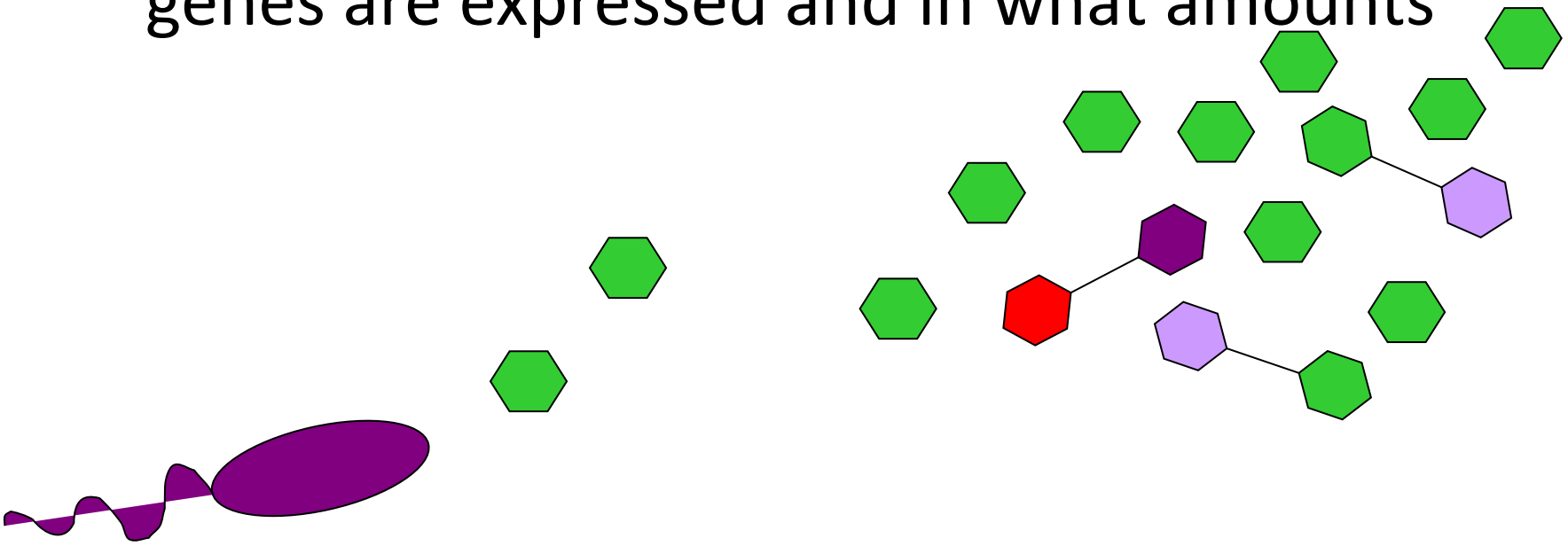
Regulation of Gene Expression in Bacteria

Transcription

- Cells are extremely selective about which genes are expressed and in what amounts



A bacterium will sense its environment at move towards nutrients

Which genes will the bacteria need to express to break down all of these sugars to make ATP. ?

Will some genes be constitutively expressed?

Will some gene expression be regulated – turned on & off?

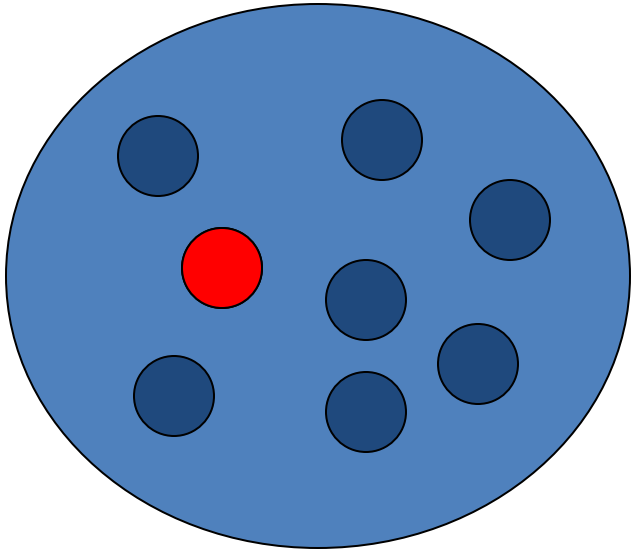
Metabolizing Lactose – A Model System

- B-galactosidase is a bacterial enzyme which is at high levels in the cell when lactose is present
- Jacques Monod & Francois Jacob hypothesized the lactose might be an inducer
  - A molecule which can stimulate gene expression

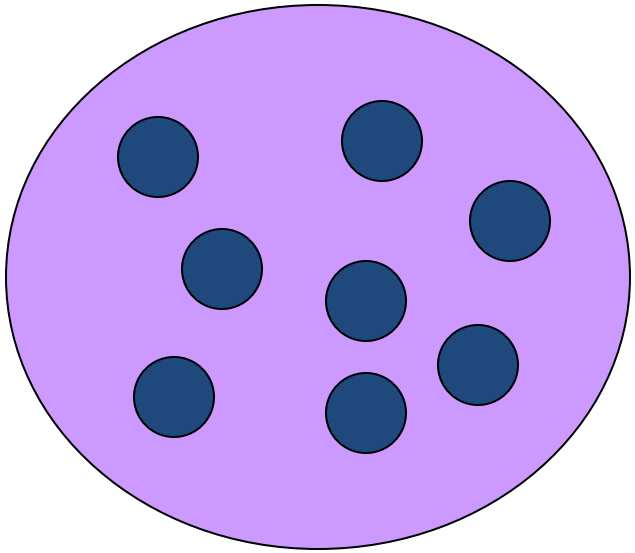
Identifying the Genes Involved

- Monod and Jacob preformed a ***genetic screen***
- Step 1
  - Generate a large pool of random mutants
    - X-rays, UV light, or chemicals
- Step 2
  - Screen the mutants to find individuals with defects in the biochemical pathway you are studying

