بسم الله الرحمن الرحيم

MOLECULAR BIOLOGY

FINAL – Lecture 12

Transcription regulation pt.1

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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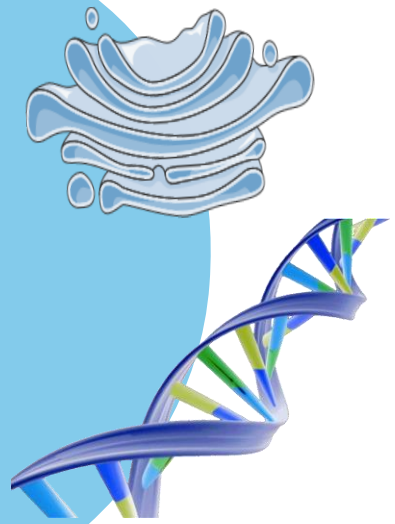


اللهم إنّي أسألك أن ترزقني علمًا نافعًا، وأن تنفعني بما علّمتني وأنت تزيدني علمًا، وأن تهبني من لدنك عقلًا منيرًا، ونفسًا منشرحة مقبلة على الدراسة والعلم برغبةٍ وحب، واجعلني يا ربّي سريع الحفظ حاد الذهن، واجعل ما رزقتني من العلم حجةً لي لا عليّ يا كريم يا رب.

Quiz on the previous lecture (actually it is the introduction that Prof. Mamoun start with in this lecture, in addition to some other information)

Transcription-Regulation

Prof. Mamoun Ahram

School of Medicine

Second year, First semester, 2024-2025

Brief introduction

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

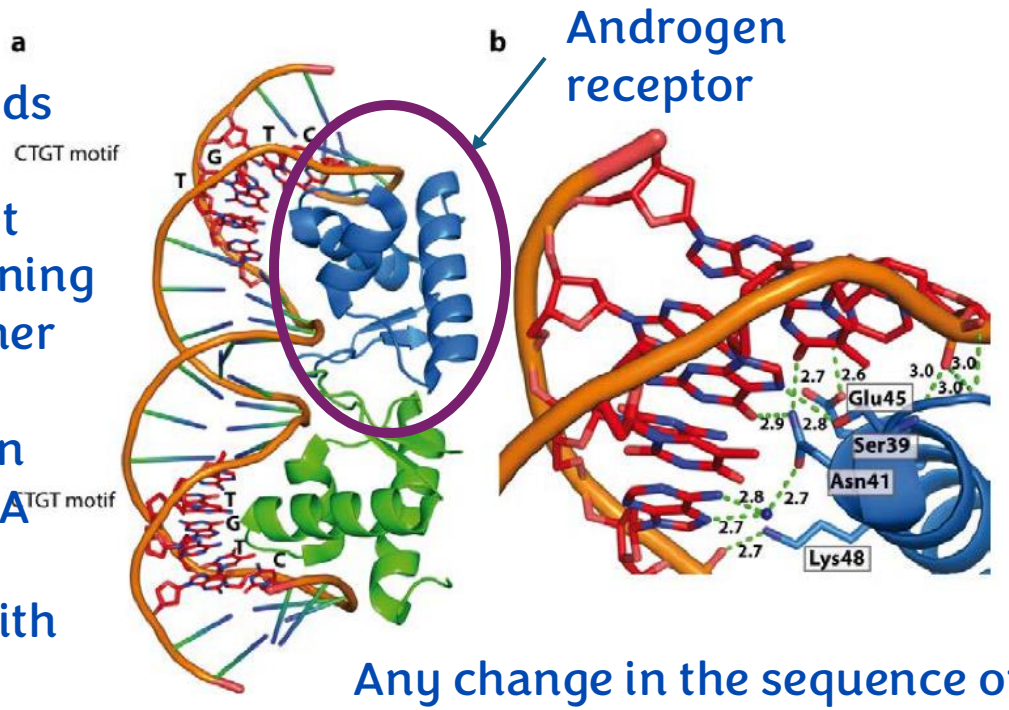
- Transcription: The process of making mRNA from DNA via RNA polymerase.
- It's mediated by regulatory elements (e.g. PPE, enhancers, silencers).
- Proteins interact with DNA in the Major groove through non covalent interactions between the DNA nucleotides and the amino acids of the proteins.
- If we 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?

A.A stands for amino acids.

e.g. Androgen receptor element:

- A sequence (*consensus sequence) of the DNA which has specific nucleotides that can interact with specific amino acids of the receptor.
- Both (A.A and nucleotides) should be specific in order to get the proper interaction, but minor variations may occur, meaning not 100% identical (some nucleotides are essential, while other may differ).
  - Some genes have an optimal element sequence resulting in very strong interaction between the receptors and the DNA (the gene is highly regulated by the AR**).
  - While other genes have ARE*** but a weaker interaction with the AR is noticed due to non-optimal nucleotide sequence (ARE is somewhat/ mildly regulated by the AR)
- The sequence determine the strength of the interaction and the regulation.



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
** Androgen Receptor
*** Androgen Response Element

Remember

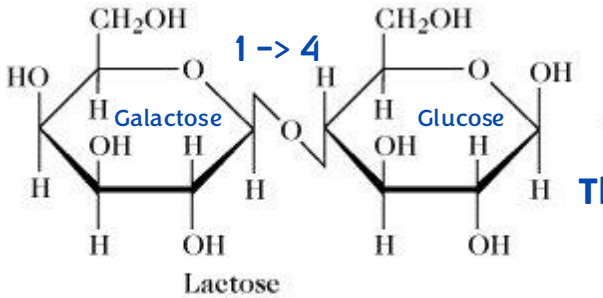
Operon:A Polycistronic genetic unit that exists in **prokaryotic** cells. It is transcribed into a **single mRNA**, and different regions of this mRNA can produce **different proteins** that work together in related mechanisms .

Regulation of transcription in prokaryotes

The lac operon (Proteins that participate in lactose metabolism.)

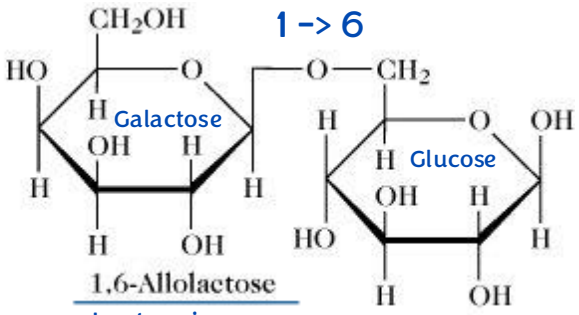
Metabolism of lactose

- 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.



