Metabolic Engineering
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Lecture - 09
Review of Cellular Metabolism - Part D

In today's lecture on metabolic engineering, we are going to continue our discussion on the reviewing the cellular metabolism.

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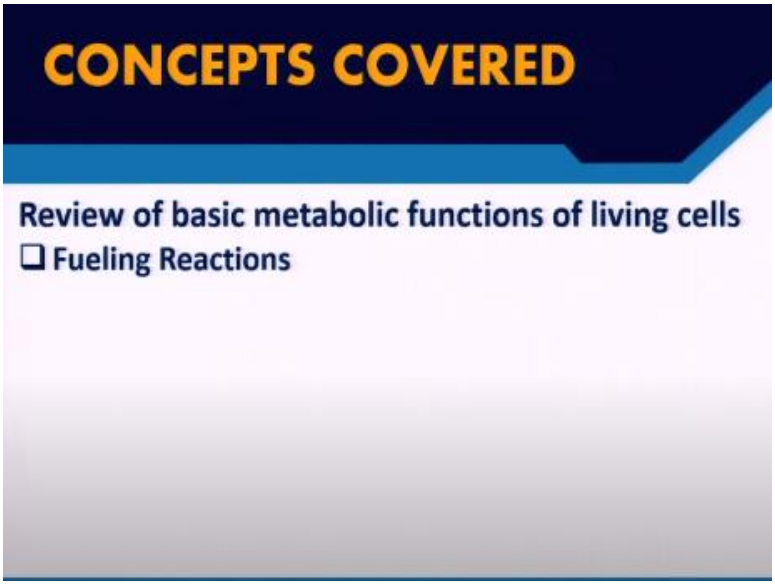

In today's lecture fueling reactions will be discussed.

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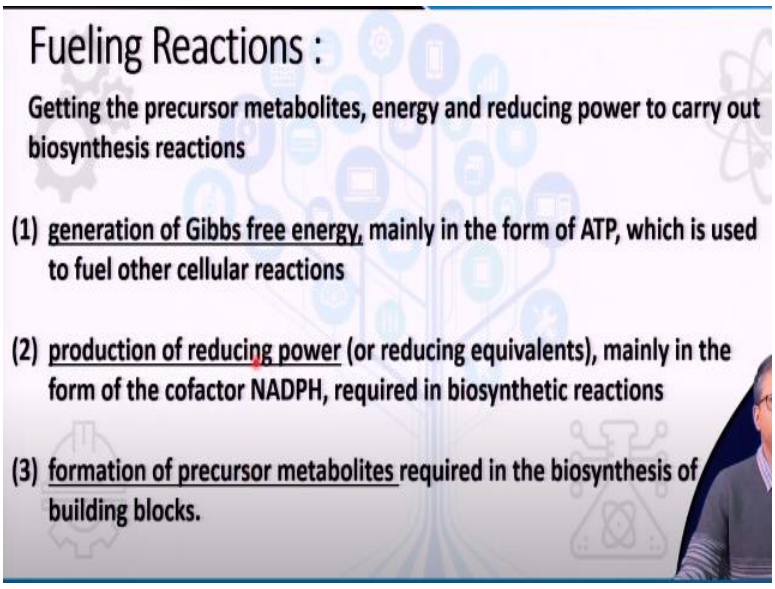

Now, what are these fueling reactions? Fueling reactions are those biochemical pathways which enable the cell to produce the precursor metabolites for all the building blocks synthesis and then synthesis of the macromolecules from the building blocks, the required free energy and the reducing power to carry out all the biosynthetic reactions.

Now categorically, these fueling reactions are considered to be having the following very important outputs. Firstly, the generation of Gibbs free energy mainly in the form of ATP or adenosine triphosphate which is used to fuel all other cellular reactions. The second one is the production of reducing power or reducing equivalents that is mainly used for all the biosynthetic reactions or electron requiring reactions and the production of the cofactors like NADPH or NADH or FADH2 are performed.

The third major type of reactions are the formation of the precursor metabolites, which are the small intermediate products of fueling reactions or products of these biochemical reactions which are required in the biosynthesis of the building blocks. So essentially, as we have also discussed in our earlier lectures, that in the cellular metabolic processes, these fueling reactions are the fundamental types of reactions which provide the all necessary resources and raw materials and energy.

So the raw materials are the small carbon metabolites which are to be used for making the building blocks and the reducing power that is the electrons which are necessary for synthesizing all complex molecules and also the energy which is required to perform all the energy seeking reactions.

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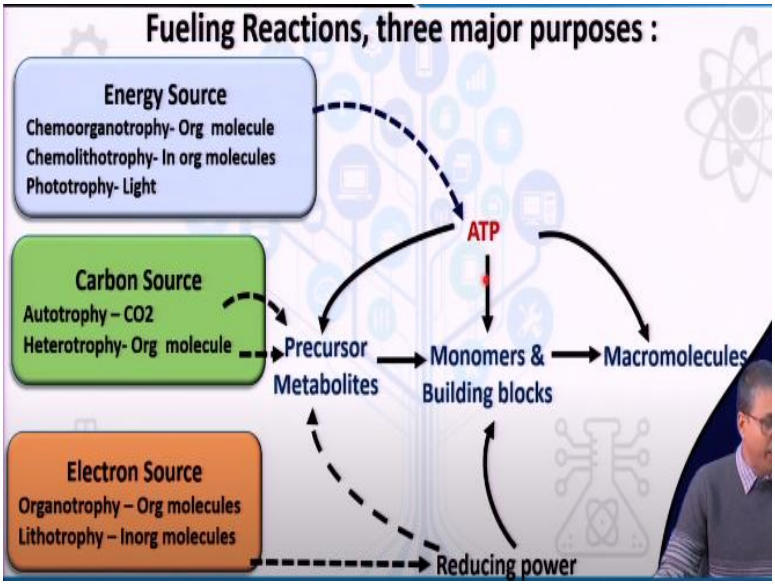

Now, there are three major purposes of this fuel reaction. The first one is the type of energy sources which are available to the cellular systems are utilized through a set of fueling reactions and there could be organic molecules providing the energy or there could be inorganic molecules providing the energy or there could be light for phototrophic organisms.

So there are organisms like different type of algae and other bacterial species who might be requiring light as a source of energy. So considering different suitable energy sources, we know that these organism's metabolic properties are very well classified as chemoorganotrophy or chemolithotrophy or phototrophy.

So basic objective of utilizing these energy sources through the fueling reactions are to produce the Gibbs free energy or the ATP. The second major purpose is to facilitate the use of different carbon source because the carbon source is the fundamental carbon source material which is supposed to provide all the carbon backbone of the molecules.

So again, there we can see that either inorganic carbon like carbon dioxide can be used by the autotrophic organism or the organisms which carry out this type of metabolism, we call them autotroph or it is called autotrophy. Organic molecules can similarly be used by all the heterotrophs or this type of metabolism is known as heterotrophy.

So basic objective of this type of reactions or the fueling reactions, which enable the cells to utilize the different type of carbon sources are to produce the precursor metabolites. So in our earlier lectures, we have seen that how a diverse array of substrates which are available to the cellular system, no matter whether those substrates are inorganic or organic, they are all converted to the required state of precursor metabolites.

And these precursor metabolites are very well defined as the major 12 metabolites we have identified in the biochemical reaction. So the fueling reactions, they enable the cells to produce the major 12 or so precursor metabolites. The third type of reactions which are there, these are called the utilization of energy sources.

So cells must be requiring energy for carrying out all the biosynthesis reaction wherever a simple molecule or a oxidized compound is converted to a reduced compound that what we see in case of the anabolic reactions or anabolism the electron sources are must. So there could be again organic molecules which could provide the electrons.

And there could be inorganic molecules, many microorganisms can utilize a number of inorganic molecules to as a source of their electrons and those organisms are considered to having the metabolism, which is called the lithotrophic metabolism. So the basic objective of this utilizing these electron sources are to produce the reducing power which we know that this reducing power that means, it is the production of the NADH or the NADPH or the FADH2.

So all these are basically the reducing power. That means, they contain the electrons within them and these electrons can be provided to any reactions which are requiring these electrons. So now, the ATPs or the Gibbs free energy is coming from the energy sources. The precursor metabolites are coming from the carbon sources. And the reducing powers are coming from the electron sources.

Now, if we look into the framework of the basic metabolic pathways as we have seen earlier, so these precursor molecules are metabolites which are derived as the products or as the intermediate of the fueling reactions where different carbon sources are used

as a substrate. They are reduced to produce different type of monomers and building blocks.

And these building blocks are eventually converted, assembled into polymerized into the macromolecules. Now, the utilization of ATP and the reducing power are involved in both the cases like conversion of the precursor molecules to the monomers or building blocks and from the monomers and building blocks to the macromolecules we need these ATP and as well as the reducing power.

