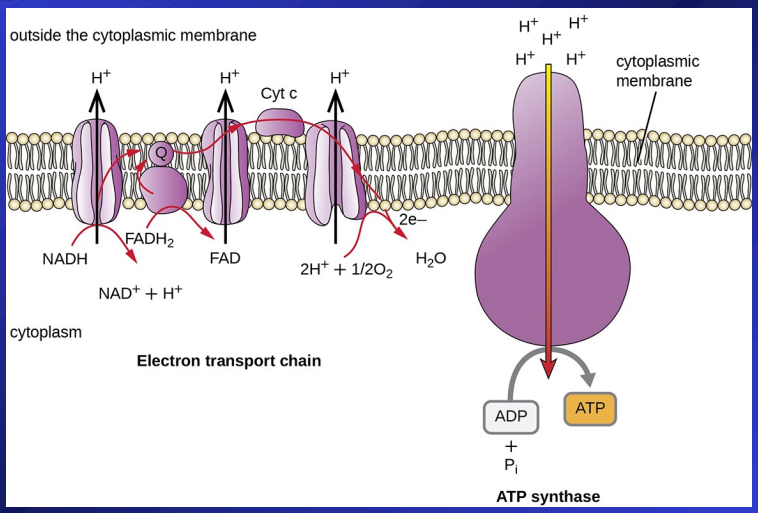
ETS / OxPhos

Electron Transport System and Oxidative Phosphorylation

- How we use the reducing equivalents (NADH or FADH2) to generate ADP + Pi -> ATP



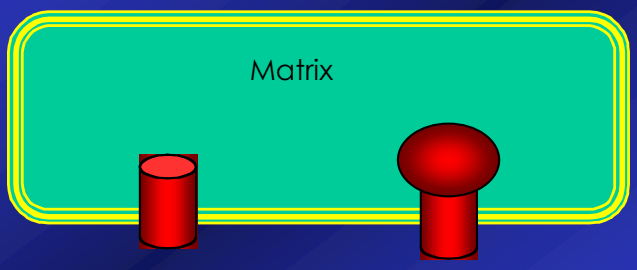
1

Electron transport and Oxidative phosphorylation or As the electron turns

Introduction:

The complete oxidation of glucose carbons by glycolysis and the citric acid cycle can be written as

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$



The reducing equivalents (electrons) are captured in the form of reduced co-enzymes (NADH and $FADH_2$) which eventually transfer electrons to molecular oxygen

$$6O_2 + 24H^+ + 24e^- \rightarrow 12\ H_2O$$

2

This process regenerates NAD^+ and FAD and generates a proton gradient across the inner mitochondrial membrane, whose dissipation provides the free energy for ATP synthesis. This process is known as oxidative phosphorylation.

Oxidative phosphorylation - the combined actions of:

- Electron transport - ETS - (the transport of e^- from reducing equivalents to O_2)
- Harnessing the chemical and electrical potential produced by the ETS
- O_2 is ultimate e- acceptor and drives ATP formation thus oxphos

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Where do the equivalents come from?

- glycolysis
- TCA
- ß oxidation of fatty acids

ETS and Oxphos tightly coupled via the H^+ gradient - can be uncoupled by poisons and inefficient coupling leads to heat

4

NADH shuttle

Much of the reducing equivalents is produced in the cytosol and needs to be shuttled into the mitochondria - this happens by one of two means

- **The malate-aspartate shuttle** allows NADH to be indirectly transported into the mitochondrion by reducing OAA to malate and transporting malate across the inner mitochondrial membrane. OAA is then transaminated to asparate and then shuttled back to the cytosol

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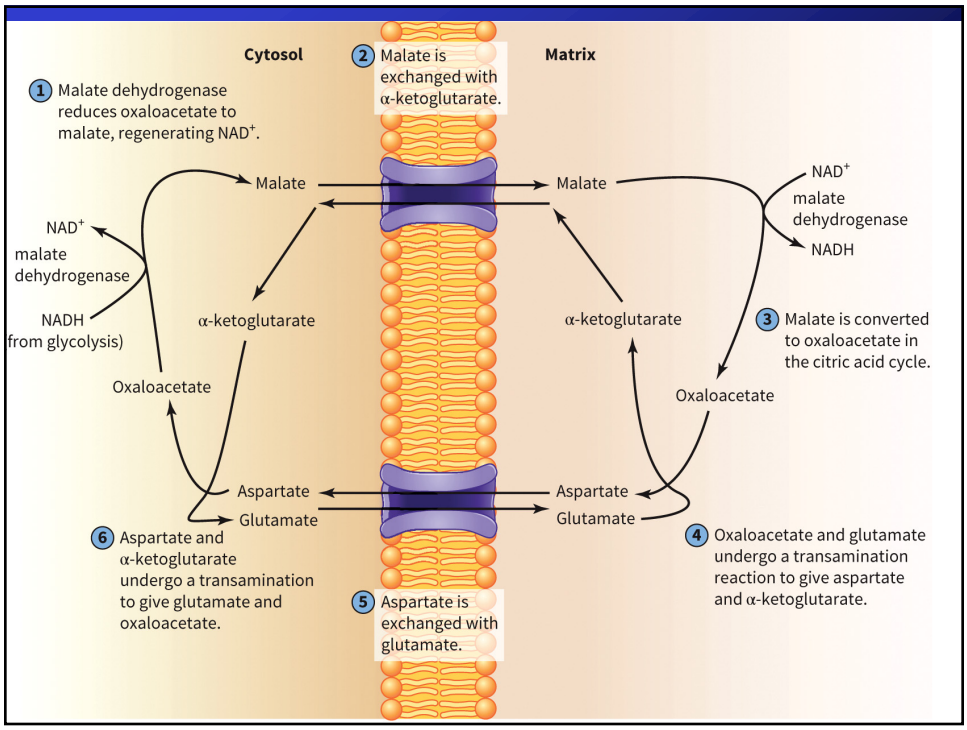

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NADH shuttle

Much of the reducing equivalents is produced in the cytosol and needs to be shuttled into the mitochondria - this happens by one of two means

- **The glycerophosphate shuttle** first reduces cytosolic dihydroxyacetone phosphate (DHAP) to 3-phosphoglycerate and NAD+. The 3-phosphoglycerate is oxidized by an inner mitochondrial membrane enzyme, flavoprotein dehydrogenase, which introduces electrons directly into the ETS via $FADH_2$.

