Gene Transcription in Prokaryotes

- Operons: in prokaryotes, genes that encode protein participating in a common pathway are organized together.
  - This group of genes, arranged in tandem, is called an OPERON.
- Genes of an operon are transcribed together into a single mRNA molecule:
  - polycistronic mRNA.
- There are two types of bacterial genes:
  - Constitutive and
  - Inducible.

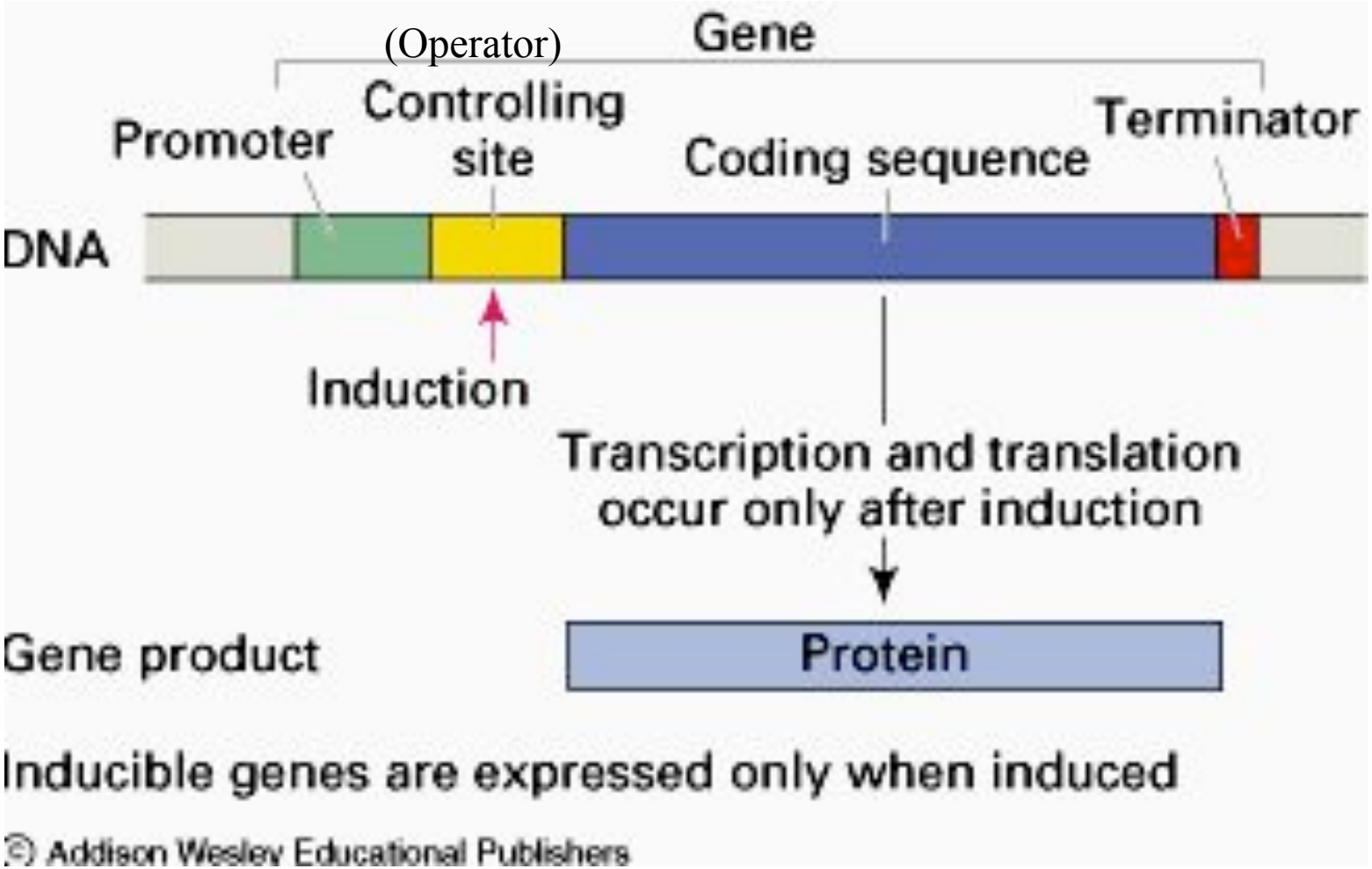
Constitutive Genes

- Gene is always on
- Remains at a constant high level
- Cannot be modulated

