W3-Note

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W3. Bistability in Biochemical Signaling Models

Q1. Some biological background

- Biological importance of bistability
- Qualitative requirements of bistability

Def of biastability: a situation where two possible steady-states are both stable.

In general, these correspond to a "**low activity**" state and a "**high activity**" state.

- **classic experiment:** add progesterone to Xenopus (frog) oocytes, measure MAPK activity
- **Population response**: gradual increase in MAPK with progesterone

What happens when MAPK activity is measured in each cell?

- With increasing progsterone, oocytes switch from **low** state to **high** state.
- An intermediate [progesterone] both high and low states are present.

Biology: generally monostable and analog response depends directly on level of stimulus.

When stimulus removed, response returns to prior level.

When is analog not good enough? Ex: fretilization, action potentials, cell division, apoptosis, differentiation, learning.

For these processes, a graded response is inadequate.

These phenomena also require persistence.

Biochemically, how does bistability arise?

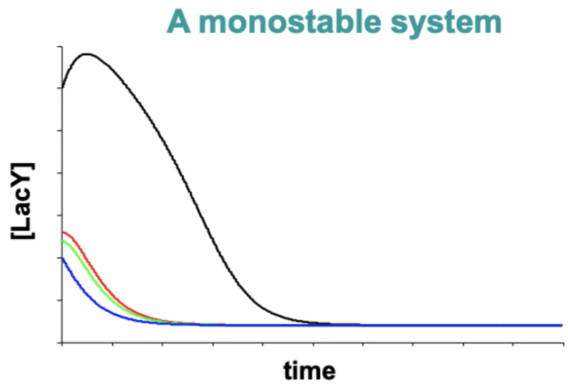
1. Mutual activation
2. Mutual inhibition

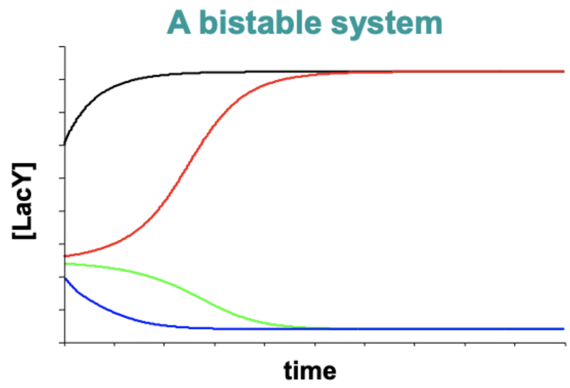
These types of circuits CAN produce bistability, but they do NOT guarantee bistability.

=> so we need quantitative analysis.

Bistability in terms of dynamical behaviour

stable & unstable, fixed points & limit cycles





Multiple steady-states are possible.

IC determine which steady-state is reached.

Thus.

- Bistability can be a useful property for biological processes that require persistence.
- Bistability means that a biological response will be essentially digital, or all-or-none, rather than graded.
- In the language of dynamical systems, bistability means that two fixed points are possible, with initial conditions determining which fixed point is reached.

Q2. How to predict if bistability will be present?

- A simple, 1D sample
- Rate-balance plots
- Ultrasensative positive feedback can create bistability
- rate-balance plots
- Example of rate-balance plots in MATLAB

Quantitative analyses of bistability

$$[A] \underset{k_{minus}}{\overset{k_{plus}}{\rightleftharpoons}} [A^*]$$

Example 1. A simple "Michaelian" system

- A* = phosphorylated A
- Total amount of [A] is constant: $[A]_{TOTAL} = [A] + [A^*]$
- To solve for [A*] in the steady-stat:

$$\frac{d[A^*]}{dt} = k_{plus}([A]_{TOTAL} - [A^*]) - k_{minus}[A^*] = 0$$

$$[A^*] = \frac{k_{plus}[A]_{TOTAL}}{k_{plus} + k_{minus}} \quad \text{or} \quad \frac{[A^*]}{[A]_{TOTAL}} = \frac{1}{1 + \frac{k_{minus}}{k_{plus}}}$$

** Rate balance plots*

Instead of solving equations, find solution graphically:

$$[A] \underset{k_{minus}}{\overset{k_{plus}}{\rightleftharpoons}} [A^*]$$

1. Forward Rate & Backward Rate

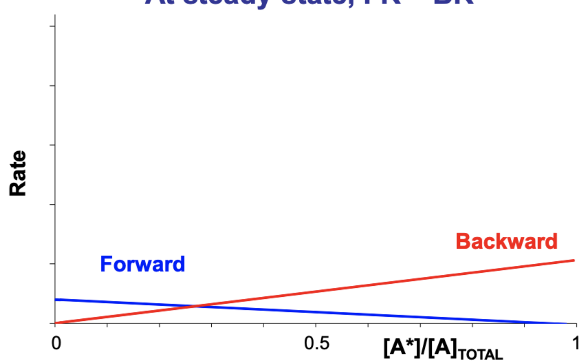
Forward Rate

$$FR = k_{plus}([A]_{TOTAL} - [A^*])$$

Backward Rate

$$BR = k_{minus}[A^*]$$

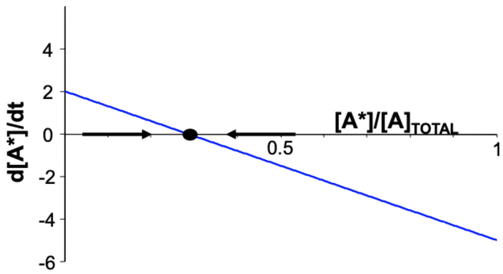
At steady-state, FR = BR



$$\frac{d[A^*]}{dt} = FR - BR$$

At steady-state, FR – BR = 0

2. dAdt = FR - BR

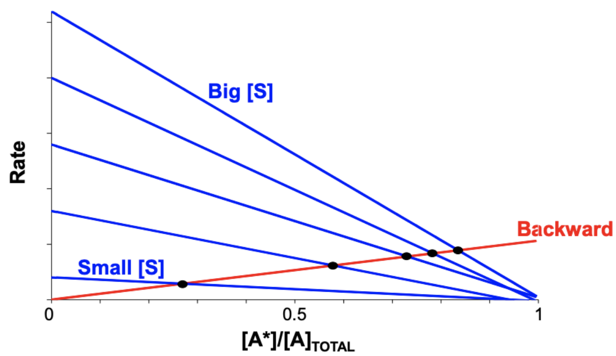


Intuitively, then, this fixed point is stable

$$k_{plus} = k_+[S]$$

3. Assume FR is a function of stimulus: $k_{plus} = k_+[S]$

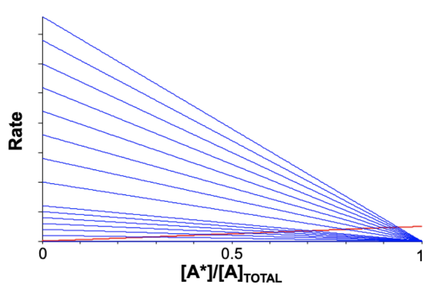
Plot rate balance for different values of stimulus [S]

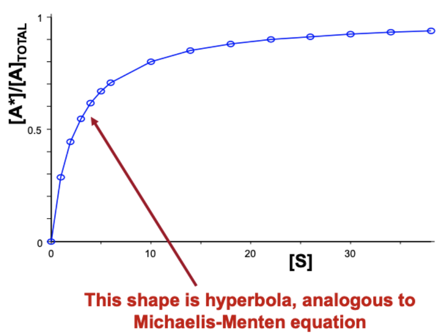


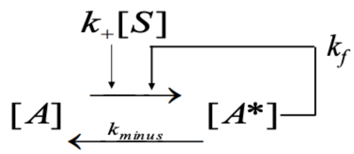
The reaction becomes:

$$[A] \underset{k_{minus}}{\overset{k_+[S]}{\rightleftarrows}} [A^*]$$

This analysis can be used to plot [S] versus $[A^*]/[A]_{TOTAL}$





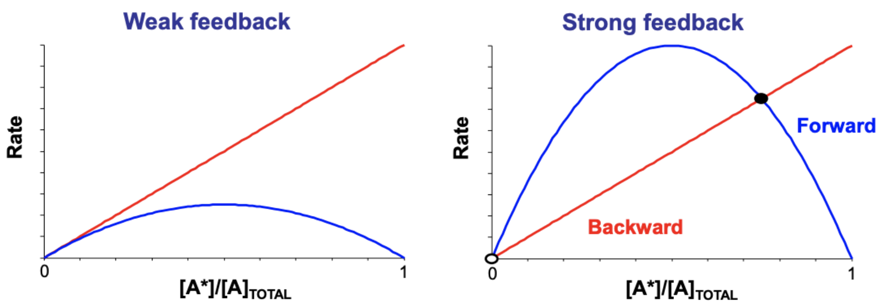


Example 2. Michaelian system with linear feedback

Forward Rates:

$$FR = \left(k_+[S] + k_f[A^*]\right)\left([A]_{TOTAL} - [A^*]\right)$$

k_f determines strength of feedback



The right plot "looks" bistable. Is it? Answer: No.

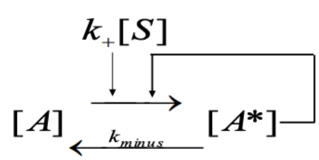
The right plot is not bistable, as:

consider a tiny deviation from A* = 0, (a spontaneous phosphorylation).

FR exceeds BR, this leads to a further increase in A*, so this steady state is unstable.

