

# Energy: Electron Transport & Oxidative Phosphorylation



In eukaryotic cells, the vast majority of ATP synthesis occurs in the mitochondria in a process called oxidative phosphorylation. Even plants, which generate ATP by photophosphorylation in chloroplasts, contain mitochondria for the synthesis of ATP through oxidative phosphorylation.

Oxidative phosphorylation is linked to a process known as electron transport (Figure 5.14). The electron transport system, located in the inner mito-

chondrial membrane, transfers electrons donated by the reduced electron carriers NADH and  $\text{FADH}_2$  (obtained from glycolysis, the citric acid cycle or fatty acid oxidation) through a series of electron acceptors, to oxygen. As

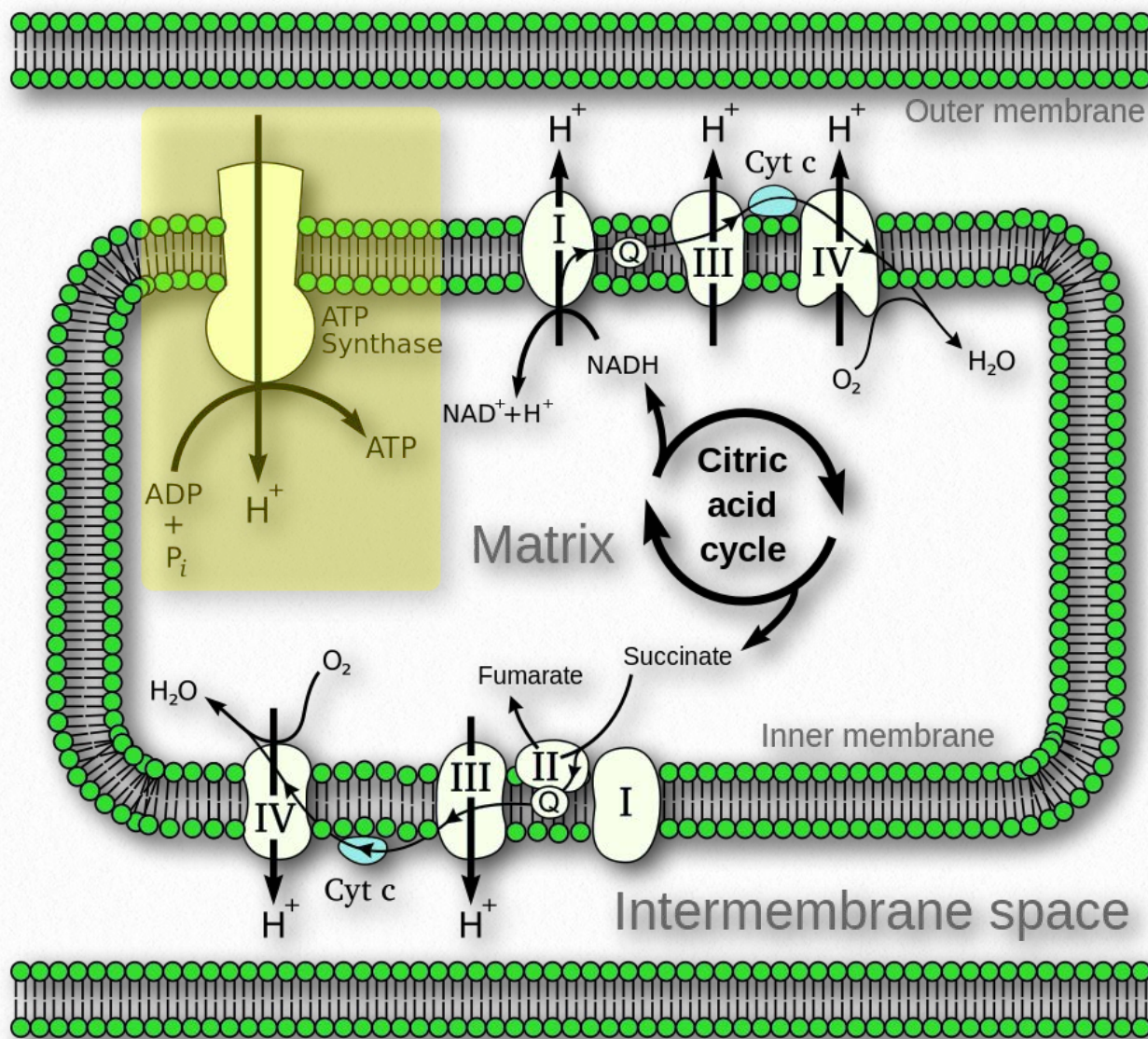
we shall see, movement of electrons

through complexes of the electron transport system essentially

“charges” a battery that is used to make ATP in oxidative phosphory-

lation. In this way, the oxidation of sugars and fatty acids is coupled to the synthesis

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**Figure 5.14 - Overview of electron transport (bottom left and top right) and oxidative phosphorylation (top left - yellow box) in the mitochondrion**

of ATP, effectively extracting energy from food.

## Chemiosmotic model

Dr. Peter Mitchell introduced a radical proposal in 1961 to explain the mechanism by which mitochondria make ATP. It is known as the chemiosmotic hypothesis and has been shown over the years to be correct. Mitchell proposed that synthesis of ATP in mitochondria depends on an electrochemical gradient, across the mitochondrial inner membrane, that arises ulti-

mately from the energy of reduced electron carriers, NADH and FADH<sub>2</sub>.

## Electron transport

Further, the proposal states that the gradient is created when NADH and FADH<sub>2</sub> transfer their electrons to an electron transport system (ETS) located in the inner mitochondrial membrane. Movement of electrons through a series of electron carriers is coupled to the pumping of protons out of the mitochondrial matrix across the inner mitochondrial membrane into the space between the inner

and outer membranes. The result is creation of a gradient of protons whose potential energy can be used to make ATP. Electrons combine with oxygen and protons at the end of the ETS to make water.

## ATP synthase

In oxidative phosphorylation, ATP synthesis is accomplished as a result of protons re-entering the mitochondrial matrix via the transmembrane ATP synthase complex, which combines ADP with inorganic phos-

phate to make ATP. Central to the proper functioning of mitochondria through this process is the presence of an intact mitochondrial inner membrane impermeable to protons.

## Tight coupling

When this is the case, tight coupling is said

to exist between electron transport and the synthesis of ATP (called oxidative phosphorylation). Chemicals which permeabilize the inner mitochondrial membrane to protons cause *uncoupling*, that is, they allow the protons to leak back into the mitochondrial matrix, rather than

through the ATP synthase, so that the movement of electrons through the ETS is no longer linked to the synthesis of ATP.

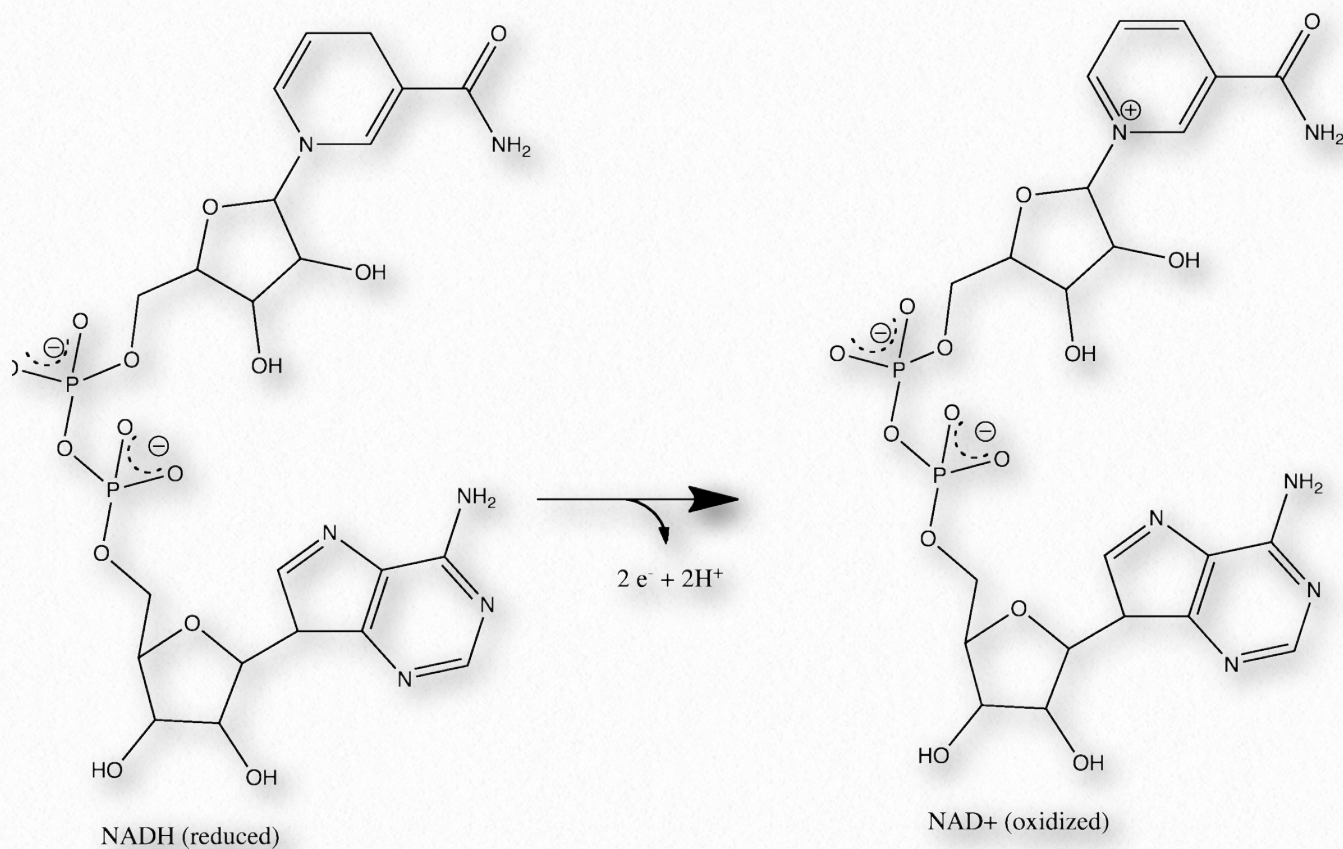
## Power plants

Mitochondria are called the power plants of the cell because most of a cell's ATP is produced there in the process of oxidative phosphorylation. The mechanism by which ATP

is made in oxidative phosphorylation is one of the most interesting in all of biology.

## Considerations

The process has three primary considerations. The first is electrical – electrons from reduced electron carriers, such as NADH and



**Figure 5.15 - Loss of electrons by NADH to form NAD<sup>+</sup>. Relevant reactions occur in the top ring of the molecule.**

FADH<sub>2</sub>, enter the electron transport system via Complex I and II, respectively. As seen in [Figure 5.16](#) and [Figure 5.17](#), electrons move from one complex to the next, not unlike the way they move through an electrical circuit. Such movement occurs as a result of a set of reduction-oxidation (redox) reactions with electrons moving from a more nega-

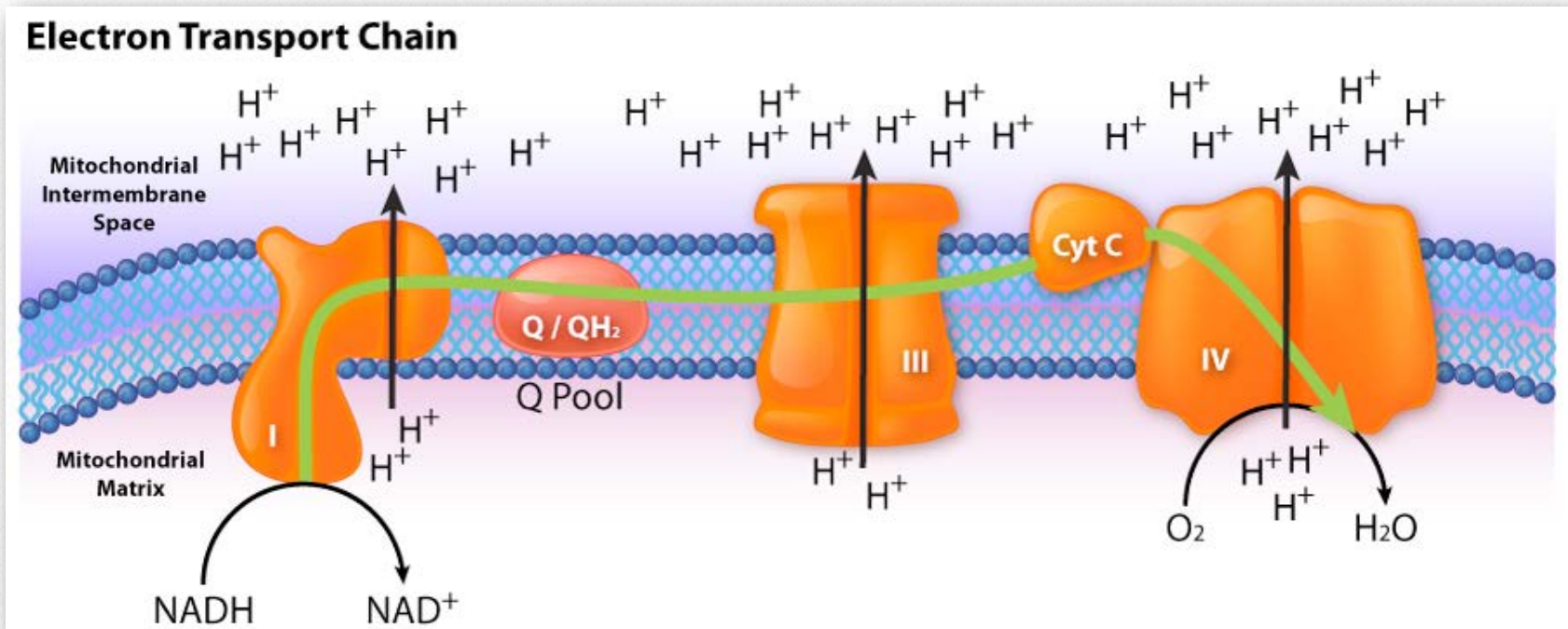


Figure 5.16 - Flow of electrons from NADH into the electron transport system. Entry is through complex I

Image by Aleia Kim

tive reduction potential to a more positive one.

One can think of this occurring as a process where carriers “take” electrons away from complexes with



lower reduction potential, much the way a bully takes lunch money from a smaller child. In this scheme, the biggest “bully” is oxygen in Complex IV. Electrons gained by a

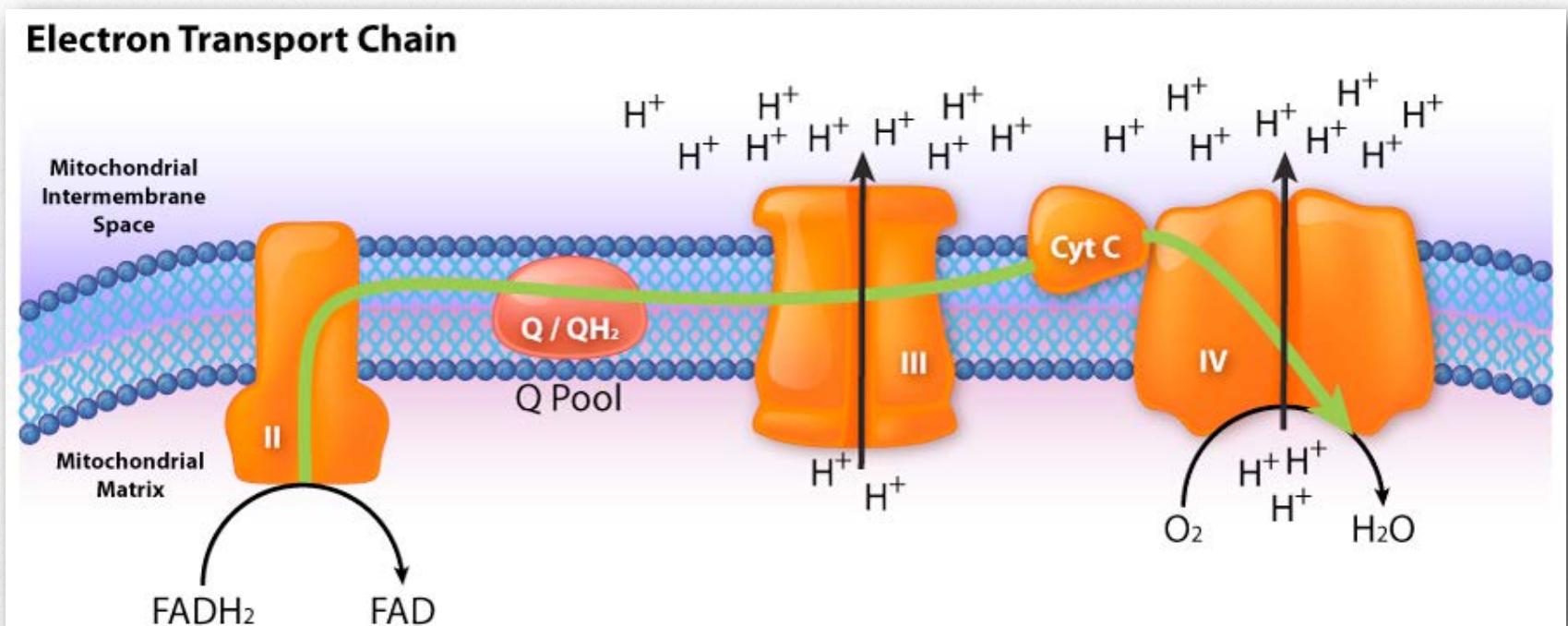
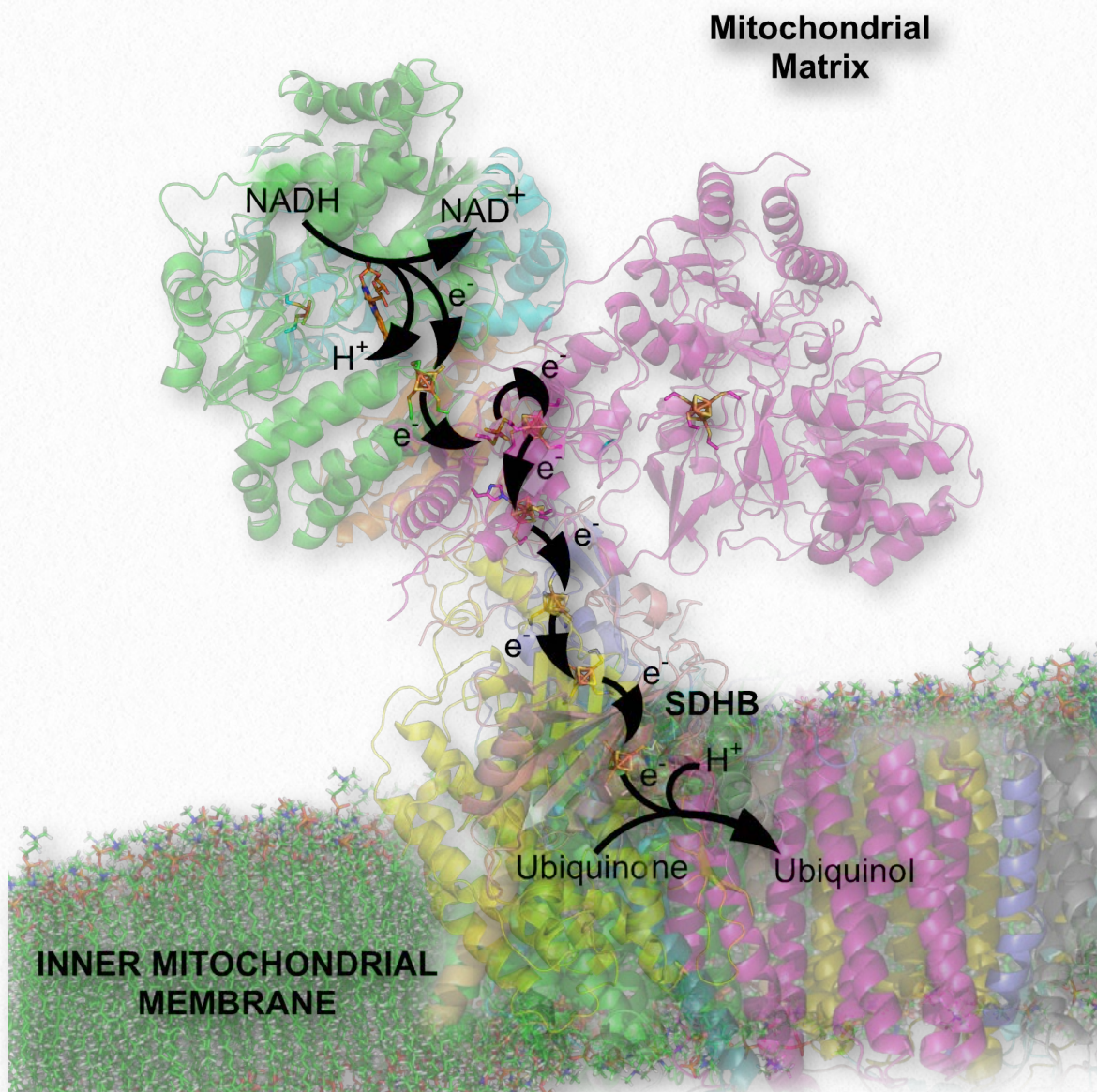


Figure 5.17 - Flow of electrons from FADH<sub>2</sub> into the electron transport chain. Entry is through complex II.

