Overview and Integration of Cellular Metabolism

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Lecture 08: Regulation of Glycolysis and Neoglucogenesis (I)

Hello everyone, welcome back to the session Overview and Integration of Cellular Metabolism today's class is about regulation of glycolysis and Neoglucogenesis. In this class we will cover the concepts like how glycolysis and Neoglucogenesis are reciprocally regulated at the level of different enzymes activity like hexokinase 4 and glucose 6 phosphatase. Hexokinase 4 is the enzyme of glycolysis and glucose 6 phosphatase is the enzyme of Neoglucogenesis, how these two are reciprocally regulated. Similarly, another level of reciprocal regulation is at the level of phosphofluctokinase present in glycolysis and fructose 1 6 bis phosphatase which is present in Neoglucogenesis. Then again how molecule fructose 6 2 6 bis phosphate regulate glycolysis and Neoglucogenesis will be discussed and also the regulation of pyruvate kinase will be discussed in this session. Now, before going to the discussion of regulation of these different enzymes if you remember from the very second class of this metabolic discussion that enzyme activity in different metabolisms are mostly regulated by two ways.

One is short term regulation another is long term regulation. Now, long term regulation always regulate the concentration or numbers of enzyme. So, basically in long term regulation synthesis and breakdown of enzyme these two activities are coordinated as per the requirement of the body. Now, the thing is that this is called long term regulation as you know that for the synthesis or breakdown of the proteins or enzymes it will take time.

Whereas, for immediate action for require for immediate requirement of body there is short term regulation of these enzymes and the short term regulation is done mostly by one is covalent modification another is allosteric regulation. Now, in glycolysis and Neoglucogenesis we will see that this short term regulation as well as long term regulation these are mostly controlled by two most important hormone one is insulin another is glucagon. So, this is the hormonal control of glycolysis and Neoglucogenesis. Here for formation of pyruvate from glucose via glycolysis insulin stimulates the

function. So, insulin stimulate glycolysis whereas, glucagon just serves the antagonistic role here.

In Neoglucogenesis the these two hormones acts just opposite to what is happening in glycolysis that is insulin here is acting as one antagonistic hormone. Whereas, glucagon as well as glucocorticoid these two hormone stimulate Neoglucogenesis. So, basically formation of glucose from pyruvate. Now, mostly these two hormones regulate the enzyme activity by covalent modification sometimes they stimulate some other molecules which can regulate these two metabolic pathway by allosteric regulation. So, this is as a whole you can see where glycolysis and Neoglucogenesis are being depicted.

So, basically from glucose to formation of pyruvate is the glycolysis again on requirement this pyruvate can synthesize can enter in fact, the TCA cycle and finally, it produce the TCA cycle intermediate oxaloacetate in Neoglucogenesis which oxaloacetate is used up to re synthesize glucose. Now, you can see there are different level of control in this two metabolic pathway and they are these two metabolic pathways somehow reciprocally regulated the molecules or the signals which stimulate glycolysis are actually inhibiting Neoglucogenesis. Similarly, signals which stimulate Neoglucogenesis is actually inhibiting glycolysis. So, this is called the reciprocal regulation of glycolysis and Neoglucogenesis. Now, one by one we will discuss different steps of the regulation different steps of regulation in these two pathways.

Now, in glycolysis one important hormone is one important enzyme is hexokinase. Now, hexokinase enzyme it has four different isoforms from isoform 1, 2, 3 and 4. Now, the type 2 or isoform 2 hexokinase 2 is the predominant hexokinase which is present in muscle myocytes whereas, the type 4 or hexokinase 4 is predominantly present in liver. Now, these two isoforms act differently or respond differently to the different stimuli. The type 2 hexokinase it has higher affinity towards glucose.

So, hexokinase 2 is easily saturated with glucose. So, basically this hexokinase type 2 is half saturated at the glucose concentration of just 0.1 millimolar whereas, if you know from the I mean from your previous chemistry concept that the level of blood glucose are mostly 4 to 5 millimolar in our circulation. Now, so, basically hexokinase 2 is easily can easily be saturated with the available blood glucose level. So, it can achieve the maximum velocity can form glucose 6 phosphate from glucose with maximum concentration.

Now, this glucose 6 phosphate molecule it inhibits hexokinase type 2. So, this exert an inhibitory effect over hexokinase type 2, but in case of the liver isoenzyme that is hexokinase type 4 it responds or acts differently like affinity towards glucose for this

hexokinase 4 is low with comparison to the isoform 2. And it is half saturated at the concentration of 10 millimolar glucose. So, basically these enzyme mostly activated after a meal when the blood glucose level is raised much higher. Now, how is this relevant with respect to the physiological condition? So, liver always act to balance or maintain homeostasis blood glucose homeostasis.

