

Oxidative Phosphorylation

Reading:

Harper's Biochemistry pp. 130-148

Lehninger Principles of Biochemistry
3rd Ed. pp. 659-690

OBJECTIVES

To understand oxidative phosphorylation, the mechanism by which living organisms utilize redox energy to synthesize ATP.

Oxidative Phosphorylation

Electron transfer through the respiratory chain releases about 200 kJ per “mole” of electron pairs.

This energy is conserved as a proton-motive force.

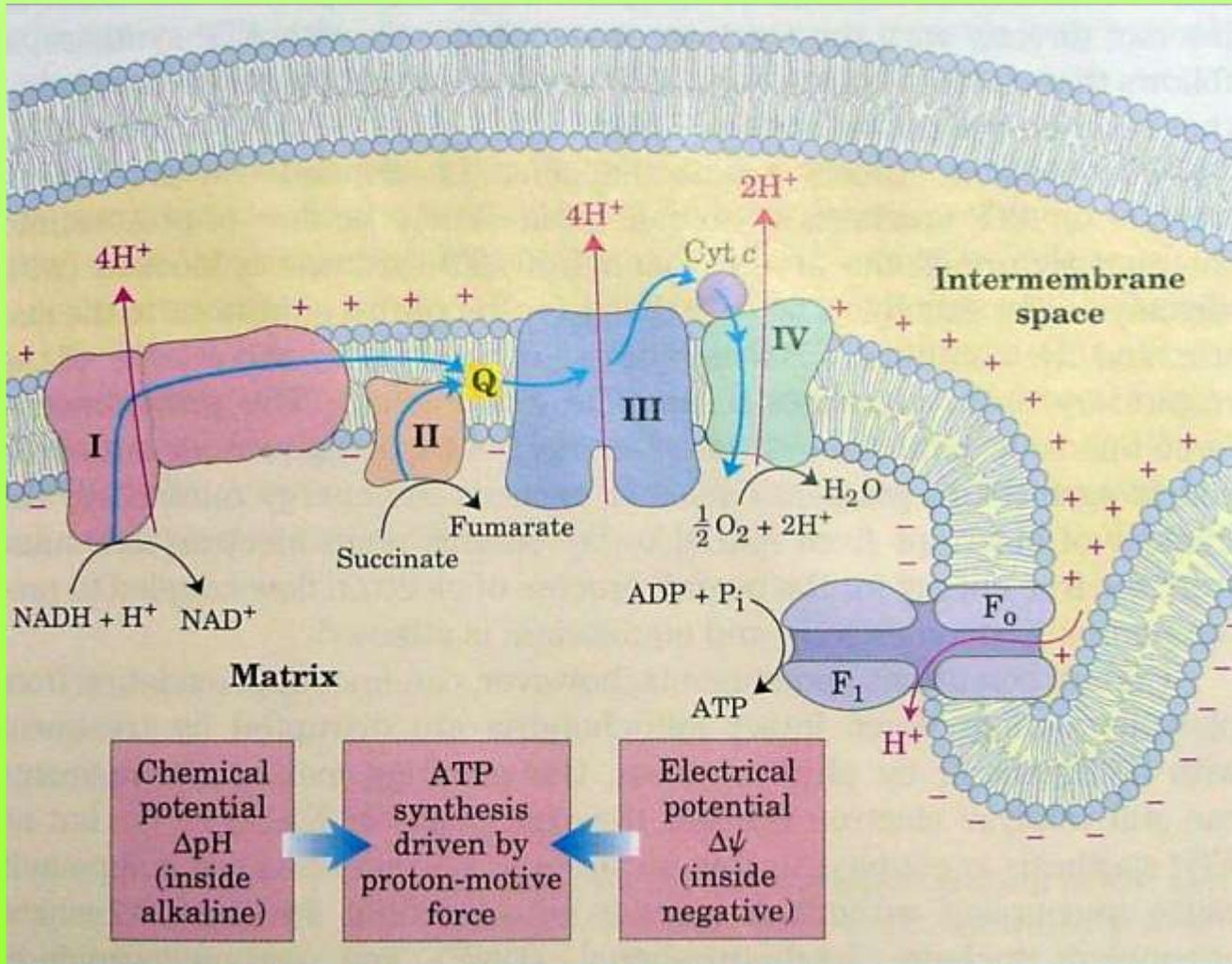
The formation of a mole of ATP from ADP and Pi requires about 30 kJ.

