

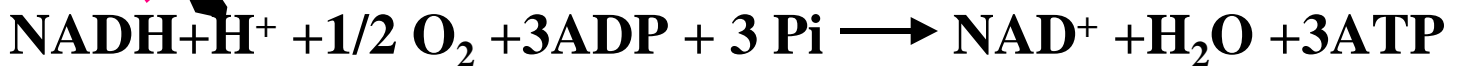
Chapter 15--Oxidative Phosphorylation and Respiration

Why do you breathe?



****TCA--to get energy by oxidizing C substrates**

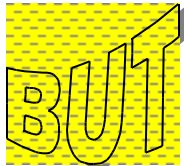
****To get lots more energy via redox.**



Summary

--2 electrons from NADH reduce O

--3 ADP are phosphorylated to ATP



BUT

This simple reaction requires

- * Reduced substrates and ADP (the reactants)**
- * Intact Membranes**
- * 5 Large Protein Complexes**
- * Many co-factors including Fe/S, Hemes, CoQ**



Understanding this reaction required many biochemists to use many experimental techniques (some new) over several decades.

Electron microscopy

Cell fractionation and purification

Reconstitution experiments

UV/visible difference spectroscopy

Measurement of O₂ consumption vs ATP production (P/O)

Measurement of reduction potentials

Use of electron donors and acceptors

X-ray diffraction

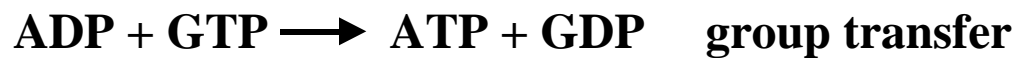


A new theory

“The chemiosmotic coupling theory”

What's direct coupling?

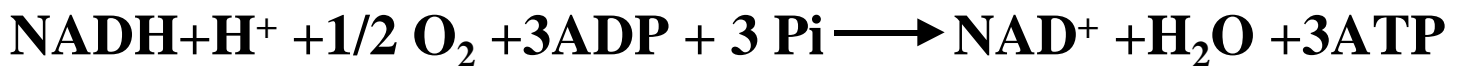
Two processes that must go together--



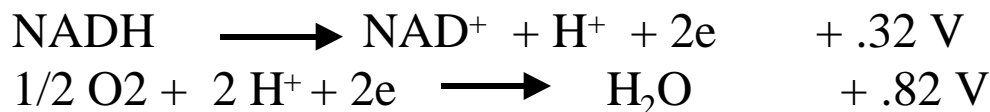
How are these reactions coupled?



Reductant	Oxidant	n	E°'
Pyruvate + CO ₂ + H ⁺	Malate	2	-0.33
NAD ⁺ + H ⁺	NADH	2	-0.32
NADP ⁺ + H ⁺	NADPH	2	-0.32
FMN (enzyme-bound) + 2H ⁺	FMNH ₂ (enzyme-bound)	2	-0.30
Cytochrome a ₃ (+3)	Cytochrome a ₃ (+2)	1	0.55
Fe (+3)	Fe (+2)	1	0.77
½O ₂ + 2H ⁺	H ₂ O	2	0.82



1/2 reactions



1.14 Volts

$$G^{\circ\prime} = -n F E^{\circ\prime} = -2 \times 96500 \times 1.14 = 220 \text{ kJ} \quad \text{OK for 3 ATP}$$

Nernst Equation

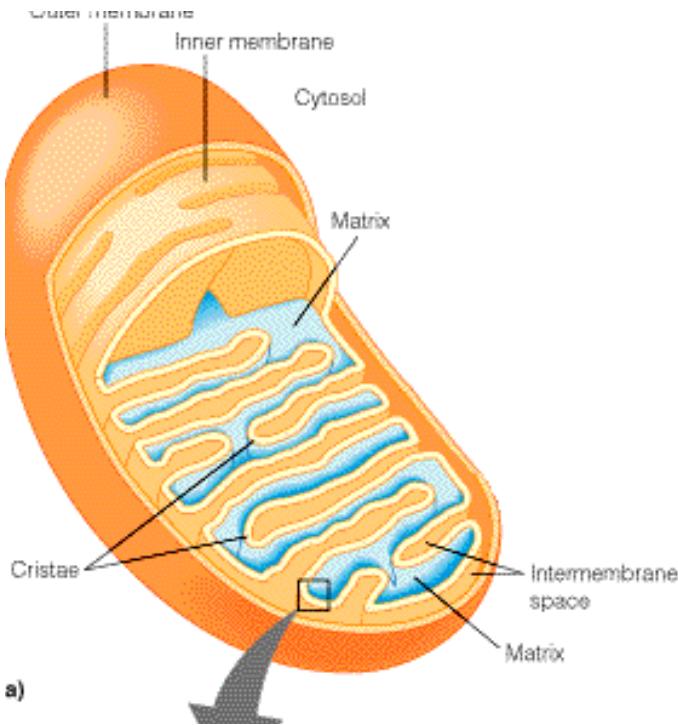
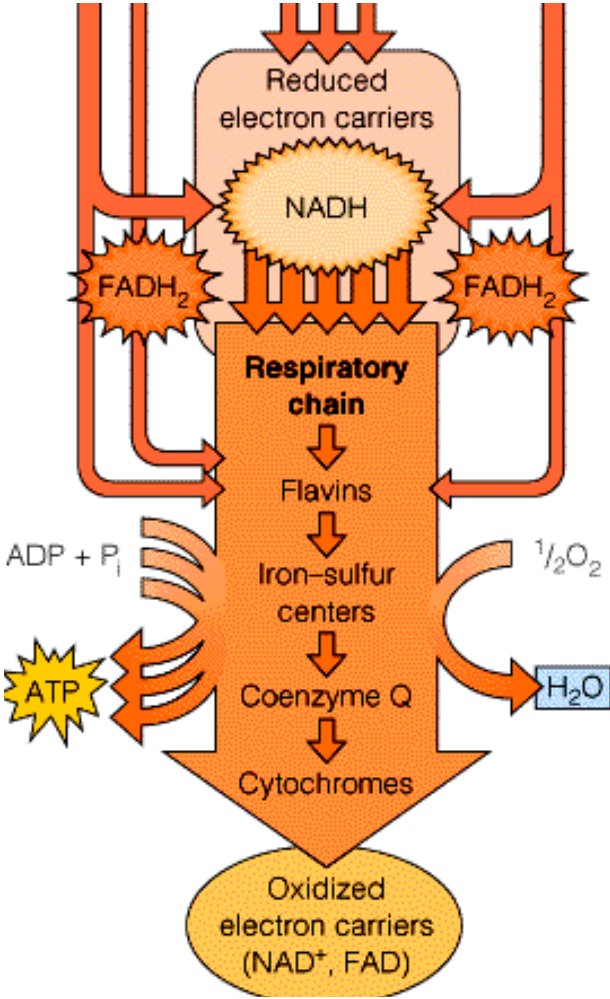
$$E' = E'_0 + \frac{2.303RT}{nF} \log \frac{[\text{electron acceptor}]}{[\text{electron donor}]}$$

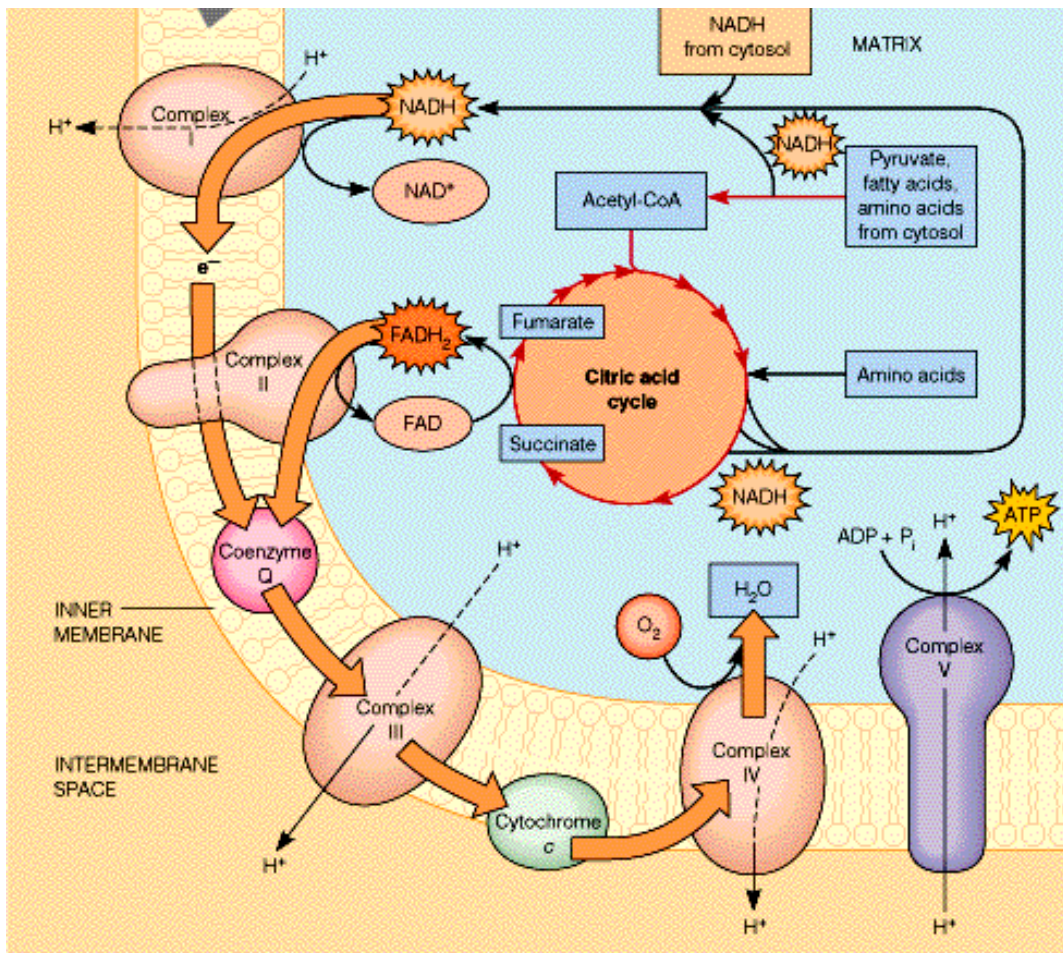
Suppose that the NAD⁺/NADH ratio is 1/10

$$= .32\text{V} + (2.3 \times 8.3 \times 310/2 \times 96500) \log 1/10$$

$$= .32\text{ V} - .03\text{ V} = +.29\text{ V}$$

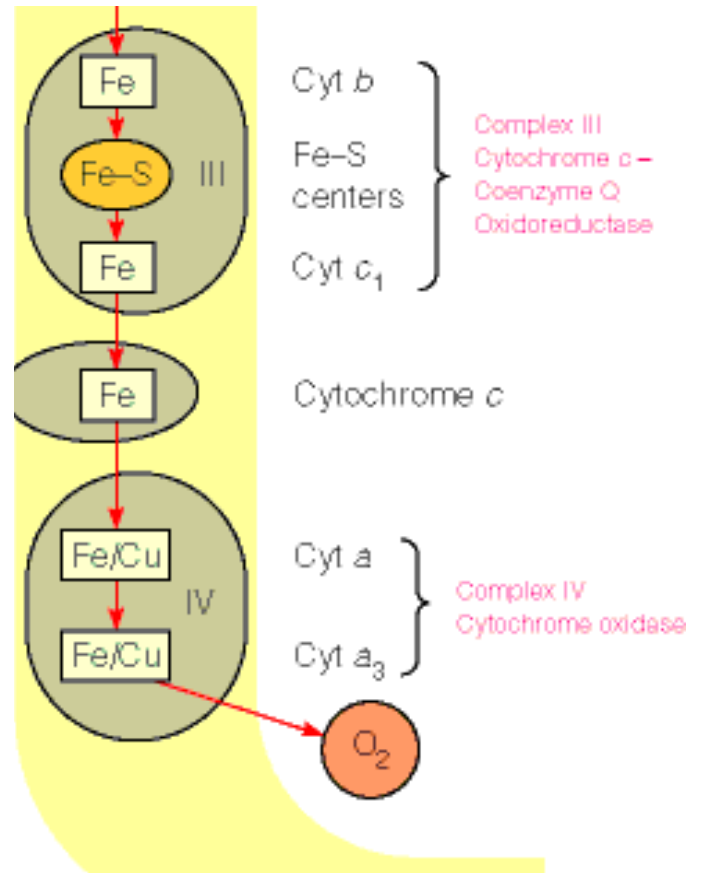
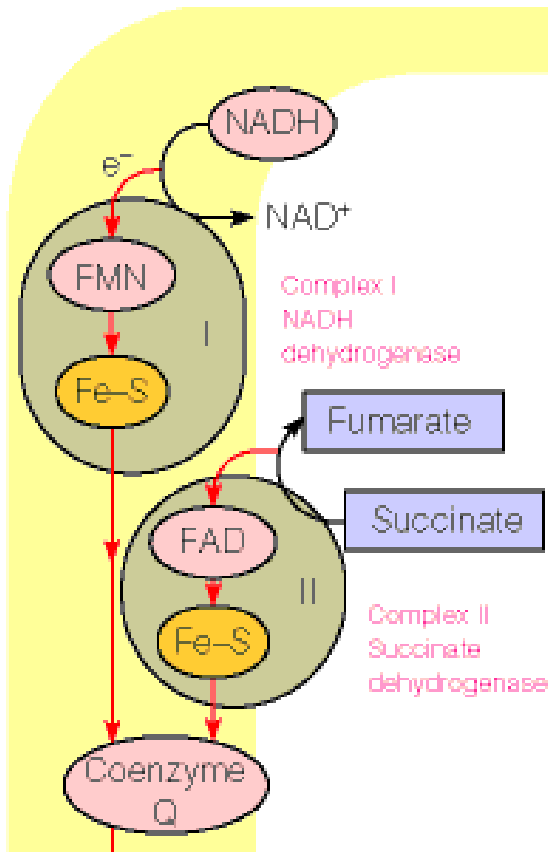
Respiration





