

BIOMENG 261

TISSUE AND BIOMOLECULAR ENGINEERING

Module I: Reaction kinetics and systems biology

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LECTURE 10: MODELLING GENE EXPRESSION AND REGULATION

- Using 'reaction' language to describe gene expression and regulation
- In particular: transcription and its regulation
- Gene regulatory states and occupancy probabilities/fractions
- Using quasi-equilibrium gene-state model to derive overall constitutive model for transcriptional flux

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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.

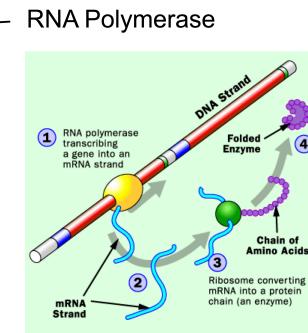
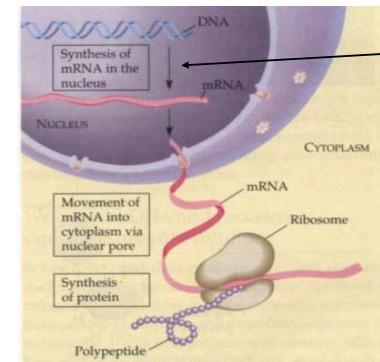
2. Systems biology I: overview, signalling and metabolic systems [3 lectures]

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.

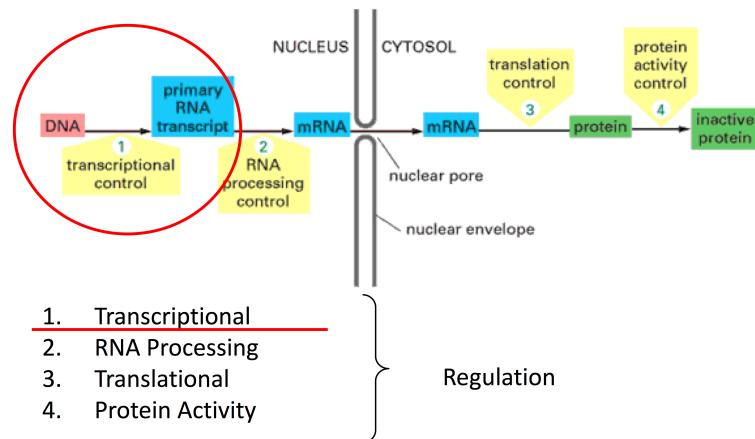
GENE EXPRESSION AND REGULATION



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GENE EXPRESSION AND REGULATION



TRANSCRIPTION REGULATION TYPES

Gene transcription is regulated by various *transcription factors*.

These can be

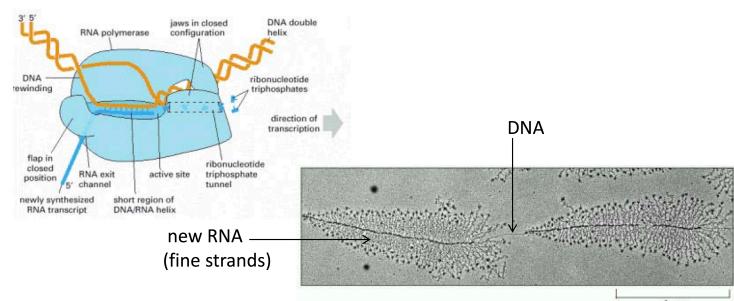
- *Activators* (increase transcription)
- *Repressors* (decrease transcription)

When a gene codes for its *own* activator/repressor we call this positive/negative *autoregulation*.

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FOCUS: GENE TRANSCRIPTION



Many molecules of RNA polymerase (beads on DNA) simultaneously transcribing each of two adjacent genes

Images from *Molecular Biology of the Cell* (4ed), "From DNA to RNA", Fig 6-8 and 6-9.
Online at www.ncbi.nlm.nih.gov/books/NBK26887/

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OVERALL BALANCE EQUATIONS

As usual, we can write overall *conservation equations*

$$\frac{dR}{dt} = v_{\text{transcription}} - v_{\text{Rdeg}}$$

$$\frac{dP}{dt} = v_{\text{translation}} - v_{\text{Pdeg}}$$

where R is mRNA, P is protein/product.

