MICROBIAL BIOTECHNOLOGY

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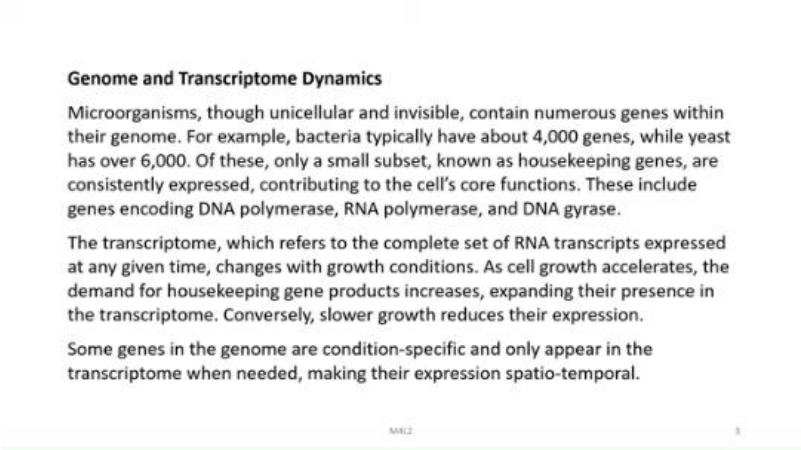
Lecture-14

Lec 14: Regulation of gene expression

Hi friends, welcome to my course on microbial biotechnology. In this lecture we will be studying about the regulation of gene expression. Briefly we will start with genome and transcription dynamics, transcriptomic dynamics, then regulation of gene expression, then what are the different types of gene regulation and certain specific cases like the lac operon and tryptophan operon and some other regulatory mechanisms involved. So, let's start with genome and transcriptome dynamics. Microorganisms are unicellular and invisible and they contain, in spite of these, numerous genes within their genome.

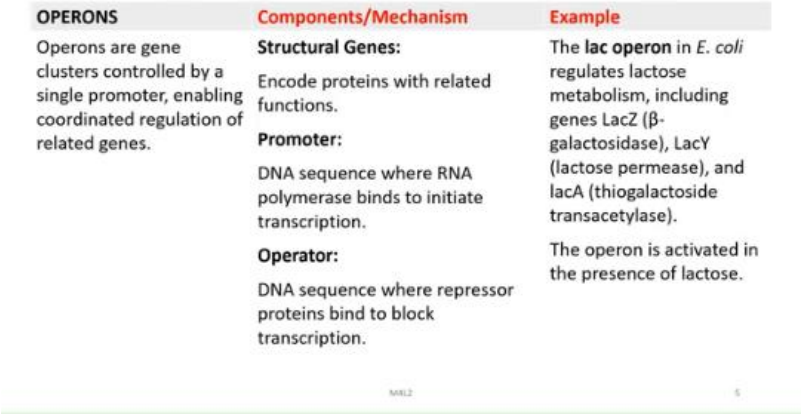
A typical bacteria may have about 4,000 genes while yeast has over 6,000. Of these, only a small subset known as housekeeping genes are consistently expressed contributing to the cell's core functions. These include genes encoding DNA polymerase, DNA RNA polymerase and DNA gyrase. Some of these we have studied in earlier lectures while studying DNA replication, transcription and translation.



The transcriptome refers to the complete set of RNA transcripts expressed at any given time and these changes with growth conditions. As cell growth accelerates, the demand for housekeeping gene products increases, expanding their presence in the transcriptome.

Conversely, slower growth reduces the expression. And if a bacteria is exposed to certain environment, some genes in the genome are condition specific and only then they will appear in the transcriptome when needed, making the expression spatio-temporal. Bacterial gene expression is tightly regulated to adapt to environmental changes only expressing certain genes when required so these helps in economizing the metabolic you know processes reducing consumption of energy in material so basically it will not only help in conserving energy but also maintaining cellular homeostasis

The key mechanisms controlling gene expression include, number one, operons, repressors, and activators; then there's a two-component system, then the core sensing, attenuation, and small RNAs, and feedback inhibition, primarily post-translational regulation. So we'll be studying some of these in detail in this lecture. So if you look into the operons, these are gene clusters controlled by a single promoter, enabling coordinated regulation of related genes, and they are basically structural genes that encode proteins with related functions. So in one go, it will express all the proteins that are required in that particular process or for adapting to that environmental condition. The DNA promoter is the DNA sequence where RNA polymerase binds to initiate transcription, which we have discussed at length in the earlier lecture.



OPERONS	Components/Mechanism	Example
Operons are gene clusters controlled by a single promoter, enabling coordinated regulation of related genes.	**Structural Genes:** Encode proteins with related functions. **Promoter:** DNA sequence where RNA polymerase binds to initiate transcription. **Operator:** DNA sequence where repressor proteins bind to block transcription.	The **lac operon** in *E. coli* regulates lactose metabolism, including genes LacZ (β-galactosidase), LacY (lactose permease), and lacA (thiogalactoside transacetylase). The operon is activated in the presence of lactose.

Then there's the operator, which is a DNA sequence where the repressor protein binds to block transcription. We have examples like the lac operon, which we'll be discussing in detail, and this regulates lactose metabolism. It includes certain genes like lacZ, lacY, and lacA. These proteins are activated in the presence of lactose. Repressors are proteins that inhibit gene transcription by binding to specific DNA regions, typically the operator. An example includes the lac repressor, which binds to the lac operon operator in the absence of lactose, preventing gene expression.

There are activators that enhance gene transcription by binding to specific DNA sequences, facilitating RNA polymerase binding. Examples include the catabolite activator protein, which binds to the lac operon promoter in the presence of low glucose levels, enhancing transcription. In a two-component system, signal transduction mechanisms help bacteria respond to environmental changes through a sensor kinase and a response regulator. The sensor kinase detects environmental signals and autophosphorylates. The response regulator receives the phosphate group from the sensor kinase and binds to DNA to regulate gene expression.

REPRESSORS	Components/Mechanism	Example
Proteins that inhibit gene transcription by binding to specific DNA regions, typically the operator.	**Binding Site:** Repressor binds to the **operator** to block RNA polymerase.	The lac repressor binds to the lac operon operator in the absence of lactose, preventing gene expression.

ACTIVATORS	Components/Mechanism	Example
Proteins that enhance gene transcription by binding to specific DNA sequences, facilitating RNA polymerase binding.	**Binding Site:** Activator binds to regions near the promoter to promote RNA polymerase binding and transcription.	The catabolite activator protein (CAP) binds to the lac operon promoter in the presence of low glucose levels, enhancing transcription.

We have examples in the case of E. coli where the EnvZ-OmpR system regulates outer membrane proteins in response to changes in osmolarity. Then there is another mechanism called quorum sensing, which is a communication system that allows bacteria to coordinate gene expression based on population density through signaling molecules. Here, the components are autoinducers, which are signaling molecules produced by the bacteria and the receptors that bind to autoinducers, initiating gene expression changes when concentrations reach a threshold. In Vibrio fischeri, quorum sensing controls bioluminescence, which occurs only when the bacterial population reaches a high density.

Attenuation is a regulatory mechanism where transcription is prematurely terminated based on specific conditions, such as the availability of amino acids, and the main components include the leader sequence, which is part of the mRNA that forms secondary structures affecting transcription continuation. The tryptophan operon in E. coli, which regulates tryptophan synthesis, leads to premature termination of transcription through attenuation when tryptophan levels are high. Small RNAs also play an important role in this aspect. They are small RNA molecules that regulate gene expression by interacting with target mRNAs to influence their stability or translation.

