

Lecture 8: Zero-order ultrasensitivity

Today's Outline

Signaling systems

- Review of kinases and phosphatases
- Cascades

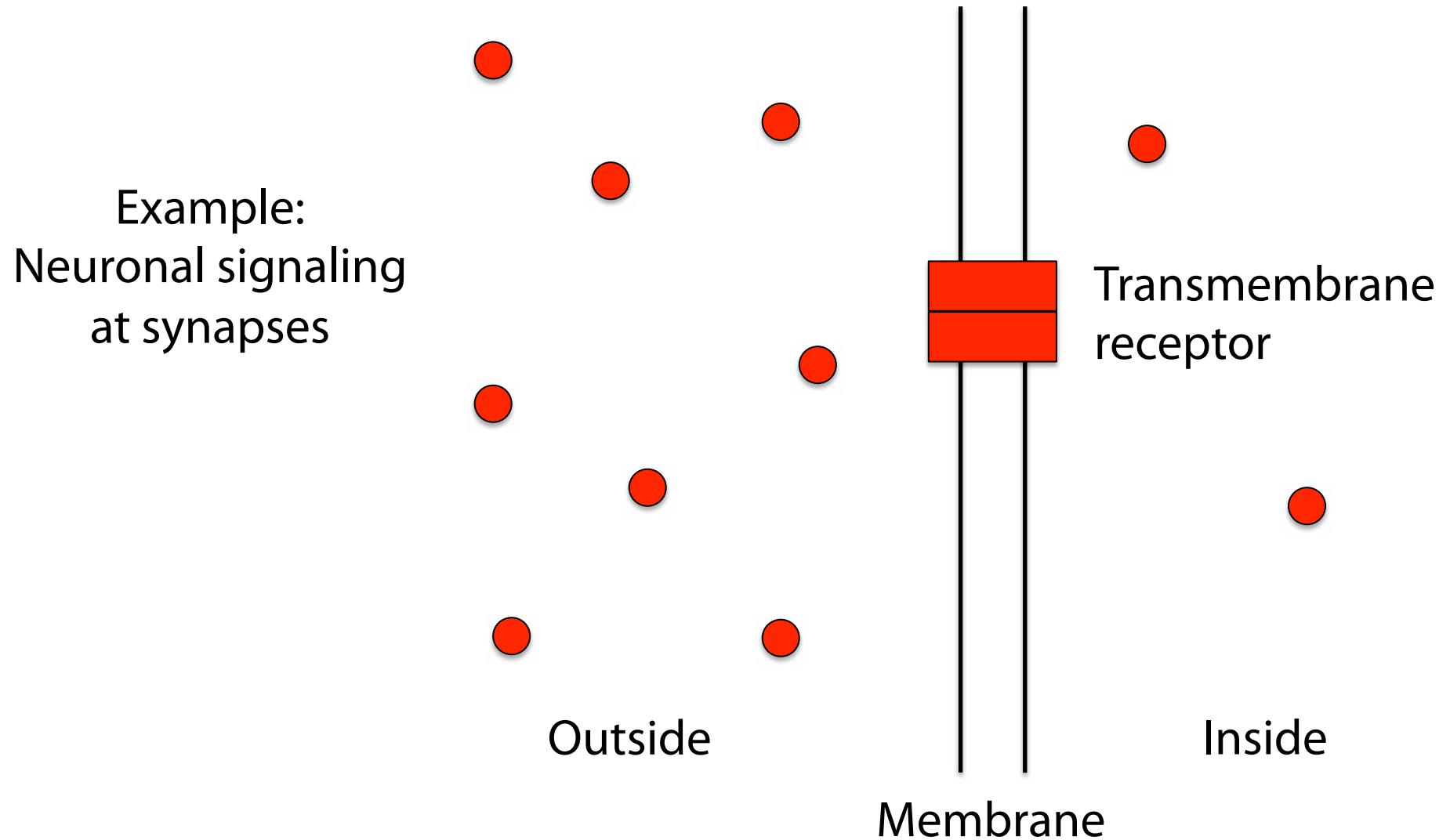
Zero-order ultrasensitivity

- Meet the players, Koshland and Goldbeter
- Sharp transitions in kinase/phosphatase loops
- Dynamics

Background info for discussion paper 2

- Fruit fly (*Drosophila melanogaster*) development
- Searching for the source of a sharp boundary

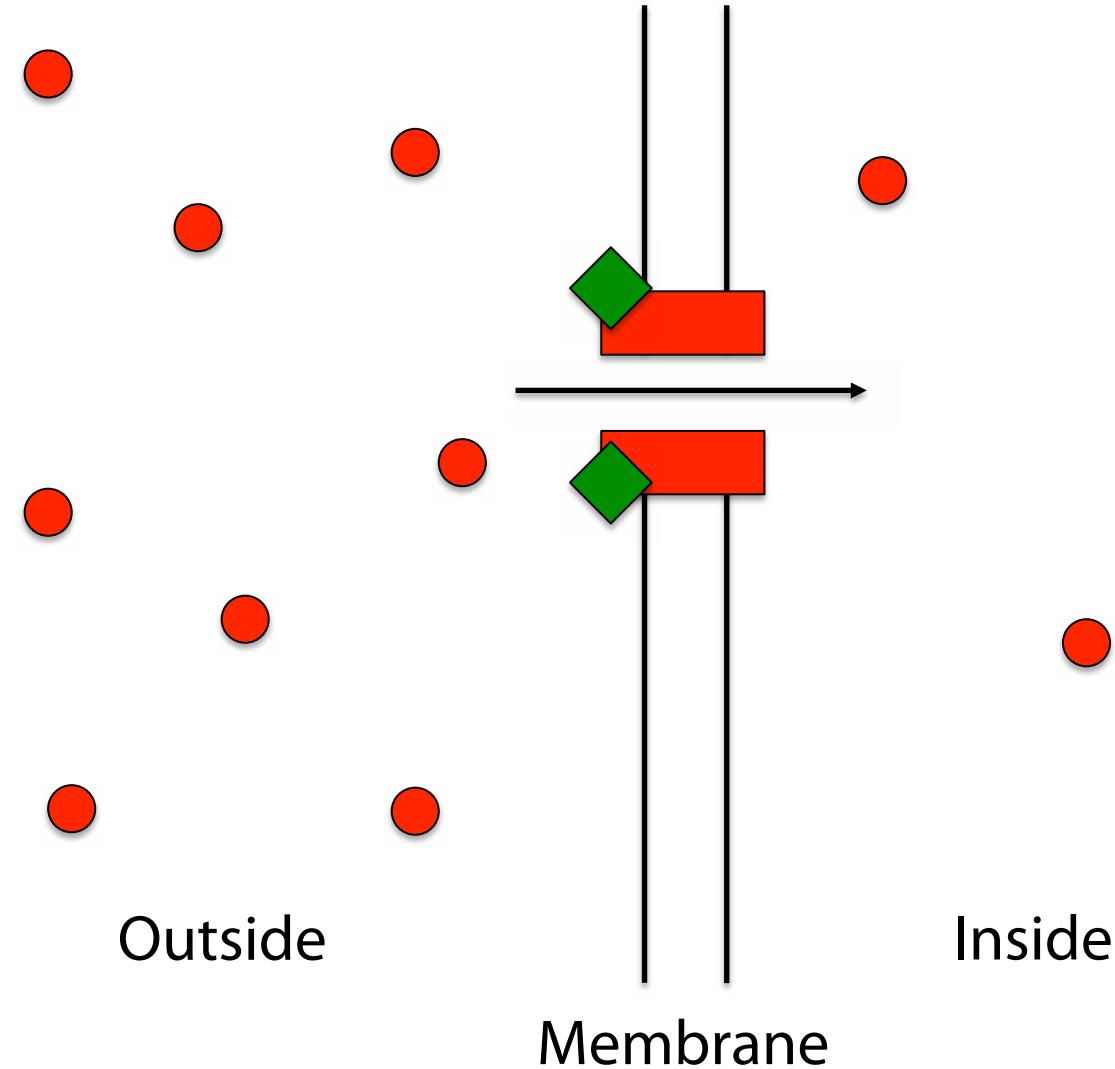
Extracellular signaling molecules bind to transmembrane receptors



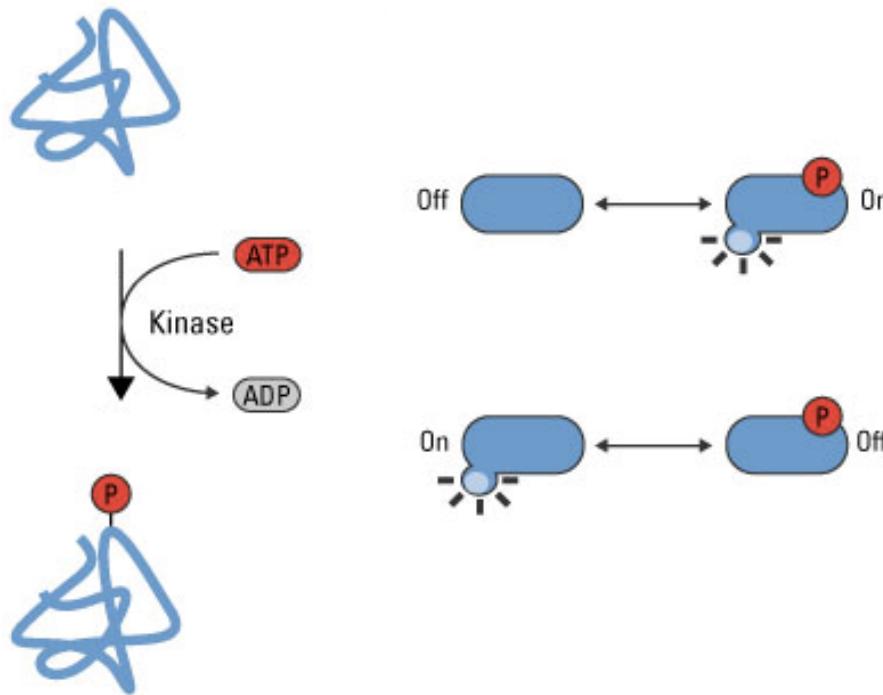
Extracellular signaling molecules bind to transmembrane receptors

Example:
Neuronal signaling
at synapses

Neurotransmitter
binding to
receptors caused a
conformational
change and
channel opening



Some receptors are kinases that are activated or inactivated by binding



Recall that kinases are enzymes which put a phosphate group on a target (often, another protein)

Extra phosphate groups can cause the target to change shape: it could become either more or less active

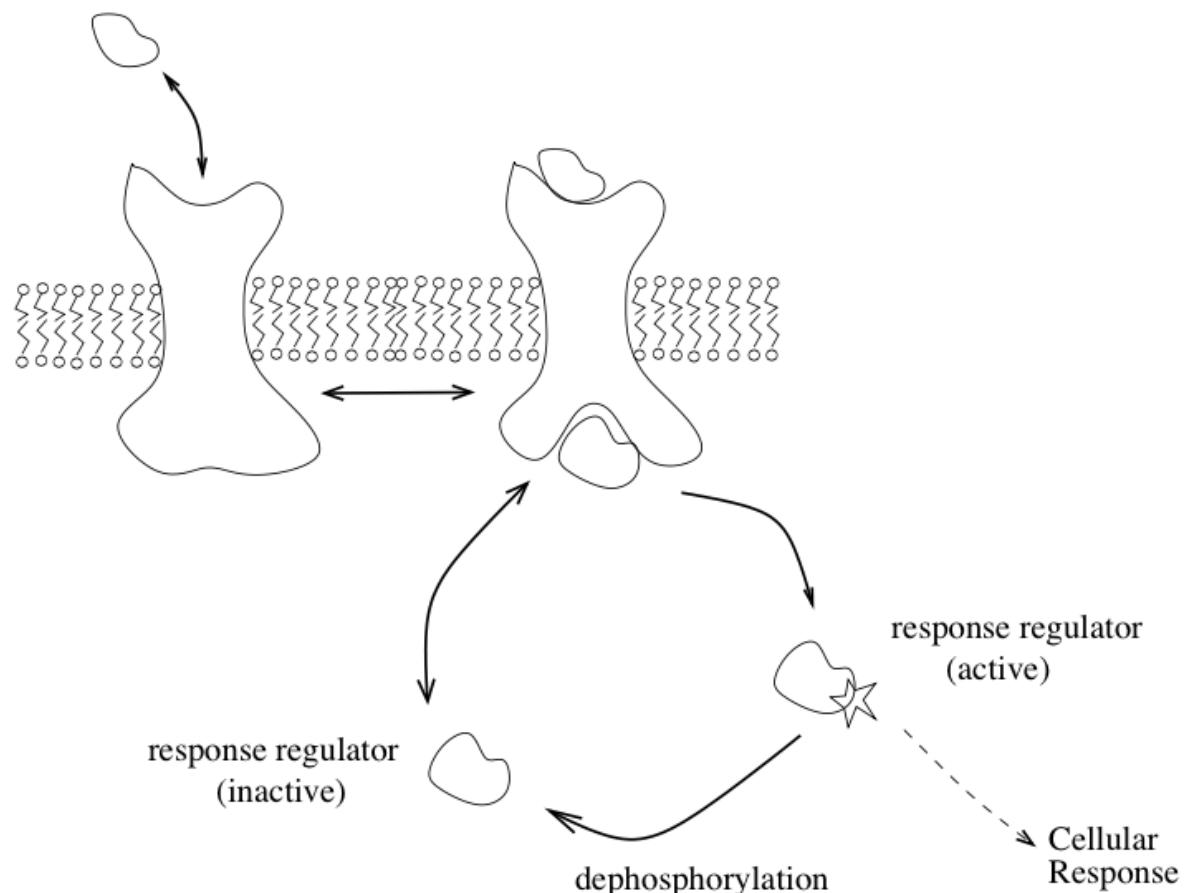
Some receptors are kinases that are activated or inactivated by binding

Example: bacterial two-component signaling.

The receptor is a kinase that becomes active only when ligand is bound.

The kinase phosphorylates a response regulator (often, a transcription factor) on the inside of the cell.

The response regulator is then active and causes a cellular response to the signaling.



