Parimal K. Khan
Department of Zoology
Patna University

Prokaryotic gene expression

- In the prokaryotic system, the expression of most genes is subject to the requirement of their products.
- These genes remain turned off when their products are not required and are switched on only when the gene products are needed by the cell.
- This regulated expression of genes in prokaryotes was first hypothesised by Jacob and Monod (1961) who named the unit of regulation “Operon”.
- An Operon is a group of genes that are located adjacent to one another in the genome and are functionally related in the sense that their products are involved in a single biochemical pathway. All such genes in an operon are expressed and regulated as a single unit.

- The expression of genes that yield enzymes involved in catabolic pathways such as in lactose and arabinose utilization is usually switched on in response to the presence of a specific substance (inducer) in their immediate surrounding. These are known as inducible genes.
- Contrary to this, some genes are switched off or their expression is repressed in the presence of a sufficient amount of their own protein products. For example, in the presence of optimal levels of tryptophan, the genes that encode for tryptophan are switched off. These are known as repressible genes. Repressible enzymes are often the components of anabolic pathways.
- Both induction and repression occur at the level of transcription.
- Lac operon in *E. coli* was the first operon to be discovered.

Positive and negative control of inducible and repressible systems

- Both of these systems involve the participation of regulator genes which encode products that regulate the expression of other genes.
- In the positive control system, the product of the regulator gene turns on the expression of the structural genes (genes involved in a metabolic pathway). The regulator protein is thus called an activator in this case.
- In the negative control system, the product of the regulator gene shuts off the expression of the structural genes. Here, the regulator protein is termed as a repressor.
- The regulator protein binds adjacent to the promoter of the structural genes at a site called regulator protein-binding site (RPBS).
- The binding of regulator protein to the RPBS depends upon the presence or absence of effector molecules which may serve as inducers or co-repressors.

