

# BIOMENG 261

## TISSUE AND BIOMOLECULAR ENGINEERING

*Module I: Reaction kinetics and systems biology*

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## MODULE OVERVIEW

Reaction kinetics and systems biology (Oliver Maclarens)  
[12 lectures/3 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.

## 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'

1

3

## 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*

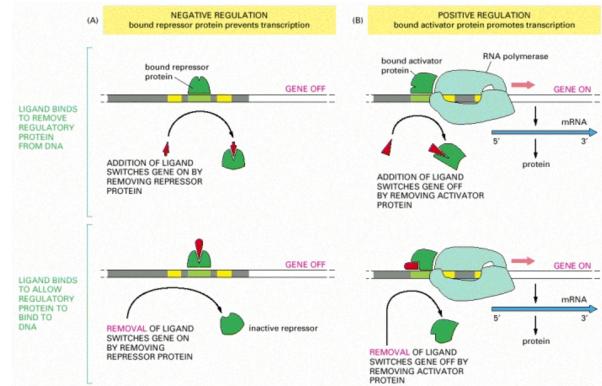
Not quite true (not just repression in general), but key ideas remain.

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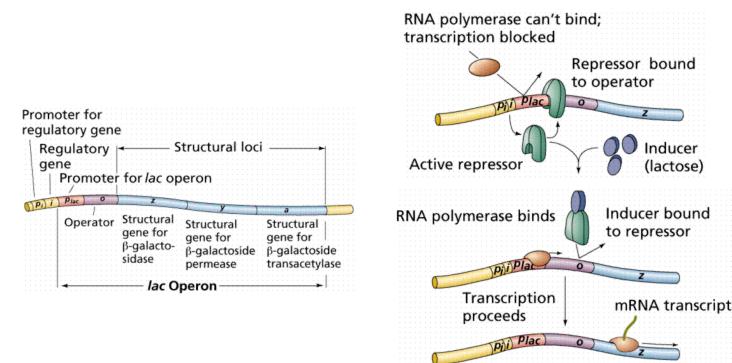
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# GENETIC SWITCHES AND REGULATION

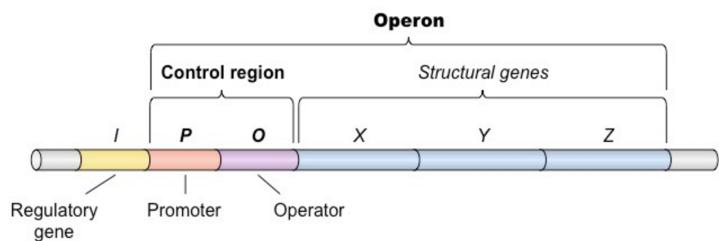
## THE LAC OPERON



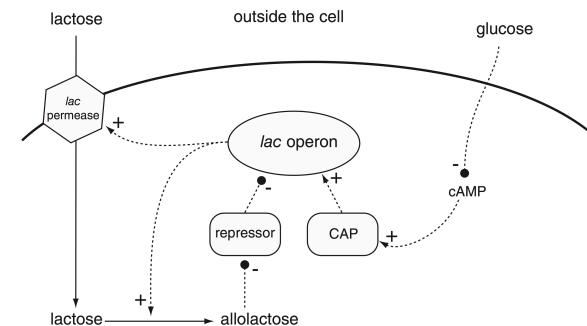
(Alberts et al. Molecular Biology of the Cell. 4th edition)



