The Electron Transport Chain

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The citric acid cycle oxidizes acetate into two molecules of CO_2 while capturing the electrons in the form of 3 NADH molecules and one molecule of $FADH_2$. These reduced molecules contain a pair of electrons with a high transfer potential. These electrons are ultimately going to be transferred by a system of electron carriers to O_2 to form H_2O. This process occurs in the mitochondria and is the major energy source used to produce ATP by oxidative phosphorylation.

Let's look at the standard free energy change for the overall reaction shown below:

$NADH + H^+ + ½O_2 \rightarrow NAD^+ + H_2O$

$NAD^+ + 2e^- + H^+ \rightarrow NADH \qquad E^{o'} = -0.315\ V$

$½O_2 + 2e^- + 2H^+ \rightarrow H_2O \qquad E^{o'} = +0.816\ V$

$\Delta E^{o'} = +0.816\ V - (-0.315\ V) = 1.136\ V$

$\Delta G^{o'} = -nF\Delta E^{o'} = -219\ kJ/mol$

$ADP + Pi \rightarrow ATP \qquad \Delta G^{o'} = +30.5\ kJ/mol$

If we could convert all of this free energy generated by reducing oxygen with NADH to synthesize ATP from ADP and Pi:

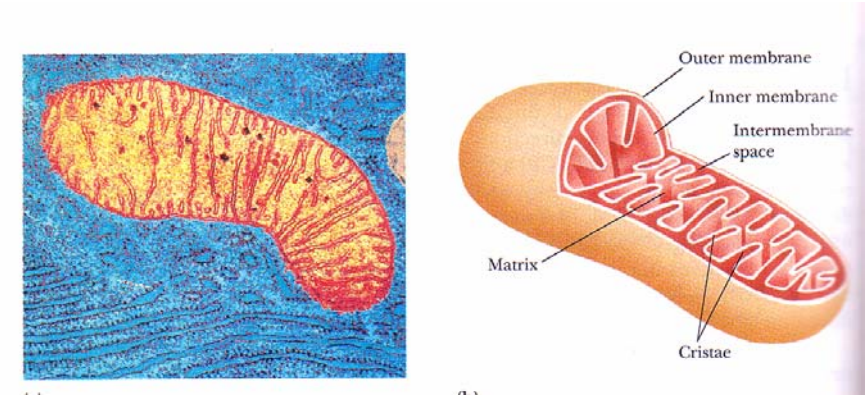
#ATP = 219/30.5 = 7.2 ATP

I. Mitochondria.

The processes involved in electron transport and oxidative phosphorylation occur within membranes. Prokaryotes do not have organelles such as mitochondria. Bacteria have a plasma membrane surrounded by a rigid cell wall. In prokaryote systems, electron transport and oxidative phosphorylation are carried out across the plasma membrane.

Eukaryotes have organelles including mitochondria. Mammalian cells have 800 to 2,500 mitochondria per cell. Remember that red blood cells do not contain mitochondria.

Mitochondria are remarkably mobile organelles. Time lapse photography shows that they are constantly moving and changing shape. In some cells they are anchored by attachment to the cell's cytoskeleton so that they remain fixed at one cellular location to target a site of high ATP utilization. In heart muscle for example the mitochondria are anchored close to the contractile muscle, in sperm they are wrapped tightly around the motile flagellum.



Shown above is a mitochondrian. The mitochondrian is enclosed by an outer membrane and a more complex inner mitochondrial membrane. The space between the inner and outer mitochondrial membranes is called the intermembrane space. With in this space we find enzymes that utilize ATP such as creatine kinase and adenylate kinase.

The outer membrane contains porins which are transmembrane proteins rich in β-sheets which allow molecules of low molecular weight (<10,000) freely diffuse in and out. As a result, the outer membrane is like a sieve that is permeable to all molecules less than 10,000 Daltons. This makes the intermembrane space chemically equivalent to the cytosol of the cell with respect to small molecules.

The inner mitochondrial membrane is packed with proteins which account for 80% of the membranes molecular weight. The inner membrane is impermeable to molecules and ions. Metabolites that must cross the inner mitochondrial membrane are carried across by specific transport proteins.

The inner membrane is folded into numerous cristae, which greatly increase the surface area of the inner membrane. Within this membrane are the proteins involved in the electron transport chain, ATP synthase and transport proteins.

The large internal space enclosed by inner mitochondrial membrane is called the matrix. It is densely packed with hundreds of enzymes, including some of our favorites such as pyruvate dehydrogenase, pyruvate carboxylase, and the soluble enzymes of the citric acid cycle. In addition there are the enzymes involved in the oxidation of fatty and amino acids. The matrix also contains the mitochondrial genome, the mitochondrial ribosomes, tRNA's and the enzymes required for the expression of mitochondrial genes.

II. The Components of the Electron Transport Chain

The electron transport chain of the mitochondria is the means by which electrons are removed from the reduced carrier NADH and transferred to oxygen to yield H_2O.

1.) NADH.

NADH is generated in the matrix by the reactions of pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehyrogenase. The electron transport chain begins with reoxidizing NADH to form NAD^+ and channeling the electrons into the formation of reduced coenzymes. Important to note that NADH transfers 2 electrons at a time in the form of a hydride.