In many cases receptor binding initializes a signaling cascade

The bound receptor activates protein A



Protein A activates protein B



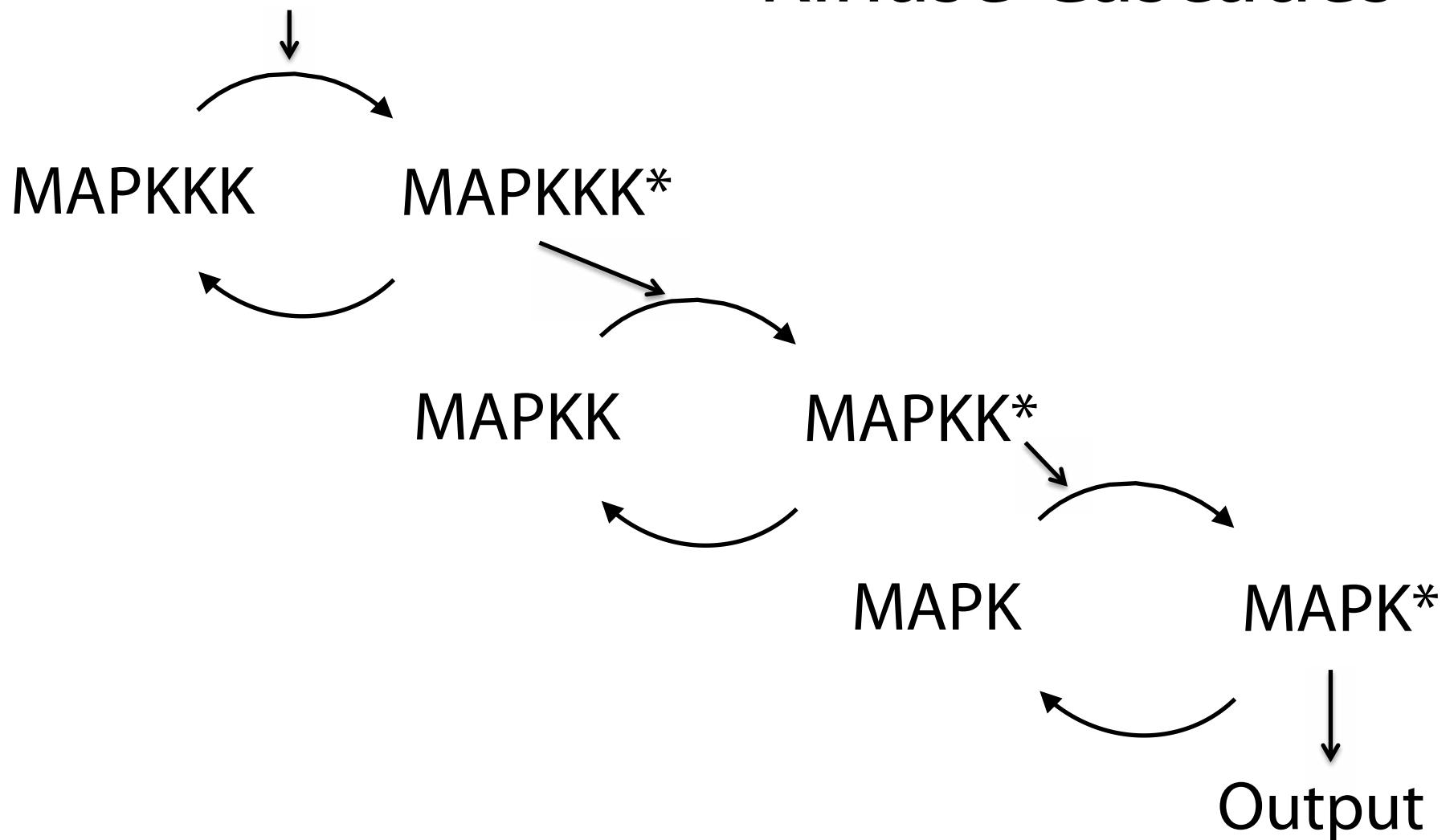
Protein B activates protein C



etc. etc.

Indirect input
from a bound
receptor

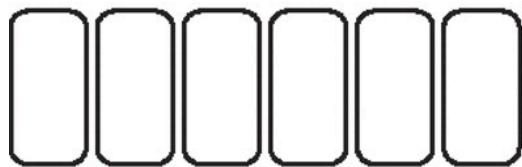
Example: MAP Kinase Cascades



Do these cascades serve a purpose?

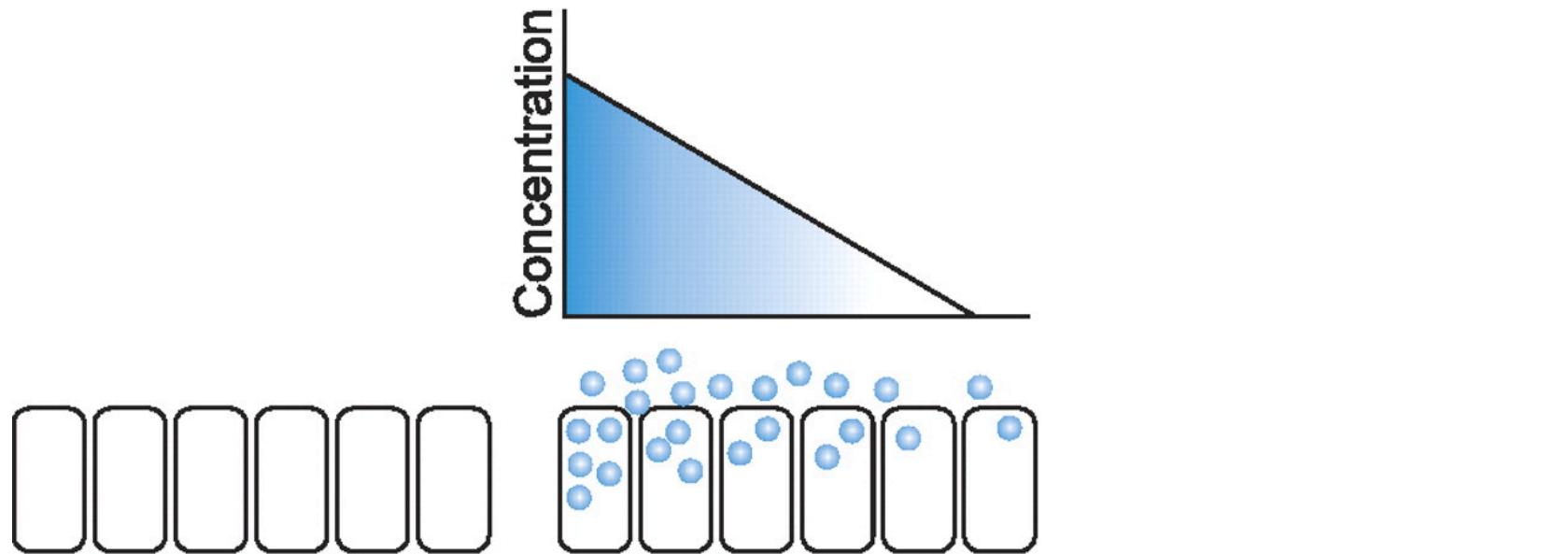
- Amplification
 - Each enzyme can activate many copies of its target before being inactivated.
- Crosstalk
 - Not always bad: can allow multiple signals to effect the same response
- Regulation
 - Each enzyme's activity could be modified by abundance, modification, pharmaceuticals...
- Converting graded signals into sharp transitions
 - Zero-order ultrasensitivity (the focus of this lecture)

Morphogens are signaling molecules
that cause different responses at >2
different concentration ranges



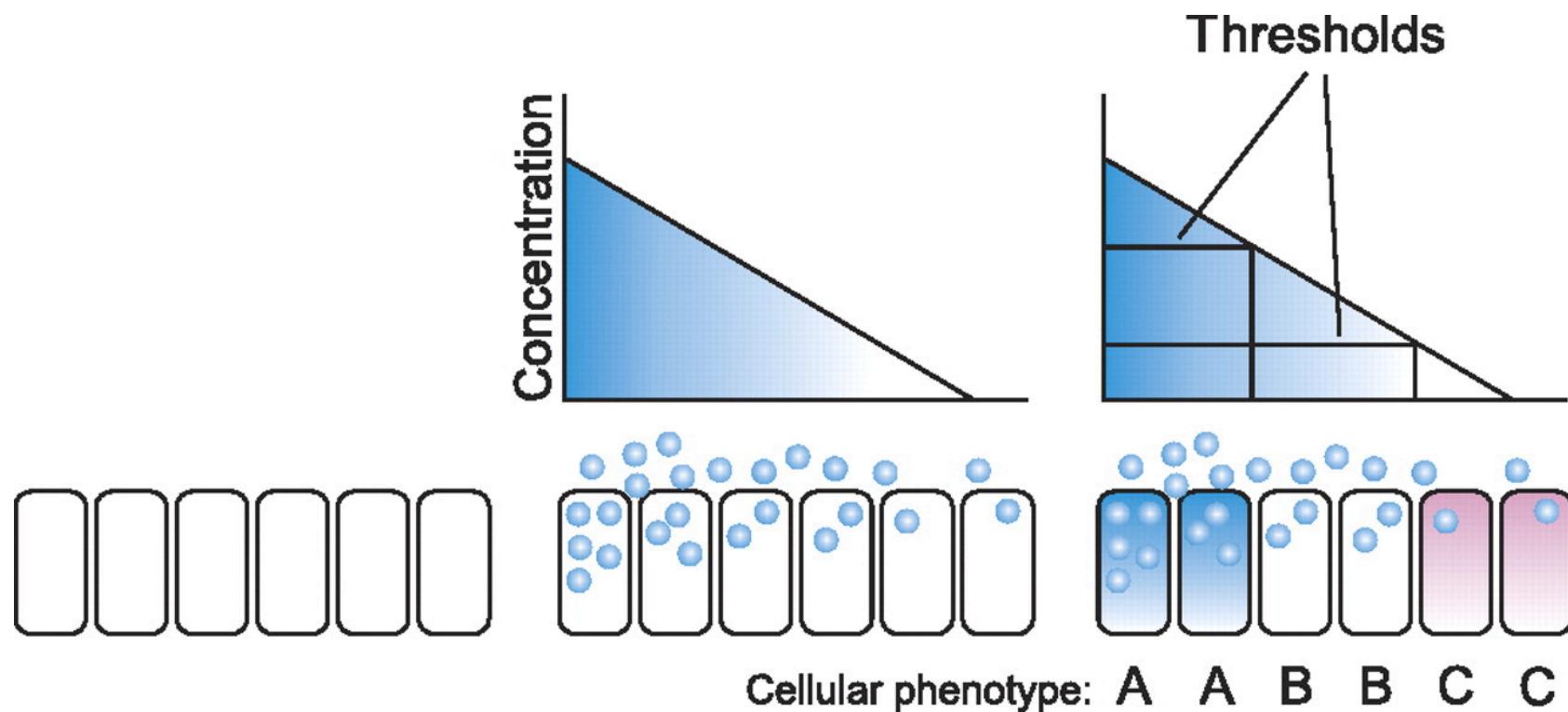
Initially
identical cells

Morphogens are signaling molecules that cause different responses at >2 different concentration ranges



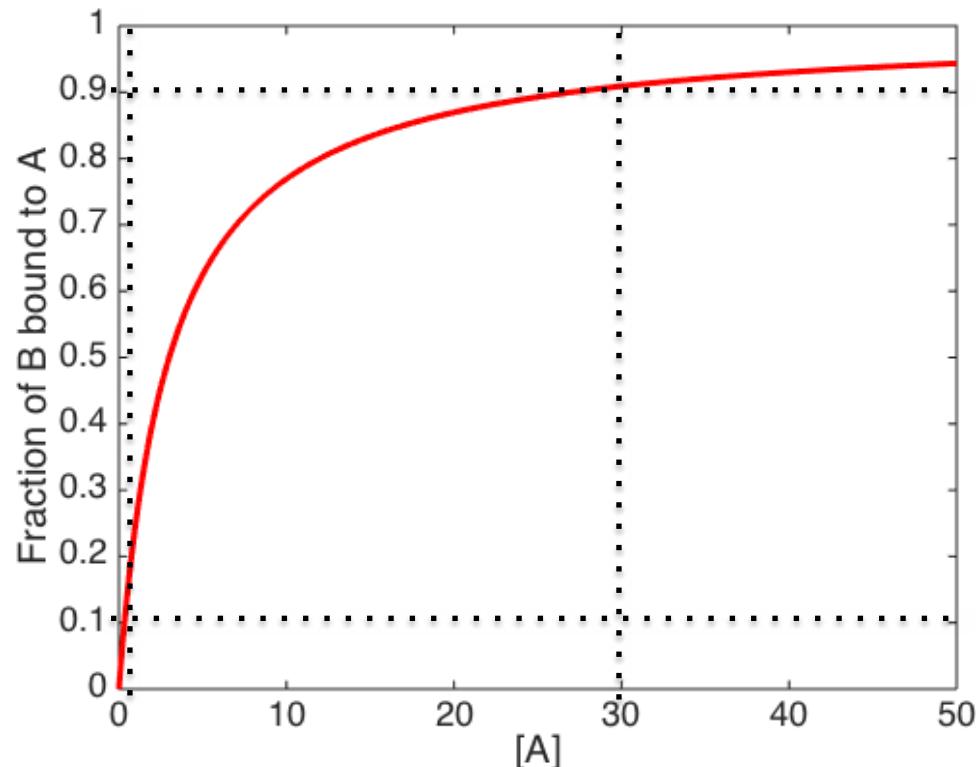
A morphogen is produced at left and diffuses

Morphogens are signaling molecules that cause different responses at >2 different concentration ranges



What is ultrasensitivity?

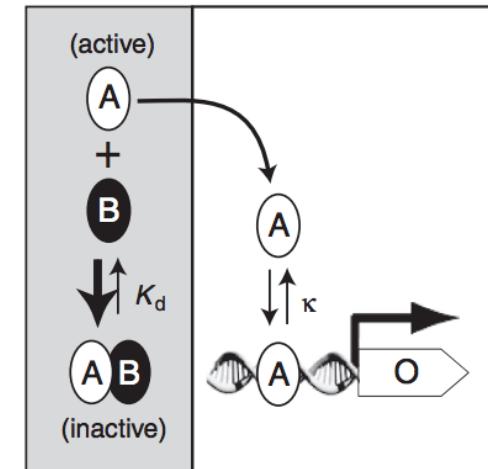
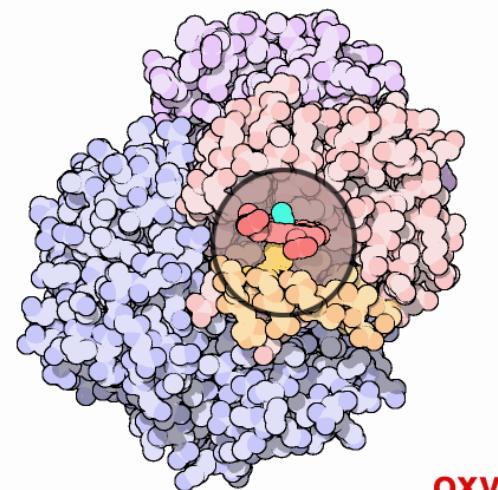
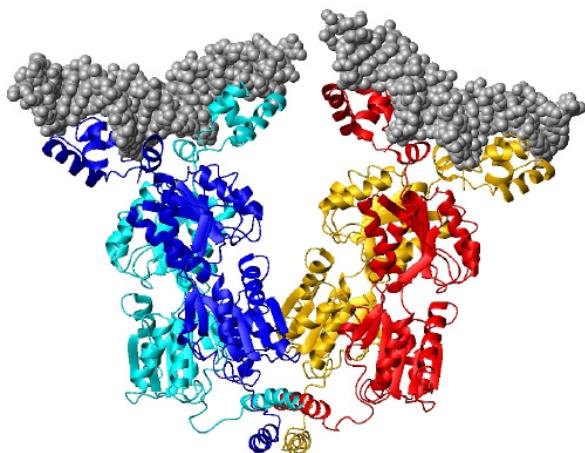
An eighty-one-fold change in concentration is required to change from 10% to 90% activity for Michaelis-Menten kinetics and simple binding curves



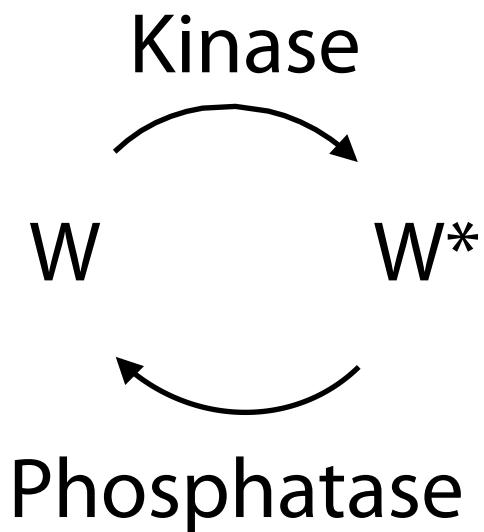
Ultrasensitivity is a general term for responses sharper than M-M kinetics

You have seen several examples already:

- Cooperativity (including through tethering)
- Concerted structural changes (MWC)
- Sequestration by an inhibitor



