

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)

[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.

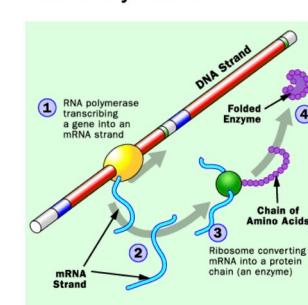
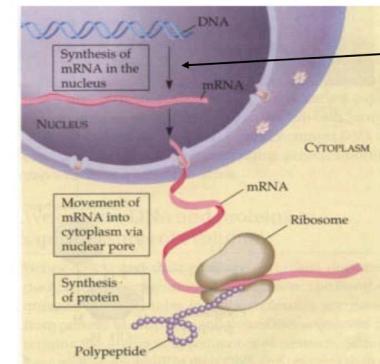
2. Systems biology I: signalling and metabolic systems [3 lectures]

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.

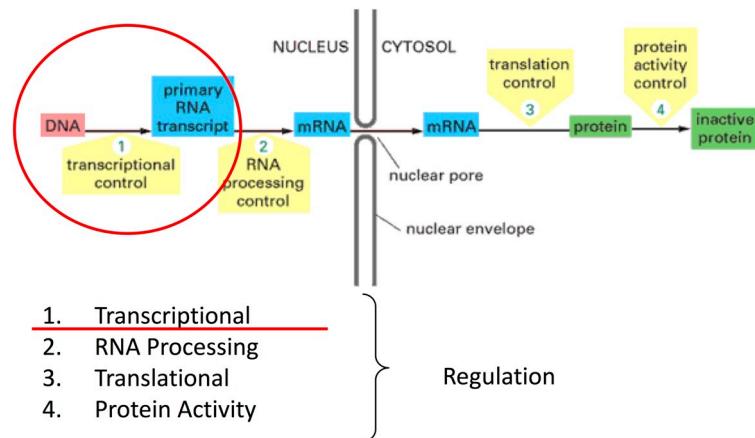
GENE EXPRESSION AND REGULATION



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GENE EXPRESSION AND REGULATION



TRANSCRIPTION REGULATION TYPES

Gene transcription is regulated by various *transcription factors*.

These can be

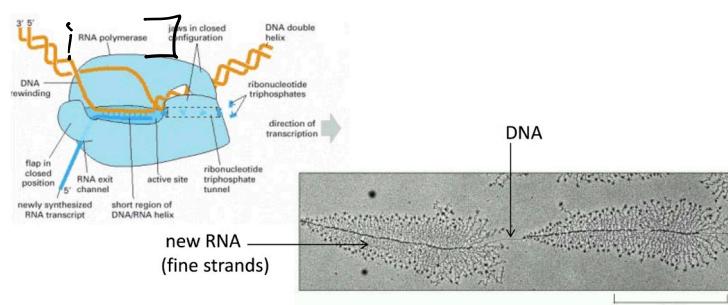
- *Activators* (increase transcription)
- *Repressors* (decrease transcription)

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/

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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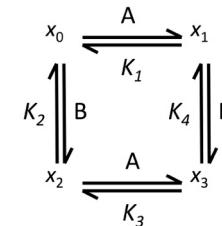
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GENE REGULATORY STATES AND FLUXES

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

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

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

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

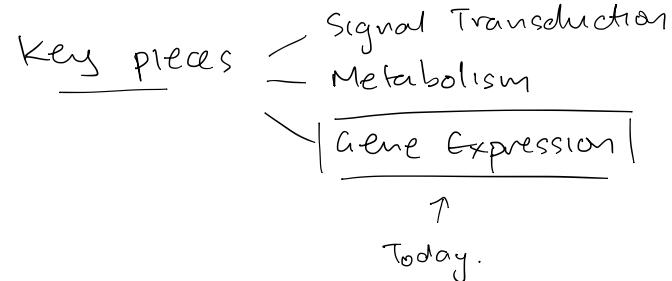
- Brief background
- Modelling via 'reaction' systems

