

1 Cell Signaling: Principles

1.0.1 The Basic Vocabulary of Cell Signaling

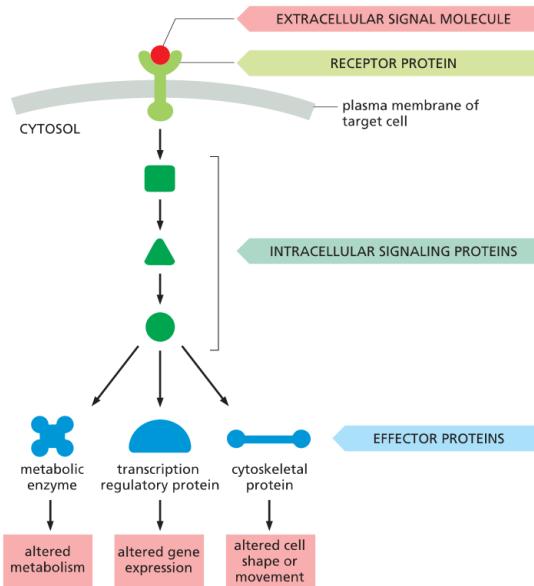


Figure 1: Basic Vocabulary of Cell Signaling

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

- i) An **Extracellular Signal 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, usually branching out.
- iv) The final protein in that cascade will then change the activity of the Effector proteins, which launches the cellular response.
- v) These **effector proteins** can be metabolic enzymes, transcription regulators, or cytoskeletal proteins.

1.0.2 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 Receptor: generally transmembrane protein, where the signaling molecule binds extracellularly.
- Intracellular Receptor: 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).

1.0.3 The Four Subtypes of Cell Signaling and Two Variations

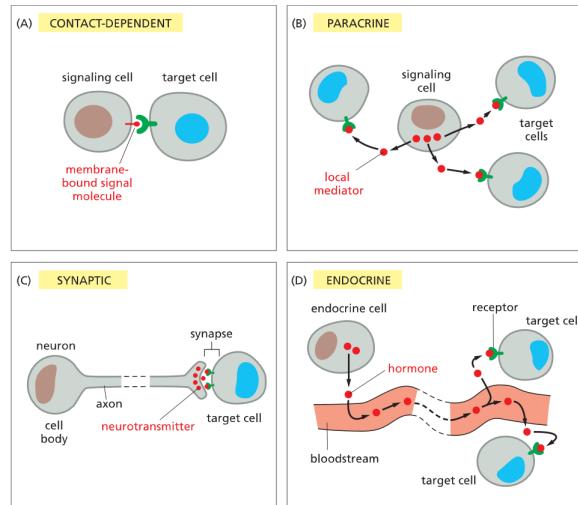


Figure 2: The Four types of Signaling

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: the cell can also have contact dependent interactions with the 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.

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.

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.

Definition 1.1 (Autocrine Signaling). *If a cell receives its own signal it is called autocrine signaling.*

Definition 1.2 (Constitutively Active). *A protein (usually a receptor or enzyme) that is always active, regardless of whether it has received a signal or a ligand is bound. This can lead to uncontrolled signaling and is often seen in cancer. The reason for this is generally a mutation to the gene of the protein.*

1.0.4 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:

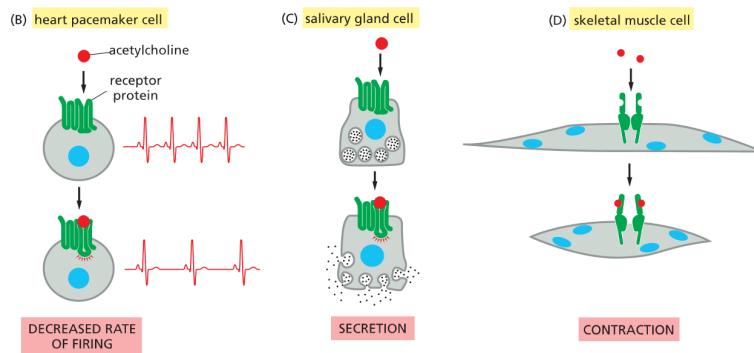


Figure 3: Examples of Acetylcholine having vastly different responses to its signal.

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.

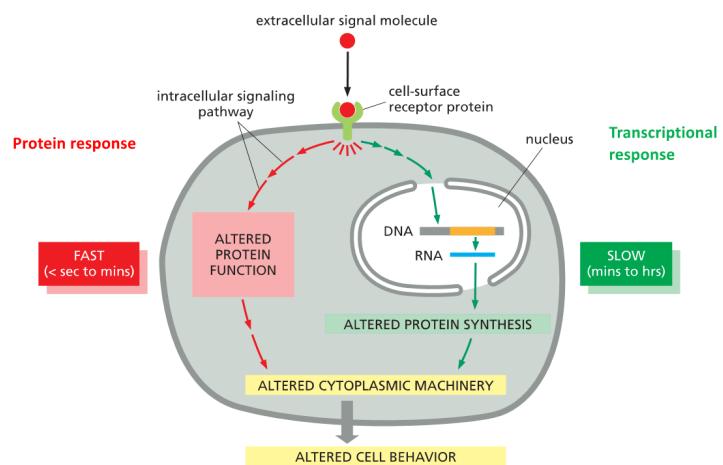


Figure 4: An overview of protein (fast) vs. transcriptional (slow) response.

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.

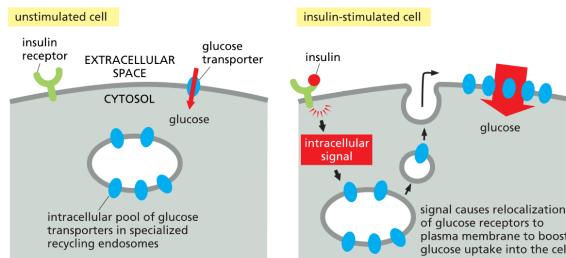


Figure 5: An example of a fast protein response with the GLUT transporter.

1.0.5 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;

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.

1.0.6 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).

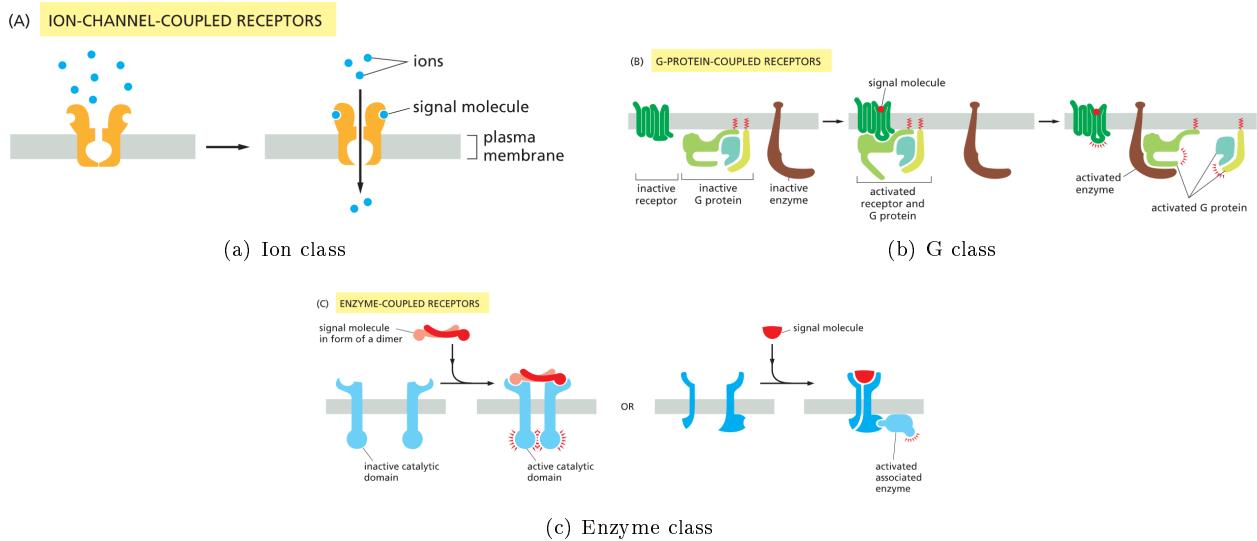


Figure 6: The three classes of cell-surface receptors

- 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 the attack on the phosphate.

ii) GTP binding

- **GTPases** are molecular switches that cycle between active (GTP-bound) and inactive (GDP-bound) states. 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.
- Since the cell has a ratio of 10:1 for GTP:GDP, the exchange of GDP to GTP is very favorable.

1.0.7 Inhibitory Signals as Activators

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

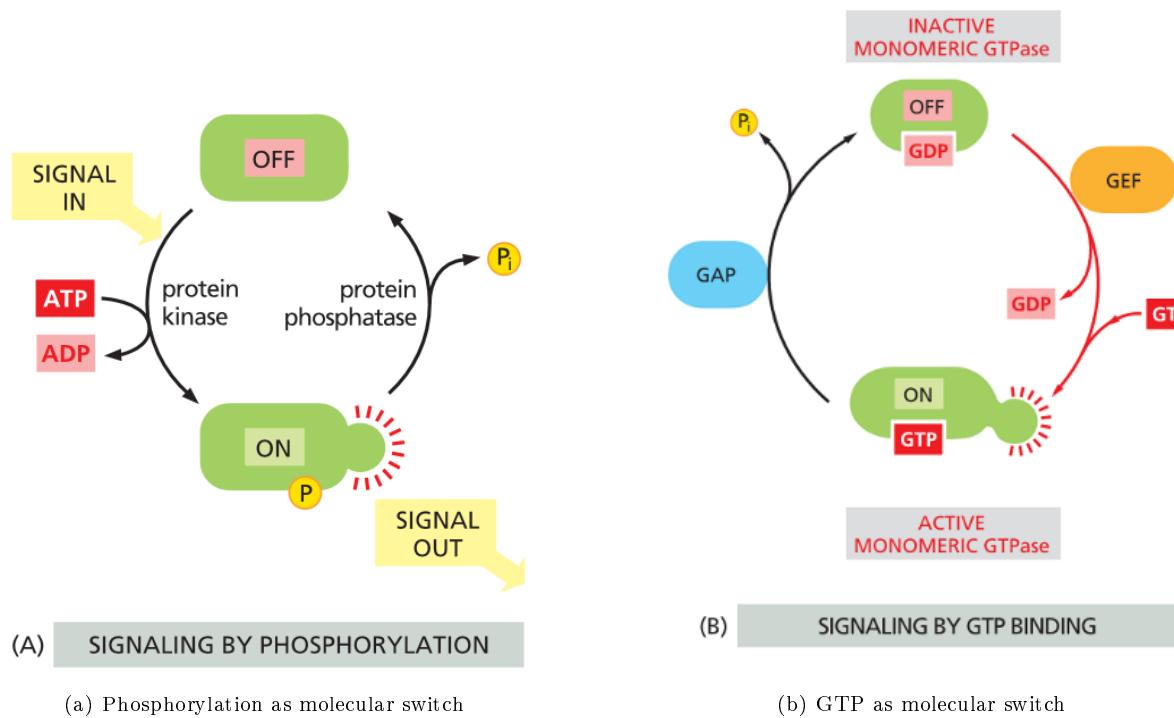


Figure 7: Two types of molecular switches in intracellular signaling

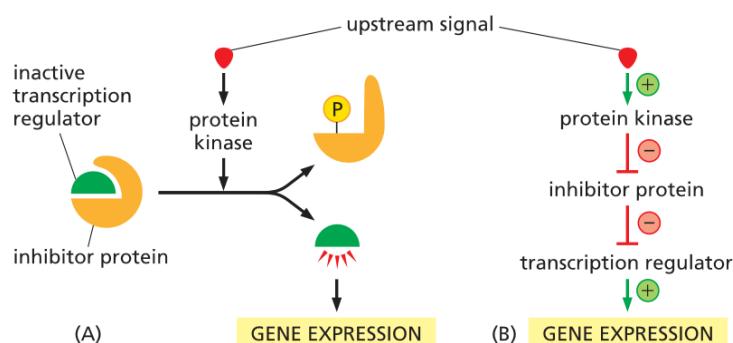


Figure 8: Example pathway of an inhibitory signal leads to activation. Note that the left and right path show the same pathway.

