Introduction to Complex Biological Systems
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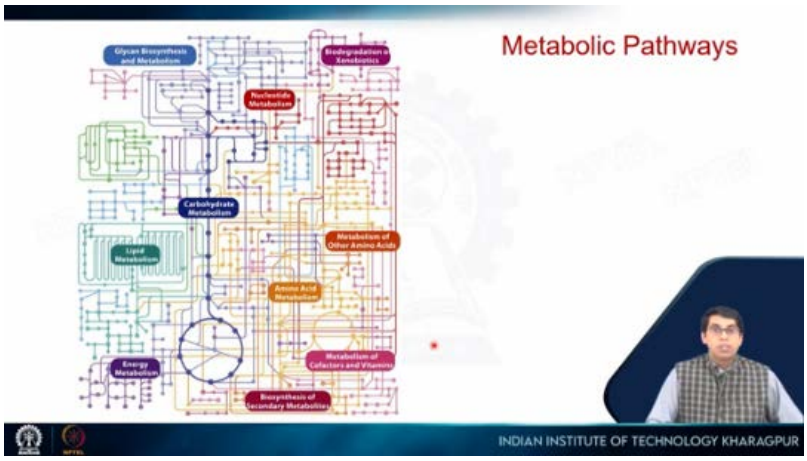
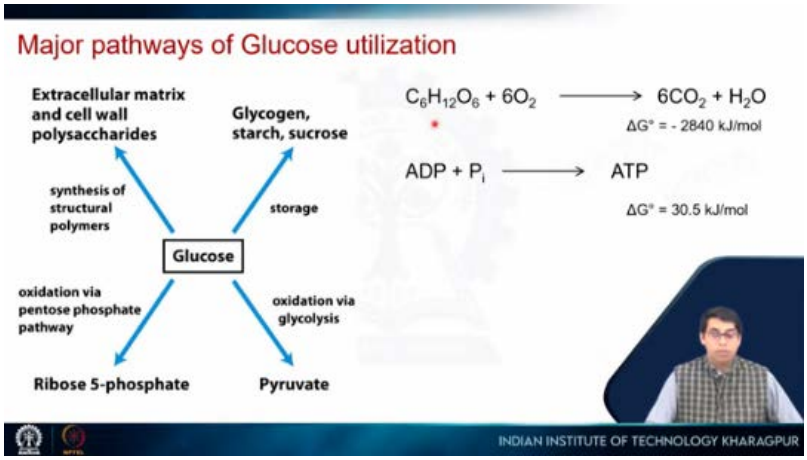
Lecture 32
Glycolysis, Gluconeogenesis and PPP

Welcome to lecture 32 in Week 7 of Introduction to Complex Biological Systems. So today, I'm going to talk about glycolysis, gluconeogenesis, and PPP, these three processes. So in the last lecture, I showed you this map, which is a metabolic pathways map.



So each of these dots represents a molecule, which is converted into some other molecule by some chemical reaction and typically, that reaction will be catalyzed by an enzyme. So I'm going to discuss glycolysis, which is represented by this straight line. So in this case, we will see glucose get converted to pyruvic acid via 10 chemical reaction steps.

So, the ultimate goal is to produce energy. So, glucose is a very high-energy molecule. If we just burn glucose, we can get 2840 kilojoules per mole of energy. But then if it is just combustion, most of that energy will be lost as heat energy.

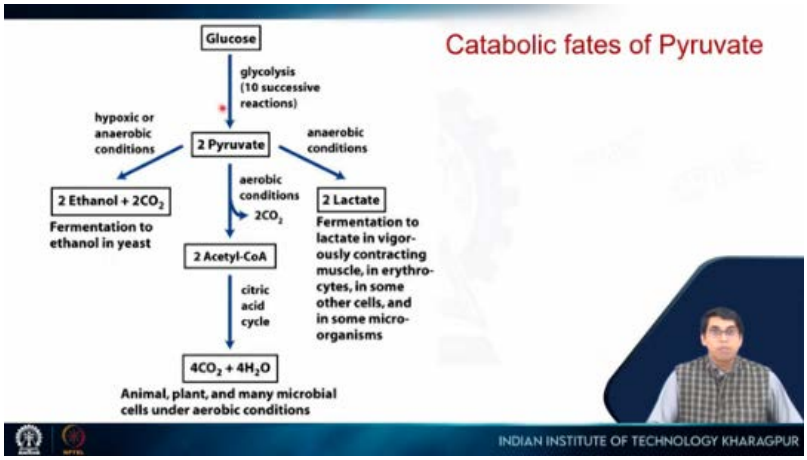


So what our body does is it performs a very controlled oxidation, where ultimately this glucose will be oxidized using oxygen to carbon dioxide and water. So this is the final reaction that you will get. However, it will go through multiple reaction steps and will produce a lot of metabolites and the energy will ultimately be harvested in the form of ATP. So when we produce ATP from ADP, we harvest around 30.5 kilojoules per mole of energy.

So the complete oxidation of glucose to carbon dioxide and water will produce ATP, approximately 32 molecules of ATP. So now, glucose undergoes all these different processes. So what we are going to look at today first is the conversion of glucose to pyruvic acid, which is oxidation via glycolysis. Glucose can also be stored as glycogen, starch, or sucrose for long-term storage.

Glucose is also oxidized via the pentose phosphate pathway or PPP to produce all sorts of nucleic acid material. So DNA, RNA, they will require this ribose sugar. So they are all produced via this pathway and it is also used to synthesize structural polymers for the extracellular matrix and cell wall polysaccharides. So glucose is used for all these different pathways.

We are going to discuss this pathway and this pathway primarily in today's lecture. So from glucose, we will see that 10 successive reactions result in the formation of two pyruvate molecules. So glucose is a six-carbon molecule. Pyruvate is a three-carbon molecule. So there is no loss of carbon from one molecule of glucose.

