Cell signaling
Chapter 15

Signaling Through G-Protein-Coupled Receptors

SIGNALING THROUGH G-PROTEIN-COUPLED RECEPTORS

- **G-protein-coupled receptors (GPCRs)** form the **largest family of cell-surface receptors**, and they mediate **most responses to signals** from the external world, as well as signals from other cells, including hormones, neurotransmitters, and local mediators.

- Our senses of sight, smell, and taste depend on them.

- There are more than **800** GPCRs in humans, and in mice there are about **1000** concerned with the sense of smell alone.

- The signal molecules that act on GPCRs are as varied in structure as they are in function and include **proteins** and **small peptides**, as well as **derivatives of amino acids** and **fatty acids**, not to mention **photons of light** and all the molecules that we can **smell** or **taste**.

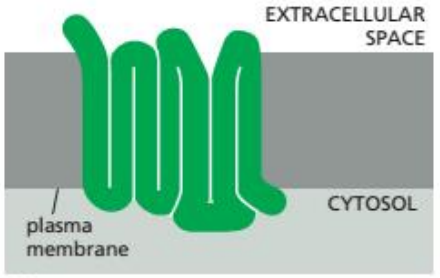
- The same signal molecule can activate many different GPCR family members:
- adrenaline activates at least 9 distinct GPCRs,
- acetylcholine another 5, and
- the neurotransmitter serotonin at least 14.

- The different receptors for the same signal are usually expressed in different cell types and elicit different responses.

SIGNALING THROUGH G-PROTEIN-COUPLED RECEPTORS

- Despite the chemical and functional diversity of the signal molecules that activate them, all **GPCRs have a similar structure**.

- They consist of a single polypeptide chain that threads back and forth across the lipid bilayer **seven times**,
forming a **cylindrical structure**,
often with a **deep ligand-binding site** at its center.



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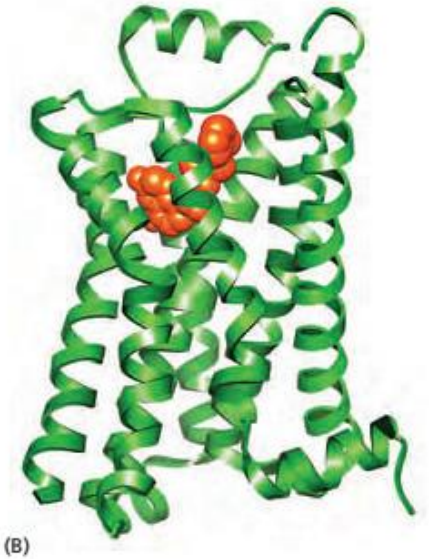
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SIGNALING THROUGH G-PROTEIN-COUPLED RECEPTORS

- In addition to their characteristic orientation in the plasma membrane, they all use G proteins to relay the signal into the cell interior.
- The GPCR superfamily includes rhodopsin, the light-activated protein in the vertebrate eye, as well as the large number of olfactory receptors in the vertebrate nose.
- Other family members are found in unicellular organisms: the receptors in yeasts that recognize secreted **mating factors** are an example.
- It is likely that the GPCRs that mediate cell–cell signaling in multicellular organisms evolved from the sensory receptors in their unicellular eukaryotic ancestors.
- It is remarkable that almost half of all known drugs work through GPCRs or the signaling pathways GPCRs activate.
- Of the many hundreds of genes in the human genome that encode GPCRs, about **150** encode **orphan receptors**, for which the ligand is unknown.
- Many of them are likely targets for new drugs that remain to be discovered.

Trimeric G Proteins Relay Signals From GPCRs

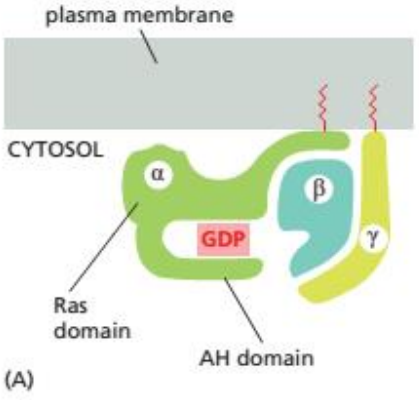
- When an extracellular signal molecule binds to a GPCR, the receptor undergoes a **conformational change** that enables it to activate a **trimeric GTP-binding protein (G protein)**, which couples the receptor to enzymes or ion channels in the membrane.
- In some cases, the G protein is physically associated with the receptor before the receptor is activated, whereas in others it binds only after receptor activation.
- There are various types of G proteins, each **specific for a particular set of GPCRs** and for a **particular set of target proteins** in the plasma membrane.
- G proteins all have a similar structure and operate similarly.
- G proteins are composed of **three protein subunits—α, β, and γ**.
- In the unstimulated state, the α subunit has GDP bound and the G protein is inactive.

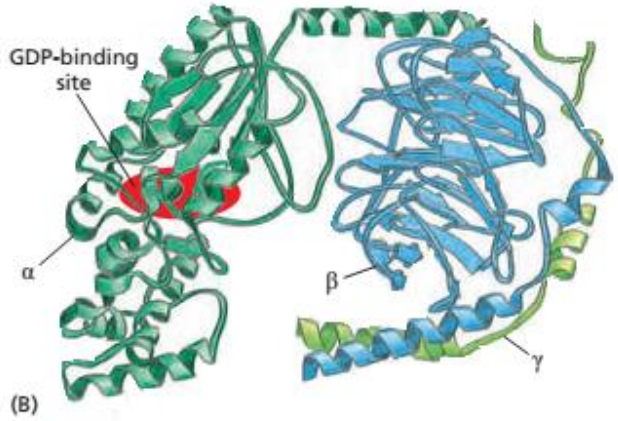
The α subunit contains the **GTPase domain** and binds to one side of the β subunit. The γ subunit binds to the opposite side of the β subunit, and the β and γ subunits together form a single functional unit.

