Biochemistry
Prof. S. Dasgupta
Department of Chemistry
Indian Institute of Technology – Kharagpur

Lecture - 24
Bioenergetics - II

We continue our discussion on biochemical energetics and what we learnt last time was 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.

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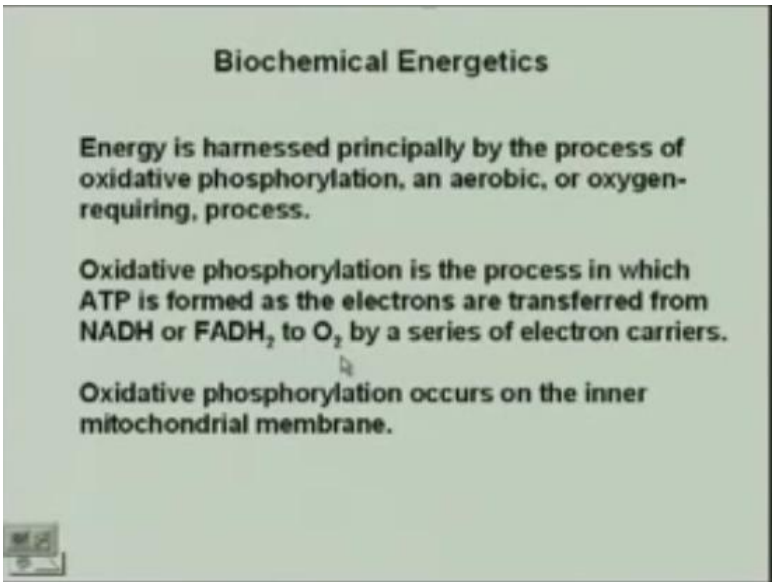

And basically what we have in an aerobic process or an oxygen requiring process is this energy harnessed by this oxidative phosphorylation. Now, what happens in oxidative phosphorylation is the formation of ATP. Now, ultimately there is going to be an electron transfer system that is actually comprised of a number of electronic systems, they are termed as different complexes, we have complex I, complex II, complex III and complex IV.

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 work out. Now, what we are going to see is how this complex actually helps in the transfer of the protons from the inner, from the inside that is the matrix side, to the inter membrane space and then we will see the action of what is called ATP synthase in the production of ATP.

So, what we are going to look at is the oxidative phosphorylation, which is the process by, which ATP is formed as the electrons are transferred from NADH or FADH2 to oxygen by a series of electro carriers.

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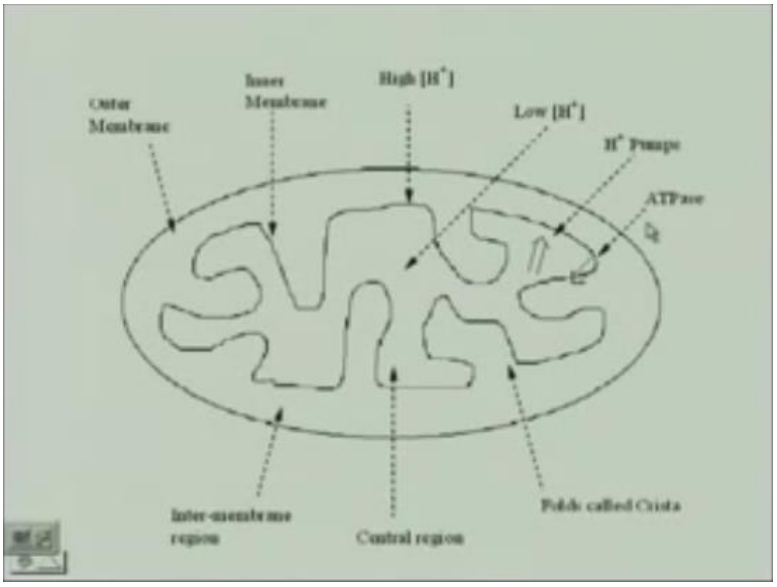

And this actually occurs in the inner mitochondrial membrane. We looked at this picture last time, where we found out that even though there is a high proton concentration in the inter membrane space, we still need to pump protons from the inside that is the matrix side to the inter membrane space, so that ATPase, which we will see later, can actually produce the ATP that is required for the energy, for functioning of all things in the body.

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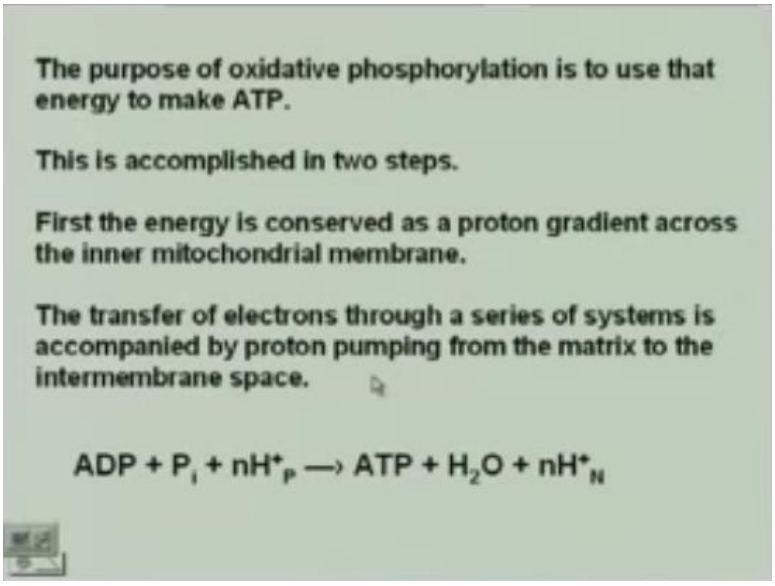

Now, so the purpose of this oxidative phosphorylation is to use the energy to make ATP. Now, the way this is accomplished is in 2 steps. First the energy is conserved as a proton

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 the outside, why because we have the proton gradient, the proton gradient giving you what?

Giving you different hydrogen ion concentrations, because you have different hydrogen ion concentrations on either side of the inner mitochondrial membrane, what you have is, you have different pH values. So, we have a free energy contribution due to the delta pH and we have a free energy contribution due to the membrane potential because we have the negative and the positive sides of the membrane.

So, 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 inter membrane space. So essentially what happens is, we have ADP+Pi go from, so where is this occurring? This is occurring in the, this is the inter membrane space and this is the matrix space. The matrix is the "n" part and the inter membrane space is referred to as the "p" part. So, this p refers to the inter membrane space and n is the matrix space where ATP is going to be produced.

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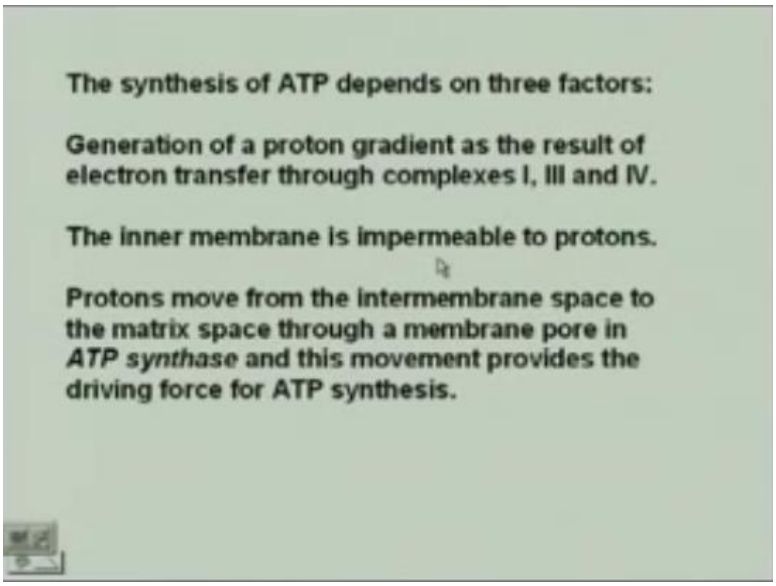

Now, we have the synthesis of this ATP that depends actually on 3 factors. There is the generation of a proton gradient as the result of the electron transfer through complexes I, III and IV. We will see exactly what these complexes are comprised of and what they mean. They are essentially enzymes along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

Another thing that we have to take into consideration is that the inner membrane is actually impermeable to protons, which mean that the higher concentration present in the inter membrane space does not diffuse into the matrix space of the mitochondria. So, the fact that the inner membrane of the mitochondria is impermeable to protons, because you remember there is a higher concentration already in the inter membrane space.

So, you would expect that the proton 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 these protons. So, what you have to do is, to maintain that high concentration for the production of ATP, you have to pump the protons from the matrix to the inter membrane space.

So, the protons actually move from the inter membrane 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 you mean by a membrane pore? Remember when we spoke about or studied membranes?

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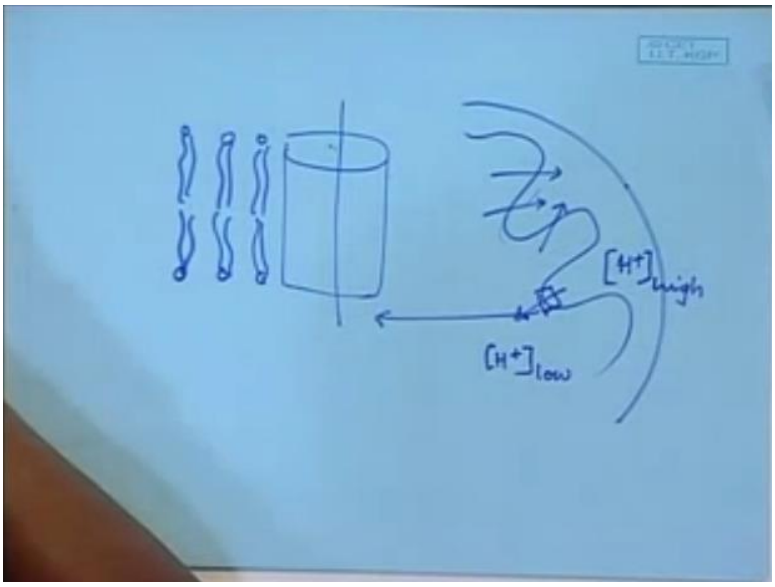

We had the lipid bi-layer. Now, in the lipid bi-layer, which is actually going to be a part of the inner mitochondrial membrane as well, we are going to have embedded certain integral proteins. Integral proteins are proteins that are embedded in the lipid protein bi-layer. So, what we need is for the channeling of the protons, we need a pore in the membrane that is going to allow the protons to pass through, because if we look at the structure of the inner

layer of the mitochondria which is something like this, so if this is the mitochondria, if this is the outer membrane and this is the inner membrane, which actually one region would blow up to look something like this, where we would have the lipid bi-layer and we have a high H+ concentration on this side, which is high.

