

BIOMENG 261

TISSUE AND BIOMOLECULAR ENGINEERING

Module I: Reaction kinetics and systems biology

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LECTURE 11: GENE REGULATION CONTINUED

- Example of modelling gene expression/regulation using reaction modelling
 - The lac operon
- Moving to larger systems of gene regulatory networks (GRNs)
 - Gene space
 - Intro to transcriptomics

Next time: data analysis for ‘transcriptomics’

MODULE OVERVIEW

Reaction kinetics and systems biology (*Oliver Maclaren*)
[12 lectures/4 tutorials/2 labs]

1. *Basic principles: modelling with reaction kinetics* [6 lectures]
Physical principles: conservation, directional and constitutive. Reaction modelling. Mass action. Enzyme kinetics. Enzyme regulation. Mathematical/graphical tools for analysis and fitting.

2. *Systems biology I: overview, signalling and metabolic systems*
[3 lectures]

Overview of systems biology. Modelling signalling systems using reaction kinetics. Introduction to parameter estimation. Modelling metabolic systems using reaction kinetics. Flux balance analysis and constraint-based methods.

3. *Systems biology II: genetic systems* [3 lectures]
Modelling genes and gene regulation using reaction kinetics. Gene regulatory networks, transcriptomics and analysis of microarray data.

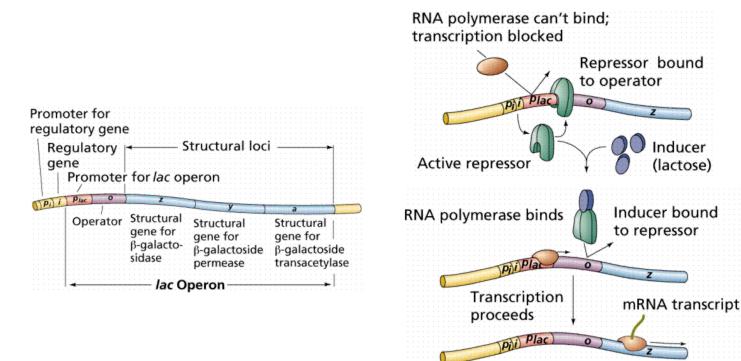
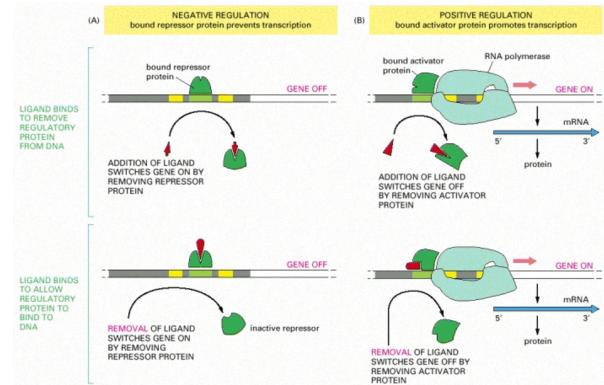
SETTING: LACTOSE METABOLISM IN E. COLI

- E. coli ‘prefers’ glucose but is capable of metabolising lactose when glucose is not available
- Jacob and Monod (1961) explained this in terms of *changes in gene expression*
 - Proposed a general theory of (prokaryotic) regulation of gene expression
 - Idea: genes are controlled in functional groups via single feedback mechanism: *control of repression*

Not quite true (not just repression in general), but key ideas remain.

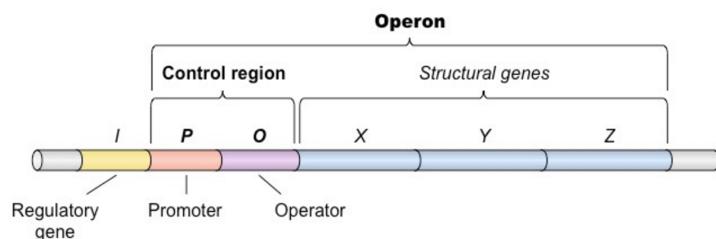
GENETIC SWITCHES AND REGULATION

THE LAC OPERON

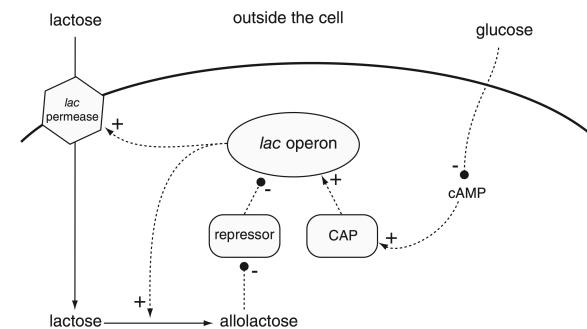


(Alberts et al. Molecular Biology of the Cell. 4th edition)