The GTPase domain of the α subunit contains two major subdomains:

the "Ras" domain, which is related to other GTPases and provides one face of the nucleotide-binding pocket; and

the alphahelical or "AH" domain, which clamps the nucleotide in place.



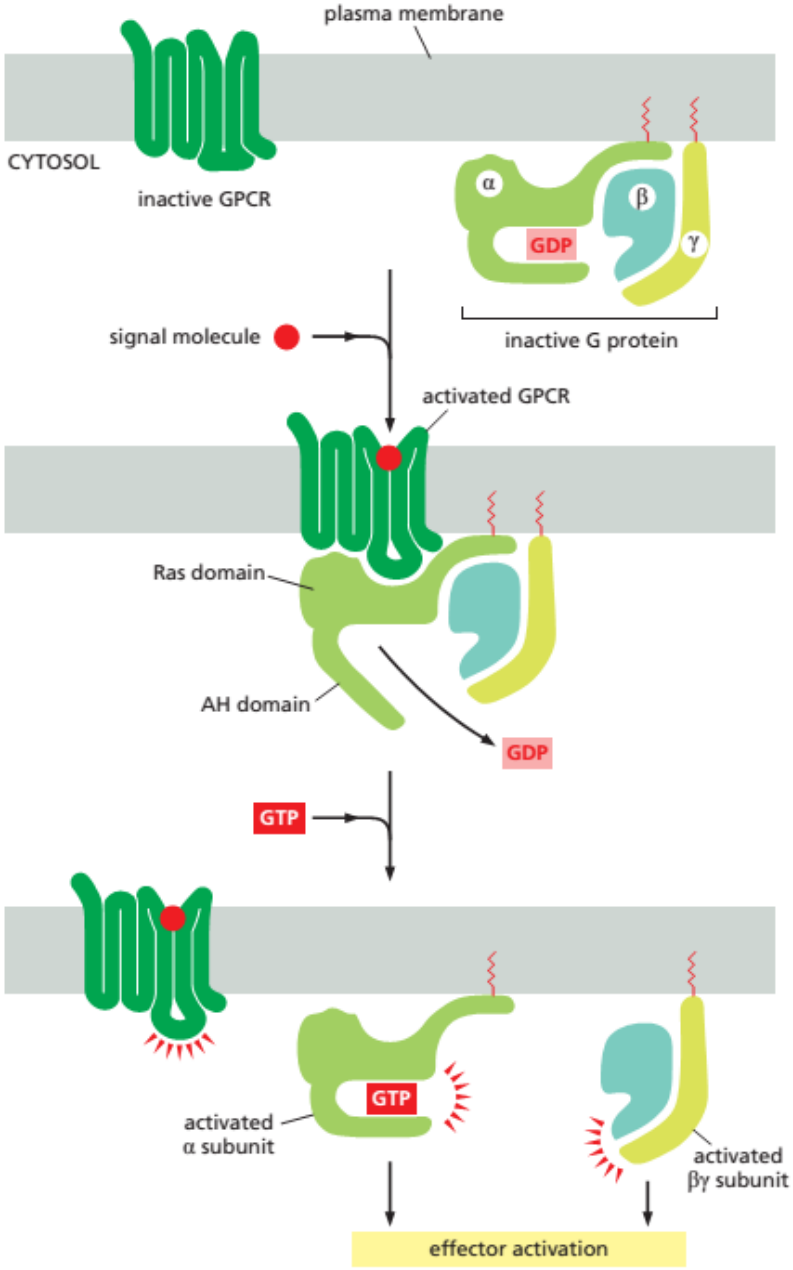


Trimeric G Proteins Relay Signals From GPCRs

1.When a GPCR is activated, **it acts like a guanine nucleotide exchange factor (GEF)** and induces the α subunit to release its bound GDP, allowing GTP to bind in its place.