Inside we have an 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. So, what you have to have is you have to have a certain pore that is going to actually pump, we look at this, these pumping systems, the pumping complexes that are going to make the protons from the inside go to the inter membrane 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. (Refer Slide Time: 04:40)

So what happens is, the protons move from the inter membrane 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. So, basically what we have is, we have the mitochondria. 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, through the intermembrane space and then this movement is possible only through the pore that is present in the protein ATP synthase. We will look at the structure of ATP synthase also and this will provide the driving force for ATP synthesis.

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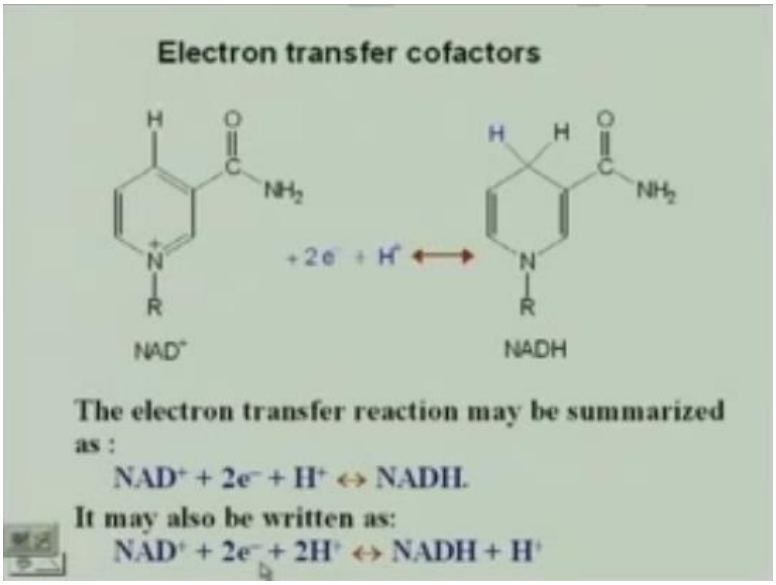

So, now before we get into the complexes I, III and IV and what they actually comprise, I said they actually make up a bunch of or rather they are made up of bunch of proteins as well as other cofactors, enzymes and prosthetic groups. But, before we do anything we have to consider the other electron 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 or electron transfers.

We have looked at 2 of these already. NAD+ going to NADH, where we have a certain electron transfer reaction that can be written as NAD+ + 2 electrons + a proton going to NADH or it can alternatively be written as NAD+ + 2 electrons + 2 protons going to NADH + H+. So, we have our basic structure of NAD+, which is made up of nothing but the nicotinamide or the niacin that we found out from, where did we get this from, from the vitamins that we actually intake as supplemental in out diet.

So, we have NAD+ and we know that this H adds on to this particular carbon atom. So, we have NADH.

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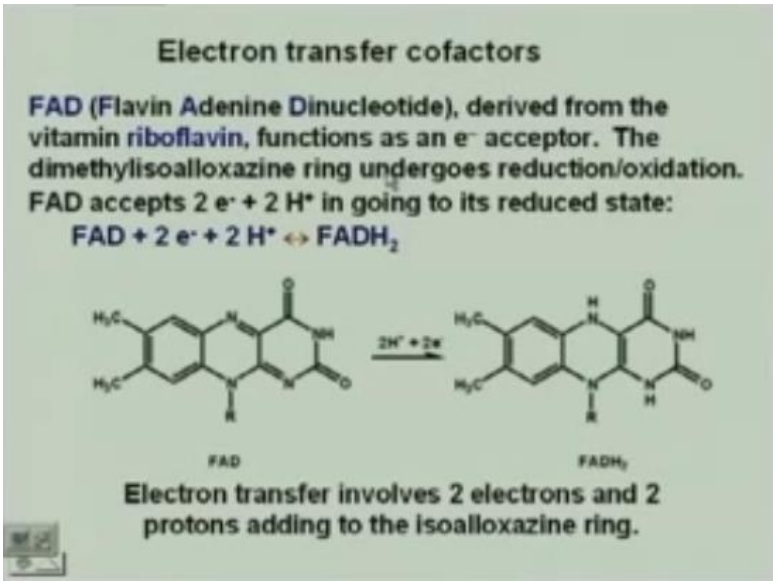

The next one we looked at was Flavin Adenine Nucleotide, FAD that goes to FADH2 where we have 2 protons add to the 2 nitrogen of this isoalloxazine ring. This isoalloxazine ring, which is the rest of this is flavin, this is flavin part we have an adenine dinucleotide part, a Flavin Adenine Nucleotide that is derived from the vitamin riboflavin. And this also functions as an electron acceptor, because when we are going to consider electrons and proton movements.

We have to consider what the factors are or what cofactors or prosthetic groups are actually going to be combined with the enzymes in taking up the protons are providing them in the process that goes on. So, we have in this electron transfer process for FAD going to FADH2 these are the 2 nitrogens that are going to take up the 2 hydrogens in becoming FADH2.

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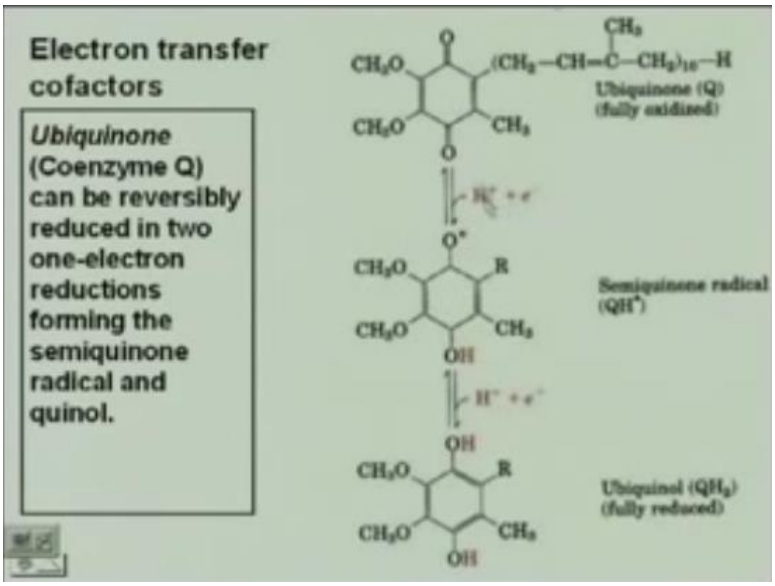

Another electron transfer cofactor that is extremely important is ubiquinone. Ubiquinone is also known as coenzyme Q. Now, this is the structure that is written on top is the total ubiquinone that is also known as coenzyme Q or referred to as just Q. What happens in this case is you have a quinone moiety 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 the protons and from the quinone, it becomes the semiquinone 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 dot and OH you have the semiquinone radical, which is going to give you, which is what, which is H+ plus electron to the ubiquinone you have the semiquinone and you have the ubiquinol.

Now, 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 2 electrons. For example, in the NAD+ going to NADH, if you go back and just look at the reaction you need 2 electrons, for the reaction to go to NADH. But in this case here again FAD going to FADH2, you again need 2 electrons for the reaction to go from FAD to FADH2.

So there is no intermediate as such. But in coenzyme Q, this intermediate semiquinone is possible. And because of this semiquinone 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. So, we have the ubiquinone go to the semiquinone, which subsequently goes to the ubiquinol because of its uptake of 2 electrons in 2 steps. That is important.

It is not like the other cases where you have to take in or it is 2 electrons that are taken in a single step. We have 2 electrons taken up in this case, but in 2 different steps. So, it is useful when we get into the reactions that are actually going to be taking up the protons and the electrons for the transfer of the protons from where, from the matrix space to the intermembrane space.

So, we have the coenzyme Q or ubiquinone, which is actually coenzyme Q or just Q as I said. This can be reversibly reduced in 2 one electron reductions forming the semiquinone radical and finally the quinol. So, that’s where it’s important lies.

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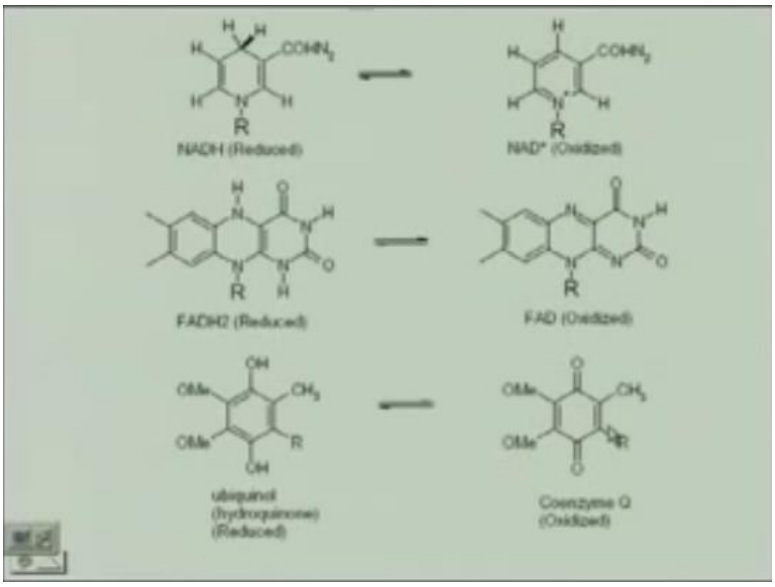

So, just summarizing what we had, we have the reduced forms of all the electron transfer cofactors here. The usual one, from NADH, NAD+ is the oxidized form. We have FADH2 the reduced form and FAD the oxidized form. We have ubiquinol the reduced form and ubiquinone or coenzyme Q as the oxidized form. So, in each case you have to know, where at which point the hydrogens are added.

