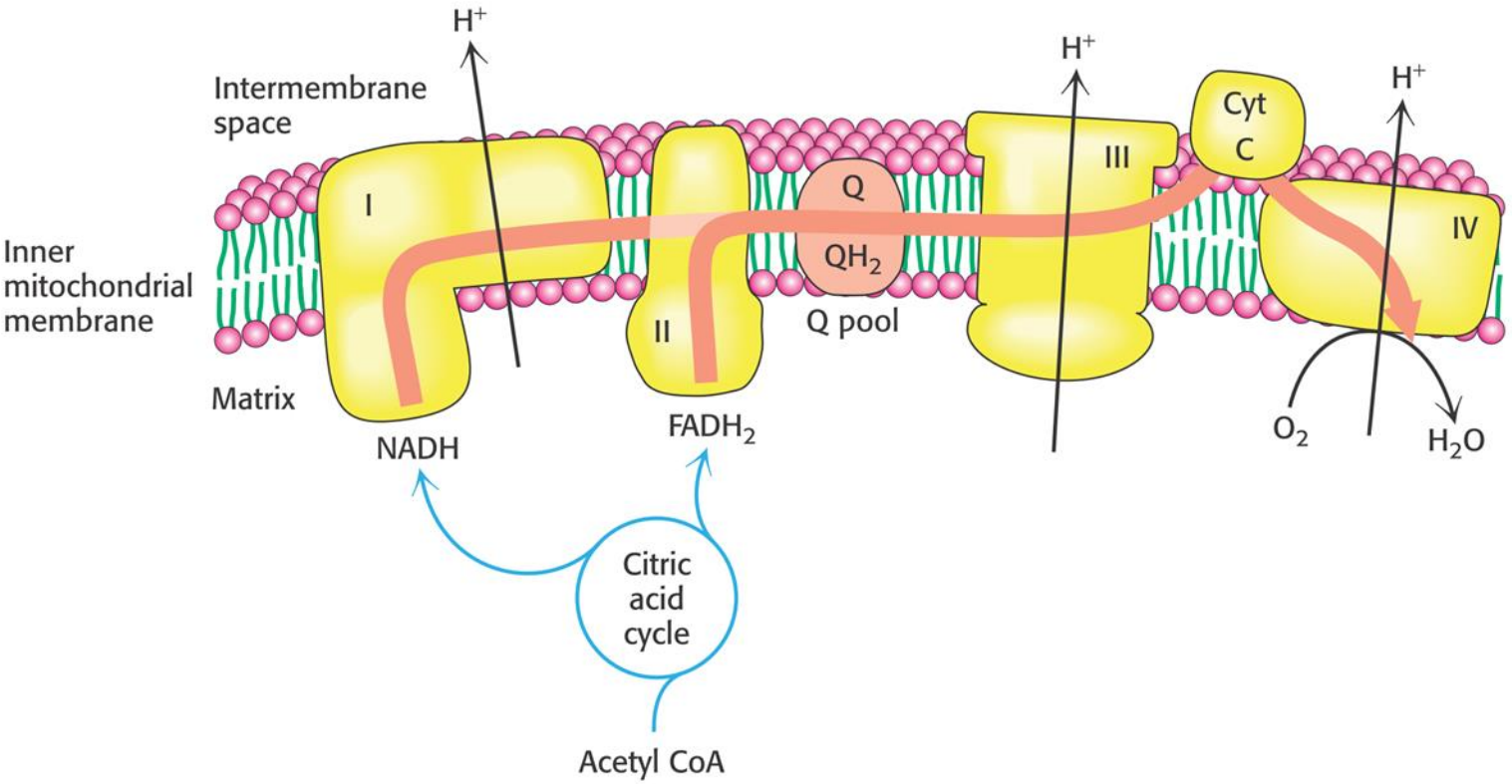
FIGURE 19–14 Path of electrons through Complex IV. The three proteins critical to electron flow are subunits I, II, and III. The larger green structure includes the other 10 proteins in the complex. Electron transfer through Complex IV begins with cytochrome *c* (top). Two molecules of reduced cytochrome *c* each donate an electron to the binuclear center Cu_A. From here electrons pass through heme *a* to the Fe-Cu center (heme a_3 and Cu_B). Oxygen now binds to heme a_3 and is reduced to its peroxy derivative (O_2^{2-}; not shown here) by two electrons from the Fe-Cu center. Delivery of two more electrons from cytochrome *c* (top, making four electrons in all) converts the O_2^{2-} to two molecules of water, with consumption of four "substrate" protons from the matrix. At the same time, four protons are pumped from the matrix by an as yet unknown mechanism.

