Systems Biology 551-1174-00L

Mechanistic Modeling Beyond Metabolism

16 Apr, 2017

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Content:

- Signaling pathways - networks (US)
- Dynamic models for cell signaling (JS)
  - MAPK cascade signaling
  - Application in drug discovery
- Exams (US/JS)

Learning Goals Lecture 7

- **Understand the consequences that kinases function in networks rather than pathways. Appreciate the role of phospho-proteomics in mapping these networks.**
- **Know main PRINCIPLES of signal transduction networks.**
- **Generalize the use of mechanistic models for quantitative analysis of dynamic signaling processes.**
- **Know examples of signaling model applications for basic biology and drug development.**

Signaling

- External signals are converted to responses within a cell
  - Nutrient information
  - Hormones
  - Neurotransmitter
  - Growth factors
  - ……..

Most cellular signals are chemical in nature

- Post-translational protein modifications (PTM) are well-suited for transmitting information because they are stable, reversible, and fast – frequently phosphorylation

Reception → **Transduction** → **Response**

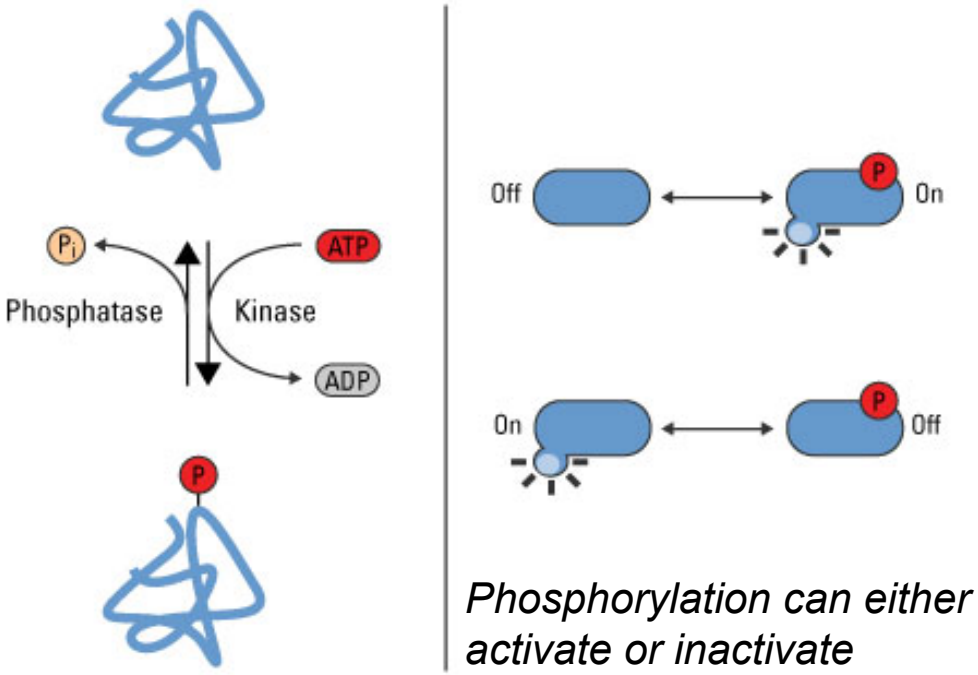
Either i) a membrane protein (receptor) that changes conformation or ii) transport of signal into cell.

eg G protein-coupled receptors, receptor tyrosin kinases, ion channels …..

Typically many consecutive PTM steps: **signal transduction pathway!**

- Metabolism
- Proliferation
- Autophagy
- Cell cycle
- Gene regulation
- ……

Protein Phosphorylation



Phosphorylation can either activate or inactivate

http://www.piercenet.com/method/phosphorylation

- Arguably most important post-translational modification.
- Transfer of phosphate group from ATP or GTP to an amino acid side-chain with a free hydroxyl group; ie **serine**, **threonine** and **tyrosine**, in eukaryotic cells.
- Usually causes a change in protein conformation that may thereby affect protein stability, localization, ability to form complexes, or activity.

