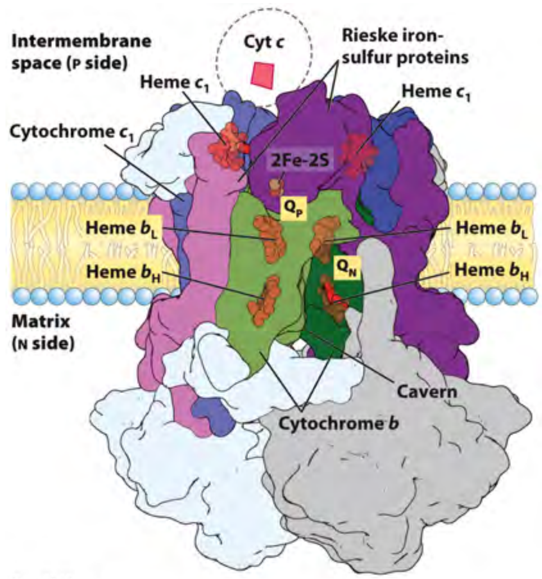
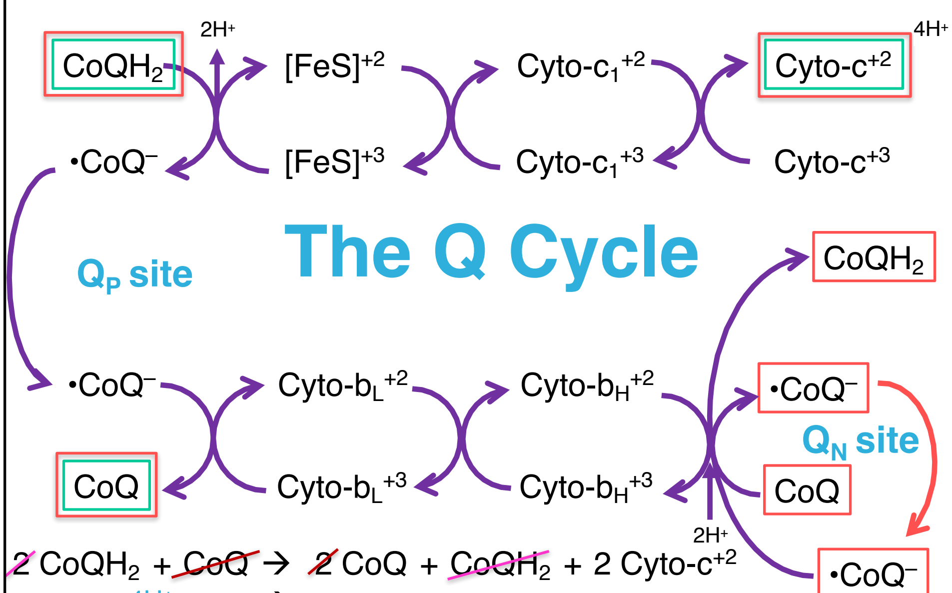
Electron Transport: Complex III

Ubiquinone:Cytochrome *c* Oxidoreductase, a.k.a. Complex III

- Uses two electrons from $CoQH_2$ to reduce two molecules of cytochrome *c*
- Additionally contains iron-sulfur clusters (Rieske protein), two different cytochrome *b's*, and a cytochrome c_1
- It's a dimer of 11 subunits (22 proteins). Three main proteins for each of the redox centers.
- Dimers create a central cavern with TWO CoQ binding sites
- Problem: How do we take the 2-electron $CoQH_2$ and get one electron at a time into cytochrome c without any flavin cofactors?



