Metabolic Engineering
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Lecture - 11
Review of Cellular Metabolism - Part F

In this part of my lecture on review of cellular metabolism

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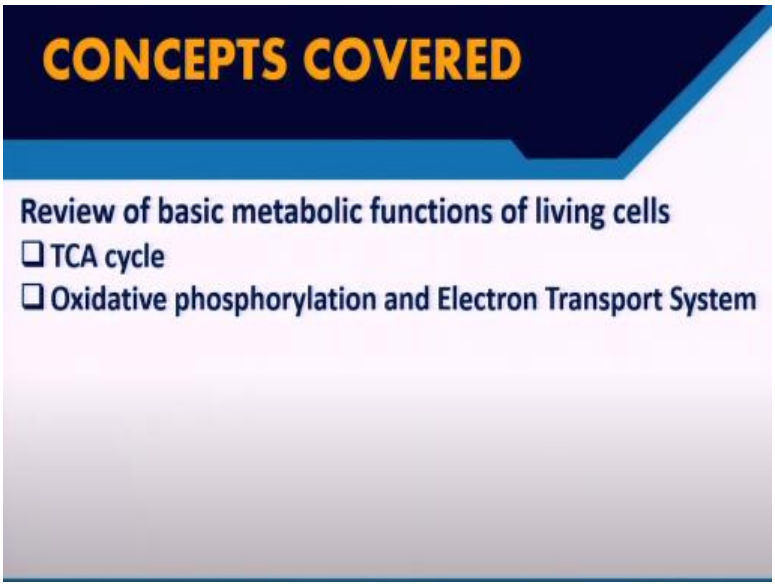

we are going to discuss about two very important components of cellular metabolism, which are the TCA cycle and oxidative phosphorylation and finally, the electron transport system.

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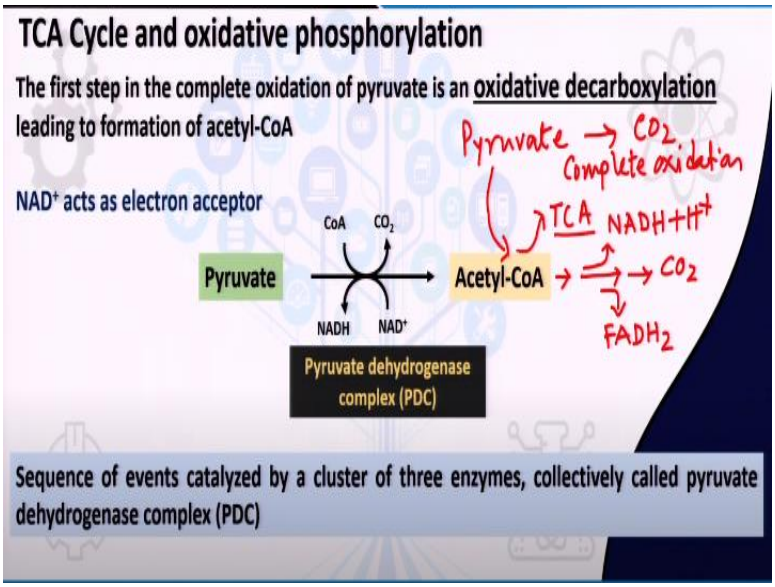

Now the TCA cycle is also known as the citric acid cycle, because one of the very important intermediate of this entire cycle is citric acid which is produced in the initial part of the complex set of biochemical reaction.

But before we go into the details of this TCA cycle or tricarboxylic acid cycle, the first step of this complex set of biochemical reaction which facilitates the oxidation of pyruvic acid to carbon dioxide and water allowing the release of electron present within the pyruvic acid and the release of the Gibbs free energy. Let us see the first step which is very critical in terms of initiating the TCA cycle event.

So the first step in the complete oxidation of pyruvic acid is an oxidative decarboxylation. So simultaneously the oxidation as well as the release of carbon dioxide molecule takes place and leading to the formation of 2 carbon acetyl-CoA molecule. So pyruvic acid or pyruvate produced as the final product of the glycolytic pathways like EMP pathway, pentose phosphate pathway or ED pathway is eventually oxidized and decarboxylated to produce the two carbon acetyl-CoA molecule.

And NAD+ is used as the electron acceptor for this oxidative reaction. This reaction is catalyzed by a very important and complex set of enzymes which is called pyruvate dehydrogenase complex or PDC. This PDC is a cluster of three enzymes and collectively they are referred as pyruvate dehydrogenase complex.

Now this acetyl-CoA which is produced following this oxidative decarboxylation is eventually converted to carbon dioxide by a set of reactions which are interconnected and from acetyl-CoA two moles of carbon dioxide is released and this process is also the oxidative process. So lot of electrons are released in terms of NADH or NADH H+ and FADH2.

The FAD, flavin adenine dinucleotide is also involved as electron acceptor in this set of reactions. So essentially the conversion of the pyruvate to carbon dioxide which is referred as the complete oxidation is facilitated only by this very fast reaction where the PDC complex facilitates the conversion of the pyruvate to first to acetyl-CoA. And then the acetyl-CoA enters into the tricarboxylic acid cycle.

So it is kind of a dedicated step. Once the acetyl-CoA is produced, it is going to react subsequently with its other substrates and then enter into the TCA cycle.

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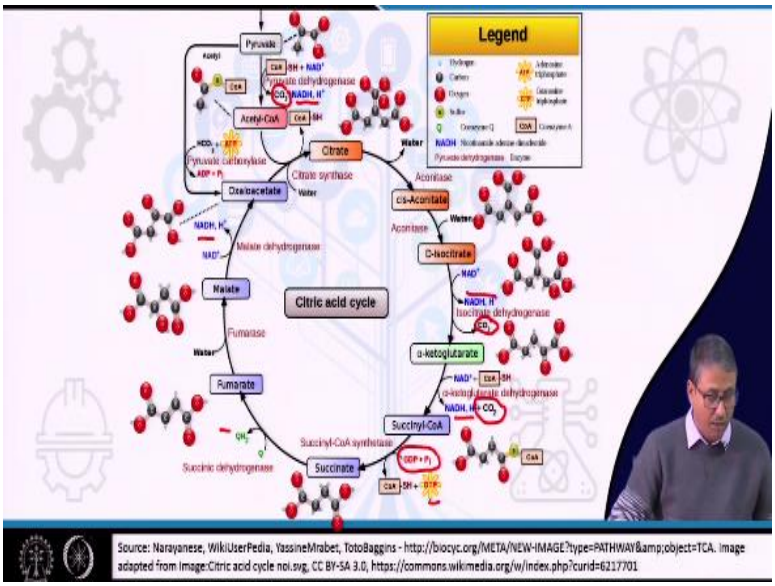

Now if we look at the TCA cycle broad overview, so we will be able to find out very interesting steps of decarboxylation where carbon dioxide molecules like as you can see over here, the carbon dioxide molecules are systematically released from the carbon backbone. So there are three decarboxylation reactions. So the all the three carbon residues within the pyruvic acids are eventually released as CO2.

So the complete oxidation of the carbon, which is entering into the TCA cycle is achieved. This TCA cycle is also developed with provisions for extracting the maximum energy out of the carbon and hydrogen bonds which are present in the substrate as we have seen in the first reaction catalyzed by the pyruvate dehydrogenase complex, the release of NADH H+.

There are also other reactions, which we are going to talk very soon where NADH H+ or the reducing equivalents are generated. And also here we have the FADH2 or the fumarate is produced by the FADH2 is produced. So a number of cofactors, which are responsible for taking the electrons from this reaction towards the other processes are generated.

One mole of GDP or GTP which is equivalent to the high energy phosphate bond containing ATP or a kind of a energy currency of the cell is also produced. So we will talk about the stoichiometry of the entire process very soon.

