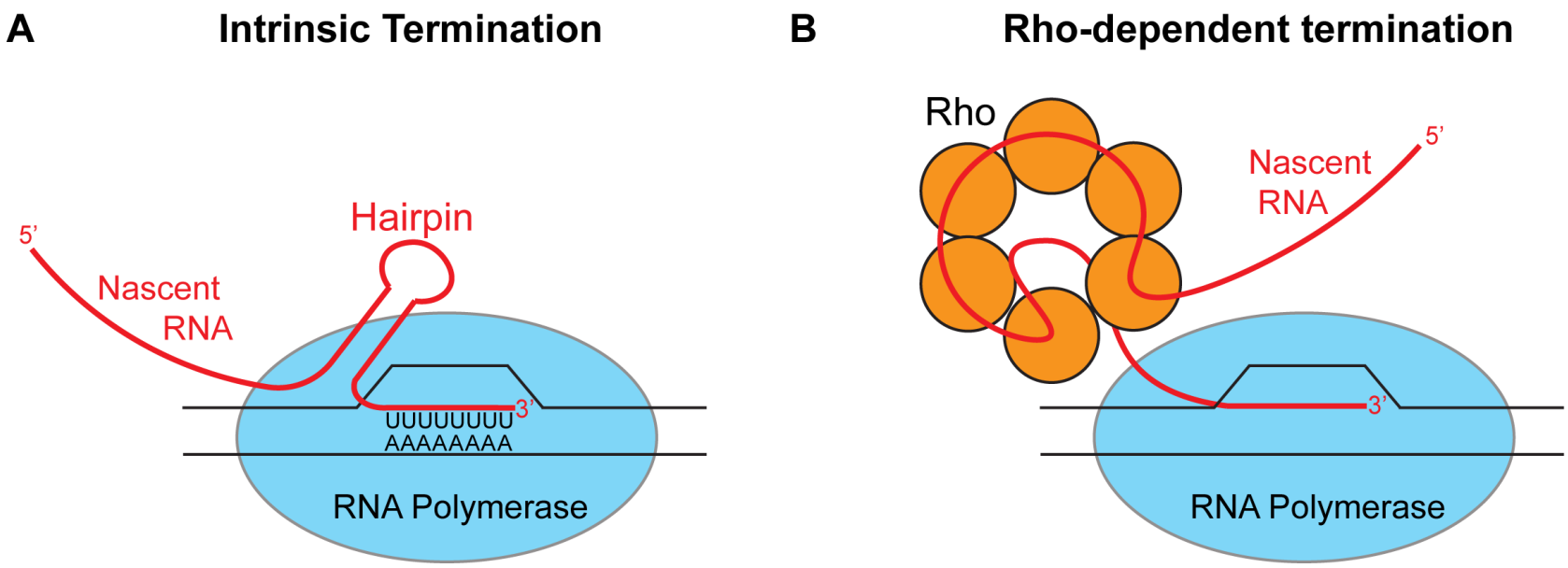
The transcription cycle:
2 possible termination mechanisms

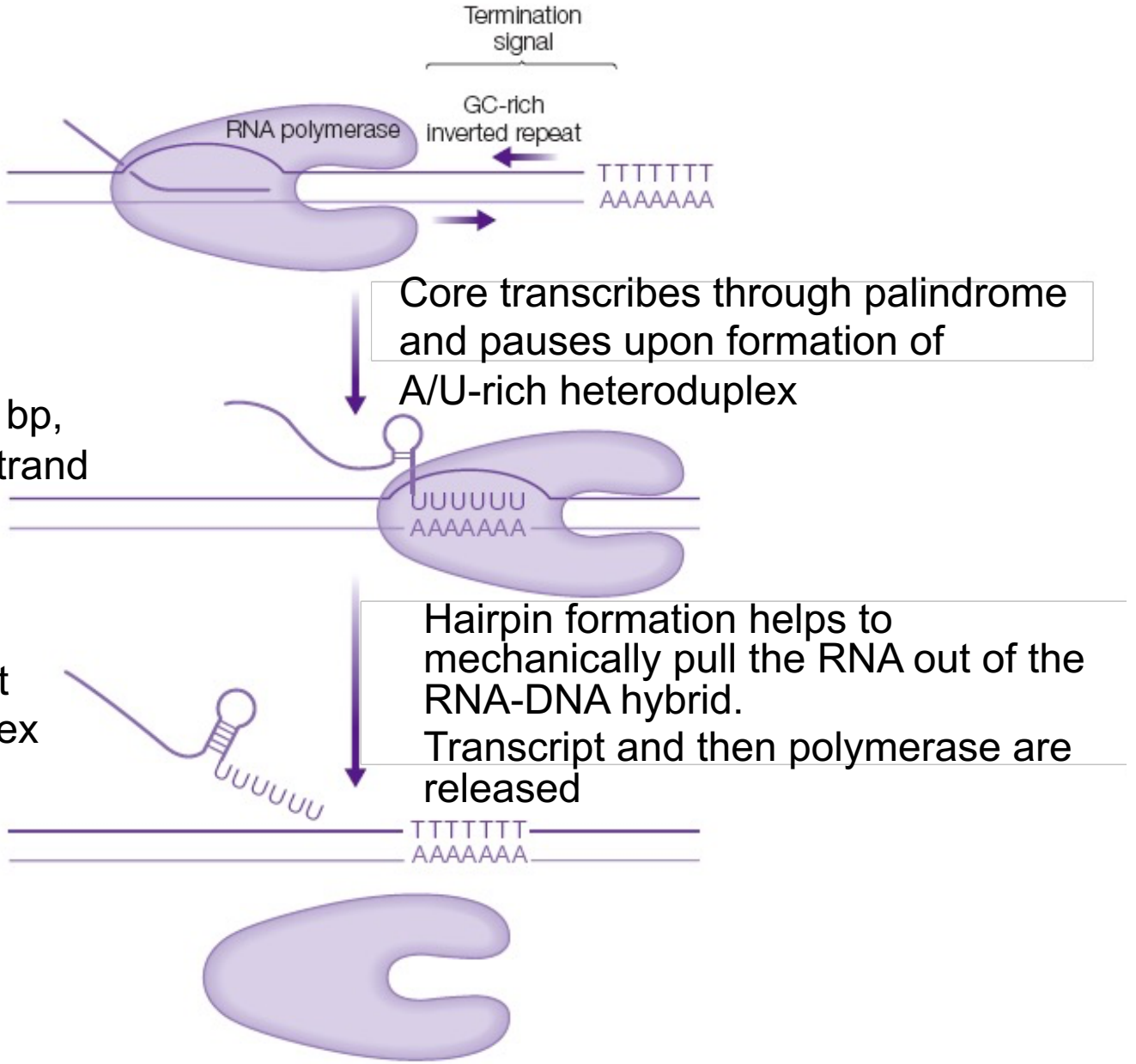


Intrinsic termination (ρ independent)

Termination sequence characterized by

a) G-C rich palindrome, followed by

b) 4-10 consecutive A-T bp, with As on template strand



Core transcribes through palindrome and pauses upon formation of A/U-rich heteroduplex

Hairpin formation helps to mechanically pull the RNA out of the RNA-DNA hybrid.

Transcript and then polymerase are released

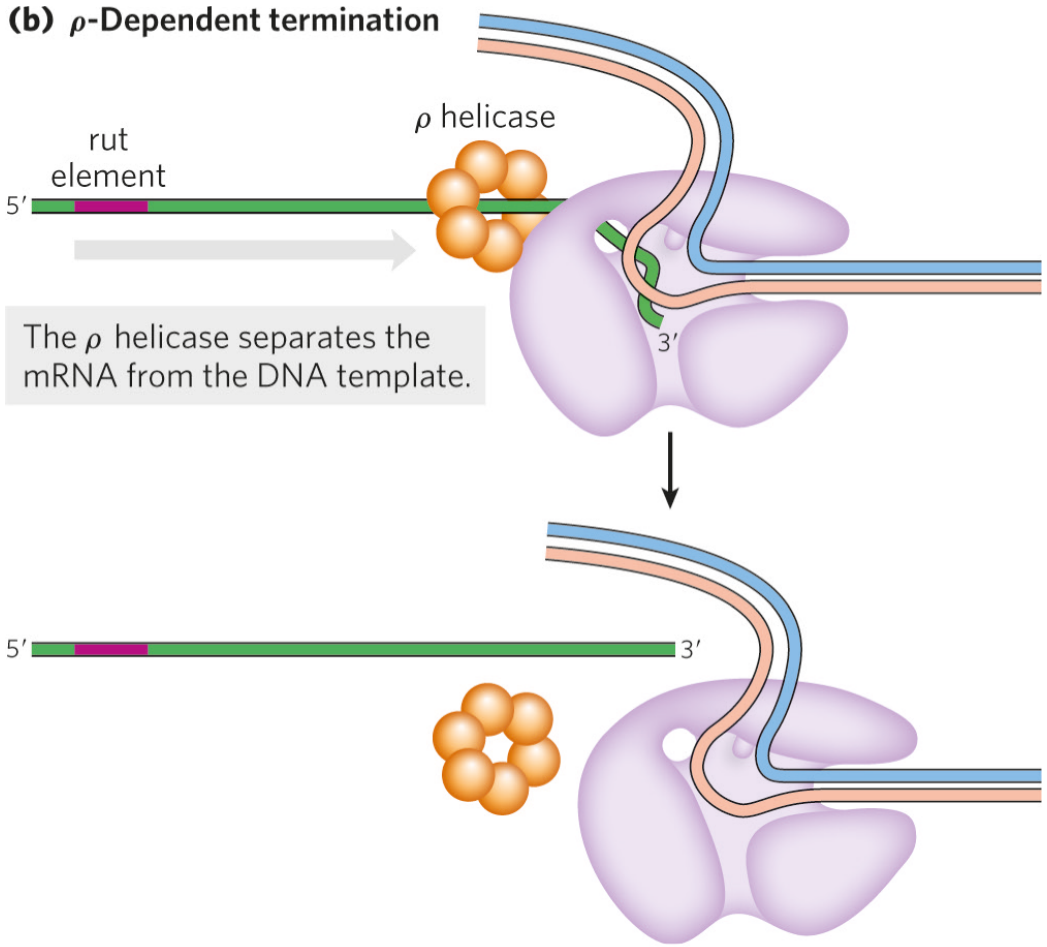
Dissociation of transcript aided by low heteroduplex stability of dA•rU region

Rho-dependent transcription termination

ρ (rho) = protein factor that has an ATP-dependent RNA-DNA **helicase** activity.

