Chapter 14 - Electron Transport and Oxidative Phosphorylation

- The cheetah, whose capacity for aerobic metabolism makes it one of the fastest animals

14.4 Oxidative Phosphorylation in Mitochondria

- Reduced coenzymes **NADH** and **QH_2** from:

  (1) Aerobic oxidation of pyruvate by the citric acid cycle

  (2) Oxidation of fatty acids and amino acids

- **Oxidative phosphorylation** is the process by which NADH and QH_2 are oxidized and ATP is formed

Mitochondrial oxidative phosphorylation

(1) **Respiratory electron-transport chain** (**ETC**) Series of enzyme complexes embedded in the inner mitochondrial membrane, which oxidize NADH and QH_2. Oxidation energy is used to transport protons creating a proton gradient

(2) **ATP synthase** uses the proton gradient energy to produce ATP

Overview of oxidative phosphorylation

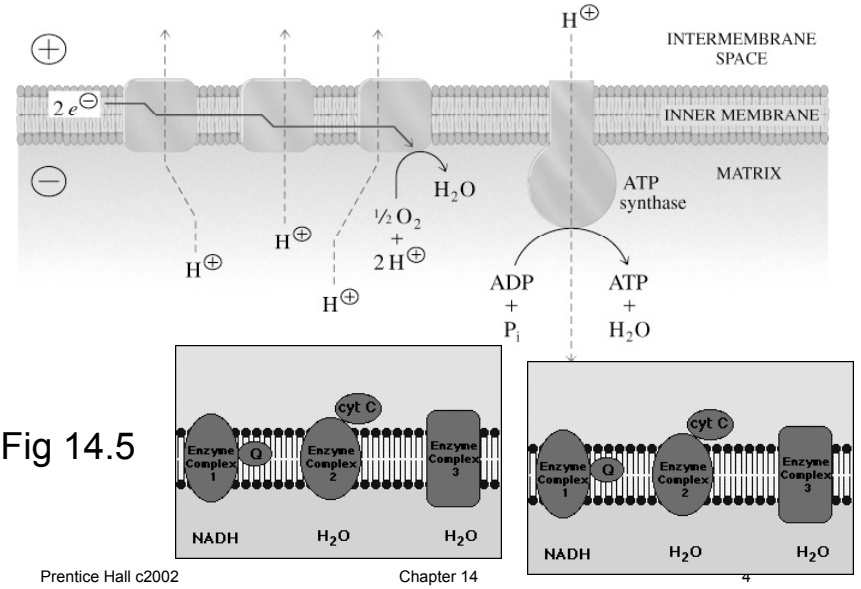
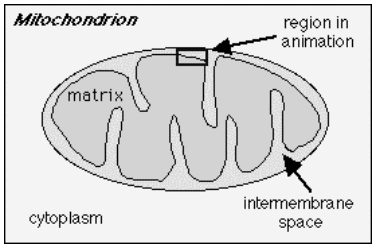
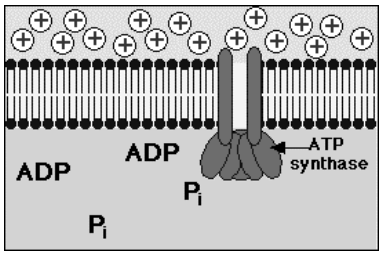


Fig 14.5





14.5 The Mitochondrion

- Final stages of aerobic oxidation of biomolecules in eukaryotes occur in the mitochondrion
- Site of citric acid cycle and fatty acid oxidation which generate reduced coenzymes
- Contains electron transport chain to oxidize reduced coenzymes

Fig 14.6 Structure of the mitochondrion

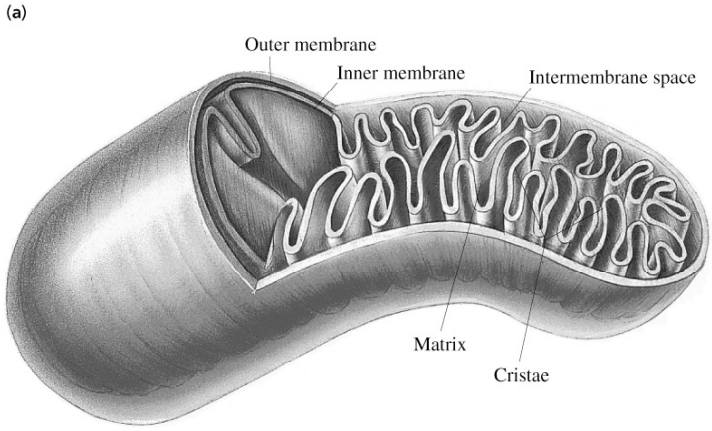


Fig 14.6 (continued)

Location of mitochondrial complexes

- Inner mitochondrial membrane:
  - Electron transport chain
  - ATP synthase
- Mitochondrial matrix:
  - Pyruvate dehydrogenase complex
  - Enzymes of the citric acid cycle
  - Enzymes catalyzing fatty acid oxidation

14.6 The Chemiosmotic Theory

- Proposed by Peter Mitchell in the 1960's (Nobel Prize 1978)
- **Chemiosmotic theory**:

  A proton concentration gradient serves as the energy reservoir for driving ATP formation

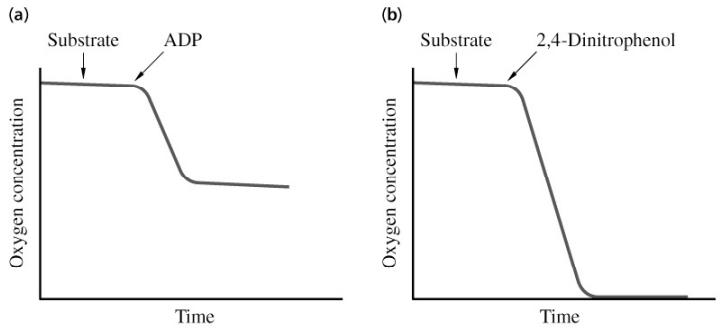
Respiration by mitochondria

- Oxidation of substrates is *coupled* to the phosphorylation of ADP
- Respiration (consumption of oxygen) proceeds only when ADP is present
- The amount of O_2 consumed depends upon the amount of ADP added

Fig 14.7 Coupled nature of respiration in mitochondria

(a) O_2 consumed only with ADP, excess P_i

(b) (+) Uncoupler DNP (O_2 consumed without ADP)

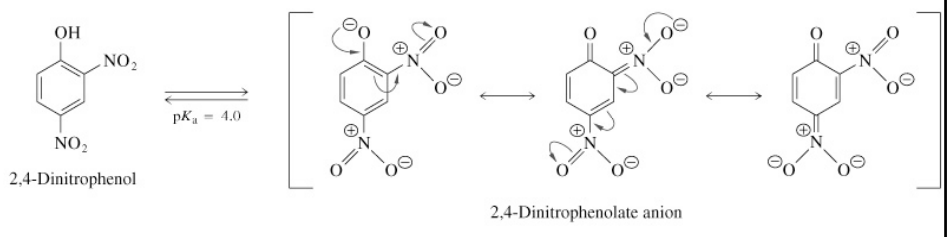


Uncouplers

- **Uncouplers** stimulate the oxidation of substrates in the absence of ADP
- Uncouplers are lipid-soluble weak acids
- Both acidic and basic forms can cross the inner mitochondrial membrane
- Uncouplers deplete any proton gradient by transporting protons across the membrane

Fig 14.8 2,4-Dinitrophenol: an uncoupler

- Because the negative charge is delocalized over the ring, both the acid and base forms of DNP are hydrophobic enough to dissolve in the membrane



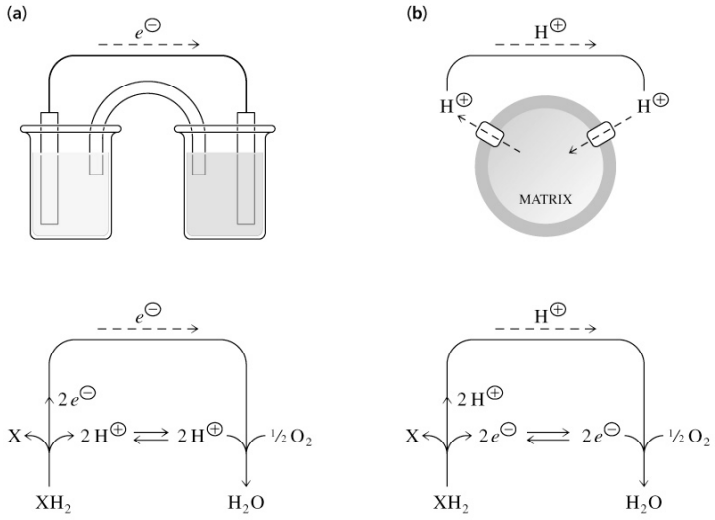
Mitchell's postulates for chemiosmotic theory

1. Intact inner mitochondrial membrane is required (to maintain a proton gradient)
2. Electron transport through the ETC generates a proton gradient (pumps H^+ from the matrix to the intermembrane space)
3. The membrane-spanning enzyme, ATP synthase, catalyzes the phosphorylation of ADP in a reaction driven by movement of H^+ across the inner membrane into the matrix

14.7 The Protonmotive Force

- **Protonmotive force** (Δ**p**) is the energy of the proton concentration gradient
- Protons that are translocated into the intermembrane space by electron transport, flow back into the matrix via ATP synthase
- H^+ flow forms a circuit (similar to an electrical circuit)

Fig 14.9 Analogy of electromotive and protonmotive force



Free-energy change from proton movement

1. *Chemical* contribution

$$\Delta G_{chem} = nRT \ln ([H^+]_{in} / [H^+]_{out})$$

(n = number of protons translocated)

2. *Electrical* contribution: $\Delta\psi$=membrane potential

$$\Delta G_{elect} = zF\Delta\psi$$

(z = charge (1.0 for H^+), F =96,485 $JV^{-1}mol^{-1}$)

Protonmotive force (Δp)