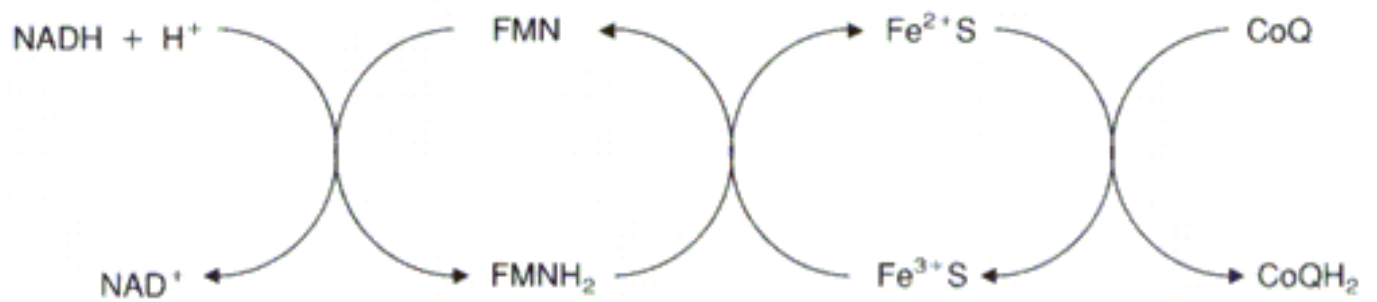
Products of citric acid cycle feed into respiration

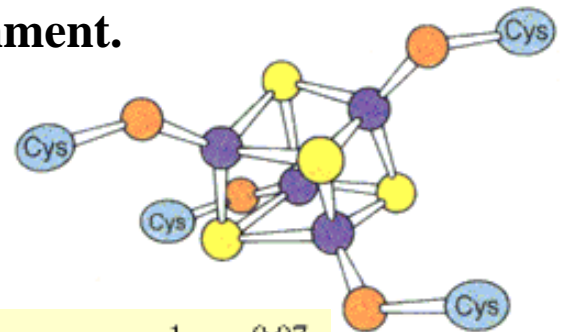
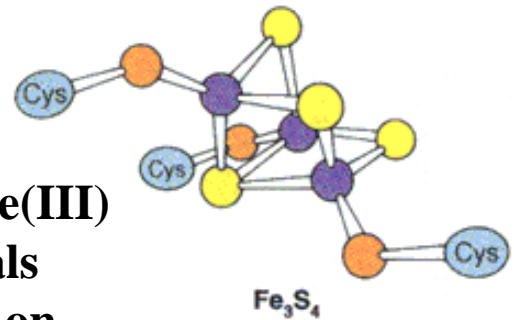
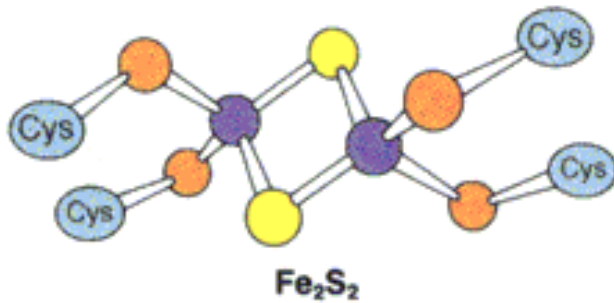
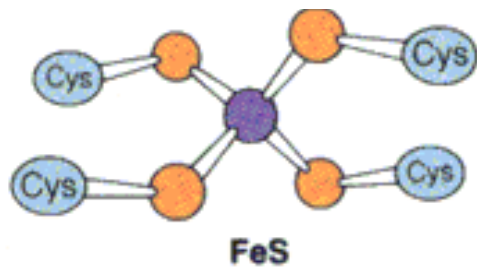
Electrons are “passed” thru five membrane protein complexes.



“Static” and “mobile” electron carriers.

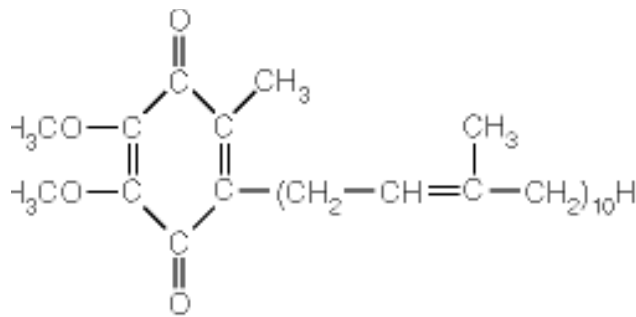
Complex I Summary



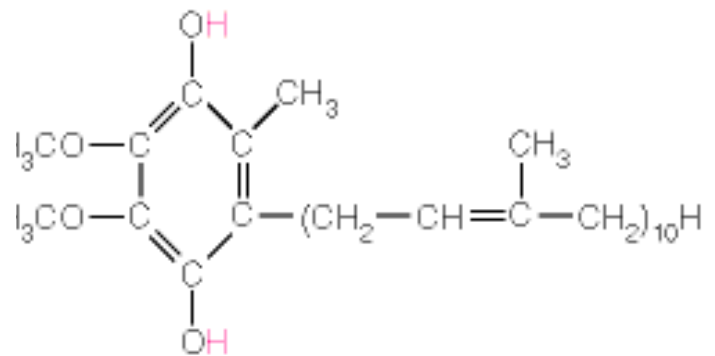
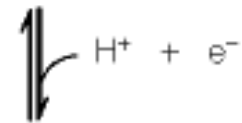


**Fe(II)/Fe(III)
Potentials
Depend on
Environment.**

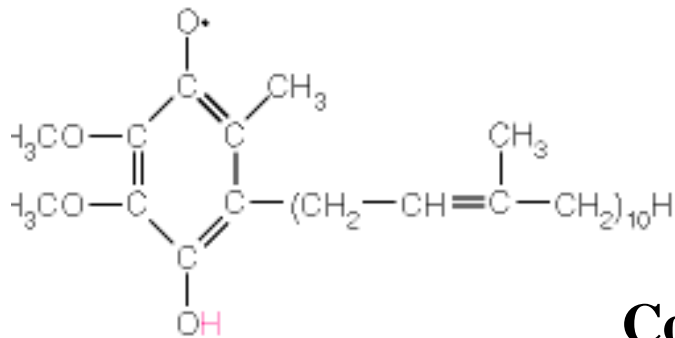
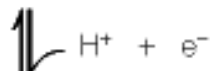
Cytochrome <i>b</i> (+3)	Cytochrome <i>b</i> (+2)	1	0.07
Dehydroascorbate + 2H ⁺	Ascorbate	2	0.08
Cytochrome <i>c</i> ₁ (+3)	Cytochrome <i>c</i> ₁ (+2)	1	0.23
Cytochrome <i>c</i> (+3)	Cytochrome <i>c</i> (+2)	1	0.25
Cytochrome <i>a</i> (+3)	Cytochrome <i>a</i> (+2)	1	0.29
½O ₂ + H ₂ O	H ₂ O ₂	2	0.30
Ferricyanide	Ferrocyanide	2	0.36
Nitrate + 2H ⁺	Nitrite + H ₂ O	1	0.42
Cytochrome <i>a</i> ₃ (+3)	Cytochrome <i>a</i> ₃ (+2)	1	0.55
Fe (+3)	Fe (+2)	1	0.77



Oxidized coenzyme Q₁₀ (CoQ)



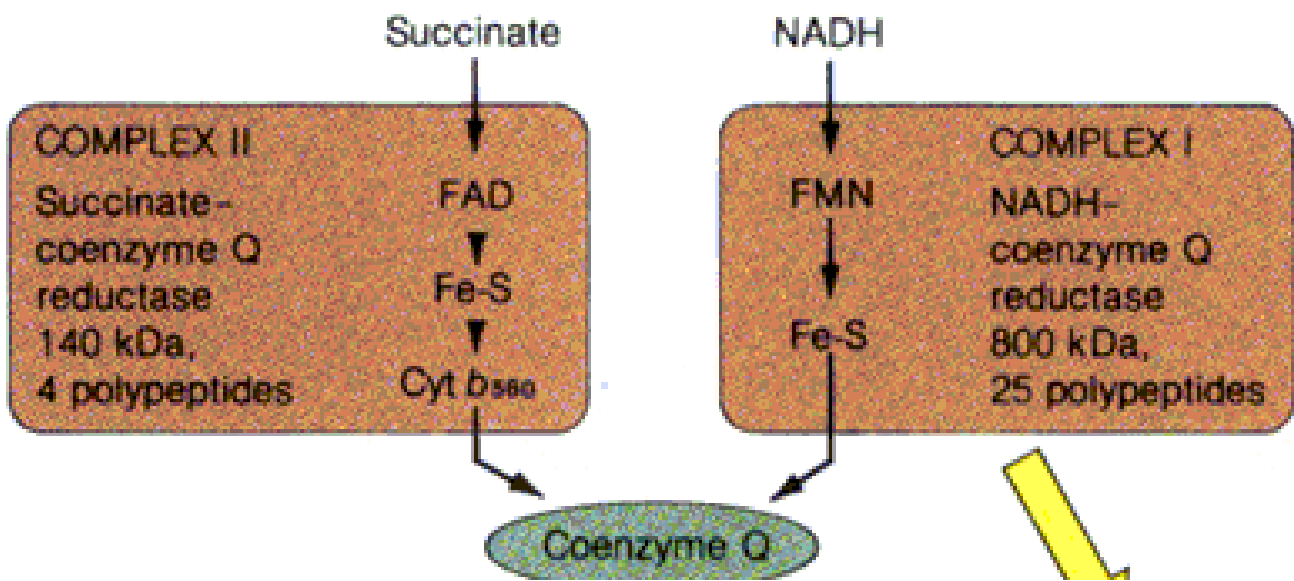
Reduced coenzyme Q₁₀ (CoQH₂)



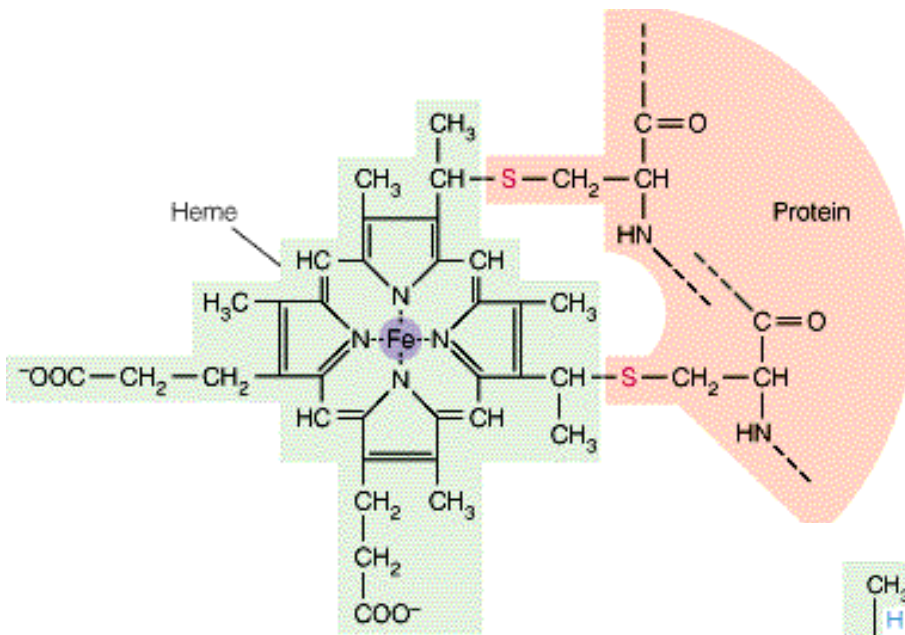
Semiquinone form of coenzyme Q

**CoQ diffuses freely in membrane.
But CoQ feeds electrons
To cytochromes 1 at a time.**

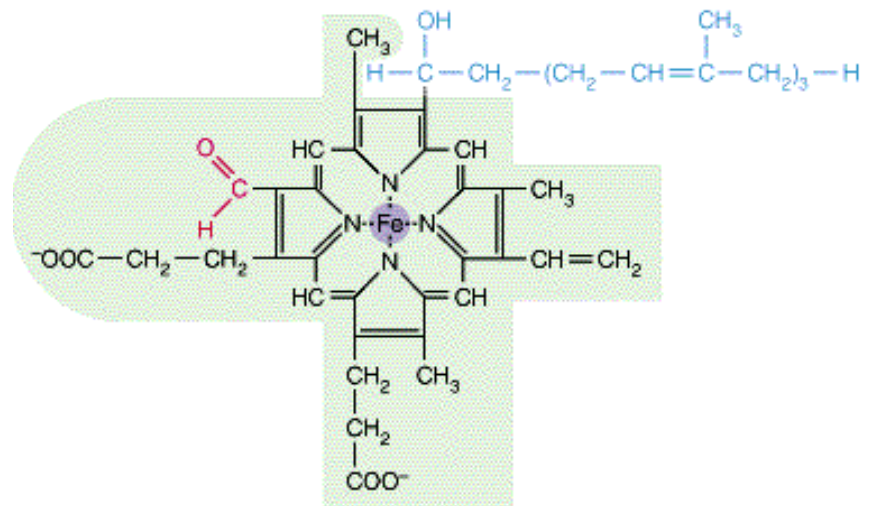
Reduced Substrates from citric acid cycle



