Environmental Chemistry and Microbiology
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Module - 9
Lecture - 46
Microbial Metabolism - III

Welcome everyone to lecture number 46, which is the third part of Microbial Metabolism in module 9.

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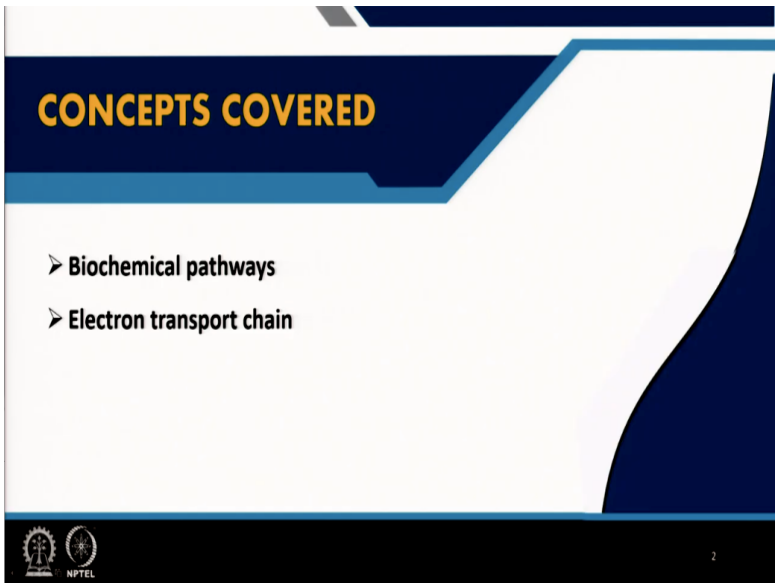

We are going to look at the electron transport chain and how the 3 different biochemical pathways; you can call them 3 or 2, respiration and fermentation; and within respiration, you have aerobic and anaerobic respiration. So, we will take a look at those pathways and how they are, how they happen within the prokaryotic cell.

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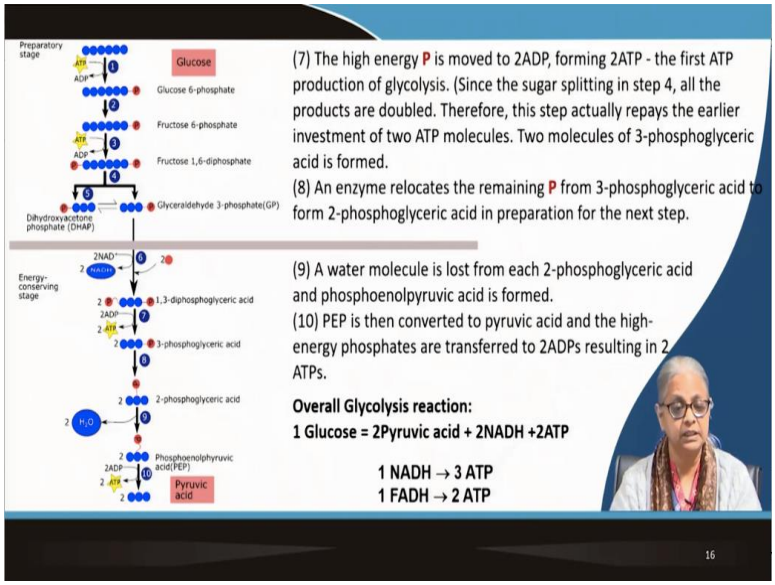

Let us go through some details about glycolysis. So, here we have the glycolysis pathway and we start with our favourite starting compound that is glucose. So, we have glucose to begin with; C-6 to begin with; 1 ATP is lost and 1 phosphate is attached to the glucose molecule. So, this is your glucose 6-phosphate. So, at the expense of 1 ATP that gets converted to ADP and you get glucose 6-phosphate. So, it is a low energy phosphate bond. And this is then rearranged. You get the isomeric form, to get fructose 6-phosphate. This fructose 6-phosphate is then converted to a diphosphate, which means phosphate is attached at both ends of the C-6 molecule. So, one more ATP is utilized and you get fructose 1,6-diphosphate.

This fructose 1,6-diphosphate is then cleaved into two C-3 compounds. So, I say C-6 to begin with and now we have 2 compounds that have been broken into (two parts). Now, each step of these reactions is mediated by a specific enzyme. We are not interested in that level of detail, but if you are interested, you can refer to the textbook. So, here we have C-6 going to C-3 compounds. We have two C-3 compounds, dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate or GP.

Now these two are interchangeable compounds. So, assuming that GP or G3P is dominant, we then go to the next step. I think GP dominates in solution, and yes; so DHAP is readily converted to GP. And the next enzyme will convert whatever GP is formed to the next compound. This compound is diphosphoglyceric acid. So, 2 things happen over here. NAD^+ is converted to NADH and a phosphate. 2 phosphates are utilized.

So, each of these C-3 compounds which had only 1 phosphate, now have 2 phosphates each. So, now we have two of the C-3 molecules. Two of these C-3 molecules, each one of them has 2 phosphates. Utilizing 2 ADP, these phosphates from 1,3-diphosphoglyceric acid are transferred to 2 ADP molecules; so they become 2 ATP and you then get 3-phosphoglyceric acid.

So, 2 phosphates have been transferred to ADP in high energy bonds along with NADH being formed. There is a rearrangement of the compound with 2-phosphoglyceric acid being formed; and water is being generated. And in the final step, we have phosphoenolpyruvic acid (PEP) which is a very important key intermediate in this entire cycle. And again, ADP, 2 more ADPs are used.

Phosphate from phosphoenolpyruvic acid is transferred to ADP and pyruvic acid is generated. So, pyruvic acid is a C-3 compound; 2 of them are formed. And in the entire process, 2 ATPs were invested, 4 were obtained. So, there is a net gain of 2 ATPs, and 2 NADH are also gained. So, that is another gain. When these 2 NADH enter the electron transport chain, each NADH will result in 3 ATPs. So, there is a net gain of 8 ATPs.