(same enzyme)
β-Galactosidase
(Transglycosylierung)

The minor reaction
Isomerization

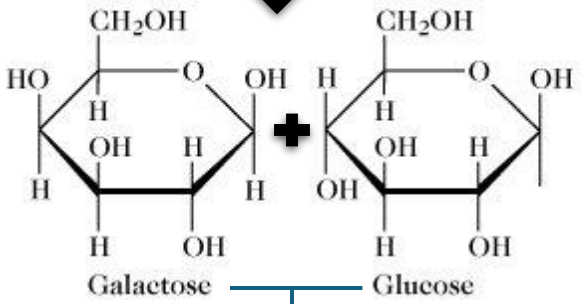


Lactose isomer

Bacteria break the glycosidic bond

(the enzyme)
β-Galactosidase
(Hydrolyse)

The major reaction



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:

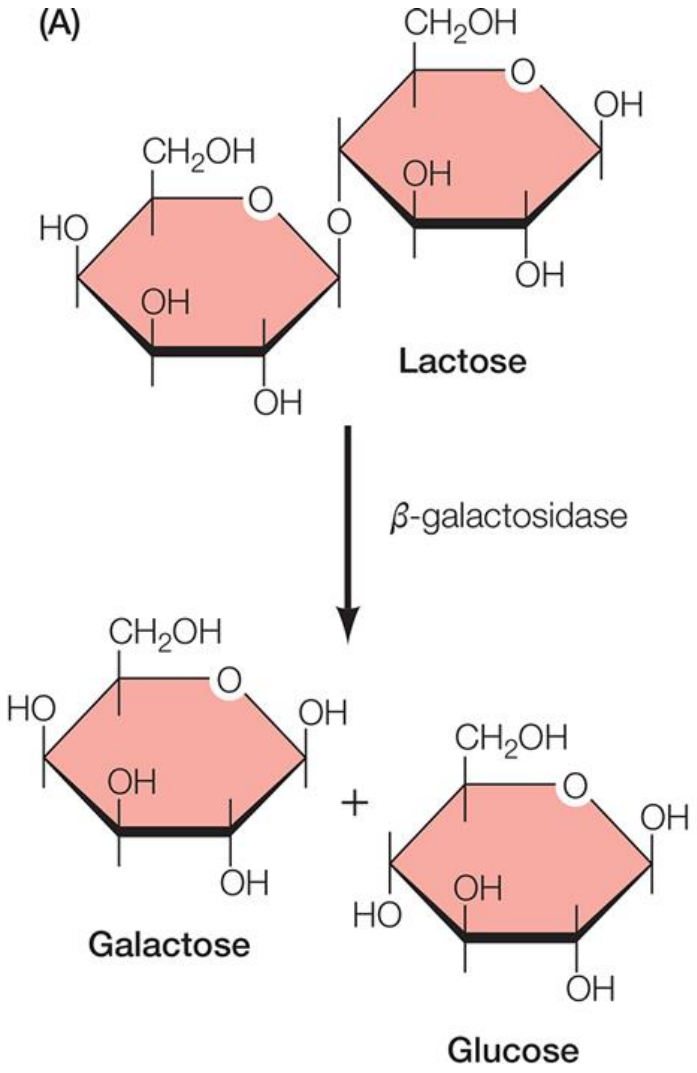
  1- β-Galactosidase (the enzyme) : Cleavage of lactose into galactose and glucose

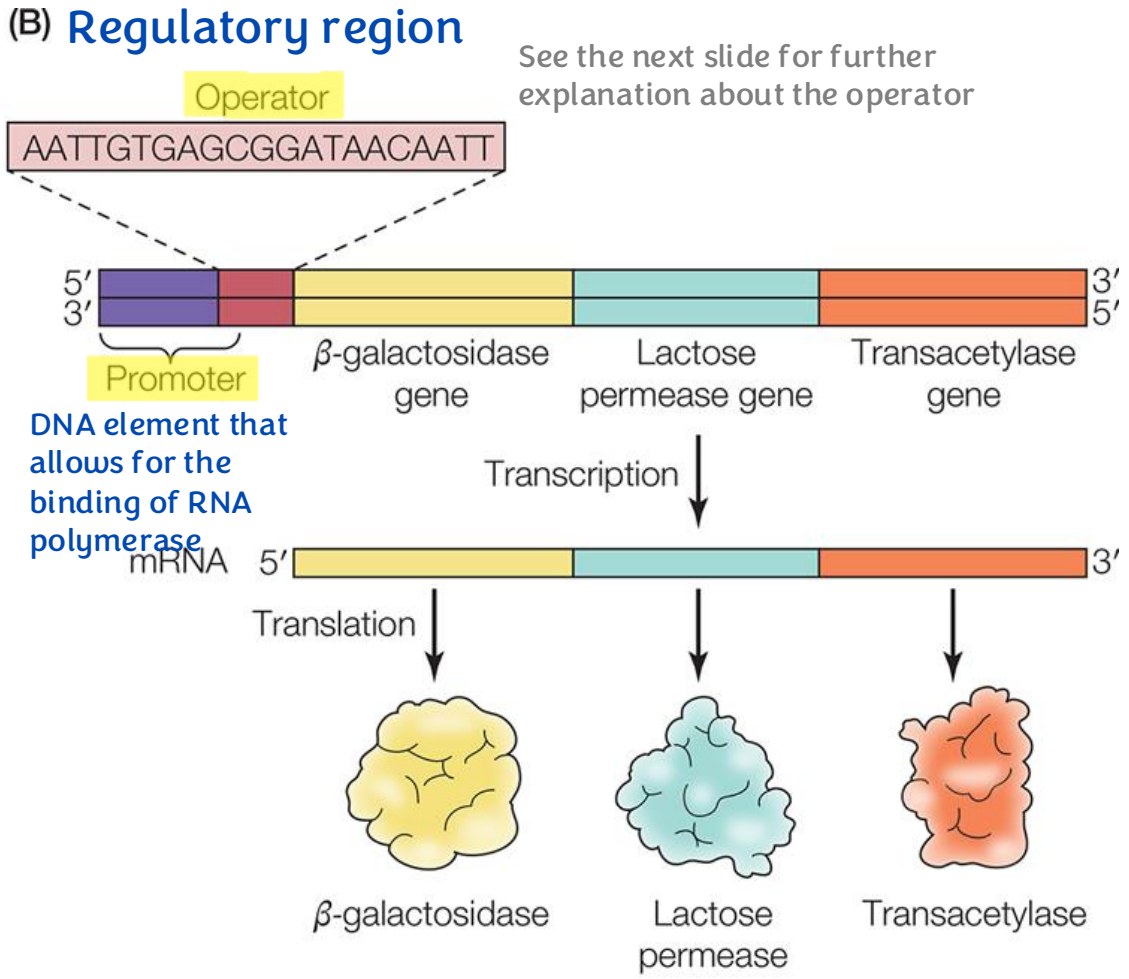
  2- Permease: Transport of lactose (a transporter that allows the entry of lac from outside to inside the cell)

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

Figure out further explanation in next slides.

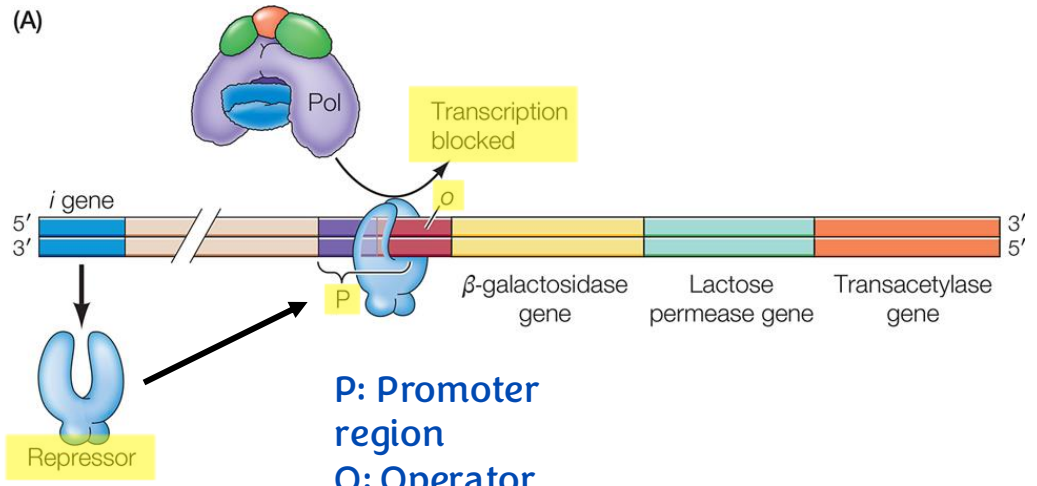
The lac operon – Con.