Electron Transport: Complex III

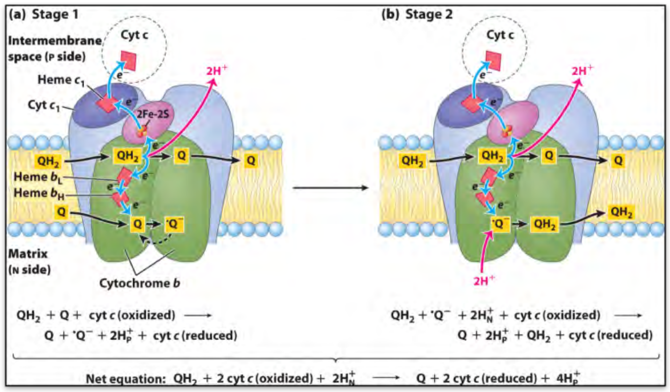


~~2~~ $CoQH_2$ + ~~CoQ~~ → ~~2~~ CoQ + ~~$CoQH_2$~~ + 2 Cyto-c^{+2}

$4H^+_{(matrix(N))}$ → $4H^+_{(intermembrane\ space\ (P))}$ − $2H^+_{(matrix(N))}$

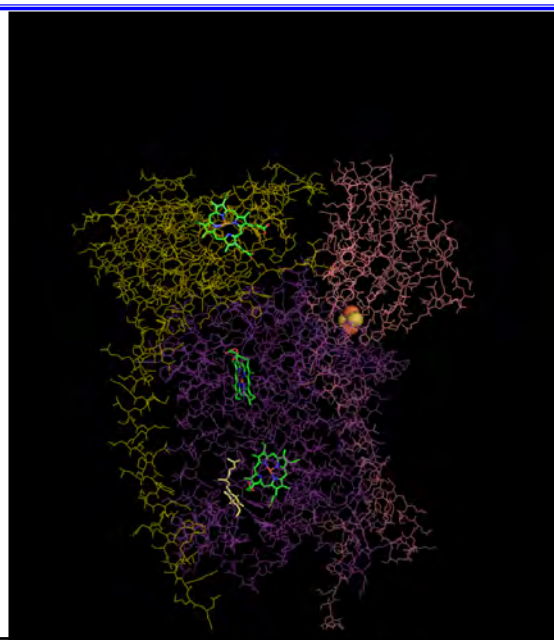
Electron Transport: Complex III

The Q Cycle



- Clearance of electrons from the reduced quinones via the Q-cycle results in translocation of four protons to the inter-membrane space.
- This cycle matches experimental evidence that four protons are transported across the membrane per two electrons that reach cyt *c*.
- The Q cycle provides a good model that explains how two additional protons are picked up from the matrix.
  - Two molecules of $CoQH_2$ become oxidized, releasing protons into the IMS.
  - One molecule becomes re-reduced, thus a net transfer of four protons per reduced coenzyme Q, plus a loss of protons from the matrix into the re-reduced CoQ adds to the membrane potential.

Electron Transport: Complex III



Electron Transport

TABLE 19-3 The Protein Components of the Mitochondrial Respiratory Chain

Enzyme complex/protein	Mass (kDa)	Number of subunits[a]	Prosthetic group(s)	Reduction potential ($\Delta E'^{\circ}$ V)	Binding sites for:	Inhibted by:
I NADH dehydrogenase	850	45 (14)	FMN, Fe-S	0.36	NADH, CoQ	amytal, rotenone
II Succinate dehydrogenase	140	4	FAD-E, Fe-S	0.09	Succinate, CoQ	
III Ubiquinone: cytochrome *c* oxidoreductase[b]	250	11	Hemes b, c_1, Fe-S	0.17	CoQ, Cytochrome c	antimycin a
Cytochrome *c*[c]	13	1	Heme			
IV Cytochrome oxidase[b]	204	13 (3–4)	Hemes a, a_3; Cu_A, Cu_B	0.57	Cytochrome c, O_2	Cyanide, azide, CO