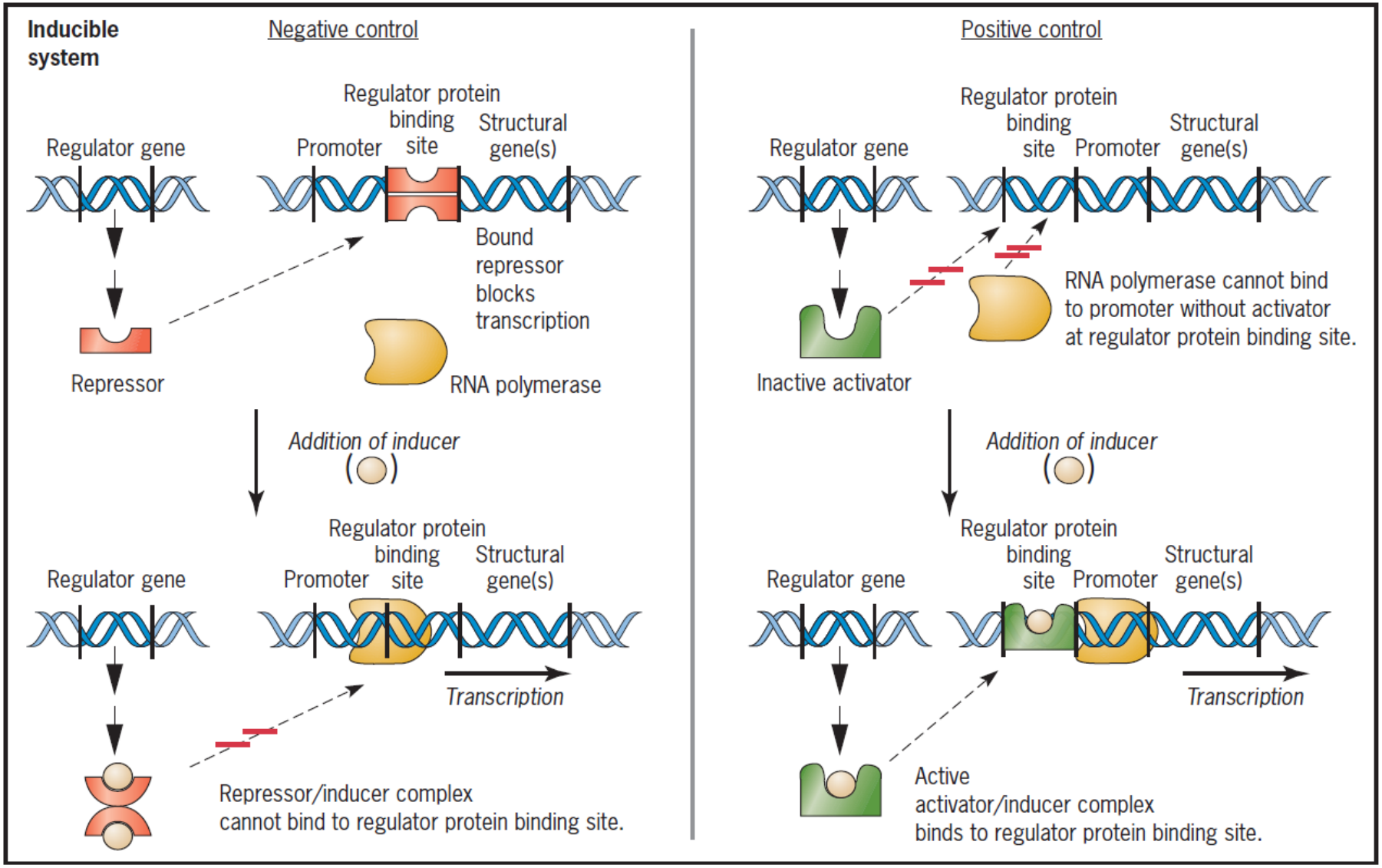


Image courtesy: Principles of Genetics, Snustad and Simmons

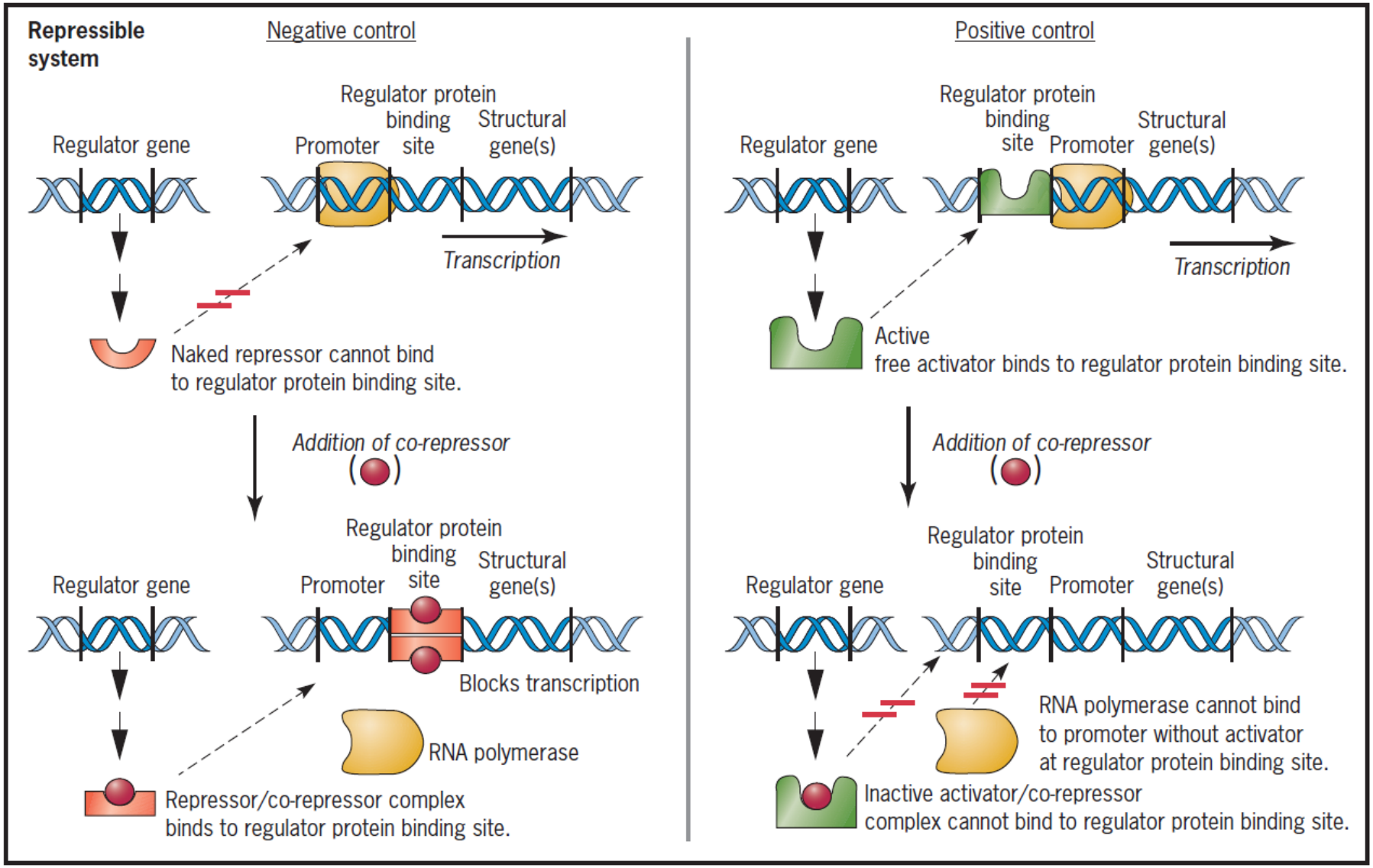
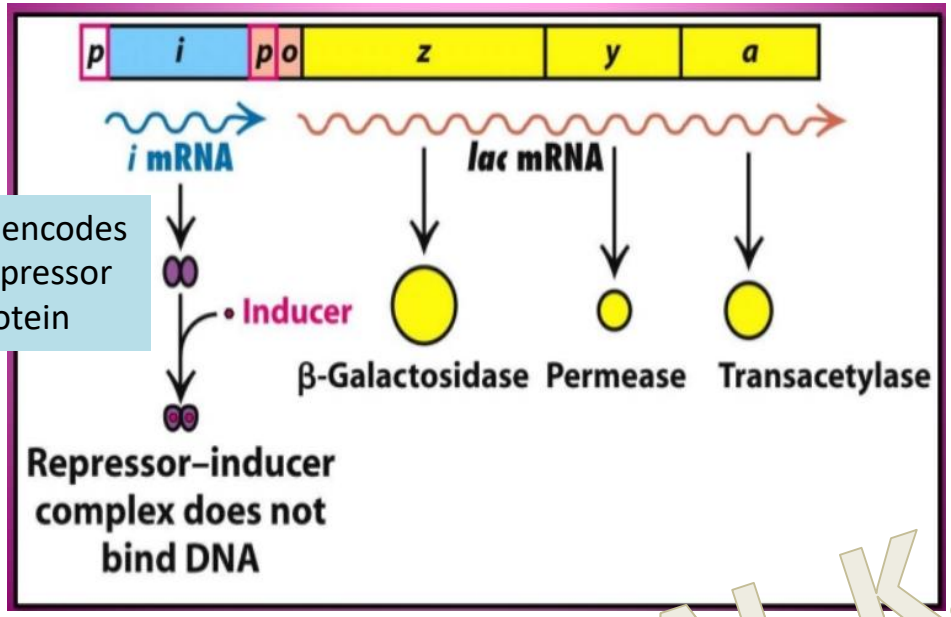
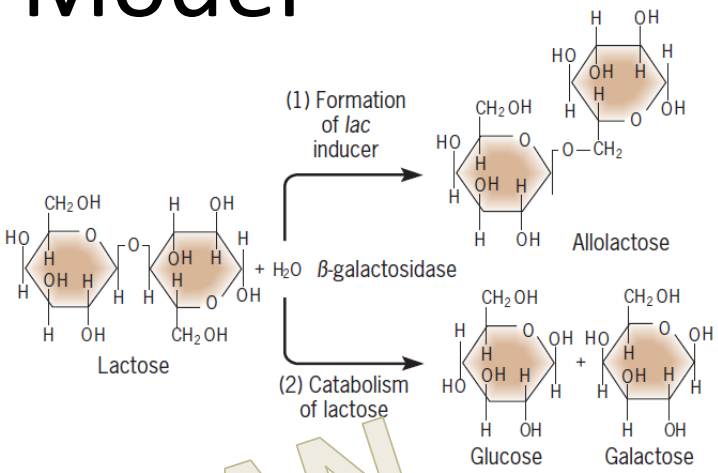


