بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 13

Transcription Regulations Pt.1

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وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: الذي سلم من العيوب والنقائص لكماله وكمال صفاته وأفعاله، وهو الذي يؤمّن الخلائق وحده ويسلّمهم.

الورود: ورد مرة واحدة في القرآن.

الشاهد: ﴿ٱلْمَلِكُ ٱلْقُدُّوسُ ٱلسَّلَٰمُ﴾ [الحشر:٢٣].

اضغط هنا لشرحٍ أكثر تفصيلًا

Transcription-Regulation

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School of Medicine

Second year, First semester, 2024-2025

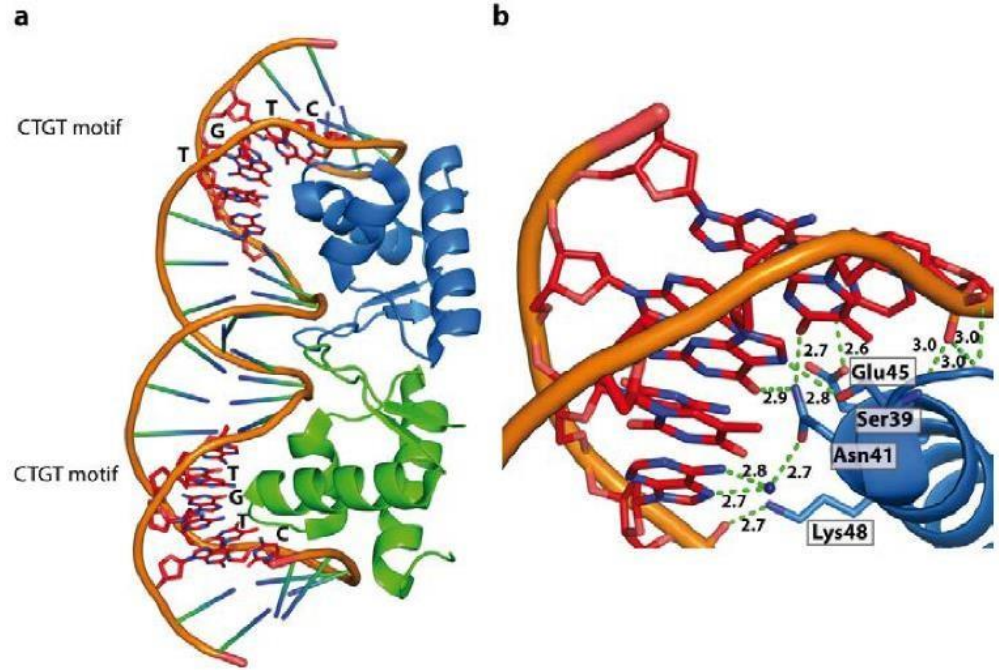
❑ Brief introduction

❖ **Before starting our lecture, there are important bullet points you need to recall:**

- Transcription: The process of making mRNA from DNA via RNA polymerase.
- It's mediated by regulatory elements (e.g. PPE, enhancers, silencers).
- Proteins interact with DNA in the Major groove through noncovalent interactions between the DNA nucleotides and the amino acids of the proteins.
- If we change an amino acid in the enzyme's active site, this may prevent the interaction between the substrate and the active site or may lead to interaction without proper catalytic reaction.

❑ How do proteins recognize/interact with DNA sequences specifically?

- Proteins bind DNA through sequence-specific interactions between amino acids of the protein and nucleotides of the DNA.
- Binding occurs at specific DNA regions called consensus sequences, which contain essential nucleotides required for interaction.
  Both the amino acids and the nucleotides must be specific for proper binding, although minor variations in non-essential nucleotides are allowed.
- DNA sequences that closely match the consensus bind proteins strongly, leading to strong gene regulation, while sequences with variations bind more weaker and are less strongly regulated.



❖ Thus, the DNA sequence determines the strength of protein binding and the level of gene regulation, and any change in the sequence of the promoter region will affect the efficiency of transcription.

▪ Consensus sequence: similar sequences that can be found in different promoter regions of different genes

❑ Regulation of transcription in prokaryotes/bacteria

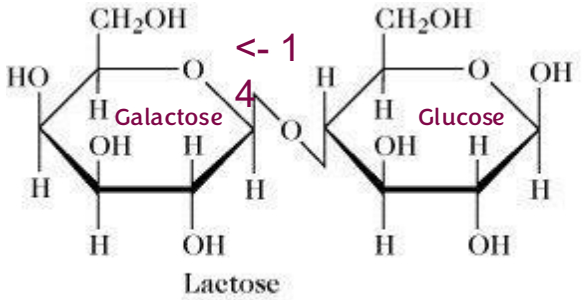
The lac operon (Proteins that participate in lactose metabolism)

- Remember: In bacteria, most genes are organized in operons and are polycistronic, meaning a single mRNA contains multiple coding regions, each producing a different protein.

- Operon: A Polycistronic genetic unit that exists in prokaryotic cells. It is transcribed into a single mRNA, and different regions of this mRNA can produce different proteins that work together in related mechanisms .

❑Metabolism of lactose

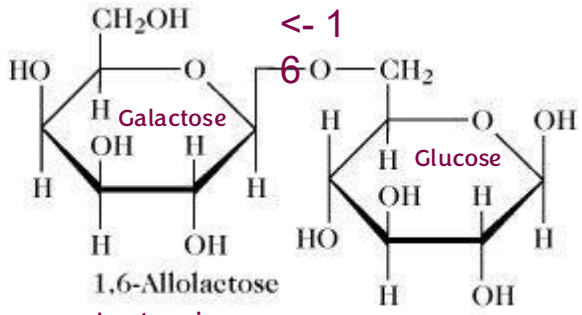
- In the 1950s, pioneering experiments were carried out by François Jacob and Jacques Monod who studied regulation of gene transcription in E. coli by analyzing the expression of enzymes involved in the metabolism of lactose.



Lactose

(same enzyme)
β-Galactosidase
(Transglycosylierung)

Isomerization
(The minor reaction)



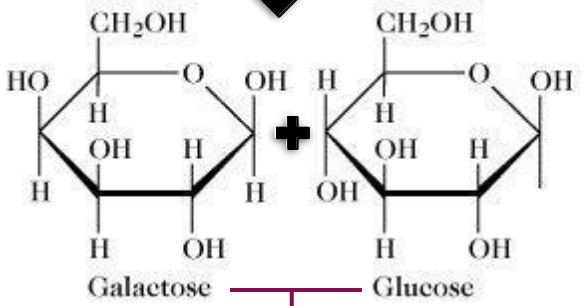
1,6-Allolactose

Lactose isomer

Bacteria break the glycosidic bond

(the enzyme)
β-Galactosidase
(Hydrolyse)

(The major reaction)



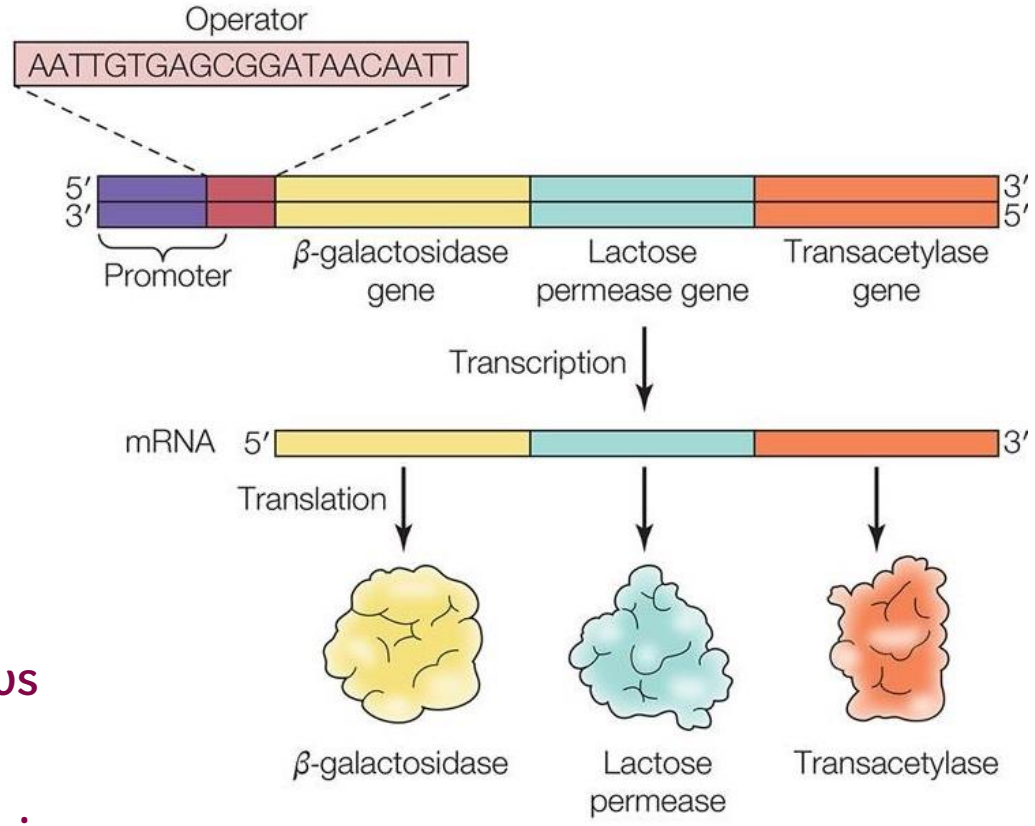
Galactose — Glucose

Utilized in energy production or other metabolic pathways.

The two French scientists
-They won Nobel prize for their work

❑The Lac operon (Lac stands for lactose)

- A cluster of genes transcribed from one promoter producing a single polycistronic mRNA that is used to make three proteins that are different in structure and function, but they participate in the same pathway (purpose/similar mechanism).