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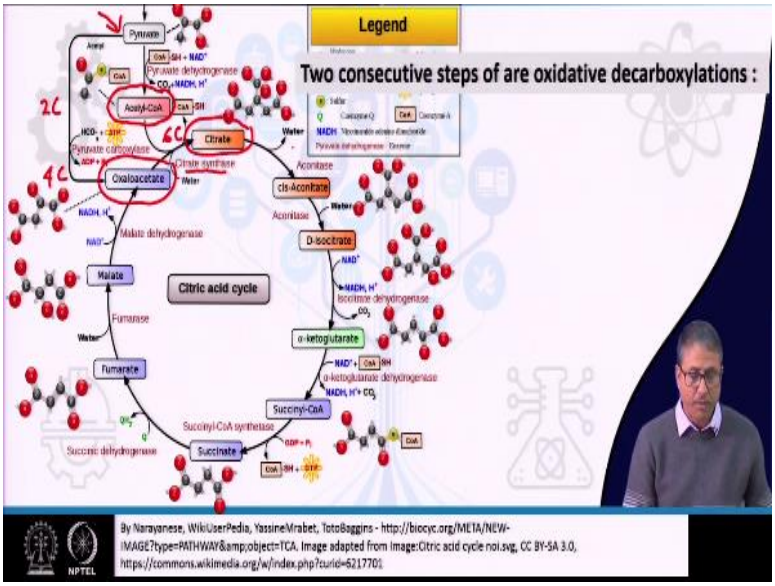

Now if we look at this TCA cycle, which is having a, it is a quite a few number of reactions starting from the acetyl-CoA. So here is the molecule acetyl-CoA, which is produced out of the conversion or the reductive or the oxidative decarboxylation of pyruvic acid. And this is the pyruvic acid, which is coming from our glycolysis pathway. Now after this acetyl-CoA is produced, the reactions are going to start.

So the carbon is entering into the TCA cycle via this acetyl-CoA. Because, pyruvate is oxidized to acetyl-CoA and this acetyl-CoA is ready to react with oxaloacetic acid and this reaction with oxaloacetic acid facilitate the formation of citric acid. So these are the two substrates which are acetyl-CoA which reacts with oxaloacetate and leads to the production of citric acid or citrate.

This reaction is catalyzed by citrate synthase. We will be discussing about the control of this TCA cycle later and we will be highlighting that this enzyme citrate synthase is one of the sites of control for this TCA cycle. Now the citrate which is produced is the first product of the TCA cycle, which is obviously a 6 carbon compound because the acetyl-CoA was the 2 carbon compound and oxaloacetate is a 4 carbon compound.

So eventually we have the 6 carbon citric acid produced. Now this citric acid which is produced over here is ready to be processed further in a state of oxidizing and decarboxylating reaction.

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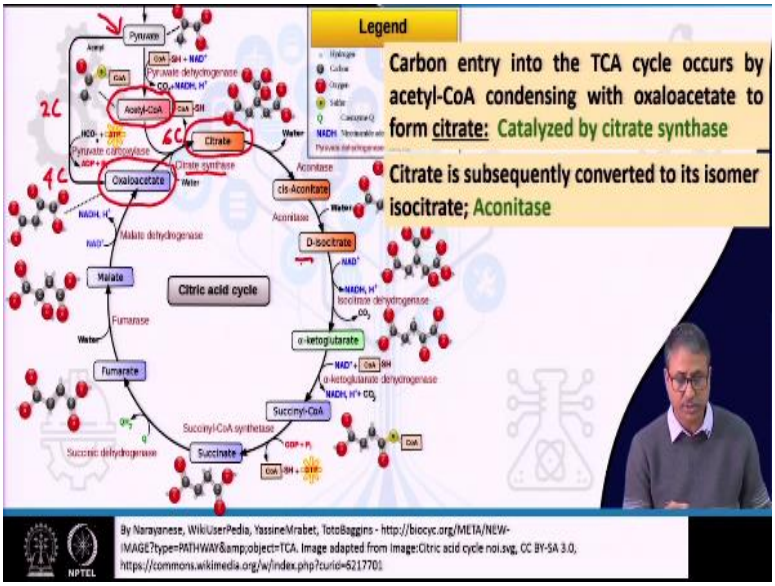

Now once the citrate is produced, it is subsequently converted to the isomer which is the isocitrate. It is the isomerization reaction catalyzed by the enzyme aconitase. So it is a two-step reaction.

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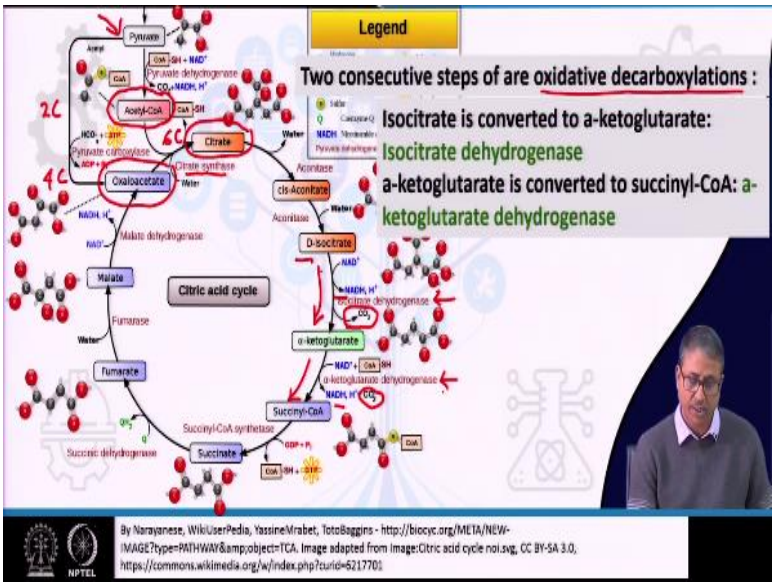

Now once the isocitrate is produced there will be two consecutive steps of oxidative decarboxylation. So simultaneously, we will be able to find out that the carbon dioxide is getting released. And also the energy of the electrons are released in terms

of producing the reduced cofactor that is NADH H+. Now the first reaction of this oxidative decarboxylation is the conversion of isocitrate to alpha-ketoglutarate.

So this is the first reaction where isocitrate is converted to alpha-ketoglutarate. It is again a decarboxylation and dehydrogenation reaction catalyzed by the isocitrate dehydrogenase enzyme. The second reaction is again the oxidative decarboxylation of alpha-ketoglutarate to succinyl-CoA. So this reaction is catalyzed by alpha-ketoglutarate dehydrogenase. Now these two enzymes are again the candidates for regulating the TCA cycle because these two reactions are irreversible.

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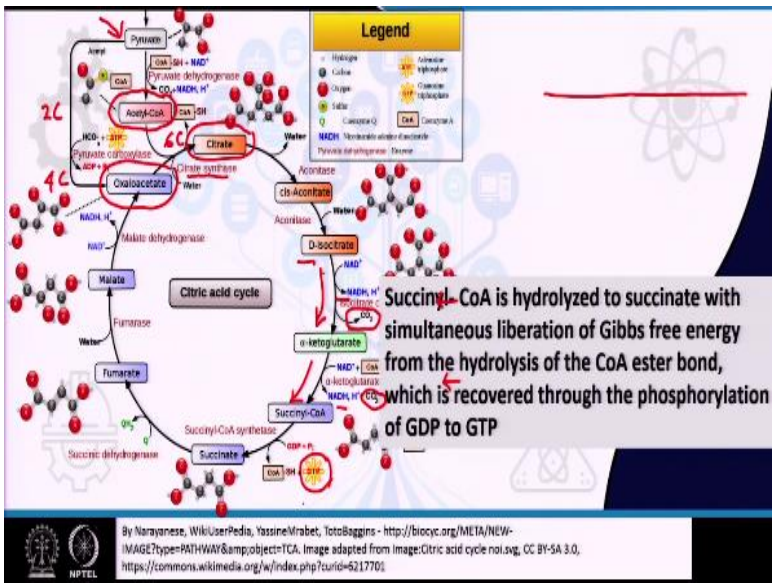

Now succinyl-CoA subsequently hydrolyzed to succinate with the release of energy in the form of the guanosine triphosphate.

