Introduction to Biomolecules
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Lecture – 23
Oxidative Phosphorylation (Part 2/2)

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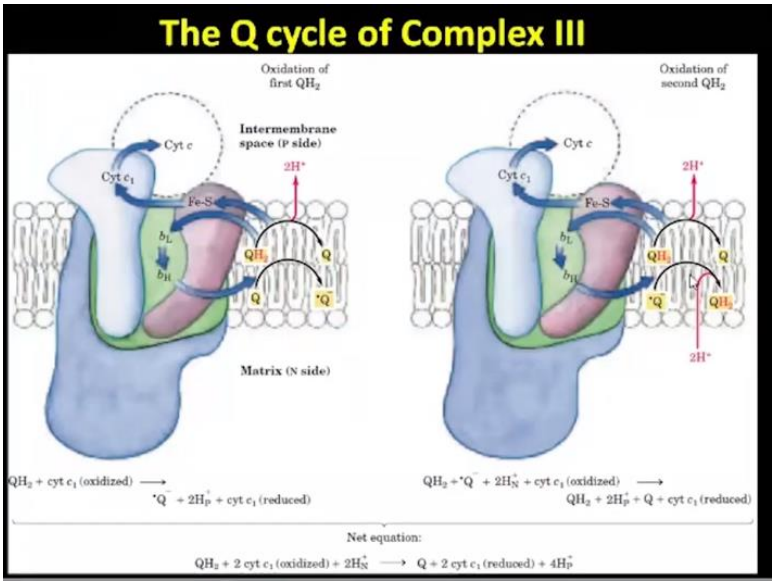

So, let us go from the first one. So here the main issue is that this fully reduced quinol, this QH 2 like where you know my cursor is, so from this one electron is transferred to cytochrome c and the other one goes back via the cytochrome b to a fully oxidized quinone and that becomes semiquinone radical. So, having given up both electrons and both protons, this becomes fully oxidized quinone.

Then in the second cycle one more molecule of fully reduced ubiquinol binds to this and from there through this iron sulfur protein one of the electrons go to the cytochrome c, just like the first one. And the other one via cytochrome b these two heme returns to reducing this semiquinone produced in the first cycle back to fully reduced ubiquinol. So while the electron has come back but the proton has not, you know it is pumped out.

And as a result, we take protons from the matrix side to fully reduce it to QH 2. So that is the reason protons are taken from here. So, here both protons are gone and here again both protons are gone and here it is not shown because this is radical and that fully to reduce you need to take the two protons.

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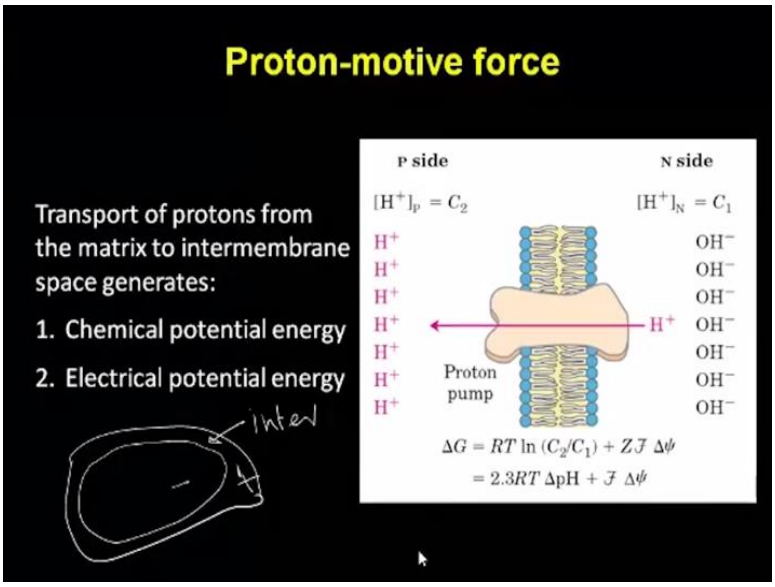

Went through these electron transfer carriers and then we saw how the electron finally goes to reduce oxygen to water. So, the electron transport part we have seen and then we saw in the process the protons are being pumped across the membrane as shown in this slide, you know it is in intermembrane space. So today what we are going to do is we are going to focus on how this proton gradient.

So, the gradient being it is a high concentration in the intermembrane space and low in the matrix of the mitochondria. So, once more to get clarity on this I am going to draw the same mitochondrial cartoon just for some of you who may not be so diligently following this. So, if this is the outer mitochondria, so you have the inner mitochondria. So, I am not going to draw all the crisp state. So, this space this is the inter-mitochondrial space.

So, this is where you have positive charge and this is where your negative charge. So, this pose in the slide cartoon the left side where the arrow points correspond to this space and where the arrow starts from the right side is this inner side. So, now the end result is we have a chemical potential energy here because the chemical species is proton, it has a concentration gradient.

Second you have a charge separation as well, therefore you have electrical potential energy. So, these two components are the energy part generated by the electron transport downhill, from a molecule with the lower reduction potential to a molecule with higher reduction

potential that downhill flow of electron resulted in generating these two components of potential energies.

So now when the proton returns back to the matrix, you are going to have a free energy change and that will equal this. So, concentration on one side; versus concentration on the other side plus you have to take the differential in the membrane potential. So, the Z is the actual electrical charge in the case of proton, for one chloride is just one. So now instead of natural log if you take log to the base 10, then it would really be pH.

