Cell and Molecular Biology
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Concepts of Genetics (Part 2)
Lecture - 30
Gene Regulation in Organism

Hello everyone, this is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering at IIT Guwahati. Now imagine that the glucose level is low or that there is no supply of glucose. For example, where will the glucose come from? The glucose will come from the food, and it will also enter the cell. It will go through with these kind of reactions. But if there is an excess of fat, then these reactions will go in this direction, resulting in less utilization of glucose.

And if there is less utilization of glucose, the enzymes present in glycolysis will be downregulated, apart from the feedback mechanism or allosteric regulation. Also, another level of regulation is that you are going to have regulation at the level of protein synthesis, which means you are actually going to make the availability of these enzymes or proteins at a lower or higher level. Depending on the amount of these proteins or the enzyme, that particular type of activity will either increase or decrease. So, these kinds of events are more relevant when you are talking about the bacterial system because the bacterial system is going to have the polycistronic transcriptional unit, right, compared to the eukaryotic system, which has the monocistronic transcriptional unit.

So, when you have a polycistronic transcriptional unit, this means you are going to have different types of enzymes produced simultaneously from the single transcript, and in that case, all the protein synthesis of these enzymes is actually going to be under tight regulation or control. So that you can have complete control over the different types of events, such as glycolysis, the Krebs cycle, and all other kinds of things. Although the Krebs cycle is not present in the bacterial or prokaryotic systems, for the sake of example, there could be many other kinds of pathways. For example, you are going to have the fatty acid synthesis pathway, other kinds of pathways like the amino acid biosynthesis pathway, and others. So all these pathways require very tight control, and one of the mechanisms through which the bacteria actually exert this control is by upregulating and downregulating the amount of protein it is actually going to synthesize.

And this has all been achieved by putting these enzymes, these genes, or the transcriptional unit under complete control mechanisms. And all these are part of the operon, which means a system that is going to operate or regulate the transcription

followed by the synthesis of these proteins. So the question is, what is an operon, right? An operon is a set of genes; it is a genetic regulatory system mostly seen in prokaryotes and bacteriophages in which a group of structural genes is transcribed together under the control of a single promoter. Technically, the operands are mostly present in the polycistronic transcriptional unit, and they will be present in prokaryotes and bacteriophages, where you will have the group of structural genes. Or I will say that the genes that are going to be transcribed for the different types of enzymes or the structural genes will be under the control of a single promoter.

This means that this single promoter is actually going to have control over the synthesis of these structural genes. So, in a typical operon, you're going to have the promoter, right? Next to the promoter, you will find the operator. And next to the operator, you're going to have this; this is going to be the coding region, right? 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're going to have the polyadenylation, right? So the coding region is going to be responsible for the synthesis of protein A, protein B, and protein C. So, generally, operons are very common in prokaryotes and bacteriophages, but they are also found in some eukaryotes. The main difference is that the expression of prokaryotic operons leads to polycistronic messenger RNA, whereas eukaryotic operons lead to monocistronic messenger RNAs. In this particular lecture, we are mostly focused on the operon present in the prokaryotic system; we have not discussed the operons present in the eukaryotic system. So the idea is that we should be able to tell you about the concept of transcriptional activation and transcriptional regulation and how things are done.

A similar kind of thing is also being done in the eukaryotic systems. Prokaryotes are single-celled organisms that lack a true nucleus and membrane-bound organelles. It adopts the operant system as a mechanism to efficiently regulate gene expression in response to changing environmental conditions. Environmental conditions refer to the requirements of the different types of metabolites, the availability of glucose, the availability of oxygen, and so on. So, the bacteria are single-celled organisms; they actually get affected very often, and they have to respond to these changes.

The Oberon system is a genetic regulatory system found in prokaryotic organisms that allows multiple genes with related functions to be controlled as a single unit. The system offers different advantages for prokaryotes, such as being energy efficient because you only need to synthesize the operator or the repressor, which is actually good enough to control and regulate the transcriptional activity of several genes. So the operon system allows them to coordinate the expression of multiple genes involved in a common

pathway and to transcribe a single messenger RNA together, saving energy and resources by producing the necessary protein only when required. Then it is actually going to have a rapid response to environmental changes. So the operon system enables them to adapt quickly to the changing conditions.

If the condition changes, the expression of the relevant gene can be turned on or off rapidly. It's simple and compact, isn't it? Superkaryotes can use a single regulatory region to control the expression of multiple genes. This is particularly advantageous in an organism with a small genome, where saving space is very crucial. So if you require multiple genes as regulatory genes and so on, you're actually going to increase the size of the genome, which the bacterial system cannot afford because it has to conserve energy, conserve space, and all those kinds of things. And that's why it is important that it actually operates and controls multiple genes with the help of the operons.

Then it has the coordinated regulations that allow the bacteria to operate with the coordinated regulation because it can regulate three genes, four genes, and five genes, which are actually present in a single pathway. For example, if you are talking about glycolysis, you are going to regulate hexokinase, pyruvate kinase, and so forth. Since all these genes are present in a single operon, it is probably easy for the bacteria to manage them all. On the other hand, it is also going to save energy. So there will be a coordinated regulation.