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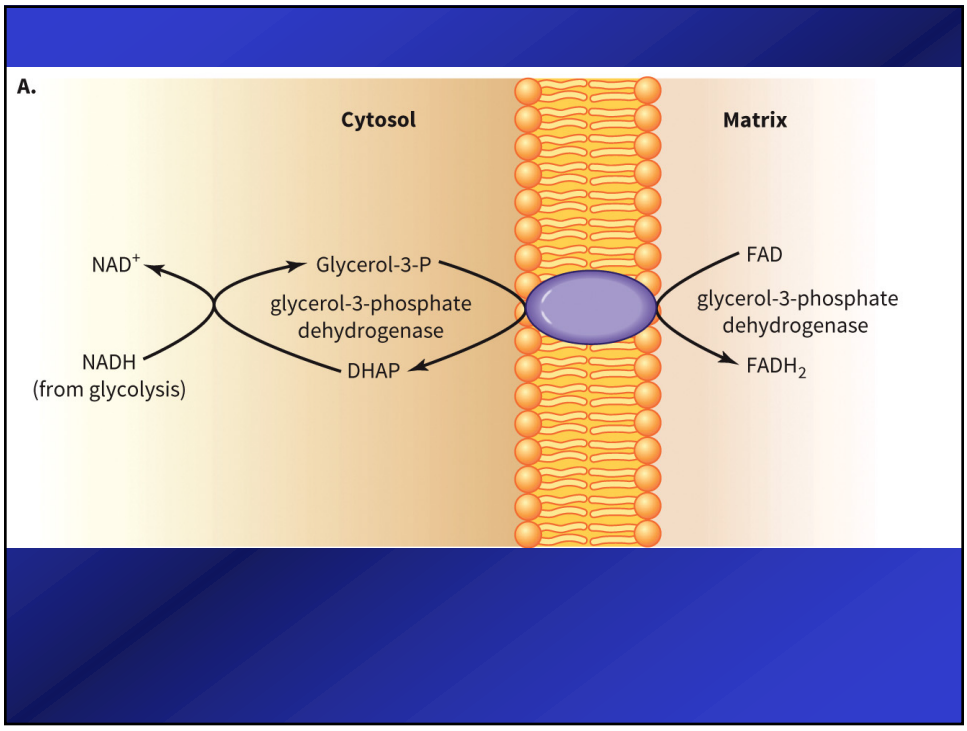

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How is ATP made from NADH/FADH/FADH$_2$?

Energy storage in the form of electrons

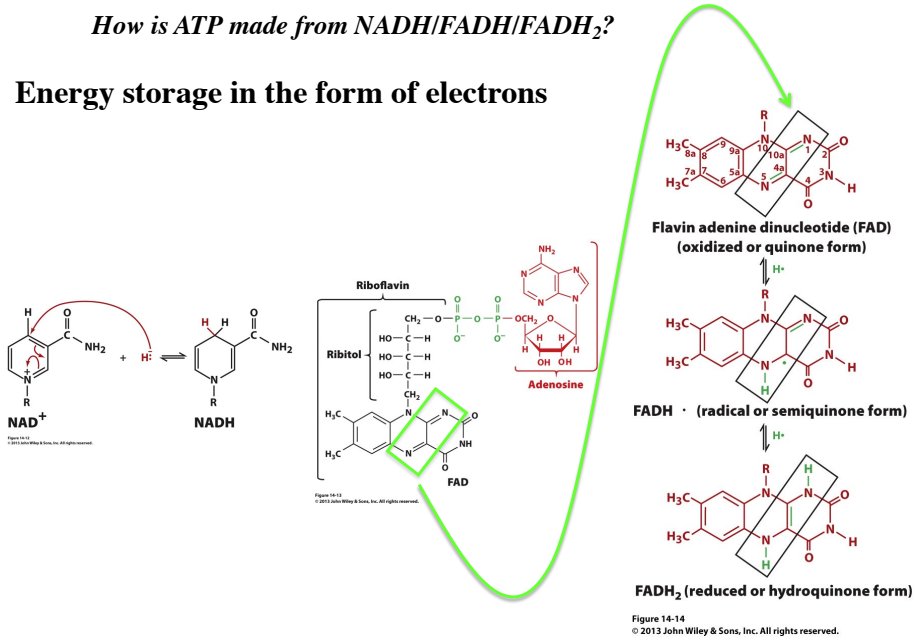

Figure 14-14
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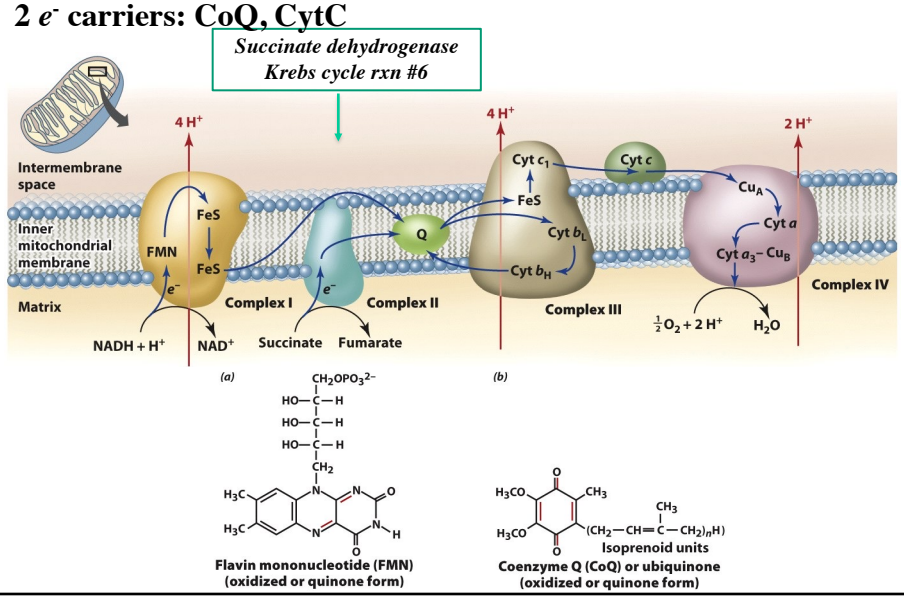
Mitochondrial electron transport chain – the players

