19 Oxidative Phosphorylation

P1 Principle 1 (1 of 5)

Mitochondria play a central role in eukaryotic aerobic metabolism. ATP production is not the only important mitochondrial function. Mitochondria play host to the citric acid cycle, the fatty acid *β*-oxidation pathway, and the pathways of amino acid oxidation. The mitochondria also act in thermogenesis, steroid synthesis, and apoptosis (programmed cell death). The discovery of these diverse and important roles of mitochondria has stimulated much current research on the biochemistry of this organelle.

P2 Principle 2 (1 of 4)

Mitochondria trace their evolutionary origin to bacteria. Over 1.45 billion years ago, an endosymbiotic relationship arose between bacteria and a primitive eukaryote or eukaryotic progenitor. Mitochondria are ubiquitous in modern eukaryotes, and their bacterial origin is evident in almost every aspect of their structure and function.

P3 Principle 3 (1 of 6)

Electrons flow from electron donors (oxidizable substrates) through a chain of membrane-bound carriers to a final electron acceptor with a large reduction potential. The final acceptor is molecular oxygen, O_2. The appearance of oxygen in the atmosphere some 2.3 billion years ago, and its harnessing in living systems via the evolution of oxidative phosphorylation, made more complex life forms possible.

P4 Principle 4 (1 of 5)

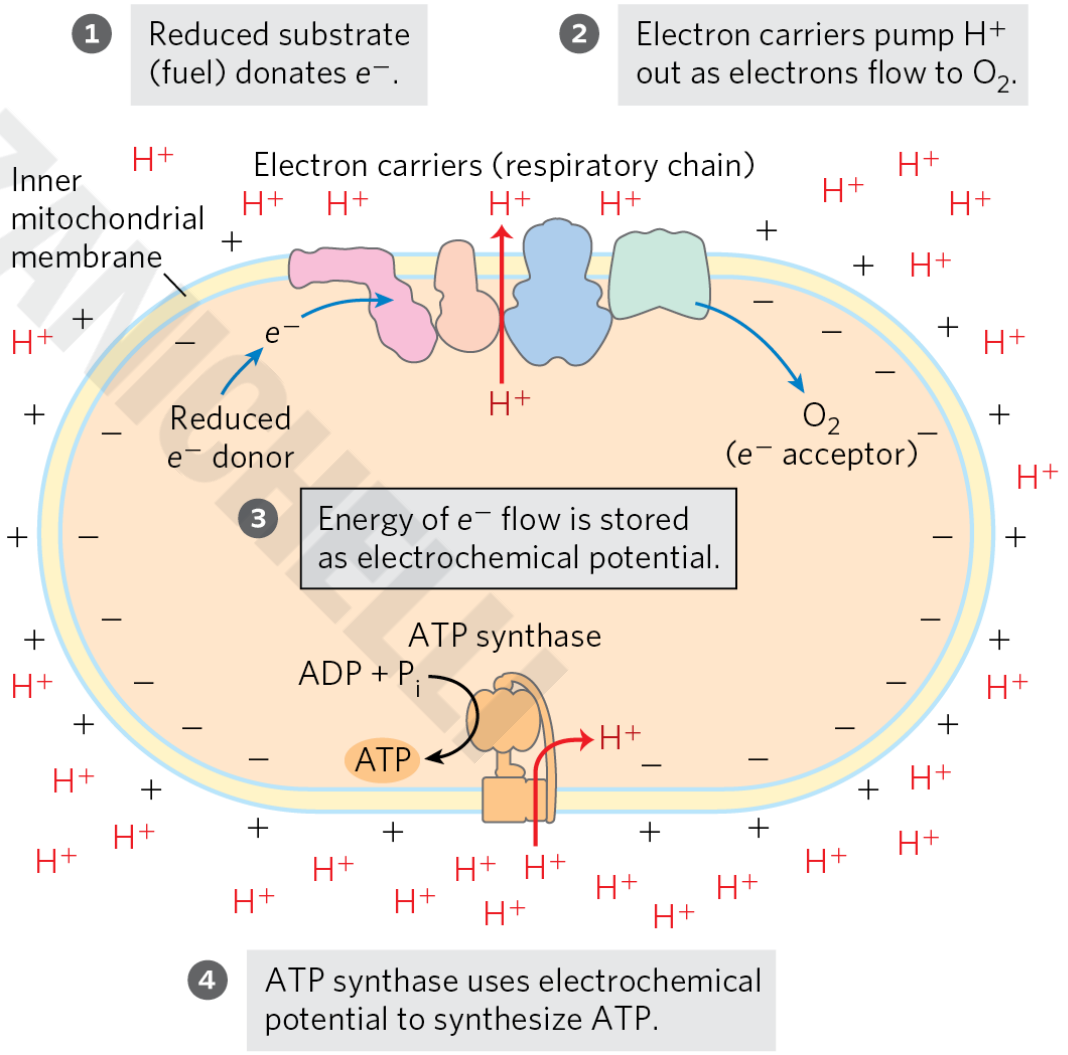
The free energy made available by "downhill" (exergonic) electron flow is coupled to the "uphill" transport of protons across a proton-impermeable membrane. The free energy of fuel oxidation is thus conserved as a transmembrane electrochemical potential.

P5 Principle 5 (1 of 7)

The transmembrane flow of protons back down their electrochemical gradient through specific protein channels provides the free energy for synthesis of ATP. This process is catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.

The Chemiosmotic Theory

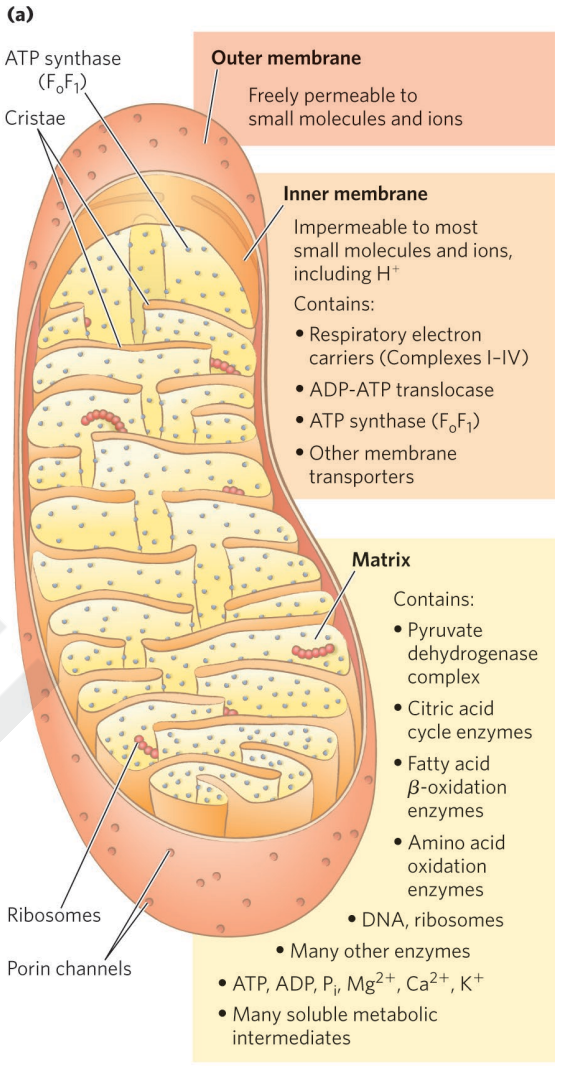
- **chemiosmotic theory =** transmembrane differences in proton concentration are the reservoir for the energy extracted from biological oxidation reactions



19.1 The Mitochondrial Respiratory Chain

Mitochondria Have Two Membranes

- outer membrane is readily permeable to small molecules and ions
  - transport occurs through porins
- inner membrane is impermeable to most small molecules and ions
  - transport requires specific transporters



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P1 Principle 1 (2 of 5)

Mitochondria play a central role in eukaryotic aerobic metabolism. ATP production is not the only important mitochondrial function. Mitochondria play host to the citric acid cycle, the fatty acid *β*-oxidation pathway, and the pathways of amino acid oxidation. The mitochondria also act in thermogenesis, steroid synthesis, and apoptosis (programmed cell death). The discovery of these diverse and important roles of mitochondria has stimulated much current research on the biochemistry of this organelle.

The Mitochondrial Matrix

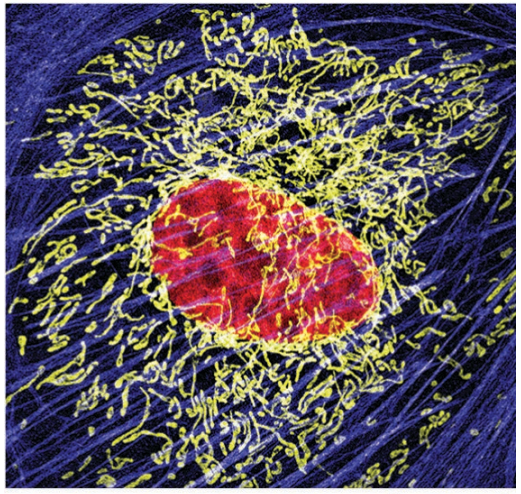
- the mitochondrial matrix contains:
  - the pyruvate dehydrogenase complex
  - enzymes of the citric acid cycle
  - enzymes of the fatty acid β-oxidation pathway
  - enzymes of the pathways of amino acid oxidation

- the inner mitochondrial membrane segregates the intermediates and enzymes of cytosolic and matrix metabolic pathways

Mitochondrial Cristae

- **cristae** = convolutions in the inner membrane of the mitochondrion
  - mitochondria of cells with high metabolic activity have more cristae

(b)



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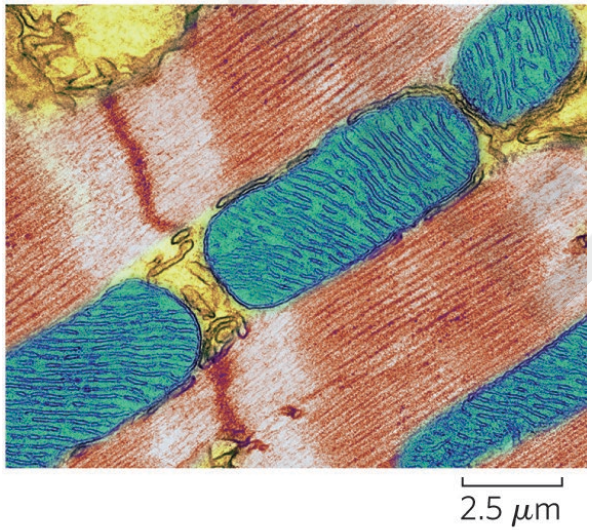

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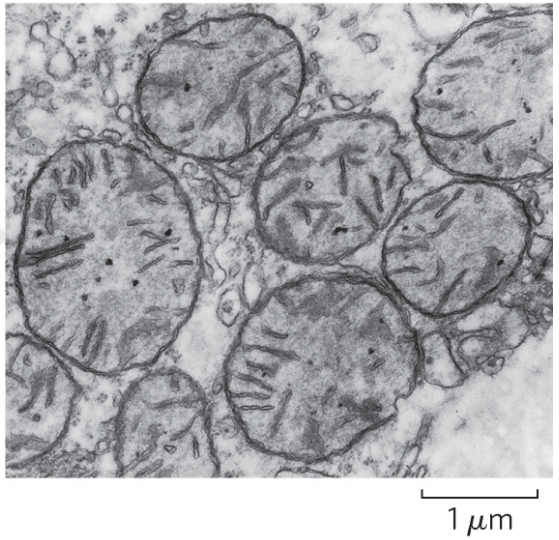

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P2 Principle 2 (2 of 4)

Mitochondria trace their evolutionary origin to bacteria. Over 1.45 billion years ago, an endosymbiotic relationship arose between bacteria and a primitive eukaryote or eukaryotic progenitor. Mitochondria are ubiquitous in modern eukaryotes, and their bacterial origin is evident in almost every aspect of their structure and function.

The Effect of Stress on Mitochondria

- during cell growth and division, mitochondria divide by fission

- stressful conditions can trigger:
  - mitochondrial fission
  - **mitophagy** = the breakdown of mitochondria and recycling of the amino acids, nucleotides, and lipids

- as stress is relieved, small mitochondria fuse to form long, thin, tubular organelles

Electrons Are Funneled to Universal Electron Acceptors

- **respiratory chain** = series of electron carriers

- dehydrogenases collect electrons from catabolic pathways and funnel them into universal electron acceptors:
  - nicotinamide nucleotides (NAD^+ or $NADP^+$)
  - flavin nucleotides (FMN or FAD)

Nicotinamide Nucleotide-Linked Dehydrogenases

- **nicotinamide nucleotide-linked dehydrogenases** = catalyze reversible reactions of the general types:

reduced substrate + NAD^+ ⇄
oxidized substrate + NADH + H^+

reduced substrate + $NADP^+$ ⇄
oxidized substrate + NADPH + H^+

- two hydrogen atoms are removed from the substrates:
  - one is transferred as a hydride ion (**:**H^-) to $NAD(P)^+$
  - one is released as H+ in the medium

Important Reactions Catalyzed by NAD(P)$^+$-Linked Dehydrogenases

TABLE 19-1 Some Important Reactions Catalyzed by NAD(P)$^+$-Linked Dehydrogenases

Reaction[a]	Location[b]
NAD$^+$-linked	
α-Ketoglutarate + CoA + $NAD^+ \rightleftharpoons$ succinyl-CoA + CO_2 + NADH + H^+	M
L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+	M and C
Pyruvate + CoA + $NAD^+ \rightleftharpoons$ acetyl-CoA + CO_2 + NADH + H^+	M
Glyceraldehyde 3-phosphate + P_i + $NAD^+ \rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	C
Lactate + $NAD^+ \rightleftharpoons$ pyruvate + NADH + H^+	C
β-Hydroxyacyl-CoA + $NAD^+ \rightleftharpoons$ β-ketoacyl-CoA + NADH + H^+	M
NADP$^+$-linked	
Glucose 6-phosphate + $NADP^+ \rightleftharpoons$ 6-phosphogluconate + NADPH + H^+	C
L-Malate + $NADP^+ \rightleftharpoons$ pyruvate + CO_2 + NADPH + H^+	C
NAD$^+$- or NADP$^+$-linked	
L-Glutamate + H_2O + NAD(P)$^+ \rightleftharpoons$ α-ketoglutarate + NH_4^+ + NAD(P)H	M
Isocitrate + NAD(P)$^+ \rightleftharpoons$ α-ketoglutarate + CO_2 + NAD(P)H + H^+	M and C

[a]These reactions and their enzymes are discussed in Chapters 14, 16, 17, and 18.
[b]M designates mitochondria; C designates cytosol.

P3 Principle 3 (2 of 6)

Electrons flow from electron donors (oxidizable substrates) through a chain of membrane-bound carriers to a final electron acceptor with a large reduction potential. The final acceptor is molecular oxygen, O_2. The appearance of oxygen in the atmosphere some 2.3 billion years ago, and its harnessing in living systems via the evolution of oxidative phosphorylation, made more complex life forms possible.

Flavoproteins

- **flavoproteins** = contain a very tightly, sometimes covalently, bound flavin nucleotide (FMN or FAD)

- the oxidized flavin nucleotide can accept either:
  - one electron (yielding the semiquinone form) or
  - two electrons (yielding $FADH_2$ or $FMNH_2$)

- electron transfer occurs because the flavoprotein has a higher $E°$ than the compound oxidized
  - $E'°$ of a flavin nucleotide depends on the protein with which it is associated

Electrons Pass through a Series of Membrane-Bound Carriers

- three types of electron transfers occur in oxidative phosphorylation:
  - direct transfer of electrons
  - transfer as a hydrogen atom ($H^+ + e^-$)
  - transfer as a hydride ion ($:H^-$)

- **reducing equivalent** = a single electron equivalent transferred in an oxidation-reduction reaction

P3 Principle 3 (3 of 6)

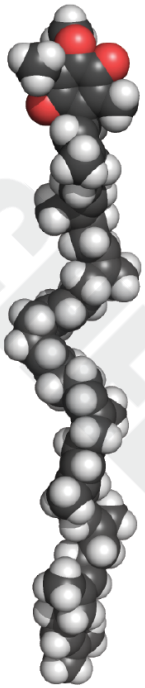
Electrons flow from electron donors (oxidizable substrates) through a chain of membrane-bound carriers to a final electron acceptor with a large reduction potential. The final acceptor is molecular oxygen, O_2. The appearance of oxygen in the atmosphere some 2.3 billion years ago, and its harnessing in living systems via the evolution of oxidative phosphorylation, made more complex life forms possible.

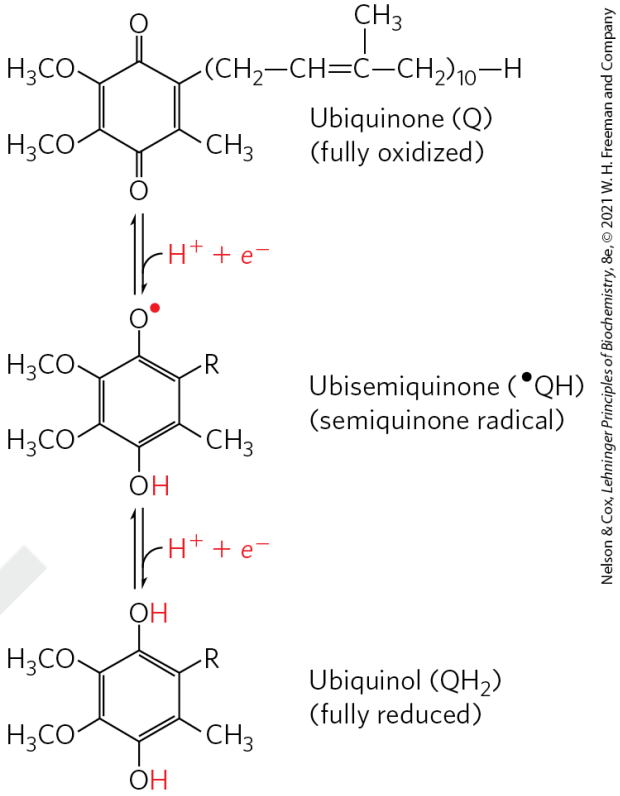
Electron-Carrying Molecules in the Respiratory Chain

- five types of electron-carrying molecules:
  - NAD
  - flavoproteins
  - **ubiquinone** (**coenzyme Q** or **Q**)
  - **cytochromes**
  - **iron-sulfur proteins**

Ubiquinone

- **ubiquinone** (**coenzyme Q** or **Q**) = a lipid-soluble benzoquinone with a long isoprenoid side chain
  - can accept one or two electrons
  - freely diffusible within the inner mitochondrial membrane
  - plays a central role in coupling electron flow to proton movement





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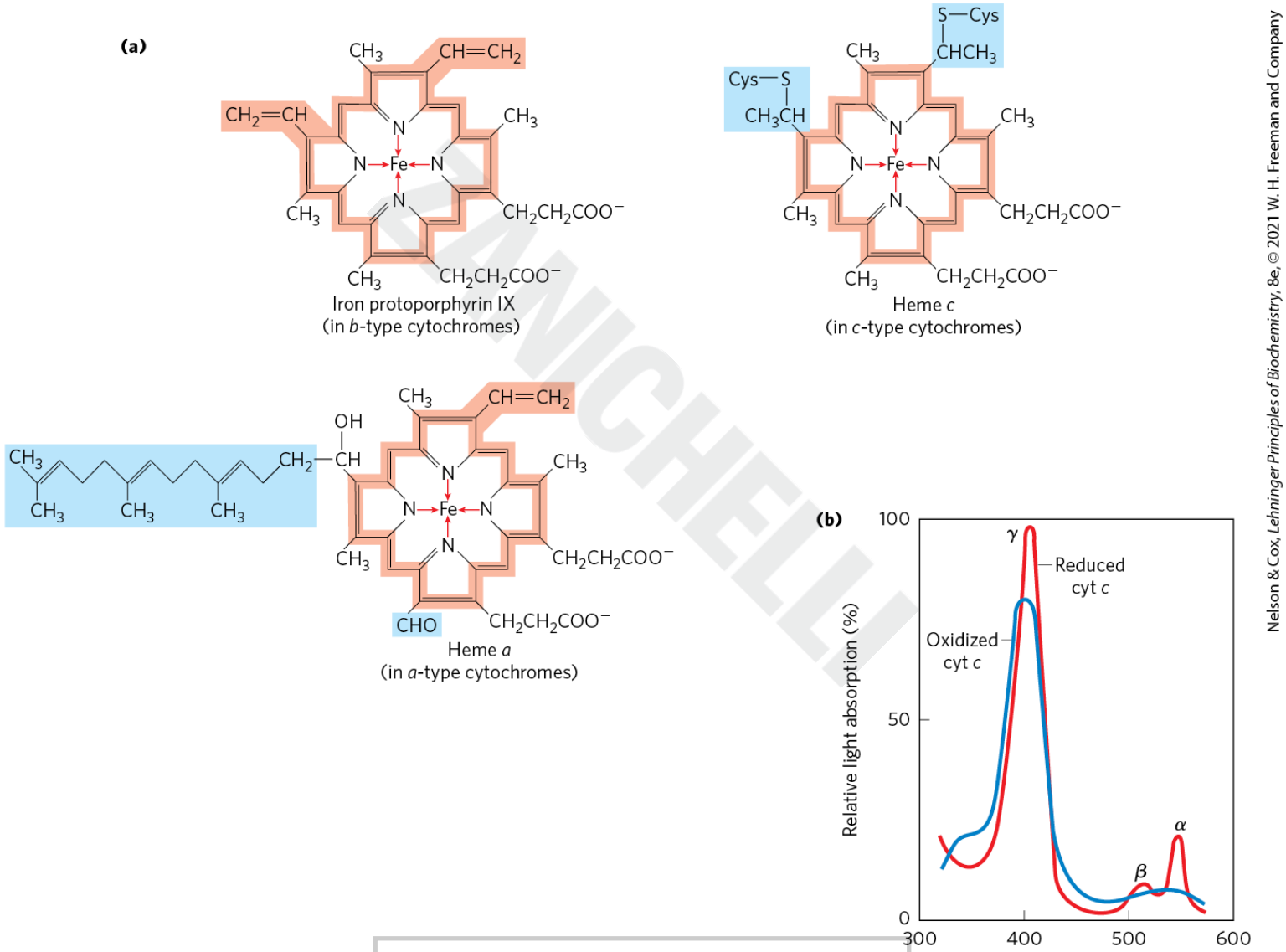
Cytochromes

