THE *lac* OPERON

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INTRODUCTION

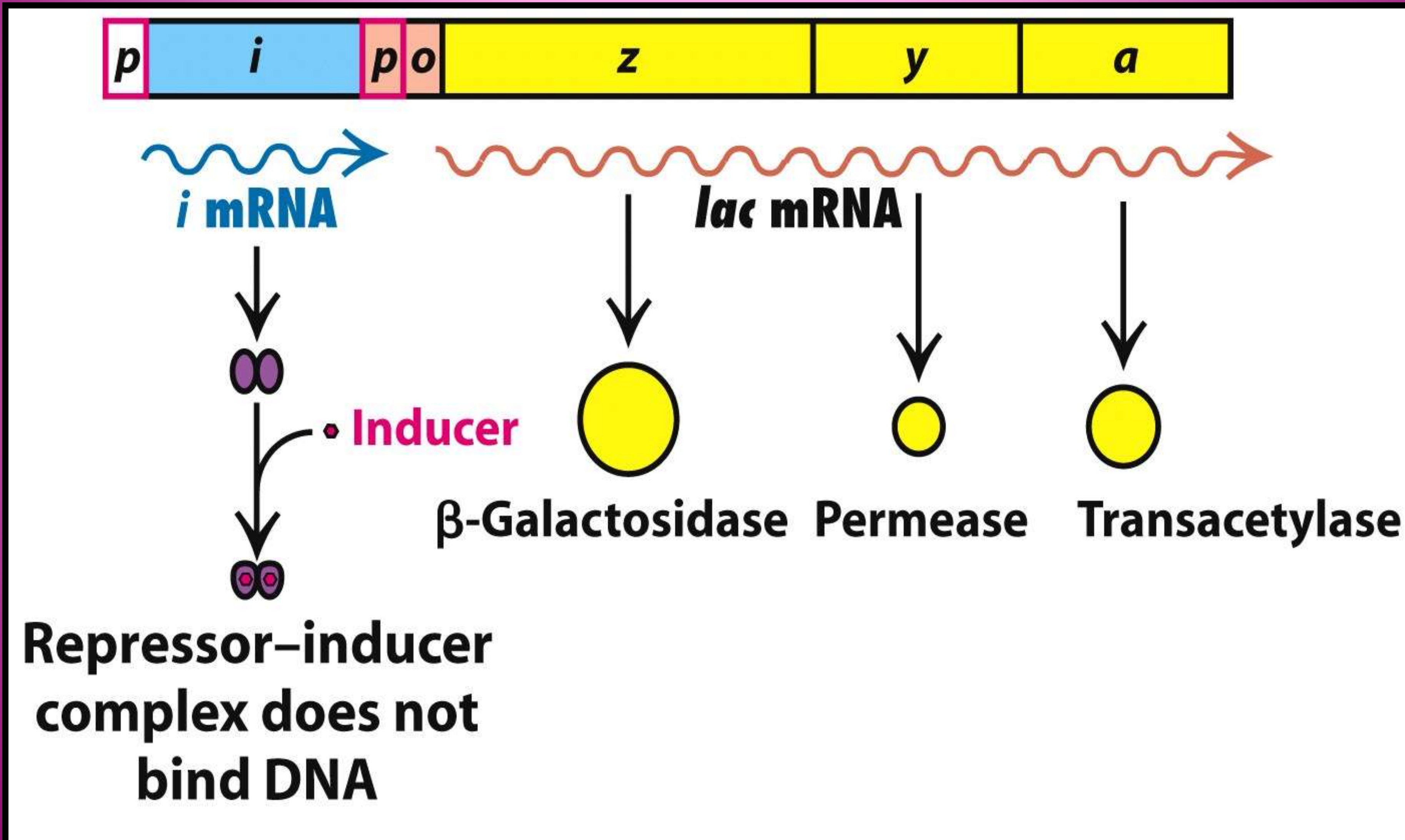
- *Operon* is operating units which can be defined as the cluster of genes located together on the chromosomes & transcribed together.
- It is group of closely linked *structure genes* & associated *control gene* which regulate the metabolic activity.
- All the genes of an operon are coordinately controlled by a mechanism 1st described in *1961* by *Francois Jacob & Jaques Monod* of the Pasture institute of Paris.

Jacob, Monod & Lwoff

The *lac* operon

- The lactose operon designated as *lac operon*.
- The lac operon codes for enzymes involved in the catabolism (degradation) of lactose.
- lactose is the disaccharide which is made up of glucose & galactose.
- It is the inducible operon since the presence of lactose induce the operon to switched on.

Operon model

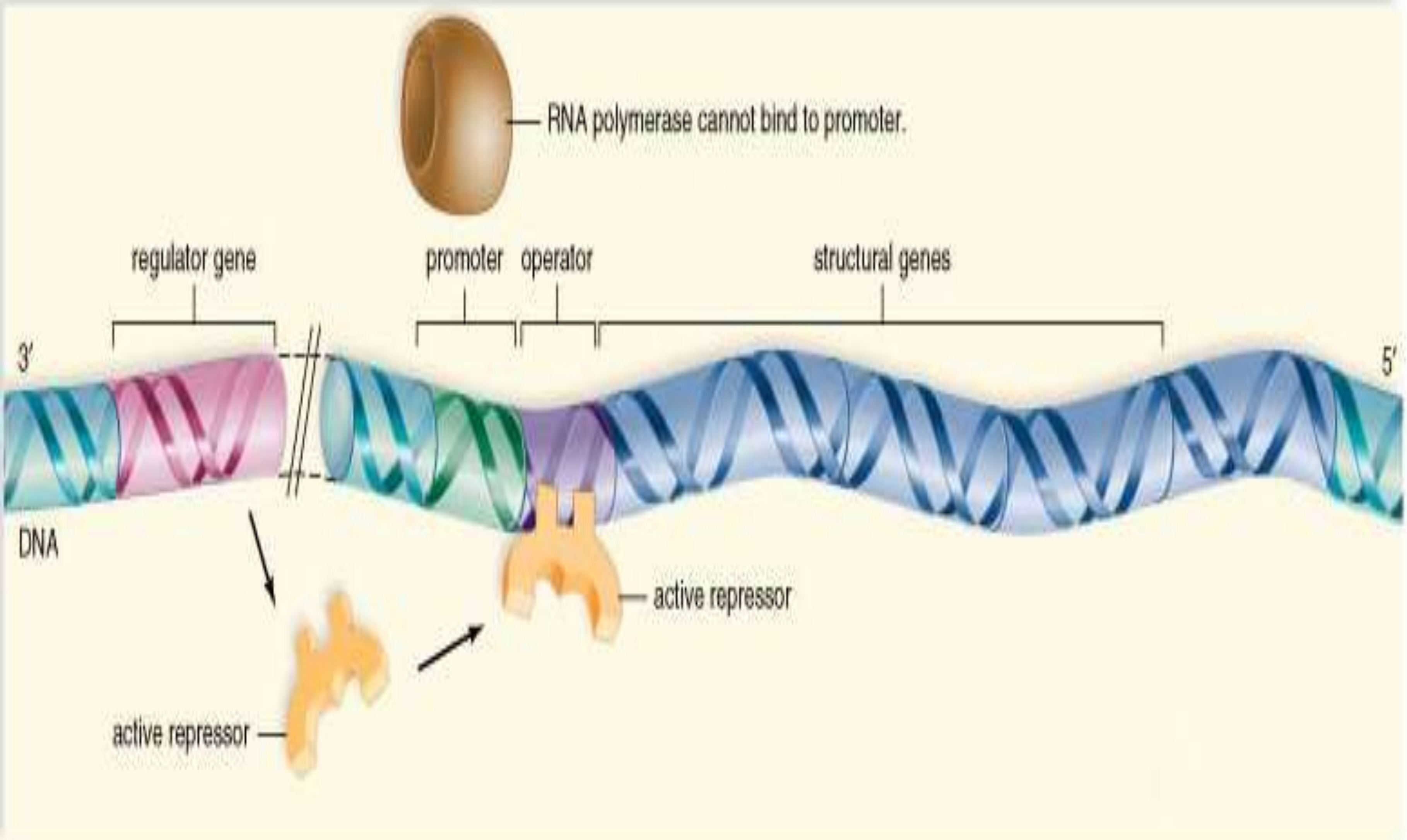


Designation of gene	Codes for enzyme	Function of the enzyme
lac Z	β-galactosidase	Breaks down lactose into glucose & galactose.
lac y	galactose permease	This protein, found in the E.coli cytoplasmic membrane, actively transports lactose into the cells
lac a	Thio-galactoside trans acetylase	The function of this enzyme is not known. It is coded for by the gene lacA.

Element	**Purpose**
Operator (lacO)	Binding site for repressor
Promoter (lacP)	Binding site for RNA Polymerase
Repressor	Gene encoding the lac repressor protein. Binds to DNA at the operator & blocks binding of RNA Polymerase at the promoter.
lacI	Controls production of the repressor protein

FUNCTIONING OF *LAC* OPERON

- In the absence of lactose(inducer), the regulator gene produce a repressor protein which bind to the operator site & prevent the transcription as a result, the structural gene do not produce mRNA & the proteins are not formed.