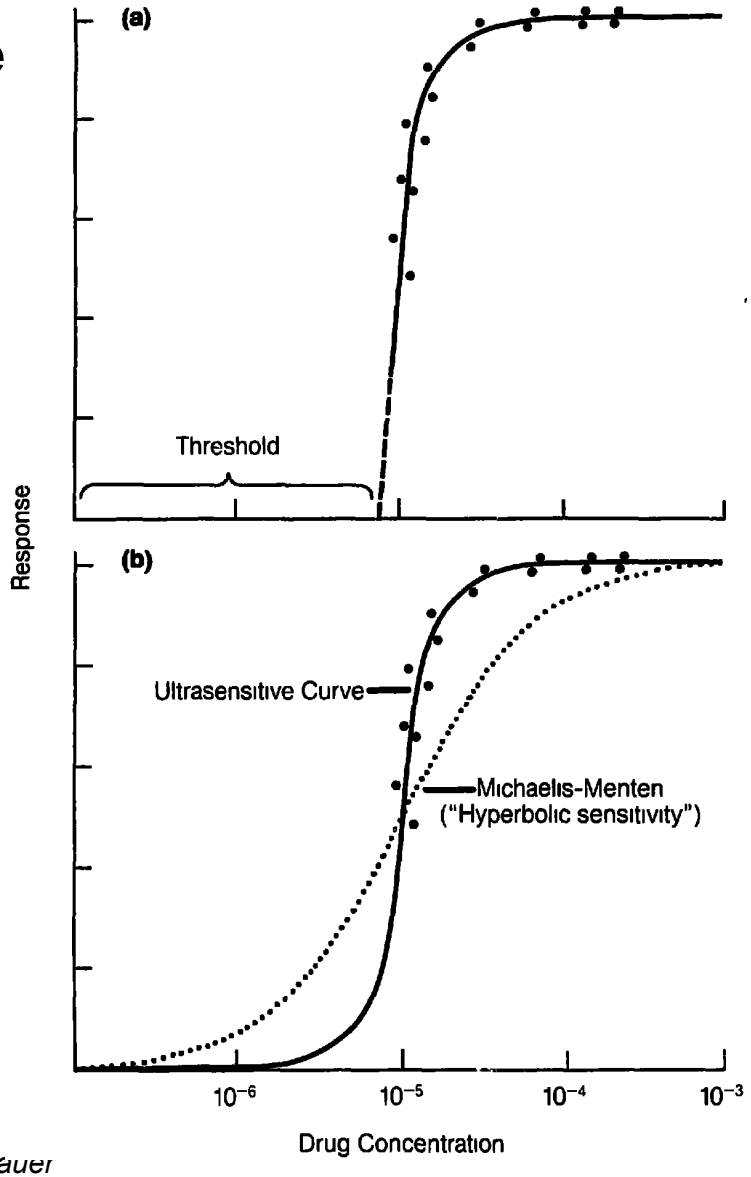
PTM are covalent and generally enzymatic modifications of specific amino acids during or after protein biosynthesis. They extend the chemical repertoire of the 20 standard amino acids by introducing new functional groups such as phosphate, acetate, amide groups, methyl, or >20 other groups.

Koshland 1987, TIBS

Signal Response Curves

Idealistic response
would be relevant for neuronal signals, hormone response, differentiation …..

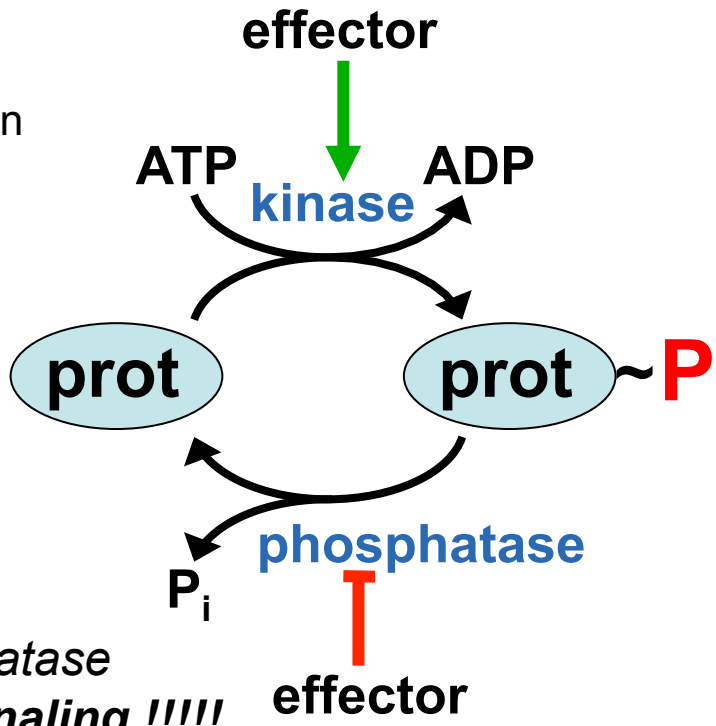
Realistic response
close to threshold



Ultrasensitivity: Response of a biological system that is more sensitive than to be expected from classical hyperbolic Michaelis-Menten kinetics.
The larger the Hill coefficient, the more ultrasensitive is max amplification

Mechanisms for Ultrasensitivity

- Cooperativity
  Where binding of a ligand to one subunit of multimeric enzymes/receptors leads to a conformational change of binding sites on other subunits, thereby either increasing or decreasing the binding affinity (e.g. oxygen binding to hemoglobin)
- Multistep ultrasensitivity
  When an allosteric effector affects more than one enzyme in a pathway (eg activation of forward reaction and inhibition of backward reaction). Higher Hill coefficients could be achieved in cascades of such reactions (*where have you seen this in biology?*)
- Others



Examples:
Phosphofructokinase and fructose-1,6-bisphosphatase
Many protein kinase/phosphatase pairs in signaling !!!!!

