Chapter 12: Regulation of Gene Expression

Transcriptional regulators:

- **Repressors: proteins that bind to DNA and prevent transcription**
  - usually bind to operator regions

- **Activators: proteins that bind to DNA and promote transcription**
  - specific sites depend on the protein- usually a site upstream from promoter

The *lac* Operon: classic example of both negative and positive transcriptional regulation

lac **operon: encodes the inducible catabolic pathway for the sugar lactose**

Contains examples of genes that are:

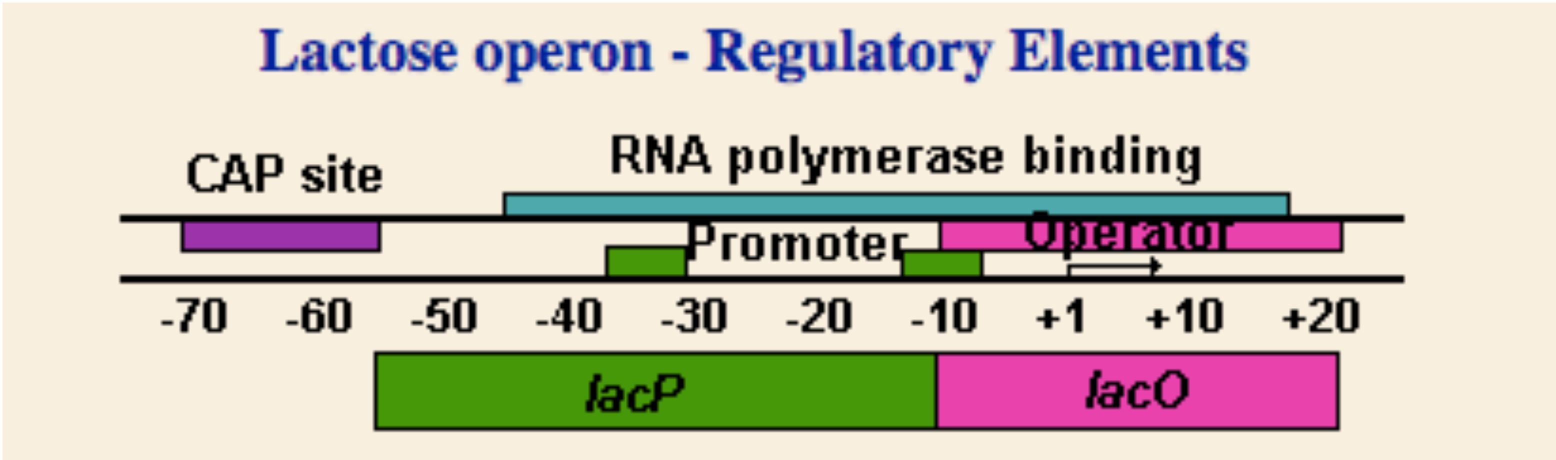
1)
2)
3)

- ***lacI*** **gene: encodes the LacI repressor protein**

  ➢ **LacI is produced constitutively and binds to the *lac* operator (*lacO*) unless bound to the inducer allolactose**

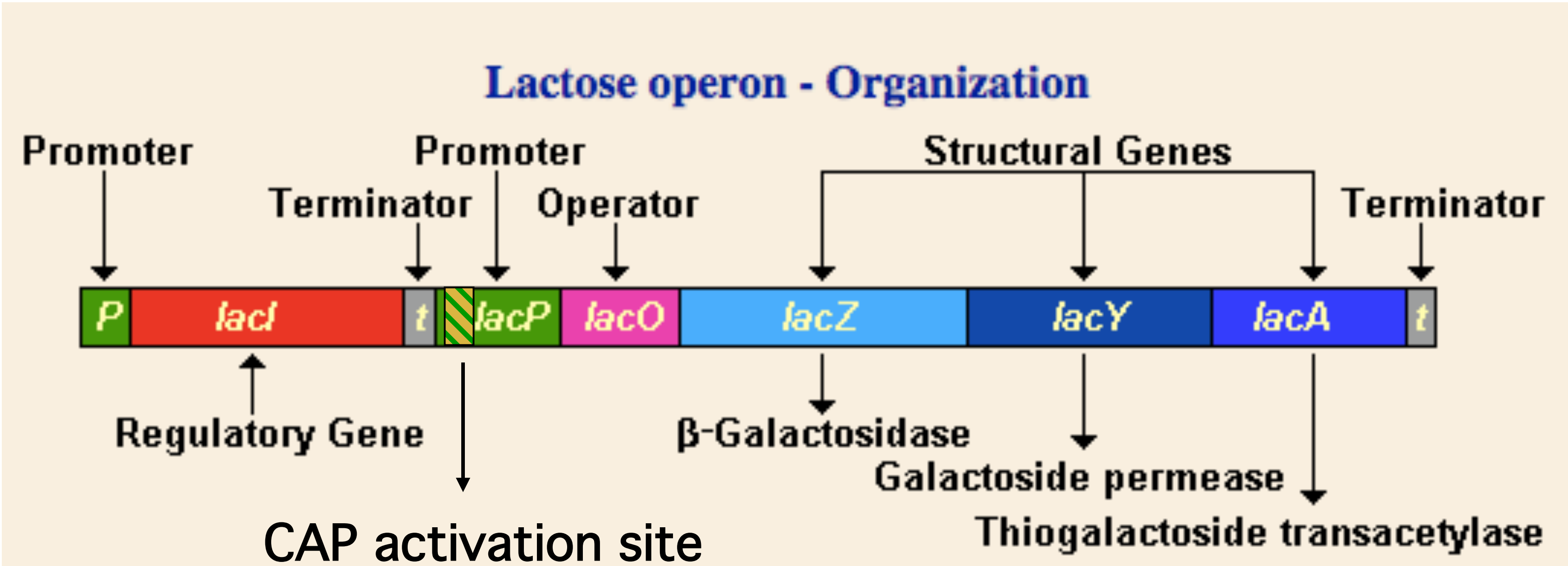
  ↳ **represses expression of the inducible genes, *lacZ*, *lacY* and *lacA*, from the promoter *lacP***