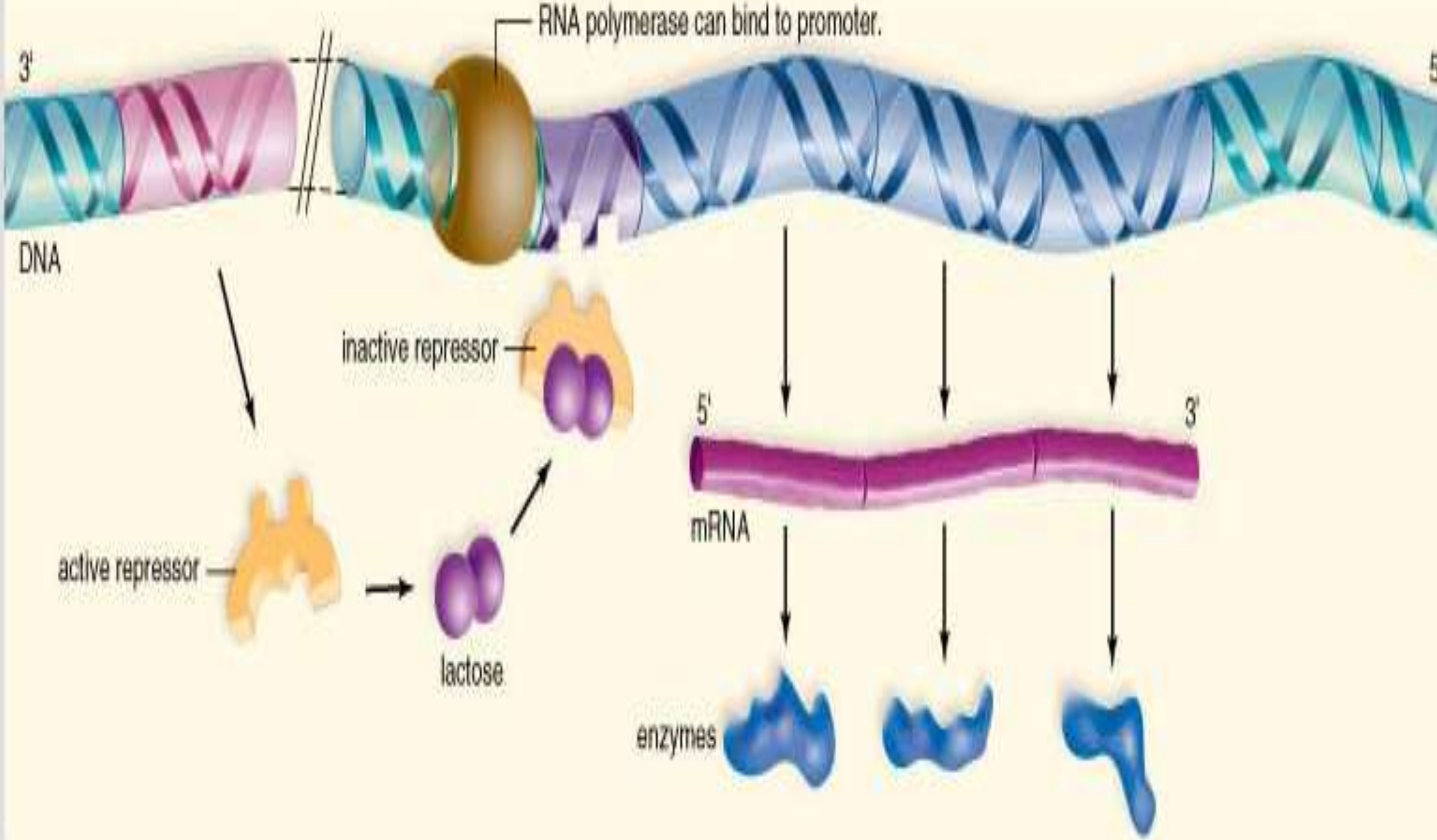


a. **Lactose absent.** Enzymes needed to take up and use lactose are not produced.

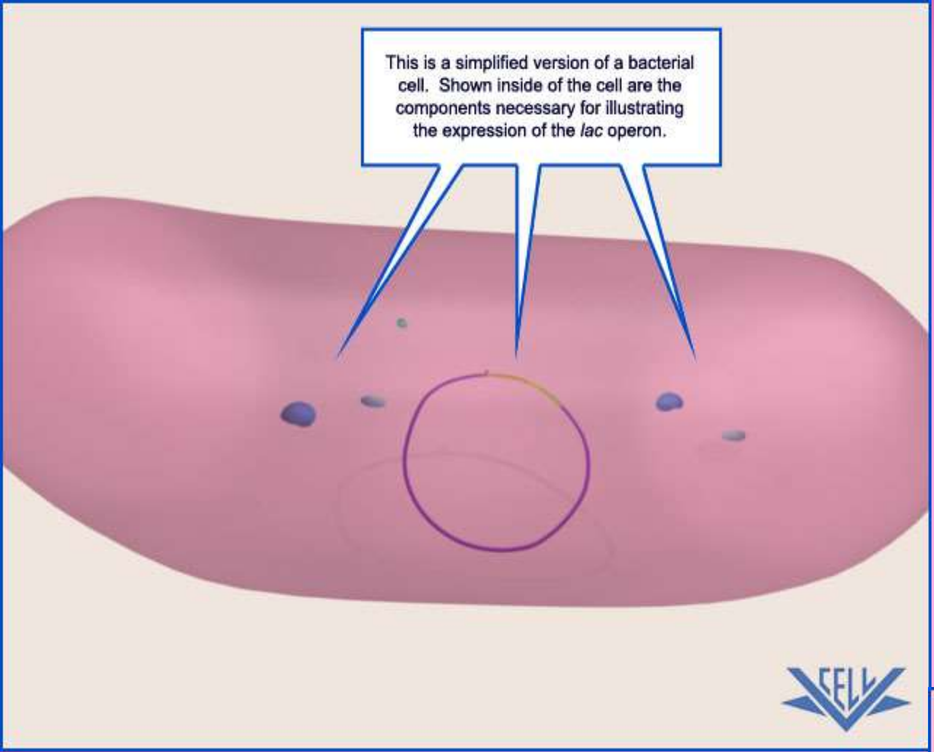
- When lactose(inducer), introduce in the medium, binds to the repressor the repressor now fails to binds to the operator.

- Therefore the operoter is made free & induces the RNA polymerase to bind to the initiation site on promoter which results in the synthesis of *lac* mRNA.

- This mRNA codes for three enzyme necessary for lactose catabolism.

