Module 3

Regulation of Gene Expression in Prokaryotes

General Questions

1. The reasons for gene expression be regulated- why??????

2. At which point does gene regulation happen – where and when???

3. The mechanisms of gene regulation - how????

Regulation of Gene Expression- "Cellular Economics" (why?)- metabolic regulation

- Microorganisms adapt to constantly changing environments – chemical, nutritional, thermal, osmotic potential, population impacts
- This depends on their ability to turn gene expression on and off in response to the environmental conditions of the time.

Regulation of Gene Expression is at several levels (where?)

- Prokaryotinc gene expression is regulated at several different levels
  - Transcription (DNA → RNA Transcript)
  - mRNA processing (RNA transcript → mRNA)
  - mRNA turnover (mRNA stability)
  - Translation (mRNA → Protein)
  - Enzyme function
- Prokarotic translation occurs while transciption progresses- more control

Regulation of Gene expression- the question of how (mechanisms)

General - Transcription, Terminologies

Specific – Lac Operon

Arabinose Operon

Tryptophan Operon

Two component System

Transcription Process- Prokaryotic RNA polymerases:

- Large 4 subunit protein- contacts major grove exposed DNA nucleotides
  - Core enzyme - β, β', α (2 copies)
  - Holozyme- core enzyme + σ
- Transcription Initiation requires proper recognition & binding of σ factor to promoter region of the gene (transparency 6.26).
  - How many σ factors are present? Why?
  - What is a promoter?
  - How do sigma factors bind to promoters?
  - Which direction along the strand does transcription occur

Terminology- Constitutive, Inducible and Repressible Gene Expression

- Gene expression can follow several patterns
  - Constitutive
  - Inducible and
  - Repressible

Constitutive, Inducible and Repressible Gene Expression

- Constitutive gene expression is not regulated, and is typical of genes whose products are essential for cellular functions (metabolic enzymes such as glycolytic enzymes).
- Called **constitutive genes.**

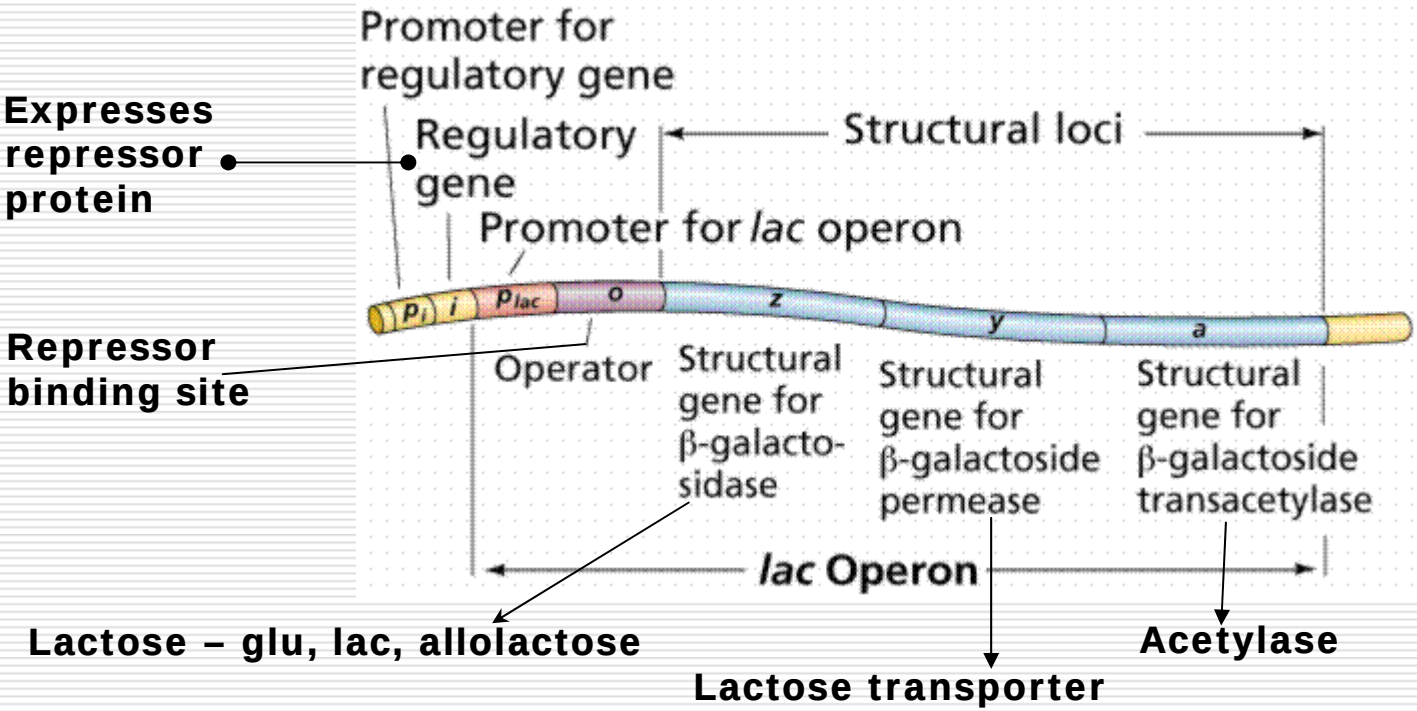
5.2 Constitutive, Inducible and Repressible Gene Expression