Replica Plating



Master Plate - Glucose



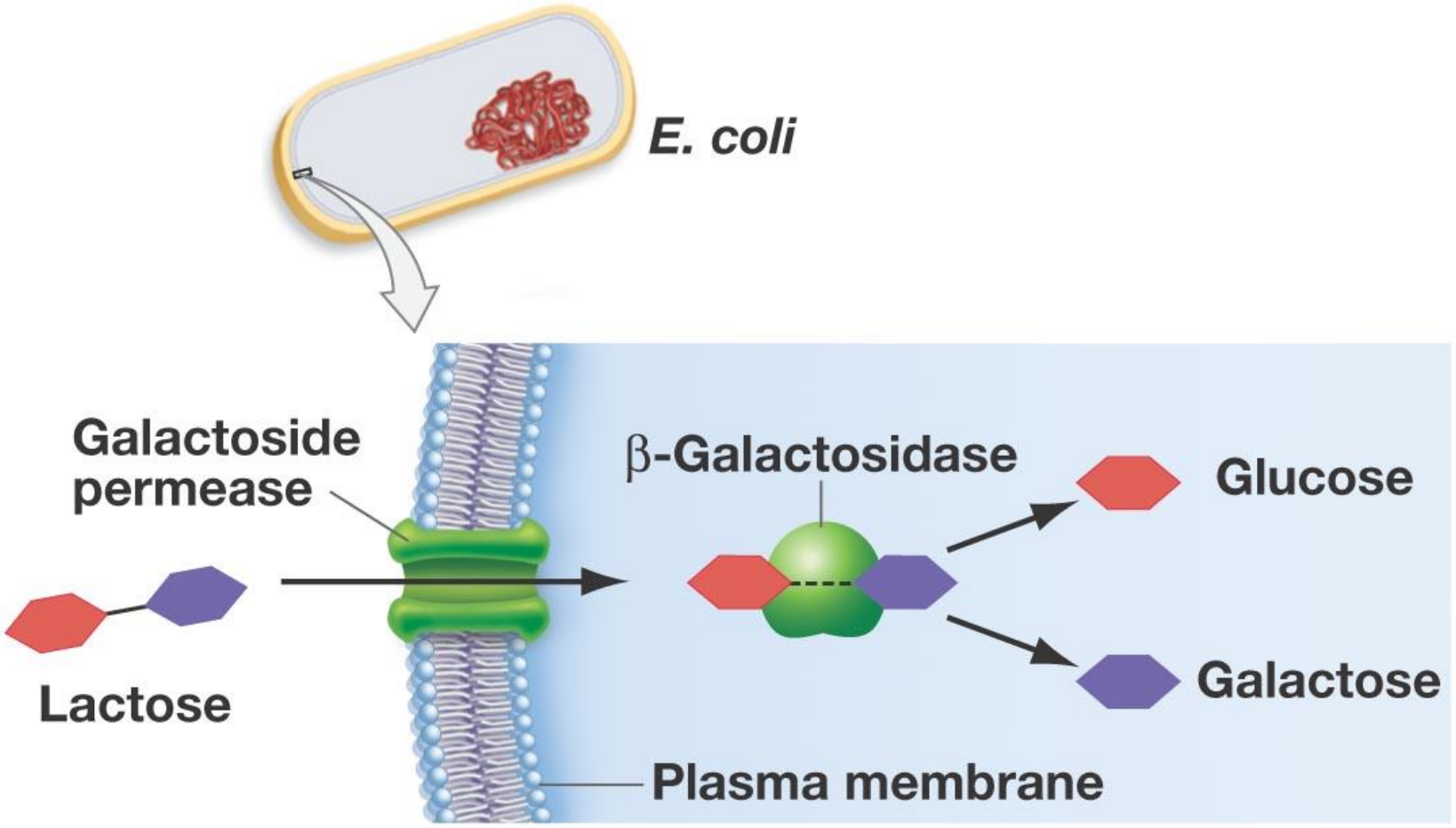
Replica Plate - Lactose

This technique ensures that the position of the bacteria is the same on both plates. When one colony does not grow on the lactose plate but does grow on the master plate then this colony has some biochemical defects in the metabolism of lactose. This colony will be isolated and characterized

Three Distinct Types of Mutants in Lactose Metabolism of *E. coli*

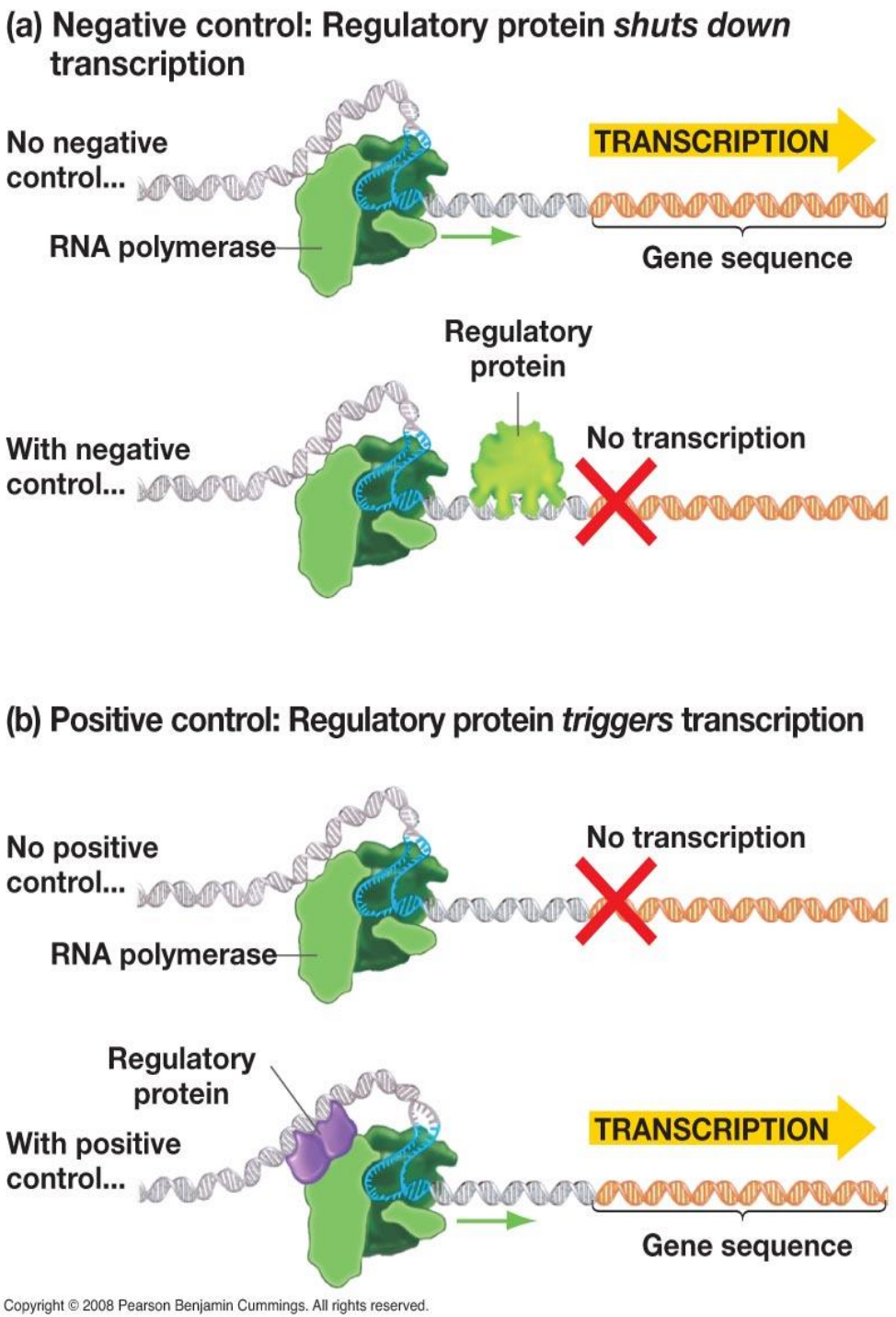
Mutant Phenotype	Interpretation	Inferred Genotype
Cells cannot cleave indicator molecule	β-galactosidase defective	*lacZ*$^-$
Lactose does not accumulate inside cells	Membrane protein for transport defective (galactoside permease)	*lacY*$^-$
Indicator molecule is cleaved even if lactose is absent	Constitutive expression of *lacZ*$^-$ and *lacY*$^-$ Regulatory gene is defective.	*lacI*$^-$

