

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/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]

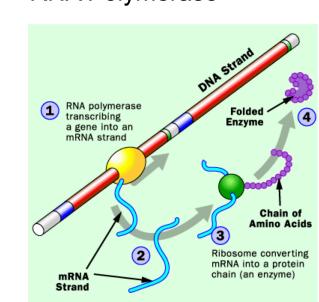
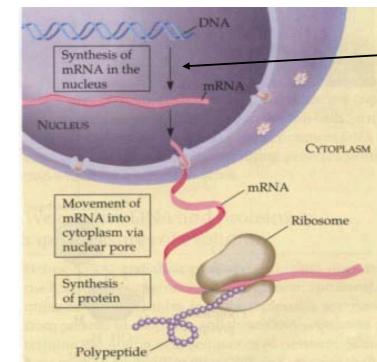
Overview of systems biology. Modelling signalling systems using reaction kinetics. Introduction to parameter estimation. Modelling metabolic systems using reaction kinetics. Flux balance analysis and constraint-based methods.

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.

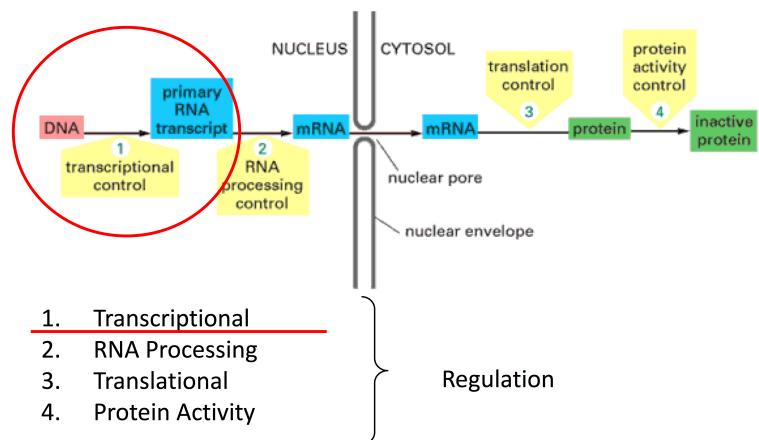
LECTURE 10: MODELLING GENE EXPRESSION AND REGULATION

- 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

GENE EXPRESSION AND REGULATION



GENE EXPRESSION AND REGULATION



1. Transcriptional
2. RNA Processing
3. Translational
4. Protein Activity

TRANSCRIPTION REGULATION TYPES

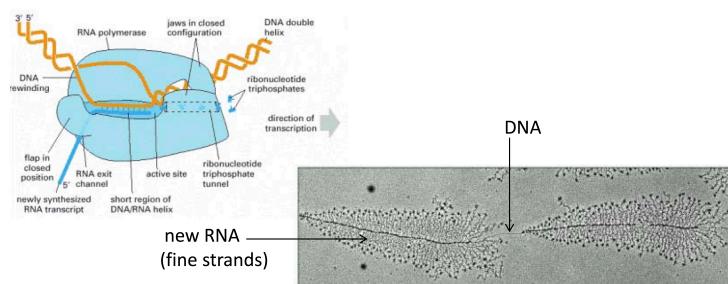
Gene transcription is regulated by various *transcription factors*.

These can be

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

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

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/

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.

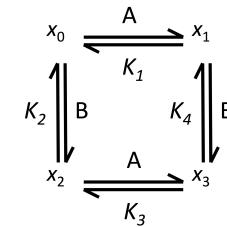
Note: using v instead of J just for consistency with typical approaches. Same basic thing - a *flux*.

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...

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...

OVERALL BALANCE 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.

EXAMPLES

See handout.

Biomeng 261 : Lecture 10

Gene expression & regulation

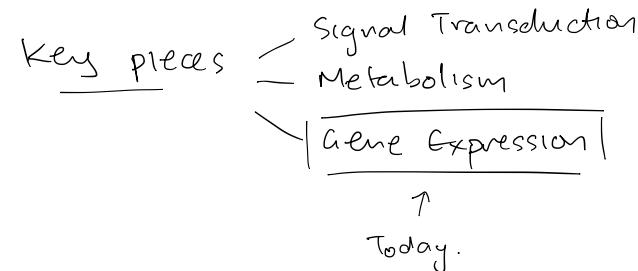
- Brief background
- Modelling via 'reaction' systems

Key idea(s)

