Introduction to Complex Biological Systems
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Lecture 33
Citric Acid Cycle and Oxidative Phosphorylation

Welcome to the third lecture of Week 7. Today, I am going to discuss the citric acid cycle and oxidative phosphorylation. So, we have already seen this in the previous lecture this week, that glucose is metabolized to pyruvate and in this process, the oxidation of glucose, we got 2 ATP molecules synthesized.

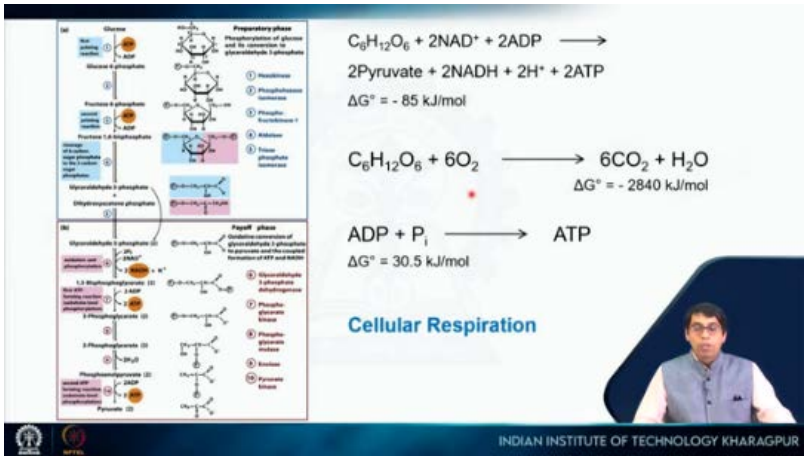
Now, glucose can be oxidized all the way to carbon dioxide and water. But in this case, there is only one oxidation step that happens here in step 6, and we get some amount of energy extracted from glucose and stored in ATP and this high-energy molecule, which is pyruvate. But the ultimate goal of oxidation is to convert glucose into carbon dioxide The electrons that come out because this is oxidation, so glucose will give up electrons, and those electrons will be accepted by oxygen to produce water.

We will see that this happens as two different sets of half-reactions. So, in one set, glucose will get converted to carbon dioxide, and in another set, oxygen will accept all the electrons and will form water and this happens in more subsequent steps. Finally, all the energy is stored in the formation of ATP. So, this slow oxidation process that happens inside the cell is called oxidative, or cellular respiration.

So, we generally call it respiration, but since this process is happening inside the cell, we call it cellular respiration and we will see that it will have two further stages. One is the citric acid cycle, and the next one is oxidative phosphorylation. It is important to point out that the subsequent steps that happen after pyruvate synthesis are much more complex. The reactions are more complex, and of course, they need an oxidative environment.

So, it is expected or hypothesized that the later part of these reactions evolved once cyanobacteria were evolved because they started producing oxygen, and then the environment became oxidizing, and only then did these more oxidative reactions evolve.

So, this is the complete summary of what we are going to study today. So, we have already seen glucose to pyruvate formation. So, that is glycolysis.



There were 10 reaction steps, and only one oxidation step was there where the electron was accepted by NAD+ to form NADH. Next, we will see that pyruvate gets converted to acetyl coenzyme A. So, this is another oxidation step, so another electron pair will be accepted by NAD+ to form NADH, and one molecule of carbon dioxide will go out. So, now we will also keep track of the carbons in the glucose. So, glucose was a 6-carbon molecule, and from one glucose, two pyruvic acids were formed.

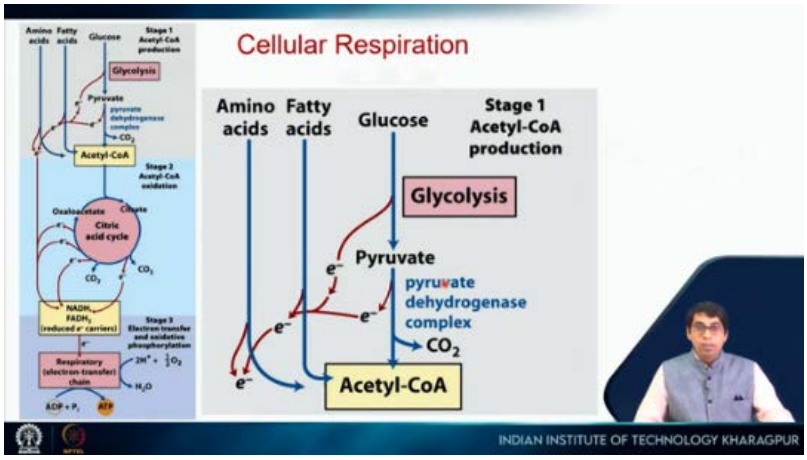
So, pyruvic acid is a 3-carbon molecule. Now, if we consider pyruvate, which is a 3-carbon molecule, in this step, one carbon goes out. So, we are left with two carbons, which is the acetyl group. Then, this acetyl group, as a thioester, acetyl-CoA, goes into the citric acid cycle and here, we will see that further oxidation steps happen, where two more carbon dioxide molecules go out. So, acetyl had two carbons, and they are now released as carbon dioxide. So, pyruvic acid gets completely oxidized into carbon dioxide: one molecule, two molecules, three molecules. So, all three carbons are released and the energy is mostly stored in different forms, majorly in the form of NADH and $FADH_2$.

So, these are the electron acceptors. So, now the remaining half reaction is still there, which is oxygen will have to accept these electrons, which are in this reduced species, to form water. So, oxygen has to form water, and that happens in stage 3, which is called

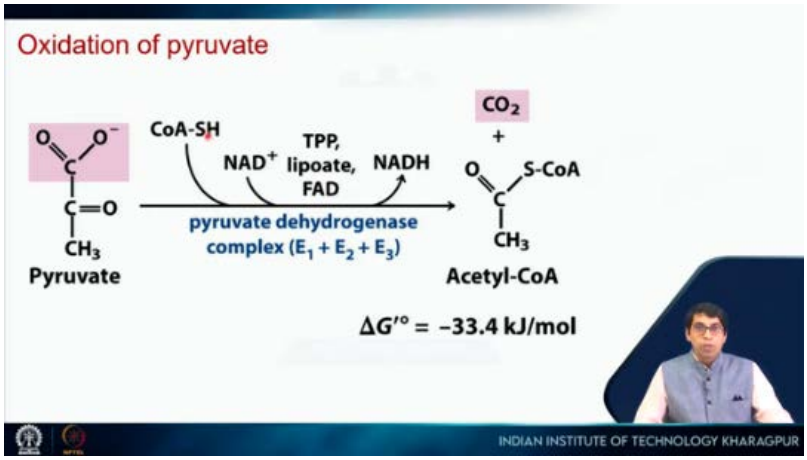
oxidative phosphorylation and we will see most of these reactions. So, starting from pyruvate to this, all of these reactions occur mostly inside the mitochondria.

So, glycolysis so glucose to pyruvate happens inside the cytosol, and from here to here, most of these reactions will occur inside the mitochondria. So, I will talk about mitochondria in more detail in the later part of this lecture. So now I am going to focus on this second part. We have already looked at stage 1, which is glucose to pyruvate. Now we are going to look at the later part of stage 1, which is the oxidation of pyruvate to form acetyl coenzyme A. The enzyme involved in this step is called pyruvate dehydrogenase.

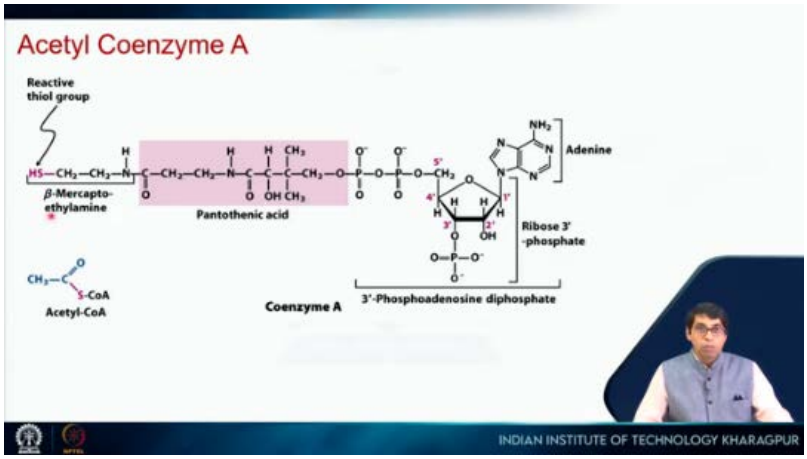
So it is not just one enzyme; there are three enzymes, E_1, E_2, and E_3. That is why it is called a pyruvate dehydrogenase complex. So this is the reaction. This is pyruvic acid. This acidic group goes out as carbon dioxide. So we are left with this acetyl group, CH_3CO, which is taken up by this coenzyme, acetyl coenzyme.



This is the coenzyme A. So when it takes up the acetyl group, it becomes acetyl coenzyme A. So that is the coenzyme A, and this is the acetyl group. Now, pyruvate dehydrogenase is the enzyme complex that catalyzes this reaction, and it consists of three different enzymes termed as E_1, E_2, and E_3 and these three enzymes use all these different cofactors. So there is coenzyme A, which becomes acetyl coenzyme A. There is NAD+, which becomes NADH, so it accepts the electrons. There is TPP, there is lipoate, and there is FAD, which will become $FADH_2$ and then it will get reduced to FAD.