Key idea(s)

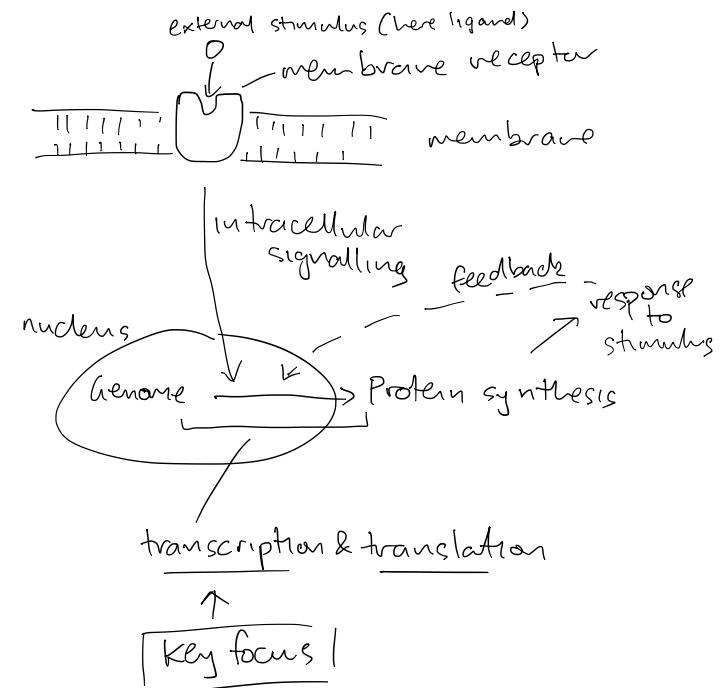
- Today -
- We can use same basic 'reaction modelling' language to model genetic expression & regulation
 - genes 'switch' between various states depending on regulatory molecules
 - ↳ use fractions/probability instead of on/off.

- Later lectures
- again encounter 'scaling up' issues when dealing with large systems

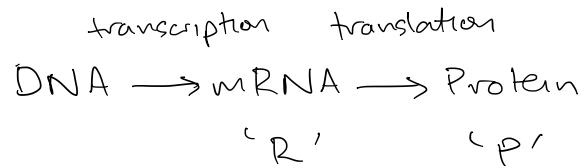
Cellular Systems Biology (Recall)



Recal signal transduction:



Simplistic overall balance equation



$$\frac{dR}{dt} = \underbrace{\mathcal{T}_{\text{transcription}}}_{\text{goal (want a constitutive eq)}} - \mathcal{T}_{R,\text{degradation}}$$

$$\frac{dP}{dt} = \mathcal{T}_{\text{translation}} - \mathcal{T}_{P,\text{degradation}}$$

Notes:

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

We'll often use \bar{n} instead of \mathcal{T}

$$\text{e.g. } \frac{dR}{dt} = \bar{n}_{\text{transcription}} - \bar{n}_{\text{deg}} \text{ etc.}$$

Main focus: Transcription & its regulation

The goal: a constitutive equation for the overall transcription rate

↳ will derive from an 'underlying' model of gene states
 ↳ like MM & enzymes.

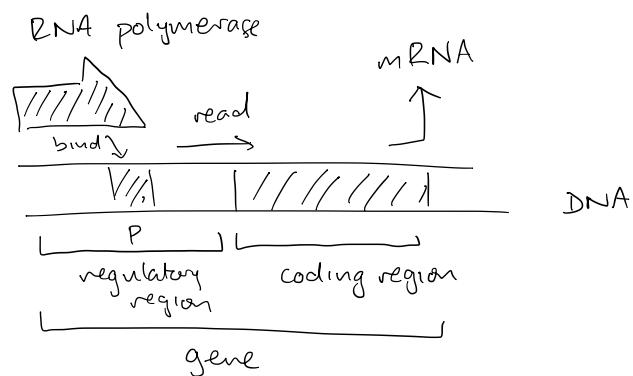
↳ will be a weighted average of transcription rate for each 'gene state'

What to include?