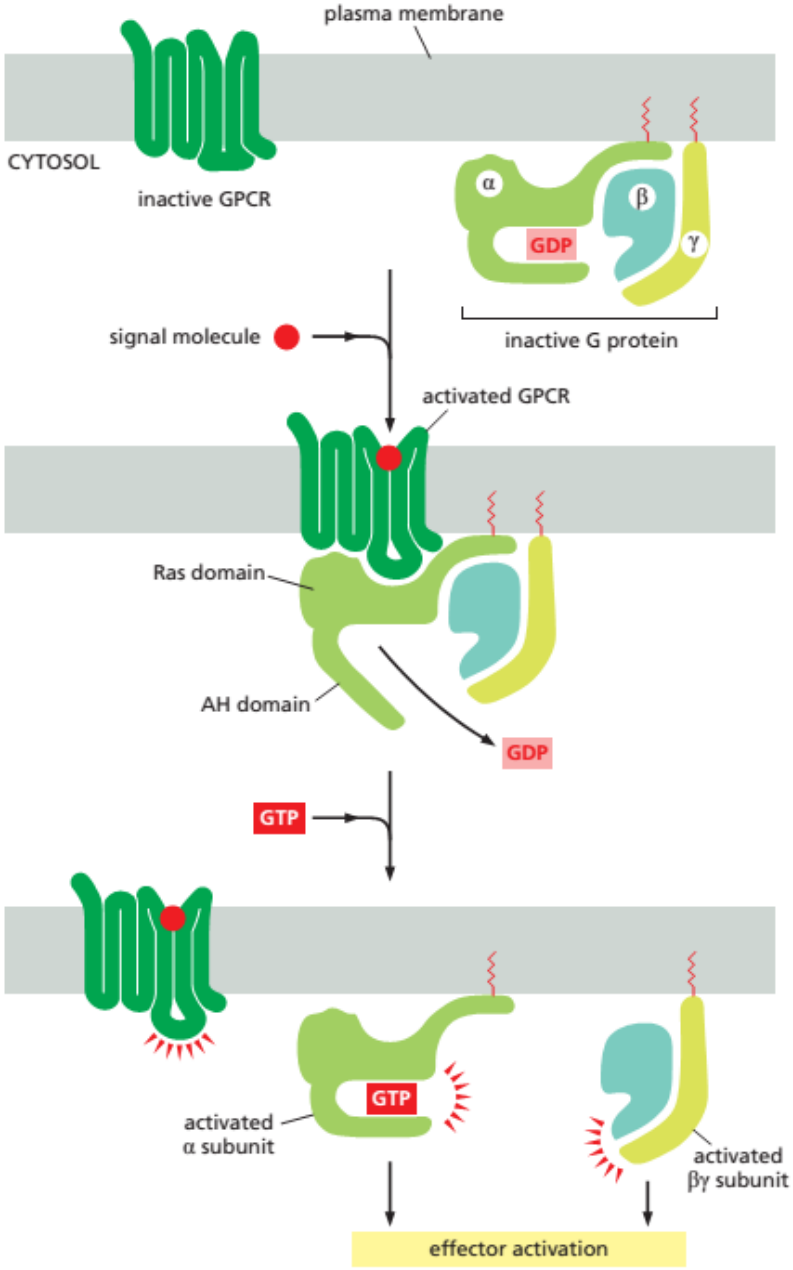
2.**GTP binding** then causes an activating conformational change in the Gα subunit,
-releasing the G protein from the receptor and -triggering dissociation of the GTP-bound Gα subunit from the Gβγ pair—both of which then interact with various targets, such as enzymes and ion channels in the plasma membrane, which relay the signal onward.

-The receptor stays active while the extracellular signal molecule is bound to it, and it can therefore catalyze the activation of many G-protein molecules.



Trimeric G Proteins Relay Signals From GPCRs

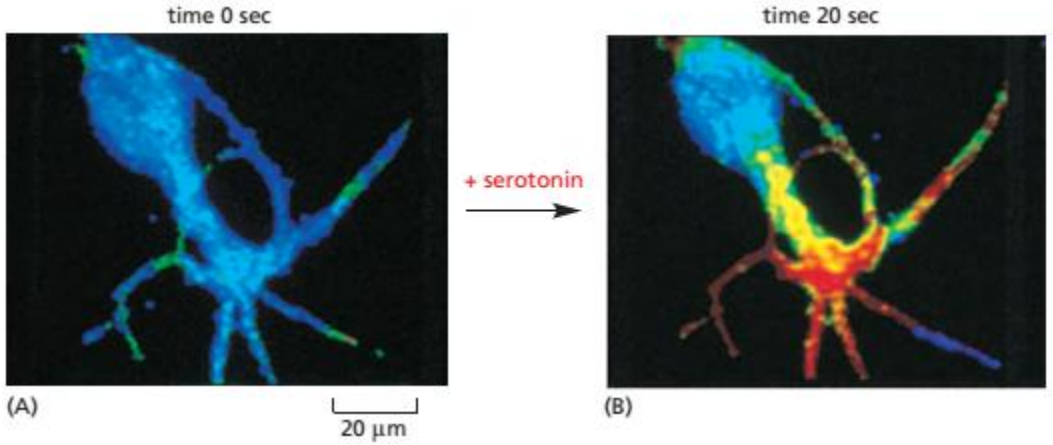
- The α subunit is a GTPase and becomes inactive when it hydrolyzes its bound GTP to GDP.
- The time required for GTP hydrolysis is usually **short** because the GTPase activity is greatly enhanced by the binding of the α subunit to a second protein, which can be either the target protein or a **specific regulator of G protein signaling (RGS)**.
- RGS proteins act as α-subunit-specific **GTPase-activating proteins (GAPs),** and they help shut off G-protein-mediated responses in all eukaryotes.
- There are about 25 RGS proteins encoded in the human genome, each of which interacts with a particular set of G proteins.



Some G Proteins Regulate the Production of Cyclic AMP

Cyclic AMP (cAMP) acts as a second messenger in some signaling pathways.

An extracellular signal can increase cAMP concentration more than twentyfold in seconds.



