SOS in Biochemistry, Jiwaji University, Gwalior

M.Sc. II Semester (2019-20)

Paper BCH 205: Fundamentals of Molecular Biology (Unit III)

lac Operon

(A negative inducible operon)

ADAPTING TO THE ENVIRONMENT

***E. coli* can use either glucose, which is a monosaccharide, or lactose, which is a disaccharide**

However, lactose needs to be hydrolysed (digested) first

So the bacterium prefers to use glucose when it can

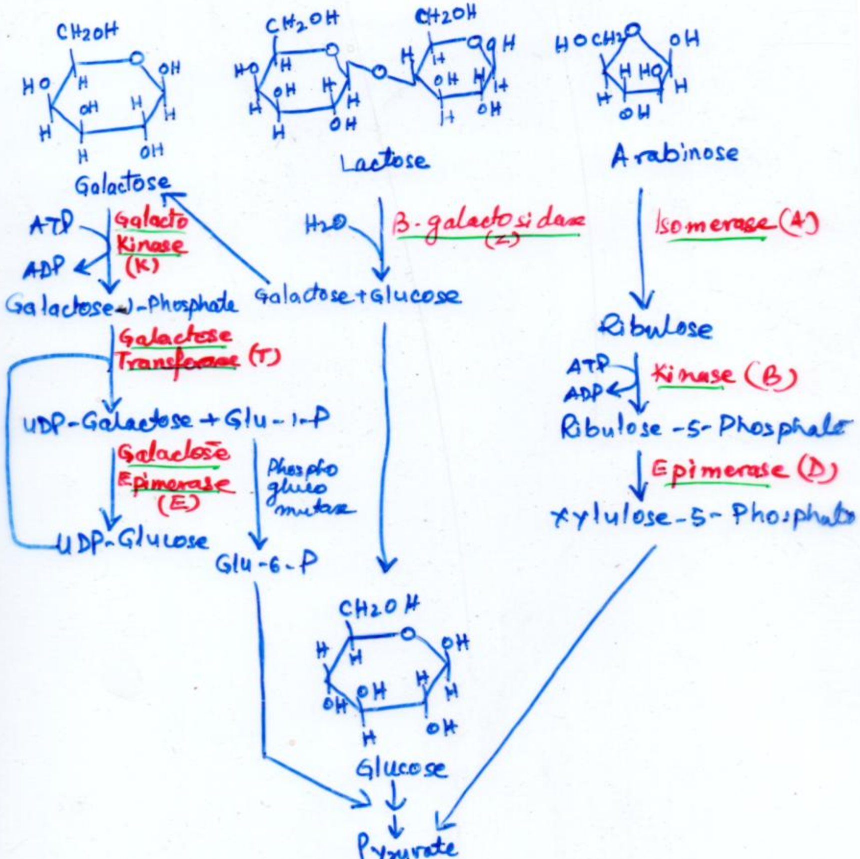
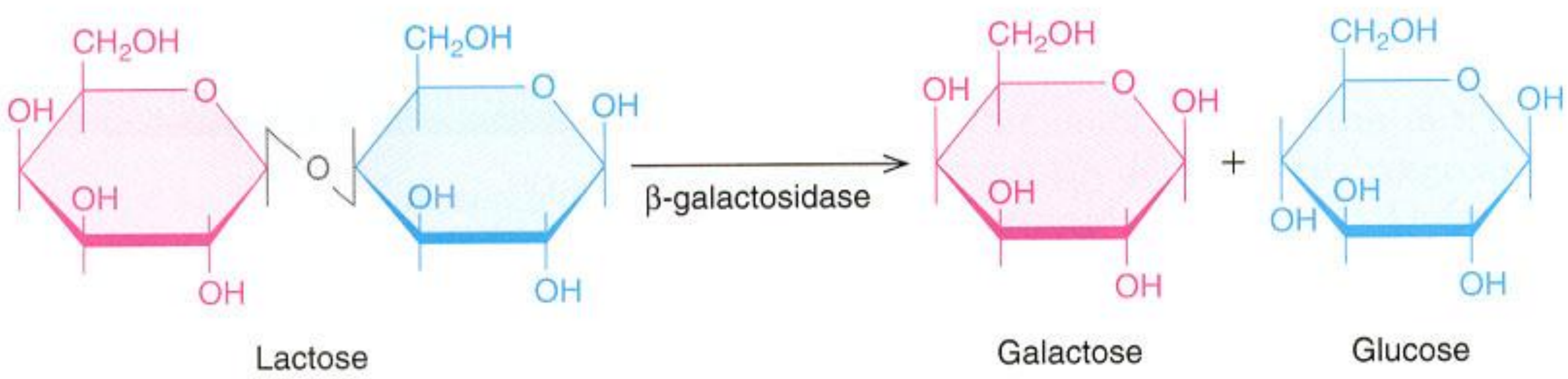


Fig.: Metabolic steps in three well studied pathways of sugar metabolism

fig.: Each set of enzymes is encoded by a cluster of genes in a single operon

The Lactose Operon

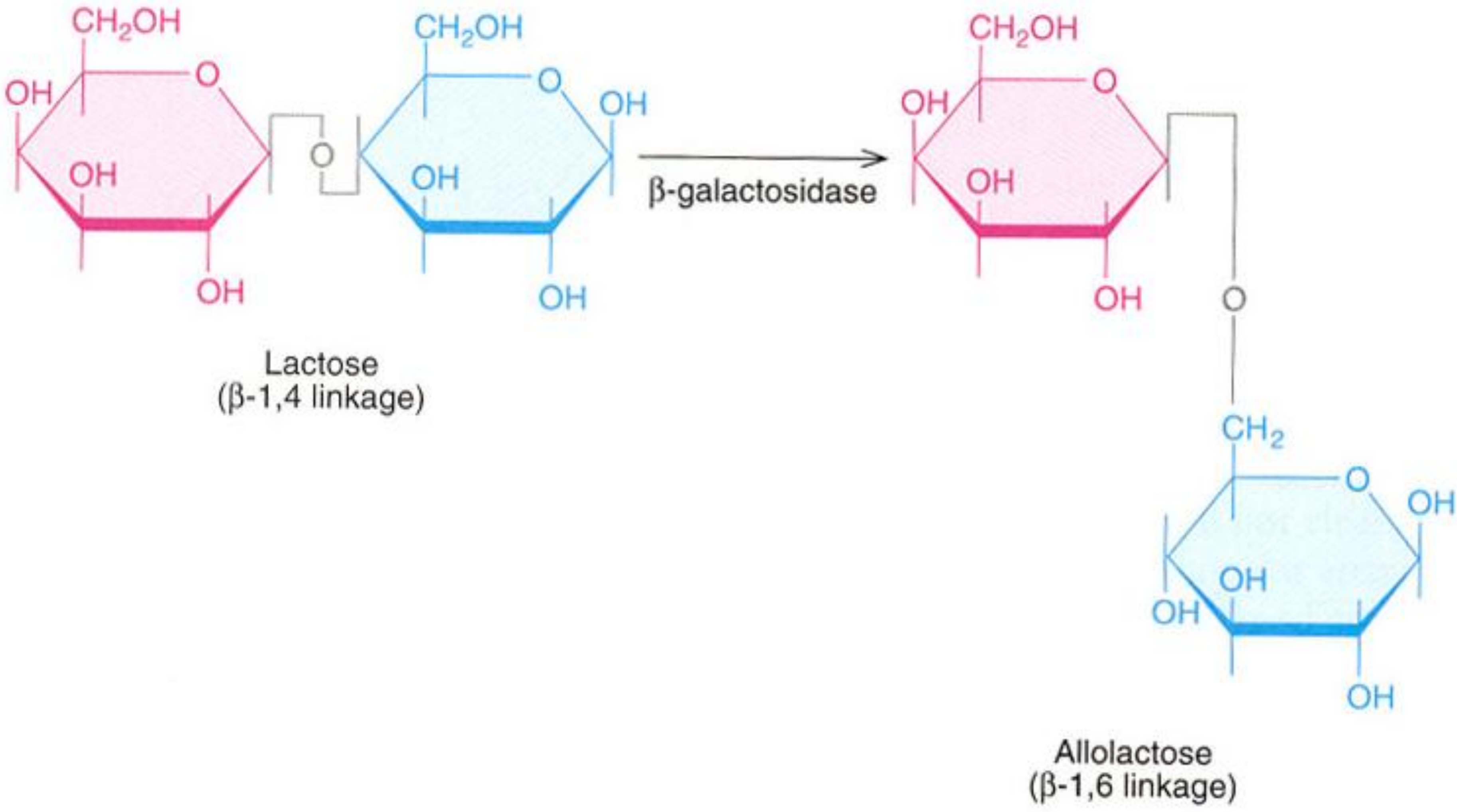


- ***lac*Z : β-galactosidase**
- ***lac*Y : lactose (galactoside) permease**
- ***lac*A : galactoside transacetylase**