4 protein complexes for e^- transfer: Complexes I-IV
2 e^- carriers: CoQ, CytC



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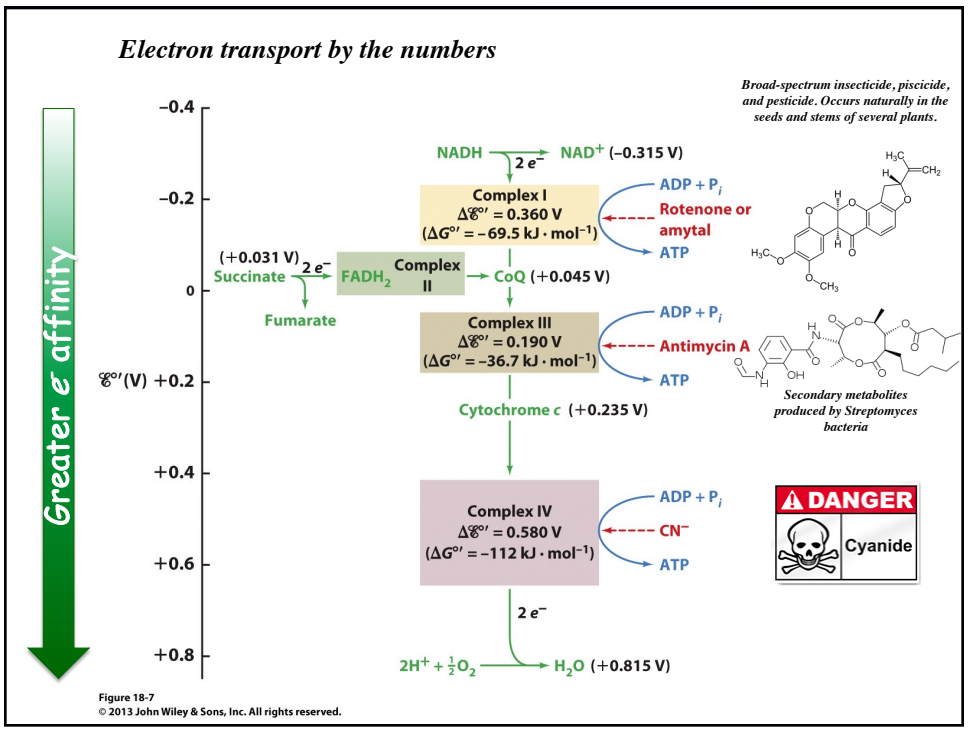

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Cytochromes - There are 7 cytochromes (heme proteins; heme = iron + porphyrin) in the ETS. All have a reddish-brown tint caused by the presence of iron. Each cytochrome has a distinct absorbance spectra which represent a structural feature of the cytochrome and is designated as a member of a, b, or c family.

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Why hemes? You must recognize the differences with the function of hemes in myoglobin and hemoglobin and the cytochromes. The metals (iron for most copper of a and a3) are used for there ability to accept and donate electrons easily. Differences in the redox state is due to the total environment of the heme/cytochrome complex.

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Iron-sulfur centers – A characteristic of the ETS is to have components with different oxidative potential placed strategically along the chain. The proteins with iron-sulfur centers are needed to provide a low oxidation potential. Thus they are present in complexes I, II and III but not in IV.

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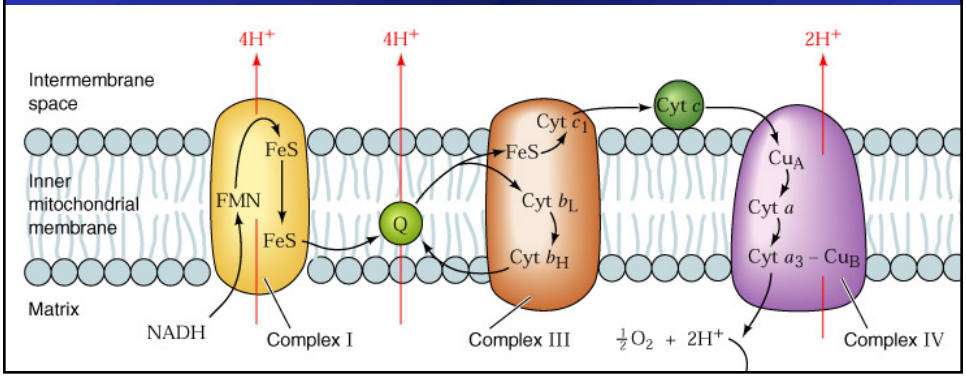
Complex I

- large complex (850 kDa) with over 30 subunits some from mitochondrial DNA.
- mediates transfer of e- from NADH to ubiquinone
- NADH is oxidized and in doing so transfer of e- to FMN
- There are several Fe-S centers held in place by cys residues (2Fe-2S, or 4Fe-4S)



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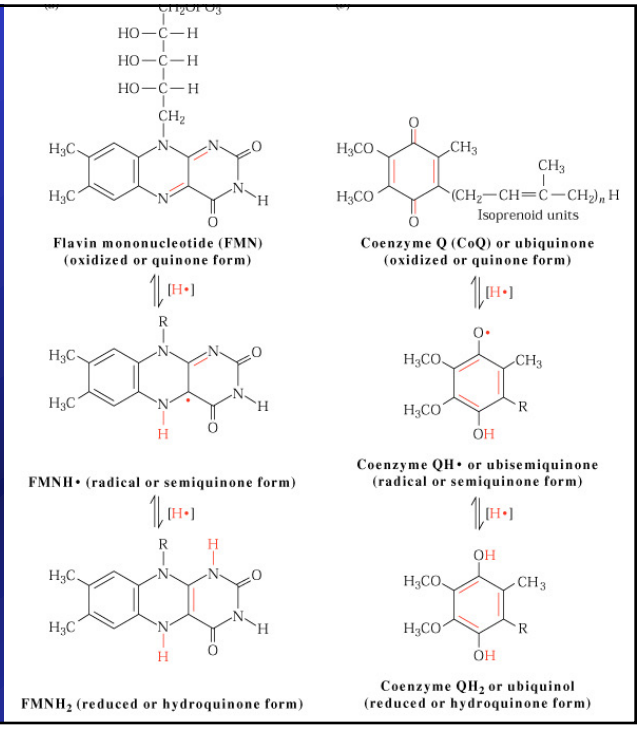
Complex I

- Coenzyme Q is ultimate acceptor of electrons in this complex
- Q and FMN can adopt 3 oxidation states



