Lecture 8

Regulation of Prokaryotic Transcription

April 21, 2016
Pyle

Regulation of Transcription Prokaryotes

Jacob and Monod

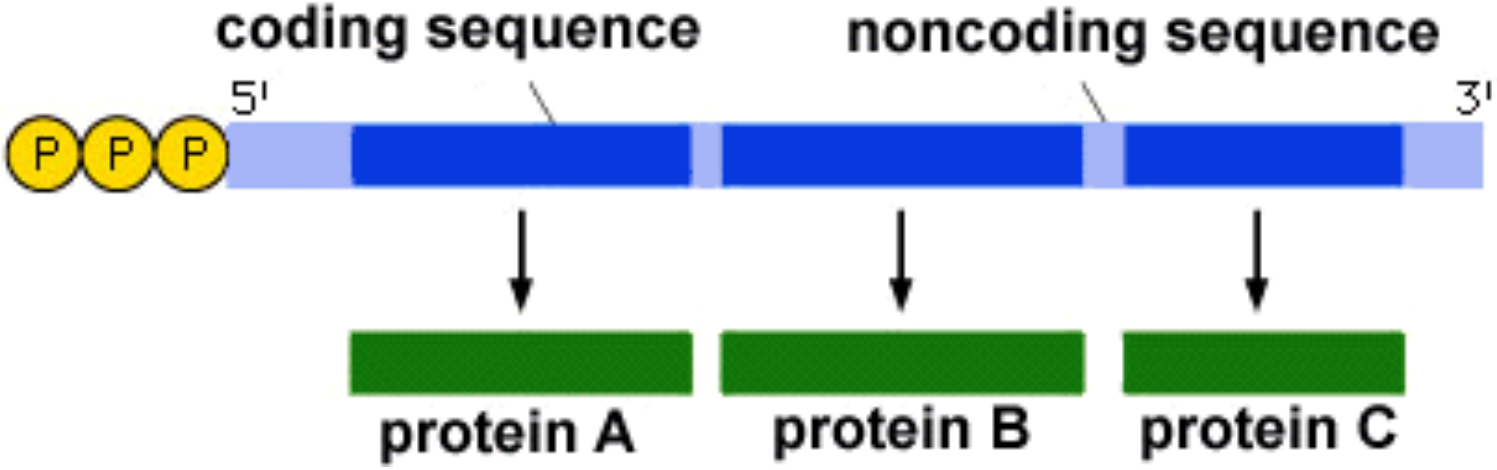
the *lac* operon

Expression of genes is transcriptionally regulated!

Novel idea that control of enzyme levels in all cells occurs through regulation of transcription. Awarded 1965 Nobel Prize in Medicine! Work is the basic underpinnings in understanding molecular and developmental biology and control of gene expression.

Related genes are often organized as operons

- Prokaryotic genes are **polycistronic-**one promoter direct the synthesis of a mRNA that can encode more than one proteins
- **Operon - an arrangement of genes in a contiguous linear array**
- **In an operon a continuous strand of mRNA carries the message for a related series of enzymes (polycistronic mRNA)**



Why are genes organized into operons?

- **Genes encoding enzymes in a common pathway can all be induced simultaneously.**

- **This type of control is called coordinate control. One mRNA expresses multiple proteins.**

Organization of a Bacterial Operon

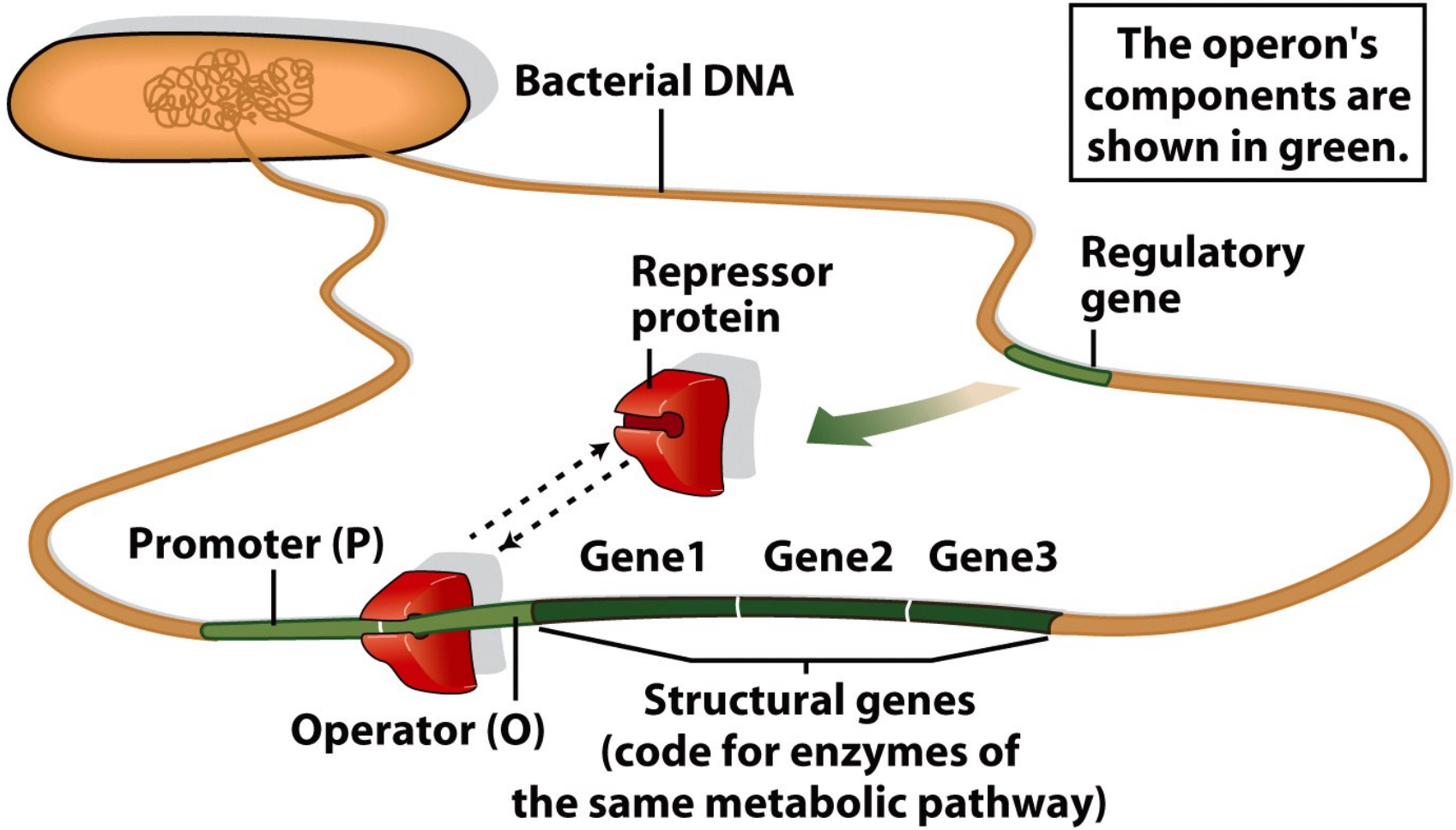
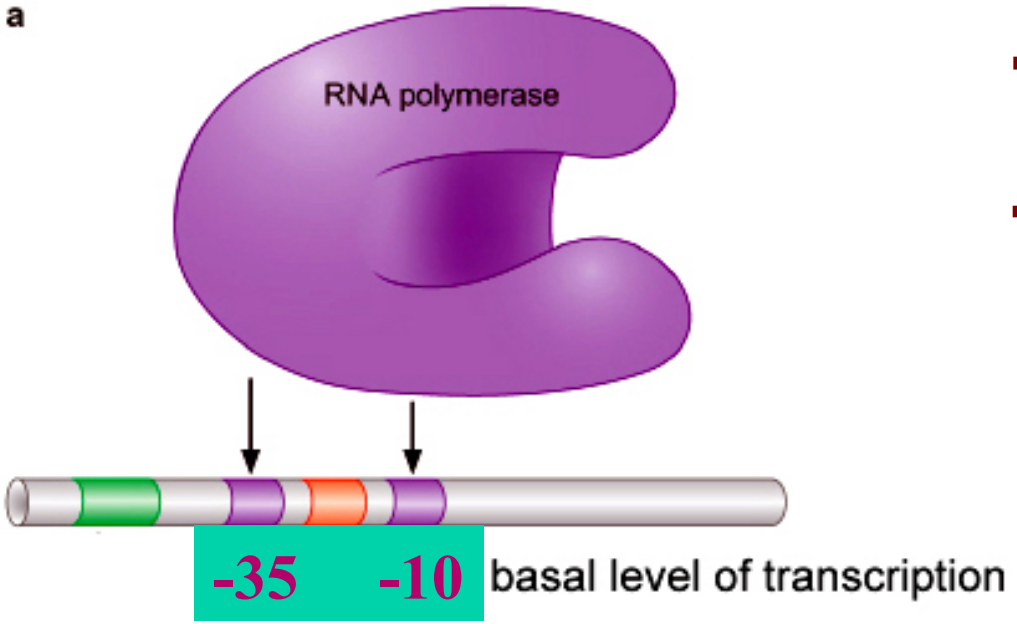