So this coordinated regulation ensures that the products of genes are produced in the appropriate stoichiometric ratio, which means that if you are processing a single glucose molecule, you require one molecule of hexokinase, one molecule of aldolase, and so on. So you can actually produce these proteins and enzymes in the right proportions so that you do not waste energy by producing one or the other in excess. And on the other hand, you should not have a lower production of any of these proteins. Then you have the resource allocation. When a particular nutrient is available, the genes required for its utilization are switched on.

Once the nutrient becomes scarce, the operon can be turned off, preventing the wasteful production of unnecessary proteins. Then we have adapted to the niche environment. So the operon system enables prokaryotes to adapt to a specialized niche by fine-tuning the expression of genes that are specifically relevant to those conditions. So this is also very, very important: that you actually have a very, very fine and regulated balance, and that is how you can achieve the fine-tuning of the expression of the genes that are relevant for those particular environmental conditions. Then you have the evolutionary advantage.

Organisms with the ability to regulate gene expression rapidly and efficiently in response

to environmental changes are more likely to survive and reproduce. So the operon, as I said, was proposed by Jacob and Monod, and it has control mechanisms through which the prokaryotic system controls the different types of genes and gene expressions within bacteria. So, in 1965, the Nobel Prize in Physiology or Medicine was awarded jointly to Jacob and Monod for their discoveries concerning the operon and viral synthesis. So these are all scientists who received the Nobel Prize in Medicine in 1965 for their concept of the operon. So, before we get into the details of the operons and how we are going to take up some examples of the operon, it is important to understand the general structure of an operon.

So, this is just a general structure of the operon, right? You're going to outline the general structure of what you will have, right? So this is the transcriptional unit, right? This is the transcriptional unit where you will find the promoters, the operators, and the structural genes. For example, in this case, this is a structural gene for A, B, and C. Apart from that, you are also going to have regulatory genes. So regulatory genes are actually going to be a part of, are going to produce the regulatory proteins, and these regulatory proteins could be activators or repressors, and the conditions of the activators and repressors will go and bind to the operator. So this region, what you see here, is actually a part of the operon, so the regulatory gene is not going to be a part of the operon, and regulatory proteins are going to either activate or facilitate the binding of RNA polymerase, or they're actually going to have the.

The other way around, right? So, the regulatory gene encodes a protein called a regulatory protein, which either acts as a repressor or an activator that controls the operon, but it is not a part of the operon because it has its own promoter, right? So, regulatory genes are not part of an operon. This is the part of the operon where you are going to have the promoters, operators, and the structural genes. Now let's talk about the regulation of an operon. So there are two types of transcriptional regulation that are possible in the operon. One is called the negative control, and the other is called the positive control.

So then the negative control, in which the regulatory protein acts as a repressor by binding to the DNA and inhibiting the transcription 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 that once it binds to the operator, it will not allow the RNA polymerase to bind to the promoter. And that's how it is actually not going to allow the transcription; so there will be no transcription of the structural genes. This is going to be called the negative control. Whereas in the case of positive control, you have the regulatory proteins that

actually bind to the operators, and that is how they facilitate the efficient binding of RNA polymerase to the promoters.

And that is how it is actually going to have more production of these particular structural genes, which are called positive regulations. When you have this, you are going to have more production, and when you have this, you are going to have lower production. This means it's going to be negative regulation; this is going to be positive regulation within this. You are going to have two different types of conditions: either it will be inducible or it will be repressible. So an operon can also be either inducible or repressible.

So inducible operons are those in which transcription is normally turned off, which is not going to take place. And it needs an inducer to induce transcription, which means it is going to be turned on. Repressible operons are those in which transcription is normally on, which means there will be a basal level of transcription taking place. Sometimes it may happen that you repress the transcription or turn it off, so you'll 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 summarized here: you're going to have the negative control, you're going to have the positive control, and within the negative control or the positive control, you can have the inducible operon or the repressible operon.

So, in the negative control, the product of the regulatory gene inhibits transcription. In the positive control, the product of the regulatory gene activates transcription. Whereas in the inducible operon, the initial condition, or I would say the basal level of transcription, is off, which means you are not going to have the transcription of that particular gene. But once this inducer is present, you are going to have the operon that will work. So, it is going to turn on the transcription; whereas, in the case of the repressible initial condition, the basal level will have transcription, but when the repressible is present, it is actually going to turn off the transcription.

So let's first discuss the negative inducible operon. So within the negative, you can have the inducible; you can actually have the inducible, or you will have the repressible. Even within the positive control, you can have either the inducible or the repressible. So there are several different conditions under which all of this has to be understood. So let's first take the initial example, which is the negative control inducible.

In a negatively inducible operon, the regulatory gene encodes a repressor that readily binds to the operator, as the operator site overlaps with the promoter site. So that the binding of the repressor physically blocks the binding of RNA polymerase to the promoter and prevents transcription. So for the initiation of transcription, something is

needed to prevent the binding of the repressor at the operator site and represent the operator site of binding, which 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 from the regulator, you will have a repressor.