lac Operon of *Escherichia coli*

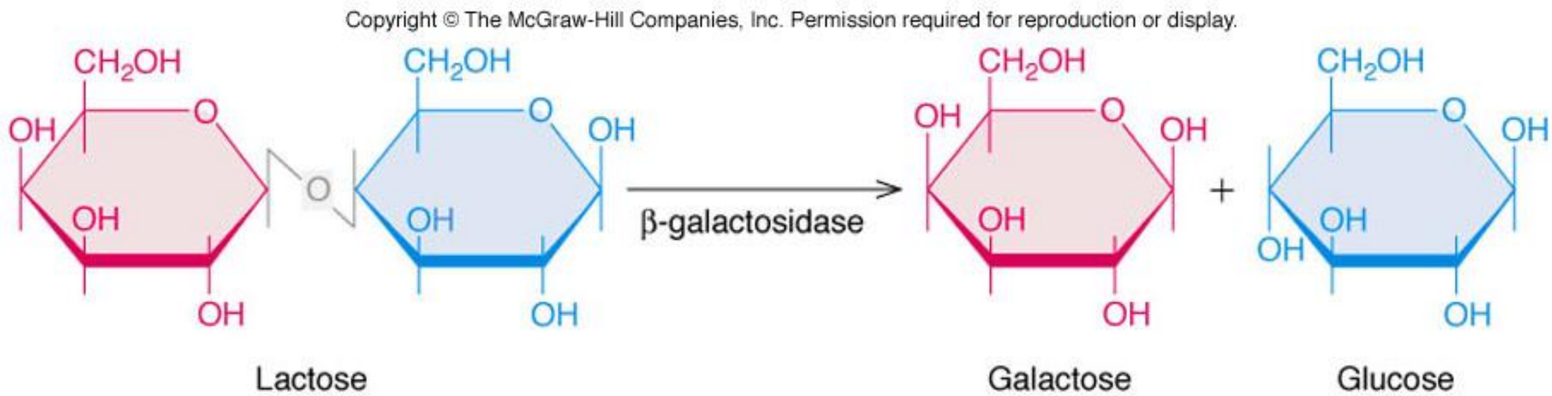
- A negative inducible Operon
- Lactose Metabolism
- Regulation of the *lac* *O*peron
- Inducer: Allolactose
  - *lacI*: Repressor encoding gene
  - *lacP*: Operon promoter
  - *lacO*: Operon operator

The nature of the physiological *lac* inducer



β-galactosidase Activity

1.



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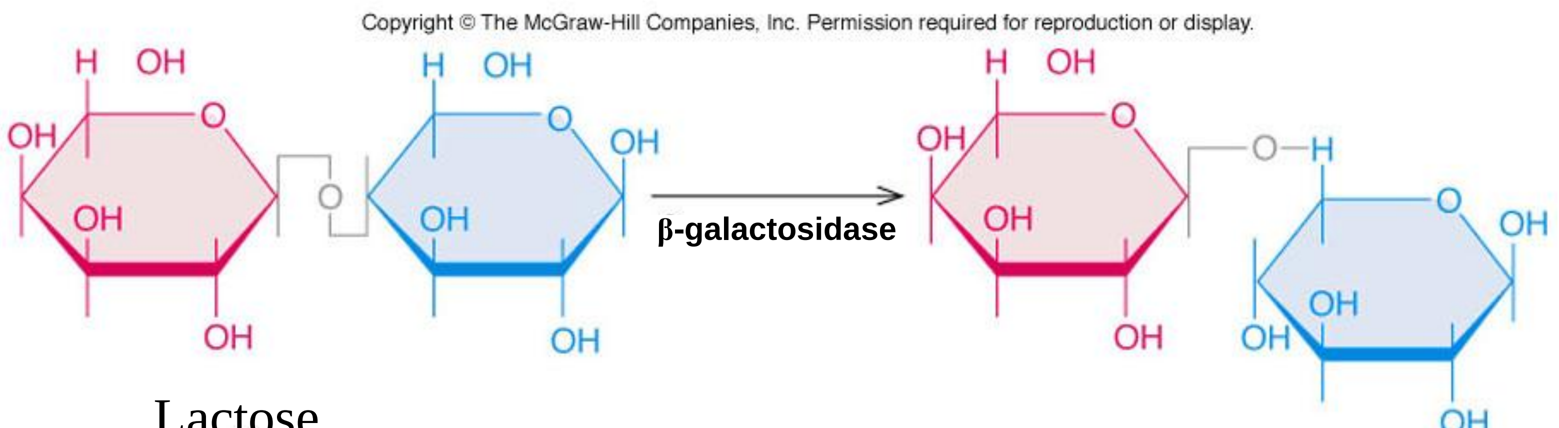
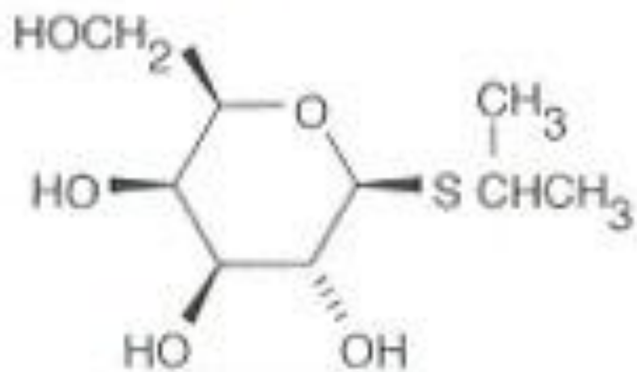


Lactose
(β -1,4 linkage)

Allolactose
(β-1,6 linkage)

