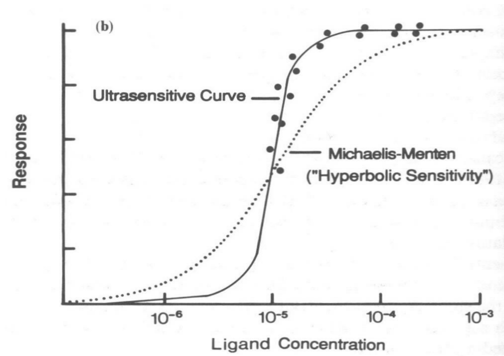
Quantifying cooperativity: Hill function and Hill coefficient

Hill equation:

$$\text{response} = \frac{[L]^n}{(K_A)^n + [L]^n}$$

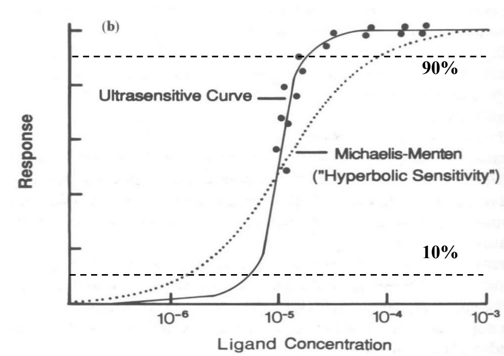


1-1

Quantifying cooperativity: Hill function and Hill coefficient

Hill equation:

$$\text{response} = \frac{[L]^n}{(K_A)^n + [L]^n}$$

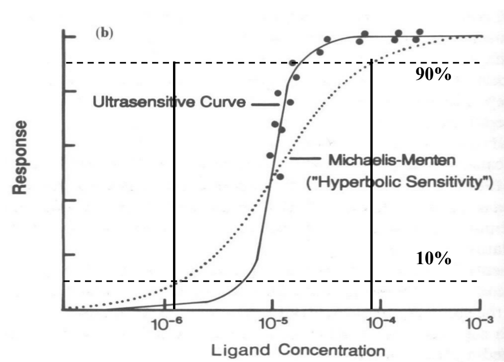


1-2

Quantifying cooperativity: Hill function and Hill coefficient

Hill equation:

$$\text{response} = \frac{[L]^n}{(K_A)^n + [L]^n}$$



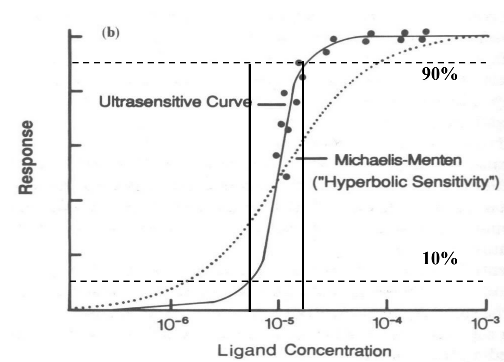
81 fold

1-3

Quantifying cooperativity: Hill function and Hill coefficient

Hill equation:

$$\text{response} = \frac{[L]^n}{(K_A)^n + [L]^n}$$



EC_{90}/EC_{10}

1-4

Apparent vs. real cooperatively

Actual (positive) Cooperativity — molecular function enhanced by binding at a molecular level.

e.g., subsequent ligand binding is enhanced by previous ligand binding.

Apparent (positive) cooperativity — no enhancement at the molecular level, but an apparent enhancement observed at the level of populations of molecules.