Electron Transport



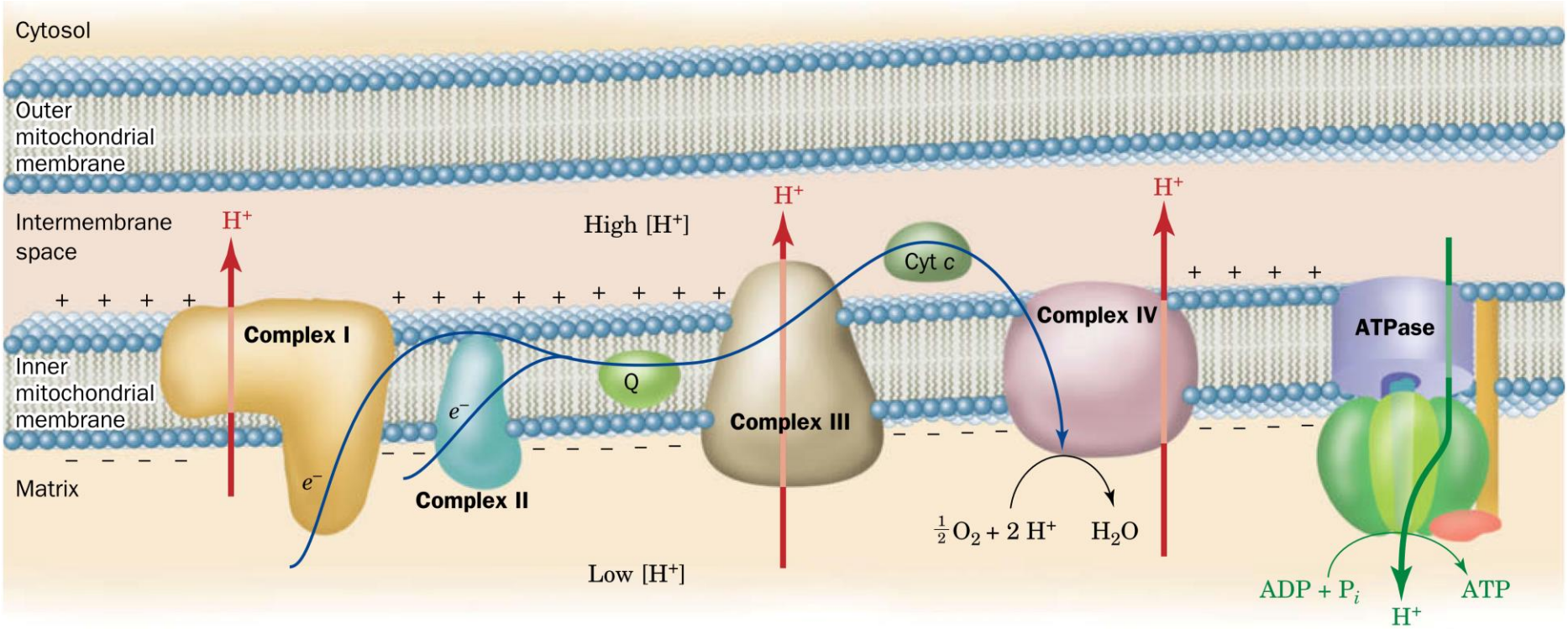
Electron Transport

- Conclusion



Oxidative Phosphorylation

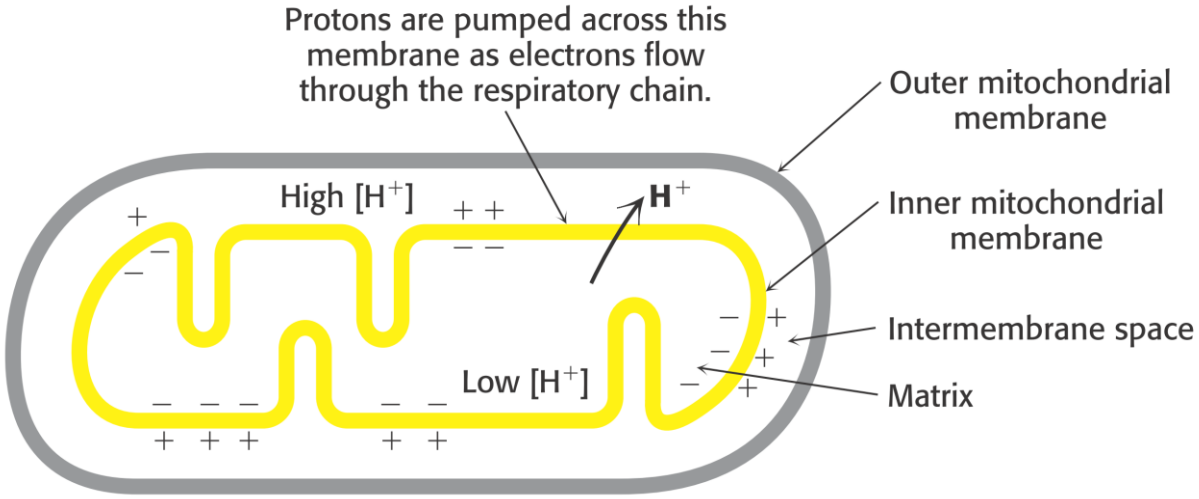
- Introduction
  - The endergonic synthesis of ATP from ADP and Pi in mitochondria is catalysed by an ATP synthase (also known as Complex V) that is driven by the electron transport process – energy coupling



Oxidative Phosphorylation

- Chemiosmotic Theory (1961)

The free energy of electron transport is conserved by pumping H^+ from the mitochondrial matrix to the intermembrane space to create an electrochemical H^+ gradient across the inner membrane. The electrochemical potential of this gradient is harnessed to synthesize ATP.



