Introduction to Biomolecules
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Lecture – 22
Oxidative Phosphorylation (Part 1/2)

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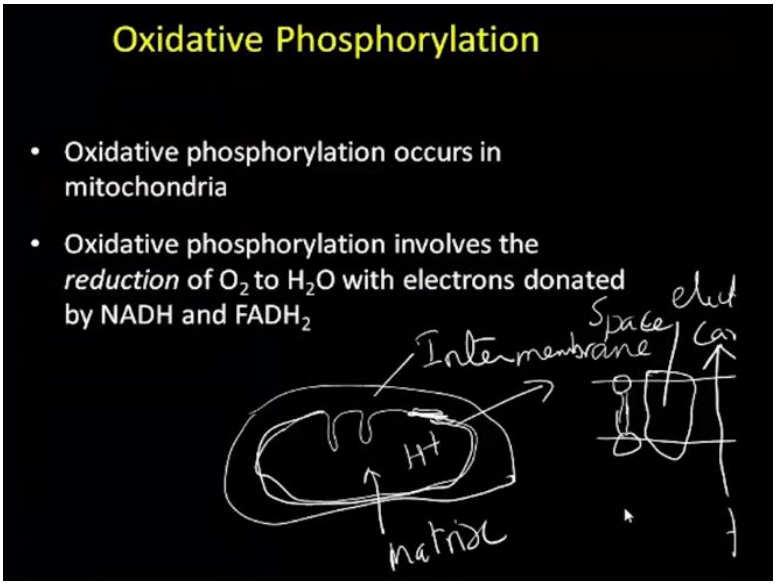

So, today we will discuss oxidative phosphorylation. So far, we have gone through the TCA cycle. So, we have seen the TCA cycle regulation as well and the main summary of the outcome of TCA cycle operation is the production of the reduced uh oxygen carrier or electron carriers like NAD and FAD in the reduced form, NADH and FASH 2. So, NAD carrier is a hydride ion so that is the form in which it carries electrons and FADH 2 carries us 2 hydrogen atoms.

So that is the outcome from TCA cycle. So, now we are going to look at what actually happens to these reduced on electron carriers. So that is the process that we call as oxidative phosphorylation. So, what it means is as these reduced electron carriers get oxidized the free energy liberated from that is used to for phosphorylating ADP into ATP. So, the oxidation is coupled to phosphorylation, so that is where this term oxidative phosphorylation comes from.

So, this process happens in mitochondria and this was I mentioned in the last class or the one before that this was discovered by, the author of this book Albert Lehninger and another scientist by name Eugene Kennedy. So today we will get into the individual steps of this

oxidation of these electron carriers in successive steps. The electron flow is in a stepwise manner and at each step we have protons getting pumped across the mitochondrial inner membrane.

So that is what we are going to see. Ultimately the electrons are going to flow through to reduce oxygen to water. So, the electrons are ultimately accepted by water and that gets reduced to water, so this is what happens. So, let us get into the discussion on this but before that I want to uh make it clear that you have a good idea of this mitochondrial structure that I think I drew last time too. So, I am just going to draw a part of it.

So, let us say in this mitochondria we have this inner membrane. I am not going to draw that to stay all through, just I am doing that a little bit. So, this membrane what is going to be critical, so this inner membrane, we are going to call this as the inner membrane. So, all the intermediate electron carriers they are all located in this. So, if I am going to take that and draw a section, so this is there all through and this is where these multi-protein complexes are located.

So, these are the intermediate electron carriers. So, their reduction potential is such that the electrons move from electron carriers of lower reduction potential to higher reduction potential so that will be the downhill flow of electrons and these carriers are located on this inner membrane. And as this happens so you are going to have a vectorial flow of protons from this inner side, this is called matrix. This is what is matrix.

So, from matrix to this is the inner membrane space or sorry it is normally called intermembrane because it is the space between two membranes, intermembrane space. So, when protons move from the matrix to the intermembrane space, you are actually going to build a proton gradient across this membrane. So, you are going to have more protons positively charged in the intermembrane space and negatively charged in the matrix so that is going to be the end result of this electron transport. So, let us go and look at individual steps.

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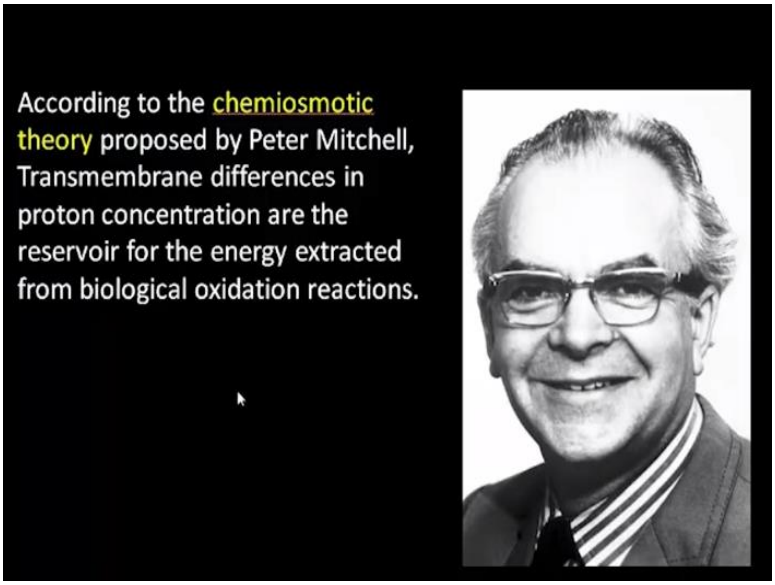

So, this proton gradient being generated on this proton gradient being responsible for the ATP synthesis, this entire thing collectively is called chemiosmotic theory. This was proposed by the British scientist Peter Mitchell shown here and he won Nobel Prize for this because this is one of the central findings in all of biology. This explains the mitochondrial oxidation and phosphorylation by which we get energy from the food that we eat.

And it also explains how energy from sunlight is used for splitting water and obtaining electrons and finally reducing carbon dioxide into carbohydrates. So, both these processes are explained by this chemiosmotic theory and so that is such a profoundly central thing to biology and that's why he won Nobel Prize for this discovery. And several experimental validations and testing of the predictions of chemiosmotic theory all of them came to be true and therefore this is a well settled fact in biology.

So, it is extremely simple. We actually have already understood what actually happens in this diagram itself. So, essentially what is happening is you have proton gradient generated by transporting or pumping protons from the matrix to the intermembrane space by the electron flow, a downhill flow of electrons. And this proton gradient when these protons flow down the gradient it precedes with a large free energy and that is used for driving the phosphorylation of ADP to ATP. So, we end up generating two gradients here.

