Bioeng 261 : Lecture 10 (Gene regulatory networks)

- Gene expression/regulation:
  - → lac operon example
  - → 'simple' gene regulatory network
  - → can use basic reaction models
- (Much) larger systems:
  - → Large gene regulatory networks (GRNs)

↳ basic ideas, terminology, methods

(Tomorrow: data analysis for GRNs etc.)

The lac operon

- Classic example of prokaryotic gene regulation
- perhaps the first well-understood genetic regulatory network (GRN)

Jacob & Monod studied/discovered (~1960)
↳ used E. coli as model system
↳ 1965 Nobel (with Lwoff)*

*For discovery of genetic control of enzyme & virus synthesis

- Math. models developed soon after (~1965)

The problem: Lactose metabolism in E. coli:

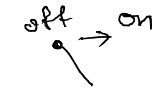
- When glucose is abundant, E. coli uses it exclusively as its food source
- When glucose is not available, E. coli can use other sugars such as lactose (lac...)

→ to switch food sources requires different enzymes for metabolism of lactose

→ Jacob & Monod realised this could be brought about through changes in gene expression!

changes in repression in particular

Genetic 'switches'



Here:

'off' state: normal glucose metabolism via expression of genes coding for standard enzymes

'on' state: switch to lactose metabolism by upregulating expression of genes for enzymes required for lactose metabolism

→ these enzymes ↑ ×1000

General idea has stood test of time! Widespread & important!

Operons

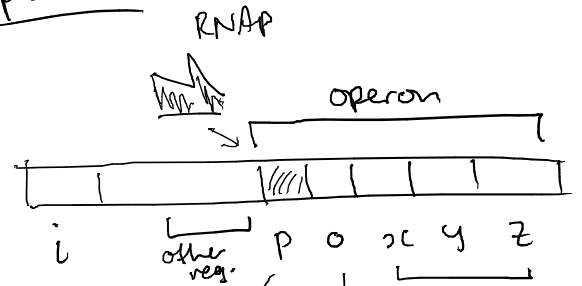
- Typical in prokaryotes for multiple genes to be grouped together

- A single promote controls the expression of the whole group } use single mRNA

- DNA sequence comprising:

operon
- promoter
- operator { where a repressor (inhibitor) binds
- cluster of related genes all controlled by same promoter (& op.) { structural genes

Operons



inhibitor / repressor gene

operator

regulation types
- negative ⊢ - -
- positive ← - -

inhibits by binding operator

→ blocks RNAP from transcribing.

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lac operon: Minimal model

- Normal conditions
  - transcription of the lac operon genes is off
    - 'lac repressor protein' { repressor is bound to operator

'negative inducible' {
- Low glucose, high lactose
  - allolactose binds to & deactivates the repressor
    - lactose 'induces' expression
      - allows further lactose uptake (+ve feedback)

Negative (repressor-based) regulation

original theory

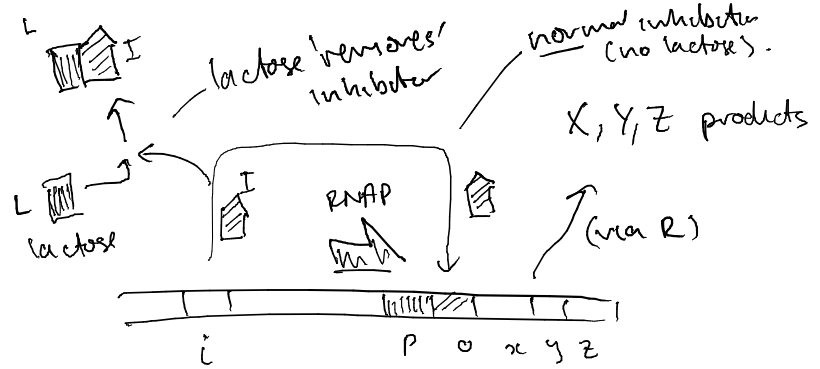
- negative inducible (eg lac)
  - usually off due to repressor; inducer inactives repressor & hence leads to activation of operon transcription
- negative repressible (eg trp)
  - usually on; repressor is present but unable to bind; corepressors can 'activate' repressor & allow repressor to bind & inactivate operon

but now know can also have { positive inducible & positive repressible }

→ but repression-based does have some advantages.

lac operon model(s)

Simple version



Overall reaction scheme

gene i → mRNA R_I → product I } production of inhibitor

$i \rightarrow i + R_I$

$R_I \rightarrow R_I + I$

competitive inhib.

$I + \text{Lactose} \rightleftharpoons I:\text{Lactose}$

$I + \text{operon} \rightleftharpoons I:\text{operon}$

transcription of structural genes

$\text{Operon} + \text{RNAP} \rightleftharpoons \text{operon}:\text{RNAP}$

$\text{RNAP}:\text{operon} \rightarrow \text{Operon} + \text{RNAP} + R$

$R \rightarrow R + X + Y + Z$

Z is enzyme for lactose metab.