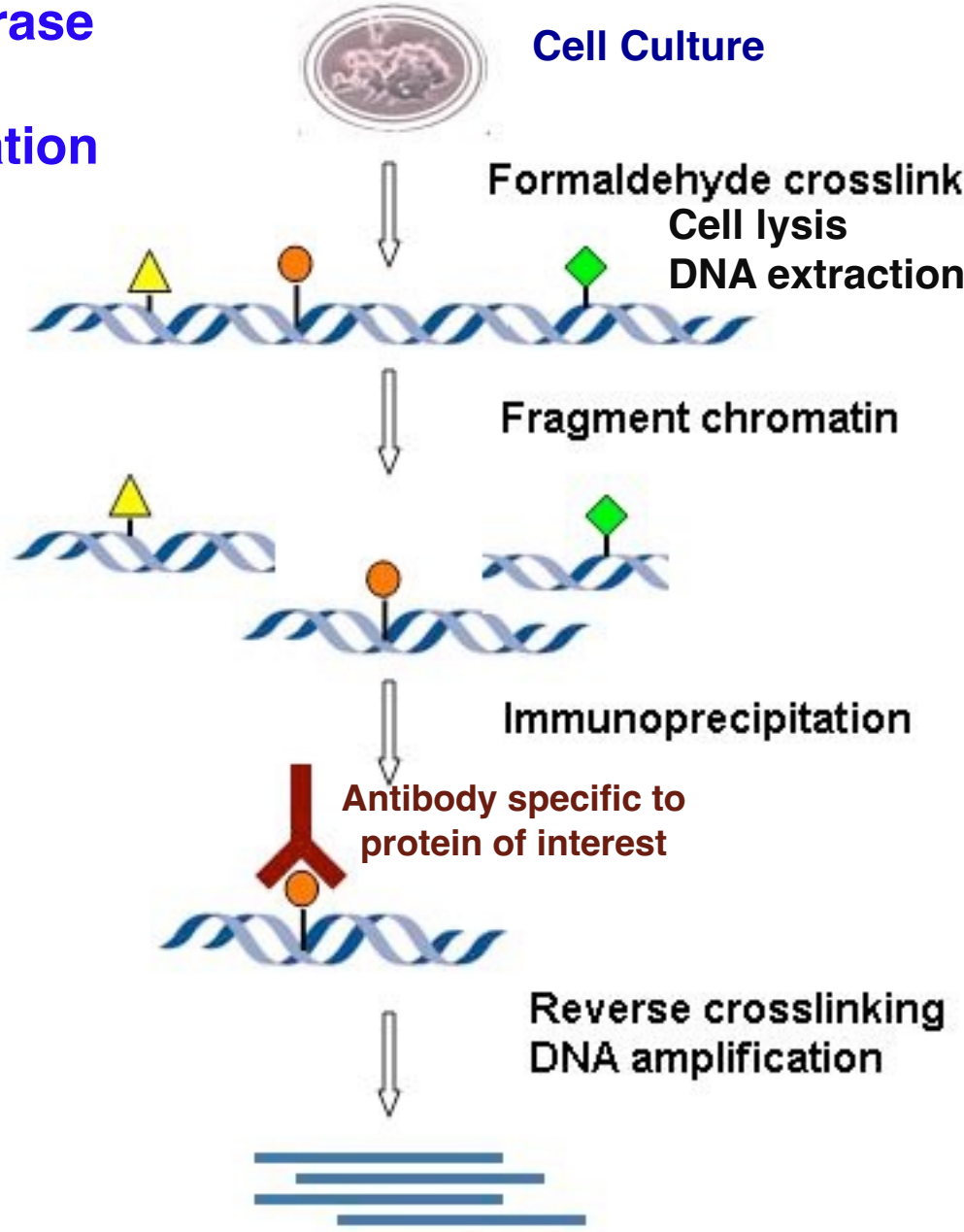
ρ-dependent terminators = class of terminators that rely on the protein rho

- Have a sequence called a *rut* (*r*ho *ut*ilization) element
- Rho promotes release of the RNA



Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

Identifying the sites of RNA polymerase binding to genes in vivo (=in cells) using "chromatin" immunoprecipitation = ChIP (technique developed in eukaryotes but adapted to bacterial cells)

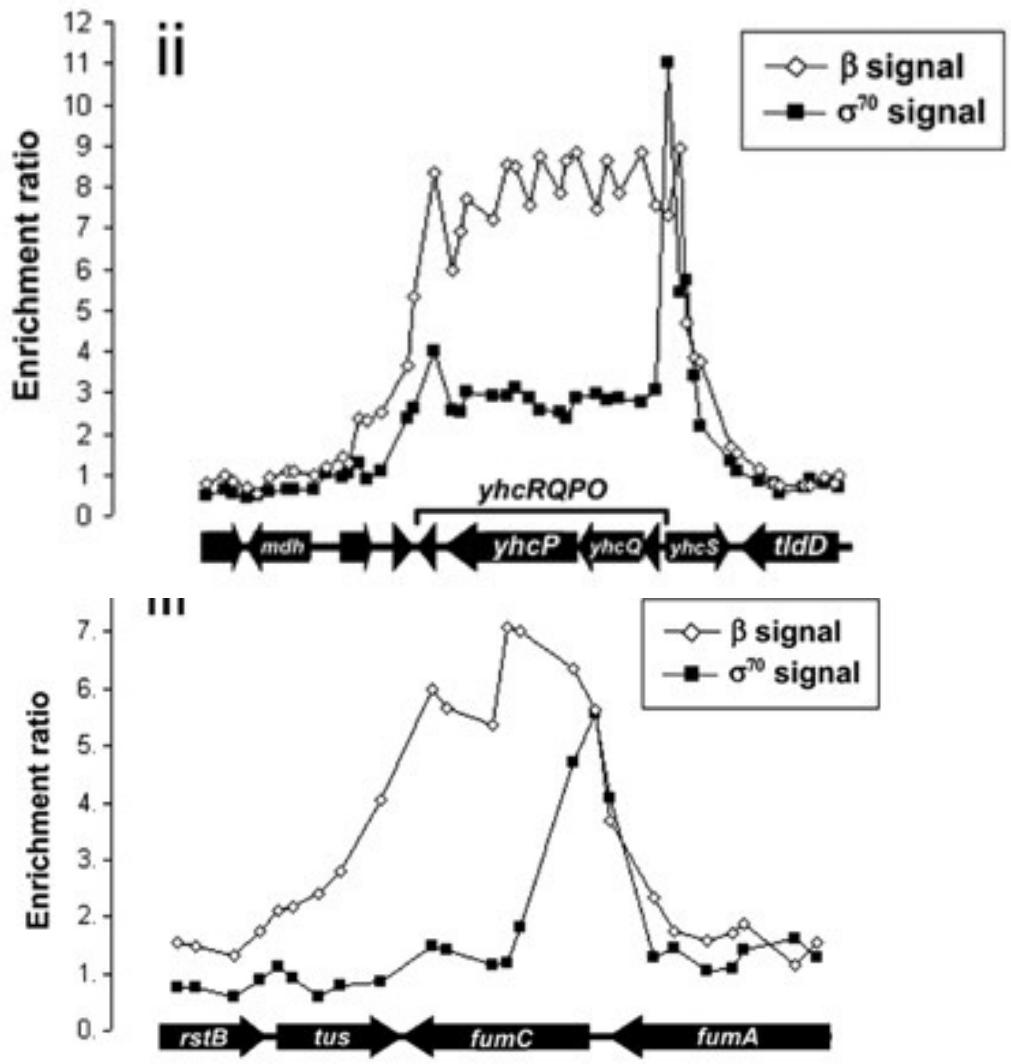


- Crosslink proteins to DNA in cells
- Lyse cells
- Fragment DNA into small fragments
- immunoprecipitate proteins of choice bound to DNA.
- Recover bound DNA, amplify and identify the DNA bound by specific proteins using sequencing technology