The three proteins are:

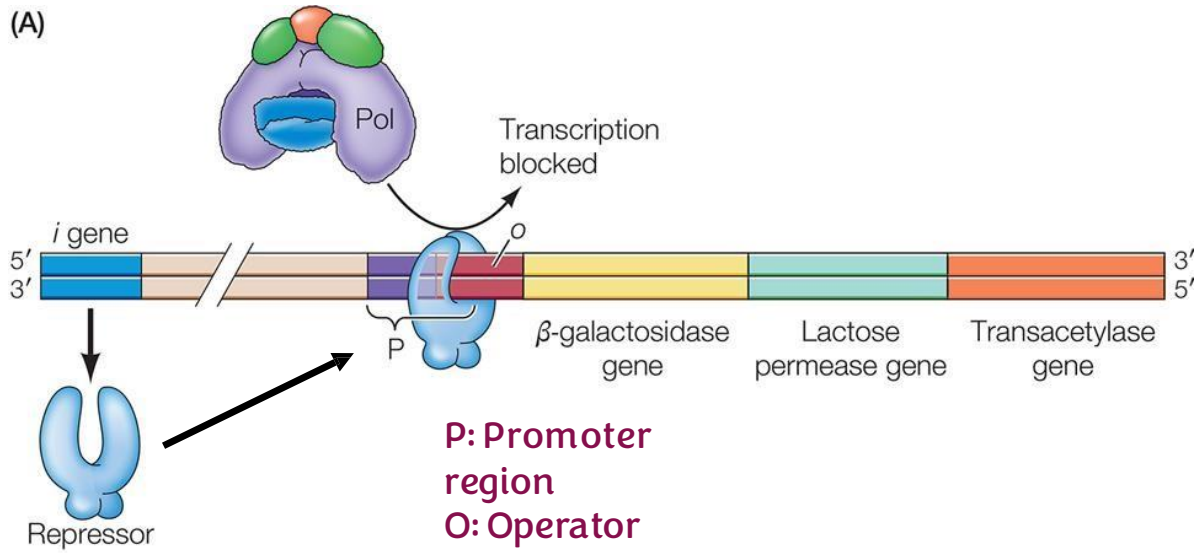
I. β-Galactosidase (the enzyme) : Cleavage of lactose into galactose and glucose

II. Permease: Transport of lactose (a transporter that allows the entry of lac from outside to inside the cell)

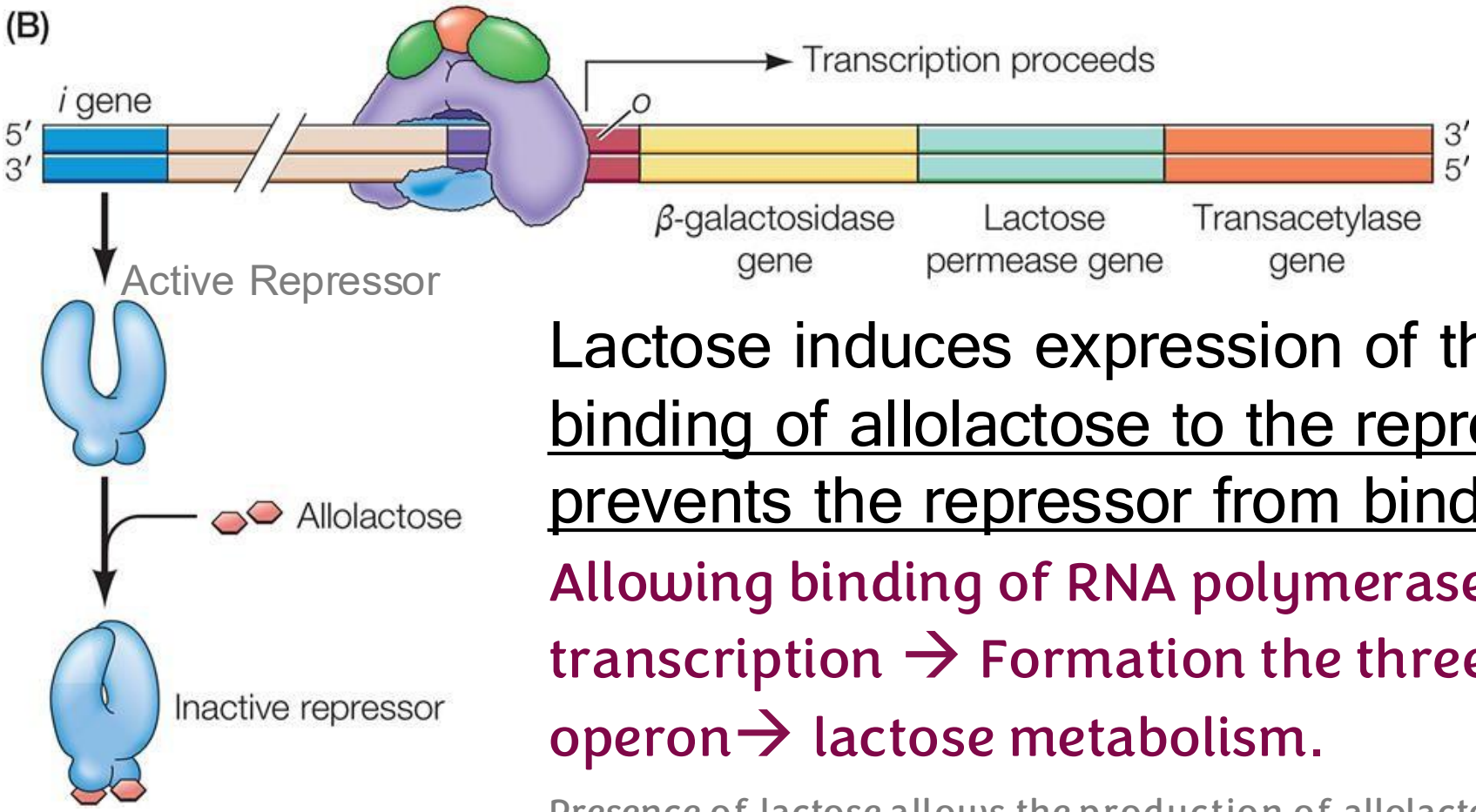
III. Transacetylase: Acetylation of toxic thiogalactosides (toxic components for bacteria, so the bacteria acetylate thiogalactosides to inhibit its toxicity and promote protection)

❑The operator

- The promoter region includes the operator region, which is a binding site of a protein called **the lac repressor.**
- The lac repressor blocks transcription by preventing (inhibiting) the RNA polymerase from binding to the promoter.
- Produced by the (I gene), " I " stands for inhibitory.
- I gene → makes lac repressor → binds to the operator → prevents the RNA polymerase from binding to the promoter → represses the transcription of the lac operon.



❑The role of allolactose

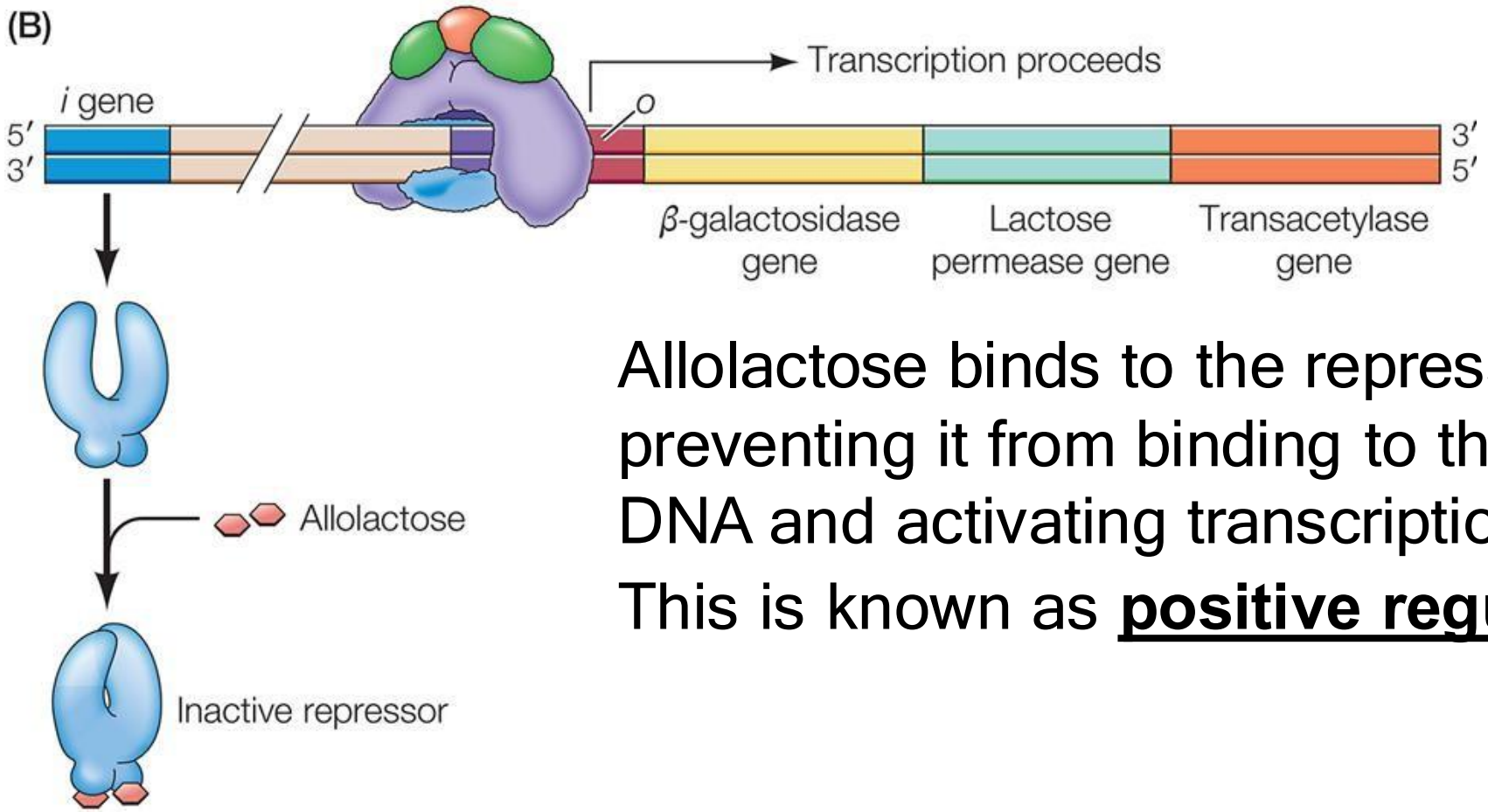


Lactose induces expression of the operon by the binding of allolactose to the repressor, which prevents the repressor from binding to the operator.