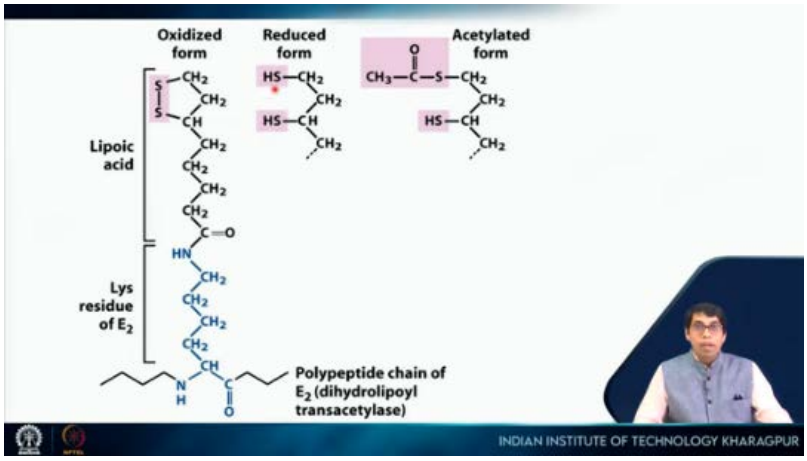
So, 1, 2, 3, 4, 5 different cofactors are used by these three enzymes in this enzyme complex. So, here is the structure of coenzyme A. This is coenzyme A. You can see that there is adenine, there is ribose 3-phosphate, and there is the phosphate group. This is called pantothenic acid and finally, there is this beta-mercaptoethylamine group. So, all of these, we will write as CoA, coenzyme A because the reaction center or the active site is this thiol group. This is the reactive thiol group. So, we will write all of this as CoA-SH and when this thiol reacts with the pyruvate to form this, this is the acetyl coenzyme A.



This is the lipoic acid, the other cofactor that we have not seen before. So, you can see that there are all these different parts. So, this is connected to the side chain. So, this is the E_2 enzyme. So, there are three enzymes: E_1, E_2, and E_3.

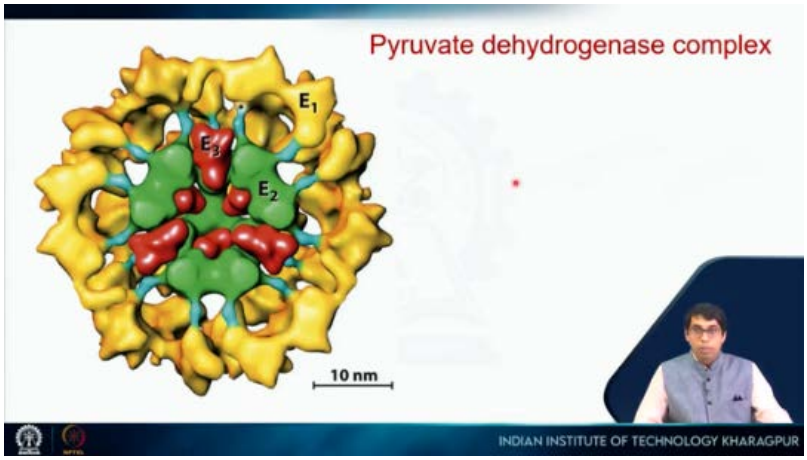
This is a lysine of the enzyme E_2. So, this is the lysine side chain, which will be NH_3^+, but it reacts with this carboxylic group. So, it forms sort of an amide bond, and there is a disulfide linkage here. So, this disulfide is very similar to the disulfide that we have seen

for cysteine, amino acid side chains. So, this is the oxidized form, and this can get reduced to form SH and SH. So, this is very similar to the disulfide oxidized and reduced form of cysteines. Now, this free SH can take up the acetyl group from the acetyl coenzyme A to form this acetylated form. So, we will see all these three in the reaction that is catalyzed by this pyruvate dehydrogenase complex.

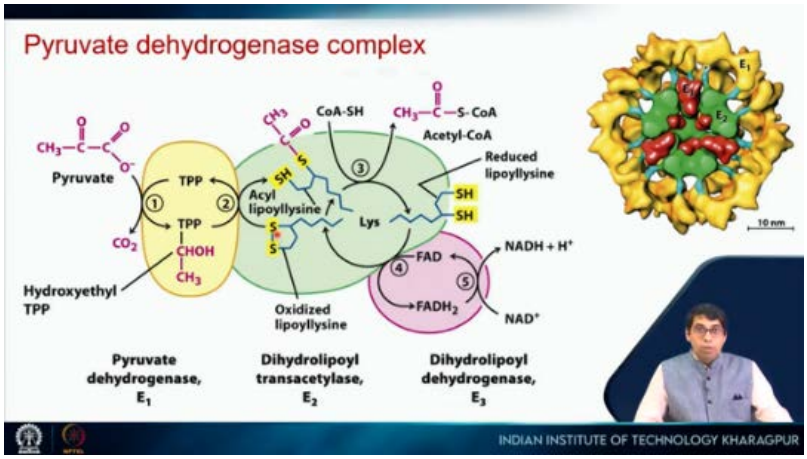


The enzyme complex looks something like this. So, E_1 is one enzyme, E_2 is the other enzyme, and E_3 is the third enzyme. So, there are multiple copies of these enzymes, and they together form this complex structure. Now, when reactions happen, E_1 takes up the substrate, it is passed on to E_2, and then finally it is passed on to E_3. In any of these steps, the substrates or the intermediates, they never diffuse out.

So they are always attached to this enzyme complex and that is something that we will refer to as substrate tunneling. So let us see what happens. This is pyruvate. So pyruvate comes in and it interacts with E_1.



So this is E_1, which is yellow, this part. The middle part is E_2, which is this green part, and the last one is E_3, which is this red part. So, E_1, E_2, and E_3. So, E_1 catalyzes the first step where TPP, thiamine pyrophosphate, interacts with pyruvic acid, carbon dioxide is released, and this acetyl group is attached to TPP as hydroxyethyl TPP. Now, TPP is regenerated, and this hydroxyethyl TPP is oxidized to acetyl and when that oxidation happens, this lipolysin, which is present in the oxidized form. So it gets reduced. So this gets reduced, and this gets oxidized. So we have this acetyl lipolysin. Now, this arm is a very flexible part, as we have seen in the structure.



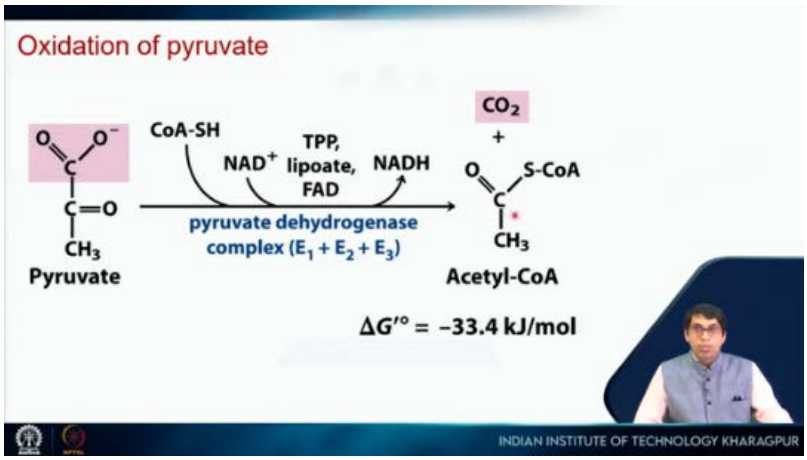
So, you can see it in this structure. So, this is a long chain. So, it is a very flexible part. So, it swings from this position to this position and when it does that, this acetyl group is handed over to coenzyme A.

So, now coenzyme A becomes acetyl coenzyme A, and we have this reduced lipolysine. Now, this reduced lipolysine has to be regenerated into the oxidized form, which is done

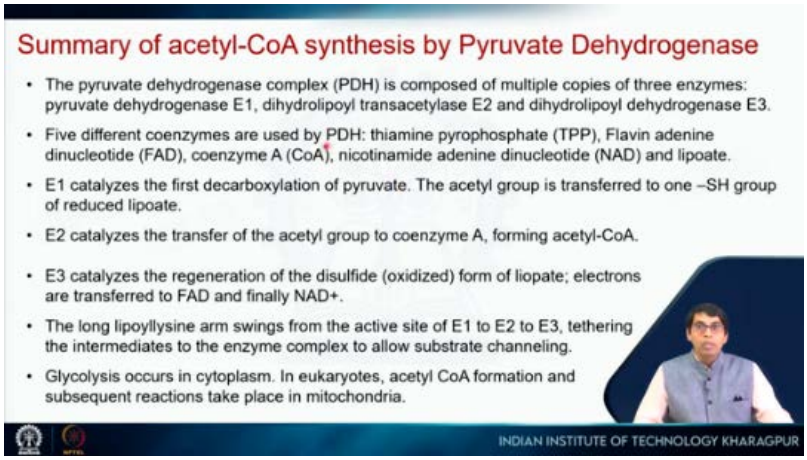
by FAD. So, FAD gets reduced to $FADH_2$, and this reduced lipolysine gets oxidized to oxidized lipolysine. So, now it is again ready for the next round of reactions. $FADH_2$ is the one that has ultimately accepted the electrons that have been released by pyruvate.

Now, it will hand over the electrons to NAD+, and it will itself get regenerated. So, this is the E_3 complex, and NAD+ gets reduced to NADH. So, this is done by the third enzyme. So, all of this TPP is regenerated, oxidized lipolysine is regenerated, FAD gets converted to $FADH_2$, and then it goes back to FAD, so it is regenerated. NAD+ gets reduced to NADH, and coenzyme A accepts the acetyl group, so it becomes acetyl coenzyme A. So, these are the two end products of this reaction.

So we get acetyl coenzyme A, carbon dioxide, and NADH. The release of carbon dioxide drives this reaction forward. So this is the summary of the acetyl coenzyme A synthesis by pyruvate dehydrogenase. The pyruvate dehydrogenase complex, in short, we will refer to it as PDH. It is composed of multiple copies of three enzymes.