- ***lacZ*** **gene: encodes the enzyme β-galactosidase**
  - **converts lactose to galactose and glucose.**
  - **also converts lactose to allolactose, which serves as a natural inducer of the *lac* operon**
    - ↳
    - ↳ **Allolactose binds to LacI preventing its ability to bind to *lacO***
- ***lacY*** **gene: encodes galactoside permease, which allows transport of lactose into the cell**
- ***lacA*** **gene: encodes thiogalactoside transacetylase, which serves as a possible detoxifier in the cell.**
- ***lacP*****:**
  -
- ***lacO*****: operator for *lacZYA* that is bound by LacI**



For optimal transcription of the *lac* operon

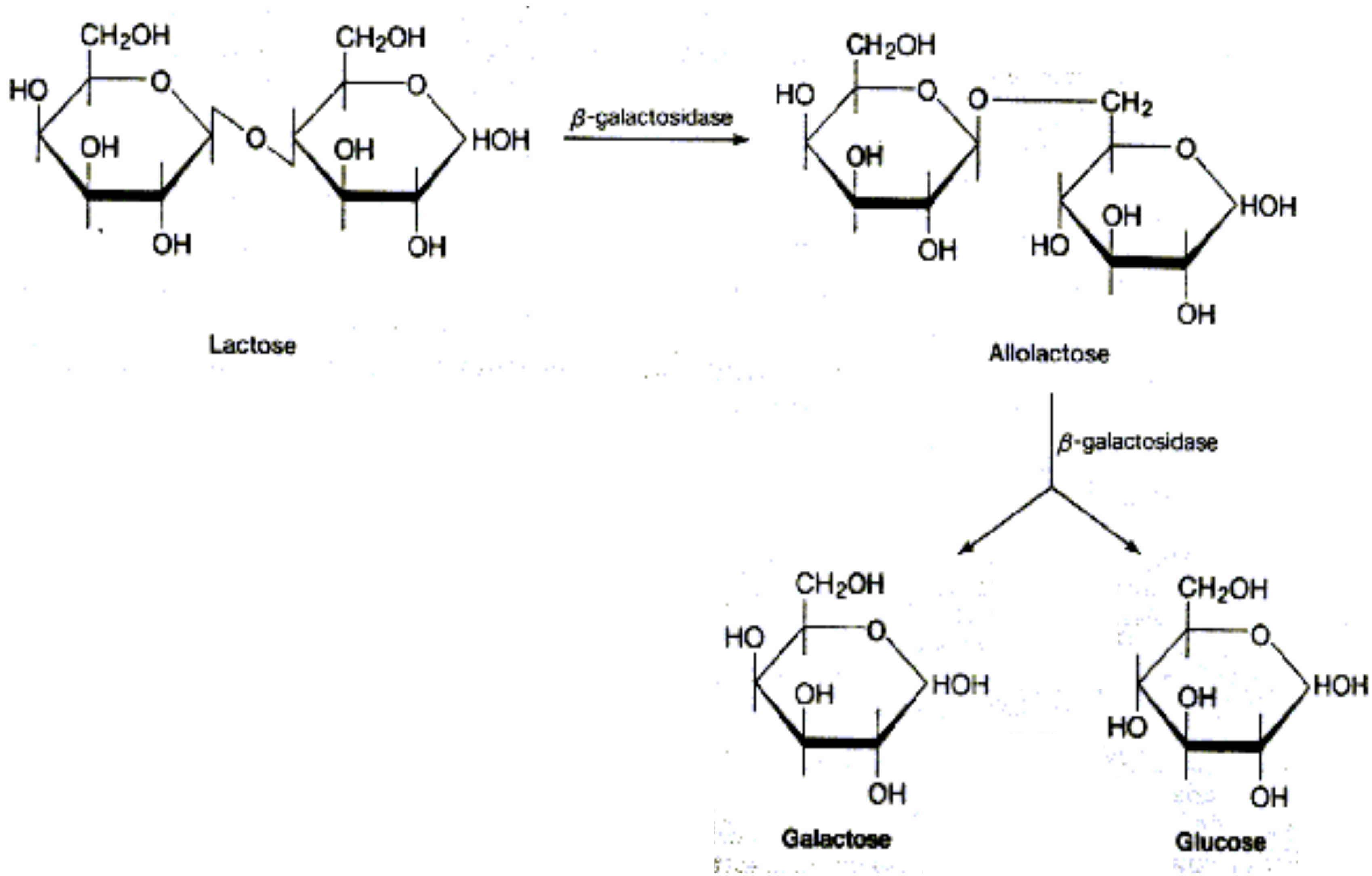
- **cAMP-CAP binds to the CAP site recruiting RNA polymerase to bind to *lacP***
  - ➢ **The binding bends the DNA, making the RNA polymerase entry site on the promoter accessible**
- **LacI must not be bound to *lacO* or transcription of the structural genes will be blocked**



- **Important to note that low level expression occurs from *lacP* all of the time regardless of the presence of lactose or the absence of glucose**

