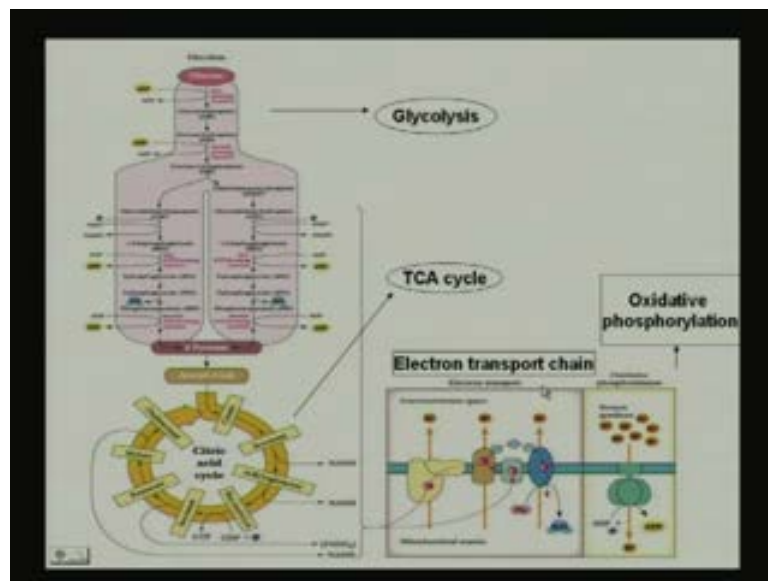


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Module No. # 01
Lecture No. # 20
Electron Transport Chain and Oxidative Phosphorylation

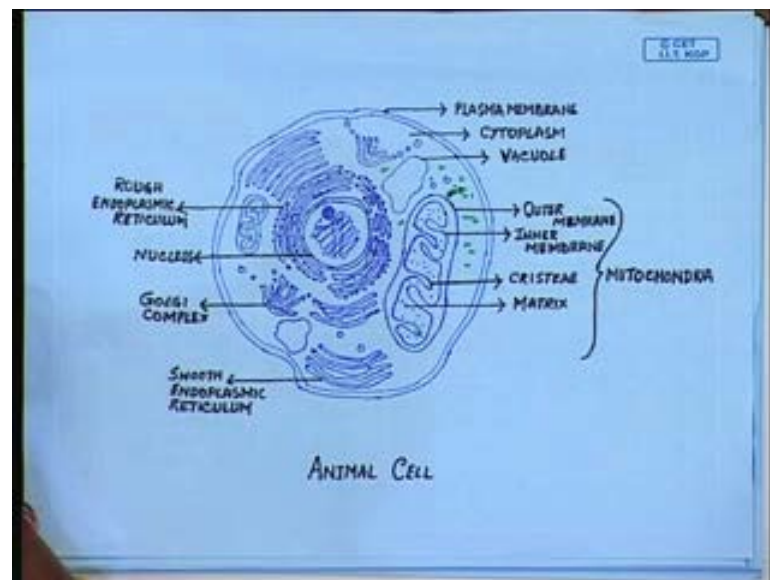
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Students, today the topic of our discussion is electron transport chain and oxidative phosphorylation, the ultimate steps for cellular respiration. Now, in our earlier classes we have already learnt that in glycolytic process that, glucose is converted to pyruvate one molecule of glucose is produced two molecules of pyruvic acid. During this process two ATP molecules were consumed in the first steps of glycolysis. And in the second step four ATP molecules along with two NADH molecules were produced. So, when we did the net energy balance we have seen that net gain of eight molecules of ATP's were there. Incase of TCA cycle, we have also learnt that how pyruvate is converted to acetyl coa. And when acetyl coa is formed one molecule of carbon dioxide and one NADH is

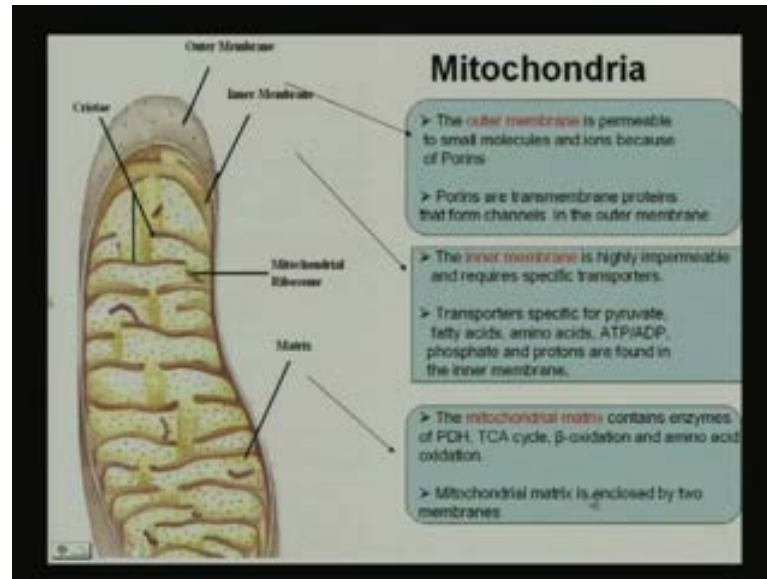
produced and acetyl coa when enters to this mitochondria of the cell. We have seen that it is the eight steps process and in during this completion. After the completion of this step we have seen that three molecules of NADH, one **molecules** molecule of FADH 2 and one molecule of GTP is produced from one molecule of pyruvate. That means when two molecules of pyruvates are being used in TCA cycle, we can get six molecules of NADH and two molecules of FADH 2 and two molecules of GTP or ATP. And total gain of 24 ATP molecules was there. We have also seen that in this process some hydride ions are being formed. And when this cycle one is further continued and it is further processed it enters to the electron transport chain and today we will be discussing on this electron transport chain.

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Now, if we see the total cell suppose this is the intact cell; glycolysis is taking place in this cytoplasmic fluid of the cell. As soon as pyruvate is produced it enters to the mitochondria. This is mitochondria of the cell. Acetal coa is produced and enters to this mitochondria. Mitochondria has got its two layers; membrane one is the outer layer another is the inner layer. Now, outer layer is the protecting layer and inner layer is just **fold** folded inside the matrix and it founds the cristae in the mitochondria.

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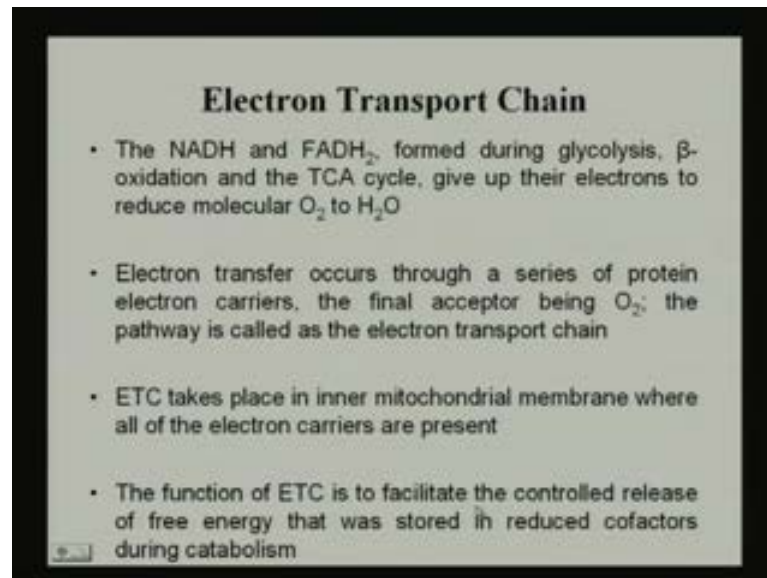


And when we see the structure of mitochondria we can find that, it has got the outer membrane, the inner membrane and mitochondrial ribosome and matrixes are there inside and this infolded that inner membrane is infolded inside and form the cristae like structure. Now, if we see the outer membrane of these mitochondria then we can find that the outer membrane is permeable to small molecules and ions because of the presence of the protein which is called the porin proteins. So porin proteins are present which helps the transportation of some ions and small molecules through this outer membrane.