You also know now that the use of coenzyme Q is more versatile because it has 2 one electron steps that can form an intermediate semiquinone. So, these are the 3 electron transfer cofactors that are going to be utilized in our complexes I, III and IV of the oxidative phosphorylation steps.

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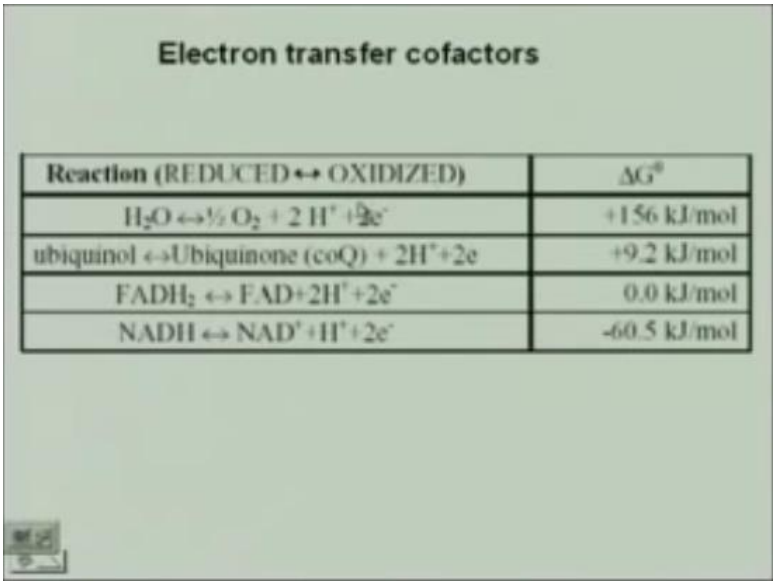

Now, here are some of the delta G zero values associated with the electron transfer cofactors. When we go from ubiquinol to ubiquinone, so quinol to quinone means I need 2 protons and 2 electrons, I have a +9.2 kJ/mol in free energy change. For H2O that is the reduced part going to half O2 + 2H+ + 2 electrons, the value of delta G zero is +156 kJ/mol. So, if I want to reduce, if I want to go from the reduced that's water to oxygen I would need this amount of free energy change.

FADH2 to FAD + 2H+ + 2 electrons is 0. NADH to NAD+ + H+ + 2 electrons is -60.5. And we will see how actually in the energetics all these combine to give you favorable energy once we complete the metabolism of carbohydrates.

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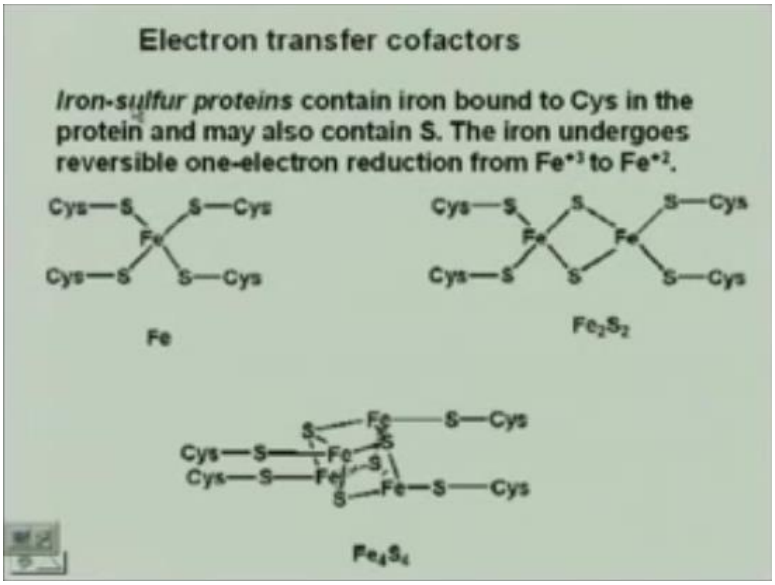

Now, we also have certain proteins that can act as electron transfer cofactors. These are mainly iron-sulfur proteins. They are just written as Fe-S proteins. They contain an iron bound to the cysteine in the protein and they may also contain additional sulfur. For example, if we look at this Fe, we have the Fe coordinated to cysteine residues in the protein into forming what are known as iron-sulfur proteins that also play a major part in the electron transfer because they can, do you know why they can do that?

Why because you can have a multiple oxidation state for the iron. It can be in the Fe2+ or Fe3+ state. So, what does that help me with? It helps in taking up an electron or giving an electron, because I am eventually looking at electron transfer I need certain moieties in the system that will be capable of electron reduction, one electron reduction, 2 electron reduction whatever. So we have certain proteins that are going to be associated with this.

These are the iron-sulfur proteins that contain iron bound to cysteine 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 irons coordinated, 2 with cysteines and 2 with sulfur atoms. So, we have a structure that looks like, we have 2 cysteines associated with, and so you would have 2 cysteines which have their sulfur atoms associated with an iron atom.

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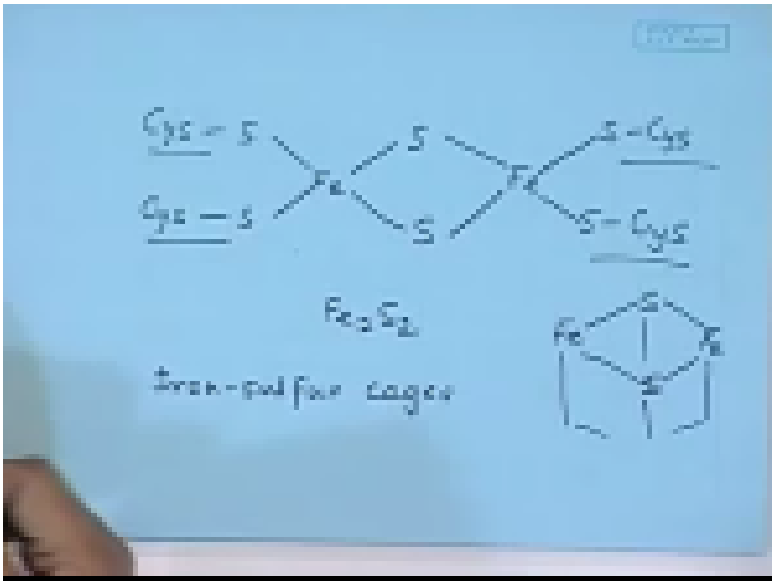

This again associated with another one. This associated with another iron. This associated with the Cys. So, that's what we are looking at. So, this would be basically a Fe2S2 system because you have additional 2 sulfurs in this case because the rest you know are coordinated to the cysteines belonging to the protein that is part of your complex system. You also, you can have Fe4, Cys4, S4 or you can also have what are called iron-sulfur cages, which literally look like cages.

So, you have like, you would have like 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.

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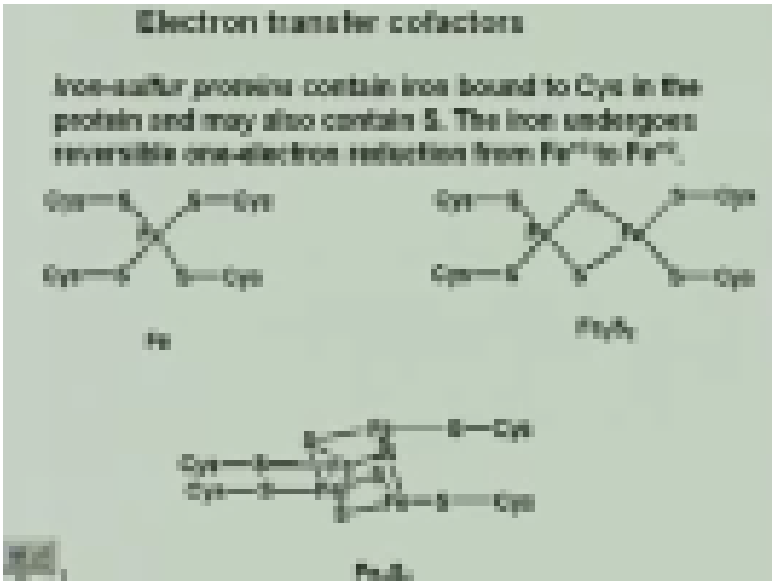

So, you can have this iron sulfur cages the ones that have been drawn here are the Fe1 with just the Fe coordinated to the Cysteine. You can have Fe2 S2, where you have the iron coordinated not only to one Cys, not only to Cysteine but also to additional sulpurs. And the iron itself can undergo a reversible one electron reduction from Fe3+ to Fe2+. So, you understand how this can be important when we considering electron transfer. We have a system that can take in an electron. And once Fe3+ does take in an electron it will convert itself to Fe2 plus.

But, we have to remember that when we considering enzymes, what do we need? We need a Cysteine that is going to get it back to Fe3+, because, it cannot just stop after one particular reaction.