Whereas, muscle in case of muscle, muscle needs more energy for its action for its contraction. So, the primary action of in liver the primary action of glucose is not to provide energy rather the blood glucose level. How? After post meal when there is a raised blood glucose concentration liver needs to balance it. So, basically what happens when there is a raised blood glucose concentration hexokinase type 4 in liver is activated easily glucose enters inside liver with the transporter GLUT 2. Now, activated hexokinase 4 convert glucose to glucose 6 phosphate and direct this glucose 6 phosphate towards different pathways like glycolysis or HMP shunt like that.

Now, importantly glucose 6 phosphate which has one inhibitory effect over this muscle isoform has no inhibitory effect in liver isoform. So, what happens even if there is formation of glucose 6 phosphate it is not able to inhibit. So, basically glucose is continuously uptaken inside the cell and is directed to different metabolic pathways till the blood glucose level comes to much lower concentration and then only the liver isoform is inactivated. Now, apart from that this liver isoform is also controlled by one regulatory protein. Now, what happens it is this regulatory presence of this regulatory protein is only specific for the liver isoform.

Now, what happens this regulatory protein is bound to hexokinase and it is present inside nucleus. Remember all the glycolytic enzymes are present in cytosol whereas, hexokinase 4 the liver isoform is present inside the nucleus. Now, when in case of raised blood glucose level glucose entered through GLUT 2 these glucose actually exert some positive effect over this hexokinase 4. How glucose after entering inside the cell it enters inside the nucleus here it dissociates this hexokinase 4 and the regulatory protein. So, hexokinase 4 easily can come out inside the cytosol here inside the cytosol this convert glucose to glucose 6 phosphate for further pathways.

Now, another product of glycolysis which is fructose 6 phosphate it has inhibitory effect over this dissociation, dissociation of hexokinase 4 and this regulatory protein. So, basically fructose 6 phosphate actually inhibits this dissociation. So, basically it actually it sends back this hexokinase 4 inside the nucleus. So, this is how the different types of hexokinase are affected based on the requirement of body whereas, liver serves as a balance acts for the balance of blood glucose level whereas, muscle isoform are mostly doing the energy supply in muscle. Then hexokinase 4 and glucose 6 phosphates they are

transcriptionally regulated.

Now, if you remember again in glycolysis hexokinase 4 is basically converting glucose to glucose 6 phosphate whereas, in neoglucogenesis formation of glucose from glucose 6 phosphate is done by glucose 6 phosphates. So, these two enzymes are transcriptionally regulated. So, this basically this is an example of long term regulation where on requirement more amount of enzymes are synthesized. Now, what happens these transcriptional regulation is mostly done by the enzyme the hormone insulin and glucagon and the signal for these conversion to the phosphorylated form or the dephosphorylated form are the signals I told you in the previous class that the signals are few molecules like ATP which signals for excess energy NADH like that energy requirement or energy require I mean the demand for energy is signaled by ADP presence of ADP AMP or NAD. So, these molecules basically signals for the transcription or synthesis of more enzymes.

So, this is how hexokinase 4 and glucose 6 phosphates are transcriptionally regulated. Then we are coming to another set of reaction that is formation of fructose 6 fructose 1 6 bisphosphate from fructose 6 phosphate which is occurring in glycolysis and it is done by the enzyme phosphofructokinase 1 whereas, in case of neo glucogenesis the reversal of this reaction is done by fructose bis 1 6 bis phosphatase and the isoform here is 1. Now, again these two enzymes are regulated by the signaling molecules like ATP ADP AMP. So, basically once again when there is excess energy as signal by presence of amount more amount of ATP. So, glycolysis is not required.

So, phosphofructokinase 1 is basically inhibited. Similarly, presence of ADP or AMP that is a hydrolyzed form of ATP it these two molecules signals for the requirement of energy means there is less amount of ATP. So, what is required? Synthesis of more ATP via glycolysis. So, ADP and AMP are basically activating phosphofructokinase 1. Similarly, these are having opposite effect over fructose 1 6 bisphosphatase.

Now, citrate also is another molecule which signals for adequate amount of energy in body means there is more glycolysis more production of pyruvate formation of acetyl coenzyme which is finally, entering in TCA cycle and forming citrate and so, there is more amount of citrate which can further produce ATP. So, basically this also citrate molecule also signals for adequate amount of energy in cell. So, it inhibits the glycolysis by inhibiting phosphofructokinase 1. Now, here you can see one very important molecule of this reciprocal regulation that is fructose 2 6 bisphosphate. Fructose 2 6 bisphosphate what this molecule does? It activates phosphofructokinase and simultaneously it inhibits fructose 1 6 bisphosphatase.