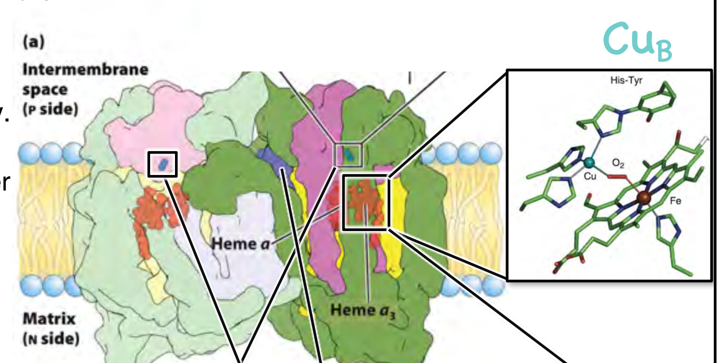
[a]Number of subunits in the bacterial complexes in parentheses.
[b]Mass and subunit data are for the monomeric form.
[c]Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

Electron Transport: Complex IV

Cytochrome Oxidase, a.k.a. Complex IV

Cu_A

- Uses electrons from FOUR reduced cytochrome *c's* to reduce dioxygen (O_2) to water.
- This reaction is among the most difficult chemically.
- The bovine complex is a dimer of 13 subunits (26 proteins). Three main proteins for each of the redox centers.



- Contains two copper proteins: one with a Cu-Cys cluster, and one with two different cytochrome a's (a & a_3) and Cu. The third main protein moves protons from the matrix through the second and protein. Function of the other 10 are unknown, although undoubtedly involved as a proton pump.

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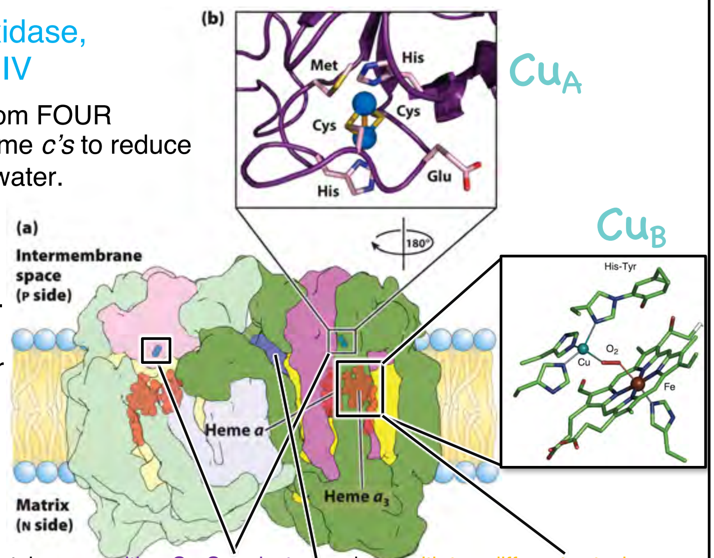

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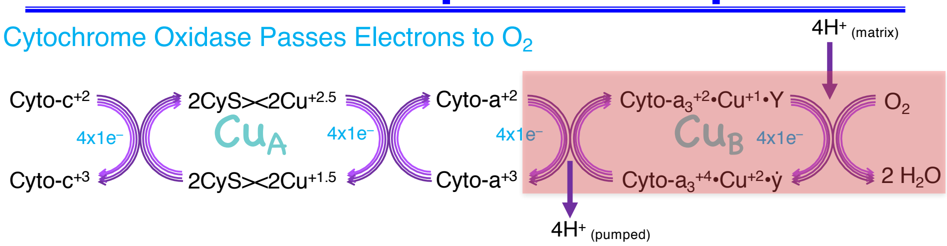
Electron Transport: Complex IV

