CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

16 (molecular)

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Before we start, we should know that molecules can't function by itself. They function by interacting with other molecules via non-covalent (electrostatic) interactions. 😉

Last time we talked about transcription in prokaryotes and eukaryotes. Today we will describe how transcription is regulated.

- As we are used to, first we start describing the mechanism in prokaryotes because it's simple, easy and similar to what happens in eukaryotes.

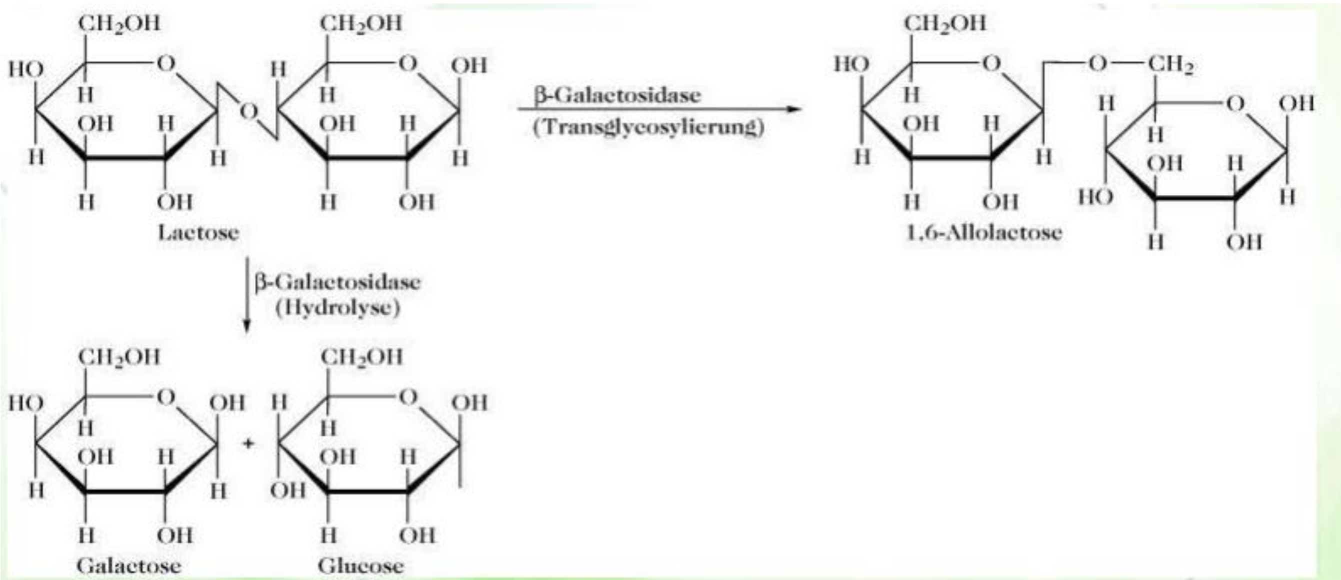
Regulation of transcription in prokaryotes

We already know what "operons" are. They are genetic units that produce a single transcriptional unit (mRNA) with different regions that produce different polypeptides, each one of them forms a protein with a specific and different function. Although they work together in the same pathway.

- one example of operons is "lac operon".

DEFINTION: lac operon: it's the operon that is responsible for the metabolism of lactose in the bacteria E.coli.

Keep in mind that lactose is a disaccharide composed of glucose and galactose.



Lactose can be used in 2 different ways:

A- In E.coli, lactose is preferred to be hydrolyzed (cleaved) to glucose and galactose. This reaction is catalyzed by the enzyme β-galactosidase.

-in general, glucose is used for the production of energy-

●Galactose can be used in different purposes:

1- it can be converted to glucose (since glucose is an isomer of galactose)

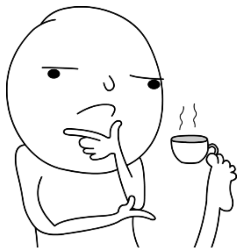
2- it can be inserted in the "cell wall" to make a thick and protective wall.

Keep in mind that bacteria in general has cell wall like plants.

B- Or lactose can be converted to 1,6-allolactose.

VERY IMPORTANT: this reaction is catalyzed by the enzyme β-galactosidase, the same enzyme that catalyzes the previous reaction!

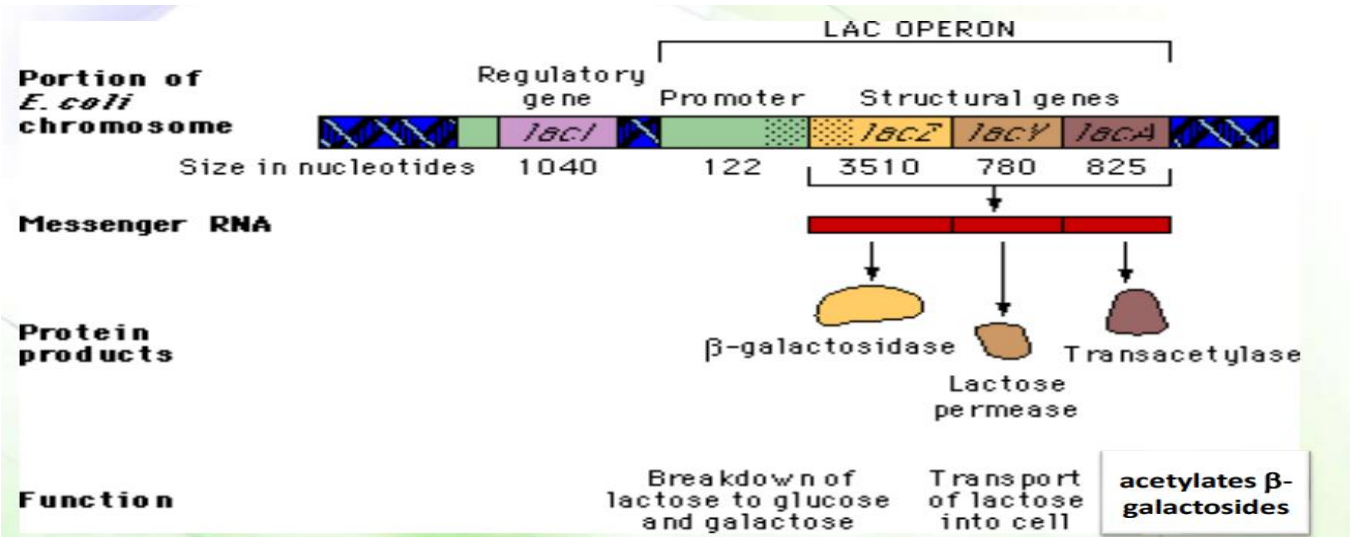
How can this enzyme catalyze 2 different reactions?