1.0.8 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 start, additionally ATP can also be required.

There are three main types of starts to signaling (see figure 9):

i) Preassembled signaling complex

- The signal complex is already assembled with all its intracellular signaling proteins, generally in the form of a **Scaffolding protein**.

ii) Protein Recruitment

- the signaling proteins are in close proximity. Once the signal molecule attaches they attach to the receptor.
- For the signal to be activated, the signaling proteins don't always need to be physically 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). PIs are part of the membrane, in close proximity to the receptor
- These phospholipids can be phosphorylated in the cell, which allows the signaling proteins to attach.

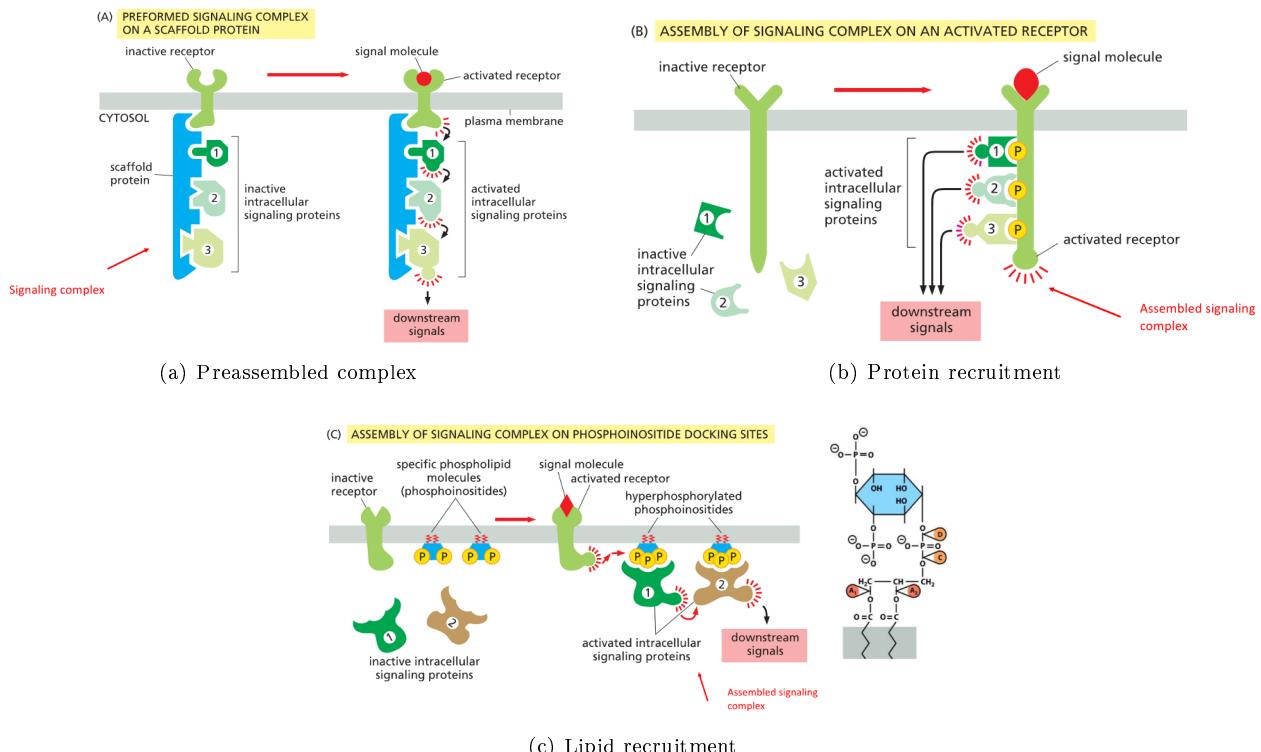


Figure 9: The three classes of cell-surface receptors

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

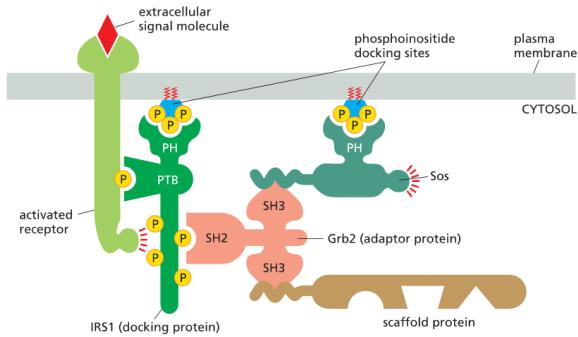


Figure 10: insulin signaling complex as an example for the complexity and modularity of a signaling complex.
startins

The shortcuts of the molecules in the figure ??:

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

1.0.9 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**.

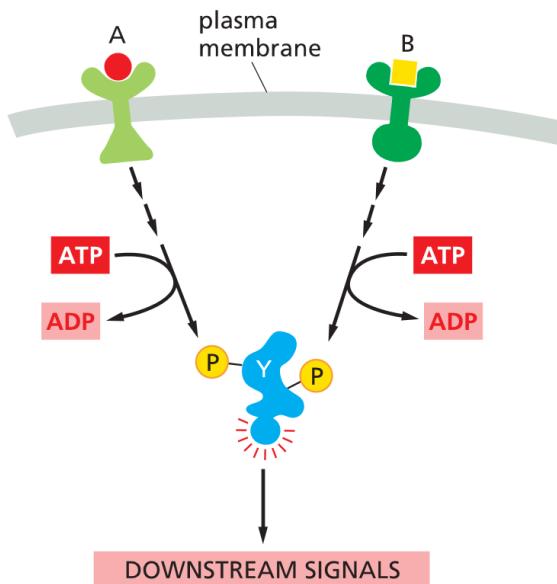


Figure 11: An example pathway showing how multiple streams need to come together to allow downstream signaling.

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.

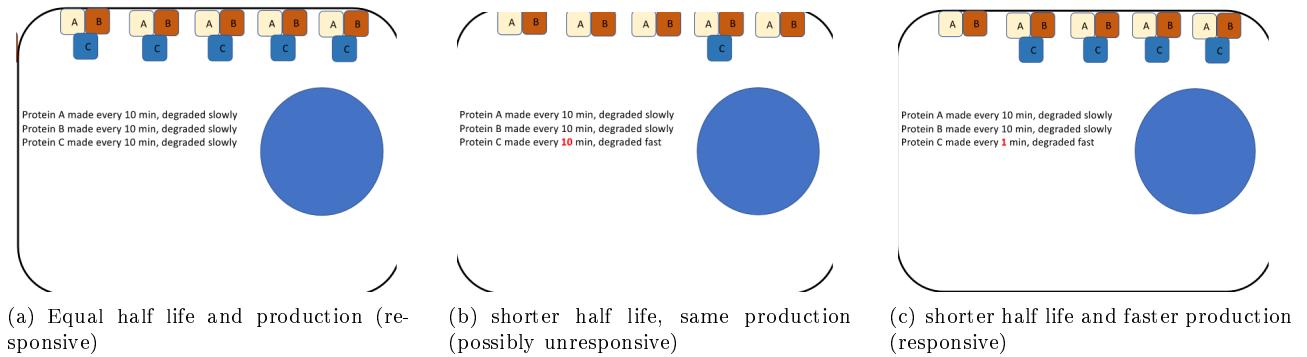


Figure 12: An example of how influencing the half life and production of certain proteins can seriously influence the responsiveness of a protein complex.

)halflife

To further show the role and importance of degradation in protein complexes here are some graphs:

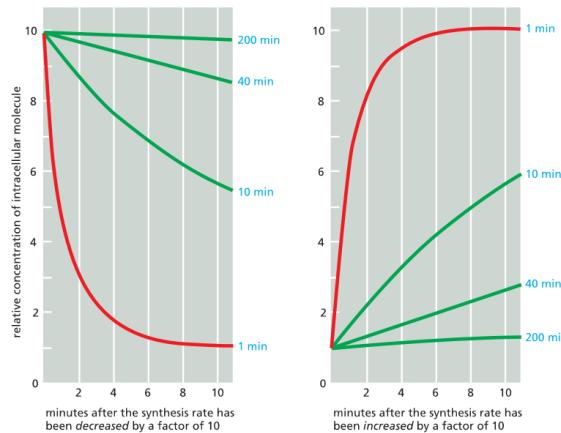
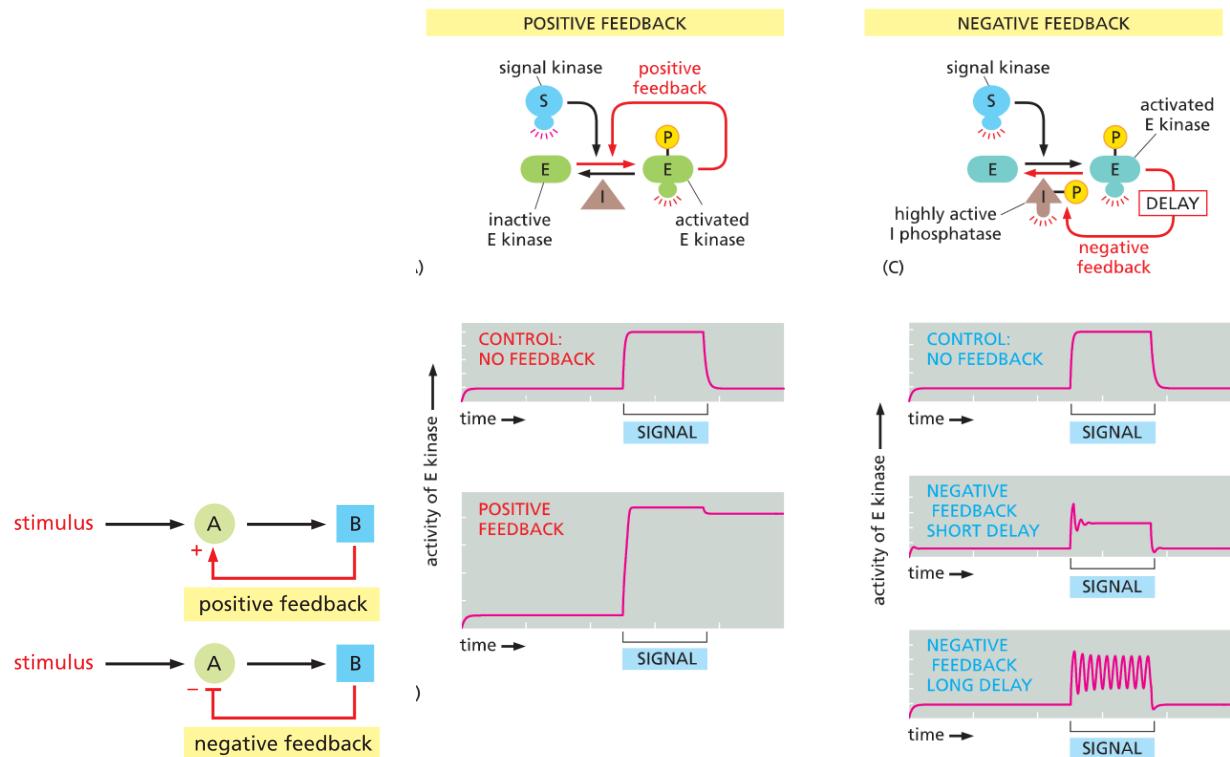


Figure 13: Shows the connection between Half-Life (blue time) and the synthesis rate. The x-axis is the time after the synthesis rate is increased by a factor 10, the y-axis the concentration of the intracellular molecule. A protein with a short half-life will probably also have a high production rate (red line) as otherwise it will be unresponsive (see fig: ??). 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 **Positive Feedback and Negative Feedback**. This is when a downstream molecule will signal upwards in the pathway to either increase or decrease its activity.