One a chemical gradient like this chemical species proton is higher on the inter membrane space and lower in the matrix. And second you also create an electrical gradient across this. It is for more positively charged than the inside and these two gradients are the driving force of

ATP synthesis. So, this is what is the proposed by chemiosmosis theory. So, it is osmotic because you have a chemical species gradient and it is also a chemical gradient as well and that is why this is called chemiosmotic theory.

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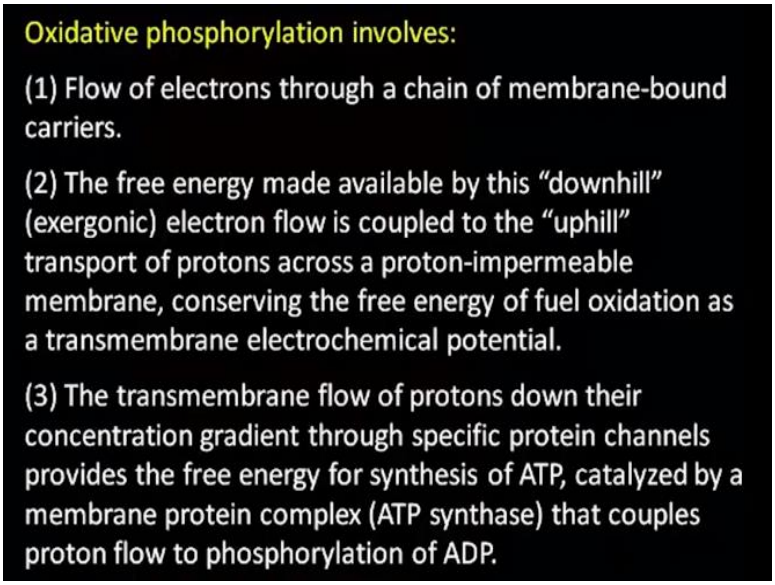

So what are the processes? We will see the chemiosmotic theory in some more detail when we actually complete this oxidative phosphorylation steps and get to the final step. And before that this is just a brief introduction to chemiosmotic theory. So, now what we will do is we will look at the individual steps involved in the oxidative phosphorylation. It is extremely simple to understand in terms of the main concept, the complexities in the details.

And you may be quickly reading and getting the summary of the process, but it actually was a monumental work and one of the phenomenal achievements in biochemistry and structural biology because these are large complex proteins and they are membrane-bound proteins, solving their crystal structures and getting the mechanism of electron flow was extremely challenging. So, this whole thing that you are learning now did not exist in the early 80s when I was learning biochemistry.

It just did not exist. We knew chemiosmotic theory, we just had an idea this is what is happening, but the details of it was not there or in the mid 90s is when the crystallographic techniques became robust and powerful enough that they could solve the structures of these proteins. So, the complexity is in those details in terms of idea it is extremely simple. So, you have electrons flowing downhill, driving uphill transport of products.

So the step 1, flow of electrons through a chain of membrane bound carriers and that flow is in this sequence free energy made available by the downhill electron flow. So, when electron carrier to another electron carrier is a downhill flow but it is in steps and each step the energy available is sufficient enough to drive a certain number of protons across the membrane. So, there are 3 steps at which protons are pumped across the inner membrane of mitochondria.

And so this is coupled to the uphill transport of protons across the proton permeable membrane. This coupling is what those carriers do, the multi-protein complexes that we are going to see as electron transport complexes. And conserving the free energy of this electron flow that is fuel oxidation into a transmembrane electrochemical potential, you have a charge gradient as well as gradient of a chemical species in this case it being proton.

And the third step the transmembrane flow of a protons the gradient being built by the step 2 now down the concentration gradient of these protons is coupled by this enzyme ATP synthase. A really fascinating enzyme and we are going to look at its structure and it is a mechanism of catalysis and that is used for making ATP by this protein ATP synthase. So these are the main things.

So, electrons flow uh from one carrier to another carrier on the membrane and that downhill flow is coupled to uphill pumping of protons, then at the third step when the protons return back down the gradient, they drive this ATP synthase to make ATP.

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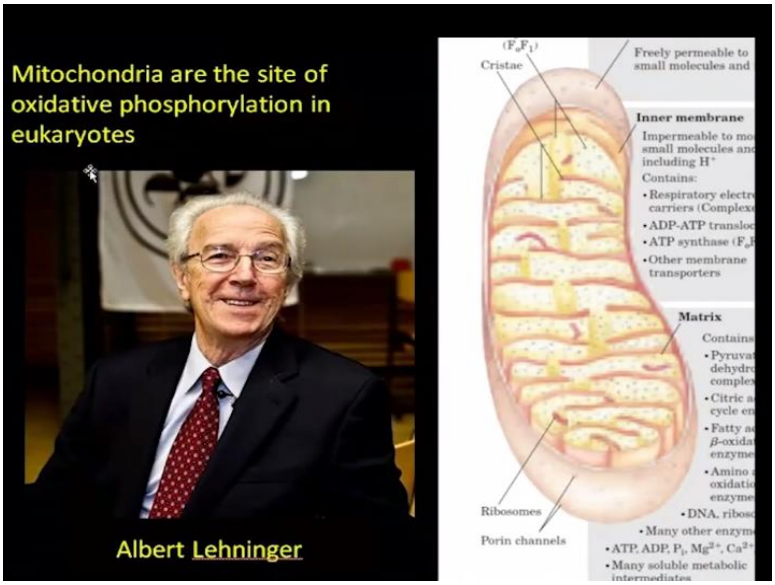

This is the first thing. I already drew this structure. I felt this was not making it easy although it is 3-dimensional and telling you the matrix space inner membrane being folded and then the outer membrane all that. So, this was identified by Albert Lehninger, he is the first one to show that the mitochondria are the site of oxidative phosphorylation in eukaryotes.

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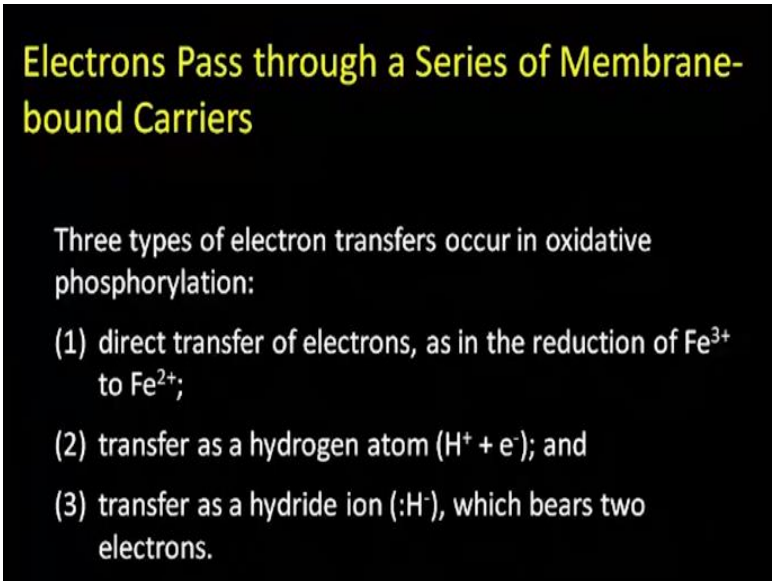

Since then, people focused on the mitochondria to work out the intermediate steps and the complexes and electron carriers involved. So how does electron pass through these membrane-bound carriers? So already we kind of know this as direct electrons, in this particular case we are going to primarily see ferric to ferrous and ferrous to ferric reversible oxidation reduction.