Note: using v instead of J just for consistency with typical approaches. Same basic thing - a *flux*.

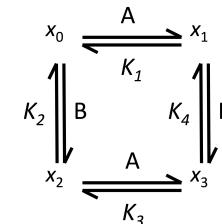
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GENE REGULATORY STATES AND FLUXES

FOCUS: TRANSCRIPTION FLUX

Our goal here is to derive a *constitutive equation* for $v_{\text{transcription}}$ in terms of underlying gene '*regulatory states*'.

- Essentially the same idea as enzyme kinetics: more *detailed model + equilibrium assumption* to get overall flux expression.



where

$$x_0 + x_1 + x_2 + x_3 = 1$$

are the *occupancy probabilities/state fractions*...which we solve for and plug into...

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GENE REGULATORY STATES

We view the gene as existing in a *number of states with rapid transitions between them*.

Each state (potentially) contributes to the *overall flux*.

Rather than 'concentrations' we use state *fractions* i.e. state *occupancy probabilities* and then use these to average the flux contributions.

We use the *equilibrium approximation* to determine the underlying probabilities to use in averaging.

OVERALL FLUX CONSTITUTIVE EQUATION

...the *overall constitutive equation* for transcriptional flux

$$v_{\text{transcription}} = x_0 v_0 + x_1 v_1 + x_2 v_2 + x_3 v_3$$

i.e.

$$v_{\text{transcription}} = \sum_{s=0}^{N_s-1} x_s v_s$$

where the choice of the v_i depends on whether the TFs are activators/repressors etc.

This plugs back into...

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OVERALL BALANCE EQUATIONS

$$\frac{dR}{dt} = v_{\text{transcription}} - v_{R\text{deg}}$$

$$\frac{dP}{dt} = v_{\text{translation}} - v_{P\text{deg}}$$

where R is mRNA, P is protein/product.

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EXAMPLES

See handout.

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Biomeng 261 : Lecture 10

Gene expression & regulation

- Brief background
- Modelling via 'reaction' systems

Key idea(s)

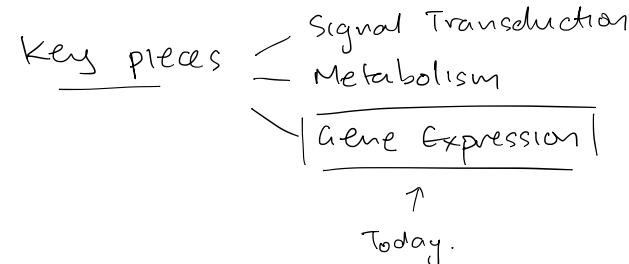
Today

- We can use same basic 'reaction modelling' language to model genetic expression & regulation
- genes 'switch' between various states depending on regulatory molecules
 - ↳ use fractions/probability instead of on/off.

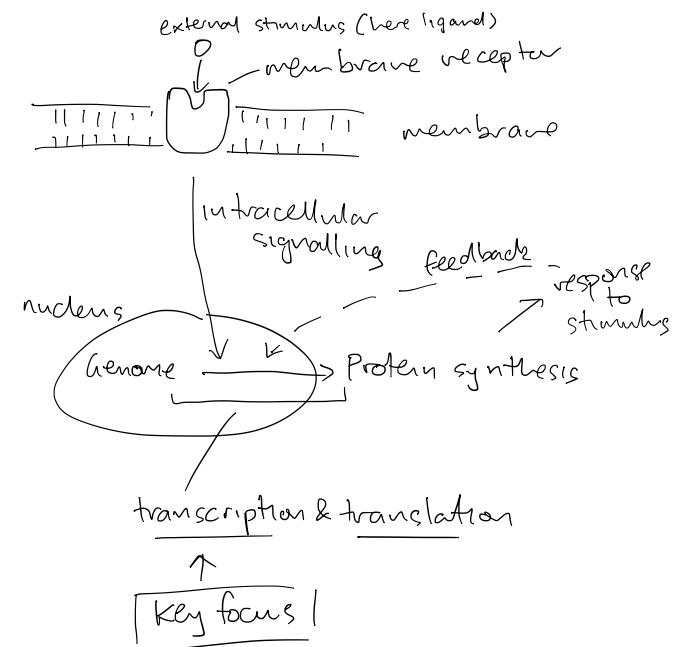
Later lectures

- more complex examples (lac operon)
- 'scaling up' issues faced when dealing with large systems