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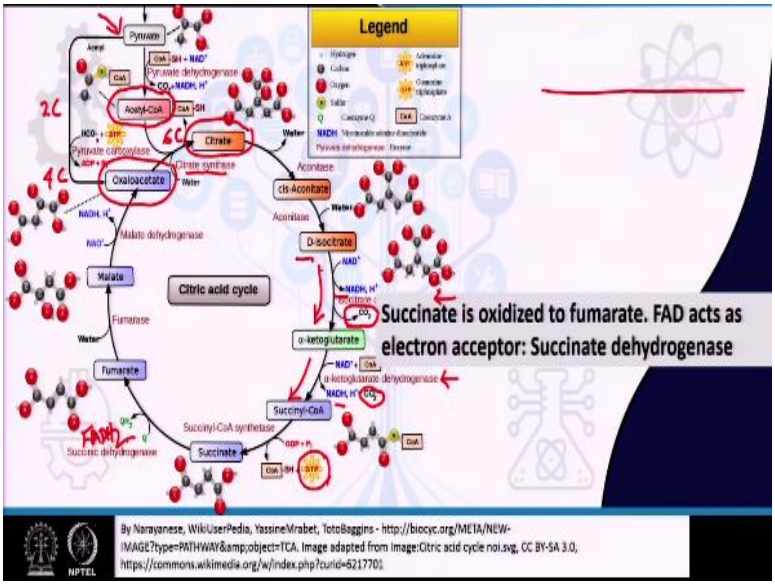

And then succinic acid is oxidized to fumarate with the succinate dehydrogenase and FAD acts as the electron acceptor. So here we gain the FADH2. There is a potentiality to gain the electrons in the form of FADH2 and fumarate is produced.

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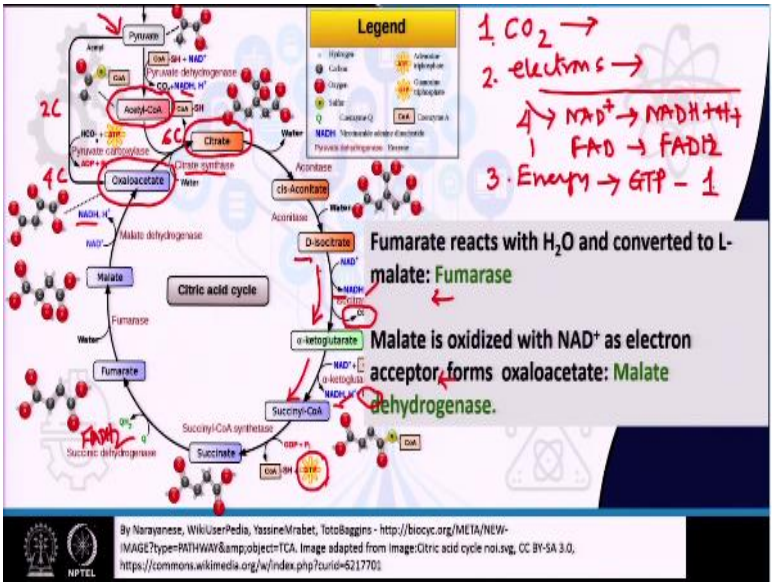

Now fumarate is reacting with one molecule of water or it is catalyzed by the enzyme fumerase to produce malate and then the malate is undergoing another round of dehydrogenation or oxidative reaction with the production of NADH H+ to give the product as oxaloacetate. So in summary, we can clearly say that the conversion of pyruvic acid to CO2 that is the complete oxidation of this pyruvic acid carbon molecules are achieved through a set of reactions.

And these set of reactions are serving two important purpose. One is the release of CO2. Another is the release of electrons because it is a oxidative process, oxidation process. So they are releasing electrons and these electrons are going to be taken by NAD+ so converting them to NADH+ H+ or to FAD and converting them to FADH2. Some amount of energy is also released.

So another is the energy, which is in the form of GTP. So three important outcomes are there. One is the carbon dioxide which is released and the complete oxidation is facilitating the breakdown of the carbon skeleton, the electrons are released which are taken by the electron carrier or reducing equivalent and thirdly, the energy is released in terms of the GTP. So how many NADH H+ are produced?

So there are actually one molecule over here. 1, 2, 3 and 4. So 4 moles of NADH H+ are produced and one mole of FADH2 are produced. So 4 plus 1 and one GTP or ATP equivalent molecule is produced per mole of pyruvic acid converted or oxidized through this TCA cycle.

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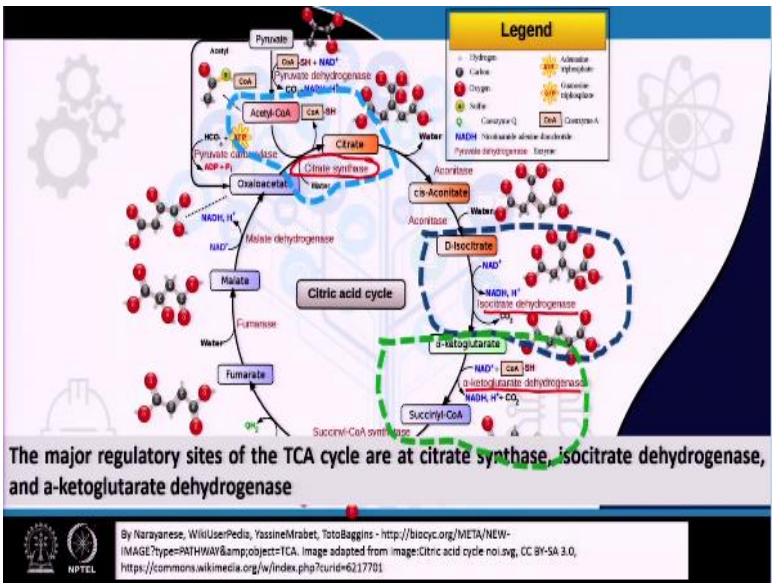

Now we will be talking about the major regulatory sites within this TCA cycle. Now there are as I mentioned earlier, there are three important enzymes which are catalyzing the irreversible reactions. These are the citrate synthase, catalyzing the conversion of oxaloacetic acid and acetyl-CoA to citrate. Then the isocitrate dehydrogenase which is catalyzing the oxidative decarboxylation of isocitrate to alpha-ketoglutarate.

And then alpha-ketoglutarate dehydrogenase which is catalyzing or oxidizing as well as decarboxylating alpha-ketoglutarate to succinic acid or succinyl-CoA. These three enzymes are the major points of regulation of the TCA cycle.

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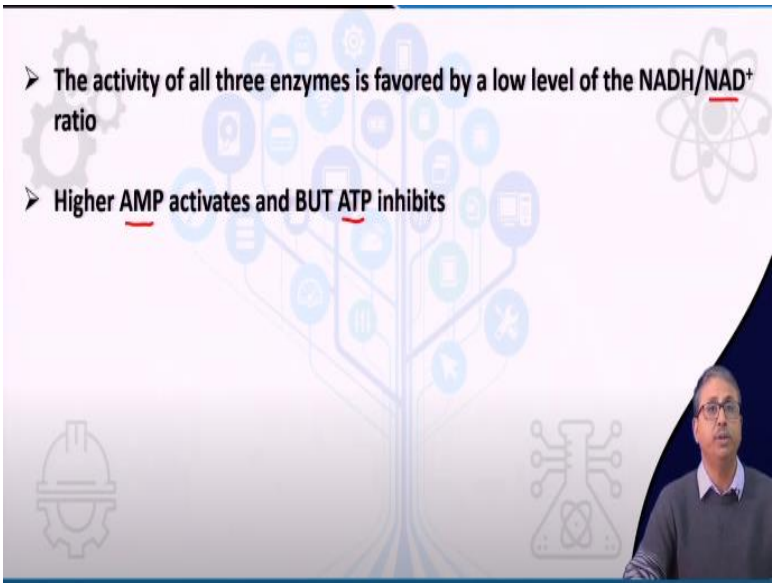

Now activity of all these three enzymes the citrate synthase, the alpha-ketoglutarate dehydrogenase and the isocitrate dehydrogenase, the activities of all three enzymes are favored by low level of NADH/NAD ratio. That means the lower the level of NADH or higher the level of NAD+, so the cell must be depleted in terms of the reducing power of the electrons which are there with NADH will actually activate these enzymes.