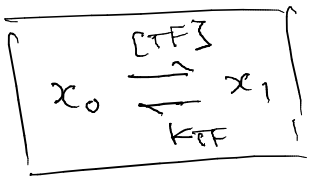
$\text{Lactose} + Z \rightarrow Z$ ('uses up'/removes)

Simple lac operon model: more details

Genetic states:

$$x_0 = \frac{K_{TF}}{K_{TF} + [TF]}$$

$$x_1 = \frac{[TF]}{K_{TF} + [TF]}$$



$$\nu = \nu_0 \frac{K_{TF}}{K_{TF} + [TF]} + \nu_1 \frac{[TF]}{K_{TF} + [TF]}$$

(ν = $\nu_{transcription}$)

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(1) TF is repressor: $\nu_1 = 0$

$$\Rightarrow \nu = \frac{\nu_0 K}{K_{TF} + [TF]}$$

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(2) Lactose L inactivates repressor TF

1. $L + TF \underset{k_-}{\overset{k_+}{\rightleftharpoons}} TF_L$ } eliminate TF_L
2. $[TF] + [TF_L] = TF_{tot}$ }

(using quasi-equil.)

$$\Rightarrow [TF] = \frac{TF_{tot}}{1 + [L]/K_L}, \quad K_L = \frac{k_-}{k_+}$$

Simple ODE model: Balance eq^n's

(mRNA) $\frac{dR}{dt} = \nu_{transcription} - \nu_{R\,deg}$ ($\nu_{R\,deg} = k_R \cdot R$)

where

$$\nu_{transcription} = \nu_0 \cdot \frac{K_{TF}}{K_{TF} + [TF]} \quad \& \quad [TF] = \frac{TF_{tot}}{1 + [L]/K_I}$$

note: $L \uparrow$, $TF \downarrow$, $\nu_{transcription} \uparrow$

+

(protein) $\frac{dP}{dt} = \nu_{translation} - \nu_{degradation}$

$\nu_{translation} = k_T R$, $\nu_{degradation} = k_P P$

very simplistic

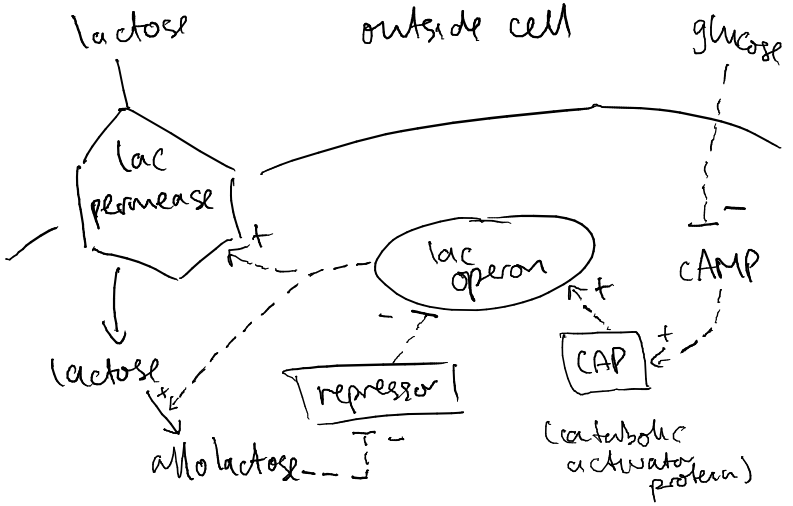
+ lactose equation $[L]$?

(see eg 2016 slides) .... & ...

More complex : [L] feedback & glucose pref.

- Glucose is preferred to lactose
  - ⇒ lactose uptake suppressed if glucose present
  - ⇒ There is an additional enhancer, cAMP/CAP
    - [without it lac is only weakly expressed] ie only weakly use lactose
  - ⇒ The concentration of cAMP is inversely prop. to glucose. [ie glucose suppresses lac]
- Also, expression of lac operon results in increased uptake of lactose: [positive feedback]

⇒ Regulation network [⊣ negative, ← positive]



We want to deal with much larger systems: [Gene regulatory networks (GRNs)]

- Even this relatively simple example can become quite complex
- What about systems with 100s...1000s...(?) of interacting parts?

