

1 Cellular Signaling

1.1 Principles of Cell Signaling

1.1.1 The Basic Vocabulary of Cell Signaling

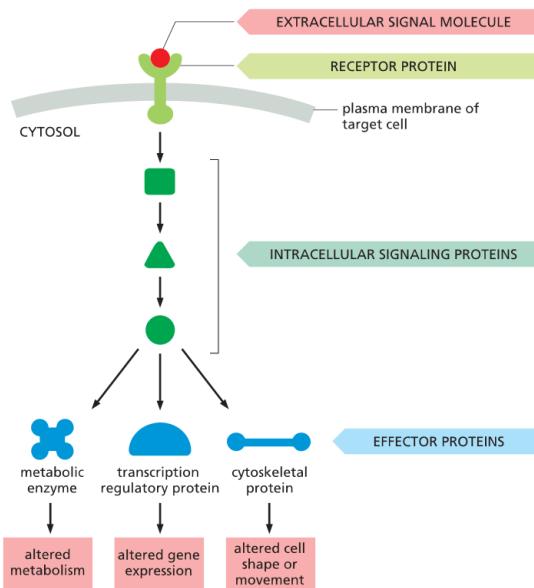


Figure 1: Basic Vocabulary of Cell Signaling

There are some key terms in cell signaling. Here's a run down of how they connect in cell signaling:

- i) An **extracellular signaling molecule** binds to a **signal receiving protein or receptor**.
- ii) That receptor is generally a transmembrane protein but can also be intracellular. This receptor is activated through the binding (generally some sort of conformational change).
- iii) This causes a **signaling cascade** through a chain of **intracellular signaling proteins** activating each other.
- iv) The final protein in that cascade will then change the activity of the effector protein, which launches the cellular response.
- v) These **effector proteins** can be metabolic enzymes, transcription regulators, or cytoskeletal proteins.

1.1.2 Cell-surface vs. Intracellular Receptors

There are two main differences between Cell-Surface and Intracellular receptors:

First, the location of the receptors (surprised you with that am I right):

- Cell-Surface: generally transmembrane protein, where the signaling molecule binds extracellularly.
- Intracellular: The receptor protein will be close or even inside the nucleus.

Then, accordingly the signaling molecule will also be different, in the case of:

- Cell-Surface, it is generally a hydrophilic signaling molecule. This means the molecule can't enter the cell, so we need the receptor to have some extracellular component.

- Intracellular, it is a small hydrophilic signaling molecule, which can transfer the cell membrane. This is necessary as it needs to reach the receptor in the nucleus. They are carrier through the blood by carrier proteins (hydrophilic).

1.1.3 The Four Subtypes of Cell Signaling

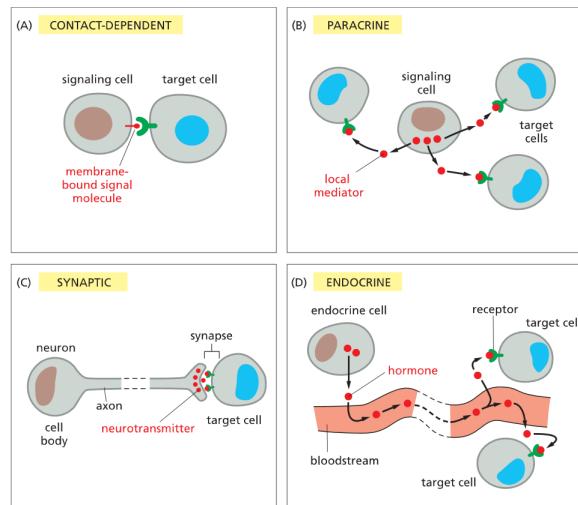


Figure 2: The Four types of Signaling

Contact Dependent Signaling:

- Area of signal: Cells are in contact.
- Form of communication: Proteins which are attached to the cells interact. One protein serves as the signal and the other as receptor.
- Variation: cell with extracellular matrix (e.g., collagen), for more details see section on ECM.

Paracrine Signaling:

- Area of signal: Cells are not in contact. This is usually a local signal, just a few cells away.
- Form of communication: One protein secreted by a cell, is the signal or ligand and attaches to the receptor of a different cell.
- Variation: If a cell receives its own signal it is called autocrine signaling.

Synaptic Signaling:

- Area of signal: Cells are not in contact. Small distance between releaser of ligand and receptor, called the synapse. Very local signal.
- Form of communication: Secretion of a ligand or neurotransmitter. Released by one cell and received by another.
- Variation: If a cell receives its own signal it is also called autocrine signaling.

Endocrine Signaling a.k.a. Hormonal Signaling:

- Area of signal: Cells are not in contact. Can be long distance and have effects from anywhere to anywhere
- Form of communication: A hormone is produced by cell A and then released into the bloodstream, where it can then leave at some point and serve as a signal to a receptor protein.
- Variation: If a cell receives its own signal it is called autocrine signaling.

1.1.4 The diversity in Signals

The same signal can cause a multitude of signals. This section will have a look of the consequences and opportunities of that.

Multiple signaling molecules, better the combination can cause very different signals: Depending on the combination of signals received a cell can kill, proliferate, or differentiate itself. Further the same signaling ligand or protein can have very different consequences depending on the receptor or cell it attaches to. See for example acetylcholine:

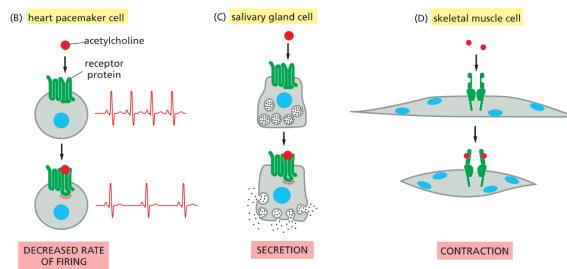


Figure 3: Examples of Acetylcholine having vastly different responses to its signal.

Speed of the response: Depending on the response path, the response by the cell can be fast or slow. If the response alters a protein it will take seconds to minutes, while if the gene has to be transcribed it takes minutes to hours. These two types are called **protein response** or **transcriptional response**. Some receptors also cause both the fast and slow response path.

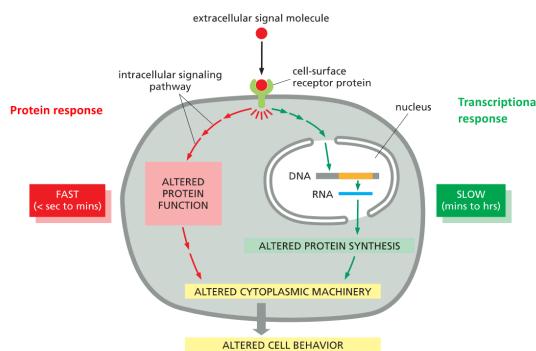


Figure 4: An overview of protein (fast) vs. transcriptional (slow) response.

Examples of a fast response: change in movement, secretion, or metabolism, caused by e.g., phosphorylation. Concretely the recruitment of GLUT transporters from recycling endosomes, has to occur very rapidly once insulin docks onto the receptor.

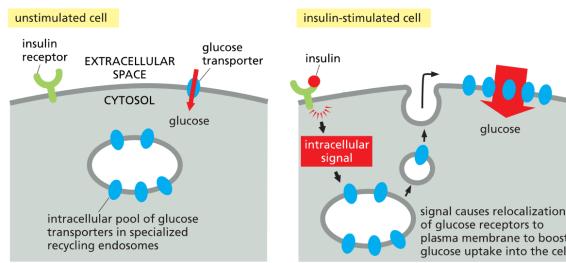


Figure 5: An example of a fast protein response with the GLUT transporter.

1.1.5 Classes of Cell-Surface Receptors

There are three main classes of cell-surface receptors, which we will all be diving into later on:

- i) Ion-Channel-Coupled Receptors a.k.a. transmitter-gated ion channels;
- ii) G-Protein-Coupled Receptors;
- iii) Enzyme-Coupled Receptors;

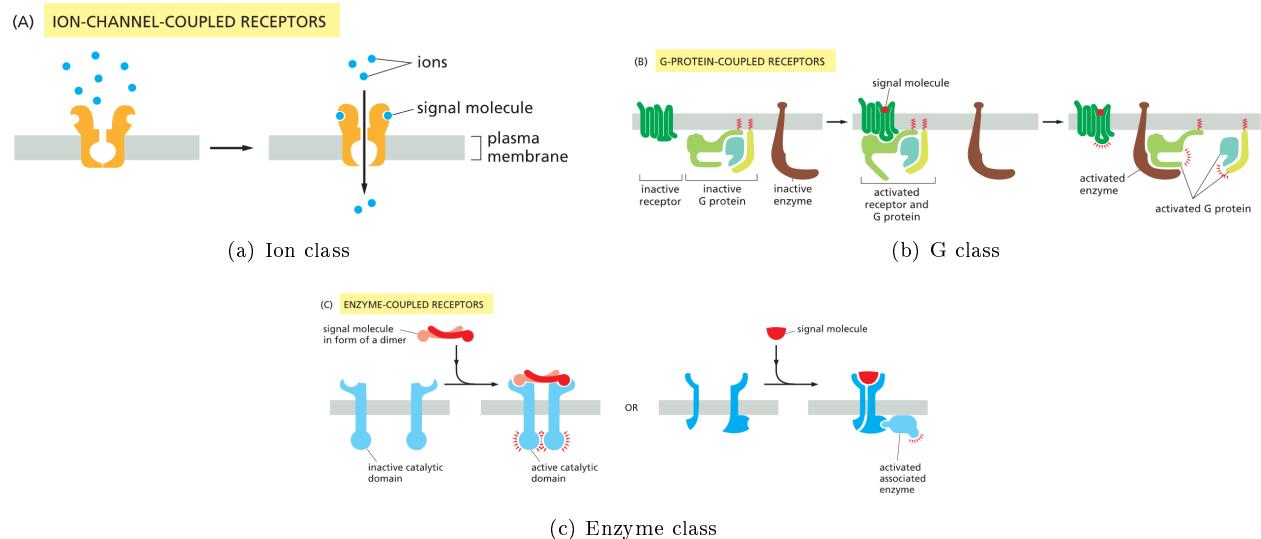


Figure 6: The three classes of cell-surface receptors

Note on the enzyme-coupled one: There are two options here: one where the enzyme is part of the receptor and another where the enzyme is recruited. Ligands activate the receptors by promoting their dimerization though, regardless if the enzyme is directly attached or not.

