A structured approach for the engineering of biochemical network models, illustrated for signalling pathways

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Tutorial outline

I. Biological introduction *Rainer Breitling*

II. Petri net introduction *Monika Heiner*

III. Biological applications *David Gilbert*

IV. Model checking *Robin Donaldson*

(each 50 min + 10 min break/discussion)

A structured approach …
Part I
Biology

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Outline

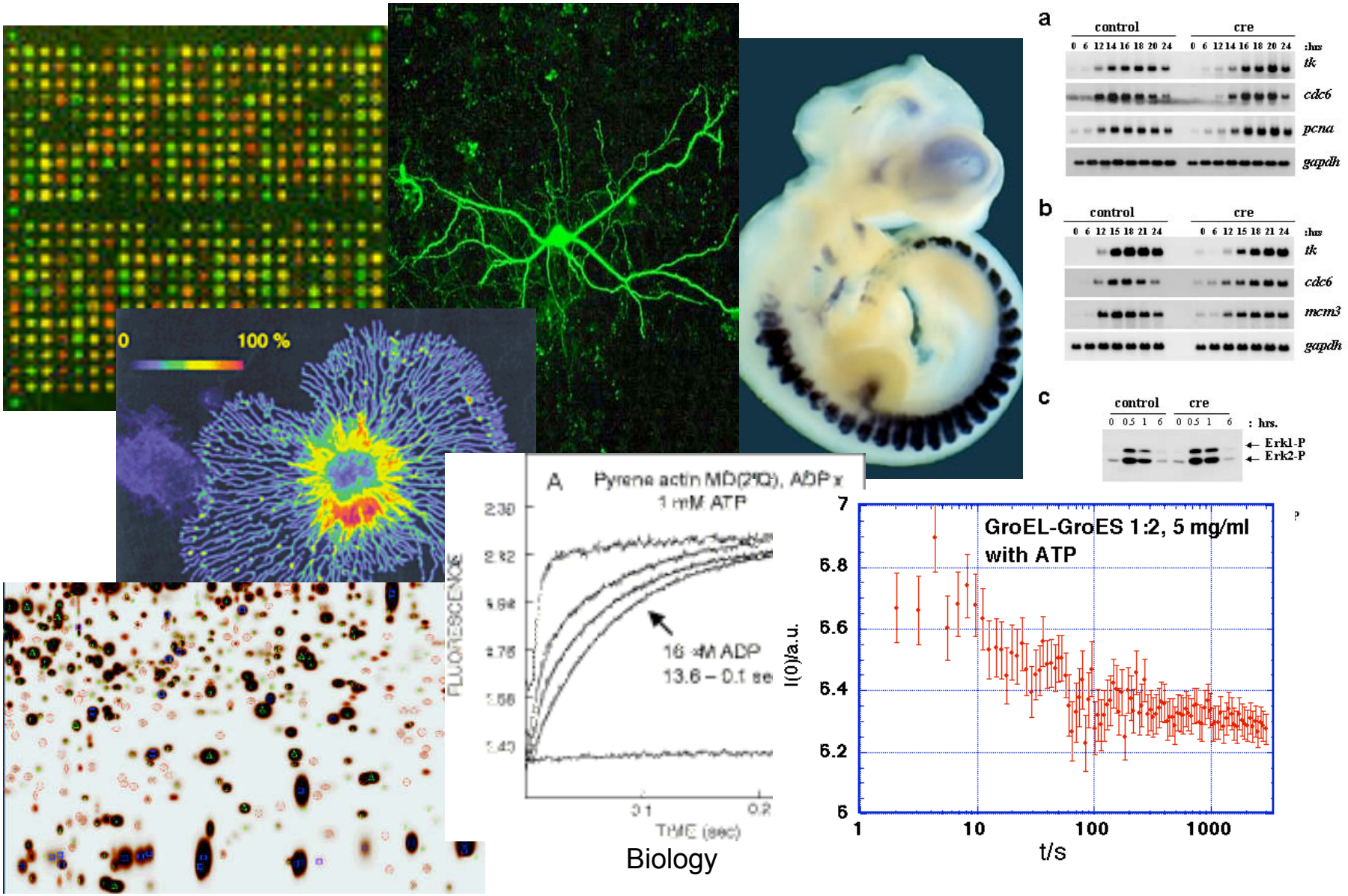
- Part 1: Why modelling?
- Part 2: The statistical physics of modelling:

  A → B

  (where do differential equations come from?)
- Part 3: Translating biology to mathematics

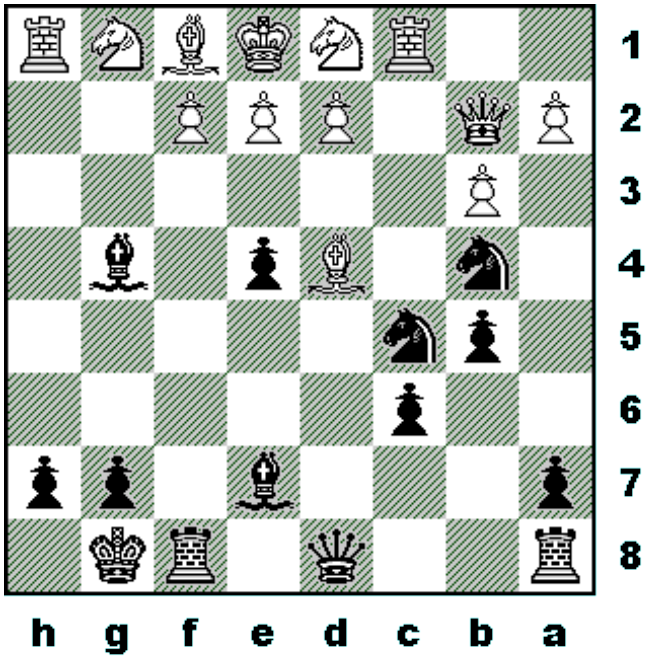
  (finding the right differential equations)

Biology = Concentrations



Humans think small-scale...

(the “7 items” rule)



- phone number length (memory constraint)
- optimal team size (manipulation constraint)
- maximum complexity for rational decision making

...but biological systems contain (at least) dozens of relevant interacting components!

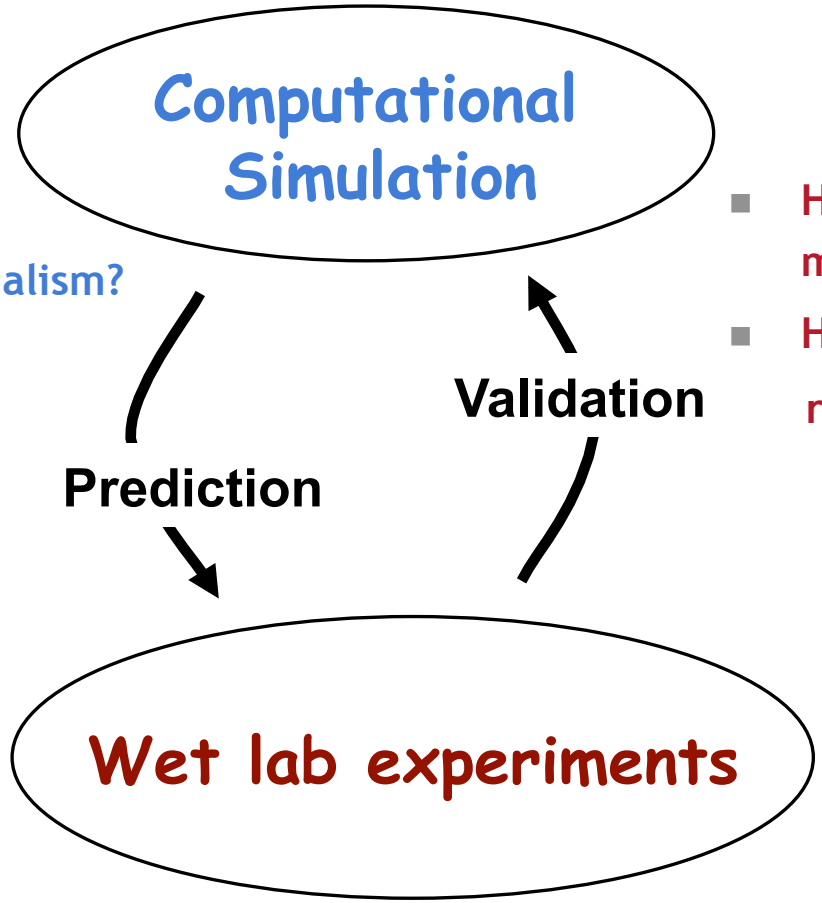
Humans think linear...