ATTENUATION	Components/Mechanism	Example
Attenuation is a regulatory mechanism where transcription is prematurely terminated based on specific conditions, such as amino acid availability.	**Leader Sequence:** Part of the mRNA that forms secondary structures affecting transcription continuation.	The **trp operon** in *E. coli* regulates tryptophan synthesis. High tryptophan levels lead to premature termination of transcription through attenuation.

Basically, sRNAs bind to complementary mRNA sequences, leading to degradation or inhibition of translation. The MicF sRNA in E. coli regulates outer membrane proteins in response to environmental conditions. Then there is feedback inhibition, a regulatory mechanism where the end product of a metabolic pathway inhibits an earlier step in the pathway to prevent excess production. Certain end products bind to an enzyme in the pathway, reducing its activity. For example, in amino acid biosynthesis, the accumulation of the end product inhibits the activity of the first enzyme in the pathway, thereby halting the production process.

Regulation of gene expression. Given the high energy demand of protein synthesis, regulating gene expression is crucial for efficiently using available energy resources. Otherwise, there will be wastage without any outcome or benefit for the cell. The production and cellular concentration of proteins are regulated through seven main processes following the central dogma of molecular biology. So, here you can see transcription happening, but then there is also mRNA degradation.

Regulation of gene expression

Given the high energy demand of protein synthesis, regulating gene expression is crucial for efficiently using available energy resources.

The production and cellular concentration of proteins are regulated through seven main processes following the central dogma of molecular biology:

a) Transcription
b) Posttranscriptional modification of mRNA
c) Messenger RNA degradation
d) Translation
e) Posttranslational modification of proteins
f) Protein targeting and transport
g) Protein degradation

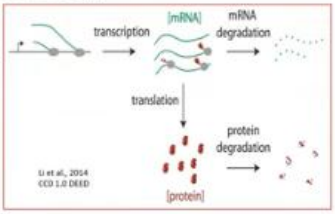
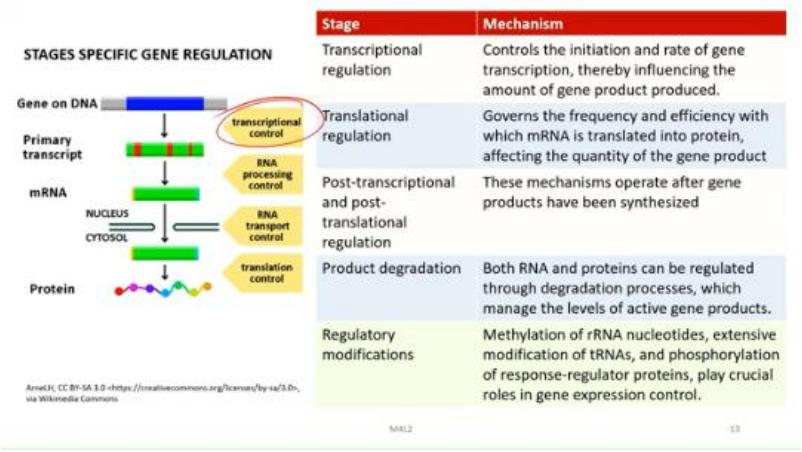


Fig. The processes regulating protein expression provides multiple points for gene regulation.

Then there is translation happening, but then there is also protein degradation. So, these main processes are transcription or post-transcriptional modification of the mRNA, messenger RNA degradation, then there is translation, post-translational modification of proteins, then protein targeting and transport, as well as protein degradation. So, what are the stages or state-specific gene regulations? So, you have this DNA with the genetic information over here, and then we have transcriptional control, which will control the transcription. And overall, even after that, the primary transcript is produced, and then this primary transcript undergoes or is under the dictum of RNA processing control. Then mRNA is produced, which is transported to be translated, and there will also be control at this stage, so this is RNA transport control.



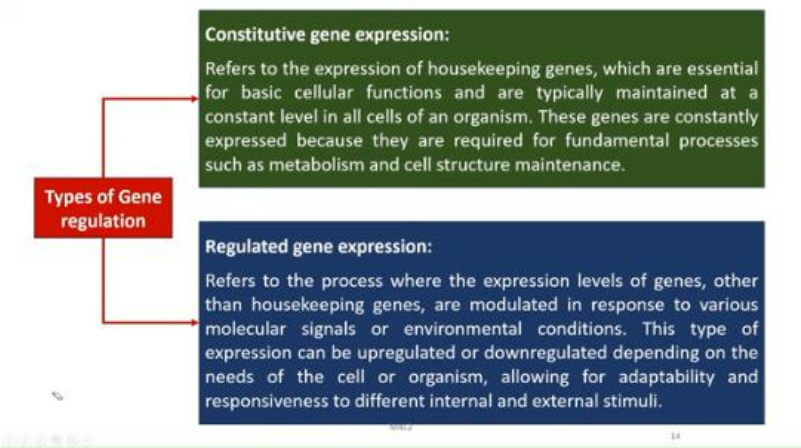
Stage	Mechanism
Transcriptional regulation	Controls the initiation and rate of gene transcription, thereby influencing the amount of gene product produced.
Translational regulation	Governs the frequency and efficiency with which mRNA is translated into protein, affecting the quantity of the gene product
Post-transcriptional and post-translational regulation	These mechanisms operate after gene products have been synthesized
Product degradation	Both RNA and proteins can be regulated through degradation processes, which manage the levels of active gene products.
Regulatory modifications	Methylation of rRNA nucleotides, extensive modification of tRNAs, and phosphorylation of response-regulator proteins, play crucial roles in gene expression control.

And then, before translation, there will also be translational control. So, there is control in each and every step involved in the production of protein from the DNA through the process of mRNA formation. So, this table you can now very easily understand: transcriptional regulation controls the initiation and rate of gene transcription. It influences the amount of gene product produced. Translational regulation governs the frequency and efficiency with which mRNA is translated into protein, affecting the quantity of the gene product.

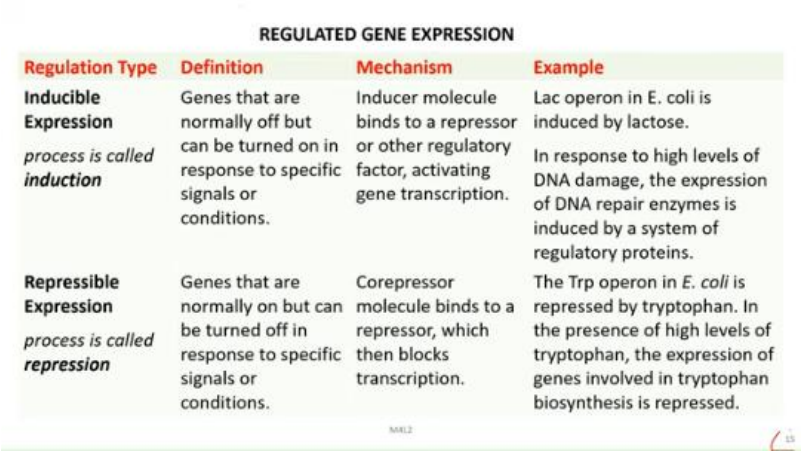
Post-transcriptional or post-translational regulation mechanisms operate after the gene products have been synthesized. Both RNN proteins can be regulated through degradation processes, which are known as product degradation, and these processes also control the level of active gene products. Then there are regulatory modifications: the methylation of RNN nucleotides, rRNA nucleotides, extensive modification of tRNA, and phosphorylation of response regulator proteins. They play a role in gene expression control.

