Signaling

- **Signal Transduction:** Once a drug binds to its receptor at the cell surface, it triggers a series of molecular events inside the cell. We call these events, signaling or signal transduction. The specific events or pathways will be different for each receptor, but we will review the common signaling pathways. The most common drug targets that trigger intracellular signaling include ion channels, G protein coupled receptors, receptor tyrosine kinases, and intracellular receptor.
- **Ion channels:** There are three types of ion channels. These include voltage-gated ion channels, channels regulated by a second messenger, and ligand-gated ion channels.
    - **Voltage-gated ion channels—sodium channel**: The cell membrane is selectively permeable, so it keeps certain types of ions in and others out. Due to this separation, the cell tends to be more negative inside and relatively positive outside. The voltage gated sodium channel is normally closed and that prevents sodium ions from entering the cell, but the channel can briefly open in response to certain stimulation. For example, the channel contains a region that serves as a voltage sensor. So when there is a change in voltage, the channel undergoes a conformation change and opens up allowing sodium ions to flow in. The entering sodium ions then change the intracellular voltage potential so that it is more positive now, which then causes the sodium channel to close.
        - **Examples of voltage-gated channels**: Sodium channels, potassium channels, chloride channels, and calcium channels.
- **Second messenger-regulated ion channels**: These are a group of channels that don't directly respond to a ligand or a change in electrical potential. Instead, they

are regulated by second messengers that are generated by activation of other receptors in the vicinity of the ion channel.

- **Ligand-gated ion channels:** Ligand-gated channels are particularly important because they can be directly regulated by many drugs. A ligand-gated channel has a specific recognition site for a ligand. Normally, the channel would be in an inactive state, but when a ligand binds to this recognition site, the channel undergoes conformational change and switches to an active state. The active channel then allows the ions to flow through.
    - Ligand-gated channels make up a large class of channels. These include channels that are activated by excitatory neurotransmitters such as acetylcholine and glutamate, as well as channels that are activated by inhibitory neurotransmitters such as Glycine and GABA.
- **G-Protein Coupled Receptors (GPCRs)**: Refer to **Figure 1**. In the inactive state, the intracellular region of the receptor is associated with the trimeric G protein complex consisting of alpha, beta, and gamma subunits. The alpha subunit is bound by GDP and the three subunits are closely associated with each other. When a ligand binds to the specific recognition site of the receptor, it causes a conformational change in the receptor. This conformation change causes the alpha subunit to exchange GDP for a GTP molecule and it dissociates from the beta and gamma subunits. The GTP-bound alpha subunit then goes on to activate a second messenger which then propagates the signal further. This interaction with the second messenger is short-lived because the alpha subunit hydrolyzes the GTP to GDP. Unlike when it's GTP-bound, the GDP bound alpha subunit is not active and dissociates from the second messenger and goes back to the receptor to regroup with its beta and gamma partners. Meanwhile, the ligand had already disengaged from the receptor, but it can bind to the receptor again and the process will repeat all over again.

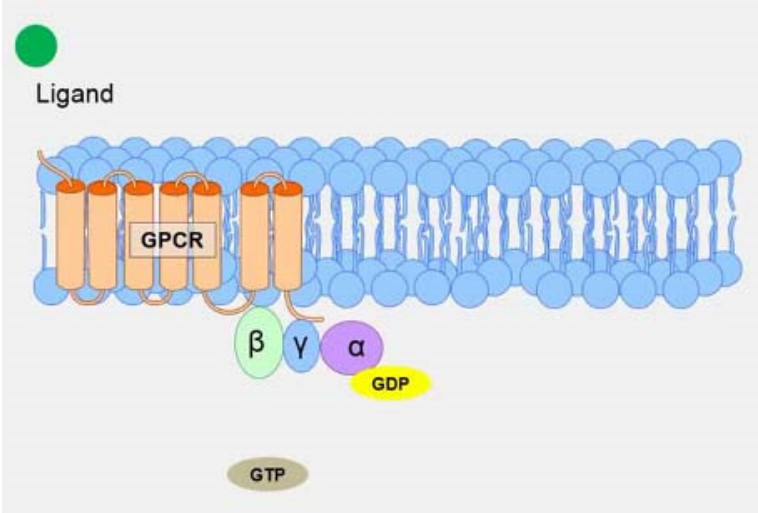

Figure 1. GPCR signaling.