Answer: Because this enzyme has a different activity or effect. In other words, it has 2 different domains, each domain catalyzes a certain reaction.

NOTE: as I mentioned, Lactose is preferred to be cleaved into glucose and galactose so I expect the first reaction to occur more than the second reaction → which is converting lactose to allolactose.

Components of the lac operon



The lac operon composes of:

a- THE PROMOTER

b- THE STRUCTURAL GENES

-notice that there are regulatory genes located outside the operon (*lacI*)-

1- Promoter region (122 nucleotides) and it includes a region called operator region.

2- three structural genes:

a- *lacZ* : it forms the protein β-galactosidase (we mentioned its function)

b- *lacY* : it forms the protein lactose permease: this protein (enzyme) transports lactose from outside the cell to inside.

c- *lacA* : it forms the protein transacetylase: it acetylates β-galactosides.

***β-galactosides: it's a sugar that looks like Lactose.**

IMPORTANT : β-galactosid<u>es</u> is different from β-galactosid<u>ase.</u> لاحظ لاحظ لاحظ

Although lac operon has been known for the past 50 years, we don't know yet what's the real function of transacetylase. We know what it does (acetylates β-galactosides) but we don't know why! ☹

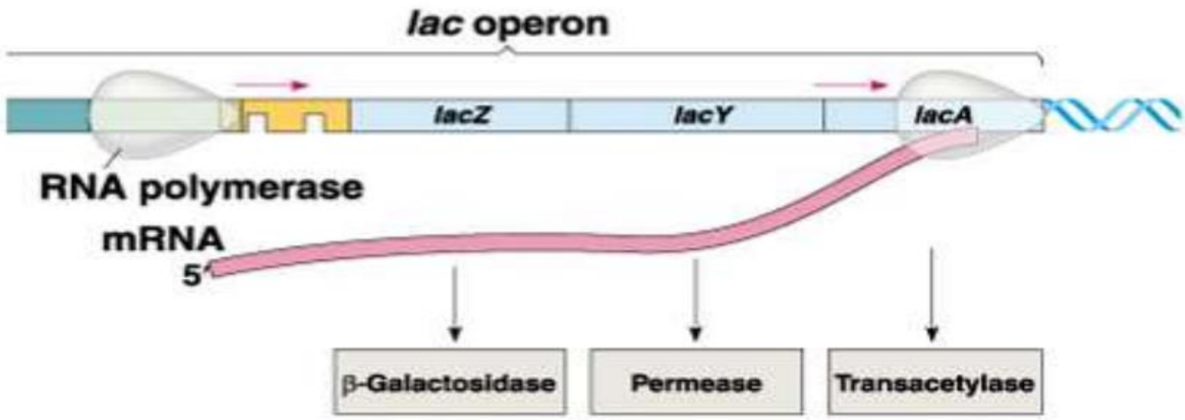
*Outside the operon and near it we have other genes:

One of these genes is *lacI : I* stands for inhibitor.

This gene has its own promoter, it produces one mRNA and one protein. So, it's monocistronic.

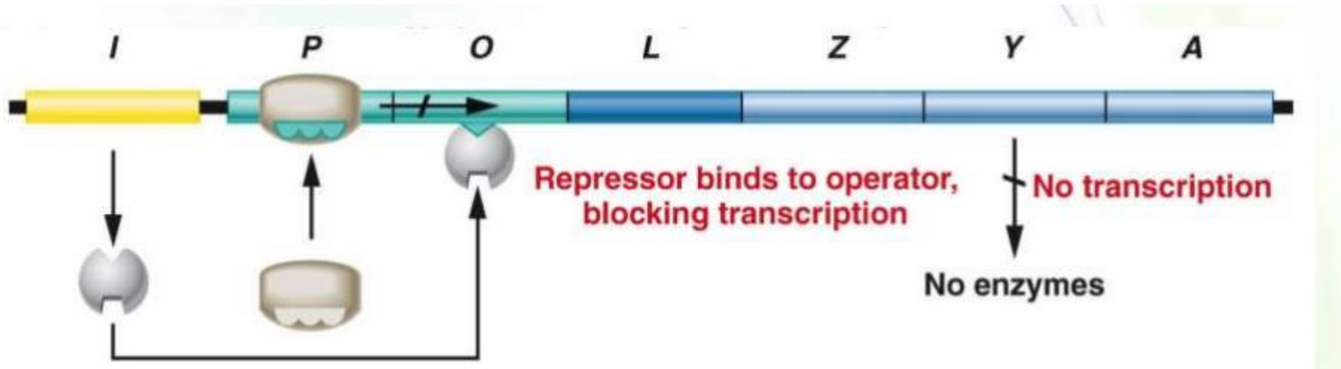
Keep in mind that bacteria can have both genes: monocistronic and polycistronic while eukaryotes can have only monocistronic genes. 😉

The figure below is taken from slides and it clarifies only the lac operon without the genes near it.



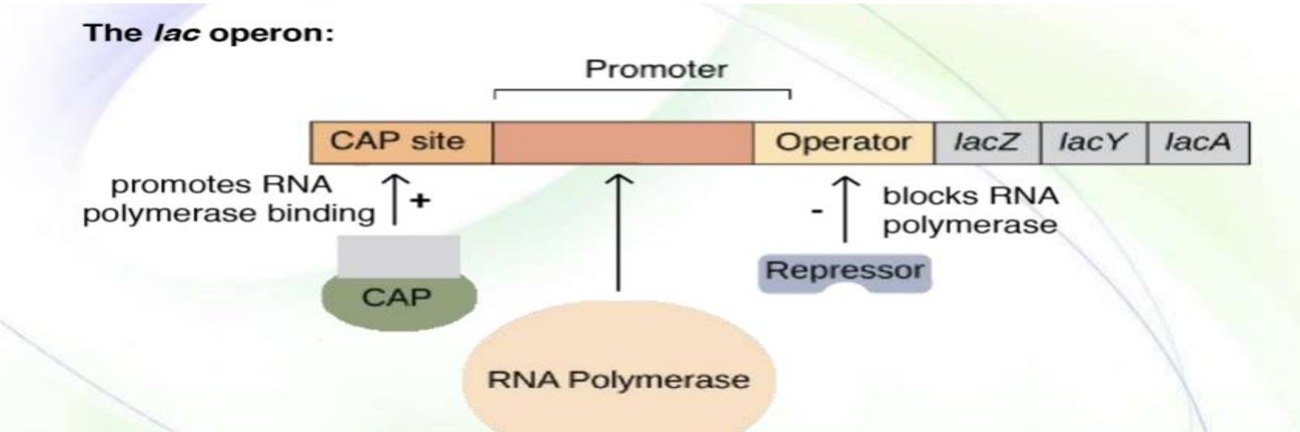
The operator: the promoter region includes the operator region, which is a binding site of a protein called the lac repressor.

