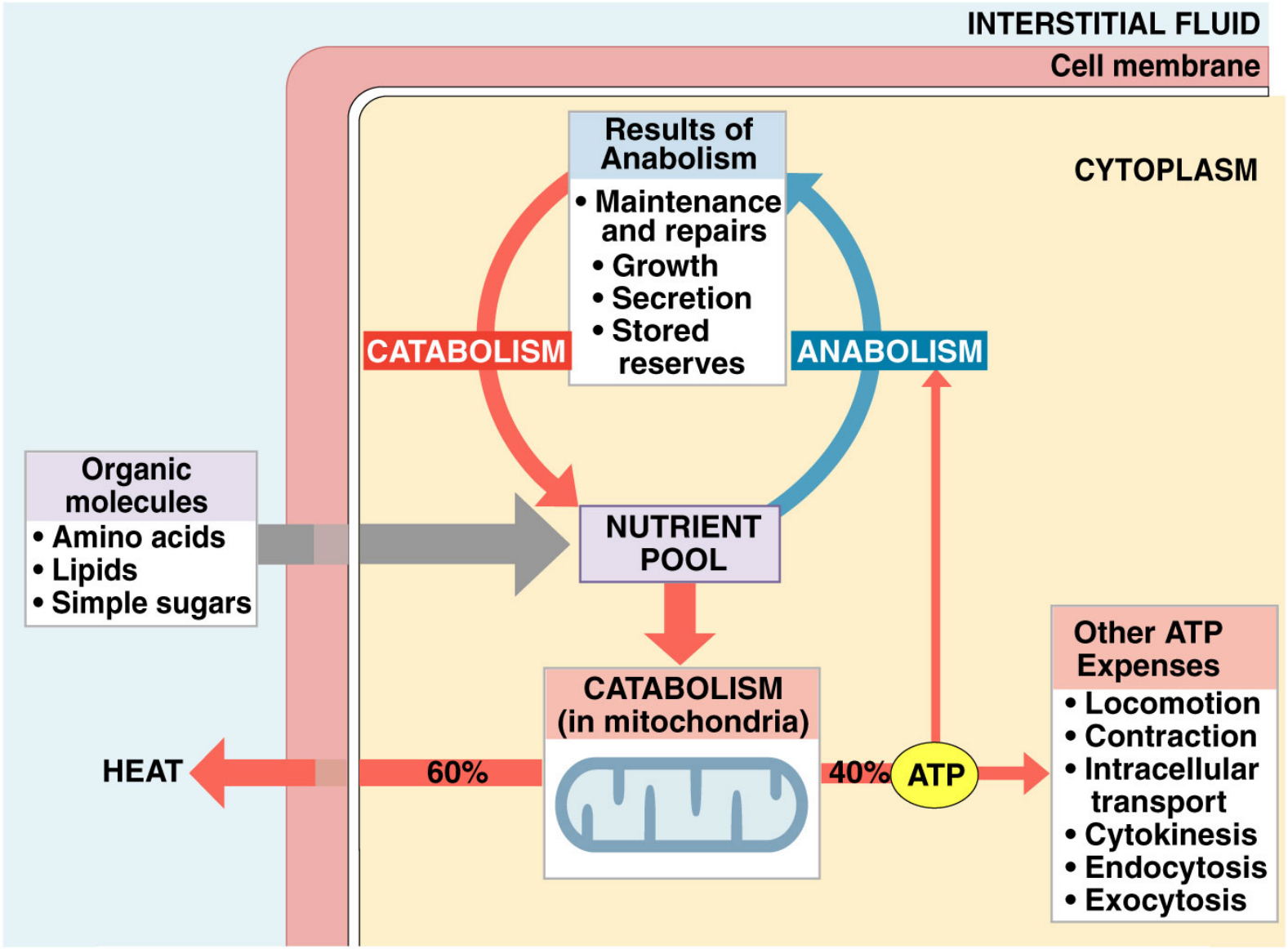
Metabolism and Energetics



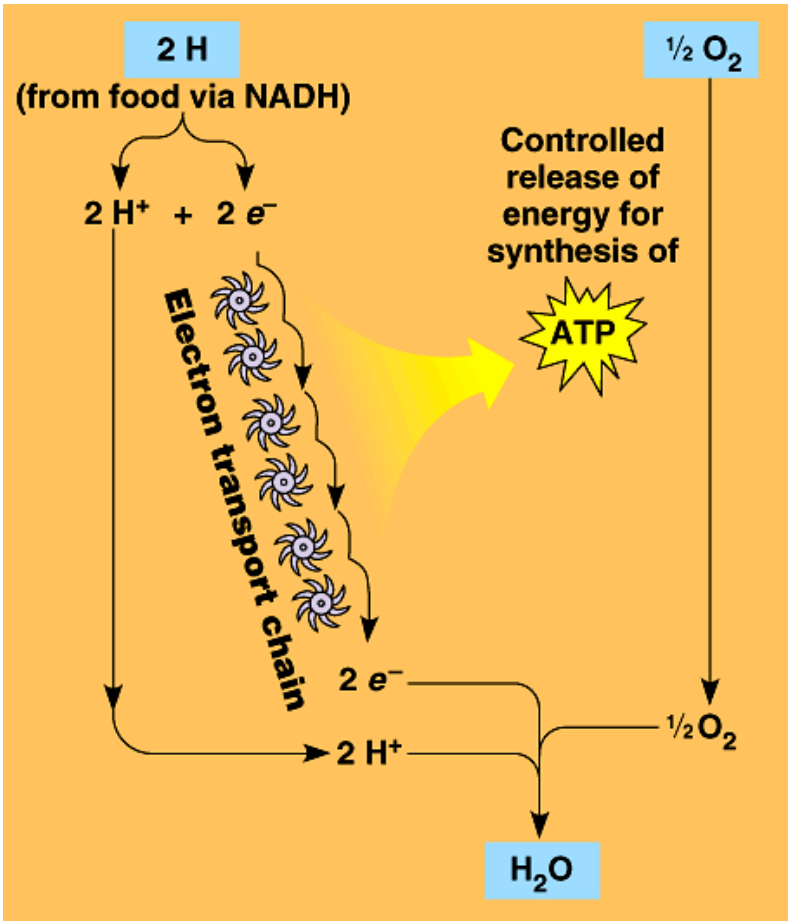
Oxidation of carbon atoms of glucose is the major source of energy in aerobic metabolism

$$C_6H_{12}0_6 + 6O_2 \text{ yields } 6\ CO_2 + H_20 + \text{energy}$$

Energy released

$$\Delta G = -\ 686\ kcal/mol$$

Glucose oxidation requires over 25 discrete steps, with production of 36 ATP.



Energy Transformations

*The mitochondrial synthesis of ATP is **not** stochiometric.*

Electron–motive force

Proton-motive force

Phosphoryl-transfer potential in the form of ATP.

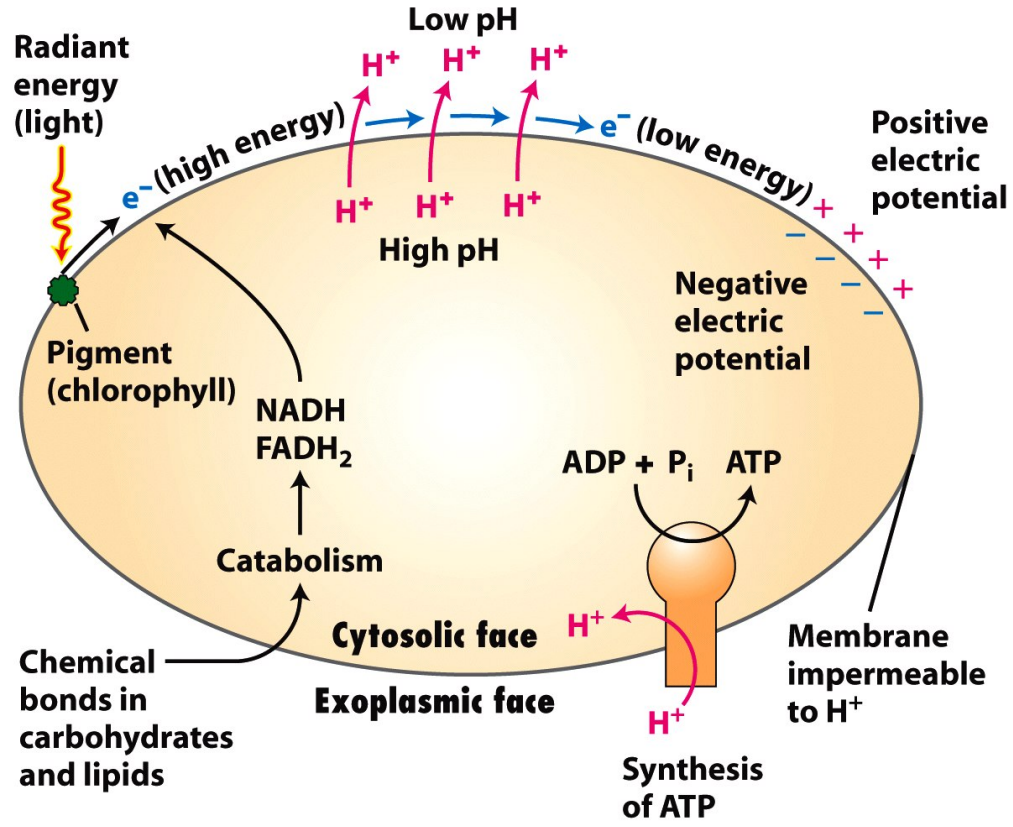
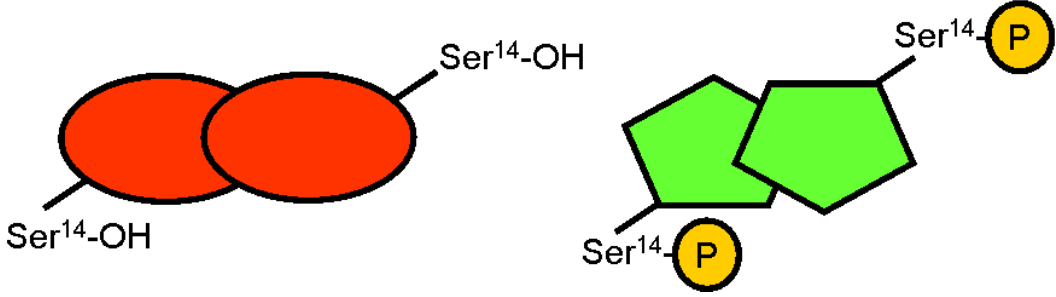
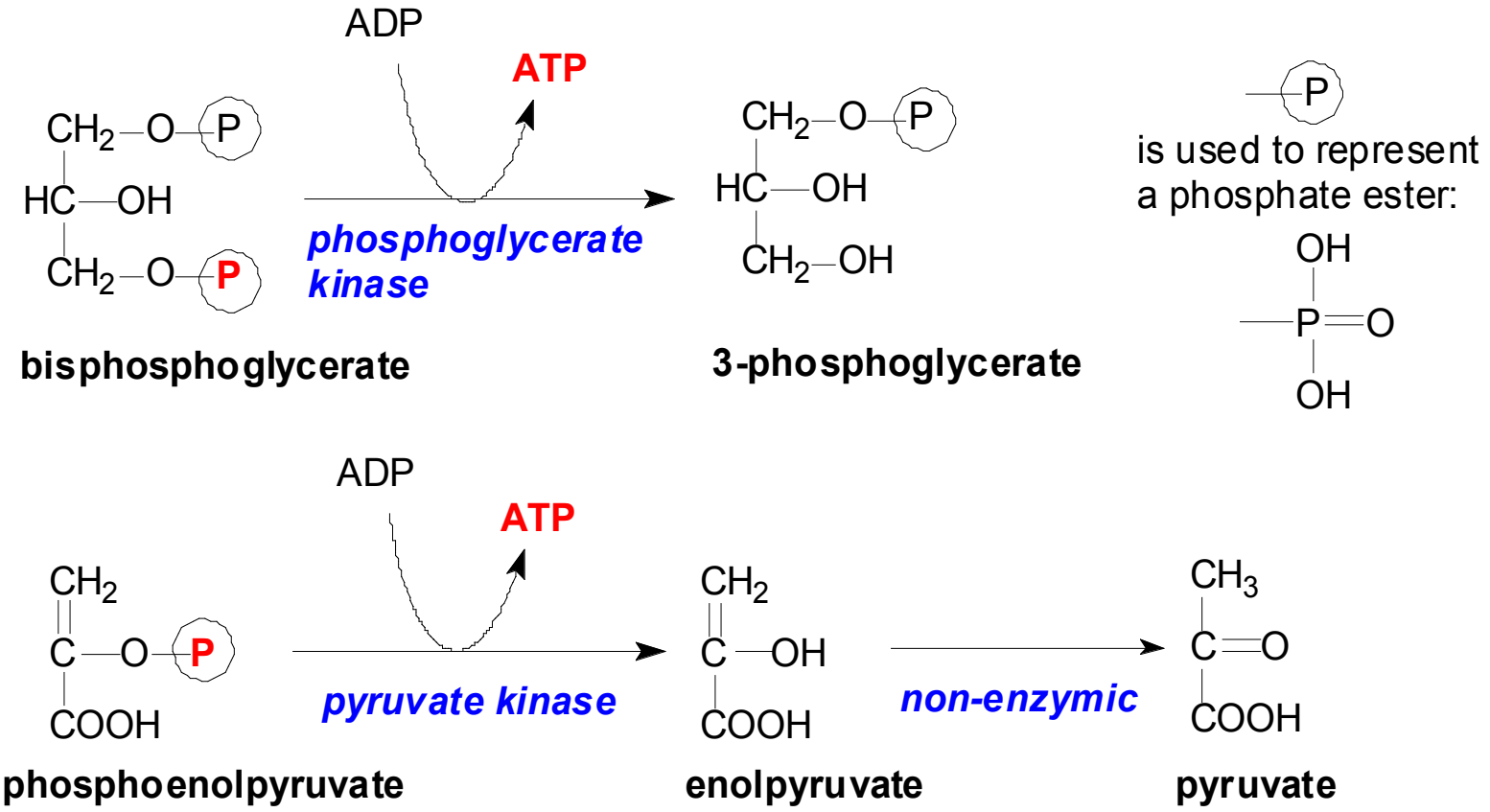


Figure 12-2
Molecular Cell Biology, Sixth Edition

Substrate level phosphorylation



The formation of ATP by substrate-level phosphorylation



Why ATP?

