Botany-UG 4th semester- paper C8T: UNIT-4- TRANSCRIPTION
Operon concept

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OPERON

The basic concept for the way transcription is controlled in bacteria is called the operon model and was proposed by Francois Jacob and Jacques Monod in 1961.

We distinguish between the components of regulatory circuits and the genes that they regulate by the terms structural gene and regulator gene.

- A structural gene is simply any gene that codes for a protein (or RNA) product. Protein structural genes represent an enormous variety of structures and functions, including structural proteins, enzymes with catalytic activities, and regulatory proteins.
- A type of structural gene is a regulator gene, which simply describes a gene that codes for a protein involved in regulating the expression of other genes (activator or repressor).

A regulator gene codes for a protein that controls transcription by binding to particular site(s) on DNA. This interaction can regulate a target gene in either a positive manner (the interaction turns the gene on) or a negative manner (the interaction turns the gene off). The sites on DNA are usually located just upstream of the target gene.

The binding of an activator protein to its target DNA site increases the rate of transcription. That is termed as **positive regulation.**

The binding of the repressor protein to its target DNA site (operator) prevents the gene from being expressed. Hence, this is called **negative regulation.**

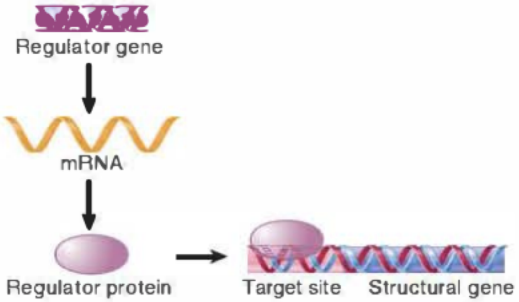


FIGURE 26.1 A regulator gene codes for a protein that acts at a target site on DNA.

General regulation of gene

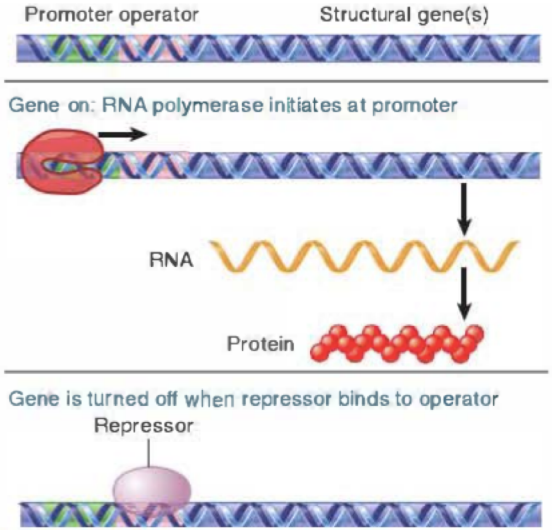


FIGURE 26.2 In negative control, a *trans*-acting repressor binds to the *cis*-acting operator to turn off transcription.

Negative regulation of gene

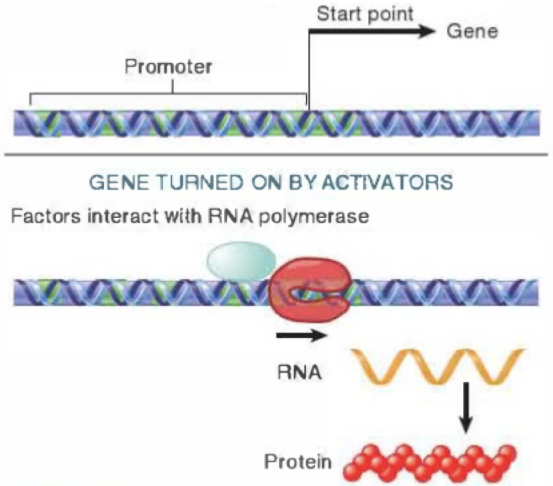


FIGURE 26.3 In positive control, a *trans*-acting factor must bind to the *cis*-acting site in order for RNA polymerase to initiate transcription at the promoter.

Positive regulation of gene

In addition to negative and positive control, a gene that encodes an enzyme may be regulated by the **concentration of its substrate or product.**

- The synthesis of enzymes in response to the appearance of a specific substrate is called induction and the gene is the **inducible gene**. For eg- when lactose (the substrate) is present in the environment the enzymes needed for utilization of lactose are also needed to be expressed. Hence the presence of lactose will induce the expression of the genes encoding the proteins required for lactose utilization.

