Genetic Engineering: Theory and Applications.
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Module II.
Basics of Biological System.
Lecture-4.
Metabolic Reactions in Biological System.

Hello everybody, this is Doctor Vishal Trivedi from Department of Biosciences and Bioengineering, IIT Guwahati. Let us continue our discussion about the different host and what we have, but before we discuss further, let us recap what we have discussed so far. So, what we have discussed so far.

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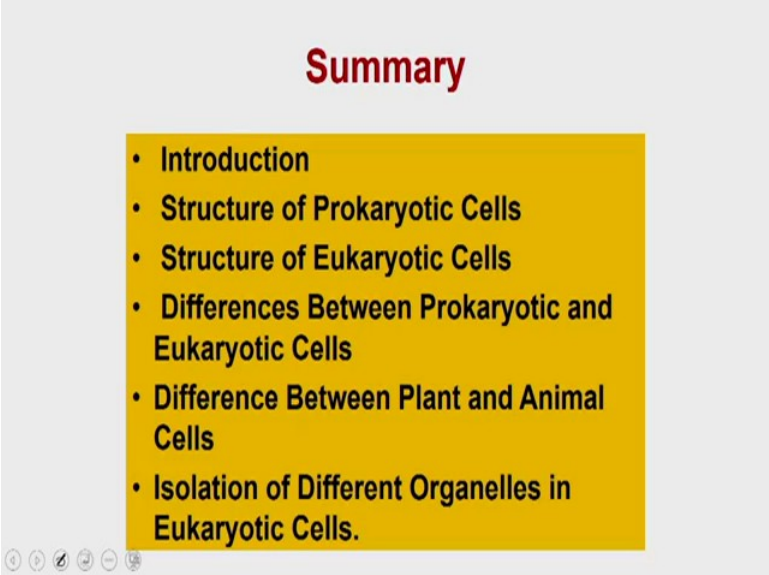

So, in the previous module we have discussed the introduction of, we have discussed about the structure of prokaryotic cell, we have discussed about the structure of eukaryotic cell, within this we have also discussed about the different organelles which are present in the eukaryotic cell, their structure, their function as well as we have also discussed what is their relevance in in in in contributing into the different activities which are which are which are which are important for the eukaryotic cell.

Then we have also discussed about the crucial and significant differences between the prokaryotic as well as eukaryotic cells. And these differences are important because once you know these differences, you could be able to exploit or you could be able to use the prokaryotic cell as well as the eukaryotic cell for for for downstream applications in the in

the in the biotechnology. Because depending on the applications you can choose the prokaryotic cell or the eukaryotic cell.

Then we have also discussed about the difference between the plant cells as well as the animal cells. And as as we discussed before, these differences are also important to know what are the limitations or what are the technological limitations you have or what are the technical challenges you are going to face when you use the plant cell or the animal cell as a source of host for for any type of biotechnology applications. And at the end we have also discussed how you can isolate the different organelles in a eukaryotic cell.

We have also discussed, in the same thing we have also discussed the isolation of the periplasmic fraction as well as the cytosol from the prokaryotic cell as well. And in in this section, in this (chap) lecture we have also discussed about the different techniques which you can use to isolate the different organelles present in a eukaryotic cell. These approaches or the techniques can be utilised even for the different, not only for isolating the organelles but also for isolating the different types of biomolecules which are varying in their sedimentation rate or which are varying in terms of their different densities.

So, these are the different things what we have discussed, so what we have discussed so far is actually enable you to understand the the information or the requirement of the host which we are going to use in the biotechnology applications. Apart from these resources, the host cell is also depends extensively on the metabolic reactions which they are going to catalysed and this metabolic reactions are being used for many purposes. So, let us see what we are, what are the different metabolic reactions are occurring in the host cells.

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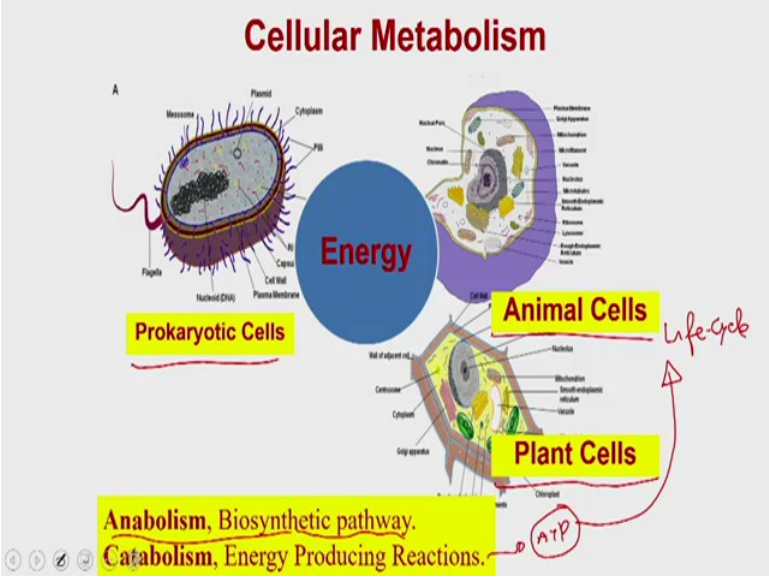

So, what we have discussed? We have discussed about the prokaryotic cell, we have discussed about the animal cell and then we have also discussed about the plant cells. And all these cells, all these host specific cells are (req) requires the (o) the energy and this energy they required to run the various metabolic reactions, to drive its life cycle or lifespans and to divide and grow from one one one cell to more many more cells. And there are two majorly there are two different types of metabolic reactions which are which are happening in a in a host cells. These are called anabolic reactions or the catabolic reactions.

These anabolic reaction as the name suggests, these are called as the biosynthetic pathway. So anabolic actions are the reactions in which the new biomolecules are being synthesised. Whereas the catabolic reactions are the energy producing reactions, which means in the catabolic reactions you are actually generating you are generating the ATP by the breakdowns of the biomolecule and this ATP you are using for running the life cycle or running the different activities within the cell.

So, there are, so these are the two majorly processes which are happening in a in any any of these prokaryotic cells any of these host cells, whether it is a prokaryotic cell, animal cell or the plant cell. So, let us discuss about these reactions. So, we will start with the glucose metabolism. So, in the glucose metabolism, as soon as glucose molecules enter into a cell, it is been it is been channelized into a in a series of metabolic reactions so that you can produce the ATP as well as reducing equivalent. And all these reducing equivalent as well as the ATP is been used to produce the energy.

