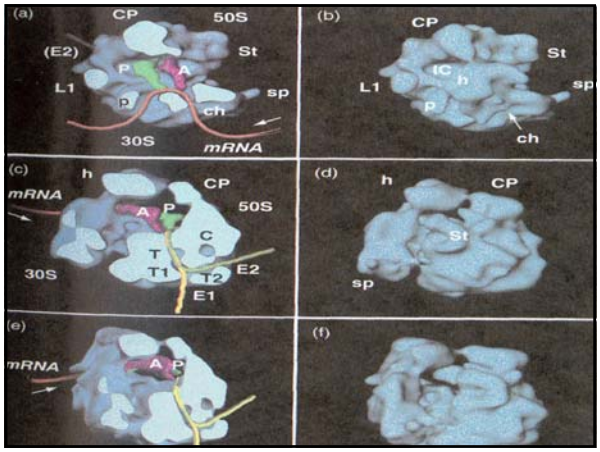


Chapter 15 Gene Regulation

- How is genetic expression regulated?
- In eukaryotic organisms, cellular differentiation is a result of genetic regulation
  - All cells have the complete set of genetic information
  - Cells perform different 'tasks' and have different functions
  - phenotypic specialization at the cellular and tissue levels
- Regulation is NOT accomplished by eliminating unused genetic information

Prokaryotic Regulation

- Enzymes can either be <u>inducible</u>: their concentration raises and lowers within the cell based on the presence or absence of an <u>inducer</u>
- Or, enzymes can be <u>constitutive</u>: their concentration is relatively constant in the cell, in other words they are expressed all the time

Prokaryotic Regulation

- Thus, the presence or absence of various molecules in the cell (amino acids, sugars etc.) can either repress or stimulate transcription of enzymes that are required to process that molecule
- This type of gene regulation (inducible vs. repressible) can be either positively regulated or negatively regulated

Positive vs. Negative Regulation

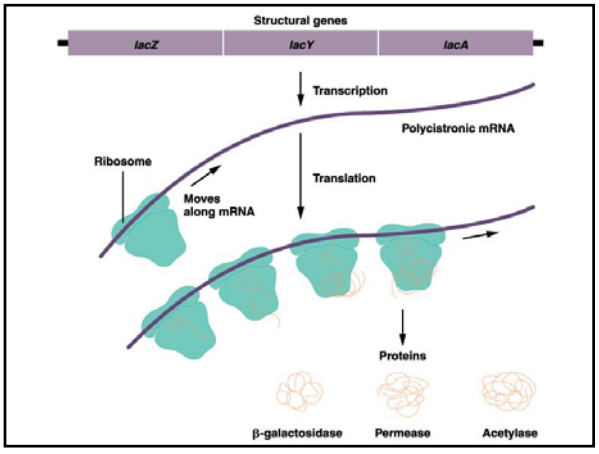
- Negative Regulation:
  - Gene Expression occurs unless it is shut off by a repressor protein.
  - Thus, repressor proteins are negative regulators since their active form shuts off transcription
- Positive Regulation:
  - Gene expression only occurs in the presence of an active regulator protein

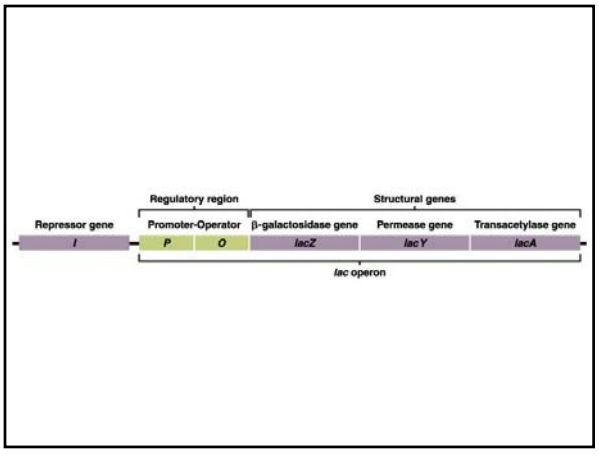
Bacterial Structural Genes

- In Prokaryotes, structural genes tend to be organized in clusters that are controlled from a single promoter
- Such a structure is called an Operon
- The promoter is said to be a *cis*-acting element
- Molecules that interact at this promoter are called *trans*-acting elements