## OPERONS

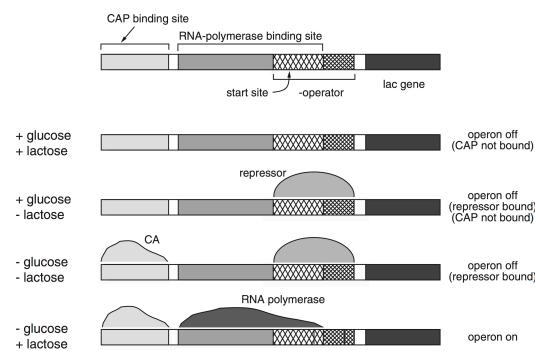


## LAC OPERON REGULATORY NETWORK

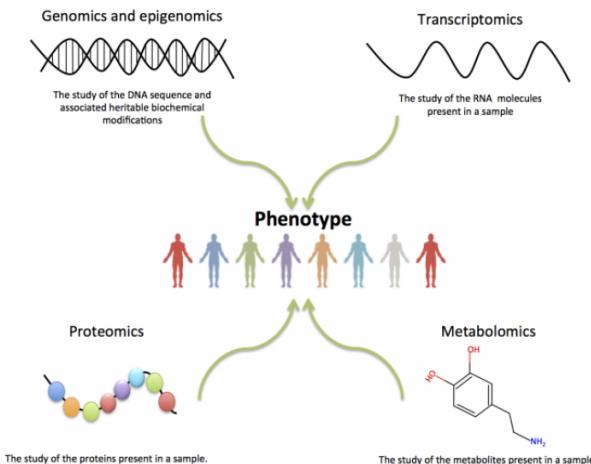


(Keener and Sneyd 2008)

## LAC OPERON REGULATION SUMMARY



## MUCH LARGER SYSTEMS - 'OMICS'



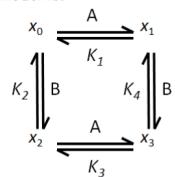
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11

## HUH? EXAMPLE QUESTION (2016)

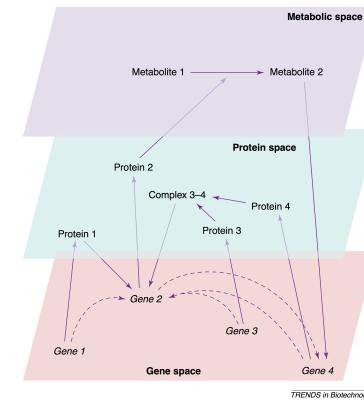
### Question 3

- (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)

10

12

# 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>

13

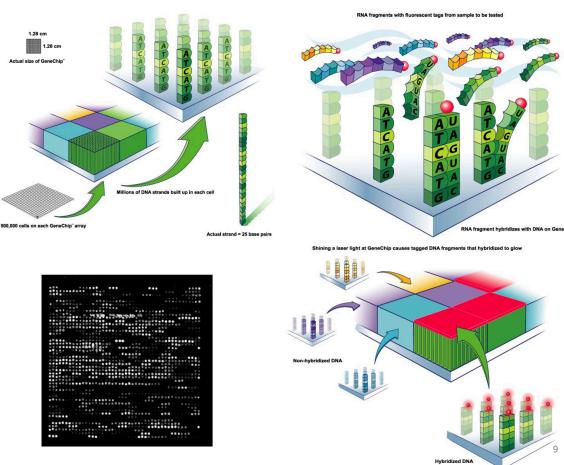
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## EXPRESSION ANALYSIS

- *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



14

16

## Bioeng 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

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

→ 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

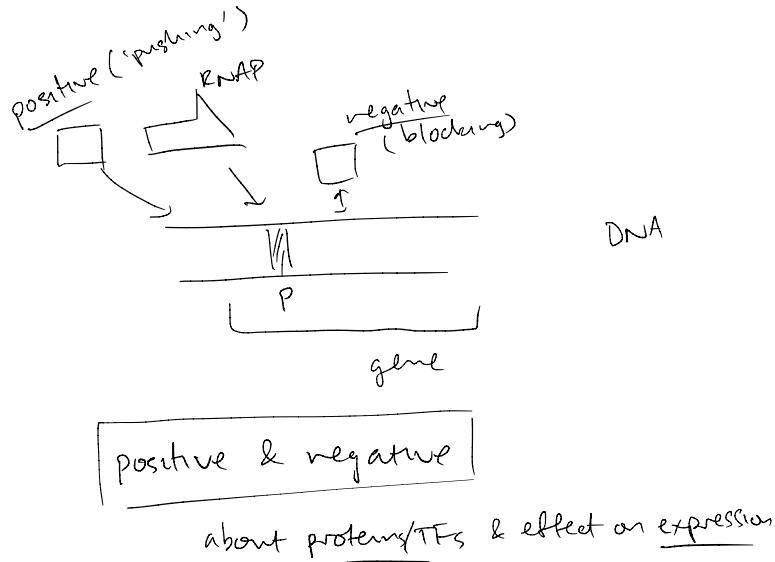
'on state' : switch to lactose metabolism by upregulating expression of genes for enzymes required for lactose metab.

