The Electron Transport and Oxidative Phosphorylation Pathways

Extracting energy from reduced coenzymes

In general, reactions that extract all of the energy from a molecule in a single step are inefficient: they waste much of the available energy. The method used for taking energy from NADH involves several steps, which allows a more efficient recovery of the energy from the molecule. The process has two phases: **electron transport** and **oxidative phosphorylation**.

The mechanism for extracting the energy from the reduced cofactors was a matter of considerable debate. The Chemiosmotic hypothesis proposed by Peter Mitchell in 1961 has the most experimental support, and is probably correct in its essential points. In essence, Mitchell proposed that the electron transport pathway conserves the energy from the electrons being transported by creating a proton gradient across the mitochondrial membrane, and that this proton gradient is then used to provide the energy required for ATP synthesis. How these processes work has been the subject of considerable research.

As mentioned in the section on Bioenergetics, the $\Delta G'^{\circ}$ for the transfer of electrons from NADH to oxygen is -219.2 kJ/mol. This is considerably larger than the $\Delta G^{\circ\prime}$ for ATP hydrolysis (-30.5 kJ/mol). Clearly NADH has a large amount of energy stored in the molecule. The task of the electron transport pathway is to conserve this energy in a form that can be used for the synthesis of more than one ATP.

Mitochondrial Structure

In order to understand how the pathways for electron transport and oxidative phosphorylation work, we need to look at the general structure of a mitochondrion. A mitochondrion contains two membranes: an **outer membrane**, which appears to largely be responsible for maintaining the shape of the organelle, and a much less permeable inner membrane. The outer membrane contains **porin**, a protein that forms pores large enough allow molecules less than ~10 kDa to diffuse freely across the membrane.
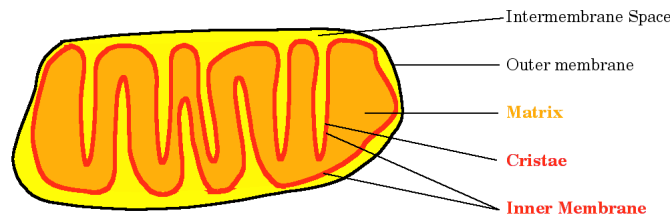
The region between the membranes is called the **intermembrane space**. The intermembrane space is occupied by soluble proteins large enough that they cannot pass through porin. For small molecules, the cytoplasm and intermembrane space are essentially contiguous regions.

The **inner membrane** acts as a barrier to prevent the movement of most molecules. A few molecules have specific transporters that allow them to enter or exit the mitochondrion. The inner membrane contains **cristae**, which are involutions in inner membrane. The function of the cristae is to increase the surface

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area of the inner membrane. The mitochondrial inner membrane may have a larger surface area than the cell plasma membrane, due to the involutions in the membrane.

Finally, within the inner membrane is the **matrix**. The matrix is a very dense protein solution (~50% protein by weight). The TCA cycle enzymes are located in the matrix, as are the enzymes for several other metabolic pathways.

Mitochondria contain a small genome (~16,500 bp). The genome contains 22 transfer RNA genes, 2 ribosomal RNA genes, and 13 polypeptide genes; the polypeptides are all involved in the electron transport pathway or oxidative phosphorylation pathway. Note that the TCA cycle enzymes (including succinate dehydrogenase) are all produced from nuclear genes; the multisubunit complexes of the electron transport pathway and ATP synthase (with the exception of succinate dehydrogenase) are made up of proteins derived from both nuclear and mitochondrial genes.

Electron transport chain

NADH and $FADH_2$ can donate electron pairs to a specialized set of proteins that act as an electron conduit to oxygen: the electron transport chain. As the electrons are passed down the chain, they lose much of their free energy. Some of this energy can be captured and stored in the form of a **proton gradient** that can be used to synthesize ATP from ADP; the remainder of the energy is lost as heat.

