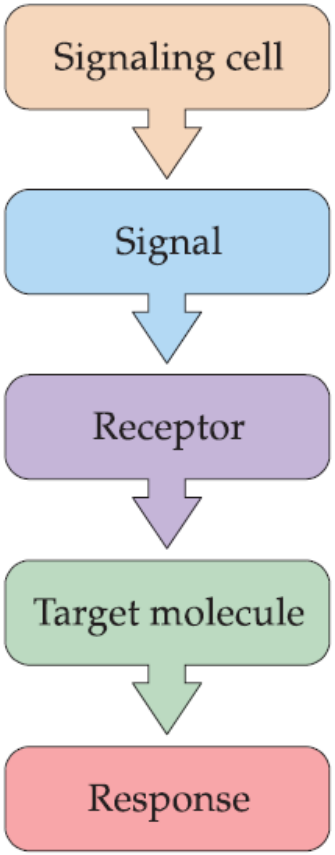
Part 3, Neural Signaling

3.5. Molecular signaling within neurons

Chemical signaling

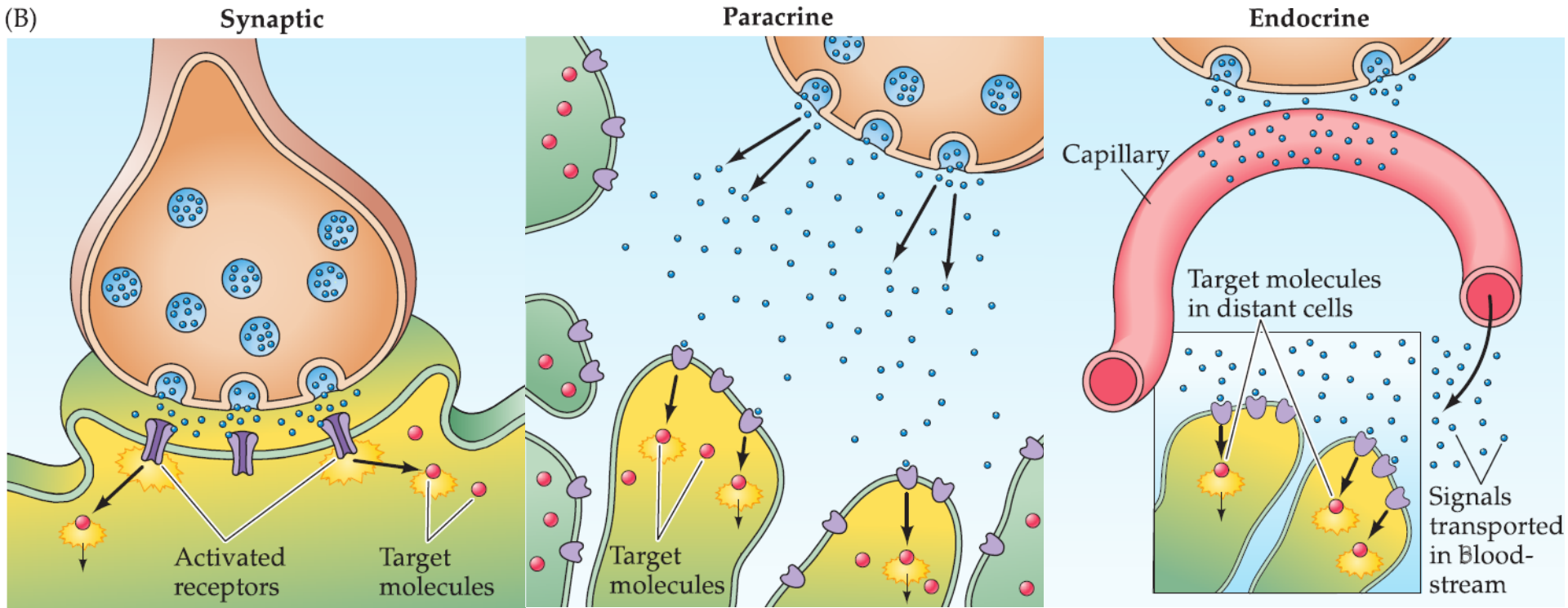
- Chemical communication coordinates the behavior of individual nerve and glial cells in physiological processes that range from neural differentiation to learning and memory.
- To carry out such communication, a series of extraordinarily diverse and complex chemical signaling pathways has evolved.
- The essential components of chemical signaling are:



- cells that initiate the process by releasing signaling molecules.
- a molecular signal that transmits information from one cell to another.
- a receptor molecule that transduces the information provided by the signal.
- a target molecule that mediates the cellular response.
- subsequent cellular responses.