-> amplify and identify the DNA bound by specific proteins by sequencing

Using ChIP to localize σ70 and β subunits on genes in the bacterial genome

Y-axis = Enrichment-> shows how much the protein analyzed by ChIP crosslinks to the DNA in the region studied

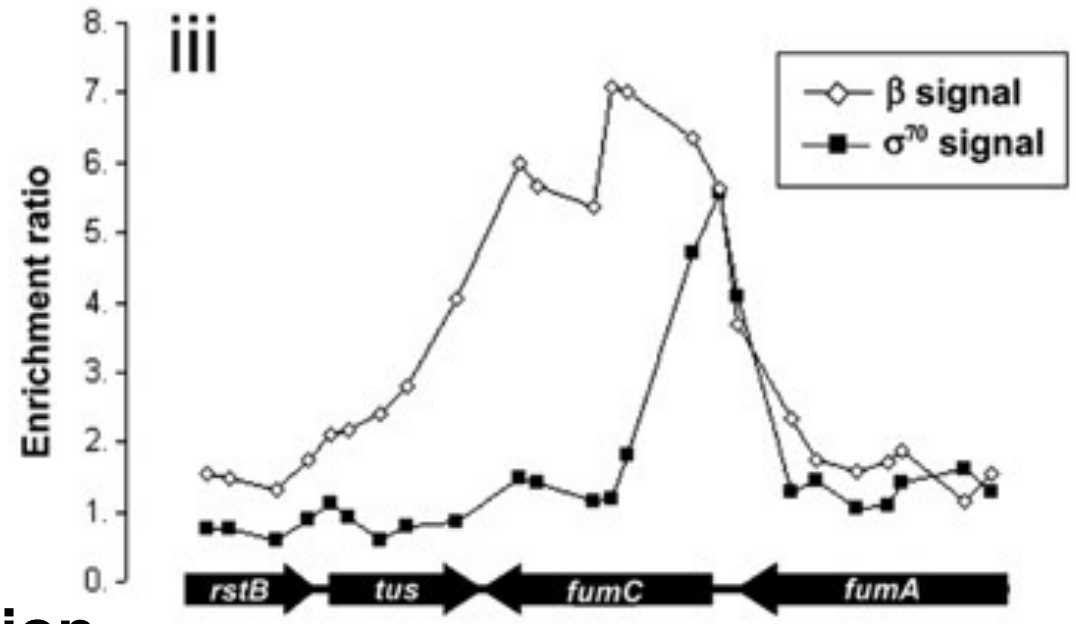


The β subunit associates with highly expressed genes/ operons (broad distribution of β along the operon)

the sigma factor mostly associates with promoters and does not travel with the core RNA polymerase (sharp peak of enrichment of σ70 in the promoter)

Grainger et. al Proc Natl Acad Sci U S A. 2005 Dec 6;102(49):17693-8

What ChIP signal would be expected for the two alpha subunits of RNAP?



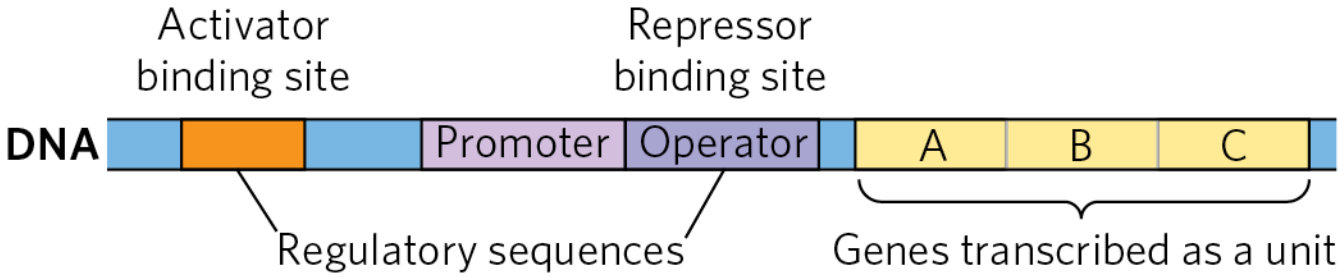
A: Same as σ70 (peaks at promoter)

B: Same as β subunit = promoter+transcribed region

C: No way to know as there are two alpha subunits on RNAP and you cannot differentiate the signal for each

D: There would be a higher enrichment in the transcribed region than in the promoter since alpha subunits join the RNA polymerase after transcription has begun

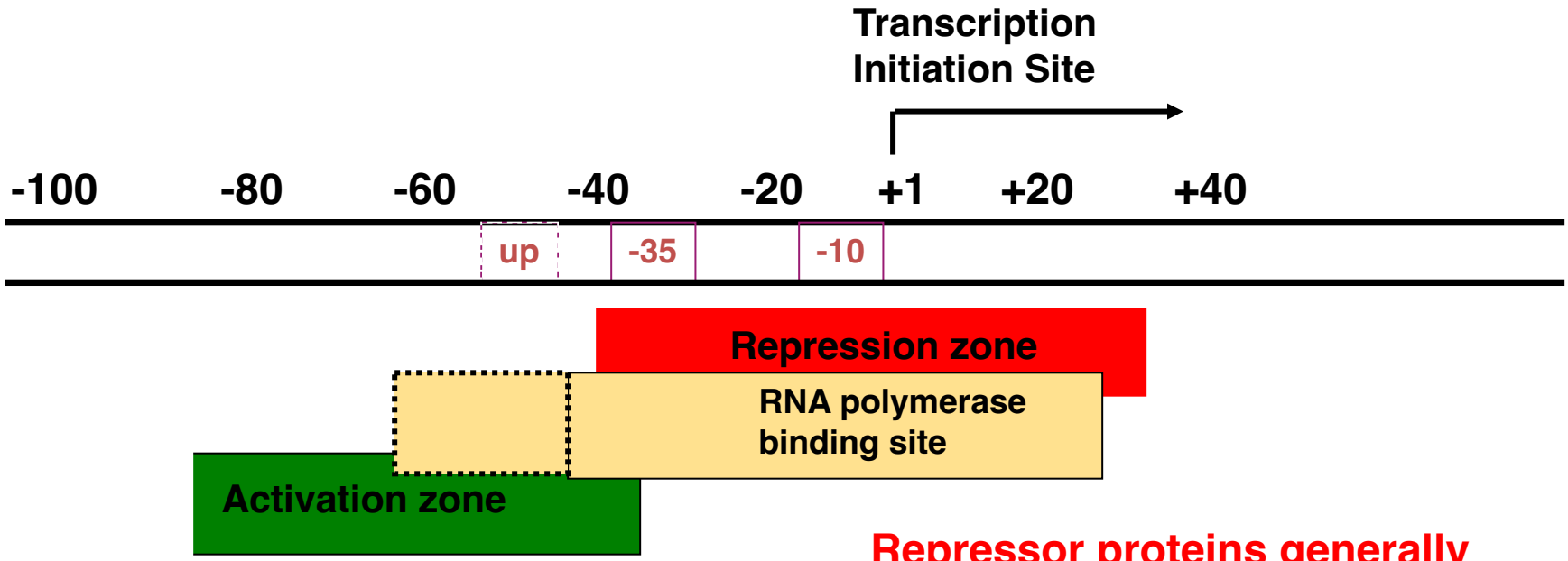
A representative bacterial operon



Some definitions:
Polycistronic mRNA: multiple genes on a single transcript
Monocistronic mRNA: single gene on a transcript
Operator: DNA sequence that a repressor binds to
Operon: gene cluster + promoter + regulatory sequences

Genes within an operon are usually related (e.g. multiple subunits of a larger complex)

Transcription Regulation by Activator and Repressor Proteins: General Principles



Activators proteins generally bind upstream of the promoter and interact with RNA Polymerase to help load it onto the promoter

Repressor proteins generally block RNA polymerase binding by competing for binding with sequences near the promoter

Negative and positive regulation

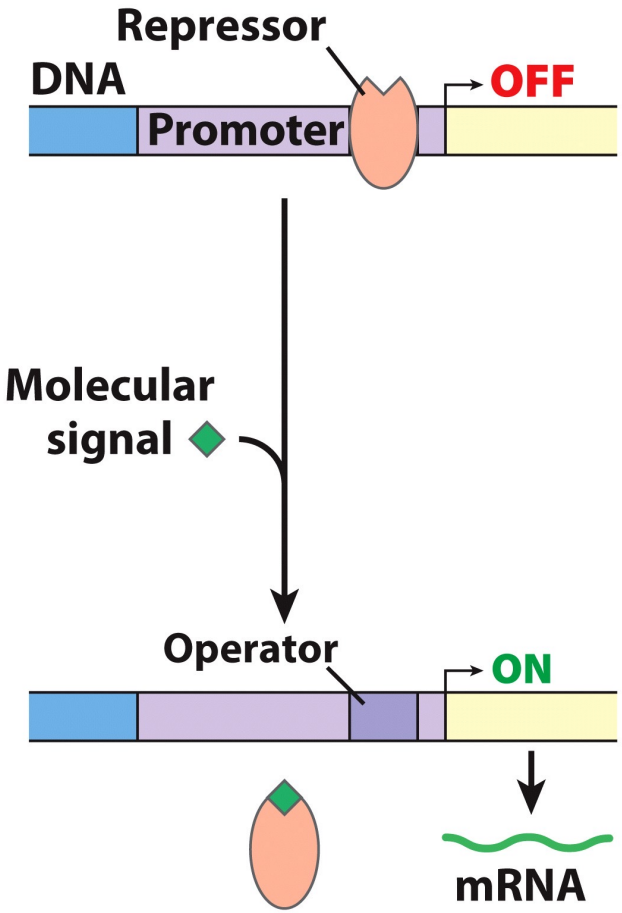
Negative regulation: regulation via a repressor (a protein that blocks transcription)