Why we need energy and reducing power because these monomers or building blocks and the macromolecules are more reduced and more complex than the precursor metabolite. So in order to reduce them, we need the source of electrons or we need to provide electrons into them and these reactions are actually not thermodynamically favorable.

So they are energy requiring reactions. So we need to provide external energy in the form of ATP in order to facilitate them.

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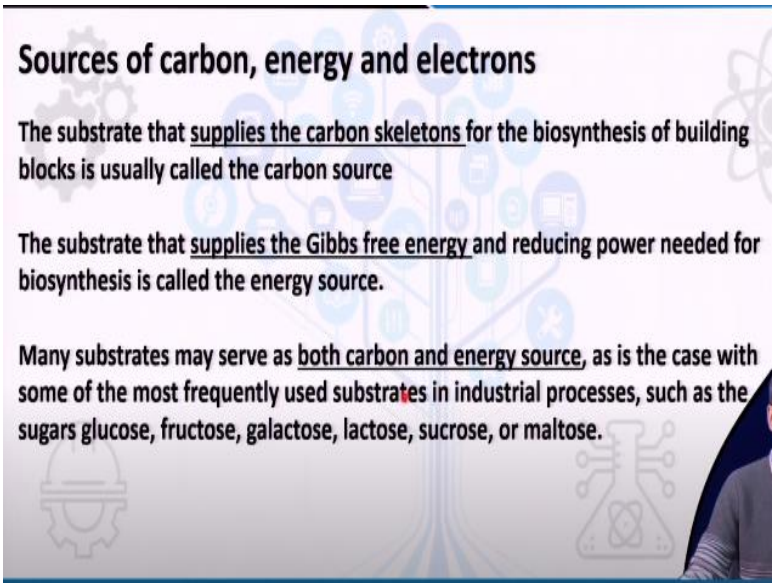

Now this source of carbon, energy and electrons can be obtained or be supplied by individual molecules. So there are events where we see that the substrate that supplies the carbon sources for the biosynthesis is called the carbon source. Similarly, the Gibbs free energy providing molecules are called the energy source. And there are many substrates.

There are many substrates like the glucose molecule for example, or most of the organic compounds. They serve both purposes like they are the substrate for the carbon source. So that means, they are the carbon substrate as well as they are the energy substrate because when we oxidize those glucose molecule or organic carbon molecules, we can or the cells can obtain the energy also.

So in case of heterotrophic microorganism or heterotrophic cellular metabolism, both carbon and energy can be obtained from the same substrate or similar type of organic substances.

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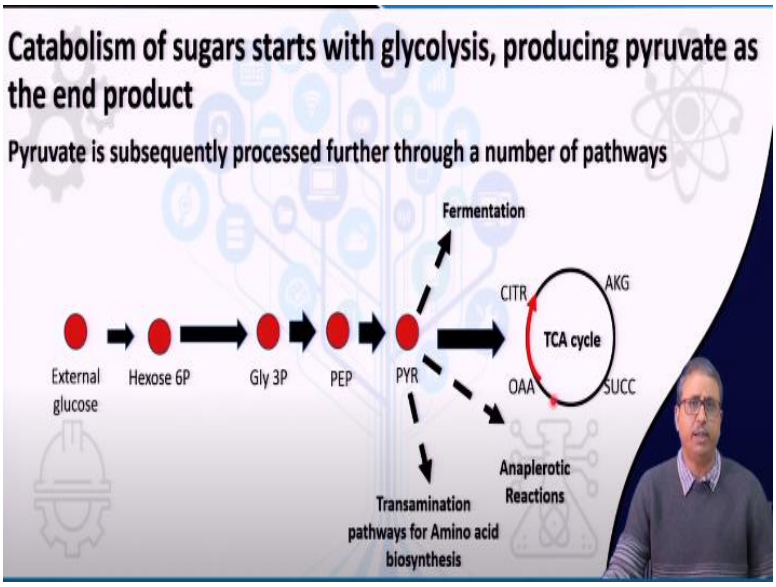

Now let us look at the catabolism of sugars. So the catabolism of sugar or the energy harvesting reactions of the sugar or organic molecule starts with the glycolysis. So that is conventionally referred as glycolysis that means, the sugar molecule is broken down or splitted into smaller molecules, okay. So this is all of the fundamental types of the fueling reactions that we are going to discuss briefly now.

Now this glycolysis reactions which are basically lysis of the carbohydrate or the sugar molecules they produce pyruvate as the end product. So the external glucose or the carbon sugar molecule which is taken up by the cell. So this is the hexose sugar phosphate which is present inside the cell and this is the external glucose molecule.

So once the sugar molecule is internalized by using different transport mechanism, they are oxidized through a set of reactions and we know these reaction sets are well known as the glycolytic reactions. So the glycolytic reactions they enable the formation of pyruvic acid over here from phosphoenolpyruvic acid to pyruvic acid.

So pyruvic acid is considered to be one of the major products of the glycolytic reaction. So there are number of glycolytic reactions that we are going to see now. But eventually, one point is common in all the glycolytic reactions or all the glycolytic pathways is that they all convert hexose sugar into pyruvic acid or pyruvate molecules.

Now pyruvate or pyruvic acid molecule which is produced by the oxidative metabolism of the hexose sugar is subsequently processed further through a number of pathways. So number of pathways means so once this pyruvic acid is produced out of this glycolytic reactions, as I mentioned there are more than one glycolytic reactions that we are going to talk today.

So this pyruvic acid is eventually processed either by the citric acid cycle where it will be reacting with the oxaloacetic acid and producing the citric acid and then alpha-ketoglutaric acid and succinic acid eventually malate and again oxaloacetate will be produced and lot of oxidation events will take place.

So almost all the carbons of the pyruvic acid will be released and lot of NADH H+ that is the energy is going to be produced out of this TCA cycle. It is a huge energy generating process for the cellular metabolic. Alternatively, this pyruvic acid can be utilized by fermentative pathway. So they lead to the fermentation process. So a number of metabolites like lactic acid, formic acid, acetic acid etc., they are all produced through this fermentative reaction.

This pyruvic acid is also transaminated and eventually lead to the leads to the production of the amino acids and amino acid biosynthesis is connected to this pyruvic acid. Now there is another very interesting type of pathways which are called anaplerotic reactions, which are basically the reactions which enable the

replenishment of the metabolites which are lost or used up by other biosynthesis reaction.

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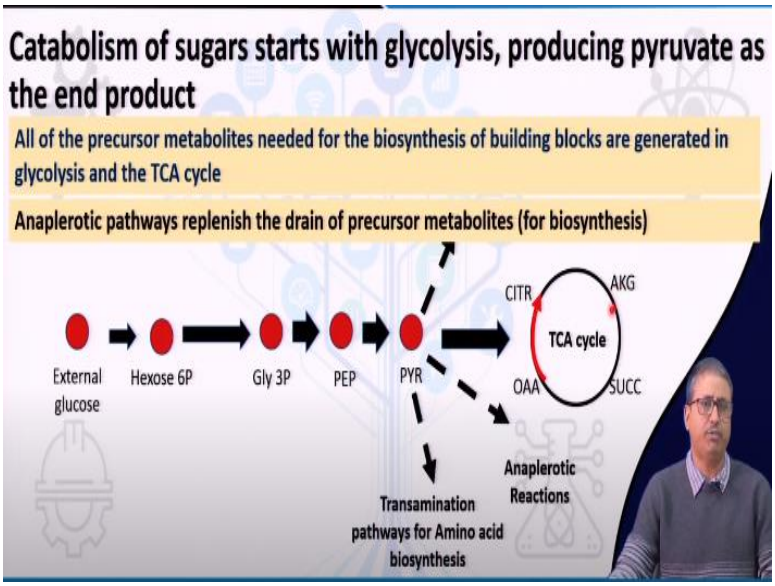

So all of these precursor metabolites needed to be used in the biosynthesis of building blocks are generated in glycolysis and the TCA cycle. Now before we talk briefly about the anaplerotic reaction, one very fundamental property or fundamental aspect of this glycolytic reaction, so no matter what type of glycolytic reaction it is, there are I said there are three types of glycolytic reactions that we are going to see today.

But all these glycolytic reactions are responsible for producing not only pyruvic acid, but also the intermediates. Either these intermediates are phosphoenolpyruvate or glyceraldehyde 3-phosphate or other intermediates that we will be seeing in pentose phosphate pathway would be the essential precursor molecules for biosynthesis of building blocks.

So almost all the 12 precursor molecules that we have seen earlier are produced from these the glycolytic reactions and the connected reactions like the TCA cycle and other glycolytic reactions, which are we are going to see now. Now anaplerotic reactions are those reactions which are responsible for replenishing the drain of precursor metabolites for biosynthesis.