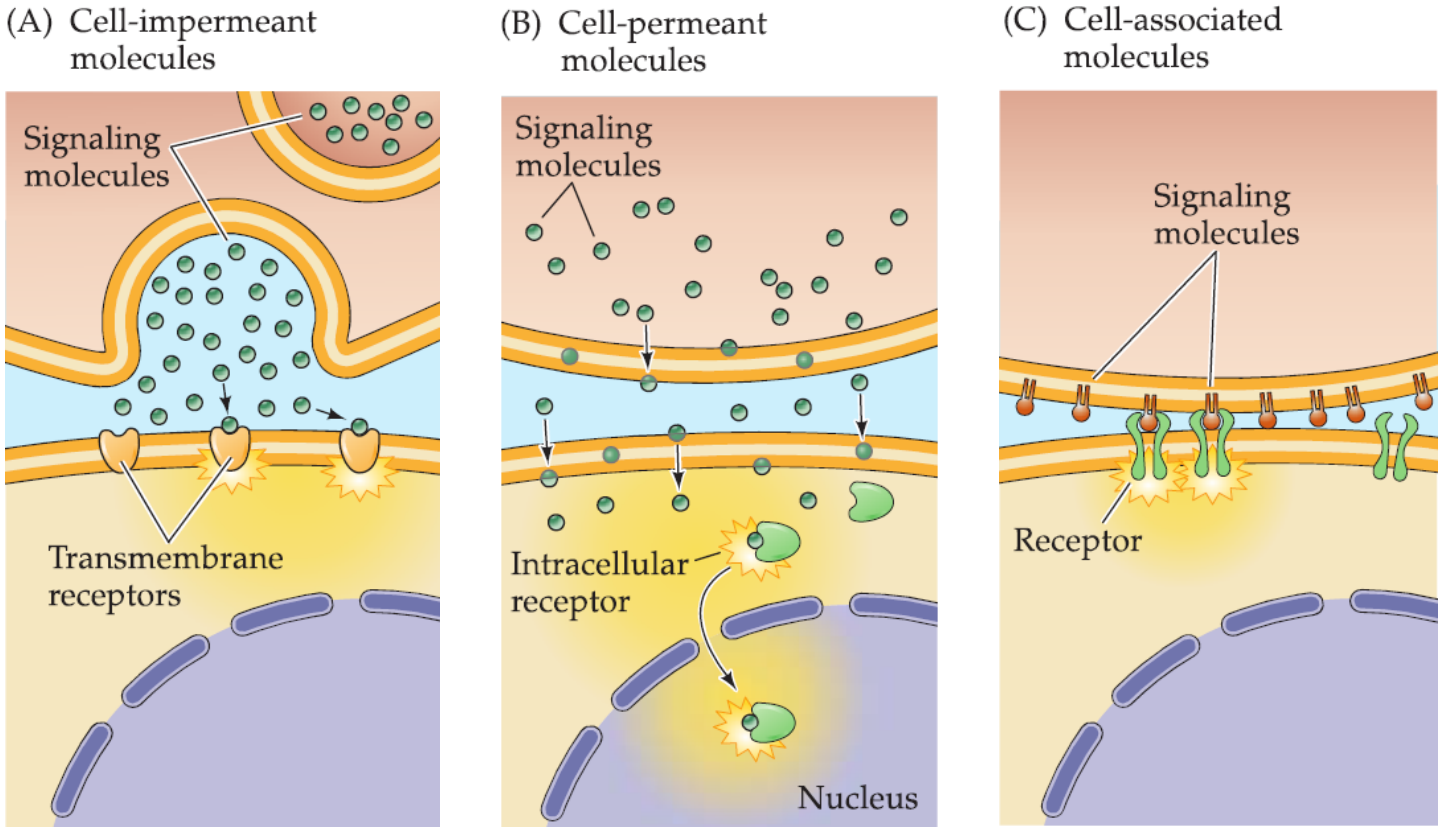
Forms of chemical signaling

- **Synaptic transmission**: a special form of chemical signaling that transfers information from one neuron to another.
- **Paracrine signaling**: acting over a longer range than synaptic transmission and involving the secretion of chemical signals onto a group of nearby target cells.
- **Endocrine signaling**: referring to the secretion of hormones into the bloodstream, where they can affect targets throughout the body.



The activation of signaling pathways

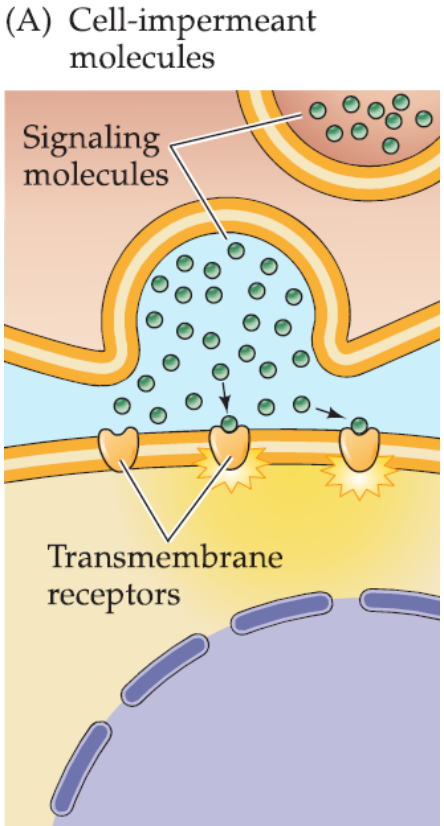
- The molecular components of these signal transduction pathways are always activated by a chemical signaling molecule.
- Such signaling molecules can be grouped into three classes: **cell-impermeant**, **cell-permeant**, and **cell-associated signaling molecules**.