$NAD^+ + 2e^- + H^+ \rightarrow NADH \qquad E^{o'} = -0.315$ V

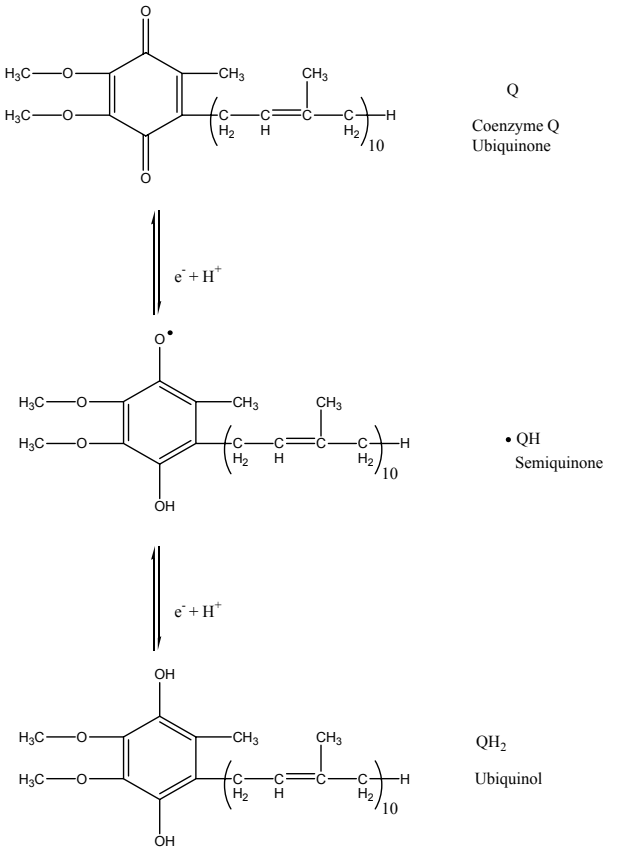
2.) Flavoproteins.

Flavoproteins have either a FAD (flavin adenosine dinucleotide) or a FMN (flavin mononucleotide) prosthetic group. Flavoproteins can accept or donate electrons one at time or two at a time. Thus they are often intermediaries between two electron acceptors/donors and one electron acceptors/donors. For flavoproteins the typical standard reduction potentials are around 0 V.

$FAD + 2e^- + 2H^+ \rightarrow FADH_2$; $FMN + 2e^- + 2H^+ \rightarrow FMNH_2 \quad E^{o'} \approx 0$ V

3.) Coenzyme Q (CoQ)

aka ubiquinone (UQ). Shown below.



CoQ has ten repeating isoprene units which make it insoluble in water, but soluble in the hydrophobic lipid bylayer.

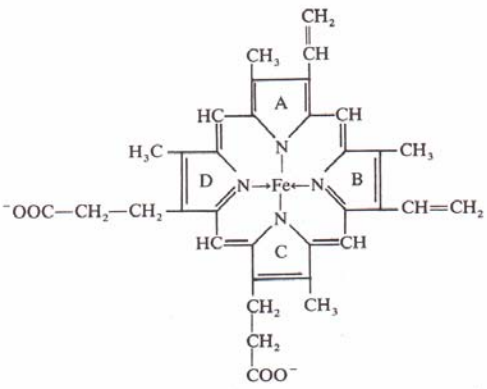
Coenzyme Q is a versatile cofactor because it is a soluble electron carrier in the hydrophobic bilipid layer of the inner mitochondrial membrane. Like flavoproteins, CoQ can accept/donate electrons one at a time or two at a time.

$Q + 2e^- + 2H^+ \rightarrow QH_2 \quad E^{o\prime} = 0.060\ V$
$Q + e^- + H^+ \rightarrow \cdot QH \quad E^{o\prime} = 0.030\ V$
$\cdot QH + e^- + H^+ \rightarrow QH_2 \quad E^{o\prime} = 0.190\ V$

3.) Cytochromes.

Cytochromes are proteins that contain heme prosthetic groups which function as one electron carriers.

The heme iron is involved in one electron transfers involving the Fe^{2+} and Fe^{3+} oxidation states.



Cytochromes are named by their absorption spectra which depends on the porphyrin structure and environment.

The example shown is the heme prosthetic group of cytochrome *b*. Cytochrome *b* contains the same iron porphyrin found in hemoglobin and myoglobin. Other cytochromes we will encounter in the electron transport complexes are cytochromes *b*, *c*, c_1, *a* and a_3.

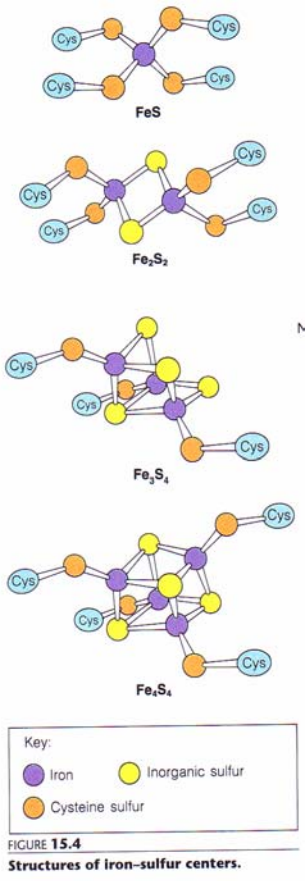


FIGURE 15.4
Structures of iron–sulfur centers.