→ These enzymes ↑ × 1000

General idea has stood test of time!

→ Widespread & important concept.

Regulation: Crude metaphors ---



But: also: control molecules

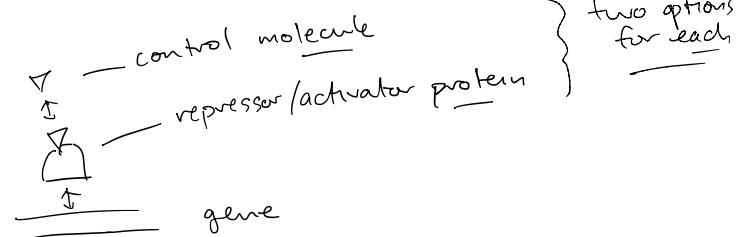
Bind to TFs:



**Inducible & repressible**

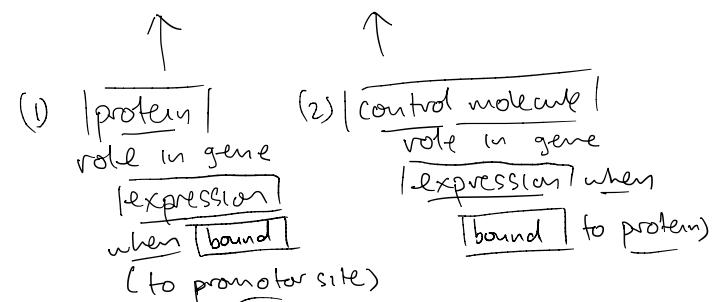
about control molecules  
& effect on expression

Types of regulation:  $2 \times 2$  types (here)



Terminology (%)

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



Note: Both about effect on  
expression when bound

## Terminology guide (attempt!)

1. Based on protein role in expression when bound
  - Negative
    - o bound repressor protein prevents transcription
  - Positive
    - o bound activator protein promotes transcription
2. Based on control molecule role in expression when bound
  - o Inducible promotes expression
    - ↳ negative inducible i.e.
      - control molecule inactivates repressor & hence promotes expression (transcription)
    - ↳ positive inducible i.e.
      - control molecule stimulates activator protein & hence promotes expression
  - o Repressible represses expression
    - ↳ negative repressible i.e.
      - control molecule activates repressor and hence represses expression
    - ↳ positive repressible i.e.
      - control molecule inactivates promoter and hence represses expression

## Negative (repression protein-based) regulation

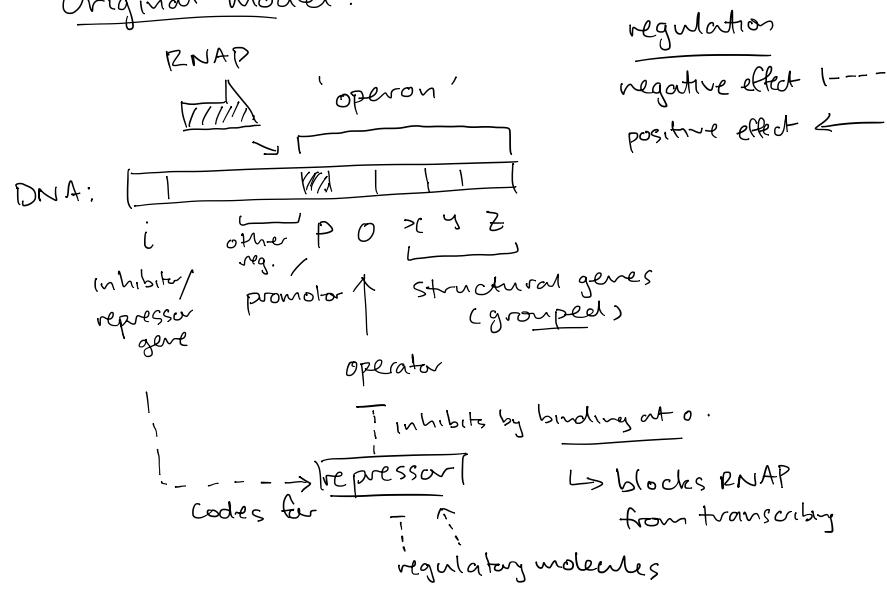
- Original theory
- later extended to allow positive regulation
- 'operons'

