Electron Transport and Oxidative Phosphorylation

- Final stages of **aerobic oxidation** of biomolecules in **eukaryotes** occur in the **mitochondrion**
- Reduced coenzymes **NADH** and **$FADH_2$** from:
  - (1) Aerobic oxidation of pyruvate by the citric acid cycle
  - (2) Oxidation of fatty acids and amino acids

Electron Transport Chain is the process by which NADH and $FADH_2$ are oxidized and a proton gradient is formed.

Mitochondrial Electron Transport

- How did we get here?
- Summary of glycolysis

$$\text{glucose} + 2\ NAD^+ + 2P_i + 2ADP \rightarrow 2\ \text{pyruvate} + 2ATP + 2NADH + 2H^+$$

- Summary of the citric acid cycle (including pyruvate dehydrogenase)

$$\text{pyruvate} + 4\ NAD^+ + FAD + GDP + 2\ H_20 \rightarrow 3\ CO_2 + 4NADH + 4H^+ + P_i + GTP + FADH_2$$

TABLE 17.1
Reactions catalyzed by NAD- and FAD-linked dehydrogenases

NAD LINKED
Glyceraldehyde 3-phosphate + P_i + NAD^+ $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+
Pyruvate + CoA + NAD^+ $\rightleftharpoons$ acetyl CoA + CO_2 + NADH + H^+
Isocitrate + NAD^+ $\rightleftharpoons$ α-ketoglutarate + NADH + H^+ + CO_2
α-Ketoglutarate + CoA + NAD^+ $\rightleftharpoons$ succinyl CoA + CO_2 + NADH + H^+
Malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH + H^+
FAD LINKED
Succinate + FAD $\rightleftharpoons$ fumarate + $FADH_2$

Oxidative phosphorylation is the process of making ATP by using the proton gradient generated by the ETC.

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

Electron Transport and Oxidative Phosphorylation

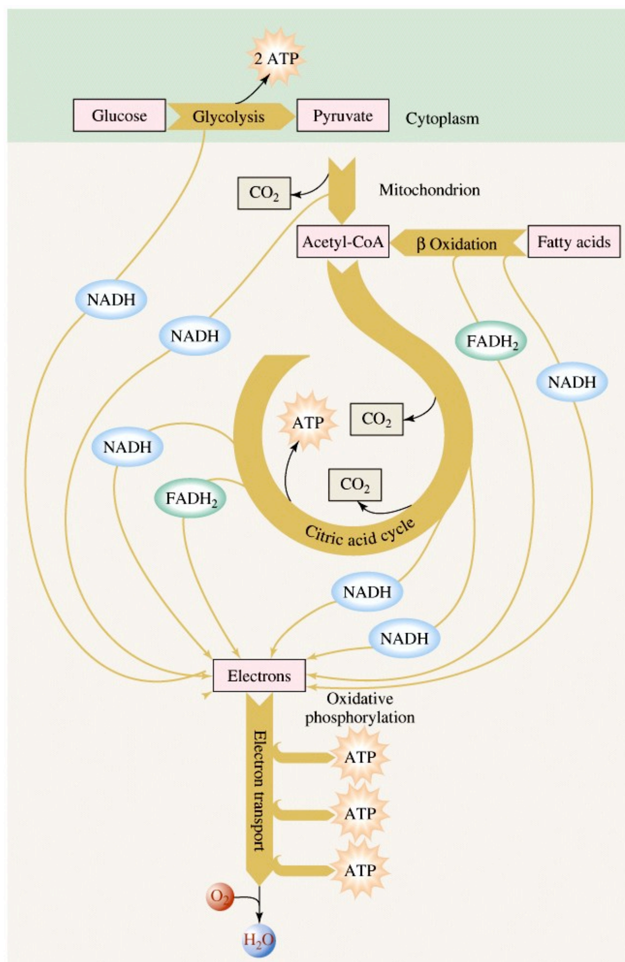

Figure 17-1 Concepts in Biochemistry, 3/e
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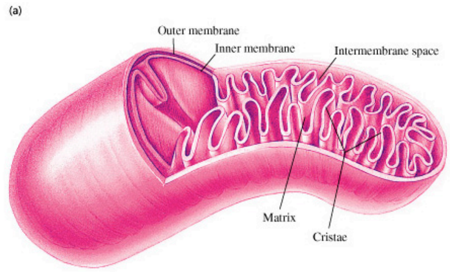
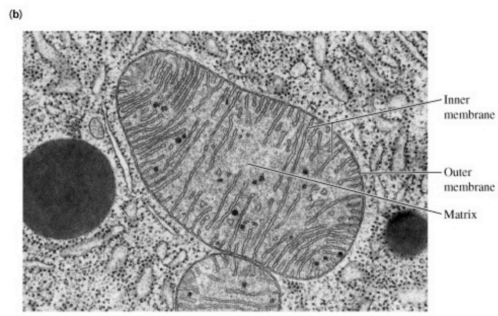
Location of mitochondrial complexes

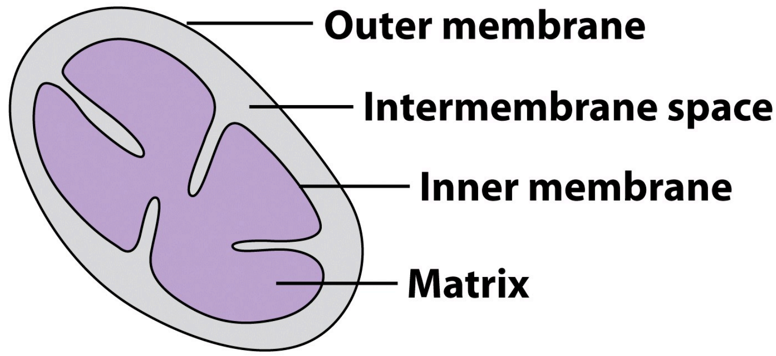
- Inner mitochondrial membrane:
  - a. **Electron transport chain:** oxidizes reduced coenzymes
  - b. **ATP synthase**: machinery to synthesize ATP



Electron transport and **oxidative phosphorylation** capture the energy in the redox potential of NADH and $FADH_2$ – 2 separate processes that are **COUPLED** to result in ATP production

Extensive folding of IMM provides a large surface area on the matrix side to form lots of assemblies of proteins to maximize ATP production





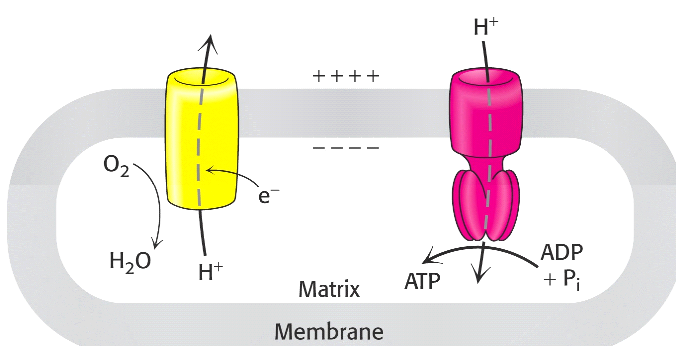
(1) **Respiratory electron-transport chain** (**ETC**) Series of enzyme complexes embedded in the inner mitochondrial membrane, which oxidize NADH and $FADH_2$. Oxidation energy is used to transport protons creating a proton gradient – protons pumped from matrix to intermembrane space across IMM

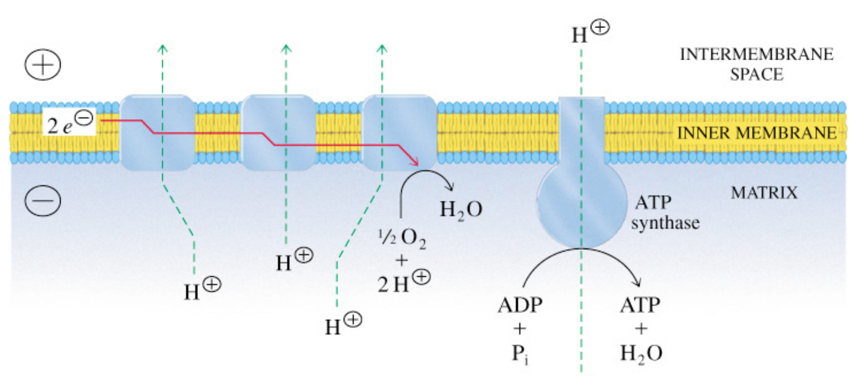
(2) **ATP synthase** uses the proton gradient energy to produce ATP; It is the release of the energy in the gradient back through the membrane through the protein **ATP Synthase** that drives ATP synthesis

Overview of electron transport chain and oxidative phosphorylation

Electron transport chain (ETC)

- Series of **sequential oxidation/reduction (redox) reactions**
- Finally see role of oxygen
- Passes **electrons** from **NADH** or **$FADH_2$** to **O_2** producing **H_2O** through a series of protein complexes (source of metabolic water!)
- Since **NAD^+ and FAD** are in **limited supply**, they must be **recycled**.