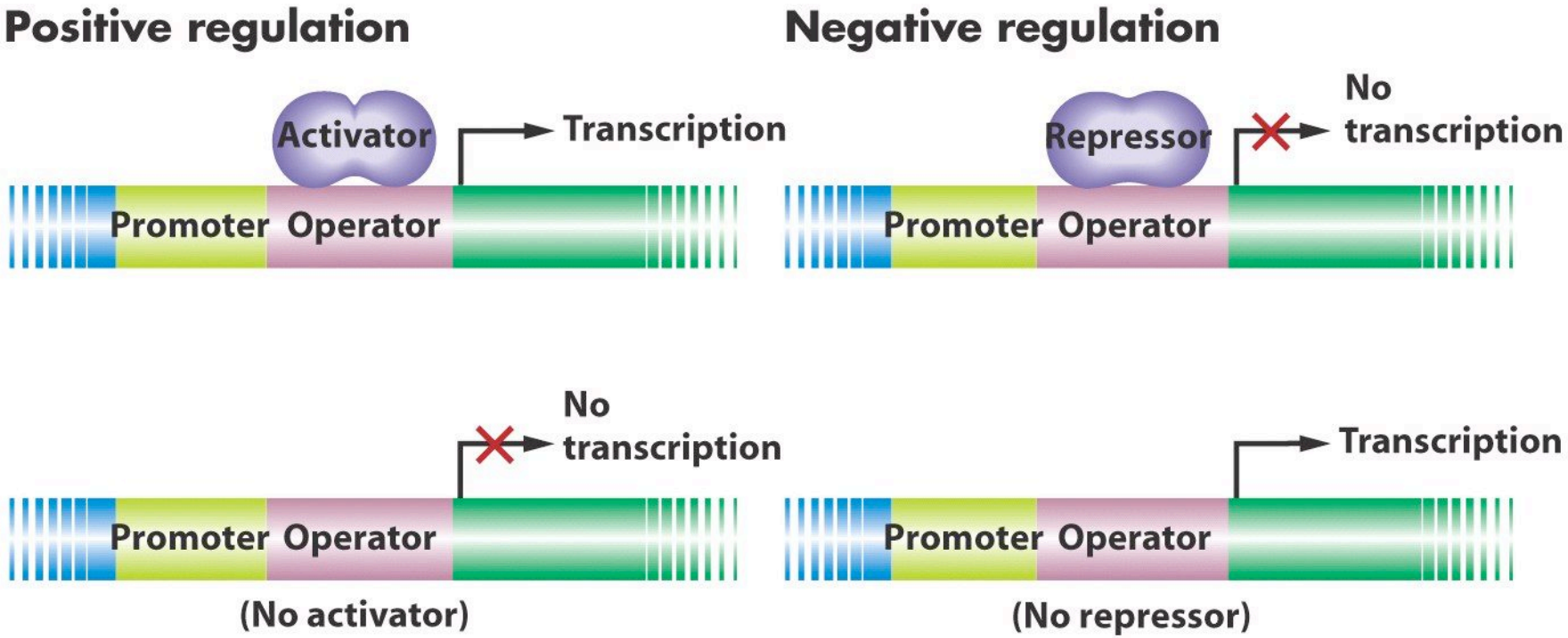
Inducible Genes

- They can be turned on or off
  - depending on the environment they are in.
- An Inducer acts as a ‘switch’ to turn the gene on or off.
  - a chemical substance in the nutrient medium
- The Inducer influences the transcription of the inducible gene(s) via controlling sites called Operators
  - on the DNA adjacent to the coding sequence of the gene(s).
- The Operatoris usually where a regulatory protein binds.

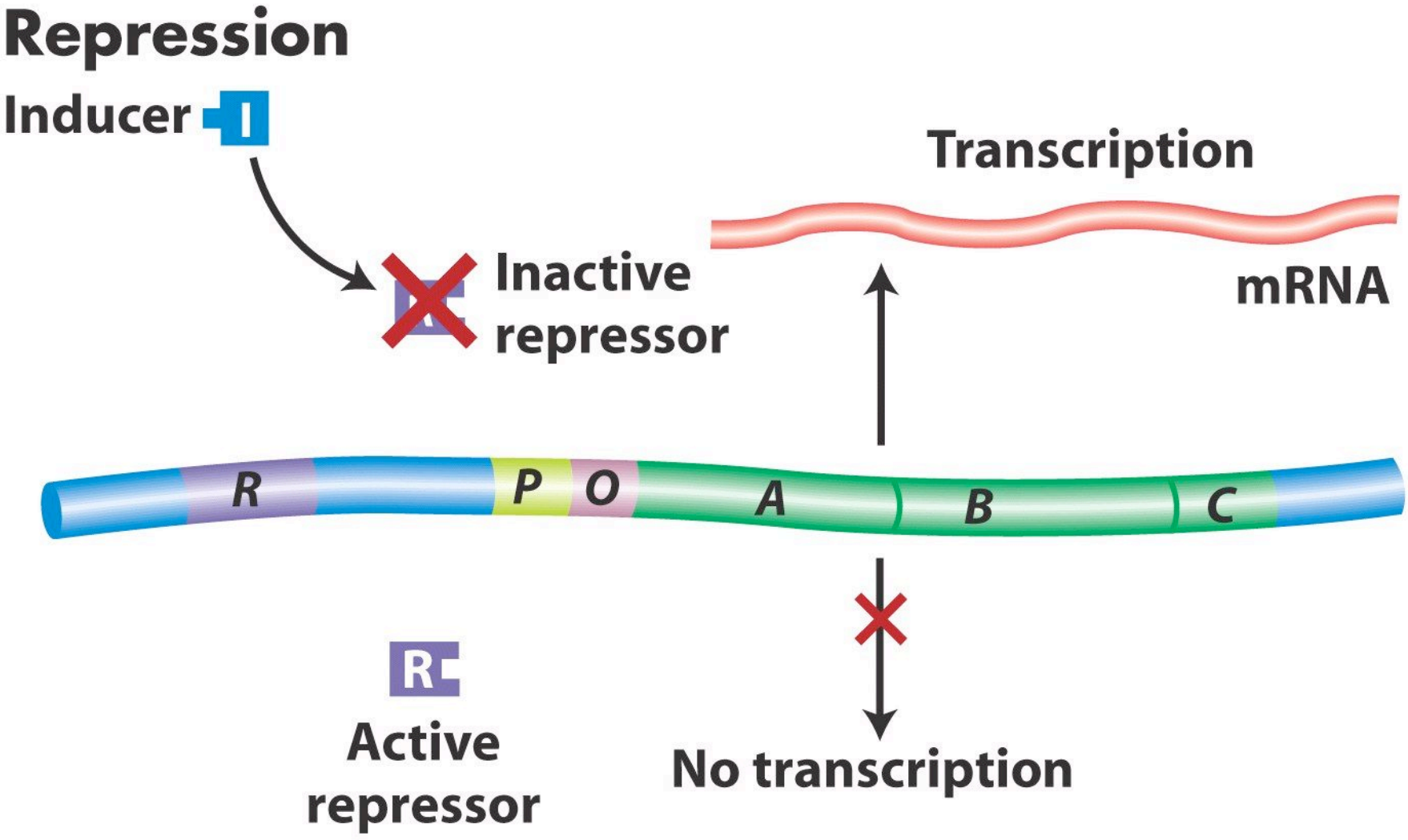
General Organization of an Inducible Gene