You know log to the base 10 of C 2 will be pH, right. The pH is the negative log of hydrogen ion concentration. So, therefore this equation will rearrange into 2.3 times RT times delta pH plus Faraday's number times the membrane potential difference. So, this is the way we will obtain the free energy change in the downward flow of the protons.

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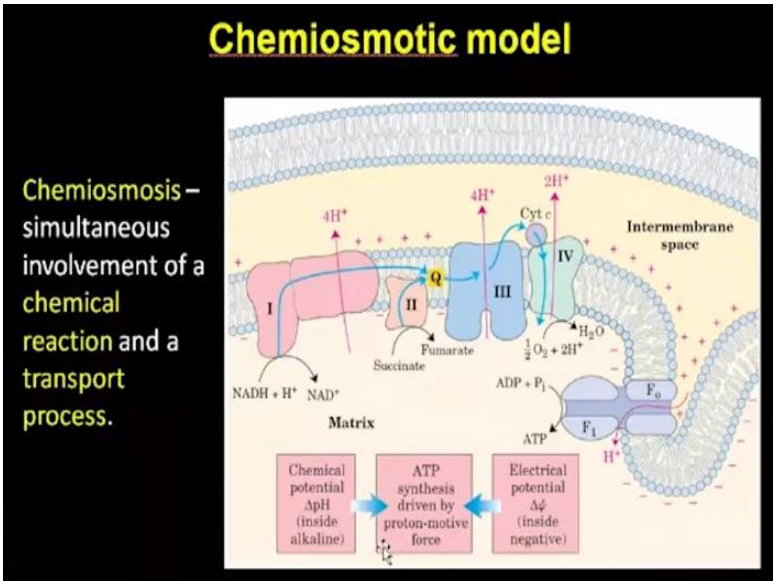

So this again brings us uh back to the chemiosmosis. So, this cartoon is much more clearly drawn here. So, this is the outer mitochondrial membrane, this is the inner mitochondrial membrane where all these electron transport complexes that we learnt are all present here. And so this is that cytochrome c that diffuses from this to this and ubiquinone is in within the membrane.

So, now as the electron flew from this to oxygen and becoming water, we have these protons pumped across by these three complexes. And so the numbers correspond to a single molecule of NADH as I told you yesterday and when you talk about molecular oxygen which

is what is normally consumed producing 2 H 2 O, then you will start with 2 NADH and the numbers will double here.

And when these protons come down, they actually come through this pump. There is no other way the protons can leak across the membrane and this pump the right analogy is like a hydroelectric power project. So, you have a dam where the water has been stored prevented from free flow down the river. So now you have generated potential energy. Now when you allow that water only through a narrow pipe, the pressure is going to be very high.

And that high pressure the potential energy difference from upstream of the dam to the downstream is now used to turn the turbine because that is the only way you are allowing the water to come out. And the turbine uh when it is in the magnetic field it generates electricity. Exactly the same thing that happens here to the last detail of the turbine. Here again you are going to have a rotation movement generated to produce the ATP molecules that we will see towards the halfway of the class today.

So, since this involves a chemical potential like pH difference and then you have a transport process, so to coming down gradient and that is why Peter Mitchell chose this word chemiosmosis to signify that the process involves a chemical reaction as well as a transport process. So, it is transported through this pump and to account for both of these, he coined this word chemiosmosis. So, both these energy components go into ATP synthesis.

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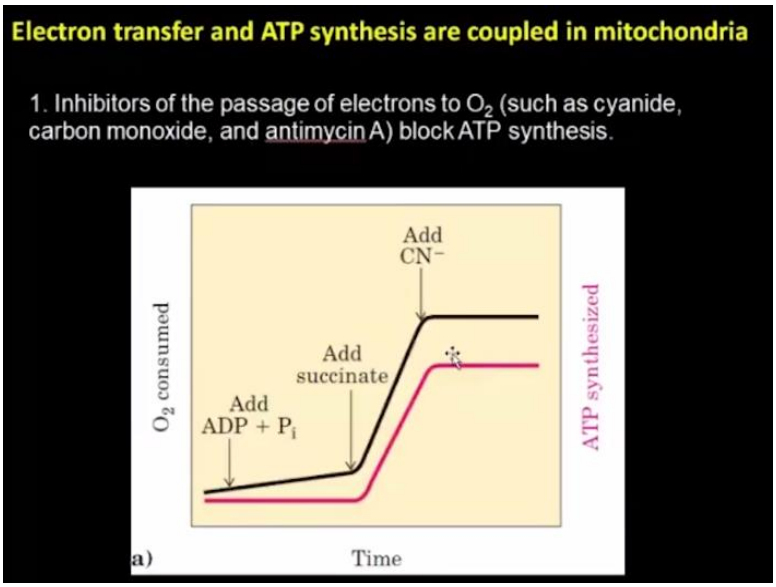

And this is readily testable by thinking about certain possibilities like prediction from this model and then testing it and then we know that it works. Like for example according to this model the electron transport or in other words oxidation and phosphorylation both will be coupled. So that is the only way the proton is going to come down. So, if we stop either one of them like for example let us say if you block this and the proton is not going to come.