e.g., T <=> R <=> R1 <=> R2

Example: Real cooperativity - hemaglobin

* four subunits, two alpha and two beta

* each subunit binds O2

* sequential binding model (below) vs. concerted binding model

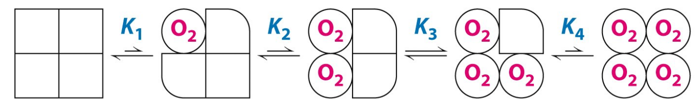


Figure 7.14
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

Implications of hemoglobin cooperativity

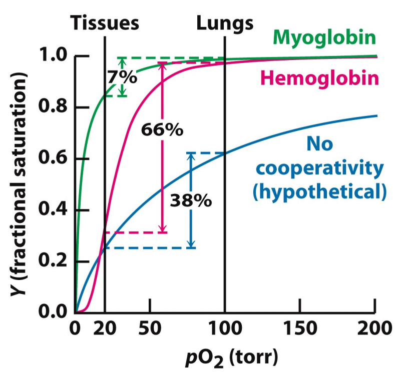


Figure 7.9
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

Physiological roles of (actual/apparent) cooperativity

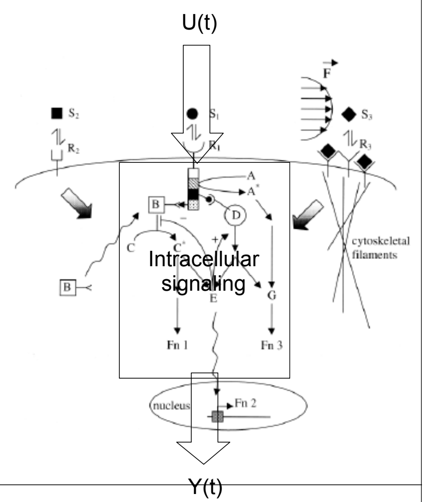
titration of binding
creating thresholds for function
regulatory switches

From environmental signals to cell responses: the role of signaling networks

Environment
* soluble and tethered ligands

Cell detection
* receptors

From Detection to Action
*** signaling networks**



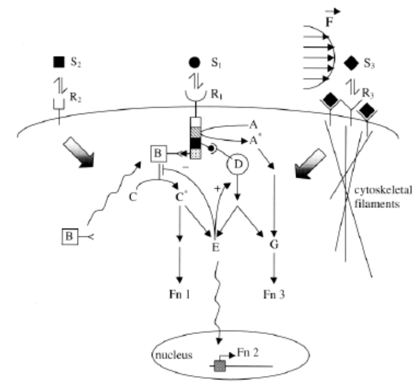
The anatomy of signaling networks

Environment
* soluble and tethered ligands

Cell detection
* receptors

From Detection to Action
* signaling networks

Concentrations of key molecules determine cell behaviors

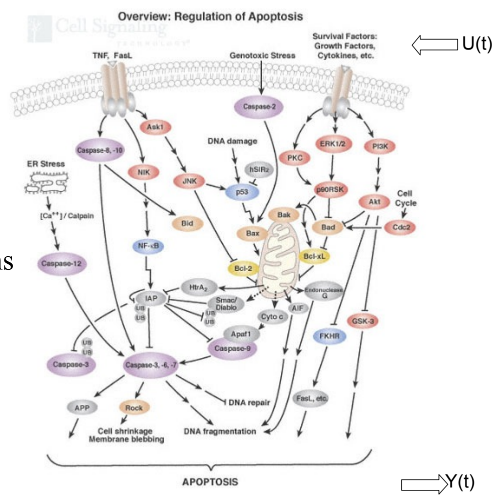


Discrete choices: To be or not to be

From analog concentrations

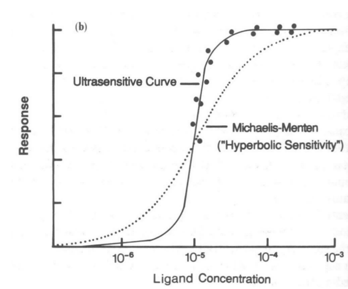
????

