Chapter 20

Oxidative Phosphorylation

Prokaryotes are bacteria containing a single chromosome and no membrane-bound organelles or nuclear envelope. Gram negative bacteria have two membranes – an inner and an outer.

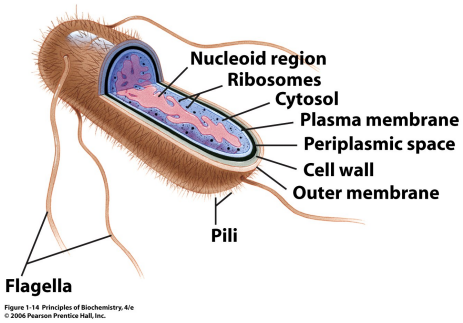

Figure 1-14 Principles of Biochemistry, 4/e
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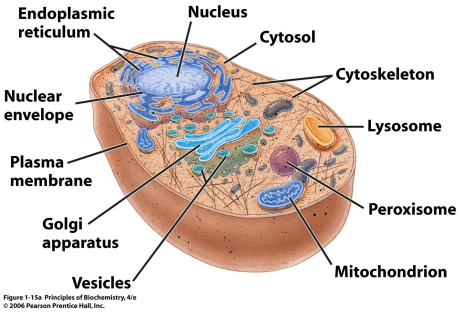
Eukaryotes contain multiple chromosomes surrounded by a membrane (nucleus) and membrane-bound organelles. Some oraganelles such as the nucleus and mitochondrion have two membranes.

Animal Cell



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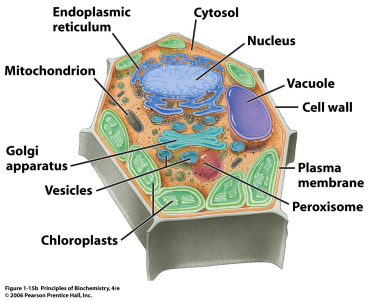
Plant Cell



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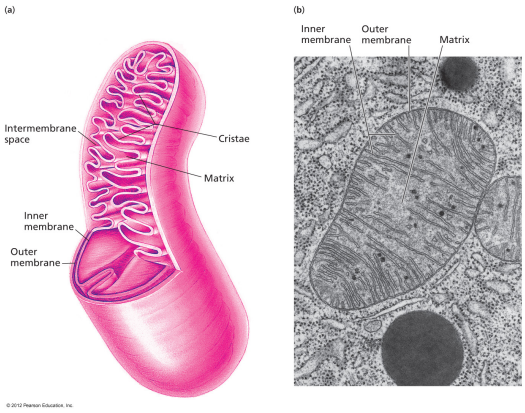
The **mitochondrion** is thought to be an ancient prokaryotic, gram negative, aerobic bacterium that took up **symbiotic** residence in a primitive, eukaryotic, anaerobic host.

Mitochondria have their own DNA, ribosomes, and transfer RNAs.

They contain an outer, highly permeable membrane and an inner impermeable membrane.



The interior **matrix** contains pyruvate deH_2ase, TCA cycle enzymes, and enzymes for oxidation of amino acids and fatty acids.

It is the **"furnace"** of the cell.

The **burning** of paper: $(C_6H_{10}O_5)_n + 6nO_2 \rightarrow + 6nCO_2 + 5nH_2O$

Part 1: Electron Flow

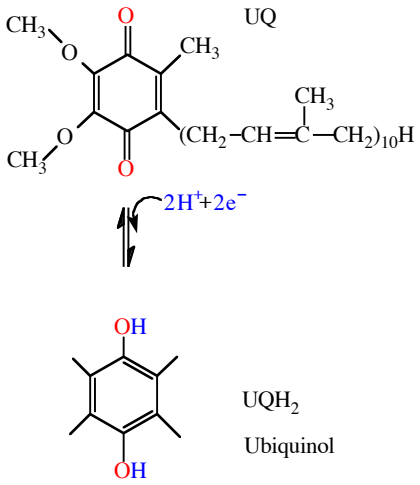
High *G* electrons from glycolysis, TCA cycle, AA, and fatty acid oxidation are funneled into universal electron carriers:

NADH / NADPH / $FADH_2$ The e^{-1} are then transferred to a chain of e^{-1} carriers in the inner membrane of the mitochondrion.