How is a concentration gradient of protons transformed to generate ATP?

Chemiosmotic Theory

Proposed by Peter Mitchell

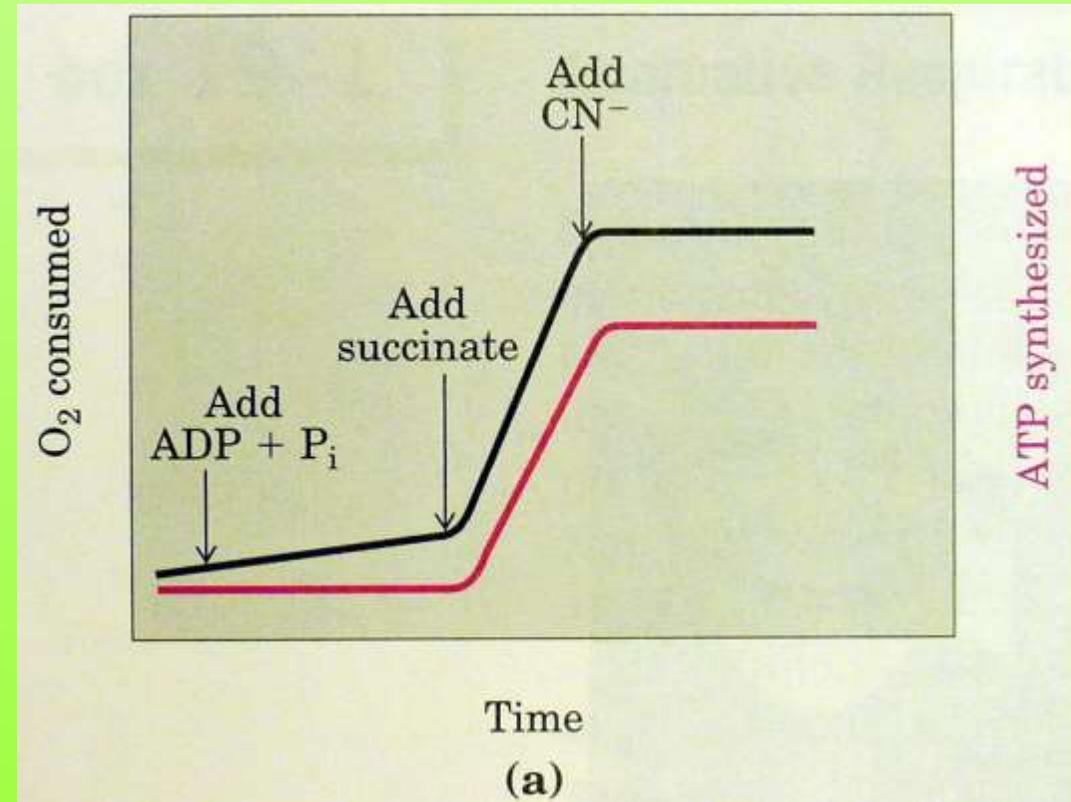
The proton-motive force, inherent in the proton gradient, drives the synthesis of ATP as protons flow passively back into the matrix through a protein pore associated with ATP synthase



Electron transport and ATP synthesis are coupled

This can be demonstrated when isolated mitochondria are incubated and O_2 consumption and ATP synthesis measured.

