BIOC 385: M12.T03-Miesfeld

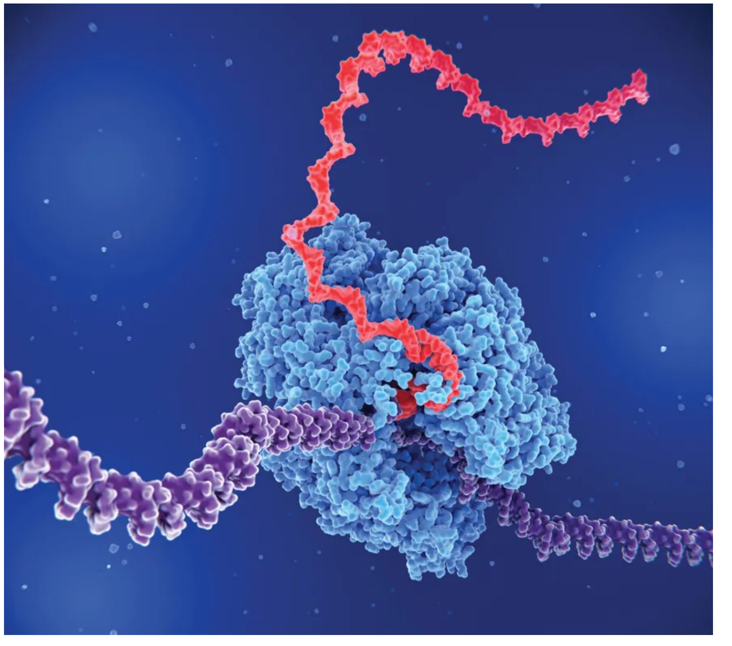
Assigned Reading: *Biochemistry* Chapter 26.2a

THE UNIVERSITY OF ARIZONA

Prokaryotic Gene Regulation: *lac* Operon

The Big Picture

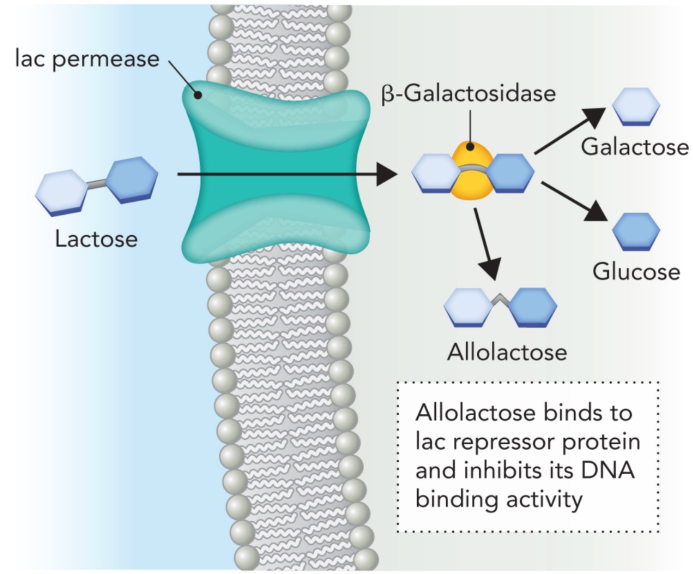
- The lac operon provides *E. coli* with a tightly regulated system to metabolize lactose only when it is available and glucose is absent.
- This system illustrates fundamental mechanisms of prokaryotic gene regulation, including repression, activation, and metabolite control.

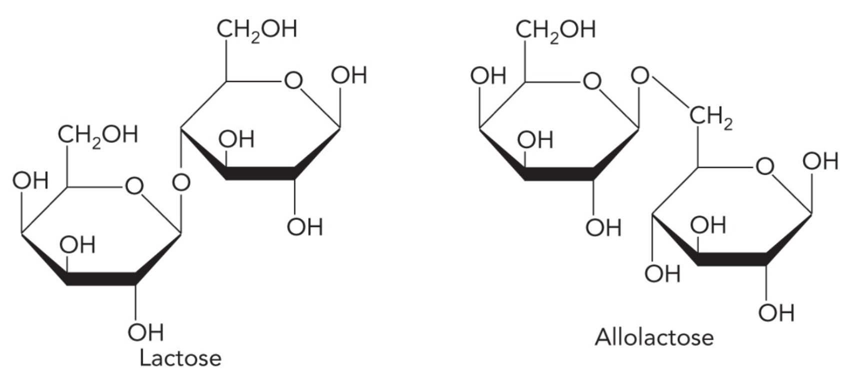


https://www.the-scientist.com/the-lac-operon-a-lesson-in-simple-gene-regulation-72357