Then as hydrogen atoms like FAD it will have in that form and the hydride when it comes from NAD it usually comes in this form. So, these are the 3 ways in which electron is going to flow through.

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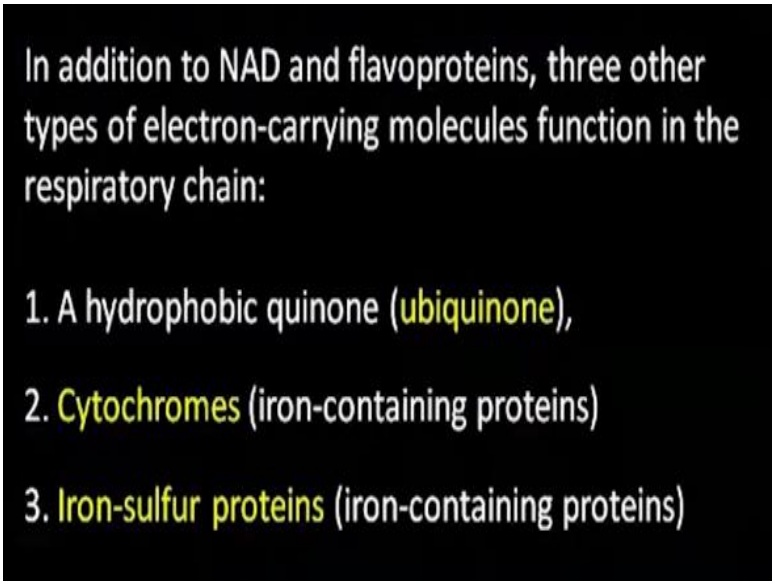

So, we will look at these electron carriers now, so that is where we are going to focus. So already we know NAD and FAD very well and where they accept the electron that also we know, so the nitrogen atoms. So now what we are going to do is we are going to look at these highlighted 3 electron carriers. One of them is already familiar to us, we have learned this ubiquinone. So, anyone remember when we discussed ubiquinone?

Alright, I am not hearing any answer, I will go ahead. So, we discussed this when we were discussing the lipid soluble vitamins A, D, E K. So, ubiquinone one of those hydrophobic pigments that we learned and this ubiquinone is freely diffusible within the inner membrane and that helps it to carry electrons from integral membrane protein kind of electron carriers from one of them to another one by freely diffusing from one complex of integral membrane proteins to another complex made of similar proteins.

So that is what ubiquinone does and cytochromes these are proteins with the heme as prosthetic group and the main moiety that gets oxidized and reduced are the iron ions Fe 2+ and Fe 3+. And then we have a third kind of proteins they contain iron instead of being as part of heme they are coordinated to sulphur atoms either the sulfur coming from the cysteine sulfhydryl group or inorganic sulfur. So, we look at the structure then it will become clear.

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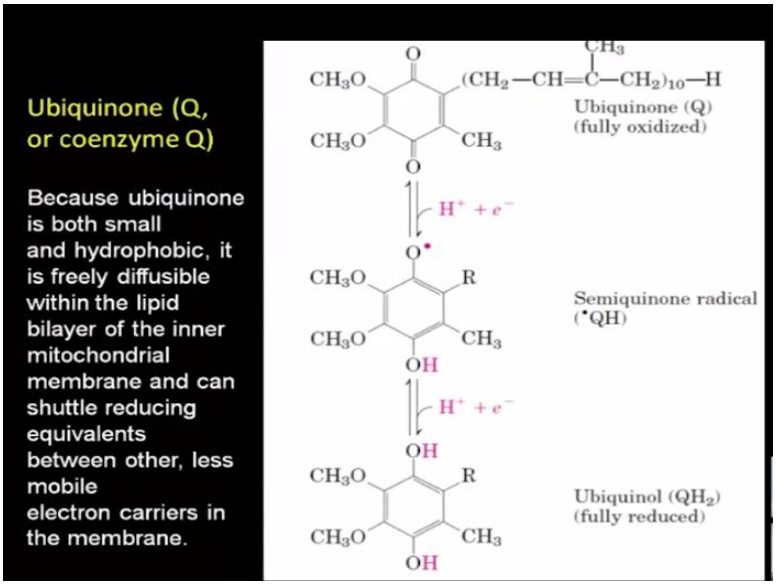

The first is the ubiquinone. So, this structure we have already seen in the last part of the lipids chapter. So, these ketone groups are the ones that are going to be reduced. So, the quinone this one comes from these ketone groups. So, you can accept one electron and one proton and get reduced to semiquinone. So, it is a free radical version, so it is not like very stable compared to the fully oxidized or fully reduced one.

Then it can accept another electron and another proton and get fully reduced to the alcohol form ubiquinol. So, because of its ability to accept 1 electron at a time this helps in bridging molecules that donate 2 electrons or 1 electron and the molecules that accept 2 electrons or 1 electron. So, it can bridge them readily. If a molecule is going to give two electrons at a time ubiquinone can accept them because it can actually accept 2 electrons to get fully reduced.

If a molecule is going to give up only 1 electron that is also fine with ubiquinone, it will accept 1 to go from ubiquinone to semiquinone or semiquinone to ubiquinol. So, that is an important feature of this molecule for coupling 1 electron or 2 electron donors or acceptors with another electron donor or acceptor of similar nature. And second important feature of ubiquinone comes from its hydrophobicity.

So, it can freely diffuse within the lipid bilayer of the inner mitochondrial membrane and shuttle reducing equivalent. Reducing equivalent meaning the electrons between less mobile electron carriers. I told you these are multi-protein complexes meaning multiple polypeptides chains in one protein complex and they are less mobile at least, you know I may not say immobile considering the fluidity of the biological membrane.

So, they are less mobile electron carriers. So, they are not going to transport from one to the other. So, usually ubiquinone is the one that does that job.

