Molecular Biology
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Module - 06
Transcription
Lecture-28 Gene Control Mechanism (Part 1)

Hello everyone, this is Dr. Vishal Tewedi from department of bios assessment by engineering IIT Guwahati. And in this particular course, we are discussing about the different aspects of the molecular biology. So, for what we have discussed, we have discussed about the basic structures of the cell. So, we have discussed about the prokaryotic structure and eukaryotic structures. Following to that, we have also discussed about the different types of biomolecules, we discussed about the different types of cellular activities.

And in the previous lecture, we have discussed about the central dogma of molecular biology and we have also discussed about the different components, which are be a part of the central dogma of molecular biology. So, we have discussed about the replication in detail about in the previous module. And we have discussed about the replication in the prokaryotes and the replication in eukaryotes. And then we also discuss about how the replication is helping the cell to recover from the different types of cellular damages or DNA damages actually.

In the current module, we are discussing about another important aspect related to the central dogma of molecular biology and that is the transcription. So, if you recall in the previous few lectures, we have discussed about the transcription in eukaryote prokaryotes followed by the transcription in eukaryotes. And in the previous lecture, we have discussed about the how the post-transcriptional modification is happening in the different types of RNA species, what is going to be produced from the DNA and then how these modifications are enabling these RNA species to work optimally for the protein production. Now, if you see the central dogma of molecular biology, what you see is that the ultimate goal of the central dogma of molecular biology is that it is going to produce a protein or I will say protein or the enzyme actually. And these proteins or the enzymes are actually going to participate into the different types of the metabolic reactions.

For example, when we are discussing about the carbohydrate metabolism in the eukaryotic cells, we discussed that the carbohydrates are actually as soon as the glucose enter into the cell, it get phosphorylated by the hexokinase followed by the phosphorylation by the different types of followed by the glucose phosphate and other

kinds of molecules and ultimately it is going to channelize that particular glucose molecule into the glycolysis. So, if the glucose if you see about glucose metabolism glucose will enter and then it is actually going to be processed by the different types of enzyme to produce the pyruvic acid. And these cascades of the reactions are going to be called as the glycolysis which occurs within the cytosol and pyruvic acid then will enter into the Krebs cycle and ultimately it is actually going to produce the different amount of energy and it is also going to produce the different types of intermediates which are actually going to be utilized for the different types of synthesis. So, Krebs cycle is going to provide the raw material for the synthesis of the different types of amino acids. Krebs cycle is going to provide the raw material to synthesize the hemine which is very important component for the blood synthesis or in general I will say hemoglobin synthesis and then Krebs cycle is also important for the synthesis of the fat and as well as the nucleic acid.

So, all these components are important because they are actually going to be required for running the different types of the life related activities. Now imagine that when the glucose level is down right or there is no supply of glucose for example, the glucose from the glucose from where your glucose will come the glucose will come from the food and as well as the glucose will enter into the cell it will go through with these kind of reactions, but if there will be access of fat then these reactions will go in this direction right and this will go in this direction and there will be less utilization of glucose and if there will be less utilization of glucose the enzymes which are being present within the glycolysis are going to be down regulated because there is a only when mechanism there are several mechanism what we have discussed when we were talking about the glycolysis or the Krebs cycle how the Krebs cycle and the glycolysis is being regulated, but apart from the feedback mechanism or allosteric regulations there is a also another level of regulation is that you are going to have the regulation at the level of the protein synthesis which means you are actually going to make the availability of these enzymes or the protein at lower level or the higher level and depending upon the amount of these proteins or the enzyme that particular type of activity is actually either will go up or will go down actually. So, these kind of events are more relevant when you are talking about the bacterial system because the bacterial system is going to have the polycystronic you know the transcriptional unit right compared to that eukaryotic system has the monocystronic transcriptional unit. So, when you have polycystronic transcriptional unit this means you are going to have the different types of enzymes being produced simultaneously from the single transcript and in that case all the protein synthesis of all these enzymes are actually going to be under tight regulation or tight control. So, that you will be able to have the complete control over the different types of events such as glycolysis, Krebs cycle and all other kinds of things right.