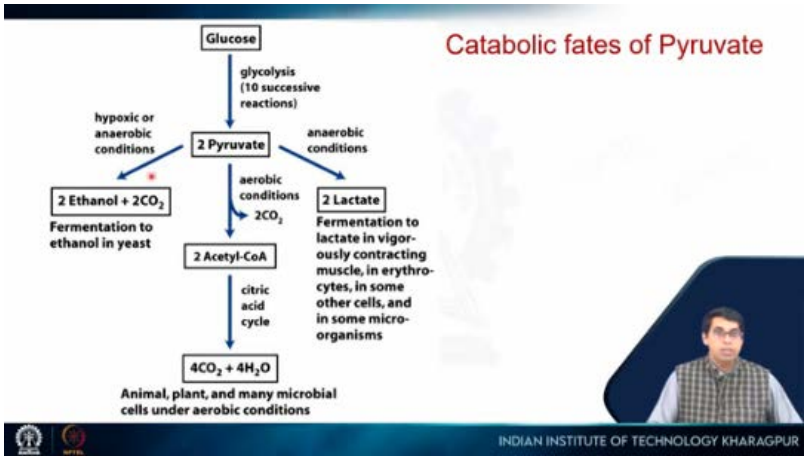


We will get two molecules of pyruvate and then in next week's lecture, we will see that this pyruvate, under aerobic conditions, so when there is oxygen will get converted to acetyl coenzyme A upon the loss of two carbon dioxide molecules. It has been reduced to 4 carbons now. So the acetyl group is 2 carbons. There are 2 acetyl groups, so there are 4 carbons and then ultimately, in the citric acid cycle, it will get converted to 4 carbon dioxide and 4 water molecules. So that is the complete oxidation of glucose. However, not all ATP is synthesized at this stage. Then there is oxidative phosphorylation that happens later on, which will result in the complete regeneration of ATP molecules. So we will discuss that, but all of this, from here to here, all of that is dependent on the presence of oxygen. So these are aerobic conditions but what happens if there is not much oxygen supply for some reason? So that will be an anaerobic condition and in that case, pyruvate gets converted to lactate.

So this happens when, let us say, we are doing some vigorous exercise. So there is not enough oxygen supply to get into this, but you need oxygen, quick generation of ATP. So a lot of glycolysis happens but then there is, of course, one problem.

I will discuss that in the coming slides. To recover from that problem we have pyruvate converted to lactate. In many microbes, something similar happens.

So instead of forming lactate, they form ethanol and carbon dioxide. So that is fermentation to ethanol in yeast. So let us look at the steps of glycolysis. So I mentioned that there are 10 steps, which means that there are 10 enzymes involved in the catalysis of these 10 steps. The first five steps of glycolysis are termed the preparatory phase.



So it prepares for this, for the payback. So why does it prepare? Because you will see that instead of producing ATP, it uses ATP. So we are actually using up some energy to prepare the reactive molecules.

So in the first step, glucose is phosphorylated by the enzyme hexokinase to produce glucose 6-phosphate and it will look something like this. So this is glucose and this is the sixth carbon. So it is numbered as 1, 2, 3, 4, 5, 6 so each of these positions is a carbon molecule. So the sixth carbon OH is phosphorylated by hexokinase, and this phosphate group comes from the ATP. So remember, ATP has three phosphate groups: triphosphate.

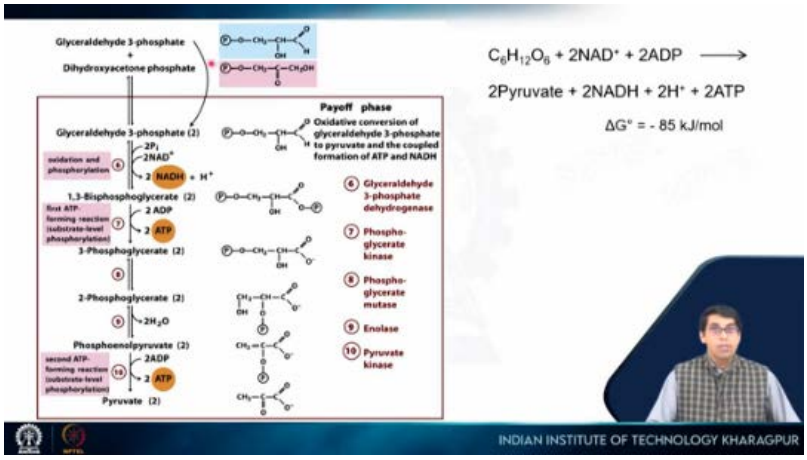
So the terminal phosphate goes here, and it becomes ADP diphosphate. Then the next step is catalyzed by phosphohexose isomerase to produce fructose 6-phosphate. So this is glucose 6-phosphate. It becomes fructose 6-phosphate. So you can see that there is a structural change.

The phosphate group is still there. Now, phosphofructokinase 1 will add another phosphate group. So it uses another molecule of ATP. So another phosphate group is added, which is added at this one position.

So now it is fructose 1,6-bisphosphate and you can see that this looks like a symmetric molecule and this is exactly what happens when this symmetric molecule is broken into two molecules. Each of those will have three carbons, one, two, and three so three carbons and one phosphate each. So this gets broken into these two molecules. Glyceraldehyde 3-phosphate and dihydroxyacetone phosphate and that is done by the enzyme aldolase.

Now, this molecule, dihydroxyacetone phosphate, is converted to glyceraldehyde 3-phosphate by the enzyme triose phosphate isomerase. So, at the end of these five steps, we get two molecules of glyceraldehyde 3-phosphate from one molecule of glucose. So glucose was a six-carbon molecule, and these are two three-carbon molecules.

That is what we get at the end of this fifth step. So that ends our preparatory phase. So now we enter into the payoff phase, where we will actually get production of certain high-energy molecules. So dihydroxyacetone phosphate gets converted to glyceraldehyde 3-phosphate by the enzyme triose phosphate isomerase. So now we end up with two molecules of glyceraldehyde 3-phosphate.



This is now oxidized. So remember that. Glucose has to be oxidized ultimately to give carbon dioxide and water. So this is the first oxidation step that we are going to see and you see that here this coenzyme NAD^+ is used.

So remember there are two molecules so you will use two NAD^+ to oxidize it. So there are two reactions which are happening in parallel. So this is oxidation and phosphorylation. So glyceraldehyde 3-phosphate gets converted to 1,3-bisphosphoglycerate.

So, there was a phosphate group here. Now, we have added another phosphate group here. So, this aldehyde group is now converted to an acidic group, and it forms this ester with the phosphate. The next step, so this reaction is catalyzed by glyceraldehyde 3-phosphate dehydrogenase. The next step is catalyzed by phosphoglycerate kinase.

So, what it does is it will transfer this phosphate group from this molecule to the ADP. So, ADP has two phosphates. It gets this extra phosphate, so it becomes ATP and we lose one of the phosphate groups. So, this becomes 3-phosphoglycerate.