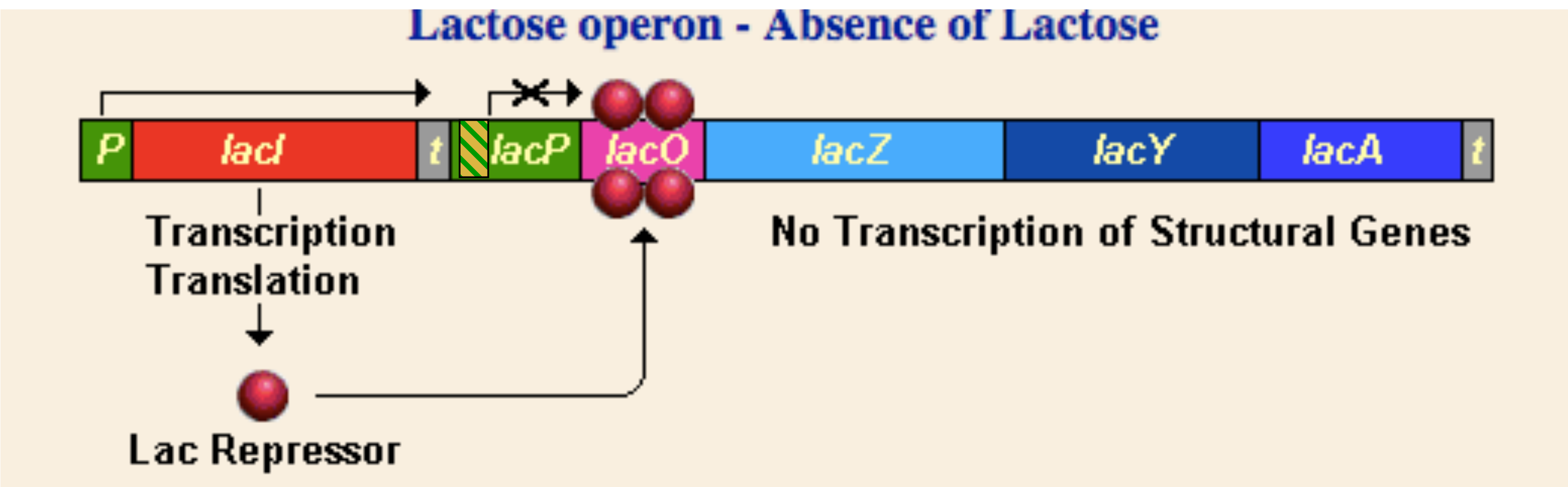
➢

- **Expression from *lacP* increases 1000 fold if lactose present and glucose absent**


CH2OH
HO
OH
OH
HOH
Lactose
β-galactosidase
Allolactose
CH2
Galactose
Glucose

If lactose is absent and glucose is present:

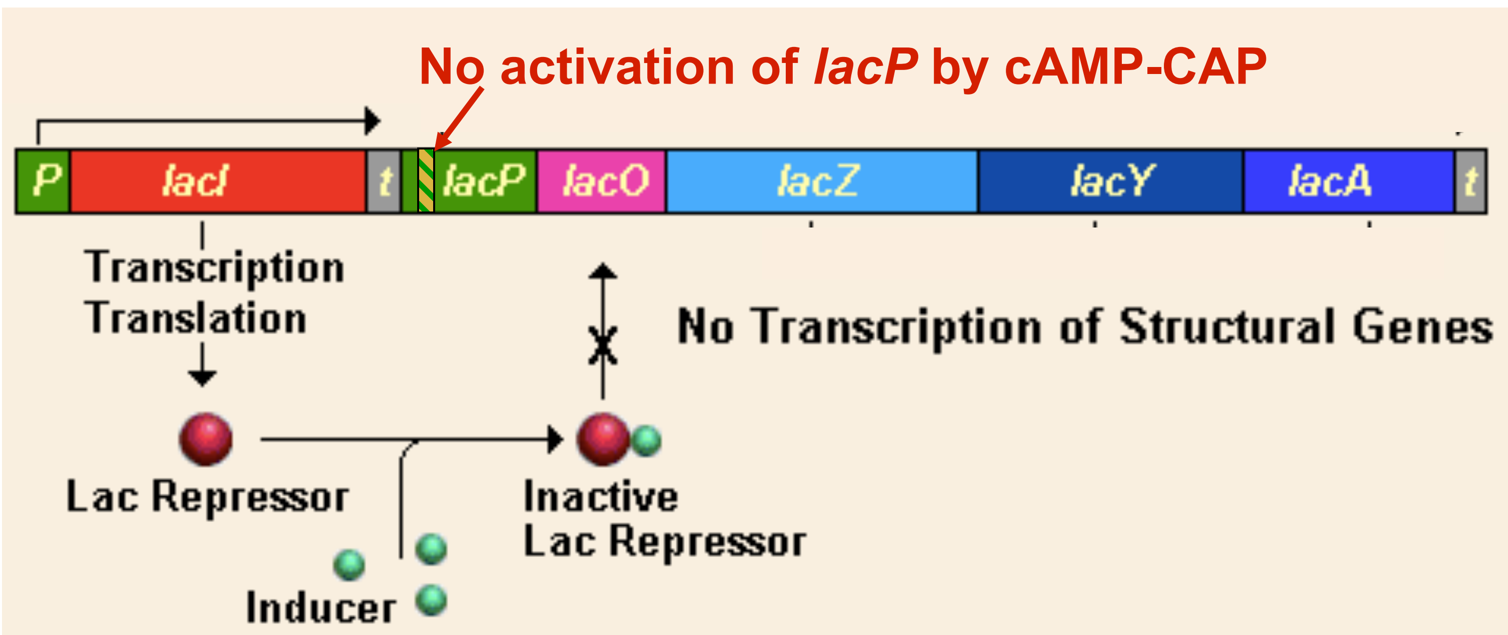
- **LacI binds to *lacO* preventing transcription from *lacP***
- **Presence of glucose leads to catabolite repression and thus no cAMP-CAP is formed to activate transcription from *lacP***
  - ➢ **Catabolite repression: some component released during catabolism of glucose results in inhibition of sugar utilization operons including lactose**



