Chapter 8

Signaling

Cells must receive and respond to signals from their surroundings. Cellular signals and the pathways through which they are passed on and amplified to produce the desired effects on their targets are the focus of this section.

Signaling

Cell Signaling

How do cells receive signals from their environment and how do they communicate among themselves? It is intuitively obvious that even bacterial cells must be able to sense features of their environment, such as the presence of nutrients or toxins, if they are to survive. In addition to being able to receive information from the environment, multicellular organisms must find ways by which their cells can communicate among themselves. Since different cells take on specialized functions in a multicellular organism, they must be able to coordinate activities perfectly like the musicians in an orchestra performing a complicated piece of music. Cells grow, divide, or differentiate in response to specific signals. They may change shape or migrate to another location. At the physiological level, cells in a multicellular organism, must respond to everything from a meal just eaten to injury, threat or the availability of a mate. They must know when to repair damage to DNA, when to undergo apoptosis (programmed cell death) and even when to regenerate a lost limb. A variety of mechanisms have arisen to ensure that cell-cell communication is not only possible, but astonishingly swift, accurate and reliable.

How are signals sent between cells?

Like pretty much everything that happens in cells, signaling is dependent on molecular

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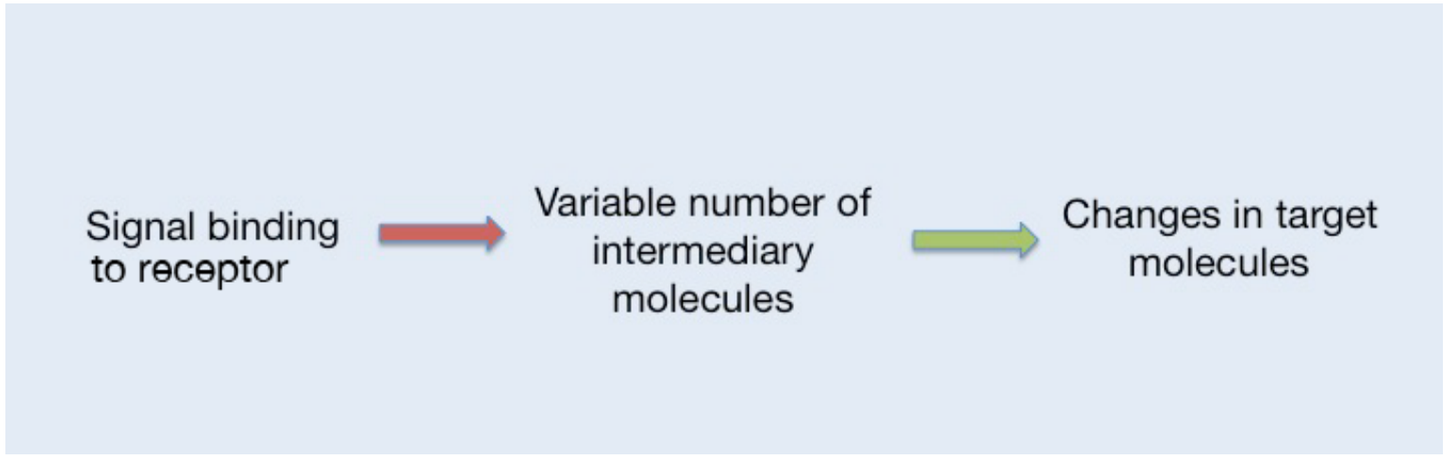
recognition. The basic principle of cell-cell signaling is simple. A particular kind of molecule, sent by a signaling cell, is recognized and bound by a receptor protein in (or on the surface of) the target cell. The signal molecules are chemically varied- they may be proteins, short peptides, lipids, nucleotides or catecholamines, to name a few. The chemical properties of the signal determine whether its receptors are on the cell surface or intracellular. If the signal is small and hydrophobic it can cross the cell membrane and bind a receptor inside the cell. If, on the other hand, the signal is charged, or very large, it would not be able to diffuse through the plasma membrane. Such signals need receptors on the cell surface, typically transmembrane proteins that have an extracellular portion that binds the signal and an intracellular part that passes on the message within the cell.



Cellular Signaling

Receptors are specific for each type of signal, so each cell has many different kinds of receptors that can recognize and bind the many signals it receives. Because different cells have different sets of receptors, they respond to different signals or combinations of signals. The binding of a signal molecule to a receptor sets off a chain of events in the target cell. These events could cause change in various ways, including, but not limited to, alterations in metabolic pathways or gene expression in the target cell.

How the binding of a signal to a receptor brings about change in cells is the topic of this section. Although the specific molecular components of the various signal transduction pathways differ, they all have some features in common:

• The binding of a signal to its receptor is usually, though not always, followed by the generation of a new signal(s) within the cell. The process by which the original signal is converted to a different form and passed on within the cell to bring about change is called signal transduction.

• Most signaling pathways have multiple signal transduction steps by which the signal is relayed through a series of molecular messengers that can amplify and distribute the message to various parts of the cell.

Talking on a Cell Phone

A little cell was waiting for a message from its friend
It couldn't text or Facebook, no e-mails could it send.
What will it do, you wonder, how will it get a clue
About the world around it and what it needs to do?
What languages are spoken by cells that have no voice?
How do they know which way to go when they must make a choice?

Cells use many signals, molecules galore
Arriving at a target cell, some slip in through the door.
Hydrophobic signals through the plasma membrane slide
They're greeted by receptors on the cytoplasmic side.
But if they're hydrophilic on the membrane they must find
A cell-surface receptor to which they soon can bind.

Receptors binding signals will cause a change in cells
Sometimes a gene is turned on, sometimes the message tells
A kinase to phosphorylate and start a big cascade
To nudge awake some enzymes, whose actions can pervade
The metabolic pathways and change the cell's routine
To death or cell division and everything between.

So cells have mechanisms to help communicate
To share a little gossip about their inner state
To tell a friend who's far away an enzyme to secrete
They do this all discreetly without the need to tweet
The chattiest of humans lie silent when in bed
But cells are always talking, unless, of course, they're dead.

Verse by Indira Rajagopal

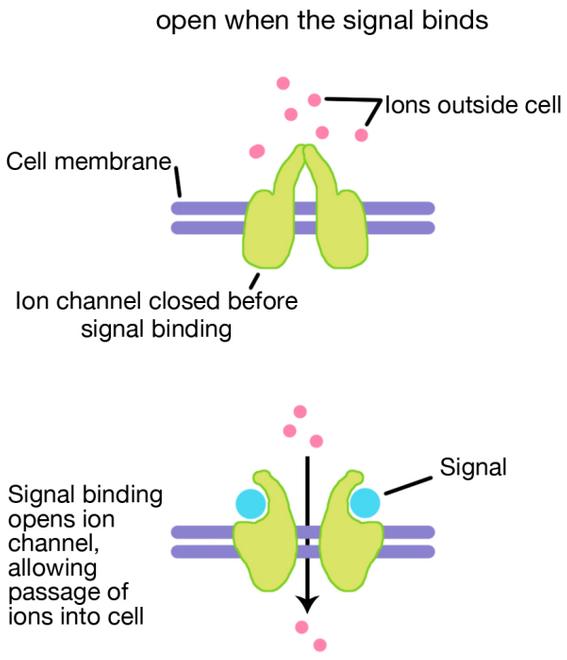
• The last of these messengers usually interacts with a target protein(s) and changes its activity, often by phosphorylation.

When a signal sets a particular pathway in motion, it is acting like an ON switch. This means that once the desired result has been obtained, the cell must have a mechanism that acts as an OFF switch.

Understanding this underlying similarity is helpful, because learning the details of the different pathways becomes merely a matter of identifying which molecular component performs a particular function in each individual case. We will consider several different signal transduction pathways, each mediated by a different kind of receptor. The first two examples we will examine are those with the fewest steps between the binding of the signal by a receptor and a cellular response.

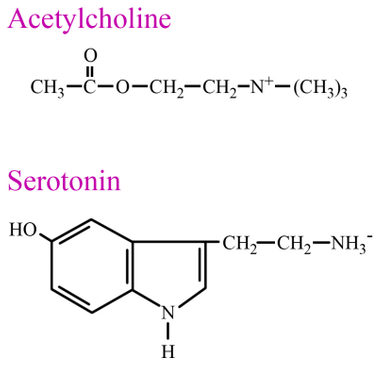
Ligand-gated Ion Channel Receptors

The simplest and fastest of signal pathways is seen in the case of signals whose receptors are gated ion channels. Gated ion channels are made up of multiple transmembrane proteins that create a pore, or channel, in the cell membrane. Depending upon its type, each ion channel is specific to the passage of a particular ionic species. The term "gated" refers to the fact that the ion channel is controlled by a "gate" which must be opened to allow the ions through. The gates are opened by the binding of an incoming signal (ligand) to the receptor, allowing the almost instantaneous passage of millions of ions from one side of the membrane to the other. Changes in the interior environment of the cell are thus brought about in microseconds and in a single step.



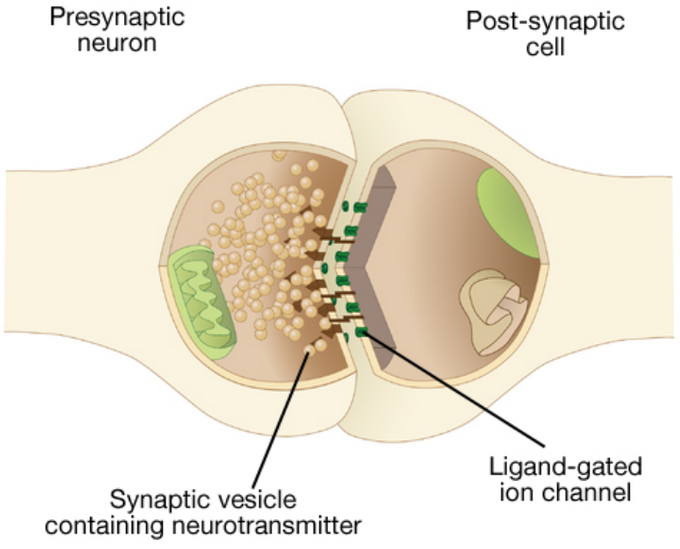
Signaling Through Gated Ion Channels

This type of swift response is seen, for example, in neuromuscular junctions, where muscle cells respond to a message from the neighboring nerve cell. The nerve cell releases a neurotransmitter signal into the synaptic cleft, which is the space between the nerve cell and the muscle cell it is "talking to". Examples of neurotransmitter signal molecules are acetylcholine and serotonin, shown above.



Neurotransmitters

When acetylcholine molecules are released into the synaptic cleft (the space between the pre- and post-synaptic cells) they diffuse rapidly till they reach their receptors on the membrane of the muscle cell. The binding of the acetylcholine to its receptor, an ion channel on the membrane of the muscle cell, causes the gate in the ion channel to open. The resulting ion flow through the channel can immediately



Signaling Across Nerve Cells

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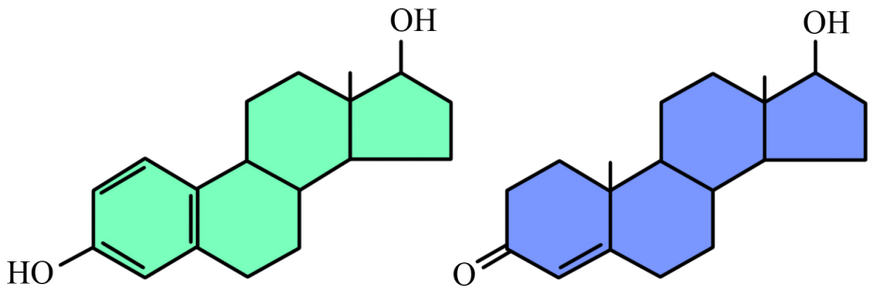
change the membrane potential. This, in turn, can trigger other changes in the cell. The speed with which changes are brought about in neurotransmitter signaling is evident when you think about how quickly you remove your hand from a hot surface. Sensory neurons carry information to the brain from your hand on the hot surface and motor neurons signal to your muscles to move the hand, in less time than it took you to read this sentence!

Nuclear Hormone Receptors

Another type of relatively simple, though much slower, signaling is seen in pathways in which the signals are steroid hormones, like estrogen or testosterone, pictured below. Steroid hormones, as you are aware, are related to cholesterol, and as hydrophobic molecules, they are able to cross the cell membrane by themselves. This is unusual, as most signals coming to cells are incapable of crossing the plasma membrane, and thus, must have cell surface receptors.



Estradiol Testosterone

By contrast, steroid hormones have receptors inside the cell (intracellular receptors). Steroid hormone receptors are proteins that belong in a family known as the nuclear receptors.

Nuclear hormone receptors are proteins with a double life: they are actually dormant transcription regulators. In the absence of signal, these receptors are in the cytoplasm, complexed with other proteins (HSP in the figure on the following page) and inactive. When a steroid hormone enters the cell, the nuclear hormone receptor binds the hormone and dissociates from the HSP. The receptors, then, with the hormone bound, translocate into the nucleus.

In the nucleus, they regulate the transcription of target genes by binding to their regulatory sequences (labeled HRE for hormone-response elements). The binding of the hormone-receptor complex to the regulatory elements of hormone-responsive genes modulates their expression. Because these responses involve gene expression, they are relatively slow.

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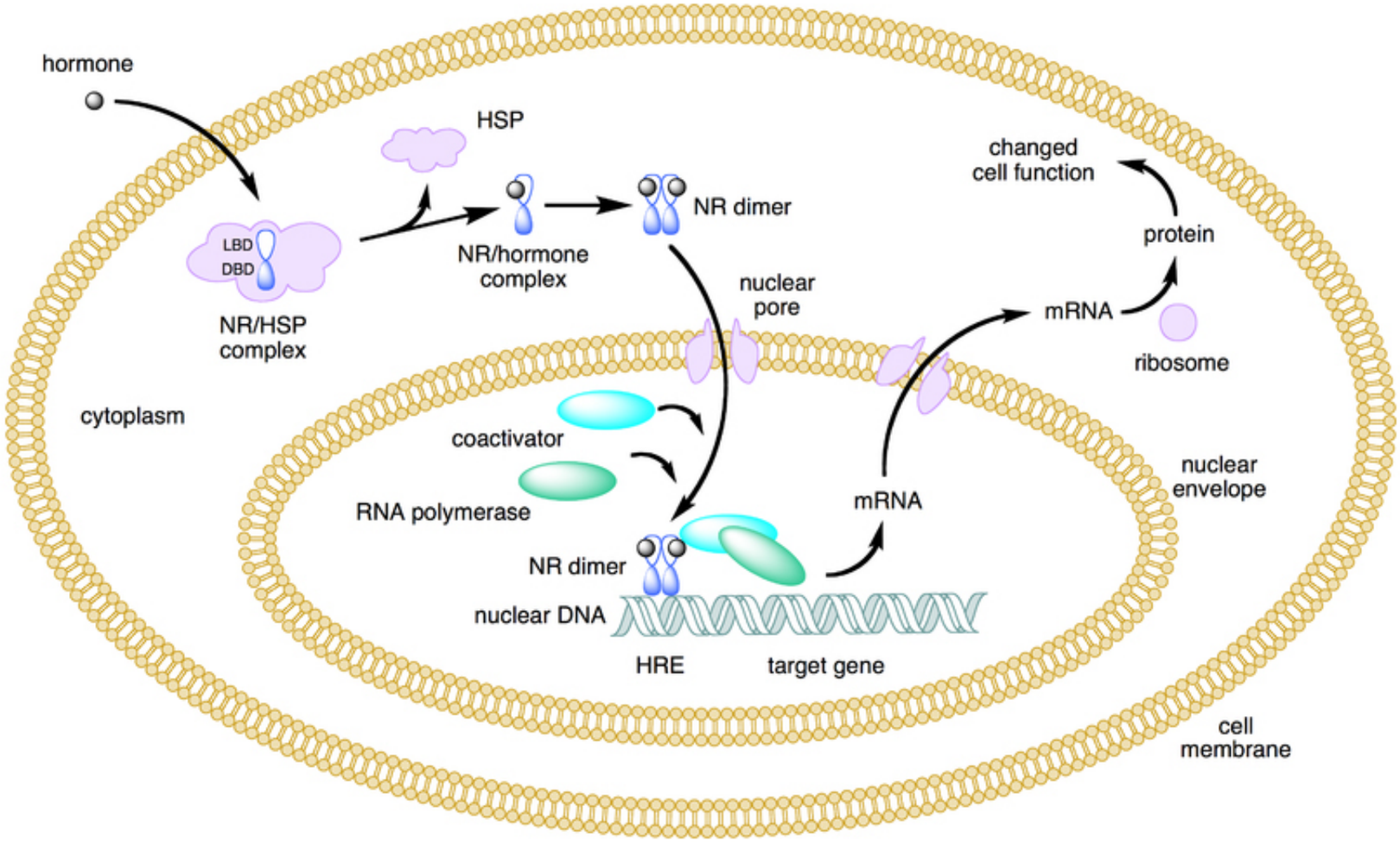
Most other signaling pathways, besides the two we have just discussed, involve multiple steps in which the original signal is passed on and amplified

through a number of intermediate steps, before the cell responds to the signal.

We will now consider two signaling pathways, each mediated by a major class of cell surface receptor- the G-protein coupled receptors (GPCRs) and the receptor tyrosine kinases (RTKs).

While the specific details of the signaling pathways that follow the binding of signals to each of these receptor types are different, it is easier to learn them when you can see what the pathways have in common, namely, interaction of the signal with a receptor, followed by relaying the signal through a variable number of intermediate molecules, with the last of these molecules interacting with target protein(s) to modify their activity in the cell.



Steroid Hormones Act by Modulating Expression of Hormone-responsive Genes

G-protein Coupled Receptors (GPCRs)

G-protein coupled receptors are involved in responses of cells to many different kinds of signals, from epinephrine, to odors, to light. In fact, a variety of physiological phenomena including vision, taste, smell and the fight-or-flight response are mediated by GPCRs.

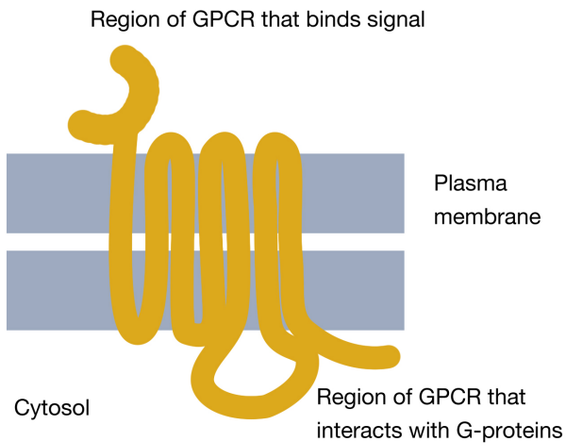
What are G-protein coupled receptors?

G-protein coupled

receptors are cell surface receptors that pass on the signals that they receive with the help of guanine nucleotide binding proteins (a.k.a. G-proteins). Before thinking any further about the signaling pathways downstream of GPCRs, it is necessary to know a few important facts about these receptors and the G-proteins that assist them.

Though there are hundreds of different G-protein coupled receptors, they all have the same basic structure:
they all consist of a single polypeptide chain that threads back and forth seven times through the lipid bilayer of the plasma membrane. For this reason, they are sometimes called seven-pass transmembrane (7TM) receptors.

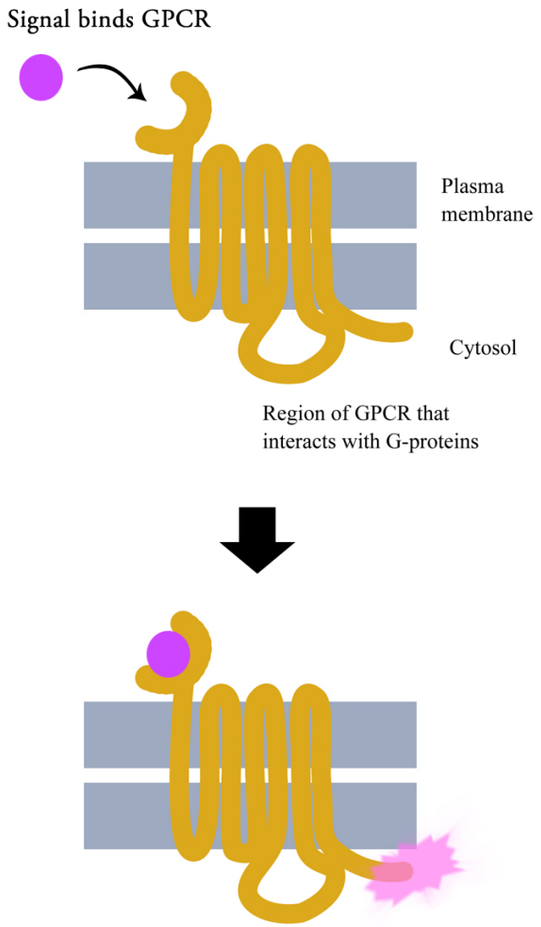
One end of the polypeptide forms the extracellular domain that binds the signal while the other end is in the cytosol of the cell.
When a ligand (signal) binds the extracellular domain of a GPCR, the receptor undergoes a conformational change that allows it to interact with a G-protein that will then pass the signal on to other intermediates in the signaling pathway.



G-protein Coupled Receptor

What is a G-protein?

As noted above, a G-protein is a guanine nucleotide-binding protein that can interact with a G-protein linked receptor. G-proteins are associated with the cytosolic side of the plasma membrane, where they are ideally



G-protein Coupled Receptor Signaling

situated to interact with the cytosolic tail of the GPCR, when a signal binds to the GPCR.

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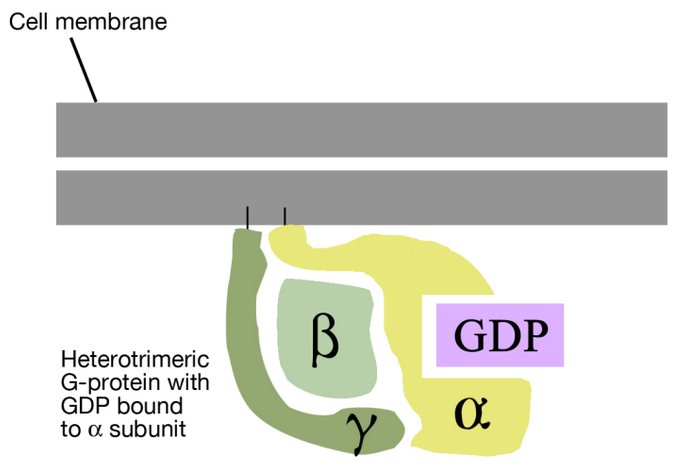
There are many different G-proteins, all of which share a characteristic structure- they are composed of three subunits called alpha, beta and gamma (αβγ). Because of this, they are sometimes called heterotrimeric G proteins (hetero=different, trimeric= having three parts). The α subunit of such proteins can bind GDP or GTP and is capable of hydrolyzing a GTP molecule bound to it into GDP. In the unstimulated state of the cell, that is, in the absence of a signal bound to the GPCR, the G-proteins are found in the trimeric form (αβγ bound together) and the α subunit has a GDP molecule bound to it.



G-protein with GDP Bound

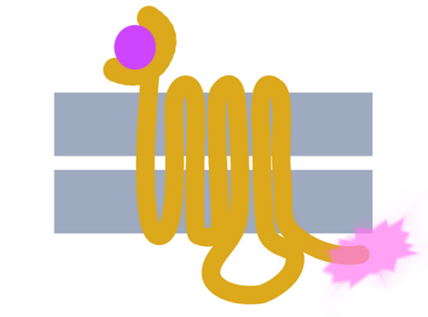
With this background on the structure and general properties of the GPCRs and the G-proteins, we can now look at what happens when a signal arrives at the cell surface and binds to a GPCR.

The binding of a signal molecule by the extracellular part of the G-protein linked receptor causes the cytosolic tail of the receptor to interact with, and alter the conformation of, a G-protein. This has two consequences:

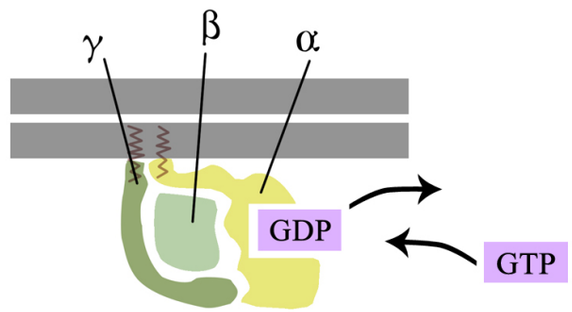
• First, the alpha subunit of the G-protein loses its GDP and binds a GTP instead.

• Second, the G-protein breaks up into the GTP-bound α part and the βγ part.

These two parts can diffuse freely along the cytosolic face of the plasma membrane and act upon their targets.



Binding of the signal to GPCR leads to a conformation change in the GPCR's tail, that results in the activation of a nearby G-protein that exchanges a GDP bound to its α subunit for a GTP.



G-protein Activation

What happens when G-proteins interact with their

target proteins?
That depends on what the target is. G-proteins interact with different kinds of target proteins, of which we will examine two major categories:

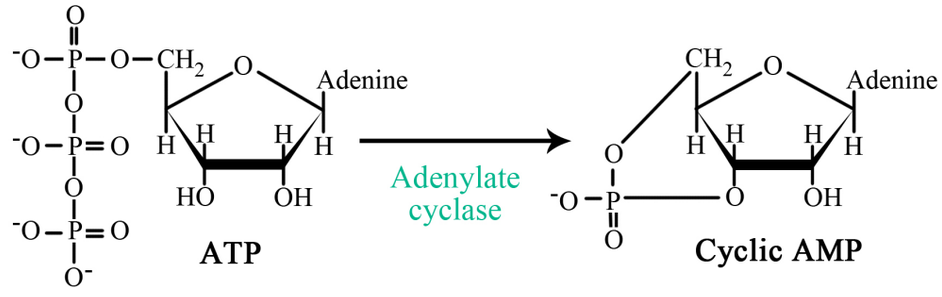
Ion Channels

We have earlier seen that some gated ion channels can be opened or closed by the direct binding of neurotransmitters to a receptor that is an ion-channel protein. In other cases, ion channels are regulated by the binding of G-proteins. That is, instead of the signal directly binding to the ion channel, it binds to a GPCR, which activates a G-protein that then binds and opens the ion channel. The change in the distribution of ions across the plasma membrane causes a change in the membrane potential.

Specific Enzymes

The interaction of G-proteins with their target enzymes can regulate the activity of the enzyme, either increasing or decreasing its activity. Often the target enzyme will pass the signal on in another form to another part of the cell. As you might imagine, this kind of response takes a little longer than the kind where an ion channel is opened instantaneously.

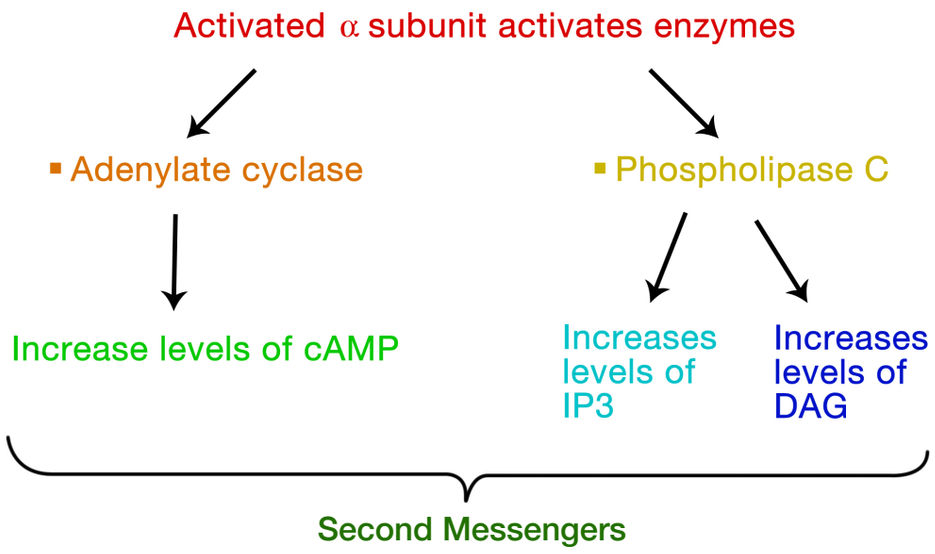
Two well-studied examples of enzymes whose activity is regulated by a G-protein are adenylate cyclase and phospholipase C. When adenylate cyclase is activated, the molecule cAMP is produced in large amounts.

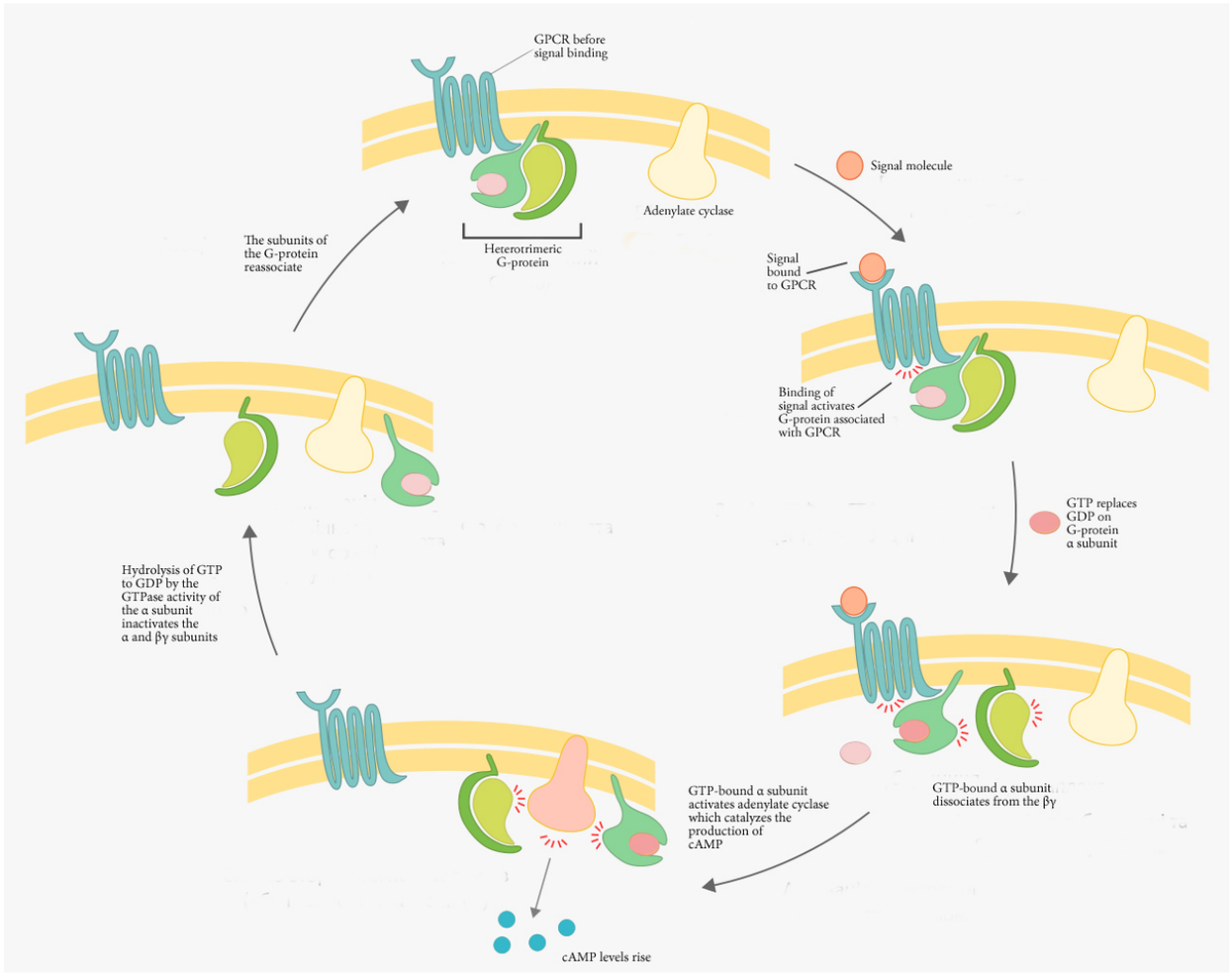


Synthesis of cAMP

When phospholipase C is activated, the molecules inositol trisphosphate (IP3) and diacylglycerol (DAG) are made. cAMP, IP3 and DAG are second messengers, small, diffusible molecules that can "spread the message" brought by the original signal, to other parts of the cell.

In these cases, the binding of a signal to the GPCR activated a G-protein, which in turn, activated an enzyme that makes a second messenger that can amplify the message in the cell.





G-protein Signaling Cycle

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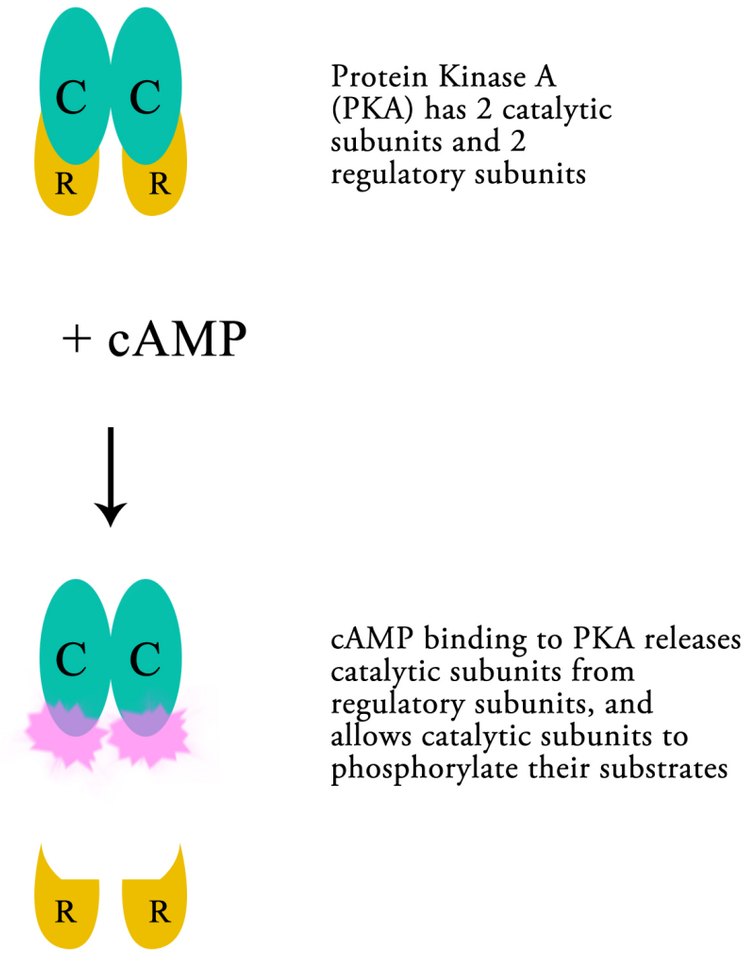


Protein Kinase A Activation

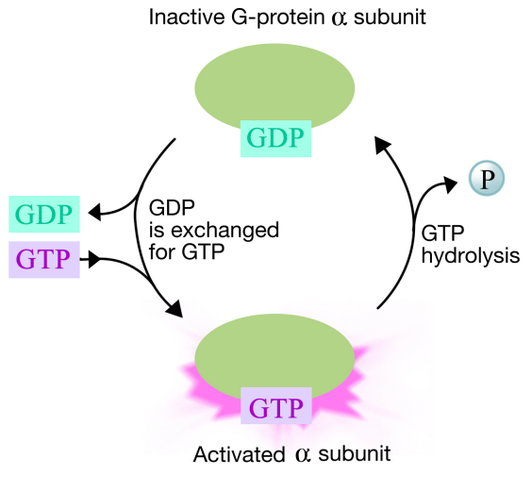
We will first trace the effects of activating adenylate cyclase and the resulting increase in cAMP.

What is the effect of elevated cAMP levels?

cAMP molecules bind to, and activate an enzyme, protein kinase A (PKA). PKA is composed of two catalytic and two regulatory subunits that are bound tightly together. Upon binding of cAMP the catalytic subunits are released from the regulatory subunits,

allowing the enzyme to carry out its function, namely phosphorylating other proteins.

Thus, cAMP can regulate the activity of PKA, which in turn, by phosphorylating other proteins can change their activity. The targets of PKA may be enzymes that are activated by phosphorylation, or they may be proteins that regulate transcription. The phosphorylation of a transcriptional activator, for example, may cause the activator to bind to a regulatory sequence on DNA and to increase the transcription of the gene it controls. The activation of previously inactive enzymes alters the state of the cell by changing the reactions that are occurring within the cell.



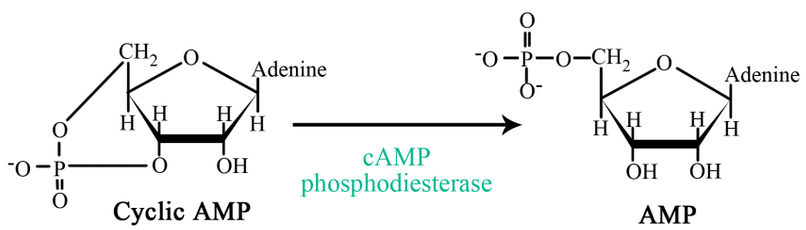
G-protein Nucleotide Swapping

For example, the binding of epinephrine to its receptor on the cell surface, activates, through the action of G-proteins, and subsequent activation of PKA, the phosphorylation of glycogen phosphorylase. The resulting activation of glycogen phosphorylase leads to the breakdown of glycogen, releasing glucose (in the form of glucose-1-phosphate) for use by the cell. Changes in gene expression, likewise, lead to changes in the cell by altering the production of particular proteins in response to the signal.

Although the steps described above seem complicated, they follow the simple pattern outlined at the beginning of this section:

- Binding of signal to receptor
- Several steps where the signal is passed on through intermediate molecules (G-proteins, adenylate cyclase, cAMP, and finally, PKA)
- Phosphorylation of target proteins by the kinase, leading to changes in the cell.

Finally, if the signal binding to the receptor serves as a switch that sets these events in motion, there must be mechanisms to turn the pathway off. The first is at the level of the G-protein. Recall that the alpha subunit of the G-protein is in its free and activated state when it has GTP bound and that it associates with the beta-gamma subunits and has a GDP bound when it is inactive. We also know that the alpha subunit has an activity that enables it to hydrolyze GTP to GDP, as shown in the figure above left. This GTP-hydrolyzing activity makes it possible for the alpha subunit, once it has completed

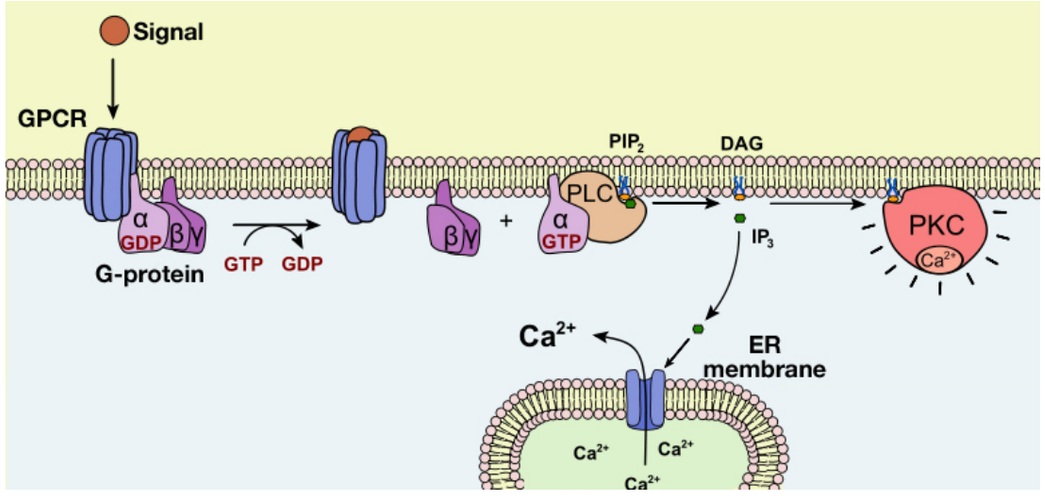


cAMP Breakdown

its task, to return to its GDP bound state, re-associate with the beta-gamma part and become inactive again.

The second "off switch" is further down the signaling pathway, and controls the level of cAMP. We just noted that cAMP levels increase when adenylate cyclase is activated. When its job is done, cAMP is broken down by an enzyme called phosphodiesterase. When cAMP levels drop, PKA returns to its inactive state, putting a halt to the changes brought about by the activation of adenylate cyclase by an activated G-protein.

Let us now examine the events that follow the activation of Phospholipase C (PLC) by a G-protein. As we noted earlier, the activation of PLC results in the production of the second messengers IP_3 and DAG. What do these molecules do?



Phospholipase C Signaling

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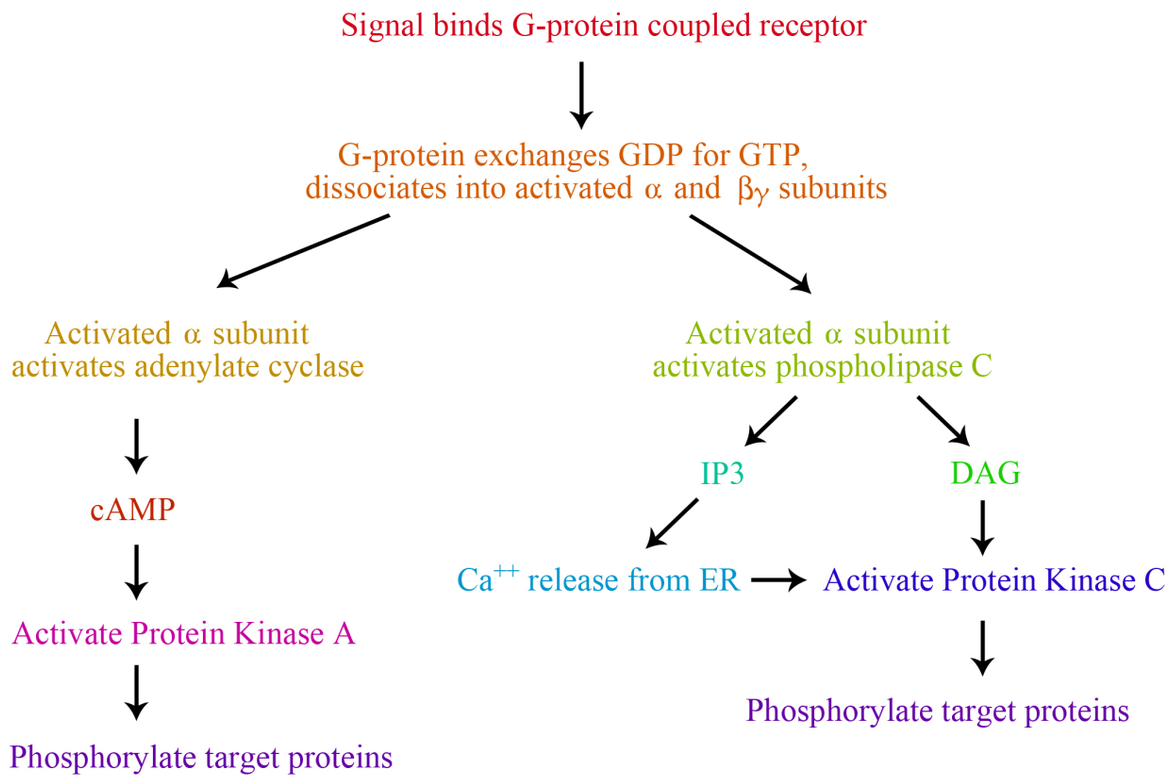

Signaling Outcomes

The IP_3 and DAG produced by activated phospholipase C work together to activate a protein kinase. First, IP_3 diffuses to the endoplasmic reticulum membrane where it binds to gated calcium ion channels. This causes calcium channels in the ER membrane to open and release large amounts of calcium into the cytoplasm from the ER lumen, as shown in the figure below.

The increase in cytosolic calcium ion concentration has various effects, one of which is to

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activate a protein kinase called protein kinase C (C for calcium), together with the DAG made in the earlier step. Like PKA, Protein kinase C phosphorylates a variety of proteins in the cell, altering their activity and thus changing the state of the cell.

The pathways leading to PKC and PKA activation following the binding of a signal to a GPCR are summarized above.

Receptor Tyrosine Kinases

Receptor tyrosine kinases mediate responses to a large number of signals, including peptide hormones like insulin and growth factors like epidermal growth factor.

Gee, I Wish I Could Do That

GPCRs, as you know,
Have a G-protein in tow.
Alpha, beta, gamma, sit
Waiting till the signals hit.

When receptors signals bind,
G-proteins respond in kind,
Swapping out their GDP
For triphosphate gleefully.

Alpha, with its GTP,
Leaves all full of energy.
A cyclase it will activate
That acts upon adenylate.

Cyclic AMPs then find
A PKA that they can bind.
With binding of cAMPs
R subunits part from Cs.

C subunits, floating free,
Are on a phosphate adding spree.
PKA phosphorylates
And target proteins activates

Another enzyme, PLC,
Awaits an alpha-GTP
To activate it, so it splits
PIP2, and makes two bits.

IP3 and DAG
Are second messengers, you see.
They work together as a team,
They've got a cunning little scheme.

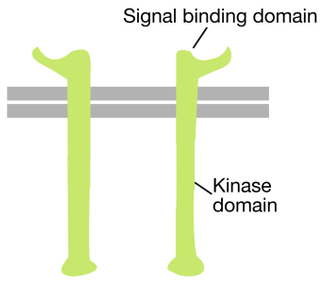
IP3 at ion gates
On ER membranes lets a spate
Of calcium into cytosol
That's bad enough, but that's not all.

The calcium binds a PKC,
And with the help of DAG,
The kinase it can activate
Proteins to phosphorylate.

The binding of a signal small
To GPCRs, caused this all.
Transducing pathways work a spell
And change the actions of a cell.

Verse by Indira Rajagopal

Like the GPCRs, receptor tyrosine kinases bind a signal, then pass the message on through a series of intracellular molecules, the last of which acts on target proteins to change the state of the cell.

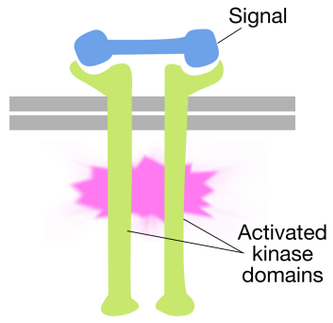


Receptor Tyrosine Kinase before signal binding

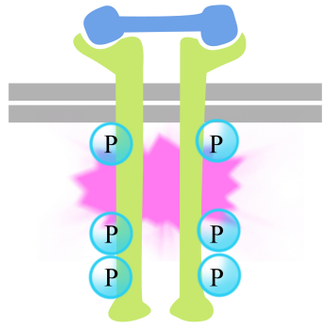
As the name suggests, a receptor tyrosine kinase is a cell surface receptor that also has a tyrosine kinase activity. The signal binding domain of the receptor tyrosine kinase is on the cell surface, while the tyrosine kinase enzymatic activity resides in the cytoplasmic part of the protein (see figure above). A transmembrane alpha helix connects these two regions of the receptor.

What happens when signal molecules bind to receptor tyrosine kinases?

Binding of signal molecules to the extracellular domains of receptor tyrosine kinase molecules causes two receptor molecules to dimerize (come together and associate). This brings the cytoplasmic tails of the receptors close to each other and causes the tyrosine kinase activity of these tails to be turned on. The activated tails then phosphorylate each other on several tyrosine residues. This is called autophosphorylation.