Goldbeter and Koshland's zero-order ultrasensitivity





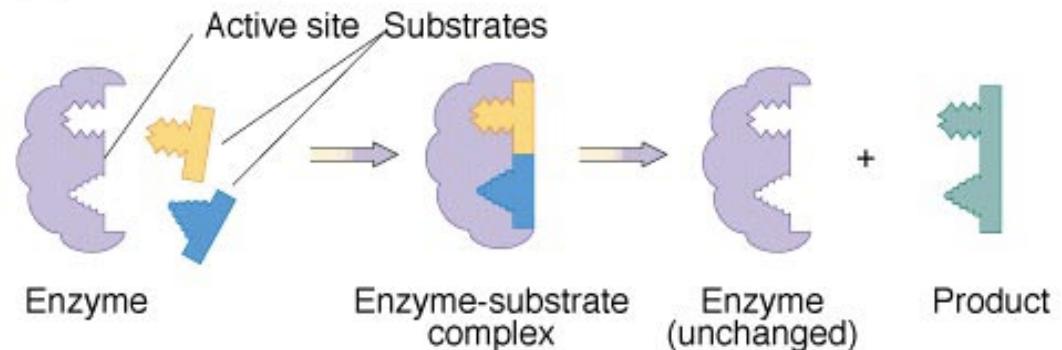
Daniel Koshland



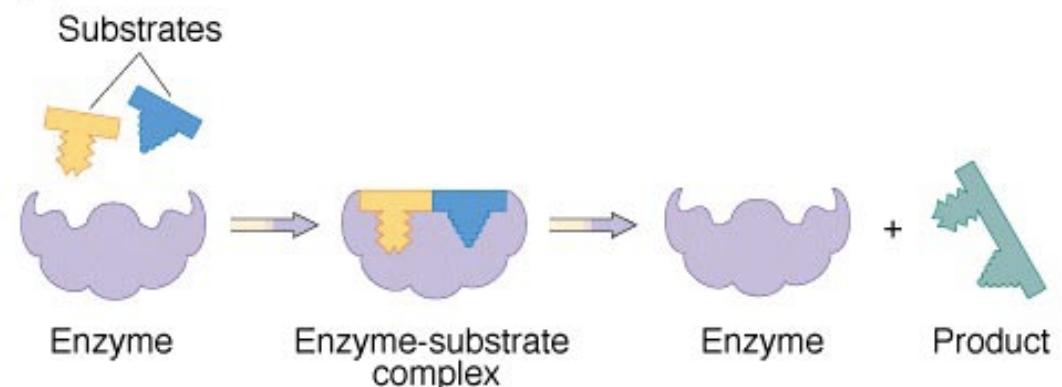


Daniel Koshland

(a) Lock-and-key model

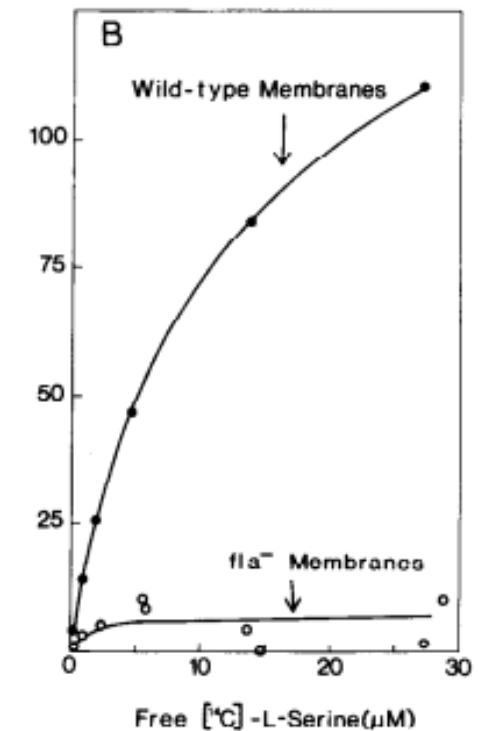
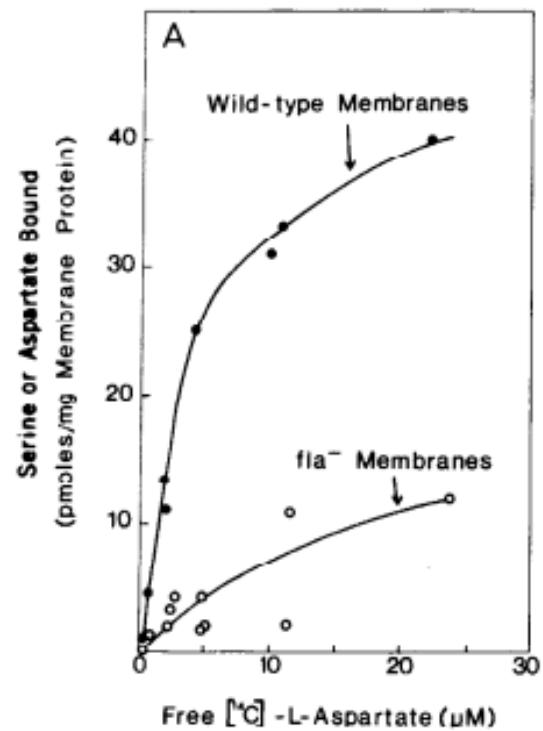


(b) Induced-fit model





Daniel Koshland





Daniel Koshland

SCIENCE

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The Addictive Personality

Science. Dr. Noitall, you are the world's greatest authority on addiction, the seer that everyone consults, the man who got Sherlock Holmes to kick his cocaine habit.

Dr. Noitall. A vast understatement of my true worth.

Science. Could you describe the addictive personality?

Dr. Noitall. An addictive person is one who has a compulsion to behave in ways that his or her family members consider detrimental to their interest. An addictive person will frequently conceal the extent of his addiction, will lie to his family about it, is immune to logical arguments to correct the error of his ways, and foregoes income that would require abandoning the addiction.

Science. Are we talking about a dope addict or alcoholic?

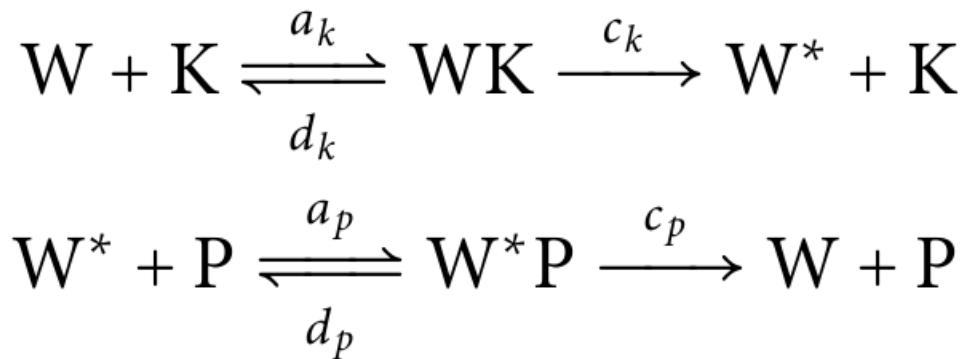
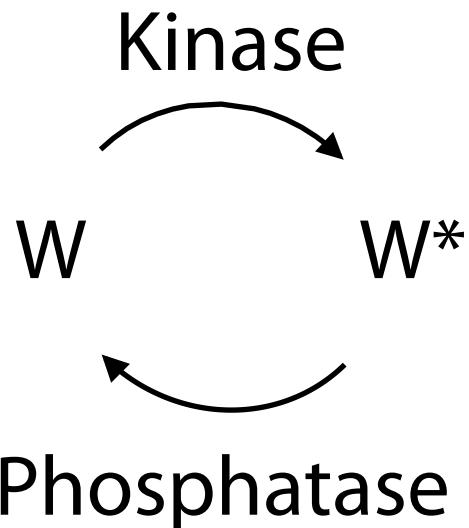
Dr. Noitall. No, I am describing a scientist. It is well known that work habits of scientists are addictive, leaving their spouses in tears, their children pleading, "Come home, Mommy (or Daddy)," and involve long hours in hostile instrument laboratories or cold rooms, exposed to noxious gases and radioactivity—conditions that no sane person would choose.



Albert Goldbeter



A kinase/phosphatase loop



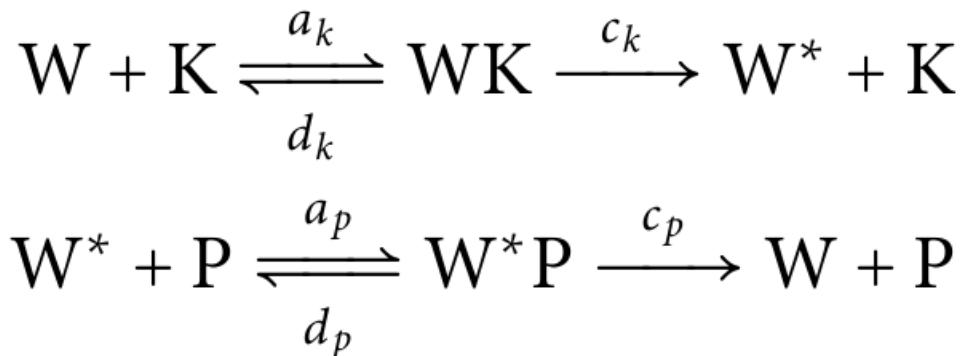
Suppose both the kinase K and the phosphate P follow Michaelis-Menten kinetics.

How will the ratio of W:W* change as $[K_{\text{tot}}]$ and/or $[P_{\text{tot}}]$ are varied?

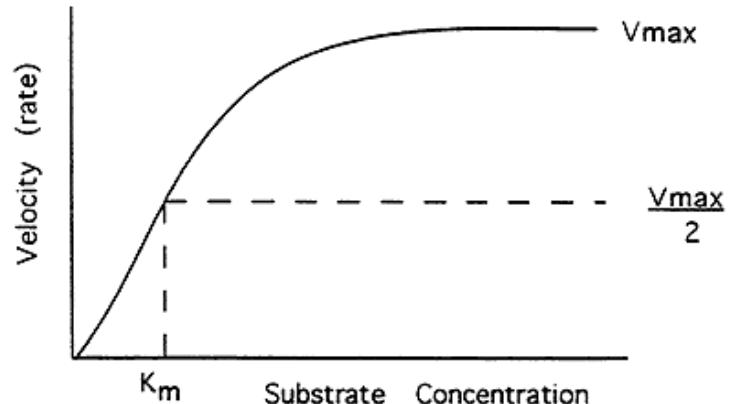
A kinase/phosphatase loop

Make one
(more) simplifying
assumption:

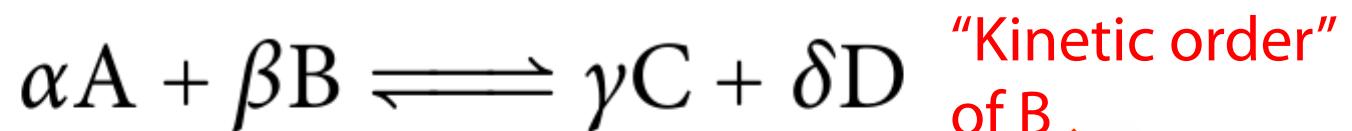
$$[W_{\text{tot}}] \gg [K_{\text{tot}}], [P_{\text{tot}}]$$



- $[W_{\text{total}}] \approx [W] + [W^*]$
- Enzymes operate at saturation (i.e., in their *zero-order* regime)

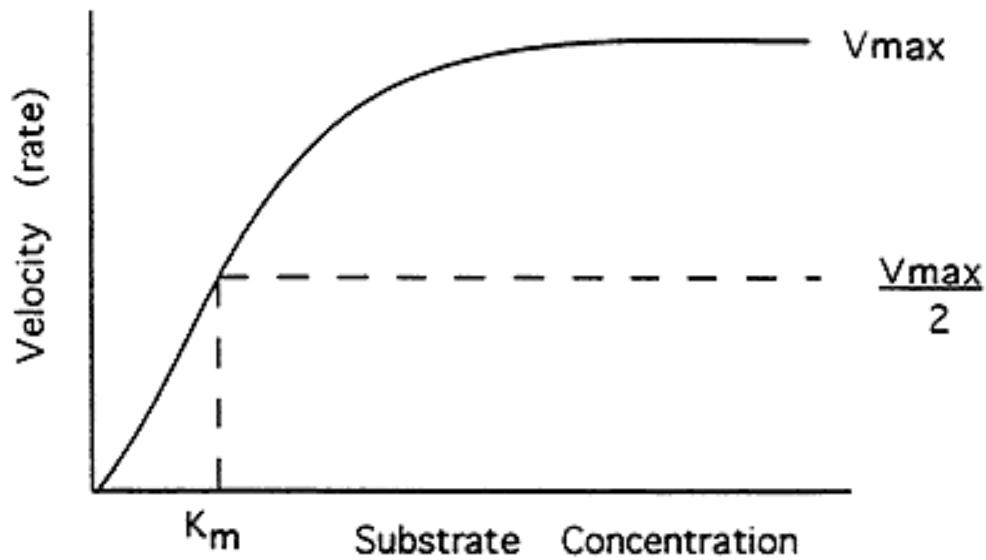


Law of Mass Action

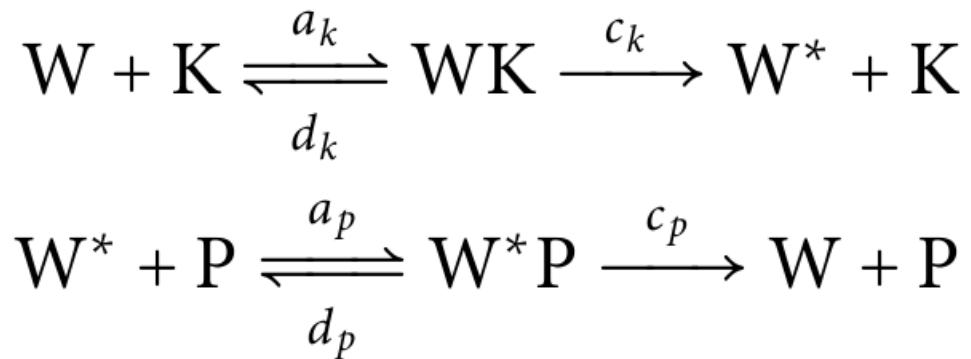
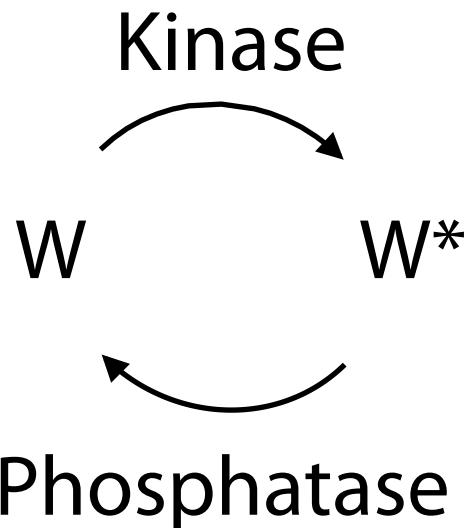


"Kinetic order" of B

Forward reaction rate = $k [A]^\alpha [B]^\beta$



A kinase/phosphatase loop



Our approach:

- Assume $[W]$ and $[W^*]$ are at steady-state
 - i.e., $c_k [WK] = c_p [W^*P]$
- Find expressions for $[WK]$ and $[W^*P]$
- Rearrange to find the fraction of W in the modified state,
 $f = [W^*]/[W_{\text{total}}]$

Find expressions for [WK] and [W*P] like we did for Michaelis-Menten kinetics



$$\begin{aligned}\frac{d[WK]}{dt} &= a_k [W][K] - (d_k + c_k)[WK] \\ &= a_k [W]([K_{\text{tot}}] - [WK]) - (d_k + c_k)[WK] \\ &= a_k [W][K_{\text{tot}}] - (d_k + c_k + a_k [W])[WK]\end{aligned}$$

Find expressions for $[WK]$ and $[W^*P]$ like we did for Michaelis-Menten kinetics