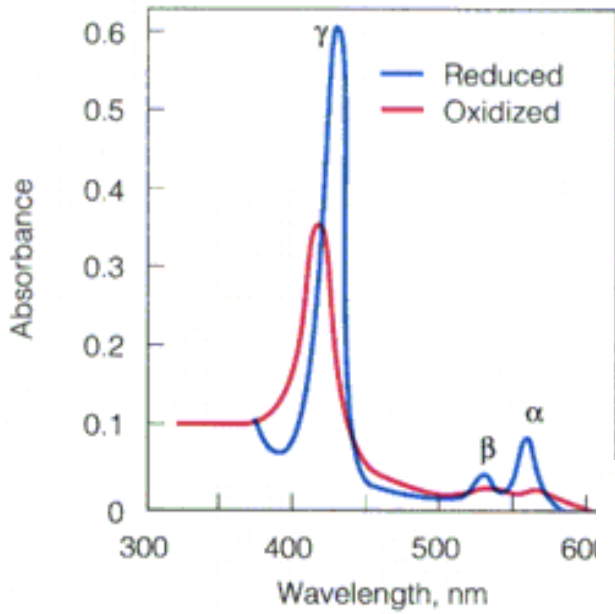
On to Complex III



**Slight HEME
and Protein
contact variations
alter E° .**

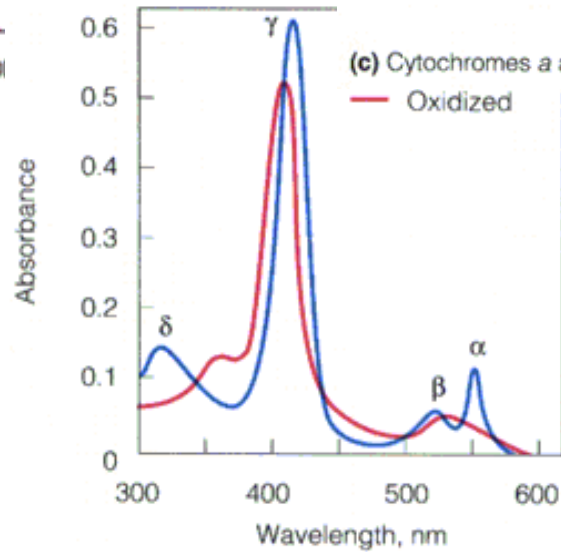
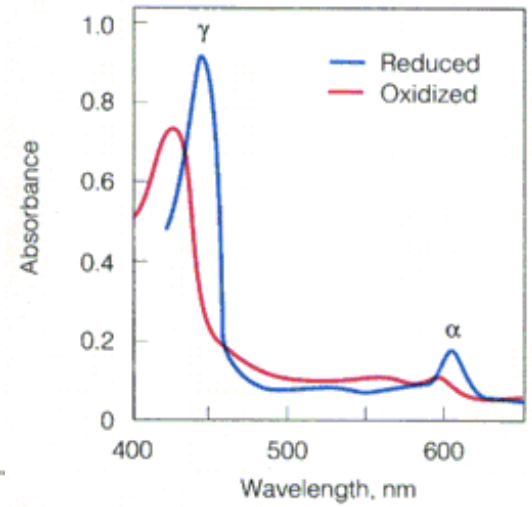


b) Heme A in cytochromes a and a_3



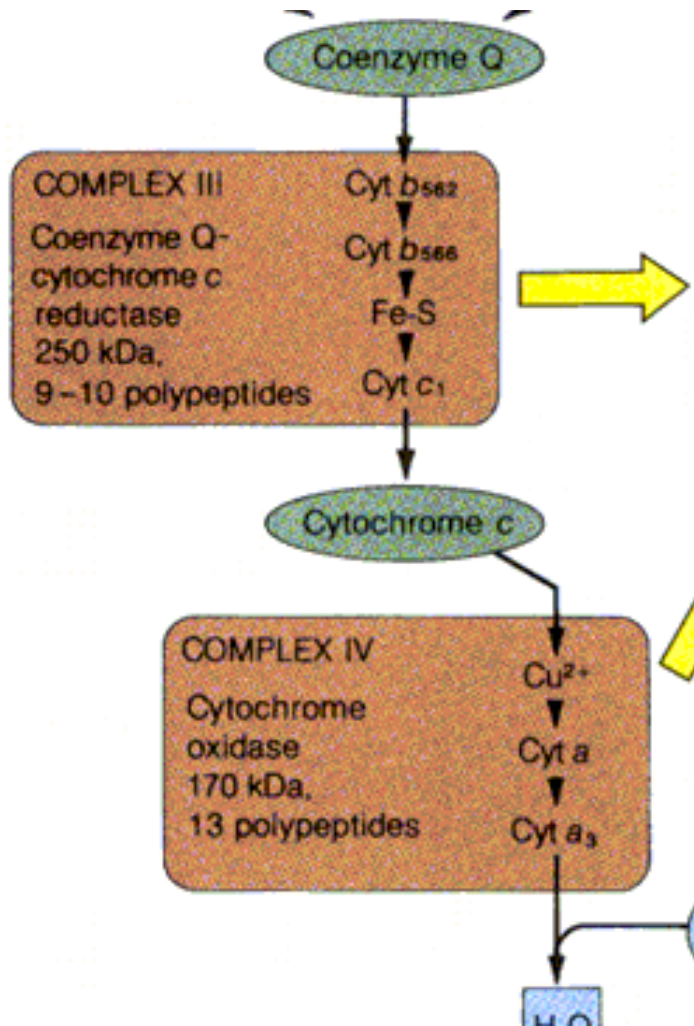
(a) Cytochrome *b*

Spectroscopic identification of oxidized and reduced cytochromes.



(b) Cytochrome *c*

(c) Cytochromes *a* and a_3



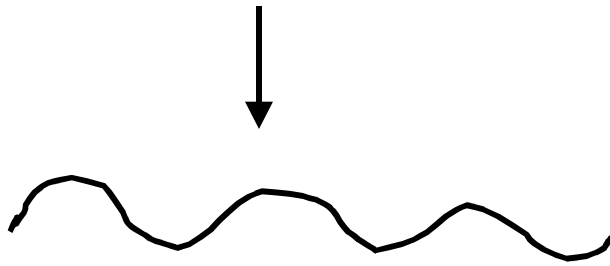
**The strange story
of cytochrome
oxidase in trypanosomes**

**Some subunit genes
nuclear, other mitochondrial
variation according to
species.**

**But *T. brucei* appeared to
be missing CO genes!!
but mRNA was found.**

**First known instance of
RNA editing.**

DNA--no start, no good ORF CO III gene, but correct
Gene neighbors on chromosome

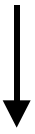


Additions
Deletions
Lots of U's

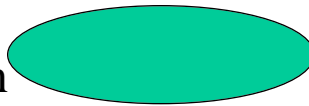


Editing
Guide RNAs to template???

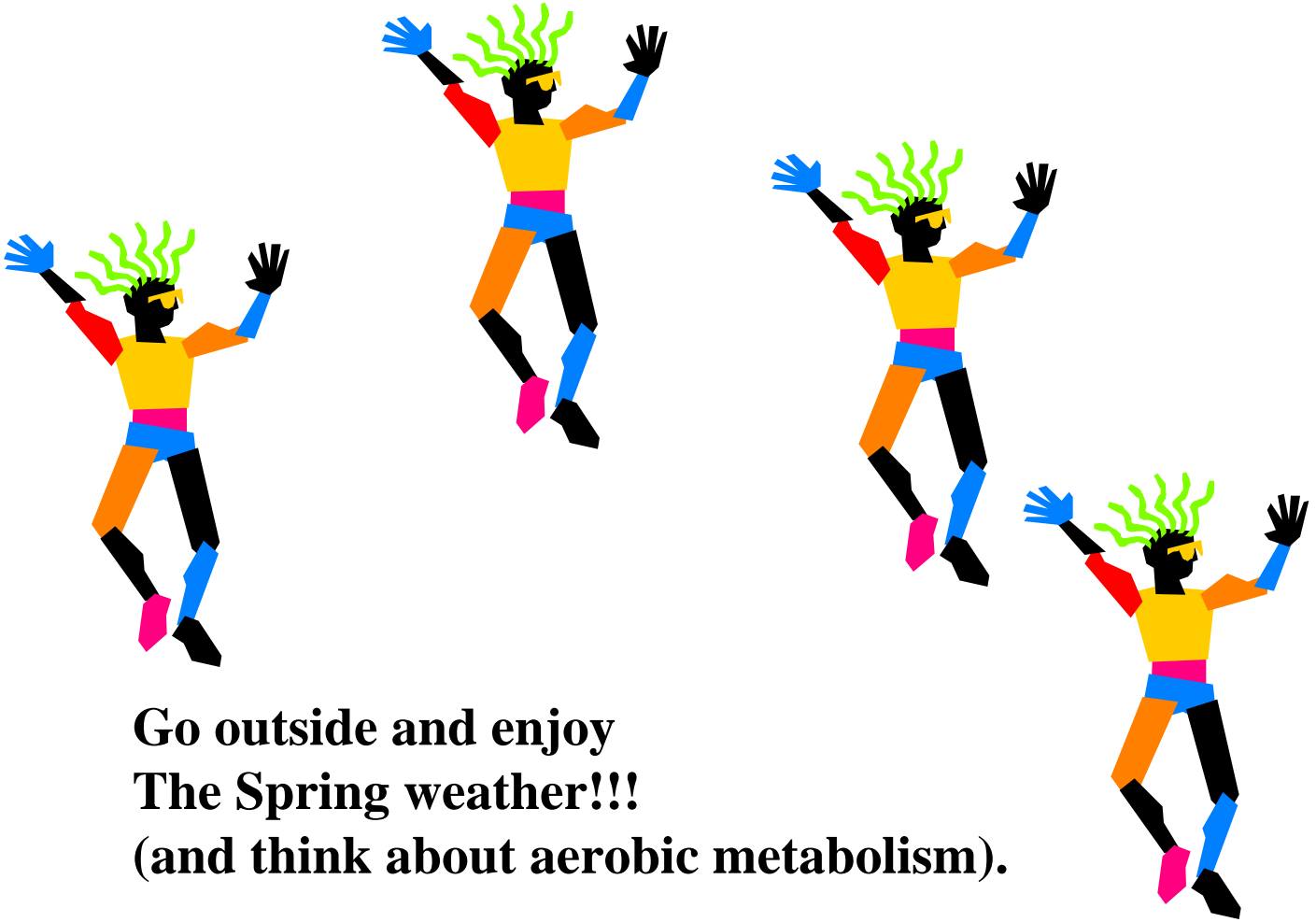
Pre-mRNA



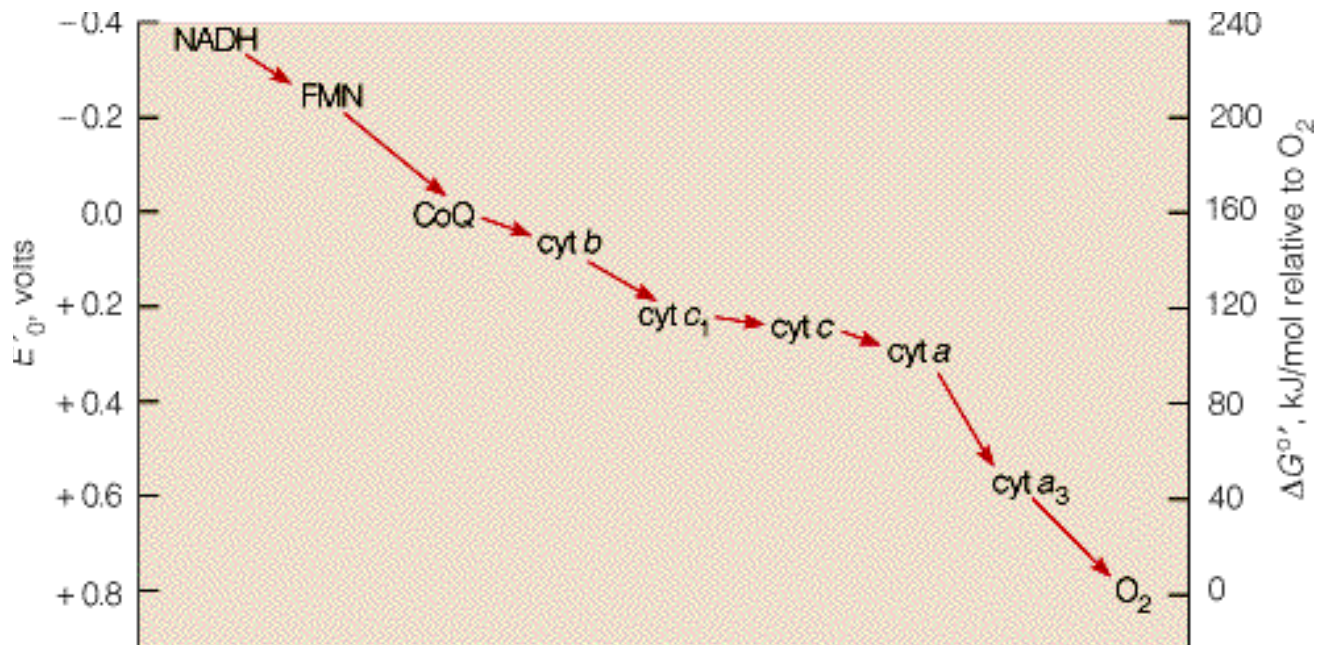
CO III protein



mRNA
Start AUG
ORF



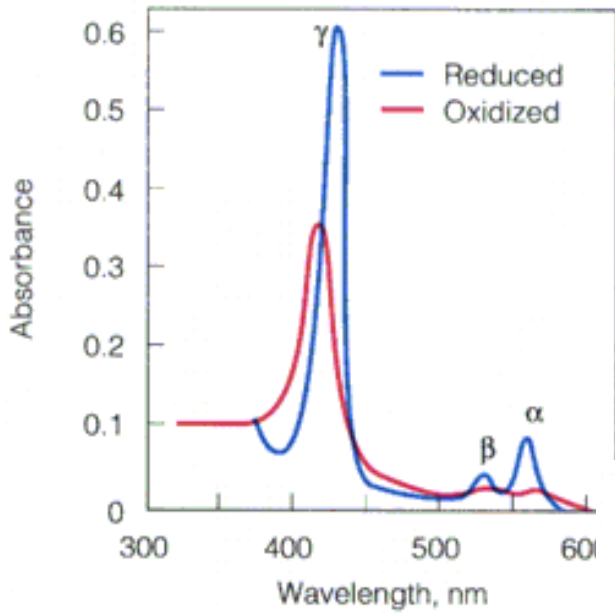
**Go outside and enjoy
The Spring weather!!!
(and think about aerobic metabolism).**



How to order reactions?