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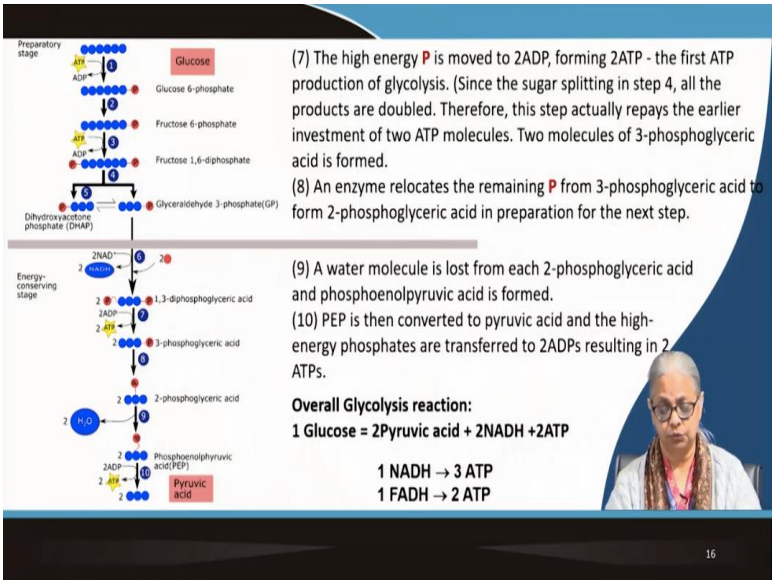

So, that is what you see over here. It is the same entire reaction. Each step is described in these 2 slides. So, the overall glycolysis reaction for us is, 1 glucose will give you 2 pyruvic acids, 2 NADH and 2 ATP. That is the net accounting of the entire reaction; or you can call it at the end of the glycolysis reaction. Do not forget that 1 NADH gives you 3 ATP. And if you have FADH, that will give you 2 ATP. And this is very important in the next step.

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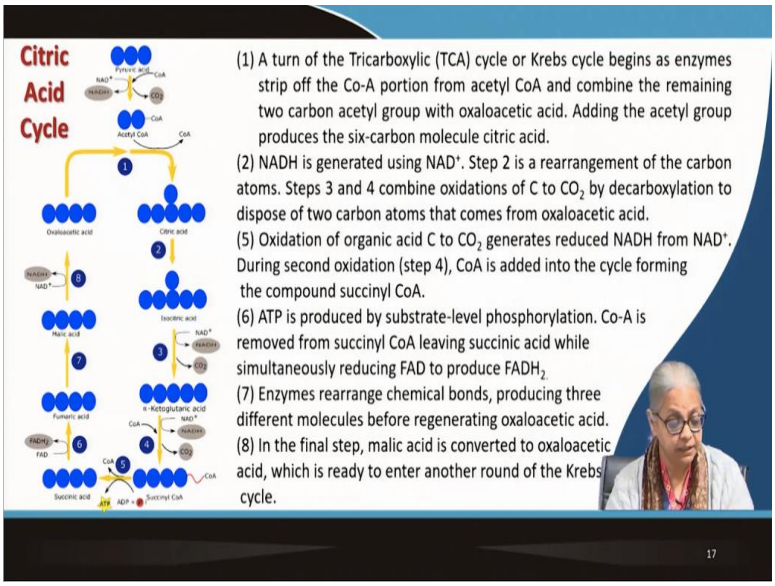

So, here we have what is called the citric acid cycle, the Krebs cycle or the tricarboxylic acid cycle (TCA cycle). So, various terms are used to describe this very crucial method of oxidizing a C-3 compound. So, here we have the beginning of the cycle. It starts with C-3 which is pyruvic acid. This pyruvic acid is converted into a key intermediate within the cycle. So, 1 carbon dioxide is lost over here. One of the carbons in pyruvic acid is converted to CO_2.

It combines; the C-2 that is left, combines with coenzyme A; so you get acetyl-CoA. Now, for the first step, NAD^+ is converted to NADH and one of the CO_2s is released. Now, this acetyl-CoA, as I said, it enters into this cyclic set of reactions which have several key intermediates. So, you have acetyl-CoA; CoA is released; it is a coenzyme; so, it has to be released without doing anything.

This acetyl group, the C-2 group will attach itself to oxaloacetic acid (OAA), which is the last step of the cycle. So, this C-4 which was the last intermediate in this cycle will attach itself to the acetyl group, and coenzyme A is released. This citric acid which is a C-6 compound will then be rearranged to form isocitric acid. This isocitric acid will again lose 1 carbon. In the process, NAD^+ is converted to NADH, you get α-Ketoglutaric acid.

α-Ketoglutaric acid is a C-5 compound. It then loses another CO_2 and is converted to succinyl-CoA when a coenzyme, again coenzyme A attaches itself to this compound to form succinyl-CoA. And in the process, CO_2 is lost and NAD^+ is converted to NADH. Now, this succinyl-

CoA is going to utilize ADP plus another phosphate to generate ATP; and coenzyme A is released back into solution.

So, this succinyl-CoA is now succinic acid. ATP has been formed. These are high energy bonds. So, in the next step, FAD^+ is utilized. It is converted to FADH. So, succinic acid is converted to fumaric acid and fumaric acid goes to malic acid; malic acid is converted to oxaloacetic acid. These are all C-4 compounds; so, they are all isomers of the same thing. So, we have NAD^+ being converted to NADH; oxaloacetic acid is the last compound in Krebs cycle; and this entire cycle continues as long as acetyl-CoA keeps entering the Krebs cycle.

So, this is all of it. And from our point of view, what is important? Something that we need to all remember is, what are the key intermediates. So, you know all the key intermediates; and the fact that a C-3 compound has been converted to three CO_2 molecules; and in the process, 1 ATP per pyruvic acid. So, here is the full account.