Peter Mitchell

Oxidative Phosphorylation

- Proton-motive Force (PMF)
  - The energy-rich, unequal distribution of protons
  - Chemical (pH) gradient vs. Electrical (charge) gradient

$$\text{PMF } (\Delta p) = \Delta H + \Delta \psi$$

  - Evidences

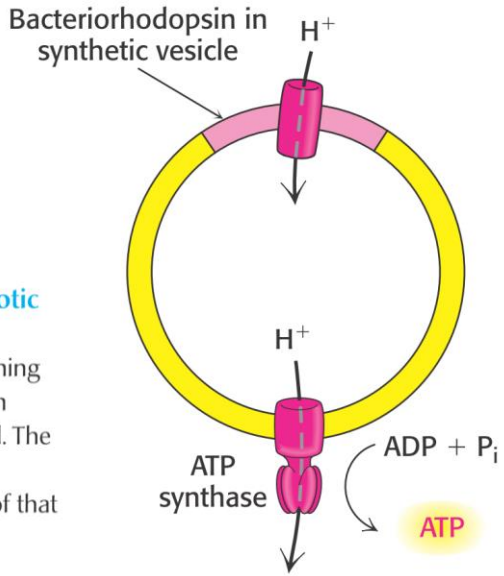


Figure 21.2 Testing the chemiosmotic hypothesis. ATP is synthesized when reconstituted membrane vesicles containing bacteriorhodopsin (a light-driven proton pump) and ATP synthase are illuminated. The orientation of ATP synthase in this reconstituted membrane is the reverse of that in the mitochondrion.