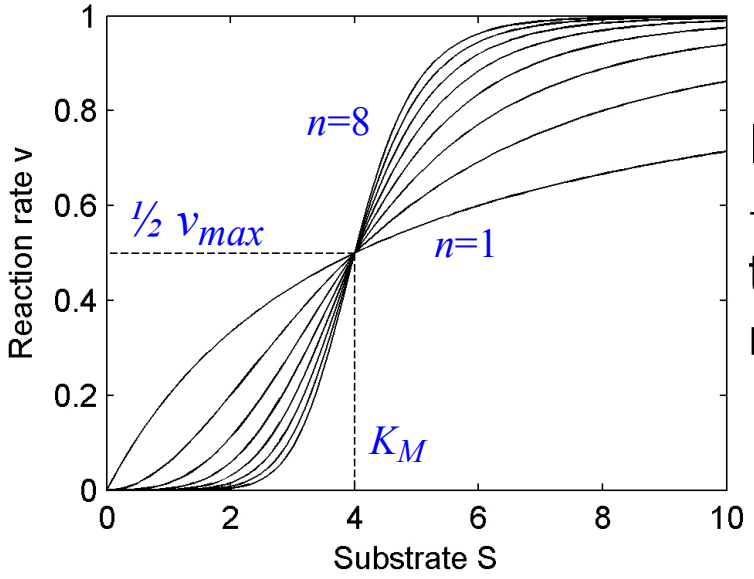
Hill Coefficient

- Quantifies cooperative binding by describing the fraction of saturated ligand binding sites as a function of the ligand concentration.

$$E + nS \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}nS \xrightarrow{k_2} E + nP$$

- A simple and widely used model is a rate law derivation analogous to Michaelis Menten. Cooperativity with a Hill coefficient n redefines K_M.

$$v = \frac{v_{max}[S]_0^n}{[S]_0^n + K_M^n}$$



Increasing cooperativity (Hill coefficient n) → Conversion from hyperbolic ('graded') to sigmoidal ('ultrasensitive') signal-response characteristic.

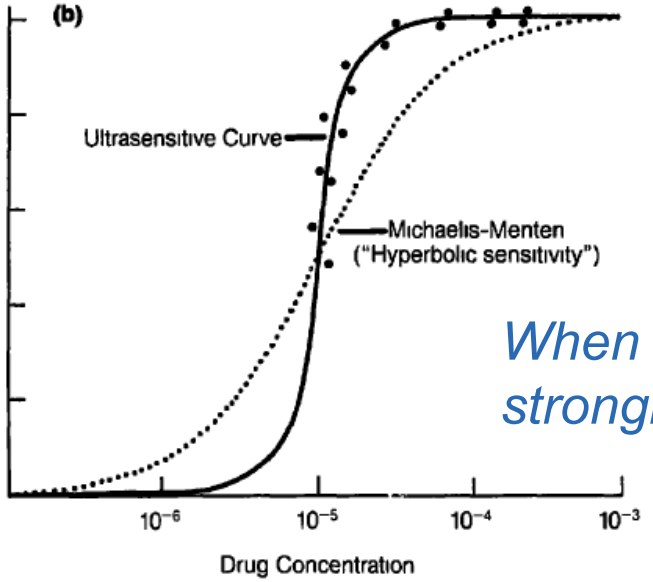
What case is n = 1?

Modulation of Proteinphosphorylation is a Key Mechanism to Achieve Ultrasensitivity

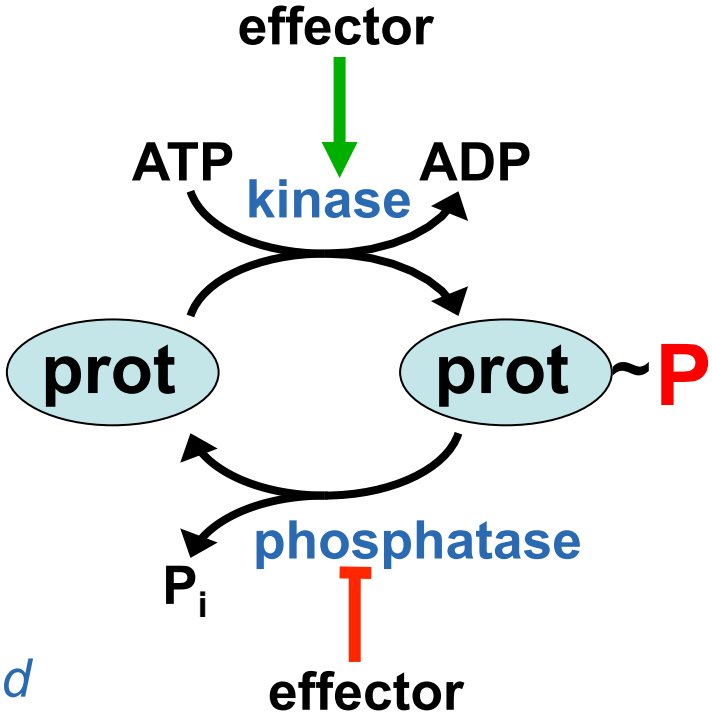
One mechanism: **Multistep ultrasensitivity**

(eg kinase cascades)

When an allosteric effector affects more than one enzyme in a pathway (eg activation of forward reaction and inhibition of backward reaction). **Higher Hill coefficients could be achieved in cascades of such reactions**.