So as we know or we can understand that there are many steps like the alpha-ketoglutaric acid or oxaloacetic acid or succinic acid. So there are other reactions,

which are trying to or will be trying to utilize these alpha-ketoglutarate or succinic acid as they are substrates. So eventually when the metabolism is going on in a full swing, so basically these precursor generation, energy generation process and the anabolic processes are going on when the cell is actually actively growing.

So what is expected, these precursor metabolites or the intermediates are withdrawing from the system. So they are continuously being taken by the different biosynthetic pathways. So under that circumstance when the metabolites are continuously being removed by the other biosynthetic reactions, who are actually utilizing these intermediates as their substrates, so replenishment of these products are required.

So cell has evolved a very interesting set of reactions, which are called anaplerotic reactions and these anaplerotic reaction help the cell metabolism to replenish those used of intermediate so that the overall metabolism process is not slowed down. So basically the anabolic reaction should match with the catabolic reaction or the energy generating reaction.

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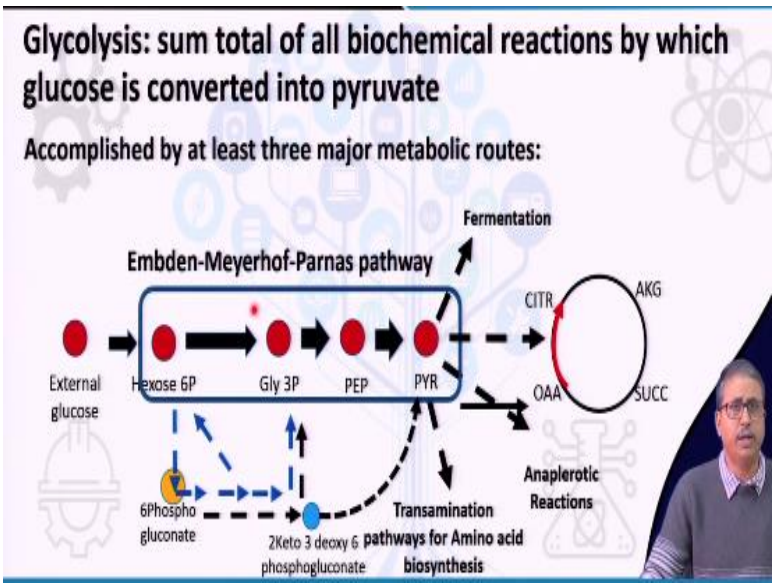

Now as we have understood that glycolysis is basically the basic carbon of the sugar metabolizing reaction and which is considered to be the sum total of all biochemical reactions by which glucose is converted into pyruvate. So this is a kind of a very general type of definition or we try to actually simplify that what is glycolysis.

The glycolysis is basically the sum total of all biochemical reactions by which the glucose can be converted into pyruvic acid. Now, this is accomplished by at least three major metabolic routes. So as I mentioned earlier that there could be at least three glycolytic pathways. So the first one that we generally discuss or we see on our screen is the Embden-Meyerhof-Parnas pathway, which is very commonly known as EMP pathway.

This EMP pathway could be a very major pathway in many of the prokaryotic and eukaryotic cellular system, where basically the external glucose is converted to the hexose sugar and then subsequently pyruvic acid is produced through this glyceraldehyde 3-phosphate and phosphoenol pyruvic acid. Lots of ATP molecule and the NADH H+ is also produced.

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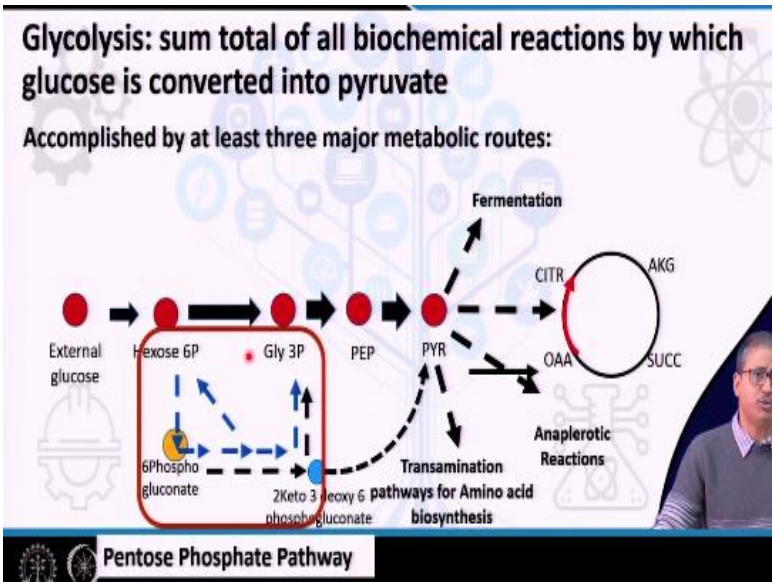

Now along with Embden-Meyerhof-Parnas pathway, the second alternative pathway is or the connected pathway is the pentose phosphate pathway. So this pentose phosphate pathway, we are going to talk or discuss about this pentose phosphate pathway, which slightly bypasses the some the reactions and it takes the some form of the hexose sugar and then the hexose glucose molecule is converted to phosphogluconate.

And the phosphogluconate is converted to a number of pentose sugars and along with that erythrose 4-carbon sugar and 7-carbon sugars and eventually, they are able to convert these sugar molecules into again back to hexose sugar or 3-carbon

glyceraldehyde 3-phosphate. So it is something like a small loop reaction or a side reactions which are having lot of different type of enzymes and different type of reactions.

And these reactions are highly controlled during the normal cellular metabolism. That we are going to study or discuss today.

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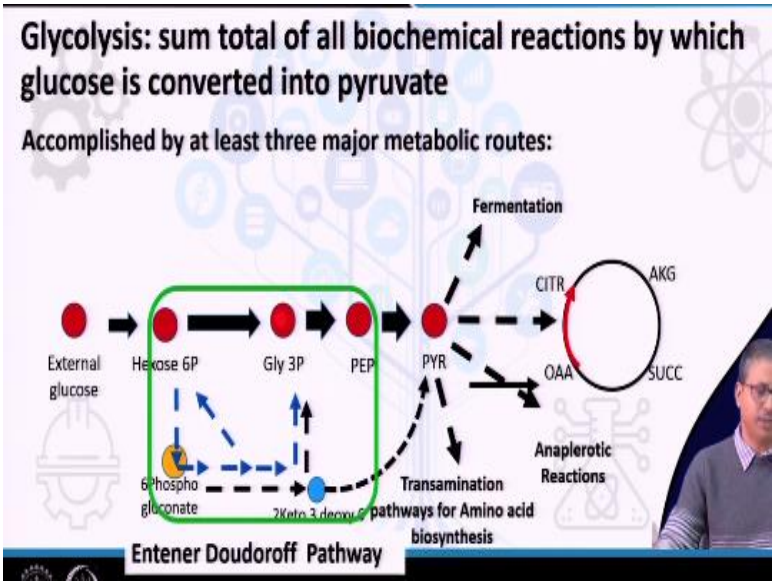

Now along with this pentose phosphate pathway, there is another third type of reaction which is common in many type of Gram negative bacteria particularly the Pseudomonas type of strains. We see that the ED pathway, okay Entener Doudoroff pathway.

This Entener Doudoroff pathway has some overlap with the pentose phosphate pathway, because this pathway also utilizes phosphogluconate and then the phosphogluconate is converted to KDPG and the KDPG is broken down into glyceraldehyde 3-phosphate and subsequently one molecular pyruvate is also produced.

So these all these three pathways may be there simultaneously occurring in a particular system or they may be having alternative operations or there may be cases where one only one of the pathways are operating. It all depends on different organisms and their requirement. Because as we will see all these three types of

glycolytic mechanisms or glycolytic processes, they have their own advantages. So that we will be discussing very shortly.

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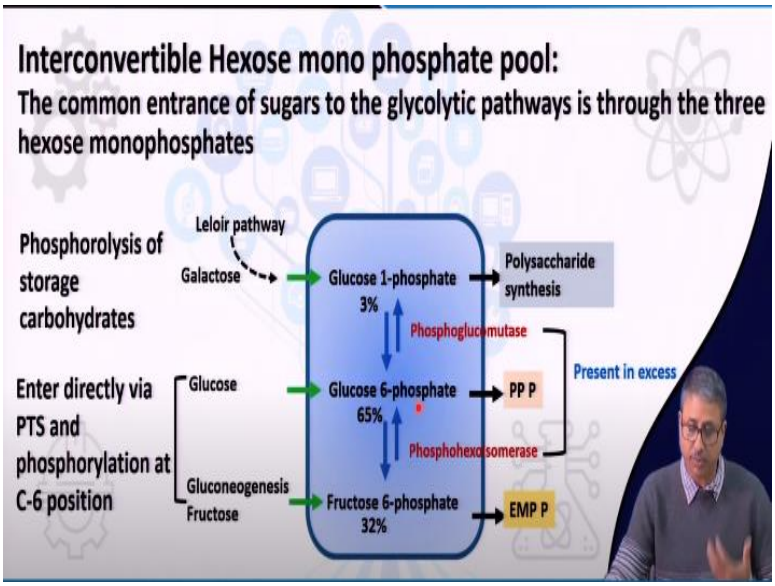

Now the next interesting point is the formation of the hexose monophosphate pool. As we understand that the glucose molecule or the sugar molecule, which is the glucose or glucose equivalent molecules like the galactose or fructose, these sugar molecules are taken up or internalized through different transport system inside the cell.