So these three enzymes are pyruvate dehydrogenase, which is E_1. Dihydrolipoyl transacetylase, which is E_2, and dihydrolipoyl dehydrogenase, which is E_3. Now, if you see the structure, you can see that there are multiple copies of these three enzymes present in the PDH complex. There are five different coenzymes used by PDH: thiamine pyrophosphate, which is TPP; flavin adenine dinucleotide, which is FAD; coenzyme A; nicotinamide adenine dinucleotide, or NAD; and lipoate. So this is a new coenzyme that we have seen here.



E1 catalyzes the first decarboxylation of pyruvate. So this is the first time carbon dioxide is produced. We will see two more carbon dioxide productions in the citric acid cycle. The acetyl group is transferred to one of the SH groups of the reduced lipoate. E_2 catalyzes the transfer of this acetyl group to coenzyme A. So from the reduced lipoate, it is transferred to coenzyme A, which results in the formation of acetyl coenzyme A. E_3 regenerates the oxidized lipoate.

So it regenerates the disulfide-oxidized form of the lipoate, and the electrons are transferred to FAD and finally to NAD+. So NADH is formed. The long lipoyl arm swings from the active site of E_1 to E_2 to E_3 and that is something that is critical for this catalytic cycle and it keeps tethering the intermediate to the enzyme complex, which is something we refer to as substrate channeling.

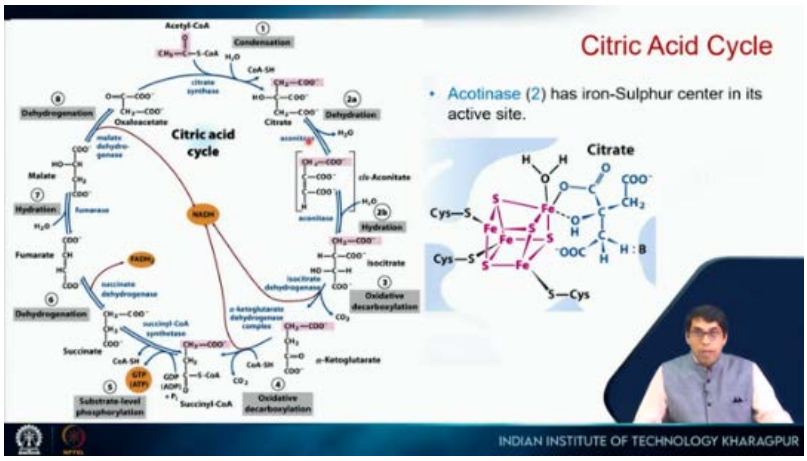
The substrate never diffuses out. So it is always bound to the enzyme complex, and this is what we call substrate channeling. Glycolysis occurs in the cytoplasm. In eukaryotes, the acetyl-CoA formation and subsequent reactions take place in the mitochondria. So this is something that I will keep coming back to again and again.

So, now the citric acid cycle. So, this is where acetyl coenzyme A has formed. Now this acetyl coenzyme A will go into these cyclic reactions, which are referred to as the citric acid cycle because the first product that is formed is citric acid and these reactions go in a cyclic fashion. So there are eight steps.

We will go through these eight steps, and we will see that it goes on in this cycle. Now, another important feature of this cycle is that many of these products that are formed are taken up by different metabolic pathways. So, I will show you one slide where all those pathways are highlighted. So, let us look at the citric acid cycle.

The first step, acetyl coenzyme A, reacts with the last step. So, the product of the last step is oxaloacetate. So, we have acetyl coenzyme A here, and we have oxaloacetate here. This condensation reaction is catalyzed by citrate synthase. It results in the formation of citrate.

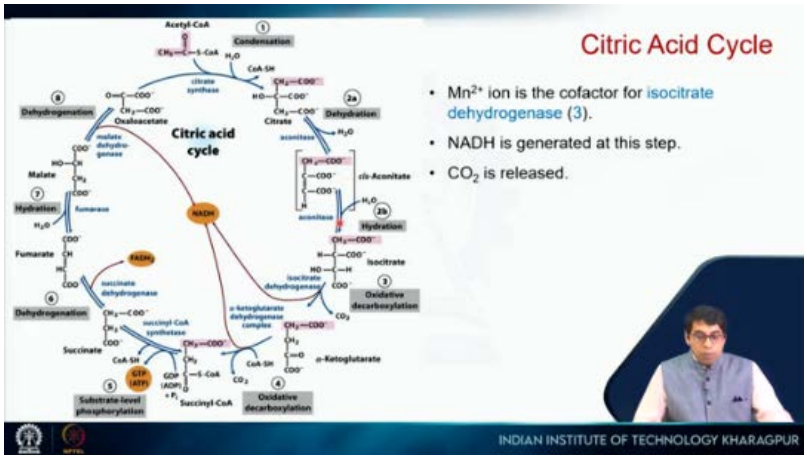
So, this is citric acid, and the coenzyme A is regenerated. So, it can again go back to the previous step to form more acetyl coenzyme A. It is recycled. After the first step, we have citrate. Citrate will be converted to isocitrate by the enzyme aconitase. Now, aconitase converts citrate to isocitrate via an intermediate, which is called cis-aconitate.



So, you see that a water molecule is taken up. So, a water molecule goes out here. So, this OH and H from here go out. So, it forms this double bond. Now, a water molecule is added, but now OH is added here and H is added here.

So, this becomes isocitrate. So, this is the second enzyme, and aconitase has an iron-sulfur center in its active site. We are going to look at this iron-sulfur center in many enzymes in the subsequent reactions. So, iron-sulfur centers look something like this. So, I have one slide later on, which will show you the different types of iron-sulfur centers.

So, in this case, we have these three inorganic ions, and also we have inorganic sulfurs plus cysteine sulfurs, which bind these iron-sulfur centers. So, once we have this isocitrate formation, then, the next step is oxidative decarboxylation. So, now the second molecule of carbon dioxide goes out.



So, we have seen the first molecule of carbon dioxide go out when acetyl-CoA forms. This is the second step of carbon dioxide going out and this is also an oxidative step. So, this is oxidative decarboxylation. So, the electrons are taken up by NADH.

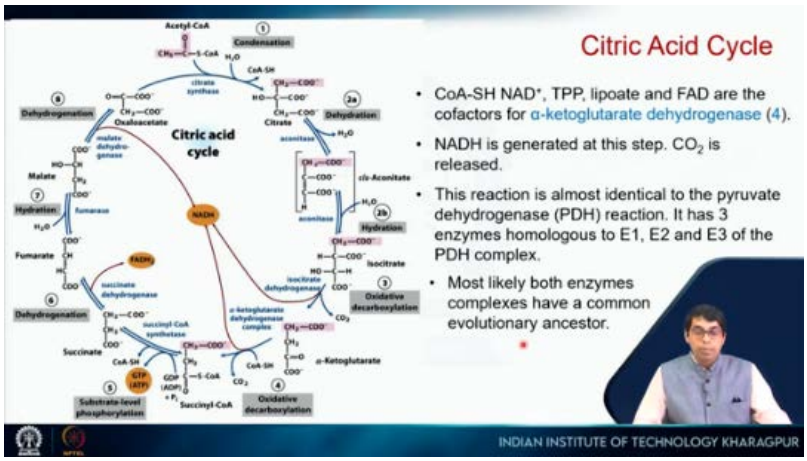
So, we have another molecule of NADH formation. Magnesium ion is the cofactor for isocitrate dehydrogenase in this step. So, in this case, we have a magnesium ion. So, from isocitrate, we have here alpha-ketoglutarate.

So, now alpha-ketoglutarate is converted to succinyl coenzyme A. So, again, coenzyme A comes in, one molecule of carbon dioxide goes out, and electrons are taken up by NADH. So, coenzyme A, NAD+, TPP, lipoate, FAD, these are the cofactors for alpha-ketoglutarate dehydrogenase. From a few slides back, these were the exact same cofactors for pyruvate dehydrogenase. So, it turns out that alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase are very similar enzyme complexes. So, in this case, again, NADH is released.

So, you see NADH is released, and carbon dioxide is also released. So, this is the third carbon dioxide that is formed. So, all three carbons of pyruvic acid or pyruvate have been converted to carbon dioxide. So, its oxidation is complete, and the electrons have been

taken up by NADH. Now, this reaction is almost identical to the pyruvate dehydrogenase reaction that we saw a few slides back.

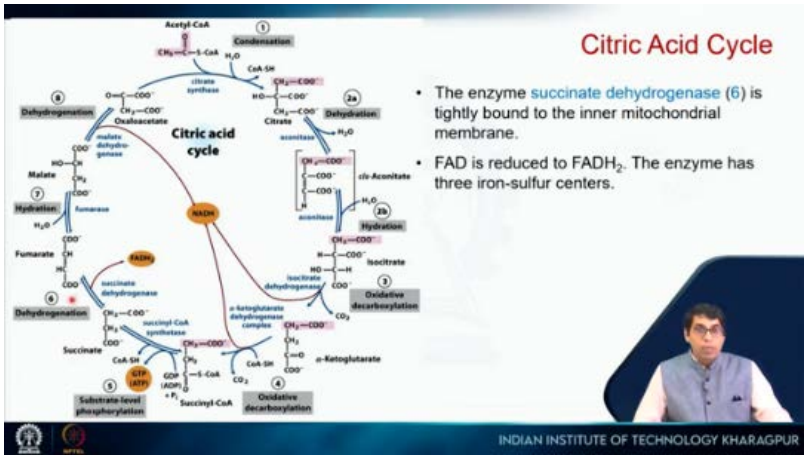
It has three enzymes which are homologous to E_1, E_2, and E_3 of the PDH complex. So, since they are very similar, it is hypothesized that most likely these enzyme complexes have a common evolutionary ancestor. So, they must have come from a common evolutionary ancestor. So, we are at this point: succinyl coenzyme A. Now, succinyl coenzyme A is converted to succinate or succinic acid by the enzyme succinyl CoA synthetase. Succinyl CoA synthetase is also called succinic thiokinase and this step drives the synthesis of GTP or ATP from GDP or ADP. So, GDP is converted to GTP, or ADP is converted to ATP. So, we have basically the synthesis of another ATP molecule at this step. Succinate is then converted to fumarate by the enzyme succinate dehydrogenase.