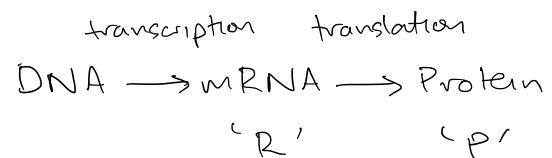
Cellular Systems Biology (Recall)



Recall signal transduction:



Simplistic overall balance equation



$$\frac{dR}{dt} = J_{\text{transcription}} - J_{R, \text{degradation}}$$

goal (want a
constitutive eqn)

$$\frac{dP}{dt} = J_{\text{translation}} - J_{P, \text{degradation}}$$

Notes:

- mRNA is 'created' but is then translated,
not used up directly
 ↳ degrades instead.

◦ We'll often use \sim instead of J

ie $\frac{dR}{dt} = \sim_{\text{transcription}} - \sim_{\text{deg}}$
 etc.

Main focus: Transcription & its regulation

T_{overall}: a constitutive equation for the overall transcription rate

↳ will derive from an 'underlying' model of gene states

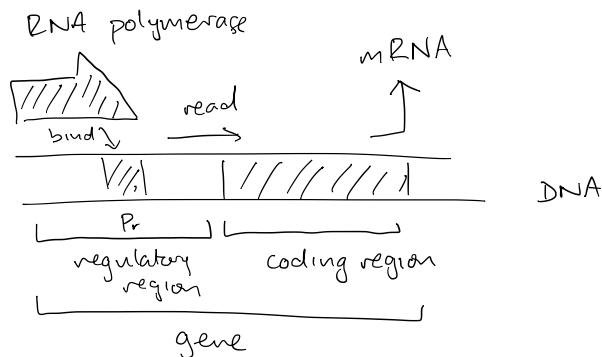
↳ like MM & enzymes.

↳ will be a weighted average of transcription rate for each 'gene state'

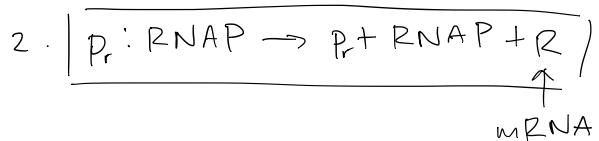
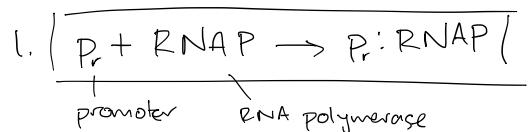
What to include?

Simple models { prokaryotic } easier
eukaryotic } same ideas,
more complicated

Transcription: Simple picture (mainly motivation)



'Reaction' steps



Note: as if R 'created out of nothing'

↳ created from other chem. species in cell.

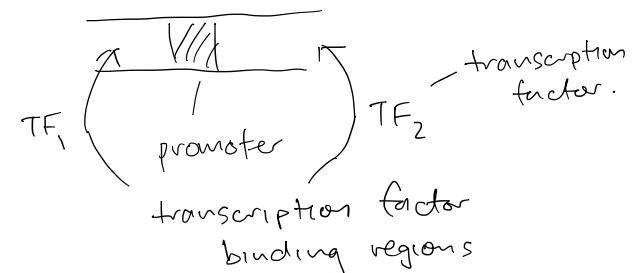
↳ depends on 'level of modelling'

Transcription: complications

(Eukaryotes:
many TFs!)

Regulation!