The reaction of ATP hydrolysis is very favorable

ΔG^o = - 30.5 kJ/mol = - 7.3 kCal/mol

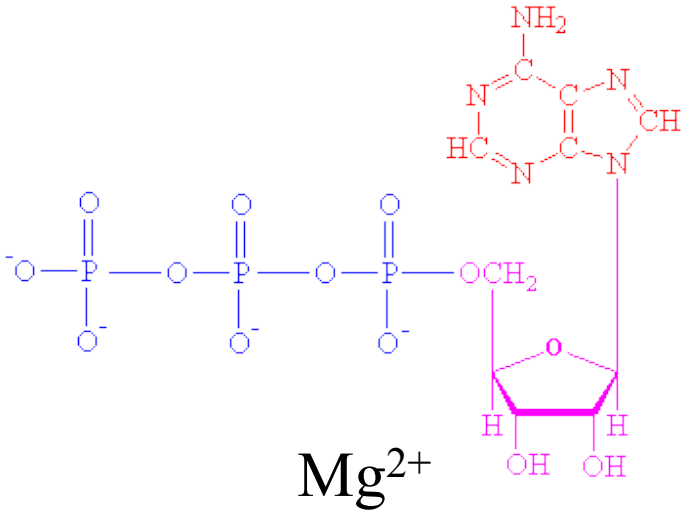
1. Charge separation of closely packed phosphate groups provides electrostatic relief.

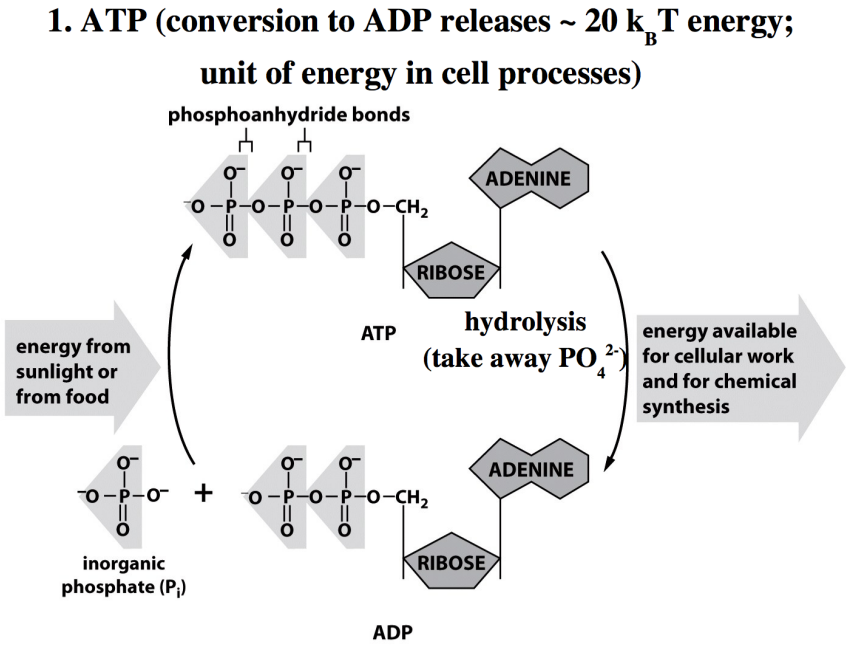
2. Inorganic P_i, the product of the reaction, is immediately resonance-stabilized (electron density spreads equally to all oxygens).

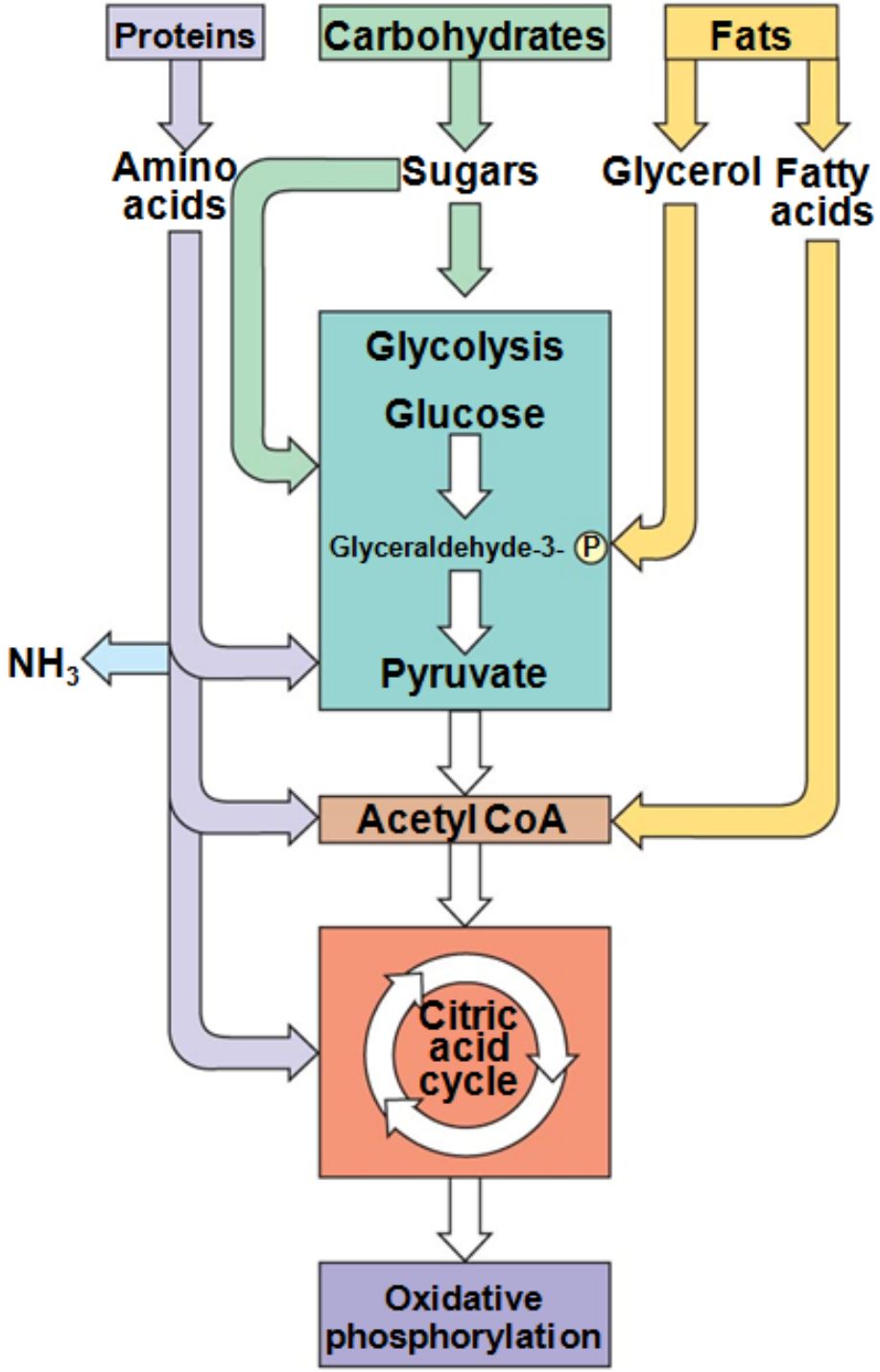
3. ADP immediately ionizes giving H^+ into a low $[H^+]$ environment (pH~7).

4. Both P_i and ADP are more favorably solvated by water than one ATP molecule.

5. ATP is water soluble.







The total body content of ATP and ADP is under 350 mmol – about 10 g,

BUT

… the amount of ATP synthesized and used each day is about 150 mol – about 110 kg.

Table 5.2 Biosynthetic cost in ATP equivalents to synthesize the macromolecules of a single *E. coli* cell.

Class	Biosynthetic cost (aerobic) – ATP equiv.
Protein	4.5×10^9
DNA	3.5×10^8
RNA	1.6×10^9
Phospholipid	3.2×10^9
Lipopolysaccharide	3.8×10^8
Peptidoglycan	1.7×10^8
Glycogen	3.1×10^7

ATP Production - stage 1 - Glycolysis

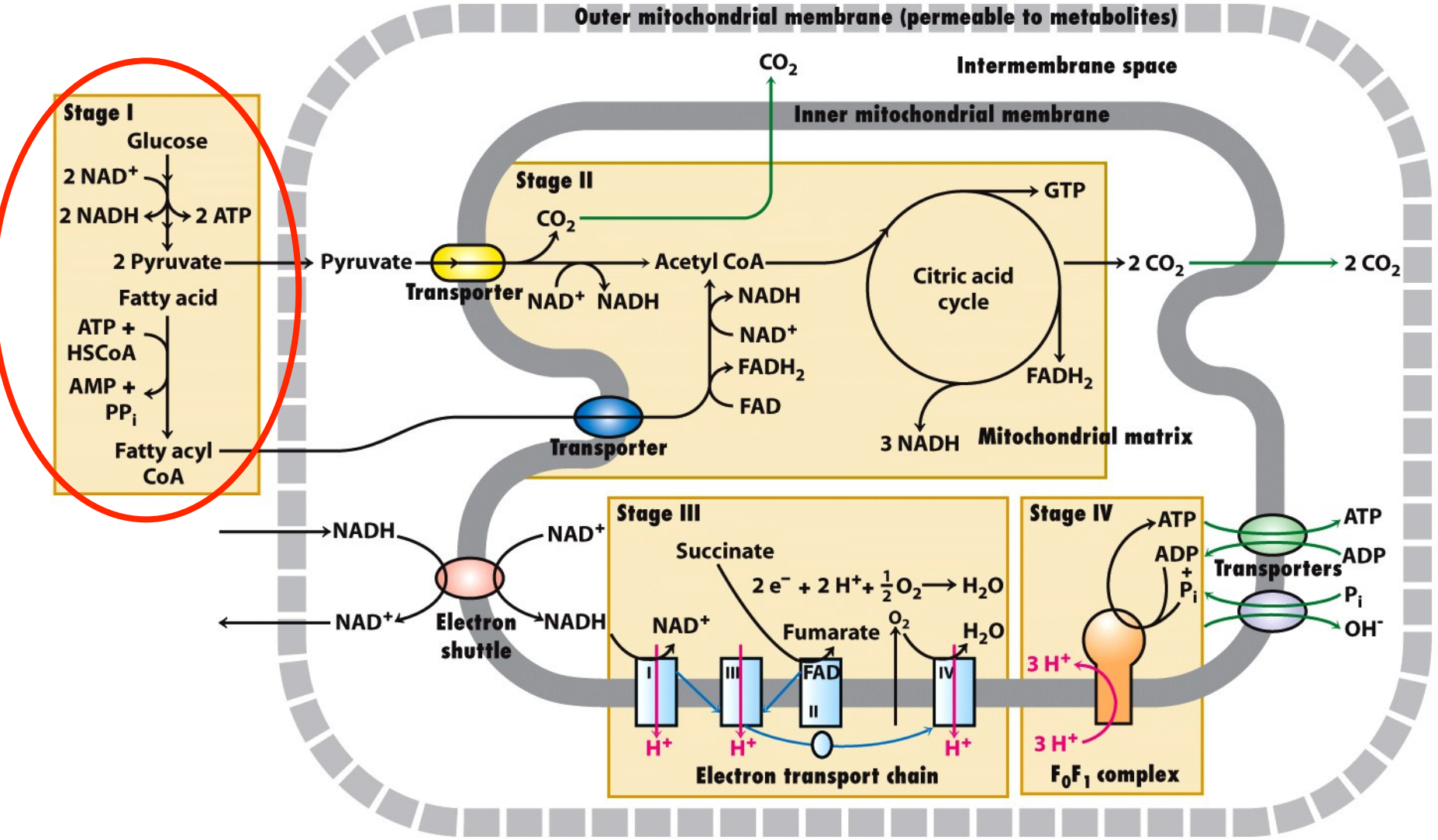


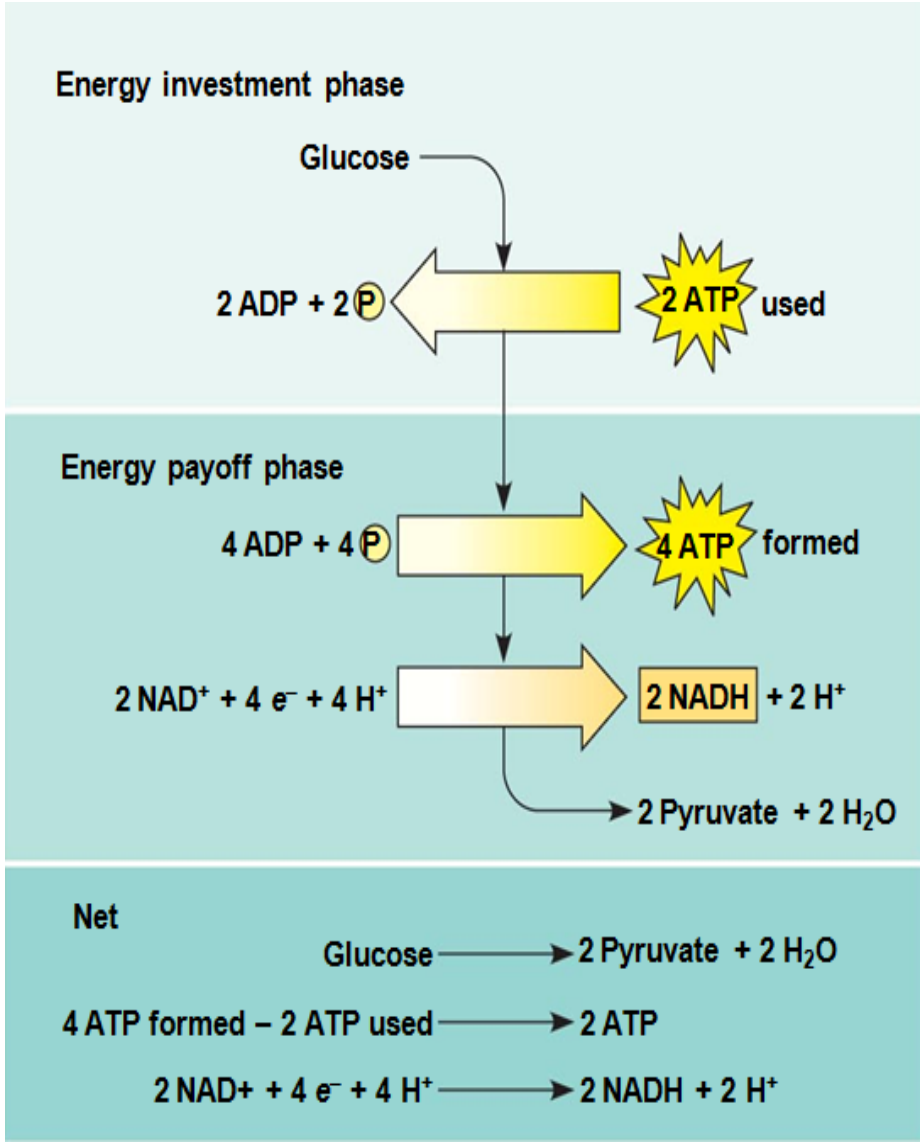
Figure 12-8
Molecular Cell Biology, Sixth Edition

Glycolysis

When rapid production of ATP is needed.