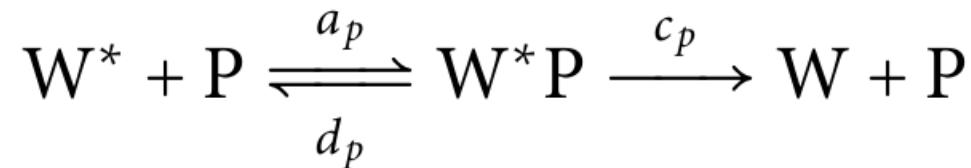
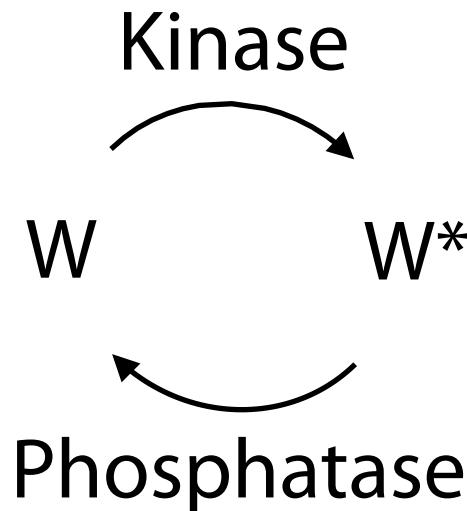
When $[WK]$ is at steady-state,

$$\frac{d[WK]}{dt} = a_k [W] [K_{\text{tot}}] - (d_k + c_k + a_k [W]) [WK] = 0$$

$$[WK] = \frac{a_k [W] [K_{\text{tot}}]}{d_k + c_k + a_k [W]} = \frac{[W] [K_{\text{tot}}]}{K_k + [W]}, \quad K_k = \frac{d_k + c_k}{a_k}$$

$$[W^*P] = \frac{[W^*] [P_{\text{tot}}]}{K_p + [W^*]}, \quad K_p = \frac{d_p + c_p}{a_p}$$

Assume $[W]$ and $[W^*]$ are
also at steady-state



$$[WK] = \frac{[W][K_{\text{tot}}]}{K_k + [W]}$$

$$[W^*P] = \frac{[W^*][P_{\text{tot}}]}{K_p + [W^*]}$$

$$c_k [WK] = c_p [W^*P]$$

$$\frac{c_k [K_{\text{tot}}]}{c_p [P_{\text{tot}}]} = \frac{[W^*](K_k + [W])}{[W](K_p + [W^*])}$$

Find an implicit expression for the fraction of W in the modified state

$$f = [W^*] / [W_{\text{tot}}]$$

Assumption: $[W_{\text{total}}] \gg [K_{\text{total}}], [P_{\text{total}}]$

$$[W_{\text{tot}}] \approx [W] + [W^*]$$

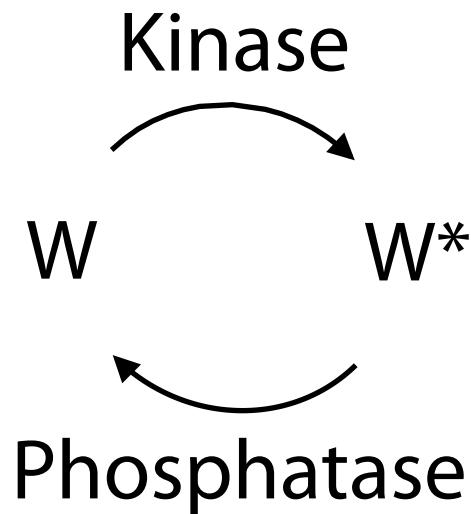
$$\frac{c_k [K_{\text{tot}}]}{c_p [P_{\text{tot}}]} = \frac{[W^*] (K_k + [W])}{[W] (K_p + [W^*])}$$

$$= \frac{f (K_1 + 1 - f)}{(1 - f) (K_2 + f)}$$

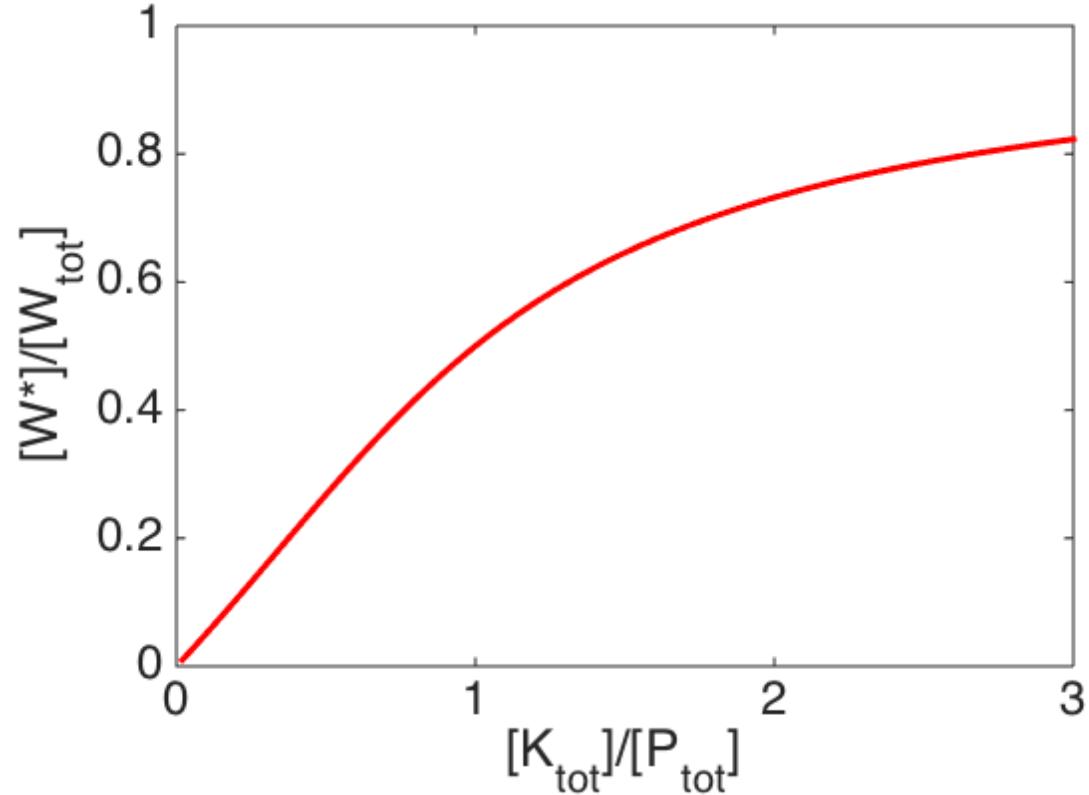
$$K_1 = \frac{d_k + c_k}{[W_{\text{tot}}] a_k}$$

$$K_2 = \frac{d_p + c_p}{[W_{\text{tot}}] a_p}$$

What do we expect to happen
when $[K_{\text{tot}}]$ and $[P_{\text{tot}}]$ are varied?



$$\frac{c_k [K_{\text{tot}}]}{c_p [P_{\text{tot}}]} = \frac{f (K_1 + 1 - f)}{(1 - f) (K_2 + f)}$$



What do we expect to happen
when K_1 and K_2 are small?

$$\frac{c_k [K_{\text{tot}}]}{c_p [P_{\text{tot}}]} = A = \frac{f (\delta + 1 - f)}{(1 - f) (\delta + f)}$$

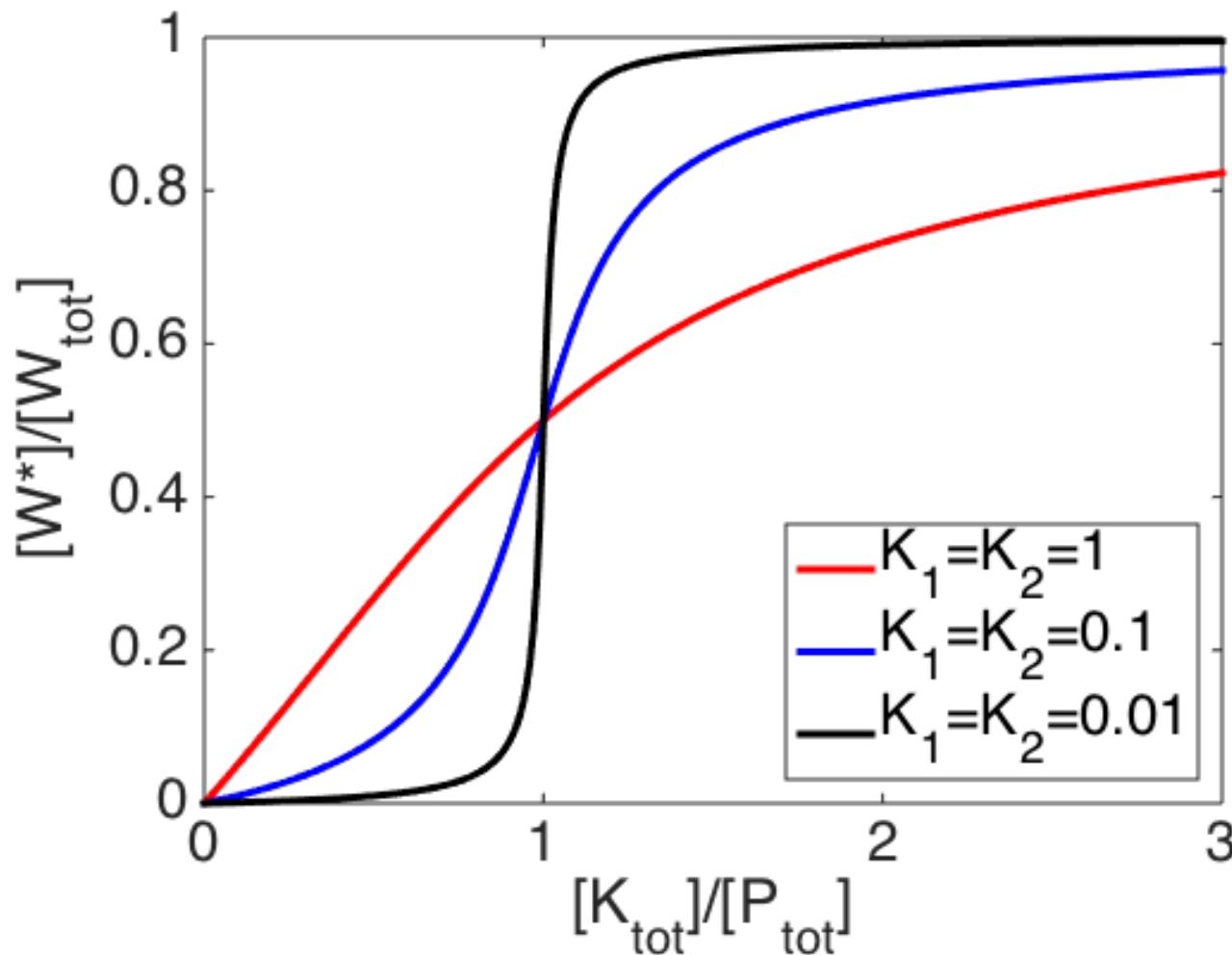
$$(1 - A) f^2 + (A - \delta A - \delta - 1) f + \delta A = 0$$

$$(1 - A) f^2 + (A - 1) f \approx 0$$

$$f (1 - f) (1 - A) \approx 0$$

$$f \approx 0 \text{ or } 1$$

What do we expect to happen
when K_1 and K_2 are small?