The term "proton gradient" means that one side of the membrane (in this case, the intermembrane space side of the mitochondrial inner membrane) has a higher concentration of protons that does the other side. Concentration gradients of any kind contain some energy; gradients of charged entities (such as protons) usually involve electrical potential gradients also, which also contain energy. The proton gradient generated by the electron transport chain has both concentration and electrical potential terms.
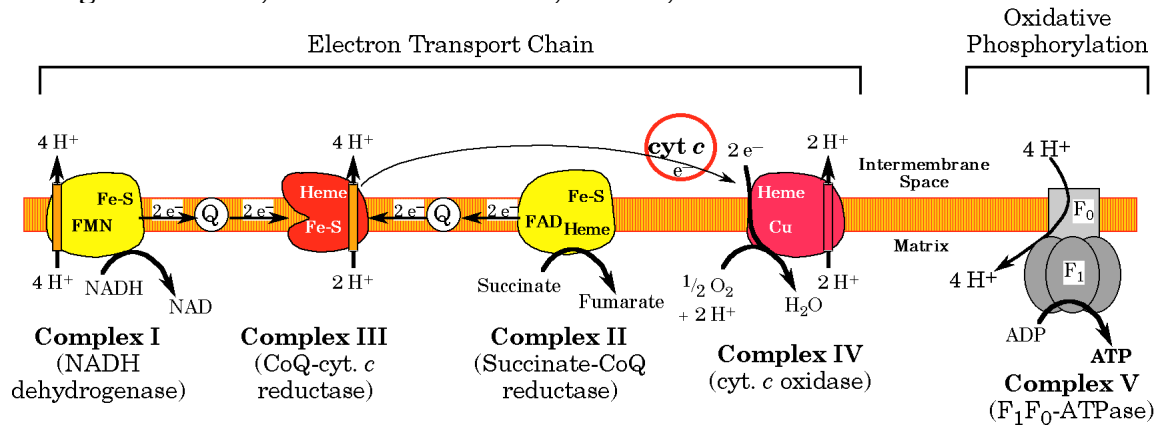
Extensive research has located a total of five protein complexes in the mitochondrial inner membrane involved in the electron transport and oxidative phosphorylation pathways. Complexes I, II, III, and IV are part of the electron transport chain. Complex V is the enzyme complex that carries out the oxidative phosphorylation reaction: the actual conversion of ADP to ATP. All of these are large multiprotein complexes.

In addition to the membrane-bound complexes, the electron transport chain requires mobile electron carriers: cytochrome *c* and Coenzyme Q. **Coenzyme Q** is not a protein, but is a membrane bound cofactor. **Cytochrome *c*** is a small soluble protein located in the intermembrane space.

The overall reaction involves the oxidation of NADH or $FADH_2$ cofactors, and results in the reduction of oxygen to water. This process is the major reason for the requirement for oxygen in most organisms. The electron transport pathway is often called the "respiratory chain", because this pathway is the major reason for respiration (= breathing in animals).

Electron transport complexes

The Complexes are proteins. Complexes I-IV have a variety of prosthetic groups including metal ions, iron-sulfur centers, hemes, and flavins.

Electron Transport Chain   Oxidative Phosphorylation

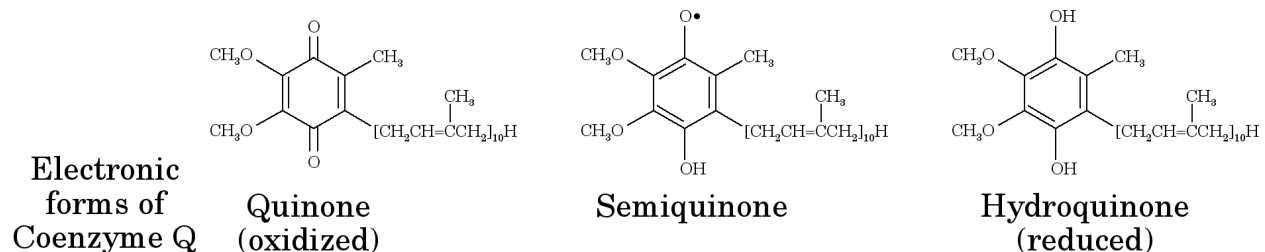| Complex I (NADH dehydrogenase) | Complex III (CoQ-cyt. *c* reductase) | Complex II (Succinate-CoQ reductase) | Complex IV (cyt. *c* oxidase) | Complex V (F_1F_0-ATPase) |

NADH dehydrogenase (Complex I)

The first complex contains a iron-sulfurs center and an FMN. Complex I **accepts electrons from NADH** to regenerate NAD. Complex I also **pumps protons**: each pair of electrons results in the movement of about 4 H^+ from the matrix to the intermembrane space. Complex I donates electrons to Coenzyme Q.

Coenzyme Q

Coenzyme Q is a non-protein electron carrier located in the inner mitochondrial membrane. Mammals use Q_{10} (note the side chain in the structures below; in mammals, the compound has ten isoprene units, while some other species use versions with 6 or 8 isoprene units). Note that Coenzyme Q can transfer one or two electrons; the structures below correspond to the fully oxidized form, the singly reduced form, and the fully reduced form of the molecule. Coenzyme Q can accept electrons from Complex I and II (and from other proteins); it donates the electrons to Complex III.