So, this is the repressor molecule. Which will go and fit and sit onto the operator; if no inducer is present, this repressor will keep binding to the operator molecule and will not allow the transcription of the structural gene because the repressor will bind to the operator and inhibit the transcription. So there will be a transcriptional off, and when the inducer is present, what will happen? Suppose insulin, for example, or I will say glucose. If glucose is present, what will happen is that glucose will bind to this repressor. And in that case, it is actually going to turn the active repressor into an inactive repressor. And then the inactive repressor would not be able to bind to the operator.

And as a result, it is actually going to allow for the transcription of these particular structural genes. So this is an example of the mechanism by which the negative inducible operon will operate. We are going to take a few examples, and then you will be able to understand this more clearly. And then we have the second condition. The second condition is that you are going to have the negatively repressible operon, right? So, the regulator gene in this type of operon synthesizes the inactive repressor that cannot bind to the operator.

So, RNA polymerase readily binds to the promoter without any inhibition and transcribes the structural genes. To turn on the transcription, something must be done to make the repressor active. A small molecule called a corepressor binds to the repressor and makes it capable of binding to the operator. So in the absence of an inducer, the regulatory genes are reducing the repressor, but these repressors are inactive, which means they will not be able to bind to the operator. And that's why there will be a transcription.

So transcription is on, right? So, the transcription is on. Sorry, transcription is right under the basal level because your RNA polymerase will go and bind to the promoter. There is no inhibition because the repressor that you are producing is inactive. And that's how there will be a production of all right. So there will be a transcription when the inducer is present; the inducer will go and bind to the repressor, and that's how it is going to convert.

The inactive repressor becomes an active repressor, and the active repressor will go and bind to the promoter, and that is how it actually inhibits transcription and turns it off. It's going to turn off the transcription. So this is another example of a different way in which

the operon can be regulated. So, this is called a negative repressible operon. Then the third condition is the positive inducible response.

Remember that in the positive, it is going to be transcriptionally off, and then it will be on when the inducer is present. So in a positive inducible operon, transcription is usually turned off because the regulatory protein, which is the activator, is produced in an inactive form. Remember that when you are talking about positive regulation, it will be an inducer. It is going to be an activator rather than a repressor. So the negative control will be done by the repressor, whereas here it will be performed by the activator.

So whatever we have discussed in the case of negative operands, it is going to be exactly the reverse; 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 takes place when an inducer has become attached to the regulatory protein, rendering it to the regulatory site. So when the inducer is not present, the regulatory region does not produce an activator. But this is an inactive regulator, which means it requires some kind of modification so that it can bind to the operator.

And that's how it actually can be. Enhance the production or enhance the transcription. So there will be a transcriptional off because this activator is neither competent enough nor efficient enough to induce the transcription. So inactive activators cannot activate the transcription and that is why there will be no transcription. But once you add the inducer, it will bind to the activators, and once the activator binds to the inducer, there could actually be structural changes within the activator, and that is how it will bind to the operator, right? The active operator stimulates the transcription correctly, and that's how you will have the transcription of the structural genes.

Then we have the positive repressible operon. This is exactly the opposite of the negatively inducible operon. So, a positive operon can also be repressible. The regulatory protein is producing an activator that will bind to the DNA, meaning transcription usually takes place and must be repressed. Transcription is inhibited when a substance becomes attached to the activator and renders it unable to bind to the DNA. So transcription is no longer stimulated; this is exactly the case of no inducer.

You are going to have the active activators, and the active activator will go and bind the operator, and that's how there will be enhanced production of these particular genes. But when the inducer is added, it will bind to the transcription factor, making it an inactive activator, and the inactive activator will actually turn off the transcription. So, these are the four different conditions in which the operon can be regulated by the activator or the repressor proteins, and it can be inducible or repressible. So these are just the summaries

of what we have discussed so far: you are going to have the repressible operon, or you are going to have the inducible operon.

The repressible operon generally operates normally. Keep the synthesis on, but it can be turned off by the repressors, whereas in the inducible operon, the genes are generally off, but transcription can be turned on by the inducer. Repressible operons are mostly present in anabolic reactions, whereas inducible operons are usually present in catabolic reactions. In repressible operons, you have the inactive form, whereas in the inducible operon, you have the active forms. The examples of the repressible operon are the tryptophan operon, whereas the inducible operon is exemplified by the lac operon. So we are going to take up these examples so that it will be easy for you to understand what is meant by the inducible operon, what is meant by the repressible operon, and so forth.

So let us take a first example, which is the lac operon, and then we will take the tryptophan operon. Example of 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 it is actually going to be related to the anabolic reactions. So let us first start with the lac operon. The lac operon, or the lactose operon, which is commonly referred to as the lac operon, contains the genes involved in lactose metabolism in E.

coli; it is expressed only when lactose is present and glucose is absent. This is very important. If glucose is present, the lac operon will no longer be active. Lactose can be broken down by E.

coli, but it is not its preferred energy source. They would much rather use glucose if it were 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 fuel. The lac operon contains three structural genes: lacZ, lacY, and lacA.

