Lecture 5

5.1. Gene Expression Regulation

Gene expression is process of convert DNA sequence to RNA to amino acid sequence of protein.

Crick and Watson in 1957 proposed that genetic information moves only in one direction and coined the term (Central Dogma of the life).

DNA ⟶ RNA ⟶ Protein

The first stage of gene expression is the conversion DNA to its RNA, this stage called (**Transcription**), and the second stage of gene expression is (**Translation**) when RNA converts to protein. The production of transcription is **mRNA**, while the production of translation is **amino acid** of protein.

In Central Dogma of life, have three main processes of transfer the genetic material are:

1- DNA replication
   DNA ⟶ DNA
2- Transcription of genetic information
   DNA ⟶ RNA
3- Translation of genetic information to protein.
   RNA ⟶ Amino acid of protein.

5.2. Prokaryote Gene Regulation

In prokaryotes the gene regulation system are adapted to provide the maximum growth rate in certain environment, about 75% of cell's gene are expressed all time. Such gene that code for RNA and poly peptides that are needed in large mount by cell, example: enzymes of glycolysis.

Gene expression is controlled primary at **transcription** level with some control of **translation** level.

Gene regulation including **transcription** and **translation** is accomplished by using **on-off mechanism**. Its means a particular gene is switched on and synthesis process will start products (mRNA , protein) will produce only when its needed. Otherwise the gene is switched off and the synthesis of its products (mRNA , protein) will stop only when is needed.

5.3. Strategy of Gene Regulation

In bacteria, the gene regulation is mostly controlled at transcription level, this control operation may be **Positive** or **Negative**.

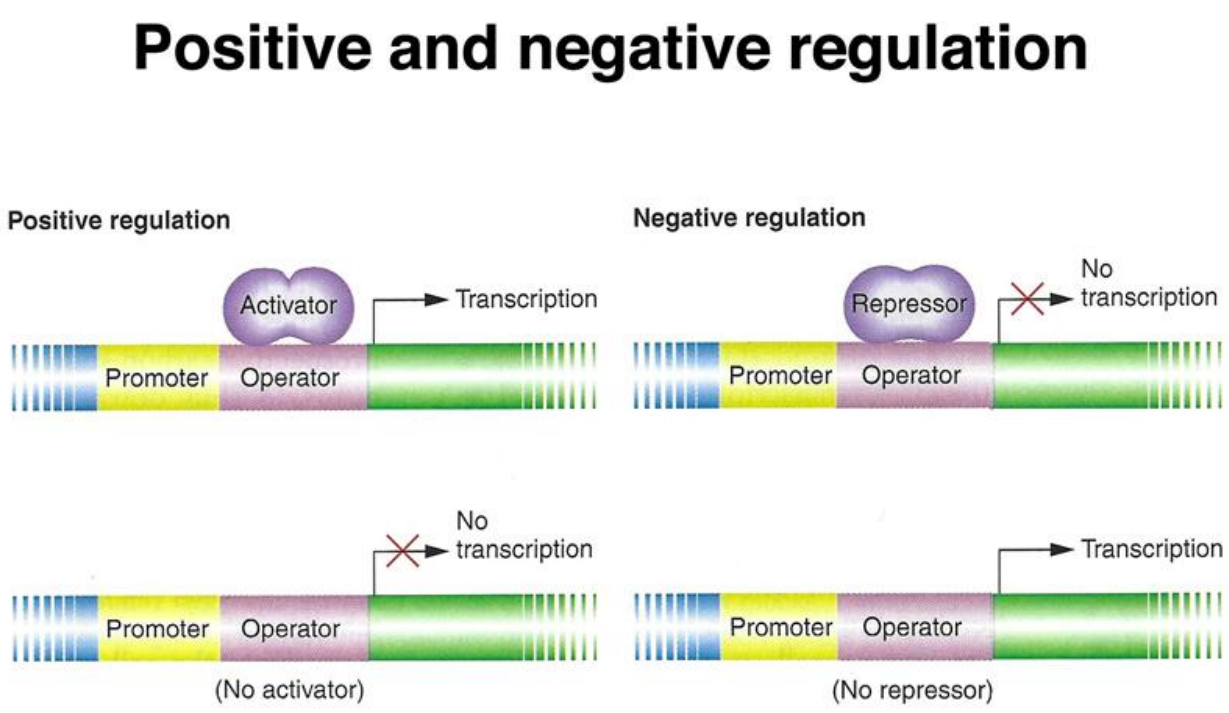
1- **Negative control**:

In negative regulation an **inhibitor** or **repressor** is present in cell. These are binding with gene to prevent the transcription, a signal molecule called **Inducer** or **Effector** is needed to react with the repressor to remove it, that allows the initiation of transcription. This negative regulation it's called also **repression** mechanism.

2- **Positive control:**

A signal molecules **inducer** activates the promoter to starts the transcription process of mRNA. This positive regulation it's called also **Induction** mechanism.

Metabolism system is a good example where regulated positively or negatively. In catabolic pathways, the effectors are substrate which is work as inducers. In anabolic pathways, effectors are usually the end product which is work as repressor.



Negative and positive regulation of mRNA transcription

Sometime there are some systems are both positively and negatively regulated, such systems used two different regulators to respond to different condition of the cell.

5.4. Induction and Repression

A set of genes will be switched on when there is necessary to metabolize a new substrate. When these genes are switched on, enzymes are produced. This process called **induction**. When metabolite products are provided in high level in medium, the bacteria will stop the synthesis and gene which is involved with its metabolism is turn off, this process called **repression**.

Difference between induction and repression

Induction	Repression
1. It turns the operon on.	1. It turns the operon off.
2. It starts transcription and translation.	2. It stops transcription and translation.
3. It is caused by a new metabolite, which needs enzymes to get metabolised.	3. It is caused by an excess of existing metabolite.
4. It operates in a catabolic pathway.	4. It operates in an anabolic pathway.
5. Repressor is prevented by the inducer from joining the operator gene.	5. Aporepressor is enabled by a co-repressor to join the operator gene.

5.5. Operon

Operon group of adjusted genes including Regulatory genes (Promoter & Operator) and Structural genes, operon was first discovered in 1961 by French biochemists Francios Jacob.

An operon is consists of promoter and series of genes that encode for enzymes and structures. Some operons are controlled by adjusted regulatory elements called **operator.**

Such operons are either repressor (turn off) or inducer (turn on) by proteins coded by regulator gene. Inducible operon are not usually work and transcript unless activated by inducer molecules, otherwise repressible operons operate in reverse fashion, they are transcription continue until deactivated by repressor molecules.

5.6. Lac operon

The lac operon is an inducible operon that is required for transport and metabolism of lactose in E.*coli* and some other enteric bacteria. Lac operon consists of promoter, operator and genes that encode the proteins involved in transport and catabolism of lactose. E.*coli* utilize the glucose and lactose as source of energy, when media contain both glucose and lactose, E.*coli* will consume the glucose first until finish and only then, bacteria will stop growing and after short lag phase of an hour, E.*coli* turn on the **lac operon** and begin to accumulate the necessary enzymes consume the lactose.

Lac operon contains two group of genes:

I- Structural genes

They are segments of DNA that hold the codes needed for production of the enzymes for utilize of lactose.

Structural genes have three type of genes are clustered together and transcribed from one promoter.

a- **Lac Z gene:** encode for β-galactosidase which is the enzyme that cleaves the disaccharide sugar lactose into glucose and galactose.

b- **Lac Y gene:** encodes for β-galactoside permease which is a membrane bound transport protein that pumps lactose into the cell.

c- **Lac A gene:** encode for β-galactoside transacetylase, enzyme that transfer an acetyl group from Acetyl-CoA to β-galactosidase.

These three enzymes are transcribed together to produce one mRNA (poly cistronic), starting from a single promoter.