- From the two previous equations the protonmotive force (Δp) is:

$$\Delta p = \Delta\psi - (0.059\ V)\ \Delta pH$$

  - $\Delta\psi$ = difference in charge across the membrane (V) in volts ($\Delta\Psi = \Delta\Psi_{in} - \Delta\Psi_{out}$)
  - $\Delta pH = pH_{in} - pH_{out}$

14.8 Overview of Electron Transport

- Five oligomeric assemblies of proteins associated with oxidative phosphorylation are found in the inner mitochondrial membrane
- **Complexes I-IV** contain multiple cofactors, and are involved in electron transport
- **Complex V** is ATP synthase

Table 14.1

TABLE 14.1 Characteristics of protein complexes of the mitochondrial respiratory electron-transport chain in bovine heart

Complex	Subunits	Molecular weight	Oxidation-reduction components
I. NADH-ubiquinone oxidoreductase	42 or 43	> 900 000	1 FMN 22–24 Fe–S in 7 or 8 clusters
II. Succinate-ubiquinone oxidoreductase	4	125 000	1 FAD 3 Fe–S clusters Cytochrome b_{560}
III. Ubiquinol–cytochrome *c* oxidoreductase	2	~250 000 (dimer of 11-chain subunits)	1 Fe–S cluster Cytochrome *b* Cytochrome c_1
IV. Cytochrome *c* oxidase	2	420 000 (dimer of 13-chain subunits)	Cytochrome *a* Cytochrome a_3 2 Copper ions

A. Complexes I-IV

- Electrons flow through the ETC components in the direction of <u>increasing</u> <u>reduction</u> <u>potentials</u>

  NADH (strong reducing agent, $E^{o'}$ = -0.32 volts)
  O_2 (terminal oxidizing agent, $E^{o'}$ = +0.82 volts)
- Mobile coenzymes: ubiquinone (Q) and cytochrome *c* serve as links between ETC complexes
- Complex IV reduces O_2 to water

Fig 14.10 Mitochondrial electron transport

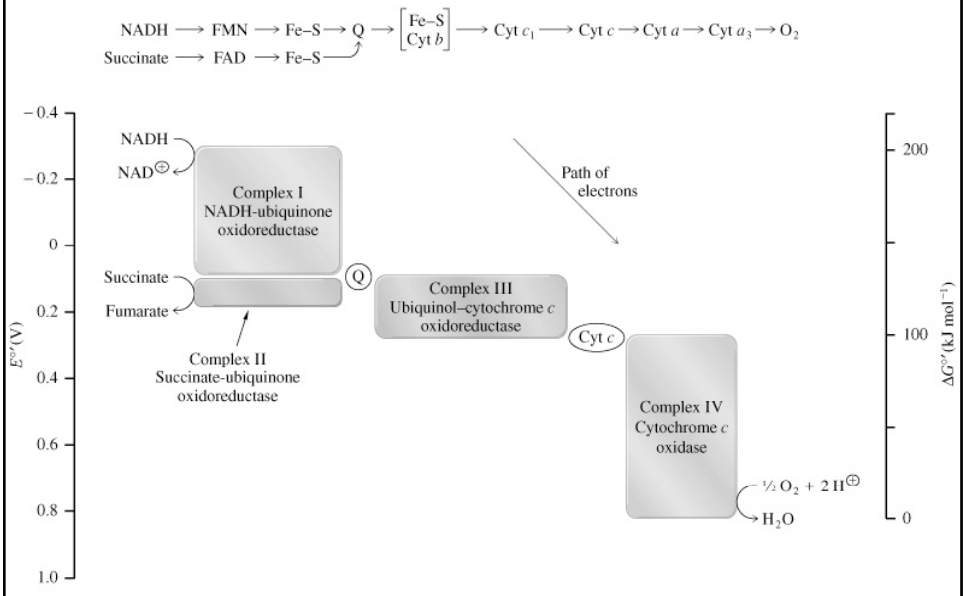


Table 14.2

TABLE 14.2 Standard reduction potentials of mitochondrial oxidation-reduction components

Substrate or complex	$E^{\circ\prime}$ (V)
NADH	−0.32
Complex I	
FMN	−0.30
Fe–Sclusters	−0.25to −0.05
Succinate	+0.03
Complex II	
FAD	0.0
Fe–Sclusters	−0.26to 0.00
QH_2/Q	+0.04
($\cdot Q^{\ominus}$/Q	−0.16)
(QH_2/$\cdot Q^{\ominus}$	+0.28)
Complex III	
Fe–Scluster	+0.28
Cytochrome b_{560}	−0.10
Cytochrome b_{566}	+0.05
Cytochrome c_1	+0.22
Cytochrome c	+0.23
Complex IV	
Cytochrome a	+0.21
Cu_A	+0.24
Cytochrome a_3	+0.39
Cu_B	+0.34
O_2	+0.82

Table 14.3

TABLE 14.3 Standard free energy released in the oxidation reaction catalyzed by each complex

Complex	$E^{\circ\prime}_{\text{reductant}}$ (V)	$E^{\circ\prime}_{\text{oxidant}}$ (V)	$\Delta E^{\circ\prime a}$ (V)	$\Delta G^{\circ\prime b}$ (kJ mol^{-1})	$\Delta G^{\circ\prime}$ (kcal mol^{-1})
I (NADH/Q)	−0.32	+0.04	+0.36	−70	−17
II (Succinate/Q)	+0.03	+0.04	+0.01	− 2	− 0.5
III (QH_2/Cytochrome *c*)	+0.04	+0.23	+0.19	−37	− 9
IV (Cytochrome *c*/O_2)	+0.23	+0.82	+0.59	−110	−

[a] $\Delta E^{\circ\prime}$ was calculated as the difference between $E^{\circ\prime}_{\text{reductant}}$ and $E^{\circ\prime}_{\text{oxidant}}$.