Image courtesy: Principles of Genetics, Snustad and Simmons

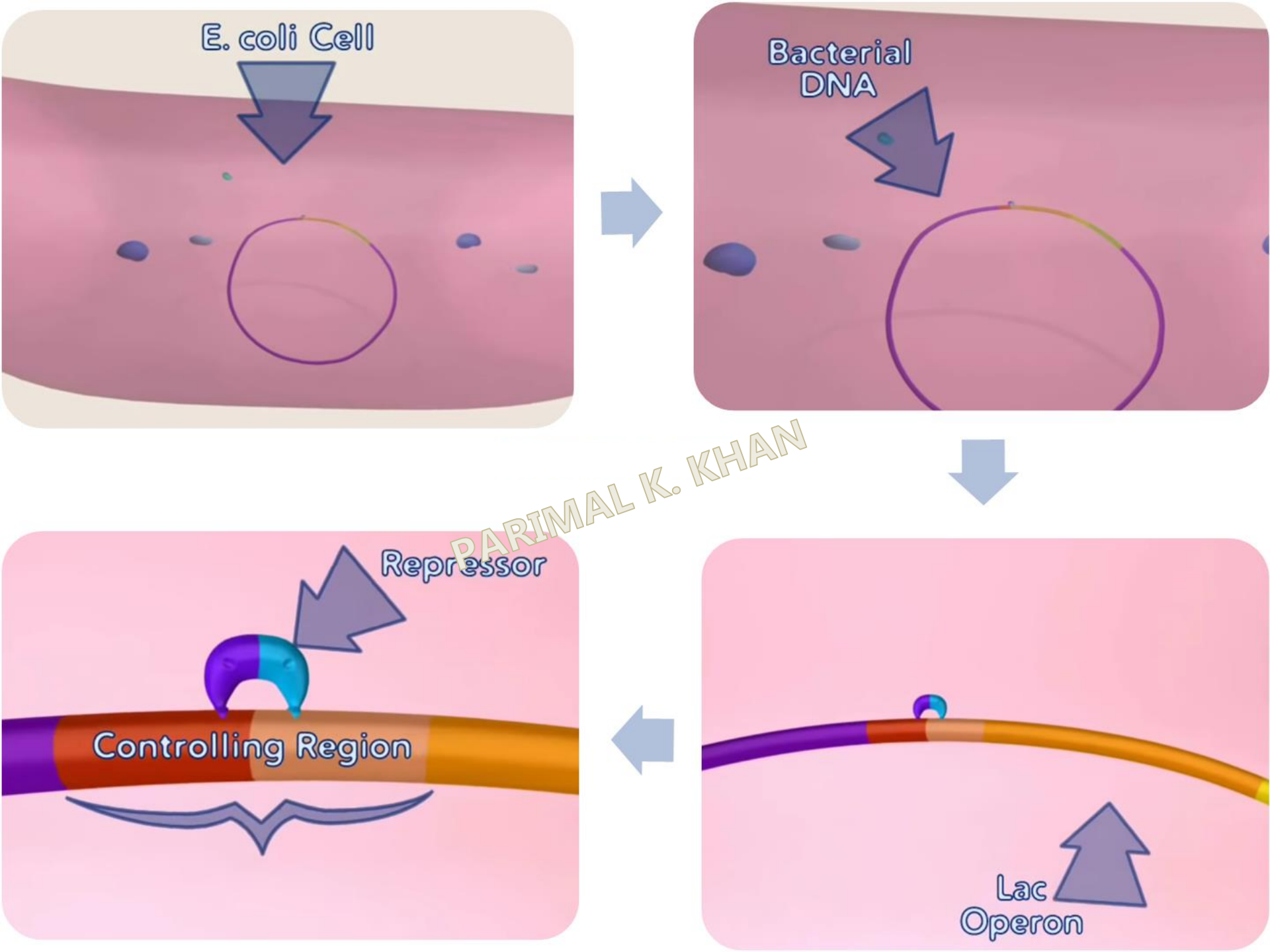
The Lac Operon Model



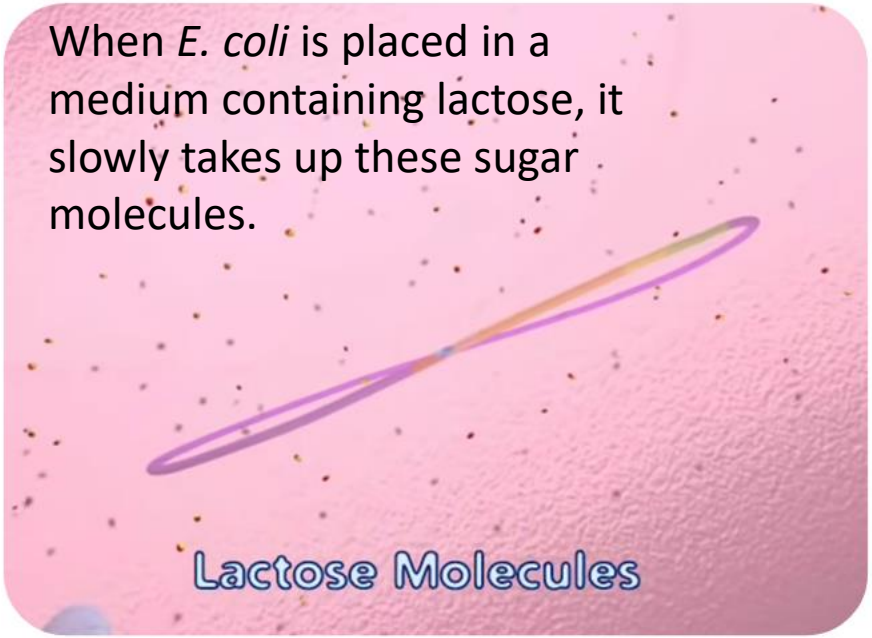
i gene encodes for repressor protein



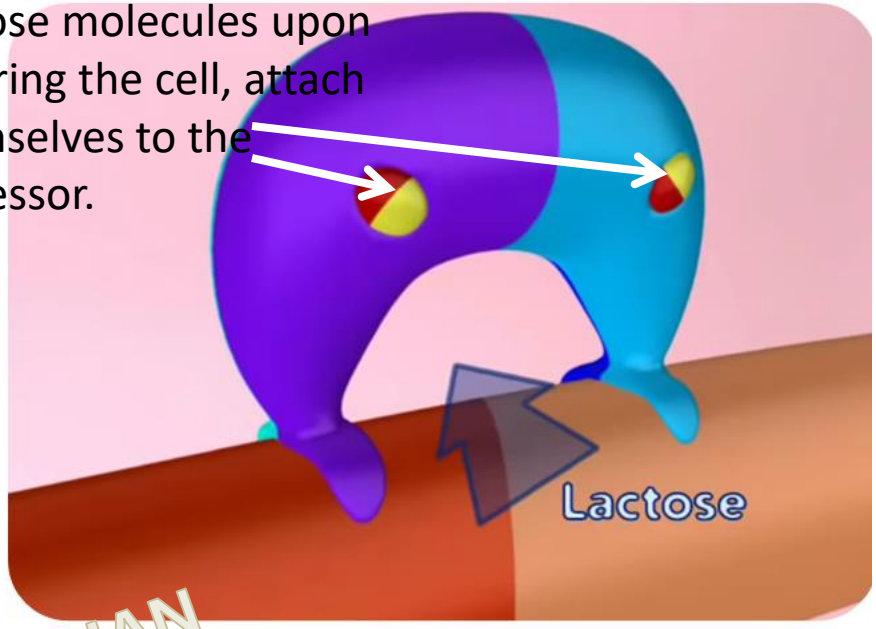
- The lac operon has 3 genes namely *lacZ, lacY* and *lacA* which encode the enzymes β-galactosidase, β-galactoside permease and β-galactoside transacetylase respectively.
- β-galactosidase breaks the disaccharide galactose into 1 molecule each of glucose and galactose. It is also responsible for changing lactose into allolactose, which specifically serves as the inducer.
- β-galactoside permease is a trans-membrane protein which transports lactose molecules from the medium into the cell.
- β-galactoside transacetylase removes toxic thiogalactosides which enter the cell along with the lactose molecules.