II- Regulatory genes:

These genes are regulating the function of the structural genes and they are adjusted to each other, also they are three types:

a- Promoter gene (P):

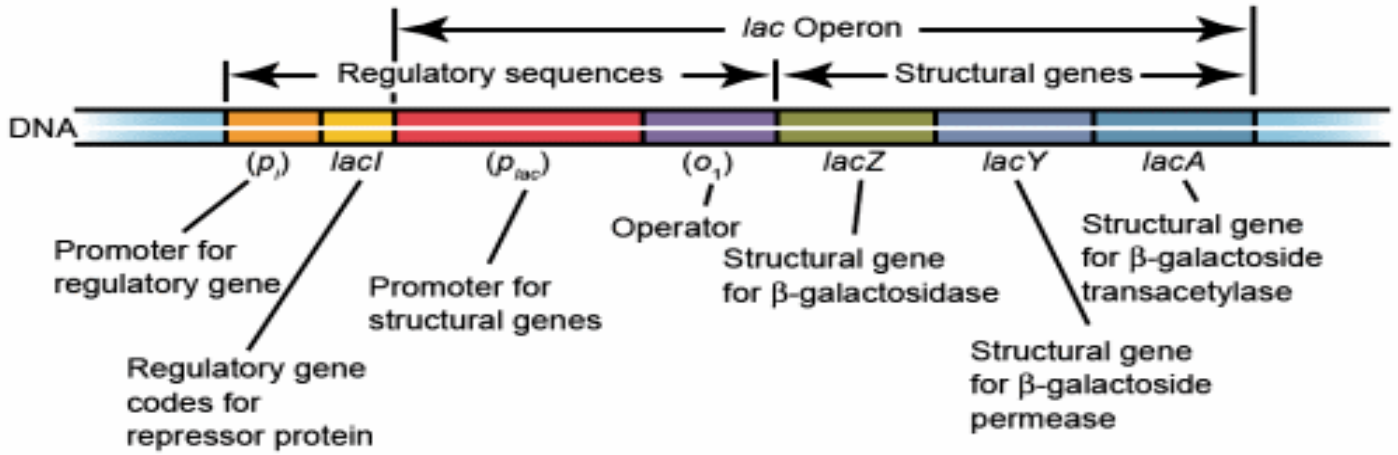
RNA polymerase binds to the promoter to start the transcription.

b- Operator gene (O):

It's located immediately to nearest of the first structural gene (Z gene), and sometimes it incorporated with the promoter. The operator gene regulates the transcription of mRNA, and its lies under the control of the repressor molecules that produces by the Repressor gene.

c- Repressor gene (L):

(L) gene is lies outside the operon and is responsible for production of repressor molecules that binds with operator gene to repress the transcription process.



Lac operon is regulated by two mechanisms:

1- Negative control that keeps lac operon repressed in absence of lactose by a protein called **lac repressor**.
2- Positive control that keeps the lac operon inactive in the presence of glucose.

I- Negative control (Repression):

Regulatory response to lactose, which uses regulatory protein called the **lactose repressors**. If there is no lactose in medium, the lactose repressor binds very tightly to operator gene (O). This binding cause repression and prevents the RNA polymerase from binding to the promoter so that the genes doesn't encode the β-galactosidase enzyme (operon turn off).

When glucose is consumed and only lactose molecules are available in medium, lac operon should work to break down that lactose; this process is done by **allosteric protein or allolactose.**

The allolactose works as an inducer to the lac operon because it binds to the lactose repressors causing change in its shape and conformation, therefor, the repressor unable to bind to the operator gene (O) any more that allowing to the RNA polymerase to bind and transcribe enzyme for lactose metabolism.

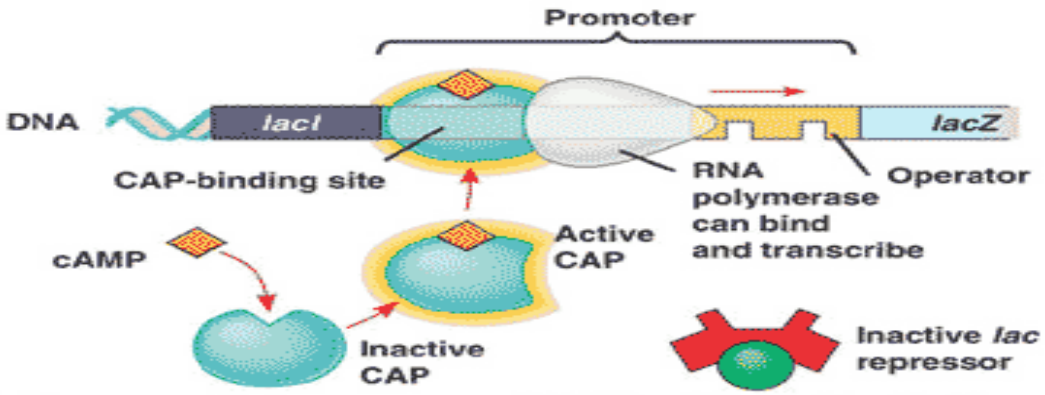
II- Positive control (CAP or Catabolism repression)

