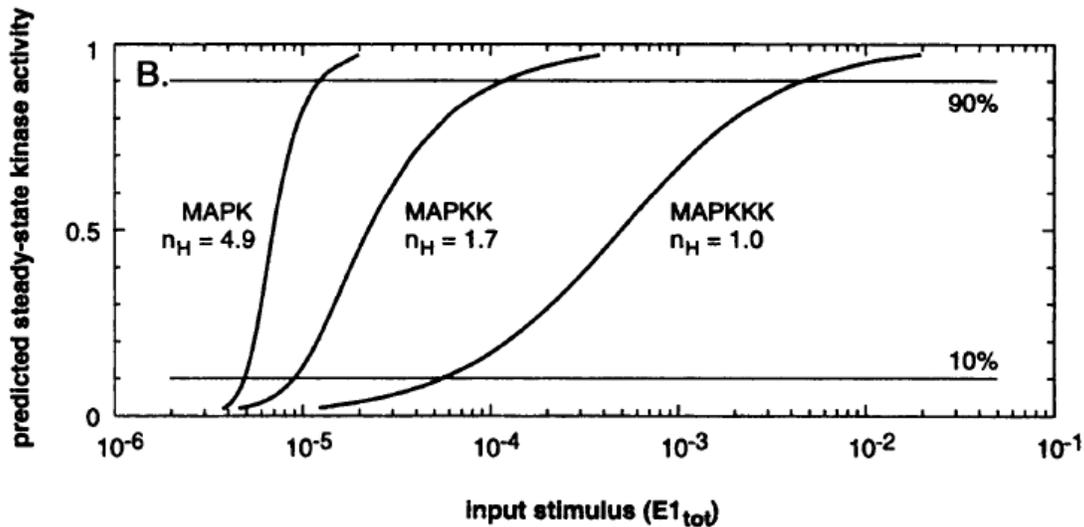


20.320, notes for 9/27

Thursday, September 27, 2012
9:35 AM

Ultrasensitivity/Amplification in the MAPK cascade

The distributive mechanism, where each player is phosphorylated in multiple steps, leads to great amplification and ultrasensitivity in the cascade.



Courtesy of the National Academy of Sciences. Used with permission. Source: Huang, Chi-Ying, and James E. Ferrell. "Ultrasensitivity in the Nitrogen-Activated Protein Kinase Cascade." *Proceedings of the National Academy of Sciences* 93, no. 19 (1996): 10078-183.

Although an 81-fold change is required for full activation of the MAPKKK, only a 13-fold change is required for activation of the MAPKK, and a 2.5 fold change is sufficient to generate a 90% activation of the MAPK. As mentioned above, this sort of strong response to small changes is absolutely critical.

But how?

1. **Double phosphorylation** at two stages.
As stimulation comes into the system, both enzyme and substrate concentrations go up. Thus, reaction rate really increases. This effect happens at two different steps of the mechanism, and the overall effect is great amplification.
2. **Increased protein concentrations** at later levels in the cascade. There's a 4000-fold difference in concentration between the signal and the downstream signaling molecules (signal molecule \ll MAPKKK $<$ MAPKK $<$ MAPK). When earlier molecules activate the later ones, they have way more to activate and the effects are amplified. Huang and Ferrel performed a sensitivity analysis, messing with different concentrations and showing that this does in fact contribute to the switch-like behavior.