- within 'regulatory region':



- Transcription factors affect ('regulate') RNAP binding/transcription rate

- Types:

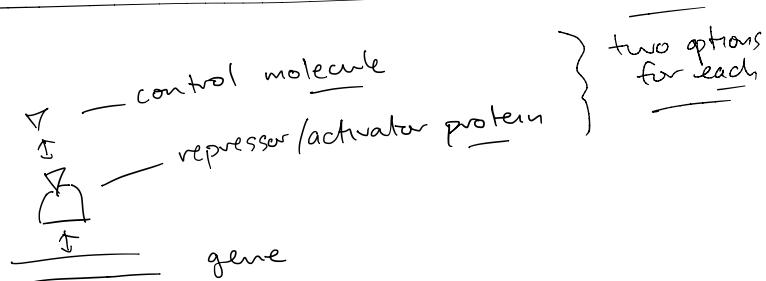
- Activator
↳ TF binding enables/helps binding of RNAP

- Repressor

- Repressor
↳ TF blocks/decreases binding of RNAP.

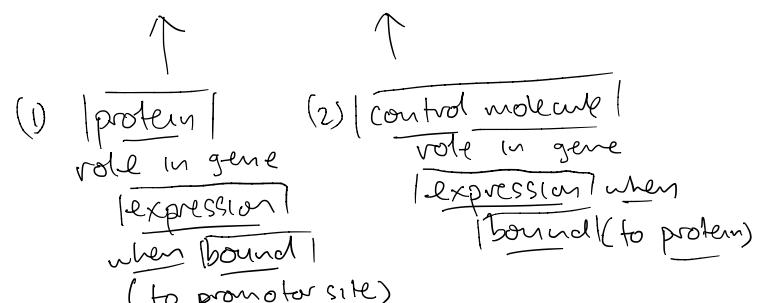
Autoregulation: gene codes for its own activators (+ve) or repressors (-ve)

Even more complication: control molecules



Terminology (%)

- negative inducible
 - positive inducible
 - negative repressible
 - positive repression
- } examples:
See
next
lecture



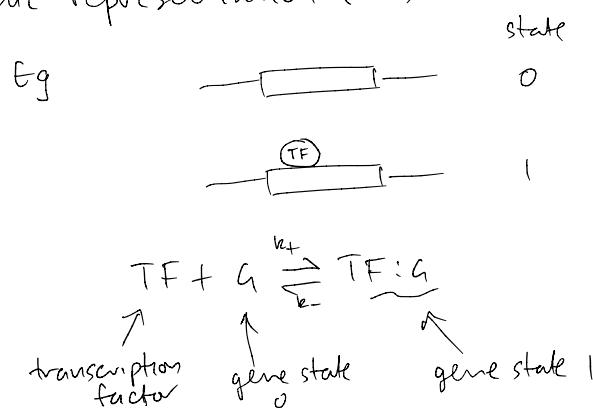
Gene regulatory states

→ In general there may be various transcription factors bound to the regulatory region of a gene

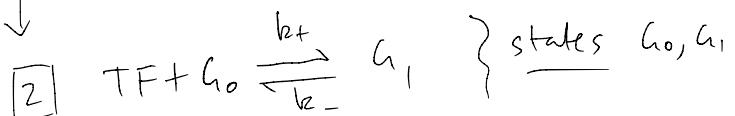
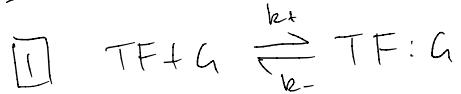
→ We model the gene as being in various ['regulatory states'] & switching between them

→ each state gives a different contribution to the overall transcription flux

State representation (PI)



Gene states: Alternative representations (PI)



Interpret:

switching states $\boxed{G_0 \leftrightarrow G_1}$

concentrations? Probabilities? \rightarrow

States: Fractional/occupancy probabilities

- Rather than concentrations, we will work in terms of the fractions / occupancy probabilities of each state of a given gene.
- Again, each state will have a different (transcription rate)