- The opposite of induction is **repression**, where the repressible gene is controlled by the amount of the product made by the enzyme. For example, E. coli synthesizes the amino acid tryptophan through the actions of an enzyme complex containing tryptophan synthetase and four other enzymes. If tryptophan is already present in the medium on which the bacteria are growing, the production of the enzyme is immediately stopeed because there is enough tryptophan. This allows the bacterium to avoid devoting its resources to unnecessary synthetic activities.

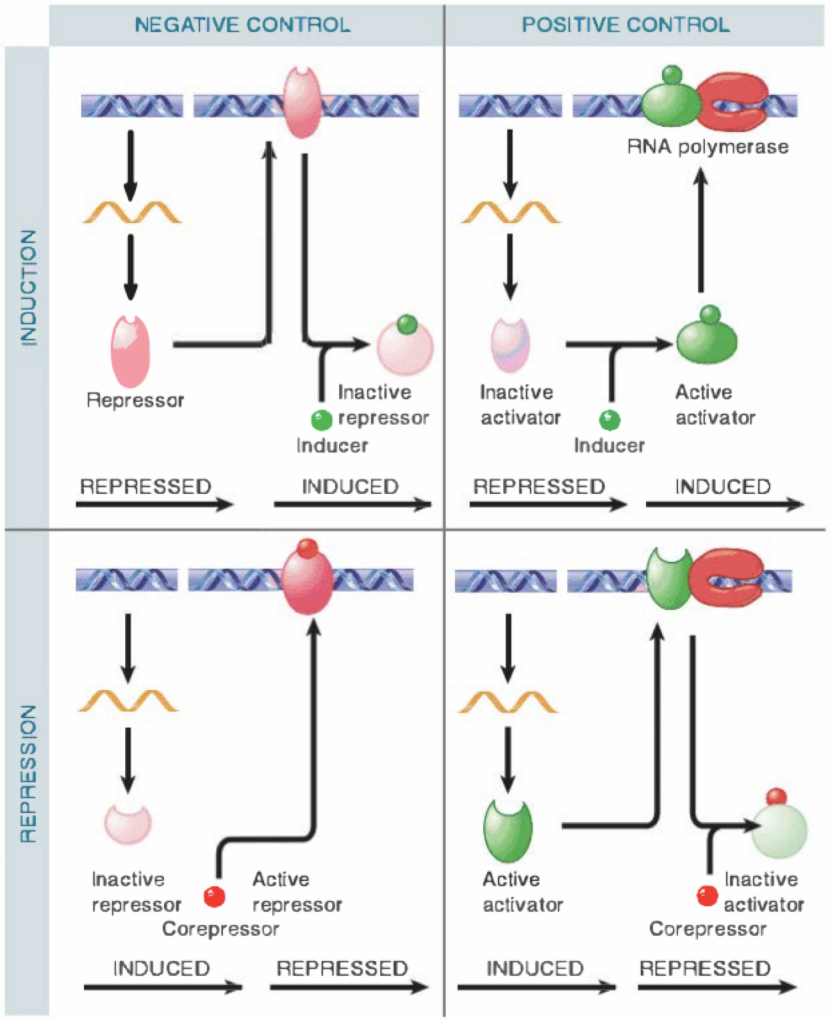
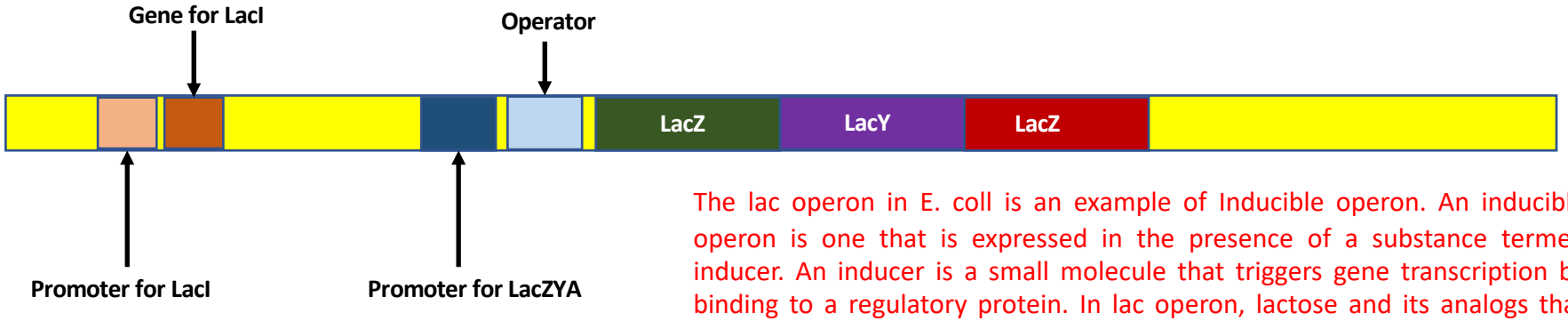


FIGURE 26.4 Regulatory circuits can be designed from all possible combinations of positive and negative control with inducible and repressible control.