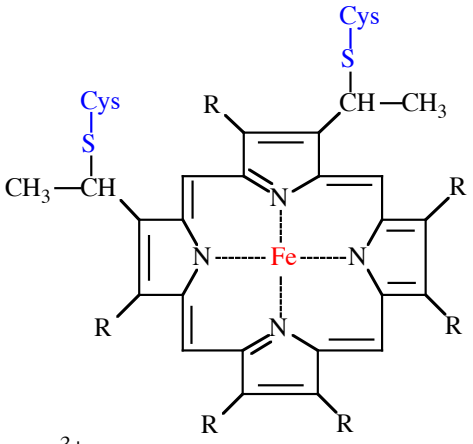
This is called the **respiratory chain**.

The electron carriers are 4 protein complexes and their coenzymes.

1. Coenzyme Q = Ubiquinone
It is lipid soluble and can diffuse through the membrane accepting and donating e^{-1}.



2. **A Family of Cytochromes** – all but cyt c are integral membrane proteins. They have different amino acid sequences and bind slightly different **iron-containing hemes**.



$Fe^{2+} \longrightarrow Fe^{3+} + e^-$

Ferrous Ferric

Cyt c is a peripheral membrane protein that binds Heme C covalently *via* Cys residues.

The **standard reduction potential**, a measure of the *G* of the e^{-1}, is different in each protein.

Table 10-4 & 14-1.

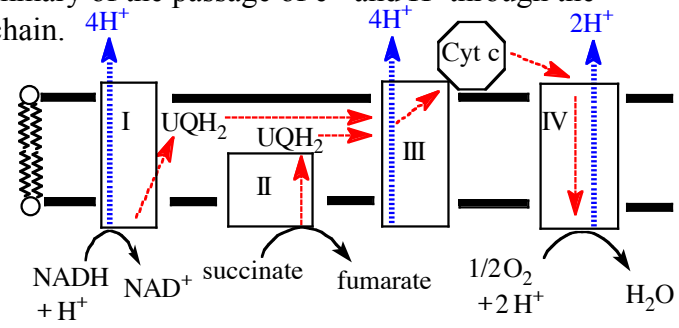
Table 14.1 Standard reduction potentials of mitochondrial oxidation–reduction components

Substrate of Complex	$E^{o\prime}$ (V)
NADH	−0.32
Complex I	
FMN	−0.30
Fe–S clusters	−0.25 to −0.05
Succinate	+0.03
Complex II	
FAD	0.0
Fe–S clusters	−0.26 to 0.00
QH_2/Q	+0.04
$(\cdot Q^{\ominus}/Q$	−0.16)
$(QH_2/\cdot Q^{\ominus}$	+0.28)
Complex III	
Cytochrome b_1.	−0.01
Cytochrome b_H	+0.03
Fe–S cluster	+0.28
Cytochrome c_1	+0.22
Cytochrome *c*	+0.22
Complex IV	
Cytochrome *a*	+0.21
Cu_A	+0.24
Cytochrome a_3	+0.39
Cu_B	+0.34
O_2	+0.82

3. Fe-S Proteins

Again, the *G* of the bound and released e^{-1} is different in different proteins.