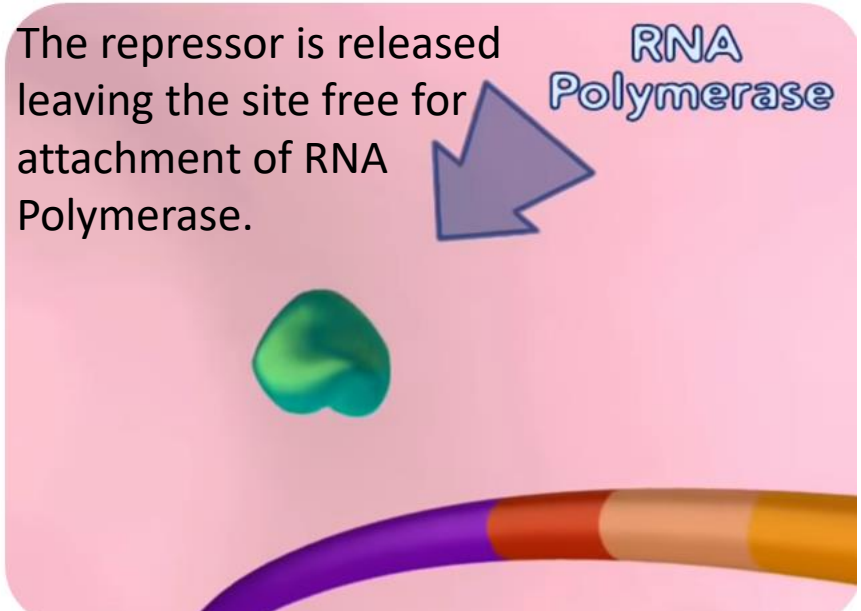
When *E. coli* is placed in a medium containing lactose, it slowly takes up these sugar molecules.



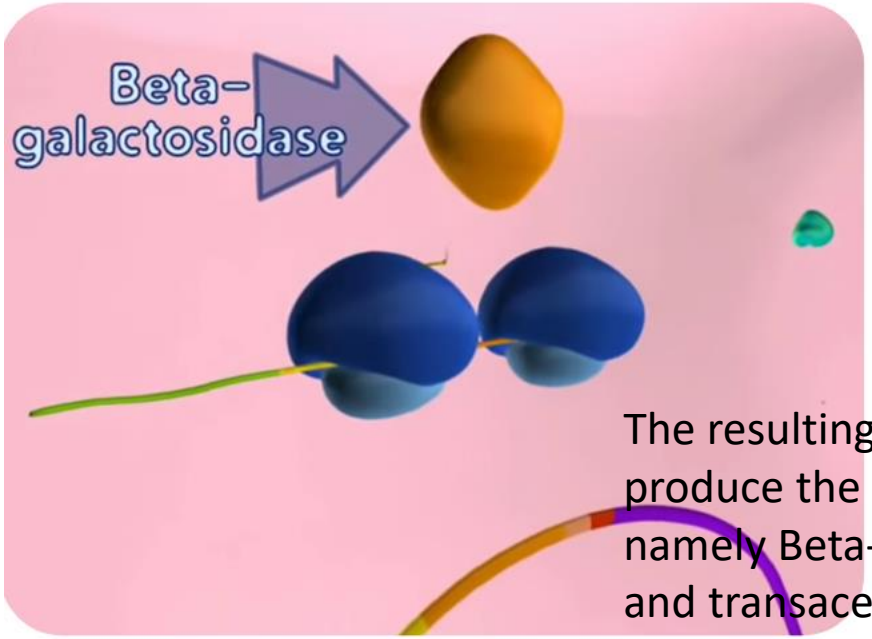
Lactose molecules upon entering the cell, attach themselves to the repressor.



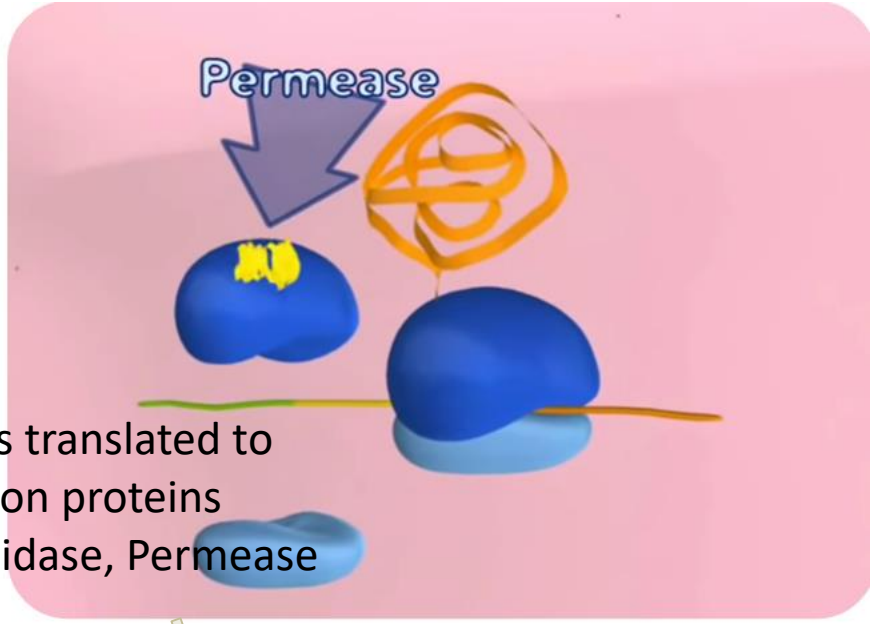
The repressor is released leaving the site free for attachment of RNA Polymerase.

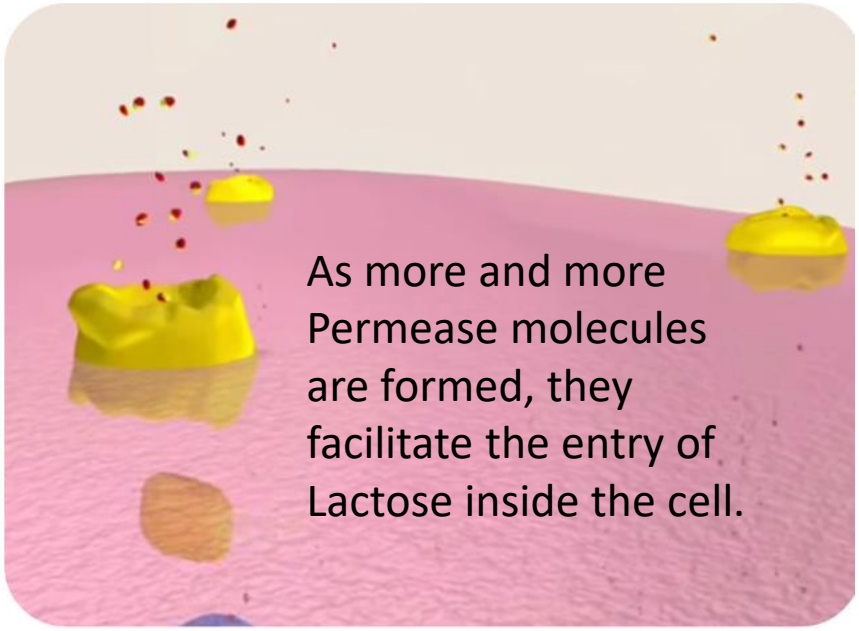


Transcription ensues.