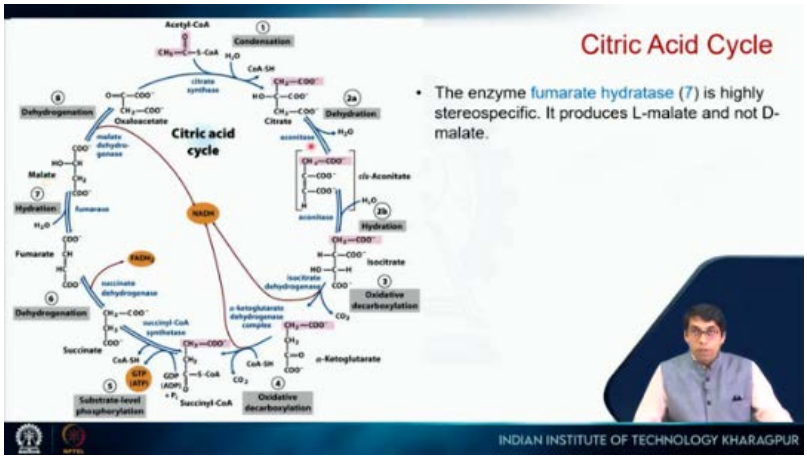
So succinic acid is converted to fumarate. This is done by the enzyme succinate dehydrogenase. Now, this succinate dehydrogenase is an enzyme that is tightly bound to the inner mitochondrial membrane. We will see this enzyme again when I talk about oxidative phosphorylation. So, we will come back to this enzyme in more detail in the next part of this lecture.

So, from succinate, fumarate is formed. Again, this is an oxidation step. The electrons are accepted by FAD, which is reduced to $FADH_2$. Fumarate, this enzyme also has three iron-sulfur centers. We have already seen one example of an iron-sulfur center.

This enzyme has three such iron-sulfur centers. Fumarate is converted to malate by the enzyme fumarase. It is also called fumarate hydratase. Now, you will see that there is a double bond here. A water molecule is added to form malate.

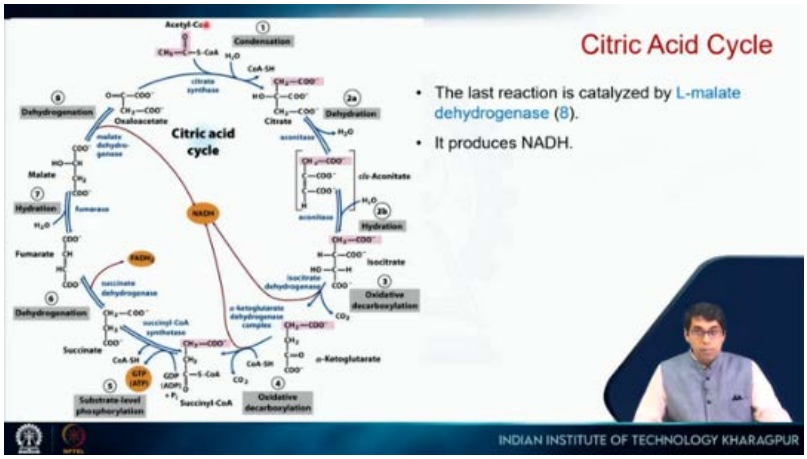


Now, this enzyme is very stereospecific. It will only take fumarate where the double bond is such that it is a trans isomer. The cis isomer will not be accepted by this enzyme, and it forms only L-malate. It will not form the D-malate. So, these are very stereospecific enzymes, and they produce only one of the two possible isomers.



The last step is catalyzed by malate dehydrogenase. So, again, this is a dehydrogenation reaction. So, malate dehydrogenase is formed; it produces electrons that are again accepted by NADH. So, three molecules of NADH are produced. One molecule of $FADH_2$ is produced, and the oxaloacetate that is formed in this step will be taken up by the next cycle of the citric acid cycle.

So, it will again go into this cycle. So, ultimately, what happens? The three carbons of pyruvic acid are converted to three carbon dioxide so its oxidation is completed. All the electrons are stored up in NADH and $FADH_2$, and also one molecule of ATP is synthesized. So, this is a summary of the citric acid cycle, where you will see that all these products are formed.

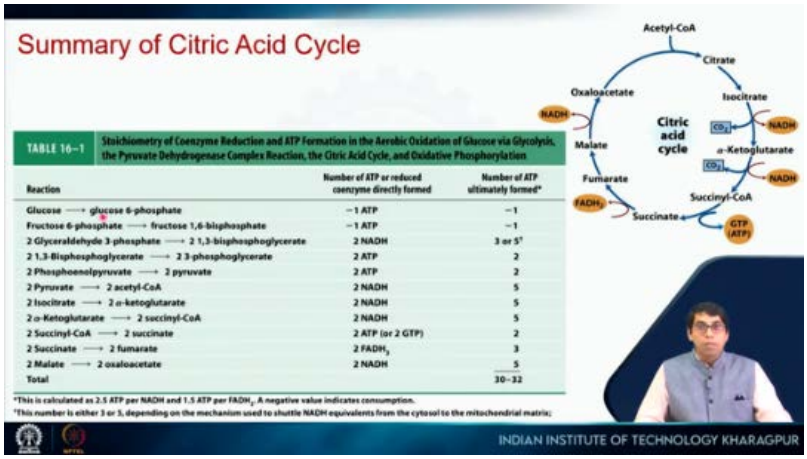


So these are the oxidation steps. These are the four oxidation steps, and in this step, ATP is synthesized. This oxaloacetate, which is formed at the last step, will again go back into the cycle to start the next round of the citric acid cycle. This table summarizes all the high-energy molecules that are produced, starting from glucose all the way to oxaloacetic. So you can see how many ATP molecules were consumed first in glycolysis. So we have minus 1 here.

Then NADH is produced, two molecules of ATP are produced, and then again, we will see we have seen all the formation of these NADH and $FADH_2$ molecules. Now, this NADH will be converted ultimately to ATP in the next part of this oxidation, which is oxidative phosphorylation, and we will see that depending on the pathway, 1 molecule of NADH can give rise to 1.5 molecules of ATP or 2.5 molecules of ATP. So, 2 NADH will give you 3 or 5 ATP molecules. So, if you do this calculation, you will get that from 1 glucose molecule, we get 30 or 32 molecules of ATP.

So, 30 to 32 molecules of ATP are synthesized from the oxidation of glucose. So, what we have seen so far is that glucose oxidation is complete into carbon dioxide. However, the NADH still has to be converted to ATP, and the electrons have to be taken up by

oxygen to form water because oxygen is the ultimate electron acceptor in this reaction. So, the citric acid cycle not only feeds into the final oxidative phosphorylation, but it also produces all these intermediates, which are taken up by different biosynthetic pathways. For example, citrate goes into the synthesis of fatty acids and steroids.

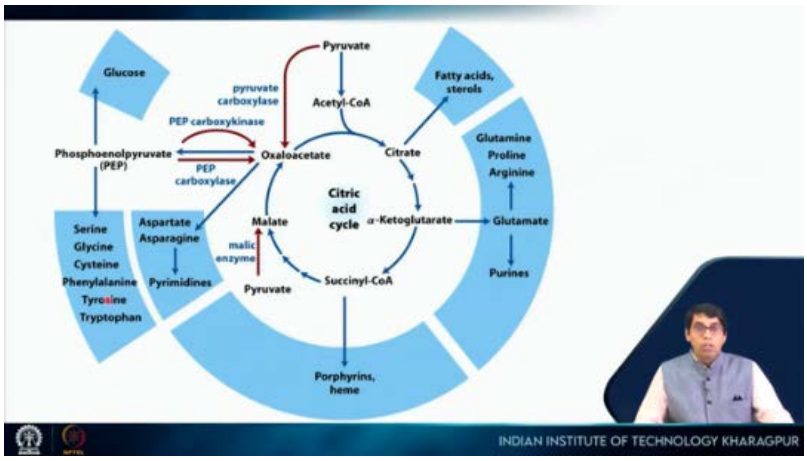


Alpha-ketoglutarate, one of the intermediate molecules, is converted to glutamate or glutamic acid. This is one of the amino acids and it is converted to arginine, purine, and glutamine. So, all these amino acids are synthesized. On the other hand, glutamate is used to produce purines.