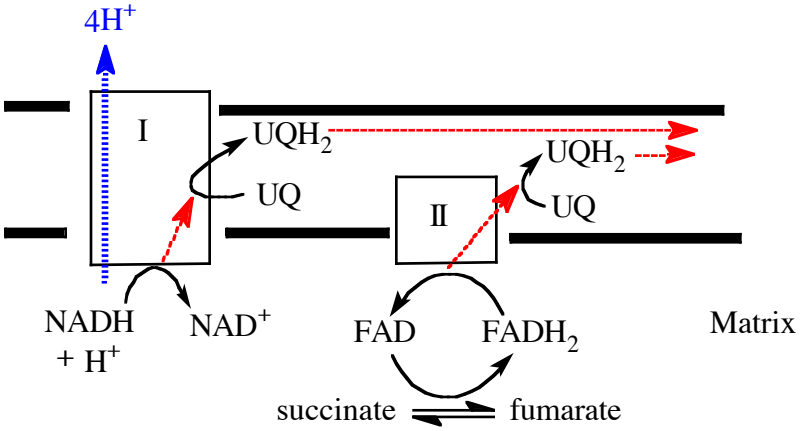
Here is a summary of the passage of e^{-1} and H^+ through the respiratory chain.



Complex I - NADH deH$_2$ase

Consists of more than 25 proteins, 7 Fe-S centres, and FMN.

Net reaction: $NADH + H^+ + UQ \rightleftharpoons NAD^+ + UQH_2$

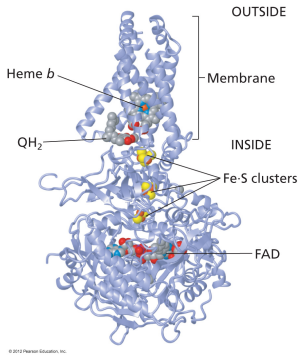


UQH_2 diffuses through the membrane to Complex III.

Notice that $4H^+$ are pumped across the membrane.

Complex II – Succinate deH$_2$ase

Consists of 4 proteins including Fe-S proteins, and covalently-bound FAD. It is the only membrane-bound enzyme (6) of the TCA Cycle.



Net reaction:

$FADH_2 + UQ \rightleftharpoons FAD + UQH_2$

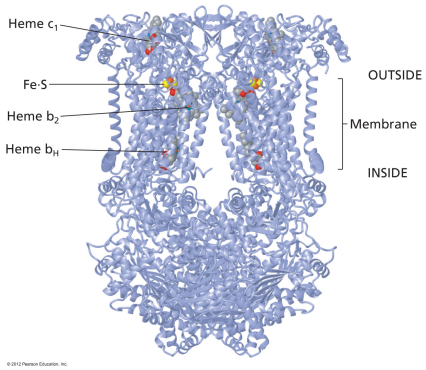
UQH_2 diffuses to Complex III.

Complex III - UQ-Cyt c Oxidoreductase

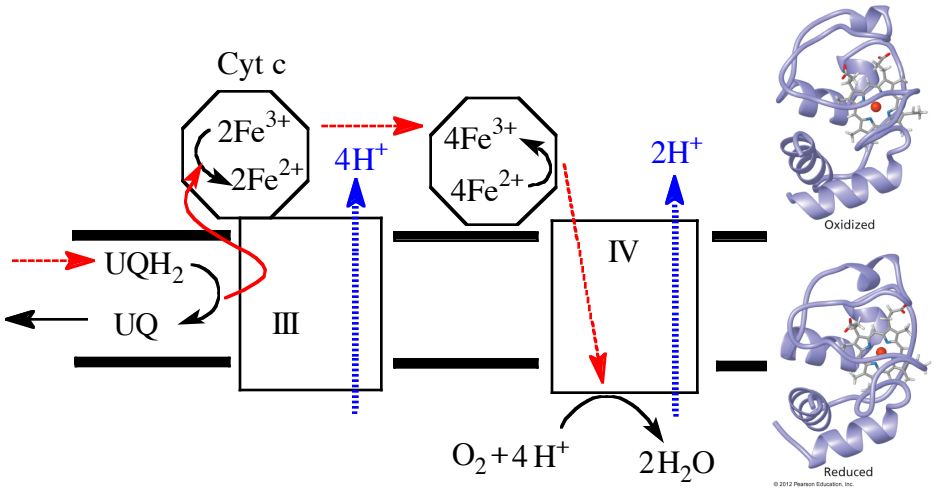
Consists of 10 proteins including Cyt b_{562}, Cyt b_{566}, Cyt c_1, Fe-S protein.

