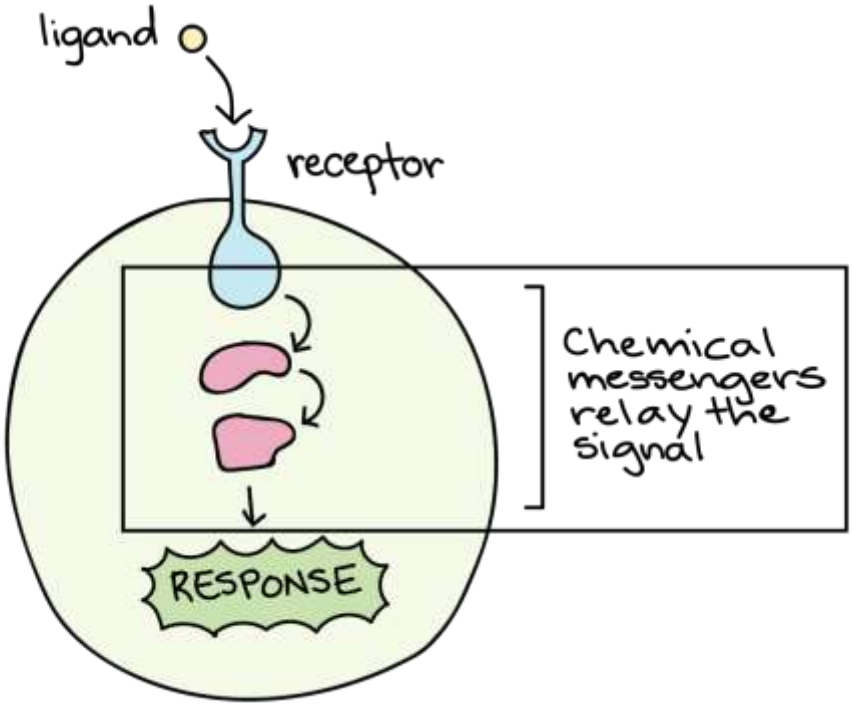
Signal relay pathways

Learn how signals are relayed inside a cell starting from the cell membrane receptor. The chains of molecules that relay intracellular signals are known as intracellular signal transduction pathways.

Introduction

Once a signalling molecule (ligand) from one cell has bound to a receptor on another cell, is the signalling process complete?

If we're talking about intracellular receptors, which bind their ligand inside of the cell and directly activate genes, the answer may be yes. In most cases, though, the answer is no—not by a long shot! For receptors located on the cell membrane, the signal must be passed on through other molecules in the cell, in a sort of cellular game of "telephone."



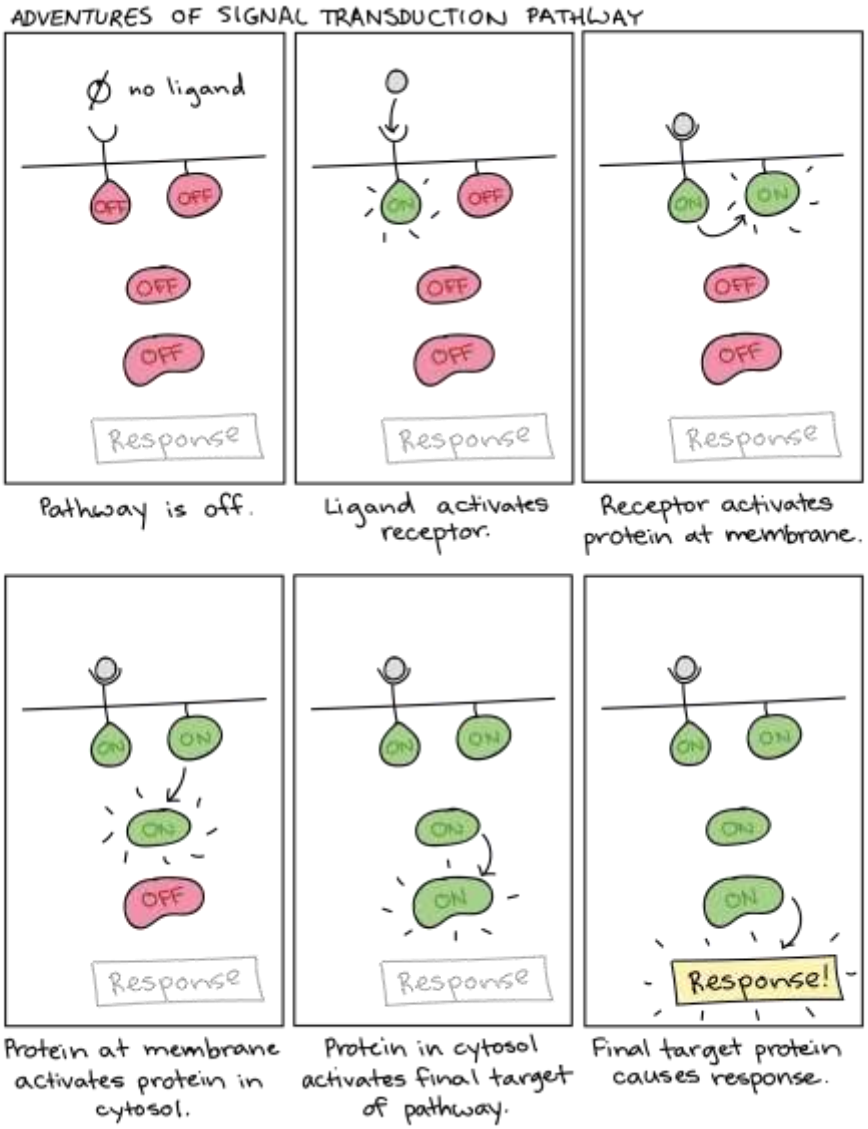
The chains of molecules that relay signals inside a cell are known as **intracellular signal transduction pathways**. Here, we'll look at the general characteristics of intracellular signal transduction pathways, as well as some relay mechanisms commonly used in these pathways.