Inhibitors of the passage of electrons to O_2 (e.g. cyanide, carbon monoxide, and antimycin A) block ATP synthesis.



Conversely, inhibition of ATP synthesis blocks electron transport

without ADP, ATP is not made, but also, oxygen is not consumed
the toxic antibiotics oligomycin or venturicidin bind to the ATP synthase and inhibit ATP synthesis and also O₂ consumption
these toxins do not interact with electron carriers
therefore, inhibition of the ATPase blocks electron transport
electron transport and ATP synthesis are obligately coupled: neither reaction occurs without the other
some compounds “uncouple” oxidation from phosphorylation, e.g. dinitrophenol- dissipates the proton gradient

Why does electron transport depend on ATP synthesis?

When ATP synthase is inhibited, no path exists for the return of protons to the matrix.

The continued extrusion of protons by the respiratory chain generates a large proton gradient - the energy required to pump protons against this gradient equals or exceeds the energy provided by electron transfer.

At this point, electron flow stops.

ATP Synthase has two functional domains

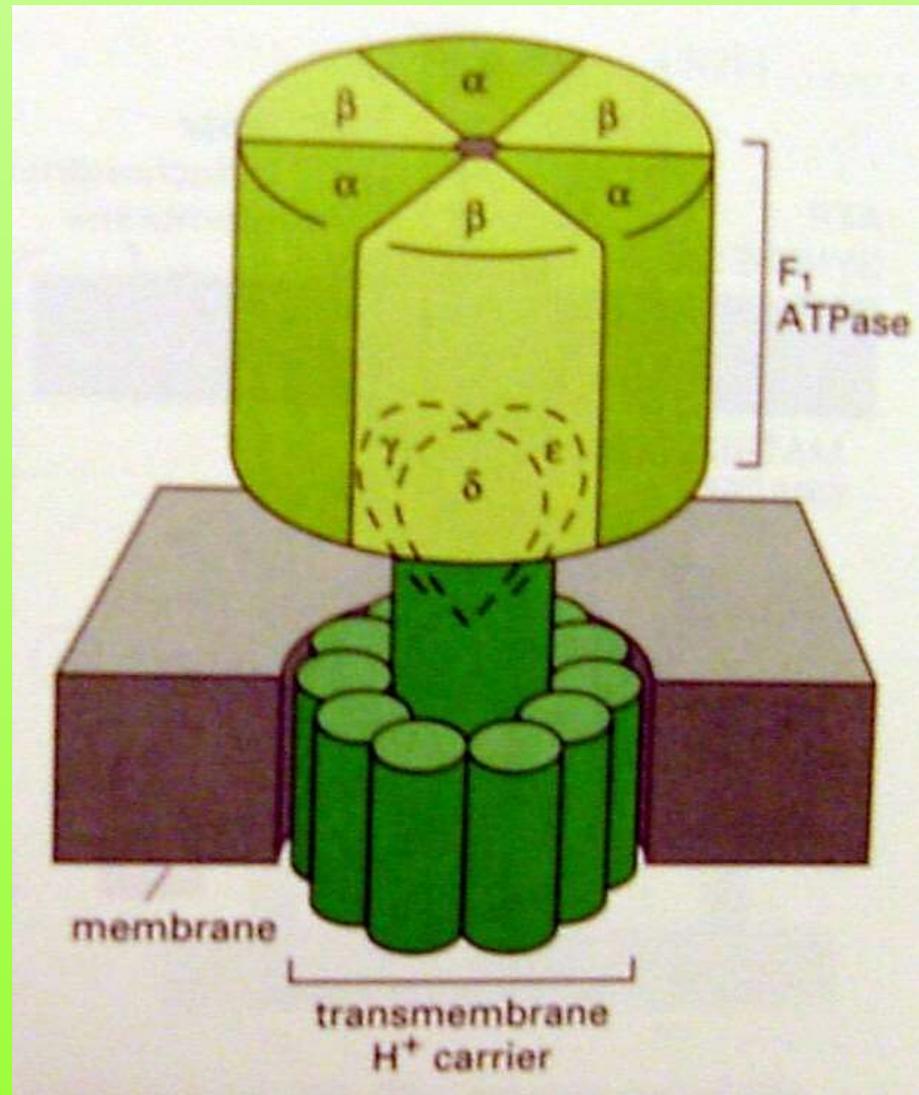
ATP synthase is a F-type ATPase

Two distinct components:

-F₁ is a peripheral membrane protein that catalyzes the rxn

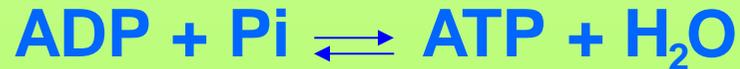


-F₀ is integral to the membrane and contains a proton pore



ATP is stabilized relative to ADP on the surface of F₁

On the enzyme's surface, the reaction

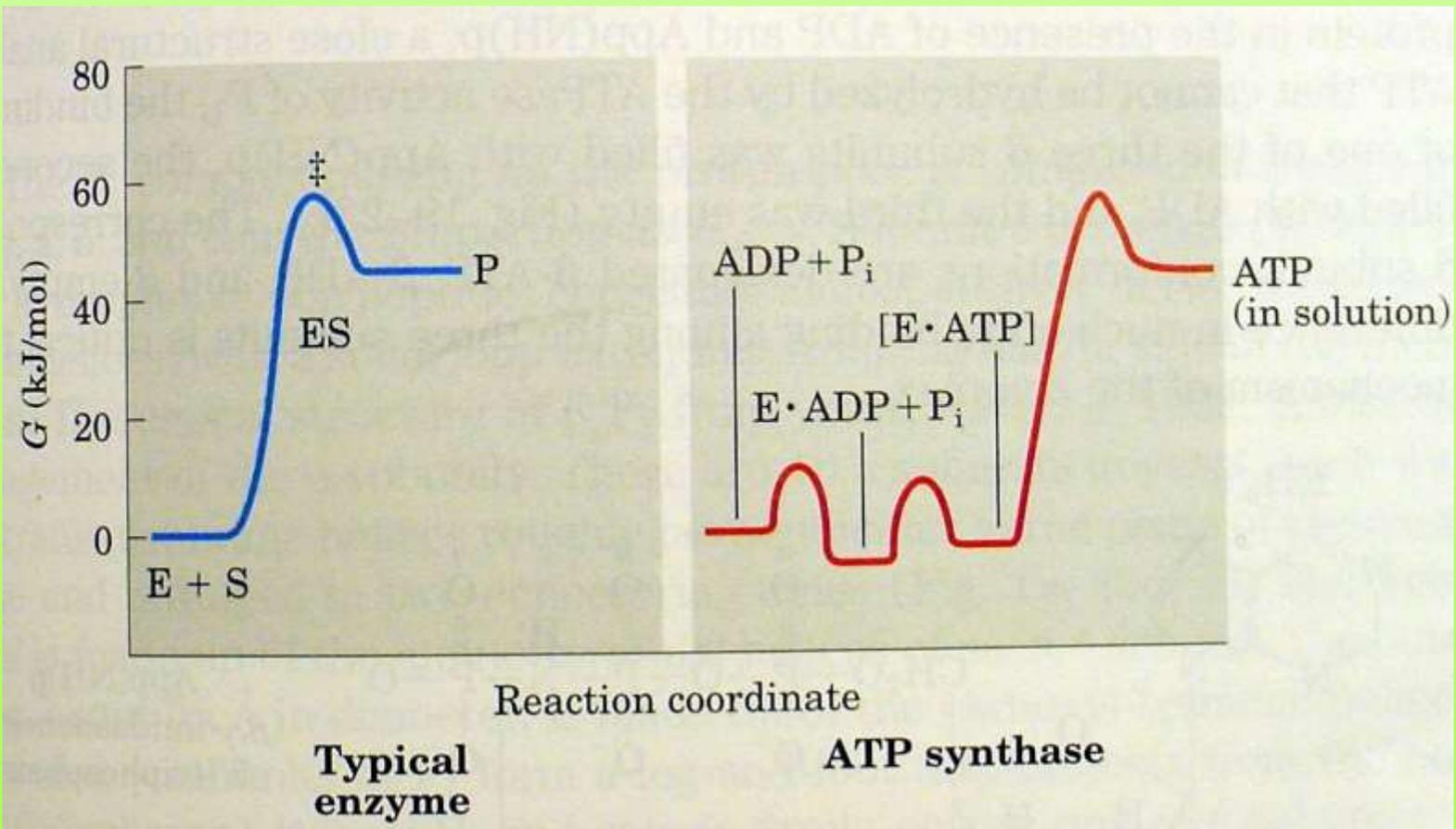


is readily reversible - the free energy change for ATP synthesis is close to zero.