Now as the electron transport is happening and as the proton concentration difference between the two sides keep increasing you will reach an equilibrium. You know as long as the proton is not going to come, you can only build a certain concentration gradient where you will reach equilibrium between this concentration difference and the energy available from the electron flow. So, as a result the electron flow will end up stopping.

So, blocking phosphorylation will lead to oxidation that is electron flow that is one prediction and that has been observed. And so we see some experimental evidence here. For example here the oxidation or the electron flow is measured by measuring the amount of oxygen consumed and also we measure the ATP produced for phosphorylation. So, this red line follows the ATP concentration.

And the meaning phosphorylation activity and the black follow the oxidation activity by measuring the oxygen consumption. So, when you have the substrate ADP plus inorganic phosphate with no electron donor, you do not have anything happening to either one of the two process. Then you provide a succinate, so remember complex two. This can transfer electron to quinone, so you can start the electron transport at this step.

And now both oxidation the electron flow as well as phosphorylation both happen. And now when you stop the electron flow by adding cyanide, this is why cyanide is poisonous. So, I did not get into all the different inhibitors, they are all listed in tables in book and you can readily see them. They really do not need explanations. So, when cyanide is added and cyanide blocks at this step a to a 3 in complex four and electron flow therefore stops.

So, oxygen consumption reduces and the important point is when you stop that electron flow or the oxidation automatically ATP synthesis also stops, so showing that these two are coupled. And this is what you would expect by this model. So, you need the electron transport to build the proton gradient and without proton gradient you are not going to do

phosphorylation and that is what this experiment. So that prediction is consistent with this observation.

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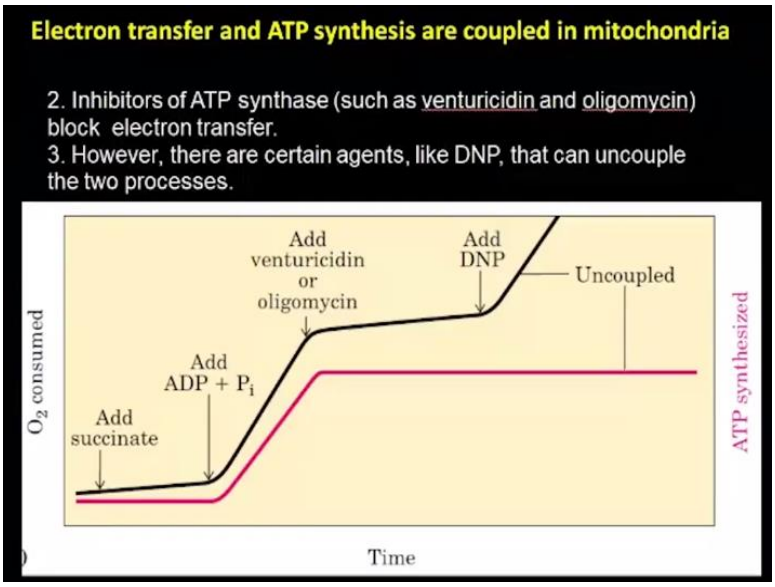

And in the next case what we are going to do is slightly complex experiment. So, you have added the substrate electron donor but nothing happens unless otherwise you allow phosphorylation possible by adding ADP and inorganic phosphate. And once you have added so like in the previous experiment when both these two are available that is the electron donor and the substrate for phosphorylation.

Now both processes start and once you inhibit the electron transport in this case instead of cyanide in this experiment oligomycin or this venturicidin are added. So now the ADP synthesis stops and oxygen consumption that is electron transport also stops. Now there are inhibitors they are called uncouplers like this DNP. When you add what actually they do is they allow a leakage of the protons across this membrane that is like short-circuiting this circuit.

And when that has been done that is allowed now electron transport continues. Oxidation happens without any phosphorylation because you do not build a gradient and without the gradient there is nothing to flow through the turbine to produce a phosphorylation. So, inhibitors of ATP synthase block electron transfer. However, there are certain agents which allow this uncoupling then this happens.

So, this uncoupling does happen in nature like for example in brown fat adipose tissue this is a lipid deposition at the back of the neck in some of the organisms that live in very cold weather. There the process is uncoupled so that the oxidation is used to generate heat energy without synthesizing ATP so that the animal can be kept warm so that uncoupling happens.

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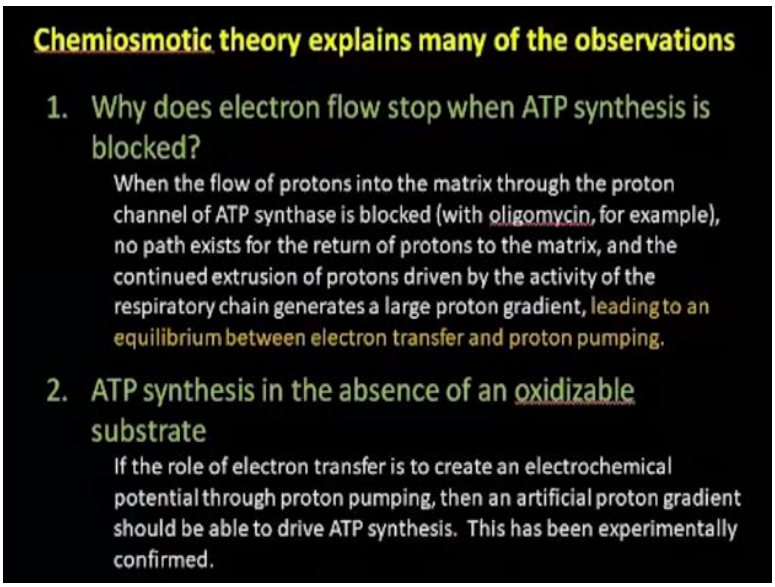

So, another important point that comes from this is the second point listed here. So, the first point I sort of already explained. Why does electron flow stop when ATP synthesis is blocked? That is because uh the proton concentration increases to a level where now the energy available from the electron transfer is only able to maintain the gradient and no more than that. So, it reaches an equilibrium with the electron transfer and proton pumping.