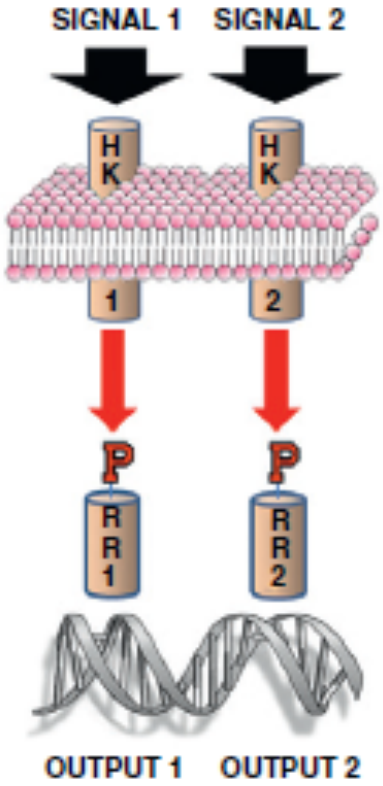
When cells need to respond strongly to small changes



Eukaryotic Signaling Pathways are Highly Interconnected – Signaling Networks

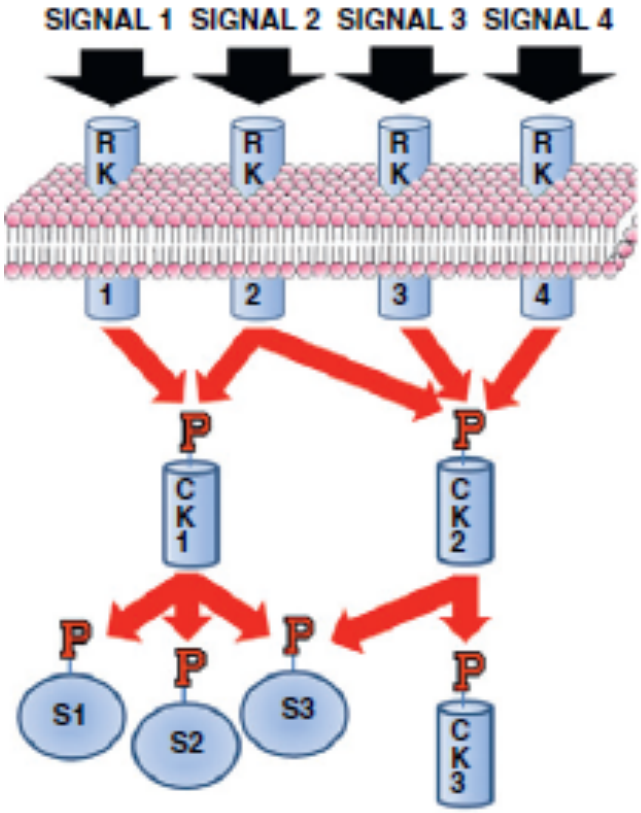
Linear bacterial 2-component signaling

Most bacterial signaling is linear with few intermediates



Branched eukaryotic signaling pathways

The reality are highly connected phosphorylation networks

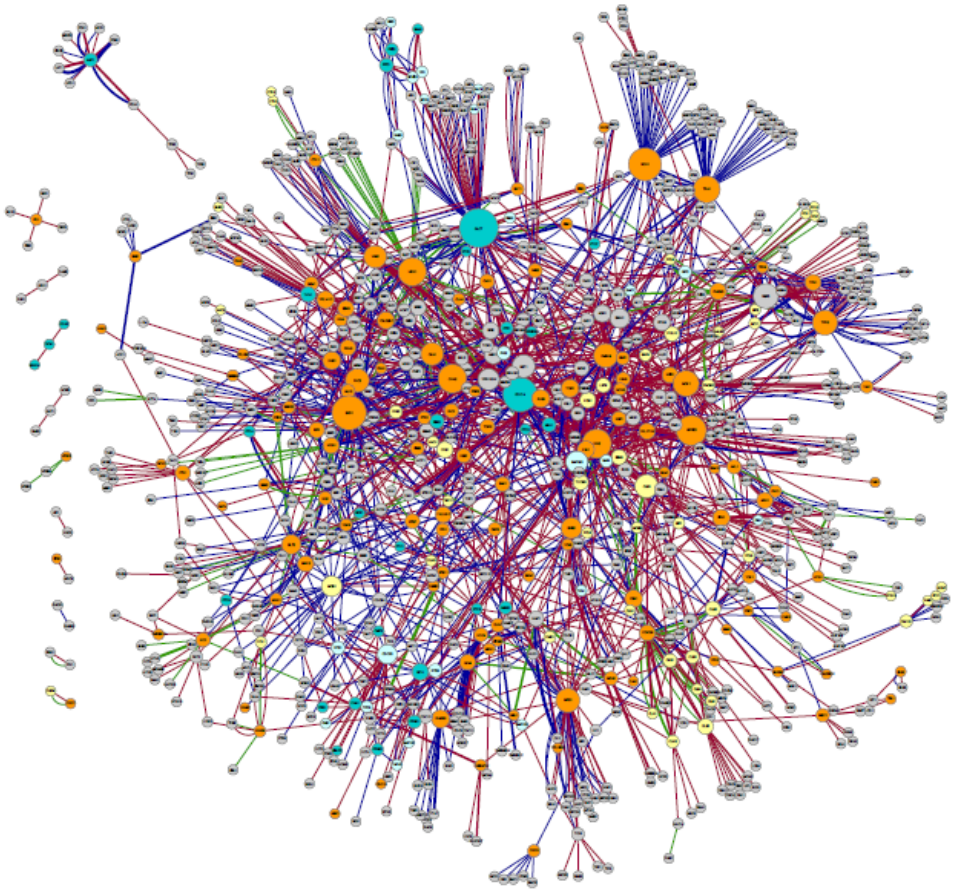


Kinase/Phosphatase Networks

- In humans > 500 kinases and >150 phosphatases, even in yeast about 300
- 80% are serine/threonine kinases (about 90% of P-sites are Ser/Thr)
- Some kinases phosphorylate only a single protein, others many (>300) substrate proteins
- More than half of the proteins are phosphorylated, most have many P-sites
- Experimental detection
  - Immuno methods: P-form specific antibodies
  - **P-proteomics**: Peptide-based methods differentiate P-peptides from non-P-peptide (typically after enrichment of P-proteins through metal oxide affinity chromatography)

