Biochemistry -I
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Lecture #24
Bioenergetics – II

We continue our discussion on biochemical energetics. What we learnt last lecture that we need the process of oxidative phosphorylation to create ATP. Now we are going to see how that process actually works in the inner mitochondrial membrane. Basically what we have in an aerobic process or an oxygen requiring process is this energy is harnessed by this oxidative phosphorylation.

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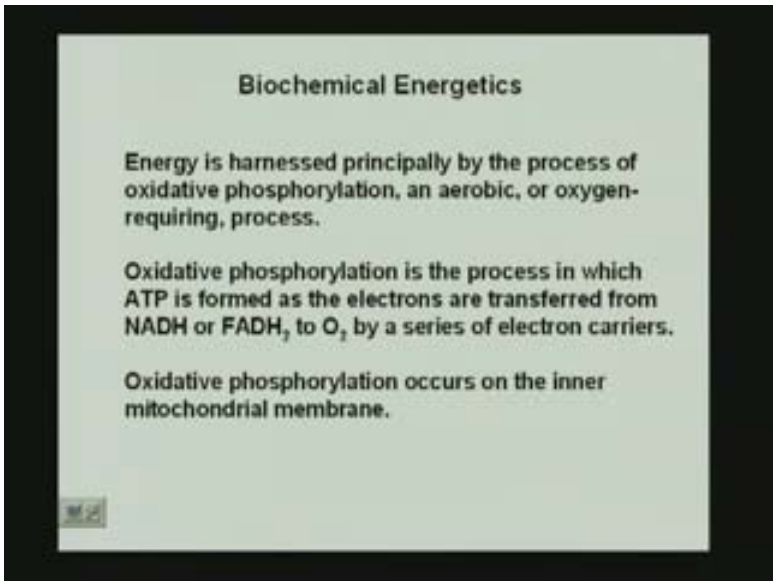

What happens in oxidative phosphorylation is the formation of ATP? Ultimately there is going to be an electron transfer system that is actually comprised of a number of electronic systems they are termed as different complexes we have complex 1, complex 2, complex 3 and complex 4. Each of these complexes are highlighted by a set of proteins and apart from the proteins there are special cofactors and prosthetic groups that are extremely essential for the process to occur. Now what we are going to see is how this complex actually helps in the transfer of the protons from the inside that is the matrix side to the intermembrane space and then we will see the action of what is called ATP synthase in the production of ATP. What we are going to look at is the oxidative phosphorylation the process by which the ATP is formed as the electrons are transferred from NADH or $FADH_2$ to oxygen by a series of electron carriers. This actually occurs in the inner mitochondrial membrane.

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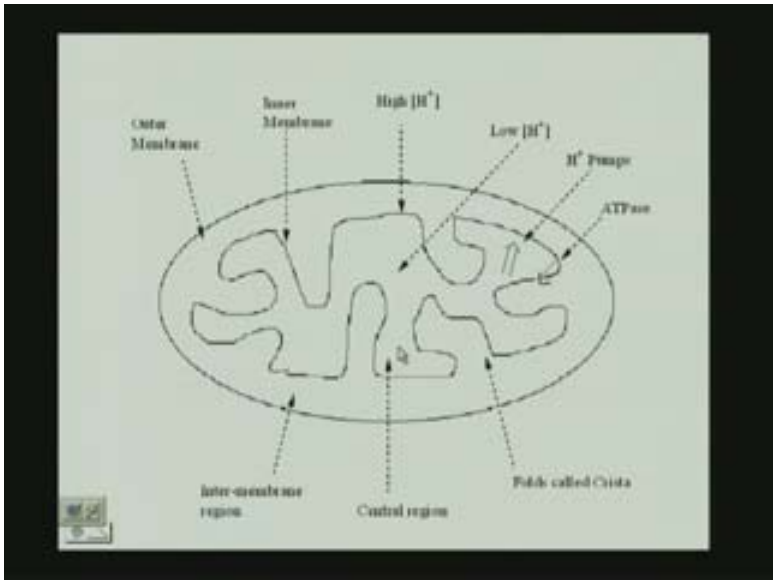

We looked at this picture last time where we found out that even though there is a high proton concentration in the intermembrane space we still need to pump protons from the inside that is a matrix side to the intermembrane space so that ATPase which we will see later can actually produce the ATP that is required for the functioning of all things in the body. The purpose of oxidative phosphorylation is to use the energy to make ATP. The way this is accomplished is in two steps. First the energy is conserved as a proton gradient across the inner mitochondrial membrane. There is this proton gradient that creates a proton motive force which we talked about the free energy last time as to how there is a membrane potential and there is also a pH gradient from the inside to outside because we have the proton gradient.

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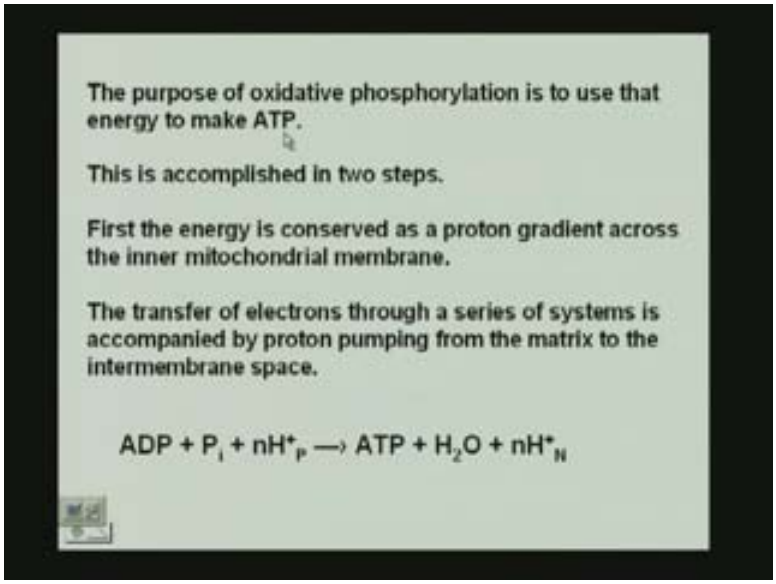

The proton gradient gives you different hydrogen ion concentration. Because you have different hydrogen ion concentrations on either side of the inner mitochondrial membrane what you have is you have different pH values. We have a free energy contribution due to the ΔpH and we have a free energy contribution due to the membrane protection because we have the negative and positive sides of the membrane.

What we have is we have the transfer of electrons through a series of systems that is accompanied by the proton pumping from the matrix to the intermembrane space. Essentially what happens is we have ADP + P_i go from, and where is this occurring? This is occurring in the intermembrane. This is the intermembrane space (Refer Slide Time: 4:27) and this is the matrix space. The matrix is the N part and the intermembrane space is referred to as the P part. So this P refers to the intermembrane space and N is the matrix space where the ATP is going to be produced.

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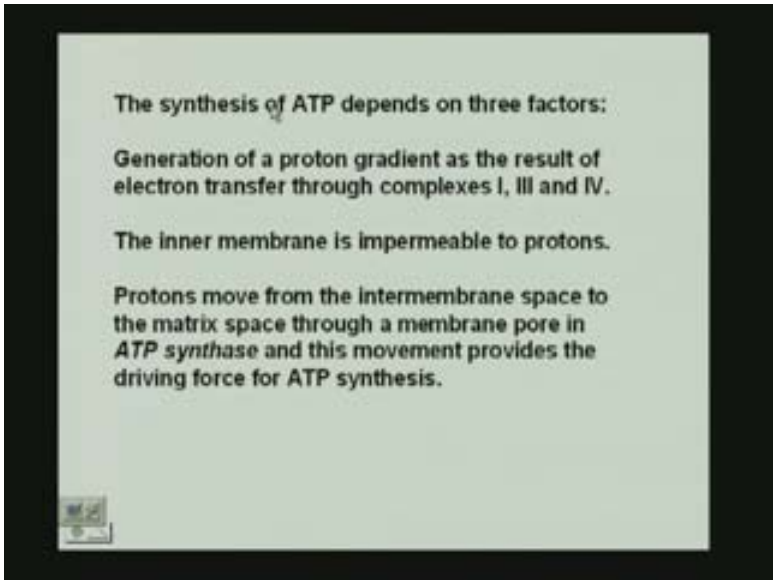

Now we have the synthesis of this ATP that depends actually on three factors. There is the generation of a proton gradient as the result of the electron transfer through complexes 1, 3 and 4. We will see exactly what these complexes are comprised of and what they mean? They are essentially enzymes along with other cofactors and prosthetic groups that permit the transfer of the protons which eventually is going to lead to ATP synthesis.

Another thing we have to take into consideration is that the inner membrane is actually impermeable to protons which means that the higher concentration present in the intermembrane space does not diffuse into the matrix space of the mitochondria. So the fact that the inner membrane of mitochondria is impermeable to protons because you remember there is a higher concentration already in the intermembrane space. Therefore you would expect that the protons would actually be able to diffuse through into the matrix but this does not happen. They do not come into the mitochondrial matrix simply because the inner membrane of the mitochondria is impermeable to this proton. So what you have to do is to maintain the high concentration for the production of ATP you have to pump the protons from the matrix to the intermembrane space.

The protons actually move from the intermembrane space to the matrix space through a membrane pore. Because the membrane itself is impermeable to protons you have to have a membrane pore. Now what do we mean by a membrane pore? Remember, when we studied membranes, we had the lipid bilayer. In the lipid bilayer which is actually going to be a part of the inner mitochondrial membrane as well we are going to have embedded certain integral proteins. Integral proteins are one protein that was embedded in a lipid bilayer. So what we need is for the channeling of the protons is we need a pore in the membrane that is going to allow the protons to pass through.