[b] The standard free energy obtained by the oxidation of one mole of NADH or the electrons derived from NADH was calculated using Equation 14.19, where $n = 2$ electrons.

Inhibitors can block electron transfer through specific complexes in the ETC

- Complex I: blocked by rotenone
- Complex III: blocked by antimycin A
- Complex IV: blocked by cyanide

B. Cofactors in Electron Transport

- Electrons enter the ETC two at a time via NADH
- Flavin coenzymes are then reduced

  (Complex I) FMN $\rightarrow$ $FMNH_2$

  (Complex II) FAD $\rightarrow$ $FADH_2$
- $FMNH_2$ and $FADH_2$ donate one electron at a time
- All subsequent steps proceed by one e^- transfers

Mobile electron carriers

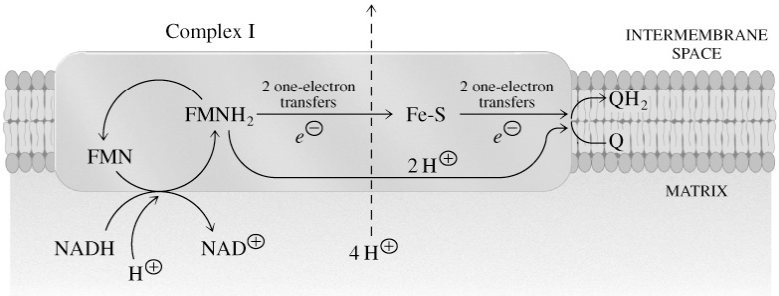
1. **Ubiquinone** (**Q**)
   Q is a lipid soluble molecule that diffuses within the lipid bilayer, accepting electrons from I and II and passing them to III
2. **Cytochrome *c***
   Associated with the outer face of the membrane, transports electrons from III to IV

14.9 Complex I

- NADH-ubiquinone oxidoreductase (NADH dehydrogenase)
- Transfers electrons from NADH to Q
- NADH transfers a two electrons as a hydride ion ($H:^-$) to FMN
- Electrons are passed through Complex I to Q via FMN and iron-sulfur proteins

Fi. 14.11 Electron transfer and proton flow in Complex I

- Reduction of Q to QH_2 requires 2 e^-
- About 4 H^+ translocated per 2 e^- transferred

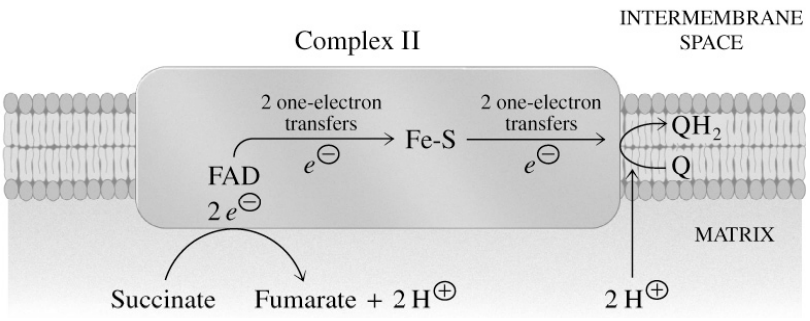


14.10 Complex II

- Succinate-ubiquinone oxidoreductase (or succinate dehydrogenase complex)
- Accepts electrons from succinate and catalyzes the reduction of Q to QH_2
- FAD of II is reduced in a 2-electron transfer of a hydride ion from succinate

Fig 14.12 Electron transfer in Complex II

- Complex II does not contribute to proton gradient, but supplies electrons from succinate

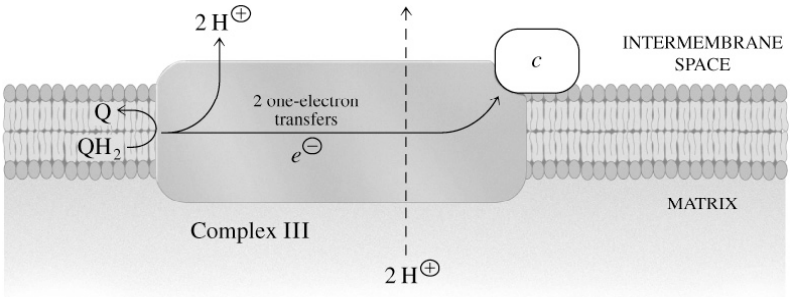


14.11 Complex III

- Ubiquinol-cytochrome *c* oxidoreductase
- Transfers electrons to cytochrome *c*
- Oxidation of one QH_2 is accompanied by the translocation of 4 H^+ across the inner mitochondrial membrane
- Two H^+ are from the matrix, two from QH_2