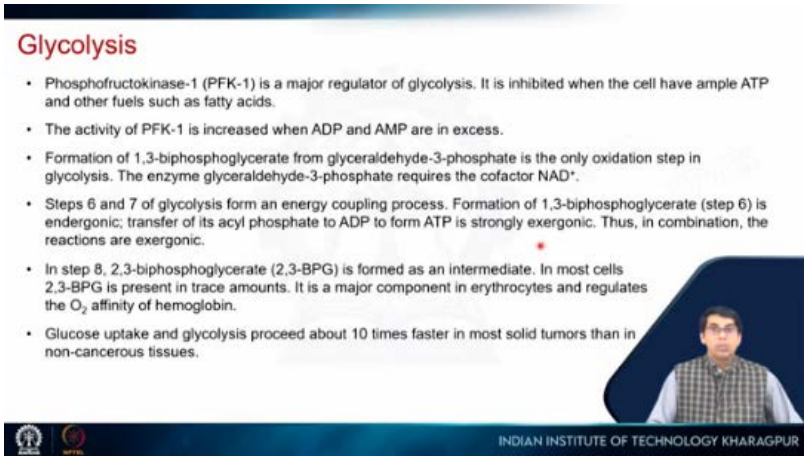
From 1,3-biphosphoglycerate, it becomes 3-phosphoglycerate. So, remember there are two molecules of this. So, we get two ATP molecules synthesized. So, we have got two NADH synthesized, and we got two ATP molecules synthesized. Then, this 3-phosphoglycerate gets converted to 2-phosphoglycerate.

So, what happens is that the phosphate group is transferred from this third carbon to this second carbon. So, on this OH group, the numbering is this is carbon 1, this is carbon 2, and this is carbon 3. So, the phosphate was on carbon 3 OH, now it is on carbon 2 OH. So, it is a mutase, phosphoglycerate mutase and then this 2-phosphoglycerate. From this, a water molecule goes out. So, this H and this OH, they will go out. So, you will get a double bond formation, which is this. So, it is a phosphoenolpyruvate.

So, it is called an enol because this is a double bond and this is an OH group. So, enol and it is catalyzed by this enzyme enolase. So, again, we have this extra phosphate group. So that is taken up by another ADP molecule to produce ATP, and we end up with pyruvate. So this is pyruvic acid, and this reaction is catalyzed by the last enzyme, which is pyruvate kinase. So at the end of this reaction, this is what we get. So this is the net reaction. Glucose, we used up 2NAD+ to produce 2NADH.

So this is a high-energy molecule. We used 2 molecules of ATP and produced 4 molecules of ATP. So the net is that we used 2 molecules of ADP and produced 2 molecules of ATP. So the net ATP formation is two molecules of ATP.

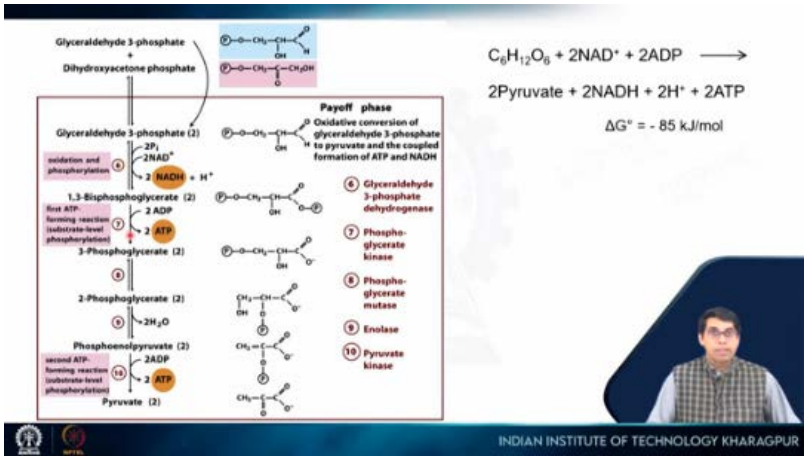
We get two NADH, two pyruvate groups, and two hydrogen ions and the ΔG of this reaction is minus 85 kilojoules per mole, which means that overall this is a very spontaneous reaction. Some of the key features of glycolysis are listed here. Phosphofructokinase-1 or PFK-1 is a major regulator of glycolysis. It is inhibited when the cell has ample ATP and other fuels, such as fatty acids.



So, what is the purpose of glycolysis? Its purpose is to give you energy to produce ATP molecules. But if there are enough ATP molecules present, then there is no need to metabolize glucose through glycolysis. So, that is why when there is enough ATP present, this enzyme, phosphofructokinase 1, is inhibited by ATP. So, this is a feedback kind of system that regulates, that tells the cells whether glycolysis is needed or not.

The activity of PFK is increased when ADP and AMP are in excess, which means that ATP is already hydrolyzed into ADP and AMP. So, when they are in excess, this enzyme is activated, and it gets into and activates glycolysis. The formation of 1,3-biphosphate glycerate from glyceraldehyde 3-phosphate is the only oxidation step in glycolysis. So, as I pointed out, it uses this NAD^+ cofactor, steps 6 and 7 of glycolysis.

So, let us go back to steps 6 and 7. So, these two steps can be considered as coupled steps. So, 6 is the formation of 1,3-biphosphoglycerate, which is step 6. It is an endergonic process.



Endergonic means that it needs energy. So it is not a favorable process. However, the transfer of its acyl phosphate to ADP to form ATP is strongly exergonic. So it means that the combination of these two makes it an exergonic reaction so if I go back again, this reaction is unfavorable. But this reaction is highly favorable.

So together, this becomes a favorable reaction going from glyceraldehyde 3-phosphate to 3-phosphoglycerate and the formation of ATP molecules. Step 8, 2,3-bisphosphoglycerate or 2,3-BPG is formed as an intermediate. So, this is step 8. Here, 2,3-bisphosphoglycerate is formed as an intermediate molecule.

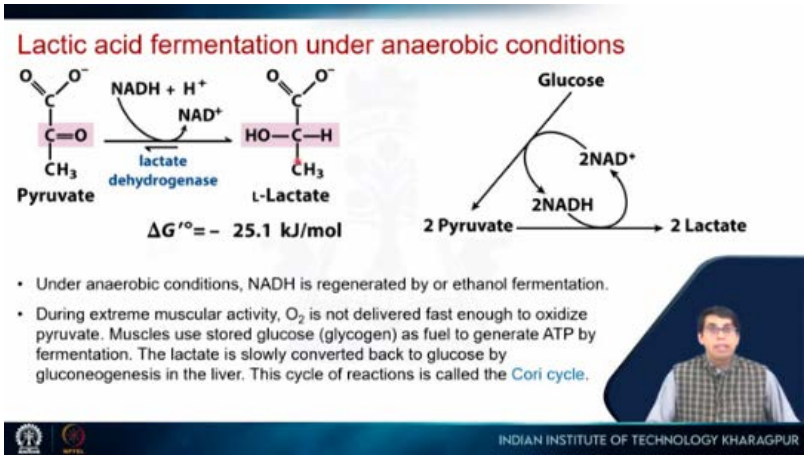
In most cells, this is present in trace amounts, so in very small amounts. But it is a major component in erythrocytes or red blood cells. We have already seen that 2,3-bisphosphoglycerate or 2,3-BPG binds to hemoglobin and regulates its oxygen affinity. Glucose uptake and glycolysis proceed almost 10 times faster in solid tumors than in non-cancerous tissues. The reason for that is that in a solid tumor or in cancerous tissues, cells are dividing much faster.