Lac repressor: it's a protein that is produced by the gene *lacI*, it represses (inhibits or blocks) transcription of the lac operon. HOW?

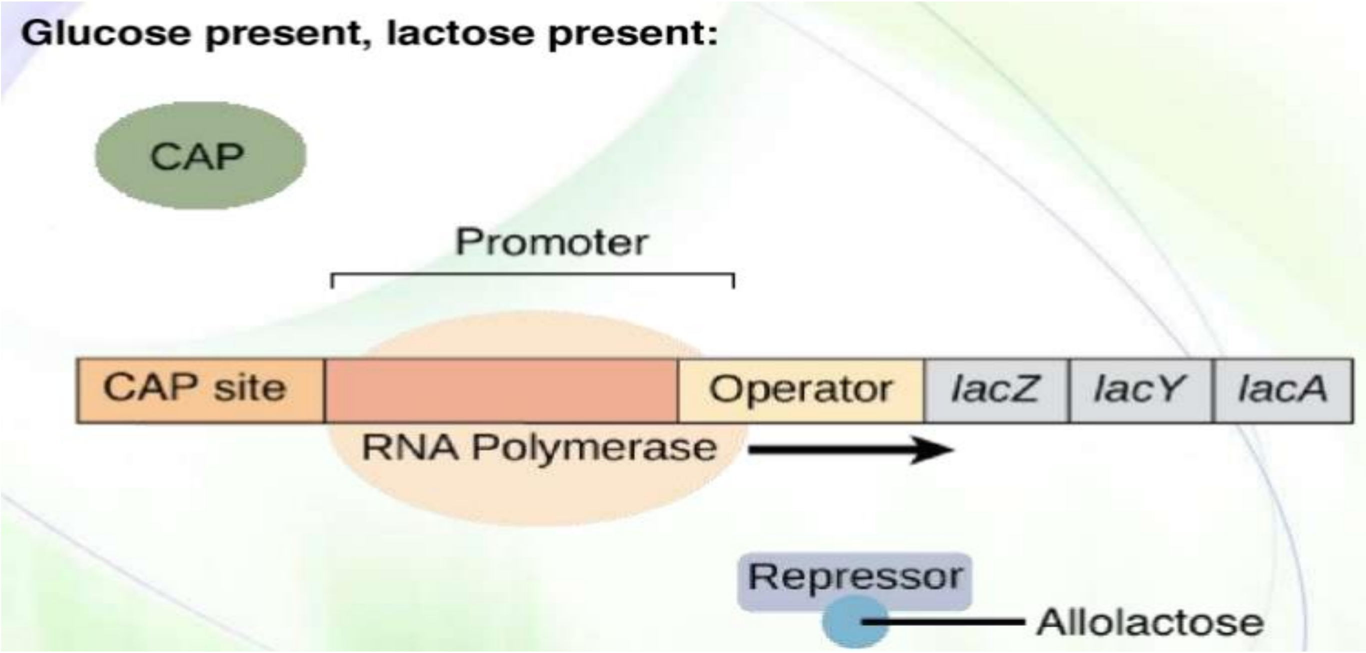


-As you see, *lacI* gene forms the repressor. The lac repressor blocks transcription by preventing the RNA polymerase from binding to the promoter.

NOTE: the promoter is the binding site of the RNA polymerase, and the operator is the binding site of the Repressor. Look at the following figure.



Sometimes, the cell needs the lac operon to be transcribed and this can't occur if the repressor is bound to the operator, so what happens is the following:

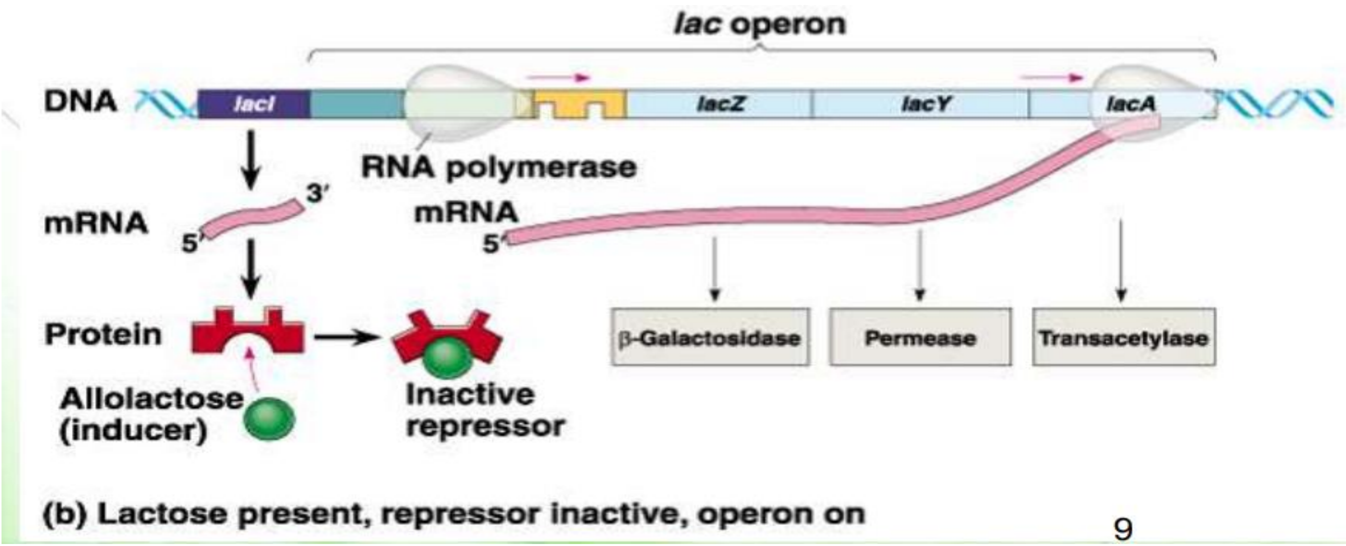


As you notice, when the repressor is bound to Allolactose, it can't bind to the operator, which allows the RNA polymerase to bind to the promoter and initiate transcription.

Summary: if there is lactose, some of it will be converted to allolactose which binds to the repressor and prevents it from binding to the operator, so the RNA polymerase can function peacefully. This is known as positive regulation (the presence of something activates transcription of a gene).

-PRESENCE OF LACTOSE → ACTIVATION OF LAC OPERON GENE-

But, how does lactose prevents the repressor from binding to operator?