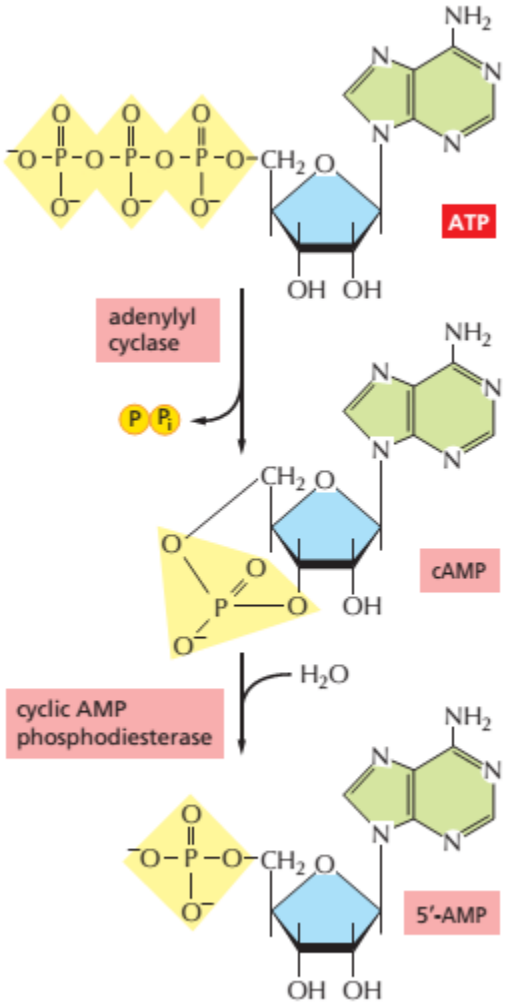
This nerve cell in culture is responding to the neurotransmitter **serotonin**, which acts through a **GPCR** to cause a rapid rise in the intracellular concentration of cyclic AMP.

To monitor the cyclic AMP level, the cell has been loaded with a fluorescent protein that changes its fluorescence when it binds cyclic AMP:
Blue indicates a low level of cyclic AMP,
yellow an intermediate level,
and red a high level.

(A) In the resting cell, the cyclic AMP level is about 5×10^{-8} M.
(B) Twenty seconds after the addition of serotonin to the culture medium, the intracellular level of cyclic AMP has increased to more than 10^{-6} M in the relevant parts of the cell, an increase of more than twentyfold.

Some G Proteins Regulate the Production of Cyclic AMP

- As explained earlier, such a rapid response requires balancing a rapid synthesis of the molecule with its rapid breakdown or removal.
- Cyclic AMP is synthesized from ATP by an enzyme called **adenylyl cyclase**, and it is rapidly and continuously destroyed by **cyclic AMP phosphodiesterases**.
- Adenylyl cyclase is a large, multipass transmembrane protein with its catalytic domain on the cytosolic side of the plasma membrane. There are at least eight isoforms in mammals, most of which are regulated by both **G proteins and Ca2+**.
- Many extracellular signals work by increasing cAMP concentrations inside the cell.
- These signals activate GPCRs that are coupled to a **stimulatory G protein (Gs)**.
- The activated α subunit of Gs binds and thereby activates adenylyl cyclase.
- Other extracellular signals, acting through different GPCRs, reduce cAMP levels by activating an **inhibitory G protein (Gi)**, which then inhibits adenylyl cyclase.



Some G Proteins Regulate the Production of Cyclic AMP

Both Gs and Gi are targets for medically important bacterial toxins:

- ✓ **Cholera toxin**, which is produced by the bacterium that causes **cholera**, is an enzyme that catalyzes the transfer of ADP ribose from intracellular NAD^+ to the α subunit of **Gs**.

This **ADP ribosylation** alters the α subunit so that it can no longer hydrolyze its bound GTP, causing it to remain in an **active state** that stimulates adenylyl cyclase indefinitely.
The resulting prolonged elevation in cAMP concentration within intestinal epithelial cells causes a large efflux of Cl^- and water into the gut, thereby causing the severe diarrhea that characterizes cholera.

- ✓ **Pertussis toxin**, which is made by the bacterium that causes **pertussis (whooping cough),** catalyzes the ADP ribosylation of the α subunit of **Gi**, preventing the protein from interacting with receptors;

as a result, the **G protein remains in the inactive GDP-bound state** and **is unable to regulate its target proteins**.

These two toxins are widely used in experiments to determine whether a cell's GPCR-dependent response to a signal is mediated by Gs or by Gi.

Some G Proteins Regulate the Production of Cyclic AMP

Some of the responses mediated by a Gs-stimulated increase in cAMP concentration are listed in Table 15–1.
As the table shows, different cell types respond differently to an increase in cAMP concentration.

Some cell types, such as **fat cells**, activate adenylyl cyclase in response to multiple hormones, all of which thereby stimulate the breakdown of triglyceride (the storage form of fat) to fatty acids.

Individuals with genetic defects in the Gs α subunit show decreased responses to certain hormones, resulting in **metabolic abnormalities**, **abnormal bone development**, and **mental retardation**.

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

Cyclic-AMP-Dependent Protein Kinase (PKA) Mediates Most of the Effects of Cyclic AMP

- In most animal cells, cAMP exerts its effects mainly by activating **cyclic-AMPdependent protein kinase (PKA).**