OPERONS



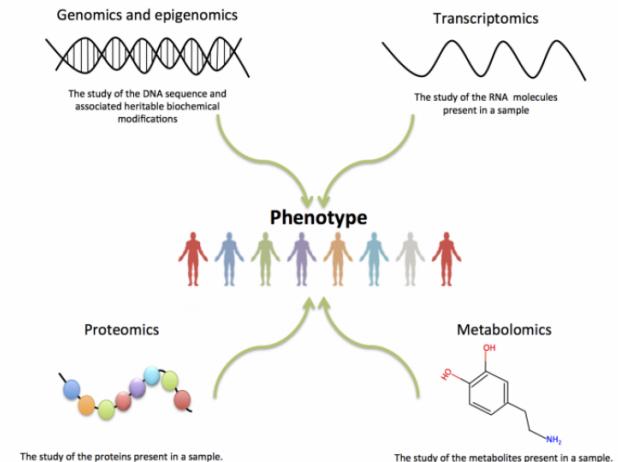
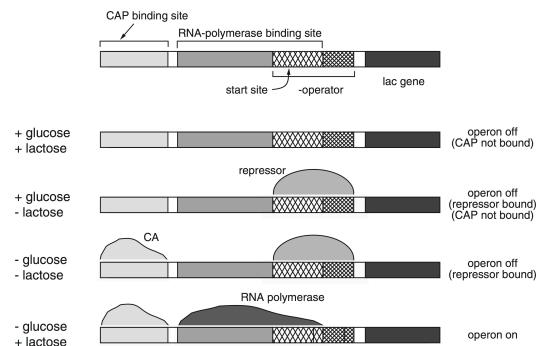
LAC OPERON REGULATORY NETWORK



(Keener and Sneyd 2008)

MUCH LARGER SYSTEMS - 'OMICS'

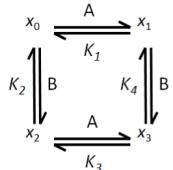
LAC OPERON REGULATION SUMMARY



HUH? EXAMPLE QUESTION (2016)

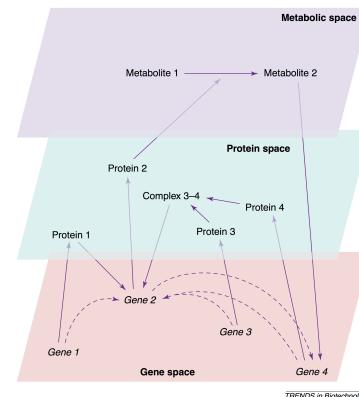
Question 3

- (a) Consider a gene regulated by two transcription factors, A and B. The schematic representation of the four state model is:



- (i) What do K_2 and K_3 represent in the sketch above?
(2 marks)
- (ii) Suppose the above scheme is used to model the regulation of the lac operon in *E. coli* where A represents the enhancer (CRP-cAMP) and B represents the repressor. If *E. coli* is grown in a medium high in both glucose and lactose, how are the concentrations of A and B affected? Why?
(4 marks)

GENE SPACE



See: Brazhnik et al. (2002) 'Gene networks - how to put the function in genomics' (on Canvas)

MICROARRAYS

TRANSCRIPTOMICS

- A subfield of *functional genomics*
 - Functional genomics: study of how genes and intergenic regions contribute to biological function
- The focus is on *gene expression*
 - In particular, via *measuring mRNA* (the transcripts)

See: Lowe et al. (2017) 'Transcriptomics technologies' (on Canvas)