Answer: when Allolactose binds to the repressor, it changes its structure so it can't bind to the operator. (it causes conformational change).

OK NOW YOU HAVE TO PAY ATTENTION CAUSE WHAT I AM GOING TO SAY IS REALLY IMPORTANT

We said that allolactose binds to the repressor, thereby preventing it from binding to the operator DNA. What does convert lactose to Allolactose?

Lactose is converted to Allolactose by the enzyme β-Galactosidase ……..

But wait … β-Galactosidase is formed by the gene *lacZ* which locates on lac operon ..

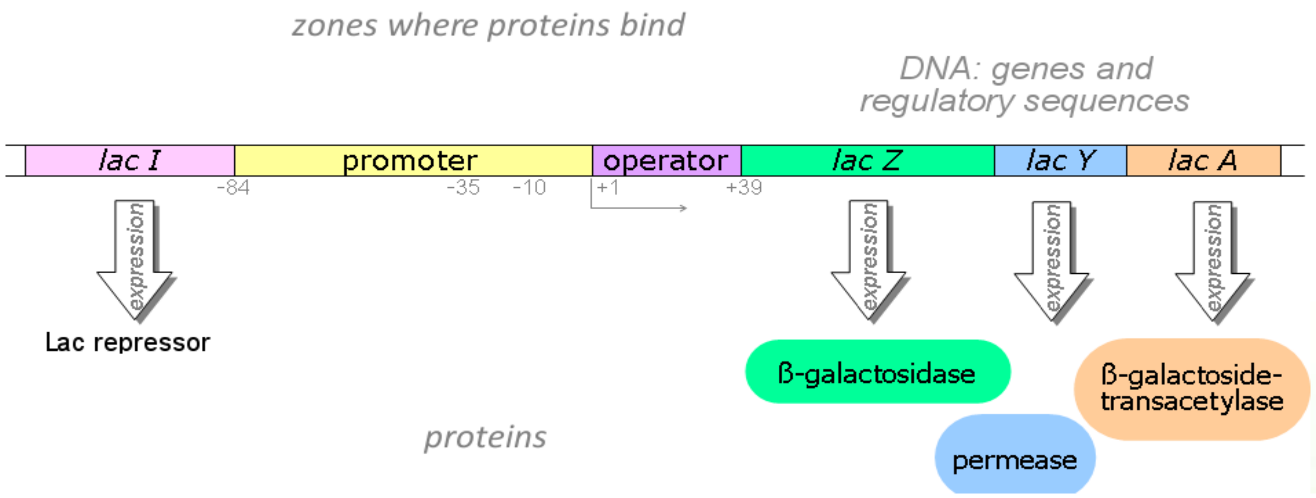
And lac operon transcription is not active because RNA polymerase can't function due to the binding of repressor to the operator. ولفي بينا يا دنيا

To explain this, imagine we have a closed faucet. Although it's closed there might be some leakage of water. The leakage isn't huge, we can have one drop that leaks every 5 minutes. The same idea here. The beauty of genetic system is that any gene isn't always "on" 100% or isn't always "off" 100%, there is some leakage.. why?

Answer: because interactions aren't fixed, you can have dissociation because they are based on non-covalent interactions which are weak reversible interactions.

Summary: there is a little amount of β-galactosidase due to leakage, so it converts Lactose to Allolactose, then it binds to the repressor and prevents it from binding to the operator so the transcription occurs. 😊

The following figure is from slides, we discussed it before:



Cis vs. trans regulatory elements

Regulatory sequences like the operator are called cis-acting elements because they affect the expression of only genes linked on the same DNA molecule.

Examples in prokaryotes: promoter, operator.

Proteins like the repressor are called transacting factors because they can affect the expression of genes located on other chromosomes within the cell. They are produced from trans-acting elements.

Example in prokaryotes: *lacI*

Keep in mind:

Element: A piece of DNA.

Factor: A Protein.

Effect of mutations

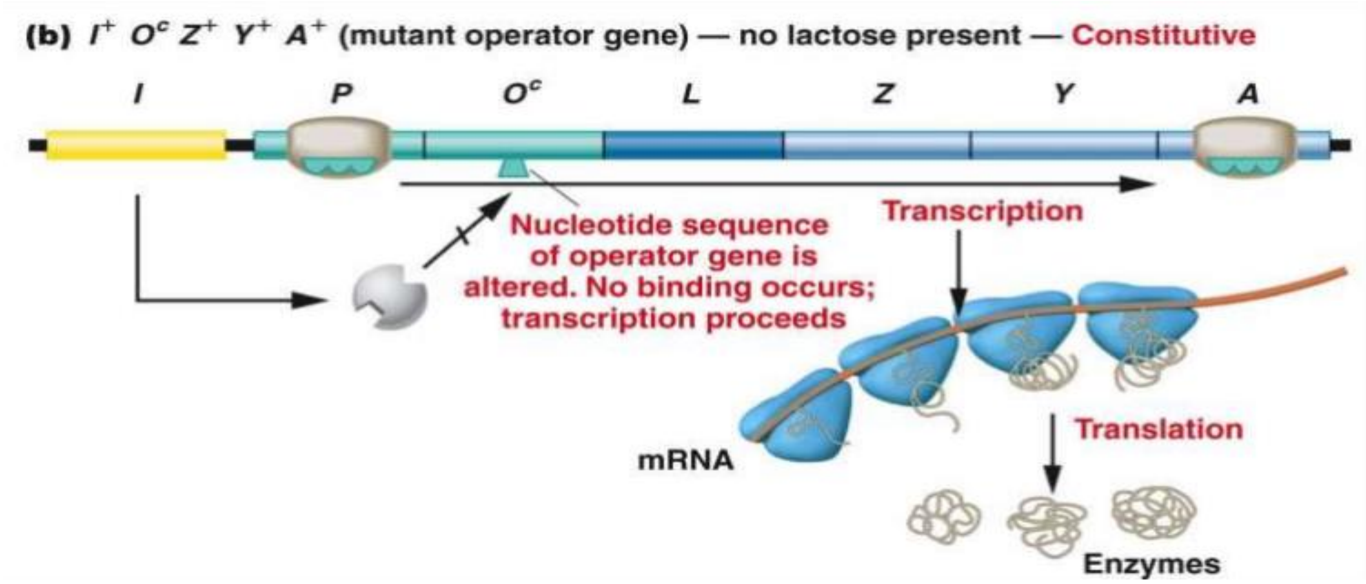
A- Some mutations result in constitutive expression (always on).

Examples:

1- mutation in the *lacI* which produces a defective lac repressor.

Defective lac repressor can't bind to the operator, so nothing prevents the binding of RNA polymerase to the promoter. The gene expression is always ON.