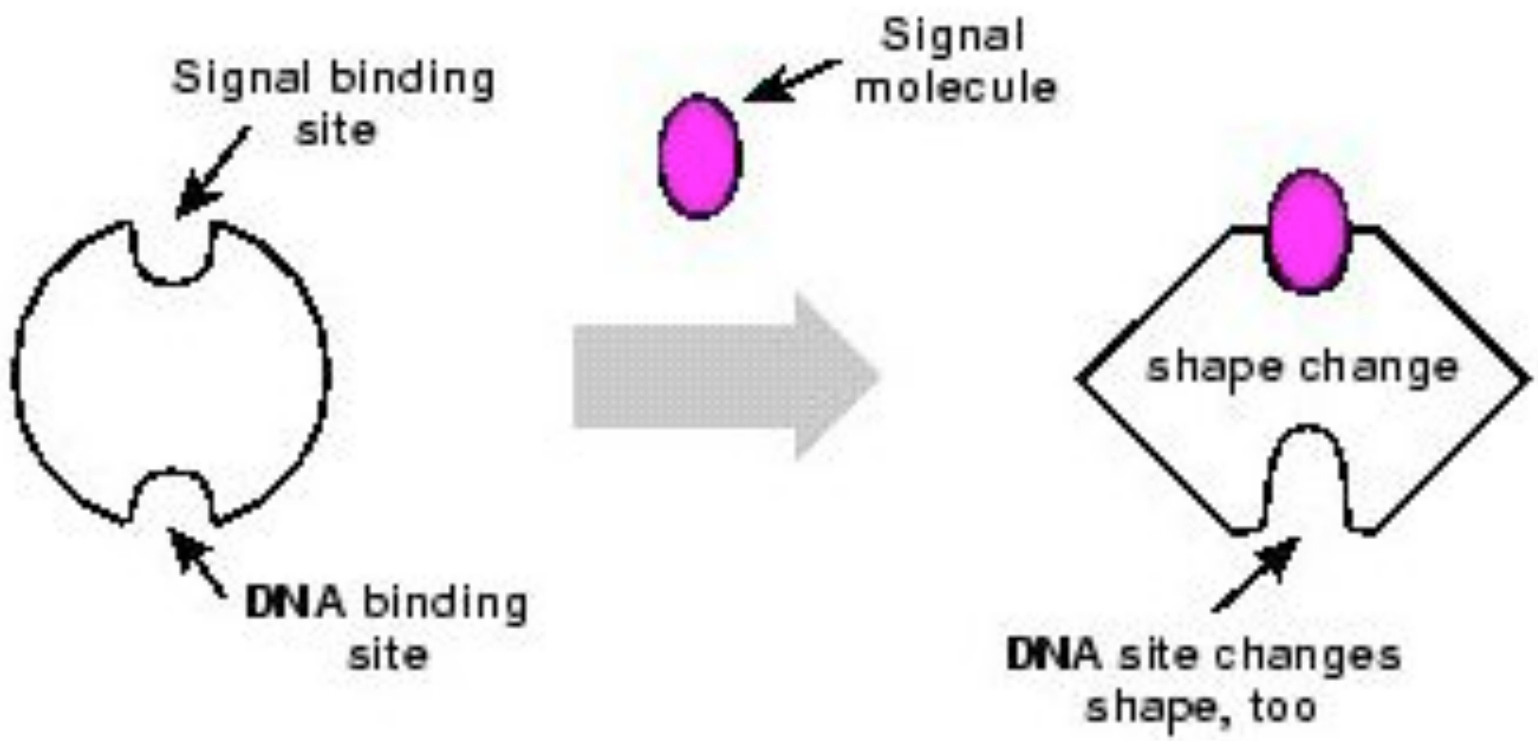
If lactose is present and glucose is present:

- **Allolactose binds to LacI preventing the repressor from binding *lacO***
  - ➢ Binding of the inducer to LacI facilitates <u>allosterism</u>

- **Presence of glucose causes catabolite repression and thus no activation of *lacP***