But the other one is if this concentration gradient only is required for ATP synthesis and not actually a substrate oxidation, then even without oxidation if you have this electrochemical gradient then ATP synthesis should happen. So that is written here if the role of electron transfer is to create an electrochemical potential through proton pumping, then an artificial proton gradient that is without using the electron transport process.

If we make an artificial proton gradient through other means that also should drive ATP synthesis and that has been experimentally confirmed. So, this is how we know chemiosmosis is what is the best explanation for what happens in our mitochondria as well as later today we will see happening in the chloroplasts in the photosynthesis. So, this is the chemiosmotic theory of oxidative phosphorylation in our mitochondria.

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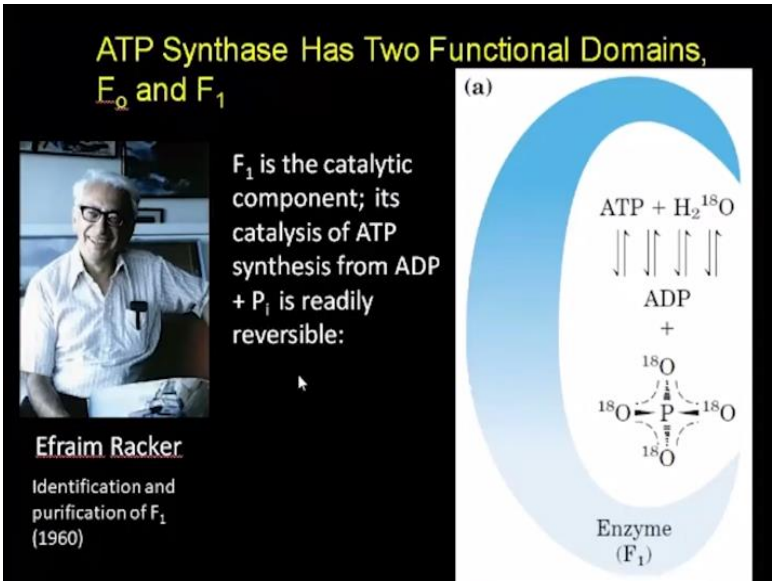

So, now let us go and see what actually this is? What is this pump and how does this make ATP? So, this has two subunits as you see here, one is F o, do not read it as F zero or F naught because this is not zero, it is o and o stands for oligomycin sensitive. So this is sensitive to oligomycin. So oligomycin blocks this proton flow and as a result ATP synthesis will not happen. So this subunit is present on the membrane.

So, it has hydrophobic residues that can interact with the hydrophobic lipid and it is anchored on the membrane. So, this is a cross section, so it is like a round structure and it is cut open to show the inner tubing or lumen. And then you have F 1, so this is the catalytic subunit, this is where ATP synthase activity is located. So, the proton flowing through this energizes this activity of F 1.

And details of these two are what we are going to see now. So, this F 1 the catalytic component was first purified and shown to have the catalytic activity by Efraim Racker and he did this as early as 1960s. And an interesting experiment that he did led to a conundrum about the way the F 1 ATP synthesis works and that was solved only in mid 90s, 35 years later that is because they needed to really crystallize.

And needed a leap in our understanding that is where you really call genius in thinking. It is not incremental process to propose a model to explain how the ATP synthesis enzyme functions. So that will be our primary focus for today's class. So, what Racker found was when you incubate the purified F 1 with ADP and inorganic phosphate which is inorganic phosphate and water with radiolabelled oxygen O 18 water.

So very quickly what he found was that all the 4 oxygens of the inorganic phosphate that would form if the original inorganic phosphate non-radiolabelled one combines the ADP and forms ATP and then that ATP hydrolyses using the oxygen from the water and this process happens multiple times. Then only this phosphate will end up having O 18 oxygen in all the 4 oxygen atoms here.

And he saw that and that indicated this explanation that is in the active side of this enzyme this ADP plus P i forming ATP and then the ATP hydrolysing back to ADP and inorganic phosphate must be happening very quickly and multiple times. In other words, this process must be existing in equilibrium so that is what he found. So, it is catalysis of ATP synthesis from ADP and P i is readily reversible. So, this led to a problem. So then why would you need energy becomes an issue?