So, these are the three genes: lacZ, lacY, and lacA. All three of these genes have their own individual roles. So lacZ is called beta-galactosidase, lacY is called beta-galactoside permease, and lacA is actually called beta-galactoside transacetylase. These genes are transcribed as a single messenger RNA under the control of a single promoter, which is this promoter. The lac operon is typically present as shut off or repressed under normal conditions but can be activated in the presence of the inducer, which is called lactose or allolactose.

The lac operon is referred to as an inducible operon. So, allolactose is a structural analog of lactose. So these are the structural genes, regulatory genes, regulatory DNA sequences,

and the regulatory proteins that are present in the lac operon. So you are going to have three different types of structural genes. LacZ, which codes for the enzyme beta-galactosidase, is important in molecular biology.

The cleaves lactose into glucose and galactose. This enzyme also converts lactose into allolactose. Then we have the lacY, which encodes the beta-galactosidase permease that transports lactose into the cell. So basically, the lacY is, if this is the cell, actually going to bring the lactose into the cell. And then lactose is going to be converted into glucose and galactose, right? By the gene product of lacZ, right? So, it is going to be called beta-galactosidase, and lacA codes for the enzyme, which is called beta-galactosidase transacetylase. It is not essential for lactose metabolism but appears to play a role in the detoxification of the compound by transferring the acetyl group.

Then we have the regulatory DNA sequences. Remember that in a transcriptional unit, you have the promoters, the coding region, and then the 3-prime poly-A tail. Apart from this, you are also going to have the operators because we are talking about the operons. So you are going to have the promoters followed by operators followed by structural genes followed by polyadenylation. So the regulatory DNA sequences you are going to have are the promoters. The promoter is the binding site for the RNA polymerase that initiates the transcription of the structural genes.

Lag promoter is A. Weak promoters. So remember that when we were talking about transcription, we discussed the weak and strong promoters. So there are compositions that make the promoter a weak promoter or a strong promoter, right? Because the big strong promoters allow for the efficient transcription of the DNA, right? And they allow the RNA polymerase to bind very efficiently. And complete the transcription, whereas in the case of weak promoters, the melting of the DNA or other kinds of activities is very difficult. And that's how it is actually going to have lower efficiency and lower production of the RNAs. Apart from that, you are also going to have the operators; the operator is a negative regulatory site bound to the lac repressor protein.

The operator overlaps 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 CAP. When the cap is bound to this site, it promotes transcription by helping RNA polymerase bind to the promoter. Apart from that, you are also going to have the regulatory gene called lacI. So the regulatory gene lacI transcribed and produced the lac repressor protein, which inhibited the transcription of the lac repressor. In order to accomplish this, it binds to the promoter, partially overlapping the operator.

When bound, the lac repressor gets in the way of RNA polymerase and prevents operon

transcription, but when the lac repressor binds with lactose, it becomes unbound. So there are multiple conditions. So you're going to have it in the absence. So, just to make it comparable to what we have discussed.

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

Since the repressor is binding to the operator, it will not allow RNA polymerase to go further to start transcription, so there will be no transcription of the structural genes of lag z, y, and z. And when allolactose is absent, the repressor binds to the operator. So the transcription cannot initiate by the RNAPL-A without any preventions. Now, when lactose or allolactose is available, what will happen is that these inducers will go and bind to the repressor. So when they bind to the repressor, allolactose or lactose, they will no longer be able to bind to the operator, and as a result, what will happen is that RNA polymerase will move and actually synthesize the structural genes.

And that's how they are actually going to produce beta-lactosidase and other kinds of enzymes from these genes. So when the allolectors bind with the lac repressors, the repressor cannot bind to an operator, allowing transcriptional initiation by RNA polymerase without any interference. Apart from lactose or allolactose, some of the allolactose analogs can also be used for the lac promoters or lac operons. One of the most popular lactose analogs is IPTG, or isopropyl beta-D-1-thiogalactoside. It is actually an inducer of protein production, and we are going to discuss that when we talk about molecular cloning.

Then we have phenyl beta-D-galactose, which is called phenyl galactose, and we also have thiomethyl galactose, or TMG. So all of these are some of the lactose analogs. So either the lactose, allolectose, or these analogs can modulate the activity of the repressor, and that's how they can actually have an effect on the lactose of RAN. Then, the lac promoter is a weak promoter.

It does not bind RNA polymerase more efficiently by itself. It won't 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 just before the lac operon. So this is the cap-binding region, and these are the cap proteins.

And cap proteins are actually going to bind to cyclic AMP. So E. coli produces cyclic AMP as a hunger signal in low glucose conditions; by attaching to the CAP, cyclic AMP modifies the structure of the CAP, enabling it to bind to the DNA and stimulate transcription. CAP is inactive without cyclic AMP, only when glucose levels are low. The cyclic AMP levels are very high; does cAMP actually activate? So in the condition of low glucose, when glucose levels are low, you're going to have a large quantity of ADP, which will be converted into AMP, and this AMP will actually be converted into cyclic AMP. And the cyclic AMP will go and bind to the CAP region, right? So it's actually going to bind the capsid proteins.