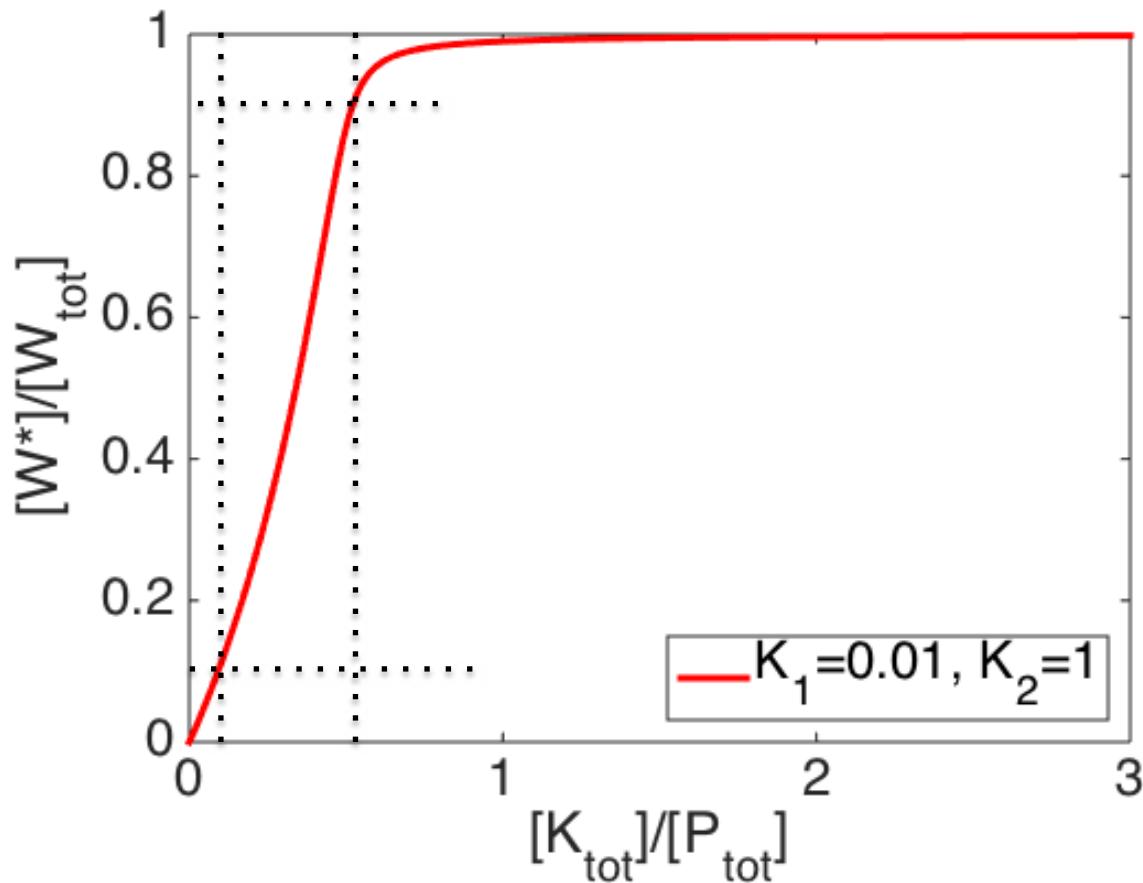


$$K_1 = \frac{d_k + c_k}{[W_{\text{tot}}] a_k}$$

$$K_2 = \frac{d_p + c_p}{[W_{\text{tot}}] a_p}$$

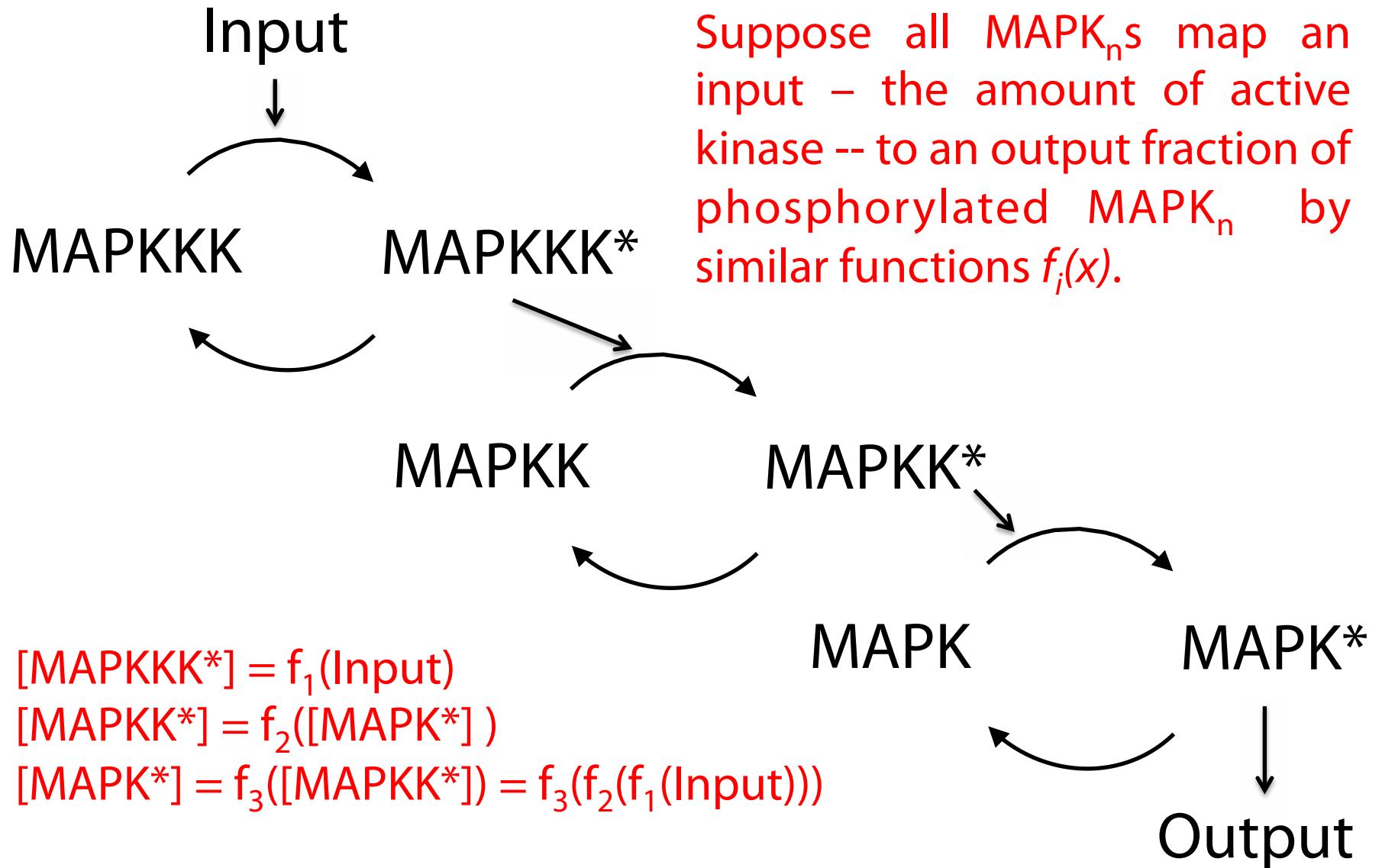
K_1, K_2 are small
when $[W_{\text{tot}}]$ is
large (which
we assumed!)

What if only one enzyme is saturated
(either K_1 or K_2 is small)?



Note that this
still meets the
definition of
ultrasensitivity

What is the effect of a cascade?



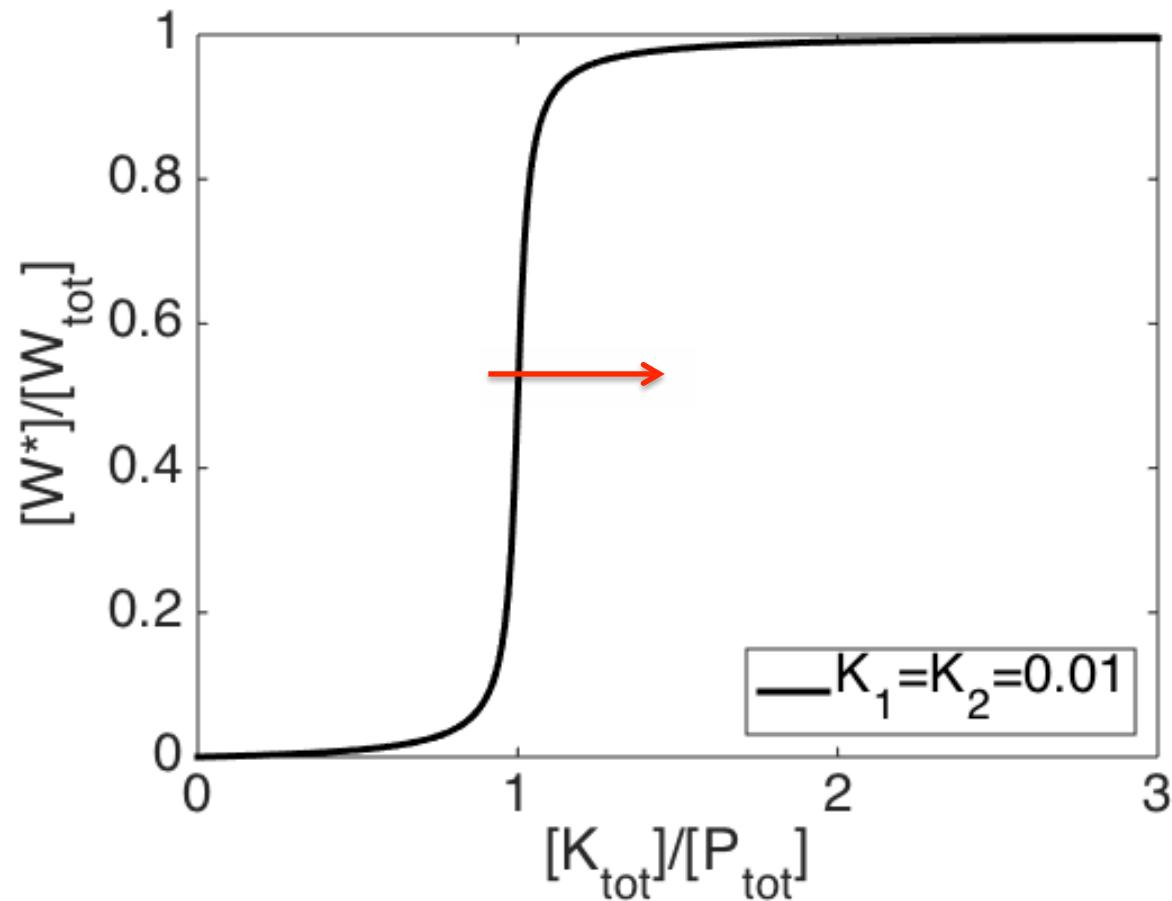
What is the effect of a cascade?

How steep is the overall output $f_3(f_2(f_1(x)))$ relative to these functions alone?

$$\frac{d}{dx} f_3(f_2(f_1(x))) = f'_3(f_2(f_1(x))) \cdot f'_2(f_1(x)) \cdot f'_1(x)$$

- When all three functions have slope greater than unity, the overall slope is even steeper
 - When all three functions have slope less than unity, the overall slope is more gradual
- This creates an even more ultrasensitive curve!

Suppose I increase $[K_{\text{tot}}]$ to move from just below to just above the threshold:



How long until the output transitions?

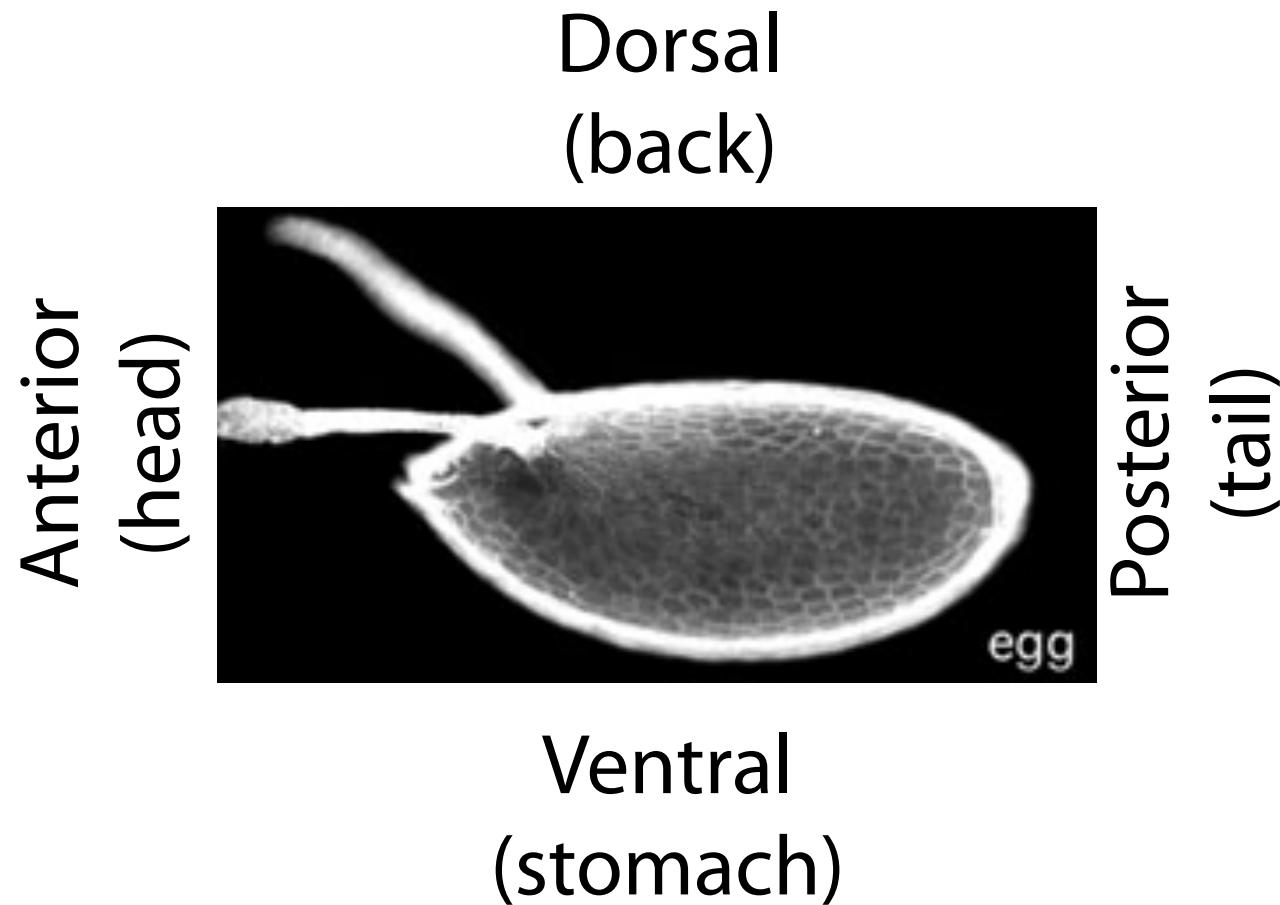
The downside (?) of zero-order ultrasensitivity: slow transitions

$$\begin{aligned}\frac{d[W^*]}{dt} &= c_k [WK] - c_p [W^*P] \\ &= \frac{c_k [W] [K_{\text{tot}}]}{K_k + [W]} - \frac{c_p [W^*] [P_{\text{tot}}]}{K_p + [W^*]} \\ &= \frac{c_k (1-f) [W_{\text{tot}}] [K_{\text{tot}}]}{K_k + (1-f) [W_{\text{tot}}]} - \frac{c_p f [W_{\text{tot}}] [P_{\text{tot}}]}{K_p + f [W_{\text{tot}}]} \\ \frac{df}{dt} &= \frac{c_k [K_{\text{tot}}] (1-f)}{K_k + (1-f) [W_{\text{tot}}]} - \frac{c_p f [P_{\text{tot}}]}{K_p + f [W_{\text{tot}}]}\end{aligned}$$

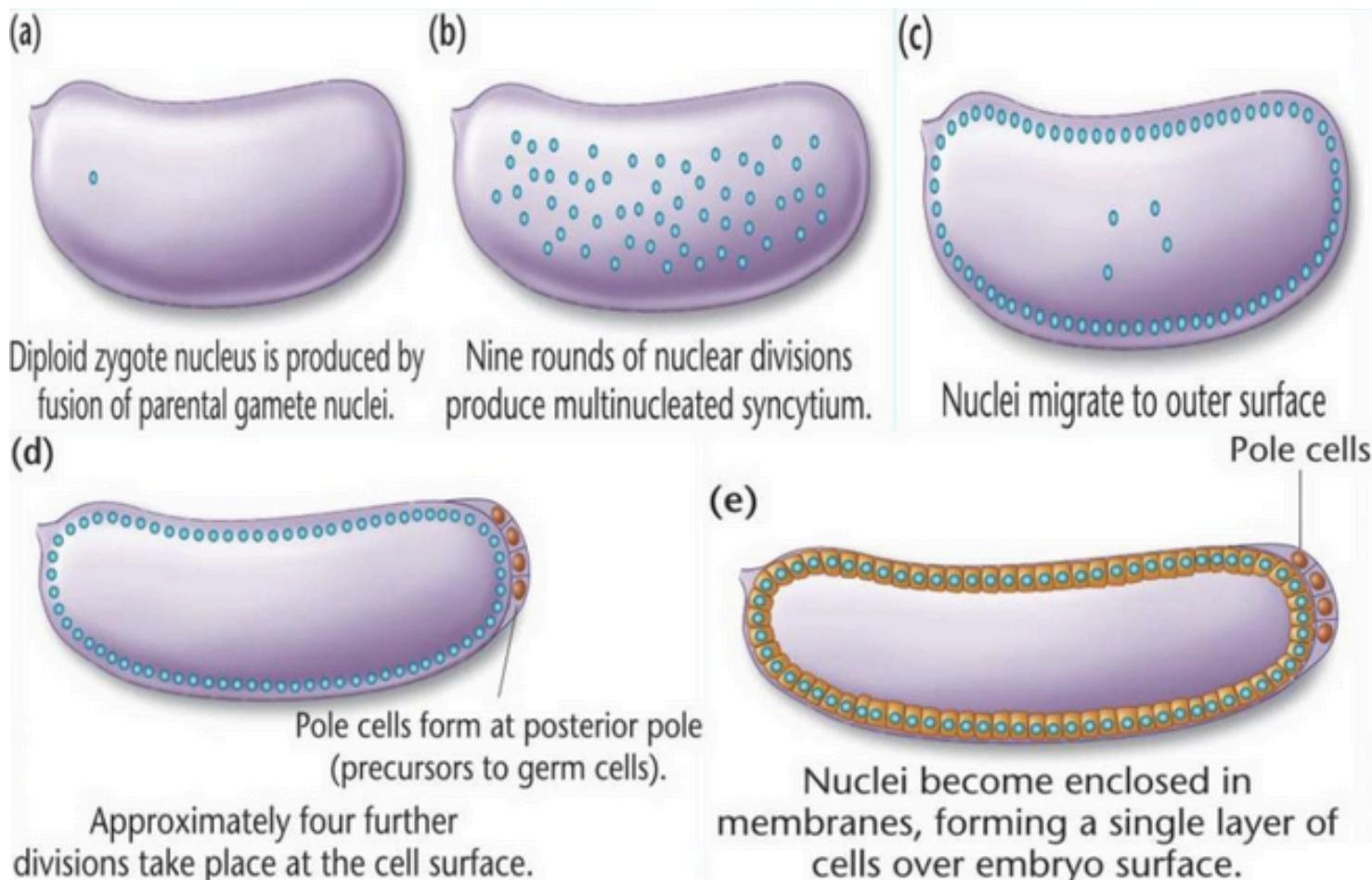
Discussion Paper #2: Melen et al., 2005

Primer on *Drosophila* (fruit fly)
development and genetics

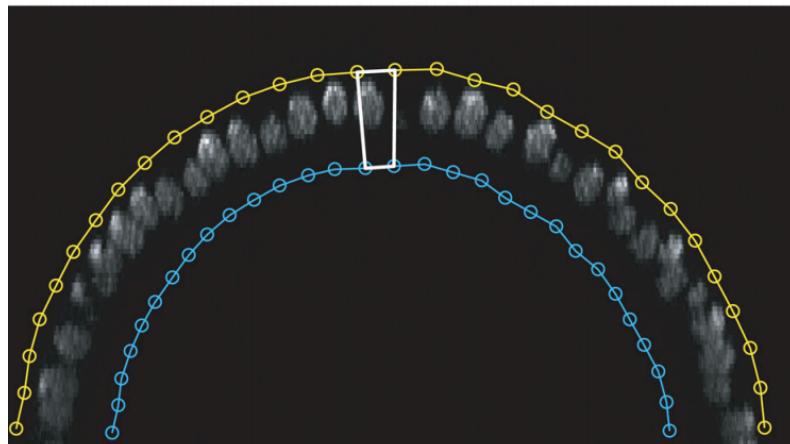
The first major problem in fruit fly embryogenesis: axis specification