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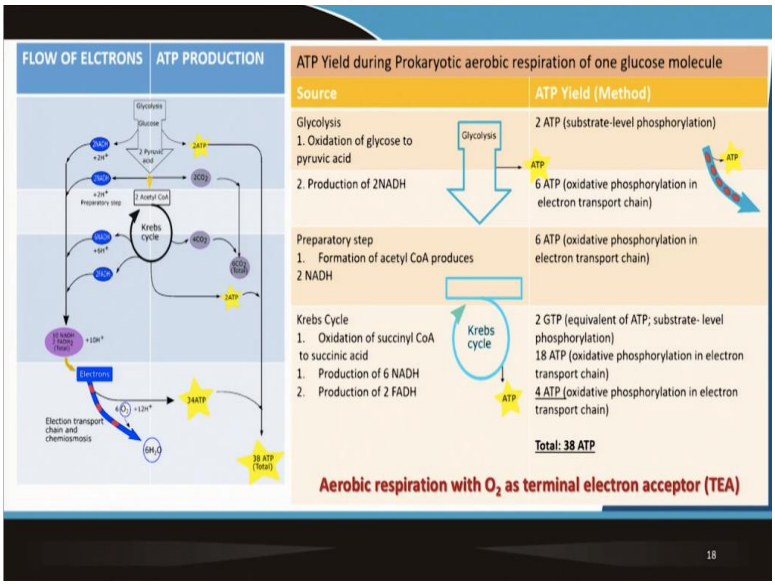

So, we have glycolysis. We have already accounted for 8 ATP in the preparatory step of the Krebs cycle, where pyruvic acid is converted to acetyl-CoA. What did we get? We got 1 NADH. Now every NADH is equivalent to 3 ATPs. So, 4 are starting compound of glucose. We have to multiply; 3 is for 1 pyruvic acid; so, 6 is for 2 pyruvic acid compounds. So, here we have 6 ATP.

Then it enters the Krebs cycle where you get 2 GTP, which are the equivalent of ATP. This is substrate level phosphorylation. Let us just take a look at it again. So, here we have the substrate

level phosphorylation, where succinyl-CoA goes to succinic acid and you get generation of ATP. So, that is 2 ATP here. Then you get production of 6 NADH for every glucose molecule; 3 NADH for pyruvic acid and 6 for glucose.

So, these 6 NADH have to be multiplied by 3. So, you get 18 ATP from oxidative phosphorylation in the electron transport chain. And finally, 2 FADH will give you 2 ATP. So, each FADH gives you 2 ATP. So, 2 FADH will give you 4 ATP. Again, that enters the electron transport chain; and you get that by oxidative phosphorylation. So, at the end of the entire process, CO_2, a C-6 molecule has been converted to 6 CO_2, and 38 ATP have been generated.

So, this is the aerobic respiration pathway with oxygen as the terminal electron acceptor. Now, this is the maximum that nature has been able to do with the best possible redox couple. So, the redox couple of glucose + oxygen is the highest energy yield; and 38 ATP is what is obtained.

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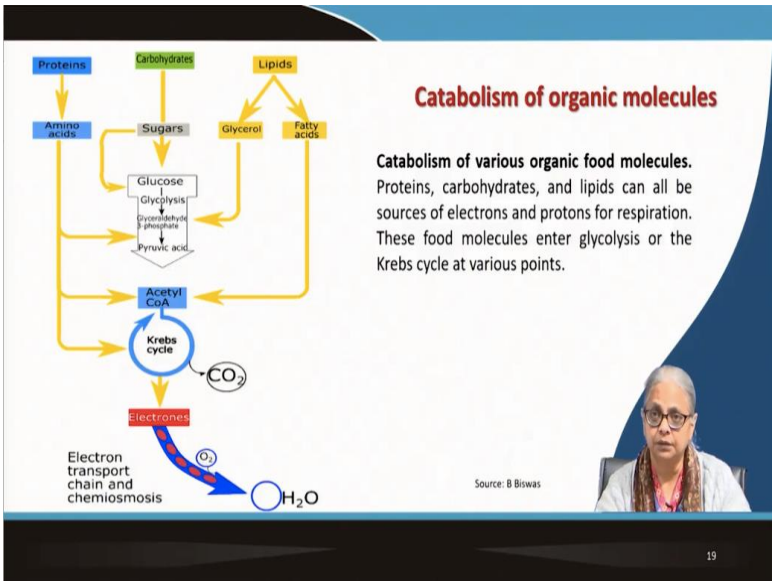

This is about the catabolism of glucose. Now, what about all the other types of compounds? So, what we just saw is carbohydrates, and that is sugar. Glucose is sugar, that we saw complete conversion of. So, let us come to the catabolism of organic molecules. So, we have looked at the catabolism of glucose going to 6 CO_2. So, that is what you see in the centre of this figure. What about the other macromolecules? What happens to proteins, lipids?

So, proteins have to be broken down into their monomeric units of amino acids. These amino acids can become part of the glycolysis step or they can enter the Krebs cycle, and from there

into the electron transport chain. So, this is one method. Then you have lipids. They get converted. Remember, the lipids are glycerols attached to fatty acids. So, the splitting of glycerol molecules and fatty acids is the first step.

And these glycerols can enter at the glycolysis step, the fatty acids can enter at the Krebs cycle. So, these are some of the points at which other organic molecules can get broken down into other intermediates or converted to CO_2.