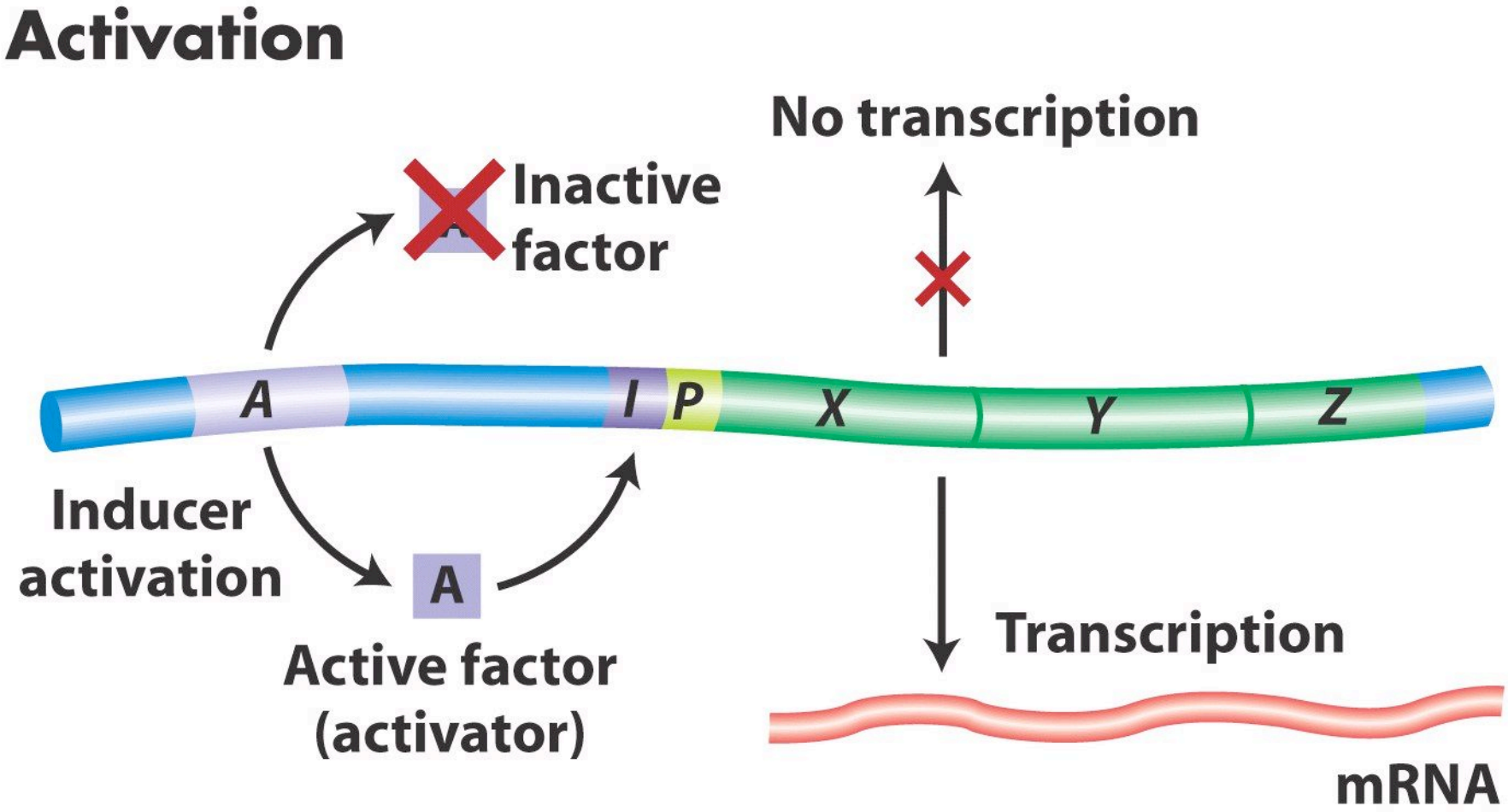
Regulatory Proteins can activate or block transcription of inducible genes



Generic Negative Regulation



Generic Positive Regulation



Inducible genes: The *lac* Operon

- E. *Coli* normally grow in a simple medium containing
  - salts,
  - a nitrogen source and
  - a carbon source.

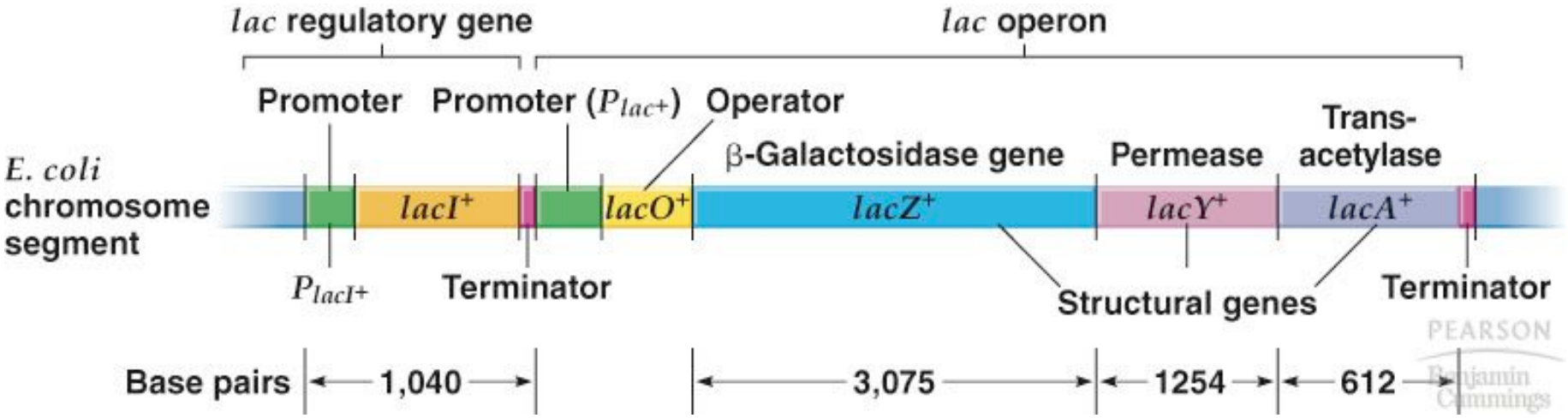
- The carbon source is usually glucose.

- The enzymes required for glucose utilization are constitutively expressed.

- If the nutrient medium contains lactose instead of glucose as the carbon source
  - the bacteria make enzymes that allow them to utilize lactose

- ***These genes are therefore inducible, and lactose is the inducer***.
  - ***These genes form the lac Operon.***