- The first two classes are secreted molecules and thus can act on target cells removed from the site of signal synthesis or release.



Cell-impermeant molecules

- Cell-impermeant molecules cannot readily traverse the plasma membrane of the target cell and must bind to the extracellular portion of transmembrane receptor proteins.

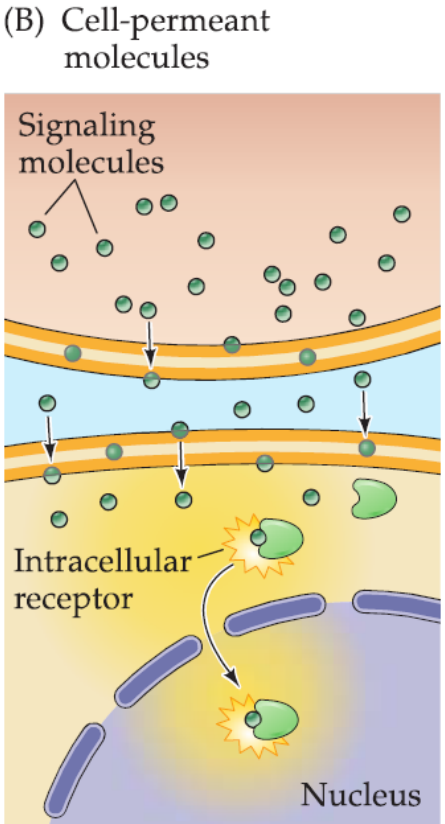
- Hundreds of secreted molecules have now been identified, including the neurotransmitters; proteins such as neurotrophic factors; and peptide hormones such as glucagon, insulin, and various reproductive hormones.



- These signaling molecules are typically short-lived, either because they are rapidly metabolized or because they are internalized by endocytosis once bound to their receptors.

