Chapter 16 Lecture

Concepts Of Genetics

Regulation of Gene Expression in Prokaryotes
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16.1 Prokaryotes Regulate Gene Expression in Response to Environmental Conditions
Section 16.1

• Gene regulation has been studied extensively in *E. coli*

• Highly efficient genetic mechanisms have evolved to turn transcription of specific genes on and off depending on a cell's metabolic need for specific gene products

• These responses can be due to changes in the environment as well as nonenvironmentally regulated cellular activity and cell division
Section 16.1

- Bacteria adapt to their environment by producing certain enzymes (inducible enzymes) only when specific substrates are present.
- Enzymes continuously produced regardless of chemical makeup of the environment are called constitutive enzymes.
- An abundance of an end product in the environment represses gene expression:
  - Repressible system
Section 16.1

- Regulation of the inducible or repressible type may be under **positive control** or **negative control**
  - Negative control: genetic expression occurs *unless it is shut off by some form of a regulator molecule*
  - Positive control: transcription occurs *only if a regulator molecule directly stimulates RNA production*
16.2 Lactose Metabolism in *E. coli* Is Regulated by an Inducible System
Section 16.2

- In the presence of lactose, the concentration of the enzymes responsible for its metabolism increases rapidly from a few molecules to thousands per cell.
- The enzymes responsible for lactose metabolism are inducible.
- Lactose is the inducer.
Section 16.2

• Genes coding for enzymes with regulatory functions are organized in clusters, and transcription is under control of a single regulatory region.

• Regulatory regions are almost always located upstream of the gene cluster they control and are cis-acting.

• The molecules that bind these cis-acting sites are called trans-acting elements.
Section 16.2

• Binding of a *trans-acting* element at a *cis-acting* site can regulate the gene cluster either negatively (by turning off transcription)
• or positively (by turning on transcription of the genes in the cluster)
Section 16.2

• Genes coding for the primary structure of an enzyme are called **structural genes**
• The **lac operon** has three structural genes, *lacZ*, *lacY*, and *lacA*, with an upstream regulatory region consisting of an operator and a promoter
• The entire gene cluster functions in an integrated fashion to provide a rapid response to the presence or absence of lactose
Section 16.2

- The *lacZ* gene encodes \(\beta\)-galactosidase, an enzyme that converts the disaccharide lactose to its monosaccharides glucose and galactose.
- This conversion is needed if lactose is to serve as the primary energy source in glycolysis.
Section 16.2

• The \textit{lacY} gene specifies the primary structure of \textit{permease}, an enzyme that facilitates the entry of lactose into the bacterial cell.

• The \textit{lacA} gene codes for the enzyme \textit{transacetylase}, which may be involved in the removal of toxic by-products of lactose digestion from the cell.
Section 16.2

• All three genes are transcribed as a single unit, resulting in a *polycistronic mRNA*
  – Cistron refers to the part of a nucleotide sequence coding for a single gene
  – A single mRNA is translated into all three gene products
Section 16.2

- Jacob and Monod (1960) proposed the operon model, in which a group of genes is regulated and expressed together as a unit.
Section 16.2

- The *lacI* gene regulates transcription of the structural genes by producing a **repressor molecule**
- The repressor is **allosteric**, meaning that it interacts reversibly with another molecule, causing both a conformational change in three-dimensional shape and a change in chemical activity
Section 16.2

• The *lac* operon is subject to negative control because transcription occurs only when the repressor fails to bind to the operator region.
(a) Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Gene/Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repressor</td>
<td>gene (I)</td>
</tr>
<tr>
<td>Promoter</td>
<td>(P)</td>
</tr>
<tr>
<td>Operator</td>
<td>gene (O)</td>
</tr>
<tr>
<td>Leader</td>
<td>(L)</td>
</tr>
<tr>
<td>Structural genes</td>
<td>Y</td>
</tr>
</tbody>
</table>

- Repressor binding site
- Lactose binding site
- RNA polymerase
- Lactose

(b) $I^+ O^+ Z^+ Y^+ A^+$ (wild type) — no lactose present — **Repressed**

- Repressor binds to operator, blocking transcription
- No transcription
- No enzymes

(c) $I^+ O^+ Z^+ Y^+ A^+$ (wild type) — lactose present — **Induced**

- No binding occurs; transcription proceeds
- Transcription
- Operator-binding region is altered when bound to lactose
- mRNA
- Translation
- Enzymes
Section 16.2

