Bioinformatics 3

V18 – Kinetic Motifs

Fri, Jan 8, 2016
Modelling of Signalling Pathways

Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell
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1) How do the magnitudes of signal output and signal duration depend on the kinetic properties of pathway components?

(2) Can high signal amplification be coupled with fast signaling?

(3) How are signaling pathways designed to ensure that they are safely off in the absence of stimulation, yet display high signal amplification following receptor activation?

(4) How can different agonists stimulate the same pathway in distinct ways to elicit a sustained or a transient response, which can have dramatically different consequences?
Linear Response

E.g., protein synthesis and degradation (see lecture V8)

S = signal (e.g., concentration of mRNA)
R = response (e.g., concentration of a protein)

\[
\frac{dR}{dt} = k_0 + k_1 S - k_2 R
\]

At steady state (which implies \( S = \text{const} \)):

\[
\frac{dR}{dt} \bigg|_{R=R_{ss}} = 0 \implies R_{ss} = \frac{k_0 + k_1 S}{k_2} = \frac{k_0}{k_2} + \frac{k_1}{k_2} S
\]

\( R_{ss} \) linearly dependent on \( S \)

\( k_0 = 1, k_1 = k_2 = 2 \)
**phosphorylation/dephosphorylation**

„forward“: R is converted to phosphorylated form RP

„backward“: RP can be dephosphorylated again to R

\[
S + R \Rightarrow RP
\]

\[
RP \Rightarrow R + T
\]

with \( \text{R}_{\text{tot}} = R + RP \)

\[
\frac{dRP}{dt} = k_1 SR - k_2 RP = k_1 S (\text{R}_{\text{tot}} - RP) - k_2 RP
\]

Find steady state for RP: linear until saturation

\[
\text{RP}_{ss} = \frac{k_1 \text{R}_{\text{tot}} S}{k_1 S + k_2} = \frac{\text{R}_{\text{tot}} S}{S + k_2/k_1} = \frac{\text{R}_{\text{tot}} S}{S + S_0}
\]

Output T proportional to RP level:

\[
\frac{dT}{dt} = k_2 RP
\]

\( \text{R}_{\text{tot}} = 1, \ S_0 = 1 \)
Enzyme: Michaelis-Menten-kinetics

Reaction rate: \[ V = k_{off}ES \]

Steady state: \[ k_{on}E \cdot S = k_{off}ES \]

\[ ES = \frac{k_{on}E \cdot S}{k_{off}} = \frac{E \cdot S}{K_M} \]

Total amount of enzyme is constant:

\[ E_T = E + ES \Rightarrow ES = E_T \frac{S}{S + K_M} \]

turnover: \[ V = V_{max} \frac{S}{S + K_M} \]
The MM-equation

Effective turnover according to MM:

\[ V = V_{max} \frac{S}{S + K_M} \]

\[ V_{max} = k_{off} E_T \]

\[ K_M = \frac{k_{off}}{k_{on}} \]

Pro:
- analytical formula for turnover
- curve can be easily interpreted: \( V_{max} \), \( K_M \)
- enzyme concentration can be ignored

Cons:
less kinetic information

\( k_{on}, k_{off}, E_T \)  \( \Rightarrow \)  \( V_{max}, K_M \)
Sigmoidal Characteristics with MM kinetics

Same topology as before with Michaelis-Menten kinetics for phosphorylation and dephosphorylation.

\[
\frac{dRP}{dt} = \frac{k_1 S (R_t - RP)}{R_0 + (R_t - RP)} - \frac{k_2 RP}{R P_0 + RP} + 0
\]

\[V = V_{max} \frac{S}{S + K_M}\]

this means that \( S = R_t - RP \)

\[K_M = R_0\]

Quadratic equation for RP

\[k_2 RP(R_o + (R_t - R_p)) = k_1 S(R_t - RP)(R P_0 + RP)\]

=> sigmoidal characteristics

(threshold behavior)

often found in signalling cascades

\[R_t = 10, \ R_0 = R P_0 = 1, \ k_1 = k_2 = 1\]
Graded Response

Linear, hyperbolic, and sigmoidal characteristic give the same steady state response independent of the previous history
=> no hysteresis