- **FOUR protein complexes in the IMM make up the ETC**
  - Complexes I, II, III, IV
  - Work together in succession to catalyze redox reactions
  - Electrons are transferred to molecular oxygen that is then reduced to water
  - Electrons move through the complexes in order
    - Electrons from **NADH** enter at **Complex I**
    - Electrons from **$FADH_2$** enter at **Complex II**

Recycling is accomplished by oxidation and transfer of electrons to oxygen.

$$NADH + H^+ + 1/2\ O_2 \xrightarrow{ADP + P_i \rightarrow ATP} NAD^+ + H_2O$$

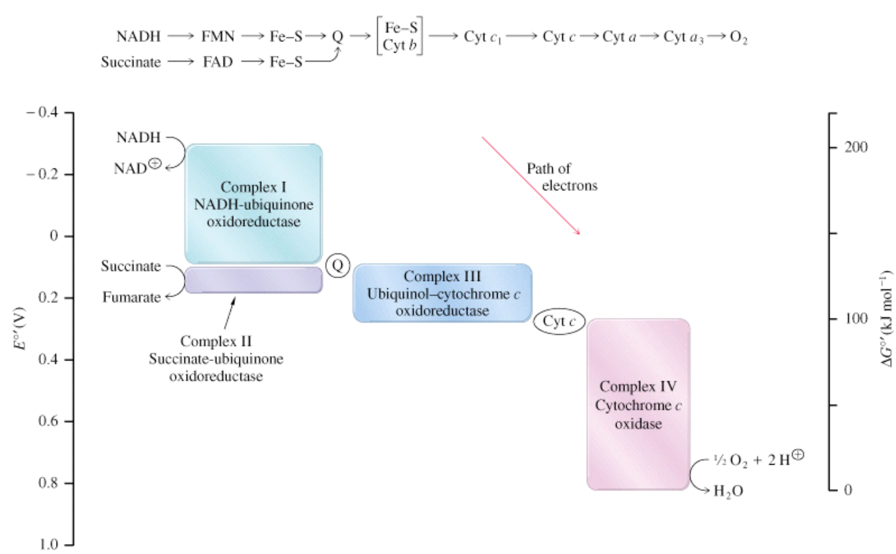
$$FADH_2 + 1/2\ O_2 \xrightarrow{ADP + P_i \rightarrow ATP} FAD + H_2O$$

NAD^+ and FAD are then available for additional oxidative metabolism. The energy released during electron transport is coupled to ATP synthesis.

- Flow of electrons is spontaneous and thermodynamically favorable because the next carrier has greater affinity for electrons than the previous
- In each reaction, an electron **donor** is **oxidized** and an electron **acceptor** is **reduced**
  – $\mathbf{A_{reduced} + B_{oxidized} \leftrightarrow A_{oxidized} + B_{reduced}}$
- **Compounds differ from one another in how readily they will be oxidized or reduced**
  – can be compared using *Eo' (volts)*
  – starting with 1 M "A" and 1 M "B", the component with most positive (low) redox potential will be reduced and the component with the most negative (high) reduction potential will be oxidized

– Electrons flow downhill – spontaneously moving from molecules that are strong electron **DONORS** to strong electron **ACCEPTORS** = move from **high** energy state to **low** energy state

–

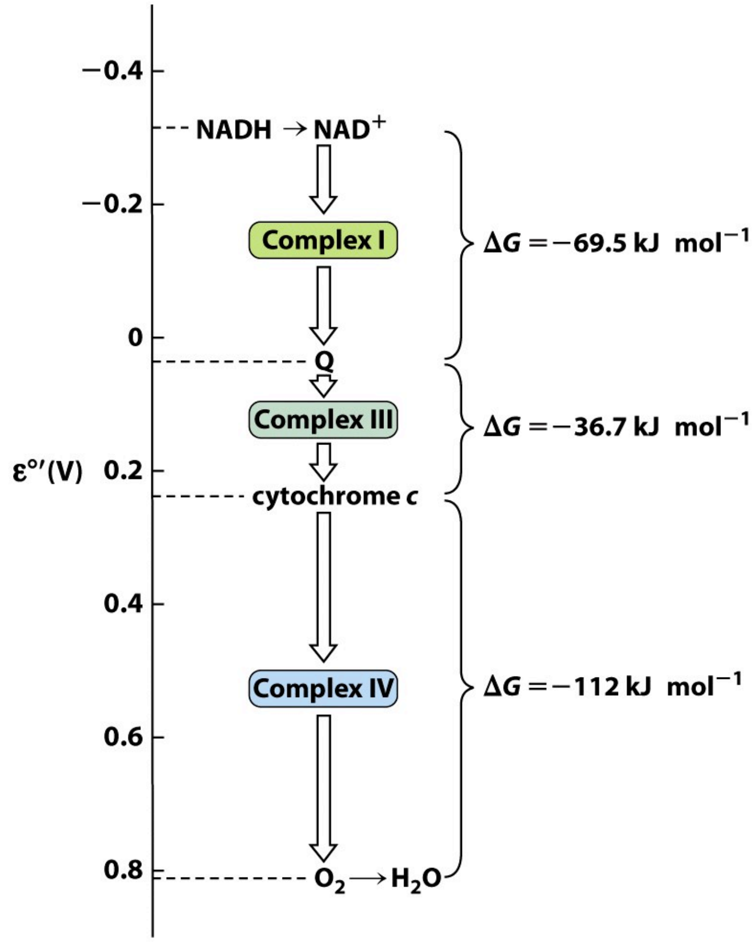


- NADH = strongest donor
- O_2 = strongest acceptor
- The redox potential energy of NADH is released **stepwise** via the electron transport chain

– The flow of electrons results in **energy** that is **released in increments** through the ETC

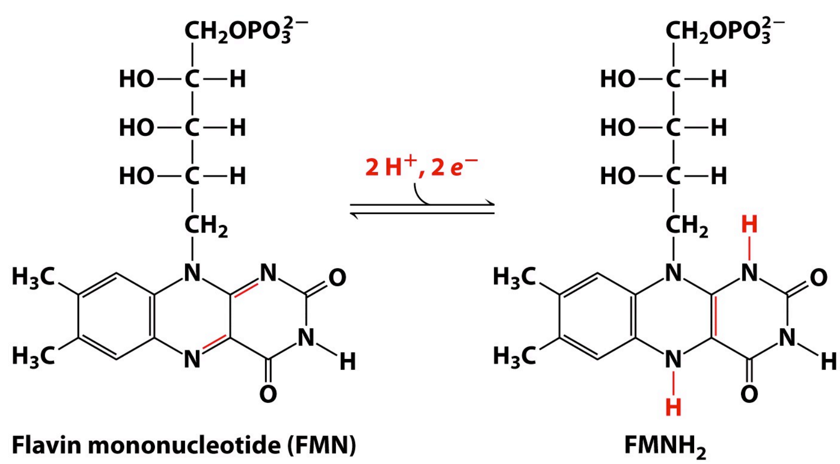
– Energy is used to pump protons (H^+) across the inner mitochondrial membrane (IMM) and set up the pH gradient