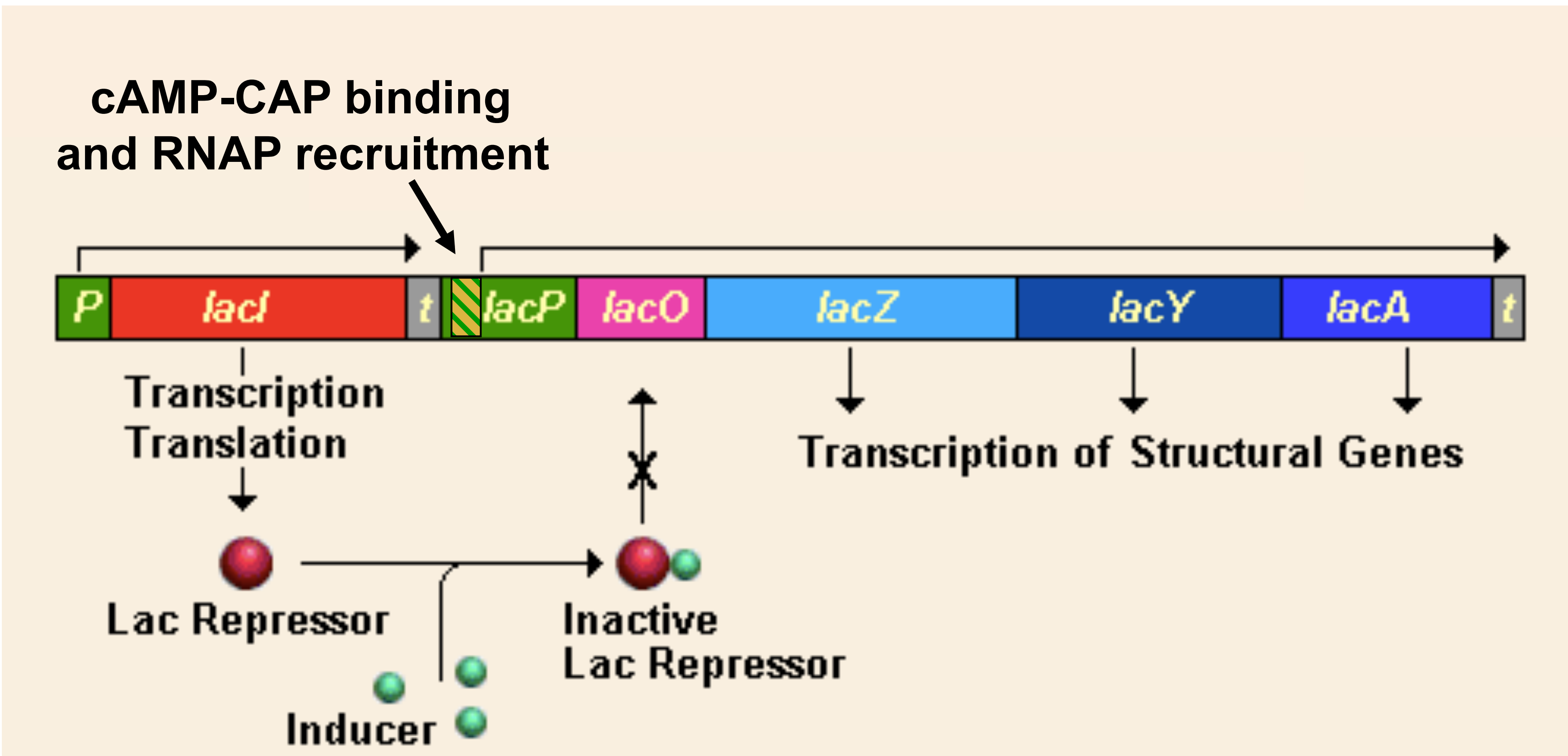


Allosterism and regulatory proteins



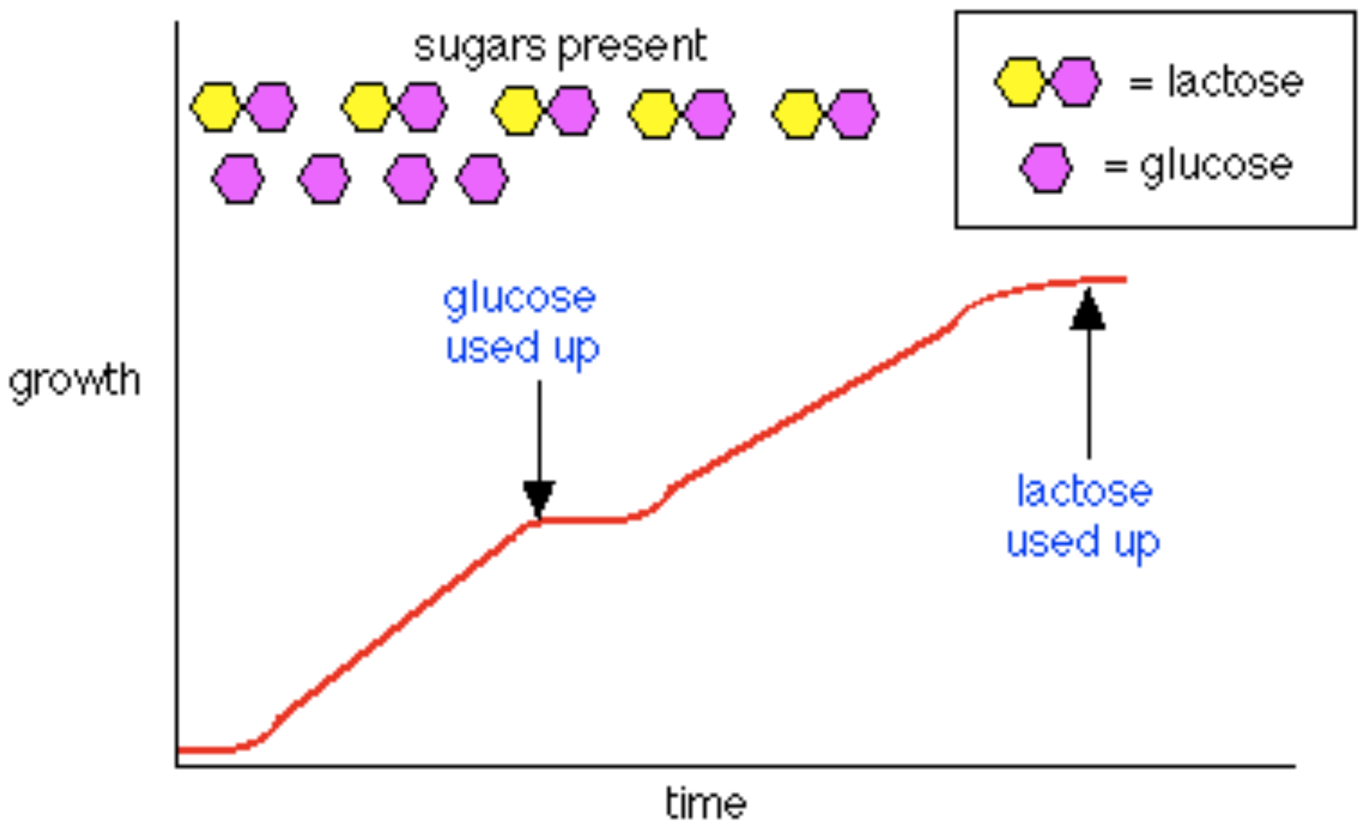
If lactose is present and glucose is absent:

- Allolactose binds to LacI preventing the repressor from binding *lacO*
- Absence of glucose allows for production of cAMP-CAP for activation of *lacP*



Presence of glucose and lactose leads to diauxic growth:

After glucose is completely degraded a pause in growth occurs while the *lac* operon is both <u>derepressed</u> and <u>activated</u>



Mutations and the *lac* Operon

If glucose is absent, will get transcription of the *lacZYA* genes with or without the presence of lactose

- ***lacO*^{C}: mutation in the operator that prevents binding of LacI**
- ***lacI*$^{-}$: LacI mutants that are unable to bind the operator**

Transcription of *lacZYA* will not happen with the following:

- ***lacI*^{S}:**
  - **Considered a superrepressor mutant b/c LacI^{S} will always be bound to *lacO* regardless of condition**
- ***lacP*$^{-}$:**

Repressor quantity mutants:

- *lacI*^{Q}: Mutation in which the concentration of LacI produced is higher

- *lacI*^{SQ}: Mutation in which the concentration of LacI produced is higher than that in a *lacI*^{Q} mutant

  - ➢ Mutations generally occur in the promoter for *lacI* resulting in increased expression

  - ➢

Cis-dominant vs. Trans-dominant Mutants

- Cis-dominant: mutations whose effects are confined only to the DNA in which they are linked

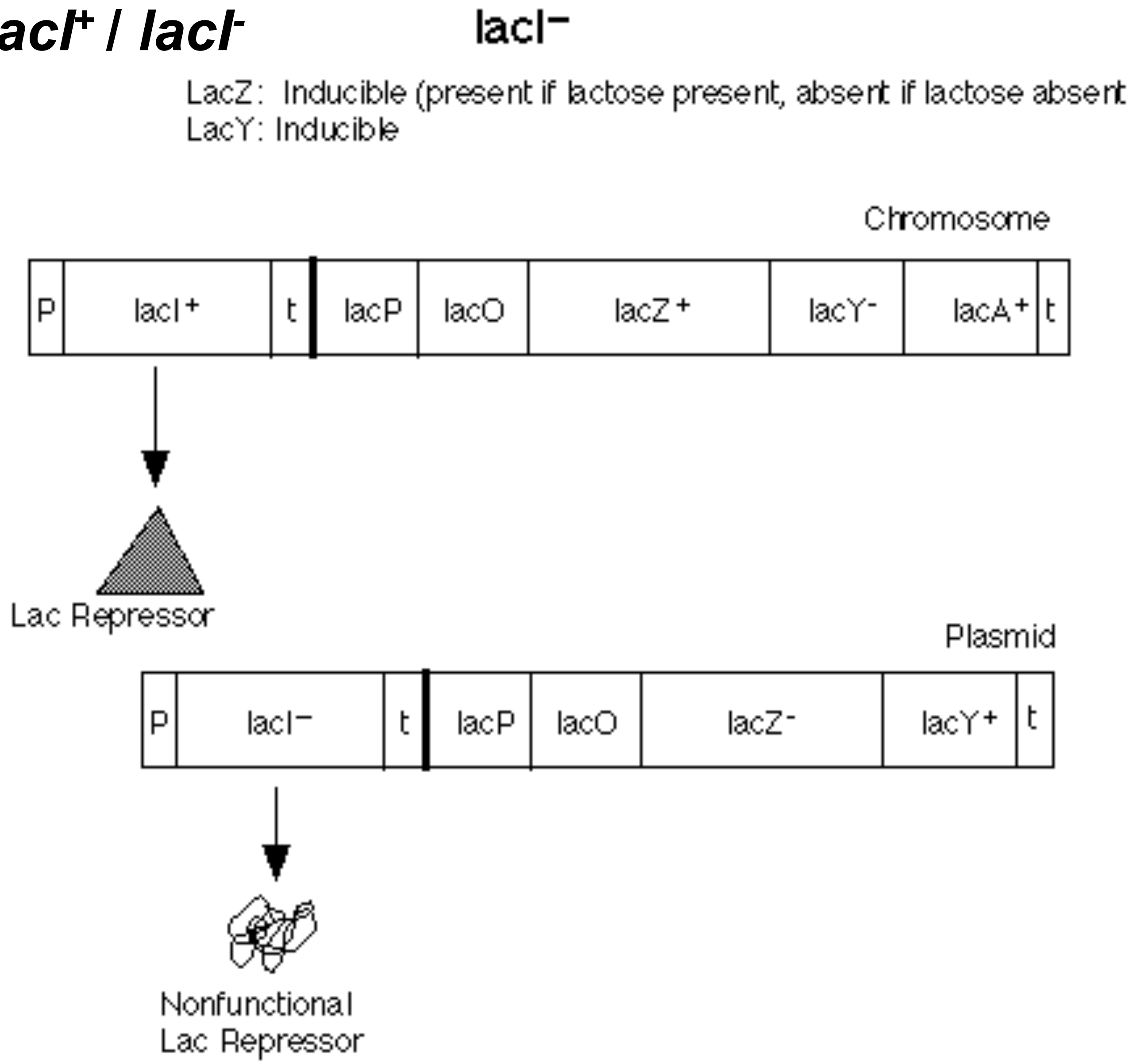
  - *lacO* and *lacP* mutations affect expression of *lacZYA* genes on the same piece of DNA