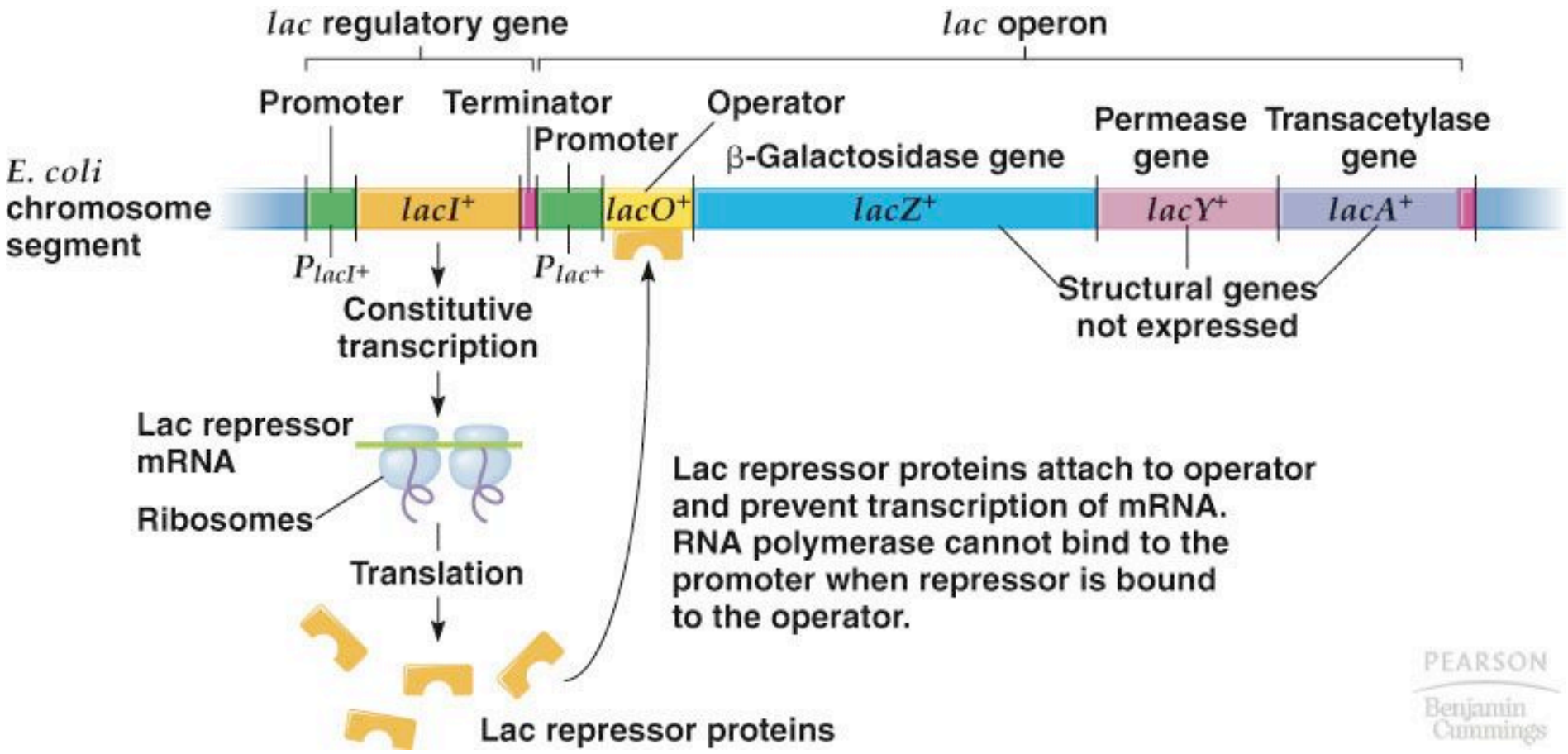
Details of Organization of the *lac* genes in E. Coli



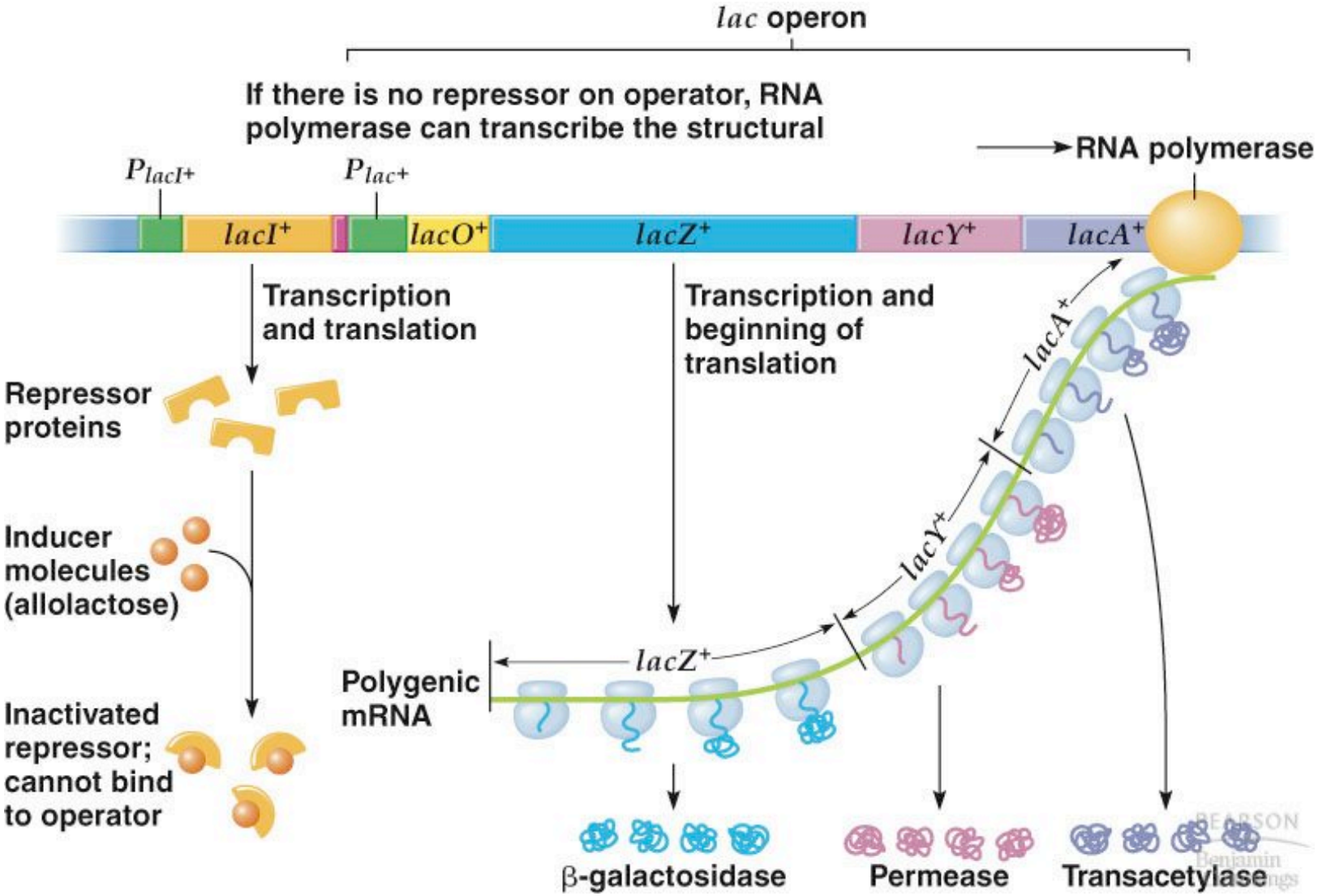
Regulation of *lac* Operon

- The *lac* operon consists of the three functional genes
- *lac Z*, *lac Y* and *lac A*, plus:
  - The operator region *lac O*, just downstream from the promoter region:
    - ***this DNA sequence directly mediates cis-regulation***
  - The repressor region, *lac I*, just upstream from the promoter region:
    - ***this region constitutively synthesizes repressor proteins***
    - ***which mediate trans-regulation by binding to the lac O sequence.***
- In the presence of lactose, allolactose is formed,
  - which binds to the repressor protein,
  - rendering it incapable of binding to the *lac O* sequence.
- This prevents repression of the lac operon
  - causing high level expression.

