GENE REGULATION IN PROKARYOTES

Course: Molecular Biology (02022312)

Instructor: Dr. M A Srour

Textbook:
Watson J, Baker TA, Bell SP, Gann A, Levine M, Losick R (2008). Molecular Biology of the Gene, 6th ed. Chap 12 pp. 377-387; Chap 16 pp. 547-87.

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Chap 16

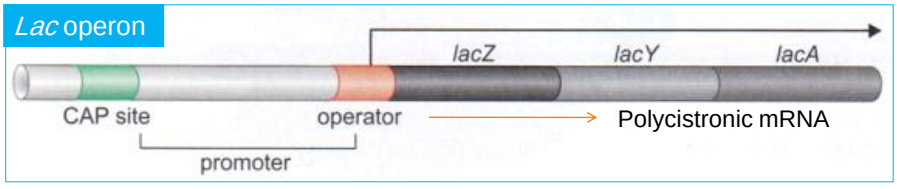
Regulation of transcription initiation: examples from prokaryotes

- Operon: a set of genes in bacteria that is coordinately regulated and contributes to a single cellular function
- Genes within a single operon when transcribed give rise to a polycistronic mRNA
- Polycistronic mRNA is translated to give different proteins

Chap 16

The *lac* Operon

- Lac genes: an activator (CAP encoded by CAP gene) & a repressor (lac repressor encoded by lacI) together control the lac genes.
- Lac genes: constitute the "*lac* Operon"
- LacZ > β-galactosidase, cleaves lactose into galactose & glucose
- LacY > lactose permease, transports lactose into the cell
- LacA > thiogalactoside transacetylase, rids the cell of toxic thiogalactosides that enter the cell via LacY

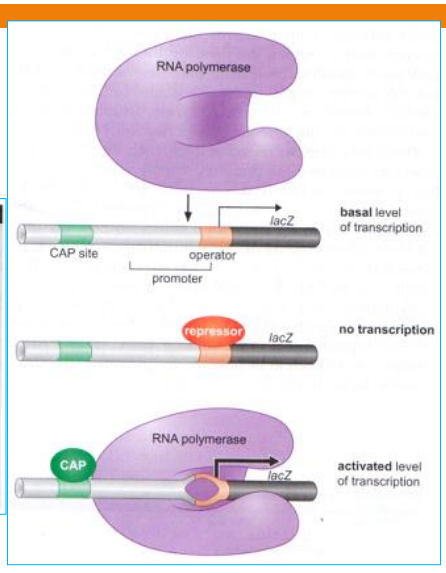


CAP & Lac Repressor have opposing effects on RNA Pol binding to the Lac promoter

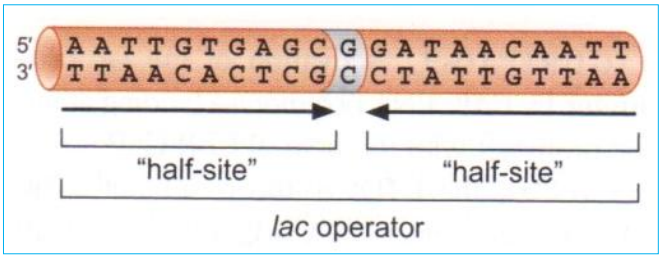
Lac operator: a 21-bp sequenence with 2-fold symmetry & recognized by two lac repressor molecules (see next slide)

RNA Pol binds the lac promoter poorly in absence of CAP, because the lac promoter 's "-35" region is not optimal for its binding & it lacks UP-element

glucose	lactose
+	+
+	−
−	+



The symmetric half sites of lac operator

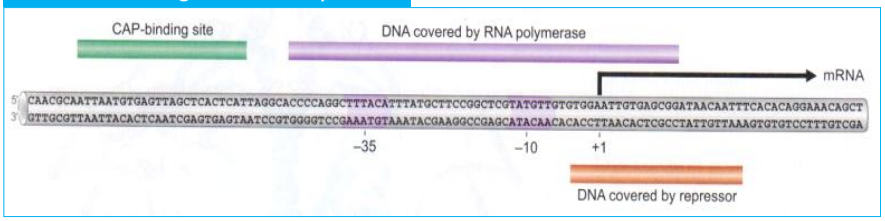


Each element of the promoter or DNA-binding sites can be mapped by DNA-foot printing or gel-mobility assay

CAP binds as a dimer to a site ~60 bp upstream of TSS & helps RNA Pol bind to the promoter

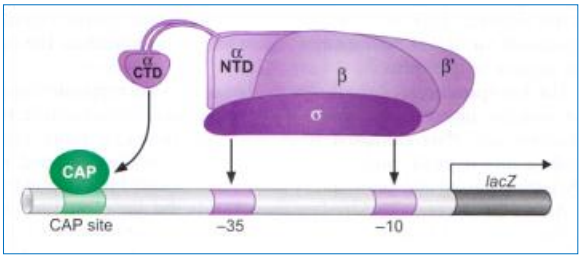
- When CAP binds its site, it recruits RNA Pol and helps it bind the promoter. The cooperative binding stabilizes the binding of Pol to the promoter.