- **cytochromes** = proteins with characteristic strong absorption of visible light due to their iron-containing heme prosthetic groups
  - one-electron carriers
  - 3 classes in mitochondria: *a, b,* and *c*
    - hemes of *a* and *b* are not covalently bound to associated proteins
    - *c* is covalently attached through Cys residues

Prosthetic Groups of Cytochromes



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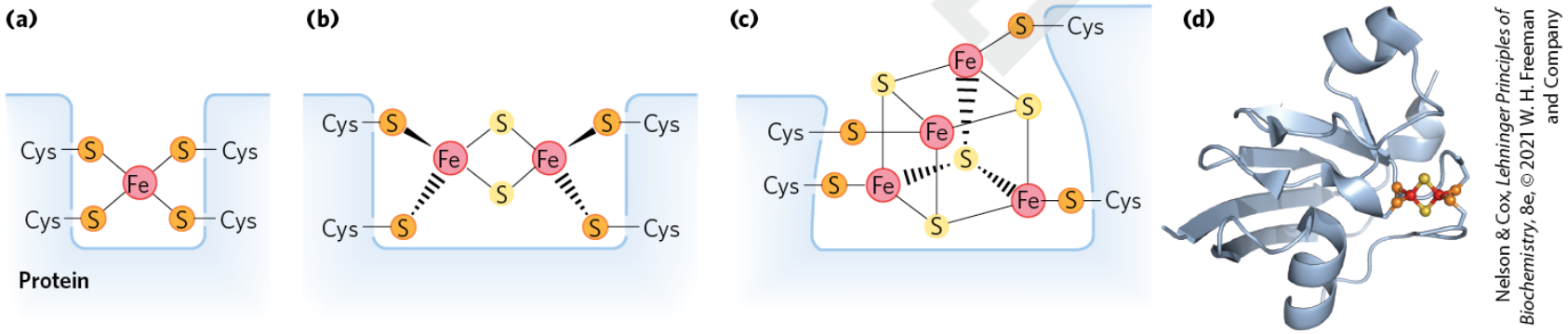
Iron-Sulfur Proteins

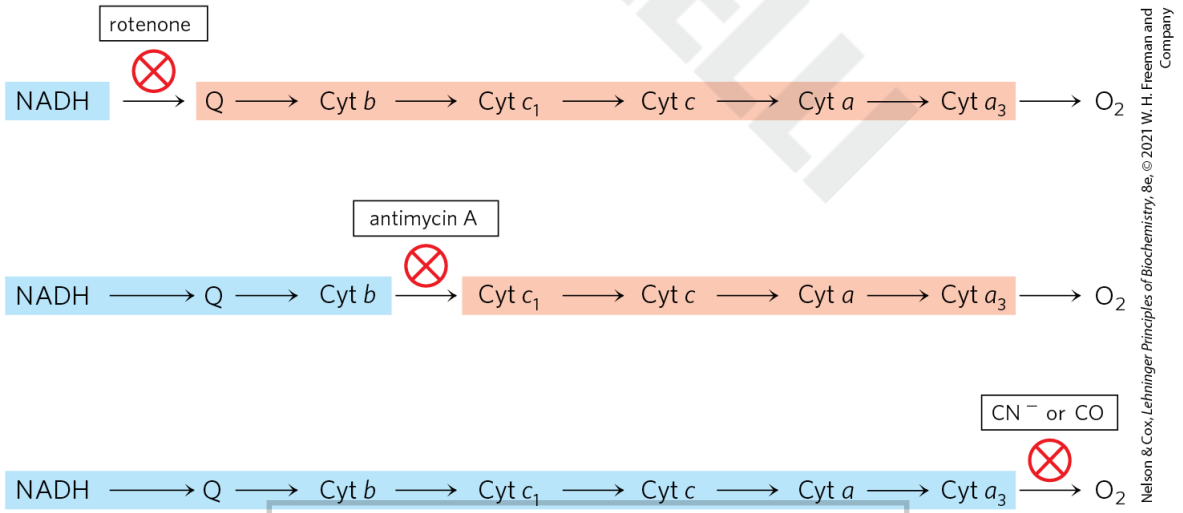
- **iron-sulfur proteins** = proteins that contain iron in association with inorganic sulfur atoms and/or with the sulfur atoms of Cys residues in the protein
  - participate in one-electron transfers

- **Rieske iron-sulfur proteins** = proteins in which one Fe atom is coordinated to two His residues



Methods for Determining The Sequence of Electron Carriers

- determine the $E'°$ of the individual electron carriers
- reduce the entire chain of carriers by omitting O_2 and then measure the oxidation rate of each electron carrier when O_2 is reintroduced
- measure the effects of inhibitors of electron transfer on the oxidation state of each carrier



Standard Reduction Potentials of Individual Electron Carriers

- electrons tend to flow spontaneously from carriers of lower $E'°$ to carriers of higher $E'°$

Table 19-2 Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

Redox reaction (half-reaction)	$E'°$ (V)
$2H^+ + 2e^- \rightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \rightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \rightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \rightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \rightarrow$ ubiquinol	0.045
Cytochrome *b* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *b* (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) + $e^- \rightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *c* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + $e^- \rightarrow$ cytochrome a_3 (Fe^{2+})	0.35
½ $O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.817

The Sequence of Electron Carriers

- the order of carriers is:

NADH → Q → cytochrome *b* → cytochrome c_1 →
cytochrome *c* → cytochrome *a* → cytochrome a_3 → O_2

- order has been confirmed by all three approaches

P3 Principle 3 (4 of 6)

Electrons flow from electron donors (oxidizable substrates) through a chain of membrane-bound carriers to a final electron acceptor with a large reduction potential. The final acceptor is molecular oxygen, O_2. The appearance of oxygen in the atmosphere some 2.3 billion years ago, and its harnessing in living systems via the evolution of oxidative phosphorylation, made more complex life forms possible.

Electron Carriers Function in Multienzyme Complexes

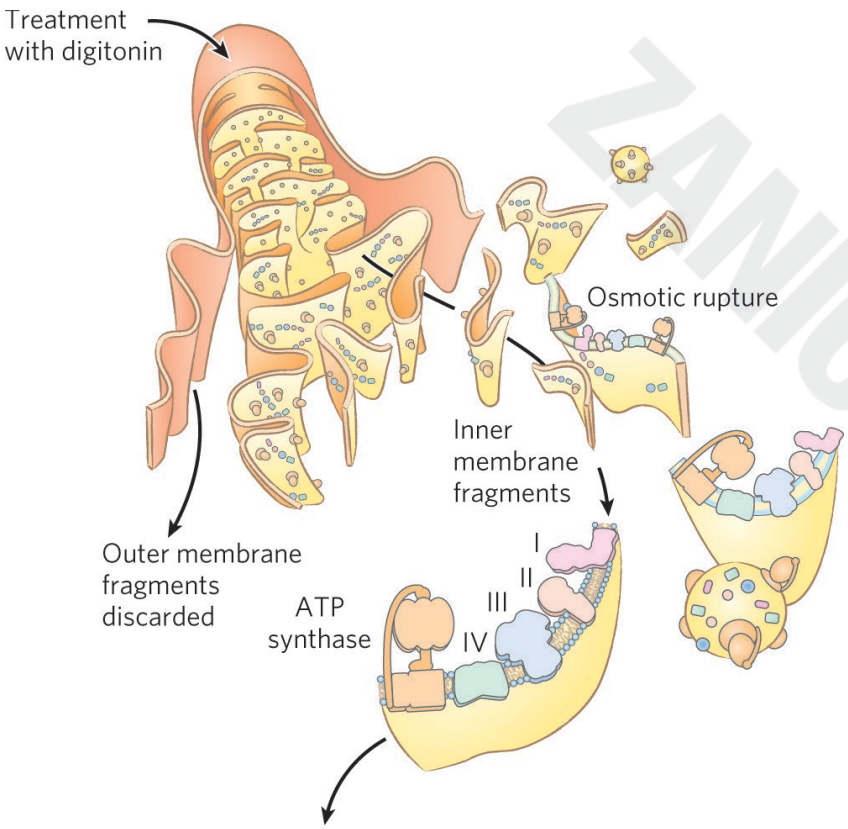
- four unique electron-carrier complexes catalyze electron transfer through a portion of the chain:
  - Complex I (from NADH to ubiquinone)
  - Complex II (from succinate to ubiquinone)
  - Complex III (from ubiquinone to cytochrome *c*)
  - Complex IV (from cytochrome *c* to O_2)

Protein Components of the Mitochondrial Respiratory Chain

Table 19-3 The Protein Components of the Mitochondrial Respiratory Chain

Enzyme complex/protein	Mass (kDa)	Number of subunits	Prosthetic group(s)
I NADH dehydrogenase	850	45 (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	204	13 (3–4)	Hemes; Cu_A, Cu_B

Separation of Functional Complexes of the Respiratory Chain



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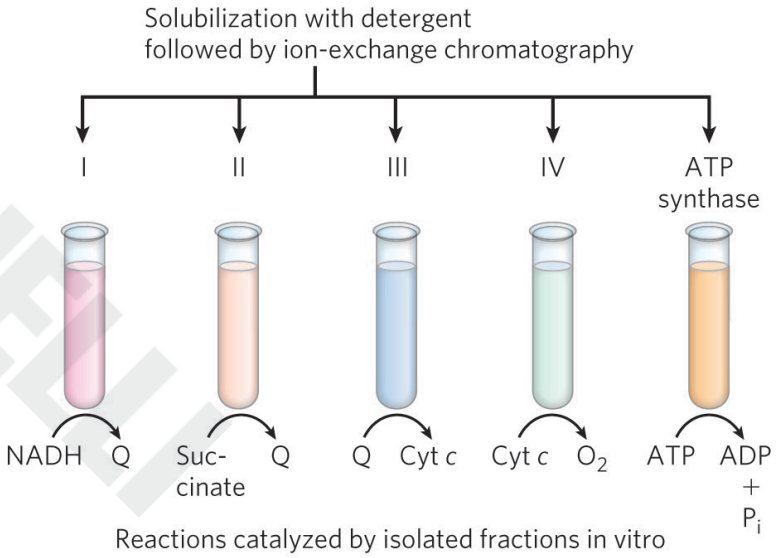

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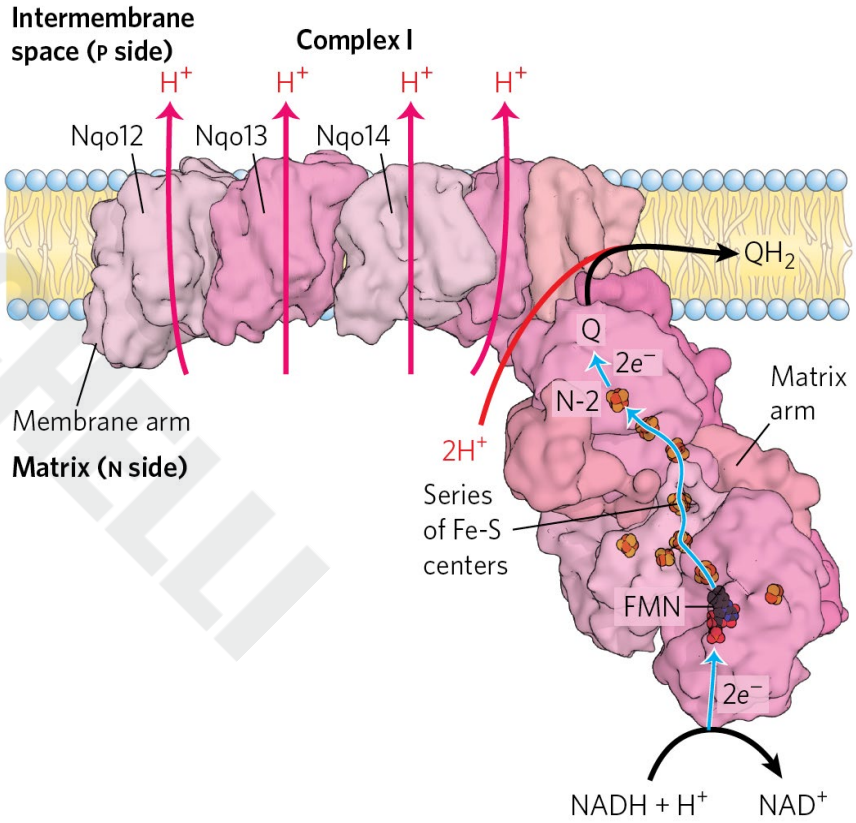
Complex I: NADH to Ubiquinone

- **Complex I (NADH:ubiquinone oxidoreductase, NADH dehydrogenase)** = large L-shaped enzyme with >40 polypeptide chains
  - FMN-containing flavoprotein accepts 2 electrons from NADH
  - 8+ Fe-S centers pass elections to ubiquinone



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Complex I Catalyzes Two Simultaneous and Obligately Coupled Processes

- Complex I catalyzes:
  - the exergonic transfer of a hydride ion from NADH and a proton from the matrix to ubiquinone

$$NADH + H^+ + Q \rightarrow NAD^+ + QH_2 \qquad (19\text{-}1)$$

  - the endergonic transfer of 4 protons from the matrix to the intermembrane space

P4 Principle 4 (2 of 5)

The free energy made available by "downhill" (exergonic) electron flow is coupled to the "uphill" transport of protons across a proton-impermeable membrane. The free energy of fuel oxidation is thus conserved as a transmembrane electrochemical potential.

Complex I Is a Proton Pump

- Complex I is a proton pump driven by the energy of electron transfer

- Complex I catalyzes a **vectorial** reaction (moves protons in a specific direction from one location to another)

The Overall Reaction of Complex I

- the overall reaction is:

$$NADH + 5H^+_N + Q \rightarrow NAD^+ + QH_2 + 4H^+_P \quad (19\text{-}2)$$

where the subscript letters indicate the location of the protons: P indicates the positive side (the intermembrane space) and N indicates the negative side (the matrix)

Agents that Inhibit Oxidative Phosphorylation

- inhibitors of Complex I:
  - amytal (a barbiturate drug)
  - rotenone (an insecticide)
  - piericidin A (an antibiotic)

TABLE 19-4 Agents That Interfere with Oxidative Phosphorylation

Type of interference	Compound[a]	Target/mode of action
Inhibition of electron transfer	Cyanide, Carbon monoxide	Inhibit cytochrome oxidase
	Antimycin A	Blocks electron transfer from cytochrome *b* to cytochrome c_1
	Myxothiazol, Rotenone, Amytal, Piericidin A	Prevent electron transfer from Fe-S center to ubiquinone
Inhibition of ATP synthase	Aurovertin	Inhibit F_1
	Oligomycin, Venturicidin	Inhibit F_o
	DCCD	Blocks proton flow through F_o
Uncoupling of phosphorylation from electron transfer	FCCP, DNP	Hydrophobic proton carriers
	Valinomycin	K^+ ionophore
	Uncoupling protein 1	In brown adipose tissue, forms proton-conducting pores in inner mitochondrial membrane
Inhibition of ATP-ADP exchange	Atractyloside	Inhibits adenine nucleotide translocase

[a]DCCD, dicyclohexylcarbodiimide; FCCP, cyanide-*p*-trifluoromethoxyphenylhydrazone; DNP, 2,4-dinitrophenol.

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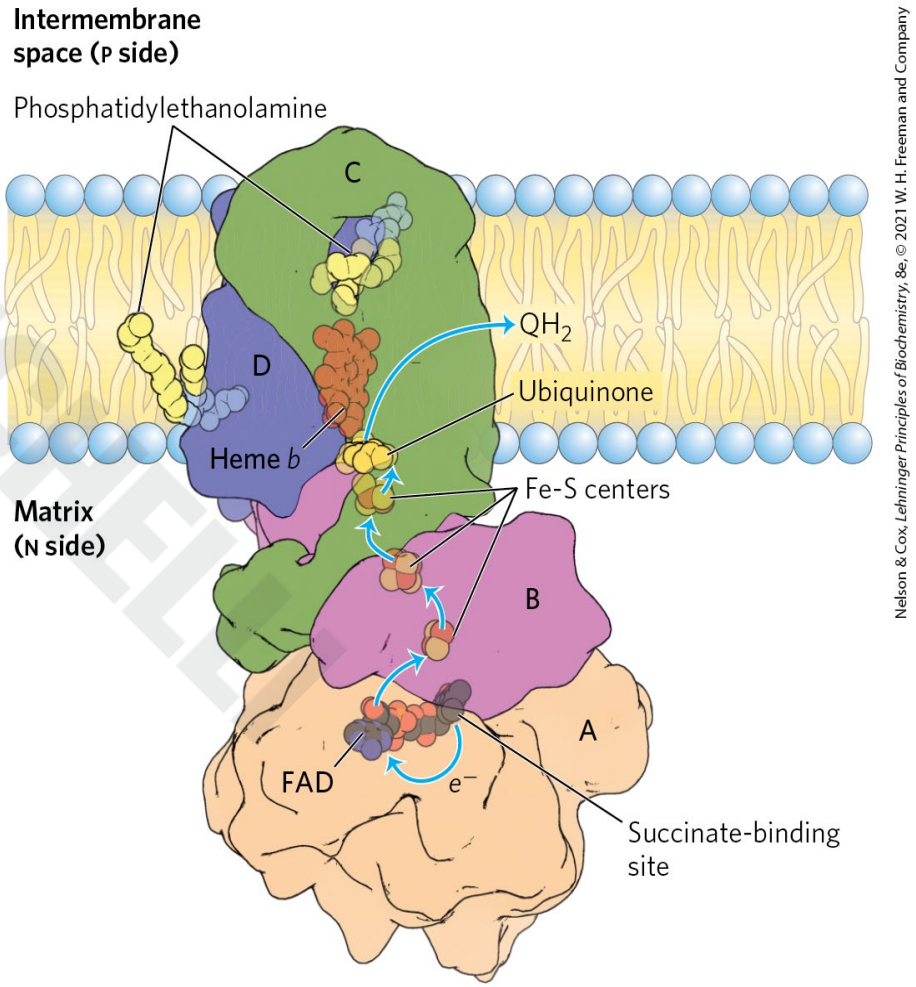
Complex II: Succinate to Ubiquinone

- **Complex II (succinate dehydrogenase)** = couples the oxidation of succinate with the reduction of ubiquinone
  - electrons pass from FAD to Fe-S centers to Q
  - no proton pumping
  - also functions to convert succinate to fumarate in the citric acid cycle



Heme *b* of Complex II

- **heme *b*** reduces the frequency with which electrons “leak” out of the system, moving from succinate to molecular oxygen to produce the **reactive oxygen species (ROS)** H_2O_2 and the **superoxide radical**

P3 Principle 3 (5 of 6)

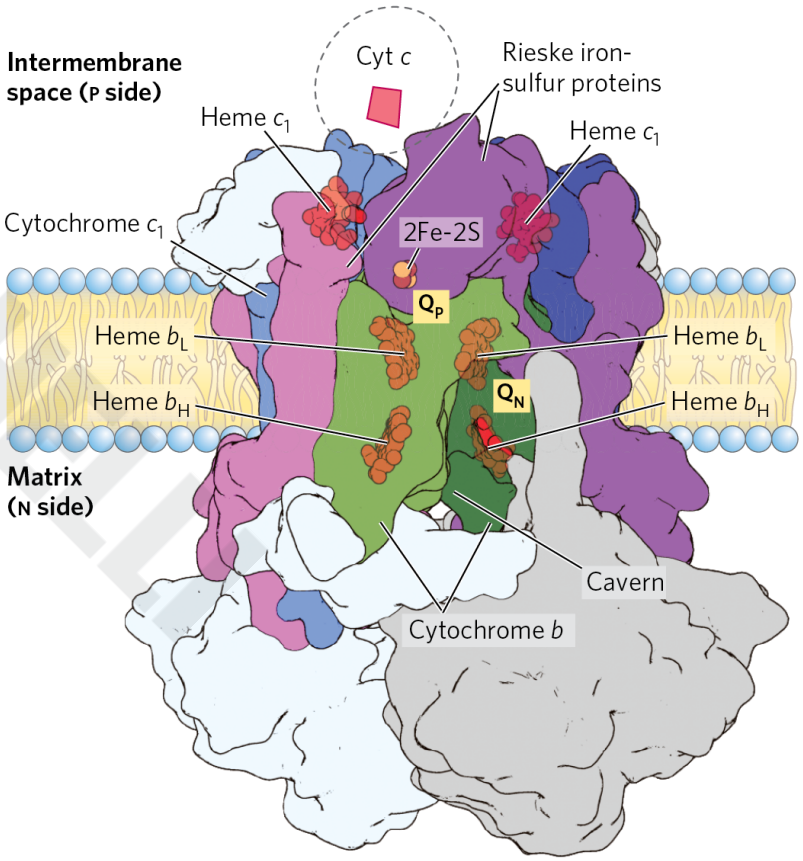
Electrons flow from electron donors (oxidizable substrates) through a chain of membrane-bound carriers to a final electron acceptor with a large reduction potential. The final acceptor is molecular oxygen, O_2. The appearance of oxygen in the atmosphere some 2.3 billion years ago, and its harnessing in living systems via the evolution of oxidative phosphorylation, made more complex life forms possible.