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**Figure 5.18 - Complex I embedded in the inner mitochondrial membrane. The mitochondrial matrix at the top**

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carrier cause it to be reduced, whereas the carrier giving up the electrons is oxidized.

### Entry of electrons to system

Movement of electrons through the chain begins either by 1) transfer from NADH to Complex I (Figure 5.16) or 2) movement of electrons through a covalently bound FADH<sub>2</sub> (Figure 5.17) in the membrane-bound succinate dehydrogenase (Complex II). (An alternate entry point for electrons from FADH<sub>2</sub> is the Electron Transferring Fla-

voprotein via the electron-transferring-flavoprotein dehydrogenase, not shown).

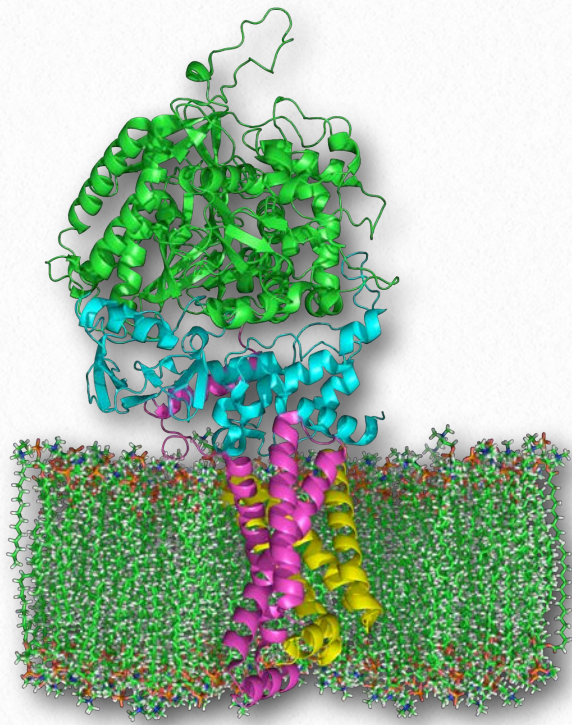
### Traffic cop

Both Complex I and II pass electrons to the inner membrane's coenzyme Q (CoQ - Figures 5.18 & 5.19). In each case, coenzyme Q accepts electrons in pairs and passes them off to Complex III (CoQH<sub>2</sub>-cytochrome c reductase) singly. Coenzyme Q thus acts as a traffic cop, regulating the flow of electrons through the ETS.

### Docking station

Complex III is a docking station or interchange for the incoming electron carrier (coenzyme Q) and the outgoing carrier (cytochrome c). Movement of electrons from Coenzyme Q to Complex III and then to cytochrome C occurs as a result of what is referred to as the Q-cycle (see below).

Complex III acts to ferry electrons from CoQ to cytochrome c. Cytochrome c takes one electron from Complex III and passes it to Complex IV (cytochrome oxidase). Complex IV is the final protein recipient of the electrons. It passes them to molecular oxygen (O<sub>2</sub>) to make two molecules of water. Making two water



**Figure 5.19 - Complex II embedded in inner mitochondrial membrane. Matrix is up.**

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molecules requires four electrons, so Complex IV must accept, handle, and pass to molecular oxygen four separate electrons, causing the oxidation state of oxygen to be sequentially changed with addition of each electron.

## Proton pumping

As electrons pass through complexes I, III, and IV, there is a release of a small amount of energy at each step, which is used to pump protons from the mitochondrial matrix (inside of mitochondrion) and deposit them in the intermembrane space (between the inner and outer membranes of the mitochondrion). The effect of this redistribution is to increase the

electrical and chemical potential across the membrane.

## Potential energy

As discussed earlier, electrochemical gradients have potential energy. Students may think of the process as “charging the battery.” Just like a charged battery, the potential arising from the proton differential across the membrane can be used to do things. In the mitochondrion, what the proton gradient does is facilitate the production of ATP from ADP and  $P_i$ . This process is known as oxidative phosphorylation, because the phosphorylation of ADP to ATP is dependent on the oxidative reactions occurring in the mitochondria.

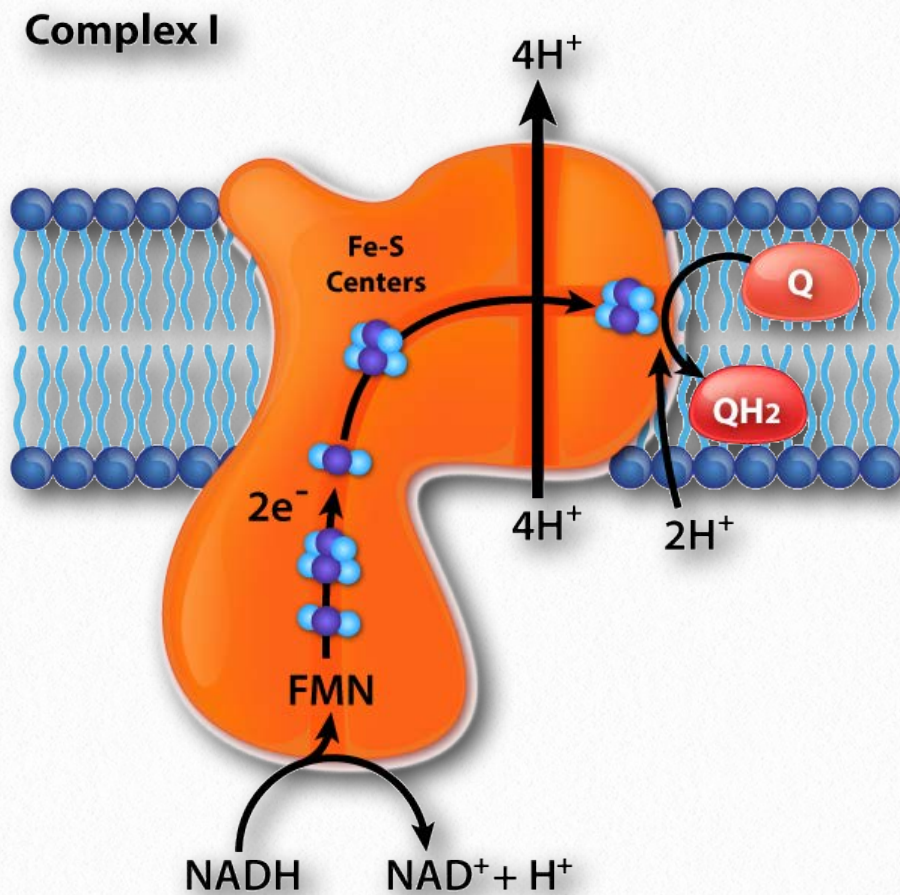
Having understood the overall picture of the synthesis of ATP linked to the movement of electrons through the ETS, we will take a closer look at the individual components of the ETS.

## Complex I

Complex I (also called NADH:ubiquinone oxidoreductase or NADH dehydrogenase (ubiquinone)) is the electron acceptor from NADH in the electron transport chain and the largest complex found in it.

Complex I contains 44 individual polypeptide chains, numerous iron-sulfur centers, a molecule of flavin mononucleotide (FMN) and has an L shape with

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**Figure 5.20 - Movement of electrons through complex I from NADH to coenzyme Q. The mitochondrial matrix is at the bottom**

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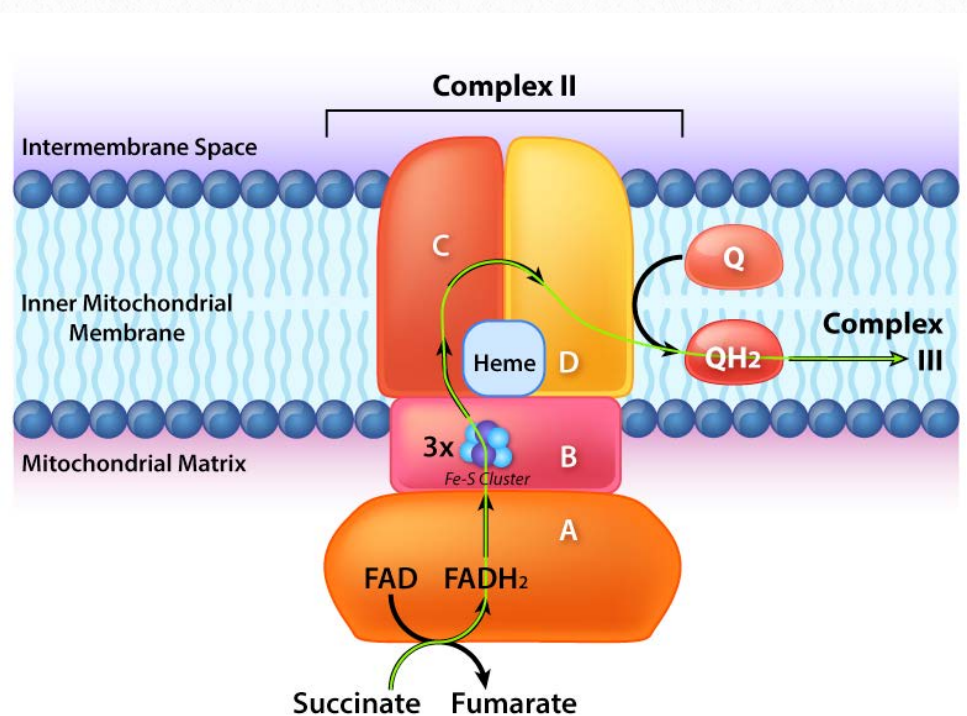
about 60 transmembrane domains. In the process of electron transport through it, four protons are pumped across the inner membrane into the intermembrane space and electrons move from NADH to coenzyme Q, converting it from ubiquinone (no electrons) to ubiquinol (gain of two electrons). An intermediate form, ubisemiquinone (gain of one electron), is found in the Q-cycle.

Electrons travel through the complex via seven primary iron sulfur centers. The best known inhibitor of the complex, rotenone, works by binding to the

CoQ binding site. Other inhibitors include ADP-ribose (binds to the NADH site) and piericidin A (rotenone analog). The process of electron transfer through complex I is reversible and when this occurs, superoxide (a reactive oxygen species) may be readily generated.

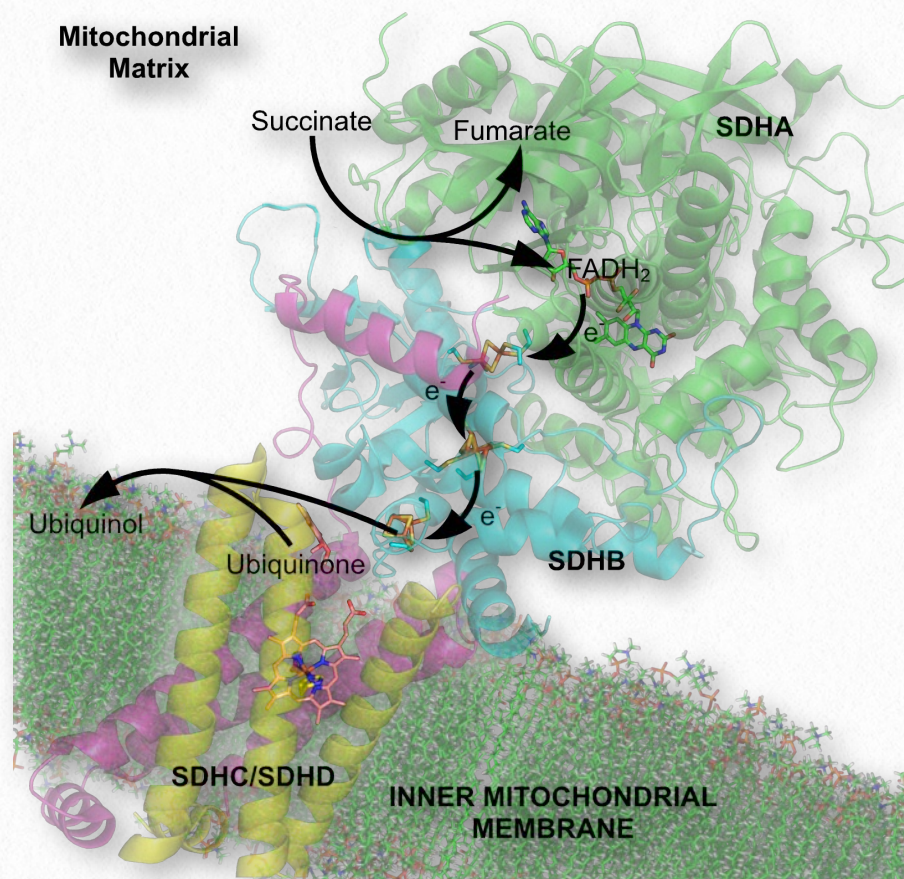
## Complex II

Complex II (also called succinate dehydrogenase or succinate-coenzyme Q reductase) is a membrane bound enzyme of the citric acid cycle that plays a role in the electron transport process, transferring electrons from its covalently bound  $\text{FADH}_2$  to coenzyme Q. The process occurs, as shown in Figure 5.20 and Figure 5.21, with transfer of electrons from succinate to FAD to



**Figure 5.21 - Movement of electrons from succinate through complex II (A->B->C->D->Q). Mitochondrial matrix on bottom.**

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**Figure 5.22 - Complex II in inner mitochondrial membrane showing electron flow. Matrix is up.**

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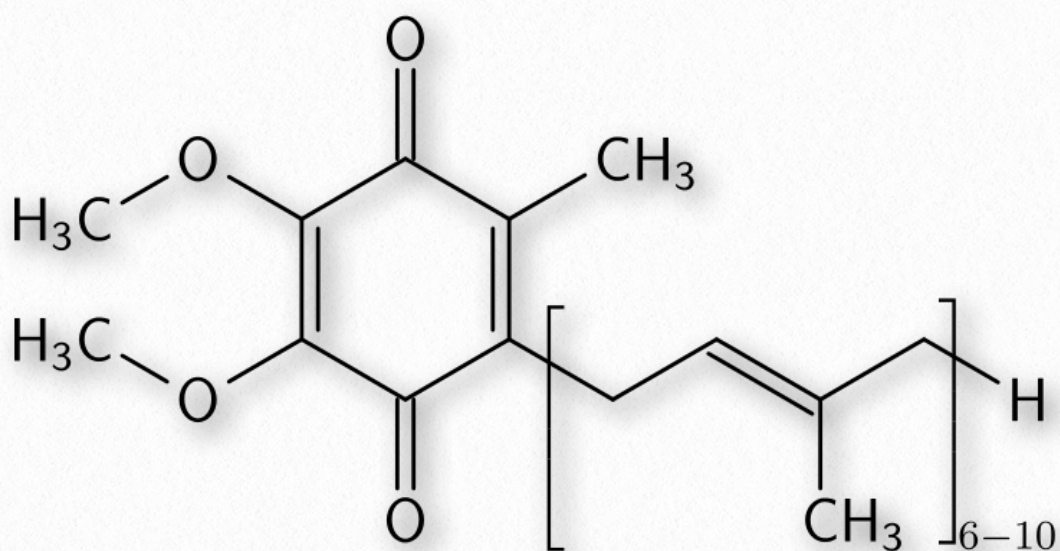
form FADH<sub>2</sub> and fumarate. FADH<sub>2</sub>, in turn, donates electrons to a relay system of iron-sulfur groups and they ultimately reduce ubiquinone (CoQ) along with two protons from the matrix to ubiquinol. The role of the heme group in the process is not clear. Inhibitors of the process include carboxin, malonate, malate, and oxaloacetate. The role of citric acid cycle intermediates as inhibitors is thought to be due to inhibition of the reversal of the transfer process which can produce superoxide.

## Coenzyme Q

Coenzyme Q (Figure 5.23) is a 1,4 benzoquinone whose name is often given as Coenzyme Q<sub>10</sub>, CoQ, or Q<sub>10</sub>. The 10 in the name refers to the number of isoprenyl units it contains that anchor it to the mitochondrial inner membrane. CoQ is a vitamin-like lipid substance found in most eukaryotic cells as a component of the electron transport system. The requirement for CoQ increases with increasing energy needs of cells, so the highest concentrations of CoQ in the body are found in tissues that are the most metabolically active - heart, liver, and kidney.

