**Fundamentals of Protein Chemistry**
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**Module - 09**
**Membrane Proteins and Transport**
**Lecture - 45**
**Electron Transport Chain**

In our final lecture in this module, that has been dealing with membrane proteins and transport, we will talk about the electron transport chain.

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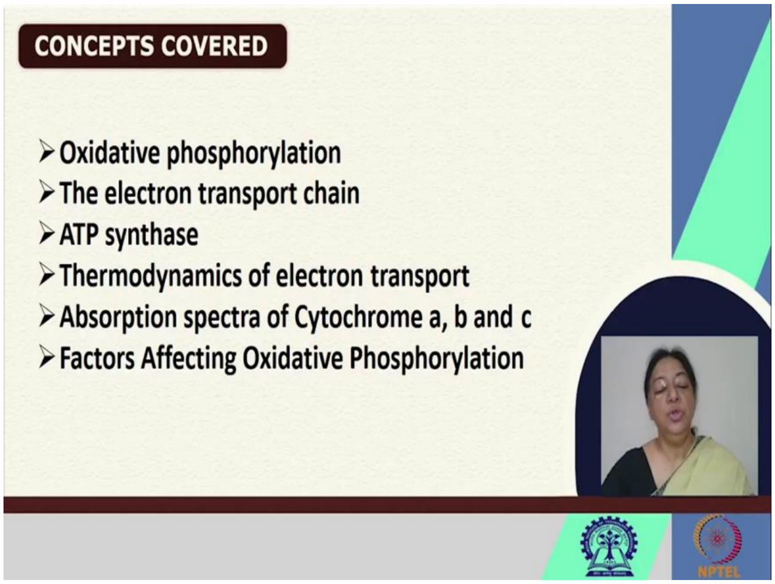


What we are going to look at is, we are going to look at oxidative phosphorylation, the electron transport chain and ATP synthase, which we have looked at in motor proteins as well and the aspects of oxidative phosphorylation.

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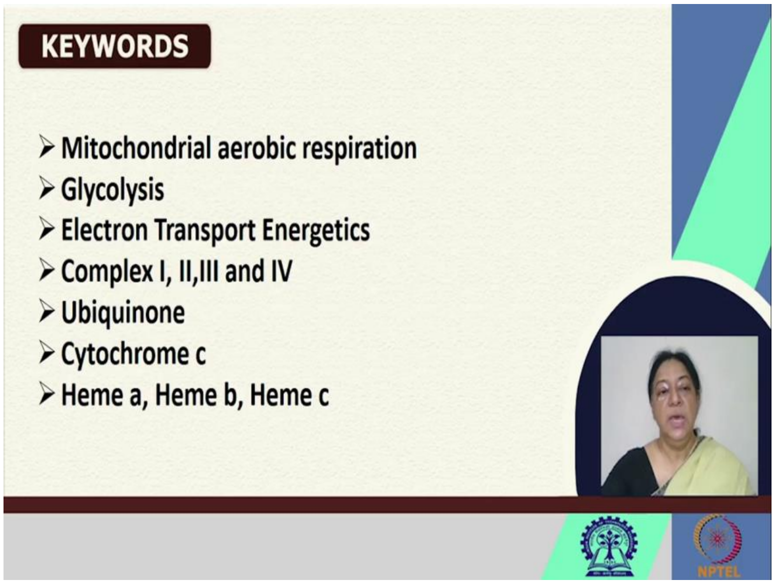


The importance in mitochondrial aerobic respiration, is evident from the fact that this is a life process involved throughout in a way that is necessary for life processes. This involves several complexes that are proteins present in the membrane. As we go forward, we will see how these proteins work and what the specific aspects of the electron transport chain are.

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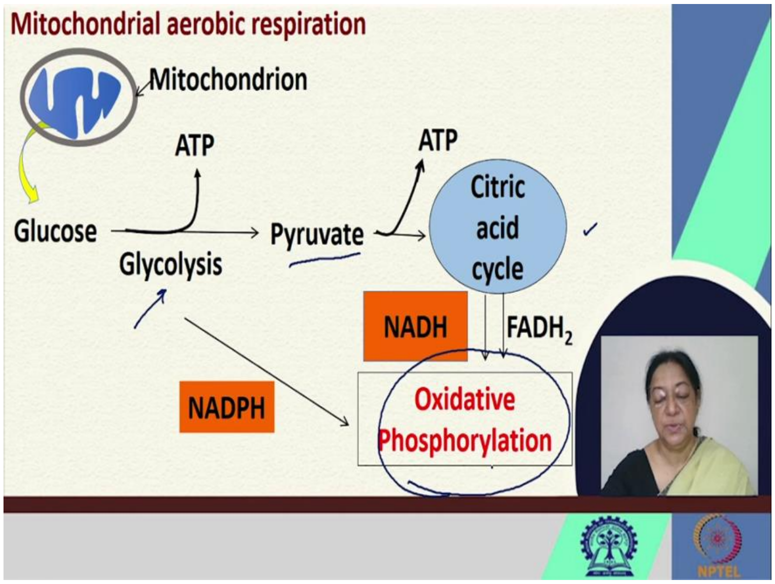


In mitochondrial aerobic respiration, we have the process of glycolysis that occurs outside in the cytoplasm. The pyruvate that is transferred into the mitochondria, the citric acid cycle that occurs in the mitochondria, followed by this important step of oxidative phosphorylation.

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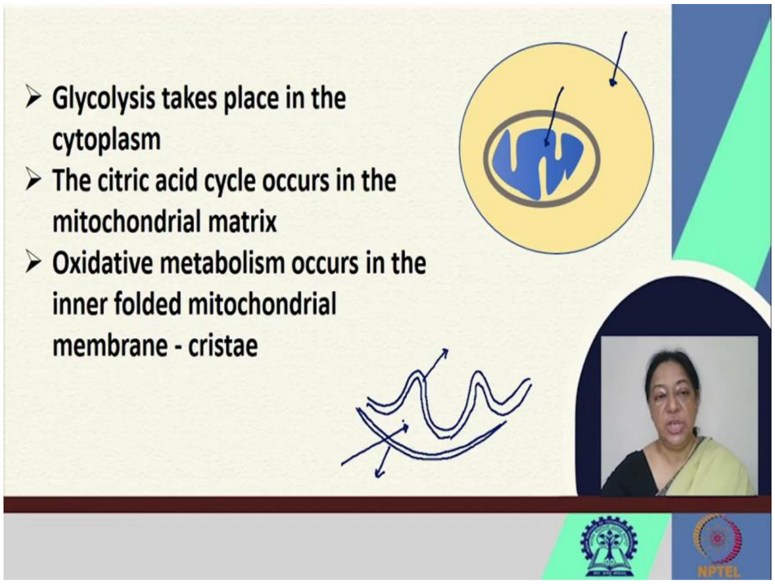


If we consider this [refer to slide] as the overall cell, where we have glycolysis that takes place in the cytoplasm, the citric acid cycle occurs in the mitochondrial matrix and oxidative metabolism occurs in these inner folded mitochondrial membrane known as the cristae.

If this is the outer membrane which we know is a lipid bilayer itself and we have the inner cristae, the folded membrane which is also a bilayer. What we have is we have this as the outer membrane, this as the inner membrane and this is the inter membrane space, where a lot of activity occurs in terms of energetics associated with the cell, in terms of oxidative phosphorylation, the electron transport chain.

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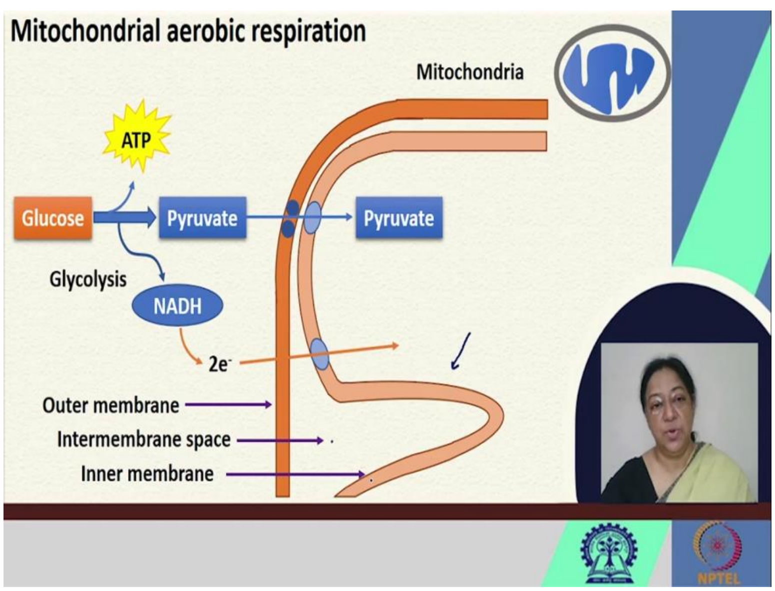


In mitochondrial aerobic respiration, the folds of the membrane are known as the cristae. We have the outer membrane, the inter membrane space and the inner membrane. We have the glucose breakdown, formation of pyruvate, that then has several electrons and with the pyruvate enters the mitochondria.

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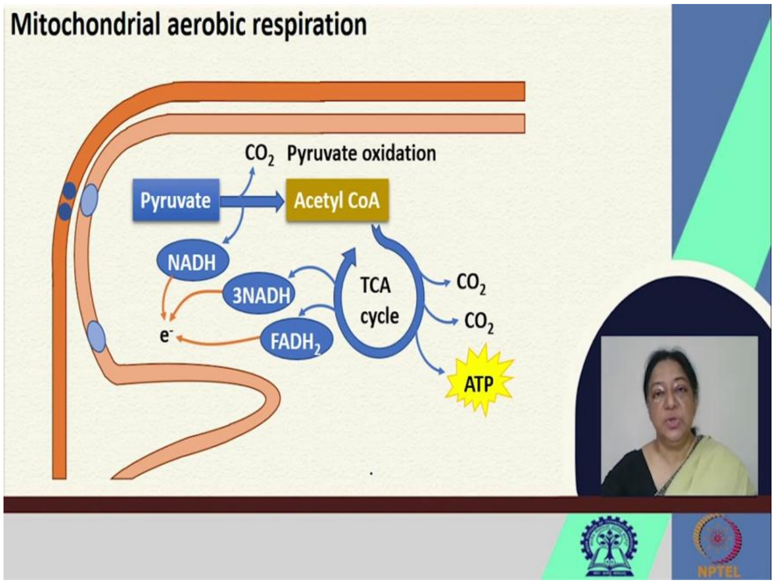


The process now occurs in a manner where we have the pyruvate release carbon dioxide, the NADH and NAD activities we will see in a moment. The acetyl coenzyme A, resulting in pyruvate oxidation that would lead to the TCA cycle in the production of carbon dioxide, ATP, $FADH_2$, and 3NADH.