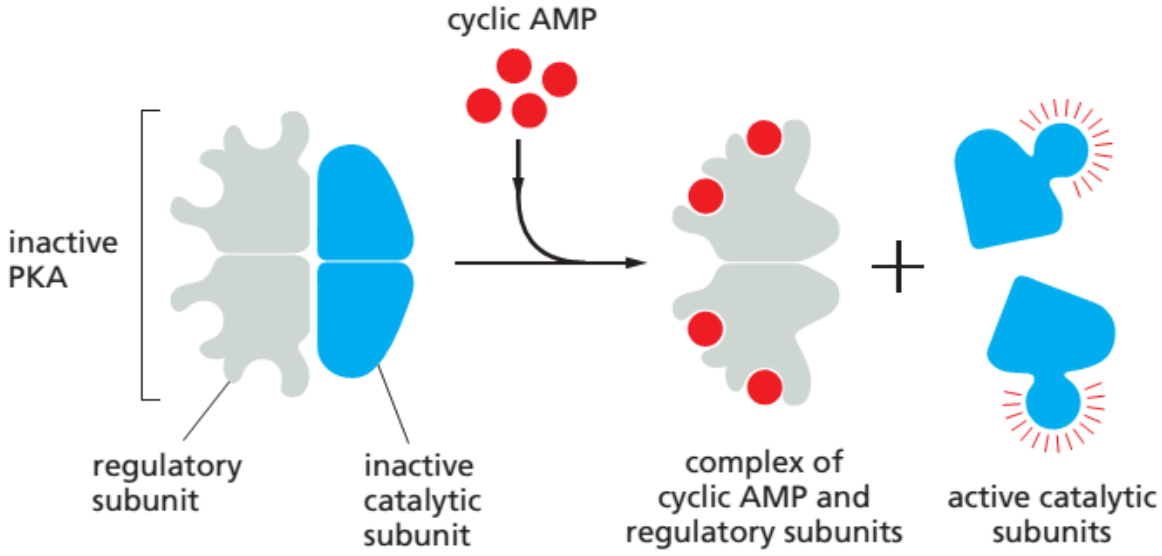
-This kinase phosphorylates specific **serines** or **threonines** on selected target proteins, including intracellular signaling proteins and effector proteins, thereby regulating their activity.

-The target proteins differ from one cell type to another, which explains why the effects of cAMP vary so markedly depending on the cell type.

-In the inactive state, PKA consists of a complex of **two catalytic subunits** and **two regulatory subunits**.

-The binding of cAMP to the regulatory subunits alters their conformation, causing them to dissociate from the complex.

-The released catalytic subunits are thereby activated to phosphorylate specific target proteins.



Cyclic-AMP-Dependent Protein Kinase (PKA) Mediates Most of the Effects of Cyclic AMP

The regulatory subunits of PKA (also called A-kinase) are important for localizing the kinase inside the cell: special **A-kinase anchoring proteins (AKAPs)** bind both to the **regulatory subunits** and to a **component of the cytoskeleton** or **a membrane of an organelle**, thereby tethering the enzyme complex to a particular subcellular compartment.

Some AKAPs also bind other signaling proteins, forming a signaling complex.

An AKAP located around the nucleus of heart muscle cells, for example, binds both **PKA** and a **phosphodiesterase** that hydrolyzes cAMP:

-In unstimulated cells, the phosphodiesterase keeps the local cAMP concentration low, so that the bound PKA is inactive;
-In stimulated cells, cAMP concentration rapidly rises, overwhelming the phosphodiesterase and activating the PKA.

Among the target proteins that PKA phosphorylates and activates in these cells is the adjacent phosphodiesterase, which rapidly lowers the cAMP concentration again.

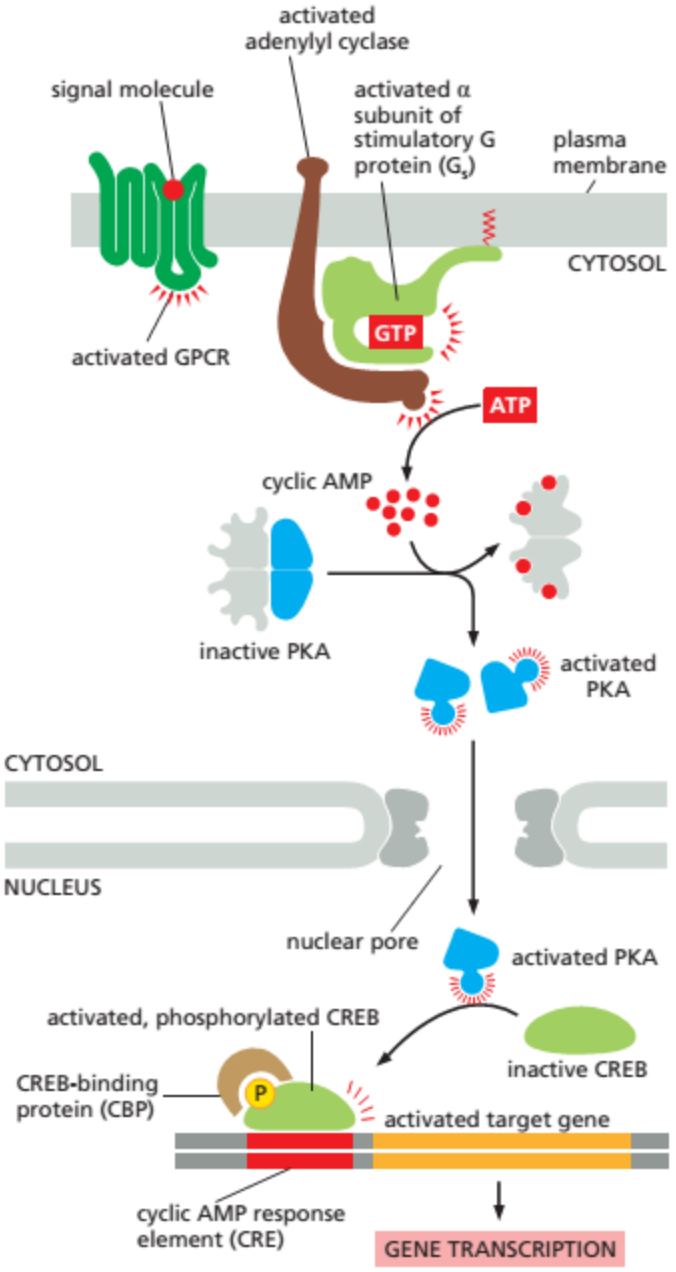
- **This negative feedback arrangement converts what might otherwise be a prolonged PKA response into a brief, local pulse of PKA activity**.

