19.1 CONSTITUTIVE, INDUCIBLE AND REPRESSIBLE GENE EXPRESSION

Constitutive Housekeeping ribosomal proteins

Inducible Catabolic (Degradative) Carbohydrate (lactose) Utilization

Repressible Anabolic (Biosynthetic) Amino Acid (Tryptophan) Biosynthesis
19.2 POSITIVE AND NEGATIVE CONTROL OF GENE EXPRESSION

**EFFECTOR (E)**
A small intracellular metabolite; often the initial substrate of a catabolic pathway or the end product of a biosynthetic pathway.

Effectors may be *inducers* or *co-repressors*.

**REGULATOR (R)**
An allosteric, sequence-specific DNA binding protein whose DNA binding is contingent on effector binding to the allosteric site, and whose binding to DNA in turn modulates the frequency of transcript initiation.

Regulators may be *repressors* or *activators*.

**OPERATOR (O)**
This is the traditional term for what your book refers to as the “RPBS”. The DNA binding sequence recognized by regulator.

Positive vs Negative Regulation is Defined by the result of $R \cdot O$ interaction.

Inducible vs Repressible is Defined by the result of $E \cdot R$ interaction.

Regulation of the Tryptophan biosynthesis genes (described in the previous section) follows the Repressible/Negative scenario as shown in Fig. 19.3 b (left). Tryptophan is the co-repressor (effector).

Induction of Lactose utilization genes is controlled by 2 separate $E \cdot R \cdot O$ interactions:

<table>
<thead>
<tr>
<th>EFFECTOR</th>
<th>REGULATOR</th>
<th>$R \cdot O$</th>
<th>Diagram</th>
</tr>
</thead>
<tbody>
<tr>
<td>allolactose/IPTG</td>
<td>Lac Repressor</td>
<td>Negative</td>
<td>Fig. 19.3 a (left)</td>
</tr>
<tr>
<td>cAMP</td>
<td>CAP</td>
<td>Positive</td>
<td>Fig. 19.3 a (right)</td>
</tr>
</tbody>
</table>
The text uses the term "multigenic mRNA" in place of the traditional term "polycistronic mRNA". In either case, this aspect of the operon facilitates coordinate expression of several genes whose functional products interact with each other.

The multigenic Lac mRNA has separate ribosome binding sites for each of the three ORF's.
19.4  THE LACTOSE OPERON IN E. COLI: INDUCTION AND CATABOLITE REPRESSION  p. 570

INDUCTION

Note (Fig. 19.5) that the gene for the Lac Repressor (Lac I) is expressed constitutively from it's own promoter $P_I$, not from $P_{Lac}$.

Typically there are only about 10 molecules of Lac Repressor present in a cell.

Lac Repressor does not inhibit binding of RNA Pol to $P_{Lac}$ but transcriptional initiation is blocked. This, RNA Pol is "pre-positioned" for transcription when the repressor leaves.

Understand the basis for the constitutive expression phenotype resulting from the lacI$^{-}$ and lacO$^c$ mutations. Also understand the basis of the Lac$^-$ phenotype resulting from the lacI$^S$ mutation. (Don't worry about the lacI$^{-}d$ mutations.)

In wild-type cells, a low background level of transcription leads to production of about 50 molecules of $\beta$-Galactosidase (and Lac Permease) per cell. This level may increase 100X after induction.

lacZ, lacY, lacA are contiguous and transcribed in the given order from the same promoter ($lacP$) to create a polycistronic mRNA.

4 classes of mutations will abolish induction of Lac mRNA transcription by Lactose: $P_{Lac}^{-}$, lacY$,^{-}$, lac Z$^{-}$ and lacI$^S$.

The artificial chemical inducer IPTG (isopropyl-thiogalactoside) will induce Lac mRNA transcription in lacY$^{-}$ and lac Z$^{-}$ mutants because it is membrane-permeable, and because it binds to repressor directly without modification by $\beta$-Galactosidase. (IPTG is not a substrate for $\beta$-galactosidase).

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>B-Galactosidase Activity</th>
<th>Lac Permease Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Lactose No Inducer</td>
<td>With Lactose With IPTG</td>
</tr>
<tr>
<td>I+ P+ O+ Z+ Y+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>I+ P+ O+ Z+ Y-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In Table 19.1 and 19.2 do not worry about the specific activity unit numbers. Just take 100-200 units as "+" (induced, high level expression) and 1-2 units as "-" (uninduced, low level expression).

CATABOLITE REPRESSION

This term is a misleading misnomer. The mechanism does not involve a "catabolite" and it does not involve repression.
The level of Lac gene expression is influenced by availability of Glucose as well as Lactose. Lac expression levels vary 1,000X due to regulation by repressor and by CAP.