1.1.6 Regulation of Intracellular Signaling Proteins

There are two main molecular switches for intracellular signaling proteins:

i) Phosphorylation

- based on a phosphate group being attached to the protein (attached means active).

- phosphorylation or dephosphorylation often leads to change in formation and to activation.
- Addition by kinases.
- Removal by phosphatases.
- This group can be added to three amino acids: Tyrosine, Threonine, or Serine. This is because they have an alcohol which works for substitution.

ii) GTP binding

- We have GTPases, whose activity is controlled through GTP/GDP. This is also a type of G-Protein, but a different class of monomeric "small" GTPases.
- A phosphate is removed from GTP to make GDP, this deactivates the molecule. GDP stays bound.
- With an incoming signal, this GDP can be exchanged for a GTP.
- GEF activate GTPases, by exchanging GDP for GTP.
- GAPs inactivate GTPases by hydrolyzing GTP and yes GAP stands for GTPase-activating protein, as it activates the inactivation.
- going from GTP to GDP is more favorable due to the ratio GTP:GDP.

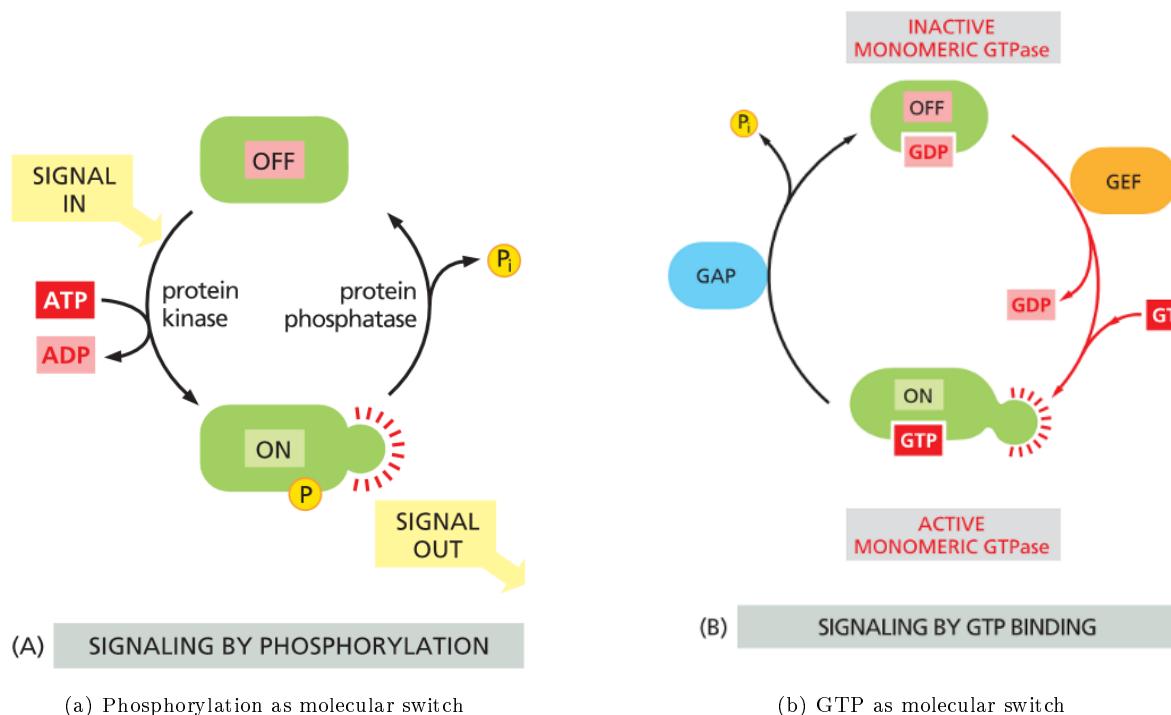


Figure 7: Two types of molecular switches in intracellular signaling

1.1.7 Inhibitory Signals as Activators

Signal transduction isn't always a positive signal, sometimes an inhibitory signal can lead to activation. Basically the idea is to inhibit the inhibitor.

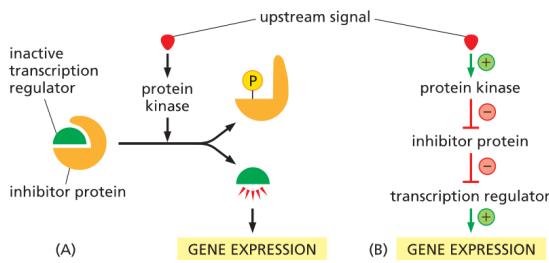


Figure 8: Example pathway of an inhibitory signal leads to activation. Note that the left and right path show the same pathway.

1.1.8 Initiating the Signal

A signal starts through a protein being in **close proximity** to the signaling compound. This proximity is key and the minimum for a signal to be start, something ATP is also required.

There are three main types of starts to signaling:

i) Preassembled signaling complex

- The signal complex is already assembled with all its intracellular signaling proteins.

ii) Protein recruitment

- the signaling proteins are in close proximity. Once the signal molecule attaches they attach to the receptor
- Sometimes the signaling proteins don't need to actually be attached, but just being in close proximity is enough.

iii) Lipid recruitment

- Instead of having the signaling proteins attach to the receptor they attach to a Phosphoinositides (PI, a type of phospholipid) in the proximity.
- These special phospholipids a.k.a. PIs a.k.a. Phosphatidylinositol are part of the membrane and in close proximity to the receptor. These phospholipids can be phosphorylated in the cell, which allows the signaling proteins to attach.

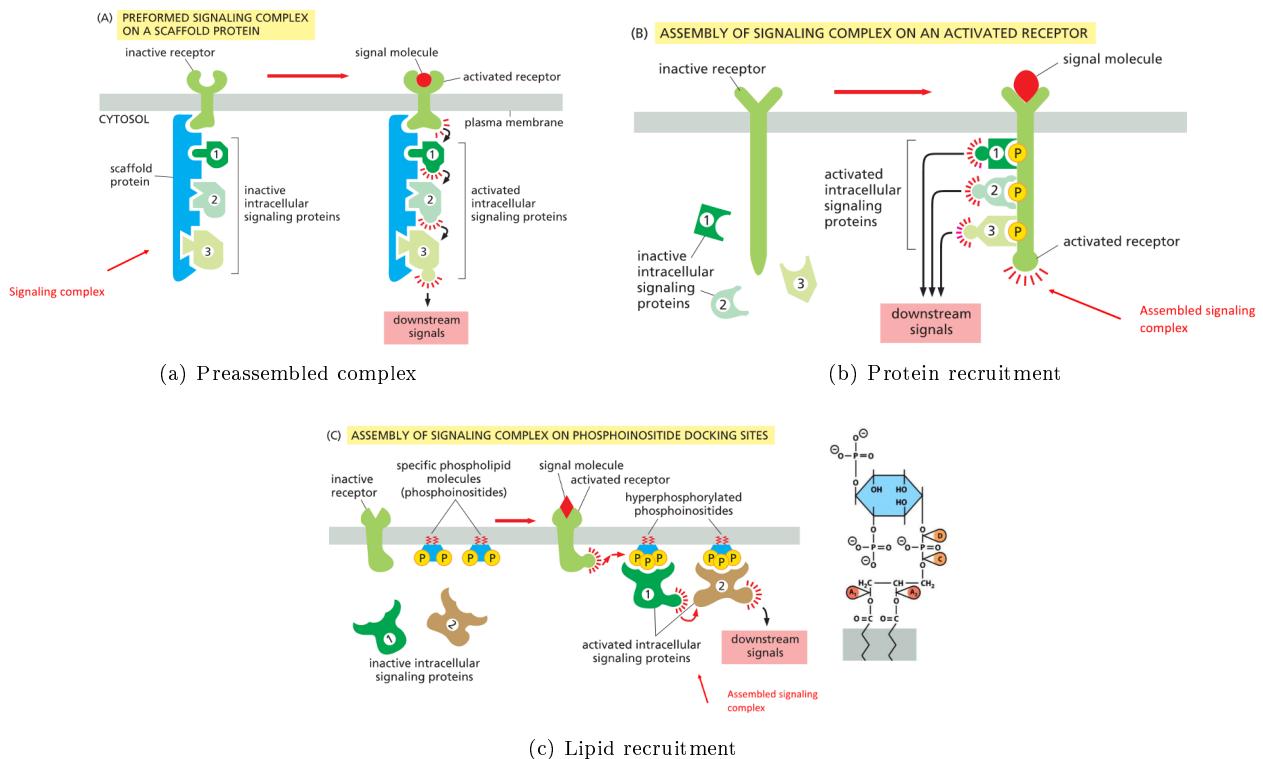


Figure 9: The three classes of cell-surface receptors

These signaling complex's got their name because they can get very complex. They are formed using **modular interaction domains**. Here is an example of an insulin receptor:

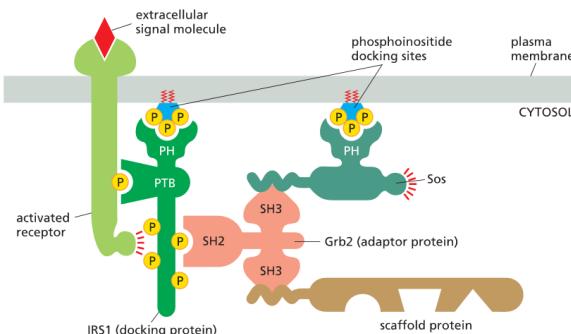


Figure 10: insulin signaling complex as an example for the complexity and modularity of a signaling complex.