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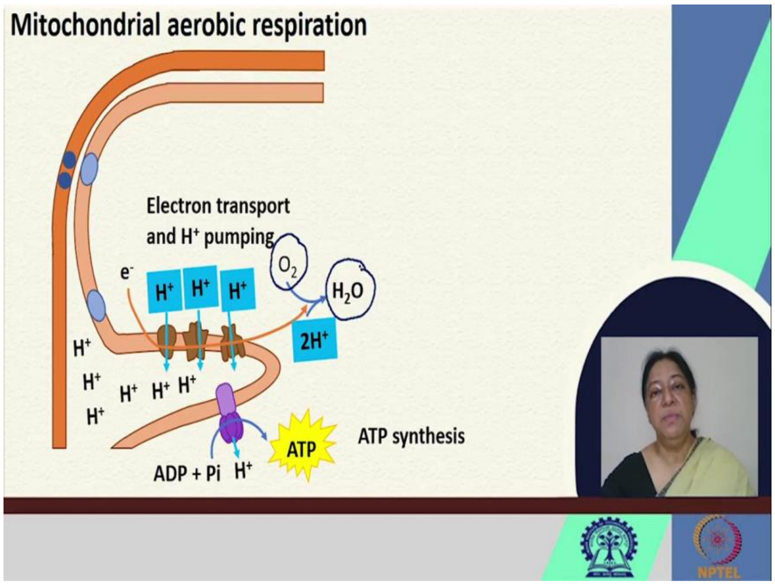

These electron transport processes are then important in the way they work with the different complexes that are embedded in the inner mitochondrial membrane, the proton transfer that occurs in several ways to finally lead from oxygen to water.

In this process we have electron transport and we have proton pumps that are embedded in the membrane. Pumps that work in a fashion that require energy to transport the proton from a lower proton concentration to a higher proton concentration; a topic that we visited when we considered transport across membranes. We have also ATP synthase, where ATP is produced from ADP + Pi, a very common motor protein that is also a membrane protein.

So, this ATP synthesis also occurs in the inner mitochondrial membrane. This membrane and the membrane proteins associated with this, are extremely important in their activity.

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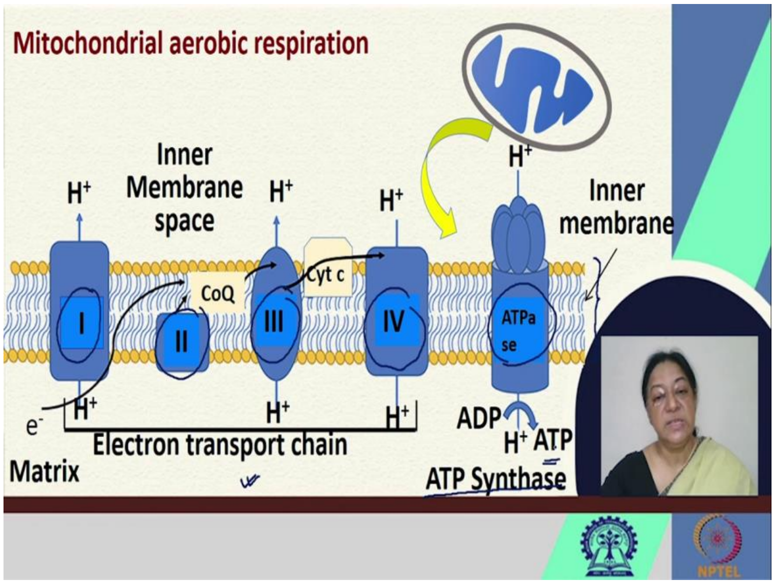


If we now look at this inner membrane in a larger perspective, we have several complexes associated with the process. The 4 complexes marked [refer to slide] complex I, complex II, complex III and complex IV, comprise what is known as the electron transport chain.

We have the ATPase involved here, where in ATP synthase, ATP is produced from ADP and Pi and all this occurs in the inner mitochondrial membrane. We will look at the complexes, the proteins involved in the complexes and we will see how the electron transport occurs in the final expression or the final production of water.

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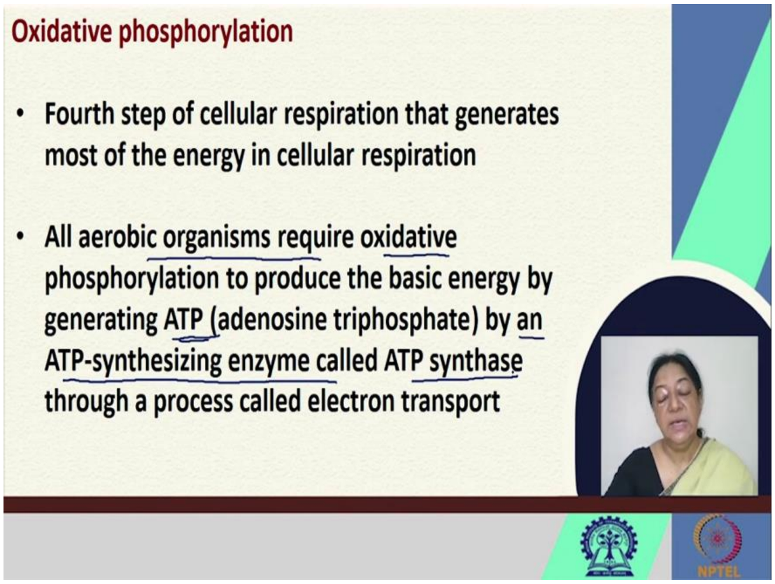


The process of oxidative phosphorylation itself, is the 4th step of the cellular respiration that generates most of the energy involved in cellular respiration. All aerobic organisms require the process of oxidative phosphorylation to produce the basic energy by generating ATP, by an ATP synthesizing enzyme called ATP synthase. We have seen the mechanism of action of ATP synthase in the previous lecture and this occurs through a process called electron transport in a series of complexes.

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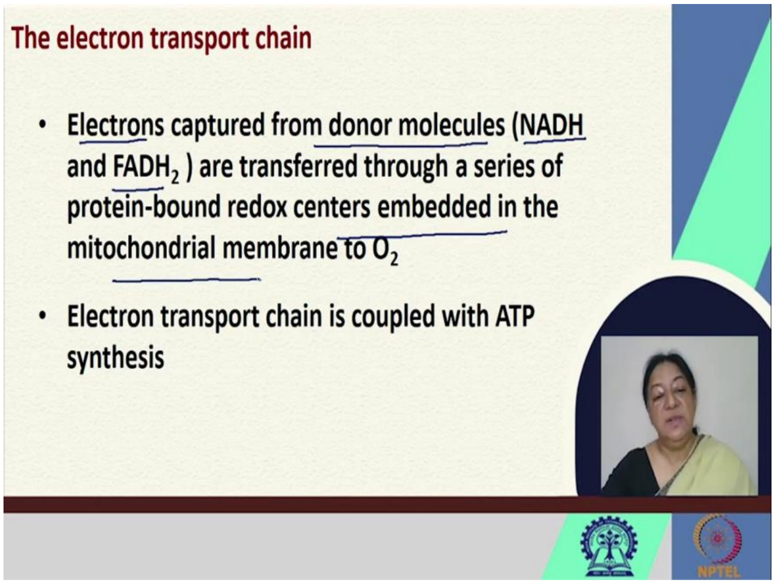


The electron transport chain itself has electrons that are captured from donor molecules. These donor molecules are NADH and $FADH_2$. These electrons are transferred through a series of protein bound redox centers that are embedded in the mitochondrial membrane and finally they will couple themselves with ATP synthase.

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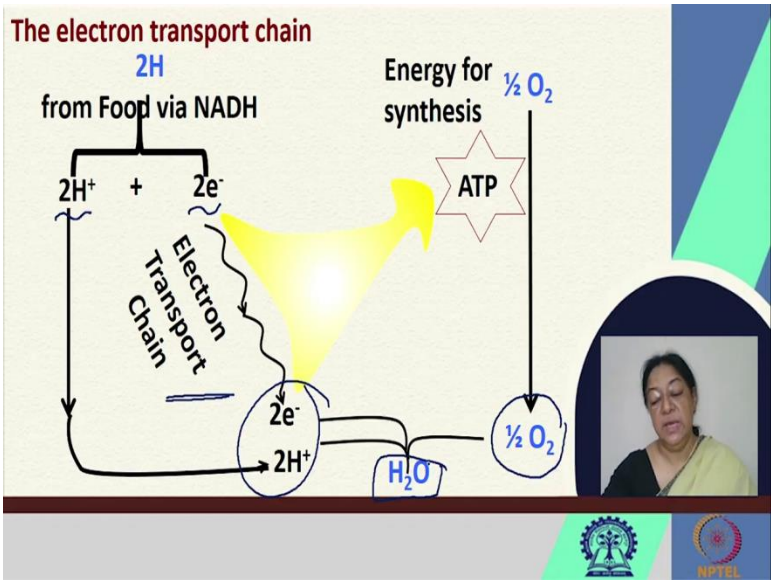


So what happens [refer to slide] is when we have the proton, we have the electrons, we have the cascade of reactions in the electron transport chain, this will then with the oxygen reduce water, in the processes involved in the electron transport chain.

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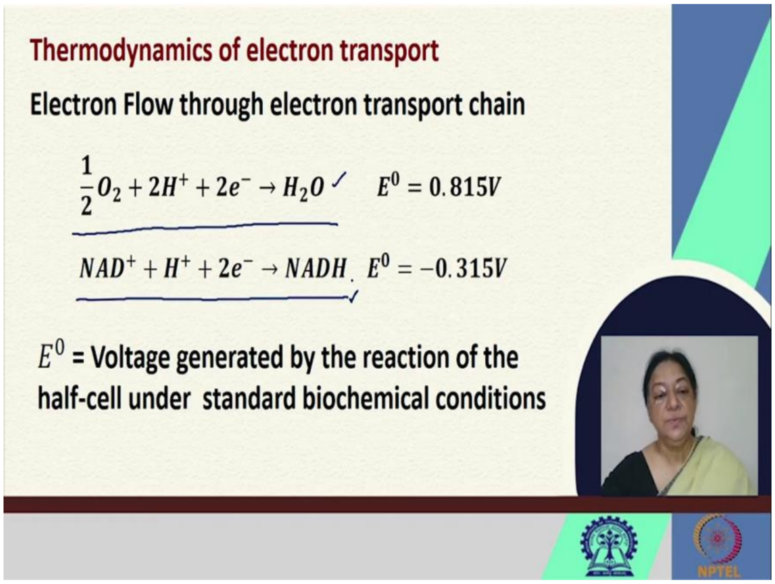


If we look at the thermodynamics of electron transport, we see that this: $\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$ is the overall reaction, but it is coupled with this reaction: $NAD^+ + H^+ + 2e^- \rightarrow NADH$ involved in the electron flow through the electron transport chain. $E^0$ is the voltage generated by the reaction of these individual half-cells, under standard biochemical conditions.