Porins are transmembrane proteins that forms the channel in the outer membrane **in the outer membrane** of the mitochondria. The inner mitochondrial membrane is highly impermeable and requires specific transporter. That means this inner membrane is highly intact and it is impermeable to any such ions or any **any any** soluble or insoluble molecules. Transporters are needed for transportation from inside to outside. The transporters specific for pyruvate fatty acid, amino acid, ATP, ADP, phosphate and protons are found in the inner membrane of this mitochondria. And when we see the inside, this inside is the matrix. The mitochondrial matrix contains **contains** enzymes that is pyruvate dehydrogenase, TCA cycle enzymes, beta oxidations and amino acid oxidations. And mitochondrial matrix is enclosed by two membranes so here, the inner membrane and the outer membrane. So, this is all about this mitochondria. And know why I am talking about this mitochondria so much, because this electron transport chain

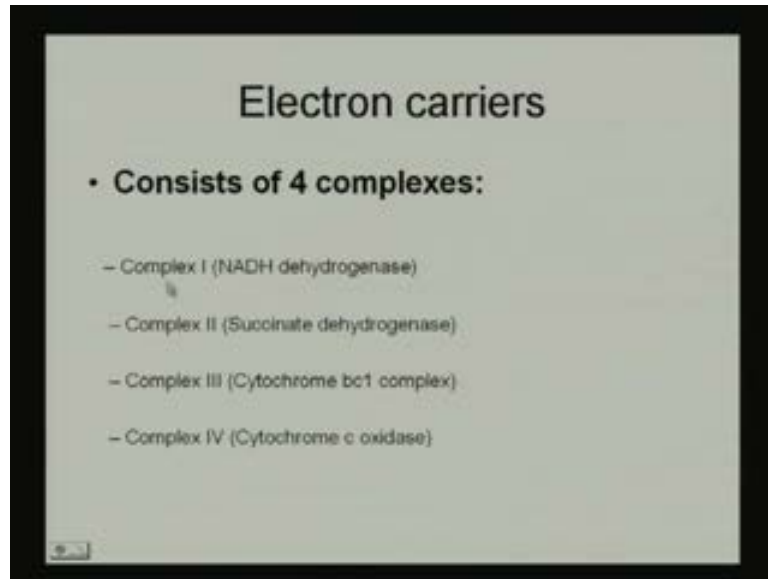
and the oxidative phosphorylation. Today the topic of our discussion what we will be discussing takes place in this mitochondria. And that is the reason why I am talking about this structure of mitochondria.

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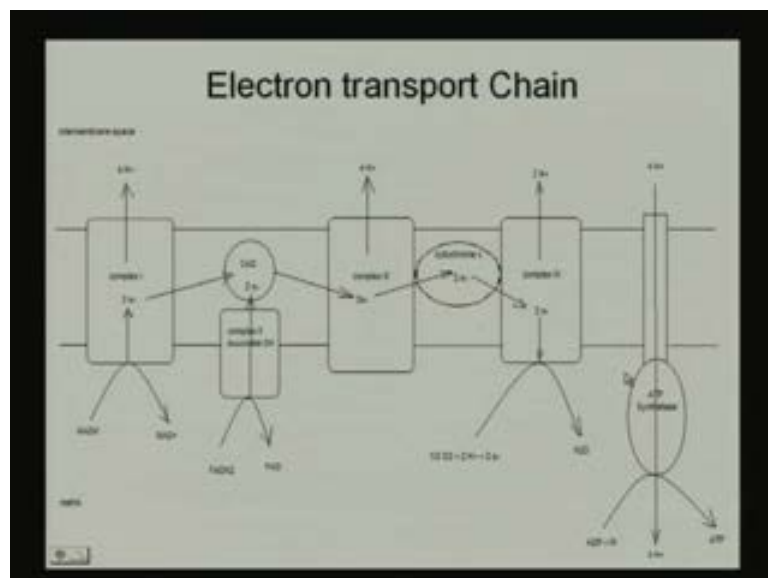
Now, coming to this electron transport chain. Now, the NADH and FADH two form during glycolysis beta oxidation and TCA cycle gives up give up their electrons to reduce molecular oxygen to water. That means this NADH and FADH 2 we have all ready seen that, NADH is formed in glycolysis, NADH is also formed in TCA cycle. FADH 2 is also formed and whatever this high energy molecules they give up their electron to reduce this oxygen molecules **the and it** and it converts to water molecule. Electron transfer occurs through a series of protein electron carriers, the final acceptor being oxygen. That means this is the oxygen which is the final electron acceptor. The pathway is called electron transport chain. Electron transport chain takes place in the inner mitochondrial membrane. So here, in this portion the here the electron transport chain is taking place. Where all the electron carriers are present, the function of electron transport chain is to facilitate the controlled release of free energy that was stored in reduced cofactors during catabolism.

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Now, here if we see the electron carriers which further help to complete this electron transport chain mainly composed of four different complexes; complex one, complex two, complex three and complex four. This complex one is NADH dehydrogenase. Complex two is succinate dehydrogenase. Complex three is cytochrome bc 1 complex. And complex four is cytochrome c oxidase. Now, let us come one by one to this each complex's which help in electron transport chain.

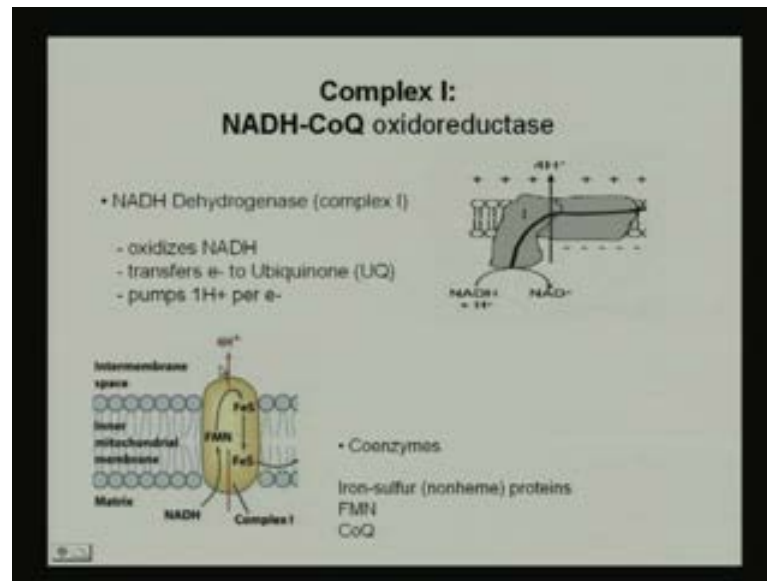
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Now, at a glance if we see that how this electron transport chain is. See here these different complexes which are there. So see, this is the matrix side of this mitochondria and this is the intermembrane space. That means the space between the space between the inner and the outer membranes. So, this is the intermembrane space of these mitochondria. So, if we are taking the view like this is the matrix side and this is the intermembrane space of mitochondria followed by the outer membrane outside. And if we see this cross sectional view, then we can find that the arrangement of complex one, complex three and complex four are there. Whereas, complex two is present in the inner mitochondrial membrane as I have already discussed that the succinate dehydrogenase the enzyme which is mainly responsible for transferring the electron from complex FADH 2 to the electron transport chain. That is the (()) is present in the inner mitochondrial membrane. That means we say that complex one, complex three and complex four are in one side and complex two is present in the inner mitochondrial membrane.

Now, here followed by and when it is getting completed complex four you see this oxygen molecule is getting this electron and it converts this oxygen to water molecule. And then this ATP synthesis is taking place and that means when this electron transport chain is getting completed then ATP synthesis is coupled up. That means this oxidation and phosphorylation is simultaneously taking place within the cell and thus it is called oxidative phosphorylation process. With the completion of oxidative phosphorylation we are getting the net production of ATP in the cell.

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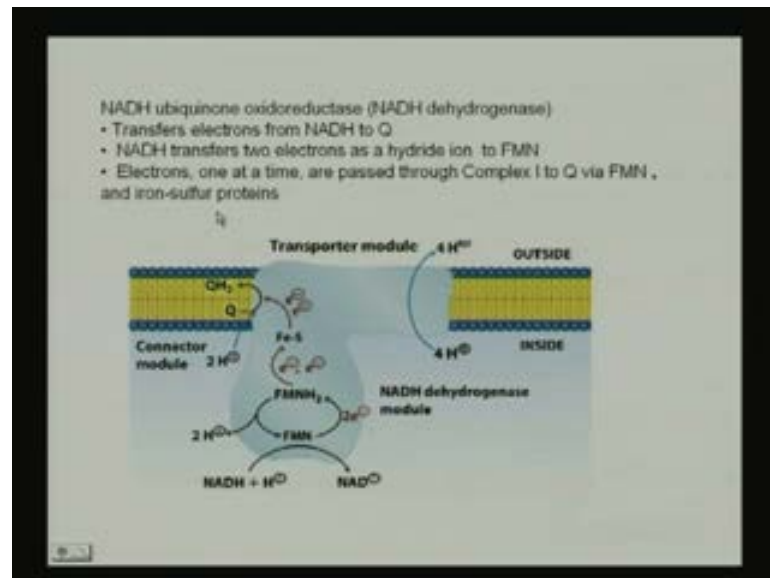


Now, coming to this complex one. Now when we are talking about this complex one this complex one is NADH coenzyme Q oxidoreductase. Now, when we are talking about this complex one, we can find that this NADH dehydrogenase is playing a significant role as per as this complex one is concerned. Now what it does? This NADH dehydrogenase oxidizes this NADH. At the same time, it transfers this electron to ubiquinone, the ultimate electron acceptor. And at the same time it pumps one proton per one electron. So, this is the function of NADH dehydrogenase. Now if we see, the structure of this particular enzyme **this** we can find that this is the enzyme which has got two side. One is the hydrophobic horizontal arm which is buried inside the membrane and another vertical arm contains the peripheral membrane proteins of the complex which is projected towards the matrix. So, this is the structure. It is the L shaped enzyme protein which helps in the catalytic activities.