Positive control of lac operon is mediated by activator protein (CAP) with help of a signal protein (cAMP).

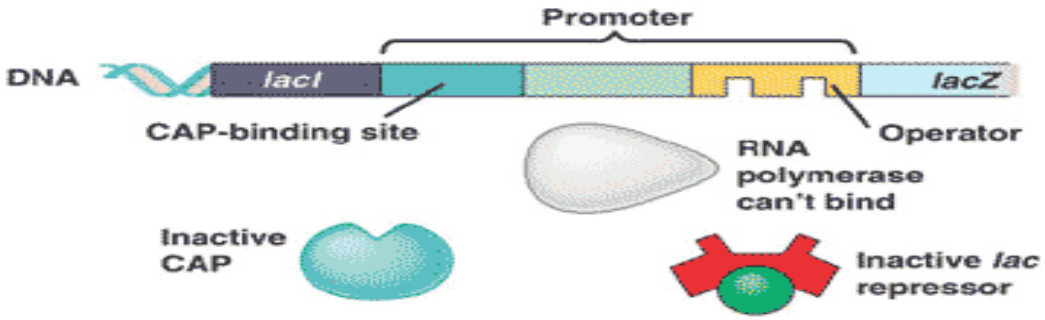
a- When glucose concentration in medium is low, (cAMP) concentration is high and there is a lot of it to bind with the (CAP) and form a complex called

CAP-cAMP complex. This complex bind CAP-cAMP binding site on lac operon which is lies left to the promoter. This helps RNA polymerase to bind to the promoter and encode the β-galactosidase and other enzymes responsible for lactose metabolism.

b- When glucose concentration in medium is high, the (cAMP) concentration is low and there is no enough (cAMP) to bind with (CAP), hence the complex of CAP-cAMP is not formed, so the RNA polymerase bind with the DNA randomly and weakly that leads to transcription of genes not complete and the enzymes responsible for lactose metabolism are not produce.