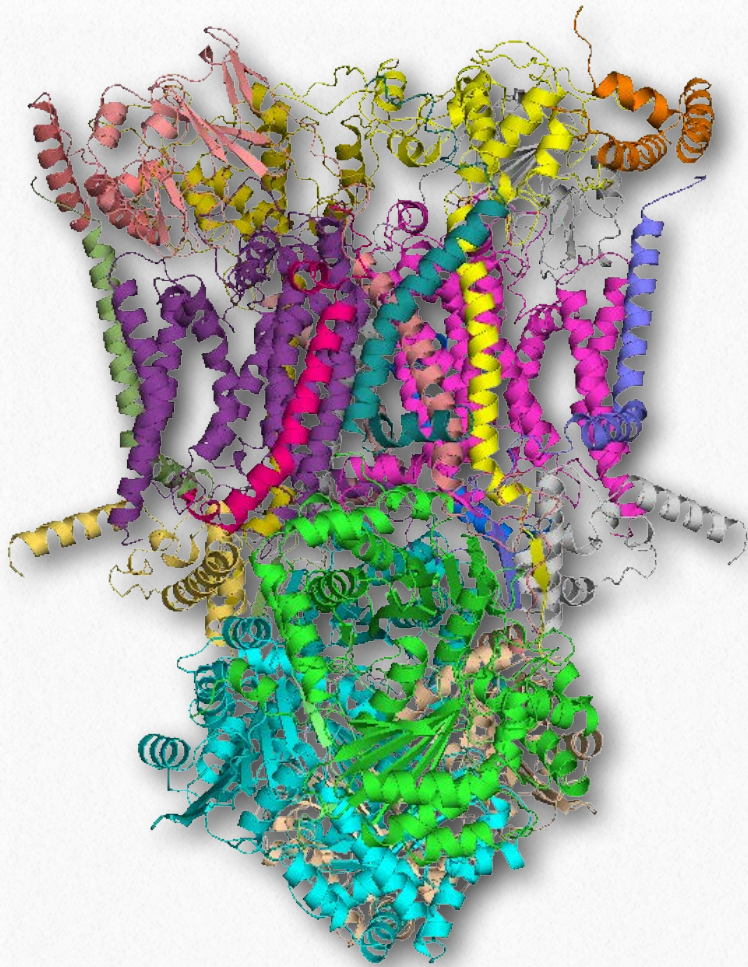
### Three forms

CoQ is useful because of its ability to carry and donate electrons and particularly because it can exist in forms with two extra electrons (fully reduced - ubiquinol), one extra electron (semi-reduced - ubisemiquinone),



**Figure 5.23 - Coenzyme Q**





**Figure 5.24 - Complex III**

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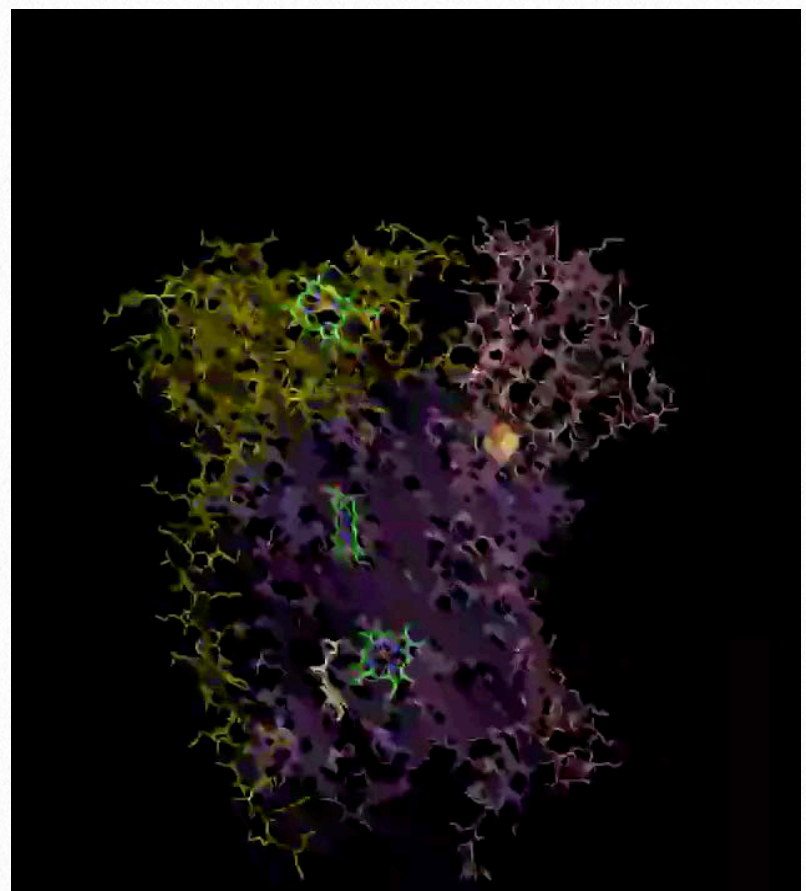
or no extra electrons (fully oxidized - ubiquinone). This ability allows CoQ to provide transition between the first part of the electron transport system that moves electrons in pairs and the last part of the system that moves electrons one at a time.

### Complex III

Complex III (also known as coenzyme Q : cytochrome c — oxidoreductase or the cytochrome  $bc_1$  complex - [Figure 5.24](#)) is the third electron accepting complex of the electron transport system. It is a transmembrane protein with multiple subunits present in the mitochondria of all aerobic eukaryotic organisms and and the cell mem-

brane of almost all bacteria. The complex contains 11 subunits, a 2-iron ferredoxin, cytochromes b and  $c_1$  and belongs to the family of oxidoreductase enzymes.

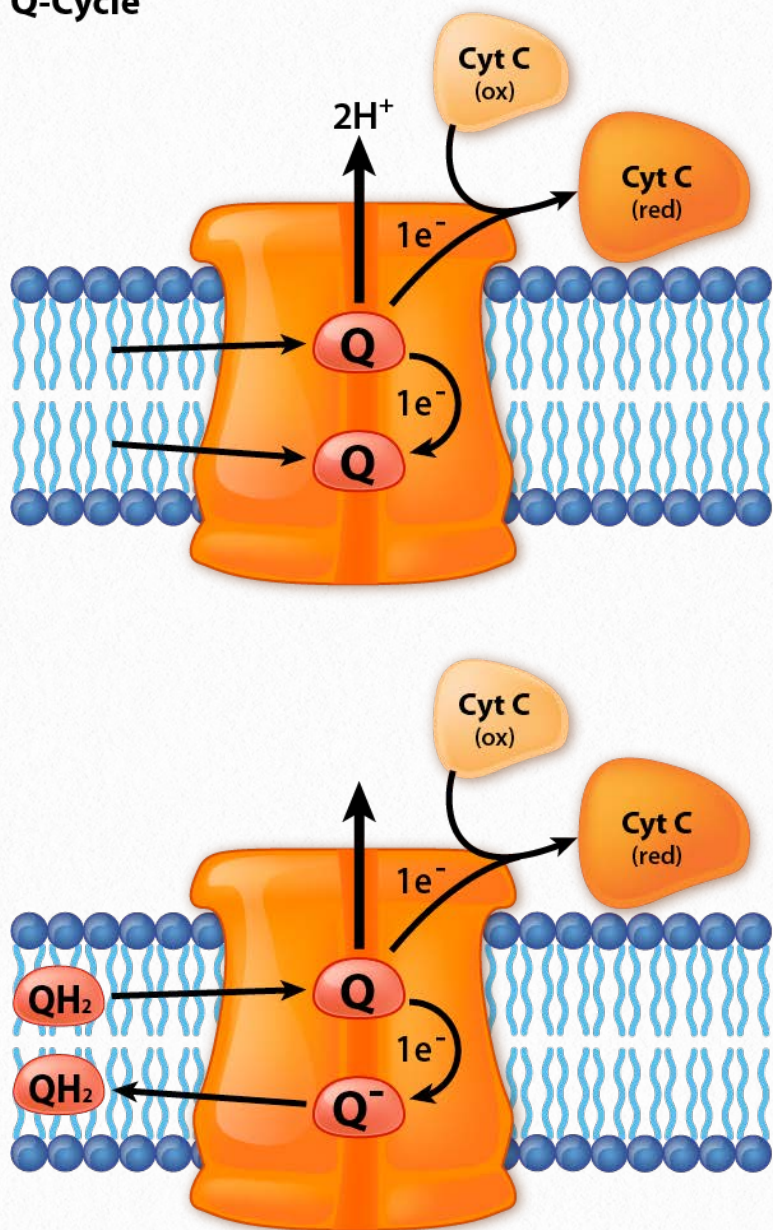
It accepts electrons from coenzyme Q in electron transport and passes them off to cytochrome c. In this cycle, known as the Q cycle, electrons arrive from CoQ in pairs, but get passed to cytochrome c individually. In the overall process, two protons are consumed from the matrix and four protons are pumped into the intermembrane space. Movement of electrons through the complex can be inhibited by antimycin A, myxothiazol, and stigmatellin. Complex III is also implicated in creation of superoxide (a reactive oxygen species) when electrons from it leak



**Movie 5.2 - The Q-cycle**

Wikipedia

## Q-Cycle



**Figure 5.25 - The Q-cycle. Matrix is down.**

Image by Aleia Kim

out of the chain of transfer. The phenomenon is more pronounced when antimycin A is present.

## Q-cycle

In the Q-cycle, electrons are passed from ubiquinol ( $\text{QH}_2$ ) to cytochrome c using Complex III as an intermediary docking station for the transfer. Two pair of electrons enter from  $\text{QH}_2$  and one pair is returned to another CoQ to re-

make  $\text{QH}_2$ . The other pair is donated singly to two different cytochrome c molecules.

## Step one

The Q-cycle happens in a two step process. First, a ubiquinol ( $\text{CoQH}_2$ ) and a ubiquinone ( $\text{CoQ}$ ) dock at Complex III. Ubiquinol transfers two electrons to Complex III. One electron goes to a docked cytochrome c, reducing it and it exits (replaced by an oxidized cytochrome c). The other goes to the docked ubiquinone to create the semi-reduced semiquinone ( $\text{CoQ}\cdot^-$ ) and leaving behind a ubiquinone, which exits. This is the end of step 1.

## Step two

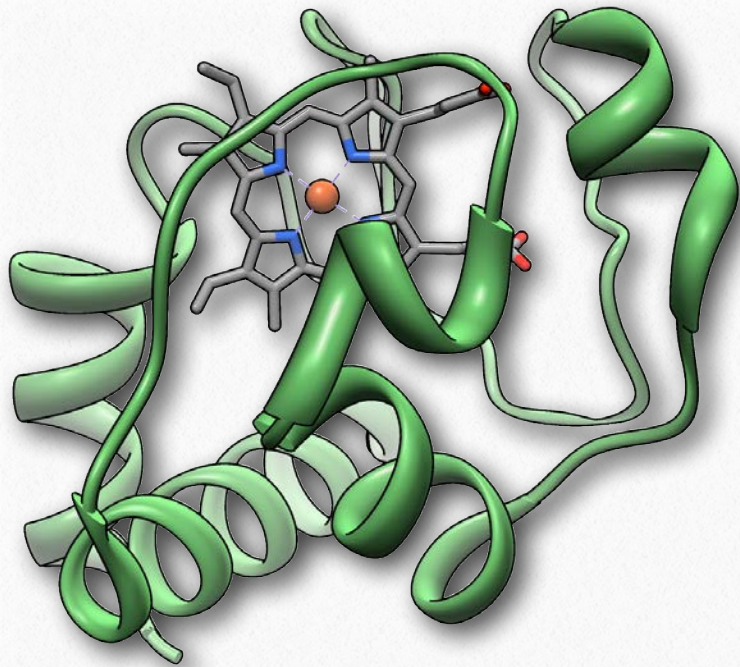
The gap left behind by the ubiquinone (Q) that departed is replaced by another ubiquinol ( $\text{QH}_2$ ). It too donates two electrons to Complex III, which splits them. One goes to the newly docked oxidized cytochrome c, which is reduced and exits. The other goes to the ubisemiquinone. Two protons from the matrix combine with it to make another ubiquinol. It and the ubiquinone created by the electron donation exit Complex III

and the process starts again. In the overall process, two protons are consumed from the matrix and four protons are pumped into the intermembrane space.

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## Cytochrome c

Cytochrome c (Figure 5.26) is a small (12,000 Daltons), highly conserved protein,



**Figure 5.25 - Cytochrome c with bound heme Group**

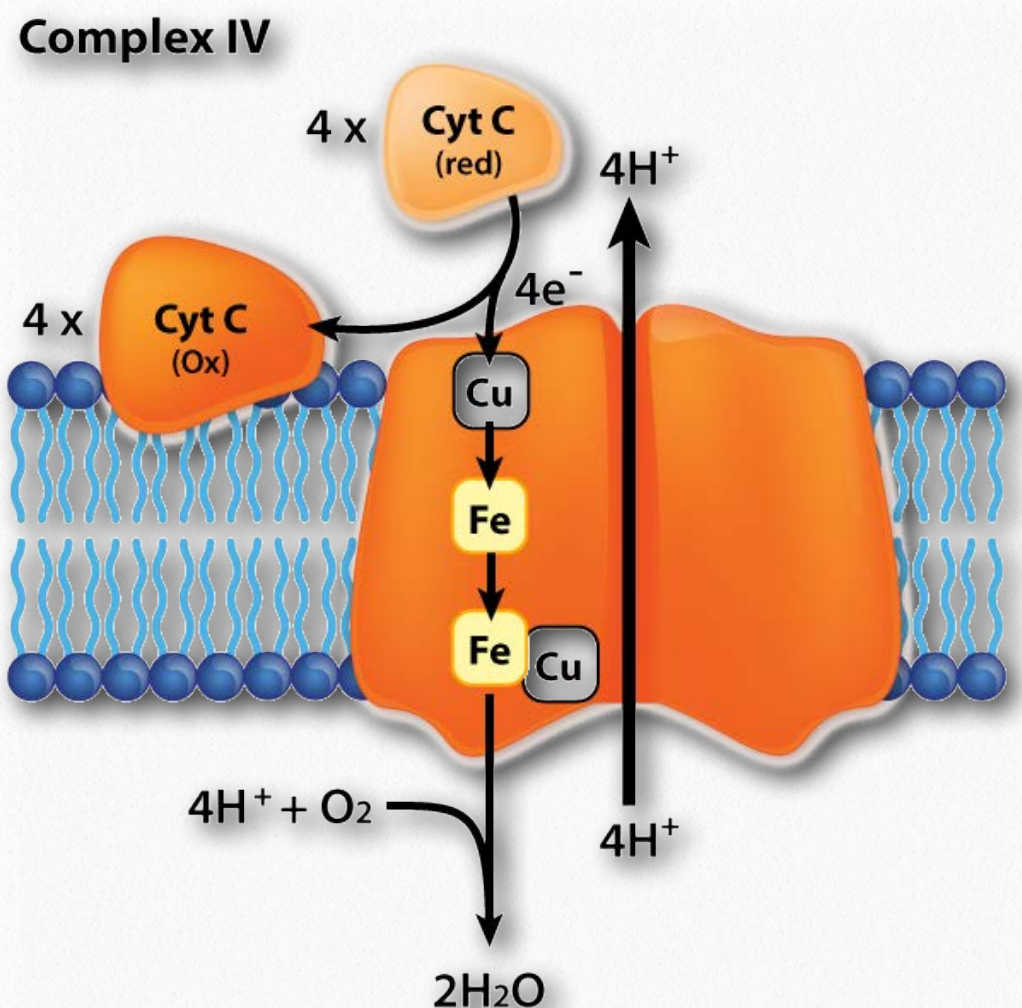
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from unicellular species to animals, that is loosely associated with the inner mitochondrial membrane where it functions in electron transport. It contains a heme group which is used to carry a single electron from Complex III to Complex IV. Cytochrome c also plays an important role in apoptosis in higher organisms. Damage to the mitochondrion that results in release of cytochrome c can stimulate assembly of the apoptosome and activation of the caspase cascade that leads to programmed cell death.

### Complex IV

Complex IV, also known as cytochrome c oxidase is a 14 subunit integral membrane protein at the end of the electron transport chain (Figure 5.27). It is responsible for accepting one electron

each from four cytochrome c proteins and adding them to molecular oxygen ( $O_2$ ) along with four protons from the mitochondrial matrix to make two molecules of water. Four protons from the matrix are also pumped into the intermembrane space in the process. The complex has two molecules of heme, two cytochromes (a and  $a_3$ ), and two copper centers (called  $Cu_A$  and  $Cu_B$ ). Cytochrome c docks near the  $Cu_A$  and donates an electron to it. The reduced  $Cu_A$  passes the electron to cytochrome a, which turns it over to the  $a_3$ - $Cu_B$  center where the oxygen is reduced. The four electrons are thought to pass through the complex rapidly resulting in com-



**Figure 5.26 - Movement of electrons and protons through complex IV. Matrix is down**

Image by Aleia Kim

plete reduction of the oxygen-oxygen molecule without formation of a peroxide intermediate or superoxide, in contrast to previous predictions.

## Respirasome

There has been speculation for many years

that a super-complex of electron carriers in the inner membrane of the mitochondrion may exist in cells with individual carriers making physical contact with each other.

This would make for more efficient trans-

fer reactions, minimize the production of reactive oxygen species and be similar to metabolons of metabolic pathway enzymes, for which there is some evidence. Now, evidence appears to be accumulating that complexes I, III, and IV form a supercomplex, which has been dubbed the respirasome<sup>1</sup>.

## Oxidative phosphorylation

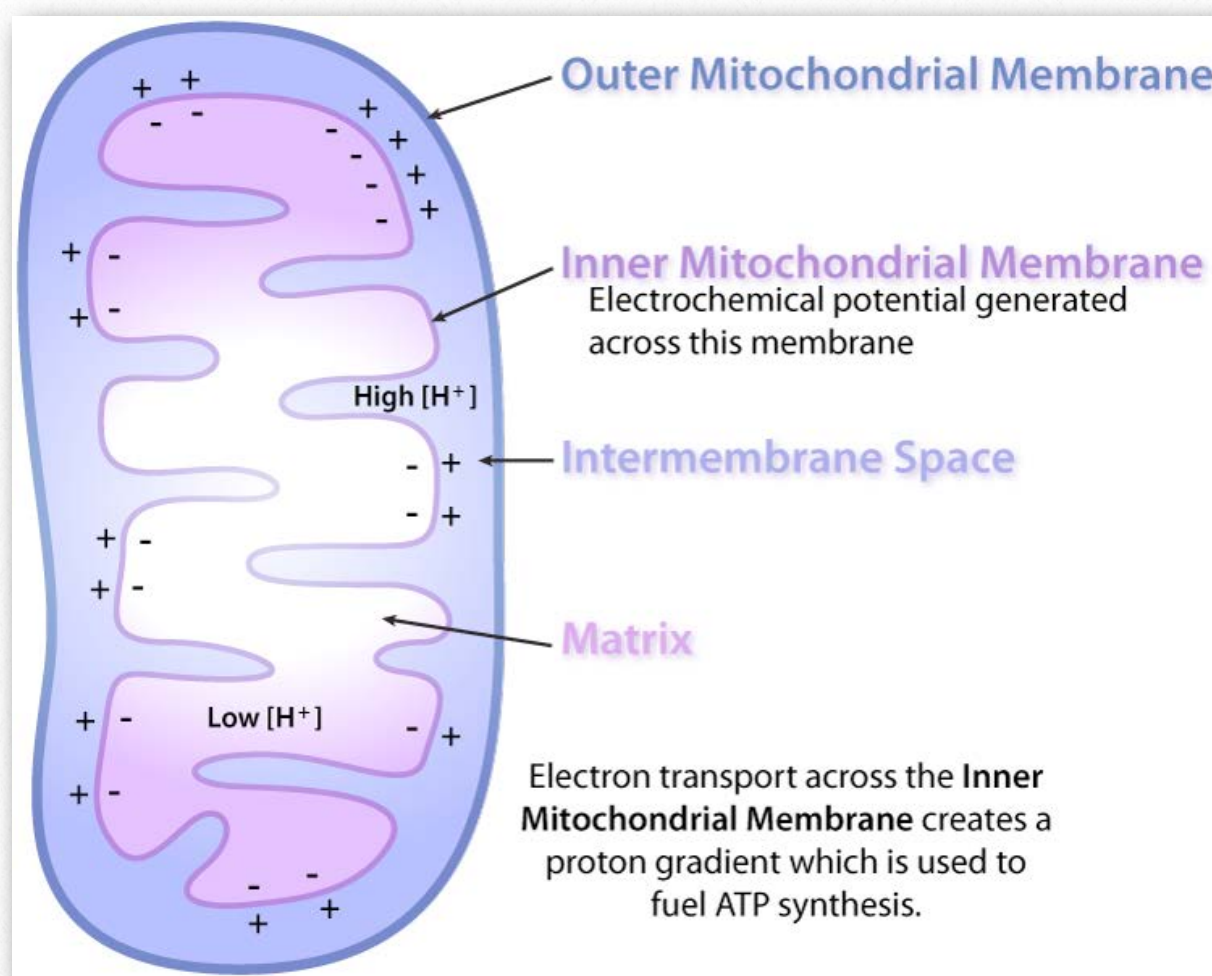
The process of oxidative phosphorylation uses the energy of the proton gradient established by the electron transport system as a means of phosphorylating ADP to make ATP. The establishment of the proton gradient is dependent upon electron trans-

port. If electron transport stops or if the inner mitochondrial membrane's impermeability to protons is compromised, oxidative phosphorylation will not occur because without the proton gradient to drive

the ATP synthase, there will be no synthesis of ATP.