An operon is a unit of bacterial gene expression and regulation, which includes structural genes and regulatory sequences recognized by regulatory gene products.
As this is prevalent in prokaryotic systems, all the genes are expressed as a single unit.

Lac Operon-

An operon consists of *cis acting* regulatory elements and proteins encoding structural genes.
The typical example is **lac operon.**



The lac operon in E. coll is an example of Inducible operon. An inducible operon is one that is expressed in the presence of a substance termed inducer. An inducer is a small molecule that triggers gene transcription by binding to a regulatory protein. In lac operon, lactose and its analogs that lead to the expression of the lac genes are termed inducers and the relief of repression for operon is termed induction

Components of Lac operon-

1. **Lac Operator-** The lac operator is a short region of DNA that lies between the promoter and the structural gene and that interacts with a regulatory protein, which controls the transcription of the operon.

2. Lac structural genes- Lactose is a β-galactoside that E. coli can use for energy and as a carbon source. Lactose uptake and degree is mediated by the products of three structural genes. A structural gene is simply any gene that codes for a protein (or RNA). The roles of the three structural genes are:

1. lacz codes for the enzyme β -galactosidase that cleaves the lactose molecule to yield glucose and galactose. This enzyme also converts lactose to allolactose.
2. lacY codes for the β -galactoside permease, which transports lactose into the cell.
3. lacA codes for β -galactoside transacetylase. The transacetylase is not essential for lactose metabolism but appears to play a role in the detoxification of compounds by transferring an acetyl group from acetyl-CoA to β -galactosides.

All three genes are transcribed into a single, multigenic messenger RNA molecule. Regulation of the production of this mRNA coordinates the regulation of the synthesis of all three enzymes.

3. Lac repressor- The Lạc repressor is the product of a trans-acting regulatory gene that regulates the transcription of the structural genes comprising lac operon. A regulatory gene simply describes a gene that codes for a protein (or an RNA) involved in regulating the expression of other genes. The regulatory gene (I gene), encodes the lac repressor protein, so named because it can block the expression of the Z, Y and A structural genes. Lac repressor molecule is a homotetramer made up of four identical subunits

the lac repressor can only bind to the lac operator. By binding to the operator, the repressor prevents transcription initiation by RNA polymerase that has bound to its lac promoter site.

The Lac repressor is a molecule with two recognition sites-a DNA-binding site that can recognize the specific operator DNA sequence for the lac operon and an allosteric site that binds the lactose allosteric effector and similar molecules (analogs of lactose). When the repressor binds to lactose or its analogs, the protein undergoes a conformational (allosteric) change; this slight alteration in shape changes the DNA-binding site so that the repressor no longer high affinity for the operator. Thus, in response to binding lactose, the repressor falls off the DNA.

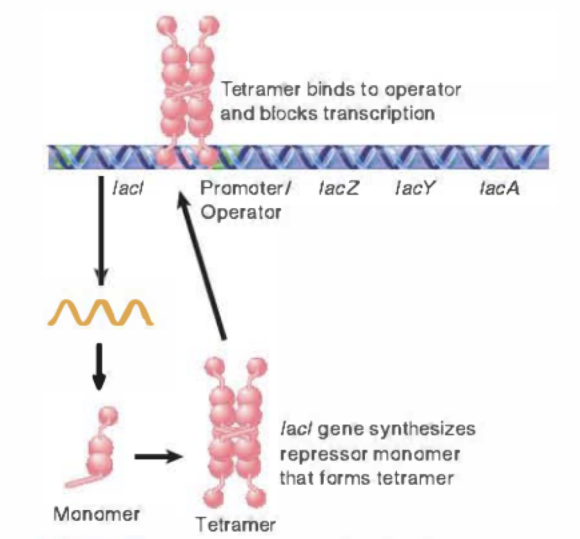


FIGURE 26.8 *lac* repressor maintains the *lac* operon in the inactive condition by binding to the operator. The shape of the repressor is represented as a series of connected domains as revealed by its crystal structure.