• Thus the operon model invokes a series of molecular interactions between proteins, inducers, and DNA for efficient regulation of structural gene expression
  – No lactose, enzymes not needed, expression of genes encoding enzymes repressed
  – Lactose present; indirectly induces activation of genes by binding to repressor
  – All lactose metabolized, none available to bind to repressor, transcription repressed
Section 16.2

• Analysis of lac expression in the absence or presence of lactose in partial diploid merozygotes was used to prove the operon model for the lac operon
A Comparison of Gene Activity (+ or −) in the Presence or Absence of Lactose for Various *E. coli* Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Presence of β-Galactosidase Activity</th>
<th>Lactose Present</th>
<th>Lactose Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I</em>⁺<em>O</em>⁺<em>Z</em>⁺</td>
<td></td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>A. <em>I</em>⁺<em>O</em>⁺<em>Z</em>⁻</td>
<td></td>
<td>−</td>
<td>−</td>
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<tr>
<td><em>I</em>⁻<em>O</em>⁺<em>Z</em>⁺</td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td><em>I</em>⁺<em>O</em>⁻<em>Z</em>⁺</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. <em>I</em>⁻<em>O</em>⁺<em>Z</em>⁺/<em>F’I</em>⁺</td>
<td></td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>I</em>⁺<em>O</em>⁻<em>Z</em>⁺/<em>F’O</em>⁺</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. <em>I</em>⁺<em>O</em>⁺<em>Z</em>⁺/<em>F’I</em>⁻</td>
<td></td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>I</em>⁺<em>O</em>⁺<em>Z</em>⁺/<em>F’O</em>⁻</td>
<td></td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D. <em>I</em>⁻<em>O</em>⁺<em>Z</em>⁺</td>
<td></td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><em>I</em>⁻<em>O</em>⁺<em>Z</em>⁺/<em>F’I</em>⁺</td>
<td></td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>
(a) $I^- O^+ Z^+ Y^+ A^+$ (mutant repressor gene) — no lactose present — **Constitutive**

No binding occurs; transcription proceeds

Operator-binding region of repressor is altered

**mRNA**

**Translation**

**Enzymes**

(b) $I^+ O^- Z^+ Y^+ A^+$ (mutant operator gene) — no lactose present — **Constitutive**

Nucleotide sequence of operator gene is altered. No binding occurs; transcription proceeds

**mRNA**

**Translation**

**Enzymes**
Lactose-binding region is altered; no binding to lactose

Repressor always bound to operator, blocking transcription

$I^s$ $O^+$ $Z^+ Y^+ A^+$ (mutant repressor gene) — lactose present — Repressed
16.3 The Catabolite-Activating Protein (CAP) Exerts Positive Control over the *lac* Operon
• The catabolite-activating protein (CAP) is involved in repressing expression of the \emph{lac} operon when glucose is present.

• This inhibition is called \textbf{catabolite repression}.
Section 16.3

- In the absence of glucose and the presence of lactose, CAP exerts positive control by binding to the CAP-binding site, facilitating RNA polymerase binding at the promoter and transcription.

- For maximal transcription, the repressor must be bound by lactose and CAP must be bound to the CAP-binding site.
(a) Glucose absent

CAP (Catabolite-activating protein) + cAMP

As cAMP levels increase, cAMP binds to CAP, causing an allosteric transition

CAP–cAMP complex binds

RNA polymerase binds

O Structural genes

Promoter region

Translation occurs

(b) Glucose present

Glucose

cAMP levels decrease

CAP cannot bind efficiently

CAP binds site

RNA polymerase seldom binds

O Structural genes

Promoter region

Translation diminished
In order to bind to the promoter, CAP must be bound to cyclic adenosine monophosphate (cAMP).

Glucose inhibits the activity of adenylyl cyclase, which catalyzes the conversion of ATP to cAMP and thus prevents CAP from binding when glucose is present.
16.4 Crystal Structure Analysis of Repressor Complexes Has Confirmed the Operon Model
• A detailed structure of the *lac* operon and its regulatory regions reveals three sites for repressor binding
• All three operators must be bound for maximum repression
• Binding of the repressor to operators $O_1$ and $O_3$ creates a *repression loop*, which prevents access of RNA polymerase to the promoter
Repres sor loop region

\[ l\alpha cI \quad O_3 \quad \text{Promoter} \quad l\alpha cO \quad O_1 \quad l\alpha cZ \quad O_2 \]

-82 -50 +11 +35 +412

CAP-binding site RNA polymerase binding site Coding sequence begins

(a) (b) (c)
16.5 The Tryptophan \((trp)\) Operon in \(E.\ coli\) Is a Repressible Gene System
Section 16.5

• There are five enzymes involved in tryptophan production, and they are part of an operon