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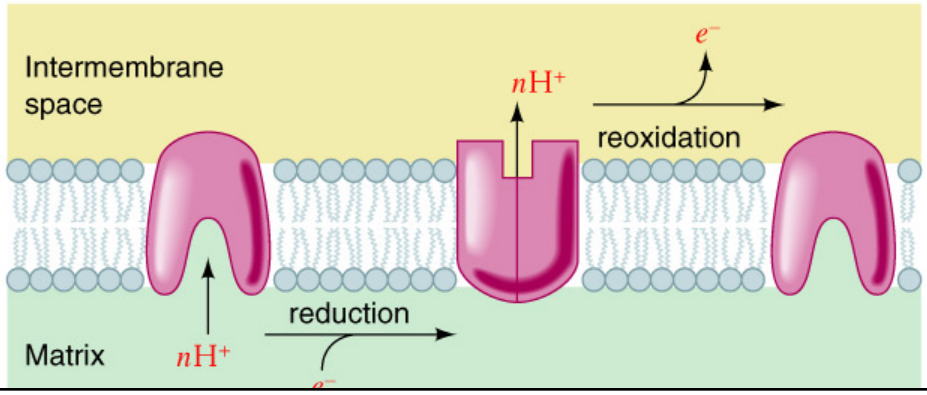
Complex I

- protein changes conformation redox state - most likely leads to proton pumping - resulting pKa changes in aas in complex I probably leads to loss and gain of protons
- 2 e- are transferred from NADH and 4 H+ are pumped



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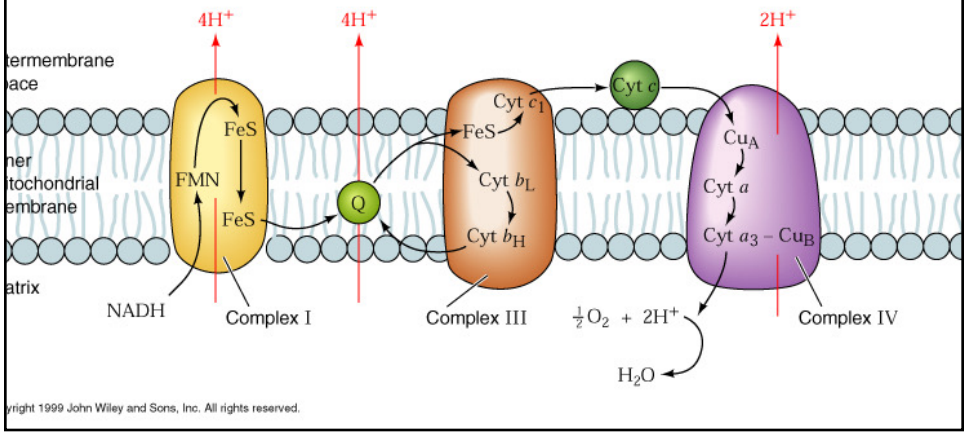
Complex II

- Succinate dehydrogenase and other FADH producing enzymes are linked to the complex - direct transfer of e- from the TCA
- FADH is part of the complex and 2e- electrons are donated here



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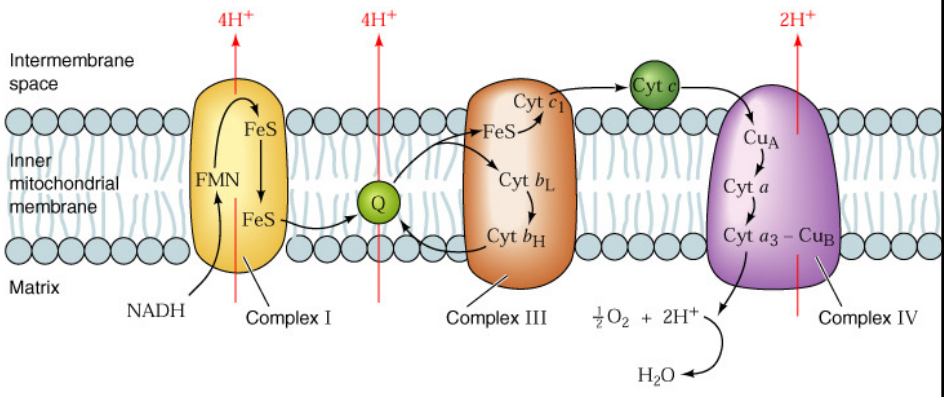
Complex II

- Transfer of 1 e- to the Fe-S center to Q
- is not the second part of the chain, rather a another entry point into the chain
- no H^+ pumped, so there is a reduced potential of H^+ gradient formed and ATP formation potential



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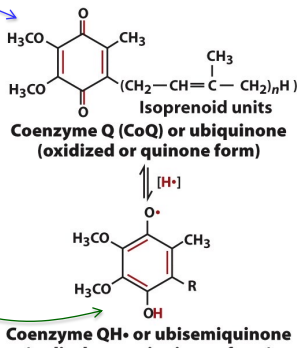
Mitochondrial electron transport chain – CoQ collection point

e^- from NADH, succinate, fatty acid oxidation, glycerophosphate shuttle



Can accept/transfer 1 or 2 e^-

- *mobile part of ETS - long hydrophobic isoprenoid chain allows movement in hydrophobic membrane core*
- *transfers one or two electrons between complexes*

Q cycle is important in transfer single e- instead of two

Figure 18-10b

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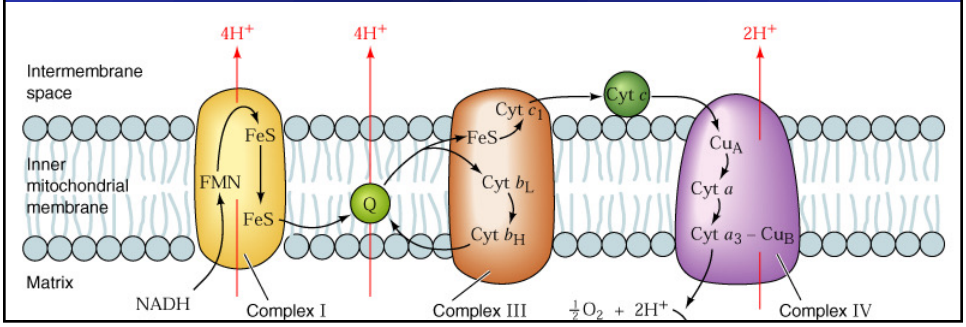
Complex III

- Accepts two one e- transfers from QH2 and eventually transferred to cytochrome C
- Fe-S center and two similar hemes B and c1 involved
- reduction potential of the b hemes is different due to membrane topology
- Important that it is two individual transfers of e- to cyto C
- Two protons are pumped - partially due to lower reduction potential at this point in the chain