Cell-permeant molecules

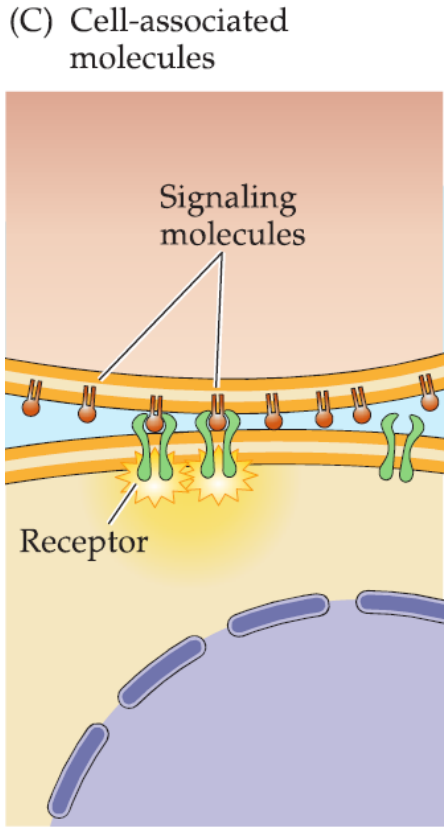
- Cell-permeant signaling molecules can cross the plasma membrane to act directly on receptors that are inside the cell.
- They include numerous steroid (glucocorticoids, estradiol, and testosterone) and thyroid (thyroxin) hormones, and retinoids.
- These signaling molecules are relatively insoluble in aqueous solutions and are often transported in blood and other extracellular fluids by binding to specific carrier proteins.



- In this form, they may persist in the bloodstream for hours or even days.

Cell-associated molecules

- These molecules are arrayed on the extracellular surface of the plasma membrane.
- As a result, these molecules act only on other cells that are physically in contact with the cell that carries such signals.
- Examples include proteins such as the integrins and neural cell adhesion molecules (NCAMs) that influence axonal growth.



- Membrane-bound signaling molecules are more difficult to study, but are clearly important in neuronal development and other circumstances where physical contact between cells provides information about cellular identities.

Receptor types

- Binding of signal molecules causes a conformational change in the receptor, which then triggers the subsequent signaling cascade within the affected cell.

- The receptors for impermeant signal molecules are membrane-spanning proteins.

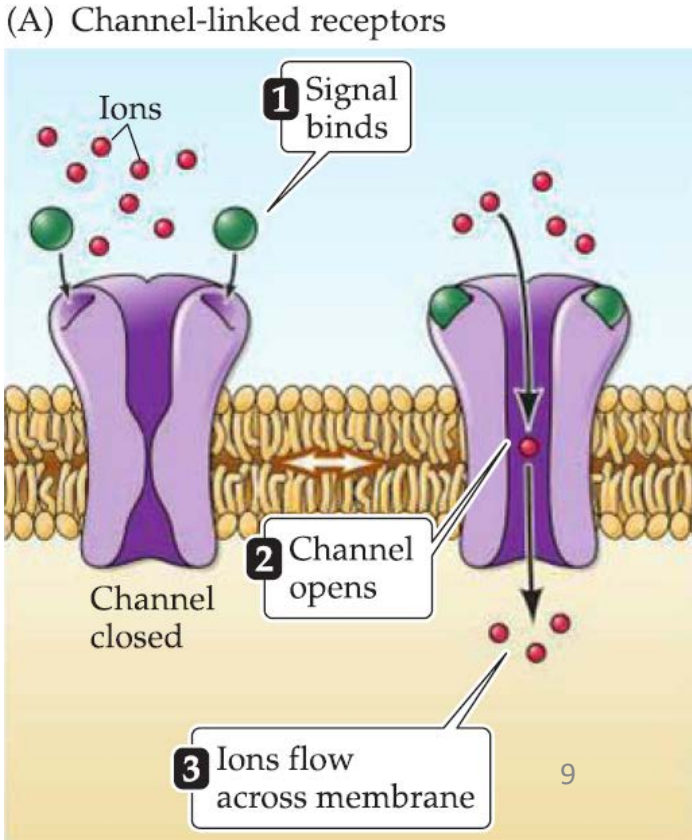
  - The extracellular domain of such receptors includes the binding site for the signal, while the intracellular domain activates intracellular signaling cascades after the signal binds.