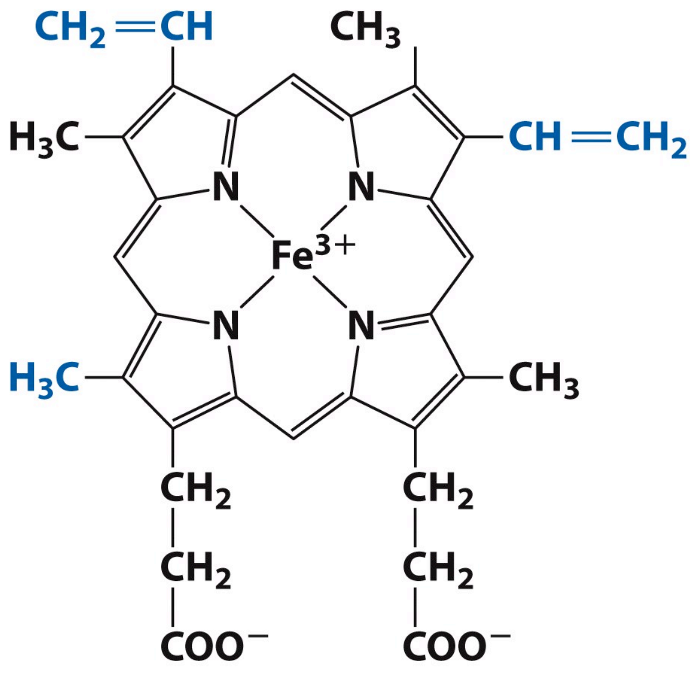
– It is the release of the energy in the gradient back through the membrane through the intergral membrane protein **ATP Synthase** that drives ATP synthesis



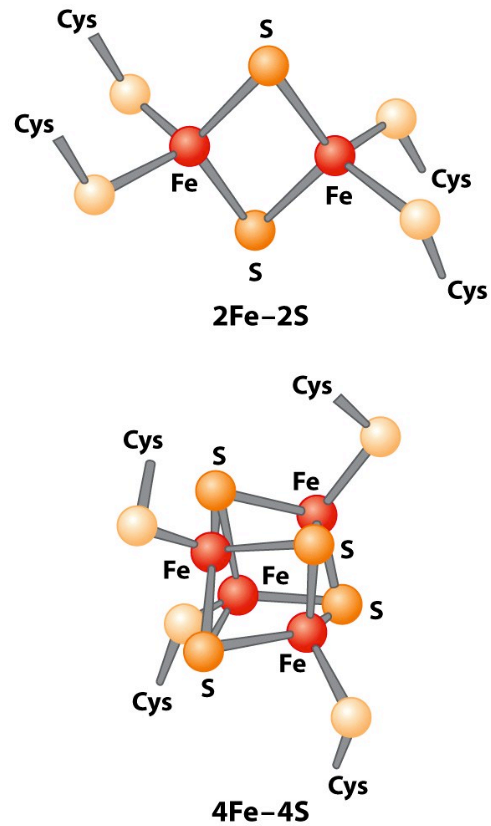
Co-factors in Electron Transport

- Complexes contain enzymes with **electron carrying groups** or **oxidation – reduction components**
- Protein components use **metal-containing prosthetic groups** or **flavins** to carry electrons
- Metal-containing groups such **as iron-sulfur clusters, copper ions, hemes**
- **Flavins**:
  - (Complex I) FMN - $FMNH_2$
  - (Complex II) FAD - $FADH_2$





Heme *b*



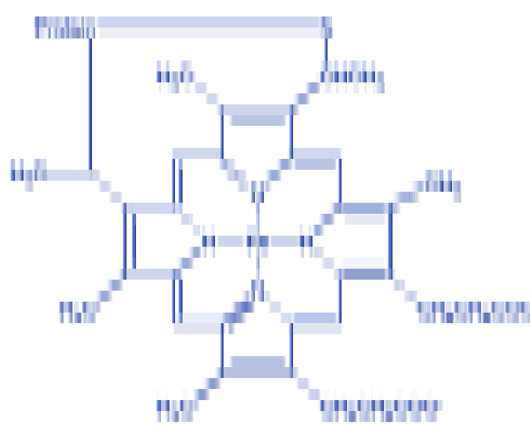
Mobile electron carriers – serve as links between ETC complexes

1. Ubiquinone (Q)

- Also called coenzyme Q
- A membrane-soluble low molecular weight compound
- Long hydrophobic tail keeps Q anchored in the mitochondrial inner membrane
- Q is a lipid soluble molecule that diffuses within the lipid bilayer, and shuttles electrons from Complexes I and II and pass them to III
- **Not** a part of any complex

2. Cytochrome *c*

- A peripheral membrane protein associated with the outer face of the membrane, transports electrons from III to IV
- Cytochromes are **heme-containing proteins** – contains Fe
- **Not** a part of any complex
- Shuttles electrons and protons from Complex III to Complex IV



Structure of cytochrome c heme group.

Overview of Electron Transport

- The electron transport chain is associated with the mitochondrial inner membrane
- **Complexes I-IV** contain multiple cofactors, and are involved in electron transport
- **OVERALL TRANSFER OF 2 ELECTRONS FROM NADH THROUGH ETC TO MOLECULAR OXYGEN:**

$$\mathbf{NADH + H^+ + \tfrac{1}{2}\, O_2 \rightarrow NAD^+ + H_2O}$$

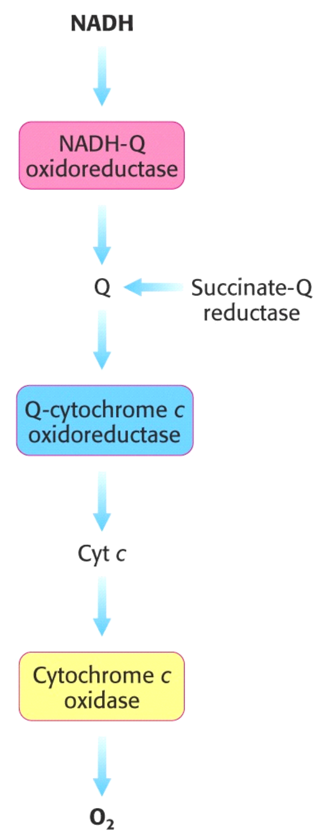


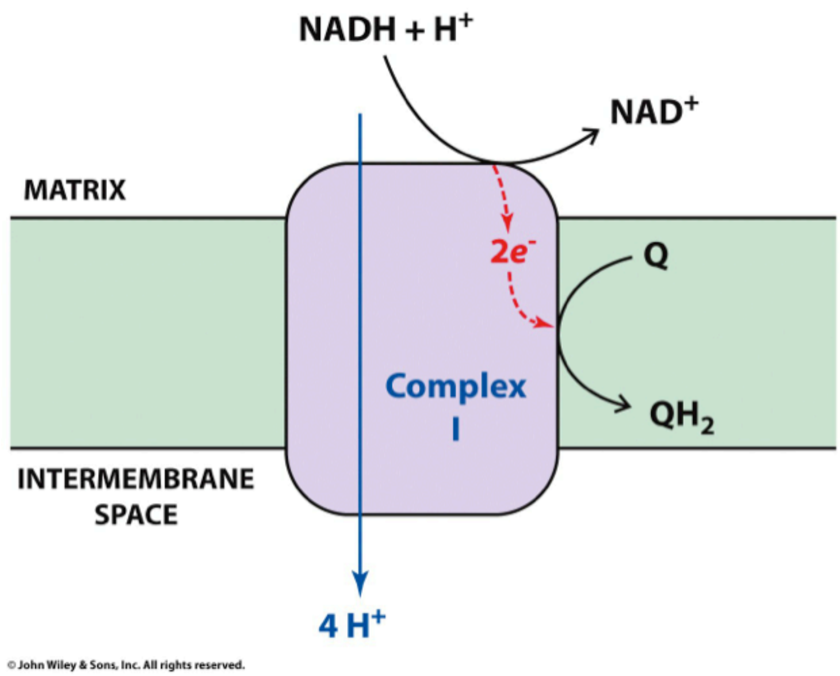
TABLE 14.1 Characteristics of protein complexes of the mitochondrial respiratory electron-transport chain in bovine heart