P4 Principle 4 (3 of 5)

The free energy made available by "downhill" (exergonic) electron flow is coupled to the "uphill" transport of protons across a proton-impermeable membrane. The free energy of fuel oxidation is thus conserved as a transmembrane electrochemical potential.

Complex III: Ubiquinone to Cytochrome *c*

- **Complex III (cytochrome bc_1 complex, ubiquinone: cytochrome *c* oxidoreductase)** = couples the transfer of 2 electrons from ubiquinol to cytochrome *c* with the vectorial transport of four protons to the intermembrane space
  - contains cytochrome *b*, cytochrome *c*1, and the Rieske iron-sulfur protein



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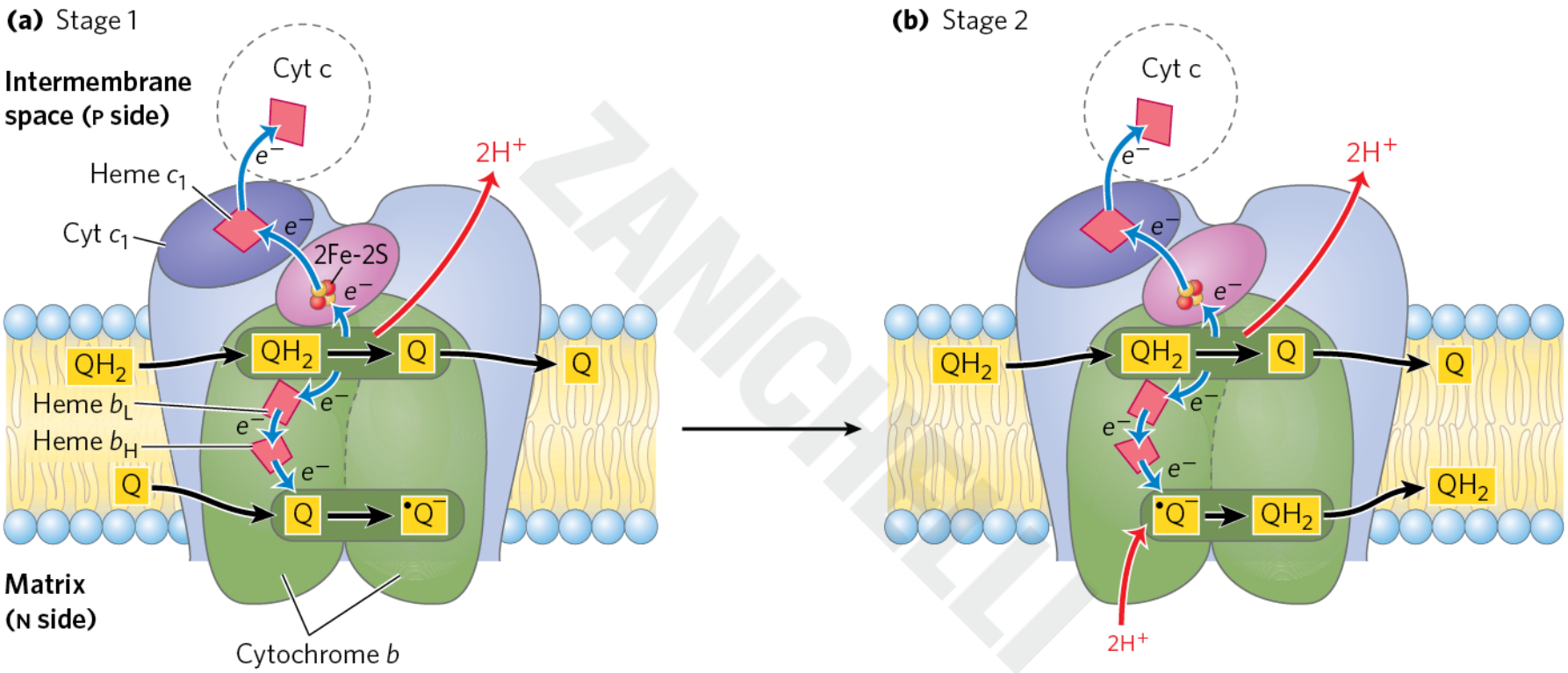
The Path of Electrons through Complex III

- ubiquinone diffuses through the inner mitochondrial membrane and transfers the electrons to cytochrome *b*
- electrons pass to an Fe-S center which passes electrons, one at a time, through cytochrome *c*

The Q Cycle



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

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

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

The Net Equation for the Redox Reactions of the Q Cycle

- the net equation is:

$$QH_2 + 2 \text{ cyt } c \text{ (oxidized)} + 2H^+_N \rightarrow Q + 2 \text{ cyt } c \text{ (reduced)} + 4H^+_P \qquad (19\text{-}3)$$

P3 Principle 3 (6 of 6)

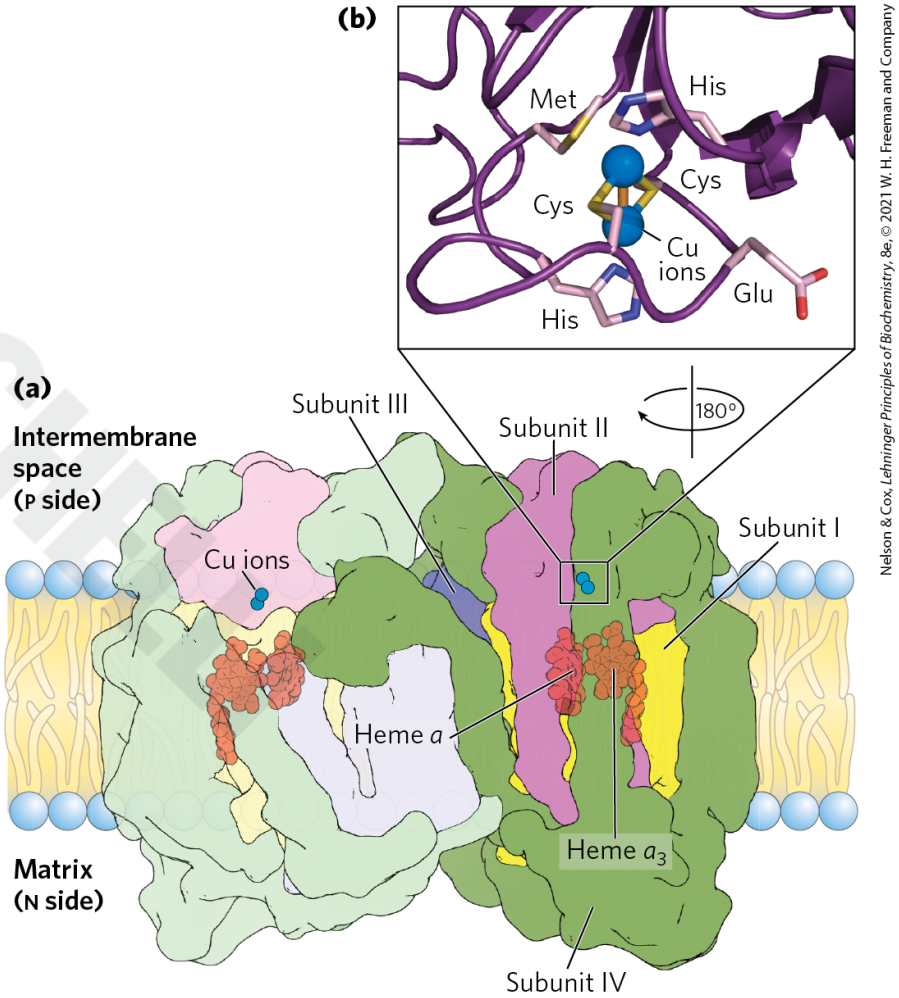
Electrons flow from electron donors (oxidizable substrates) through a chain of membrane-bound carriers to a final electron acceptor with a large reduction potential. The final acceptor is molecular oxygen, O_2. The appearance of oxygen in the atmosphere some 2.3 billion years ago, and its harnessing in living systems via the evolution of oxidative phosphorylation, made more complex life forms possible.

P4 Principle 4 (4 of 5)

The free energy made available by "downhill" (exergonic) electron flow is coupled to the "uphill" transport of protons across a proton-impermeable membrane. The free energy of fuel oxidation is thus conserved as a transmembrane electrochemical potential.

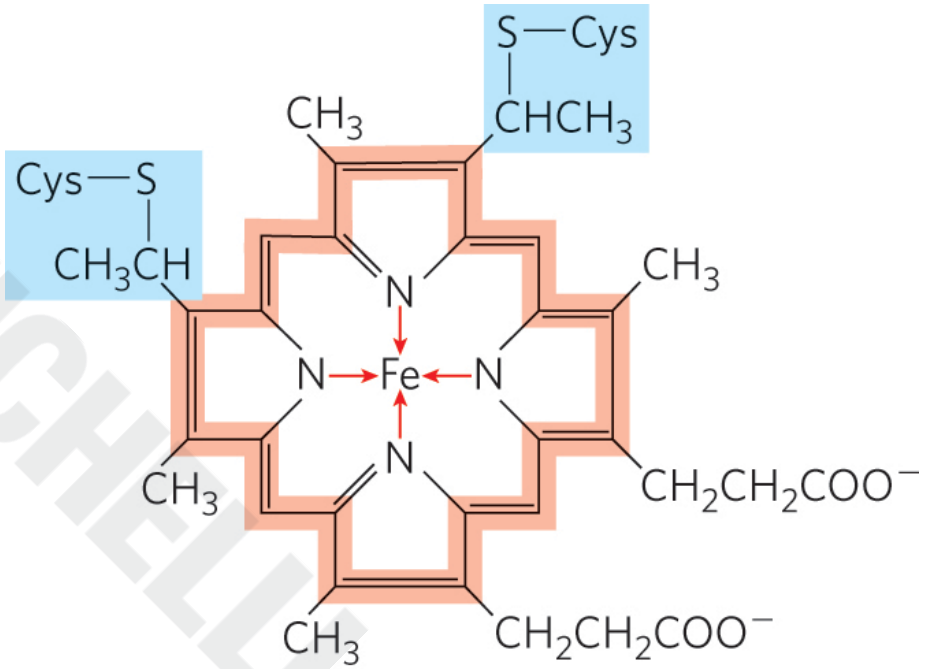
Complex IV: Cytochrome *c* to O_2

- **Complex IV (cytochrome oxidase)** = carries electrons from cytochrome *c* to molecular oxygen, reducing it to H_2O
  - large dimeric enzyme
  - 3 subunits have been conserved through evolution
  - contains cytochromes *a* and a_3
  - contains 2 Cu ions



Cytochrome *c*

- cytochrome *c:*
  - moves through the intermembrane space
  - carries a single electron from the cytochrome bc_1 complex to Complex IV
  - absorbs visible light



Heme *c*
(in *c*-type cytochromes)

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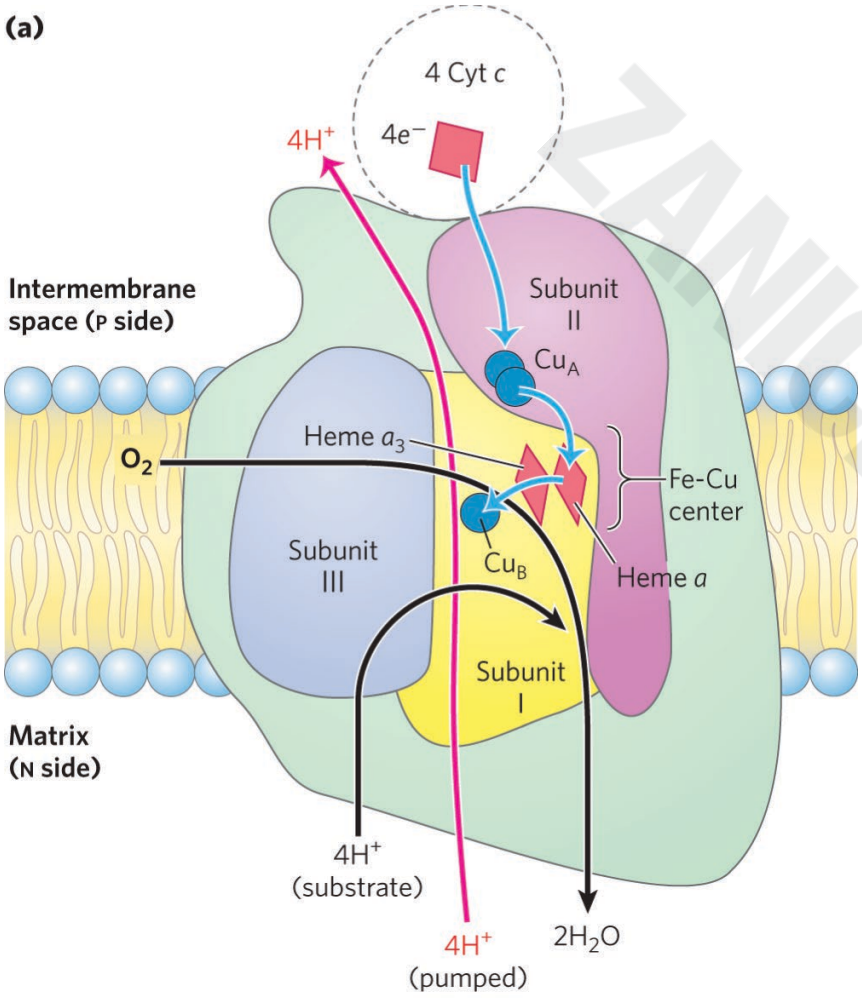
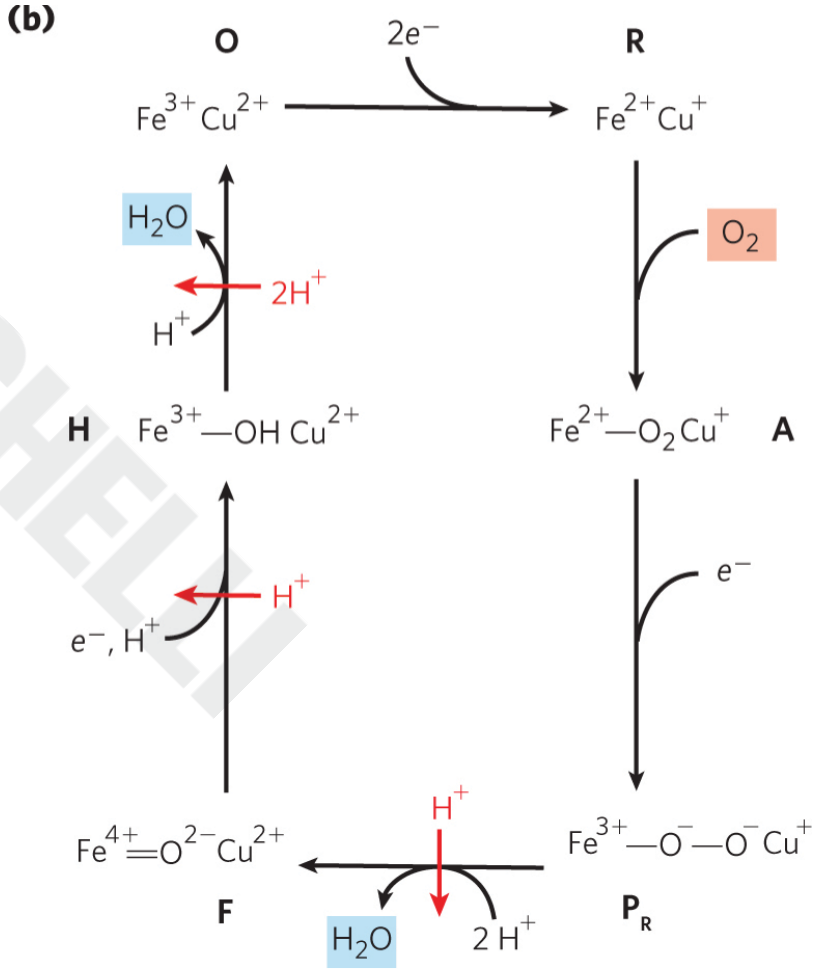
Path of Electrons Through Complex IV





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The Overall Reaction of Complex IV

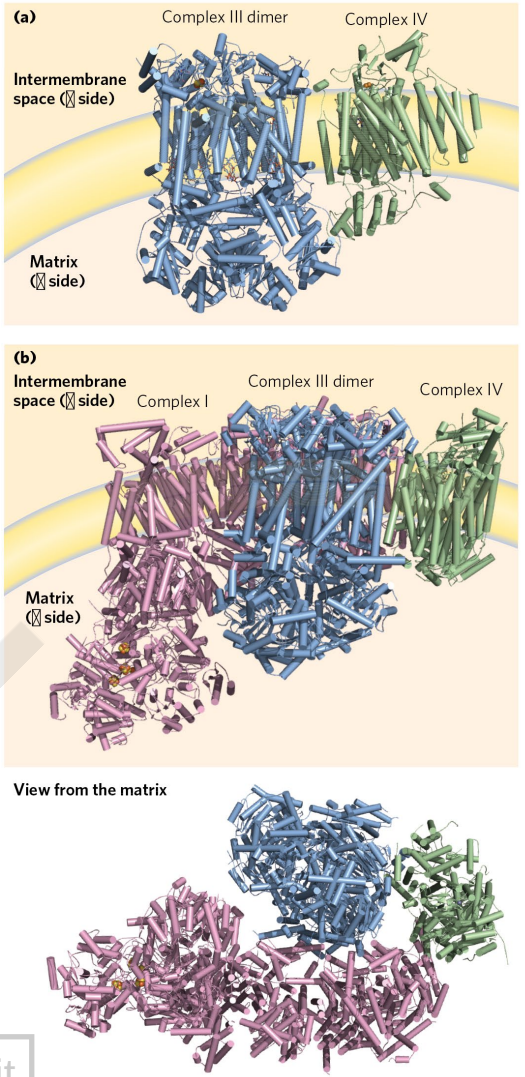
- the net equation is:

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

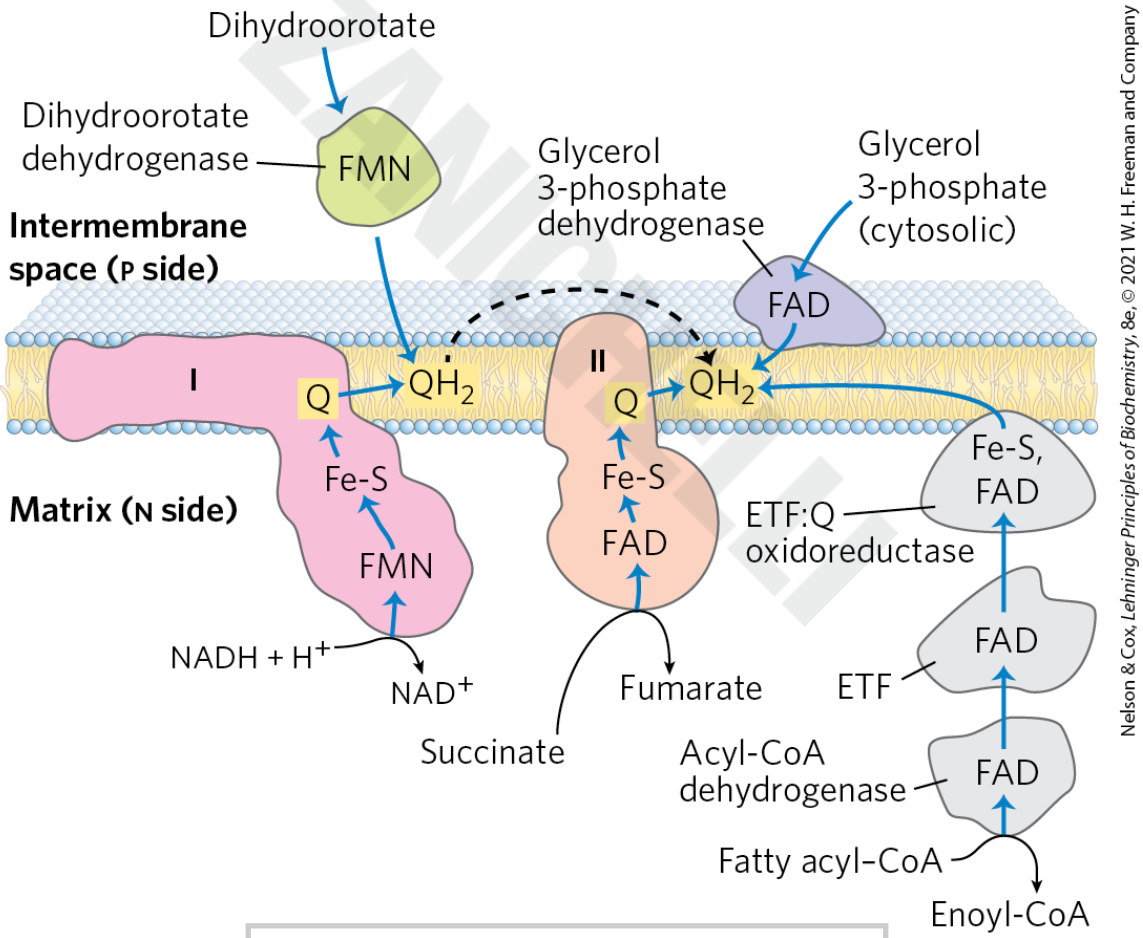
Mitochondrial Complexes Associate in Respirasomes

- **respirasome** = supercomplex containing Complexes I, III, and IV
  - may facilitate electron transfers or limit ROS production

- cytochrome *c* and ubiquinone readily diffuse between supercomplexes



Other Pathways Donate Electrons to the Respiratory Chain via Ubiquinone



Electrons Derived from the Oxidation of Fatty Acids Pass to Ubiquinone

- **acyl-CoA dehydrogenase** = flavoprotein that catalyzes the first step of the *β* oxidation of fatty acyl–CoA
  - electrons pass from the substrate to the FAD of the dehydrogenase to electron-transferring flavoprotein (ETF)

- ETF = passes its electrons to **ETF:ubiquinone oxidoreductase**
  - reduces Q in the inner mitochondrial membrane to QH_2

The Oxidation of Glycerol Phosphate and Dihydroorotate

- **glycerol 3-phosphate dehydrogenase** = flavoprotein located on the outer face of the inner mitochondrial membrane
  - Q accepts electrons, and the QH_2 produced enters the pool of QH_2 in the membrane

- **dihydroorotate dehydrogenase** = located on the outside of the inner mitochondrial membrane
  - donates electrons to Q in the respiratory chain

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

- the net equation for the transfer of two electrons from NADH through the respiratory chain to molecular oxygen is:

$$2\ NADH + 2H^+ + O_2 \rightarrow 2\ NAD^+ + 2H_2O \qquad (19\text{-}5)$$

The Change in Standard Reduction Potential

- the standard reduction potential change is:

$$\Delta E'^\circ = E'^\circ \text{ (electron acceptor)} - E'^\circ \text{ (electron donor)}$$
$$= 0.816 \text{ V} - (-0.320 \text{ V})$$
$$= 1.14 \text{ V}$$

E'° for $NAD^+/NADH$ is −0.320 V

E'° for O_2/H_2O is 0.816 V

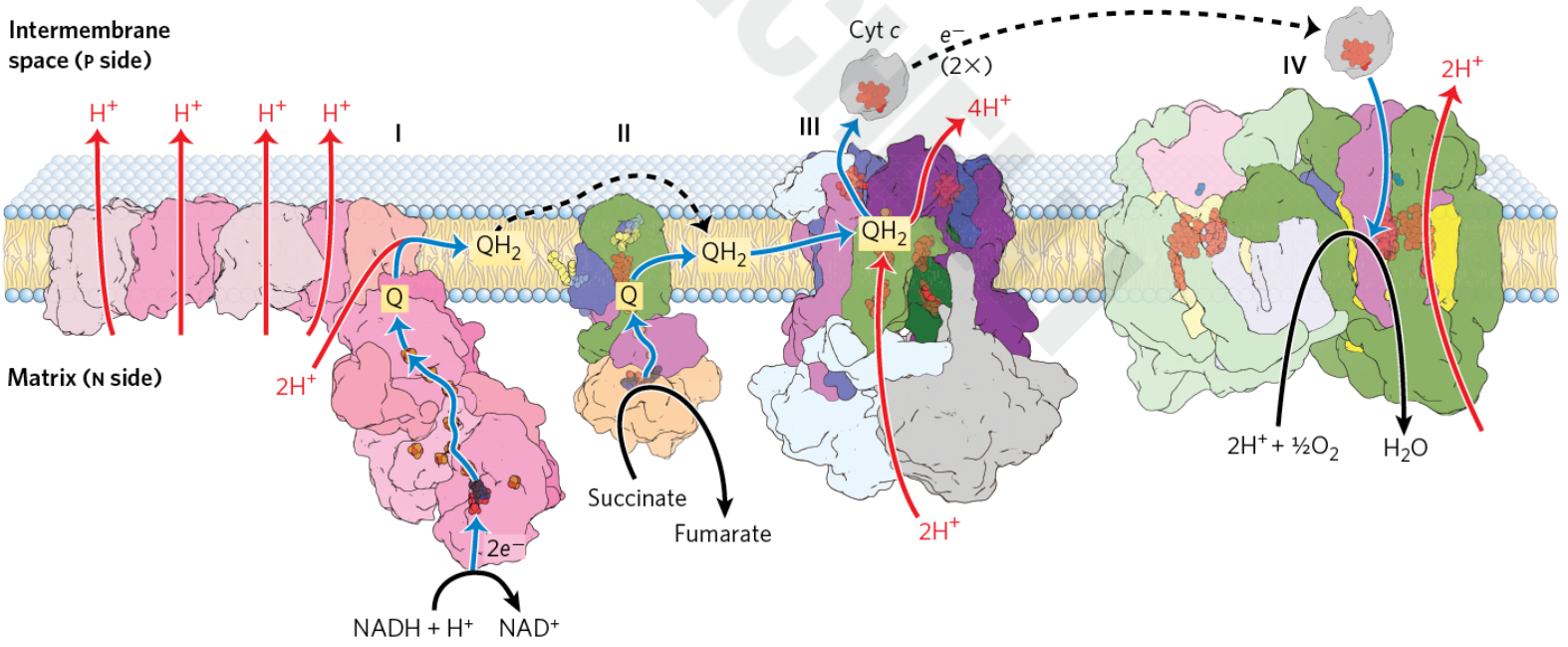
The Net Reaction is Highly Exergonic

- the standard free-energy change is:

$$\Delta G'^\circ = -nF\,\Delta E'^\circ \qquad (19\text{-}6)$$
$$= -2(96.5\ \text{kJ/V} \cdot \text{mol})(1.14\ \text{V})$$
$$= -220\ \text{kJ/mol (of NADH)}$$

Summary of the Flow of Electrons and Protons through the Respiratory Chain

- much of the free energy generated is recovered and stored in the form of an electrochemical proton gradient across the mitochondrial inner membrane



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The Net Vectorial Equation

- the net vectorial equation is:

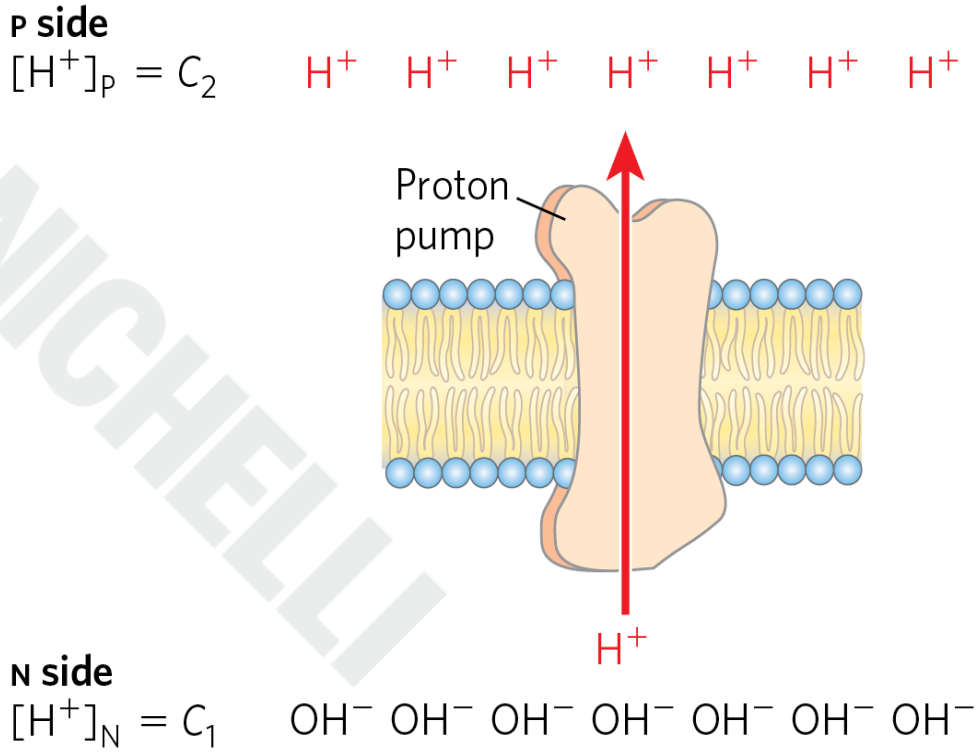
$$2\ NADH + 22H^+_N + O_2 \rightarrow 2\ NAD^+ + 20H^+_P + 2H_2O \qquad (19\text{-}7)$$

P4 Principle 4 (5 of 5)

The free energy made available by "downhill" (exergonic) electron flow is coupled to the "uphill" transport of protons across a proton-impermeable membrane. The free energy of fuel oxidation is thus conserved as a transmembrane electrochemical potential.

Proton-Motive Force

- **proton-motive force** = the energy stored in an electrochemical proton gradient across the mitochondrial inner membrane
  - composed of chemical and electrical potential energy



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The Free-Energy Change for the Creation of an Electrochemical Gradient by an Ion Pump

- the free-energy change is:

$$\Delta G = RT \ln(C_2/C_1) + ZF\,\Delta\psi \qquad (19\text{-}8)$$

where C_2 and C_1 are the concentrations of an ion in two regions, Z is the absolute value of its electrical charge, and $\Delta\psi$ is the transmembrane difference in electrical potential (in volts)

Calculating the Proton-Motive Force

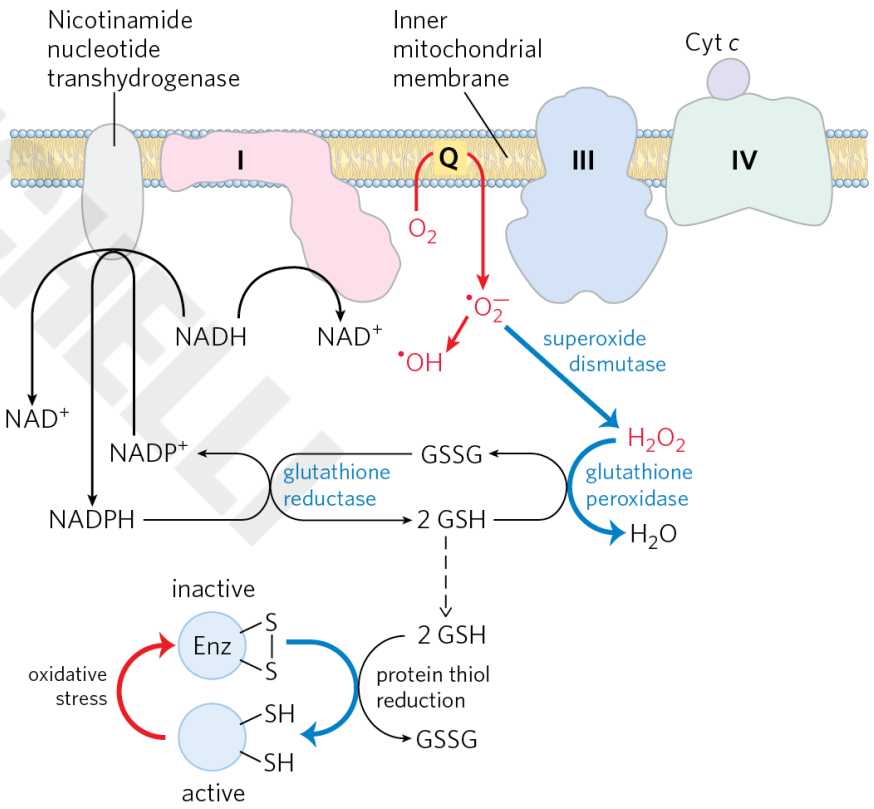
- the free-energy change is:

$$\Delta G = RT \ln(C_2/C_1) + ZF\,\Delta\psi$$
$$= RT\,(2.3(\log[H^+]_P - \log[H^+]_N) + ZF\,\Delta\psi$$
$$= RT\,(2.3(pH_N - pH_p) + ZF\,\Delta\psi$$
$$= RT\,(2.3\,\Delta pH) + ZF\,\Delta\psi$$

$$= 2.3RT\,\Delta pH + ZF\,\Delta\psi \qquad (19\text{-}9)$$

Reactive Oxygen Species Are Generated during Oxidative Phosphorylation

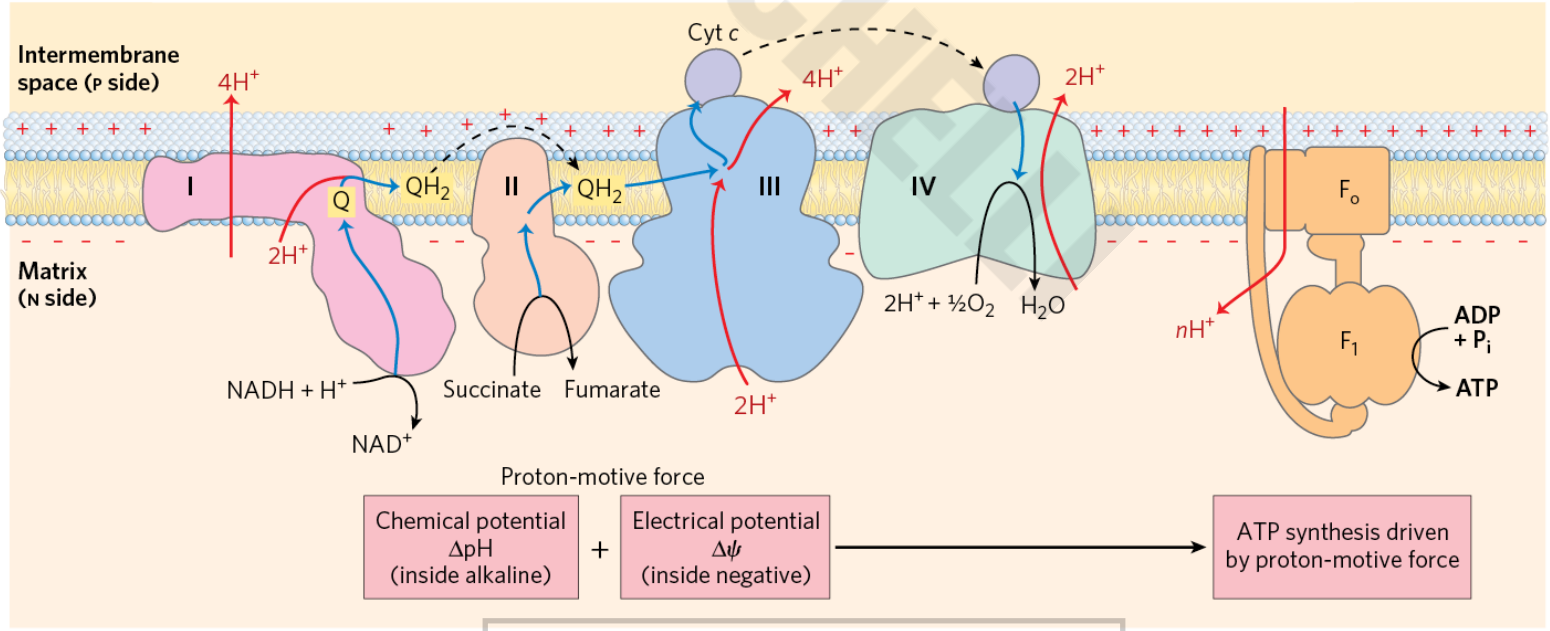
- **superoxide dismutase** = catalyzes the conversion of superoxide and two protons to H_2O_2 and O_2
- **glutathione peroxidase** = converts H_2O_2 to H_2O
- low ROS levels serve as signals reflecting insufficient O_2



19.2 ATP Synthesis

In the Chemiosmotic Model, Oxidation and Phosphorylation Are Obligately Coupled

- **chemiosmotic model** = describes the coupling of ATP synthesis to an electrochemical proton gradient (the proton-motive force)



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ATP Synthesis

- **ATP synthase** = drives the synthesis of ATP as protons flow passively back into the matrix through its proton pore

- to emphasize the role of the proton-motive force, the equation for ATP synthesis is:

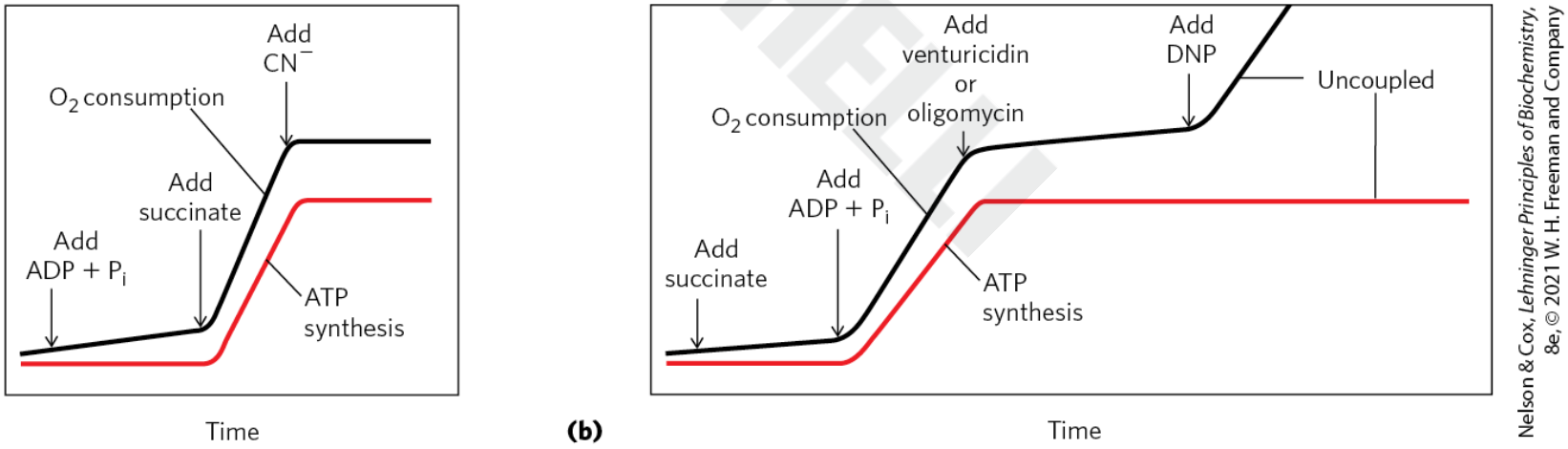
$$ADP + P_i + nH^+_P \rightarrow ATP + H_2O + nH^+_N \quad (19\text{-}10)$$

P5 Principle 5 (2 of 7)

The transmembrane flow of protons back down their electrochemical gradient through specific protein channels provides the free energy for synthesis of ATP. This process is catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.