Figure 12-28 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Transcription of the structural genes is controlled by repressor protein
When bound to operator site of DNA blocks movement of RNA polymerase

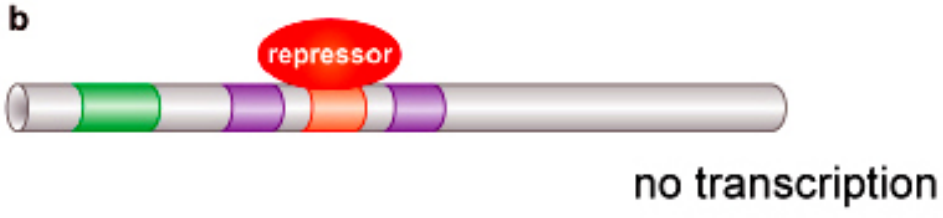
Promoter sequence determines first level of regulation

- Weak promoters have poor consensus sequences and initiate transcription infrequently
- Strong promoters generally have good consensus sequences and initiate transcription often
- Many promoters are regulated by additional regulatory proteins as well
  - Repressors inhibit transcription initiation (Lac repressor)
  - Activators increase transcription initiation (CAP)

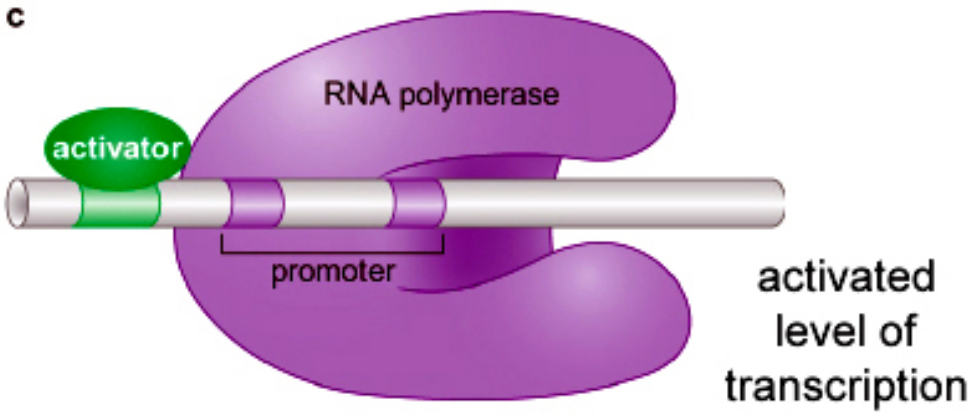


Three levels of transcription:

-basal level

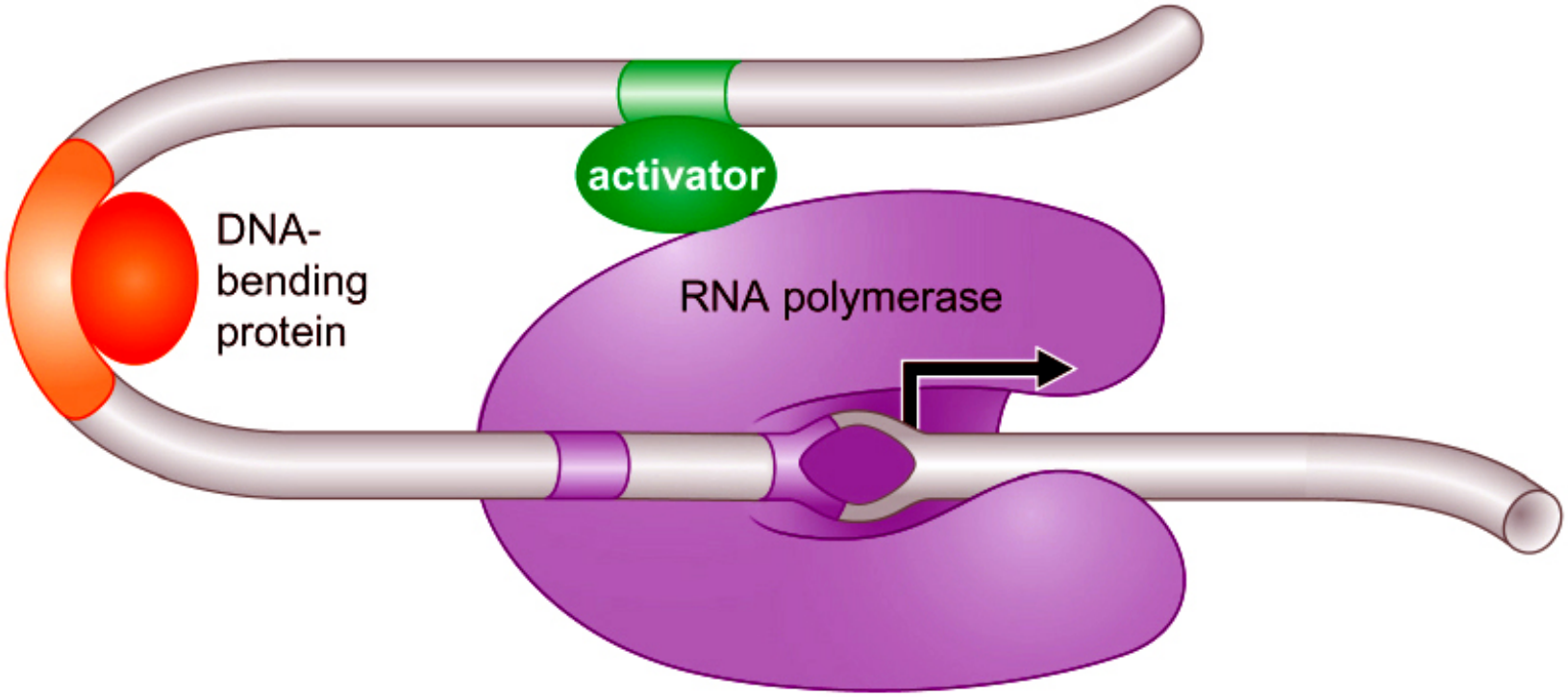


-repressed

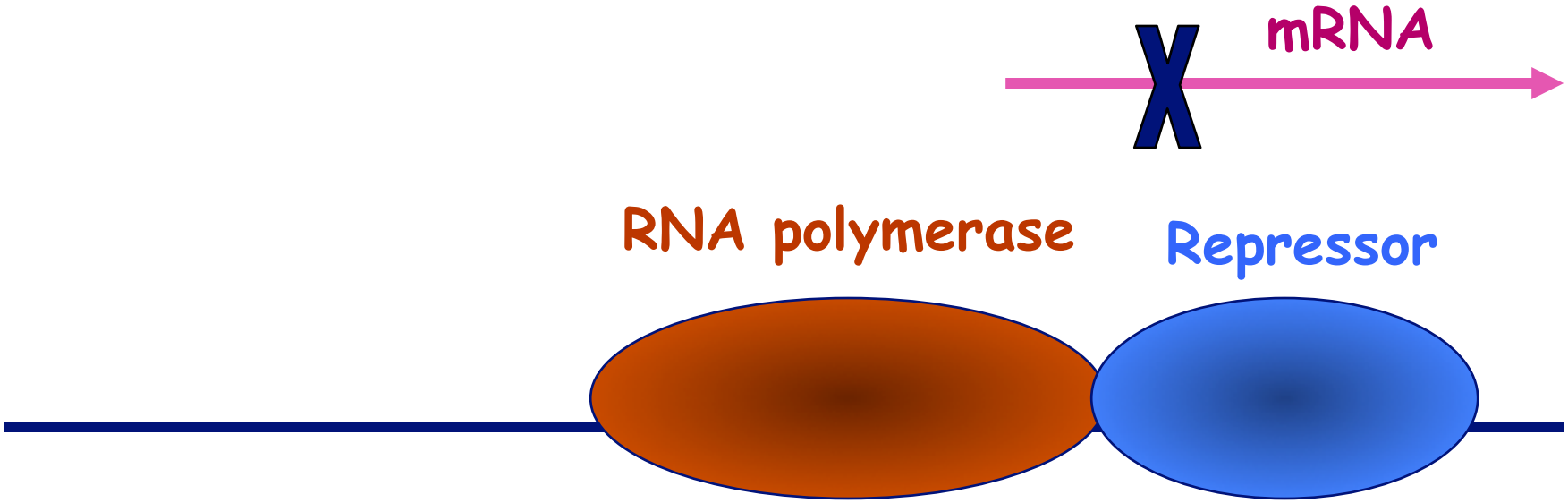


-activated

DNA bending proteins can facilitate these distal interactions



Activation by recruitment



Repressors usually block transcription initiation by:

- interfering with RNA polymerase binding or
- preventing open complex formation

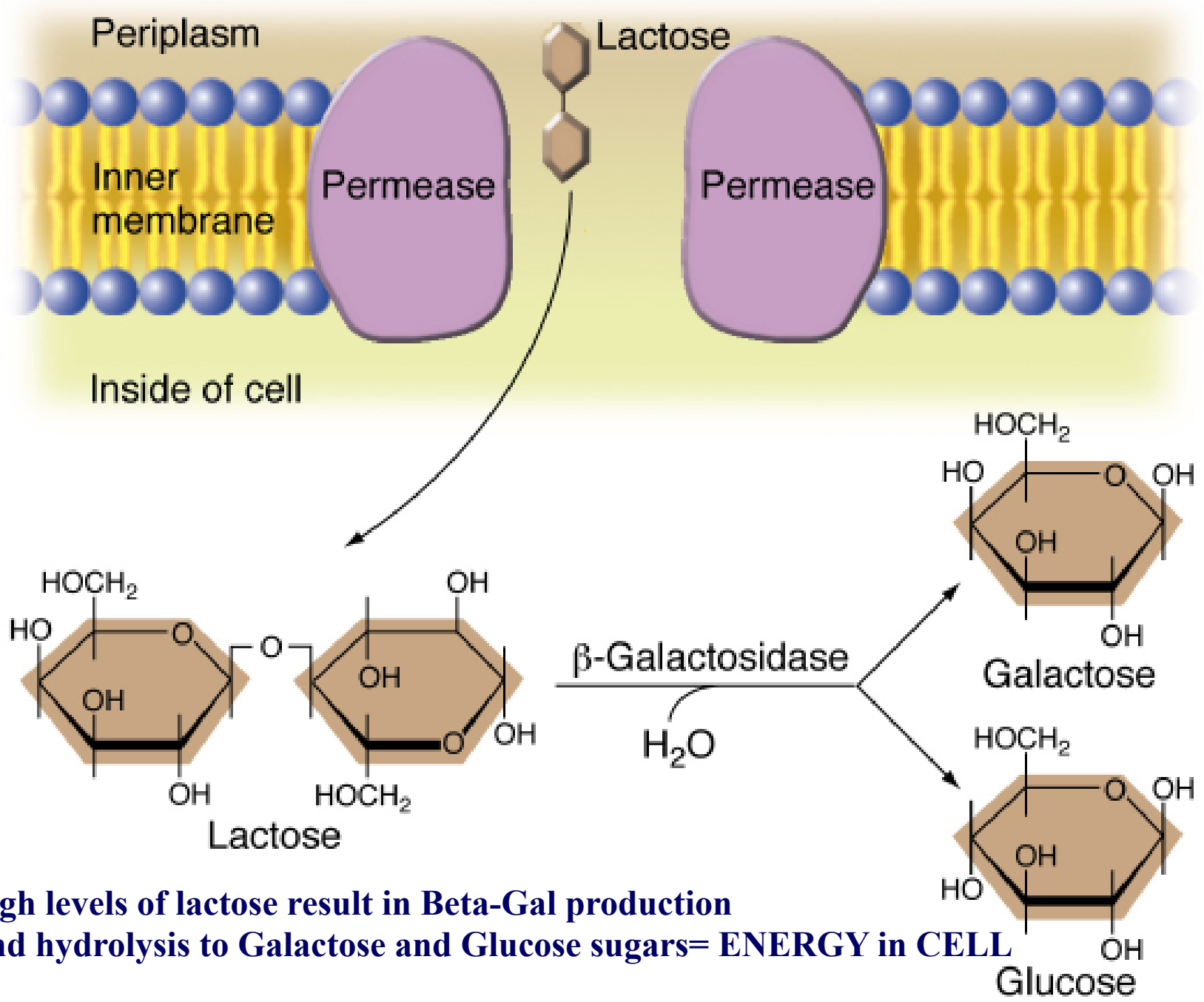
The lac operon consists of three genes under the control of a single promoter.

- *lacZ* encodes β-galactosidase, an enzyme that catalyzes the hydrolysis of lactose
- *lacY* encodes the lactose permease, required for transport of lactose into the cell
- *lacA* encodes a transacetylase enzyme that transfers an acetyl group from acetyl-CoA to β-galactosides

The cell can use lactose as an energy source by producing the enzyme β-galactosidase to digest lactose into glucose and galactose.

Levels of b-galactosidase and lactose permease vary depending on the growth medium

- **Cells grown in the absence of lactose have very little β-galactosidase and lactose permease activity**
  - **These enzymes are not needed by the cell under these conditions**
- **Addition of lactose causes "induction" of β-galactosidase and lactose permease activity, up to 1000 fold**



High levels of lactose result in Beta-Gal production
And hydrolysis to Galactose and Glucose sugars= ENERGY in CELL

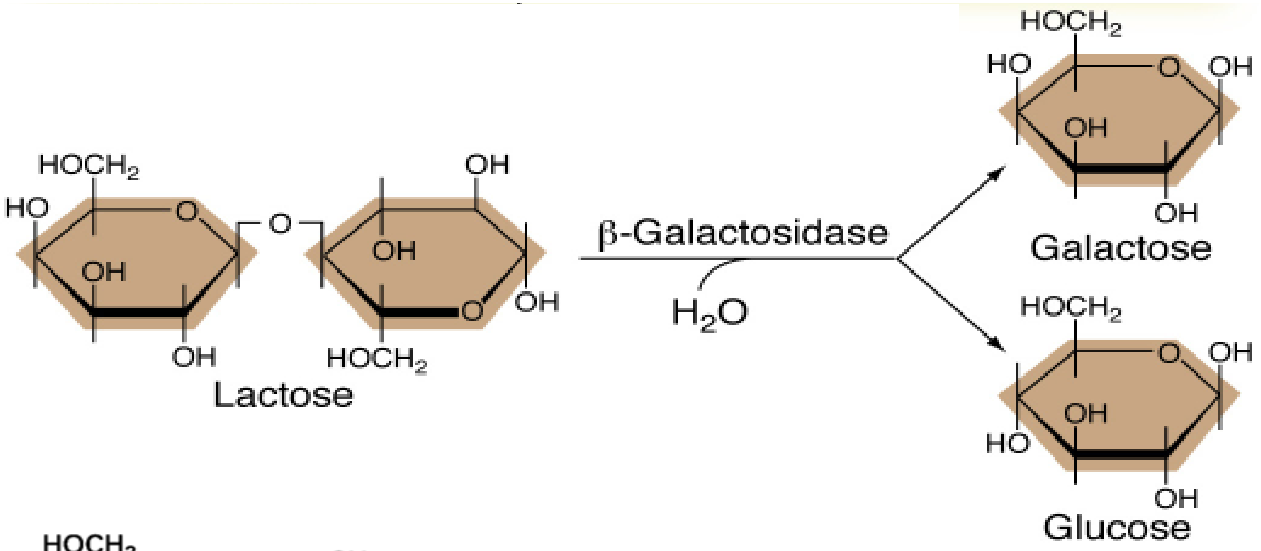
How do bacteria know which enzymes to induce?