Although the Krebs cycle is not present in the bacterial system or the prokaryotic system but for the sake of examples there could be say many more other kinds of pathways for example you are going to have the fatty acid synthesis pathway and you are going to have the other kinds of pathways like amino acid biosynthesis pathway and so on. So, all these pathways require a very tight control and one of the mechanism through which the bacteria is actually bringing the control is by up regulating and down regulating the amount of protein what it actually going to synthesize. And this is all been achieved by putting these enzymes or putting these genes or putting the transcriptional unit under a complete control mechanism right and all these are been a part of the operon which means a system which actually is going to operate or going to regulate the transcription of these particular transcription followed by the synthesis of these proteins. So, a typical bacterial cell contains several thousand genes some genes carry out the universal task and are constantly active these are called as the housekeeping genes. For example, the housekeeping genes include those that facilitate the synthesis of the protein and the ribosomal RNA.

The majority of the genes however only become active when their byproducts are needed such genes should not be expressed constitutively because the energy could be could be used for more productive task. So, what is mean by the constitutively means that it is actually going to be expressed throughout the life cycle it is not going to be induced right. So, you can actually have the two different two modes of the expression one is called as the constitutive the other one is called as the induced. So, induced means in case you have some action some kind of thing right for example, when you stand in the sun you are going to have the sweating that. So, that sweating reaction are induced reaction because they are being induced by the sunlight whereas, you are going to have the constitutive reactions constitutive reactions means you are going to have the irrespective of whether you are in sun or light or whatever you are going to have that for example, the running of our heartbeat right.

So, our heartbeat is going to be constitutive reactions. So, such gene expression is controlled. So, the induced gene expression is controlled. So, their products are only produced when they are required in accordance with the need of the cell the phase at which the gene expression can be controlled are numerous. So, in prokaryotes the most common step at which the regulation of gene expression occur is the transcriptional initiation right.

You remember that we have said that in the prokaryotes the transcription and the translation go together so, there is no regulation at the translational level in the in the case of prokaryote as soon as the RNA is being produced it is been present in the cytosol right. So, and it is actually going to be taken up by the translational machinery and there

will be a synthesis of the protein. And what you can actually control is the initiation part right as if you do not allow the RNA polymerase to go and bind to the promoter and sit and start the transcription then it is actually going to be controlled. So, it is energetically the most efficient step to regulate the gene expression. The transcriptional regulation occur in step after initiation specifically during elongation and termination.

Prokaryotic transcriptional regulation is accomplished by the gene regulating protein that bind with the regulatory sequence near the transcription site of the transcriptional unit. Gene regulatory protein the product of gene regulatory protein are of 2 types they can be activators and they can be repressor. So, gene regulatory proteins or the gene regulatory components are actually going to be the regulatory units which are going to regulate the efficiency of the RNA polymerase to go and sit on to the transcriptional initiation site and that is how they are actually going to control the transcription. These gene regulatory proteins or the protein gene products could be activator. So, that actually going to activate.

So, they can actually be able to facilitate the RNA polymerase to go and bind to the initiation site or they could be repressor. So, that if they are actually going to block. So, activator means they are actually going to activate repressor which means they are actually going to block or they are going to inhibit the process. In the absence of both activator and repressor transcription carried out by RNA polymerase is called as the basal level of transcription. The binding of a repressor decreases the transcription less than the basal level.

So, repressor is inhibitor right it is going to inhibit whereas, the binding of an activator increases the transcription which is the above the base level. If both the both repressor and activator represent and functional the action of the repressor typically overtake over that the transcription. The basic concept of how gene regulation occur at the transcriptional initiation in bacteria are provided by the classical model called the operon model. This is formulated by the Jacobson Monnet in the 1961. So, how these repressor and the activators are regulating the gene expression profiling and how they are actually regulating the transcriptional activities within the bacteria is being provided by a classical models and these models are called as the operon model and these models are hypothesized and formulated by the Jacobson Monnet in the year of 1961.

So, the question comes what is operon? So, operon is a set of genes. So, operon is a genetic regulatory system mostly seen in the prokaryotes and the bacteriophage in which a group of structural gene are transcribed together under the control of a single promoter, which means the operon technically the operons are over sleeve in present in the polycistronic transcriptional unit and they are going to be present in the prokaryotes and

the bacteriophage where you are going to have the group of structural genes or I will say the genes which is going to be transcribed for the different types of enzymes or the structural genes and they will be under the control of a single promoter. This means this single promoter is actually going to have the control over the synthesis of these structural genes. So, you are going to in a typical operon you are going to have the operon promoter next to the promoter are going to have the operator and the objects to the operator you are going to have this is, this is going to be the coding region. So, this is going to be the coding region and this coding region is going to be responsible for.