Lactose Metabolism in *E. coli*

- Lactose metabolism in *E. coli* is controlled by the Lac Operon which is an inducible System
- The Operon consists of 3 genes that encode 3 proteins which are involved in the catalytic degradation of the disaccharide lactose (galactose-Beta 1,4 – glucose)
- All three genes transcribed as a single unit
  - polycistronic mRNA
- Operon is regulated by absence or presence of lactose = the inducer





Lac Operon

- *Lac Z* gene encodes for β-galactosidase, an enzyme that converts lactose into monosaccharides glucose and galactose
- *Lac Y* gene codes for β-galactoside permease, a membrane bound protein that helps lactose enter the cell.
- *Lac A* gene, codes for transacetylase whose role is yet to be determined in this process

Regulatory Genes

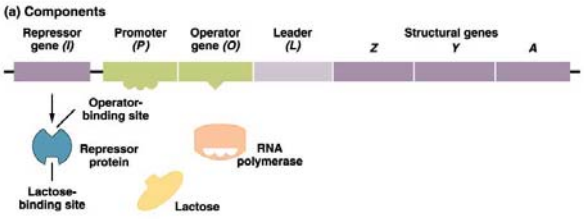
- *Lac I* gene codes for a repressor molecule that regulates the transcription of the structural genes.
- The repressor interacts with the DNA sequence at the Operator region (O) of the operon
- When the repressor binds to the Operator, Transcription is inhibited

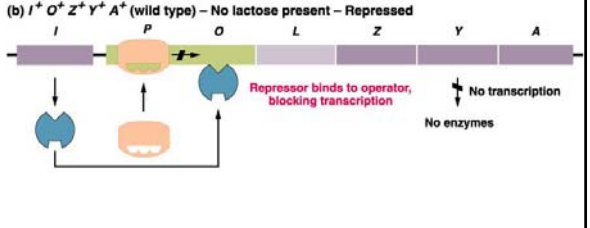
Lac Operon Control

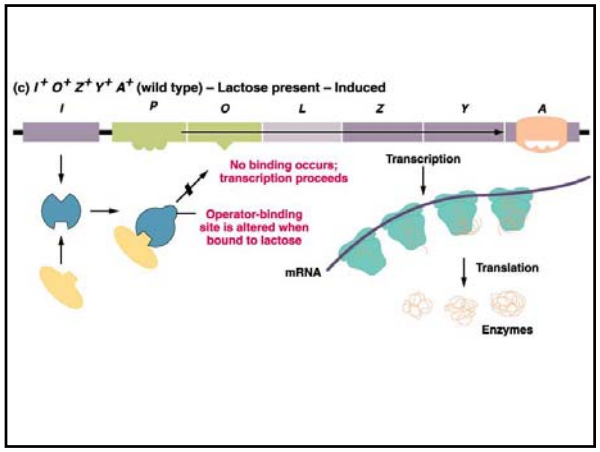
- However, when lactose is present in the cell, it interacts with the repressor molecule, changing its structure which prevents it from binding to the operator.
- Thus, transcription can then proceed.
- Since transcription occurs only when the repressor fails to bind to the operator, negative control is exerted.