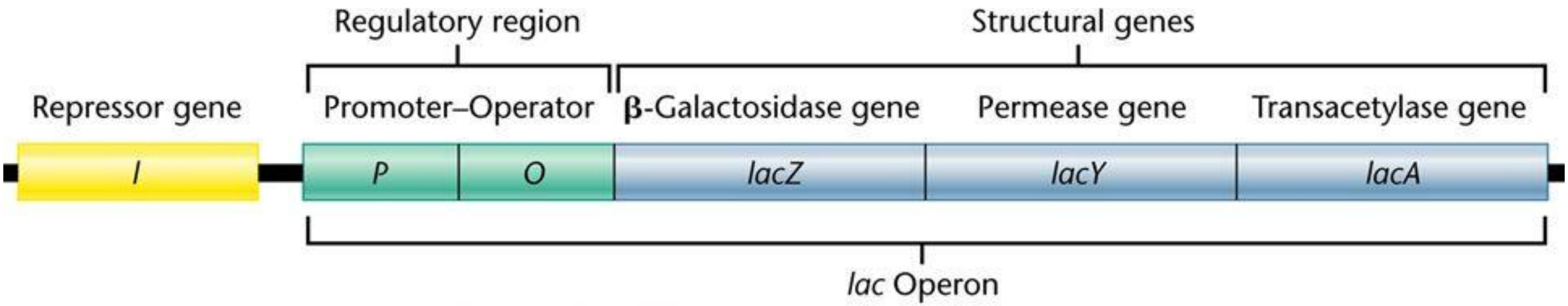
Non-metabolizable analogue of lactose

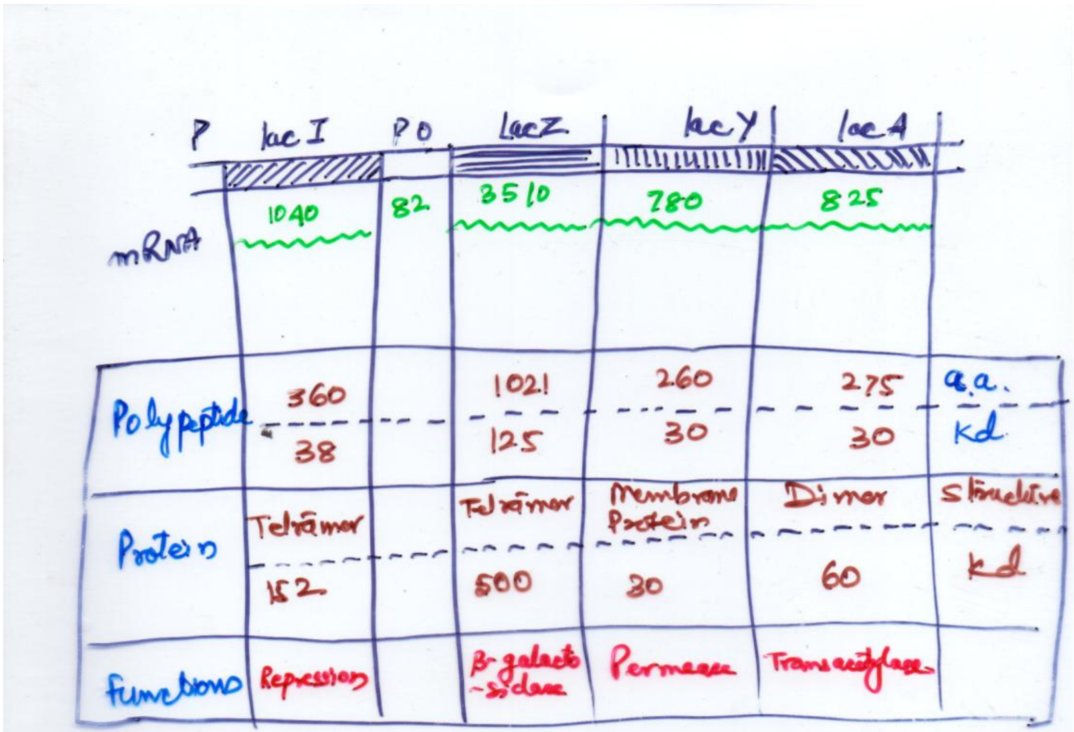
ISOPROPYL β-D-THIOGALACTOPYRANOSIDE
(Isopropyl β-D-thiogalactoside; IPTG)



lac Operon

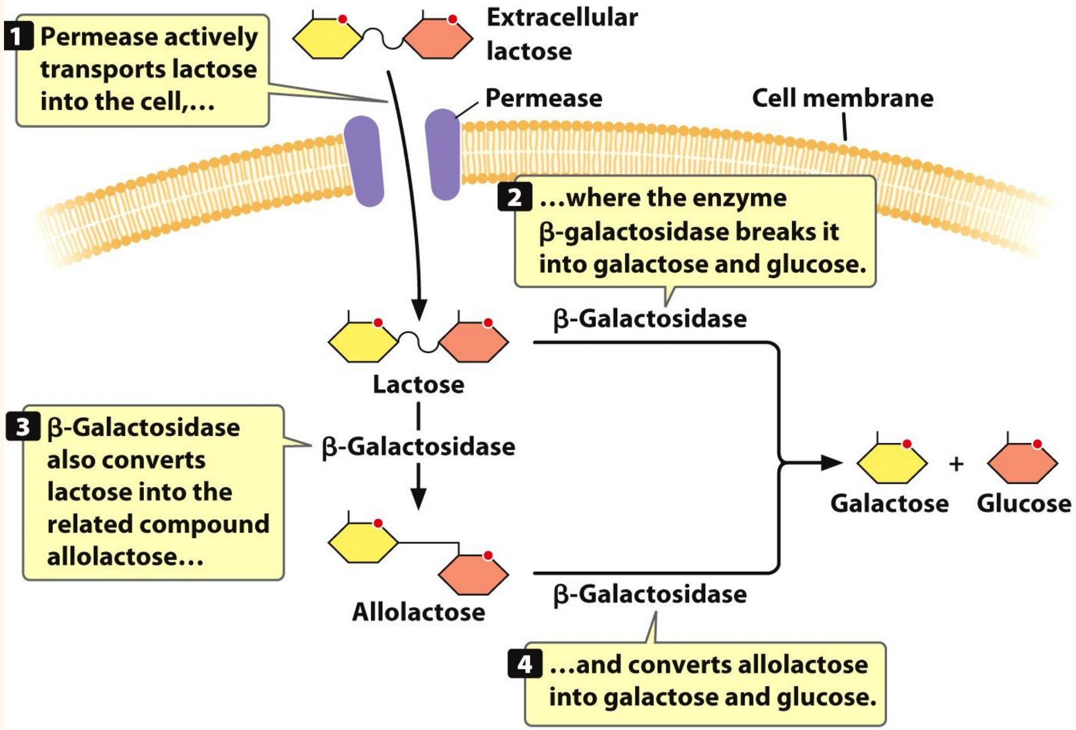
Structure **~6,000 bp**





lac Operon of *Escherichia coli*

- **Structural genes**
  - ***lacZ*: encoding β-galactosidases**
  - ***lacY*: encoding permease**
  - ***lacA*: encoding transacetylase**
- **The repression of the *lac* operon never completely shuts down transcription.**



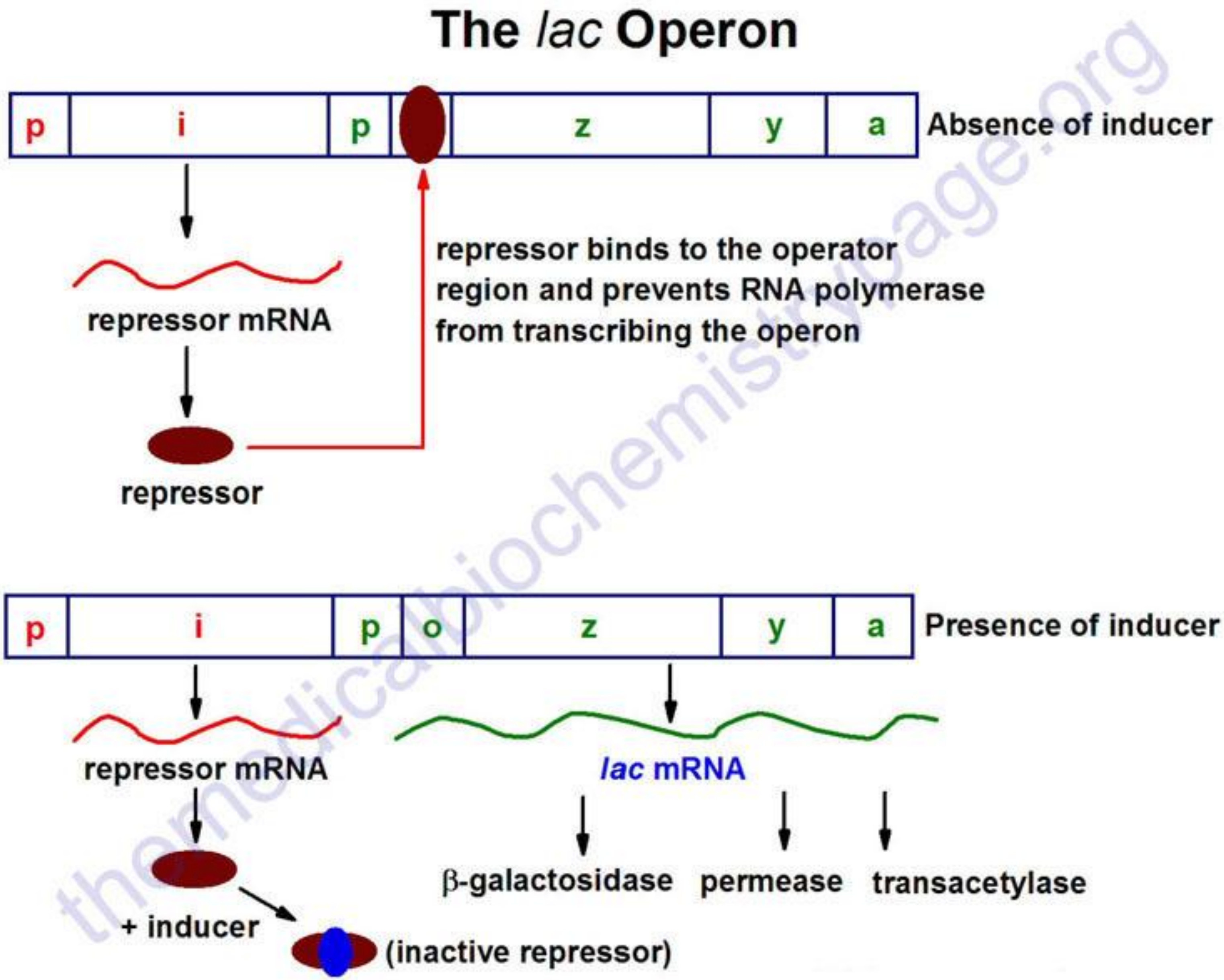
Jacob and Monod's Operon Hypothesis

(Based on Genetics)

1. **There are two key control elements of the operon: the repressor gene and the operator to which the repressor gene product binds.**
2. **There is a specific interaction between the inducer and repressor that prevents the repressor from binding to the operator.**
3. **All three *lac* genes are clustered under a single control unit.**
4. **Subsequent deletion analysis showed that there is a promoter necessary for the expression of all three *lac* genes.**

Biochemical studies confirm all of the tenets of Jacob's and Monod's hypothesis.