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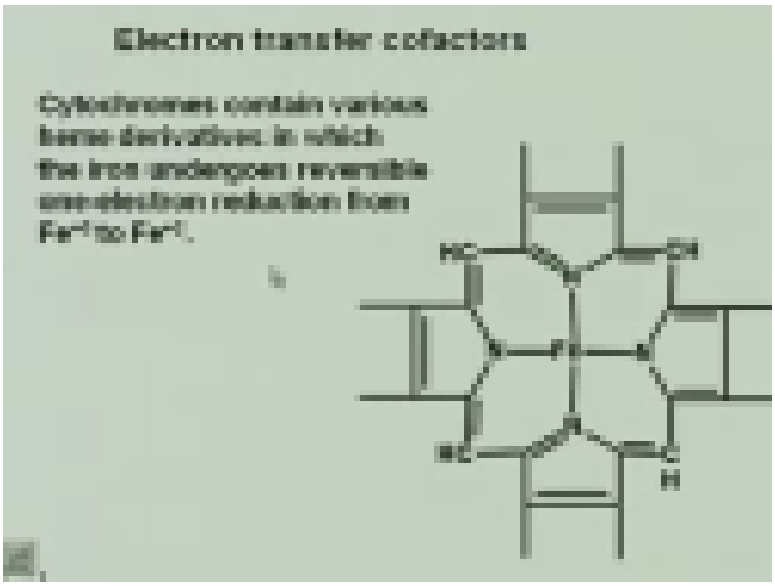

So, now that we know some of the electron transfer cofactors. This protein cytochrome, the cytochromes they contain various heme derivatives, we studied heme, when we did hemoglobin and myoglobin, this heme also coordinates an iron in the proto (()) (21:16) system. Similarly, cytochromes contain various heme derivatives, which actually differed in their substituents in the rings, which makes them different from the heme that is probably in hemoglobin or myoglogin.

So, there are different substituents in the hemes that make different cytochromes also. So, you have cytochrome A, cytochrome B, 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 two hemes associated with it. And, now in this case the iron is coordinated to the nitrogen of the(()) (21:57) rings. Now the coordination here also allows for a one electron reduction.

Because you have to remember when 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. So, if we take in a proton, if we take an H plus, we have to have 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 plus, we have associated with the H plus, what? the electrons. So, 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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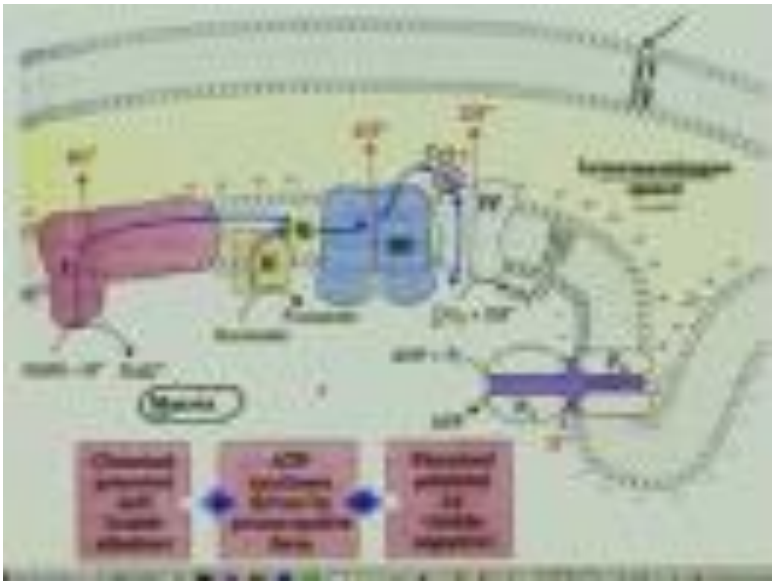

Now, this is what we have in our system. We have, this is the outer mitochondrial membrane. I am going to go this little bit slowly. The circles that you see there, what are those circles? Those are the polar head groups of the lipid bilayer. This is the inner mitochondrial membrane. Now in the inner mitochondrial membrane, so, here we have our polar head groups.

So, 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. Now, what we have here? What is this space? This space is our inter membrane space. Here is the matrix of the mitochondria. Now, what you have in this matrix is remember, here, what do we have? We have a high H+ concentration. Inside here we have a low H+ concentration.

What are we doing? We want to pump H+ to the other side. Why? to make ATP. This is the system, the pore, remember I mentioned, a pore in the mitochondrial membrane that is eventually going to provide the H plus or transfer the H plus into this matrix, so that we can produce ATP. Now, we are going to look at this system in a bit detail. What we have is, we have here certain complexes. Each of these complexes is what?

It is a kind of integral membrane protein. It is comprised of a protein and along with the protein we have prosthetic groups or other electron transfer cofactors, the ones that we had been mentioning that are going to help us in the transform of the protons from the inside to the inter membrane space. Now, we are going to look at the constituents of each of the complexes. But, this is sort of the pictorial representation of how it actually looks.

So what we have? Is we have in the membrane, now across the membrane what do 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? 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 delta PH which gives a chemical potential.

We also have an electrical potential due to the charge difference at both ends inside, inside meaning the matrix and the inter membrane space. If we look at this both actually are inside.

Both of these are actually inside the mitochondria. But, this is the inter membrane space and this is the matrix, the (()) (26:47).

Then, we have 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 this complexes eventually giving you sufficient H plus concentrations in the inter membrane space that is going to result in ATP synthesis. Let us go back to our slides.

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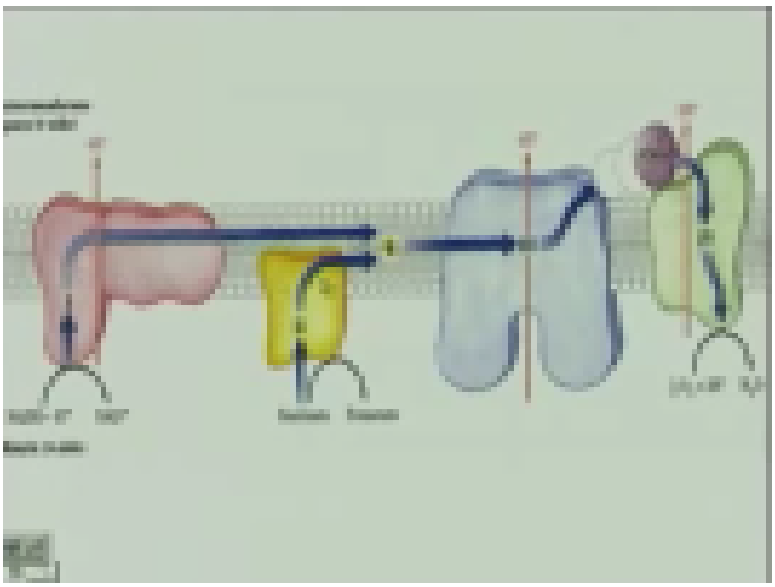

So, this is basically a pictorial representation without all the (()) (27:33) of the inter membrane space of the whole mitochondria. So, we have complex 1, complex 2, complex 3, complex 4. Now, Complex 2 actually is not always mentioned because it does not involve proton transfer. We will see that in a moment.

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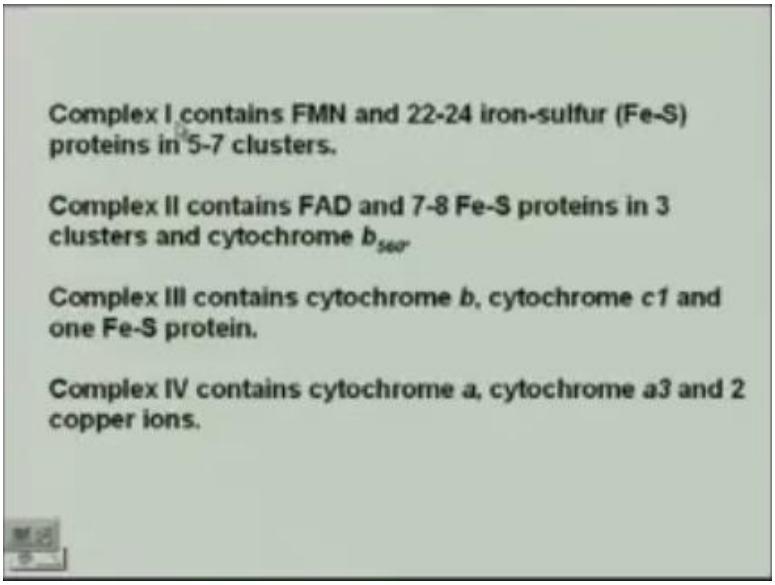

Complex 1 contains FMN. What is that FMN? FMN is flavin mononucleotide. And it has 22 to 24 iron sulfur proteins present in clusters. So, that is what complex 1 is. And apart from that we are going to see what else it contains. So, these are the electron transfer cofactors present in complex 1. So, Complex 1 has FMN and iron sulfur. Complex 2 has FAD that is flavin adenine dinucleotide, and iron sulfur and cytochrome B.

Complex 3 has cytochrome B, cytochrome C1 and iron sulfur protein. Complex 4 has two cytochromes and two copper irons. Why do we need copper again? It would be the same thing. The copper also has multiple valency so it can take up or give electrons. So, we have, what do we have here? We have these electron transfer cofactors associated with complex 1, 2, 3 and 4 that are going to assist in the electron transfer.