Regulation of *lac* operon in Wild-Type E.*Coli* in the absence of Lactose



Regulation of *lac* operon in the presence of Lactose



How are the *lac* operon genes regulated?

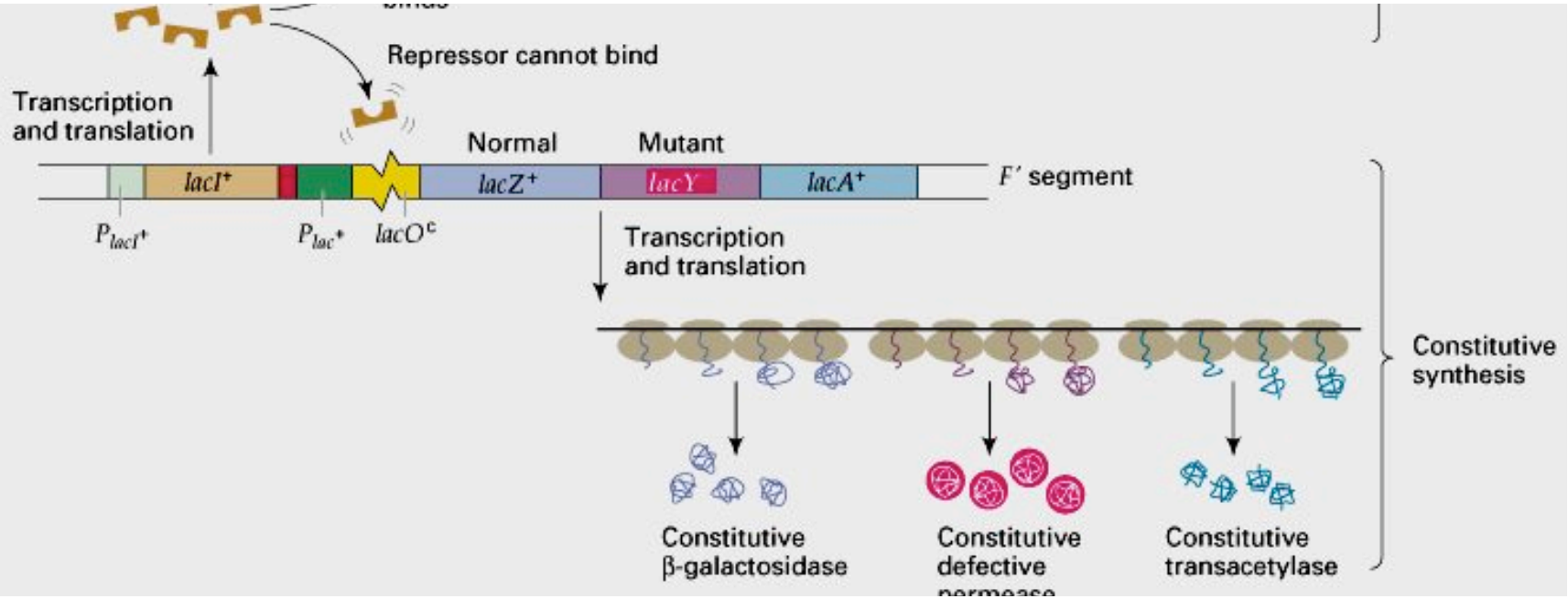
- Two scientists, Jacob and Monod, developed mutant strains of E. *Coli* in which the lac operon genes were constitutively expressed.

- They identified two types of mutants:
  - Mutation in a sequence termed *lac O*,
    - upstream of the *lac* Z gene.
    - Now we know as the Operator
  - Mutation in a sequence further upstream termed *lac I*.

- If either of these regions was mutated
  - there was constitutive expression of all of the *lac* operon genes.
  - THEY MUST BE IMPORTANT FOR REGULATION!

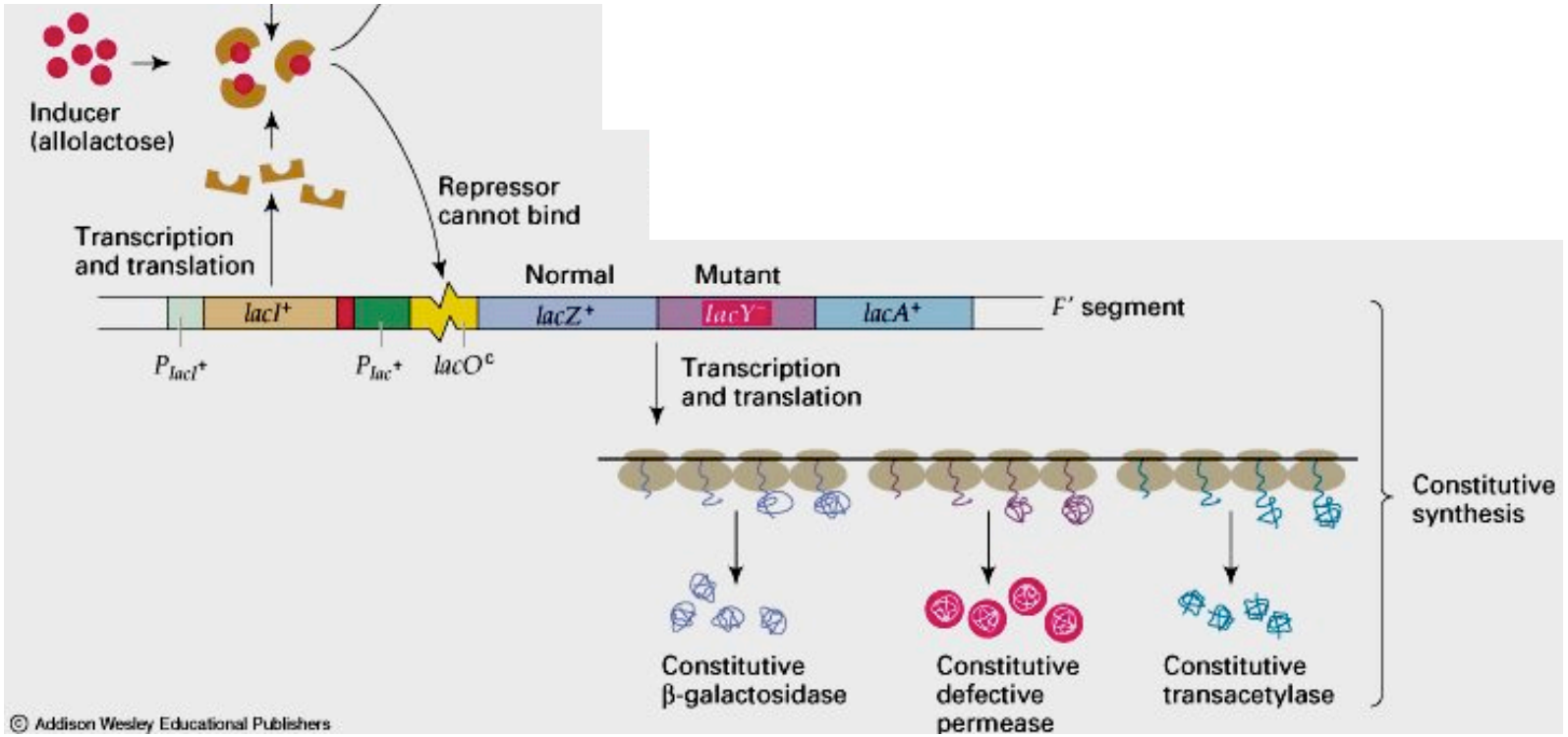
lac O^c (constitutive mutant)