Allowing binding of RNA polymerase to the promoter → transcription → Formation the three proteins of the lac operon → lactose metabolism.

Presence of lactose allows the production of allolactose by isomerization, therefore increasing the formation of lac operon proteins which have an impact on increasing lactose metabolism, which really makes sense.

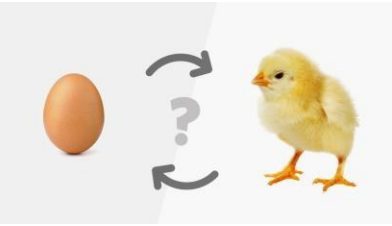
❑The role of allolactose



Allolactose binds to the repressor, thereby preventing it from binding to the operator DNA and activating transcription.

This is known as **positive regulation**.

Wait...

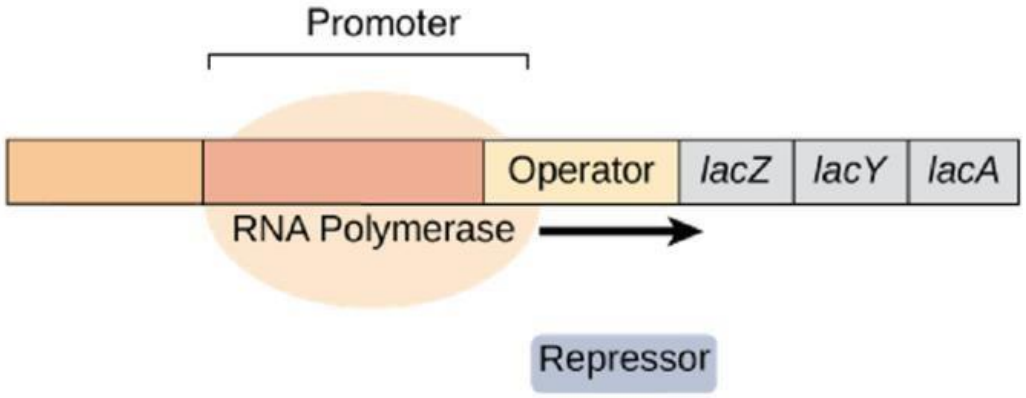


- So, we need allolactose to make β-galactosidase (via the positive regulation mentioned previously), but we need β-galactosidase to make allolactose (recall the minor reaction). Which one comes first?
- ANSWER: some promoters are leaky.

  - The type of the interaction is non-covalent and reversible, so we may find some repressor released (not bound to the operator although there is no allolactose).
  - So, the RNA polymerase take advantage of that and binds to the promoter, then forming – galactosidase (which produces allolactose from lactose by isomerization) and permease (which allows lactose to get inside the cell).
  - After having small amount of lactose, stimulation of expression occur.

Note that this is not always the case

Not all promoters are leaky and the ones that are leaky are not leaky in all types of cells.
e.g.: Insulin is produced by – cells of the Pancreas, but it is NEVER leaky in brain cells, because regulation is very tightly controlled .



❑ Cis vs. trans regulatory elements

Cis: (At the same Level of the gene) (Needs to present in specific place to act)

Trans: (At different level) (Works regardless of where you put the gene)

Remember:

- Factors are proteins.
- Elements are DNA or RNA sequences.

- DNA regulatory sequences like the operator are called cis-acting elements because they affect the expression of only genes linked on the same DNA molecule or close-by.
  - Mention other examples of cis-acting elements. e.g.: The promoter region, enhancers, silencers.
  - If the cis regulatory element is affected the gene will not function.
  - The cis regulatory element must exist on the same chromosome /domain of the DNA molecule that it will regulate (at the same level).

❑Cis vs. trans regulatory elements

- Proteins (usually) 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 (that is, genes). e.g.: I gene.
  - Mention other examples of trans-acting elements.
  - If the place of trans acting factors are manipulated (change its place – other domain, gene...) it will still be functional (affective) = (it will still affect the transcription).
  - It is all about changing the site of the DNA or RNA sequence or the protein.

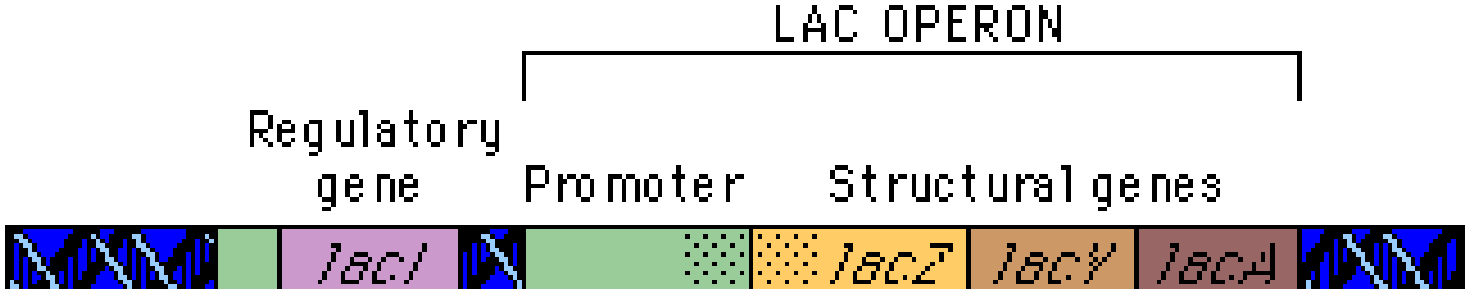
❑ Effect of mutations (Creating a mutation clarifies the function)

- Some mutations result in **constitutive** expression (always on).
  - Mention examples: mutations in operator/ repressor (can't bind)

  → the lac Operon is expressed even in the absence of lactose .

- Other mutations cause **non-inducible** or repressed expression (always off).
- Mention examples: mutations in promoter region/ repressor (always bound) / I gene / RNA polymerase.

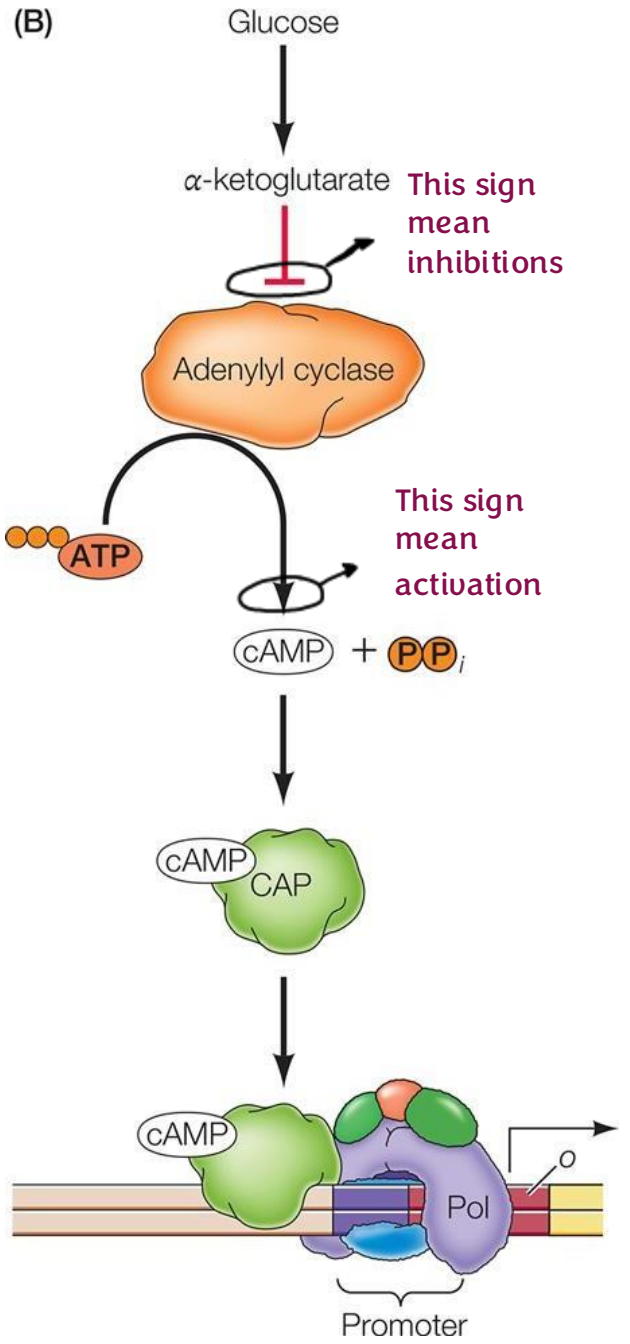
  → the lac Operon is never transcribed even in the presence of lactose .