2- mutation in the operator: the repressor is functional but it can't bind to the operator due to mutation in the operator, so nothing prevents the binding of RNA polymerase to the promoter. The gene expression is always ON.



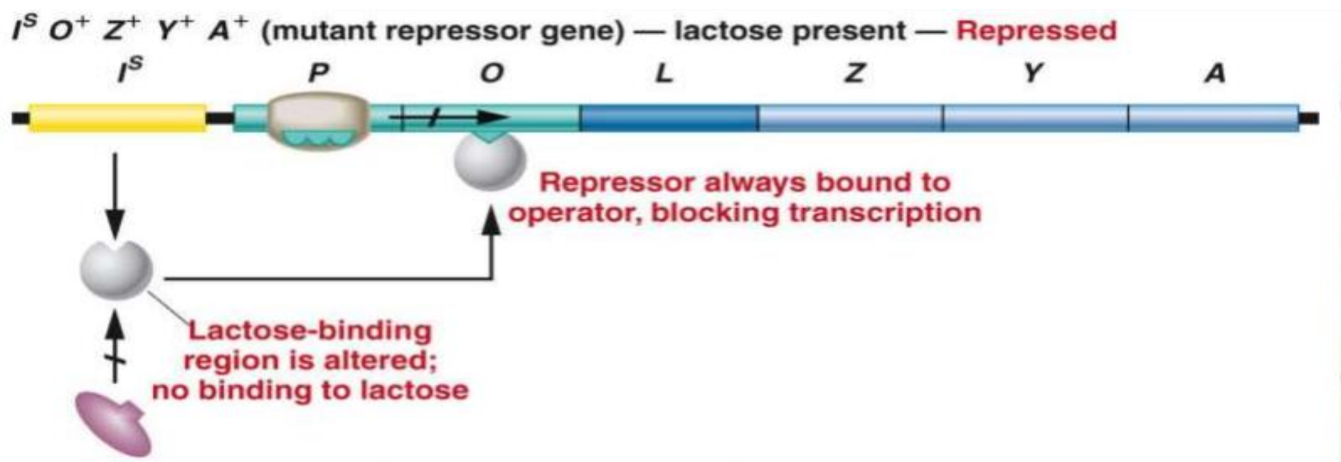
B- Other mutations cause non-inducible or repressed expression (**always off**).

Examples:

1- a mutation in the promoter: RNA polymerase can't bind even if the operator is free of lac repressor. Transcription is always OFF.

2- a mutation in *lacZ* : non-functional β-galactosidase is produced, so it won't convert Lactose to Allolactose. Transcription is always OFF.

3- a mutation in *lacI* : when a mutation happens in *lacI* it may produce a defective repressor (as I mentioned earlier in A-1 constitutive expression). Or it can produce a repressor with altered Allolactose-binding region, so Allolactose can't bind anymore to the repressor. Transcription is always OFF.



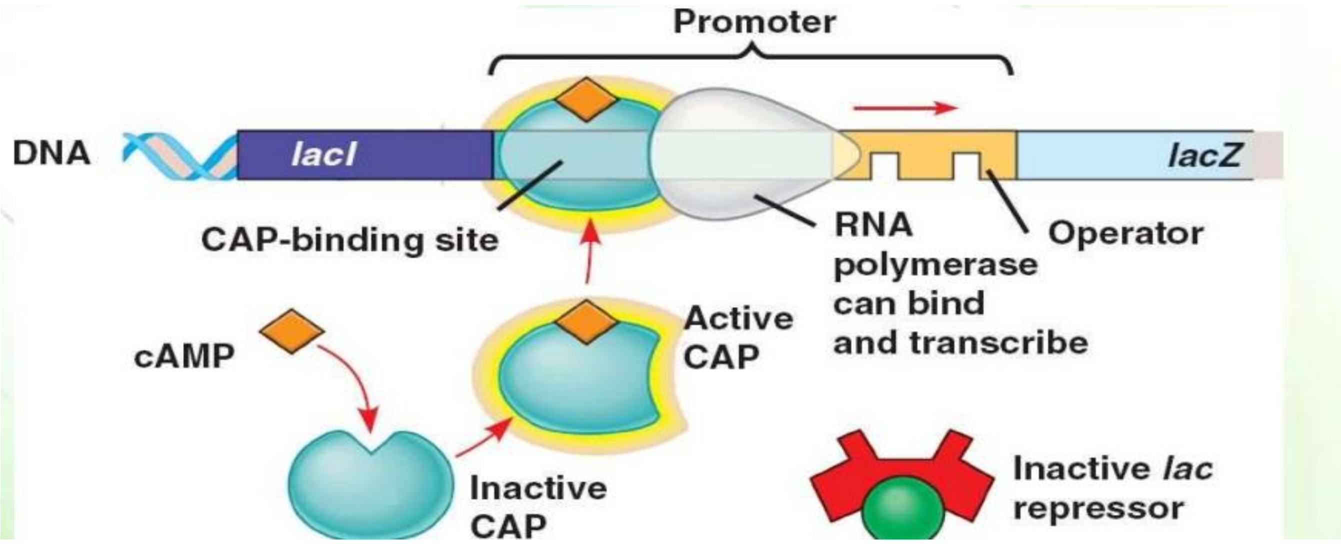
Another level of regulation

Another regulator is cAMP, which binds to a protein known as catabolite activating protein (CAP).

What happens is, cAMP binds to CAP and activates it. Then CAP binds to a region upstream the promoter.

CAP function: it activates transcription and makes it really fast and efficient.

VERY IMPORTANT NOTE: without CAP, RNA polymerase can still do transcription, but it's lazy.



***How can we regulate this mechanism?**

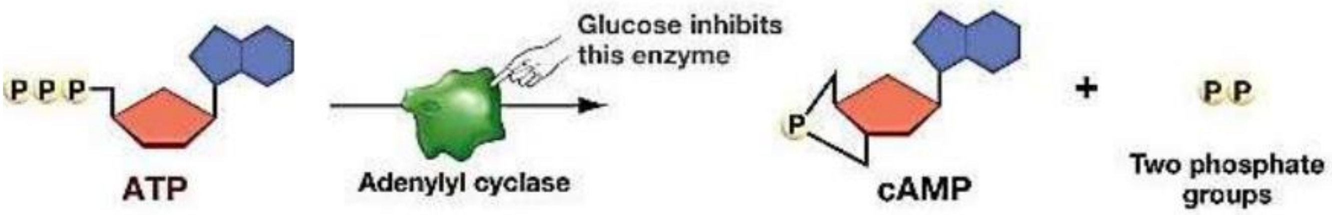
-ATP is converted to cAMP by an enzyme known as adenylyl cyclase (or adenylate cyclase), this enzyme is regulated by glucose. Glucose inhibits adenylyl cyclase activity.