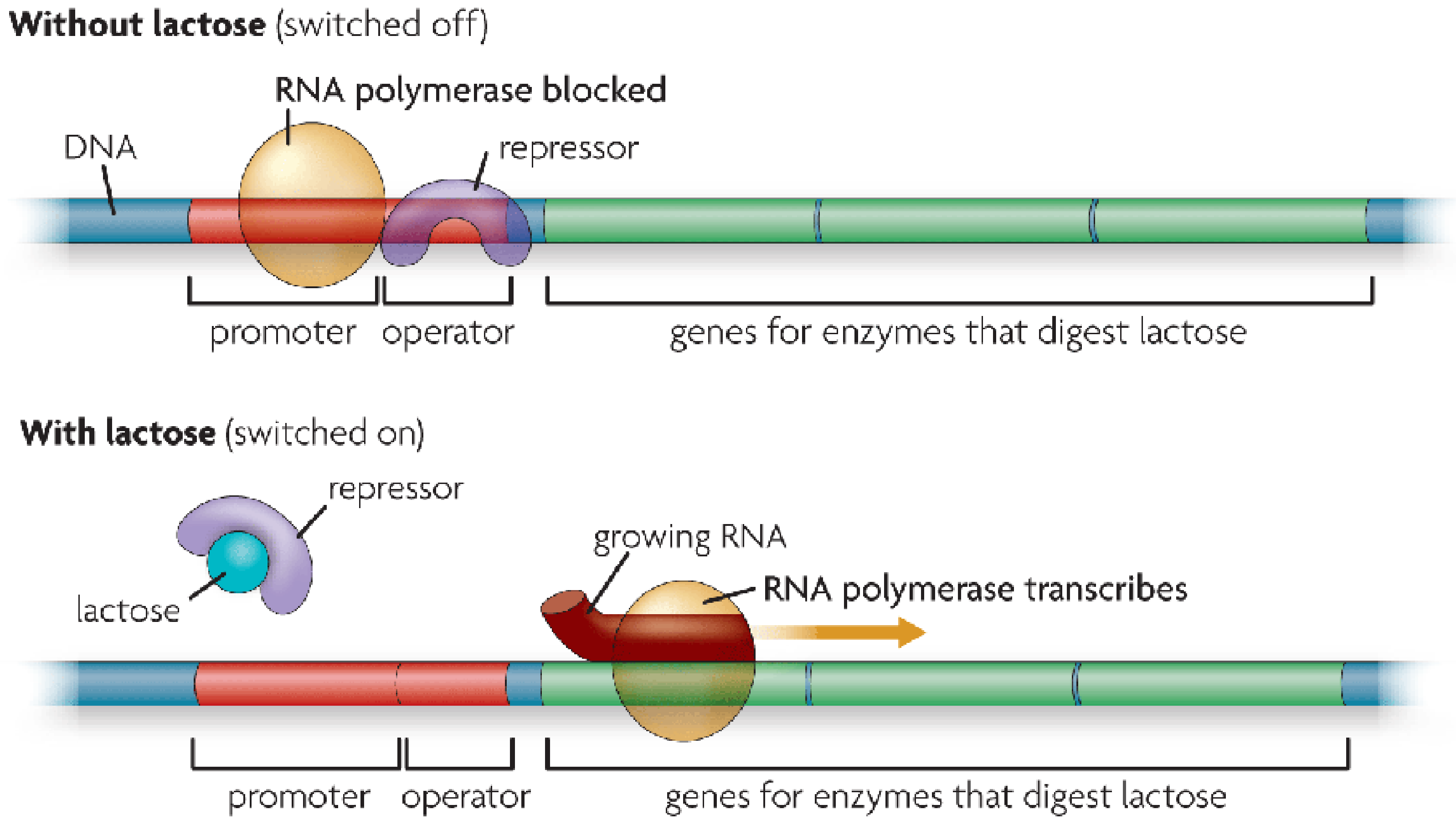
Function


The lac Operon
p
i
p
z
y
a
Absence of inducer
repressor binds to the operator
region and prevents RNA polymerase
from transcribing the operon
repressor mRNA
repressor
p
i
p
o
z
y
a
Presence of inducer
repressor mRNA
lac mRNA
β-galactosidase
permease
transacetylase
+ inducer
(inactive repressor)

Prokaryotic cells turn genes on and off by controlling transcription

The *lac* operon acts like a switch.

- **The *lac* operon is "off" when lactose is not present.**
- **The *lac* operon is "on" when lactose is present.**



Arrangement of the promoter and operator sequences that control the lac operon in E. coli

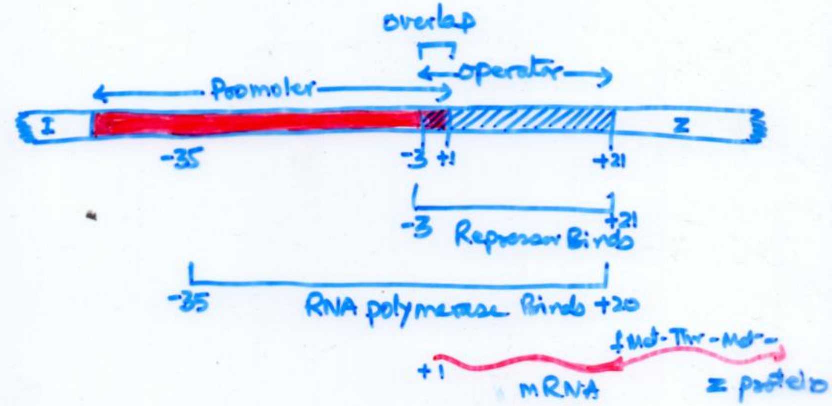
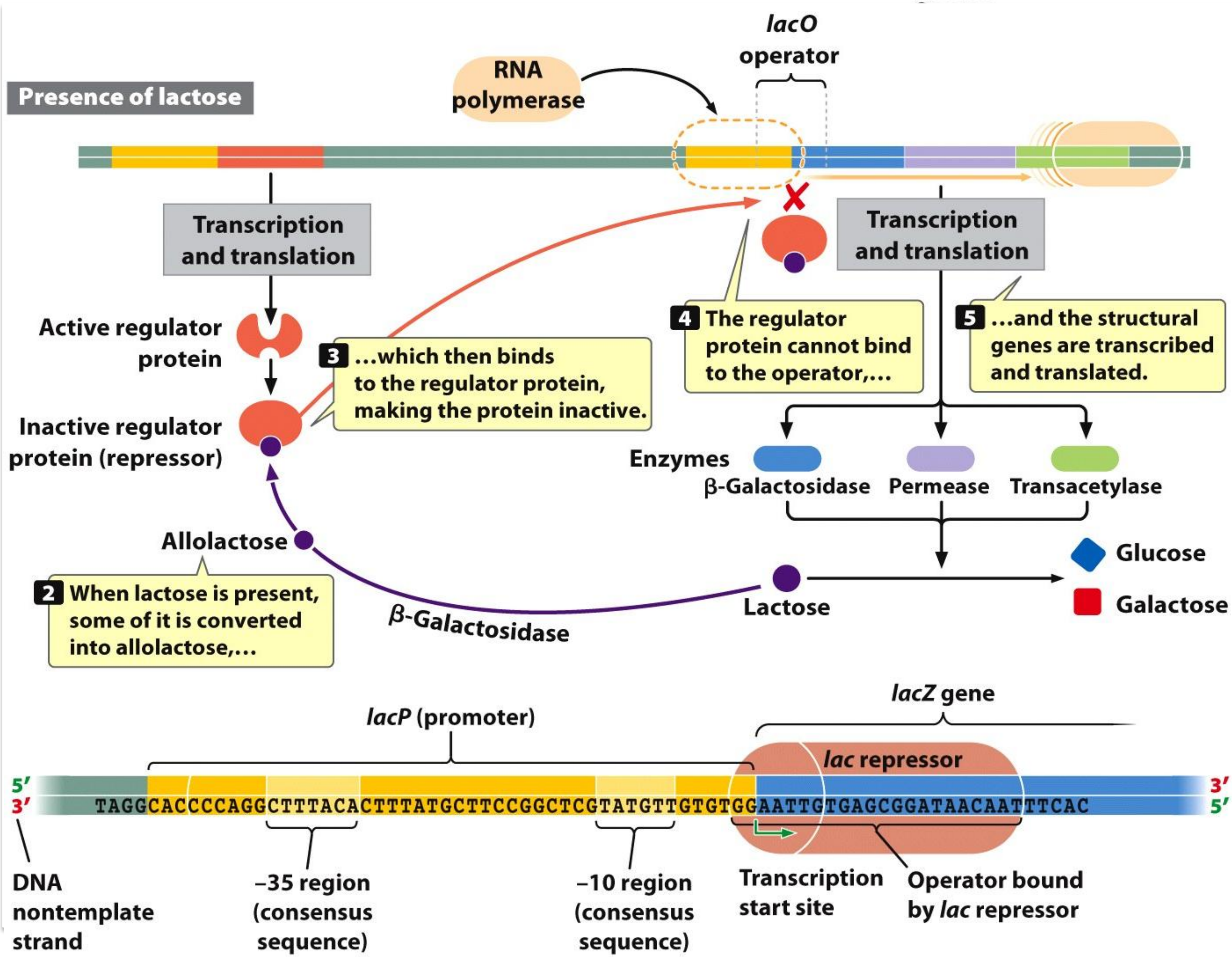