The control region of lac operon



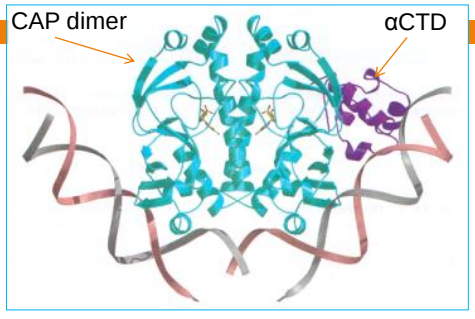
CAP has separate activating & DNA-binding surfaces

- Where does the activating region of CAP touch RNA Pol when activating the lac genes?
- The αCTD binds the CAP and adjacent DNA, because the lac promoter lacks the UP-element

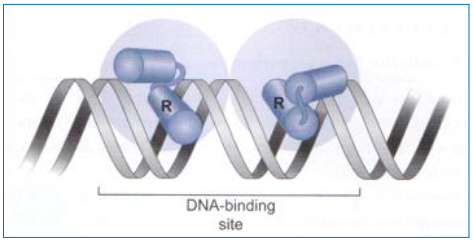


CAP & lac Repressor bind DNA using a common structural motif

- Recognition of specific DNA sequence by the CAP protein is achieved via a conserved region of secondary structure called " helix-turn-helix"

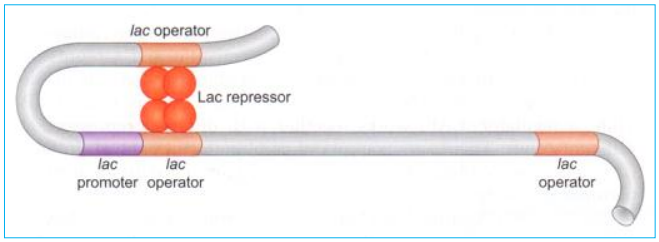


Binding of a protein with "helix-turn-helix" domain to DNA



The activities of *lac* Repressor & CAP are controlled Allosterically by their signals

- β-galactosidase (encoded by *lacZ* gene) converts **Lactose** to **Allolactose,** which then controls the lac Repressor (?)
- The expression of *lac* genes is leaky!
- CAP effect: glucose lowers the intracellular levels of cAMP, which is when complexed with CAP, the CAP proteins adopts its DNA-binding conformation



Combinatorial control: CAP controls many genes >> *gal* genes in *E. coli*

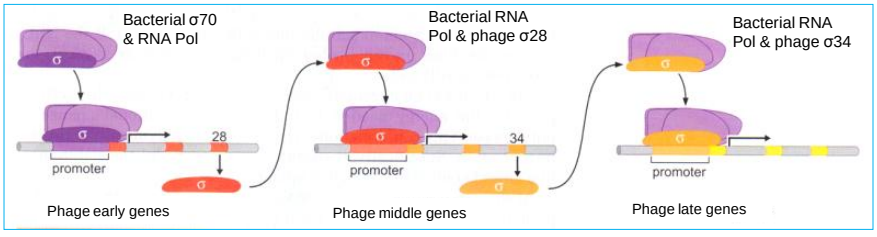
- Gal genes are controlled by galR (Repressor) and CAP (activator)
- galR mediates the effect of inducer galactose
- CAP works together with different repressors at different genes > an example of combinatorial control
- CAP works with >100 genes in *E. coli*
- When same signal controls multiple genes , it's typically communicated to each of those genes by the same regulatory protein.

Alternative "σ" factors direct RNA Pol to alternative sets of promoters

- *E. coli* encodes several σ factors that can replace σ^{70} under certain conditions
- The heat shock σ factor (σ^{32}), is induced by heat shock, replaces a proportion of σ^{70} factor, and directs RNA Pol to transcribe genes that protect the cell from the effects of heat shock
- The level of (σ^{32}) factor is increased by
  - Its translation is stimulated
  - Its protein is stabilized
- The σ^{54} factor directs RNA Pol involved in nitrogen metabolism

Alternative "σ" factors direct RNA Pol to alternative sets of promoters

- Sometimes, a series of σ factors directs a particular program of gene expression
- Example: alternative factors control the ordered expression of genes in the phage SPO1, that infects *Bacillus subtilis:* bacterial σ^{70}, phage σ28 & σ34 factors



