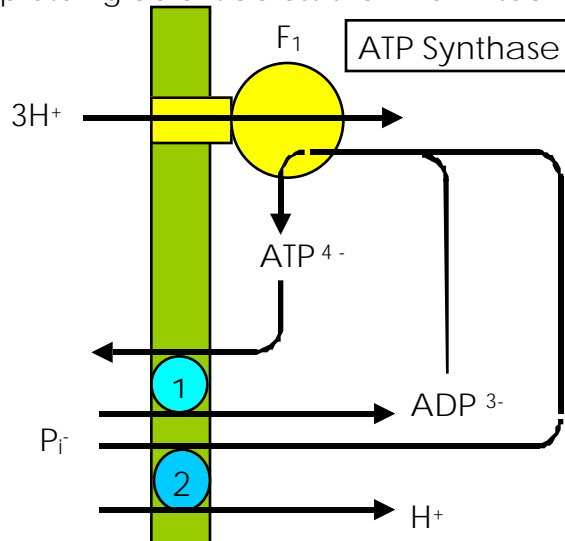


Electron Transport Chain III

ATP synthase (F_1F_0) - also known as the ATPase for the reverse direction. Without a proton gradient, the reverse reaction is spontaneous. Also called complex V in some books.

ATP synthase phosphorylates ADP by a mechanism driven by the free energy of electron transport, which is conserved in the formation of an electrochemical proton gradient across the inner mitochondrial membrane.



The protonmotive force results from the difference in pH and the difference in charge on both sides of the inner mitochondrial membrane.

$$G = 2.3 RT [\text{pH}(\text{in}) - \text{pH}(\text{out})] + ZF$$

The controversy comes down to thermodynamics - the free energy of ATP synthesis from ADP is about 51.6 kJ/mol. Yet the actual G for one H^+ returned to the matrix is much less.

Mitchel chemiosmotic theory - oxidative phosphorylation

$ADP + P_i \rightarrow ATP \Rightarrow$ ATPase found on inner mitochondria

the proton motive force \rightarrow that force generated by the unbalance of hydrogen ions across the inner mitochondrial membrane

- combination of pH and membrane potential (0.14 V and 1.4 pH units) drive ATP synthesis = 0.224V

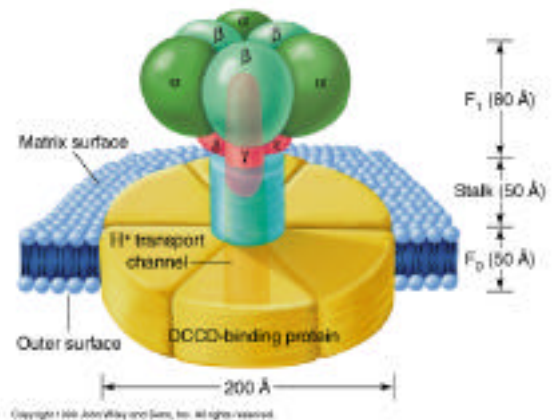
Evidence for the theory - bacteriorhodopsin, artificial pH gradient, broken mitochondria, uncouplers

H^+ pumped per 2 e^- transferred from NADH or $FADH_2$ is not certain

- anywhere from 6 to 10 total protons pumped when starting from site I and four less than that when electrons enter from site II
- about 2.5 to 3 protons / ATP generation - controversial
- Some leakage of the proton gradient creates inefficient coupling - thus a more realistic interpretation is that there are 2.5 ATP produced for each NADH

ATP synthase has two functional units each with several subunits.