Now, here is the binding side when NADH is coming and it is donating its electron to this particular enzyme. It immediately takes up and it starts its function. How it functions? There are different helping hands. That is the coenzymes which are present inside this particular complex one and this coenzymes are iron sulfur nonheme protein, FMN and coenzyme Q. This iron sulfur nonheme protein is one of the cofactor that is otherwise known as Fe-s complex. Flavin mononucleotide is another cofactor and coenzyme Q is another cofactor. These three cofactors are helping to transfer the electron from NADH to the ultimate side that is coenzyme Q that is ubiquinone.

Now, here if we see this transmembrane space and this matrix, see NADH is donating its electron and it is getting transferred. At the same time we can find that some protons are getting pumped from inside matrix to the outside of this intermembrane space.

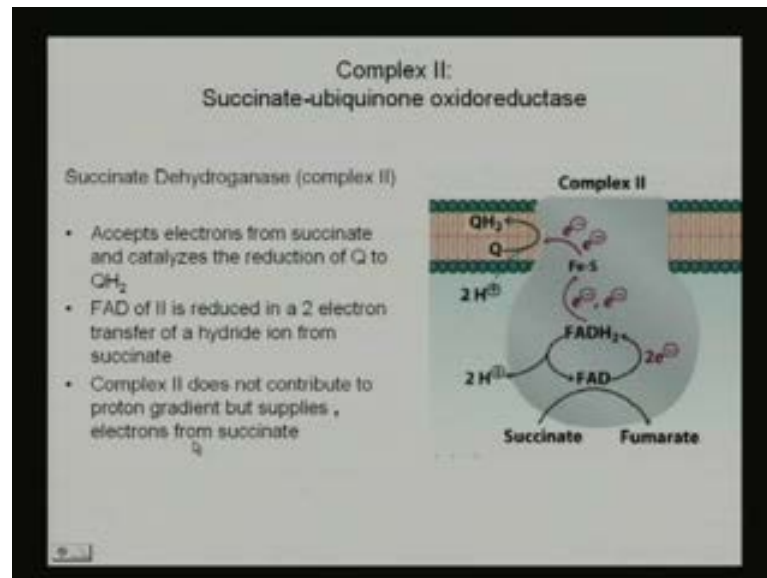
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Now see here, NADH is leaving this electron to flavin mononucleotide and it is further passing to this Fe-s complex. That is iron sulfur protein. Ultimately it transfers this electron to ubiquinol and it forms ubiquinol Q H 2. When this process of transfer of electron is going on, at the same time these protons are getting pumped out from inner matrix to the intermembrane space.

Now, see here this NADH ubiquinone (()) oxidoreductase that is NADH dehydrogenase transfers this electron from NADH to Q that is ubiquinone. NADH transfers two electrons as per hydride ion to flavo mononucleotide. Electrons one at a time are passed through the complex one to ubiquinone via FMN and iron sulfur protein. And in this way this function of complex one is getting completed.

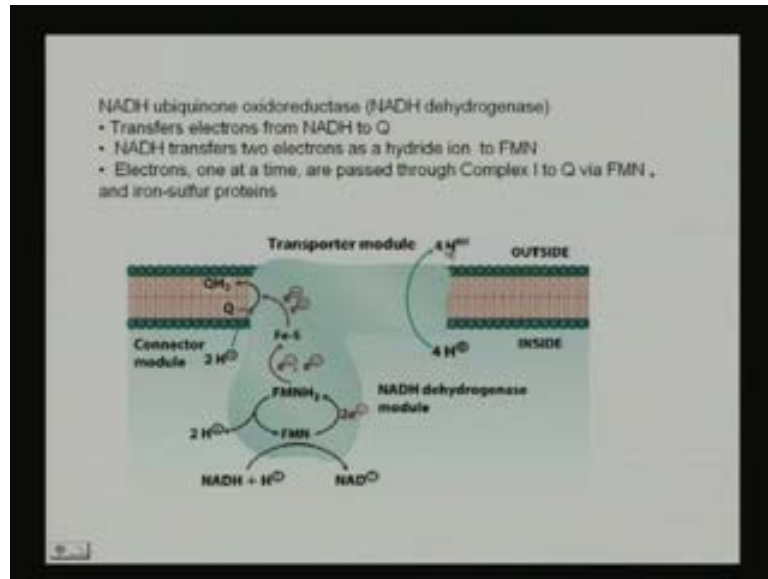
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Now this electron has come up to this ubiquinone and ubiquinone is converted to ubiquinol. That Q becomes QH₂.

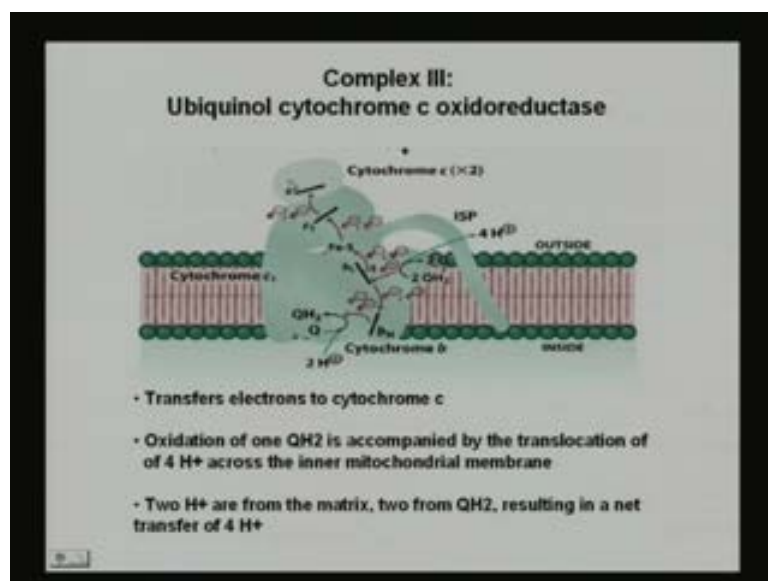
Now, coming to this complex two. As I have told you, the location wise that complex two is in the inner mitochondrial membrane as this succinate dehydrogenase this enzyme which is helping to transfer this electron in complex two is present in the inner mitochondrial membrane. It accepts the electron from the succinate and catalyzes the reduction of Q that ubiquinone to QH₂. FAD of complex two is reduced in a two electron transfer of a hydride ion from succinate. Complex two does not contribute to proton gradient but, supplies electron from succinate.

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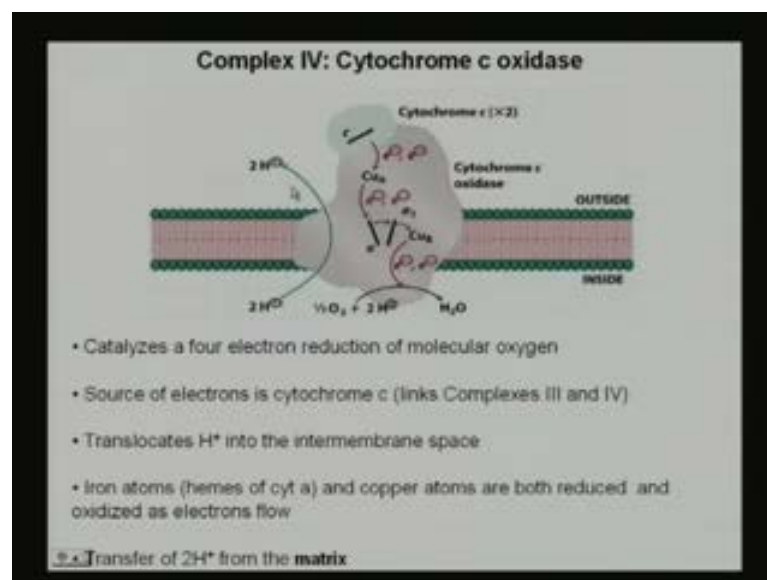
That means here in case of complex one, if you remember here this proton gradient is form this pumping of this proton from inside matrix to this outside this intermembrane space is taken place. But, here in this case only the transfer of electrons are taking place and here also the ultimate acceptor of electron is ubiquinone. And it is converted to ubiquinol. So, Q H₂ is the ultimate **this** ubiquinol is the ultimate acceptor of electron. Now, in this way this FADH₂ and NADH is donating the electron to the electron transport chain.