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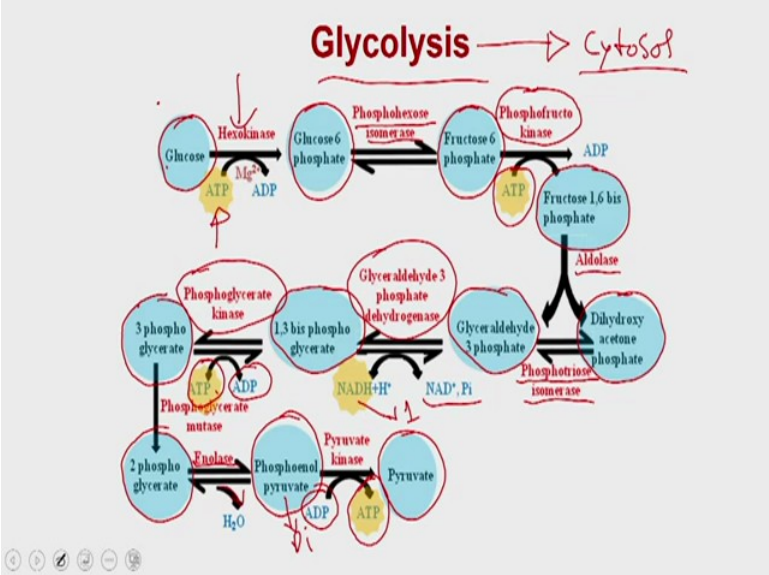

So, the first metabolic pathway what we are going to discuss is called as the glycolysis. Glycolysis is operating in a in cytosol of the cell, whether it is the bacterial cell or the (eukaryo) or the eukaryotic cell. So, first reaction which is required to channelize the glucose molecules into the glycolysis is, which is catalysed by the enzyme known as the hexokinase. So, the hexokinase (uti) utilises the glucose, it can utilise any 6 carbon (glu) 6 carbon sugar and with the help of ATP and magnecium as as the cofactor, hexokinase generates the glucose-6-phosphate and then glucose-6-phosphate is been isomerised in the fructose 6 phosphate.

So, irrespective of whether you have a glucose, fructose, mannose or maltose, all these sugar molecules are being accepted by the hexokinase to generate their corresponding the 6 phosphate. For example in the case of fructose it will generate the fructose 6 phosphate, in the case of glucose it will generate the glucose-6-phosphate. So, if and after that there is the isomerase reactions. This isomerase will convert all these forms to the fructose 6 phosphate. So, if you remember in this place you are actually spending 1 ATP to commit the glucose molecule into the glycolysis.

Once a fructose 6 phosphate is generated, it is again getting phosphorylated on the other carbon and then you are generating the fructose 1, 6-bis phosphate, the enzyme is phospho fructo kinase. And one other molecule of ATP is being utilised in this process. Once the fructose 1, 6 bis phosphate is being generated, then the fructose 1, 6 bis phosphate is been split by the aldolase in the 2 molecules, one is called Glyceraldehyde 3 phosphate or the other molecule is called as the dihydroxy acetane phosphate.

These 2 molecules, like Glyceraldehyde 3 phosphate or the dihydroxy acetone phosphate are the isomers. So, these molecules can convert to each other by the with the help of an enzyme known as phosphotrios isomerase. Irrespective of this the formation of any of these molecules, the Glyceraldehyde 3 phosphate is being further processed to form the 1, 3 1, 3 bis phospho glycerate and the enzyme is known as the Glyceraldehyde 3 phosphate dehydrogenase. And in this process you are generating, you are using the NAD plus and P I and you are generating the first molecule of NADH.

These NADH molecules are been are are called as a reducing equivalent, they will be further used in the downstream reactions to generate the ATP. Then 1, 3 bisphosphate glycerate is being converted into the 3 phospho glycerate and the enzyme name is the phospho glycero kinase. This means you are removing the one phosphate molecule from this from this compound and this phosphate molecule is being received by the ATP, should generate the ATP. So, since you are generating the 2 molecule of Glyceraldehyde 3 phosphate, you are going to generate the 2 molecules of ATP.

Then the (phos) 3 phosphoglycerate is being converted into 2 phospho glycerate, which is actually just the just the change in position of the phosphate within the molecule. And then from this molecule you are generating the phosphoenol pyruvate, this is a dehydration reaction where you are going to have a loss of water and the enzyme name is enolase. And then from the phosphoenol pyruvate,it will get converted into the pyruvate which means again you are going to lose another phosphate molecule. And this phosphate molecule is going to be received by the ADP to generate the ATP.

So, now (will a) if you see very carefully, you are having the multiple places. So, (in mul) in in initially when you started with the glucose, you are spending 2 ATPs, one at this place, the other one is at this place. So, you are spending 2 ATP molecule to channelize the (mole) to channelize the (Com) theglucose into the into the glycolysis. But you are generating the 2 molecules of glyceraldehyde 3 phosphate and at the end the Glyceraldehyde 3 phosphate is getting converted into the NADH and this and NADH is going to be into the electron transport chain and the (ele) in within the electron transport chain, it will going to generate the ATP, which means you have spend 2 ATPs in the initial reactions and with the generation of NADH you have produced the ATP.

and in the second reaction, (you) with the with the section of phosphate molecule from the 1, 3 bisphospho glycerate, you have generated 2 molecules of ATP and and in the last step

when the phosphoenol pyruvate is also losing another phosphate molecule, you are generating another 2 molecules of ATP. Which means if you see the balance sheet of ATP production from a glycolysis, you will see that the with the glycolysis you are gaining many ATP by spending 2 ATP in the first reaction.

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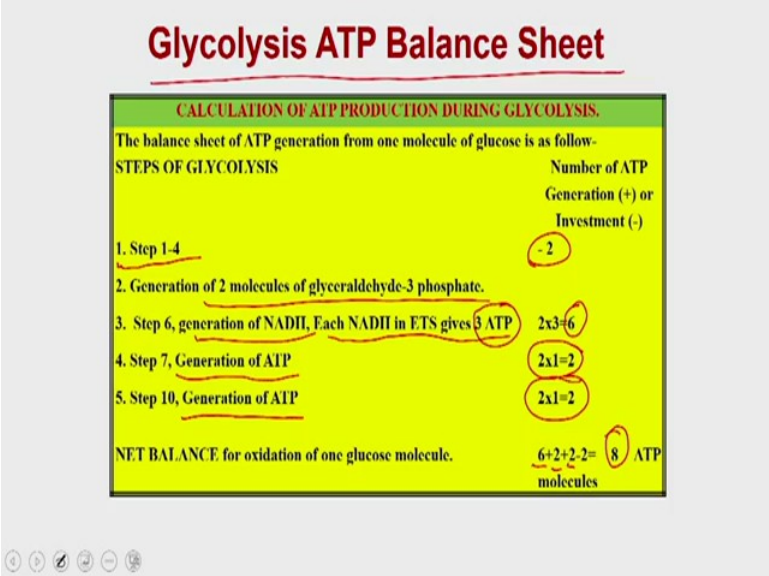

So, let us see the glycolysis, the balance sheet. The balance sheet is like this, as we discussed, in step 1 to 4, you are actually spending 2 ATPs and then in the in the subsequent step when you are you are generating 2 molecule of glyceraldehyde 3 phosphate and in the step 6 you are generating the NADH and each NADH molecule, when it goes for the electron transport chain, it generates 3 ATP. Which means from the 2 (AT) from the 2 NADH molecule are going to get the 6 ATPs, then in the step 7, when there was loss of phosphate, you are going to generate 2 ATP and in the last step when the pyruvate is being generated, you are going to generate another 2 ATP.

So, what is the net balance? If you take the single molecule of glucose, what you have generated is the 6+2+2, which is 10 and 2 ATP molecule you have generated in the initial 4 steps, so that you are activating the glucose and that is being the consumption. So, at the end of the 1 glucose molecule, when it is getting oxidised in the glycolysis, you are generating the 8 molecules of ATP. So, when a glucose molecule enter into the glycolysis, you can see that you are actually spending 2 ATP molecules but in return you are getting the 8 molecules back.