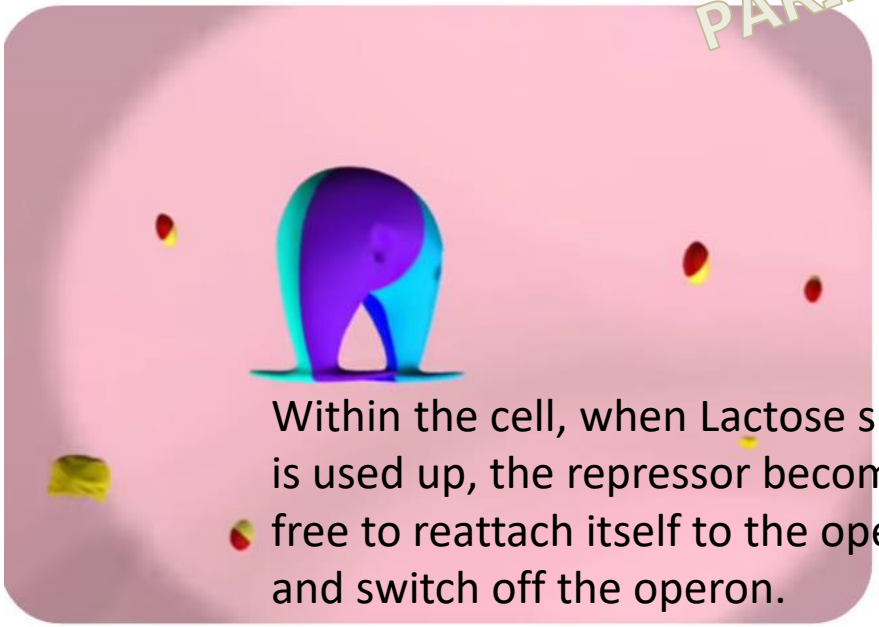


The resulting mRNA is translated to produce the Lac operon proteins namely Beta-galactosidase, Permease and transacetylase.





As more and more Permease molecules are formed, they facilitate the entry of Lactose inside the cell.

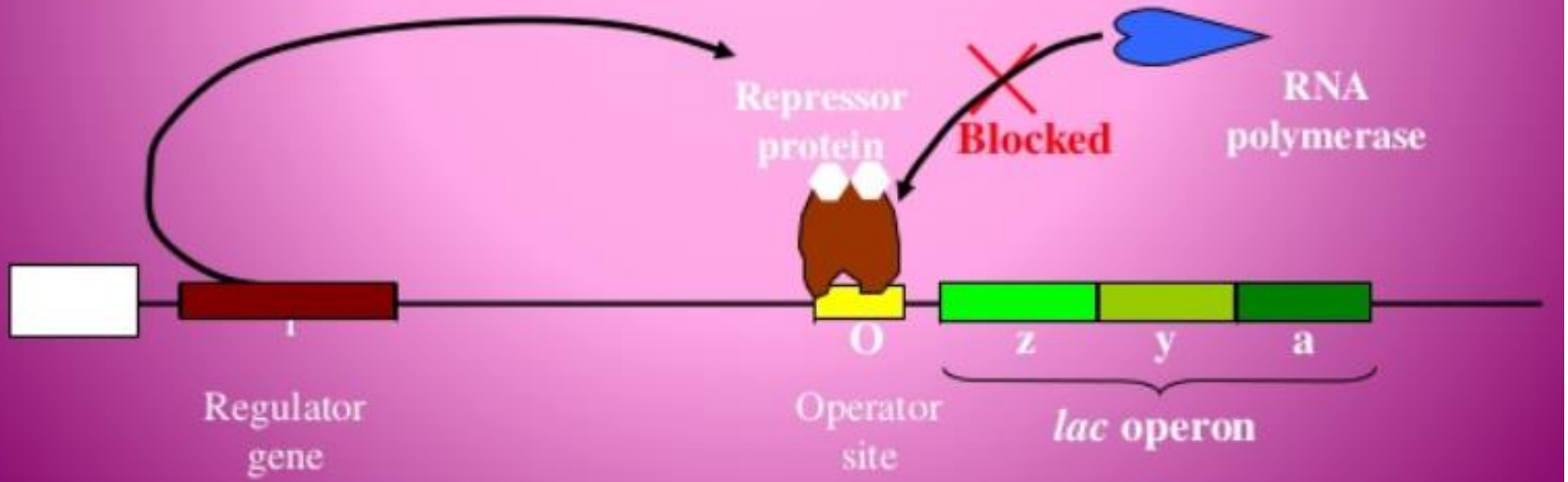


Within the cell, when Lactose supply is used up, the repressor becomes free to reattach itself to the operator and switch off the operon.

NEGATIVE CONTROL OF LAC OPERON

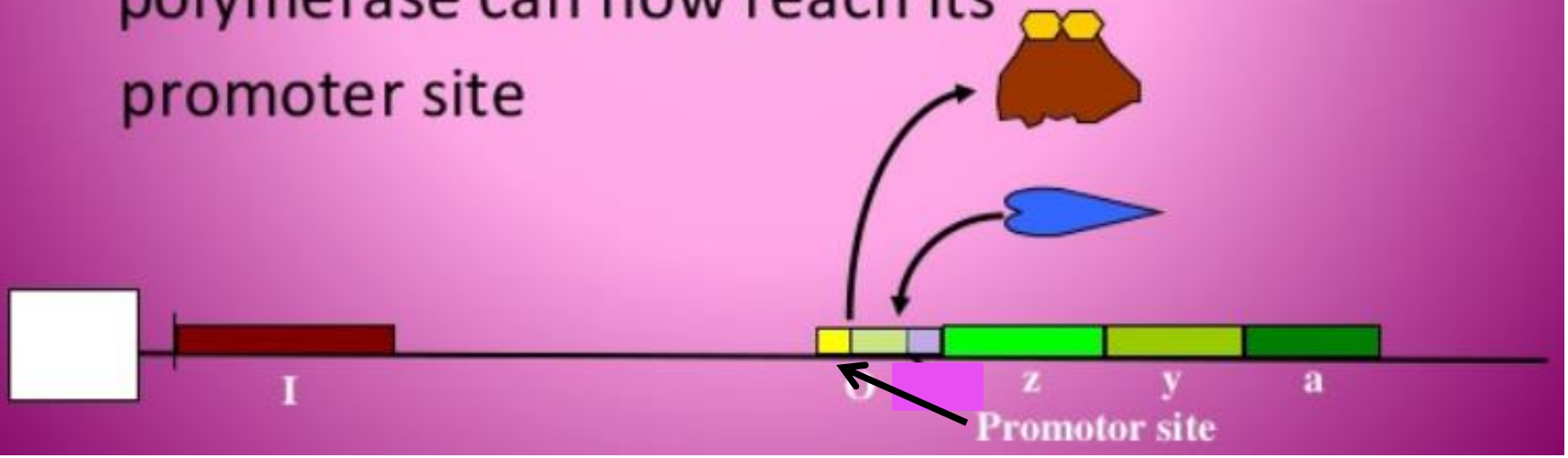
1. When lactose is absent

- A repressor protein is continuously synthesised. It sits on a sequence of DNA just in front of the *lac* operon, the **Operator site**
- The **repressor protein** blocks the **Promoter site** where the RNA polymerase settles before it starts transcribing