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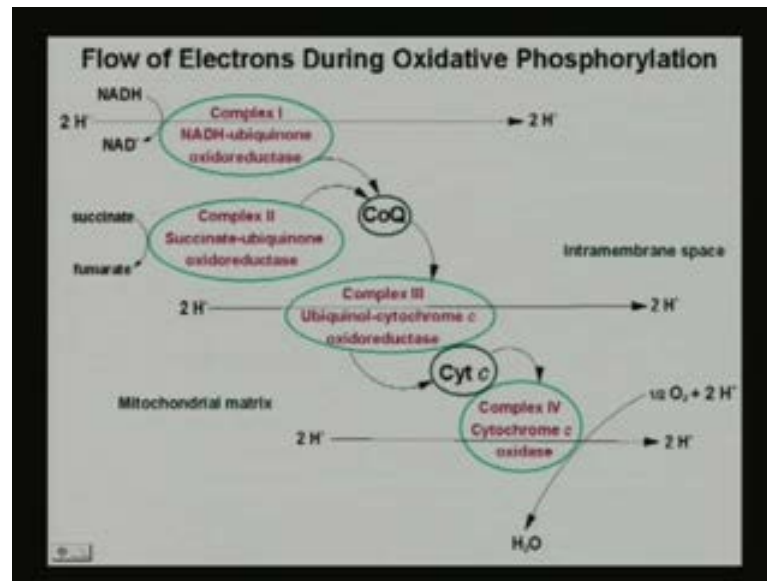
It is further carried over to complex three. In complex three, it is the ubiquinol cytochrome c oxidoreductase enzyme which is playing a significant role and it transfers this ubiquinol to cytochrome c. And here, with it transfer this electron it goes via the different coenzyme, cofactors like cytochrome b, ferrous iron protein, cytochrome c one and ultimately it reaches to cytochrome c. The transfer of electron to cytochrome c from ubiquinol is taking place. Oxidation of one QH_2 ubiquinol is accompanied by the translocation of four hydrogen ions across the inner mitochondrial membrane. Two hydrogen ions are from the matrix, two from ubiquinol resulting in a net transfer of four hydrogen ions from matrix side to intermembrane space.

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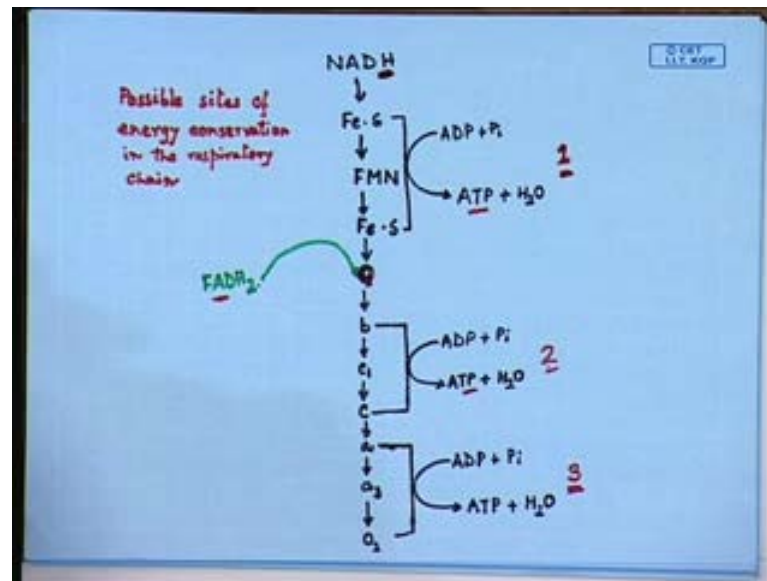
Now, when it comes up to that cytochrome c, this cytochrome c is accepting this electron. Then it catalyzes a four electron reduction of molecular oxygen. Now, this source of electron is cytochrome c. This cytochrome c is **farther** further donating this electron via this copper complex and then it comes to cytochrome a a three and ultimately it reduces this oxygen to water molecule. During this process these hydride ions are also getting pumped out from the matrix side to the intermembrane space. Translocation of hydrogen ions into the intermembrane space is taking place. Iron atoms that is hemes of cytochrome a and copper atoms are both reduced and oxidized as electron flows. And transfer of two hydrogen ions from the matrix side to the intermembrane space is also taking place simultaneously.

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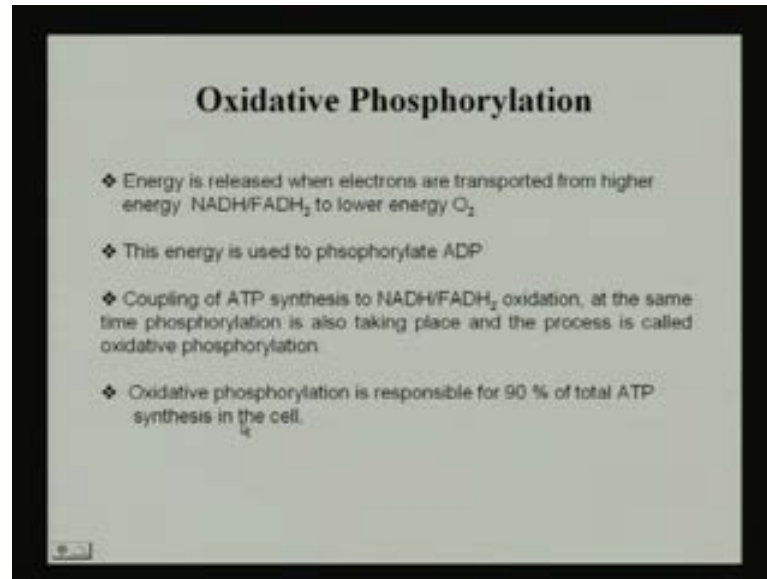
Now, if we see the flow of electron during this electron transport chain, we can find that we can sum up what we have learnt till now. That NADH is donating the electron to complex one; from complex one ultimately it is coming the acceptor of electron is the ubiquinone. Ubiquinone is transferring this electron to this complex three. Here FADH₂ is also transferring the electron to ubiquinone. And ubiquinone is once again transferring to complex three. From complex three it comes to cytochrome c. Cytochrome c to the complex four and complex four here this oxygen molecule is getting reduced and water is produced. And here during this process, you see in complex one these protons are being pumped out. In complex three also this protons are getting pumped out and complex four the protons are getting pumped out. We do not see any proton pumping in case of complex two.

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Now, if we sum up this the particular studies what we have learned that, **then** I have already told you that one molecule of NADH is equivalent to three molecules of ATP. Now, how it is? How it can be proved? So see here, this NADH. NADH is donating its electron and the possible sites of energy conservation in respiratory chain are this ATP and P_i is getting converted to ATP and water molecule. So, this is the first molecule of ATP which is being generated during this electron transport. Now, when this chain is continuing FADH₂ is from the complex two. We have seen that they are also contributing this electron, they are also donating this electron and ultimate electron accepted is the ubiquinone. And this Q is accepting this electron. Now, you see here it is coming from the inner mitochondrial membrane. Now, when it enters to this particular electron transport chain, by this time this NADH which its electron or reproduced one molecule of ATP. Now, this chain is continuing and when it is going through the complex b and c cytochrome b and cytochrome c the second molecule of ATP is getting generated. And when it is coming to a and this molecular oxygen, another molecule of ATP is being synthesized. So, we can tell that one molecule of NADH is producing three molecules of ATP. And one molecule of FADH₂ is producing two molecules of ATP. So, this is the production pattern of ATP from one molecule of NADH and FADH₂.

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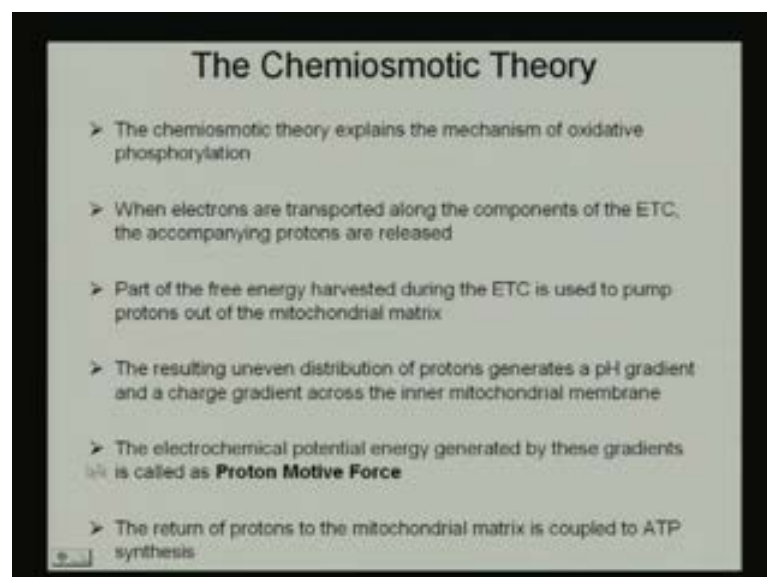


Oxidative Phosphorylation

- ◆ Energy is released when electrons are transported from higher energy NADH/FADH₂ to lower energy O₂
- ◆ This energy is used to phosphorylate ADP
- ◆ Coupling of ATP synthesis to NADH/FADH₂ oxidation, at the same time phosphorylation is also taking place and the process is called oxidative phosphorylation
- ◆ Oxidative phosphorylation is responsible for 90 % of total ATP synthesis in the cell.

Now, if we couple up this as per as electron transport chain is concerned. Now, when we are coupling up this electron transport chain and oxidative phosphorylation then, we can find that energy is released when electrons are transported from higher energy. That is NADH or FADH₂ to lower energy oxygen. The energy is used to phosphorylate ADP. Coupling of ATP synthesis to NADH or FADH₂ oxidation at the same time phosphorylation is also taking place in the same place. And this process is called as oxidative phosphorylation process. Oxidative phosphorylation is responsible for ninety percent of the total synthesis of ATP in the cell.