Lactose Metabolism

- Lactose is imported into the cell by the lac permease protein; the enzyme β-galactosidase cleaves lactose into glucose and galactose.
- Allolactose is an alternate product of the β-galactosidase activity that catalyzes the isomerization of lactose to form allolactose.
- Allolactose binds to the lac repressor protein causing it to dissociate from DNA and de-repress transcription.





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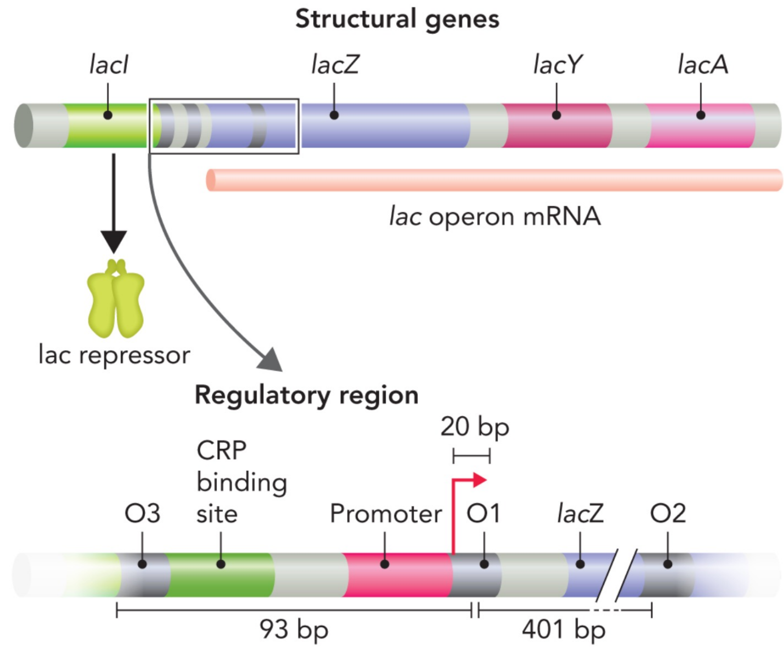
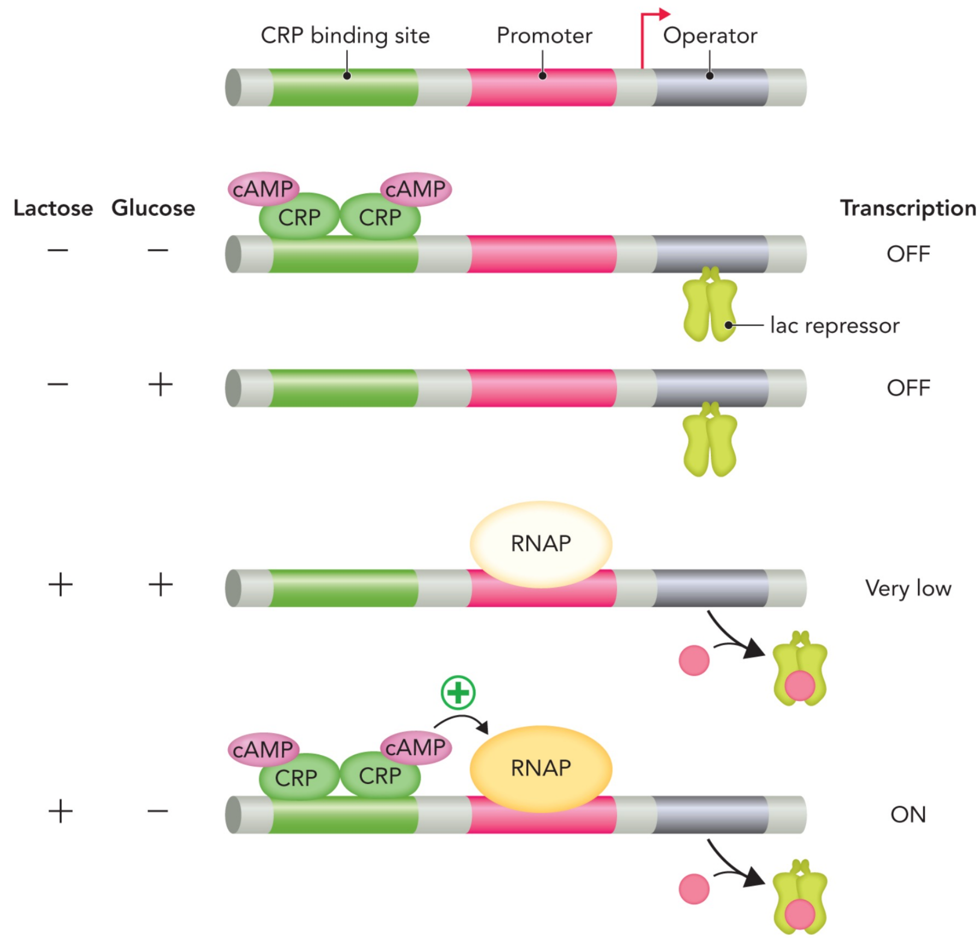
Organization of the *lac* Operon

