Gene Expression
Prokaryotes and Viruses

BIT 220
Chapter 23
Types of Regulatory Mechanisms

• Rapid turn-on and turn off of gene expression (responds to some external source)

• Expression of a cascade of gene expression
Constitutive Gene Expression

• “housekeeping genes” – rRNA, tRNA, polymerase

• Expressed most of the time in most cells

• Carry out important cellular functions
Inducible Gene Expression

- Gene products needed only at certain times
- Wasteful for the cell to always be making gene product
- Substance turning gene on is called induction (inducible expression)
Positive and Negative Gene Expression

- Regulator genes- genes that control other genes
- Positive – regulator gene turns on expression of other gene
- Negative - regulator gene turns off expression of other gene
- Figure 23.4 – general operon; (Also see Figure 23.3)
- Activators and repressors
Some terms

- **RPBS** – regulator protein binding site – adjacent to promotor of structural gene
- Allosteric interactions – changes in protein structure caused by binding of small molecules
Inducible/repressible operons

• Inducible operon: turned off in the absence of an effector (inducer) molecule
• Repressible operon: turned on in the absence of an effector (co-repressor) molecule
Operons

• Jacob and Monod (1961)
• Repressible model
• lac operon – lactose utilizing genes
• Includes structural genes, operator and promotor genes
• Figure 23.7 – also go over handout I gave you and next slide
• Don’t need to know all the genotypes (skip pp. 580-584)
lac operon genes

- *lac Z, Y and A* – respectively $\beta$-galactosidase, $\beta$-gal permease and $\beta$-gal transacetylase
- Promotor (P)
- Operator (O)
- Regulator gene (I) – encodes a 360 aa repressor
- Negatively controlled inducible operon
trp operon

- Repression and attenuation – requires nucleotide sequences present at a specific part of the operon
- *trp* synthesizing genes
- Negative repressible operon
- 5 structural genes- biosynthesis of tryptophan
- **Figures 23.11 – 23.13** (as an FYI- don’t know these figures in detail)
trp operon

- Second level of control attenuation
- Control of termination of transcription
- G:C rich palindrome followed by several A’s and Ts
- Happens only in presence of tRNA\textsuperscript{trp} (presence of tryptophan)
\( \lambda \) phage

- When it infects bacteria, can follow 2 paths:
  - Lytic cycle – replicates and causes bacterial cells to burst
  - Lysogenic cycle – becomes integrated as a prophage (chap. 17)

- Figure 23.14
Manipulation of Gene Expression in Prokaryotes

- How used in biotech industry
- How used to help produce proteins
Promotors

Characteristics of a Promotor:
1. strong
2. regulatable
3. constitutive or inducible (can be repressed or turned on)
Some promoters

**lac Promoter**
induce (derepress) with IPTG or lactose

**trp Promoter**
induce in absence of tryptophan

dual plasmid systems
Best features of 2 promoters
Reporter genes

• A gene whose phenotypic expression is easy to monitor; used to study promoter activity in different tissues or developmental stages
• Recombinant DNA constructs are made in which the reporter gene is attached to a promoter region of particular interest and the construct transfected into a cell or organism
Reporter genes

Reporter genes can report:
• the strength of promoters, whether native or modified for reverse genetics studies
• the efficiency of gene delivery systems
• the efficiency of translation initiation signals
• the success of molecular cloning efforts.
# Examples of Reporter Genes

<table>
<thead>
<tr>
<th>Protein</th>
<th>Activity &amp; Measurement</th>
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<tbody>
<tr>
<td><strong>CAT</strong> (chloramphenicol acetyltransferase)</td>
<td>Transfers radioactive acetyl groups to chloramphenicol; detection by thin layer chromatography and autoradiography</td>
</tr>
<tr>
<td><strong>GAL</strong> (β−galactosidase)</td>
<td>Hydrolyzes colorless galactosides to yield colored products.</td>
</tr>
<tr>
<td><strong>GUS</strong> (β−glucuronidase)</td>
<td>Hydrolyzes colorless glucuronides to yield colored products.</td>
</tr>
<tr>
<td><strong>LUC</strong> (luciferase)</td>
<td>Oxidizes luciferin, emitting photons.</td>
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<tr>
<td><strong>GFP</strong> (green fluorescent protein)</td>
<td>Fluoresces on irradiation with UV.</td>
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Imaging of luciferase expression in tissue sections of the brain of a transgenic mouse. With the powerful overlay technique, luminescence signals can be superimposed onto the brightfield image, to localize gene expression. (Courtesy B. Hengerer, H. Berns, Ciba-Geigy, Swiss).
Reporter genes

- Reporter genes can be attached to other sequences so that only the reporter protein is made or so that the reporter protein is fused to another protein (fusion protein).
Fusion proteins

• fusion proteins (a combination of the protein of interest tagged with the known protein or peptide) can be produced in culture by microorganisms in large quantities.
Why use a fusion protein

• protein of interest is present in very small quantities insufficient to characterize or to raise antibody against

• Types:
  – GFP
  – Epitope tagging
Plasmid Containing Tag sequence

Promoter → MCS → Tag sequence

Restriction endonuclease
Ligate with insert

ORI

Antibiotic resistance gene

Express protein

Gene I

Recombinant plasmid