**The lac operon regulatory network**

**Gene expression**: A process that takes gene info & synthesizes (=creates) a functional gene product (e.g., a protein).

Some genes code for proteins

Others (e.g., rRNA, tRNA) code for functional RNA

**Summary**: Gene expression is a 2-step process

(i) **transcription** of genes (messenger RNA synthesis)

(ii) **translation** of genes (protein synthesis).

**Transcription**: DNA is copied into mRNA (inside cell nucleus)

```
GAC TGAGCA ACTC TTC
CTG ACTC CTCGAGAAG
```

Helicase enzyme binds to and "unzips" DNA to read it:

Output: A complementary strand of mRNA: \text{...CUGACUCCUGAGAAG...}

Note: DNA consists of base pairs A, C, G, T. RNA consists of A, C, G, U.
Segments of RNA strand not needed for protein coding are removed.

RNA then leaves the nucleus.

**Translation**: RNA read by ribosomes.

Each triple of RNA bases code for an amino acid.

A protein is a chain of amino acids.

```
GUG CAU CUG AC U CCU GAG AAG
```

```
V H L T P E K
```

The protein then folds into a 3D shape.

The expression level is the rate at which the gene is being expressed.

Housekeeping genes are continuously expressed, as they are essential for basic life processes.

Regulated genes are expressed only under certain outside factors (environmental, physiological). Expression is controlled by the cell.
Easiest to control gene regulation by affecting transcription.
Certain repressor proteins bind to sites on DNA or RNA.

Goal: Understand this complex cell behavior.

Mathematical models need to reflect dependencies between a system components.

* Types of models
  - Deterministic vs. stochastic
  - Dynamic vs. static
    - time-continuous vs. time-discrete

Continuous models (e.g., DE's) need knowledge of interactions, rates, concentrations (constancy). These models are quantitative.

Discrete-time, discrete-space models are called algebraic models. They are qualitative.

Boolean models (state space = \{0, 1\}) were introduced in 1969 by S. Kauffman for gene regulatory network models.
A classic example: the lactose (lac) operon - controls transport & metabolism of lactose in E. coli.

One of the most widely studied mechanisms of gene regulation used as a "test system" for models of gene regulation.

E. coli: Bacterium in the intestines of mammals & birds, (affected by animal's diet!)

DNA replication & gene expression all studied in E. coli before they were studied in Eukaryotic cells.

Physiology well-understood, genome has been sequenced.

Host consumes milk $\Rightarrow$ E. coli exposed to lactose (milk sugar)

Lactose consists of one glucose sugar linked to one galactose sugar.

Glucose can be used as an energy source

So can galactose, but an enzyme is needed.

* To transport sugars in/out of cell, sugar transport proteins are required.
• Once imported, specific enzymes act on the sugar. Either:
  - Use it to make a cellular molecule (anabolism)
  - Break it down to harvest energy

Glucose is the preferred energy source for all cells.

Interactions between sugars, transport proteins, enzymes are specialized.

Ex: E. coli: won’t make lactose transport protein if no lactose present
  won’t make enzyme to break lactose \( \xrightarrow{\text{glucose}} \) galactose

Cells only make most proteins when needed: Inducible proteins.

Inducible genes are regulated genes.

Ex: lac operon:
  • lac permease (transporter protein)
  • \( \beta \)-galactosidase (enzyme) to be utilized

These are produced by the lac operon:

\[
\text{promoter} \quad \text{operator} \quad (\text{DNA strand})
\]

\[
\text{lac} \quad P \quad O \quad \text{lac} \quad \text{lac} \quad \text{lac} \quad \text{lac}
\]

\[
\text{lac repressor gene} \quad \beta\text{-galactosidase gene} \quad \text{lactose permease gene} \quad \text{transacetylase gene (not involved)}
\]
All three of these genes (lac I, lac Z, lac A) are encoded by a single mRNA strand.

**No lactose present**

Lac I gene codes for the lac repressor protein which binds to the operator (controlling region) and prevents mRNA transcription. This "turns off" the operon.

**Lactose present**

Lactose is brought into cell by lac permease protein.

β-galactosidase converts lactose into allolactose.

Allolactose binds to lac repressor, so it can't bind to operator.

Transcription continues! mRNA encoding lac genes is produced.

Summary:

- Lactose is brought into cell by lac permease protein.
- β-galactosidase converts lactose into allolactose.
- Allolactose binds to lac repressor, so it can't bind to operator.
- Transcription continues! mRNA encoding lac genes is produced.
• lac proteins are produced, more lactose is brought into cell.
  (operon is on)

• Eventually, all lactose is used up, so there will be no more allolactose.

• The lac repressor binds to operator, mRNA transcription stops.
  (the operon has turned itself off)

Case 3: Glucose and lactose are both available:

  Glucose (preferred energy source) is used up first.

  This is controlled by mechanism of catabolite repression.

  Without it, the steps outlined in the "lactose present" case
  would take place (lac repressor protein doesn't bind, transcription occurs)

• Catabolite repression represses this process.

• Needs DNA binding protein, "catabolite activator protein" (CAP).

  Product of cAMP receptor protein (crp) gene

• cAMP produced by adenylate cyclase (enzyme).

  \[ \text{if and only if no glucose present!} \]
So if no glucose is present:

\[
\begin{align*}
\text{CAMP} & \rightarrow \text{CAP-CAMP complex} \\
\text{CAP} & \rightarrow \text{lac RNA can no be made}
\end{align*}
\]

If glucose is present, E. coli uses it. Keeps CAMP levels low.

When glucose is used up, CAMP levels rise.

The CAP-CAMP facilitates attachment of RNA polymerase.

Network interactions & motifs (Summary)

- Lac \( Y \) expression creates lac permease, transports lactose into cell.

- Lac \( Z \) expression creates \( \beta \)-galactosidase from \( \lambda \)
  
  Lac \( Z \) polypeptides. \( \beta \)-gal converts:
  
  \( + \) lactose into allolactose (Allo)
  
  \( + \) lactose into glucose & galactose (Gal)

- \( \beta \)-gal also converts
  
  \( + \) allolactose into glucose & galactose
• Aldolase binds to repressor protein (R), inhibiting it.

• When not bound by Aldolase, R binds to lac operon genes, preventing transcription.

• External glucose inhibits production of cAMP.

• When cAMP binds to CRP protein (CAP), it forms CAP-cAMP complex, activating the lac operon.

• External glucose inhibits uptake by permease proteins.
We can put these motifs together for a "cartoon" of the lac operon regulatory mechanism.

Next: How to design a mathematical model that can reflect its qualitative behavior.