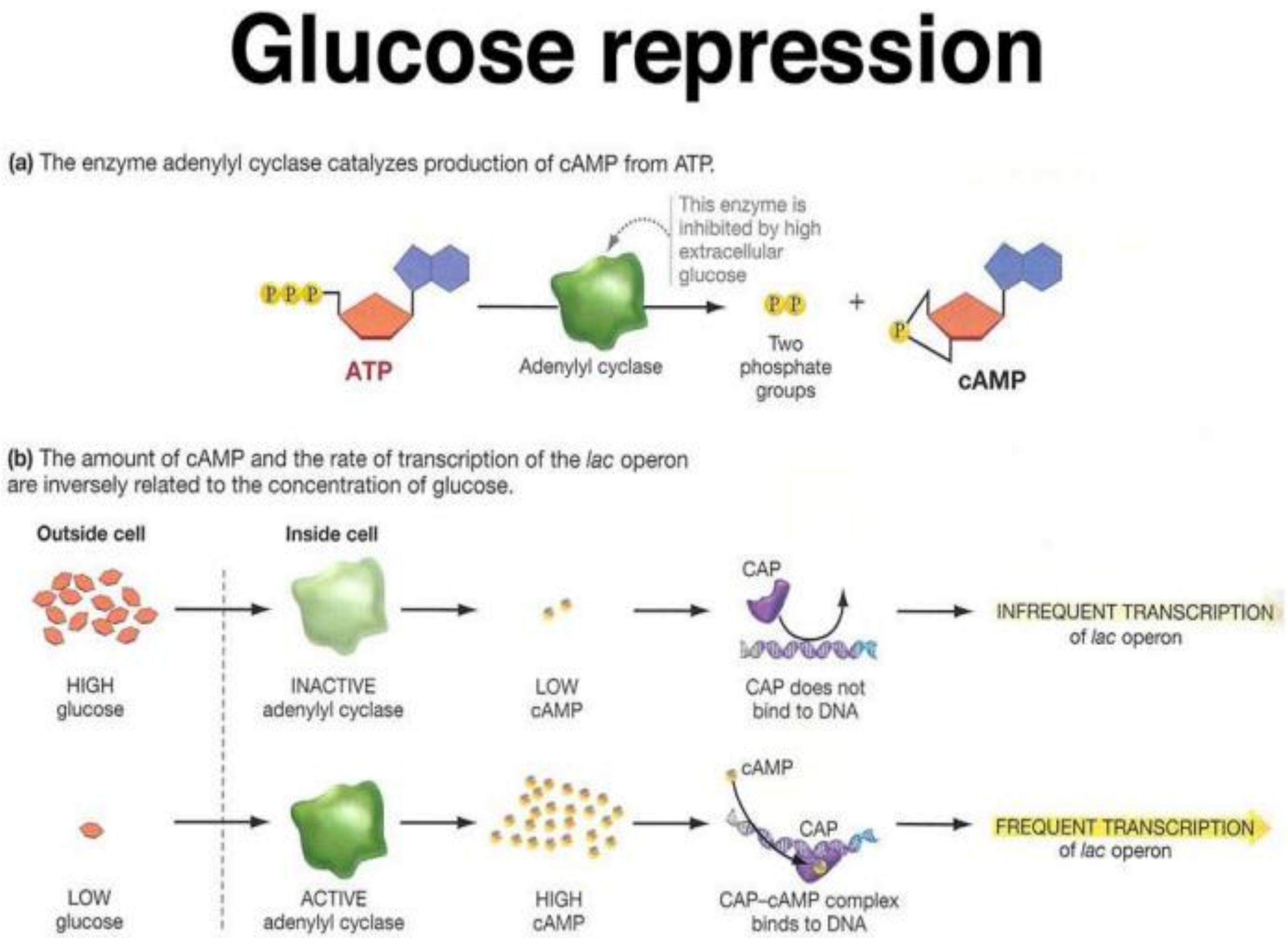
-The ability of CAP to bind to the promoter is influenced by how much cAMP is in the cell is produced by adenylyl cyclase, which is inhibited by high level of glucose.

-Glucose is preferentially utilized by bacterial cells and it represses the lac operon even in the presence of the normal inducer (lactose).

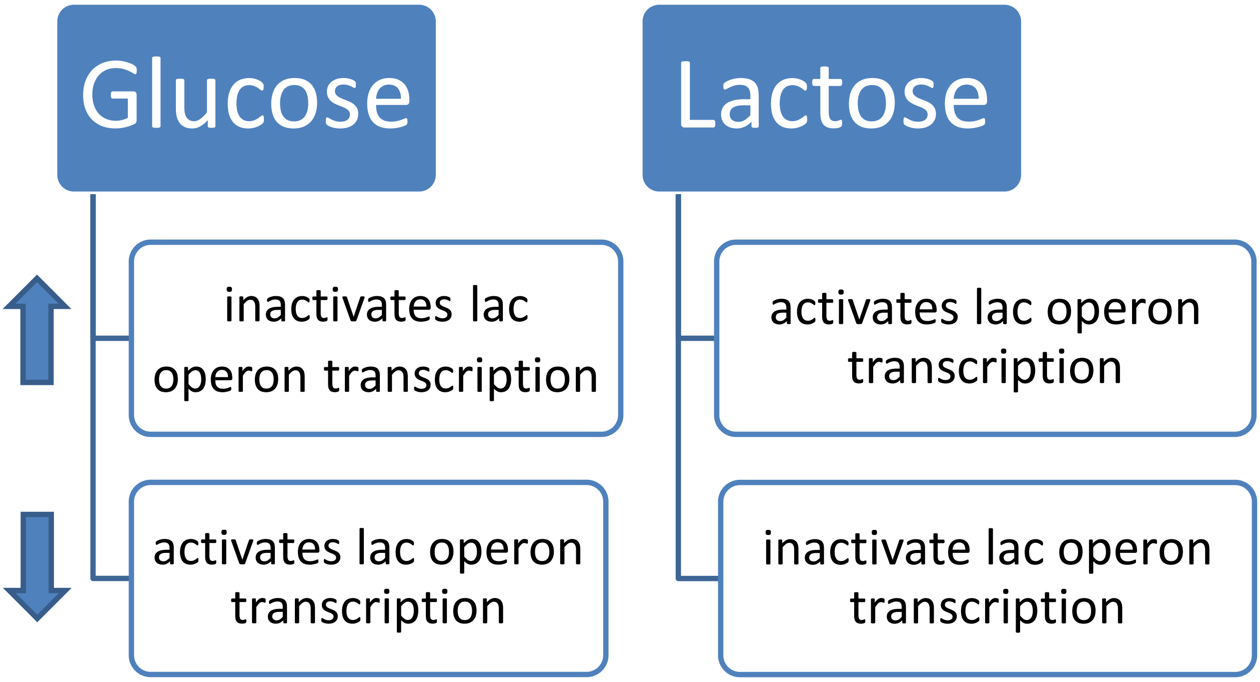
*Summary: if there is glucose → it inhibits adenylyl cyclase → no conversion of ATP to cAMP → no activation of CAP → transcription is slow and not efficient.