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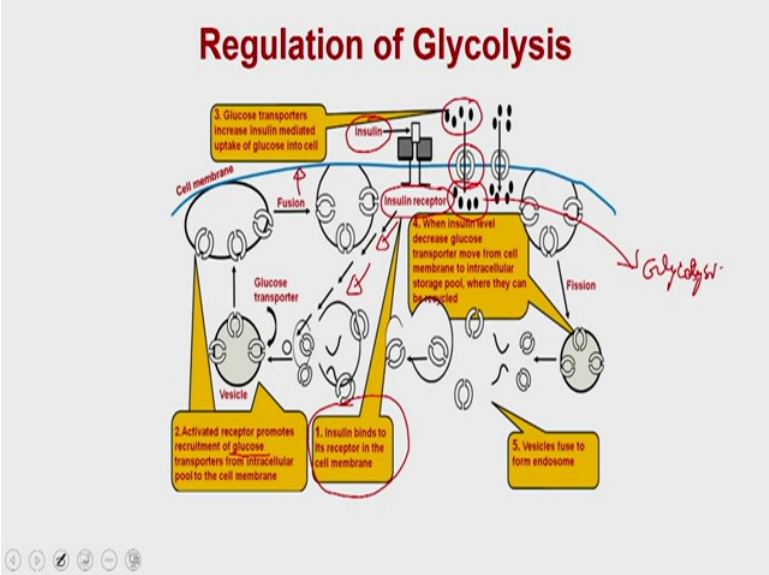

But as the glucose enters into the cell and the glycolysis operates, all these events are under the tight control. So let us see what factors are controlling the glycolysis reactions within the cell. So, (Gla) within the within the cell, you have the transporters which are present on the cell surface and one of the hormone, which is called as the insulin and the insulin hormone, when it binds to the insulin receptor, it drives the downstream event or the downstream signalling and because of that it actually control, it regulates the glucose metabolism within the cell.

And how it happen is that in the first event the (gluco) insulin binds to its receptor in the cell membrane and that actually activates the insulin on the insulin insulin mediated downstream signalling. Once the insulin is insulin mediated downstream signalling is activated, it actually up regulates the recruitment of glucose transporter, which is glut 3 and glut 4 and these transporters then goes on to the cell membrane and they are available for the glucose transport. So, as soon as they are available on the plasma membrane, they allow the glucose molecule to becoming inside the cell.

And as soon as these cells these these glucose molecules are available, they are being used or they will be available for the glycolysis. But you can imagine that if the glucose level is going, is very high, and the insulin level is going down, then in that case all these events are going to be reversed, the the the glucose transporter which are present on the cell surface are being internalised into the vesicles and then ultimately they are being going to be degraded to form into the endosomal (re) endosomal system and that is how the level of insulin glucose receptors on the cell surface is going to be reduced.

Once the glucose receptors on the cell surface is going to be reduced, the entry of the glucose is going to be less and as a result the downstream glycolysis is going to be less. Apart from these glucose mediated control, you also have the control at the within the insulin, within the glycolysis reaction which are operating in the cell. And these regulations are mostly either by the feedback mechanism or the by covalent modification.

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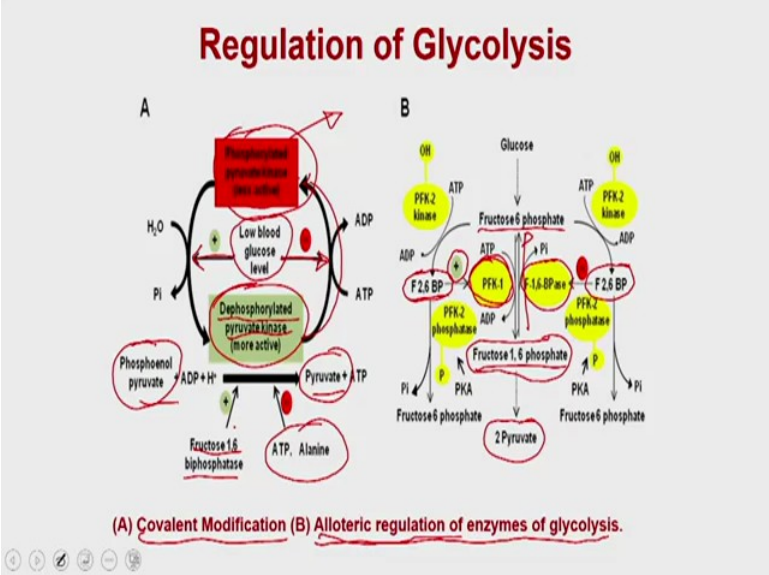

So, in the in the at the enzymatic level, you have 2 different types of two different types of regulations, one is called the covalent modifications and the other one is called as the allostericallosteric regulations. So, let us discuss about the covalent modifications. So, in the covalent modification, the example is the pyruvate kinase, the pyruvate kinase which is actually which is actually (take) taking up the phosphate from the phosphoenol pyruvate and it is generating the pyruvate. And you can see that as the enzyme is, phosphor enzyme or this pyruvate kinase could be present in 2 form, one is phosphorylated form, the other one is the un-phosphorylated or the native form.

So, dephosphorylated form is more active and whereas the phosphorylated form is less active. So, in the event of the so what happen is that when there is a when there is a low glucose, when there is a low glucose in the in the cell, it actually (in it) it actually channelizes the (phosphorylash) (phos when) it channelizes the dephosphorylation of this enzyme. So, because of that the enzyme get converted from phosphorylated pyruvate kinase to the dephosphorylated pyruvate kinase. And because of that it actually activates the conversion of phosphoenol pyruvate to pyruvate and that is how you actually producing the energy.

But if the glucose level goes up into the blood, which means this the cells have the sufficient sufficient energy, then the these glucose molecules are catalysing the phosphorylation of pyruvate kinase and once the phosphorylated pyruvate kinase is being generated, that actually is going to be lower down or slow down the activity of, slow down the convergen of phosphoenol pyruvate to pyruvate.

Apart from this regulation, which is actually a covalent modification, the fructose 1, 6 bisphosphate, if you remember in the initial 1 to 4 reactions, fructose is being phosphorylated by the phosphorylated by another round and that has generated the fructose 1, 6 bisphosphate, that actually is positively regulating the convergen of phosphoenol pyruvate to pyruvate. Whereas if there is sufficient quantity of ATP or the Alanine, is present inside the cell, it is negatively regulating.

Which means if you have enough amount of ATP in the cell, it actually inhibits these reactions or it will block the convergen of phosphoenol pyruvate to pyruvate. Which means if you have sufficient energy, there is no reason that you actually utilise the glucose which is present inside the cell through glycolysis. The other kind of modification which is called allosteric regulations. So, in the allosteric regulation is where the molecule is regulated in a different mechanism. So, every enzyme has an allosteric site and in the allosteric site, the (sub) the substrate or the another molecule actually goes and bind and that is how it actually modulates their activity.

So, you can imagine this is that the fructose 6 phosphate is getting converted into fructose 1, 6 bisphosphate and this reaction is catalysed by the phosphofructo kinase. So, phosphofructo kinase has the allosteric binding site for fructose to 2 6 bisphosphate. Which is neither a substrate or nor the product of this reaction. And because of that it actually modulates its activity. Similarly the fructose 1, 6 bisphosphatase which is actually going to drive the reverse reaction is also been having the allosteric binding site for fructose 1, 6 bisphosphate.

And fructose 2, 6 bisphosphate is positively regulating the activity of fructose phosphofructo kinase 1and that means if the fructose 2, 6 bisphosphate is present, it will it will activate the reaction or it will enhance the catalytic conversion rate of phosphofructo kinase. And as a result you are going to have more level of the fructose 1, 6 bisphosphate, which will eventually get converted into the pyruvate. Whereas if there is a if there is a sufficient quantity, then the the fructose 2, 6 bisphosphate is going to be negatively (regu) So, at the at

the same time, fructose 1, 6, fructose 2, 6 bisphosphate negatively regulate or (nega) will reduce the activity of fructose 1, 6 bisphosphatase.