## ATP synthase

The protein complex harvesting energy from the proton gradient and using it to make ATP from ADP is an enzyme that has several



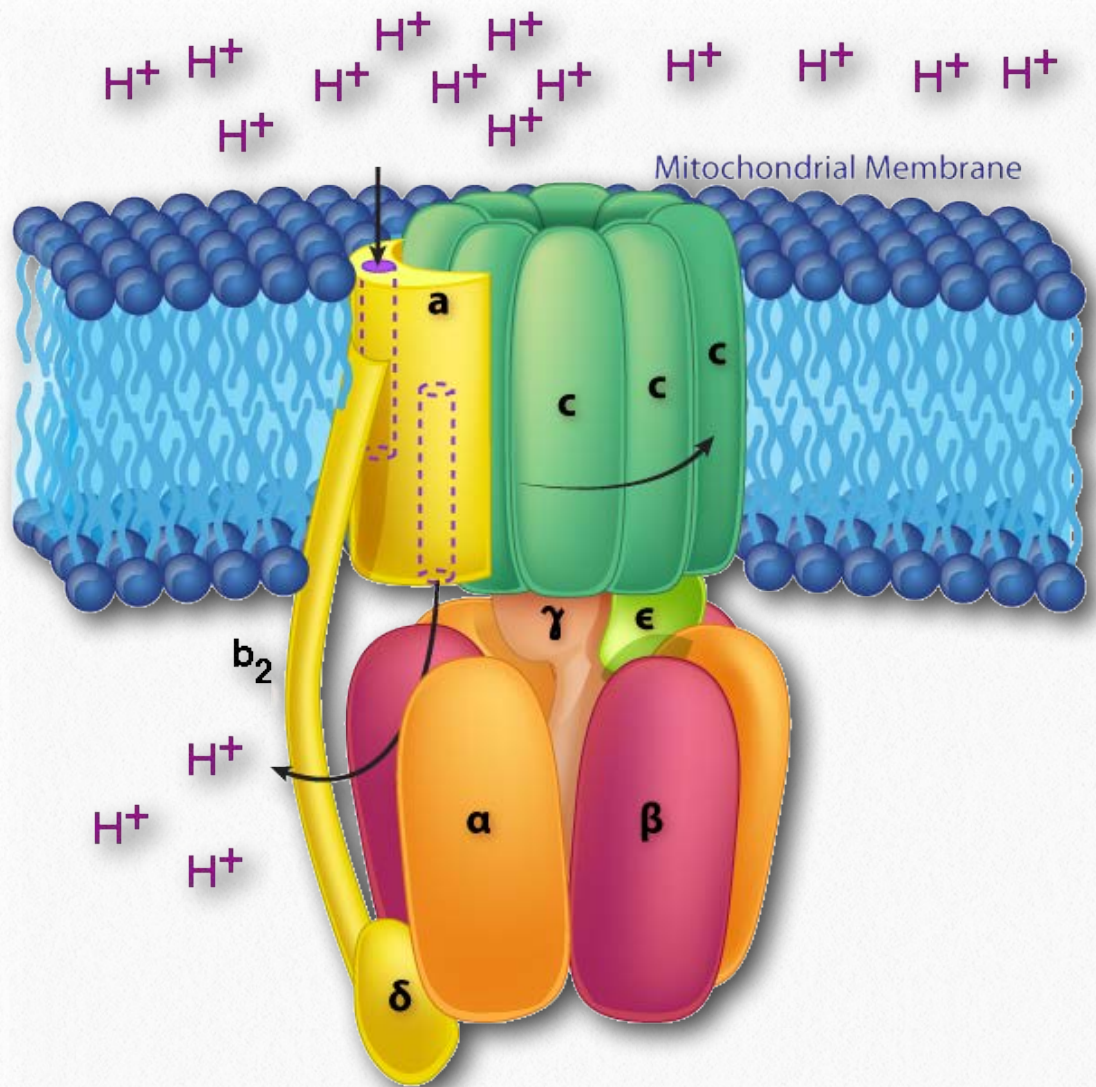
**Figure 5.27 - Mitochondrial anatomy. Electron transport complexes and ATP synthase are embedded in the inner mitochondrial membrane**

Image by Aleia Kim

names - Complex V, PTAS (Proton Translocating ATP Synthase), and ATP synthase (Figure 5.29). Central to its function is the movement of protons through it (from the intermembrane space back into the matrix). Protons will only provide energy to make ATP if their concentration is greater in the intermembrane space than in the matrix and if ADP is available.

It is possible, in some cases, for the concentration of protons to be greater inside the matrix than outside of it. When this happens, the ATP synthase can run backwards, with protons moving from inside to out, accompanied by conversion of ATP to ADP + P<sub>i</sub>. This is usually not a desirable circumstance and there are some controls to reduce its occurrence.

Normally, ATP concentration will be higher inside of the mitochondrion and ADP concentration be higher outside the mitochondrion. However, when the rate of ATP synthesis exceeds the rate of ATP usage, then ATP concentrations rise outside the mitochondrion and ADP concentrations fall everywhere.



**Figure 5.28 - ATP synthase. Protons pass from intermembrane space (top) through the complex and exit in the matrix (bottom).**

Image by Aleia Kim

This may happen, for example, during periods of rest. It has the overall effect of reducing transport and thus lowering the concentration of ADP inside the matrix. Reducing ADP concentration in the matrix reduces oxidative phosphorylation and has effects on respiratory control (see [HERE](#)).

Another important consideration is that when ATP is made in oxidative phosphorylation, it is released into the mitochondrial matrix, but must be trans-

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ported into the cytosol to meet the energy needs of the rest of the cell. This is accomplished by action of the adenine nucleotide translocase, an antiport that moves ATP out of the matrix in exchange for ADP moving into the matrix. This transport system is driven by the concentrations of ADP and ATP and ensures that levels of ADP are maintained within the mitochondrion, permitting continued ATP synthesis.

One last requirement for synthesis of ATP from ADP is that phosphate must also be imported into the matrix. This is accomplished by action of the phosphate translocase, which is a symport that moves phosphate into the mitochondrial matrix along with a proton.

There is evidence that the two translocases and ATP synthase may exist in a complex, which has been dubbed the ATP synthasome.

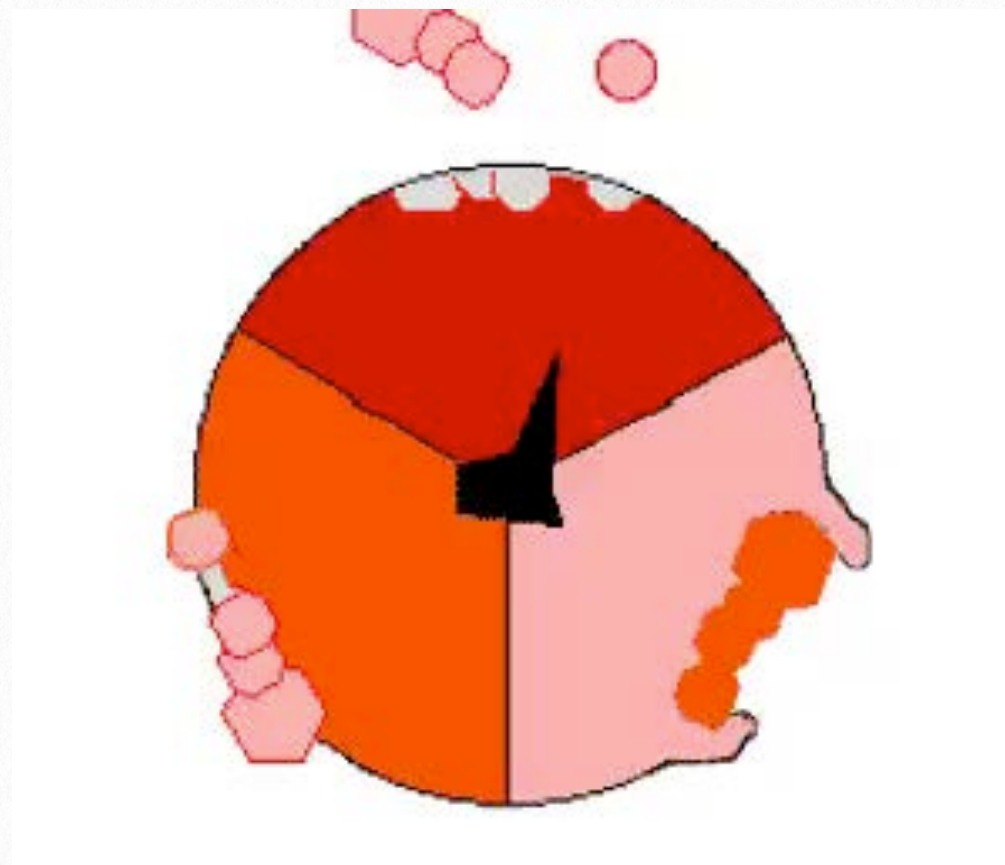
In summary, the electron transport system charges the battery for oxidative phosphorylation by pumping protons out of the mitochondrion. The intact inner membrane of the mitochondrion keeps the protons out, except for those that re-enter through ATP Synthase. The ATP Synthase allows protons to re-enter the mitochondrial matrix and harvests their energy to make ATP.

### ATP synthase mechanism

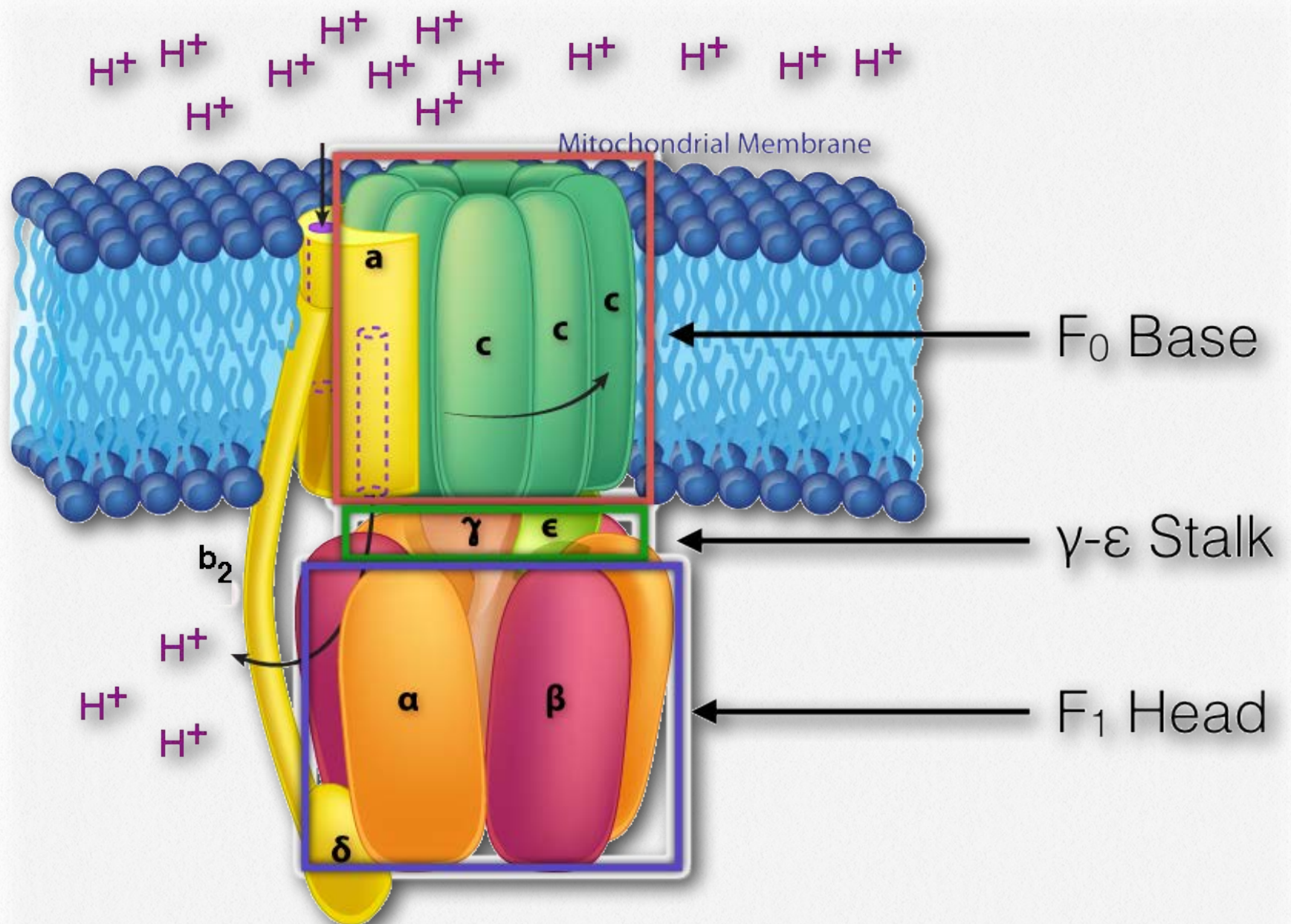
The ATP Synthase itself is an amazing

nanomachine that makes ATP using a gradient of protons flowing through it from the intermembrane space back into the matrix. It is not easy to depict in a single image what the synthase does. [Figure 5.31](#) and [Figure 5.32](#) illustrate the multi-subunit nature of this membrane protein,

which acts like a turbine at a hydroelectric dam. The movement of protons through the ATP Synthase c-ring causes it and the  $\gamma$ - $\epsilon$



**Movie 5.3 - ATP Synthase - ADP + Pi (pink) and ATP (red). The view is end-on from the cytoplasmic side viewing the  $\beta$  subunits**



**Figure 5.29 - Important structural features of the ATP synthase**

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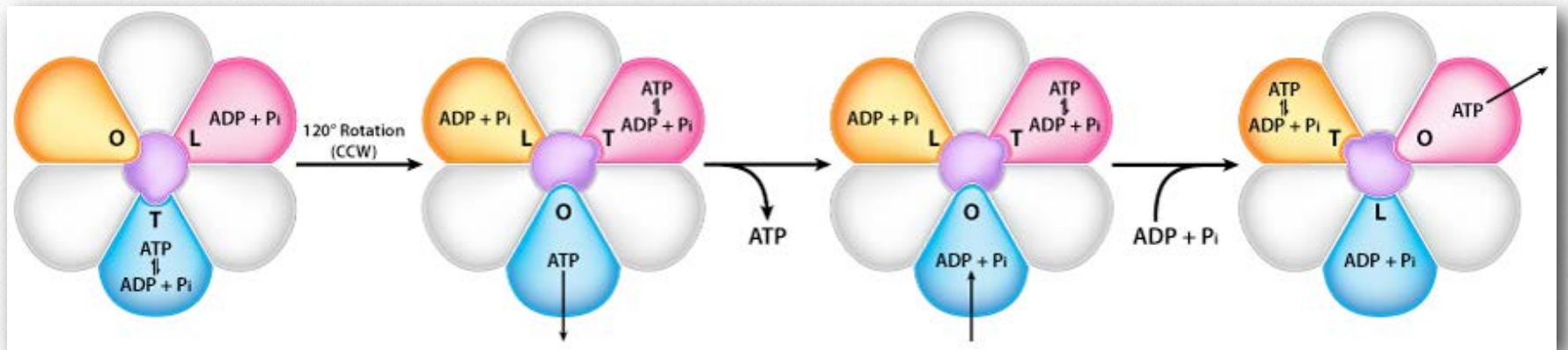
stalk attached to it to turn. It is this action that is necessary for making ATP.

In ATP Synthase, the spinning components, or rotor, are the membrane portion (**c** ring) of the **F<sub>0</sub>** base and the **γ-ε** stalk, which is connected to it. The **γ-ε** stalk projects into the **F<sub>1</sub>** head of the mushroom structure. The **F<sub>1</sub>** head contains the catalytic ability to make ATP. The **F<sub>1</sub>** head is hexameric in structure with paired **α** and **β** proteins arranged in a trimer

of dimers. ATP synthesis occurs within the **β** subunits.

### Rotation of **γ** unit

Turning of the **γ** shaft (caused by proton flow) inside the **α-β** trimer of the **F<sub>1</sub>** head causes each set of **β** proteins to change structure slightly into three different forms called Loose, Tight, and Open (L,T,O - [Figure 5.31](#)). Each of these forms has a function.



**Figure 5.30 - Loose (L), Tight (T), and Open (O) structures of the F<sub>1</sub> head of ATP synthase. Change of structure occurs by rotation of  $\gamma$ -protein (purple) in center as a result of proton movement. Individual  $\alpha$  and  $\beta$  units do not rotate**

Image by Aleia Kim

The Loose form binds ADP + P<sub>i</sub>. The Tight form “squeezes” them together to form the ATP. The Open form releases the ATP into the mitochondrial matrix. Thus, as a result of the proton flow through the ATP synthase, from the intermembrane space into the matrix, ATP is made from ADP and P<sub>i</sub>.

## Respiratory control

When a mitochondrion has an intact inner membrane and protons can only return to the matrix by passing through the ATP synthase, the processes of electron transport and oxidative phosphorylation are said to be tightly coupled.

## Interdependence

In simple terms, tight coupling means that the processes of electron transport and oxidative phosphorylation are interdependent. Without electron transport going on in the cell, oxidative phosphorylation will soon stop.

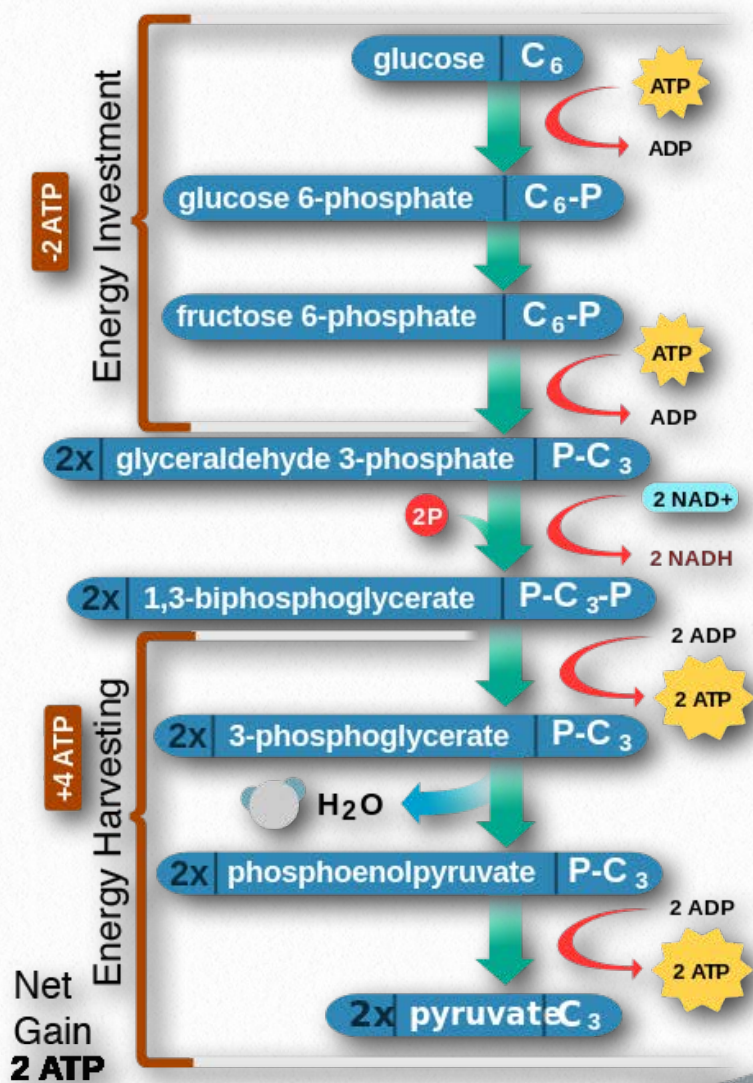
The reverse is also true, because if oxidative phosphorylation stops, the proton gradient will not be dissipated as it is being built by the electron transport system and will grow larger and larger. The greater the gradient, the greater the energy needed to pump protons out of the mitochondrion. Eventually, if nothing relieves the gradient, it becomes too large and the energy of electron transport is insufficient to perform the pumping. When pumping stops, so too does electron transport.

## ADP dependence

Another relevant point is that ATP synthase is totally dependent upon a supply of ADP. In the absence of ADP, the ATP synthase stops functioning and when it stops, so too does movement of protons back into the mitochondrion. With this information, it is possible to understand the link between energy usage and metabolism. The root of this, as noted, is respiratory control.