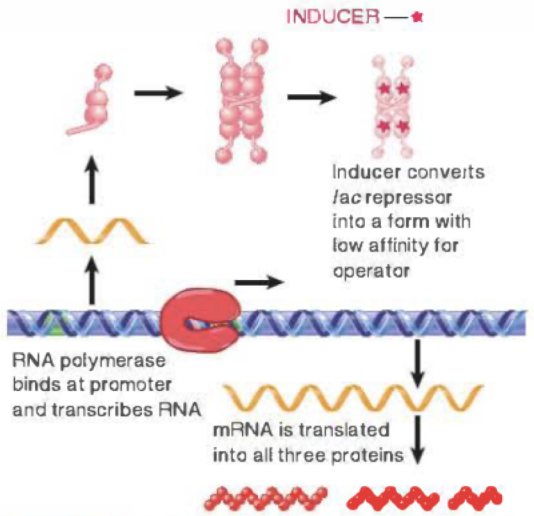


FIGURE 26.9 Addition of inducer converts repressor to a form with low affinity for the operator. This allows RNA polymerase to initiate transcription.

The lac operon is controlled by both positive and negative regulation.

Negative regulation of lac operon-

In the absence of an inducer (lactose or an analog), the lac repressor binds to the lac operator site and prevents transcription of the lac operon. Because binding of a repressor prevents the transcription, it is termed negative regulation. In this sense, the lac repressor molecule is a negative regulator and acts as a roadblock on the DNA. Consequently, all of the structural genes of the lac operon (the Z, Y and A genes) are repressed, and there is no B-galactosidase, β -galactoside permease or transacetylase in the cell.

When an inducer is present, it binds to the allosteric site of the Lac repressor, thereby inactivating the operator binding site of the Lac repressor protein. However lactose itself does not have this effect: rather, allolactose does. Allolactose is thus referred to as the actual inducer of the lac operon. Formation of allolactose is also catalyzed by the β -galactosidase. Inactivation of Lac repressor permits the induction of transcription of the structural genes of the lac operon and after translation of the genes, the enzymes required for utilization of the lactose are synthesized.

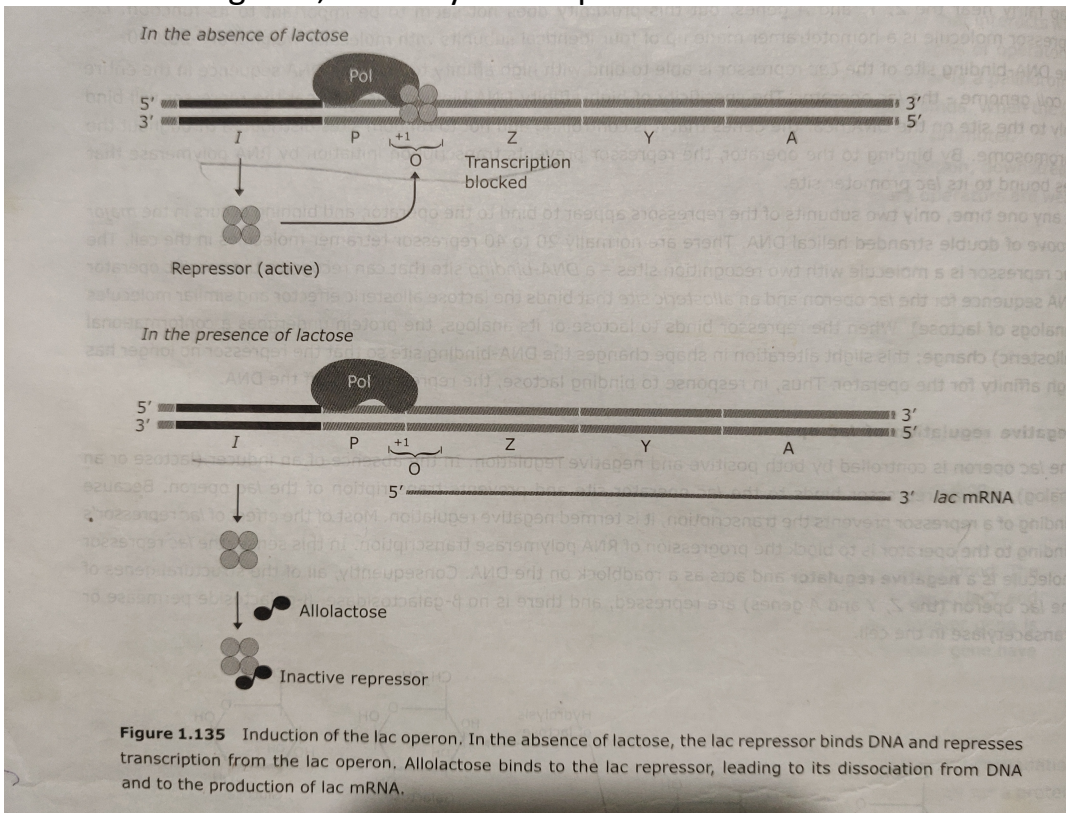
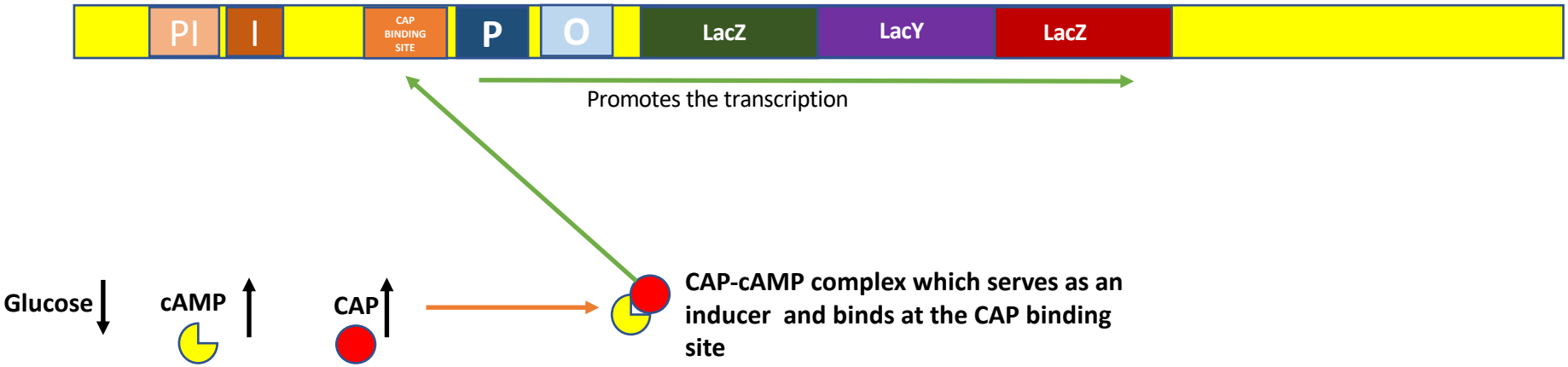