How to enable the state stable 1). Non-linear ("ultrasensitive") feedback

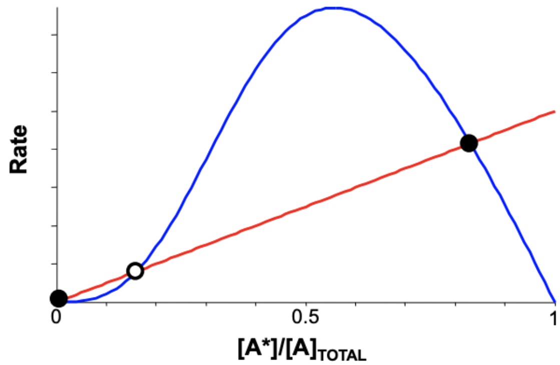
2). Partial saturation of the back reaction



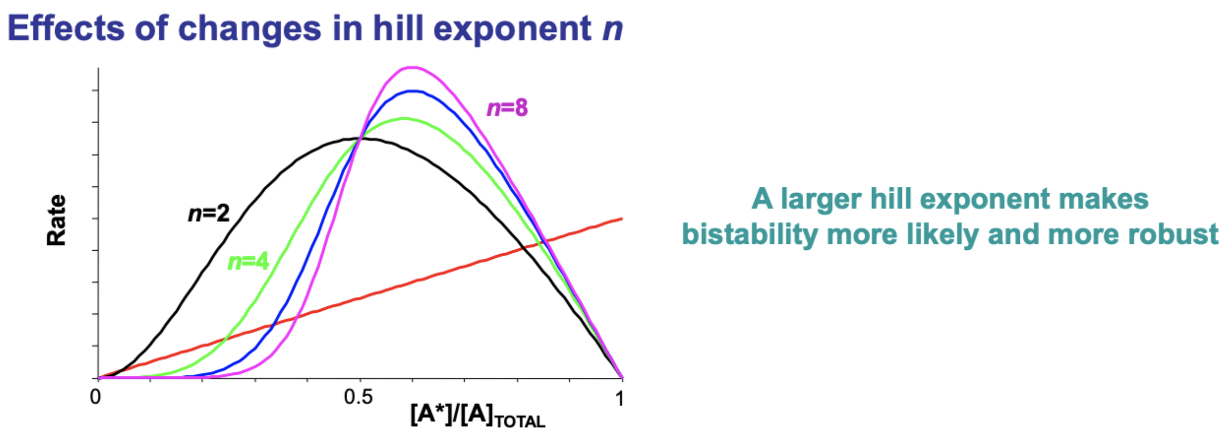
Example 3. Michalian system with ultrasensitive feedback

FR:

$$FR = \left(k_+[S] + k_f \frac{[A^*]^n}{[A^*]^n + K_{mf}^n}\right)\left([A]_{TOTAL} - [A^*]\right)$$

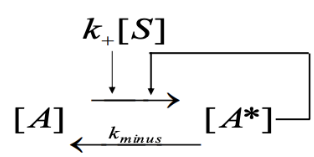


Change in hill exponent n: $n\uparrow$ **bistability likely & robust** $\uparrow$



- Rate-balance plots are useful for assessing whether bistability may occur in one-variable systems.
- Ultrasensitive positive feedback can produce bistability in a onevariable system.

Example 4. Linear feedbacks plus saturating back reaction

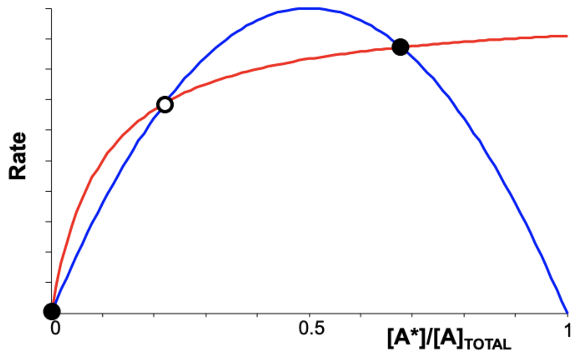


FR:

$$FR = \left(k_+[S] + k_f[A^*]\right)\left([A]_{TOTAL} - [A^*]\right)$$

BR:

$$BR = k_{minus}\left(\frac{[A^*]}{[A^*] + K_{mb}}\right)$$

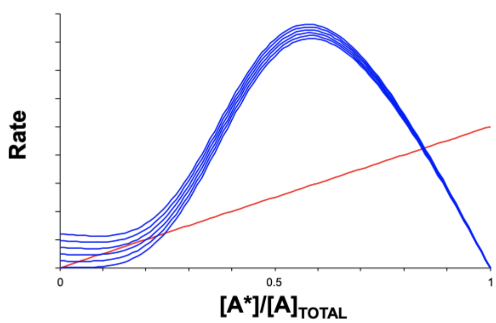


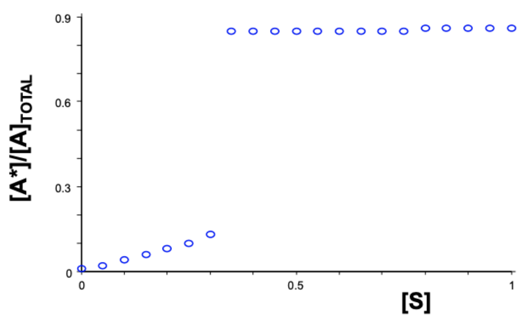
How can the cell change states?