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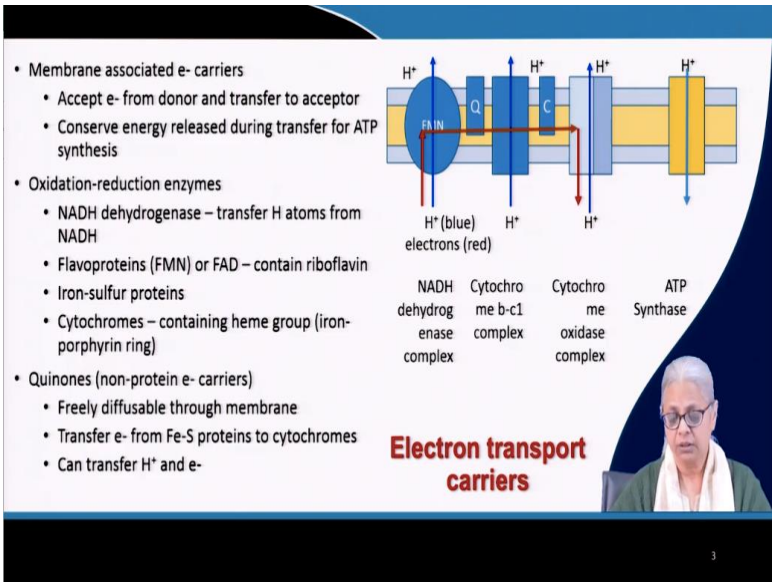

So, let us first look at the electron transport carriers. I already mentioned in a previous topic that this is your plasma membrane. So, here are your hydrophilic portions. You have your hydrophobic portion and you have the remaining hydrophilic portion of the phospholipid bilayer membranes. Now, there are several membrane spanning proteins that are a part of the electron transport chain.

So, the bottom part of this layer faces the cytoplasm. So, you have your periplasmic space. Now, you have membrane associated electron carriers. So, the first thing that is going to happen is, let us say you have; let me also back up here and say that there are 3 complexes that are shown in the textbook; and we are following the textbook by Tortora, Funke and Case. So, they have gone with 3 complexes. So, I am sticking to that.

There are other sources in the literature which show 4 complexes. Either way, the endpoints remain the same. There are no major differences. So, here we have our 3 major complexes. And we have membrane associated electron carriers which are accepting electrons from the

donor and transferring them to the acceptor. So, in the first case, we have NADH. Now, NADH is going to give up a hydrogen atom. So, 1 proton and 1 electron are going to pass from NADH into FMN, which is the first complex. And here, the protons are being pumped out of the cell. So, a proton motive force is being generated. So, the concentration of protons outside the cell (membrane) is going to be higher than the concentration of protons from the inside of the membrane, and are being pumped out of the cytoplasm. These protons are going to be transferred along with electrons.

So, the red line represents electrons and the blue line represents protons. So, these electrons are not going to be pumped out. Instead, they are going to be passed from one complex to another through the plasma membrane, through these complexes which are associated with the plasma membrane. Eventually, these electrons will be transferred at this end point to our terminal electron acceptor (TEA). So, our terminal electron acceptor can be oxygen.

That oxygen gas will accept these electrons and convert to water; or you can have carbonate, nitrate, sulphate and so on. So, all that will happen at the last point. So, here we have our membrane-associated complexes. So, you have complex I, complex II and complex III. So, complex I is NADH dehydrogenase complex; complex II is cytochrome b-c1 complex; and the last one is cytochrome oxidase complex.

Now, out of these, as the electrons are picked up by the terminal electron acceptor, in the last part of the entire process, the proton motive force; remember, in all 3 cases, in all 3 complexes, protons are being pumped out. These protons will then be utilized in the ATP synthesis process. So, at this end, at the inner side, inside the cytoplasm, ADP, adenosine diphosphate will pick up one more phosphate utilizing the proton motive force that already exists across the membrane, and this protein or enzyme, ATP synthase will generate ATP molecules.

So, first NADH is involved with generating the proton motive force. This proton motive force is then utilized for generating ATP. And we already went through the fact that 1 NADH molecule will give you 3 ATP; and 1 FADH molecule will give you 2 ATP. So, these are some of the things that you need to remember to understand how energy is generated during the electron transport process.

So, these membrane-associated electron carriers will conserve the energy released by helping in the synthesis of ATP. These are oxidation-reduction enzymes. NADH dehydrogenase is transferring the entire hydrogen atom from NADH; so, you have a split between the protons and the electrons. The electrons are transferred from 1 complex to the other; and the protons are pumped out of the cell (across the plasma membrane).

Then you have the flavoproteins or FAD. They contain riboflavin; and they are also oxidation reduction enzymes, iron-sulphur proteins and cytochromes. All of them contain heme groups or other methods of transferring electrons. So, we will take a look at some of them.

And then we come to non-protein electron carriers. The non-protein electron carriers are quinones. These quinones are not attached to the membrane. They are freely diffusible through the membrane. They can transfer electrons from the iron-sulphur proteins to the cytochromes. And they can also transfer both protons as well as electrons.

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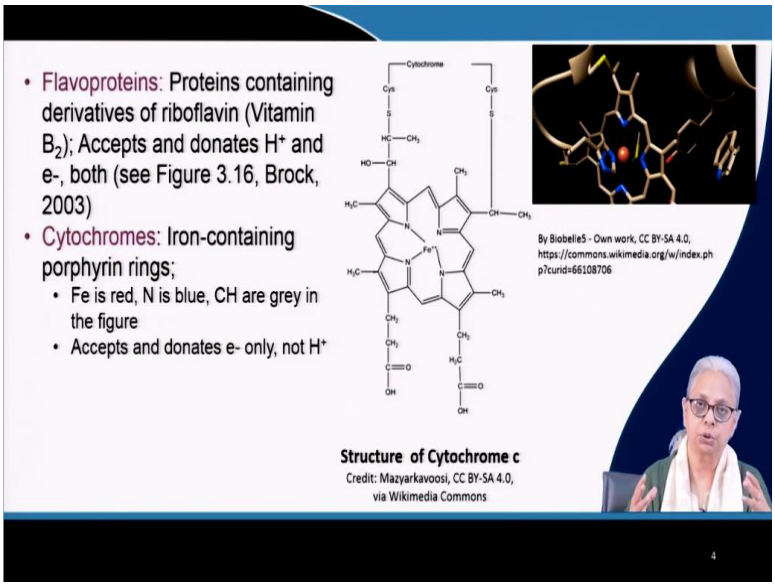

So, let us see if we can take a closer look at some of them. I have already mentioned that flavoproteins are proteins that are derivatives of riboflavin, which is vitamin B2; and they are capable of accepting and donating both protons as well as electrons. And I will refer you to the textbook. Any of the textbooks will give you the structure of flavoproteins.