(a) Shows both positive and negative feedback. (b) Shows both a positive and negative feedback loop and corresponding time vs. enzyme activity graph.

Discussion of the fig: 1.0.9: in feedback the positive one is pretty straightforward. You add positive feedback the signal gets extended. For negative feedback the type of delay which is in effect 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 feedback delay: in this case after a short pretty strong response it finds a stable damped state pretty quickly.
- Long feedback delay: here the signal becomes strong, meaning 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, its quantity and function. It is often done through phosphorylation or ubiquitylation of the receptor proteins. Some are also cases of feedback:

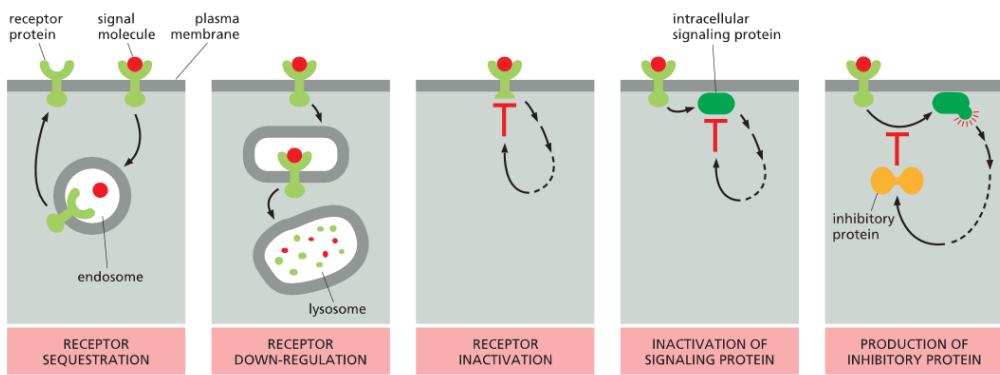


Figure 14: A bunch of ways the extracellular signal molecule's strength on the pathway can be adapted. This happens mainly by messing around with the receptor protein, it's quantity and function.

1.0.10 All or nothing, Hyperbolic, Sigmoidal Signals

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

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

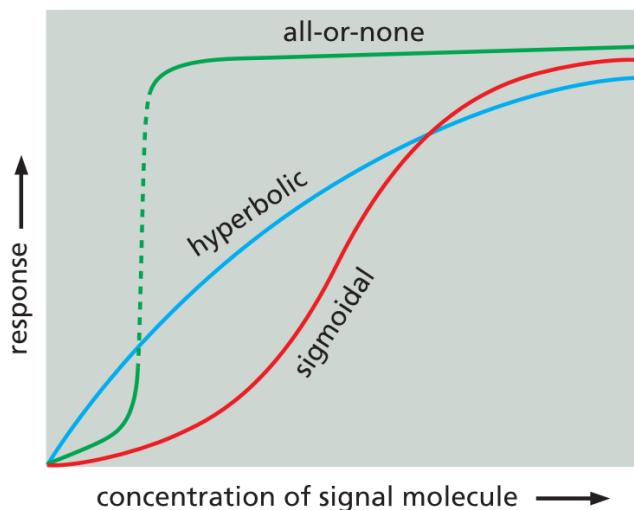


Figure 15: The three shapes a response tends to take in reaction to the signal. This is determined by how it is processed.

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:

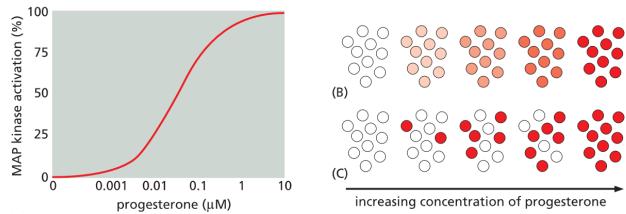


Figure 16: What appears to be sigmoidal may actually be all or nothing.

2 Cell Signaling 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-coupled-receptor (GPCR) 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.

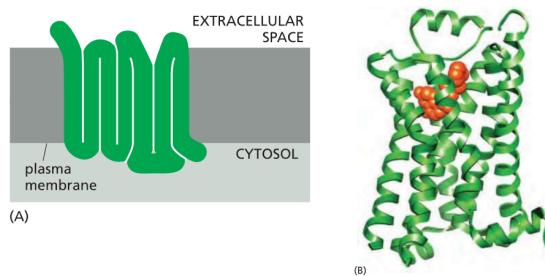


Figure 17: Simplified image of GPCR in membrane and its 3D structure.

2.1.2 Heterotrimeric G Protein

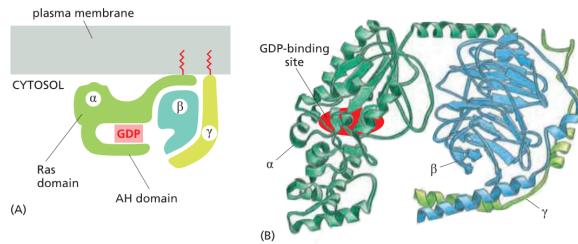


Figure 18: Simplified image of G-Protein in membrane and its 3D structure.

Some quick facts:

- 3 proteins that make up the G-protein
- The G-proteins Alpha Subunit is a GTPases.
- GPCR uses trimeric G-proteins.
- at least 20 different alpha subunits exist.
- there are numerous different Beta Complex and Gamma Complex, 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 major 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
	G_t (transducin)	α and $\beta\gamma$	Activates phospholipase C- β
III	G_q	α	Activates phospholipase C- β
IV	$G_{12/13}$	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 19: 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:

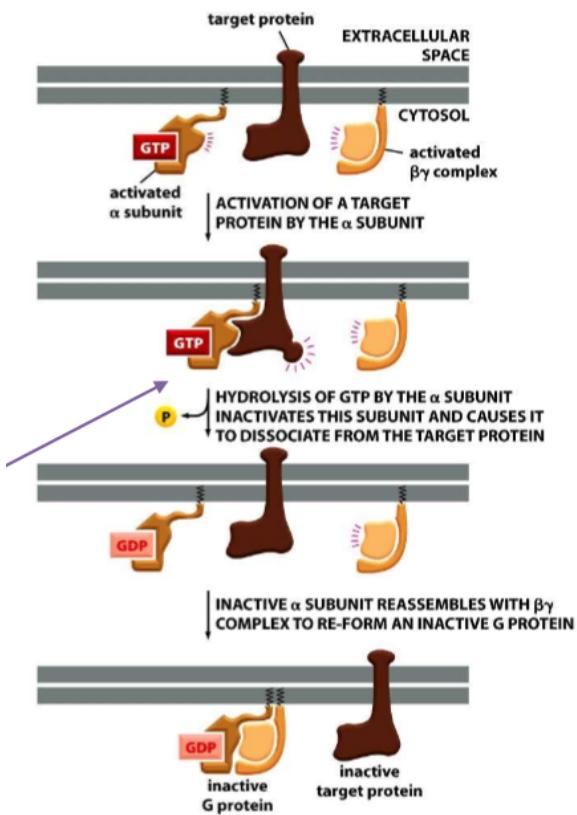


Figure 20: Once we have activated the G-protein and the subunits split, once they are used and the GTP is converted to GDP the inactive subunits merge back together and the cycle can start again.

The main **downstream targets**:

- **Adenylate Cyclase**, which in turn increases or decreases **cAMP** (very common target);

- Channels;
- PLC, which in turn generates IP₃ and diacylglycerol.

2.1.4 Stopping GPCR signaling

The signalling of GPCR can be stopped through **GPCR Kinases (GRKs)** (**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. Note therefore GRK can only phosphorylate activated receptors.
- ii) This allows the Arrestin to prevent the receptor from binding to its G-protein and directs the receptors endocytosis.

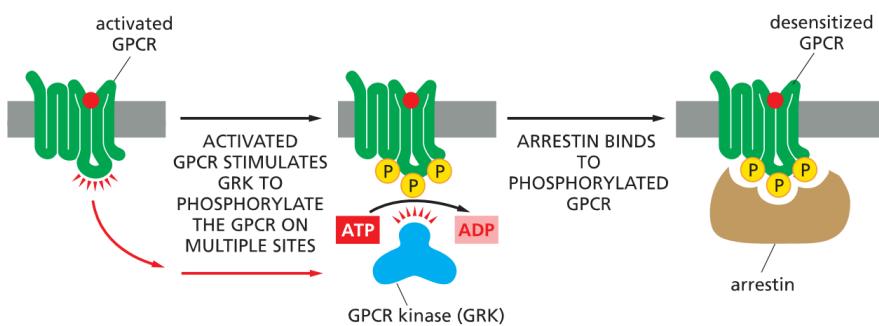


Figure 21: How GRK is a negative regulator, through negative feedback, for GPCR.

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

2.2.1 cAMP

Cyclic AMP a.k.a. cAMP is a derivate of ATP. Two phosphates are replaced by a sugar bond, by enzyme **Adenylate Cyclase**. cAMP is a shortlived molecule which is "uncycled" to 5'-AMP, by enzyme **cAMP Phosphodiesterase**. The fact the molecule is so shortlived makes it great as a signaling molecule.

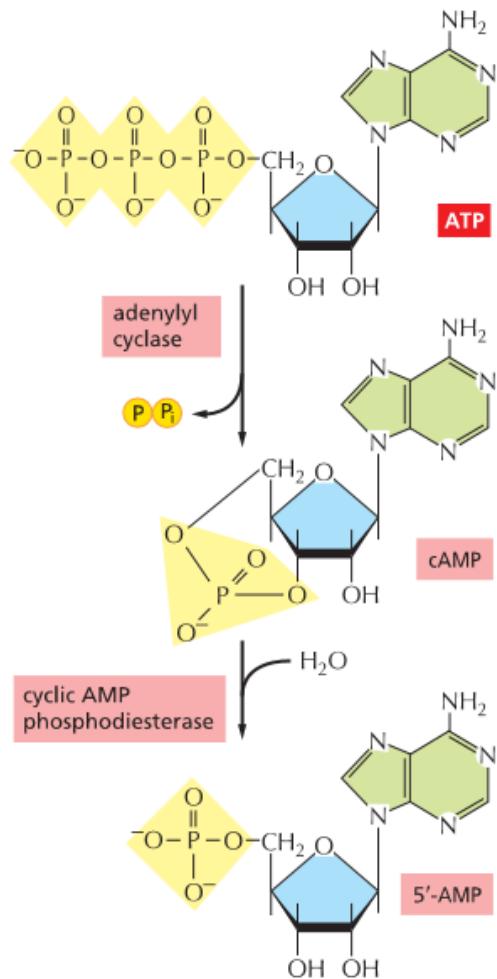


Figure 22: The production of cAMP with the enzymes adenylyl cyclase and cAMP phosphodiesterase.

Remark 2.1 (cAMP jumping between cells). cAMP can be transported to other cells via **GAP Junctions**.