Now, if we look into the different types of gene regulation, they can be broadly divided into two types.

One is constitutive gene expression, which refers to the expression control of housekeeping genes that are essential for basic cellular functions and are typically maintained at a constant level in all cells of an organism. These genes are constantly expressed because they are required for fundamental processes such as metabolism and cell structure maintenance. So that's why they are called constitutive. Then there is regulated gene expression. This refers to the process where the expression levels of genes other than housekeeping genes, which are under the control of constitutive gene expression,



are modulated in response to various molecular signals or environmental conditions. This type of expression can be upregulated or downregulated depending on the needs of the cell or organism to suit or adapt to the environment, thereby allowing for adaptability and responsiveness to different internal as well as external stimuli. Let us now look into the types of regulated gene expression. Here, in inducible expression, genes are normally off but can be turned on in response to specific signals or conditions. This process is known as induction.



REGULATED GENE EXPRESSION

Regulation Type	Definition	Mechanism	Example
Inducible Expression *process is called* ***induction***	Genes that are normally off but can be turned on in response to specific signals or conditions.	Inducer molecule binds to a repressor or other regulatory factor, activating gene transcription.	Lac operon in E. coli is induced by lactose. In response to high levels of DNA damage, the expression of DNA repair enzymes is induced by a system of regulatory proteins.
Repressible Expression *process is called* ***repression***	Genes that are normally on but can be turned off in response to specific signals or conditions.	Corepressor molecule binds to a repressor, which then blocks transcription.	The Trp operon in *E. coli* is repressed by tryptophan. In the presence of high levels of tryptophan, the expression of genes involved in tryptophan biosynthesis is repressed.

The inducer molecule binds to a repressor or other regulatory factor, activating the transcription. Examples are leucoperin, and also in response to high levels of DNA damage, the expression of DNA repair enzymes is induced by a system of regulatory proteins. Then we have repressible expression, and this process is known as repression. Here, genes are normally on but can be turned off in response to specific signals or conditions, just opposite to the earlier one. Core repression molecules bind to a repressor, which then blocks transcription.

The tryptophan operon in E. coli is repressed by tryptophan. In the presence of high levels of tryptophan, the expression of genes involved in tryptophan biosynthesis is repressed. Then we have positive regulation, negative regulation, and feedback regulation. In positive regulation, gene expression is enhanced by the binding of an activator protein. Activator proteins bind to DNA sequences, increasing the likelihood of transcription.

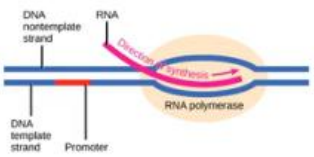
Examples like the CAP, catabolite activator protein in E. coli, enhance lac operon transcription when glucose is low. In negative regulation, gene expression is inhibited by the binding of a repressor protein. Repressor proteins bind to operator regions, blocking transcription. The lac repressor in E. coli inhibits lac operon transcription in the absence of lactose. In feedback regulation, the end product of a pathway regulates the expression of genes involved in its own production.

Regulation Type	Definition	Mechanism	Example
Positive Regulation	Gene expression is enhanced by the binding of an activator protein.	Activator proteins bind to DNA sequences, increasing transcription likelihood.	CAP (catabolite activator protein) in E. coli enhances lac operon transcription when glucose is low.
Negative Regulation	Gene expression is inhibited by the binding of a repressor protein.	Repressor proteins bind to operator regions, blocking transcription.	Lac repressor in E. coli inhibits lac operon transcription in the absence of lactose.
Feedback Regulation	End product of a pathway regulates the expression of genes involved in its own production.	Product inhibits an upstream step in its biosynthesis.	Tryptophan biosynthesis is regulated by feedback inhibition from high levels of tryptophan.

Here, the product inhibits an upstream step in its biosynthesis. Tryptophan biosynthesis is regulated by feedback inhibition from high levels of tryptophan. Then, we have the transcription regulation, and we know that transcription occurs in four steps. The first stage is promoter recognition, then initiation, elongation, and termination, which we have discussed at length in the earlier lecture. Here, we will focus on the regulation of this whole process.

So, we know that RNA polymerases recognize the specific promoter sequence near the transcription start site, forming the transcription initiation complex or TIC. Regulatory proteins can enhance or inhibit RNA polymerase binding, and the strength of the promoter affects how efficiently transcription is initiated. For housekeeping genes with constant expression, RNA polymerase-promoter interactions regulate gene product levels, while variations in promoter sequences control expression levels. Expression of non-housekeeping genes also varies due to promoter differences and is regulated by specific proteins. Eukaryotic promoters are more variable and require general transcription factors, in contrast to prokaryotic expression, which is primarily transcriptional.

Transcriptional regulation



Transcription is initiated by RNA polymerase and other proteins binding at the promoter region

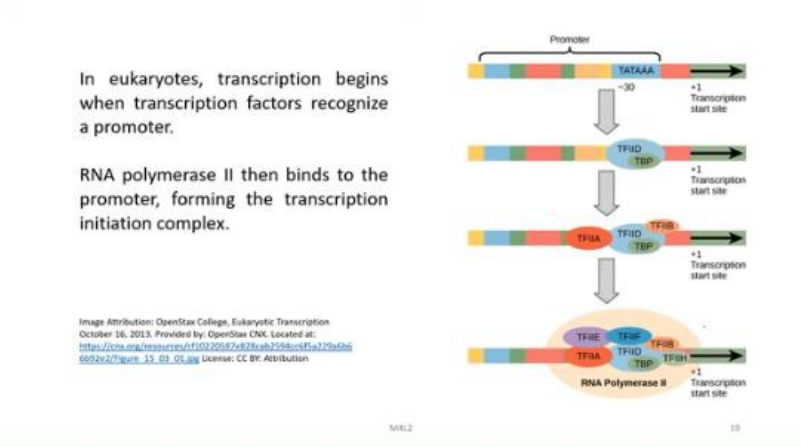
Transcription occurs in four steps:

1. Promoter Recognition,
2. Initiation,
3. Elongation, and
4. Termination.

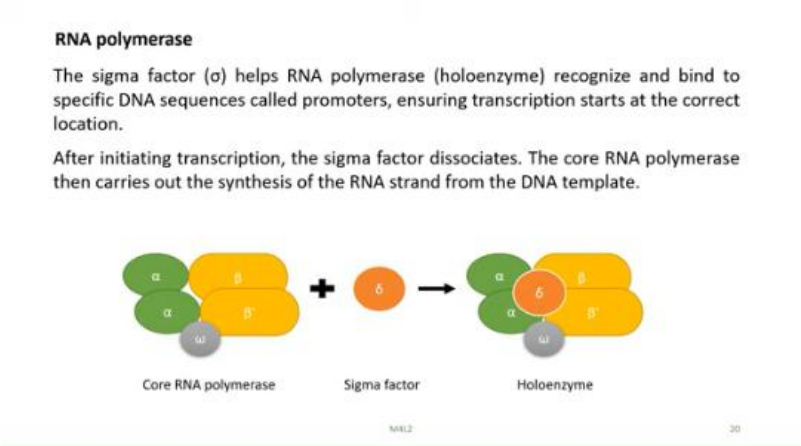
In prokaryotes, RNA polymerase recognizes specific promoter sequences near the transcription start site, forming the transcription initiation complex.

Regulatory proteins can enhance or inhibit RNA polymerase binding, and the strength of the promoter affects how efficiently transcription is initiated.