Which means (it) on the on one one hand it increases the activity of phosphofructo kinase, so that will increase the phosphorylation reaction. On the other hand it will reduce the activity of phosphatase. And by doing so fructose 2, 6 bisphosphate allosterically favours the formation of fructose 1, 6 bisphosphate. Once the fructose 2, 6 bisphosphate function is over, then fructose 2, 6 bisphosphate get converted into fructose 6 phosphate. And that is how it actually, its effects are going to be controlled.

Now let us move on to the next reaction and next reaction, once you generated the pyruvate, that pyruvate will enter into the next next catalytic reaction or next metabolic reactions. So, pyruvate, once it is generated into the cytosol, it will supported to the mitochondria. This transporter, this transport is never been in the pyruvate form. The pyruvate getconverted into the acetyl CoA and then acetyl CoA moved into the mitochondria and catalyse downstream reactions.

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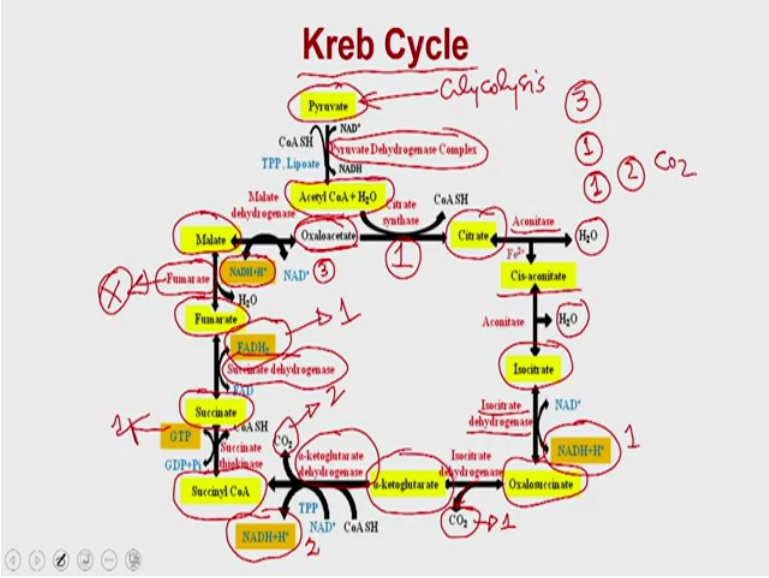

And the, these reactions which are occurring inside the mitochondria are called as the Kreb cycle. So, let us see what are reactions are happening inside the Kreb cycle. So, as we discussed thepyruvate which is actually being generated from the glycolysis is getting converted into the acetyl CoA, the enzyme name is pyruvate dehydrogenase complex and once this acetyl CoA is being generated, it is being used to catalyse the first reaction of Kreb cycle. As the name suggests, the Kreb cycle is being discovered by a scientist named as the

Hans Kreb and it is actually the multiple reactions which are which are being used to catalysed the the catalyse the (diff) different steps to generate the generate the ATP as well as reducing equivalent.

So, the oxaloacetate, which is being present, combined with the acetyl CoA in the first reaction, the enzyme name is citrate synthase to generate the citrate. The citrate is being utilised by the aconitase (to) and it is a dehydration reaction, so one loss one water one one water molecule is been lost. And that is how it generates and produces the cis aconitase. Cis aconitase is also loses another round of water to generate the isocitrate. Now, this isocitrate is being used by the isocitrate dehydrogenase, isocitrate dehydrogenase and in this reaction the first time the isocitrate is converted into the oxalosuccinate and one molecule of NADH is being produced.So, this is the first NADH which is being produced in the Kreb cycle.

After the oxalosuccinate, (it) there is the decarboxylation reaction and it reduce it loses its one (amou) one molecule of carbon and it get converted into the alpha keto glutarate. Alpha keto glutarate is being converted into the succinyl CoA and the one molecule of NADH is being produced. This is the second NADH molecule and and the succinyl the enzyme name is Alpha keto glutarate dehydrogenase and another second molecule of carbon dioxide is being released. So, this is the second molecule of carbon dioxide, this is the first molecule of carbon dioxide.

Now, the succinyl CoA is being converted into the succinate and if you see very carefully, in this step the GTP is being produced instead of ATP. So, the first molecule of GTP is being produced. Now the succinate is being converted into the fumarate with the help of enzyme known as the succinate dehydrogenase. And the another molecule of FADH is being produced, which is actually the first molecule of the FADH2, which is reducing equivalent. So, so far we have generated in the Kreb cycle (we) (the) (they have) they have generated 2 molecules of NADH. Now,and the 1 molecule of FADH2 and the one molecule of GTP.

Now the fumarate is getting converted into the malate, with the help of an enzyme known as fumarase and the malate is getting converted into the oxaloacetate and the third molecule of NADH is being produced. So, this is the third molecule of NADH. Which means you are generating 3 molecules of NADH, one molecule of FADH 2 and one molecule of GTP. So, at the end, the oxaloacetate which is being generated again goes through the cycle and combine with the acetyl CoA to generate the citrate. And that is how this is called the Kreb cycle because this is a cyclic reactions occurring in a in in one one after other.

How people know that this is a cyclic reaction? So the Hence Kreb has done many carbon many experiments and ultimately he has figured out that this is a cyclic event occurring because if you block any of these substrates and you does not allow or suppose you inhibit the fumarase, you could be able to block the Kreb cycle or you disrupt the the the convergence of one product into another product. So, at the end what you are getting out of the Kreb cycle? What you are getting is 3 molecules of NADH, one molecule of FADH, one molecule of the GTP and 2 molecules of carbon dioxide.

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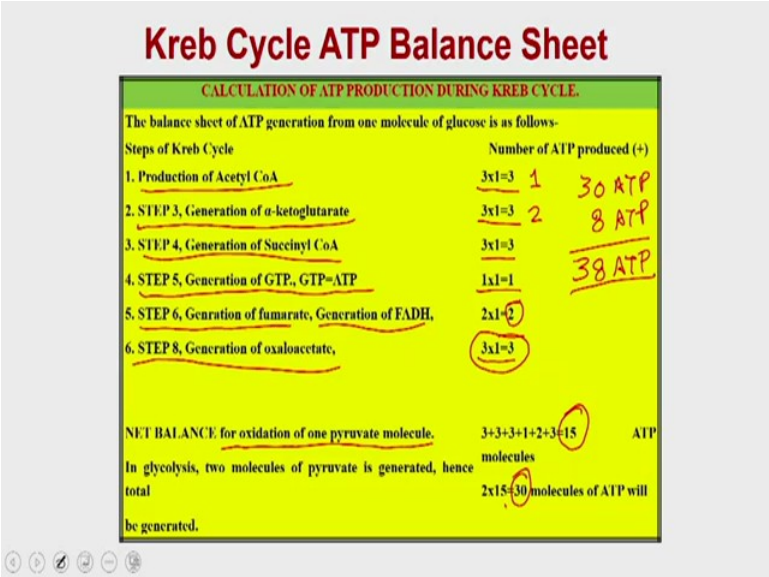

Let us see what is the balance sheet of the Kreb cycle. So, in a balance sheet of one carbon Kreb cycle, you always remember that you have generated the 2 molecule of pyruvate from the one molecule of glucose. So, the initial reaction is that in the first acetyl CoA is generated. So, you have seen that when the pyruvate got converted in the acetyl CoA, it has generated the one molecule of NADH. Then in the step 3 when there was a generation of Alpha keto glutarate, another NADH is being generated. So, this is the first NADH, this is the second NADH.