So they have a very high requirement for energy. But since the cells are dividing, it does not give enough time for the blood vessels to form. There is not enough time for proper oxygen supply. So, it means that the reactions are happening in anaerobic conditions, in the lack of oxygen. So, you cannot get ATP from the further downstream metabolic processes.

So, you try to produce as much ATP as possible through glycolysis. So, that is why glycolysis proceeds much faster in solid tumors compared to non-cancerous tissues. So again, let us look at this chart. So what we have seen so far is from glucose, we have formed two pyruvate molecules via these 10 reaction steps.

So glucose is a six-carbon molecule, and we have produced pyruvate, which is a three-carbon molecule. So we have produced two pyruvate molecules. So in the next lecture, we will discuss this. But today I am going to show you this anaerobic condition. So when there is a lack of oxygen or when, let us say, you are doing some vigorous exercise or you are sprinting, you are going through a 100-meter sprint, then your body needs a huge burst of energy. So, in those cases, there is not enough time to produce ATP via these processes, and ATP is produced by glycolysis. Then, the pyruvate goes into this lactate formation. So, why is this needed? The reason this is needed is that, in the case of glycolysis, this is glucose, and two pyruvate molecules are formed. It uses up two molecules of NAD to form two molecules of NADH.

Now, NAD and NADH, these molecules are not abundant. They have a limited supply. So, if we are going through this anaerobic condition, we are only producing pyruvate. Then, we are using up NAD to produce NADH. When you go from pyruvate to the further reaction steps and you go to this oxidative phosphorylation, the NAD+ is regenerated. But we are not going through all those processes.



We are stopping here because we are producing ATP by these fast reactions. So, all the NAD+ will be used up. So, you cannot proceed with glycolysis, and also, many other processes will stop. So, somehow, you have to regenerate it, and that is what this does.

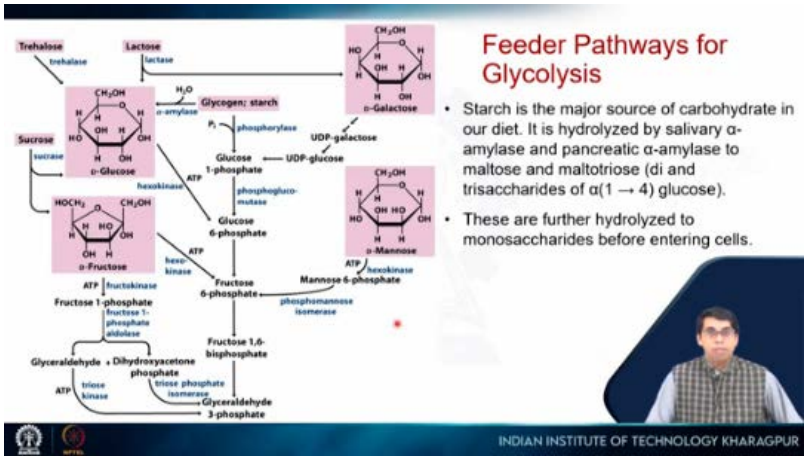
So pyruvate gets converted to lactate. So this is again a reduction reaction. So this was oxidation, and this was reduction. So in this reduction, NADH is used up as a reducing agent to produce NAD+.

So you regenerate NAD+. So under anaerobic conditions, NADH is regenerated by lactate or ethanol fermentation. So during extreme muscular activity, oxygen is not delivered fast enough to oxidize pyruvate. So muscles use stored glucose or glycogen as fuel to generate ATP.

So there is glycogen which is stored in the muscle, and that is used up as fuel. So only these steps happen and from that one glucose you will get two molecules of ATP. But that will result in the formation of lactate.

So once you are done and you go into the resting period, this lactate in the bloodstream is released. It is carried to the liver, and there it is converted back to glucose via the process called gluconeogenesis. So this lactate is converted back to glucose via gluconeogenesis in the liver, and then it is converted to glycogen, which is again put back into muscles and other places as stored fuel. So this cycle of reactions is called the Cori cycle.

So now what we are going to do is we are going to look into more details, examining gluconeogenesis that happens. Before I get into gluconeogenesis, let us look at where glucose comes from. So what are the feeder pathways for glycolysis? So we have seen that glucose forms glucose-1-phosphate or glucose-6-phosphate and then this whole process happens. So glucose comes from glycogen or starch. So it turns out that starch is the major source of carbohydrate in our diet.

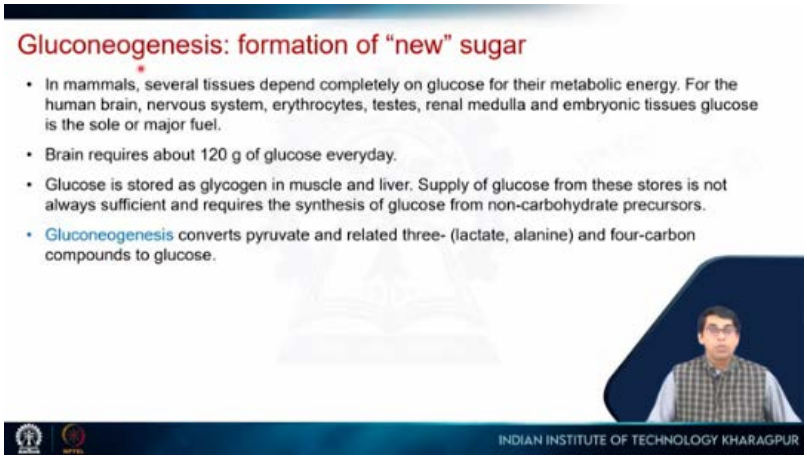


So when you eat something like rice. It has a lot of starch. That starch is hydrolyzed by enzymes called alpha-amylase. So in our saliva, there is alpha-amylase, salivary alpha-amylase, it will break down this polymeric starch into smaller fragments and then those go into our stomach. There the pH is low, and in that condition, this salivary alpha-amylase is actually deactivated and there, new amylase, the pancreatic amylase, is secreted, and it will further chop up these polysaccharides into smaller fragments like maltose or maltotriose. So these are di- and trisaccharides of alpha 1-4 glucose so two molecules of glucose or three molecules of glucose. Then there are other enzymes which will further hydrolyze these into monosaccharides or single molecules of glucose and then that will be taken up by the cells via various receptors. So you have that glucose, and then it goes into glycolysis. So the lactose which is present in milk that is a disaccharide. So it is formed of glucose and galactose. So there are enzymes which will break it down into glucose and galactose.