Another level of regulation (negative regulation)

Negative regulation: the presence of something that shuts off transcription. In our case this –something– is glucose.

- Another regulator is catabolite activator protein (CAP) which binds to regulatory sequences upstream of the promoter.
- CAP can then interact with the RNA polymerase to facilitate its binding to the promoter (P).
- CAP binding to DNA is influenced by cAMP, which is produced by adenylyl cyclase, which is inhibited by high level of glucose.
- If **glucose** is present, it is **preferentially utilized** by bacterial cells and it represses the lac operon even in the presence of the normal inducer (lactose).
- This is known as negative regulation.



Another level of regulation (negative regulation)

There are two proteins that regulate the transcription of lac operon:

1. Repressor
   - Binds **downstream** of the promoter
2. Catabolite activator protein (**CAP**)
   - Binds **upstream** of the promoter, It binds to cAMP and activates the RNA polymerase
   - The activity of CAP is affected by the presence of glucose

In the case of glucose absence :

Adenylyl cyclase is active → cAMP → CAP → RNA polymerase → Expression of lac operon

- If you give bacteria glucose and lactose, it would prefer glucose over lactose, this means that there would not be a need for the expression of lac operon.

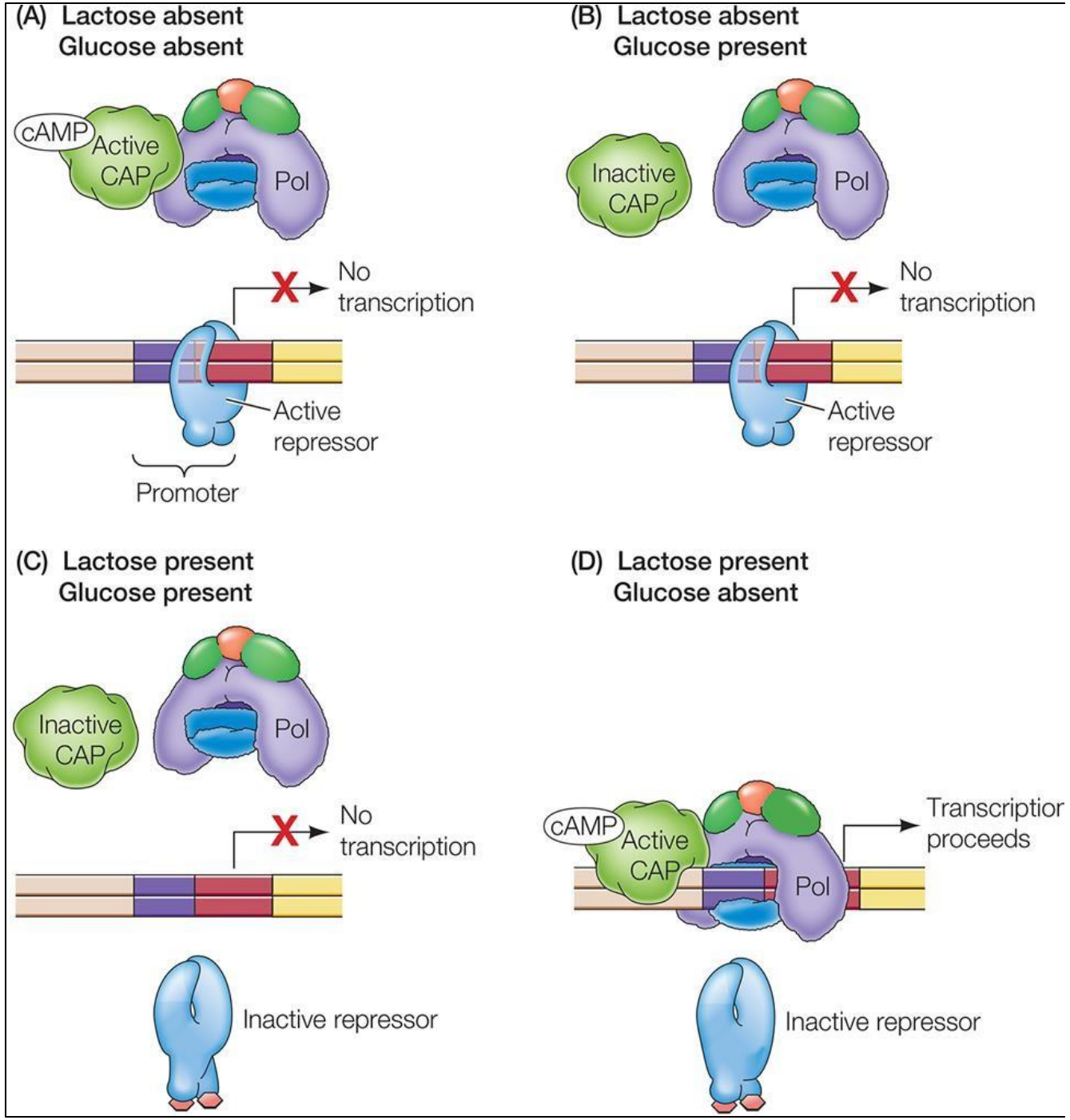
When present, Glucose inhibits adenylyl cyclase, no cAMP production, CAP won't be activated, RNA polymerase won't be activated, **no lac operon expression**.

This is called **negative regulation**

Glucose VS Lactose

- There are four possibilities but remember **the main principles are:**

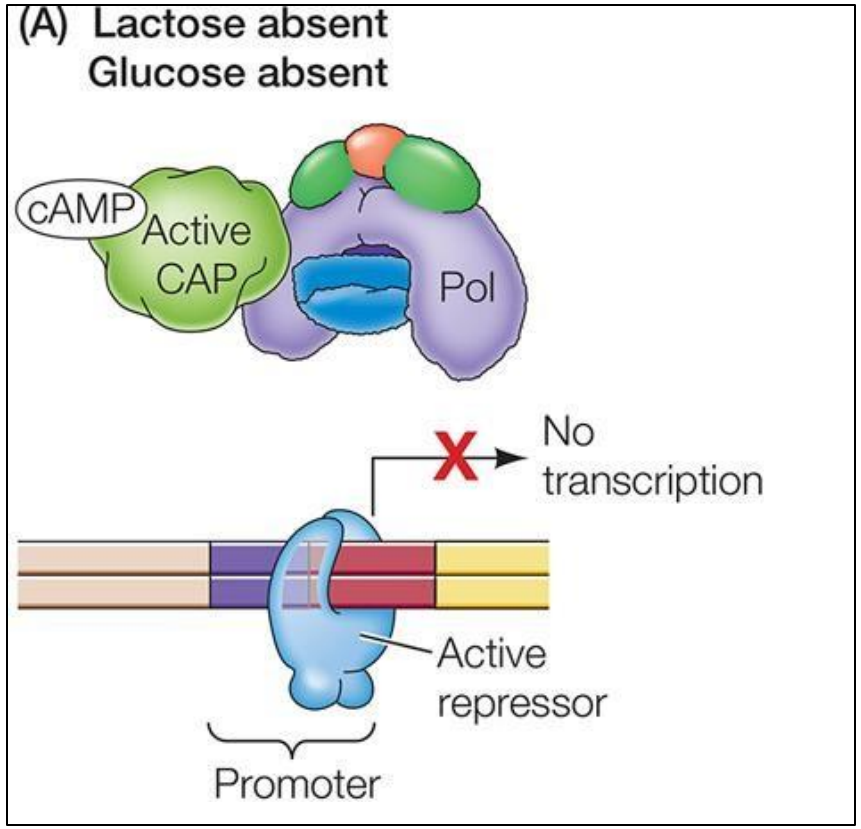
- ✓ Lactose inactivates the repressor.
- ✓ Glucose inhibits the production of cAMP, which inactivates CAP.



The four cases scenarios

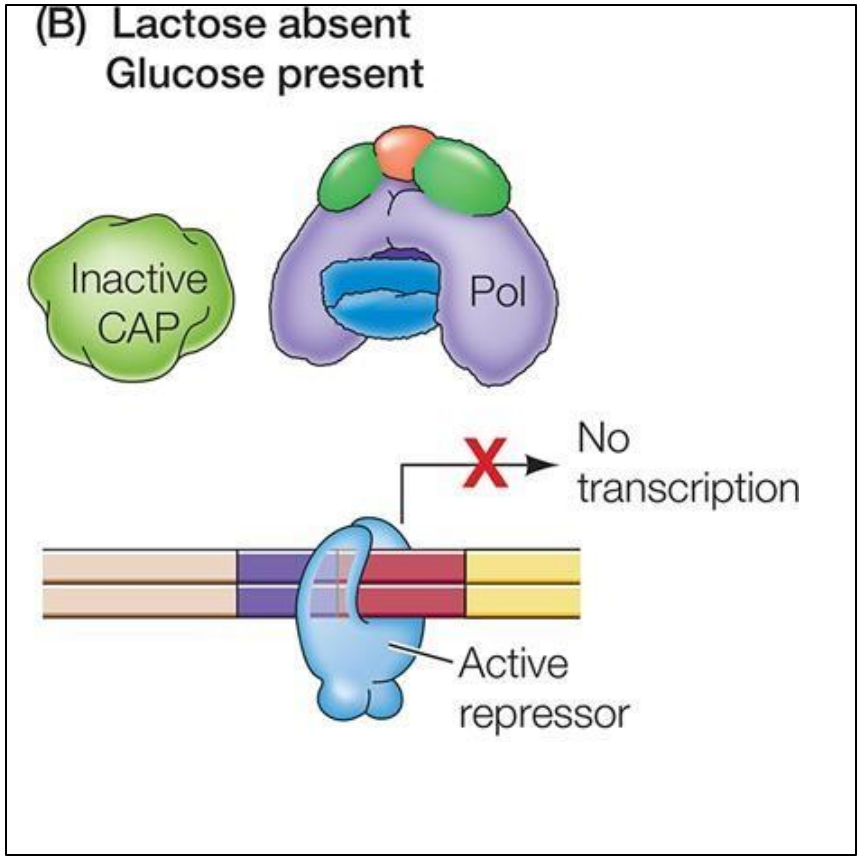
1) Lactose ✗
Glucose ✗

No expression of lac operon, because CAP is bound to RNA polymerase but the polymerase can not bind to the promoter because of the repressor.