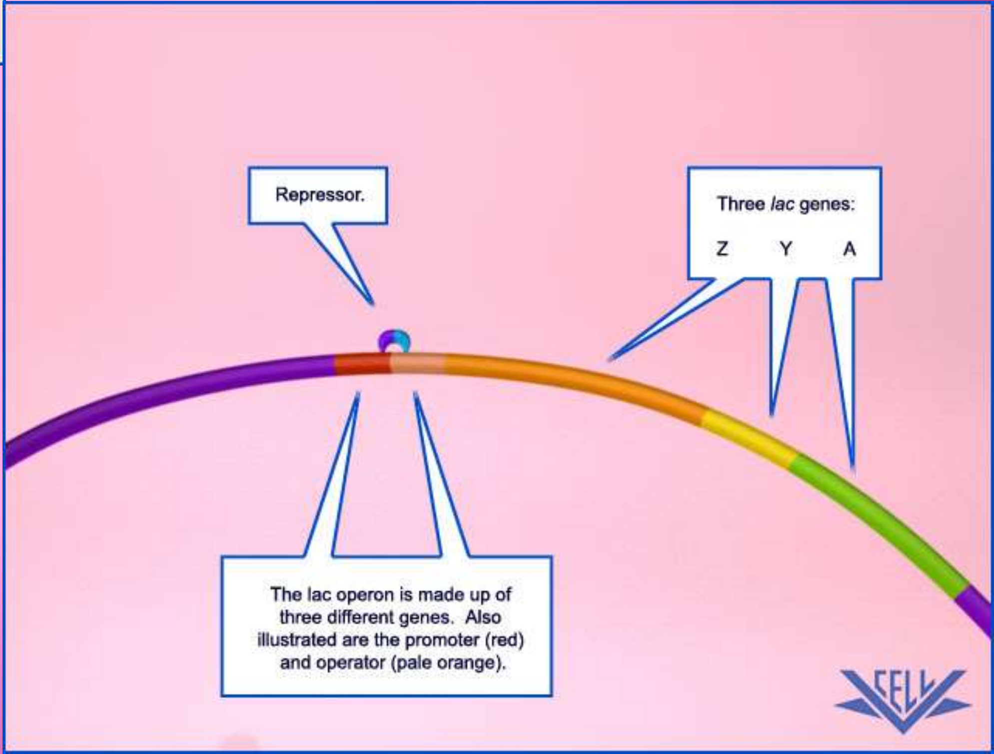


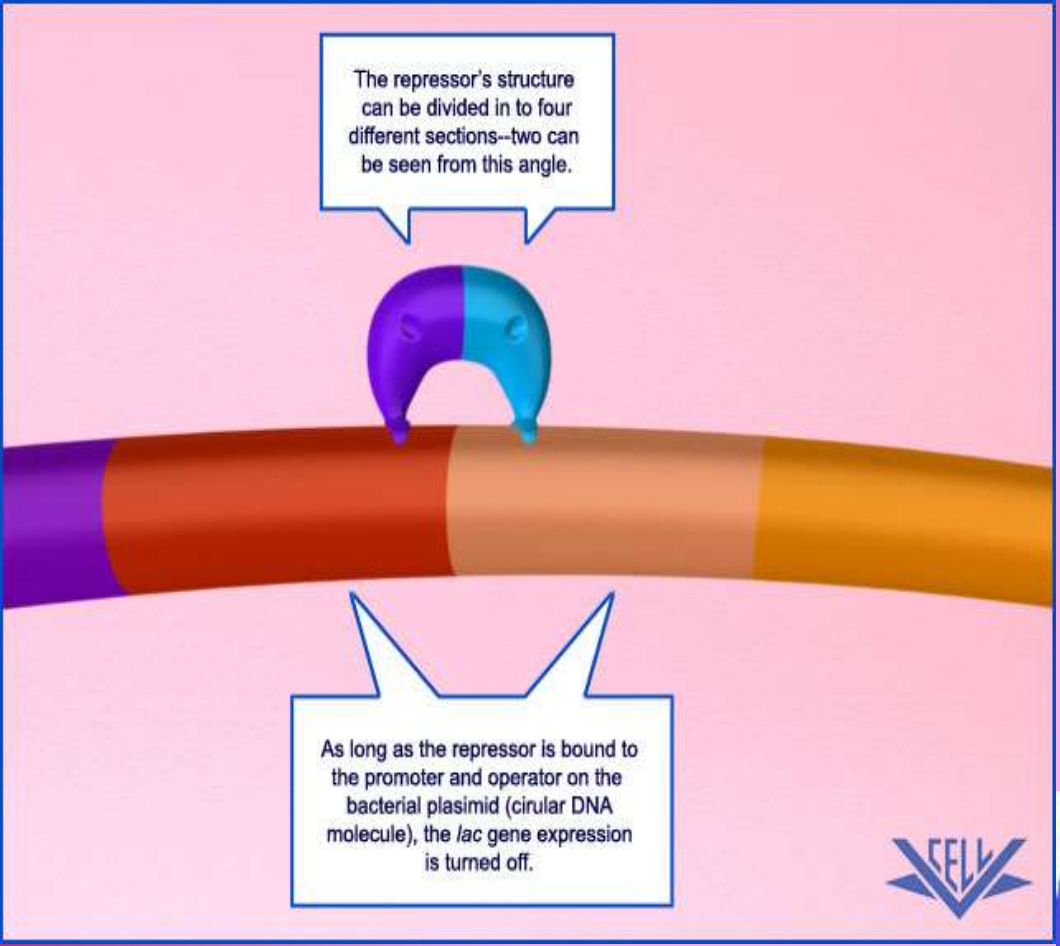
b. **Lactose present.** Enzymes needed to take up and use lactose are produced only when lactose is present.



A simplified ***E. coli bacterial cell.***

The ***lac operon*** gene sequence.





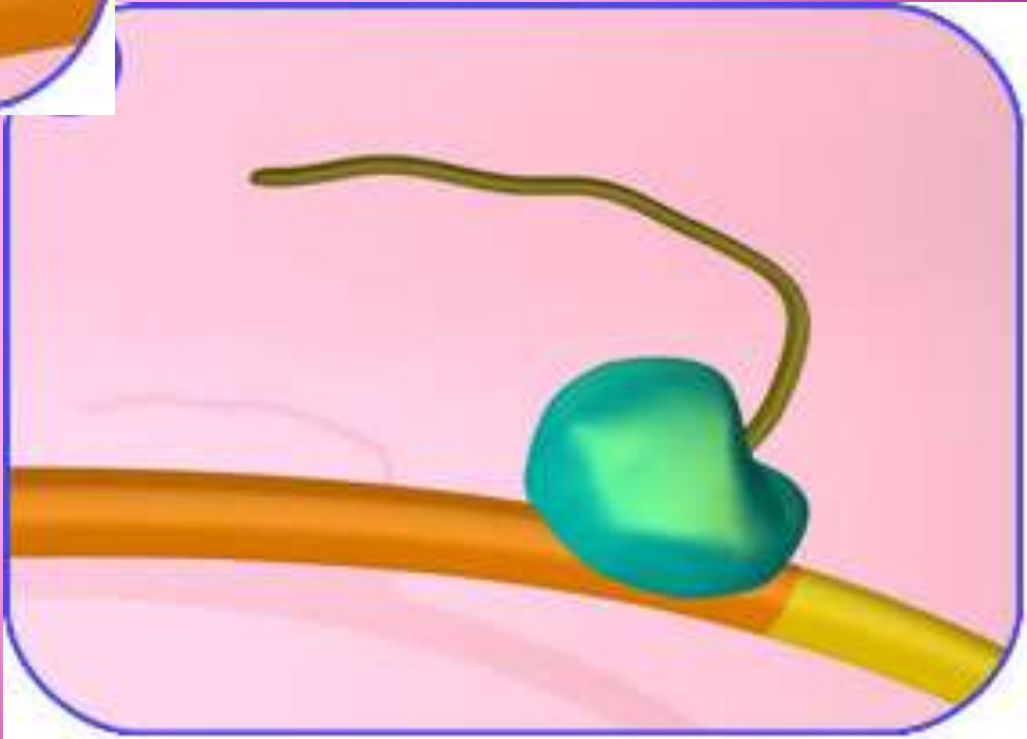
The ***repressor*** molecule, bound to the ***controlling region***.

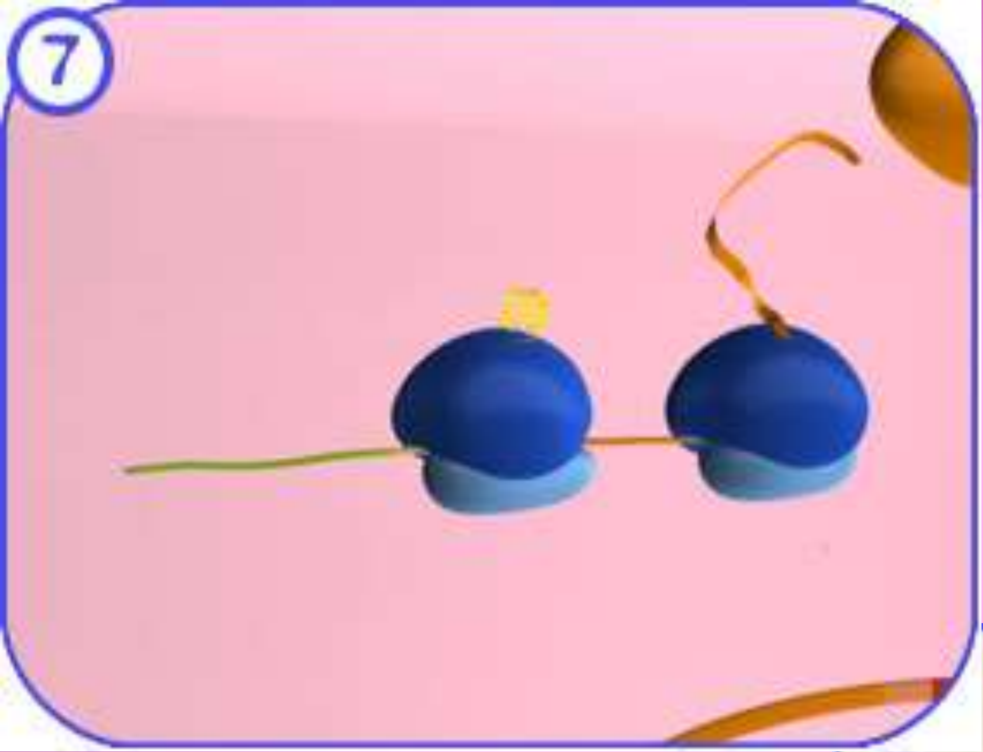
Lactose molecules added to the environment outside of the cell.

Lactose molecules bound to the ***repressor***.

This releases the repressor from the DNA.

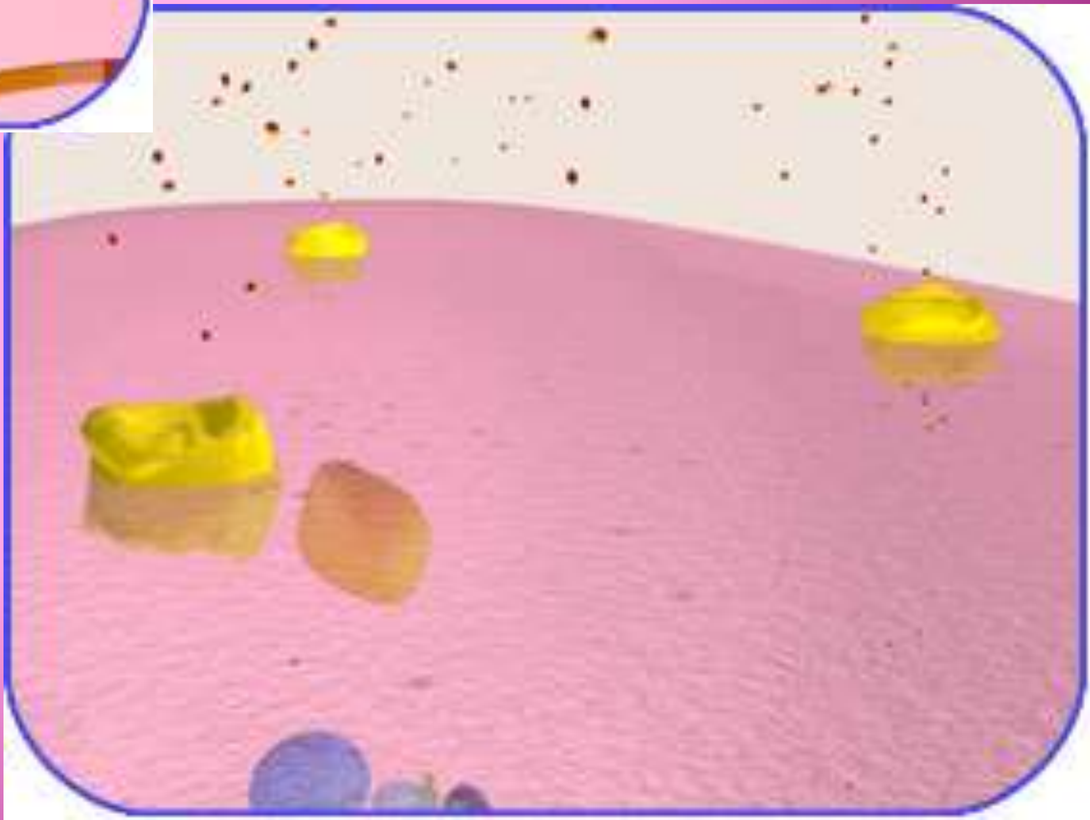
RNA polymerase transcribing the genes in the ***lac operon*** into ***mRNA.***