And once they bind to the cap protein, they actually block its activity. So when the cap is attached to the mRNA, cyclic AMP attaches to the cap and activates it, allowing it to bind to the DNA; the cap helps RNA polymerase 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 converted into AMP, and then the AMP is 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 is actually going to activate and allow it to bind to the DNA, and CAP is actually going to help the promoter bind to the promoter, and that's how they are actually going to have a high level of transcription of these genes. When there is high glucose, there will be no production of cyclic AMP, and that's how there will be no binding of cyclic AMP to the cap. And as a result, the cap will not help the RNA polymerase bind to the promoter, and that is how there will be a low level of transcription.

So a high amount of lac operon transcription is only possible without glucose. This method ensures that the bacteria activate the lac operon and begin using lactose only after exhausting their primary energy source, which is glucose. So, if glucose is present, it actually blocks or inhibits lac operon activity simply because it does not allow the production of cyclic AMP, and cyclic AMP binds to the cap region or cap proteins. and that's how they are actually going to facilitate the binding of RNA polymerase to the promoter. So, if we summarize all these conditions, what are the conditions under which you will have glucose absent and lactose absent, right? So, there will be no transcription, right? So, there are going to be four conditions.

In four conditions, in the first condition, you will have both glucose and lactose absent. In those conditions, there will be no transcription of the lac operon, nor will there be any

lac operon activity because the lac operon inducer is absent and glucose is also absent. So they are still there, although there will be a production of cyclic AMP because glucose is absent; but since lactose is also absent, the repressor protein will actually repress the activity of RNA polymerase or repress the production from it. Now there will be a second condition. So the second condition would be that glucose is absent but lactose is present. In that condition, there will be high transcription because, in the absence of glucose, we have already discussed that ADP will actually form AMP, and AMP will actually form cyclic AMP, and cyclic AMP will go into the cap.

It will go and bind to the cap region of the DNA, and that's how it will actually facilitate the promoter; since lactose is present, it will also bind to the repressor. And that's how it is actually going to remove the repression, and as a result, it is actually going to allow the RNA polymerase to proceed with transcription, and that's why there will be a high transcription level. Now the third condition is that you are going to have glucose present and lactose absent. So if glucose is present, it actually does not allow the production of cyclic AMP, and there will be a low level of cyclic AMP so that the CAP proteins will not be able to bind to the CAP region. And that's how the RNA polymerase will not be able to efficiently bind to the promoter; on the other hand, since lactose is absent, the repressor will actually bind, allowing the operator, right? So, it's going to allow the repressor to bind to the operator, and that's why there will be no transcription.

In the fourth condition, both biomolecules are present, which means that glucose and lactose are present as well. In that case, there will be a low level of transcription because, since lactose is present, there will be no cyclic AMP if glucose is present. So it's not going to efficiently allow the binding of the cap proteins to the cap region of the DNA. And that's how there will be a very low level of transcriptional activity by RNA polymerase. Because lactose is present, it will actually bind to the repressor, and that is how it will destroy the inhibition of the operator and allow RNA polymerase to proceed.

But this level of RNA transcription would be lower compared to the transcription activity that we have just observed in condition number 2. So, if we summarize all these activities, the summary would be that the lac operon contains the genes involved in lactose metabolism. The lac operon is a negatively inducible operon. The genes are expressed only when lactose is present and glucose is absent.

Remember, this is very important because glucose is the primary metabolite. The primary metabolite preferred by the cell is glucose, whereas lactose is a secondary metabolite and is not preferred by the cell. So it will not utilize the lactose until glucose is absent. We have already discussed that when glucose is absent, it leads to the synthesis of cyclic AMP, which then binds to the CAP proteins. And that's how the cap protein is

actually going to help in the binding of RNA polymerase to the promoter site, and that's how it is going to lead to higher production of protein synthesis.

The operon is turned off under normal conditions. The operon is turned on and off depending on the glucose and lactose levels, the catabolite activator protein, and the lac repressor. The lac repressor blocks the transcription of the operon by binding to the operator. In the presence of lactose, it ceases to act as a repressor. So, catabolite activator protein acts as an enhancer to activate the transcription of the operon, but only when the glucose levels are low.

These are the four conditions. What we have already discussed is that if we have glucose and no lactose, then there will be no transcription, right? Because glucose is present, it is going to inhibit the production of cyclic AMP. Then you can have both glucose and lactose present; it will have a low level of transcription. In your third condition, where glucose is absent and lactose is also absent, there will be no transcription. And then the fourth condition is that glucose is absent but lactose is present; so in that case, there will be a production of cyclic AMP.

And the cycling MV will allow the binding of the cap proteins to the cap region. And that's how it is actually going to facilitate the binding of RNA polymerase to the promoter. And that's why there will be a high level of transcription. And because the lactose is also present, it will actually block the repressor and lacI, and that's how it will have a high level of transcription.

So this is the summary of the lac operon. Now let's move on to the next operon, which is the tryptophan operon. The tryptophan operon is a part of the anabolic operon; compared to that, the lac operon is a catabolic operon. So, the tryptophan operon is found in E. coli. It is a group of genes that encodes enzymes for the synthesis.

So remember that the first operon we have discussed is for the breakdown of lactose. It is going to break down lactose into glucose and galactose. And that's how it is going to derive energy from the lactose molecule. Whereas here, you are actually going to consume the energy. So, this is actually an anabolic pathway. It is an anabolic or negatively controlled operon; it always remains on under normal conditions and off when the tryptophan level is high, so this is exactly the reverse of what we have just discussed for the lac operon.