...but biological systems contain:

- non-linear interaction between components
- positive and negative feedback loops
- complex cross-talk phenomena

Biochemical Pathway Simulation

- What is the best formalism?
- How to deal with lack of information?
- Predictions on what?



- How to collect quantitative measurements in vivo?
- How to manipulate regulatory mechanisms?

The simplest chemical reaction

$$A \rightarrow B$$

- irreversible, one-molecule reaction
- examples: all sorts of decay processes, e.g. radioactive, fluorescence, activated receptor returning to inactive state
- any metabolic pathway can be described by a combination of processes of this type (including reversible reactions and, in some respects, multi-molecule reactions)

The simplest chemical reaction

$$A \rightarrow B$$

various levels of description:

- homogeneous system, large numbers of molecules = ordinary differential equations, **kinetics**
- small numbers of molecules = probabilistic equations, **stochastics**
- spatial heterogeneity = partial differential equations, **diffusion**
- small number of heterogeneously distributed molecules = single-molecule tracking (e.g. cytoskeleton modelling)

Kinetics Description

Main idea: Molecules don't talk

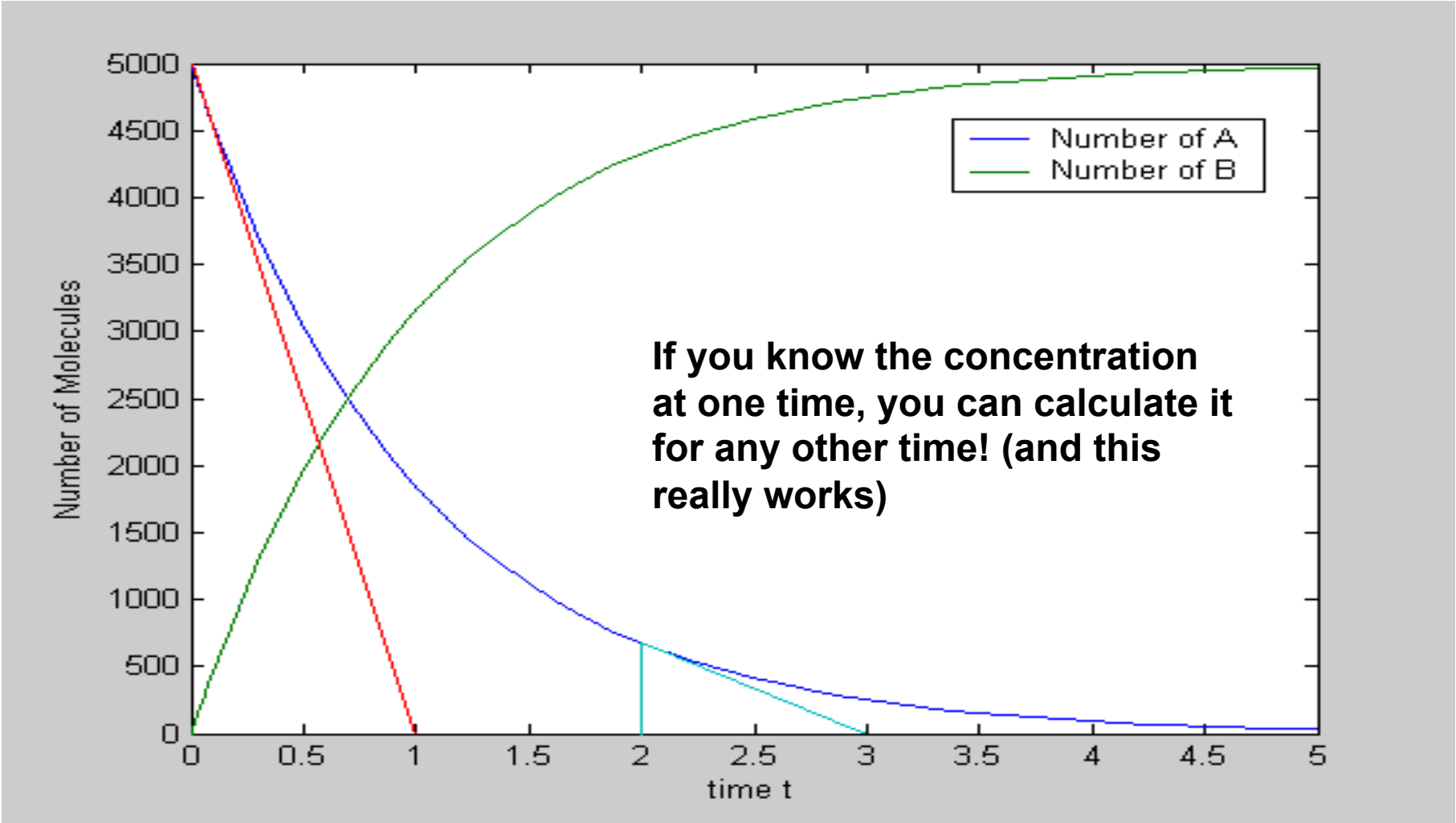
- Imagine a box containing N molecules.
  How many will decay during time t? **k*N**
- Imagine two boxes containing N/2 molecules each.
  How many decay? **k*N**
- Imagine two boxes containing N molecules each.
  How many decay? **2k*N**
- In general:

$$-\frac{dn(t)}{dt} = \lambda * n(t) \quad \Leftrightarrow \quad n(t) = N_0 e^{-\lambda t}$$

differential equation (ordinary, linear, first-order)

exact solution (in more complex cases replaced by a numerical approximation)

Kinetics Description



Probabilistic Description

Main idea: Molecules are isolated entities without memory

Probability of decay of a single molecule in some small time interval:

$$p_1 = \lambda \Delta t$$

Probability of survival in Δt:

$$p_2 = 1 - p_1 = 1 - \lambda \Delta t$$

Probability of survival for some time t:

$$p = \lim_{x \to \infty} (1 - \lambda \frac{t}{x})^x = e^{-\lambda t}$$

Transition to large number of molecules:

$$n(t) = N_0 e^{-\lambda t} \quad \text{or}$$

$$\frac{dn(t)}{dt} = -\lambda N_0 e^{-\lambda t} = -\lambda n(t)$$

Probabilistic Description – 2

Probability of survival of a single molecule for some time t:

$$p = \lim_{x \to \infty} (1 - \lambda \frac{t}{x})^x = e^{-\lambda t}$$

Probability that exactly x molecules survive for some time t:

$$p_x = (e^{-\lambda t})^x (1 - e^{-\lambda t})^{N_0 - x} \begin{pmatrix} N_0 \\ x \end{pmatrix}$$

Most likely number to survive to time t:

$$\max(x \mid p_x) = N_0 e^{-\lambda t}$$

Probabilistic Description – 3

Markov Model (pure death!)