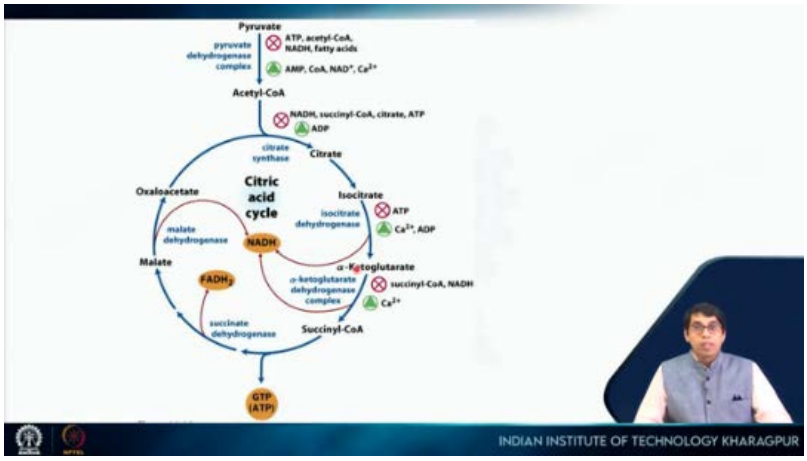
So, this will go into the formation of DNA and RNA. Succinyl coenzyme A is used to produce porphyrins and heme. So, we have seen this heme that was also present in hemoglobin. Oxaloacetate goes into the formation of aspartate, asparagine, or pyrimidine.

So, purine is here, and pyrimidine is here. So, two more amino acids and also one of the building blocks of DNA and RNA. We have already seen this phosphoenolpyruvate formation, from which glucose is synthesized on one side, and on the other side, these other remaining amino acids are synthesized. So, all these very important molecules are synthesized from these different molecules that are generated in the citric acid cycle. So, the citric acid cycle, just like glycolysis, is also highly regulated and the basic theme that you will see here is that whenever these high-energy molecules like ATP, acetyl coenzyme A, NADH, or fatty acids are present, they will inhibit these steps. So this step, this step, this step, and this step, all these steps are inhibited by these high-energy molecules. On the other hand, when these molecules are present in abundance. So AMP,

coenzyme A, NAD+, or calcium ions then it will activate these enzyme complexes. So they will activate these reaction steps.



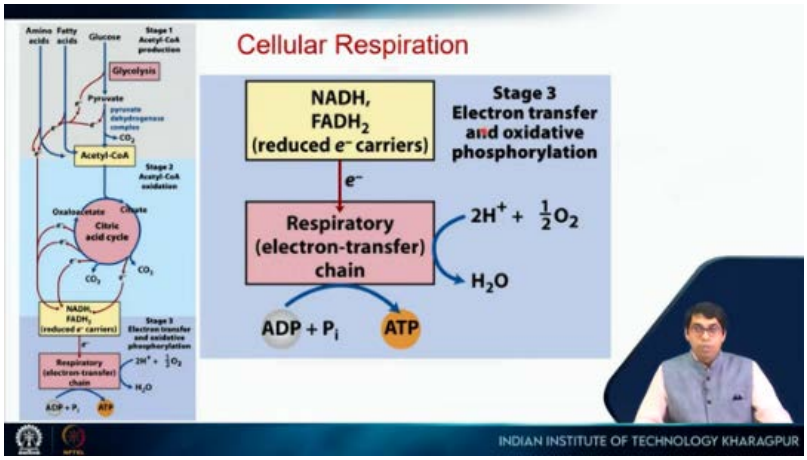
So these are mostly allosteric regulations and the important factor that is needed here is the ratio of ATP to AMP, NADH to NAD+, or acetyl coenzyme A to coenzyme A. So the ratio of these molecules determines whether pyruvic acid will enter the citric acid cycle or not. So now we are going into the third stage, or the last stage, of this cellular respiration. So, what we have shown so far is that in pyruvic acid glucose is converted into 2 pyruvic acids and these pyruvic acid carbons are oxidized to carbon dioxide. So, that step is complete.



The electrons have been taken up by NAD+ and FAD, resulting in the formation of NADH and $FADH_2$. So, these are the reduced electron carriers. Now, these electrons have to be ultimately given up to oxygen to form water, and the energy released will be used to

synthesize ATP from ADP. So this is phosphorylation, and this is oxidation. So these two steps are coupled together, resulting in the process called oxidative phosphorylation.

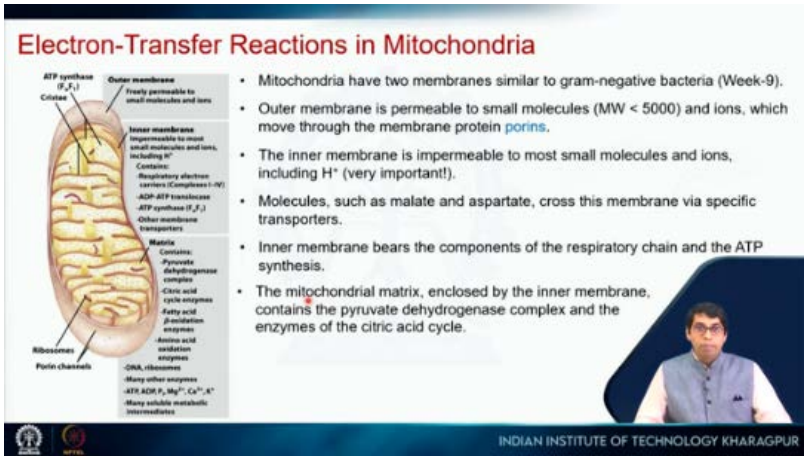
So, electron transfer and oxidative phosphorylation are what we are going to look at. So, as I mentioned earlier, most of these things are happening inside the mitochondria. So, this is one of the organelles in eukaryotic cells. So, let's look at the structure of mitochondria. Mitochondria have two membranes, very similar to gram-negative bacteria.



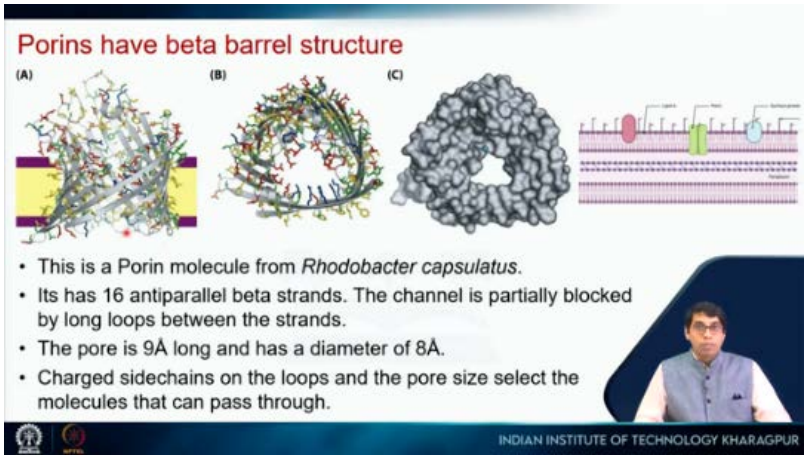
So, you can see there is an outer membrane here and an inner membrane here. So, we'll talk about gram-negative bacteria in more detail in week 9 lectures. The outer membrane is permeable to small molecules, which are less than 5000 Daltons, and ions, which means that small molecules can easily pass through this membrane, and that is channelized by these membrane proteins called porins. We have already seen porins in previous lectures. The inner membrane is more impermeable, which means that it will not allow small molecules, ions, or even hydrogen ions to pass through, and this is something that is very important that even hydrogen ions should not pass through this inner membrane.

Molecules such as malate and aspartate cross this membrane via specific transporters. So, any molecule that passes through the inner membrane is transported via specific transporters, which will allow only those molecules. The inner membrane bears components of the respiratory chain and ATP synthesis. So, we will see this in more detail. The mitochondrial matrix, which is the liquid or soluble solution part that is inside,

is enclosed by the inner membrane and contains the pyruvate dehydrogenase complex and the enzymes of the citric acid cycle.



So whatever you have seen in the previous slides, they all occur inside this mitochondria. So this is just a reference back to porins. This is something that I have discussed earlier. So this is the membrane, and you have this porin, porins. So these are membrane-bound proteins, and they have a very large diameter so that they can allow molecules of a certain size to pass through.

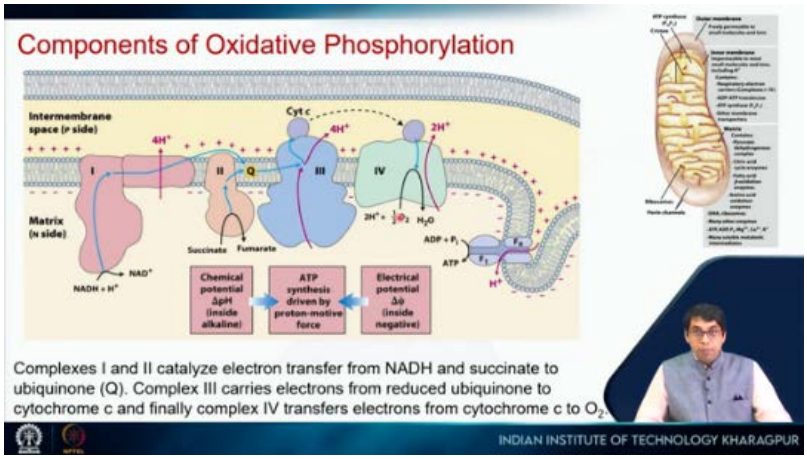


So, let us look at the components of oxidative phosphorylation. So, again, this is the mitochondria, and what we are looking at are the two membranes. So, this is the outer membrane. So, you will have porins in this outer membrane. So, big molecules or molecules which are less than 5000 Daltons can easily pass through.

This is the inner membrane, and this is something that is very tightly regulated. So, not even hydrogen ions can pass through this inner membrane and this is where the matrix is. So this matrix is also referred to as the N side because it will have a net negative charge, and this intermembrane space will be called the P side because it will have a net positive charge, and that net positive charge will be because we will see ultimately that hydrogen ions will be pumped out from here to here by these different parts of this complex.