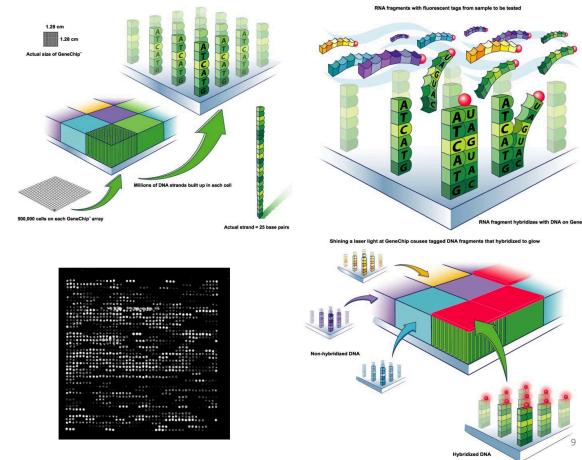
For video intros: see e.g. <https://youtu.be/0ATUjAxNf6U> or <https://youtu.be/VNsThMNjKhM>

EXPRESSION ANALYSIS

- *Microarrays*
 - Mature technology
 - Relatively well-established data analysis methods
- *RNA-seq*
 - Newer technology, rapidly overtaking microarrays
 - Less standardisation of analysis methods
 - Much more computationally/storage intensive

But: *microarrays still relevant and useful*: we will consider these
(easier and better understood)

MICROARRAYS



Biomeng 261: Lecture 11 : control of gene expression cont'd

Gene regulatory systems &

gene 'regulatory networks' ('GRNs')

- lac operon (often refers to 'gene's-eye view')

↳ 'simple' gene regulatory system/network

↳ can understand via basic reaction modelling

- Much larger systems

↳ Large GRNs

↳ Overview of basic ideas, terminology etc

→ (Tomorrow: intro to data analysis for GRNs)

The lac operon

- classic example of prokaryotic gene regulation
- Perhaps the first well-understood GRN/system

Jacob & Monod

- * For discovery of genetic control of enzyme & virus synthesis
- ↳ studied/discovered (~1960)
 - ↳ used E.coli as model system
 - ↳ 1965 Nobel (with Lwoff)*

→ Math models developed soon after (~1965)

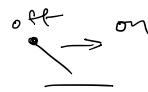
The problem: Lactose metabolism in E. coli.

- when glucose is abundant, E. coli use it exclusively as a food source
- when glucose is not available, E. coli can use other sugars such as Lactose (lac-)

So?

- 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 normal gene expression of genes coding for standard enzymes

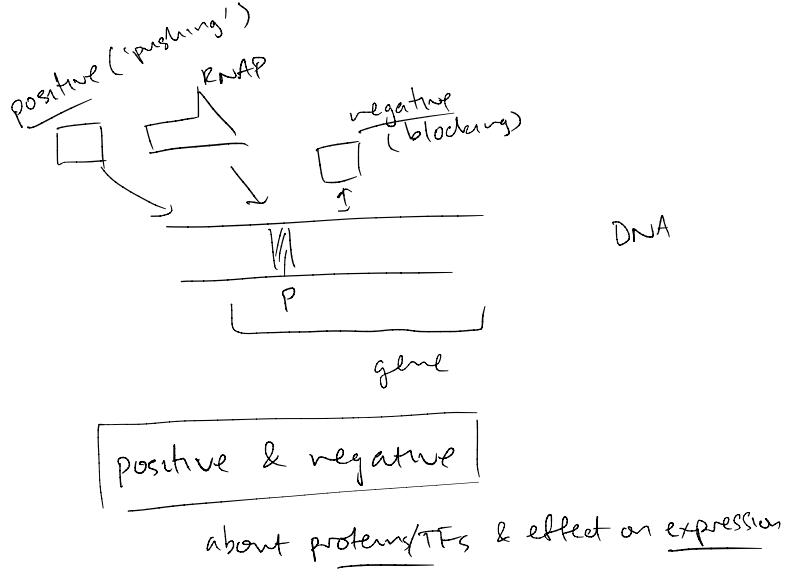
'on state' : Switch to lactose metabolism by upregulating expression of genes for enzymes required for lactose metab.

→ These enzymes ↑ x 1000

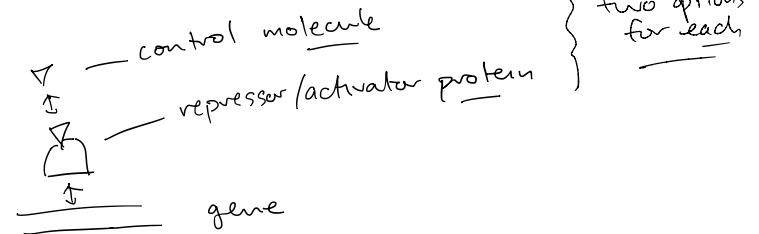
General idea has stood test of time!