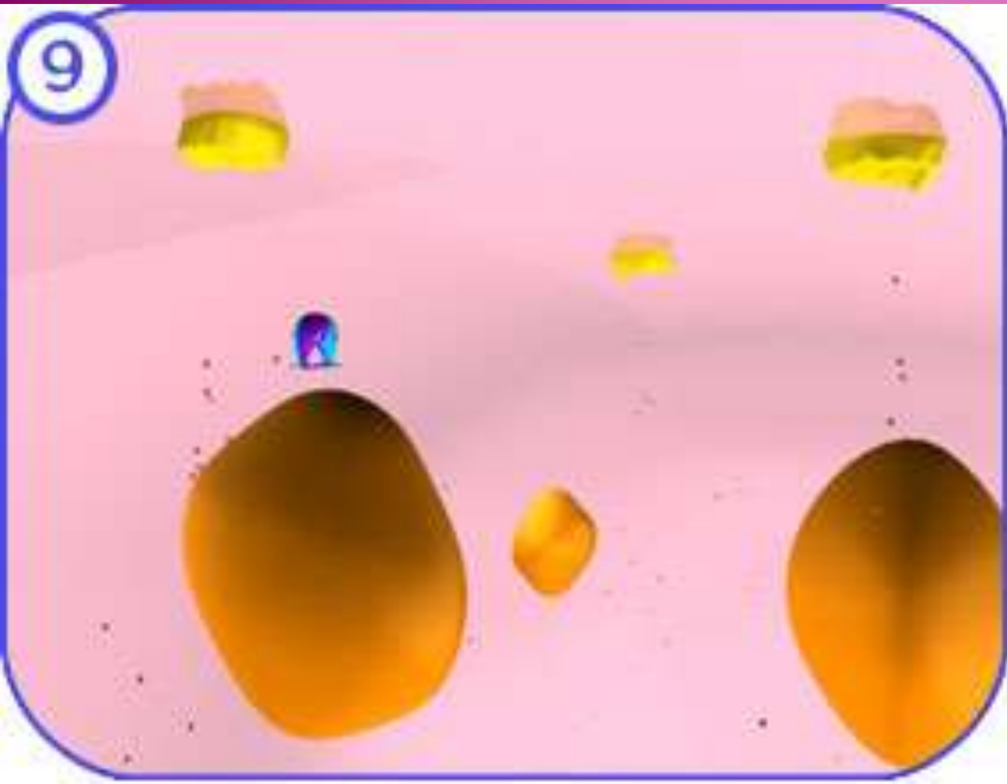




Ribosomes translating the ***mRNA*** into proteins.

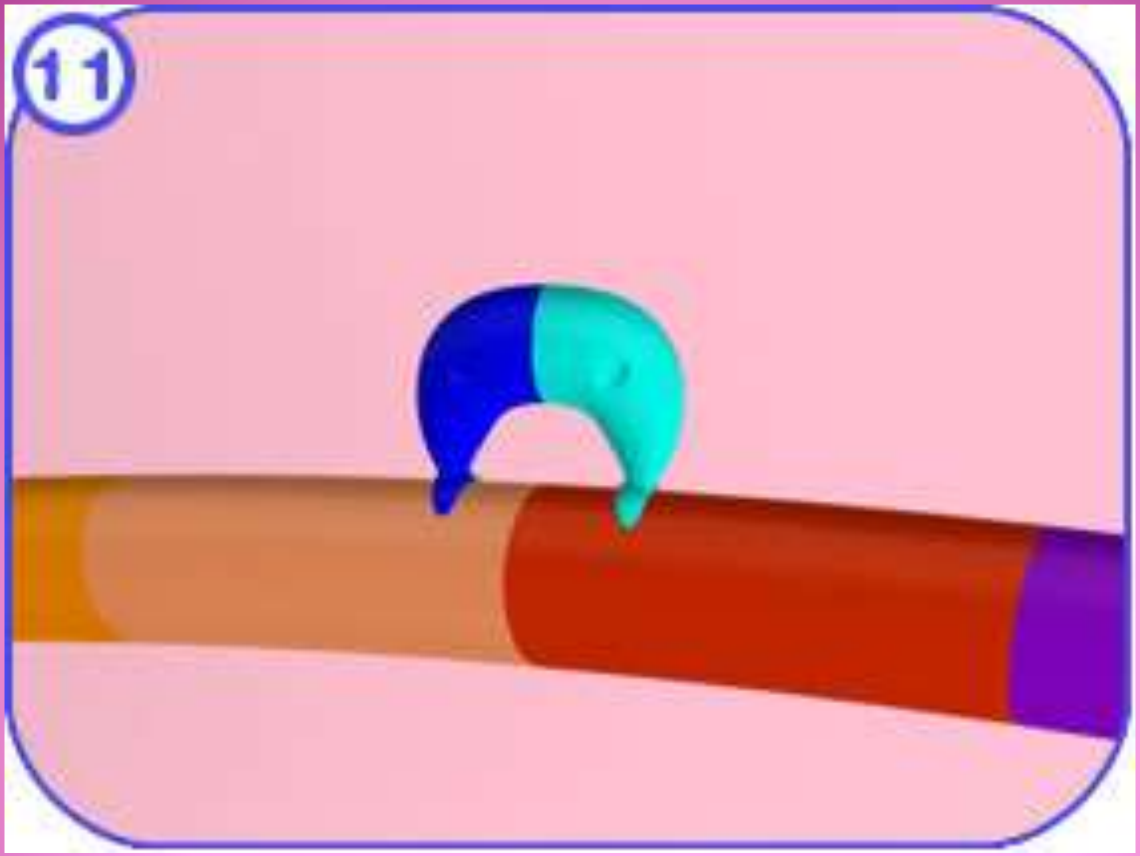
One of the proteins (yellow) encoded by the ***lac operon*** allows ***lactose*** to enter the cell at a high rate.





A second protein (orange) digests the ***lactose*** as it enters the cell.

The ***lactose*** molecules bound to the ***repressor*** are released.



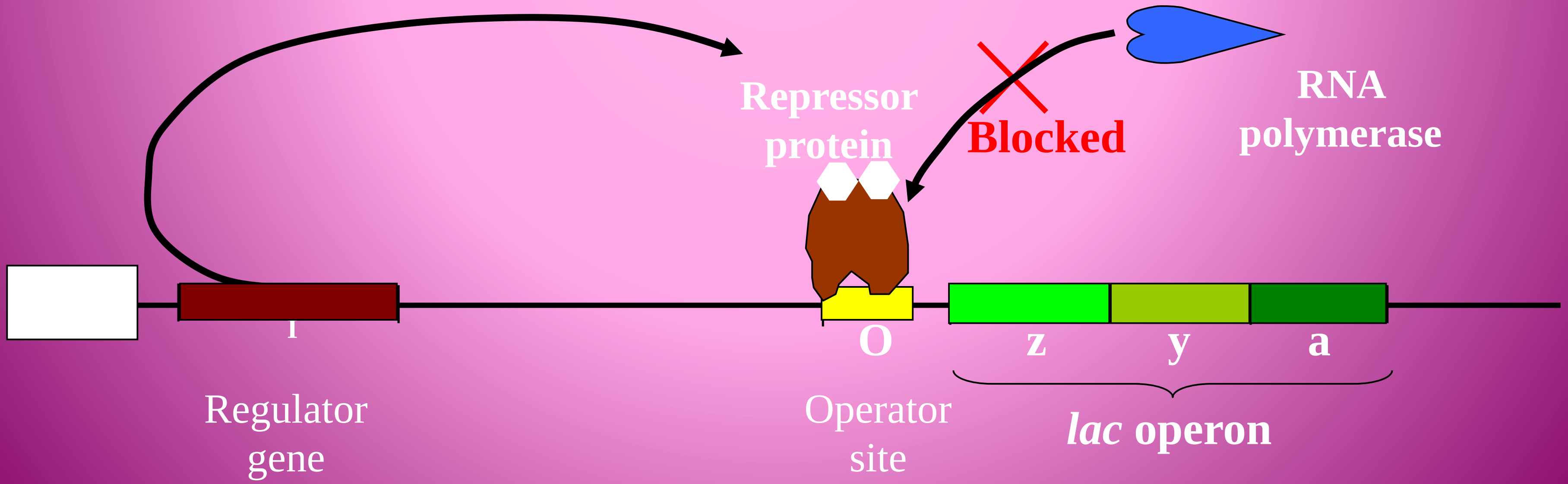
Repressor again binds to the ***controlling region*** of the DNA.