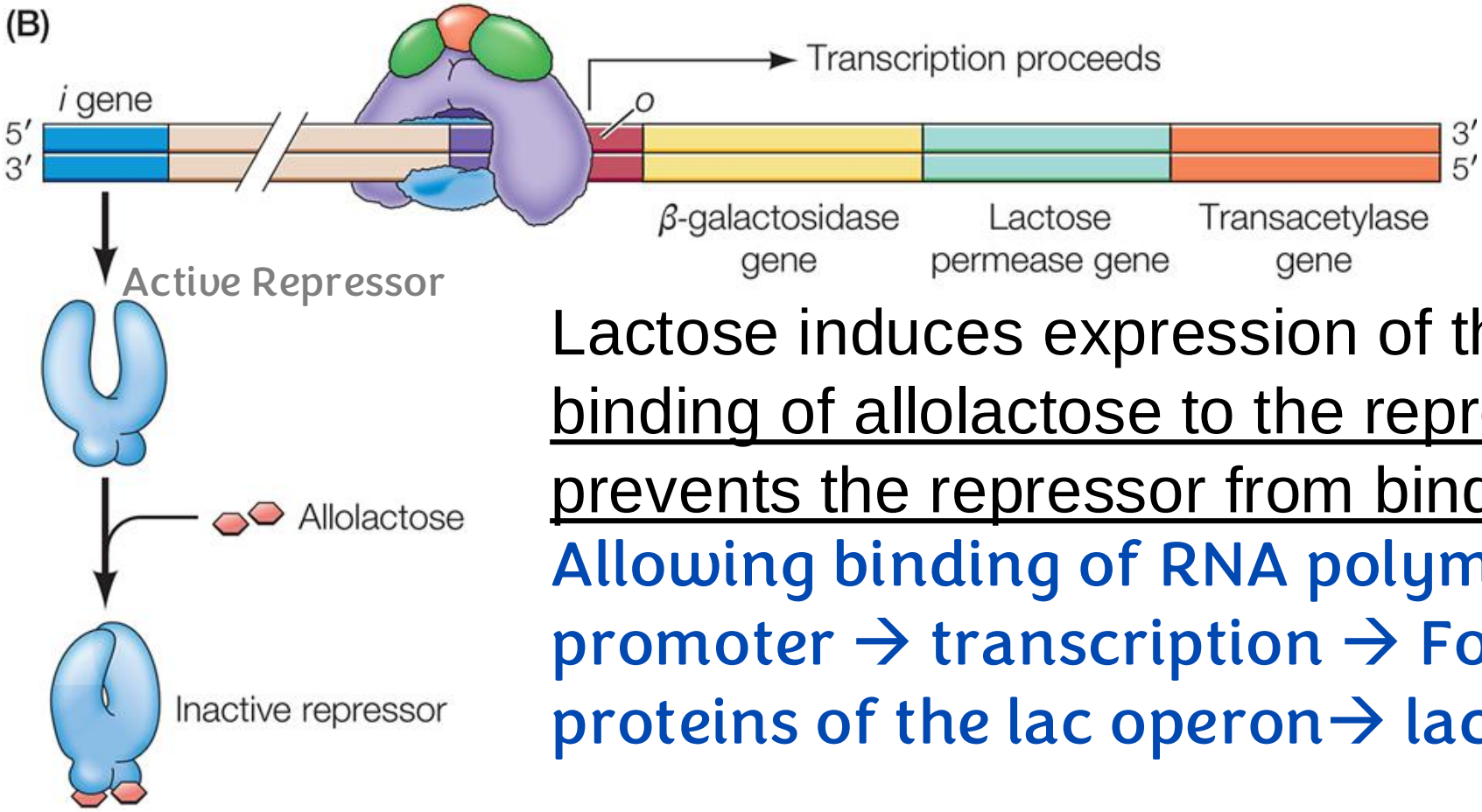


The operator

- 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

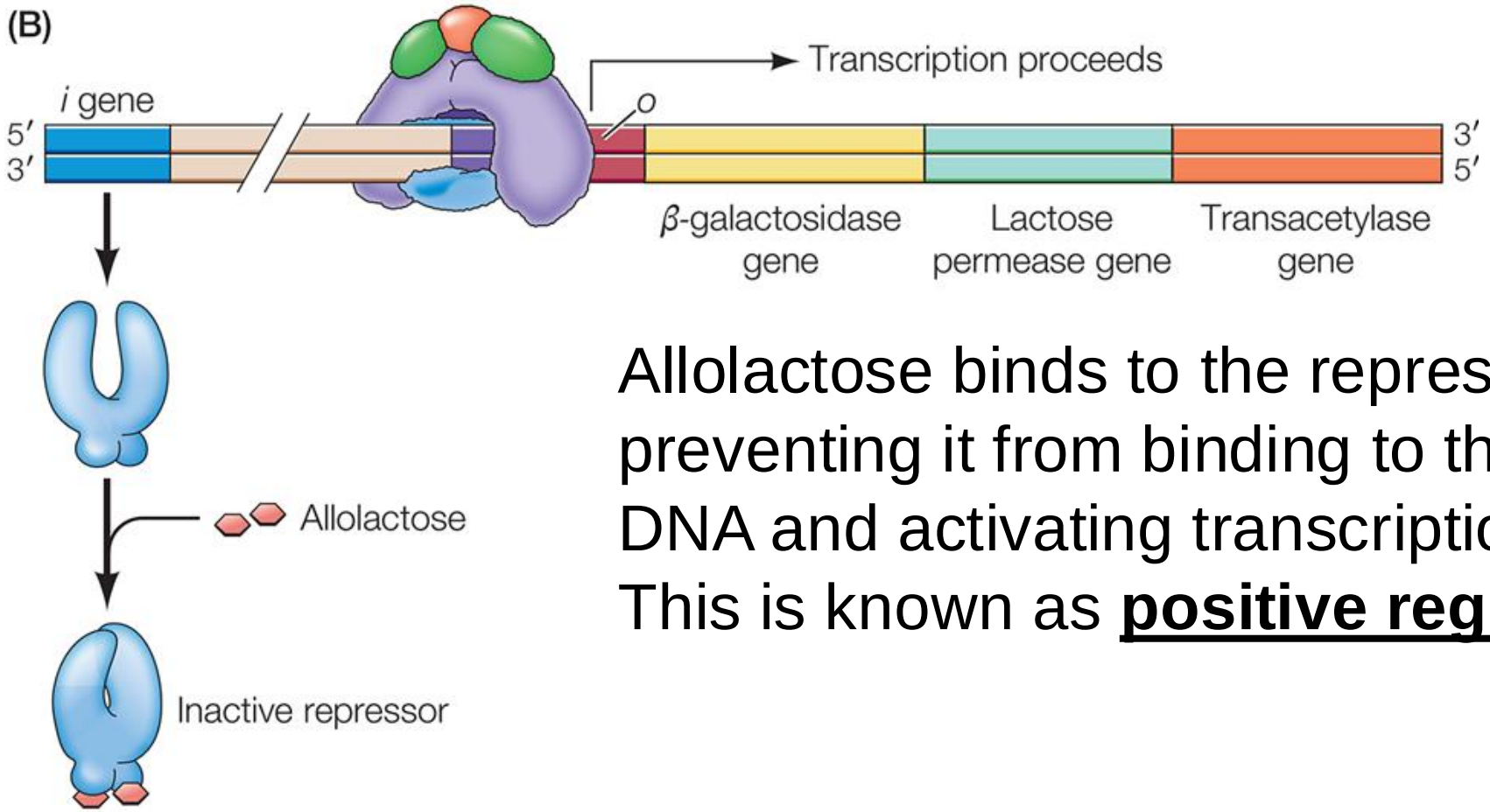


Lactose induces expression of the operon by the binding of allolactose to the repressor, which prevents the repressor from binding to the operator.

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

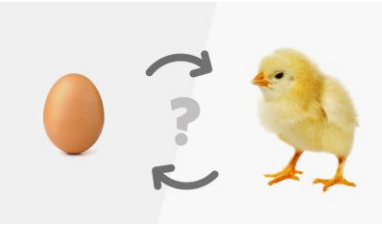
Presence of lactose allows the production of allolactose by isomerization, therefore increasing the formation of lac operon proteins which have an impact on increasing lactose metabolism, which really makes sense.

The role of allolactose



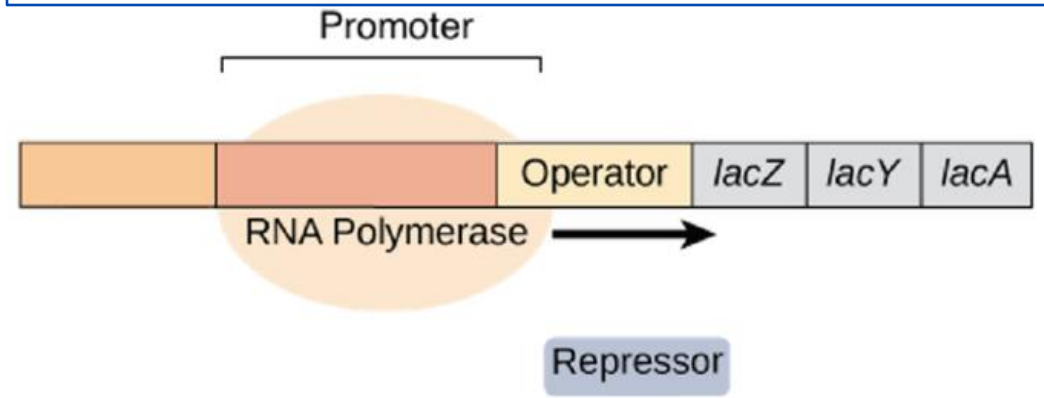
Allolactose binds to the repressor, thereby preventing it from binding to the operator DNA and activating transcription.
This is known as **positive regulation**.

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 β-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 β- cells of the Pancreas, but it is NEVER leaky in brain cells, because regulation is very tightly controlled .

Cis vs. trans regulatory elements

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

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

- Enhancers & silencers are cis acting elements.