So, basically fructose 2 6 bisphosphate this molecule is actually activate glycolysis and inhibits neoglucogenesis. Now, let us see how this reciprocal regulation by fructose 2 6 bisphosphate is delivered. Now, fructose 2 6 bisphosphate is basically a byproduct of glycolysis. So, while forming fructose 1 6 bisphosphate which occurs in glycolysis with the help of the enzyme phosphofructokinase 1, fructose 6 phosphate actually . The fructose 6 phosphate while forming fructose 1 6 bisphosphate from phosphofructokinase 1, it also forms certain amount of fructose 2 6 bisphosphate with the enzyme phosphofructokinase 2.

Now, this phosphofructokinase 2 enzyme is basically a bifunctional enzyme. So, in one single enzyme molecule there are two different form to there are two different types of enzyme activity. One is the kinase activity another is the phosphatase activity on requirement either of the one activity is present in the cell. So, you can see this is a bifunctional enzyme it has kinase activity in the form of phosphofructokinase 2 as well as as well as it has phosphatase activity in the form of fructose bisphosphatase 2 isoform. It is the fructose bisphosphatase 2 remember the bisphosphatase 1 is present in neo glucosinases.

Now, when there is glucose is lower in circulation there is release of glucagon. Now, this glucagon hormone actually activate the cyclic AMP mediated pathway which activate cyclic AMP dependent protein kinase. Now, this protein kinase definitely phosphorylates phosphorylates whom this bifunctional enzyme. Now, when this bifunctional enzyme is phosphorylated the phosphatase form is activated and the kinase form is deactivated. So, with the help of cyclic AMP dependent protein kinase there is phosphorylation of this bifunctional enzyme.

So, basically the phosphatase iso the phosphatase function of the bifunctional enzyme is activated. Now, because the active and because phosphatase is activated fructose 2, 6 bisphosphate is hydrolyzed. So, the level of fructose 2, 6 bisphosphate is low and so, there is inhibition of glycolysis by inhibiting phosphofructokinase 1. But then when there is increased blood glucose level which release the hormone insulin, now insulin activates phosphatase phosphoprotein phosphatase and it dephosphorylates this bifunctional enzyme. On dephosphorylation what happens the phosphatase remains inactive whereas, the phosphofructokinase 2 function is activated.

On activation phosphofructokinase 2 it forms fructose 2, 6 bisphosphate and this fructose 2, 6 bisphosphate it stimulates glycolysis by inducing phosphofructokinase 1 or as you can see fructose 2, 6 bisphosphate it like it activate the phosphofructokinase 1 it deactivate the fructose bisphosphatase type 1 which is actually converting fructose 1, 6 bisphosphate to fructose 6 phosphate in neo glucogenesis. So, basically once again to

remind that fructose 2, 6 bisphosphate is an allosteric regulator it activates glycolysis by activating the enzyme phosphofructokinase 1 and it deactivates the fructose bisphosphatase 1 in neo glucogenesis. Next is pyruvate kinase now pyruvate kinase enzyme has different isoform again to remind that pyruvate kinase actually it converts phosphanol pyruvate to pyruvate. Now, pyruvate kinase isoforms are mostly regulated once again by the energy signaling molecules like ATP or acetyl coenzyme A or long chain fatty acid from where we get acetyl coenzyme A. So, these are the deactivator of glycolysis.

So, basically it these molecules actually inhibit glycolysis. Now, alanine which is formed from pyruvate by transamination these also inhibit the enzyme pyruvate kinase. Similarly, fructose 1, 6 bisphosphate which is an intermediate of glycolysis excess presence of fructose 1, 6 bisphosphate indicates there is adequate supply of glucosin cell which induces the pyruvate kinase enzyme to form more amount of pyruvate. Now, amongst the different isoform only the liver isoform is regulated by covalent modification and this covalent modification once again is done by phosphorylation and dephosphorylation that is a reversible phenomena. Now, when phosphorylated this pyruvate kinase remain inactive again when there is low blood glucose level there is release of glucagon.

Glucagon activates the protein kinase cyclic AMP dependent protein kinase. Now, these kinase actually phosphorylate the pyruvate kinase. Similarly, with the help of the hormone insulin there is activation of protein phosphoprotein phosphatase and there is dephosphorylation on dephosphorylation protein pyruvate kinase is activated and rendering is function for formation of pyruvate for phosphanol pyruvate. So, this is how pyruvate kinase is allosterically regulated. Then the conversion of pyruvate to phosphanol pyruvate is also regulated by multiple methods.

So, one important molecule is acetyl coenzyme A. Now, acetyl coenzyme A inhibits the enzyme pyruvate dehydrogenase which is forming acetyl coenzyme A form pyruvate and it activates the enzyme pyruvate carboxylase which is present in neo glucogenesis and converts pyruvate to oxaloacetate. Now, if you remember pyruvate after it enters inside the mitochondria to be converted to oxaloacetate also in mitochondria only this pyruvate is converted to acetyl coenzyme A. So, basically it is the metabolic status of the cell which decides the fate of pyruvate whether it will go towards neo glucogenesis or it will go towards glycolysis. So, after entering inside the mitochondria there are certain signals which decides the fate of pyruvate.