Binding initiates a signalling pathway

When a ligand binds to a cell-surface receptor, the receptor's intracellular domain (part inside the cell) changes in some way. Generally, it takes on a new shape, which may make it active as an enzyme or let it bind other molecules.

The change in the receptor sets off a series of signalling events. For instance, the receptor may turn on another signalling molecule inside of the cell, which in turn activates its own target. This

chain reaction can eventually lead to a change in the cell's behaviour or characteristics, as shown in the cartoon below.



Cartoon-style schematic showing how the components of a hypothetical signalling pathway are activated sequentially, with one turning on the next to produce a cellular response.

Because of the directional flow of information, the term **upstream** is often used to describe molecules and events that come earlier in the relay chain, while **downstream** may be used to describe those that come later (relative to a particular molecule of interest). For instance, in the diagram, the receptor is downstream of the ligand but upstream of the proteins in the cytosol. Many signal transduction pathways amplify the initial signal, so that one molecule of ligand can lead to the activation of many molecules of a downstream target.

The molecules that relay a signal are often proteins. However, non-protein molecules like ions and phospholipids can also play important roles.

Phosphorylation

The cartoon above features a bunch of blobs (signalling molecules) labelled as “on” or “off.” What does it actually mean for a blob to be on or off? Proteins can be activated or inactivated in a variety of ways. However, one of the most common tricks for altering protein activity is the addition of a phosphate group to one or more sites on the protein, a process called **phosphorylation**.

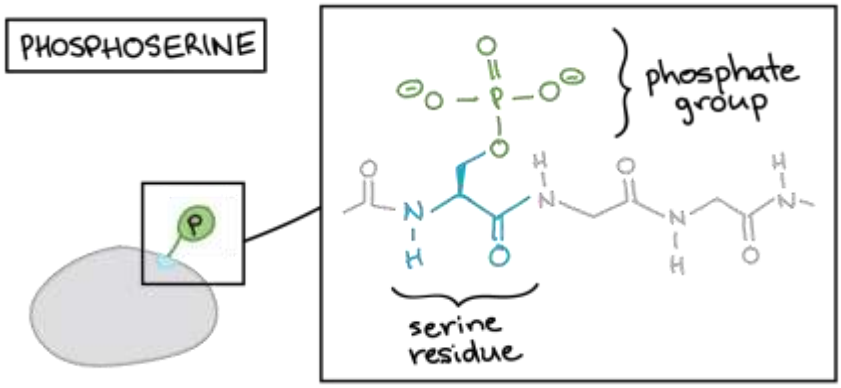


Diagram of a phosphorylated protein bearing a phosphate group attached to a serine residue, showing the actual chemical structure of the linkage.

Phosphate groups can’t be attached to just any part of a protein. Instead, they are typically linked to one of the three amino acids that have hydroxyl (-OH) groups in their side chains: tyrosine, threonine, and serine. The transfer of the phosphate group is catalysed by an enzyme called a **kinase**, and cells contain many different kinases that phosphorylate different targets.