BUT: In fast time-dependent scenarios, delay may lead to a modified response
Time-dependent Sigmoidal Response

Direct implementation:

\[ v_1 = \frac{S k_1 R}{R_0 + R} \quad v_2 = \frac{k_2 R P}{R P_0 + R P} \]

Parameters: \( k_1 = 1 \text{ (mol s)}^{-1}, \ k_2 = 1 \text{ s}^{-1}, \ R_0 = R P_0 = 1 \text{ mol} \)

Initial conditions: \( R = 10 \text{ mol}, \ R P = 0 \)

Time courses for \( S = 1, \ 1.5, \ \text{and} \ 2, \ R P(0) = 0: \)

equilibrium is reached faster for stronger signal
Adaption - „sniffer“

Linear response modulated by a second species X

\[
\frac{dX}{dt} = k_3 S - k_4 X \quad \quad \quad \frac{dR}{dt} = k_1 S - k_2 X R
\]

Steady state: \( R_{ss} \) independent of \( S \)

\[
X_{ss} = \frac{k_3}{k_4} S \quad \quad \quad R_{ss} = \frac{k_1 k_4}{k_2 k_3}
\]

\( R \) changes transiently when \( S \) changes, then goes back to its basal level.

found in smell, vision, chemotaxis, …

Note: response strength \( \Delta R \) depends on rate of change of \( S \).

\( \Rightarrow \) non-monotonous relation for \( R(S) \)

\( k_1 = 30, k_2 = 40, k_3 = k_4 = 5 \)
Positive Feedback

Feedback via R and EP
=> high levels of R will stay

"one-way switch" via bifurcation

Found in processes that are "final": frog oocyte maturation, apoptosis, …
Mutual Inhibition - Toggle Switch

\[
\frac{dR}{dt} = k_1 S - k_2 R - k_4 E(R)
\]

\[
\frac{dEP}{dt} = \frac{k_3 R E}{EP_0 + EP} - \frac{k_5 EP}{E_0 + E}
\]

Sigmoidal "threshold" in E <=> EP leads to bistable response (hysteresis): **toggle switch**

Converts continuous external stimulus into two well defined stable states:

- lac operon in bacteria
- activation of M-phase promoting factor in frog eggs
Negative Feedback

S controls the "demand" for R

=> homeostasis
found in biochemical pathways,
no transient changes in R for steps in S
(cf. "sniffer")
Negative Feedback with Delay

Cyclic activation $X \Rightarrow YP \Rightarrow RP \Rightarrow X \Rightarrow$ Oscillations (in a range of $S$)

\[
\frac{dX}{dt} = k_0 + k_1 S - k_2 X - k_7 RP \cdot X
\]

\[
\frac{dYP}{dt} = \frac{k_3 X \cdot Y}{Y_0 + Y} - \frac{k_4 YP}{YP_0 + YP}
\]

\[
\frac{dRP}{dt} = \frac{k_5 YP \cdot R}{R_0 + R} - \frac{k_6 RP}{RP_0 + RP}
\]

Proposed mechanism for circadian clocks
Circadian Clocks

Figure 1. A network of transcriptional–translational feedback loops constitutes the mammalian circadian clock.

PER: period
CRY: cryptochrome

CK1: casein kinase
Rev-erb, ROR: retinoic acid-related orphan nuclear receptors
Cdg: clock-controlled gene(s)

Ko & Takahashi Hum Mol Genet
15, R271 (2006)
Substrate-Depletion Oscillations

R is produced in an **autocatalytic** reaction from X, finally **depleting** X…

Similar to Lotka-Volterra system (autocatalysis for X, too):
The Cell Cycle

DNA replication

DNA separation (mitosis)

M-phase

G1-phase

G2-phase

S-phase

Cell division (cytokinesis)

cell growth

When to take the next step???
Cell Cycle Control

Oscillatory networks underlie the
- circadian clock,
- the beating of our hearts, and
- the cycle of cell division, which creates two cells from one, driving the reproduction and development of living systems.

Already simple genetic circuits can give rise to oscillations.