Positive regulation: regulation via an activator (a protein that facilitates/enhances transcription)

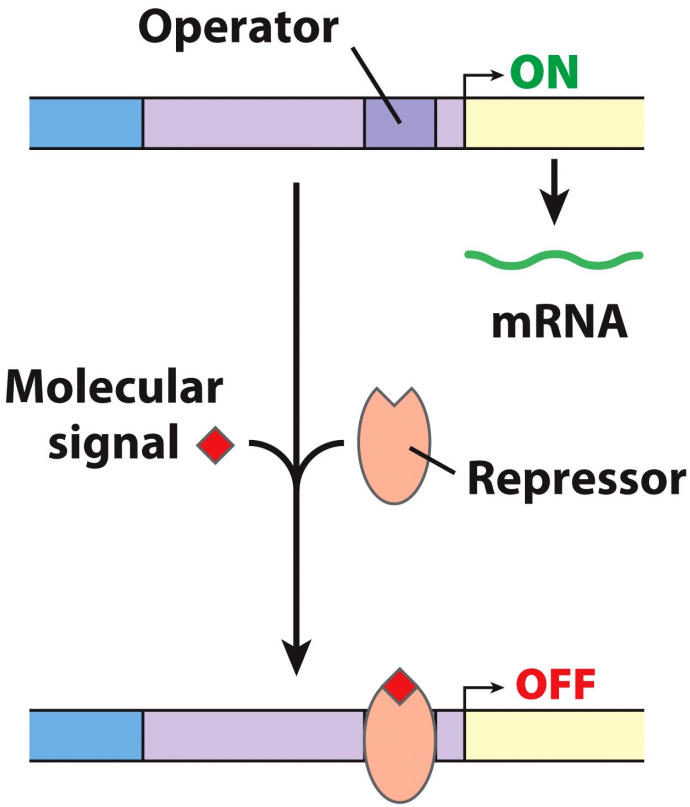
Transcriptional Regulatory Models:

Negative Regulation

Negative regulation
Molecular signal causes dissociation of repressor from DNA, inducing transcription.



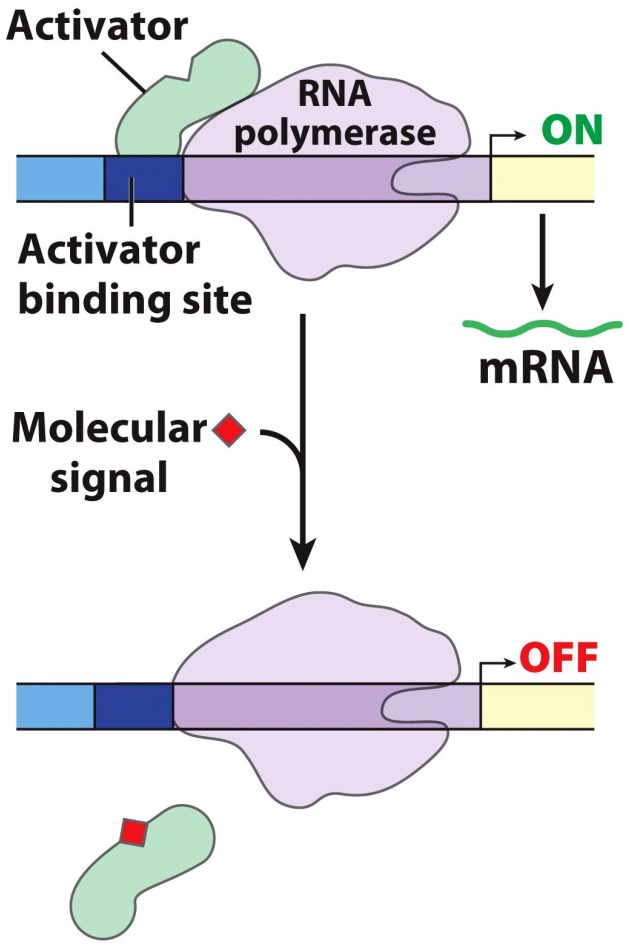
Negative regulation
Molecular signal causes binding of repressor to DNA, inhibiting transcription.



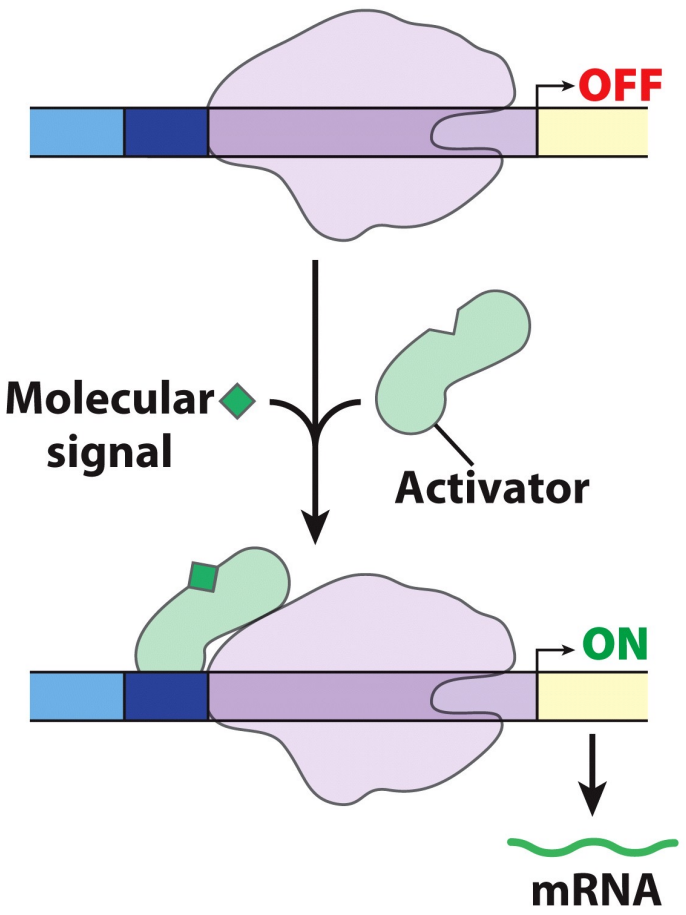
Transcriptional Regulatory Models:

Positive Regulation

Positive regulation
Molecular signal causes dissociation of activator from DNA, inhibiting transcription.



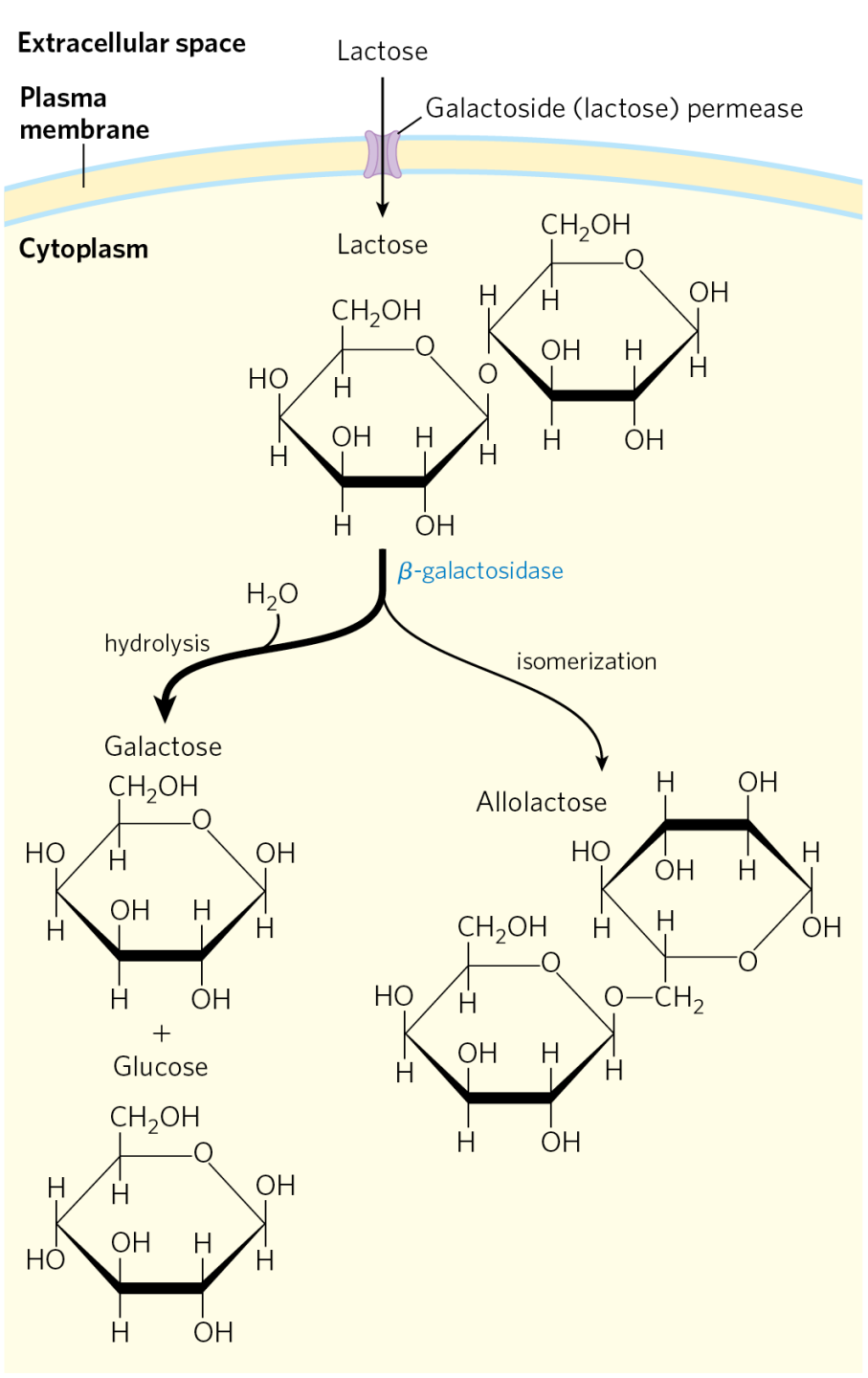
Positive regulation
Molecular signal causes binding of activator to DNA, inducing transcription.