Critical Proteins for Lactose Metabolism



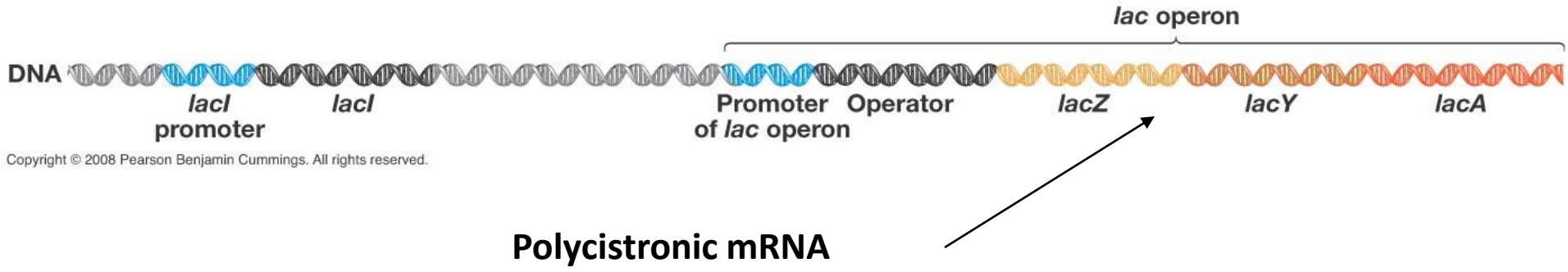
Transcription Regulation

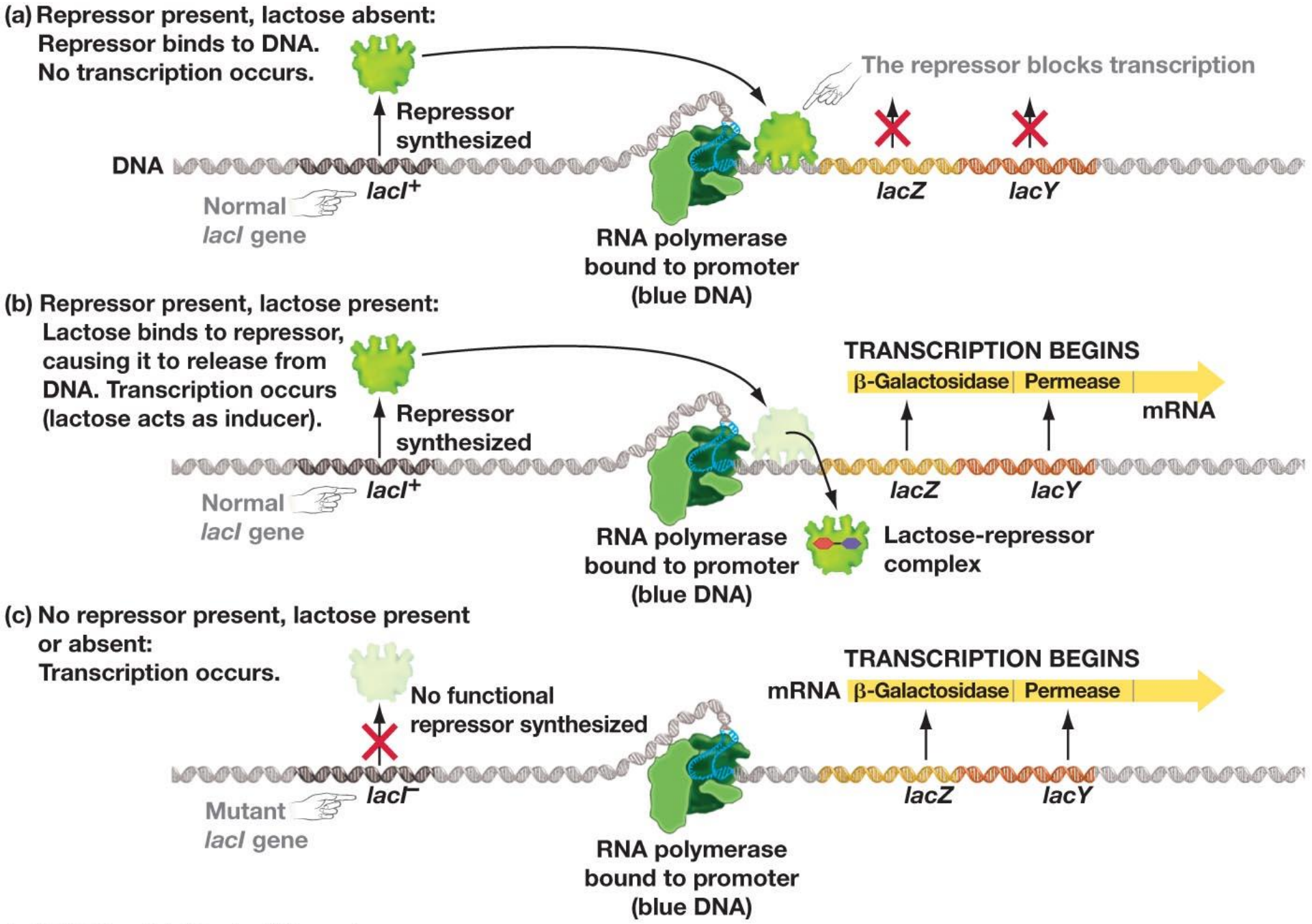
- Repression
  - Transcription factors and regulatory proteins bind to DNA and block transcription
- Induction
  - Transcription factors and regulatory proteins bind to DNA and allow transcription



The *lac* Operon

- Jacob and Monod summarized their results in 1961 with a comprehensive model of negative control
- They coined the term ***operon*** for a set of coordinately regulated bacterial genes that are transcribed together into one mRNA



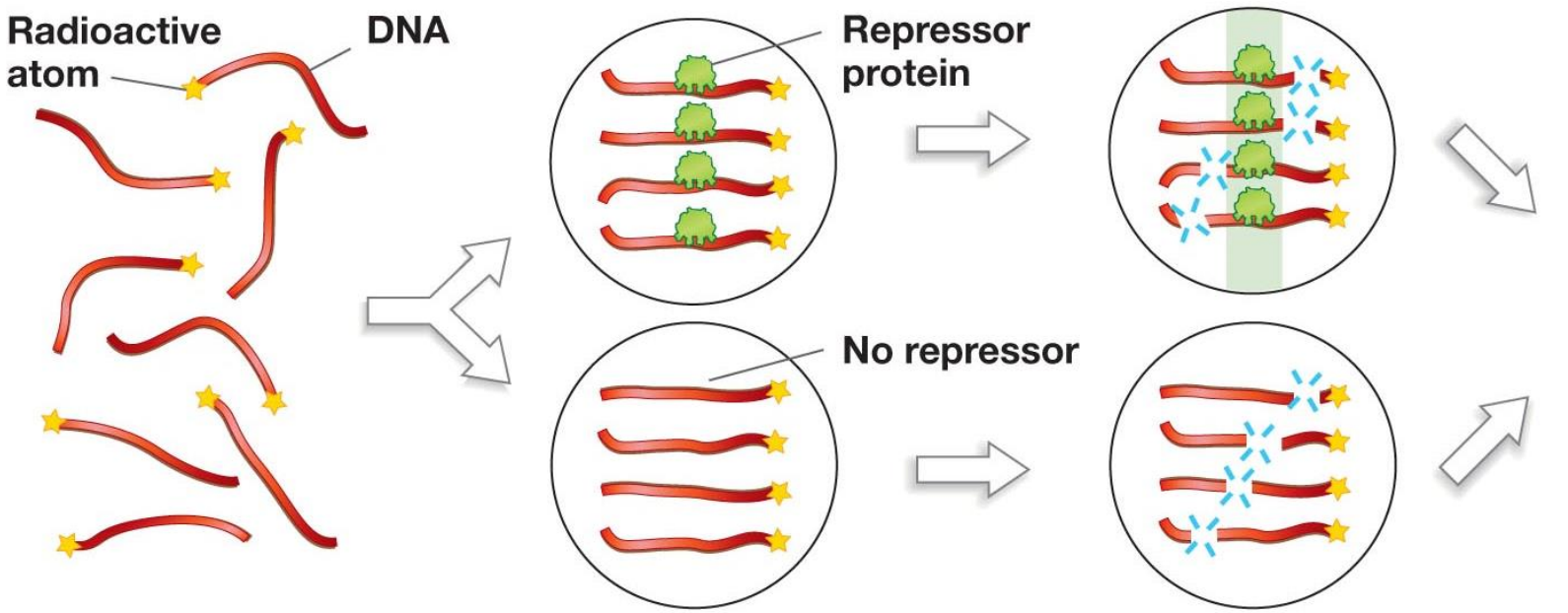


The Jacob-Monod Model

- *lacZ, lacY*, and *lacA* genes are adjacent and are transcribed into one mRNA initiated from a single promoter
  - Coordinated gene expression
- Repressor protein is encoded by *lacI,* binds to operator and prevents transcription of *lacZ, lacY* and *lacA*
  - lacI is expressed constitutively
- Lactose is the inducer and binds to repressor
  - Allosteric regulation of repressor