4.) Iron-Sulfur Proteins

In the electron transport chain we will encounter many iron-sulfer proteins which participate in one electron transfers involving the the Fe^{2+} and Fe^{3+} oxidation states.

These are non-heme iron-sulfur proteins.

The simplest iron-sulfer protein is FeS in which iron is tetrahedrally coordinated by four cysteines.

The second form is Fe_2S_2 which contains two irons complexed to 2 cysteine residues and two inorganic sulfides.

The third form is Fe_3S_4 which contains 3 iron atoms coordinated to three cysteine residues and 4 inorganic sulfides.

The last form is the most complicated Fe_4S_4 which contains 4 iron atoms coordinated to 4 cysteine residues and 4 inorganic sulfides.

5.) Copper Proteins

Copper bound proteins participate in one electron transfers involving the Cu^{+} and Cu^{2+} oxidation states.

III. Overview of the Electron Transport Chain.

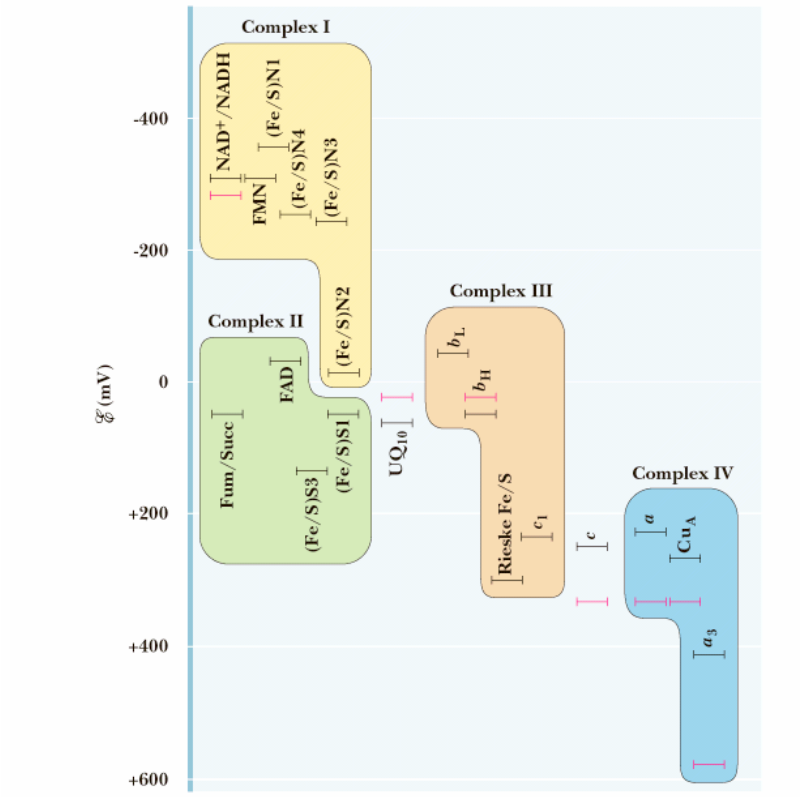
Electrons move along the electron transport chain going from donor to acceptor until they reach oxygen the ultimate electron acceptor. The standard reduction potentials of the electron carriers are between the $NADH/NAD^{+}$ couple (-0.315 V) and the oxygen/H_2O couple (0.816 V) as on the next page.

The components of the electron transport chain are organized into 4 complexes. Each complex contains several different electron carriers.

1. **Complex I** also known as the NADH-coenzyme Q reductase or NADH dehydrogenase.
2. **Complex II** also known as succinate-coenzyme Q reductase or succinate dehydrogenase.
3. **Complex III** also known as coenzyme Q reductase.
4. **Complex IV** also known as cytochrome c reductase.

Complex I accepts electrons from NADH and serves as the link between glycolysis, the citric acid cycle, fatty acid oxidation and the electron transport chain.

Complex II includes succinate dehydrogenase and serves as a direct link between the citric acid cycle and the electron transport chain.



Complexes I and II both produce reduced coenzyme Q,$CoQH_2$ which is the substrate for Complex III.

Complex III transfers the electrons from $CoQH_2$ to reduce cytochrome *c* which is the substrate for Complex IV.

Complex IV transfers the electrons from cytochrome *c* to reduce molecular oxygen into water.

Each of these complexes are large multisubunit complexes embedded in the inner mitochondrial membrane.

Protein Complexes of the Mitochondrial Transport Chain

Complex	Mass kD	Number of Subunits	Prosthetic Groups	Substrate binding sites.
I	850	>30	FMN Fe-S	NADH (matrix side) CoQ (lipid core)
II	140	4	FAD Fe-S	Succinate (matrix side) CoQ (lipid core)
III	248	11	Heme b_L Heme b_H Heme c_1 Fe-S	CoQ (lipid core) Cyt *c* (intermembrane side)
Cytochrome *c*	13	1	Heme *c*	Cyt c_1 Cyt *a*
IV	162	>10	Heme *a* Heme a_3 Cu_A Cu_B	Cyt *c* (intermembrane side) O_2 (matrix side)