Different Scenarios

1. Lactose (-)
2. Lactose (+)
3. Lactose (+) and glucose (+)
4. Lactose (+) and glucose (-)

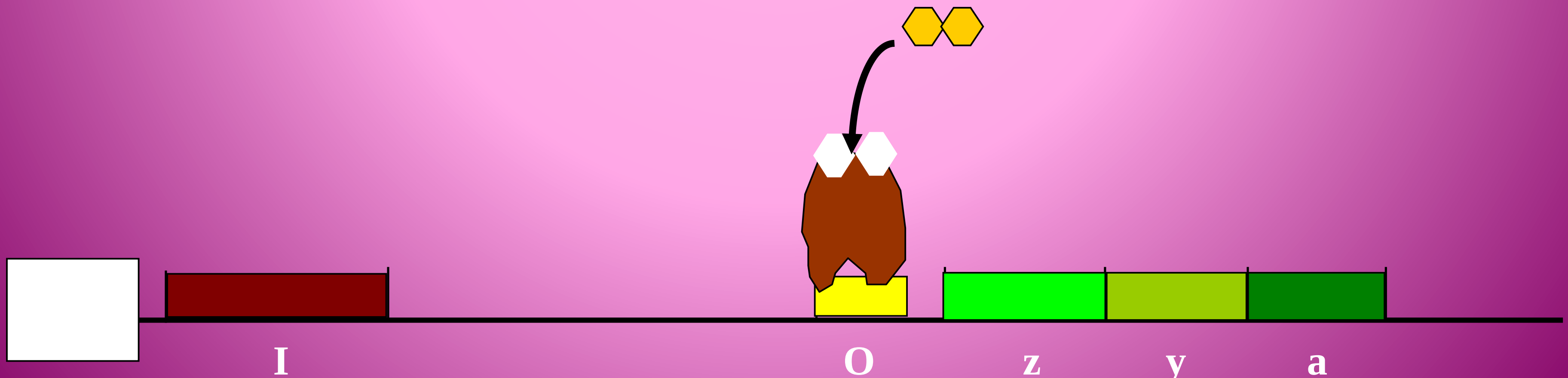
1. When lactose is absent

- 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



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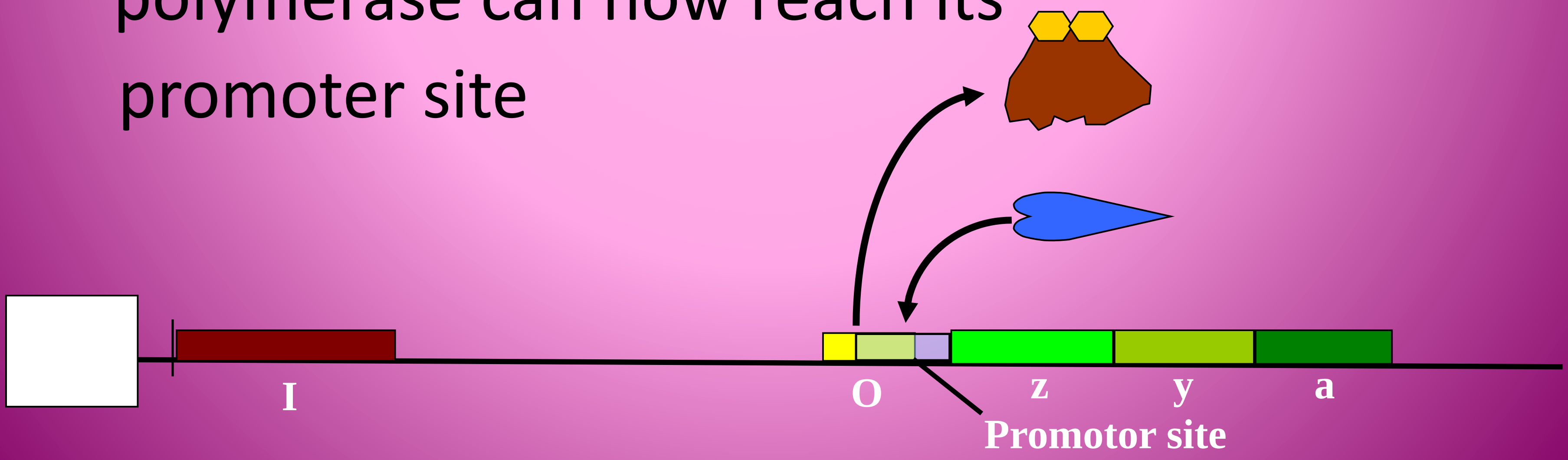
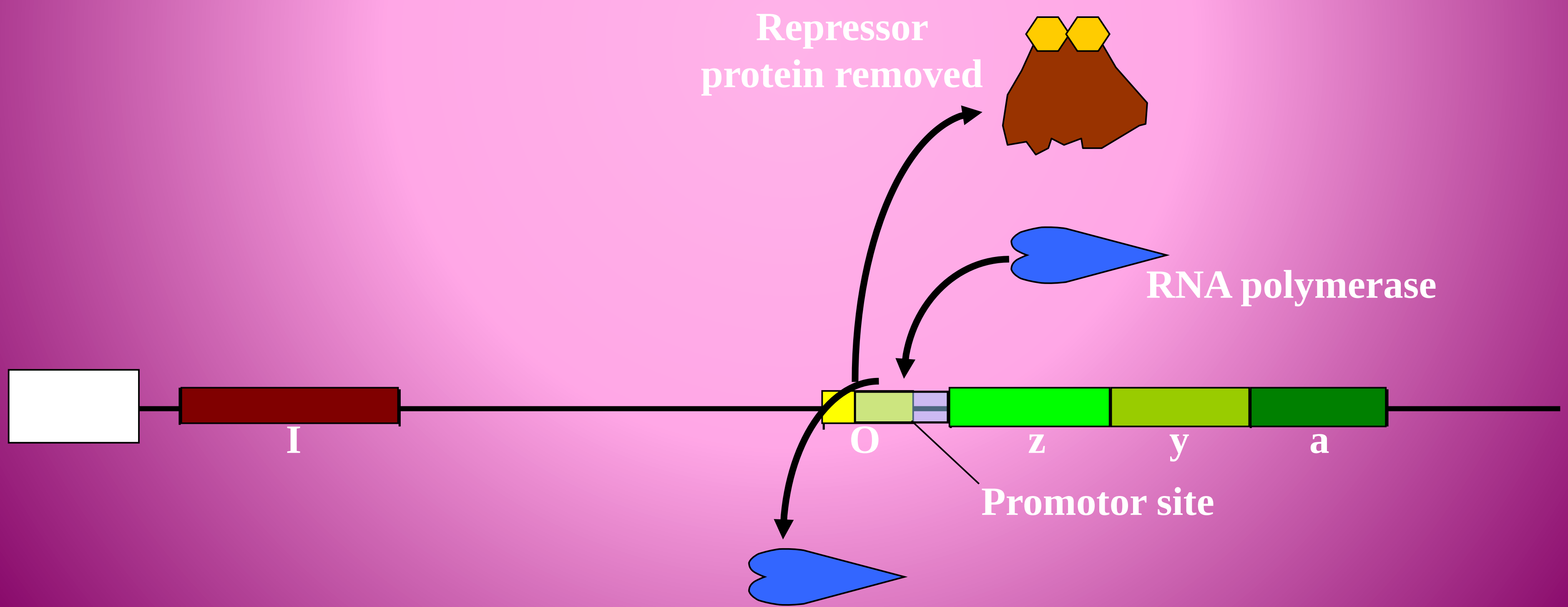


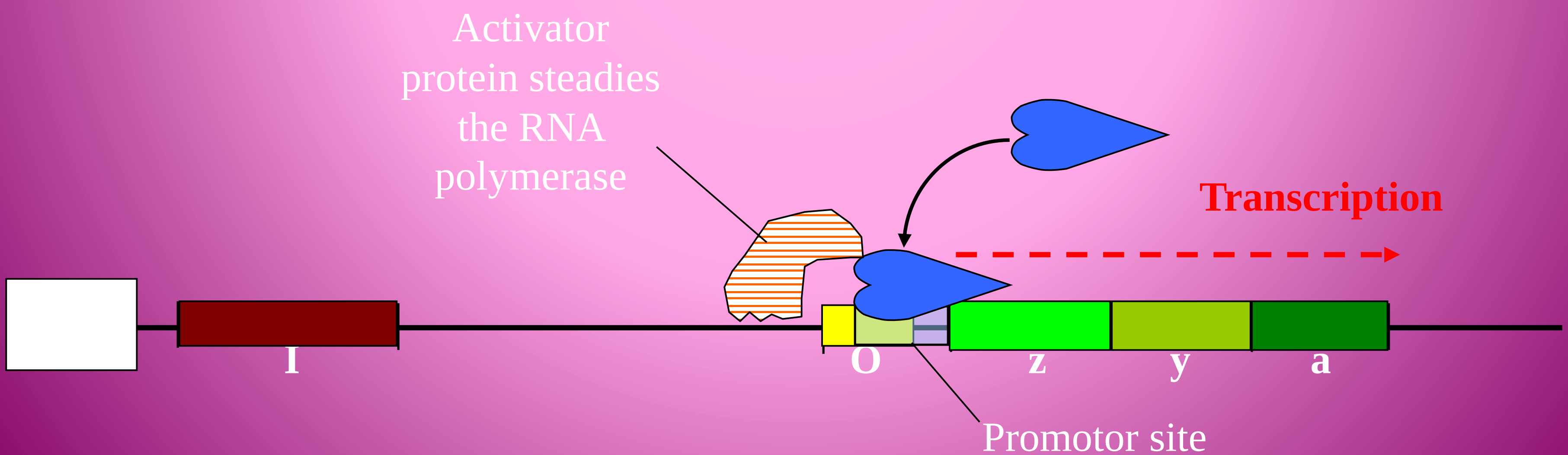
3. When both glucose and lactose are present