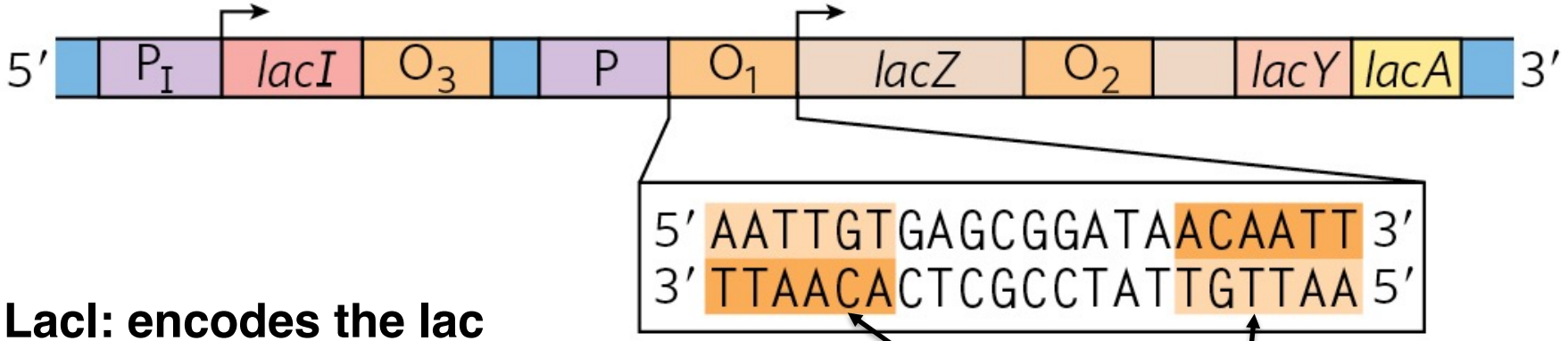
A case study in bacterial gene regulation: Catabolism of lactose in *E. coli*

<u>Galactoside permease (LacY)</u>: transports lactose into the cell

<u>β-galactosidase (LacZ)</u>: cleaves lactose to galactose and glucose



Structure of the *lac* operon



LacI: encodes the lac repressor

LacZ: β-galactosidase

LacY: Galactoside permease

(We will not discuss LacA)

P: promoter for lac genes

P_I: promoter for lacI

O_1: main operator

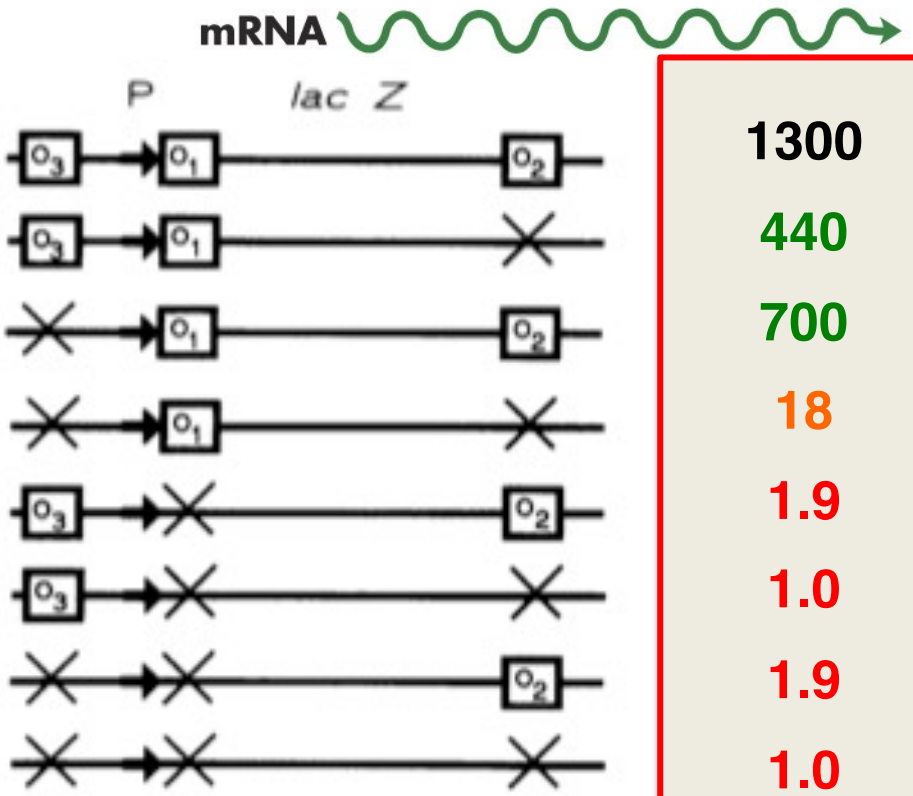
O_2 and O_3: additional operator sites

Specific DNA sequences that the lac repressor binds to

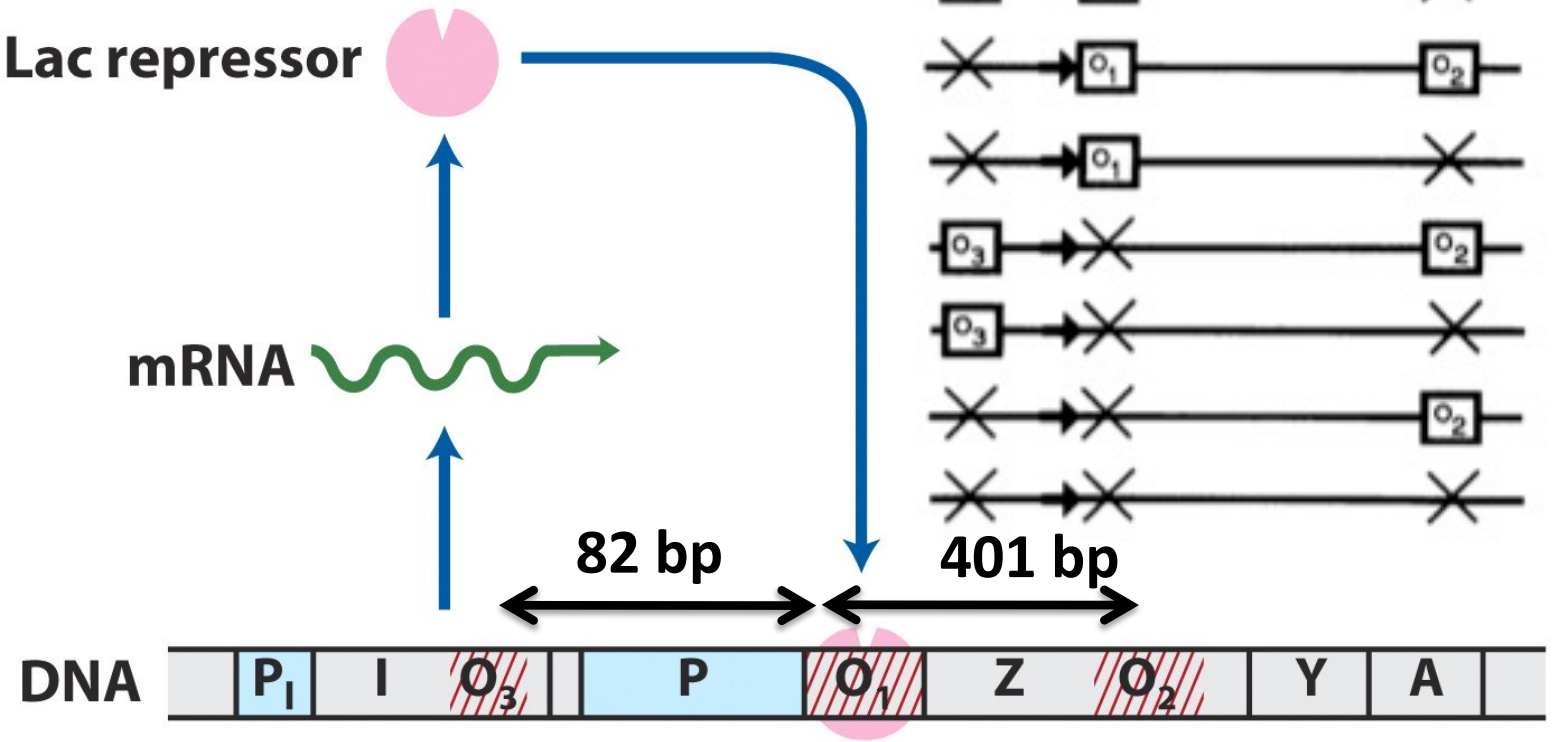
How does repression of the *lac* operon actually work?

