Gene expression responds to environmental conditions

- Some regulatory proteins present at 5-10 copies per cell, some enzymes for glycolysis present at 100,000 copies/cell
- Genes whose products are presently unneeded or at acceptable levels are turned off
- Mechanisms to achieve proper mix of gene expression are varied
Lactose metabolism in E. coli

- Jacob and Monod (1946) studied as model system, many others followed...
- Enzyme \( \beta \)-galactosidase only expressed when lactose present in the medium
  - Enzyme said to be expressed in inducible fashion with lactose as inducer
- Identified cis-acting elements (operator, promoter) and trans-acting factors

Lactose Hydrolysis

- Enzyme encoded by lacZ gene
- Glucose and galactose products
- Enzyme cleaves broad range of \( \beta \)-galactosides
  - Including synthetic analogs such as X-gal

Bacterial Genes and Operons

- Related genes are often clustered and expressed as a unit on a single mRNA
  - Operon
  - Polycistronic mRNA
E. coli lac operon

- lacI has a constitutive promoter and is expressed separately from the lac operon
  - Encodes lac repressor
  - Low level of expression

lac operon expression

- lac operon encodes polycistronic mRNA giving rise to 3 different enzymes

Structural Genes of lac Operon

- Structural genes encode the primary structure of the enzymes/proteins
  - For lac operon these are lacZ, lacY and lacA
  - Enzymes encoded are β-galactosidase, lactose permease and transacetylase, respectively
  - Genes are also coordinately regulated
The lac operon includes cis-acting regulator elements and protein-coding structural genes

<table>
<thead>
<tr>
<th>P</th>
<th>P_O</th>
<th>lacI</th>
<th>lacZ</th>
<th>lacY</th>
<th>lacA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>1040</td>
<td>82</td>
<td>3510</td>
<td>780</td>
<td>825</td>
</tr>
<tr>
<td>mRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>Repressor</td>
<td>β-galactosidase</td>
<td>Permease</td>
<td>Trans-acetylase</td>
<td></td>
</tr>
</tbody>
</table>

A regulator binds a target site on DNA

Regulator protein

mRNA

Regulator gene

Target site

Structural gene

Lac Operon Expression

(a) Components

Repressor gene (I)
Promoter (P)
Operator gene (O)
Leader (L)
Structural genes

Operator-binding site
Repressor protein
Lactose-binding site
lac Operon Expression

• In absence of inducer, repressor binds to operator and blocks RNAP from binding to promoter.

lac Operon Expression

• Binding of inducer to repressor causes conformational change in protein, preventing interaction with operator.
• RNA polymerase binds to promoter and expresses operon.

The promoter and operator overlap

Startpoint

Unwinding

Promoter binds RNA polymerase

Operator binds repressor
Gratuitous Inducers

- Lactose is normal inducer (actually allolactose for the pure at heart)
  - But other β-galactosides also work
- Isopropylthiogalactoside (IPTG) also acts as an inducer but is not metabolized
  - Shows induction does not involve interaction with the actual enzyme being synthesized

Isopropylthiogalactoside (IPTG)

- Gratuitous inducer
- Not metabolised
  - Level remains constant
The lac promoter is quite weak...

- **Problem:** That’s OK if some glucose is around but:
  - if lactose is available (and glucose isn’t available) cells could get very hungry!
- **Solution:** when cells are hungry:
  - make the lac promoter work more effectively
- **Mechanism:** CAP (catabolite activator protein) – also called CRP (cAMP receptor protein)

---

**Cyclic AMP has 5’-P-3’ bonds**

---

**cAMP is an inducer that activates CRP**

("CAP" and "CRP" are the same)
Not enough glucose…

CAP binds cAMP then binds the promoter

With glucose…

CAP needs cAMP to enable it to bind promoter (conformation is incorrect for binding)

CAP binds to a consensus sequence

Transcription

AANTGTGANNTNNNTCAANTTTNNTNNACACANTNNNAGTNTAANN

Highly conserved pentamer Less conserved pentamer
Xgal is incredibly useful!