2.2.2 cAMP as a signaling molecule

The pathway is as follows:

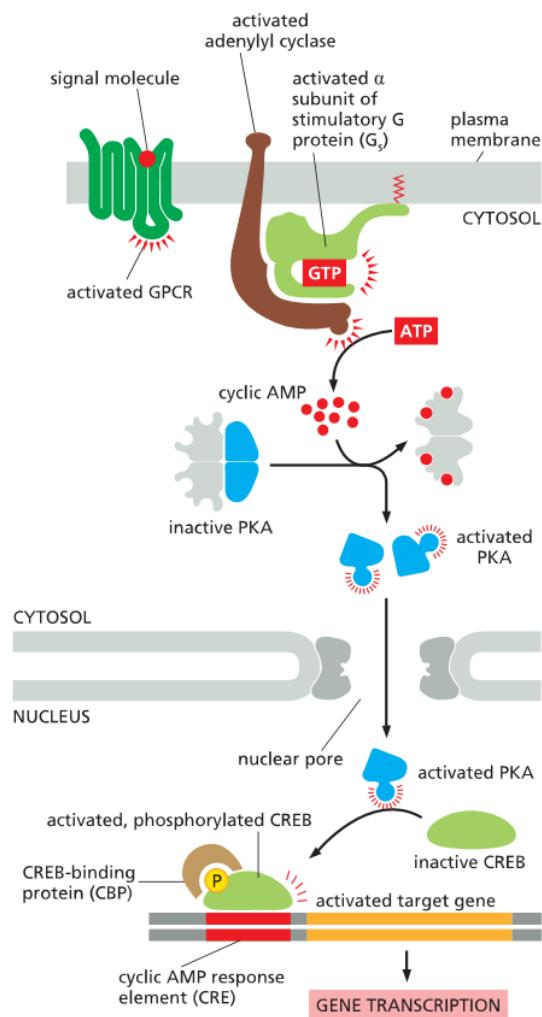


Figure 23: The production of cAMP with the enzymes adenylyl cyclase and cAMP phosphodiesterase.

- Activation of GPCR:** GPCR gets activated, which in turn activates the adenylyl cyclase and 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.

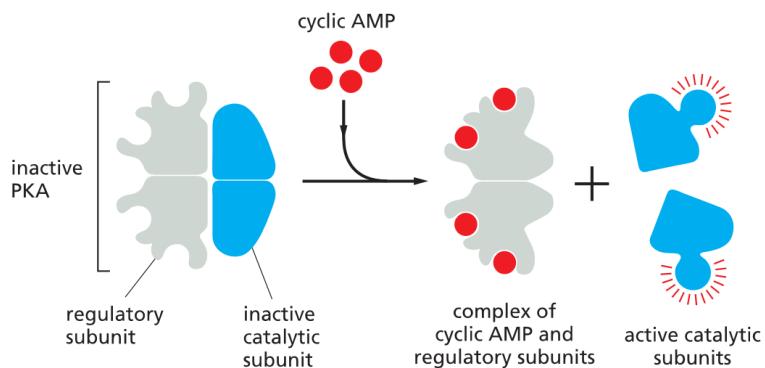


Figure 24: Shows the activation of PKAs by cAMP.

- 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.

The activation of GPCR by a ligand, say serotonin, increasing the signal strength 20fold in a matter of seconds. Depending on the cell and the ligand we will get very different cell responses. The PKA however will always be the same and it is the substrate of the PKA that changes. Hence we can receive so many different 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 25: The expression using different hormones in different cells. Vasopressin is also a "love" hormone, meaning 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 adenine. The enzyme is called **Guanylate Cyclase**.

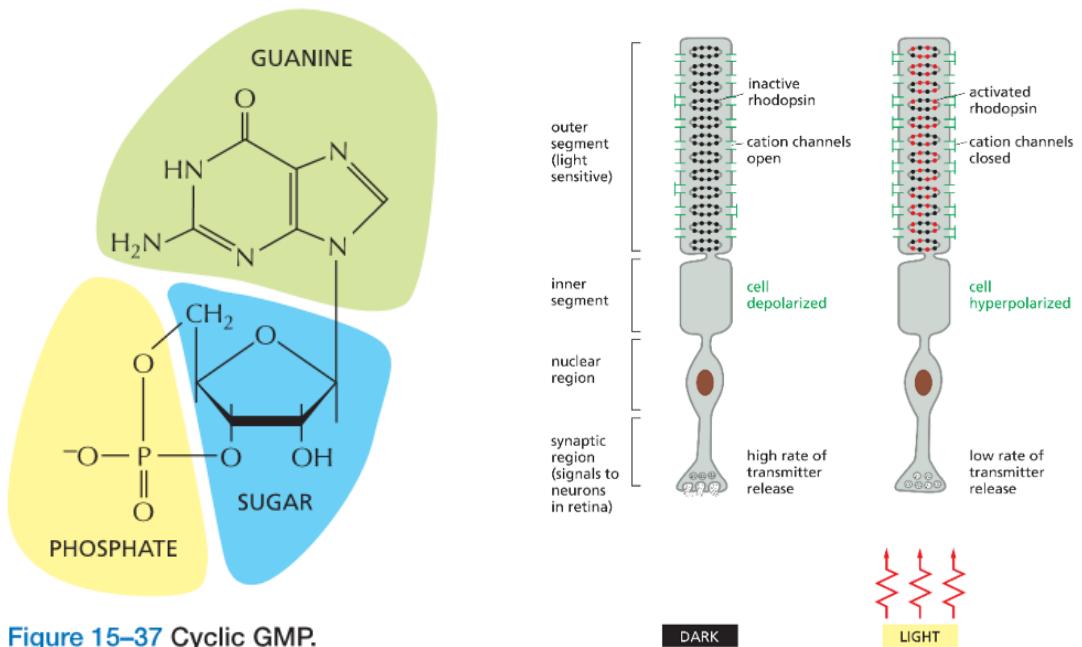


Figure 15-37 Cyclic GMP.

- (a) The structure of cGMP, where the only difference to (b) How the cell changes when light is received. Note that cAMP is the guanine for adenosine.
 (b) 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).

Figure 26: cGMP

2.2.4 Case study with response to light

The process of recognition is as follows (see fig:26):

- 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 cGMP 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.

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 27: 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

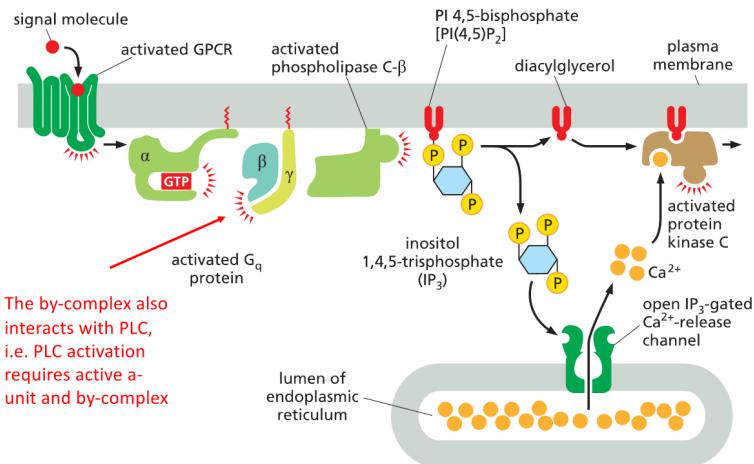


Figure 28: Shows the pathway of GPCR activating Cytosolic Ca $^{2+}$.

Rundown of the pathway:

- The GPCR activates the PLC β via a G-protein called G_q. The G_q Beta-gamma complex and the alpha-complex activates the PLC β .
- PLC β hydrolyzes PI(4,5)P₂, causing it to split into two messengers
- IP₃ diffuses through the cytosol and releases Ca²⁺ from the ER by binding to the IP₃-gated Ca²⁺ channels.
- Then the diacylglycerol, DAG, (other part of PI(4,5)P₂), remaining in the membrane, together with the Ca²⁺ and Phosphatidylserine activate the protein Kinase C (PKC). Of the min. 10 forms of PKC at least 4 are activated by DAG.

2.3.2 Ca $^{2+}$ feedback waves and oscillations

The concentration of Ca²⁺ plays a big role in activating or inactivating. So, giving itself positive or negative feedback. Here's how:

- Activation:** At low concentrations, Ca²⁺ goes to neighboring channels and activates them, causing the release of more Ca²⁺ 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.

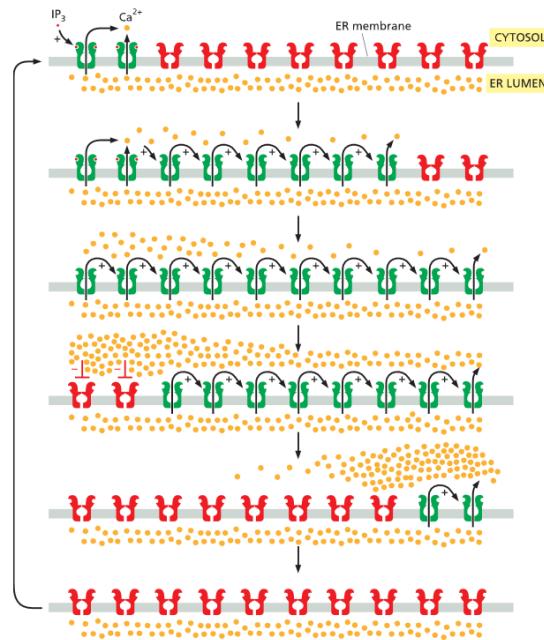


Figure 29: Shows how Ca^{2+} influences the activity of its own channels

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.

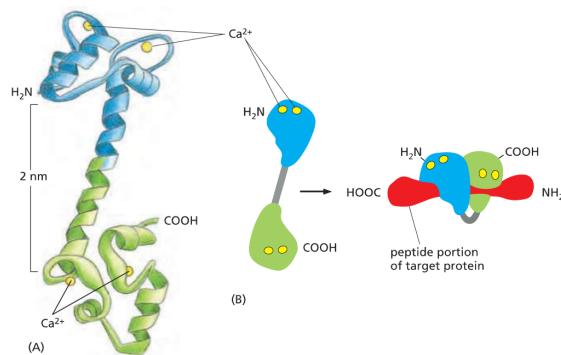


Figure 30: On the left the structure of calmodulin and on the right an example of how it can bind to target proteins (this move is called the jackknife).

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

- i) 6 CaM-Kinase II (green) form a ring.
- ii) The kinase domains pop in and out naturally.
- iii) Calmodulin can bind the popped-out domain in place when it is bound to Ca^{2+}
- iv) Then that kinase domain gets Autophosphorylation, 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.

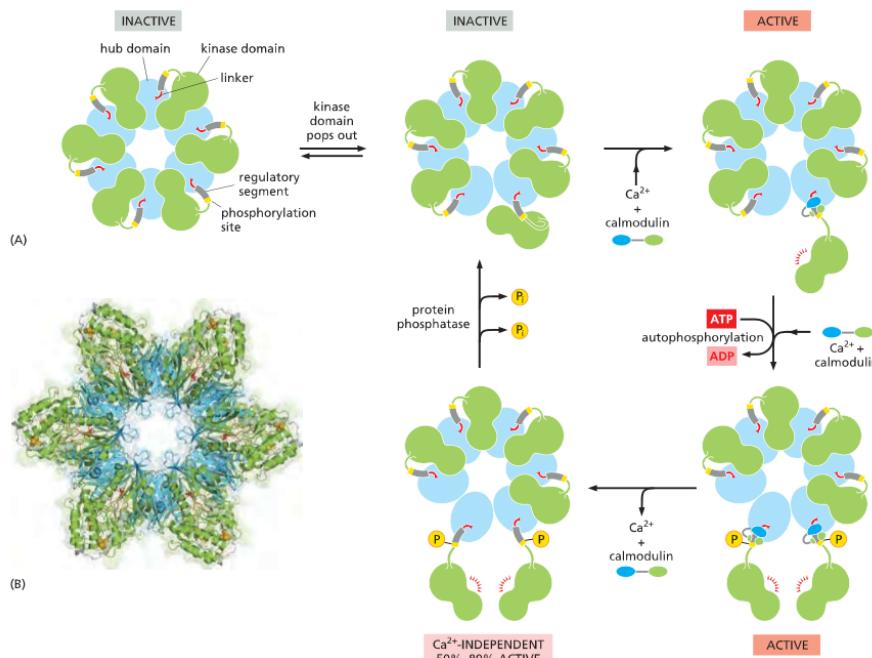


Figure 31: Shows how Ca^{2+} /calmodulin activate enzymes, in this case CaM-KII.