Then in the step 4, when there was succinyl CoA is generated, then also there was a generation of the reducing equivalents and then in the step 5 there was a generation of GTP. One GTP is equivalent to ATP, so that is how you can have one ATP or GTP. Then when there was a generation of fumarate, you have generated the FADH. So, if one molecule of FADH when it goes through the ETS, ETS Oxidation or when it goes for the oxidation into the electrons transport chain, it generates the 2 molecule of ATP. And then the last step you

generated the oxaloacetate and that actually also given the one NADH. So, that is how you have generated 3 ATP.

So, let us see what is the net balance. So, net balance of one molecule of pyruvate molecule, when it got into the Oxidation, you have generated 15 ATP molecule. And in the glycolysis you have generated 2 molecules of pyruvate, that means from one molecule of glucose you have generated the 30 ATP molecule. Which means you have generated 30 ATP from the Kreb cycle and you have generated the 8 ATP from the glycolysis. Which means if you if you oxidise one molecule of glucose, you will get the total 38 ATP, if (the) all these reducing equivalent will go for the complete oxidation.

Similar to (ka) similar to glycolysis, the Kreb cycle is also under the tight control. It is being regulated by at many steps at many by many enzymes and by different types of mechanisms. So, let us see what are these mechanisms to control the Kreb cycle.

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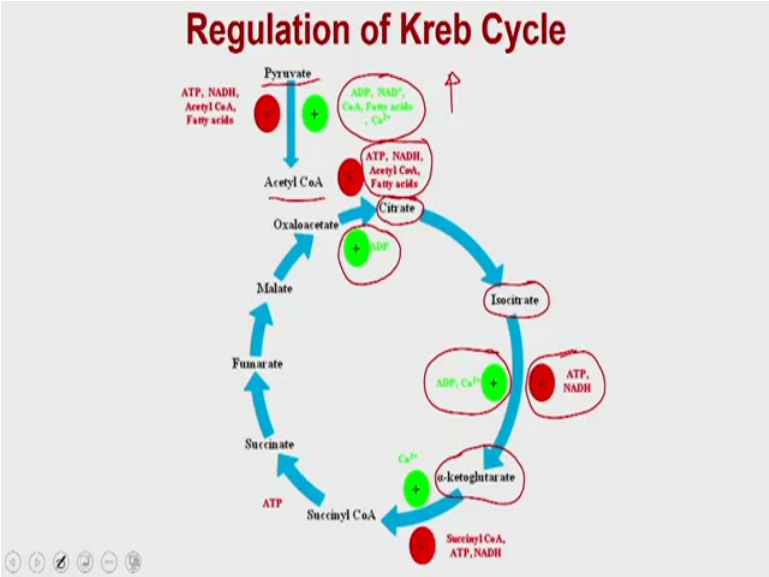

So, the Kreb cycle is always or mostly been regulated by a feedback mechanism, where the the the substrates such as for example for the first reaction you are converting the pyruvate to the acetyl CoA. In this cases the pyruvate, the ADP, NAD plus, Co enzyme, Fatty acid, calcium, these are going to be increased this activity. So, if there is an increase in the level of NAD plus or if there is an increase in the level of ADP, what this mean is that the cell is having the less energy. Which means you need to run the Kreb cycle to produce the energy.

So, that is why these factors are going to enhance the activity of or enhance the activity of the enzyme which will catalyse the conversion of pyruvate to acetyl CoA. Once the acetyl CoA

will get (con) will will combine with the oxaloacetate to to form the citrate, at this step also, the ADP, which will actually the significance of the loss of ATP or low level of ATP in the cell is going to enhance or (catla) will activate these reactions. Whereas, if you have the large quantity of ATP, NADH, acetyl CoA or the fatty acid, that is going to go (the) that is going to inhibit these reactions. Same is true for when you do the convergen of isocitrate to Alpha keto glutarate that time also ADP and calcium are going to be the positive modulator they will enhance the activity, whereas the ATP and NADH is going to inhibit or will going to do a inhibition of these activities. Same is true for the other step as well. So, the Kreb cycle is mostly been regulated by the feedback mechanism which if the ATP is being generated, you have large quantity of ATP, then the ATP will go and will go and inhibit the reactions.

But if the there is a large quantity of NAD or there is a large quantity of (AT) ADP in the cytosol, which will signify that the cell is undergoing the low-energy state, then that actually is going to up regulate the activities of different enzymes and that is how it is going to enhance the different reactions and that is how you are going to see the more activity or more Kreb cycle running.

So, let us continue our discussion about the Kreb cycle. And, what we were discussing that the Kreb cycle is under the tight control and where the various substrate as well as the products are causing the the feedback inhibitions. And the question comes, why the Kreb cycle is so much tightly controlled. The reason behind the tight control of Kreb cycle is that the Kreb cycle is central metabolic pathway. So, just after the glycolysis, which actually wear the glucose is being break down and get converted into the pyruvic acid, the pyruvic acid get enters into the mitochondria and then it actually enters into the central metabolic pathway which is actually called as the Kreb cycle.

So, in the Kreb cycle, the the the pyruvate which is being generated from the glycolysis is being is processed further and then several molecules of NADH, FADH 2, as well as the molecule of GTP is being generated. So, apart from the purpose of Kreb cycle to metabolise the carbohydrate molecules, to provide the energy in the form of reducing equivalent, whether it is the NADH or the FADH 2, the the purpose of Kreb cycle is also to coordinate with many other metabolic pathways. So, let us see how the Kreb cycle plays central role within the metabolism of an organism.

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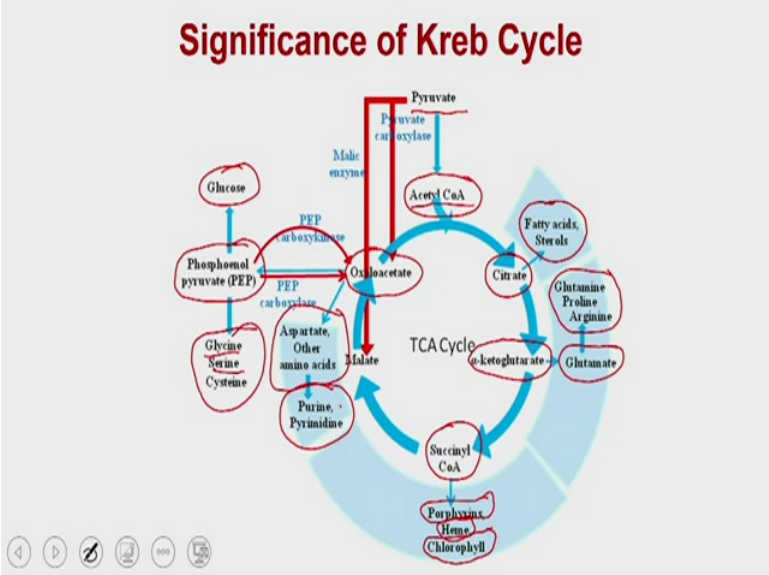

So, as you can see the Kreb cycle, it is receiving the pyruvate from the glycolysis and then the pyruvate is getting converted into acetyl CoA and this acetyl CoA is actually entering into the Kreb cycle and then it is getting processed (win) (with) with the help of several intermediates and with these intermediates it is producing the reducing equivalent as well as the free energy which is in the form of GTP. And what you can see now is that the oxaloacetate. So, oxaloacetate is being also being channelized with the phosphoenol pyruvate.