1 glucose + 2ADP + 2P_i + 2NAD ⇔ 2pyruvate + 2ATP + 2$NADH_2$

GLYCOLYSIS



The role of ATP in transport of materials across cell membranes:

metabolic trapping

Oxidative Phosphorylation

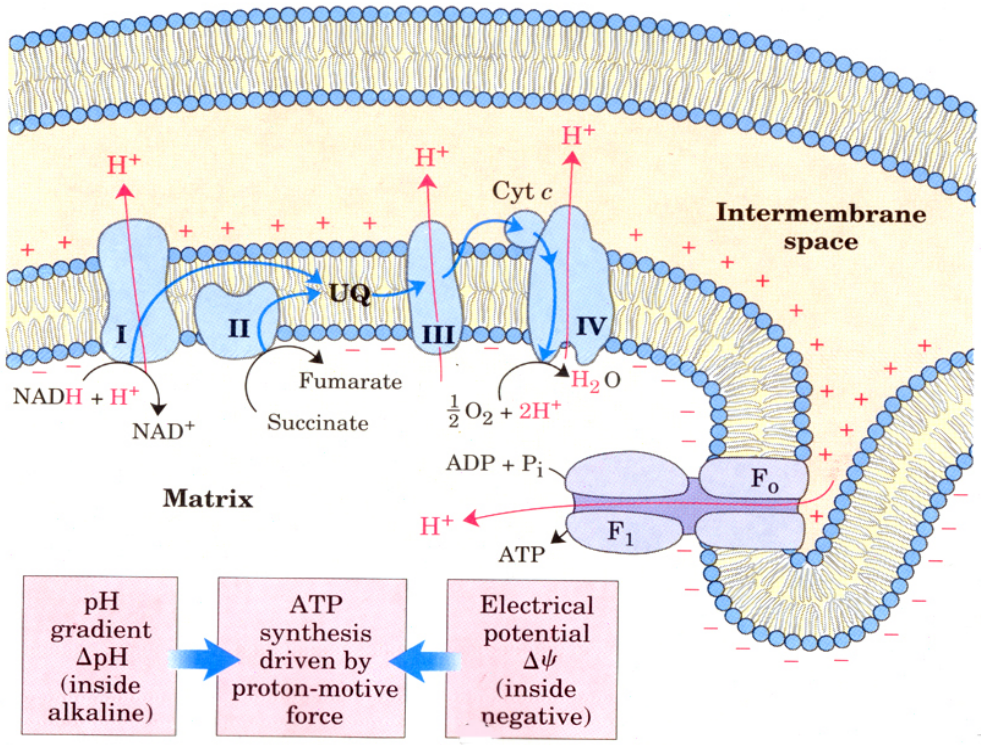
1) The flow of electrons through a chain of membrane-bound carriers

$$O_2 + 4H^+ + 4e^- + nH^+_{in} \rightarrow 2H_2O + nH^+_{out} + \Delta\varphi$$

*2) The free energy made available by the "downhill" **electron flow is coupled to** the "uphill" **transport of protons** across a proton-impermeable membrane.*

*3) The trans-membrane **flow of protons** down their concentration gradient through specific protein channels provides the free energy for synthesis of ATP.*

$$ADP + P_i + nH^+_i \rightarrow ATP + nH^+_o$$



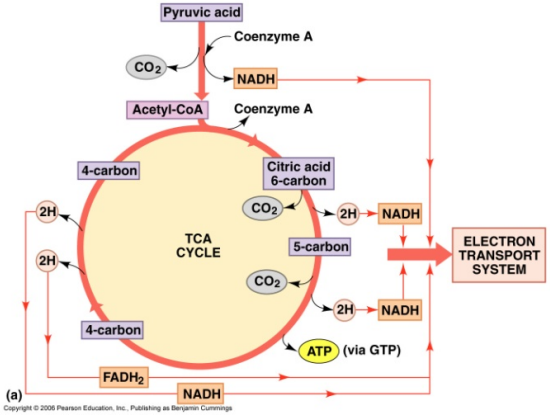
Reduction potentials are a measurement of electron affinity.

Compounds with a very high affinity for electrons are oxidants, e.g., O_2, and have a positive reduction potential ($E^{o'} > 0$).

Very strong reductants are compounds that readily give up electrons, e.g., NADH, and have a negative reduction potential ($E^{o'} < 0$).

Electrons flow from reductants to oxidants (electrons flow toward compounds with higher $E^{o'}$ values).

Redox reactions (oxidation-reduction) in the citrate cycle are a form of energy conversion involving the transfer of electron pairs from organic substrates to the carrier molecules NAD^+ or FAD.



Transfer of electrons

(redox reaction)

Reducing agent

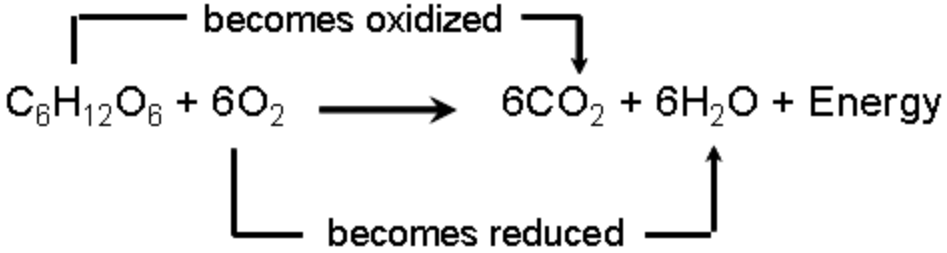
$$Xe^- + Y \longrightarrow X + Ye^-$$

Oxidizing agent

Electron ***acceptor*** (oxidizing agent, oxidant) is itself reduced – energy increase.

Electron ***donor*** (reducing agent, reductant) is itself oxidized – energy decrease.

During cellular respiration glucose is oxidized and oxygen is reduced



Redox potential - *empirical measure of tendency to gain e's measured in Volts*

$$E = E^{o'} - \frac{RT}{nF} \ln \frac{[A_{red}]}{[A_{ox}]}$$

if $[A_{red}] = [A_{ox}]$, $E = E^{\circ\prime}$

E°′ *is the standard redox potential, the potential at which* ***[oxidant] = [reductant]****.*

A more negative **$E^{o'}$** ***indicates a strong tendency to donate electrons, to reduce, and to become oxidized.***

For an electron transfer:

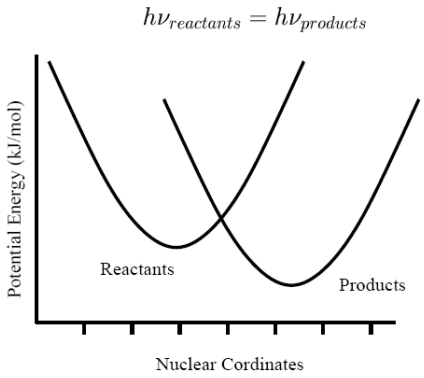
$$\Delta E^{o'} = E^{o'}_{(oxidant)} - E^{o'}_{(\text{reductant})} = E^{o'}_{(acceptor)} - E^{o'}_{(donor)}$$

$$\Delta G^{o'} = -nF\Delta E^{o'}$$ *n = # electrons transferred = 1,2,3*

An electron transfer reaction is ***spontaneous*** *(negative* ***ΔG****) if* ***ΔE°′*** *> 0.*

ΔE, unlike ΔG, is not a state function and depends on the path of the reaction.

Electron & proton movements



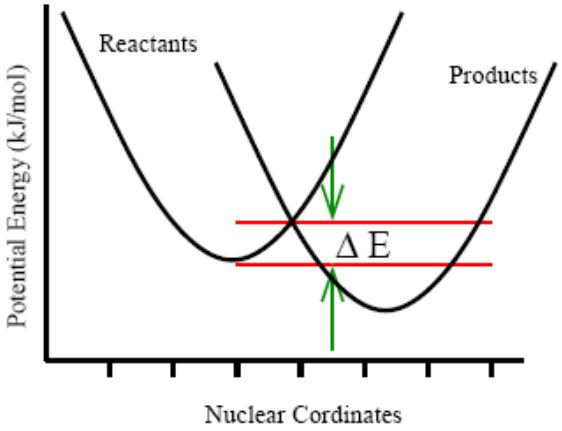
Electrons (protons) sit within potential energy wells.

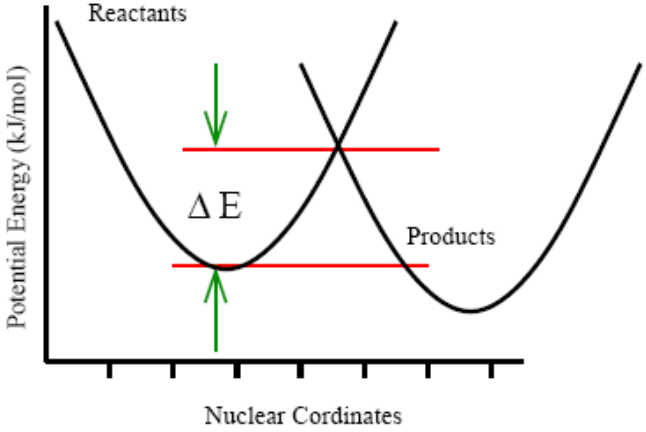
The electron/proton movement depends on:

- *the total potential energy change,*

  (they should match $h\nu_{reactants} = h\nu_{products}$)

- *centre-to-centre distance*

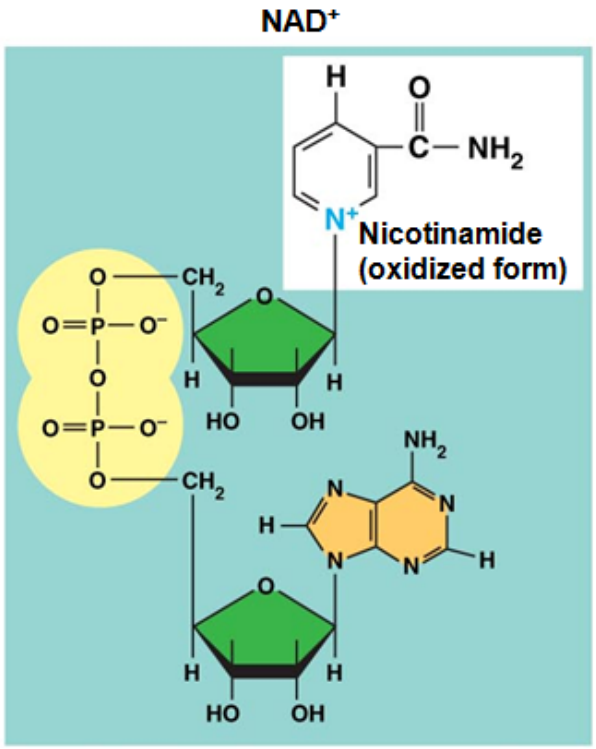


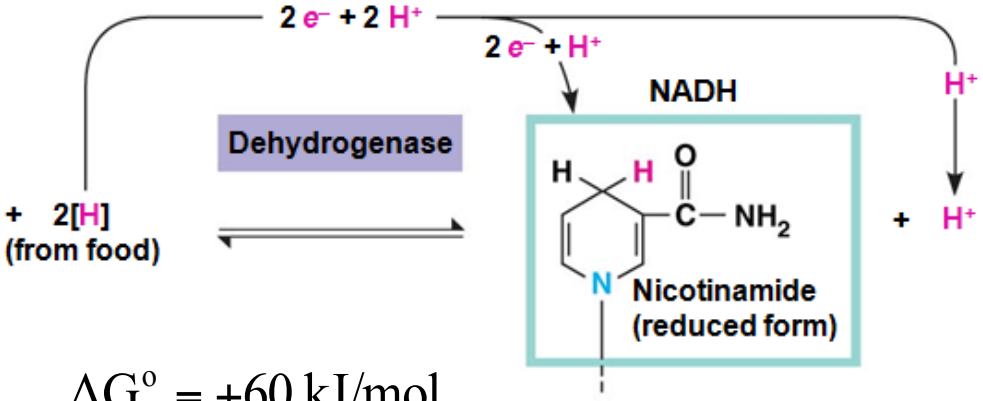


As distance increases energy barrier goes up!