Figure 1.135 Induction of the lac operon. In the absence of lactose, the lac repressor binds DNA and represses transcription from the lac operon. Allolactose binds to the lac repressor, leading to its dissociation from DNA and to the production of lac mRNA.

Positive regulation and catabolite repression– (in presence of glucose)

The bacteria have distinct preference among potential carbon sources when they are offered a choice. When glucose is available as an energy source, it is preferred as an energy source because it is the simplest form. So when *E.coli* finds both glucose and lactose in the medium, it metabolizes the glucose and represses the use of lactose. This phenomenon is due to the repressive effect of glucose on the synthesis of enzymes required for the metabolism of other sugar. Hence this is also known as catabolite repression.

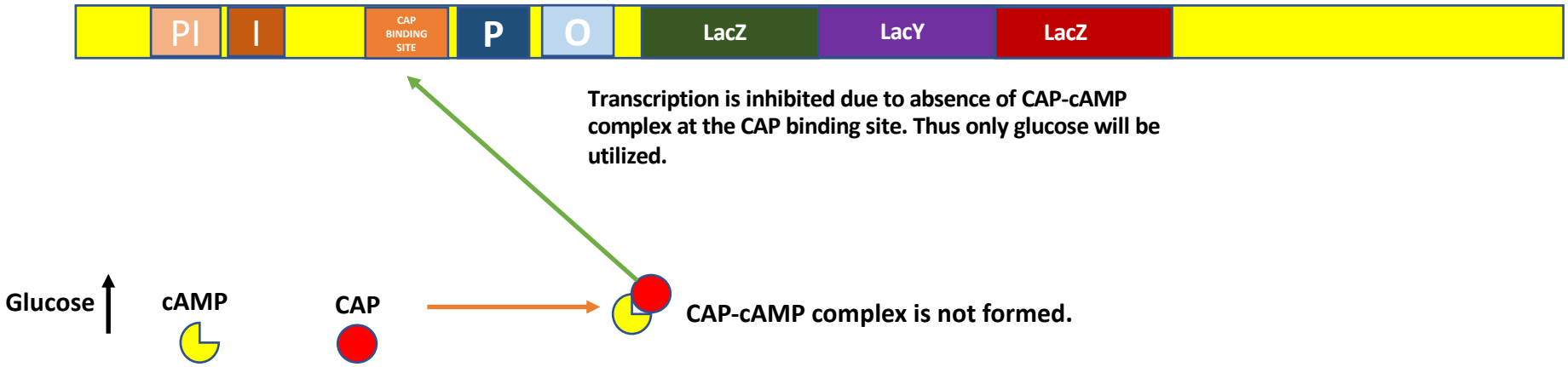
In a situation when there is no glucose, only lactose is present (glucose -, lactose +)