And as soon as they enter inside the cell they are converted into a phosphate compound like as we know through the glucose is often converted or brought in the cell. All the galactose is brought in the cell through the PTS system. In the last class we have talked about that. So through PTS system when this the sugar molecules are taken inside the cell or transported inside the cell, they are phosphorylated.

Otherwise, by spending an additional ATP molecule the sugar molecule can be converted to like in eukaryotic cell we see that there is a specific enzyme called hexokinase which is responsible for converting glucose into glucose 6-phosphate by spending or utilizing one mole of ATP. Now the common entrance of sugar to the glycolytic pathway is through this 3-hexose monophosphates.

Now there are three types of hexose monophosphates, these are all monophosphates, because they consist of only one phosphate groups. Because in the cellular system we see there are also bisphosphates or maybe diphosphates are also there where within a

carbon backbone or sugar backbone there are more than one phosphate residues are attached. But we are not talking about them.

We are talking about the monophosphate sugar molecules. Now these monophosphate sugar molecules together they represent a common pool of hexose 1-phosphate, hexose monophosphate. And interestingly, any kind of sugars whether it is a storage carbohydrate or it is the glucose molecule, which is available in the outside whenever they are entering into the cellular system.

For example, the galactose might enter into the cell through direct transfer and then the Leloir pathway will convert the galactose into glucose 1-phosphate by phosphorylating in the carbon one position. Or if the glucose molecule is coming inside the cell through direct or transport process like the through the PTS or the phosphorylation at C6 position happens, mostly as I mentioned the glucose is transported inside the cell through PTS system.

Galactose is also transported through PTS system, but we will see the galactose is converted to glucose 1-phosphate mostly when it is transported through Leloir pathway. But in case of glucose, it is the carbon 6 position which is phosphorylated. Similarly, fructose can also be converted to fructose 6-phosphate by specific reactions.

Now the interesting point about this that these three sugar monophosphates are individually responsible for producing different or providing the carbon resource for the individual biosynthetic pathway like the glucose 1-phosphate is responsible for the polysaccharide synthesis, whereas the glucose 6-phosphate is represented is basically responsible for the main pentose phosphate pathway.

And when it is converted to fructose 6-phosphate it is responsible for the Embden-Meyerhof-Parnas pathway. So and ED pathway also. So the essentially it is not the fact that they are responsible for providing the precursor molecule or providing the resources carbon backbone for building synthesis of the building blocks for different other biosynthetic reaction.

The point of interest over here is that these three sugar monophosphates are interconvertible. Interconvertible by virtue of two enzymes, which are the phosphoglucomutase, which basically converts the glucose 1-phosphate to glucose 6-phosphate and this is a reversible reaction. Similarly phosphohexoisomerase this enzyme is responsible for the reversible conversion of glucose 6-phosphate to fructose 6-phosphate.

Now, the interesting point is continued by the fact that these two enzymes are mostly present in excess. So in a cellular system, we often observe that these three these two enzymes are often is excess. And these three hexose monophosphates are actually in equilibrium, okay. As they are in equilibrium that means, from any kind of substrates like whether it is provided, whether the cell is provided with galactose or provided with glucose or provided with fructose, the cell is able to metabolize any of the required thing.

So no matter whether the cell is fed with galactose or cell is fed with glucose or cell is fed with fructose, the cell will produce anything that the downstream reactions would require. And under this or at this equilibrium situation, the concentration of these or the relative concentration of these three monophosphates are little different like in glucose 6-phosphate it is around 65%, fructose 6-phosphate is 32% and glucose 1-monophosphate it is 3%.

So under during the equilibrium state, we see that the concentration regime is like this, glucose 6-phosphate is relatively higher. But anytime these reactions are reversible.

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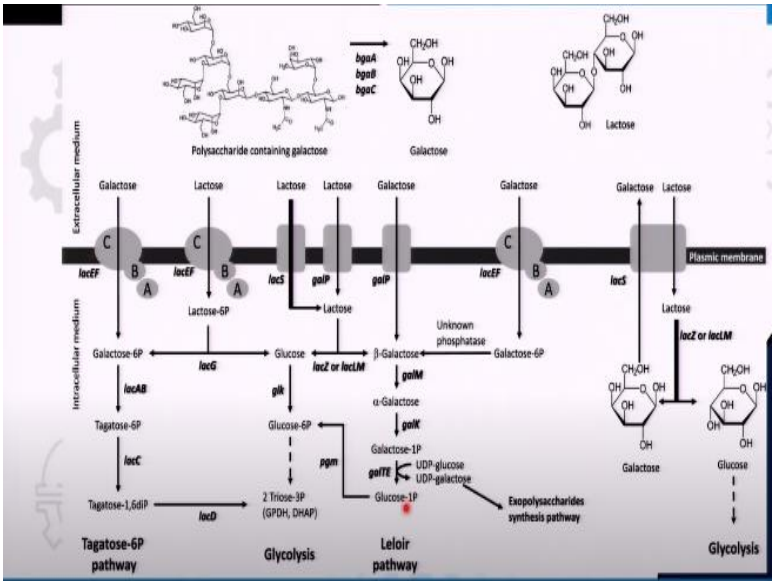

Now this is very, little bit complicated, but this diagram represent the galactose transport and how the external galactose can be taken up inside the cell by direct transporter and then converted to glucose 1-phosphate, that is the Leloir pathway. Alternatively, the galactose can be also transported through PTS and when it is transported through PTS, it is mostly phosphorylated at the 6 carbon position.

And in that case, generally it is converted to triosephosphate. But it can be converted to again the glucose and glucose to beta galactose and then essentially from beta galactose to glucose 1-phosphate can be produced. So essentially, it means that even if the galactose is transported by either the direct transport or the PTS based transport, the glucose 1-phosphate can be produced.

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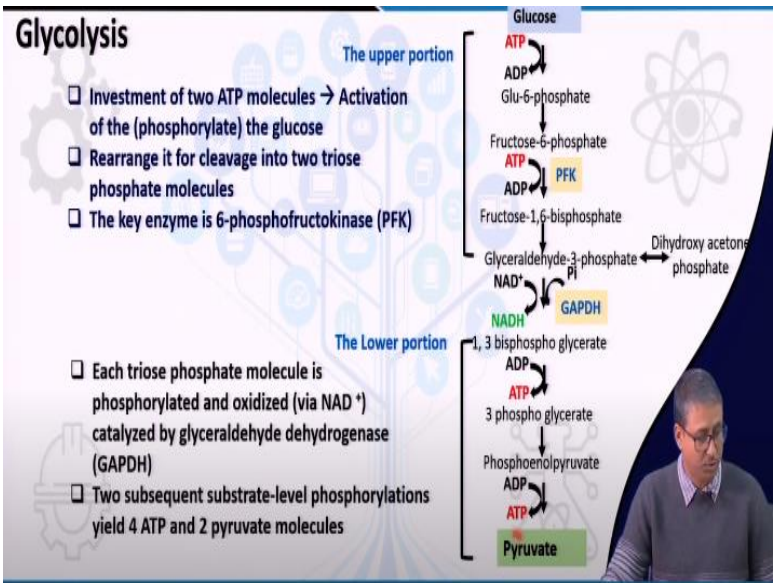

Now let us look at the basic structure of the glycolysis which is basically the Embden-Meyerhof-Parnas pathway or EMP pathway. As we can notice here, so glucose is energized to produce the glucose 6-phosphate with the expenditure of ATP and then isomerization reaction helps to convert the glucose 6-phosphate to fructose 6-phosphate. The second the energy requiring step again activates the fructose 6-phosphate into fructose 1,6 bisphosphate.

And then this actually undergoes the reaction where it is splitted into 2,3 carbon molecules the glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Now subsequently, these are the glyceraldehyde 3 phosphates are oxidized and two substrate level phosphorylation events happen and pyruvic acid, two moles of pyruvic acid are produced from one moles of one mole of glucose.

Now there are actually two very interesting part of this entire Embden-Meyerhof-Parnas pathway. It is that the upper portion and the lower portion. The upper portion is basically the reactions where we see the investment of the ATP molecules particularly if we see these reaction, investment of two ATP molecules are visible. And these investment or the expenditure of ATP molecules are basically to activate the glucose molecule.