So, this is the coding region, and then you are going to have the poly A site. So, the coding region is going to be responsible for for the synthesis of the A protein, B protein and the C protein. So generally operands are very common in prokaryotes and the bacteriophage but it is also found in some eukaryotes. The main difference is that the expression of prokaryotic operand leads to the polycystronic messenger RNA whereas the eukaryotic operands leads to the monocystronic messenger RNAs. In this particular lecture we are mostly been focused on to the operand what is being present in the prokaryotic system.

We have not discussed about the operands in the present in the eukaryotic system. So the idea is that you should we should be able to tell you the concept of the transcriptional activations and transcriptional regulations and how the things are been done similar kind of things are also been done in the eukaryotic system. So prokaryotes are the single cell organisms lacking a true nucleus and the membrane bond organelles. It adopt the operand system as a mechanism to efficiently regulate the gene expression in response to the changing environment conditions. Environment condition means the requirements of the different types of metabolites, availability of glucose, availability of oxygen and so on.

So the bacteria has since bacteria is a single cell organisms it actually gets affected very oftenly and it is actually has to respond to these changes. The operand system is a genetic regulatory system found in the prokaryotic organism that allowed the multiple gene with the related function to be controlled as a single unit. The system offers different advantage for prokaryotes like it is energy efficient because you are supposed to only synthesize the operator or the repressor and that actually is good enough to control and regulate the active transcriptional activity of several genes. So operand system allows them to coordinate the expression of multiple gene involved in a common pathway and transcribe together a single messenger RNA saving the energy and resources by producing the necessary protein only when required. Then it is actually going to have a rapid response to the environmental changes.

So the operand system enable them to adapt to the changing conditions quickly. If condition changed the expression of the relevant gene can be turned on or off rapidly. It is simple and compact right. So prokaryotes can use a single regulatory region to control the expression of multiple genes. This is particularly advantage in a small genome where saving space is very crucial.

So if you require a multiple genes as a regulatory genes and so on you are actually going to increase the size of the genome and that the bacterial system cannot afford because bacterial system has to conserve the energy, conserve the space and all those kind of things and that is why it is important that it should actually operate and control the multiple genes with the help of the operands. Then it has the coordinated regulations. It allows the bacteria to go with the coordinated regulation because it can regulate these three genes four genes five genes which are actually been present in the single pathway. For example if you are talking about the glycolysis you are going to regulate the hexokinase you are going to regulate the pyruvate kinase and so on. So since the all these genes are present in a single operand probably it is easy for the bacteria to manage all these and on the other hand it is also going to save the energy.

So there will be a coordinated regulation. So this coordinated regulation ensure that the product of genes these are produced in the appropriate stoichiometric ratio which means if you are processing the single glucose molecule you require one glucose molecule of hexokinase you require the one glucose molecule of aldolase and so on. So you can actually be able to produce these proteins and enzymes in a right proportion so that you should not waste the energy by producing some one or other in excess amount and on the other hand you should not have the lower production of any of these proteins. Then you have the resource allocations when a particular nutrient is available the genes required for the utilization are switched on. Once the nutrient becomes scarce the operation operand can be turned off preventing the wasteful production of the unnecessarily proteins.

Then we have the adaptation to the niche environment so the operand system enable the prokaryotes to adopt in a specialized niche by turning fine tuning the expression of genes that are specifically relevant to those conditions. So this is also very very important that you are actually having a very very fine and regulated balance and that is how you can be able to have the fine tuning of the expression of genes that are relevant that particular for that particular environmental conditions. Then you have the evolutionary advantage organisms with ability to regulate gene expression rapidly and efficiently in response to the environmental changes were more likely to survive and reproduce. So operand as I said you know is been proposed by the Jacobin monit and it is been control mechanisms through which the prokaryotic system is controlling the different types of gene and gene

expressions within the bacteria. So in 1965 the Nobel Prize in physiology and medicine was awarded jointly to the Jacobs Monet for discovery concerning the operon and the viral synthesis.