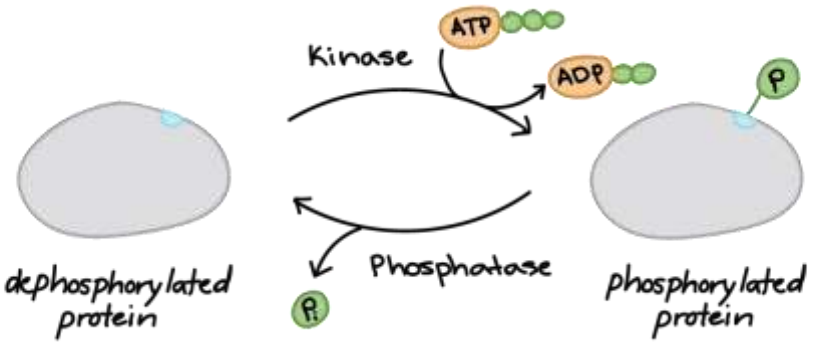
Phosphorylation often acts as a switch, but its effects vary among proteins. Sometimes, phosphorylation will make a protein more active (for instance, increasing catalysis or letting it bind to a partner). In other cases, phosphorylation may inactivate the protein or cause it to be broken down.

How can adding one little phosphate group have such a big effect on protein behaviour? The answer comes down to basic biochemistry: adding a phosphate group attaches a big cluster of negative charge to the surface of the protein.

This negative charge may attract or repel amino acids within the protein itself, changing its shape. Because a protein’s function depends on its structure, changing the shape of the protein may alter its ability to work as an enzyme, either increasing or decreasing activity. Alternatively, phosphorylation may provide a docking site for an interaction partner (say, one with a bunch of positive charges), or prevent another partner from binding.

These are just a few examples, but they give a sense of how the phosphate group can directly affect the chemical behaviour of a protein.

In general, phosphorylation isn’t permanent. To flip proteins back into their non-phosphorylated state, cells have enzymes called **phosphatases**, which remove a phosphate group from their targets.

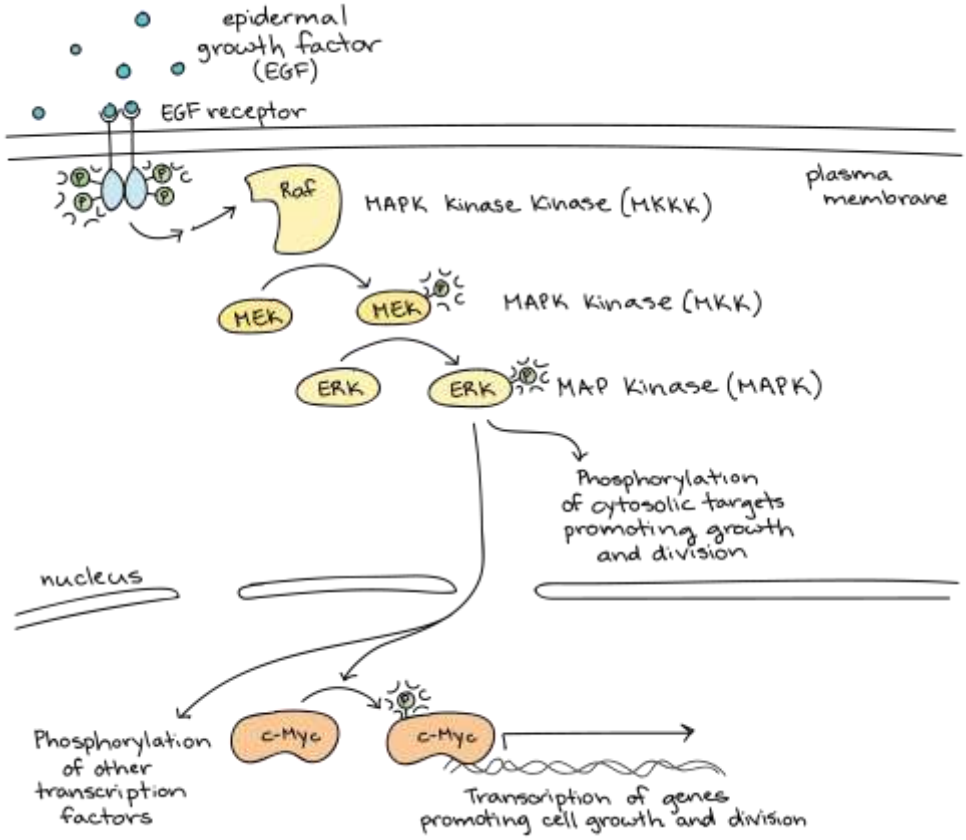


Cartoon-style diagram showing how a protein is phosphorylated by a kinase through the addition of a phosphate from ATP, producing ADP as a by-product, and dephosphorylated by a phosphatase, releasing Pi (inorganic phosphate) as a by-product. The two reactions make up a cycle in which the protein toggles between two states.

Phosphorylation example: MAPK signalling cascade

To get a better sense of how phosphorylation works, let’s examine a real-life example of a signalling pathway that uses this technique: growth factor signalling. Specifically, we'll look at part of the epidermal growth factor (EGF) pathway that acts through a series of kinases to produce a cellular response.