When there is no glucose, the concentration of cAMP increases as it has inverse relationship with glucose. There is a specific site adjacent to the promoter in the operon called CAP binding site. Now when there is no glucose, the cAMP concentration is high and *E.coli contains* another protein called CAP (catabolite gene activator protein also known as CRP). CAP and cAMP bind together to form cAMP- CAP complex which acts as an inducer and binds at the CAP binding site beside the promoter region. This interaction triggers the binding of RNA polymerase at the promoter region and helps in promoting transcription of the lac structural genes.

This serves as a dual regulation apart from the normal lac operon system. Since lactose is present, it is not allowing the inhibitor to bind at the promoter. Hence this is dual degree of positive signal for transcription

In a situation when glucose is present, but lactose is also present (glucose +, lactose +)



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Here since lactose is present, lac operon should be turned on, but since glucose is also present, the regulation will be different. Because since glucose is present, bacteria will try to utilize glucose as their preferred source of energy and they will not turn on the lac operon until all the glucose is completely depleted. If glucose is present, concentration of cAMP is less and thus cAMP-CAP complex cant be formed. So the inducer cannot bind at the CAP binding site and thus it will not get the positive signal for the RNA polymerase to continue transcription. Since Lactose is also present, lacI repressor cant inhibit the transcription. But since CAP binding site is empty, the transcription will be very slow and very low concentration of protein will be translated. Now after utilizing all the glucose, the concentration of glucose will be depleted, the concentration of cAMP will increase, this will again inhibit the lac operon.

Tryptophan operon

- The tryptophan operon, required for the synthesis of the amino acid tryptophan, is an example of a negatively controlled repressible operon. A negatively controlled operon is one that is inhibited by a regulatory protein such as a repressor. This operon is also anabolic type that means many proteins will be required to produce tryptophan. These genes are under one promoter called Tryp promoter and the operon is known as tryptophan operon.

- The tryptophan operon consists of five structural genes: *trpE, trpD, trpC, trpB* and *trpA* that code for enzymes involved in the conversion of chorismic to tryptophan. There is a promoter site *trpP* , an overlapping operator site *trpO* and a leader region *trpL* that codes for a leader peptide and an RNA attenuator. The leader peptide is involved in the attenuation mechanism.

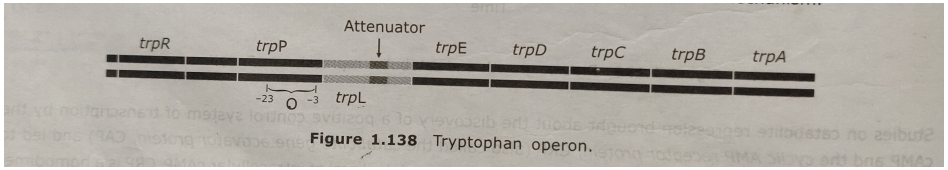


Figure 1.138 Tryptophan operon.

- In this repressible operon, there is another regulatory sequence called repressor gene called *trpR*. The product of this gene is inactive by itself called aporepressor. The aporepressor cannot recognize the operator sequence of the *trp* operon.
- The repressor becomes active only when it combines with tryptophan.
- Hence, in the absence of tryptophan, the aporepressor (inactive) has no effect on tryptophan operon. Consequently, the enzymes for synthesizing the amino acid are produced and the cell makes tryptophan.
- If tryptophan concentration exceeds beyond a certain level, the tryptophan behaves as an corepressor as it converts the aporepressor into the active repressor.
- When tryptophan binds to the aporepressor, it induces conformational change that converts the aporepressor to holorepressor and it can bind at the operator region and block the attachment of RNA pol to the promoter thus it can inhibit the transcription of the structural genes.