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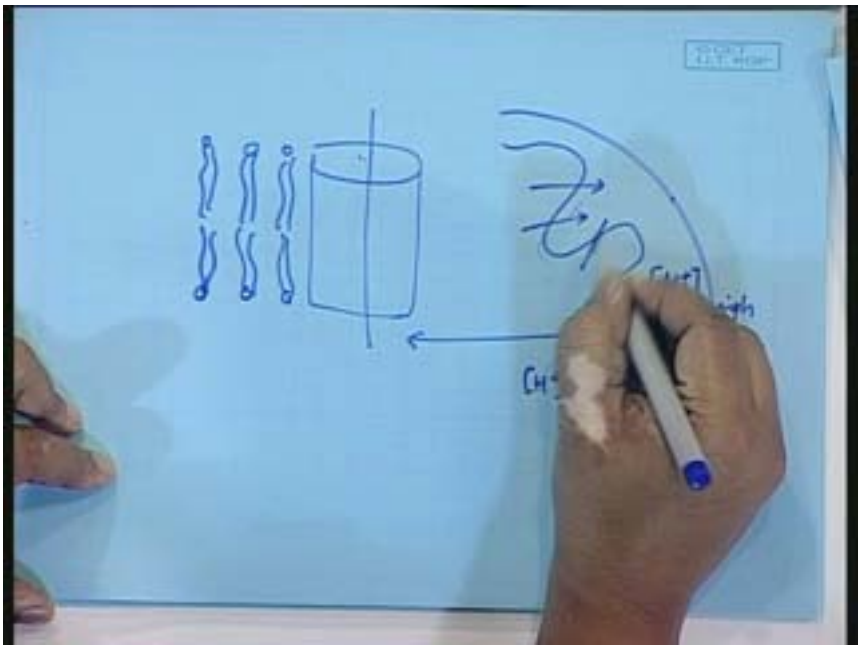

Because if we look at the structure of the inner layer of the mitochondria which is something like this, if this is the mitochondria, if this the outer membrane and this is the inner membrane where actually one region would blow up to look as something like this where we would have the lipid bilayer and we have a high H^+ concentration on this side which is high, on the inside we have a H^+ concentration which is low so ideally you would expect just the protons to diffuse through. But that does not happen because this inner membrane is impermeable to protons. Hence what you have to have is, you have to have a certain pore that is going to actually pump, we look at these pumping systems (Refer Slide Time: 7:45) the pumping complexes that are going to make the protons from the inside go to the intermembrane space and then they come back through the pore with the help of what is called ATP synthase, ATPase and by doing that they produce ATP, so that is the whole procedure.

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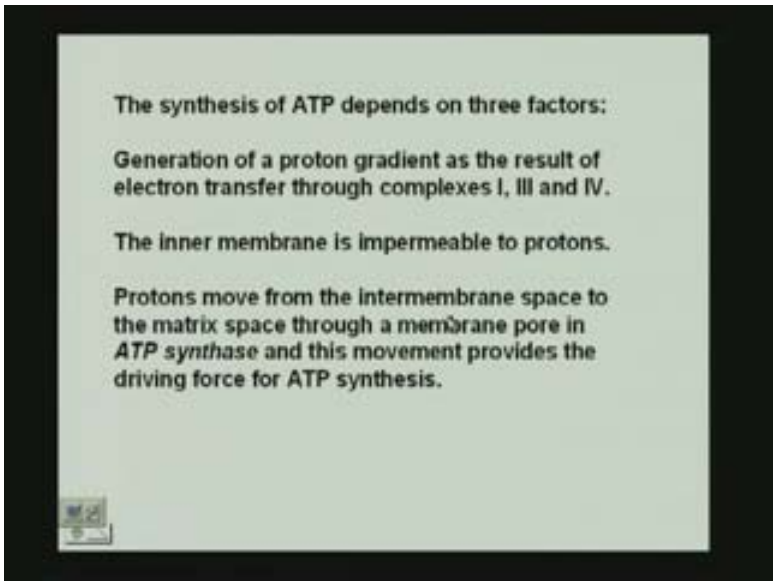

Therefore what happens is the protons move from the intermembrane space to the matrix space that is from the inter area between the 2 membranes to the matrix space through a membrane pore in ATP synthase and this movement provides the driving force for ATP synthesis. Thus basically what we have is we have the mitochondria, and in the mitochondria we have a series of complexes which we are going to look at in a moment and we have the inner membrane impermeable to protons. So essentially since we need the protons for the ATP synthesis they have to be pumped from the inside of the matrix the matrix of the mitochondria to the intermembrane space and then this movement is possible only through the pore that is present in the protein ATP synthase and this will provide for the driving force for ATP synthesis. We will look at the structure of the ATP synthase also.

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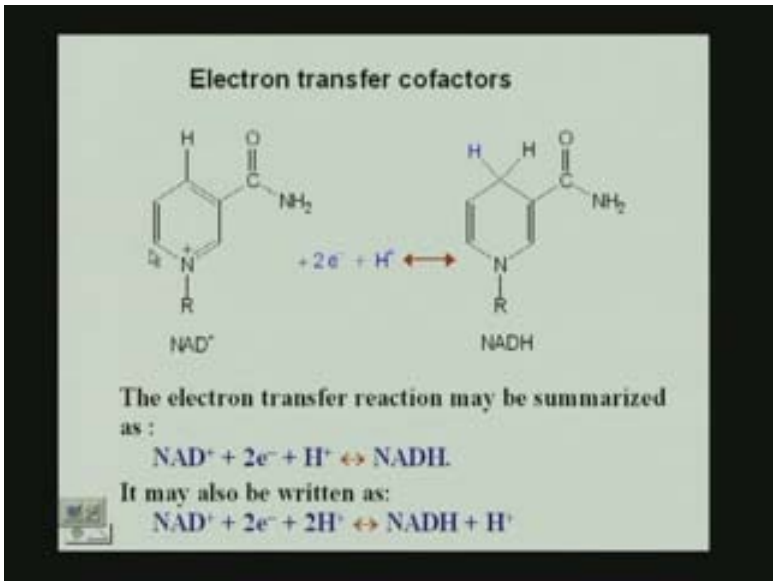

Now before we get into the complexes 1, 3 and 4 and see what they actually comprise of as I said they are actually made up of a bunch of proteins as well as other cofactors, enzymes and prosthetic groups. But before we do anything we have to consider the other electron transfer cofactors because we are speaking about electron transfer and proton transfer we have to have intermediate electron carriers that are going to assist in the proton and electron transfers. we have looked at the two of these already NAD^+ going to NADH where we have a certain electron transfer reaction that can be written as NAD+ plus two electrons plus a proton going to NADH or it can alternatively be written as $NAD^+ + 2e^- + 2\ H^+ \rightarrow NADH + H^+$.

We have our basic structure of NAD^+ which is made up of nothing but nicotinamide or the niacin that we found out from, where we got this from? We got it from the vitamins that we actually intake as supplements in our diet. We had NAD^+ and we know that this H adds on to this particular carbon atom so we have NADH.

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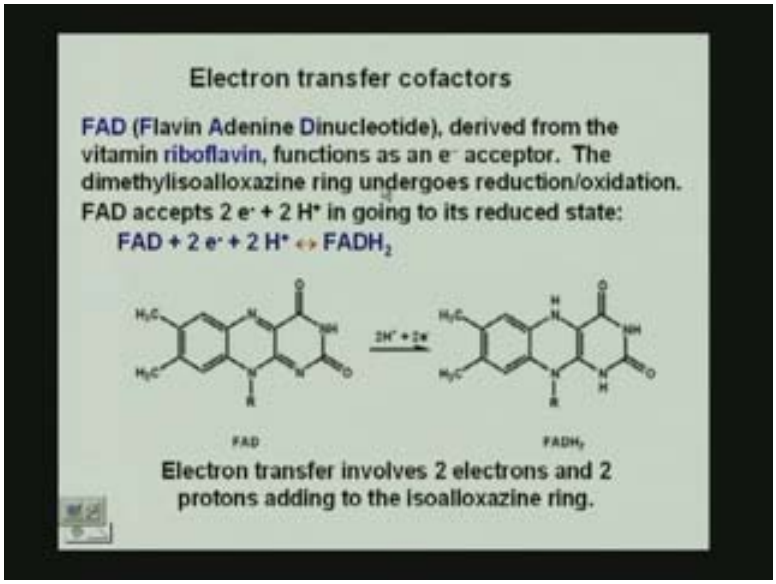

The next one that we looked at was Flavin Adenine Dinucleotide FAD that goes to $FADH_2$ where we have 2 protons add to the 2 nitrogens of this isoalloxazine ring. This is the Flavin part, we have an Adenine Dinucleotide part, Flavin Adenine Dinucleotide that is derived from the vitamin riboflavin and this also functions as an electron acceptor. Because when we are going to consider electrons and protons movements we have to consider what the factors are, what are the cofactors or prosthetic groups are actually going to be combined with the enzymes in taking up the protons or providing them in processing the protons. So we have in this electron transfer process for FAD going to $FADH_2$. These are the two nitrogens that are going to take up the two hydrogens in becoming $FADH_2$.

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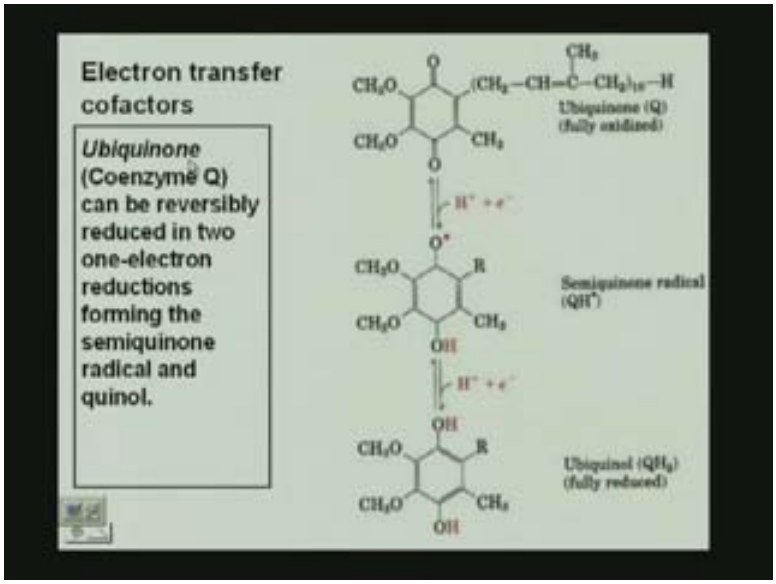

Another electron transfer cofactor that is extremely important is Ubiquinone.
Ubiquinone is also known as coenzyme Q. The structure that is written on the top is the total ubiquinone that is also known as coenzyme Q or referred to as just Q. What happens in this case is you have quinonemoiety as you can see here the rest of the structure is as in the other cases is referred to as R because this is the part which is going to take up the electrons or protons and from the quinone it becomes the semi-quinone radical and after that it becomes ubiquinol which is the fully reduced form of the quinone.

So the fully oxidized form is when it has double bonded O on both sides. If you have a radical O* and OH you have the semi-quinone radical which is H^+ + e to the ubiquinone. You have the semi-quinone and the ubiquinone. The use of coenzyme Q is its efficiency in being able to accept one electron one proton at a time. In the other cases you may need two electrons. For example; NAD^+ going to NADH if we go back and just look at the reaction (Refer Slide time: 13:29) you need two electrons for the reaction to go to NADH. But in this case here again for the FAD going to $FADH_2$ you again need two electrons for the reaction to go from FAD to $FADH_2$ so there is no intermediate as such. But in coenzyme Q this intermediate semi-quinone is possible and because of this semi-quinone radical you realize that it is possible for it to pick up a single electron and a single proton which makes it more versatile in its use.