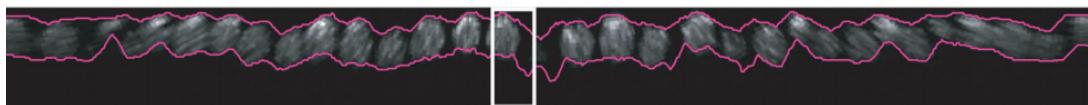
Following fertilization, a surface layer of cells forms



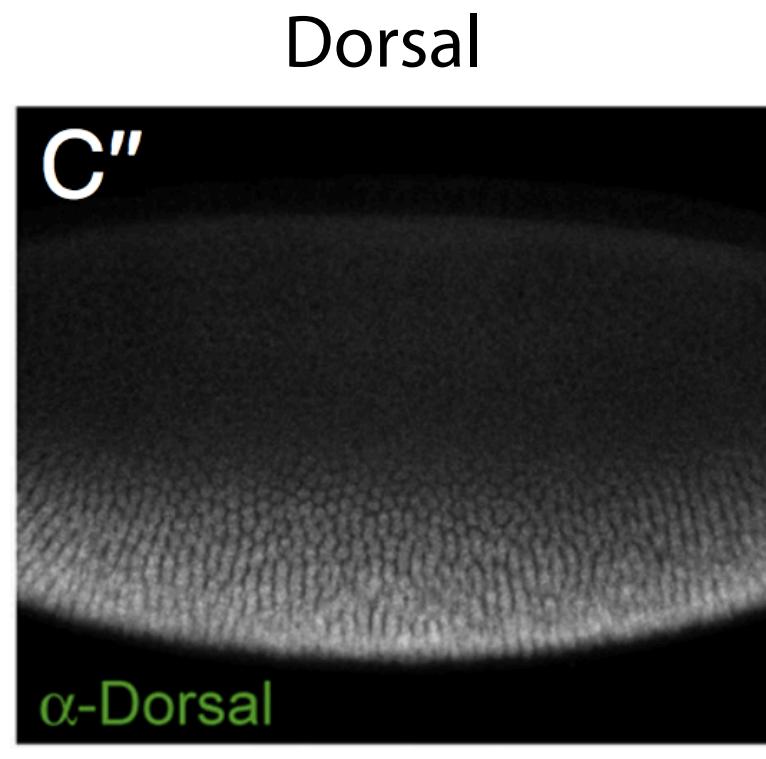
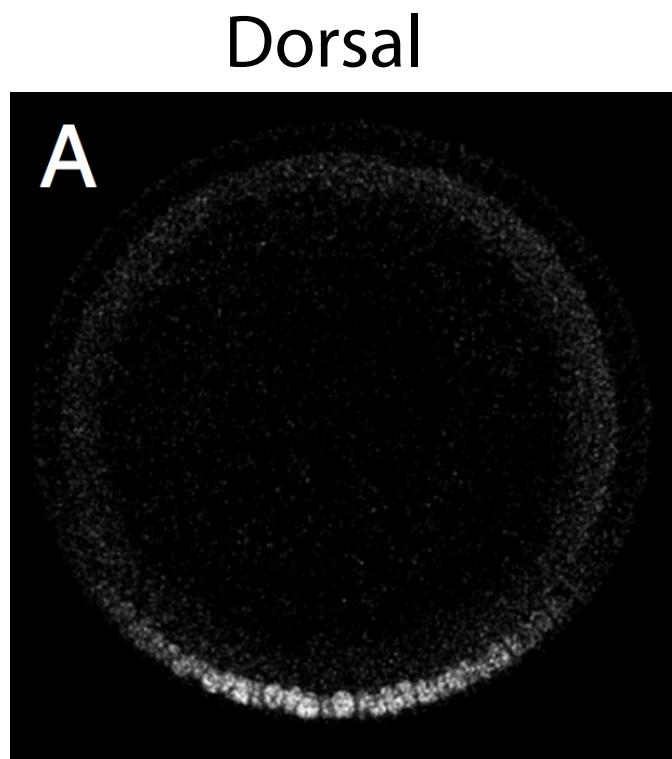
Why is *Drosophila* DV patterning a convenient model system?



Simple geometry and
easy imaging

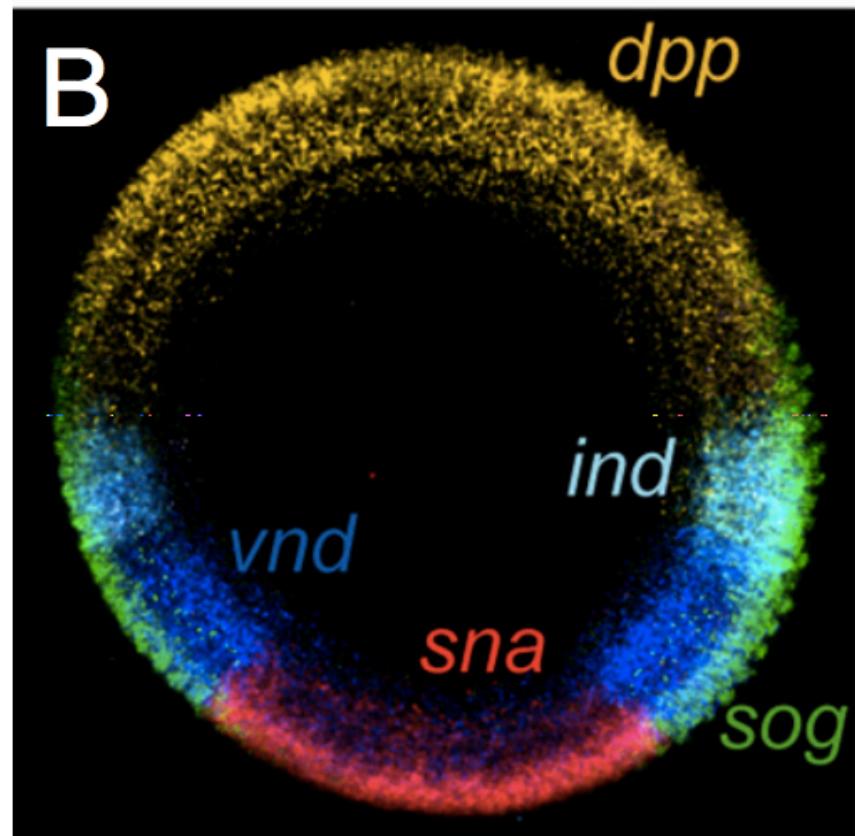


Dorsoventral gradient of active Dorsal (a transcription factor) in the early embryo



Liberman et al., 2009

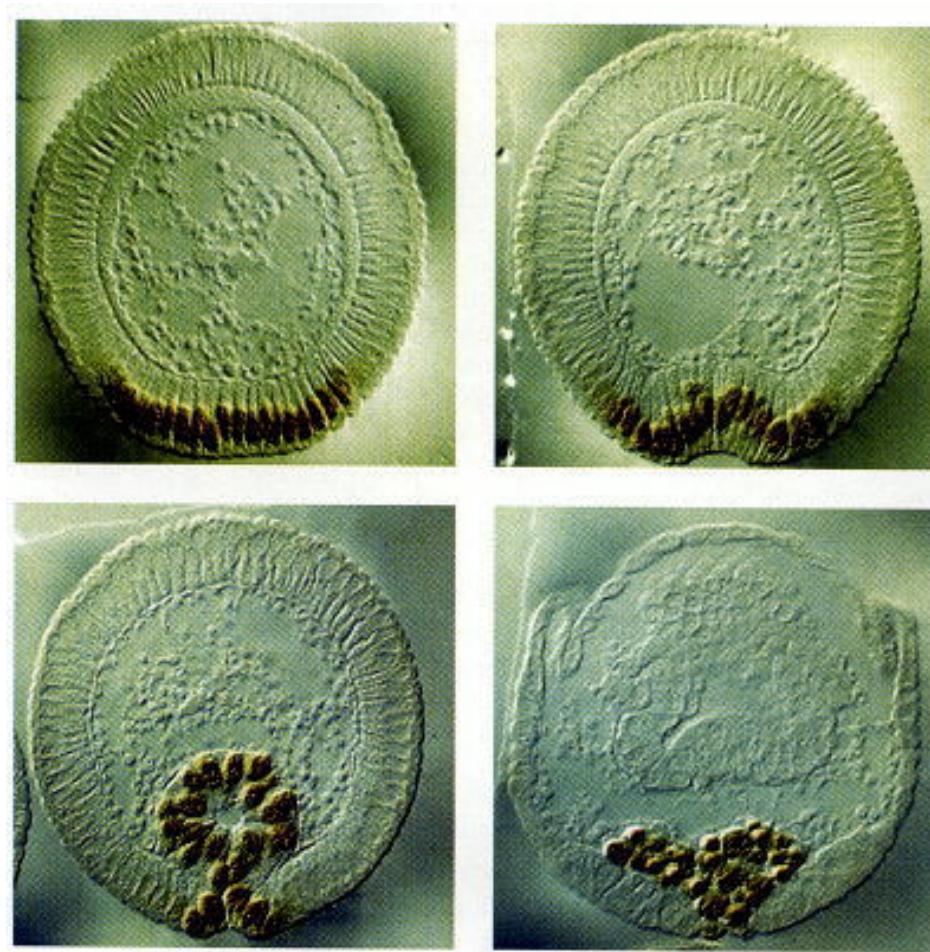
Dorsal has many target genes, and their activation thresholds differ



Liberman et al., 2009

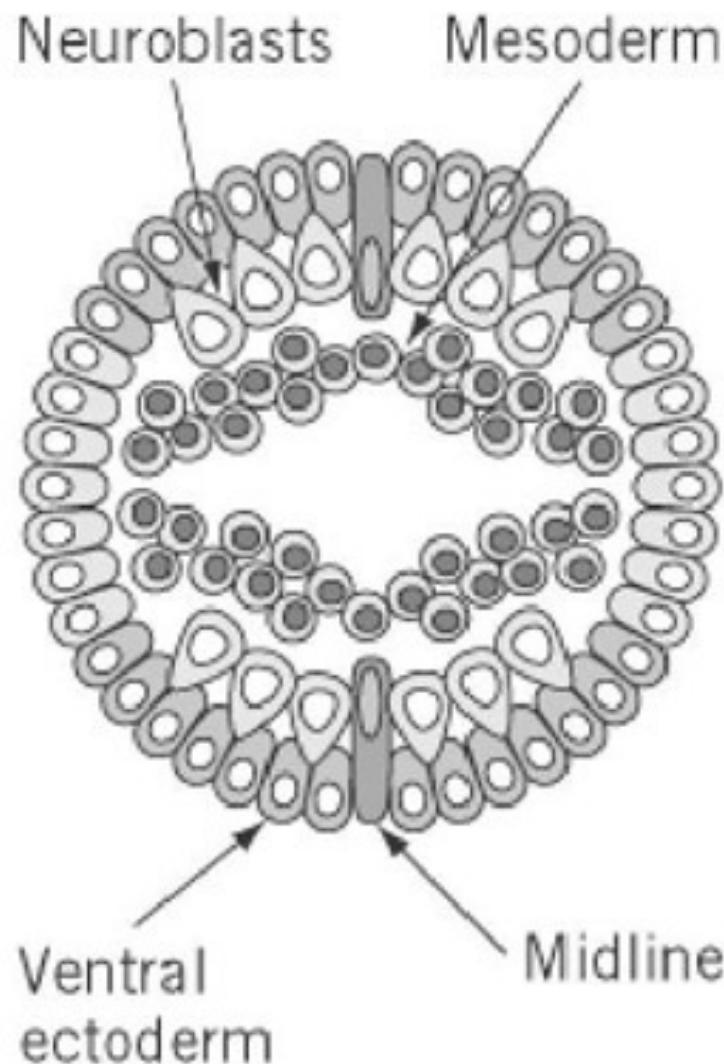
Sharp expression boundaries are created through cooperativity in Dorsal binding (and downstream interactions)

The most ventral cells buckle inward and become mesoderm, forming muscles, heart, etc.



Gilbert 7th ed.

Cells that remain at the surface form ectoderm,
e.g. the nervous system and epidermis (skin)

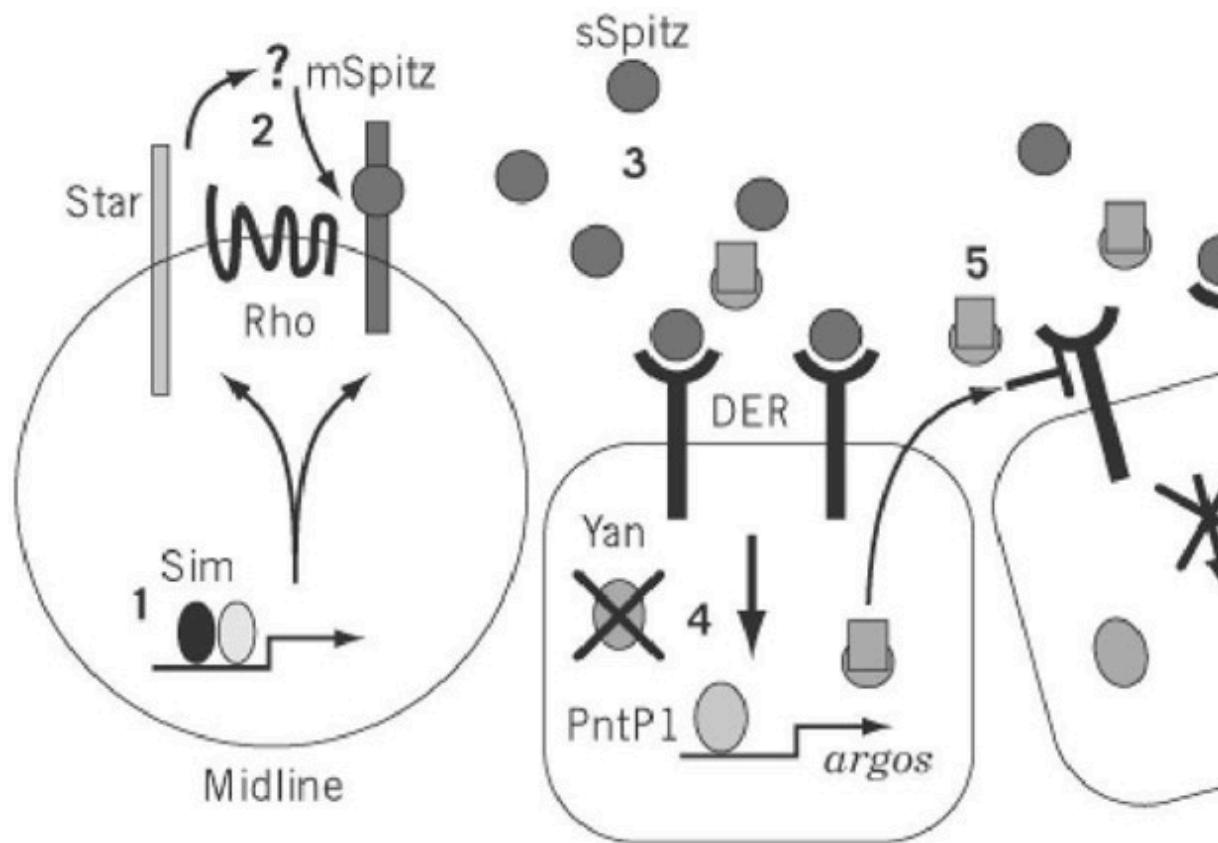


The ventral midline cells secrete a *morphogen* called Spitz.

