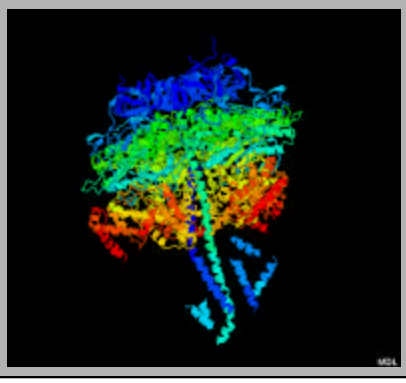
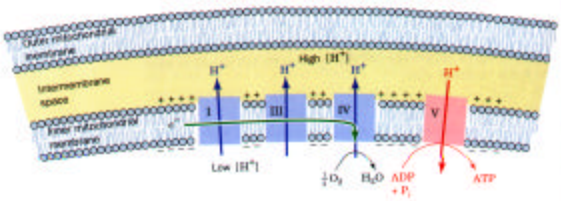
11: ATP Synthesis

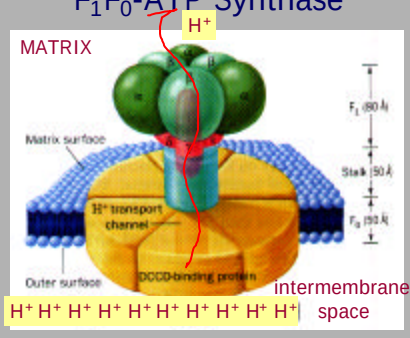


Chemiosmotic Gradient



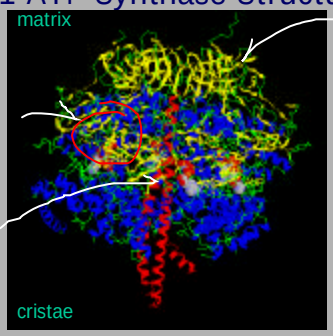
- Proton translocation is endergonic, requiring the input of energy to pass H^+ against a concentration gradient.
- The movement of a H^+ with that gradient is exergonic, releasing free energy for ADP phosphorylation.

F_1F_0-ATP Synthase



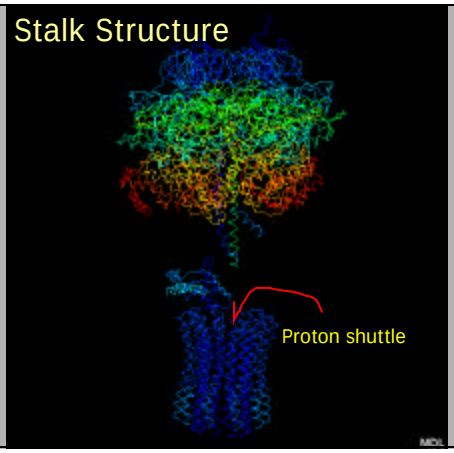
- F_0 hydrophobic; F_1 hydrophilic

F1 ATP Synthase Structure

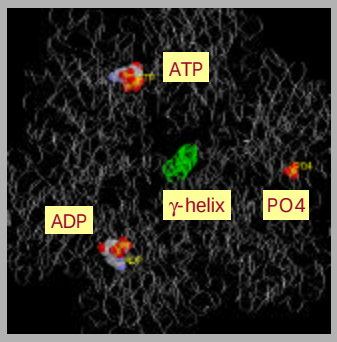


- Model File: "F1 ATP Synthase.HTML"

Stalk Structure

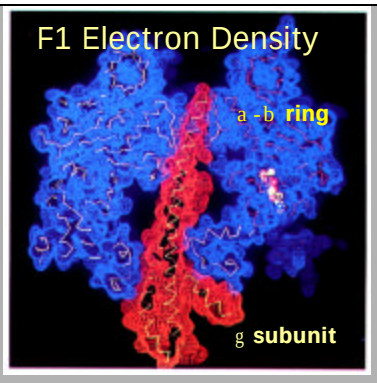


F1 Structure



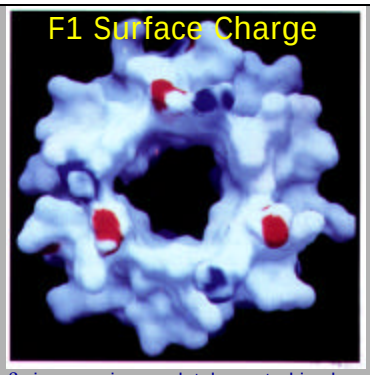
- Subunits:
  - 3 α
  - 3 β
  - γ, δ, ε
- The α & β units alternate in a hexagonal array
- The γ, δ, ε units form a base
- Note the 90-A helix of the γ unit