<table>
<thead>
<tr>
<th>Lactose</th>
<th>Glucose</th>
<th>+</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>+</td>
<td>LOW</td>
<td>INTERMEDIATE</td>
<td>HIGH</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>INTERMEDIATE</td>
<td>HIGH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This leads to *diauxic* pattern of growth in laboratory media containing both sugars.

The Lac promoter is intrinsically "weak" because of a poor match to consensus sequence of the sigma factor. This is important for positive regulation by CAP.

The mechanism by which glucose regulates adenyl cyclase activity in this case is not yet known. It is interesting to note that cAMP is found as a "second messenger" is many gene expression regulation systems in eukaryotic cells.
Focus on Protein-DNA Interactions That Control Transcription of the Lac Operon  p. 574

**CAP**

CAP is a homodimer with 2-fold (dyad) symmetry

CAP/cAMP binds to an inverted repeat located between the carboxy-terminal end of *lacI* and the -35 region of *P_Lac*.

DNA bends 90° while bound to CAP/cAMP.

Increased efficiency of transcriptional initiation at *P_Lac* may be due to both altered base pair accessibility due to bending, and to direct contacts between CAP/cAMP and RNA Pol.

**Lac Repressor**

The Lac Repressor is a homotetramer.

Each dimer has dyad symmetry, so the protein binds 2 copies of the inverted repeat recognition sequence (the "operator") independently.

The DNA between the two bound operators (O1 and O2 or O1 and O3) forms a loop.

When the repressor is bound to O1 and O2, the -10 and -35 regions of *P_Lac* are in the loop. These regions are shown in green in Figure 1 of the Focus Box, but they are not specifically labeled as such.
Focus on Problem Solving: Testing Your Understanding of the Lac Operon  p. 577

Again, do not worry about the unit activity numbers. Just use + for anything over 50 units.

However, you should assume that the “inducer” is IPTG!

A Milestone in Genetics: Jacob, Monod, and the Operon Model  p. 586

The classic genetic analysis by Jacob and Monod:

<table>
<thead>
<tr>
<th></th>
<th>in CIS</th>
<th>in TRANS</th>
</tr>
</thead>
<tbody>
<tr>
<td>o^c and z^+</td>
<td>i^+ o^c z^+ y^+</td>
<td>i^+ o^c z^- y^+</td>
</tr>
<tr>
<td></td>
<td>i^+ o^+ z^- y^+</td>
<td>i^+ o^+ z^+ y^+</td>
</tr>
<tr>
<td></td>
<td>= Lac^+ constitutive</td>
<td>= Lac^+ regulated</td>
</tr>
<tr>
<td>i^c and z^+</td>
<td>i^c o^+ z^+ y^+</td>
<td>i^c o^+ z^- y^+</td>
</tr>
<tr>
<td></td>
<td>i^+ o^+ z^- y^+</td>
<td>i^+ o^+ z^+ y^+</td>
</tr>
<tr>
<td></td>
<td>= Lac^+ regulated</td>
<td>= Lac^+ regulated</td>
</tr>
</tbody>
</table>
Justifications for Studying the Lac Operon

Lac gene regulation is a classic case because the Operon Model was the first example of the molecular basis for environment-genome interaction.

Substrate recognition and catalysis by E. coli β-galactosidase does not depend on recognition of the glucose moiety of lactose. In fact, β-galactosidase from E. coli will catalyze hydrolysis of a plethora of natural and artificial β-D-galactopyranosides. This is one of several pieces of circumstantial evidence that fuels speculation that this enzyme did not evolve to metabolize lactose as its natural substrate originally, and that it may have evolved in a lineage other than the ancestors of contemporary E. coli (see below). Lactose, after all, is a very rare sugar in nature, since the only place it is synthesized in appreciable quantity is the mammary gland.

Alternate substrates for β-Galactosidase include the great variety of secondary metabolites that are synthesized by plants as β-D-galactoside derivatives. There is circumstantial evidence that glycosidases in general (and β-D-galactosidases in particular) made by fecal bacteria (including E. coli) contribute to colon and rectal cancer. For more on this provocative notion see Tamura, G. et al (1980) Proc. Natl. Acad. Sci. USA 77: 4961. This and other studies implicate glycosidase activity of intestinal microfloras as a mechanism for metabolic activation of natural dietary pre-carcinogens.

Portions of the Lac operon, specifically the “alpha complementing fragment” of β-Galactosidase, are used as reporter genes in a wide array of cloning strategies. The most ubiquitous application is “blue/white” screening with the chromogenic β-Galactosidase substrate called X-Gal.

The Lac operon has several features that suggest it has recently invaded the E. coli genome by horizontal gene transfer

- The Transacetylase is non-essential in E. coli.
- The Lac operon is not found in all Enterobacteriaceae.
- The broad substrate specificity of β-galactosidase suggests that the original substrate was not lactose.