- *an extent of electron wave-functions overlap is better at favored orientations H-bonds.*

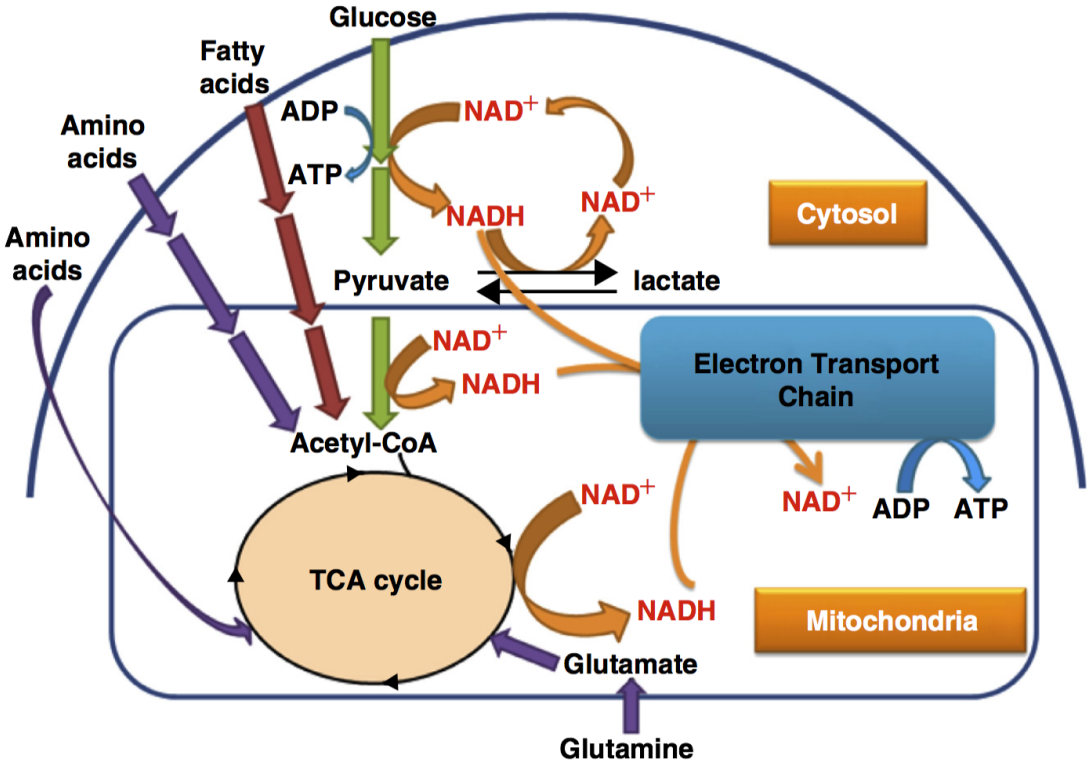
Electron carrier in aqueous phase

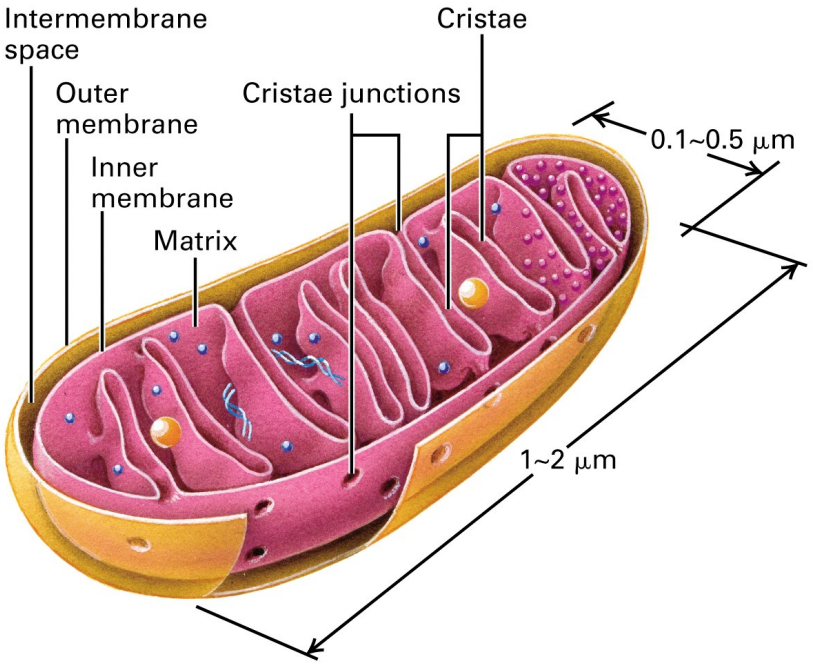


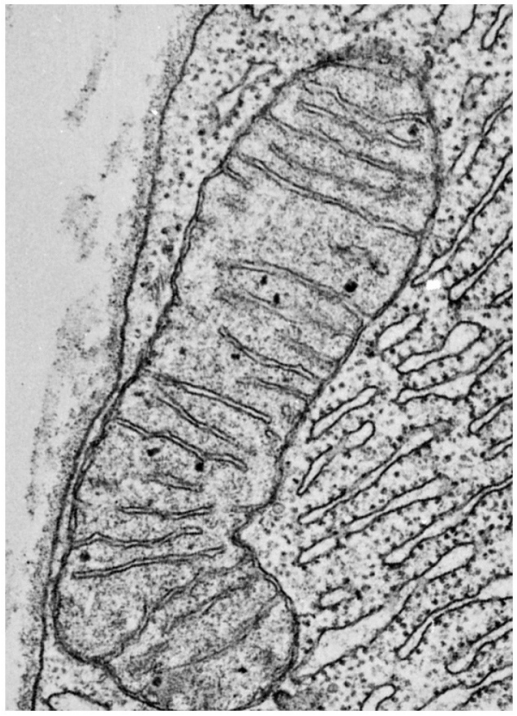


$\Delta G^{o} = +60\ \text{kJ/mol}$

NADH is never covalently bound



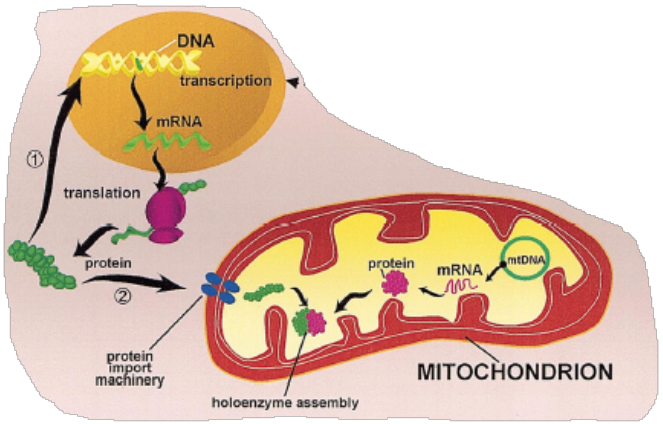




The infrastructure Mitochondria

mito-DNA... 16,500+ np's...

codes for 20% of mitochondrial proteins (13).



Functions

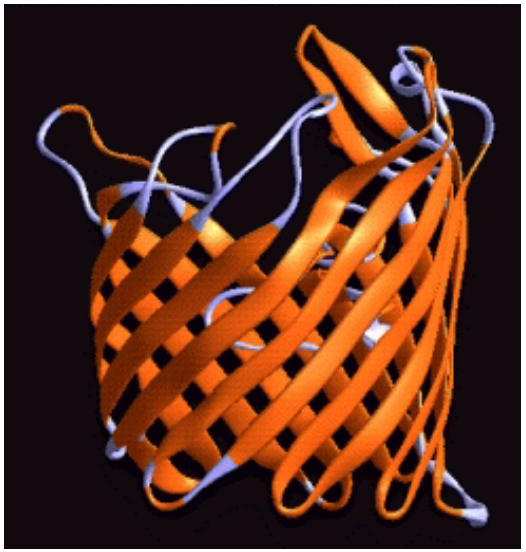
The primary sites for ATP synthesis.

A key role in <u>apoptosis</u> - programmed cell death.

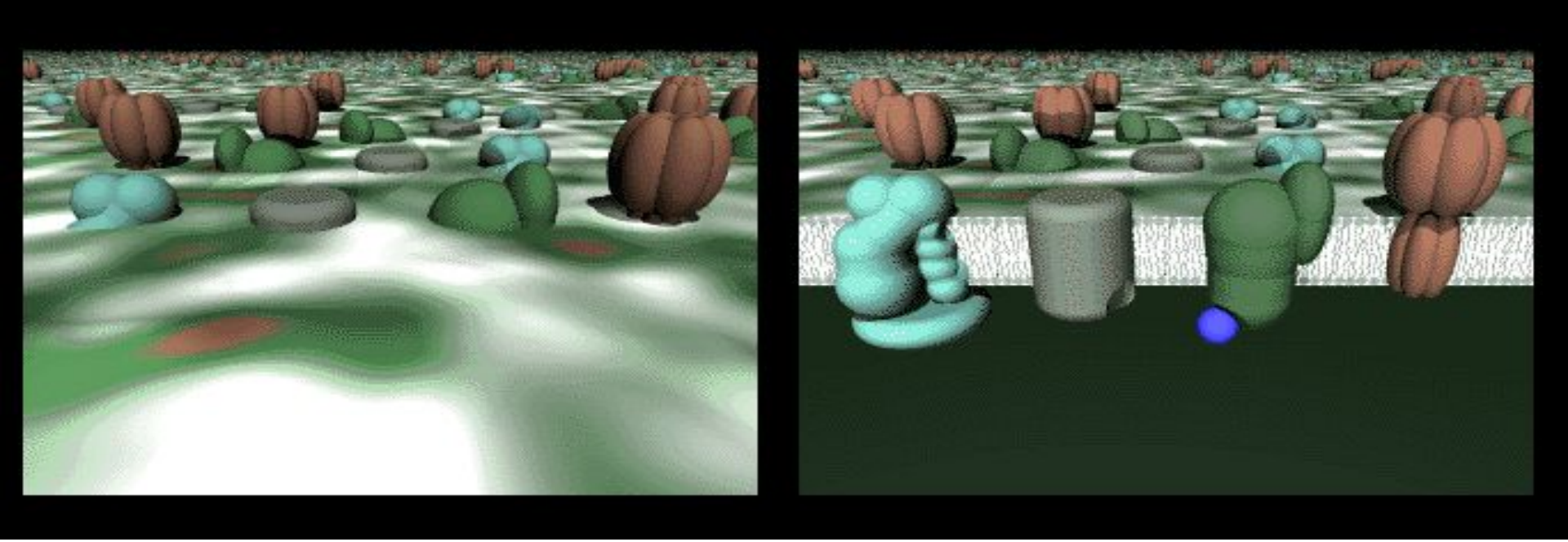
The outer membrane contains porins, anion channels which regulate metabolite flux, ie., phosphate, Cl^-, adenine nucleotides and organic anions.

- *porin proteins → large pores (5,000 -10,000 D)*

- *permeable to ions and molecules < 1 kDa*



There are no pH and potential gradients across the mitochondrial outer membrane.



The inner membrane is impermeable to ions and polar molecules. Specific transporters shuttle metabolites such as ATP, pyruvate, and citrate.

Functions in energy generation.

- ***electric device - capacitor*** – charge separation between the cytoplasm and the outside of the cell
- ***structural*** – the membrane holds many of the components involved in electron transport in the an exact conformation necessary to enable them to perform their tasks correctly