Oxidative Phosphorylation

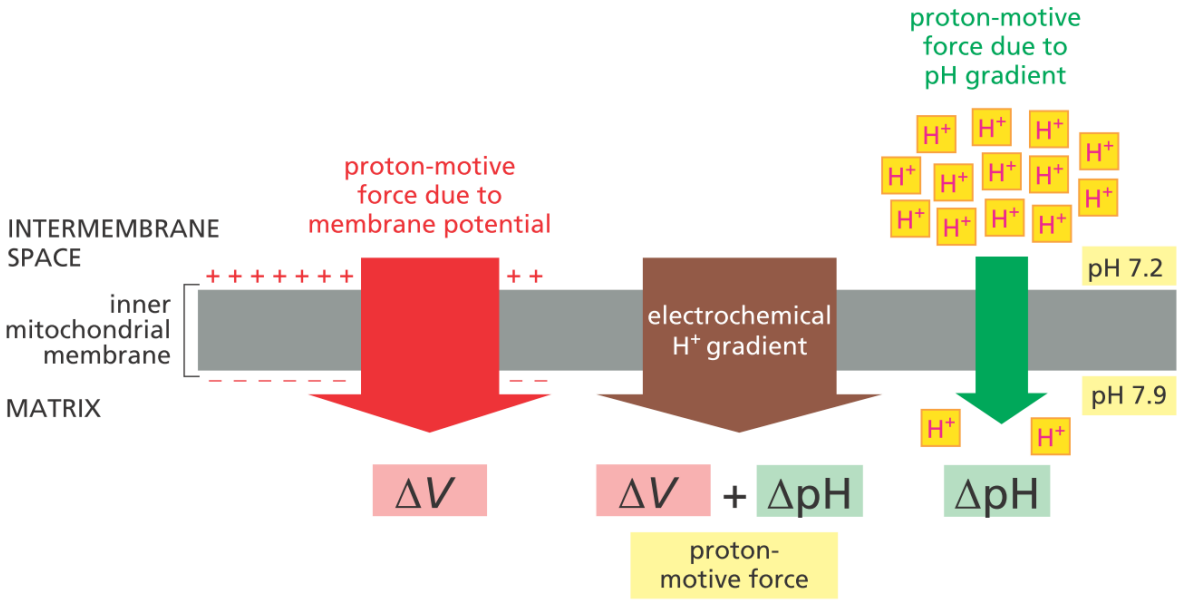