2) Lactose ✗
Glucose ✓

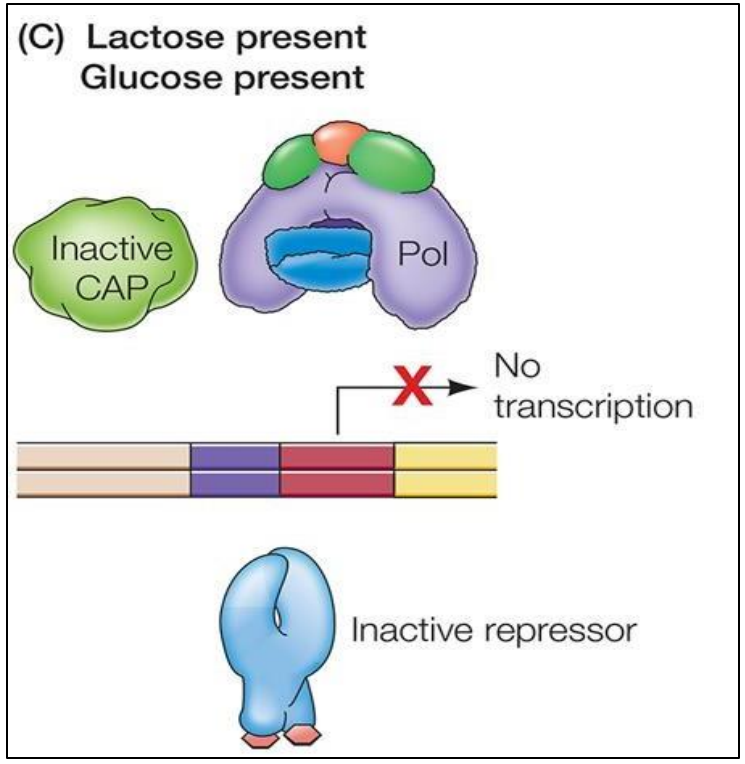
No expression of lac operon, CAP and RNA polymerase are not bound because there is no cAMP, and the repressor is active.



The four cases scenarios

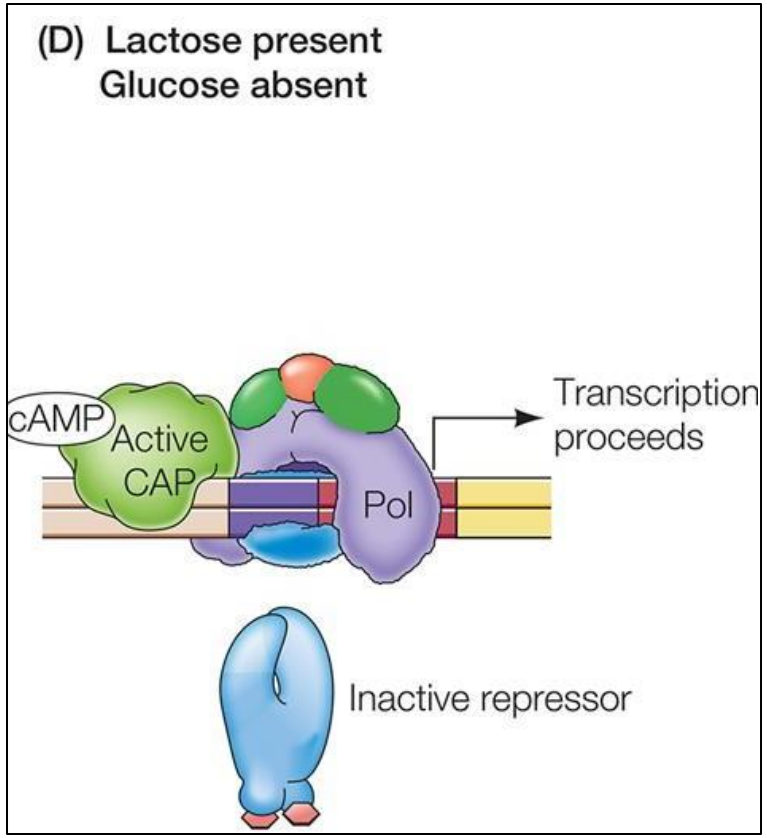
3) Lactose ✅
Glucose ✅

Lactose is present and glucose is present. Allolactose binds to the repressor, causing the repressor to dissociate from the DNA operator. As a result, RNA polymerase can bind to the promoter and initiate transcription. However, because glucose is present, cyclic AMP levels are low and CAP does not bind to the DNA. Therefore, transcription occurs, but at a very low level



4) Lactose ✅
Glucose ❌

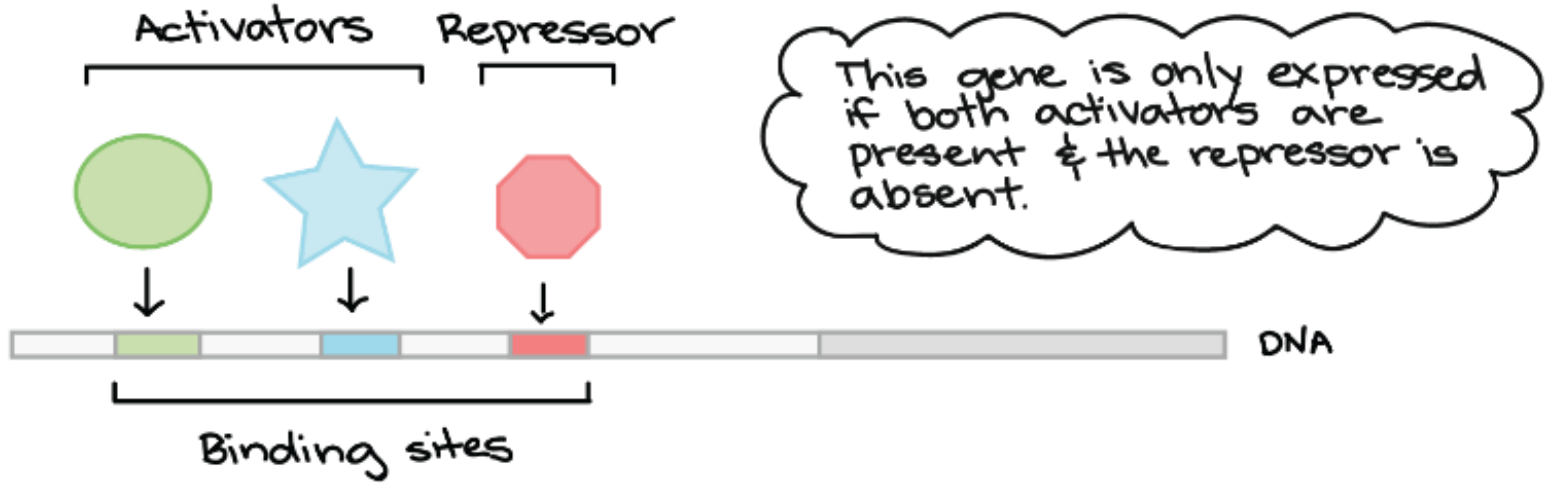
Lac operon is expressed, cAMP present,CAP is bound to RNA polymerase, lac operon transcription proceeds and the repressor inactive .