This diagram shows part of the epidermal growth factor signalling pathway:



Phosphorylation (marked as a P) is important at many stages of this pathway.

- When growth factor ligands bind to their receptors, the receptors pair up and act as kinases, attaching phosphate groups to one another's intracellular tails. Read more in the article on receptors and ligands.
- The activated receptors trigger a series of events (skipped here because they don't involve phosphorylation). These events activate the kinase Raf.
- Active Raf phosphorylates and activates MEK, which phosphorylates and activates the ERKs.
- The ERKs phosphorylate and activate a variety of target molecules. These include transcription factors, like c-Myc, as well as cytoplasmic targets. The activated targets promote cell growth and division.

Together, Raf, MEK, and the ERKs make up a three-tiered kinase signalling pathway called a **mitogen-activated protein kinase** (**MAPK**) cascade. (A *mitogen* is a signal that causes cells to undergo *mitosis*, or divide.) Because they play a central role in promoting cell division, the genes encoding the growth factor receptor, Raf, and c-Myc are all proto-oncogenes, meaning that overactive forms of these proteins are associated with cancer.

MAP kinase signalling pathways are widespread in biology: they are found in a wide range of organisms, from humans to yeast to plants. The similarity of MAPK cascades in diverse organisms suggests that this pathway emerged early in the evolutionary history of life and was already present in a common ancestor of modern-day animals, plants, and fungi.

Second messengers

Although proteins are important in signal transduction pathways, other types of molecules can participate as well. Many pathways involve **second messengers**, small, non-protein molecules that pass along a signal initiated by the binding of a ligand (the "first messenger") to its receptor.

Second messengers include Ca_2+ ions; cyclic AMP (cAMP), a derivative of ATP; and inositol phosphates, which are made from phospholipids.

Calcium ions

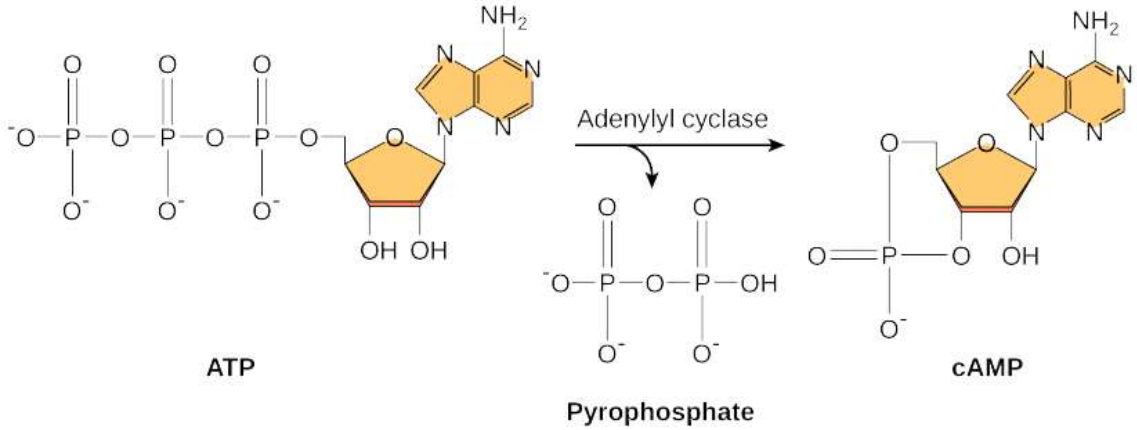
Calcium ions are a widely used type of second messenger. In most cells, the concentration of calcium ions (Ca_2+) in the cytosol is very low, as ion pumps in the plasma membrane continually work to remove it. For signalling purposes, Ca_2+ may be stored in compartments such as the endoplasmic reticulum.

In pathways that use calcium ions as a second messenger, upstream signalling events release a ligand that binds to and opens ligand-gated calcium ion channels. These channels open and allow the higher levels of Ca_2+ that are present outside the cell (or in intracellular storage compartments) to flow into the cytoplasm, raising the concentration of cytoplasmic Ca_2+.

How does the released Ca_2+ help pass along the signal? Some proteins in the cell have binding sites for Ca_2+ ions, and the released ions attach to these proteins and change their shape (and thus, their activity). The proteins present and the response produced are different in different types of cells. For instance, Ca_2+ signalling in the β-cells of the pancreas leads to the release of insulin, while Ca_2+ signalling in muscle cells leads to muscle contraction.