And then we come to cytochromes. Now, cytochromes, as I said, are iron containing porphyrin rings. I think, in a previous topic, I have already shown this. So, here you have Fe^{2+} at the centre of a porphyrin ring, which is an large organic molecule; and it is bound by nitrogen

atoms. So, you can see it in pictorial form. Here is our iron and it is bound by the nitrogens in blue; and the grey colours are for the carbon, the hydrocarbon part. Now, these cytochromes can accept and donate electrons; only they do not transfer protons.

So, I refer back again to this. So, these are the quinones and these are the cytochrome pools. So, you can see that some of them are involved in proton, in pumping out the protons and in other cases, just the electrons are passed from one complex to another.

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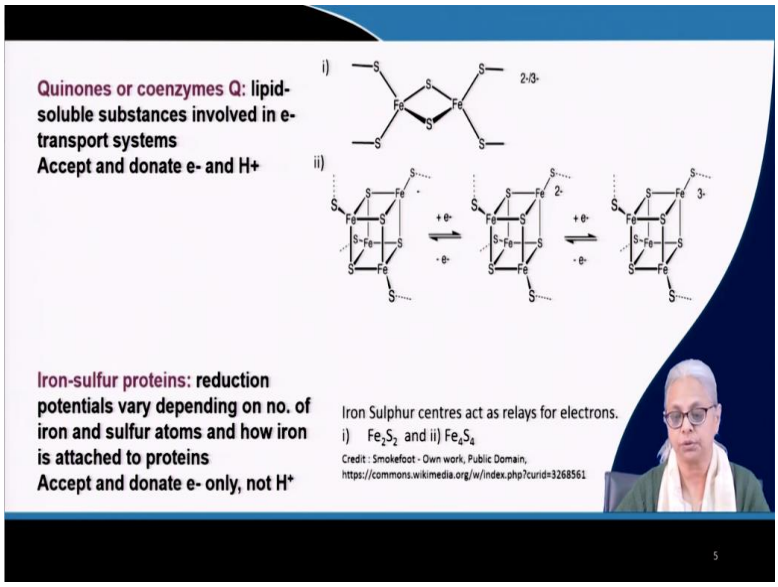

So, I already mentioned that we have quinones, which are also called coenzymes Q. These are lipid soluble substances. So, you can understand that the lipids within the plasma membrane are where these enzymes can be found literally; and they are involved in the electron transport system. So, they can accept and donate both electrons and protons. And then we have the iron-sulphur proteins.

Now, these iron-sulphur proteins act as relays for the electrons. So, you can see different examples, Fe_2S_2, Fe_4S_4. And change in the reduction potential will depend on the number of iron and sulphur atoms in these proteins, and how many iron atoms are attached to these proteins. They are capable of accepting and donating only electrons, not protons. So, these are schematics to show you the different types of iron-sulphur proteins.

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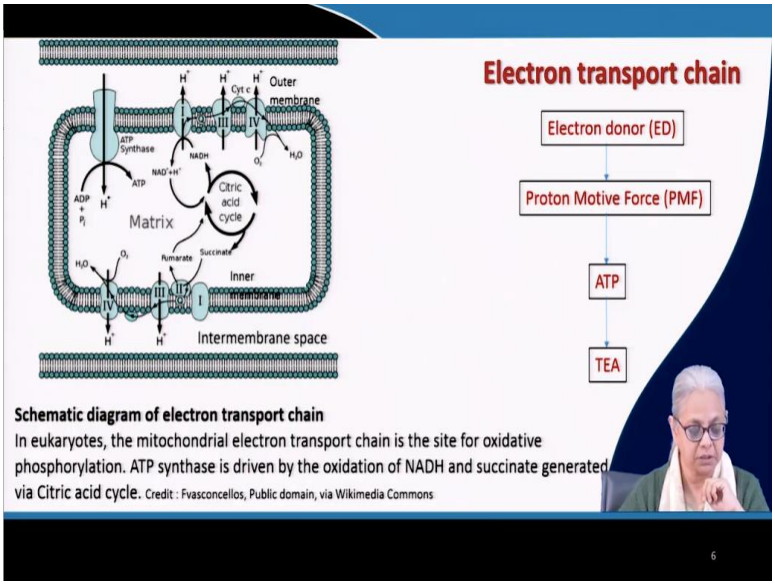

This electron transport chain is applicable to prokaryotes only. In eukaryotes, the same process happens at the mitochondria, which is the site for oxidative phosphorylation. So, it is important to keep the definition of oxidative phosphorylation in mind. It is defined quite simply as the synthesis of ATP coupled with the electron transport chain. Now, it is important to remember that this electron transport process happens in prokaryotes at the plasma membrane. And whether the outside of the plasma membrane faces another outer membrane or whether it faces the cell wall, regardless, the same process is happening.

In eukaryotes, this process happens at the mitochondrial membrane. So, that is at the inner membrane of the mitochondria. And like I said, this is the argument used in favour, rather to explain the endosymbiotic theory. So, we will come back to this point later again. So, that is about it over here.