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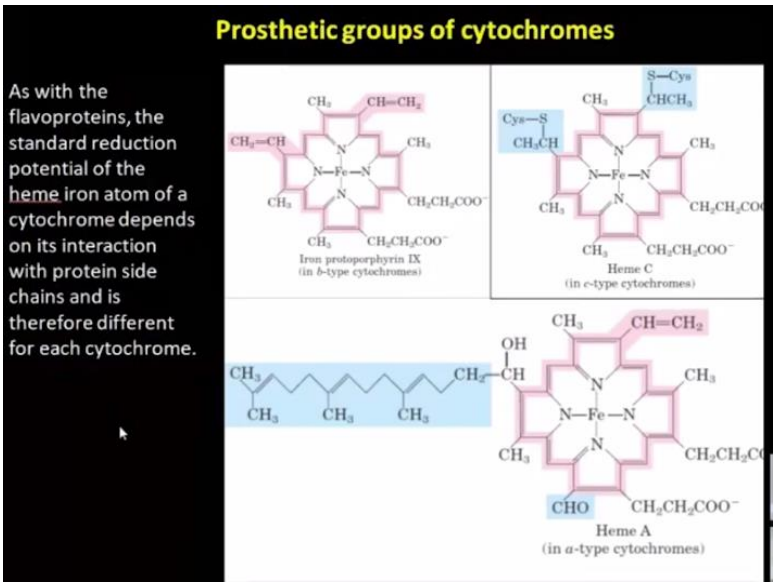

So the next set of electron carriers we are going to look at are the cytochromes. They are 3 types, cytochrome A and then you have cytochrome B and then cytochrome C. So, these proteins contain the structure shown here, the porphyrin rings the pink color conjugated double bond structure. This is what we call as the heme moiety. So, this is present in these cytochromes as prosthetic groups. They are usually tightly bound.

For example, if you look at cytochrome C this particular version of the porphyrin enduring structure it is covalently attached to the sulfhydryl group of cysteine of the cytochrome protein, you can see two such attachments. So, it is covalently attached. So, it is really tightly bound and they vary a little bit like for example this is covalently attached, this has a long isoprenoid hydrophobic side chain and so this has two double bond structures in B type.

So, this is protoporphyrin 9 and this is heme C and this is heme A, slight variations in their structures. And the main point is in all of them these nitrogen atoms or the porphyrin ring structure coordinates an iron ion Fe and that is the one that is going to be oxidized and reduced. So, this is what we meant when we thought of this step that is direct electron transfer from ferric to ferrous and that happens in these heme moieties in cytochromes.

And so just like flavin nucleotides here since this heme is tightly bound the reduction potential of the different types of cytochromes is completely dependent on the interactions. So depends on the heme ion atom of a cytogram depends on its interaction protein side chains and is therefore different from one side acronym to another cytochrome. So, there are more details, I am not going to go into those details.

So whatever detail I am going through is just sufficient for an introductory class, any more reduction in this then you would not be learning these topics. So it is reduced to that, you will know that when you read the book. So, some of you are motivated to learn more about these processes please make sure you read the book.

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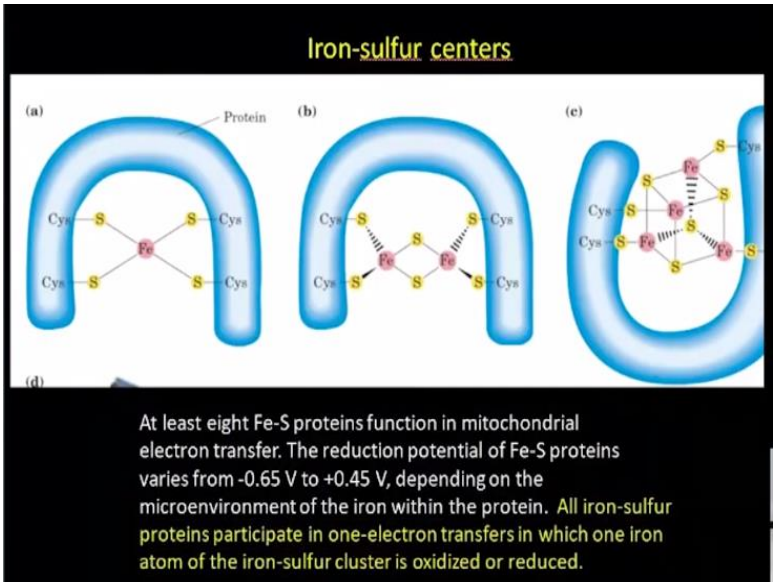

So, the third kind of electron carriers that we are going to learn are the iron-sulfur centers. So, they are very much like cytochromes in that the iron ion is the one that is going to be oxidized and reduced that is the one that is going to be the electron carrier. But in cytochrome we saw the ion is coordinated to the nitrogen atoms of the porphyrin rings, here instead they are coordinated to the sulfur from cysteine residues of the protein or in a more complicated way you might have inorganic sulfurs as well as cysteine sulfurs.

And you may have more than one iron ion. In this case you see two and in more complex structures you see four of them. So, these are the Fe S proteins, iron-sulfur proteins. And there again the reduction potential varies depending on the micro environment of where this ion is located in the protein. And these proteins are highlighted here, they participate in one electron transfer. They accept one, donate one.

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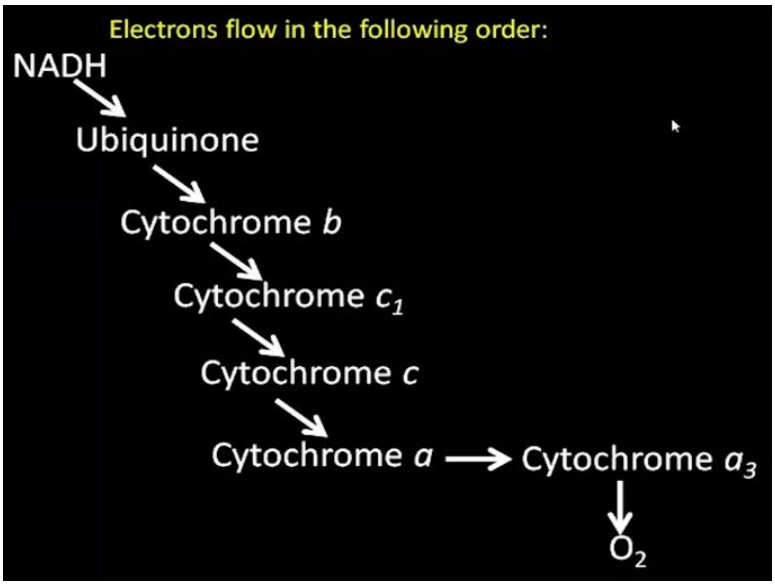

So now by looking at the relative reduction potentials of these complexes and also by looking at in an experimental way like for example you allow them to be reduced but you do not supply oxygen, then when you suddenly allow oxygen which ones get oxidized first and what is the order of oxidation of these that is another method. And third by using inhibitors that inhibit electron flow in the chain of electron carriers.