Electronic forms of Coenzyme Q

Quinone (oxidized)   Semiquinone   Hydroquinone (reduced)

Succinate Dehydrogenase (Complex II)

Succinate dehydrogenase is one of the enzymes in the TCA cycle (enzyme #6 in the TCA cycle diagram). It is the only TCA cycle enzyme embedded in the mitochondrial inner membrane. The conversion of succinate to fumarate results in reduction of the enzyme-bound FAD. The oxidation of the reduced flavin requires the donation of electrons to Coenzyme Q; in other words, the enzyme cannot carry out the reaction if the electron transport is non-functional.

Succinate dehydrogenase does **not** pump protons, and therefore any proton gradient formed as a result of the donation of electron to succinate dehydrogenase by succinate occurs only due to later steps in the electron transport pathway.

### Coenzyme Q-dependent cytochrome *c* reductase (Complex III)
Complex III accepts electrons from Coenzyme Q (and therefore, indirectly, from both Complex I and Complex II). Like Complex I, Complex III is also a proton pump. Complex III contains several heme prosthetic groups. Different heme domains have different absorbance spectra. The different spectral species are sometimes referred to as cytochrome *b* and cytochrome c_1; these are all part of the same protein complex.

The electrons that Complex III receives are donated one at a time to a soluble heme-containing electron carrier protein called cytochrome *c*. Cytochrome *c* is located in the intermembrane space. Although cytochrome *c* is a relatively small protein (~12 kDa), it is too large to pass through the porin molecules in the outer membrane.

Cytochrome *c* oxidase (Complex IV)
Cytochrome *c* oxidase, as the name implies, accepts electrons from cytochrome *c*. Complex IV is the terminal part of the electron chain and transfers electrons directly to oxygen. Like Complexes I and III, Complex IV is a proton pump. Cytochrome *c* oxidase is sometimes referred to as the cytochrome a-a_3 complex.

The cytochrome *c* oxidase three-dimensional structure has been solved. The complex contains a total of four hemes as well as copper and magnesium ions. In the representation at right, hydrophobic residues are shown in yellow, while charged residues are shown in red (negative) and blue (positive). Note the yellow band across the middle of the structure, which interacts with the hydrophobic interior of the membrane.
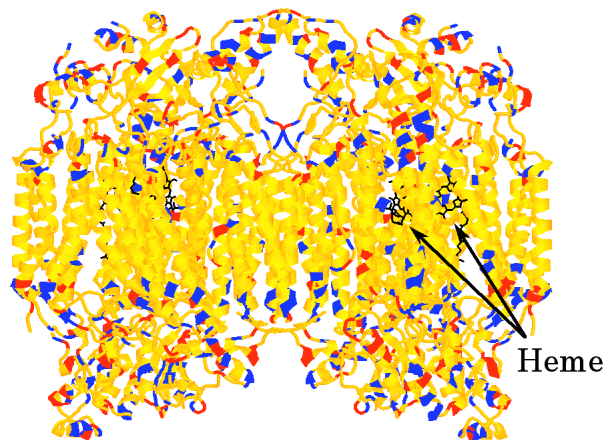

Heme

The electron transport chain uses a number of heme prosthetic groups; cytochrome *c* oxidase is the only one that is capable of binding oxygen; all of the others have both of the axial heme-iron binding sites occupied by amino acid side-chains.

Proton pumping
The exact mechanism for the proton pumping remains a matter of debate. The number of protons pumped as a result of a pair of electrons entering the chain from NADH also remains a matter of debate. Current estimates suggest that the equivalent of about 4 protons are pumped by each of the three proton pumps (Complex I, III, and IV).

(Note: a "proton equivalent" is an electron being pumped in the opposite direction. Every electron moving from outside to inside is the equivalent of a proton moving from outside to inside. In each case, the net result is an increase in the number of positive charges on the outside of the membrane.)
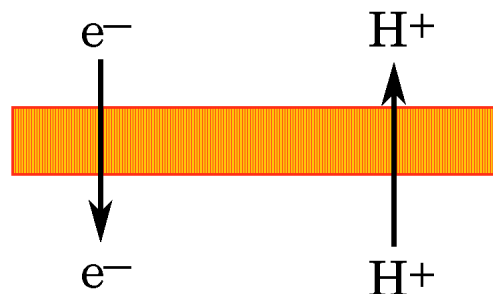
F_1F_0-ATPase = ATP Synthase (Complex V)

The final complex required for the synthesis of ATP is the ATP synthase enzyme complex itself. Complex V uses the proton gradient generated by the proton pumps to synthesize ATP. Once again, the exact number of protons required to synthesize a molecule of ATP are a matter of some controversy. Current estimates seem to indicate that about 4 H^+ must move down the gradient for each ATP produced. This value (if correct) applies to optimum conditions; under different conditions (such as a different ADP/ATP ratio), the value may be different.