E.g., a negative feedback loop \( X \rightarrow R \rightarrow X \) can yield oscillations (\( X \) activates \( R \), which inhibits \( X \), so that \( R \) goes down, so that \( X \) goes back up...).

Such a circuit requires significant non-linearity or a time delay to keep from rapidly settling to a constant steady state.

An oscillator of this sort is thought to be the core of many eukaryotic cell cycles.

Cell Cycle Control System

cdc = "cell division cycle"

Feedback loops control cell cycle

A negative feedback loop can give rise to oscillations. Here, such an oscillator forms the core of eukaryotic cell cycles.

Cyclin–CDK acts as activator, and APC-Cdc20 acts as repressor.

Non-linearity in APC-Cdc20 activation prevents the system from settling into a steady state.

- CDKs require the binding of a cyclin subunit for activity. These cyclin partners can also determine the localization of the complex and its specificity for targets.
- At the beginning of the cell cycle, cyclin–CDK activity is low, and ramps up over most of the cycle. Early cyclins trigger production of later cyclins and these later cyclins then turn off the earlier cyclins, so that control is passed from one set of cyclin–CDKs to the next.
- The last set of cyclins to be activated, the G2/M-phase cyclins, initiate mitosis, and also initiate their own destruction by activating the APC-Cdc20 negative feedback loop. APC-Cdc20 targets the G2/M-phase cyclins for destruction, resetting the cell to a low-CDK activity state, ready for the next cycle.

G1 $\Rightarrow$ S — Toggle Switch

Mutual inhibition between Cdk1-CycB and CKI (cyclin kinase inhibitor)

Mutual Inhibition

Assume: CycB:Cdk1:CK1 is stable  \( \iff \) dissociation is very slow

\[ \Rightarrow \text{same topology} \]

\[ \iff \text{same bistable behavior (?)} \]
Rate Equations: Toggle Switch

Stoichiometric matrix
"(C)" = catalyst

\[
\frac{dR1}{dt} = k_1 \ A \ S \\
\frac{dR2}{dt} = k_2 \ R \ E \\
\frac{dR3}{dt} = k_3 \ R \ E \\
\frac{dR4}{dt} = \frac{V_4 \ EP}{EP_0 + EP}
\]

\[
\frac{dR}{dt} = \frac{dR1}{dt} - \frac{dR2}{dt} = k_1 \ A \ S - k_2 \ R \ E
\]

\[
\frac{dE}{dt} = \frac{dR4}{dt} - \frac{dR3}{dt}
\]

\[
\begin{array}{|c|c|c|c|}
\hline
 & R1 & R2 & R3 & R4 \\
\hline
A & -1 &  &  &  \\
S & (C) &  &  &  \\
R & 1 & -1 & (C) &  \\
E & (C) & -1 & 1 &  \\
EP & 1 & -1 &  &  \\
X & 1 &  &  &  \\
\hline
\end{array}
\]
Rate Equations: G1/S Module

\[
\begin{align*}
\frac{dR_1}{dt} &= k_1 \, [\text{CycB}] \, [\text{Cdk1}] \\
\frac{dR_2}{dt} &= k_2 \, [\text{CycB}:\text{Cdk1}] \, [\text{CKI}] \\
\frac{dR_3}{dt} &= \frac{k_3 \, [\text{CycB}:\text{Cdk1}] \, [\text{CKI}]}{K_3 + [\text{CKI}]} \\
\frac{dR_4}{dt} &= \frac{V_4 \, [\text{CKI}:\text{P}_3]}{K_4 + [\text{CKI}:\text{P}_3]} \\
\end{align*}
\]

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
& R_1 & R_2 & R_3 & R_4 & R_5 & R_6 \\
\hline
\text{CycB} & -1 & & & & & \\
\text{Cdk1} & -1 & & & & & \\
\text{CycB}:\text{Cdk1} & 1 & -1 & (C) & 1 & & \\
\text{CKI} & -1 & -1 & 1 & 1 & & \\
\text{CKI}:\text{P}_3 & 1 & -1 & & & & \\
\text{CycB}:\text{Cdk1}:\text{CKI} & 1 & & & & -1 & \\
\hline
\end{array}
\]

\[
\begin{align*}
\frac{d[\text{CycB}:\text{Cdk1}]}{dt} &= \frac{dR_1}{dt} - \frac{dR_2}{dt} + \frac{dR_6}{dt} \\
\frac{d[\text{CKI}]}{dt} &= \frac{dR_4}{dt} - \frac{dR_3}{dt} - \frac{dR_2}{dt} + \frac{dR_6}{dt} \\
\end{align*}
\]
Comparison: Matrices