The shortcuts of the molecules in the picture:

- PH = Pleckstrin Homology - binds to phosphorylated PI's
- PTB = Phosphotyrosine Binding - binds phosphotyrosine
- SH = Src Homology, Src is on the first signaling proteins identified in a viral induced chicken sarcoma.
- IRS = Insulin Receptor Substrate

1.1.9 Regulating and Dampening the Signal

One way a cell can add extra regulation to a pathway is to require multiple independent pathways to integrate for them to signal downstream, called **signal integration**.

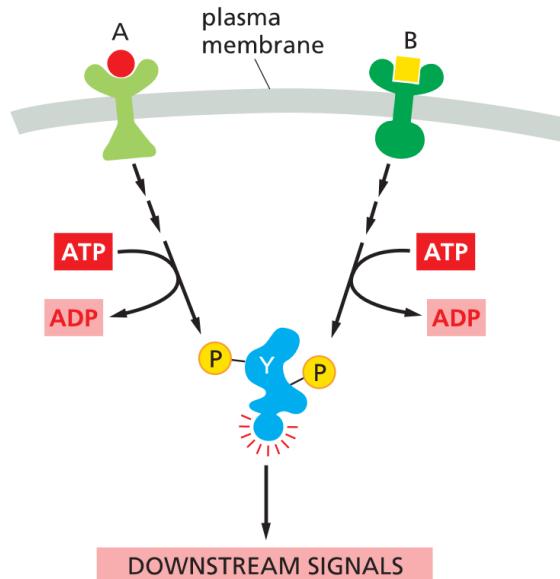


Figure 11: An example pathway showing how multiple streams need to come together to allow downstream signaling.

Now, this need for multiple proteins gives the cell the power to change the response duration and strength depending on how it changes the production and degradation rate of each protein.

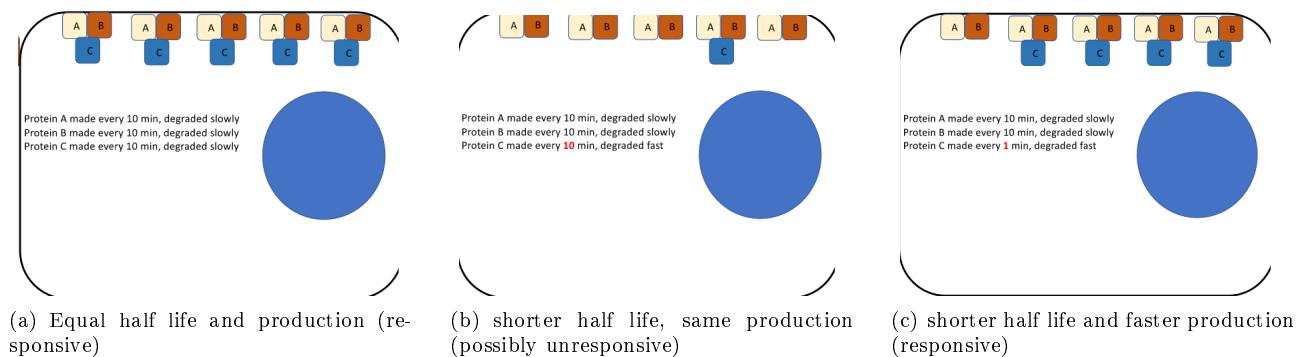


Figure 12: An example of how influencing the half life and production of certain proteins can seriously influence the responsiveness of a protein complex.

To further show the role and importance of degradation in protein complexes here are some graphs we're gonna digest:

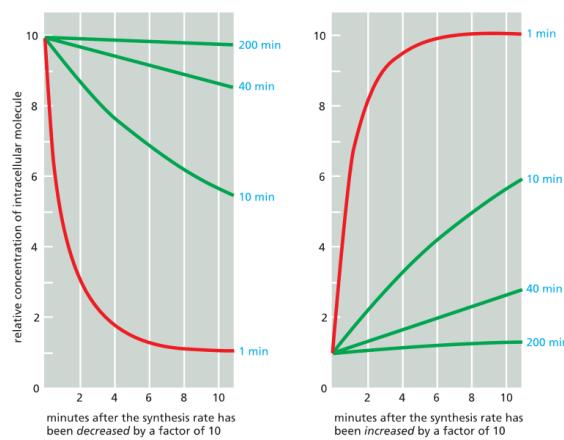


Figure 13: Shows the connection between half-life (blue time), with it degradation, and synthesis rate (y-axis). The x-axis is the time after the synthesis rate is increased by a factor 10. A protein with a short half-life will probably also have a high production rate (red line) as otherwise it will be unresponsive (see picture above). Hence it will react much more strongly to a signal than a protein with a long half-life and slow production rate (green line).

The next key concept is **negative and positive feedback**. This is when a downstream molecule will signal upwards in the pathway to either increase or decrease its activity.

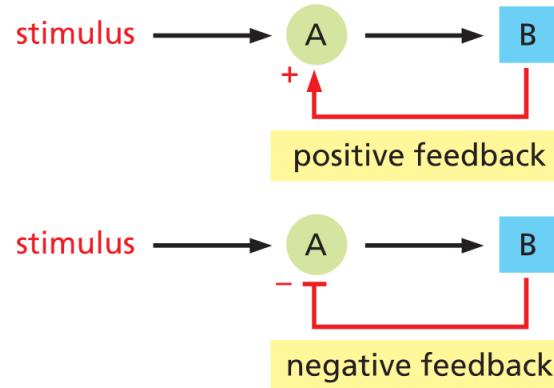


Figure 14: Shows both positive and negative feedback. Note that B is often not directly after A but somewhere down the chain (so T not B)

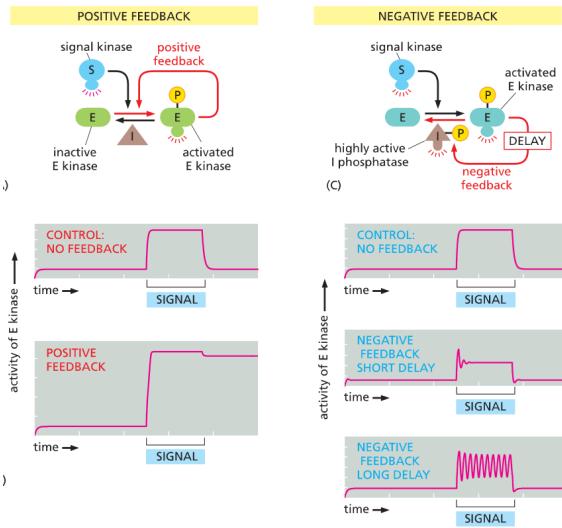


Figure 15: Shows both a positive and negative feedback loop. Below the pathway it also shows the effects of the feedback in a time vs. enzyme activity graph.

Discussion of the image: in feedback the positive one is pretty straightforward. You add positive feedback the signal gets extended. For negative feedback the delay with which the delay arrives plays a major role, as the dampening gets stronger the more is being produced upstream. So, the reaction is stronger there more upstream there is.

- Short delay: in this case after a short pretty strong response it finds a stable damped state pretty quickly.
- Long delay: here the signal becomes strong, meanign we get a strong but delayed feedback reaction, once that feedback hits, it kills the signal too strongly, so the feedback gets turned back really strongly. That again allows the signal to become strong and we start over again.

Next up is **adapting the extracellular signal molecule**. This will lead to the desensitization or sensitization of the signal molecule. This happens mainly by messing around with the receptor protein, it's quantity and function. It is often done through phosphorylation or ubiquitylation of the receptor proteins. Some are also cases of feedback:

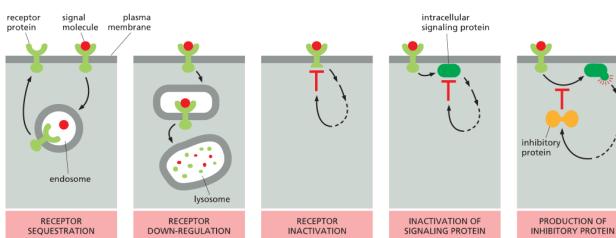


Figure 16: A bunch of ways the extracellular signal molecule's strength on the pathway can be adapted. This happens mainly by messing around with the receptor protein, it's quantity and function.