Along with that higher concentration adenosine monophosphate as we have already learnt AMP is a very good marker for the energy depleted condition of the cellular system rather than ADP. So AMP activates all these enzymes, but ATP inhibit these three enzymes vary significantly.

Because when the cell is having higher level of ATP or higher level of NADH H+ the cell gets the kind of signal that the cell is actually rich in energy and reducing equivalent, so there may not be enough requirement for carrying out the TCA cycle.

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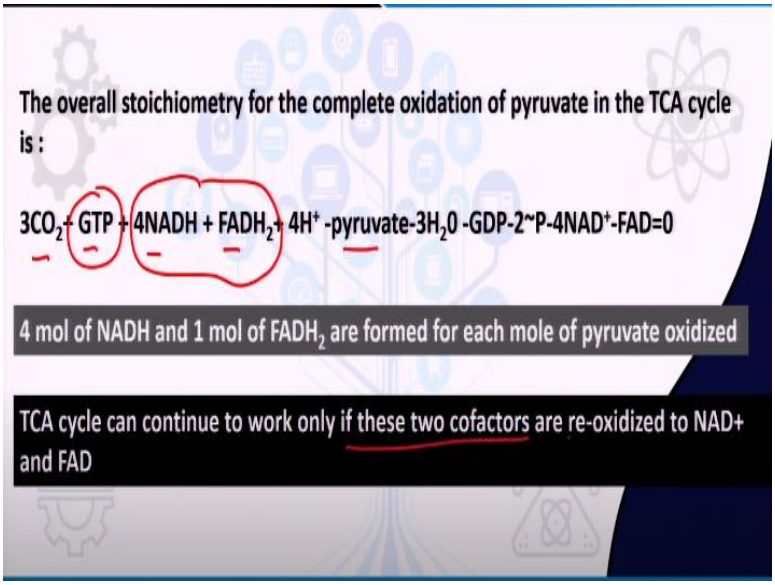

Now the overall stoichiometry for this complete oxidation of pyruvate is the pyruvate molecule is completely oxidized to 3 carbon compound, 3 CO2 molecules and one mole of ATP equivalent GTP the high energy compound, 4 moles of NADH H+ and one mole of FADH2 H+ is generated from this reaction.

Now it is important to understand that this TCA cycle, which is allowing release of a high amount of energy as well as reducing equivalent, facilitating the complete oxidation of the pyruvic acid will continue to work only if these two cofactors are reoxidized to NAD+ and FAD+. Now why it is so?

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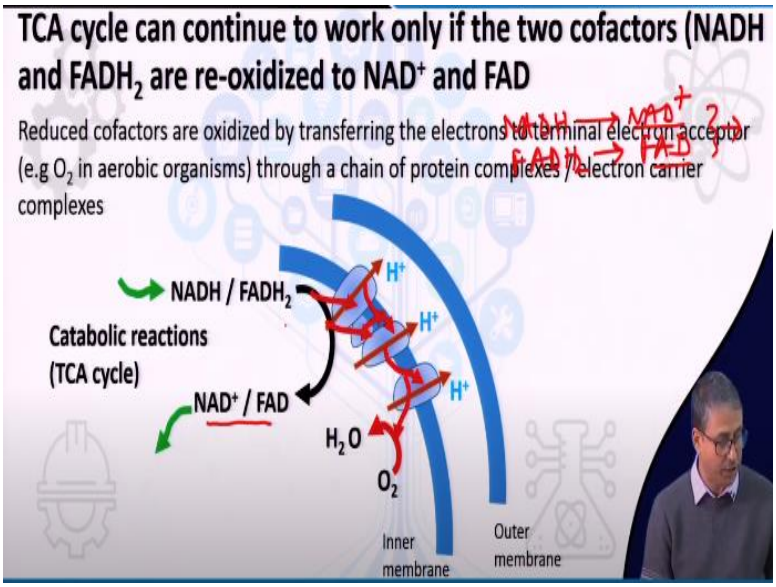

That the TCA cycle will continue only if this NADH and FADH2 are reoxidized to NAD+ and FAD. That means we will have to have some mechanism by which the

NADH to be converted back to NAD+ and if FADH2 needs to be converted to FAD, why? Because these two electron carriers will be necessarily be requiring by the oxidizing reactions, like the PDC complex, like the pyruvate is getting oxidized to acetyl-CoA.

The isocitrate is oxidized to alpha-ketoglutarate or alpha-ketoglutarate is oxidized to succinyl-CoA. All these reactions will require NAD+. Similarly, in the next reaction where FAD is required and FADH2 is prepared. So FAD is required. So the reoxidation of this NADH and FADH2 to this is essential because this will be required by the oxidative reactions.

Now the reduced cofactors are oxidized by transferring the electrons to terminal electron acceptors that is in aerobic organisms through a chain of protein complexes and electron carrier complexes. So we will be talking about or discussing this in a little bit more detail that how this is achieved. So let us look at this schematic diagram.

So as we have seen just now the NADH and FADH2 are produced by the catabolic reactions of TCA cycle or maybe we can consider even the EMP pathway as well, that glycolytic pathway which facilitate the production of NADH. So the point is here is this that these need to be reoxidized and they need to be converted to the oxidized form.

That is the NAD+ or FAD because these are required essentially for these oxidative reactions and cell generally maintains a steady pool of the NADH and NAD+ or FADH to FAD ratio. So that is to be maintained, okay. Now how this reoxidation event occurs? The reoxidation events occur basically by a series of reactions, where the electrons are transferred from this reduced cofactor like NADH or FADH2 to a number of electron carriers which are coupled with protein complexes present within the inner membrane.

So in case of prokaryote it is the inner membrane is very much there in case of Gram negative bacteria or it is the plasma membrane in case of Gram positive bacteria or in case of a mitochondria it is again the inner membrane. And this flow of electrons

allow the oxidation of the reduced cofactor like NADH FADH2 and also facilitate a very important process which is called oxidative phosphorylation. We are going to study that.

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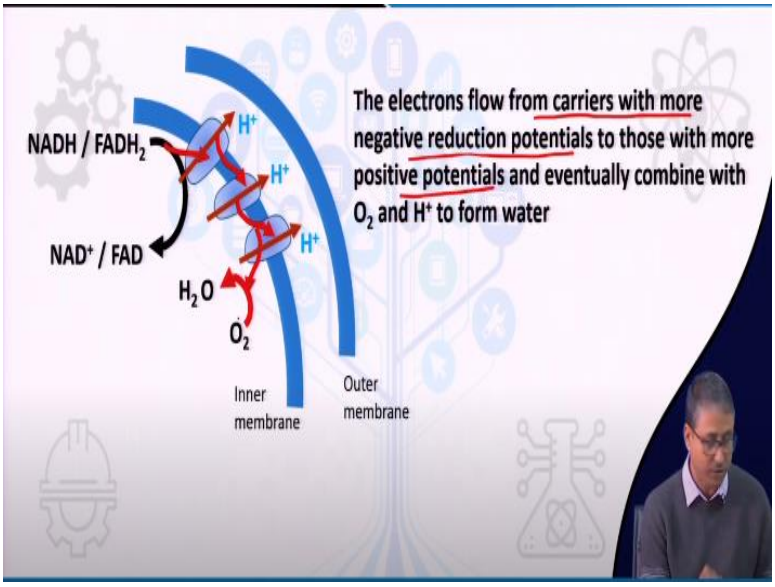

Now the electrons which are originating from the reduced cofactor like NADH or FADH2, they eventually flow from carriers with more negative reduction potential to those with more positive reduction potential.

That means these carriers, the carriers which are capable of ferrying or carrying the electrons to the electron acceptor, terminal electron acceptor like oxygen in case of aerobic organisms are arranged within the membrane or inner membrane in a particular way, so that they carry the electrons from a more negative potential like NADH H+ or FADH2 will be having a more negative potential compared to the electron acceptor that is the final electron acceptor that is the oxygen.