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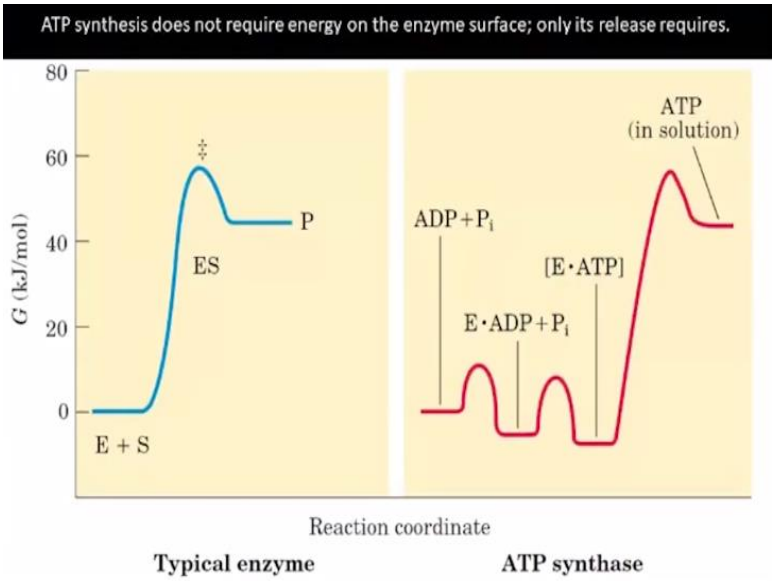

So that is explained in this reaction coordinate graph. So for a normal enzyme, an enzyme substrate combines this ES complex formation is the one that requires lot of energy that is where all the binding energy, non-covalent interaction everything comes. On the other hand with the ATP synthase, this particular ATP synthase, this mitochondrial F 1 ATP synthase on that complex formation like ES.

So, ES and then EP so; these are not having any big energy difference. It is nearly the equilibrium constant is 1, but on the other hand for the ATP to dissociate away from the enzyme requires a lot of energy. So that is an interesting difference from this and why is this?

And what is the explanation for this took a lot of time. So, what eventually people found was that the enzymes active site very tightly bound ATP.

And that itself was enough to drive the reaction from ADP plus inorganic phosphate to ATP, but the energy was actually required to release the ATP from the enzymes like active site. And a mechanism for this how this happens was proposed by Paul D Boyer who won Nobel Prize for that really great discovery so that we come to it in a minute after we understand the crystal structure of the F 1 ATPS.

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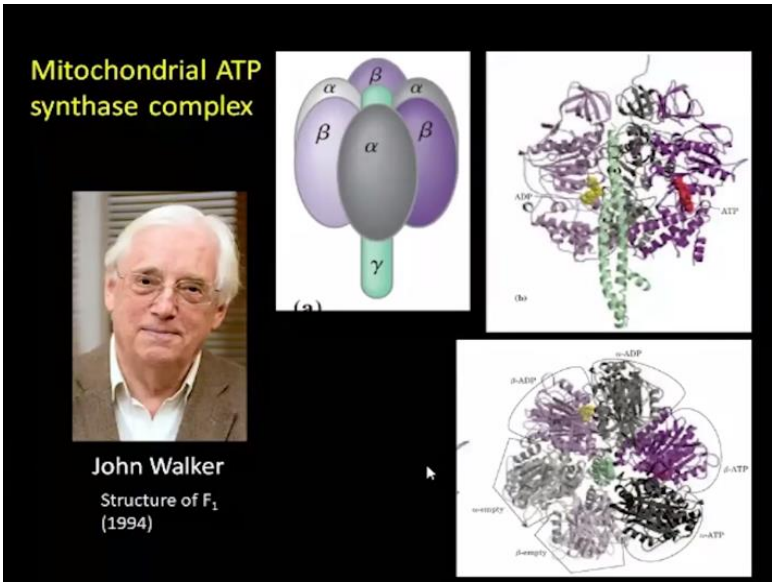

And that was solved by John Walker in 1994. So, the enzyme was purified 1960 and its crystal structure became available in 94 because it is not that solving this crystal structure took 34 years, on the crystallographic techniques became advanced enough to attempt these kinds of complex structures that process took that much time and definitely this was one of the complex structures and it required a lot of effort to solve the structure.

So, this is the model of the solved structure. So where you have the subunits here, so you have an alpha subunit and beta subunit and they exist as dimers and three such heterodimers are present and then the gamma subunit forms a rod shaped structure in the middle of the circular arrangement of these hetero-tridimers. These are like 1, 2, 3 so you have three heterodimers arranged in a circular form.

And in the middle like a shaft you have the gamma subunit and this is the ball and stick model of this. And here other subunits there are a few more subunits and they are not shown

here and F o is also not shown. And here you can see the beta subunit of the ATP here and then here to another beta subunit you have the ADP bound. So, these are shown in yellow and red colours.

So, this is a side view like as shown in this model and this one is as if you are viewing from top down in this direction. So you have these arrangements. And this what is alpha empty beta empty all these will become clear when we go and consider the Boyer's mechanism. So, pay attention in any case to these. These two have a confirmation that is different from the other two indicated by this outer line.

So alpha beta, but the confirmation of the three heterodimers are distinct, each one is different from the other two. So, this is sort of indicated in this cartoon by this round shape and this is little sharpened and this is with these sharp ends. So, they have distinct confirmation and that becomes critical.