Vary the amount of stimulus [S]

Most plots have assumed [S] = 0

$$FR = \left(k_+[S] + k_f \frac{[A^*]^n}{[A^*]^n + K_{mf}^n} \right)\left([A]_{TOTAL} - [A^*]\right)$$

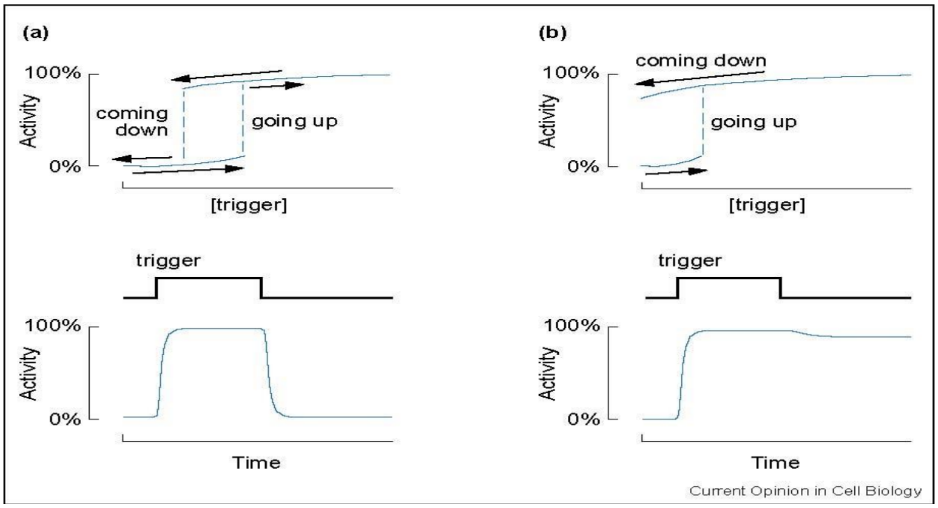




Where the system switches between 3 and 1 steady-states is a bifurcation

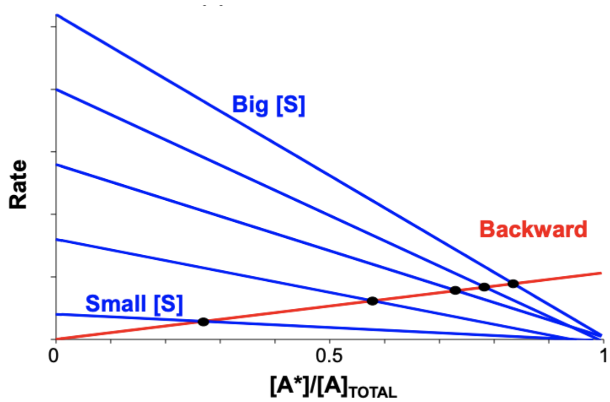
Switching can be reversible or irreversible

In either case, transition on the way up is higher than transition on the way down.

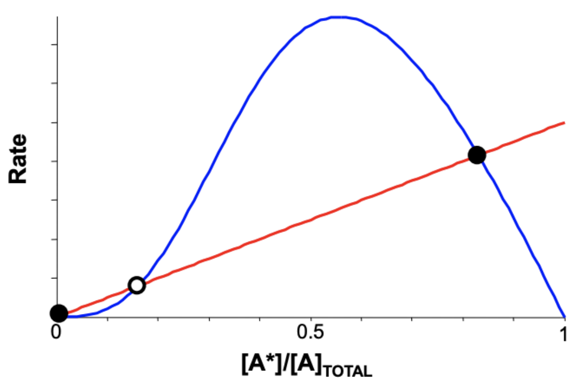


--- rate-balance plots in MATLAB —

1. Condition 1. No feedback



2. Condition 2. Ultrasensative positive feedback



- In a one-variable system, bistability can be produced by:
  - ultrasensitive positive feedback
  - a back reaction that saturates
- Analysis of rate-balance plots can generate a bifurcation diagram showing a transition from monostability

to bistability.

- Array arithmetic in MATLAB can be used to produce helpful rate balance plots.

Q3. Bistability in 2 variables systems

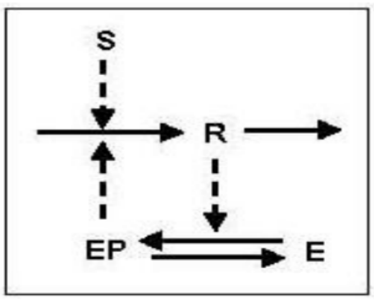
- Can occur by mutual activation or mutual repression
- Dynamic simulations can demonstrate bistability
- Bifurcation plots establish bistable regime

How to predict where bistability will be present?

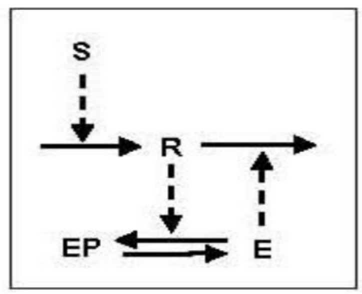
-- 1). Plot nullclines in the phase planes

Analysis of 2 variables systems

R = response
S = stimulus
E = enzyme



R = response
S = stimulus
E = enzyme



- **R causes phosphorylation of E**
- **EP leads to synthesis of R**

$$\frac{d[R]}{dt} = k_{1R}\left([E]_{TOTAL} - [E]\right) + k_{1R}[S$$

$$\frac{d[E]}{dt} = -k_{2E}[R]\frac{[E]}{[E]+K_{m2E}} + k_{1E}\frac{[I}{[E]_{TO}}$$