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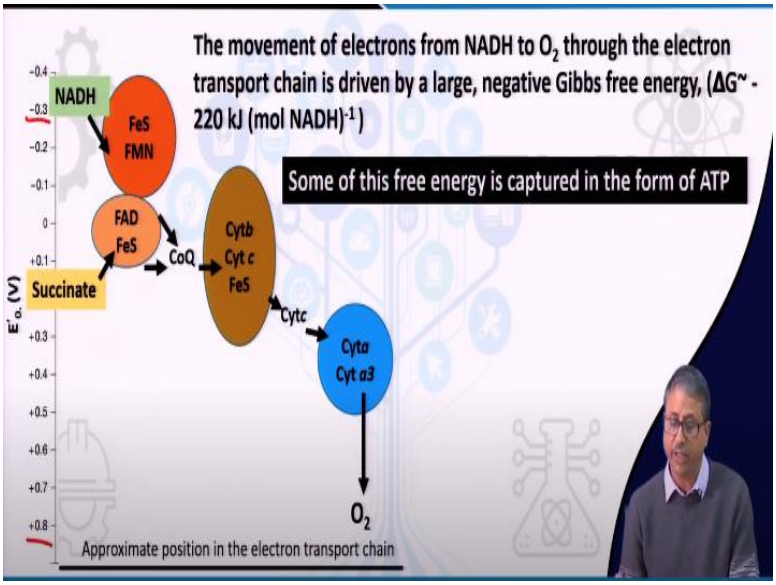

Now if we look at the position of these electron carriers, position of the electron carriers with respect to the electron donor that is the NADH or the succinate, which actually donates the electrons to FAD and FAD turns into FADH2. So the movement of the electrons from NADH to the terminal electron acceptor that is oxygen through the electron transport chain is driven by a large negative Gibbs free energy change.

Because as it is mentioned, that from a large negative potential to a large positive potential, the electrons are allowed to move and this movement of electrons from a strongly negative redox potential to a positive redox potential facilitates a generation of huge amount of free energy change, which is equivalent to minus 220 kilojoule per mole of NADH.

Now some of this free energy which is available because of this potential difference is captured in the form of ATP or in the form which is capable of helping the cell in doing some useful work. So we will be discussing that now.

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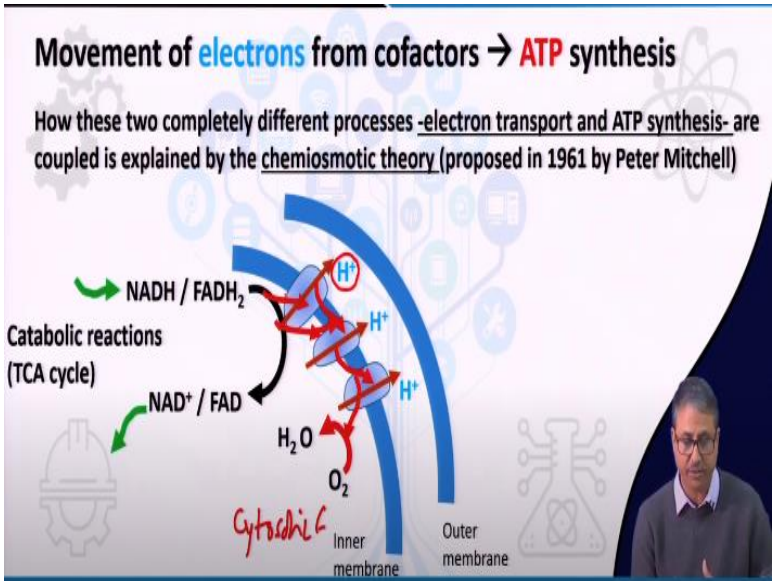

Now there are two different or two distinct aspects. One is the movement of electrons from the cofactors that is the NADH or FADH2 to the membrane electron carriers and the other fact is the ATP synthesis. Now how these two things are the completely relatively they are quite different because ATP synthesis is a high energy kind of reaction which phosphorylation happens.

ADP is phosphorylated to produce the ATP and another is from the negative potential to a positive potential flow of electrons within the electron transport chain. Now how these two completely different processes of electron transport and ATP synthesis are coupled is explained by the process of chemiosmotic theory which is proposed in 1961 by Peter Mitchell.

Now if we look at the same schematic diagram very carefully, we will be able to see that during this process of electron carrying, so electrons are being transported from the reduced cofactor to the terminal electron acceptor oxygen a number of special events which are called the efflux or pumping out of protons are occurring.

So basically protons are pumped out from the internal side of the cell that is the cytosolic side of the cell that is here is the inner membrane. So this is the cytosolic side. So from the cytosolic side to the outside or in case of a Gram negative bacteria, we can consider the periplasmic space, so or intermembrane space.

So a number of protons are pumped out by the same carrier complexes or some of the carrier complexes, which are responsible for creating a kind of electrochemical gradient. So this is the point about the chemiosmotic theory.

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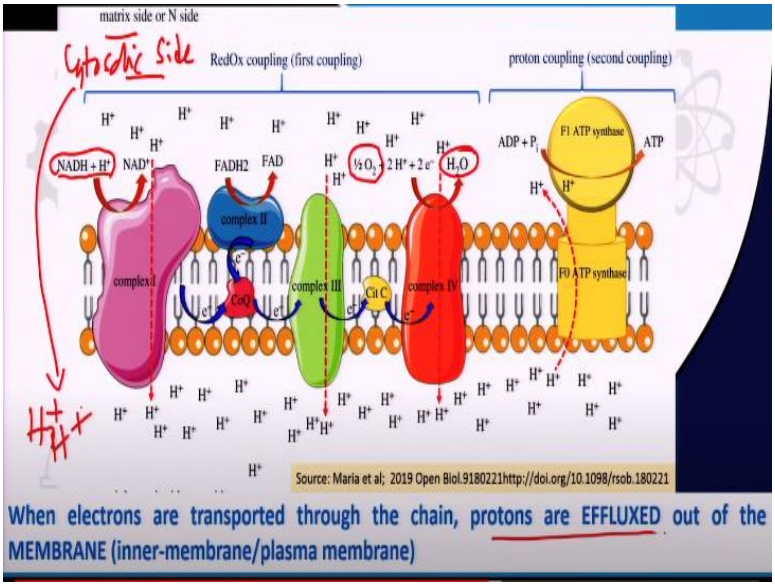

Now this is best illustrated by a kind of a schematic diagram where we can see it is based on the mitochondrial system where we can identify the number of complexes. These are all the carrier complexes like complex I, complex II complex III complex IV and other complexes. So all these complexes are capable of transporting the electrons or ferrying the electron. So here is the metric side or the cytosolic site.

So this is the cytosolic site. In case of the prokaryotic system or in case of a mitochondrial system we can consider this as a matrix site. So all our reactions are happening over here like TCA cycle is happening over here. And this is the NADH H+ which is ready to donate the electrons to the carrier complexes. So they donate the electron and then the electron eventually flows from one carrier to the other and finally, to the oxygen to produce the water molecules.

Oxygen is the final electron acceptor. It has around +0.8 millivolt or so the potential from NADH is around -0.3 point something. So it has a huge potential difference that we just mentioned some time ago. Now during this course of journey of the electrons from this NADH H+ to the oxygen. So this is a kind of a very complicated journey through a number of electron carrying molecules.

So these are actually carrier complexes, which are associated with different proteins, iron sulfide protein, flavin mononucleotides, flavin dinucleotide and cytochrome based complex. So when the electrons are transported through the chain, protons are effluxed out of the membrane. So this is one of the very important or interesting aspect of the entire the chemiosmosis process.

That when the electrons are transported from a negative potential towards a positive potential, that is the terminal electron acceptor a number of carrier complexes, which are responsible for carrying the electrons towards the final electron acceptor, they are also acting as efflux pump that means, they are capable of pumping out protons from the cytosolic side to the outside.

So from the cytosolic side to the outside, the protons are effluxed out. So protons are effluxed continuously as the electrons pass through one complex to the other.