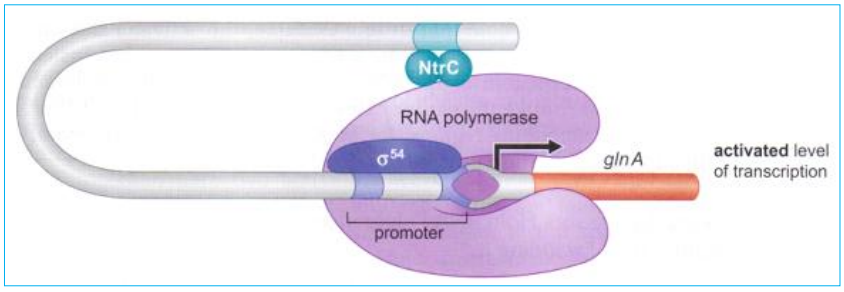
NtrC & MerR: transcriptional Activators that work by Allostery rather than Recruitment

- Activators that work by recruitment: simply bring an active form of RNA Pol to the promoter
- Activators that work by allosteric mechanism: Pol initially binds the promoter in an inactive complex, To activate transcription the activator triggers an allosteric change in that complex
- Examples:
- NtrC: controls genes involved in N2 metabolism such as glnA
  - It induces conformational change in Pol that is prebound to promoter and induce transition to open complex
- MerR: controls a gene merT, that encodes an enzyme which makes cells resistant to toxic effects of mercury
  - It induces a conformational change in DNA and induce transition to open complex

NtrC has ATPase activity and works from DNA sites Far from the gene

- NtrC has separate activating and DNA-binding domains and binds DNA only in the presence of a specific signal
- At low N2 levels, NtrC is phosphorylated by a kinas NtrB & undergoes conformational change that reveals the activator's DNA-binding domain>>> binds promoter as a dimer >>> binds RNA Pol & σ54 & transcribe the glnA gene
- NtrC hydrolyze ATP and use the energy to induce conformational change in Pol, which in turn triggers Pol to initiate transcription
- At some genes controlled by NtrC, there is another binding site for another protein called IHF
- IHF bends the DNA & hence brings the DNA-bound activator closer to the promoter

Activation by NtrC

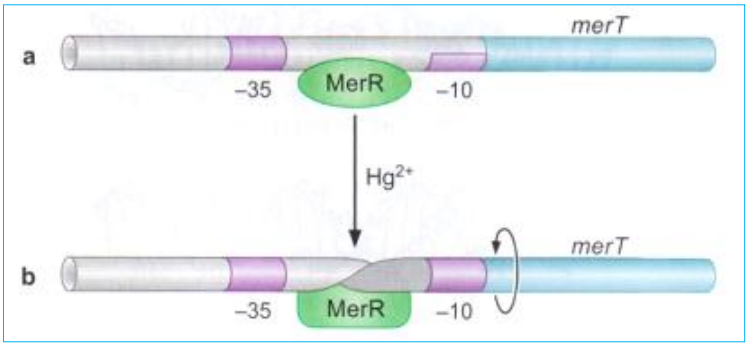


Although not specified in the figure, NtrC contacts the σ54–containing holoenzyme

MerR activates transcription by twisting promoter DNA

- When bound to a single DNA-binding site, in the presence of mercury, MerR activates the merT gene
- merR binds a sequence between -10 & -35 regions of merT promoter
- The -10 & -35 regions are separated by 19 bp & thus these 2 elements are not optimally separated nor aligned
- When MerR binds Hg+, it undergoes a conformational change and induce a conformational change that causes DNA center of the promoter to twist

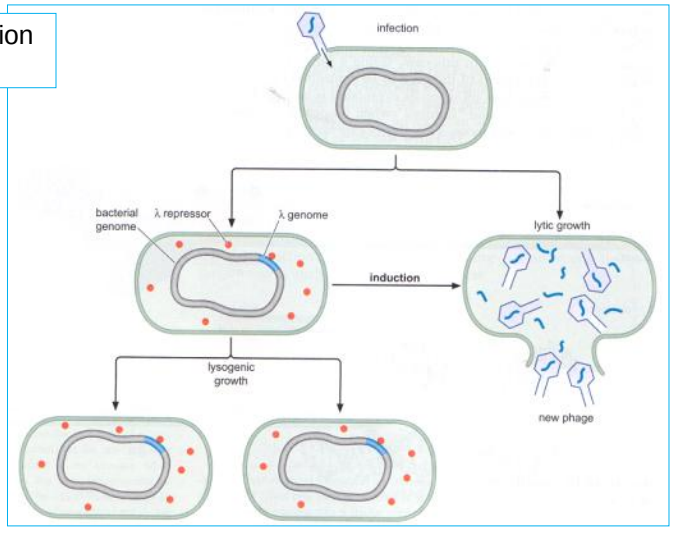
Activation by merR



(a) In the absence of mercury, MerR binds & stabilizes the inactive form of the promoter
(b) in the presence of mercury, MerR twists DNA so as to properly align promoter elements