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. For instance at low frequency the autophosphorylation induced by the Ca^{2+} /calmodulin binding does not maintain the enzyme's activity long enough for the enzyme to remain active until the next Ca^{2+} spike arrives. At a higher spike frequencies, however, the enzyme fails to deactivate completely between the spikes, therefore its activity ratchets up with each spike. Hence CaM-KII is a good mechanism of decoding the frequencies of oscillations in a cell. Here's a figure to visualize:

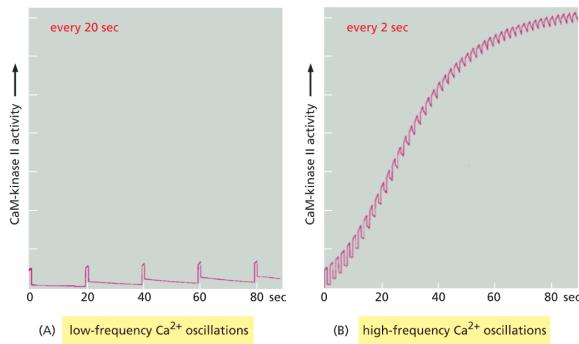


Figure 32: How different frequencies of oscillations cause major differences in enzyme activity.

3 Cell Signaling: Receptor Tyrosine Kinase a.k.a. RTKs

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 33: A bunch of different RTK groups

RTK 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** important for interactions with other proteins.

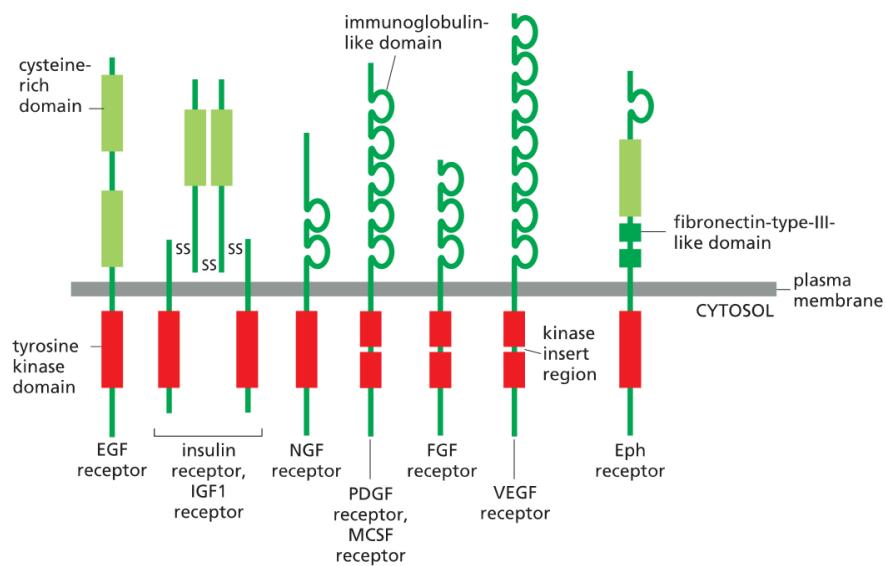


Figure 34: A bunch of different RTK types, with the core features.

3.1 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 **Autophosphorylation**.
- iii) Once the first phosphorylation has happened that initiates Transphosphorylation of several Tyrosines.
- iv) Phosphotyrosine sites recruit and/or activate downstream signaling proteins.

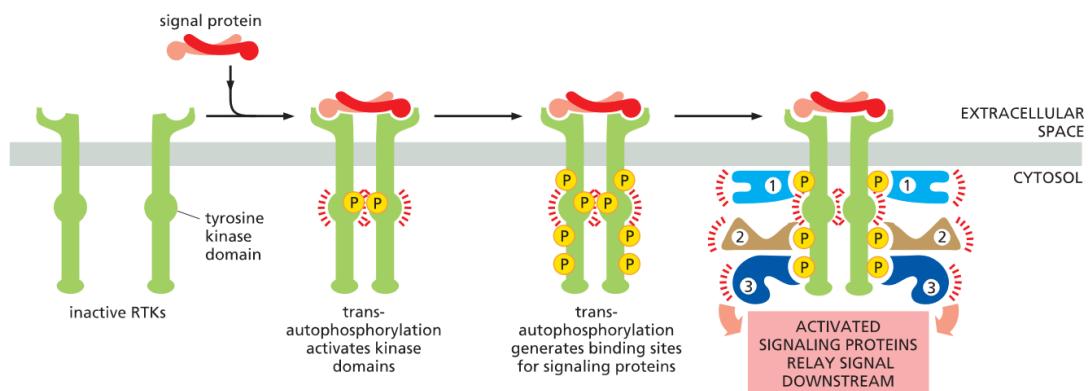


Figure 35: Activation of a RTK dimer

3.2 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, which makes it active.
 - iii) The receiver Kinase domain then phosphorylates the tyrosines on both receptors.

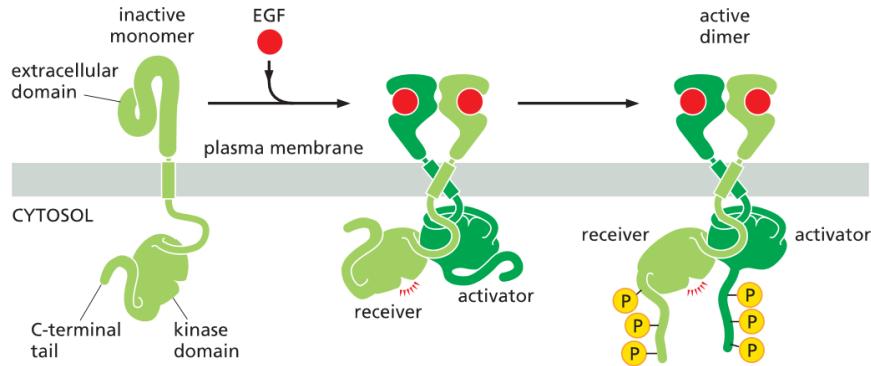


Figure 36: The activation of a EGF receptor kinase.

Remark 3.1 (Cancerous EGF Kinase). If the receiver domain mutates in such a way that it is constitutively active, then Ras MPK and PI3K are also always activated resulting in uncontrolled cell growth.

3.3 Tyrosine Kinase Associated Signaling

3.3.1 JAK-SAT

JAK stands for JANUS Kinases, which are part of **Kinase cytokine receptors**.

They work very similar to a regular RTK, with the JAK-STAT being cytokine receptors:

- i) once the ligand connects the cytokine receptors get together and become active.
- ii) The activation of the JAKs, which are associated with the receptors, happens through cross phosphorylation of each other.
- iii) Then the JAKs phosphorylate the receptors at a Tyrosine, on the receptor.
- iv) Said phospho-Tyrosine can then recruit **STAT** proteins.
- v) Once they have docked they get phosphorylated by the JAKs, making them active.
- vi) Then they enter the cytosol and translocate to the nucleus.
- vii) In the nucleus they associate with a complex and activate transcription.

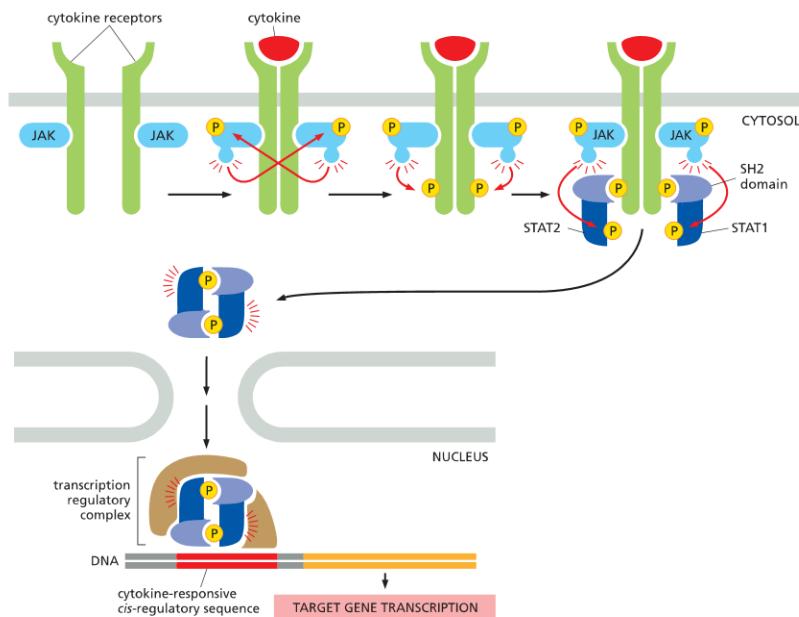


Figure 37: The JAK-SAT pathway. The exact details on the complex for transcription are beyond this course.

There are 4 different JAKs and 7 STATs, which are very important and especially known for immune responses. They are the following:

- JANUS Kinases:
 - i) JAK1
 - ii) JAK2
 - iii) JAK3
 - iv) TYK2 (you thought this was about to say JAK 4 didn't you)
- STATS
 - i) STAT1
 - ii) STAT2
 - iii) STAT3
 - iv) STAT4
 - v) STAT5a
 - vi) STAT5b (gotcha again, not 6 just yet)
 - vii) STAT6

It is a very diversely used pathway, which over 128 extracellular signaling proteins and their receptors using JAK-STAT. Here are some signaling proteins:

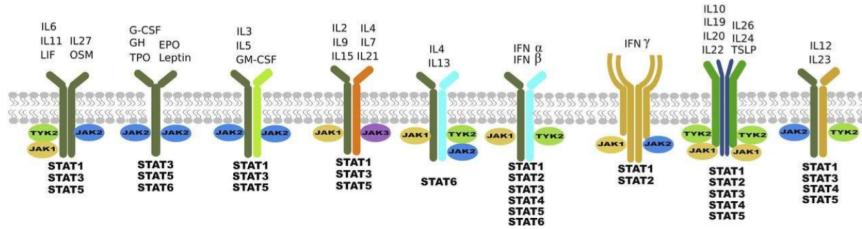


Figure 38: A bunch of signaling proteins which use JAK-STAT.

As per usual here a bunch of different JAK-STATs in the body:

TABLE 15-6 Some Extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK-STAT Signaling Pathway			
Signal protein	Receptor-associated JAKs	STATs activated	Some responses
Interferon- γ (IFN γ)	JAK1 and JAK2	STAT1	Activates macrophages
Interferon- α (IFN α)	Tyk2 and JAK2	STAT1 and STAT2	Increases cell resistance to viral infection
Erythropoietin	JAK2	STAT5	Stimulates production of erythrocytes
Prolactin	JAK1 and JAK2	STAT5	Stimulates milk production
Growth hormone	JAK2	STAT1 and STAT5	Stimulates growth by inducing IGF1 production
Granulocyte-Macrophage-Colony-Stimulating Factor (GMCSF)	JAK2	STAT5	Stimulates production of granulocytes and macrophages

Figure 39: A bunch of different JAK-STATs in humans

Also JAK-STAT and FGFR signaling inhibition can restore sensitivity to anti-hormonal drugs in prostate cancer [Editor's note: I have no idea how important this is, guessing this is to some degree his research or something.]