to digital cell response

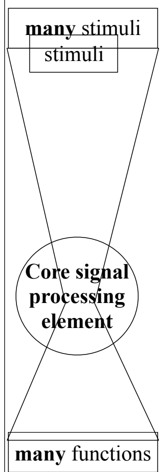


www.cellsignal.com, *Cell Signaling Technology*

Cooperativity provides a way to achieve discrete responses to analog inputs

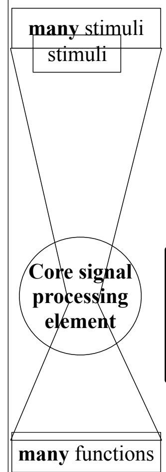


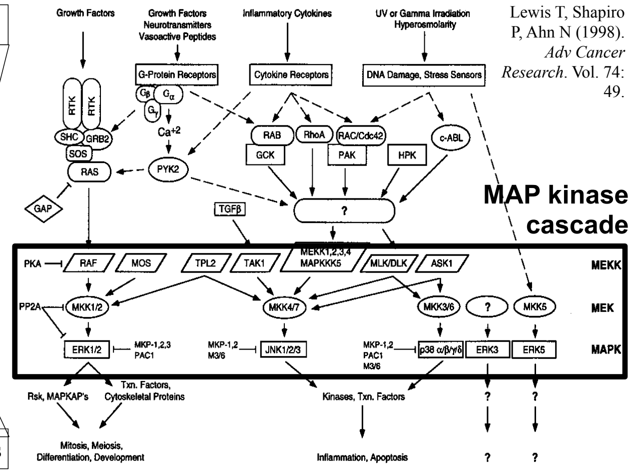
Core signaling module: the MAP kinase cascade



10-1

Core signaling module: the MAP kinase cascade





Lewis T, Shapiro P, Ahn N (1998). *Adv Cancer Research*. Vol. 74: 49.

10-2

From Yeast to Humans

Yeast MAPK

Stimulus	a or α factor	Starvation	high osmolarity	Hypotonic shock	C and N depletion
MAPKKK	STE11	STE11	STE11 SSK2/22	BCK1	???
MAPKK	STE7	STE7	PBS2	MKK1/2	???
MAPK	FUS3	KSS1	HOG1	MPK1	SMK1
Response	Mating	Filamentation	Osmolyte synthesis	Cell wall remodeling	Sporulation

11-1

From Yeast to Humans

Yeast MAPK

Stimulus	a or α factor	Starvation	high osmolarity	Hypotonic shock	C and N depletion
MAPKKK	STE11	STE11	STE11 SSK2/22	BCK1	???
MAPKK	STE7	STE7	PBS2	MKK1/2	???
MAPK	FUS3	KSS1	HOG1	MPK1	SMK1
Response	Mating	Filamentation	Osmolyte synthesis	Cell wall remodeling	Sporulation

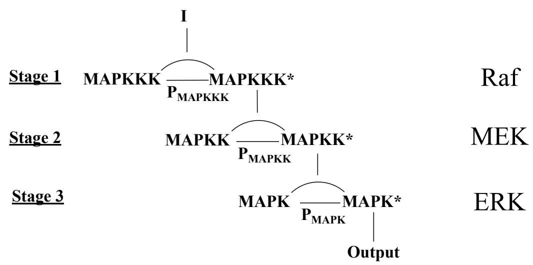
Mammalian MAPK

Stimulus	Growth Factors	Inflammatory cytokines cellular stress		???	Serum Stress
MAPKKK	Mos Raf TPL2	TPL2 MEKK	MLK/DLK TAK ASK	???	???
MAPKK	MEK1/2	MKK4/7	MKK3/6	???	MKK5
MAPK	ERK1/2	JNK	P38 MAPK	ERK3	ERK5
Response	Proliferation, Differentiation, Development	Inflammation, Apoptosis, Development		???	Proliferation?

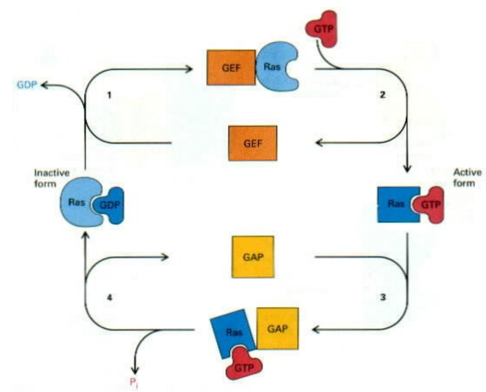
11-2

The MAPK cascade is composed of "futile cycles" in series

a well-conserved mechanism of signaling…
"futile cycles" in series…

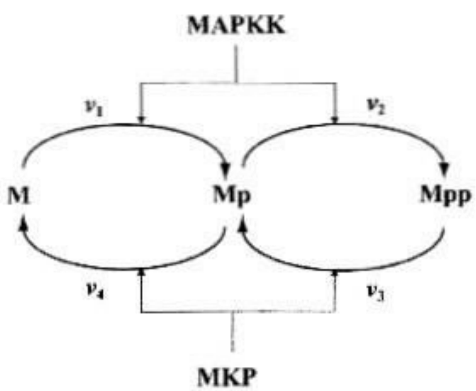


Futile Cycles upstream of MAP kinase cascade



Futile cycles are manifested in many ways
- G protein cycling

Futile Cycles of Phosphorylation in MAP kinase cascade



Futile cycles are manifested in many ways
- G protein cycling
- Phosphorylation

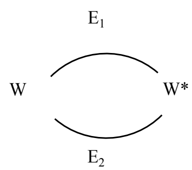
State of the system is described by the substrate