IV. Complex I

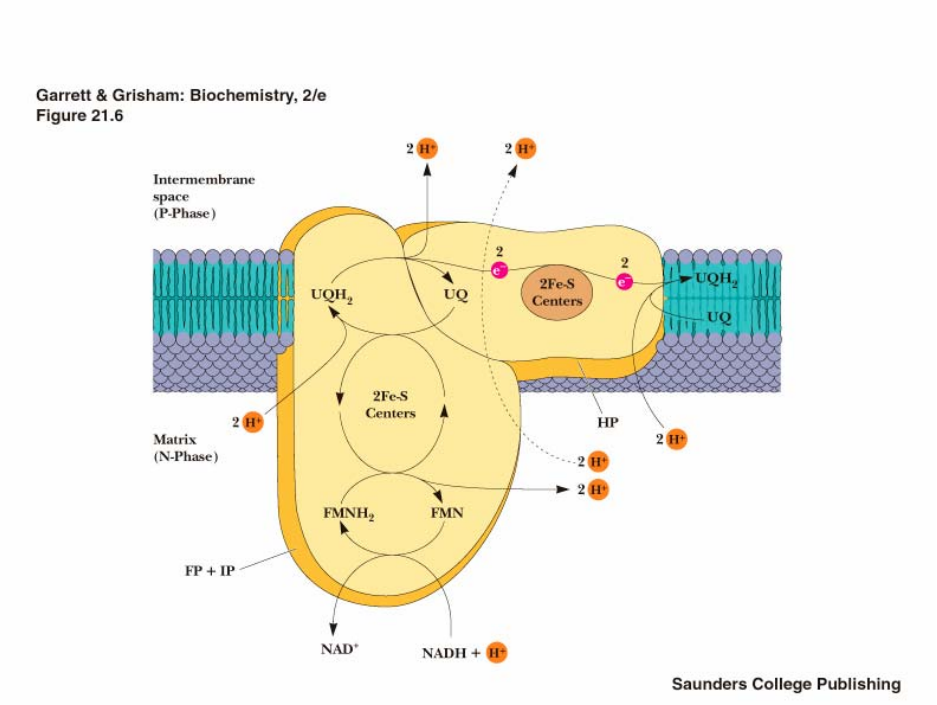
Complex I is also called NADH-Coenzyme Q reductase because this large protein complex transfers 2 electrons from NADH to coenzyme Q. Complex I was formerly known as NADH dehydrogenase. Complex I is huge, 850,000 kD and is composed of more than thirty subunits. It contains a FMN prosthetic group and seven or more Fe-S clusters. This complex has between 20-26 iron atoms bound.

The prosthetic group FMN is absolutely required for activity. Therefore this complex is a flavoprotein.

This complex binds NADH, transfers two electrons in the form of a hydride to FMN to produce NAD^+ and $FMNH_2$. The subsequent steps involve the transfer of electrons one at a time to a series of iron-sulfer complexes that includes both 2Fe-2S and 4Fe-4S clusters.

Note the importance of FMN. First it functions as a 2 electron acceptor in the hydride transfer from NADH. Second it functions as a 1 electron donor to the series of iron sulfur clusters. FMN and FAD often play crucial links between 2 electron transfer agents and 1 electron transfer agents.

The final step of this complex is the transfer of 2 electrons one at a time to coenzyme Q. CoQ like FMN and FAD can function as a 2 electron donor/acceptor and as a 1 electron donor/acceptor. CoQ is a mobile electron carrier because its isoprenoid tail makes it highly hydrophobic and lipophillic. It diffuses freely in the bilipid layer of the inner mitochondrial membrane.

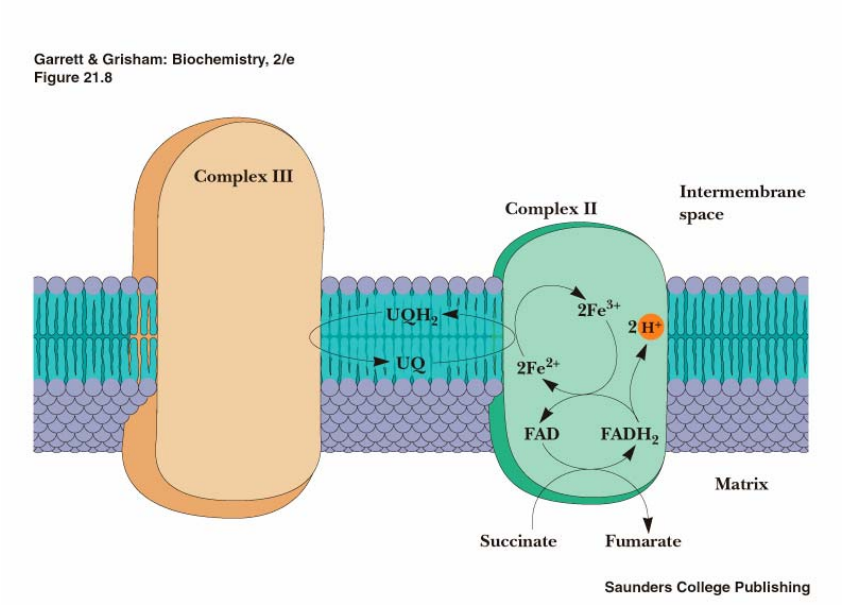


The process of transferring electrons from NADH to CoQ by complex I results in the net transport of protons from the matrix side of the inner mitochondrial membrane to the inter membrane space where the H^+ ions accumulate generating a proton motive force. The intermembrane space side of the inner membrane is referred to as the **P face** (P standing for positive). The matrix side of the inner membrane is referred to as the the **N face**. The transport of electrons from NADH to CoQ is coupled to the transport of protons across the membrane. This is an example of active transport. The stiochiometry is 4 H^+ transported per 2 electrons.