The second event is the rearrangement of these activated sugar molecules which is the fructose 1,6 bisphosphate so that it is now cleaved into 2,3 carbon compound. And one of the key enzymes in this set of reaction that is the upper portion is the phosphofructokinase PFK. We will discuss about the role of PFK in flux distribution afterwards.

In the lower portion of the reactions, each of these three triosephosphate we call the three carbon compounds glyceraldehyde 3-phosphate or dihydroxyacetone phosphate. And we know that dihydroxyacetone phosphate are interconvertible. So these two compounds are interconvertible.

And they undergo the phosphorylation and oxidation and they eventually produce the NADH which is the reducing power and the 1,3 bisphosphoglycerate molecule which is having two phosphate group because of the incorporation of one phosphate group

form here. And then two subsequent substrate level phosphorylation reactions happen because these 1, 3 bisphosphoglycerate is highly energized and it is ready to be oxidized and ready to be giving the energy.

So eventually this bisphosphoglycerate is converted to 3-phosphoglycerate and then from them to phosphoenolpyruvate and phosphoenolpyruvate undergoes the second substrate level phosphorylation to produce the pyruvic acid molecule.

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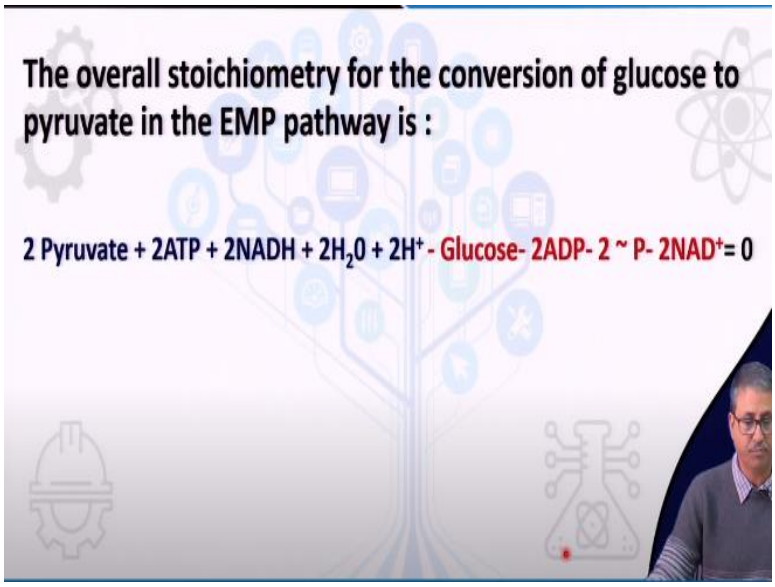

And this is the overall stoichiometry of the reaction.

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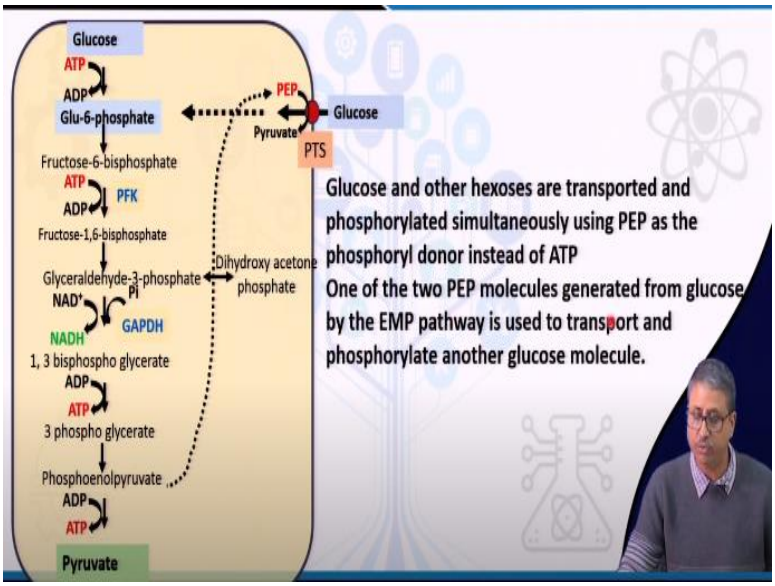

Now there are two interesting aspects of this EMP pathway with respect to metabolic engineering or in order to improve or in order to work with this EMP pathway or in

order to work with the glycolysis in general. Now although we considered in a previous discussion that two moles of ATPs are required to activate the glucose molecule, in case of prokaryotic system, glucose and other hexoses are transported and phosphorylated simultaneously using the PEP as the phosphoryl donor instead of ATP.

So we have seen the group translocation mode of transport where PTS type of transporters are involved in our earlier lecture. And where we have observed that phosphoenolpyruvate acts as the high energy compound and donates the phosphate group, while the glucose is transported or the sugar is transported inside the cell. So whether it is glucose or its mannose or it is galactose, they are internalized in the form of glucose phosphate itself.

So when it comes inside the cell, it is the glucose 6-phosphate. So directly the ATP expenditure is saved. So in case of prokaryotes, actually this expenditure is not there. However, one mole of PEP is used. Now if we look at this PEP, which is phosphoenolpyruvate, during the subsequent steps of the breakdown of the glyceraldehyde 3-phosphate, the triose sugar, where the oxidation and then substrate level phosphorylation events occur, we can see that there is a formation of phosphoenolpyruvate over here before the second substrate level phosphorylation.

So we need to understand that this phosphoenolpyruvate is a high energy compound. And if it is processed appropriately like the phosphoenolpyruvate kinase, then it can actually donate the energy in the form of another ATP molecule and then pyruvic acid can be produced. But if we take this phosphoenolpyruvate to be utilized in the transport of glucose, then possibly we miss out one ATP generation over here, okay.

So one of the two PEP molecules generated from glucose by the EMP pathway is used to transport and phosphorylate another glucose molecule.

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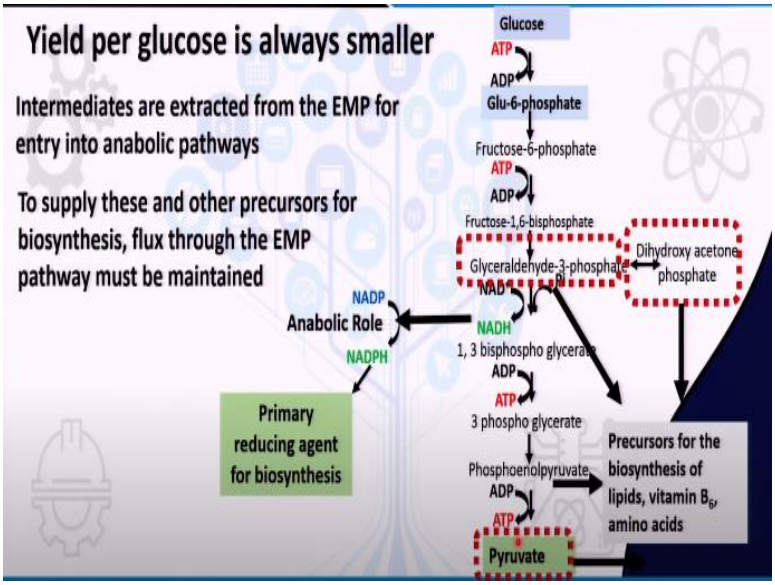

So thereby, the actual calculation of the energy generation or ATP generation might be varying depending upon how much glucose is transported by PTS. And the yield per glucose is not only constrained by the energetics of the overall process, because the amount of energy that is the ATP is, how much ATP is produced, is under the control of how glucose is transported inside the cell. But also the yield per glucose is always smaller, okay.

Now if we look at this pathway, which is the Embden-Meyerhof-Parnas pathway, now intermediate, there are a number of intermediates. We have been talking about this precursor synthesis of the metabolites which are used for synthesis of different building blocks. So here we can see that a number of intermediates including the dihydroxyacetone phosphate, glyceraldehyde three phosphate or even the pyruvic acid, they can be extracted from the main pathway of EMP.

That is why the thick arrows are indicating that any point of time the cell is feeling that they need more biosynthesis reactions, because often the biosynthesis reactions will be going on hand in hand with these anabolic catabolic reactions. So the intermediates can be extracted or removed from these main pathway so that they can be entered into the building block synthesis reactions.

Because they are the precursor for different amino acids, lipid or even the vitamin production. Now to supply these and other precursors for biosynthesis, flux through the EMP pathway must be maintained. Now since continuously there is a depletion of

the intermediates, okay from this EMP pathway, because the cell would always be requiring the intermediates rather than only pyruvate.

Because we know that if pyruvate is produced pyruvate will be then feeding a number of other pathways including the TCA cycle, fermentation and amino acid biosynthesis. Those are also very much required. But at the same time, the synthesis of some other amino acids, lipid and vitamins are directly dependent on these intermediates also. So cell needs to maintain a balance between these two events.