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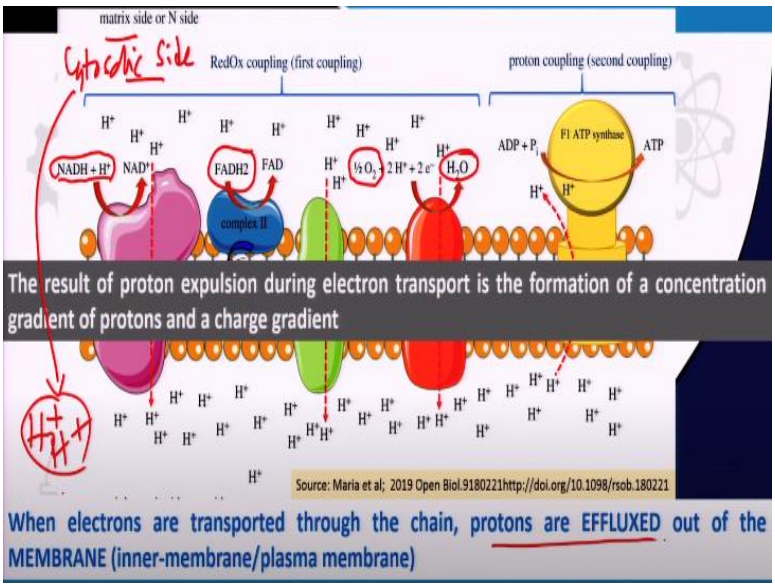

Now if this proton expulsion continues, during this electron transport system, eventually it leads to the formation of a formation of a concentration gradient of proton because the electrons are continuously being shuttled, because this NADH H+ or FADH2 these are continuously being generated by TCA cycle. So there is a continuous flow of electrons through these NADH H+ and FADH2 to the, to this electron carrier complex.

And when it continues, a proton gradient builds up, okay. And it is not only a proton gradient, it is also a gradient of the charge because the protons are positively charged. So it is a chemical species as well as a charged molecule the protons.

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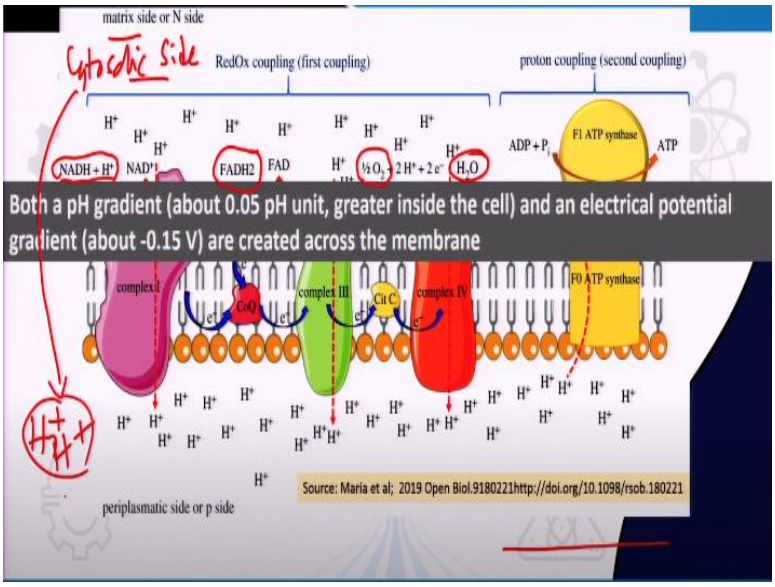

Now it leads to a change in pH that is a pH gradient is produced as well as an electrical potential gradient of around -0.15 volt is created across the membrane. So this efflux of protons through some of the electron carriers of the electron transport chain during this oxidation of reducing power or cofactor to transfer of electrons to terminal electron acceptor, eventually a pH gradient and a electrical potential gradient is created.

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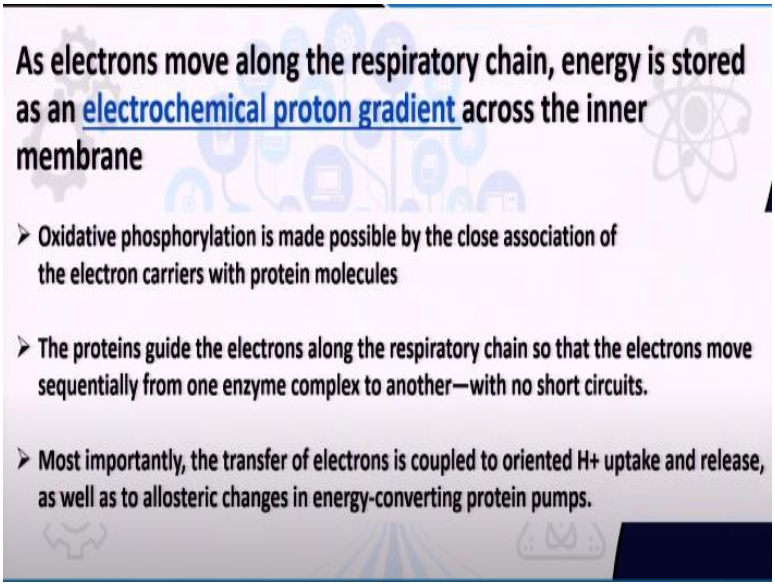

Now as electrons move from or through the respiratory chain, the energy is stored because they are moving, the electrons are moving from a strongly negative potential to a strong positive potential. So as the electrons are moving down the gradient, an electrochemical proton gradient is created. Now oxidative phosphorylation, when we will see that phosphorylation of ATP is happening is made possible by the close association of the electron carriers and with the protein molecules.

The proteins guide the electrons along with the respiratory chain, so that the electrons move sequentially from one enzyme complex to the another. It is highly organized and highly directed. According to the flow of obeying the obeying the electron potential gradient the electrons are directed by the protein molecules which are associated with these carrier complexes within the membrane, so that there is no short circuit or the electrons they do not fly here and there within the membrane complex.

Now most importantly, the transfer of electrons is coupled to oriented proton uptake. So the transfer of electrons is coupled to oriented proton uptake and release as well as allosteric change in the energy-converting proton pump. So we will be able to see how this proton gradient is eventually utilized to do some useful work like the formation or the synthesis of the ATP molecule or towards performing some other useful work.

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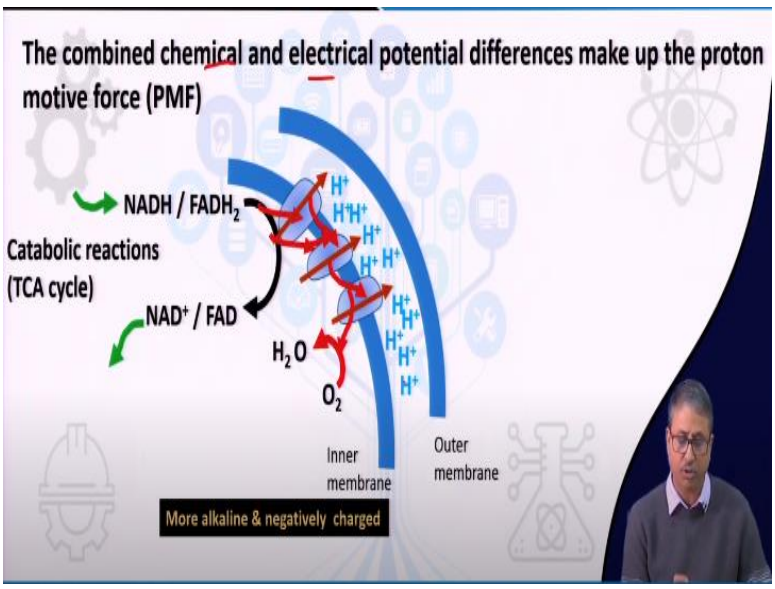

So here is the complete picture now that the combined the chemical as well as electrical potential difference happens because of the efflux of the protons. So lot of

protons accumulate in the intermembranous space or the periplasmic space making the cytosolic side more alkaline or relatively alkaline and relatively more negatively charged. So that is how it is called a kind of electrochemical gradient.