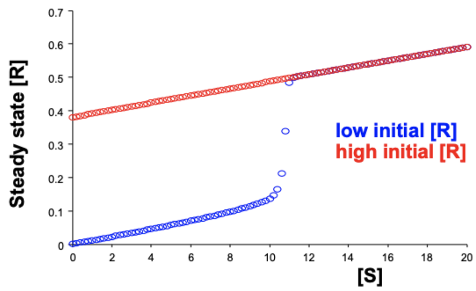
- **R causes phosphorylation of E**
- **E (not EP) leads to degradation of R**

$$\frac{d[R]}{dt} = k_0 + k_1[S] - \left(k_2 + k_2'[E]\right)[R]$$

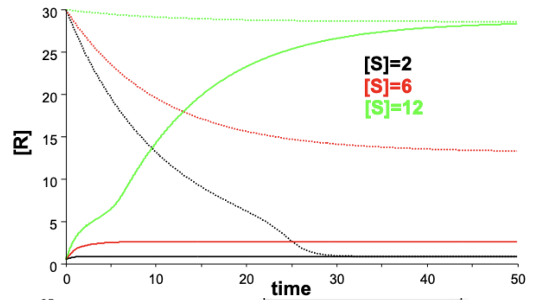
$$\frac{d[E]}{dt} = -k_{2E}[R]\frac{[E]}{[E]+K_{m2E}} + k_{1E}\frac{[E]_{TOTAL} - [E]}{[E]_{TOTAL} - [E] + K_m}$$

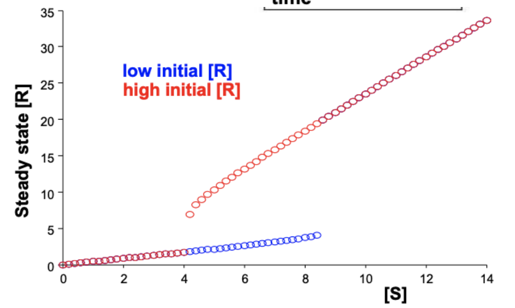
For [S] less than ~11, two steady states are possible.

Time course of [R} at different values of [S]



Initial Conditions determine which steady-state is reached





Nullclines: How to determine that bistability will occur at only some values of [S]?

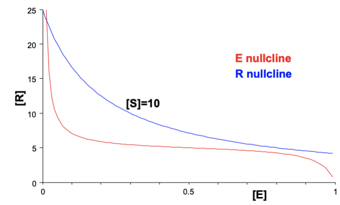
=> Plot **nullclines**, i.e. points where either $\frac{d[R]}{dt} = 0$ or $\frac{d[E]}{dt} = 0$

$$\frac{d[R]}{dt} = k_0 + k_1[S] - \left(k_2 + k_2'[E]\right)[R] = 0$$

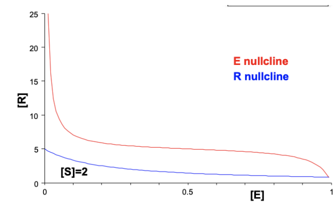
$$\frac{d[E]}{dt} = -k_{2E}[R]\frac{[E]}{[E]+K_{m2E}} + k_{1E}\frac{[E]_{TOTAL} - [E]}{[E]_{TOTAL} - [E] + K_{m1E}} = 0$$

First equation: equally easy to solve for [E] in terms of [R] or vice-versa

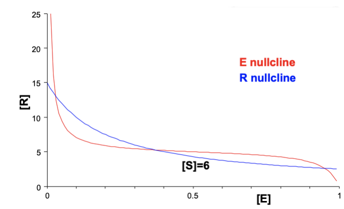
Second equation: MUCH easier to solve for [R] as function of [E]

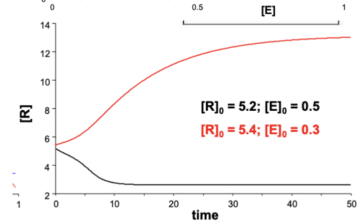


[S] = 10
One intersection: monostable.



[S] = 2
One intersection: monostable.





[S] = 6
Two intersections: this suggests (but does not prove) the middle fixed point is unstable

- In a two-variable system, bistability can be produced by:
  - mutual activation
  - mutual repression
- Bifurcation diagrams summarize which regions of particular
  - parameters are associated with bistability.
- Plotting nullclines in the phase plane is the first step towards
  - predicting whether bistability is present.

-- 2). Mathematically rigorous: Jacobian & eigenvalues To understand stable & unstable fixed points mathematically:

- Step 1. we compute the **Jacobian matrix.**

Based on:

$$\frac{d[E]}{dt} = -k_{2e}[R]\frac{[E]}{[E]+K_{m2e}} + k_{1e}\frac{[E]_{TOTAL}-[E]}{[E]_{TOTAL}-[E]+K_{m1e}} = f$$

$$\frac{d[R]}{dt} = k_{0r} + k_{1r}[S] - \left(k_{2r} + k_{3r}[E]\right)[R] = g$$