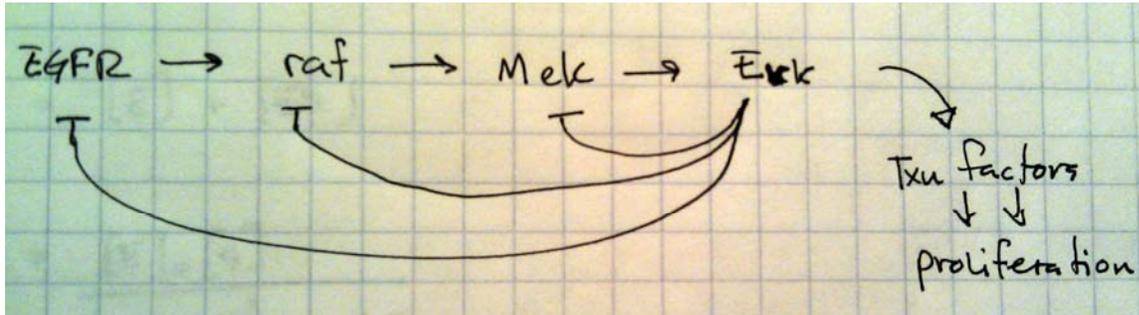
Control of Ultrasensitivity

You don't always want a hair-trigger. There are several ways of controlling these responses:

1. Feedback inhibition.

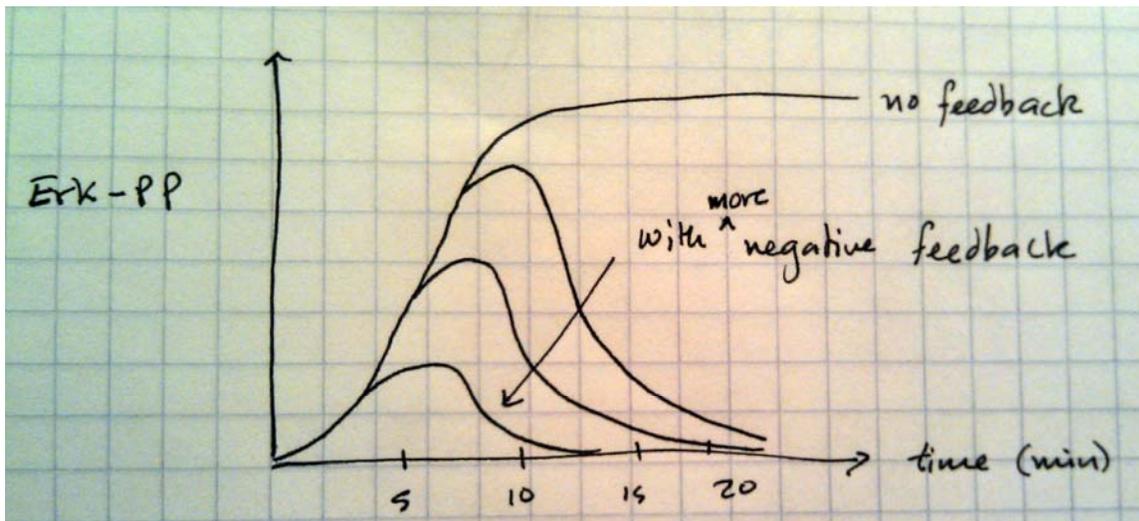
One real-world example of MAPK cascade is:

EGFR → raf → Mek → Erk → transcription factors, proliferation



The main mechanism for this negative feedback is Dual-Specificity Phosphatases (DUSP's), which are also created by the transcription factors activated by Erk. They are the phosphatases that dephosphorylate MAPK-PP's, which are the final element in the cascade and the thing whose level determines the signal. In other words, the whole cascade is just creating MAPK-PP's, and DUSP's have the power to dial them back. That's how you get negative regulation.

In the absence of negative feedback, Erk gets phosphorylated and just stays in that state. If its negative feedback kicks in and it stops being activated, the phosphatases (which are always active) kick in and turn it off. The net result is that Erk activation goes up and then comes back down. And this can be regulated quantitatively by changing the level of inhibition.



People have thought of inhibiting Mek to treat cancer. If you remove Mek you remove Erk, but then all the inhibitory effects of Erk also go away. The result is EGFR overexpression, and EGFR activates other oncogenic pathways than this one (example: PI3K → Akt → anti-apoptosis). Net result: cancer drugs don't work.

The cell uses at least three different **mechanisms for feedback inhibition**.