-this is known as negative regulation. (the presence of something inactivates gene expression).





Until now, we have taken 2 substances that regulate transcription of lac operon.



Note that when we increase the concentration of lactose, transcription is activated (positive regulation), and when we increase the concentration of glucose, transcription is inactivated (negative regulation)

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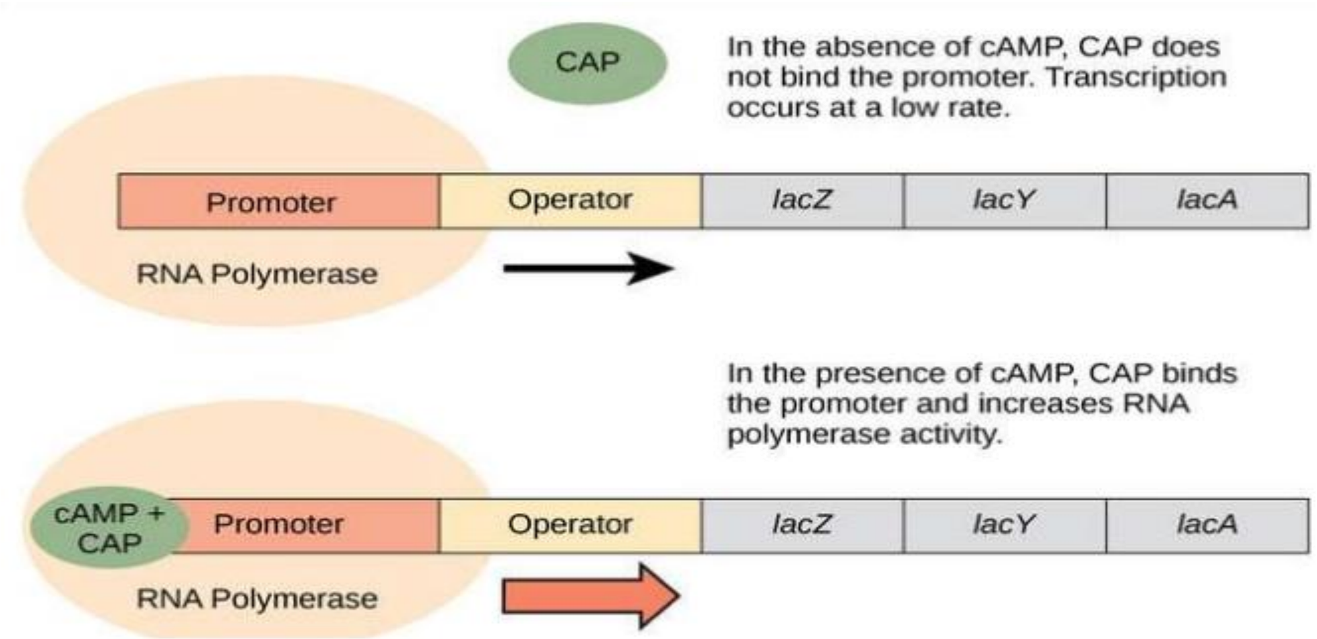
cAMP and CAP influence on lac operon transcription



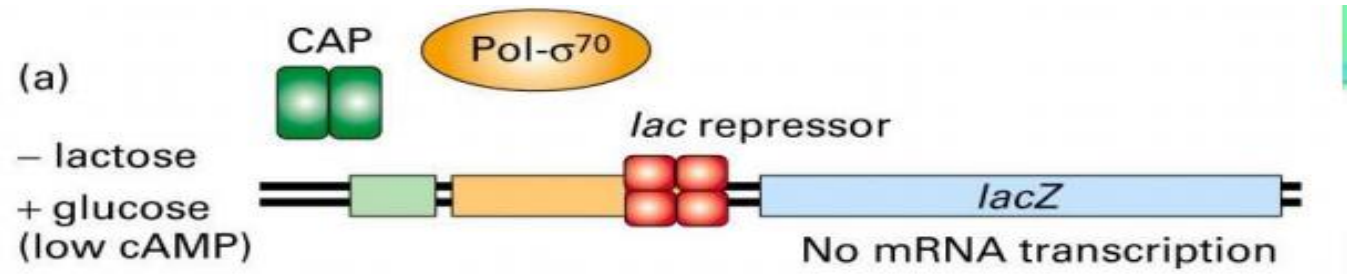
Since lactose and glucose regulate lac operon transcription, there are 4 scenarios of what will happen in E.coli bacteria:

1- first case: NO Lactose, NO Glucose:

NO transcription because the repressor is always bound to the operator and RNA polymerase can't do transcription.

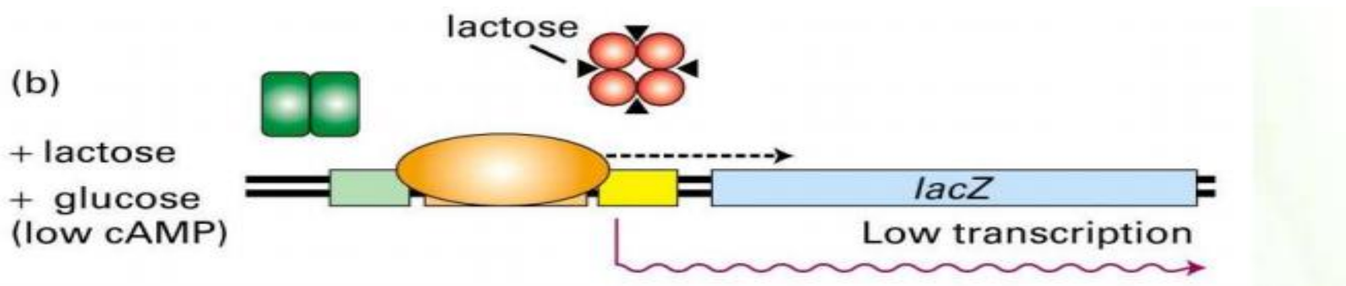
2- second case: NO Lactose, YES Glucose:

NO transcription because the repressor is always bound to the operator and RNA polymerase can't do transcription.