- two complexes - F_1 and F_0
- F_0 complex
- transmembrane pore or channel for protons to move through
- H^+ ions build up at junction of the two subunits
 - increases in H^+ concentration may lead to protonization of critical amino acids (asp)



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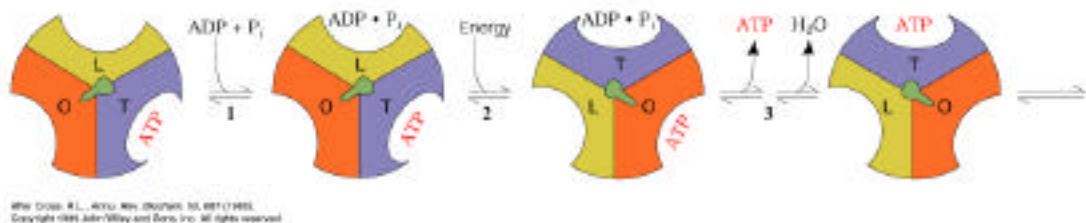
- Asp-H shifts rotor to open position and new aa interactions ionizes the asp and releases proton out of matrix
- This is shown by the inhibition of a reactive glutamate residues with the compound dicyclohexylcarbodiimide.

F₁ complex catalyzes ATP synthesis

- five subunits - on the matrix side of the inner membrane
- alpha and beta are nearly identical but the beta subunit contains the active site
- when separated the F₁ complex is an ATPase - hydrolysis of the gamma phosphate of ATP

The binding change mechanism - Paul Boyer - U of M and John Walker determined much of the ATPsynthase - Nobel Prize winners 1997 for this work

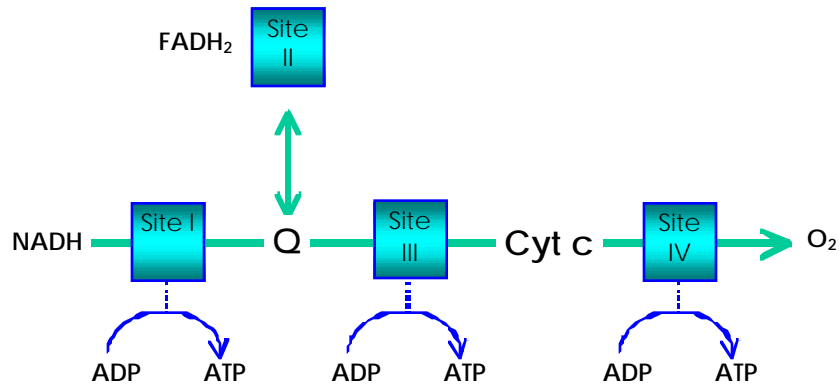
- H⁺ gradient leads to conformational changes of the F₁ complex
- Both ATP and ADP bind to the three beta subunits
- Three conformation changes for the whole enzyme (F₁F₀) the open (O), loose (L) and tight (T) binding sites
- Proton flux through pore shifts beta subunit conformations
- As the conformation of each of the subunits change a phosphoanhydride bond is formed with P_i and ADP
- The membrane potential helps to create high concentration gradient within the F₀ pore.
- The free energy of the proton concentration gradient converts the T state to the O state, thereby releasing ATP.