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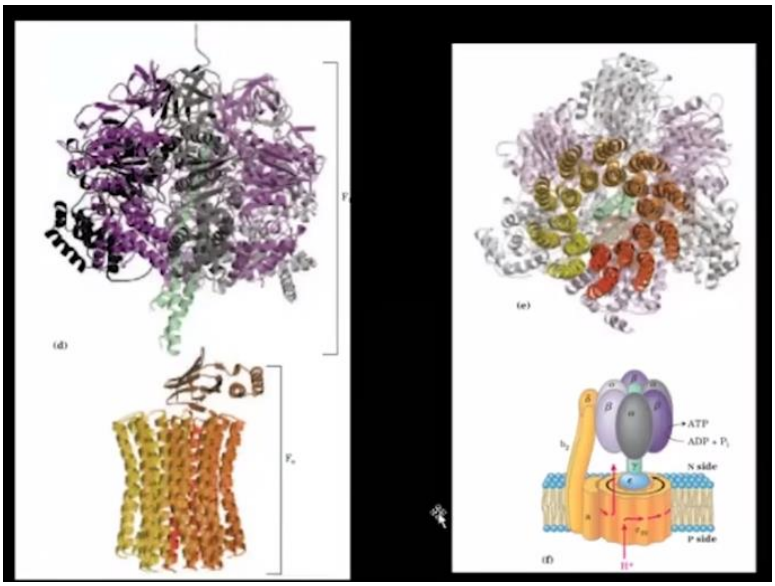

So, this is shown along with the F o subunit which is like a barrel shaped c subunit, so there we are going to use a, b, c like the c sub unit that 10 of them form the cylinder like structure on the membrane and then you have the a subunit and then the b, b is the one that contacts this F 1 by attaching to the delta subunit which is not shown in the crystal structure of F 1 and thereby it anchors this circular structure to the membrane. So, this does not have any ability to rotate or move because this anchors tightly to the membrane.

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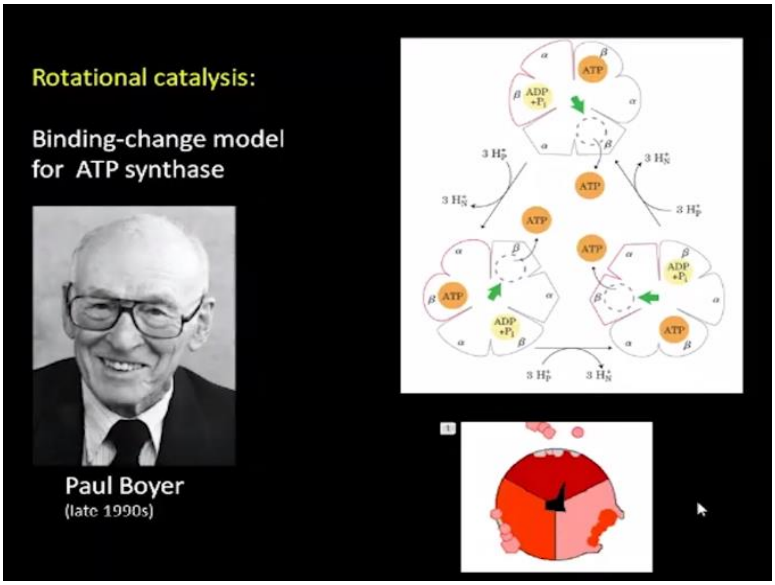

And what Paul Boyer proposed, let me show this picture here. So, he died a I think 3 years ago or maybe 2 years ago, very recently he passed away. He is one of the pioneering biochemists, he edited methods in enzymology several volumes of that book. So, he proposed a model whereby as the yellow protons flow through this cylinder that is the F o's c subunit that starts to rotate.

And to that via this epsilon subunit the gamma subunit of the shaft of F 1 is attached and due to that along with the cylinder the shaft also rotates. And the shaft rotating so this has specific contacts to the beta subunit and as it rotates it sequentially changes the confirmation of the 3 beta subunits, so that is his proposal. This is called the rotational catalysis and that rotation cartooned here so where you have this interacting with a particular beta subunit.

This now undergoes the confirmation change that releases the ATP out and the interactions among them are such that when the gamma subunits interact with the given beta subunit changing its confirmation to empty meaning there is no ADP or ATP bond that is why it is called beta empty, alpha empty and when it does that adjacent to one, one of them confirmation changes such that it binds ADP and inorganic phosphate.

And since this readily becomes ATP from prime reactors experiment, we know then this change is confirmation to this one. And when it again rotates, then as shown here the next one becomes empty, the ATP gets released and when it rotates to the next one that also releases. So when this gamma subunit finishes one 360 degree rotation, each subunit have catalysed the formation of an ATP and released it out as well.

So, the phosphorylation really happens and the energy required is only to drive the confirmation to the empty confirmation by the ATP is thrown away and that is what happens. So, that is shown in this animation of which I will see whether I can play this, it does not play. So, essentially what is going to see is this black arrow is the gamma subunit. So, when it interacts with one given beta subunit the ATP is released.

And then now that is ready to bind ADP and P i and that is what has happened here and when it shifts to this then this is going to become now the beta empty and this will be the ADP binding one. And then ATP forms and when it comes again that is going to release. So, this needle like structure you will see it rotating in jerky motion, every emotion is 120 degrees.

So, the 360 is equally divided into three parts for the interaction from shifting from one beta to another beta to another beta. So, it is not like a smooth rotation, instead it goes in steps. It is a three-step rotation and that is what happens.