- **Second Messengers in GPCR signaling**: There are two major second messengers involved in GPCR signaling. These are Adenylate Cyclase and Phospholipase C gamma.
  - **Adenylate Cyclase (AC)**: Refer to **Figure 2**. Adenylate cyclase (AC) is an enzyme that is a critical regulator of a variety of cellular functions, and GPCR signaling is one way in which it is regulated. When activated by the GTP-bound alpha subunit, adenylate cyclase converts ATP to cyclic AMP (cAMP). Increased cAMP levels in turn cause activation of a serine threonine kinase called Protein Kinase A or PKA. Activated PKA then phosphorylates numerous proteins that can influence all aspects of cell function.

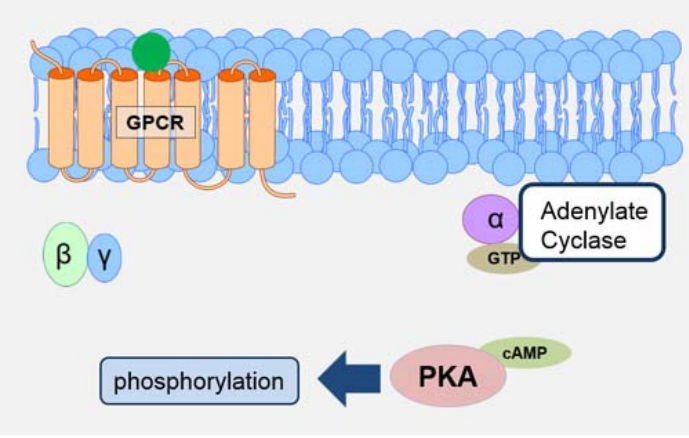

Figure 2. Adenylate Cyclase: The alpha subunit activates AC. AC converts ATP to cAMP. cAMP activates PKA. PKA phosphorylates numerous proteins.

- **Phospholipase C-gamma (PLCγ)**: PLCγ is an enzyme that is normally associated with the cell membrane and is usually inactive until activated by an upstream signal. GPCR is one of the receptor pathways that can activate PLCγ. Once activated by the GTP bound alpha subunit, PLCγ hydrolyzes membrane phospholipids into diacylglycerol (DAG) and inositol triphosphate (IP_3). Both DAG and IP_3 can then activate protein kinase C or PKC, a serine/threonine kinase. Activated PKC can then phosphorylates and activates several proteins that then directly affect cellular processes. IP_3 can also trigger the release of intracellular calcium storage vesicles. The increase in intracellular calcium concentration can also serve as a second messenger to regulate proteins that can affect these cellular processes.

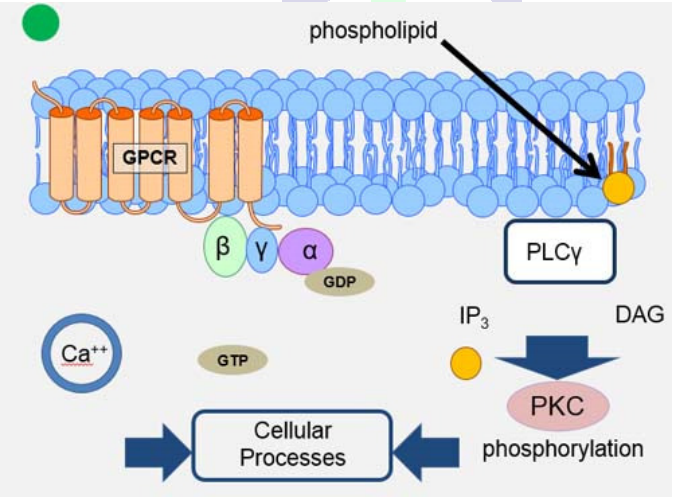

Figure 3. Phospholipase-gamma-mediated signaling.

- **Receptor Tyrosine Kinases (RTK)**: See **Figure 4.** Unlike PKA and PKC, which phosphorylate proteins on serine and threonine residues, tyrosine kinases selectively phosphorylate tyrosine residues within their target proteins. Receptor tyrosine kinases have a ligand binding domain at the surface of the cell but the intracellular region of the receptor is contains the enzymatic activity (tyrosine kinase domain). In addition, RTKs also have tyrosine residues in a region within the cytoplasmic tail that serves as its own substrate. Thus, these receptors have 3 parts:

1)Ligand binding domain, 2) tyrosine kinase domain, and 3) tyrosines that serve as substrates. When a dimeric ligand binds, it forces receptor dimerization or aggregation. This aggregation brings receptors in closer proximity which causes the kinases to become active. Once activated, the tyrosine kinase phosphorylates the tyrosine residues in the tail region in cross phosphorylation manner, in a sense that one receptor phosphorylates tyrosine residues of the other receptor and vice versa.