F1 Electron Density



- The γ subunit inserts through the central core of the hexagonal α–β ring structure

F1 Surface Charge



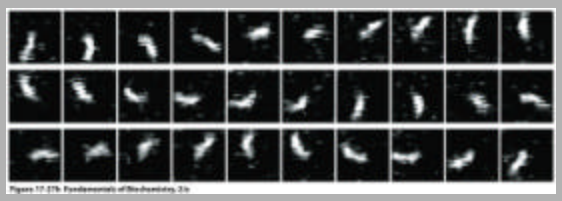
- The α–β ring core is completely neutral in charge, indicating that there are no ionic interactions with the γ helices that protrude through the center.

Movement Models:

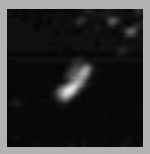
http://www.stolaf.edu/people/giannini/flashanimat/metabolism/atpsyn1.swf

http://nature.berkeley.edu/~hongwang/Project/ATP_synthase/

http://www.biologie.uni-osnabrueck.de/biophysik/junge/overheads.html



F1 ATPase: molecular machine

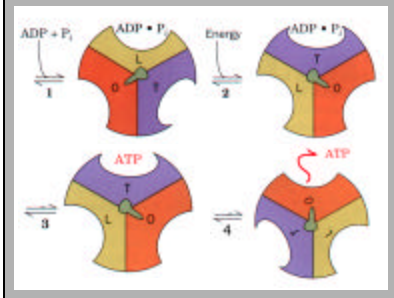


http://www.k2.ims.ac.jp/Movies.html

F1 Reaction Mechanism

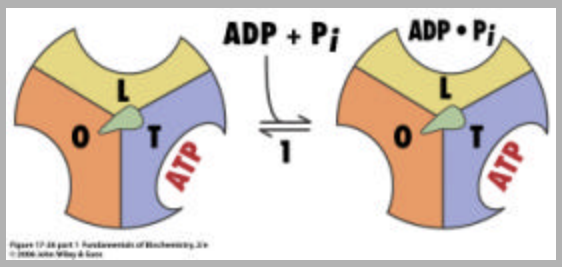
- The αβ units each form a catalytic domain between their juncture forming establishing a 3-site system with the same catalytic function
- However, these dimer units each have different conformational structures depending on their orientation to the γ-helix running through the core
  - T: tight binding conformation
  - L: loose binding conformation
  - O: open conformation (no binding)
- The free energy released by proton transfer is harnessed to change the conformation of a binding site

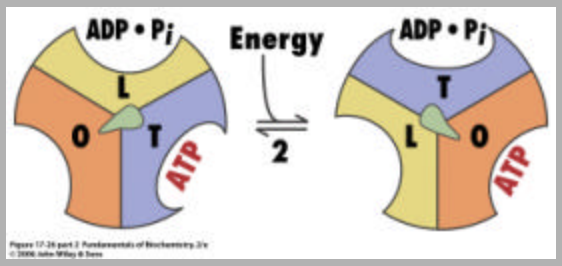
F1 Reaction Steps*

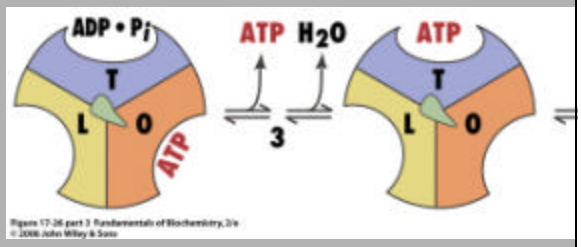


- L: bind reactants
  - ADP, Pi
- H^+ → T: reaction
  - ADP + Pi
- T: bound ATP
- H^+ → O: open
  - ATP release
- O: no binding
- H^+ → L: binding

Thus, the free energy released by the translocation of 3 H^+ are required to phosphorylate ADP → ATP



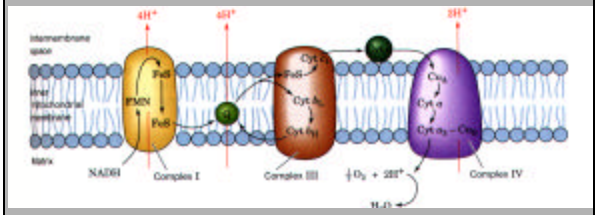




Reaction Stoichiometry

- 1 360-degree revolution
- 3 ATP
- 10 H+

Sequential *e*⁻ transfers



- ATP synthesis is not directly coupled to the cytochromes
- H^+ translocation at the cytochromes generates the chemioosmotic gradient necessary for ADP phosphorylation