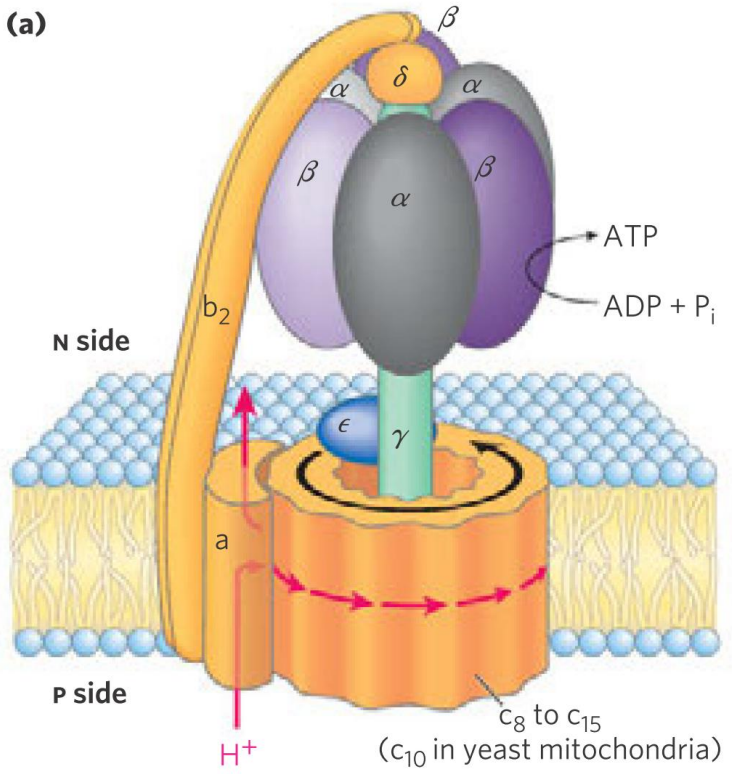
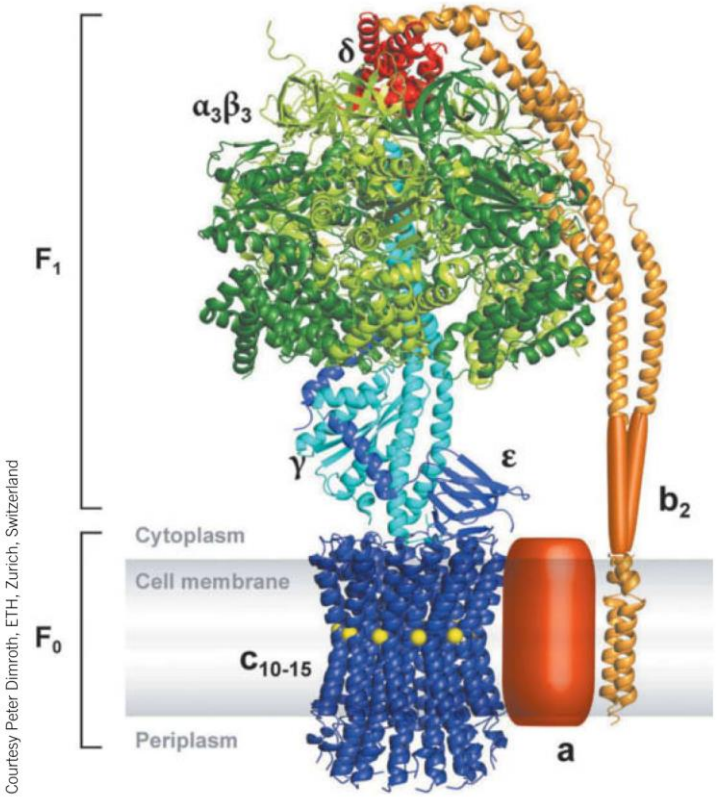