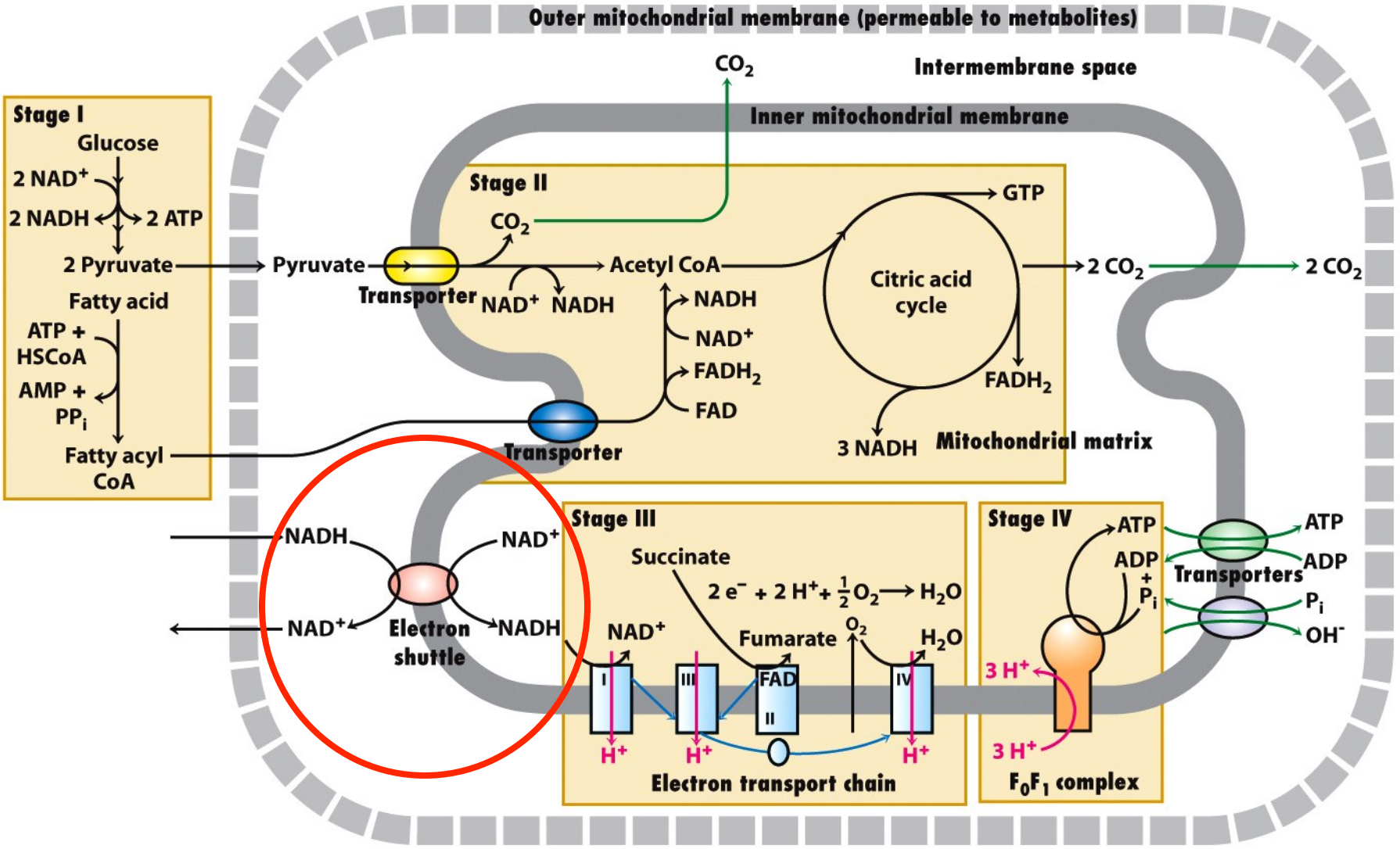


Figure 12-8
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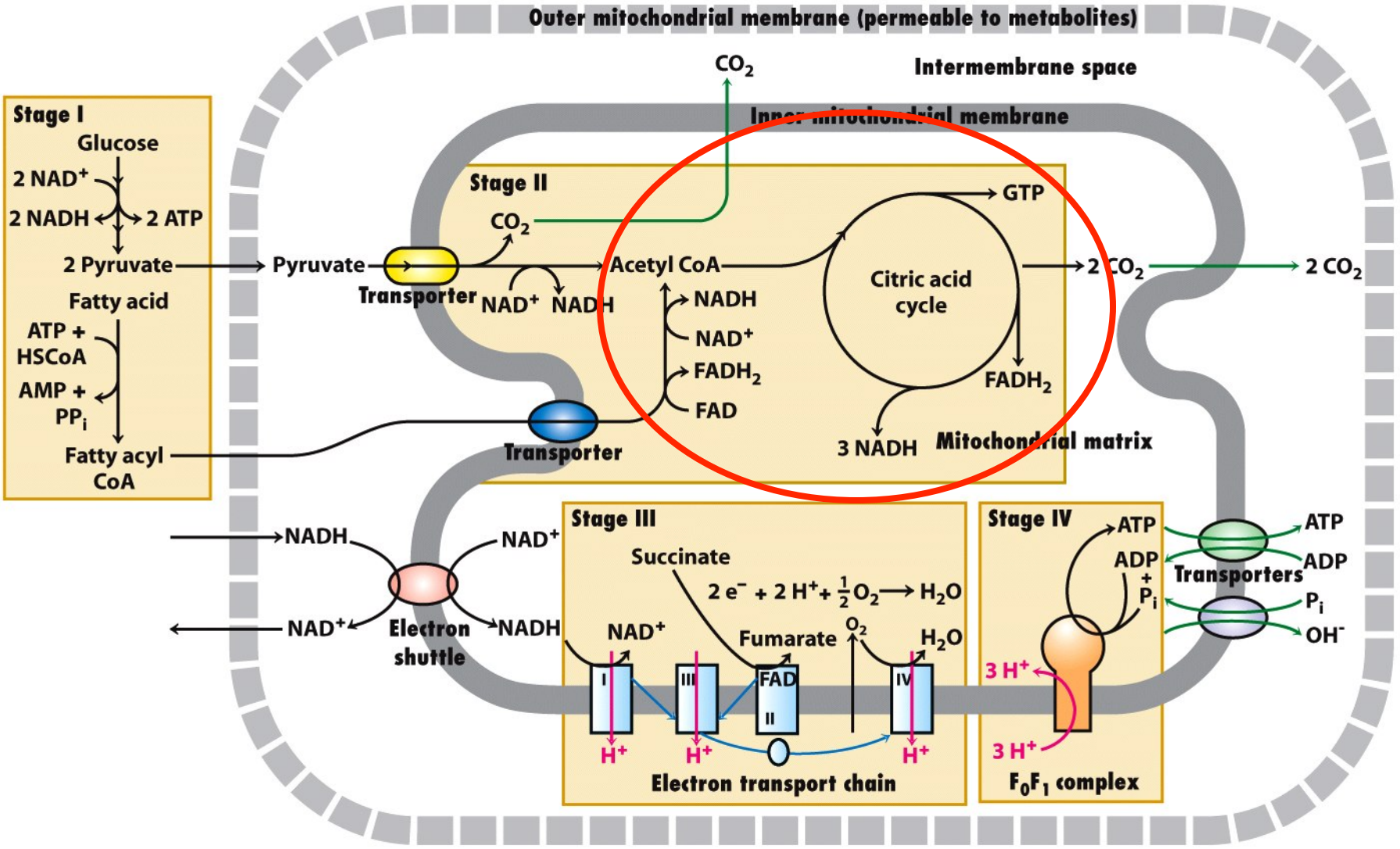


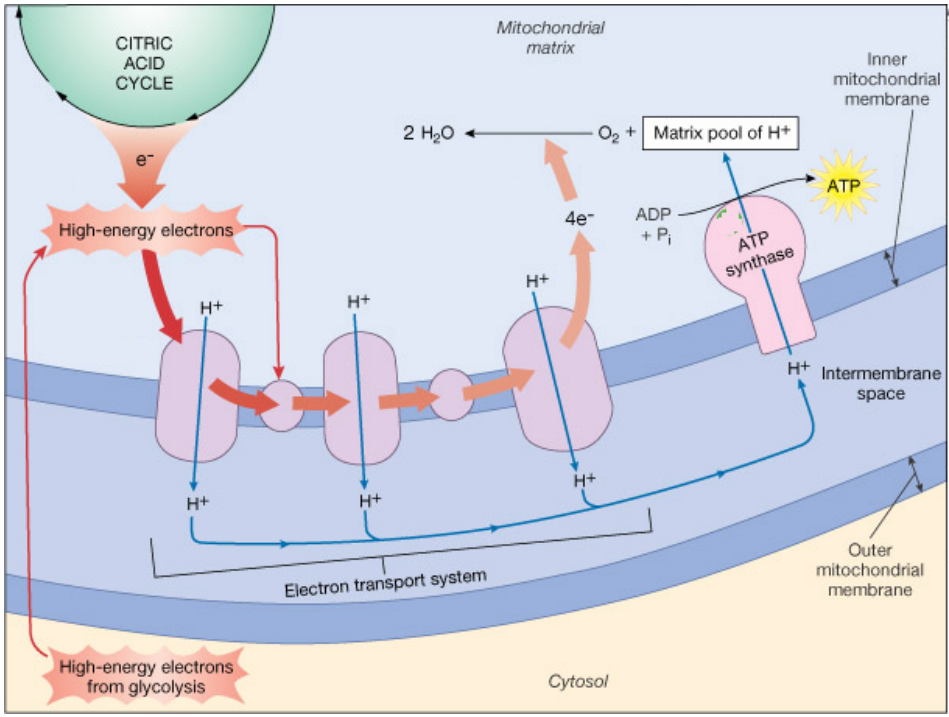
Figure 12-8
Molecular Cell Biology, Sixth Edition

Redox reactions in the citrate cycle involve the transfer of *e-p* pairs to generate NADH and $FADH_2$

The reduction of NAD^+ to NADH involves the transfer of a **hydride ion ($:H^-$)**, which contains 2 *e*- and 1 H^+, and the release of a **proton (H^+)** into solution

$$NAD^+ + 2\ e^- + 2\ H^+ \leftrightarrow NADH + H^+$$

FAD is reduced by *sequential addition* of one **hydrogen (1 *e*- and 1 H^+)** at a time to give the fully reduced $FADH_2$ product



$$FAD + 1\ e^- + 1\ H^+ \leftrightarrow FADH + 1\ e^- + H^+ \leftrightarrow FADH_2$$

The Electron Transport System (ETS) is a series of electron carriers

As the electrons pass through the chain of reactions release small amounts of energy in a series of steps as a result a proton gradient is generated.

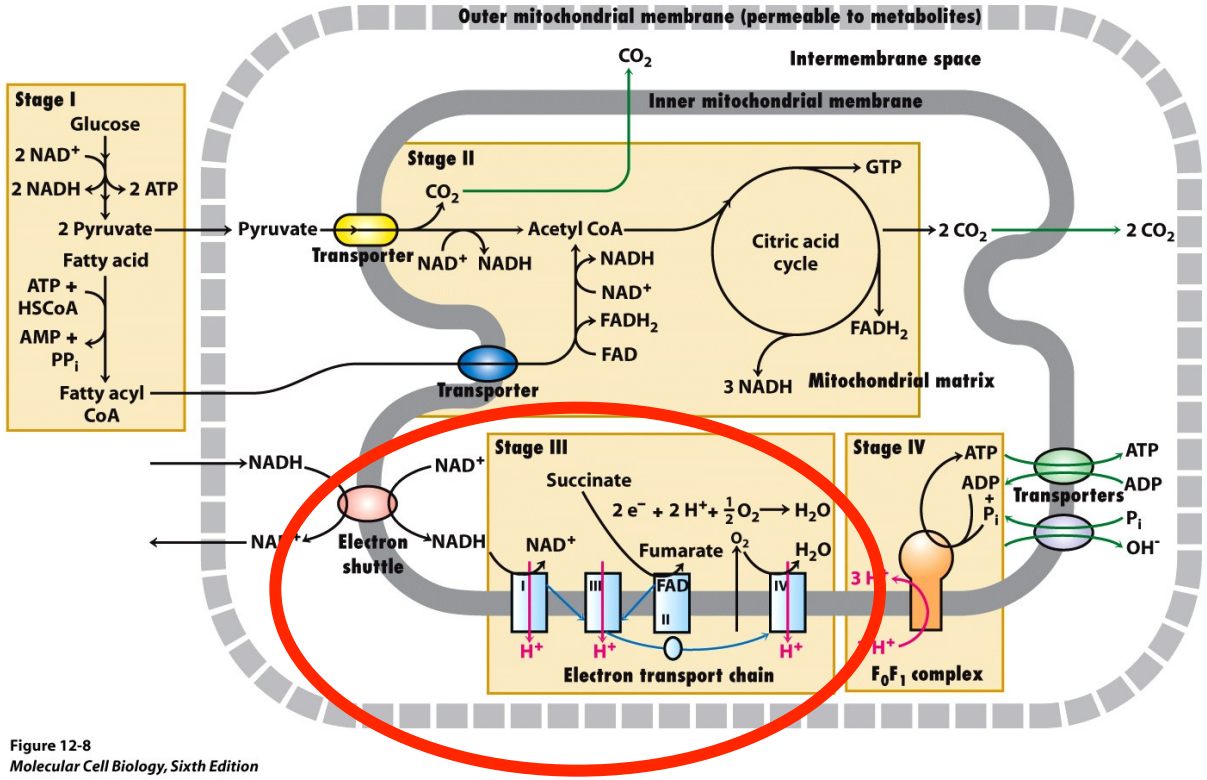


Figure 12-8
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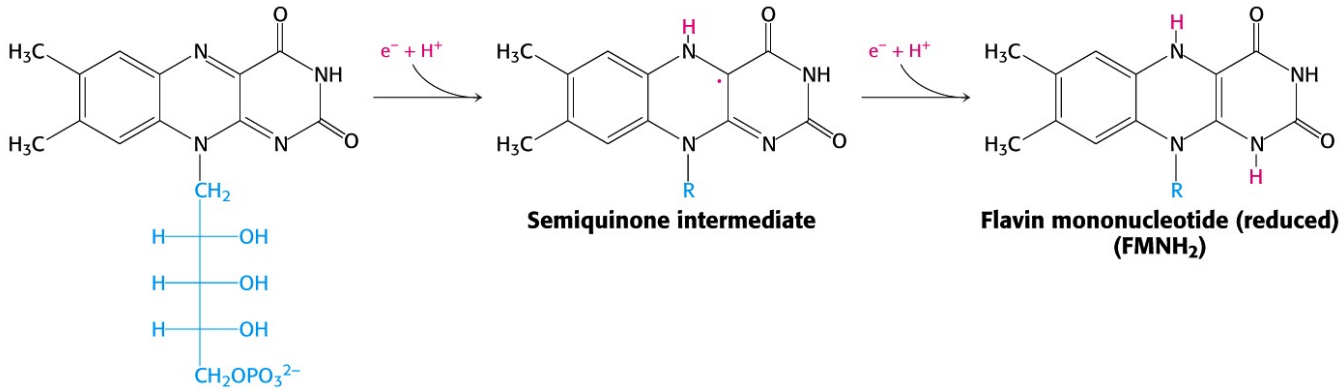
Why protons ?

*P*rotons because one single **Histidine**, **Glutamate** or **Aspartate** residue furnish a simple and tunable binding site for H^+.

pKa of these groups can vary by 2-3 units depending on the environment. These sites do not bind metal ions tightly unless work in concert.

It is much more difficult to build a selective binding site that would discriminate between Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Hg^{2+}, Fe^{2+}, Fe^{3+}, etc .