Coupling of Electron Transfer and ATP Synthesis in Mitochondria

- inhibitors of electron transfer block ATP synthesis
- inhibitors of ATP synthesis block electron transfer (unless oxidation and phosphorylation are uncoupled)



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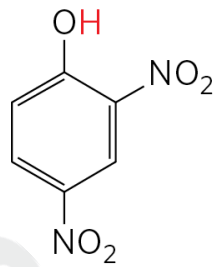
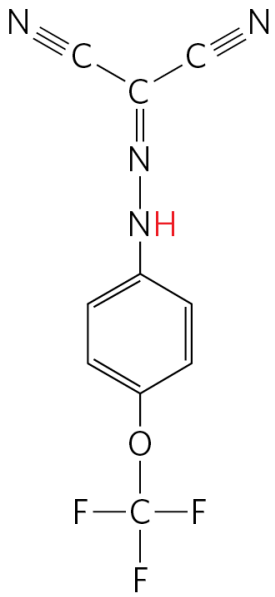
Chemical Uncouplers of Oxidative Phosphorylation

- physical shear or detergent uncouples electron transfer from ATP synthesis

- DNP and FCCP = weak acids that carry protons across the inner mitochondrial membrane
  - dissipates the proton gradient



2,4-Dinitrophenol (DNP)



Carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP)

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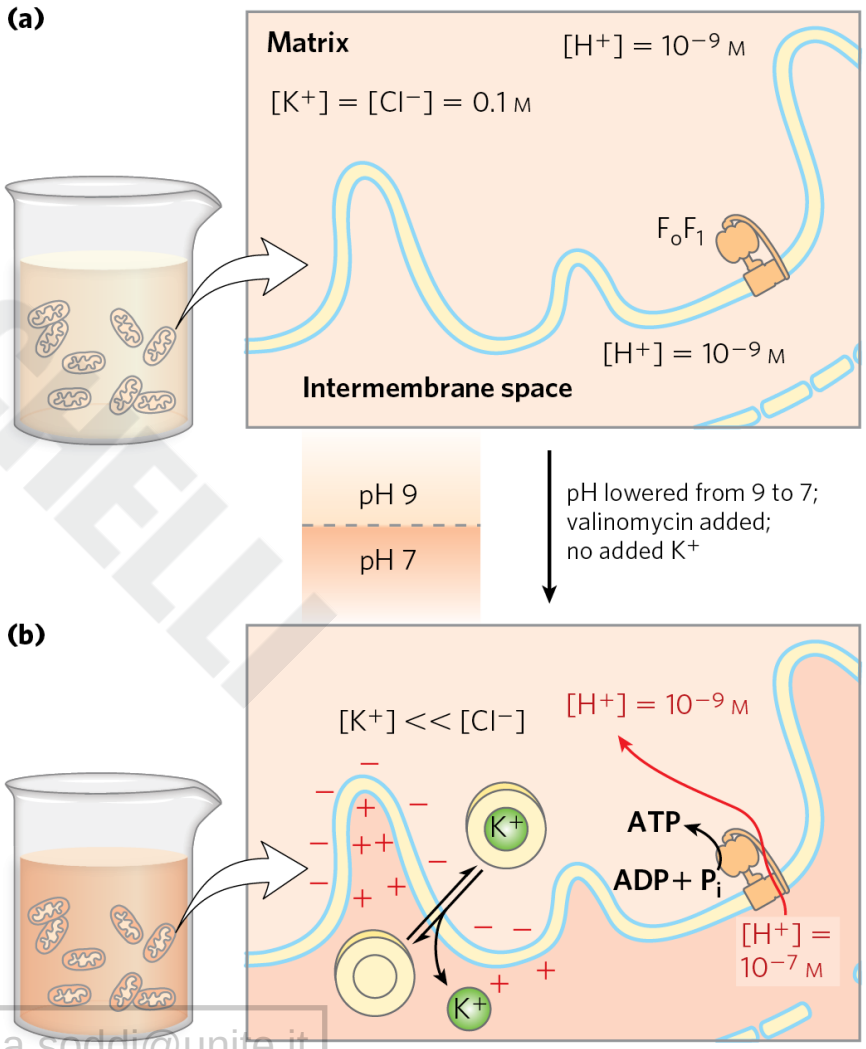
Proton-Motive Force Alone Drives ATP Synthesis

- in the absence of an oxidizable substrate, the proton-motive force alone drives ATP synthesis



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P2 Principle 2 (3 of 4)

Mitochondria trace their evolutionary origin to bacteria. Over 1.45 billion years ago, an endosymbiotic relationship arose between bacteria and a primitive eukaryote or eukaryotic progenitor. Mitochondria are ubiquitous in modern eukaryotes, and their bacterial origin is evident in almost every aspect of their structure and function.

ATP Synthase Has Two Functional Domains, F_o and F_1

- mitochondrial ATP synthase is an F-type ATPase
  - similar in structure and mechanism to the ATP synthases of bacteria and chloroplasts

P5 Principle 5 (3 of 7)

The transmembrane flow of protons back down their electrochemical gradient through specific protein channels provides the free energy for synthesis of ATP. This process is catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.

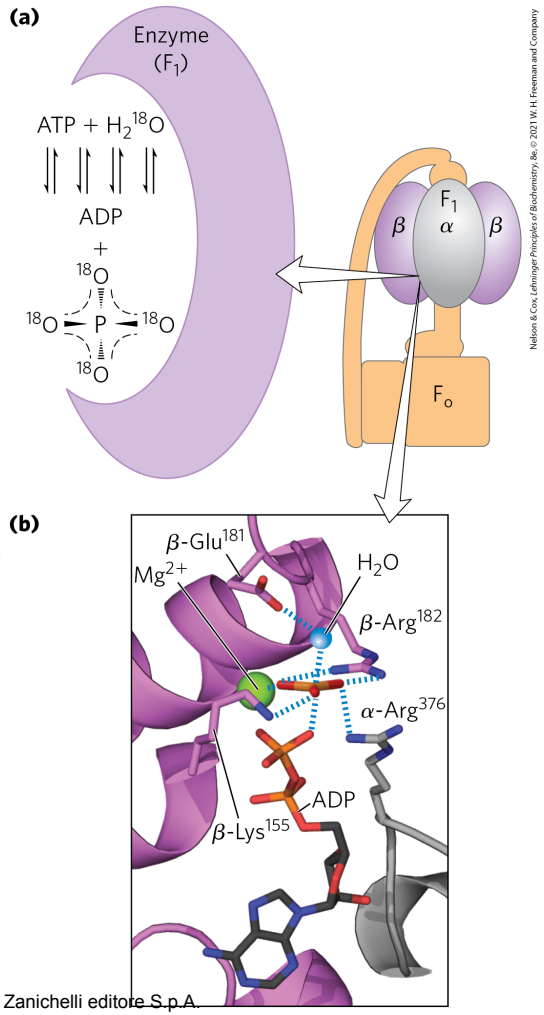
Mitochondrial ATP Synthase

- contains two distinct components:
  - F_o = an integral membrane protein with a proton pore
  - F_1 (originally **F_1 ATPase**) = a peripheral membrane protein
    - catalyzes hydrolysis of ATP when isolated

ATP Is Stabilized Relative to ADP on the Surface of F_1

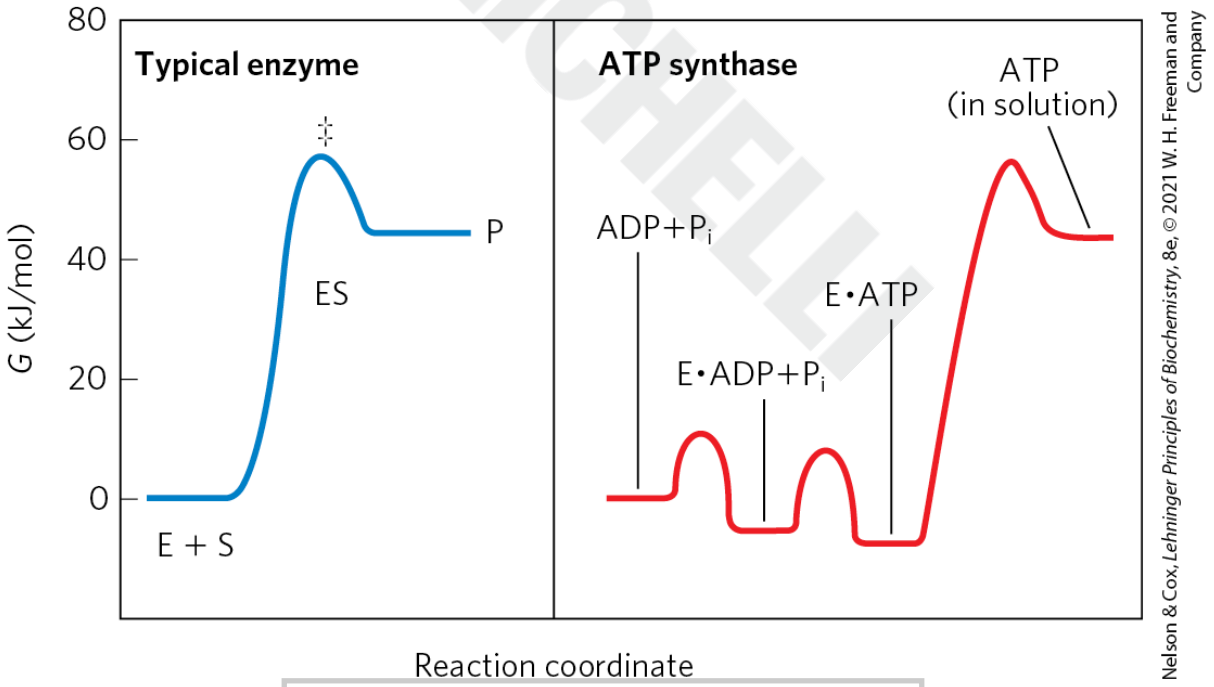
- on the surface of F_1, the reaction ADP + P_i ⇌ ATP + H_2O is readily reversible

- ATP synthase stabilizes ATP relative to ADP + P_i by binding ATP more tightly, releasing enough energy to counterbalance the cost of making ATP



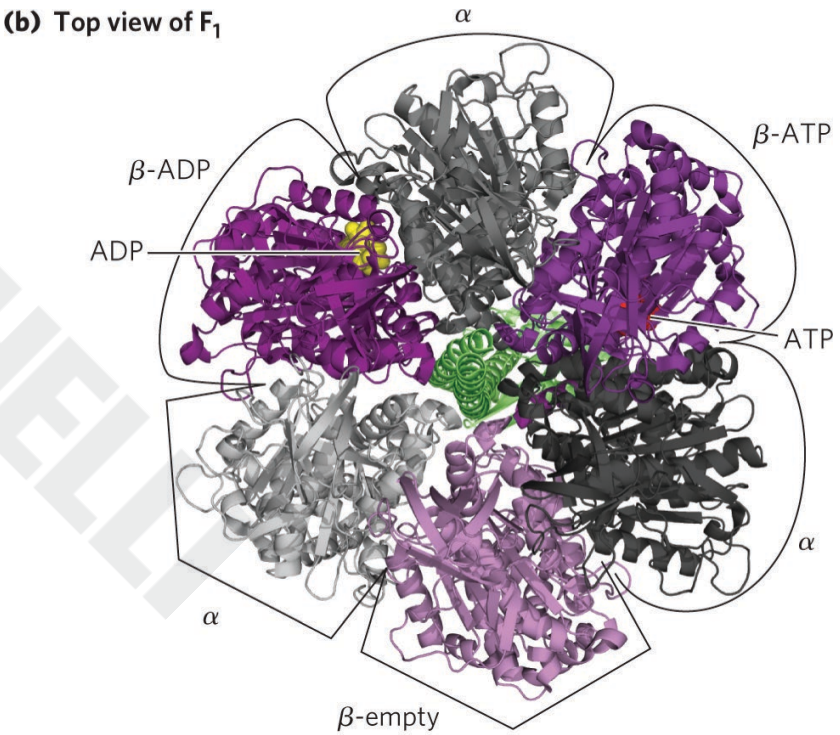
The Proton Gradient Drives the Release of ATP from the Enzyme Surface

- the free energy required for the release of ATP is provided by the proton-motive force



Each β Subunit of ATP Synthase Can Assume Three Different Conformations

- mitochondrial F_1 has the composition $\alpha_3\beta_3\gamma\delta\varepsilon$
  - each of the three β subunits has one catalytic site for ATP synthesis
  - each β subunit has a different conformation:
    - β-ATP
    - β-ADP
    - β-empty



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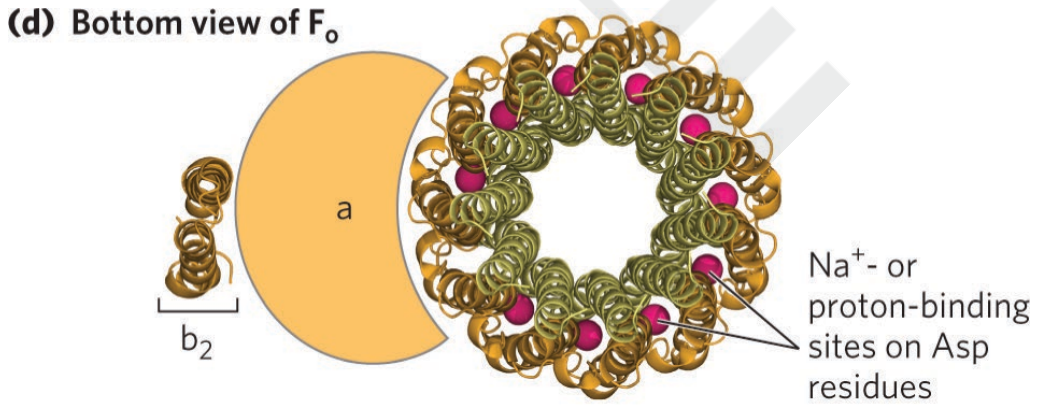
The Structure of the F_o Complex

- mitochondrial F_o has the composition ab_2c_n
  - *n* ranges from 8 to 17 depending on the species
- **c ring** = arrangement of c subunits into two concentric circles
  - the c subunits rotate together as a unit



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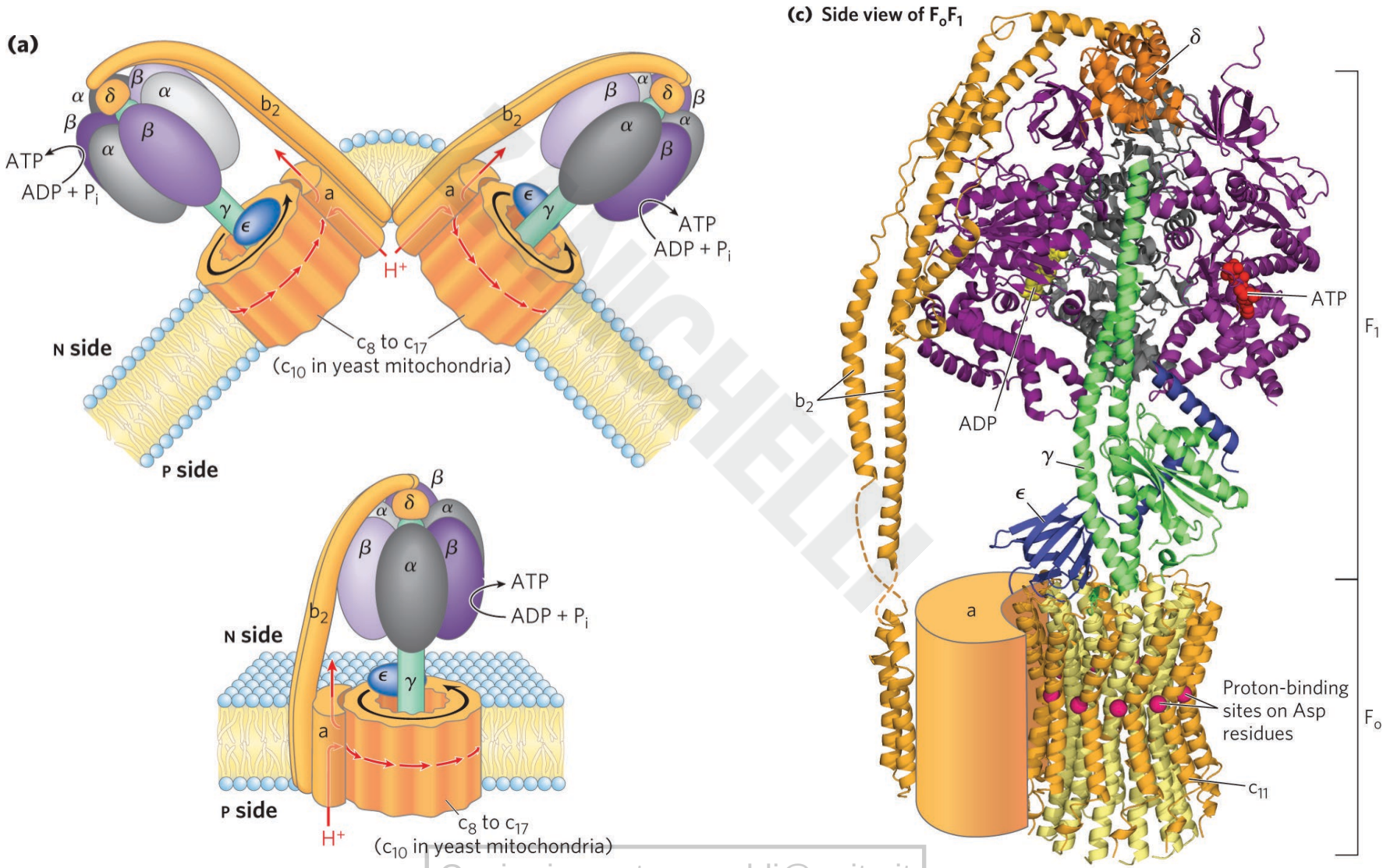
The Structure of the F_oF_1 Complex



P5 Principle 5 (4 of 7)

The transmembrane flow of protons back down their electrochemical gradient through specific protein channels provides the free energy for synthesis of ATP. This process is catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.