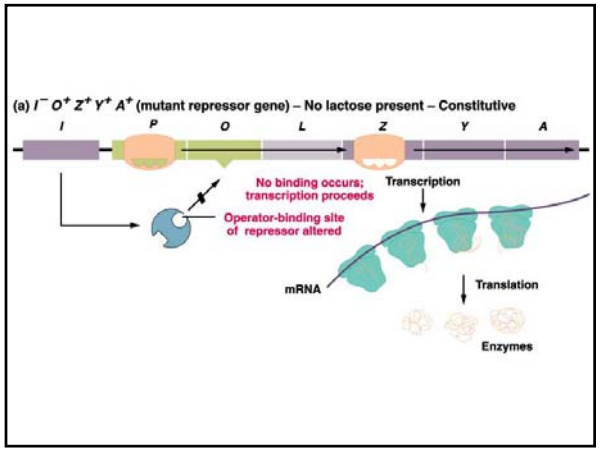
Genetic Proof of the *Lac* Operon

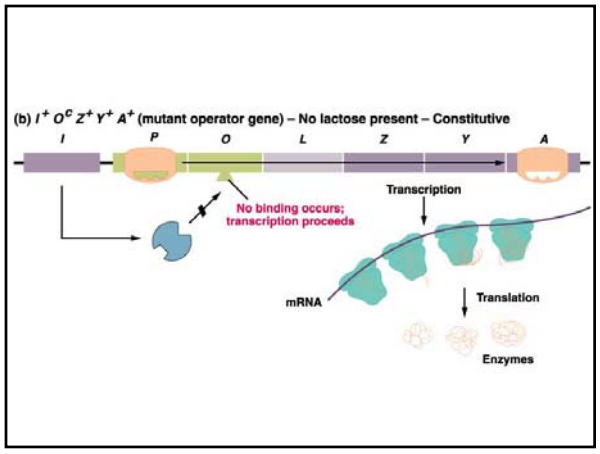
- Mutants can be created in each of the genes present in the *lac* operon allowing us to test the model
- "Extra" genes can be inserted into the bacteria via a plasmid called the F-Factor
- Let's look at the *lac* operon and some mutant cells to see how things work

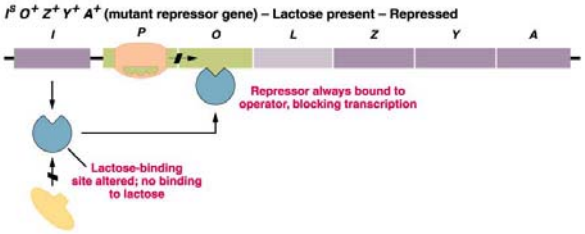





(c) $I^+ O^+ Z^+ Y^+ A^+$ (wild type) – Lactose present – Induced
I
P
O
L
Z
Y
A
No binding occurs; transcription proceeds
Operator-binding site is altered when bound to lactose
Transcription
mRNA
Translation
Enzymes


(a) $I^- O^+ Z^+ Y^+ A^+$ (mutant repressor gene) – No lactose present – Constitutive
I
P
O
L
Z
Y
A
No binding occurs; transcription proceeds
Operator-binding site of repressor altered
Transcription
mRNA
Translation
Enzymes


(b) $I^+ O^C Z^+ Y^+ A^+$ (mutant operator gene) – No lactose present – Constitutive
I
P
O
L
Z
Y
A
No binding occurs; transcription proceeds
Transcription
mRNA
Translation
Enzymes

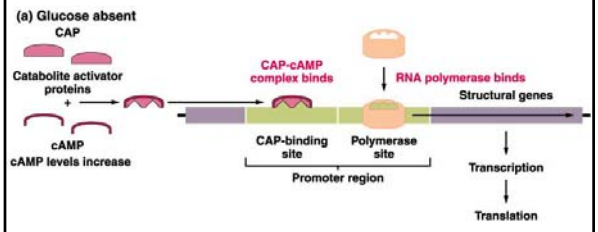


Positive Control of the *Lac* Operon

- Polymerase binding to the *lac* operon promoter is not very efficient
- Another protein, catabolite activating protein (CAP) helps facilitate RNA Pol binding
- If glucose is present in the cell, regardless of whether lactose is present or not, transcription is turned off = catabolite repression

Catabolite Repression

- CAP exerts a positive control on the *lac* operon by binding to the promoter facilitating RNA Pol binding and thus transcription
- When glucose is present however, CAP does not bind to the promoter efficiently, and transcription does not proceed