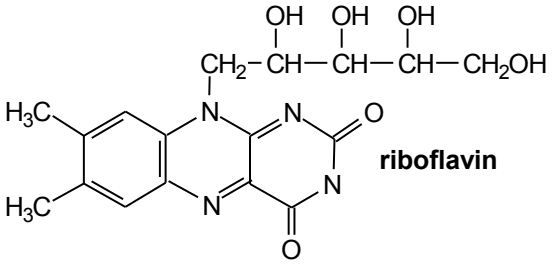
The proton delivery is highly efficient



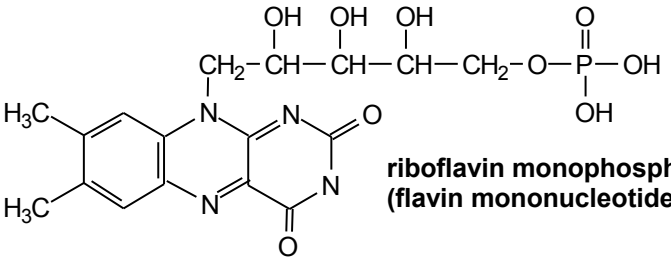
Flavin mononucleotide (oxidized)
(FMN)

Semiquinone intermediate

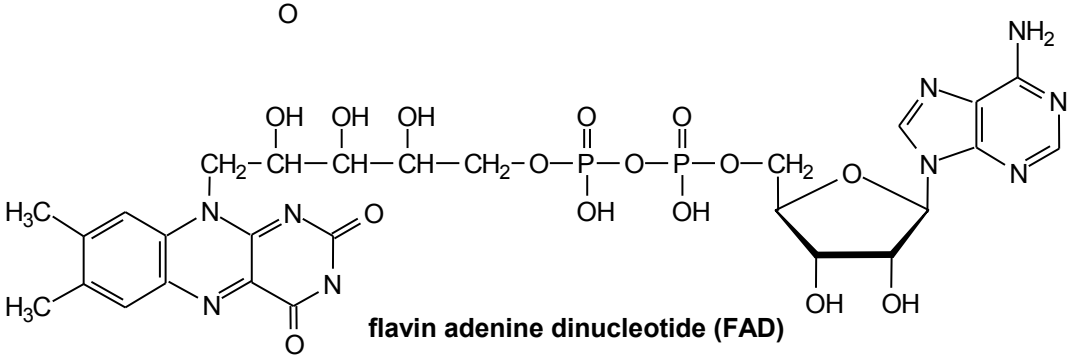
Flavin mononucleotide (reduced)
($FMNH_2$)



riboflavin



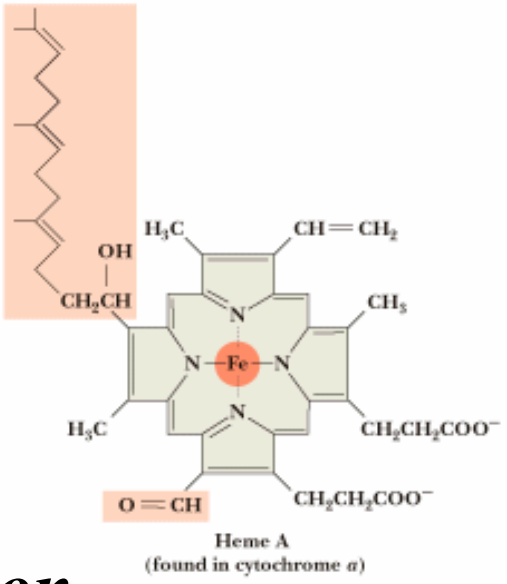
riboflavin monophosphate
(flavin mononucleotide, FMN)



flavin adenine dinucleotide (FAD)

Protein bound – interphase located electron carrier.

Intra-protein electron curriers.

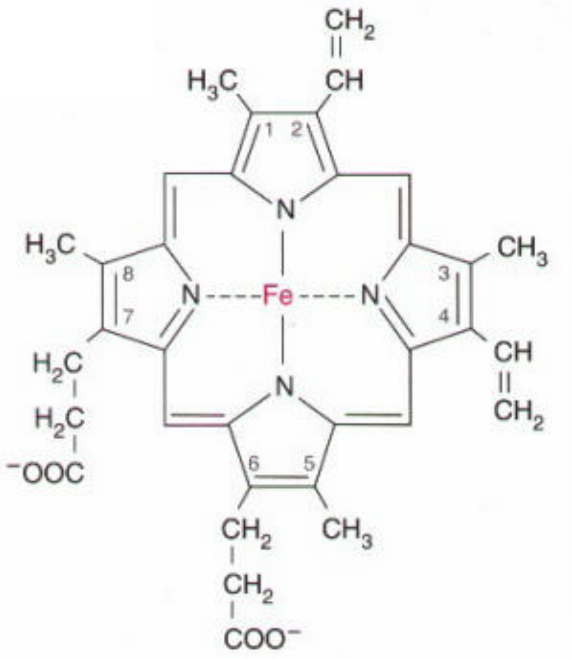


Heme A
(found in cytochrome *a*)

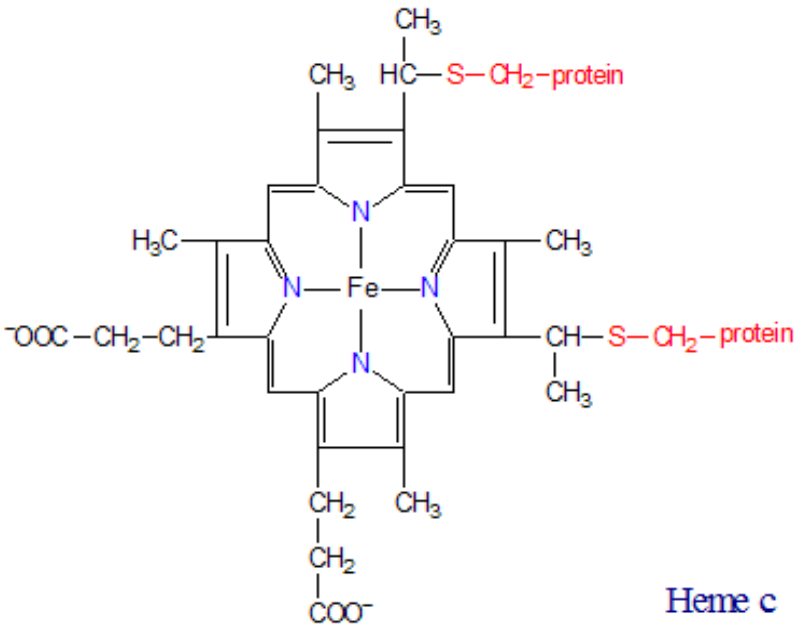
Cytochromes – *are based on porphyrins with iron in center, as Fe(II) tightly bound at sides.*

The Fe^{+3} -cytochrome accepts only one electron.

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



Cytochrome *b* heme group



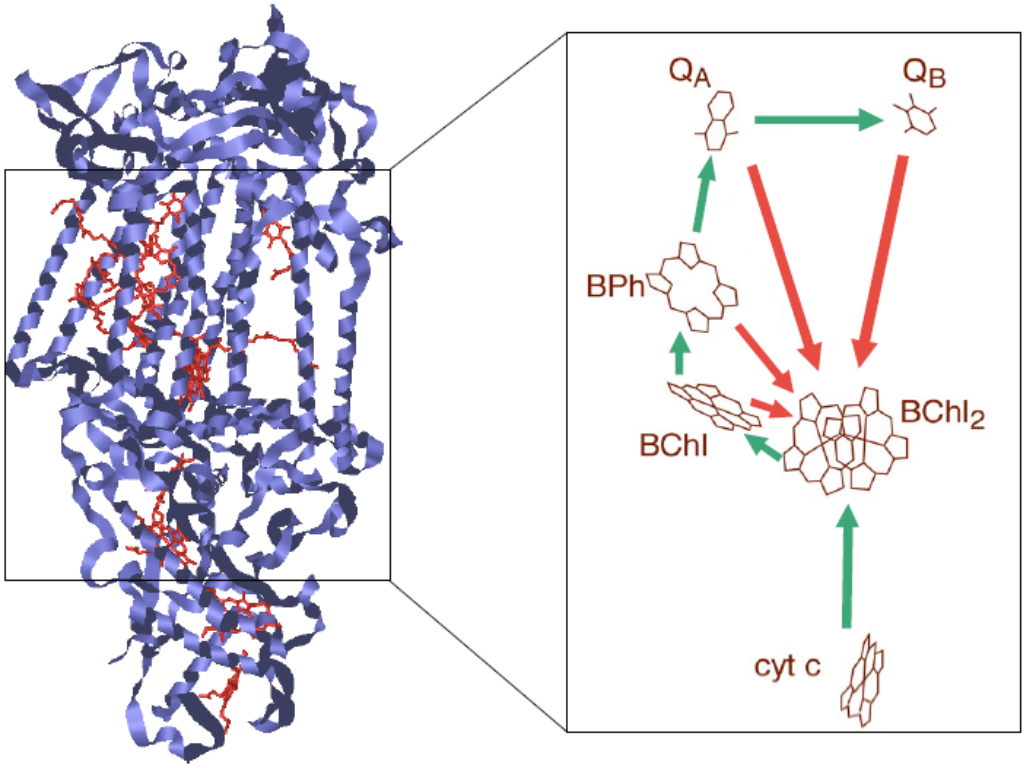
Heme c

Chain of electron carriers within the protein

Rps. viridis reaction center C subunit - heme chain

Electron transfer by tunneling is intrinsically very fast at short distances.

Nature uses distance as the primary design criterion - short for **good**, long for **bad**.

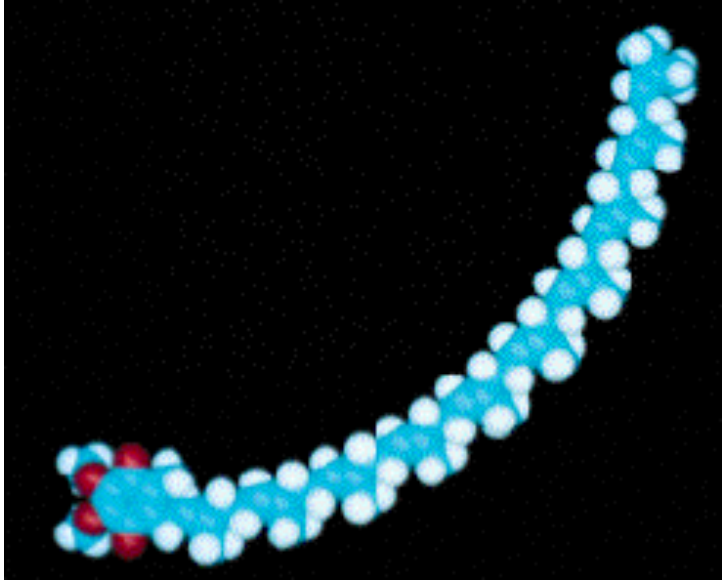


→ Physiologically productive, beneficial, good

→ Physiologically unproductive, deleterious, bad

Natural proteins act as scaffolds to hang cofactors at a distance R ≤ 14Å, sufficiently short that ET is not rate-limiting.

Hydrophobic electron carrier

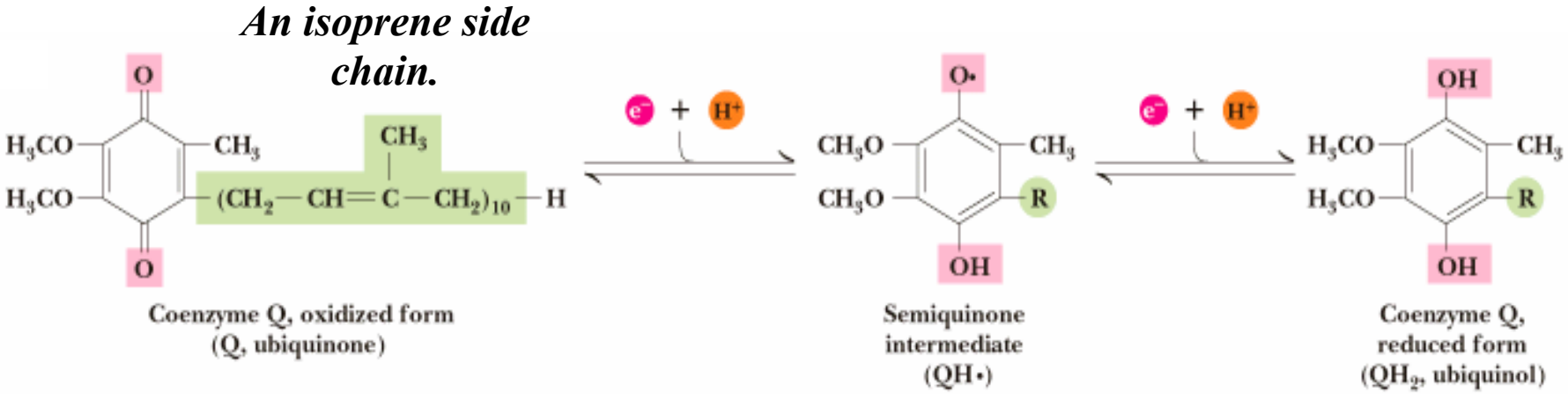


Coenzyme Q = Ubiquinone

- accepts 2 protons and 2 electrons
- is hydrophobic

$$CoQH_2 + 2Fe^{+3}\text{-complex} \rightarrow CoQ + 2Fe^{+2}\text{-complex}$$

Oxidation States of Coenzyme Q

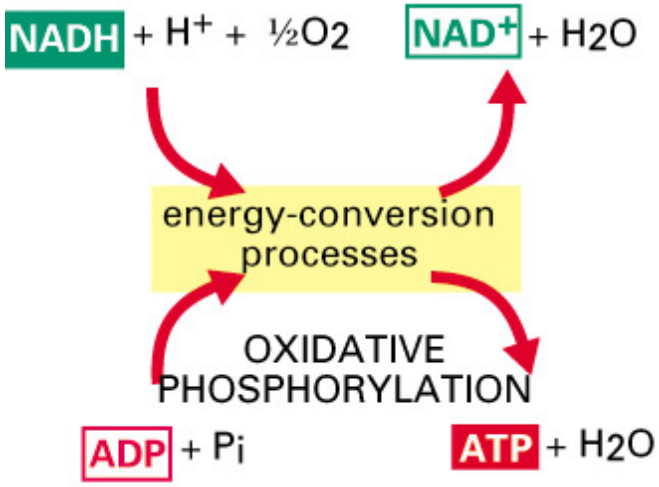


Peter Mitchell

Nobel Prize in Chemistry, 1978

"For his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory"

Chemiosmotic theory



Oxidation and phosphorylation are coupled through a ***proton – motive force***.

$$\Delta p = \frac{\Delta\tilde{\mu}_{H^+}}{F} = \Delta\Psi - \frac{2.3RT}{F}\Delta pH$$

One of the great unifying principles of 20th century biology.

