Regulation of Gene Action

The basis of cell differentiation is gene regulation: different sets of genes are turned on and off in different cells. (There are other mechanisms as well but this is our focus.)

E.g. globin genes are expressed only in erythroblasts and are turned off in muscle cells. Myosin genes are on in muscle cells but off in erythrocytes.

Progression through the cell cycle also requires turning different sets of genes on and off at different stages.

Bacteria and single-celled eukaryotes undergo cell differentiation. This includes responding to the availability of different nutrients.

I will discuss some of the most basic aspects; Dr. Restifo will give more detail.
The expression of any gene begins with transcription which can be regulated. The expression of protein-coding genes requires several additional steps and can be turned off at any step. However, I will focus only on the control of transcription.
Transcription Control in Prokaryotes

Negative Regulation

Negative regulation involves a protein repressor that binds to a repressor binding site and prevents binding of the transcription complex.

Inducible system: **off** unless inducer molecule binds to and inactivates repressor.

Repressible system: **on** unless co-repressor binds to inactive aporepressor to form active repressor.
Negative Regulation Examples

Inducible system: lactose operon in *E. coli*

*E. coli* can cleave lactose into glucose + galactose to use for carbon and energy sources. This requires the enzyme β-galactosidase, and also galactoside permease to import the lactose into the cell.

If there is no lactose in the medium, *E. coli* does not make either β-galactosidase or galactoside permease. Synthesis is blocked at the transcription level. If lactose is added to the medium, the synthesis of both molecules is *induced*. 
lac Operon

Operon encodes
• *lacZ*: β-galactosidase which cleaves lactose into glucose and galactose.
• *lacY*: lactose permease which brings lactose into the cell
• *lacA*: thiogalactoside transacetylase, not required for growth on lactose, unknown function but sequence is conserved so it is important.

Other important players:
• *lacP*: Promoter, binds RNA polymerase to start transcription
• *lacO*: Operator, binds repressor
• *lacI*: Repressor gene, encodes repressor protein

β-galactosidase and lactose permease are made only if lactose is present; only if they are needed. They are induced by their substrate.
Simple Model of *lac* System

(A) Repressor gene
(B) mRNA
(C) Transcription allowed

- Operator
- Structural genes

*Direction of transcription*

Repressor prevents transcription

Inducer-repressor complex

Transcription allowed

- *β*-galactosidase
- Permease
- Transacetylase

Repressor protein

Inducer

RNA polymerase
lac Operon Mutants

Basic features of lac operon were deduced from mutant phenotypes by Francois Jacob, Jacques Monod, and collaborators in 1960s by studying the phenotypes of mutants.
# lac Operon Mutants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>lacZ</em>&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Null mutation in <em>lacZ</em></td>
</tr>
<tr>
<td><em>lacY</em>&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Null mutation in <em>lacY</em></td>
</tr>
<tr>
<td><em>lacO</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td><em>lacO</em> can’t bind repressor; constitutive (always on)</td>
</tr>
<tr>
<td><em>lacP</em>&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Promoter can’t bind RNA polymerase; operon not transcribed</td>
</tr>
<tr>
<td><em>lacI</em>&lt;sup&gt;s&lt;/sup&gt;</td>
<td>Repressor can’t bind inducer; super-repressor</td>
</tr>
</tbody>
</table>
Studying Interactions of *lac* Operon Mutants

Studied the interactions of different mutant alleles in partial diploids which have the bacterial chromosome plus a plasmid with some genes. Plasmids = small DNA molecules that use their own replication origins to replicate independently of the cell chromosome; have the own origin of replication. Usually not required for cell function; some may be present in many copies.