3.3.2 TGF Beta Signaling

While Karthaus doesn't put it in the tyro-kinase associated box, I feel like it fits, so we putting it here :)

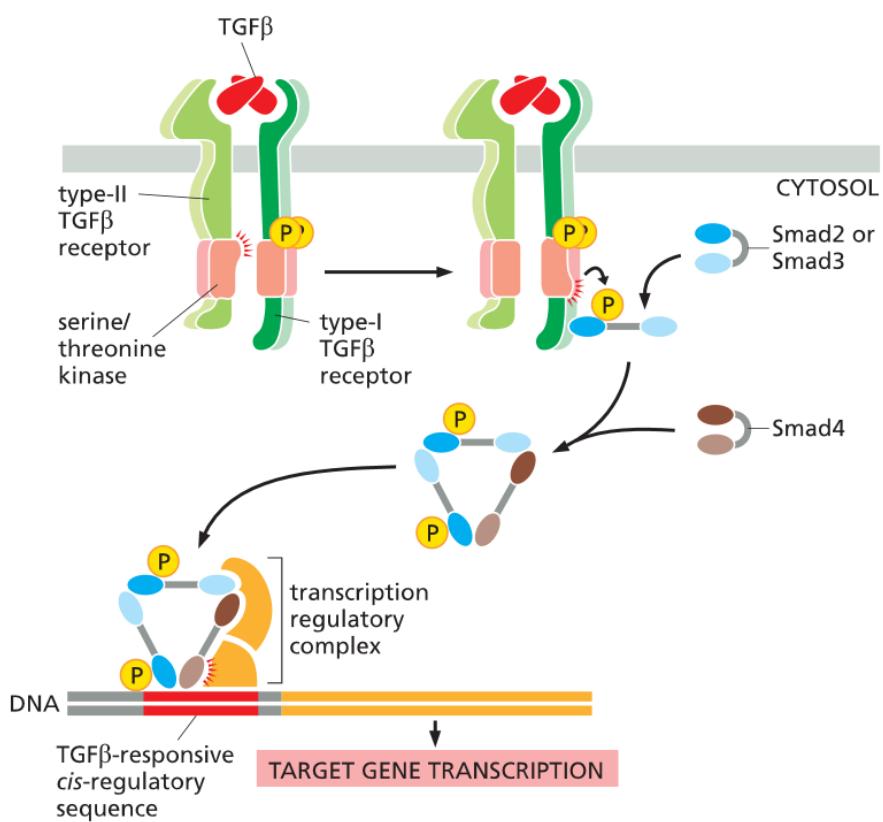


Figure 40: The TGF-beta pathway, using Smad

- i) The TGF beta dimer promotes the assembly of a tetrameric receptor complex of TGF β 's containing two copies of the Type I receptor and Type II receptor.
- ii) type-II receptors phosphorylate type-I receptors, which activate their kinase activity.
- iii) type-I receptors then activate R-Smad, such as Smad2 or Smad3.
- iv) This leads p-Smads to open up and be exposed to dimerization of the phosphorylated surface
- v) This leads to trimerization, with the co-Smad, Smad 4.
- vi) This Smad complex then enters the nucleus, and joins an even bigger transcription complex

4 RTK and G-Protein: Downstream and Similarities

4.1 Checking out how GPCRs and RTKs are intertwined

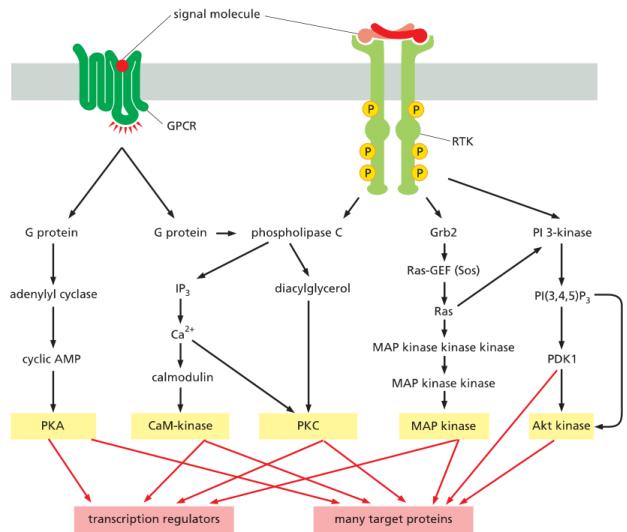


Figure 41: Compares the pathways caused by GPCRs and RTKs, also shows which are shared. All of them end with a Kinase, which then causes a reaction chain downstream

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

4.2 Binding to the Receptor

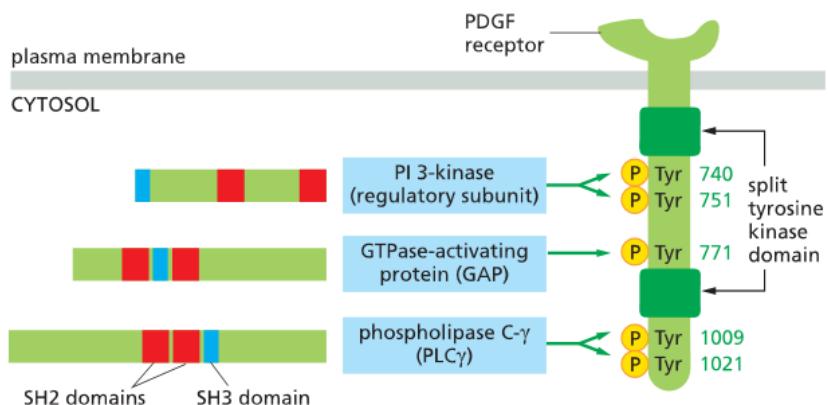


Figure 42: Phospho-tyrosine on PDGF receptors being docking-sites for proteins containing SH2 or PTB domains.

The phospho-tyrosines are docking sites for proteins containing:

- i) Src Homology (SH), Proto-oncogene

- ii) Phosphotyrosine Binding (PTB), Proto-oncogene
- iii) PLC

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

4.2.1 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 1.0.6.

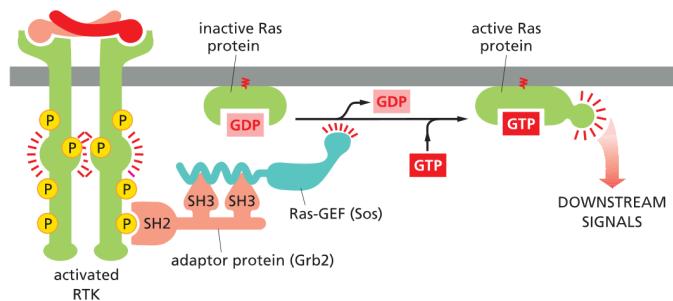
Here are some groups:

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.

(a) Ras groups in our body.



(b) The pathway for Ras.

Activation of Ras by an RTK:

- i) Adaptor protein Grb2 docks to RTK with Src Homology (SH)
- ii) Ras-GEF then interacts with Grb2
- iii) Ras-GEF then exchanges the GDP for a GTP
- iv) Ras is activated.

Remark 4.1 (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.

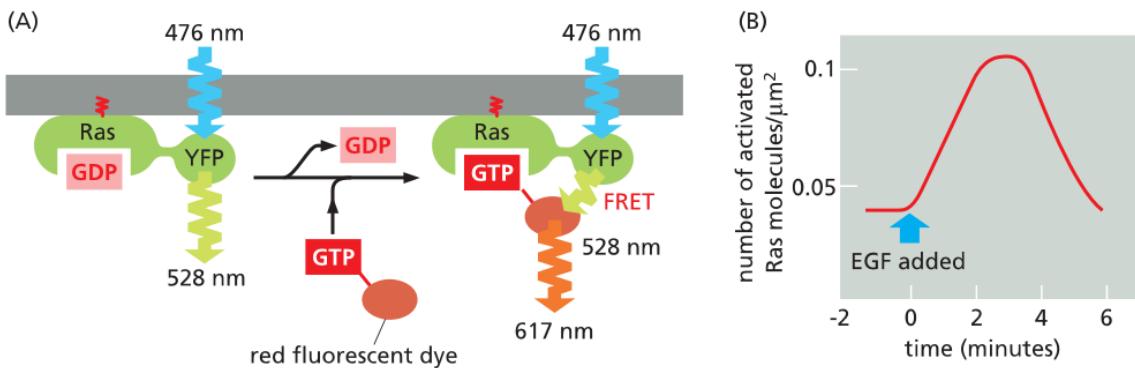


Figure 43: How FRET can be used to detect the activity of Ras.

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.

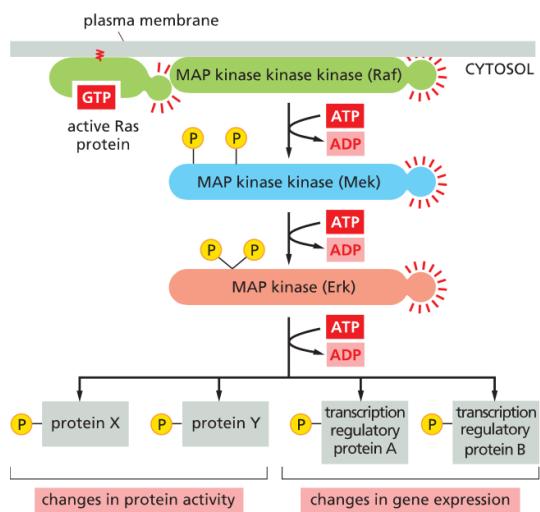


Figure 44: How the MAP kinase module is activated by Ras.

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. GAP is a Tumor Suppressor, EGF-R a Proto-oncogene.

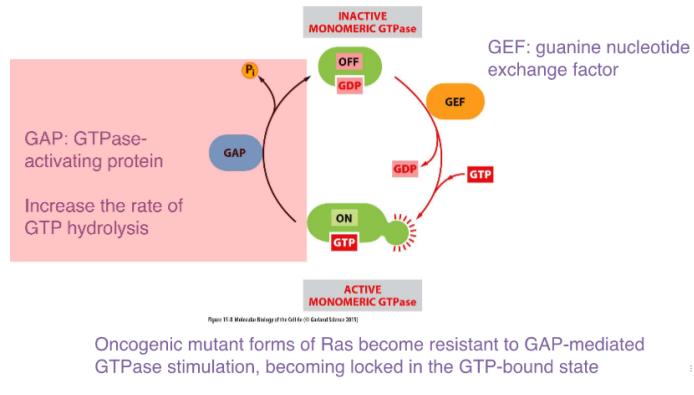


Figure 45: Showing how mutating the GAP messes with the regulation of Ras.

4.2.2 PI3K signaling

The PI3-Kinase phosphorylates the 3-carbon of a given PI molecule (can already be phosphorylated at other carbons or not). This then creates docking sites for downstream proteins, prominently AKT. In an alternative pathway the PI can also be activated by PLC causing it to go down the PLC pathway (see section 2.3). However, these are two independent pathways, even if the starting substrate PI is the same!

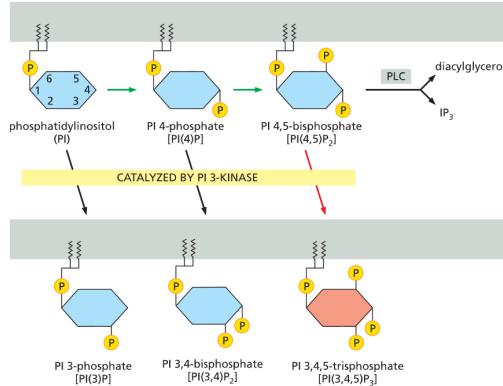


Figure 46: Phosphorylation of the 3-carbon activates the PI.

PI 3-Kinase activates AKT:

- PI3K is recruited by RTK
- PI3K creates docking sites on the PI where proteins with a PH domain can dock.
- PDK1 and mTORC2 activate AKT a.k.a. Protein Kinase B by phosphorylation at two different sites, allowing p-AKT to disassociate from the membrane.
- 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.

The fig: 47 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.