Rotational Catalysis Is Key to the Binding-Change Mechanism for ATP Synthesis

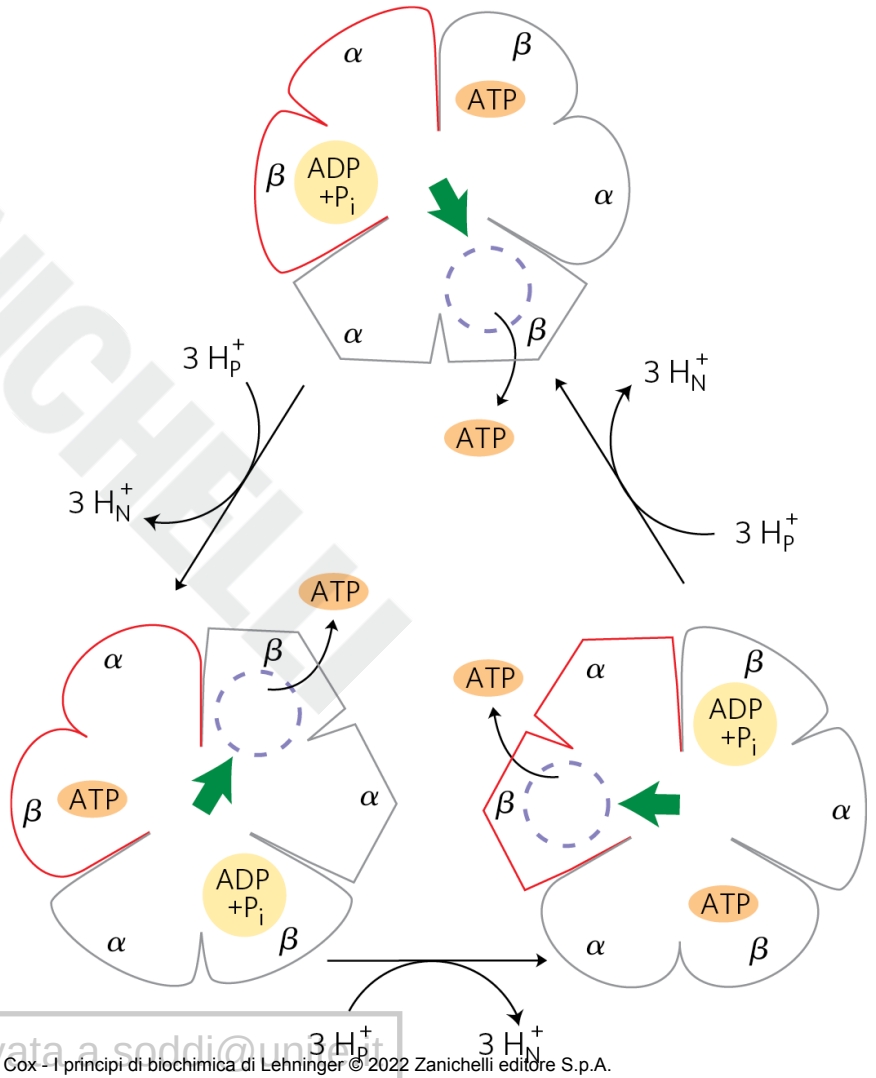
- **rotational catalysis** = mechanism by which the flow of protons through F_o causes the c ring to rotate and, in turn, trigger the subunit conformational changes in F_1

The Binding-Change Model

- **binding-change model** = the active site of each β subunit cycles through the three conformations:
  - β-ATP – tight binding
  - β-ADP – loose binding
  - β-empty – very loose binding

Binding-Change Model for ATP Synthase

- the three active sites of F_1 take turns catalyzing ATP synthesis
- translocation of three protons fuels synthesis of one ATP
- each complete rotation synthesizes three ATP



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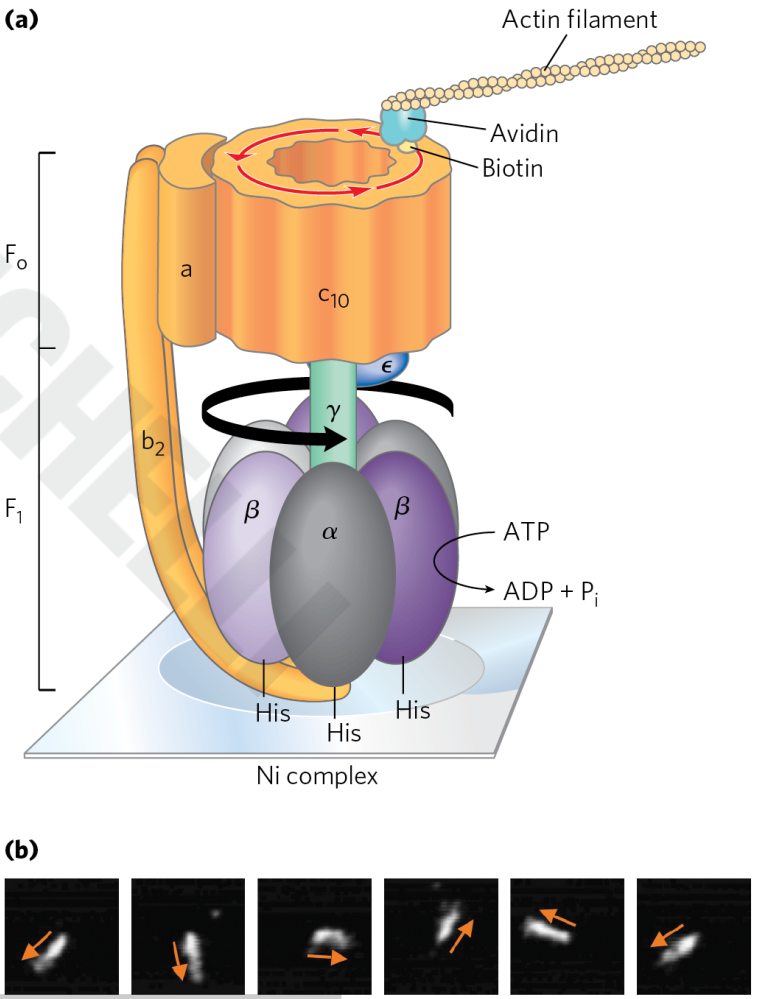
Coupling Proton Translocation to ATP Synthesis

- proton translocation causes a rotation of F_0 and γ
  - causes a conformational change within all the three $\alpha\beta$ pairs, one of which promotes condensation of ADP and P_i into ATP.

Experimental Demonstration of Rotation of F_o and γ

- the γ subunit rotates in one direction when F_oF_1 is synthesizing ATP and in the opposite direction when hydrolyzing ATP



(b) Courtesy Hiroyuki Noji.
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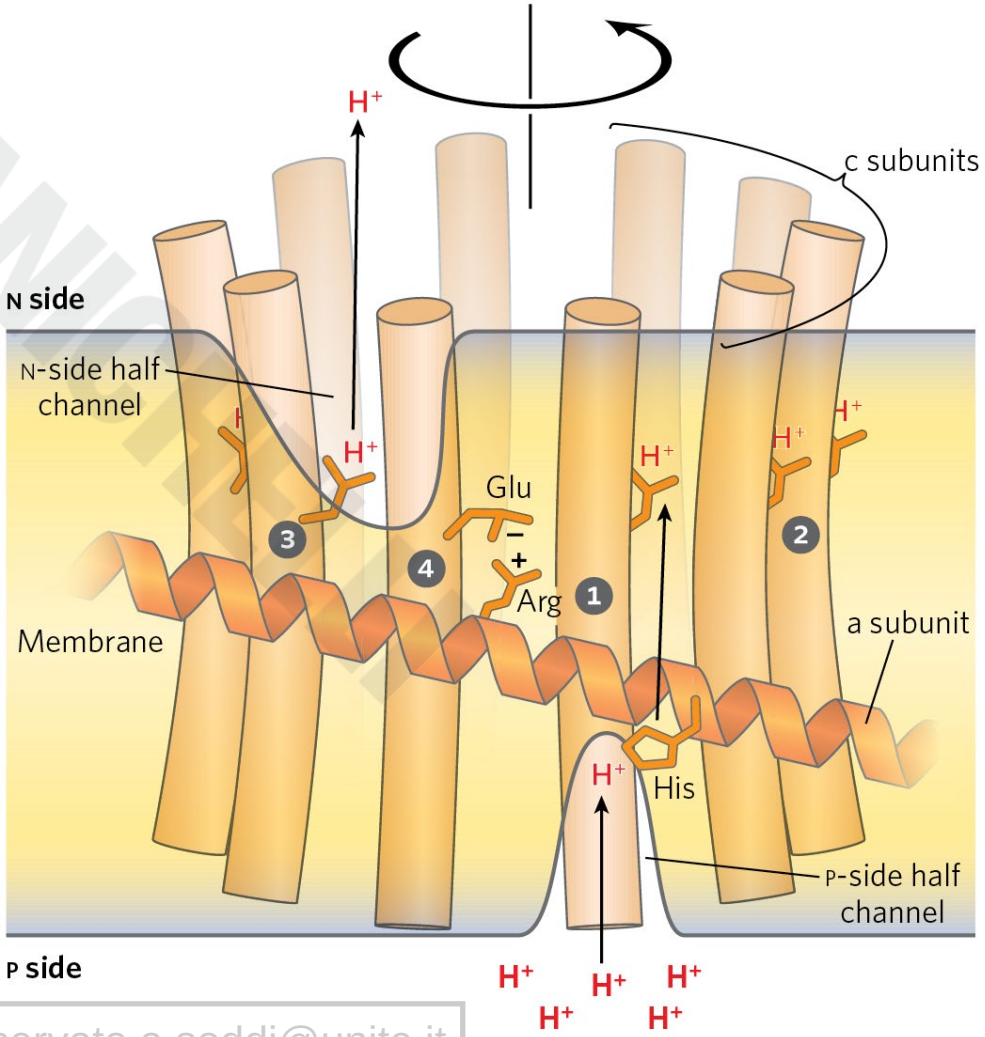
P5 Principle 5 (5 of 7)

The transmembrane flow of protons back down their electrochemical gradient through specific protein channels provides the free energy for synthesis of ATP. This process is catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.

A Model for Proton-Driven Rotation of the c Ring

- the a subunit is stationary and the c ring rotates
- transient protonation of a key Glu residue in each c subunit elicits conformation changes that drive rotation and transmit protons



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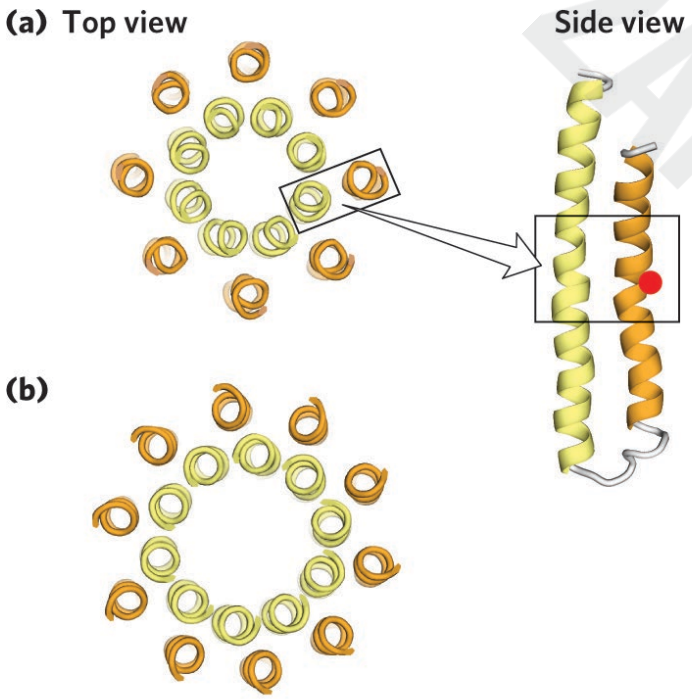
Species Differences in Number of c Subunits in the c Ring of the F_o Complex



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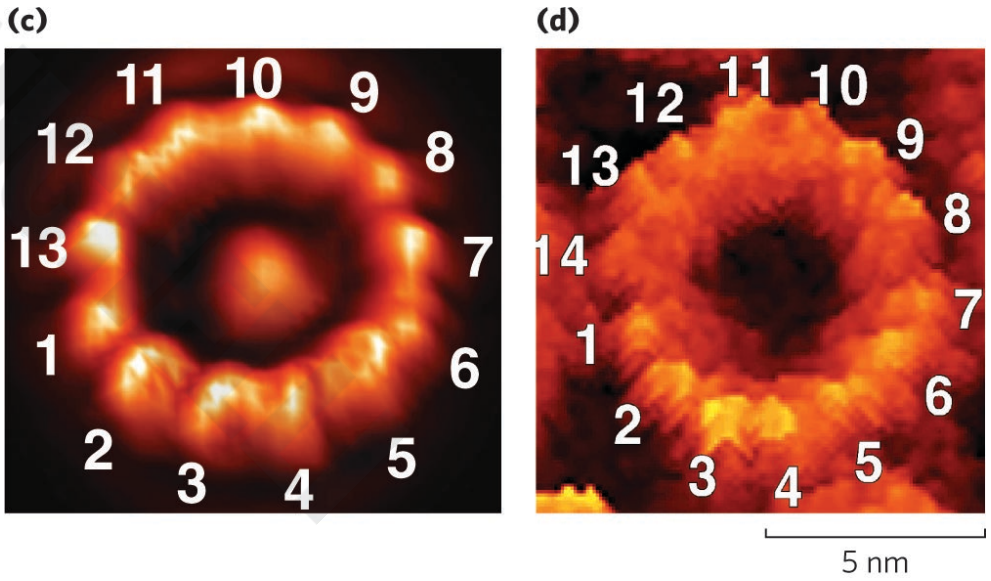

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Chemiosmotic Coupling Allows Nonintegral Stoichiometries of O_2 Consumption and ATP Synthesis

- the overall reaction equation for ATP synthesis is:

$$x\ \text{ADP} + x\text{P}_i + \tfrac{1}{2}\ \text{O}_2 + \text{H}^+ + \text{NADH} \rightarrow x\ \text{ATP} + \text{H}_2\text{O} + \text{NAD}^+ \qquad (19\text{-}11)$$

- **P/O ratio (P/2e^- ratio)** = the value of *x*
  - there is no theoretical requirement for P/O to be integral

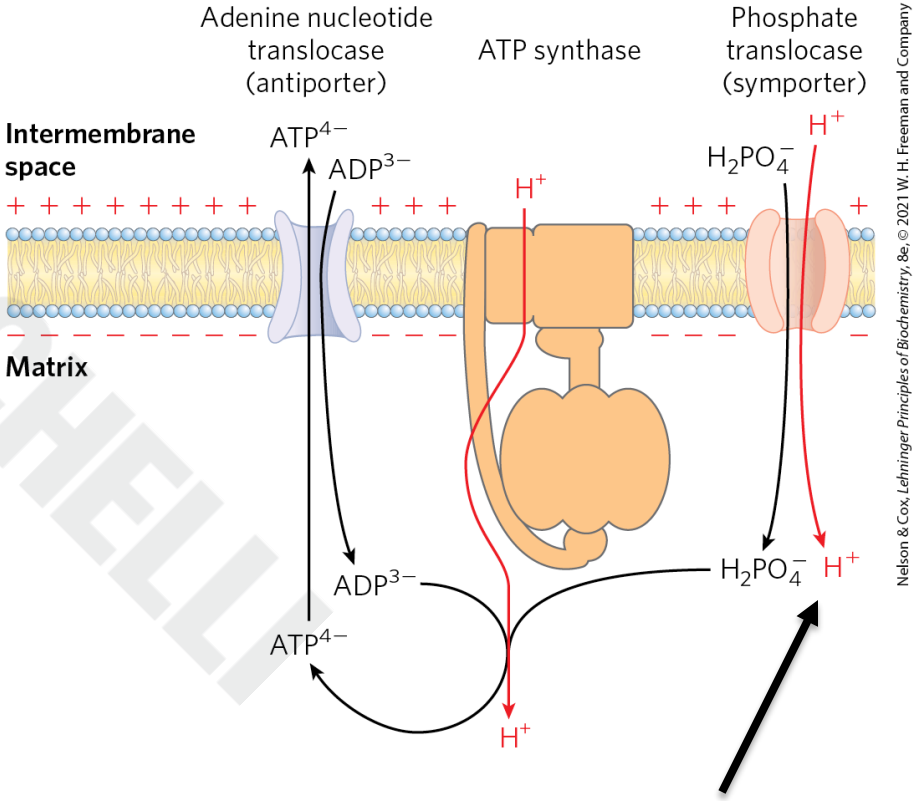
The P/O Ratio

- the P/O ratio is:
  - ~2.5 when electrons enter the respiratory chain at Complex I
  - ~1.5 when electrons enter at ubiquinone

- the ratio varies among species, depending on the number of c subunits in the F_o complex

The Proton-Motive Force Energizes Active Transport

- **adenine nucleotide translocase** = antiporter that moves ADP into the matrix and ADP out
- **phosphate translocase** = promotes symport of one $H_2PO_4^-$ and one H^+ into the matrix
- **ATP synthasome** = a complex of ATP synthase and both translocases



translocation of a fourth proton per ATP is required to facilitate cotransport

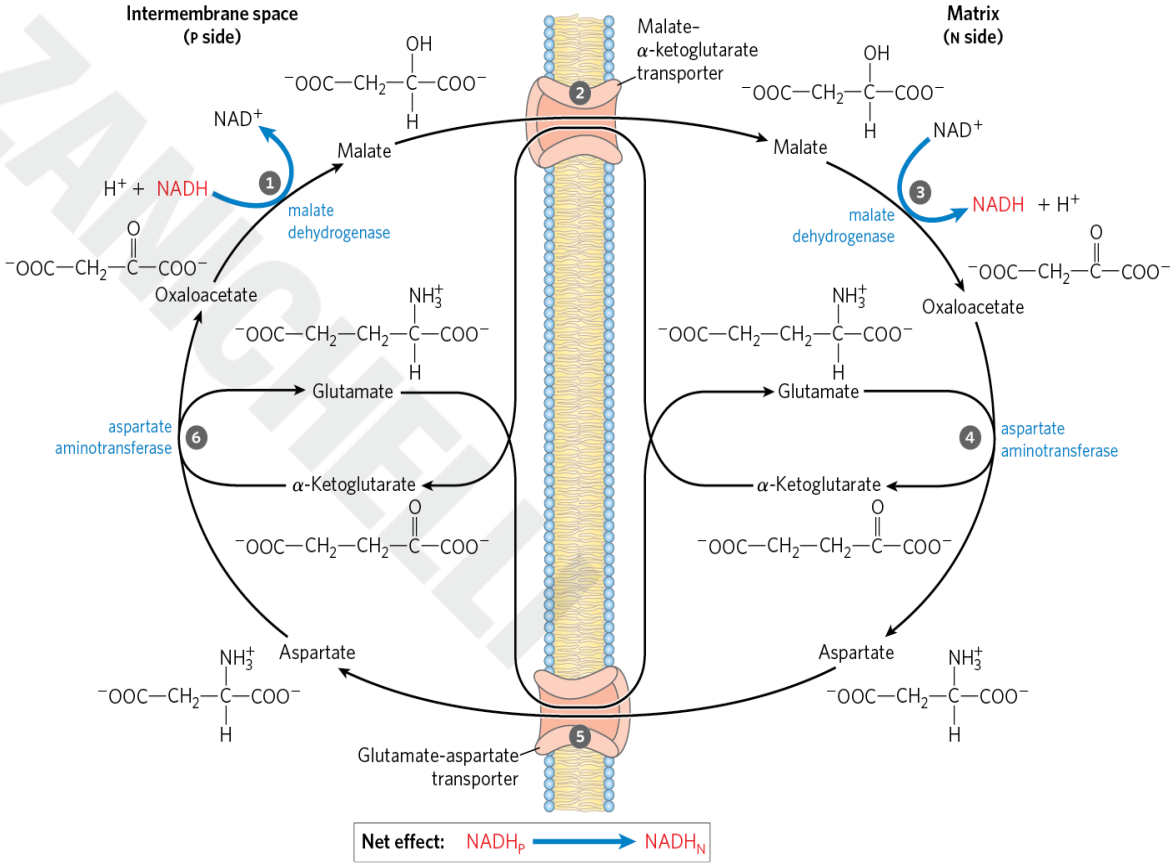
Shuttle Systems Indirectly Convey Cytosolic NADH into Mitochondria for Oxidation

- the inner mitochondrial membrane is impermeable to NADH and NAD

- NADH equivalents are moved from the cytosol to the matrix by:
  - **the malate-asparate shuttle**
  - **the glycerol 3-phosphate shuttle**