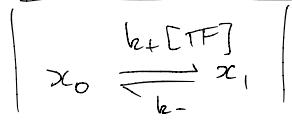
\rightarrow we will be interested in relative time spent in each state & use to 'average over' to get an overall flux ('long term average')

So get
$$\left[\begin{array}{c} k_+ [\text{TF}] \\ x_0 \xrightleftharpoons[k_-]{ } x_1 \\ \hline x_0 + x_1 = 1 \end{array} \right] \quad \left. \begin{array}{l} x_0 = \text{prob. of} \\ \text{gene being} \\ \text{in state } 0 \\ \text{etc} \\ \vdots \end{array} \right\}$$

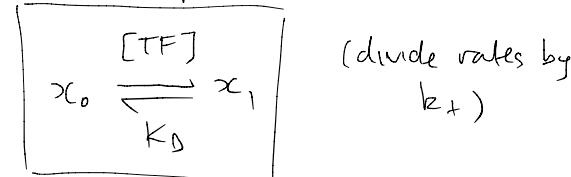
(Basically: divide everything by $G_0 + G_1 + \dots = \text{constant}$) \rightarrow

- - More representations - -

Finally, instead of just



we will also picture this as



where $\boxed{K_D = k_- / k_+}$ = $\boxed{\text{equilibrium/dissociation constant}}$

Note: only relative rates (& proportions) matter here

→ we will be using quasi-equilibrium analysis, & 'long time averages', so only ratios will matter.

states & transcription rates : picture

- gene can be in multiple states
- rapidly switches between
 - ↳ interested in 'long term' relative time in each state
 - ↳ will assume quasi-equilibrium
 - ↳ gives equilibrium occupancy probabilities

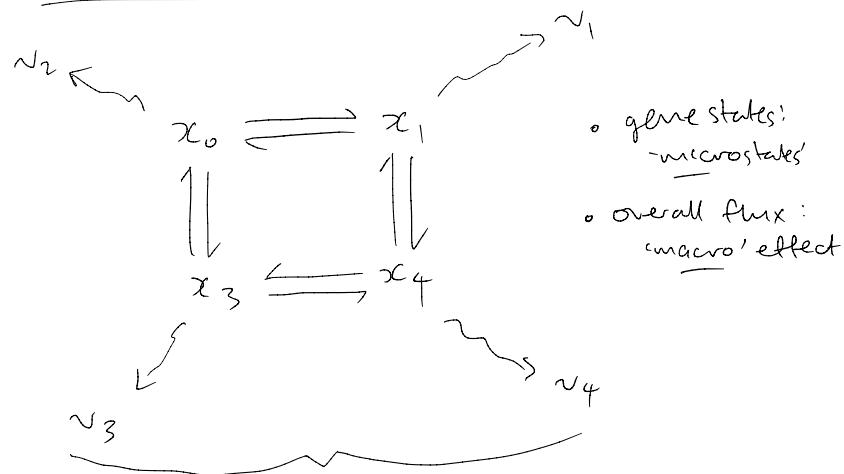
→ $\boxed{\text{add up weighted contributions of each state to total transcription flux } \sim}$

Combining contributions to overall flux gives:

$$\boxed{U_{\text{transcription}} = \sum_{s=0}^{m-1} x_s v_s}$$

where:
 x_s : probability of gene being in state s
 v_s : transcription rate (flux) in state s .

Picture of state switching:



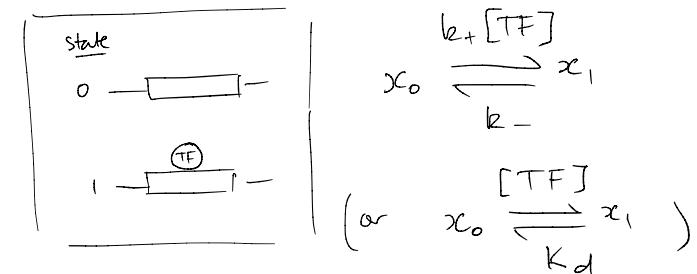
average/overall behaviour:

$$v = \sum x_i v_i$$

- Multiple states with different occupancy probabilities x_i } find using quasi-equil.
- each state has transcription rate v_i
- overall transcription rate = $\sum x_i v_i$
via 'averaging over' all states

Example! A single TF.