Impact of Phospho-Proteomics

- Most of what we know about the small-scale connections of important signaling networks was done through traditional biochemistry. *But different from metabolism, the* ***knowledge is very fragmented****!*
- Advances in mass spec instruments, P-peptide enrichment and data processing, have made P-proteomics possible, providing a much broader view of the **cell-wide network topology of kinases, phosphatases and their targets**.
- Example of kinase-phosphatase network in yeast published in 2010
  Bodenmiller et al. 2010
  Breitkreutz et al. 2010
  Signaling breakthrough paper 2010



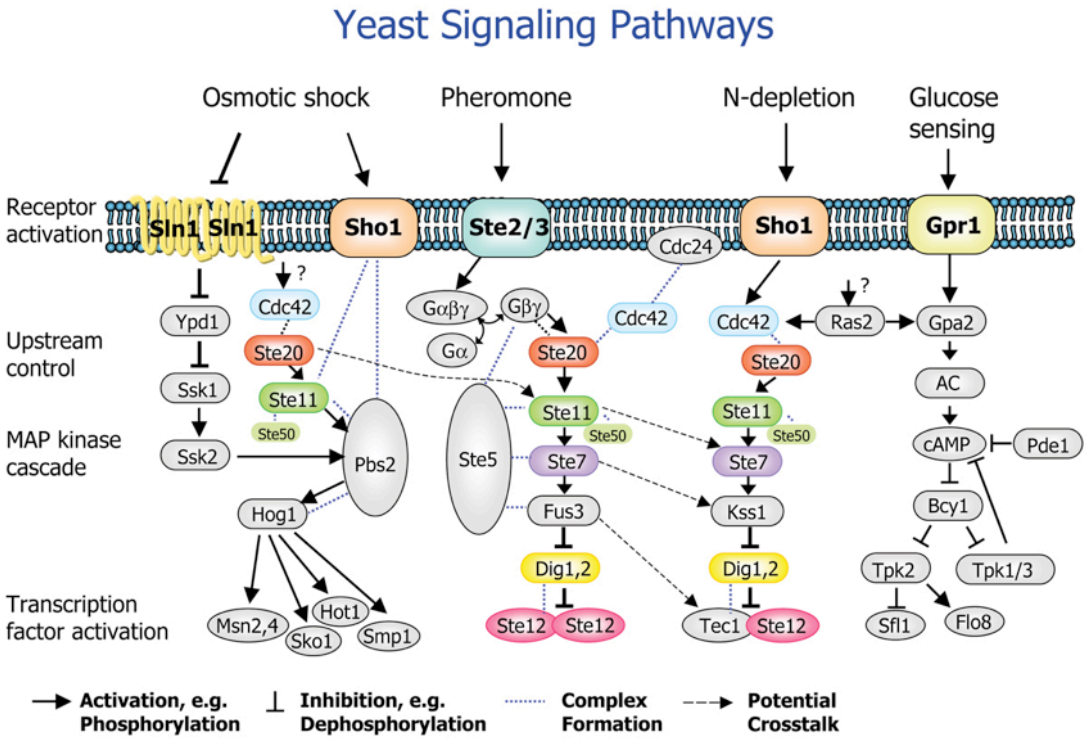
120 kinases
40 phosphatases
887 proteins
1844 interactions

How could one use P-proteomics to learn about signaling?

Pathways vs Networks

For practical (and didactic) reasons and to mechanistically capture signaling dynamics we work mostly with the concept of “pathways”