The last e^{-1} acceptor is Cyt c which dissociates from Complex III and carries one e^{-1} to Complex IV.

This is an ancient protein with homologs in chloroplasts and bacteria.



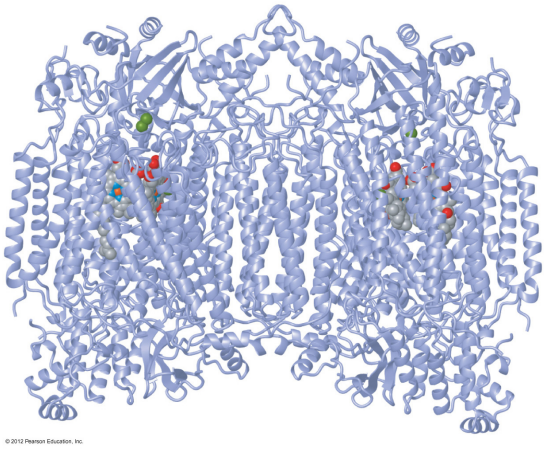
Net reaction: $UQH_2 + 2Cyt\text{-}Fe^{3+} \rightleftharpoons UQ + 2Cyt\ c\text{-}Fe^{2+}$



Notice that $2H^+$ are pumped across the membrane.

Complex IV - Cytochrome Oxidase

Consists of about 10 proteins including cytochromes a and a_3.



Net reaction: $4\text{Cyt c-Fe}^{2+} + O_2 + 4H^+ \rightleftharpoons 4\text{Cyt c-Fe}^{3+} + 2H_2O$

$4e^{-1}$ pass from 4 cyt c through Complex IV to O_2.

The $4e^{-1}$ come from 2 NADH.

$2H^+$ are pumped across the membrane for each $2e^{-1}$ transferred.

Energetics:

e^{-1} in NADH have high *G*;
e^{-1} in water have low *G*;
thus e^{-1} flow is down the *G* hill.

$NADH + H^+ + ½\ O_2 \rightarrow H_2O + NAD^+$

$\Delta\mathcal{E}^o = 0.816 - (-0.320) = +1.14$ Volts

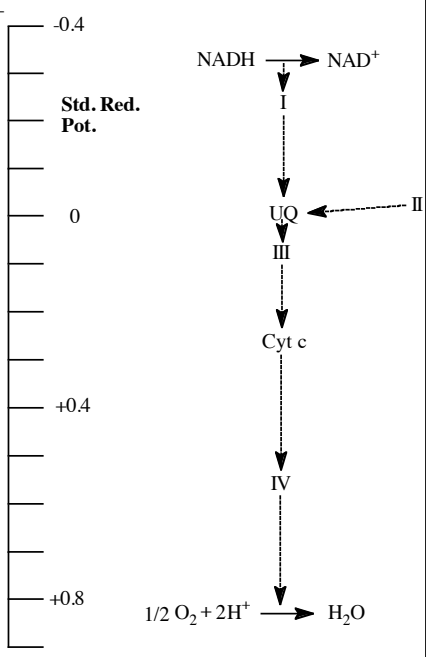
$\Delta G'^o = -n\mathcal{F}\Delta\mathcal{E}^o = -220$ kJ/mol

This is more than enough *G* to make 1 ATP from ADP + P_i

$\Delta G'^o = 30.5$ kJ/mol;

$\Delta G \sim +50$ kJ/mol

Succinate oxidation yields –150 kJ/mol.