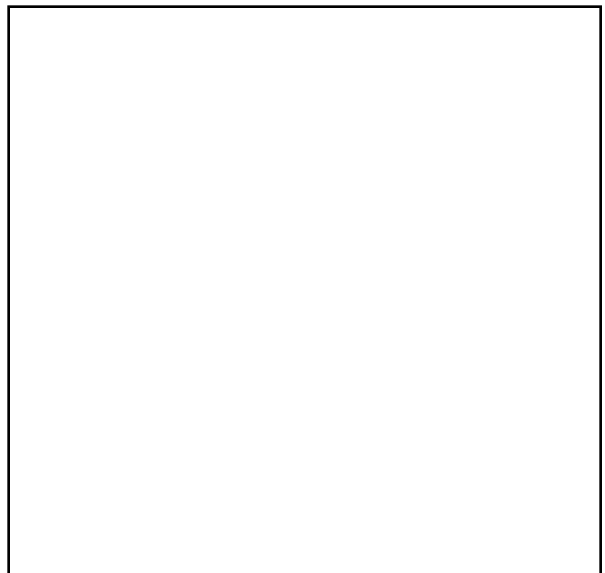
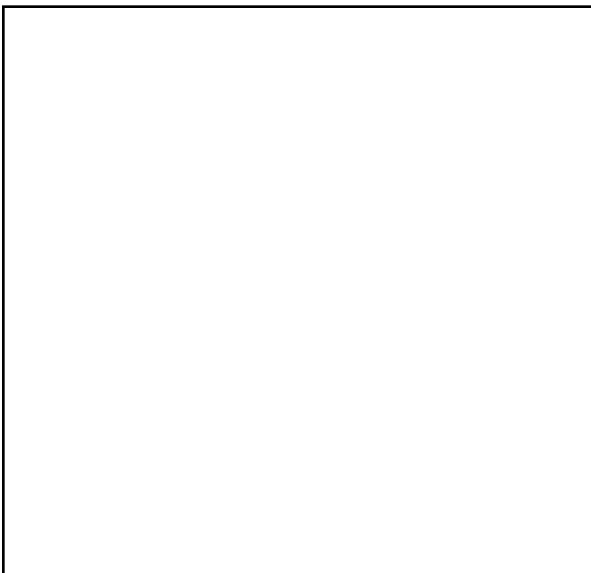
The amount of ATP produced to the amount of O₂ consumed is the P/O ratio (atomic O not molecular O₂).

ETS Poisons and Inhibitors - Specific inhibitors and uncouplers change the electron transfer and ATP production - respiration - O₂ + H⁺ -> H₂O

- Think of this a pipeline, if the middle is blocked no ATP production and O₂ used. If the end is blocked and a hole is punched in the hose you lose O₂ but no ATP production. Uncouplers "punch holes" in the ets. Some inhibitors act as blockers in the hose itself or at the end of the hose.



- Rate of system depends on oxygen to accept electrons
- Without ADP - ATPsynthase is stopped and electrons do not flow back into mitochondrial matrix and respiration stops
- uncouplers, degrade proton gradient. Transfer though membrane reversing H⁺ gradient. No ATP produced but lots of electrons transferred to try and restore H⁺ gradient - heat is produced - thus oxidation (e⁻ transfer) without phosphorylation
 - 2,4 DNP is an uncoupling agent that can transverse the inner mitochondria membrane dissipating the H⁺ gradient
- Respiration poisons - block at complex I, III and IV Effect is to stop the flow of e⁻ through the chain. - when added to the effect of uncouplers can lead to interesting studies
 - Carbon monoxide (CO), cyanide, and hydrogen disulfide (H₂S) – inhibit cytochrome C oxidase (site IV)
 - The fish poison and insecticide rotenone and barbiturates inhibit the oxidation of NADH (site I)
 - Antimycin A inhibits the cytochromes b and C (site III)
- If site 1 is blocked site II can still input electrons, ATP is formed and oxygen consumed. However if site two is blocked then no e⁻ are transferred and O₂ is not consumed.
- Think of what happens if various combinations of these inhibitors are used.



Brown fat (thermogenesis) - regulated by fatty acids, leads to uncoupling. High amounts of thermogenin (uncoupling protein) are found in babies and some women (lower back). The protein thermogenin is responsible for the uncoupling and is under the control of free fatty acids and nucleotides. It acts as a channel to allow H^+ to re-enter the mito.

Hormone sensitive lipase

- Uncoupling protein -inhibited by adenine and guanine nucleotides.
- Norepinephrine -> [cAMP] and activates hormone sensitive lipase
- Increase in [FA] reverse nucleotide inhibition of uncoupling channel

Oxygen Radicals

- **Addition of electrons to oxygen -> superoxide $O_2^{\cdot-}$**

- **Highly reactive oxidants - results in damage of mitochondria and surrounding tissue - example of this in Parkinson's Alzheimers and Huntington's disease.**

- **Apoptosis (programed cell death) can be activated by leaking damaged mitos**

- **Antioxidants limit damage SOD reduces superoxide**

- **Mutations associated with Lou Gehrig's disease**