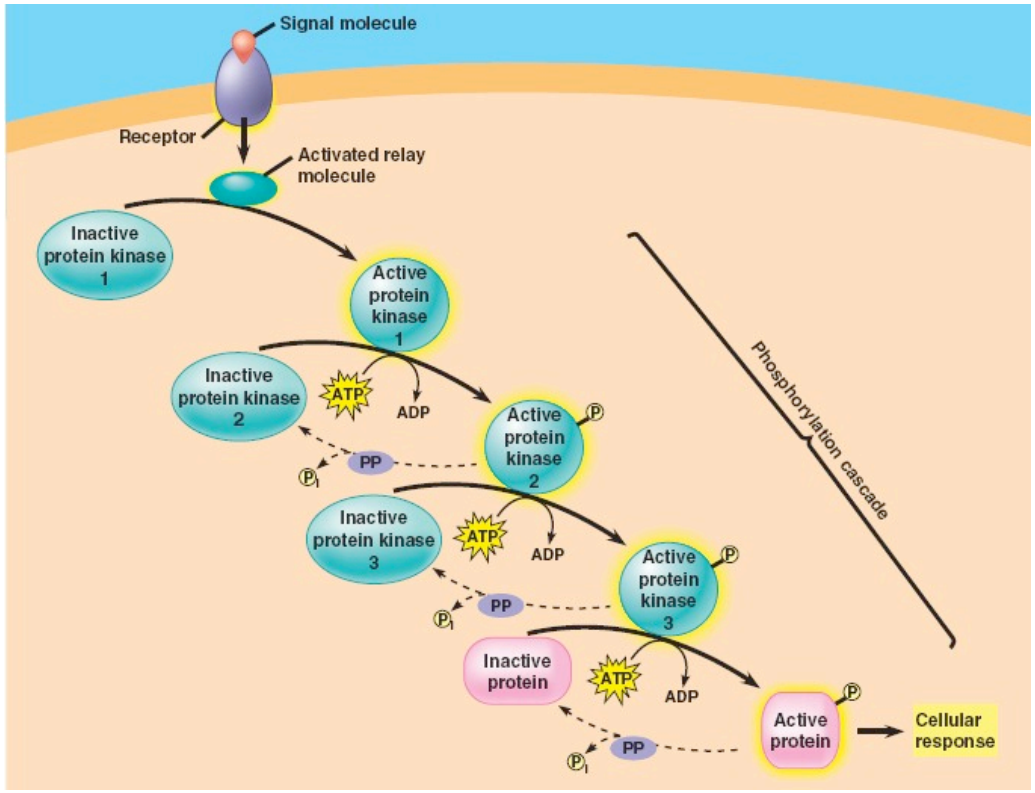
Kinases participate often in multiple pathways. There is much more cross-connection that apparent from pathway diagrams



Signaling Pathways in baker's yeast *S. cerevisiae*. Most of these pathways comprise a MAP kinase cascade and transcription factors that regulate the expression of target genes.
Klipp and Liebermeister 2006

Signaling Cascades

- Many classes of receptors bind their ligand and activate protein kinases inside the cell.
- Activation frequently leads to a series of protein phosphorylation/ dephosphorylation events – a cascade
- Responses to signals must be reversible. **Phosphatases** that remove phosphates are one example of a specific mechanism to reverse a signal response. **Autodephosphorylation** is another removal mechanism.



Why Kinase Cascades ?

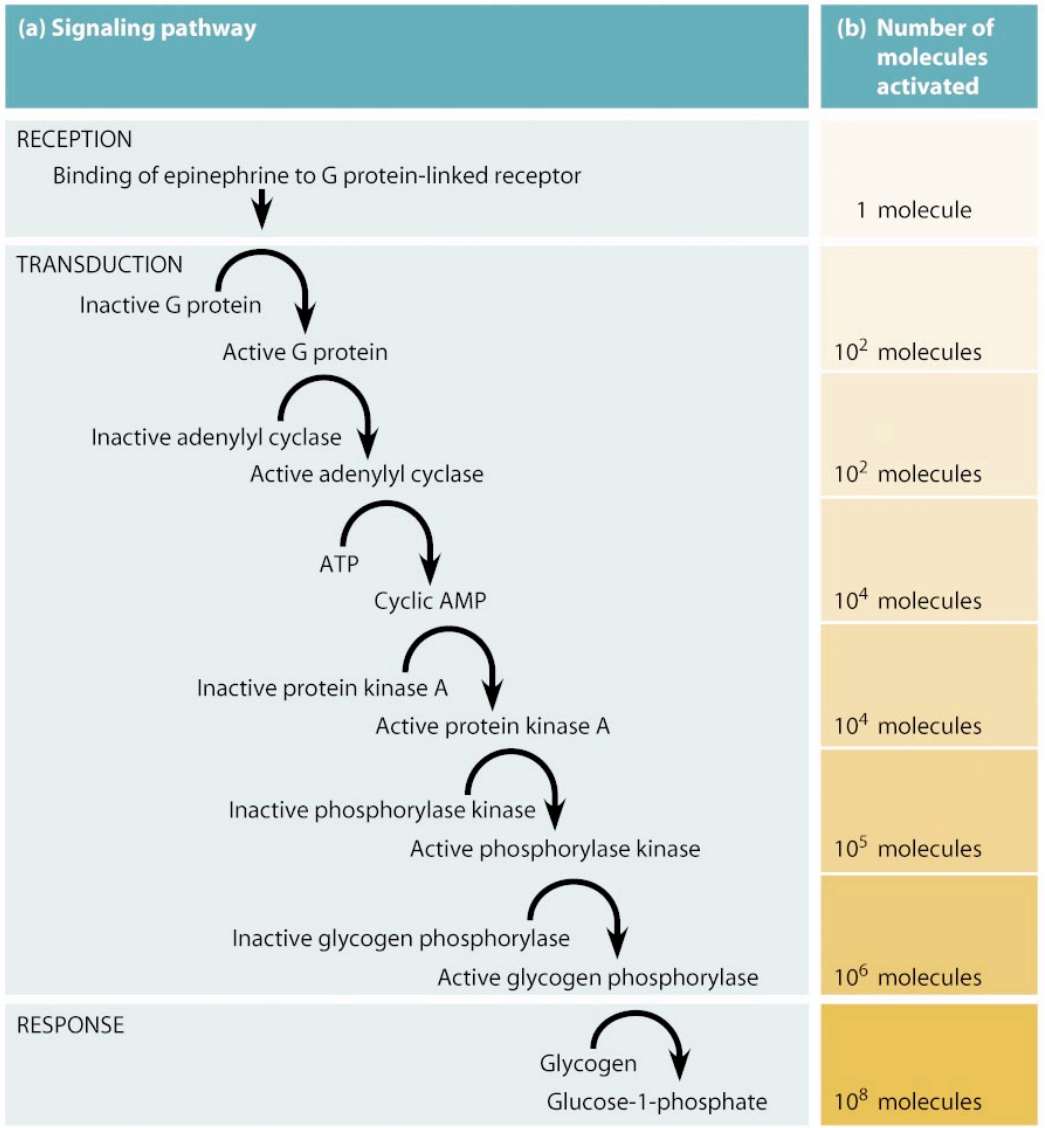
General assumption:

Signal amplification

- Dramatically speeding up a cell response.
- Many target proteins can be reached. Example liberation of glucose from glycogen.

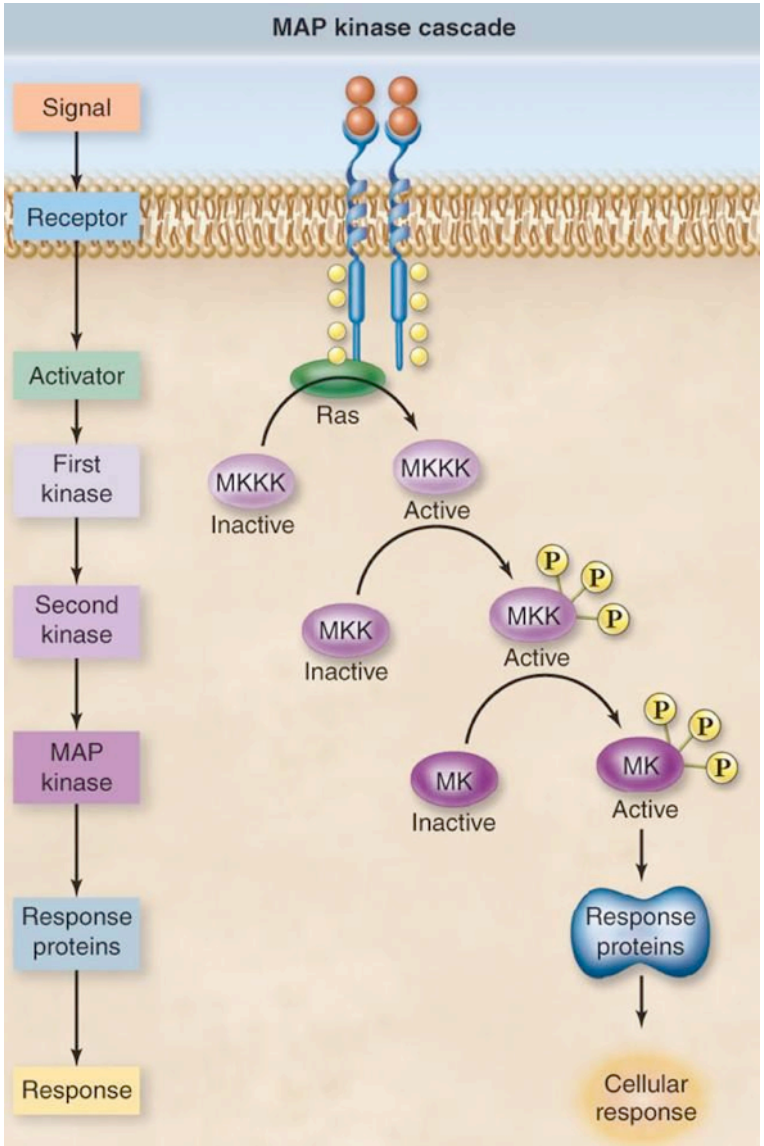
Modulation of specificity

Can also dampen the signal, given appropriate parameters



MAP Kinase Cascades

- Mitogen-activated protein kinases (MAPKs)
- Family of Ser/Thr kinases that transduce signals from membrane cell to nucleus
- Many different MAPK Cascades that participate in many different signaling responses to a wide range of stimuli,
- control many processes (eg growth, differentiation, apoptosis ….)
- Conserved from yeast to humans
- Dephosphorylation is assumed to occur continuously
- **Signal flow through MAP cascades appears only linear when taken out of context of other interactions !!**



What Influences Signal Processing?

- Parameters
  - ultrasensitivity
  - amplification/dampening (eg MAP kinase cascade
  - dynamics
  - dynamic filtering
- Regulation structure
  - feedback/feed forward
  - ……
- Pathway interaction
  - multiple inputs
  - competition of targets for a kinase ….
  - .....