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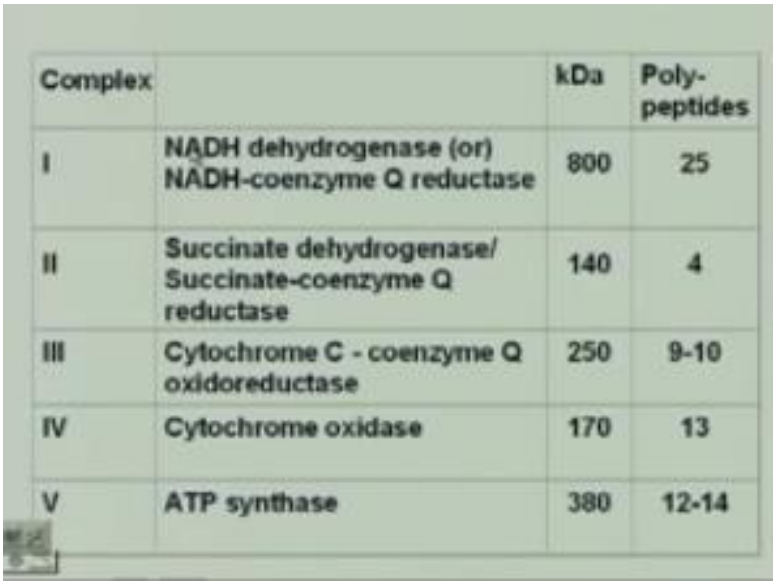

Complex		kDa	Poly-peptides
I	NADH dehydrogenase (or) NADH-coenzyme Q reductase	800	25
II	Succinate dehydrogenase/ Succinate-coenzyme Q reductase	140	4
III	Cytochrome C - coenzyme Q oxidoreductase	250	9-10
IV	Cytochrome oxidase	170	13
V	ATP synthase	380	12-14

Now, the enzymes involved. Apart from the cofactors, we now have to look at the enzymes involved. For complex one, which is associated with what? FMN + Fe-S, what are these, what are these that I mentioned here, these are electron transfer cofactors. They are not the enzymes. They are the cofactors that do what, are going to assist the enzymes into performing its function. So, now we have to look at the enzymes.

The enzymes here are NADH dehydrogenase. As soon as we see NADH dehydrogenase, we know what the enzyme is actually going to do. Complex-II has Succinate dehydrogenase, so again from the name of the enzyme, we can say what that is going to do. Complex-III is Cytochrome C or it is coenzyme Q oxidoreductase. Complex-IV is Cytochrome oxidase. Complex-V 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 to as Complex-V.

But, these are the four complexes that are actually going to take place, take part in the electron or rather proton transfer that is going to result in oxidative phosphorylaration that is going to be finally, oxygen that is going to be required, for with the proton motive force that is going to give us ATP.

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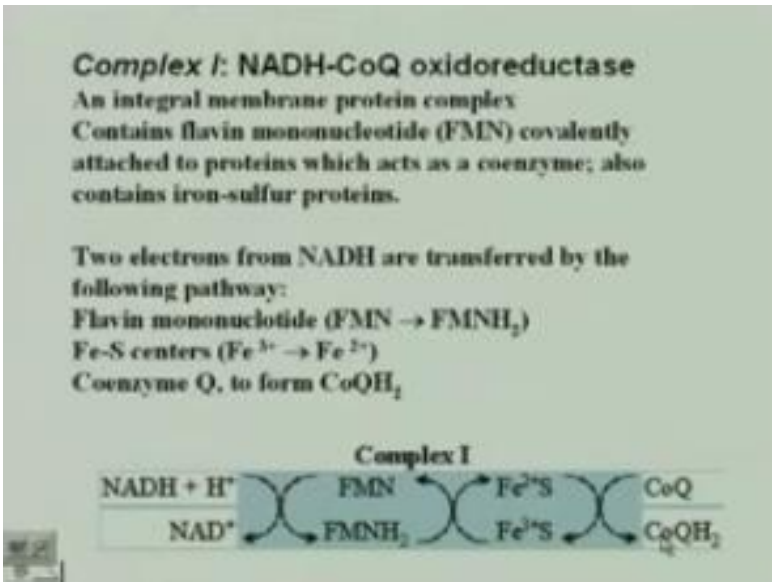

So complex-I, so Complex I is the integral membrane protein complex, that contains FMN that is covalently attached to the proteins, which acts as a coenzyme and it also contains the iron-sulfur proteins. So, what does complex-I have, it has the NADH dehydrogenase aqua CoQ oxidoreductase and apart from that, it has FMN and Fe-S clusters. That is what comprises complex-I

Now, what happens in complex-I, there are two electrons from NADH that have transferred through flavin mononucleotide, the iron centers and coenzyme Q. Now, I have on the next slide, I have just compacted the whole thing. It is rather here only. We have, this is complex-I. These are the cofactors that actually takes part in the reaction because you have to remember, the enzyme actually gets back to where it was.

So, 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+. Now, what is happening here then? There has to be some cofactor that is going to take up the hydrogens. FMN takes that up. Now, in the process 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 also.

If you are going to have H 2, what is H 2? 2Hplus + 2 electrons. So, when you have the two H atoms taken up here, the two electrons are taken up by Fe 3+. Then what happens to Fe 3+? It forms Fe 2+. Then, what did we learn about coenzyme Q? Coenzyme Q can take up these two electrons and form coenzyme QH 2, which is the quinol form.

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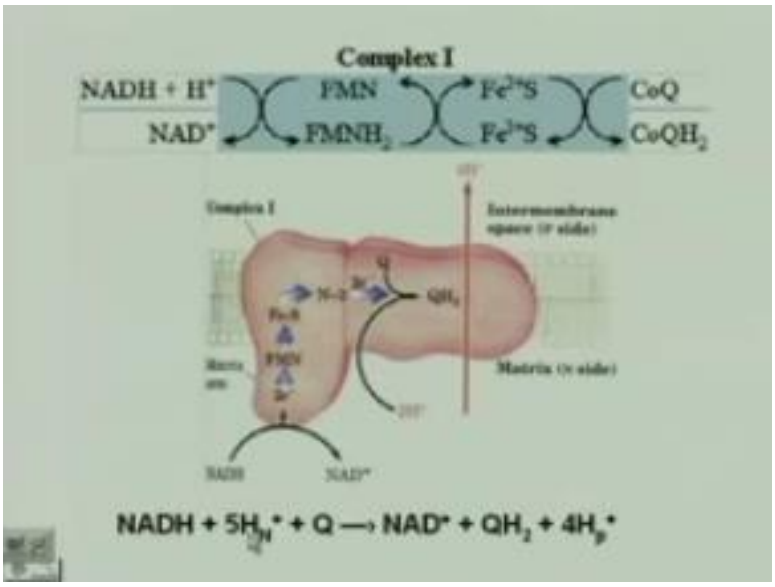

So, we have basically an overall reaction. This is actually what you need to know. NADH + 5 protons from the matrix side + Q forming, NADplus + QH 2 + 4 protons in the inter membrane space. So, this is our final reaction for complex one. And remember the complex one, it has this whole enzyme that is an oxygen, all of these enzymes are going to be dehydrogenases or oxidoreductases. Why? because all of them are involved in redox

reactions. Since all of them are involved in redox reactions, they have to be either dehydrogenases, oxidases, oxidoreductases, whatever. So, this is what is, complex-I. So, we have one reaction that has now transferred some protons.

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Complex-II, Complex-II does not transfer any protons. It is, this is one example that 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.

But it can also; 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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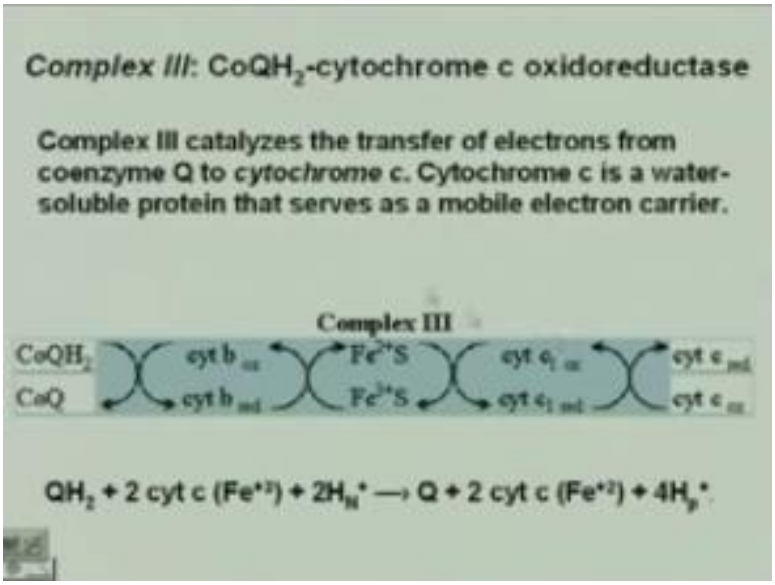

In complex-III, the use of cytochromes comes into the picture. What are cytochromes? Cytochromes are proteins that have heme components to them. These hemes have coordinated iron and the coordinated iron has multiple valency. So, it can take up the electrons. You have to remember that in each of these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

Our ultimate aim is to get protons to the inter membrane space. Once we get these protons to the inter membrane space, we know that ATP can be synthesized. That is our ultimate aim. So, these are the different complexes involved that 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. Now, if you remember, in the first complex is, what happened? Q formed QH 2.

In the first complex, when we had, let me just show you, I have that reactions together later on, coenzyme Q actually formed CoQH 2. So, what is it? This Q formed QH 2. Now, what has to happen? The QH 2 has to get back to Q. Otherwise this reaction, you cannot have another NADHs go to NAD pluses.

It is simple as that. 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 two protons from the matrix side that is the N side, to form Q, the two cytochromes are now, it was Fe3+, it is going to form Fe2+. And, we have 4 H + in the inter membrane space. So what have I done, in this complex-III, I have reduced Cytochrome c.

I have oxidized Q. And I have transferred in effect two protons to the inter membrane space. So, this is the overall reaction of complex-III. Now, what you need to remember is, which of the complexes actually help in the proton transfer, the over all of each of the complexes. That is what you have to remember. So, we know that the QH 2 + 2 cytochrome c in this set is going to give Q + 2 cytochrome c reduced + 4H P. What is 4H P? It means 4H P+ means four protons in the inter membrane space.