Two gene-state model



Steps

1. Assume gene-state model in (quasi-) equilibrium
 2. Find fractions x_0, x_1, \dots
 3. Find overall transcription
- $$v = v_0 x_0 + v_1 x_1 + \dots$$
4. Determine v_0, v_1 etc depending on whether TF is activator/inhibitor

1.a). Equilibrium

$$x_0 \cdot k_+ [TF] = x_1 \cdot k_-$$

forward reverse

$$\Rightarrow \left| \frac{x_1}{x_0} = \frac{k_+}{k_-} [TF] = \frac{[TF]}{K_d} \right|$$

b). Total fraction = 1

$$\Rightarrow \underline{x_0 + x_1 = 1}$$

2. Two equations, two unknowns x_0, x_1

$$\Rightarrow \text{Combine: } x_0 + x_0 \frac{[TF]}{K_d} = 1$$

$$\Rightarrow x_0 \left[1 + \frac{[TF]}{K_d} \right] = 1$$

$$x_0 \left[\frac{K_d + [TF]}{K_d} \right] = 1 \Rightarrow \text{solve for } x_0$$

use $x_1 = 1 - x_0$

$$\Rightarrow \left| x_0 = \frac{K_d}{K_d + [TF]} \right|$$

$$\Rightarrow \left| x_1 = \frac{[TF]}{K_d + [TF]} \right|$$

$$3. \text{ Utranscription} = x_0 v_0 + x_1 v_1$$

$$= \frac{K_d}{K_d + [TF]} \cdot v_0 + \frac{[TF]}{K_d + [TF]} \cdot v_1$$

4. Specify v_0, v_1 based on [TF] type.

by suppose [TF] is a perfect activator

→ increases transcription

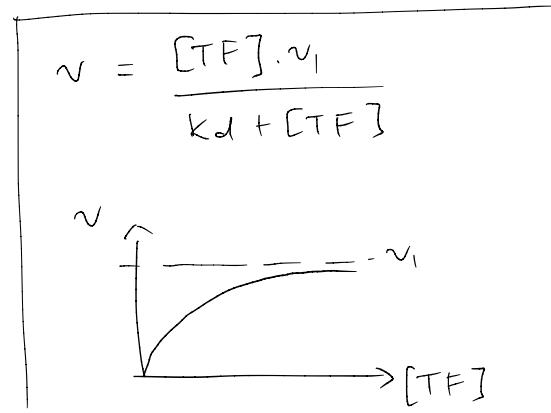
→ no transcription without

$$\Rightarrow \text{set } \underline{v_0 = 0}$$

so

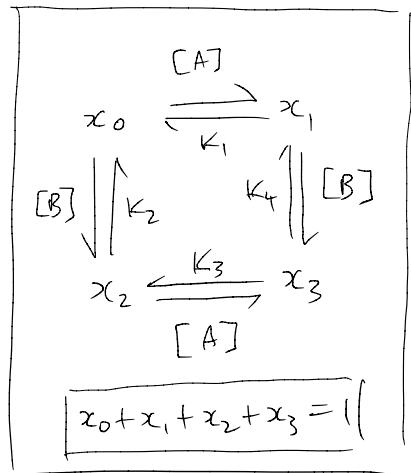
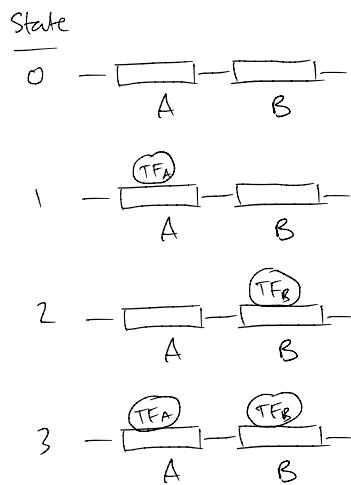
$$v = \frac{[TF] \cdot v_1}{K_d + [TF]}$$

ie



[Exercise: what if TF is a (perfect) repressor?]