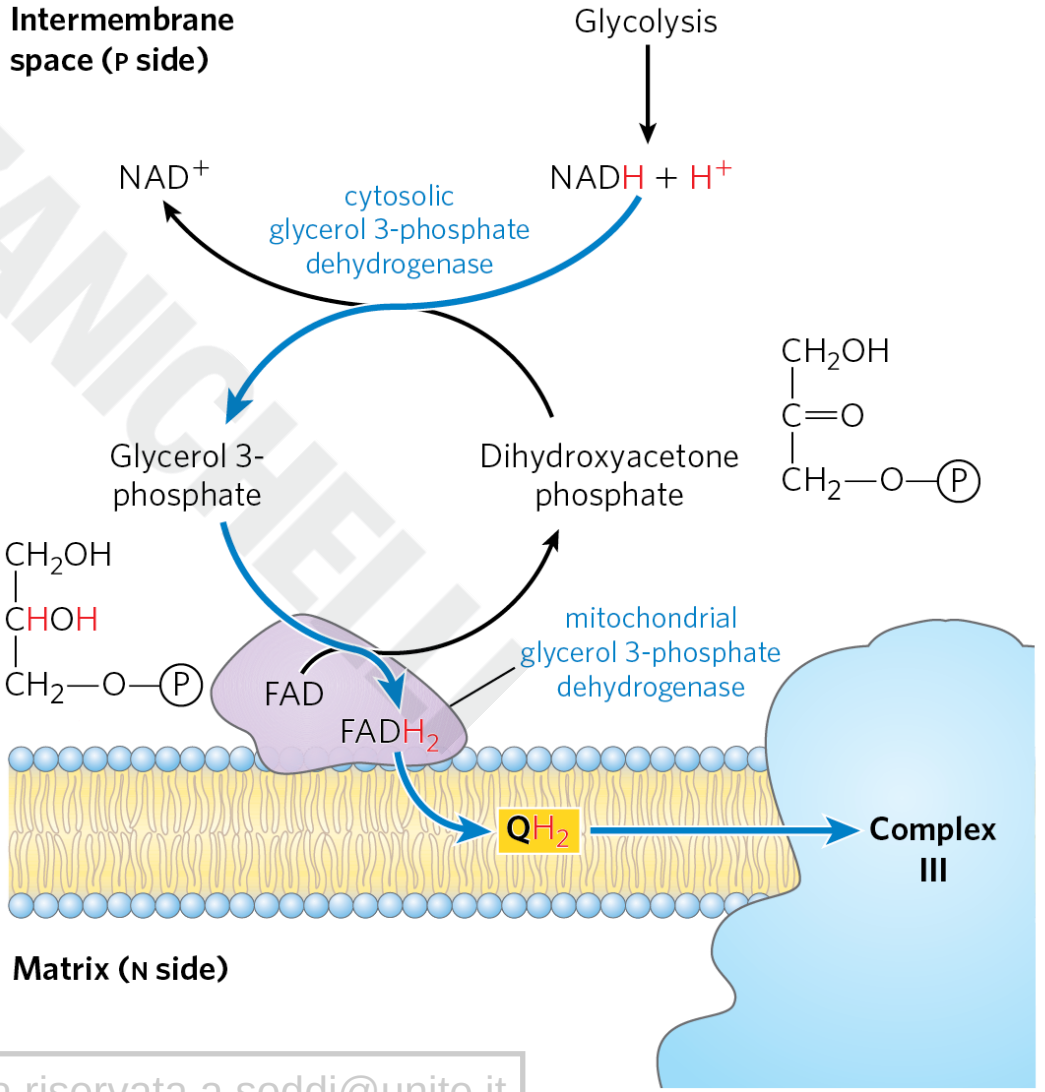
The Malate-Aspartate Shuttle

- NADH equivalents moved in by the **malate-aspartate shuttle** enter the respiratory chain at Complex I and yield a P/O ratio of 2.5



The Glycerol 3-Phosphate Shuttle

- NADH equivalents moved in by the **glycerol 3-phosphate shuttle** enter the respiratory chain at Complex III and yield a P/O ratio of 1.5



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19.3 Regulation of Oxidative Phosphorylation

ATP Yield from Complete Oxidation of Glucose

Table 19-5 ATP Yield from Complete Oxidation of Glucose

Process	Direct product	Final ATP
Glycolysis	2 NADH (cytosolic) 2 ATP	3 or 5 2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix) 2 $FADH_2$ 2 ATP or 2 GTP	15 3 2
Total yield per glucose		30 or 32

- glycolysis under anaerobic conditions (lactate fermentation) yields only 2 ATP per glucose

Oxidative Phosphorylation Is Regulated by Cellular Energy Needs

- the **acceptor control** of respiration = ADP
  - the rate of O_2 consumption depends on the availability of ADP, the P_i acceptor

- the **acceptor control ratio** = the ratio of the maximal rate of ADP-induced O_2 consumption to the basal rate in the absence of ADP
  - at least 10 in some animal tissues

The Mass-Action Ratio is a Measure of a Cell's Energy Status

- **mass-action ratio** = [ATP]/([ADP][P_i])
  - usually this ratio is very high
  - when lowered (and more ADP is available), the rate of respiration increases

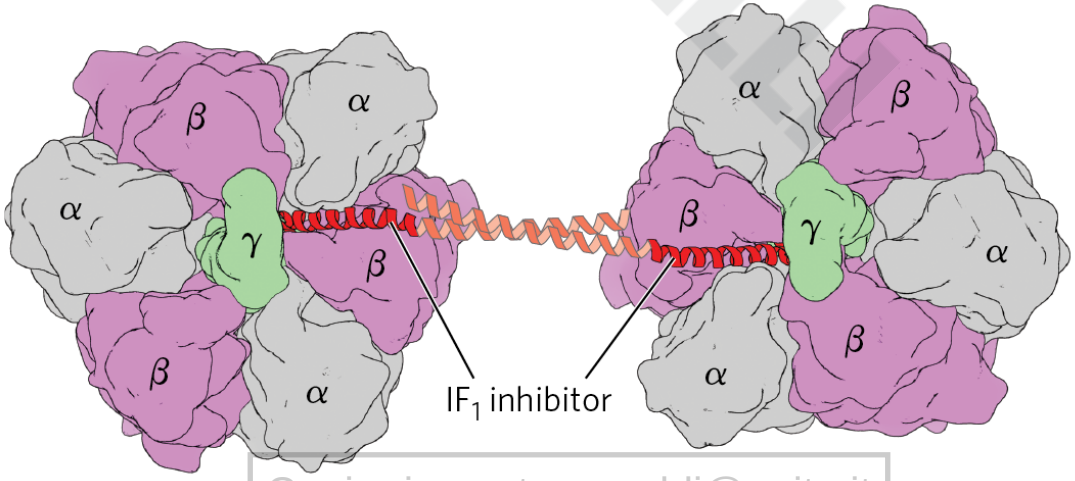
P5 Principle 5 (6 of 7)

The transmembrane flow of protons back down their electrochemical gradient through specific protein channels provides the free energy for synthesis of ATP. This process is catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.

An Inhibitory Protein Prevents ATP Hydrolysis

- IF_1 = protein inhibitor that simultaneously binds 2 ATP molecules to inhibit enzyme activity in both directions
  - active in its dimeric form, which occurs when pH < 6.5

- in hypoxic (oxygen-deprived) cells, IF_1 binds and prevents a drastic drop in [ATP].



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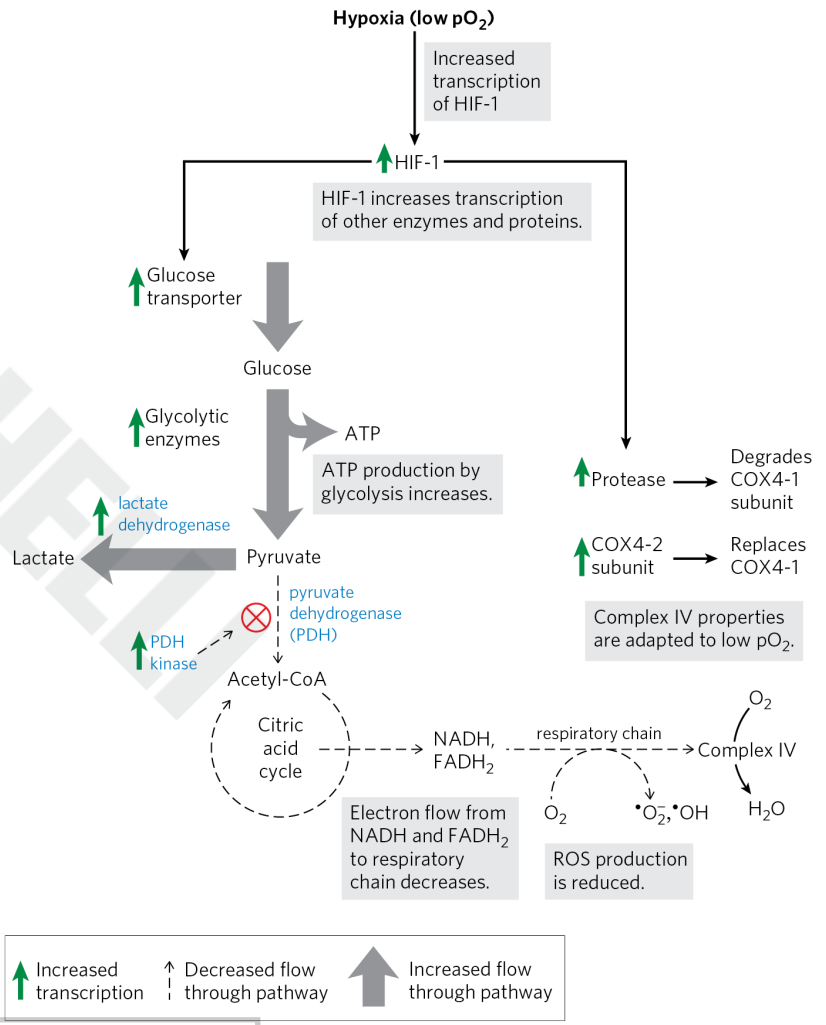
Hypoxia Leads to ROS Production and Several Adaptive Responses

- defenses against ROS:
  - glutathione peroxidase system
  - regulation of pyruvate dehydrogenase
    - phosphorylated (inactive) under hypoxic conditions
  - modification of a subunit of Complex IV (COX4-1 to COX4-2)
    - acts more efficiently under hypoxic conditions

Regulation of Gene Expression by HIF-1

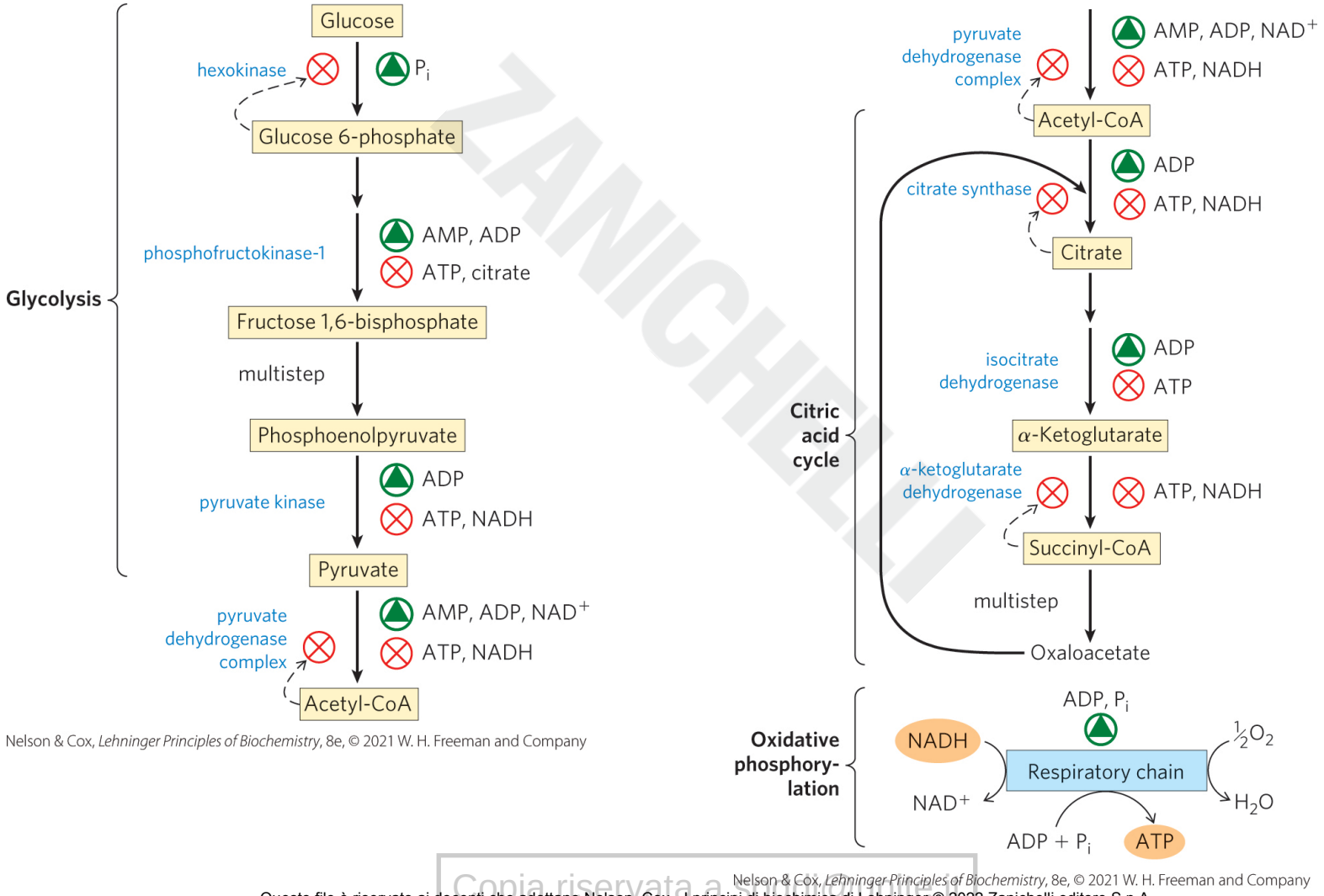
- HIF-1 = the hypoxia-inducible factor
  - accumulates in hypoxic cells
  - acts as a transcription factor
  - master regulator of O_2 homeostasis
  - mediates changes in glucose transport and glycolytic enzymes that produce the Warburg effect



ATP-Producing Pathways Are Coordinately Regulated

- ATP and ADP relative concentrations control the rates of:
  - electron transfer
  - oxidative phosphorylation
  - the citric acid cycle
  - pyruvate oxidation
  - glycolysis

Regulation of ATP-Producing Pathways



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19.4 Mitochondria in Thermogenesis, Steroid Synthesis, and Apoptosis

P1 Principle 1 (3 of 5)

Mitochondria play a central role in eukaryotic aerobic metabolism. ATP production is not the only important mitochondrial function. Mitochondria play host to the citric acid cycle, the fatty acid *β*-oxidation pathway, and the pathways of amino acid oxidation. The mitochondria also act in thermogenesis, steroid synthesis, and apoptosis (programmed cell death). The discovery of these diverse and important roles of mitochondria has stimulated much current research on the biochemistry of this organelle.

P5 Principle 5 (7 of 7)

The transmembrane flow of protons back down their electrochemical gradient through specific protein channels provides the free energy for synthesis of ATP. This process is catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.

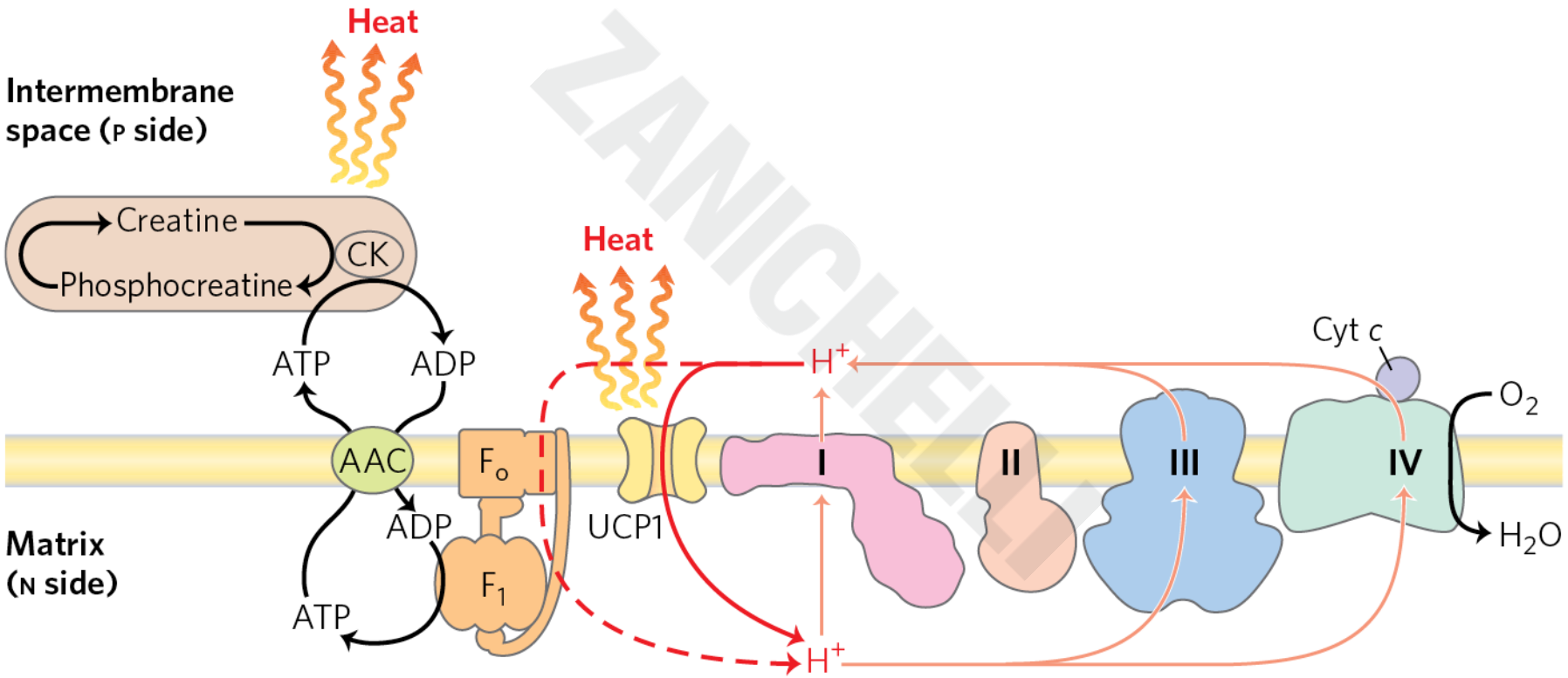
Uncoupled Mitochondria in Brown Adipose Tissue Produce Heat

- **brown adipose tissue (BAT)** = adipose tissue in newborn mammals that functions to generate heat through fuel oxidation

- **uncoupling protein 1 (UCP1)** = a long-chain fatty acid/H^+ symporter that provides a path for protons to return to the matrix without passing through the F_oF_1 complex
  - results in energy of oxidation being dissipated as heat

- hydrolysis of phosphocreatine also liberates heat

Two Mechanisms of Mitochondrial Thermogenesis



Hibernating Animals

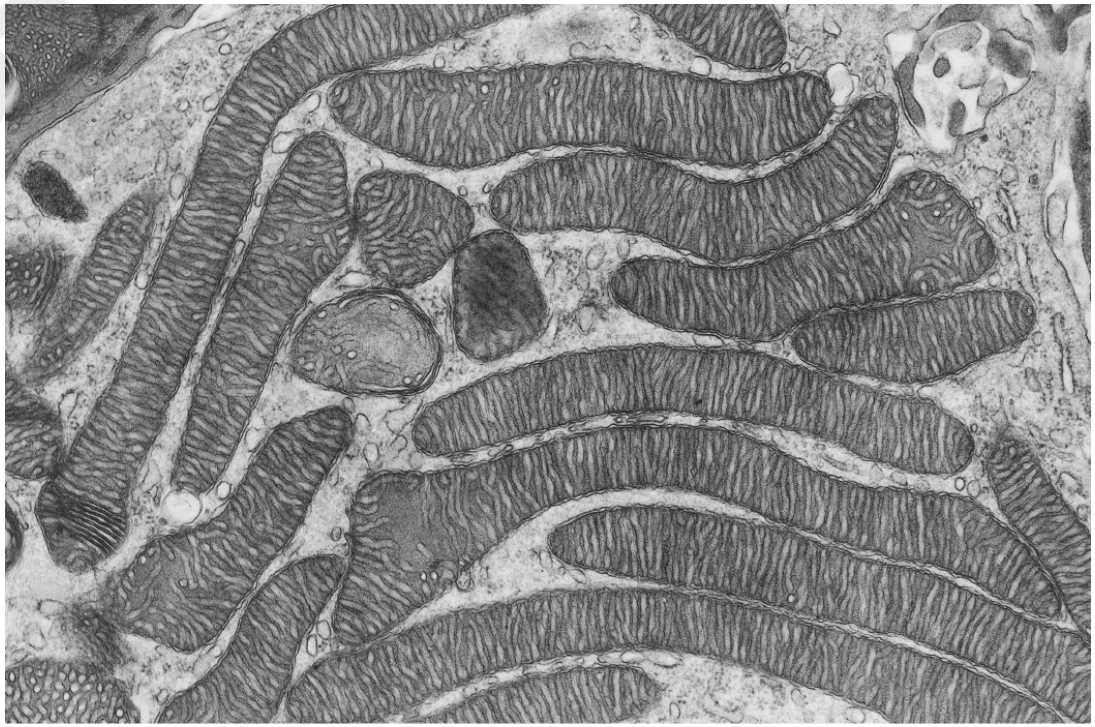
- hibernating animals depend on the activity of uncoupled BAT mitochondria to generate heat during their long dormancy

P1 Principle 1 (4 of 5)

Mitochondria play a central role in eukaryotic aerobic metabolism. ATP production is not the only important mitochondrial function. Mitochondria play host to the citric acid cycle, the fatty acid *β*-oxidation pathway, and the pathways of amino acid oxidation. The mitochondria also act in thermogenesis, steroid synthesis, and apoptosis (programmed cell death). The discovery of these diverse and important roles of mitochondria has stimulated much current research on the biochemistry of this organelle.