Part II Chemiosmotic Coupling

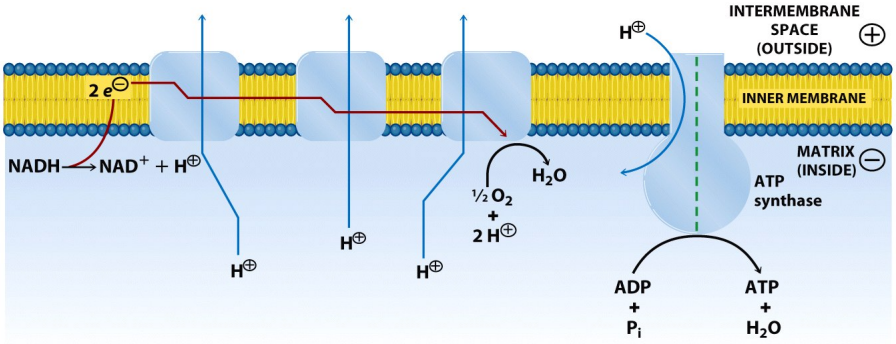
But how is the *G* of e^{-1} flow converted into ATP synthesis?

During the transport of 2 e^{-1}, 10 H^+ are removed from the matrix and transported into the space between the inner and outer mitochondrial membranes by Complexes I, III, IV.

This **electrochemical work** is done using the G released during e^{-1} flow.

A **chemical / pH gradient** is built up across the inner membrane with OH^- in the matrix and H^+ in the space.

An **electrical gradient** is also built up across the inner membrane as positive and negative charges are separated. The matrix becomes negative, the inter-membranous space, positive.



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This results in an electrical potential difference of 0.15 – 0.2 Volts ($\Delta\psi$).

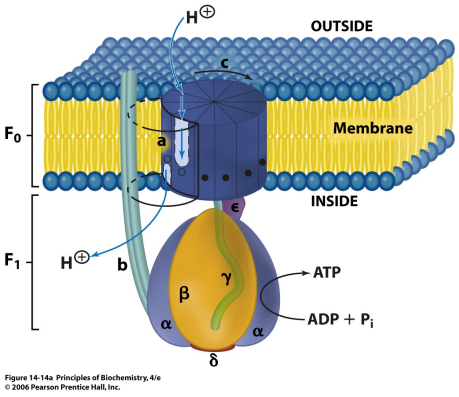
Combining the pH and electrical gradients, about 200 kJ of the 220 kJ available is stored in the electrochemical gradient.

The e^{-1} from succinate result in the pumping of $6H^+$ and the storage of about 120 kJ.

The *G* stored in the electric and chemical gradients could be released if H^+ were permitted to diffuse back into the mitochondrion down the *G* hill.

The return of $4H^+$ into the matrix would provide enough *G* for the synthesis of 1ATP, so 10 H^+ would yield 2.5 ATP and $6H^+$ yield 1.5 ATP.

The H^+ gradient is coupled to ATP synthesis by the **F_oF_1 ATP Synthase**.



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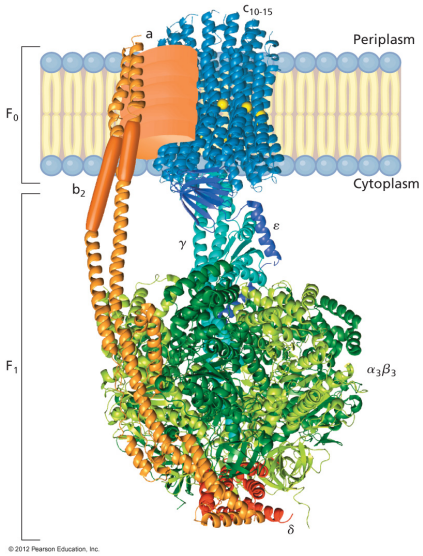