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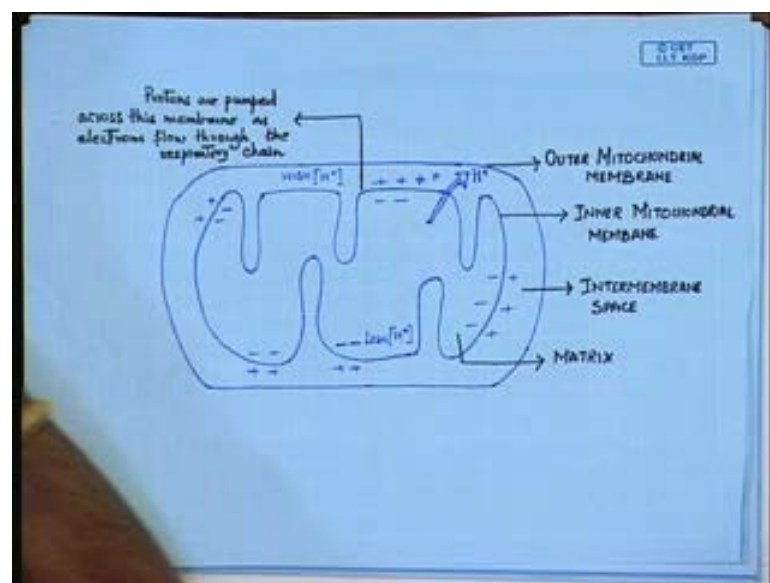


The Chemiosmotic Theory

- The chemiosmotic theory explains the mechanism of oxidative phosphorylation
- When electrons are transported along the components of the ETC, the accompanying protons are released
- Part of the free energy harvested during the ETC is used to pump protons out of the mitochondrial matrix
- The resulting uneven distribution of protons generates a pH gradient and a charge gradient across the inner mitochondrial membrane
- The electrochemical potential energy generated by these gradients is called as **Proton Motive Force**
- The return of protons to the mitochondrial matrix is coupled to ATP synthesis

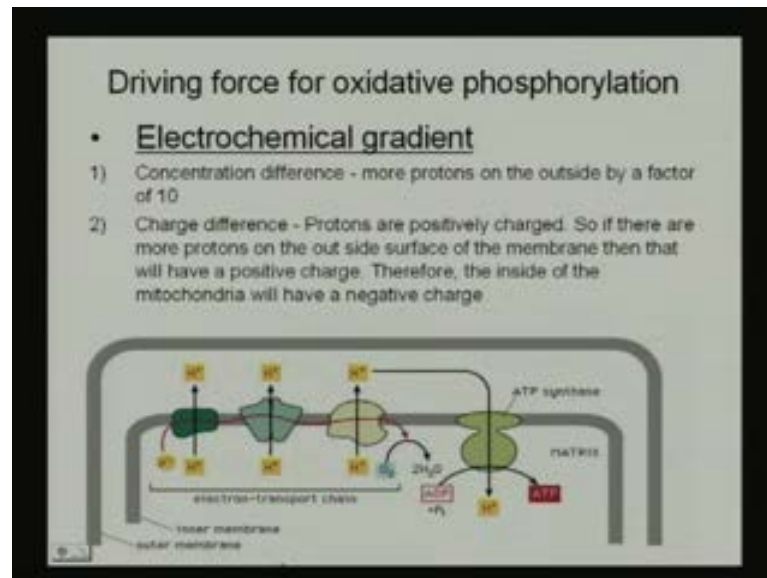
If we see the energy that proton pumping during this electron transport chain; then we can find that there is the chemiosmotic theory which is there which is established by the scientist Mitchell and it is one of the theory which explain the mechanism of oxidative phosphorylation. When electrons are transported along with the component of electron transport chain, the accompanying protons are released. The part of the free energy harvested during the electron transport chain is used to pump the proton out of this mitochondrial matrix. The resulting uneven distribution of the proton generates the p H gradient and a charge gradient across the inner mitochondrial membrane.

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Now, here what is happening when this process, electron transport chain processes going on, if this is the structure of mitochondria protons are being pumped out from matrix to the intermembrane space. And here what is **what is** happening, the concentration of this H plus ion is more in this particular place. Then what is there inside? So, in this way one gradient is formed. That gradient is either electrochemical gradient or the p H gradient which is formed in inside the matrix and the intermembrane space. The electrochemical potential energy generated by these gradients is called proton motive force. The return of protons to this mitochondrial matrix is coupled to the ATP synthesis.

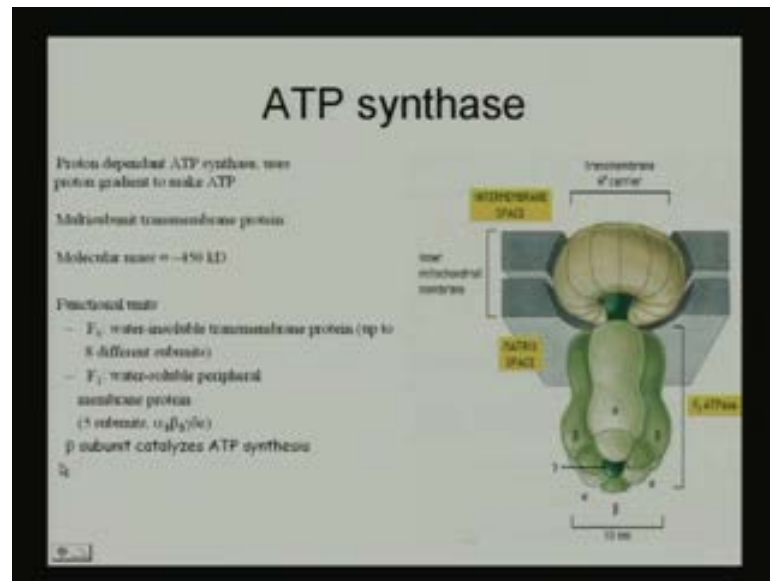
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Now you see now this is the **this is the** electron transport chain. Say protons are getting pumped out from inside matrix to the outside. This is complex one, complex two, complex three. Electrons are being transferred and oxygen is reduced to water molecule. Now, these protons are being pumped out of this inner mitochondrial membrane to the intermembrane space.

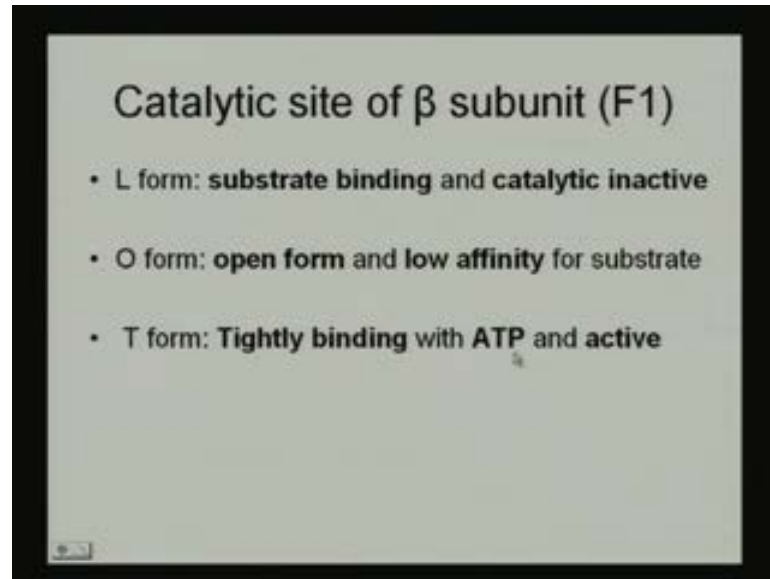
Now, when it is farther coupled to the ATP synthase, the enzyme ATP synthase is playing a significant role. ATP synthase has got two moieties; one is the F₀ another is the F₁. And when this ATP synthase is taking place inside this matrix, then protons are being pumped in the matrix and ADP and P_i is phosphorylated and it forms this ATP molecule. And this with this concentration difference **the** then we are considering this electro chemical gradient, the concentration difference is the because of the more proton on the outside and this factor is **this** the concentration difference is **this** more protons on the outside by a factor of 10. The charge differences are because of this protons are positively charged which are more on the outer side than this inner mitochondrial matrix. So, there are more protons on the outside surface. That means more positively charged on the outside and negatively charged by inside.