- I gene (with its promoter = the whole sequence for transcription) is a trans regulatory element.

- 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.
  - 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).

Remember:

Factors are proteins.

Elements are DNA or RNA sequences.

Cis vs. **trans** regulatory elements – Con.

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

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

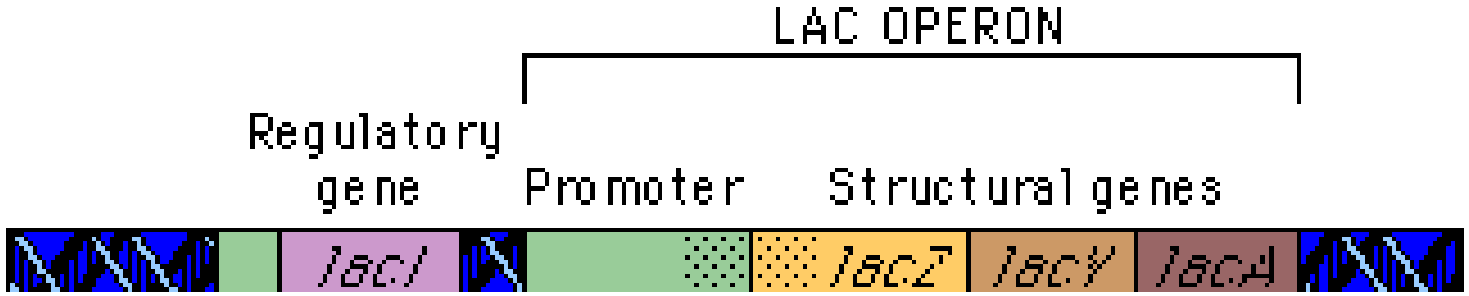
- Proteins (usually) like the repressor are called transacting factors because they can affect the expression of genes located on other chromosomes within the cell. They are produced from trans-acting elements (that is, genes). e.g.: I gene.
  - Mention other examples of trans-acting elements.
  - If 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

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

  –> the lac Operon is expressed even in the absence of lactose .
- Other mutations cause **non-inducible** or repressed expression (<u>always off</u>).
- Mention examples: mutations in: promoter region/ repressor (always bound) / I gene/ RNA polymerase.

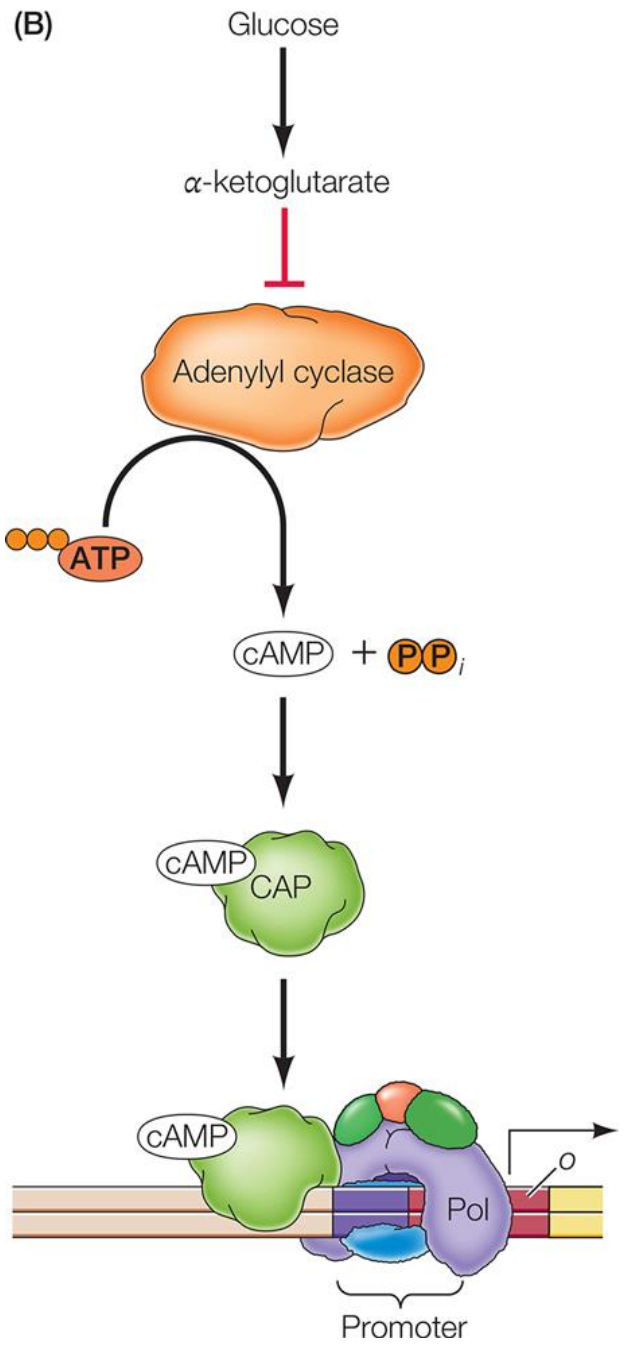
  –> the lac Operon is never transcribed even in the presence of lactose .