Fig - Repressor Binding site

The positions in which constitutive mutations have been found are marked with 'X'



Presence of lactose
RNA polymerase
lacO operator
Transcription and translation
Active regulator protein
Inactive regulator protein (repressor)
3 …which then binds to the regulator protein, making the protein inactive.
4 The regulator protein cannot bind to the operator,…
5 …and the structural genes are transcribed and translated.
Transcription and translation
Enzymes
β-Galactosidase
Permease
Transacetylase
Allolactose
2 When lactose is present, some of it is converted into allolactose,…
β-Galactosidase
Lactose
Glucose
Galactose
lacZ gene
lacP (promoter)
lac repressor
5′
3′
TAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCAC
3′
5′
DNA nontemplate strand
–35 region (consensus sequence)
–10 region (consensus sequence)
Transcription start site
Operator bound by lac repressor

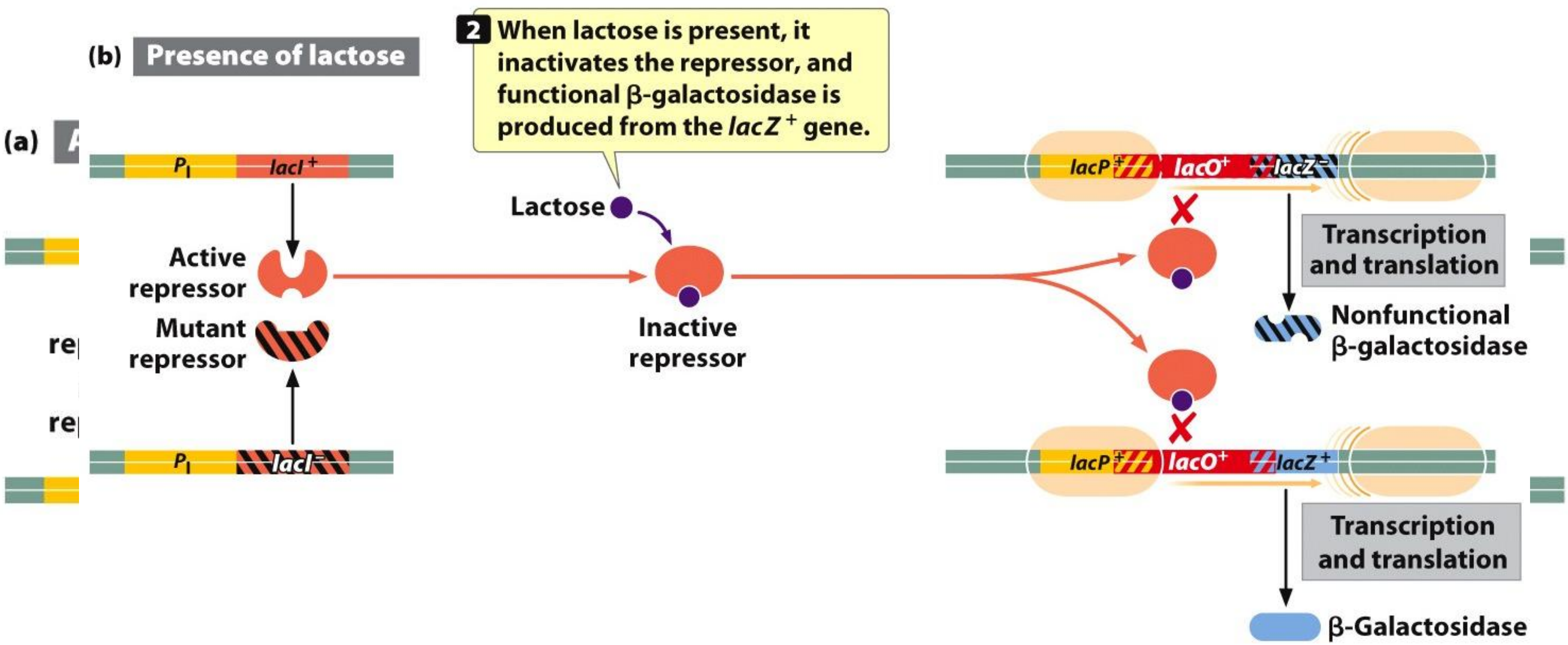
MUTATIONS IN *LAC*

- **Partial diploid: full bacterial chromosome + an extra piece of DNA on F plasmid**
- **Structural-gene mutations: affect the structure of the enzymes, but not the regulations of their synthesis**

- ***lacZ*$^{+}$*lacY*$^{-}$ / *lacZ*$^{-}$*lacY*$^{+}$ produce fully functional β-galactosidase and permease.**