At this moment, these two events mean one event is the utilization of the pyruvate. That is let the glucose be converted to pyruvate and then the pyruvate will be converted to a number of other important resources or the building blocks. Or while the pyruvate synthesis is continuing some of the intermediates will be taken out and will be put into the biosynthesis of the other things.

Because as we can see, even the NADH which is a very important electron carrier and this electron carrier is important because once it is oxidized through electron transport system that is the oxidative phosphorylation, it produces a number of important things. One is the proton motive force and the second is the ATP generation.

Now even this NADH is also kind of required item for the anabolic reactions. But if the anabolism is going on in a very high rate, sometimes this NADH can be converted to NADPH by utilizing NADP. So NADH is converted to NADPH. We will talk about these reactions little later. And then this NADPH will be the primary reducing agent for biosynthesis.

So although we understand or we look at the EMP pathway as a very flat simple biochemical conversion of glucose into two moles of pyruvate, but in reality within a cellular system, which is actively metabolizing the glucose molecules and trying to grow or trying to produce some of the important metabolites of our interest, the actual metabolic behavior of this EMP pathway is under the control of three factors.

One is how the glucose is transported. Second, how this intermediates are being taken up by different biosynthetic reactions. And number three, how this NADH is allowed

to be oxidized through electron transport system that is the oxidative phosphorylation or it is converted to NADPH and this NADPH is used up in the biosynthetic reactions.

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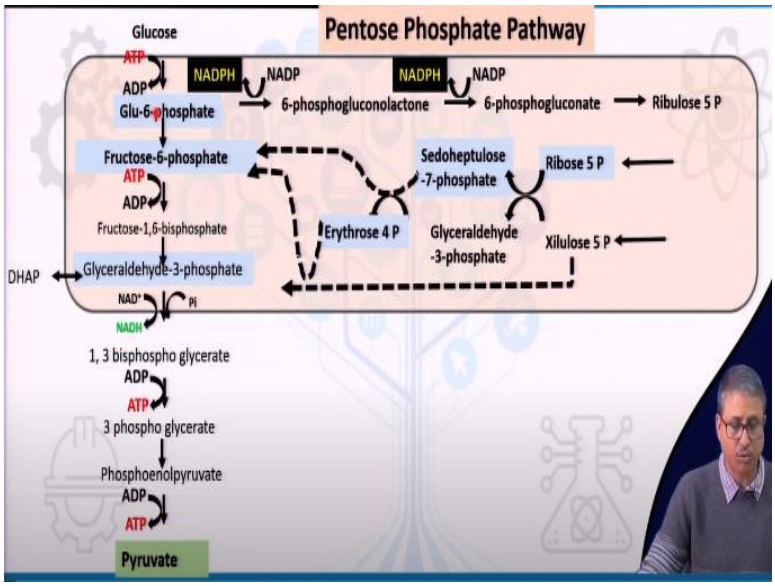

Now this next glycolytic reaction is the pentose phosphate pathway, which is a very interesting reaction and it is connected to the EMP pathway because it starts with the glucose 6-phosphate.

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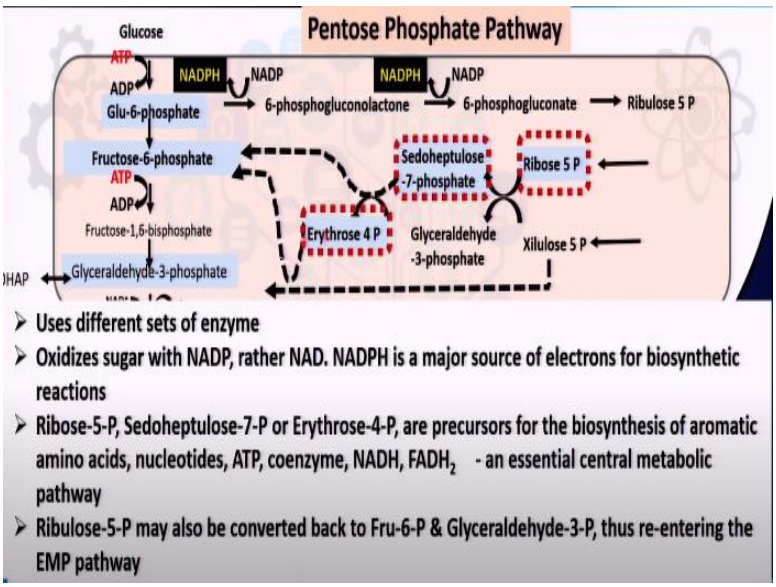

And if we look into these reactions, it is having very characteristic properties because it is composed of a number of enzymes. We are not going to talk about all the details, but we will simplify it that a number of almost all the enzymes in the pentose phosphate pathway are different from the EMP pathway. However, it oxidizes sugar obviously.

And this oxidation of sugar is accomplished by a set of very specific enzymes and with the recruitment of NADP as the electron acceptor not NAD as electron acceptor. So unlike the EMP pathway, which is utilizing NAD plus as electron acceptor pentose phosphate pathway uses NADP phosphorylated form of nicotinamide adenine dinucleotide and thereby allowing the production of NADPH.

And this NADPH is demarcated for utilization in the biosynthetic reaction. Now these first two reactions are the oxidative reactions or dehydrogenation reactions, which are very specific. So glucose 6-phosphate is converted to ultimately to 6-phosphogluconate. And 6-phosphogluconate is converted to ribulose 5-phosphate. So sugar following a decarboxylation reaction.

So that means one mole of carbon dioxide is released and the phosphogluconate 6 carbon compound is converted to 5 carbon ribulose 5-phosphate. Now ribulose 5-phosphate can undergo a number of transformation following the formation of ribose and xylulose. And eventually, the transaldolase and transketolase type of reactions enable these transformation, a number of transformations.

And thereby producing quite a number of interesting metabolites or intermediates including the sedoheptulose 7-phosphate the 7 carbon compound, glyceraldehyde 3-phosphate and erythrose 4-phosphate. Now, while glyceraldehyde 3-phosphate is the substrate again for the EMP pathway, so it can be processed directly.

This erythrose 4-phosphate sedoheptulose 7-phosphate and ribose 5-phosphate, they are the precursor for the biosynthesis of aromatic amino acids, nucleotides, ATP, coenzyme, NADH, FADH2, etc. And therefore pentose phosphate pathway represents one of the very essential central metabolic pathways just like the Embden-Meyerhof-Parnas pathway or the EMP pathway, which we just discussed.

Now when there is not a very high demand or parallely along with the supplying some of the molecules of these sedoheptulose or erythrose towards the biosynthetic side, some molecules of ribulose 5-phosphate can be converted back to fructose 6-

phosphate or glyceraldehyde 3-phosphate as we mentioned earlier, and then processed through the EMP pathway for energy generation.

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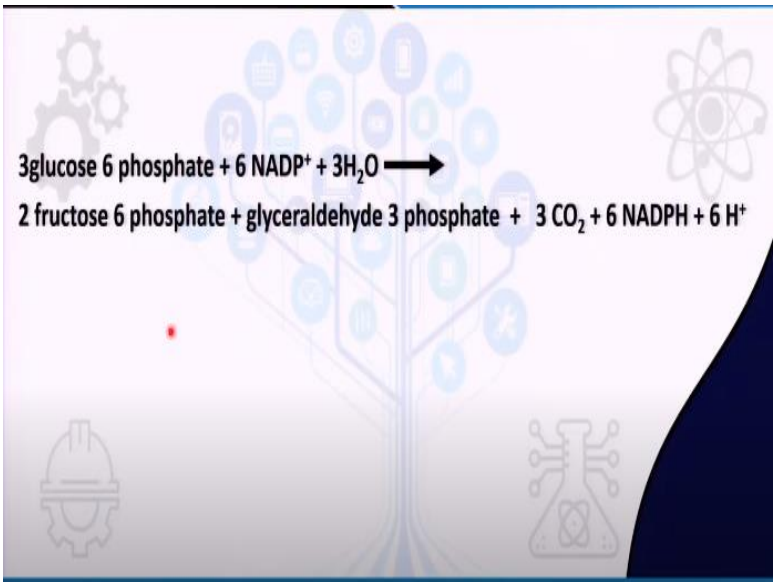

So this is the stoichiometry under the normal circumstances when the all the reactions are happening in this condition.