→ Widespread & important concept.

Regulation: Crude metaphors ...

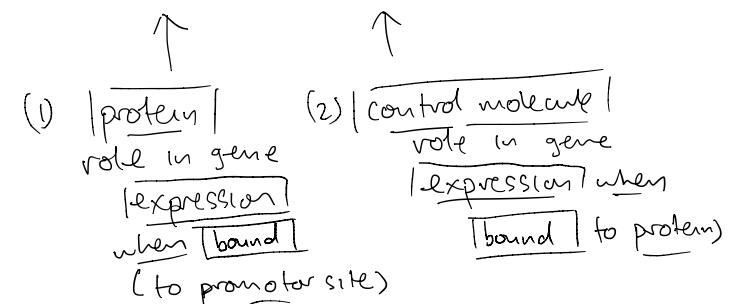


Types of regulation: 2×2 types (here)



Terminology (')

- negative inducible] original lac model
- positive inducible] extended lac model
- negative repressible
- positive repression



But: also: control molecules

Bind to TFs:

control molecule



Inducible & repressible

about control molecules
& effect on expression

Note: Both about effect on
expression when bound

Terminology guide (attempt!)

1. Based on protein role in expression when bound

→ Negative | Positive
o bound repressor protein prevents transcription o bound activator protein promotes transcription

2. Based on control molecule role in expression when bound

- o inducible promotes expression

↳ negative inducible ie
→ control molecule inactivates repressor & hence promotes expression (transcription)

↳ positive inducible ie
→ control molecule stimulates activator protein & hence promotes expression

- o repressible - represses expression

↳ negative repressible ie
→ control molecule activates repressor and hence represses expression

↳ positive repressible ie
→ control molecule inactivates promoter and hence represses expression

Negative (repression protein-based) regulation

→ Original theory

→ later extended to allow positive regulation

→ 'operons'

Examples: two key examples (both negative)

- o negative inducible

→ eg lac operon (original model)

→ expression usually off due to repressor

→ inducer inactivates repressor & hence leads to activation of operon transcription

- o negative repressible

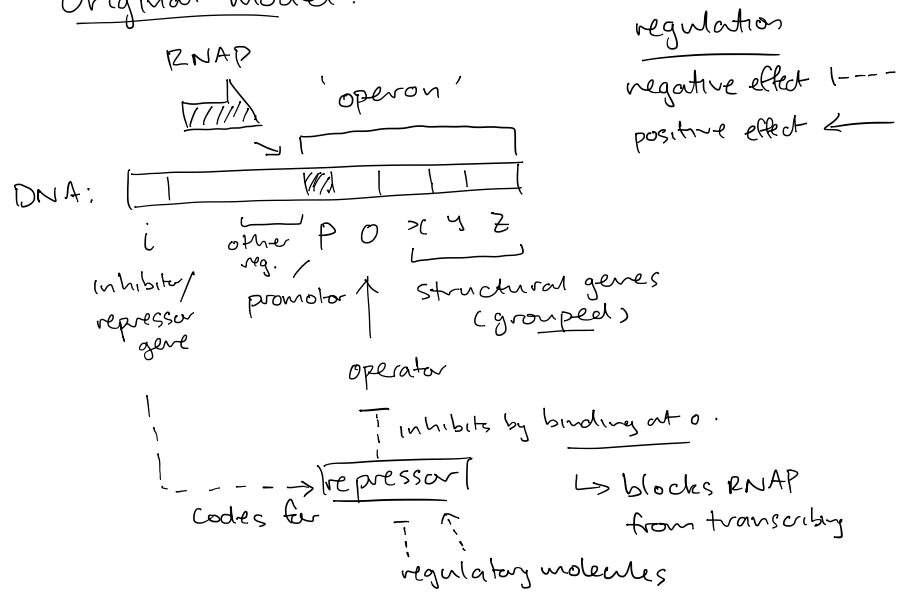
→ eg trp operon (tryptophan)

→ expression usually on
→ repressor present but unable to bind

→ corepressor enables binding of repressor & hence represses expression.