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

- **Complexes I – where NADH electrons enter the chain**
- **Complex II – Where $FADH_2$ electrons enter the chain**
- Electrons passed from **Complex I or II to Coenzyme Q**
- **Coenzyme Q** shuttles electrons to **complex III**
- **Complex III** shuttles electrons to **cytochrome C**
- **Cytochrome C** shuttles electrons to **Complex IV**
- **Complex IV** transfers electrons to **O_2** which is then reduced to **water**
- Flow through **Complexes I, III and IV** release energy which is used to pump protons across the IMM and form a "proton gradient"
- Proton gradient has lots of potential energy
- When the energy is released (protons flow back into matrix through ATP synthase), the energy drives ATP synthesis

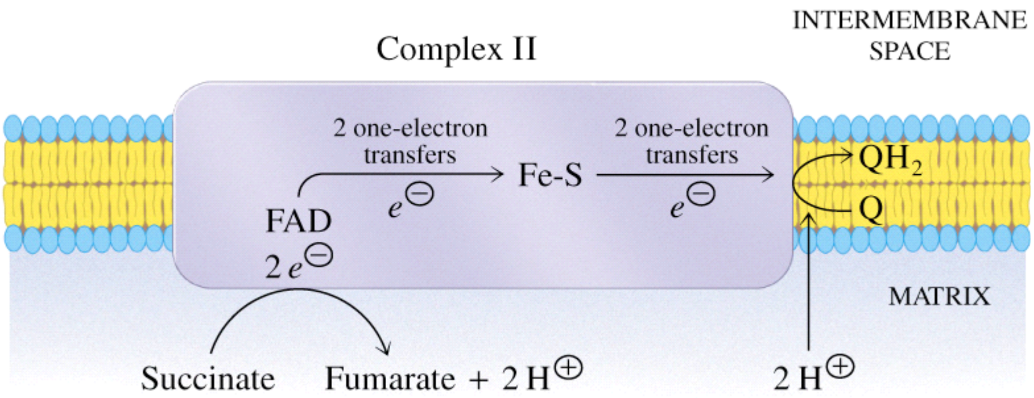
Electron transfer and proton flow in Complex I

- Also called NADH-ubiquinone oxidoreductase
- Complex I includes a **flavoprotein** (contains FMN – related to FAD) and proteins with **Fe-S centers (iron-sulfur clusters)**
    - These proteins provide two centers for oxidation reduction reactions
- Transfers electrons from NADH to Coenzyme Q via FMN and iron-sulfur proteins
- NADH transfers a two electrons as a hydride ion (H:-) to FMN
- Reduction of Q to QH_2 requires 2 e-
- **About 4 H+ translocated per 2 e- transferred**



Electron transfer in Complex II

- **Succinate-ubiquinone oxidoreductase**
- Same as **succinate dehydrogenase**, a component of the **TCA cycle**
- **Succinate dehydrogenase**
    - Directs transfer of electrons from succinate to CoQ via $FADH_2$.
    - Catalyzes the reduction of Q to QH_2
- **Acyl-CoA dehydrogenase**
    - From β-oxidation of fatty acids. It also transfers electrons to CoQ via $FADH_2$.

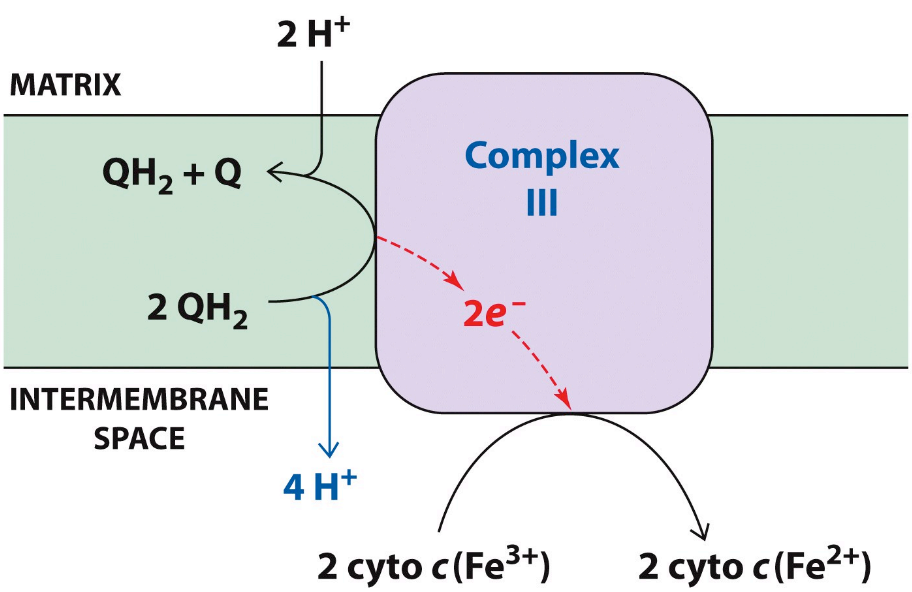


- Complex II proteins provide **two** centers for **oxidation reduction** reactions
  - FAD → $FADH_2$
  - Fe^{3+} → Fe^{2+} (iron-sulfur cluster)
- FAD of Complex II is reduced in a 2-electron transfer of a hydride ion from succinate
- **Complex II** does **NOT** contribute to **proton gradient**, but supplies electrons from succinate

  Note that **all electrons from $FADH_2$ and NADH must pass through CoQ.**

Electron transfer and proton flow in Complex III

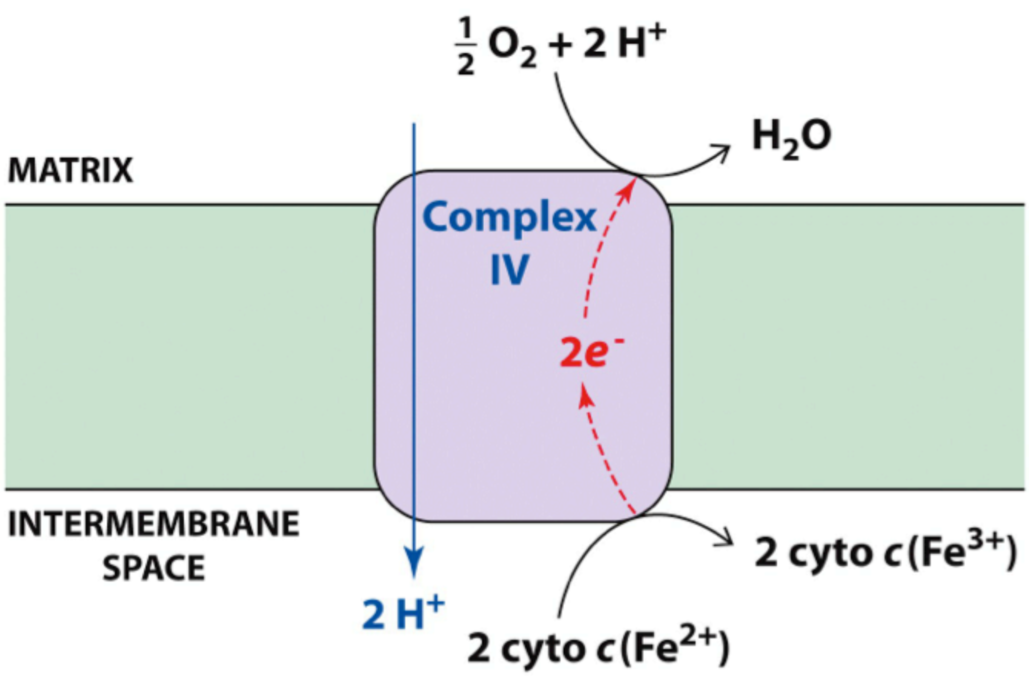
- **Ubiquinol-cytochrome *c* oxidoreductase**
- Transfers electrons to **cytochrome *c***



- Complex III contains several cytochromes (heme prosthetic group) and Fe-S center proteins which provide several centers for oxidation reduction reactions
- 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**
    - Regenerates Q for next round