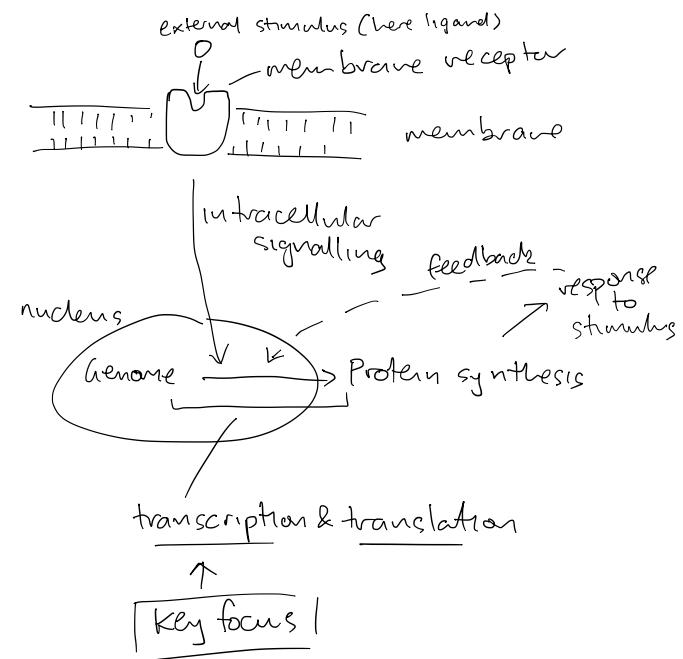
- Today
- We can use same basic 'reaction modelling' language to model genetic expression & regulation
 - genes 'switch' between various states depending on regulatory molecules
 - ↳ use fractions/probability instead of conc.

- 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

transcription translation

DNA → mRNA → Protein

‘R’ ‘P’

$$\frac{dR}{dt} = \underbrace{J_{\text{transcription}} - J_{R,\text{degradation}}}_{\begin{array}{l} \text{goal (want a} \\ \text{— constitutive expr)} \end{array}}$$

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

Notes: • mRNA is 'created' but is
then translated,
| not used up directly |
↳ degrades instead.

- We'll often use ∇ instead of \vec{J}

$$ce \frac{dR}{dl} = v_{\text{transcription}} - v_{\text{deg}}$$

etc.

Main focus: Transcription & its regulation

Goal: a constitutive equation for
the overall transcription rate

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

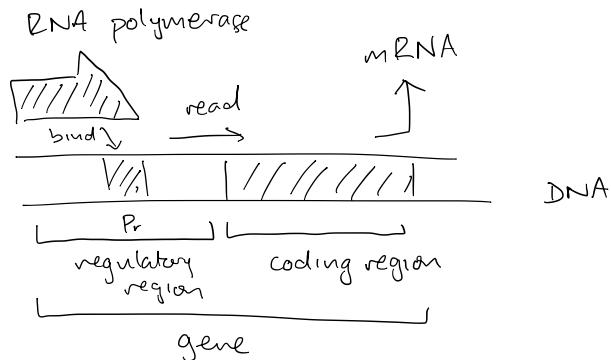
↳ will be a weighted average
of transcription rate
for each 'gene state'

What to include?

