

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*)
[11-12 lectures/3 tutorials/2 labs]

1. Basic principles: modelling with reaction kinetics [5-6 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: 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'

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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*

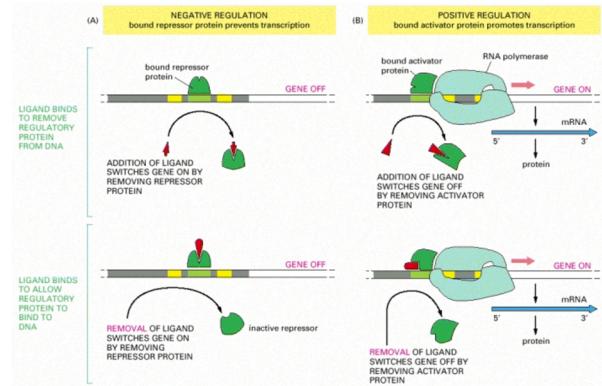
Not quite true (not just repression in general), but key ideas remain.

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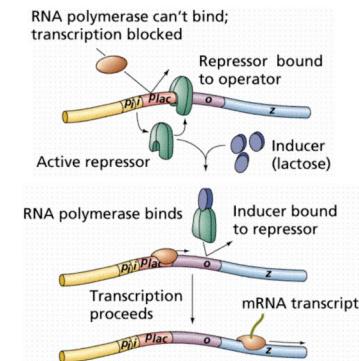
GENETIC SWITCHES AND REGULATION

THE LAC OPERON



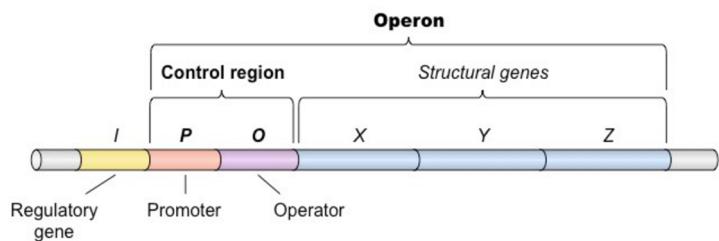
(Alberts et al. Molecular Biology of the Cell. 4th edition)

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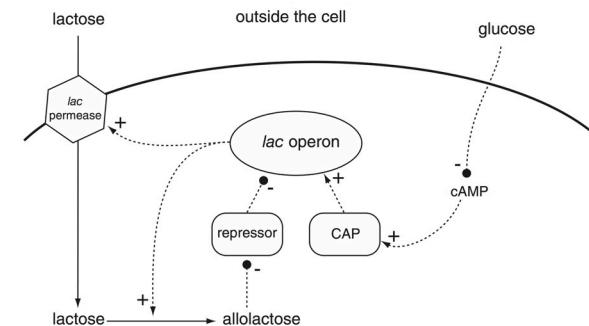
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OPERONS



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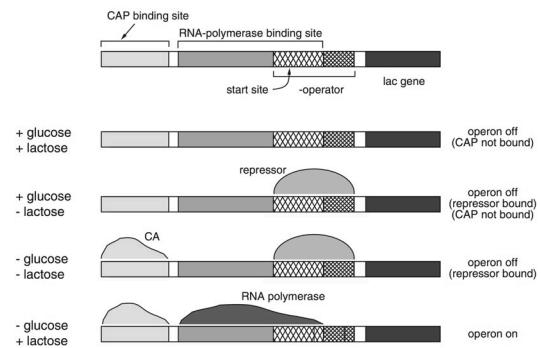
LAC OPERON REGULATORY NETWORK



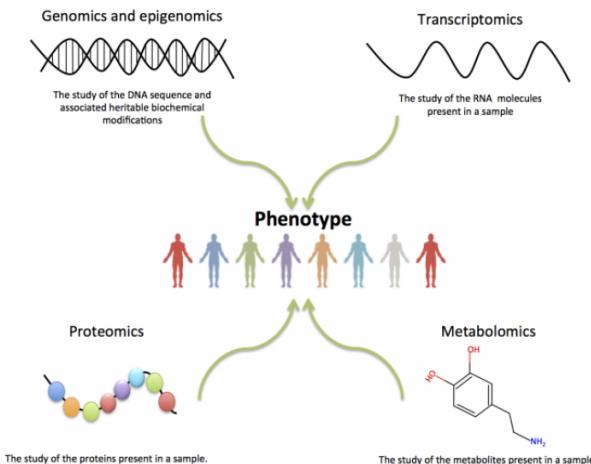
(Keener and Sneyd 2008)

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LAC OPERON REGULATION SUMMARY