The phosphoenol pyruvate can be converted back to the glucose and it also can be used to produce the the different types of amino acids. And so as you can see the Kreb cycle is indirectly connected to the phosphoenol pyruvate or the carbohydrate metabolism as well as the amino acid biosynthesis pathway with the help of oxaloacetate. In the in a in a alternate pathway, the oxaloacetate is also being used to synthesise the the aspartate and other amino acid. And these amino acids, the carbohydrate which is present in these amino acids can be used even to synthesise the purine as well as the pyrimidine.

Which means the oxaloacetate is which is actually the part of the Kreb cycle is playing central role in connecting the Kreb cycle to the amino acid pathway, the nucleotide pathway and the as well as the resynthesis of glucose molecules. Similarly what you can see is the citrate, which is being synthesised in the Kreb cycle is utilised for the biosynthesis of fatty acid as well as the sterol. Similarly the Alpha keto glutarate, the Alpha keto glutarate is used to synthesise the glutamate and the glutamate is ultimately producing the different types of amino acids which are glutamine, proline and arginine.

And what you can see also is the succinyl CoA the succinyl CoA is another intermediate which is present in the Kreb cycle and that actually can be a precursor for synthesis of chlorophyll, Heme as well as the porphyrins. So, different types of iron containing proteins could be synthesised using the succinyl CoA, which is actually the Heme bound proteins. So, as you can see the the TCA cycle is is a central pathway, it is on one side it is converted to the amino acid pathway, on other side it is converted, it is linked to the it is linked to the nucleotide (bio) biosynthesis pathway and on the other hand it is (syn) it is linked to the fatty acid synthesis, Alpha keto glutarate is is linking the TCA cycle to the another type of amino acid synthesis and as well as the succinyl CoA is connecting the TCA cycle to the heme biosynthesis of heme or the porphyrins.

Which means that if there is a there is a requirement of these intermediates, like for example if there is a requirement of glycine or serine, it the Kreb cycle can donate or can utilise or can channelize some of these oxaloacetate molecule for the synthesis of glycine or the serine molecules. Similarly the other intermediate also can be used to to channelize the carbohydrate molecule which are present in the TCA cycle to some of these derivatives. Which means the Kreb cycle can actually regulate the different (in bio) different metabolic intermediates which are being generated in the metabolic which are being generated within the cell and it actually makes the tight control between the these intermediates.

And because of this important role which Kreb cycle play within the within the metabolism, the Kreb cycle is under tight control by all these intermediates and also as well as the intermediate which are being present in the Kreb cycle itself. But these are the situation happens and these are the things which Kreb cycle is doing, when the oxygen is present. Because Kreb cycle is producing the large quantity of NADH, one molecule of FADH and the GTP.

This NADH and FADH which are being generated in the Kreb cycle are need to be oxidised in the in the electron transport chain. So, all this NADH and FADH are going to go to the electron transport chain where the different types of electron receptors are going to receive these molecules and then they will, they will they are going to process the electron which have been, they are going to process the NADH or FADH 2 to generate the ATP. Which means and in this electron transport chain, the oxygen is required.

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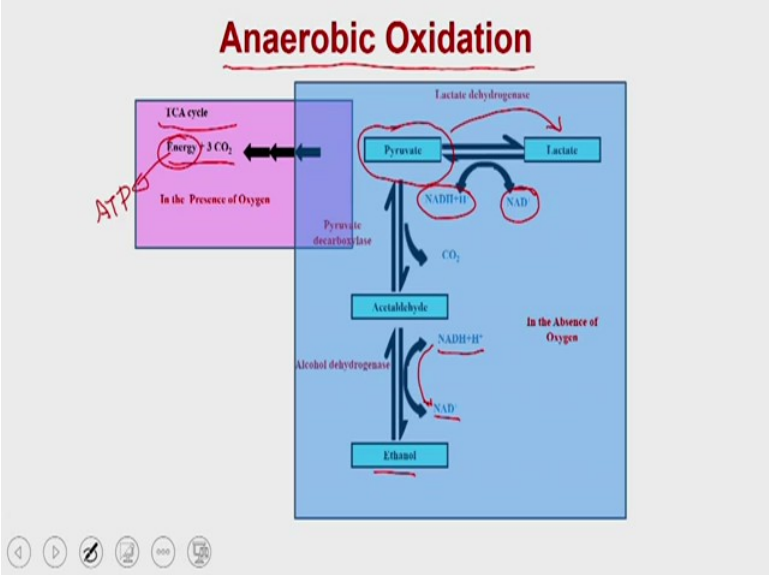

So as a result the if there is oxygen is present, the Kreb cycle will will run these intermediates and as well as there will be energy generation. But what will happen, there will be no oxygen in the environment or there is a low oxygen in the environment. So, as we discussed, if there is oxygen, the TCA cycle will run and it actually will produce the carbon dioxide and the energy which has been in the form of ATP. So, if oxygen is present, the TCA cycle will run, it will generate the 3 molecules of carbon dioxide and it will going to process the reducing equivalents to generate the ATP.

But once the oxygen is not present, the pyruvate which is being generated from the glycolysis is being channelized into two different pathways. In on pathway it is going to be converted into the lactate and in this process the NADH which has been generated by the cell is going to be utilised to generate the NAD+. In the other pathway where it utilises the two enzyme, one is called pyruvate decarboxylase, the other one is called as the alcohol dehydrogenase. It actually do the decarboxylation reaction first to generate the acetaldehyde from the pyruvate.

And then from the acetaldehyde it generates it generates the ethanol where a one molecule of NADH is being used and the NADH is getting converted into NAD+. So, let us see and this these pathway which are operating in the absence of oxygen are called as the Anaerobic Oxidation. Let us discuss these two reactions separately and understand what is the significance of anaerobic oxidation in the metabolic pathway.

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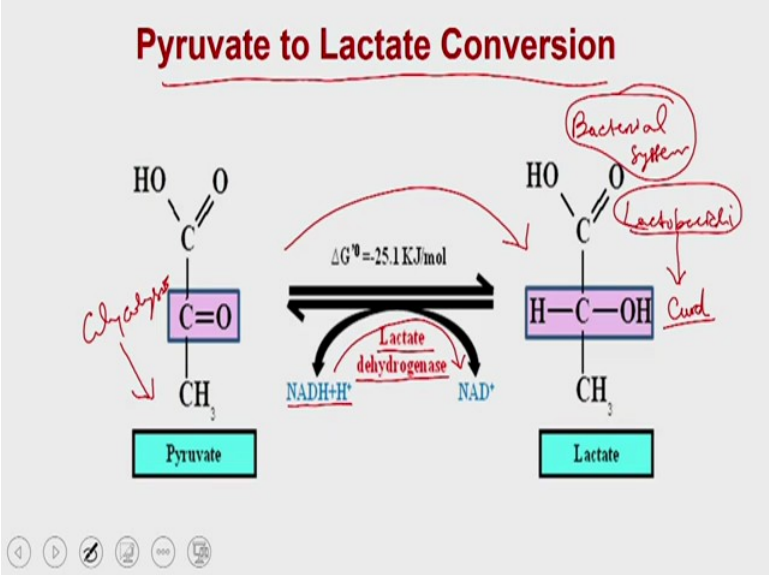

So, let us start first the first reaction which is the conversion of pyruvate to lactate. So, as as we said, this pyruvate is being generated from the glycolysis. So, in the from the glycolysis you have utilised the glucose molecule and then you have produced one molecule of pyruvate. In the absence of oxygen the pyruvate will not enter into the Kreb cycle, instead the pyruvate will get converted into the lactate or the lactic acid. This (path) and in this process the one molecule of NADH is being converted into the NAD+ and the enzyme which catalyses this reaction is called as the lactate dehydrogenase.