- 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



Summary

Carbohydrates	Activator protein	Repressor protein	RNA polymerase	lac Operon
+ GLUCOSE + LACTOSE	Not bound to DNA	Lifted off operator site	Keeps falling off promoter site	No transcription
+ GLUCOSE - LACTOSE	Not bound to DNA	Bound to operator site	Blocked by the repressor	No transcription
- GLUCOSE - LACTOSE	Bound to DNA	Bound to operator site	Blocked by the repressor	No transcription
- GLUCOSE + LACTOSE	Bound to DNA	Lifted off operator site	Sits on the promoter site	**Transcription**

LAC MUTATIONS

- Jacob & Monod workout the structure & function of *lac* operon by analyzing mutations that affects lactose metabolism.

- To help define the role of the different components of the operon, they use partial diploid stain of *E.coli.*

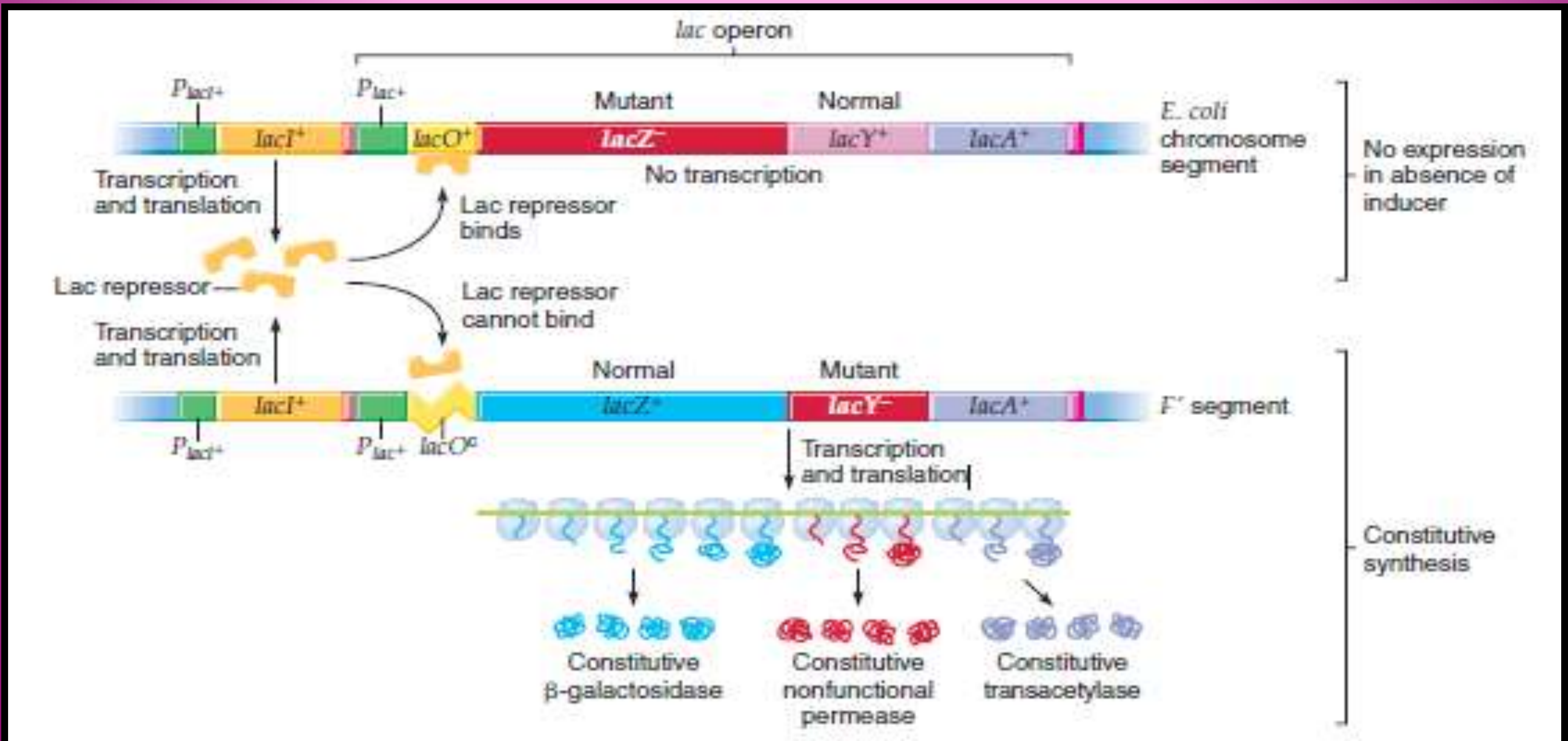
- They determine that some part of the *lac* operon are *cis* acting where other are *trans* acting.

STRUCTURAL-GENE MUTATION

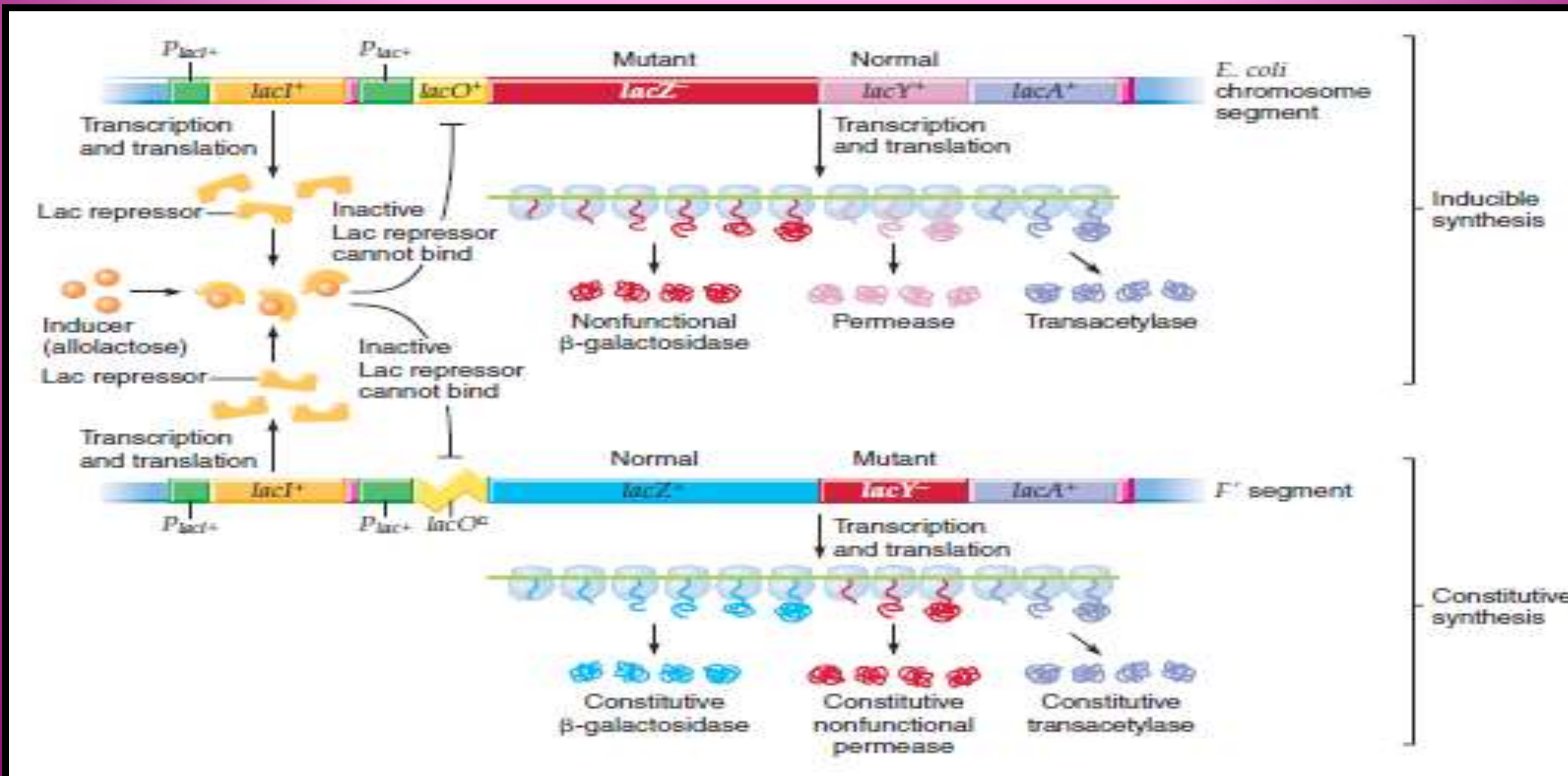
- Jacob and Monod first discovered some mutant strains that had lost the ability to synthesize either β-galactosidase or permease.