MUCH LARGER SYSTEMS - 'OMICS'



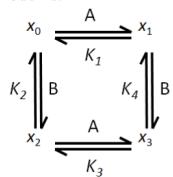
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HUH? EXAMPLE QUESTION (2016)

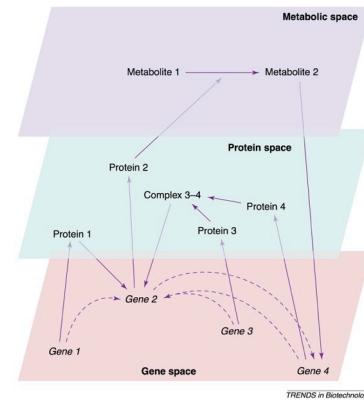
Question 3

- (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)

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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)

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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)

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Biomeng 261: Lecture 11

Gene 'regulatory networks' ('GRNs')
↳ (control of expression)

- o lac operon
 - ↳ 'simple' gene regulatory network
 - ↳ can understand via basic reaction modelling

- o 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.

Jacob & Monod

↳ studied/discovered (~1960)
↳ used E. coli as model system
↳ 1965 Nobel (with Monod)*
↳ 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 use it exclusively as a food source
- When glucose is not available, E. coli can use other sugars such as Lactose (bc...)

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

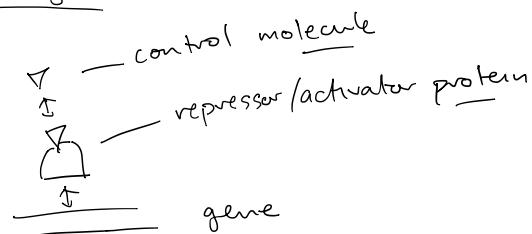
'on state' : switch to lactose metabolism by upregulating expression of genes for enzymes required for lactose metab.

→ These enzymes ↑ x 1000

General idea has stood test of time!

→ Widespread & important concept.

Types of regulation



Terminology (%)

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

↑ ↑

<u>protein</u> <u>role in gene</u> <u>expression</u> <u>when bound</u> (to promoter site)	<u>control molecule</u> <u>role in gene</u> <u>expression when</u> <u>bound (to protein)</u>
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Terminology guide (attempt!)

- Based on protein role in expression when bound
 - Negative | → positive
 - bound repressor protein prevents transcription
 - bound activator protein promotes transcription
- Based on control molecule role in expression when bound
 - 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
 - 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:

o Negative inducible

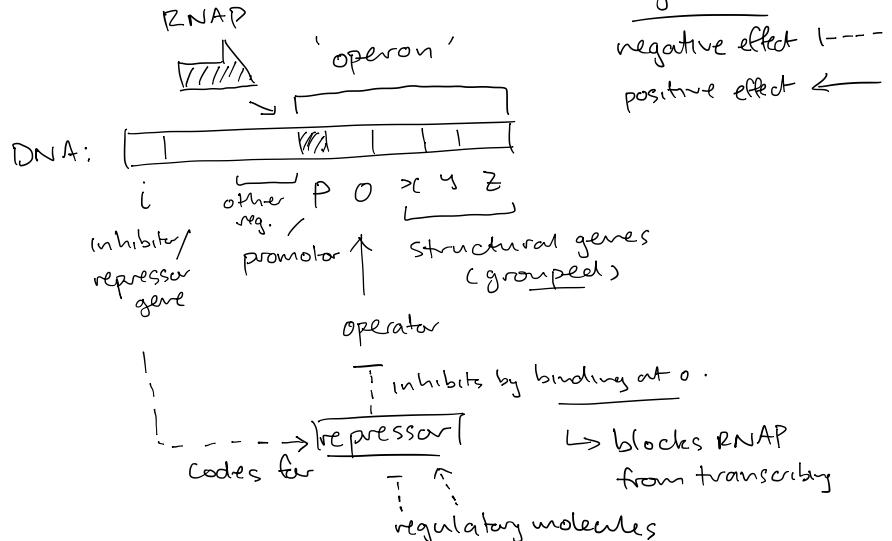
- eg [lac operon] (original model)
- expression usually off due to repressor
- inducer inactivates repressor & hence leads to activation of operon transcription

o Negative repressible

- eg [trp operon] (tryptophan)
- expression usually on → repressor present but unable to bind
- co-repressor enables binding of repressor & hence represses expression.