We have the Ubiquinone go to the semi-quinone which subsequently goes to the ubiquinol because of its up take of two electrons in two steps, this is important. It is not like the other cases where two electrons are taken up in a single step. We have two electrons taken up in this case but in two different steps so it is useful when the reactions are actually going to be taking up the protons and the electrons for the transfer of the protons from the matrix space to the intermembrane space.

So we have the coenzyme Q or the ubiquinone which is actually coenzyme Q or Q as I said, this can be reversibly reduced in two one electron reductions forming the semi quinone radical and finally the quinol so that is where its importance lies.

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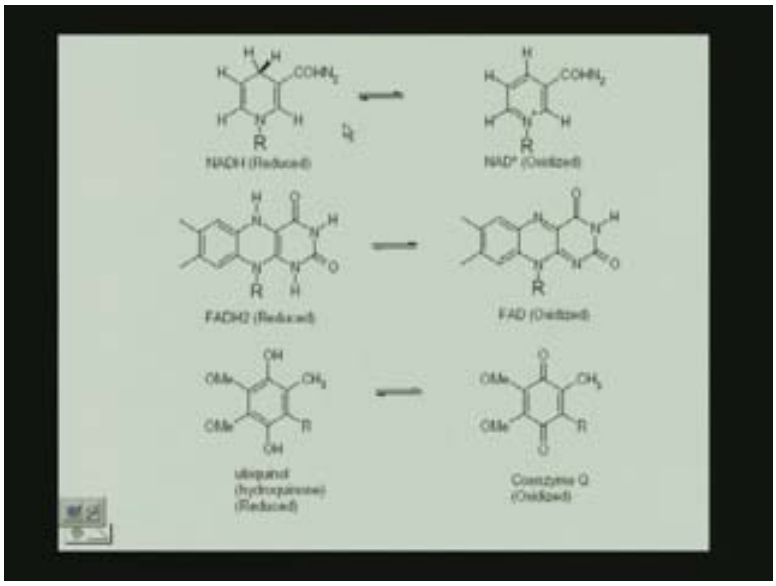

Let us summarize what we have. We have the reduced forms of all the electron transfer co factors here, the usual ones from NADH NAD^+ is the oxidized form, we have the $FADH_2$ the reduced form and FAD the oxidized form, we have the ubiquinol the reduced form and the ubiquinone or coenzyme Q as the oxidized form. In each case you have to know where and at which point the hydrogens are added. You also know now that the use of coenzyme Q is more versatile because it has two one electron steps that can form intermediate semi quinone. Hence these are the 3 electrons transfer cofactors that are going to be utilized in our complexes 1, 3 or 4 of the oxidative phosphorylation steps.

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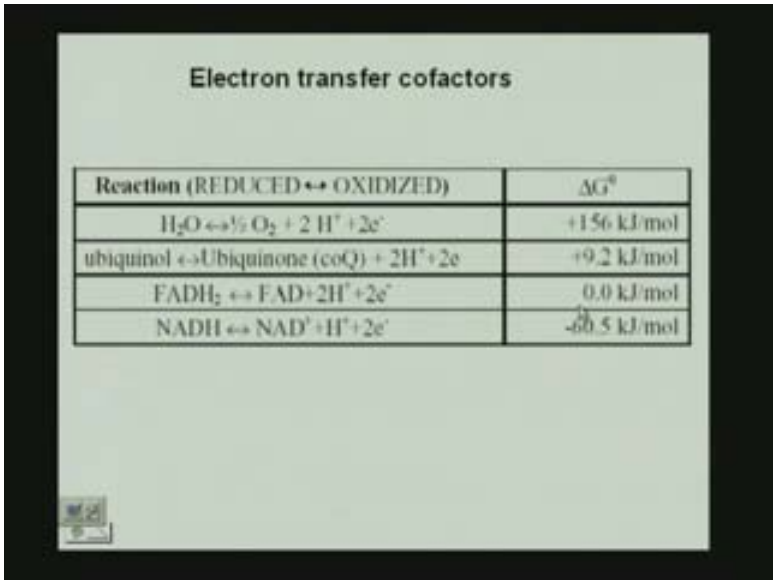

Here are some of the ΔG_0 values associated with the electron transfer cofactors. When we go from ubiquinol to ubiquinone, by quinol to quinine I mean two protons and two electrons. I have a +92 kJ mol^{-1} in free energy change. For H_2O that is the reduced part going to ½ O_2 + $2H^+$ + 2e the value of $\Delta G = +156$ $kJmol^{-1}$. If I want to reduce water to oxygen I would need this amount of free energy change. $FADH_2$ to FAD + $2H^+$ +2e is 0.0 kJ mol^{-1}. ΔG value for the NADH → NAD + $2H^+$ +2e is -60.5 kJ mol^{-1}. We will see how actually in the energetics all these combine to give you favorable energy once you complete the metabolism of carbohydrates.

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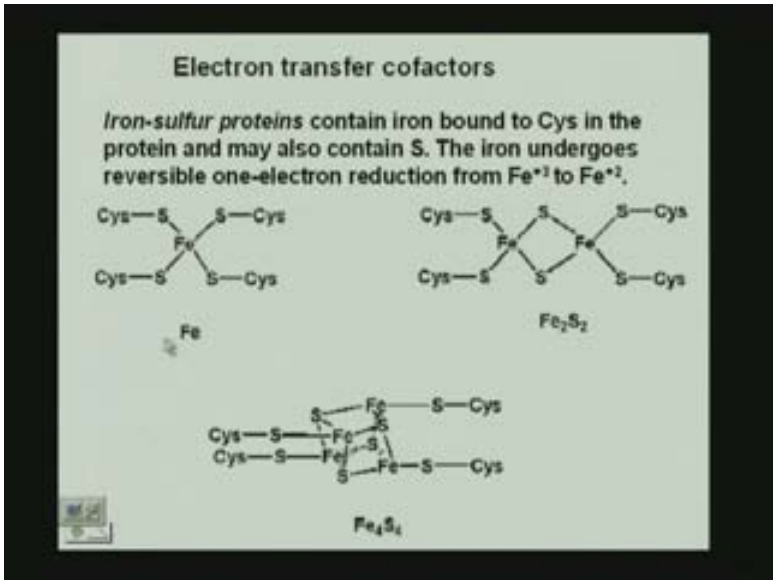

Now we also have certain proteins that can act as electron transfer co factors. These are mainly iron sulfur proteins. They are just written as FeS proteins. They contain an iron bound to cystine in the protein and they may also contain additional sulfur. For example; if we look at this Fe, we have the Fe coordinated to cystine residues in the protein into forming what are known as iron sulfur proteins that also play a major part in electron transfer and they can do that because you can have a multiple oxidation state for the iron, it can be on the Fe^{+2} and Fe^{+3} states so what does that help me? It helps me in taking up an electron or giving an electron. Because eventually on looking at electron transfer I need certain moieties in the system that will be capable of electron reduction like one electron reduction, two electron reduction or whatever.

We have certain proteins that are going to be associated with this. These are the iron sulfur proteins that contain iron bound to cystine in the protein and may also contain sulfur. For example, the one that is shown on the right hand here is you see that 2 Fe coordinated with 2 Cys and 2 S atoms. Therefore we have two cystines associated with so we would have 2 Cys which have their S atoms associated with a Fe atom, this again associated with another Fe, this associated with the Cys so that is what we are looking at.

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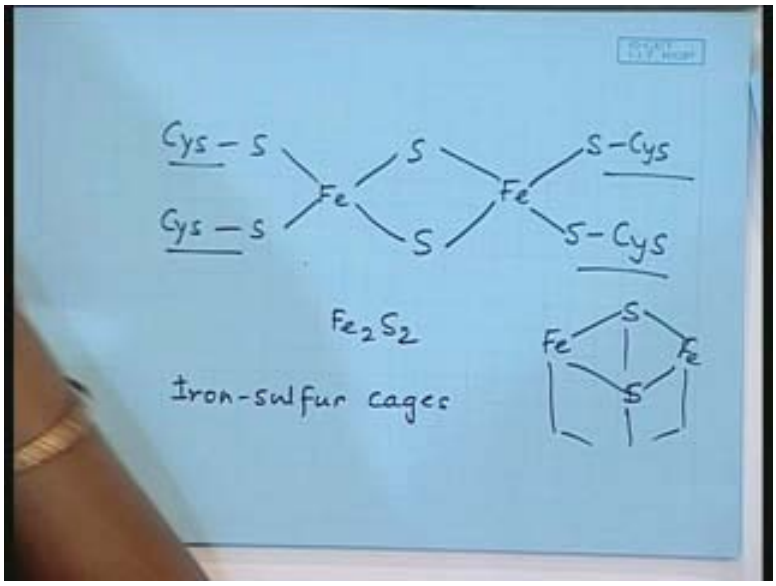

This would be basically a Fe_2S_2 system because you have an additional 2 S atoms in this case because the rest you know you know are coordinated to the cystines belonging to the protein that is part of your complex system. You can have Fe_4CysS_4 or you can also have what are called FeS cages which literally look like cages. You would have a cube like that where you would have all these connected together and then you would have something like that so they actually look like cages. You can have these FeS cages and the ones that have been drawn here are the Fe one with just the Fe coordinated to the cystines. You can have Fe_2S_2 where you have the Fe coordinated not only to Cys but also to additional sulfurs.

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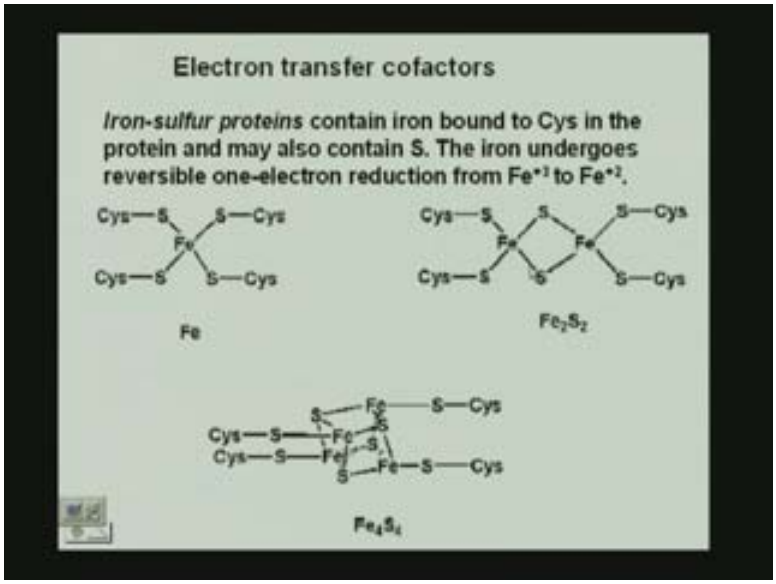

The Fe itself can undergo a reversible one electron reduction from Fe^{+3} to Fe^{+2} so you understand how this can be important when we are considering electron transfer. We have a system that can take in an electron. And once Fe^{+3} has taken an electron it will convert itself to Fe^{+2}. But we have to remember that when we are considering enzymes we need a system that is going to get it back to Fe^{+3} because it cannot just stop after one particular reaction.