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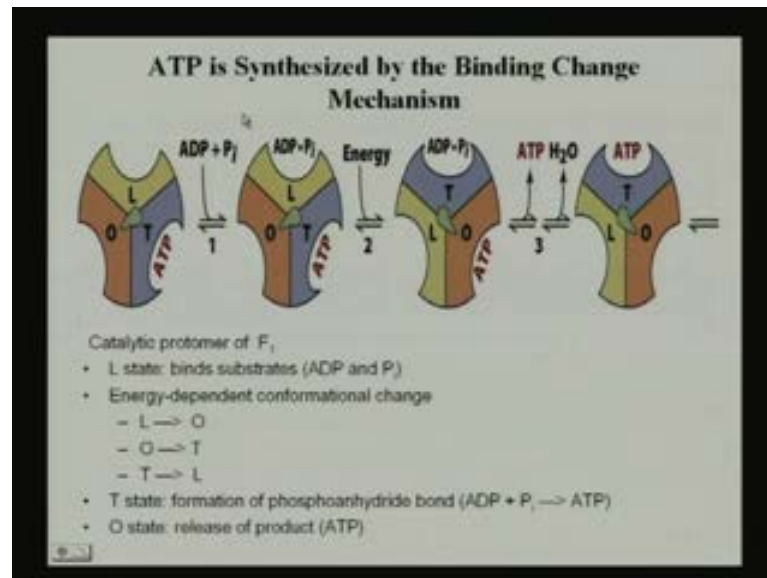
Now, if we come to this ATP synthase and if we learn little more about this particular enzyme, we will find that this enzyme is a very interesting enzyme. You know ATP synthase, it is the proton dependant ATP synthase and it uses the proton gradient to synthesize ATP. It is the multisubunit transmembrane protein and molecular masses mass of this particular enzyme is around four fifty kilo Dalton. As I have already told you that it has got two moieties of units; one is the F 0 another is the F 1. F 0 is the water insoluble transmembrane protein and it has got eight different sub units. F1 is the water soluble peripheral membrane protein and it has got five different subunits among which beta subunit is actually participating. It is catalyzing a significant role as per as ATP synthesis is concerned.

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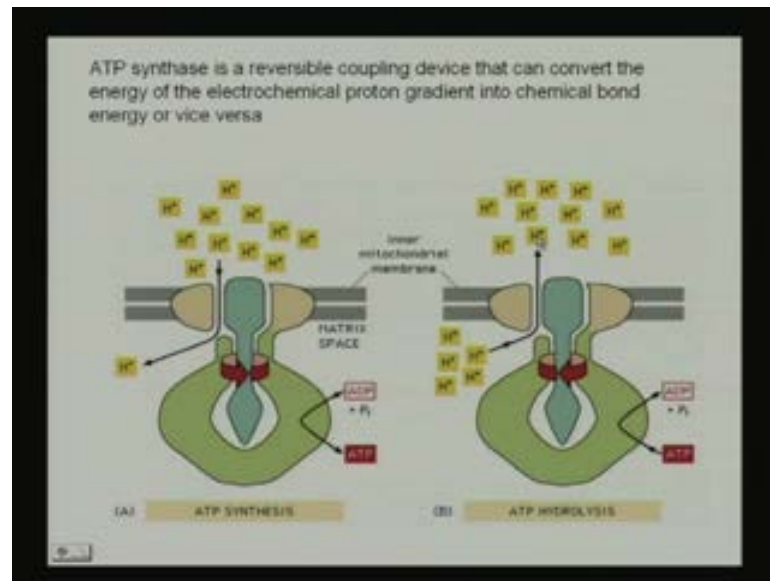
Now, this beta subunit is also very interesting. Now, here if we see this beta subunit that is, in the F 1 portion F 0 and F 1. It is the F 1 portion of this particular ATP synthase. Now, if we see this beta subunit we can find that it has got L form, O form and T form three catalytic sites are there. What are the functions of these three catalytic site? The L form is the substrate binding and catalytical substrate binding site but, catalytically it is inactive. O form is the open form and it has a very low affinity for the substrate. That means it does not have any affinity for substrate binding. And T form is the tightly binding and ATP with ATP that means it has got very strong affinity for ATP and it is also active.

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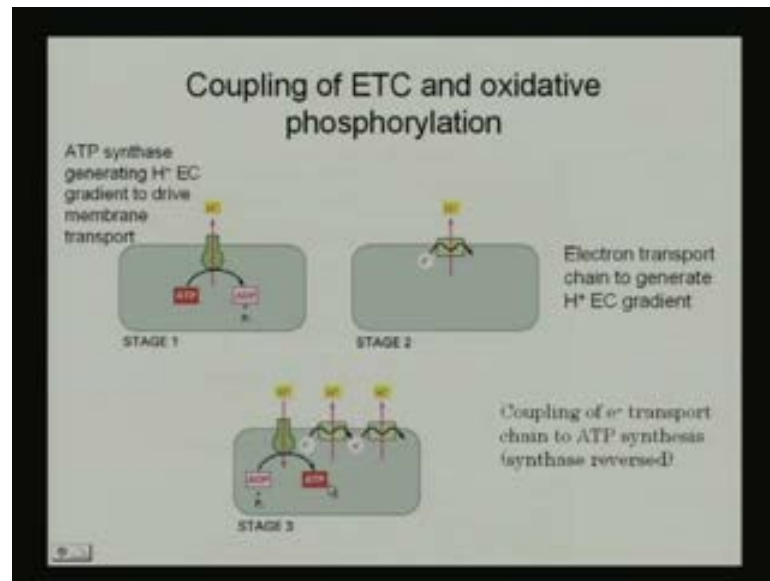
Now, this if we are symbolizing this L form T form and O form then, we can find that this L form is having this is the substrate binding affinity but, catalytically it is inactive. That means it can only bind the substrate but, it does not have any catalytic activity. This T form, it has got very strong affinity for its substrate binding and also it is **it is** active in nature. So, it can bind this ATP molecule to this and O form is does not have any substrate binding affinity and it is the open form. So, it is present in this. Now, when pumping of proton starts the beta subunit gradually changes its confirmation and this is the beauty of this mechanism of this particular beta subunit of ATP synthase. Now, here when this ADP and P_i is getting binded with the L site and when electron flow starts, then it starts changing its orientation. This conformational changes takes place. So, L state it binds the substrate ADP and P_i . So, energy dependent conformational changes when takes place, this L site become O site, O site become T site and T site becomes L site. And this way it starts changing its conformation. And in the T state formation of phosphoanhydride bonds are taking place resulting in the formation of ATP molecule. And as soon as the O state is coming and this O state is releasing this ATP molecule and in this way ATP synthesis is taking place in this particular, with this help of this particular enzyme ATP synthase.

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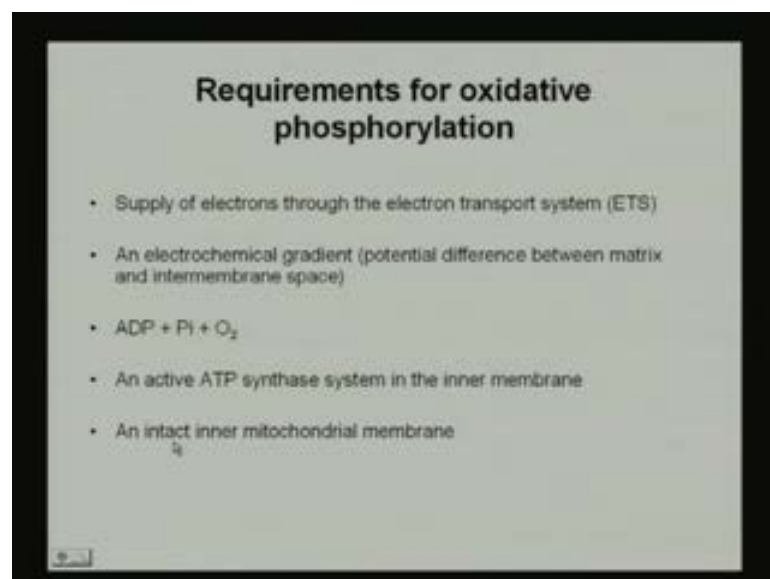
Now if we see, this is the ATP synthase this is the F₀F₁ form, this is F₁. And when we see this proton concentration the gradient outside of this, of the intermembrane is more than the inner matrix membrane then protons are being pumped in the inside in the matrix zone of these particular mitochondria. And during that time ATP synthesis is going on that means when these protons are getting pumped in and concentration of protons inside is less outside is more ATP synthesis is taking place. Whereas, when protons are getting pumped out during this electron transport chain, the ATP hydrolyses is taking place and when ATP is giving rise to ADP and P_i and the system is getting energy and protons are being pumped out from inner mitochondrial membrane to the intermembrane space. And in this way, the ATP synthesis and ATP hydrolyses are taking place simultaneously along with the electron transport chain.