labeling experiments have shown that the terminal pyrophosphate bond of ATP is cleaved and re-formed repeatedly before P_i leaves the enzyme surface.

ATP synthase binds ATP tightly, and the free energy of enzyme-bound ATP is close to that of ADP + P_i - on the enzyme surface, the reaction is reversible and the equilibrium constant close to 1.

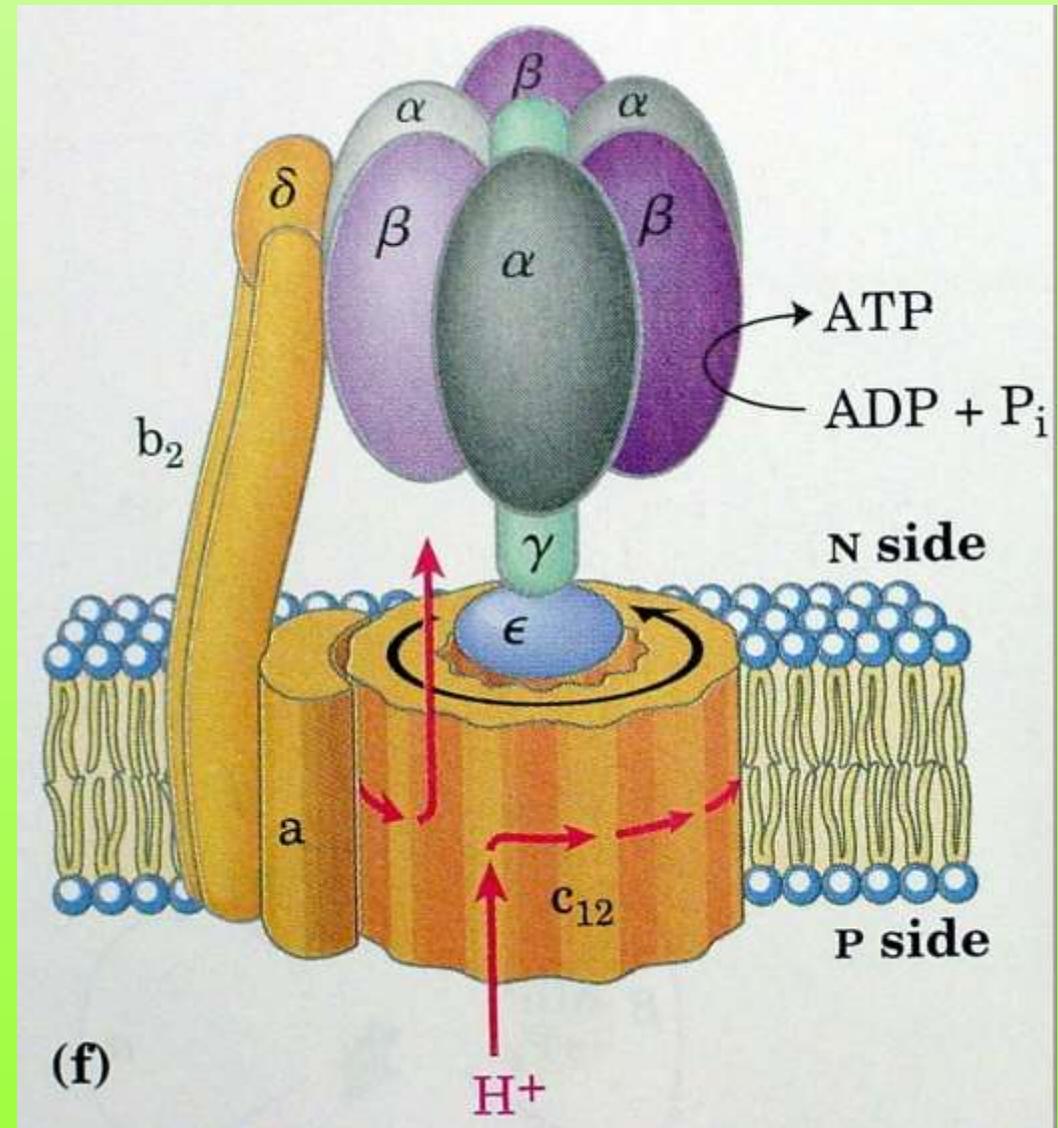
The energy consuming step is release of the bound ATP, and this is provided by the proton-motive force.