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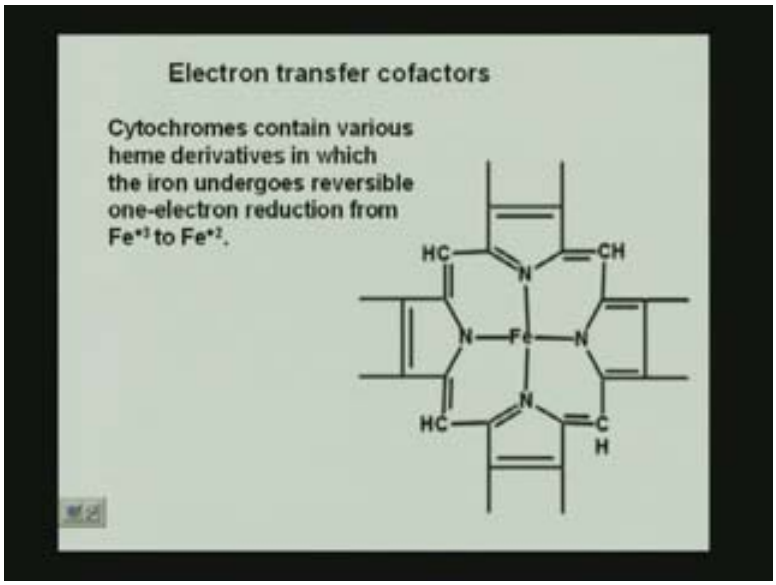

So now that we know some of the electron transfer co factors this protein cytochrome, the cytochromes contain various heme derivatives. We studied heme and we did hemoglobin

and myoglobin, this heme also coordinates an ion in the protocol fire in nine systems. Similarly, cytochromes contain various heme derivatives which actually differ in their substituents in the rings which make them different from the heme that is probably in the hemoglobin or myoglobin. So there are different substituents in the heme that make different cytochromes also. You have cytochrome A, cytochrome B and cytochrome C but the difference lies in the fact that you have different substituents in the heme. And there is one cytochrome that even has 2 hemes associated with it.

Now in this case the Fe is coordinated to the nitrogen of the pyrrole rings. The coordination here also allows for a one electron reduction. Because you have to remember when you are considering these electron transfer steps we are interested in finding systems that are going to take or provide these electrons because eventually we have to transfer protons and electrons. If we have taken H^+ an electron associated with it that is going to balance the charge and we have to have something that is going to take-up this electron. So when we are pumping in protons it is not just sufficient to put in the H^+. We have associated with the H^+ the electrons. Therefore we have to have systems that are going to allow or take up these electrons and then provide a balance in the whole system of events.

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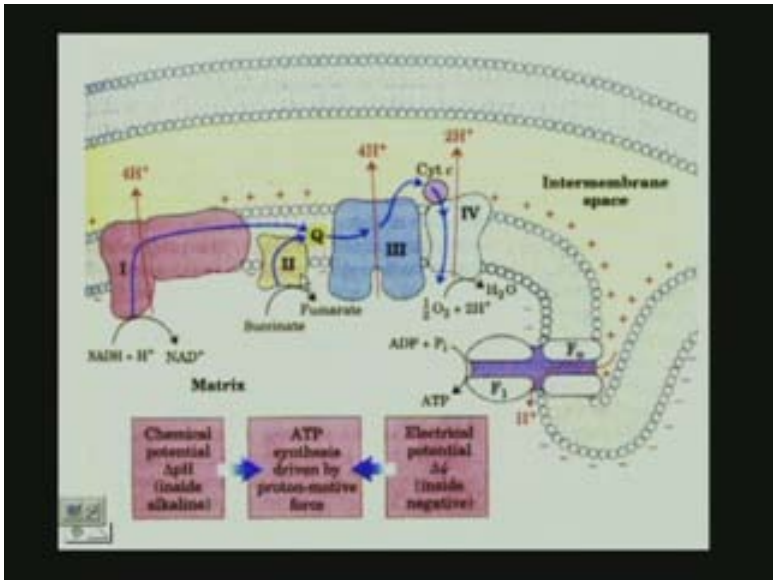

This is what we have in our system. We have this as the outer mitochondrial membrane. The circles that you see there what are those circles? Those are the polar head groups of the lipid bilayer. This is the inner mitochondrial membrane. Here we have our polar head groups, dangling here we would have lipid bilayers. These are the lipid chains. Similarly, here also we have our fatty acid chains hanging from our polar head group. What is this space here, this is intermembrane space, here is the matrix of the mitochondria. What you have in this matrix is, remember? What do we have? Here we have a high H^+ concentration and inside here we have low H^+ concentration. What we are doing? We want to pump H^+ to the other side to make ATP. This is the system the pore, remember I

mentioned the pore in the mitochondrial membrane that is eventually going to provide the H^+ or transfer the H^+ into this matrix so that we can produce ATP.

We are going to look at this system in a bit detail. Here we have certain complexes. Now each of these complexes as you can see is kind of an integral membrane protein. It is comprised of a protein and along with the protein we have the prosthetic groups or other electron transfer co-factors the once that we have been mentioning that are going to help us in the transfer of the protons from the inside to the intermembrane space.

Now we are going to look at the constituents of each of these complexes. This is the pictorial representation of how it actually looks. So what we have now is, we have in the membrane, so across the membrane what we have? We have a chemical potential generated due to the pH difference on both sides of the membrane. Why do we have a pH difference? It is because we have different concentrations of hydrogen ions on either side of the inner membrane. So the inner mitochondrial membrane has different pH values or different hydrogen ion concentrations on either side of the membrane which means that there is a ΔpH which gives a chemical potential. We also have an electrical potential due to the charge difference at both ends inside and inside meaning the matrix and the intermembrane space.

Now, if we look at these both actually are inside, both of these are actually inside the mitochondria but this is the intermembrane space and this is the matrix the cytosome. Then based on the chemical potential and the electrical potential we have ATP synthesis driven by the proton motive force. Now what we are going to look at is we are going to look at each of these complexes and see what it actually does. There are certain reactions associated with each of these complexes eventually giving you sufficient H^+ concentration in the intermembrane space that is going to result in ATP synthesis.

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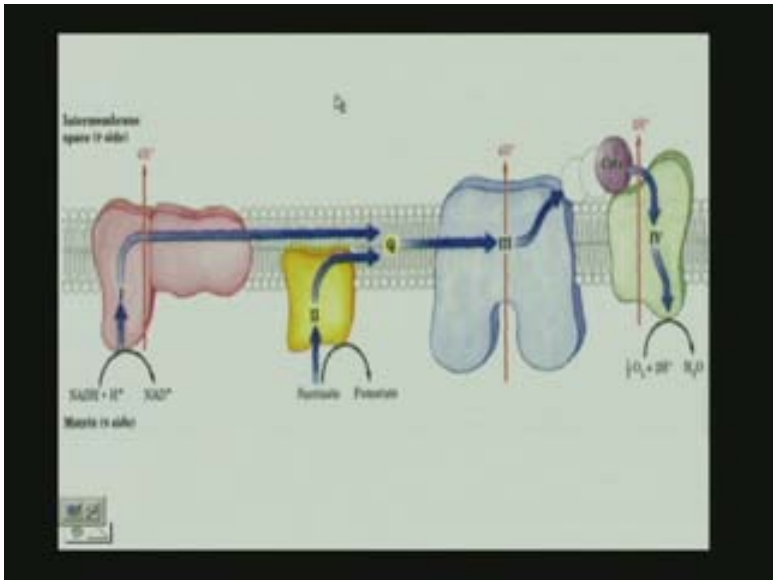

Let us go back to our slides. This is basically a pictorial representation without all the paraphernalia of the intermembrane space or the whole mitochondria. We have complex 1, complex 2, complex 3 and complex 4. Two actually is not always mentioned because it does not involve proton transfer. We will see that in a moment.

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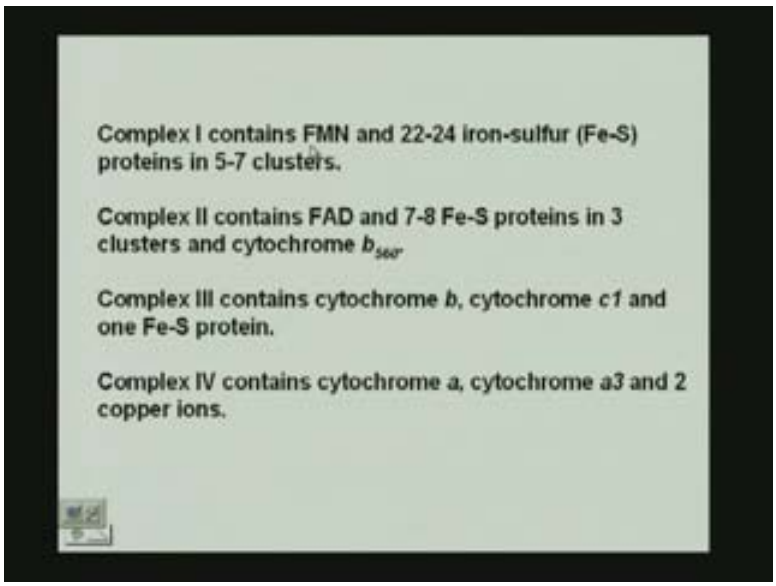

Complex 1 contains FMN. What is FMN? FMN is Flavin Mono Nucleotide and it has 22 to 24 Fe S proteins present in a cluster so that is what complex 1 is. And apart from that we are going to see what else it contains. These are the electron transfer co-factors present in complex 1. So complex 1 has FMN and Fe S. Complex 2 has FAD that is

Flavin Adenine Dinucleotide and Fe S and cytochrome B. Complex 3 has cytochrome B, cytochrome C_1 and Fe S protein. Complex 4 has 2 cytochromes and 2 copper ions. Why do we need copper (Cu) again? It would be the same thing. The copper also has multiple valency so it can take up electrons. Therefore what do we have here? We have these electron transfer co-factors associated with complex 1, 2, 3 and 4 that are going to assist in the electron transfer.