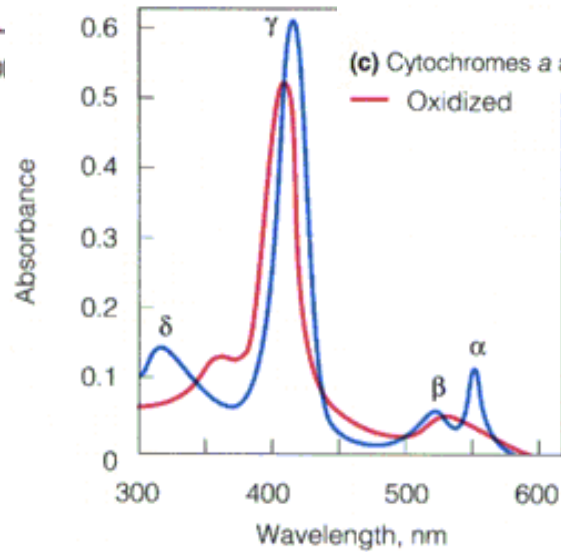
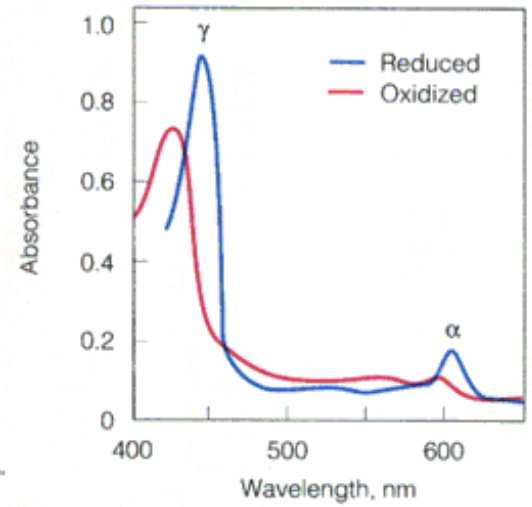
**** arrange in order of potential --all rxns exergonic (but only know standard states...)**

****Inhibit/activate pathway and see what is ox'd or reduced.**



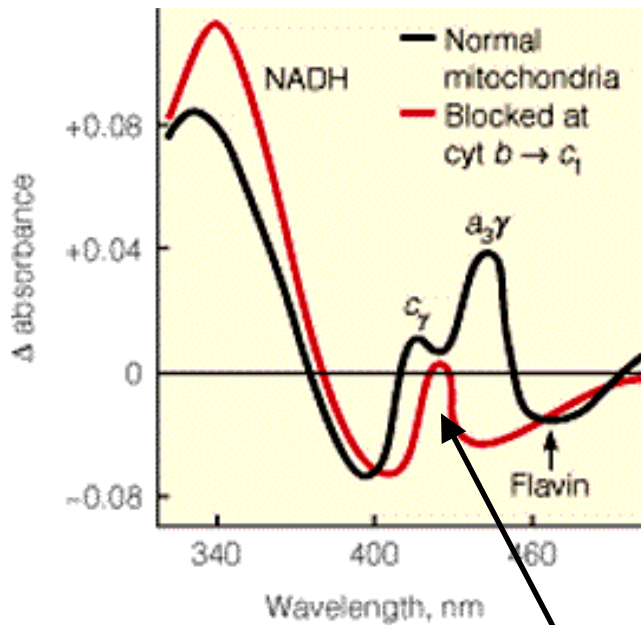
(a) Cytochrome *b*

Spectroscopic identification of oxidized and reduced cytochromes.

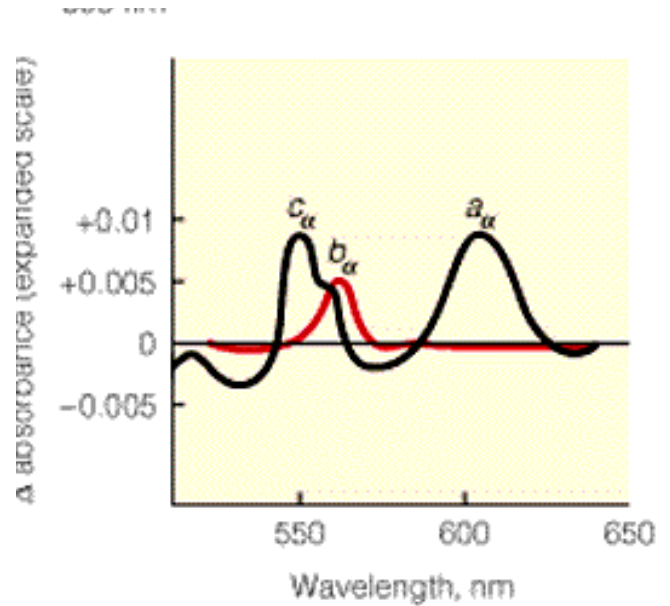


(b) Cytochrome *c*

(c) Cytochromes *a* and a_3

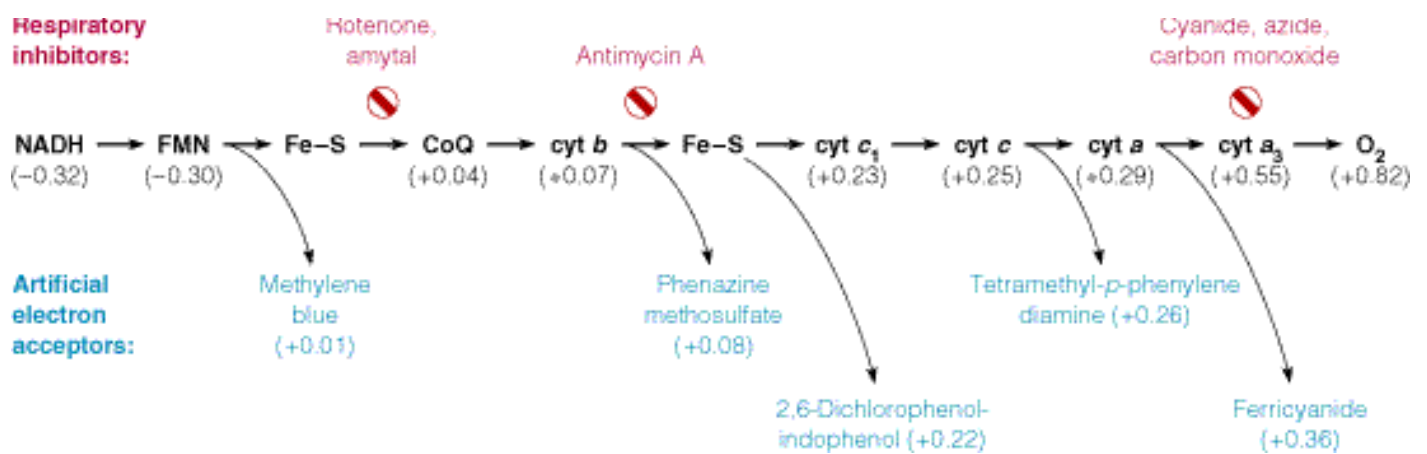


(a) Difference spectra for wavelengths below 500 nm



(b) Difference spectra continued with extended scale

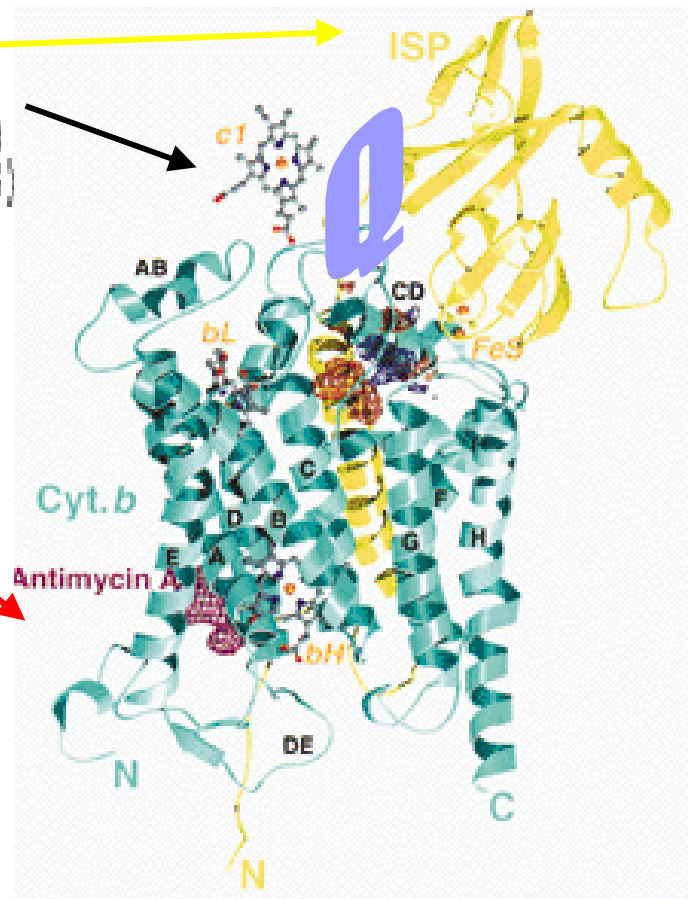
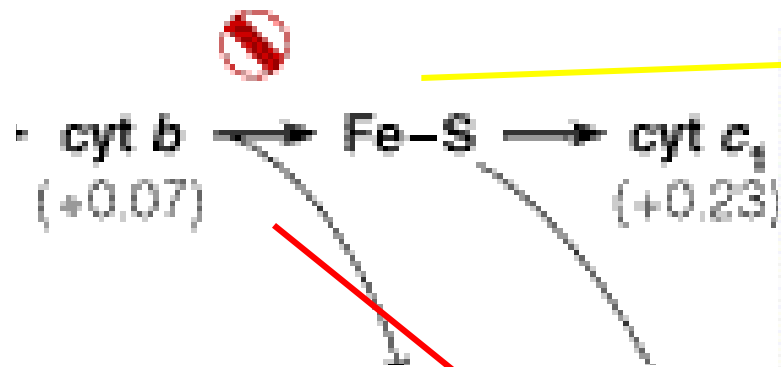
Technique invented by Britton Chance (UPENN)
 Goal--Find order of redox reactions from NADH to H₂O.
 Cyt b is reduced and c1 oxidized (see 410 nm)
 Black line is fully reduced compared to fully oxidized.



Add antimycin A-- NADH etc will be reduced, all acceptors following block will be oxidized.

Add methylene blue--NADH etc will be reduced, all electrons Diverted to methylene blue to remaining acceptors are oxidized.