## Glycolysis in the Cytoplasm



## Citric Acid Cycle in the Mitochondria

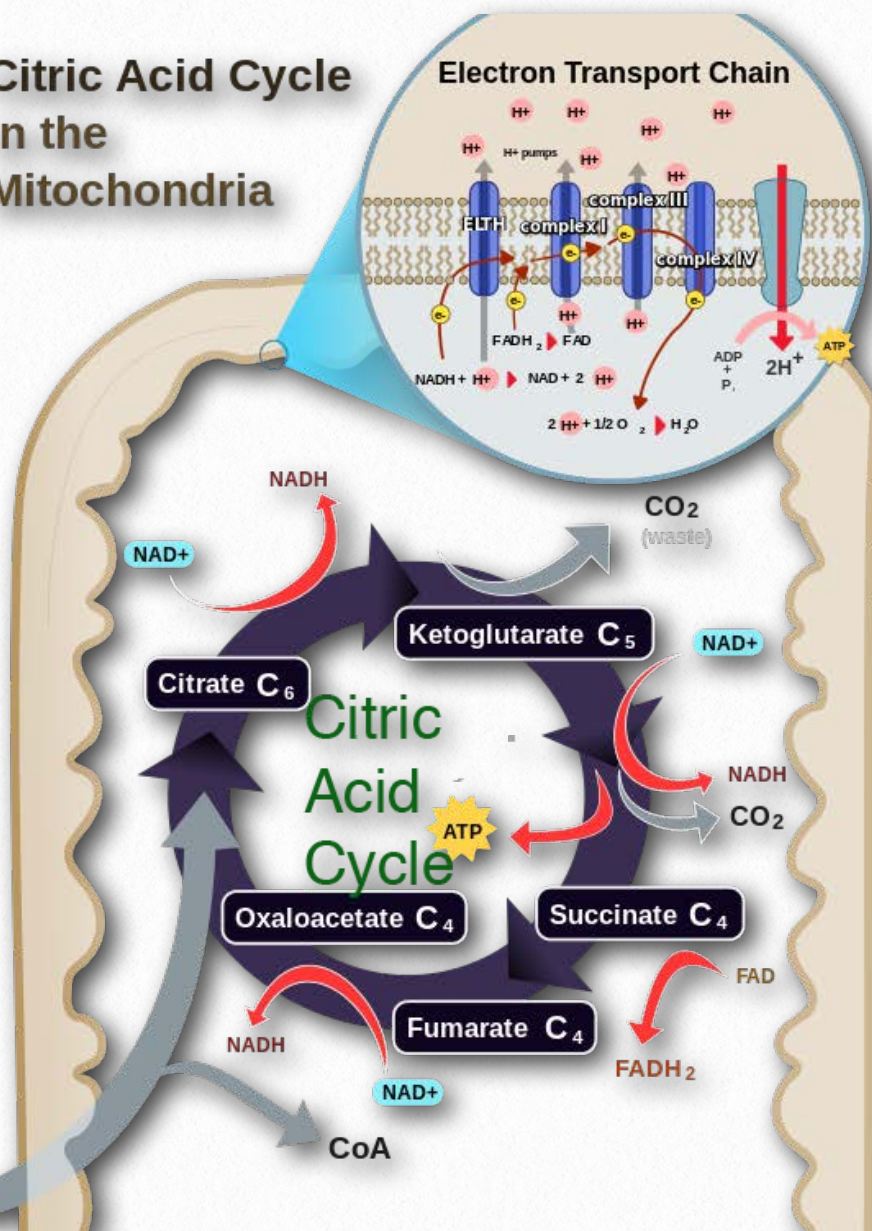


Figure 5.31 - Respiration overview in eukaryotic cells

Wikipedia

### At rest

To illustrate these links, let us first consider a person, initially at rest, who then suddenly jumps up and runs away. At first, the person's ATP levels are high and ADP levels are low (no exercise to burn ATP), so little oxidative phosphorylation is occurring and thus the proton gradient is high. Electron transport is moving slowly, if at all, so it is not using oxygen and the person's breathing is slow, as a result.

### Exercise

When running starts, muscular contraction, which uses energy, causes ATP to be converted to ADP. Increasing ADP in muscle cells favors oxidative phosphorylation to attempt to make up for the ATP being burned. ATP synthase begins working and protons begin to come back into the mitochondrial matrix. The proton gradient decreases, so electron transport re-starts.

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Electron transport needs an electron acceptor, so oxygen use increases and when oxygen use increases, the person starts breathing more heavily to supply it. When the person stops running, ATP concentrations get rebuilt by ATP synthase. Eventually, when ATP levels are completely restored, ADP levels fall and ATP synthase stops or slows considerably. With little or no proton movement, electron transport stops because the proton gradient is too large. When electron transport stops, oxygen use decreases and the rate of breathing slows down.

### Electron transport critical

The really interesting links to metabolism occur relative to whether or not electron transport is occurring. From the examples, we can see that electron transport will be relatively slowed when not exercising and more rapid when exercise (or other ATP usage) is occurring. Remember that electron transport is the way in which reduced electron carriers, NADH and FADH<sub>2</sub>, donate their electrons to the ETS, becoming oxidized to NAD<sup>+</sup> and FAD, respectively.

Oxidized carriers, such as NAD<sup>+</sup> and FAD are needed by catabolic pathways, like glycolysis, the citric acid cycle, and fatty acid oxidation. Anabolic pathways, such as fatty acid/fat synthesis and gluconeogenesis rely on reduced electron carriers, such as

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FADH<sub>2</sub>, NADH, and the related carrier, NADPH.

### Links to exercise

High levels of NADH and FADH<sub>2</sub> prevent catabolic pathways from operating, since NAD<sup>+</sup> and FAD levels will be low and these are needed to accept the electrons released during catabolism by the oxidative processes.

Thanks to respiratory control, when one is exercising, NAD<sup>+</sup> and FAD levels increase (because electron transport is running), so catabolic pathways that need NAD<sup>+</sup> and FAD can function. The electrons lost in the oxidation reactions of catabolism are captured by

#### Rest

ATP **High** / ADP **Low**  
Oxidative Phosphorylation **Low**  
Electron Transport **Low**  
Oxygen Use **Low**  
NADH **High** / NAD<sup>+</sup> **Low**  
Citric Acid Cycle **Slow**

#### Exercise

ATP **Low** / ADP **High**  
Oxidative Phosphorylation **High**  
Electron Transport **High**  
Oxygen Use **High**  
NADH **Low** / NAD<sup>+</sup> **High**  
Citric Acid Cycle **Fast**

NAD<sup>+</sup> and FAD to yield NADH and FADH<sub>2</sub>, which then supply electrons to the electron transport system and oxidative phosphorylation to make more needed ATP.

Thus, during exercise, cells move to a mode of quickly cycling between reduced electron carriers (NADH/FADH<sub>2</sub>) and oxidized electron carriers (NAD<sup>+</sup>/FAD). This allows rapidly metabolizing tissues to transfer electrons to NAD<sup>+</sup>/FAD and it allows the reduced electron carriers to rapidly become oxidized, allowing the cell to produce ATP.

## Rest

When exercise stops, NADH and FADH<sub>2</sub> levels rise (because electron transport is slowing) causing catabolic pathways to slow/stop. If one does not have the proper amount of exercise, reduced carriers remain high in concentration for long periods of time. This means we have an excess of energy and then anabolic pathways, particularly fatty acid synthesis, are favored, so we get fatter.

## Altering respiratory control

One might suspect that altering respiratory control could have some very dire consequences and that would be correct. Alterations can take the form of either inhibiting electron transport/oxidative phosphorylation or uncoupling the two. These alterations can be achieved using compounds with specific effects on particular components of the system.

All of the chemicals described here are laboratory tools and should never be used by people. The first group for discussion are the inhibitors. In tightly coupled mitochondria, inhibiting either electron transport or oxidative phosphorylation has the effect of inhibiting the other one as well.

## Electron transport inhibitors

Common inhibitors of electron transport include rotenone and amytal, which stop movement of electrons past Complex I, malonate, malate, and oxaloacetate, which

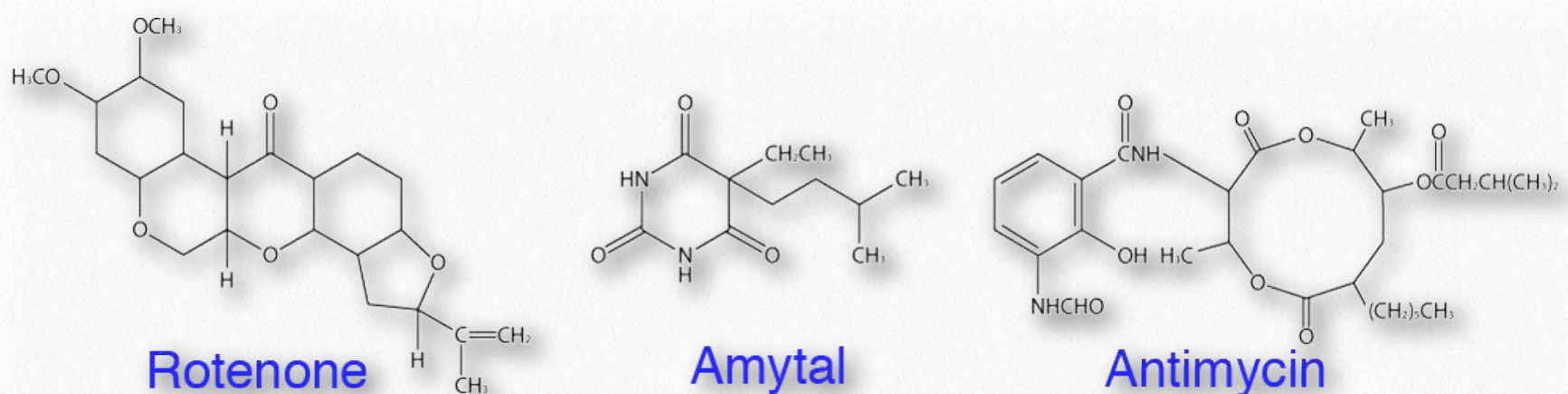
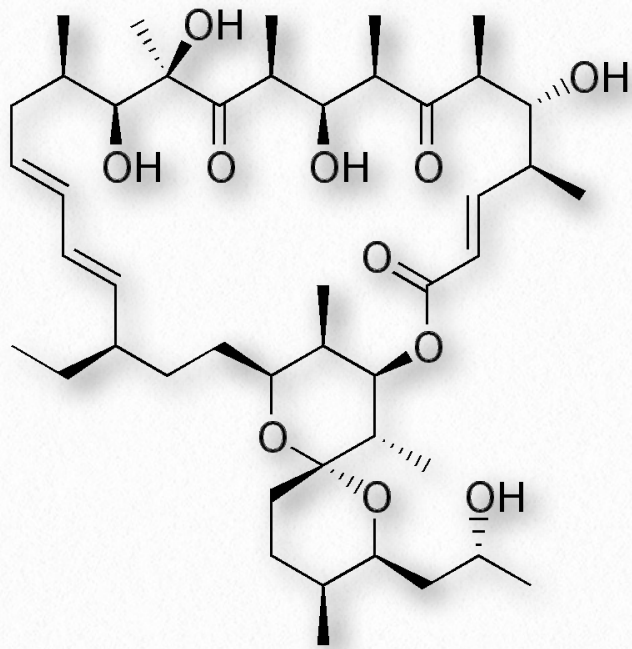


Figure 5.32 - Three inhibitors of electron transport

Image by Aleia Kim



**Figure 5.33 - Oligomycin A - An inhibitor of ATP synthase**

hibit movement of electrons through Complex II, antimycin A which stops movement of electrons past Complex III, and cyanide, carbon monoxide, azide, and hydrogen sulfide, which inhibit electron movement through Complex IV (Figure 5.33). All of these compounds can stop electron transport directly (no movement of electrons) and oxidative phosphorylation indirectly (proton gradient will dissipate). While some of these compounds are not commonly known, almost everyone is aware of the hazards of carbon monoxide and cyanide, both of which can be lethal.

### ATP synthase inhibitor

It is also possible to use an inhibitor of ATP synthase to stop oxidative phosphorylation directly (no ATP production) and electron transport indirectly (proton gradient not relieved so it becomes increasingly difficult to

in-

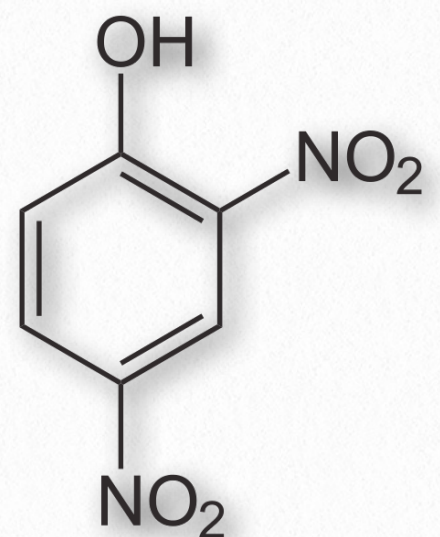
pump protons out of matrix). Oligomycin A (Figure 5.34) is an inhibitor of ATP synthase.

### Rotenone

Rotenone, which is a plant product, is used as a natural insecticide that is permitted for organic farming. When mitochondria are treated with this, electron transport will stop at Complex I and so, too, will the pumping of protons out of the matrix. When this occurs, the proton gradient rapidly dissipates, stopping oxidative phosphorylation as a consequence. There are other entry points for electrons than Complex I, so this type of inhibition is not as serious as using inhibitors of Complex IV, since no alternative route for electrons is available. It is for this reason that cyanide, for example, is so poisonous.

### 2,4-DNP

Respiratory control can be completely destroyed by using a reagent that permeabilizes the inner mitochondrial membrane to protons. There are several such reagents, but the best known one is 2,4



**Figure 5.34 - 2,4 DNP - an uncoupler of respiratory control**

dinitrophenol (2,4 DNP - [Figure 5.35](#)).

Treatment of mitochondria with 2,4 DNP makes the mitochondrial inner membrane “leaky” to protons. This has the effect of providing an alternate route for protons to reenter the matrix besides going through ATP synthase, and uncouples oxidative phosphorylation from electron transport.

Imagine a dam holding back water with a turbine generating electricity through which water must flow. When all water flows through the turbine, the maximum amount of electricity can be generated. If one pokes a hole in the dam, though, water will flow through the hole and less electricity will be created. The generation of electricity will thus be uncoupled from the flow of water. If the hole is big enough, the water will all drain out through the hole and no electricity will be made.

### **Bypassing ATP synthase**

Imagine, now, that the proton gradient is the equivalent of the water, the inner membrane

is the equivalent of the dam and the ATP synthase is the turbine. When protons have an alternate route, little or no ATP will be made because protons will pass through the membrane’s holes instead of spinning the turbine of ATP synthase.

It is important to recognize, though, that uncoupling by 2,4 DNP works differently from the electron transport inhibitors or the ATP synthase inhibitor. In those situations, stopping oxidative phosphorylation resulted in indirectly stopping electron transport, since the two processes were coupled and the inhibitors did not uncouple them. Similarly,

stopping electron transport indirectly stopped oxidative phosphorylation for the same reason.

Such is not the case with 2,4 DNP.

Stopping oxidative phosphorylation by destroying the proton gradient allows electron transport to continue unabated (it actually stimulates it), since the proton gradient cannot build no matter how much electron transport runs. Consequently, electron trans-

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#### **In Cells With Tight Coupling**

1. O<sub>2</sub> use depends on metabolism
2. NAD<sup>+</sup> levels vary with exercise
3. Proton gradient high with no exercise
4. Catabolism depends on energy needs
5. ETS runs when OxPhos runs and vice versa

#### **In Cells That Are Uncoupled**

1. O<sub>2</sub> use high
2. NAD<sup>+</sup> Levels high
3. Little or no proton gradient
4. Catabolism high
5. OxPhos does not run, but ETS runs rapidly

port runs like crazy but oxidative phosphorylation stops. When that happens,  $\text{NAD}^+$  and FAD levels rise, and catabolic pathways run unabated with abundant supplies of these electron acceptors. The reason such a scenario is dangerous is because the body is using all of its nutrient resources, but no ATP is being made. Lack of ATP leads to cellular (and organismal) death. In addition, the large amounts of heat generated can raise the temperature of the body to unsafe levels.

## Thermogenin

One of the byproducts of uncoupling electron transport is the production of heat. The faster metabolic pathways run, the more heat is generated as a byproduct. Since 2,4 DNP causes metabolism to speed up, a considerable amount of heat can be produced. Controlled uncoupling is actually used by the body in special tissues called brown fat. In this case, brown fat cells use the heat created to help thermoregulate the temperature of newborn children.

Permeabilization of the inner membrane is accomplished in brown fat by the synthesis of a protein called thermogenin (also known as uncoupling protein). Thermogenin binds to the inner membrane and allows protons to pass through it, thus bypassing the ATP synthase. As noted for 2,4 DNP, this results

in activation of catabolic pathways and the more catabolism occurs, the more heat is generated.

## Dangerous drug

In uncoupling, whether through the action of an endogenous uncoupling protein or DNP, the energy that would have normally been captured in ATP is lost as heat. In the case of uncoupling by thermogenin, this serves the important purpose of keeping newborn infants warm. But in adults, uncoupling merely wastes the energy that would have been harvested as ATP. In other words, it mimics starvation, even though there is plenty of food, because the energy is dissipated as heat.

This fact, and the associated increase in metabolic rate, led to DNP being used as a weight loss drug in the 1930s. Touted as an effortless way to lose weight without having to eat less or exercise more, it was hailed as a magic weight loss pill. It quickly became apparent, however, that this was very dangerous. Many people died from using this drug before laws were passed to ban the use of DNP as a weight loss aid.

## Alternative oxidase

Another approach to generating heat that doesn't involve breaking respiratory control is taken by some fungi, plants, and protozoa. They use an alternative electron transport.



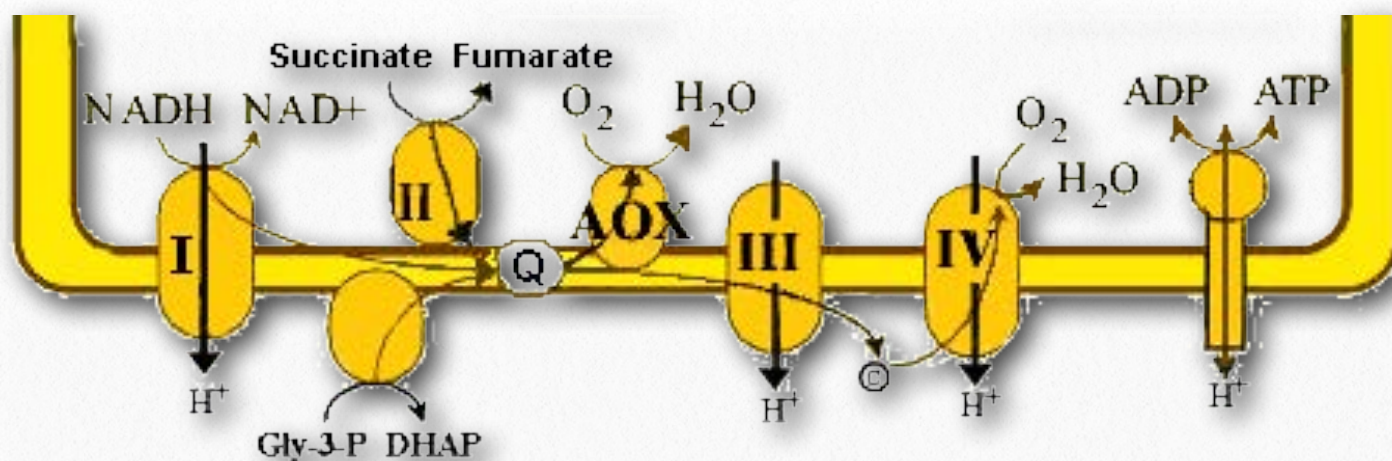
In these organisms, there is an enzyme called alternative oxidase (Figure 5.36). Alternative oxidase is able to accept electrons from CoQ and pass them directly to oxygen.