So this is the all scientists who have got the Nobel Prize in medicine in the 1965 for their concept of the operon. So before we get into the detail of the operons and how we are going to take up some of the examples of the operon it is important to understand what will be the structure of the general structure of an operon. So this is just a general structure of the operon you are going in the general structure what you are going to have. So this is the transcriptional unit right this is the transcriptional unit where you are going to have the promoters you are going to have the operators you are going to have the structural genes for example in this case this is a structural gene for A B and C and apart from that you are also going to have the regulatory genes. So regulatory genes are actually going to be a part of is going to produce the regulatory proteins and going to produce the regulatory proteins and these regulatory proteins could be activators right or it could be repressor and condition of the activator and repressor will go and bind to the operator right and so this region what you see here is actually a part of operon.

So regulatory gene is not going to be a part of operon and regulatory proteins are going to either activate or either going to facilitate the binding of RNA polymerase or it is actually going to have the other way around right. So regulatory gene encodes for a protein called regulatory protein which either act as a repressor or activator which control the operon but it is not a part of operon because it has its own promoter right. So regulatory genes are not a part of operon this is the part of operon where you are going to have the promoters operators and genes for the structural genes. Now let us talk about the regulation of an operon. So there are two types of transcriptional regulation which is going to be possible in the operon one is called as the first is called as the negative control and the second is called as the positive control.

So then the negative control in which the regulatory protein is act as a repressor which binding to the DNA and inhibiting the transcriptional of the protein right. So and then you have a positive control in which the regulatory protein is acting as an activator which is stimulate for the transcription. So this is the regulatory gene from which you are going to have the regulatory proteins and regulatory proteins could either be a repressor which means once it goes and bind to the operator it will not allow the RNA polymerase to go and bind to the promoter and that is how it is actually not going to allow the transcription of so there will be no transcription of the structural genes. This is going to be called as the negative control whereas in the case of positive control you are going to have the regulatory proteins which are actually go and bind to the operators and that is how they are actually going to facilitate the efficient binding of the RNA polymerase to

the promoters and that is how it is actually going to have the more production of the particular structural genes and these are called as the positive regulations. It means when you have this you are going to have the more production when you have this and you have going to have the lower production this means it is going to be negative regulation this is going to be a positive regulations.

Within this you are going to have the two different types of conditions either it going to be inducible or it going to be repressible. Then you going to have so operon can also be either inducible or repressible. So inducible operons are those in which the transcription is normally been off which is not going to take place and it needs inducer to induce the transcription which means it is going to be transcom it is going to be turn on. Repressible operons are those in which the transcription is normally been on which means there will be a basal level of transcription which is going to take place. Sometimes it may happen to repress the transcription or turn it off.

So you are going to have the positive control, negative control and then you can also have the inducible operon or the repressible operon. So this is what I have summarize here. So you are going to have the negative control. You are going to have positive control. And within the negative control or the positive control you can be having the inducible operon or the repressible operon.

So, in the negative control the product of the regulatory gene inhibits the transcription in a positive control the product of regulatory gene is going to activate the transcription. Whereas in the inducible operon your initial condition or I will say the basal level of transcription is going to be off which means you are not going to have the transcription of that particular gene. But once this inducible inducer is present then you are going to have the operon which is going to work. So, it is going to have the turn on the transcription whereas, in the case of repressible initial condition the basal level there will be a transcription, but when the repressible is present there it is actually going to turn off the transcription. So, let us first discuss about the negative inducible operon.

So, within the negative you can have the inducible you can actually having the so you are going to have the inducible or you are going to have the repressible. Even within the positive control also you can have the inducible or the repressible. So, there are several different conditions in which all these has to be understood. So, let us first take the first example that is the negative congruent inducible. So, in a negative so negative inducible operon in a negative inducible operon the regulatory gene encode a repressor which readily binds to the operator as operator side overlap with the promoter side.

So, that the binding of the repressor physically block the binding of RNA polymerase on

the promoter and prevent the transcription. So, for the initiation of transcription something is needed to prevent the binding of the repressor at the operation side and represent the operators site of binding that is the inducer. This type of system is said to be inducible since transcription is usually off and must be turned on. So, in this negative inducible operon what will happen is that you are going to from the regulator you are going to have the repressor. So, this is the repressor molecule which will go and fit and sit on to the operator.