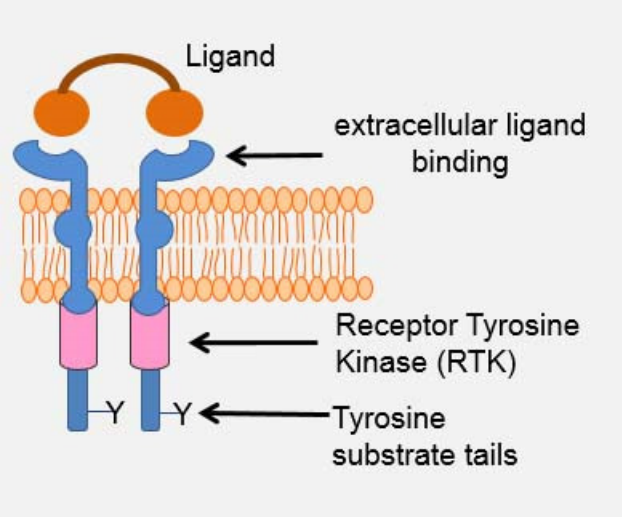

Figure 4. Receptor Tyrosine Kinase.

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  - **RTK & RAS/MAPK Signaling Pathway**: RTKs are involved in many signaling pathways. One common pathway that is activated by receptor tyrosine kinases is known as the RAS/MAPK pathway (**Fig. 5**). Once activated by the ligand, RTK autophosphorylates tyrosine residues within the tail. These phosphorylated tyrosines serve as docking site for the adapter protein Grb2. Grb2 docks with the phosphorylated receptor via its SH2 domain, which is a specific phosphotyrosine-binding pocket. Grb2 also has SH3 domain, which binds to SOS. SOS is an activator of RAS. RAS is a small G-protein that is normally bound to GDP (off position). Once activated by SOS, RAS exchanges GDP for GTP and becomes active. Activated RAS then activates the serine/threonine kinase Raf. Once activated, RAS activates another kinase called RAF. In turn, RAF activates a kinase called MEK. MEK, which is a mixed kinase that can phosphorylate proteins on serine/threonine as well as

tyrosine residues, then goes on to activate MAPK. MAPK phosphorylates numerous proteins, including transcription factors that play a role in changes in transcription of the cell.

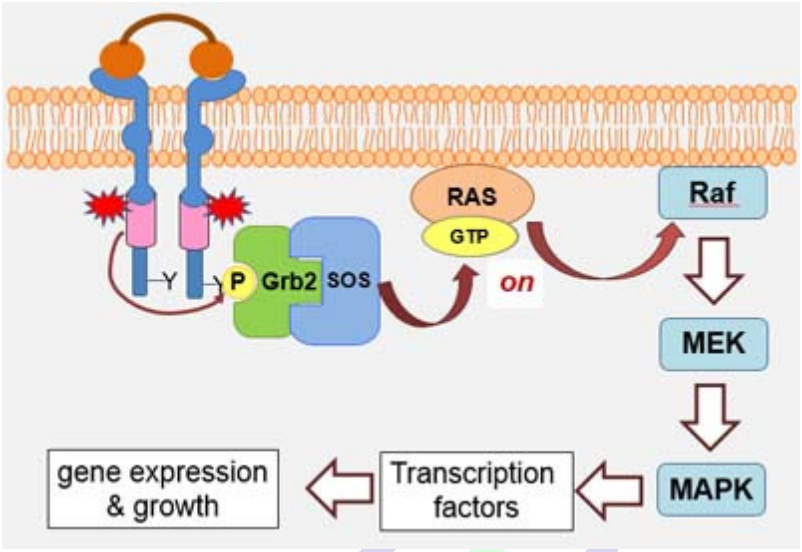


Figure 5. RTK signaling through the RAS-MAPK pathway.

- **Non-Receptor Tyrosine Kinases**: See **Figure 6**. There is a group of receptors that are related to RTK and share many functionalities, including signaling through the RAS/MAPK pathway. However, a key distinction is that these receptors do not have intrinsic tyrosine kinase activity. Instead, they signal through closely associated non-receptor tyrosine kinases (NRTK). Once the receptors are activated through ligand-induced aggregation, the closely associated NRTKs are also activated. NRTK then phosphorylate tyrosine residues within the receptor tails. These tyrosine residues serve as docking sites for Grb2 and other signaling proteins that activate the RAS/MAPK pathway as well as other pathways in the same way that RTKs signal.