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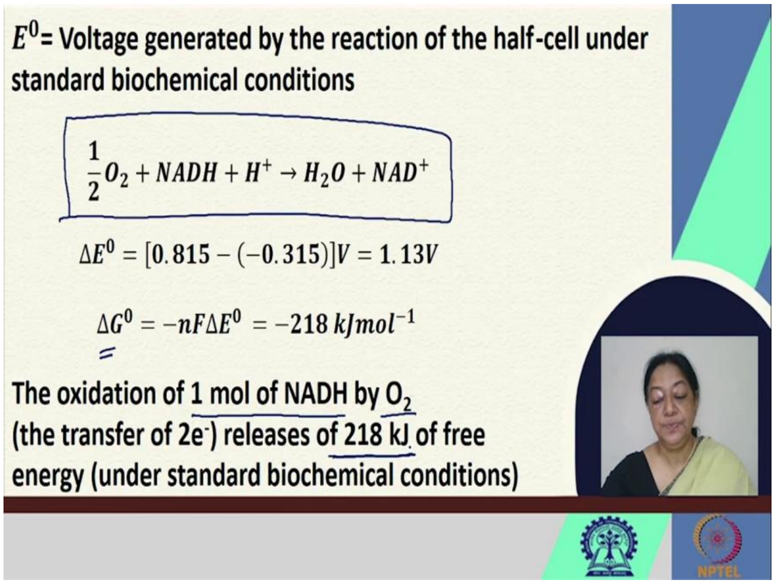


This ½$O_2$ + NADH + $H^+ \rightarrow$ $H_2O$ + $NAD^+$ is our overall reaction when we consider the different half-cells and we get a value of free energy change associated with this; a favorable value. So, the oxidation of one mole of NADH by oxygen, that is the transfer of 2 electrons as we saw in the previous slide, releases 218kJ amount of free energy under standard biochemical conditions. So the change in free energy is this amount, -218 $kJmol^{-1}$ under standard biochemical conditions.

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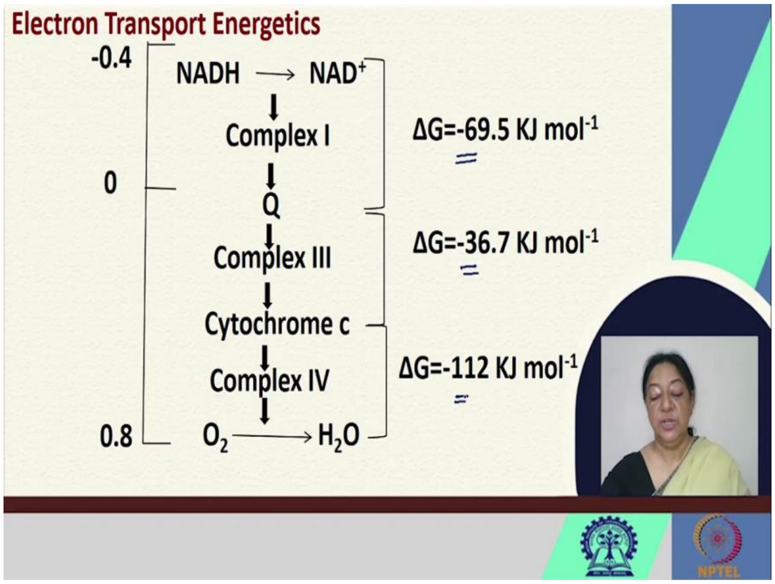


The associated energetics with the different complexes are given here [refer to slide] and we can see that we have favorable cases, as we go along in the energetics.

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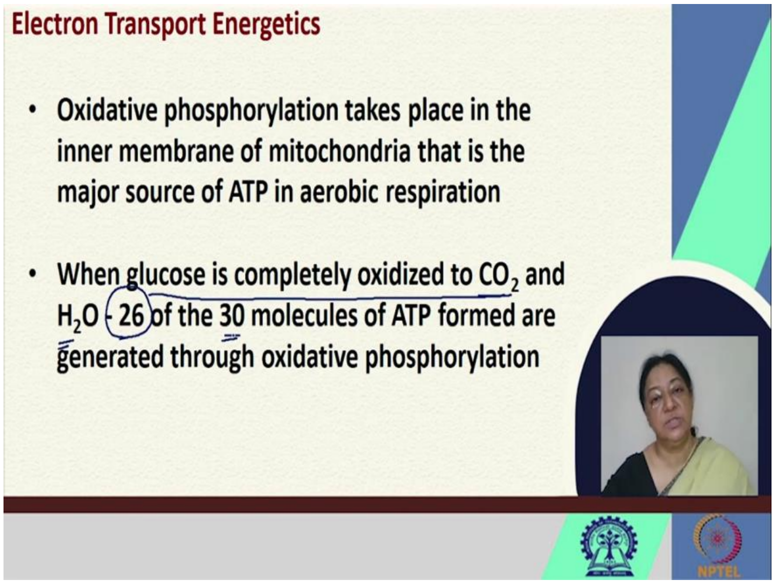


In the electron transport oxidative phosphorylation that takes place in the inner membrane of the mitochondria, this is as we realize, a major source of ATP in aerobic respiration and thus, is an extremely important process that occurs.

When glucose is completely oxidized to carbon dioxide and $H_2O$, 26 of the 30 molecules of ATP formed are generated through this process of oxidative phosphorylation. So we go back to our complex system, where we have our electron transport chain comprised of the 4 complex systems, complex I, II, III, and IV and finally ATP synthase that is going to generate the ATP; all embedded as membrane proteins in the inner membrane of the mitochondria.

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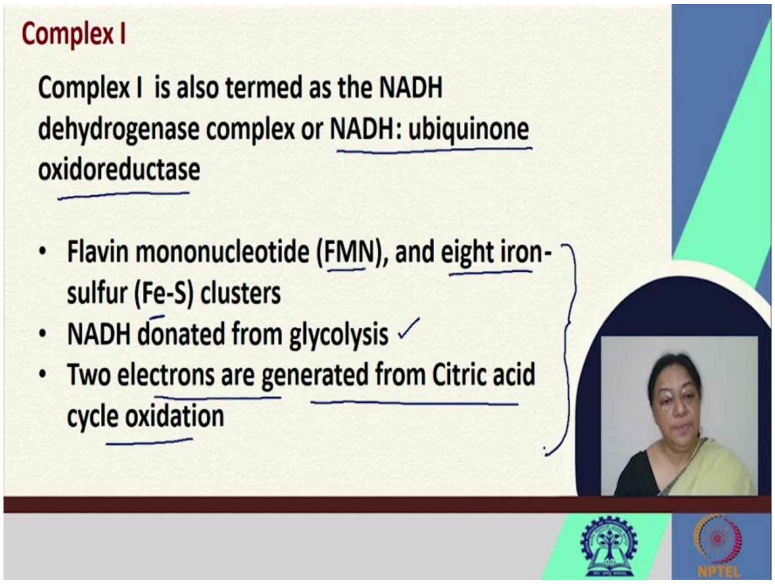


Complex I is NADH dehydrogenase complex or also known as the NADH ubiquinone oxidoreductase. In this complex, there is the molecule flavin mononucleotide FMN and 8 iron sulfur clusters, which we came across in our metal proteins lecture. NADH that is donated from glycolysis and 2 electrons that are generated from the citric acid cycle oxidation that occurs in the mitochondria.

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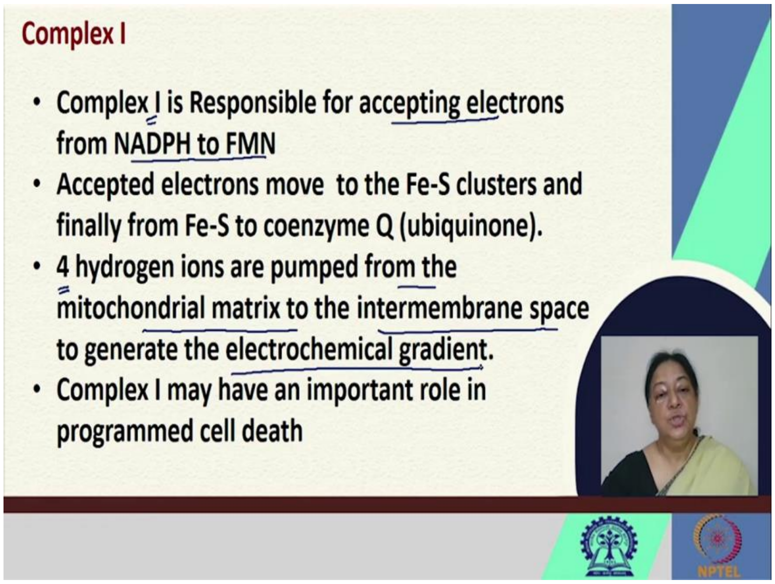


So all this together gives us our complex I and what happens in this process is, the complex is responsible for accepting electrons from NADPH, that is transferred to FMN. The accepted electrons then move to the iron sulfur clusters and finally from the iron sulfur cluster, to the coenzyme Q. 4 hydrogen ions are pumped from the mitochondrial matrix to the inter membrane space, that generates the electrochemical gradient and this complex I is known to have a role in programmed cell death.

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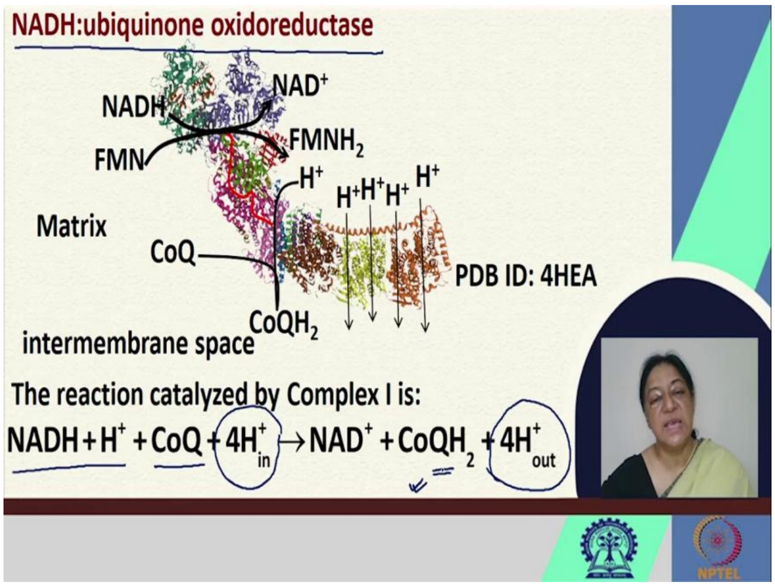

What happens in complex I that is NADH ubiquinone oxidoreductase is, a series of processes occur and the overall reaction is this $NADH + H^+ + CoQ + 4H^+_{in} \rightarrow NAD^+ + CoQH_2 + 4H^+_{out}$ and we have a reduction of coenzyme Q to coenzyme $QH_2$.

Now, without going into the details of the mechanisms involved, the idea here is to realize the complexity of these membrane proteins and the way they act in conjunction with other cofactors and protons, creating this transport across the membrane in terms of a proton pump, in terms of several activities, cascades of reactions that occur.