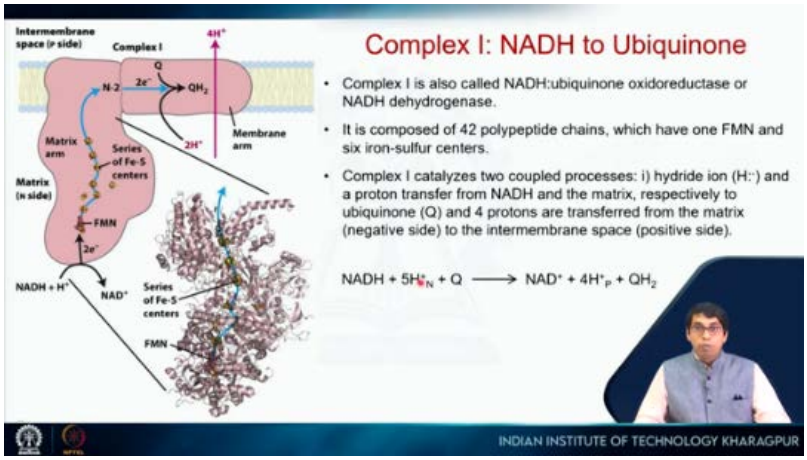
So, there are 4 complexes: 1, 2, 3, and 4. Out of these, 1, 3, and 4 pump out hydrogen ions, which is coupled to the oxidation-reduction reaction that happens here. So, complexes 1 and 2 catalyze electron transfer from NADH and succinate to ubiquinone. So, this is succinate dehydrogenase, the same enzyme that we saw in the citric acid cycle. So, it is a membrane-bound enzyme. So, when it oxidizes succinate to fumarate, the electrons are transferred ultimately to NADH, which is finally transferred to ubiquinone.

So, ubiquinone will become ubiquinol. Once this ubiquinone or ubiquinol is formed, complex III carries electrons from this reduced ubiquinone, which is actually ubiquinol, to cytochrome C. So, this will transfer to cytochrome C and finally, cytochrome C, which is a soluble protein present in the intermembrane space, will go and bind to complex IV, and it will transfer the electrons to complex IV, where the electrons will be finally transferred to oxygen. So, the electrons will be taken up by oxygen to produce water. So, the electron transfers are completed from NADH to oxygen via these four different complexes.



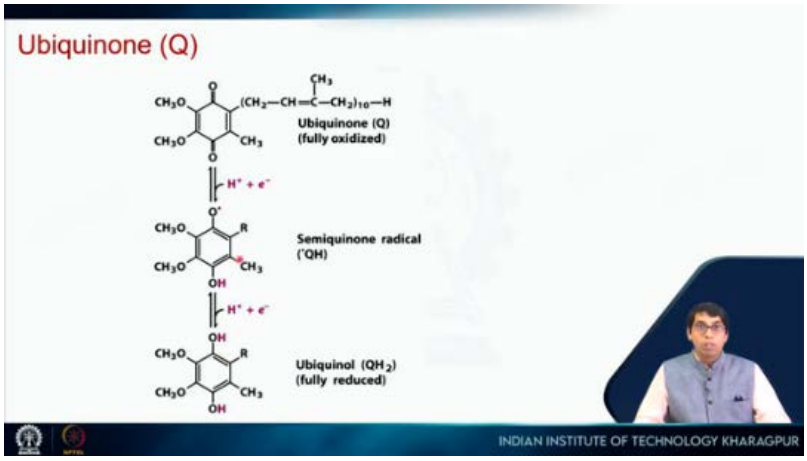
So, let us look at complex I. which is this one. So, what it does is it transfers electrons from NADH to ubiquinone. So, complex I is also called NADH-ubiquinone oxidoreductase or NADH dehydrogenase. It's a big protein complex.

It has 42 polypeptide chains, of which one FMN and six iron-sulfur centers are present. So these polypeptide chains bind to these different cofactors. So there is one FMN and there are six iron-sulfur centers. Complex 1 catalyzes two coupled processes. The first one is hydride ion transfer and proton transfer to the matrix, so the hydride ion, H- comes out from here and H^+ from the matrix; these are transferred to ubiquinone. So this Q becomes QH_2. So this is one reaction. So the two electrons are taken up by the ubiquinone. So this is the reduction. This gets oxidized, and ubiquinone gets reduced to ubiquinone. The second step is four protons are transferred from the matrix to the intermembrane space, which is shown by this red arrow. So four protons are taken up from here and transferred to this intermembrane space. So this is the N side, and this is the P side. So these two reactions are coupled by this complex one and we can write the net reaction like this: NADH plus 5 protons plus ubiquinone results in the formation of NAD+. Four of these protons are transferred out from the N side to the P side, and two protons so one from here and one from here are taken up by ubiquinone to form QH_2 or ubiquinone. So, this is the detailed structure of ubiquinone. So, this is ubiquinone. So, you can see two keto groups here.



When it takes up one electron, then it forms this semiquinone radical. So, if you put an electron here and then you can push the arrows, this will form an O dot, and this will

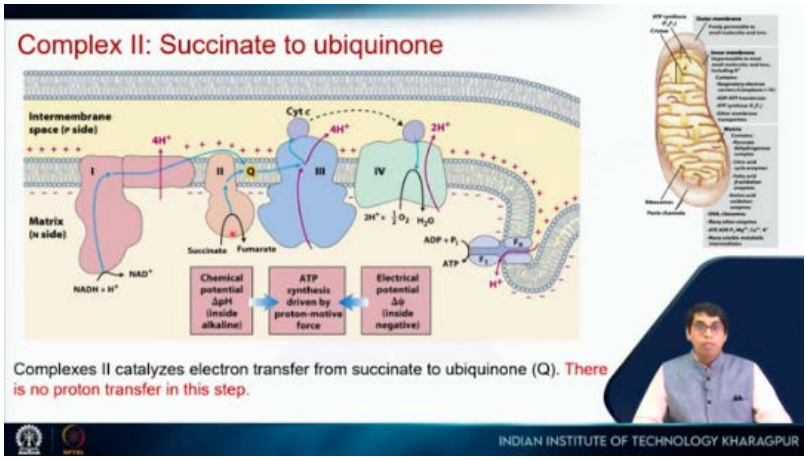
become OH. Now, you can add another electron, so this dot becomes O^-, and it can bind to this H^+ to form OH and OH. So now, these two keto groups have converted to alcohol groups. So, ubiquinol, which is fully oxidized, becomes ubiquinol, which is fully reduced. So, these are the examples of iron-sulfur centers. So, we have already seen something like this.



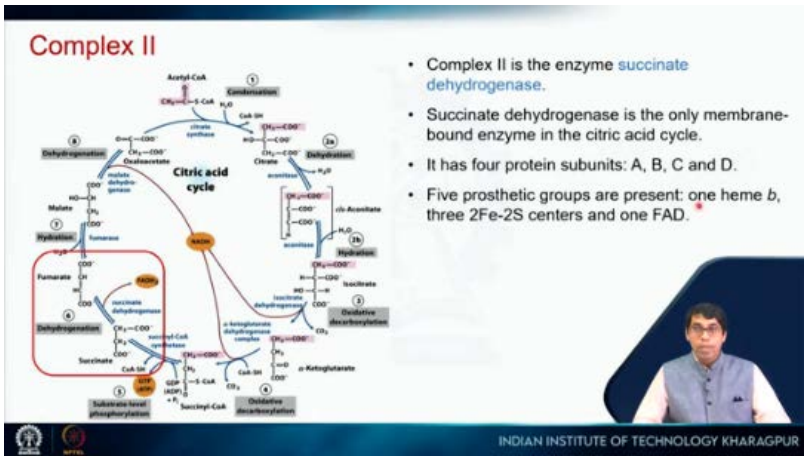
So, this is a more complex iron-sulfur center, but it can also exist in these simpler forms. So, iron is coordinated by cysteines or inorganic sulfurs. The standard reduction potential of these iron-sulfur centers varies from -0.65 volts to +0.45 volts. So, it will also depend on the environment that is provided by the enzyme.

So we are done with complex I; now we are looking at complex II. We have already seen this. So, complex II catalyzes the electron transfer from succinate to ubiquinone. Just like this, here the electron transfer is from NADH to ubiquinone; here it is from succinate to ubiquinone. In this case, there is no proton transfer.

So, this is the only complex that does not result in any proton transfer. So, this is succinate dehydrogenase. We have seen this in the citric acid cycle and as mentioned earlier, it is a membrane-bound enzyme complex. So, complex II is the enzyme succinate dehydrogenase.



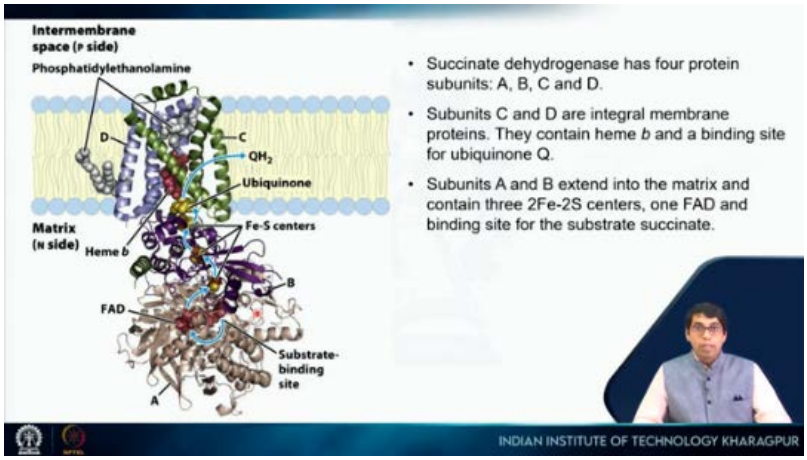
It is the only membrane-bound enzyme in the citric acid cycle. It has four protein subunits: A, B, C, and D. So, this succinate dehydrogenase has four subunits and uses these five prosthetic groups. One heme *b*, three iron-sulfur centers, and one FAD. So, this will be the structure of succinate dehydrogenase. So, there are four subunits: A, B, C, and D, and you can see that these subunits are labeled here.