Multiple TFs : two TFs \Rightarrow four gene states



Steps

$$x_1 = \frac{[A]x_0}{K_1}$$

$$x_2 = \frac{[B]x_0}{K_2}$$

all at
equal.
(solve for
 x_1, x_2, x_3
in
terms of
 x_0)

(3 eqn) $x_3 = \frac{[B]x_1}{K_4} \Rightarrow x_3 = \frac{[B]}{K_4} \cdot \frac{[A]x_0}{K_1}$

lb. sub into $x_0 + x_1 + x_2 + x_3 = 1$

(1 eqn) $\Rightarrow x_0 + x_0 \frac{[A]}{K_1} + x_0 \frac{[B]}{K_2} + \frac{[B][A]}{K_4 K_1} = 1$

$$\Rightarrow x_0 \left(1 + \frac{[A]}{K_1} + \frac{[B]}{K_2} + \frac{[B][A]}{K_4 K_1} \right) = 1$$

\Rightarrow

$$\Rightarrow x_0 = \frac{1}{1 + \frac{[A]}{K_1} + \frac{[B]}{K_2} + \frac{[A][B]}{K_4 K_1}}$$

$$K x_1 = \frac{[A]}{K_1} x_0$$

$$x_2 = \frac{[B]}{K_2} x_0$$

$$x_3 = \frac{[B][A]}{K_4 K_1} x_0$$

$$\Rightarrow v = x_0 v_0 + x_1 v_1 + x_2 v_2 + x_3 v_3$$

$$= x_0 \left[v_0 + \frac{[A]}{K_1} v_1 + \frac{[B]}{K_2} v_2 + \frac{[A][B]}{K_4 K_1} v_3 \right]$$

where $x_0 = \frac{1}{1 + \frac{[A]}{K_1} + \frac{[B]}{K_2} + \frac{[A][B]}{K_4 K_1}}$

\Rightarrow this is our 'constitutive' eqn
for vtranscription!

\rightarrow

complete the model of dynamics

ie sub into

$$\frac{dR}{dt} = \boxed{\text{transcription}} + v_{R,\deg}$$

$$\frac{dP}{dt} = v_{\text{translation}} - v_{P,\deg}$$

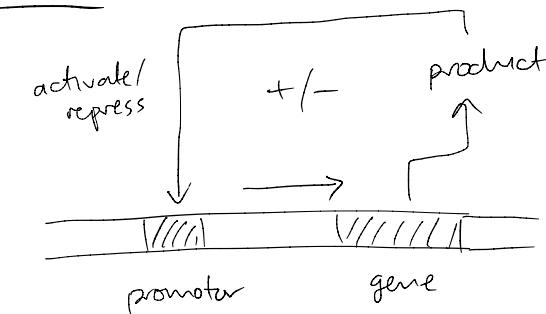
simplistic extras to 'complete'

$$\text{eg } v_{R,\deg} = k_{R,\deg} \cdot R$$

$$v_{P,\deg} = k_{P,\deg} \cdot P$$

actually
much more
complicated → $v_{\text{translation}} = k_{\text{translation}} \cdot R$

Feedback



here product affects own transcription !

⇒ autoregulation { positive (\uparrow transcription)
self negative (\downarrow transcription)

→ will briefly look at example next time

+
look at large scale expression regulation in last lecture.

Appendix

Genes & all that

In all cells, the 'info' required for regulating cell function is stored or coded in its genome

↳ genome: all genetic material
eg DNA/RNA etc

Languages

<u>Genetic</u>	<u>Protein I</u>	<u>Protein II</u>
Letters: DNA (ACGT)	Letters: amino acids	Letters: primary structure
Words: codons (triplets)	Words: peptides	Words: secondary structure
Sentences: genes	Sentences/parag. polypeptides	Sentences: tertiary struc. Paragraphs quaternary

Appendix Cont'd

Gene expression

DNA → Protein / Polypeptide
?
↳

needs:

- transcription &
- translation } from one language to another } uses RNA

Transcription & Translation