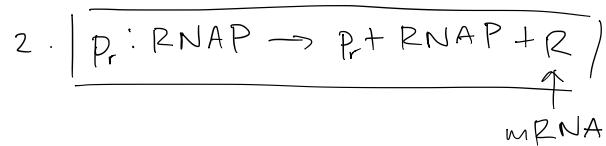
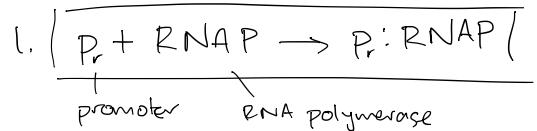
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graph LR
    A[Simple models] --> B[prokaryotic]
    A --> C[eukaryotic]
    B --- D[easier]
    C --- E["same ideas,  
more complicated"]
  
```

Transcription: Simple picture (mainly motivation)



'Reaction' steps



Note: as if R 'created out of nothing'

↳ created from other chem. species in cell.

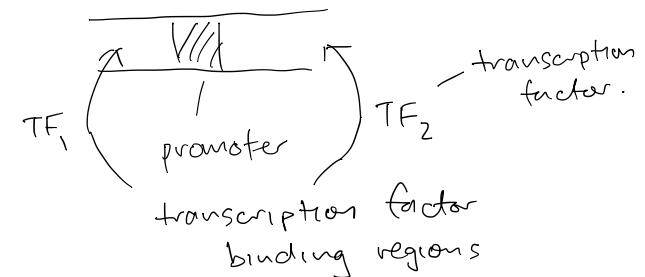
↳ depends on 'level of modelling'

Transcription: complications

(Eukaryotes:
many TFs!)

Regulation!

- within 'regulatory region':



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

- Types:

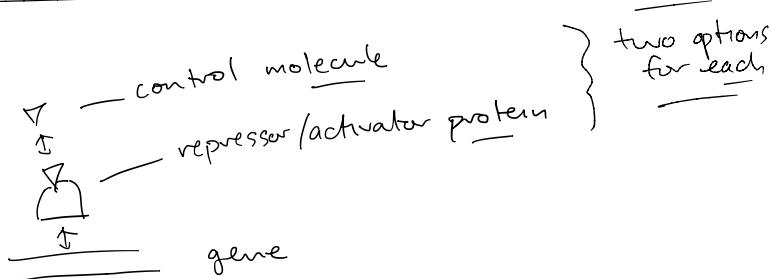
• Activator
↳ TF binding enables/helps binding of RNAP

Repressor

↳ TF blocks/decreases binding of RNAP.

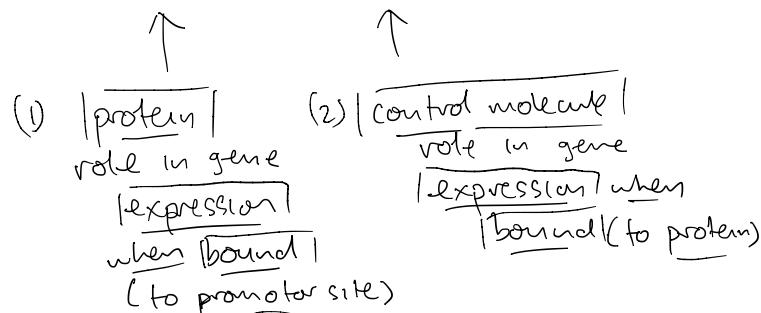
Autoregulation: gene codes for its own activators (+ve) or repressors (-ve)

Even more complication: control molecules



Terminology (%)

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



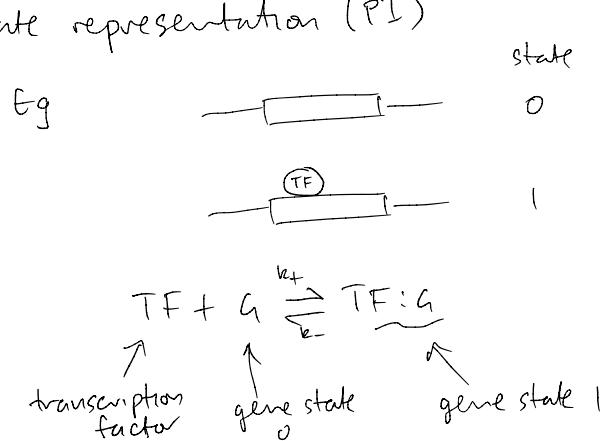
Gene regulatory states

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

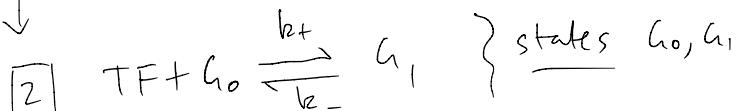
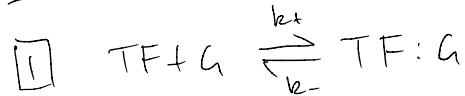
→ we model the gene as being in various ['regulatory states'] & switching between them

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

State representation (PI)



Gene states: Alternative representations (PI)



using $J_+ = \frac{k_+ [\text{TF}][\text{G}_0]}{\text{combine}}$
 $J_- = k_- [\text{G}_1]$



Interpret:

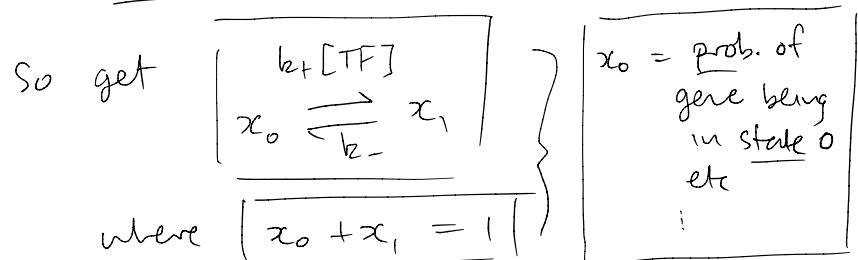


Concentrations? Probabilities? →

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

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