Cytochrome Oxidase Passes Electrons to O_2

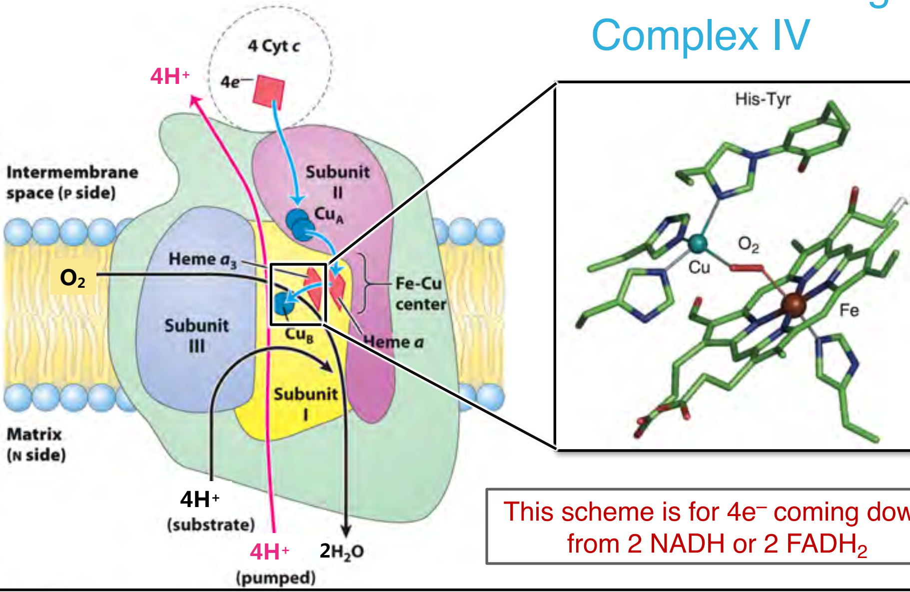


- Contains 6 copper ions per complex (3/monomer$_{13}$)
- There are two types, sites for the copper:
  - Cu_A: two ions that accept electrons from cyt *c* (looks like a 2Fe-2S cluster)
  - Cu_B: bonded to heme a_3, forming a binuclear center that transfers four electrons to oxygen.
- Four electrons are used to reduce one oxygen molecule into **two** water molecules.
- Four protons are picked up from the matrix in this process.
- Four additional protons are pumped from the matrix to the intermembrane space.