Figure 14–14 The electrochemical proton gradient across the inner mitochondrial membrane. This gradient is composed of a large force due to the membrane potential (ΔV) and a smaller force due to the H^+ concentration gradient—that is, the pH gradient (ΔpH). Both forces combine to generate the proton-motive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

Oxidative Phosphorylation

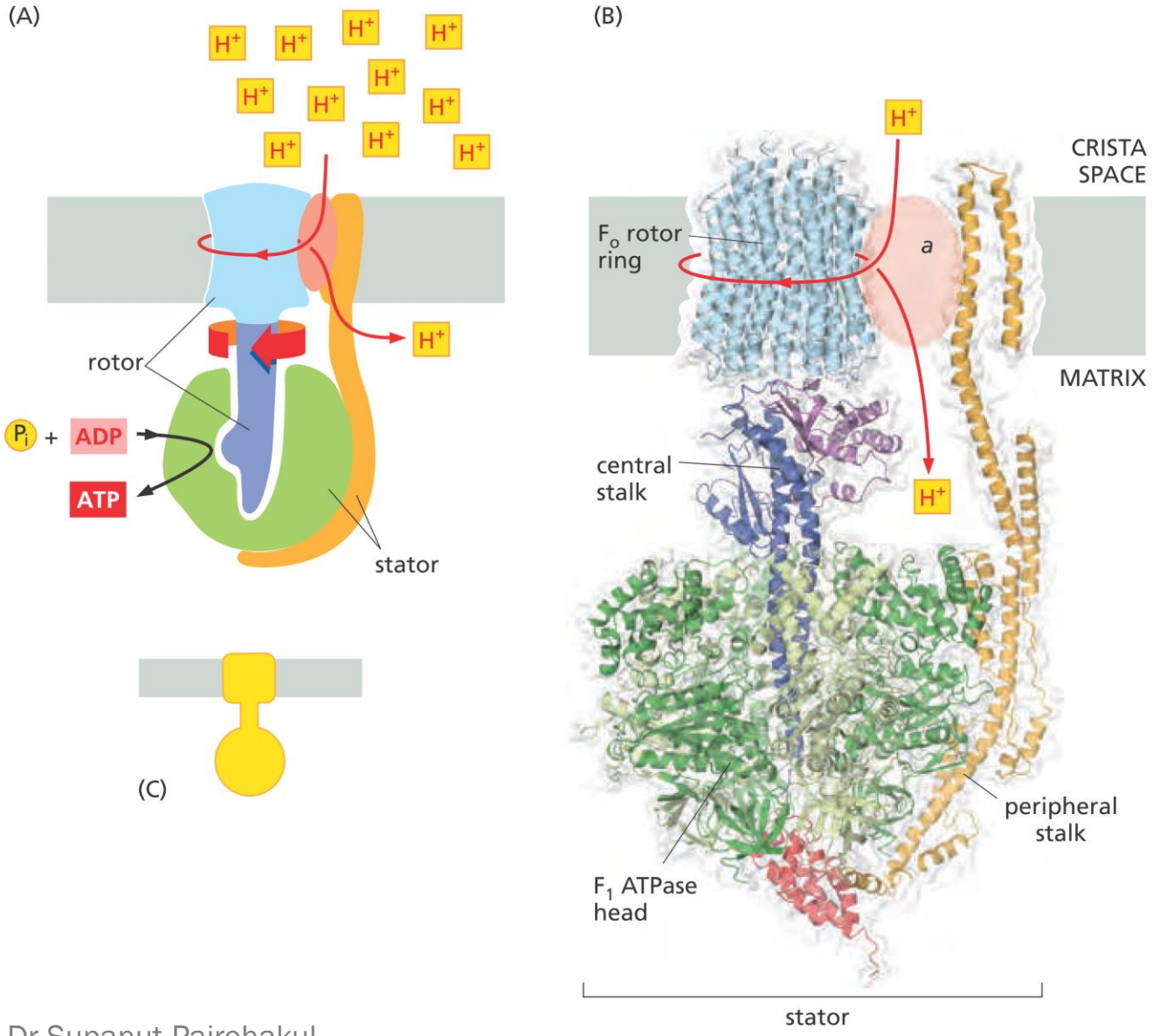
- ATP Synthase
  - A large, complex enzyme that looks like a ball on a stick
  - F_0 subunit and F_1 subunit (catalytic activity)