So, if you inhibited a step, then what happens is all the electron carriers before that will all be reduced and all the ones after that will be oxidized. So, by these three experimental means people have deduced the sequence shown in this slide. So, the electrons flow from NADH to ubiquinone, so this is complex 1 where this happens NADH ubiquinone oxidoreductase. And from there by complex 3, complex 2 I will tell you separately, complex 3 transfers ubiquinone to cytochrome c via these intermediates.

Complex 3 is going to have these proteins as part of it. And complex 4 transfers from cytochrome c to oxygen via this a and a 3. So, this is the sequence scientists have deduced by those three means that I just listed. One of them is simply comparing the reduction potentials. So, the electron flows from lower reduction potential carrier to the higher reduction potential. So, purely by knowing the reduction potential of them this series was predicted.

And then the second was an experiment where you allow the electron transport to happen so that all the electron carriers get reduced but then oxygen is not provided. So the ultimate acceptor is not there. Now when you allow oxygen, then this is the one that is going to be

oxidized first, meaning this is the downstream most and then the next will be this and so on. So that also gave the same sequence as predicted from the reduction potential.

And the third experiment was using inhibitors. For example if an inhibitor blocks the electron transfer from cytochrome b to c 1, then NADH ubiquinone cytochrome b these will be reduced as long as substrate from which electrons are available and when oxygen is available these are going to be oxidized. And by using variety of inhibitors blocking at different steps that again suggested in the same step. So, from all three put together we have this as the sequence of the electron carriers via which the electrons flow from NADH to oxygen.

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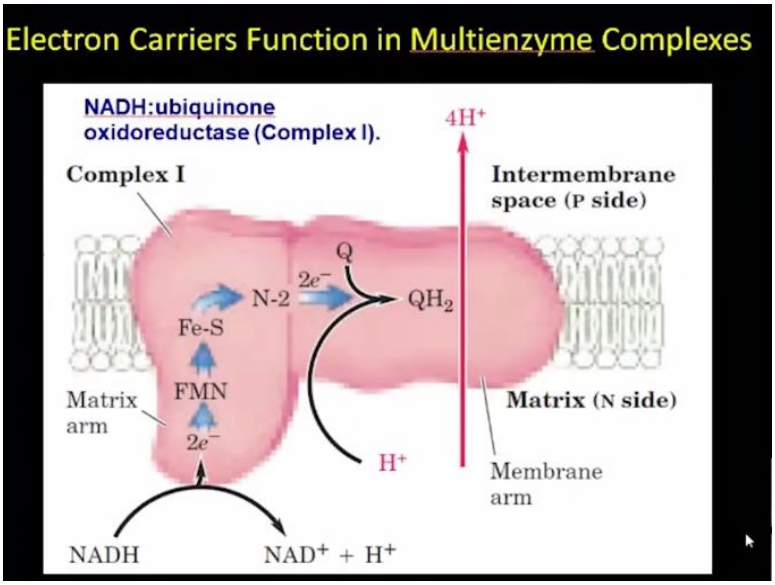

So now to the actual structure. So, this is the multienzyme complex called complex 1. It is also known as NADH ubiquinone oxidoreductase, so that is the job it does. It transfers electrons or it oxidizes NADH, reduces the ubiquinone. So that is the oxidoreductase. So here the orientation is important. So, this is an L-shaped structure, So, this is not a single polypeptide you need to remember.

This is a cartoon shown for the simplicity so that we readily understand, but it is a multiple polypeptide containing protein complex. This is the matrix side. So, remember the structure of the mitochondria that I drew in the first slide. So, the inner most space is the matrix. So, this is the inner membrane. So, this is the intermembrane space. P side meaning positive side because proton gets transferred across.

And because of that this is relatively negative to this intermembrane space so that is why it is called N side. So, in this what we are focusing on is the direction of electron flow. So, from NAD electrons flow to a flavin mononucleotide prosthetic group containing protein, from there to an iron-sulfur protein and then finally to ubiquinone. In this process, ubiquinone gets reduced to QH 2 because this is going to give you a hydride, 2 electrons and 1 hydrogen and one proton taken from the matrix.

So, you have a net 2 hydrogen atoms are transferred here so you get the reduced QH 2. And the free energy available from this drives the pumping of 4 protons across the membrane. So, essentially five protons are taken from here, one went into reducing QH 2 and the 4 pumped across. So that is what the structure of this molecule achieves. When it allows this electron to flow whatever conformation changes that happen that end up pumping this. So, this is complex 1.

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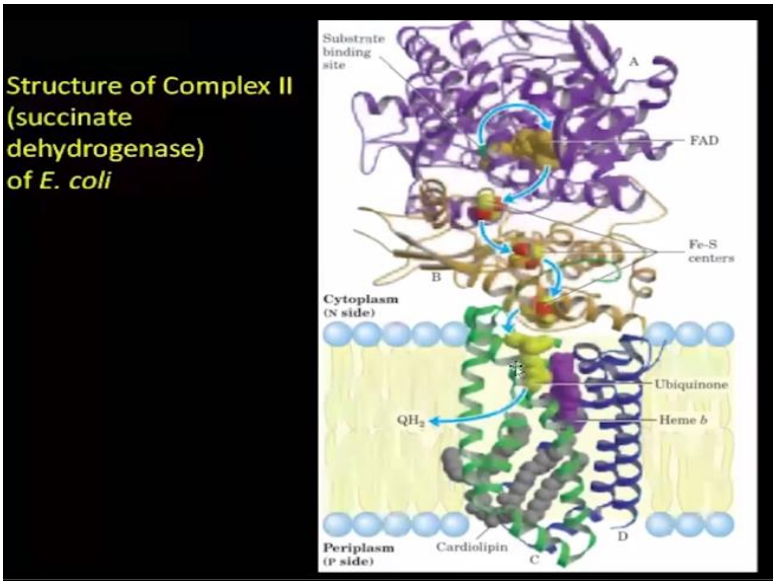

Now let us look at complex 2 that I did not mention in the initial introduction so that is because it actually comes from a sort of a loop or a bypass sort of thing or when we see the overall structure will understand. So, this complex 2 is something that we already know. So, in TCA cycle we saw succinate dehydrogenase which oxidizes succinate into fumarate and there I told you succinate dehydrogenase is a membrane-bound enzyme.

While all other enzymes of the TCA cycle are in the mitochondrial matrix. So that membrane bond that membrane is what is the inner membrane shown here this lipid bilayer. So, this is in the cytoplasmic side when you look at E. coli, E. coli does not have internal organelles and it

does not have mitochondria. So, it is a cytoplasm behaves like the matrix of the mitochondria in eukaryotic cells. So that is why it is written cytoplasm, this is not typographical error.