So, since the repressor is sitting on the so if the no inducer is present this repressor will be keep binding to the operator molecule and it will not allow the transcription of the structural gene because repressor will bind to the operator and it will inhibit the transcription. So, there will be a transcription offs. And when the inducer is present what will happen is that the inducer is suppose the insulin for example, or I will say glucose. If the glucose is present what will happen is that the glucose will go and bind to this repressor. And in that case it is actually going to make the active repressor to inactive repressor and then the active repressor would not be able to bind the operator and as a result it is actually going to allow the transcription of these particular structural genes.

So, this is an example of or the mechanism in which the negative inducible operon is going to operate. We are going to take the few examples and then you will be able to understand this more nicely. And then we have the second condition. Second condition is that you are going to have the negative repressible operon. So the regulator gene in this type of operon synthesizes and the inactive operon that cannot bind to the operator.

So, RNA polymerase readily bind to the promoter without any inhibition and transcribe the structural genes. To turn the transcription something must be needed to make the repressor active. A small molecule called a corepressor binds to the repressor and make it capable of binding to the operator. So, in the absence of inducer the regulatory genes are producing the repressor but these repressors are inactive which means they will not be able to bind the operator and that side there will be a transcription.

So, transcription is on right. So transcription is on sorry transcription is on right under the basal level because you RNA polymerase will go and bind to the promoter there is no inhibition because the repressor what you are producing is inactive and that is how there will be a production of on right. So, there will be a transcription on. When the inducer is present the inducer will go and bind to the repressor and that is how it is going to convert the inactive repressor into active repressor and active repressor will go and bind to the promoter and that is how it is actually going to inhibit the transcription and that is how it is actually going to have the turn off it is going to turn off the transcription. So,

this is the another example or another way in which the operand can be can be regulated.

So, this is called as negative repressible operand. Then the third condition is the positive inducible response right. Remember that in the positive it is going to be transcriptionally off and then it is going to be on when the inducer is present. So in a positive inducer inducible operand transcription is usually turn off because the regulatory proteins that is the activator is produced in an inactive form right. Remember that when you are talking about the positive regulation it is going to be inducer it is going to be activator rather than repressor. So, the negative control it is going to be done by the repressor whereas, here it is going to be activator.

So, whatever we have discussed in the case of negative operands it is going to be exactly the reverse. So, in this case the activator is produced, but that activator is in the inactive form which means it cannot activate the transcription right. So, transcription take place when an inducer has become attached to the regulatory protein rendering to the regulatory side. So, when the inducer is not present the regulatory region is producing an activator, but this is a inactive regulator which means it requires some kind of modification. So that it will go and bind to the operator and that is how it actually can enhance the production or enhance the transcription.

So, there will be a transcriptional off right because the this activator is not competent enough or efficient enough to induce the transcription. So, inactive activators cannot activate the transcription and that is why there will be no transcription. But once the you add the inducers these inducer will go and bind to the activators and once the activator binds to the inducers they will actually going to have there could be a structural changes within the activator and that is how they will be actually go and bind to the operator right. And the active operator is stimulate the transcription right and that is how you are going to have the transcription with of the structural genes. Then we have the positive repressible operand this is exactly the opposite of the negative inducible operand.

So a positive operand can also be repressible the regulatory protein is producing an activator and that will bind to the DNA meaning the transcription usually take place and has to be repressed. Transcription is inhibited when a substance become attached to the activator and render it unable to bind the DNA. So transcription is no longer stimulated. So this is exactly the in the in the case of no inducer you are going to have the active activators and active activator will go and bind the operator and that is how there will be enhanced production of the these particular genes. But when the inducer will be in added inducer will go and bind to the transcription making them inactive transcription activator and inactive activator will actually going to turn off the transcription.

So these are the four different conditions in which the operand can be regulated by the repressor or the repressor proteins and it could be inducible or it could be repressible. So these are the just the summary of what we have discussed so far. So you going to have the repressible operand or you are going to have the inducible operand. Repressible operand generally in normally keep that GLS synthesis on but can be turned off by the repressors whereas in the inducible operand generally the genes are tough with that means the transcription is off but can be turned on by the inducer. Repressible operand are mostly been present in the anabolism reactions or anabolic reactions whereas the inducible operands are always been present in the catabolic reactions.