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**

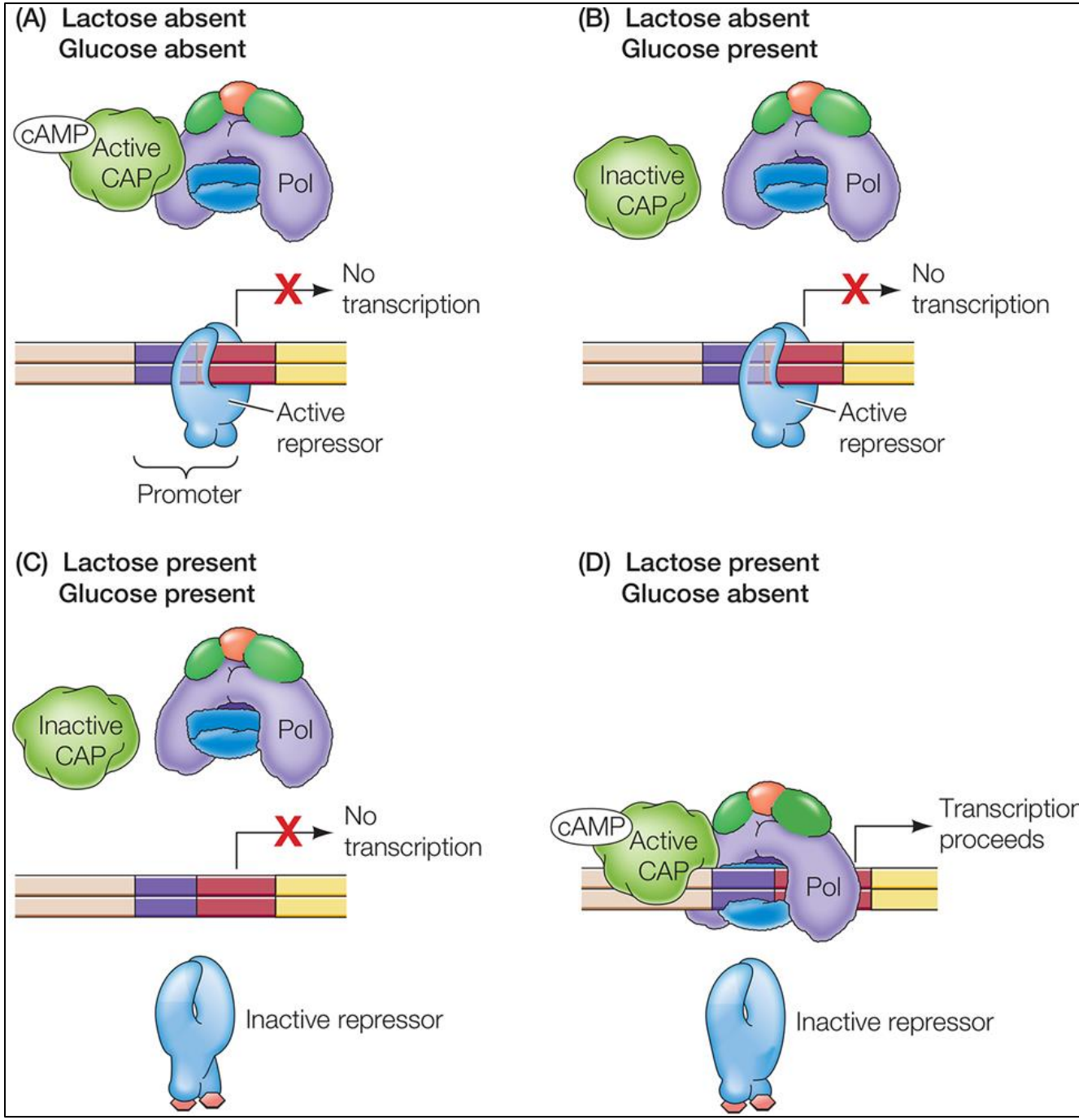
Glucose VS Lactose

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

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

So to ensure the lac operon transcription, two conditions must meet:

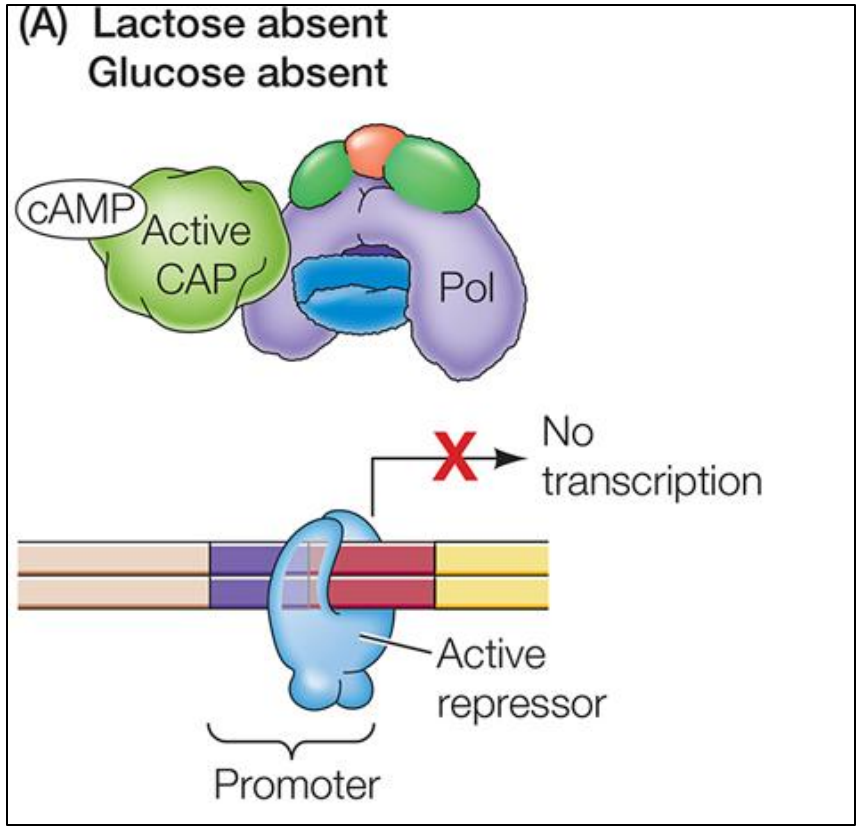
1. Unbound repressor
2. Active CAP



The four cases scenarios

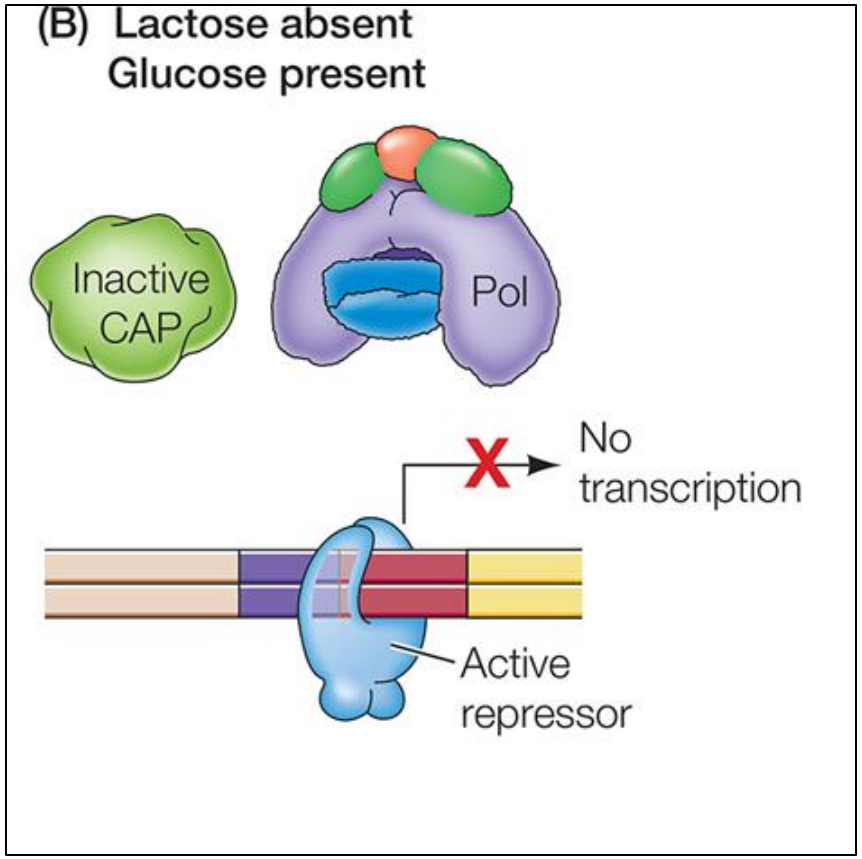
1) Lactose ✗
Glucose ✗

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 ✓

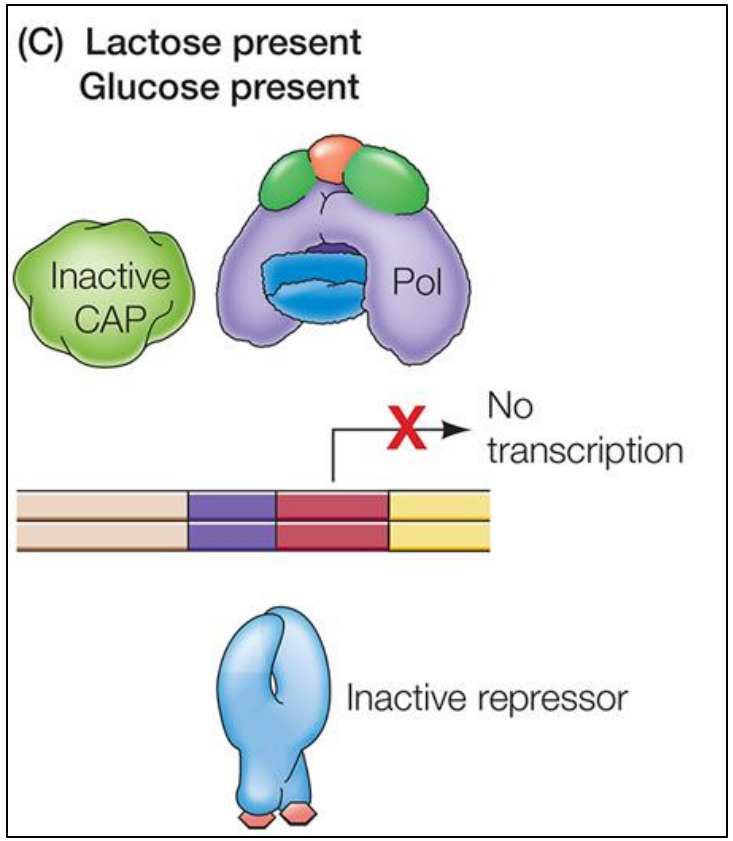
No expression of lac operon, CAP and RNA polymerase are not bound because there is no cAMP, and the repressor is active.



The four cases scenarios

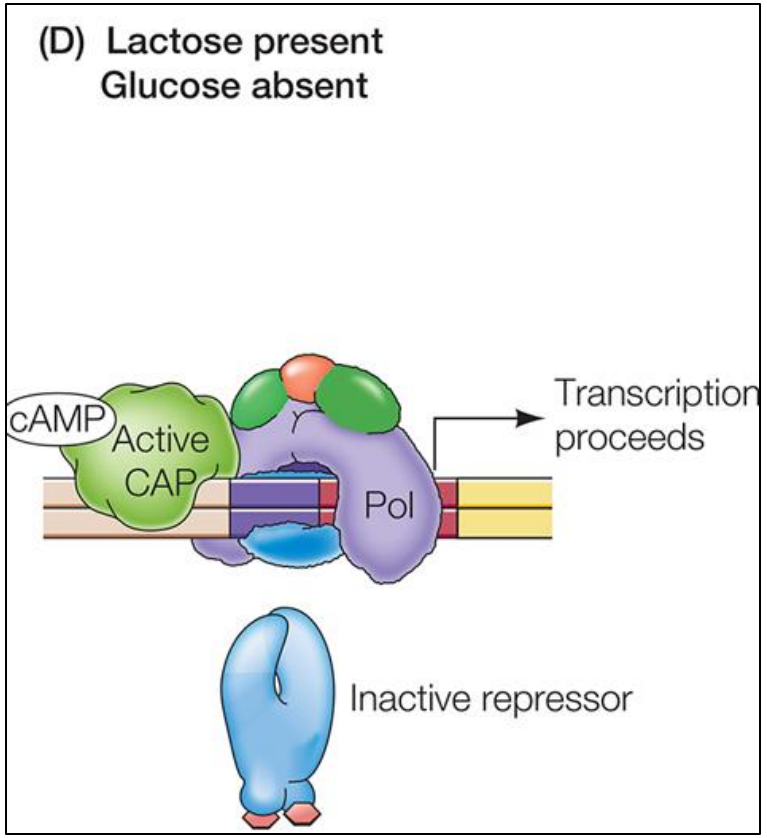
3) Lactose ✅
Glucose ✅

No expression of lac operon, why? CAP is inactive because there is no cAMP, although the repressor is unbound .
(the bacteria prefer glucose utilization in the presence of both)