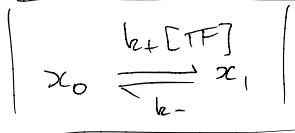


(Basically: divide everything by $\text{G}_0 + \text{G}_1 + \dots = \text{constant}$)

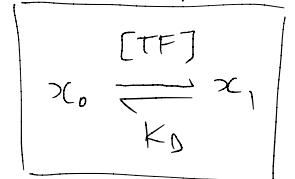


- - More representations - -

Finally, instead of just



we will also picture this as



(divide rates by
 k_+)

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

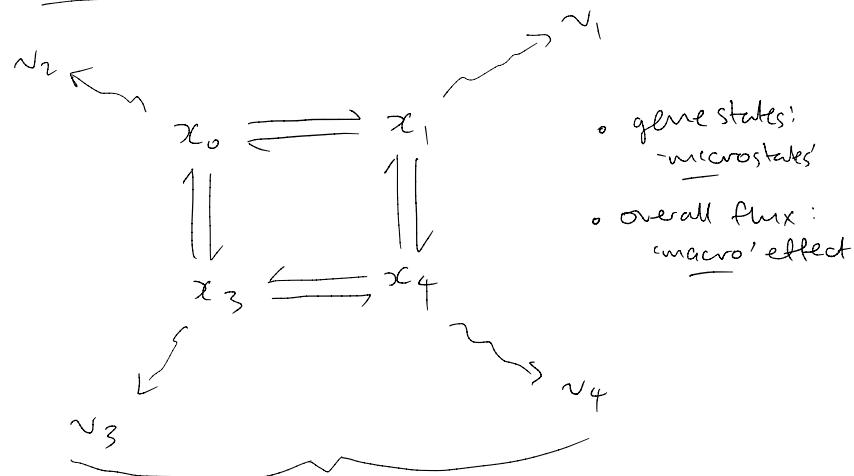
→ $\boxed{\text{add up weighted contributions of each state to total transcription flux} \sim}$

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:



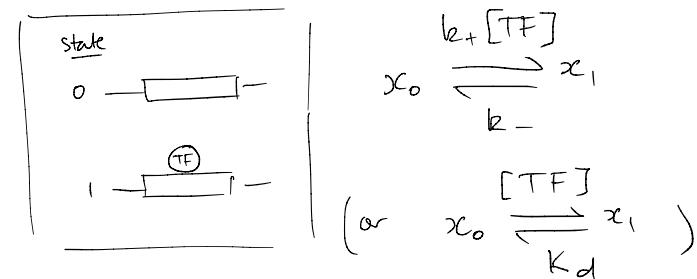
average/overall behaviour:

$$v = \sum x_i v_i$$

- Multiple states with different occupancy probabilities x_i } find using quasi-eqnl.
- 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

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

b). Total fraction = 1

$$\Rightarrow \boxed{x_0 + x_1 = 1} \quad |$$

2. Two equations, two unknowns x_0, x_1

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

& solve

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

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

use $x_1 = 1 - x_0$

$$\Rightarrow \boxed{x_0 = \frac{K_d}{K_d + [TF]}} \quad |$$

$$\Rightarrow \boxed{x_1 = \frac{[TF]}{K_d + [TF]}} \quad |$$

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

$$= \frac{K_d}{K_d + [TF]} \cdot \nu_0 + \frac{[TF]}{K_d + [TF]} \cdot \nu_1$$

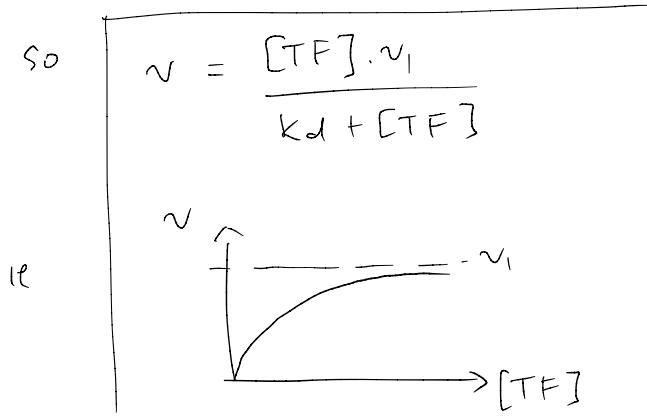
4. Specify ν_0, ν_1 based on [TF] type.

eg suppose [TF] is a perfect activator

→ increases transcription

→ no transcription without

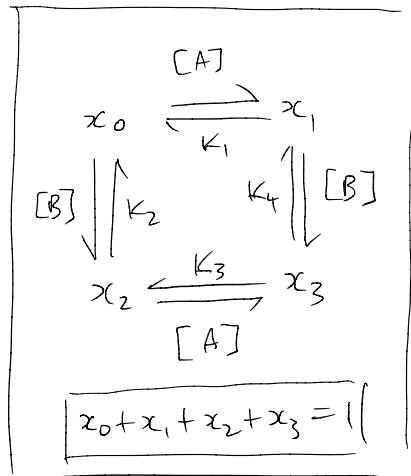
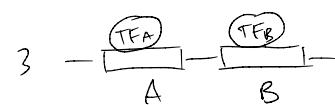
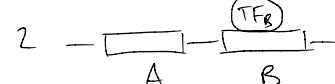
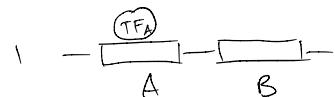
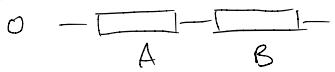
$$\Rightarrow \text{set } \underline{\nu_0 = 0}$$