- The mutation which occurred on lacZ and LacY structural genes altered the amino acid sequences of the proteins encoded by the genes.

a) In the **absence of inducer**, the *lac*O^+ operon is turned off, whereas the *lacOc* operon produces functional β-galactosidase from the ***lac***Z^+ gene and nonfunctional permease molecules from the ***lac***Y^- gene with missense mutation.



b) In the **presence of inducer** the functional β-galactosidase and defective permease are produce from the *lac*O^c operon, whereas the *lac*O^+ operon produces nonfunctional β-galactosidase from the *lac*Z^- gene & functional permease from *lac*Y^+ gene.



OPERATOR MUTATIONS

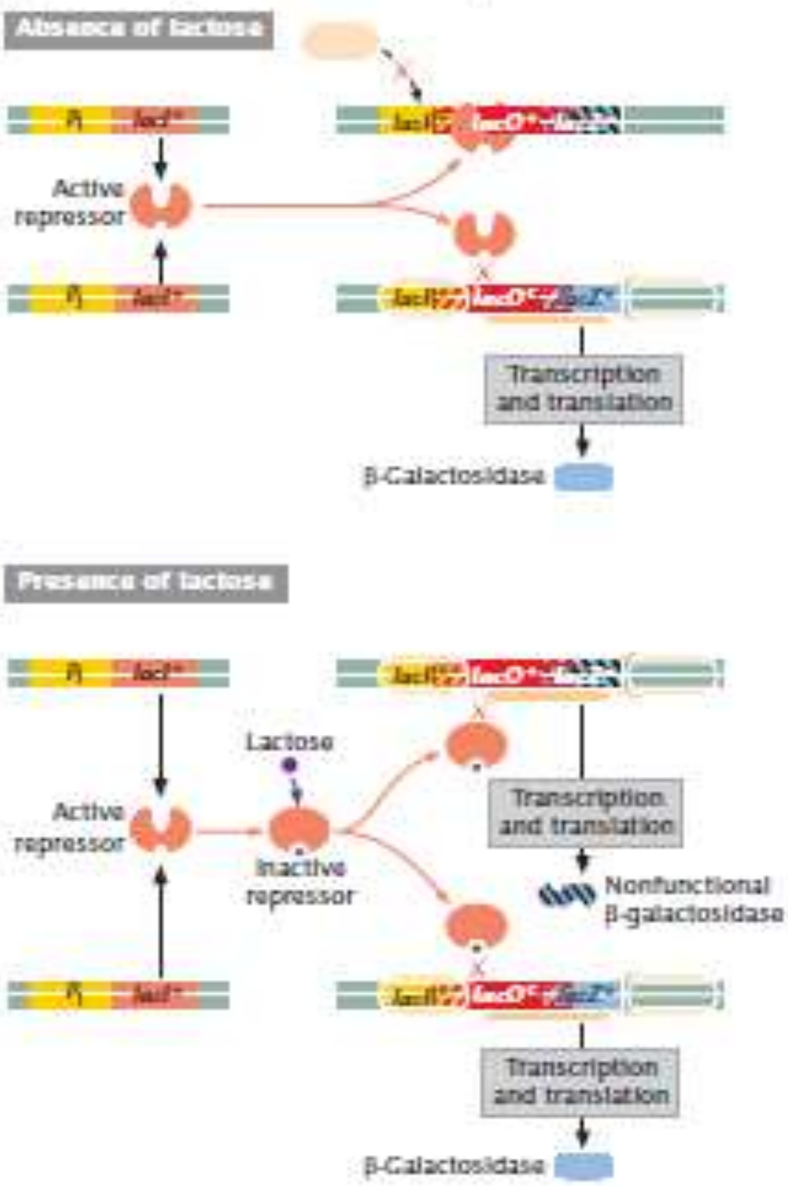
- Jacob & Monod find another constitutive mutants to a site adjacent to *lacZ.*

- This mutations occurred at the operator site & were referred to as *lacO*$^{c.}$

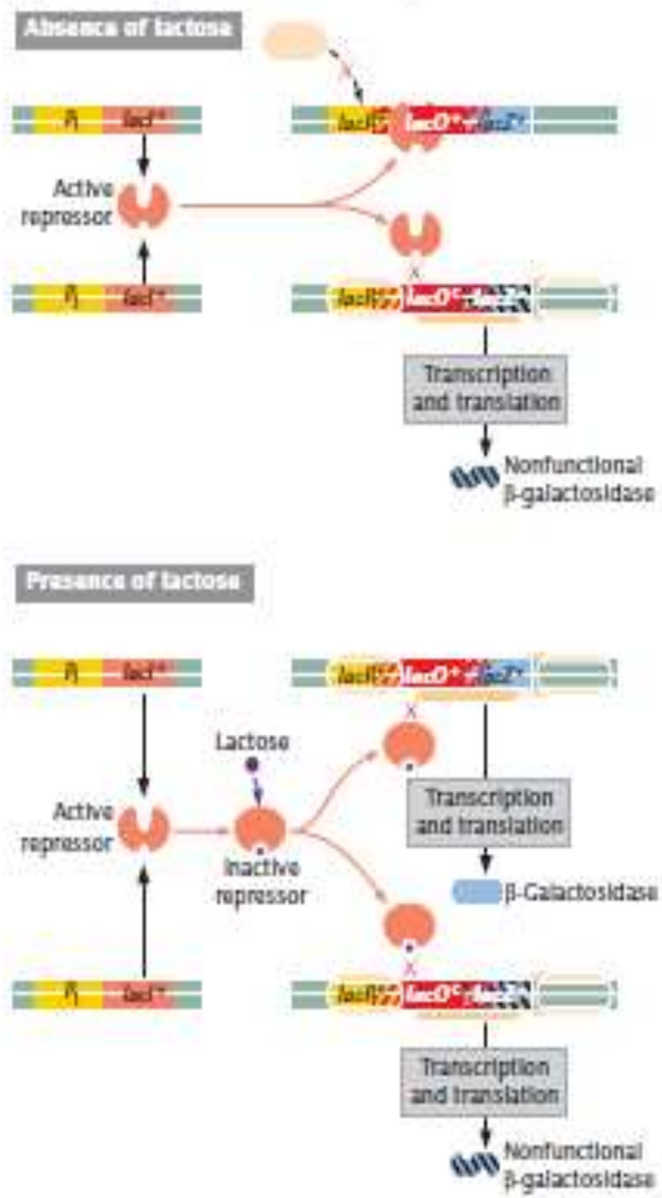
- The *lacO*^{c} mutations altered the sequence of DNA at the operator so that the repressor protein was no longer able to bind.

- A partial diploid with genotype *lac*I^{+} *lacO*^{c} *lac*z^{+} /*lac*I^{+} *lacO*$^{+}$ *lac*z^{+} exhibited constitutive synthesis of β-galactosidase, indicating that *lacO*^{c} is dominant over *lacO*$^{+}$.

(a) Partial diploid *$lacI^+$ $lacO^+$ $lacZ^-$ / $lacI^+$ $lacO^C$ $lacZ^+$*



(b) Partial diploid *$lacI^+$ $lacO^+$ $lacZ^+$ / $lacI^+$ $lacO^C$ $lacZ^-$*



PROMOTER MUTATION:

- Mutations affecting lactose metabolism have also been isolated at the promoter site; these mutations are designated *lac*P^- ,and they interfere with the binding of RNA polymerase to the promoter.

- This binding is essential for the transcription of the structural gene.

- E.coli strain with *lac*P^- mutation does not produce *lac* proteins either in a presence or absence of lactose.

- *lac*P^- mutations are cis acting.

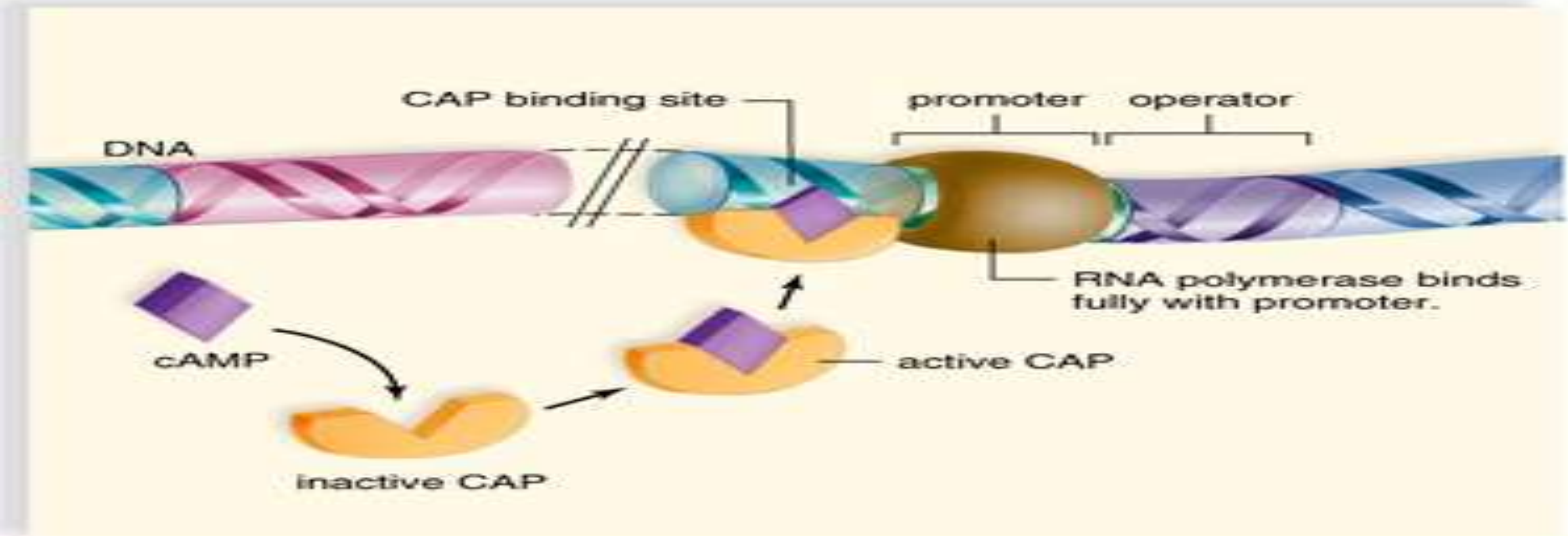
- The lac operon is under two forms of control; positive and negative control.