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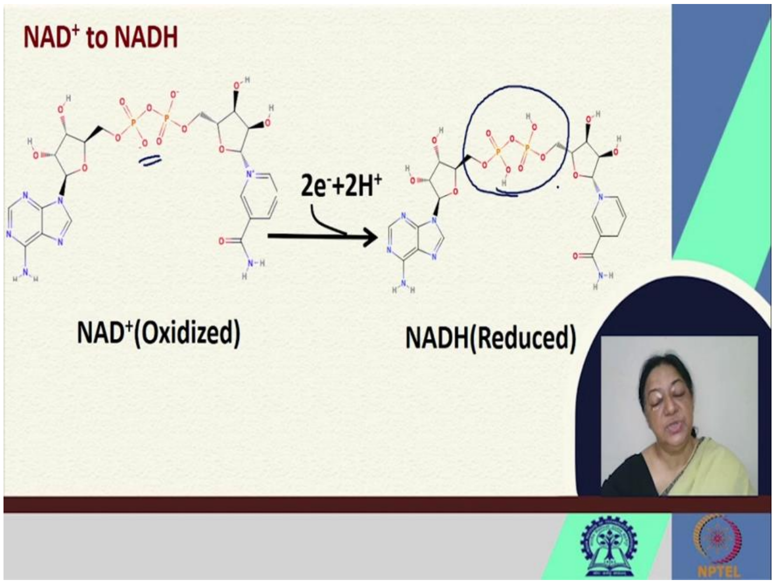


So when we look at $NAD^+$ oxidized, we see that we can have the 2 electron $2H^+$ system resulting in our change at this point here, where we have reduced NADH.

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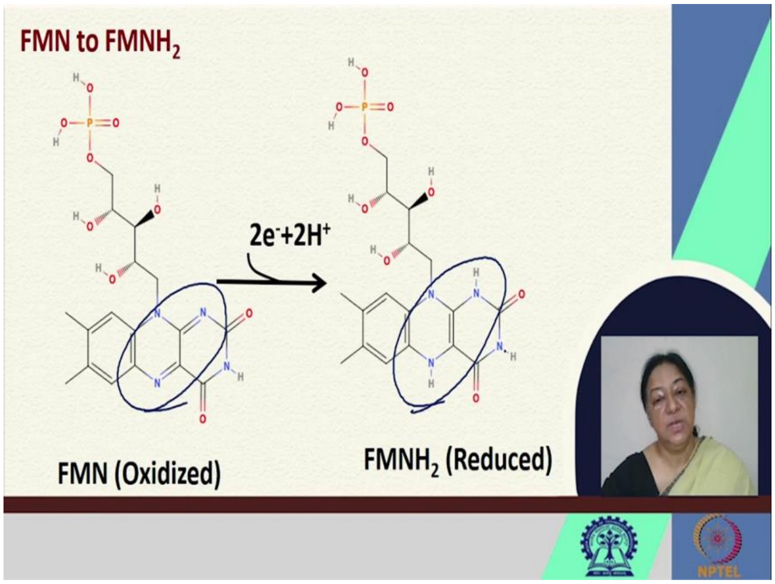


When we see FMN, the oxidized part of FMN is going to get reduced, where we see the reduction over here [refer to slide]; the nitrogens that have accepted the protons.

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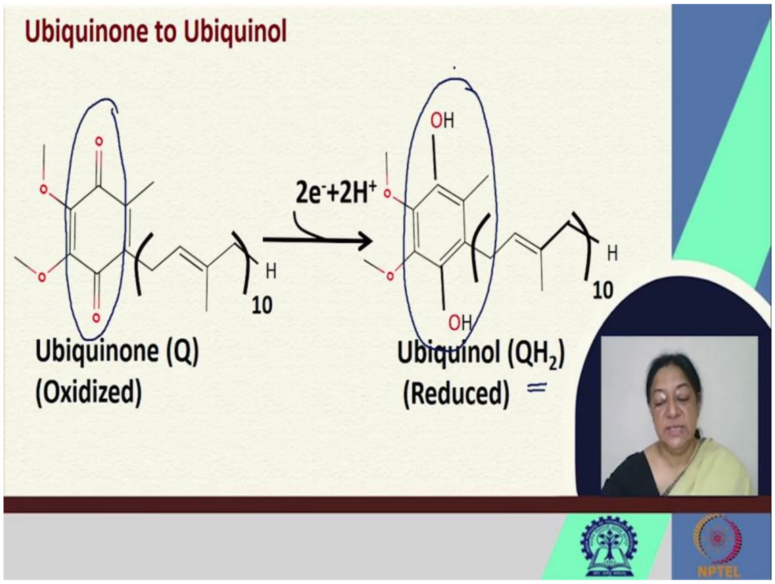


The other process where we have ubiquinone to ubiquinol; Q going to $QH_2$ that we looked at, where we have the quinon moiety, which is this [refer to slide] moiety form the quinol moiety. These are the reactions that generally occur in this specific complex I.

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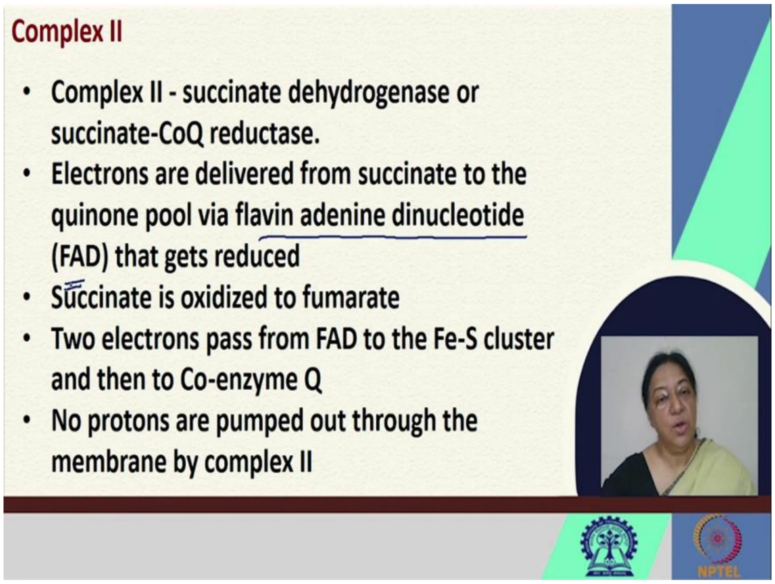


In complex II, we are looking at a protein succinate dehydrogenase or also known as succinate-CoQ reductase. In this case, electrons are delivered from succinate to the quinone pool via flavin adenine dinucleotide FAD, that in the process gets reduced.

So succinate is oxidized to fumarate and 2 electrons pass from FAD to the iron sulfur cluster and then to coenzyme Q. There are no protons pumped out through the membrane in complex II.

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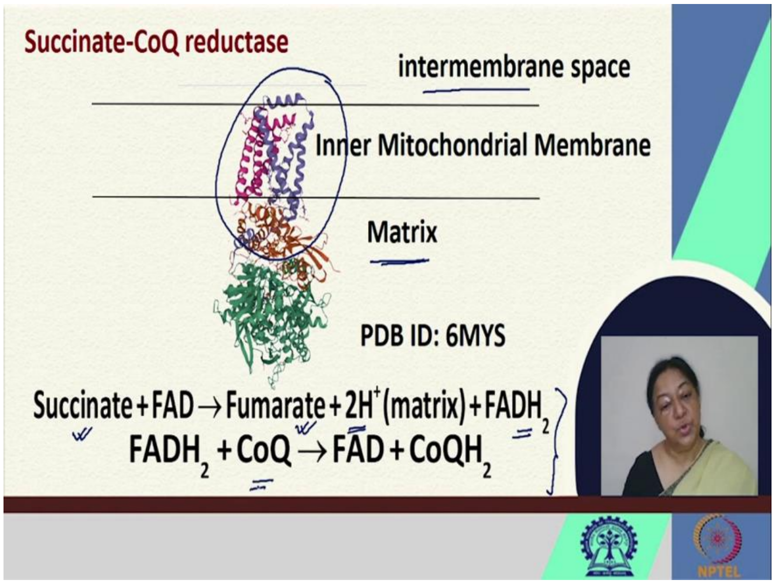


So again we have our inter membrane space, the matrix of the mitochondria and this [refer to slide] is where we have our embedded protein in the inner mitochondrial membrane. The overall reaction is where we have the succinate form the fumarate, in the process generate protons in the matrix, that are then taken up by $FADH_2$ to reduce coenzyme Q.

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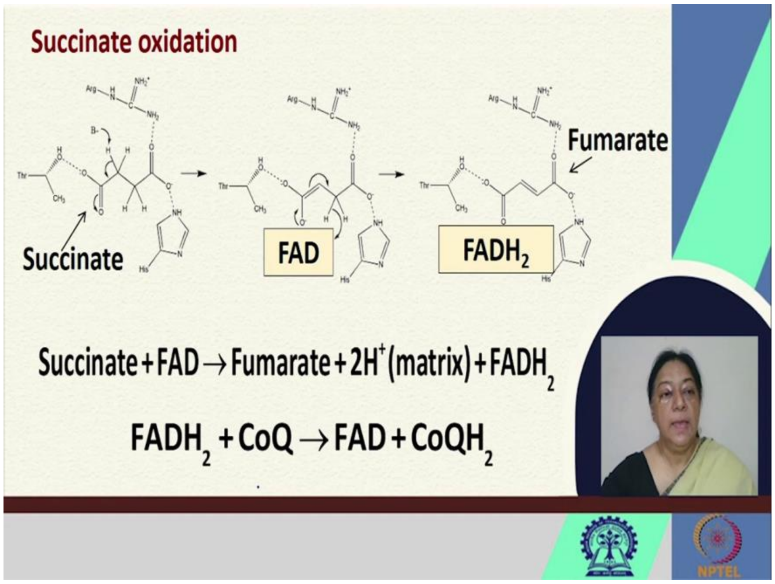


So, this is the overall process where we have succinate go to fumarate, in the event FAD goes to $FADH_2$.

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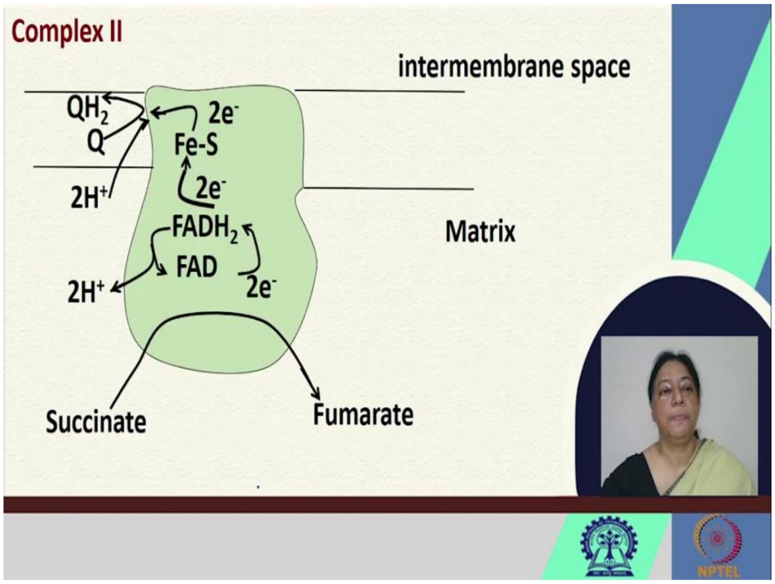


This is the series of reactions that occur where we have the Fe-S, the iron sulphur complex assist in the electron transfer for the process to occur in complex II.