Decay rate: $\Lambda(n,t) = n\lambda$

Probability of decay: $p = \Lambda(n,t)dt$

Probability distribution of n surviving molecules at time t: $P(n,t)$

Description:

Time: `t -> wait dt -> t+dt`

Molecules:

`n -> no decay -> n`

`n+1 -> one decay -> n`

$$P(n,t+dt) =$$

$$P(n+1,t)\Lambda(n+1,t)dt$$

$$+ P(n,t)[1-\Lambda(n,t)dt]$$

Final Result (after some calculating): The same as in the previous probabilistic description

Spatial heterogeneity

- concentrations are different in different places, n = f(t,x,y,z)
- diffusion superimposed on chemical reactions:

$$\frac{\partial n(t)_{xyz}}{\partial t} = -\lambda n(t)_{xyz} \pm \text{diffusion}$$

- partial differential equation

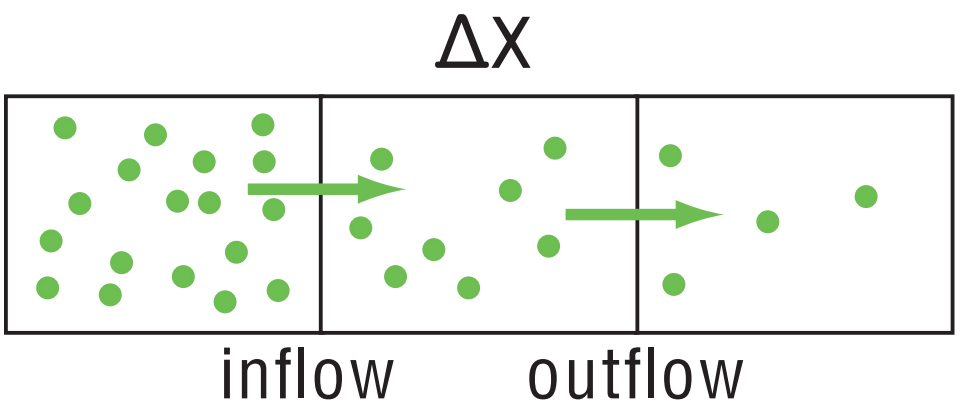
Spatial heterogeneity

- one-dimensional case (diffusion only, and conservation of mass)

$$\frac{\partial n(t,x)}{\partial t}\Delta x = \text{inflow} - \text{outflow}$$

$$\text{outflow} = -K\frac{\partial n(t,x+\Delta x)}{\partial x}$$

$$\text{inflow} = -K\frac{\partial n(t,x)}{\partial x}$$



Spatial heterogeneity – 2

$$\frac{\partial n(t,x)}{\partial t}\Delta x = K\frac{\partial n(t,x+\Delta x)}{\partial x} - K\frac{\partial n(t,x)}{\partial x}$$

Transition to differential equation to get diffusion equation :

$$\frac{\partial n(t,x)}{\partial t} = K\frac{\partial^2 n(t,x)}{\partial x^2}$$

Shorthand for three dimensions :

$$\frac{\partial n(t,x,y,z)}{\partial t} = K\nabla^2 n(t,x,y,z)$$

Combination with chemical reaction :

$$\frac{\partial n(t)}{\partial t} = -\lambda n(t) + K\nabla^2 n(t)$$

Summary of Physical Chemistry

- Simple reactions are easy to model accurately
- Kinetic, probabilistic, Markovian approaches lead to the same basic description

$$\frac{dn(t)}{dt} = -\lambda n(t) \Leftrightarrow n(t) = N_0 e^{-\lambda t}$$

- Diffusion leads only to slightly more complexity
- Next step: Everything is decay...

Some (Bio)Chemical Conventions

Concentration of Molecule A = [A], usually in units mol/litre (molar)

Rate constant = k, with indices indicating constants for various reactions (k_1, k_2...)

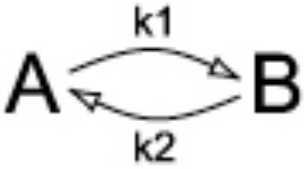
Therefore:

$$A \rightarrow B$$

$$\frac{d[A]}{dt} = -\frac{d[B]}{dt} = -k_1[A]$$

Reversible, Single-Molecule Reaction

A ←→ B, or A → B || B → A, or



Differential equations:

forward reverse

$$\frac{d[A]}{dt} = -k_1[A] + k_2[B]$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B]$$

Main principle: Partial reactions are **independent**!

Reversible, single-molecule reaction – 2

Differential Equation:

$$\frac{d[A]}{dt} = -k_1[A] + k_2[B]$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B]$$

Equilibrium (=steady-state):

$$\frac{d[A]_{equi}}{dt} = \frac{d[B]_{equi}}{dt} = 0$$

$$-k_1[A]_{equi} + k_2[B]_{equi} = 0$$

$$\frac{[A]_{equi}}{[B]_{equi}} = \frac{k_2}{k_1} = K_{equi}$$

Irreversible, two-molecule reaction

The last piece of the puzzle

A+B→C

Differential equations:

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -\frac{d[C]}{dt}$$

$$\frac{d[A]}{dt} = -k[A][B]$$

Non-linear!

Underlying idea: Reaction probability = Combined probability that both [A] and [B] are in a "reactive mood":

$$p(AB) = p(A)p(B) = k_1^*[A]k_2^*[B] = k[A][B]$$

A simple metabolic pathway

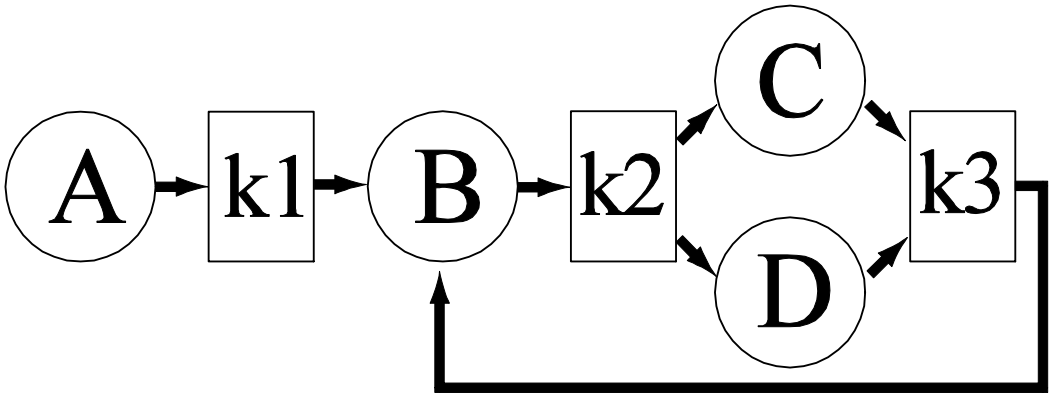
A→B←→C+D

Differential equations:

d/dt	decay	forward	reverse
[A]=	`-k1[A]`		
[B]=	`+k1[A]`	`-k2[B]`	`+k3[C][D]`
[C]=		`+k2[B]`	`-k3[C][D]`
[D]=		`+k2[B]`	`-k3[C][D]`