Cyclic AMP (cAMP)

Another second messenger used in many different cell types is **cyclic adenosine monophosphate** (**cyclic AMP** or **cAMP**), a small molecule made from ATP. In response to signals, an enzyme called **adenylyl cyclase** converts ATP into cAMP, removing two phosphates and linking the remaining phosphate to the sugar in a ring shape.



Reaction that converts ATP to cAMP. Shows the structures of the molecules.

Once generated, cAMP can activate an enzyme called **protein kinase A** (**PKA**), enabling it to phosphorylate its targets and pass along the signal. Protein kinase A is found in a variety of types of cells, and it has different target proteins in each. This allows the same cAMP second messenger to produce different responses in different contexts.

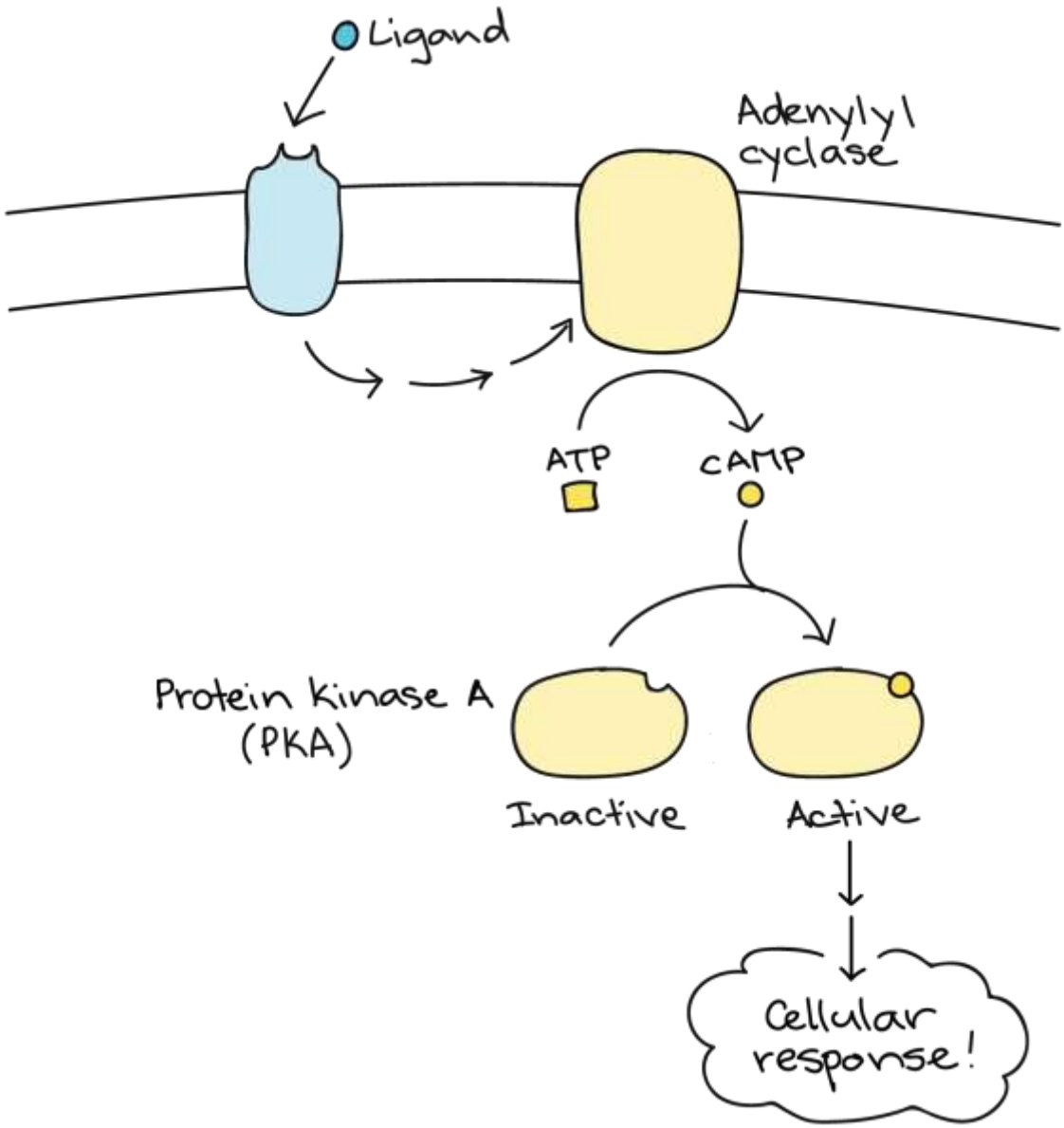


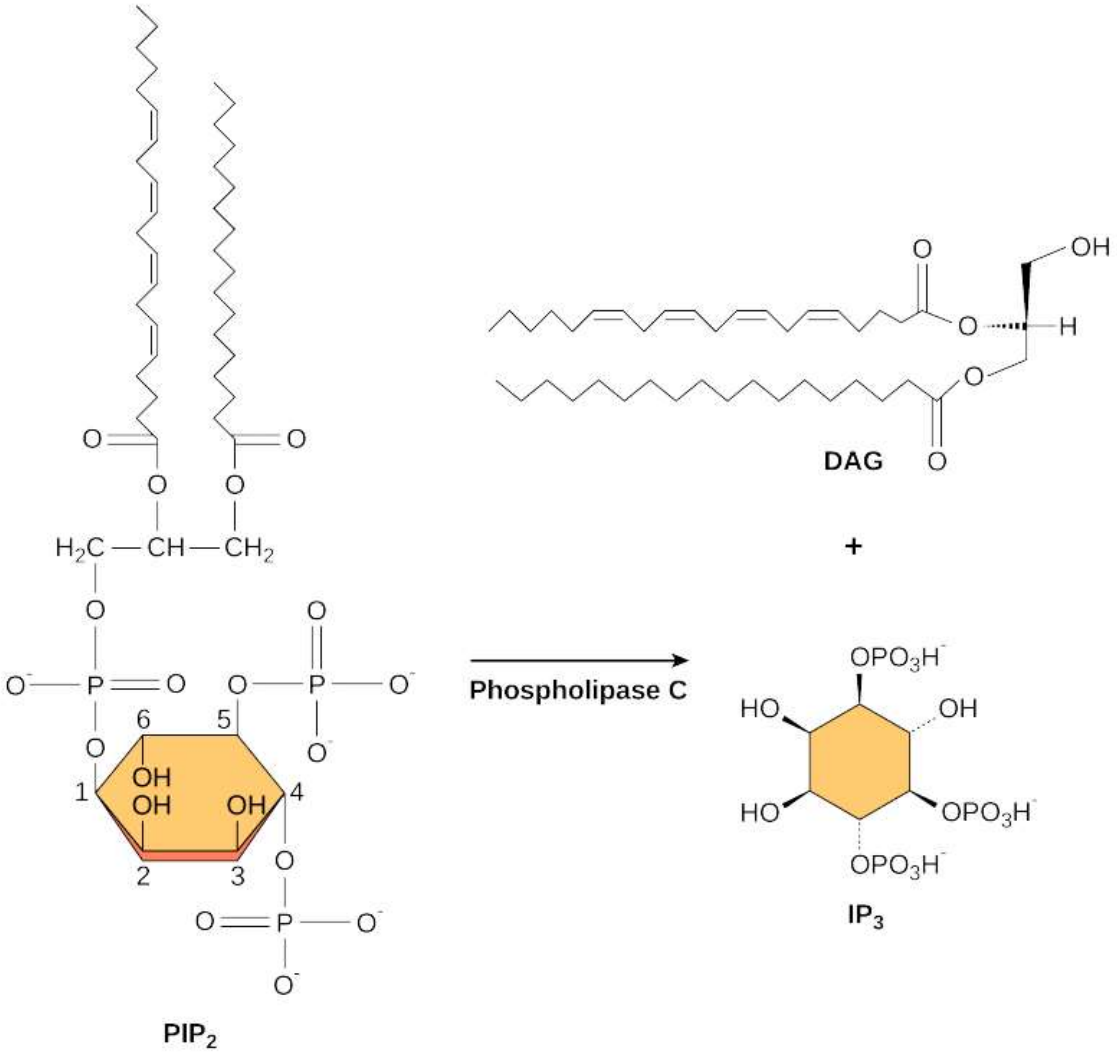
Diagram of a pathway that uses cAMP as a second messenger. A ligand binds to a receptor, leading indirectly to activation of adenylyl cyclise, which converts ATP to cAMP. cAMP binds to protein kinase A and activates it, allowing PKA to phosphorylate downstream factors to produce a cellular response.

cAMP signalling is turned off by enzymes called **phosphodiesterases**, which break the ring of cAMP and turn it into adenosine monophosphate (AMP).

Inositol phosphates

Although we usually think of plasma membrane phospholipids as structural components of the cell, they can also be important participants in signalling. Phospholipids called **phosphatidylinositols** can be phosphorylated and snipped in half, releasing two fragments that both act as second messengers.

One lipid in this group that's particularly important in signalling is called $PIP2$. In response to a signal, an enzyme called phospholipase C cleaves (chops) $PIP2$ into two fragments, DAG and $IP3$. These fragments made can both act as second messengers.



Cleavage of PIP2 to produce DAG and IP3. Shows the structures of the various molecules.