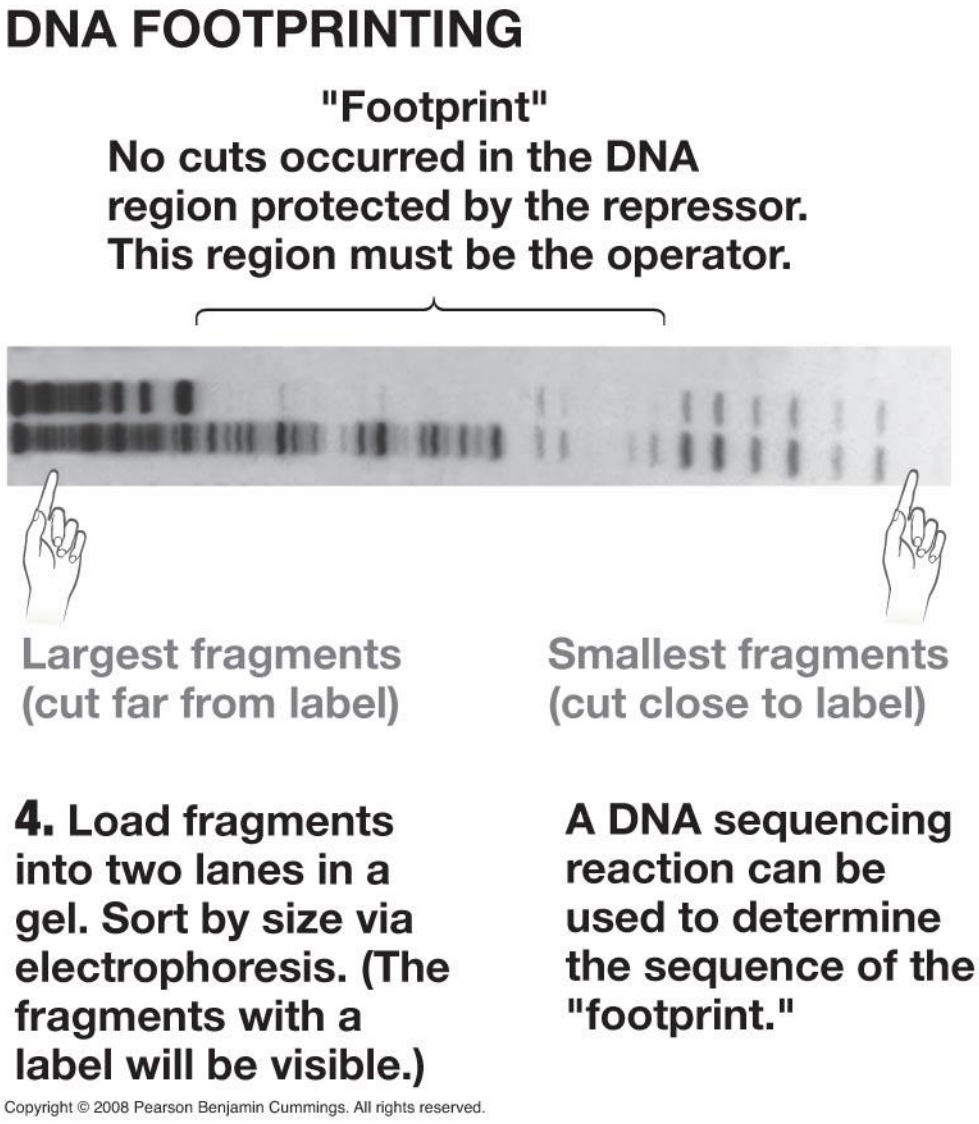
DNA Footprinting

In 1967, six years after the Jacob and Monod paper Walter Gilbert confirmed the hypothesis of Jacob and Monod by fully characterizing the operator



DNA Footprinting

- These experiments showed that the repressor interacted physically with the DNA sequences of the operator
- The operator was not a protein or a mRNA molecule



Importance of *lac* operon

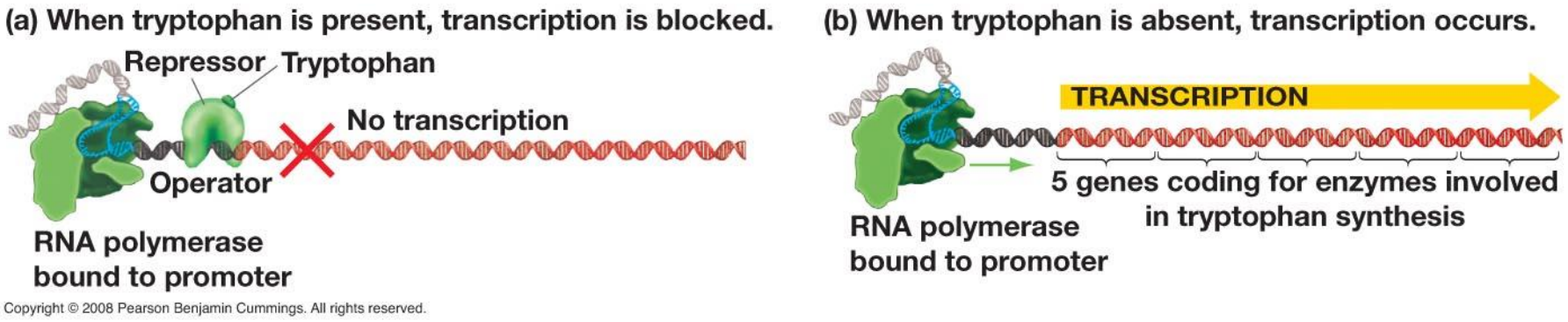
- Confirmed the presence of regulatory sequences of DNA
- Confirmed the presence of regulatory DNA binding proteins
- Post translational modification (binding of lactose to an existing protein)-the activity of existing protein is altered as the environment changes.