The first is **signaling feedback**, when members of the cascade gain or lose phosphates through their interactions with other kinases and phosphatases. This is quick (~1-5 min) and readily *reversible* (put them on, take them off). Erk, say, phosphorylates raf, Mek, EGFR, etc.

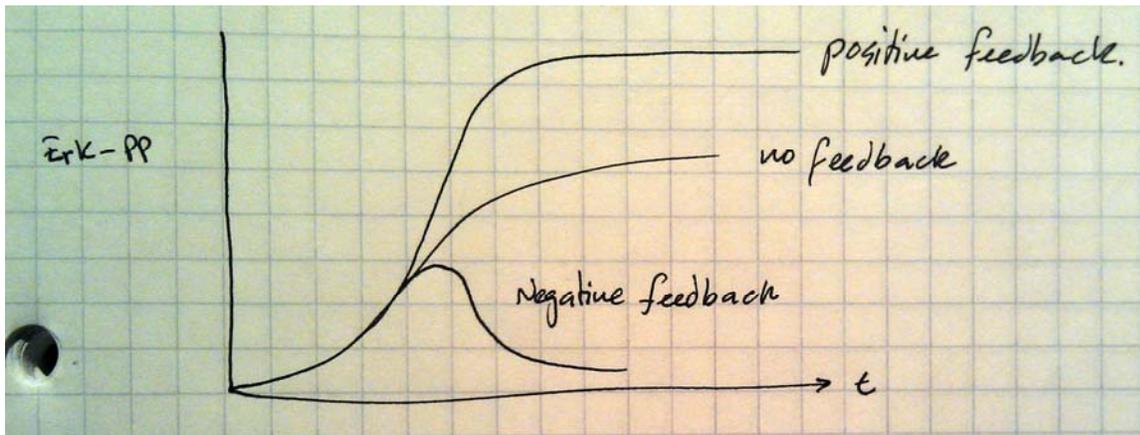
The second is through **phosphatase feedback**, which refers to changing the concentrations of phosphatase. It's slower (at least 15min) because it requires translation to make them and protein degradation to remove them. This kind of regulation changes the *state* of the system, providing a form of memory. When phosphatase concentrations are different, the system will respond to different levels of signal. This is a way for the system to acclimate to noise and not freak out when it doesn't need to. This system is not nearly as reversible.

There is also **transcription-factor feedback**

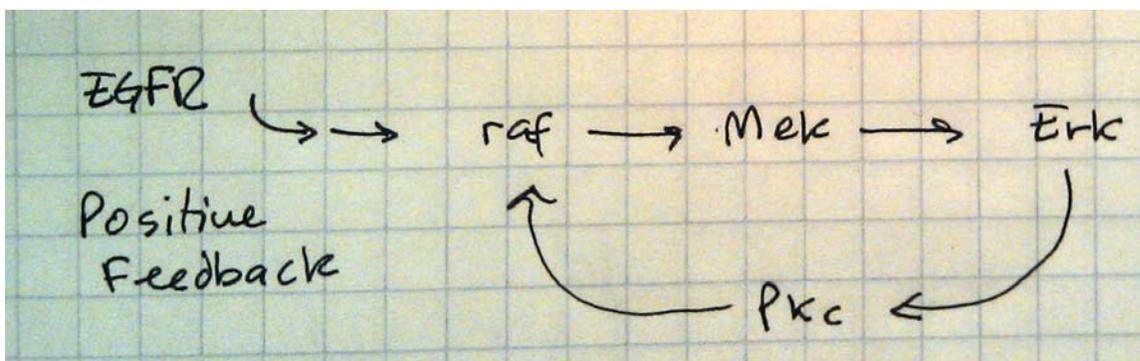
ETS is a family of transcription factors that normally sit on DNA and block the transcription of thousands of genes. Erk activates them (by phosphorylating them) and results in the activation of thousands of genes. One of these genes is that for ETS transcription factors, so they are produced in response to their own activation. The net result is pulses of signal. They help regulate the duration of signals from the network. There are multiple, overlapping ways like this in which the cell regulates response to the external signal.