In a typical enzyme-catalyzed reaction, reaching the transition state (F) between substrate and product is the major energy barrier. For ATP synthase, release, not formation, of ATP is the major energy barrier

For the continued synthesis of ATP in this way, ATP synthase must cycle between a form that binds ATP very tightly and a form that releases ATP.

As protons flow, the cylinder (c_{12} subunits) and shaft (γ subunit) rotate, and the β subunits of F_1 , which are fixed in place relative to the membrane, change conformation as the γ subunit associates with each in turn



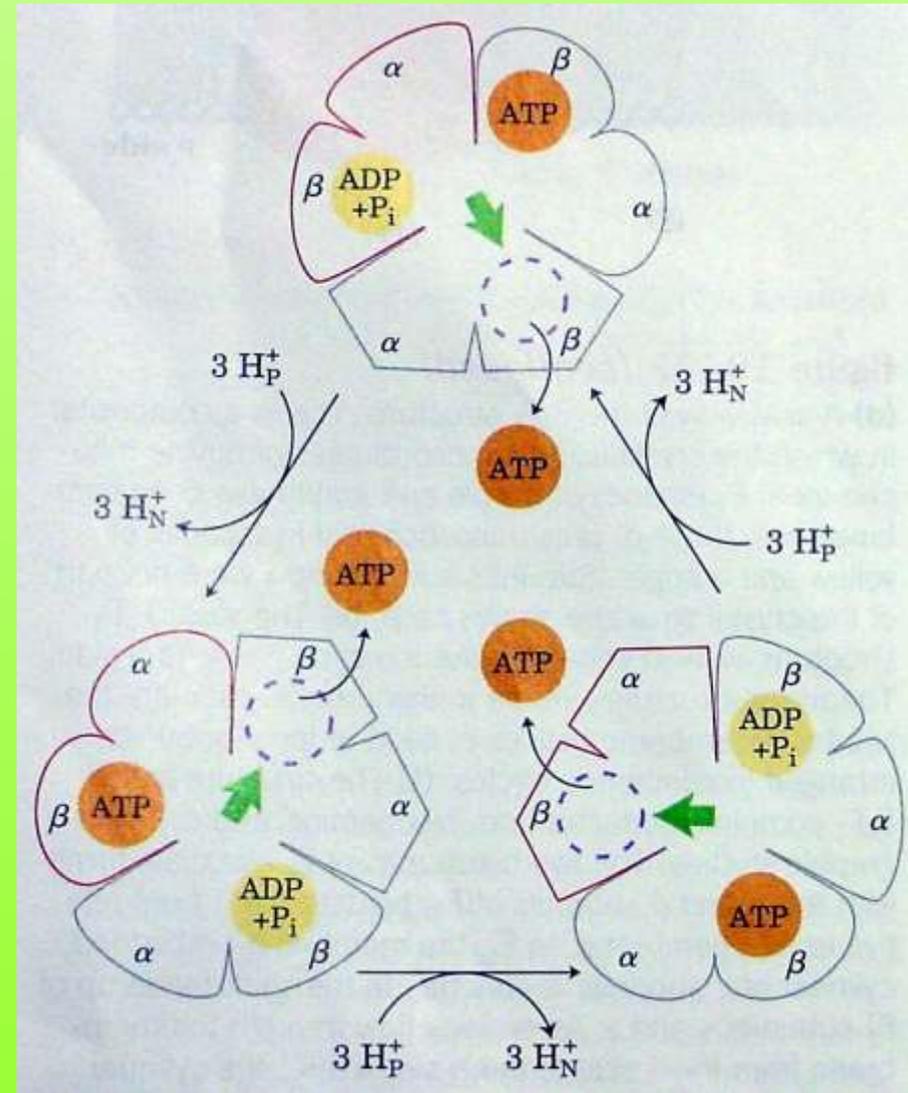
Binding-change model for ATP synthase

The F_1 complex has three non-equivalent adenine nucleotide binding sites, one for each pair of α and β subunits. Rotation of the central shaft converts the sites as follows:

β -ATP \rightarrow β -empty, ATP dissociates

β -ADP \rightarrow β -ATP, promotes ATP formation

β -empty \rightarrow β -ADP, loosely binds ADP + Pi



Electrons, protons, and ATP- what's the stoichiometry?

How many protons are pumped outward by electron transfer from one NADH to O₂?

Consensus values for protons pumped:

10 for NADH

6 for succinate

How many protons must flow inward through the F₀F₁ complex to drive the synthesis of one ATP?

Consensus value for number of protons for one ATP = 4

□ P/O values (# NADH's or succinate/ATP)

10/4 = 2.5 for NADH

6/4 = 1.5 for succinate

Complete oxidation of a molecule of glucose to CO_2 yields 30 to 32 ATP molecules.