Glucose can go directly here. Galactose is further converted by other enzymes into glucose 1-phosphate, which is converted to glucose 6-phosphate by phosphoglucomutase. So that's another enzyme, and then it goes into glycolysis. If you have mannose, then that is converted to mannose 6-phosphate, and then phosphomannose isomerase will convert it into fructose 6-phosphate, and that goes into glycolysis. Fructose itself can become fructose 6-phosphate, or it can go through other pathways to form glyceraldehyde 3-phosphate. Sucrose can get converted to glucose or fructose and get into the glycolysis. So these are all the different pathways from which all these sugar molecules can get into glycolysis. So these are called the feeder pathways for glycolysis. So what we have seen is glucose to pyruvic acid formation via glycolysis. If you need a very quick burst of energy, then

glucose gets converted to pyruvate, pyruvate gets converted to lactate, and then this lactic acid gets converted back to glucose via gluconeogenesis. So that is what we are going to see now.

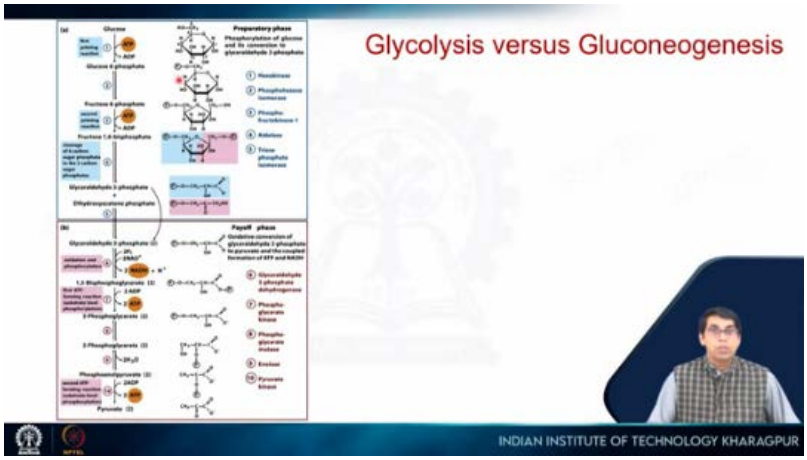
So what is gluconeogenesis? It is the formation of new sugar. So in mammals, there are several tissues which depend completely on glucose for their metabolic energy.



For example, our brain, nervous system, erythrocytes, red blood cells, testes, renal medulla, embryonic tissues, they are all dependent on glucose as the sole or major fuel. In fact, our brain uses almost 120 grams of glucose every day. You will see that if you are doing some intense study for your exams and things like that, you will become very hungry because your brain is using up all this glucose. So it's a very energy-intensive organ.

Glucose is stored as glycogen in muscle and liver. Supply of glucose from the stores is not always sufficient and requires the synthesis of glucose from non-carbohydrate precursors. So gluconeogenesis is a process, which converts pyruvate and related three or four carbon compounds. So three-carbon compounds like lactate or alanine and four-carbon compounds into glucose. So gluconeogenesis results in the formation of glucose.

So what we are going to do is we are going to see this simpler process where pyruvate is converted to glucose. So if we look at it like that, then what we are going to do is we are going to synthesize glucose from pyruvic acid. So this is glycolysis in one slide. So, you have all the 10 reactions listed here.



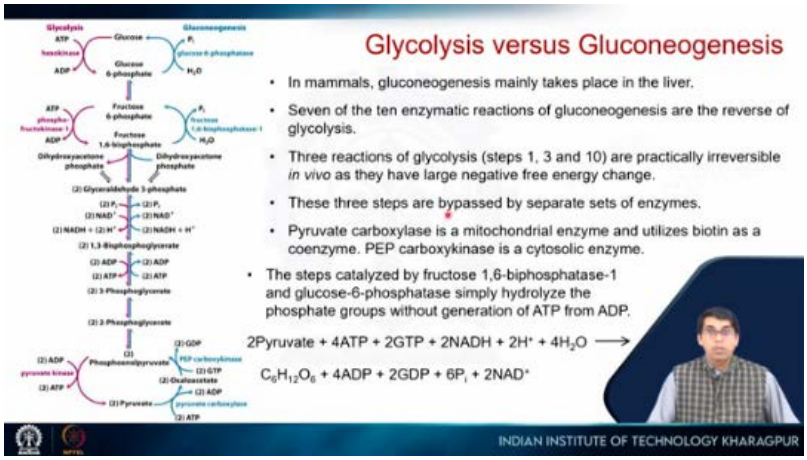
Now, if we put glycolysis and gluconeogenesis side by side, it will look something like this. So, glucose to pyruvate is the left-hand side, and then pyruvate to glucose is the right-hand side. So, both have exactly 10 reactions. So, actually, pyruvate to glucose will have one additional reaction which is here.

So in mammals, gluconeogenesis mainly takes place in the liver, 7 out of 10 enzymatic reactions of gluconeogenesis are the reverse of glycolysis. So if we talk about glycolysis, going from glucose to pyruvate, there were 10 reaction steps so 7 reaction steps are exactly the same in gluconeogenesis only these three, 1, 2, and 3. These three are the ones that are different. So, three reactions of glycolysis, that is step 1, which is this, step 3, which is this. So, this is step 2, it is the same, step 3 is this, and the last one, which is step 10. So, these are practically irreversible reactions in vivo as they have a large negative energy change. So, if I go back to this slide, you will see that they are listed like this. So, that is step 1. So, you see it is an irreversible arrow. This is reversible.

Then step 3 is this, and step 10 is this. Now, you might notice that step 7 is also something that is irreversible. I have already discussed this that step 6 and step 7, they act as a coupled reaction. So together, they become a reversible reaction because this is favorable and this is unfavorable.

So if you take them together, they compensate for each other, which means you can go in either direction using the same enzymes. So practically, it is step 1 where ATP is used, step 2 where ATP is used, and step 10 where ATP is produced. These are the three steps that are

irreversible. So since these are irreversible, you have to find a different way of going through this. So these are bypassed by a separate set of enzymes for gluconeogenesis.



So the first one, pyruvate carboxylase, is a mitochondrial enzyme and utilizes biotin as a coenzyme. So when you go from pyruvate to phosphoenolpyruvate, it happens in two steps. In the first step, pyruvate carboxylase uses ATP. So it hydrolyzes ATP to produce ADP and forms oxaloacetate from pyruvate so oxaloacetate is produced here and then phosphoenolpyruvate carboxykinase converts oxaloacetate to phosphoenolpyruvate and here it uses GTP so two molecules of pyruvate form two molecules of phosphoenolpyruvate. Two ATP molecules are consumed, and two GTP molecules are consumed in this step. So instead of producing ATP, here we have consumed ATP and GTP. Then these steps are all very similar.