Now, one such signal is acetyl coenzyme A. Now, if there is huge amount of acetyl coenzyme A which is supplied by beta oxidation of fatty acid it signals that there is

enough amount of energy in cell enough amount of acetyl coenzyme A which can enters in TCS cycle. So, no further requirement of formation of acetyl coenzyme A from pyruvate. So, there is inhibition of the enzyme pyruvate dehydrogenase whereas, these acetyl coenzyme A also signals for activation of neo glucogenesis that there is huge amount of acetyl coenzyme A which can reform glucose from acetyl coenzyme A. Now, another enzyme phosphanol pyruvate carboxy kinase it signals it is also activated by different signals and this is required for formation of oxaloacetate to phosphanol pyruvate. Now, phosphanol pyruvate carboxy kinase are also transcriptionally regulated and also say and signal by the energy signal molecules like ATP AMP or NADH NAD.

Next is different transcriptional regulation. So, basically these enzymes are regulated transcriptionally for long term regulation. Now, in long term regulation definitely there is on requirement there is more synthesis of enzymes. Now, there are different transcription factors which actually signals for synthesis of different enzymes of glycolysis and neo glucogenesis. Now, you can see here xylulose 5 phosphate is one such signal molecule which can activate the transcription of different enzyme and these xylulose 5 phosphate comes from HMP shunt. Remember when there is excess amount of glucose these glucose enters cell and it is for it is converted to glucose 6 phosphate.

Now, this glucose 6 phosphate it can either it can go towards glycolysis as well as it can enter HMP shunt. Now, with HMP shunt there is xylulose 5 phosphate formation which activates the phosphoprotein phosphatase 2 A. Remember it causes dephosphorylation phosphoprotein phosphatase. Now, what happens there is one transcription factor carbohydrate responsive element binding protein. It is mostly expressed in liver adipose tissue kidney.

Now, this carbohydrate response element binding protein is activated by xylulose 5 phosphate. How? CHRAPP is mostly present in cytosol in phosphorylated condition and there are 2 phosphoryl group present in this transcription factor. Now, xylulose 5 phosphate activate phosphoprotein phosphatase 2 A which dephosphorylates and one molecule one phosphoryl group is basically hydrolyzed. Now, this dephosphorylated form it enters nucleus through nuclear pore. In nucleus pore there in nucleus there is nuclear form of phosphoprotein phosphatase 2 A which is also activated by xylulose 5 phosphate.

So, there is another level of dephosphorylation. Now, the this dephosphorylated CHRAPP can bind with MLX and it forms the activated transcription factor which actually binds in the carbohydrate response element that is a that is a this signal is present in DNA basically. So, this acts as a promoter for mRNA synthesis and CHRAPP MLX this transcription factor activates the transcription of different enzymes which is

present in glycolysis like hexokinase, phosphofructokinase 1, pyruvate kinase. So, by xylulose 5 phosphate actually glycolysis is activated and this actually inactivate the other enzymes like neoglucogenesis enzymes of neoglucogenesis are inactivated. Similarly, there are other transcription factor present like SREBP 1C which is Terol response element binding protein also CREB is a transcription factor CREB stands for cyclic AMP response element binding protein. So, this is the long term regulation of neoglucogenesis and glycolysis.

So, these are the different inducer and repressor or activator or inhibitor of different enzymes present in glycolysis and neoglucogenesis like hexokinase, phosphofructokinase 1, pyruvate kinase, pyruvate dehydrogenase these are mostly activated or induced by insulin hormone and repressed by glucagon as well as there are the list of activator I have already discussed. Similarly, in neoglucogenesis pyruvate carboxylase, phosphanol pyruvate carboxykinase, glucose 6 phosphatase these enzymes are induced by glucocorticoid, glucagon epinephrine and inhibited by insulin and also these are the activators and inhibitors of neoglucogenetic enzymes. So, at the end of this session these are the key points we need to remember that in glycolysis and neoglucogenesis there are 3 irreversible steps present which are catalyzed by different enzymes and those are the 3 points where basically these 2 pathways are regulated. Then to limit cycling between glycolysis and neoglucogenesis these 2 pathways are actually reciprocally regulated by allosteric mechanism which is mainly achieved by the opposing effects of fructose 2, 6 bisphosphate enzyme and this fructose 2, 6 bisphosphate basically regulate phosphofructokinase 1 and fructose bisphosphatase 1 activity. Then there are different level of transcriptional regulation where transcription factors like CHREBP, CREB, SRDP they regulate the synthesis of different enzymes of glycolysis and neoglucogenesis and all these regulations are mostly done by hormones insulin and glucagon.

These are my references. Thank you all. See you in the next class. Thank you.