Metabolic Networks as Bigraphs

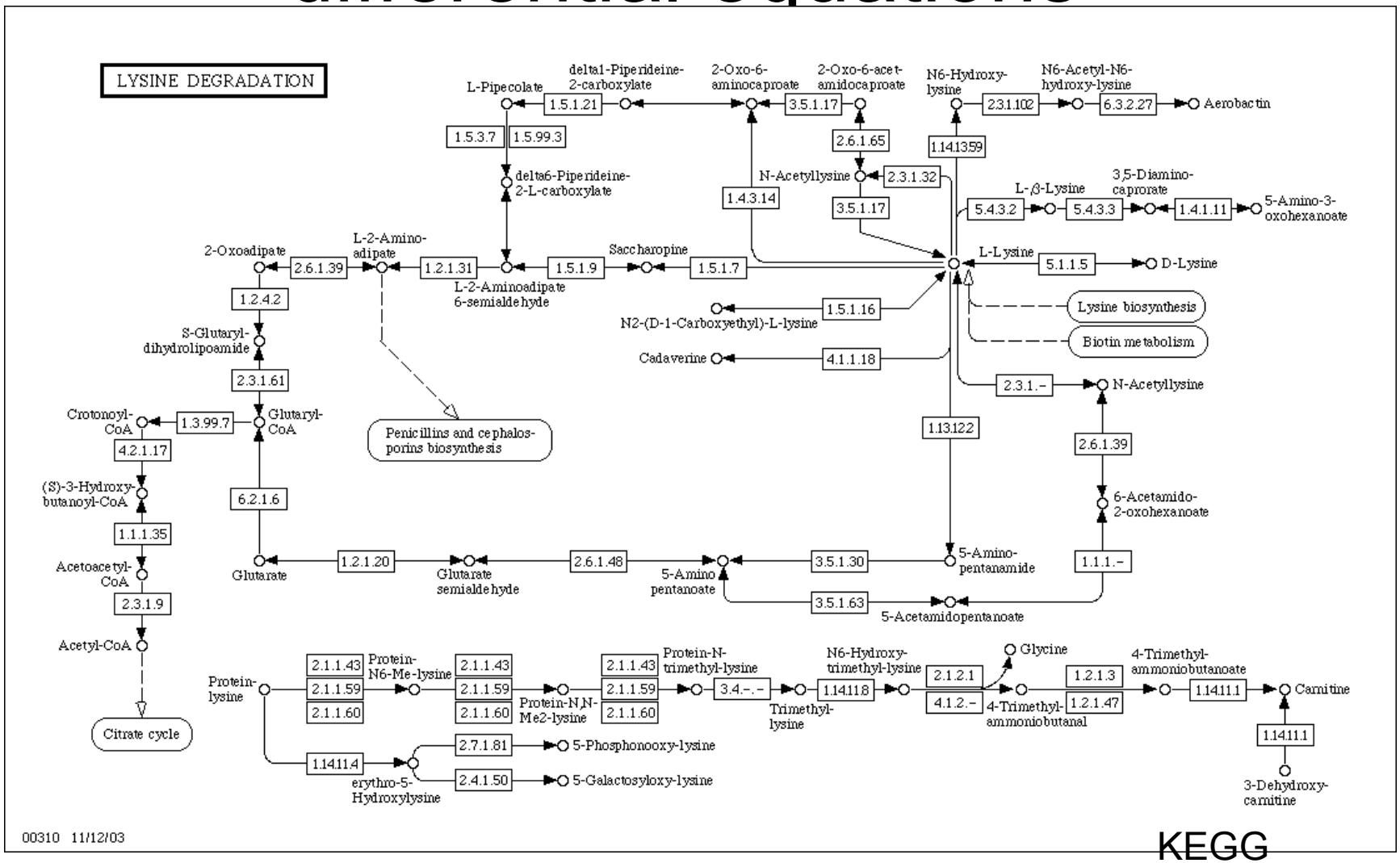
A→B←→C+D



	k1	k2	k3
A	-1	0	0
B	1	-1	1
C	0	1	-1
D	0	1	-1

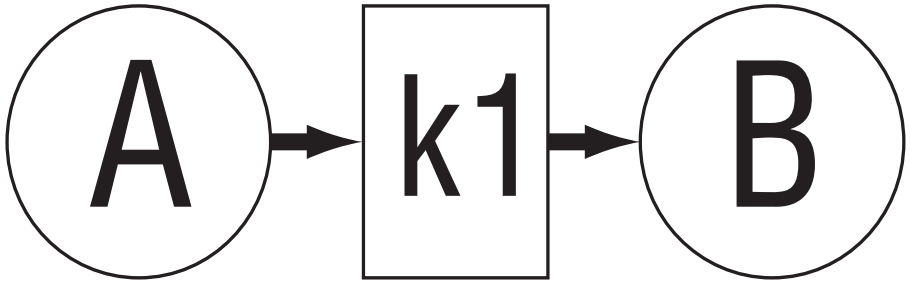
d/dt	decay	forward	reverse
[A]	-k1[A]		
[B]	+k1[A]	-k2[B]	+k3[C][D]
[C]		+k2[B]	-k3[C][D]
[D]		+k2[B]	-k3[C][D]

Biological description → bigraph → differential equations

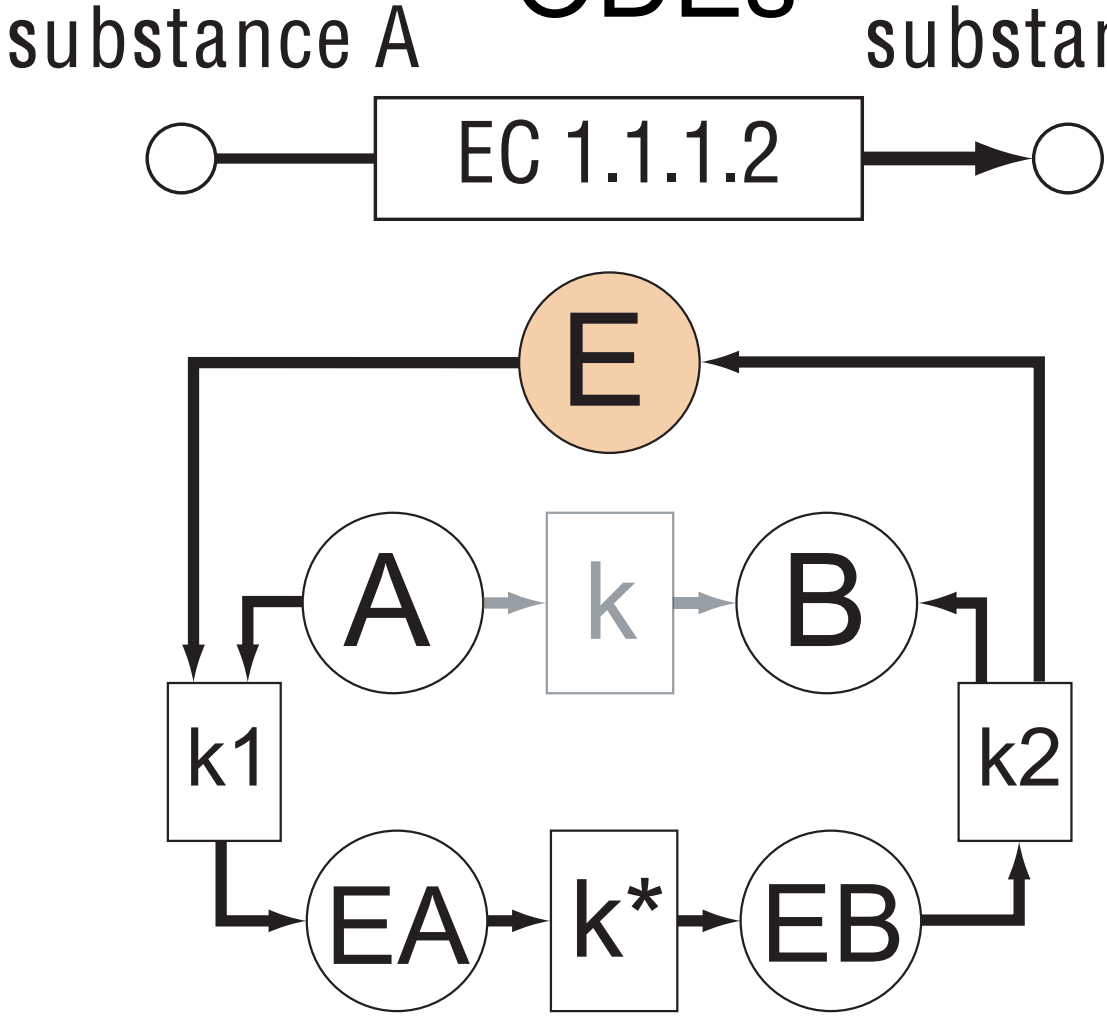


KEGG

Biological description → bigraph → ODEs



Biological description → bigraph → ODEs



A special case: enzyme reactions

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

In a **quasi steady state**, we can assume that [ES] is constant. Then:

$$[ES] = \frac{k_1[E][S]}{k_{-1} + k_2}$$

If we now define a new constant $\mathbf{K_m}$ (Michaelis constant), we get:

$$[ES] = \frac{[E][S]}{K_m} \qquad K_m = \frac{k_{-1} + k_2}{k_1}$$

A special case: enzyme reactions

Substituting [E] (free enzyme) by the total enzyme concentration we get:

$$[ES] = \frac{([E_0] - [ES])[S]}{K_m}$$

$$[ES] = [E_0]\frac{1}{1 + \frac{K_m}{[S]}}$$

Hence, the **reaction rate** is:

$$V = \frac{d[P]}{dt} = k_2[ES]$$

$$\frac{d[P]}{dt} = k_2[E_0]\frac{[S]}{K_m + [S]} = V_{max}\frac{[S]}{K_m + [S]}$$

A special case: enzyme reactions

Underlying assumptions of the Michaelis-Menten approximation:

- Free diffusion, random collisions
- Irreversible reactions
- Quasi steady state

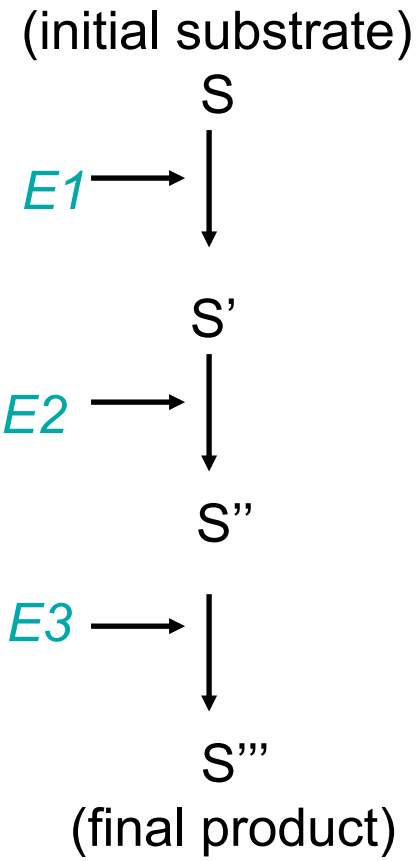
In **cell signaling pathways**, all three assumptions will be frequently violated:

- Reactions happen at membranes and on scaffold structures
- Reactions happen close to equilibrium and both reactions have non-zero fluxes
- Enzymes are themselves substrates for other enzymes, concentrations change rapidly, $d[ES]/dt \approx d[P]/dt$

Metabolic pathways vs Signalling Pathways

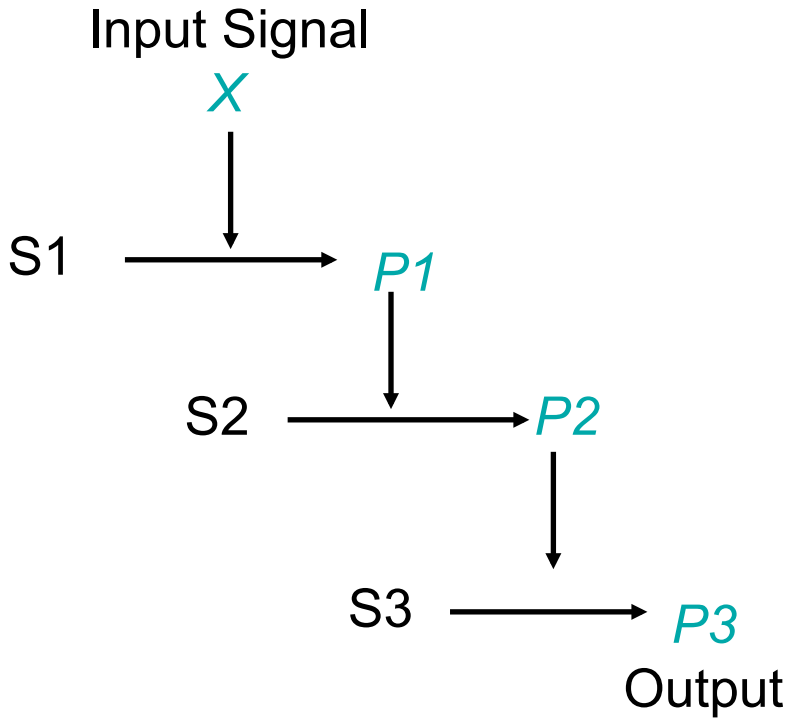
(can you give the mass-action equations?)