- Inducible and repressible gene products are only required under certain circumstances.
- Inducible genes are turned on in response to the presence of a substance in the environment eg: lactose
- Repressible genes are turned off in response to an environmental signal eg:the simultaneous presence of lactose / glucose or xylose / glucose (genes for utilization of lactose and xylose are repressed)

5.3 Operons: Coordinately regulated units of gene expression

- The operon model was put forward to explain the coordinate regulation of genes encoding for enzymes needed for lactose utilisation in *E. coli*- Jacob & Monod (1961)
- Consists of three basic groups of elements
  - control region, contains operator and promoter
  - structural genes, encoding for series of enzymes for a metabolic or anabolic pathway
  - Repressor gene – encodes a repressor protein. The repressor is able to bind to the operator DNA under certain conditions
  - The repressor protein is modified by effector molecules in the environment.

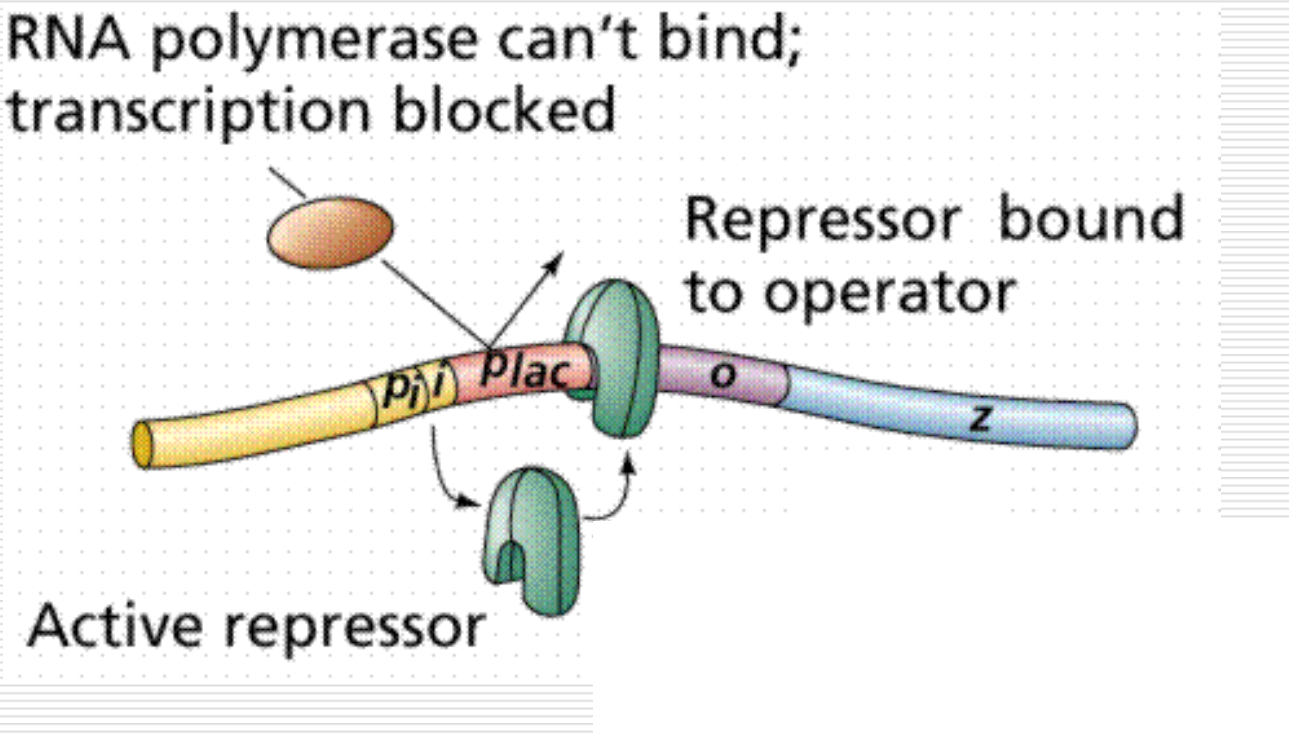
5.4 Lactose operon- polycistronic



5.4 Lactose (*Lac*) operon

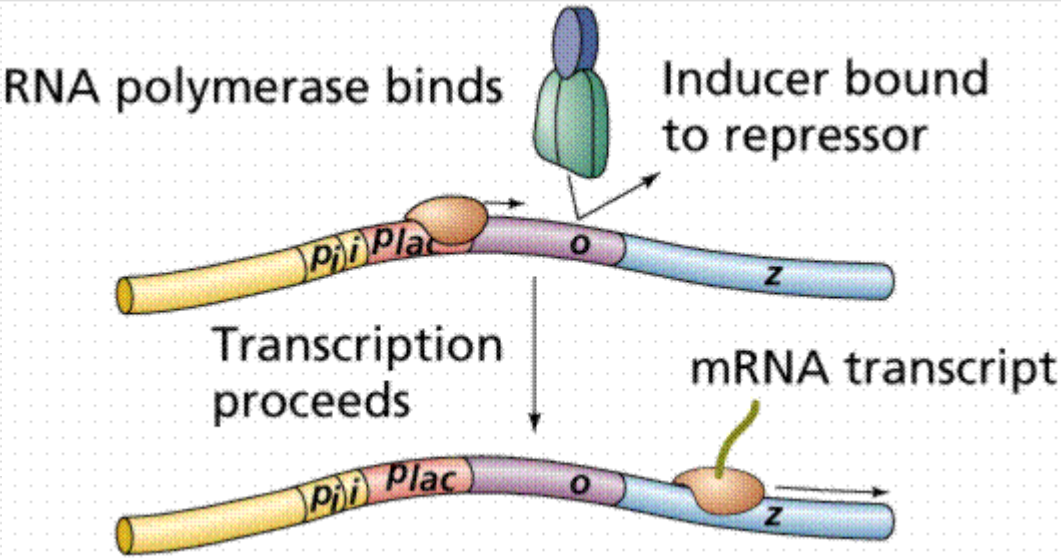
- *Organisation:*
  - *LacZYA* genes are under the control of a single P_{lac} promoter- encodes a catabolic pathway – breakdown of environmental lactose into glucose and galactose
  - The *I* (aka *LacI*) gene product, the lac repressor, is expressed from a separate transcription unit upstream from $P_{lac.}$
- Mechanism:
  - No lactose than the operon normal state is "off" (how?): Lac repressor protein is made up of 4 identical protein subunits (symmetrical structure) & binds to a palindromic (symmetrical) 28 bp operator DNA sequence O_{lac} that overlaps the *lacZYA* RNA start site. Bound repressor blocks transcription from P_{lac}- No transcription.
  - Lactose present: lac repressor protein binds to lactose, the inducer molecule (forms allolactose) and therefore repressor cannot bind to operator region. However, RNA polymerase can bind to promoter, leading to transcription and translation.
- Repressor protein is allosteric, and undergoes a conformational change on binding allolactose. After allolactose concentration drops (all lactose metabolised), repressor protein returns to original conformation and binds to operator again.