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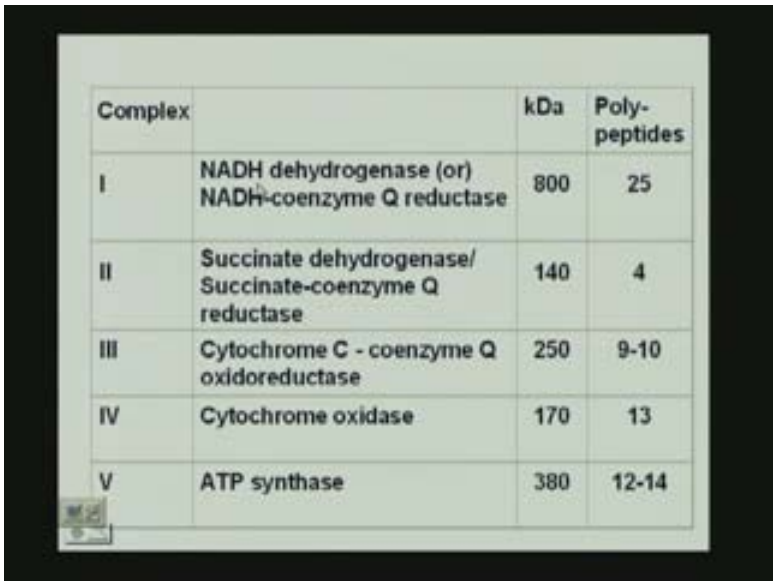

Now, the enzymes involved; apart from the co-factors we now have to look at the enzymes involved. For complex 1 which is associated with FMN + FeS, what are tthese that I mentioned here? These are the electron transfer co-factors they are not the enzymes. They are the co-factors that are going to assist the enzymes into performing its function.

Now we have to look at the enzymes. The enzymes are NADH dehydrogenase. As soon as we see NADH dehydrogenase we know what the enzymes are actually going to do. Complex 2 has succinate dehydrogenase. Again from the name of the enzyme we can say what that is going to do. Complex 3 is cytochrome C or it is coenzyme Q oxidoreductase. Complex 4 is cytochrome oxidase and complex 5 is usually not mentioned but that is the ATP synthase that is the last part in this step that is going to actually produce the ATP. It is sometimes referred as complex 5. But these are the complexes 4 that are actually going to take place or take part in the electron or rather proton transfer and that is going to result in oxidative phosphorylation where finally oxygen is going to be required or with the proton motive force that is going to give us ATP.

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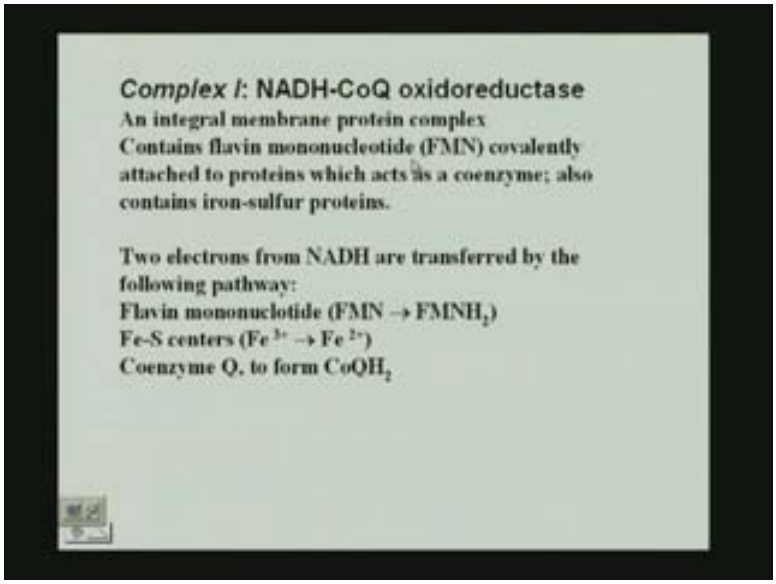

Complex 1: Complex 1 has is the integral membrane protein complex that contains FMN that is covalently attached to the proteins which acts as a coenzyme and it also contains the Fe S proteins. Therefore what does complex 1 have? It has the NADH dehydrogenase or CoQ oxidoreductase and apart from that it has FMN and FeS clusters. That is what comprises complex 1. Now what happens in complex 1? There are 2 electrons from NADH that are transferred through Flavin mononucleotide, the Fe centers and coenzyme Q.

Now we have, this is complex 1. These are the cofactors that actually takes part in the reaction because you have to remember that the enzyme actually gets back to where it was. It is the cofactors that actually finally have to get back to where they started from. So what we are looking at here is we are looking at NADH + H^+ going to NAD^+.

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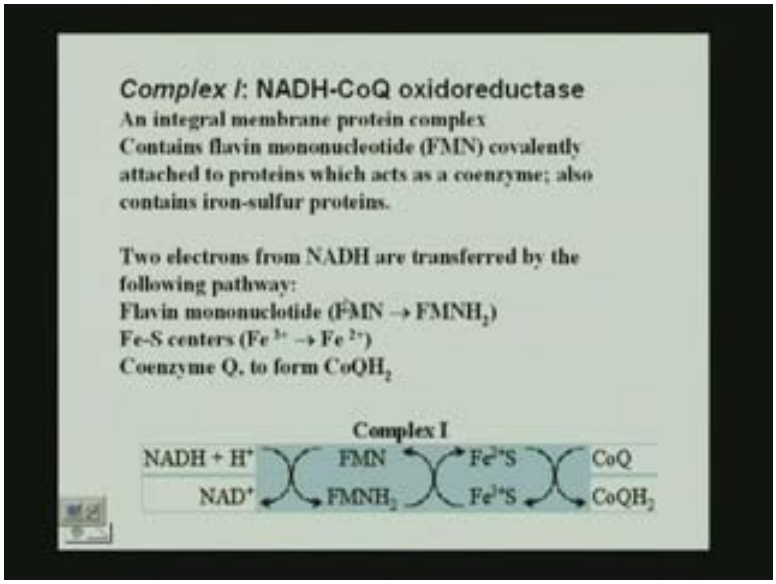

What is happening here then? There has to be some co-factor that is going to take up the hydrogens FMN takes that up. Now in the processes of taking up the hydrogens then Fe^{+3} has to go to Fe^{+2} because the H^+ is going to be associated with an electron. If you are going to have H_2 where H_2 is $\rightarrow$ 2 H^+ + 2e so when you have the 2H atoms taken up the 2 electrons are taken up by Fe^{+3} then what happens to the Fe^{+3} it forms Fe^{+2}.

What did we learn about coenzyme Q? Coenzyme Q (CoQ) can take up these 2 electrons and form $CoQH_2$ which is the quinol form. We have basically an overall reaction. This is actually what you need to know NADH + 5 H^+ from the matrix side + Q forming NAD^+ + QH_2 + 4 H^+ in the intermembrane space. This is our final reaction for complex 1. Complex 1 has this whole enzyme that is oxygen. All of these enzymes are going to be dehydrogenases or oxidoreductase because all of them are involved in redox reactions. Since all of them are involved in redox reactions they have to be dehydrogenases, oxidases, oxidoreductases or whatever. This is what complex 1 is. We have one reaction that has now transferred some protons.

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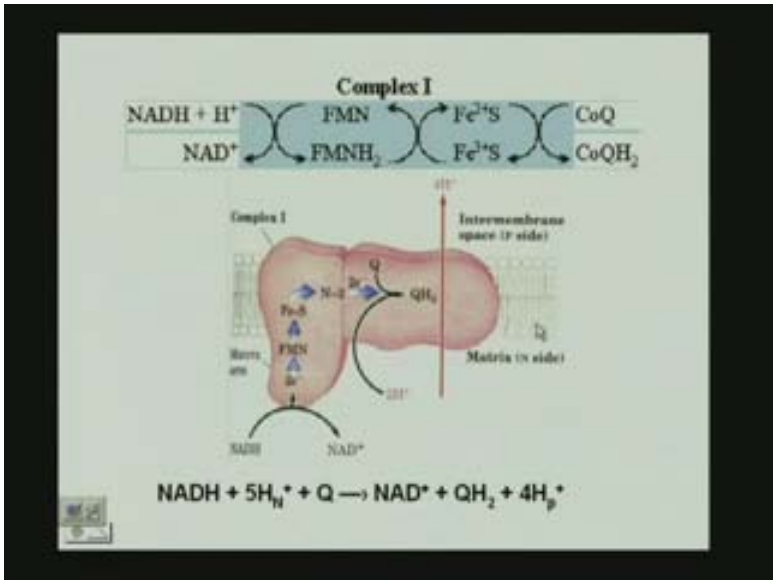

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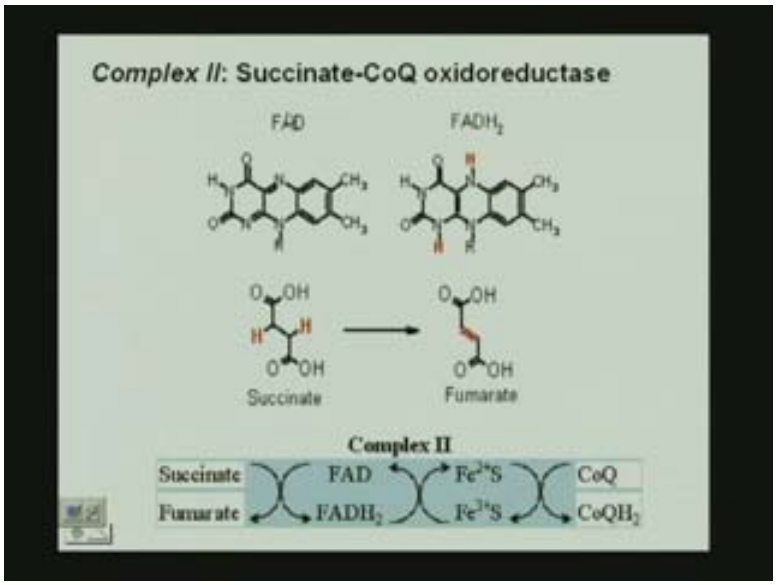

Complex II: Complex II does not transfer any protons. This is one example I think I mentioned when we did FAD and $FADH_2$. In the use of FAD and $FADH_2$ succinate goes to fumarate. So we have a double bond formation here in succinate either dehydrogenase because you are removing the hydrogens from succinate or it is also known as succinate CoQ oxidoreductase. This enzyme can also be mentioned as succinate dehydrogenase because it is taking up the hydrogens from succinate and forming fumarate. This is actually complex II but there are no protons transferred.