Courtesy Peter Dimroth, ETH, Zurich, Switzerland

Oxidative Phosphorylation



Oxidative Phosphorylation

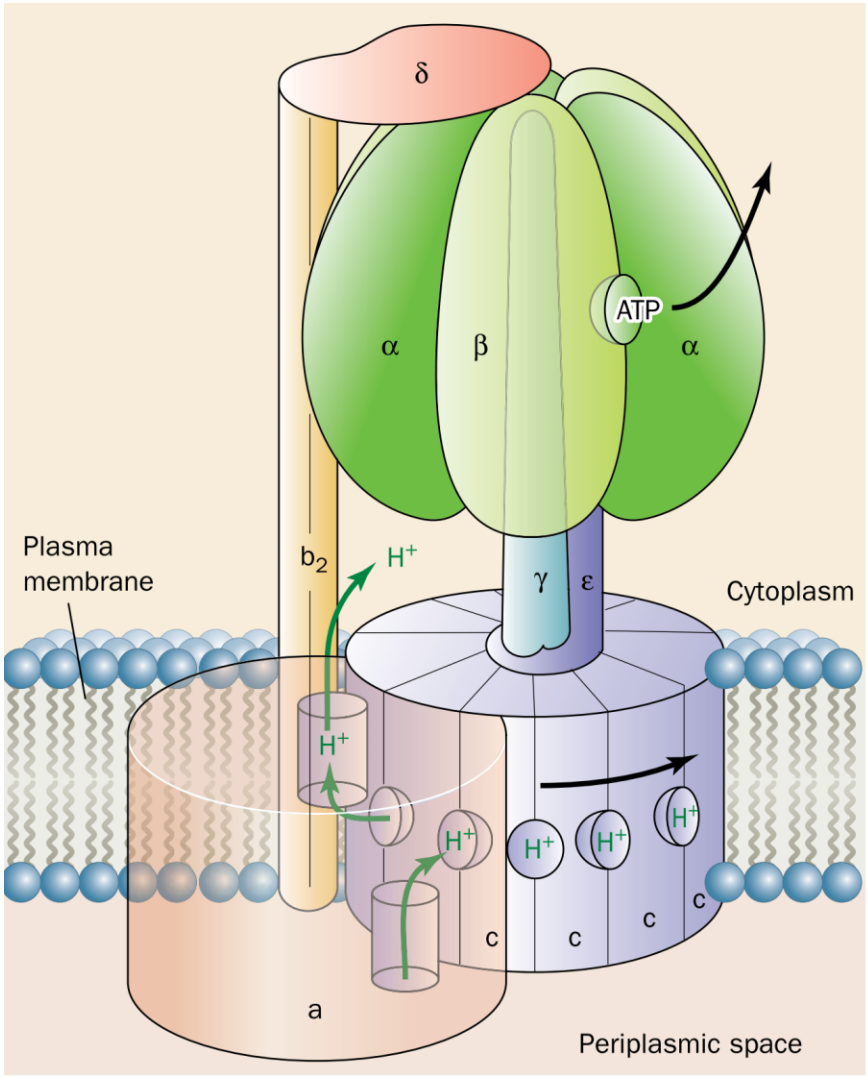


FIG. 18-26 Model of the *E. coli* F_1F_0-ATPase. The $\gamma\varepsilon$–c_{12} ring complex is the rotor and the ab_2–$\alpha_3\beta_3\delta$ complex is the stator. Rotational motion is imparted to the rotor by the passage of protons from the outside (periplasmic space, *bottom;* equivalent to the mitochondrial intermembrane space) to the inside (cytoplasm, *top*; equivalent to the mitochondrial matrix). Protons entering from the outside bind to a *c* subunit where it interacts with the *a* subunit, and exit to the inside after the *c*-ring has made a nearly full rotation as indicated (*black arrows*), so that the *c* subunit again contacts the *a* subunit. The $b_2\delta$ complex presumably functions to prevent the $\alpha_3\beta_3$ assembly from rotating with the γ subunit. [After a drawing by Richard Cross, State University of New York, Syracuse, New York.]

? Which parts of the complex rotate and which remain stationary?

Oxidative Phosphorylation

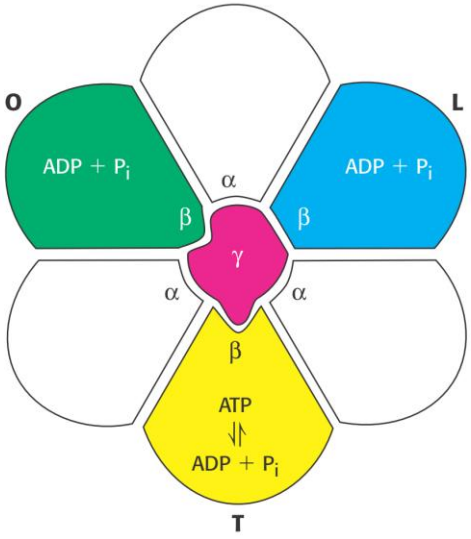


Figure 21.5 ATP synthase nucleotide-binding sites are not equivalent. The γ subunit passes through the center of the $\alpha_3\beta_3$ hexamer and makes the nucleotide-binding sites in the β subunits distinct from one another.

Progressive alteration of the forms of the three active sites of ATP synthase
Subunit 1 L → T → O → L → T → O ⋯
Subunit 2 O → L → T → O → L → T ⋯
Subunit 3 T → O → L → T → O → L ⋯