- The regulatory region contains a promoter, operator sites for the lac repressor protein, and a binding site for the activator protein CRP.
- Structural genes *lacZ*, *lacY*, and *lacA* are co-transcribed as one mRNA, with β-galactosidase and permease essential for lactose utilization.



lac Operon Regulation

- In the absence of lactose, the lac repressor binds the operator to block RNA polymerase, preventing unnecessary enzyme synthesis.
- When lactose is present and glucose is absent, allolactose inactivates the repressor and CRP–cAMP recruits RNA polymerase, fully activating transcription.



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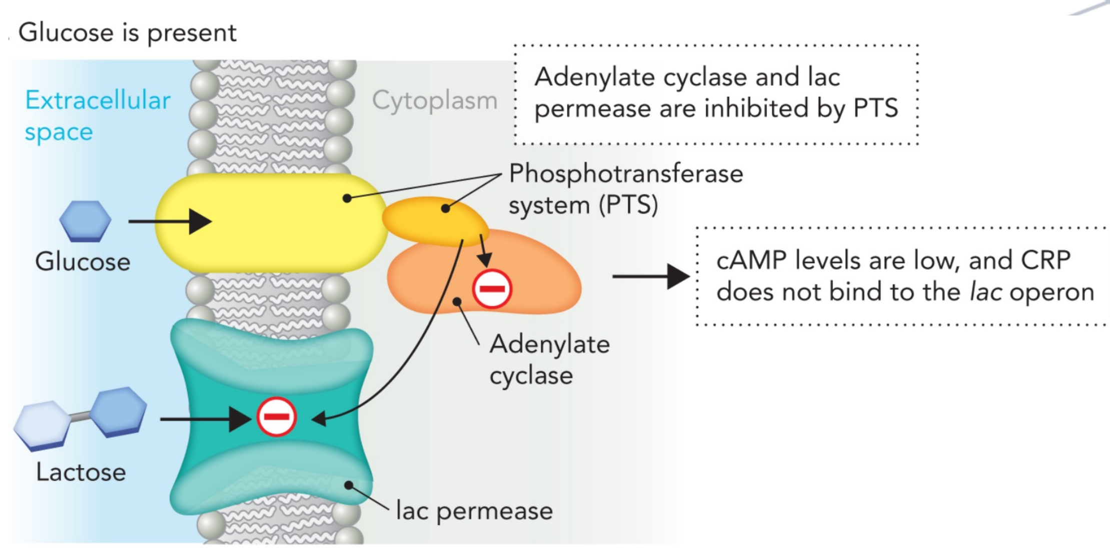
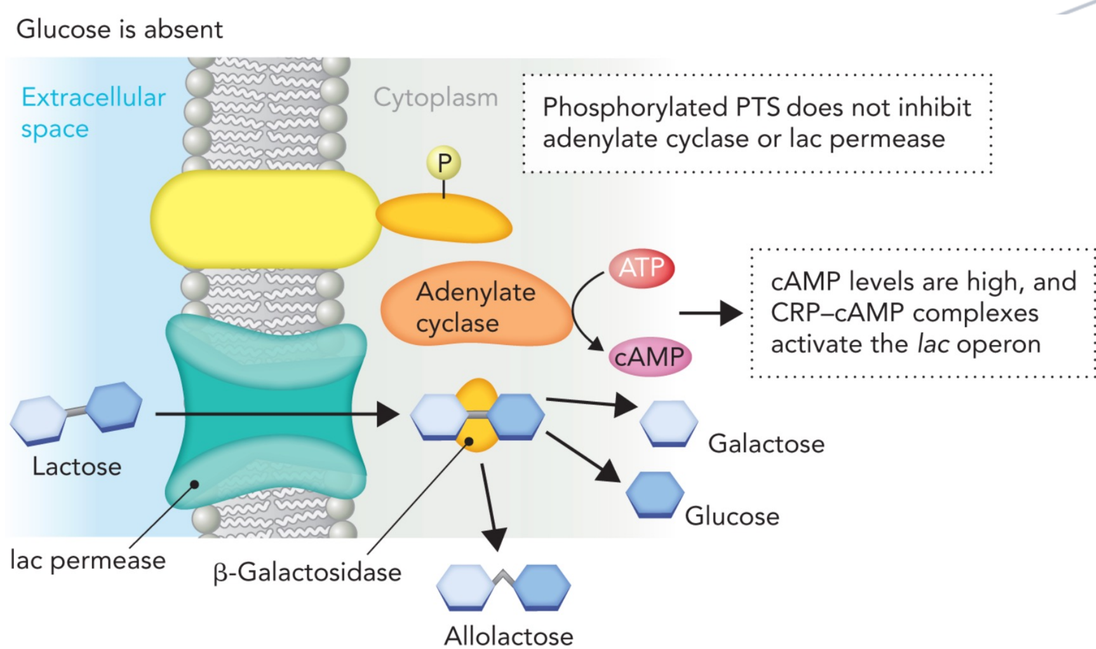
Glucose Repression of *lac* Expression by PTS

- When glucose is abundant, the phosphotransferase system (PTS) remains dephosphorylated, inhibiting adenylate cyclase and lowering cAMP levels.
- Low cAMP prevents CRP activation and also reduces lactose uptake, ensuring glucose is used preferentially over lactose.



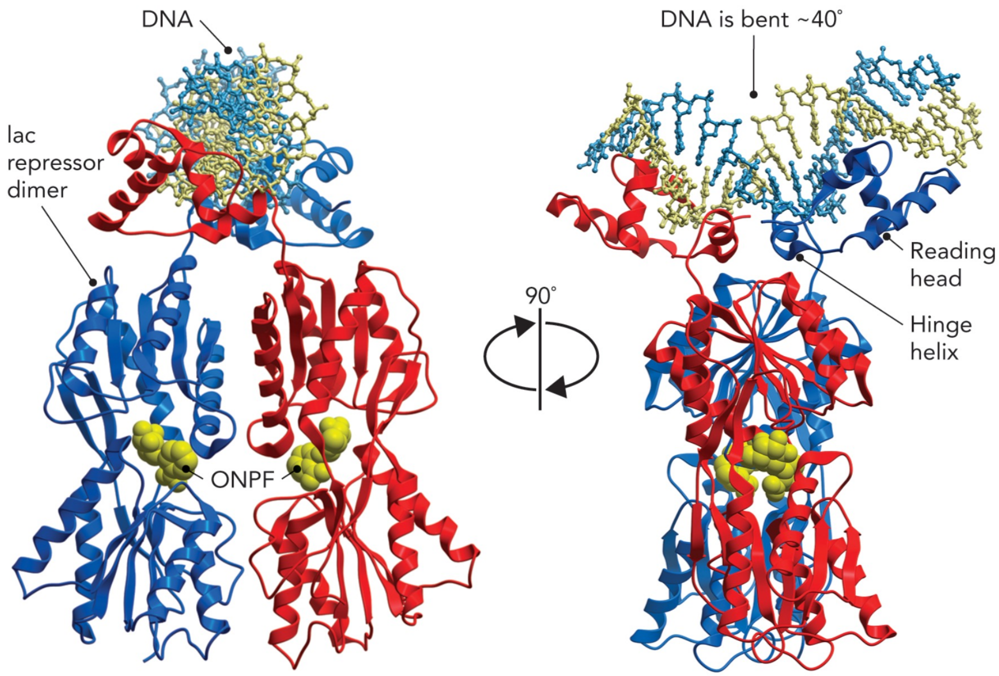
Glucose Depletion: cAMP Activation of *lac*