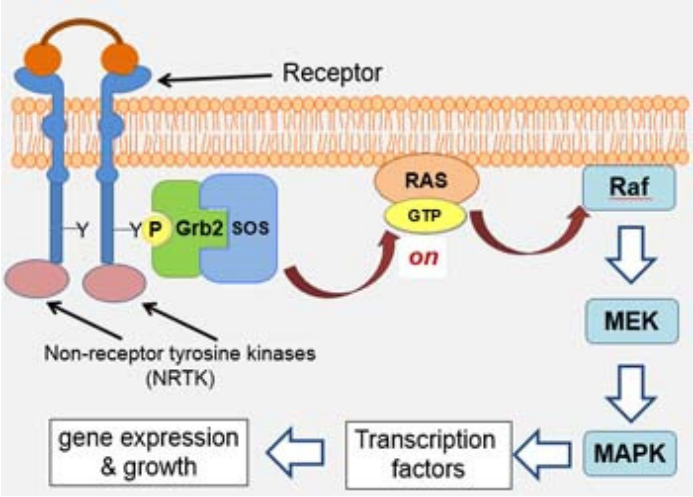


Figure 6. NRTK-mediated signaling

- **Nuclear Hormone Receptors**: Nuclear Hormone Receptors are responsible for mediating the effects of different types of hormones. There are at least 40 different types of receptors that fall into this category, so it's considered to be a Superfamily. The most common ligands that bind to these receptors include, steroidal hormones such as corticosteroid, estrogen, and androgens. Other ligands include the thyroid hormone, vitamin D, certain lipids, and even bile acids. Of course, each receptor in this family is going to be different, but there are two common features that all these receptors share. One is that all these receptors have hormone-binding domains. The second feature is that these receptors have the ability to bind to specific regions of the DNA and influence transcription. This way, the receptors can regulate transcription in different ways, depending on whether it's bound to hormone or not.
    - **Mechanism**: See **Figure 7**. Ligands for nuclear receptors are usually lipophilic, and are usually bound to hormone-binding carrier proteins or albumin. However, once the ligand is close to a cell, it can easily traverse the cell membrane and enter the cell. Once inside the cytosol, the ligand finds its specific hormone receptor and binds to it. The receptors are normally excluded from the nucleus. However, ligand-bound receptors are dimerized,

which masks the nuclear exclusion signal present within the receptors, allowing the dimerized receptors to enter the nucleus. Since the receptor has a DNA binding domain, it would bind to specific DNA sequences in the regulatory region of a gene and cause changes in the transcription of that gene. However, this is a minimal requirement, because usually the ligand-receptor complex also binds to other important proteins that regulate transcription. The final nuclear receptor complex can either increase or decrease the transcription rate of a specific gene. The genes that contain these receptor-binding sequences are known as hormone-responsive genes, because they are affected by the hormones that actually penetrate the cell.

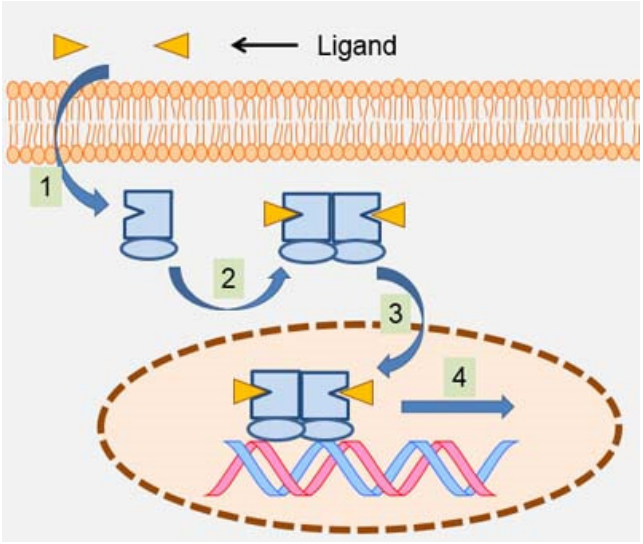
- 

Figure 7. Nuclear Hormone Receptor Signaling: **1**. Ligand enters the cell & binds to the receptor. **2**. Receptor dimerizes. **3**. Dimerized receptor enters the nucleus. **4**. Receptor associates with other regulatory proteins and modulates transcription of hormone responsive genes.