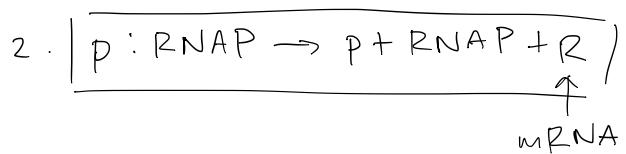
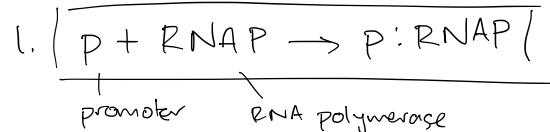
Simple models

- prokaryotic } easier
- eukaryotic } same ideas, more complicated

Transcription: Simple picture



'Reaction' steps



Note: as if R 'created out of nothing'

↳ created from other chem.
species in cell.

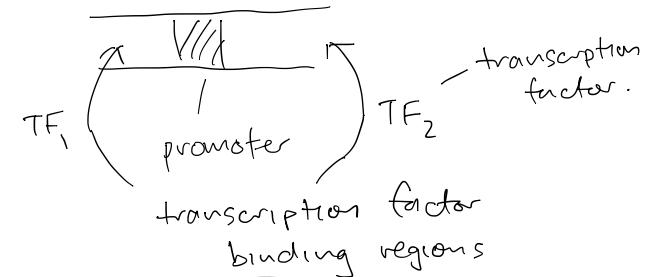
↳ depends on 'level of
modelling'

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.

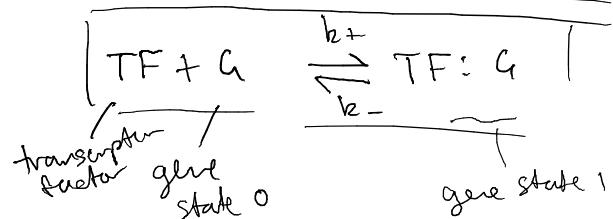
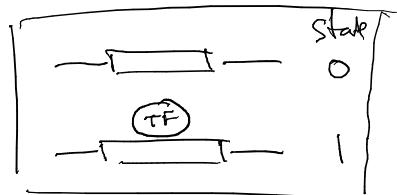
Autoregulation: gene codes for its own activators (+ve) or repressors (-ve)

Gene regulatory States

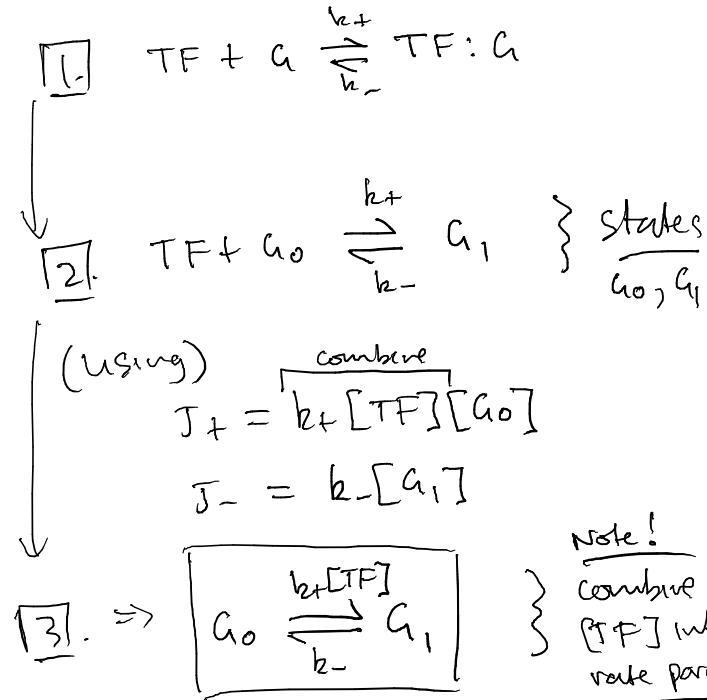
- In general there may be various transcription factors bound to the regulatory region of a gene
- We model the gene as being in various 'regulatory States' & switching between them
- each state has a different contrb. to overall transcription flux

State-P.I.