- Trans-dominant: mutations that exert control over the operon even the if the mutation not located on the same piece of DNA

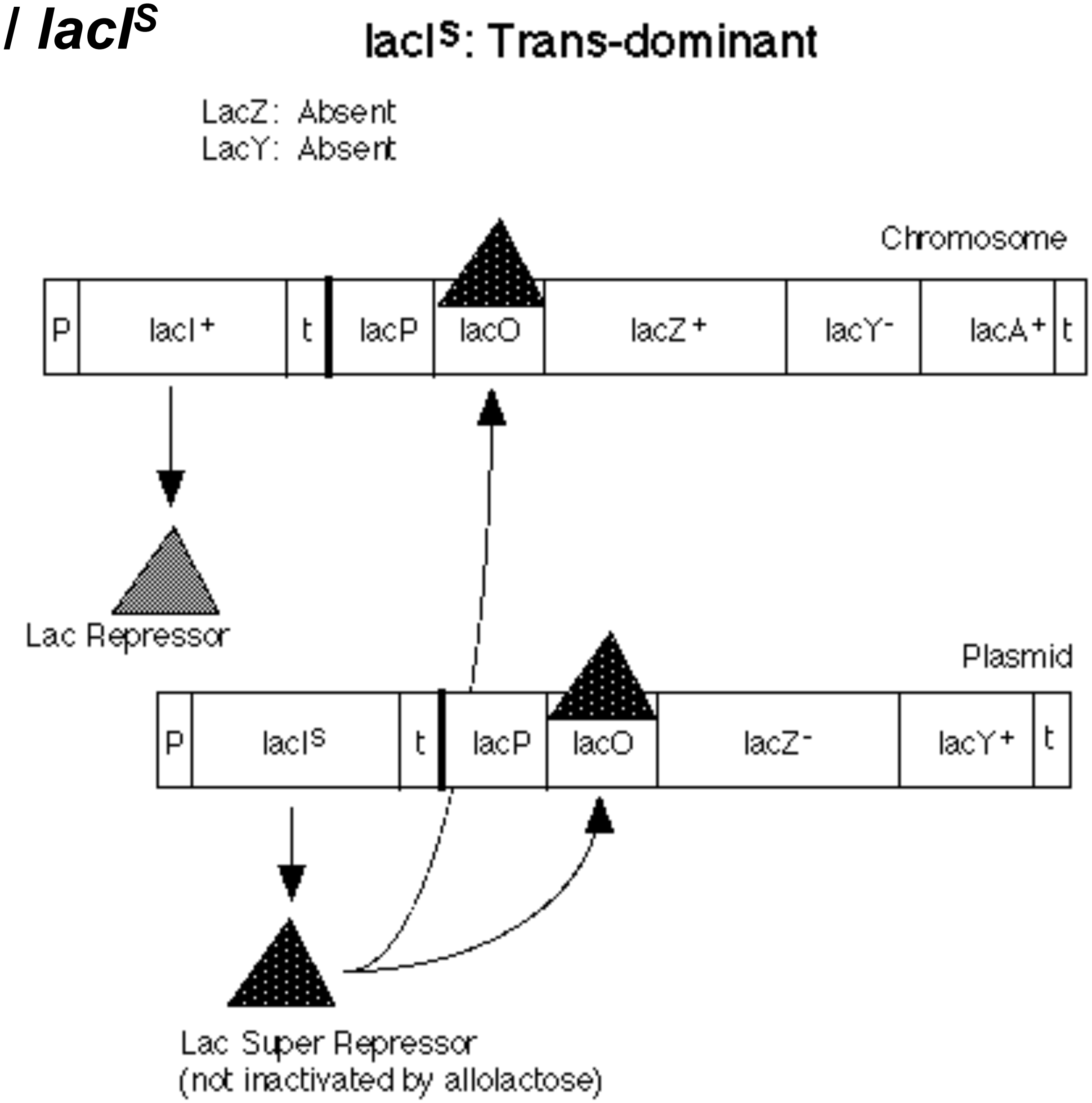
  - To determine if a mutation is trans-dominant, a merodiploid must be constructed

Examples of trans-dominant mutations:

- **Merodiploid *lacI*$^+$ / *lacI*$^-$**



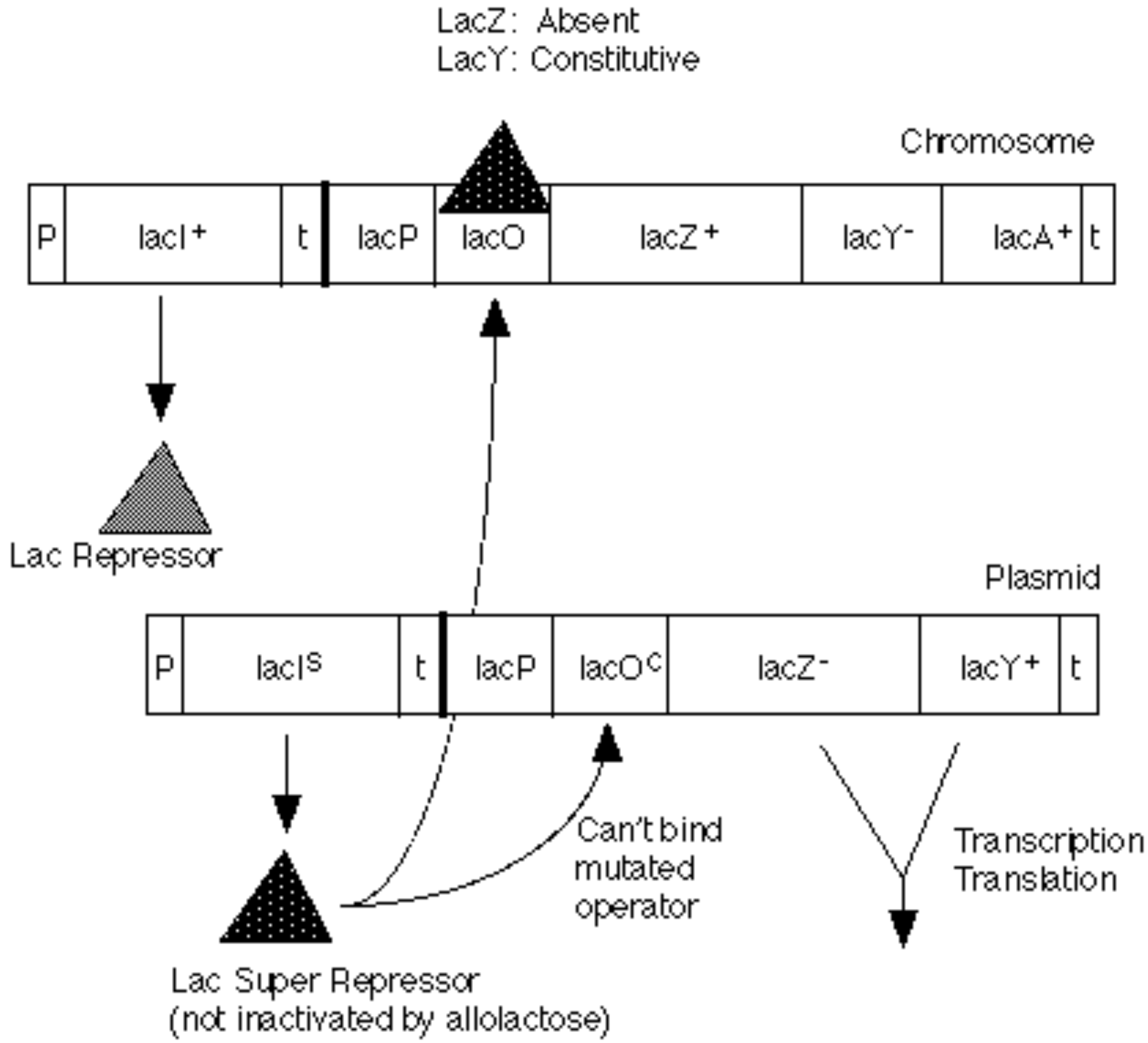
• **Merodiploid *lacI*$^{+}$ / *lacI*^{S}**



• **Merodiploid *lacI*^{S} / *lacO*^{C}**

lacI^{S}: Trans-dominant

lacO^{C}: Cis-dominant



Determining the expression of *lac* genes in merodiploids

For each of the following examples determine whether the expression of *lacZ* and *lacY* is inducible, constitutive, or absent. Assume that no glucose is present and wild type for those genotypes not given.

1) $lacO^{C}\ lacZ^{+}\ lacY^{-}\ /\ lacO^{-}\ lacZ^{-}\ lacY^{+}$

2) $lacP^{-}\ lacI^{+}\ lacZ^{+}\ lacY^{-}\ /\ lacP^{+} lacI^{-}\ lacZ^{-}\ lacY^{+}$

3) $\boldsymbol{lacI^{S}\ lacZ^{-}\ lacY^{-}\ /\ lacI^{-}\ lacZ^{+}\ lacY^{+}}$

4) $\boldsymbol{lacI^{-}\ lacO^{C}\ lacZ^{+}\ lacY^{-}\ /\ lacI^{Q}\ lacO^{+}\ lacZ^{-}\ lacY^{+}}$

5) $\boldsymbol{lacP^{-}\ lacI^{SQ}\ lacZ^{+}\ lacY^{-}\ /\ lacP^{+}\ lacI^{-}\ lacZ^{-}\ lacY^{+}}$

6) $\boldsymbol{lacI^{S}\ lacO^{C}\ lacZ^{-}\ lacY^{+}\ /\ lacI^{-}\ lacO^{+}\ lacZ^{+}\ lacY^{-}}$

7) $\boldsymbol{lacP^{-}\ lacI^{S}\ lacZ^{-}\ lacY^{+}\ /\ lacP^{+}\ lacI^{-}\ lacZ^{+}\ lacY^{-}}$

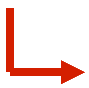
8) $lacI^- \ lacO^C \ lacZ^- \ lacY^+$ / $lacI^+ \ lacO^+ \ lacZ^+ \ lacY^-$

9) $lacI^+ \ lacP^- \ lacO^C \ lacZ^+ \ lacY^-$ / $lacI^- \ lacP^+ \ lacO^+ \ lacZ^- \ lacY^+$

10) $lacI^S \ lacO^+ \ lacZ^+ \ lacY^-$ / $lacI^- \ lacO^C \ lacZ^- \ lacY^+$

Experimental Uses of the *lac* Operon

- ***lacZ* gene is the most widely used reporter gene**
  - ➢ **Look for activation of specific promoters or expression of specific genes by inserting the *lacZ* gene directly downstream**



- **MCS of a vector within the *lacZ* gene for recombinant transformant selection**
  - ➢ **Use a cell line that is *lacI*$^-$ and a promoter that does not require the presence of cAMP for activity**
- ***lacP* in expression vectors**
  - ➢ **Expression of cloned genes can be controlled by addition of the inducer IPTG**

Review