Metabolic



Classical enzyme-product pathway

Signalling cascade



Product become enzyme at next stage

Cell signaling pathways

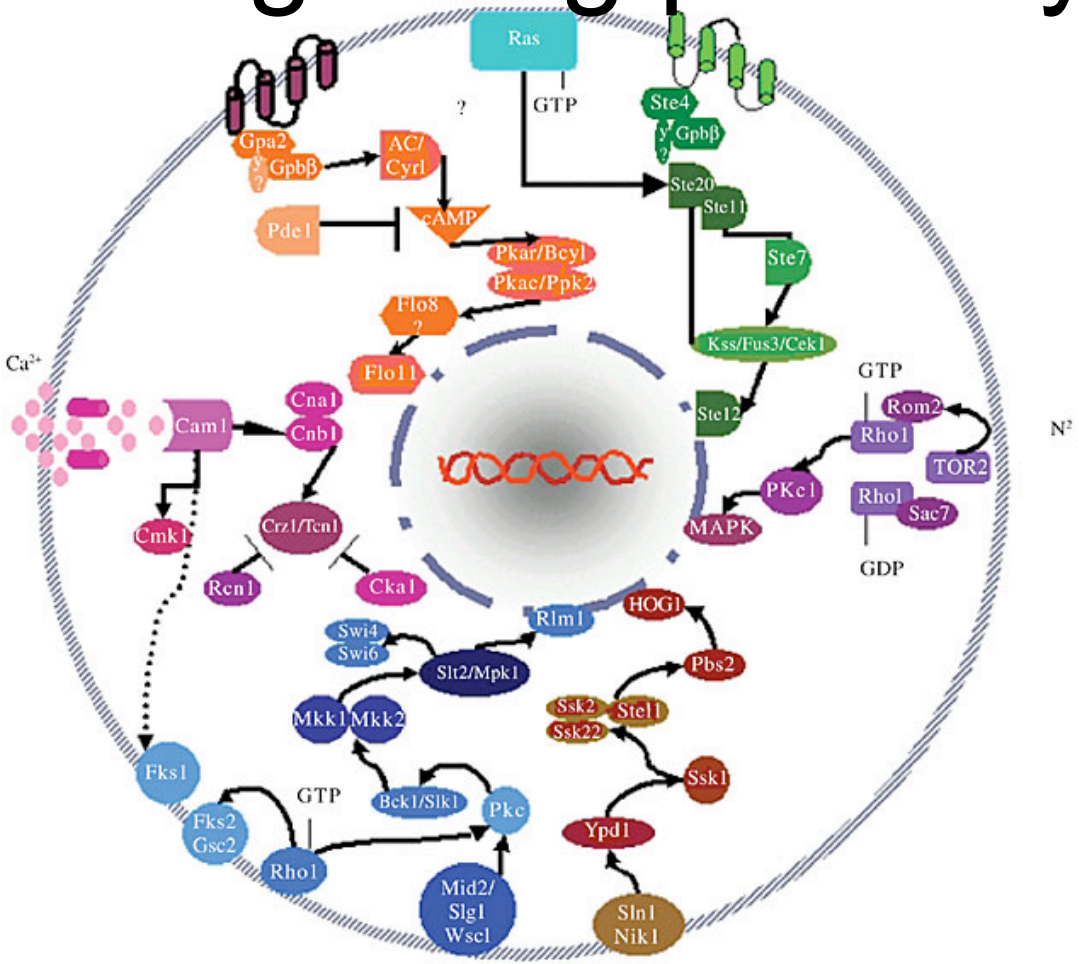
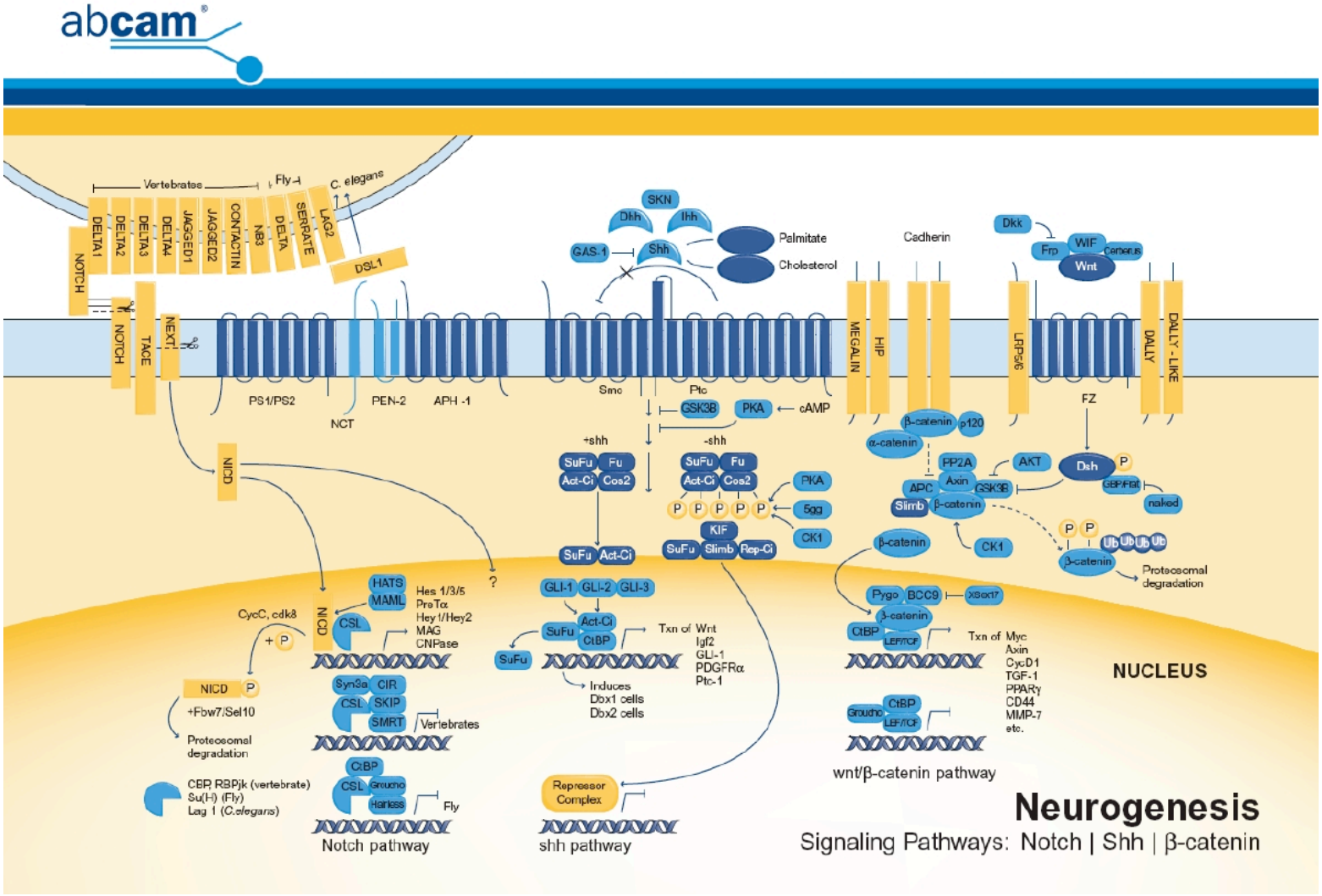
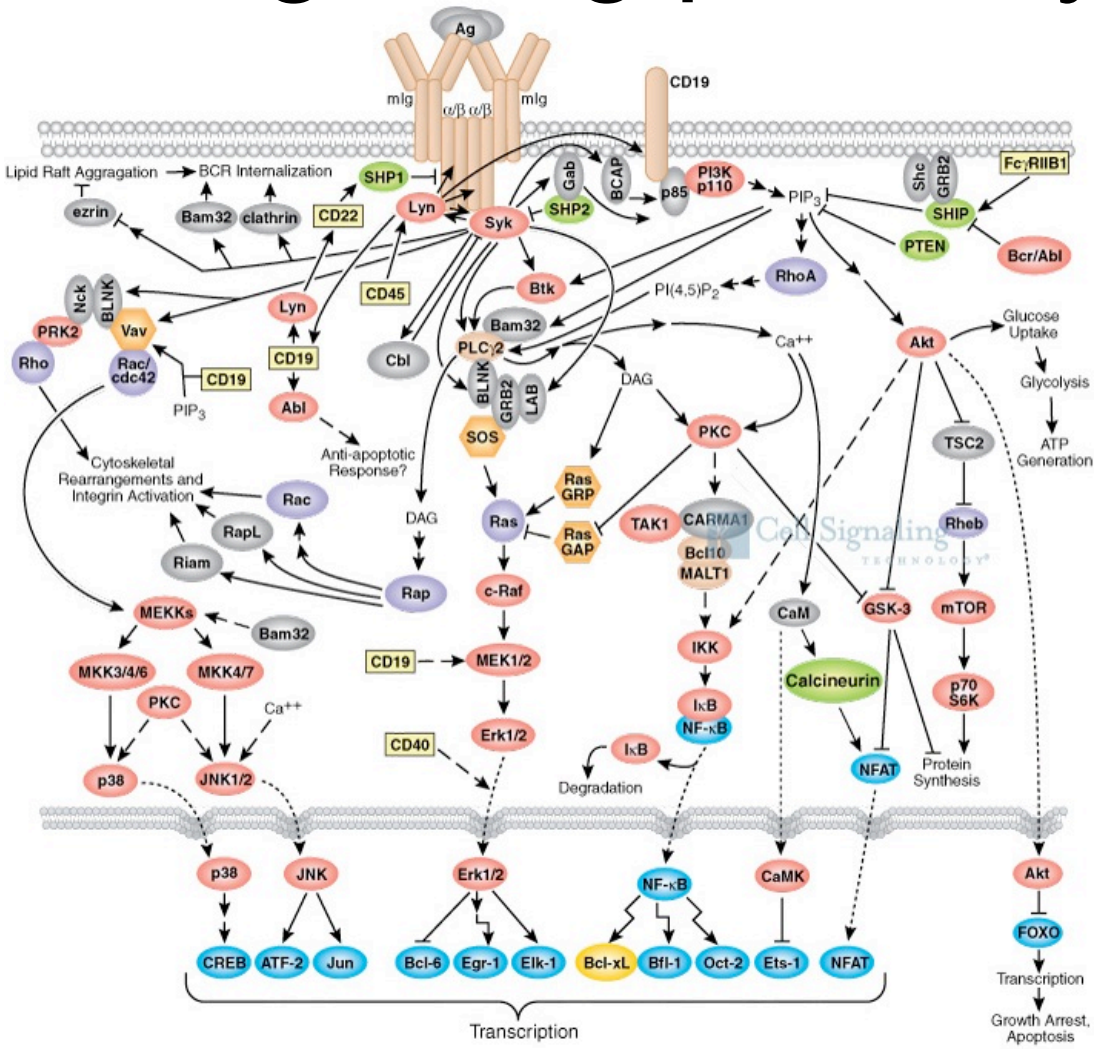


Figure 1. Signal transduction pathways in *Paracoccidioides brasiliensis*. Cell adhesion (orange), pheromone response (green), calcium/calmodulin (pink), cell integrity (blue), high osmotic growth stress response (brown), and TOR (purple) pathways are depicted.