Eukaryotic gene expression is regulated at multiple levels. So, in eukaryotes, transcription begins when transcription factors recognize a promoter. RNA polymerase II then binds to the promoter, forming the transcription initiation complex. So, you can see here the RNA polymerase with many of these factors that are involved. The sigma factor helps RNA polymerase recognize and bind to specific DNA sequences called promoters.



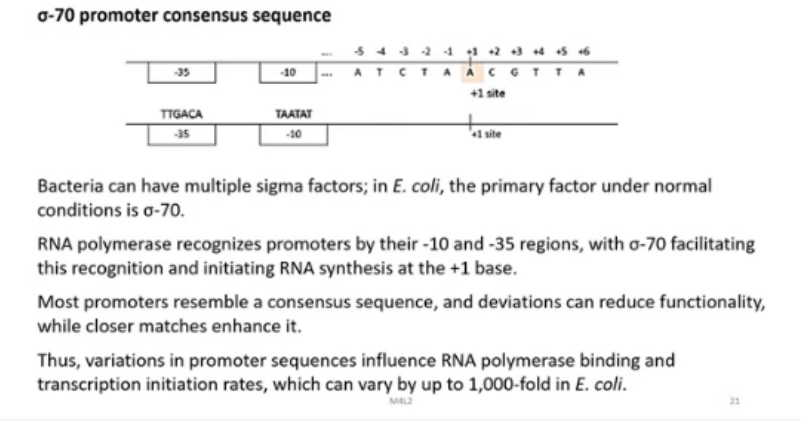
Transcription starts at the correct location. As we discussed in the last lecture, after initiating transcription, the sigma factor dissociates. The core RNA polymerase then carries out the synthesis of the RNA strand from the DNA template. This is all known to you. There are certain consensus sequences in the sigma 70 promoter.



Bacteria can have multiple sigma factors. For example, the primary factor under normal conditions is sigma 70. The RNA polymerase recognizes promoters in their minus 10 and minus 35 regions, with sigma 70 facilitating this recognition and initiating RNA synthesis at the plus 1 base. Most promoters assemble a consensus sequence, and deviations can

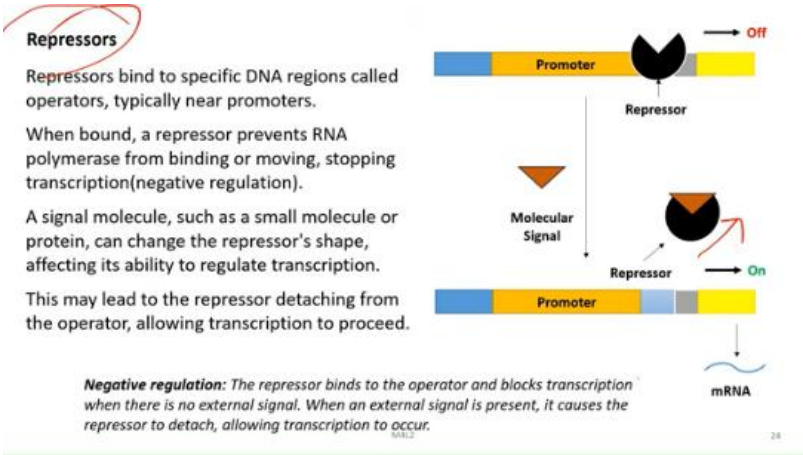
reduce functionality while closer matches enhance it. So, the sequence of these promoters also plays a critical role in this regulation.

Variations in promoter sequences influence RNA polymerase binding and transcription initiation rates, which can vary by up to 1000-fold in the case of E. coli. What are the regulatory proteins of transcription initiation? We know that the sequence plays an important role, but let us look into the regulatory proteins. There are the specificity factors. After the specificity of RNA polymerase for a given promoter, this alters the specificity of RNA polymerases for a given promoter or set of promoters.



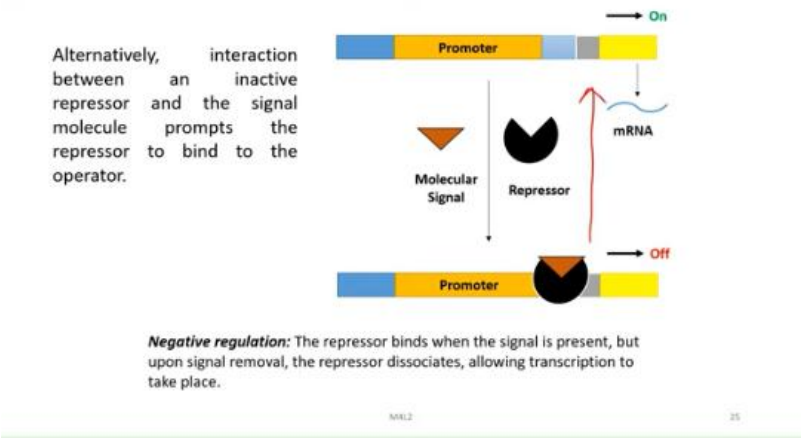
Then there are repressors. These are regulatory proteins binding to their control region, prevents transcription, they excess of RNA polymerase to the promoter, then there are activators, regulatory proteins binding to the control regions, promote RNA polymerase binding to the promoter, they enhance the RNA polymerase promoter interaction. Then the specificity factor, as we have discussed, sigma 70 subunit is a specificity factor under heat stress Sigma 70 is replaced by sigma 32, which directs RNA polymerase to different promoters with a distinct consensus sequence.

In eukaryotic cells, the TATA binding protein serves a similar role among general transcription factors. Now let us look into repressors. Here is a promoter and you can see a repressor getting bound over here and a molecular signal comes and binds to a repressor and this repressor gets detached. from the promoter and in this case the gene will be switched on and as long as the repressor binds to the promoter the gene will be switched off. When bound, a repressor prevents RNA polymerase from binding or moving, stopping transcription.



This is the negative regulation. The signal molecules of this small molecular protein can change the repressor shape affecting its ability to regulate transcription. This may lead to the repressor detaching from the operator allowing transcription to produce and thereby producing mRNA. Alternatively, interaction between inactive repressor and the signal molecule prompts the repressor to bind to the operator and in this case the mRNA production will be switched off.

And in this case, because there is no any repressor binding to it, because there is no any presence of the signal molecule, the mRNA is getting produced. So this is negative regulation, the repressor binds and the signal is present, but upon signal removal, the repressor dissociates, allowing transcription to take place. So this can be also reported. Shown in the reverse way to show the detachment of the signal molecule from the repressor. Then there is activators.

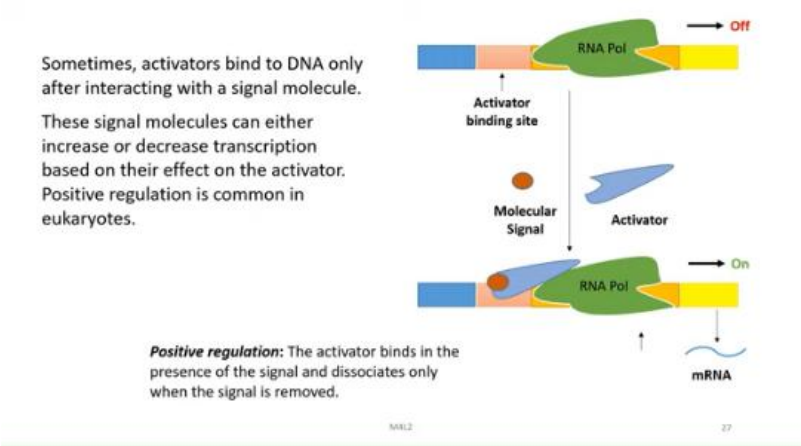


These bind to DNA to boost RNA polymerase activity at a promoter representing positive regulation. So this is the RNA molecule. To these, the activator has bound and which switch

on the process and mRNA is produced then there is a signal molecule binds to the activator binding site and then due to this, this activator is removed and this switch off the process. So, in bacteria, activators are often near weak promoters, increasing transcription.