V. Complex II

Believe it or not, you are already familiar with Complex II. It is none other than succinate dehydrogenase. The only enzyme of the citric acid cycle that is an integral membrane protein. This complex is composed of four subunits. 2 of which are iron-sulfur proteins and the other two subunits together bind FAD through a covalent link to a histidine residue. These two subunits are called flavoprotein 2 or FP_2. Complex II contains 3 Fe-S centers, 1 4Fe-4S cluster, 1 3Fe-4S cluster and 1 2Fe-2S cluster.

In the first step of this complex, succinate is bound and a hydride is transferred to FAD to generate $FADH_2$ and fumarate. $FADH_2$ then transfers its electrons one at a time to the Fe-S centers. Thus once again FAD functions as 2 electron acceptor and a 1 electron donor. The final step of this complex is the transfer of 2 electrons one at a time to coenzyme Q to produce $CoQH_2$.



The overall reaction for this complex is:

$Succinate + CoQ \rightarrow Fumarate + CoQH_2$ $\quad \Delta E^{o'} = 0.060\ V - (+0.031V) = 0.029\ V$

$\Delta G^{o'} = -nF\Delta E^{o'} = -5.6\ kJ/mol$

Compare this with complex I

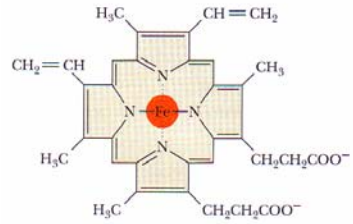
$NADH + H^+ + CoQ \rightarrow NAD^+ + CoQH_2$ $\quad \Delta E^{o'} = 0.060\ V - (-0.315V) = 0.375\ V$

$\Delta G^{o'} = -nF\Delta E^{o'} = -72.4\ kJ/mol$

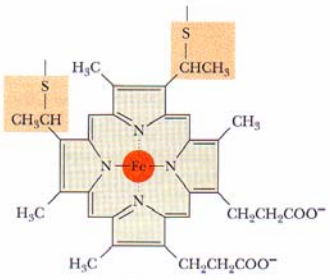
For complex II the standard free energy change of the overall reaction is too small to drive the transport of protons across the inner mitochondrial membrane. This accounts for the 1.5 ATP's generated per $FADH_2$ compared with the 2.5 ATP's generated per NADH.

V. Complex III

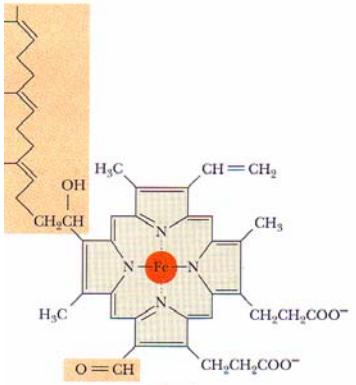
This complex is also known as coenzyme Q-cytochrome *c* reductase because it passes the electrons form $CoQH_2$ to cyt *c* through a very unique electron transport pathway called the Q-cycle.



Iron protoporphyrin IX
(found in cytochrome *b*, myoglobin, and hemoglobin)



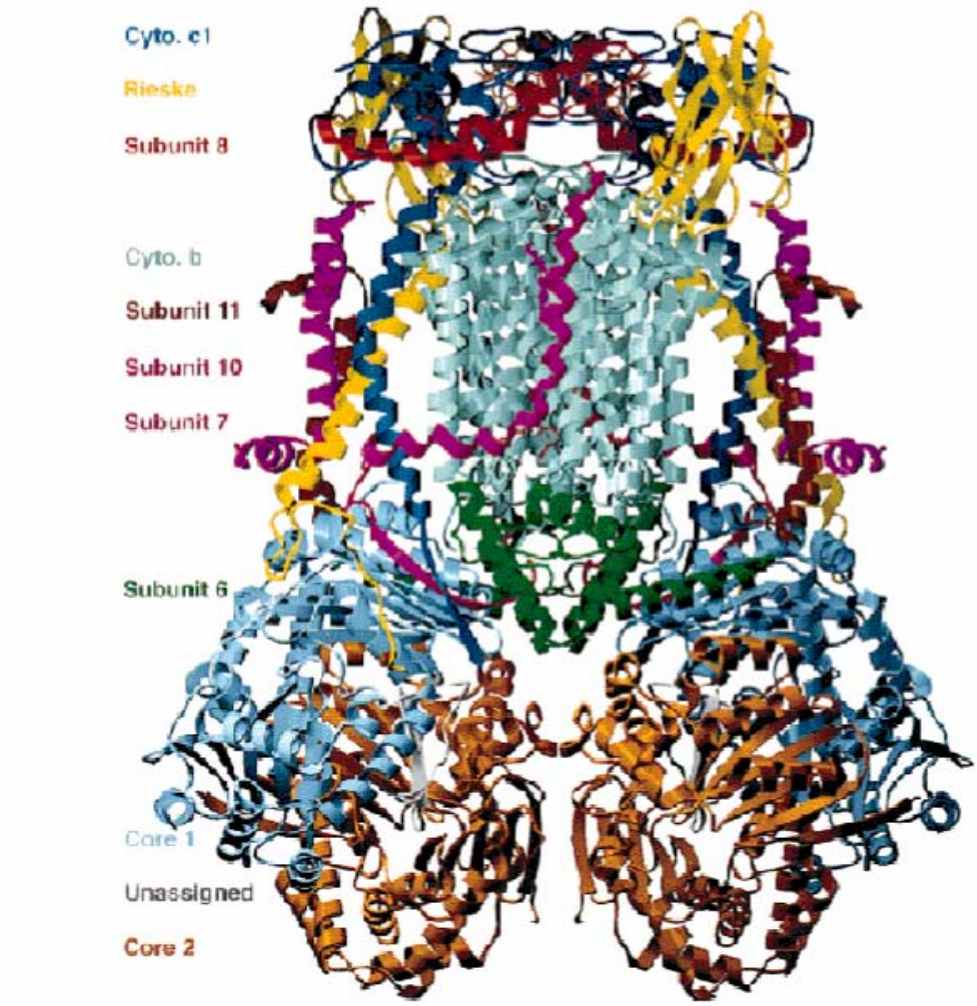
Heme C
(found in cytochrome *c*)