The ATP synthase is a complicated protein complex, known to be comprised of two separate multisubunit proteins: F_0 (the transmembrane part of the complex that allows protons to move down their gradient) and F_1 (the part of the complex that extends into the matrix and acts as the ATP synthesis enzyme. A few years ago, the structure of the F_1 complex was solved. Based on the structure and on some other evidence, it is thought that the red "axle" in the representation below is attached to the F_0 complex, that the remainder of the F_1 protein rotates around the axle, and that this rotation results in formation of ATP from ADP+P_i.

Two views of the
ATP Synthase
(F_1)

Adenosine
nucleotides

"Axle"
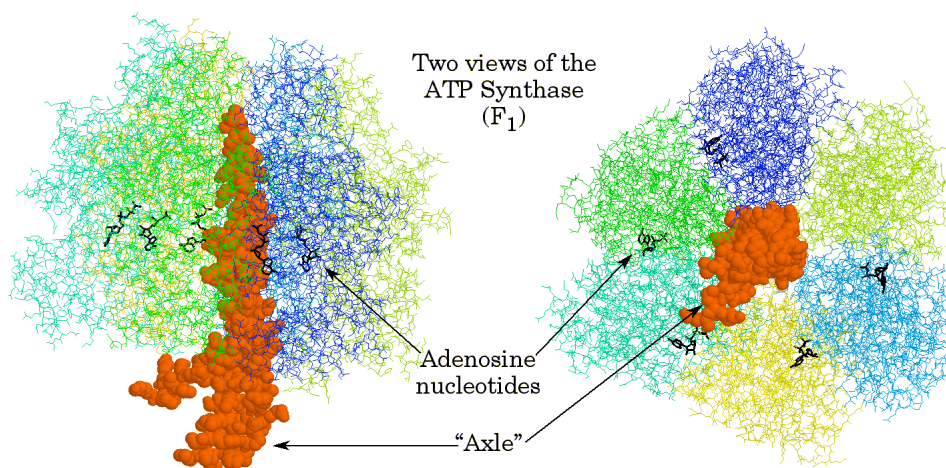
Inhibitors of ATP Synthesis

The role of the different protein complexes in the electron transport chain was worked out using compounds that specifically inhibit each of the steps in the pathway. Some of these compounds are well-known drugs or poisons. A few examples are given below.

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Barbiturates such as amytal and the plant toxin **rotenone** inhibit transfer of electrons to Coenzyme Q from Complex I. The antibiotic **Antimycin A** inhibits transfer from Complex III to cytochrome c.

Cyanide and H_2S inhibit cytochrome c oxidase because they bind to the oxygen-binding site on the heme prosthetic group. **Carbon monoxide** can also inhibit cytochrome c oxidase, but the main action of carbon monoxide *in vivo* is inhibition of hemoglobin oxygen binding. In contrast, cyanide is much more effective cytochrome c oxidase inhibitor because it has a high affinity for cytochrome c oxidase, and a relatively low affinity for hemoglobin.

Atractyloside: inhibits ADP/ATP exchange transporter. Atractyloside indirectly inhibits Complex V because it reduces the amount of free ADP substrate available; the effect is an inhibition both of ATP synthesis and of electron transport.

2,4-Dinitrophenol uncouples proton gradient from ATP synthesis, because it can carry protons across the mitochondrial inner membrane. Dinitrophenol was used as a weight-loss drug. Although dinitrophenol was highly effective, it was also potentially lethal, and it is no longer used for dieting.
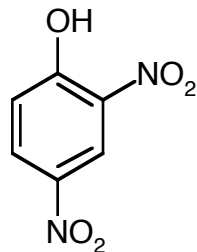
2,4 Dinitrophenol

Uncoupling protein (UCP) is a controllable proton channel present in brown adipose tissue. The brown color of brown adipose tissue is due to the presence of large numbers of mitochondria. UCP (and 2,4-dinitrophenol) allows electron transport without ATP generation, with the energy released as heat.

Regulation of the electron transport pathway
The synthesis of ATP by electron transport and oxidative phosphorylation appears to be regulated essentially exclusively by substrate availability. The pathway cannot proceed without ADP+P_i or NADH; if both are available, then the pathway will result in ATP synthesis.