In eukaryotes, activators bind to distant enhancer regions and affect promoters far away. Some activators remain bound to DNA enhancing transcription until a signal molecule causes them to detach. Sometimes, activators bind to DNA only after interacting with a signal molecule. These signal molecules can either increase or decrease transcription based on their effect on the activator.

This positive regulation is common in eukaryotes, and you can see here that due to the activation binding of this molecular signal to the activator, which together binds to the RNA polymerase, the process is switched on. Let us look into the overall prokaryotic gene regulation. Genes that code for products involved in a group of similar processes are regulated by a basic generic mechanism in bacteria. These genes are grouped together on the chromosome and are also transcribed together under the control of a single promoter. Numerous mRNAs found in bacteria are polycistronic, meaning they contain multiple genes on a single transcript.



The cluster's single promoter, which starts transcription, is where all of the cluster's genes are regulated in terms of expression. An operon is made up of the promoter, gene cluster, and other sequences that work together to regulate the gene. Common operons consist of 2 to 6 genes that are transcribed together. However, some operons include 20 or more genes. So, let us try to learn about the work of François Jacob and Monod in brief.

In E. coli, the genes encoding enzymes for lactose utilization are clustered in the lactose operon, regulated by a single promoter known as the lac promoter. Jacob and Monod

pioneered the discovery of operons through their work on the lac operon, proposing the operon model for bacterial gene regulation in 1961, and were awarded the Nobel Prize in Physiology in 1965 for their contribution. So in prokaryotes, structural genes with similar functions are often grouped in the genome, as we have mentioned several times in this lecture and in earlier lectures, and these are transcribed together under a single promoter.

In *E. coli*, the genes encoding enzymes for lactose utilization are clustered in the lactose (lac) operon, regulated by a single promoter known as the lac promoter.

French scientists François Jacob and Jacques Monod pioneered the discovery of operons through their work on the lac operon, proposing the operon model for bacterial gene regulation in 1961.

They were awarded the Nobel Prize in Physiology or Medicine in 1965 for their contributions.

Francois Jacob

Jaques Monod

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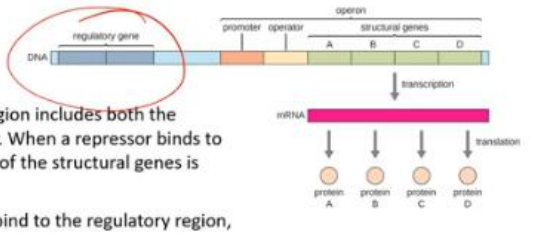
Okay, so these are the structural genes A, B, C, D, for example. So together these constitute an operon, the promoter, operator, and also the structural genes. The operon's regulatory region includes both the promoter and the operator. When the repressor binds to the operator, transcription of the structural genes is inhibited.

In prokaryotes, structural genes with similar functions are often grouped in the genome and transcribed together under a single promoter.



The operon's regulatory region includes both the promoter and the operator. When a repressor binds to the operator, transcription of the structural genes is inhibited.

Conversely, activators can bind to the regulatory region, enhancing transcription.

Figure: Polycistronic transcript

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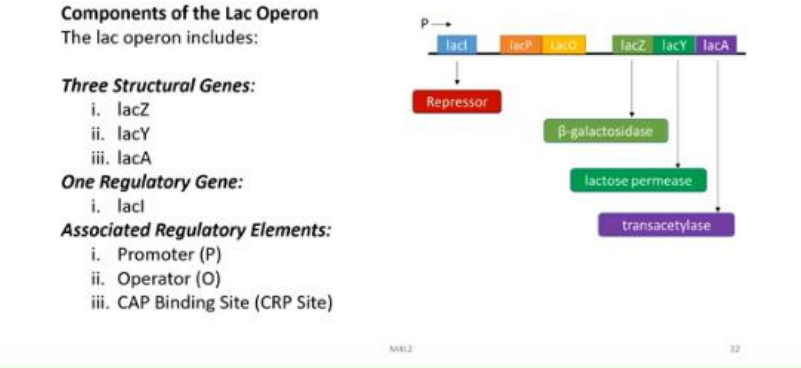
Conversely, activators can bind to the regulatory region, enhancing transcription. So, if transcription happens, this mRNA is produced which contains A, B, C, D continuously, and these are translated later into individual proteins. This is what we call a polycistronic transcript. The lac operon is a well-studied genetic system in Escherichia coli that provides

insight into the regulation of gene expression. It consists of a set of genes responsible for the metabolism of lactose, a sugar found in milk.

The lac operon serves as a model for understanding how cells adapt to environmental changes and regulate gene expression in response to nutrient availability. So, what are the different components of the lac operon? It has three structural genes: the lacZ, lacY, and lacA genes. Then it has one regulatory gene, the lacI gene, and then it has associated regulatory elements: the promoter P, operator O, and CAP-binding site or CRP.

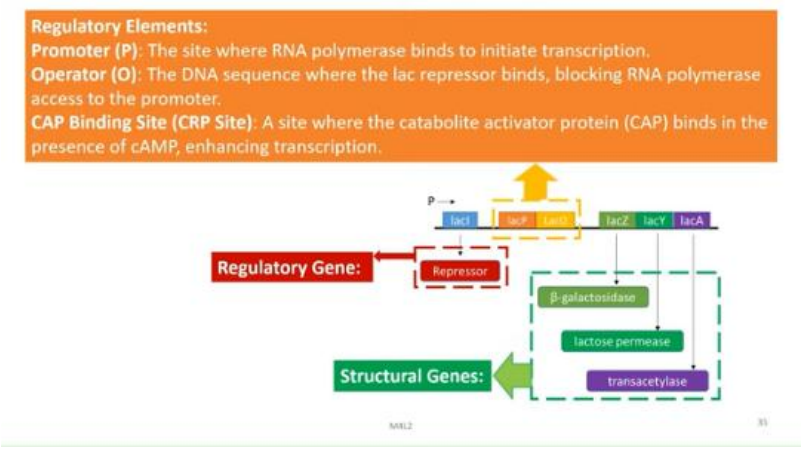
So, this picture shows that Lac-Z produces the protein beta-galactosidase, Lac-Y produces lactose permease, and Lac-A produces transacetylase. So, this shows us the regulatory elements, the structural genes, and then the binding of the repressor to the regulatory gene. LacA encodes the lac repressor protein. This protein binds to the operator region and inhibits transcription of the lac operon genes in the absence of lactose. The structural genes, as we have discussed, produce beta-galactosidase, lactose permease, and transacetylase.