1.1.10 All or nothing, Hyperbolic, Sigmoidal Signals

There are three main shapes a signal response will take on:

- **hyperbolic**: a gradually increasing cell response to a gradually increasing signal, eventually reaching a plateau.
- **sigmoidal**: it takes a while for the signal to take effect, but then results in a steeper reaction at some intermediate concentration
- **All or nothing**: extreme form; nothing happens until a certain concentration threshold is reached and then we get a full signal.

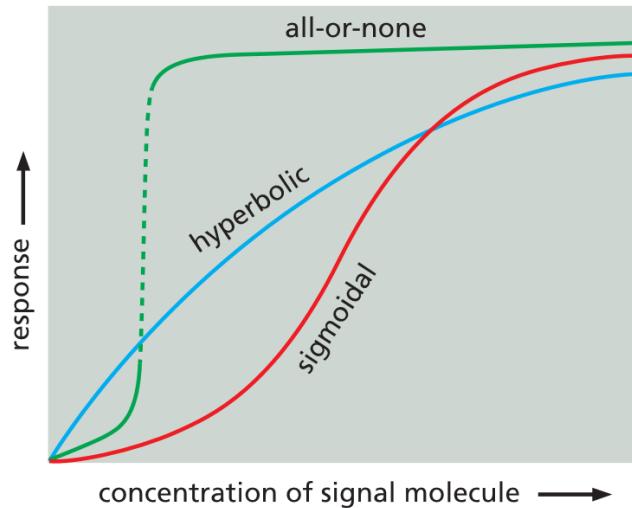


Figure 17: The three shapes a response tends to take in reaction to the signal. This is determined by how it is processed.

When we analyze cells it is important to remember that we are taking an average response of all cells. While a hyperbolic response average is probably hyperbolic in all cells, what appears to be sigmoidal could actually be a all or nothing response with some cells firing and others doing nothing. Hence, it is important to analyze the individual cells too. Here is a visualization:

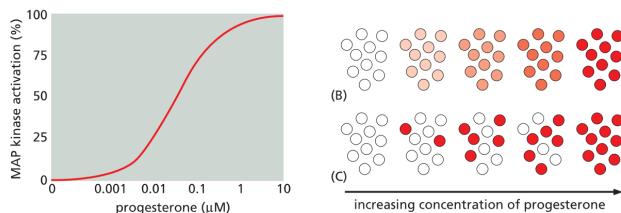


Figure 18: What appears to be sigmoidal may actually be all or nothing.

2 The World of G-proteins

2.1 The Components of a G-Protein Pathway

Guanine nucleotide-binding proteins or G-proteins are a major type of cell-surface receptor. There are many different types of G proteins.

2.1.1 G-Protein-Coupled Receptor or GPCR

the G-protein is the place the ligand attaches too. Then the GPCR will activate the G-protein. GPCR uses trimeric G-proteins.

Structure: A GPCR has seven transmembrane regions, composed of 7 alpha helices and 6 loops. It has a N-terminal extracellular region and a C-terminal intracellular region. The alpha helices form a pocket for the ligand to bind. Depending on the size of the ligand GPCR will have a differently sized extracellular domain to accomodate for the ligand, while remaining specific. There are over 700 different GPCR in humans.

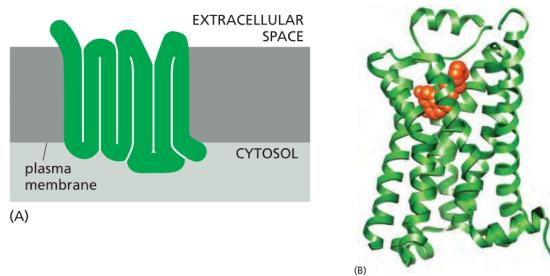


Figure 19: Simplified image of GPCR in membrane and its 3D structure.

2.1.2 Heterotrimeric G Protein

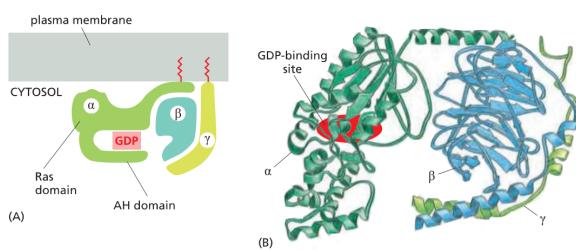


Figure 20: Simplified image of G-Protein in membrane and its 3D structure.

Some quick facts:

- 3 proteins that make up the complex that makes up the G-protein.
- GPCR uses trimeric G-proteins.
- at least 20 different alpha subunits exist.
- there are numerous different beta and gamma complexes, meaning we have quite a number of different G-proteins out there.

There are a bunch of different trimeric G-proteins, which are split into four families, which all have different functions:

TABLE 15–3 Four Major Families of Trimeric G Proteins*			
Family	Some family members	Subunits that mediate action	Some functions
I	G_s	α	Activates adenylyl cyclase; activates Ca^{2+} channels
	G_{olf}	α	Activates adenylyl cyclase in olfactory sensory neurons
II	G_i	α	Inhibits adenylyl cyclase
		$\beta\gamma$	Activates K^+ channels
	G_o	$\beta\gamma$	Activates K^+ channels; inactivates Ca^{2+} channels
		α and $\beta\gamma$	Activates phospholipase C- β
III	G_t (transducin)	α	Activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
	G_q	α	Activates phospholipase C- β
	$G_{12/13}$	α	Activates Rho family monomeric GTPases (via Rho-GEF) to regulate the actin cytoskeleton

*Families are determined by amino acid sequence relatedness of the α subunits. Only selected examples are included. About 20 α subunits and at least 6 β subunits and 11 γ subunits have been described in humans.

Figure 21: Shows the four major families of trimeric G-proteins.

Function: The Ras domain is part of the alpha subunit and is related to GTPases and provides a face for GDP/GTP to bind too. The alpha helix (AH) domain binds it in place. Activation from a GPCR triggers the release of GDP from the alpha subunit followed by the binding of GTP

2.1.3 Activation of G-Protein by GPCR

Here is how GPCR activates a G-protein:

- i) An extracellular signal molecule binds to the GPCR molecule;
- ii) The GPCR molecule changes conformation, which allows it to bind to the Ras domain of the G-protein;
- iii) This alters the conformation of the alpha subunit, specifically the alpha helix subunit, releasing the GDP.
- iv) The binding of GTP then promotes the closing of the subunit
- v) This triggers conformational changes causing the alpha subunit to dissociate from both the GPCR as well as the beta-gamma subunit.
- vi) Both the alpha and the beta-gamma subunit then become active in downstream pathways.
- vii) GPCR stays active as long as the ligand is bound to it, meaning it can activate many G-proteins.

How an activated G-protein starts the downstream cascade:

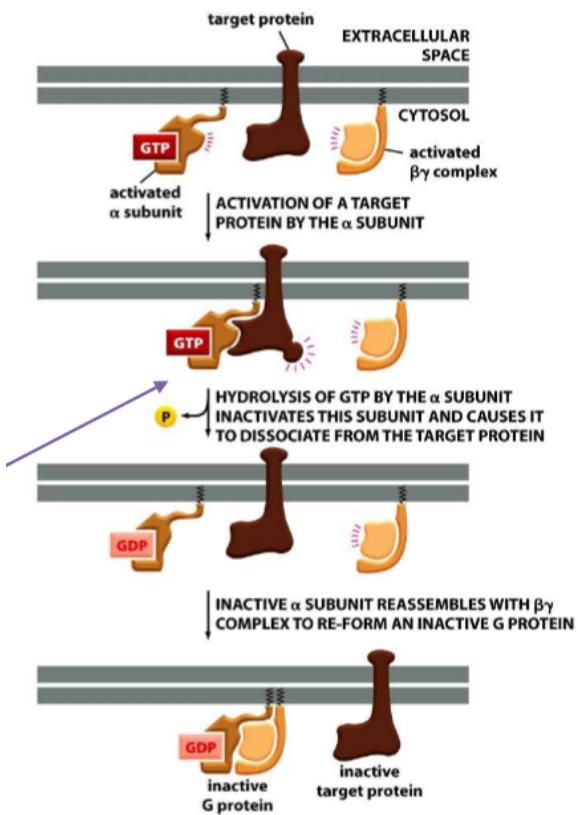


Figure 22: Once we have activated the G-protein and the subunits split, once they are used and the GTP converted to GDP the inactive subunits merge back together and the cycle can start again.

The main **downstream targets**:

- Adenylate (adenylyl) cyclase, which in turn increases or decreases cAMP (very common target);
- Channels;
- Phospholipase C, in turn generates IP₃ and diacylglycerol.

2.1.4 Stopping GPCR signaling

The signalling of GPCR can be stopped through GPCR kinases (GRKs) and arrestins, as they cause desensitization of the GPCR. The process:

- i) **Negative feedback:** Activated GPCR stimulates GRKs which phosphorylate the GPCR on multiple sites.
- ii) This then allows the arrestin to bind to the GPCR desensitizing it.

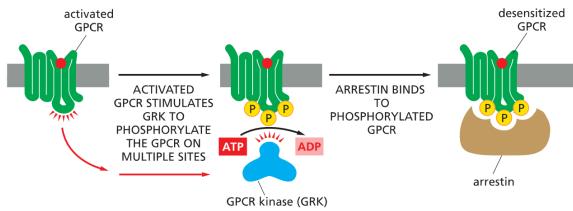


Figure 23: How GRK is a negative regulator, through negative feedback, for GPCR.

2.2 GPCR signaling through Cyclic AMP a.k.a. cAMP

2.2.1 cAMP

Cyclic AMP a.k.a. cAMP is a derivative of ATP. Two phosphates are replaced by a sugar bond (with enzyme adenylyl cyclase). cAMP is a shortlived molecule which is "uncycled" to 5'-AMP (with enzyme cAMP phosphodiesterase). The fact the molecule is so shortlived makes it great as a signaling molecule.

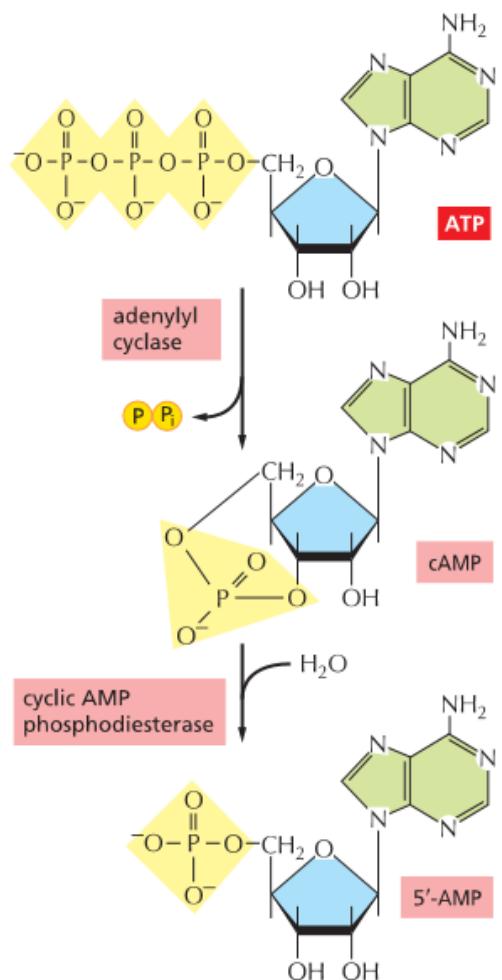


Figure 24: The production of cAMP with the enzymes adenylyl cyclase and cAMP phosphodiesterase.

2.2.2 cAMP as a signaling molecule

The pathway is as follows:

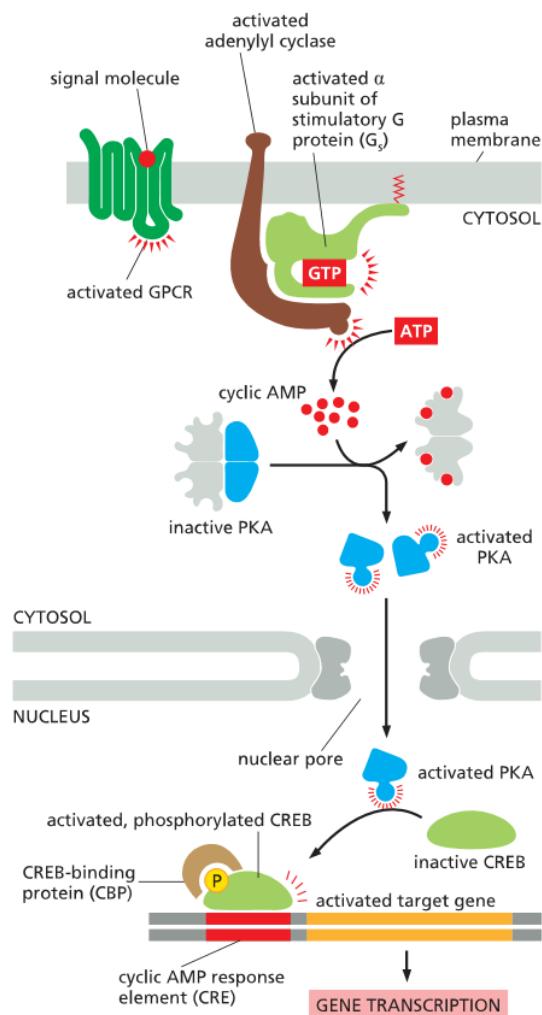


Figure 25: The production of cAMP with the enzymes adenylyl cyclase and cAMP phosphodiesterase.

- Activation of GPCR:** GPCR gets activated, which in turn activates the G-protein.
- cAMP produced:** The activated adenylyl cyclase converts ATP into cAMP.
- Activation of PKA:** The main role of cAMP is the activation cAMP-dependent protein kinases (PKAs). By binding to the regulatory subunits of the PKA tetramer induces a conformational change, which makes the regulatory subunits to dissociate from the catalytic subunits activating them. This release requires multiple cAMPs per regulatory unit. This means a lot of cAMP is required, as cAMP quickly decays, so we get a pretty sharp response.

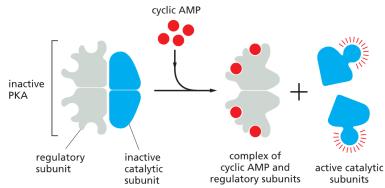


Figure 26: Shows the activation of PKAs by cAMP.

- iv) The active PKA is then translocated to the nucleus, where it activates a transcription factor CREB (cAMP response binding protein) through phosphorylation.
- v) CREB interacts with CREB-binding protein and activates transcription on the cAMP response element.

In a cell it will respond to activation of GPCR by a ligand, say serotonin, increasing the concentration 20fold in a matter of seconds. Depending on the cell and the ligand we will get very different cell responses. Here are some examples with hormones:

TABLE 15-1 Some Hormone-induced Cell Responses Mediated by Cyclic AMP		
Target tissue	Hormone	Major response
Thyroid gland	Thyroid-stimulating hormone (TSH)	Thyroid hormone synthesis and secretion
Adrenal cortex	Adrenocorticotrophic hormone (ACTH)	Cortisol secretion
Ovary	Luteinizing hormone (LH)	Progesterone secretion
Muscle	Adrenaline	Glycogen breakdown
Bone	Parathormone	Bone resorption
Heart	Adrenaline	Increase in heart rate and force of contraction
Liver	Glucagon	Glycogen breakdown
Kidney	Vasopressin	Water resorption
Fat	Adrenaline, ACTH, glucagon, TSH	Triglyceride breakdown

Figure 27: The expression using different hormones in different cells. Vasopressin is also a "love" hormone, meanign that when you are in love GPCR is active

2.2.3 cGMP

Cyclic-Guanine-Mono-Phosphate or cGMP is an alternative in the cAMP pathway. So, sometimes cGMP is activated by GPCR not cAMP. The only difference is the guanine instead of adenosine. The enzyme is called guanylate cyclase.

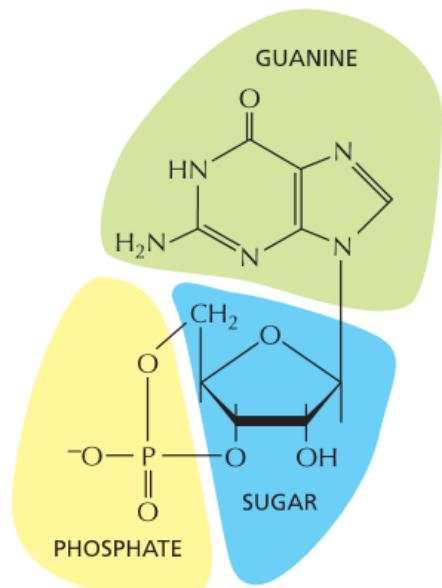


Figure 15–37 Cyclic GMP.

Figure 28: The structure of cGMP, where the only difference to cAMP is the guanine for adenine.

2.2.4 Case study with response to light

The process of recognition is as follows:

- i) a **rhodopsin** molecule absorbs a photon;
- ii) 500 G-proteins molecules (transducin) are activated (signal is amplified);
- iii) 500 cGMP phosphodiesterase molecules are activated;
- iv) 10^5 cGMPs are hydrolyzed (signal has been amplified);
- v) They block 250 cation channels;
- vi) $10^6 - 10^7 Na^+$ -ions per second are prevented from entering the cell for a period of around a second (signal has been amplified);
- vii) The membrane potential is altered by 1mV, which in turn relays a signal to the brain.

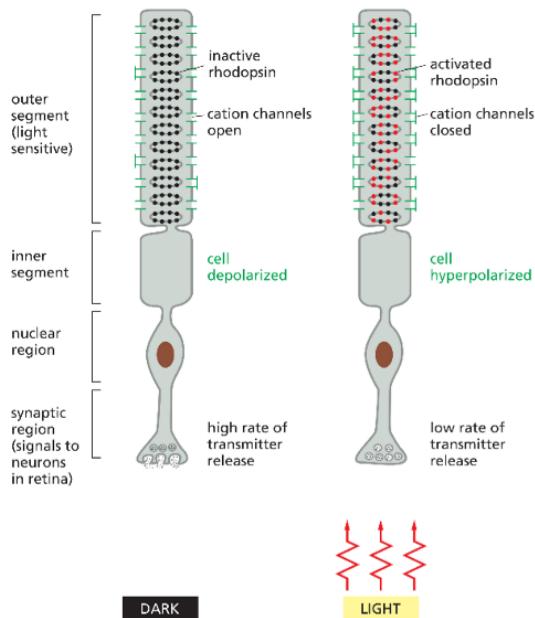


Figure 29: How the cell changes when light is received. Note that the signal sent to the brain is inverted of how a normal neuron is fired (channels close instead of open).

2.3 GPCR Signaling through phosphlipase C

Here are some example cell responses where GPCRs activate PLC β

TABLE 15-2 Some Cell Responses in Which GPCRs Activate PLC β		
Target tissue	Signal molecule	Major response
Liver	Vasopressin	Glycogen breakdown
Pancreas	Acetylcholine	Amylase secretion
Smooth muscle	Acetylcholine	Muscle contraction
Blood platelets	Thrombin	Platelet aggregation

Figure 30: Some cell responses where GPCR activates PLC β , shoutout to Vasopressin for all the lovin'.

2.3.1 Case Study: GPCRs activating Cytosolic Ca^{2+} and activating protein Kinase C

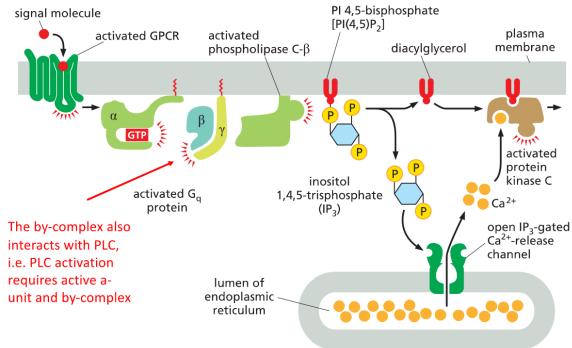


Figure 31: Some cell responses where GPCR activates PLC β , shoutout to Vasopressin for all the lovin'.

Rundown of the pathway:

- i) The GPCR activates the PLC β via a G-protein called G_q. The G_q Beta-gamma complex and the alpha-complex activate with the PLC β .
- ii) PLC β hydrolyzes PI(4,5)P₂, causing it to split into two messengers
- iii) IP₃ diffuses through the cytosol and releases Ca^{2+} from the ER by binding to the IP₃-gated Ca^{2+} channels.
- iv) Then the diacylglycerol (other part of PI(4,5)P₂), remaining in the membrane, together with the Ca^{2+} and phosphatidylserine activate the protein Kinase C (PKC). Of the min. 10 forms of PKC at least 4 are activated by diacylglycerol.

2.3.2 Ca^{2+} feedback waves and oscillations

The concentration of Ca^{2+} plays a big role in activating or inactivating. So, giving itself positive or negative feedback. Here's how:

- **Activation:** At low concentrations Ca^{2+} goes to neighboring channels and activates them, causing the release of more Ca^{2+} and a wave like reaction across receptors (first couple pics). This means that the channels can stay active even without any IP₃ being present.
- **Inactivation:** When Ca^{2+} is present at very high concentrations it inactivates the channels. That means that now we create a wave of inactivation.
- **Oscillation:** In the continued presence of the ligand activator, or even without, this mix of feedback can cause oscillations in Ca^{2+} excretion.

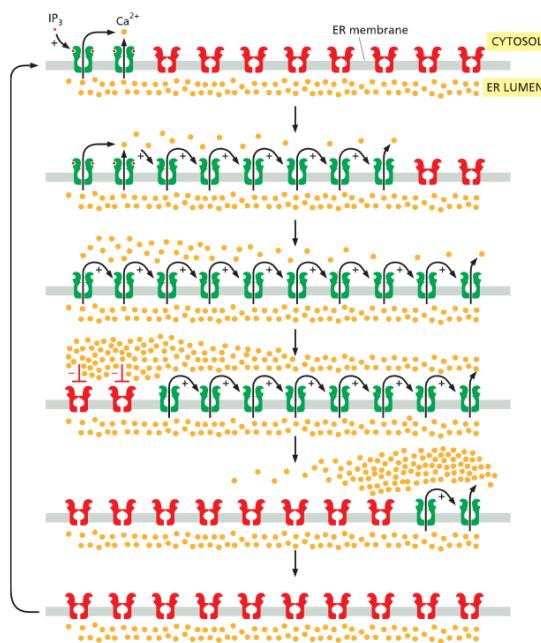


Figure 32: Shows how Ca^{2+} influences the activity of its own channels

2.3.3 How Ca^{2+} plays an important role in regulating and relaying signals

Ca²⁺ and calmodulin: With the help of Ca^{2+} /calmodulin, Ca^{2+} is able to bind to target proteins and with that relay the signal. The dumbbell shape of calmodulin and alpha-helix allows it to take on numerous different conformations.

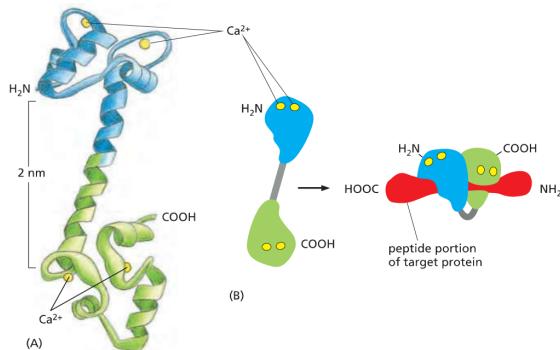


Figure 33: On the left the structure of calmodulin and on the right an example of how it can bind to target proteins (this move is called the jackknife).

CaM-Kinase II is regulated by calmodulin. Here's how that runs down:

- 6 CaM-KII (green) form a ring.
- The kinase domains pop in and out naturally.
- Calmodulin can bind the popped-out domain in place when it is bound to Ca^{2+}

- iv) Then that kinase domain gets phosphorylated, making it active.
- v) In the continued presence of calmodulin it is even more active.
- vi) Becomes inactive through dephosphorylation
- vii) The more domains are active, the more active the enzyme as a whole.

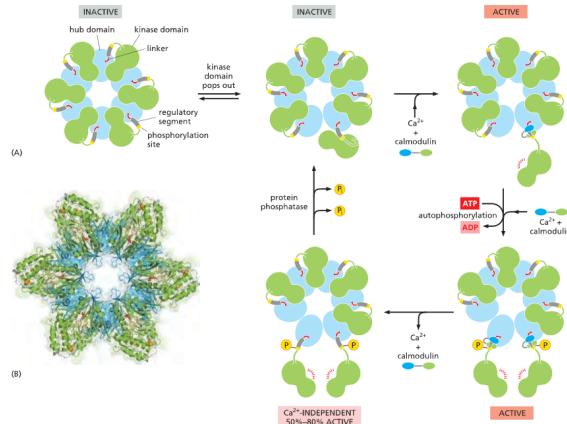


Figure 34: Shows how Ca^{2+} /calmodulin activate enzymes, in this case CaM-KII.

Depending on the frequency of the Ca^{2+} oscillations, the activity of the enzyme is influenced in major ways. The more frequent the oscillations the more the activity as a whole will rise. Hence CaM-KII is a good mechanism of decondign the frequencies of oscillations in a cell. Here's a figure to visualize:

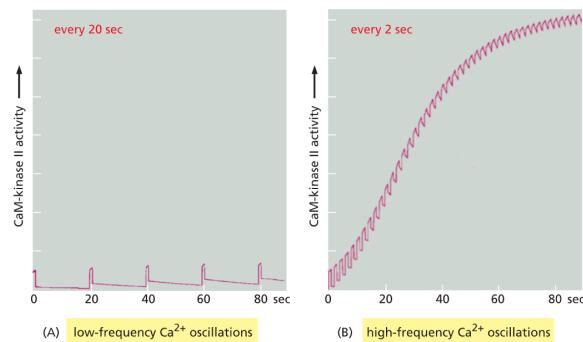


Figure 35: How different frequencies of oscillations cause major differences in enzyme activity.

3 Other Types of Signaling

3.1 Receptor Tyrosine Kinase a.k.a. RTK signaling

Receptor Tyrosine Kinases are a large group, here are some of them:

TABLE 15-4 Some Signal Proteins That Act Via RTKs		
Signal protein family	Receptor family	Some representative responses
Epidermal growth factor (EGF)	EGF receptors	Stimulates cell survival, growth, proliferation, or differentiation of various cell types; acts as inductive signal in development
Insulin	Insulin receptor	Stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factor (IGF1)	IGF receptor-1	Stimulates cell growth and survival in many cell types
Nerve growth factor (NGF)	Trk receptors	Stimulates survival and growth of some neurons
Platelet-derived growth factor (PDGF)	PDGF receptors	Stimulates survival, growth, proliferation, and migration of various cell types
Macrophage-colony-stimulating factor (M-CSF)	M-CSF receptor	Stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factor (FGF)	FGF receptors	Stimulates proliferation of various cell types; inhibits differentiation of some precursor cells; acts as inductive signal in development
Vascular endothelial growth factor (VEGF)	VEGF receptors	Stimulates angiogenesis
Ephrin	Eph receptors	Stimulates angiogenesis; guides cell and axon migration

Figure 36: A bunch of different RTK groups

RTKs are connected by the fact that they all have an intracellular kinase domain which can phosphorylate a Tyrosine. The extracellular domain on the other hand is completely variable. the kinase region can also have a kinase insert region emerging from it, which is relevant for interactions with other proteins.

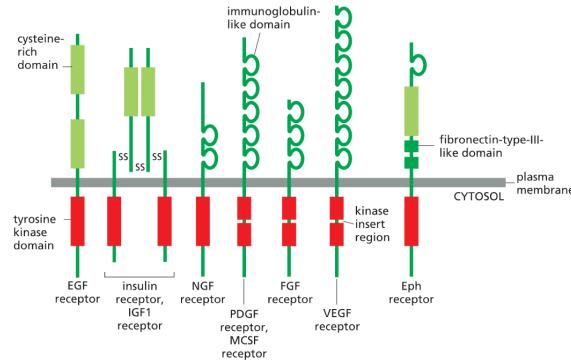


Figure 37: A bunch of different RTK types, with the core features.

3.1.1 Activation of RTKs by Dimerization

- i) Two RTKs are initially inactive until some type of ligand arrives to bring them together.
- ii) In this proximity the RTKs dimerize and make an initial Tyrosine auto-phosphorylation.
- iii) Once the first phosphorylation has happened that initiates trans-phosphorylation of several Tyrosines.
- iv) Phospho-Tyrosin sites recruit and/or activate downstream signaling proteins.

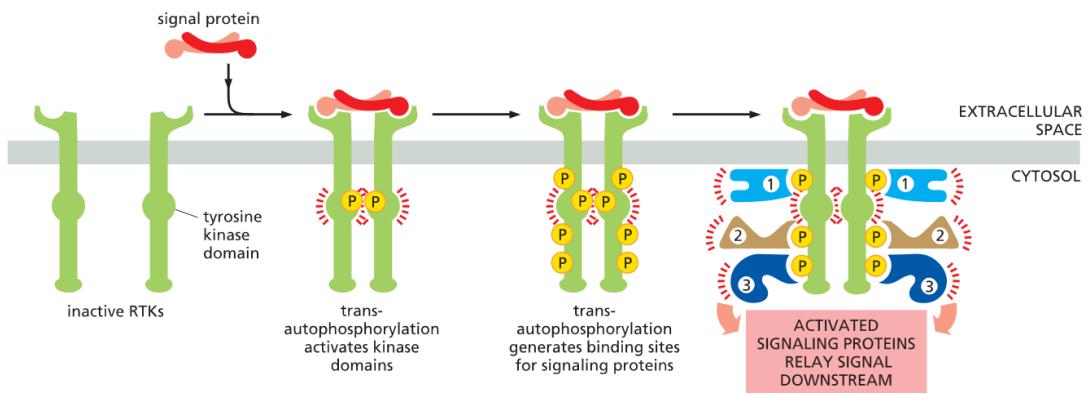


Figure 38: Activation of a RTK dimer

3.1.2 An exception to the rule: Activation of EGF Kinase

Compared to the regular activation the kinase domains are not both auto-phosphorylated to be activated. We still have two identical domains, but one takes on the role of activator, while the other is the receiver.

- i) Both domains are activated through EGF.
- ii) Then the activator pushes on the receiver, causing a conformational change in the receiver domain, activating it.
- iii) The activated Kinase domain then phosphorylates the tyrosines on both receptors.

3.2 Binding to the Receptor

The phospho-tyrosines are docking sites for proteins containing:

- i) SH2 (Src Homology, the sarcoma), cancer bro
- ii) PTB (PhosphoTyrosine Binding), cancer bro
- iii) PLC

Because of the multitude of phospho-tyrosines many different proteins, and consequently different pathways, can interact with the receptors.

3.2.1 Ras signaling

Ras is essentially a monomeric GTPase. Ras is anchored to the membrane through a lipid modification. For a refresher on how GTP can be regulated please refer to Section 1.1.6.

Here are some groups:

TABLE 15-5 The Ras Superfamily of Monomeric GTPases		
Family	Some family members	Some functions
Ras	H-Ras, K-Ras, N-Ras	Relay signals from RTKs
	Rheb	Activates mTOR to stimulate cell growth
	Rap1	Activated by a cyclic-AMP-dependent GEF; influences cell adhesion by activating integrins
Rho*	Rho, Rac, Cdc42	Relay signals from surface receptors to the cytoskeleton and elsewhere
ARF*	ARF1–ARF6	Regulate assembly of protein coats on intracellular vesicles
Rab*	Rab1–60	Regulate intracellular vesicle traffic
Ran*	Ran	Regulates mitotic spindle assembly and nuclear transport of RNAs and proteins

*The Rho family is discussed in Chapter 16, the ARF and Rab proteins in Chapter 13, and Ran in Chapters 12 and 17. The three-dimensional structure of Ras is shown in Figure 3-67.

Figure 39: Ras groups in our body.

Activation of Ras by an RTk:

- Adaptor protein Grb2 docks to RTk with SH2
- Ras-GEF then interacts with Grb2
- Ras-GEF then exchanges the GDP for a GTP
- Ras is activated.

Detecting Ras activity: We use FRET (Fluorescence resonance energy transfer), by attaching a yellow fluorescent protein (YFP) to the gene of Ras. Then we add a red fluorescent dye to GTP. That way when no GTP is there (Ras inactive), it emits yellow light, but when GTP is attached to the Ras (active) red light is emitted.

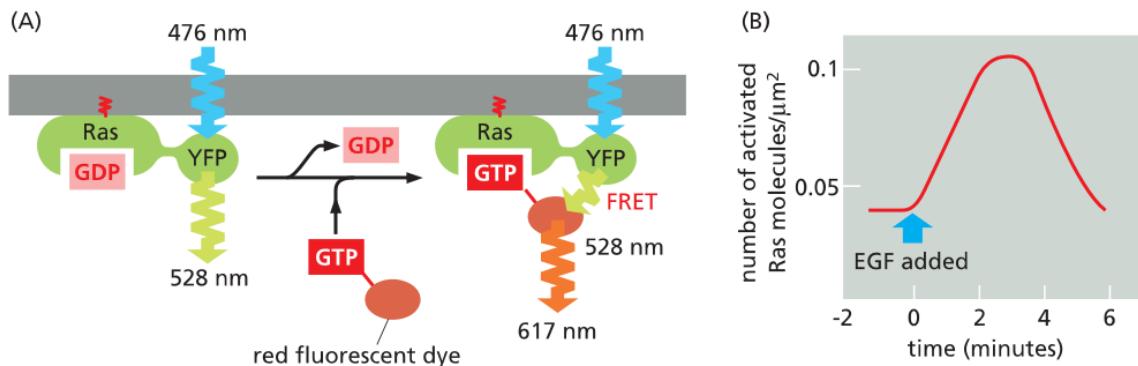


Figure 40: How FRET can be used to detect the activity of Ras.

Case study: MAP kinase module is a module activated by Ras. This is done the following way: Ras activates Raf to the membrane, which in turn activates Mek, which in turn activates Erk, which phosphorylates a bunch of downstream proteins, such as further kinases, and transcription regulators. The resulting activations cause complex changes in the cell.

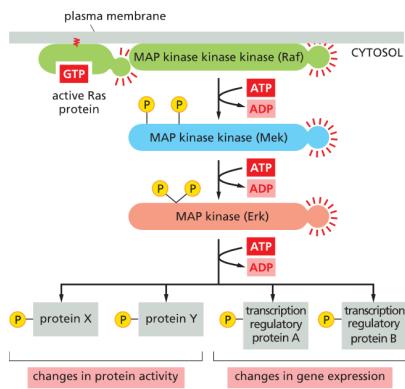


Figure 41: How the MAP kinase module is activated by Ras.

How cancer changes Ras: By changing certain amino acids (G12, G13, Q61), the mutants show impaired GTPase activity, leading to a gain-of-function. So, the GAP proteins no longer work as well. Of the three types of famous Ras (K,H,N-Ras) it seems mostly the mutant K-Ras is found in cancers. Mutant Ras probably important in the initiation of tumors.

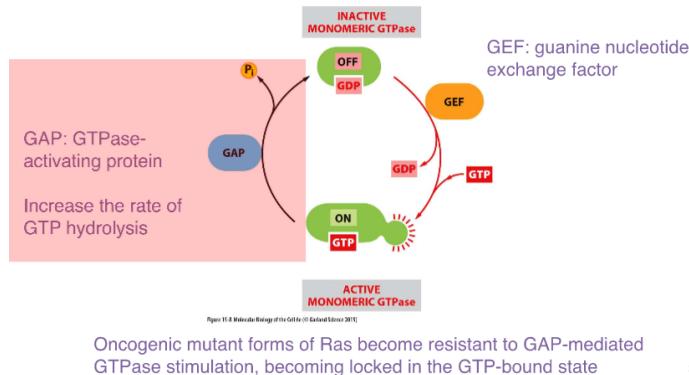


Figure 42: Showing how mutating the GAP messes with the regulation of Ras.

3.2.2 PI3K signaling

In order to kick off the splitting PLC to diacylglycerol and IP₃, it first needs to be phosphorylated at the 3-carbon of the PI, by a PI 3-Kinase (hmmmm I wonder where that name comes from). It can also create docking sites for downstream proteins.

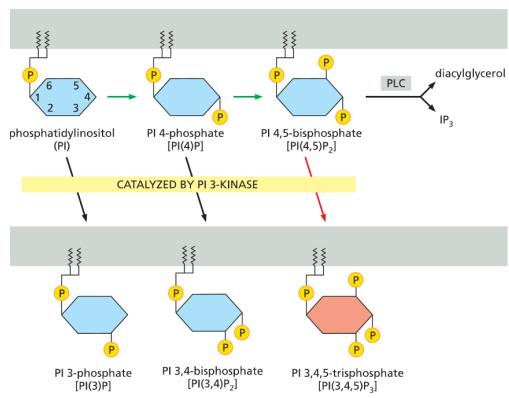


Figure 43: Phosphorylation of the 3-carbon activates the PLC.