Energetics of the TCA cycle and glucose oxidation
The number of ATP produced per NADH oxidized depends on the number of protons pumped by each complex, and on the number of protons required by the ATP synthase. Some research indicates ~3 ATP/NADH, while other studies suggest somewhat fewer (~2.5). Possible causes of discrepancies include:
1) The number of protons pumped at each stage may vary somewhat.
2) The mitochondrial inner membrane is not a perfect barrier: a few protons leak through the membrane, partially uncoupling the system.

3) The proton gradient is used to pump other molecules (*e.g.*, protons drive the pumping of pyruvate into the mitochondria).
4) Many of the measurements (for example, of the exact proton gradient existing in cells, and of the ADP concentration in the mitochondrion) are difficult to perform.

For the purposes of the following discussion, we will assume that, *under optimum conditions*, NADH can be converted to about three ATP, because Complex I, III, and IV are each thought to pump four protons, and ATP synthesis is thought to require four protons. Electrons from $FADH_2$ enter at the level of Complex II, which does not pump protons, but instead hands them to Complex III; this suggests that $FADH_2$ results in formation of about two ATP. Using these estimates of the number of ATP produced, and the net reaction for TCA cycle suggests that the TCA cycle can result in the production of 12 ATP: three ATP for each of the three NADH, two ATP for the $FADH_2$, and one substrate level phosphorylation.

During the conversion of pyruvate to acetyl-CoA, one NADH is generated, resulting in an additional three ATP; each complete oxidation of pyruvate to carbon dioxide therefore results in 15 ATP. Glucose conversion to pyruvate produces 2 NADH and 2 ATP, adding a total of 8 ATP, and therefore resulting in 38 ATP (under optimum conditions) for complete aerobic glycolysis. (Note once again, that this represents the **maximal** amount of ATP obtainable from complete oxidation of glucose; the value is more for the purposes of comparison than for claiming that each molecule of glucose will *always* result in 38 molecules of ATP).

How efficient is the conversion of glucose into usable energy?

Energy efficiency is the ratio of the amount of energy extracted from a compound relative to the amount of energy actually present in the compound. The $\Delta G'^{\circ}$ for glucose combustion = –2870 kJ/mol, while the $\Delta G'^{\circ}$ for ATP hydrolysis = –30.5 kJ/mol. Assuming that glucose oxidation results in 38 ATP formed, the efficiency is:

$$\frac{(38 \text{ ATP formed})\left(-30.5 \frac{kJ}{mol} \text{ for each ATP}\right)}{-2870 \frac{kJ}{mol} \text{ for each Glucose}} \; 100 = 40\% \; efficiency$$

This is an underestimate, because the actual ΔG values under cellular conditions are somewhat different (the actual ΔG for ATP hydrolysis is ~–50 kJ/mol); in cells, the efficiency of energy recovery is actually ~60-65%. Some of the unrecovered energy is lost due to the losses that always occur in real systems; some of the energy goes into the irreversible steps in the pathway (for example, some energy is lost in the pyruvate kinase step, which is why this step is physiologically irreversible).

Summary
Reduced cofactors contain significant stored energy. In most cases, nicotinamide cofactors contain more energy than flavin cofactors. The mitochondria contain specialized protein complexes involved in transferring electrons from the reduced cofactors to molecular oxygen. The energy from the electrons is stored in the form of a proton gradient, which can be used to generate ATP.

Three protein complexes (Complex I [NADH dehydrogenase], Complex III, [cytochrome c reductase], and Complex IV, [cytochrome c oxidase]) each pump enough protons to result in generation of approximately one ATP. NADH electrons enter at the level of Complex I, while for electrons derived from $FADH_2$ the first proton pump is Complex III. The proton gradient is used by the ATP synthase to produce the ATP.

ATP synthesis can be inhibited by inhibitors that affect the electron transport chain, or by inhibitors of the transport of ADP into the mitochondrion, or by compounds that allow the protons to cross the membrane freely.

Under cellular conditions, conversion of glucose to lactate results in formation of two ATP, while conversion of glucose to carbon dioxide and water results in **considerably** more ATP (up to a maximum of 38 ATP under optimum conditions).

The glycolytic pathway is much faster than the TCA cycle and electron transport/oxidative phosphorylation, and therefore pyruvate production may exceed the capacity of the cell to oxidize all of the pyruvate produced. Metabolism under conditions when oxygen is limiting, or when the glycolytic throughput exceeds the rate of pyruvate oxidation results in pyruvate reduction to lactate or ethanol.

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