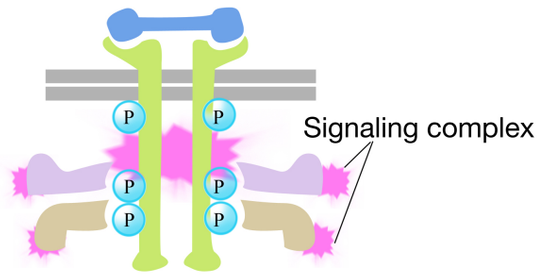
Signal binding causes dimerization of receptor and activation of tyrosine kinase domains



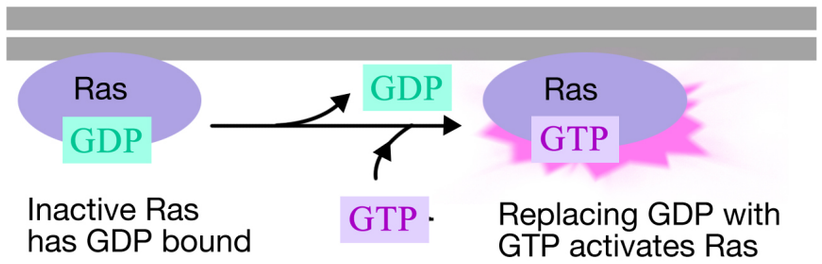
Activated tyrosine kinase domains add phosphate onto each other

The phosphorylation of tyrosines on the receptor tails triggers the assembly of an intracellular signaling complex on the tails. The newly phosphorylated tyrosines serve as binding sites for signaling proteins that then pass the message on to yet other proteins. An important protein that is subsequently activated by the signaling complexes on the receptor tyrosine kinases is called Ras.

The Ras protein is a monomeric guanine nucleotide binding protein that is associated with the cytosolic face of the plasma membrane (in fact, it is a lot like the alpha subunit of trimeric G-proteins). Just like the alpha subunit of a G-protein, Ras is active



Complex of signaling proteins assembles on phosphorylated RTK tails. This complex can activate Ras.



Ras Activation

when GTP is bound to it and inactive when GDP is bound to it. Also, like the alpha subunit, Ras can hydrolyze the GTP to GDP.

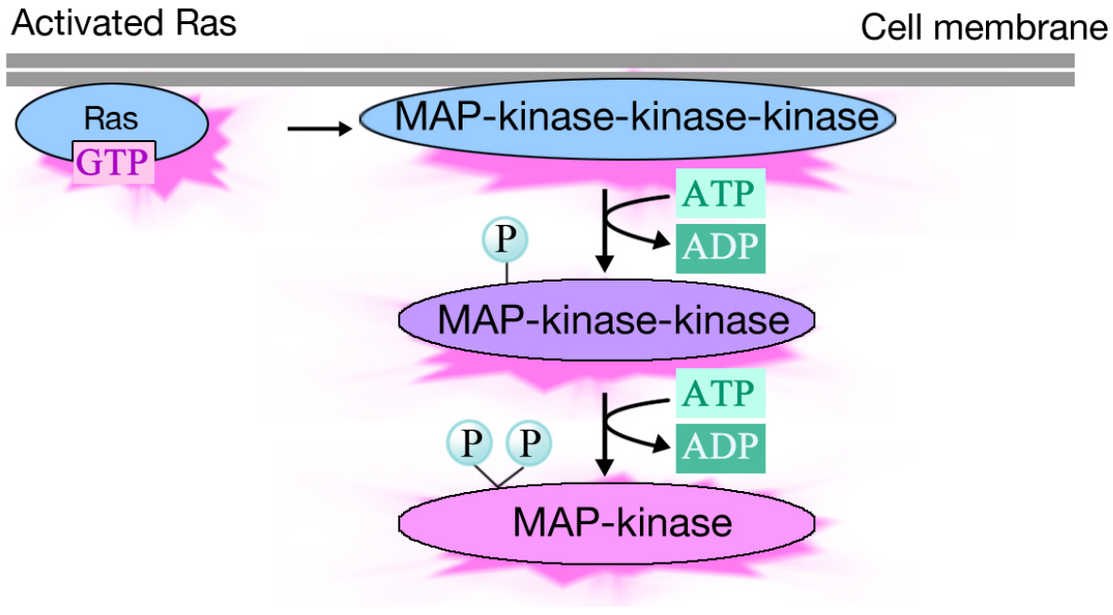
When a signal arrives at the receptor tyrosine kinase, the receptor monomers come together and phosphorylate each others' tyrosines, triggering the assembly of a complex of proteins on the cytoplasmic tail of the receptor. One of the proteins in this complex interacts with Ras and stimulates the exchange of the GDP bound to the inactive Ras for a GTP. This activates the Ras.

Activated Ras triggers a phosphorylation cascade of three protein kinases, which relay and distribute the signal. These protein kinases are members of a group called the MAP kinases (Mitogen Activated Protein Kinases). The final kinase in this cascade phosphorylates various target proteins, including enzymes and transcriptional activators that regulate gene expression.

The phosphorylation of various enzymes can alter their activities, and set off new chemical reactions in the cell, while the phosphorylation of transcriptional activators can change which genes are expressed. The combined effect of changes in gene expression and protein activity alter the cell's physiological state.

Once again, in following the path of signal transduction mediated by RTKs, it is possible to discern the same basic pattern of events: a signal is bound by the extracellular domains of receptor tyrosine kinases, resulting in receptor dimerization and autophosphorylation of the cytosolic tails, thus conveying the message to the interior of the cell.

The message is passed on *via* a signalling complex to Ras which then stimulates a series of kinases. The terminal kinase in the cascade acts on target proteins and brings about in changes in protein activities and gene expression.



Activated Ras Cascade

The descriptions above provide a very simple sketch of some of the major classes of receptors and deal primarily with the mechanistic details of the steps by which signals received by various types of receptors bring about changes in cells. A major take-home lesson is the essential similarity of the different pathways.

Another point to keep in mind is that while we have looked at each individual pathway in isolation, a cell, at any given time receives multiple signals that set off a variety of different responses at once. The pathways described above show a considerable degree of "cross-talk" and the response to any given signal is affected by the other signals that the cell receives simultaneously. The multitude of different receptors, signals and the combinations thereof are the means by which cells are able to respond to an enormous variety of different circumstances.

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The Tao of Hormones

To the tune of "*The Sound of Silence*"

Biochemistry my friend
It's time to study you again
Mechanisms that I need to know
Are the things that really stress me so
"Get these pathways planted firmly in your head,"
Ahern said

Let's start with ep-inephrine
Membrane proteins are well known
Changed on binding this hormone
Rearranging selves without protest
Stimulating a G alpha S
To go open up and displace its GDP
With GTP

Because of ep-inephrine
Active G then moves a ways
Stimulating ad cyclase
So a bunch of cyclic AMP
Binds to kinase and then sets it free
All the active sites of the kinases await
Triphosphate

Because of ep-inephrine
Muscles are affected then
Breaking down their glycogen
So they get a wad of energy
In the form of lots of G-1-P
And the synthases that could make a glucose chain
All refrain

Because of ep-inephrine
Now I've reached the pathway end
Going from adrenalin
Here's a trick I learned to get it right
Linking memory to flight or fright
So the mechanism that's the source of anxious fears
Reappears
When I make ep-inephrine

Recorded by Tim Karplus
Lyrics by Kevin Ahern