Tiptofon does not need to be synthesized by E. coli bacteria when it is present in the environment. Hence, the transcription of a gene in the tip operon is turned off on the other side. When the availability of tiptofon is low, the operon is turned on, and the genes

are transcribed for the biosynthetic enzyme for tiptofon synthesis. Tip repressor does not always attach to DNA; instead, it binds and inhibits transcription only in the presence of tryptophan. Tryptophan binds to the repressor molecule and alters its structure, which switches an inactive repressor into an active state; thus, tryptophan acts as a co-repressor. Remember that this is very important: tryptophan acts as a co-repressor because it enhances the repressive activity of the repressor, converting the inactive repressor into an active one.

Because it enhances the repressive activity of the repressor, it converts the inactive repressor into an active repressor. One unique feature of the tryptophan repressor is attenuation. So, like regulation by the tryptophan repressor, attenuation is a mechanism for reducing the expression of a tryptophan operon when the level of tryptophan is high. However, rather than blocking the initiation of transcription, attenuation prevents the completion of transcription.

So this is a unique feature of the tryptophan operon. Now the structure of the tryptophan operon shows that there are five structural genes: type E, D, C, B, and A that code for the enzymes involved in the conversion of chorismic acid to tryptophan. Remember that we have already discussed tryptophan biosynthesis when we were talking about amino acid metabolism. So, tip E actually codes for the enzyme anthranilate synthase 1. Tip D actually codes for the enzyme anthranilate synthase II.

Tip C encodes the enzymes 5-fervazole anthranilate isomerase and indole-3-glycerol phosphate synthase. Then tip B encodes the enzyme tryptophan synthase B subunits, and tip A is actually going to encode the enzyme tryptophan synthase A subunit. The controlling site in tryptophan lies next to tip E and consists of a promoter, an overlapping operator, and a leader region, or tip L. So, these are the regulatory regions, right? So, this is the operon, right? This is the operon.

This is the regulatory region. You're going to have a promotion. You're going to have the operators, the leader region, which is called trip L, and then you're going to have structural genes like trip E, trip D, trip C, trip B, and trip A, and they are actually going to give you a polycistronic messenger RNA. So it also contains a repressor regulatory gene called trpR, and when tryptophan is present, the trpR protein binds to the operator, blocking the transcription of the tryptophan operon by inhibiting RNA polymerase's binding. So, this is the repressor regulatory gene TRIP-R. So, when it is produced, it will bind the tryptophan, and that is how it will actually make the active repressor, which is how it will allow the binding of the repressor to the operator region.

and that is how it is actually going to block the transcription of these genes, structural

genes. Reactions catalyzed by the enzyme are synthesized from the tryptophan operon. So this is anyway that we have discussed the biosynthesis in detail. Ultimately, from the indole, you are going to have the synthesis of tryptophan, where you will have the activity of anthranilate synthase, anthranilate transferase, PRA isomerase, IGP synthase, and so on. So all these genes are actually going to be part of the tryptophan operon. This we are not going to discuss in detail because we have already discussed these things when we talked about tryptophan biosynthesis.

Now tryptophan operon regulations. So in the absence of tryptophan, it means that the bacteria actually require the synthesis of tryptophan. So there will be low levels of tryptophan in the environment. When there is a little tryptophan or an absence of tryptophan in the cell, in this condition the tryp repressor is inactive because there is no tryptophan available to bind to the repressor and activate it through a conformational change. So the inactive repressor cannot bind to the DNA at the operator or block transcription, which allows tryptophan to be transcribed by RNA polymerase. So, once there is an absence of tryptophan, which means there is low tryptophan present, the repressor is not going to be active because it has to bind the tryptophan molecule to become an active repressor.

And that is how it will not be able to bind to the operator; as a result, the RNA polymerase will go and bind. It is actually going to do the transcription, and it will produce the polycistronic messenger RNA, so there will be a high level of gene production or transcripts from the operon now in the presence of tryptophan. When there is high tryptophan, things are going to be reversed because if tryptophan is present, it will bind to the repressor proteins, and as a result, it will actually form the active repressor. And if the active repressor is present, it will bind to the operator, and that is how it will prevent the RNA polymerase from entering the promoter and completing the process. The transcription, and that is how there will be a low transcription in the case of tryptophan being present, okay.

Then we have the transcriptional attenuation of the tryptophan operon. So it is possible to obtain stricter regulation in E. coli by repressing the transcription initiation alone, but translation-mediated transcriptional attenuation offers additional regulation. The attenuation site in the tryptophan operon is situated after the start site. More transcription stops here when tryptophan levels are high. When the tryptophan levels are low or sparse, transcription continues to produce functional protein.

So in this case, you are actually going to have the attenuation site. So this is actually going to be the attenuation site that is present. So, at high tryptophan levels, it is actually going to be transcribed, and it is actually going to produce messenger RNA, which is for

this particular protein. So, when the tryptophan concentration is low, the entire operon, including the ligase sequence, is transcribed into messenger RNA. When the tryptophan concentration is high, only the 140 nucleotides, which are part of the sequence that precedes the attenuator, are transcribed into messenger RNA, and the structural genes are not transcribed.