<u>lectron transfer and proton flow in Complex IV</u>

- **Cytochrome *c* oxidase -** Combination of cytochromes
    - A complex of 10 protein subunits that contains **2 cytochromes** (a and a_3) and proteins with copper centers that provide multiple centers for **oxidation-reduction**
        - Consists of, 2 types of prosthetic groups - 2 heme and 2 Cu.
            - $Fe^{3+} \rightarrow Fe^{2+}$
            - $Cu^{2+} \rightarrow Cu^{1+}$
    - Source of electrons is **cytochrome *c*** (links Complexes III and IV)
    - Catalyzes a **four-electron reduction** of molecular **oxygen** (O_2) to **water** (H_2O)
        - Cytochromes a and a_3 are the only species capable of direct transfer of electrons to oxygen.
    - Translocates **H^+** into the intermembrane space and contributes to the proton gradient



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)

HOWEVER, FOR **EACH PAIR OF ELECTRONS** (e.g. NADH), ONLY GET **$2H^+$ TRANSFERRED** TO INTERMEMBRANE SPACE IN **COMPLEX IV**

SUMMARY OF ELECTRON TRANSPORT CHAIN

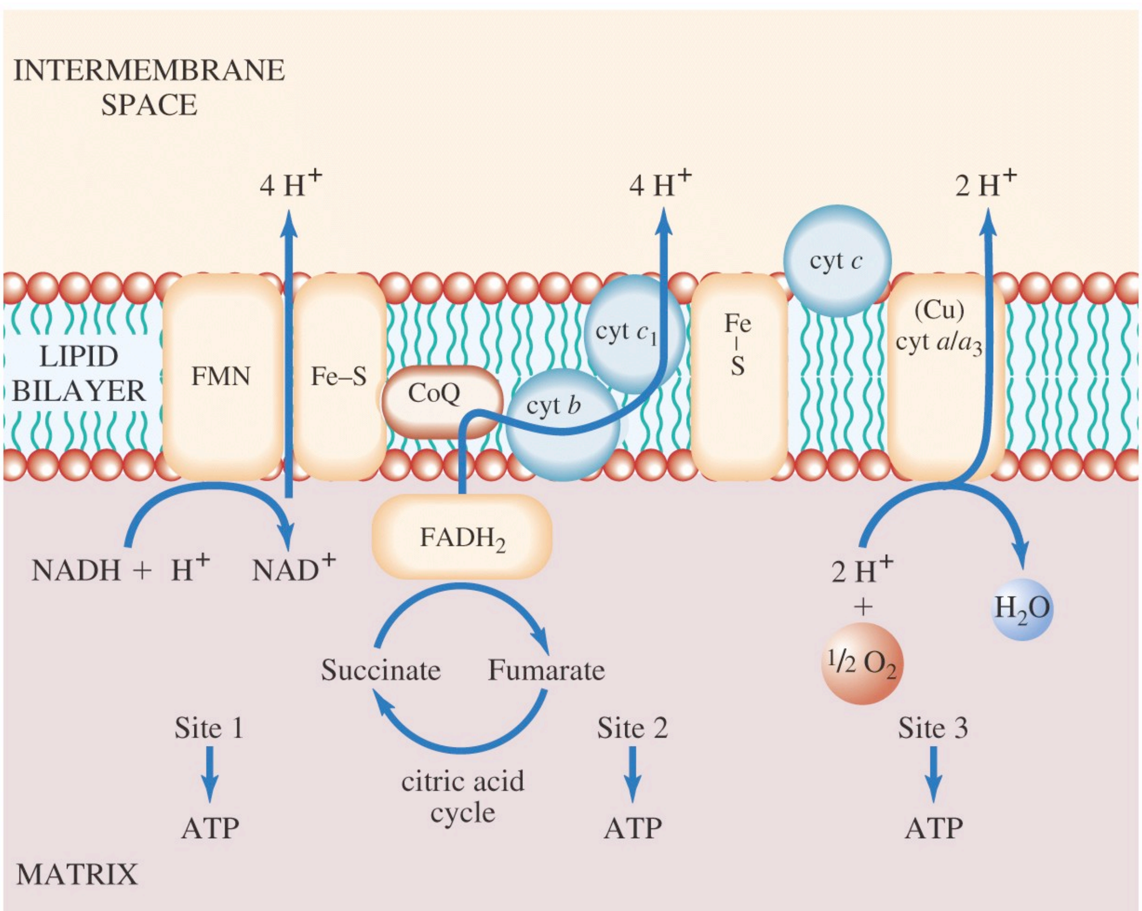
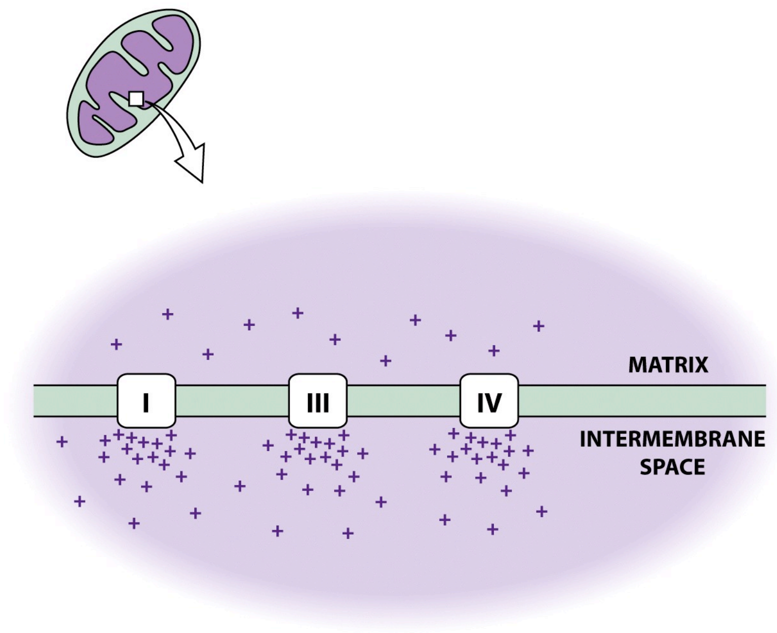


Figure 17-12 Concepts in Biochemistry, 3/e



Mitochondrial Electron Transport

NADH is oxidized by Complex I
Complex I is reduced
Complex I is oxidized by Ubiquinone
Ubiquinone is reduced

Succinate is oxidized by Complex II
Complex II is reduced
Complex II is oxidized by Ubiquinone
Ubiquinone is reduced

Ubiquinone is free to diffuse through the mitochondrial inner membrane

Ubiquinone is oxidized by Complex III
Complex III is reduced
Complex III is oxidized by Cytochrome C
Cytochrome C is reduced

Cytochrome C is free to diffuse through the mitochondrial inter-membrane space

Cytochrome C is oxidized by Complex IV
Complex IV is reduced
Complex IV is oxidized by oxygen
Oxygen is reduced

Electron Transport and Oxidative Phosphorylation

ENERGETICS:

- **Complex I**, **Complex III** and **Complex IV** pump **protons** across the inner mitochondrial membrane
  - pumping uses the energy liberated from the oxidation of NADH and $FADH_2$
  - pumping generates a membrane potential because it generates an electrochemical gradient
    - negative inside, positive outside
    - alkaline inside, acidic outside