• In the presence of tryptophan, the operon is repressed and none of the enzymes are produced

• When tryptophan (corepressor) is present, the system is repressed and enzymes are not made
(a) Components

Promoter  Operator  Leader  Attenuator

trpP  trpO  L  A

trpR  P  O  L  A  trpE  trpD  trpC  trpB  trpA

5'  Repressor  gene  Regulatory  region  Structural  genes  3'

Tryptophan binding site  Repressor protein

(b) Tryptophan absent

Repressor alone cannot bind to operator  Transcription proceeds  Polycistronic mRNA

(c) Tryptophan present

Repressor binds to trypophan, causing allosteric transition  Repressor-trypophan complex binds to operator  Transcription blocked
Section 16.5

- The *trp* structural genes are preceded by a leader sequence containing a regulatory site called an **attenuator**
16.6 Attenuation Is a Process Critical to the Regulation of the trp Operon in \textit{E. coli}
Section 16.6

- Transcription of the leader region of the *trp* operon can occur even when the operon is repressed in the presence of tryptophan (attenuation)
- In the absence of tryptophan, transcription is not terminated in the leader region and proceeds through the entire operon
Section 16.6

• The leader region can form two different conformations, depending on the presence or absence of tryptophan.

• In the presence of tryptophan, the hairpin structures formed act as a transcriptional terminator.

• In the absence of tryptophan, a different hairpin forms and acts as an antiterminator, and transcription proceeds...
(a) Transcription of trp Operon (DNA)

(b) Stem-loop structures in leader RNA sequence

(c) Alternative secondary structures of leader RNA
Section 16.6

- The **leader region** contains two tryptophan codons.
- The **antiterminator hairpin** structure forms in the absence of tryptophan because the ribosome stalls at these codons because there is not adequate charged tRNA$_{trp}$.
- In the presence of tryptophan, the ribosome proceeds through this sequence and the terminator hairpin can form.
Section 16.6

• The attenuation mechanism is common to several operons for enzymes responsible for synthesis of other amino acids
• These include threonine, histidine, leucine, and phenylalanine
• *Bacillus subtilis* uses just attenuation and hairpins to regulate its *trp* operon

• Instead of ribosome stalling, the mechanism of attenuation for *trp* operon involves TRAP (*trp* RNA-binding attenuation protein)

• **TRAP** has 11 subunits, each of which can bind one molecule of tryptophan

• The fully saturated TRAP can then bind to the 5' leader sequence to form the terminator hairpin and prevent formation of the antiterminator hairpin
(a)

(b)

Terminator hairpin
(Tryptophan abundant)
Section 16.6

• Uncharged tRNA\textsubscript{trp} induces expression of the \textbf{anti-TRAP (AT)} gene

• The AT protein associates with TRAP in the tryptophan-activated state and inhibits binding to the target leader RNA sequence
16.7 Riboswitches Utilize Metabolite-Sensing RNAs to Regulate Gene Expression
Section 16.7

- Numerous cases of gene regulation depend on alternative forms of mRNA secondary structure and involve riboswitches
- Riboswitches are mRNA sequences present upstream from the coding sequence
- mRNA responds to the environment of the cell and regulates its own expression
Section 16.7

• All riboswitches possess a metabolite-sensing RNA sequence that allows transcription of that RNA to either proceed or not proceed.

• Two important domains within a riboswitch are the aptamer, which binds to the ligand, and expression platform, which is capable of forming the terminator structure.
16.8 The *ara* Operon Is Controlled by a Regulator Protein That Exerts Both Positive and Negative Control
Section 16.8

• The arabinose (ara) operon is subject to both positive and negative regulation by the AraC protein

• The metabolism of arabinose (sugar) is governed by the enzymatic products of three structural genes _ara B, A, and D_
Section 16.8

- Transcription is controlled by the regulatory protein AraC, which interacts with two regulatory regions, \textit{araI} and \textit{araO}_2
- AraC binds to \textit{araI} in the presence of arabinose and CAP-cAMP to induce expression
- In the absence of arabinose and CAP-cAMP, AraC binds to both \textit{araI} and \textit{araO}_2 to form a loop that causes repression
(a) Components of ara operon

Regulatory gene  Operator region  Inducer site  Structural genes
araC  O2  O1  CAP site  l  araB  araA  araD

Regulator protein  CAP–cAMP

(b) Arabinose present; operon is induced – positive regulation

C  O2  O1  B  A  D

CAP site bound  Inducer site bound  Transcription

(c) Arabinose absent; operon is repressed – negative regulation

O2 site bound  Dimers interact

CAP site unbound  Inducer site bound  No transcription