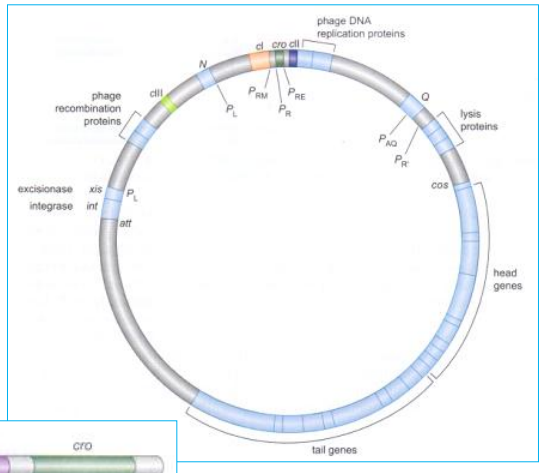
The Case of Bacteriophage λ: Layers of Regulation

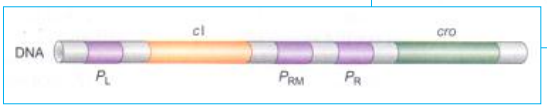
Growth & induction of λ lysogen



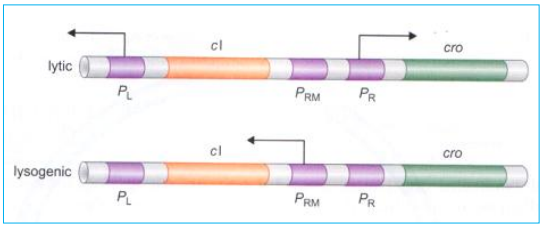
Alternative patterns of gene expression control lytic & lysogenic growth

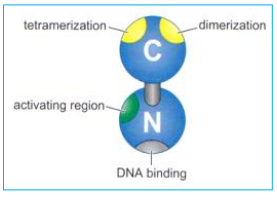
- Phage λ has a 50-kb genome and approx 50 genes, including regulatory proteins





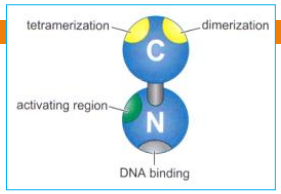
Alternative patterns of gene expression control lytic & lysogenic growth





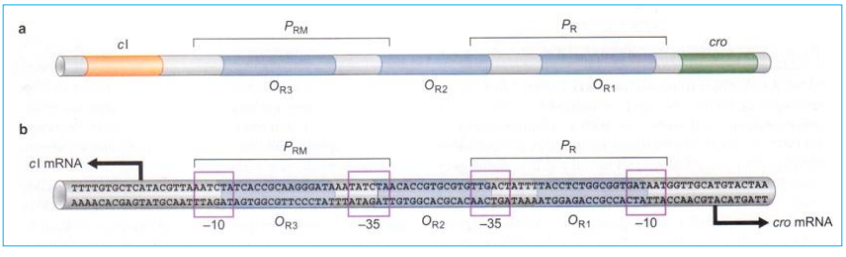
- Most phage genes are transcribed directly from P_R or P_L (rightward & leftward) or from other promoters whose activities are controlled by products of gens transcribed from P_L or P_R
- PR & PL are constitutive promoters
- P_{RM} (promoter for repressor maintenance) transcribes only cI gene. It is a weak promoter and directs efficient transcription only when an activator is bound just upstream

Regulatory proteins and their binding sites



- The cI gene encodes λ repressor;
- The λ repressor can work as repressor & as activator
- As a repressor, it works similar to the lac repressor & as activator similar to CAP, by recruitment
- Cro (stands for control of repressor & other things) only represses transcription like the lac repressor
- Cro & λ repressor bind to any one of 6 Operators; 3 located in left-hand & 3 in right-hand control region (O_{R1}, O_{R2}, O_{R3})

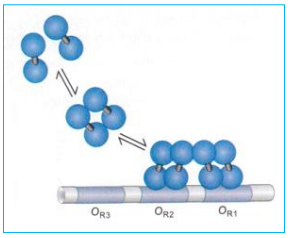
Relative positions of promoter & operator sites in O_R



Lambda repressor binds OR1 10X better than it binds OR2 or OR3. Cro binds OR3 with affinity 10X that of binding to OR1 or OR2.

λ repressor binds to operator sites cooperatively

- Cooperative binding: repressor at OR1 helps repressor bind to low-affinity site OR2 by cooperative binding



Repressor & cro bind in different patterns to control lytic & lysogenic growth

- Lytic phase: Cro bind O_{R3} and overlaps P_{RM}>> represses the promoter while RNA Pol binds OR1 & OR2 and transcribes cro & other lytic genes
- Lysogenic phase: lambda repressor is on and binds OR1 & OR2 >> represses transcription from PR & PL, while PRM is on