This scheme is for $4e^-$ coming down from **2** NADH or **2** $FADH_2$

Electron Transport: Complex IV

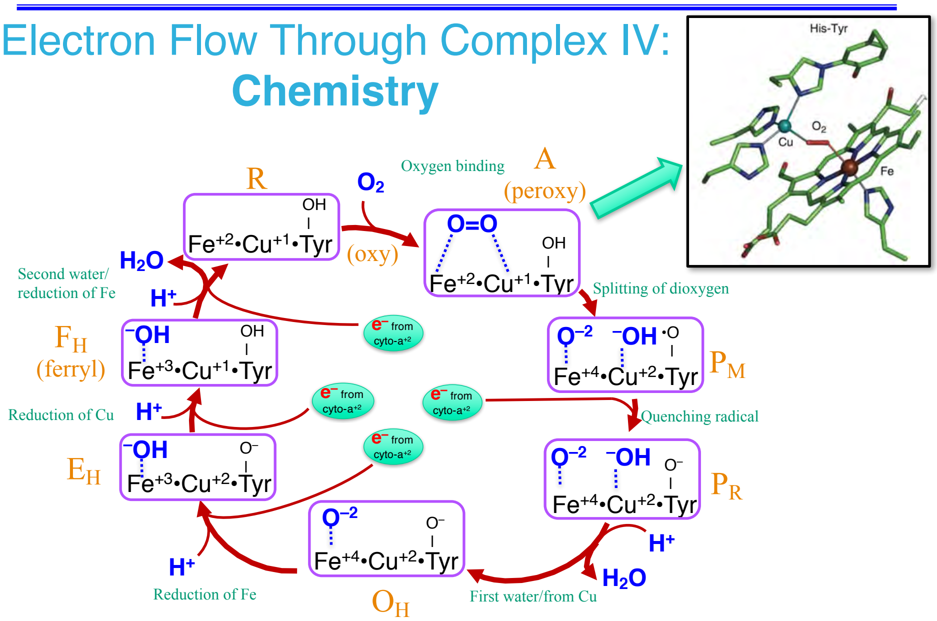
Electron Flow Through Complex IV



This scheme is for 4e⁻ coming down from 2 NADH or 2 $FADH_2$

Electron Transport: Complex IV

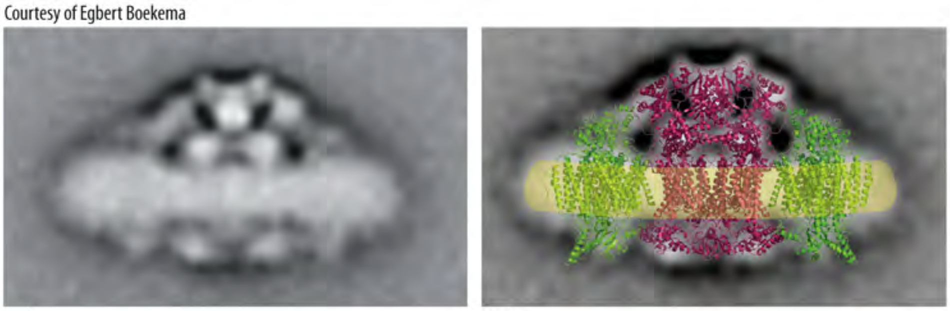
Electron Flow Through Complex IV: **Chemistry**



Electron Transport

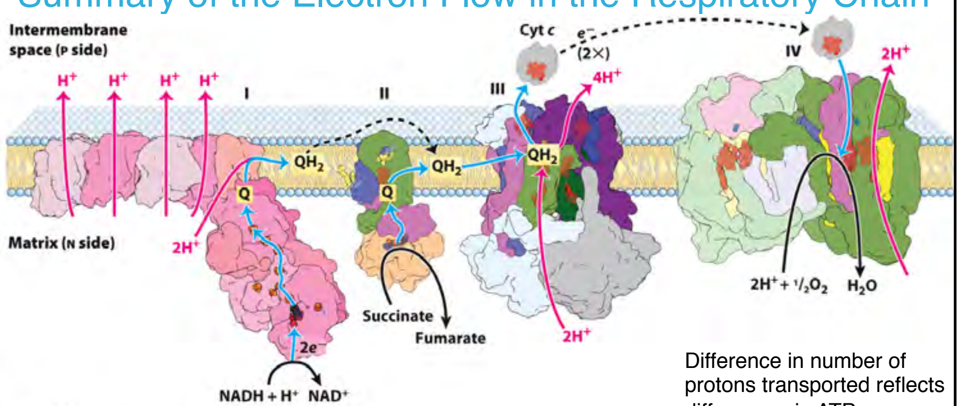
Multiple Complexes Associate Together to Form a "Respirasome"

Courtesy of Egbert Boekema



Electron Transport

Summary of the Electron Flow in the Respiratory Chain



This scheme is for 2e⁻ coming down from **1** NADH or **1** $FADH_2$

Complex I → Complex IV

$$1NADH + 11H^+_{(N)} + \frac{1}{2}O_2 \rightarrow NAD^+ + 10H^+_{(P)} + H_2O$$

Complex II → Complex IV

$$FADH_2 + 6H^+_{(N)} + \frac{1}{2}O_2 \rightarrow FAD + 6H^+_{(P)} + H_2O$$

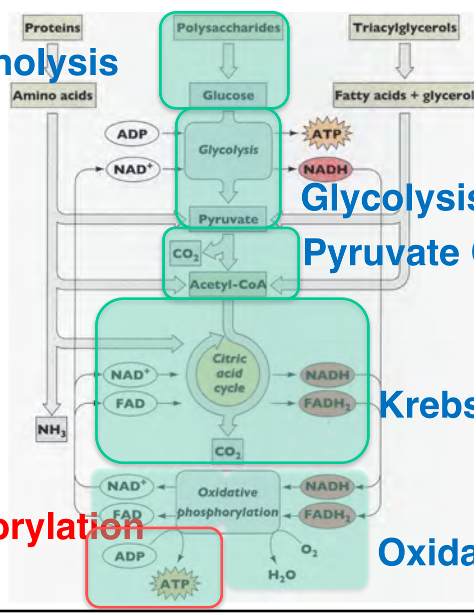
Difference in number of protons transported reflects differences in ATP synthesized=P/O ratios:

Substrate	P/O ratio
Pyruvate	3
Acetyl CoA	3
NADH	3
Succinate	2

Phosphorylation (The Chemiosmotic Theory)

Dr. Kornberg: **Lecture 02.17.17 (45:00-48:25)- mitochondria coupling (3.5 min)**

Oxidative Phosphorylation



Glycogenolysis

Glycolysis

Pyruvate Oxidation

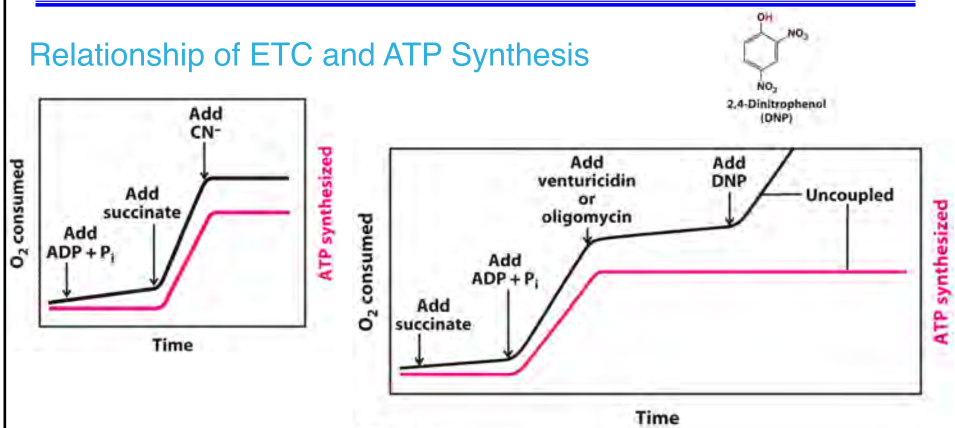
Krebs' Cycle

Phosphorylation

Oxidative

Oxidative Phosphorylation

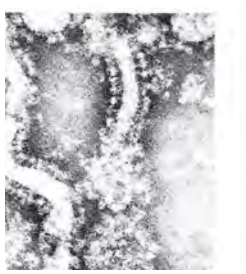
Relationship of ETC and ATP Synthesis

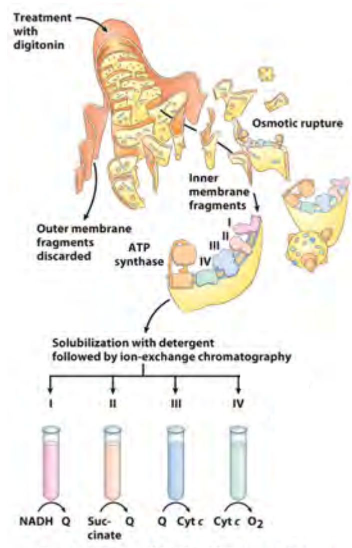


- As described, ATP synthesis requires electron transport.
- But electron transport does not requires ATP synthesis.

Phosphorylation

How Does Oxidative Phosphorylation Form ATP?





- Before the 1970's, it was thought that the ET chain used the energy of redox to make a "high-energy" intermediate for "substrate-level phosphorylation, as occurs in glycolysis and TCA cycle.
- It was clear that one of the most abundant proteins on the inner-mitochondrial membrane had a curious mushroom-shaped structure.
- It was purified, along with the other Complexes I-IV, initially called Complex V.