5.4 Lactose operon



Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates

5.4 Lactose operon



Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates

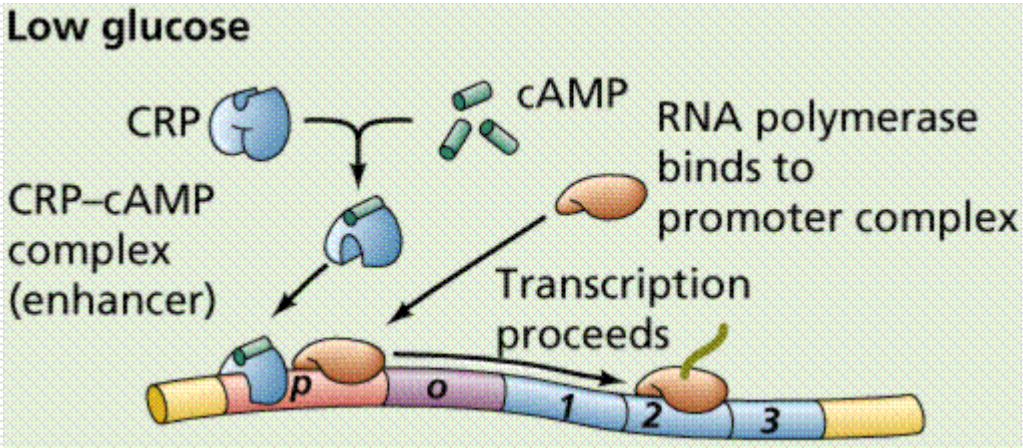
5.4 Catabolite repression

- A diauxic growth curve results when two sugars are present –e.g. Glucose is used first followed by other sugars such as lactose or xylose.
- Glucose has been shown to block the expression of a number of operons controlling the catabolism of particular sugars such as lactose (ie. *lac* operon).
  - In the presence of both glucose and lactose, *lac* operon is expressed at very low levels only.
  - Glucose does not act upon *lac* operon transcription directly, but via cyclic AMP (cAMP).
- The P_{lac} promoter is not a strong promoter and for high level expression requires a specific activator protein – cAMP receptor protein (CRP), aka catabolite activator protein (CAP).
  - CRP exists as a dimer – cannot bind to DNA nor regulate transcription
  - CRP activated by binding to cAMP- binds to a site upstream from the P_{lac} promoter inducing a 90º bend, improving access to RNA polymerase for binding-

5.4 Catabolite repression

- High glucose in the cell leads to low cAMP concentration. Low cAMP means CAP does not bind to CAP-binding site and transcription is not stimulated. Lactose is not catabolised.
- Low glucose leads to high cAMP, and CAP-cAMP complex binding to CAP binding site, stimulating transcription. Lactose may be catabolised if *lac* operon is derepressed (presence of inducer allolactose inactivates repressor protein)
- Glucose is preferred carbon source, and will be used before operons allowing the catabolism of other carbon sources (sugars) are expressed.