Did the substrate somehow instruct the synthesis of the enzyme or activate an enzyme already present ?
-presence or absence of sugars/energy in the cell

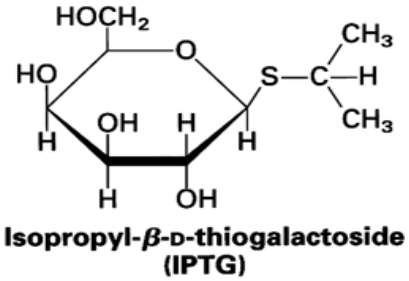
- **Though induction was observed with many enzymes, lactose metabolism was studied first:**
  - **Good induction**
  - **Substrates and analogs could be easily synthesized**

Substrate & Inducer used in Lac Operon Studies

Substrate & Inducer



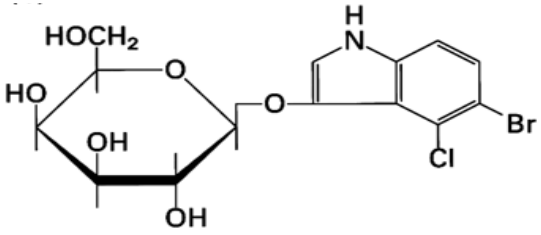
Inducer



Isopropyl-β-D-thiogalactoside (IPTG)

IPTG will induce the lac operon but is not metabolized, so its concentration stays constant during an experiment.

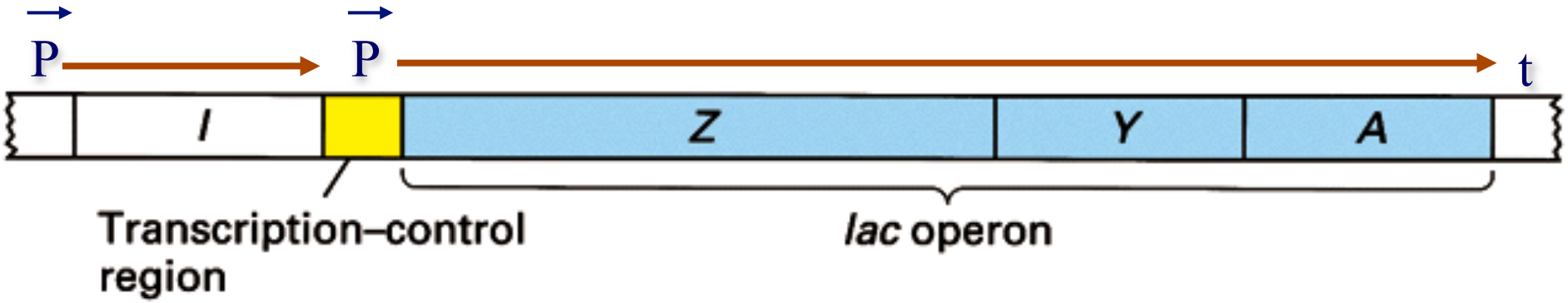
Substrate



X-gal

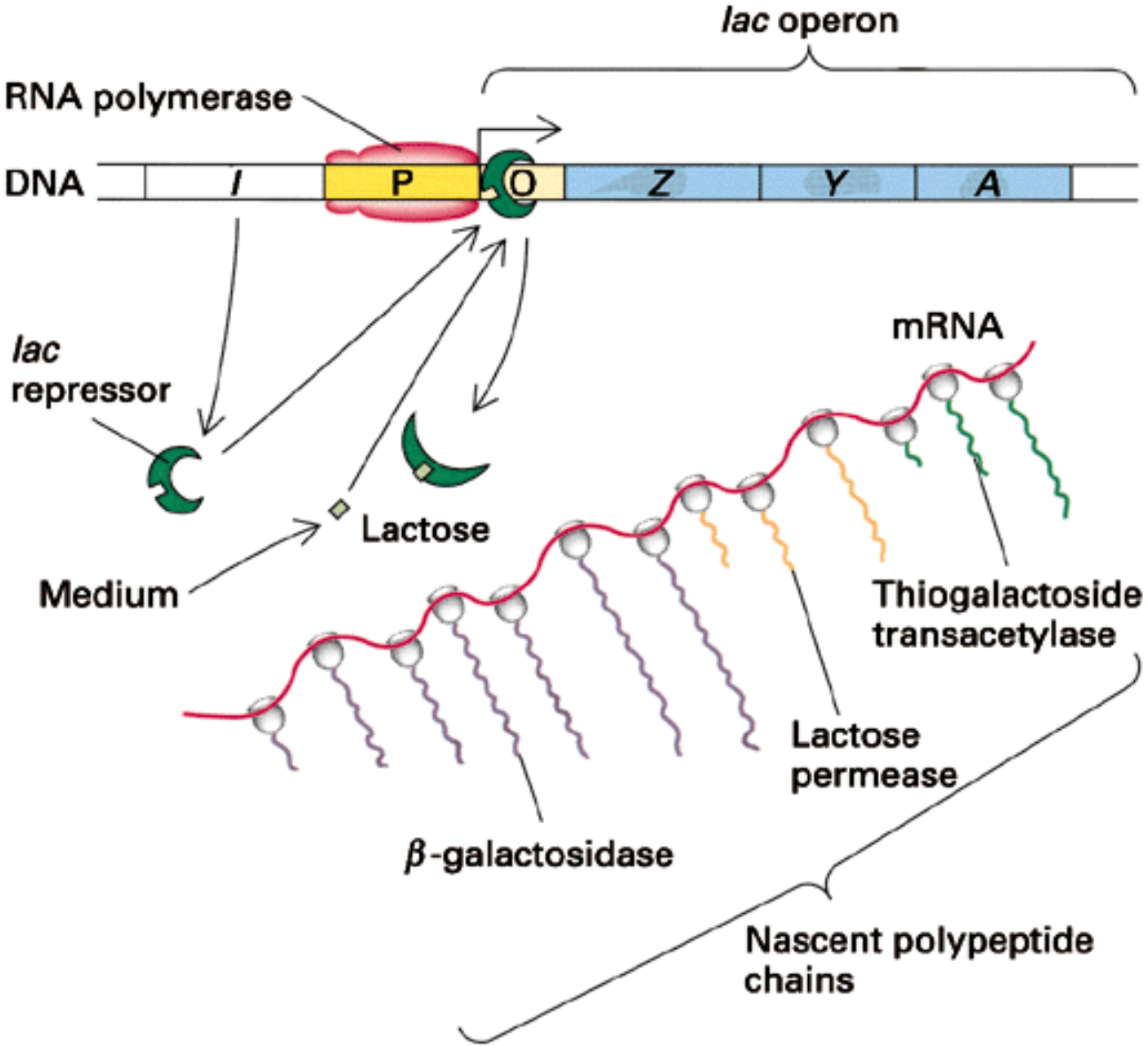
Bacterial colonies expressing β-galactosidase turn blue on agar plates containing X-gal, which is a substrate for β-galactosidase but is not an inducer

lac operon



lacI is not part of the *lac* operon but codes for repressor. It has a separate promoter and is transcribed independently of *lacZ*, *lacY* and *lacA*.

Jacob and Monod model of Lac Operon



Genetic studies identified the elements responsible for induction

The first understanding of how these enzymes are coordinately regulated came from analysis of E. coli mutants that do not regulate the synthesis of these enzymes normally.

Mutations are changes in the normal DNA sequence.