Now we reach fructose 1,6-biphosphate. So in this case, fructose 1,6-biphosphatase 1 is the enzyme which catalyzes this reaction. So the reverse process would be the formation of ATP, but that is not what happens because this direction is not favorable, so we are not getting the synthesis of ATP. What happens is simply the phosphate group is hydrolyzed by this enzyme, so there are two phosphates on this fructose molecule. One of the phosphate groups, the phosphate on carbon 1, is hydrolyzed.

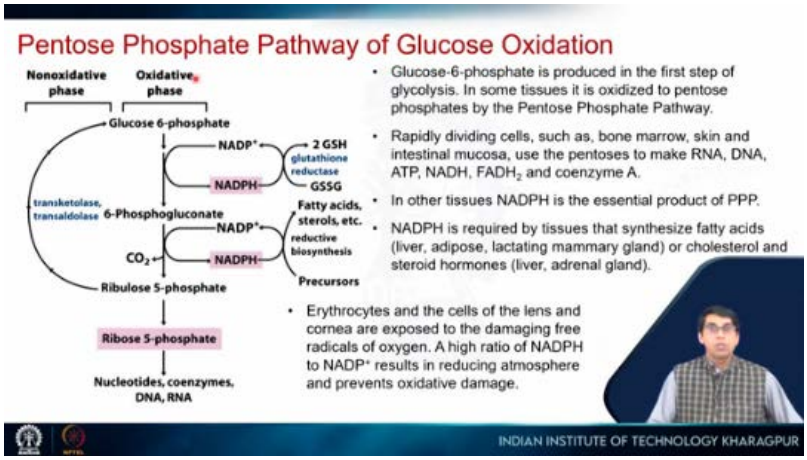
So you get fructose 6-phosphate and the same thing happens here. So from glucose 6-phosphate, you just hydrolyze the phosphate group by this enzyme, glucose 6-phosphatase, and you get glucose. So again, there is no generation of ATP in this case. So it is not the reverse of this step.

So we are not generating ATP in these two steps. However, we have consumed ATP here and consumed another high-energy molecule, which is GDP here. So in this case, as I point out here, pyruvate kinase carboxylase, so this reaction happens in the mitochondria, whereas this reaction happens in the cytosol. So there is some transport that goes on between the mitochondria and the cytosol. So if you are interested, you can look that up.

The steps catalyzed by fructose 1,6-bisphosphatase 1, which is this, and glucose 6-phosphatase simply hydrolyze the phosphate groups without the generation of ATP from ATP. So no ATP generation is happening here. So this will be the overall reaction. From two pyruvate molecules, we produce one glucose molecule. However, we end up using four ATP molecules.

So we end up hydrolyzing 4 ATP molecules, so 2 here and 2 here, and 2 GTP molecules, and we also convert 2 NADH to 2 molecules of NAD+. So in essence, gluconeogenesis is consuming more energy than the amount of energy that was produced in glycolysis. So now we have looked at this glucose to pyruvate formation, pyruvate to lactate formation, and then lactate to glucose formation. So next, I am going to talk about the oxidation of glucose via the pentose phosphate pathway.

So pentose phosphate pathway of glucose oxidation, glucose 6-phosphate is produced in the first step of glycolysis. In some tissues, it is oxidized to pentose phosphates via the pentose phosphate pathway. So after the formation of glucose 6-phosphate, instead of going into glycolysis, it can go into this alternative pathway, which is the pentose phosphate pathway. So rapidly dividing cells such as bone marrow, skin, and intestinal mucosa use this pentose phosphate pathway to produce the raw materials that are needed for producing RNA, DNA, ATP, NADH, $FADH_2$, and coenzyme A so all these molecules require the formation of this ribose sugar or ribose 5-phosphate, and glucose then feeds into this pathway.

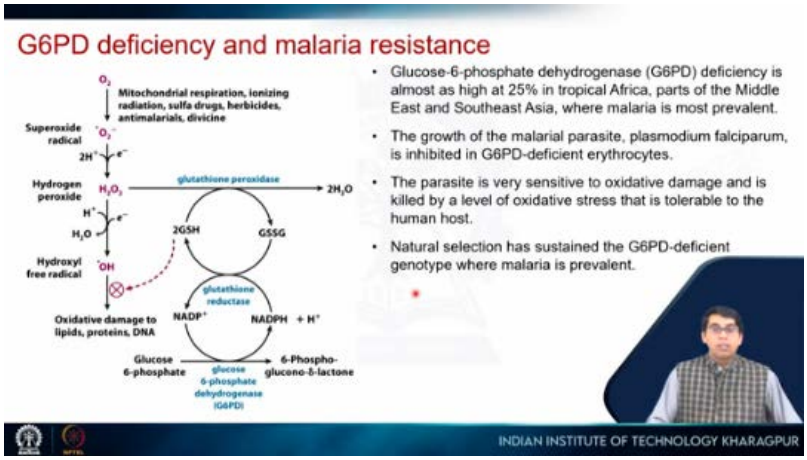


So in other tissues, NADPH is an essential product of the pentose phosphate pathway. So the production of NADPH is also something that is very important. Remember that when normal glycolysis is happening, we are using up NAD+, and we are producing NADH but in other processes, NADPH is something that is used up. So we want to produce this NADPH, and that is produced via this pathway.

So not only ribose 5-phosphate, but the production of NADPH is also an important product of this pathway. NADPH is required by tissues that synthesize fatty acids, cholesterol, and steroid hormones. So for the synthesis of these molecules, you need NADPH. Now, NADPH is not only important for the synthesis of these molecules, but it is also important for the creation of a reducing environment.

So if we think about our red blood cells or erythrocytes, or the cells in the lens of our eye, these are in direct contact with oxygen. So in this case, we have this highly reactive molecule, oxygen, and it can produce further reactive oxygen species like free radicals of oxygen and these free radicals of oxygen or superoxide of oxygen, these are reactive oxygen species, and they can cause a lot of damage to the cells. So these reactive oxygen species have to be quickly neutralized, and that is done by NADPH.

So, a high ratio of NADPH to $NADP^+$ results in a reducing atmosphere and prevents oxidative damage. So, this is something that is very important. So, the production of NADPH is important, which is done by this particular pathway. So, it turns out that this function has certain important consequences.



So, what we see here is oxygen. So, mitochondrial respiration, ionization, radiation, different drug molecules that we take, pollutants like herbicides, all these different things can result in the production of superoxide radicals. So, this is a very reactive oxygen species, which gets converted to hydrogen peroxide. So, this is also something that is a highly reactive molecule. The moment you produce hydrogen peroxide or the superoxide, these molecules are quickly neutralized by glutathione peroxidase.

So, they are neutralized by glutathione. So, glutathione itself gets oxidized and will reduce these molecules and then, glutathione is regenerated by glutathione peroxidase. So, these molecules help in consuming these reactive species. Now, glutathione reductase is the enzyme that will result in the production of glutathione from the oxidized glutathione.