- In the absence of glucose, the PTS becomes phosphorylated, stimulating adenylate cyclase to increase cAMP production.
- High cAMP activates CRP binding to DNA, enabling transcription of the lac operon and other operons required for alternative sugar metabolism.



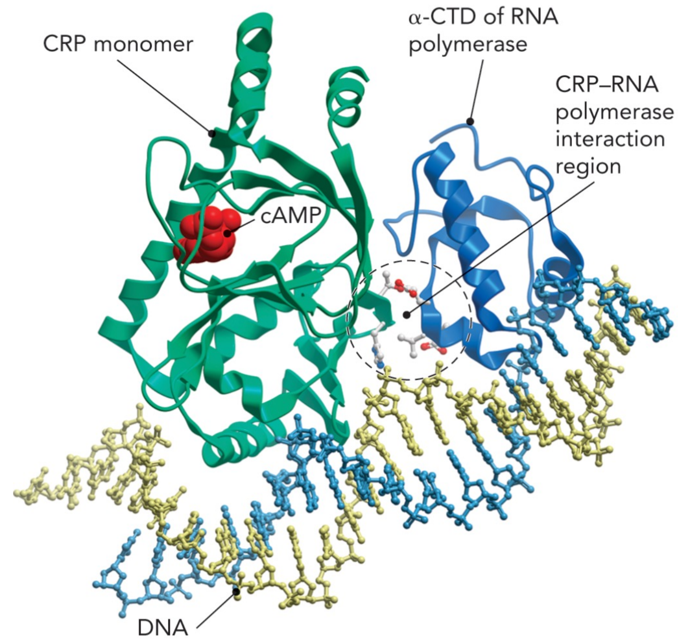
Lac Repressor Protein Distorts the DNA Helix

- The structure of the lac repressor dimer–DNA complex reveals that the DNA is bent ∼40° in the complex.
- Such distortion of the DNA is often seen in DNA binding proteins to facilitate binding of multiprotein complexes to DNA by bringing DNA-bound proteins closer together.



CRP Binds Directly to RNA Polymerase

- CRP makes direct contact with the C-terminal domain of the RNA polymerase α subunit (α-CTD), enhancing cooperative promoter binding.
- This protein–protein interaction demonstrates how activators stabilize transcription complexes to increase initiation efficiency.



Key Concepts to Guide Your Learning

- Prokaryotic genes encoding proteins with related functions are often organized into single transcription units called operons; expression of the entire set of genes in the operon can then be regulated by action at a single promoter.
- Genes encoding proteins required for catabolic pathways are expressed only when substrates are available; genes encoding proteins required for biosynthetic pathways are expressed only when the end product is at low levels in the cells.
- The *lac* operon is expressed only when lactose is present in the environment and when glucose is absent; in the presence of lactose, the lac repressor dissociates from a site near the promoter, allowing RNA polymerase access to the promoter.
- In the absence of glucose and presence of lactose, cAMP levels increase resulting in the formation of an active cAMP–CRP complex, which then binds to the *lac* promoter and stimulates expression owing to the absence of lac repressor binding.