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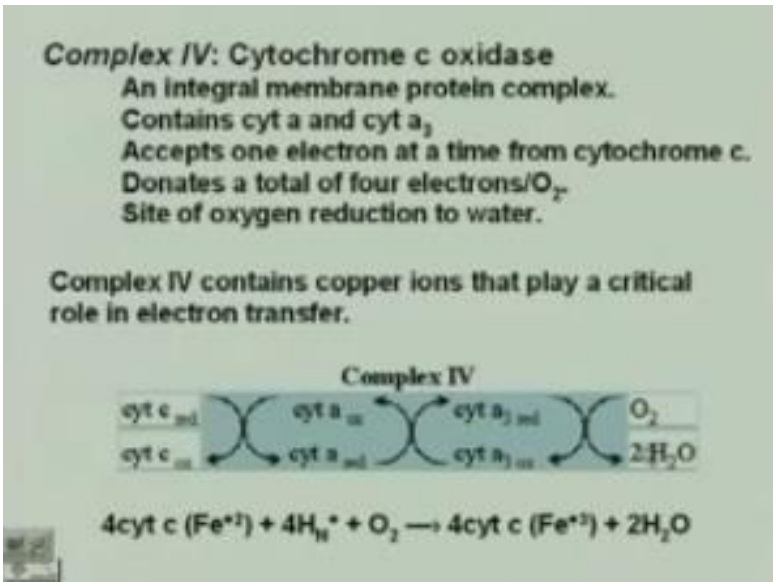

Then, we have complex-IV. That is actually again cytochrome c. And, in this case what happens, a total of four electrons go to water, and this to oxygen, and this is the site of oxygen reduction to water. And in this, we have a one electron system also here, in the form of cytochromes. The cytochromes have one iron in them in the heme, they can also take up one electron at a time. Like which system? Ubiquinone.

Ubiquinone will form a semi quinone, then form a quinol. So we have one electron at a time, to cytochrome c. We have four electrons given to oxygen. And there is one interesting thing about the proteins here, at one point, there is actually an oxygen radical formed. And this oxygen radical can be extremely damaging to the membrane. So, what happens is, the protein actually holds the radical extremely tightly, it has an 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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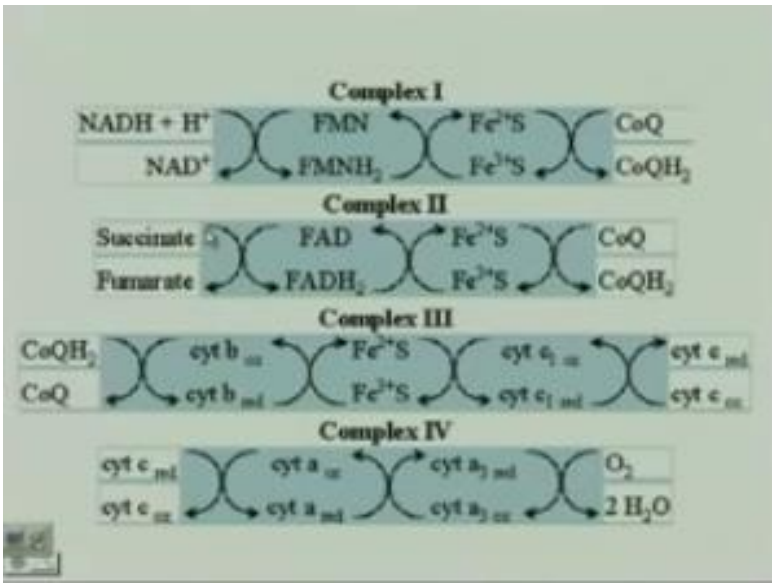

So, this is actually a summary of all the complexes that we just considered, where we have, we look at the final equation in a moment that is eventually what we want to do. We have NADH+, H+ going to NAD+, in this case CoQ forms CoQH 2. In Complex-II, we have succinate going to fumarate, which actually forms again CoQ going to CoQH 2. In complex-III, 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-IV? We have to get everything back to normal. So in complex-IV, cytochrome c, the reduced form of cytochrome c is oxidized to cytochrome c the oxidized form, where it can take up again the electrons to form the reduced form. What actually we have? the picture of electrons being taken up, protons being transferred.

The electron transfer cofactors be it coenzyme Q, be it NAD+, FAD. They help in the transfer of these protons and electrons. That is essentially what we were looking at. So we have, finally the oxygen reduction to 2H 2 O.

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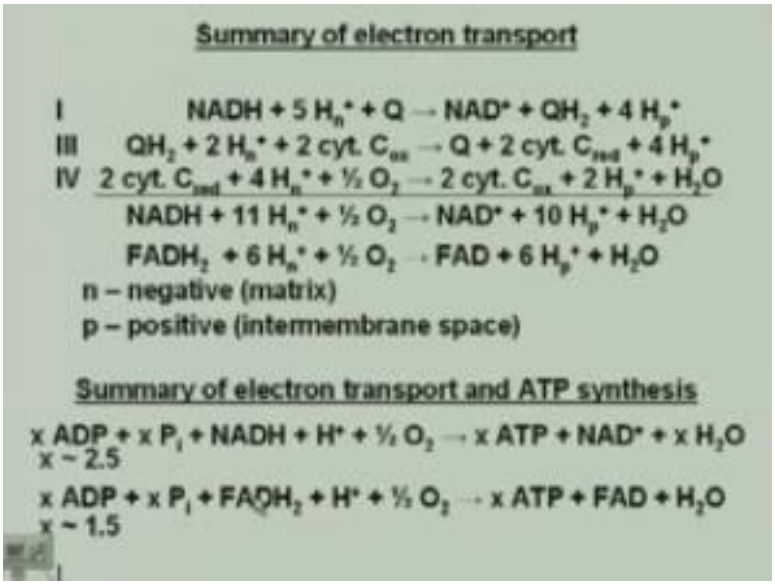

This is the summary of all the complexes that we have. We have eventually NADH + 11H n plus + ½ oxygen, give you NAD plus + 10H plus p side + H 2 O. And FADH 2 + 6 n plus + ½ O 2, go to FAD + 6 H p plus. Now, if we consider actually the summary of the electron transport and ATP synthesis, we actually have xADP + xPi with NADH + H plus, give you x number of ATP. In terms of NADH and NAD plus, in this system, for every NADH, you get 2.5 moles of ATP.

For ADP and Pi for the system FADH 2, 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 + Pi, if it is an NADH, NAD system, you will get 2.5 moles of ATP. And for the ADP set, you will get 1.5, so many ATPs synthesized.

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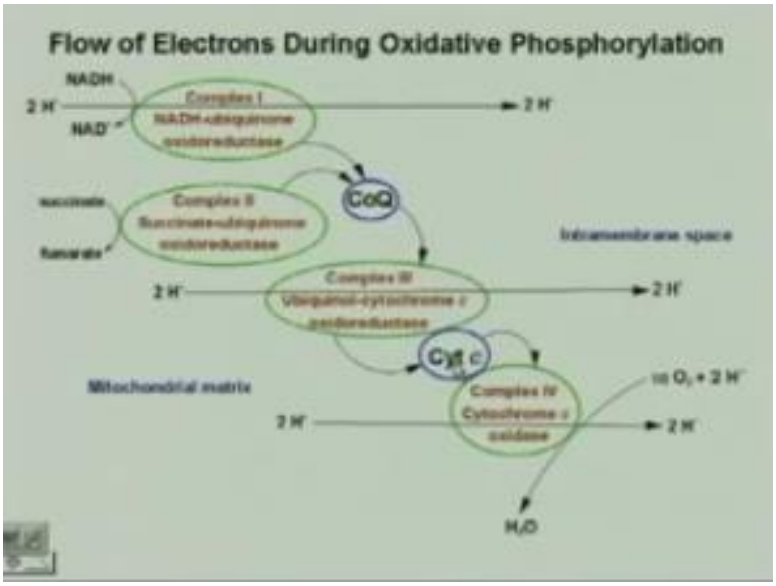

So, eventually what is happening is, you have the mitochondrial matrix and you have the inter membrane space, where you have done a transfer of protons. Then these protons, with the electrons, will form water when it reduces oxygen. But, in that formation,

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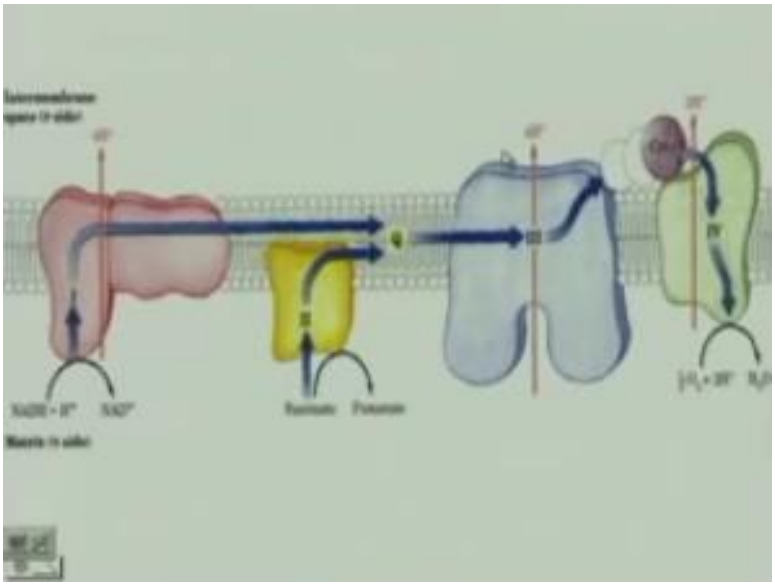

But in that formation, we eventually have to form ATP.