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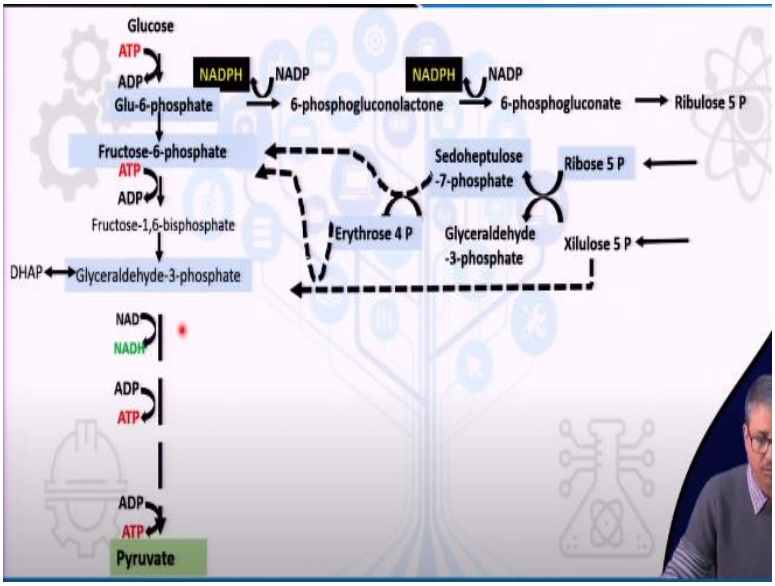

However, if we look at this pentose phosphate pathway, and this EMP pathway, the interconnections

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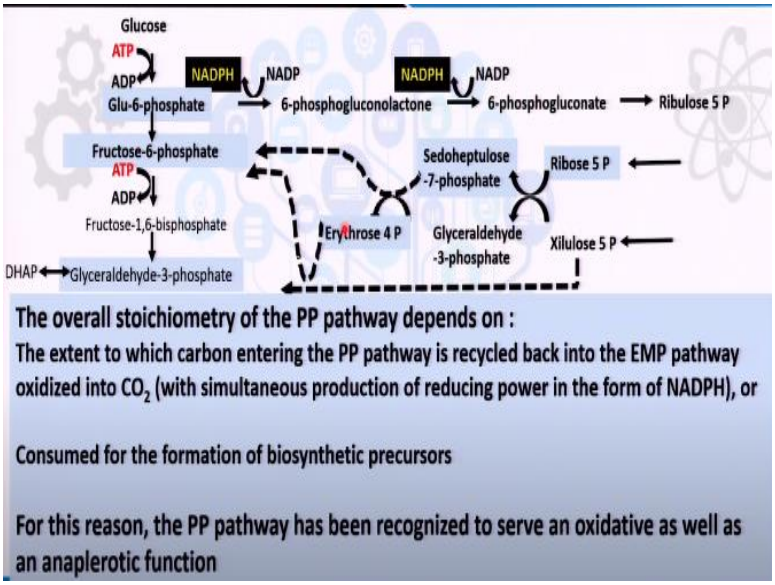

The stoichiometry is not always very simple that the glucose molecule which is entering into this pathway. Because the glucose is entering, if we consider PTS, then it is converted to glucose 6-phosphate and then glucose 6-phosphate is available for both the EMP pathway as well as the pentose phosphate pathway.

Now the stoichiometry of this pathway pentose phosphate pathway depends on number one, the extent to which the carbon entering into the pathway is recycled back into the EMP pathway. That means the fructose 6-phosphate and glyceraldehyde 3-phosphate whether the pentose phosphate pathway is able to convert all the molds of glucose 6-phosphate that is converted to 6-phosphogluconate back to the fructose 6-phosphate and glyceraldehyde.

So if we have a carbon budget, so if we have n mole of glucose 6-phosphate taken and it is 6 carbon, so 6n carbon molecules are taken by the pentose phosphate pathway. So all these 6n carbon molecules should be counted back as fructose 6-phosphate or glyceraldehyde 3-phosphate. But often that is not going to happen. Because why that is not going to happen?

Because the cell will always have high demand for example ribose 5-phosphate, because that is the precursor for nucleotide synthesis. Erythrose phosphate for many amino acids are produced from that. So cell will always be removing some carbons out of this entire flow of metabolites as its precursor molecules. So ultimately the

carbons to be available for coming back to these and producing energy will be reduced or changed.

And for this reason, the PPP pathway has been recognized to serve both as an oxidative pathway as well as the anaplerotic function because it is replenishing. It is anabolic pathway, it is at the same time allowing the production of helping the anabolic reactions. At the same time it is a part of the catabolic reaction where oxidation of a complex molecule is happening.

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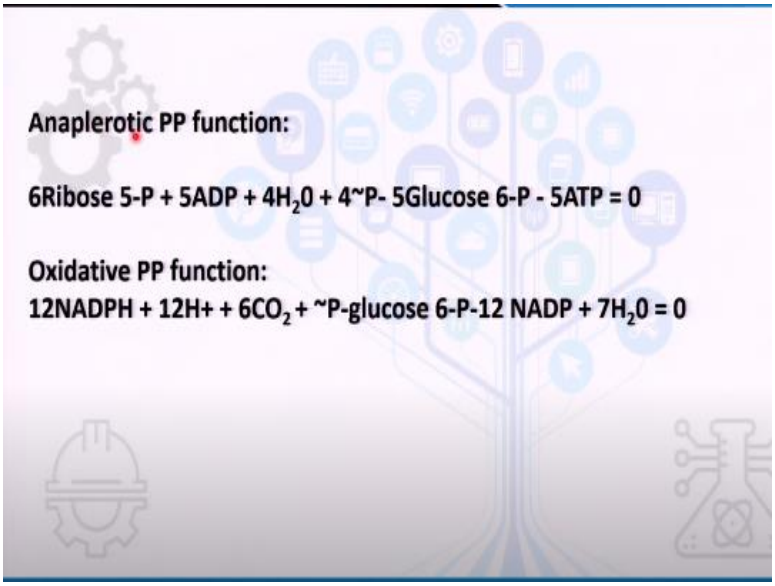

Now we can see that the stoichiometry is altered in case of anaplerotic reaction and in case of the oxidative reaction.

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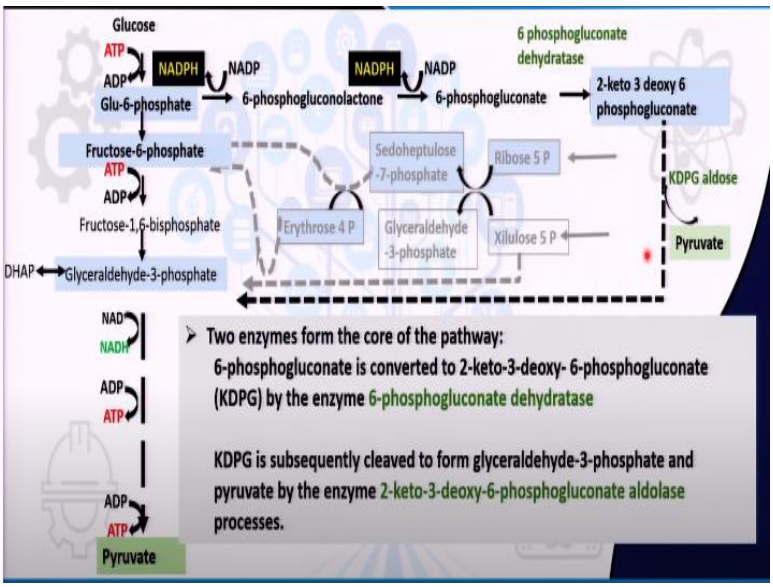

Now we are going to talk about this the third type of glycolytic reaction, which is called the ED pathway. Now during the ED pathway, this the phosphogluconate which is produced following the oxidation of glucose 6-phosphate it is converted to a very specific substrate, specific product which is the 2-keto-3-deoxy-6-phosphogluconate by the 6-phosphogluconate dehydratase.

And this enzyme along with the next enzyme which is the KDPG aldolase, these two enzymes are again very unique to this ED pathway and they are involved in converting very specifically this phosphogluconate, which is again a common molecule between these pentose phosphate pathway and the ED pathway, okay.

Now the KDPG which is produced over here is subsequently cleaved by this KDPG aldolase into two molecules. One is the pyruvic acid molecule another is the glyceraldehyde 3-phosphate molecule. Now this pyruvic acid and glyceraldehyde 3-phosphate can again be oxidized further because we know that pyruvic acid is the major product of the glycolytic reaction.

So you obtain the pyruvic acid directly from here or the glyceraldehyde 3-phosphate can be processed further by the normal EMP pathway. The residual reactions can still occur with the glyceraldehyde 3-phosphate and pyruvic acid can be produced.