Operon : negative regulation

Original model:

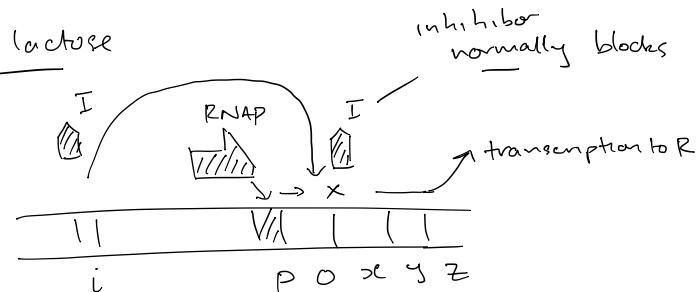


- Notes:
- Typical in prokaryotes for multiple genes to be grouped together (x, y, z)
 - A single promoter (& operator) controls expression of whole group (uses single mRNA to rep.). grouped
 - The promoter + operator + structural genes are called an 'operon'
 - Operator is site where a repressor can bind

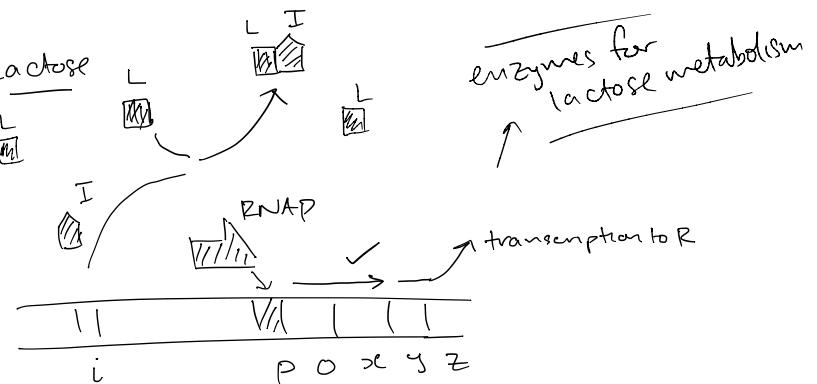
Lac operon : Simple model.

First consider the 'negative inducible' effect of lactose

No lactose



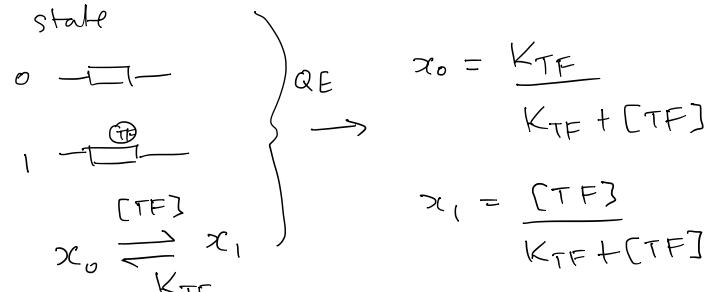
Lactose



Translate to math?

Simple version of simple model:

1. Gene reg.



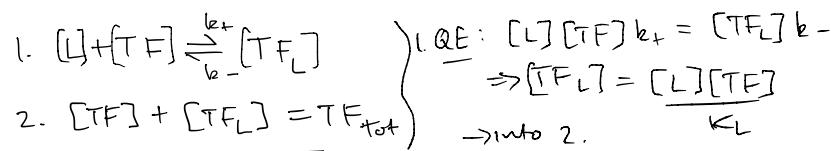
$$\nu = \nu_{\text{transcription}} = \nu_0 x_0 + \nu_1 x_1$$

2. TF is repressor

$$\rightarrow \nu_1 = 0$$

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

3. Lactose L inactivates repressor TF



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

Combine:

$$\nu_{\text{transcription}} = \nu_0 x_0$$

$$\Rightarrow \nu_{\text{transcription}} = \nu_0 \frac{K_{TF}}{K_{TF} + [TF]}$$

where $[TF] = \frac{[TF]_{\text{tot}}}{1 + [L]/K_L}$

$$\Rightarrow L \uparrow \Rightarrow TF \downarrow \Rightarrow \nu_{\text{transcription}} \uparrow$$

→ as desired!

(but very simplified)

More complex model

unfortunately, not the full story

→ what if both lactose & glucose present (etc) ?

→ E. coli prefer glucose

Observations: (key table -->

Glucose	Lactose	lac transcription
+	-	No
+	+	No/Low level
-	-	No
-	+	Yes! ← only case

- Glucose must be absent } for lac
- Lactose must be present } expression

→ Lactose uptake suppressed in presence of glucose.
→ preference for glucose.