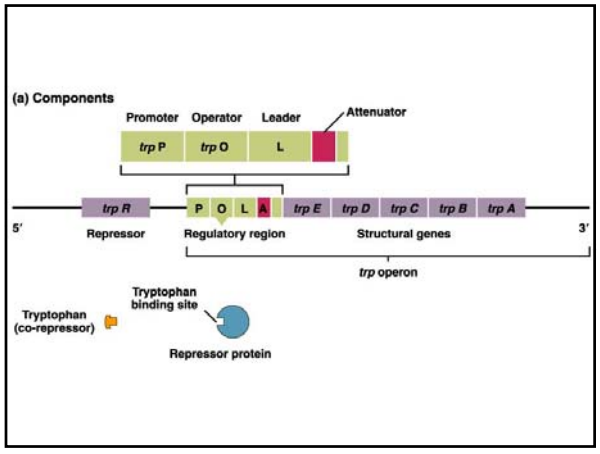


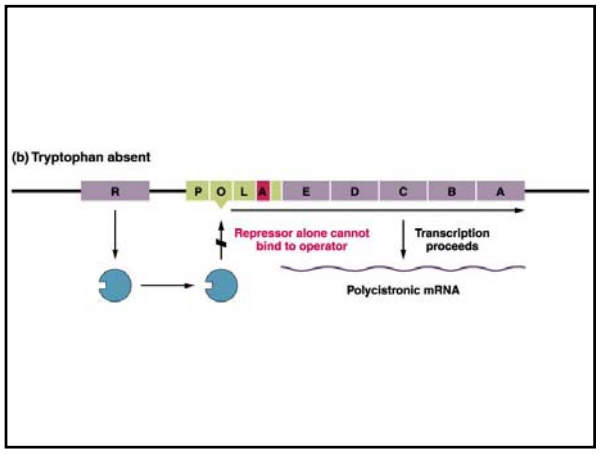
Tryptophan Metabolism: A repressible System

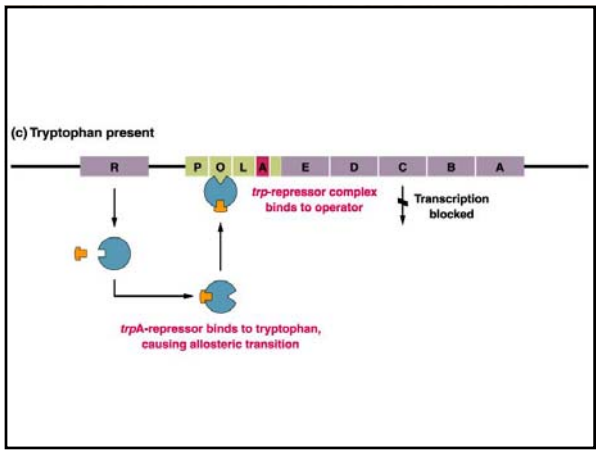
- Wild type *E. coli* can produce the enzymes necessary for the biosynthesis of amino acids and other essential macromolecules
- If the amino acid tryptophan is present in enough quantity in growth medium for *E. coli*, then the enzymes necessary for its production are repressed
- 5 genes are involved in the *trp* operon
- In the presence of tryptophan, all 5 genes are repressed (not expressed)

Trp Operon

- An inactive repressor is normally made that cannot bind to the operator region
- However, when trp is present, it interacts with this repressor, allowing it to bind to the operator and repress transcription
- This system, like the *lac* system is under negative control since the regulatory complex inhibits transcription


(a) Components
Promoter
Operator
Leader
Attenuator
trp P
trp O
L
trp R
P
O
L
A
trp E
trp D
trp C
trp B
trp A
5′
3′
Repressor
Regulatory region
Structural genes
trp operon
Tryptophan (co-repressor)
Tryptophan binding site
Repressor protein


(b) Tryptophan absent
R
P
O
L
A
E
D
C
B
A
Repressor alone cannot bind to operator
Transcription proceeds
Polycistronic mRNA


(c) Tryptophan present
R
P
O
L
A
E
D
C
B
A
trp-repressor complex binds to operator
Transcription blocked
trpA-repressor binds to tryptophan, causing allosteric transition