Heme A
(found in cytochrome *a*)

Shown to the left are the porphyrins found in cytochromes. Cytochrome *b* contains the same iron protoporphyrin as hemoglobin and myoglobin. The *c* cytochromes contain heme *c* through covalent attachment by cysteine residues. Cytochrome *a* is found in two forms in complex IV.

In complex III we find two *b*-type cytochromes and one *c*-type cytochrome. Complex III is complex and we have a crystal structure.

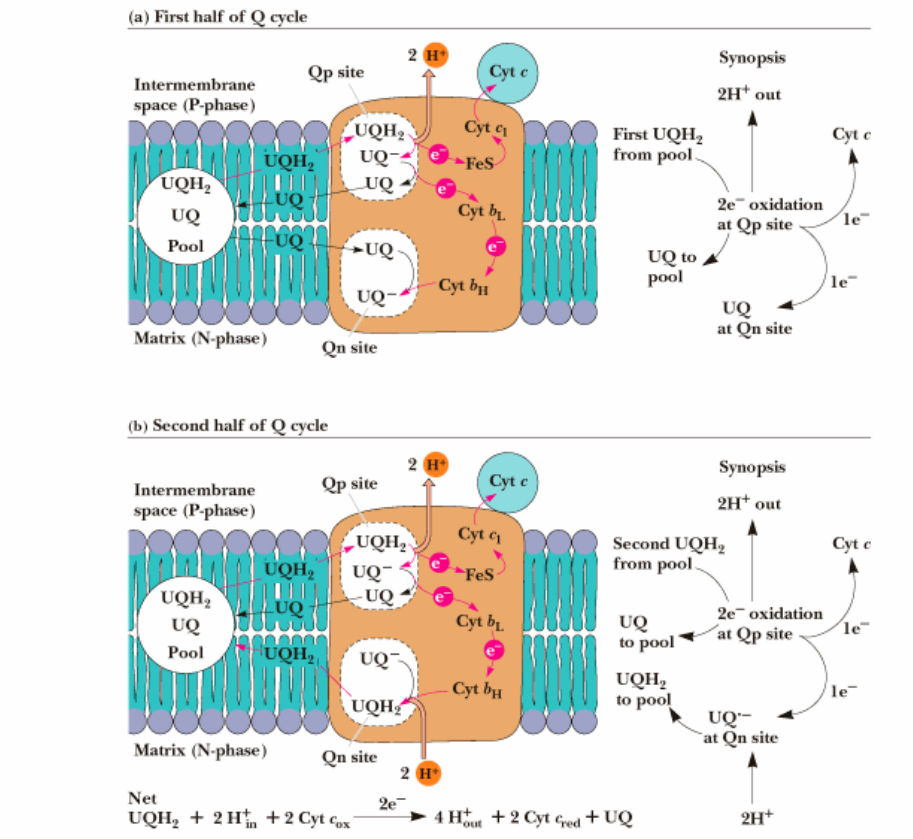


Complex III has a beautiful dimeric structure
The bottom of the structure extends 75 Å into the mitochondrial matrix, while the top of the structure extends 38 Å out into the intermembrane space.
Shown in pale green are the α–helices of cytochrome b which define the transmembrane portion of the complex.
Shown in bright yellow is the Reiske protein which is an iron-sulfur protein that is mobile in the crystal structure. This motility is required for the electon transfer function of this protein.

Q-Cycle

The Q-cycle is initiated when $CoQH_2$ diffuses through the bilipid layer to the $CoQH_2$ binding site which is near the intermembrane face. This $CoQH_2$ binding site is called the $\mathbf{Q_P}$ site. The electron transfer occurs in two steps. First one electron from $CoQH_2$ is transferred to the Rieske protein (a Fe-S protein) which transfers the electron to cytochrome c_1. This process releases 2 protons to the intermembrane space.

Coenzyme Q is now in a semiquinone anionic state, $CoQH^{\bullet -}$ still bound to the Q_P site. The second electron is transferred to the b_L heme which converts $CoQH^{\bullet -}$ to CoQ. This reoxidized CoQ can now diffuse away from the Q_P binding site. The b_L heme is near the P-face. The b_L heme transfers its electron to the b_H heme which is near the N-face. This electron is then transferred to second molecule of CoQ bound at a second CoQ binding site which is near the N-face and is called the Q_N binding site. This electron transfer generates a $CoQ^{\bullet -}$ radical which remains firmly bound to the Q_N binding site. This completes the first half of the Q cycle.