3 operator sequences in the Lac Operon

- O1 has an essential function
- Needs two operator sites : O1+O2 or O1+O3 for optimal repression



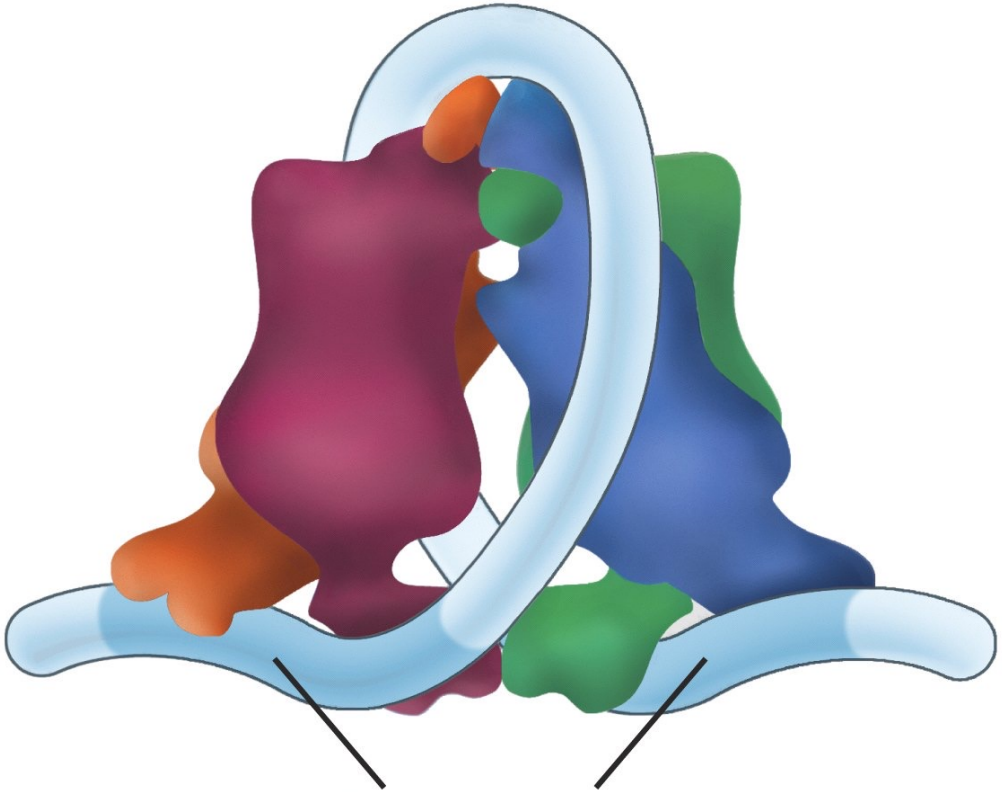
Fold Repression
1300
440
700
18
1.9
1.0
1.9
1.0



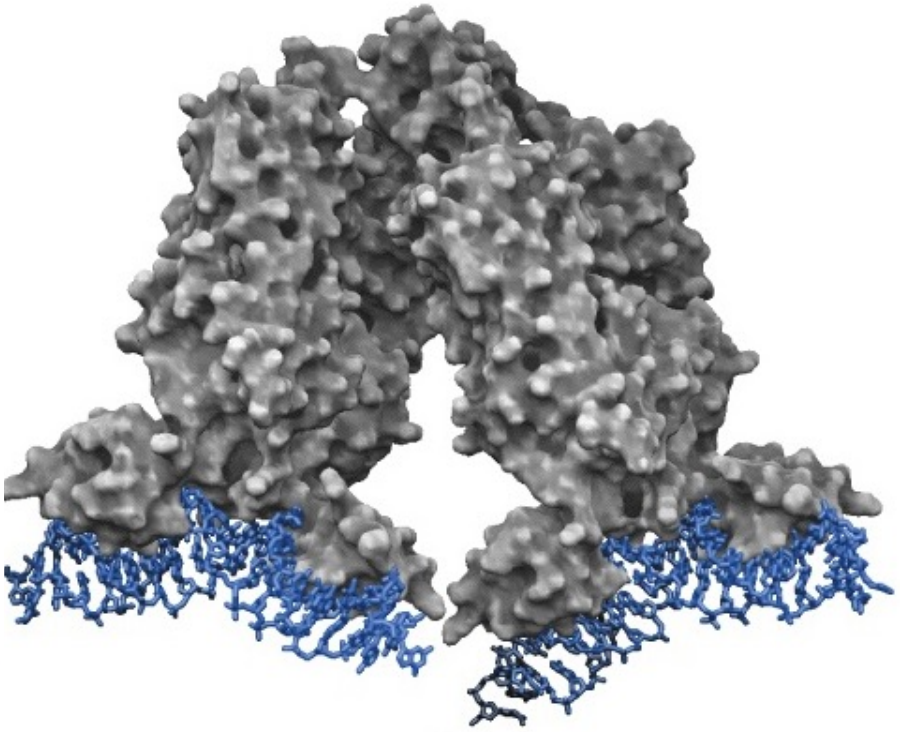
Structural study of the Lac Repressor

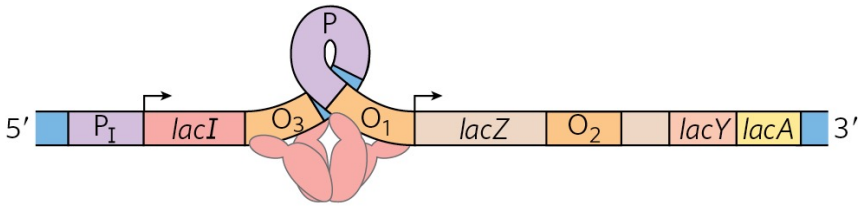
A tetramer of Lac Repressor complexed to looped operator DNA