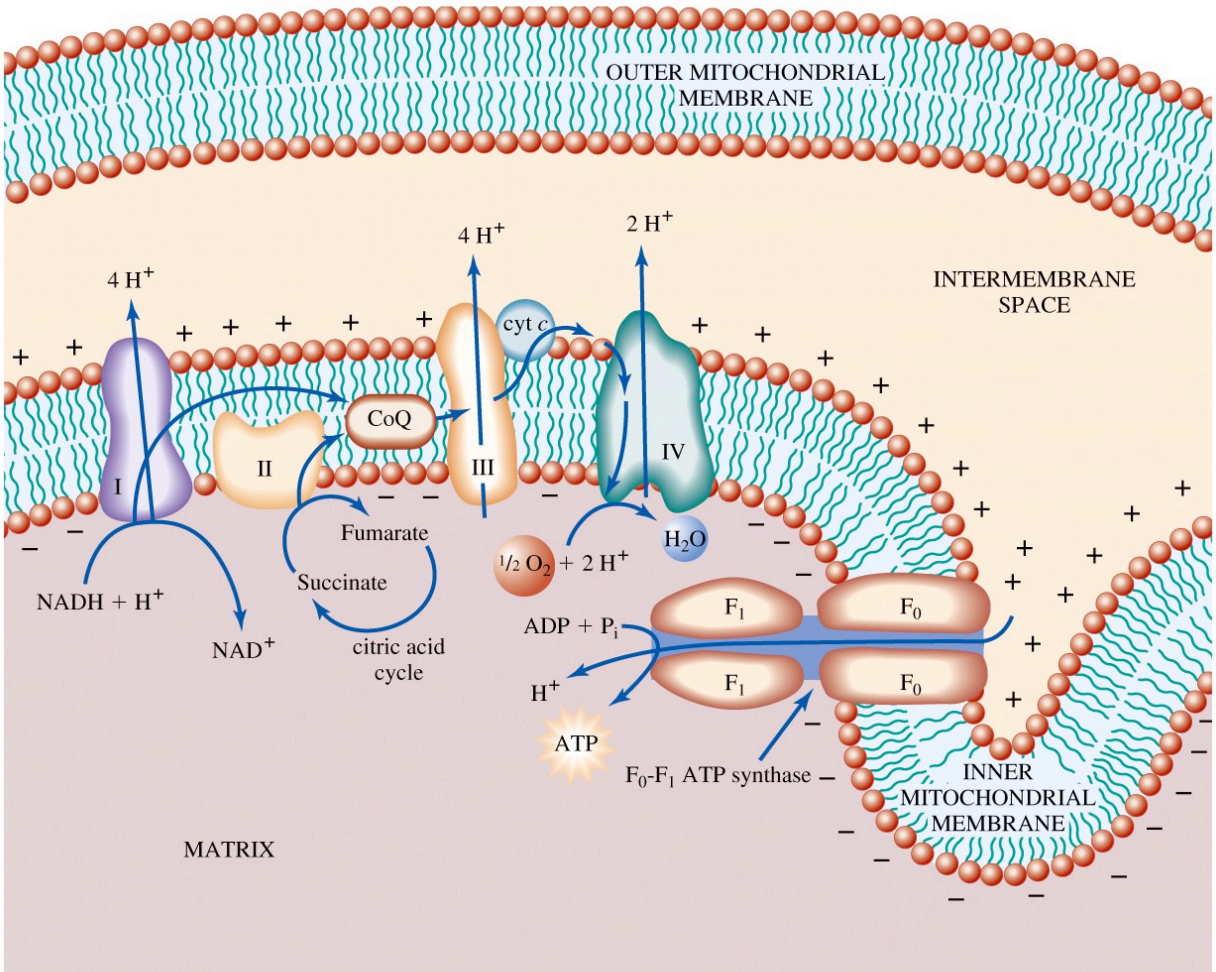
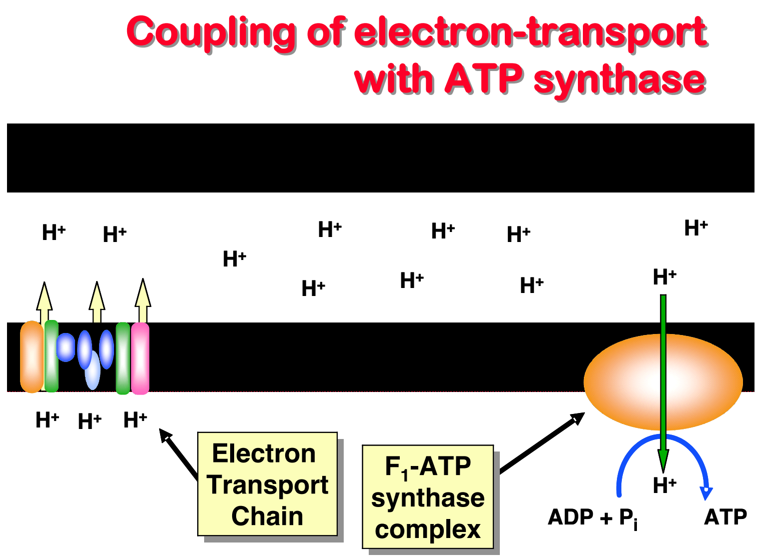


Figure 17-13 Concepts in Biochemistry, 3/e

The Chemiosmotic Hypothesis

- Proposed by Peter Mitchell in the 1960's (Nobel Prize in 1978)
- **A proton concentration gradient serves as the energy reservoir for driving ATP formation**
- Electron transport through the ETC generates a proton gradient (pumps H+ from the matrix to the intermembrane space)
- **Protonmotive force** (**Δp**) 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)
- The transmembrane protein, **ATP synthase**, catalyzes the phosphorylation of ADP in a reaction driven by movement of H^+ across the inner membrane into the matrix
- As **protons** move **back** into the **matrix** through **ATP Synthase**, the **energy stored in the electrochemical proton gradient is used to make ATP**



ANIMATION

http://www.science.smith.edu/departments/Biology/Bio111/etc.html

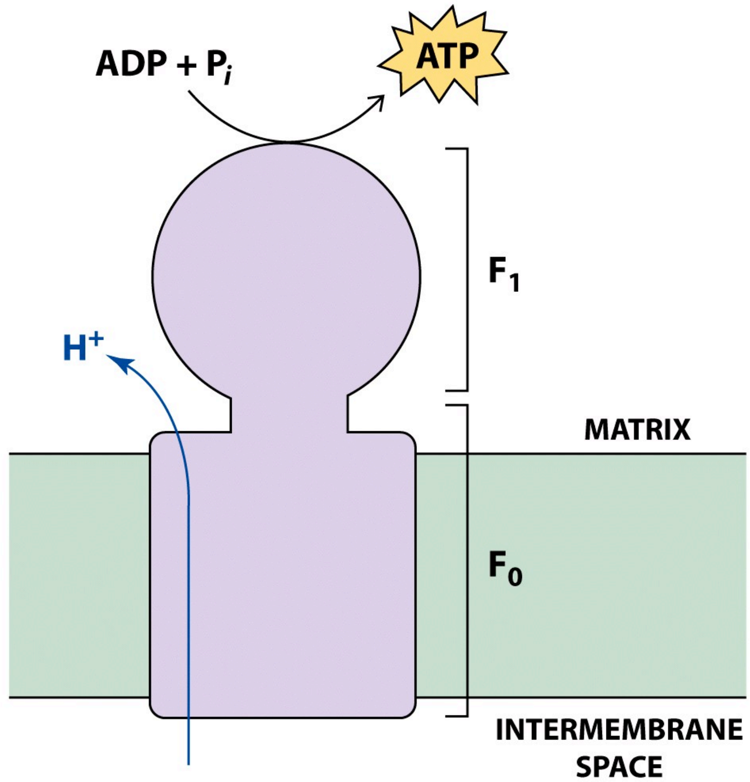
http://www.wiley.com/college/fob/anim/

Chapter 17

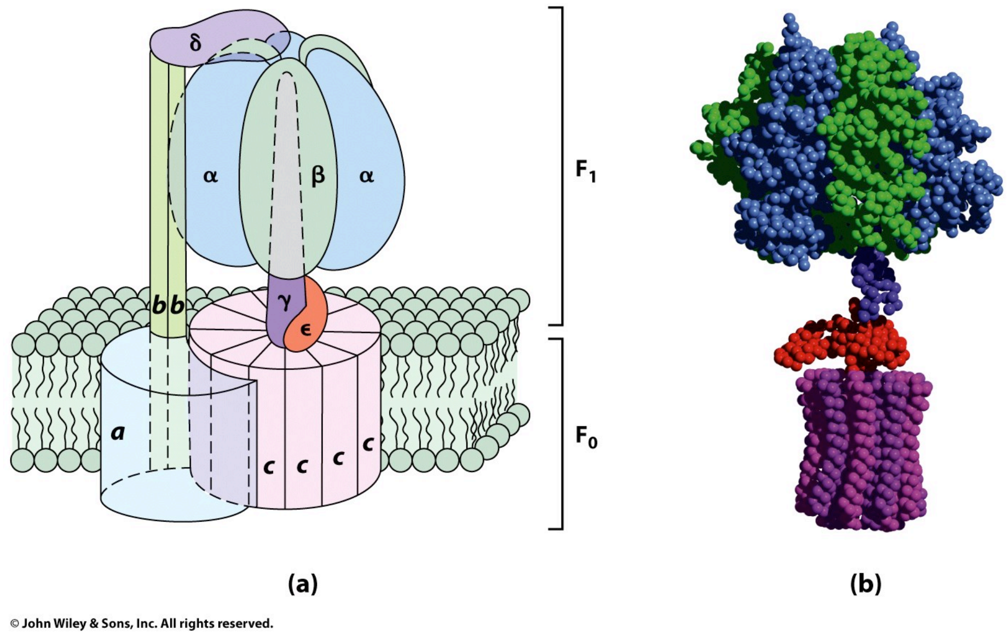
- Fig. 17-8 -- The Mitochondrial Electron Transport Chain