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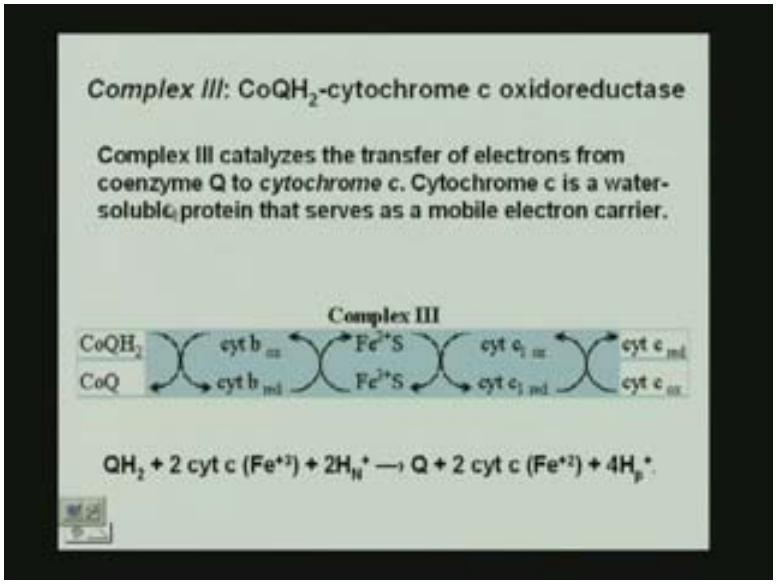

In complex III the use of cytochromes comes into the picture. What are cytochromes? Cytochromes are proteins that have heme components to them. These hemes have coordinated iron and the coordinated iron has multiple valency of Fe. It can take up the electrons. You have to remember that in each of these processes what we are actually doing is transferring protons and electrons.

Our ultimate aim is to get protons to the intermembrane space. Once we get these protons to the intermembrane space, we know that ATP can be synthesized. That is our ultimate aim. Hence these are the different complexes involved that are actually going to help or bring about that process. Now here we have complex III that catalyzes the transfer of electrons from coenzyme Q to cytochrome C.

If you remember in the first complexes what happened? Q formed QH_2. I have the reactions altogether later on. Coenzyme Q actually formed CoQH 2 so this Q formed QH_2 now what has to happen is the QH_2 has to get back to Q otherwise you cannot have another NADH go to NAD^+. So what we have to do is we have to have another system with the cytochromes in this case that is going to take QH_2 with the help of cytochrome C with $2H^+$ from the matrix side that's the N side form Q. The two cytochromes was Fe^{+3} it is going to form Fe^{+2} and we have $4H^+$ in the intermembrane space. What have I done in this complex 3? I have reduced cytochrome C and oxidized Q. Also, I have transferred in effect to protons to the intermembrane space. So this is the overall reaction of complex 3. What you need to remember now is which of the complexes actually help in the proton transfer? The overall reactions of each of the complexes are what you have to remember. We know that the QH_2 + 2 cytochrome C in this set is going to give Q + 2 cytochrome C reduced + 4 H_p. What is $4H_p$? So $4H_p$+ means 4 protons in the intermembrane space.

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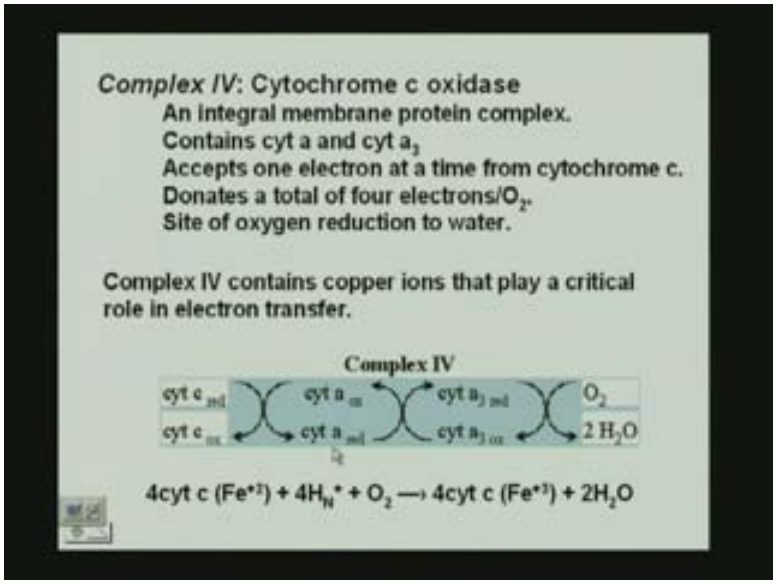

Then we have complex 4 that is actually again cytochrome C. In this case what happens? A total of 4 electrons go to water and this is the site of the oxygen reduction to water. Here we also have 1 electron system also here in the form of cytochromes. The cytochromes have 1 Fe in them in the heme, they can also take up 1 electron at a time like which system ubiquinone. So ubiquinone will form a semi-quinone then form a quinol. We have 1 electron at a time to cytochrome C. We have 4 electrons given to oxygen and there is one interesting thing about the proteins here that at one point there is actually an oxygen radical form and this oxygen radical can be extremely damaging to the membrane. Therefore what happens is this protein actually holds the radical extremely tightly. It has extreme high affinity for the radical so that it does not destroy the membrane. Until it is reduced to water it is held on extremely tightly.

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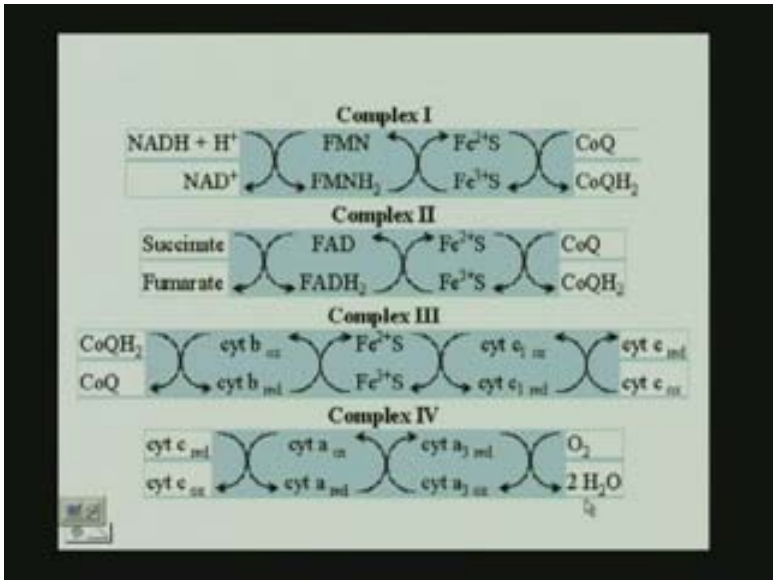

This is actually a summary of all the complexes that we just considered. We will look at the final equation in a moment that is eventually what we want to do. We have NADH + H^+ going to NAD^+ in this case CoQ forms $CoQH_2$. In complex 2 we have succinate going to fumarate which actually forms again CoQ going to $CoQH_2$. In complex 3 we have $CoQH_2$ form CoQ back again which then can again be utilized in complex I and we have cytochrome c the reduced form being oxidized. So what has to happen in complex 4 is we have to get every thing back to normal. In complex 4, the reduced form of cytochrome C is the oxidized form where it can take up again the electrons to form the reduced form. Therefore actually we have the picture of electrons being taken up protons being transferred. The electron transfer cofactors be it coenzyme Q, be it NAD+ or even FAD but they help in the transfer of these protons and electrons which is essentially what we are looking at. So we finally have the oxygen reduction to 2 H_2O.

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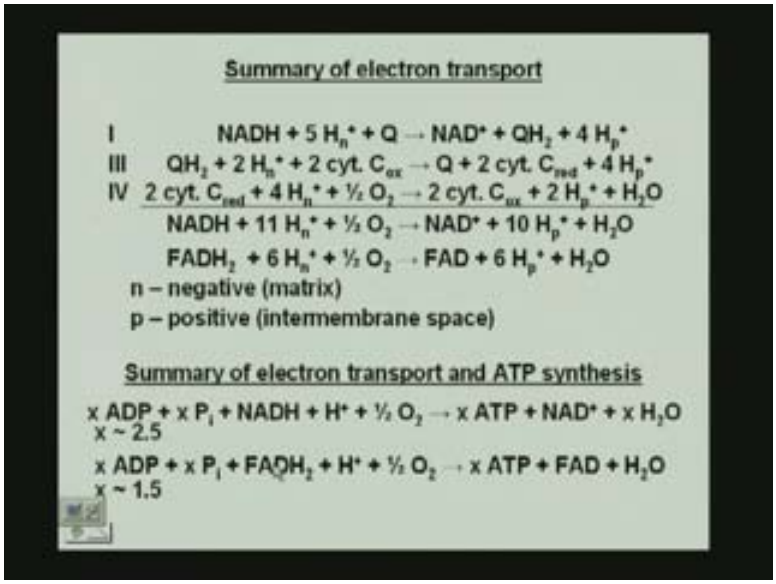

This is the summary of all the complexes that we have. We have eventually NADH + 11 Hn^+ + ½ O_2 → NAD^+ + 10 Hp^+ + H_2O and FADH2 + 6 Hn^+ + ½ O_2 → FAD + 6 Hp^+ + H_2O. If we consider actually the summary of the electron transport and ATP synthesis, we actually have xADP + x P_i + NADH + H^+ + ½ O_2 → x number of ATP in terms of NADH and NAD^+ in this system for every NADH you get 2.5 moles of ATP. For ADP and P_i for the system FADH2 to FAD you get 1.5 moles of ATP. In older books you will see that this is 3 and 2 but now the convention is that for ADP + P_i if it is an NADH, NAD system you will get a 2.5 moles of ATP and for the ADP set you will get 1.5 so many ATP's synthesized.

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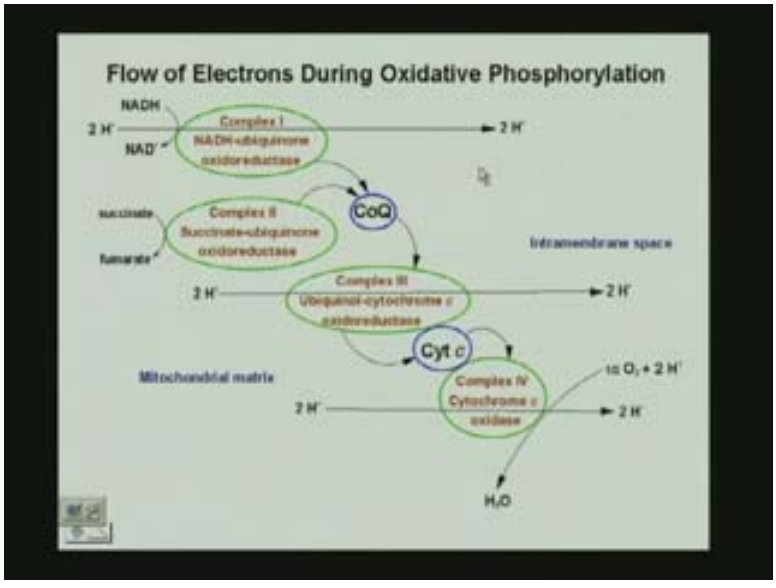

Eventually what is happening is you have mitochondrial matrix and you have the intermembrane space where you have done a transfer of protons and then these protons with the electrons will form water when it reduces oxygen. But in that formation we eventually have to form ATP so we have to get to ATP synthase.