F1 Conformational Changes

- What is the specific mechanism by which the movement of H^+ can alter the conformational structure of the 3 active sites?
- The conformational changes are driven by the rotational movement of the $\alpha_3\beta_3$ catalytic ring assembly around the helical axis of the γ-subunit.
- Note that the contacting hydrophobic surfaces in this assembly are devoid of hydrogen bonding and ionic interactions that would restrict any rotational movement.
- Note that the γ-subunit is not symmetrical - the ring will be unevenly 'torqued' as it rotates establishing differential contact to alter the binding site conformations.
- F1 rotation can be directly observed *in vivo*.

P/O Ratio

- For one full rotation of the catalytic ring (360º), we would generate 3 ATP
- But how many H^+ equal 1 ATP?
- The problem with the 120º model is that it was derived *a posteriori* to balance the general assumption that the free energy of 3 H^+ are required to phosphorylate 1 ADP → ATP
- This ratio of H^+ to ADP phosphorylation is called the P:O ratio (ADP oxidation : O_2 reduction)

P/O Ratio = ~2.5

- The transfer of 1 e- pair from NADH through the ETS system (I, III & IV) results in the translocation of 10 protons
- The measured rates of ADP phosphorylation indicate that for the reduction of 1 O_2 (each e- pair), 2.5 ATP are synthesized
- This yields a ratio of 4 protons per ATP
- One 360º turn of the F_1 $\alpha_3\beta_3$ catalytic ring would produce 3 ATP, so more than 10 protons are likely needed to make the full cycle.

Environment Matters

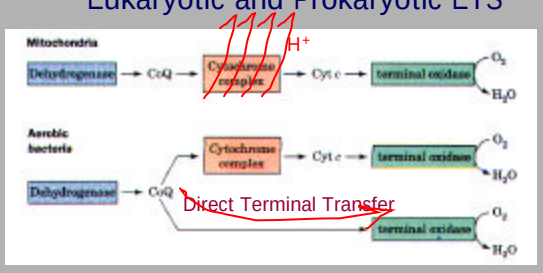
- Schulten, SCIENCE 290:61 (PDF file available)
  - What are the mechanisms that make protein function so robust against thermal motion?
  - Electron transfer occurs through a web of potential "tunneling" pathways
- In prokaryotes, different ETS schemes most likely maintain oxidative phosphorylation under a variety of environmental conditions:
  - Temperature
  - External ionic conditions
  - Availability of metabolic substrates

Photophosphorylation

Chemiosmotic gradient alters reaction ΔG

- At pH 7:
  - $HATP^{3-} + H_2O \rightarrow HADP^{2-} + H_2PO_4^-$
  - $\Delta G^o = -32.9$ kJ mol^{-1}
- At pH > pKa for ATP (7.1):
  - $ATP^{4-} + H_2O \rightarrow ADP^{3-} + HPO_4^{2-} + H^+$
  - $\Delta G^o = +5.4$ kJ mol^{-1}
- pH-dependent equilibrium constant K':
  - $K' = \frac{[ADP^{3-}][HPO_4^{2-}][H^+]}{[ATP^{4-}]}$

Eukaryotic and Prokaryotic ETS

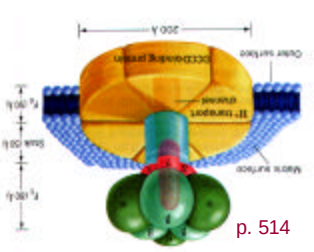


- Prokaryotic ETS systems located on plasma membrane
- Protons translocated to periplasmic space
- CoQ system is highly conserved
- Prokaryotes evidence greater substrate diversity

Regulation of Oxidative Phosphorylation

- NADH + 2Cyt-c(Fe^{3+}) + 5ADP + 5Pi ←→
- NAD^+ + 2Cyt-c(Fe^{2+}) + 5 ATP
- $\Delta G^{o'} \sim 0$
- The pathway is thus readily reversed by the addition of ATP
- F1 subunit does demonstrate ATPase activity
- ETS can (to some degree) be used to generate NADH by providing an electron source to Cyt-c

ETS and PS



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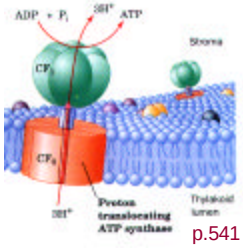

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