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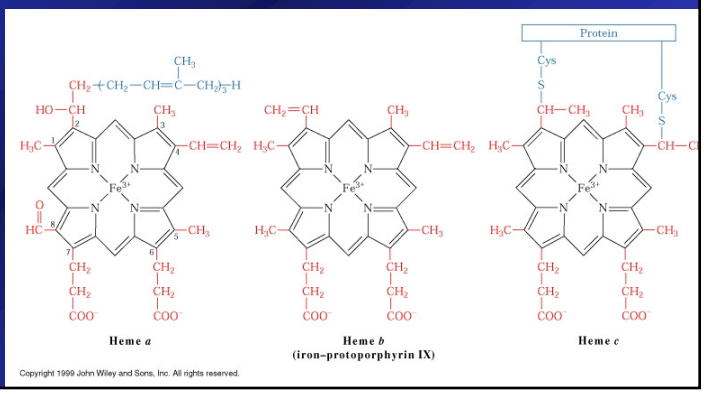
Complex III

Two protons are pumped - partially due to lower reduction potential at this point in the chain

- Hemes
  - same porphyrin ring as in hemoglobin
  - cytochromes b and c are covalently attached through cysteines via thioester linkages

- Heme A has a long hydrophobic side chain
- How are these held in place differently than hemoglobin heme?



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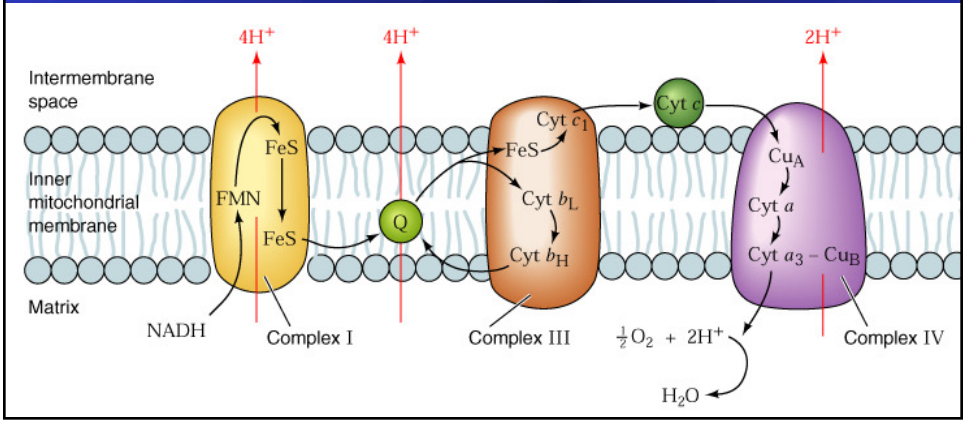
Cytochrome C

- Only water soluble cytochrome
- loosely associated with inner mitochondrial membrane (cytosolic side)
- migrates in the reduced state carrying 1e- to complex IV
- highly conserved through evolution



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Complex IV

- Differs in copper ions in e- transfer not Fe-S.
- e- transferred from cytochrome C to molecular oxygen, one at a time
- one proton pumped per e- transferred - two for O_2, two cytosolic side
  - Cytochrome C to Cu_A- $Heme_a$ -> $Heme_b$-Cu_B -> O_2
  - This complicated system is to prevent the formation of oxygen radicals and superoxide anions
  - Controlled by transfer of e- to oxygen while bound to Fe and Cu complex

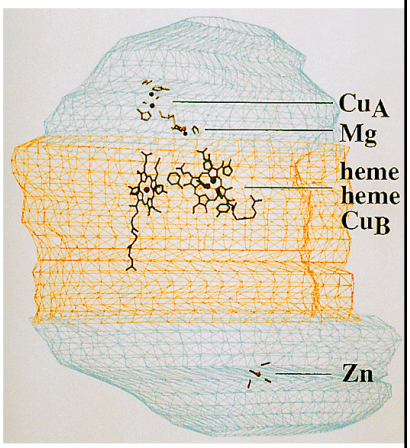

Figure 17-16. Locations of redox centers in beef heart cytochrome *c* oxidase. [Courtesy of Shinya Yoshikawa, Osaka University, Japan.]

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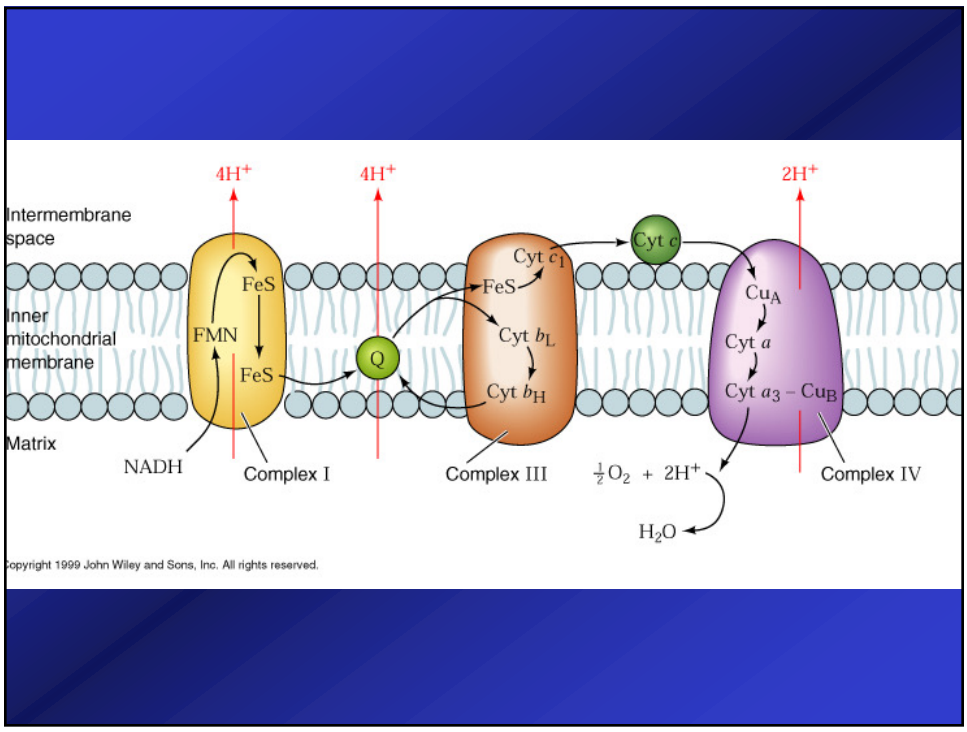