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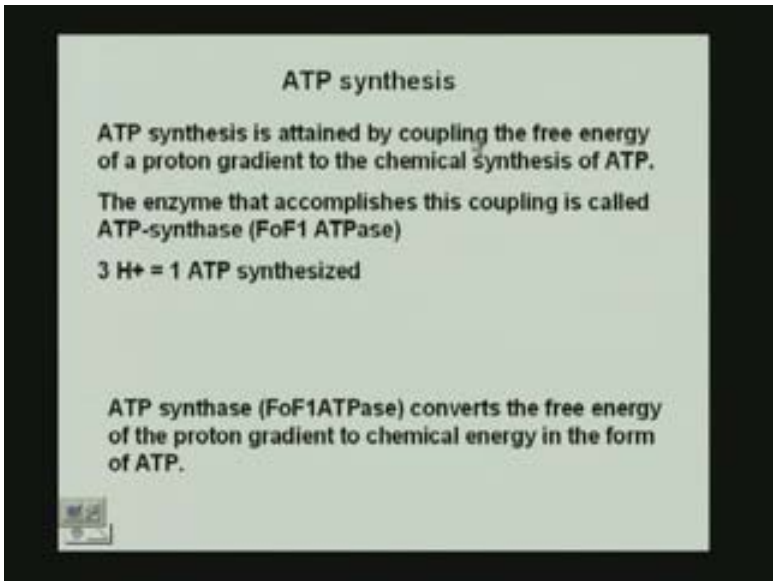

This ATP synthesis is attained by coupling the free energy of the proton gradient to the chemical synthesis of ATP and the enzyme that actually accomplished is this called F_0F_1 ATPase and this is also called ATPsynthase because it works in a beautiful manner. This converts the free energy from the proton gradient to the chemical energy in the form of ATP, which is what we just looked at.

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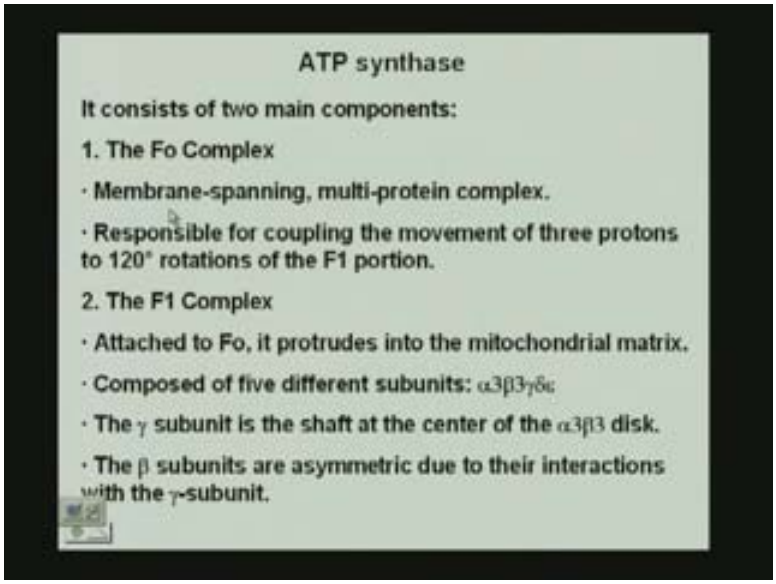

The protein itself is composed of two main components. You have the F_0 complex that is a membrane spanning a multi-protein complex. It is responsible for coupling the movement of 3 protons to 120 degree rotations of the F_1 portion. We will see exactly what that means in a moment. What we need to know now is that in ATP synthase there are two main components. The two main components are an F_0 complex and an F_1 complex. The F_0 complex is the membrane spanning complex it is a multi-protein or multi-subunit complex and it is responsible for the motion of the protein. We will see what that is in a moment. The F_1 complex is attached to F_0 and it protrudes into the mitochondrial matrix. It is composed of 5 different subunits named as α β γ Δ ε. There are 3 α subunits, 3 β subunits, γ, Δ and ε. The γ subunit is the shaft at the center of α 3 β 3 disk and the beta subunits are asymmetric due to their interactions with the γ subunit.

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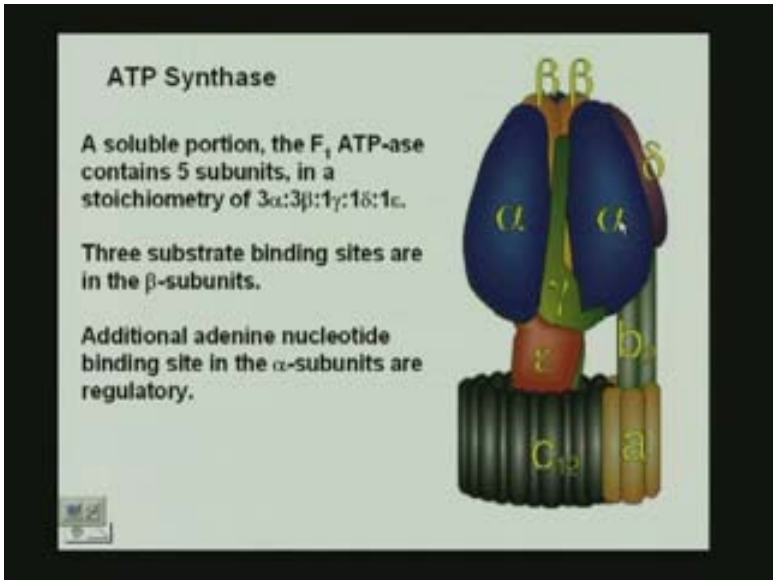

Let us see what each of these things mean? This is a picture of protein. You see there are α subunits, β subunits, γ subunits, Δ and ε subunits. This part that is the bottom part is the integral part to the membrane. So outside what sort of residues would we see? They are hydrophobic residues that would interact with the long fatty acid chains. There is a γ shaft here that actually holds these subunits together. It looks like a stock. So you have a stock where you have 6 subunits, 3 of the α type, three of the β type that is with this stock. Now the β subunit is the catalytic subunit that is actually going to produce the ATP. So this part is the F_1 part and the F_0 part the part that is connected with the membrane. Every time three protons are pumped the F_1 subunit rotates by 120 degrees.

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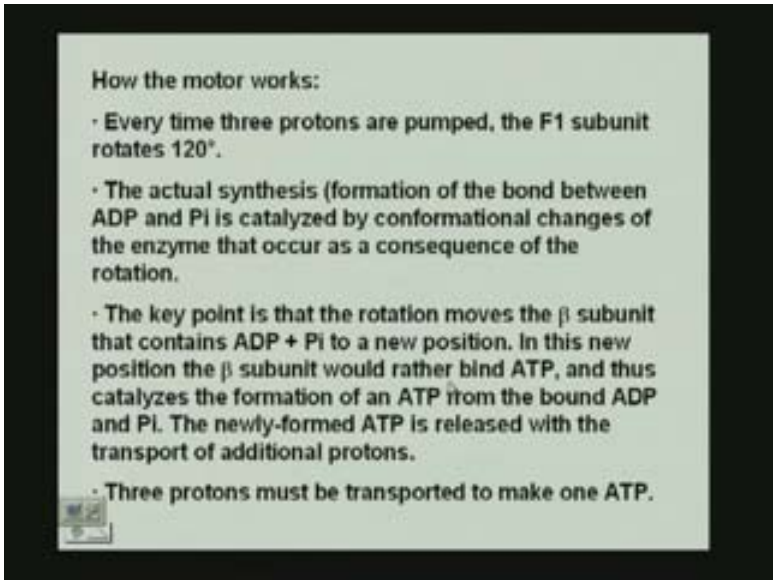

Basically it looks as something like this. If we just look at this we are looking top down we have a central part here, (Refer Slide Time: 47:21) we have something like this.

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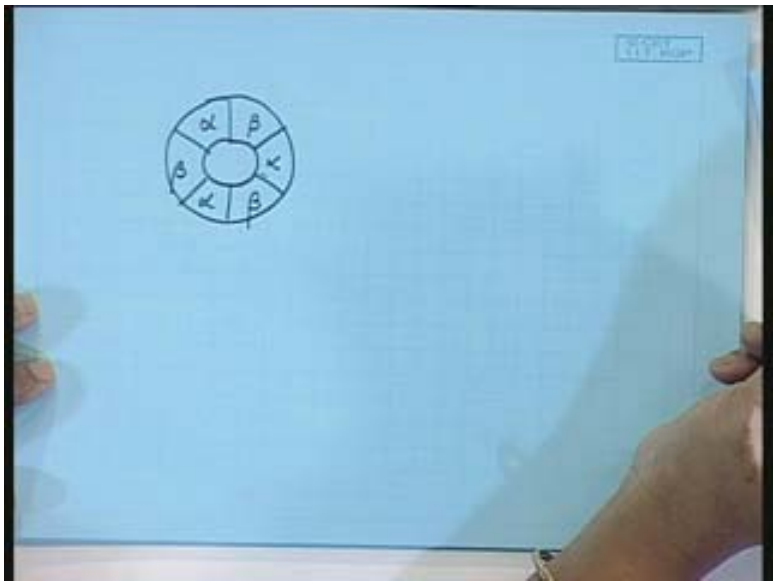

I have a β and α subunits here, a β here, a α here, a β here, a α here. It looks something like that. What is going to happen now is there going to be rotation. This is connected to a stock down at the membrane. So this looks actually like this where you have a stock and this part that is connected to the membrane. So this is your membrane and (Refer Slide Time: 47:58) this part is F_0 part and this is your F_1 part that is inside the mitochondrial matrix. We have now a motor that works and every time we have 3 protons

pumped, but how are these being pumped? They are being pumped by the whole electron carrier system that we just looked at. All these complexes together are going to pump the protons and for every 3 electrons rather protons pump the F_1 subunit rotates a 120 degrees. You see now that as it rotates 120 degrees each β sub unit will come into the picture. Because if you are hooked on a β subunit, you rotate a 120 degrees it will be onto another β subunit. This is the γ unit that actually acts as a stock and rotates and the β subunit which is the catalytic unit is squeezed.

Suppose you have a stock like this we have the α units interspersing. Now had we all 6 β units all of them catalytic the rotation would have to be 60 degrees. Now we have alternating α and β so we have a β subunit that is held in something called open, loose and tight which we will see in a moment so I have an open form now. As I rotate 120 degrees this β form comes here now which makes the other one tight and makes this one loose. Therefore we have open, tight and loose conformations depending on where you are located in with respect to the γ stock that is rotating it.