4) Lactose ✅
Glucose ❌

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

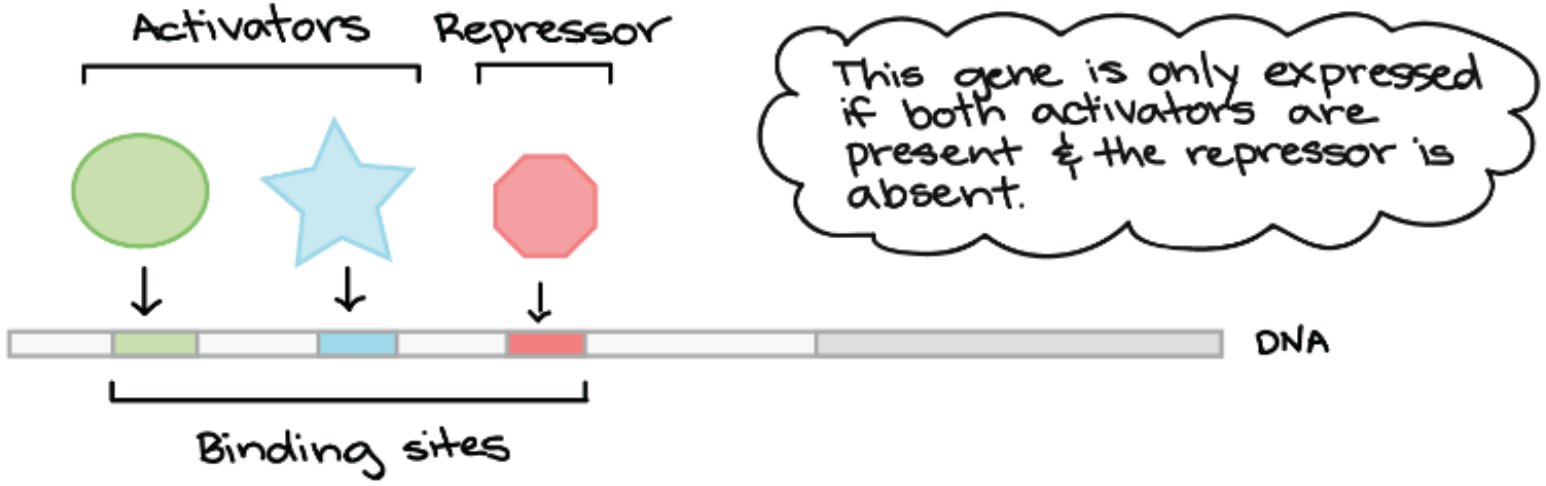


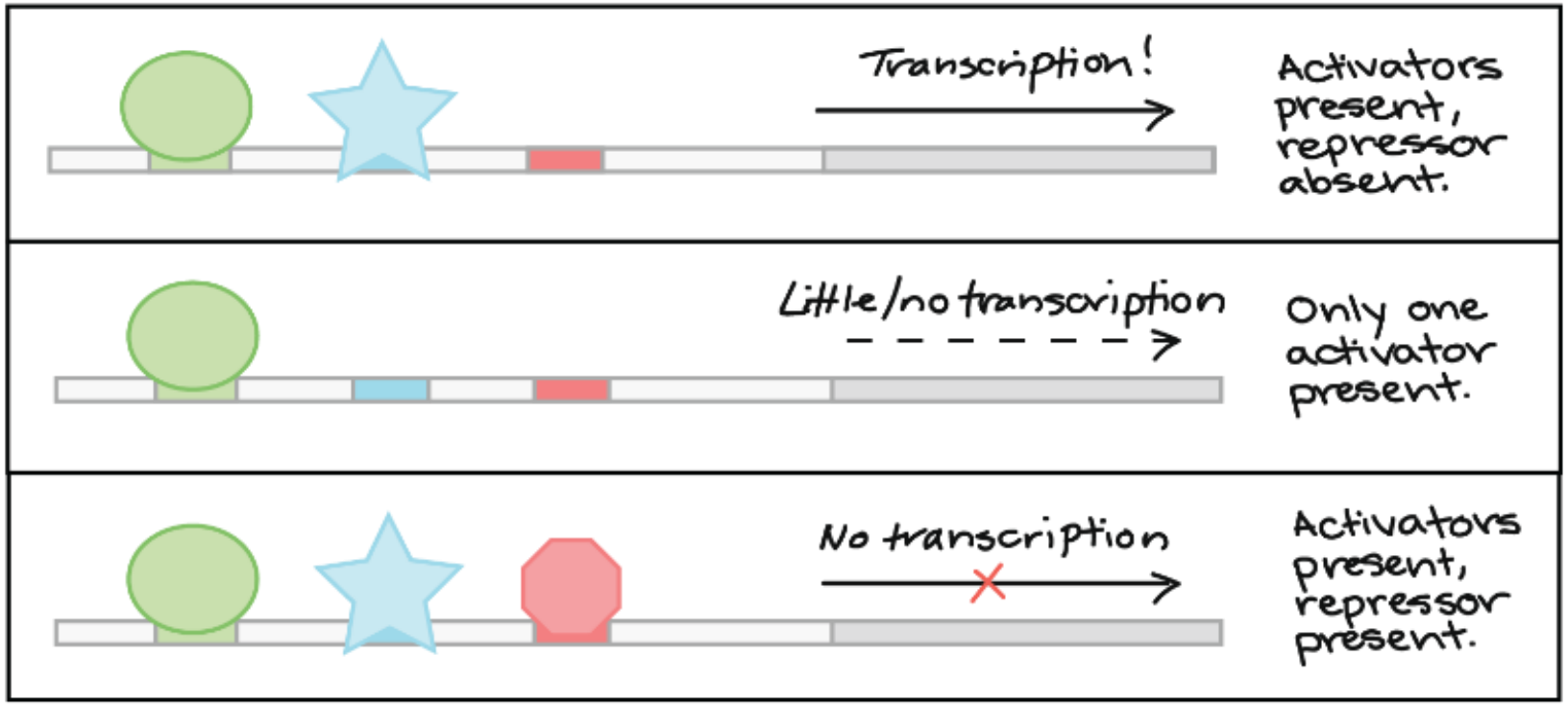
Regulation of transcription in eukaryotes

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

Cis-acting elements





Control whether the transcription is on or off or a gradient of activity

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.

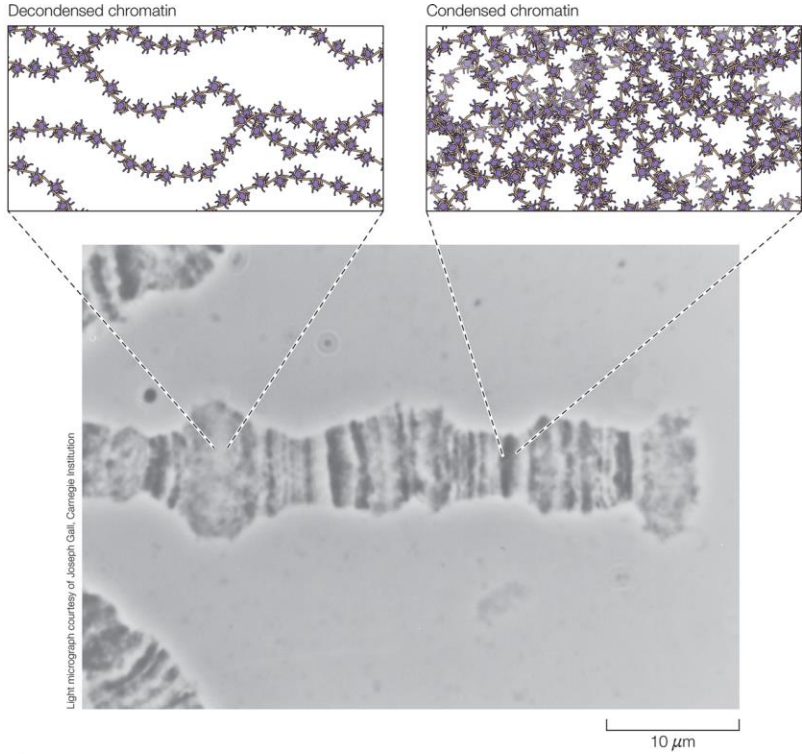
Modifying the DNA structure or chemistry without changing the sequence