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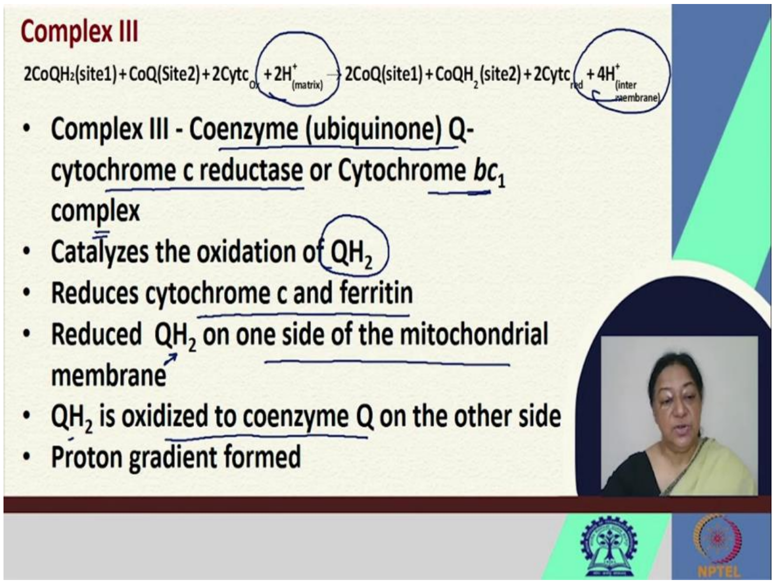


In complex III, a coenzyme Q cytochrome c reductase or cytochrome $bc_1$ complex, as it is called. This catalyzes the oxidation of $QH_2$, it reduces cytochrome c and ferritin, a molecule involved in iron transfer and the reduced $QH_2$ on one side of the mitochondrial membrane. The $QH_2$ is oxidized to coenzyme Q on the other side and as a result, there is a proton gradient formed. This is then utilized later on in the next complex.

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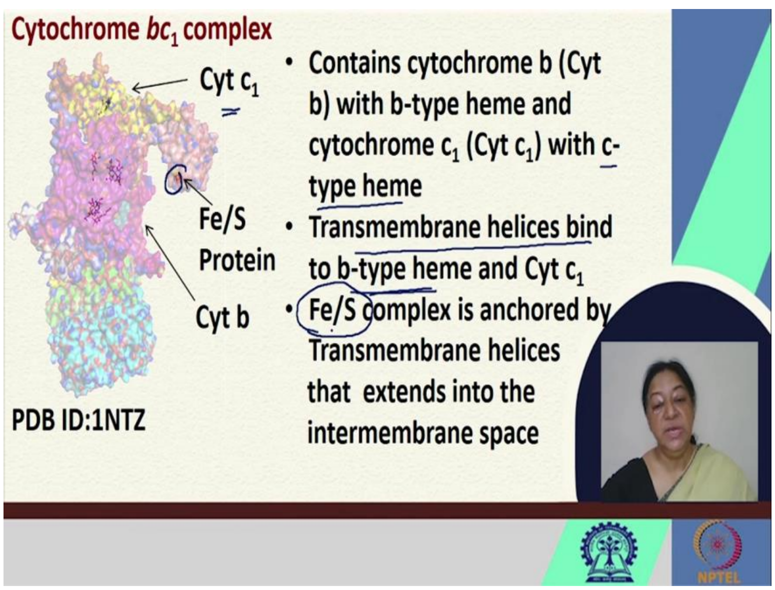


So we have the iron sulfur protein, we have the cytochrome complexes, the cytochrome b with a specific b type heme as it is called, a cytochrome $c_1$ with the c type heme. Now these transmitted helices bind to the b type heme and what happens is the Fe-S complex is anchored by the transmembrane helices, that extend into the inter membrane space.

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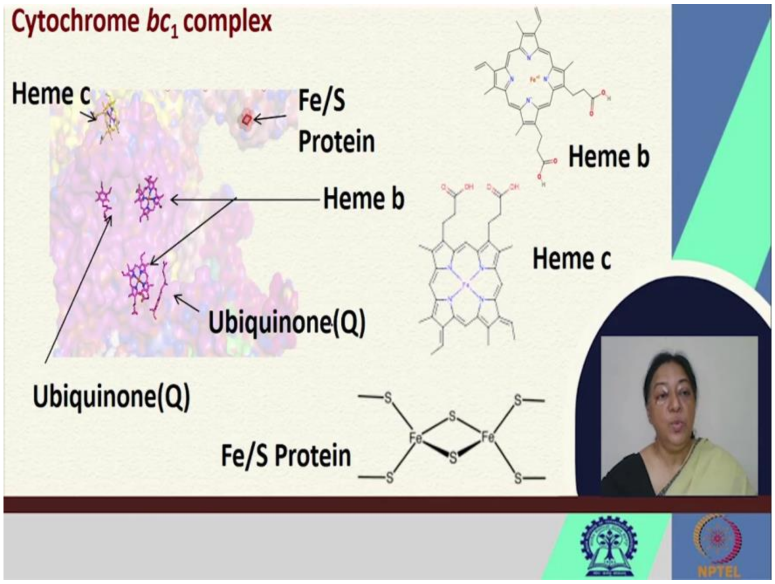


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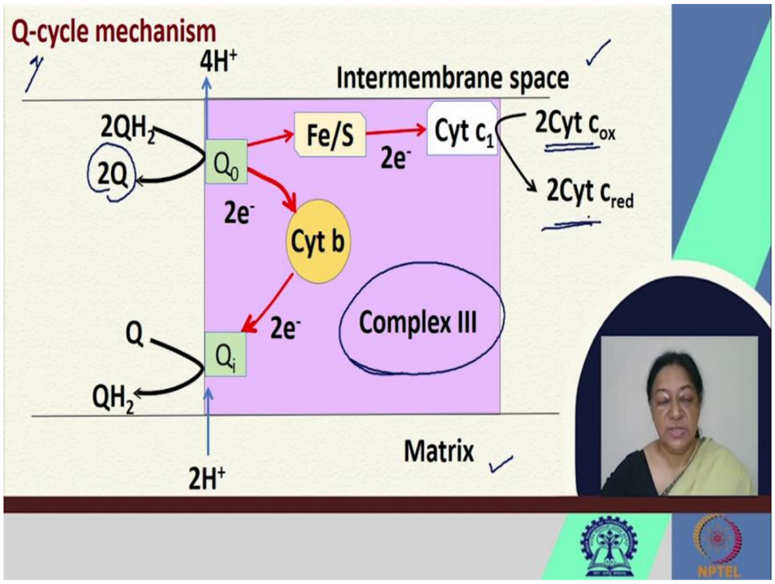


Following this we have the process occur, where we have a series of reactions known as the Q-cycle mechanism, where we have the inter membrane space, the matrix, complex III embedded in the inner mitochondrial membrane and we have the formation of Q from the $2QH_2$. And what we have is the involvement of the cytochrome c proteins, where we have the electron transfer.

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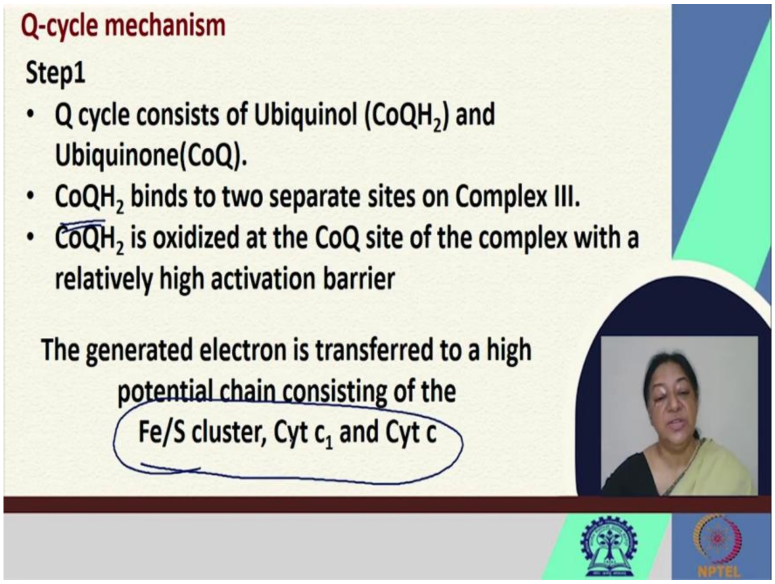


In the Q-cycle mechanism, there are two steps where we have the Q-cycle consisting of ubiquinol, that is $CoQH_2$ and ubiquinone CoQ. We have this $CoQH_2$ bind to two separate sites on complex III. As a result there is a $CoQH_2$ oxidized at the site of the complex, with a very high activation barrier. The generated electron is then transferred to a high potential chain that consists of these iron sulfur cluster and the two cytochromes involved.

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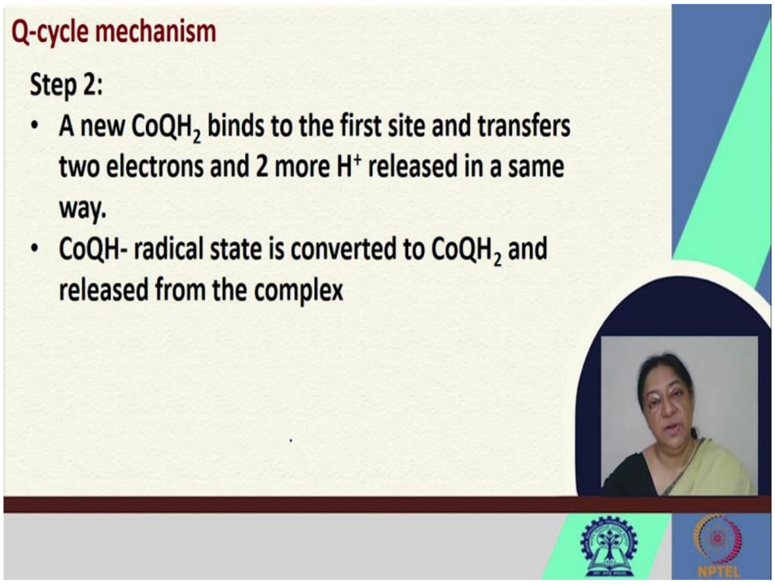


In step 2, we have a new $CoQH_2$ bind to the first site and transfer 2 electrons and 2 more protons are released in a similar fashion. Now, given that this course is rather related to protein chemistry, we would expect an idea related to the membrane proteins involved here and their activity related in this case.