Energy is consumed – careful with microscopic reversibility

What are the quantitative implications of a futile cycle 'design'?

Covalent modification systems – Koshland-Goldbeter Switch

Model:

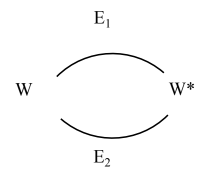


Goldbeter, A. and Koshland, D. (1981) PNAS **78**(11), 6840-6844.

16-1

Covalent modification systems – Koshland-Goldbeter Switch

Model:



$$\mathrm{W} + \mathrm{E}_1 \underset{k_{u1}}{\overset{k_{c1}}{\rightleftharpoons}} \mathrm{U}_1 \xrightarrow{\mathrm{k}_{a1}} \mathrm{W}^* + \mathrm{E}_1$$

$$\mathrm{W}^* + \mathrm{E}_2 \underset{k_{u2}}{\overset{k_{c2}}{\rightleftharpoons}} \mathrm{U}_2 \xrightarrow{\mathrm{k}_{a2}} \mathrm{W} + \mathrm{E}_2$$

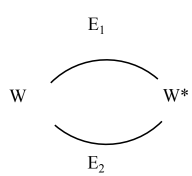
Goldbeter, A. and Koshland, D. (1981) PNAS **78**(11), 6840-6844.

16-2

Covalent modification systems – Koshland-Goldbeter Switch

Model:

Mass-action kinetics.
For example…



$$\mathrm{W} + \mathrm{E}_1 \underset{k_{u1}}{\overset{k_{c1}}{\rightleftharpoons}} \mathrm{U}_1 \xrightarrow{\mathrm{k}_{a1}} \mathrm{W}^* + \mathrm{E}_1$$

$$\mathrm{W}^* + \mathrm{E}_2 \underset{k_{u2}}{\overset{k_{c2}}{\rightleftharpoons}} \mathrm{U}_2 \xrightarrow{\mathrm{k}_{a2}} \mathrm{W} + \mathrm{E}_2$$

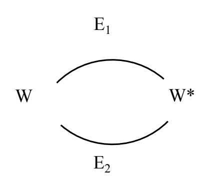
Goldbeter, A. and Koshland, D. (1981) PNAS **78**(11), 6840-6844.

16-3

Covalent modification systems – Koshland-Goldbeter Switch

Model:

Mass-action kinetics.
For example…



$$\mathrm{W} + \mathrm{E}_1 \underset{k_{u1}}{\overset{k_{c1}}{\rightleftharpoons}} \mathrm{U}_1 \xrightarrow{\mathrm{k}_{a1}} \mathrm{W}^* + \mathrm{E}_1$$

$$\mathrm{W}^* + \mathrm{E}_2 \underset{k_{u2}}{\overset{k_{c2}}{\rightleftharpoons}} \mathrm{U}_2 \xrightarrow{\mathrm{k}_{a2}} \mathrm{W} + \mathrm{E}_2$$

$$\frac{\mathrm{d}W}{\mathrm{d}t} = -k_{c1} W E_1 + k_{u1} U_1 + k_{a2} U_2$$

$$\mathbf{E_{1T} = [E_1] + [WE_1]}$$
$$\mathbf{E_{2T} = [E_2] + [W^*E_2].}$$

$$\mathbf{W_T = [W] + [W^*] + [WE_1] + [W^*E_2]}$$

Goldbeter, A. and Koshland, D. (1981) PNAS **78**(11), 6840-6844.