Mutant cells have mutations in the normal DNA sequence found in cells isolated from the wild.

The DNA sequence found in cells isolated from natural sources in the wild is called the "wild-type" sequence.

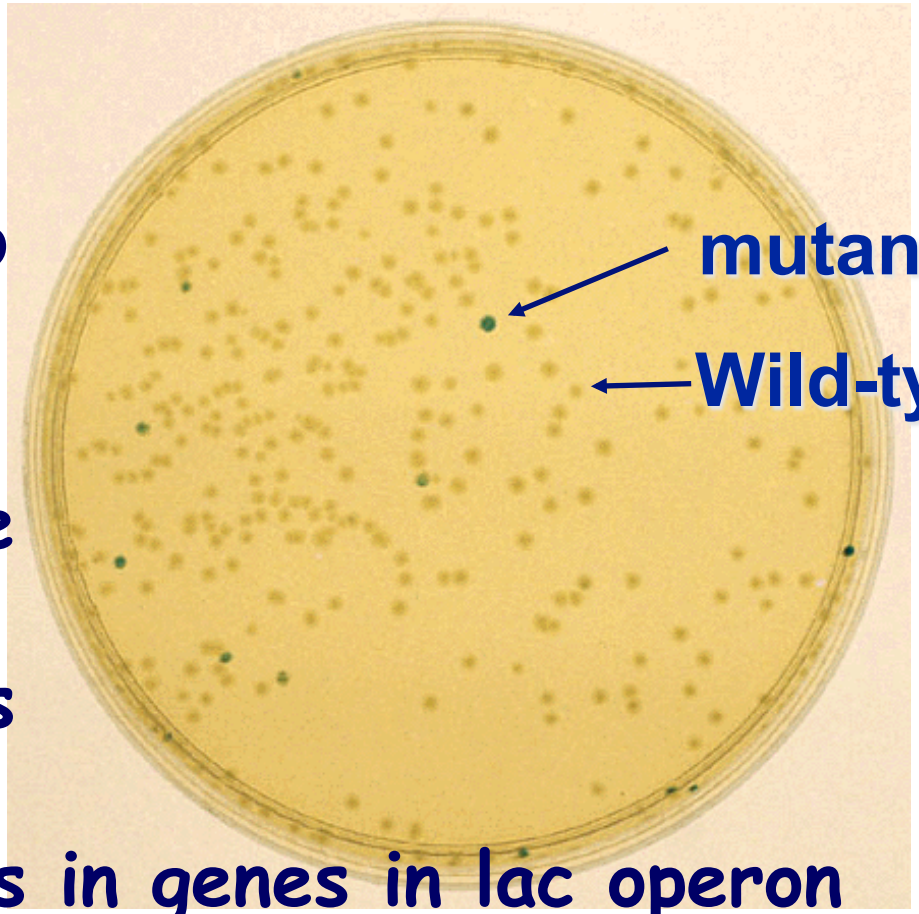
Terminology

- Inducible synthesis - synthesis that is turned on by an inducer
- Constitutive synthesis - synthesis at a constant level in the presence or absence of inducer

E. coli was treated with a mutagen and then plated on agar media containing X-gal

Media:
Glucose,
no lactose= no inducer

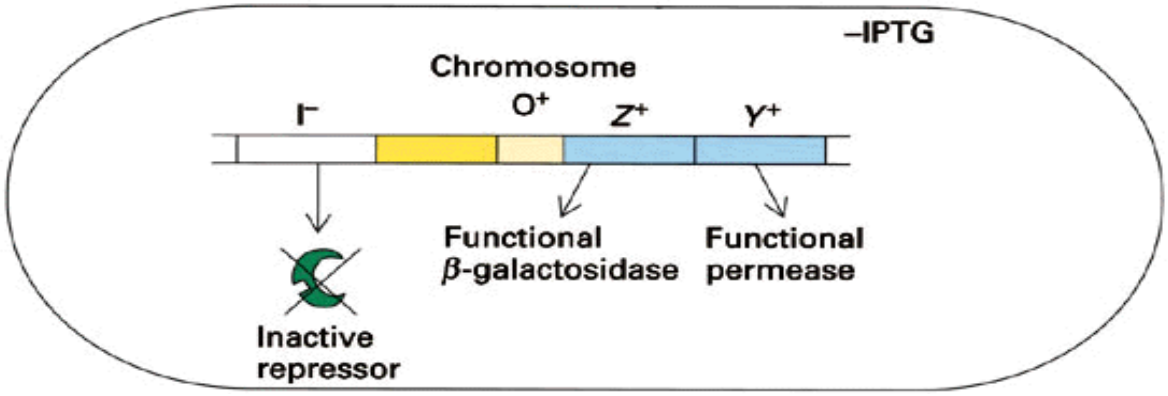
Blue colonies:
β-galactosidase
Synthesized in
Mutant colonies