Fig 14.13 Electron transfer and proton flow in Complex III

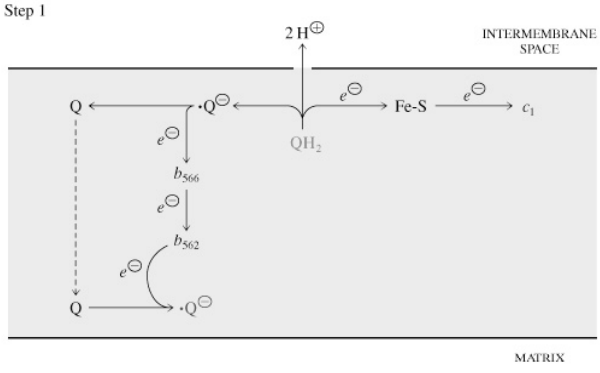
- Four H^+ are translocated, two from the matrix and two from QH_2



Proposed Q cycle.

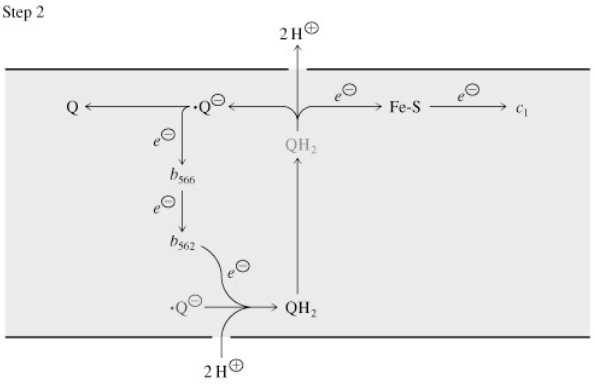
- Proposal for electron movement between between QH_2 and cytochrome *c* in Complex III
- One e^- transferred to cytochrome *c* via the Fe-S protein
- Second e^- transferred to cytochrome *b* then Q
- Three forms of Q are involved: QH_2, Q and the semiquinone anion $\cdot Q^-$

Fig 14.14 Q cycle: Step 1



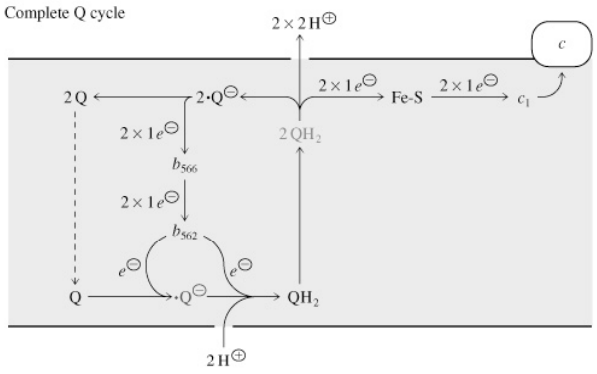
In the first step, QH_2 at the cystolic face of the membrane donates an electron to the Fe–S protein of Complex III, forming $\cdot Q^{\ominus}$ and releasing 2 $H^{\oplus}$ to the cytosol. The Fe-S protein moves from the site of its reduction to transfer an electron to cytochrome c_1. From cytochrome c_1, the electron continues down the respiratory chain. $\cdot Q^{\ominus}$ donates an electron to the b_{566} heme group, with formation of Q, which diffuses to the matrix face of the membrane. Reduced b_{566} donates an electron via b_{562} to Q at the matrix face of the membrane to form $\cdot Q^{\ominus}$ (red).

Q cycle: Step 2



Completion of the cycle requires a second molecule of QH_2 (blue) to repeat the first part of the cycle, producing another reduced cytochrome c_1, another 2 $H^{\oplus}$, another Q, and reduced b_{562}, which this time reduces the $\cdot Q^{\ominus}$ formed in the first step of the cycle (red) to QH_2, consuming 2$H^{\oplus}$ from the matrix.

Q cycle: Step 3



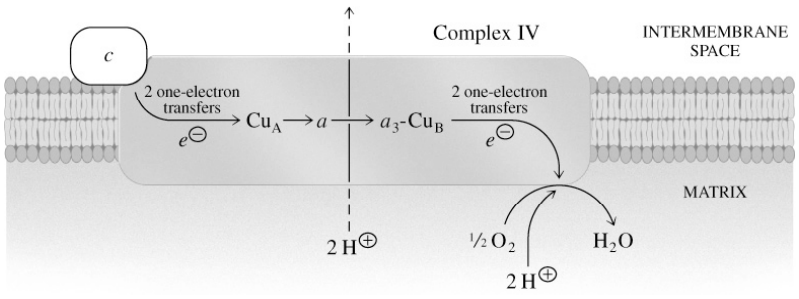
The result of the two steps is the oxidation of two QH_2 molecules and the formation of one QH_2 molecule. Electrons from Complex III are donated to the mobile carrier cytochrome *c*. [Adapted from Trumpower, B. L. (1990). The protonmotive Q cycle: energy transduction by coupling of proton translocation to electron transfer by the cytochrome bc_1 complex. *J. Biol. Chem.* 265:11 409-11 412.]