Example:



States : various representations



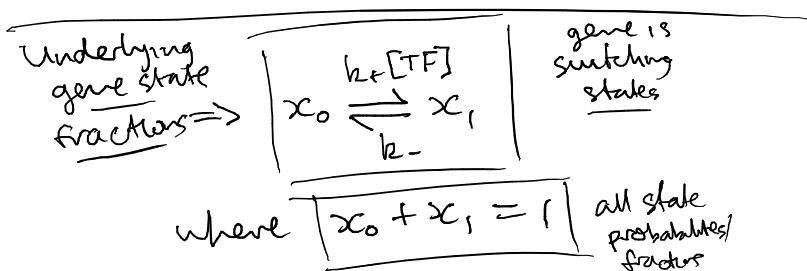
⇒ switching states $G_0 \leftrightarrow G_1$

Concentrations? →

States: Fractional (Occupancy) Probabilities

- Rather than concentrations we will work in terms of the Fractional/occupancy probabilities of each state of a given gene.
- then average over many cases to get overall flux -

- Again, each state will then have a different transcription rate

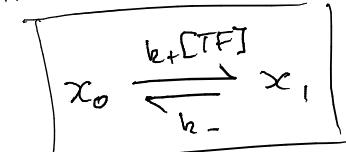


(Basically: divide everything by $k_0 + k_+ = \text{const.}$)

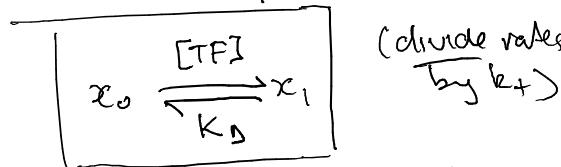
Interpretation: $x_0 = \text{probability}$ of gene being in state 0
etc \rightarrow will then 'average' over.

More state representations ...

Finally ... instead of just



we will also picture this as:



where $K_D = \frac{k_-}{k_+}$ (equil.) dissociation constant

Note: \rightarrow same relative rates!

(we will only consider equilibrium, so units issue not import!)
only relative rates matter here

States & transcription rates

- gene can be in multiple states
- rapidly switches between
 - will assume quasi-equl.
 - gives equilibrium occupancy probabilities
- add up contributions of each state to ν

Combining contributions to overall flux:

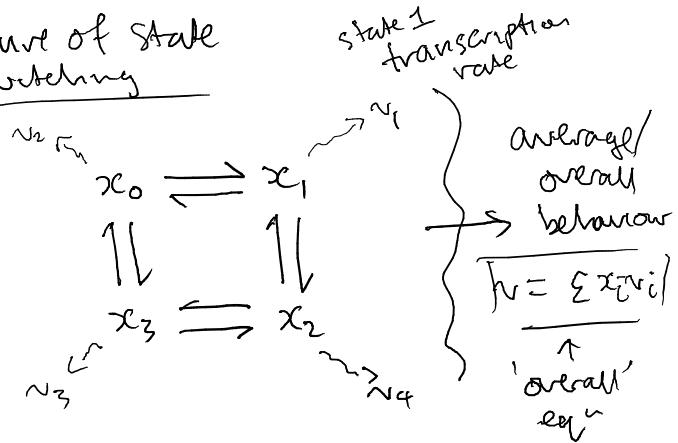
overall constn.
eqn.

$$\nu_{\text{transcriber}} = \sum_{s=0}^{m-1} x_s \nu_s$$

where x_s : probability of gene being in state s

ν_s : transcription rate (flux) for state s .