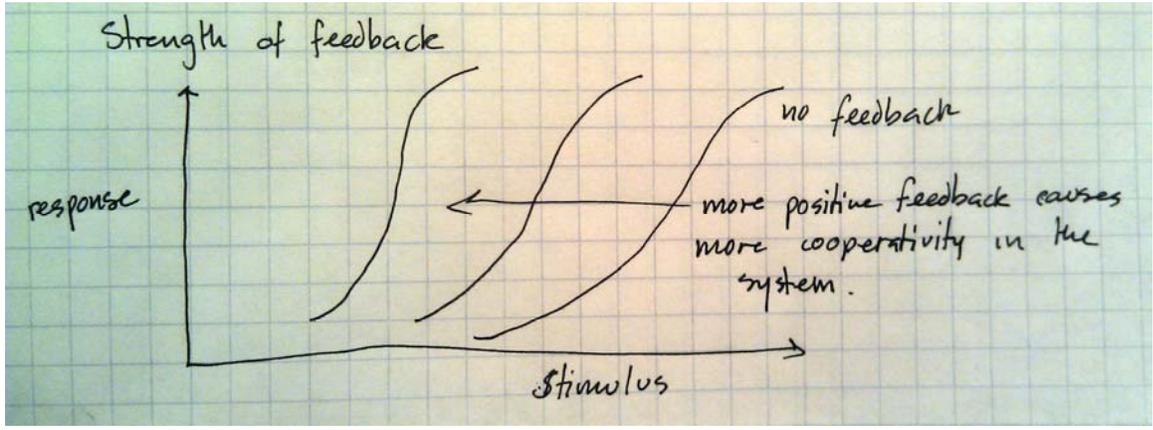
2. Positive Feedback.

There are cases where what you want is not a transient response, but a long-standing order. Differentiating stem cells into epithelials, for example, takes in the order of 10 days. This is a problem if our signal shuts down in 5 minutes. The path to standing orders is positive feedback. There are a few ways to do this:

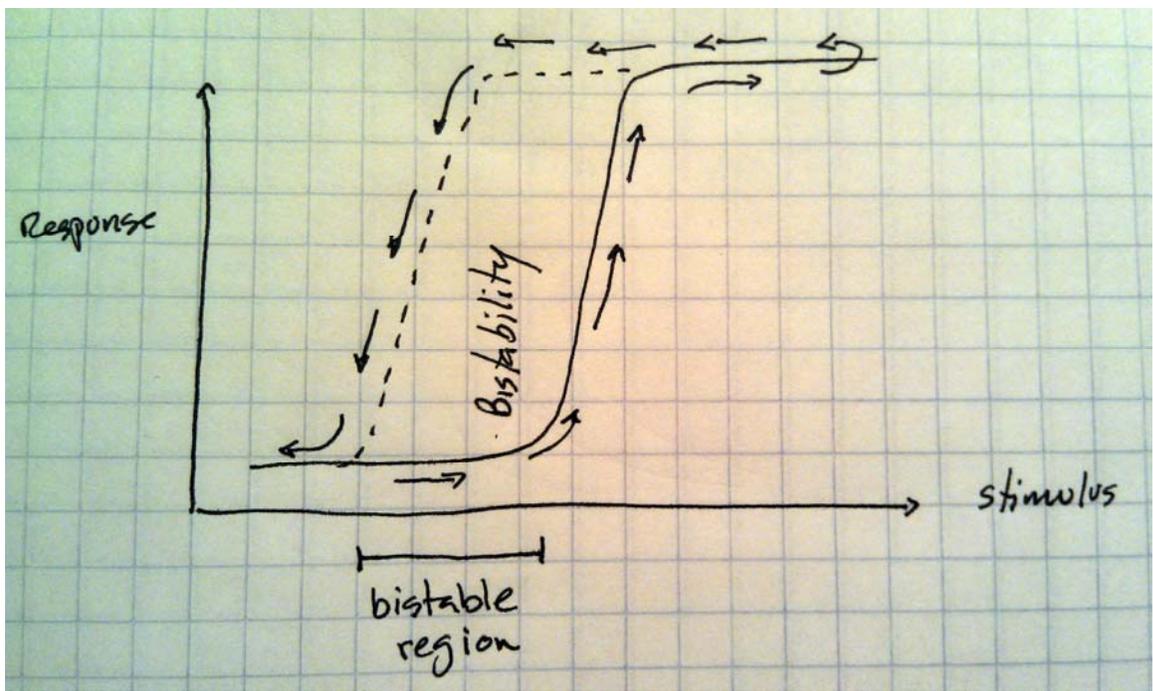


Activating phosphorylation sites are one way to lead to a sustained, stronger response. As with negative feedback, this effect is quantitative and modulatable. More feedback results in greater cooperativity and switch-like behavior.





However, positive feedback also results in an interesting effect. After the system goes up to a high level, it needs a level of signal somewhat lower than the original trigger to come back to baseline response. The system develops hysteresis. This results in a region (a level of signal) where the system is bistable: it could either not respond or respond like crazy, depending on what the history of the cell is. If the cell was recently inactive, it may not yet respond. If it was recently active, this same level will not be enough to switch it off. Cell's history then starts to matter.

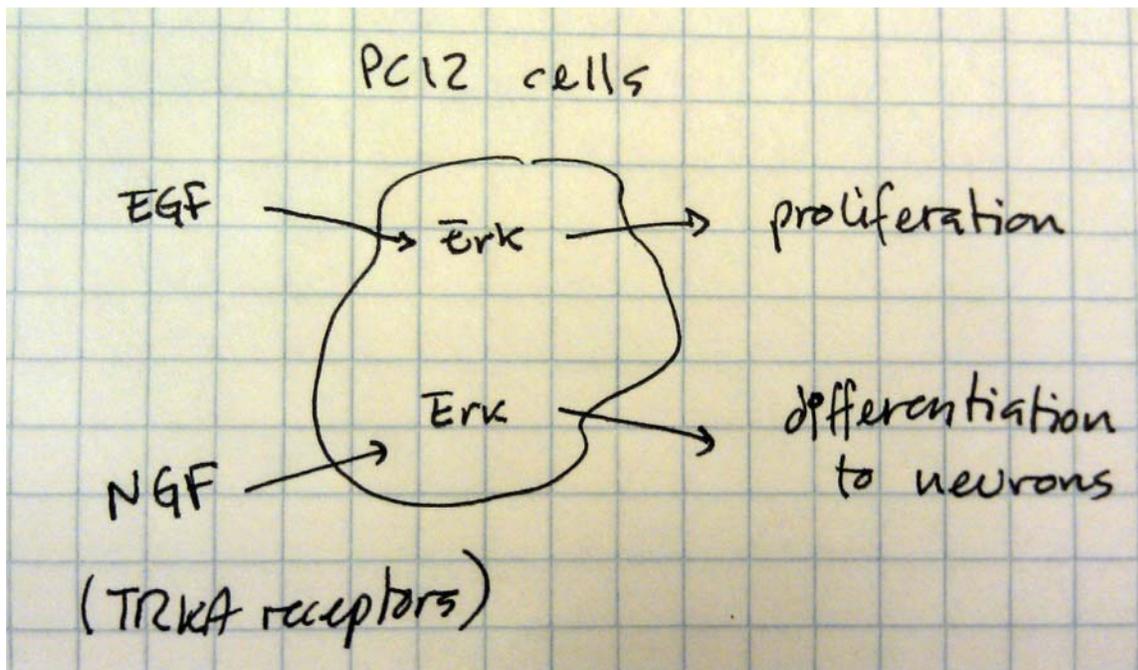


The arrows indicate the cell's path.