So, this cytoplasm of E. coli behaves like our mitochondrial matrix. And this periplasmic space that is the space outside of the plasma membrane of E. coli but still not to the outside medium, it still has cell wall etc. So that space between the cell wall and the membrane is the periplasmic space. This periplasmic space behaves like a mitochondrial intermembrane space. So, the intermembrane space equals the periplasmic E. coli.

So here the P side does not mean periplasmic side it means positive side like the charge is positive and this is negative. So here the succinate dehydrogenase so here you can see you can actually count by the color it is the ribbon model of the protein, so you have a purple 1 and this orangish yellow 2, then you have this blue 3 and then this green 4, so 4 different polypeptide chains. So, you have A, B and then C and D. So this is what forms this complex.

So, in this complex you have here the substrate binding side. So what is the substrate? Substrate is succinate, from there the electron transfer is to the FAD, from there it goes to this iron-sulfur centers, 3 of them and it goes this blue arrow tracks the direction of electron flow to finally to the ubiquinone and this reduced ubiquinone diffuses into the membrane. And this heme b located here that is not accepting or donating electron.

It is not in the path of the electron flow. People believe this prevents leakage of the electrons in this pathway and if that leakage is not blocked looks like the free radicals formed through the free availability of electron that ends up creating damages to the membrane and other proteins. So, for example mutations in these protein domains that affect this heme binding have been shown to have mild problems like benign tumor formation, etc.

So, this is the electron flow diagram in the complex 2. So, the complex 2 essentially transfers the electron from succinate to ubiquinone reducing into QH 2. Complex 1 did similar thing from NADH to ubiquinone.

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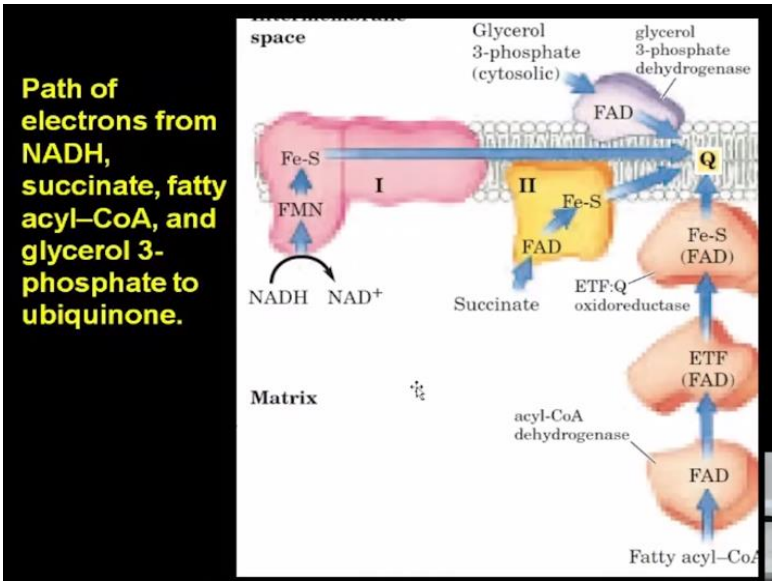

So, this sort of puts all of them together. So, this is the NAD, NADH that is getting oxidized by complex 1 and this is the succinate like if you are looking at eukaryotic cell and this is mitochondria where TCA cycle operates. So further from other enzymes you have the NADH and from the succinate dehydrogenase you have the complex 2 which is a membrane bound enzyme that is directly transferring to Q, it does not go via this.

And then there are other electron transfer complexes as well like for example glycerol 3-phosphate produced from cytoplasm. It comes from a variety of sources, one of them easy to understand is when you have a triacylglycerol where the acyl groups are removed you get glycerol and dihydroxyacetone phosphate when that gets oxidized you get a glycerol 3-phosphate. And from such a source of glycerol phosphate, this glycerol 3-phosphate dehydrogenase enzyme so that is bound to the membrane.

But on the other side like the cytoplasmic side compared to like in contrast to the succinate deodorants that is on the matrix side. And that again transfer to the ubiquinone. See now you understand the importance of the ubiquinone molecule. And the third source of alternative source like this I am taking as the main one because NADH is what is the primary one because most of the enzymes in TCA cycle three of the dehydrogenases produce NADH and in glycolysis we have one producing NADH.

So, this I am saying as the main and these are the two alternative roots and the third one is fatty acid oxidation. So only in terms of catabolism we are focused so far only on carbohydrates going from glucose to acetyl-CoA and in the interest of time I am not sure

whether we will have enough time to go into fatty acid catabolism. So, we have directly moved on to the oxidative phosphorylation.

But when we get time we will go and visit how fatty acids are oxidized as well, but for now we need to focus on what happens to the electrons from the fatty acid oxidation. So, this is the first enzyme of that fatty acid breakdown acyl-CoA dehydrogenase that is an FAD containing enzyme that transfers electron to the intermediate to one electron transfer protein ETF and that is also an FAD containing protein, it is transferred to that.

And that then transfers to this ETF ubiquinone oxidoreductase, this is iron-sulfur protein which transfers from ETF to the ubiquinone. So, these 3 protein complexes ultimately transfer the electrons abstracted from fatty acid oxidation to ubiquinone. So, now we have seen 4 routs by which electrons from substrate oxidation are transferred to ubiquinone in the inner membrane of mitochondria.

So, now we will follow the journey of electrons from ubiquinone. So, ubiquinone as we have seen can accept 1 or 2 electrons at a time. It can also donate 1 or 2 electrons at a time that becomes crucial for the next steps. So, the complex 3 is going to transfer electrons to cytochrome c and that accepts only one electron. So, this QH 2 having 2 protons and 2 electrons will have to do one at a time and that is accomplished by a process called Q cycle.

I will explain that while we are discussing the complex 3. So, complex 3 is going to oxidize ubiquinone and reduce cytochrome c. So, one important thing I did not mention about cytochromes is cytochrome c alone is attached to the surface of the membrane in the intermembrane space site of the membrane and it can freely diffuse within that space. While the other cytochromes are integral membrane proteins forming part of complexes. They are in complex 3 and 4, we will see them as we go.