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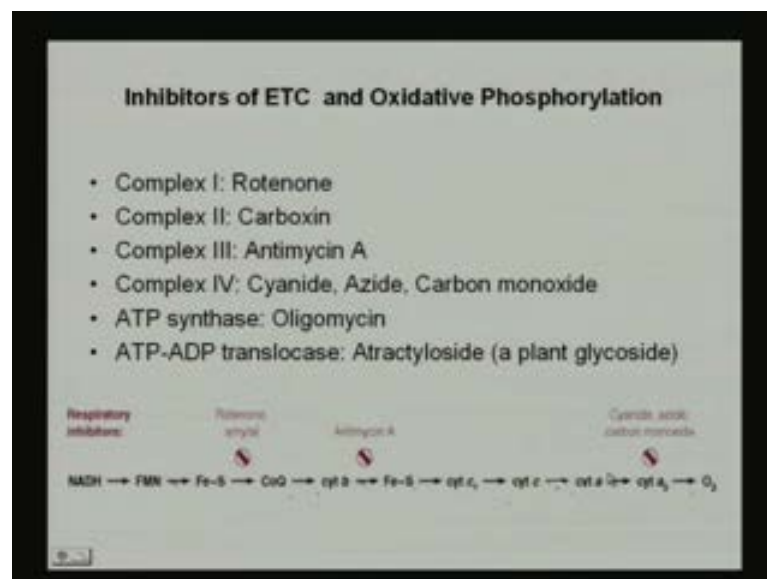
And now, if we see sum of this electron transport chain and oxidative phosphorylation then we can coupled up both the things that when ATP is converted to ADP protons are being pumped out from inner mitochondria matrix to the intermembrane space. When electrons are getting flow, the protons are being pumped out from inner mitochondrial membrane to outer mitochondrial membrane. And in stage three, this ADP and P_i is once again synthesizing ATP. Electrons are flowing and protons are being pumped in the matrix and ATP synthesis is taking place. What I have all ready mentioned in electron transport chain coupling with oxidative phosphorylation process.

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Now, what are the minimum requirements for carrying out this oxidative phosphorylation in the mitochondria? Here the supply of electron through the electron transport chain is essential and electrochemical gradient. That is the potential difference between the matrix and intermembrane space is essential. ADP P_i and O₂ should be there and active ATP synthase system in the inner membrane of mitochondria is very, very essential and most important part is an intact inner mitochondrial membrane and this is playing a significant role.

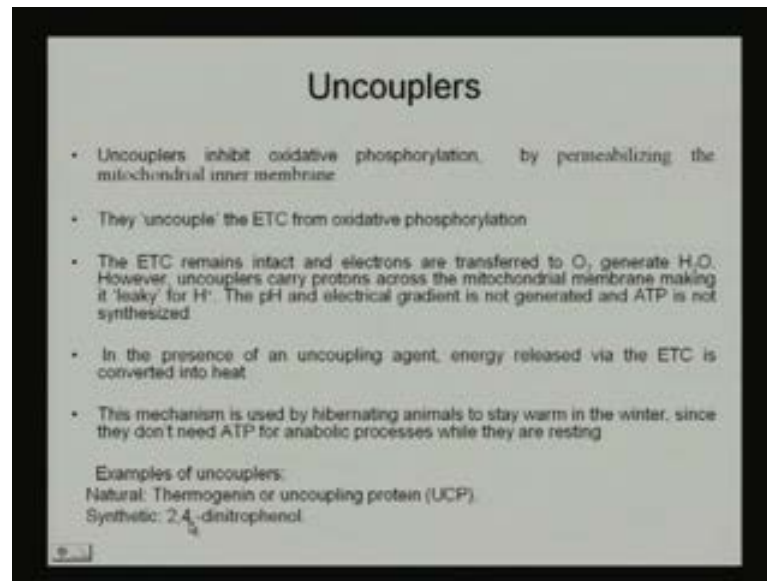
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If there is any breakage or anything there should, will not be any gradient formation resulting in the **resulting in the** discontinuation of the oxidative phosphorylation process. If we see different inhibitors of electron transport chain and oxidative phosphorylation we can find that in electron transport chain that rotenone, carboxin, antimycin a, cyanide, azide, carbon monoxide etc etc are the inhibitor of this electron transport chain.

Here, in the plain straight electron transport chain we are considering complex one, complex three and complex four. We can see that complex one is getting inhibited with rotenone. Antimycin a is inhibiting this portion and further continuation of this chain length is getting stopped. And in the eight cyanide or Azide they have stopped in this cytochrome a three. And here it **it it** is getting stopped and similarly, ATP synthase is also getting stopped by **the** a series of this inhibitors which inhibits the enzyme.

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Uncouplers

- Uncouplers inhibit oxidative phosphorylation by permeabilizing the mitochondrial inner membrane
- They 'uncouple' the ETC from oxidative phosphorylation
- The ETC remains intact and electrons are transferred to O_2 generate H_2O . However, uncouplers carry protons across the mitochondrial membrane making it 'leaky' for H^+ . The pH and electrical gradient is not generated and ATP is not synthesized
- In the presence of an uncoupling agent, energy released via the ETC is converted into heat
- This mechanism is used by hibernating animals to stay warm in the winter, since they don't need ATP for anabolic processes while they are resting

Examples of uncouplers:
Natural: Thermogenin or uncoupling protein (UCP)
Synthetic: 2,4-dinitrophenol

The role of uncoupler, inhibit oxidative phosphorylation is already there and they uncouple, mainly they uncouple the electron transport chain from oxidative phosphorylation. The electron transport chain remains intact and electrons are transferred to oxygen generating water. However, uncoupler carries the proton across the mitochondrial membrane making it leaky for hydride ion. The p H of the electrical gradient is not generated and ATP is not getting synthesized. In the presence of an uncoupler agent energy release via electron transport chain is converted into heat. The mechanism is used by hibernating **animal** animals to stay warm in the winter since they do not need ATP for anabolic process while they are resting. And there are different uncouplers and most important natural uncoupler is the thermogenins or some uncoupling proteins and for synthetic uncoupling reagent that is 2 4 dinitrophenol is one of the potent inhibitor uncoupling agent.

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Electron shuttle systems : Transport of NADH into mitochondria

- The mitochondrial membrane is impermeable to NADH, thus, a transport system would be required to allow entry to NADH into the mitochondrial matrix
- Electron shuttle systems that accept electrons from cytosolic NADH, enter mitochondria, and give up the electrons to electron acceptors in the mitochondrial matrix
- Two shuttle system are there :
 - Glycerol-3-Phosphate Shuttle
 - Malate-Aspartate Shuttle

Now, as I have told you that inner mitochondrial membrane are not permitting anything for going out of this system. Now, when we are talking about this electron transport this electron shuttle system, it helps in the transportation of NADH to the mitochondria. And when we are talking about this mitochondrial membrane, it is impermeable to NADH and thus transportation. Because flow of electron is essential so whatever this NADH or FADH₂ is produced in the cytosol of the cell is being transported inside this mitochondria. And for this particular activity, the two shuttle systems are existing. One is the Glycerol-3-Phosphate Shuttle another is the malate aspartate shuttle.

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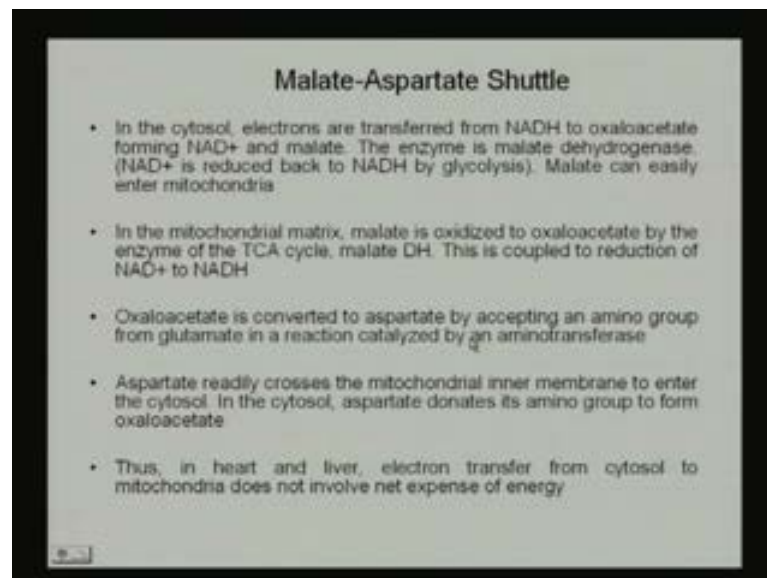
Glycerol-3-Phosphate Shuttle: Functions in the skeletal muscle and brain

- NADH on the cytoplasmic side is oxidized to NAD⁺ with coupled reduction of DHAP to glycerol-3-phosphate
- The oxidation of glycerol 3-phosphate back to DHAP is catalyzed by a mitochondrial membrane bound isoenzyme of glycerol-3-phosphate dehydrogenase
- The oxidation is coupled to reduction of a FAD prosthetic group of the mitochondrial enzyme to FADH₂
- Reduced FADH₂ transfers its electrons to CoQ via the ETC
- Thus, in muscle and brain, even though 2 NADH are produced by glycolysis, actually 2 FADH₂ are available for entry into the ETC

The diagram illustrates the Glycerol-3-Phosphate Shuttle mechanism across the mitochondrial membrane. In the cytosol, NADH + H⁺ is oxidized to NAD⁺ by the enzyme cytosolic glycerol-3-phosphate dehydrogenase, which reduces dihydroxyacetone phosphate to glycerol-3-phosphate. Glycerol-3-phosphate then enters the mitochondrion and is oxidized back to dihydroxyacetone phosphate by the enzyme mitochondrial glycerol-3-phosphate dehydrogenase, which reduces FAD to FADH₂. FADH₂ then transfers electrons to ubiquinone (Q) in the electron transport chain.