  - Cell-impermeant signaling molecules can bind to and activate either **channel-linked receptors**, **enzyme-linked receptors** or **G-protein-coupled receptors**.

- **Intracellular receptors** are activated by cell-permeant or lipophilic signaling molecules.

Channel-linked receptors

- **Channel-linked receptors** (also called ligand-gated ion channels) have the receptor and transducing functions as part of the same protein molecule.
  - Interaction of the chemical signal with the binding site of the receptor causes the opening or closing of an ion channel pore in another part of the same molecule.
  - The resulting ion flux changes the membrane potential of the target cell and, in some cases, can also lead to entry of Ca^{2+} ions that serve as a second messenger signal within the cell.
  - Examples?

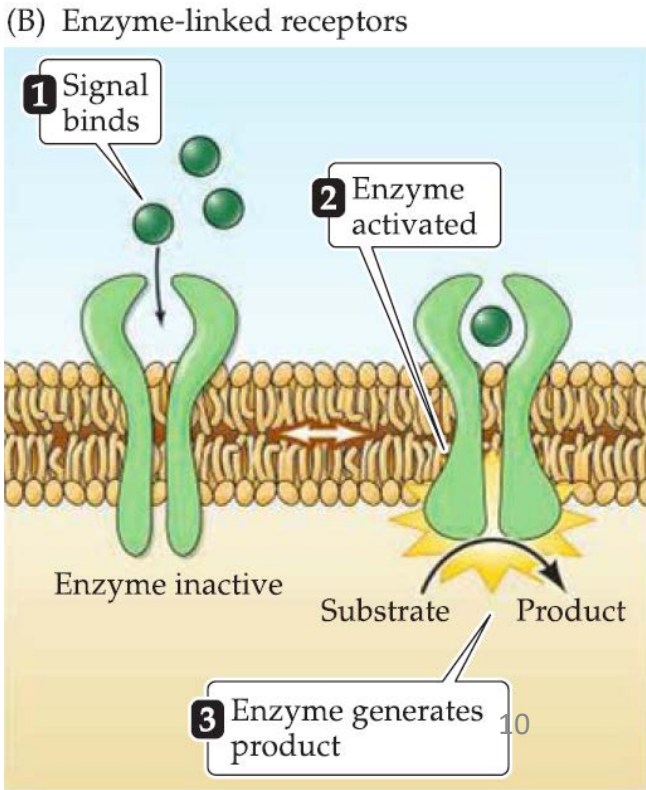


Enzyme-linked receptors

- The intracellular domain of such receptors is an enzyme whose catalytic activity is regulated by the binding of an extracellular signal.

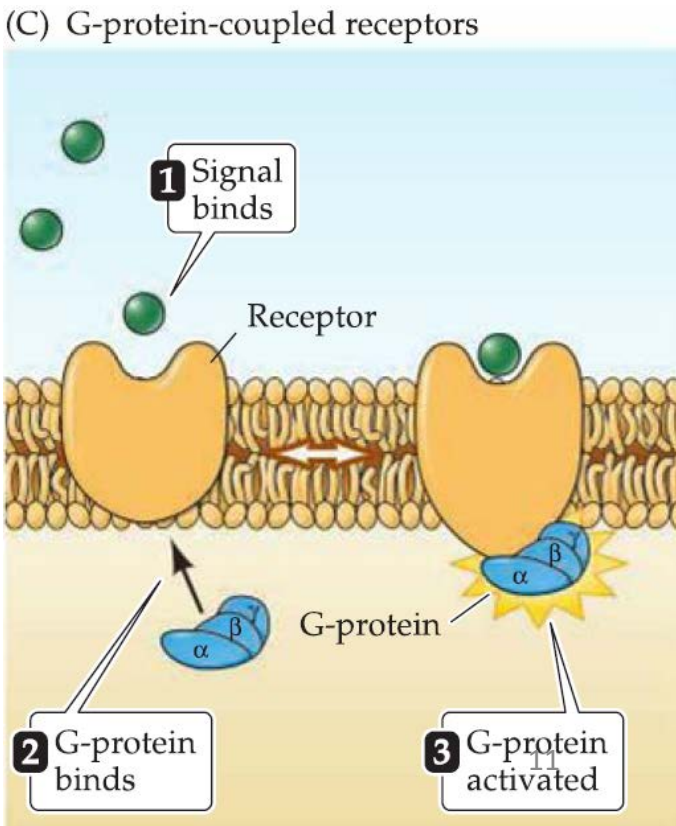
  - The great majority of these receptors are **protein kinases**, often tyrosine kinases, that phosphorylate intracellular target proteins, thereby changing the physiological function of the target cells.