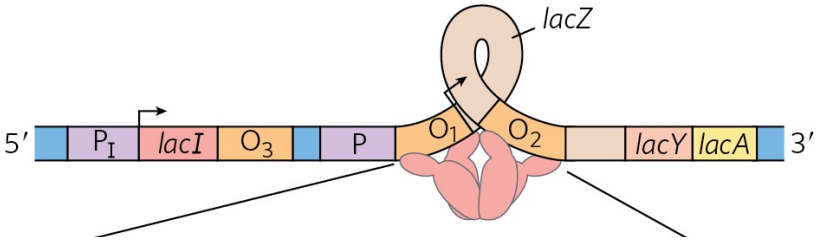
Cartoon



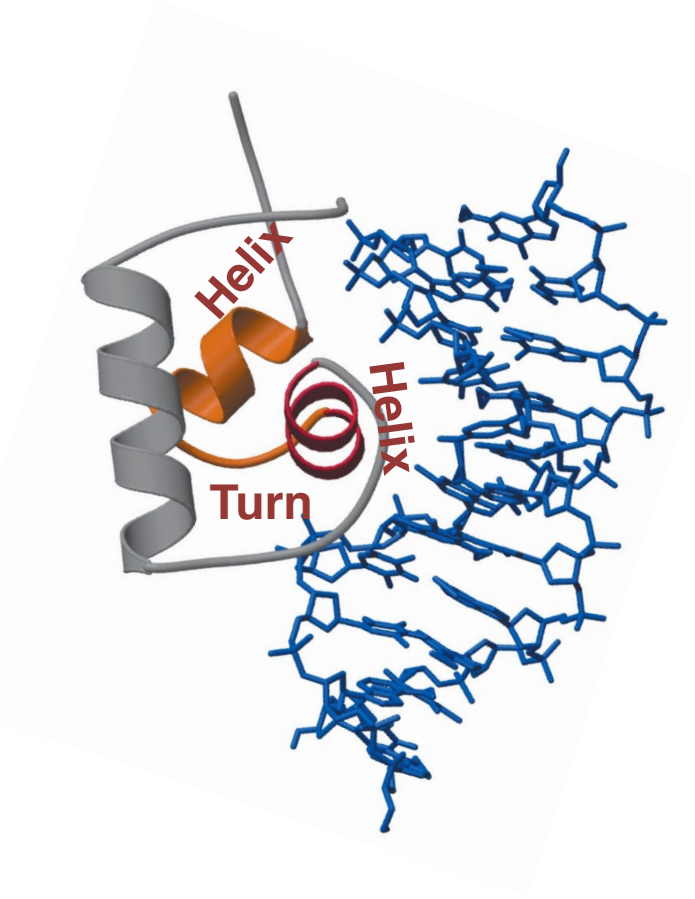
Space filling view

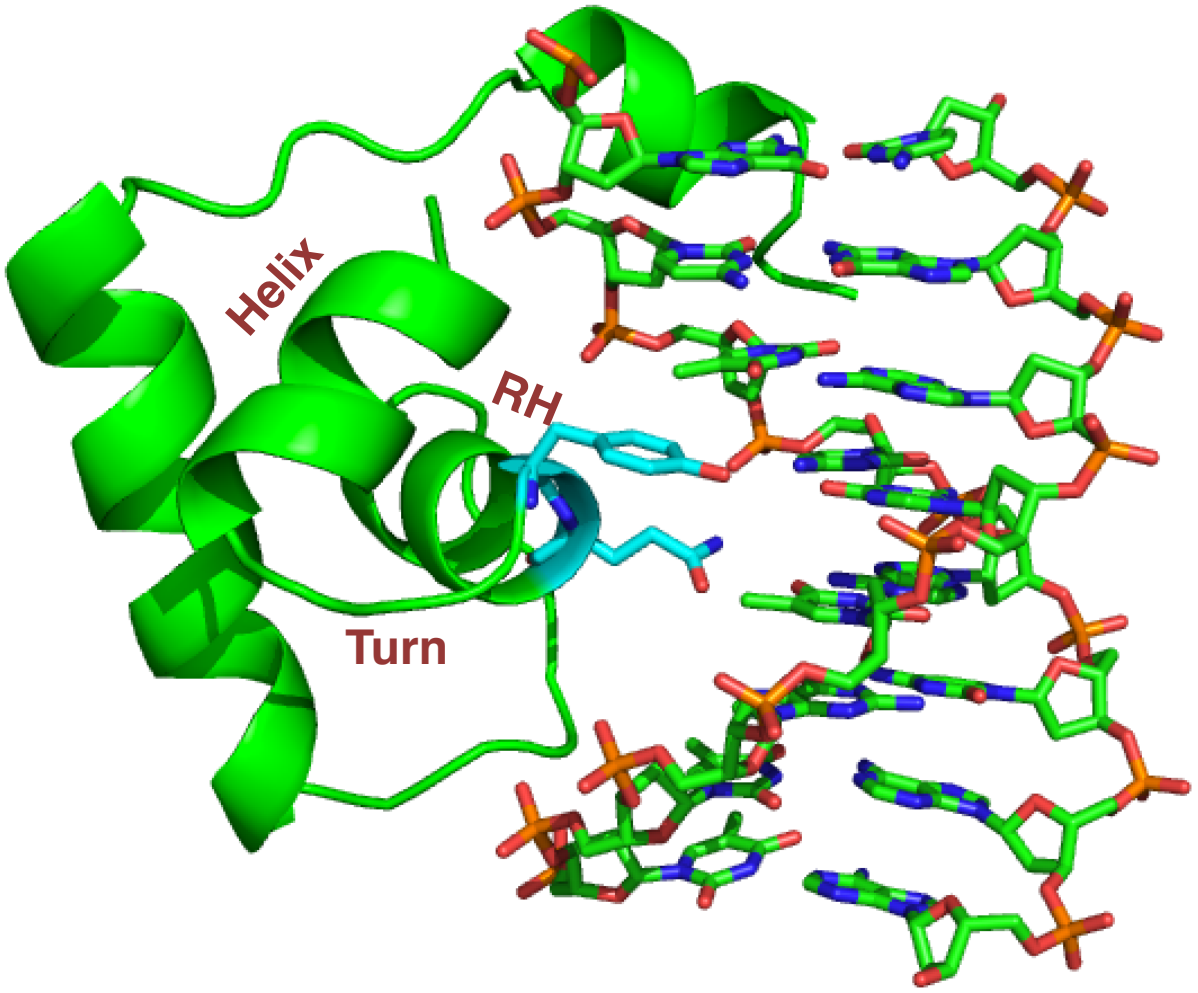






Interaction of one helix of the Helix-Turn-Helix domain of the Lac repressor with the operator DNA





Blue Amino acids of the Recognition Helix (RH) of the Lac Repressor HTH insert onto the major groove of Operator DNA to read base sequences (see DNA structure chapter)

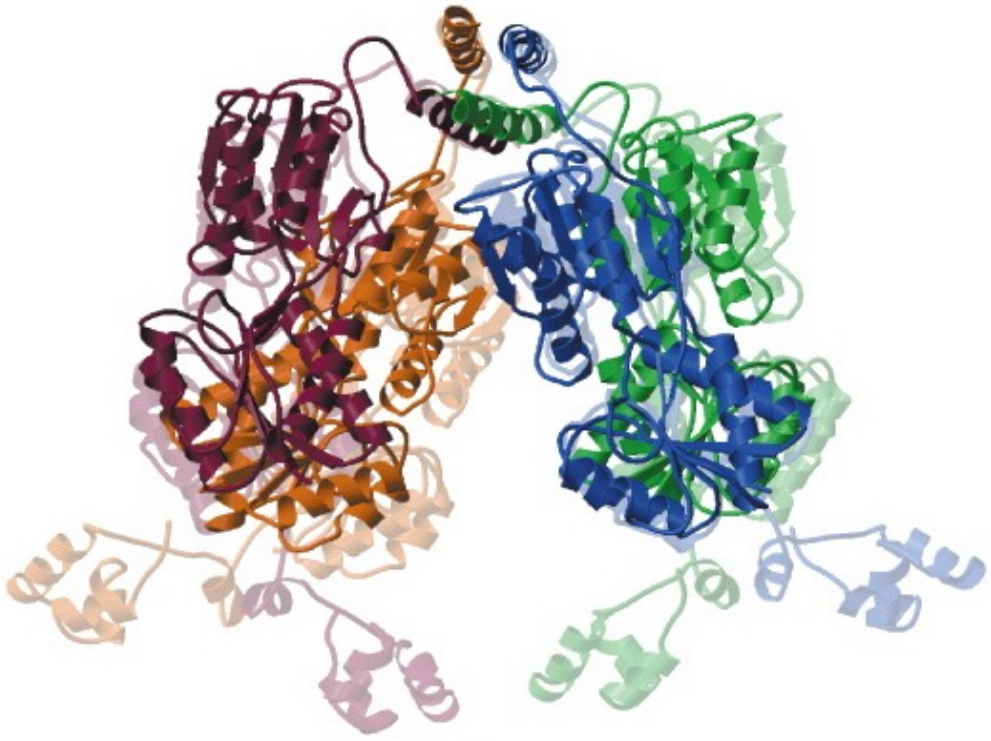
PDB ID = 1CJG

How is the binding of the repressor to the operator sequence controlled?

Conformational change of the Lac Repressor so it can no longer bind the operator

Inducer = allolactose (or here, IPTG = allolactose mimic)
Without inducer: transparent image
With Inducer (inducer not depicted here): overlaid solid image

PDB IDs:
1LBG = Lac + DNA
1LBH = Lac + IPTG
1LBI = Lac alone



What is the consequence of binding of the inducer on the structure of the Lac Repressor?

Without inducer: transparent
With Inducer: solid

A: It inhibits dimerization resulting in 4 monomers and loss of DNA binding

B: There is a conformational change in the loop regions towards the top that change tetramer structure

C: The Helix-Turn-Helix Domains become flexible and lose their DNA binding capacities – they are no longer "seen" in the structure because they are disordered

D: The inducer activates a protease that cleaves the Helix-Turn-Helix Domains resulting in loss of DNA binding

Regulation via the lac repressor = *negative regulation*

The lac operon also undergoes *positive regulation*.

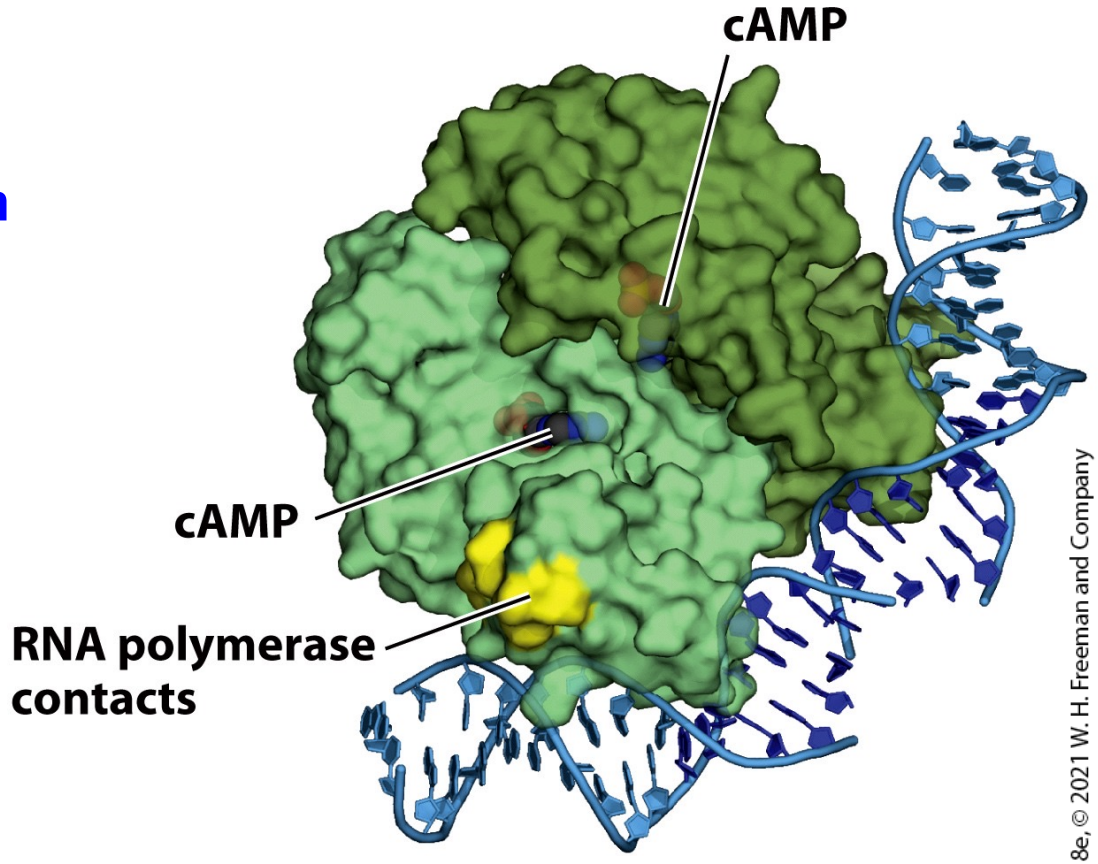
- **Glucose is the preferred energy source in E. coli**
- **If glucose is abundant, we don't want to express the genes that metabolize other sugars**