14.12 Complex IV

- Cytochrome *c* oxidase
- Catalyzes a four-electron reduction of molecular oxygen (O_2) to water (H_2O)
- Source of electrons is cytochrome *c* (links Complexes III and IV)
- Translocates H^+ into the intermembrane space

Fig 14.15 Electron transfer and proton flow in Complex IV

- Iron atoms (hemes of cyt *a*) and copper atoms are both reduced and oxidized as electrons flow



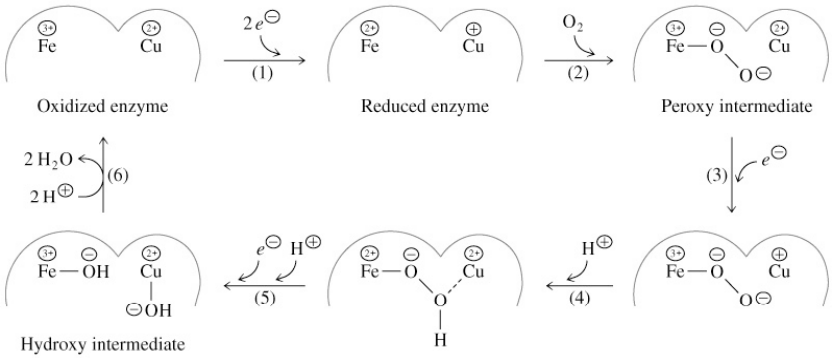
Complex IV contributes to the proton gradient

Net effect is transfer of four H^+ for each pair of e^-

$$O_2 + 4\ e^- + 4H^+ \longrightarrow 2\ H_2O$$

1. Proton translocation of 2 H^+ for each pair of electrons transferred (each O atom reduced)
2. Formation of H_2O removes $2H^+$ from the matrix (contributes to Δp even though no proton translocation occurs)

Fig 14.16 Proposed mechanism for reduction of molecular oxygen by cytochrome oxidase



14.13 Complex V: ATP Synthase

- **F_0F_1 ATP Synthase** uses the proton gradient energy for the synthesis of ATP
- An F-type ATPase which generates ATP
- Composed of a "knob-and-stalk" structure
- **F_1** (knob) contains the catalytic subunits
- **F_0** (stalk) has a proton channel which spans the membrane.

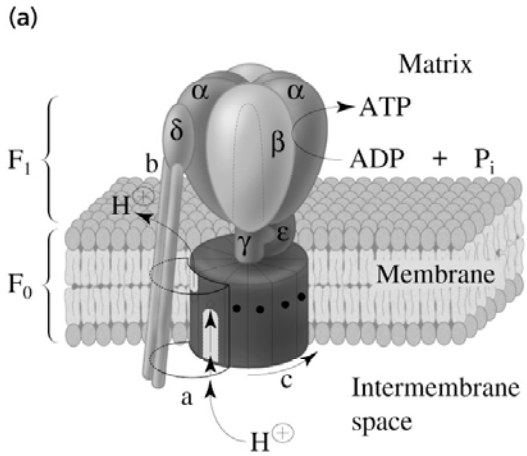
ATP Synthase components

- Passage of protons through the F_o (stalk) into the matrix is coupled to ATP formation
- Estimated passage of **3 H^+ / ATP** synthesized
- F_o is sensitive to oligomycin, an antibiotic that binds in the channel and blocks H^+ passage, thereby inhibiting ATP synthesis

Structure of ATP synthase

- F_1 knobs: inner face of the inner mitochondrial membrane (subunit composition: $\alpha_3\beta_3\gamma\delta\varepsilon$)
- F_o subunit composition: $\mathbf{a_1b_2c_{9\text{-}12}}$ (c subunits form cylindrical, membrane-bound base)
- $\alpha_3\beta_3$ oligomer of F_1 is connected to c subunits by a multisubunit stalk of γ and ε chains

Fig 14.17 (a) Knob-and-stalk structure of ATP synthase



Mechanism of ATP Synthase

- There are 3 active sites, one in each β subunit
- The c-ε–γ unit forms a "rotor"
- Rotation of the γ subunit inside the $\alpha_3\beta_3$ hexamer causes domain movements in the β-subunits, opening and closing the active sites

ATPase mechanism (continued)

- The a-b-γ–$\alpha_3\beta_3$ unit is the "stator" (the F_o channel is attached to $\alpha_3\beta_3$ by the a-b-δ arm)
- Passage of protons through the F_o channel causes the rotor to spin in one direction and the stator to spin in the opposite direction

Fig 14.17 (b)

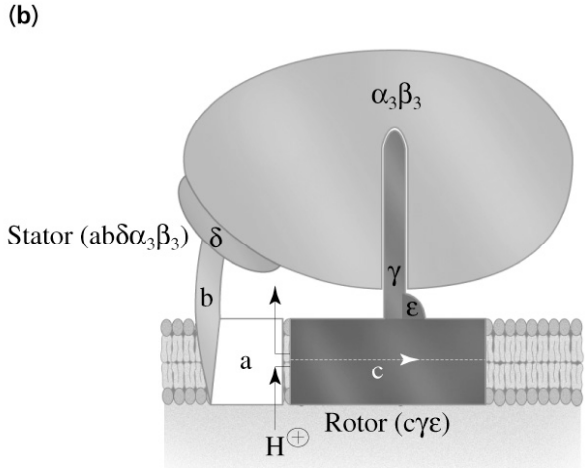


Fig 14.17 (c)