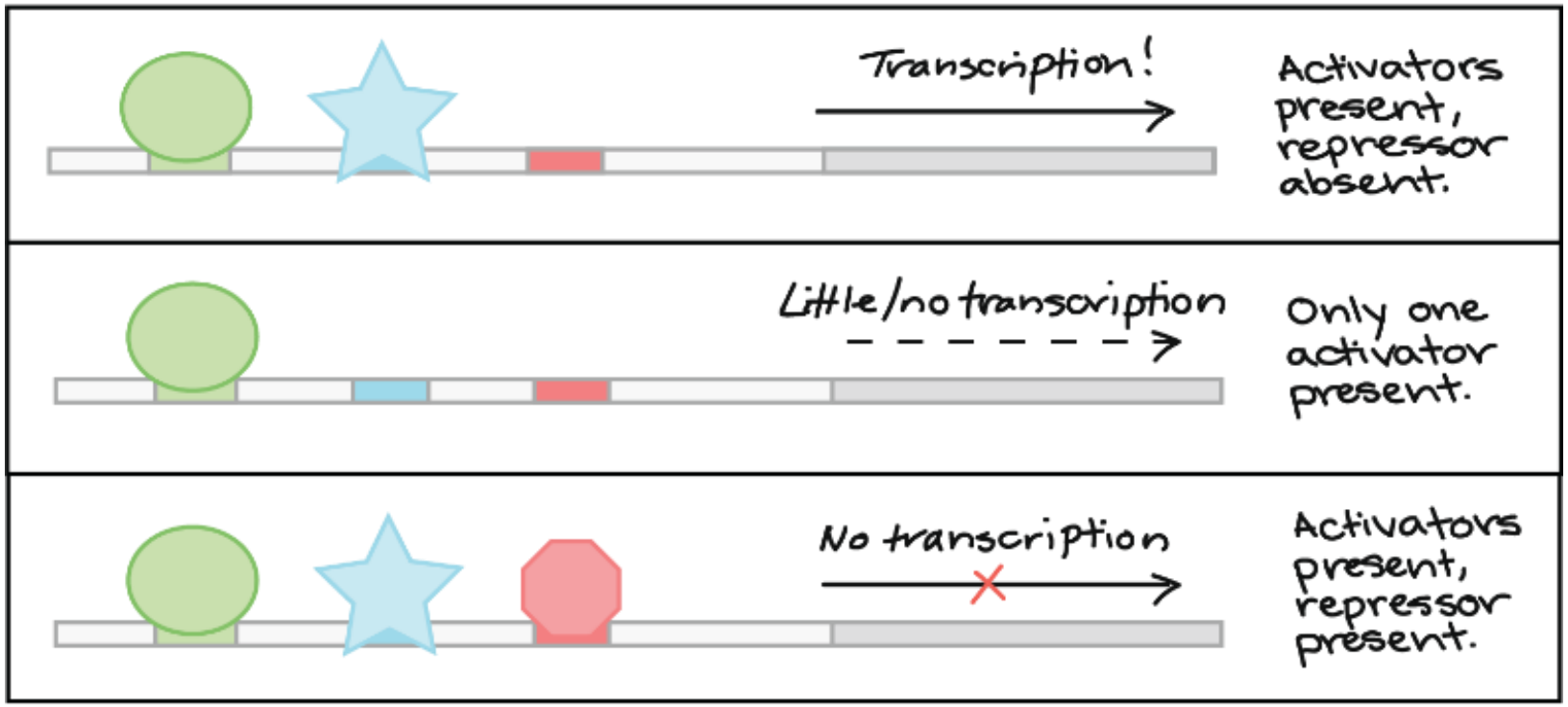


Regulation of transcription in eukaryotes

Regulatory mechanisms

- 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:
  - Cis-acting elements (location sensitive)
    - Promoters, promoter proximal elements, enhancers, and silencers
  - Trans-acting factors They can act on more than one chromosome
    - transcriptional regulatory proteins (activators, repressors)
      - DNA and chromatin structural modification → By targeting the histones
      - DNA chemical modification (example: methylation of cytosine)
      - Noncoding RNA molecules


Activators
Repressor
This gene is only expressed if both activators are present & the repressor is absent.
DNA
Binding sites


Transcription!
Activators present, repressor absent.
Little/no transcription
Only one activator present.
No transcription
Activators present, repressor present.

How do TFs regulate gene expression?

- Transcription factors cause epigenetic/epigenomic changes in DNA and chromatin. One gene Many genes

- What is epigenetics? Higher level of control than simple changes in nucleotide sequence
  - Epi: “above” or “in addition to”
  - It indicates **alterations** in gene expression **without a change in the DNA sequence**, but through DNA modification via internal or external factors.

Modifying the DNA structure or chemistry without changing the sequence

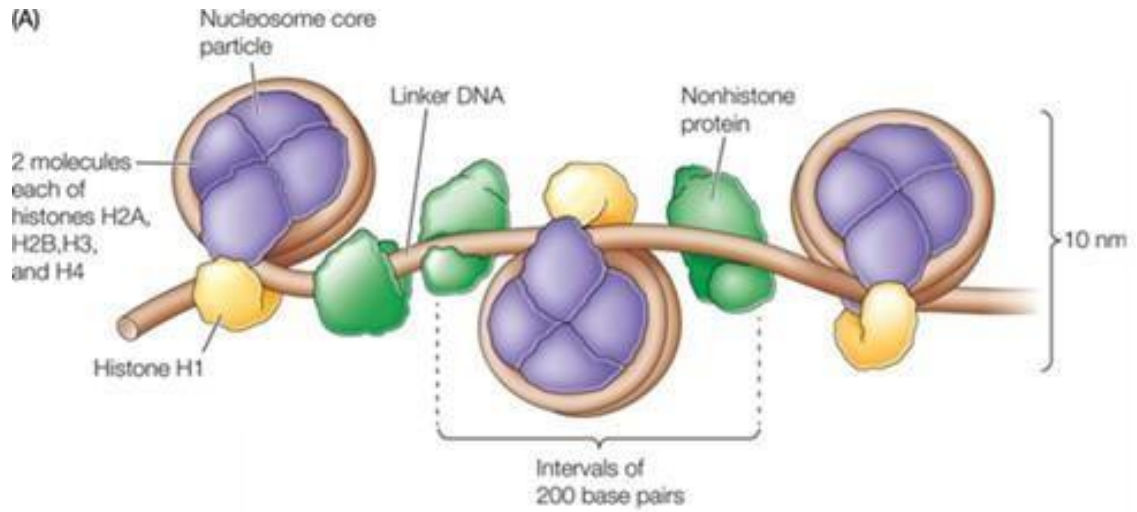
- Internal factor such as stress and mood
- External factors such as the sun exposure, nutrition

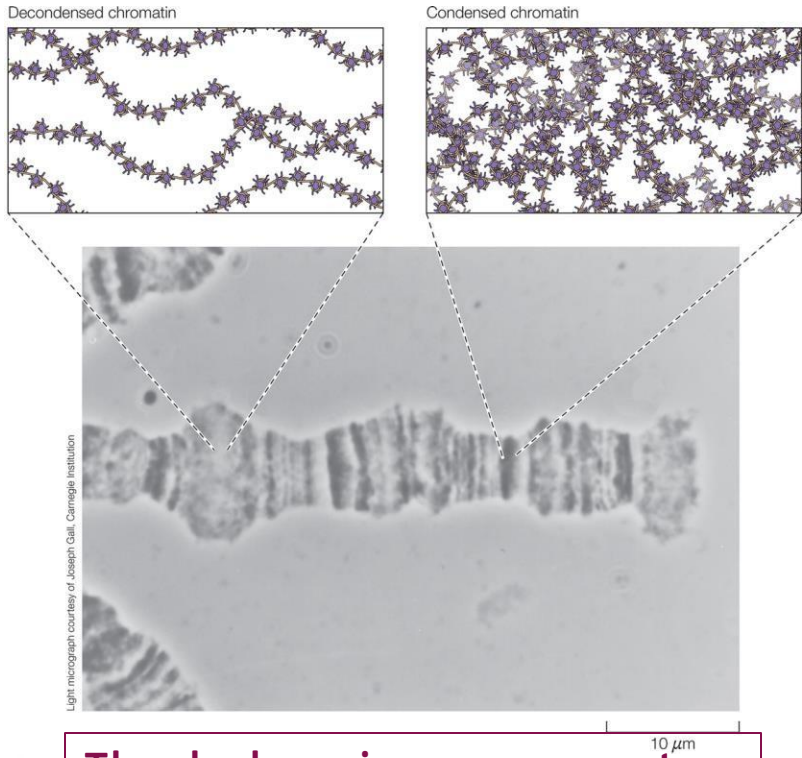
Nucleosome

- DNA exists as chromatin (mixture of DNA and Proteins), which is DNA wrapped around an octamer of histone proteins (H2A, H2B, H3, and H4) as a nucleosome core particle. Histone 1 can also bind to the DNA outside the nucleosome core. There is a free linker DNA between every two nucleosome core particles.
- DNA can either be loosely or tightly condensed, that is as euchromatin or heterochromatin, respectively.

(accessible) (Not accessible) (When the DNA مدحش)

(When the DNA relaxed)





Telomeres and the centromeres are composed of heterochromatin .

The dark regions represent condensed DNA , while the light regions correspond to euchromatin , where genes are active .

Modulation of chromosomal structure

- Active genes exist in euchromatin.

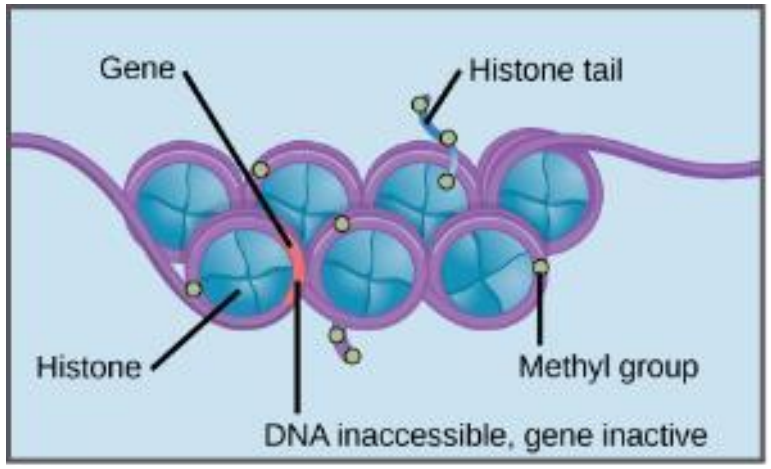
Accessible for transcription factor to easily find the DNA sequences.

- Inactive genes exist in heterochromatin.

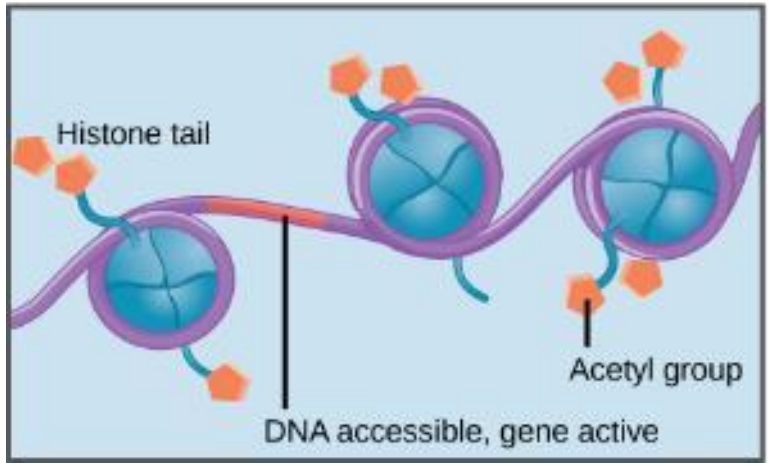
Inaccessible hidden DNA

- The packaging of eukaryotic DNA in chromatin can regulate transcription.
- Regulatory proteins switch between the two structures of chromatin.

