Gene Control

Lactose
INTRODUCTION TO CONTROL OF MACROMOLECULAR SYNTHESIS

Although there are exceptions, control of metabolic processes usually occurs at the initiation stage.

A. Replication

Replication of the chromosome is tightly regulated such that replication is begun once and only once per cell cycle and at the right time in the cell cycle. This regulation is exerted by a regulatory protein or proteins acting at the replication origin to control the initiation stage. Once DNA synthesis has begun, it normally proceeds to complete duplication of the entire chromosome.

B. Gene Expression

Gene expression generally means all the processes necessary to produce a functional product. That is, both transcription and translation (except for genes which do not produce protein products, such as tRNA and rRNA genes, in which case gene expression would involve transcription and processing of the RNA products). Gene expression also is tightly regulated, usually by regulating transcription. Transcription usually is regulated by controlling the initiation of transcription. Genes are subject to negative, positive, or negative and positive controllers. (Some genes are not regulated; they are transcribed at a constant, usually low, level and are said to be expressed constitutively.)

**Negative control** is exerted by repressors which bind operators and inhibit initiation of transcription. Repressors can be either active or inactive, depending on the presence or absence of low molecular weight compounds in the environment (or other environmental factors).

**Positive control** is exerted by activator proteins which bind activator binding sites near promoters and stimulate initiation of transcription. Activator proteins can be either active or inactive, depending on the presence or absence of low molecular weight compounds in the environment (or other environmental factors).

Repressors and activators act together to turn genes or operons on or off; they form a molecular switch. These switches function to turn genes on or off over one to two generations. That is, about thirty minutes to a couple of hours can be required for this switch to change completely from one to off or off to on.

**Fine tuning** of gene expression is done by the process of attenuation. That is, if a gene or operon is turned on, the level of expression can be adjusted within seconds to produce more or less product. A gene or operon which is being transcribed can stop transcription near the promoter (that is, after polymerization of about 200 nucleotides) if the entire messenger is not needed momentarily. This is economical because it saves the polymerization of several thousand nucleotides in the complete messenger, at the cost of several hundred nucleotides at the 5' end. Attenuation depends on a signal to terminate transcription early; this signal depends on the intracellular concentration of some low molecular weight compound. An example is attenuation of an amino acid biosynthesis operon. The intracellular concentration of the amino acid acts as a signal for the growing mRNA to form a terminator stem-loop near its 5' end; this terminates the synthesis of messenger early. Attenuation is another form of molecular switch.
MIC 226

GENE EXPRESSION CONTROL

OPERON STRUCTURE

Sites on DNA where proteins bind - Promoter, Operator
Structural genes which encode proteins (e.g., enzymes)
   Some genes encode stable RNA products
Cistron, polycistronic mRNA
Repressor, Negative control
Activator, Positive control

LACTOSE OPERON (catabolic)

Lactose (glucose, galactose, beta galactoside, beta galactosidase)
Lactose operon genes –
   Beta galactosidase, permease
   Control Sites (promoter, operator, activation site)
Repressor gene, repressor, active repressor, inducer, inactive repressor
Positive control, cAMP, Catabolite Activator Protein

TRYPTOPHAN OPERON (anabolic)

Structural genes
Control sites (promoter, operator, attenuator site)
Repressor - inactive
   - co-repressor, active repressor
Attenuation
   coupled transcription and translation
   fine tuner of gene expression
   leader peptide, trp codons
   alternative structures of mRNA, molecular switch
TRANSCRIPTION CONTROL (INITIATION)
REPRESSOR CONTROLS NEGATIVELY

REPRESSOR GENE

RNA POLYMERASE

+1 POLYCISTRONIC mRNA

OPERATOR

GENE A

GENE B

SITES

STRUCTURAL GENES

REPRESSOR mRNA

ACTIVE/INACTIVE DEPENDS ON ENVIRONMENT
**Active Repressor** - binds operator, inhibits transcription (initiation)

- **Promoter**
- **Operator**
- **Gene A**
- **Gene B**

Little or no transcription

**Inactive Repressor** - cannot bind operator

RNA polymerase transcribes

- **Promoter +1**
- **RNA polymerase**
- **Operator**
- **Gene A**
- **Gene B**

**Operon**; **Operator**

**Cistron**; **Polycistronic mRNA**
Activator proteins control positively.

Activator protein gene

<table>
<thead>
<tr>
<th>Activator binding</th>
<th>Promoter +1</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>Gene A</td>
<td>Gene B</td>
</tr>
</tbody>
</table>

5' --- 3'

Activator mRNA

Active/inactive depends on environment.
ACTIVE ACTIVATOR - BINDS ACTIVATOR SITE
STIMULATES RNA POLYMERASE

ACTIVATOR SITE [PROMOTER] +1 LOTS OF mRNA ...
... GENE A GENE B ...