The process occurs in coupled mitochondria. Its mechanism of action is to reduce the yield of ATP, since fewer protons are being pumped per reduced electron carrier. Thus  $\text{NAD}^+$  concentrations increase, oxygen consumption increases, and the efficiency of ATP production decreases.

Organisms using this method must activate catabolic pathways by the increase in  $\text{NAD}^+$  concentration. This, in turn produces quantities of NADH and  $\text{FADH}_2$  necessary to make sufficient amounts of ATP. The byproduct of this increased catabolism is more heat. Not surprisingly, the alternative oxidase pathway can be activated by cold temperatures.

## Energy efficiency

Cells are not 100% efficient in energy use. Nothing we know is. Consequently, cells do not get as much energy out of catabolic processes as they put into anabolic processes. A good example is the synthesis and breakdown of glucose, something liver cells are frequently doing. The complete conversion of glucose to pyruvate in glycolysis (catabolism) yields two pyruvates plus 2 NADH plus 2 ATPs. Conversely, the complete conversion of two pyruvates into glucose by gluconeogenesis (anabolism) requires 4 ATPs, 2 NADH, and 2 GTPs. Since the energy of GTP is essentially equal to that of ATP, gluconeogenesis requires a net of 4 ATPs more than glycolysis yields. This difference must be made up in order for the organism to meet its energy needs. It is for this reason that we eat. In addition, the inefficiency of our capture of energy in reactions results in the production of heat and helps to keep us warm, as noted.



**Figure 5.35 - Alternative oxidase (AOX) of fungi, plants, and protozoa bypasses part of electron transport by taking electrons from CoQ and passing them to oxygen.**

You can read more about glycolysis ([HERE](#)) and gluconeogenesis ([HERE](#)).

## Metabolic controls of energy

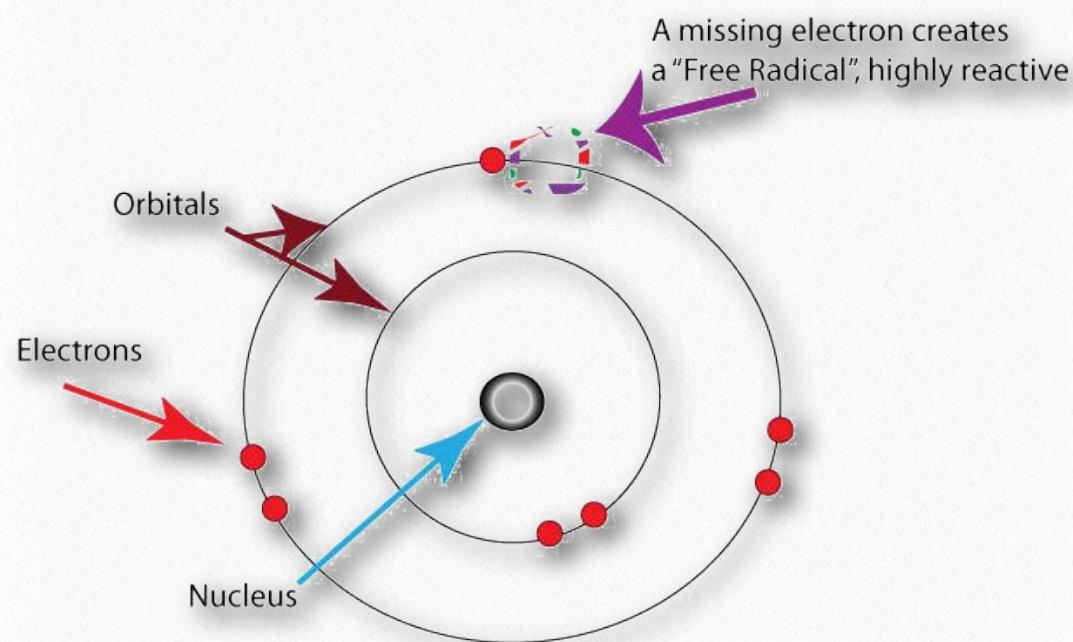
It is also noteworthy that cells do not usually have both catabolic and anabolic processes for the same molecules occurring simultaneously inside of them (for example, breakdown of glucose and synthesis of glucose) because the cell would see no net production of anything but heat and a loss of ATPs with each turn of the cycle. Such cycles are called futile cycles and cells have controls in place to limit the extent to which they occur. Since futile cycles can, in fact, yield

heat, they are used as sources of heat in some types of tissue. Brown adipose tissue of mammals uses this strategy, as described earlier. See also [HERE](#) for more on heat generation with a futile cycle.

## Reactive oxygen species

Reactive oxygen species (ROS - [Figure 5.37](#)) are oxygen containing molecules, such as peroxides, hydroxyl radical, superox-

ide, peroxyxynitrite, and others that are very chemically reactive. Though some ROS, such as peroxide and nitric oxide have important biological functions in signaling, increases in reactive oxygen species in times of stress can cause significant damage in the cell. Exogenous sources of ROS, such as pollution, tobacco, smoke, radiation or drugs can also cause significant problems.

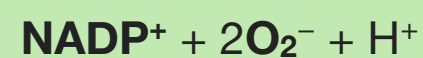


**Figure 5.36 - Structure of an oxygen free radical**

Wikipedia

Endogenous production of ROS is directed towards intracellular signaling ( $H_2O_2$  and nitric oxide, for example) and defense. Many cells, for example, have NADPH oxidase ([Figure 5.38](#)) embedded in the exterior

portion of the plasma membranes, in peroxisomes, and endoplasmic reticulum. It produces superoxides in the reaction below to kill bacteria.

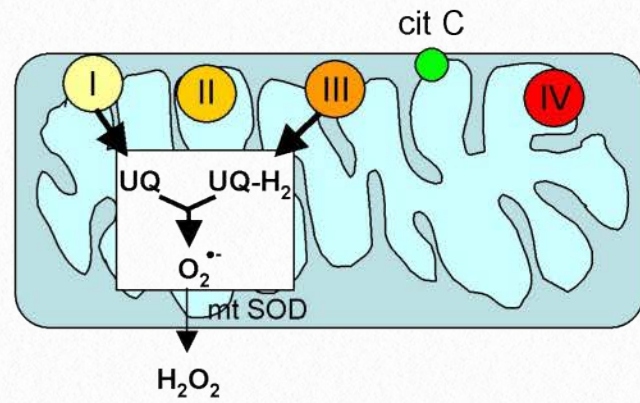




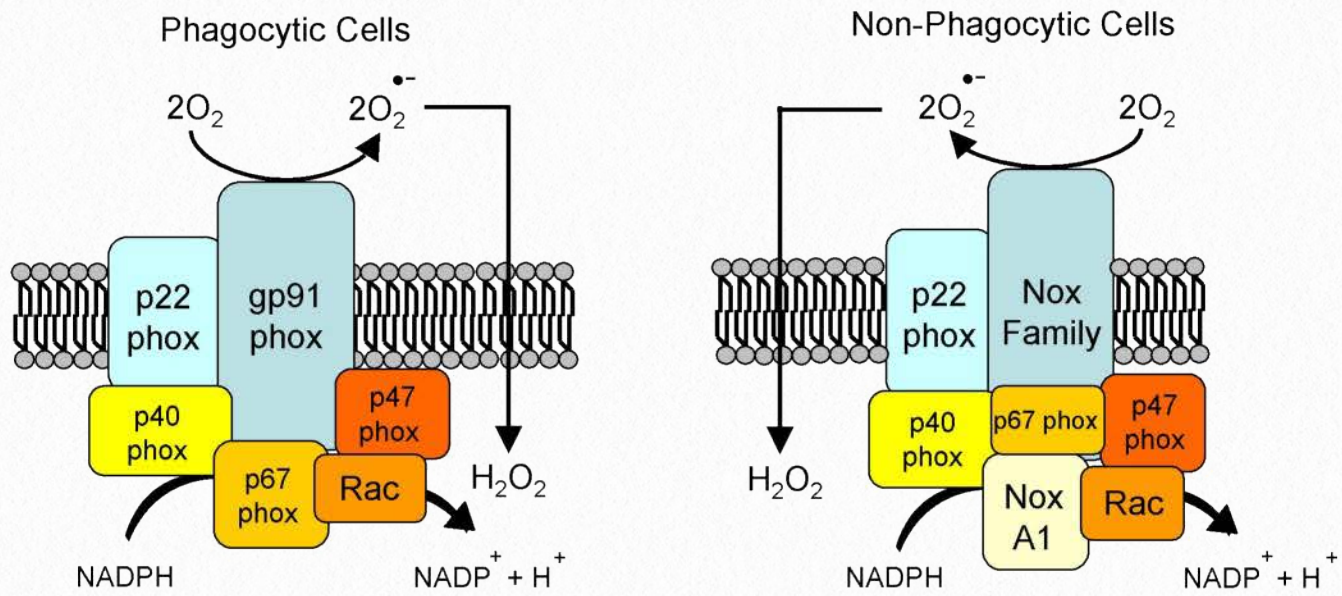
### a) Mitochondria

Stimuli inducing increased mitochondrial generation of ROS:

- serum deprivation
- integrin signalling
- apoptosis
- $\text{TNF}\alpha$
- hypoxia
- ceramide
- p53
- oncogenic Ras



### b) NADPH oxidase



Stimuli for activation of NADPH oxidase and 5-lipoxygenase

- integrin signalling
- growth factors
- cytokines/hormones
- immunological stimuli
- hypoxia
- oncogenic Ras

### c) 5-lipoxygenase

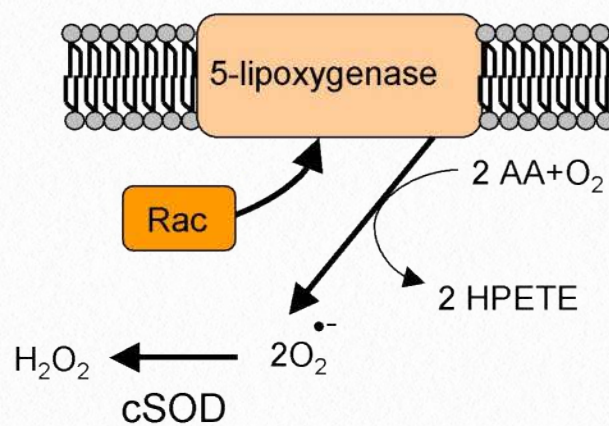


Figure 5.37 - Three sources of reactive oxygen species (ROS) in cells

In the immune system, cells called phagocytes engulf foreign cells and then use ROS to kill them. ROS can serve as signals for action. In zebrafish, damaged tissues have increased levels of  $H_2O_2$  and this is thought to be a signal for white blood cells to converge on the site. In fish lacking the genes to produce hydrogen peroxide, white blood cells do not converge at the damage site. Sources of hydrogen peroxide include peroxisomes, which generate it as a byproduct of oxidation of long chain fatty acids.

## Aging

Reactive oxygen species are at the heart of the free radical theory of aging, which states that organisms age due to the accumulation of damage from free radicals in their cells. In yeast and *Drosophila*, there is evidence that reducing oxidative damage can increase lifespan. In mice, increasing oxidative damage decreases life span, though in *Caenorhabditis*, blocking production of superoxide dismutase actually increases lifespan, so the role of ROS in aging is not completely clear.

It is clear, though, that accumulation of mitochondrial damage is problematic for individual cells. Bcl-2 proteins on the surface of mitochondria monitor damage and if they detect it, will activate proteins called Bax to

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stimulate the release of cytochrome c from the mitochondrial membrane, stimulating apoptosis (programmed cell death). Eventually the dead cell will be phagocytosed.

A common endogenous source of superoxide is the electron transport chain. Superoxide can be produced when movement of electrons into and out of the chain don't

match well. Under these circumstances, semi-reduced CoQ can donate an electron to  $O_2$  to form superoxide ( $O_2^-$ ). Superoxide can react with many molecules, including DNA where it can cause damage leading to mutation. If it reacts with the aconitase enzyme, ferrous iron

( $Fe^{++}$ ) can be released which, in turn, can react in the Fenton reaction to produce another reactive oxygen species, the hydroxyl radical (Figure 5.39).

Countering the effects of ROS are enzymes, such as catalase, superoxide dismutase, and anti-oxidants, such as glutathione and vitamins C and E.

Glutathione protects against oxidative damage by being a substrate for the enzyme glutathione peroxidase. Glutathione peroxidase catalyzes the conversion of hydrogen peroxide to water (next page).

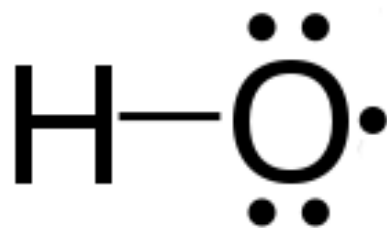
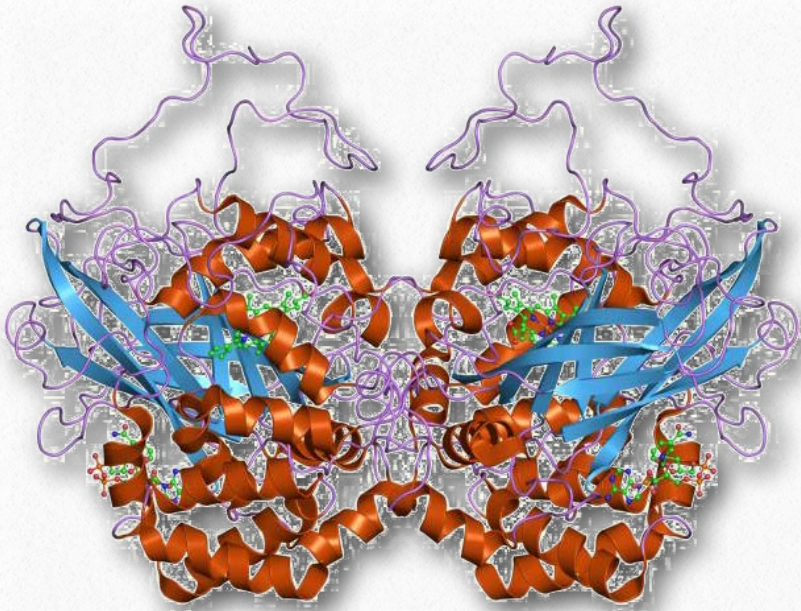
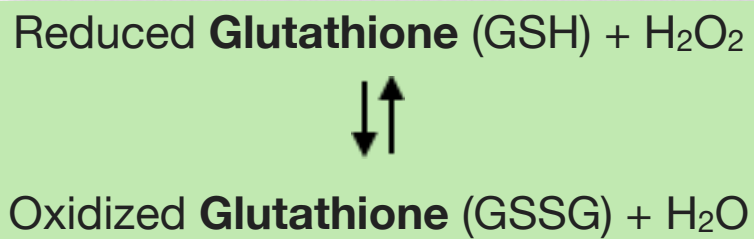


Figure 5.38 A hydroxyl radical

Wikipedia

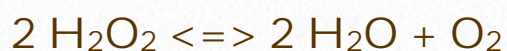


**Figure 5.39 - Catalase**



## Catalase

Catalase (Figure 5.40) is an important enzyme for cells of all types that live in an oxygen environment. A first line of defense against reactive oxygen species, catalase catalyzes the breakdown of hydrogen peroxide into water and oxygen.



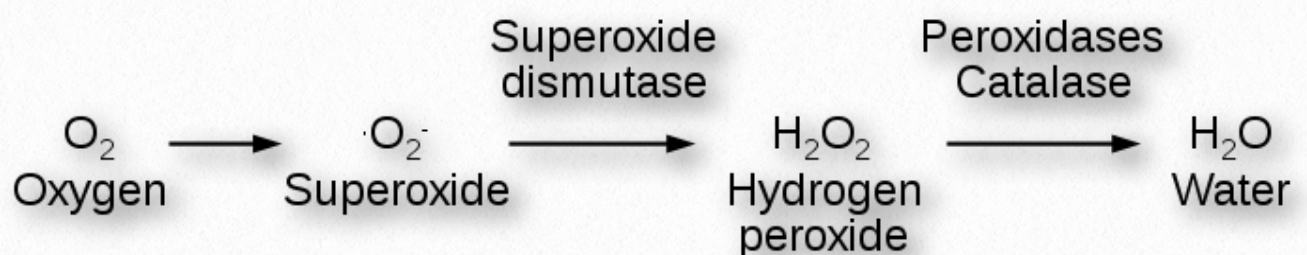
The enzyme, which employs four heme groups in its catalysis, works extremely rapidly, converting up to 40,000,000

molecules of hydrogen peroxide to water and oxygen per enzyme per second. It is abundantly found in peroxisomes.

In addition to catalase's ability to break down hydrogen peroxide, the enzyme can also use hydrogen peroxide to oxidize a wide variety of organic compounds, including phenols, formic acid, formaldehyde, acetaldehyde, and alcohols, but with much lower efficiency.

## Health

The importance of catalase for health is uncertain. Mice deficient in the enzyme appear healthy and humans with low levels of the enzyme display few problems. On the other hand, mice engineered to produce higher levels of catalase, in at least one study, lived longer. The ability of organisms to live with lower levels or no catalase may arise from another group of enzymes, the peroxiredoxins, which also act on hydrogen peroxide and may make up for lower quantities of catalase. Last, there is evidence that reduced levels of catalase with aging may be responsible for the graying of hair. Higher levels of H<sub>2</sub>O<sub>2</sub> with



**Figure 5.40 - Detoxifying reactive oxygen species**

reduced catalase results in a bleaching of hair follicles.

## Superoxide dismutase

Another important enzyme for protection against reactive oxygen species is superoxide dismutase (SOD), which is found, like catalase, in virtually all organisms living in an oxygen environment. Superoxide dismutase, also like catalase, has a very high  $K_{cat}$  value and, in fact, has the highest  $K_{cat}/K_m$  known for any known enzyme. It catalyzes the reactions at the top of the next column (superoxides shown in red):

The enzyme thus works by a ping-pong (double displacement) mechanism (see [HERE](#)), being converted between two different forms.