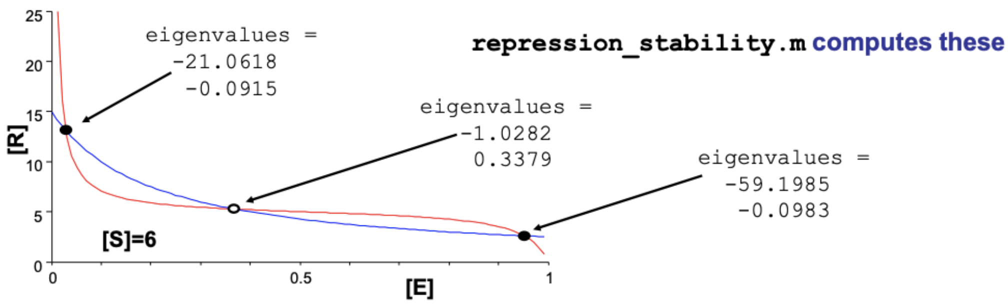
Jacobian matrix:

$$J = \begin{bmatrix} \frac{\partial f}{\partial [E]} & \frac{\partial f}{\partial [R]} \\ \frac{\partial g}{\partial [E]} & \frac{\partial g}{\partial [R]} \end{bmatrix} = \begin{bmatrix} \frac{-k_{2e}[R]K_{m2e}}{([E]+K_{m2e})^2} - \frac{k_{1e}K_{m1e}}{([E]_{TOTAL}-[E]+K_{m1e})^2} & \frac{-k_{2e}[E]}{[E]+K_{m2e}} \\ -k_{3r}[R] & -(k_{2r}+k_{3r}[E]) \end{bmatrix}$$

- **Step 2. Evaluate this at the fixed points defined by [E*], [R*]**

$$J = \begin{bmatrix} \frac{-k_{2e}[R^*]K_{m2e}}{([E^*]+K_{m2e})^2} - \frac{k_{1e}K_{m1e}}{([E]_{TOTAL}-[E^*]+K_{m1e})^2} & \frac{-k_{2e}[E}{[E^*]+K} \\ -k_{3r}[R^*] & -(k_{2r}+k_{3r} \end{bmatrix}$$

- Step 3. The **eigenvalues** of the Jacobian (at the fixed points) determine stability:
  - The real part of either is positive: **the fixed point is unstable**
  - Real parts of both are negative: **the fixed point is stable**



-- 3). Qualitative and graphical: direction arrows In 2D phase plane, direction determined by:

$$\begin{bmatrix} d[E]/dt \\ d[R]/dt \end{bmatrix}$$

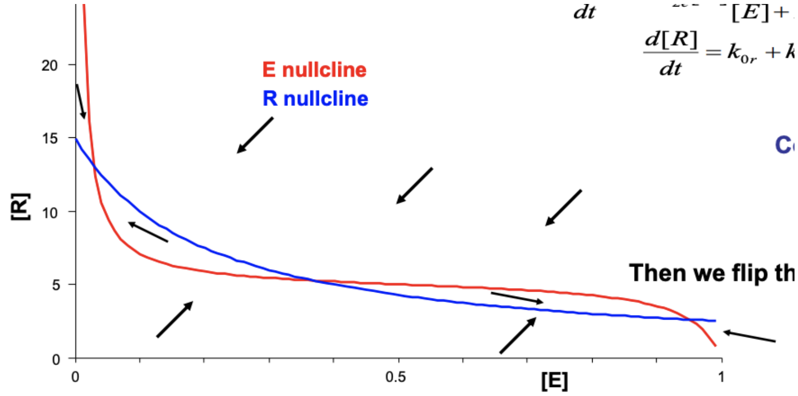
$$\frac{d[E]}{dt} = -k_{2e}[R]\frac{[E]}{[E]+K_{m2e}} + k_{1e}\frac{[E]_{TOTAL}-[E]}{[E]_{TOTAL}-[E]+K_{m1e}}$$

$$\frac{d[R]}{dt} = k_{0r} + k_{1r}[S] - \left(k_{2r} + k_{3r}[E]\right)[R]$$

Consider **[E] big and [R] big,**

$$\frac{d[E]}{dt} < 0; \frac{d[R]}{dt} < 0$$

Then we flip the arrow each time we cross a nullcline.



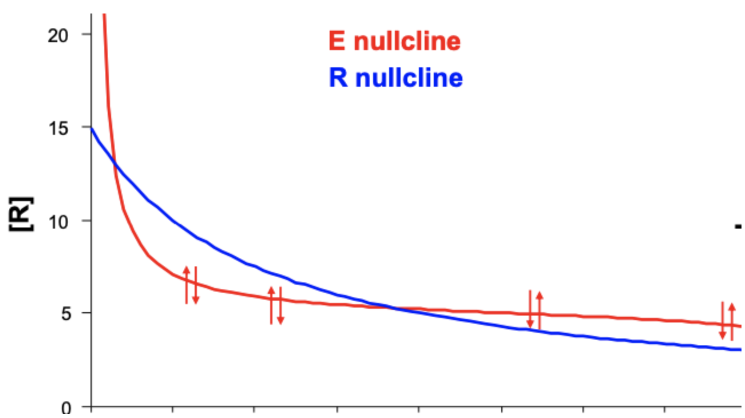
With these simple rules, we can often determine stability.