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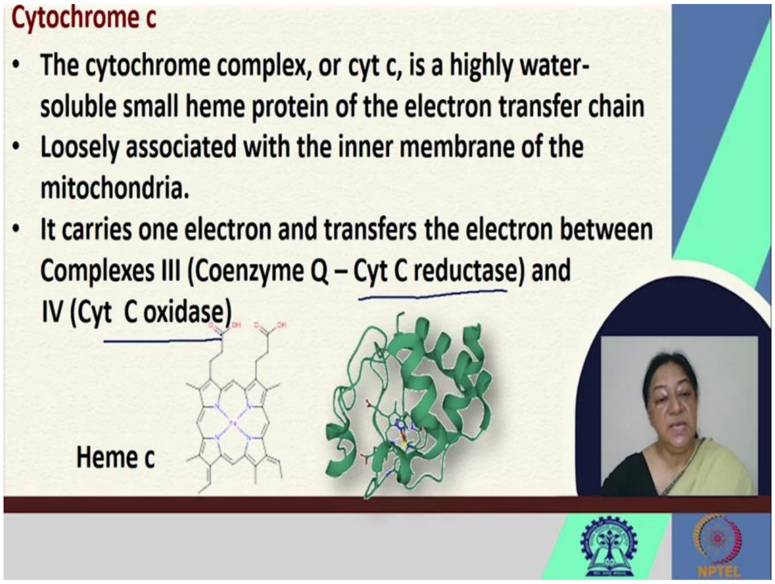


So in the cytochrome c protein, is a water soluble small heme protein of the electron transfer chain. It is loosely associated with the inner membrane of the mitochondria and it carries 1 electron and transfers the electron between complexes 3 and 4. As a result, we have cytochrome c reductase, cytochrome c oxidase. The heme c is involved in the cytochrome c protein.

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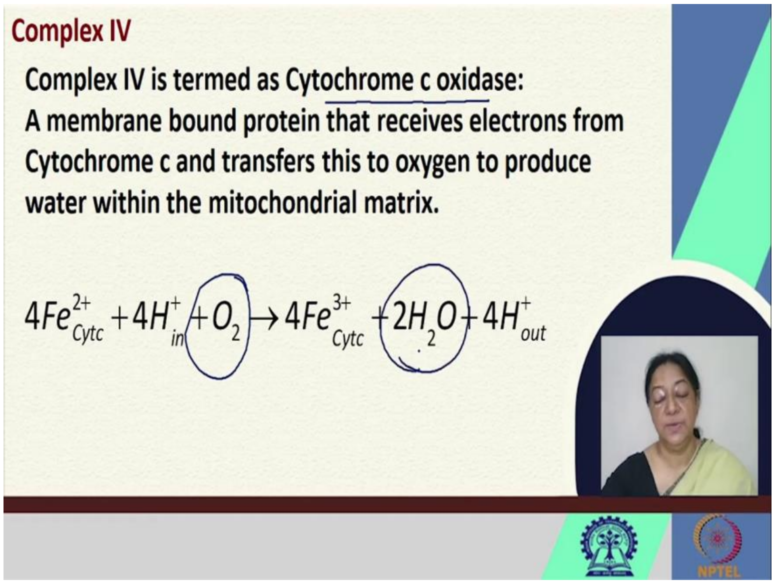


In complex IV which is cytochrome c oxidase, we have again a membrane bound protein that receives electrons from cytochrome c and transfers this to oxygen to produce the water within the mitochondrial matrix. This is what we observe here [refer to slide] where we have our water are formed from the oxygen.

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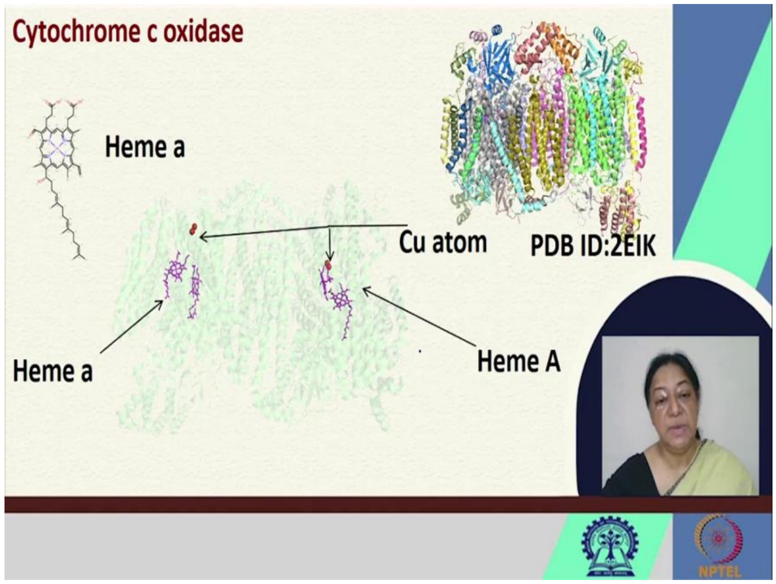


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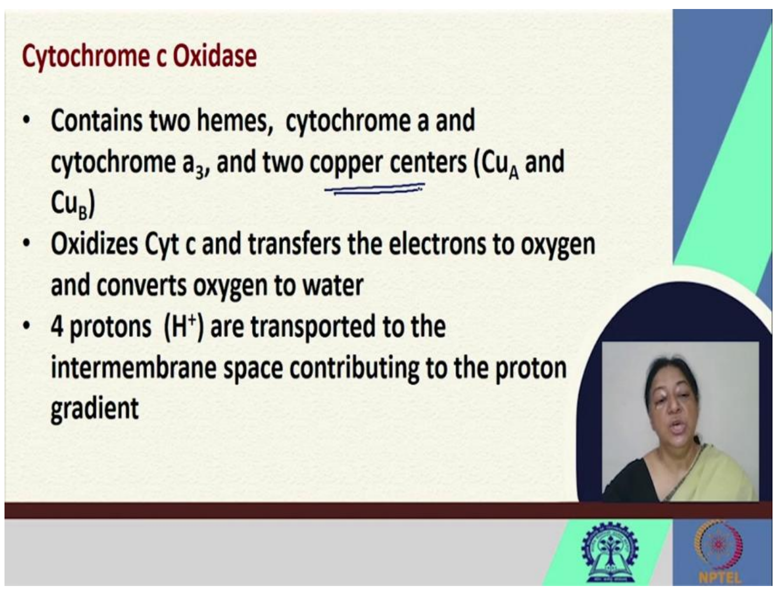


So, we have the different aspects of cytochrome c oxidase, where we have two hemes, cytochrome a and cytochrome $a_3$ and two copper centers $Cu_A$ and $Cu_B$, which we looked at in metalloproteins. This oxidizes cytochrome c and transfers the electrons to oxygen and converts oxygen to water and the 4 protons are transported to the inter membrane space, that contribute to the required proton gradient.

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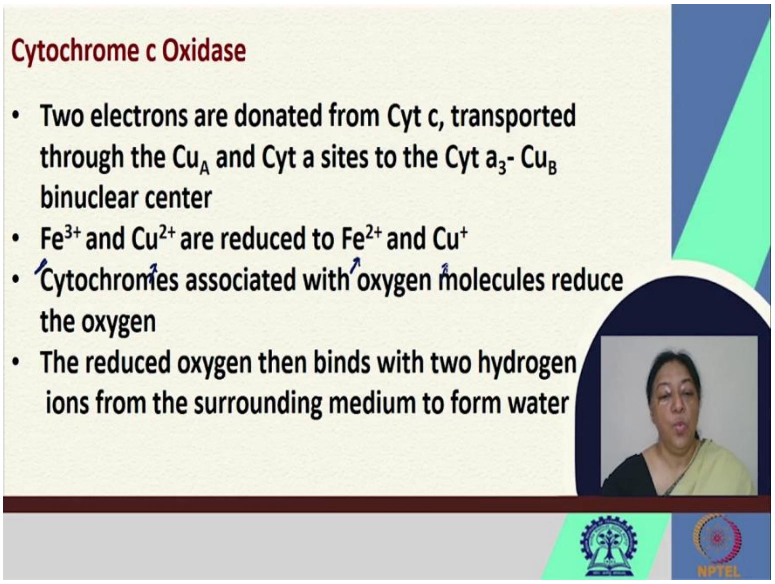


Following this we have two electrons that are donated, transported through the copper A and the cytochrome a sites to a specific center, where we have the reduction of $Fe^{3+}$ to $Fe^{2+}$ and $Cu^{2+}$ to $Cu^{+}$, in a unique mechanism, therefore the cytochromes associated with oxygen molecules are reduced to form water.

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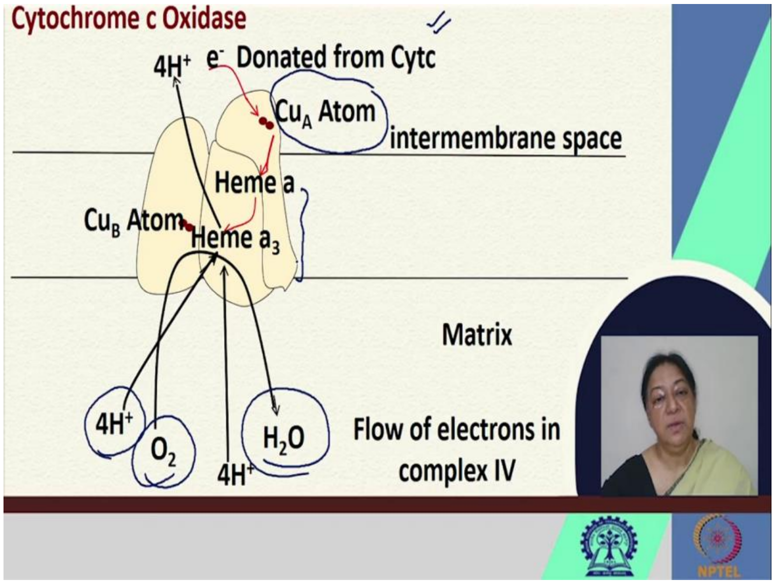


Therefore the oxygen, the protons and finally the electron transfer that is going to lead to the formation of water and the electrons donated from the cytochrome c, come through the copper system. And we have the two hemes associated with this protein, in the inner membrane of the mitochondria.