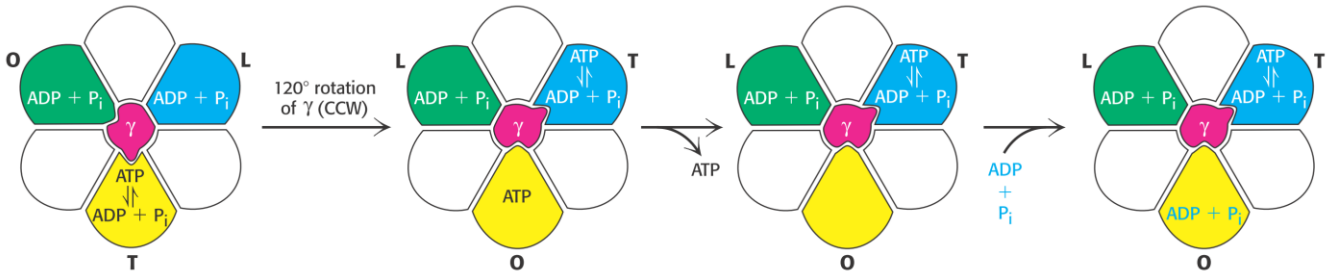


Figure 21.6 A binding-change mechanism for ATP synthase. The rotation of the γ subunit interconverts the three β subunits. The subunit in the T (tight) form converts ADP and P_i into ATP but does not allow ATP to be released. When the γ subunit is rotated counterclockwise (CCW) 120 degrees, the T-form subunit is converted into the O form, allowing ATP release. New molecules of ADP and P_i can then bind to the O-form subunit. An additional 120-degree rotation (not shown) traps these substrates in an L-form subunit.

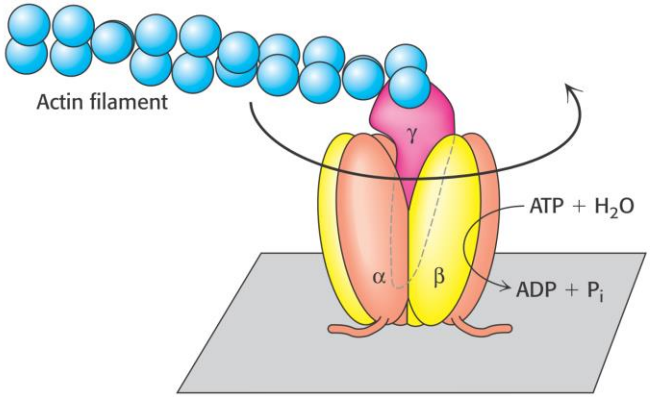


Figure 21.7 Direct observation of ATP-driven rotation in ATP synthase. The $\alpha_3\beta_3$ hexamer of ATP synthase is fixed to a surface, with the γ subunit projecting upward and linked to a fluorescently labeled actin filament. The addition and subsequent hydrolysis of ATP result in the counterclockwise rotation of the γ subunit, which can be directly seen under a fluorescence microscope.

Oxidative Phosphorylation

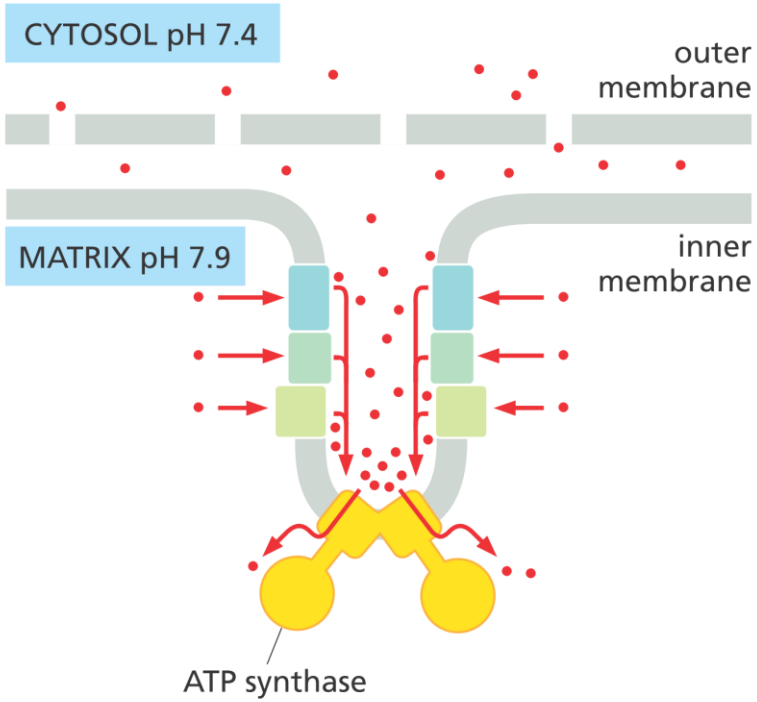


Figure 14–33 ATP synthase dimers at cristae ridges and ATP production. At the crista ridges, the ATP synthases *(yellow)* form a sink for protons *(red)*. The proton pumps of the electron-transport chain *(green)* are located in the membrane regions on either side of the crista. As illustrated, protons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

Oxidative Phosphorylation