Arrows on the nullclines:

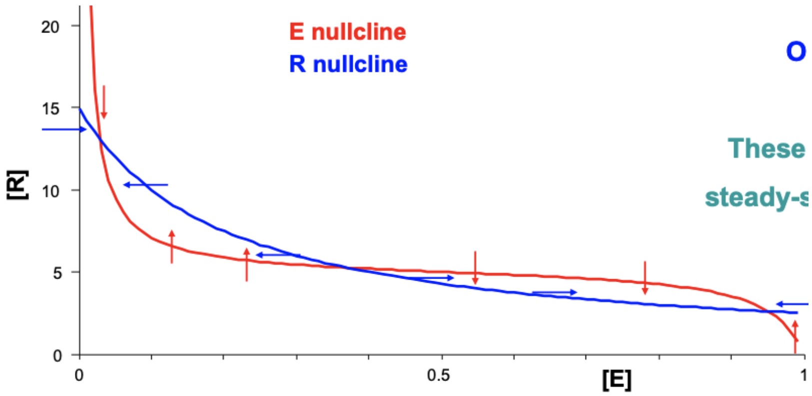
- On the E nullcline, dE/dt = 0, direction of movement is up/down
- On the R nullcline, dR/dt = 0, direction of movement is left/right

The direction changes when a nullcline is crossed

dR/dt = 0: on E nullcline



dE/dt = 0 on R nullcline



These considerations suggest that

- middle steady-state is unstable.
- left and right steady-states are stable.

Summary

- In a two-variable system mutual activation or mutual repression can produce bistability.
- When nullclines intersect 3 times, bistability may be present.
- Stability of fixed points can be determined graphically by:
  - plotting direction arrows for extreme values of the two variables
  - flipping arrows in one direction each time a nullcline is crossed

Bistable systems produce digital, all or none, rather than graded responses.

Bistability is biologically useful when persistence is required: apoptosis, cell division, differentiation, etc.

Bistability is produced by complex regulation, eg: mutual activation ot inhibition

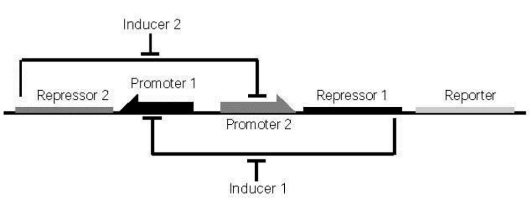
The presence or absence of bistability can be assessed mathematically or graphically (rate balance plots, nullclines in the phase plane).

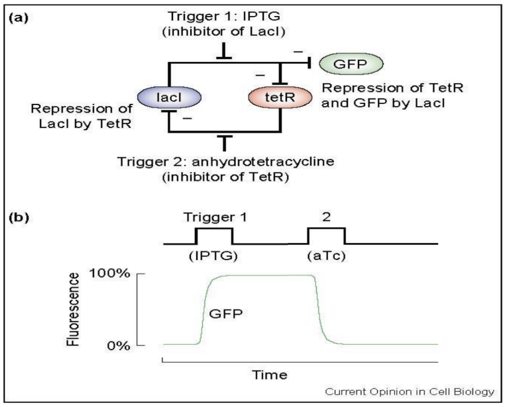
Q4. Example of bistability:

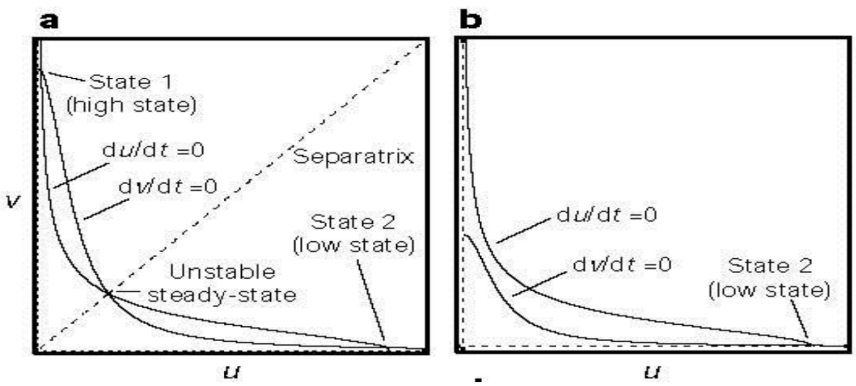
Example 1. An artificial genetic "toggle switch"

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^{\beta}} - u$$

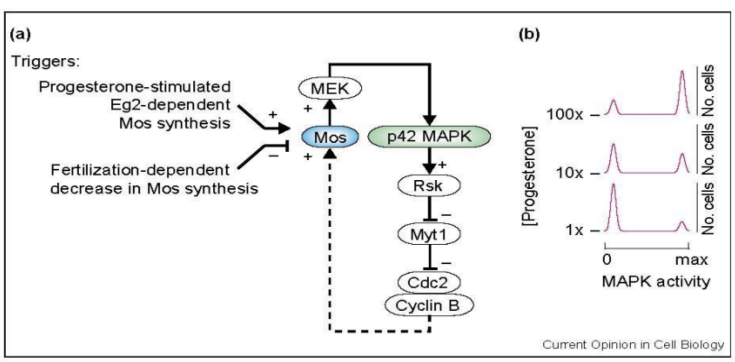
$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^{\gamma}} - v$$