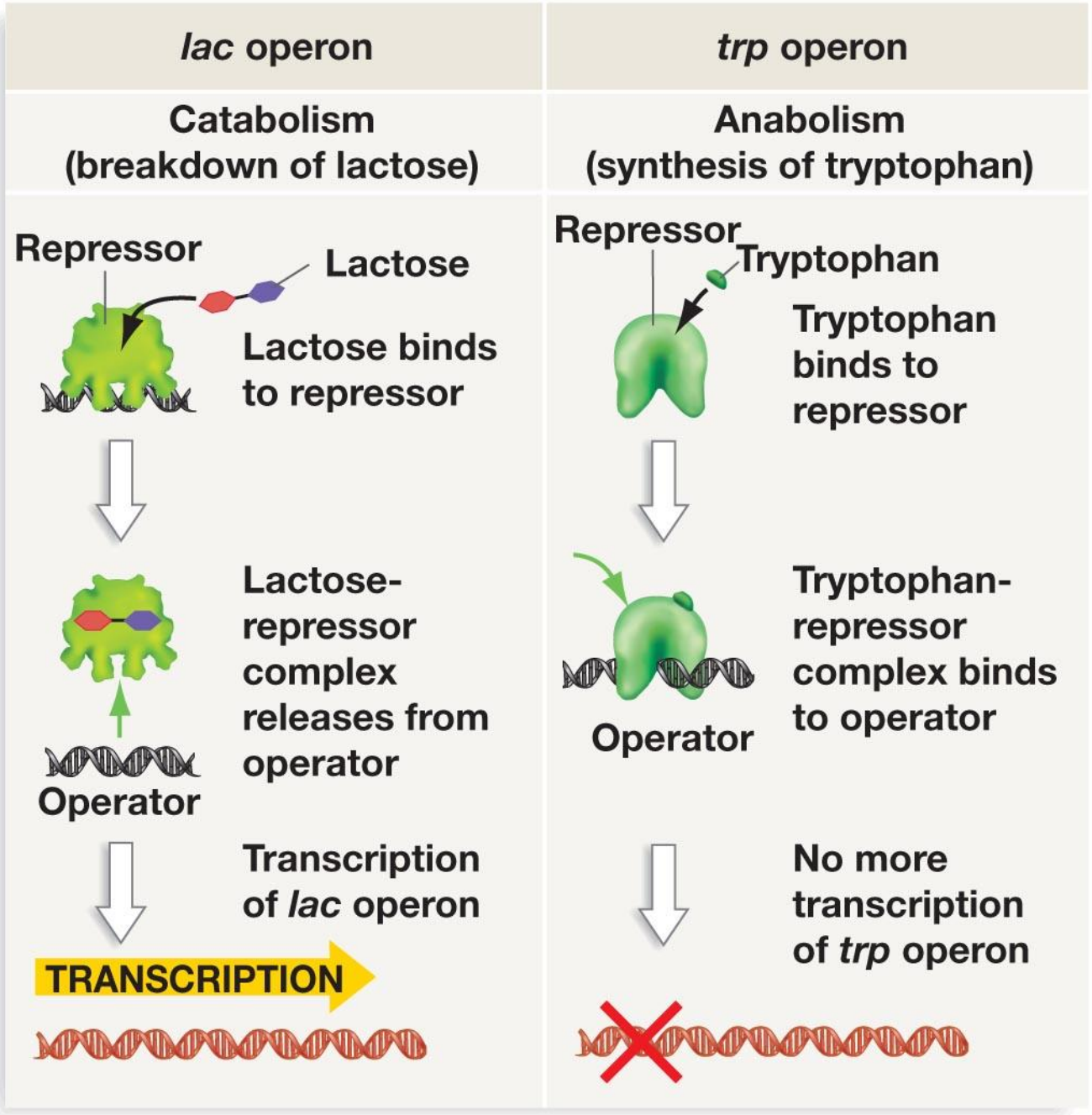
Other Operons

- The *lac* operon system is an ***inducible system***
  - This means that the genes under control of this promoter are always "OFF" and must be regulated "ON"
  - Many other genes in *E. coli* are regulated by an inducible system
- However further work has shown that ***repressible systems*** also exist in *E. coli*
  - This means that the genes under control of this promoter are always "ON" and must be regulated "OFF"

trp Operon

- The *trp* operon codes for an enzymes needed for the synthesis of tryptophan
- This is an anabolic pathway as opposed to the catabolic pathway for the *lac* operon
- Bacterial cells should have the *trp* operon "ON" in the absence of tryptophan and "OFF" in the presence of tryptophan





Catabolite Repression

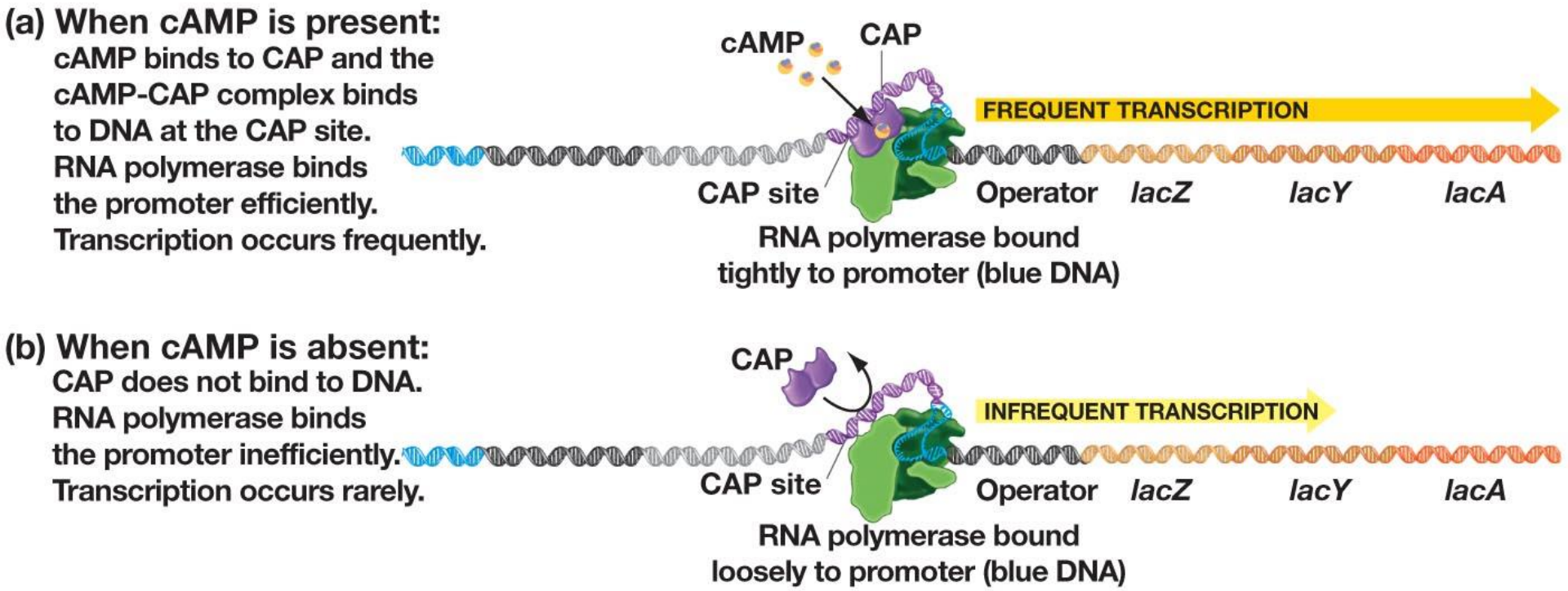
- Glucose is the preferred fuel source for bacterial cells
- In the presence of both glucose and lactose very little β-galactosidase is present
- How can this result be explained?
  - Lactose is present so the repressor is not bound to the operator transcription should occur?