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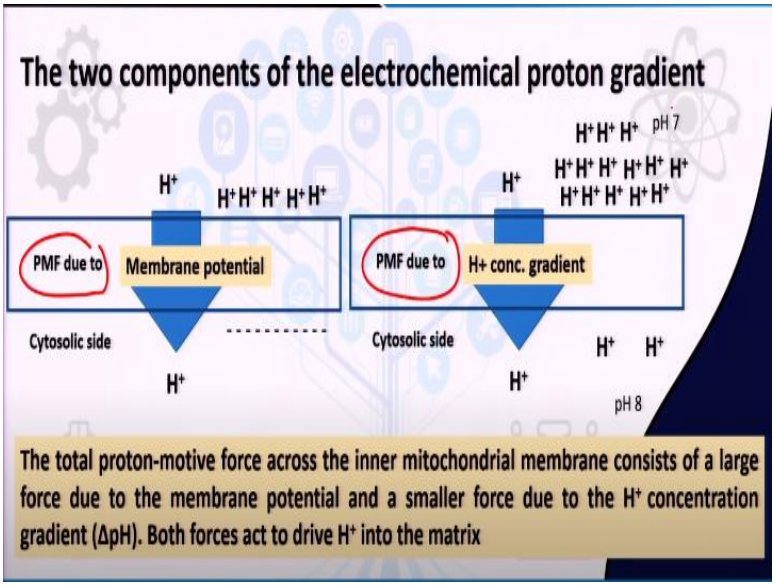

And which is actually responsible for creating certain more important events or further important events. So that event is referred as the proton motive force. The proton motive force is basically these protons, which are effluxed out of the system are able to are trying to come inside and establish the equilibrium and they create a kind of a pressure on the membrane.

This potential difference is leading to the formation of the proton gradient. Now there are two important components of this electrochemical proton gradient. One is the membrane potential. So one driver for the PMF is proton motive force is basically the membrane potential. The outside of the membrane is positively charged and the inside of the membrane or the cytosolic side is negatively charged.

And another cause of the proton motive force is the nature of the positive nature of the hydrogen ion concentration. So outside of the periplasmic side or the intramembranous face side, it is more proton concentration leading to the pH to be slightly acidic compared to the cytosolic site. Now the total proton motive force across the inner mitochondrial membrane consist of a large force due to the membrane potential and a smaller force due to the proton concentration within this.

Now both forces act as a drive to proton in the matrix. So this membrane potential and the hydrogen proton concentration gradient both are responsible for driving the inward entry of the proton into the matrix.

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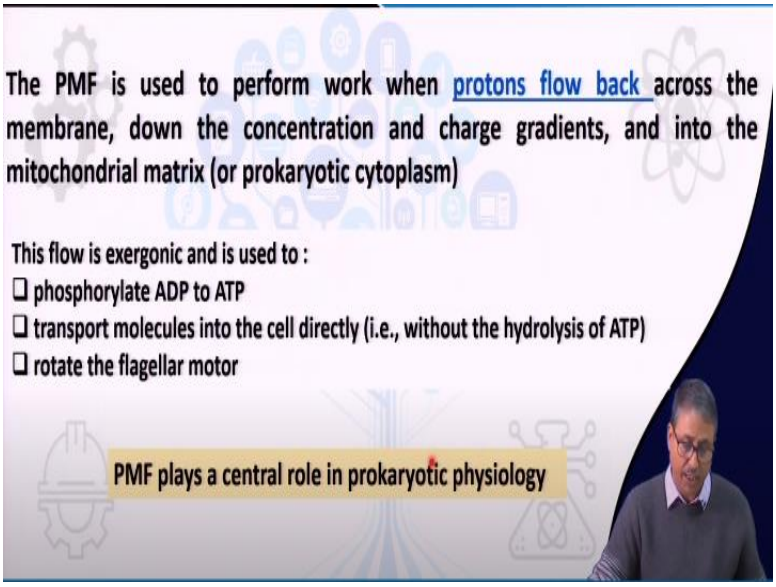

So now we will be talking about that because of this electrochemical gradient the protons would like to come now inside the cell. How that is achieved? Now the PMF, which is used to perform work when protons flow back, so proton motive force is first generated through the creation of the electro chemical gradient.

Now once the PMF is there that is proton motive force is there by virtue of a large electrical and potential, electrical potential and the chemical species wise potential difference that is used to perform work only when the protons are able to flow back into the system across the membrane. And down the concentration gradient, because outside now are the intermembranous space that has more concentration of proton compared to the cytosolic site.

Now this flow of protons now from the outside to the inside of the membrane, outside to the inside because the large concentration of proton was moved outside through the efflux pumps, which are connected to the electron transport system. Now the protons would like to flow back to the cytosolic side or the inside of the membrane.

Now this flow of protons, if they are allowed to flow, would be exergonic, would release some amount of energy and that energy can be utilized to phosphorylate

adenosine diphosphate to ATP that is making the phosphodiester bond. Transport molecules into the cell directly because that is exergonic. So the flowing, when the protons want to flow inside back this is energy yielding reaction.

So some amount of energy can be harvested by coupling a transport of some kind of molecules which is otherwise difficult to transport because they might be against the concentration gradient. Or doing some useful work for particularly for cells, which are having prokaryotic cell in particular who are having flagella because flagellar motor requires large amount of this proton motive force or energy.

Now PMF or proton motive force plays a central role in prokaryotic physiology. It is not only the flagellar movement, but there are other transport events other than the generation of ATP directly.

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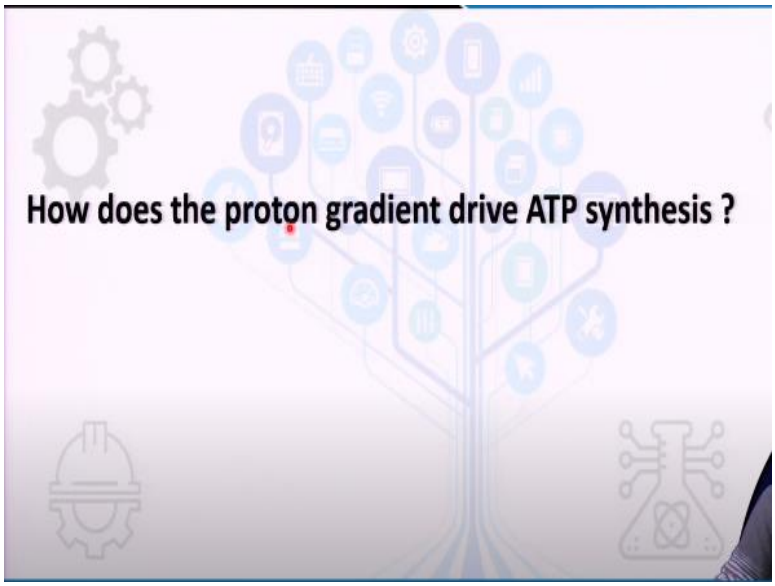

Now how does the proton gradient drive the ATP synthesis?

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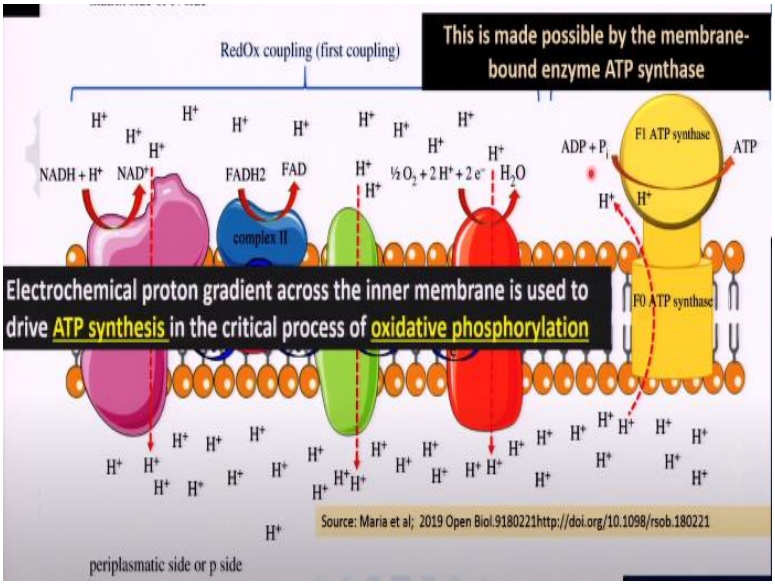

Now we have seen this electron transport system where a number of carrier complexes are there which are responsible for carrying the electrons from the reduced cofactors like NADH or FADH2 to the terminal electron acceptor that is the oxygen and which is responsible for building a high concentration of proton. So which is the concentration of proton as well as the concentration or the charge.