Proteins in electron transfer

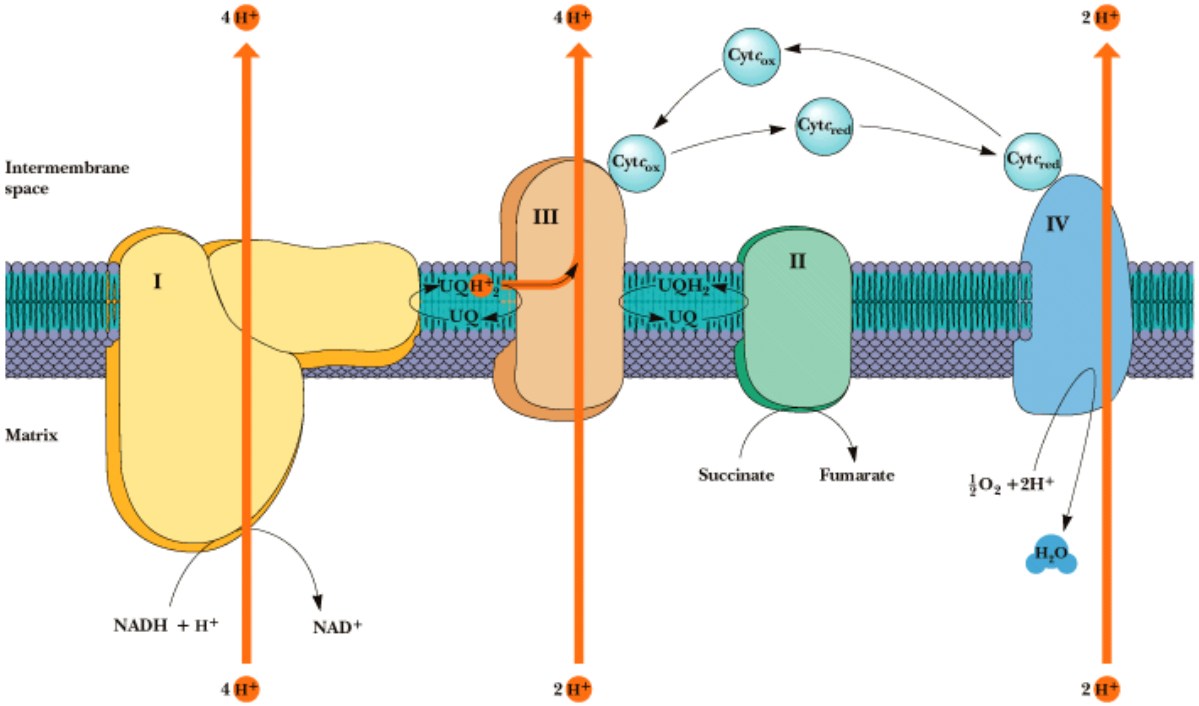
Membrane proteins

Complex I - Transfers e^- from NADH to quinone pool & pumps H^+.

Complex II - Transfers e^- from succinate to quinone pool.

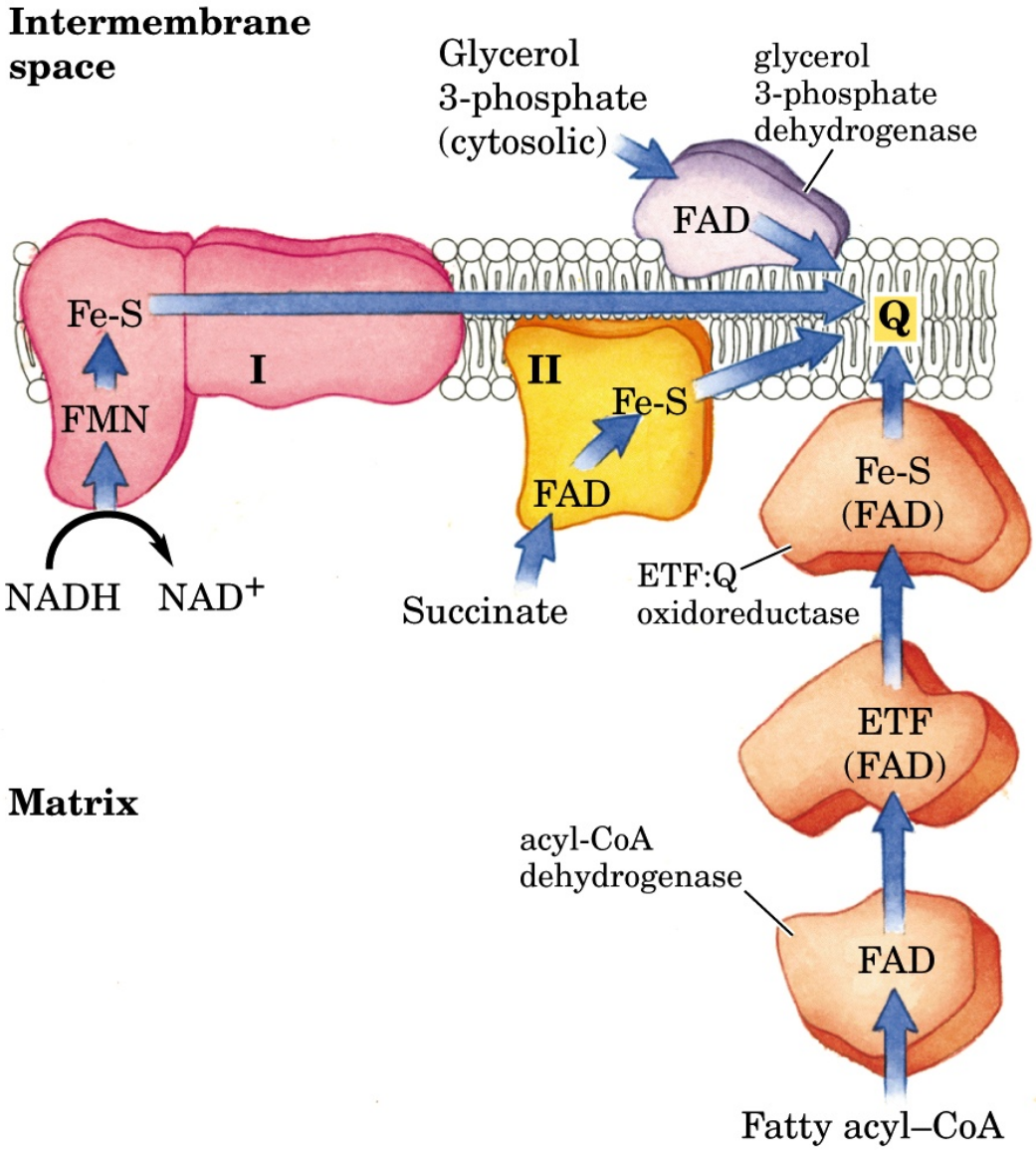
Complex III - Transfers e^- from quinol to cyt. c & pumps H^+.

Complex IV - Accepts e^- from cyt. c, reduces O_2 to H_2O & pumps H^+.

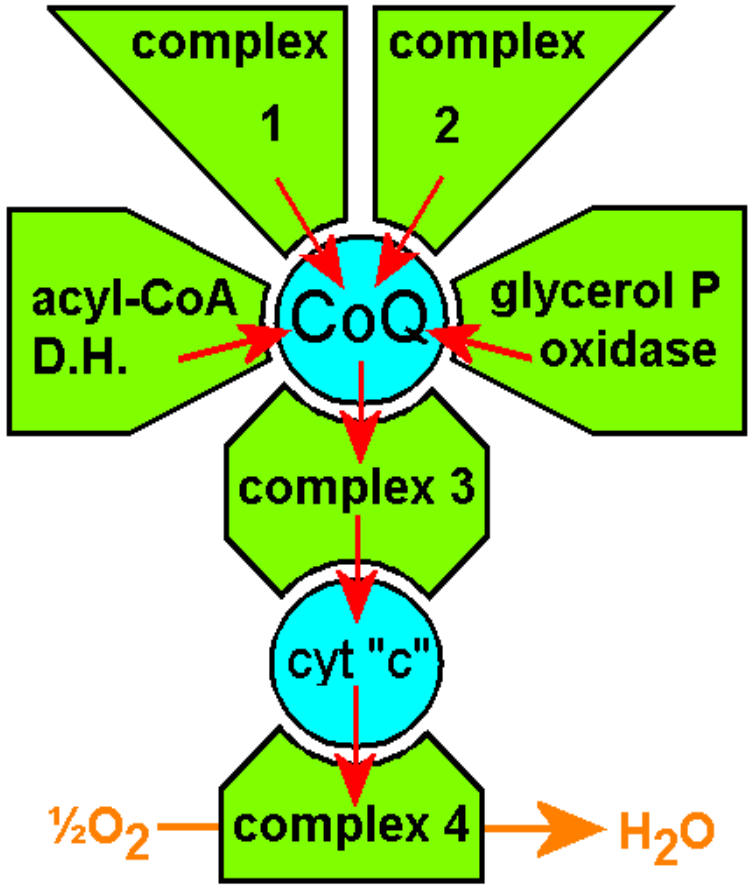


Cytochrome c is small, highly mobile hydrophilic electron carrier.

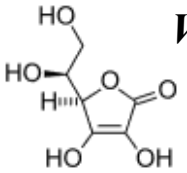
Several pathways feed electrons directly or indirectly into ubiquinone.



Functioning of the system



Partial reduction of oxygen generates dangerous molecules such as superoxide radicals and hydrogen peroxide which are highly toxic to cells.

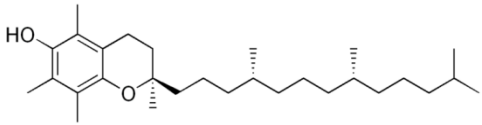


Vitamin C

Pathological conditions that may entail free-radical injury:

- *Parkinson disease*
- *Cervical cancer*
- *Diabetes*
- *Down syndrome*
- *Cerebrovascular disorders*

Vitamins E and C are antioxidant.
Vitamin C is hydrophilic.
Vitamin E is lipophilic.



Vitamin E

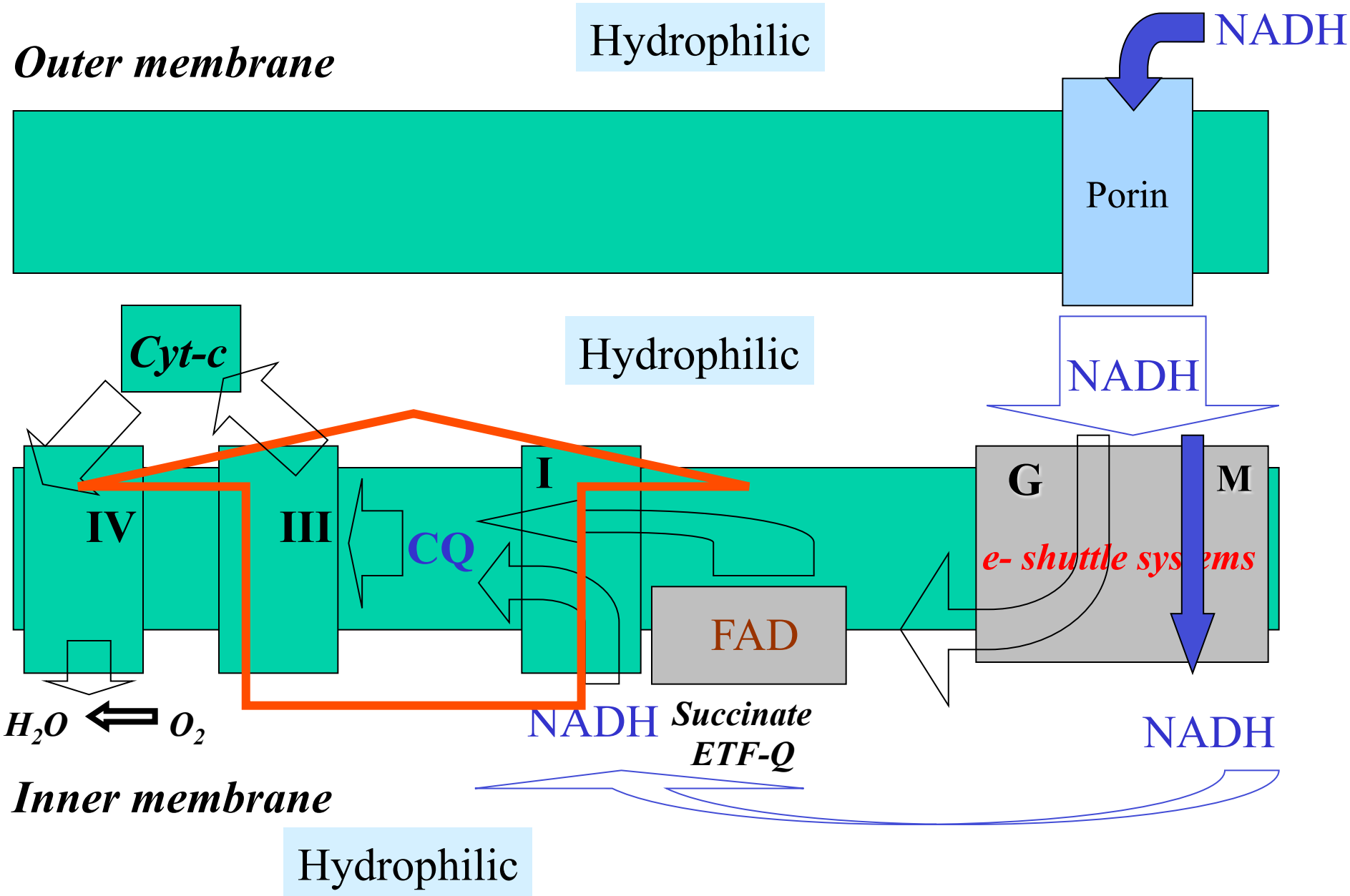
All aerobic organisms have superoxide dismutase to rid themselves of the damaging free radical type molecules.