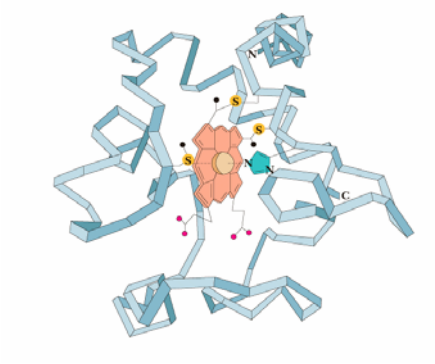
Happily the second half of the Q-cycle is similar to the first half. A second molecule of $CoQH_2$ binds to the Q_P site. In the next step, one electron from $CoQH_2$ (bound at Q_P) is transferred to the Rieske protein which transfers it to cytochrome c_1. This process releases another 2 protons to the intermembrane space. The second electron is transferred to the b_L heme to generate a second molecule of reoxidized CoQ. The b_L heme transfers its electron to the b_H heme. This electron is then transferred to the $CoQ^{\bullet -}$ radical still firmly bound to the Q_N binding site. The take up of two protons from the N-face produces $CoQH_2$ which diffuses from the Q_N binding site. This completes Q cycle.

The net result of the Q-cycle is $2e^-$ transported to cytochrome c_1. Two protons were picked up from the N-face in the second half of the Q-cycle and 4 protons total were released into the intermembrane space.

The two electron carrier $CoQH_2$ gives up its electrons one at a time to the Rieske protein and the b_L heme both of which are 1 electron carriers.

The electrons that end up on cytochrome c_1 are transferred to cytochrome *c*. Cytochrome *c* is the only water soluble cytochrome. Cytochrome *c* is coordinated to ligands that protect the iron contained in the heme from oxygen and other oxidizing agents. Cytochrome c is a mobile electron carrier that diffuses

through the intermembrane space shuttling electrons from the c_1 heme of complex III to Cu_A site of complex IV.

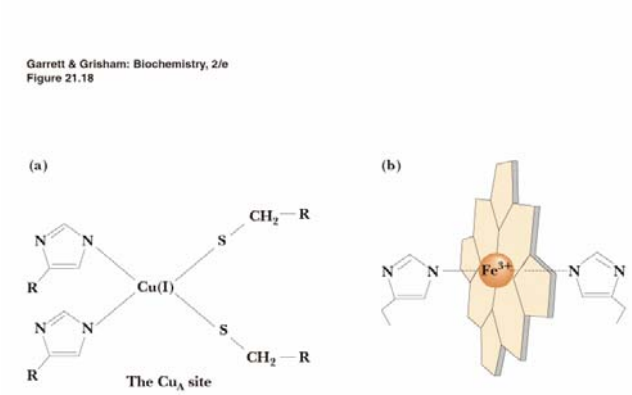


Cytochrome *c* shown to the left. The heme is linked to the protein by 4 cysteine linkages shown in yellow. A methionine sulfur atom is coordinated to the iron compexed in the heme. A histidine residue protects the iron from oxygen and other potential ligands.

VI. Complex IV.

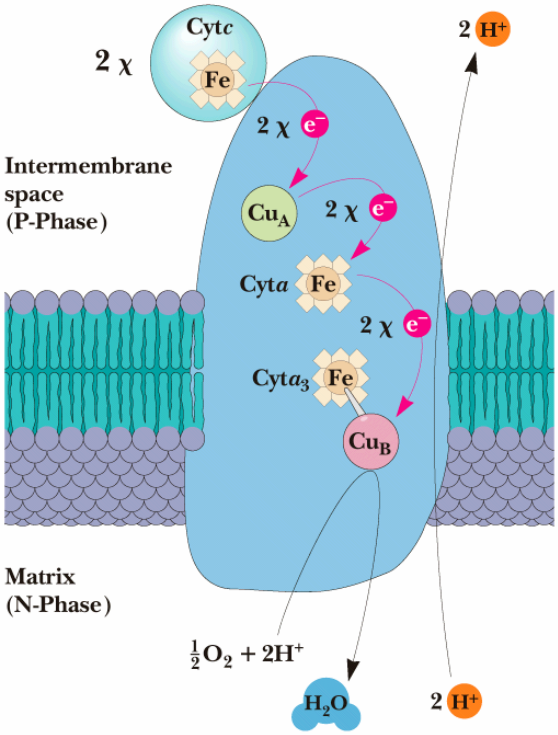
Complex IV is also known as cytochrome *c* oxidase because it accepts the electrons from cytochrome *c* and directs them towards the four electron reduction of O_2 to form 2 molecules of H_2O.

4 cyt *c* (Fe^{2+}) + 4 H^+ + O_2 → 4 cyt *c* (Fe^{3+}) + $2H_2O$

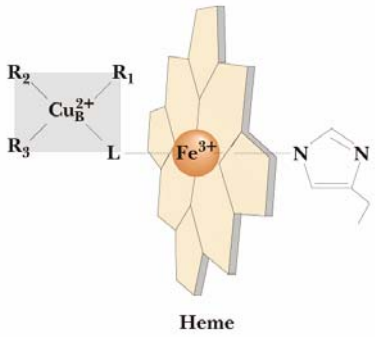


Cytochrome *c* oxidase contains 2 heme centers, cytochrome *a* and cytochrome a_3 and two copper proteins. Each of the protein bound coppers are associated with one of the cytochromes, The copper sites are called Cu_A and Cu_B. Cu_A is associated with cytochrome *a* and is shown to the left.