Absence of Inducer (No Lactose):

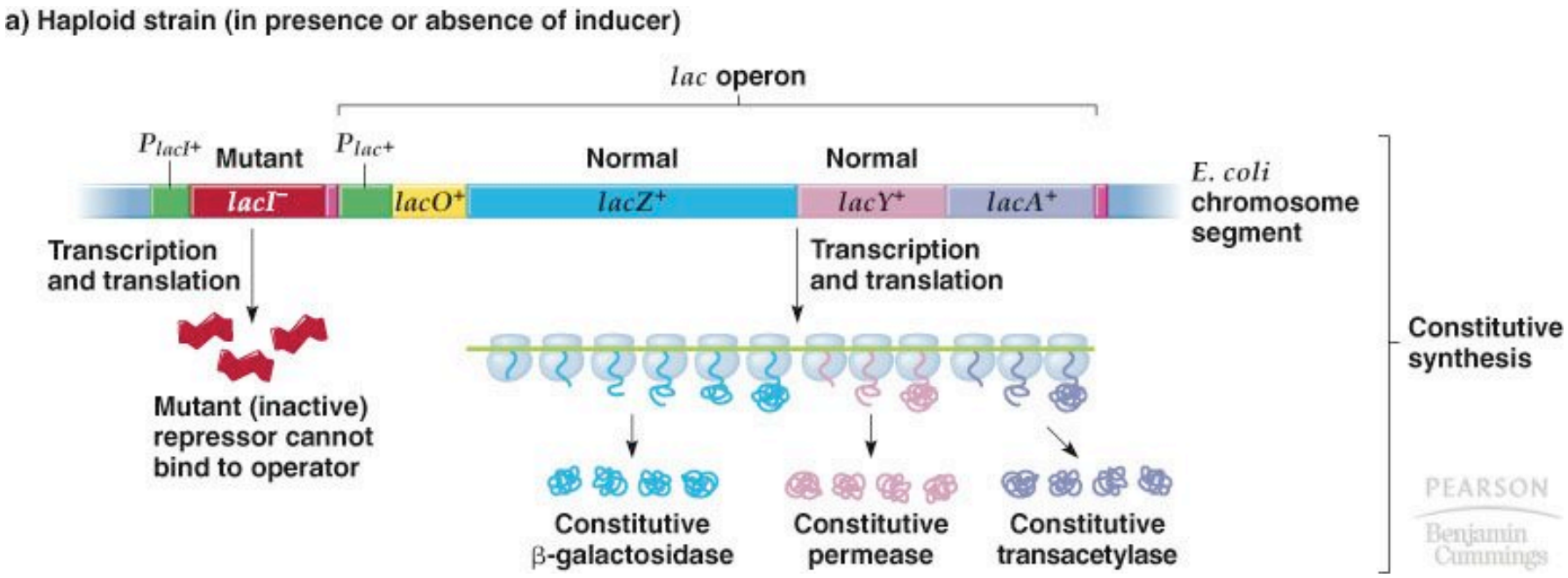


lac O^c mutation

Presence of Inducer (Lactose!):

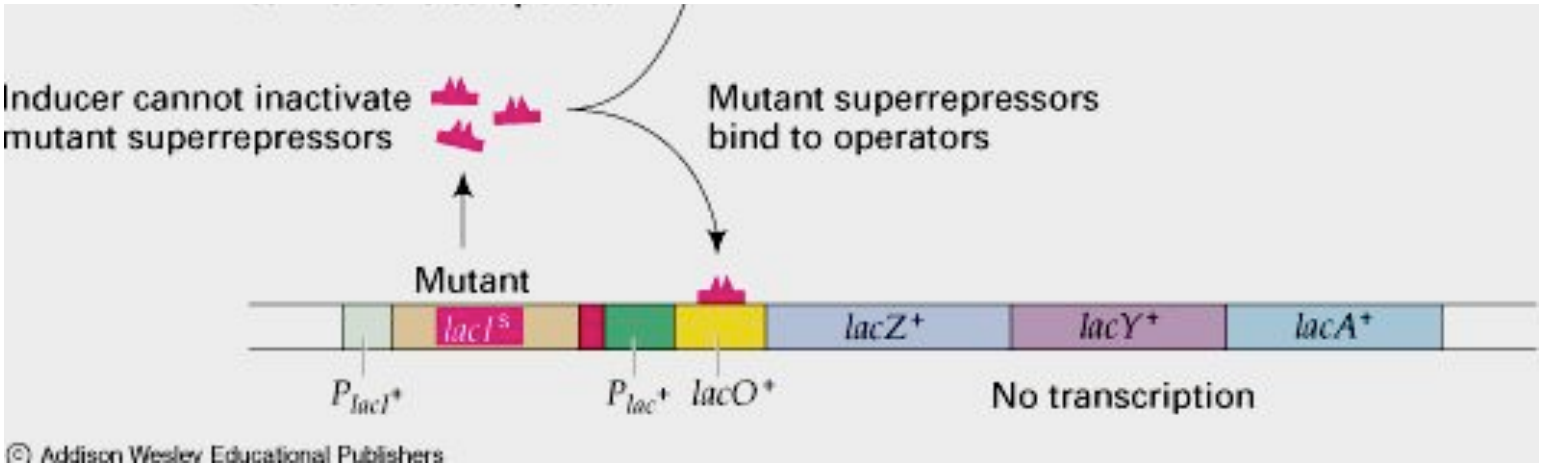


lac I$^-$ mutation
+ or - Inducer (lactose)



Trans-dominant effect of *lac* I^S mutation (super repressor)

- Show no production of any of the operon genes either in
  - the presence or absence of the inducer, lactose.
- The mutation causes a conformational change in the repressor protein
  - it cannot bind to lactose,
  - but can bind to the operator sequence.