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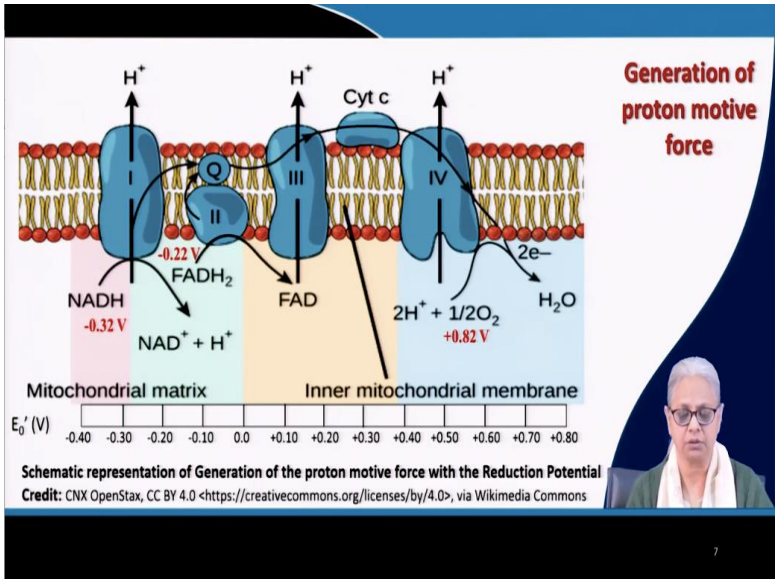

And more of the same. So, if you want to look in terms of reduction potentials, you can see how the reduction potential from complex I to IV is going from negative E_0' values to positive E_0' values. So, that is, if you remember the electron tower, this is similar to the electron tower. And that tells you how the electrons that are available from NADH, they are passing on all the way to the terminal electron acceptor. That is helping to generate proton motive force, which in turn will be used for ATP generation.

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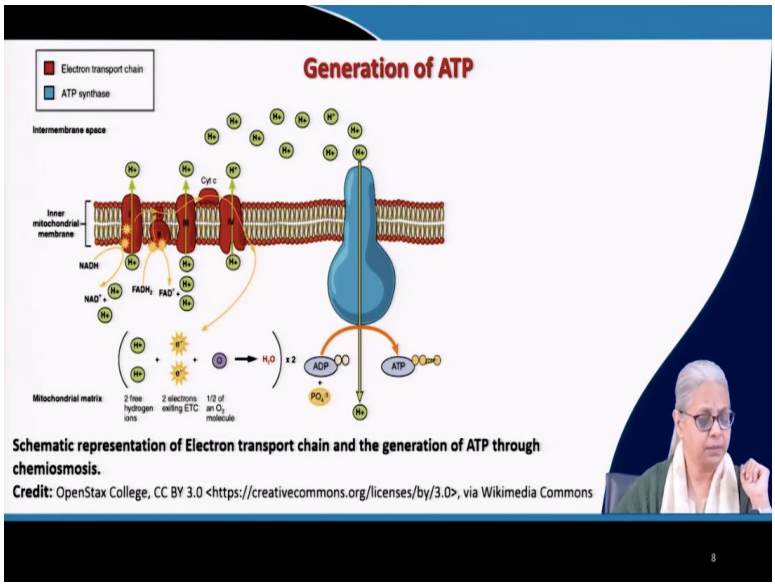

More of the same. So, here you can see, this is an even better schematic. So, you can see the protons from the cytoplasm or from the mitochondrial matrix. They are being pumped out of the mitochondria or the prokaryotic cell, either way. So, here we have the 4 complexes. You can see the generation of the proton motive force and the utilization of NADH.

It gets converted to NAD^+ which will go back to the glycolysis step and so on. And the same thing with $FADH_2$. That will be converted to FAD^+ and again it will be sent back to the citric acid cycle. So, here is the dissipation of the proton motive force and the generation of ATP utilizing ADP. So, this entire process is oxidative phosphorylation.

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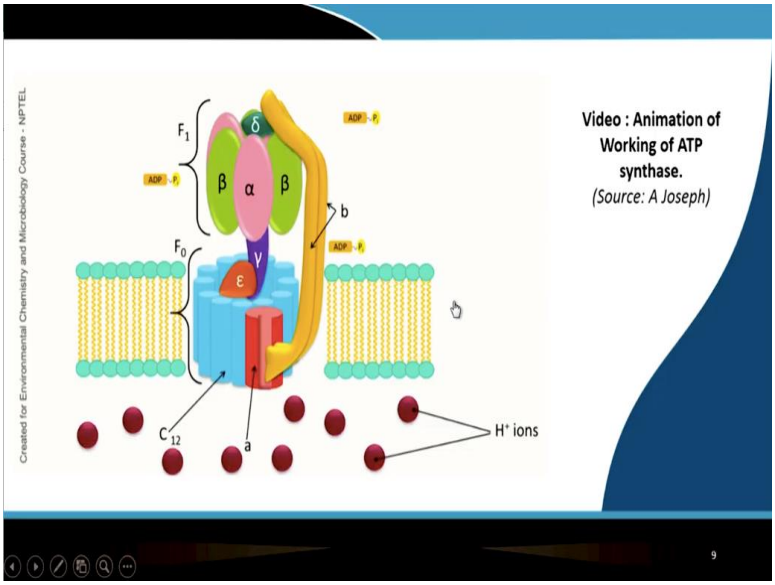

Here we have an animation of ATP synthase and how it works in helping to use the proton motive force and create ATP. **(Video Starts: 24:30)** So, the top part of the phospholipid bilayer is the inside of the cell and the bottom part is where the proton motive force is. So, these red circles represent the protons. They are being taken in through ATP synthase, within the cytoplasm, you have ADP. And along with a phosphate, these protons, along with the ADP molecules will be used to generate ATP using the phosphates. **(Video Ends: 25:01)**