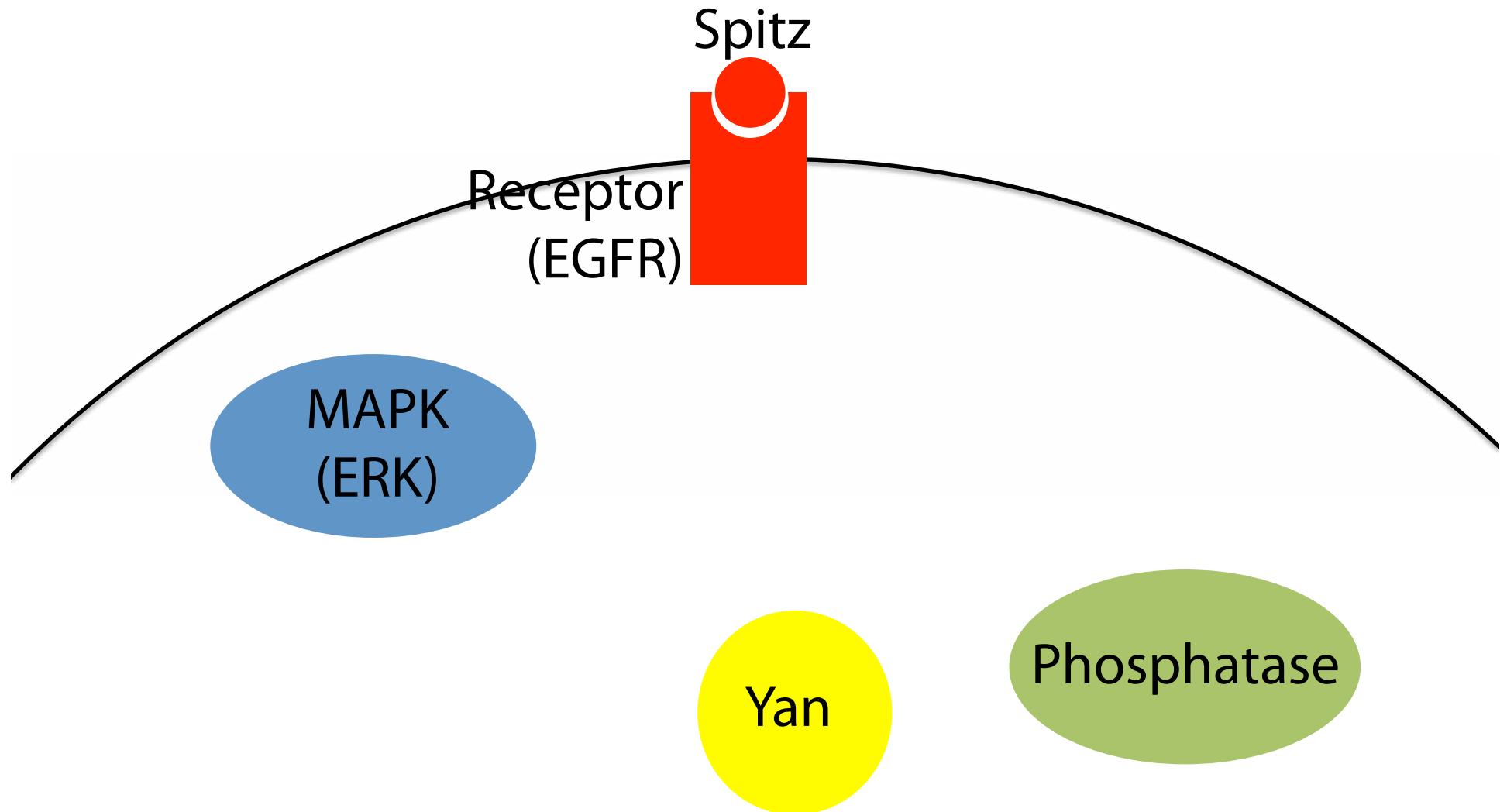
Spitz reaches different concentrations depending on the distance from the midline.

Cells determine their fate based on the [Spitz] they detect.

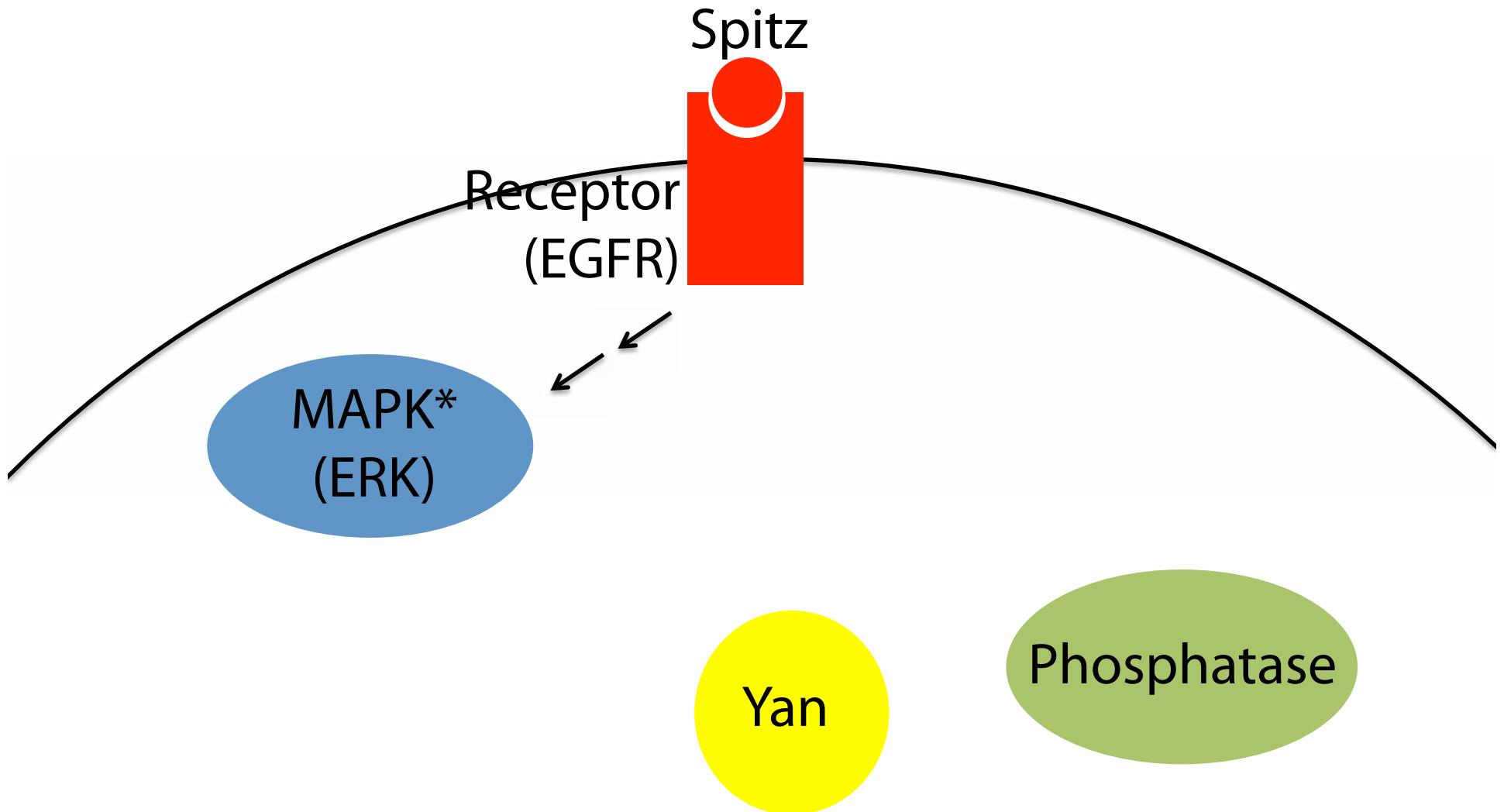
A transcriptional repressor, Yan, is degraded when [Spitz] is high



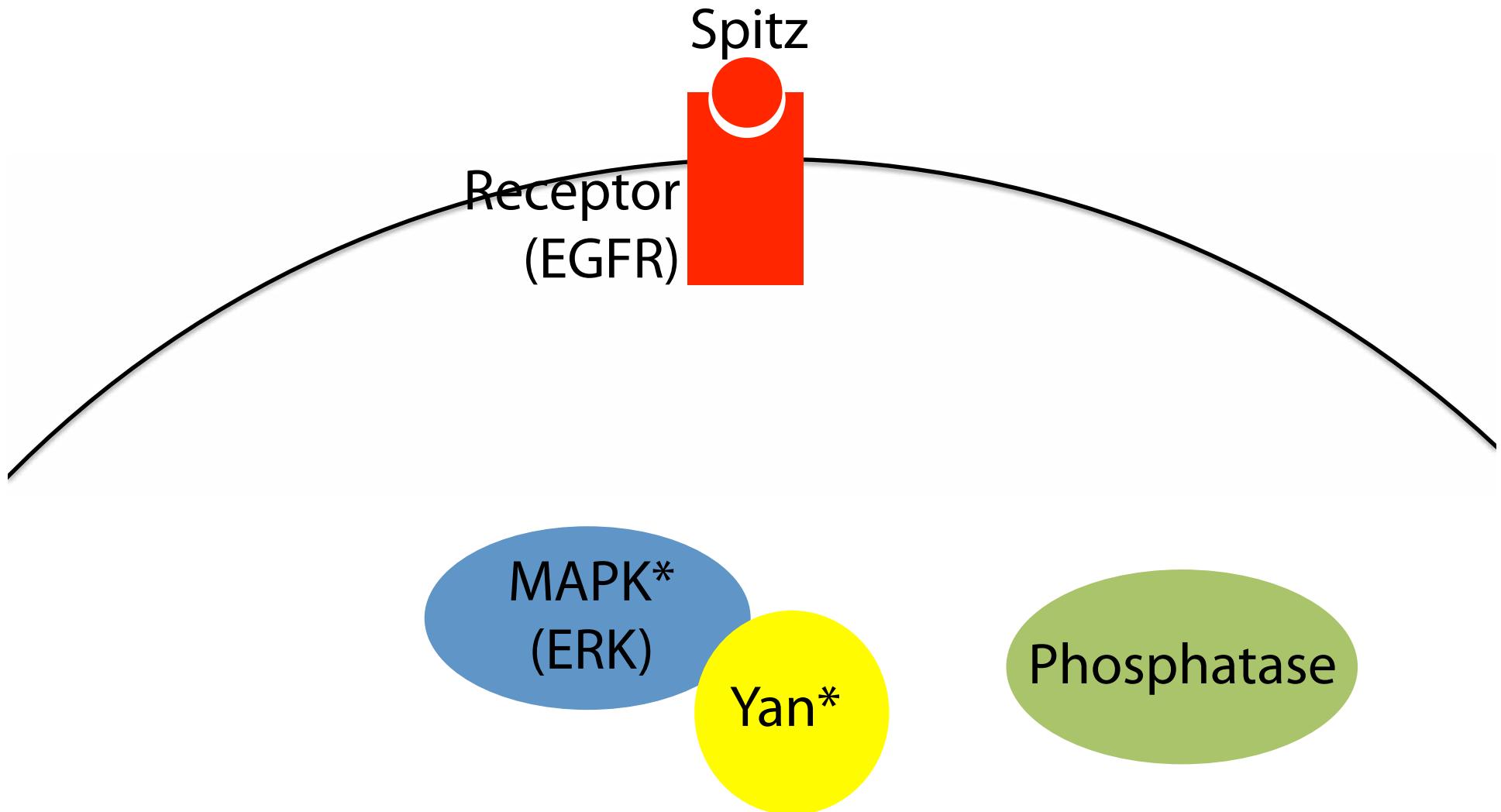
[Spitz] binds its receptor



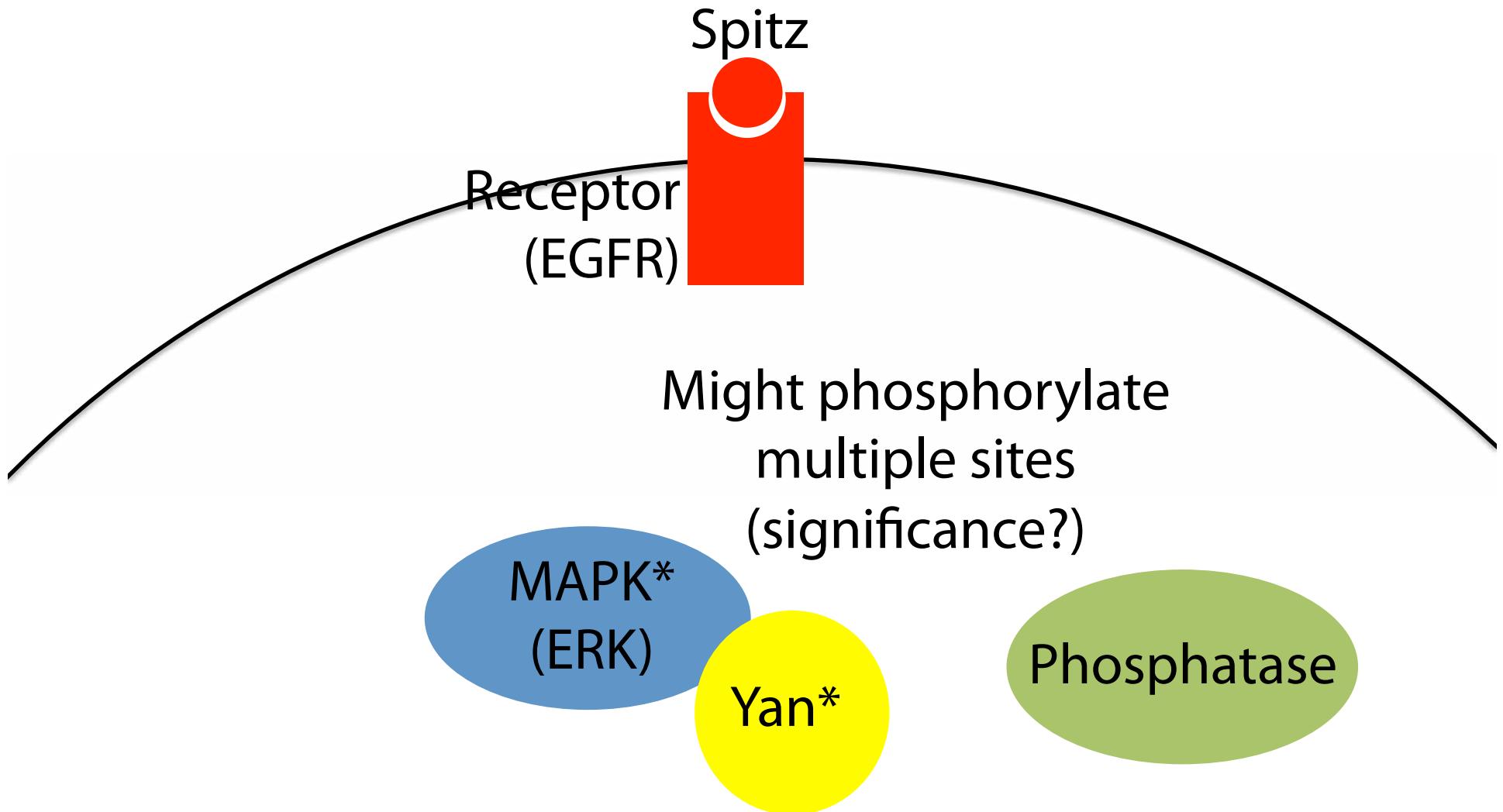
Bound receptor activates MAPK (ERK) through a MAP kinase cascade