2. When lactose is present

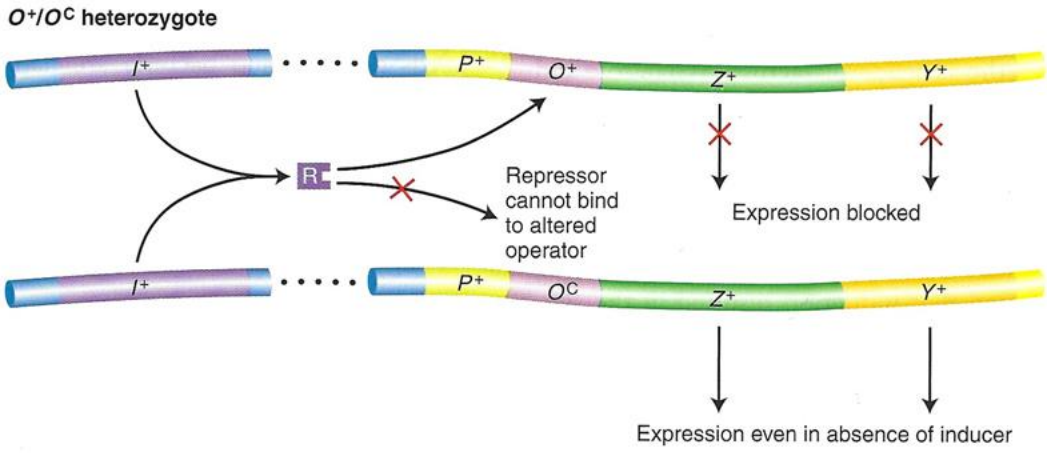
- A small amount of a sugar allolactose is formed within the bacterial cell. This fits onto the repressor protein at another active site (**allosteric site**)
- This causes the repressor protein to change its shape (a **conformational change**). It can no longer sit on the operator site. RNA polymerase can now reach its promoter site

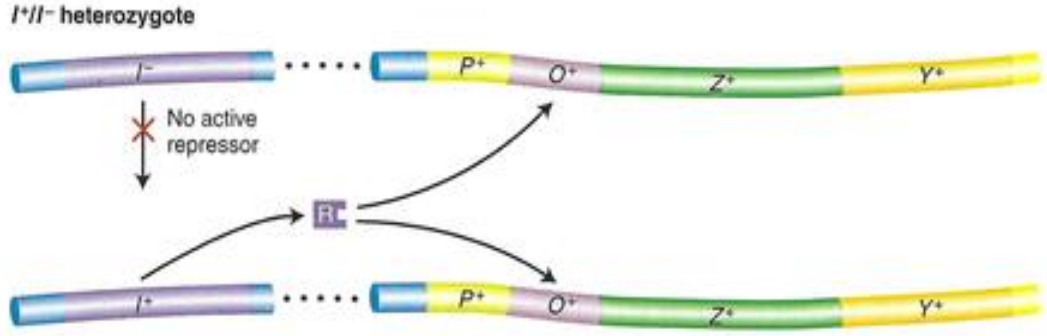


EFFECT OF MUTATION OF CONTROLLING ELEMENTS

- Jacob and Monod identified several mutants of *E. coli* in which expression of Lac operon had turned constitutive *i.e.* the enzymes encoded by the genes of lac operon are synthesized irrespective of the presence or absence of lactose.
- This suggested mutation in the control system of the operon. Two such mutations have been identified; the operator constitutive mutation (O^c) and mutation in the Regulatory gene (I^-).
- In partial diploids, the *cis/trans* arrangement of the operator (o) and the regulatory gene (i) control the expression of the structural genes of the operon.
- The operator was found to be functional only in *cis* condition or *cis* dominative. This means that the wild type operator must lie adjacent to the structural genes to be able to control their expression. In other words, the strand that contains the genes to be controlled/regulated, must contain the operator or switch for normal phenotype.
- the regulatory gene, however, is *trans* dominative and remains functional even when present on a separate strand. The regulatory gene may be present elsewhere in the genome and may still function.

Operators are cis-acting while Regulatory gene is trans-acting



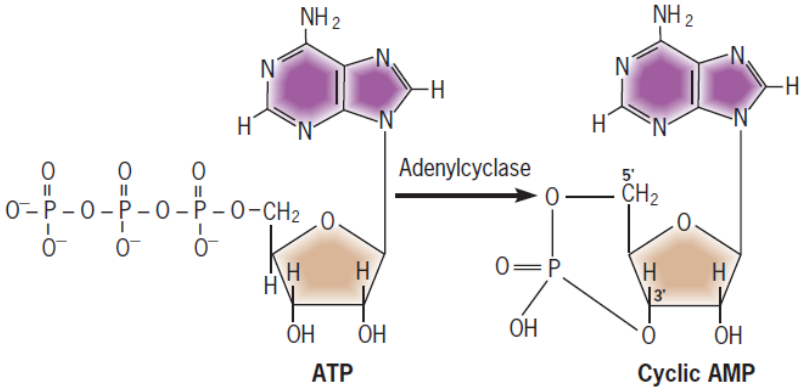


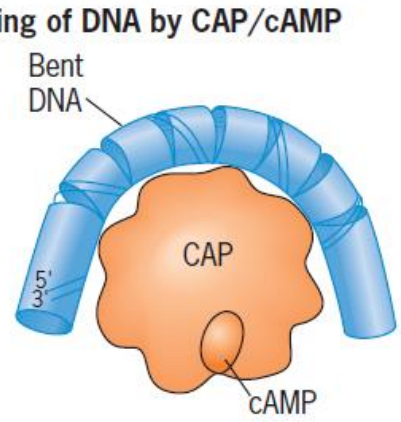
Some lac operon genotypes

Genotype	if lactose is not present then:	if lactose is present then:
$I^+ O^+ Z^+$	**No beta galactosidase**	**beta galactosidase produced**
$\frac{I^+ O^+ Z^-}{I^- O^+ Z^+}$	**No beta galactosidase**	**beta galactosidase produced**
$\frac{I^+ O^C Z^+}{I^+ O^+ Z^-}$	**beta galactosidase produced**	**beta galactosidase produced**

POSITIVE CONTROL OF LAC OPERON

- The removal of repressor from operator isn't always enough to activate the operon, rather an additional factor called activator is needed for positive control.
- This activator responds to low levels of glucose by stimulating transcription of lac operon.
- Alternatively, the presence of high levels of glucose molecules lead to a decline in the concentration of the activator.
- In this manner, *E. coli* ensures that in the availability of glucose (the preferred source of energy generation), the lac operon remains switched off.
- The activator in this case is cAMP which along with a Catabolite Activator Protein (CAP) binds to DNA just upstream to the promoter. This leads to the recruitment of RNA Polymerase to the promoter site leading to the formation of an open promoter complex which facilitates transcription.