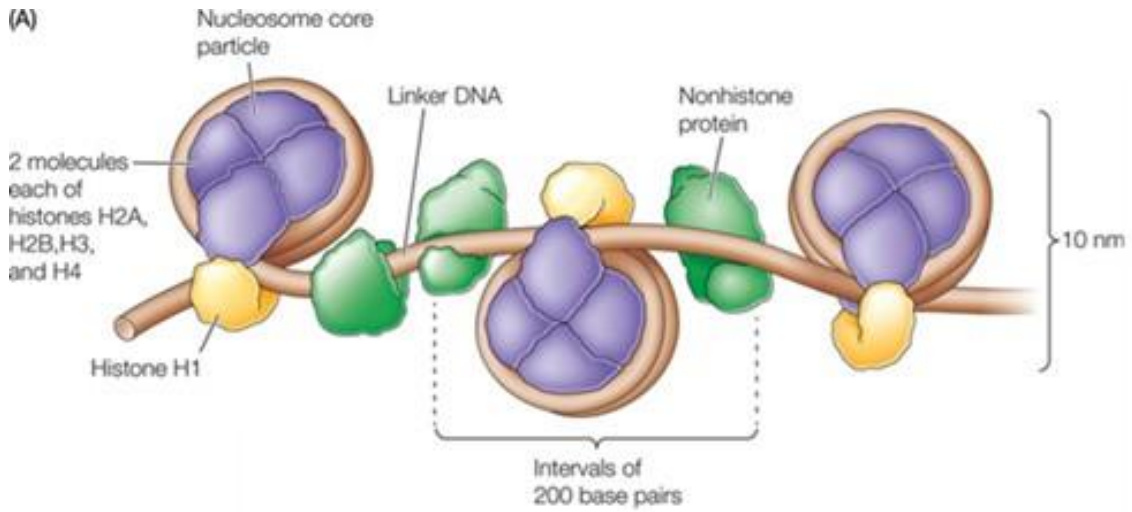
- Internal factor such as stress and mood
- External factors such as the sun exposure, nutrition

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)





Modulation of chromosomal structure

- Active genes exist in euchromatin.

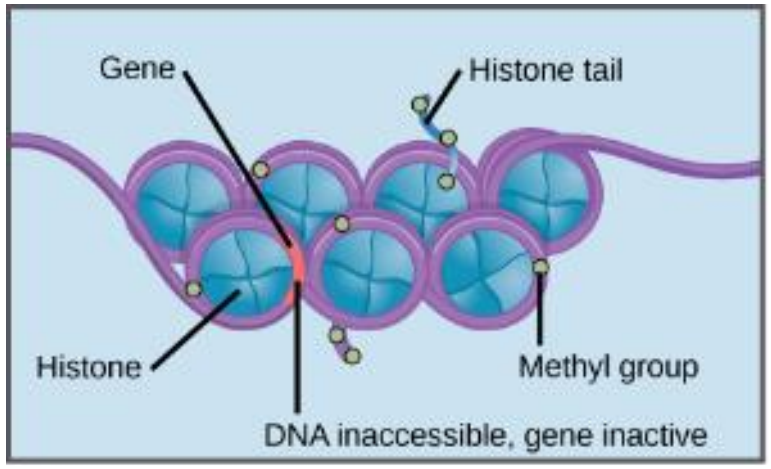
Accessible for transcription factor to easily find the DNA sequences.

- Inactive genes exist in heterochromatin.

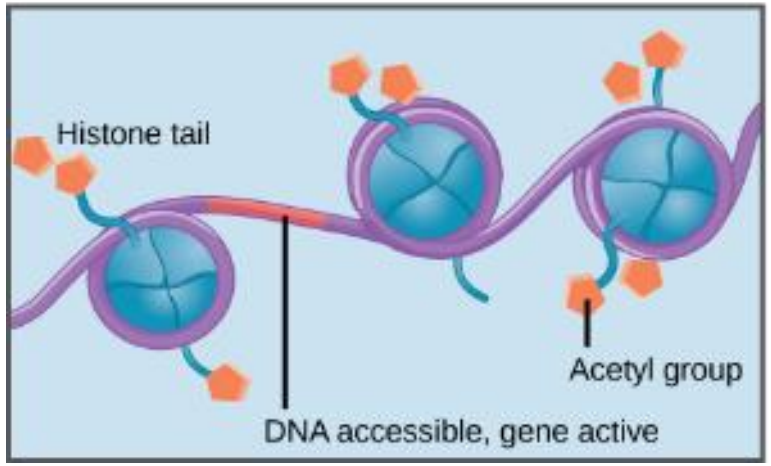
Inaccessible hidden DNA

- 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.

To allow DNA polymerase access to the DNA for synthesis, the DNA must detach from the histones. A similar process occurs for RNA polymerase during transcription.

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 → DNA contains cytosine bases, which can either be methylated or unmethylated. When cytosine is methylated, a small methyl group is added to it, but the actual DNA sequence remains unchanged
  4) Binding of noncoding RNAs to DNA

These four mechanisms will be further explained in the next lecture

For any feedback, scan the code or click on

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	25	• Internal factor such as when you eat something and it affects the regulatory elements in the promoter region • External factors such as the sun, your mood, stress	• Internal factor such as stress and mood • External factors such as the sun exposure, nutrition
V1 → V2			

رسالة من الفريق العلمي: Additional Resources:

- اللّهم علّمنا ما ينفعنا وانفعنا بما علّمتنا وزدنا علمًا وعملًا متقبّلا يا أكرم الأكرمين، أرِنا الحقّ حقّا وارزقنا اتّباعه وأرنا الباطل باطلًا وارزقنا اجتنابه، نسألك علم الخائفين منك وخوف العالمين بك.

تلك البلادُ استمدّت من حضارتنا ما أَبدعَته وأولته أَيادينا
فيها النفائسُ جاءت من صناعَتِنا ومن زراعتِنا صارت بساتينا

قال ابن القيم -رحمه الله-: "وقد أجمع عقلاء كل أمة على أن النعيم لا يدرك بالنعيم، وأن من آثر الراحة فاتته الراحة، وأن بحسب ركوب الأهوال واحتمال المشاق تكون الفرحة واللذة؛ فلا فرحة لمن لا هم له، ولا لذة لمن لا صبر له، ولا نعيم لمن لا شقاء له، ولا راحة لمن لا تعب له؛ بل إذا تعب العبد قليلا استراح طويلا، وإذا تحمل مشقة الصبر ساعة قاده لحياة الأبد، وكل ما فيه أهل النعيم المقيم صبر ساعة"