Whereas some responses mediated by cAMP occur within seconds, others depend on changes in the transcription of specific genes and take hours to develop fully. In cells that secrete the peptide hormone somatostatin,

Cyclic-AMP-Dependent Protein Kinase (PKA) Mediates Most of the Effects of Cyclic AMP

Whereas some responses mediated by cAMP occur within seconds, others depend on changes in the transcription of specific genes and take hours to develop fully:

- In cells that secrete the **peptide hormone somatostatin**, for example, cAMP activates the gene that encodes this hormone.
- The regulatory region of the somatostatin gene contains a short cis-regulatory sequence, called the **cyclic AMP response element (CRE),** which is also found in the regulatory region of many other genes activated by cAMP.
- A specific transcription regulator called **CRE-binding (CREB) protein** recognizes this sequence.
- When PKA is activated by cAMP, it phosphorylates CREB on a single serine; phosphorylated CREB then recruits a transcriptional coactivator called **CREB-binding protein (CBP)**, which stimulates the transcription of the target genes.
- Thus, CREB can transform a short cAMP signal into a long-term change in a cell, a process that, in the brain, is thought to play an important part in some forms of **learning and memory**.



Some G Proteins Signal Via Phospholipids

Many GPCRs exert their effects through G proteins that activate the **plasma-membrane-bound enzyme phospholipase C-β (PLCβ)**.
Table 15–2 lists some examples of responses activated in this way.

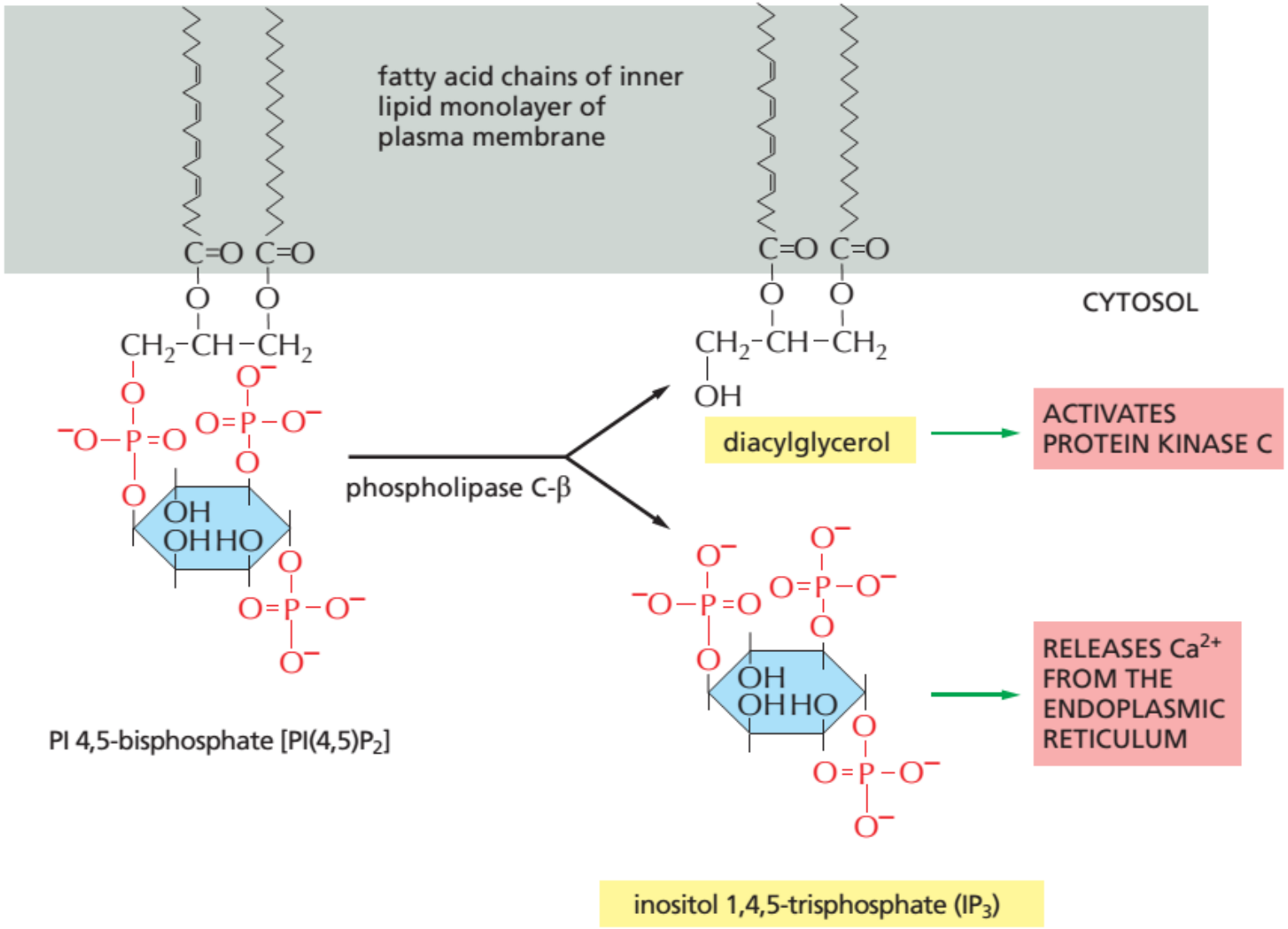
TABLE 15–2 Some Cell Responses in Which GPCRs Activate PLCβ