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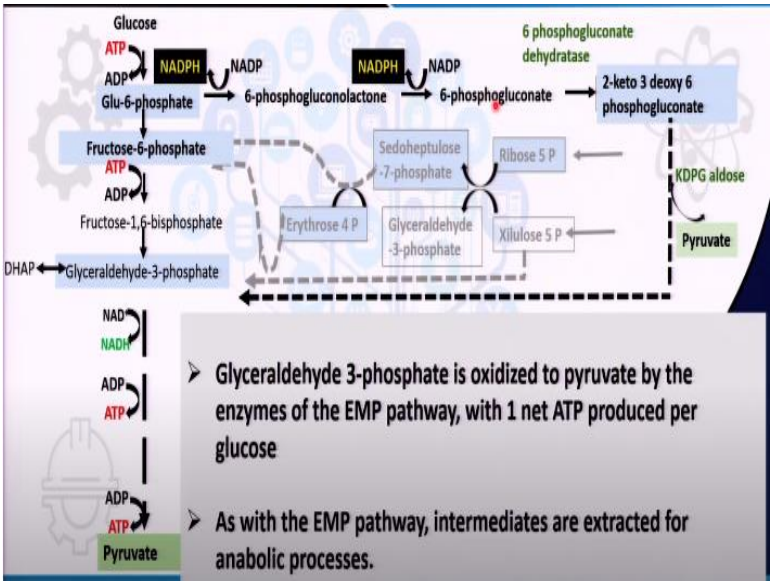

Now glyceraldehyde 3-phosphate when it is oxidized to pyruvic acid it can produce the one mole of ATP because why one mole of ATP because another mole of ATP is

compensated with the ATP used over here. And as with the EMP pathway intermediates are extracted for anabolic processes. So many of the intermediates could because one of the major intermediate could be the phosphogluconate, which is supposed to feed the pentose phosphate pathway also.

So there is actually big flux distribution issue with these three pathways happening together, okay. One is this EMP pathway trying to take the flux of the glucose 6-phosphate. Another is the pentose phosphate pathway, which is again or the ED pathway, both of them are responsible for taking the flux of the glucose 6-phosphate.

And then within pentose phosphate pathway and ED pathway there could be a flux distribution with respect to this molecule 6-phosphogluconate because both PPP and ED pathway both of them would require this as the molecule of their interest.

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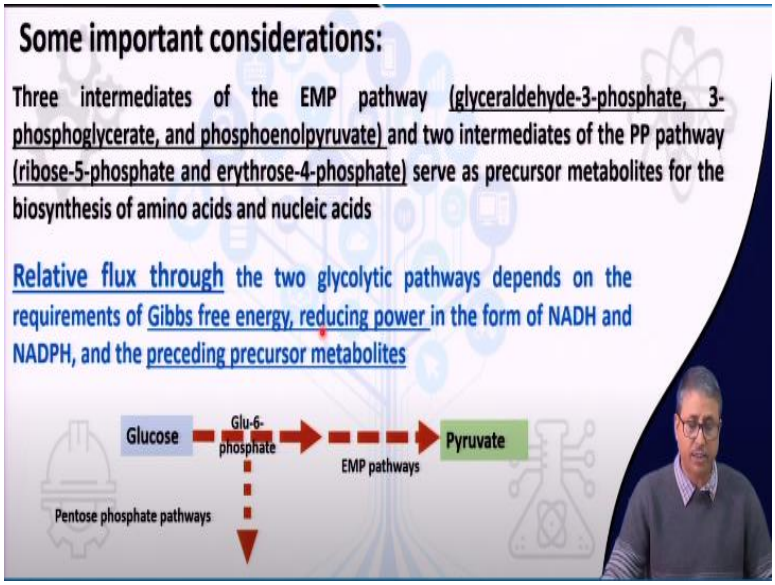

Now, some important consideration with respect to these glycolytic reactions are there are three intermediates of the EMP pathway, mainly the glyceraldehyde 3-phosphate, the 3-phosphoglycerate and phosphoenolpyruvate and two intermediate of the PPP, that is the ribose 5-phosphate and erythrose-4-phosphate serve as the major precursor molecules for the biosynthesis of amino acid and nucleic acid.

In addition to this there could be Sedoheptulose 7-phosphate, which is also an important precursor. Now while these different intermediate metabolites are used or required for the biosynthetic reactions or the anabolic reactions, the relative flux through these two main reactions that is the EMP pathway, which directly converts the glucose to pyruvic acid.

And the pentose phosphate pathway, which enables the cell to carry out a number of reactions to number of metabolites production including the NADPH and the pentose sugar, the 4 carbon erythrose sugar, but at the same time, keeps the option open for returning the carbons back to the EMP pathway through either the fructose 6 phosphate or the glyceraldehyde 3 phosphate.

So this juncture between these pentose phosphate pathway and EMP pathway is considered to be a very important point for the flux distribution. And the relative flux through these two glycolytic pathways actually depend on the requirement of three things. One is the Gibbs free energy, reducing power, and the requirement for the precursor metabolites.

What are the demand for these three that actually regulate the entire process of the flux distribution.

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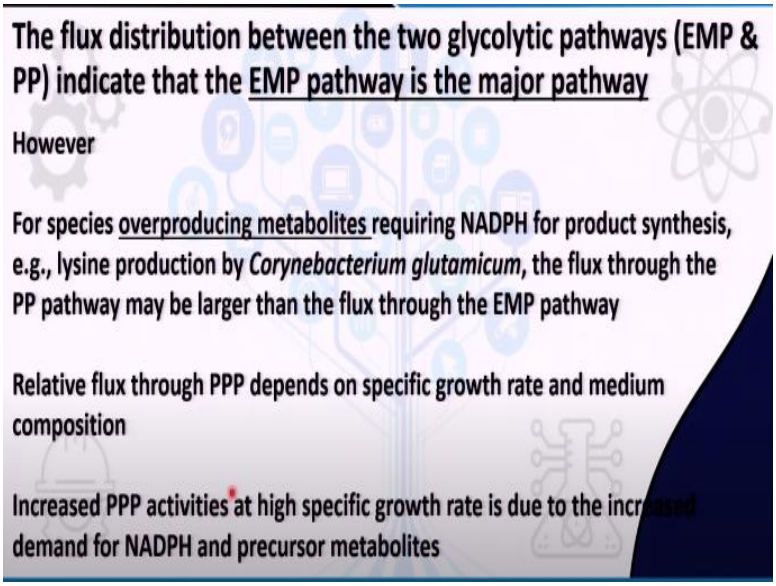

Now the flux distribution between the two pathways indicate that the EMP pathway in general what we have found, that is the major. And almost like 60, 65% of the carbon flux is generally passes through the EMP pathway.

However, when the organisms are over producing certain metabolites, for example lysine amino acid over production by the bacterium Corynebacterium glutamicum, we can find out that a high flux through the pentose phosphate pathway, because they need lot of NADPH.

So it is not only that during lysine production, but also we have seen during any type of microbial growth, where a specific growth rate is very high and medium composition is also suitable to allow the sustained specific growth rate and this specific growth rate, high specific growth rate means a high supply or increased supply of NADPH and the precursor metabolites.

Then naturally, we find that high PPP activities and so the flux will be the more towards the PPP pathway.

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So with this we complete this part of the fueling reaction. And for today's lecture, we have covered most of the points from the metabolic engineering textbook and some other very interesting resources was used. One of them is this Glycolysis for Microbiome Generation, which is a basically ASM press publication. And then some other review and book articles.

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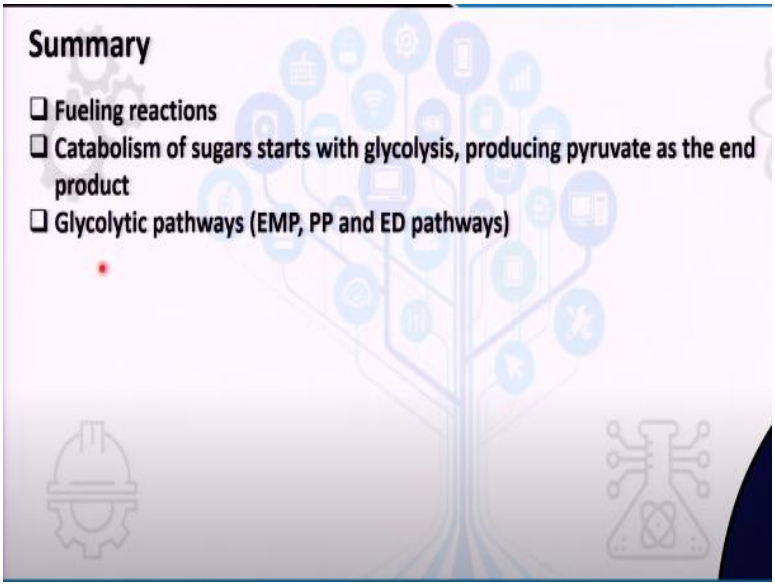

So overall, in today's lecture, we have discussed about the fueling reactions and catabolism of sugar that starts with glycolysis producing pyruvic acid as the end product and the different type of pathways, glycolytic pathways that the EMP pentose phosphate and ED pathways. We briefly discussed about the flux distribution between these two.

But in subsequent classes we will be continuing more discussion on how these metabolic flux is actually distributed and controlled while the carbon is processed through this glycolytic pathway which is the major fueling reaction. Thank you.