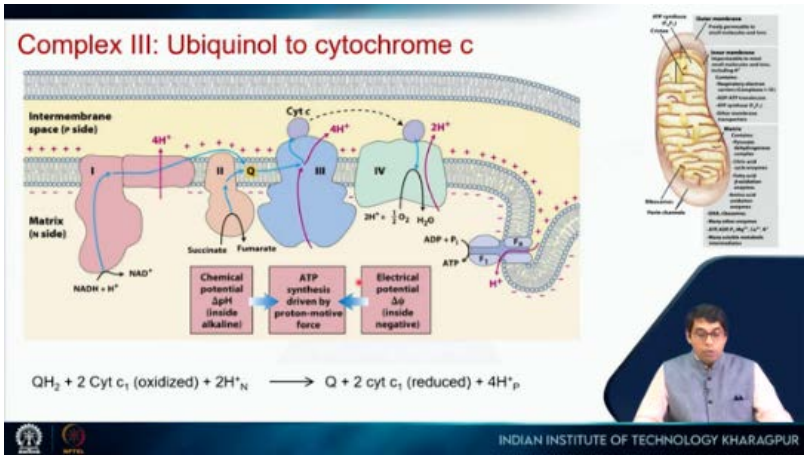
So this is A, B is here, and C and D are here. So C and D are integral membrane proteins. So you can see that they are embedded in the inner membrane of the mitochondria. They contain heme B and a binding site for ubiquinone. So, ubiquinone is bound to these subunits C and D, and they also have heme b. So, heme b, this red molecule that you see, is heme b. Subunits A and B extend into the matrix.

So, this is A and B, and they are more in the matrix than in the membrane and they contain three iron-sulfur centers and one FAD bound to them and they also have the

binding site for the substrate. So the substrate will bind somewhere here. So electrons are transferred to ubiquinone at this point. Now complex III comes.



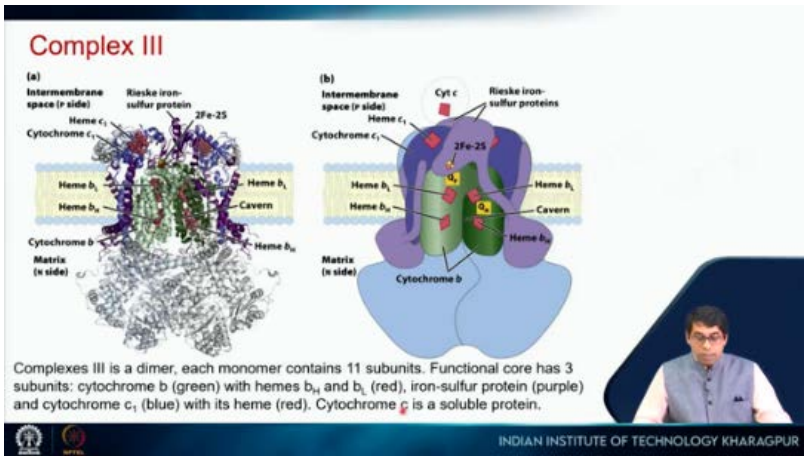
So ubiquinone has been reduced to ubiquinol. Complex III comes. It will take the electrons from ubiquinone and transfer them to cytochrome c. So ubiquinol will be converted back to ubiquinone. The oxidized cytochrome c_1 becomes reduced cytochrome c_1. So cytochrome c_1 is a cytochrome which is part of complex III, and cytochrome c is a soluble protein which will shuttle between these two complexes, III and IV and while this happens, four protons are again pumped out from the N side to the P side. So, four protons from this matrix or the N side will be pumped out into this intermembrane space, which is the P side. Complex III is a dimer. So it looks like this.



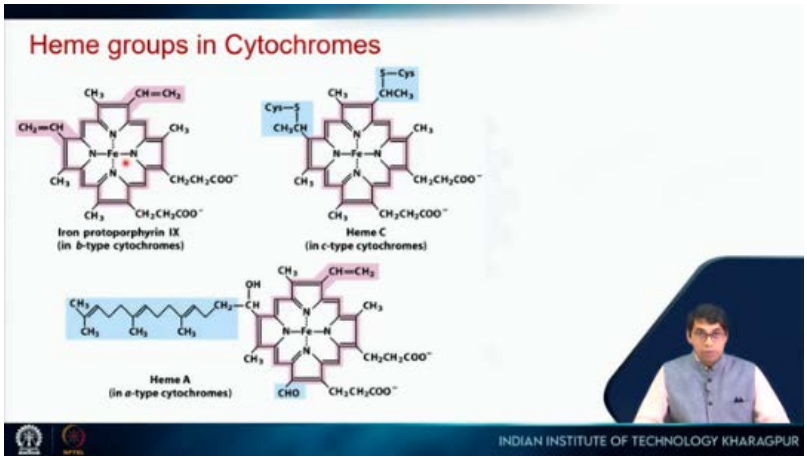
You can see that it is a symmetric dimer and each of these monomers, so if I draw a straight line here and if I just look at the left half, that is the monomer. So this monomer contains 11 subunits. The functional core is also shown here. It has three subunits, the

cytochrome b, which is shown in green, which have the hemes b_H and b_L. So you see these hemes b_H and b_L shown here. So that is cytochrome b. There is an iron-sulfur protein, which is purple. So the iron-sulfur cluster is shown here and cytochrome c_1, which is here, also has a heme, which is shown in red.

So, this is cytochrome c_1. Now, this cytochrome c_1 is bound to this complex, and this cytochrome c is soluble, which will move out. Cytochrome c is a soluble protein. Now, these cytochromes have these different types of hemes. So, this is something that we have already seen before in the case of hemoglobin.



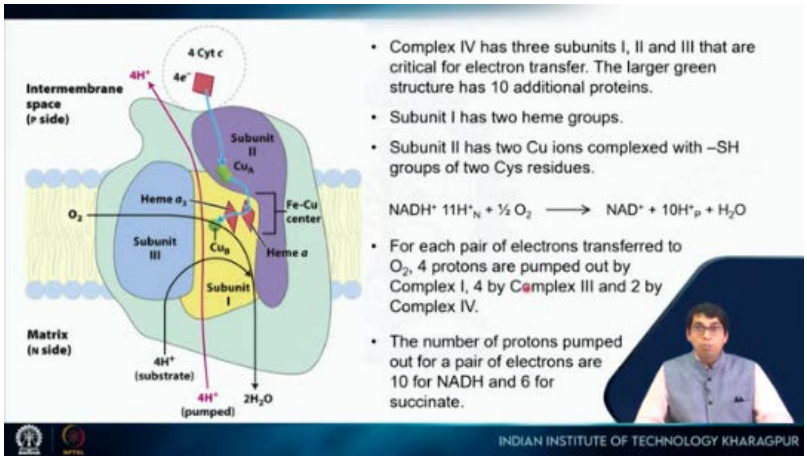
So, in the case of hemoglobin, this heme is optimized for binding oxygen. But in this case, the heme is present to accept electrons. So, it will get oxidized and reduced. So, it will shuttle between Fe^{+2} and Fe^{+3} states. Now, let us look at the fourth complex, which is where this cytochrome c, electrons will be taken up from the cytochrome c, and they will be passed into oxygen here, resulting in the formation of water. So, cytochrome c, which is the reduced form, plus protons from this matrix side, plus oxygen, this will get oxidized, 4 protons are pushed out, and oxygen is reduced to water. Now, if you do your balance, you will see that ultimately two protons are pushed out from this N side to the P side because some protons will be taken up by the water molecule. So, this is complex IV.



So, this is the detail of complex IV. It has 3 subunits: 1, 2, and 3, which are critical for this electron transfer. The larger green part shown here is a structural protein, and it has 10 additional proteins. Subunit 1 has 2 heme groups. So, this is subunit 1, shown in yellow; it has 2 heme groups.

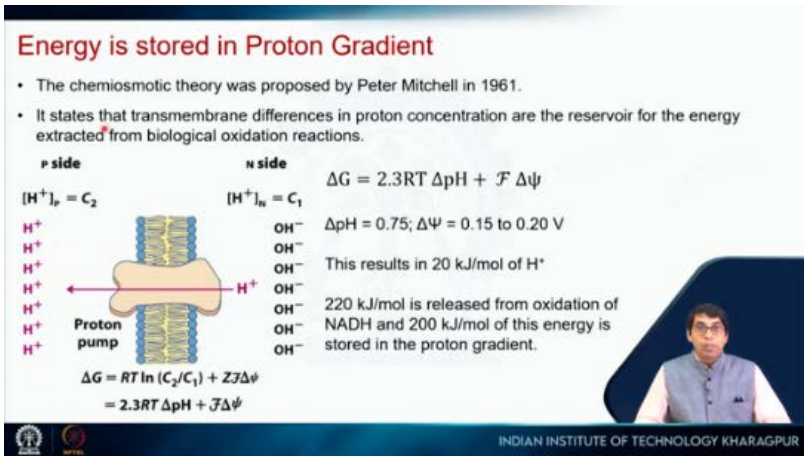
Subunit 2, which is shown in purple, has two copper ions complexed with the thiol group of two cysteine residues. So, there is a copper ion. So, in this case, the aqua factor is copper. The reaction for this is shown here. NAD plus 11 protons plus half oxygen will give you NAD.