The actual synthesis is the formation of ADP + Pi and that is catalyzed by conformational changes of the enzyme that occurs as a consequence of the rotation. Now what happens is the β subunit contains the ADP + P_i and it is loosely connected. Then we have at one point ATP being formed that is still within the β subunit, at the third rotation it has very low affinity for the ATP so what it does is that it throws that out. So at one point it has high affinity for the ADP + P_i forms the ATP but the ATP sits there, it doesn't move off but as soon as the stock rotates again it spurs it out, so this is exactly what happens.

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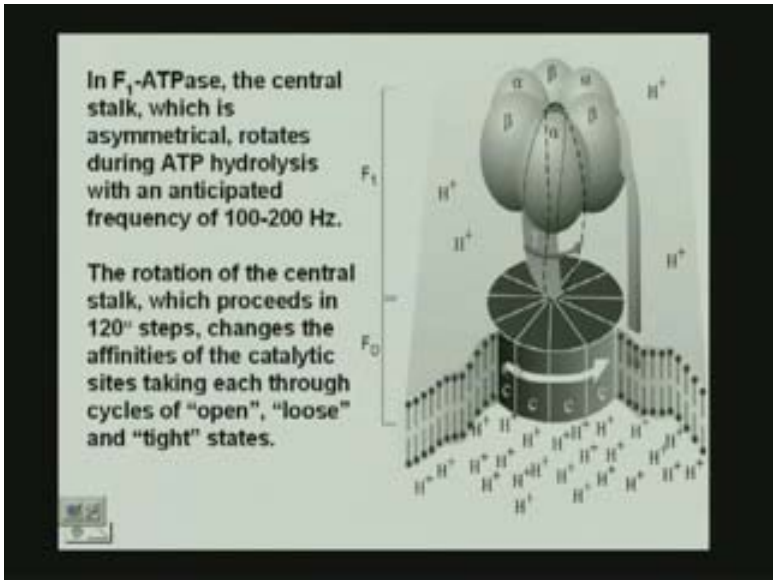

We have this rotation of the c units that are connected with the membrane and with this rotation, now you can see the γ stalk rotating so what is that going to rotate? That is

going to rotate these β beta subunits and as the β subunits rotate there are 3 states that it gets into called the open, loose and tight states. One β subunit has low affinity for both ADP and ATP so they will be reversibly associated with the unit.

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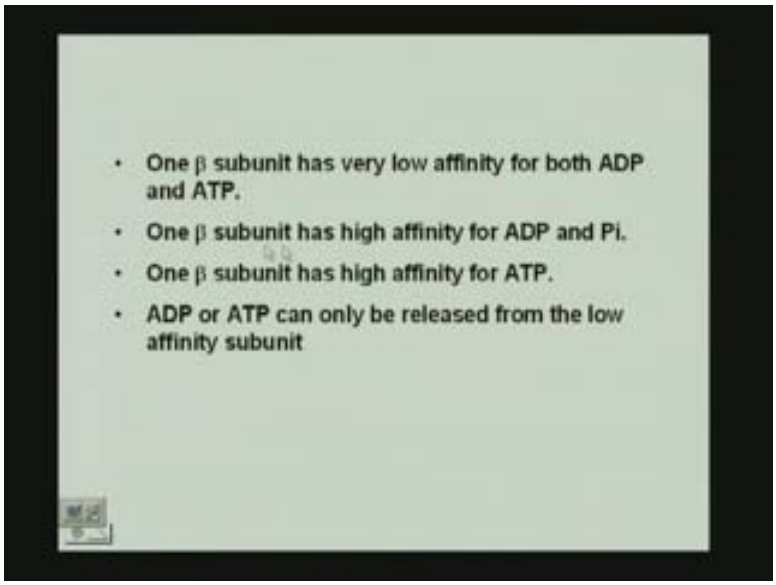

One β subunit has high affinity for ADP and P_i so it is going to take in the substrates. The other β subunit has high affinity for ATP therefore it will not release the ATP and will hold on to it. It will make the ATP but it will hold on to it. As soon as it rotates then it will be released only from the low affinity subunit. So as the γ stock shifts it changes it from an open, tight, loose state, once it is connecting or once it is collecting, once it is making, once it is releasing so it goes on that way.

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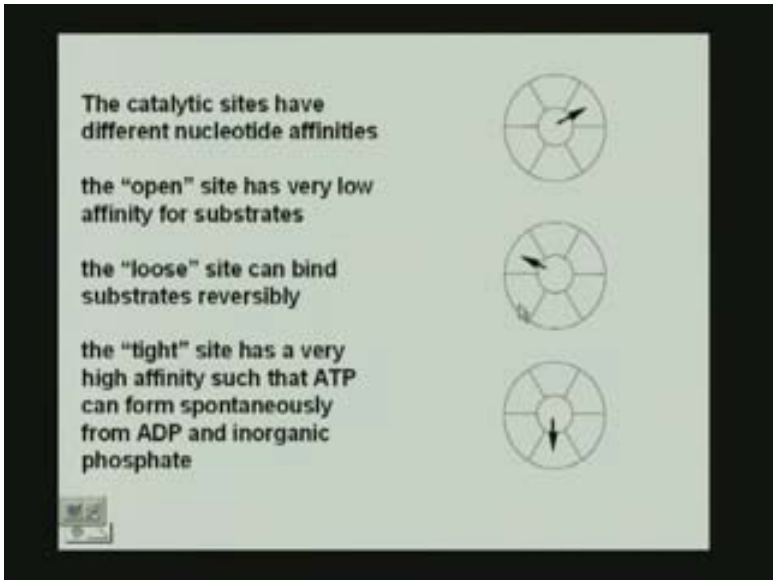

We have here different nucleotide affinities. The open site that has the low affinity for the substrates, the loose site that can bind it reversibly and the tight site which has high affinity such that ATP forms spontaneously from this and it goes in the anticlockwise direction so we have the β subunit shift in this direction each time so eventually we have the ATP being produced.

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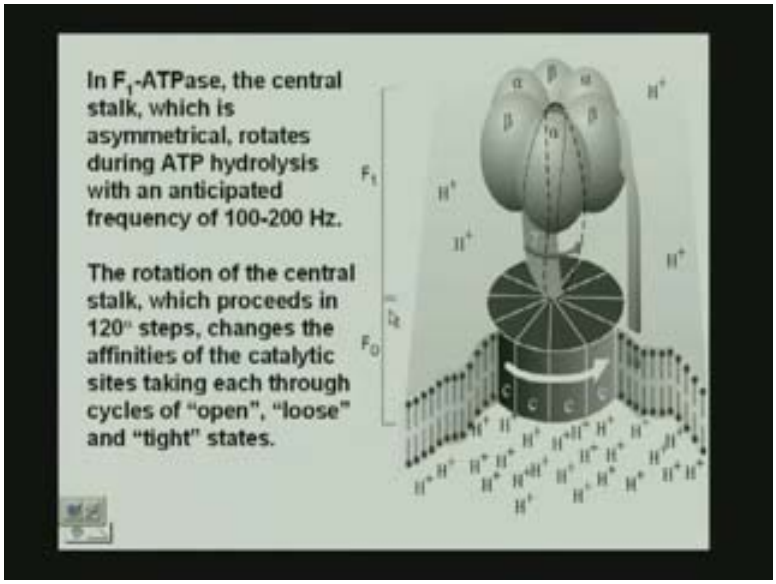

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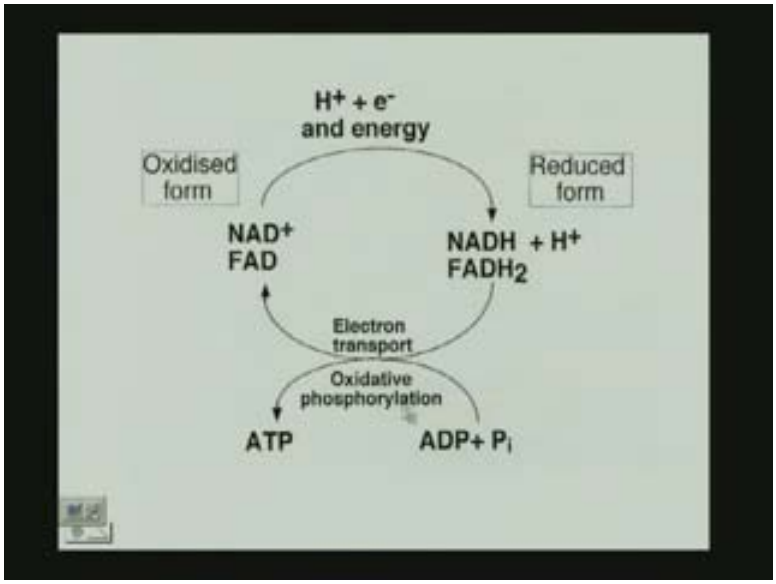

Basically what we have is we have this process that is called the oxidative phosporylation. In the whole set of electron transport systems we have all set, the oxidized forms the NAD^+ and FAD going to NADH and $FADH_2$ to the reduced forms in the event of creating a proton transfer, a proton force a proton motive force then with the oxidative phosporylation it creates ATP which eventually gives us the overall reactions in oxidative phosporylation that provide us with ATP synthesis.

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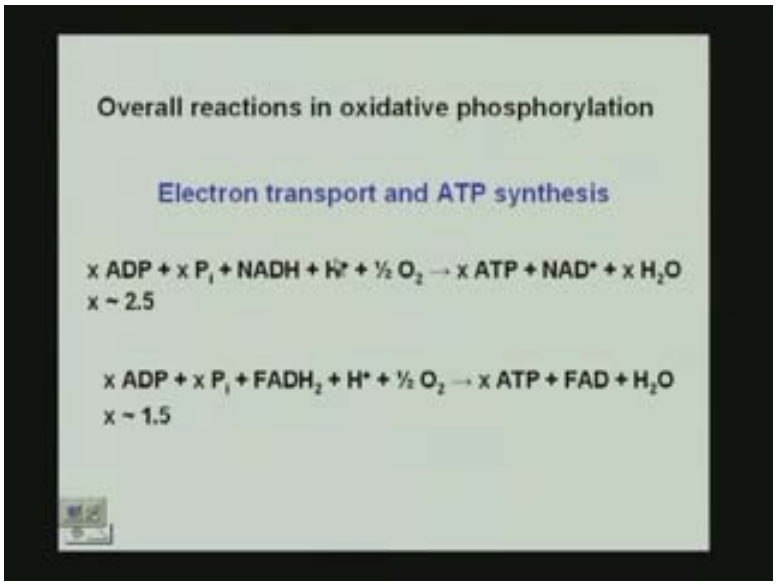

So what we learnt is how we can actually synthesize ATP from the protons so for every proton that comes in here $3H^+$ we will have the synthesis of ATP. This completes our

discussion on oxidative phosphorylation. We will start metabolism in our next class, thank you.