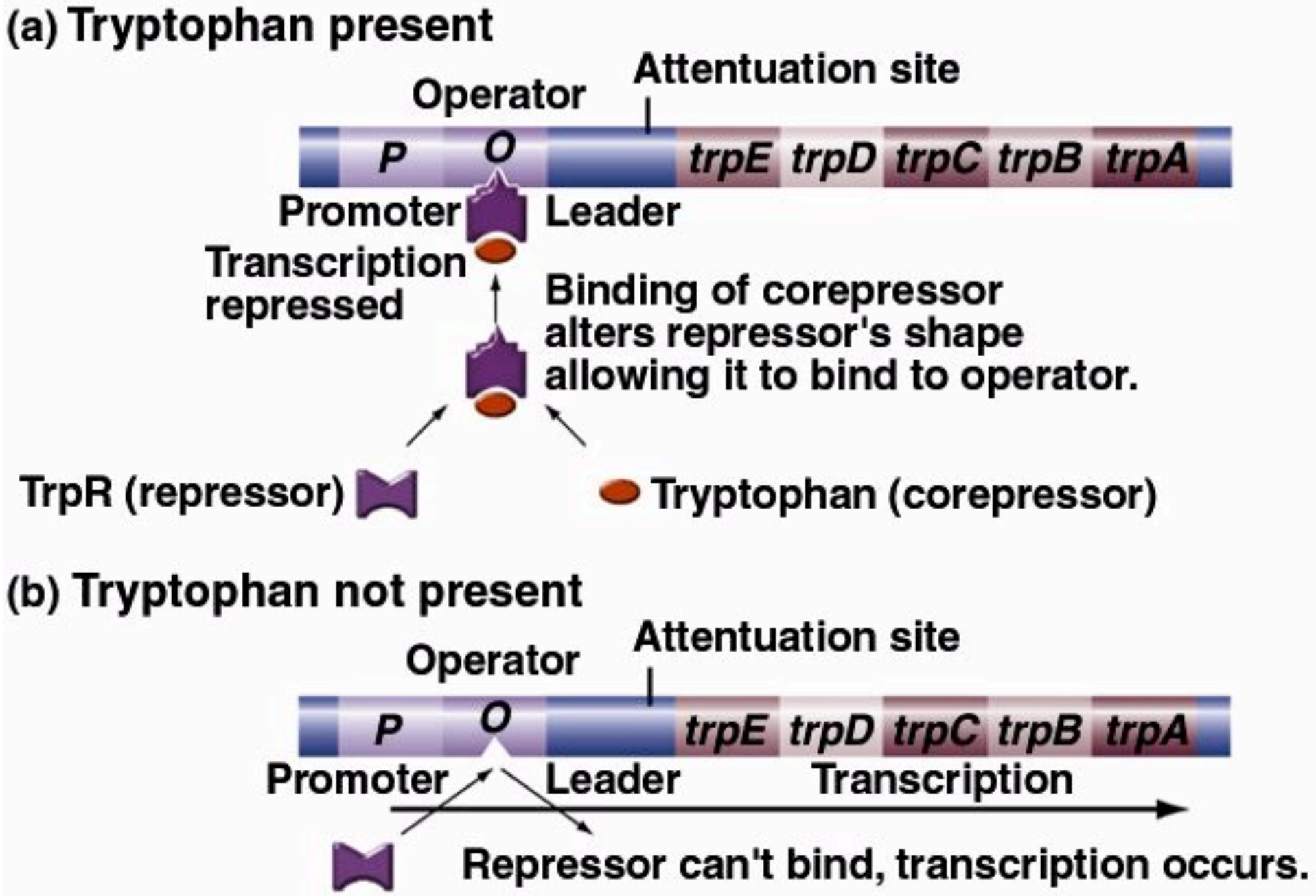


The attenuation of gene expression:

- The presence of tryptophan activates a repressor of the trp operon
  - trpR gene produces repressor

- Corepressor – tryptophan binds to trp repressor
  - allowing it to bind to operator DNA and
  - inhibit transcription

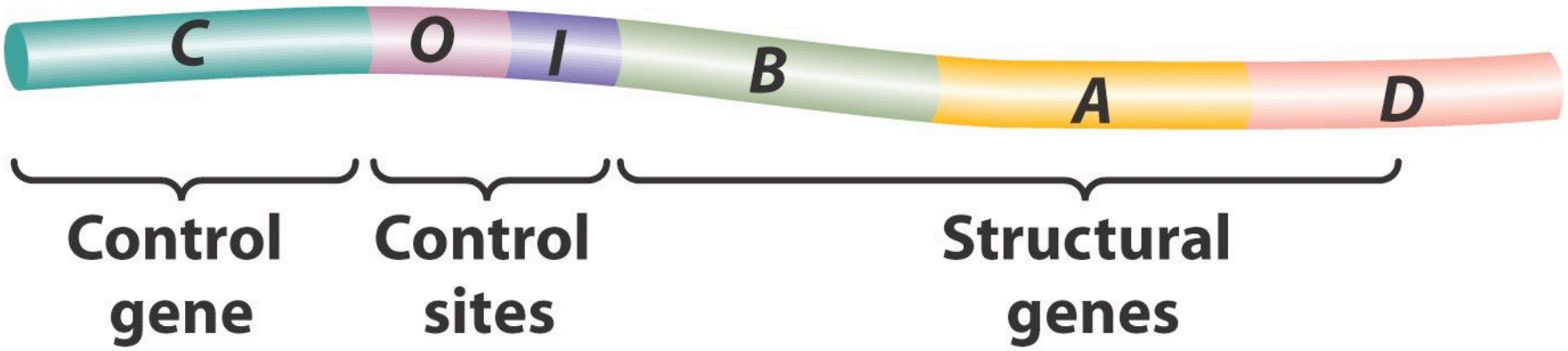
Tryptophan as a corepressor



Arabinose Operon

- Dual Positive and Negative Control
- Single DNA-binding protein may act as either a repressor or an activator
  - araC protein
- Different from Lac Operon where
  - 1 protein for neg reg
    - Repressor protein
    - (there is also a different protein for positive reg, but we are not going to talk about that!!!)