How? Mutations in genes in lac operon

Two possible mutations result in constitutive expression of the lac operon

- **I^- mutants: Mutation in I gene, resulting in a defective repressor.**

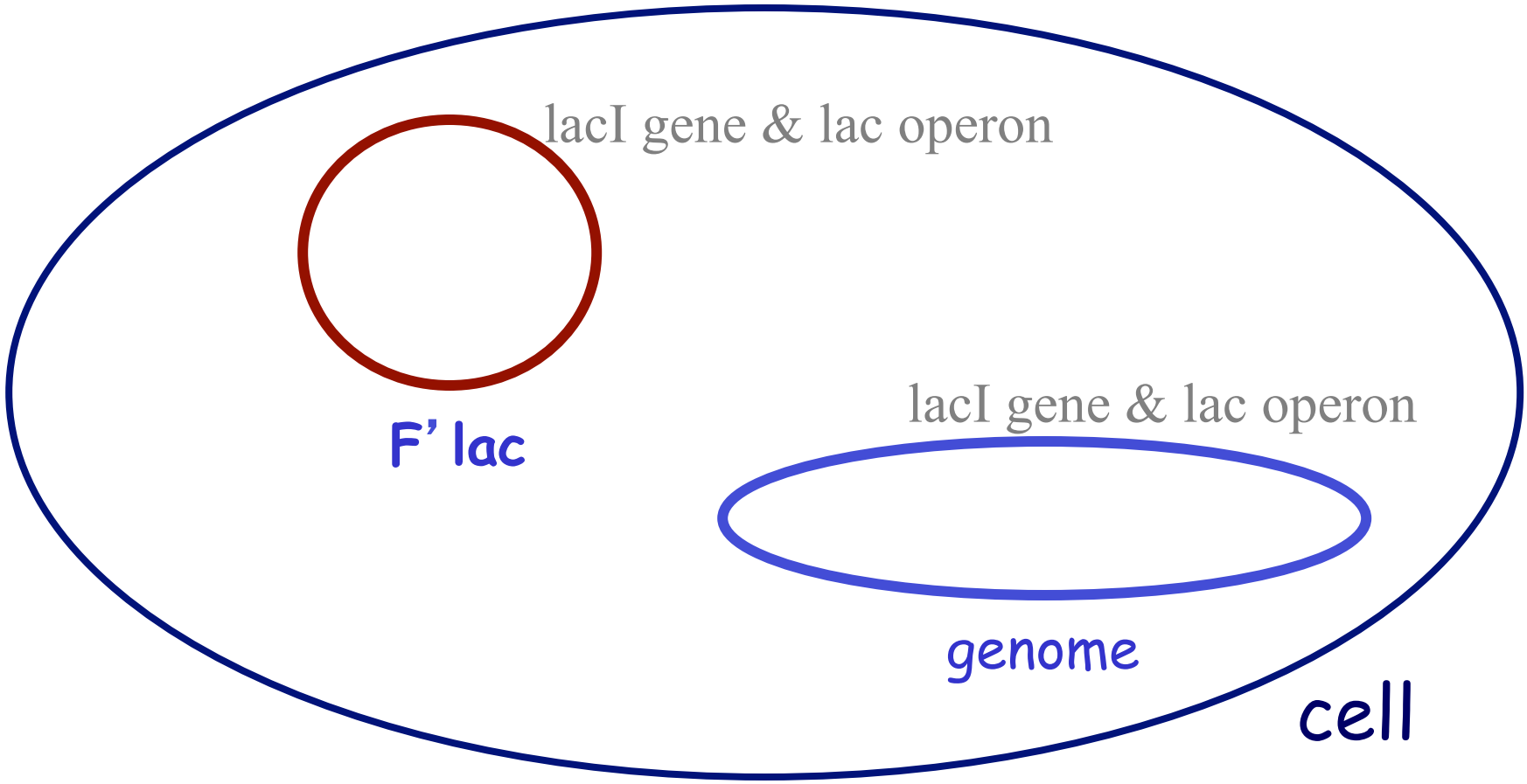


- **O^c mutants: mutation in the binding site (operator) for the repressor.**

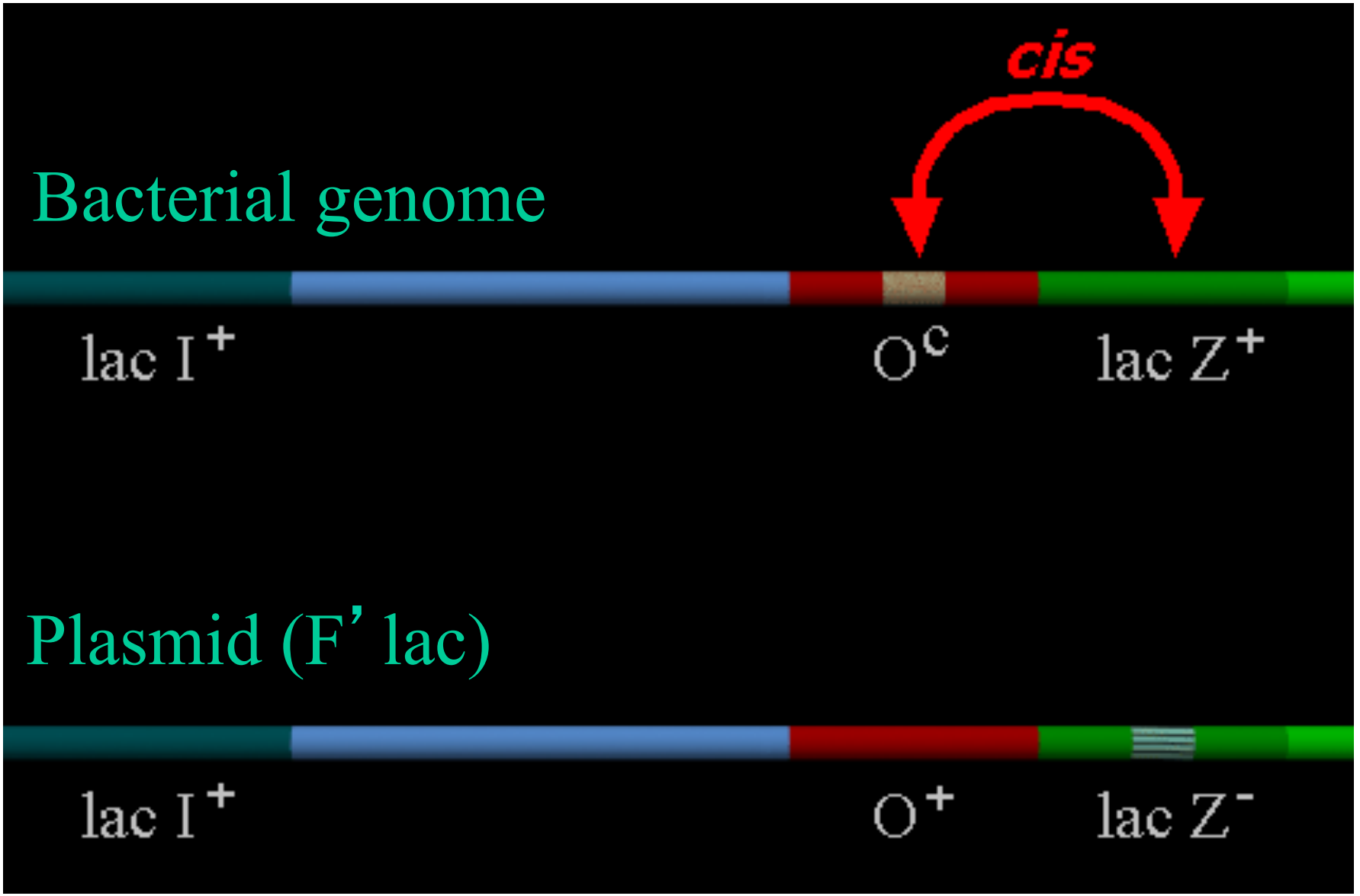
I^- and O^c mutants can be distinguished by analyzing partial diploids

- Both result in constitutive expression of β-galactosidase
- Since bacteria are haploid, a plasmid can be used to introduce a second copy of the lac operon into the cell
- I^- and O^c mutations behave differently in the cis versus trans arrangement

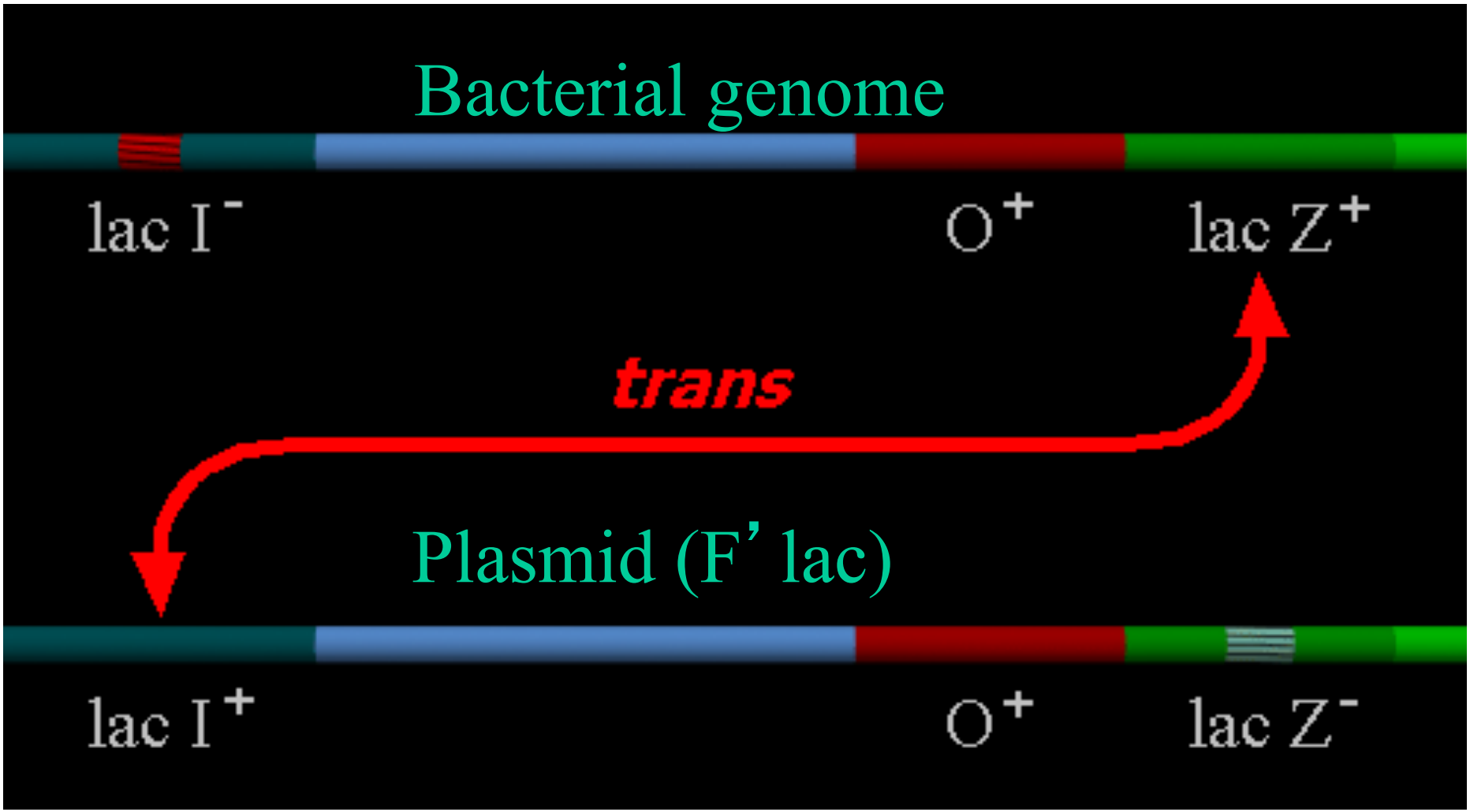
Partial Diploid



O^c mutation is physically linked to the Lac Z gene (*Cis*)



The Lac I Repressor is the protein product of the I gene. It is diffusible within the cell (Trans)



β-galactosidase activity

F' lac	Chromosome	-inducer	+inducer
-------	I+O+Z+	------	++++

Wildtype situation, lac operon is transcribed only in the presence of an inducer.