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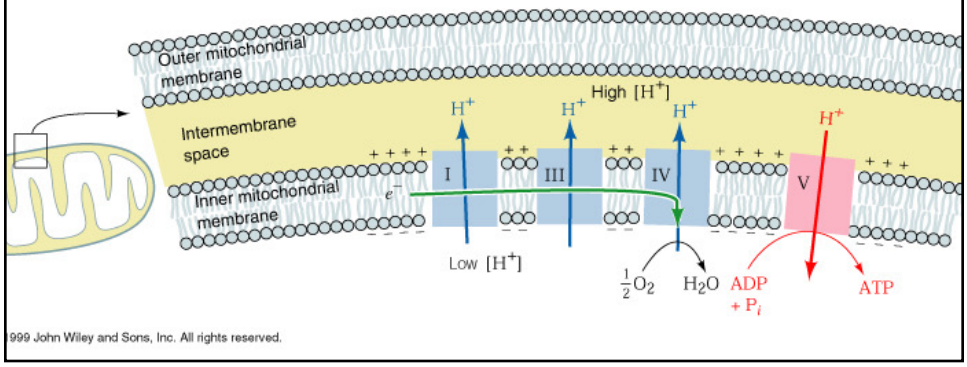
ATP synthase (F_1F_0)- also known as the ATPase for the reverse direction. Without a proton gradient, the reverse reaction is spontaneous. Also called complex V in some books.

- 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.



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

$$\Delta G = 2.3\ RT\ [pH(in) - pH(out)] + ZF\Delta\Psi$$

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

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Mitchel chemiosmotic theory - oxidative phosphorylation

ADP + Pi -> ATP

The ATPase is found on inner mitochondria

- The proton motive force -> 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 and uncouplers

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Pump you up.

Number of 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

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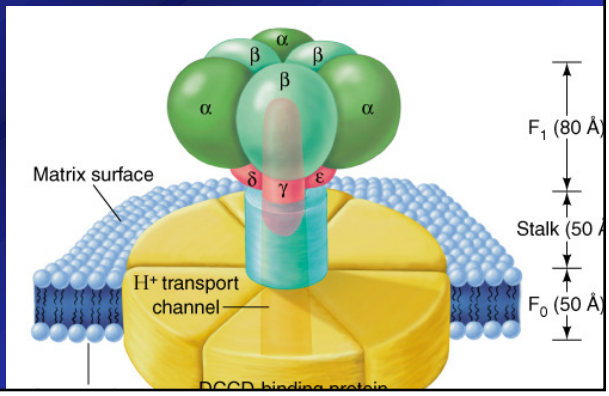
ATP synthase has two functional units each with several subunits.

- 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)

- 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



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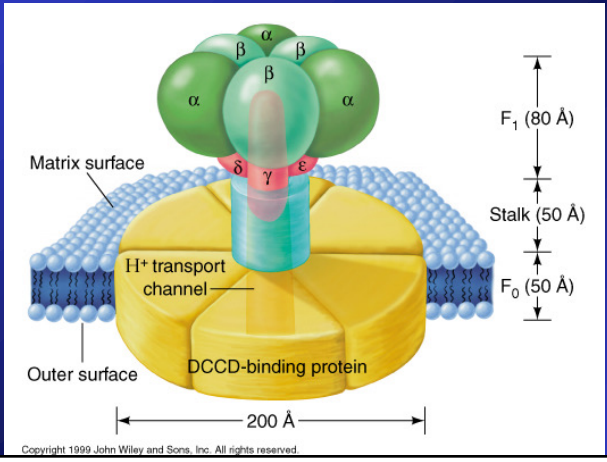
F_1 complex catalyzes ATP synthesis

- 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 F_1 complex is an ATPase - hydrolysis of the gamma phosphate of ATP



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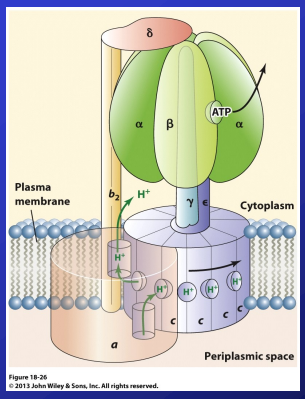
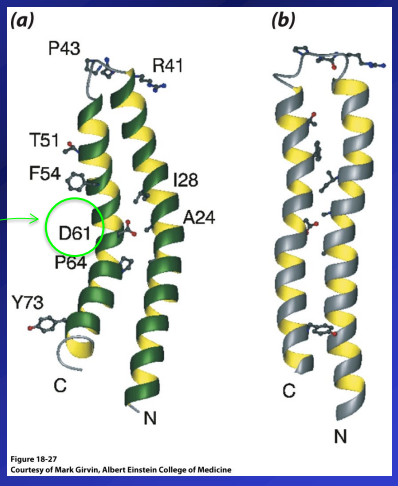
F_1F_o – ATPase model



***3 phases of ATP synthesis*:**
Translocation of H^+ by F_o
Formation of phosphoanhydride bond of ATP by F_1
Coupling of proton gradient dissipation with ATP synthesis



Change in protonation state leads to rotation of F_o

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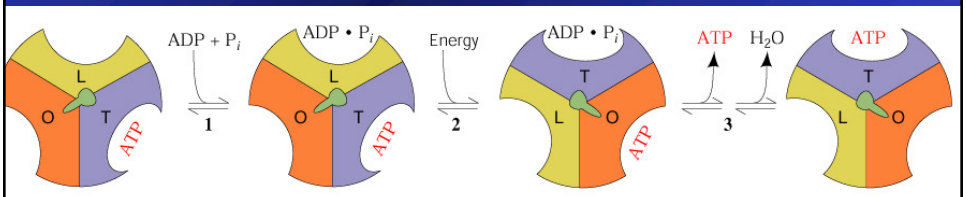
The binding change mechanism - Paul Boyer - U of M and John Walker determined much of the ATPsynthase - Nobel Prize winners 1997 for this work

- H+ gradient leads to conformational changes of the F_1 complex
- Both ATP and ADP bind to the three beta subunits
- Three conformation changes for the whole enzyme (F_1F_0) the open (O), loose (L) and tight (T) binding sites



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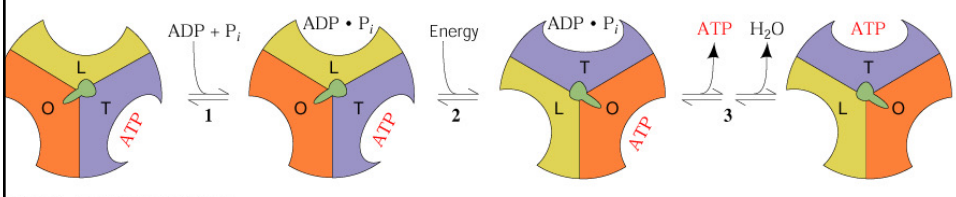
The binding change mechanism

- Proton flux through pore shifts beta subunit conformations
- As the conformation of each of the subunits change a phosphoanhydride bond is formed with Pi and ADP
- The membrane potential helps to create high concentration gradient within the F_0 pore.
- The free energy of the proton concentration gradient converts the T state to the O state, thereby releasing ATP.