16-4

Covalent modification systems – Koshland-Goldbeter Switch

Steady-state solution:

$$W^* = \frac{\left(\frac{V_1}{V_2}-1\right) - K_2\left(\frac{K_1}{K_2}+\frac{V_1}{V_2}\right) + \left(\left[\frac{V_1}{V_2}-1-K_2\left(\frac{K_1}{K_2}+\frac{V_1}{V_2}\right)\right]^2 + 4K_2\left(\frac{V_2}{V_2}-1\right)\left(\frac{V_1}{V_2}\right)\right)^{1/2}}{2\left(\frac{V_1}{V_2}-1\right)}$$

(in implicit form…)

$$V_1 = k_1E_{1T},\ V_2 = k_2E_{2T},\ K_1 = \frac{d_1+k_1}{a_1W_T} = K_{m1}/W_T,$$

$$\text{and } K_2 = \frac{d_2+k_2}{a_2W_T} = K_{m2}/W_t\,.$$

$$\frac{V_1}{V_2} = \frac{w^*(1-w^*+K_{M1})}{(1-w^*)(w^*+K_{M2})}$$

$$W + E_1 \underset{d_1}{\overset{a_1}{\rightleftarrows}} WE_1 \xrightarrow{k_1} W^* + E_1$$

$$W^* + E_2 \underset{d_2}{\overset{a_2}{\rightleftarrows}} W^*E_2 \xrightarrow{k_2} W + E_2.$$

Goldbeter, A. and Koshland, D. (1981) PNAS **78**(11), 6840-6844.

17-1

Covalent modification systems – Koshland-Goldbeter Switch

Steady-state solution:

$$W^* = \frac{\left(\frac{V_1}{V_2}-1\right) - K_2\left(\frac{K_1}{K_2}+\frac{V_1}{V_2}\right) + \left(\left[\frac{V_1}{V_2}-1-K_2\left(\frac{K_1}{K_2}+\frac{V_1}{V_2}\right)\right]^2 + 4K_2\left(\frac{V_2}{V_2}-1\right)\left(\frac{V_1}{V_2}\right)\right)^{1/2}}{2\left(\frac{V_1}{V_2}-1\right)}$$

(in implicit form…)

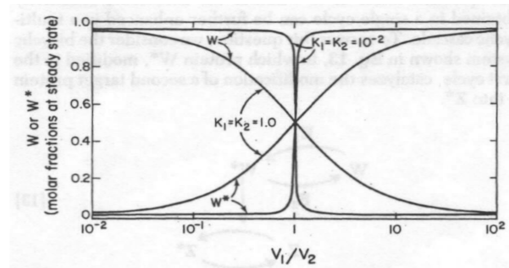
$$V_1 = k_1E_{1T},\ V_2 = k_2E_{2T},\ K_1 = \frac{d_1+k_1}{a_1W_T} = K_{m1}/W_T,$$

$$\text{and } K_2 = \frac{d_2+k_2}{a_2W_T} = K_{m2}/W_t\,.$$

$$\frac{V_1}{V_2} = \frac{w^*(1-w^*+K_{M1})}{(1-w^*)(w^*+K_{M2})}$$

$$W + E_1 \underset{d_1}{\overset{a_1}{\rightleftarrows}} WE_1 \xrightarrow{k_1} W^* + E_1$$

$$W^* + E_2 \underset{d_2}{\overset{a_2}{\rightleftarrows}} W^*E_2 \xrightarrow{k_2} W + E_2.$$



Goldbeter, A. and Koshland, D. (1981) PNAS **78**(11), 6840-6844.

17-2

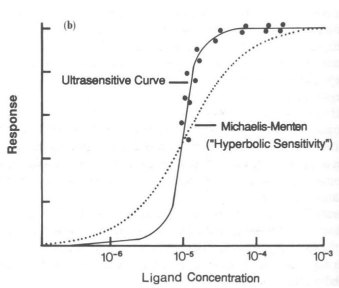
Ultrasensitivity – a switch-like response

Ultrasensitivity refers to the notion of a steep dose-response curve

Goldbeter-Koshland switch exhibits zero-order ultra sensitivity

Study questions:

- under what condition does G-K switch occur?
- is this apparent or real cooperativity?