Mitochondrial P-450 Monooxygenases Catalyze Steroid Hydroxylations

- mitochondria are the site of biosynthetic reactions that produce steroid hormones
  - occur in steroidogenic tissues (adrenal gland, gonads, liver, and kidney)



Don Fawcett/Science Source.

The Cytochrome P-450 Family

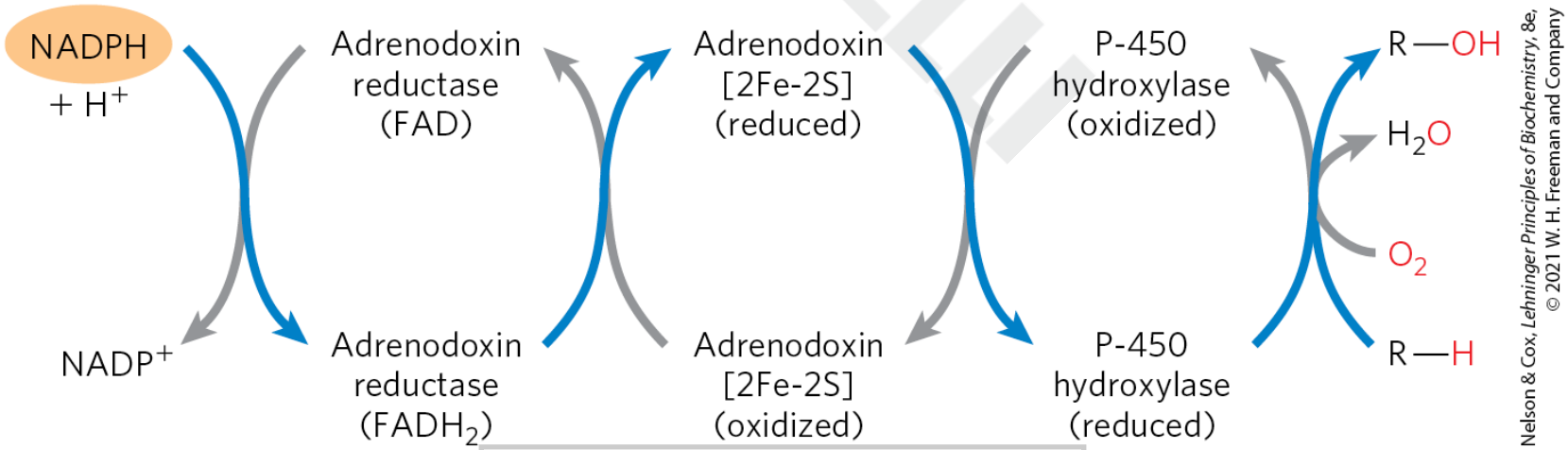
- **cytochrome P-450** family = monoxygenases that catalyze a series of hydroxylation reactions to synthesize steroid hormones from a sterol
  - have a critical heme group

- cytochrome P-450 enzymes catalyze the reaction

$$R–H + O_2 + NADPH + H^+ \rightarrow R–OH + H_2O + NADP^+$$

Path of Electron Flow in Mitochondrial Cytochrome P-450 Reactions

- a flavoprotein and an iron-sulfur protein carry electrons from NADPH to the P-450 heme
- all P-450 enzymes have a heme that interacts with O_2 and a substrate-binding site that confers specificity



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P-450 Enzymes in the ER of Hepatocytes

- catalyze similar reactions with hydrophobic compounds, including xenobiotics

- **xenobiotics** = compounds not found in nature but synthesized industrially

Mitochondria Are Central to the Initiation of Apoptosis

- **apoptosis (programmed cell death)** = process in which individual cells die for the good of the organism

- can be triggered:
  - during normal embryonic development
  - in response to an external signal
  - by internal events (DNA damage, viral infection, oxidative stress from the accumulation of ROS, heat shock)

P1 Principle 1 (5 of 5)

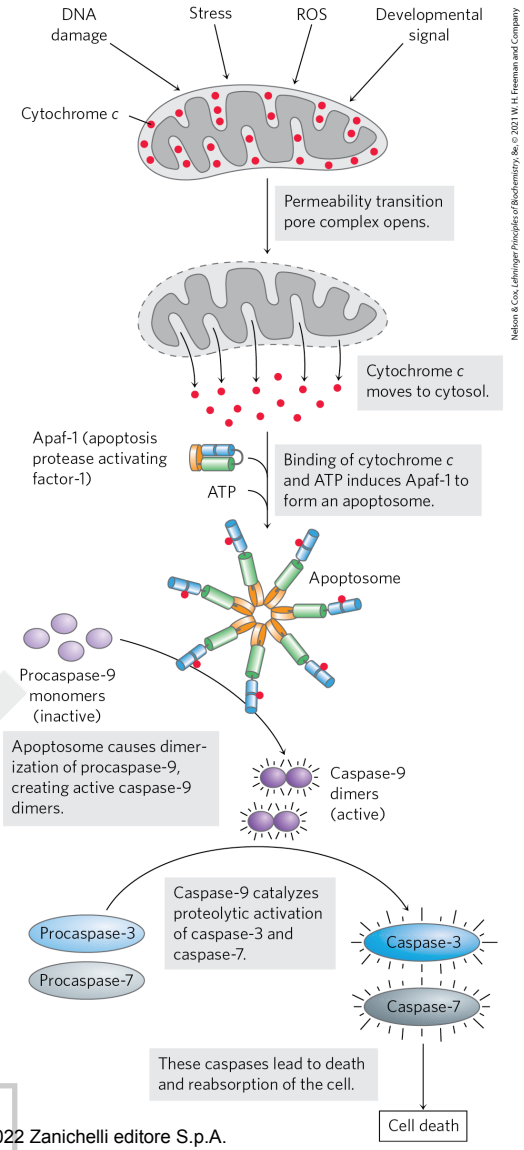
Mitochondria play a central role in eukaryotic aerobic metabolism. ATP production is not the only important mitochondrial function. Mitochondria play host to the citric acid cycle, the fatty acid *β*-oxidation pathway, and the pathways of amino acid oxidation. The mitochondria also act in thermogenesis, steroid synthesis, and apoptosis (programmed cell death). The discovery of these diverse and important roles of mitochondria has stimulated much current research on the biochemistry of this organelle.

Role of Cytochrome *c* in Apoptosis

- **permeability transition pore complex (PTPC)** = a multisubunit membrane protein that opens to increase permeability

- **Apaf-1 (apoptosis protease activating factor-1)** = monomers that interact with cytochrome *c* molecules to form an **apoptosome**

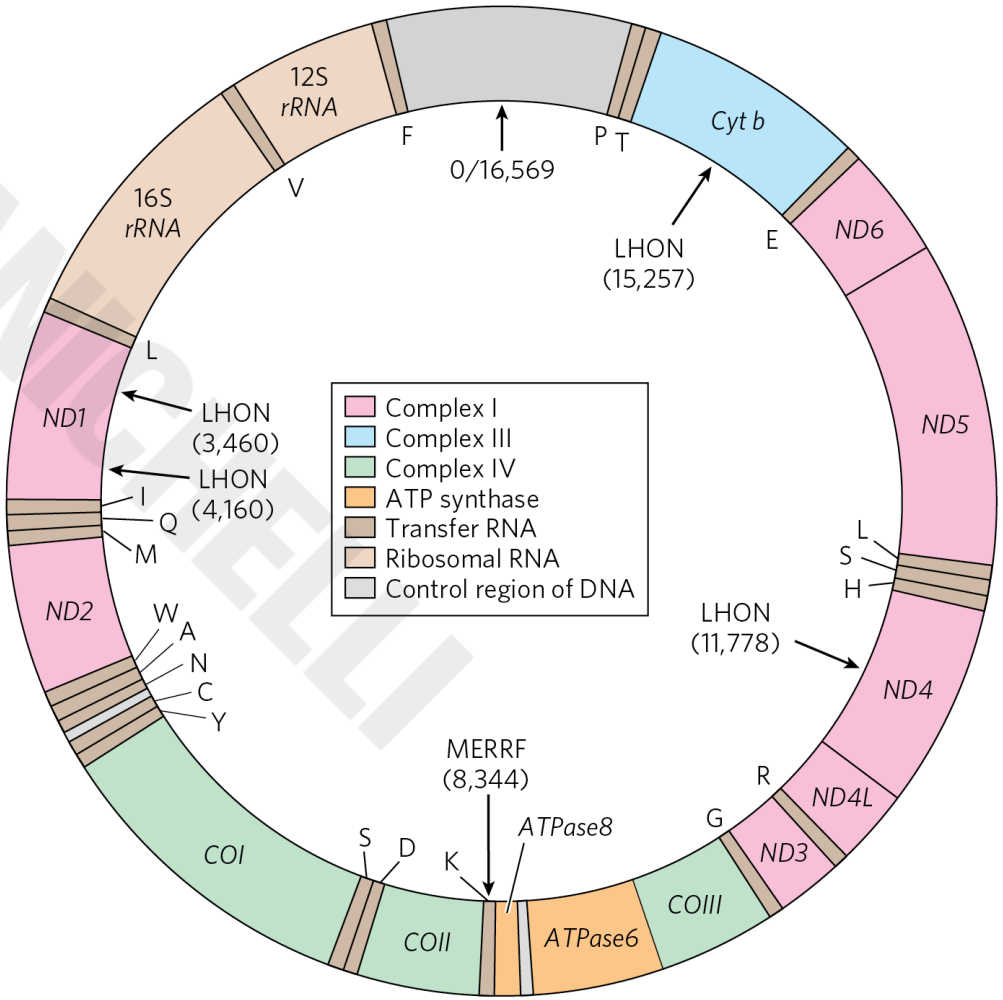
- **caspases** = proteases involved in apoptosis



19.5 Mitochondrial Genes: Their Origin and the Effects of Mutations

Mitochondrial Genes and Mutations

- 13 mitochondrial proteins are encoded by the mitochondrial genome and synthesized in mitochondria
- ~1,200 mitochondrial proteins are encoded by nuclear genes and imported into mitochondria



Respiratory Proteins Encoded by Mitochondrial Genes in Humans

TABLE 19-6 Respiratory Proteins Encoded by Mitochondrial Genes in Humans

Complex	Number of subunits	Number of subunits encoded by mtDNA
I NADH dehydrogenase	45	7
II Succinate dehydrogenase	4	0
III Ubiquinone:cytochrome *c* oxidoreductase	11	1
IV Cytochrome oxidase	13	3
V ATP synthase	8	2

P2 Principle 2 (4 of 4)

Mitochondria trace their evolutionary origin to bacteria. Over 1.45 billion years ago, an endosymbiotic relationship arose between bacteria and a primitive eukaryote or eukaryotic progenitor. Mitochondria are ubiquitous in modern eukaryotes, and their bacterial origin is evident in almost every aspect of their structure and function.

Mitochondria Evolved from Endosymbiotic Bacteria

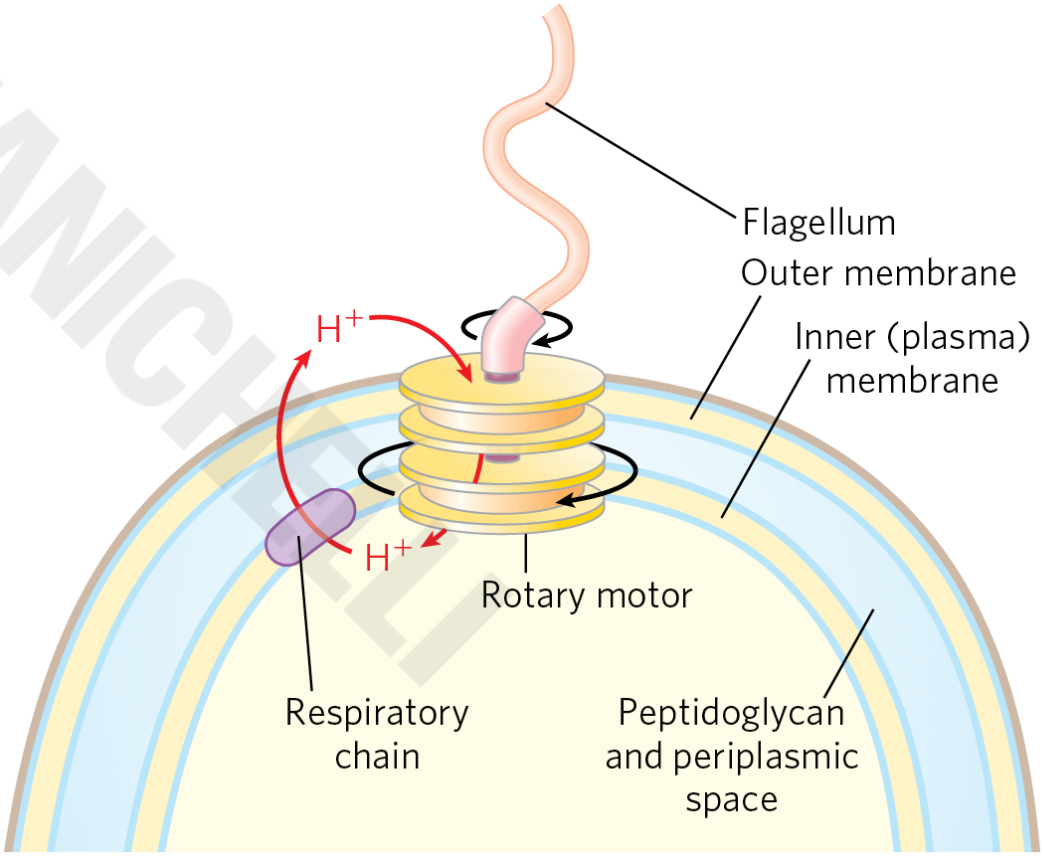
- mitochondria arose from aerobic bacteria that entered into an endosymbiotic relationship with primitive eukaryotes
  - endosymbiotic bacteria eventually became mitochondria

- the chemiosmotic mechanism likely evolved before the emergence of eukaryotes

Respiration-Linked Extrusion of Protons Drives Other Processes

- uptake of extracellular nutrients against a concentration gradient occurs in symport with protons

- the rotary motion of bacterial flagella is driven by the transmembrane electrochemical potential



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Mutations in Mitochondrial DNA Accumulate throughout the Life of the Organisms

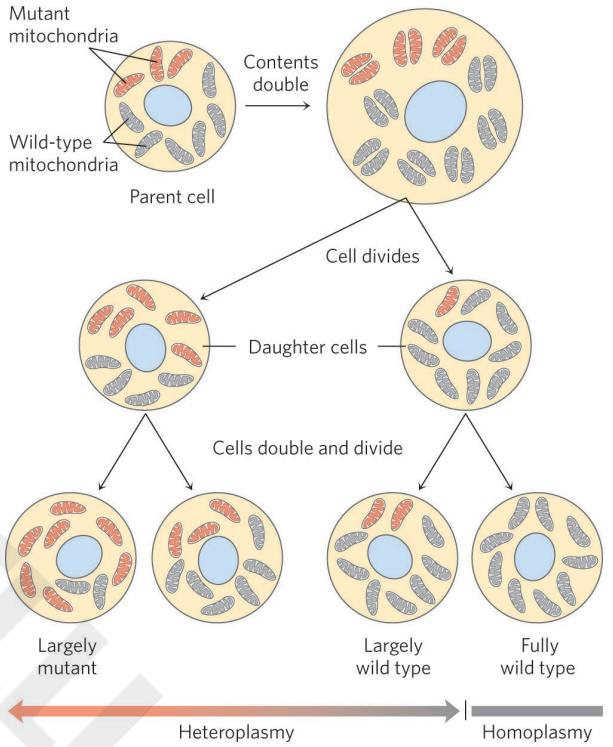
- defects in mtDNA occur over time because:
  - of the greatest exposure to ROS
  - the mtDNA replication system is less effective than the nuclear system at correcting mistakes and damage

- animals inherit essentially all mitochondria from the female parent

Heteroplasmy and Homoplasmy

- **heteroplasmy** = different types of mitochondrial genomes within a cell
  - results in mutant phenotypes of varying degrees of severity

- **homoplasmy** = the mitochondrial genome is the same within a cell



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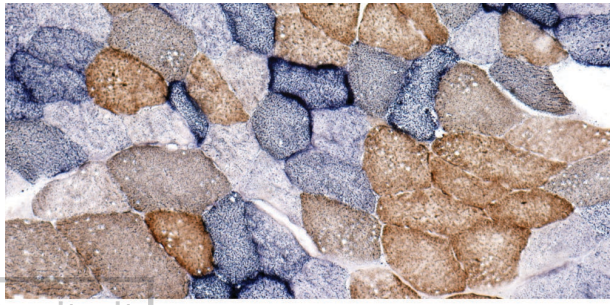
(b) Courtesy of Rob Taylor.



Some Mutations in Mitochondrial Genomes Cause Disease

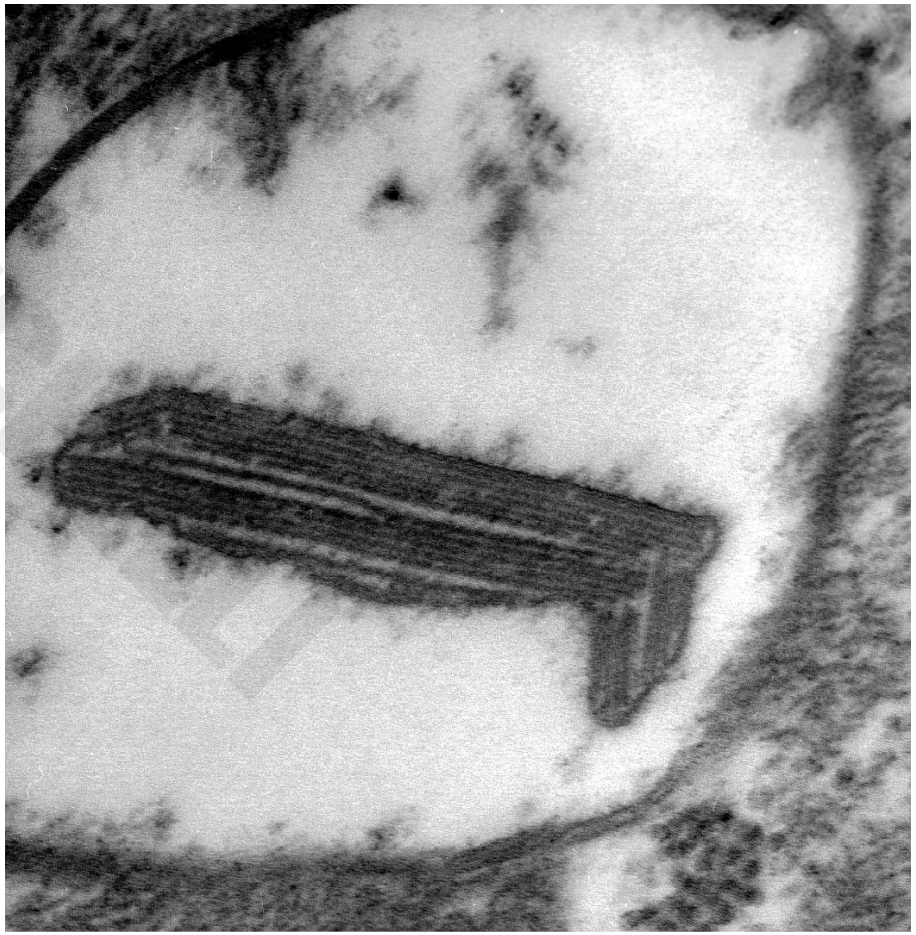
- **mitochondrial encephalomyopathies** = a group of genetic diseases that primarily affect the brain and skeletal muscle
  - inherited from the mother

- **Leber hereditary optic neuropathy (LHON)** = rare disease that affects the central nervous system
  - single amino acid substitution in Complex I causes the mitochondria to be partially defective in electron transfer from NADH to ubiquinone

Myoclonic Epilepsy with Ragged-Red Fibers (MERRF) Syndrome

- **myoclonic epilepsy with ragged-red fibers (MERRF) syndrome =** caused by a mutation in the gene that encodes a tRNA specific for lysine

- skeletal muscle fibers of affected individuals have abnormally shaped mitochondria that sometimes contain paracrystalline structures



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Mitochondrial Donation

- mitochondrial donation = transplantation of a prospective mother's nuclear genes into an enucleated ovum from a donor with healthy mitochondria
  - creates an ovum free of a mutation that would have led to a mitochondrial disease
  - raises ethical issues

A Rare Form of Diabetes Results from Defects in the Mitochondria of Pancreatic β Cells

- mitochondrial defects in pancreatic β cells can limit ATP production when glucose levels are high
- compromises insulin release and gives rise to a form of type 2 diabetes