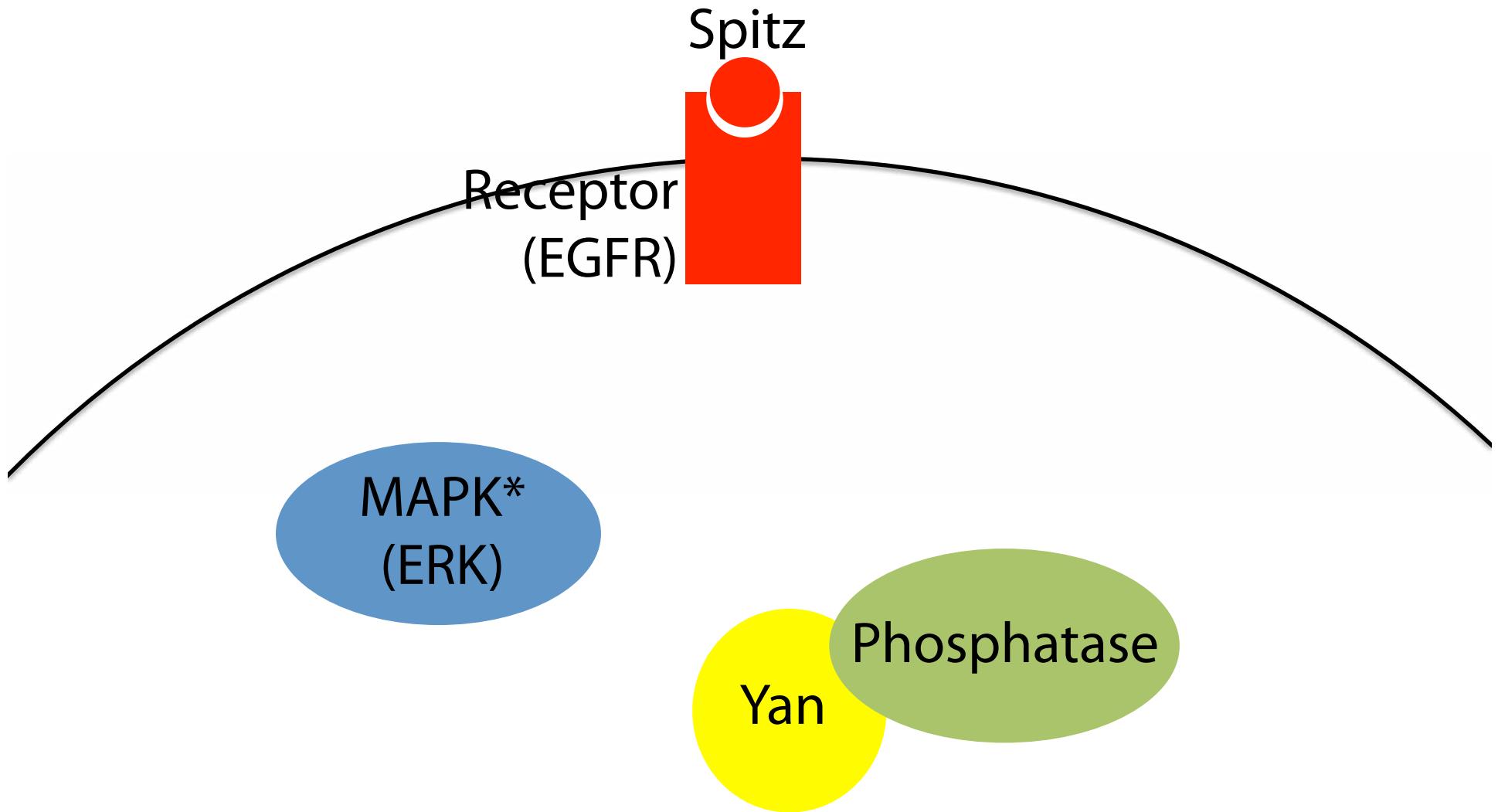
Active MAPK (MAPK*) binds Yan and phosphorylates it



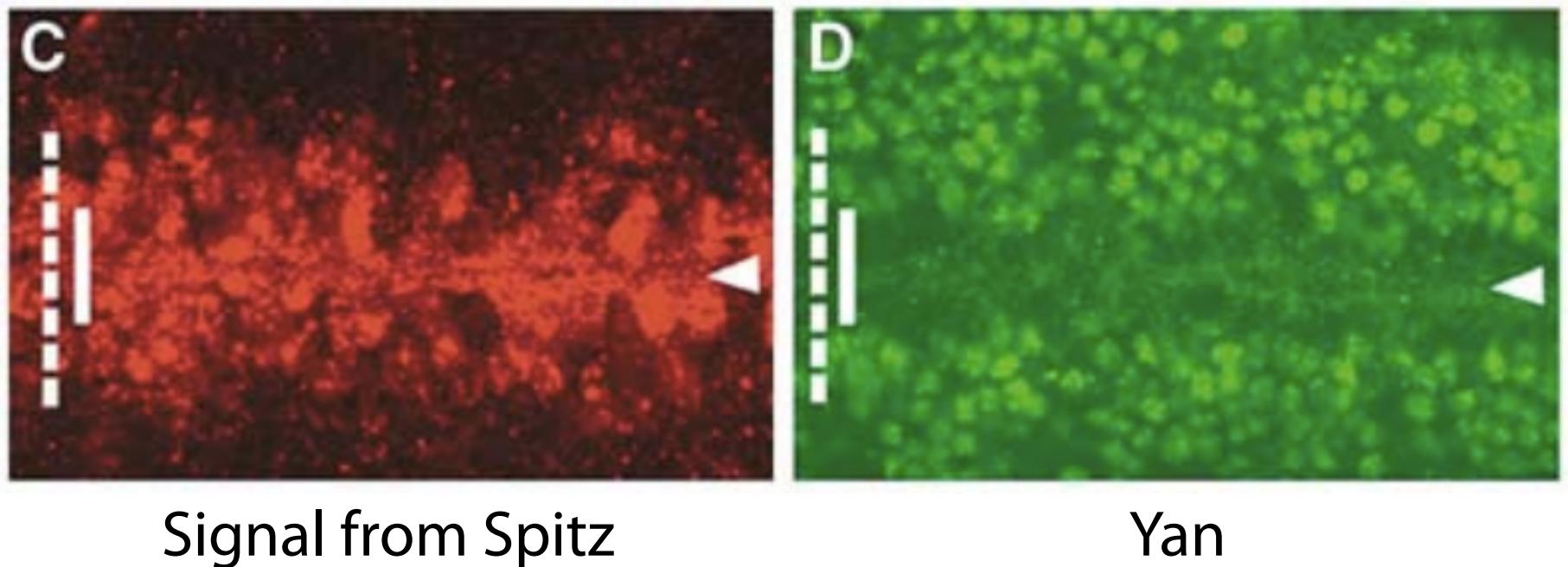
Active MAPK (MAPK*) binds Yan and phosphorylates it (Yan*)



Yan* is degraded (unless a phosphatase removes the phosphate)



The [Spitz] gradient is broad, but the pattern of Yan degradation is sharp

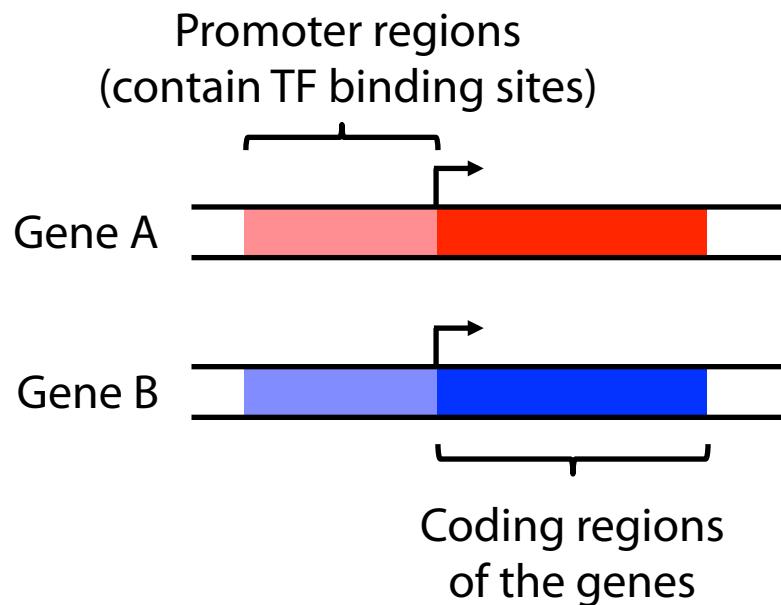


Melen et al. consider several potential sources of ultrasensitivity

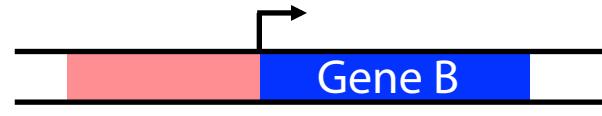
- First-order (enzymes in excess)
 - One phosphorylation is enough for Yan degradation
- Zero-order ultrasensitivity (Yan in excess)
 - One phosphorylation is enough for Yan degradation
- Cooperativity
 - need multiple phosphates on Yan for degradation
- Unidentified source of positive feedback

Why is *Drosophila* DV patterning a convenient model system?

The classic way of expressing a gene at an unusual location or time during development



Create new, hybrid gene with
Gene A's promoter and Gene B's
coding sequence

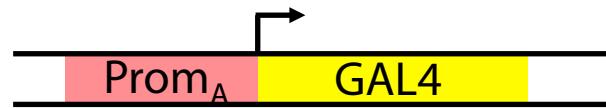


The protein B will be expressed at the same time and place as protein A

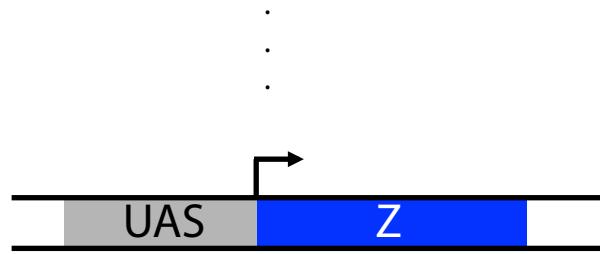
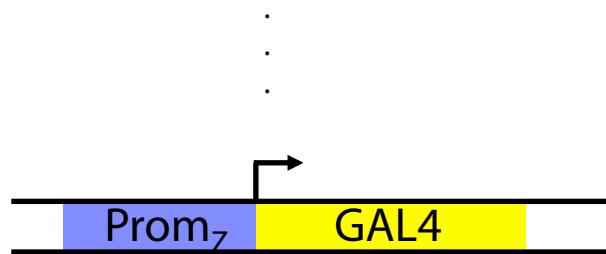
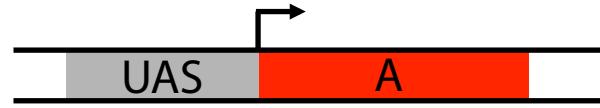
Why is *Drosophila* DV patterning a convenient model system?

Can create these combinations quickly using a synthetic gene library in *Drosophila*

The Gal4 protein is a transcriptional activator which binds a site called "UAS".



Similarly, many hybrid genes exist with UAS upstream of different coding regions



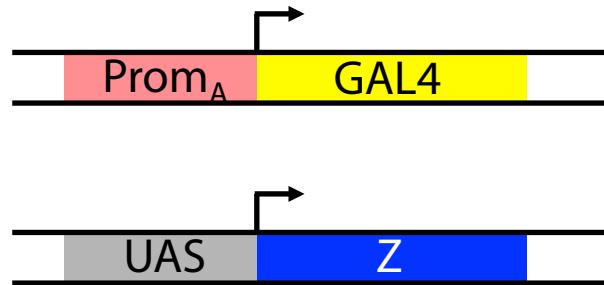
Many hybrid genes exist with GAL4's coding region under different promoters

Why is *Drosophila* DV patterning a convenient model system?

Can create these combinations quickly using a synthetic gene library in *Drosophila*

Just breed flies with the GAL4 gene and UAS gene of your choice

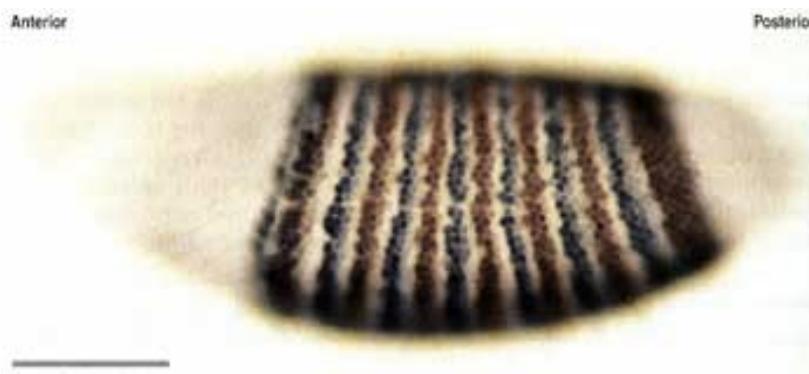
Gal4 protein is expressed wherever gene A is expressed.



Gal4 binds UAS and activates gene expression.

Therefore, protein Z is expressed wherever gene A is expressed.

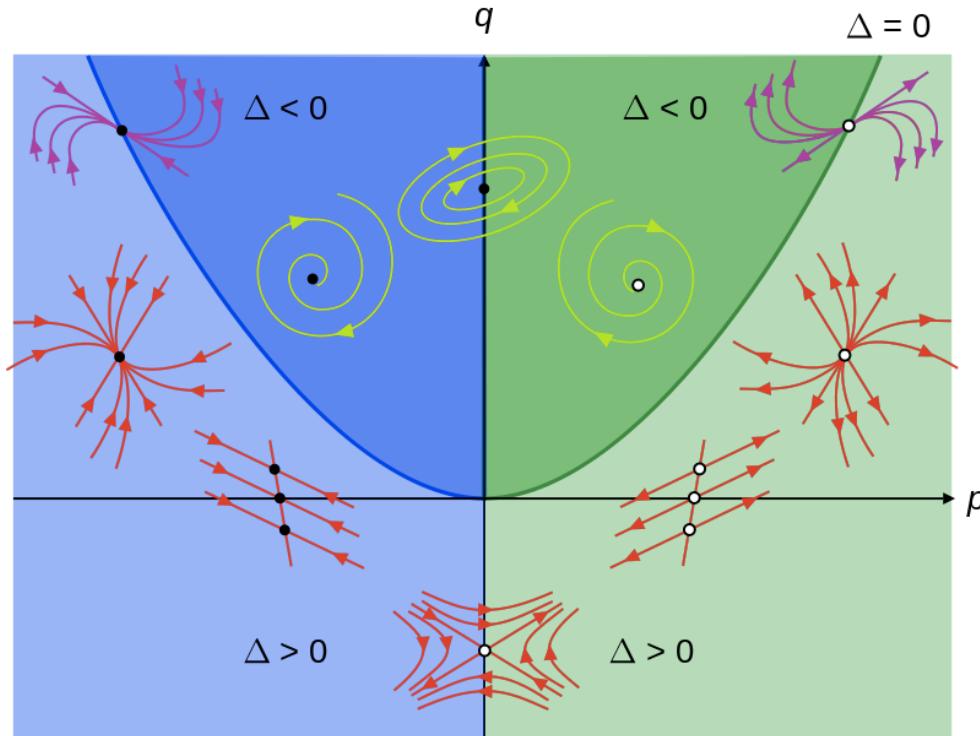
Why is *Drosophila* ventral patterning a convenient model system?



For example, we can test hypotheses by expressing a gene in stripes.

We look for differences between the gaps (where everything should be normal) and the stripes.

Too many biological minutiae for your taste?
Great news!



$$\frac{dx}{dt} = Ax + By$$

$$\frac{dy}{dt} = Cx + Dy$$

$$p = A + D$$

$$q = AD - BC$$

$$\Delta = p^2 - 4q$$

Next time: Phase plane and stability analysis