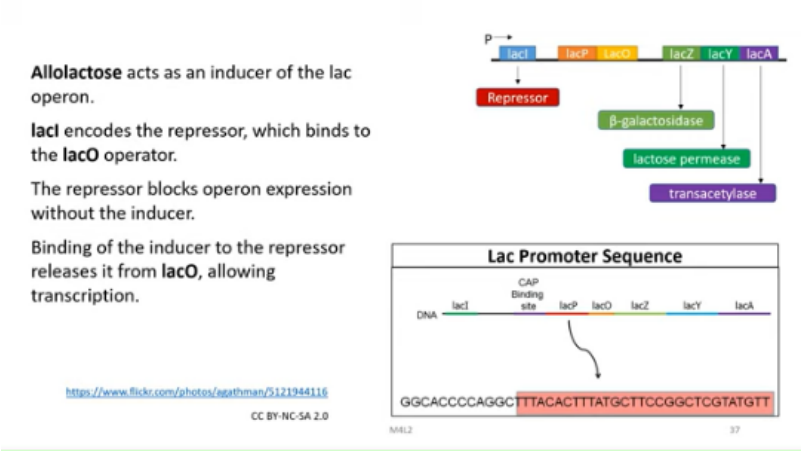
The beta-galactosidase hydrolyzes lactose into glucose and galactose. Then, permease is a membrane protein that facilitates the entry of lactose into the cells. Then, beta-galactosidase is involved in detoxifying byproducts of lactose metabolism. Let us look into the regulatory elements. The promoter site is where RNA polymerase binds to initiate transcription.

The operator site (O) is the DNA sequence where the lac repressor binds, blocking RNA polymerase access to the promoter. The CAP binding site is a site where the catabolite activator protein binds in the absence of cAMP, enhancing transcription. Let us now look into lactose metabolism in E. coli. Galactoside permease and beta-galactosidase activities

are necessary for the uptake and metabolism of lactose. So, this lactose has come inside through these permeases.

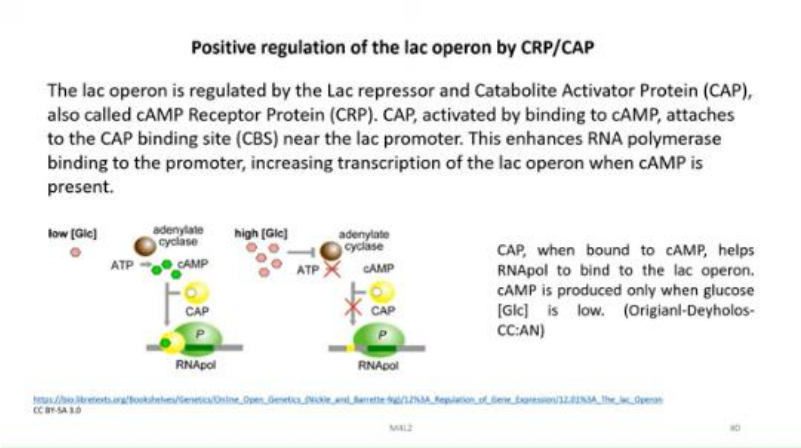


Beta-galactosidase also catalyzes the transglycosylation of lactose to allolactose, as you can see over here, and it also produces glucose and galactose from lactose. Allolactose acts as an inducer of the lac operon. LacI encodes the repressor, which binds to the lacO operator. The repressor blocks operon expression without the inducer. Binding of the inducer to the repressor releases it from lacO, allowing transcription to proceed.



The lac operon is inducible and can be activated without glucose. Lac gene products break down lactose into glucose and galactose. In the absence of lactose, the lac repressor binds to the operator, blocking RNA polymerase from transcribing the genes. The lac operon is expressed only when lactose is present, preventing wasteful enzyme production. Lactose is converted to allolactose, an inducer that binds to the repressor, altering its shape so it cannot bind the operator.

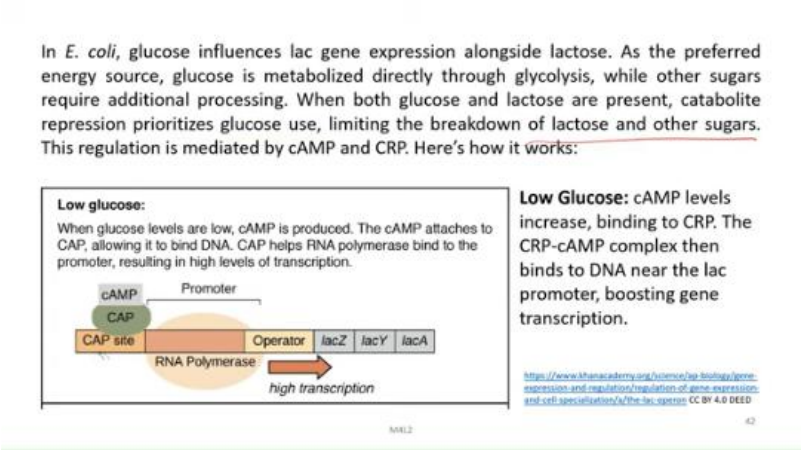
This allows RNA polymerase to transcribe the structural genes. Free to transcribe these structural genes. Positive regulation of the lac operon by CRP or CAP. The lac operon is regulated by the lac repressor and catabolite activator protein, also called cAMP receptor protein or CRP. CAP, activated by binding to cAMP, attaches to the CAP binding site near the lac promoter.



This enhances RNA polymerase binding to the promoter, increasing transcription of the lac operon when cAMP is present. CAP, when bound to cAMP, helps RNA polymerase bind to the lac operon. cAMP is produced only when glucose is low. Positive regulation of the lac operon occurs when glucose levels drop. E. coli may use other sugars for energy, which requires new genes to be transcribed.

As glucose decreases, cAMP levels rise. cAMP binds to the CAP protein. It then attaches to a region upstream of the genes needed for using other sugars, enhancing their transcription. Low glucose influences lac gene expression alongside lactose as the preferred energy source. Glucose is metabolized directly through glycolysis, while other sugars require additional processing.

When both glucose and lactose are present, catabolite repression prioritizes glucose use, limiting the breakdown of lactose and other sugars. This regulation is mediated by cAMP and CRP. In the case of low glucose, cAMP levels increase, binding to CRP. The CRP-cAMP complex increases

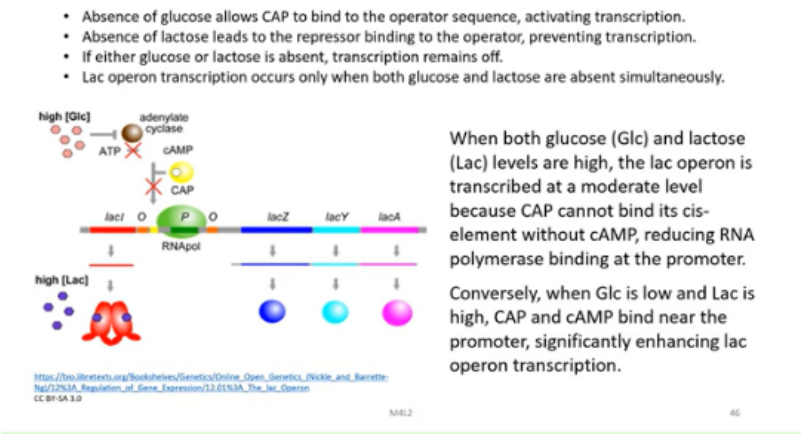


then binds to DNA near the leg promoter boosting gene transcription. Under high glucose condition camp levels drop releasing CRP binding to DNA you can see here and lowering the lac operon expression the transcription will be low in this case and here the transcription is high because the cap is binding to this side but the here cap is not binding to this cap side CRP camp and lac operon regulation CRP camp function CRP acts as a positive regulator when glucose is low and High camp levels allow CRP to bind DNA and enhance expression operants like the lac operon. Lac repressor function.

The lac repressor is a negative regulator that prevents gene expression in the absence of lactose. For maximum lac operon expression, both lactose and low glucose are required. Effect of glucose in camp. When glucose is present, camp levels decrease, inhibiting CRP binding to DNA and lowering lac operon expression. Operon regulation.