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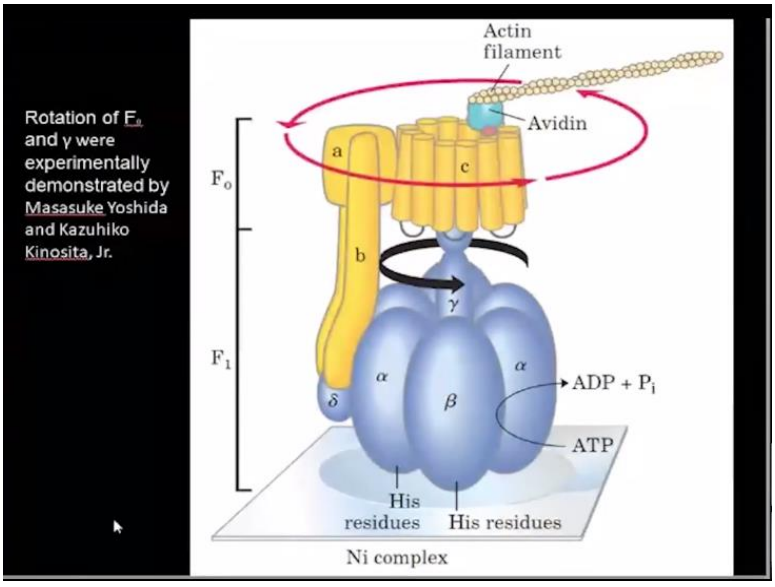

This is what Paul Boyer proposed and this was confirmed later by really elegant experiments that this Yoshida and Kinosita devised. So, what these people actually devised is they made transgenic proteins alpha beta with the histidine residues and the histidine because of their negative charge can bind to a glass slide this is microscope slide coated with a nickel. So, this is anchored now to the glass slide.

And you have the gamma subunit connecting to this cylinder c of this F o and to that they have added biotin this red circle here. And to that they had a long-acting filament with orbiting attached to it and due to the orbiting biotin affinity this is now in interacting with this. So now when you add ATP and when this hydrolyzes it, so you have reverse rotation compared to the ATP synthesis and that also would be predicted by Boyer's model and that is exactly what happens.

So, now as it rotates, they had a fluorochrome attached to acting. So, using a fluorescence microscope on time lapse video, they were able to monitor the fluorescence shifting in a circular fashion and they could see that it shifted in 120 degree at a time and that is how they were able to observe how this happens. And here the energy available from the proton gradient and the energy required for the rotation are nearly perfectly matched.

Such that the machine this motor is at the theoretical maximum of energy efficiency in converting the proton gradient potential energy available in making this motion and that is what made Boyer to call this as a splendid machine. So, this is the amazing thing about an enzyme catalysis where you have rotation of a turbine like motion leading to the catalysis of ADP to ATP synthesis.

So, the main point is the energy required here is not for the reaction itself, but it is actually to release the product from the enzyme active site. So, I hope this is clear. So, with this we are actually finishing our oxidative phosphorylation. So, we saw electron transfer that is oxidation caused proton gradient and also a charge separation and when that potential energy difference is allowed to dissipate via a turbine.

That is the F 1 ATP synthase linked to this F o membrane bound actual turbine, we end up making the phosphorylation reaction that is ADP plus inorganic phosphate becoming ATP. So, this completes our understanding of starting from glucose how we get energy in the form of ATP molecules and we know why ATP is high energy molecule. And we also know how the carbohydrate molecule has been oxidized to carbon dioxide and in the same process oxygen being reduced to water.

So, carbon dioxide and water go in, in the photosynthesis and carbon assimilation reaction that we are going to see next and then finally it all comes back to carbon dioxide and water

through the oxidative phosphorylation. So, we have actually traveled with the electrons all the way from glucose to oxygen becoming water. So, this completes one sequence of events.

And through the process of this we have understood quite a few of the central concepts of biochemistry including catalysis, bioenergetics, regulation or many aspects of enzyme mechanisms and so on. So next we switch gears to considering a proton gradient formation where energy is actually input in the form of sun's light energy. Here energy is released through the process.

Because we have electrons falling from up to a down level that is from lower reduction potential to higher reduction potential and therefore energy is available. Now what we are going to do is we are going to provide energy and force electrons to flow through to make the proton gradient again, so that is what is photosynthesis.

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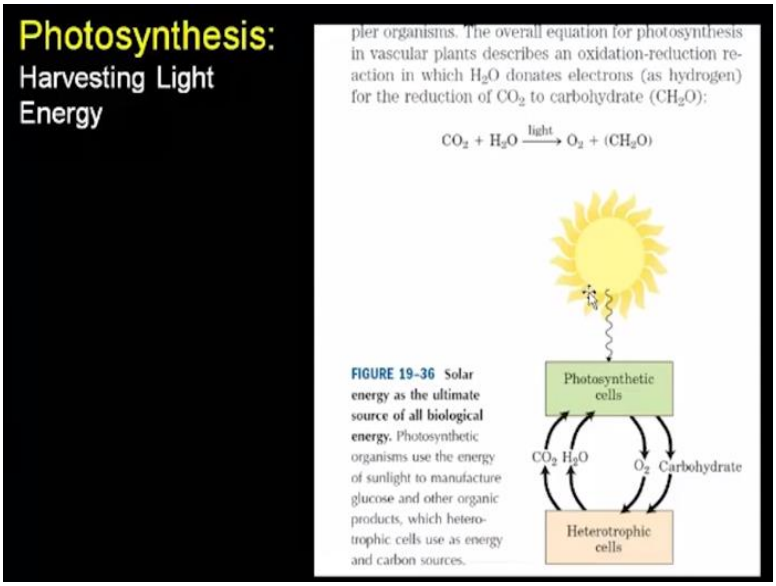

So, before we get into those details let us have a global picture of matters cycling among the biological systems that is between photosynthetic organisms and heterotrophs like us so that what we will look at in this slide. So, this is extremely simple. You probably learnt in high school but probably did not look at it from a philosophical angle so that is what I want you to do here.