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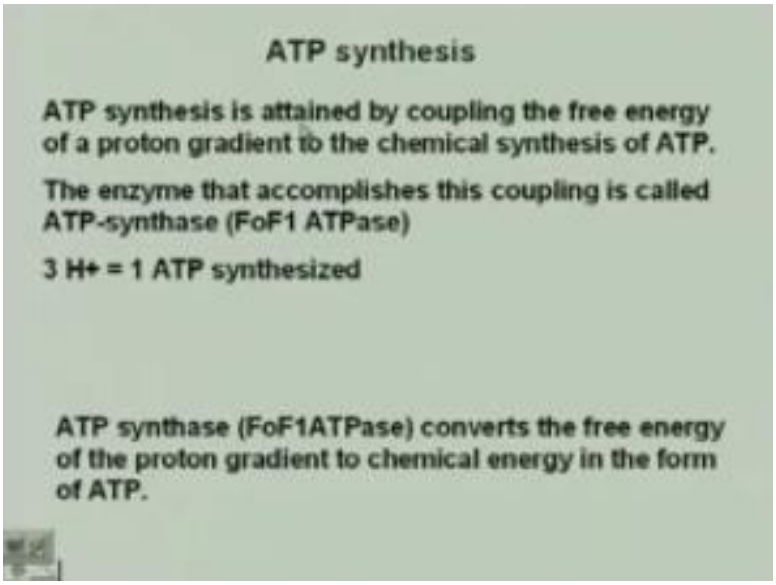

So, we have to get to ATP synthase. Now, this ATP synthesis is attained by coupling the free energy of the proton gradient to the chemical synthesis of ATP. And the enzyme that actually accomplishes this is called F0F1 ATPase and this is also called ATP synthase, because it works in a beautiful manner. Now, this converts the free energy from the proton gradient, which we just looked at, to the chemical energy in the form of ATP.

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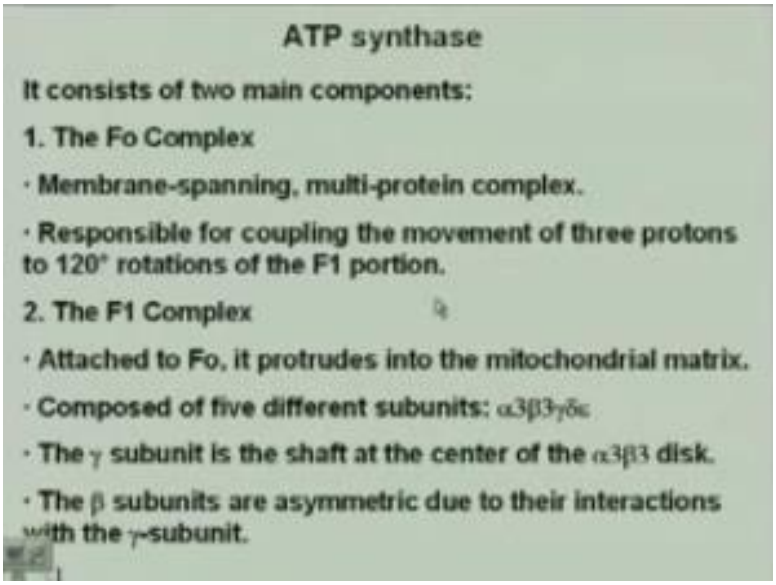

Now, the protein itself, is composed of two main components. You have the F0 complex that is a membrane spanning, multi-protein complex. It is responsible for coupling the movement of three protons to 120degree rotations of the F1 portion. You will see exactly what that means, in a moment. What we need to know now is that in ATP synthase, there are two main components. The two main components are an F0 complex and an F1 complex.

The F0 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. In the F1 complex, it is attached to F0 and it protrudes into the mitochondrial matrix. It is composed of five different subunits, named as alpha, beta, gamma, delta, epsilon. There are three alpha subunits, three beta subunits, a gamma, a delta and an epsilon. The gamma subunit is the shaft at the center of the alpha 3, beta 3 disk. And the beta subunits are asymmetric due to their interactions with the gamma subunit.

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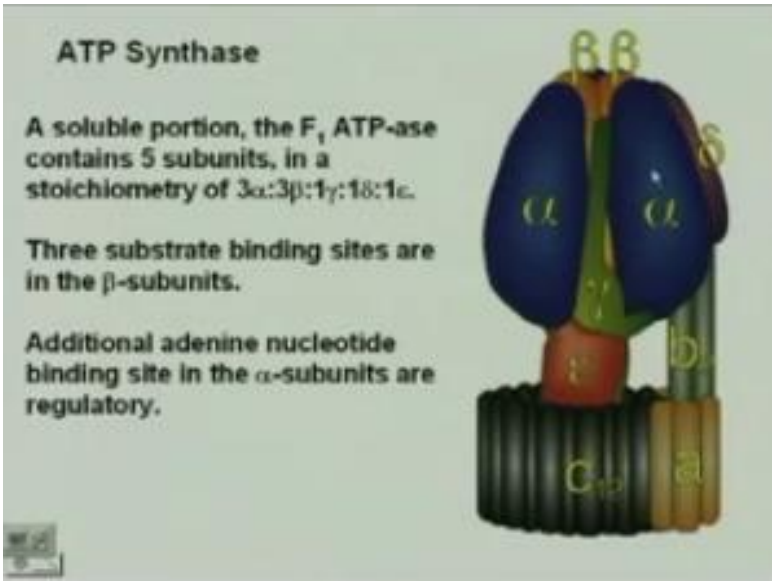

Now, let us see what each of these things mean. This is the picture of the protein. You see, there are alpha subunits, beta subunits; you have delta subunits and epsilon subunits. This part that is the bottom part is what the integral part to the membrane is. So, outside what sort of residues would we see? hydrophobic residues that would interact with the long fatty acid chains. There is a gamma shaft here that actually holds this subunit or these subunits together.

So actually it looks a stock. So, you have a stock, where you have six subunits, three of the alpha type, three of the beta type that are with this stock. Now, the beta subunit is, what the catalytic subunit is. That is actually going to produce the ATP. So we have this, this part is the F1 part and this is the F0 part the part that is connected with the membrane.

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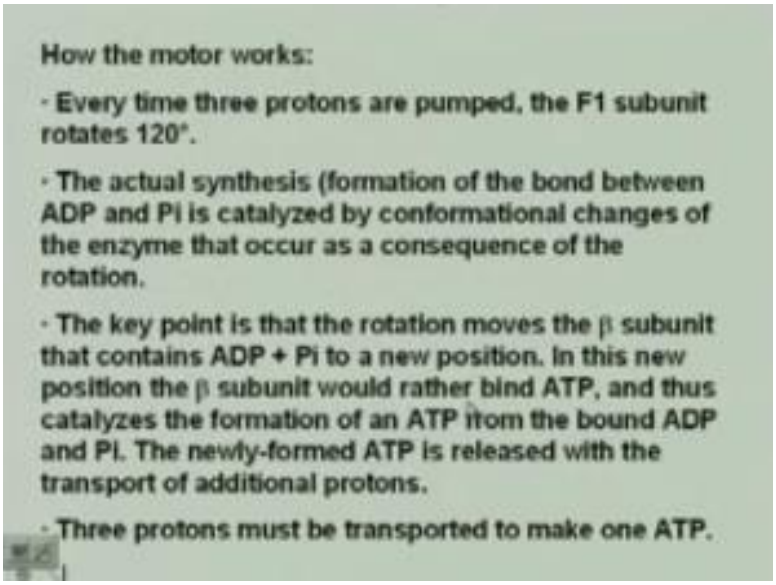

Now, every time three protons are pumped, the F1 subunit rotates by a 120degrees.

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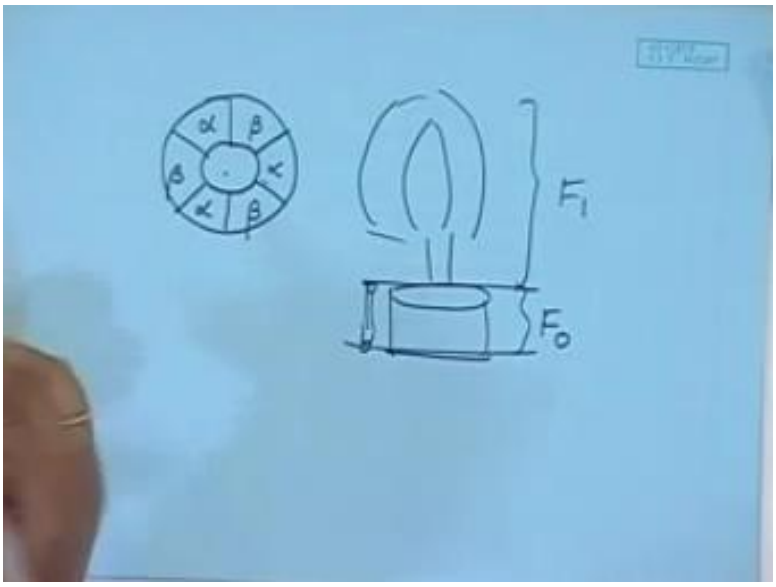

So, basically it looks something like this. If we just look at this, we are looking top down, we have a central part here, we have something like this say, so, I have a beta subunit here, an alpha here, a beta here, an alpha here, a beta here and an alpha here. It looks something like that. What is going to happen now is, there is going to be a rotation. This is connected to a stock down at the membrane.

So this looks like, actually like this, where you have a stock and this is the part that is connected to the membrane. So, this is your membrane and this part is your F0 part and this is your F1 part that is inside the mitochondrial matrix. So we have now a motor that works and every time we have three protons pumped, how are these being pumped now? 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 three electrons, rather protons pumped, the F1 subunit rotates a 120degrees. You see now that, as it rotates a 120degrees, each beta subunit will come into the picture, because if you are hooked on a beta subunit, you rotate a 120degrees, it will be on to a another beta subunit. And this gamma unit that actually acts as a stock and rotates and the beta subunit which is the catalytic unit is squeezed actually.