- Negative control occurs when the binding of a protein prevents an event.

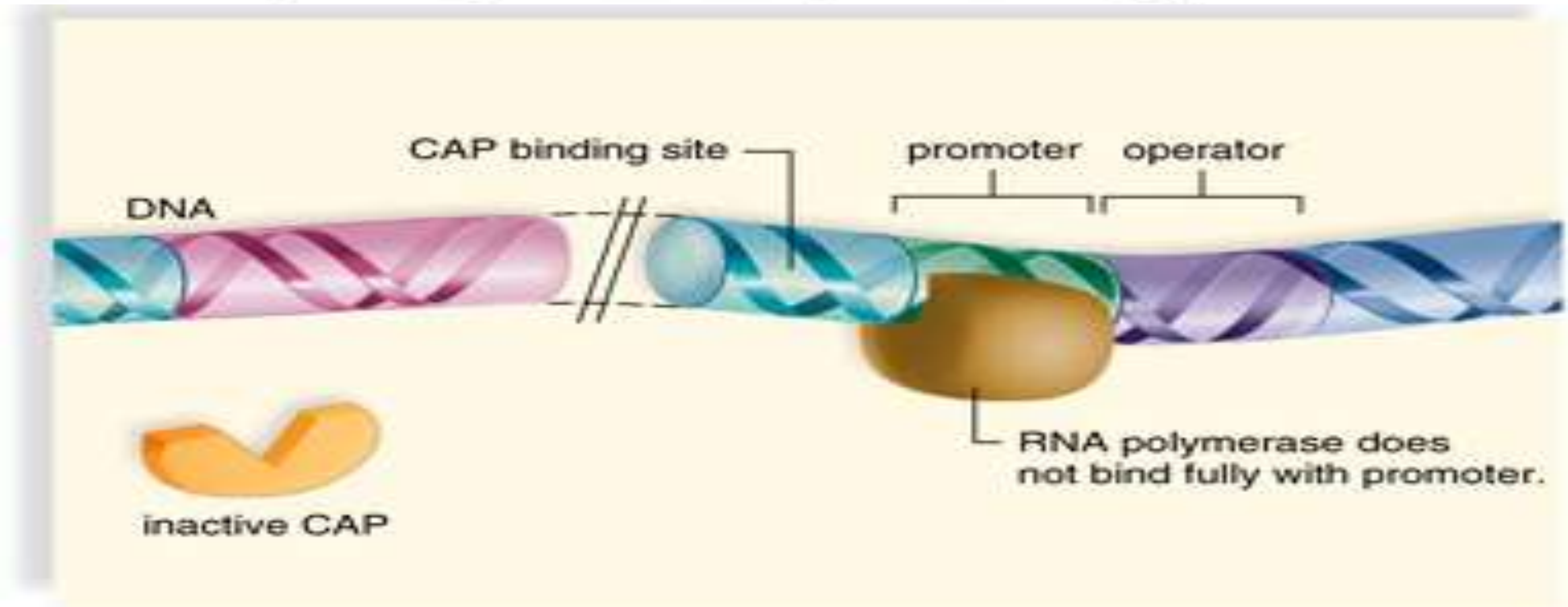
- Positive control is when the binding causes the event.

POSITIVE CONTROL

- When glucose is available, gene that participate in the metabolism other sugars are repressed, in a phenomenon known as catabolite repression.
- Catabolite repression Is a type of +ve control in the lac operon.
- The catabolite activator protein(CAP), complex cAMP, binds to a site near the promoter & stimulates the binding of RNA polymerase.
- A cellular level of cAMP are controlled by glucose; allolactose level increases the abundance of cAMP & enhance the transcription of the lac structural genes.



a. **Lactose present, glucose absent (cAMP level high)**

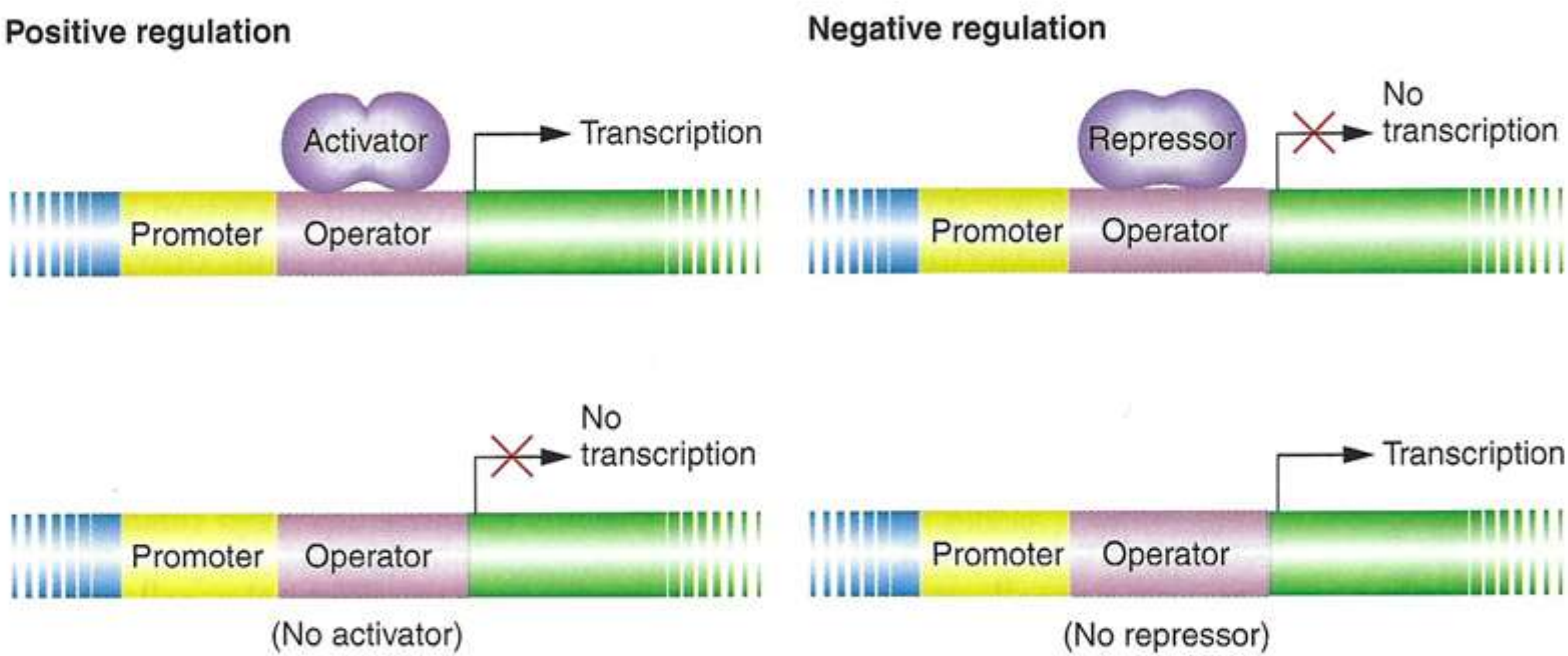


b. **Lactose present, glucose present (cAMP level low)**

NEGATIVE CONTROL

- The lac repressor bind to the operator.
- The DNA sequence cover by the repressor overlaps the DNA sequence recognized by the RNA polymerase.
- Therefore, when the repressor is bound to the operator, RNA polymerase cannot bind to the promoter & transcription can not occur, the *lac* operon is said to be under –ve control.

Positive and negative regulation



POSITIVE VS NEGATIVE CONTROL

	Regulatory protein is **present**	Example of regulatory **protein**	Mutate regulatory gene to lose **function**
Positive control	Operon ON	Activator	Operon OFF
Negative control	Operon OFF	Repressor	Operon ON

REFERENCE

Books :

- **Genetics by *Benjamin Pierce***
- **iGenetics by Peter J.Russell**

Internet :

- **Www.google.com**
- **https://www.google.co.in/search?q=The+lac+operon+in+e.coli.ppt&client=opera&hs=OtG&biw=1366&bih=586&source=lnms&tbm=isch&sa=X&ei=OzQ0VJu1N42xuATqjIH4BQ&ved=0CAYQ_AUoAQ**

Thanks!