Examples: two key examples (both negative)

- o Negative inducible
  - e.g. lac operon (original model)
  - expression usually off due to repressor
  - inducer inactivates repressor & hence leads to activation of operon transcription
- o Negative repressible
  - e.g. trp operon (tryptophan)
  - expression usually on
  - repressor present but unable to bind
  - corepressor enables binding of repressor & hence represses expression.

## Operon : negative regulation

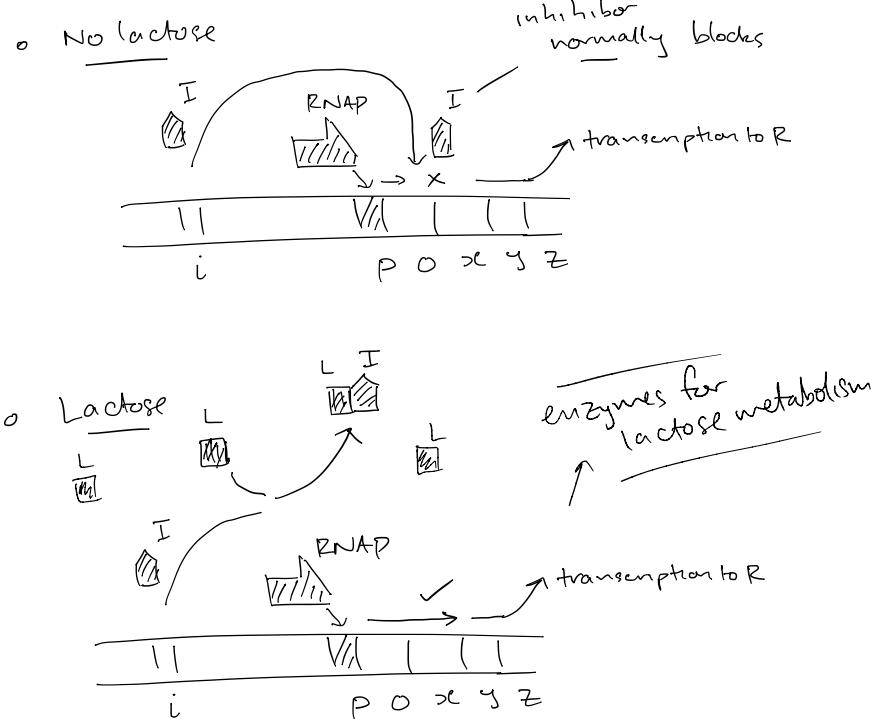
Original model:



- Notes:
- Typical in prokaryotes for multiple genes to be grouped together ( $x, y, z$ )
  - A single promotor (& 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.

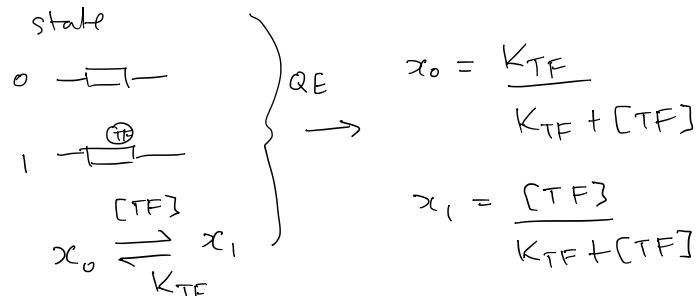
First consider the 'negative inducible' effect of lactose



Translate to math?