In case of Glycerol-3-Phosphate shuttle it functions mainly the skeletal muscle and brain. As we have all ready learned that NADH on the cytoplasmic side is oxidized to NAD with coupled reduction of dihydroxyacetone phosphate and glycerol three phosphate. The oxidation of glycerol three phosphate back to this dihydroxyacetone phosphate is catalyzed by a mitochondrial membrane bound isoenzyme. That is the glycerol three phosphate dehydrogenase. This enzyme is there and this oxidation is coupled to a reduction of FAD, a prosthetic group of the mitochondrial enzyme to FADH 2. And this FADH 2 which is now produced is transferring this electron to the ubiquinone and it is converting this ubiquinone to ubiquinol. And this way this it is helping the electron the supply of electron to this electron transport chain. And thus, in muscle and brain, even though two NADH are produced by glycolysis. Actually two FADH 2 are available for the entry into the electron transport chain. Another very interesting shuttle which is taking place in the electron transport chain is the malate aspartate shuttle.

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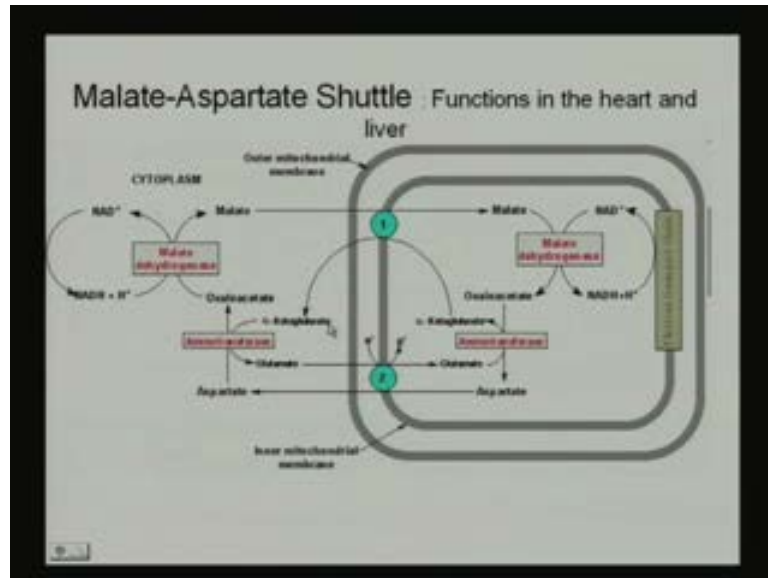
Malate-Aspartate Shuttle

- In the cytosol, electrons are transferred from NADH to oxaloacetate forming NAD⁺ and malate. The enzyme is malate dehydrogenase. (NAD⁺ is reduced back to NADH by glycolysis). Malate can easily enter mitochondria
- In the mitochondrial matrix, malate is oxidized to oxaloacetate by the enzyme of the TCA cycle, malate DH. This is coupled to reduction of NAD⁺ to NADH
- Oxaloacetate is converted to aspartate by accepting an amino group from glutamate in a reaction catalyzed by an aminotransferase
- Aspartate readily crosses the mitochondrial inner membrane to enter the cytosol. In the cytosol, aspartate donates its amino group to form oxaloacetate
- Thus, in heart and liver, electron transfer from cytosol to mitochondria does not involve net expense of energy

In the cytosol electrons are transferred from NADH to oxaloacetate forming NADH and malate. The enzyme is malate dehydrogenase which is one of the very important enzymes as I have all ready discussed in TCA cycle. Malate can easily enter mitochondria. In the mitochondria matrix, the malate is oxidized to oxaloacetate by the enzyme of this TCA cycle. That is the malate dehydrogenase this is coupled to the reduction of NAD to NADH. Oxaloacetate is converted to aspartate by accepting the

amino group from glutamate in the reaction catalyzed by this aminotransferase. So, this aspartate is converted to, oxaloacetate is converted to aspartate.

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So this is the oxaloacetate which is getting converted to aspartate. Oxaloacetate is converted to aspartate and aspartate as soon as it is produced so it is coming to the cytosol. And here in presence of this aminotransferase once again oxaloacetate is being formed and it is converted to malate and malate is going inside this mitochondria. And in this way TCA cycle is getting continued and the glutamate which is there. This glutamate is donating its amino group and alfa keto glutarate is formed. And this alfa keto glutarate is once again coming to the cytosol of this particular cell and it is converted to glutamate and this way this shuttle is going on.

The aspartate readily crosses the mitochondrial inner membrane into and entered the cytosol. In cytosol aspartate donate its amino group to form oxaloacetate. Thus in heart and liver electron transfer from cytosol to mitochondria and it does not involve any expense of energy.

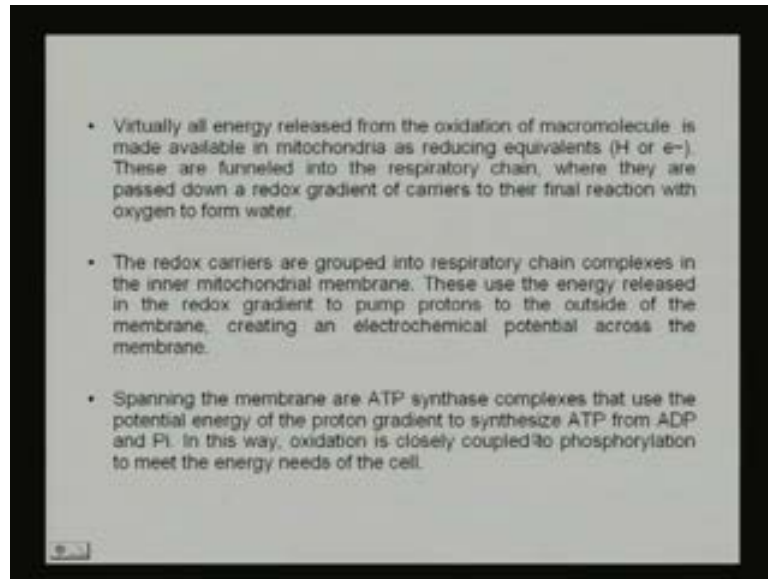
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Pathway	ATP	NADH	FAD	TOTAL ATP
Glycolysis	-2 4	2	0	
PDH	0	2	0	
TCA	2	6	2	
Glycerol-3-P shuttle	0	-2	2	
	4	8	4	
ATP Harvested	4	14	8	36

And this is the shuttle system in case of this electron transport chain and oxidative phosphorylation. And here, if we see the total energetics how many ATP molecules are being produced from glycolytic process because this is the ultimate steps of respiration. It starts from glucose to pyruvate, pyruvate and it enters to pyruvate is converting converted to acetyl coa enters to TCA cycle and then it flows to electron transport chain followed by oxidative phosphorylation. And here the cellular respiration **respiration** is getting completed.

So, if we see the total this process we can find that, incase of glycolysis the first step two molecules of ATP is being consumed so minus 2. 2 molecules of NADH is produced, four molecules of ATP is produced in case of pyruvate dehydrogenase, two molecules of NADH being produced in TCA cycle, 2 ATP, 6 NADH and 2 FAD molecules are produced. In glycerol three phosphate shuttle two molecules of NADH is being consumed and two molecules of FADH is produced. FADH 2 is produced. So, total gain if we see we can get that four molecules of ATP, eight molecules of NADH and 4 molecules of FAD is produced which is giving these four molecules of ATP, 24 molecules of NADH. Because one **n ad** NADH is equivalent to three molecules of ATP, we have all ready seen so 8 molecules of NADH means 24 molecules of ATP and 4 molecules of FAD means 8 molecules of **n a** ATP. So, all together we can see that there is a net gain of thirty six ATP molecules. When they enter catabolic process, the recellular respiration process is getting completed.

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Now, in conclusion what we can tell that virtually all energy released from the oxidation of macromolecule is made available in mitochondria as reducing equivalent. That means the hydride ions and the electrons these are funneled into the respiratory chain where they are passed down a redox gradient of carriers to their final reaction with oxygen to form water. The redox carriers are grouped into respiratory chain complexes in the inner mitochondrial membrane. These use the energy release in the redox gradient to pump protons to the outside of the membrane creating an electrochemical potential across the membrane. Spanning the membrane are ATP synthase complexes that use the potential energy of the proton gradient to synthesize ATP from ADP and inorganic phosphate. In this way oxidation is closely coupled to the phosphorylation to meet the energy need of the cell. So, with this I think we have all ready learned the cellular respiration of any living cell. So, how this catabolic mechanism is going on inside the cell and how this glucose to oxidative phosphorylation is continuously carrying out in any aerobic system. With this I think we have all ready learned. Now, with this I think this respiration incase of any aerobes are all ready discussed in details. In my next class, we will discuss some other metabolic activities which are going on in any living system. Thank you very much.