Target tissue	Signal molecule	Major response
Liver	Vasopressin	Glycogen breakdown
Pancreas	Acetylcholine	Amylase secretion
Smooth muscle	Acetylcholine	Muscle contraction
Blood platelets	Thrombin	Platelet aggregation

The phospholipase acts on a phosphorylated inositol phospholipid (a phosphoinositide) called **phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2]**, which is present in small amounts in the inner half of the plasma membrane.

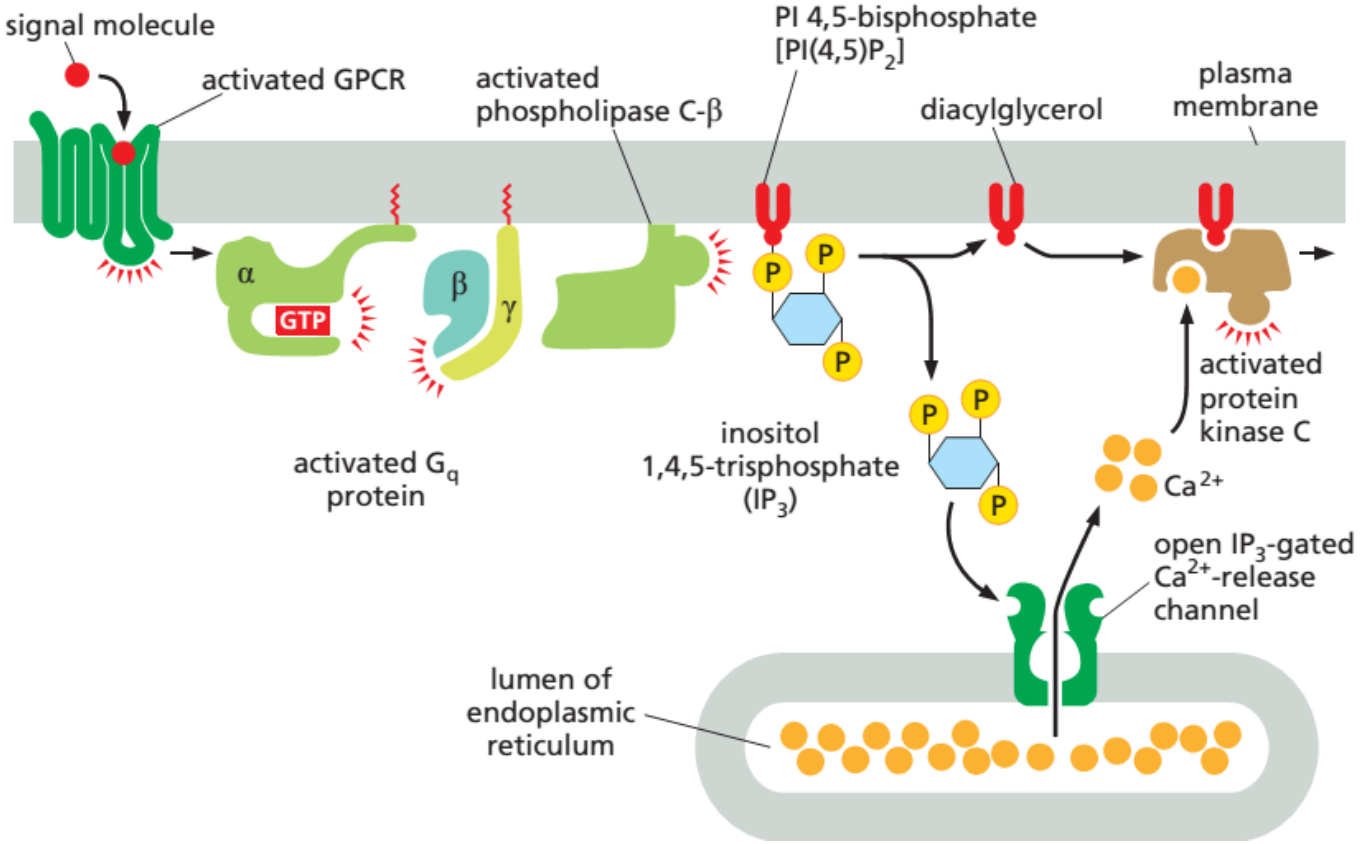
Some G Proteins Signal Via Phospholipids

The phospholipase acts on a phosphorylated inositol phospholipid (a phosphoinositide) called **phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2]**, which is present in small amounts in the inner half of the plasma membrane.