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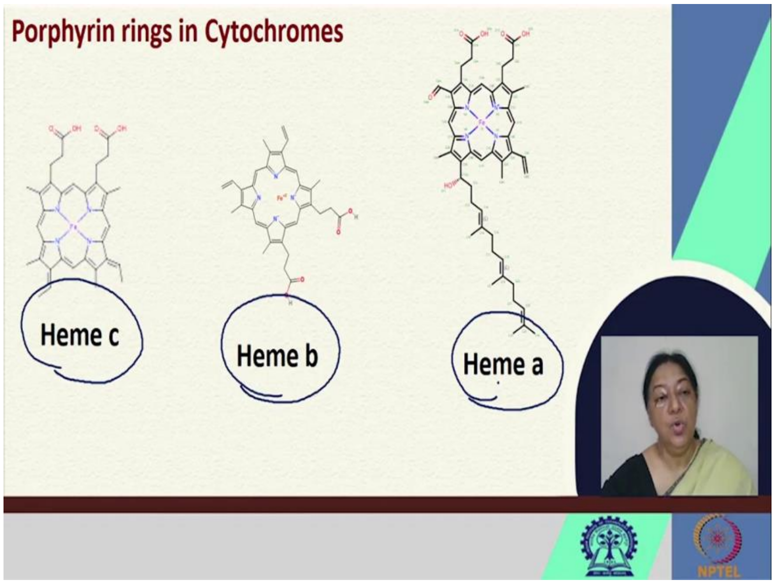


When we look at the different porphyrin rings in the cytochromes, we see there is heme c, heme b and heme a. Each of them have their characteristics that can be studied to look at the specific aspects of the complex systems.

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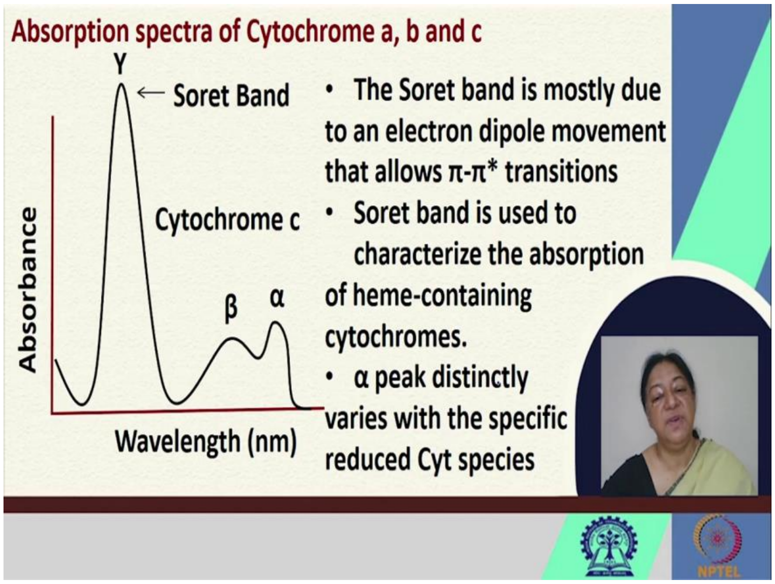


The absorption spectra that we see, has a specific characteristic. One characteristic for example, in cytochrome c shown here [refer to slide] is the Y band, the β and the α. The Y band is known as the soret band. The soret band is mostly due to an electron dipole movement that permits π-π* transitions and it is used to characterize the absorption of heme containing cytochromes. The α peak distinctly varies with the specific reduced cytochrome species.

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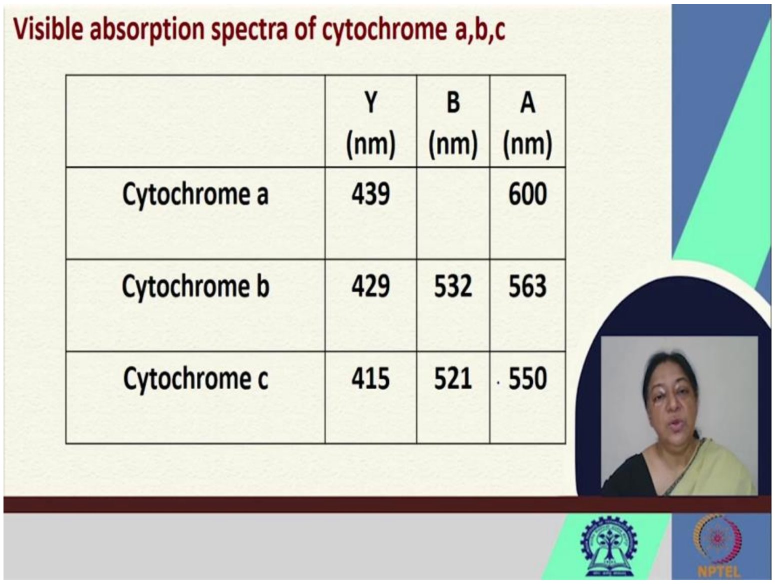


If we look at the different types, we have the soret band, the β, and the α and these are the absorption spectra that are observed for the specific types of cytoplasms.

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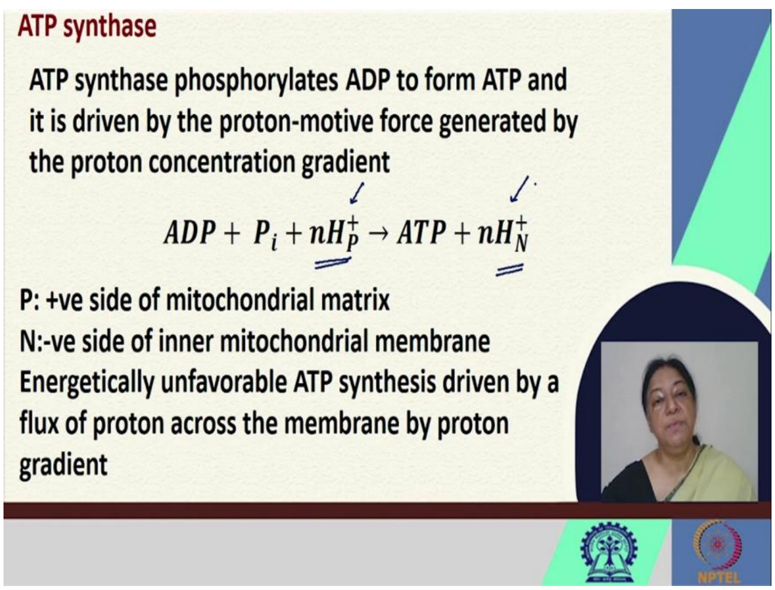


For ATP synthase, this phosphorylates ADP to form ATP and is driven by the proton motive force that is generated by the proton concentration gradient, that is a consequence of our electron transport chain. So what we have is, we have the positive side of the mitochondrial matrix and we have the negative side of the inner mitochondrial membrane. And then what happens is, the energetically unfavorable ATP synthesis is driven by this flux of protons across the membrane, by the proton gradient.

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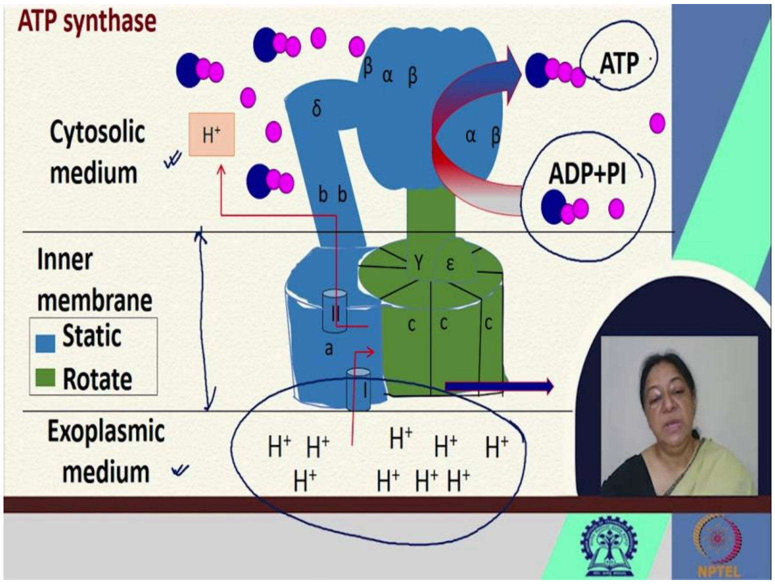


This [refer to slide] is our series of steps that occur in the proton pump that we observe. This is our inner mitochondrial membrane, the cytosolic medium and the exoplasmic region. What happens in this specific process, is we have the proton transfer in this case and as the proton moves, we have specific movement occur. This proton transfer then occurs, resulting in the formation of ATP through the ATP pump from ADP and Pi.

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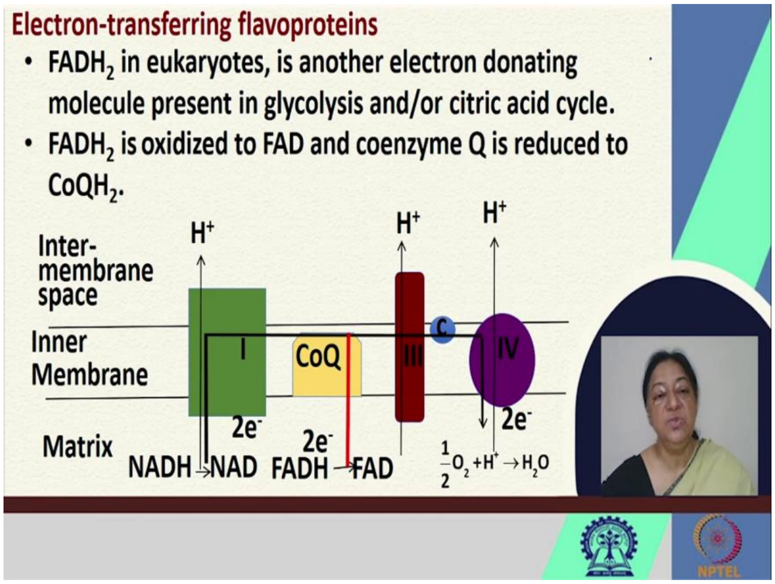


So, in the electron transferring flavoproteins that we see in this whole process, we have the different complexes I, II, III and IV and we looked at $FADH_2$ that in eukaryotes, is another electron donating molecule, that is present in the glycolysis and of the citric acid cycle. It is oxidized to FAD and coenzyme Q; in the process it is reduced to $CoQH_2$.

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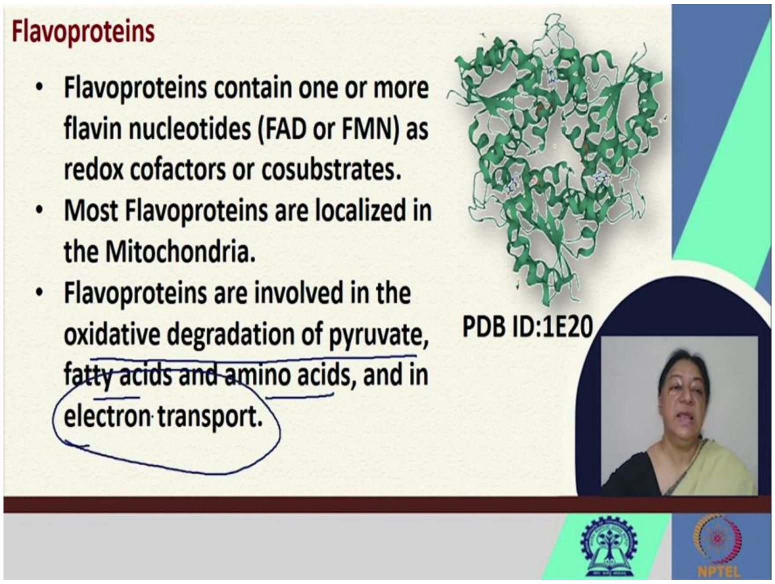


When we look at flavoproteins in general, they contain one or more flavin nucleotides FAD or FMN, as redox factors or cosubstrates. They are localized in the mitochondria for their specific activity and they are involved in the oxidative degradation of pyruvate fatty acids, amino acids and most importantly in electron transport.