It is positively charged compared to the inner side or the cytosolic site which is negatively charged. Now the electro chemical proton gradient which is created because of this efflux of these protons across the inner membrane is used to drive ATP synthesis.

Now this proton motive force which is in place now because of the formation or establishment of these electrochemical gradient because the functioning of this efflux pump of the electron carrier would drive the ATP synthesis and that is a critical component of the oxidative phosphorylation. Now how this PMF can drive ATP synthesis?

This PMF which is basically connected to again these electrochemical gradient can drive ATP synthesis by a special very special enzyme complex which is called ATP synthase which is located in the membrane itself. So that means, the membrane contains a number of very important molecules or carrier complexes, a set of carrier complexes which are responsible for the redox coupling is the first phase of coupling

which are actually responsible for transferring the electrons from the donor to the receiver through the redox potential gradient.

And the second one is this large F0 F1 ATP synthase complex which is capable of making use of this electrochemical gradient or the proton motive force.

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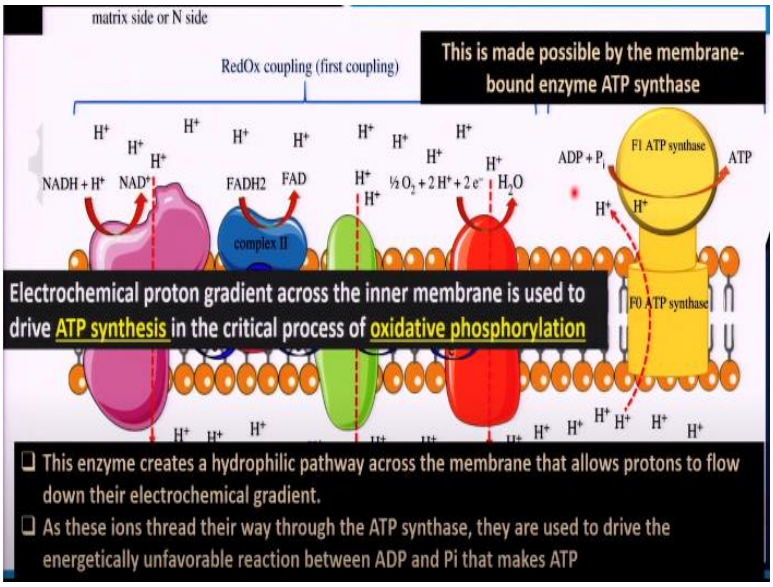

So this enzyme which is located on the inner membrane or the plasma membrane in case of a microbial system and it is allowing or it creates a hydrophilic pathway across the membrane that allows the protons to flow down the electrochemical gradient. We have earlier noticed the high concentration of protons are built up over here. So these protons want to flow back or come back.

So this enzyme complex facilitates or creates a hydrophilic pathway so that the charged protons can move back into the cytosolic site very favorably. And it is a favorable process because from the higher concentration to the lower concentration, they will move. But it is not only a favorable process for them, but it is also an exergonic process. That means it leads to release of energy.

So as these ions or the protons thread their way through the ATP synthase they are used to drive energetically unfavorable reaction. Because as I mentioned that these this movement is exergonic from a high concentration to low concentration so there could be a gain in energy. So that energy which is released because of this transfer of

the high protons from a higher concentration to the lower concentration is utilized to phosphorylate ADP to produce ATP.

That is the part of the phosphorylation part. So one ADP molecule can be phosphorylated to produce the ATP molecule.

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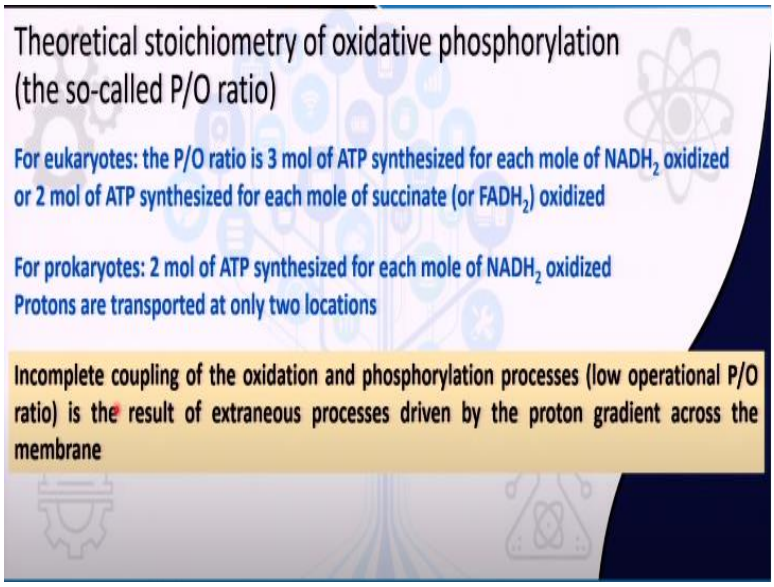

Now if we look at the theoretical stoichiometry of this oxidative phosphorylation, it is also referred as the P by O ratio. For eukaryote the P by ratio is around three moles of ATP synthesized per mole of NADH H+ oxidized or two moles of ATP synthesized for each mole of succinic acid or FADH2 oxidized. For prokaryotes it is less. It is two mole of ATP synthesized for each mole of NADH2 oxidized.

And the protons are because protons are transported only two locations. In prokaryotic system the electron transport system is more complex and it is branched also. So maybe because of that and the point of efflux of protons as electrons flow through the electron transport system are relatively less.

So because of that the amount of the ATP generation or the theoretical yield of the ATP per mole of NADH2 oxidized is significantly less in case of prokaryotic organisms. Now it is also true that in prokaryotes the incomplete coupling of the oxidation and phosphorylation is because of the extraneous processes which are driven by a proton gradient.

Because a number of transport processes particularly and flagellar movement for example, these are all directly connected to the proton gradient or proton motive force. So it is not that all the energy which is created because of the electrochemical gradient is utilized to drive the ATP synthesis by F0 F1 ATPase.

In case of prokaryotes, a large number of, a large portion of the proton motive force is actually dedicated for other functions, which are also very important like the cellular transport or the physiological, other physiological functions like the helping in the movement of the cells through flagellar motor function.

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So in this part of the lecture, we have used mainly the metabolic engineering textbook as well as two important books, one is the molecular biology of the cell that is by Alberts and the Prescott Microbiology book.

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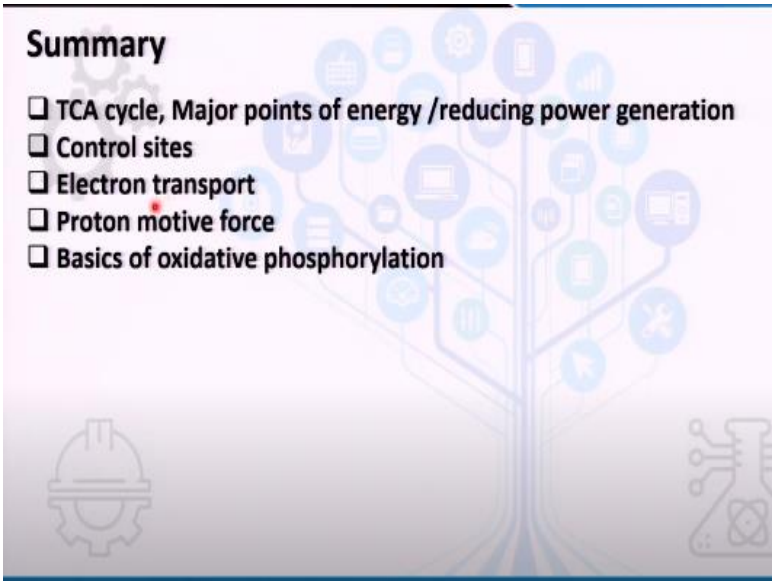

So in summary today, we have covered in this part of the lecture, the TCA cycle and major points of energy and reducing power generation, the control sites and the factors which could control the TCA cycle, the electron transport chain and how electron transport chain is facilitating the generation of proton motive force and how proton motive force is connected to the oxidative phosphorylation. Thank you.