Phosphorylation

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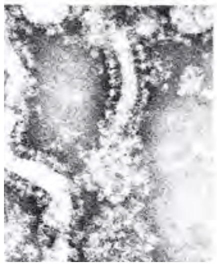

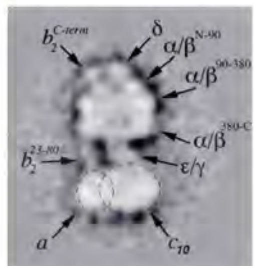

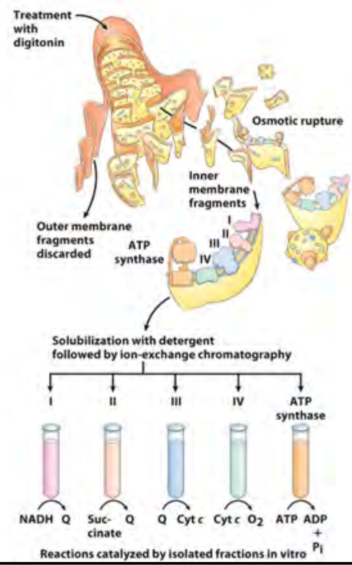

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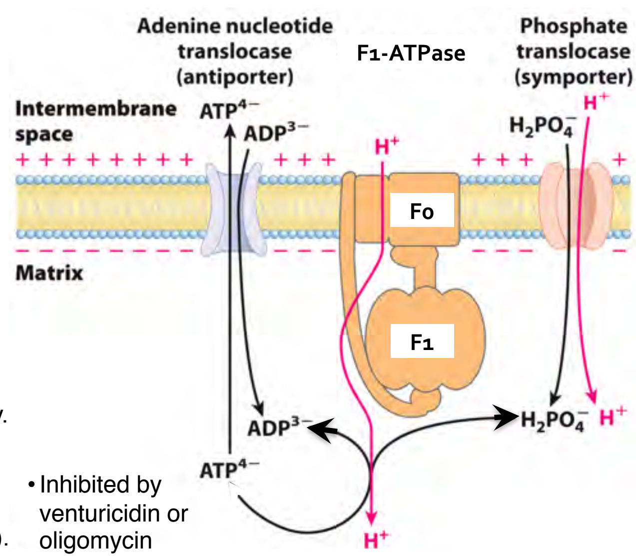
Phosphorylation

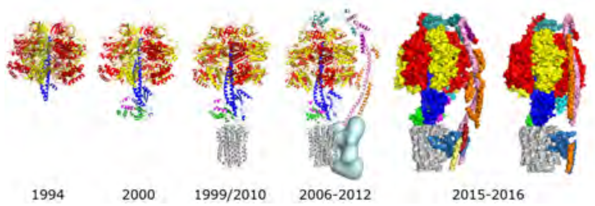
How Does Oxidative Phosphorylation Form ATP?

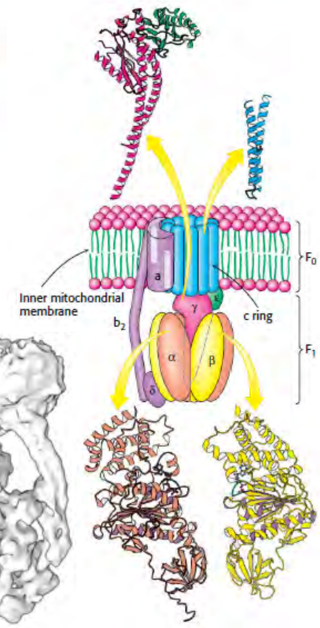
- Complex V, when purified separately from the membrane is an effective ATPase enzyme, hence it was initially called the F_1-ATPase.
- The F_1 part had 9 subunits ($\alpha_3\beta_3\gamma\delta\varepsilon$)
- When more careful purifications were performed, it was clear that its activity was closely coupled to an intact inner membrane with little activity.
- When membrane proteins were isolated, the F_0 part had 15 subunits of 3 different proteins (a, b_2, c_{10}).



- Inhibited by venturicidin or oligomycin

Phosphorylation





Contains two functional units:

- F_1
  - soluble complex in the matrix
  - individually catalyzes the hydrolysis of ATP
- F_0
  - integral membrane complex
  - transports protons from IMS to matrix, dissipating the proton gradient
  - energy transferred to F_1 to catalyze phosphorylation of ADP.
  - This mechanism was only fully accepted in late 1970s.