Promoter Strength

- Not all promoters are created equal
- Strong promoters
  - RNA polymerase and sigma strongly bind to promoter and initiate transcription without additional factors
- Weak promoters
  - Additional factors are necessary to for the promoter to strongly bind to RNA polymerase

lac Promoter

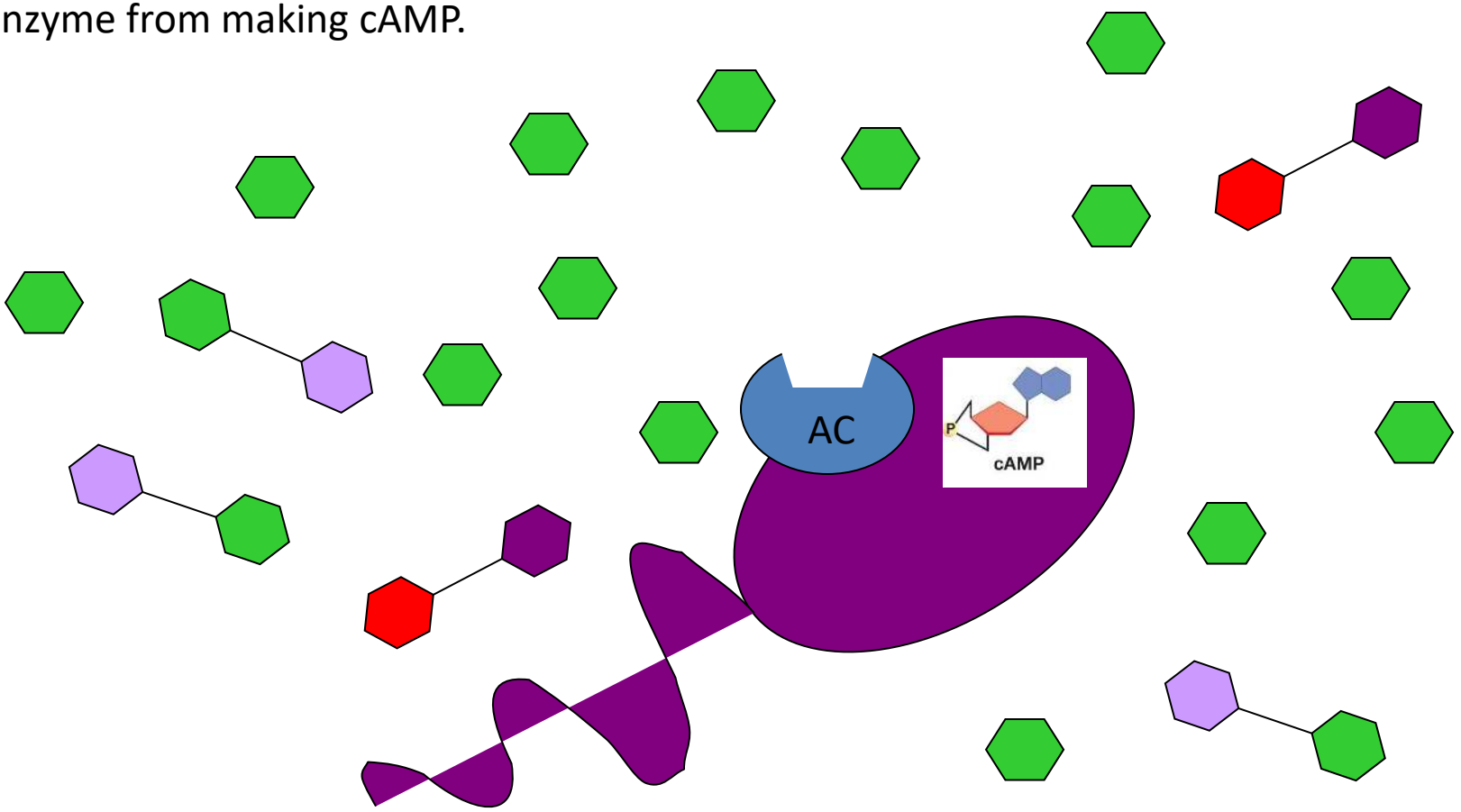
- *lac* promoter is a weak promoter
  - *lac* promoter has an additional regulatory DNA sequence known as the CAP binding site
    - Catabolite activator protein
- CAP binding greatly increases the strength of the lac promoter
  - CAP can only bind when its allosteric molecule cAMP is present
- How this all works ??????



But how does a bacteria cell decide when to make cAMP?