Operon : negative regulation

Original model:



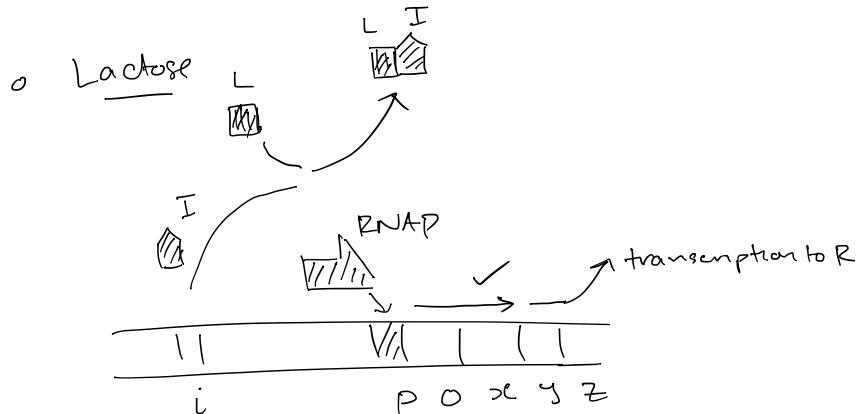
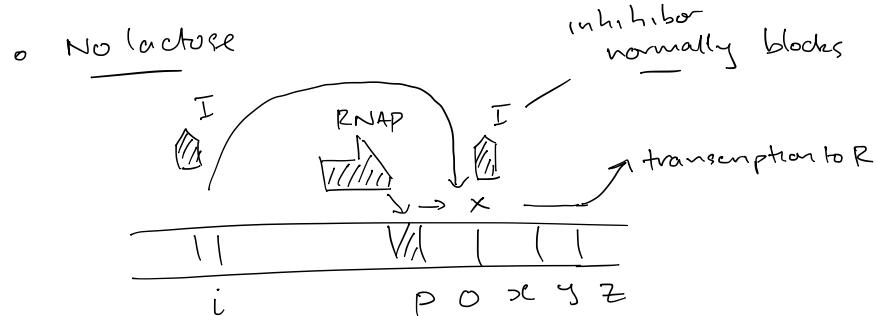
Notes:

- 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 promotor + 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.

state

$$0 \xrightarrow{\quad} \left\{ \begin{array}{l} 0 \\ 1 \end{array} \right. \xrightarrow{\text{QE}} \begin{array}{l} x_0 = \frac{K_{TF}}{K_{TF} + [TF]} \\ x_1 = \frac{[TF]}{K_{TF} + [TF]} \end{array}$$

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

2. TF is repressor

$$\rightarrow \nu_1 = 0$$

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

3. Lactose L inactivates repressor TF

$$\left. \begin{array}{l} 1. L + TF \xrightleftharpoons[k_2]{k_1} TF_L \\ 2. [TF] + [TF_L] = TF_{\text{tot}} \end{array} \right\} \text{Quasi: elim. } TF_L$$

$$\Rightarrow [TF] = \frac{TF_{\text{tot}}}{1 + [L]/K_L}, K_L = \frac{k_1}{k_2}$$

Combine:

$$v_{\text{transcription}} = v_0 x_0$$

$$= v_0 \frac{K_{TF}}{K_{TF} + [TF]}$$

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

$$\Rightarrow \boxed{L \uparrow \Rightarrow TF \downarrow \Rightarrow v_{\text{transcription}} \uparrow}$$

→ as desired!

More complex model

unfortunately, not the full story

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

→ E. coli prefer glucose

Observations:

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

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

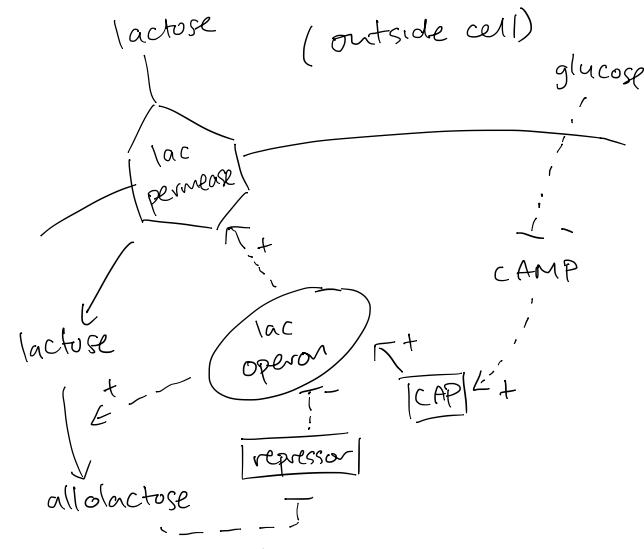
→ Lactose uptake suppressed in presence of glucose.

↳ preference for glucose.

Idea:

- 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:



what about even more complex networks?!

→ tommorrow

→ some terminology: