

20.320, notes for 10/2

Tuesday, October 02, 2012
9:37 AM

Roadmap

Cue --> Signals --> Responses

We talked about signals the last 2 classes. We'll talk about cues for the following two, and about responses (transcriptional, mostly) during the last 2 classes in this module.

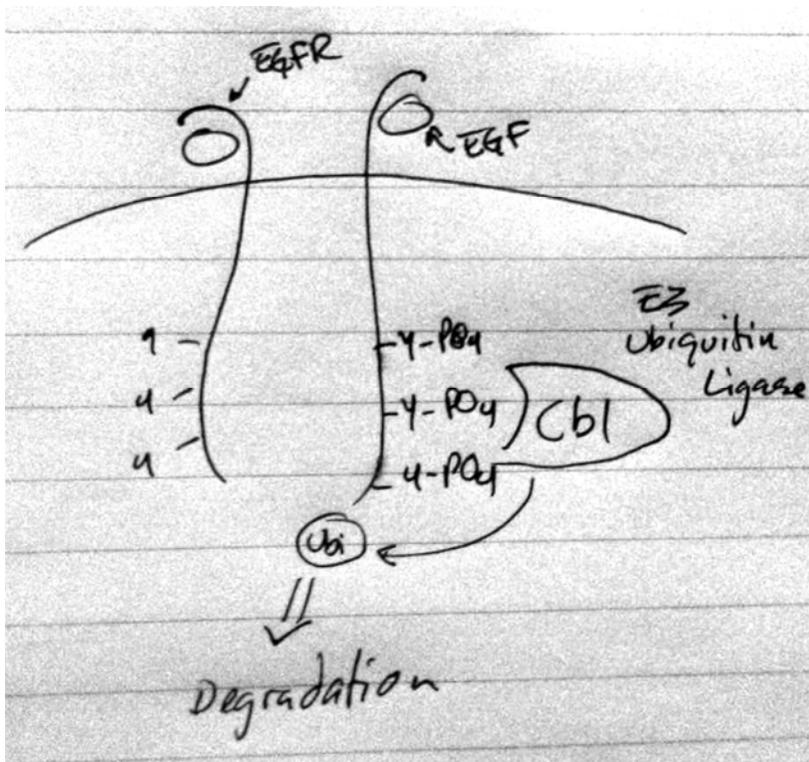
Signal shut-down

Aberrant receptor activation leads to oncogenesis and tumor progression. How does the cell prevent this from happening? There's a few ways.

1. Feedback inhibition
 - a. Example: Erk ----| EGFR
2. Receptors are endocytosed and degraded.
 - a. This happens to both activated and non-activated receptors.
 - b. This endocytosis is part of the procedure by which the cell samples the medium around it. The stuff that gets drawn in is degraded to peptides, yes, but also eventually presented to the immune system through MUC class I/class II surface presentation (where peptides are exposed on the surface for the immune system to know what the cell has been exposed to).

Deactivation of activated receptors

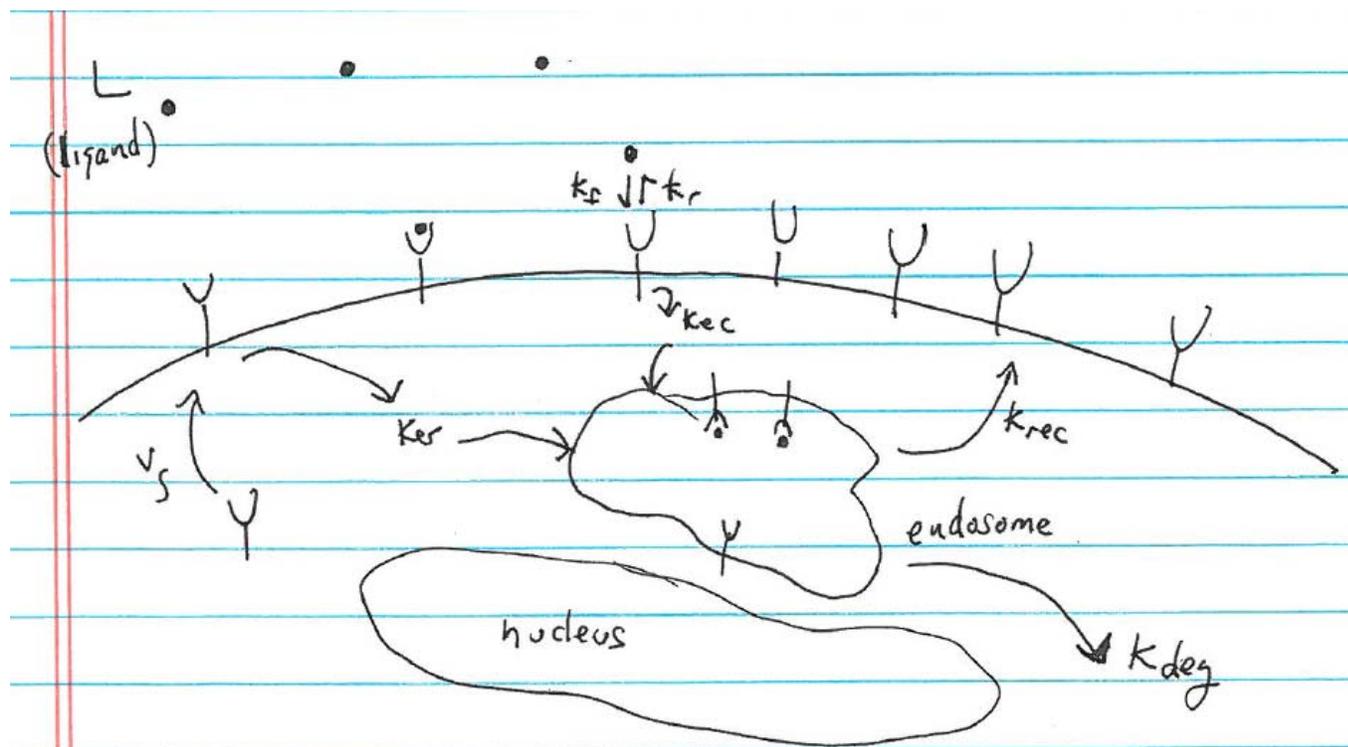
You have the phosphorylated EGFR. Its phosphorylated tyrosines are recognized by Cbl, a ubiquitin ligase that slaps ubiquitin tags on the whole EGFR and marks it for degradation. Remove the tyrosines, and you don't have Cbl recognition anymore. This happens in a lot of cancers.



Modeling signal shut-down

We will now try to model this process of down-regulating signals. We'll start with a simple case and then add complexity as needed. How does the context/environment affect the # of cell surface receptors? Let's build a model.

We start with a cell membrane, receptors, and a rate of receptor synthesis. This is all we need for the first iteration.



Nomenclature

L	Ligand
R_s	Receptors on surface
V_s	Rate of synthesis
K_{deg}	Rate of degradation
R_i	Receptors internal
C_i	Complexes internal
C_s	Complexes on surface
K_{er}	Rate of receptor endocytosis
R_T	Total receptors

Note that $K_{ec} > K_{er}$

Assumptions of our model

1. Complexes in the endosome do not unbind
 - a. Note that the endosome pH is about 5.5, very different from the rest of the cell. We can expand the model to include this un-binding.

2. Internalized complexes recycle at the same rate as receptors
 - a. This is probably a bad assumption
3. Ligand is only internalized when it's in complex.

If the rate of receptor production is constant, is the number of receptors also constant? Is this a good approximation?

We're modeling transcription and translation as constants, but in fact they are regulated by receptor activation pathways. We'll ignore that for now.

Given this model, we can now ask questions like "How many receptors are there at the surface in the absence of ligand?".

How many receptors on surface in absence of ligand?

$$\frac{dR_s}{dt} = V_s - k_{er}R_s + k_{rec}R_i - k_f[L]R_s + k_r[C_s]$$

$$\frac{dC_s}{dt} = k_f[L]R_s - k_rC_s - k_{rec}C_s + k_{rec}C_i$$

$$\frac{dR_i}{dt} = k_{er}R_s - k_{rec}R_i - k_{deg}R_i$$

$$\frac{dC_i}{dt} = k_{rec}C_s - k_{rec}C_i - k_{deg}C_i$$

Absence of ligand $L=0, C_s=0, C_i=0$
 no recycling

$$\frac{dR_s}{dt} = V_s - k_{er}R_s$$

$$\frac{dR_i}{dt} = k_{er}R_s - k_{deg}R_i$$

At steady state $\frac{dR_s}{dt} = \frac{dR_i}{dt} = 0$

$$R_{s0} = \frac{V_s}{k_{er}} \quad R_{i0} = \frac{k_{er}}{k_{deg}} R_{s0} \quad R_{s1} = \frac{V_s}{k_{deg}}$$

$$R_{T0} = R_{s0} + R_{i0} = V_s \left(\frac{1}{k_{er}} + \frac{1}{k_{deg}} \right)$$

$$\frac{R_{s0}}{R_{T0}} = \left(1 + \frac{k_{er}}{k_{deg}} \right)^{-1} \quad \text{fraction of receptors on surface}$$

ⓐ T_0 , absence of ligand

Plug in representative #s
 $k_{er} = 2 \times 10^{-2} \text{ min}^{-1}$ $k_{deg} = 1 \times 10^{-2} \text{ min}^{-1}$ $v_s = 100 \text{ min}^{-1} \text{ cell}^{-1}$
 $\Rightarrow R_{s0} = 5 \times 10^3 \text{ copies/cell}$
 $R_{i0} = 1 \times 10^4 \text{ copies/cell}$
 $R_{s0}/R_{i0} = 0.3$
 - What does this tell us?
 - In the absence of ligand, only 1/3 of receptor is on the surface. Not available for binding ligand, less sensitivity to signal.

But how do we measure the amount of surface receptors? We could do FACS with anti-EGFR antibodies tagged with fluorophores. This is great, and measures just the surface receptors. Most people for a long time just did Western blots, which are misleading because (unless you're isolating the membrane) you're measuring total receptors from the whole cell.

What happens to R_s , R_T when we add ligand? We can make a few different assumptions

1. Assumption: $k_{deg} \gg k_{er}, k_{rec}$.
 - a. When receptors or complexes are internalized, they are immediately degraded.

What happens to R_s, R_T when we add ligand
 1. $k_{deg} \gg k_{er}, k_{rec} \Rightarrow$ When receptors or complexes are internalized, they are immediately degraded
 Initial conditions, @ $T_0 = 0$ $L = 0$
 2. $\frac{dR_s}{dt} = v_s - k_{er} R_s$ $\frac{dR_s}{dt} = 0$ @ steady state
 $\frac{dR_{s0}}{dt} = \frac{v_s}{k_{er}}$ $R_{s0} = R_{T0}$ - starting condition

Thus, our starting condition is that $R_{s0} = R_{T0}$. We can further assume that $[L]_0 \gg R_s$, so that there will be minimal ligand depletion. We will also assume that ligand binding and complex formation happens much faster than internalization. Thus, it is at equilibrium.

Look at steady state after ligand binding

$$\frac{d(R_s + C_s)}{dt} = 0$$

$$V_s - k_{er} R_s - k_{ec} C_s = 0$$

Relate # complexes to # receptors

$$k_d = \frac{[L]_0 R_s}{C_s}$$

$$C_s = \frac{[L]_0 R_s}{k_d}$$

$$R_s = \frac{V_s}{(k_{er} + k_{ec} \frac{[L]_0}{k_d})}$$

$$C_s = \frac{[L]_0}{k_d} \left(\frac{V_s}{k_{er} + k_{ec} \frac{[L]_0}{k_d}} \right)$$

$$R_T = R_s + C_s$$

$$R_T = \frac{(1 + \frac{[L]_0}{k_d}) V_s}{k_{er} + k_{ec} \frac{[L]_0}{k_d}}$$

from no ligand starting concentration

$$\frac{R_T}{R_{T0}} = \frac{1 + \frac{[L]_0}{k_d}}{1 + \frac{[L]_0}{k_d} \frac{k_{ec}}{k_{er}}}$$

What can we learn from this equation?

What controls the amount of receptor that's there after you add ligand? The dominating term is k_{ec}/k_{er} .

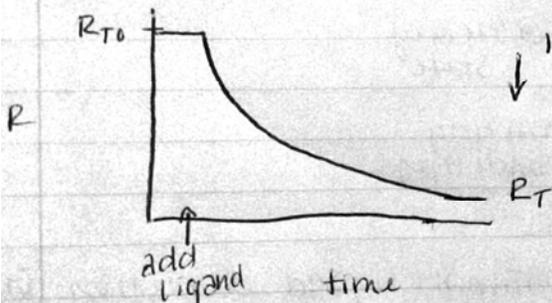
This controls the amount of receptor that's there after ligand is added. If $k_{ec} > k_{er}$, as we'd assumed earlier, then

$R_T < R_{T0}$.

What can you learn from this?

k_{ec}/k_{er} controls amount of receptor after ligand stimulation

If $k_{ec} > k_{er}$ then $R_T < R_{T0}$



↓ If we signal here, there will be less signal due to less receptor

And thus, the cell's response level depends on how long it's been since it started signaling. Same ligand

has less effect later on. This protects the cell from ongoing, aberrant signaling.

How do you regulate the system?

1. Increase k_{ec}
2. Increase k_{er}
 - a. This is counterintuitive. Doesn't that move our crucial control term the wrong way? Because it controls the total receptor concentration. Remember that

$$R_{To} = \frac{V_s}{k_{er}}$$

And so increasing k_{er} will result in fewer receptors that can be activated at all. This is a really clever approach. People have taken multiple anti-EGFR antibodies and used them to trigger greater endocytosis without added signaling. By using at least two different kinds of antibodies, you can basically cross-link bunches of receptors together and trigger endocytosis. Dane Wittrup showed that messing with k_{rec} and k_{er} has similar effects. In other words, you can either increase internalization or block recycling.

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