Idea: also positive repressible component!

- There is an additional activator (positive) regulation protein: CAMP/CAP
 - CAP: catabolite activator protein

↳ without it, lac is only weakly expressed

↳ glucose suppresses it

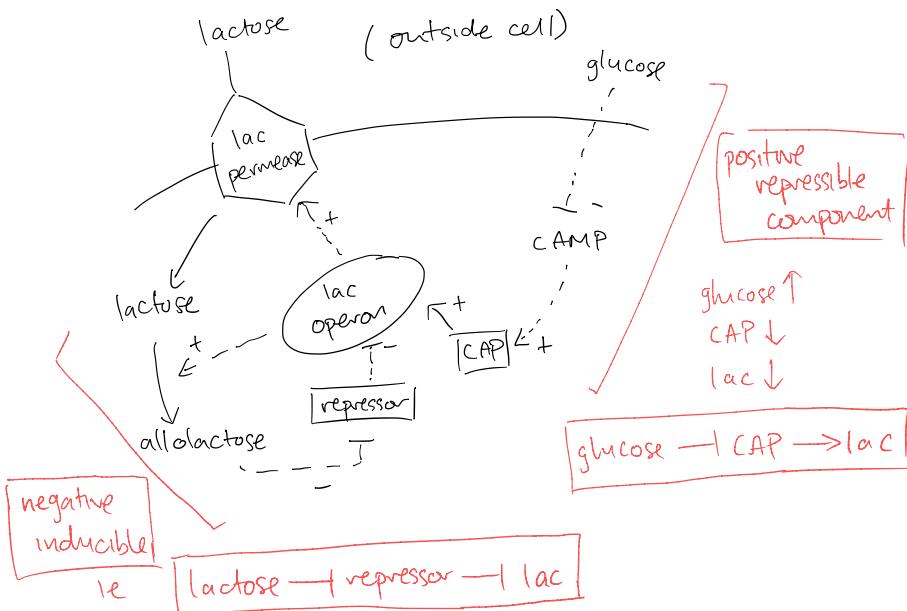
→ 'positive repressible' regulation

- There is also a positive feedback loop

→ expression of lac leads to increase in lactose uptake.



Regulation Network:

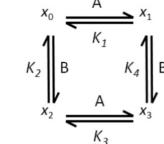


Example questions

(2016)

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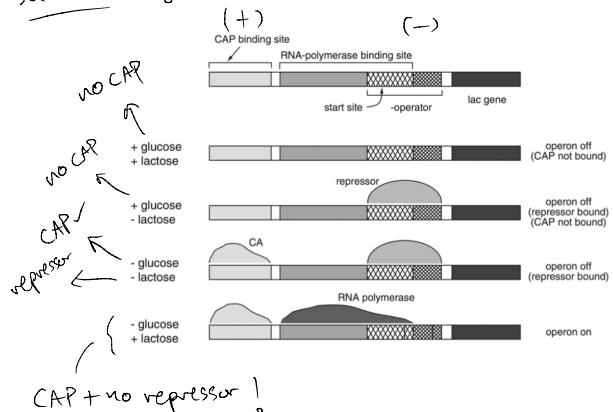
(or dissociation)
^ equilibrium constant
for binding of
B when A
isn't bound
(K_3 : exercise!)

Not best Q, but:

glucose ↑ \Rightarrow CAP ↓ ie A low

lactose ↑ \Rightarrow repressor B ↓ ie low

Same thing: (Keener & Sneyd book)