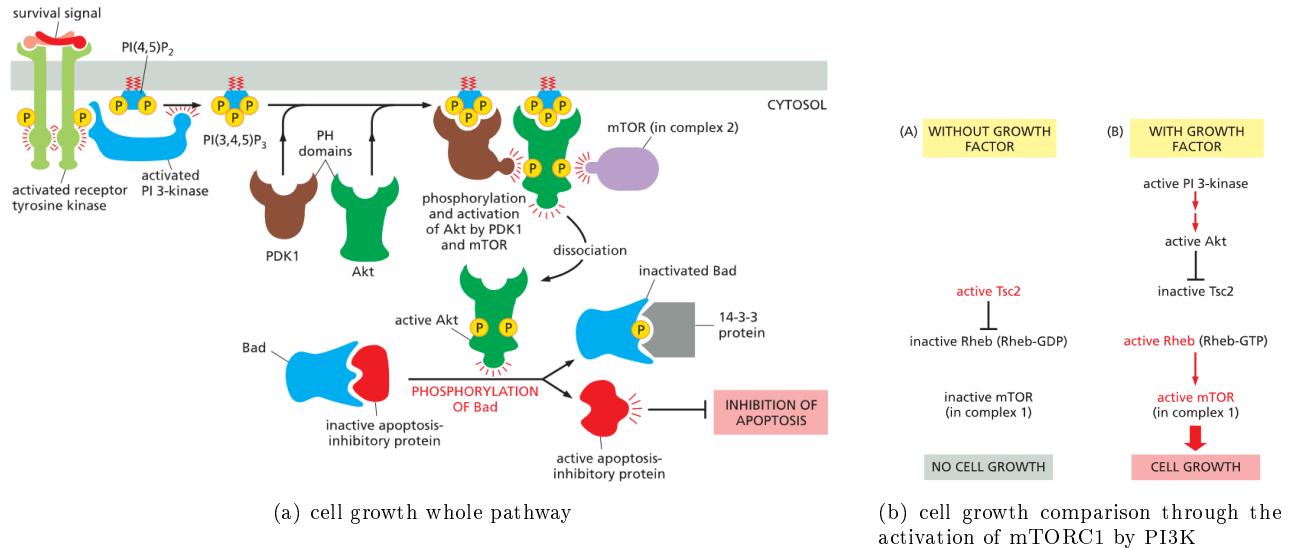


Figure 47: Note: (a) mTORC2 is in action, activating ATK, while in (b) mTORC1 is in action, about controlling protein synthesis, growth, and metabolism (further downstream of ATK).

4.3 EGF Receptors in cancer

In cancer EGFR can become over-activated, making it a Proto-oncogene.

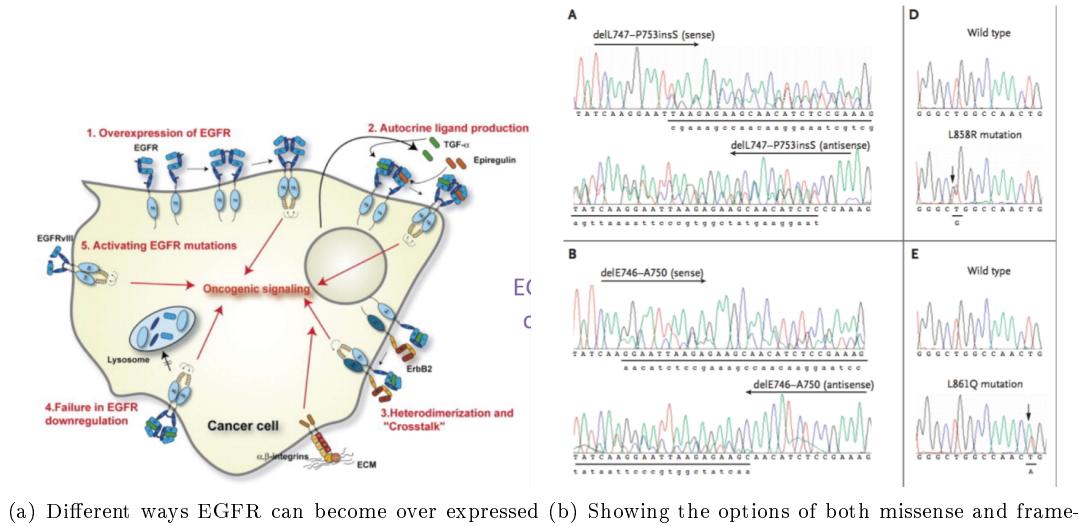


Figure 48:

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 49:

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

5 Cell Signaling: Alternative Signaling

5.1 Signaling with Regulated Proteolysis

The namesake for all these beauties comes from how the fruit fly looked when we fucked it up. Details at the start of each section.

5.1.1 Notch

Notch = V-shaped indentation in the wing.

The idea here is that instead of having the receptor cause a phosphorylation or similar, we cleave the receptor so that the intracellular part becomes the downstream signal, entering the nucleus. Here is the detailed process, oriented around the red numbered arrows in the picture:

- i) First Proteolytic Cleavage (red arrow 1): inside the trans Golgi network NOTCH is cut to become a mature version where the two parts are connected noncovalently.
- ii) these parts then migrate to the cell membrane.
- iii) Once the Notch complex binds to the Delta, through its repeating EGF regions on a neighboring cell, the two parts are split by endocytosis (red arrow 3).
- iv) This split then exposes the cleavage site (red arrow 3) to make the cut, allowing the Notch tail (a.k.a. notch intracellular domain a.k.a. NICD) to migrate the nucleus.
- v) the tail binds to Rbpsuh protein, which then converts from a repressor to an activator.

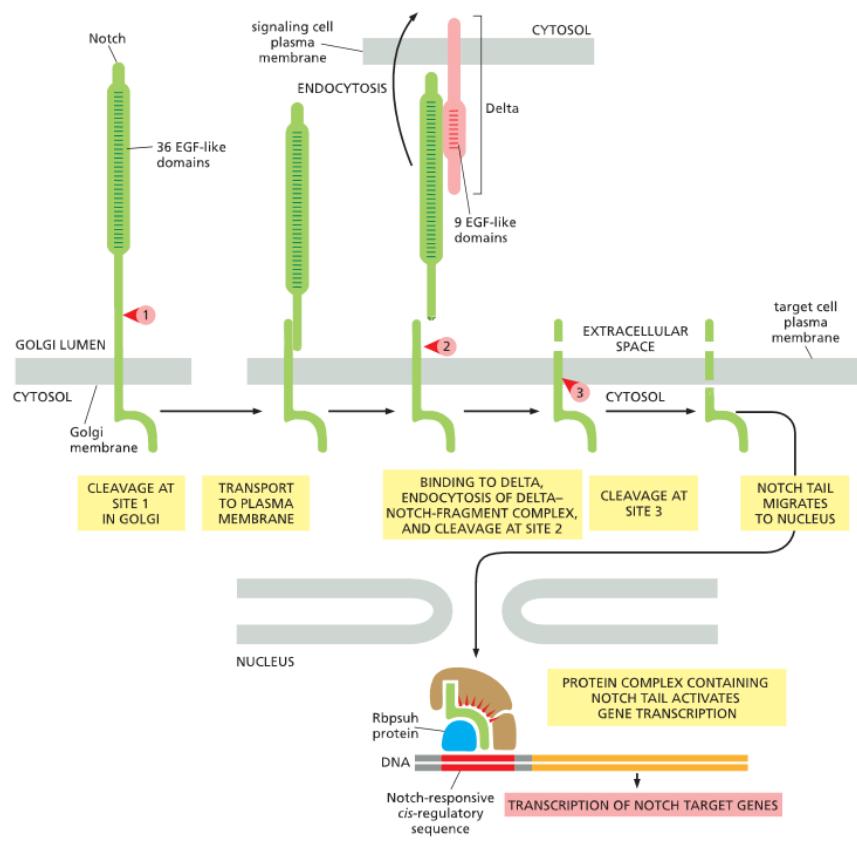


Figure 50: The Notch pathway, note that red arrows are cleavage sites.

Definition 5.1 (Lateral Inhibition in Notch). *Notch is contact-dependent and not autocrine. This allows for one cell to become excited and in the process inhibiting the neighboring one, which is called lateral inhibition.*

Looking at the example of neural cell development. Initially all Epithelial cells want to become neural cells, however we don't want that many. So, instead if a **cell expresses Delta than the neighboring ones know not to become a neural cell anymore**. Since, they all want to be neural cells though a competition starts to appear of who can produce the most delta ligands and the winner becomes the neural cell.

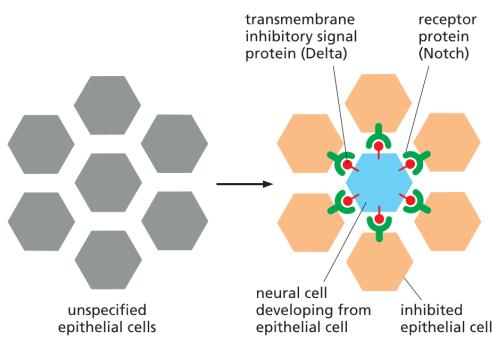


Figure 51: Case study of Notch, showing how Notch and Delta help in cell development.

5.1.2 WNT

$\text{Wnt} = \text{Wingless (WG)} + \text{Integration (t)}$.

Wnt is a special secreted ligand, that through a bunch of lipid modifications, **associates to the plasma membrane** and doesn't travel very far. That means that the spread of Wnt is very limited to the neighboring cells.

Looking at the Wnt ligand pathway: the broad idea is that we want to stop the degradation of the anti-repressor.

Here are the details, first in the absence of the Wnt ligand:

- i) Beta Catenin interacts with degradation complex.
- ii) In this complex it is phosphorylated first by CK1, then GSK3, triggering its ubiquitylation and then degraded.
- iii) The degraded form prohibits it from binding to LEF1/TCF, meaning it can't kick out the repressor Groucho.

Now, in the presence of the Wnt ligand:

- i) Wnt binds to frizzled and LRP, clustering the two co-receptors together
- ii) The tail of LRP is phosphorylated by GSK3 and then CK1. Further Disheveled is recruited to the Frizzled site. The exact role of disheveled isn't known.
- iii) The Axin, of the degradation complex, is then recruited by the disheveled and then bound to the phosphorylated LRP.
- iv) This results in the disassembly of the degradation complex.
- v) This means the β -catenin stays stable, attaches to the LEF1/TCF and kicks out Groucho.

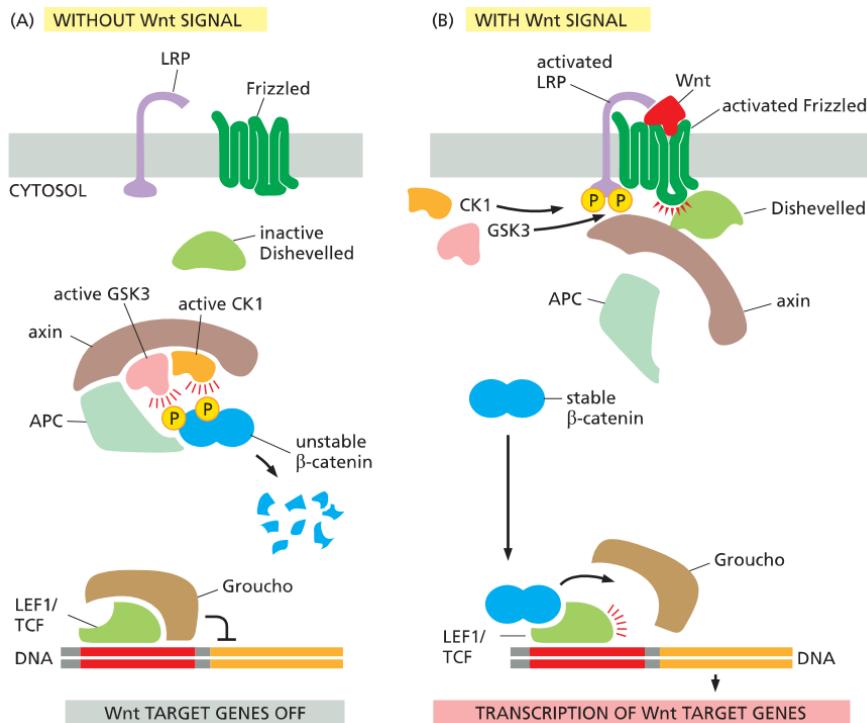


Figure 52: The Wnt pathway, on the left without Wnt, leading to no expression, while on the right it has Wnt, meaning the gene is expressed.