β-galactosidase activity

F' lac	Chromosome	-inducer	+inducer
-------	I+O+Z+	------	++++
-------	I-O+Z+	++++	++++

Mutation in the lac repressor gene (lacI) results in constitutive expression b/c no repressor is made

β-galactosidase activity

F' lac	Chromosome	-inducer	+inducer
-------	$I^+O^+Z^+$	------	++++
-------	$I^-O^+Z^+$	++++	++++
-------	$I^+O^cZ^+$	++++	++++

Mutation in the operator also results in constitutive expression.
b/c operator site is lost
And repressor can't bind

β-galactosidase activity

F' lac	Chromosome	-inducer	+inducer
-------	I+O+Z+	------	++++
-------	I-O+Z+	++++	++++
-------	$I^+O^cZ^+$	++++	++++
I+O+Z-	I-O+Z+	-----	++++

When the repressor is supplied in trans, regulation is observed again.

β-galactosidase activity

F' lac	Chromosome	-inducer	+inducer
-------	$I^+O^+Z^+$	------	++++
-------	$I^-O^+Z^+$	++++	++++
-------	$I^+O^cZ^+$	++++	++++
$I^+O^+Z^-$	$I^-O^+Z^+$	-----	++++
$I^+O^+Z^-$	$I^+O^cZ^+$	++++	++++

When the operator is supplied in trans, there is no effect (i.e. expression remains constitutive b/c functional operator is needed to bind repressor)

Box 18-2 Jacob, Monod and Gene Regulation

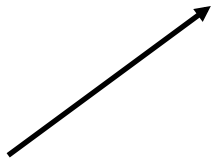
These and other results led them to propose that genes
Were expressed from specific sites called promoters
And
Expression was regulated by repressors that act through
Operator sites located on DNA beside promoter

Cis versus Trans regulation is a key paradigm in gene regulation

lac operon is also under positive control

Observation:

Lactose media: β-gal activity high
Glucose media: β-gal activity low
Glu+lac media: β-gal activity low



***lac* repressor is not binding to operator and yet β-gal is not being expressed,**
Why? Positive control by glucose

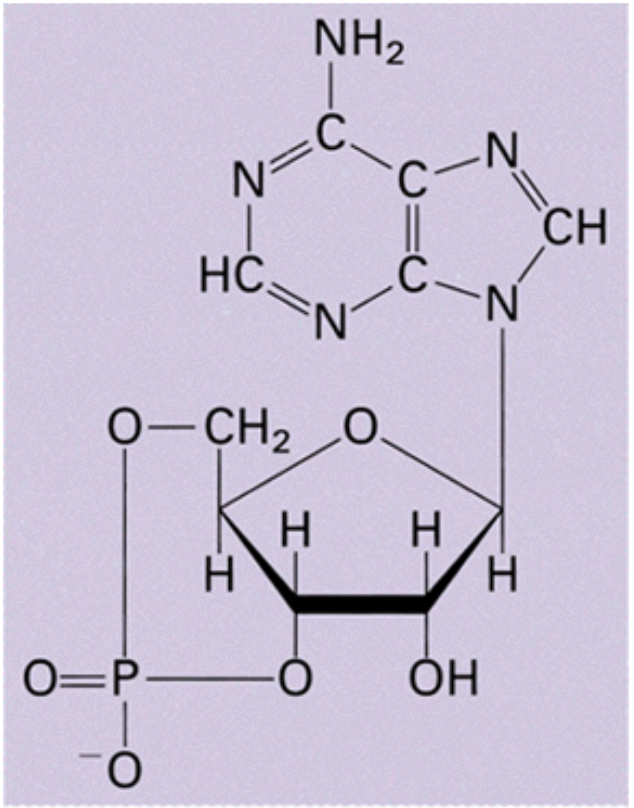
Many operons are under general control referred to as catabolite repression

Glucose metabolism is favored over lactose metabolism (or any other disaccharide)