INACTIVE ACTIVATOR:
CANNOT BIND ACTIVATOR SITE
CANNOT STIMULATE RNA POLYMERASE

ACTIVATOR SITE [PROMOTER] LITTLE OR NO mRNA ...
... GENE A GENE B ...
BACTERIAL GENES ARE SUBJECT TO:

- NEGATIVE CONTROL
  REPRESSOR - ACTIVE/INACTIVE

- POSITIVE
  ACTIVATOR PROTEIN - ACTIVE/INACTIVE

- BOTH POSITIVE + NEGATIVE
  FINAL RESULT DEPENDS ON INTERACTION OF BOTH MECHANISMS

- NEITHER POSITIVE NOR NEGATIVE ALWAYS TRANSCRIBED
  USUALLY AT LOW LEVEL - CONSTITUTIVE -
**Catabolic Operon - Lactose**

**Lactose**

- Lactose
- β-Galactosidase
- Glucose + Galactose

**Proteins Needed for Growth**
- Lactose Transport
- β-Galactosidase
- Etc, etc...

**Lactose Genes On**

**Glucose**

**Proteins Needed:**
- Lactose Transport
- β-Galactosidase
- Etc, etc...
LACTOSE UTILIZATION

LACTOSE = \( \beta \)-GALACTOSIDE

\( \beta \)-1,4 LINKAGE

\( \beta \)-GALACTOSIDE

\( \beta \)-GALACTOSE

\( \beta \)-GLUCOSE

EPIMERS-DIASTEREO ISOMERS

Glycolysis
GALACTOSE \[\rightarrow\] GLUCOSE

GALACTOSE OPERON
3 GENES
3 PROTEINS

GALACTOSE + ATP \[\rightleftharpoons\] GLUCOSE-1-P04 + ADP
3 REACTIONS
3 ENZYMES
**Lactose Genes (Operon)**

- **I**: Repressor Gene
- **C**ap Site
- **O**perator

**Operator Levels**
- **Z**: β Galactosidase
- **Y**: Lactose Transport

**Repressor Protein**
- Active

**Operon Regulation**
- **No Lactose (No Inducer)**: Active repressor binds operator, little or no transcription, ~10 molecules β-galactosidase/cell
- **Lactose Medium (Inducer)**: Inducer binds repressor, repressor inactivated, cannot bind operator
TRANSCRIPTION PERMITTED

$> 10^3$ MOLECULES $\beta$-GALACTOSIDASE/CELL

NUEL
JACOB
MONOD
LOWF
LACTOSE OPERON IS SUBJECT TO
POSITIVE CONTROL
GROW IN LIMITING CONCENTRATIONS OF
GLUCOSE AND LACTOSE

LOG
TURBIDITY

TIME
Lactose operon is subject to positive control. Grow in limiting concentrations of glucose and lactose.

Log turbidity

Time

β-galactosidase level

Cells use glucose first. No β-galactosidase in presence of glucose.

Glucose prevents positive control from activating.

Glucose inhibits cAMP synthesis.

cAMP = cyclic adenosine monophosphate — needed to activate.

CAP = catabolite activator protein.
POSITIVE CONTROL OF LAC

CAMP BINDS CAP; ACTIVATES CAP
CAMP-CAP BIND CAP SITE TO STIMULATE TRANSCRIPTION

\[
\begin{align*}
\text{GLUCOSE INHIBITS ADENYLATED CYCLASE} \\
\text{Cyclic AMP} + \text{PPi}
\end{align*}
\]
Maximum Lac Transcription

Repressor + inducer is inactive - cannot bind operator

Catabolite activator protein
Cap binds cAMP, becomes active
Cap·cAMP bind cap site - stimulate RNA polymerase

Positive + negative control systems allow cells to use more efficient carbon/energy source first
That is: choose between carbon sources
Maximum Lac expression requires:

Inducer - to inactivate repressor

(Induction)

And

Positive activation

CAMP·CAP (Catabolite activator protein)

Must bind Cap site to stimulate transcription.
For cells growing in the following conditions, the lac operon will be controlled in the following ways:

<table>
<thead>
<tr>
<th>Growth Medium</th>
<th>Negative Control Repressor-Operator</th>
<th>Positive Control C AMP - CAP Activator</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>Glucose (only)</td>
<td>LAC repressor is synthesized; is active; binds operator; inhibits RNA polymerase</td>
<td>Glucose inhibits adenylate cyclase; no C AMP synthesis; C AMP - CAP cannot bind activator site</td>
<td>LAC is off</td>
</tr>
<tr>
<td>Lactose (only)</td>
<td>LAC repressor is synthesized; is inactivated by lactose; (allo-lactose); cannot repress</td>
<td>C AMP is synthesized; C AMP and CAP bind activator site; stimulate RNA polymerase to transcribe</td>
<td>LAC is on</td>
</tr>
<tr>
<td>Glucose and lactose</td>
<td>LAC repressor is synthesized; is inactivated by lactose; (allo - lactose); cannot repress</td>
<td>Glucose inhibits adenylate cyclase; no C AMP synthesis; C AMP - CAP cannot bind activator site</td>
<td>LAC is off (until all the glucose is used)</td>
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