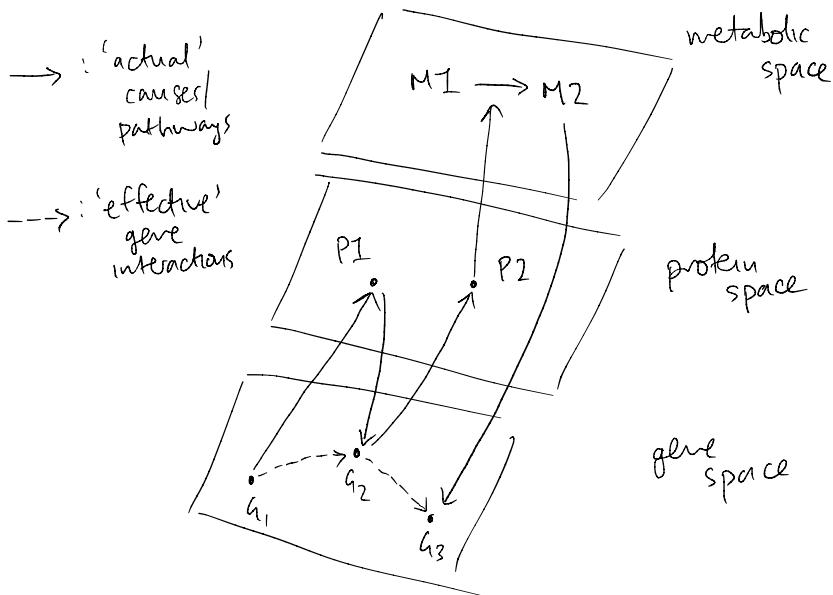
What about even more complex networks ?!

→ tomorrow

→ some terminology (if time):

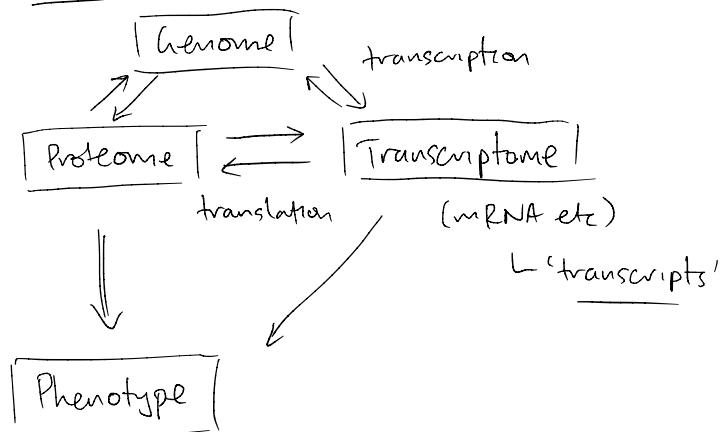
Gene Space

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

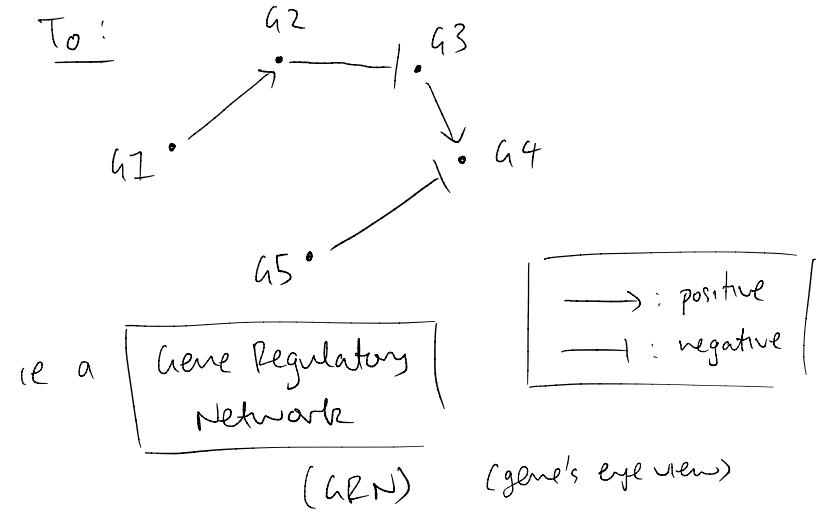


Gene Regulatory Networks

From:



To:



Transcriptomics

- A subfield of 'functional genomics'
 - ie the study of how genes & intergenic (between gene) regions contribute to biological function
- Transcriptomics focuses on
gene expression levels
 - In particular as measured via the levels of the transcripts (mRNA) associated with genes
 - ↳ mRNA easier to measure etc than proteins, but see 'proteomics'

Ideas:

- does expression go up or down under treatment?
- do groups of genes go up/down together

Expression Analysis

Two key approaches:

- Microarrays

↳ uses 'probes' (eg cDNA)

↳ samples 'hybridise' if complementary to probes

↳ amplify & quantify via qPCR

↳ see lab. tec. lectures.

- 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 overtaking!

(see Lowe et al. 2017
'Transcriptomics technologies')