Simple version of simple model:

1. Gene reg.



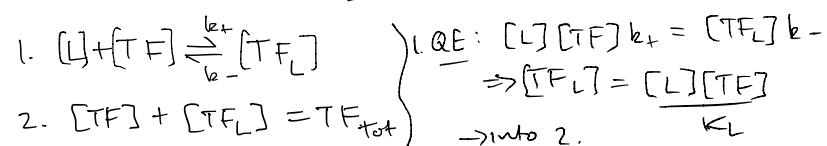
$$\nu = \nu_{\text{transcription}} = \nu_0 x_0 + \nu_1 x_1$$

2. TF is repressor

$$\rightarrow \nu_1 = 0$$

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

3. Lactose L activates 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

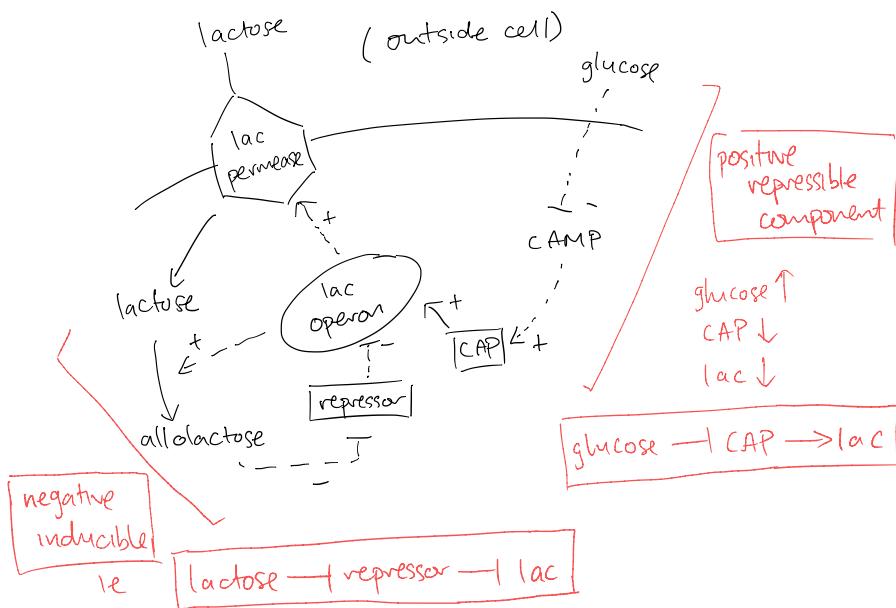
→ 'positive repressible' regulation

- There is also a positive feedback loop

→ expression of lac leads to increase in lactose uptake.



## Regulation Network:

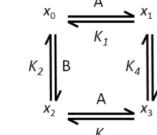


## Example questions

(2016)

**Question 3**

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

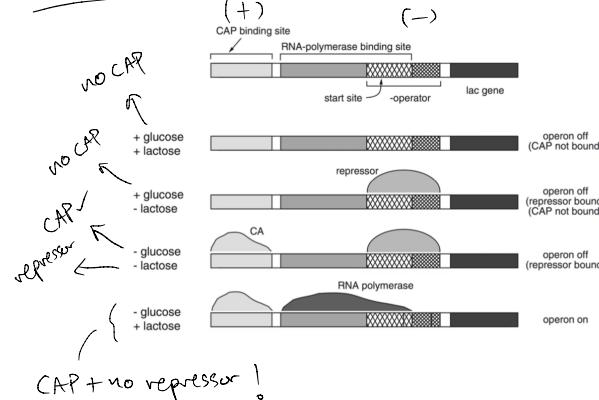


(or dissociation)  
^ equilibrium constant  
for binding of B when A isn't bound  
( $K_3$ : exercise!)