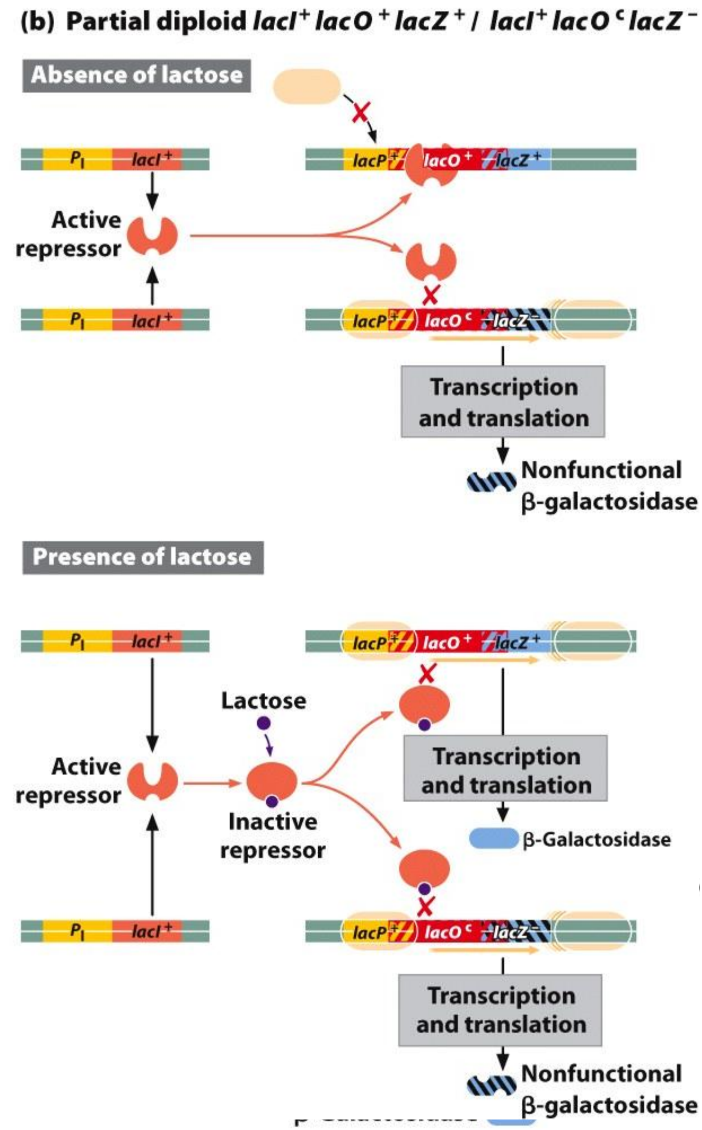
MUTATIONS IN *LAC*

- **Regulator gene mutations**: *lacI*$^{-}$ leads to constitutive transcription of three structure genes.
  - *lacI*$^{+}$ is dominant over *lacI*$^{-}$ and is trans acting. A single copy of *lacI*$^{+}$ brings about normal regulation of *lac* operon.
  - *lacI*$^{+}$*lacZ*$^{-}$ / *lacI*$^{-}$*lacZ*$^{+}$ produce fully functional β-galactosidase.



MUTATIONS IN *LAC*

- **Operator mutations:** $lacO^c$: C = constitutive
  - $lacO^c$ is dominant over $lacO^+$, which is cis acting.
  - $lacI^+ lacO^+ Z^- / lacI^+ lacO^c lacZ^+$ produce fully functional β-galactosidase constitutively.



MUTATIONS IN *LAC*

- **Promoter mutations**
  - ***lacP*$^{-}$: cis acting**

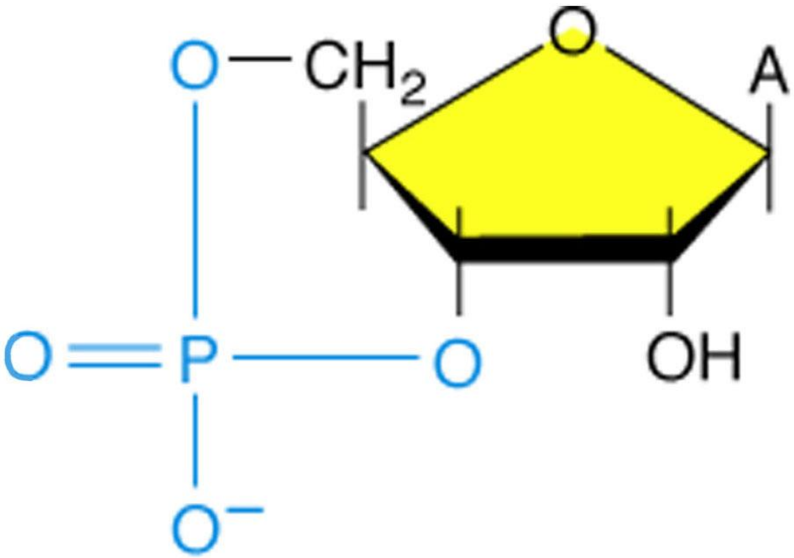
  - ***lacI*$^{+}$*lacP*$^{-}$*lacZ*$^{+}$ / *lacI*$^{+}$*lacP*$^{+}$*lacZ*$^{-}$ fails to produce functional β-galactosidase.**

POSITIVE CONTROL AND CATABOLITE REPRESSION

- **Catabolite repression: using glucose when available, and repressing the metabolite of other sugars.**

- **The positive effect is activated by catabolite activator protein (CAP). cAMP is bound to CAP, together CAP–cAMP complex binds to a site slightly upstream from the *lac* gene promoter.**

Positive control of the *lac* operon



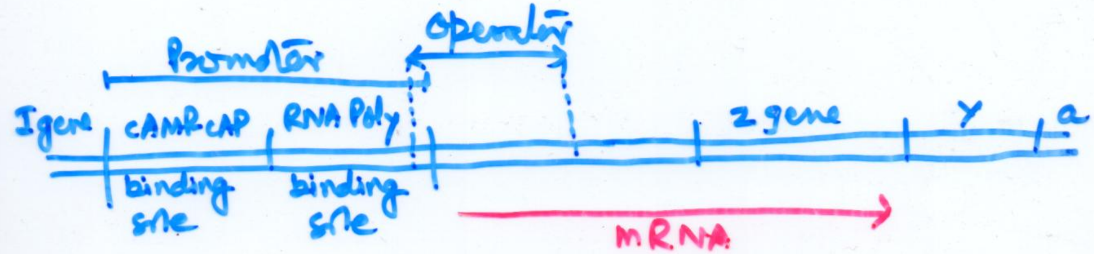
Cyclic-AMP

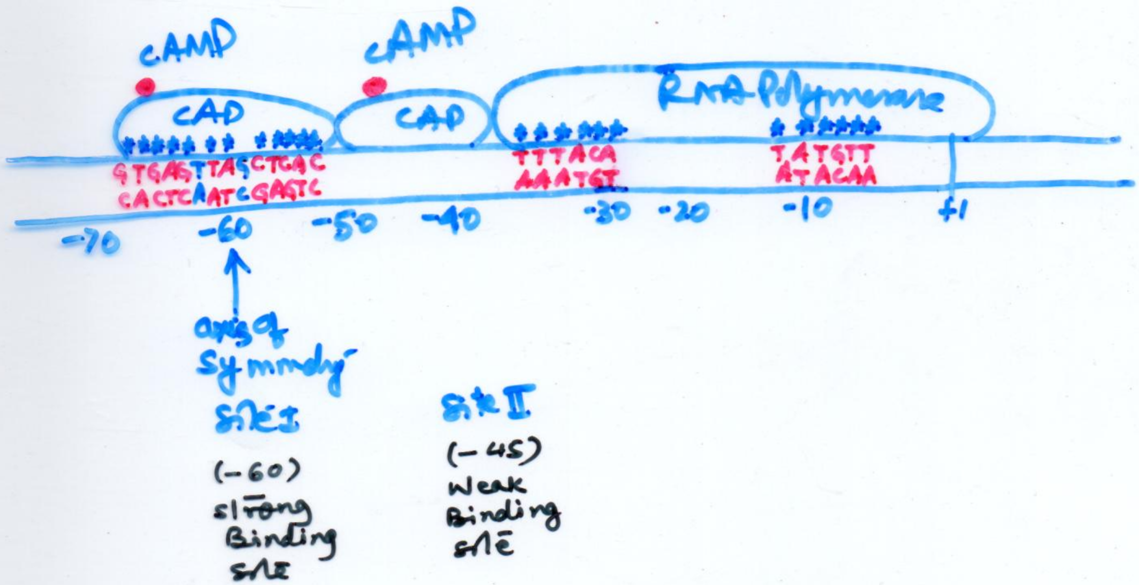
+ **CAP**

(Catabolite Activator Protein)

(These are trans-acting factors)