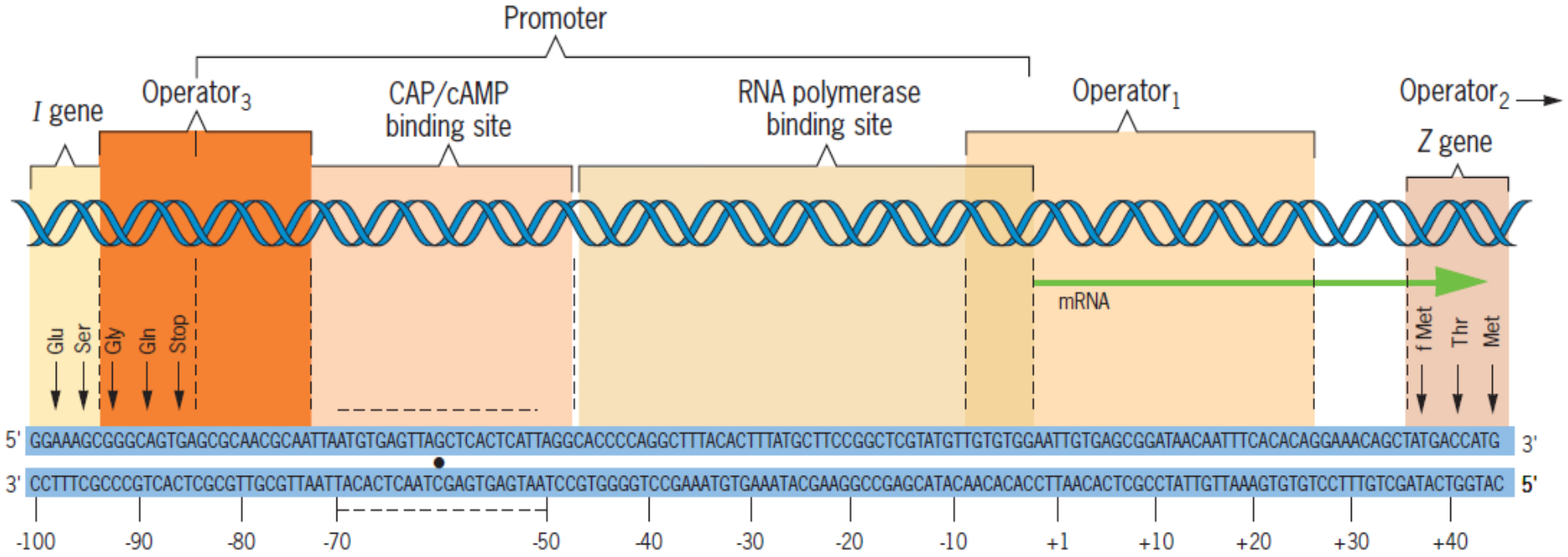
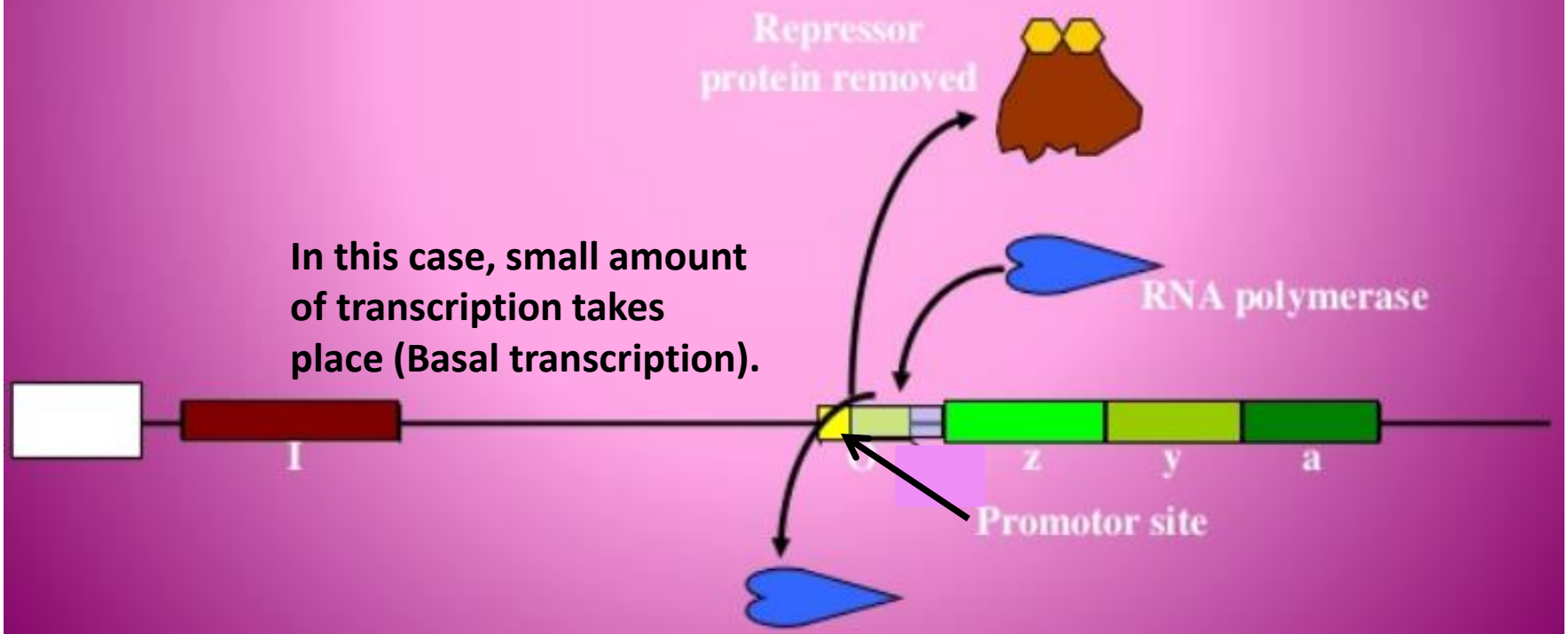