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Potential Exam (Knowledge) Question

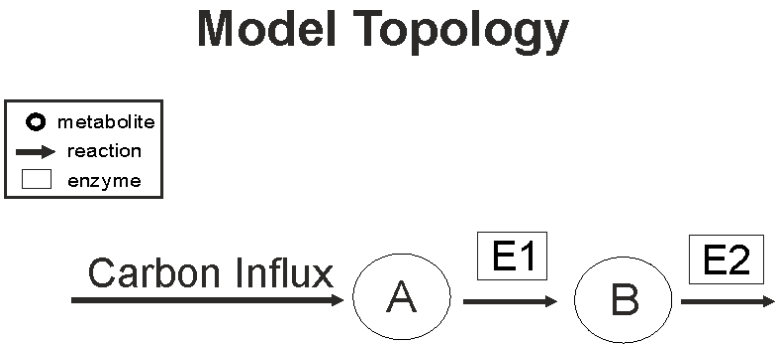
A) Was ist Ultrasensitivität und wie unterscheidet sie sich von einer Michaelis-Menten Kinetik? (1 P)

B) Nennen Sie ein Beispiel wie eine ultrasensitive Systemantwort erreicht werden kann. (1 P)

A) Response that is more sensitive than to be expected from classical hyperbolic Michaelis-Menten kinetics.
The larger the Hill coefficient, the more ultrasensitive is max amplification.

B) i) cooperativity or ii) Multistep ultrasensitivity:

Potential Exam (Transfer ODE) Question



Ein biologisches System mit irreversiblen Reaktionen hat die in der Abbildung gezeigte Topologie (nehmen Sie Michaelis-Menten Kinetik für Enzyme E1 und E2 und einen Carbon Influx mit konstante Rate r_{in} an).

A) Schreiben Sie die Bilanzgleichungen für die Metabolitkonzentrationen. (1 P)

B) Ändern sich die Steady State Konzentrationen von Metabolit A und/oder B wenn die Startkonzentration von Metabolit A von 4 mM auf 0.01 mM geändert wird? (1 P)

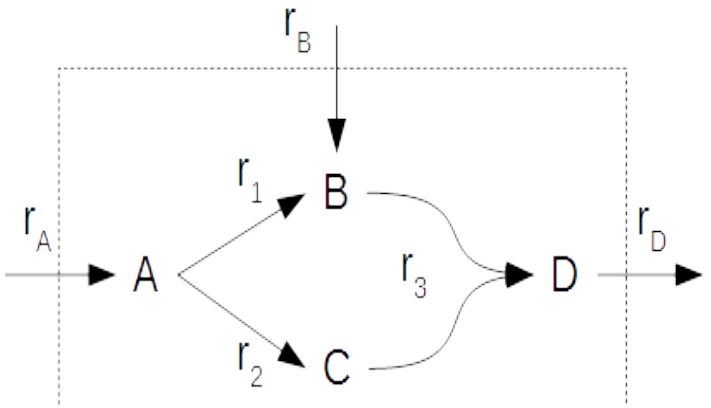
C) Ändern sich die Steady State Konzentrationen von Metabolit A und/oder B wenn der K_m-Wert von Enzym 2 von 0.5 mM auf 0.25 mM geändert wird? (1 P)

D) Der Carbon Influx r_{in} ist konstant und der Fluss durch Enzym E1 wird mit r_1 bezeichnet. Skizzieren Sie graphisch die Raten r_{in} und r_1 als Funktion der Konzentration von Metabolit A. (2 P)

E) Existieren Fälle, in denen das System keinen stationären Zustand erreichen kann? Wenn ja, wann (Argumentieren Sie mit der Skizze aus D)? (1 P)

Potential Exam (Transfer FBA) Question

Sie analysieren ein metabolisches Netzwerk im stationären Zustand. Das Netzwerk hat vier interne Metabolite A-D, drei interne Reaktionen R_1-R_3 mit Flüssen r_1-r_3, drei externe Reaktionen. Alle Reaktionen sind irreversibel.



Hinweis: Die Lösung von A) ist nicht Voraussetzung für die anderen Teile der Aufgabe.

A) Geben Sie die stöchiometrische Matrix des Netzwerkes an. (2 P)

B) Welche Reaktionen sind essentiell um einen Steady State zu erhalten in dem nicht alle Flüsse null sind (begründen Sie die Antwort)? (1 P)

C) Sie haben den Fluss r_D = 1 mmol/h gemessen. Welche anderen stationären Flüsse können Sie direkt bestimmen und welche Werte haben diese? (1 P)

D) Mit dem Messwert aus C), wie müssen sich r_A und r_B zueinander im Steady State verhalten? (1 P)

Potential Exam (Exercise) Question: Pseudo Code

Problem

Assume you have the implementation of a model and you are asked to 1) simulate and 2) plot the dynamic behavior of the system, in the case where we change the initial concentrations of one species (species A). The range of initial concentrations that we want to change (and afterwards simulate the system and plot the results) is: [10, 20, 30,…,100]. Write a small script in pseudo-code, that solves this problem (3-4 pt).

Solution

Line 1 for *concentrationValue* = 10 until 100 with step 10
Line 2 in the model, change initial concentration of species A to *concentrationValue*;
Line 3 simulate the model and store the results;
Line 4 plot the results of the simulation in a figure;
Line 5 end

Pseudo code does not run on any computer, but displays the logic of programing the individual steps without requiring the synthax of a programming language (of course you could write the actual Matlab code if you want….). The formulation is free, but the logic must hold.