So, it is actually when you have a low level of tryptophan that transcription starts from here and goes all the way up to the end. So, it is actually going to have the full-length messenger RNA, where you will have the leader sequences as well as the RNA sequences of the structural genes. Whereas when you have a very high level of tryptophan, transcription will start from here. But it will only end up here, and you are only going to have the leader sequence of 140 nucleotides, and that is how you are actually going to stop this in the transcription of these particular genes. And this is a very unique phenomenon that is only happening in the tryptophan operon, which is called transcriptional attenuation. What is the mechanism? So the operation leader sequence has a 14-codon open reading frame that codes for a leader peptide of 14 amino acids, which contains two tryptophan codons.

The mechanism of translation-mediated attenuation depends on the fact that translation in bacteria is coupled to transcription. So the ribosome begins translating the 5' end of the messenger RNA while it is still being synthesized. Thus, the translation rate can affect the structure of a growing RNA chain, which determines whether transcription can continue or not. The function of the leader sequence is to fine-tune the expression of the tryptophan operon based on the availability of tryptophan in the cell. The two tryptophan codons for the leader sequence lie within region 1, and the translational stop codon lies between regions 1 and 2. The leader sequence contains four regions, which are regions 1 to 4, and can form various base-paired stem loops or hairpin-like secondary structures.

So regions are like you have region 1, region 2, region 3, and region 4, and region 3 is complementary to both region 1 and region 4; as a result, it will actually form a hairpin-like structure. So, it is actually behaving exactly the same as we have discussed about intrinsic transcriptional stop site. So, it is actually going to form a loop-like structure, and as a result, it is going to stop the growth of RNA polymerase. If regions 3 and 4 base pair with each other, they form a loop-like structure called an attenuator and function as a transcriptional terminator.

If pairing occurs between regions 2 and 3, then no such attenuation forms and transcription continues. So this is exactly the site we have just discussed, where the ribosome binds to the poly-tryptophan polycystin messenger RNA that is being translated when the tryptophan levels are high and starts the translation of the leader sequence. The

translation stop codons are present between regions 1 and 2, and the two tryptophan codons for the leader sequence are in region 1. The ribosome follows the messenger RNA closely during translation and creates the leader peptide. This peptide, the moving ribosome, completes the translation of the leader peptide and pauses at the stop codon, blocking region two.

At this point, the ribosome prevents region two from interacting with sequence three. So sequence three base pairs with the region four to form a three-four stem-loop, which serves as a transcriptional terminator, and as a result, tryptophan prevents the tryptophan operon from continuing to be transcribed. So this is exactly what happens when you have a high level of tryptophan transcription. So there will be a two tryptophan coding region that has been present, and it is actually going to allow the formation of a loop-like structure, which will stop the progression of RNA polymerase; as a result, it will halt transcription. So this is exactly what we discussed.

So if this tryptophan is in short supply, then the ribosome will pause at the two tryptophan codons contained in the sequence. This leaves sequence 2 free to base pair with sequence 3 to form the 2-3 structure, also called an anti-terminator. So the 3-4 structure cannot form, and transcription continues to the end of the tryptophan operon. So when the tryptophan levels are low, there will be base pairing of 2 and 3, and these 2 and 3 are called the anti-terminator because they will not be able to find a strong loop structure. And that's how the transcription will continue, and that's how it is actually going to involve the synthesis of messenger RNA for tryptophan synthesis.

Now let's move on to the third operon, which is called the arabinose operon or the ara operon. So the five-carbon sugar L-arabinose must be broken down by an operon known as the L-arabinose operon, also known as the ara or the araBAD operon in E. coli. The three structural genes, era B, era A, and era D, are found in the L-arabinose of the RAND code for the three metabolic enzymes needed for the breakdown of L-arabinose. These genes generate the enzyme called Arabinose or the ribulokinase Era A, which is an isomerase, and the Era D, which is an epimerase, that catalyzes the conversion of L-Arabinose into the.

D-xylose 5-phosphate is an intermediate in the pentose phosphate pathway. So L-erbinose is also part of the catabolic pathway, right? It is going to follow exactly the same mechanism that we have just discussed regarding the lac operon. So a single transcript and messenger RNA are produced from the transcription of all structural genes in the L-Auburn operon. The catabolite activator protein, or the CAP cyclic AMP complex, which is produced by the regulatory gene RRC, regulates the expression of the L-Auberon operon as a whole. The protein that codes for RAC controls the expression of

Arabidopsis by acting as both an activator when arabinose is present and a repressor when arabinose is absent.

RAC is sensitive to the level of arabinose at high RAC levels. The RAC protein not only regulates the expression of Arabidopsis but also controls its own expression. So, these are the metabolic pathways of L-arabinose through the action of three enzymes. So, when you have L-arabinose, it is going to act through L-arabinose isomerase, which will convert L-arabinose into L-ribulose, and then L-ribulose will act through L-ribulose kinase. And that is how it is going to form L-arabinose 5-phosphate, and L-arabinose 5-phosphate is going to be isomerized.