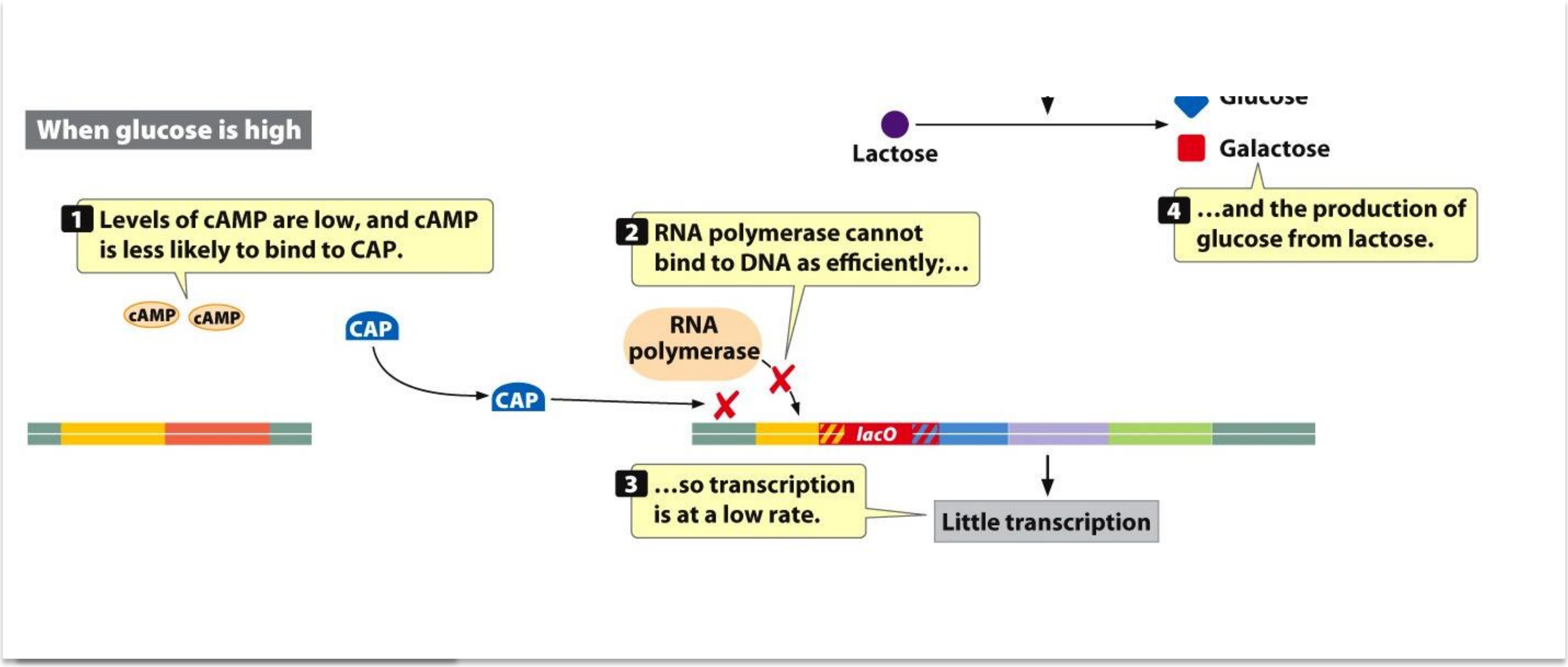
Organization of lac Operon





POSITIVE CONTROL AND CATABOLITE REPRESSION

- **cAMP**—adenosine-3′,5′-cyclic monophosphate
- The concentration of cAMP is inversely proportional to the level of available glucose.



FOUR SITUATIONS ARE POSSIBLE

1. When glucose is **present** and lactose is **absent** the *E. coli* does **not** produce β-galactosidase.

2. When glucose is **present** and lactose is **present** the *E. coli* does **not** produce β-galactosidase.

3. When glucose is **absent** and lactose is **absent** the *E. coli* does **not** produce β-galactosidase.

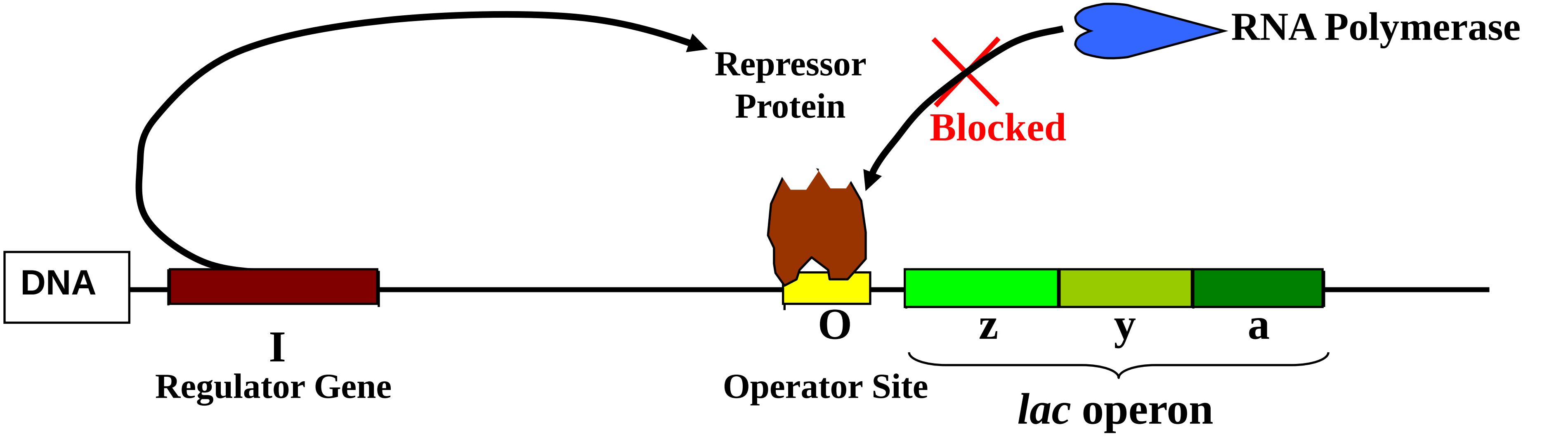
4. When glucose is **absent** and lactose is **present** the *E. coli* **does** produce β-galactosidase

THE CONTROL OF THE *LAC* OPERON

1. WHEN LACTOSE IS ABSENT

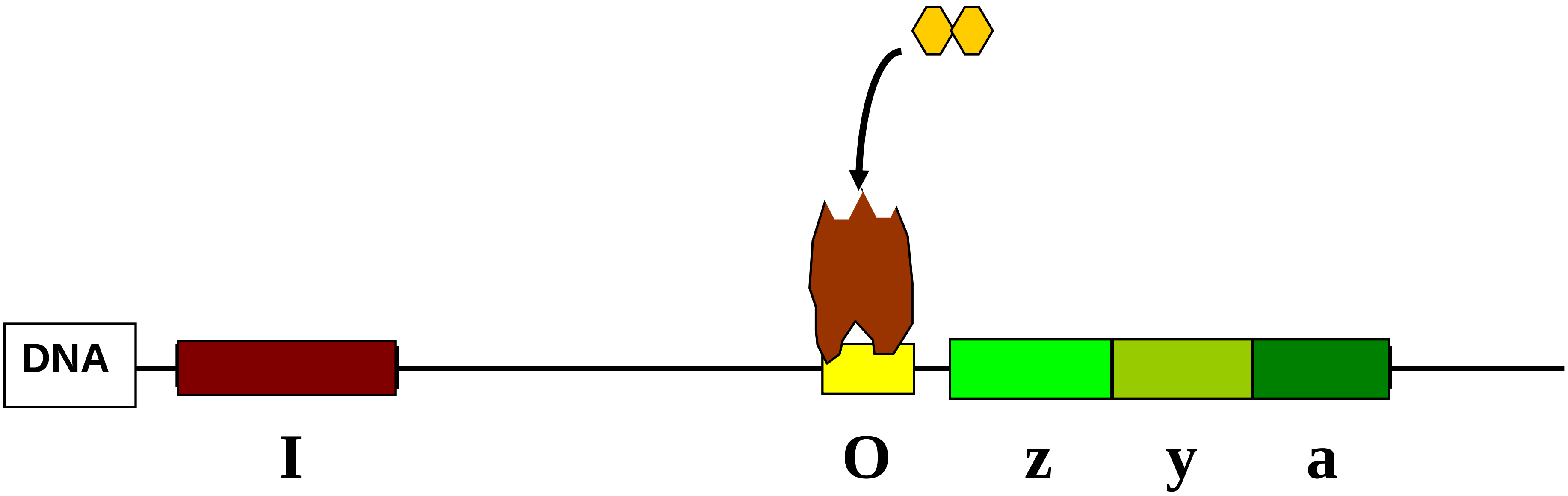
A repressor protein is continuously synthesised. It sits on a sequence of DNA just in front of the *lac* operon, the Operator site