ATP Synthase

- **F_0F_1 ATP Synthase** uses the proton gradient energy for the synthesis of ATP
- Large transmembrane protein complex
- Faces into the mitochondrial matrix – spans the IMM
- Composed of a "knob-and-stalk" structure
- **F_0 (stalk) has a proton channel which spans the membrane.**
    - Forms a proton pore
    - Membrane-spanning portion – integral membrane protein
    - Made up of 4 different subunits
    - F_o subunit composition: $a_1b_2c_{9\text{-}12}$ (c subunits form cylindrical, membrane-bound base)



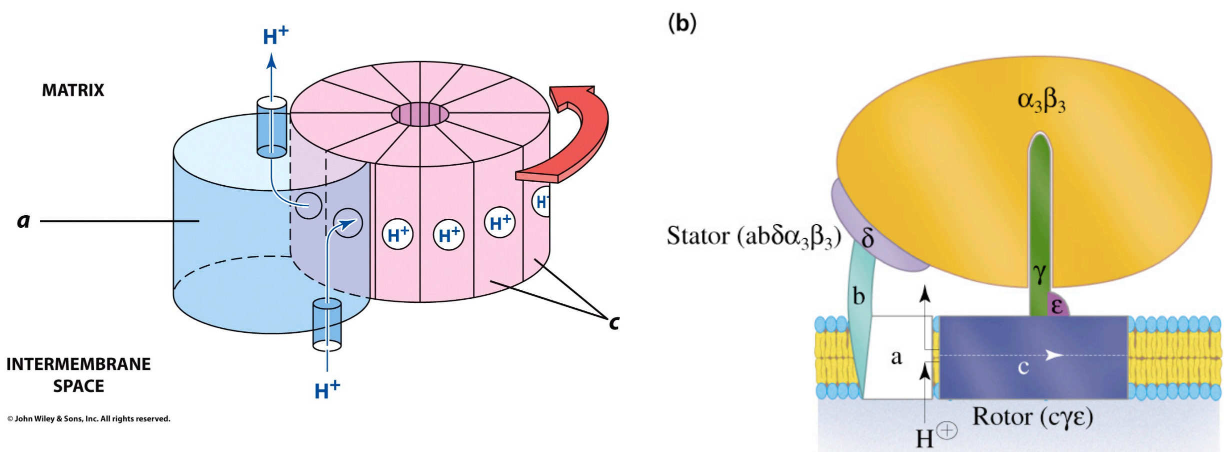
- **F_1 (knob) contains the catalytic subunits (ATP-synthesizing subunits)**
    - Where ATP synthesis takes place
    - F_1 knobs: inner face of the inner mitochondrial membrane
        - (subunit composition: $\alpha 3\beta_3\gamma\delta\varepsilon$)
        - $\alpha_3\beta_3$ oligomer of F_1 is connected to c subunits by a multisubunit stalk of γ and ε chains
- 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



Mechanism of ATP Synthase

- F_1-F_0 complex serves as the molecular apparatus for coupling H+ movement to ATP synthase.
- There are 3 active sites, one in each β subunit
- Passage of protons through the Fo channel causes the rotor to spin in one direction and the stator to spin in the opposite direction

Proton flow → C unit rotates → γ rotates → conformation change → ATP synthesized



Animation:

http://www.stolaf.edu/people/giannini/biological%20anamations.html
mitochondrial electron transport
ATP synthase
ATP synthase mechanism

Binding-change mechanism of ATP synthase (This page is NOT for the Exam)

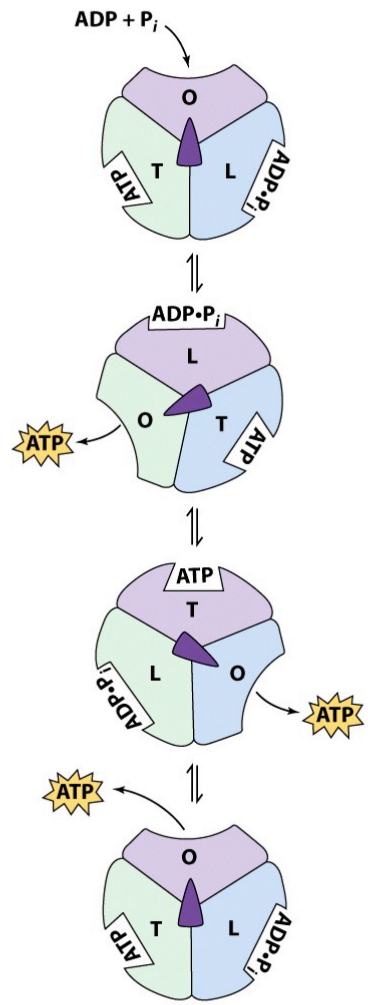
1. ADP, Pi bind to an open site

2. Inward passage of protons, cause a conformational change, and ATP is synthesized from ADP and Pi

3. ATP released from open site, ADP and Pi form ATP in the tight site

α/β subunits are asymmetric:
T is catalytically active, binds to ADP tight;
O has low affinity for substrate
L is also inactive, but can bind ADP

ADP+Pi first binds to L state; rotation of α/β subunits converts L to T, T to O and O to L states, one ATP is made.



The binding change mechanism. F_1 has three chemically identical but conformationally distinct interacting αβ protomers: O, the open conformation, has very low affinity for ligands and is catalytically inactive; L binds ligands loosely and is catalytically inactive; T binds ligands tightly and is catalytically active. ATP synthesis occurs in three steps: (**1**) ADP and P_i bind to site L. (**2**) An energy-dependent conformational change converts binding site L to T, T to O, and O to L. (**3**) ATP is synthesized at site T and ATP is released from site O. The enzyme returns to its initial state after two more passes of this reaction sequence. The energy that drives the conformational change is apparently transmitted to the catalytic $\alpha_3\beta_3$ assembly via the rotation of the γδε assembly, here represented by the centrally located asymmetric object (*green*). [After Cross, R.L. *Annu. Rev. Biochem.* **50**, 687 (1980).]

Animation:

http://www.wiley.com/college/fob/anim/

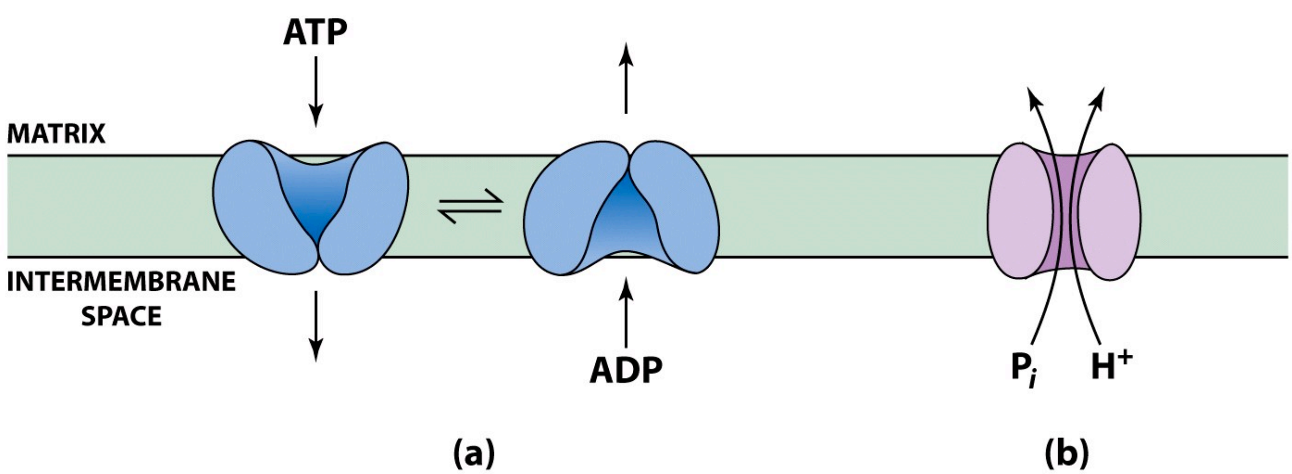
Chapter 17: **Fig. 17-21** -- The Binding Change Mechanism for ATP Synthesis