By the l-arabinose epimerase, that's how it produces d-xylose 5-phosphate. So, what is the structure of the arabinose operon? You will have the regulatory genes like era C, and you will have the promoter, which is called para C. And then you are going to have the different types of structural genes like era B, era A, and era D. Apart from that, you are going to have some of the regulatory proteins and all that. So this is the region of the arabinose operon.

Where the era B is going to be produced by UH, era A is going to form the isomerase, and then era D is going to form the epimerase. The l-arabinose operon consists of three structural genes and the regulatory region with the operator regions called o1 and o2, and the initiation regions called i1 and i2, so these are the regions, right? The structural genes are era B, era A, and era D. There is also a cap binding site where the cyclic cap and cyclic MP complex bind and facilitate the catabolic repression, resulting in the positive regulation of era B when the cells lack glucose. The regulatory gene ARAC is located upstream of the L-arabinose operon and encodes the arabinose-responsive regulatory protein ARAC. Both ARAC and ARABATE have a specific promoter where RNA polymerase binds and initiates the transcription.

ARABATE and ARAC are transcribed in the opposite direction from the ARABATE promoters and the ARAC promoter, respectively. Now, arabinose operon regulation, in addition to being under the control of the CAP cyclic AMP activator, the alerobinose system is also positively or negatively regulated by the binding of RAC proteins. So, RAC performs as homodimers and interacts with the operator and initial range region or initiation range of the alerobinose operon to control the transcription of arabid, arabad. DNA binding domain and a dimerization domain make up such a monomer to the domain. So this is the DNA binding domain, this is the Arabidopsis binding site, and this is the structure of ERa from one over, and there will be dimerization.

So you are going to have the arabinose-binding site, and you are going to have the DNA-

binding site. The binding of arabinose is carried out by the dimerization domain. Upon binding to arabinose, ERASI undergoes an integrational shift and adopts two different conformations. The binding of the allosteric inducer, arabinose, is also the only factor that affects the conformation. When the concentration of ERASI rises too high, ERASI can potentially adversely auto-regulate its own expression by attaching dimer ERASI to the operator region, and ERASI production is inhibited.

So now you have the negative regulation of the arabinose. So cells do not require the arabinose product to metabolize arabinose when it is not present. So, in the absence of arabinose, you're going to have negative regulation. So dimeric era c therefore functions as a repressor: one monomer binds to the arabic genes operator while the other monomer binds to the remote DNA half site called arab. A DNA loop is created as a result. The arabat promoter cannot be bound by the RNA polymerase while in this operational orientation; as a result, the structural derivative transcription is blocked. So this is exactly what is going to happen: you are going to have the operators, which are actually going to dimerize, and that's how you're going to have the binding of the operators onto the arabin 2 and arabin 2 onto the DNA, and that's how it is actually going to form a loop-like structure, and in this loop-like structure, the RNA polymerase will not be able to bind.

Then you have the positive regulation of arabinose, so both in the absence and in the presence of arabinose and the lack of glucose, the arabinose operator is an activator. Uh, activated for expression, so when you have low glucose, you are going to have the ADP followed by AMP followed by the production of cyclic AMP, and that's how you're going to have the cyclic AMP CAP proteins for complex formation, and that complex is going to bind the CAP region of the DNA. And on the other hand, when the aerobinose is present, it will go and bind to the operators, and that is how it will not allow the interaction of the operators to form the loop-like structure, and that is how there will be a transcription of the structural genes from the operons. So era C and CAP cooperate and act as activators when aerobinose is present.

So when glucose is absent, a high level of the CAP protein-cyclic AMP complex binds to the CAP region side. Binding of cyclic AMP is responsible for opening up the DNA loop between era one and era two. O2 increases the binding affinity of era C protein for era I2 and thereby promotes RNA polymerase to bind to the ERABAT promoter to switch on the expression of the ERABAT required for L-urbanose metabolism. So this is all that we have discussed in relation to the regulation of transcriptional regulation of the operons. Now what we have discussed is that the operons are functional mostly in prokaryotic structures, but they are also present in eukaryotic structures. In a typical operon, what you have is a promoter followed by the operators and then followed by the structural genes, and these operons can be positively regulated, negatively regulated,

inducible, or repressible.

So in this context, we have discussed the two operons from the catabolic reactions and one operon from the anabolic reactions. So in a lac operon, it is a catabolic operon where the operon is mostly present as a negatively regulated operon. So there will be no production of the proteins, and there will be no production of enzymes, but when there is an absence of glucose and a presence of lactose, then the bacteria will actually have the transcription of these genes. Because the repressor is going to be bound by the lactose, that is how it is actually going to relieve the inhibition, and that is how there will be transcription of the gene, which is actually going to act on the lactose molecule. And that is how the lactose is going to be converted into glucose and galactose, and that is how that glucose will be utilized by glycolysis to produce energy.

Apart from that, we have also discussed the tryptophan operon and we have also discussed the arabidone subclone. So, this is all about the discussion of the operons and how the operons actually regulate the transcriptional activity within the prokaryotic system. So, with this, I would like to conclude my lecture. Thank you.