  - Noteworthy members of this group of receptors are the Trk family of neurotrophin receptors and other receptors for growth factors.



G-protein-coupled receptors

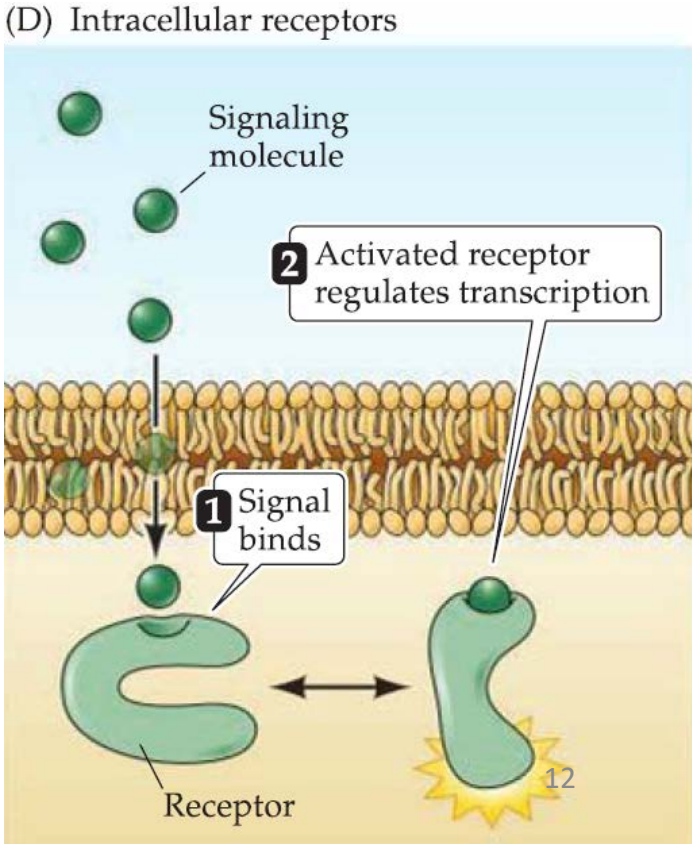
- **G-protein-coupled receptors** regulate intracellular reactions by an indirect mechanism involving an intermediate transducing molecule, called the **GTP-binding proteins** (or **G-proteins**).
  - Because these receptors all share the structural feature of crossing the plasma membrane seven times, they are also referred to as 7-transmembrane receptors (or metabotropic receptors).
  - Well-known examples include the β-adrenergic receptor, the muscarinic type of acetylcholine receptor, and metabotropic glutamate receptors, as well as the receptors for odorants in the olfactory system, and many types of receptors for peptide hormones.
  - Rhodopsin, a light-sensitive, 7-transmembrane protein in retinal photoreceptors, is another form of G-protein-linked receptor whose signal is light photons.