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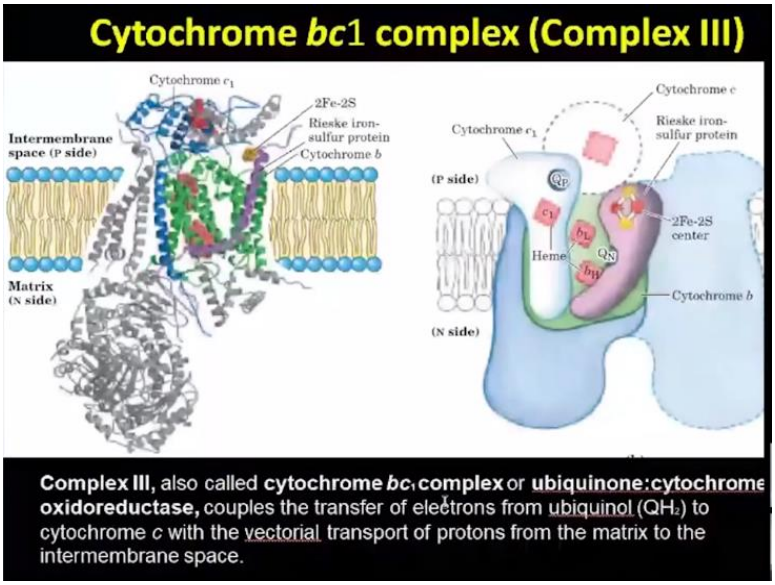

So, this is the complex 3. One of the biggest complexes of this electron transport chain. The book tells you this is one of the landmark achievements of structural biology during 95 to 98. Fortunately, I graduated 10 years before that and therefore I did not need to go through the structures of this, but it is really fascinating to understand how electrons flow through and know the mechanisms of how these complexes function.

So, this is the summary structure of that sort of a landmark structural biology work. So, this contains 60 polypeptide chains and we are not going to look at anything other than these colored ones that is the business part of it, meaning these are the ones that are directly involved in electron flow. So, we are not going to talk about these gray colored ones. And this whole structure is monomer of this complex, so do not get confused this monomer to be synonymous with a single polypeptide.

This is a multi-polypeptide complex which exists in two such units and this is the structure of one of them. So, in this cartoon diagram the other one is shaded out here, so it is this side of a structure two of them together form this complex 3. And it has multiple cytochromes you can see cytochrome b is shown here in green color and then you have cytochrome c 1. Then here this blue color one, sorry the blue color one is the cytochrome c 1 and you have this purple one which is the iron-sulfur protein.

So, I did not talk about this Rieske iron-sulfur protein primarily because I thought if you know the iron sulfur that we saw is good enough. So, instead of cysteine residues histidine residues are involved in this. So that is the prosthetic group for this protein. So, the electron

flow is best understood by looking at this cartoon. So, you have in the cytochrome b the green color one you have the ubiquinone binding site.

So, you have two sides, one in the c 1 that is Q P meaning the positive side and here the negative side look at the matrix side Q N. And within this you have these cytochrome c 1 heme moieties, 3 heme moieties shown here. And this is the iron-sulfur center of the Rieske iron protein. So, these are the ones that are involved in the electron flow. So where is electron coming from and where is it flowing to it is from the QH 2 which we saw in the previous step and ultimately it goes to the cytochrome c.

So, this is the only protein, this is not part of the complex. It is shown here for clarity of the substrate. So here cytochrome c acts as the, so cytochrome c is not shown here, do not think cytogram c and c 1 are identical. So, c 1 is part of the complex while c is not and the c is the ultimate acceptor of the electron coming from QH 2. So, this couples electron transfer from ubiquinol that is fully reduced ubiquinone to cytochrome c.

And just like what we saw in complex 1 here again this electron transfer leads to a vectorial transport meaning in one particular direction from matrix to inner membrane you have proton transfer.

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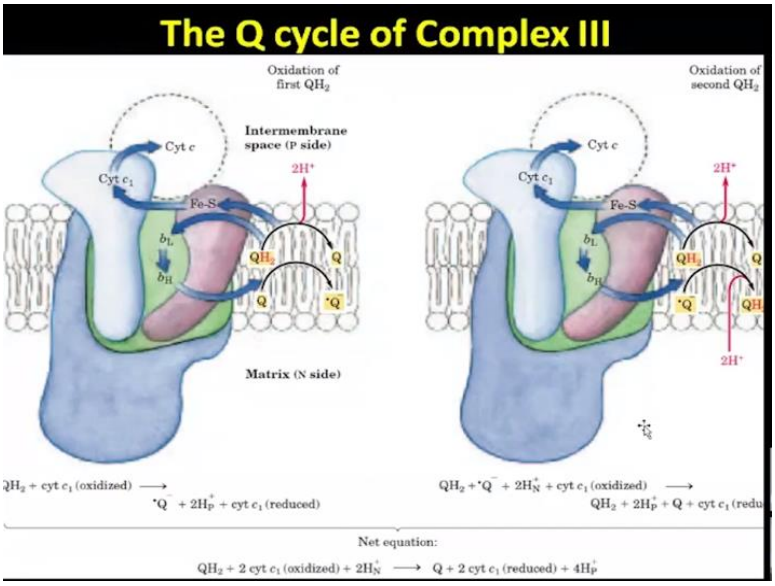

And so this is best understood by considering the Q cycle which is shown here. So, I will walk you through this step by step so that it is not very complex. So, essentially this complexity comes from one simple problem. The problem I already actually explained that is

QH 2 has 2 electrons and cytochrome c will accept only one at a time. So, we have to transfer this electron from QH 2 one at a time, we cannot take both of them and that is made possible by the Q cycle shown here.

So, let us start with the first one. So here you have QH 2 the first ubiquinone is going to bind here. From there one electron this blue arrow that points, transfers to the iron-sulfur center of this Rieske iron-sulfur protein to cytochrome c 1 and to cytochrome c, so one electron goes to the ultimate substrate. The other electron from this Q is transferred to these two heme moieties within cytochrome b back to ubiquinone, oxidized ubiquinone.

So, in this process QH 2 having donated the 2 electrons has become Q, it is fully oxidized. And this 1 electron that came back reduces a ubiquinone to a semi-quinone radical. So, this is the first step of the cycle. So here electron goes in cycle and that is why it is called Q cycle. Now in the second step, a second reduced ubiquinone binds to it, from there again one electron via this route goes to the cytochrome c.

So the second cytochrome c is reduced. And the other electron goes back to this radical semiquinone produced in the oxidation of the first molecule of UH 2, now this is reduced to UH 2. So, the end result is actually one QH 2 becoming Q, but actually we have taken 2 QH 2, but we returned one of it back. So, 4 electrons taken from the matrix, 2 gets used for this and only 2 are pumped out and this is what is Q cycle.

So, if you consider one at a time it is not very difficult. All that you need to remember here is out of the 2 electrons taken from fully oxidizing or fully reduced ubiquinol is only transferred to cytochrome c. The other electron is shuttled back by the cytochrome b to a fully oxidized ubiquinone and that is reduced to the semiquinone radical. And when you oxidize another fully reduced ubiquinol 1 electron goes to cytochrome c just like the first one.