- Molecular structure of rotor and stator (part) of ATP synthase

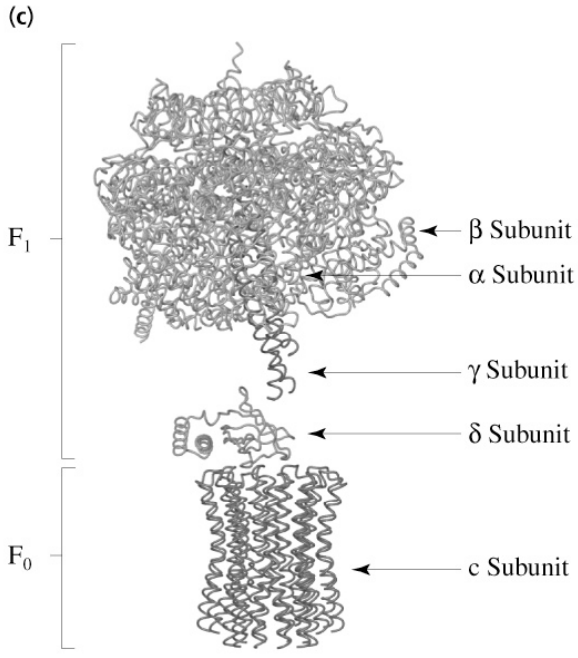


Fig 14.18 Binding-change mechanism of ATP synthase

1. ADP, P_i bind to an open site
2. Inward passage of protons, conformation change, ATP synthesis from ADP and P_i
3. ATP released from open site, ADP and P_i form ATP in the tight site

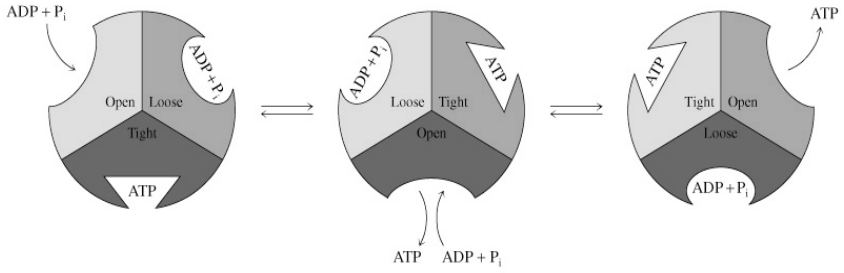
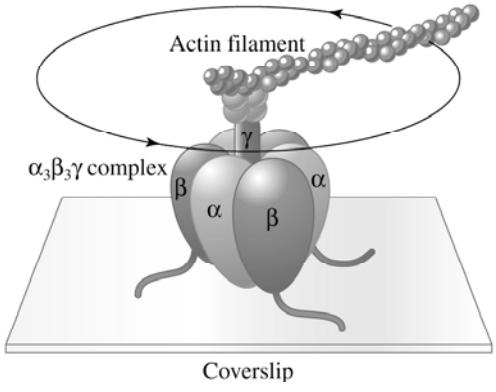


Fig 14.19 Experimental observation of ATP synthase rotation

- Fluorescent protein arm (actin) attached to γ subunits
- $\alpha_3\beta_3$ subunits bound to a glass plate
- Arm seen rotating when MgATP added

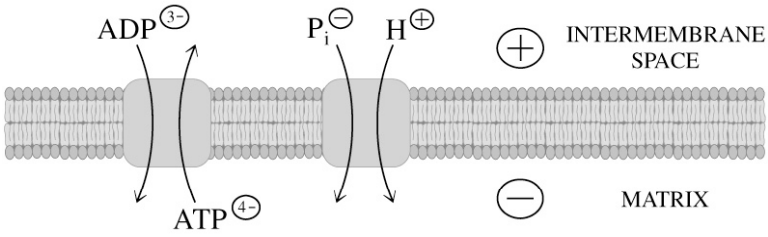


14.14 Active Transport of ATP, ADP and P_i Across the Mitochondrial Membrane

- ATP is synthesized in the mitochondrial matrix
- ATP must be transported to the cytosol, and ADP and P_i must enter the matrix
- ADP/ATP carrier exchanges mitochondrial ATP^{4-} for cytosolic ADP^{3-}
- The exchange causes a net loss of -1 in the matrix (draws some energy from the H^+ gradient)

Fig 14.20 Transport of ATP, ADP and P_i across the inner mitochondrial membrane

- Adenine nucleotide translocase: unidirectional exchange of ATP for ADP (antiport)
- Symport of P_i and H^+ is electroneutral



14.15 The P:O Ratio

$$\textbf{P:O ratio} = \frac{\text{molecules of ADP phosphorylated}}{\text{atoms of oxygen reduced}}$$