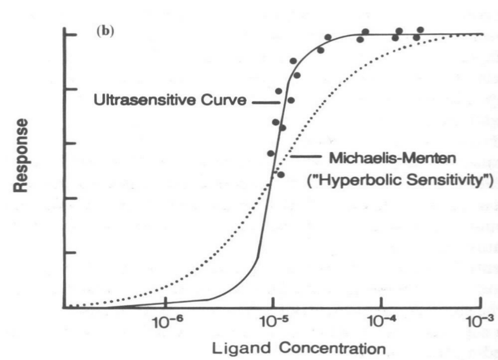


18

Quantifying ultrasensitivity: Hill coefficient

Hill equation:

response $\frac{[L]^n}{K_d + [L]^n} = \frac{[L]^n}{(K_A)^n + [L]^n}$

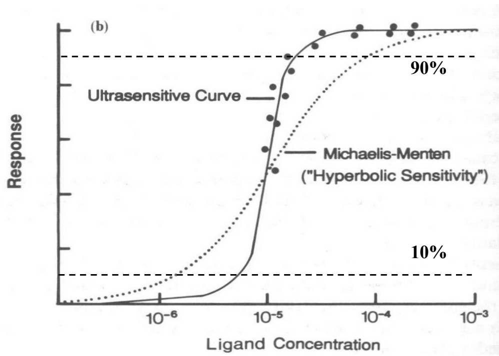


19-1

Quantifying ultrasensitivity: Hill coefficient

Hill equation:

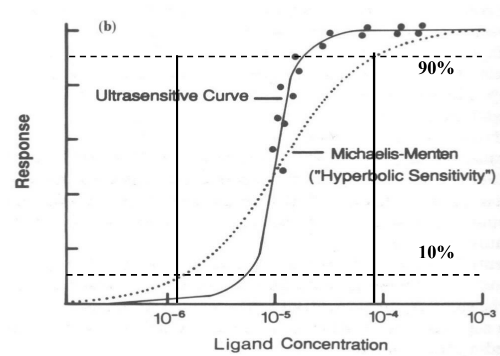
$$\text{response} = \frac{[L]^n}{K_d + [L]^n} = \frac{[L]^n}{(K_A)^n + [L]^n}$$



Quantifying ultrasensitivity: Hill coefficient

Hill equation:

$$\text{response} = \frac{[L]^n}{K_d + [L]^n} = \frac{[L]^n}{(K_A)^n + [L]^n}$$

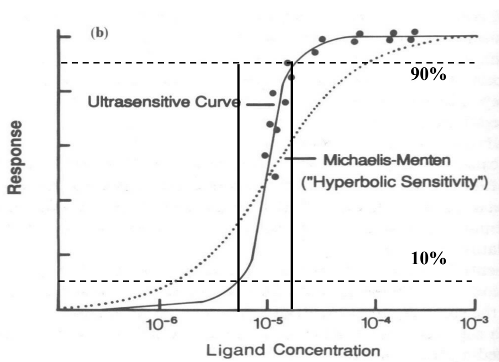


81 fold

Quantifying ultrasensitivity: Hill coefficient

Hill equation:

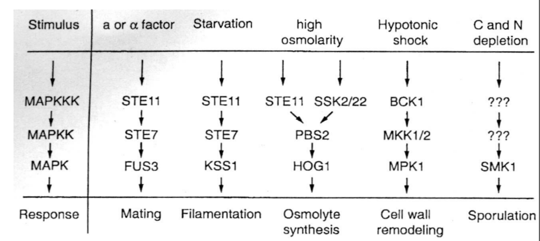
$$\text{response} = \frac{[L]^n}{K_d + [L]^n} = \frac{[L]^n}{(K_A)^n + [L]^n}$$



EC_{90}/EC_{10}

Recall: Futile cycles are in series

Yeast MAPK



Recall: Futile cycles are in series

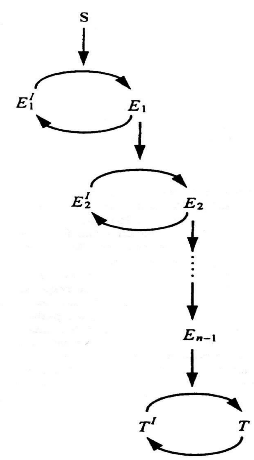
Yeast MAPK

Stimulus	a or α factor	Starvation	high osmolarity	Hypotonic shock	C and N depletion
MAPKKK	STE11	STE11	STE11 SSK2/22	BCK1	???
MAPKK	STE7	STE7	PBS2	MKK1/2	???
MAPK	FUS3	KSS1	HOG1	MPK1	SMK1
Response	Mating	Filamentation	Osmolyte synthesis	Cell wall remodeling	Sporulation