So, this NADH becomes NAD^+. One proton from here is taken up, one proton from here, and 11 protons from here. So, they are all taken up by this oxygen to produce water. So, you can see this is not a balanced reaction because we have to count for all the number of protons that are transferred from the inner side to the outer side. So, for each pair of electrons transferred to the oxygen, 4 protons are pumped out by complex 1, 4 protons are pumped out by complex III, and 2 protons are pumped out by complex II, which is shown here. So, for each electron that is transferred from here to here, here to here, and finally here to oxygen. So, the electrons are coming from NADH to oxygen. So, for each pair of electrons going from NADH to oxygen, 4 electrons are pumped out by complex 1, 4 electrons are pumped out by complex 2, and 2 protons are pumped out by complex 3 and complex 4, so a total of 10. However, for 2 electrons that are transferred from succinate to fumarate.



4 plus 2, 6 protons are pumped out. So, for 2 electrons transferred from succinate to oxygen, 6 protons are pumped out. For 2 electrons transferred from NADH to oxygen, 10 protons are pumped out and that is what is written here. This is something that will be important to calculate the number of ATP molecules that are ultimately synthesized from the oxidation of glucose. So, what we have done so far is we have pumped out protons from the N side to the C side. So, we have a huge proton gradient. The chemiosmotic theory states that these proton gradient and electric field gradient is the one that stores energy.

So, this theory was proposed by Peter Mitchell in 1961. So, it states that the transmembrane differences in proton concentration are the reservoir for the energy extracted from biological oxidation reactions. So, the energy that is extracted from the oxidation of glucose is now stored as this transmembrane difference in proton concentration and we can calculate that using this equation: $\Delta G = 2.3RT\Delta pH + F\Delta\Psi$.



So, now if we plug in these values, where ΔpH, the difference in pH between or the proton concentration between these two sides, is 0.75, and the $\Delta\Psi$, the difference in electropotential, is 0.15 to 0.2 volts. So, if you plug in these numbers, you get 20 kilojoules per mole of proton. So, for 1 mole of proton, the ΔG is 20 kilojoules

Now, when NADH, 2 electrons are transferred from NADH to oxygen, 10 protons are pumped out. So, 10 times 20, that is 200 kilojoules of this energy, is stored in the proton gradient, 220 kilojoules is the energy that is released from the oxidation of NADH. So, it means that out of 220 kilojoules of energy that is released when NADH is oxidized to NAD+, 200 kilojoules of that energy is stored in this proton gradient, which means that this is a very efficient process. So, 200 out of 220 kilojoules of energy is actually efficiently stored. Only 20 kilojoules are lost. So now we have this proton gradient. Glucose is oxidized to carbon dioxide. Electrons are accepted by oxygen. However, the energy is stored as this proton gradient across the membrane.

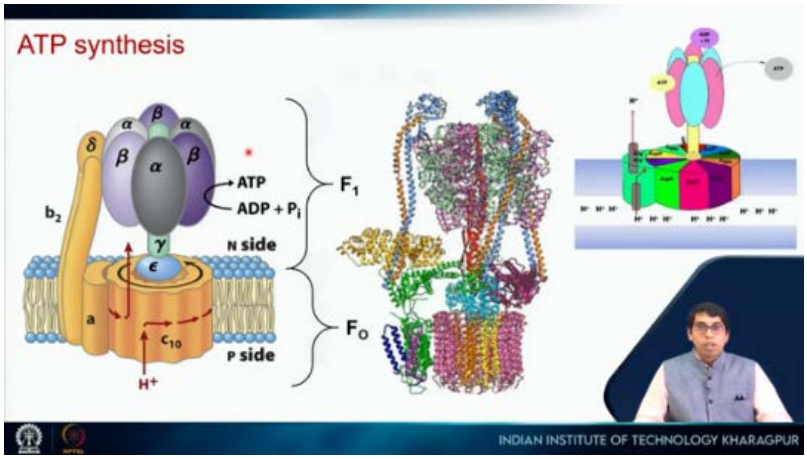
Now, we have to use this stored energy to produce ATP. So, ADP plus inorganic phosphate will form ATP, and this is how it is made. So, this is not a favorable reaction. To drive this reaction, we will use the energy stored across the membrane.

So, there is this enzyme or enzyme complex called ATP synthase, which will result in ATP synthesis. So, it will allow the passing of protons along the gradient and use that energy to synthesize ATP. So, the ATP synthase looks something like this. So, there is a transmembrane region and this part, which is on the N side. This is on the matrix side.

So, it will allow proton diffusion from this side to this side. So, this is along the gradient and while this happens, these beta subunits will synthesize ATP. So, when a proton goes in, it drives the rotation of this motor. So, this is a very fantastic molecular machine and this energy is used to convert ADP into ATP.

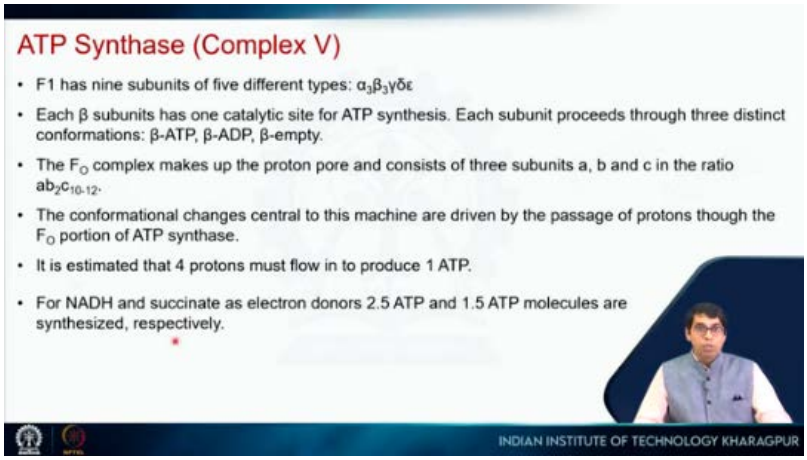
So, protons are going in, there is rotation here, and that energy is used to synthesize ATP from ADP. Now, based on the structure that is known to date, this machine is grouped into two, F_1 and F_2. So, F_1 and F_0. So, F_0 is the part which is the integral membrane part, and this F_1 part is where the ATP synthesis happens, and it is in the matrix of

mitochondria. F_1 has 9 subunits of 5 different subtypes, alpha(α) 3, beta(β) 3, gamma(γ), delta(Δ) and epsilon(ε). So, you can see that there are 3 alpha(α) subunits, 1, 2, 3, and 3 beta(β) subunits and then there is gamma(γ), delta(Δ) and epsilon(ε).

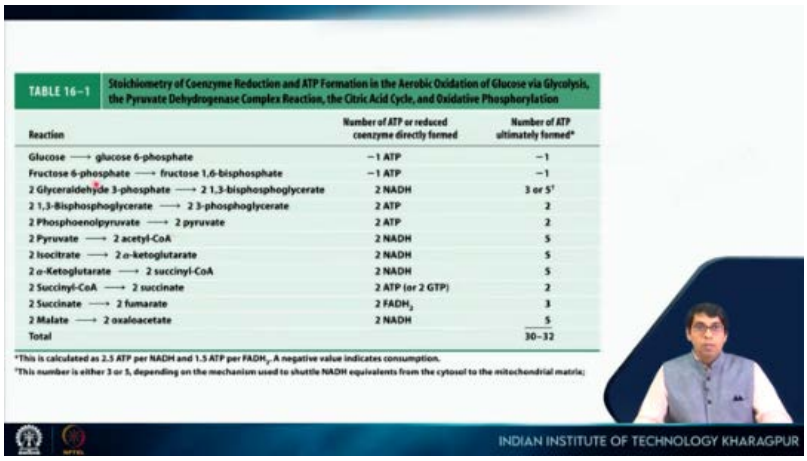


So, these are the parts of this F_1. Each β subunit has one catalytic site for ATP synthesis. Each subunit proceeds through three distinct conformations: β ATP, β ADP, and β MT. So, you can see that when this rotates, these three subunits have three different conformations. One conformation binds ATP, one conformation binds ADP, and the other one is MT and by rotation, they will bind ADP, catalyze the formation of ATP, and then release that ATP. So, this rotation results in the conversion of these 3 conformations and drives the reaction. The F_O complex makes up the proton pore and consists of 3 subunits, a, b, and c in the ratio of 1a, 2b, and 10 to 12c. Now, this 10 to 12c depends on different homologs.

The conformational changes central to this machine are driven by the passage of protons through the F_O portion of ATP synthase. It is estimated that 4 protons must flow in to produce 1 ATP. So, when 4 protons or 4 hydrogen ions flow in, 1 ATP molecule is synthesized. So, now we can do some calculations. When 2 electrons are transferred from NADH to produce NAD+, 10 protons flow out, 4 protons flow in to produce 1 ATP. So, 10 divided by 4 is 2.5. So, the oxidation of NADH to NAD+ will result in the synthesis of 2.5 ATP molecules. For succinate, when it goes from succinate to fumarate, 6 protons flow out.



Now, 4 protons flow in to form 1 ATP. So, 6 divided by 4 is 1.5. So, the oxidation of one succinate molecule will result in the formation of 1.5 molecules of ATP. So, that is where we get these numbers: 2.5 ATP and 1.5 ATP. So again, if we go back to this table, you see that from NADH, all the succinate is converted to NADH.



We will get 3 or 5 because it is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$, which comes from succinate. So, the oxidation of glucose will result in the formation of 30 to 32 molecules of ATP. So, for this part, you can follow any biochemistry book. Primarily, Lehninger Principles of Biochemistry is a very good book for this particular lecture. Thank you.

REFERENCES

Following books may be referred to

- Lehninger Principles of Biochemistry
- Biochemistry (Lubert Stryer)
- Molecular Biology of the Cell (Alberts)
- Molecular Cell Biology (Lodish)