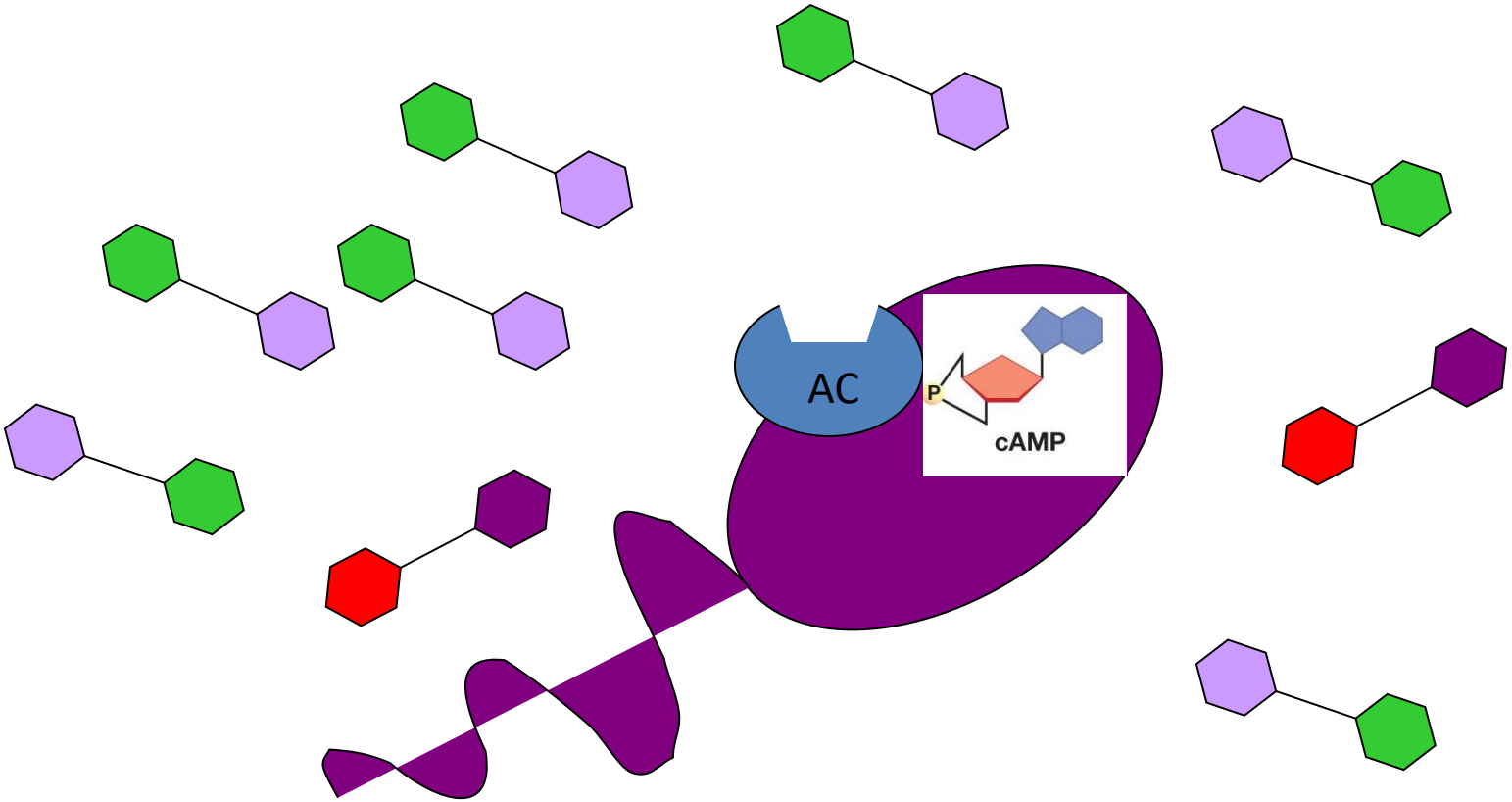
Blocking Production of cAMP

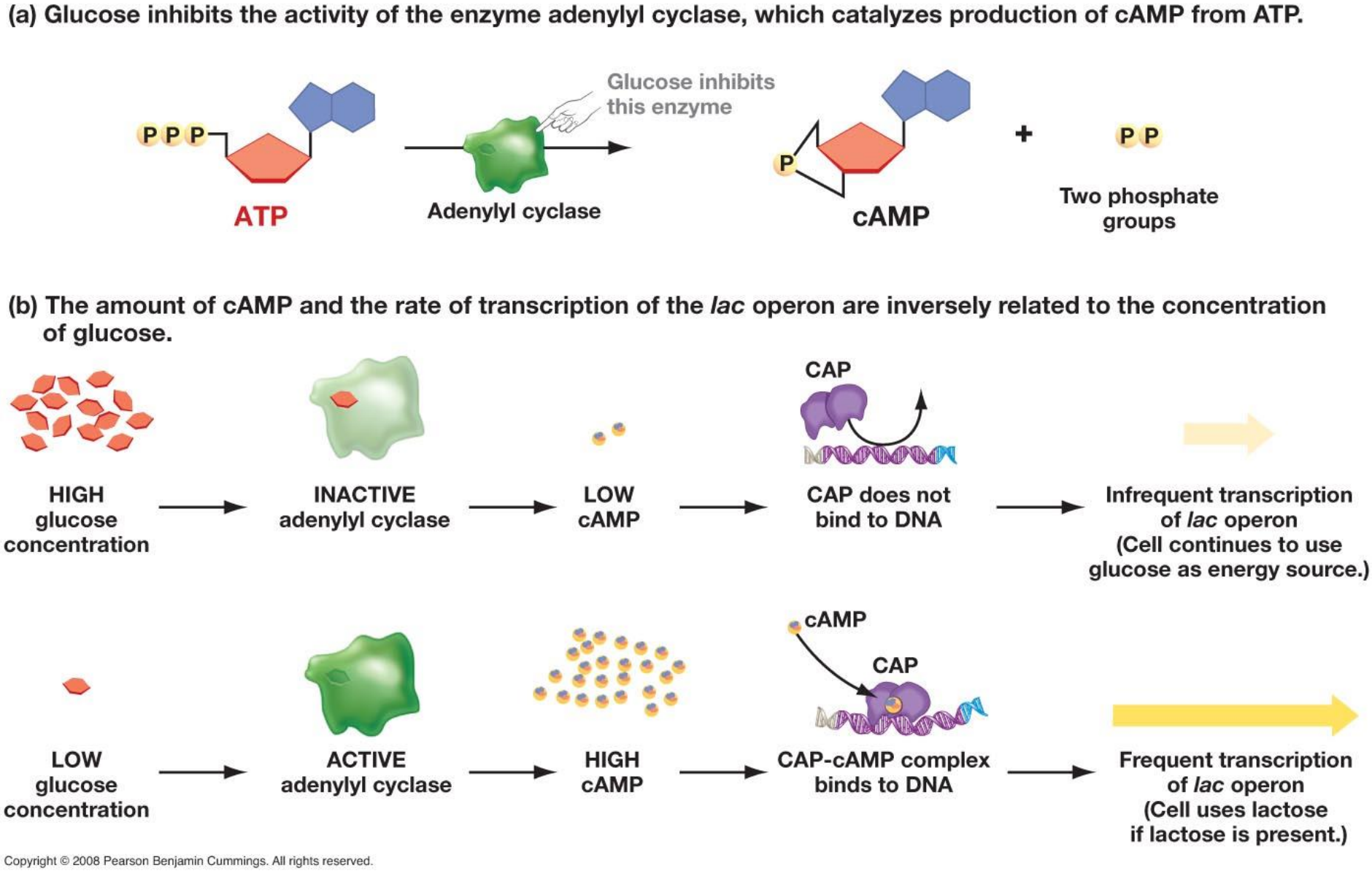
In the original example the bacteria has many sugars to choose from. Glucose is known to be the fuel source of choice. Under these conditions glucose levels outside the cell will bind to adenylyl cyclase and inhibit this enzyme from making cAMP.