Mammalian MAPK

Stimulus	Growth Factors	Inflammatory cytokines cellular stress		???	Serum Stress
MAPKKK	Mos Raf TPL2	TPL2 MEKK MLK/DLK	TAK ASK	???	???
MAPKK	MEK1/2	MKK4/7	MKK3/6	???	MKK5
MAPK	ERK1/2	JNK	P38 MAPK	ERK3	ERK5
Response	Proliferation, Differentiation, Development	Inflammation, Apoptosis, Development		???	Proliferation?

Futile cycles in sequence compound overall sensitivity



$$R_S^T = \lim_{\Delta S\to 0}\left(\left(\frac{\Delta T}{T}\right)/\left(\frac{\Delta S}{S}\right)\right) = d\ln T/d\ln S.$$

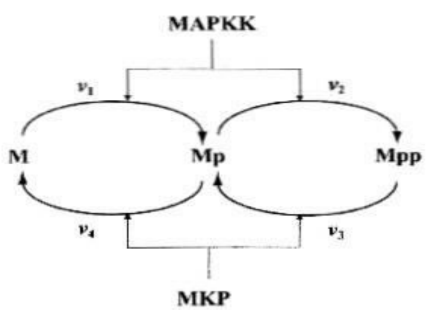
$$r_{i-1}^i = \frac{d\ln E_i}{d\ln E_{i-1}},\ E_j = \text{const.}$$

$$R_S^T = r_S^1 \cdot r_1^2 \cdot \ldots \cdot r_{n-1}^T = \Pi(\text{path}).$$

Overall response factor is a product of individual stages

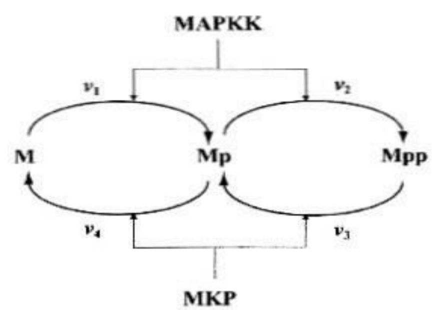
Brown, Hoek and Kholodenko. (1997) *TIBS*

Each stage of cascade may involve 2 futile cycles



Huang, C and Ferrell, J. (1996) *PNAS* 93, 10078-10083.

Each stage of cascade may involve 2 futile cycles

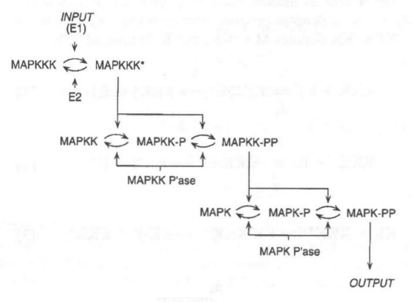


Processive: E + S $\rightleftharpoons$ ES $\rightarrow$ ESp $\rightleftharpoons$ ESpp $\rightarrow$ E + Spp

Distributive: E + S $\rightleftharpoons$ ES $\rightarrow$ E + Sp
E + Sp $\rightleftharpoons$ ESp $\rightarrow$ E + Spp

Huang, C and Ferrell, J. (1996) *PNAS* 93, 10078-10083.

Each stage of cascade may involve 2 futile cycles



Processive: E + S $\rightleftharpoons$ ES —— ESp $\rightleftharpoons$ ESpp —— E + Spp

Distributive: E + S $\rightleftharpoons$ ES —— E + Sp
E + Sp $\rightleftharpoons$ ESp —— E + Spp

Huang, C and Ferrell, J. (1996) *PNAS* 93, 10078-10083.