PI 3-Kinase activates AKT:

- i) PI3K is recruited by RTK
- ii) PI3K creates docking sites where proteins with a PH domain can dock.
- iii) PDK1 activates AKT by phosphorylation
- iv) p-AKT activates many cellular programs including cell growth and anti-apoptosis (hinting that cancer may be interested here).

Negative regulation: Phosphatase PTEN removes phosphate from PI(3,4,5)P₃, making it a negative regulator of the PI3K.

Case study: how PI 3-kinase promotes cell survival:

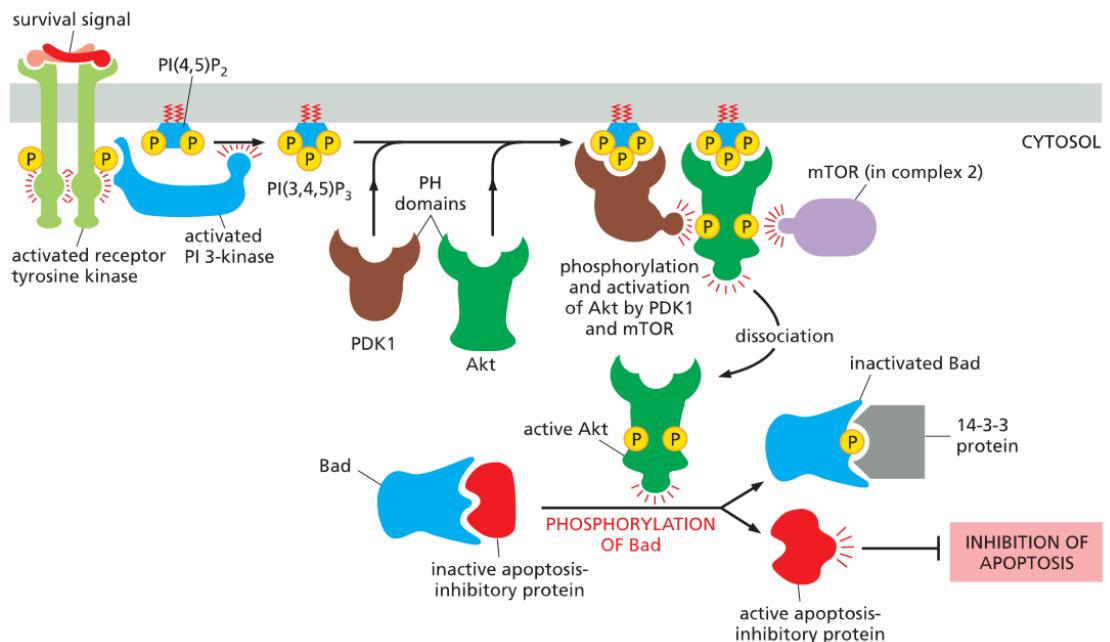
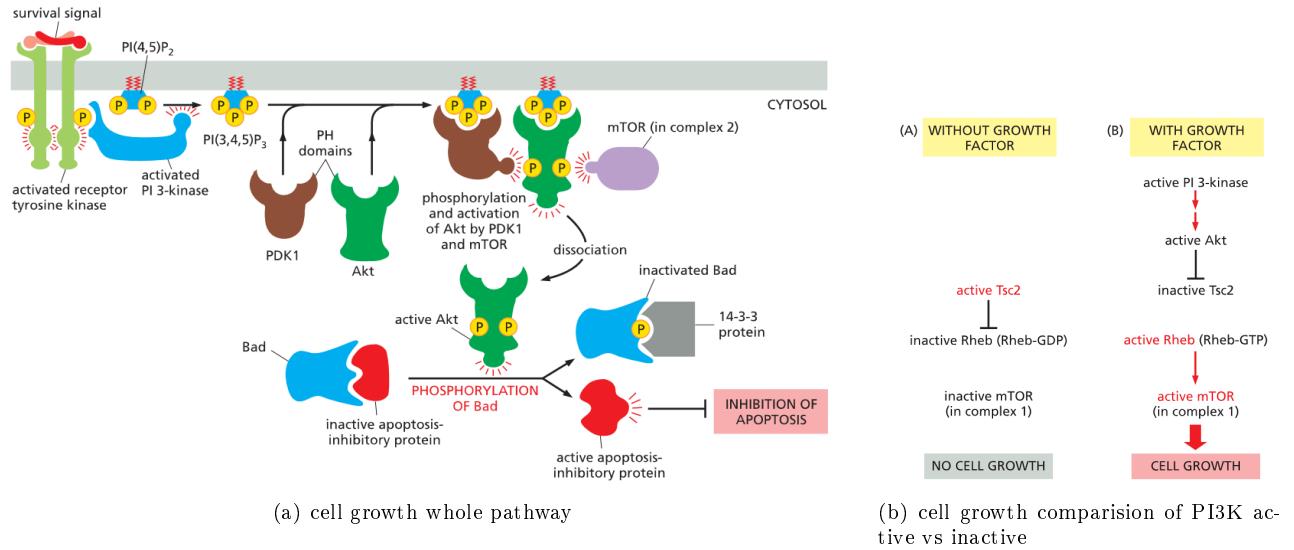


Figure 44: How PI3K inhibits cell death.

The figures below shows on the left the entire pathway which promotes cell growth. The one on the left shows how the chain on the left continues leading to cell growth. The MAP kinase can also go down the pathway on the right, meaning bot can promote cell growth.



3.2.3 Checking out how GPCRs and RTKs are intertwined

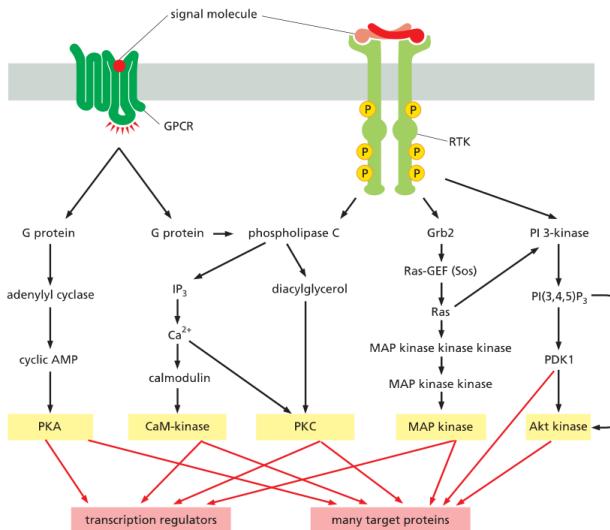


Figure 45: Compares the pathways caused by GPCRs and RTKs, also shows which are shared. All of them end with a Kinase, which then causes a reaction chain downstream

Both GPCR and RTK have a PLC pathway, called beta and gamma respectively. The effect is very similar.

3.3 EGF Receptors in cancer

In cancer EGF-R can become over-activated.

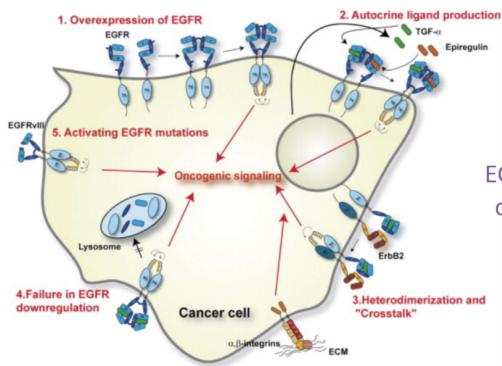


Figure 46: Different ways EGFR can become overexpressed in cancer

Mutations in cancer occur at the kinase domain of the receptor, leading to the domain being permanently active. Thus Ras-MAP Kinase and PI3K are always active, leading to uncontrolled cell growth a.k.a. cancer. These mutations will be deletions which keep the reading frame or missense mutations:

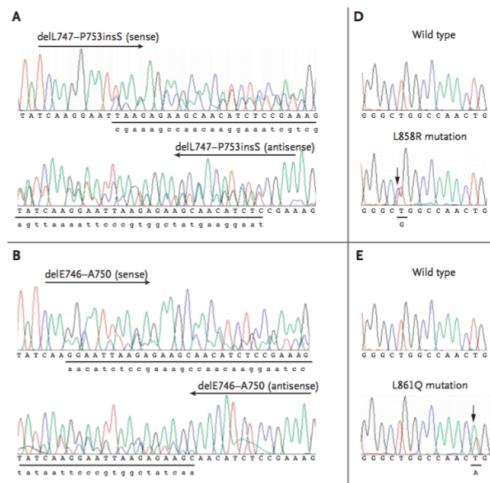


Figure 47: Showing the options of both missense and frame-keeping deletions, found through Sanger Sequencing.

Inhibitors can target always active EGFR. Through this some pathways are now slowed down, leading to more normalized cell growth.

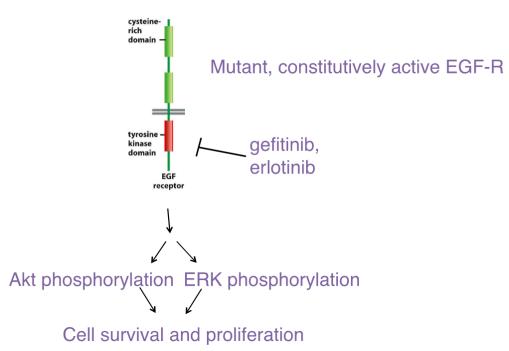


Figure 48: How an inhibitor restore normal cell growth.