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



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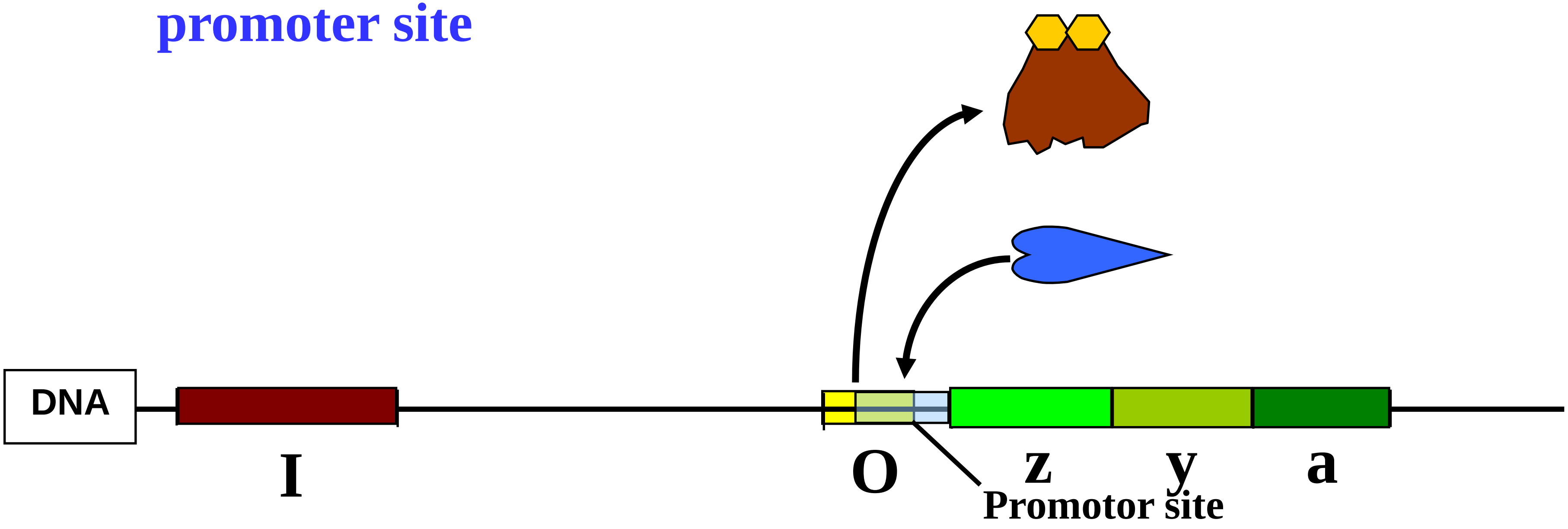
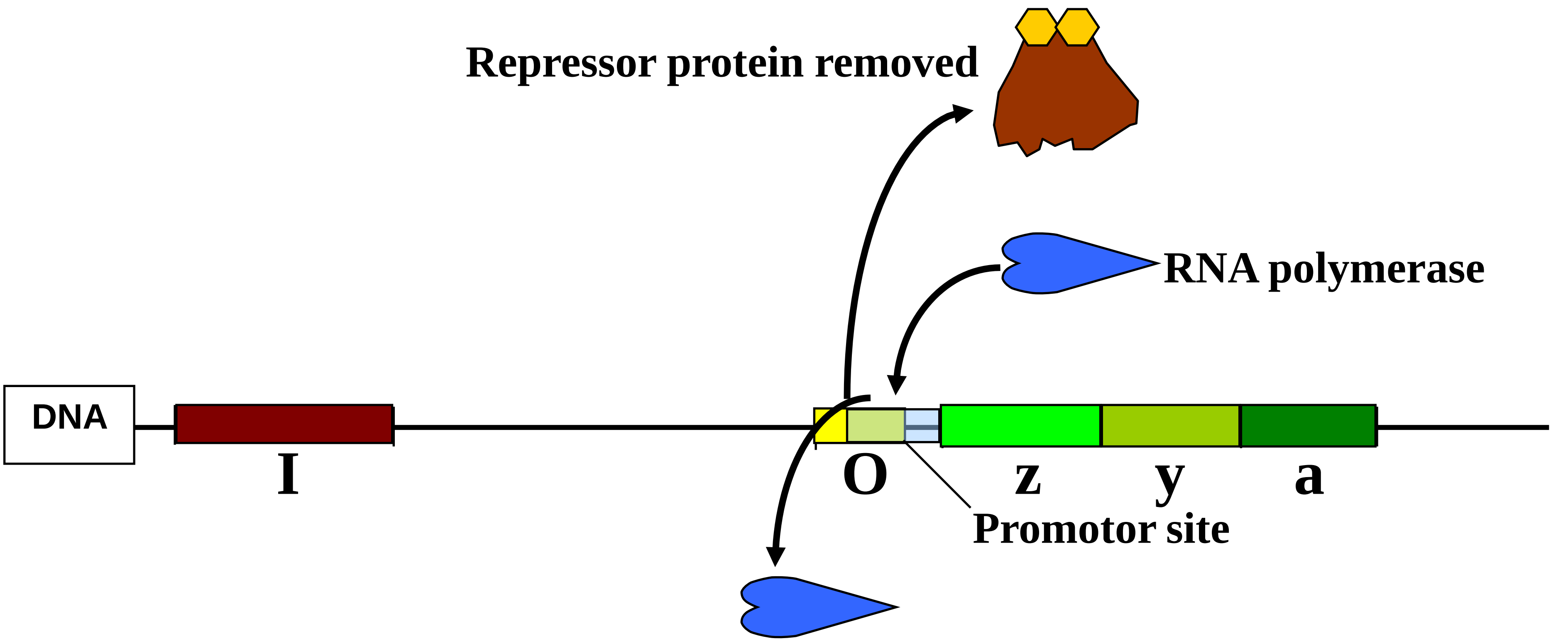


3. WHEN BOTH GLUCOSE AND LACTOSE ARE PRESENT

- **This explains how the *lac* operon is transcribed only when lactose is present.**

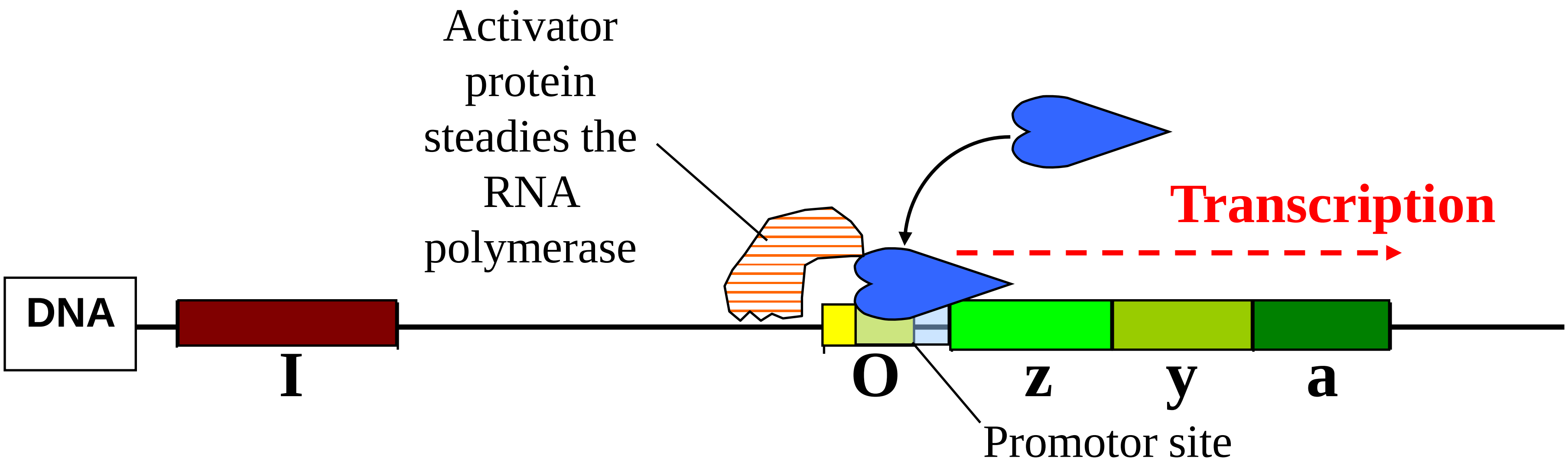
- **BUT….. this does not explain why the operon is not transcribed when both glucose and lactose are present.**

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**