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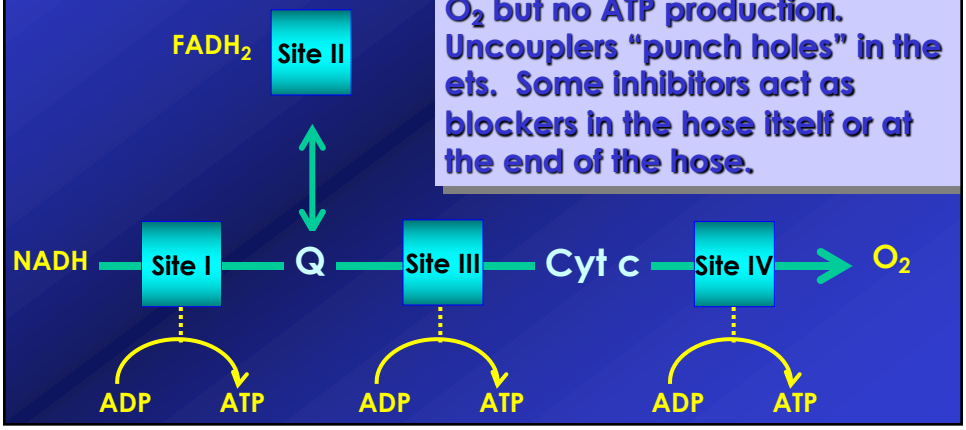
ETS Poisons and Inhibitors Specific inhibitors and uncouplers change the electron transfer and ATP production - respiration

Think of this a pipeline, if the middle is blocked no ATP production and O_2 used.

If the end is blocked and a hole is punched in the hose you lose O_2 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.



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ETS Poisons

- 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

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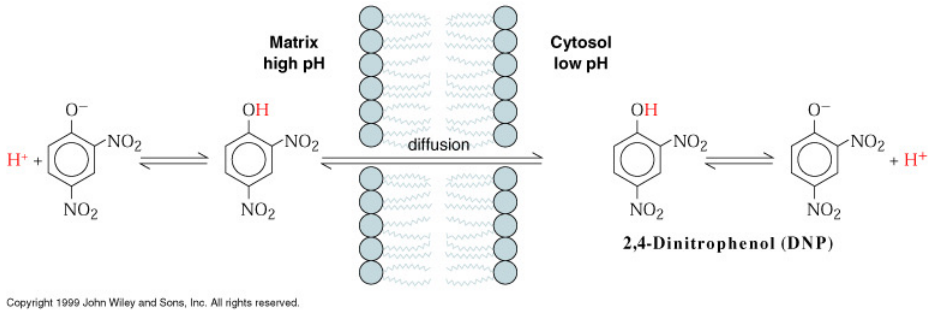
•2,4 DNP is an uncoupling agent that can transverse the inner mitochondria membrane dissipating the H+ gradient



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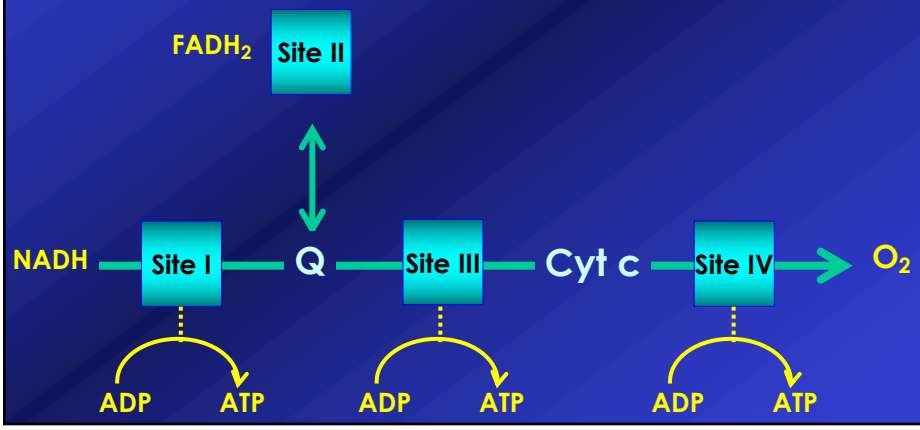
Respiration poisons - block at complex I, III and IV
Effect is to stop the flow of e- through the chain. - when added to the effect of uncouplers can lead to interesting studies

- Carbon monoxide (CO), cyanide, and hydrogen disulfide (H_2S) – inhibit cytochrome C oxidase (site IV)



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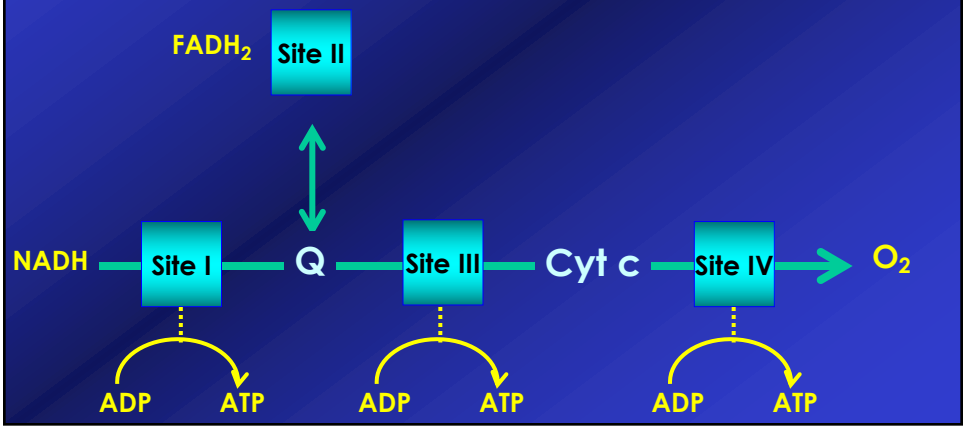
Respiration poisons - block at complex I, III and IV Effect is to stop the flow of e- through the chain. - when added to the effect of uncouplers can lead to interesting studies