Overall efficiency = 68%

table 19-5

ATP Yield from Complete Oxidation of Glucose

Process	Direct product	Final ATP
Glycolysis	2 NADH (cytosolic)	3 or 5*
	2 ATP	2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 FADH_2	3
	2 ATP or 2 GTP	2
Total yield per glucose		30 or 32

*The number depends on which shuttle system transfers reducing equivalents into mitochondria.

Cyanide Poisoning

- A. 22 year old comatose man had odor of almonds and severe metabolic acidosis.
- B. A presumptive diagnosis of cyanide poisoning was made. The symptoms tend to be non-specific, and blood cyanide is not easy to measure. The almond odor is however characteristic of gaseous cyanide. Later confirmed that he has taken a massive dose of amygdalin, obtained from almonds and containing a cyanide derivative.
- C. Treatment: Nitrites, followed by infusion of thiosulfate, 100% oxygen, and sodium bicarbonate. The patient recovered.

Cyanide Poisoning

Discussion. Cyanide binds to the heme of cytochrome oxidase, inhibiting the enzyme and blocking respiration. Nitrites induce the synthesis of methemoglobin and increase serum levels. Cyanide will also bind to methemoglobin decreasing the levels available to react with cytochrome oxidase. Thiosulfate combines with cyanide to produce thiocyanate which does not react with the free oxidase. This reaction is mediated by the mitochondrial enzyme rhodanese. Using this rationale, cyanide poisoning, while potentially fatal, can be successfully treated if diagnosed early.