Image courtesy: Principles of Genetics, Snustad and Simmons

3. When both glucose and lactose are present

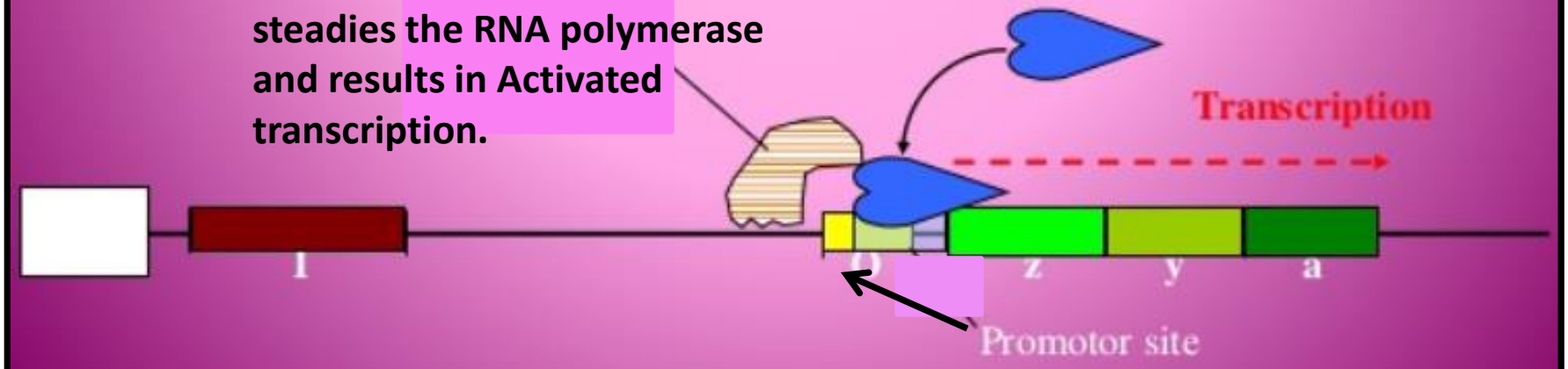
- When glucose and lactose are present RNA polymerase can sit on the promoter site but it is unstable and it keeps falling off.

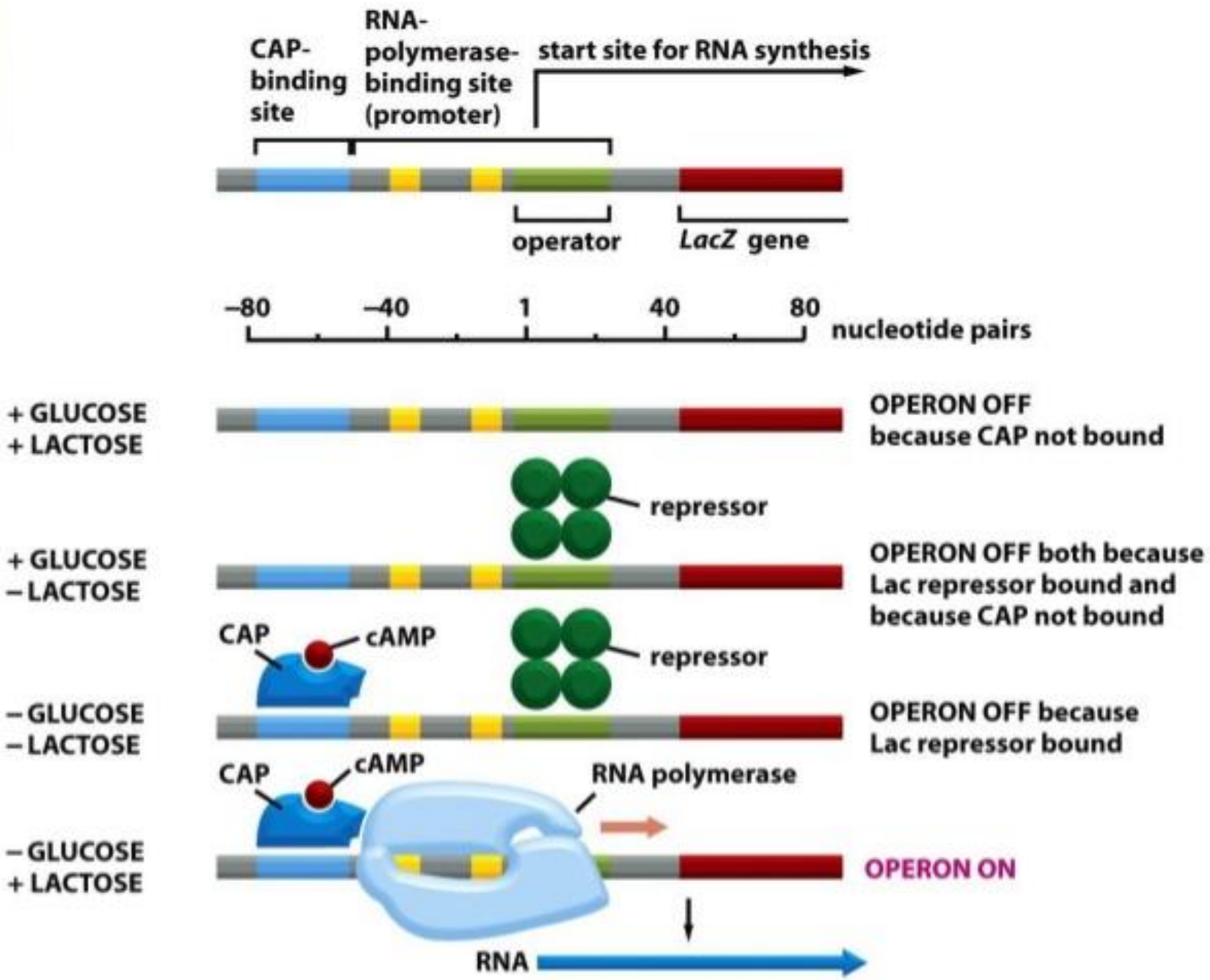


4. When glucose is absent and lactose is present

- Another protein is needed, an **activator protein**. This stabilises RNA polymerase.
- The activator protein only works when glucose is absent
- In this way *E. coli* only makes enzymes to metabolise other sugars in the absence of glucose

The CAP-cAMP complex formed when glucose is absent steadies the RNA polymerase and results in Activated transcription.




CAP-binding site
RNA-polymerase-binding site (promoter)
start site for RNA synthesis
operator
LacZ gene
–80
–40
1
40
80
nucleotide pairs
+ GLUCOSE
+ LACTOSE
OPERON OFF because CAP not bound
repressor
+ GLUCOSE
– LACTOSE
OPERON OFF both because Lac repressor bound and because CAP not bound
CAP
cAMP
repressor
– GLUCOSE
– LACTOSE
OPERON OFF because Lac repressor bound
CAP
cAMP
RNA polymerase
– GLUCOSE
+ LACTOSE
OPERON ON
RNA

Thank you

PARIMAL K. KHAN

Image courtesy: Google images