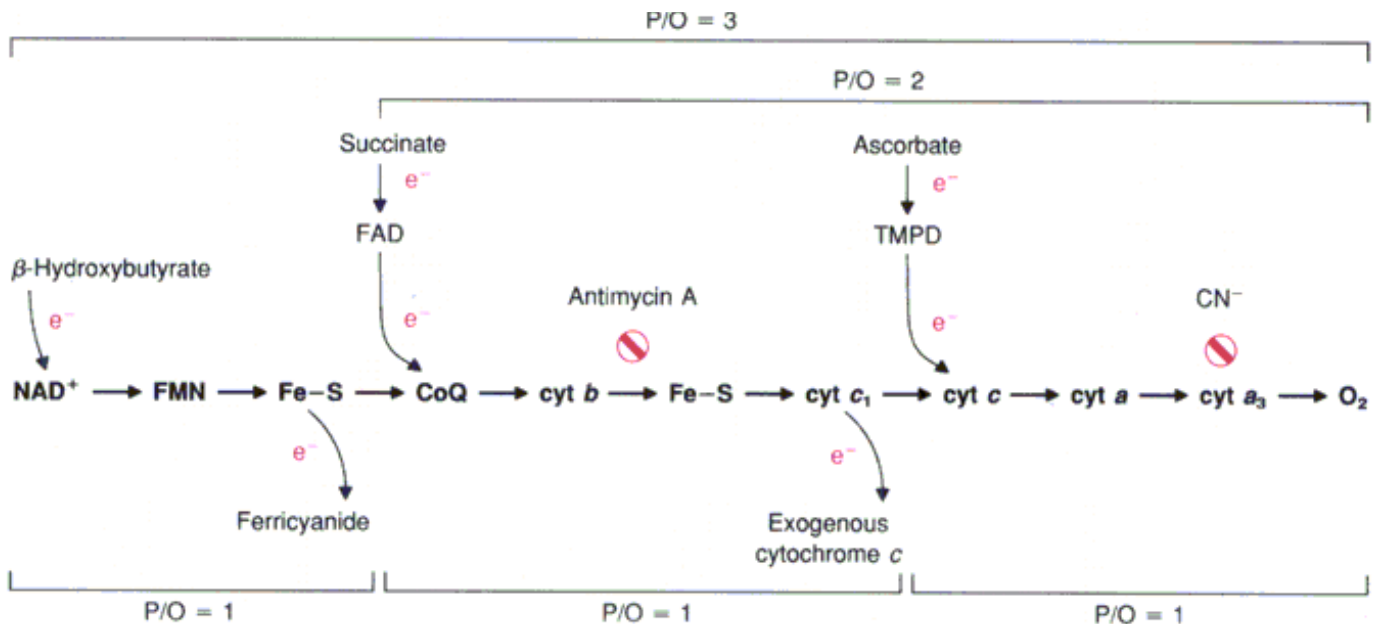
Antimycin A



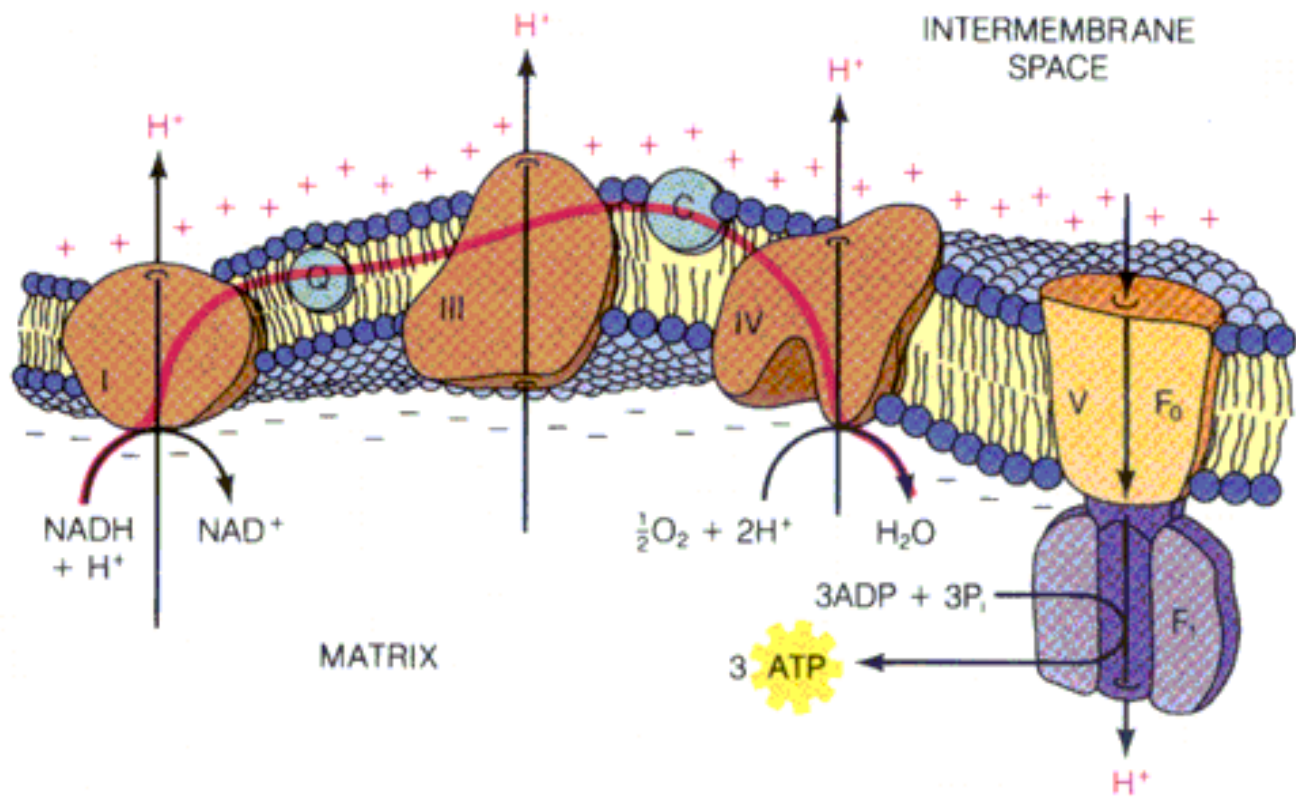
Decades of Fruitless Biochemistry

The Search for ATP “coupling sites”

Reconstitution Experiments--Complexes I, III, IV alone carry out enough respiration to form 1 ATP / electron pair



In real life these numbers need not be integers



Chemiosmotic Coupling Hypothesis (Mitchell 1961, Nobel 1978)

Chemiosmotic Coupling Hypothesis

Complexes I, II, III, & IV are asymmetric within membrane.

Complexes I-IV take protons from the mt. matrix and deposit them in the intermembrane space.

An electro ($\Delta\Psi$) chemical (ΔpH) gradient is created.

Energy is “stored” in the proton gradient.

Protons exergonically enter the mt. only thru Complex V driving ATP production.

Proton Pumping

Proton Motive Force = PMF

$$\begin{array}{rcc} +.224 \text{ V} & & .14 \text{ V} & & +.084 \text{ V} \\ \Delta\mu_{\text{H}} & = & \Delta\psi & - & 2.3RT \Delta\text{pH}/F \\ \text{Electrochemical} & & \text{Membrane} & & \text{pH gradient} \\ \text{H}^+ \text{ gradient} & & \text{potential} & & \end{array}$$

The pH is 1.4 units lower in the intermembrane space.

**+ .224 V corresponds to 21 kJ per mole of protons out >> in
2-12 protons may cross the membrane for each ATP synthesized.**

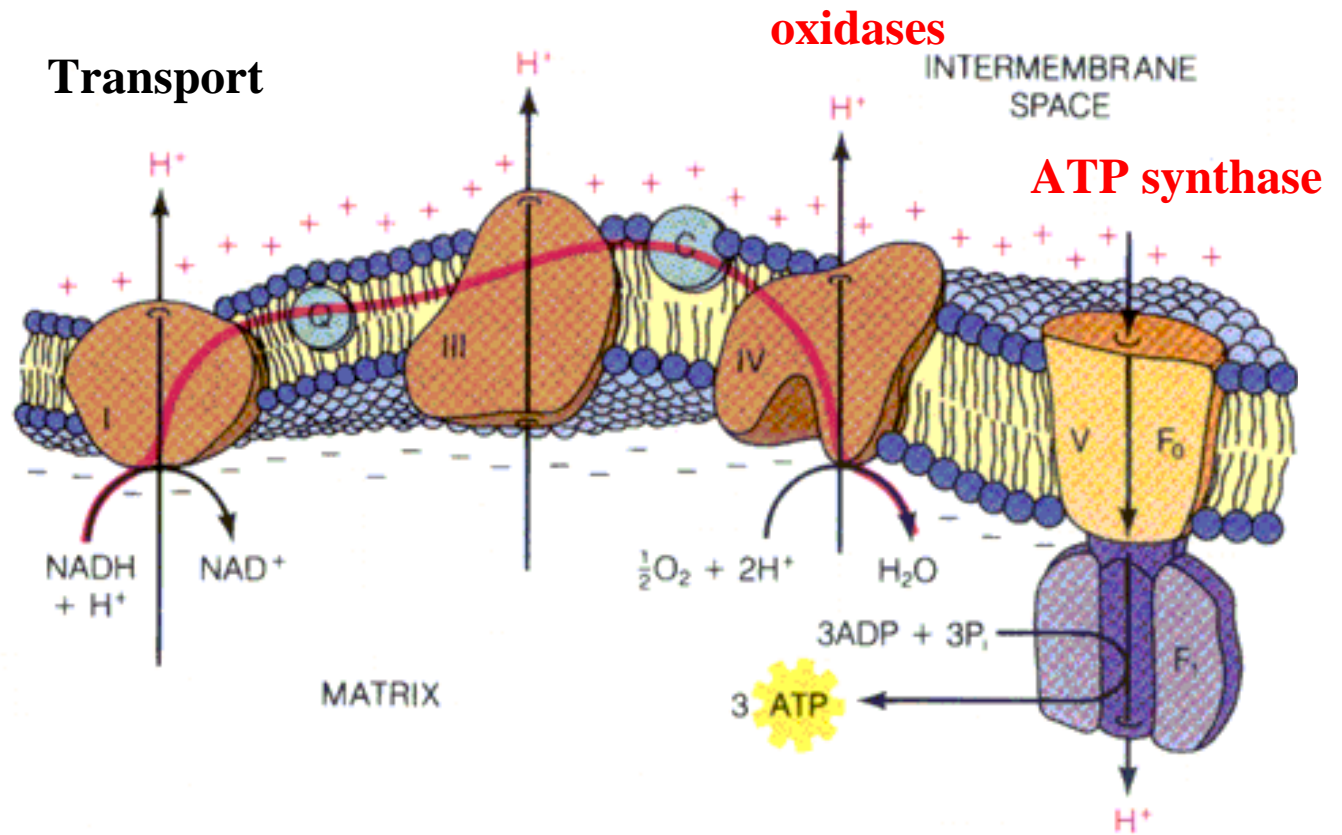
Predictions and Experiments

If a proton gradient is made in the absence of respiration, ATP synthesis should work.

Yes!

Yes!

If either the membrane potential or the pH gradient is reduced, less ATP synthesis will occur.



Chemiosmotic Coupling Hypothesis (Mitchell 1961, Nobel 1978)

Glycolysis thru TCA cycle yield

Net:



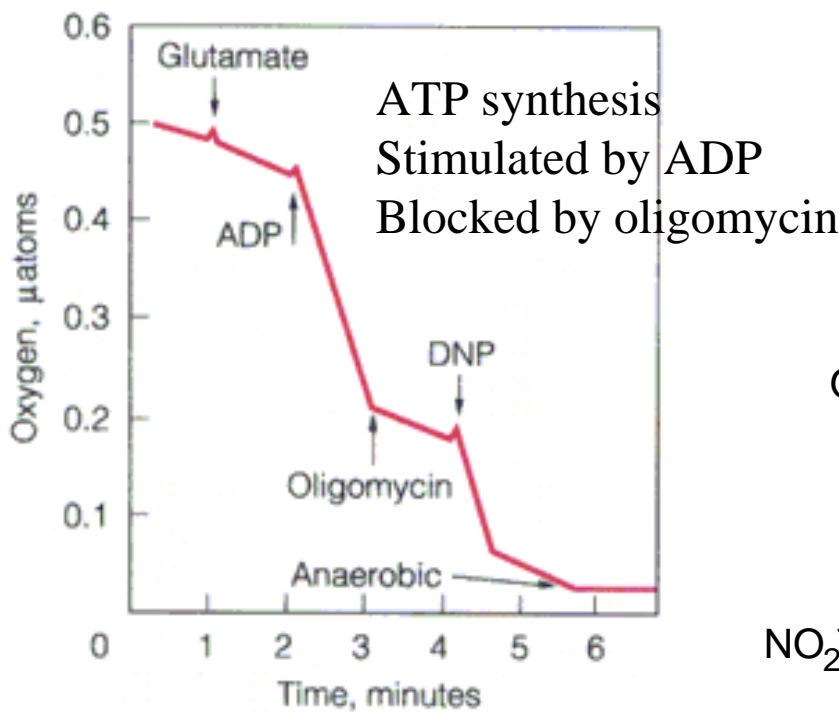
↑
About 3 ATP from each

↑
About 2 ATP from each

Glycolysis thru respiration yield.

About 38 moles ATP per mole glucose

(about 40% efficient assuming standard states)



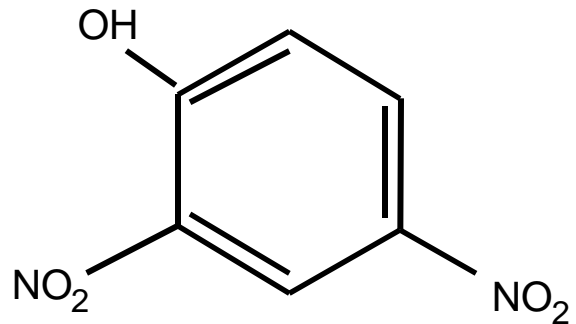
ATP synthesis
 Stimulated by ADP
 Blocked by oligomycin

Decouplers

Valinomycin K^+

Nigericin K^+/H^+

Dinitrophenol H^+



Protonated form is lipophilic
 and traverses membrane bearing proton.

Production of a proton gradient (one example)

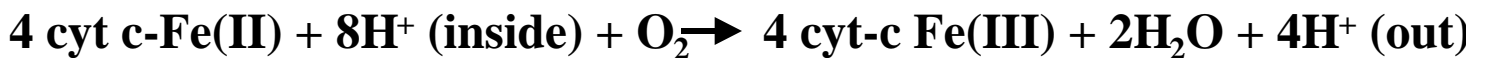
**Cytochrome oxidase (based on xtal structures
Of oxidized and reduced proteins.)**

Asp 51 delivers proton to “outside”

Ser 441 and Asp 51 sidechains rotate

Tyr 54 takes in a proton from the inside

How to “prove” this mechanism?