22-3

Futile cycles in sequence compound overall ultrasensitivity

Table 3. Predicted Hill coefficients for MAPK cascade components assuming one-step (processive) or two-step (distributive) models for the phosphorylation of MAPK and MAPKK

	Effective Hill coefficient (nH) predicted for:		
Model	MAPKKK	MAPKK	MAPK
One-step phosphorylation for MAPKK activation; One-step phosphorylation for MAPK activation	1.0	1.3	1.5
One-step phosphorylation for MAPKK activation; Two-step phosphorylation for MAPK activation	1.0	1.3	2.0
Two-step phosphorylation for MAPKK activation; One-step phosphorylation for MAPK activation	1.0	1.7	3.7
Two-step phosphorylation for MAPKK activation; Two-step phosphorylation for MAPK activation	1.0	1.7	4.9

Model predicts that the two-step, double hit mechanism produces more ultrasensitive response

Huang, C and Ferrell, J. (1996) *PNAS* 93, 10078-10083.

23-1

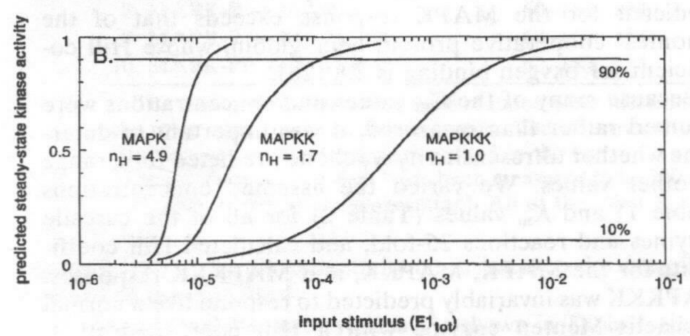
Futile cycles in sequence compound overall ultrasensitivity

Model predicts that the two-step, double hit mechanism produces more ultrasensitive response

Huang, C and Ferrell, J. (1996) *PNAS* 93, 10078-10083.

23-2

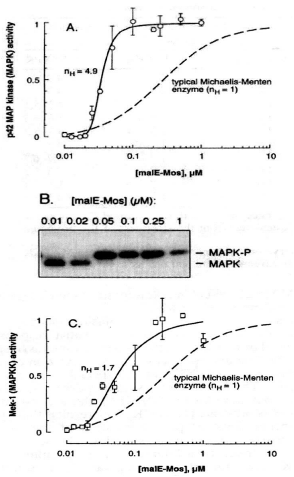
Futile cycles in sequence compound overall ultrasensitivity



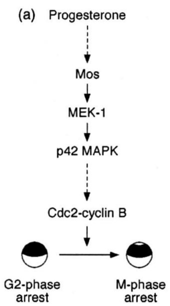
Huang, C and Ferrell, J. (1996) *PNAS* 93, 10078-10083.

23-3

Experimental validation of switch-like behavior of MAPK cascade



Experimental studies in *Xenopus* oocyte extracts display an ultrasensitive dose response



Huang, C and Ferrell, J. (1996) *PNAS* 93, 10078-10083.

From in vitro to in vivo (i.e., in cells)

Is the MAPK cascade a switch *inside* cells?

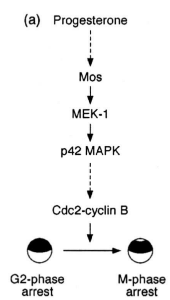
Background on Xenopus oocytes

Xenopus oocyte maturation:
- Fully grown oocytes are arrested in G_2
- Progesterone initiates oocyte maturation: first meiotic division and arrest in metaphase of meiosis II

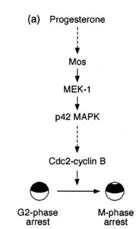
MAPK activation is necessary for maturation

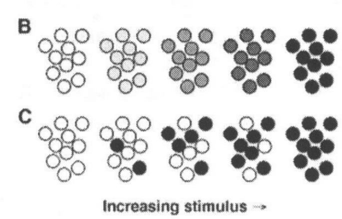
Xenopus oocyte maturation is an all-or-none process, a discrete transformation

Maturation is an irreversible process



Single-cell vs. Population view





Consider two extremes…
Graded response vs *switch-like response* to graded stimulus, progesterone
How does individual cell proceed from $G_2 \rightarrow$ M ?

Ferrell, J. and Machleder, E. (1998) *Science* 280, 895-898.