This is the reduced glutathione; this is the oxidized glutathione. So that is regenerated by glutathione reductase and it uses NADPH. So, in essence, we need NADPH as a cofactor for the neutralization of these reactive oxygen species and this NADPH is supplied by this PPP pathway. So, glucose 6-phosphate gets converted to 6-phosphoglucono-delta-lactone, and that is where NADPH is produced, which is shown in the previous slide. So, to this, you get NADPH production, which helps in the regeneration of glutathione and then another molecule of NADPH is produced.

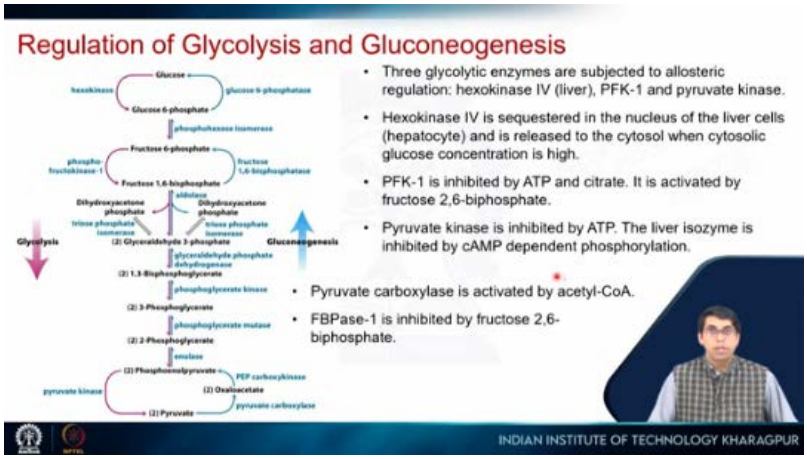
Now, it turns out that this enzyme, glucose 6-phosphate dehydrogenase, its deficiency is almost as high as 25% in tropical Africa, parts of the Middle East, and Southeast Asia. So, these are the regions where malaria is most prevalent, the people who are residing in these regions for a long time. In those populations, almost 25% of them have been found to have

a deficiency of this gene, which means that there is some mutation in the gene so that this enzyme is not functional. So, what does that mean?

How does such a high proportion of people have that? The hypothesis that people have come up with is that in these regions, malaria is prevalent. Now, the growth of the malarial parasite, which is Plasmodium falciparum, so this particular parasite is inhibited in G6PD or glucose-6-phosphate dehydrogenase deficient erythrocytes. Why? Because this parasite is very sensitive to oxidative damage so, it is killed by a certain level of oxidative stress. So, if these types of species are produced, they will kill the parasite. However, that level of stress is tolerable to the human host. So if there is enough oxidative stress, which is tolerable to us, but not tolerable to the parasite, then since we do not have this, we are not producing enough NADPH, which means we are not neutralizing enough of this molecule.

So there is oxidative stress, but it is not too much. That it will kill the host. So it only kills the parasite. So natural selection, it seems like it has sustained the glucose-6-phosphate dehydrogenase deficient genotype where malaria is prevalent and this is something again we will see a lot when we discuss evolution.

So coming back to glycolysis and gluconeogenesis, again, if we look at glycolysis and gluconeogenesis, it seems like we are going from glycolysis, glucose to pyruvate via glycolysis, and pyruvate to glucose via gluconeogenesis. Seven out of ten enzymes are the same. So it seems like there might be a futile cycle that can go on between these two.



So if that happens, then we will keep on hydrolyzing ATP because you consume more ATP and GTP during gluconeogenesis than you produce during glycolysis. So that will be detrimental, which means that there has to be some control so that when you need glycolysis, there should not be gluconeogenesis, or when you need gluconeogenesis, there should not be glycolysis. So both pathways should not occur simultaneously at the same time in the same place. and that is exactly what happens.

The three glycolytic enzymes are subjected to allosteric regulation. So what are these three enzymes? Hexokinase, this one. So remember that gluconeogenesis happens in the liver.

So the hexokinase that is present in the liver is the one that is allosterically regulated. So, it turns out that there are four isoforms of hexokinase: 1, 2, 3, and 4, and isoform 4 is present in the liver. So, what is an isoform? It is the same enzyme, but the amino acid sequence is slightly different, which will result in slightly different behavior of that protein. So, they carry out the same kinetic reaction.

So, hexokinase 4 is present in the liver. It is allosterically regulated. PFK-1, phosphofructokinase 1, is also allosterically regulated and pyruvate kinase is also allosterically regulated. So these are the exact three steps that are irreversible.

So those three steps are allosterically regulated. So what is the regulation? Hexokinase 4 is sequestered in the nucleus of the liver cells. So the liver cells are also called hepatocytes. When there is no need for glycolysis and it is released into the cytosol when cytosolic glucose concentration is high. So when there is a lot of glucose, like after a healthy lunch, there is a lot of glucose production. So there is a lot of glucose in the cytosol. This enzyme will be released. Otherwise, there is a protein that binds to hexokinase and transports it into the nucleus so that it is not performing its reaction. Phosphofructokinase 1, or PFK-1, is inhibited by ATP and citrate. So again, if you have enough ATP, it means that you have an abundance of high-energy molecules, so you do not need glycolysis so high ATP molecules or a high concentration of ATP will inhibit this. A high concentration of citrate, which is formed from the citric acid cycle, the first step of the citric acid cycle, will also inhibit this PFK.

So these are indications that you have enough of these molecules, so you don't need to go into glycolysis. However, it is activated by fructose 2,6-bisphosphate. So, remember that this is just a note that this is a different molecule. So, the one that is produced here during glycolysis is fructose 1,6-bisphosphate. However, this is fructose 2,6-bisphosphate.

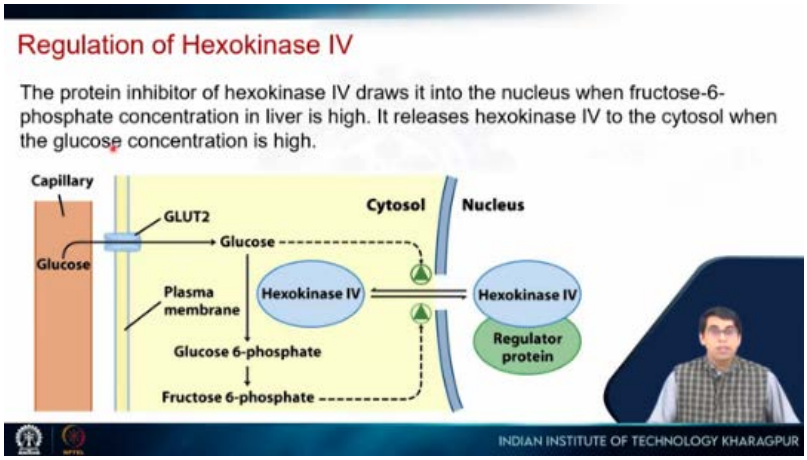
So, I will come to this later in a subsequent slide. Finally, pyruvate kinase, the last enzyme, is also inhibited by ATP. So, if you have enough ATP, it will be inhibited. The isozyme that is present in the liver is inhibited by cyclic AMP-dependent phosphorylation. So, I will show you that in more detail in a subsequent slide.