Cell signaling pathways



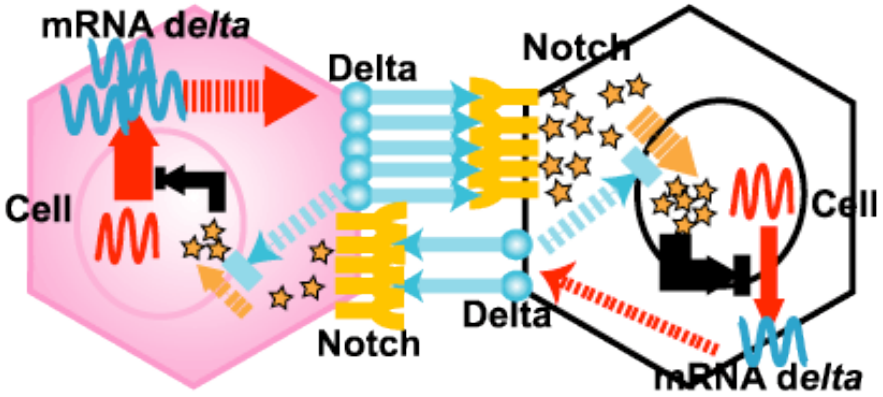
Cell signaling pathways

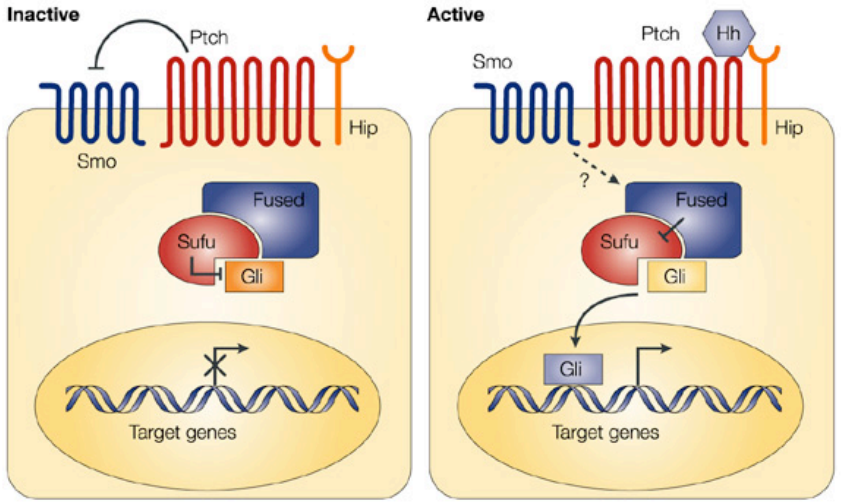


Cell signaling pathways

- Common components:
  - Receptors binding to ligands
    - R(inactive) + L → RL(active)
  - Proteins forming complexes
    - P1 + P2 → P1P2-complex
  - Proteins acting as enzymes on other proteins (e.g., phosphorylation by kinases)
    - P1 + K → P1* + K