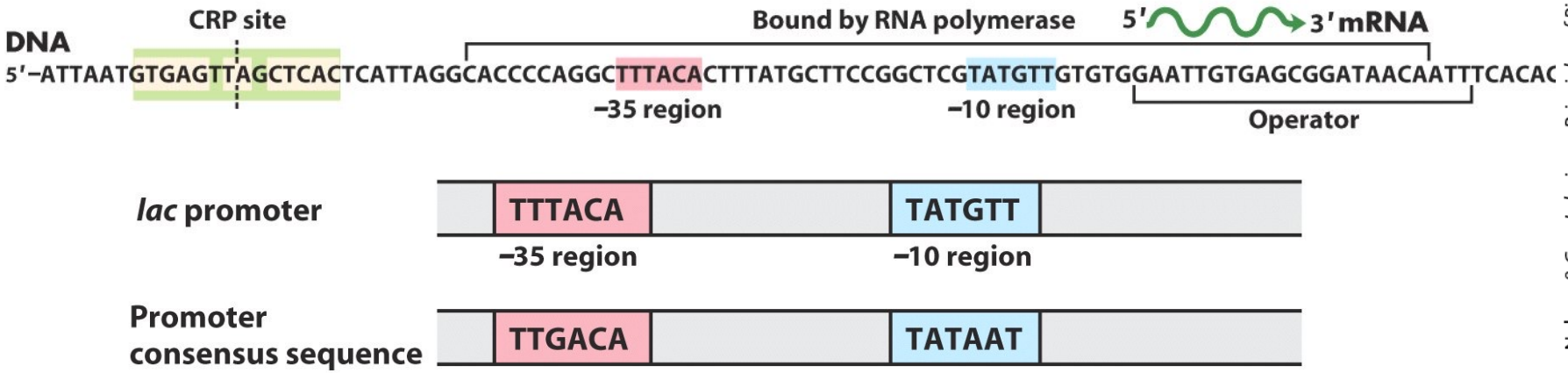
DNA Binding by CAP a.k.a.CRP
Catabolite Activator Protein
= Cyclic AMP Receptor Protein

High Glucose -> low cAMP
-> No binding of CAP

Low Glucose -> High cAMP
Binding of CAP to Lac promoter

CAP facilitates binding of the RNA polymerase to the Lac Promoter





When lactose is absent → very little transcription

Whether [glucose] is high or low, <u>if lactose is absent</u>:

- **repressor stays bound**
- **no transcription even when CRP-cAMP binds DNA**

Glucose high, cAMP low, lactose absent

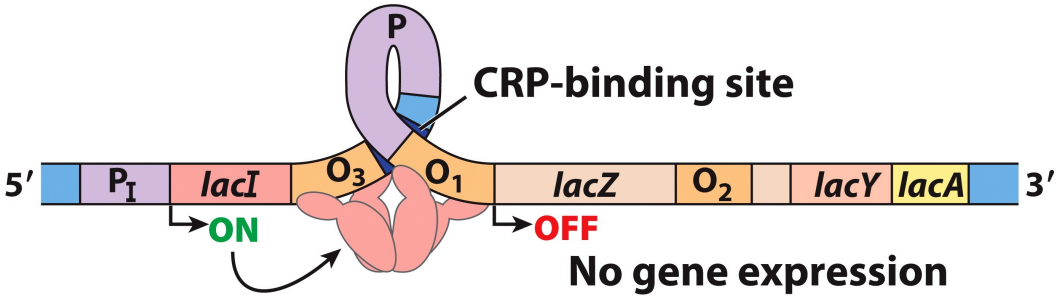


Figure 28-17a
Lehninger Principles of Biochemistry, Sixth Edition

Glucose low, cAMP high, lactose absent

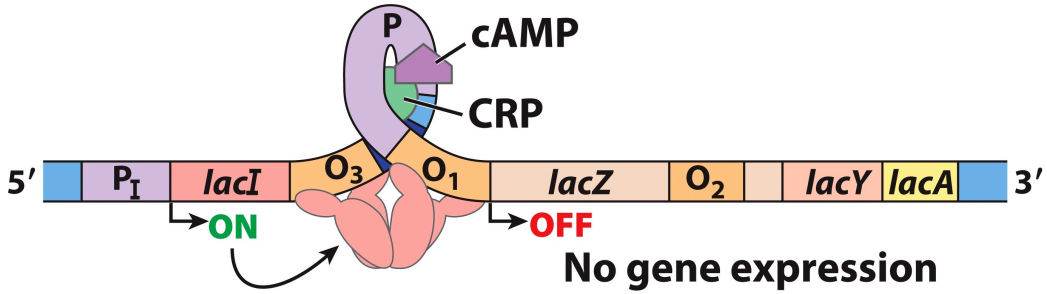


Figure 28-17b
Lehninger Principles of Biochemistry, Sixth Edition

When lactose is present, transcription levels depend on glucose:

- **Repressor dissociates, but transcription only stimulated significantly if cAMP rises due to low glucose levels:**

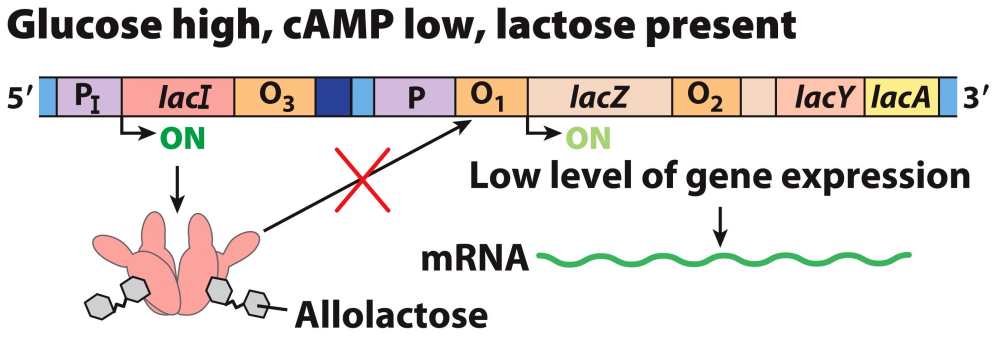


Figure 28-17c
Lehninger Principles of Biochemistry, Sixth Edition

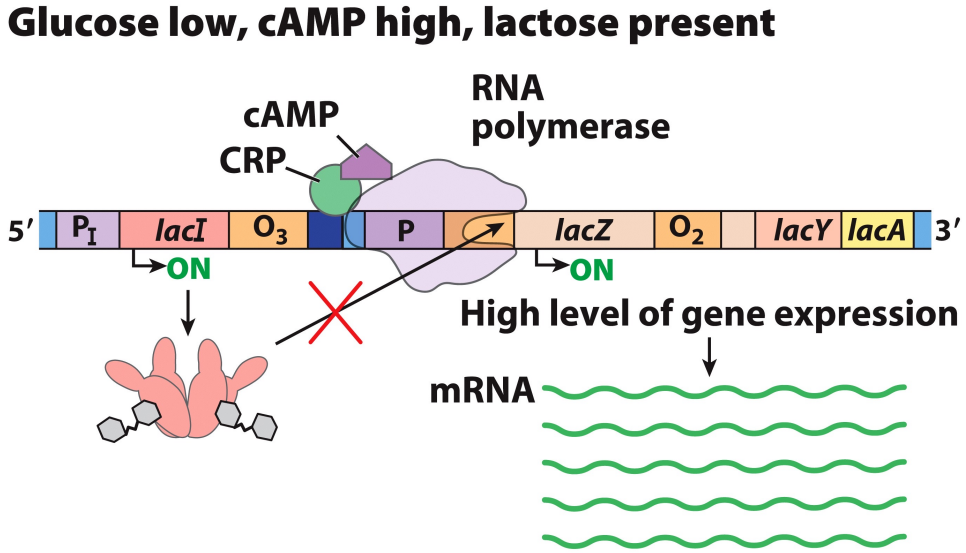


Figure 28-17d
Lehninger Principles of Biochemistry, Sixth Edition