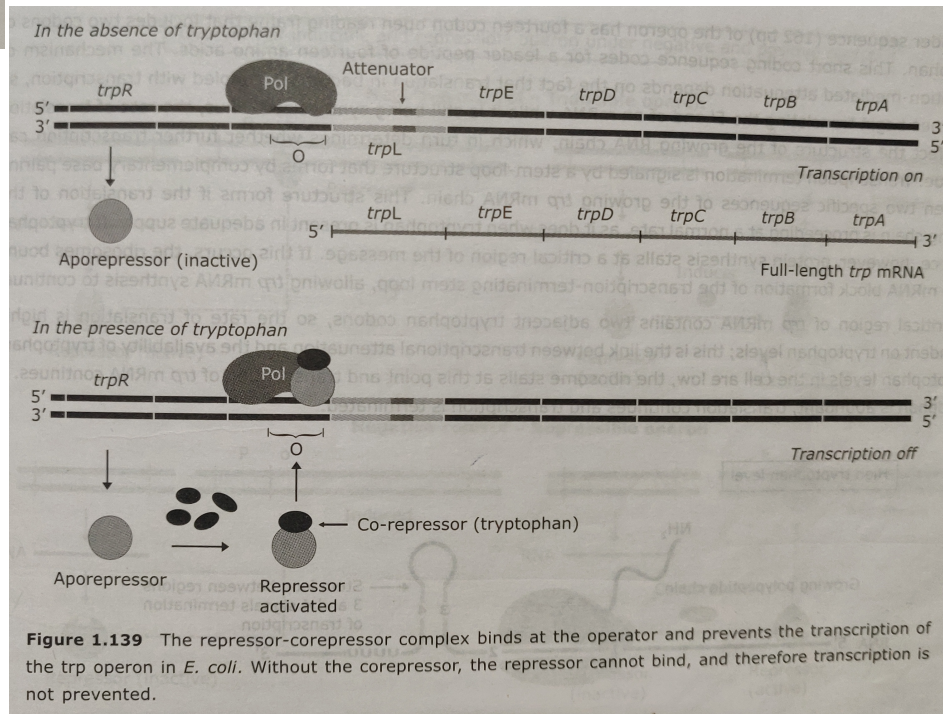


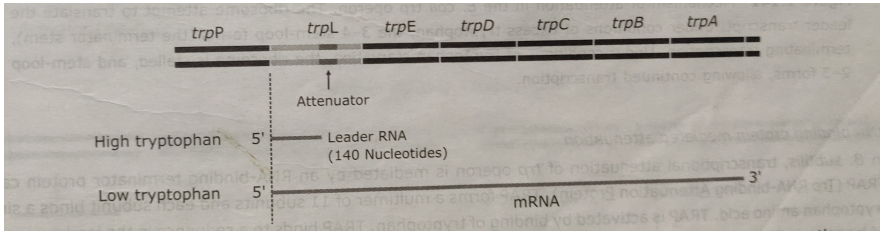
Figure 1.139 The repressor-corepressor complex binds at the operator and prevents the transcription of the trp operon in *E. coli*. Without the corepressor, the repressor cannot bind, and therefore transcription is not prevented.

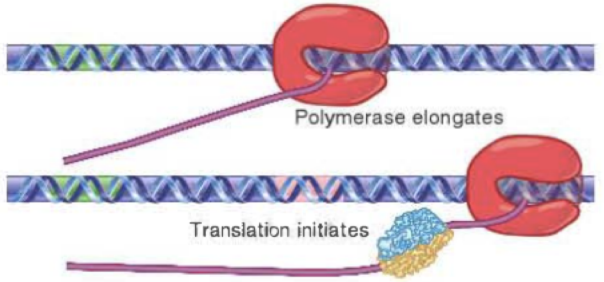
Transcriptional attenuation-

An additional mechanism termed transcriptional attenuation also negatively regulates the expression of operons by controlling the ability of RNA polymerase to continue elongation past specific site. This is carried out following while translation continues while transcription is occurring.

Translation mediated transcriptional attenuation

- In *E.coli,* translation mediated transcriptional attenuation provides an additional level of control that results in more stringent regulation. In *trp* operon, the site of attenuation is located downstream of the transcription start site. If tryptophan is abundant, most transcription terminates at this site; only if tryptophan is scarce does transcription continue to yield functional *trp* mRNA.
- The leader sequence of the operon has a fourteen codon open reading frame that includes two codons of tryptophan. This short coding sequence codes for a leader peptide of fourteen amino acids.
- The mechanism of translation-mediated attenuation depends on the fact that translation in bacteria is coupled with transcription, so ribosomes begin translating the 5'end of an mRNA while it is still being synthesized. Thus the rate of translation can affect the structure of growing RNA chain which is turn can determine whether further transcription can continue.