Arabinose Operon



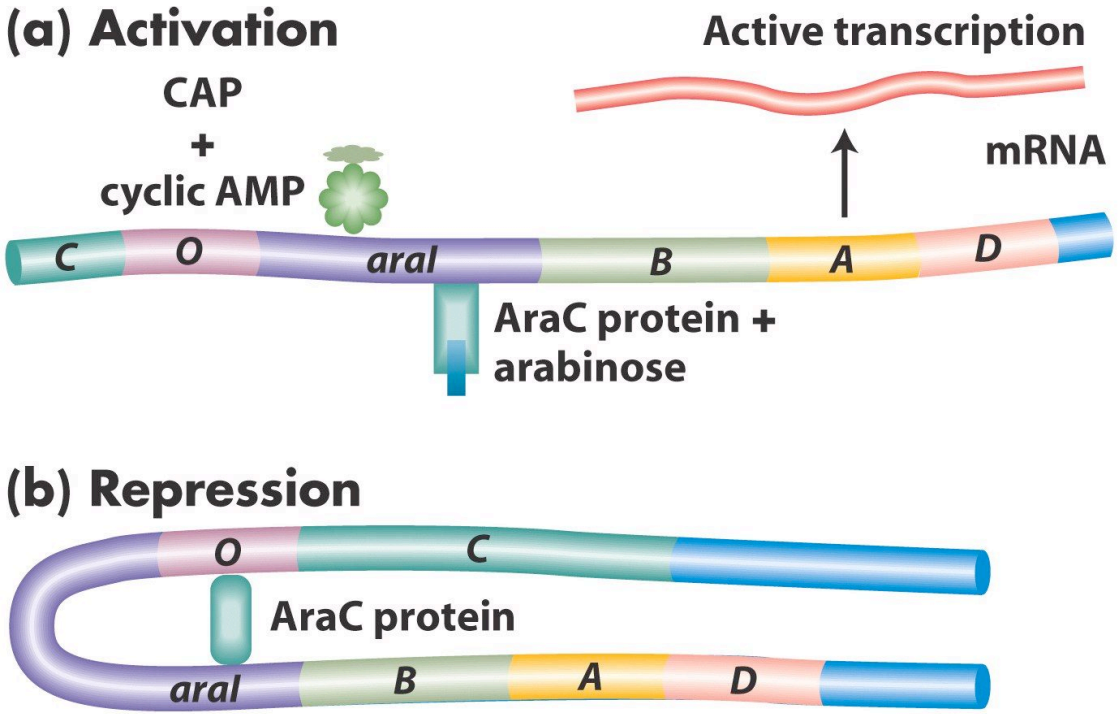
araC - constitutively active control gene

O- operator

araI - initiator containing the promoter

araB, araA, araD - inducible polycistronic genes

The looping of DNA is a common feature of regulatory proteins



araC acts as both a repressor and activator

- (a) Arabinose present
  - Arabinose binds to *araI*
  - CAP-cAMP binds to *araI*
  - Nothing binds to *araO*.
  - RNA polymerase interacts with *araC* at the *araI* sites and transcribes genes
- (b) No arabinose
  - Binding of araC to *araO* and *araI* causes looping and prevents RNA from transcribing

Global regulatory mechanisms coordinate the expression of many genes

- Normal Temperature:
  - Normal sigma factor (σ^{70}) binds to RNA polymerase and
  - recognizes sequence in promoter to initiate transcription
- High temperature:
  - Disables normal sigma factor (σ^{70})
  - Alternate sigma factor (σ^{32}) binds to RNA polymerase and
  - recognizes a different sequence in promoters of heat induced genes (heat shock genes) to initiate transcription
  - Heat shock genes enhance survival at high temps

The Prokaryotic Transcription Process: Initiation of Transcription

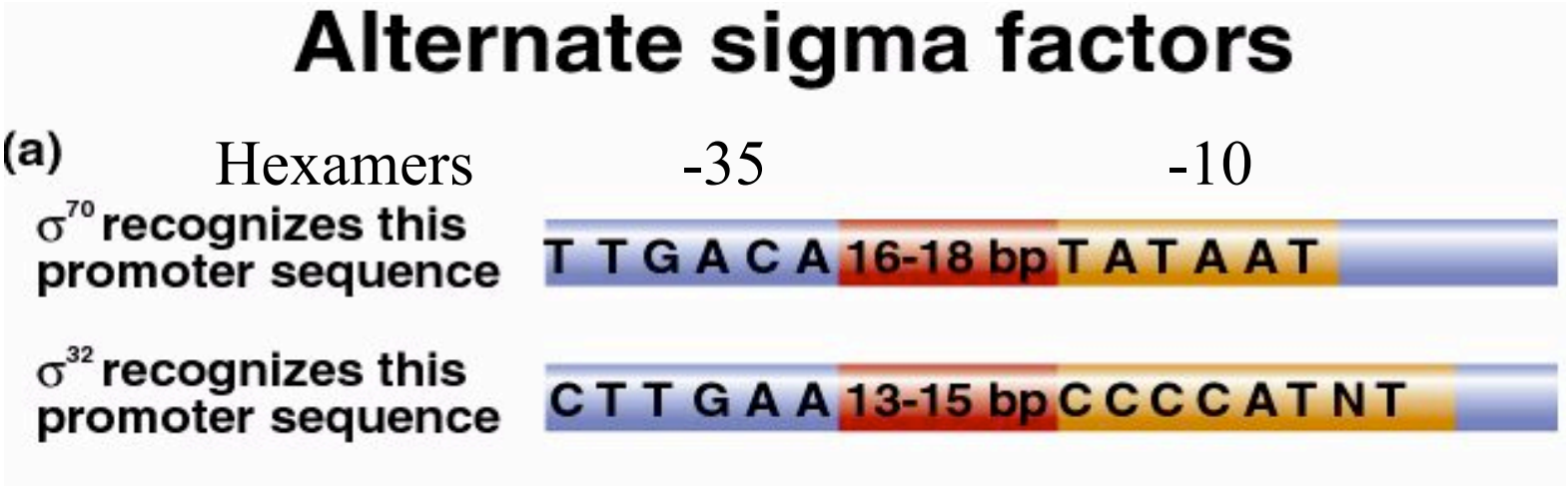
- Most *E. coli* genes have a σ^{70} promoter, the most abundant sigma factor in the cell.
- Other sigma factors may be produced in response to changing conditions:

1. σ^{70} recognizes the sequence TTGACA at –35, and TATAAT at –10.

2. σ^{32} recognizes the sequence CCCCC at –39 and TATAAATA at –15. Sigma^{32} arises in response to heat shock and other forms of stress.

3. σ^{23} recognizes the sequence TATAATA at position –15. Sigma^{23} is present in cells infected with phage T4.

Global regulatory mechanisms coordinate the expression of many genes



Homework Problems

Chapter 19

DON'T forget to take the online QUIZ!
DON'T forget to submit the online iActivity:
"Mutations and Lac"