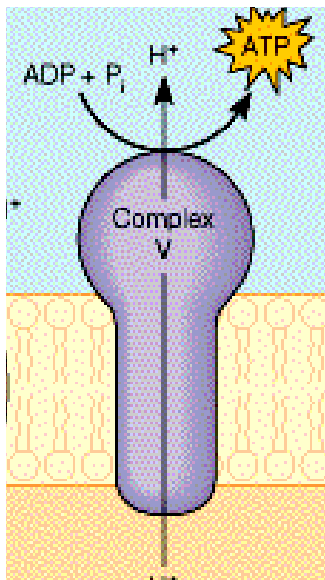


ATP Synthase

EM--"knobs" on matrix side of inner mt. Membrane

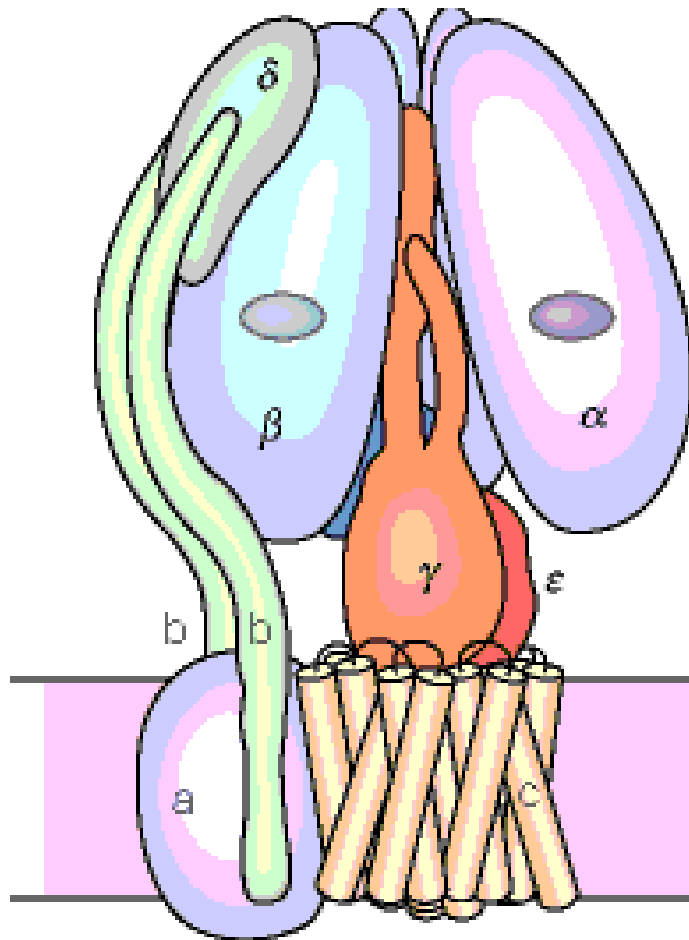
Removal--no ATP synthesis

Put them back--ATP synthesis restored.



Knobs = F1 subunit

Base= F0 subunit



Complex V ATP synthase

F₁

Knobs = F1 subunit
 $\alpha_3\beta_3 \gamma\delta\epsilon$
 $\alpha_3\beta_3$ “6-fold symmetry”
Stalk - $\gamma\epsilon$
Base- $a b_2 c_{12}$

Coupling

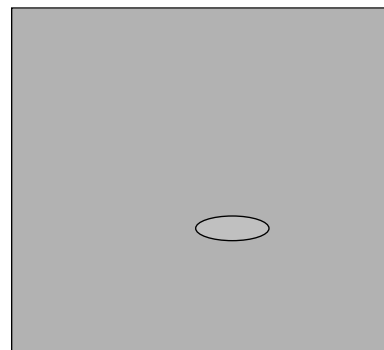
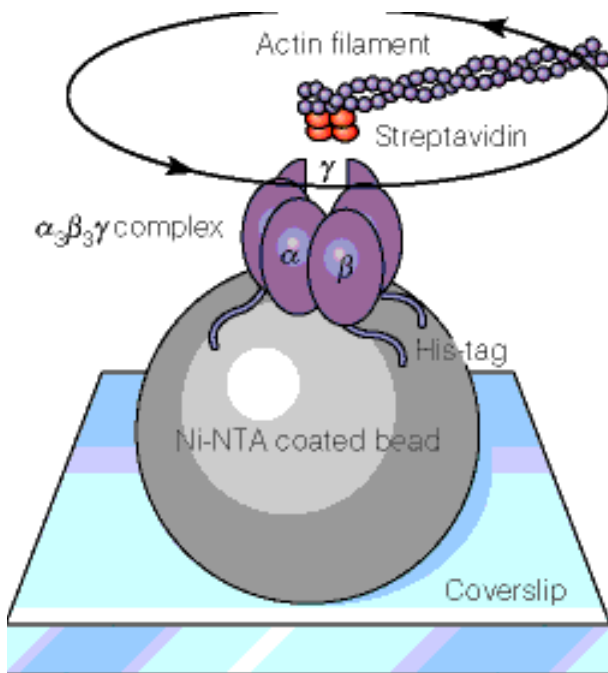
Protons passing thru F₀
Drive Δ conformation
Rotation of γ
 Δ conformation of ADP
Binding sites.

F₀

Evidence that Complex V is a rotating motor.

1) There are lots of movies on the WWW.

2) Biotin labeled γ protein /streptavidin/ long fluorescent actin His-tagged β bound to big Ni-bead immobilized.



What the movies look like
(uncolorized)

Rotating Actin Observations

Rotation is quantized in 120° steps.

**If ATP is used rotation is one way
(and hydrolysis occurs)**

Original experimental observation

**If ADP is used rotation is in the opposite direction
(and ATP synthesis occurs)**

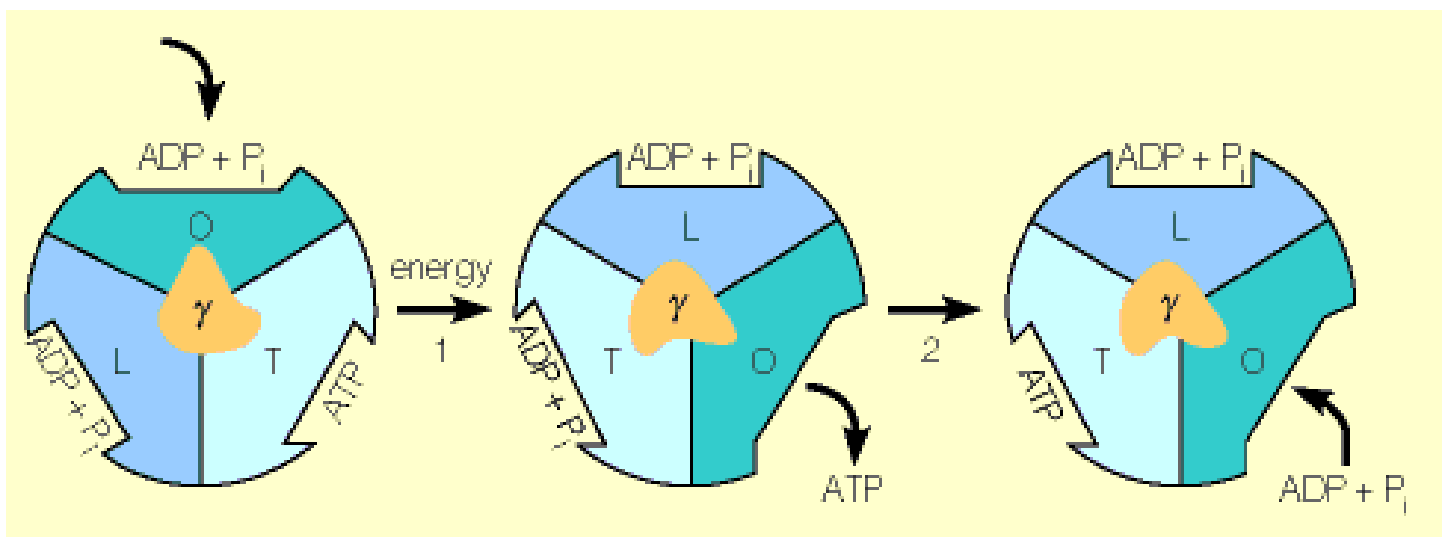
ATP synthase conformational changes

3 $\alpha\beta$ sites--1 each in Loose, tight, open conformations

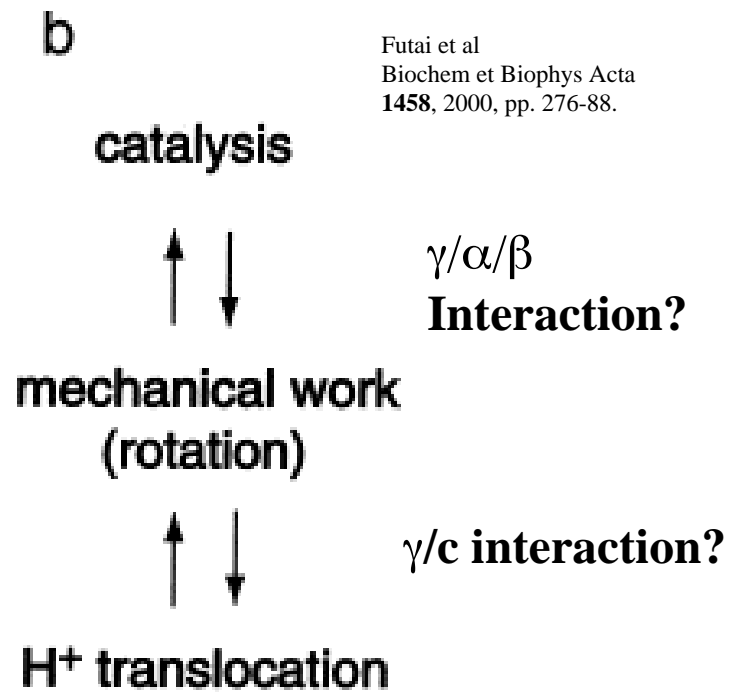
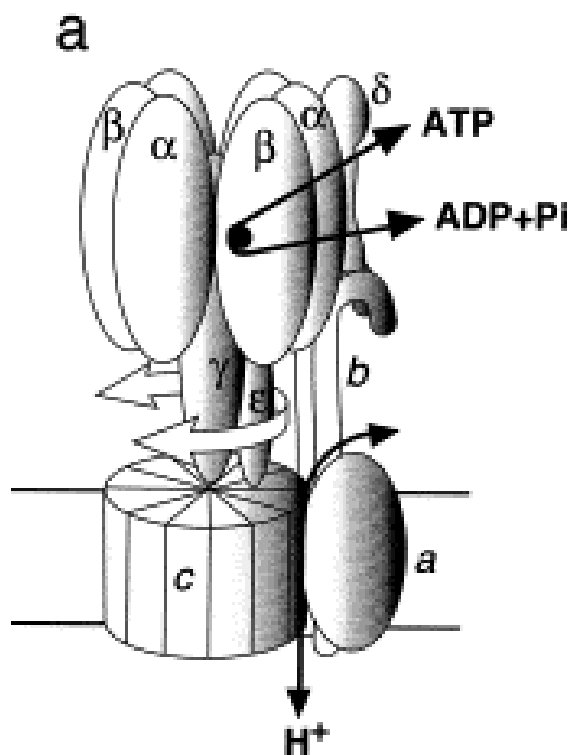
Proton movement drives γ rotation, ATP release (T to O Δ conf.)

Rotation of γ subunit causes sequential $\alpha\beta$ conformational Δ 's.

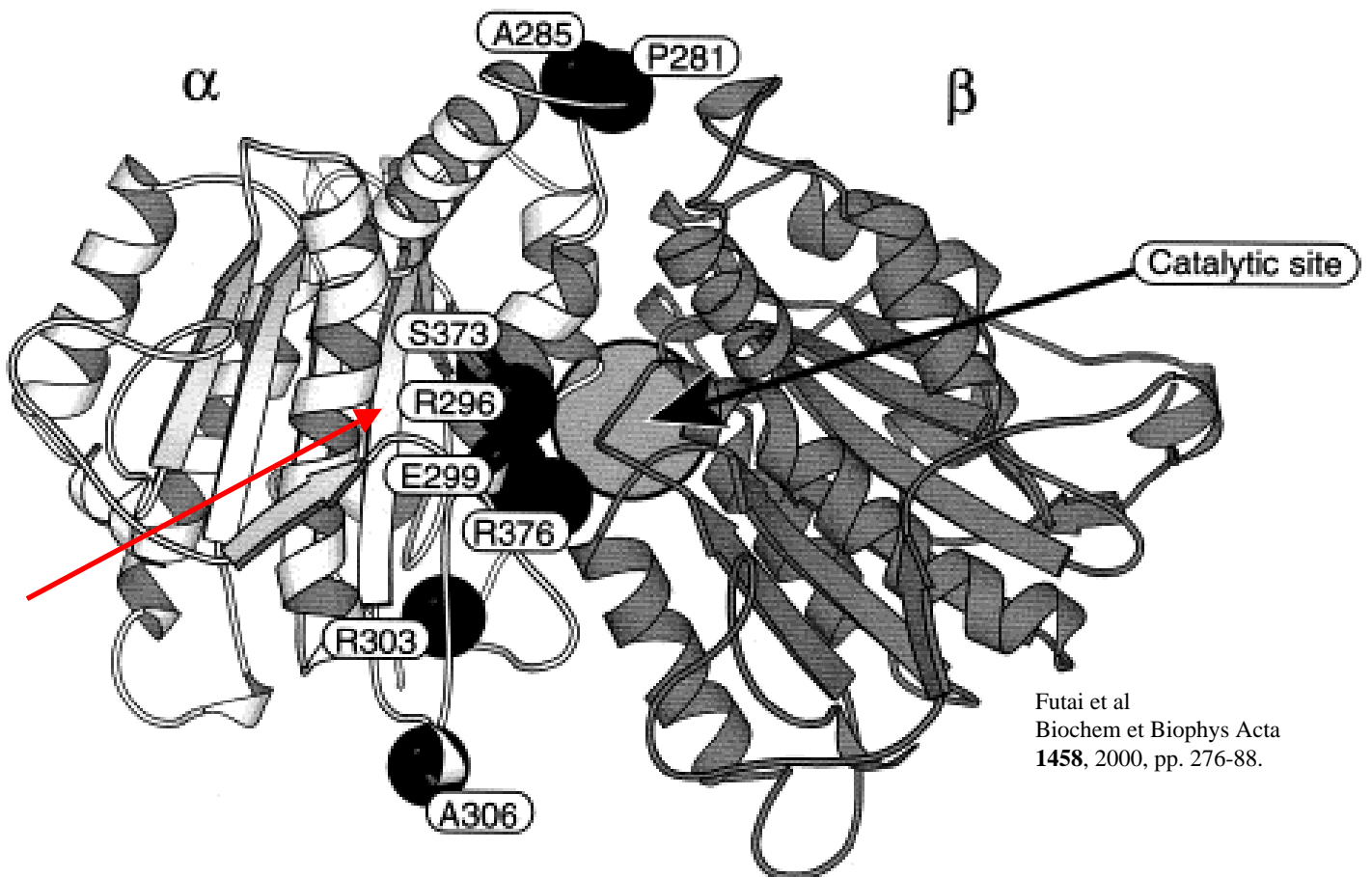
ATP release requires energy (not ATP synthesis).