- (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 (CAP-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)

Same thing: (Keener & Sneyd book)



not best Q, but:

glucose ↑  $\Rightarrow$  CAP ↓ ie A low

lactose ↑  $\Rightarrow$  repressor B ↓ ie low

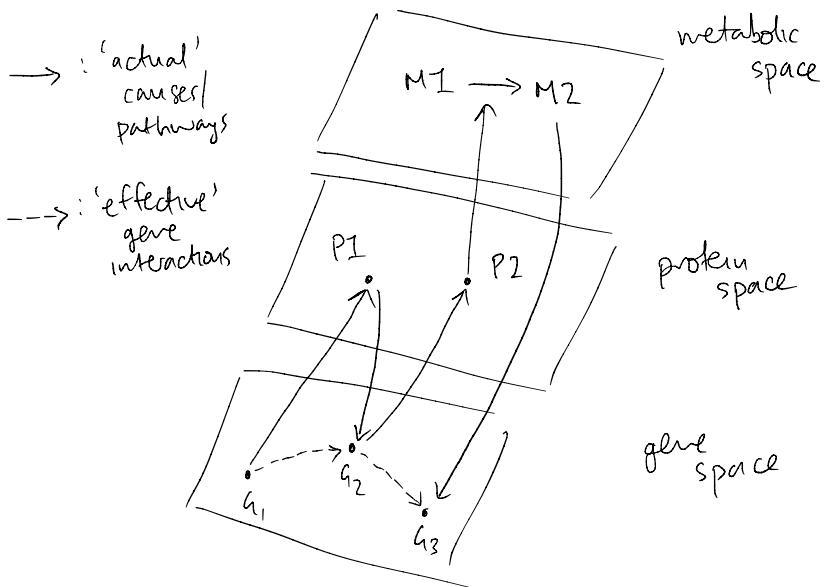
What about even more complex networks?!

→ tomorrow

→ some terminology (if time):

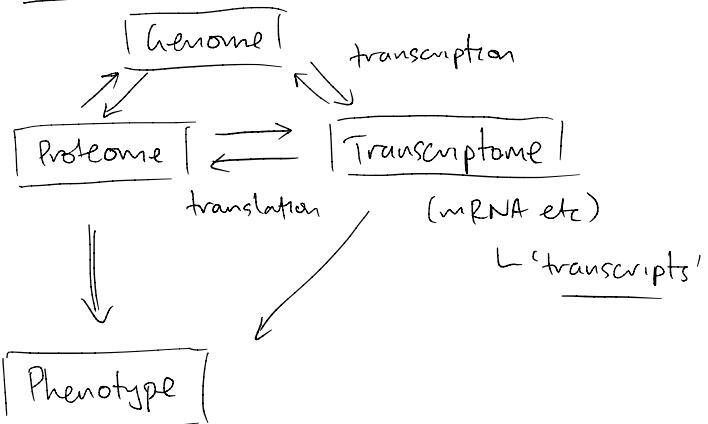
### Gene Space

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

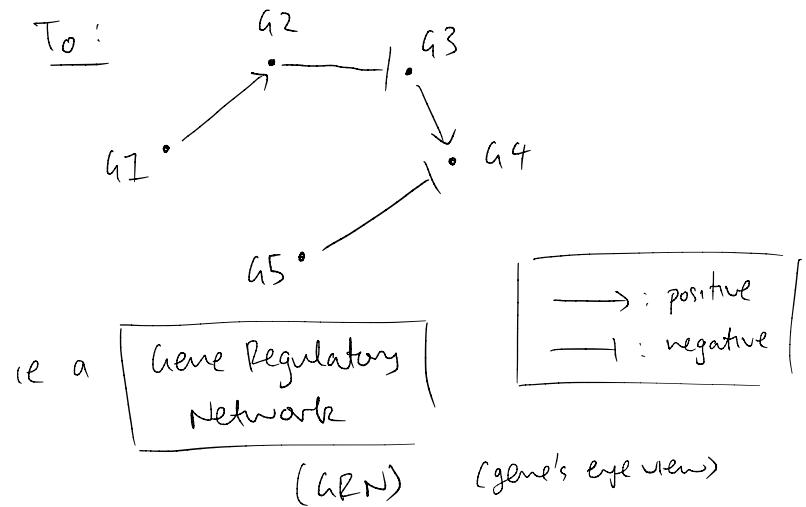


### Gene Regulatory Networks

From:



To:



## Transcriptomics

- A subfield of 'functional genomics'
  - ↳ 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. tech. 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')