In repressible operands you are going to have the inactive form whereas in the inducible operand you are going to have the active forms and the examples of the repressible operand is the tryptophan operand whereas inducible operand which is the example of the lac operand. So we are going to take up these examples so that it will be easy for you to understand what is mean by the inducible operand what is mean by the repressible operand and so on. So we have taken first example that is the lac operand and then we are going to take the tryptophan operand. So this is the example where you are going to have the synthesis whereas here you are going to have the breakdown of the substance. So this is going to be related to the catabolic reactions and this is actually going to be related to the anabolic reactions.

So let us first start with the lac operands. So lac operand or the lactose operand which is in short is called as lac operand. So the lac operand of the E. coli contains the gene which are involved into the lactose metabolism. It is expressed only when the lactose is present and the glucose is absent.

This is very important. If you have a glucose in the lac operand will no longer be active. Lactose can be broken down by the E. coli but it is not their preferred energy source. They would much instead use glucose if it is available.

Lactose can be broken down more slowly and with less energy than glucose. However if lactose is the only sugar present E. coli will immediately use it as a fuel. The lac operand contains the 3 structural genes lacZ, lacY and lacA. So these are the 3 genes lacZ, lacY and lacA and all these 3 genes have their own individual roles. So lacZ is called as the beta galactosidase, lacY is called as beta galactosidase permease and lacA is actually been called as beta galactosidase trans-acetylase.

These genes are transcribed as single messenger RNA under the control of the single promoter that is the this promoter. The lac operand is typically been present as a shut off or repressed in a normal condition but can be activated in the presence of the inducer

which is called as lactose or allolactose. Plus the lac operand referred to be an inducible operand. So allolactose is a structural analog of the lactose. So these are the structural genes, regulatory genes and the regulatory DNA sequences and the regulatory gene which are present in the lac operands.

So you are going to have the 3 different types of structural genes lacZ which codes for the enzyme beta galactosidase, the cleaves lactose into the glucose and galactose this enzyme also converts the lactose into allolactose. Then we have the lacY which encodes for the beta galactosidase permease which transport the lactose into the cell. So basically the lacY is so this is if this is the cell the lacY is actually going to bring the lactose into the cell. Okay and then lactose is going to be get converted into glucose and galactose right by the enzyme which is called as lac by the gene product of lacZ right. So it is going to be called as beta galactosidase and lacA codes for the enzyme which is called as beta galactosidase trans-tylase.

It is not essential for the lactose metabolism but appears to be play a role in the detoxification of the compound by transferring and the acetyl group. Then we have the regulatory DNA sequences remember that in a transcriptional unit you have the promoters you are going to have the coding region and then you are going to have the 3 prime poly A tail right. Apart from this you are also going to have the because we are talking about the operons. So in this case you are going to have the operators. So you are going to have the promoters followed by operators followed by structural genes followed by the poly A tail right.

So the regulatory DNA sequences you are going to have the promoters. The promoter is the binding site for the RNA polymerase which initiated the transcription of the structural gene. Lac promoter is a weak promoter. So remember that when we were talking about the transcription we discuss about the weak and a strong promoters. So there are compositions which actually becomes the which makes the promoter as a weak promoter or the strong promoters right. Because a strong promoters allows the efficient transcription of the DNA right and it allows the efficient very efficiently the RNA polymerase to go and bind the and complete the transcription whereas in the case of weak promoters the melting of the DNA or the other kinds of activities is very difficult and that is how it is actually going to have the lower efficiency and lower production of the RNAs.

Apart from that you are going to have the operators. So the operator is a negative regulatory site bound with the lac repressor protein. The operator overlap with the promoters. Then we have the cap binding site. The cap binding site is a positive regulatory site that is bound by the catabolite activator protein or the cap. When the cap

is bound to this site it first promotes the transcription by helping the RNA polymerase bind to the promoter.

Apart from that you are also going to have the regulatory genes which is called as lac I. So the regulatory gene lac I transcribed and produced the lac repressor protein and inhibited the lac repressor transcription in order to accomplish this it binds to the promoter partially overlapping the operator. When bound the lac repressor gets into the way of RNA polymerase and prevents the operon transcription but when the lac repressor binds with the lactose it becomes the repressed. There are multiple conditions. So you are going to have the in the absence of so just to make it comparable what we have discussed.