And the other electron comes to reducing the semiquinone free radical to the fully reduced ubiquinone. So, this is the ultimate summary of the reaction, but these are the individual ones. So, this is how this coupling happens between 2 electron donors and 1 electron acceptor. So now when you are thinking of ubiquinone, semiquinone or ubiquinol this is not very difficult. But consider electrons going from FADH, even FADH 2 let us not take.

Let us take NADH, it accepts and donates in hydride ion format. Meaning forget about that 1 proton there, but it is taking 2 electrons at a time and it is giving up 2 electrons at a time and the ultimate substrate here is cytochrome c which will accept only 1 electron. So, what do you do with the other electron coming from the NADH? And that is handled by this specific chemical nature of ubiquinone and by the operation of complex 3.

So, this is all extremely sophisticated molecular machinery in biological systems is built by using those few elements and organizing them and bonding them in a very smart way. This is where the 3.5 billion years of experimentation has gone in. So, this is the way I want you to look at the molecular structure when you learn biochemistry. So that is when biochemistry is not a confusing complex, instead it is a fascinating biological phenomenon.

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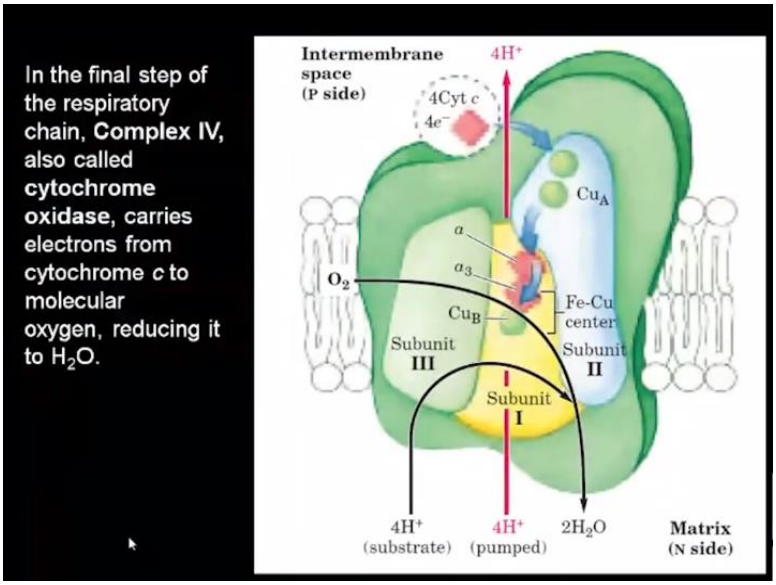

Alright, so now we have moved the electrons from NADH to cytochrome c and once reduced cytochrome c freely diffuses within the intermembrane space and binds to the final complex and that final complex is complex 4. So, this is cytochrome c oxidase because the other product here is oxygen directly. So, the electrons are transferred directly to oxygen. So, for every 4 electrons transferred from 4 cytochrome c an oxygen molecule is reduced by taking 4 protons from the matrix to 2 water molecules.

So, the water molecules split by the chlorophyll which we will learn later when we go to learning photosynthesis and those water molecules are now returned by cycling the electrons through the biological systems and making life possible and finally to water. So, let us look at

the electron flow in the complex 4. So here again like complex 1 and the complex 3, you have proton pumping happening.

For every 4 electrons you have 4 protons pumped across, and for the first time we are encountering a new metal here copper. So here you have 3 main proteins. We are ignoring this big portion, so it is part of the complex, we are only looking at the polypeptide chains directly involved in the electron flow subunit 1, 2 and 3. So it flows to the subunit 2 where the metal ion that is oxidized and reduced is copper and to iron-copper center in subunit 1.

And then finally in that so you have this, sorry before that you have this heme groups a and a 3. These are the cytochromes. So we have encountered cytochromes in complex 3 and now in complex 4. So in complex 3 we saw cytochrome b and cytochrome c 1, I am not talking about cytochrome c that is freely diffusible within the intermembrane space. And then here we are seeing the cytochrome a and a 3 and these are the two heme moieties.

And finally to another copper ion in subunit 1 and from there it is transferred to oxygen and producing water molecules. And as this electron transport happens 4 protons are pumped across for every 4 electrons and this is how electrons abstracted from food materials like carbohydrates, fatty acids and so on are finally used to reduce oxygen to water and the free energy made available by the electron flow from glucose to oxygen.

Glucose being lower reduction potential to oxygen being the higher reduction potential. So in that downhill flow, the free energy liberated is now conserved in the form of electrical gradient across this membrane and the chemical gradient across the membrane. So, you have proton concentration different and charge difference between the two so that is what has been accomplished. So, I will stop here today and then the more theoretical considerations of this process that is the chemiosmotic theory we will do it tomorrow

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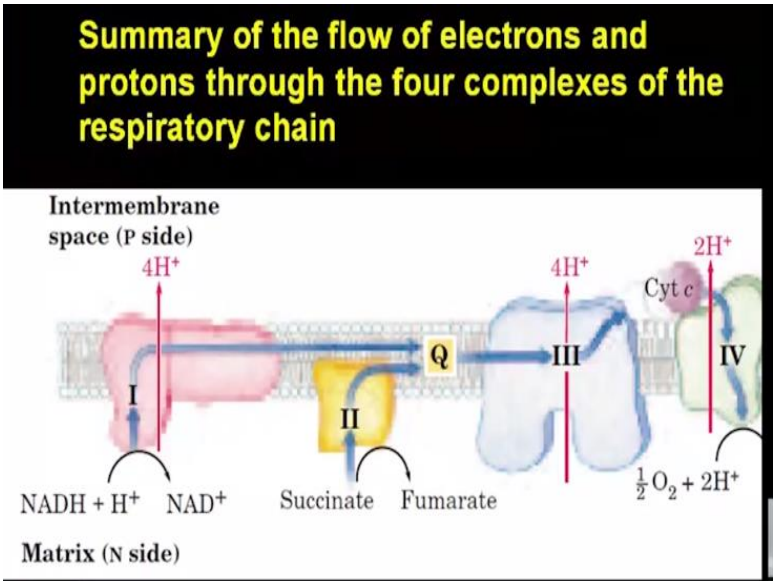

So just this is the summary of whatever we have learned so far. For every NADH that is 2 electrons so you have totally 10 protons pumped out, pumped into the intermembrane space and you have one water molecule produced from that. So, this summary is purely considering one NADH, but if you want to look at one molecular oxygen getting converted then it will be 2 H 2 O and in that case it will be 20 protons and we would have consumed 2 NADH.

So, this is the electron transport chain. So, we have not looked at the phosphorylation yet, we have only looked at the oxidation meaning the electron transport from lower reduction potential to higher reduction potential is what is oxidation. So, we have seen the oxidation, but tomorrow we will consider the chemiosmotic theory in little bit more detail and along with that we will also see the phosphorylation. So, I will stop here.