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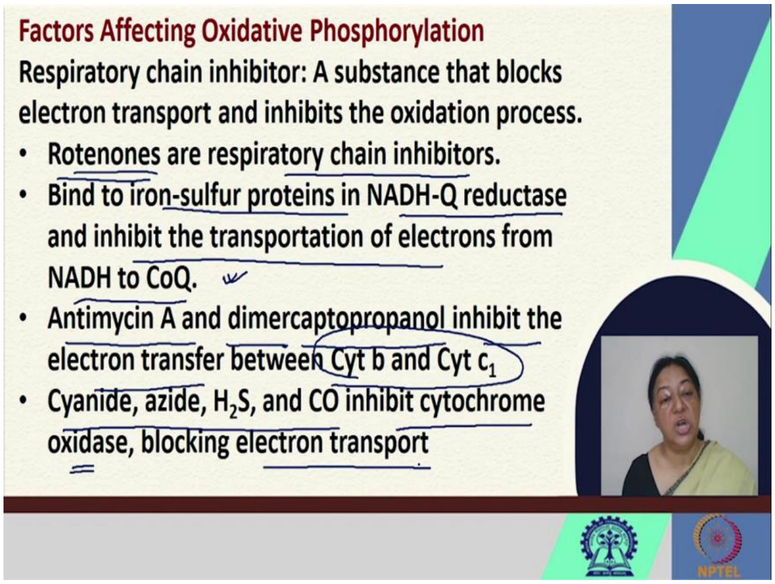


If we look at the specific factors that affect oxidative phosphorylation, we have respiratory chain inhibitors. A substance that can block the electron transport; in event inhibit the oxidation process. Rotenones are known as respiratory chain inhibitors. They can bind to the iron-sulfur proteins in NADH-Q reductase and inhibit the transportation of electrons from NADH to CoQ in complex I.

Antimycin A and dimercaptopropanol can inhibit the electron transfer between cytochrome b and cytochrome $c_1$. So, this will in turn infect complex III and complex IV and then, we can also have other small molecules that inhibit cytochrome oxidase, thus blocking electron transport.

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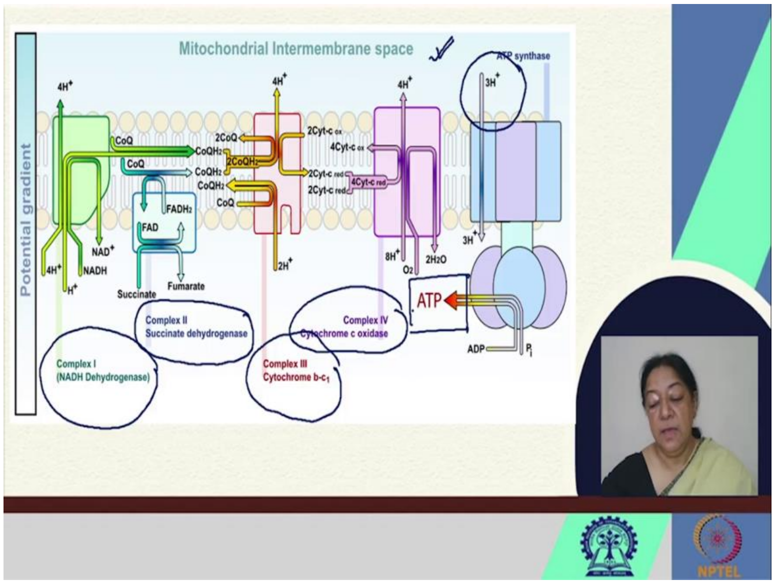


In the whole scheme of things that we have seen, we look at a specific potential gradient, complex I involving the NADH dehydrogenase, complex II the succinate dehydrogenase, complex III the cytochrome $bc_1$ and complex IV the cytochrome c oxidase. All this occurrence leads to proton pumping. This proton pumped into the inter membrane space of the mitochondria, is then used up by the proton pump in ATP synthase that results in our production of ATP.

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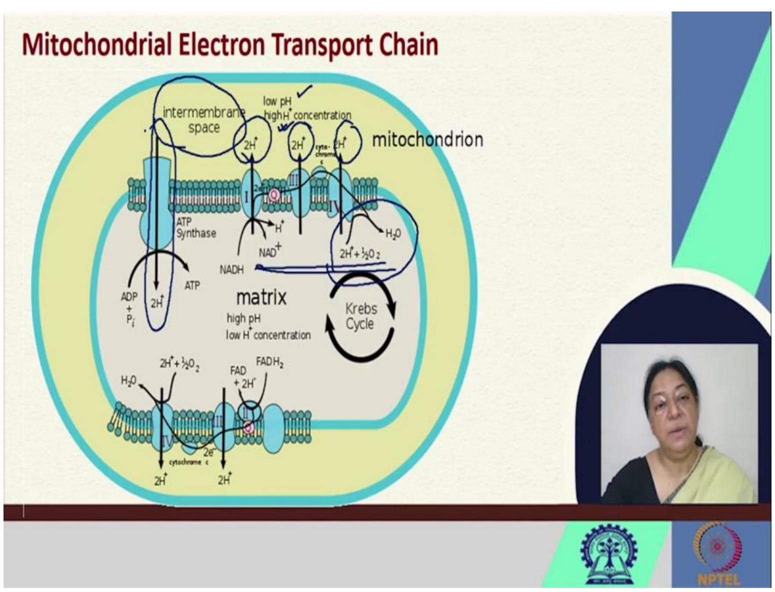

If we go to the grand scheme of things in the mitochondria, we see that we have the inter membrane space here [refer to slide] where we have a low pH, a high $H^+$ concentration. Nevertheless, this electron transport chain that results in the final reaction forming $H_2O$ from water, pumps these protons into the inter membrane space, that is then utilized by our ATP synthase in the production of ATP.

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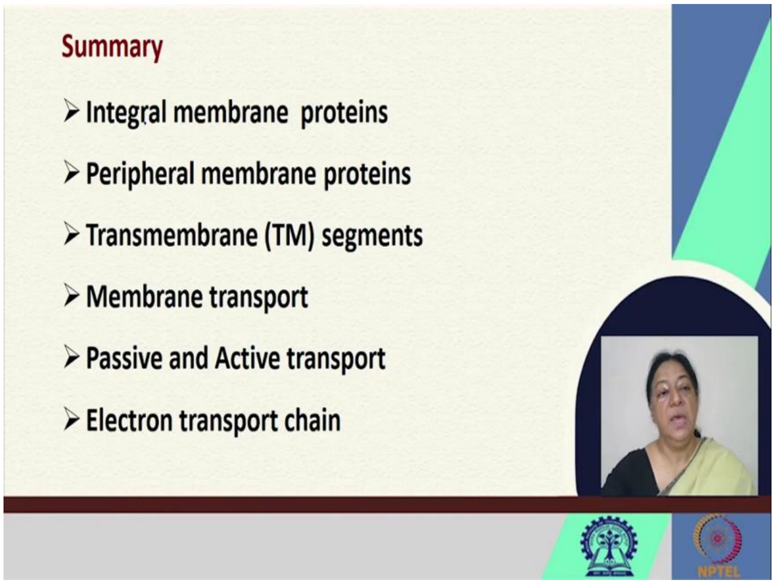


So, we looked at a series of membrane bound proteins. In our module that dealt with membrane proteins, we looked at integral membrane proteins, peripheral membrane proteins, what we meant by transmembrane segments, membrane transport, passive and active transport and finally,the electron transport chain. This is an extremely important topic for membrane proteins, for protein chemistry in general because it involves the very important process of oxidative phosphorylation.

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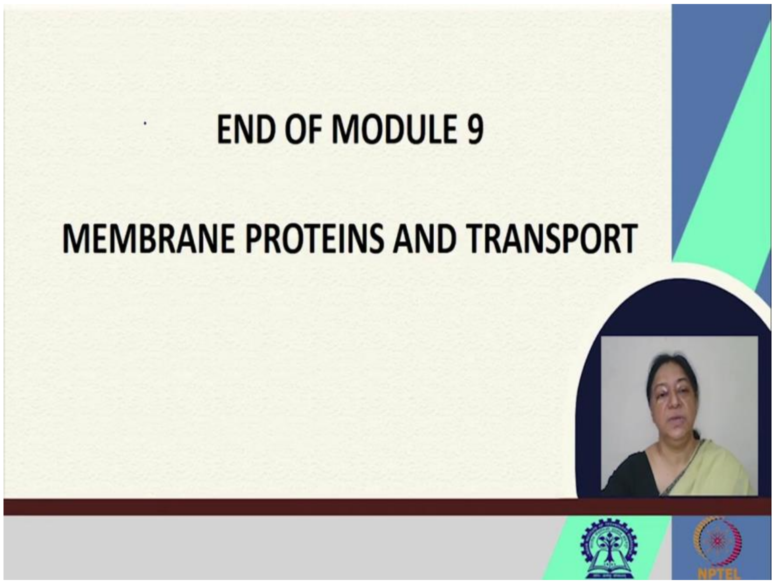


This is the end of module 9, membrane proteins and transport.

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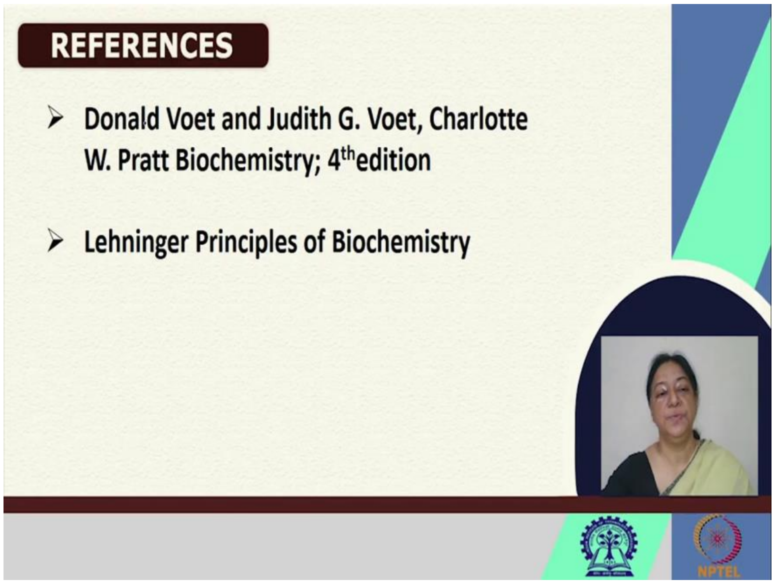


These [refer to slide] are the books.

Thank you.