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

Multiple TFs : two TFs \Rightarrow four gene states

State



Steps

$$x_1 = \frac{[A]x_0}{k_1}$$

$$x_2 = \frac{[B]x_0}{k_2}$$

$$(3 \text{ eqn}) \quad x_3 = \frac{[B]x_1}{k_4} \Rightarrow x_3 = \frac{[B]}{k_4} \cdot \frac{[A]x_0}{k_1}$$

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

$$\text{lb. sub into } x_0 + x_1 + x_2 + x_3 = 1$$

$$(1 \text{ eqn}) \quad \Rightarrow x_0 + x_0 \frac{[A]}{k_1} + x_0 \frac{[B]}{k_2} + \frac{[B][A]}{k_4 k_1} = 1$$

$$\Rightarrow x_0 \left(1 + \frac{[A]}{k_1} + \frac{[B]}{k_2} + \frac{[B][A]}{k_4 k_1} \right) = 1$$

\Rightarrow

$$\Rightarrow x_0 = \frac{1}{1 + \frac{[A]}{k_1} + \frac{[B]}{k_2} + \frac{[A][B]}{k_4 k_1}}$$

$$x_1 = \frac{[A]x_0}{k_1}$$

$$x_2 = \frac{[B]x_0}{k_2}$$

$$x_3 = \frac{[B][A]}{k_4 k_1} x_0$$

} sub x_0
in.

or just jump
to

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

$$= x_0 \left[v_0 + \frac{[A]}{k_1} v_1 + \frac{[B]}{k_2} v_2 + \frac{[A][B]}{k_4 k_1} v_3 \right]$$

$$\text{where } x_0 = \frac{1}{1 + \frac{[A]}{k_1} + \frac{[B]}{k_2} + \frac{[A][B]}{k_4 k_1}}$$

\Rightarrow this is our 'constitutive' eqn
for $v_{\text{transcription}}$!

\rightarrow

complete the model of dynamics

ie sub into

$$\frac{dR}{dt} = \overbrace{\nu_{\text{transcription}}} + \nu_{R,\deg}$$

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

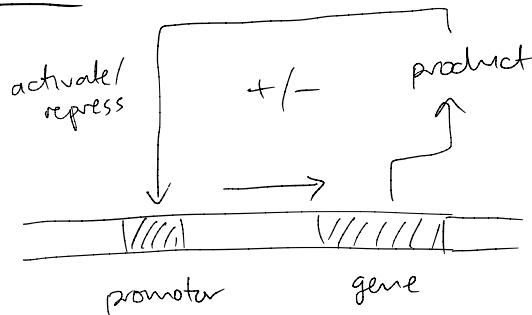
simplistic extras to 'complete'

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

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

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

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

L 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. poly peptides	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