DAG stays in the plasma membrane and can activate a target called protein kinase C (PKC), allowing it to phosphorylate its own targets. $IP3$ diffuses into the cytoplasm and can bind to ligand-gated calcium channels in the endoplasmic reticulum, releasing Ca_2+ that continues the signal cascade.

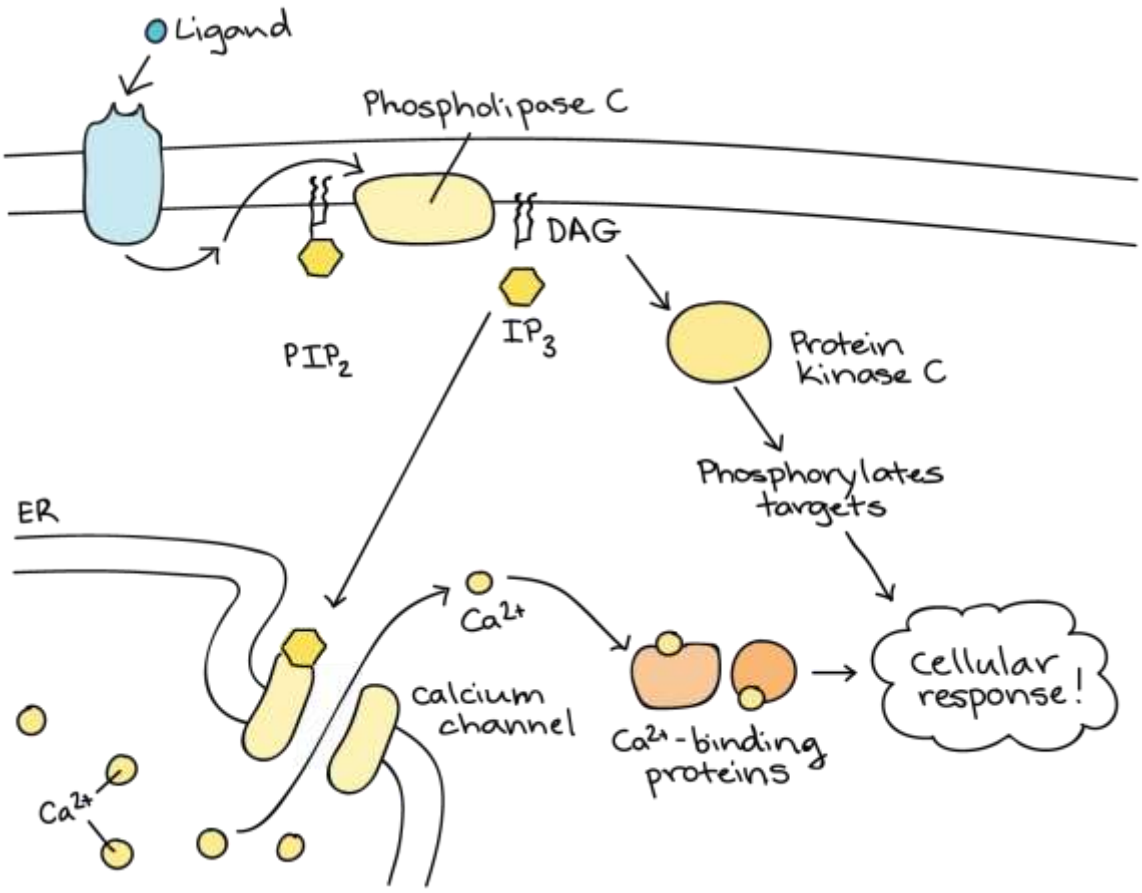
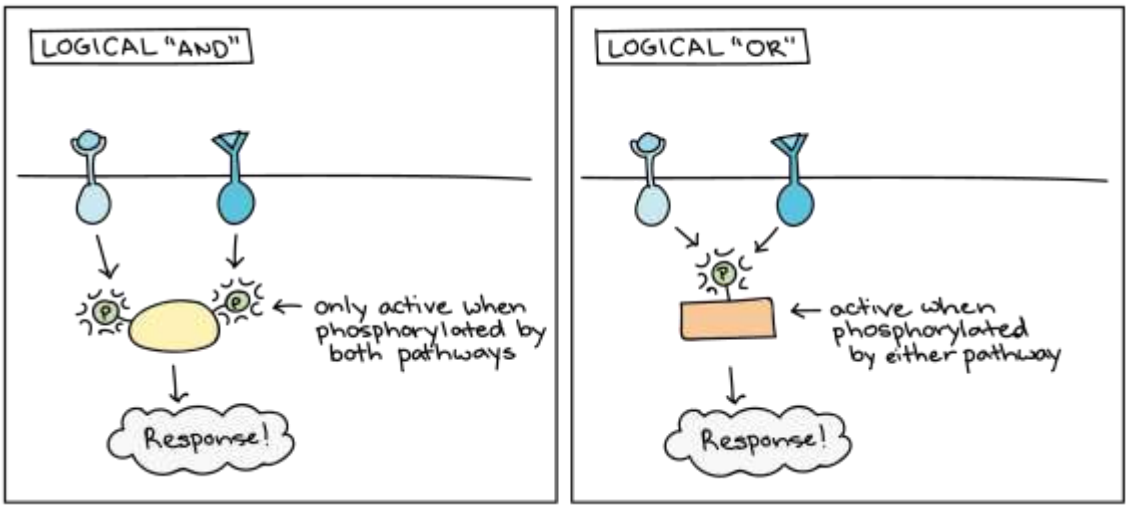


Image of a signalling pathway that uses inositol triphosphate and calcium ions as second messengers. After a ligand binds to a receptor at the membrane, phospholipase C is indirectly activated. It cleaves PIP2 to produce IP3 and DAG. DAG stays in the membrane and activates protein kinase C, which phosphorylates its targets. The IP3 is released into the cytosol and binds to a calcium ion channel in the endoplasmic reticulum, causing the channel to open. Calcium ions stored in the endoplasmic reticulum rush into the cytosol, where they bind to calcium-binding proteins. The calcium-binding proteins trigger a cellular response.

And...it's even more complicated than that!

Signalling pathways can get very complicated very quickly. For instance, the full version of the epidermal growth factor signalling pathway we saw earlier looks like a huge hairball and takes up an entire poster if you try to draw it out! You can see this for yourself in Sal's video on the MAPK pathway.

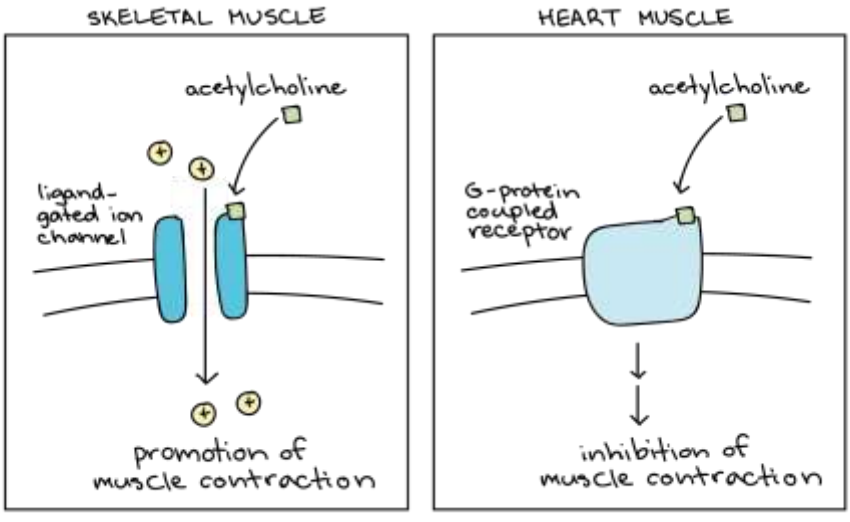
This complexity arises because pathways can, and often do, interact with other pathways. When pathways interact, they basically allow the cell to perform logic operations and "calculate" a best response to multiple sources of information. For instance, signals from two different pathways may be needed to activate a response, which is like a logical "AND." Alternatively, *either* of two pathways may trigger the same response, which is like a logical "OR."



Left diagram: logical "AND" in a cell signalling pathway. An intermediate must be phosphorylated on two different residues, one targeted by each of two pathways, in order to become active and produce a response. The response only occurs if the first pathway AND the second pathway are active.

Right diagram: logical "OR" in a cell signalling pathway. An intermediate must phosphorylated on a single residue in order to become active and produce a response, and either of two pathways can phosphorylate the same residue. The response occurs if the first pathway OR the second pathway is active.

Another source of complexity in signalling is that the same signalling molecule may produce different results depending on what molecules are already present in the cell. For example, the ligand acetylcholine causes opposite effects in skeletal and heart muscle because these cell types produce different kinds of acetylcholine receptors that trigger different pathways.



Cell type specificity in response to acetylcholine.

Left panel: skeletal muscle cell. The acetylcholine molecule binds to a ligand-gated ion channel, causing it to open and allowing positively charged ions to enter the cell. This event promotes muscle contraction.

Right panel: cardiac muscle cell. The acetylcholine molecule binds to a G protein-coupled receptor, triggering a downstream response that leads to inhibition of muscle contraction.

These are just a few examples of the complexities that make signalling pathways challenging, but also fascinating, to study. Cell-cell signalling pathways, especially the epidermal growth factor pathway we saw earlier, are a focus of study for researchers developing new drugs against cancer.