These kind of reactions are occurring in the mammalian cells as well as these reactions are occurring in the bacterial system or prokaryotic system. So, in the bacterial system, what happen is that when the bacteria is growing under the anaerobic conditions, it cannot utilise the it cannot utilise the the pyruvate into the downstream by metabolic pathways. And once the pyruvate accumulates into the cell, that actually drives this reactions and the pyruvate get converted into the lactate, lactic acid in the presence of lacto dehydrogenase.

One of the classical example of the bacterial species, which is utilising this reaction very optimally and (synthesi) (and) and producing the lactate or the lactic acid is called lactobacilli. These lactobacilli are the bacteria which are responsible for generation of curd. So, these are the things which any way we are going to discuss in a subsequent slide. Now let us go to the second reaction.

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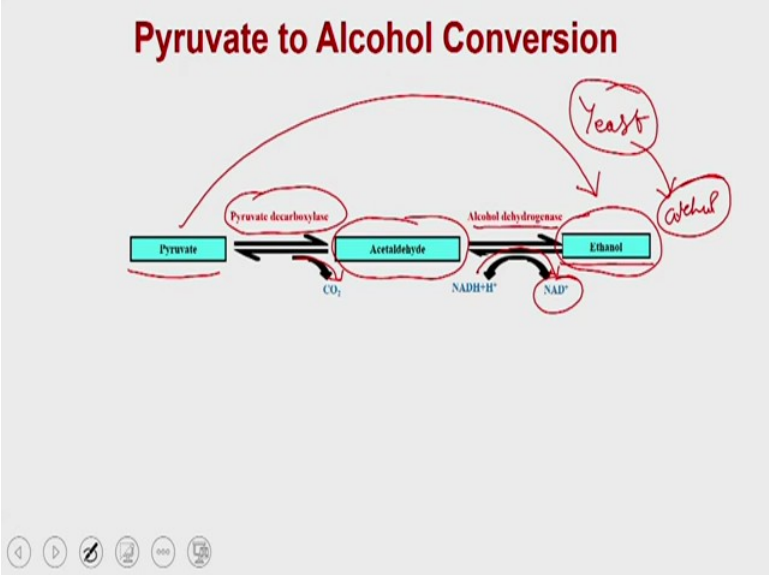

In the second reaction, the pyruvate is getting converted into the alcohol or the ethanol. In this in this you have the two different reactions which are coupled and the first reaction is being catalysed by the pyruvate decarboxylase. As a name suggest, it is actually going to remove one (oxi) one molecule of carbon dioxide from the pyruvate molecule and that actually will generate the one molecule of acetaldehyde and once acetaldehyde is being generated, then the acetaldehyde is being processed by the alcohol dehydrogenase and the one molecule of NADH is being used to produce the NAD+ and ultimately acetaldehyde is getting converted into the ethanol.

As so, in this process the pyruvate is getting converted into the ethanol. And these are the reactions which are very oftenly and occurring in the yeast and that is how the people are using the yeast to produce the alcohol. And this so let us see what what are the different intermediates or what are the different, what is the mechanism of the pyruvate to alcohol conversion.

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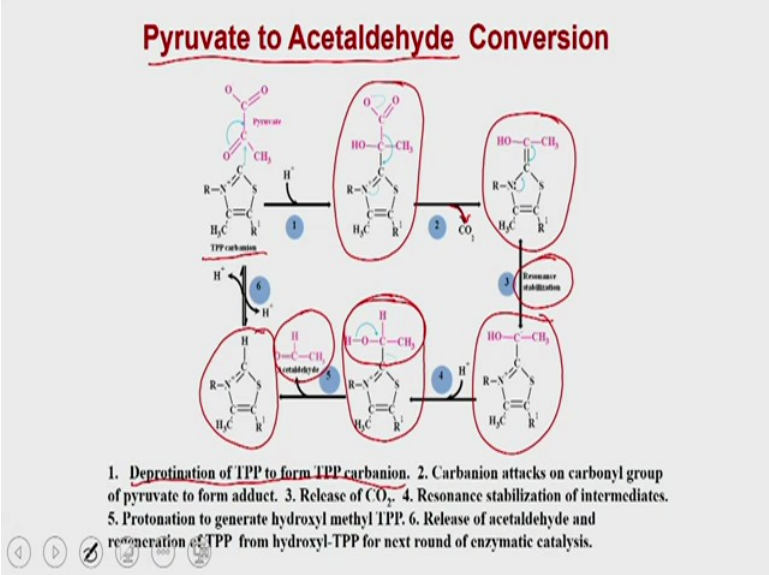

So, as I as I said, this is a two-step reaction, in the first step the pyruvate is getting converted into acetaldehyde. In this what will happen is the the the pyruvate decarboxylase has the TPP or the TPP as a as a cofactor. So, TPP is present in the form of a carboanaion and that actually binds this this molecule of the pyruvate. So, the first reaction is the deprotination of TPP to form the carbanion and that actually binds the the pyruvate, so it attacks on the pyruvate molecule and that is how you are going to have the the complex.

Once this complex is formed, the one molecule of carbon dioxide is being removed and that is how you are going to have the the the intermediate. This intermediate is then being is is being stabilised by a Resonance to generate this intermediate and ultimately in the step 4, (the) from this intermediate you are going to have the proto-nation to generate the methyl derivative of the TPP and at the end the acetaldehyde is being generated.

So, this acetaldehyde is being generated from this reaction and the TPP is being regenerated on the enzyme which actually catalyses the same reaction again and again. So, that is how the pyruvate is getting converted into the acetaldehyde, following these kind of complicated direction mechanism. Which is (the) and the reaction, (which) the enzyme which catalyses this reaction is called as the pyruvate decarboxylase.

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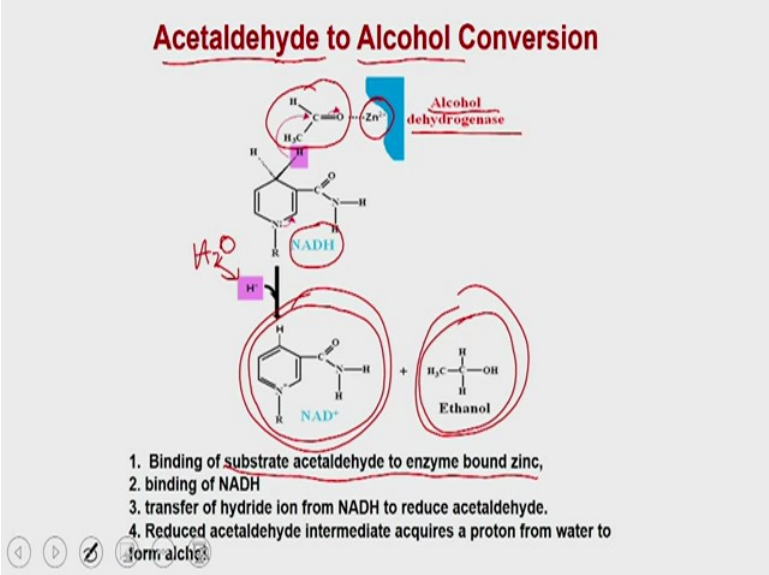