Intracellular receptors

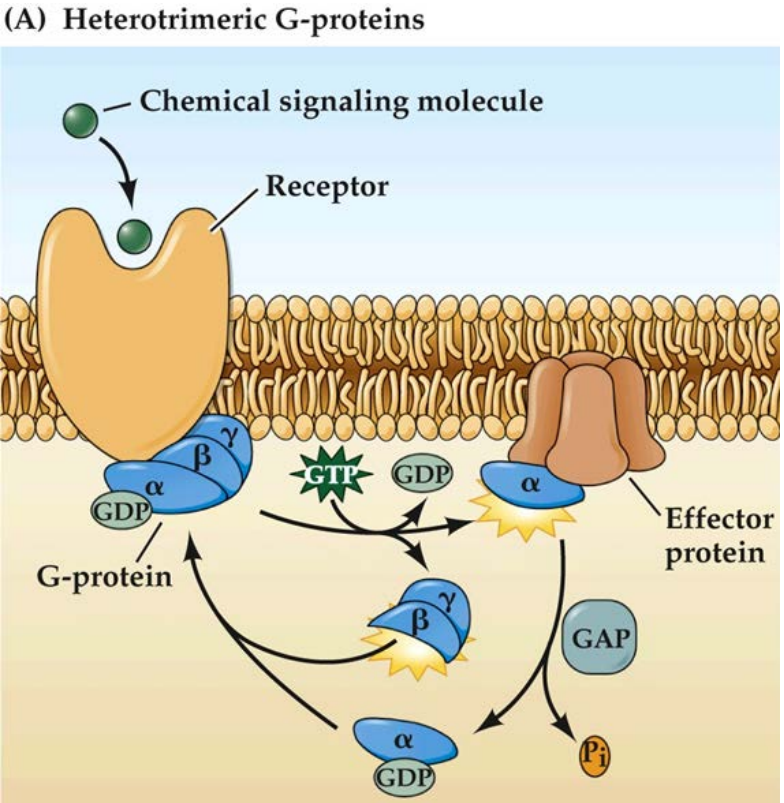
- **Intracellular receptors** are activated by cell-permeant or lipophilic signaling molecules.

- Many of these receptors lead to the activation of signaling cascades that produce new mRNA and protein within the target cell.
  - Often such receptors comprise a receptor protein bound to an inhibitory protein complex.
  - When the signaling molecule binds to the receptor, the inhibitory complex dissociates to expose a DNA-binding domain on the receptor.
  - This activated form of the receptor can then move into the nucleus and directly interact with nuclear DNA, resulting in altered transcription.



G-proteins and their molecular targets

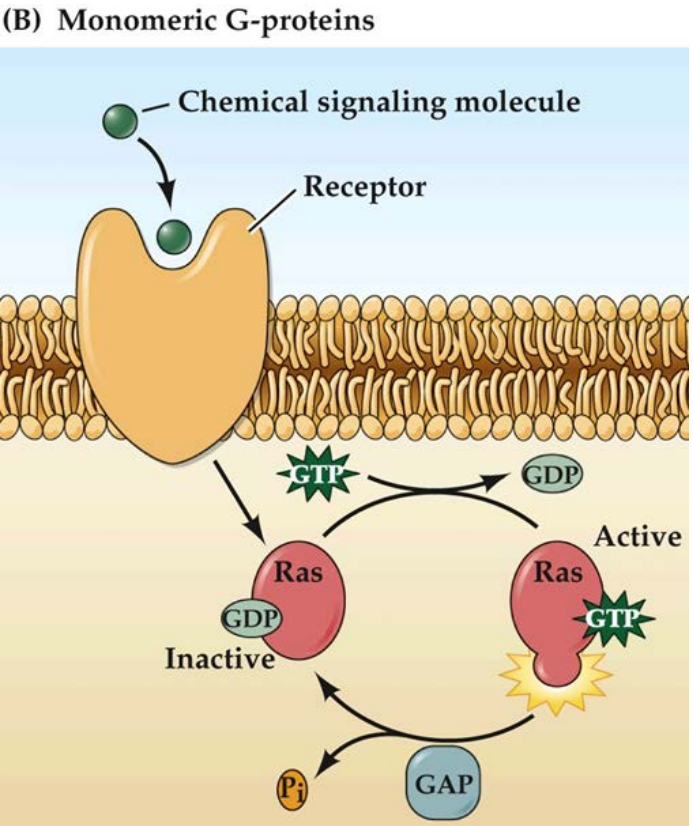
- There are two general classes of GTP-binding proteins.
- **Heterotrimeric G-proteins** are composed of three distinct subunits: α, β, γ.
  - There are many different α, β and γ subunits, allowing a bewildering number of G-protein permutations.
  - Regardless of the specific composition of the heterotrimeric G-protein, its α subunit binds to guanine nucleotides, either GTP or GDP.
  - Binding of GDP allows the α subunit to bind to the β and γ subunits to form an inactive trimer.