For gluconeogenesis, pyruvate carboxylase, this enzyme is activated by acetyl coenzyme A. So again, the presence of acetyl coenzyme A means that there is enough energy. So these are high-energy molecules. You don't need to go through glycolysis. You can actually store pyruvate as glucose. So you can now store this as glucose.

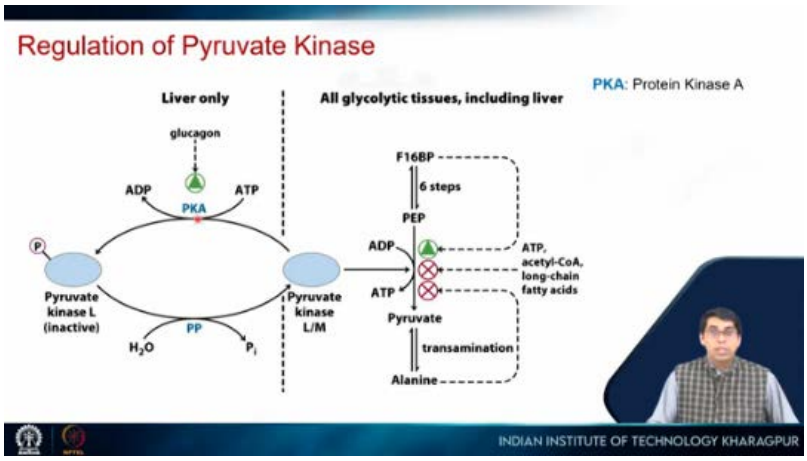
Convert it into glycogen and store it in the muscles for future use. FBPase-1, fructose 1,6-bisphosphatase 1. It is inhibited by fructose 2,6-bisphosphate.

So you see that this enzyme is activated by fructose 2,6-bisphosphate and the corresponding enzyme is inhibited by fructose 2,6-bisphosphate. So this is activated by fructose 2,6-bisphosphate, this is inhibited by fructose 2,6-bisphosphate. So the presence of fructose 2,6-bisphosphate will inhibit gluconeogenesis and activate glycolysis. So this is how we get regulation between these two processes.

So let us look at some of these in more detail. Regulation of hexokinase 4. So hexokinase is present in the cytosol when there is enough glucose so that it will trigger glycolysis. This is in the liver cell. However, when glucose is not present, it will bind to this regulator protein and get transferred to the nucleus.



So if you have enough fructose 6-phosphate, it will trigger this so it will transfer hexokinase into the nucleus to stop further glycolysis. When there is less fructose 6-phosphate and more glucose, then it will trigger the dissociation and it will transport this hexokinase into the cytosol so that glycolysis can proceed. Pyruvate kinase, this happens only in the liver so there is this enzyme protein kinase A. So we have learned that kinases are enzymes which use ATP and phosphorylate other protein molecules.



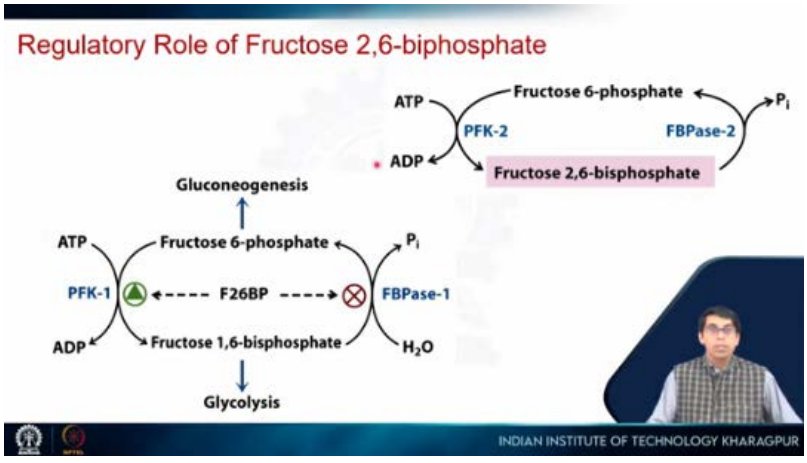
So when there is a lot of glucagon, it will result in the production of the secondary metabolite, which is cyclic AMP. Cyclic AMP activates this protein kinase A and that will phosphorylate pyruvate kinase in the liver. So once it gets phosphorylated, it will become inactive. So no more pyruvate is formed.

However, when cyclic AMP levels are low, then these Phosphatase is activated, so it will remove the phosphate group, and this becomes activated. So pyruvate kinase is also activated by other molecules and inactivated by other molecules. So it is inactivated by

ATP. So again, if you have enough ATP or acetyl coenzyme A or long fatty acid chains, these are all high-energy molecules, you don't need glycolysis.

So it will prevent this step, phosphoenolpyruvate to pyruvate conversion. However, if there is fructose 1,6-bisphosphate, one of the early steps, so there is an accumulation of this molecule, then that will come and activate pyruvate kinase. Also, pyruvate is used to produce alanine so, just one step, transamination, and you get alanine. If you have enough or a decent amount of alanine, so high levels of alanine, it will also go and Inhibit this enzyme so no more pyruvate formation. So, all these different metabolic products which are there, when they are present in abundant amounts, you shut down this process. When they are not present in abundant amounts and you have an accumulation of this molecule, you activate this process. So, all of these steps are highly regulated.

So, let's look at fructose 2,6-biphosphate. So, fructose 1,6-biphosphate is something that is produced in the normal pathway. But fructose 2,6-biphosphate is also produced from fructose 6-phosphate by a different enzyme, which is phosphofructokinase 2. So, remember phosphofructokinase 1 produces fructose 1,6-biphosphate, and phosphofructokinase 2 produces fructose 2,6-biphosphate. So, once you have this molecule, it will activate PFK-1, so it will activate glycolysis, and it inactivates FBPase-1, so it will inactivate gluconeogenesis. So, this molecule allows glycolysis and stops gluconeogenesis when you do not have this, then you will have gluconeogenesis and no glycolysis So, it will not allow both steps, and that is this futile cycle to occur.



So, these are again the books that you can refer to. So, you can go through any biochemistry book; you can look at Lehninger's Principles of Biochemistry as the primary reference. Thank you.