5.4 Catabolite repression



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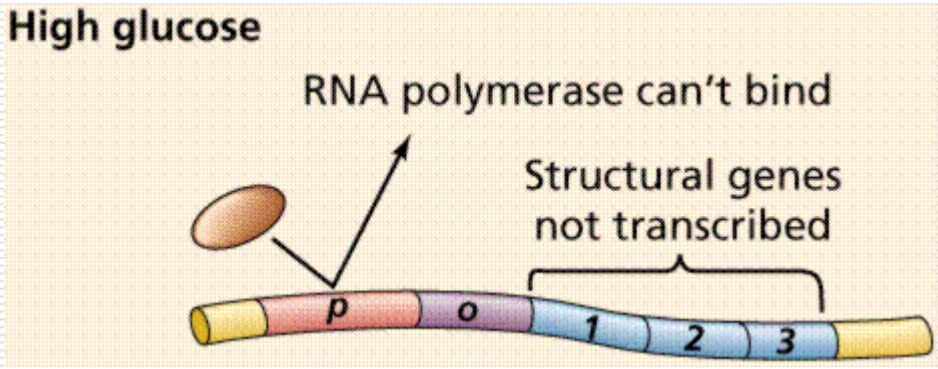

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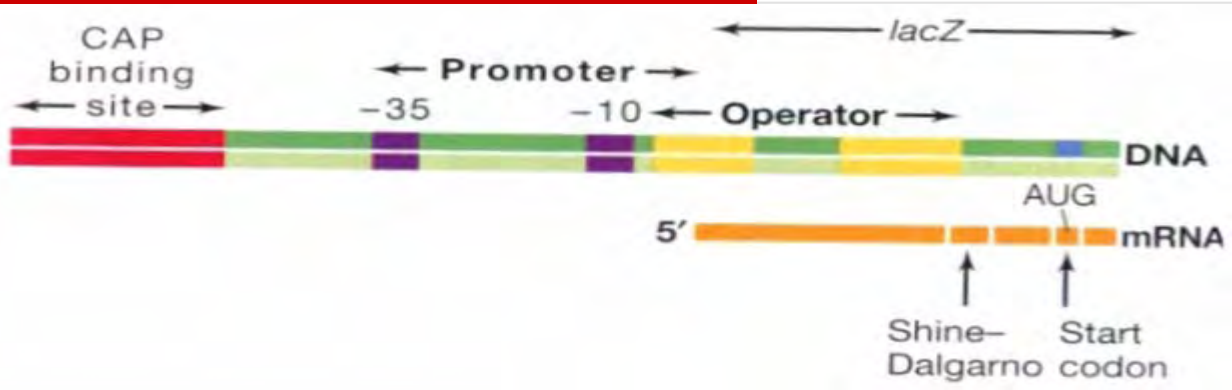
5.4 Catabolite repression

- Four basic situations:
- 1: High Glu/low Lact ⇒ low cAMP/low allolactose ⇒ CAP free and repressor bound ⇒ **No transcription**
- 2: Low Glu/low Lact ⇒ high cAMP/low allolactose ⇒ CAP-cAMP bound and repressor bound ⇒ **No transcription**
- 3:High Glu/high Lact ⇒ low cAMP/high allolactose ⇒ CAP free and repressor not bound ⇒ **low transcription**
- Low Glu/high Lact ⇒ high cAMP/high allolactose ⇒ CAP-cAMP bound and repressor not bound ⇒ **high transcription**

Recap- the Lac operon regulation



Overall regulation of the lactose operon. The first structural gene in this operon, *lacZ*, encodes the enzyme β-galactosidase, which breaks down lactose (see Figure 8.14). The operon contains two other genes that are also involved in lactose metabolism. The two halves of the operator (where the repressor would bind) are almost perfect inverted repeats. There are also inverted repeats in the CAP binding site, although these are less perfect. The transcriptional start site would be located on the DNA exactly at the 5′-end of the mRNA. The location of the −35 sequence and the Pribnow box, which are part of the promoter (are also shown. In addition, the location of the base pairs encoding the Shine–Dalgarno sequence and the start codon are also given. These two sequences are critical sequences on the mRNA

Lac Operon Videos

- http://www.maxanim.com/genetics/Lac%20operon/Lac%20operon.htm

- http://www.maxanim.com/genetics/The%20Lac%20Operon%20Induction/The%20Lac%20Operon%20Induction.htm