Regulation:

- Electrons do not flow unless ADP is present for phosphorylation
- **Increased ADP** levels cause an **increase** in **catabolic** reactions of various enzymes including:
    a. glycogen phosphorylase
    b. phosphofructokinase
    c. citrate synthase

Active Transport of ATP, ADP and Pi Across the Inner Mitochondrial Membrane

- ATP is synthesized in the mitochondrial matrix
- ATP must be transported to the cytosol, and ADP and Pi 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)
- **Adenine nucleotide translocase**: unidirectional exchange of ATP for ADP (antiport)
- Symport of Pi and H^+ is electroneutral



The P:O Ratio

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

- 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	4	2

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

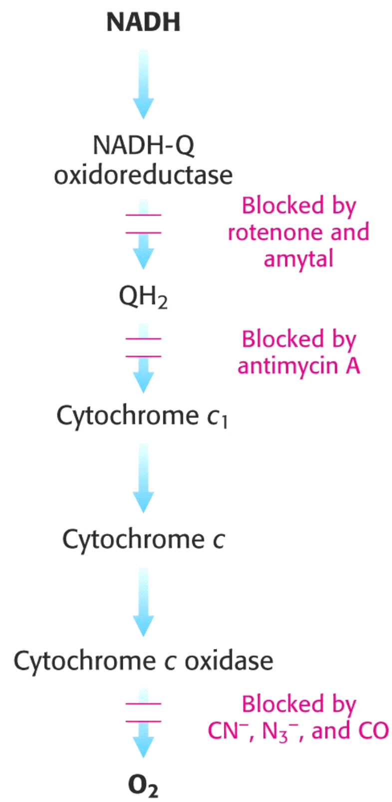
For **NADH**: 10 H^+ translocated / O ($2e^-$)

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**

RESPIRATORY INHIBITORS & UNCOUPLERS:



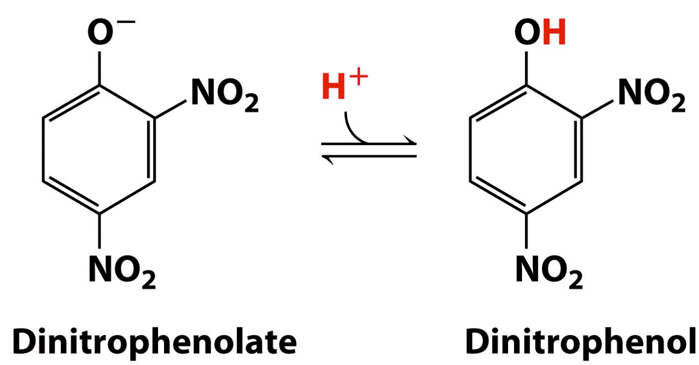
<u>Inhibitors</u> are chemicals that can block electron transfer through specific complexes in the ETC

- **Complex I**: blocked by rotenone, barbiturates
- **Complex III**: blocked by antimycin A
- **Complex IV**: blocked by cyanide, azide, carbon monoxide

<u>Uncouplers</u>

- In some special cases, the coupling of the two processes can be disrupted.
- **Uncouplers** stimulate the oxidation of substrates in the <u>absence of ADP</u>
- Large amounts of O_2 are consumed but no ATP is produced.
- 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
- Do NOT affect electron transport
- Allow protons back into the matrix without making ATP
- Stimulate oxygen consumption

<u>2,4-Dinitrophenol: an uncoupler</u>



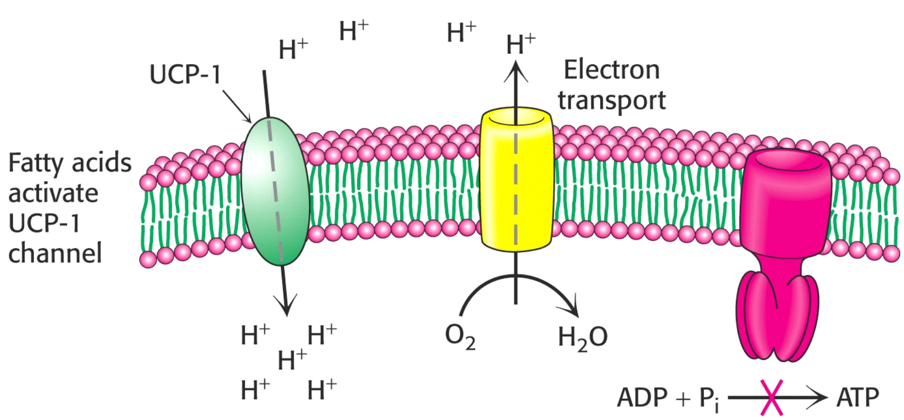
- Used as a diet/weight loss drug
- Hydrophobic low molecular weight substance that can diffuse through the mitochondrial inner membrane
- Shuttles protons across the membrane and dissipates proton gradient
- ATP synthesis goes down
  - ADP concentration in cells goes up and acts as a stimulator
  - Signals to turn on pathways to make ATP
  - Therefore, electron transport and O_2 consumption turned on fully and is NOT regulated

- Energy produced by electron transport released as HEAT rather than harnessed into ATP synthesis
- Fuels (carbs and fats) are consumed at great rates and get quick weight loss BUT
  - Get heavy breathing – using lots of oxygen
  - Excessive fever (heat generation)
  - BIG problem – no control over uncoupling
  - Brain, heart and muscles are affected as well
- 2,4-dinitrophenol is **extremely toxic** and pulled from the market

NATURAL UNCOUPLERS

- In newborn and hibernating animals, brown fat oxidizes large amounts of substrate (mostly fatty acids) to generate heat
- 'Brown fat'- brown because of the large number of mitochondria and their associated cytochromes
- In brown fat mitochondria **oxidation** of NADH and $FADH_2$ is **uncoupled** from **ATP synthesis**
  - Mitochondria contain **thermogenin** (uncoupling protein).
  - Thermogenin allows the release of energy as heat instead of ATP.
  - ***Thermogenin*** dissipates proton electrochemical gradient

- By providing another channel for return of protons - **bypasses** ATP synthase
- Also called ***uncoupling protein (UCP)***
- **In brown fat mitochondria, the energy that would have been used to make ATP is liberated as heat**



Electron Transport Chain and Oxidative Phosphorylation Animation Websites:

1. http://www.cat.cc.md.us/biotutorials/cellresp/etsar.html

2. http://wunmr.wustl.edu/EduDev/LabTutorials/Cytochromes/etc_movie.html

3. http://faculty.nl.edu/jste/electron_transport_system.htm

4. http://cwx.prenhall.com/horton/chapter14/deluxe.html
   Live Figures:

Figure 14.10	Mitochondrial electron transport.
Figure 14.19	Demonstration of the rotation of a single molecule of ATP synthase

5. http://www.wiley.com/college/fob/anim/

   Chapter 17

   - **Fig. 17-8** -- The Mitochondrial Electron Transport Chain
   - **Fig. 17-18** -- The Coupling of Electron Transport and ATP Synthesis
   - **Fig. 17-21** -- The Binding Change Mechanism for ATP Synthesis

6. Great site with lots of information:
http://www.brookscole.com/chemistry_d/templates/student_resources/shared_resources/animations/oxidative/oxidativephosphorylation.html

7. http://www.science.smith.edu/departments/Biology/Bio111/etc.html **

8. http://www.stolaf.edu/people/giannini/biological%20anamations.html **

   mitochondrial electron transport
   ATP synthase
   ATP synthase mechanism