So suppose you have a stock like this and you have the three, we have the alpha units interspersing, now had we had the all six beta units, all of them catalytic, the rotation would have to be 60degrees. Now, we have alternating alpha and beta, so we have a beta subunit

that is held in a, it is called open type, loosen type, we will see that in a moment. I have an open form now.

As I rotate 120degrees, this beta form comes here now, which now makes this, the other one set tight and make this one loose, So we have open, tight and loose conformations, depending on where you are located in, with respect to the gamma stock that is rotating it. So, the actual synthesis is the formation ADP + P i and that is catalyzed by conformational changes of the enzyme that occur as the consequence of the rotation.

Now, what happens is, the beta subunit contains the ADP + P i. It is, say loosely connected. Then we have, at one point ATP being formed that is still within the beta subunit. At the third rotation, it has very low affinity for the ATP. So what does it do? It throws it out. So at one point, it has high affinity for the ADP + the P i, forms the ATP, but the ATP sits there. It does not move off. But as soon as the stock rotates again, it squeezes it out. So, this is exactly what happens.

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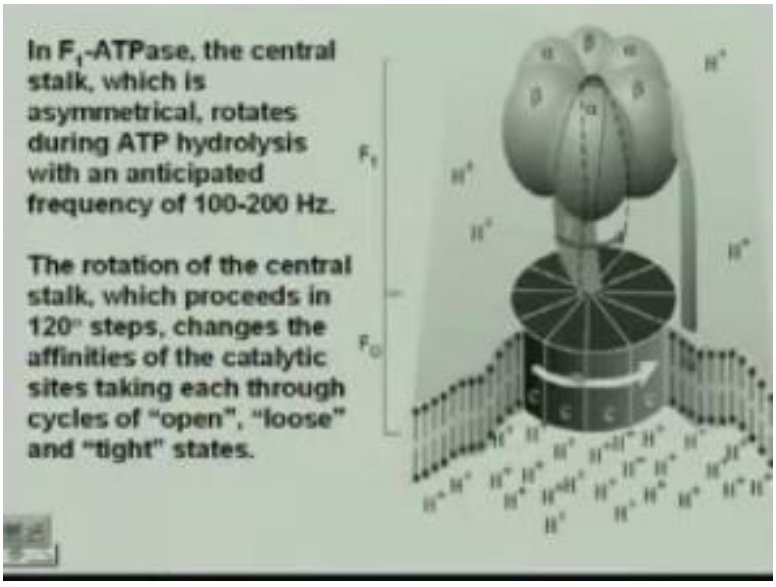

We have this rotation of the c units that are connected with the membrane and with this rotation we have the, see the gamma stock rotating, so what is that going to rotate? That is going to rotate these beta subunits. And as the beta subunits rotate, there are three states that it gets into, called the open, loose and tight states.

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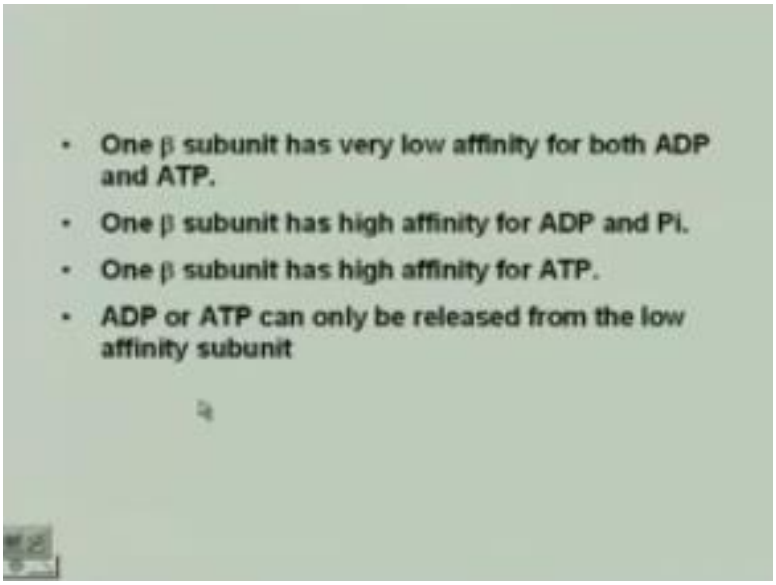

Now, one beta subunit has low affinity for both ADP and ATP. So, they will be irreversibly associated with the unit. One beta subunit has high affinity for ADP and P i. So, it is going to take in the substrates. The other beta subunit has high affinity for ATP. So, it will not release the ATP. It will 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 gamma stock shifts, it changes it from open, tight, loose state. So, 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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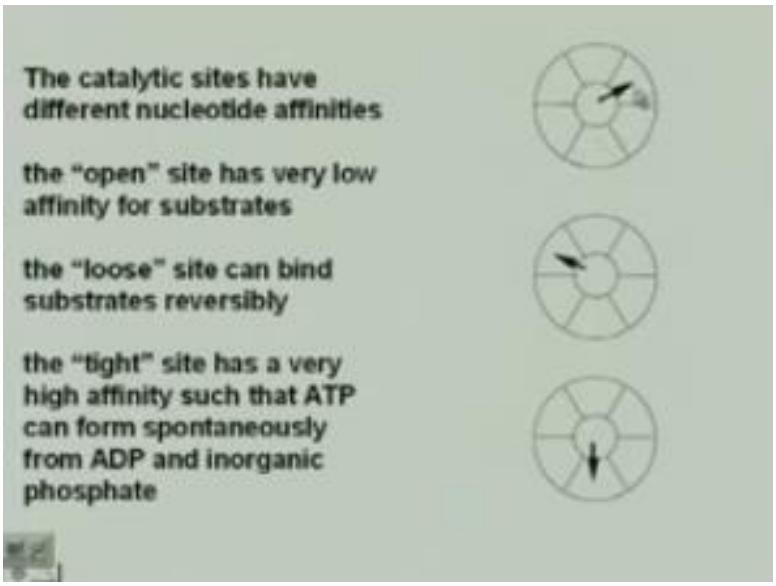

So we have here, so we have different nucleotide affinities, the open site that has low affinity for substrates, the loose site that can bind it reversibly and the tight site, which has high

affinity such that ATP forms spontaneously from this. So, this would be the shifting of the, it goes in anticlockwise direction, so we have the beta subunit shift and this deduction each time. So, eventually we have the ATP being produced.

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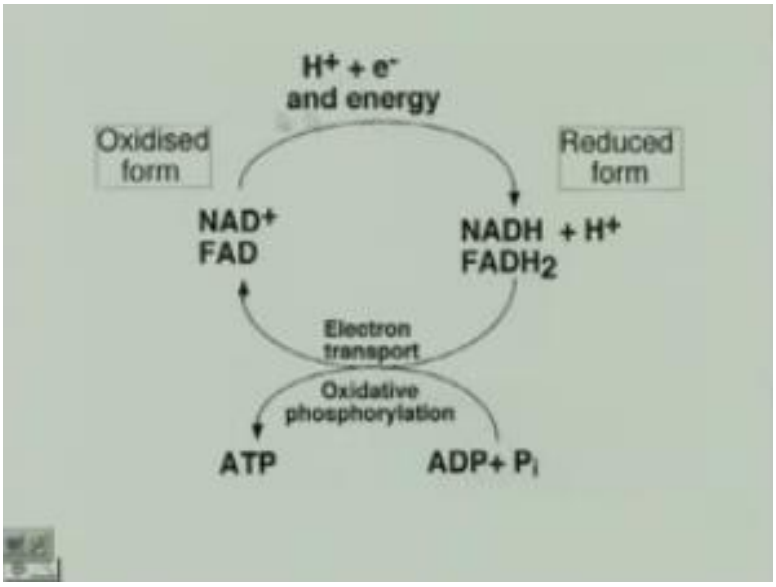

So basically what we have is, we have this process that is called oxidative phosphorylation. In the whole set of electron transport systems, we have the, all set the oxidized forms, the NAD plus and FAD going to NADH and FADH 2 the reduced forms, in the event of creating a proton transfer, a proton force, proton motive force.

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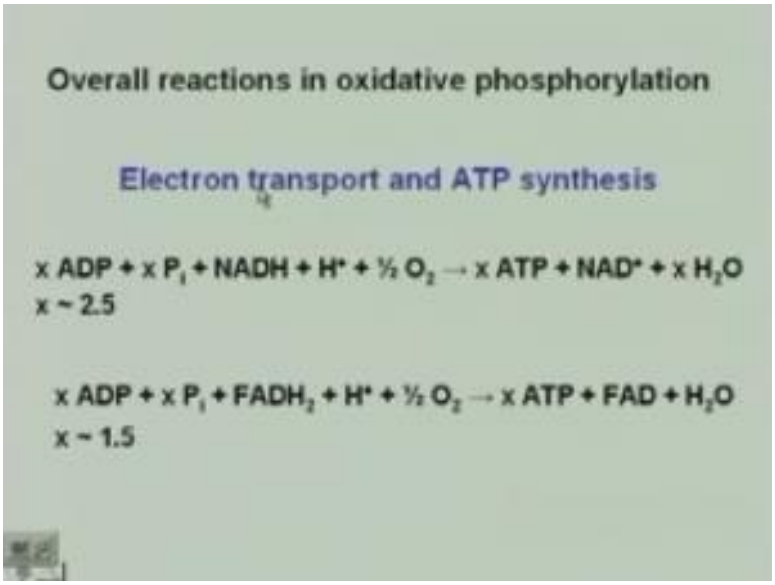

Then with the oxidative phosphorylation, it creates our ATP, which eventually gives us the overall reactions in oxidative phosphorylation that provide us with ATP synthesis. So what we learnt is how we can actually synthesize ATP, from the protons. So every three protons

that come in here, we will have the synthesis of ATP. This completes our discussion on oxidative phosphorylation. We will start metabolism in our next class. Thank you.