So in absence of inducer so in absence of inducer so remember that lac operon is lactose is going to be an inducer. So in absence of inducer so in absence of inducer or in presence of inducer. So when the lactose is not available which means the inducer is not available the lac repressor strongly bind with the operator and it stop the RNA polymerase from the initiation of transcription. However, lac repressor loses its capability to bind DNA when lactose is present it leaves the operator and float away making it possible for RNA polymerase to transcribe the gene. So if the lactose is not present then the what will happen is that the repressor will not be able to bind the operator.

And so if the lactose is not available the repressor will go and bind to the operator and since the repressor is binding to the operator it will not allow the RNA polymerase to go further to start the transcription. So there will be no transcription of the structural gene of lacZ Y and Z and when the allolactose is absent the repressor bind with the operator so the transcription cannot initiated by the RNA polymerase without any prevention. Now when the lactose or the allolactose is available so what will happen is these inducer will go and bind to the repressor. So when they will go and bind to the repressor allolactose or the lactose it will they will no longer be able to bind the operator and as a result what will happen is that the RNA polymerase will move and it will actually going to do the synthesis of the structural genes and that is how they are actually going to produce the beta lactosidase and other kinds of enzyme from these genes. So when the allolactose bind with the lac repressor, the repressor cannot bind an operator so transcription initiation by RNA polymerase without any prevention.

So some of the apart from the lactose or the allolactose some of the allolactose analogs can also be used for the for the lac promoters or the lac operons one of the very popular lactose analog is the IPTG or isopropyl beta D1 thioglycosidase it is actually a inducer for the protein production and that we are anyway going to discuss when we are going to

discuss about the molecular cloning. Then we have the phenyl beta D galactose which is called as phenyl galactose and then you also have thiomethyl galactose or the TMG. So all these are some of the lactose analog so either the lactose allolactose or these analogs can be able to modulate the activity of the repressor and that is how they can actually be able to have the effect on the into the lactose operon. Then the lac promoter is a weak promoter it does not bind RNA polymerase more efficiently on its own it would not be able to accomplish much more without the help of the catabolite activator protein. High transcriptional levels are facilitated by the caps binding to a stretch of DNA right before the lac operon.

So this is the cap binding region and this is the cap proteins and cap proteins are actually going to bind by the cyclic AMP. So E.coli produces a cyclic AMP as a hunger signal in the low glucose conditions by attaching to the cap cyclic AMP modifies the structure of the cap enabling it to bind the DNA and stimulate the transcription. Cap is inactive without cyclic AMP only when the glucose levels are low the cation MP levels are very high does camp actually activate. So in the condition of low glucose when the low when the condition is low glucose you are going to have the large quantity of the ADP and ADP is going to be get converted into AMP and this AMP is actually going to be get converted into cyclic AMP.

And the cyclic AMP will go and bind to the cap region right. So it is actually going to bind the cap proteins and once they bind to the cap protein they are actually going to you know they are actually going to block the activity. So when the cap attached to the cap cyclic AMP attached to the cap and activate it allowing it to bind the DNA cap helps RNA polymerase to bind to the promoter resulting in a high level of transcription right. So in a state of starvation you are going to have a very high amount of ADP and that ADP is getting converted into AMP and then the AMP is getting converted into cyclic AMP and in the case of low glucose this cyclic AMP will go and bind to the cap and as a result it actually going to activate and allowing it to bind the DNA and cap is actually going to help the promoter to bind to the bind to the promoter and that is how they are actually going to have the high level of transcription of these genes. When there will be a high glucose so in the case of high glucose there will be no production of cyclic AMP and that is how there will be no binding of cyclic AMP to the cap and as a result the cap will not going to help the RNA polymerase to bind to the promoter and that is how there will be a low level of transcription.