Picture of state switching



- multiple states with different occupancy probabilities x_i

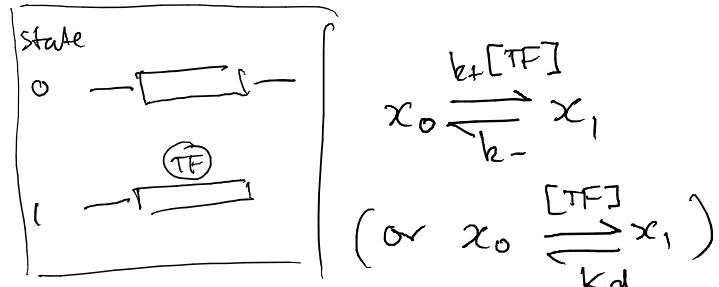
[we will find quasi-equl.]

- each state has transcription rate v_i
- overall transcription rate = $\sum x_i v_i$ } average, over, will use quasi-equl.

[gene states: 'microstates'
overall flux: 'macro' effect]

Example! Single TF.

Two gene state model



Steps

1. Assume gene state model
in equilibrium (\Rightarrow total fraction = 1)

2. Find fractions x_0, x_1, \dots

3. Find overall transcription

$$v = v_0 x_0 + v_1 x_1 + \dots$$

4. Det. v_0, v_1 , etc
depending on whether TF
is activator / inhibitor etc

1a) Equilibrium

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

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

1b). Total fraction = 1 (recall enzymes & E_0)
 $\Rightarrow x_0 + x_1 = 1$

2. [Two equations for x_1 & x_2]

combine:

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

$$\Rightarrow \begin{cases} x_0 = \frac{K_d}{K_d + [\text{TF}]} \\ x_1 = \frac{[\text{TF}]}{K_d + [\text{TF}]} \end{cases}$$

$$3. \quad v_{\text{transcription}} = x_0 v_0 + x_1 v_1$$

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

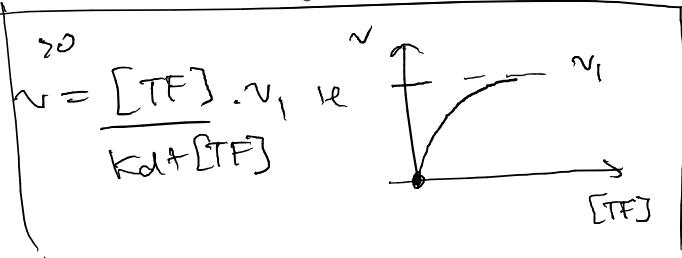
4. Suppose TF is activator (for example)

→ increases transcription.

& suppose is required for transcription

→ no transcription without .

⇒ set $v_0 = 0$

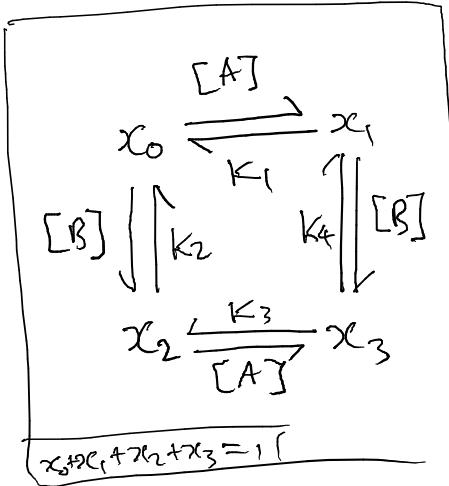
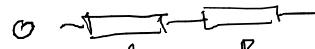


Exercise: what if TF is a repressor?

connected to switch scheme open

Multiple TFs : two TFs \Rightarrow Four gene states.