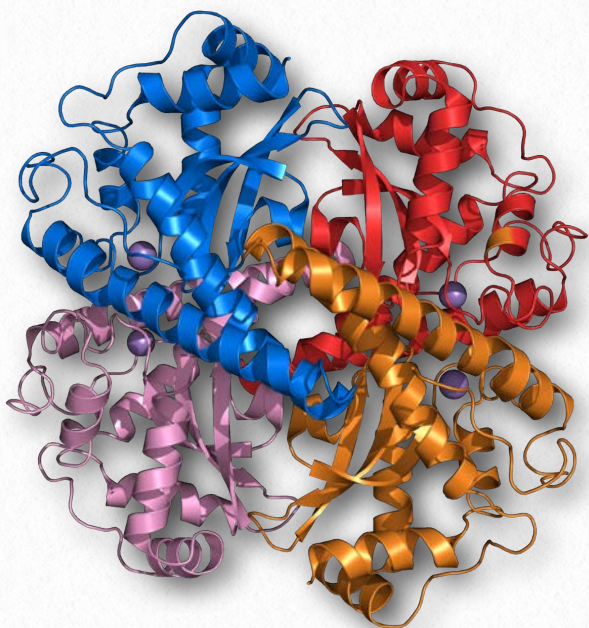


Figure 5.41 - SOD2 of humans

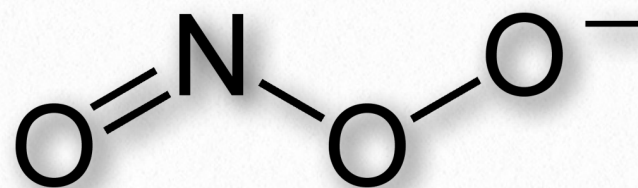
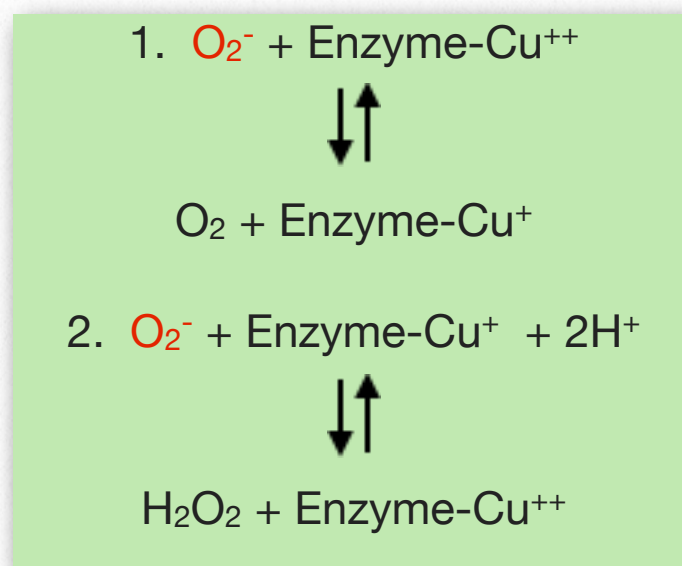
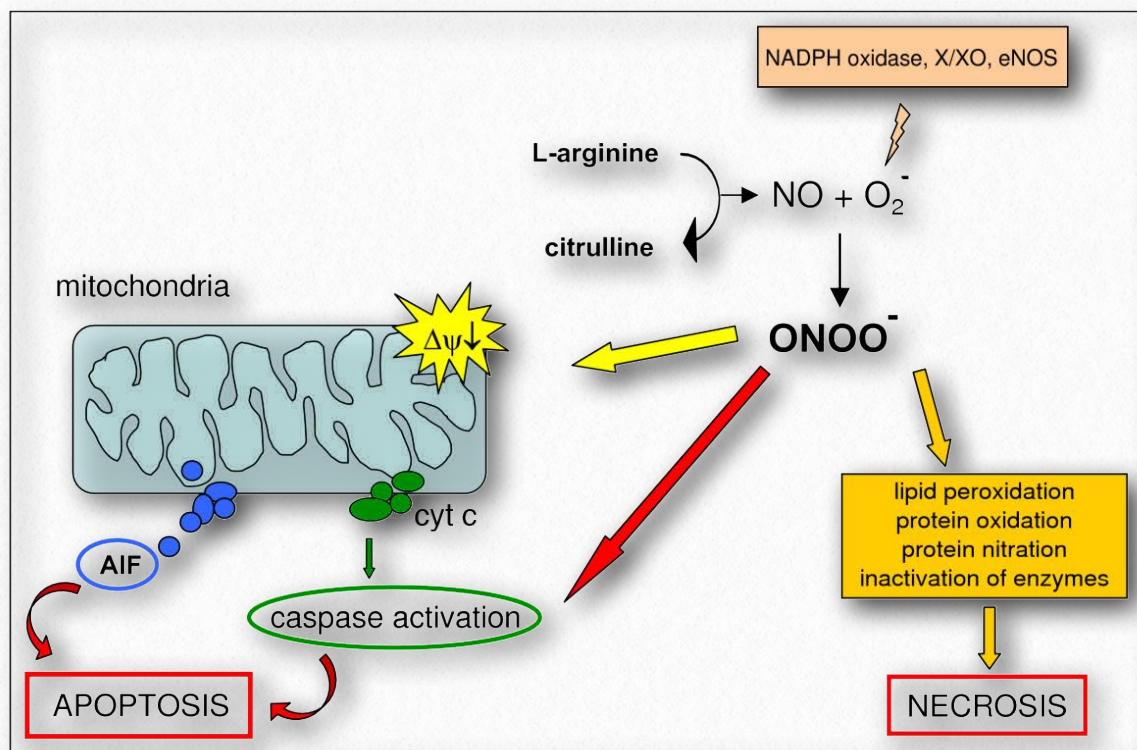


Figure 5.42 3 - Peroxynitrite Ion



The hydrogen peroxide produced in the second reaction is easily handled by catalase and is also less harmful than superoxide, which can react with nitric oxide (NO) to form very toxic peroxynitrite ions ([Figure 5.43](#)). Peroxynitrite has negative effects on cells, as shown in [Figure 5.45](#).

In addition to copper, an ion of  $Zn^{++}$  is also bound by the enzyme and likely plays a role in the catalysis. Forms of superoxide dismutase that use manganese, nickel, or iron are also known and are mostly found in prokaryotes and protists, though a manganese SOD is found in most mitochondria. Copper/zinc enzymes are common in eukaryotes.



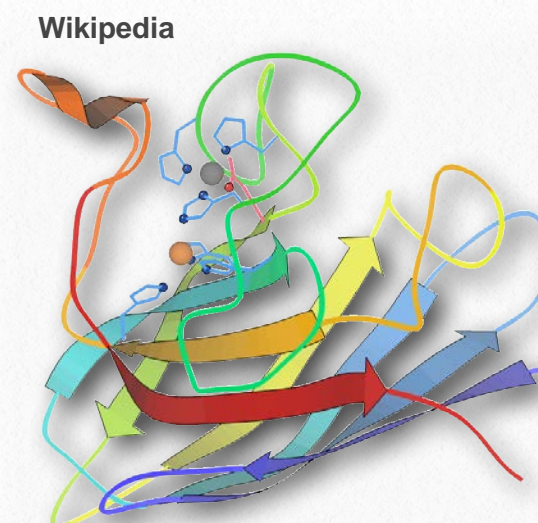
## Mixed function oxidases

Other enzymes catalyzing reactions involving oxygen include the mixed function oxidases. These enzymes use molecular oxygen for two different purposes in one reaction. The mixed function part of the name is used to indicate reactions in which two different substrates are being oxidized simultaneously.

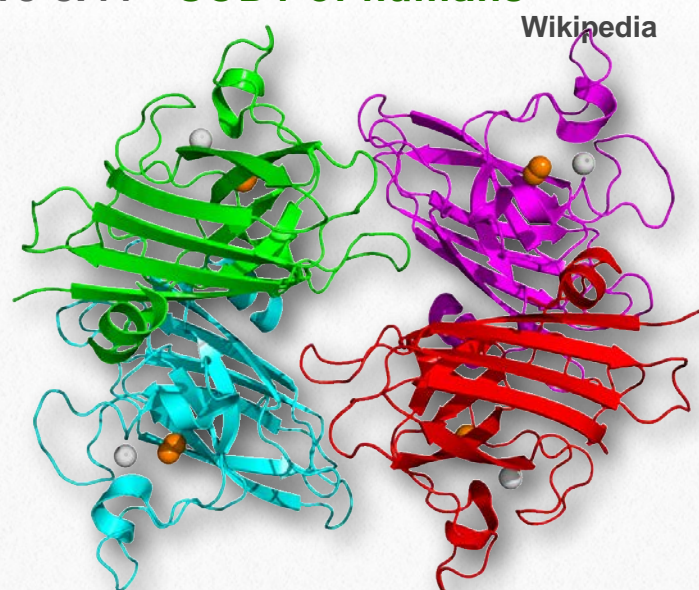
**Figure 5.43 - Peroxynitrite's effects on cells lead to necrosis or apoptosis**

Three forms of superoxide dismutase are found in humans and localized to the cytoplasm (SOD1 - Figure 5.45), mitochondria (SOD2 - Figure 5.46), and extracellular areas (SOD3 - Figure 5.47). Mice lacking any of the three forms of the enzyme are more sensitive to drugs, such as paraquat. Deficiency of SOD1 in mice leads to hepatocellular carcinoma and early loss of muscle tissue related to aging. *Drosophila* lacking SOD2 die before birth and those lacking SOD1 prematurely age.

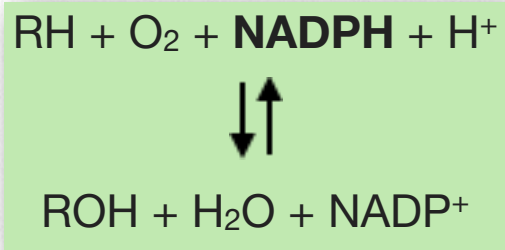
In humans, superoxide dismutase mutations are associated with the genetically-linked form of Amyotrophic Lateral Sclerosis (ALS) and over-expression of the gene is linked to neural disorders associated with Down syndrome.



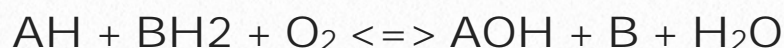
**Figure 5.44 - SOD1 of humans**



**Figure 5.45 - SOD3 of humans**



Monooxygenases are examples of mixed function oxidases. An example of a mixed function oxidase reaction is shown below.



In this case, the oxygen molecule has one atom serve as an electron acceptor and the other atom is added to the AH, creating an alcohol.

## Cytochrome P<sub>450</sub> enzymes

Cytochrome P<sub>450</sub> enzymes (called CYPs) are family of heme-containing mixed function oxidase enzymes found in all domains of life. Over 21,000 CYP enzymes are known. The most characteristic reaction catalyzed by these enzymes follows

Monooxygenase reactions such as this are relatively rare in the cell due to their use of molecular oxygen. CYPs require an electron donor for reactions like the one shown here and frequently require a protein to assist in transferring electrons to reduce the heme iron. There are six different classes of P<sub>450</sub> enzymes based on how they get electrons

1. Bacterial P<sub>450</sub> - electrons from ferredoxin reductase and ferredoxin

2. Mitochondrial P<sub>450</sub> - electrons from adrenodoxin reductase and adrenodoxin

3. CYB5R/cyb5 - electrons come from cytochrome b5

4. FMN/Fd - use a fused FMN reductase

5. Microsomal P<sub>450</sub> - NADPH electrons come via cytochrome P<sub>450</sub> reductase or from cytochrome b<sub>5</sub> and cytochrome b<sub>5</sub> reductase

6. P<sub>450</sub> only systems - do not require external reducing power

The CYP genes are abundant in humans and catalyze thousand of reactions on both cellular and extracellular chemicals. There are 57 human genes categorized into 18 different families of enzymes. Some CYPs are specific for one or a few substrates, but others can act on many different substrates.

CYP enzymes are found in most body tissues and perform important functions in synthesis of steroids (cholesterol, estrogen, testosterone, Vitamin D, e.g.), breakdown of endogenous compounds (bilirubin), and in detoxification of toxic compounds including drugs. Because they act on many drugs, changes in CYP activity can produce unexpected results and cause problems with drug interactions.

Bioactive compounds, for example, in grapefruit juice, can inhibit CYP3A4 activity, lead-

ing to increased circulating concentrations of drugs that would normally have been acted upon by CYP3A4. This is the reason that patients prescribed drugs that are known to be CYP3A4 substrates are advised to avoid drinking grapefruit juice while under treatment. St. Johns Wort, an herbal treatment, on the other hand, induces CYP3A4 activity, but inhibits CYP1A1, CYP1B1, and CYP2D6. Tobacco smoke induces CYP1A2 and watercress inhibits CYP2E1.

## Cytochromes

Cytochromes are heme-containing proteins that play major roles in the process of electron transport in the mitochondrion and in photosynthesis in the chloroplast. They exist either as monomers (cytochrome c) or as subunits within large redox complexes (Complex III and Complex IV of electron transport). An atom of iron at the center of the heme group plays a central role in the process, flipping between the ferrous ( $\text{Fe}^{++}$ ) and ferric ( $\text{Fe}^{+++}$ ) states as a result of the movement of electrons through it.

There are several different cytochromes. Cytochrome c (Figure 5.47) is a soluble pro-

tein loosely associated with the mitochondrion. Cytochromes a and  $a_3$  are found in Complex IV. Complex III has cytochromes b and  $c_1$  and the plastoquinol-

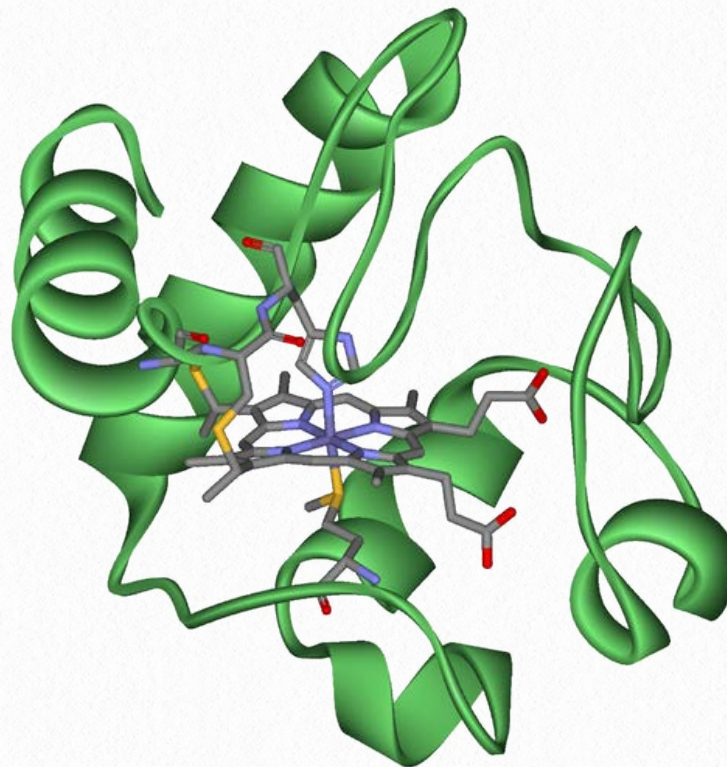


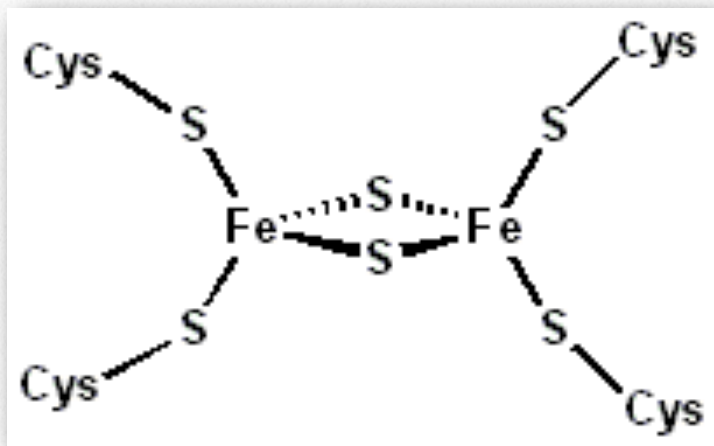
Figure 5.46 - Cytochrome c with its heme group

plastocyanin reductase of the chloroplast contains cytochromes  $b_6$  and f. Another important class of enzymes containing cytochromes is the cytochrome  $P_{450}$  oxidase group (see above). They get their name from the fact that they absorb light at 450 nm when their heme iron is reduced.

## Iron-Sulfur Proteins

Iron-sulfur proteins contain iron-sulfur clusters in a variety of formats, including sulfide-linked di-, tri-, and tetrairon centers existing in different oxidation states (Figures 5.48 & 5.49). The clusters play a variety of roles, but the best known ones are in electron transport where they function in the redox reactions involved in the movement of electrons.

Complexes I and Complex II contain multiple Fe-S centers. Iron-sulfur proteins, though, have many other roles in cells. Aconitase uses an iron-sulfur center in its catalytic ac-



**Figure 5.47 - Fe<sub>2</sub>S<sub>2</sub> Cluster**

tion and the ability of the enzyme to bind iron allows it to function as a barometer of iron concentration in cells. Iron-sulfur centers help to generate radicals in enzymes using S-Adenosyl Methionine (SAM) and can serve as a source of sulfur in the synthesis of biotin and lipoic acid. Some iron-sulfur proteins even help to regulate gene expression.

### Ferredoxin

Ferredoxins are iron-sulfur containing proteins performing electron transfer in a

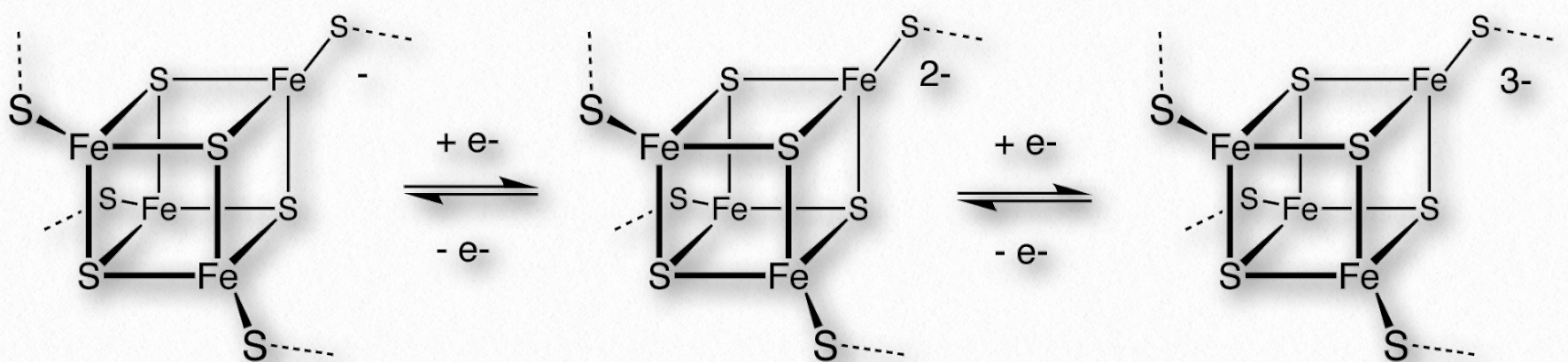
wide variety of biological systems and processes. They include roles in photosynthesis in chloroplasts. Ferredoxins are classified structurally by the iron-sulfur clustered centers they contain. Fe<sub>2</sub>S<sub>2</sub> clusters (Figure 5.50) are found in chloroplast membranes and can donate electrons to glutamate synthase, nitrate reductase, and sulfite reductase and serve as electron carriers between reductase flavoproteins and bacterial dioxygenase systems. Adrenodoxin is a soluble human Fe<sub>2</sub>S<sub>2</sub> ferredoxin (also called ferredoxin 1) serving as an electron carrier (to cytochrome P<sub>450</sub>) in mitochondrial monooxygenase systems. Fe<sub>4</sub>S<sub>4</sub> ferredoxins are

subdivided as low and high potential ferredoxins, with the latter ones functioning in anaerobic electron transport chains.

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### Ferritin

Ferritin is an intracellular iron-storage protein found in almost all living organisms, from bacteria to higher plants and animals. It is a globular protein complex with 24 subunits



**Figure 5.48 - Redox reactions for Fe<sub>4</sub>S<sub>4</sub> clusters**



and is the primary intracellular iron-storage protein in eukaryotes and prokaryotes. Ferritin functions to keep iron in a soluble and non-toxic form. Its ability to safely store iron and release it in a controlled fashion allow it to act like the prime iron buffer and solubilizer in cells - keeping the concentration of free iron from going too high or falling too low. Ferritin is located in the cytoplasm in most tissues, but it is also found in the serum acting as an iron carrier. Ferritin that doesn't contain any iron is known as apo-ferritin.

## Monoamine oxidases

Monoamine oxidases are enzymes that catalyze the oxidative deamination of monoamines, such as serotonin, epinephrine, and dopamine. Removal of the amine with oxygen results in the production of an aldehyde and ammonia. The enzymes are found inside and outside of the central nervous system.

There are two types of monoamine oxidase enzymes - MAO-A and MAO-B. MAO-A is particularly important for oxidizing monoamines consumed in the diet. Both MAO-A and MAO-B play important roles in inactivating monoaminergic neurotransmitters. Both enzymes act on dopamine, tyramine (Fig-

ure 5.50), and tryptamine. MAO-A is the primary enzyme for metabolizing melatonin, serotonin, norepinephrine, and epinephrine, while MAO-B is the primary enzyme for phenethylamine (Figure 5.51) and benzylamine. MAO-B levels have been reported to be considerably reduced with tobacco usage.