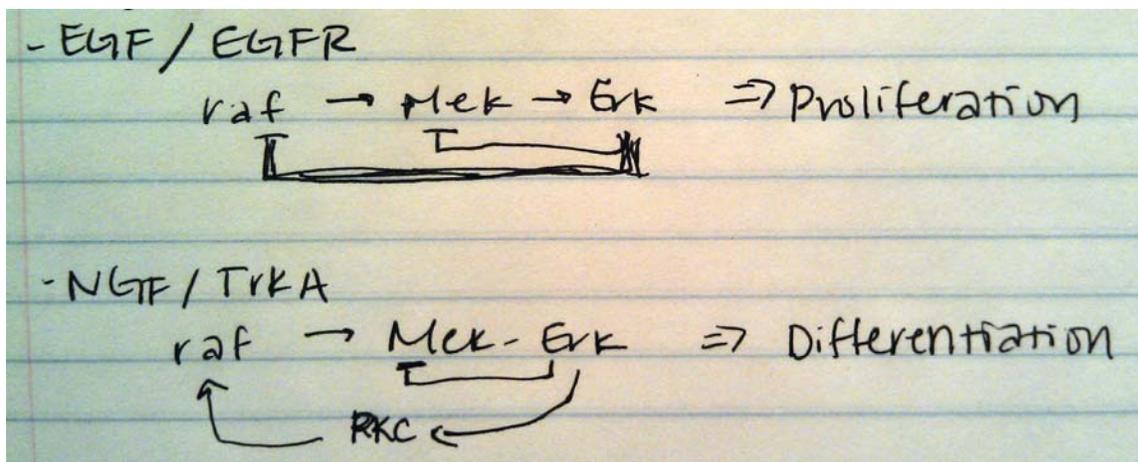
This is very useful for processes where it's important for the response to stay on once it's been turned on, even if the original signal disappears. Imagine apoptosis, which must continue once it starts to avoid the creation of undead cells.

Example: PC12 responses to EGF and NGF.

One interesting example of feedback regulation is the case of PC12 cells and their different responses to EGF and NGF. They both work through Erk, but lead to very different responses.



The difference is caused by an intermediate player, PKC, whose presence changes the type of feedback from strongly negative to positive.



How does the cell know whether to activate PKC? How do you change the substrate of Erk? Regulation here works by a combination of availability and kinetics. As you might remember, when there's a limited amount of enzyme available it will preferentially work on the substrates with the greatest affinity for it. In the case of this pathway, Erk has much lower affinity for PKC than for raf; therefore, in cases where Erk is limited most of its time is spent inhibiting raf. That's why there's negative regulation in the context of EGF.

When you apply NGF, however, something interesting happens. The cell has many more TRKA receptors than EGFR, and so a similar amount of NGF results in a much higher level of Erk inside the cell. As a result, there's now enough Erk going around to activate PKC, activate raf, and lead to the differentiation pathway. It's like substrate specificity is different for different levels of the enzyme. And that's just one of the neat ways in which protein networks can achieve very precise control with very humble means.

= Experimental validation

1) NGF, block PKC \Rightarrow proliferate

2) EGF, + PMA (phorbol ester, activates PKC) \Rightarrow differentiate

- How does the cell know when to activate PKC?

- The level of the signal

less EGFR \Rightarrow less Erk-PP, less substrates

more TRKA \Rightarrow more Erk-PP \Rightarrow additional substrates

* Erk chooses substrates by those with high concentrations and low K_m values

- Now test it:

siRNA on TRKA \Rightarrow decrease expression \Rightarrow NGF \rightarrow proliferation

\uparrow EGFR \rightarrow differentiation

Evidence was shown for this, I might add, by knocking out TRKA and showing that NGF leads to regular proliferation in that case. Isn't science awesome?

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20.320 Analysis of Biomolecular and Cellular Systems
Fall 2012

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