- Translocation of $3H^+$ required by ATP synthase for each ATP produced
- 1 H^+ needed for transport of P_i, ADP and ATP
- **Net: 4 H^+ transported for each ATP synthesized**

Calculation of the P:O ratio

	Complex I	III	IV
#H^+ translocated/$2e^-$	4	2	4

Since 4 H^+ are required for each ATP synthesized:

For **NADH**: 10 H^+ translocated / O ($2e^-$)

P/O = (10 H^+/ 4 H^+) = **2.5 ATP/O**

For **succinate** substrate = 6 H^+/ O ($2e^-$)

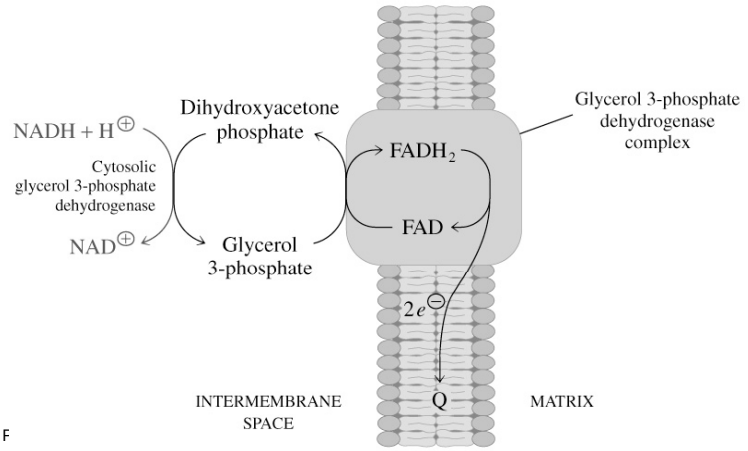
P/O = (6 H^+/ 4 H^+) = **1.5 ATP/O**

14.16 Aerobic Oxidation of Cytosolic NADH

- Cytosolic NADH must enter the mitochondria to fuel oxidative phosphorylation, but NADH and NAD^+ cannot diffuse across the inner mitochondrial membrane
- Two shuttle systems for reducing equivalents:
  (1) Glycerol phosphate shuttle: insect flight muscles
  (2) Malate-aspartate shuttle: predominant in liver and other mammalian tissues

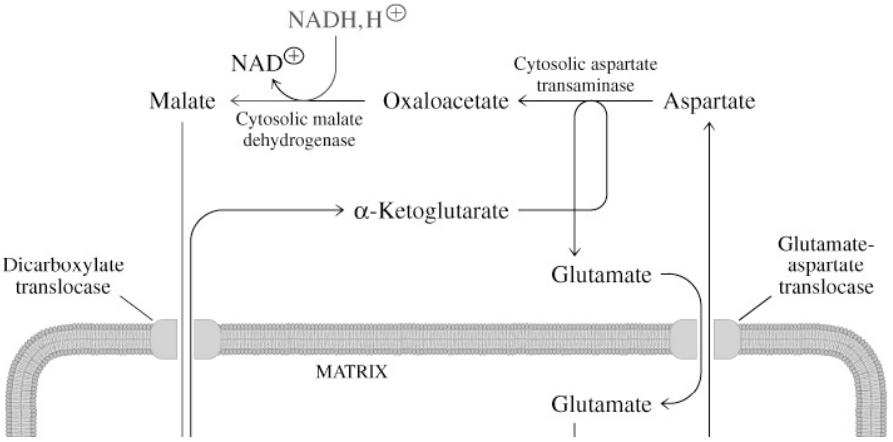
Fig 14.21 Glycerol phosphate shuttle

- Cytosolic NADH transfers 2 e^- to FAD, then Q



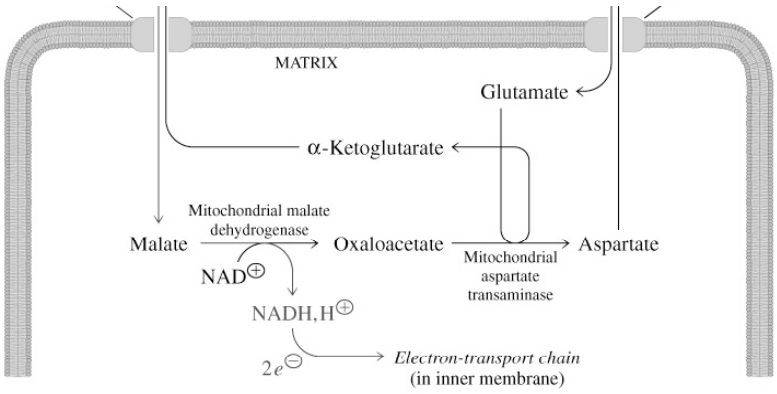
F

Fig. 14.22 Malate-aspartate shuttle



(continued next slide)

Fig 14.22 (continued)



14.17 Regulation of Oxidative Phosphorylation

- Overall rate of oxidative phosphorylation depends upon substrate availability and cellular energy demand
- Important substrates: **NADH, O_2, ADP**
- In eukaryotes intramitochondrial ratio ATP/ADP is a secondary control mechanism
- High ratio inhibits oxidative phosphorylation as ATP binds to a subunit of Complex IV