Actions of monoamine oxidases thus affects levels of neurotransmitters and consequently are thought to play roles in neurological and/or psychiatric disorders. Aberrant levels of MAOs have been linked to numerous

psychological problems, including depression, attention deficit disorder (ADD), migraines, schizophrenia, and substance abuse. Medications targeting MAOs are sometimes used to treat depression as a last resort - due to potential side effects. Excess levels of catecholamines, such as epinephrine, norepineph-

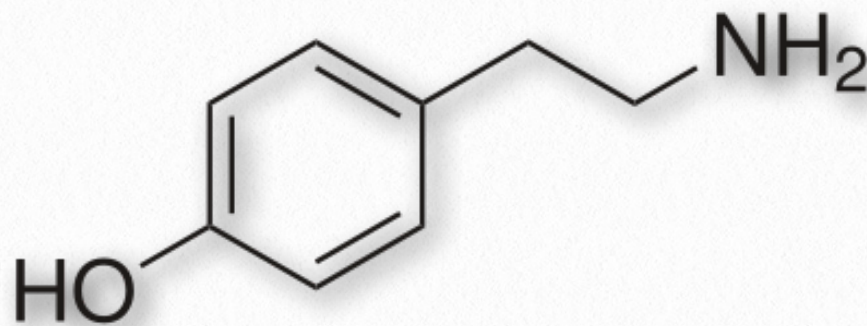


Figure 5.49 - Tyramine

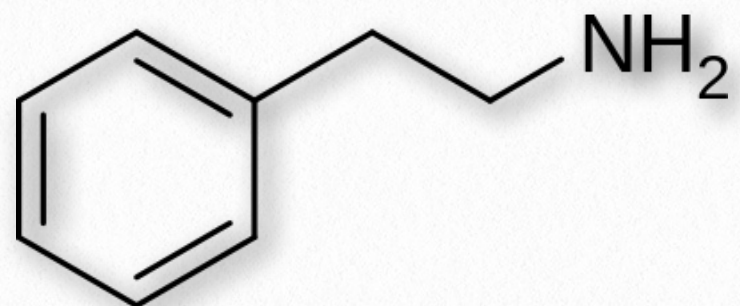


Figure 5.50 - Phenethylamine

rine, and dopamine, can result in dangerous hypertension events.

## DNA damage theory of aging

The DNA Damage Theory of Aging is based on the observation that, over time, cells are subject to extensive oxidative events. As already noted, these afford opportunities for the formation of ROS that can damage cellular molecules, and it follows that accumulation of such damage, especially to the DNA would be deleterious to the cell. The build-up of DNA damage could, thus, be responsible for the changes in gene expression that we associate with aging.

## Numerous damage events

The amount of DNA damage that can occur is considerable. In mice, for example, it is estimated that each cell experiences 40,000 to 150,000 damage events per day. The damage, which happens to nuclear as well as to mitochondrial DNA, can result in apoptosis and/or cellular senescence. DNA repair systems, of course, protect against damage to DNA, but over

time, unreparable damage may accumulate.

## Oxidative damage

DNA damage can occur in several ways. Oxidation can damage nucleotides and alter their base-pairing tendencies. Oxidation of guanine by reactive oxygen species, for example, can produce 8-oxo-guanine (Figures 5.52 and 5.53). This oxidized nucleobase commonly produced lesion in DNA arising from action of reactive oxygen species

like superoxides. 8-oxoguanine is capable of forming a stable base pairing interaction within a DNA duplex with adenine, potentially giving rise to a mutation when DNA rep-

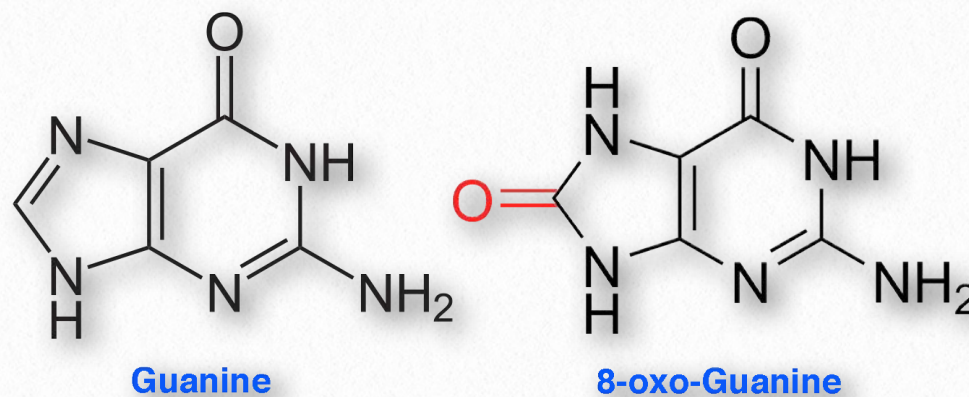


Figure 5.51 - Guanine and 8-oxo-guanine

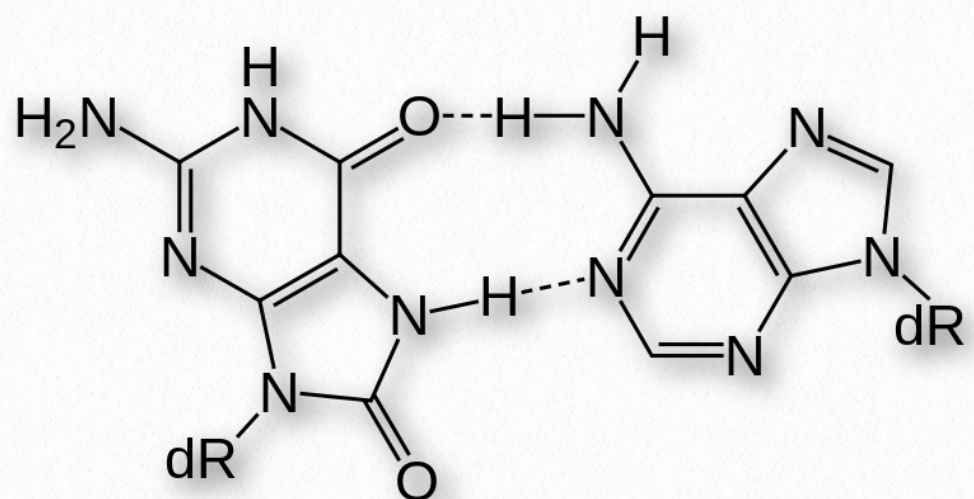


Figure 5.52 - Adenine-8-oxo-guanine base pair. dR = deoxyribose



**Figure 5.53 - Good antioxidant sources**

lication proceeds. 8-oxoguanine can be repaired if recognized in time by a DNA glycosylase, which acts to clip out the damaged base and it can then be replaced by the proper one. Polycyclic aromatic hydrocarbons from cigarette smoke, diesel exhaust, or overcooked meat can covalently bind to DNA and, if unrepaired, lead to mutation. Chemical damage to DNA can result in broken or cross-linked DNAs.

## Diseases of DNA repair

The importance of DNA repair in the aging process is made clear by diseases affecting DNA repair that lead to premature aging. These include Werner syndrome, for whom the life expectancy is 47 years. It arises as a result of loss of two enzymes in base excision repair. People suffering from Cockayne syndrome have a life expectancy of 13 years due to mutations that alter transcription-coupled nucleotide excision repair, which is an important system for fixing oxidative damage.

Further, the life expectancies of 13 species of mammalian organisms correlates with the level of expression of the PARP DNA repair-inducing protein. Interestingly, people who lived past the age of 100 had a higher level of PARP than younger people in the population.

## Antioxidants

There is a growing interest in the subject of antioxidants because of health concerns raised by our knowledge of problems created as a result of spontaneous oxidation of biomolecules by Reactive Oxygen Species (ROS), such as superoxide. Antioxidants have the chemical property of protecting against oxidative damage by being readily oxidized themselves, preferentially to other biomolecules.

Biologically, cells have several lines of antioxidant defense. They include molecules, such

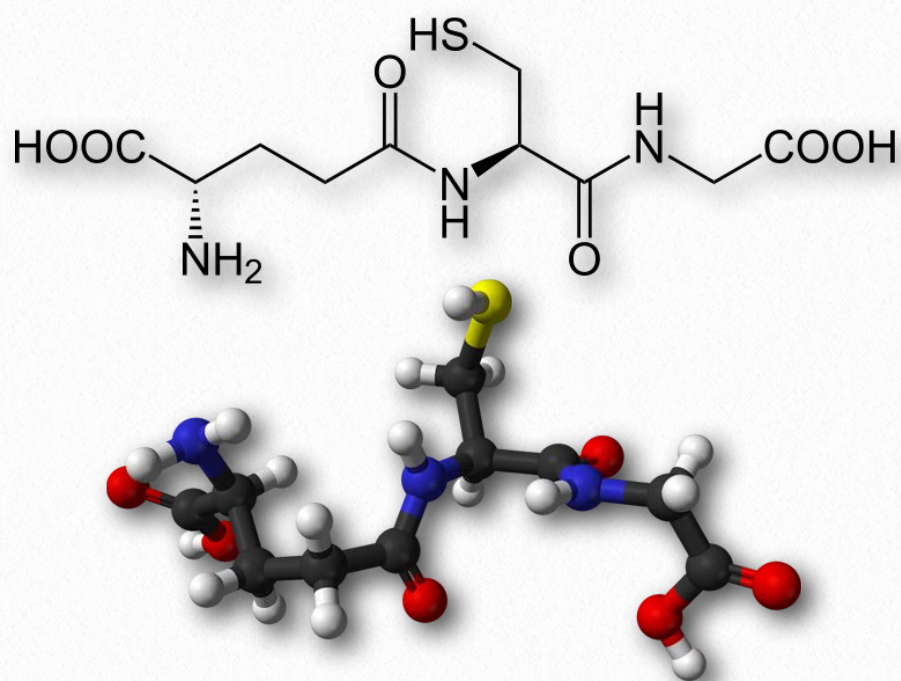
as vitamins C, A, and E, glutathione, and enzymes that destroy ROS such as superoxide dismutase, catalase, and peroxidases.

### Health effects

Oxidation by ROS is mutagenic and has been linked to atherosclerosis. Nonetheless, randomized studies of oral supplementation of various vitamin combinations have shown no protective effect against cancer and supplementation of Vitamin E and selenium has revealed no decrease in the risk of cardiovascular disease. Further, no reduction in mortality rates as a result of supplementation with these materials has been found, so the protective effects, if any, of antioxidants on ROS in human health remain poorly understood.

### Glutathione

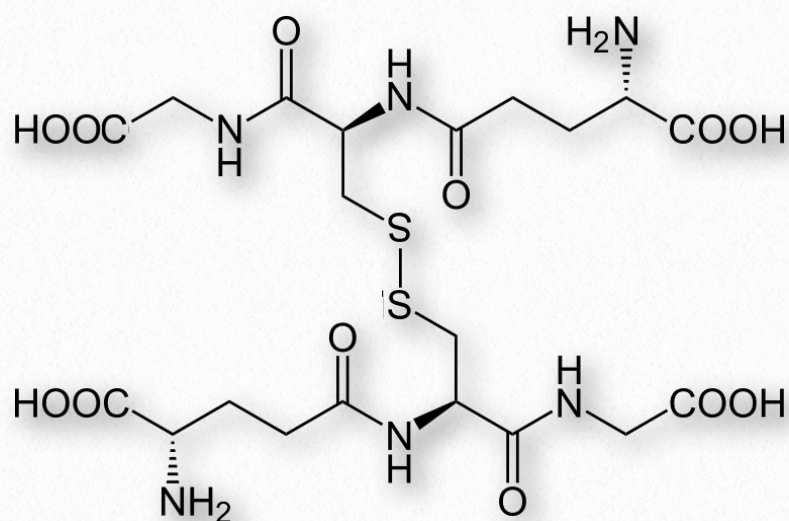
The major endogenous antioxidant found in cells spanning most living systems, glutathione is a tripeptide protecting cells against damage caused by reactive oxygen species and heavy metals (Figures 5.55 & 5.56). The three amino acids in glutathione (glutamate, cysteine, and glycine) are joined in an unusual fashion.



**Figure 5.54 - Structure of reduced glutathione (GSH)**

The glutamate is joined to the center cysteine by a peptide bond between the R-group carboxyl of glutamate and the  $\alpha$ -amine of cysteine. The bond between cysteine and glycine is a normal peptide bond between the  $\alpha$ -carboxyl of cysteine and the  $\alpha$ -amine of glycine.

The thiol group of cysteine is a reducing agent that reduces disulfide bonds to sulfhydryls in cytoplasmic proteins. This, in turn, is the bridge when two glutathiones get oxidized and form a disulfide bond with each other (Figure 5.56). Glutathione's two oxidative states are abbreviated as follows: GSH (reduced) and GSSG (oxidized).



**Figure 5.55 - Oxidized glutathiones (GSSG) joined by a disulfide bond**

Wikipedia

Disulfide-joined glutathiones can be separated by reduction of their bonds with glutathione reductase, using electrons from NADPH for the reduction.

### Non-ribosomal synthesis

Glutathione is not made by ribosomes. Rather, two enzymes catalyze its synthesis. The enzyme  $\gamma$ -glutamylcysteine synthetase catalyzes the joining of the glutamate to the cysteine and then glutathione synthetase catalyzes the peptide bond formation between the cysteine and the glycine. Each step requires energy from ATP.

### Essential for life

Glutathione is important for life. Mice lacking the first enzyme involved in its synthesis in the liver die in the first month after birth. In healthy cells, 90% of glutathione is in the GSH state. Higher levels of GSSG correspond to cells that are oxidatively stressed.

Besides reducing disulfide bonds in cells, glutathione is also important for the following:

- Neutralization of free radicals and reactive oxygen species.
- Maintenance of exogenous antioxidants such as vitamins C and

E in their reduced forms.

- Regulation of the nitric oxide cycle

### Resveratrol

Categorized as a stilbenoid, resveratrol (Figure 5.57) is a phenolic compound produced in the skin of plants such as grapes, raspberries, and blueberries, in response to injury or when they are being attacked by pathogens.

Numerous health benefits are claimed for the compound, though evidence of such benefits is in short supply. Resveratrol is metabolized rapidly in the body, so it is difficult to maintain levels of it.

Some data indicates resveratrol may improve the functioning of mitochondria. It also acts as an antioxidant and causes concentration of another anti-oxidant, glutathione, to increase. The compound appears to induce expression of manganese superoxide dis-

YouTube Lectures  
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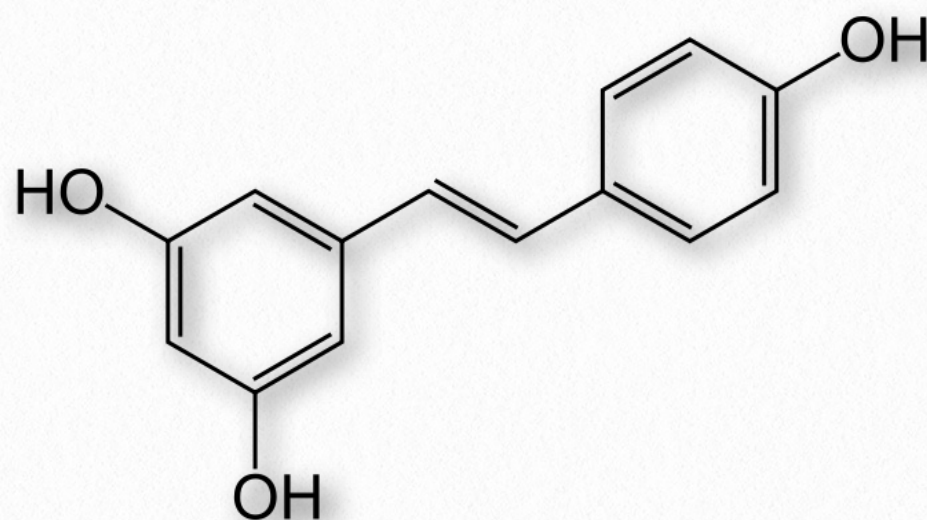


Figure 5.56 - Resveratrol

mutase (protects against reactive oxygen species) and inhibits several phosphodiesterases. This causes an increase in cAMP which results in increases in oxidation of fatty acids, mitochondria formation, gluconeogenesis, and glycogen breakdown. It has been claimed to be the cause of the French Paradox in which drinking of red wine is supposed to give protection for the cardiovascular system. Research data is lacking in support of the claim, however. Resveratrol is known to activate Sirtuin proteins, which play roles in gene inactivation.

## Summary

In summary, energy is needed for cells to perform the functions that they must carry out in order to stay alive. At its most basic level, this means fighting a continual battle with entropy, but it is not the only need for energy that cells have.

## References

1. Winge, D.R., *Mol Cell Biol.* 2012 Jul; 32(14): 2647–2652. doi: 10.1128/MCB.00573-12

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# I'm a Little Mitochondrion

To the tune of "I'm a Lumberjack"

**Metabolic Melodies** Website [HERE](#)

I'm a little mitochondrion  
Who gives you energy  
I use my proton gradient  
To make the ATPs

*He's a little mitochondrion  
Who gives us energy  
He uses proton gradients  
To make some ATPs*

Electrons flow through Complex II  
To traffic cop Co-Q  
Whenever they arrive there in  
An FADH-two

*Electrons flow through Complex II  
To traffic cop Co-Q*

*Whenever they arrive there in  
An FADH-two*

*Tightly coupled is my state  
Unless I get a hole  
Created in my membrane by  
Some di-ni-tro-phe-nol*

*Yes tightly coupled is his state  
Unless he gets a hole  
Created in his membrane by  
Some di-ni-tro-phenol*

*Both rotenone and cyanide  
Stop my electron flow  
And halt the calculation of  
My "P" to "O" ratio*

*Recording by Tim Karplus  
Lyrics by Kevin Ahern*



# N-A-D

To the tune of "Penny Lane"

**Metabolic Melodies** Website [HERE](#)

In the catabolic pathways that our cells employ  
Oxidations help create the ATP  
While they lower Gibbs free energy  
Thanks to enthalpy

If a substrate is converted from an alcohol  
To an aldehyde or ketone it is clear  
Those electrons do not disappear  
They just rearrange – very strange

N-A-D is in my ears and in my eyes  
Help-ing mol-e-cules get oxidized  
Making N-A-D-H then

And the latter is a problem anaerobically  
'Cuz accumulations of it muscles hate  
They respond by using pyruvate  
To produce lactate

Catalyzing is essential for the cells to live  
So the enzymes grab their substrates eagerly  
If they bind with high affinity  
Low  $K_m$  you see, just as me

N-A-D is in my ears and in my eyes  
Help-ing mol-e-cules get oxidized  
Making N-A-D-H then

Recorded by Tim Karplus  
Lyrics by Kevin Ahern

# Superoxide Dismutase

To the tune of "Supercalifragilisticexpialidocious"

**Metabolic Melodies** Website [HERE](#)

When oxygen's electrons all are in the balanced state  
There's twelve of them for oh-two. The molecule is great  
But problems sometimes happen on the route to complex IV  
Making reactive species that the cell cannot ignore

Oh superoxide dismutase is super catalytic  
Keeping cells from getting very peroxy-nitric  
Faster than a radical, its actions are terrific  
Superoxide dismutase is super catalytic

Enzyme, enzyme deep inside  
Blocking all the bad oxides

The enzyme's main advantage is it doesn't have to wait  
By binding superoxide in a near-transition state  
It turns it to an oxygen in mechanism one  
Producing "h two oh two" when the cycle is all done

Oh superoxide dismutase you're faster than all them  
You've got the highest ratio of  $k_{cat}$  over  $K_M$   
This means that superoxide cannot cause too much mayhem  
Superoxide dismutase is faster than all them

Superoxide dismutase  
Stopping superoxide's ways

The enzyme's like a ping-pong ball that mechanistic-ly  
Bounces between two copper states, plus one and two you see  
So S-O-D behaves just like an anti-oxidant  
Giving as much protection as a cell could ever want

Oh superoxide dismutase, the cell's in love with you  
Because you let electron transport do what it must do  
Without accumulation of a radical oh two  
Superoxide dismutase - that's why a cell loves you

Lyrics by Kevin Ahern

No Recording Yet For This Song