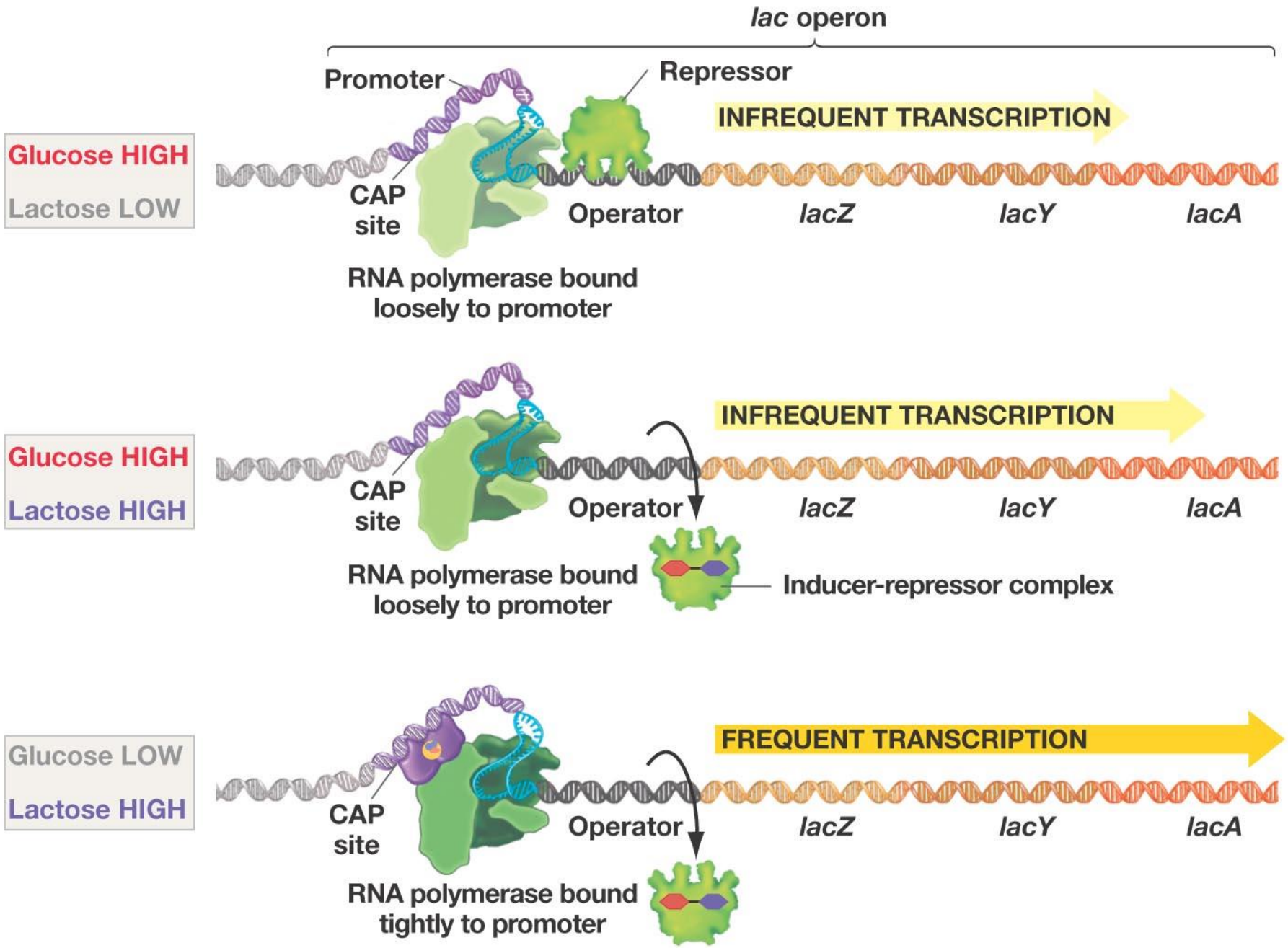
Production of cAMP

However, if environmental conditions change then no glucose will bind to adenylyl cyclase. Therefore, cAMP levels will increase and if lactose is Present the lac operon will be turned "ON". However, if no lactose is present The repressor will still be bound to the operator an no transcription will occur.



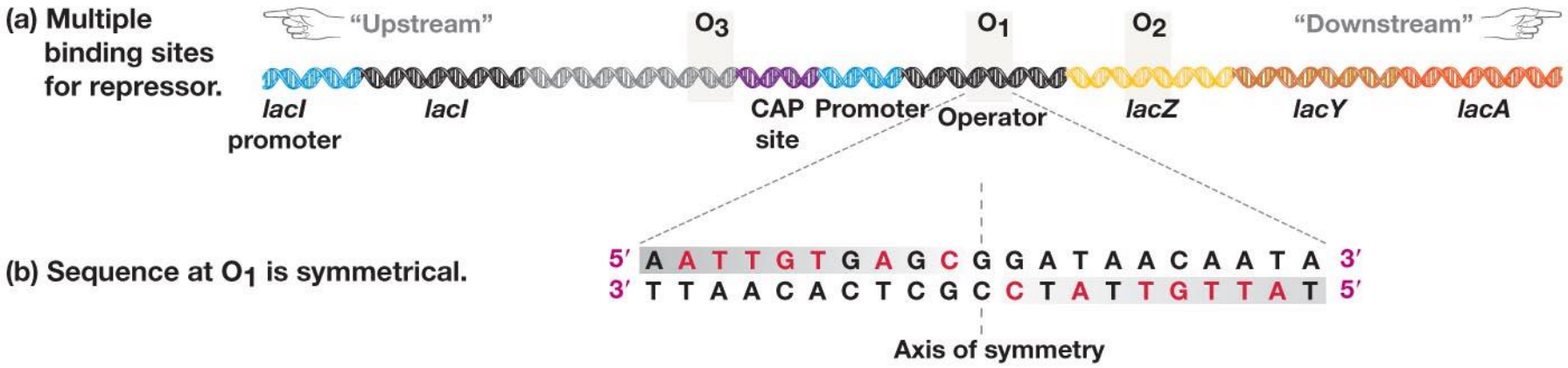


CAP-cAMP system influences many genes in addition to the *lac* operon.
Cap sites are found adjacent to the promoters for several operons that are required for the metabolism of sugars other than glucose



Characterization of the Operator

- The sequences of many DNA regulatory elements have dyad symmetry
- These sequences are now known to interact with specific amino acid sequences in the DNA binding protein

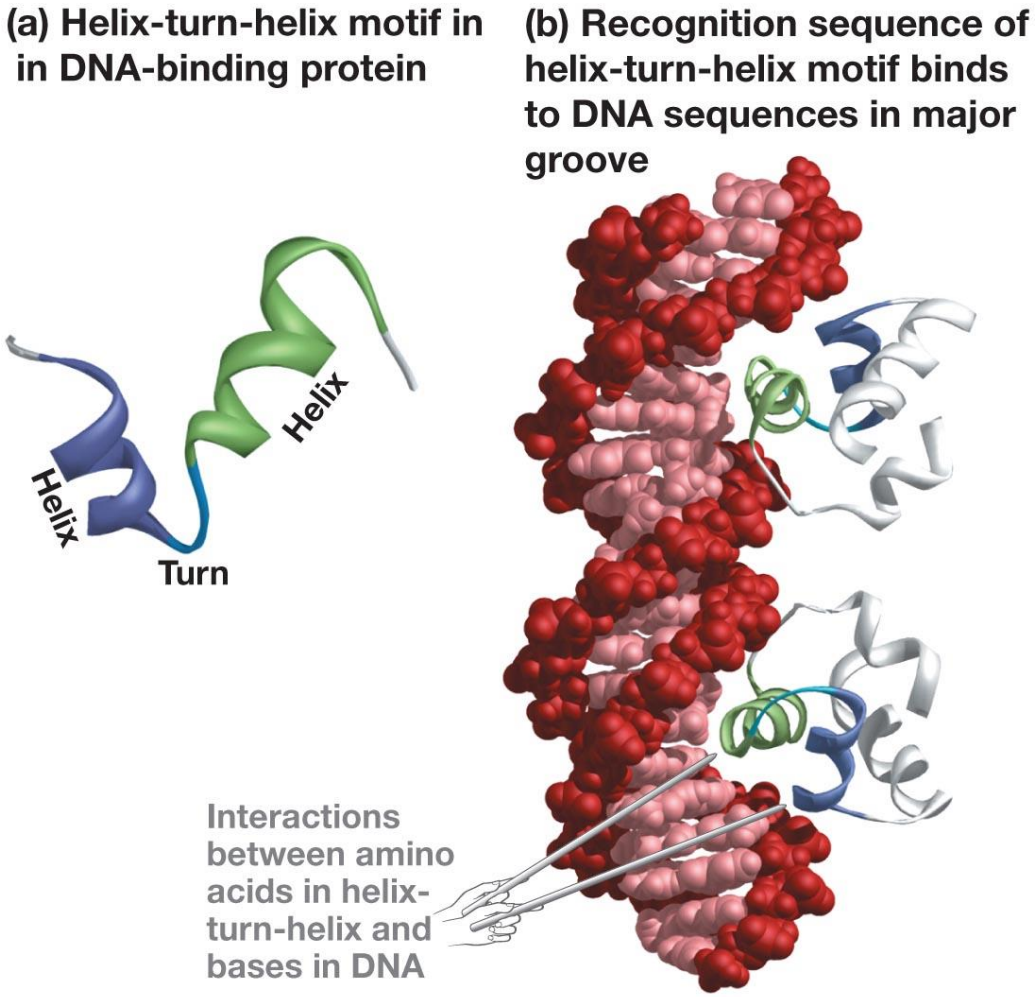


Characterization of DNA Binding Proteins

- Most DNA binding proteins have distinct domains which are characterized by specific motifs
  - Each domain has a specific job
    - Bind to DNA
    - Bind to inducer
  - Each motif is a specific 3D structure
    - Helix turn helix
    - Leucine zipper
    - Barrel motif

Helix Turn Helix

- One helix interacts with sugar phosphate backbone
- One helix interacts with base pairs in the major groove
  - Recognition sequence



Recognition Sequence

- The amino acid sequences of recognition sequences vary
- Each regulatory protein has a unique recognition sequence and a unique regulatory sequence of DNA
- As with many DNA regulatory proteins, binding of the allosteric molecule causes a conformational change in structure
  - Cause the protein to bind
  - Cause the protein to fall off

(b) Interaction of inducer and repressor