Walker and Boyer 1997 Nobel Prize

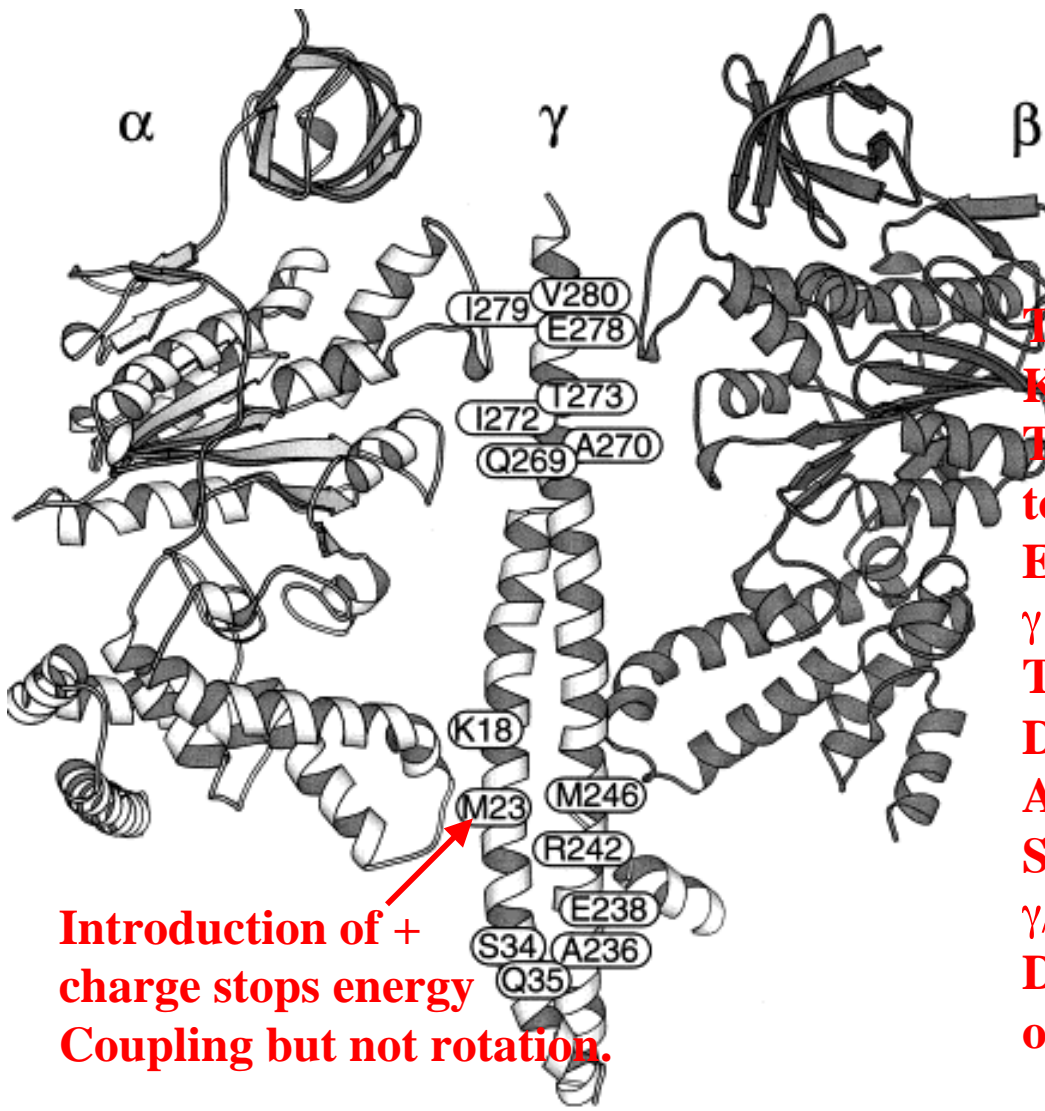


Three processes are normally coupled, but mutation can disrupt this coupling.



Futai et al
 Biochem et Biophys Acta
 1458, 2000, pp. 276-88.

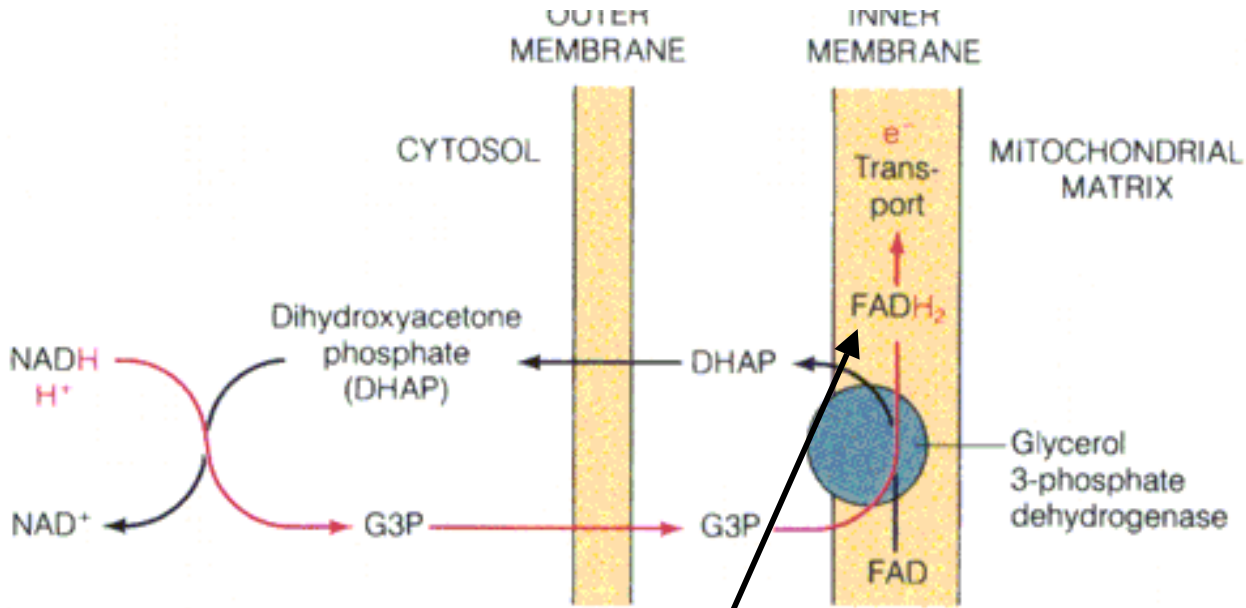
Mutants Ser 174 Phe and Arg 296 Cys catalyse but do not transmit conformation and don't couple with proton translocation. Both mutants together restore subunit cooperativity and coupling.



**Introduction of +
 charge stops energy
 Coupling but not rotation.**

**The g subunit is
 Key to holding
 The complex
 together.
 Early evidence for
 γ rotation--form
 Then remove γ/β
 Disulfide link. Allow
 ATP hydrolysis or
 Synthesis. Reform
 γ/β S-S link. Find
 Different
 orientation.**

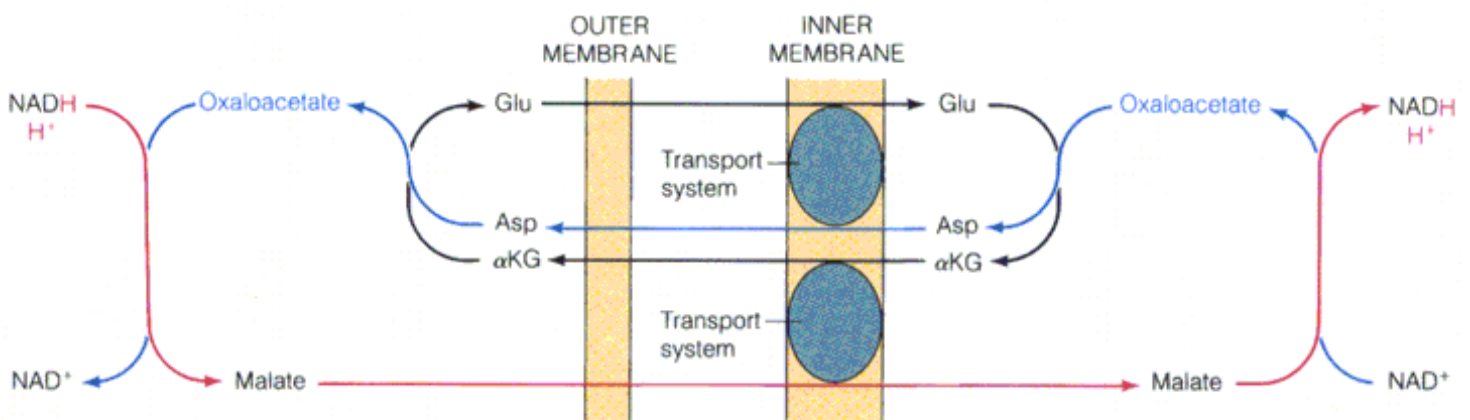
**Mitochondria import electron pair of NADH indirectly
Glycerol 3 phosphate is imported and reoxidized.**



(a)

Loss of efficiency
NADH to FADH₂

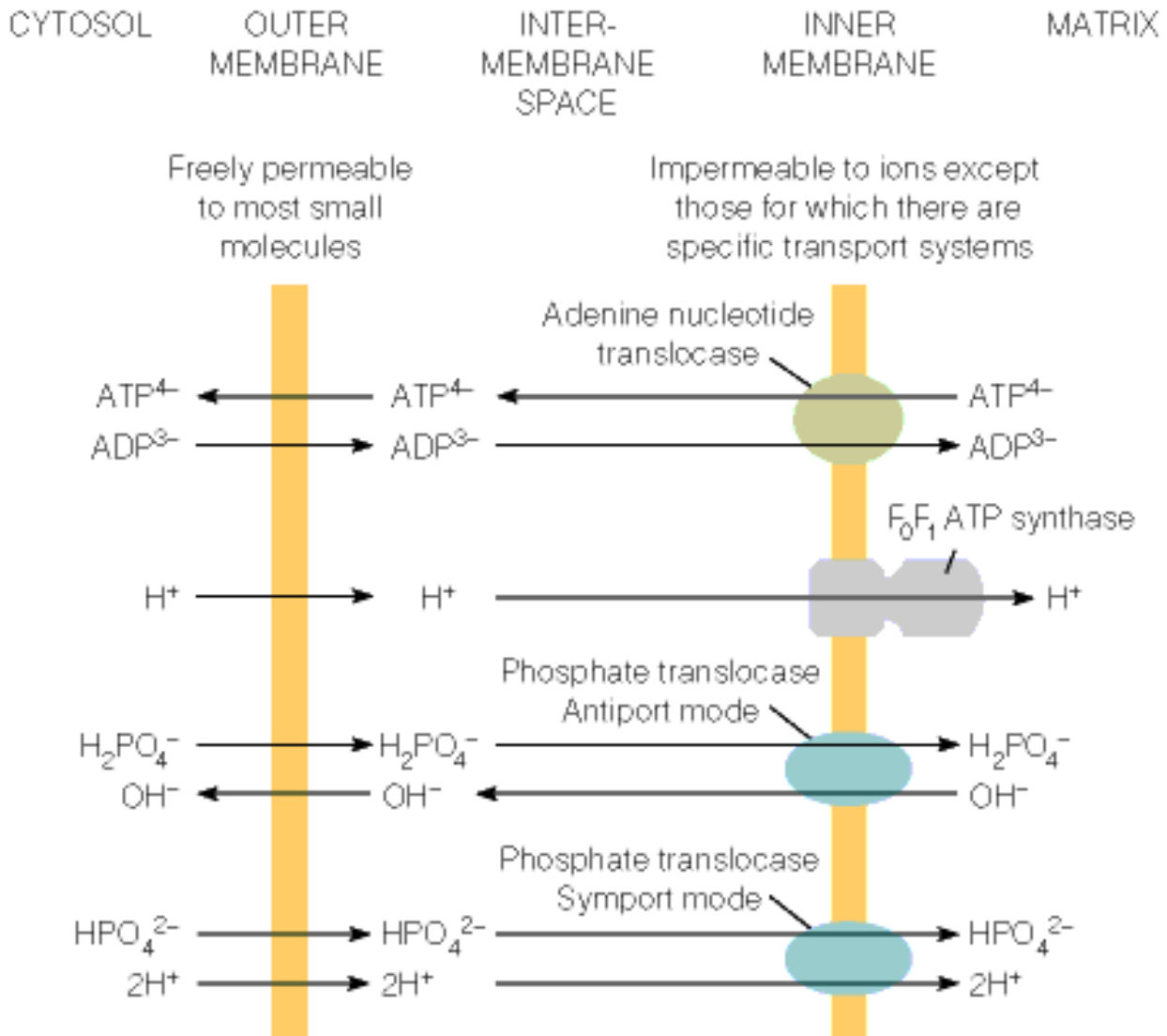
Transport systems for malate (in) and aspartate (out)