State



$$\text{Steps: } \frac{x_1}{x_0} = \frac{[A]}{K_1}; \frac{x_2}{x_0} = \frac{[B]}{K_2}$$

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

$$\begin{aligned} &\text{Solve for } x_0 \\ &\Rightarrow x_0 = \frac{1}{1 + \frac{[A]}{K_1} + \frac{[B]}{K_2} + \frac{[A][B]}{K_1 K_4}} \end{aligned}$$

$$\begin{aligned} &(\text{then } x_1 = -x_0, x_2 = -x_0) \\ &x_3 = -x_0 \end{aligned}$$

So

$$\begin{aligned}N_{\text{transcription}} &= x_0 v_0 + x_1 v_1 + x_2 v_2 + x_3 v_3 \\&= x_0 \left[v_0 + v_1 \frac{[A]}{K_1} + v_2 \frac{[B]}{K_2} + v_3 \frac{[A][B]}{K_1 K_2} \right]\end{aligned}$$

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

| use as 'constit' eq.

Recall:

$$\frac{dR}{dt} = N_{\text{transcription}} - N_{\text{deg}}$$
$$\frac{dP}{dt} = N_{\text{translation}} - N_{\text{pdeg.}}$$

Simplistic: eg $N_{\text{deg}} = k_{\text{deg}} \cdot R$

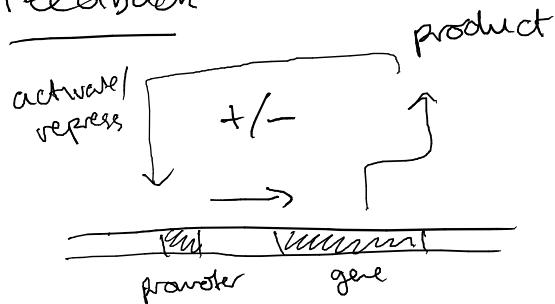
actually much more complicated!

$$N_{\text{deg}} = k_{\text{deg}} \cdot P$$

actually much more complicated!

$$N_{\text{translation}} = k_{\text{translation}} \cdot R$$

Feedback



Here product affects own transcription

↓
auto regulation! { positive (\uparrow transcript)
negative (\downarrow transcript)}

will briefly look at example next time

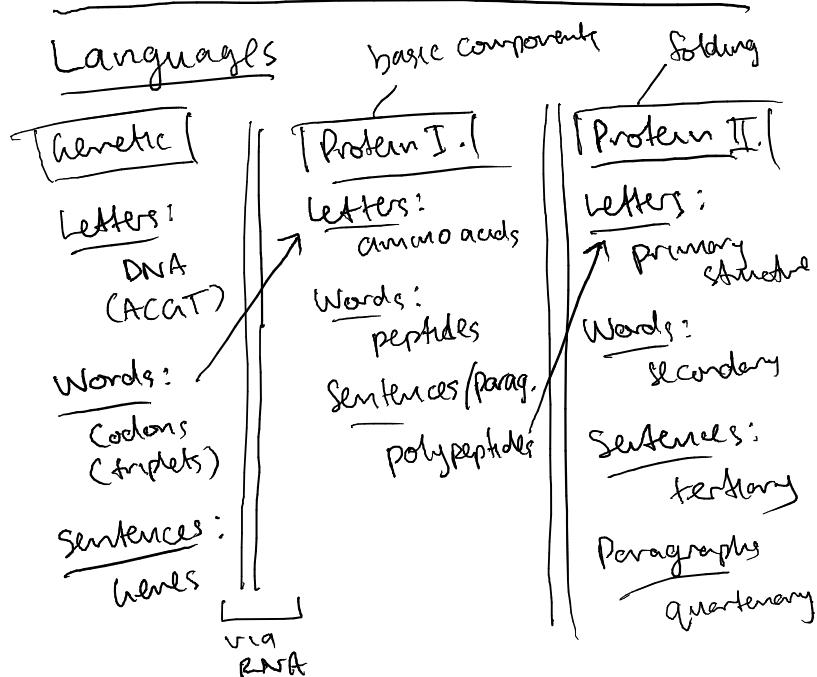
+
look at larger scale expression/regulation

Appendix

Genes & all that...

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

- all genetic material
 - eg DNA, RNA etc



Appendix Cont'd.

Gene expression

DNA → Protein / Polypeptide

?

Needs:

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

Transcription & Translation