Superoxide dismutase catalyzes the reaction:

$$2O_2^{\cdot -} + 2H^+ \leftrightarrows O_2 + H_2O_2$$ *peroxide*

Catalase converts: $2H_2O_2 \leftrightarrows O_2 + 2H_2O$

Electrons flow



Free energy of protons

$$\Delta G = RT \ln \frac{[H^+]_{in}}{[H^+]_{out}} + F\Delta\Psi_{in-out}$$

CHEMICAL - ΔpH (0.5) = 60 mV– 40%

ELECTRICAL - membrane potential ΔΨ (140mV) – 60%

Total proton motive force = 200 mV

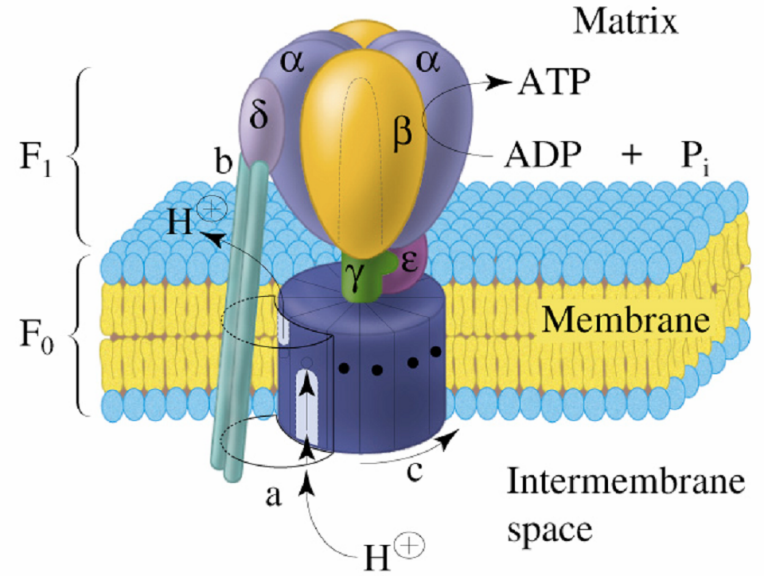
The voltage gradient is about 30 x 10^6 volts/m.

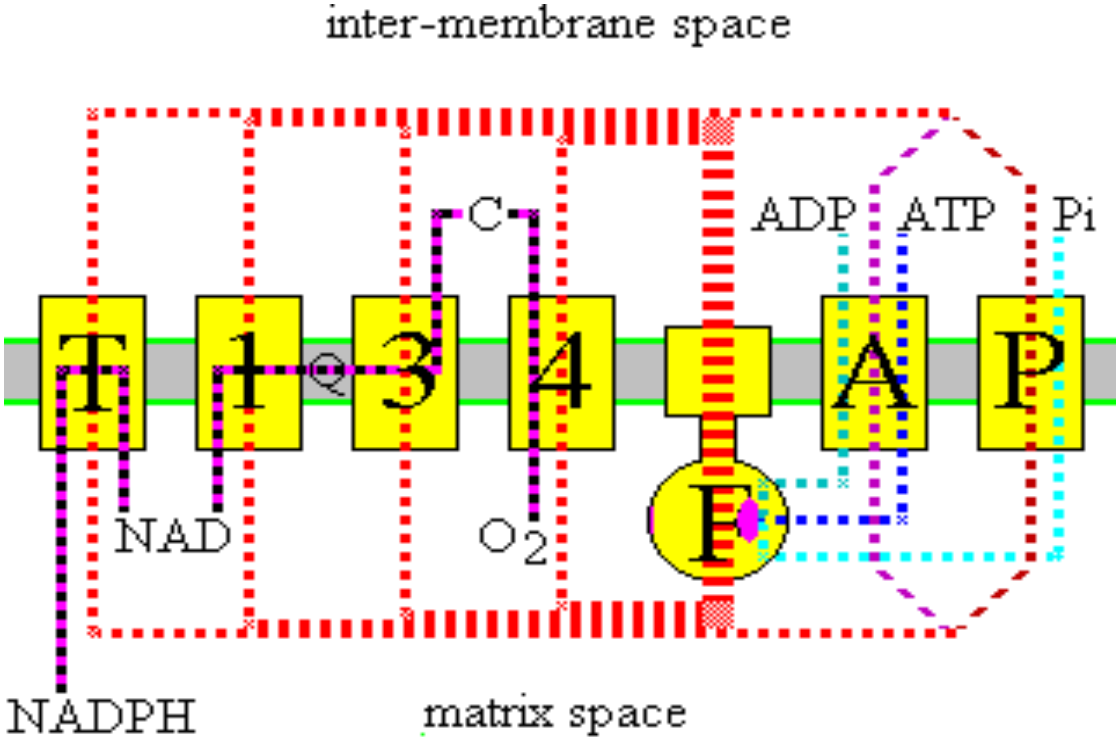
ATP synthesis

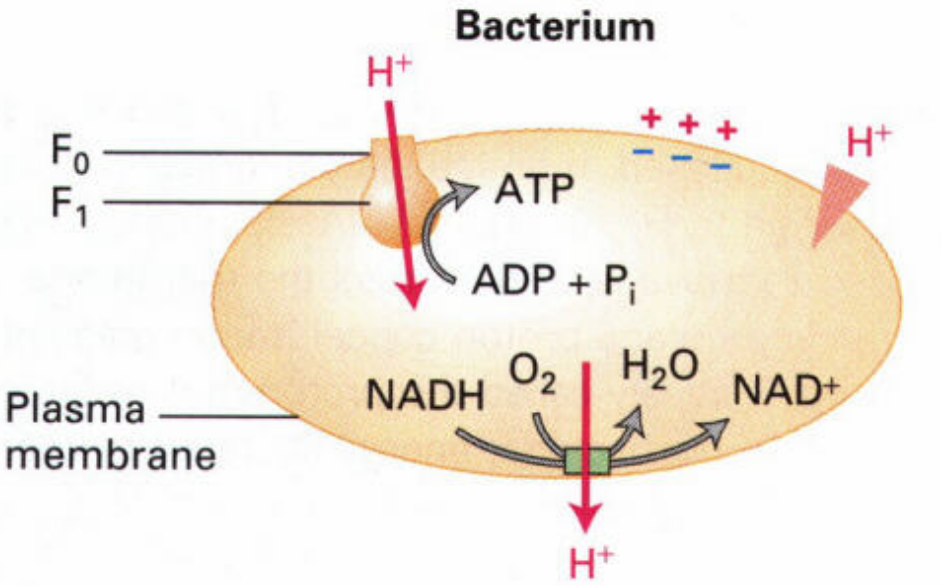
Synthesis of ATP from ADP and orthophosphate is coupled to a proton flux.

$$ADP + P_i + nH_p^+ \rightarrow ATP + H_2O + nH_n^+$$

ATP Synthase - makes 100 ATP per 300 H^+ per sec

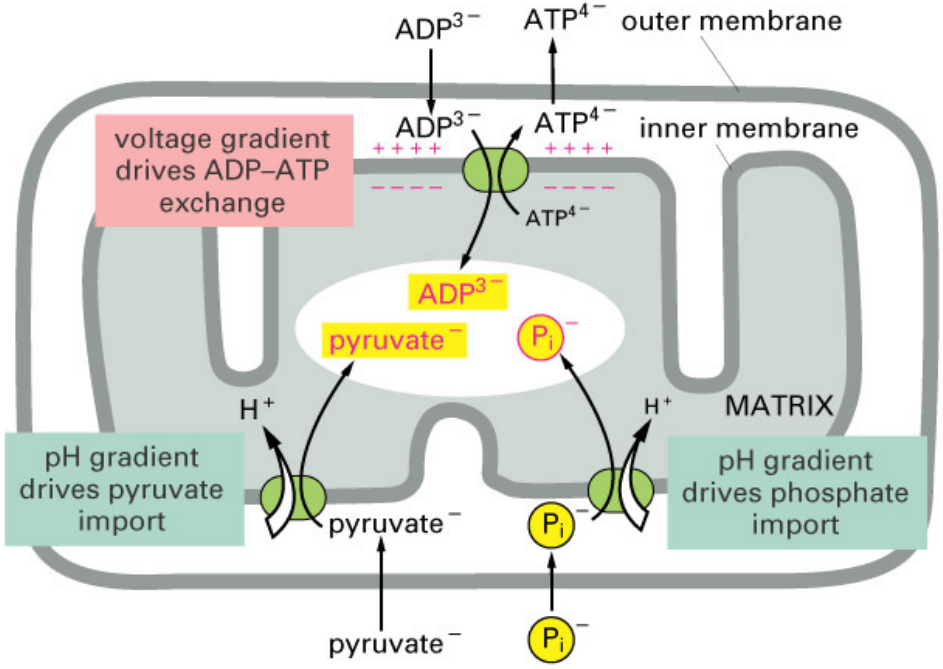






In bacteria - no mitochondria – ATP is in the cytosol.

Transport through the mitochondrial membranes

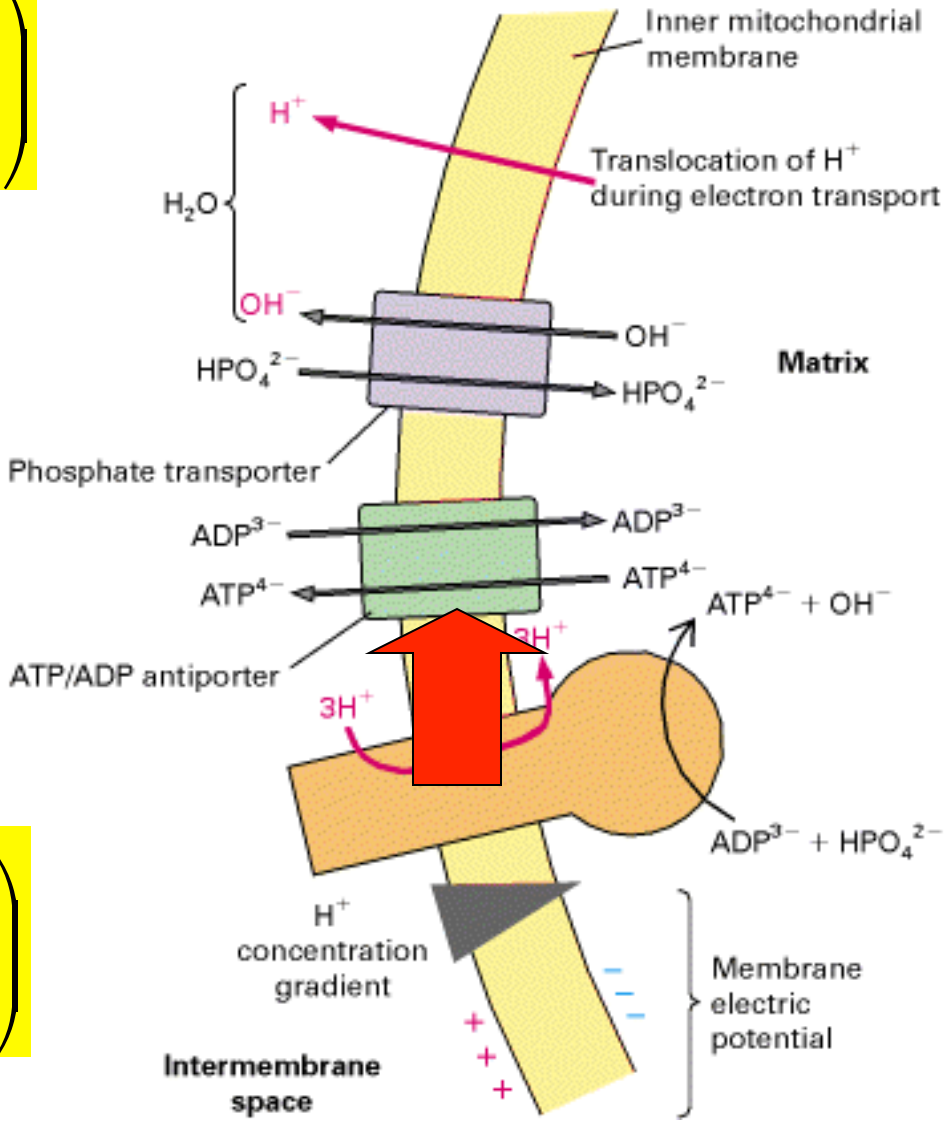


Adenine nucleotide translocase *catalyzes 1:1 exchange of ADP for ATP.*

$$\Delta\mu_{H^+} = \Delta\psi + \frac{RT}{F}\ln\left(\frac{[H^+]_{in}}{[H^+]_{out}}\right)$$

The membrane potential drives ATP/ADP exchange in the direction of ATP efflux and ADP influx – electrically dissipative.

$$\Delta\psi = \frac{RT}{F}\ln\left(\frac{[ATP]_{out}[ADP]_{in}}{[ATP]_{in}[ADP]_{out}}\right)$$



PO_4^- – enters mitochondria via PO_4^-/OH^- exchange (electroneutral).

$$\Delta\mu_{H^+} = \Delta\psi + \frac{RT}{F}\ln\left(\frac{[H^+]_{in}}{[H^+]_{out}}\right)$$

PO_4^- will accumulate in mitochondria because of the higher internal OH^- concentration.

$$\frac{[PO_4^-]_{in}}{[PO_4^-]_{out}} = \frac{[OH^-]_{in}}{[OH^-]_{out}} = \frac{[H^+]_{out}}{[H^+]_{in}}$$