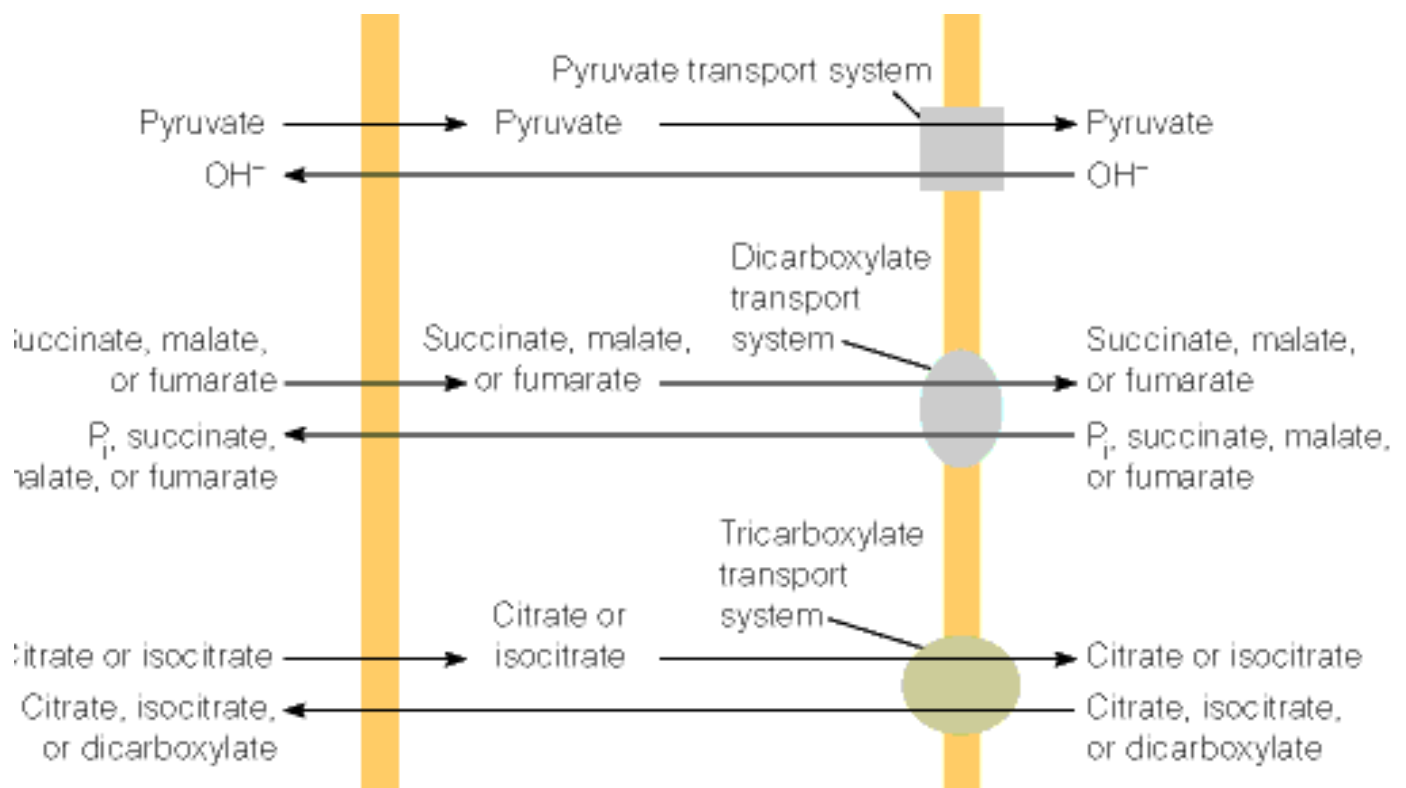
NADH reduces oxaloacetate to form malate.

Malate is transported in, then re-oxidized to form oxaloacetate and NADH.

Oxaloacetate is transaminated & the resulting Aspartate is transported out.



**Outer membrane is very leaky, but inner membrane is not--
Membrane proteins must transport small molecules.**



90% of O₂ used in respiration

10% used to incorporate O

Monooxygenases--1 O (other to H₂O)

Dioxygenases--both O's

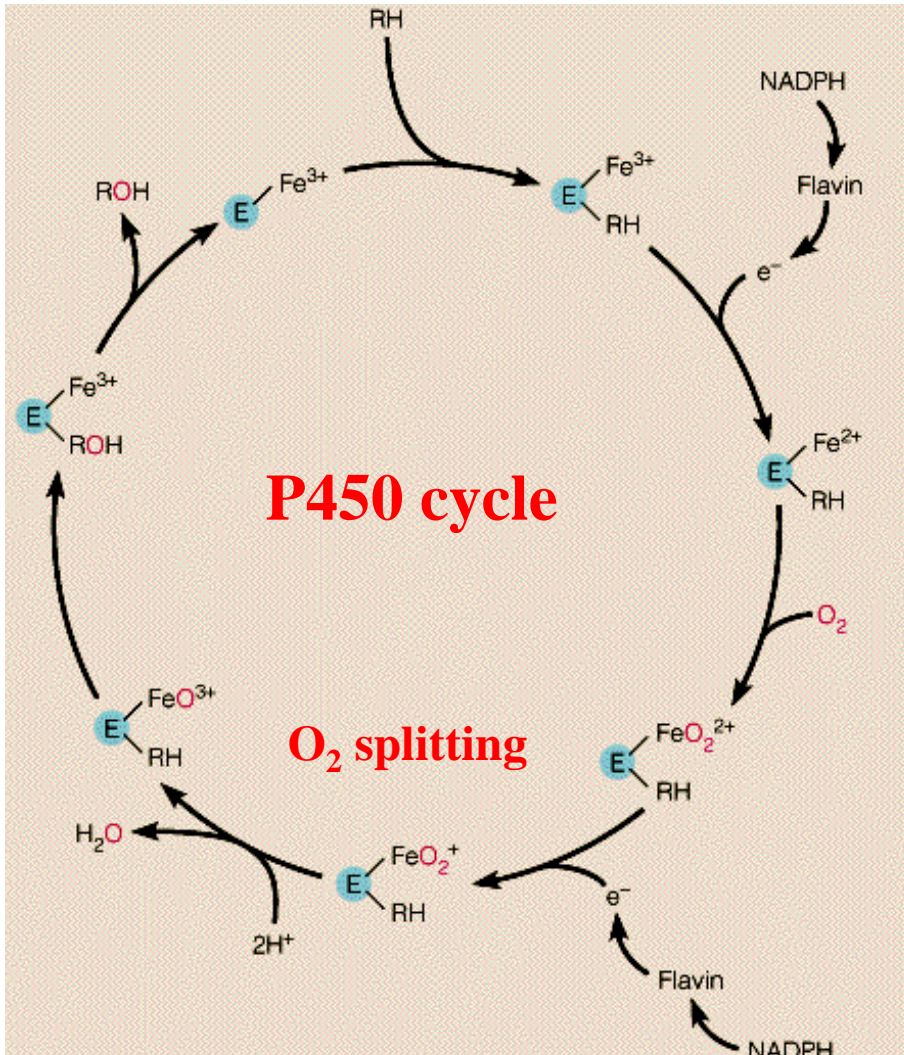
Cytochrome P450's

Family absorbs 450 nm

Binds O₂ and CO

Heme with Cys-S⁻ (thiolate)

**Hydroxylate normal metabolites (steroids)
and foreign molecules (aflatoxin)**



RH → ROH

Why flavin?

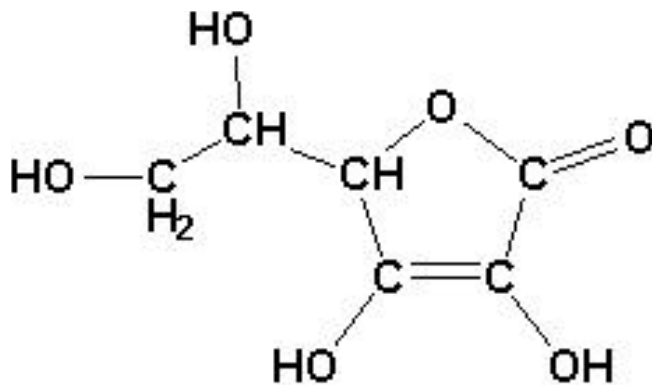
Oxidative Damage to membranes, proteins, DNA

**~ 1-2% of respiration electrons detour to form
Reactive, potentially dangerous free radicals (HO·,
Peroxides (HOOH), superoxides O₂⁻)**

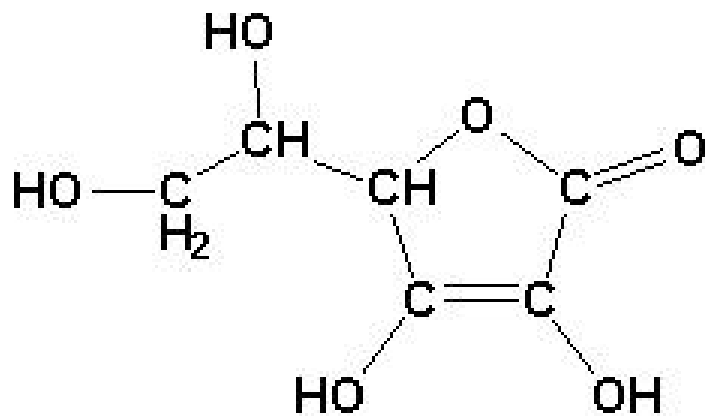
Chemical Defenses

Glutathione--oxidizes to -S-S-

Ascorbic Acid (vitamin C)--oxidizes OH to =O



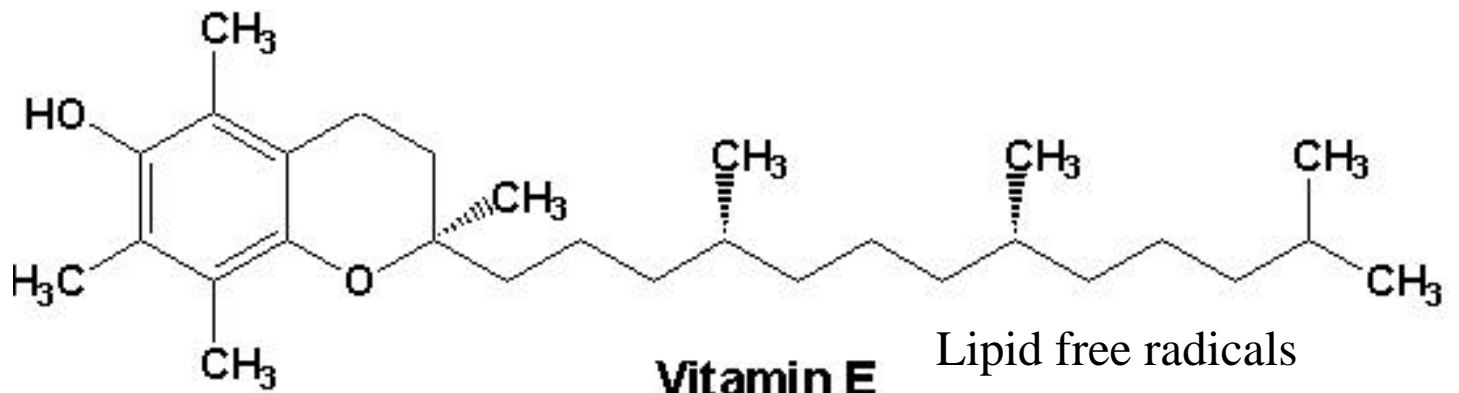
Ascorbate



Ascorbate

Major antioxidant

Collagen synthesis
(hydroxyproline)

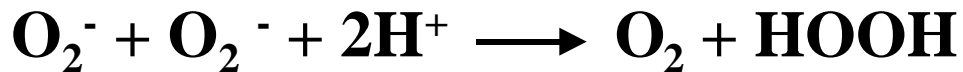


Vitamin E

Lipid free radicals

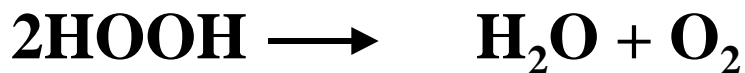
Enzymatic Defenses

Superoxide Dismutase



Versatile co-factors according to species Fe, Mn, Cu, Zn

Peroxidases and catalases



**Phagocytosis (white blood cells killing the bad guys bacteria)
Uses free radical species, superoxide and respiratory burst
(to get lots of O₂)**