⇒ just like before, we usually must accept trade-offs in order to scale up

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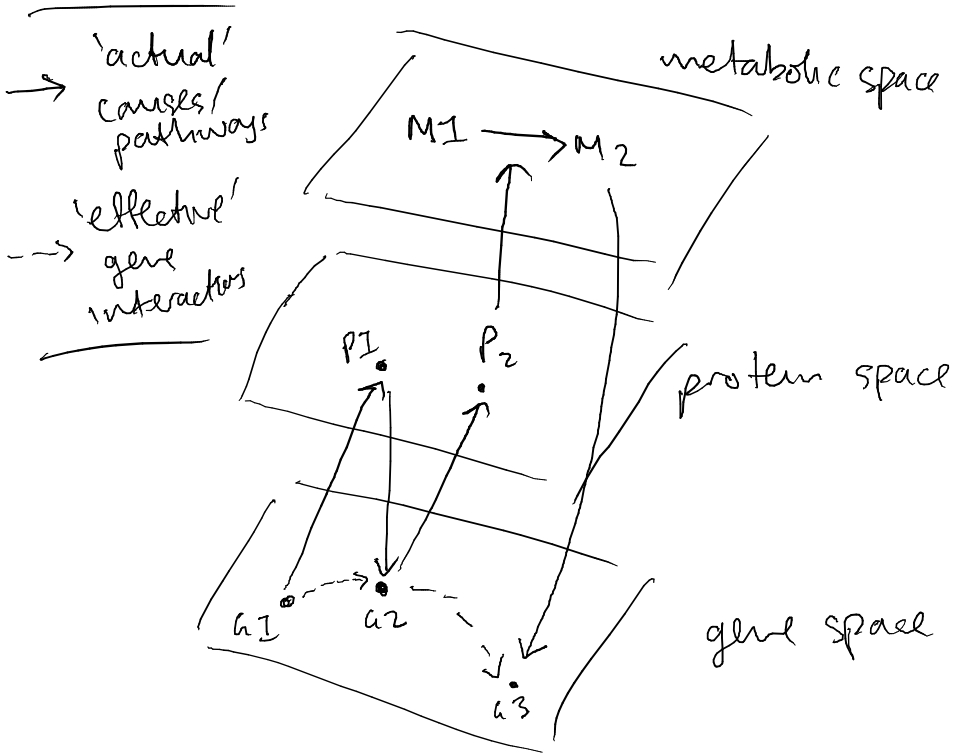
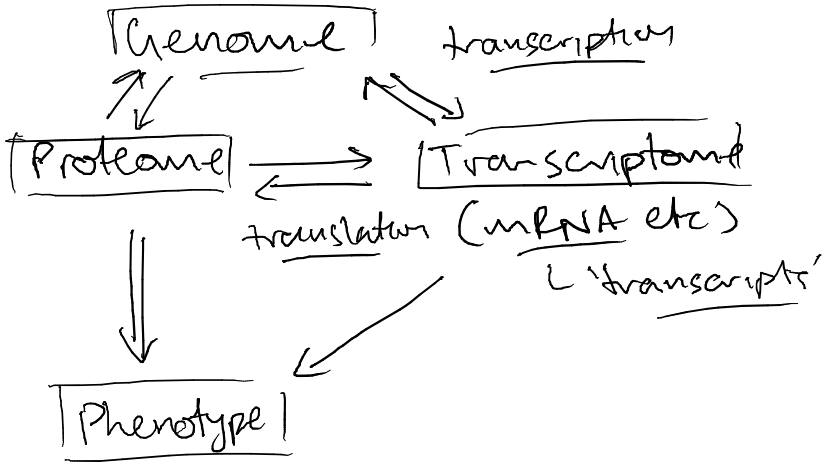
Gene space

- A way of 'projecting all the action' into interactions between [genes]



Gene Regulatory Networks

From:



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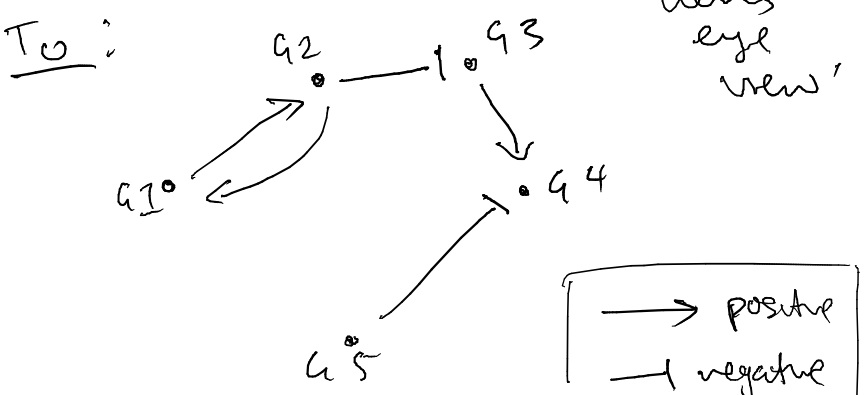

Gene regulatory Network (GRN)

Transcriptomics

- Subfield of 'functional genomics'
  - the study of how genes & intergenic regions contribute to biol. function
- Transcriptomics focuses on gene expression levels, in particular on the levels of the transcripts (mRNA) associated with genes.

mRNA easier to measure etc than proteins, but see 'proteomics'!

→ idea: (under treatment)
- does expression go up or down?
- do groups of genes go up/down together?

Expression Analyses

Two key approaches

- Microarrays
  - uses 'probes' (eg cDNA)
  - samples 'hybridise' if complementary to probes
  - amplify & quantify via qPCR
- RNA-seq
  - direct sequencing of transcripts.
  - 'next gen' high-throughput sequencing

We will discuss Microarrays

→ well understood
→ more mature & easier to analyse
→ still used & useful...

...but RNA-seq rapidly overtaking!