Control of Gene Expression: Prokaryotic

Control points for gene expression

- **DNA rearrangements** - expression depends on the position of DNA sequences in genome
- **Transcriptional regulation** - RNA synthesis controlled by regulating initiation or termination of RNA
- **RNA processing** - regulation through splicing

Control points continued

- **Translational control** - protein synthesis is regulated by altering the translational process
- **Stability of mRNA** - the effect of the RNA on the cell is relative to the length of time it persists
- **Post-translational control** - a variety of mechanisms that affect enzyme activity, activation or stabilization
Goals of gene regulation

- Prokaryotes
  - Maximum growth rate in a particular environment
- Eukaryotes
  - Growth and division in concert with other cells
  - Maintenance of specialized characteristics

Gene Regulation in Prokaryotes

- Transcriptional Control Genes are “on” when its product is needed and “off” when it is not
- Coordinated Regulation
  - All enzymes in a pathway are either produced or not

Coordinated Regulation

- Enzymes acting in sequence in a single metabolic pathway are often regulated together
- Polycistronic mRNAs encode all of the gene products that function in the same pathway
- Mechanism depends on pathway - catabolic or anabolic
Catabolic Regulation

- Availability of molecule to be degraded regulates pathway
- “Inducible system” - presence of molecule (inducer) leads to synthesis of enzymes in pathway (induction)

Anabolic pathways

- Final product of pathway is frequently the regulatory molecule
- “Repressible system” - presence of the final product molecule (co-repressor) results in failure to synthesize enzymes

Molecular Mechanisms

- Negative regulation - a repressor protein present in cell prevents transcription
  - Inducible system - Inducer antagonizes the repressor
  - Repressible system - an aporepressor combines with the co-repressor to form a functional repressor
Molecular mechanisms

• Positive Regulation-Activator binds to regulatory site to stimulate transcription; Gene is off in absence of activator
Allosteric Effectors

- Both activator and repressor proteins must respond to environmental conditions.
- Must exist in “on” and “off” state
- An allosteric site interacts with DNA binding domain to determine
- Allosteric effector binds to allosteric site to determine the interaction

Molecular Mechanisms

- Negative regulation and positive regulation are not mutually exclusive
  - Utilizes both systems to respond to different conditions in the cell
- Negative regulation - more common in prokaryotes
- Positive regulation - more common in eukaryotes
Metabolism of Lactose

- **Permease** - product of Y gene, this enzyme transports lactose into cell
- **β-galactosidase** - product of Z gene, this enzyme adds water to the linkage to break lactose into glucose and galactose
- **Transacetylase** - product of A gene, this enzyme's exact function is unknown and is not required for lactose metabolism

### Lac Repressor
- Product of I gene, this protein can block the expression of Z, Y and A genes
- **DNA binding site** - binds, binds operator site
- **allosteric site** - binds lactose or inducer analog
- **lac promoter site** - site at which RNA polymerase binds
- **lac operator site** - site on DNA which Lac repressor binds
Operon: genetic unit of coordinate expression

Isopropyl-β-D-thiogalactoside (IPTG)

F’ factors
Origin of F’ lac

Constitutive Mutants

• Mutants that produced all three enzymes even in the absence of an inducer
• Defect not in enzymes themselves but in the way they are produced.
• One set of mutations mapped to another locus - the I locus or the repressor gene
• A second set mapped to a region of DNA near the beginning of the set of genes called the operator

Oc mutant

• Changes in the bases of the operator make it incapable of binding repressor
• Protein binding site - no gene product
I- mutant

- I- mutant produces a nonfunctional repressor
- Can be overridden by the product of another functional I gene = there is a protein product

New terms

- Trans acting or trans dominance - Action of a diffusible product (e.g. a protein)
- Cis acting or Cis dominance - Physical interaction between the element and the genes that it is in contact with is required

Allostery

- I- mutations (superrepressor) cause repression even in the presence of the inducer
**Promoter**

- Initiation site for transcription - located between $I$ and $O$
- Mutations are cis dominant

**RNA polymerase binding is prevented by Lac repressor bound to the operator**

**Repressor has a helix-turn-helix motif**
Operator Structure

Repressor is a tetramer with HTH in each subunit
Binds two operator sites
Very stable interaction between repressor and operator

Positive Control: Catabolite Repression

- Keeps lactose system turned off in the presence of glucose
- Breakdown product of glucose prevents lac operon from being turned on
- Effect is through cAMP
  - High glucose = low cAMP
  - Low glucose = high cAMP
CAP-cAMP complex

- Glucose metabolite inhibits formation
- CAP bound to DNA causes promoter to bend increasing the efficiency of RNA polymerase to promoter
- Positive control

CRP-cAMP contact with RNA polymerase

- CRP binds as a dimer
- cAMP functions as effector whose binding to CRP allows it to function as positive regulator
Lac operon - the summary

(a) Glucose present (CAP), no lactose, no lac mRNA

(b) Glucose present (CAP), lactose present

(c) No glucose present (LMP), lactose present

(d) No glucose present (LMP), lactose present

Very little lac mRNA
More complex: Tryptophan Operon

- Repressible system - excess of tryptophan shuts down the system by binding repressor and activating it so that it binds the operator
- A second system called feedback inhibition. Activity of the first enzyme in the biosynthetic pathway is inhibited by tryptophan
- Attenuation - a third mechanism of control

Trp operon and its products
Attenuation

- The 141 bases are transcribed irregardless of the tryptophan levels.
- This leader has two codons for tryptophan
- When tryptophan is abundant, there is sufficient Trp-tRNA to allow translation of the codons.