Some G Proteins Signal Via Phospholipids

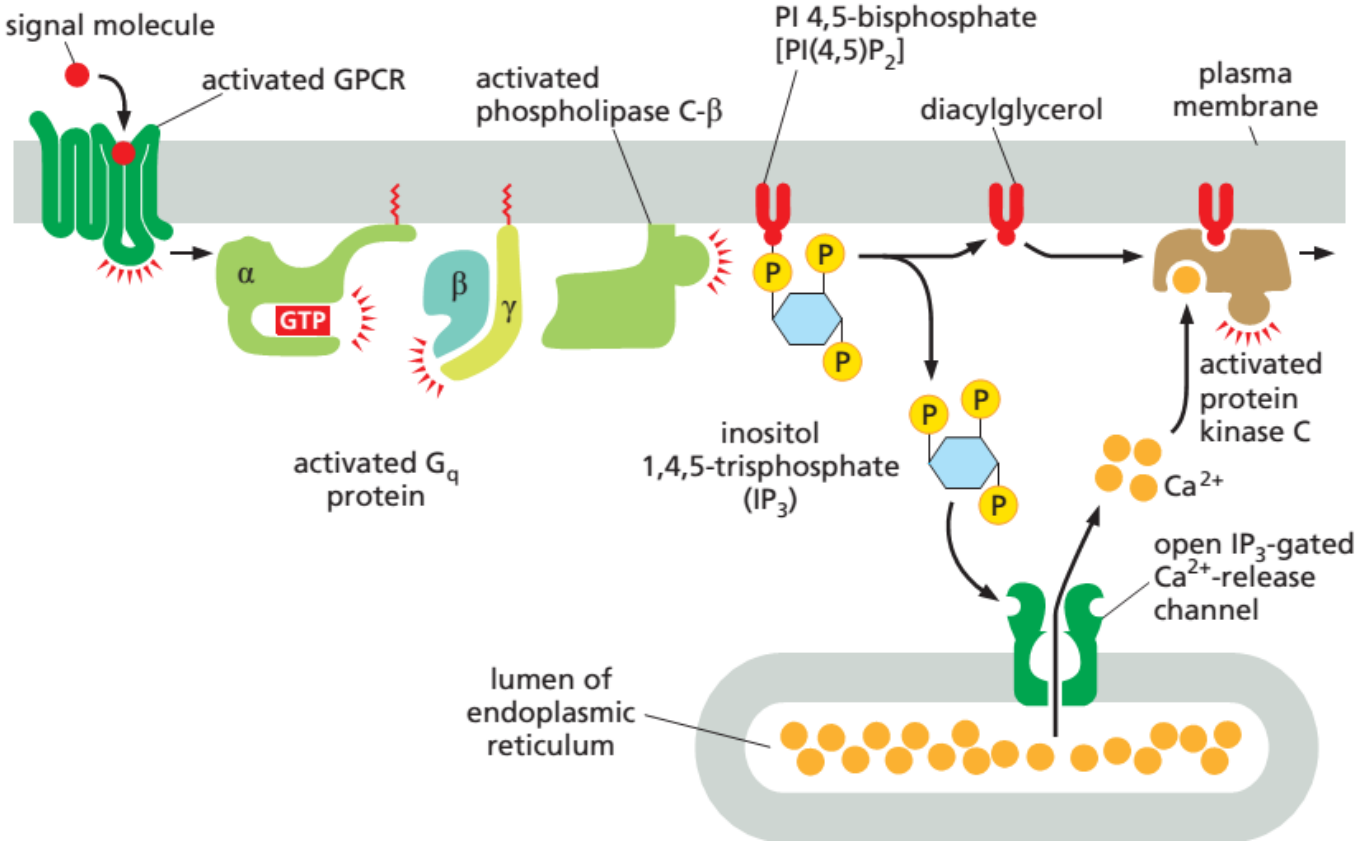
- Receptors that activate this inositol phospholipid signaling pathway mainly do so via a **G protein called Gq**, which activates phospholipase C-β in much the same way that Gs activates adenylyl cyclase.
- The activated phospholipase then cleaves the PI(4,5)P2 to generate two products: **inositol 1,4,5-trisphosphate (IP3)** and **diacylglycerol**.
- At this step, the signaling pathway splits into two branches:
- **IP3** is a water-soluble molecule that leaves the plasma membrane and diffuses rapidly through the cytosol. When it reaches the endoplasmic reticulum (ER), it binds to and opens IP3-gated Ca^{2+}-release channels (also called IP3 receptors) in the ER membrane. Ca^{2+} stored in the ER is released through the open channels, quickly **raising the concentration of Ca^{2+} in the cytosol**. The increase in cytosolic Ca^{2+} propagates the signal by influencing the activity of **Ca^{2+}- sensitive intracellular proteins**.



Some G Proteins Signal Via Phospholipids

- The other cleavage product of the PI(4,5)P2, diacylglycerol, also acts as a second messenger, but it remains embedded in the plasma membrane, where it has **several potential signaling roles**.
- One of its major functions is to activate a protein kinase called **protein kinase C (PKC),** so named because it is Ca^{2+} -dependent. The initial rise in cytosolic Ca^{2+} induced by IP3 alters the PKC so that it translocates from the cytosol to the cytoplasmic face of the plasma membrane.
- There it is activated by the combination of **Ca^{2+}**, **diacylglycerol**, and **the negatively charged membrane phospholipid phosphatidylserine**: Once activated, PKC phosphorylates target proteins that vary depending on the cell type.

- The principles are the same as discussed earlier for PKA, although most of the target proteins are different.



Some G Proteins Signal Via Phospholipids

- Diacylglycerol can be further cleaved to release **arachidonic acid**, which can either act as a signal in its own right or be used in the synthesis of other small lipid signal molecules called **eicosanoids.**

- Most vertebrate cell types make eicosanoids, including **prostaglandins**, which have many biological activities.

- They participate in pain and inflammatory responses, for example, and many anti-inflammatory drugs (such as aspirin, ibuprofen, and cortisone) act in part by inhibiting their synthesis.