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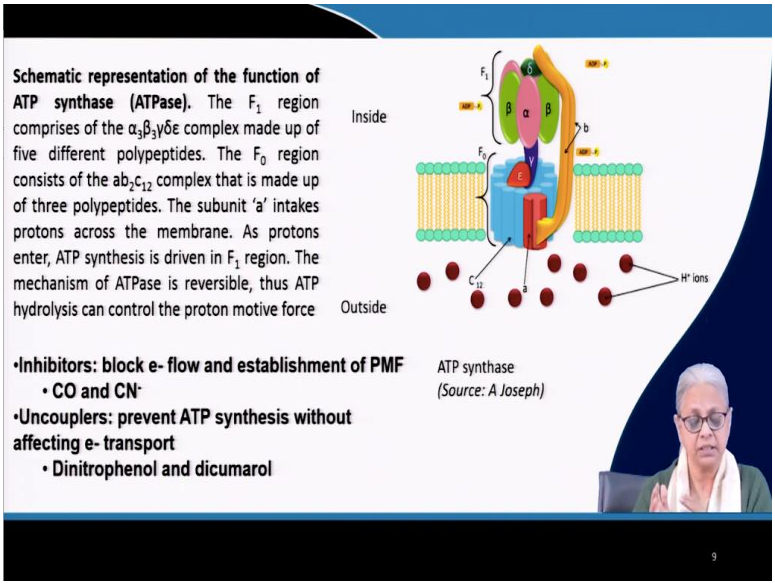

So, here we have the schematic representation of the ATP synthase enzyme. It is also called ATPase. It has 2 regions, the F_1 region and the F_0 region. F_1 comprises 5 different polypeptide strands. So, you can see them here. And the F_0 region has 3 polypeptide strands. Now, there is a subunit in the F_0 region which will pick up protons that have been concentrating outside the membrane, on the outer side of the membrane.

Now, as these protons enter the membrane, this ATP synthesis will happen on the other side. So, here you have ADP. ADP will be attached to a phosphate molecule. And in that process, the proton motive force will be utilized and ATP hydrolysis. So basically, water molecules are lost; ATP is generated from the ADP molecules; and that is how the entire thing happens.

Now, there are 2 things from the environmental microbiology point of view, we have 2 compounds, 2 groups of compounds that are very important. One is inhibitors – these inhibitors are molecules or rather compounds that block the flow of electrons, and the establishment of the proton motive force. So, you have carbon monoxide and cyanide. We know that both of them are toxic compounds. So, the way they act, the nature of their toxicity is to prevent the electron transport chain from proceeding; they prevent PMF from being established; and therefore, ATP generation stops. Then we have uncouplers – uncouplers are compounds that prevent ATP synthesis without impacting the electron transport chain. So, there are couple of examples here. We have dinitrophenol and dicumarol.

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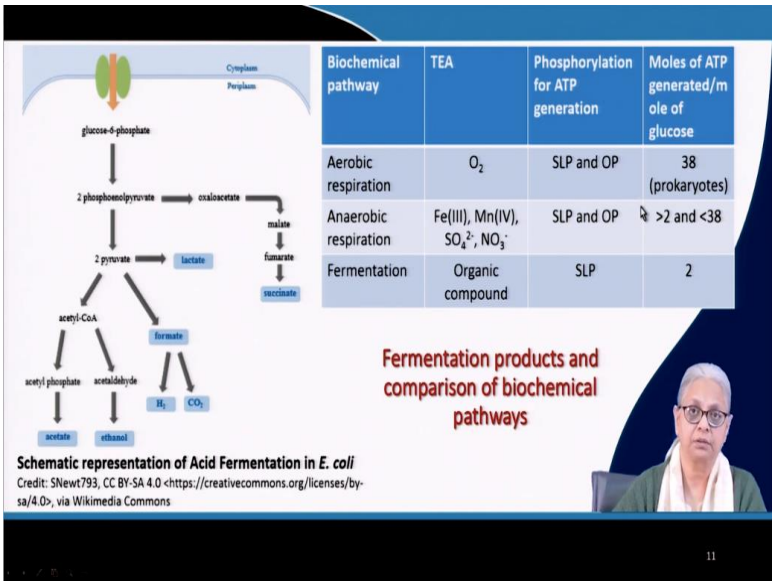

Then, here we have an example of fermentation in *E. coli*. So, let us say we start with glucose. Glucose is phosphorylated. It becomes glucose-6-phosphate, that is substrate level phosphorylation. This glucose 6-phosphate is converted to phosphoenolpyruvate. So, this phosphoenolpyruvate can be converted to oxaloacetate, and pyruvate can be converted to lactate.

Now, phosphoenolpyruvate can be directly converted to oxaloacetate, malate, fumarate and succinate. This is an acidic form - succinic acid. Pyruvate on the other hand will be converted to lactate or lactic acid and formate or formic acid. And then, that will further be converted to hydrogen gas and carbon dioxide. Then we have pyruvate being converted to acetyl-CoA.

This acetyl-CoA can be converted to acetyl phosphate, acetaldehyde; and the endpoints are acetate and ethanol. So, these are just examples of the fermentation end products of the same starting compound, depending on the organism that is doing it. I have already given examples in the previous topic. So, like I said, every different organism is going to generate different products depending on the starting compound and the nature of the organism.

Now, in a nutshell, literally, to summarize all 3 processes. So, 3 biochemical pathways, aerobic respiration, anaerobic respiration and fermentation. These are our terminal electron acceptors. So, for aerobic respiration, it is oxygen. Phosphorylation happens by a combination of substrate level phosphorylation. So, this is substrate level phosphorylation. And there is no other phosphorylation for fermentation. This is fermentation.

In aerobic respiration and anaerobic respiration, you get both; substrate level phosphorylation, (SLP) and OP which is oxidative phosphorylation. We have already gone through the accounting, where we saw that 38 ATP is the maximum number of ATP molecules that can be generated per molecule of glucose. And this is for prokaryotes only. In anaerobic respiration; let me go to the next one which is fermentation.

In fermentation, if you remember the glycolysis process, only 2 ATP are generated directly, and the remaining is NADH, which has to go to the electron transport process. So, in fermentation, you are left with only 2 ATP, because that is the end point. There is no further generation of ATP, because that reducing power which happens through the electron transport chain does not happen in the fermentation reaction.