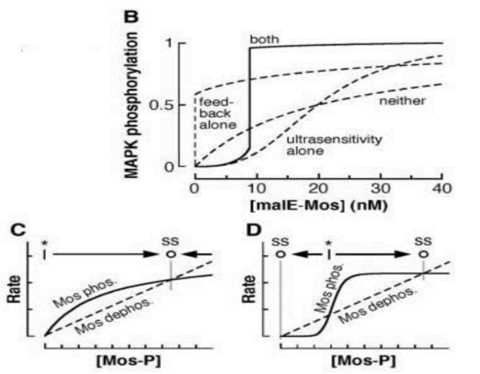




Example 2. MAPK cascade in oocyte maturation

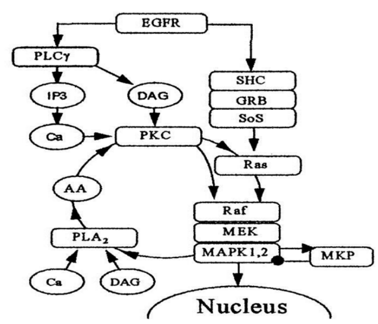


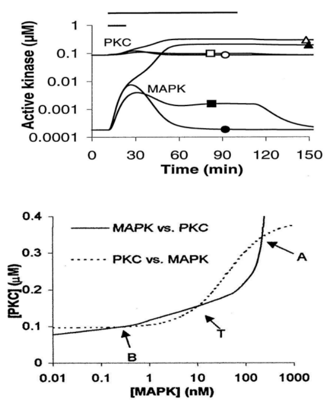
Ferrell (2002) *Curr. Op. Cell Biol.* 14:140–148.



Ferrell & Machleder (1998) *Science* 280:895-898

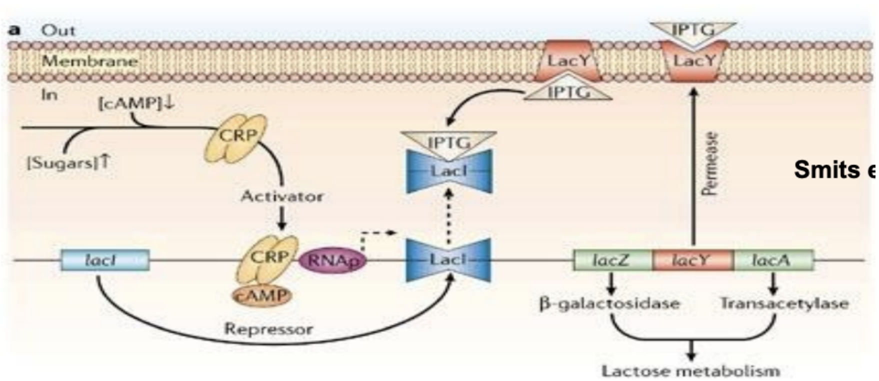
Example 3. MAP-kinase pathway in mammalian cells





Bhalla & Iyengar (1999) *Science* 283:381-387

Example 4. Lac operon in E. coli



- With low nutrient levels, LacI will repress transcription of the LacA, LacY, and LacZ genes.
- Lactose, allolactose, or IPTG will bind to LacI, relieve repression.
- LacY encodes a “permease” which allows lactosse into the cell.

$$\frac{dl}{dt} = \beta l_{ext} LacY - \gamma l$$

$$\frac{dLacY}{dt} = \delta + p\frac{l^4}{l^4 + l_0^4} - \sigma LacY$$

l **= intracellular lactose**

LacY **= expression of LacY/permease**

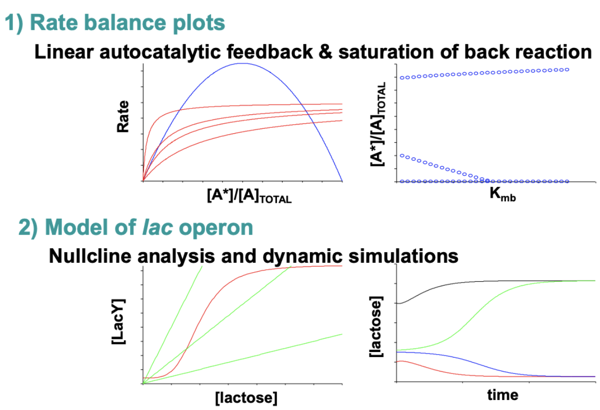
β, γ, δ, σ, p, $\boldsymbol{l_0}$ **= constants**

$\boldsymbol{l_{ext}}$ **= external lactose**

Example of bistable systems: A minimal model of the lac operon

(Note: in most models, dLacY/dt depends on [lac

dependence on [lactose]^4 to improve the nullcl



- Bistability is observed in biological systems when mutual activation or mutual inhibition is present
  - MAP-kinase signaling
  - The lac operon in E. coli
- Mutual activation/inhibition can occur through post-translational modifications (e.g. phosphorylation) or through changes in gene expression.
- Mutual activation/inhibition can be direct or can occur through intermediates.