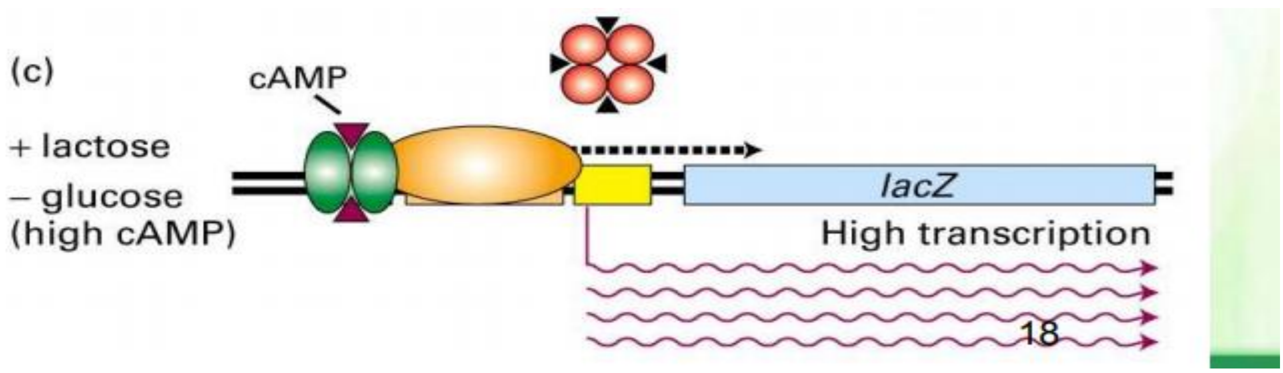
3- third case: YES Lactose, YES Glucose:

THERE IS TRANSCRIPTION, but it's slow.



4- fourth case: YES Lactose, NO Glucose:

THERE IS TRANSCRIPTION, and it's high.



Regulation of transcription in eukaryotes

We studied together the lac operon and how it's regulated in bacteria (E.coli). Now, let's talk about the regulation of transcription in eukaryotes.

Although the control of gene expression is far more complex in eukaryotes than in bacteria, the same basic principles apply.

Transcription in eukaryotic cells is controlled by:

1- Cis-acting elements: Promoters, PPEs, enhancers and silencers.

2- Transcriptional regulatory proteins (factors): Activators and repressors.

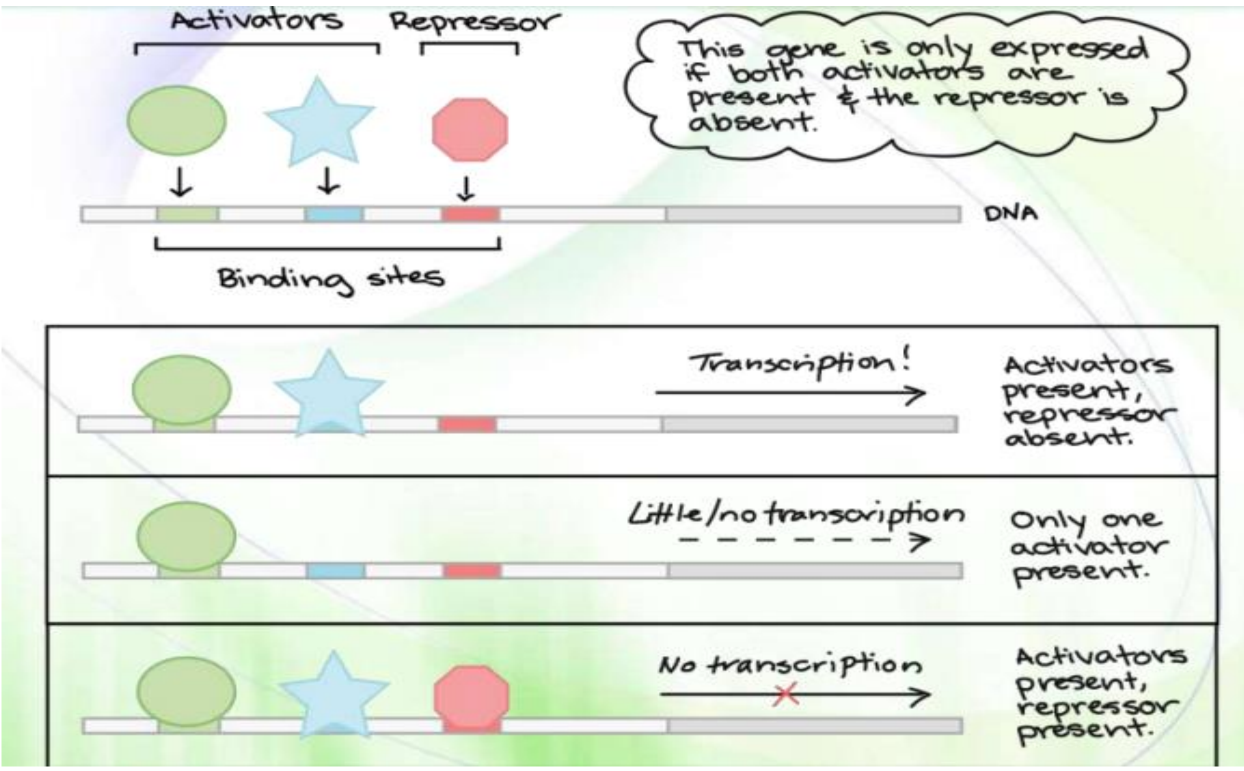
3- chromatin remodeling. (How the structure of the DNA looks like "compact, packaged and unpackaged")

4- DNA modification. (methylation of cytosine)

VERY IMPORTANT: when we talk about chromatin remodeling, we mean histone remodeling. But when we talked about DNA modification, we mean nucleotides modification.

5- Noncoding RNA molecules.

NOTE: there can be more than one PPE near one gene.



Notes about the figure above:

-If there is only one activator, there is little transcription.

-If there are 2 or more activators, the transcription is fast.

-If there are one or more repressors, no transcription will occur even though there are activators.

How do Transcription Factors (TFs) regulate gene expression?

Answer: By causing epigenetic/epigenomic changes in DNA.

-What is epigenetics?

Epi means "above" or "in addition to".

It indicates genetic alteration in gene expression without a change in DNA sequence. HOW?

1- change chromatin packaging.

2- chemical modification of histones.

3- chemical modification of DNA. (for example, methylation of cytosine)

NOTE: chemical modification of DNA doesn't mean changing DNA sequence.

ONCE YOU FLY, YOU WILL WALK WITH EYES SKYWARD.

Da Vinci 1452-1519