Level of glucose in media is detected by cAMP

glucose ↑ cAMP ↓

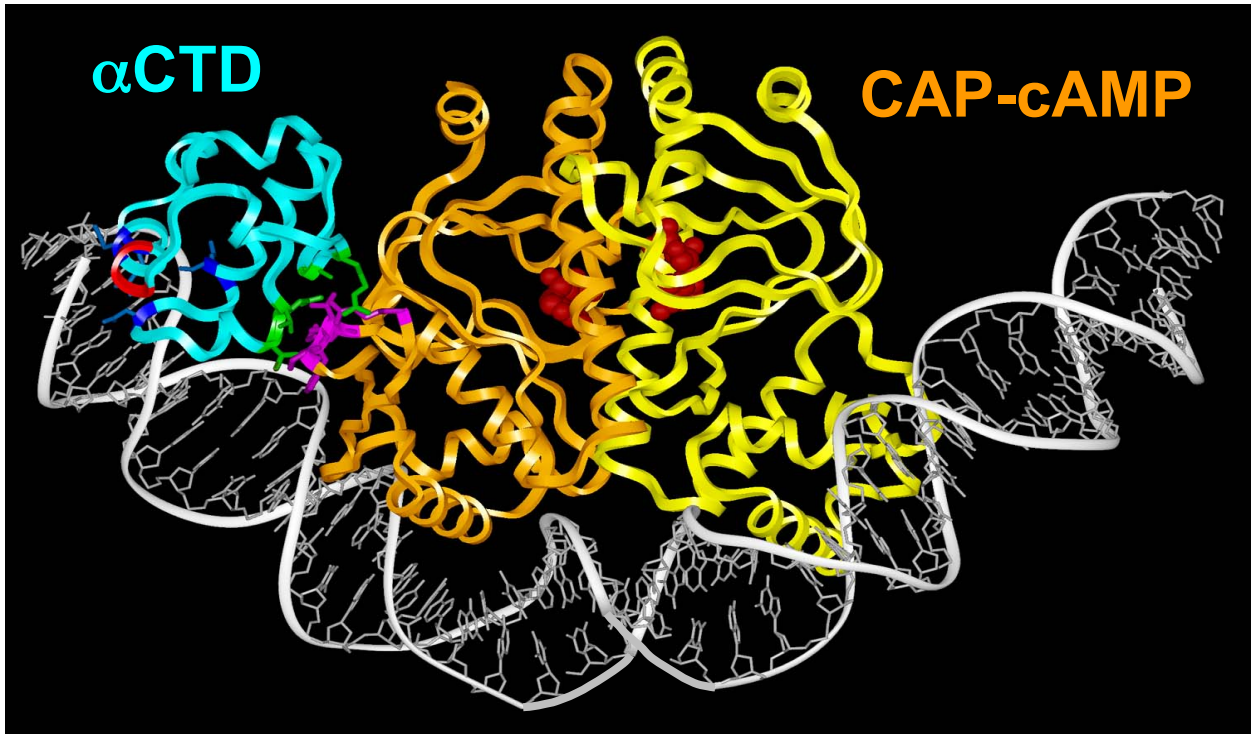
glucose ↓ cAMP ↑



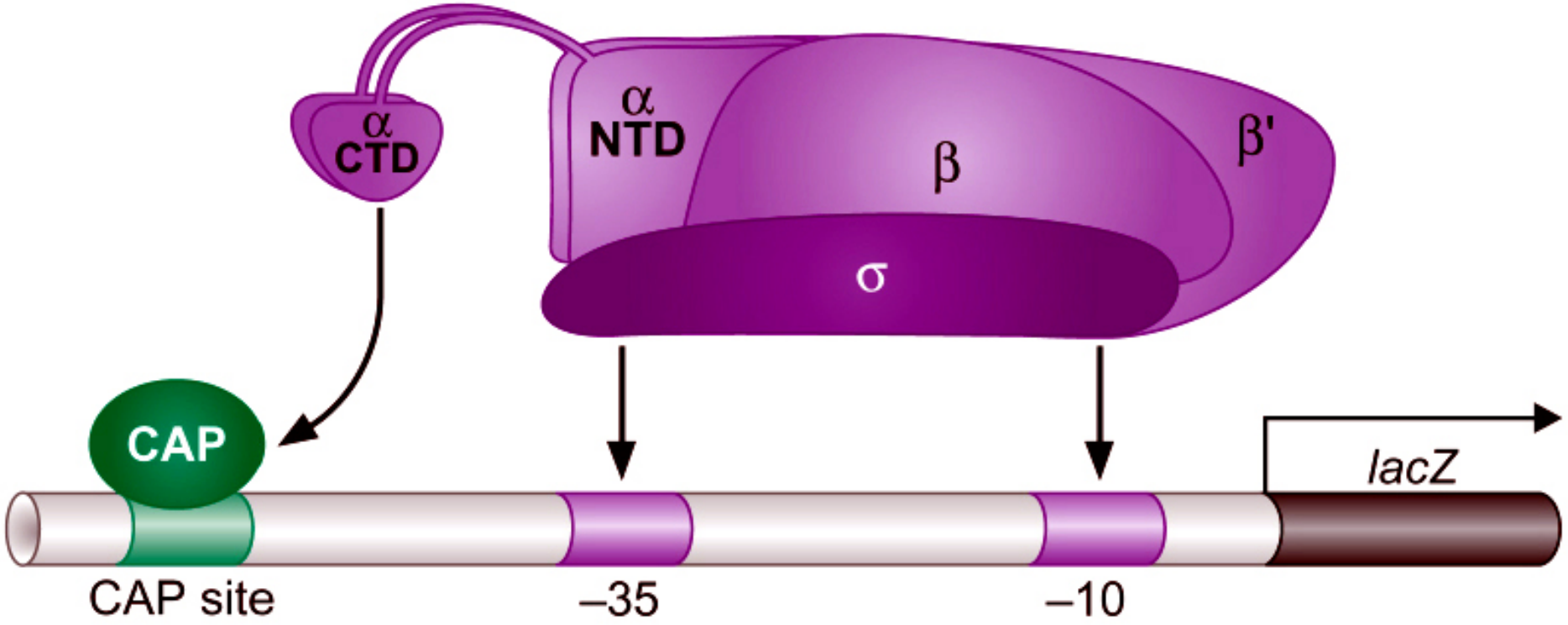
Cyclic AMP

CAP (Catabolite Activator Protein)

- CAP-cAMP binds to specific DNA sequences
- Binding results in a bend in the DNA
- CAP-cAMP also interacts directly with the RNA polymerase to stimulate binding to weak promoter sequences



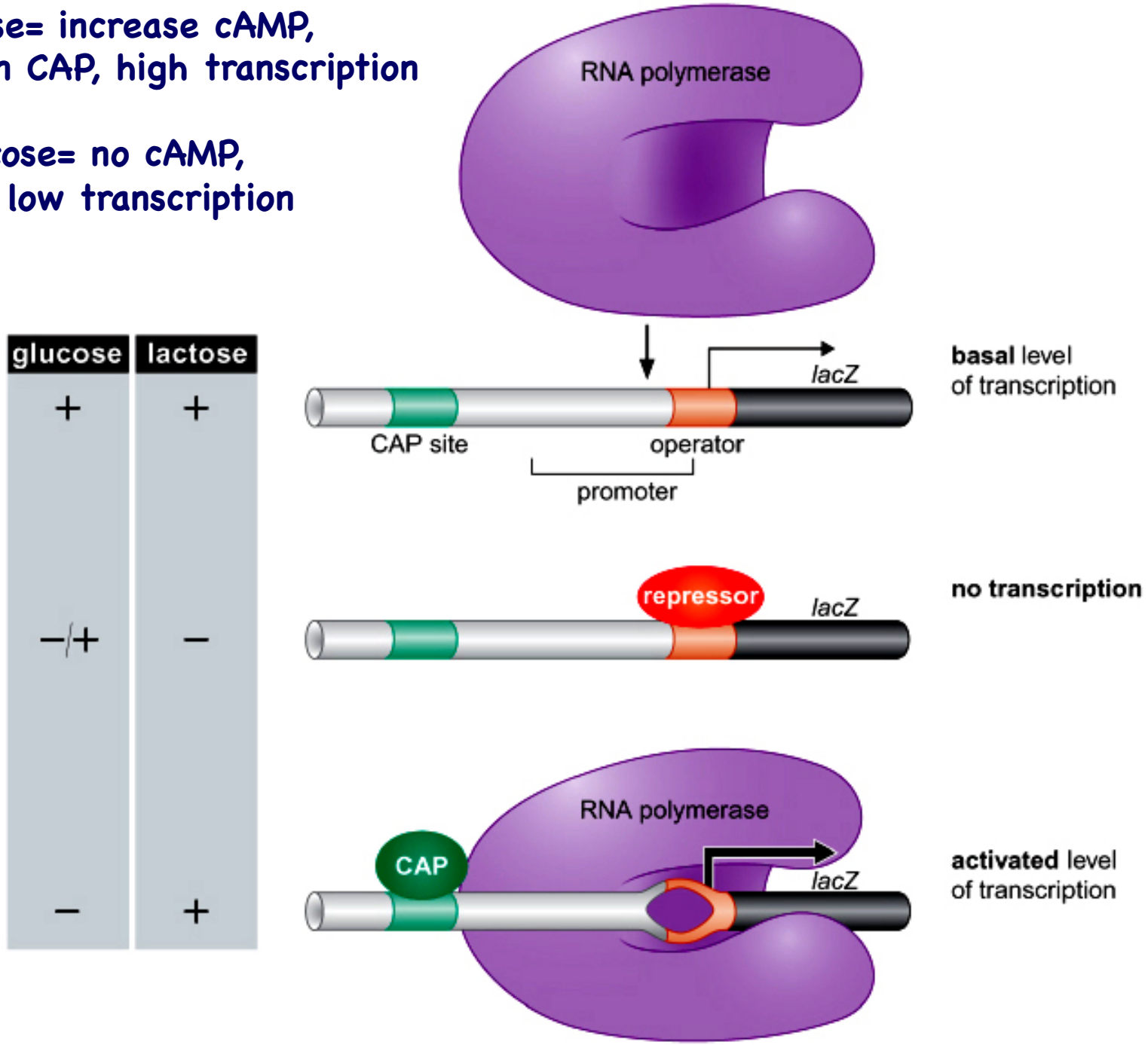
The DNA-bound CAP is able to interact physically with RNA polymerase and essentially increase the affinity of RNA polymerase for the lac promoter.



RNA polymerase binding at lac promoter with the help of CAP.
CAP is recognized by the CTD (carboxy terminal domain) of polymerase.
CAP is activator which increases transcription.

No glucose= increase cAMP,
= bring in CAP, high transcription

High glucose= no cAMP,
=no CAP, low transcription



Implications and Critical Thinking-8

- At promoters activated by recruitment alone polymerase binds very poorly, yet once it is bound the polymerase spontaneously undergoes transition to the open complex and transcription ensues.
- Because binding is the limiting step for transcription, such promoters can be regulated simply at the level of polymerase binding.
- Activators can therefore act by recruiting the polymerase to the promoter simply by binding near the promoter DNA with one surface, and, with another, binding to polymerase.
- How can one determine how well a promoter will interact with polymerase?