So, in fermentation, you are left with only 2 ATP per mole of glucose. So, 2 moles of ATP per mole of glucose. The terminal electron acceptor in this case as well as the electron donor is the same organic compound. So, you have substrate level phosphorylation, but no oxidative phosphorylation.

Now, in anaerobic respiration; we are going to go into more details about these 3 pathways in the next topic. We will be doing a lot of quantification of all these compounds; how much energy is released, how do the bacteria utilize that energy and so on. So, these are the possible terminal electron acceptors. We have Fe(III), Mn(IV), sulphate, nitrate and so on. So, SLP and OP; basically, substrate level phosphorylation and oxidative phosphorylation will happen. 38 is our maximum; 2 is our minimum; so, depending on the nature of the terminal electron acceptor, we will get a number between 2 and 38 for anaerobic respiration.

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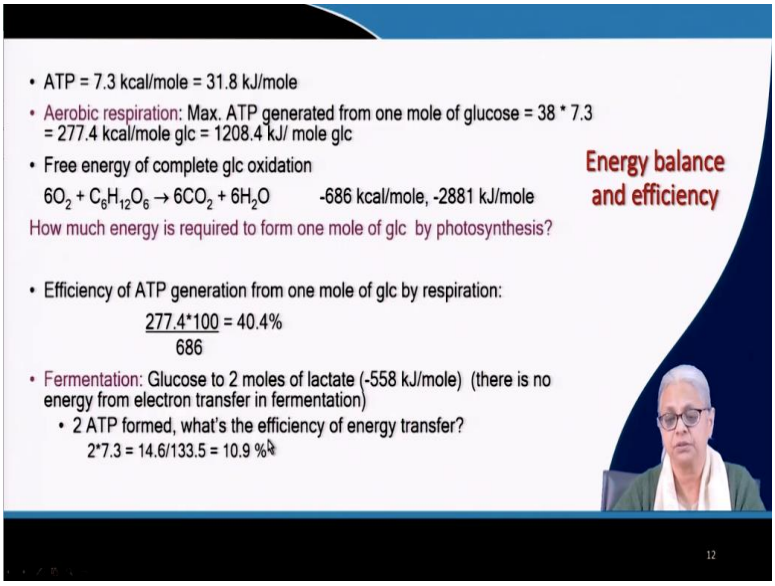

Before we end this topic, we will do one more thing, and that is to understand the efficiency of the natural process. So, 1 ATP, we know is equivalent to 7.3 kilocalories/mole or 31.8 kilojoules/mole. Aerobic respiration where we have already seen that the maximum ATP that you can generate from 1 mole of glucose is 38 ATP, 38 moles of ATP/mole of glucose.

So, if I multiply it; I have used kilocalories/mole, but it is better to use kilojoules/mole. So, in terms of either kilocalories or in terms of kilojoules, this is what you get. So, in terms of kilojoules/mole, you get 1208.4 kilojoules/mole of glucose, or you can say 277.4

kilocalories/mole of glucose. Now, what is the free energy of glucose, the free energy of formation of glucose?

So, how much of the energy that is available from the complete oxidation of glucose is being trapped in the form of ATP. So, here we have our free energy of complete glucose oxidation. So, if we write it as $C_6H_{12}O_6 + O_2 \rightarrow CO_2 + H_2O$, our ΔG_0 for this reaction is -686 kilocalories /mole or -2881 kilojoules/mole. So, we will come to this question later. So, let us take a look at the efficiency of ATP generation.

What is nature's efficiency in generating 38 ATP from this available energy? So, if this is what is available, how much is being utilized as ATP? So, we get 40.4%. So, that is pretty good from nature's perspective literally.

So, we come to fermentation where glucose is converted to various end products. So, in this example, the end product is lactate. In this process, 2 ATP are also formed.
So, if I were to write the chemical reaction for 1mole of glucose → 2 moles of lactate, then the ΔG_0 for that reaction is -558 kilojoules per mole. And the 2 ATP that are formed can be accounted for and what is the efficiency of energy transfer? So, obviously, the efficiency of energy transfer in terms of ATP only is very poor. It is close to 11%.

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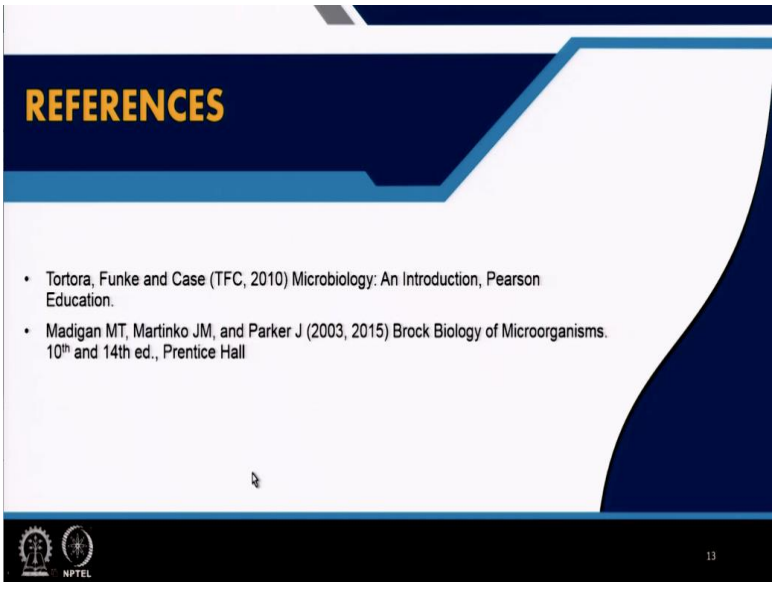

I will end this topic over here; and we will look at other aspects of the energetics of microbial reactions in the next topic. Thank you.