5.1.3 Hedgehog

Hedgehog = Larva looked like a hedgehog.

The game is all about Cubitus Interruptus (Ci) (Ci). Without Hedgehog it get cleaved to a repressor, with Hedgehog it is an activator.

Here is what happens when we have no hedgehog:

- Through the absence of Hedgehog, Patched is allowed to exist, which means it inhibits Smoothened.
- The lack of Smoothened which causes Ci to be caught in a degradation complex.
- This degradation complex includes a Fused kinase and a scaffold protein Costal2. Costal2 recruits three other kinases (PKA, GSK3, CK1), which phosphorylate Ci.
- That P-Ci is ubiquitylated and cleaved in proteasomes.
- the cleaved Ci then forms a repressor and moves to the nucleus to do just that.

Now, when we have hedgehog:

- Hedgehog binding to iHog as Patched is removed and degraded, stopping the inhibition of Smoothened.
- Smoothened is then phosphorylated by PKA and CK1 and translocated to the plasma membrane.
- There it recruits Fused, Costal2, which are forced to let go the Ci they were holding on to.
- The Ci enters the nucleus in its complete form and activates the hedgehog genes.

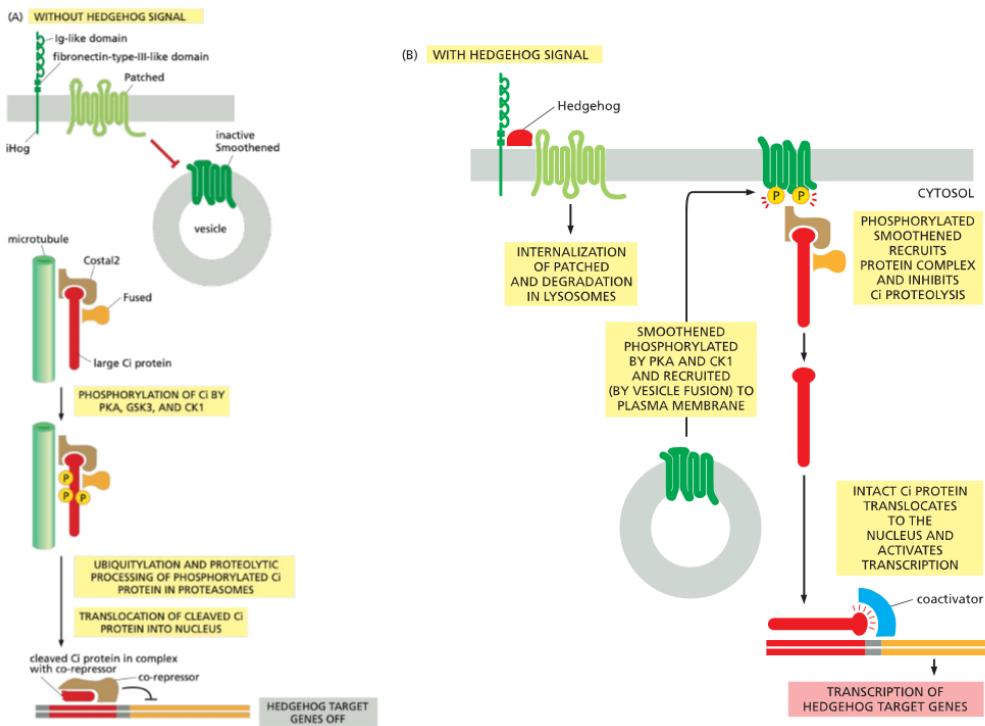


Figure 53: Shows the pathway of Hedgehog; on the left without Hedgehog, meaning gene repression; and on the right with Hedgehog and activation.

5.1.4 NF κ B pathway

Core concept - Negative Feedback Loop: What this causes is that the moment a gene is expressed it immediately inhibits its own transcription. That leads to a short expression, which is quickly blocked out.

Remark 5.2 (Application to NF κ b). Activated NF κ B increases expression of the I κ B α gene, and I κ B α then binds to NF κ B and inactivates it, thereby shutting off the response. If the initial activating signal persists, then additional cycles of NF κ B act on and inactivation may follow.

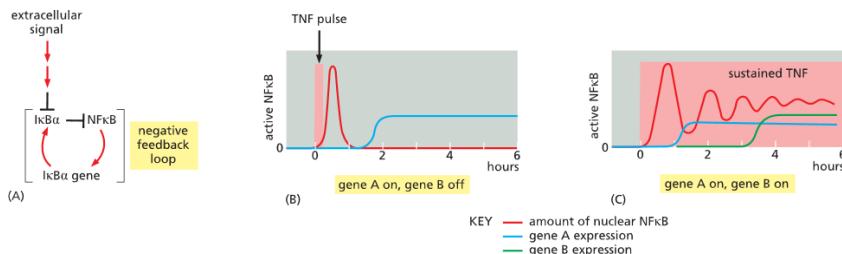


Figure 54: The left shows the idea behind a negative feedback. The middle has a short TNF pulse, while the one on the right has a sustained one.

For the short TNF pulse: Gene A gets its transcription turned on by this while gene B does not. For the prolonged TNF exposure: gene B will also be transcribed. Why gene B needs that prolonged exposure isn't understood. Note that due to the negative feedback loop we get an oscillation of TNF until it finds the right

concentration steady state with the gene.

NF- κ B can be activated by many signals, a lot of them being **inflammatory signals** (infections, Bacteria, and immune cells secreting signals).

Now an example of the NF κ B pathway, activated by TNF α :

- i) TNF α is a trimer, as are its receptors. The binding of TNF α causes a rearrangement of the clustered tails of the receptors.
- ii) These tails can now recruit a bunch of signaling proteins.
- iii) This in turn activates the protein kinase which activates I κ B kinase kinase (IKK). IKK is a heterotrimer composed of three subunits (IKK alpha and IKK beta (catalytic subunits), and NEMO (regulatory subunit)).
- iv) IKK β then phosphorylates two serine of I κ B, which marks it for ubiquitylation and consequent degradation.
- v) The released NF κ B translocates to into the nucleus, where it with co-activators stimulates transcription.

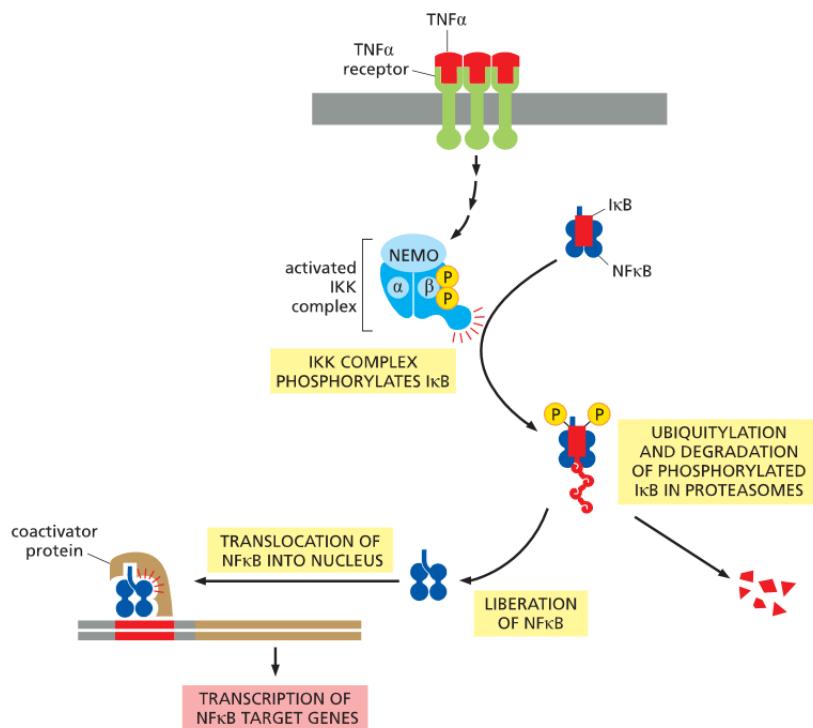


Figure 55: This shows the NF κ B pathway.

In cells lacking NEMO, TNF triggers cell death.

5.2 Nuclear Receptor Signaling

Some signaling molecules can bind to intracellular molecules. This means they need to get through the membrane, so they got to be lipophilic. **Cholesterol is kinda the God of precursors**, as its already in the

membrane so must be popular. However, most of the signaling molecules will be more hydrophilic than cholesterol making them better for exiting membranes and transport throughout body.

The signaling with these molecules is incredibly simple. A dimer molecule comes in, reaches the nucleus and

- changes the factors to activate gene expression. In the this process the receptors are already on the gene. This binding can also kick out inhibitors and often binds further activators.
- changes the factors to repress gene expression. This really is really just the same thing but opposite: activators are cleared out and repressor recruited.

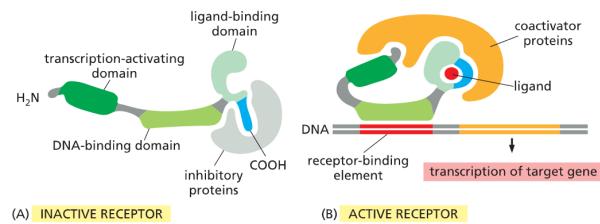


Figure 56: How nuclear receptors attach right to the DNA, at the repressor (not shown) or activator site

Depending on the cell we will get completely different reactions and gene expressions.

Remark 5.3 (Use study - Prostate Cancer): In prostate cancer, Androgen activate the Androgen receptor (AR) (AR), which then turns on genes that support tumor growth and survival. In some therapeutic or experimental contexts, researchers can design genes controlled by androgen-responsive promoters so that their expression is turned on in the presence of androgen.

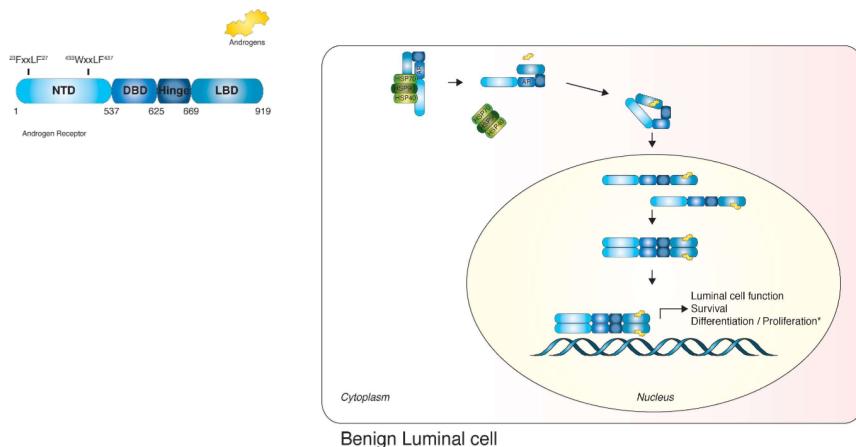


Figure 57: Yh I'm done with this, this is an example of Androgen being used to reprogram prostate cancer.