Insulin gene in pancreatic cells is in the euchromatic form, but in nerve cells it's in the heterochromatic form.
Cells exchange DNA structures between the euchromatin and the heterochromatin depending on their needs. This is **epigenetic control**.



Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.



Histone acetylation results in loose packing of nucleosomes. Transcription factors can bind the DNA and genes are expressed.

How are chromosomal structures altered?

How to alternate between euchromatin and heterochromatin and vice versa

- Change of compactness of the chromatin by:
  1) Change the structure and position of nucleosomes
  2) Chemically modify histones
     - Acetylation, methylation, and phosphorylation
  3) Chemically modify cytosine
  4) Binding of noncoding RNAs to DNA

These four mechanisms will be further explained in the next lecture

Change the structure and position of nucleosomes

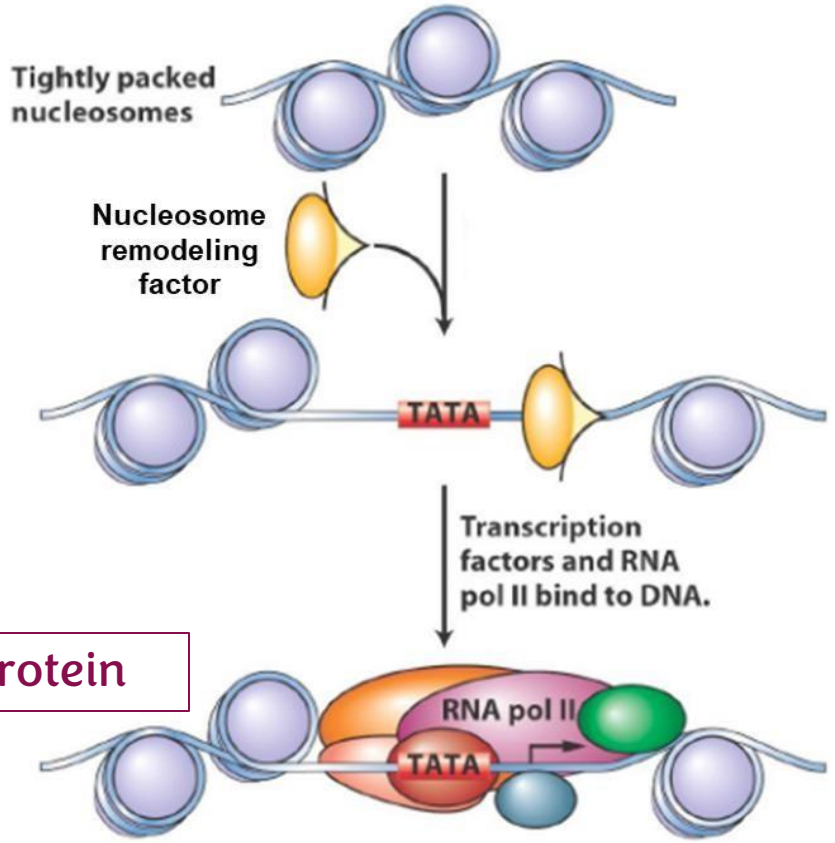
Chromatin remodeling factors

Cells can reposition nucleosomes to expose DNA wrapped around histones. This can reveal regulatory sequences in the DNA, like promoters, proximal elements, and enhancers. They can also remove histones, making the DNA accessible. Alternatively, they can loosen DNA–histone interactions, reducing the number of DNA turns around histones, from two turns to one.

- They facilitate the binding of transcription factors by
  - Removing histones from DNA
  - Repositioning nucleosomes making DNA sequences accessible
  - Altering nucleosome structure allowing protein bindi n to DNA

  – The proteins that are responsible for these changes are called:

- Chromatin (nucleosome, protein) remodeling factors can be associated with transcriptional activators and repressors. These aren't found in bacteria

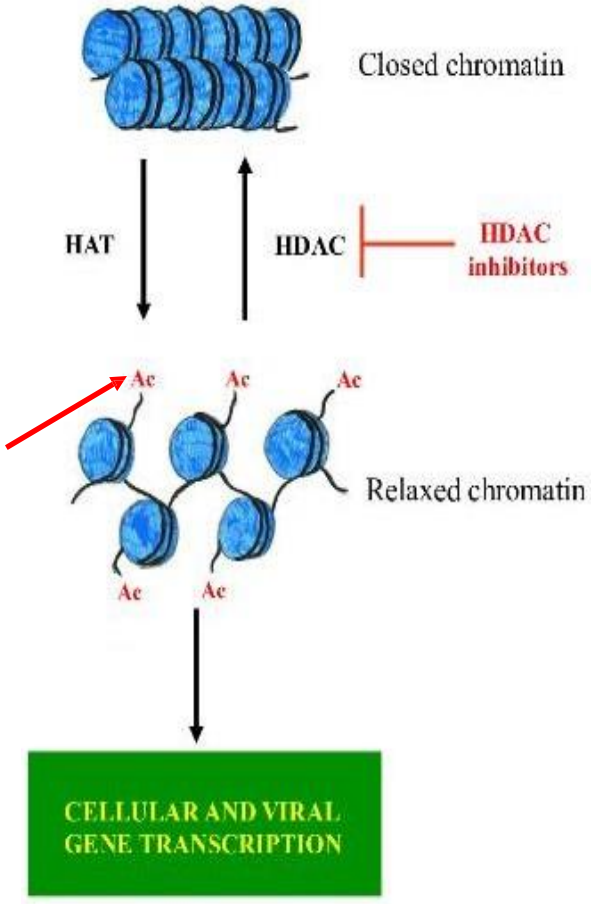


Chemical modification of histones

Histone acetylation

The function of HAT is to transfer acetyl groups and convert heterochromatin into euchromatin ,so its activators of transcription

- Every regulatory proteins can bind to DNA at a specific sequences.
- Histones bind non-specifically to DNA due to its negative charge.
- The components that are repeated in all nucleotides are the negatively charged phosphate groups. You can expect that histones have positively charged amino acids (lysine and arginine).

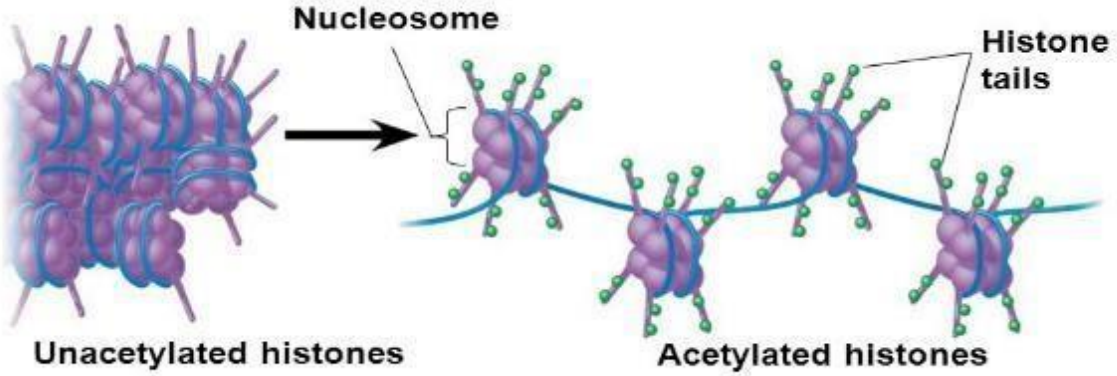


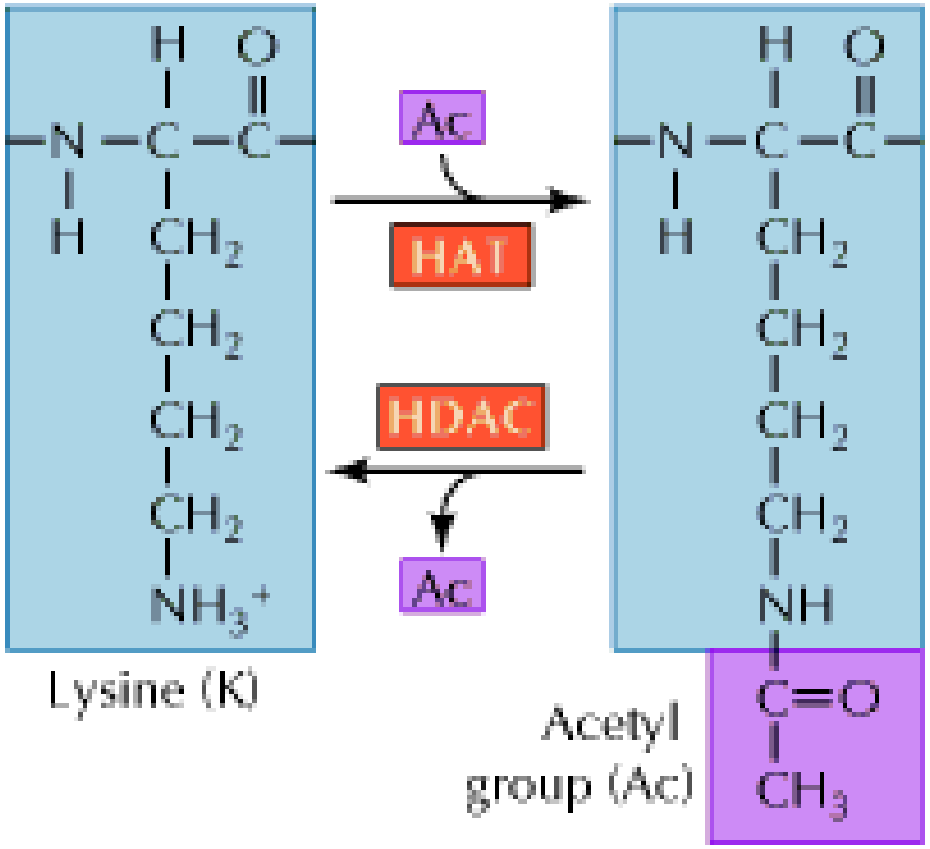
- he core histones (H2A, H2B, H3, and H4) have two domains (internal 3-dimensional structures):
  - A histone-fold, which is involved in interactions with other histones and in wrapping DNA around the nucleosome core particle.
  - An amino-terminal tail (indicated by a red arrow), which extends outside of the nucleosome, and is rich in lysine (positively charged amino acid)

Acetylation of lysine

The function of HDAC is to remove acetyl groups from lysin residues, restoring their positive charge. This strengthens the interaction between histones and DNA , converting euchromatin into heterochromatin , thereby inhibiting transcription.