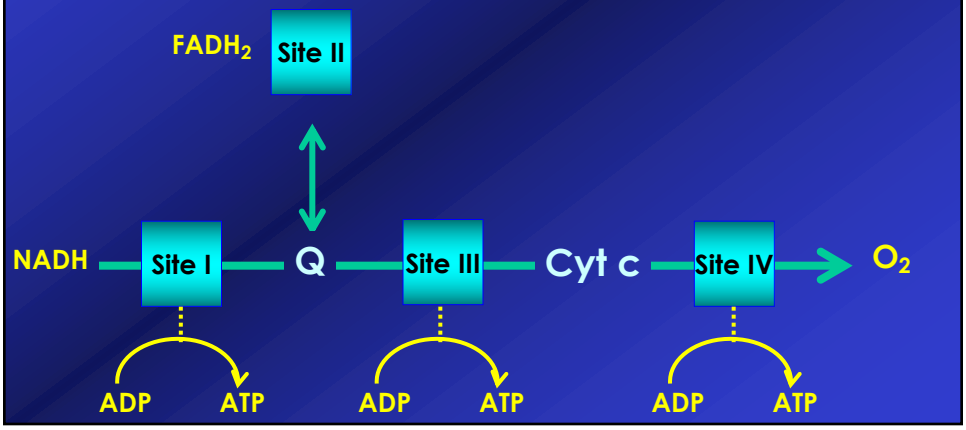
- The fish poison and insecticide rotenone and barbiturates inhibit the oxidation of NADH (site I)



39

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

- Antimycin A inhibits the cytochromes b and C (site III)



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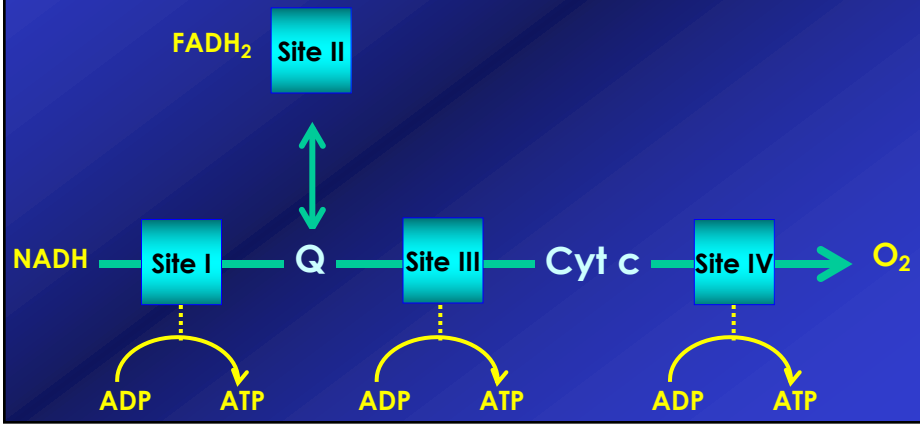
- If site 1 is blocked site II can still input electrons, ATP is formed and oxygen consumed. However if site two is blocked then no e- are transferred and O_2 is not consumed.
- Think of what happens if various combinations of these inhibitors are used



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Brown fat (thermogenesis) - regulated by fatty acids, leads to uncoupling. High amounts of thermogenin (uncoupling protien) 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.
- Norepiephrinbe -> [cAMP] and activates hormone sensitive lipase
- Increase in [FA] reverse nucleotide inhibition of uncoupling channel

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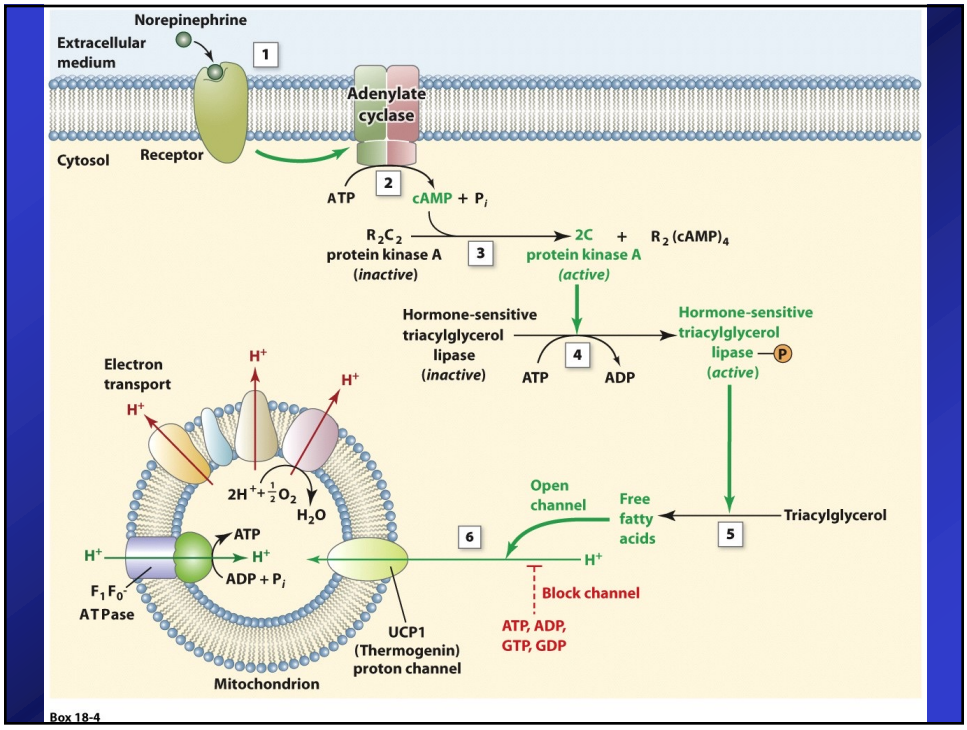
Norepinephrine
Extracellular medium
1
Adenylate cyclase
Cytosol
Receptor
ATP
2
cAMP + P_i
R_2C_2
protein kinase A
(inactive)
3
2C + $R_2(cAMP)_4$
protein kinase A
(active)
Hormone-sensitive triacylglycerol lipase (inactive)
4
ATP
ADP
Hormone-sensitive triacylglycerol lipase (active)
P
Electron transport
H^+
$2H^+ + \frac{1}{2}O_2$
H_2O
ATP
ADP + P_i
F_1F_0 ATPase
Open channel
Free fatty acids
5
Triacylglycerol
6
Block channel
ATP, ADP, GTP, GDP
UCP1 (Thermogenin) proton channel
Mitochondrion
Box 18-4

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