The lac operon is most active when lactose is available and glucose is scarce. CRP camp regulates other operons for secondary sugar metabolism like arabinose. A regulon is a group of operons controlled by the same regulatory protein coordinating expression across multiple operons. In eukaryotic systems, similar gene regulation principles apply involving complex networks controlled by various factors. E. coli prefers glucose, thus the like operon is highly expressed.

Only when glucose is low and lactose is present, ensuring efficient gene expression based on nutrient availability. The absence of glucose allows CAP to bind to the operator sequence, activating transcription. The absence of lactose leads to the repressor binding to the operator, preventing transcription. If either glucose or lactose is absent, transcription remains off. Lack of operon transcription occurs only when both glucose and lactose are absent simultaneously.



When glucose and lactose levels are high, the lac operon is transcribed at a moderate level because CAP cannot bind its cis element without cAMP, reducing RNA polymerase binding at the promoter. Conversely, when glucose is low and lactose is high, CAP and cAMP bind near the promoter, significantly enhancing lac operon transcription. So in this table, we can see conditions that affect transcription of the lac operon as a summary. So glucose high or positive and glucose negative. In the first case, CAP will bind.

That's not binding. But in the case of low, it is binding. So when lactose is absent, the repressor binds. So in this case, there is no transcription.

Now, in the case of the presence of glucose and lactose, CAP cannot bind, the repressor cannot bind, and there will be a little bit of transcription. And in the other two cases where glucose is present and lactose is absent, CAP binds, the repressor binds, and there is no transcription. And then, in the case where glucose is absent, CAP is binding; lactose is present, then the repressor is binding, and transcription is happening. Let us now discuss the tryptophan operon. So, tryptophan is an aromatic amino acid that is synthesized through the activity of several key enzymes, such as anthranilate synthetase.

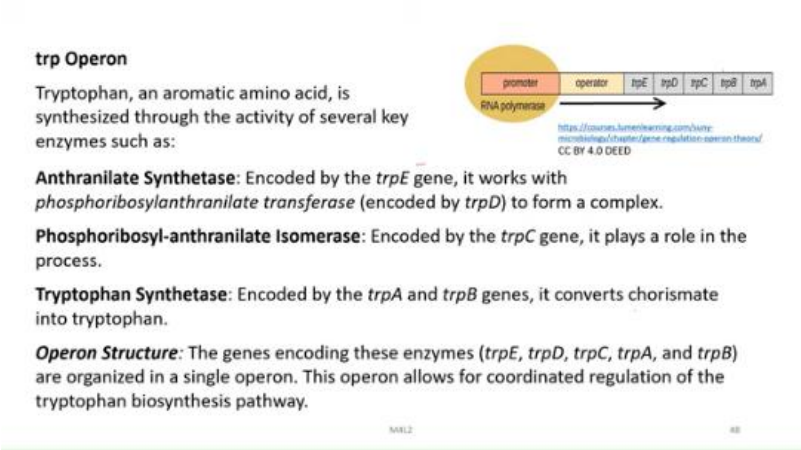
Table: Conditions Affecting Transcription of the *lac* Operon

Glucose	CAP binds	Lactose	Repressor binds	Transcription
+	–	–	+	No
+	–	+	–	Some
–	+	–	+	No
–	+	+	–	Yes

Then you have phosphoribosyl anthranilate isomerase and tryptophan synthetase. These different enzymes are encoded, for example, by the TRPE gene. It works with phosphoribosyl anthranilate transferase. This is encoded by the TRPD gene. So, this is the TRPE gene, as you can see.

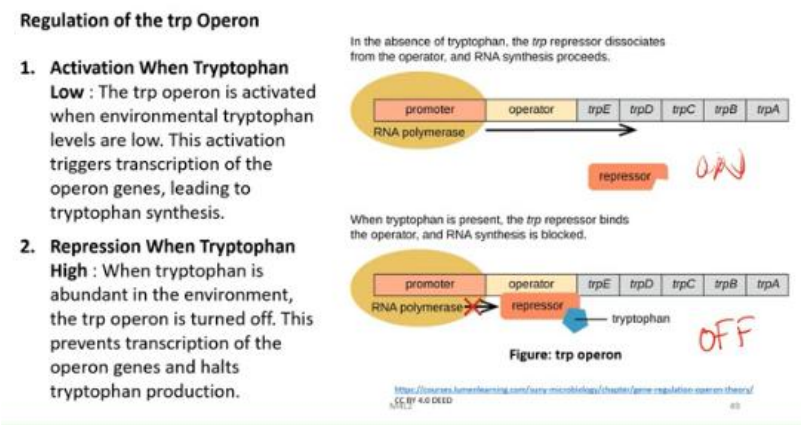
And then there is the TRPC gene, which produces the next enzyme here. This plays a crucial role, and then there are the TRP-A and TRP-B genes here, which produce tryptophan synthase, and this converts chorismate into tryptophan. Now, all these genes of the tryptophan operon are under a single controlling element, the promoter and operator, as you can see. So, they are a polycistronic gene.

Now, this is the operant structure as we have already discussed and because they are located under single promoter operator, it allows for coordinated regulation of the tryptophan biosynthesis pathway. So, whenever tryptophan is required, all these enzymes required in the process will be produced together and because absence of one single enzyme will stop the process of tryptophan production. So, this ensures that tryptophan production happens as and when needed by the cell. So, these arrangement of all the genes in a single operon.



Now, how these particular operon is regulated? We will try to understand here. So, in the absence of tryptophan, the tryptophan repressor will dissociate from the operator and RNA synthesis will proceed. When tryptophan is present, we do not need tryptophan.

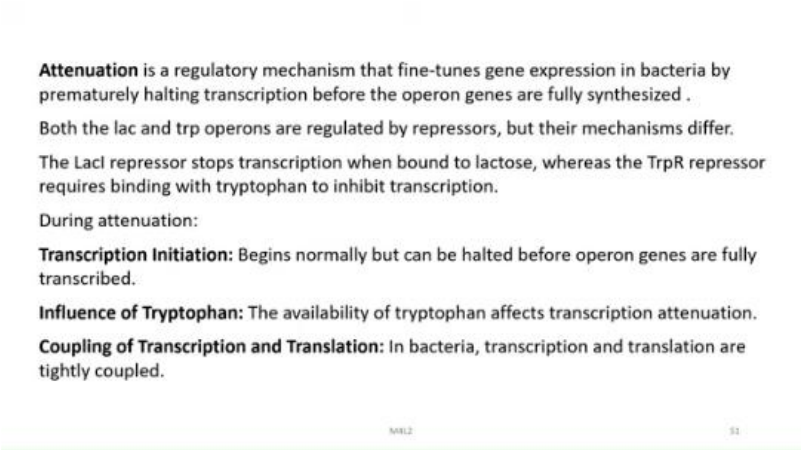
So, the repressor will bind to the operator and the RNA synthesis is blocked. So, this is a simple switching on and switching off mechanism. So, in a way the repressor gets encode to the regulatory site, the tryptophan operon is switched off. So, none of those enzymes which are required in the tryptophan synthesis are allowed to produced. But when there is demand for tryptophan, the repressor is detached and the operand is switched on.



So activation occurs when tryptophan is low, and there is repression when tryptophan is high. So, the tryptophan R function—tryptophan R is a translational repressor that binds the TRP operon when tryptophan levels are high in the cell—and the TRP R gene encodes the TRP R repressor, which is located away from the tryptophan operon. So, this is not a part of this. Tryptophan operon. This repressor gene, which is encoded by the TRPR gene, is located away from the tryptophan operon.