### Comparison Table

<table>
<thead>
<tr>
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<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
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</thead>
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<td></td>
<td></td>
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<tr>
<td>S</td>
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<td>(C)</td>
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<tr>
<td>R</td>
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<td>−1</td>
<td>(C)</td>
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<td>E</td>
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<td>EP</td>
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<tr>
<td>X</td>
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### Difference Table

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
</tr>
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<tbody>
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<tr>
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<tr>
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<td>−1</td>
<td>(C)</td>
<td></td>
<td>1</td>
<td></td>
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<tr>
<td>CKI</td>
<td>−1</td>
<td>−1</td>
<td>1</td>
<td></td>
<td>1</td>
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<tr>
<td>CKI:P₃</td>
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<td>−1</td>
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<tr>
<td>CKI:P₃</td>
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<td>−1</td>
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<tr>
<td>CycB:Cdk1:CKI</td>
<td>1</td>
<td></td>
<td></td>
<td>−1</td>
<td></td>
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</tr>
</tbody>
</table>

**Difference:** catalysts vs. substrates
Comparison: Equations

\[
\begin{align*}
\frac{dR_1}{dt} &= k_1 \, A \, S \\
\frac{dR_2}{dt} &= k_2 \, R \, E \\
\frac{dR_3}{dt} &= k_3 \, R \, E \, \frac{E_0 + E}{E_0 + E} \\
\frac{dR_4}{dt} &= \frac{V_4 \, EP}{EP_0 + EP}
\end{align*}
\]

\[
\begin{align*}
\frac{dR}{dt} &= \frac{dR_1}{dt} - \frac{dR_2}{dt} = k_1 \, A \, S - k_2 \, R \, E \\
\frac{dE}{dt} &= \frac{dR_4}{dt} - \frac{dR_3}{dt} = \frac{k_3 \, R \, E \, E_0 + E}{E_0 + E} - \frac{V_4 \, EP}{EP_0 + EP}
\end{align*}
\]

Rename species => same rate equations => same behavior
Predicted Behavior: \( G1 \Rightarrow S \)

Signal: cell growth = concentration of \( \text{CycB, Cdk1} \)

Response: activity (concentration) of \( \text{CycB:Cdk1} \)

Toggle switch:
=> above critical cell size \( \text{CycB:Cdk1} \) activity will switch on

G2 => M

**Toggle switch:**
- **mutual activation** between CycB:Cdk1 and Cdc25 (phosphatase that activates the dimer)
- **mutual inhibition** between CycB:Cdk1 and Wee1 (kinase that inactivates the dimer)

=> when the cell **grows** further during the second gap phase G2, the activity of CycB:Cdk1 will **increase** by a further step

Negative feedback loop oscillator

i) CycB:Cdk1 activates anaphase promoting complex (APC)
ii) APC activates Cdc20
iii) Cdc20 degrades CycB

Behavior:
at a critical cell size
CycB:Cdk1 activity increases and decreases again
=> at low CycB:Cdk1 level, the G1/S toggle switches off again,
  => cell cycle completed

Overall Behavior

Cell divides at size 1.46
=> daughters start growing from size 0.73
=> switches to replication at size 1.25

G1/S toggle => bistability
M/G1 oscillator

G2/M toggle => bistability

Preventing Cross-Talk

Many enzymes are used in multiple pathways

=> how can different signals cross the same kinase?

=> different temporal signature (slow vs. transient)

=> Dynamic modelling!
Summary

Today:

Behavior of cell cycle control circuitry from its modules:
two toggle switches + one oscillator
=> map biological system onto motif via
  • stoichiometric matrices
  • rate equations