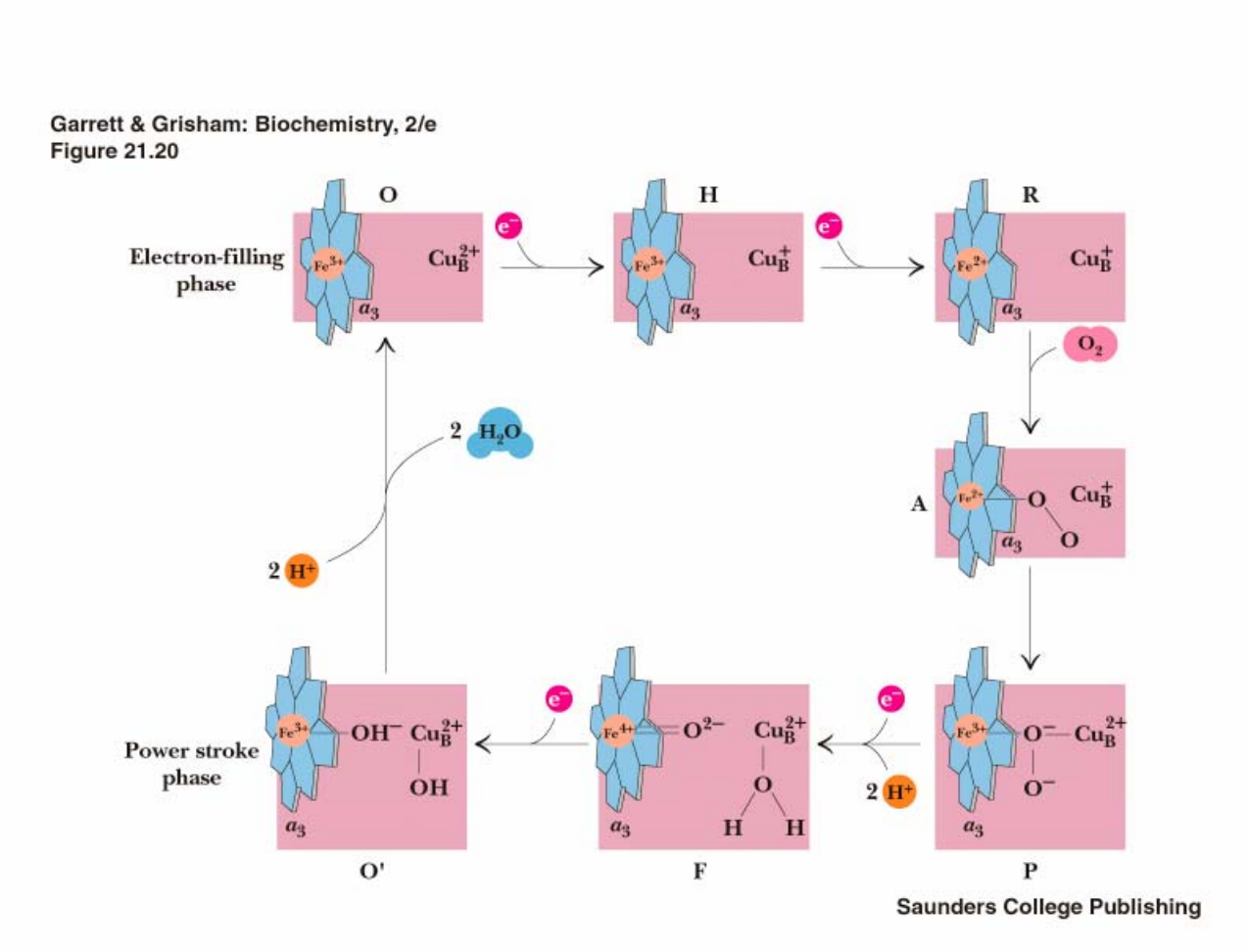
Cu_B is associated with cytochrome a_3.
The copper sites function as 1 electron carriers cycling between the cuprous state Cu^+ and the cupric state Cu^{2+}. Just like iron containing proteins they transfer electrons one at a time.



Cytochrome *c* is bound on the P-face of the membrane and transfers its electron to Cu_A. The oxidized cytochrome *c* dissociates. Cu_A then transfers the electron to cytochrome *a*. The protein bound Cu_A and the iron bound in cytochrome *a* are 15 Å apart. In contrast the Cu_B and the iron bound in cytochrome a_3 are very close to each other forming a binuclear metal center shown below.



Cytochrome *a* transfers the electron to Cu_B. A second cytochrome *c* binds and transfer its electron to Cu_A which is subsequently transferred to cytochrome *a* which in turn is transferred to cytochrome a_3. The binuclear metal center now has two electrons bound allowing the binding of O_2 to binuclear center. The next step involves the uptake of two protons and the transfer of yet another electron through the same pathway which leads to cleavage of the O--O bond and the generation of a Fe^{4+} metal center. The fourth electron is transferred to form a hydroxide at the heme center which becomes protonated and dissociates as H_2O. The mechanism is shown below.



The reduction of oxygen by complex IV involves the transfer of four electrons. Four protons are abstracted from the matrix and two protons are released into the intermembrane space.