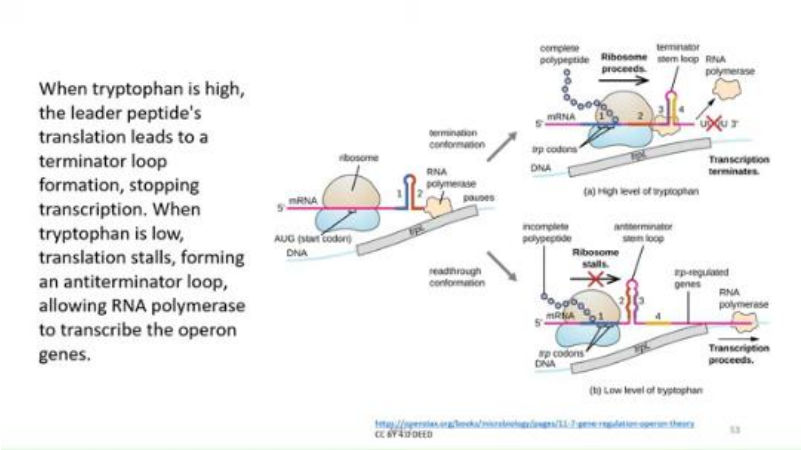
So when tryptophan is low, TRPR does not bind to the operator. So the operator is active, and tryptophan is synthesized. At high levels, it binds to TRP-R, causing a shape change in the repressor, allowing TRP to bind to the operator, blocking RNA polymerase from transcribing the operator genes. The overall expression of the tryptophan operator is regulated by the presence of tryptophan, the end product of the pathway it controls. Attenuation is a regulatory mechanism that fine-tunes gene expression in bacteria by prematurely halting transcription before the operon genes are fully synthesized.

Both the LAC and TRP operons are regulated by repressors, but their mechanisms differ. The LAC-I repressor stops transcription when bound to lactose, whereas the TRP-R repressor requires binding with tryptophan to inhibit transcription. During attenuation, transcription initiation begins normally but can be halted before operon genes are fully transcribed. Then there is the influence of tryptophan. The availability of tryptophan affects transcription attenuation.



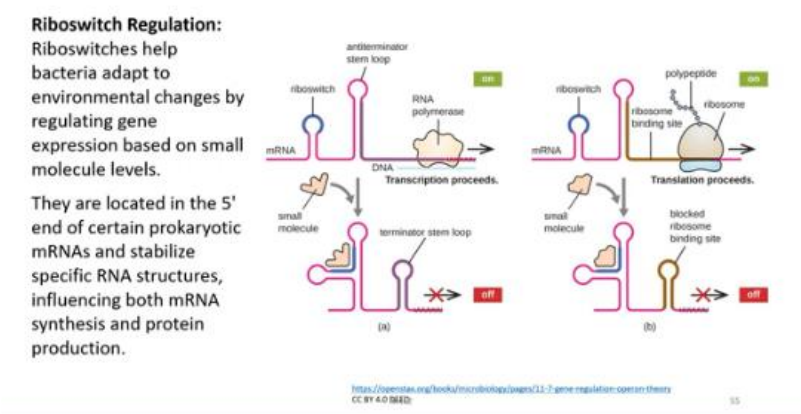
In bacteria, transcription and translation are tightly coupled. In a TRP operon, the leader sequence, TRP-L, has regions that can form different tRNA structures. For example, the terminator loop forms when tryptophan is abundant, causing transcription to stop. Then, there is an anti-terminator loop that forms when tryptophan is low, allowing transcription to continue. When tryptophan is high, the leader peptide's translation leads to terminator loop formation, stopping transcription.

When tryptophan is low, Translation stalls, forming an anti-terminator loop, allowing RNA polymerase to transcribe the operon genes. There are other regulatory mechanisms, like the riboswitch. Riboswitches are regulatory elements in some bacterial mRNAs. They control gene expression by changing shape in response to small molecules' structure and function.



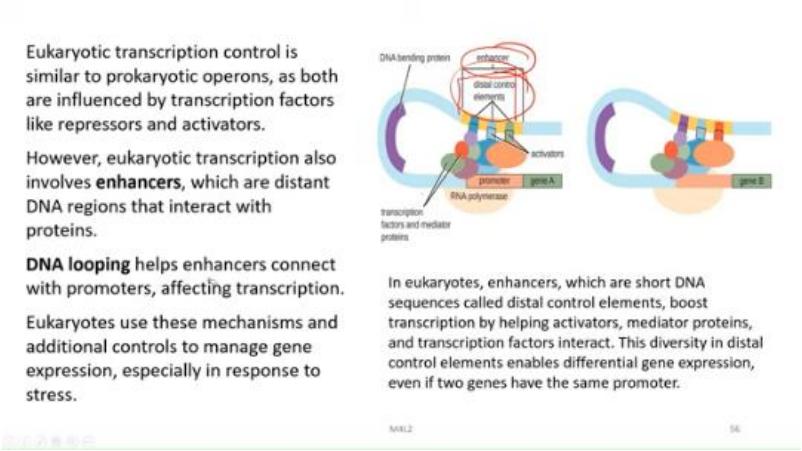
A riboswitch is an mRNA segment that alters its shape when binding to a specific small molecule. How does it work? It blocks transcription. The riboswitch can form a structure that prevents RNA polymerase from continuing transcription or blocks translation. It forms a structure that hides or exposes the ribosome binding site, affecting translation.

Ribose switches help bacteria adapt to environmental changes by regulating gene expression based on small molecule level. They are located in the 5' end of certain prokaryotic mRNAs and stabilize specific RNA structures influencing both mRNA synthesis and the protein production. So, in this picture you can see that this is the riboswitch in the mRNA and here there is an empty terminator stem loop and these RNA polymerase binding over here and it allows the transcription to proceed. But whenever a small molecule binds to it and you can see here it is binding between the anti-terminator stem and riboswitch here. So the structural change is happening and the process is therefore stopped and this cannot proceed.



In another case also you can see here is the riboswitch and there is the translation machinery. So here similar small molecule binds and these blocks the ribosome binding site as a result of which the translation is switched off. Eukaryotic transcription control is similar to prokaryotic operants as both are influenced by transcription factors like repressors and activators. However, Eukaryotic transcription also involves enhancers which are distant DNA regions that interact with proteins. So, you can see here a gene A under control of a promoter and these are the transcription factors and mediators which have bound and then these are the activators and this is the enhancer with distal control animal.

So, if you open it up, it actually will be located in another region over here. But these DNA binding has brought it near to the promoter region of the gene which is to be expressed or repressed. So DNA looping here, it helps enhancers connect with promoters affecting transcription. Eukaryotes use these mechanisms and additional control to manage gene expression especially in response to various stress.



So here in eukaryotes, enhancers with short DNA sequences called distal control elements boost transcription by helping activators, mediator proteins, and transcription factors interact. This diversity in distal control elements enables differential expression even if two genes have the same promoter. So finally, let us discuss eukaryotic epigenetic regulation. In other words, epigenetics stands for 'beyond genes.' In eukaryotes, epigenetic regulation involves chemical modifications of DNA and histones that affect transcription, such as methylation of specific cytosine

nucleotides, often triggered by environmental factors, which generally reduce gene expression. Histones can also undergo modifications like acetylation and deacetylation,

which alter DNA packaging and its accessibility for transcription. So with this, we come to the end of our lecture on gene regulation. Thank you for your patient hearing. Amen.