- Transcription termination is signaled by a stem-loop structure that forms by complementary base pairing between two specific sequences of the growing *trp* mRNA chain.
- The leader sequence has 5 segments. Of which 2 and 3 can form stem loop and 3 and 4 can form stem loop. The stem loop formed by **2 and 3, it is anti terminator loop**, ribosome can break that loop and continue translating Whereas if **3 and 4 form stem loop, it is called terminator loop** and once this is formed, ribosome cant break it and falls off.
- When tryptophan concentration is low ribosome waits at the 1st segment for tRNA to bring the specific tryptophan codon, this stalling of ribosomes induces 2 and 3 segment to form loop, this loop can be easily broken by ribosome and hence transcription and translation is continued.
- When tryptophan concentration is high the ribosome wont stop at the leader sequence as tRNA can bring tryptophan codon very fast and thus it rapidly moves to the 2nd segment. So 3rd and 4th segment form loop which is the terminator loop and ribosome will fall off and proteins will not be formed.
- This regulation occurs at the level of translation in addition to the normal regulation during transcription.

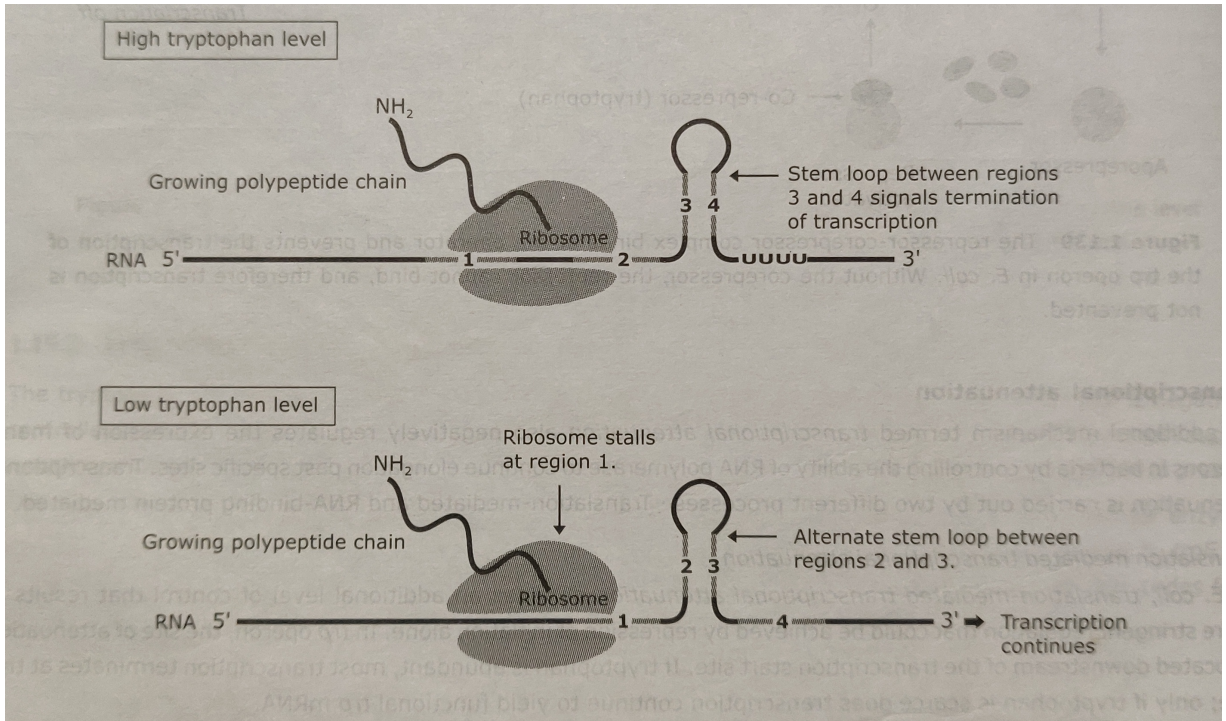


Figure 1.141 Mechanism of attenuation in the *E. coli* trp operon. The ribosome attempt to translate the leader transcript. Under conditions of excess tryptophan, the 3–4 stem-loop forms (the terminator stem), terminating transcription. Under conditions of tryptophan starvation, the ribosome is stalled, and stem-loop 2–3 forms, allowing continued transcription.

References-
1.Lewin’s Gene XI- Lewin, B., Krebs, J. E., Goldstein, E. S., & Kilpatrick, S. T. (2014).
2.Pathfinder study material part 2