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

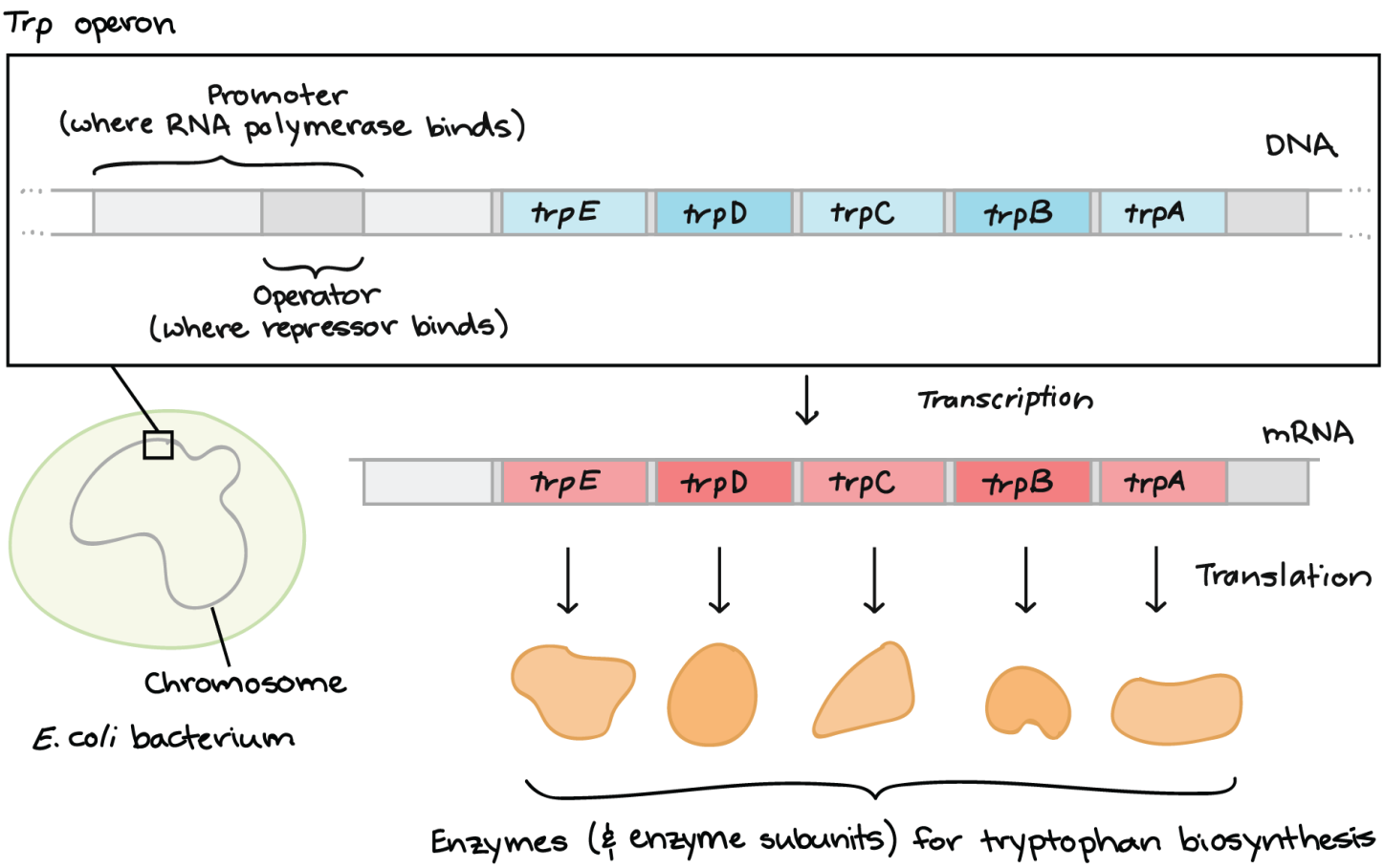
The (CAP) molecule function as a **positive regulator** that enhances the transcriptional activity of RNA polymerase at the promoter, while (cAMP) is an **effector** that binds to the (CAP) to enable it to bind near the promoter and continue its regulatory function.

The overall effect of glucose in preventing lac operon genes transcription is known as catabolite repression because the presence of catabolite (glucose) represses transcription of operon.

5.7. Trp Operon

Tryptophan operon required to control the synthesis of amino acid (tryptophan). Trp operon is consisting of **Regulatory gene (promoter, an operator) and Structure gene (five structural genes)** that encode enzymes involved in the synthesis of amino acid.

Structural genes are (**trpE, trpD, trpC, trpB and trpA**) these genes are located adjacent to one beside one and gene trpE is first gene that translated. There are two regions called **Leader** and **Attenuator** located close to gene trpE.



Trp Operon Structure

Trp operon is regulated by two mechanisms:

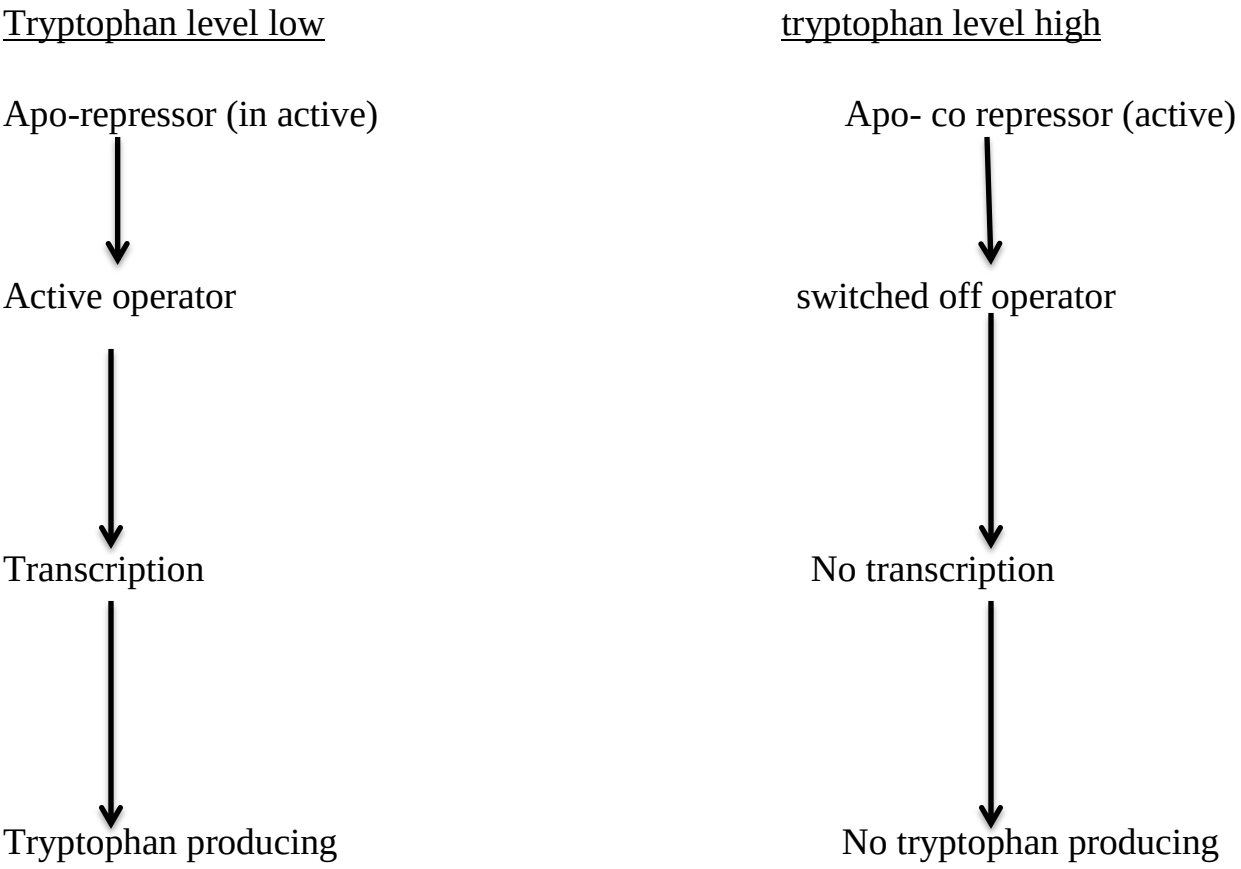
I- Negative Mechanism (Repression regulation)

The trp operon is regulated by protein repressor, in contrast to the lac operon where lactose acts as an inducer that prevents the repressor from binding to the operator, tryptophan amino acid acts as **co-repressor.**

The repressor is inactive in the absence of tryptophan and it is called **apo-repressor**, when the tryptophan concentration is high that cause to plenty of **co-repressor** is available to bind with the apo-repressor to form **apo-co repressor complex** which causes an allosteric alteration in the repressor's shape and produce the active form of the repressor so it can bind to the operator site of trp

operon and inhibits the transcription of the genes in the operon, so the tryptophan amino acid is no more produced.

When the tryptophan amino acid level decrease in around, it splits from the apo-repressor and cause return back to its inactive form so no binding with operator gene. So RNA polymerase will bind to the promoter and starts to transcribing the five structural genes to produce tryptophan.



II- attenuation mechanism (Control by attenuation):

In addition to the standard negative control, the trp operon employs another mechanism of control called **attenuation mechanism**. This extra control is needed because the repression of trp operon is much weaker than lac operon.

It was found sometimes initiation at the promoter leads to the transcription of truncated mRNA. There is a region of DNA in trp operon immediately proceeding the first structural gene (trpE) called **leader-attenuator**, its purpose to attenuating or weaken the transcription of the operon when tryptophan level is high in the medium. The attenuator causes premature termination of transcript mRNA because it contains a transcription stop signal (terminator).