In **histone acetylation**, acetyl groups are attached to positively charged lysines in histone tails. This generally loosens chromatin structure, promoting the initiation of transcription.





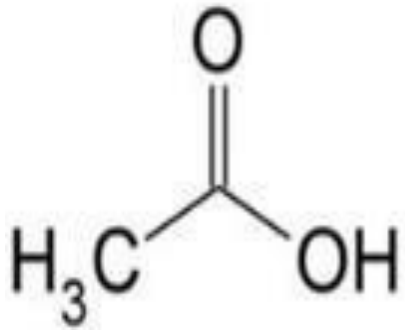
Histones have two domains:

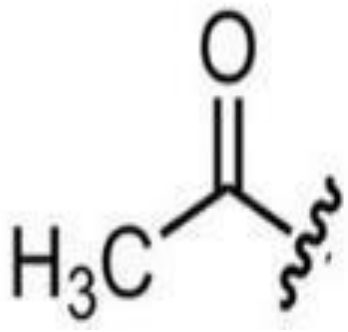
1. histone fold which makes the interaction with the DNA
2. amino terminus which is rich in lysine

These lysine groups will be acetylated → Acetyl group will bind to the positive R group of lysine

- The function of the positive charge in the R group is to make a further interaction with the DNA.

After acetylation, the positive charge will gone making the interaction weaker and the DNA become loose.



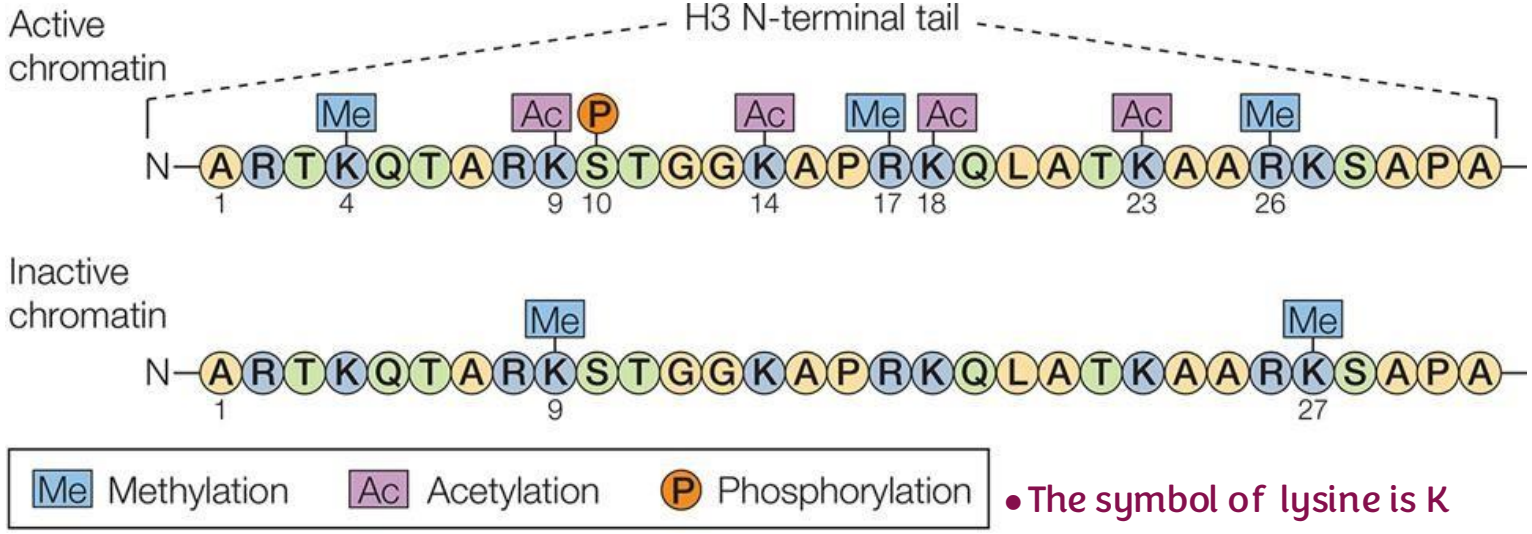


Other modifications of histones

- Histone can also be methylated (it is like putting a mask on the positive charge), which modulates interactions with DNA, or phosphorylated, adding a negative charge that repels DNA due to the negative phosphate groups.
- The effect, whether transcriptional activation or repression, depends on the modification sites.
- Histone modifications can: (1) alter chromatin structure and (2) provide binding sites for other proteins that can either activate or repress transcription.

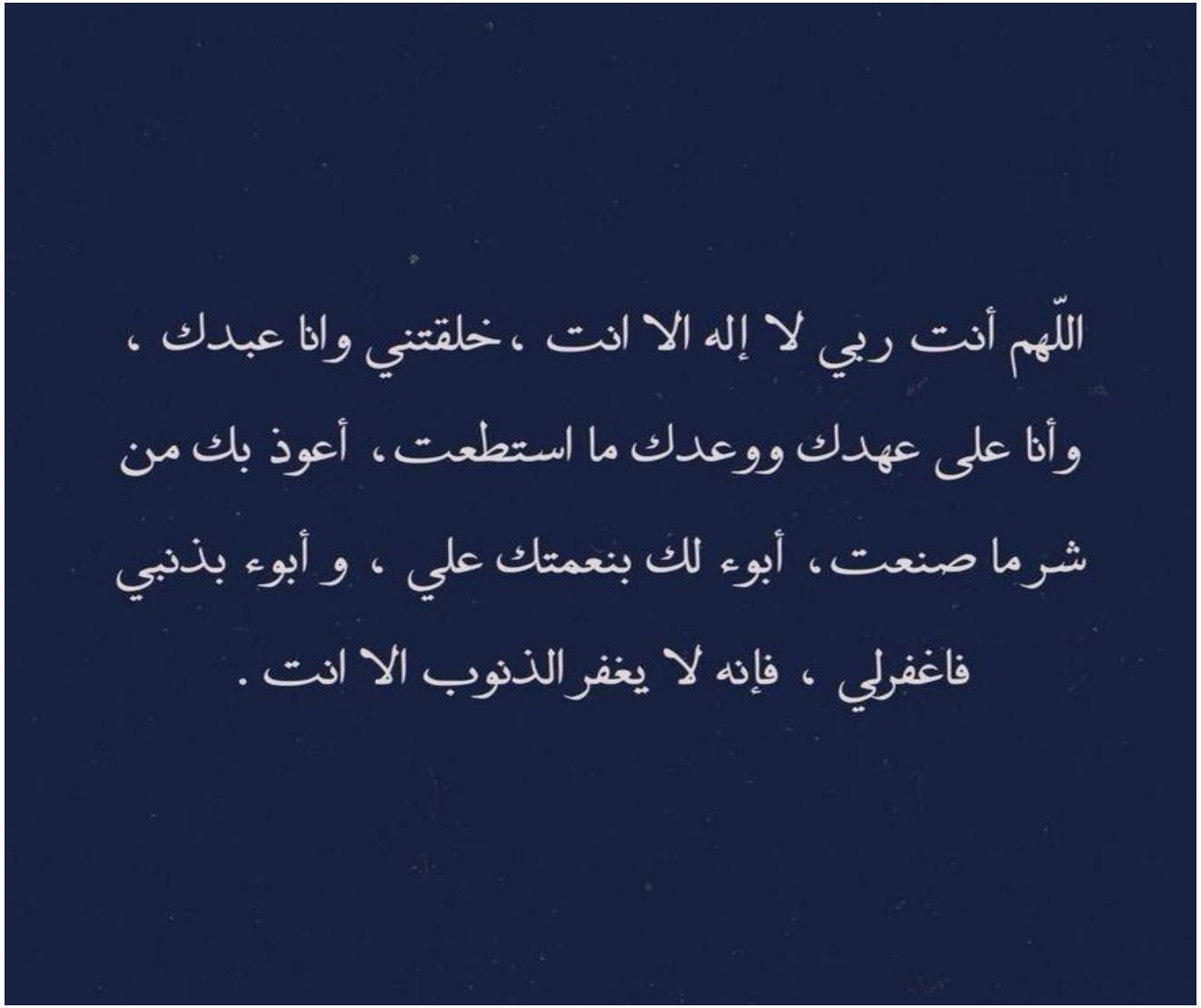
– this modification can change chromatin structure by loosening or tightening the wrapping between DNA and histones.

– As we said, phosphorylating the cytosolic domain of the receptor creates additional binding sites for other molecules (docking sites).



• The symbol of lysine is K

رسالة من الفريق العلمي:



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			