Cell signaling pathways





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Cell signaling pathways

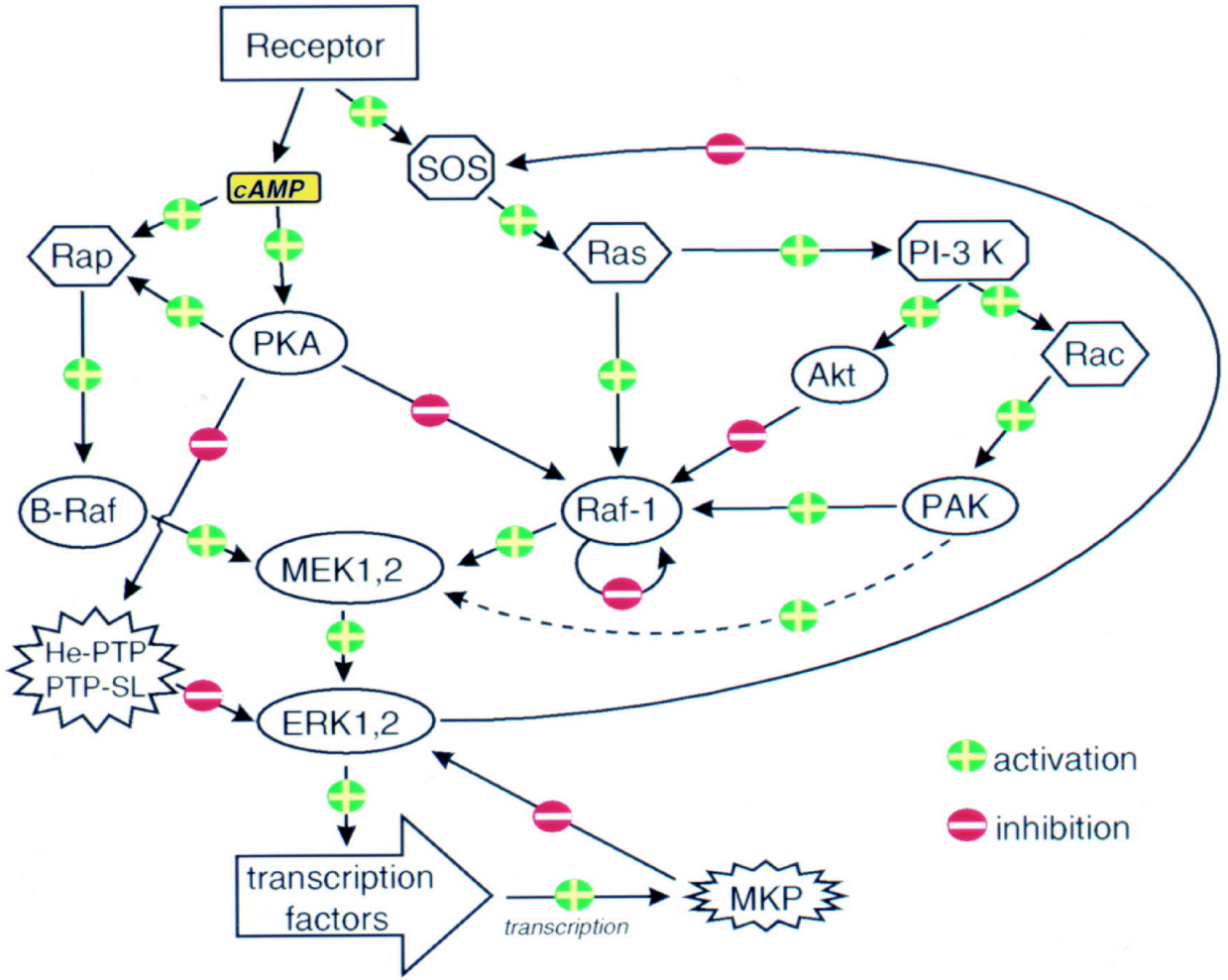


Fig. courtesy of W. Kolch

Cell signaling pathways

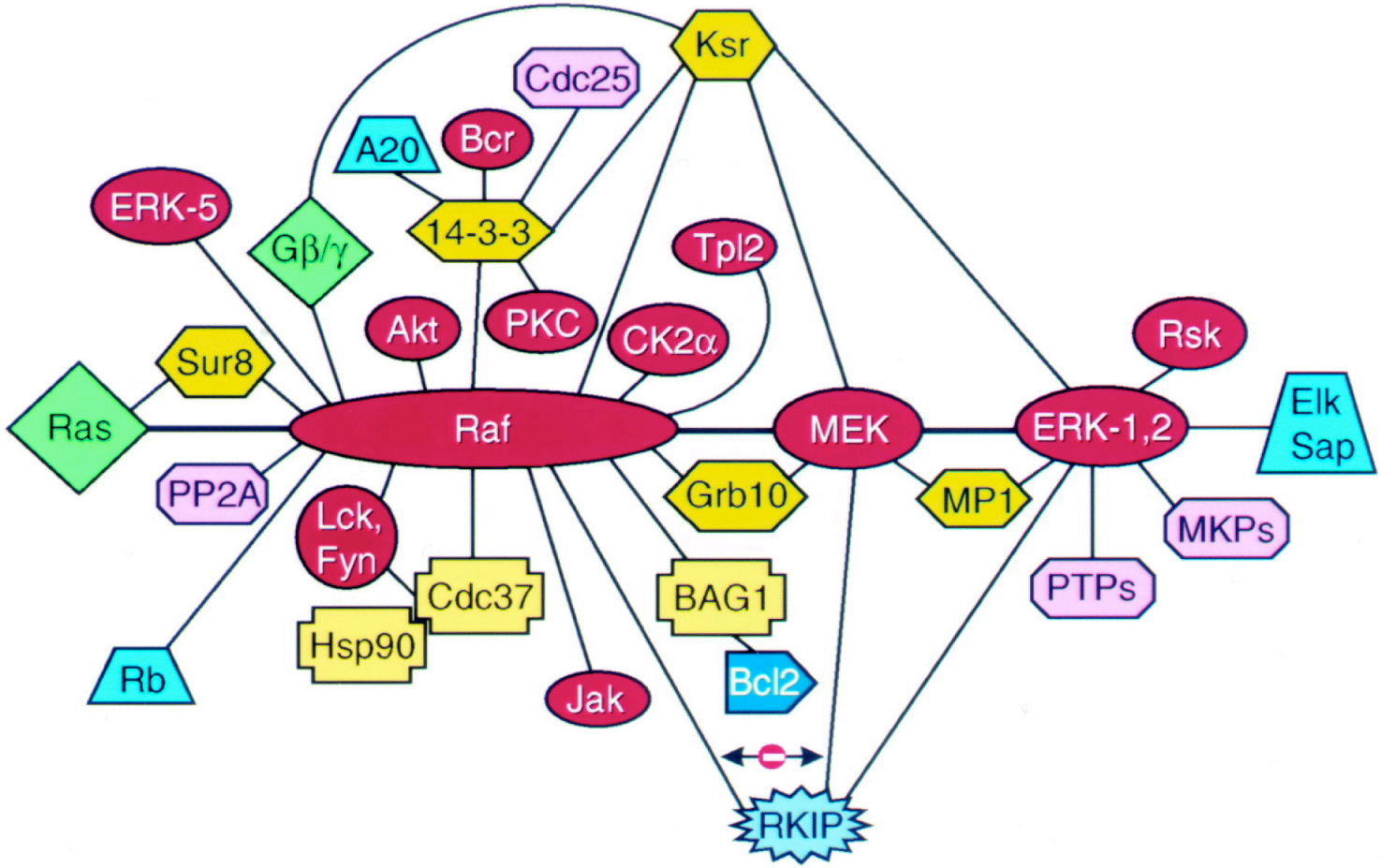
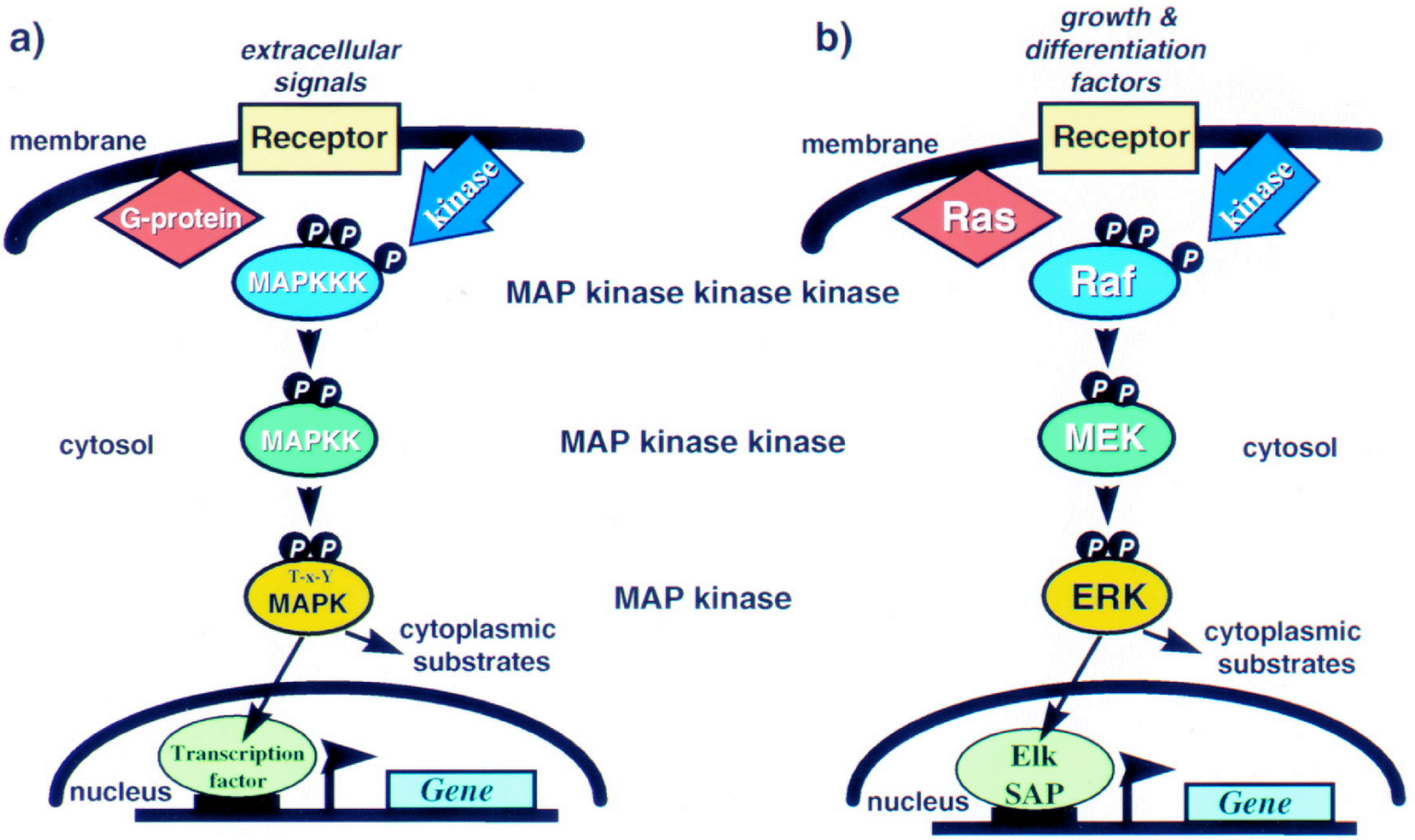


Fig. courtesy of W. Kolch

Cell signaling pathways



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