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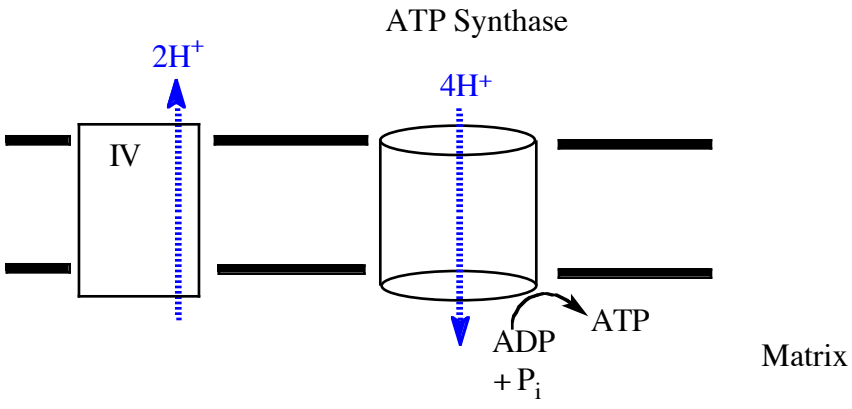
F_o is a tetrameric integral membrane protein channel for H^+.

F_1 is a peripheral membrane protein with subunits $\alpha_3\beta_3\gamma\delta\varepsilon$. It contains the ATP synthesis activity.

H^+ flow through F_o releases *G* that is used to make ATP.

Electron transport and oxidative phosphorylation are tightly **coupled** so inhibition of one, shuts down the other process.



Oxidative phosphorylation is regulated by the supply of ADP and phosphate.

The enzyme ***ATP / ADP translocase*** moves ATP into the cytoplasm and ADP into the mitochondrion.

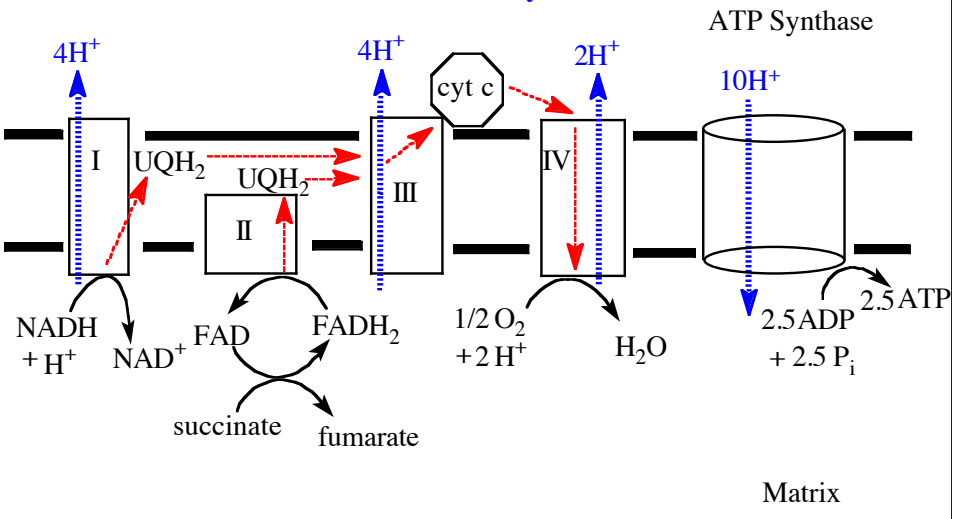
ATP Yield from Complete Oxidation of Glucose

Glycolysis	2NADH 2ATP	7ATP*
Pyruvate Oxidation	2NADH	5ATP
Acetyl CoA Oxidation	6NADH 2$FADH_2$ 2GTP	20ATP
Total:		32ATP

So complete oxidation of glucose conserves: 32x30.5 = 976 kJ/mol or 34% of the 2840 kJ/mol available. Actual *G* calculations suggest that up to 65% of the *G* is conserved.

*Note that cytosolic NADH does not always generate 2.5 ATP.

Summary



Stoichiometry is for 2 electrons from NADH.

THE END!