![Diagram of F' lac plasmid and Chromosome with lac operon, lacZ^− lacY^+ and lac operon, lacZ^+ lacY^−](DNA molecules not to scale)

**F' lac plasmid**
- *lac operon*
- *lacZ^− lacY^+*

**Chromosome**
- *lac operon*
- *lacZ^+ lacY^−*

**Cell genotype**  
F’ *lacZ^− lacY^+ / lacZ^+ lacY^−*

**Cell phenotype** Lac^+  
*(NOTE: other genes assumed to be wild type if not specified.)*
Jacob, Monod, and collaborators deduced how the lac operon is controlled from these data and from the map position of the mutants. Note lacO and lacP mutants only affect expression of lac genes on the same chromosome, while lacI mutants can operate at a distance, from another chromosome.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Synthesis of lac mRNA</th>
<th>Lac phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. F' lacO&lt;sup&gt;c&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacO&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Constitutive</td>
<td>+</td>
</tr>
<tr>
<td>2. F' lacO&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacO&lt;sup&gt;c&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Constitutive</td>
<td>+</td>
</tr>
<tr>
<td>3. F' lacI&lt;sup&gt;-&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacI&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Inducible</td>
<td>+</td>
</tr>
<tr>
<td>4. F' lacI&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacI&lt;sup&gt;-&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Inducible</td>
<td>+</td>
</tr>
<tr>
<td>5. F' lacO&lt;sup&gt;c&lt;/sup&gt; lacZ&lt;sup&gt;-&lt;/sup&gt;/lacO&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Inducible</td>
<td>+</td>
</tr>
<tr>
<td>6. F' lacO&lt;sup&gt;c&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacO&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Constitutive</td>
<td>+</td>
</tr>
<tr>
<td>7. F' lacI&lt;sup&gt;s&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacI&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Uninducible</td>
<td>-</td>
</tr>
<tr>
<td>8. F' lacI&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacI&lt;sup&gt;s&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Uninducible</td>
<td>-</td>
</tr>
<tr>
<td>9. F' lacP&lt;sup&gt;-&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacP&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Inducible</td>
<td>+</td>
</tr>
<tr>
<td>10. F' lacP&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacP&lt;sup&gt;-&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Inducible</td>
<td>+</td>
</tr>
<tr>
<td>11. F' lacP&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;-&lt;/sup&gt;/lacP&lt;sup&gt;-&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Uninducible</td>
<td>-</td>
</tr>
<tr>
<td>12. F' lacP&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;+&lt;/sup&gt;/lacP&lt;sup&gt;-&lt;/sup&gt; lacZ&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Inducible</td>
<td>+</td>
</tr>
</tbody>
</table>
lac Operon: Second Level of Regulation
(Things are always more complicated than we’d like.)

When glucose is present, β-galactosidase etc. are not made.

$cAMP = \text{cyclic AMP}$

CRP = cAMP receptor protein

Glucose inhibits synthesis of $cAMP$.

$cAMP + CRP$ must be bound to promoter in order for $lac$ transcription

Synthesis of $lac$ mRNA?

NO

[Diagram showing the regulation process]

YES

[Diagram showing transcription]

NO
lac Operon Structural Details

Protected by RNA polymerase

-10

Protected by repressor

Symmetrical operator halves

Ribosome binding site

Beginning of lacZ coding sequence

Met  Thr

TGCTTCGGCCTCGTATGTTGTTGAGCAGGATTACAATTGTACAGAACACCATTGACC
ACGAAGCCGACGATAACAACACACCTTAAACACTCGCCATTGTTAAAGTCTTTGTCGATACCTG

mRNA

+1

lacO

lacZ
Inducible lac operon: operon turned on only when lactose is available as a carbon and nitrogen source.

**Repressible system: tryptophan operon in E. coli**

Tryptophan is needed all the time by growing cells, so *trp* genes should be on all the time, until cells have made sufficient tryptophan (or it is provided in the medium).
Negative Regulation Examples

Repressible system: tryptophan operon in *E. coli*

Tryptophan is needed all the time by growing cells, so *trp* genes should be on all the time, until cells have made sufficient tryptophan.

*trpA-E* genes are in an operon. OK if only needed to make tryptophan. Has operator *trp o* and promoter *trp-p* and attenuator *trp a.*
Negative Regulation of *trp* Operon

Tryptophan levels low: aporepressor protein complex (encoded by distant genes) can’t bind to promoter and transcription is on.

Tryptophan levels high: tryptophan binds to aporepressor to form active repressor which binds to promoter and shuts off transcription.

Attenuation mechanism stops transcription in leader region unless there is sufficient Trp-tRNA in the cell, provides fine-tuning of tryptophan synthesis.