So there will be high amount of lac operon transcription are only possible without glucose. This method ensure that the bacteria only activate the lac operon and begin using the lactose after exhausting their primary energy source that is the glucose. So if the glucose is present it is actually going to block or it is going to inhibit the lac operon

activity simply because it is actually going to does not allow the production of cyclic AMP and cyclic AMP is going to bind the cap region or the cap proteins and that is how they are actually going to facilitate the binding of RNA polymerase to the promoter. So if we summarize all these conditions what is the conditions you are going to have the glucose absent lactose absent. So there will be no transcription. So there are going to be four conditions in four conditions you are going to have in first condition you are going to have the glucose absent lactose absent in those conditions there will be no transcription of the RNA the lac operon or there will be no lac operon activity because the lac operon inducer is also absent and the glucose is also absent.

So they still there although there will be a production of cyclic AMP because the glucose is absent but since the lactose is also absent the repressor protein will actually going to repressor repress the activity or repress the production of the production of RNA from the RNA from the RNA polymerase. Now the there will be a second condition. So second condition would be that the glucose is absent but the lactose is present in that condition there will be a high transcription because in the absence of glucose we discussed already that from the ADP it is actually going to form the AMP and from the AMP it is actually going to form the cyclic AMP and the cyclic AMP will go into the cap and it will go and bind to the cap region of the DNA and that is how it is actually going to facilitate the promoter. And since the lactose is present lactose will also going to bind the repressor and that is how it is actually going to remove the repression and as a result it is actually going to have the allow the RNA polymerase to go for the transcription and that side there will be a high transcription level.

Now the third condition is that you are going to have the glucose present and lactose absent. So if the glucose is present it is actually not going to allow the production of cyclic AMP and there will be a low level of cyclic AMP so that the cap will not going cap proteins will not be able to bind to the cap region and that is how the RNA polymerase will not be efficiently be able to bind to the promoter and that is how and on the other hand since the lactose is absent the repressor will actually going to bind and it is going to you know allow the operator right. So it is going to allow the repressor to bind to the operator and that side there will be no transcription. In the fourth condition fourth condition both the biomolecules both the molecules are present which means the glucose is also present and lactose is also present in that case there will be a low level of transcription because since the lactose is so if the glucose is present there will be no cyclic AMP so it is not going to efficiently allow the binding of the cap proteins to the cap region of the DNA and that is how there will be a very low level of transcriptional activity from the RNA polymerase because the lactose is present it will actually going to bind the repressor and that is how it is actually going to destroy the inhibition of the operator and that is how it is going to allow the RNA polymerase to go. But this level of

RNA transcription would be less compared to the transcription activity what we have just observed in the condition number two. So if we summarize all these activities summary would be that the lactoperon contains the gene those are involved into the lactose metabolisms.

Lactoperon is a negative inducible operon the genes are expressed only when the lactose is present and the glucose is absent remember this is very important because the glucose is the primary metabolite and or primary metabolite preferred by the cell whereas the lactose is a secondary metabolite and is not been preferred by the cell. So it will not going to utilize the lactose until the glucose is absent and we have already discussed that when the glucose is absent it is going to have the synthesis of the cyclic AMP and the cyclic AMP will go and bind to the cap proteins and that is how the cap protein is actually going to help into the binding of RNA polymerase to the promoter site and that is how it is actually going to have the higher production of the protein synthesis. The operon is turned off in a normal conditions the operon is turned on and turned off depending upon the glucose and lactose level the catabolic activator protein and the lactoperon. The lactepressor blocks the transcription of the operon by binding with the operator in the absence in the presence of lactose it stops acting as a repressor. So catabolite activator protein act as a enhancer activate the transcription of the operon but only when the glucose levels are low.

So these are the four conditions what we have already discussed if we have the glucose we have the no lactose then there will be no transcription because the glucose is present and it is going to inhibit the activity of the cyclic production of cyclic AMP. Then you can have the both glucose and lactose present then it will be having the low level of transcription then you third condition where the glucose is also absent and lactose is also absent then there will be no transcription and then the fourth condition is that the glucose is absent but the lactose is present. So in that case there will be a production of cyclic AMP and the cyclic AMP will allow the binding of the cap proteins to the cap region of cap region and that is how it is actually going to facilitate the binding of the RNA polymerase to the promoter and that is why there will be a high level of transcription. And because the lactose is also present it will actually going to block the repressor and lac I and that is how it is actually going to have the high level of transcription.

So this is the summary of the lac operon. So with this I would like to conclude my lecture here in a subsequent lecture we are going to discuss some more aspects related to molecular biology. Thank you.