So, the Sun's energy drives the photosynthetic cells to combine carbon dioxide and water to produce carbohydrates and evolve oxygen in the process. So, carbohydrate you have the 3 elements required. It is amazingly simple, only 3 elements that we put together to make life

happen. So, the energy production, liberation and most of the storage all are involving only these 3 elements, extremely simple system.

So, photosynthetic cells put these together to make this and we just saw the heterotrophs consume these and return these back. So, therefore in living system by the action of Sun's energy this is the sole source of energy. If you are burning fossil fuel, fossil fuel is carbohydrate made millions of years ago or thousands of years ago and that was made by photosynthetic cells.

So, Sun's energy is the only source of energy in the biosphere in the biological systems for the carbon, hydrogen, oxygen cycling that is shown here. This cycling is made possible by the energy from sun and sun alone. So, the equation above summarizes the process. So, the energy of light CO 2 + H 2 O gives you CH 2 O + O 2. So, now let us get into some of the details in the next few minutes of time we have.

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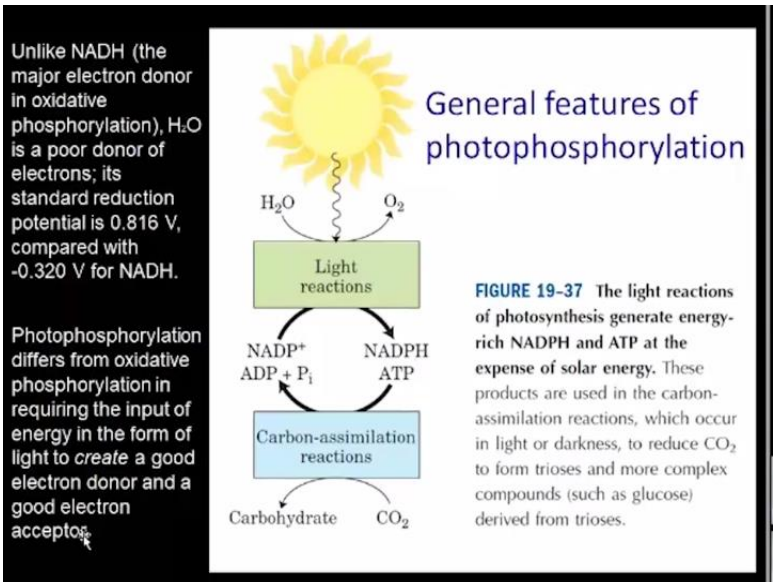

So, this slide summarizes the general features like when you have light through light reactions because it is light dependent so we call them light reactions, water is split and electrons are taken up so oxygen is returned. So, the water is here oxidized. Just now in the previous one we saw the opposite, so here this is one process. Second you have these oxidized coenzymes like NAD photosystem uses the phosphorylated version of NAD, so NADP.

Otherwise here also the electron transfer is in the hydride form gets reduced to NADPH. So, oxygen is evolved and reducing equivalents are produced. And the third is ATP formation as well. So, these are the things the light reaction produces and carbon assimilation reactions do not call them dark reactions because they can happen even in the presence of light. It is just that these reactions are not dependent on light.

So, therefore the correct way of calling them is carbon assimilation reaction because it assimilates carbon dioxide. So, by using the reducing equivalence and the energy in ATP these reactions reduce carbon dioxide into carbohydrates. So, therefore you have two components, one light reaction producing ATP and reducing equivalence, oxygen evolution is incidental in terms of our energy here.

And these two are used to convert carbon dioxide to carbohydrate. So, essentially whatever is the end product of TCA cycle decarboxylation we saw producing carbon dioxide and here now we take the carbon dioxide and make carbohydrate. So, these are the two major components of the photophosphorylation reaction. So, two points I want to draw your attention to, one is in the mitochondrial oxidative phosphorylation the electron donor is NADH.

So that is a very willing donor of electrons compared to the molecule that is going to donate here which is a water. So, therefore to get electrons out of water you really need energy so that is one important difference here and that energy is what is coming from Sun. So how is that energy used, abstracted to do this work is one important thing we are going to see. And the second is that you need which I just mentioned as first part of it itself.

The first part therefore is just that NADH is a good electron donor and water is not so and water is the electron donor here in the light reaction. And the second as a result of that you need energy input. So, photo phosphorylation differs in that respect from the oxidative phosphorylation. Oxidative phosphorylation did not require energy input. We did not hydrolyze any ATP to move the electrons from one complex to another complex.

Whereas here energy will be required to generate good electron donor as well as good electron acceptor, to do that you need energy input. So, these are the two main differences we

need to keep in mind. So, I will stop here with these in a bird's eye view of photophosphorylation and we will get into the details tomorrow.

So, the first thing is we are going to look at how is light energy taken up to really generate a good electron donor and a good electron acceptor if possible that we may not be able to finish tomorrow so that is where we are going to focus tomorrow.