Now let us go to the next reaction. So, once acetaldehyde is being generated, it is getting converted into the ethanol or the alcohol. And the enzyme which catalyses this reaction is called as the alcohol dehydrogenase. So, alcohol dehydrogenase is a Zinc bound enzyme. So, in the in the in the in the alcohol dehydrogenase, the active side think is actually binding the your acetaldehyde and the it is taking up the, so in the first step what happen is the substrate acetaldehyde is binding to the enzyme bound Zinc with a coordinate bond and then the the NADH is also binding the enzyme in the second step and then in the third step there will be a transfer of hydride ion from the NADH to reduce the to reduce the acetaldehyde and once the acetaldehyde is reduced, it actually generates intermediate and that intermediate is getting the proton from the water molecules and the ultimately the NADH is getting converted into NAD+ and the acetaldehyde is getting converted into the ethanol.

So, let us recap again. The reaction of acetaldehyde to alcohol is generated is catalysed by the enzyme called alcohol dehydrogenase, which is a Zinc bound enzyme. So, the active side Zinc is in the first step, the active side zinc is binding the acetaldehyde and it is also binding the NADH and then there is a transfer of hydride ion , this transfer of hydride ion from the NADH to the acetaldehyde. And that actually generates a intermediate and then this intermediate is getting the proton or it is actually taking up the proton from the water and with the with the transfer of proton, it actually generates the alcohol as well as the NAD+ as well as the NAD.

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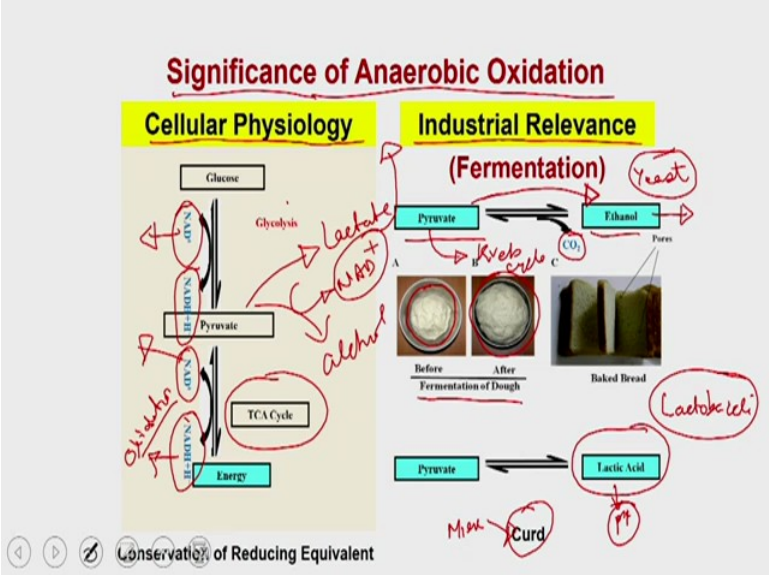

So, by doing this, pyruvate is getting converted into the alcohol. So, what is the significance of anaerobic oxidation? What you can see is that in the anaerobic oxidation, so anaerobic oxidation has the significance, both for driving the cellular physiology as well as it has the industrial relevance. So, let us discuss first the the (cellular) role in the cellular physiology. So, under the normal conditions or the under the (deox) oxygen in the presence of oxygen, the glucose is getting converted into the pyruvate and the pyruvate is getting converted into the energy with the help of TCA cycle.

Which means when (we) it is getting converted into energy, the NAD plus is getting converted into the NADH. Where and in the (presen) whereas in the in the glycolysis also, NADH is getting converted into NADH. But, when there is no oxygen, this NADH cannot go for the oxidation, which means there will be a shortage of the NAD+. And if there is a shortage of NAD+, the ultimately there will be an accumulation of NADH in the cell and if that happens, if that happens the NADH, the the the cell cannot produce the energy as well as cell cannot actually run many of the metabolic reactions where the NADH is required.

So, to avoid these circumstances, because you definitely need a pool of NAD+, so that you can actually run the metabolic reactions which are which are important for the cell apart from producing the energy. So, because of that, what happen is the cell is going with a with a loss-making loss making policies where the pyruvate is getting converted into the lactate and the pyruvate is getting converted into alcohol. And in these two processes, what is happening, you are actually generating the NAD+, because you are utilising the NADH which is already being preformed in the cell.

And because of that you are going to generate a pool of NAD+ and the this pool of NAD+ can be used for the metabolic reactions or alternate metabolic reactions where to the to the some extent until the oxygen is not available for the cell. And that actually helps to the cell to survive for a longer period of time and also it actually (which is) allows the cell to the to withstand the stress which are being regenerated in the in the absence of oxygen. Now, let us discuss about the industrial relevance or the industrial importance of this anaerobic oxidation.

In the first step the pyruvate is getting converted into ethanol with the help of the carbon dioxide. So, there will be a removal of carbon dioxide. So, the the classical example in this case is the yeast. We have already discussed that this is this (pro) this reaction is very very very up regulated in the case of yeast. So, that can be used for two purposes, one which we have already discussed that if you trying to make the bread, what you have to do is you have you take the small amount of dough and then mix it along with the yeast molecules or the yeast organism.

So what will happen is because it is it dough and there is anaerobic condition inside the dough, the yeast will run its glycolysis to produce the pyruvate but it cannot mobilise that pyruvate to (gen) to (gen) to generate the energy in the Kreb cycle. Instead it will (u) it will run this reaction which means it will run the pyruvate to alcohol convergen and in this process it is going to produce the carbon dioxide. And once the carbon dioxide is trapped inside the dough, it will make the dough is very fluffy and that is how you are going to have the bread which is having the pores.

In the alternate pathway, you can also you can also convert the pyruvate into the alcohol and that alcohol can be used for producing the different types of beverages. Similarly, in other pathway the when the pyruvate is getting converted into the lactic acid, this is very often found in the case of another bacteria which is called as the lactobacilli. So, in the case of lactobacilli, you are actually converting the pyruvate to the lactic acid and lactic acid is actually reducing the pH of media. And because of that it actually converts the milk to the curd and that is how the lactic acid bacteria or lactobacilli has a relevance in converting the milk to curd and production of the curd.

So, the the anaerobic oxidation is having a very very huge industrial relevance. We have only discussed the two examples, one is the production of ethanol or the production of the bread, the other one is the production of curd. But apart from these two pathways, there are many many pathways which are actually being found in many other different organisms to generate

the different types of metabolite and these metabolites have a very high economical values in the in the in the industrial context.

So, what we have discussed so far, we have discussed about the cellular metabolism in the in the in the (eu) in the mammalian cells and what we have also discussed the control as well as the relevance of these metabolic pathways in terms of driving the different types of reactions as well as the in terms of running the life cycle of the particular organism. We have also discussed about the anaerobic oxidation and its relevance in sustaining the life of the organism and at the end we have also discussed about the industrial relevance or the physiological relevance of these anaerobic pathways.

So, with this we would like to conclude the lecture here and we we will continue our discussion about the growth media as well as the different types of nutrient media which people use to grow the prokaryotic cells as well as the eukaryotic cell in the subsequent lectures. Thank you.