- Binding of an extracellular signal to a G-protein-coupled receptor in turn allows the G-protein to bind to the receptor and causes GDP to be replaced with GTP.
- When GTP is bound to the G-protein, the α subunit dissociates from the βγ complex and activates the G-protein.
- Following activation, both the GTP-bound α subunit and the free βγ complex can bind to downstream effector molecules that mediate a variety of responses in the target cell.

G-proteins and their molecular targets

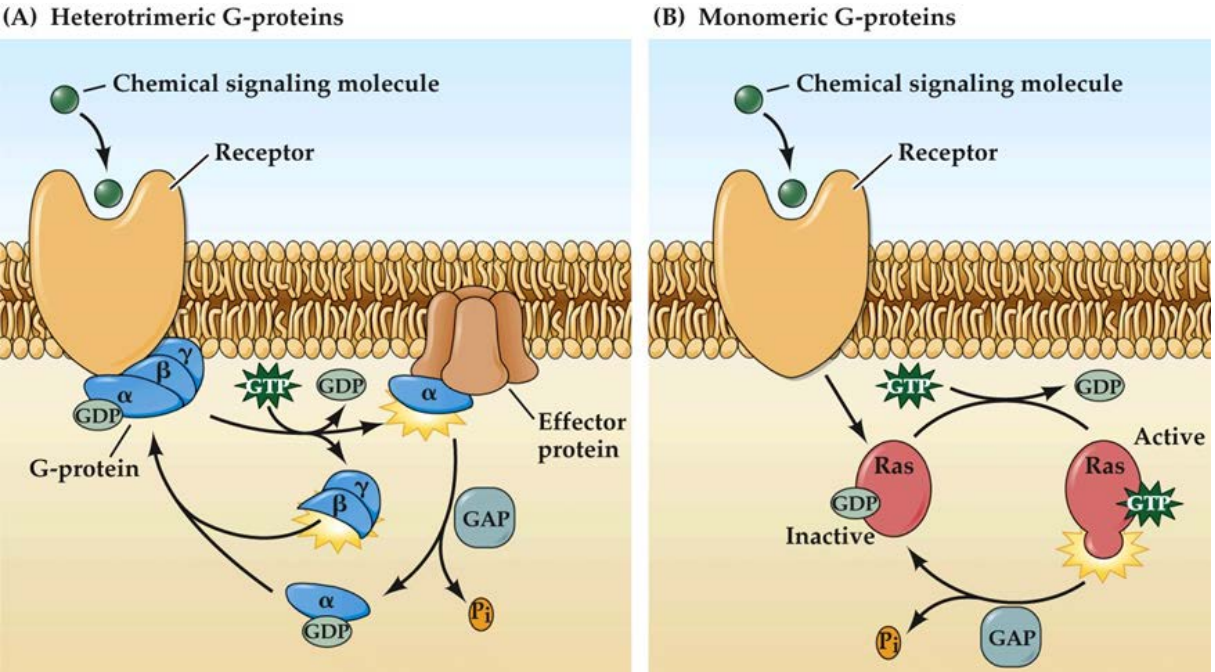
- The second class of GTP-binding proteins are the **monomeric G-proteins** (also called **small G-proteins**).
- These monomeric GTPases also relay signals from activated cell surface receptors to intracellular targets such as the cytoskeleton and the vesicle trafficking apparatus of the cell.
- The first small G-protein was discovered in a virus that causes *ra*t sarcoma tumors and was therefore called **ras**.



- Ras is a molecule that helps regulate cell differentiation and proliferation by relaying signals from receptor kinases to the nucleus.
- Ras is known to be involved in many forms of neuronal signaling.
- Since the discovery of ras, a large number of small GTPases have been identified and can be sorted into five different subfamilies with different functions.
- For instance, some are involved in vesicle trafficking in the presynaptic terminal or elsewhere in the neuron, while others play a central role in protein and RNA trafficking in and out of the nucleus.

G-proteins and their molecular targets

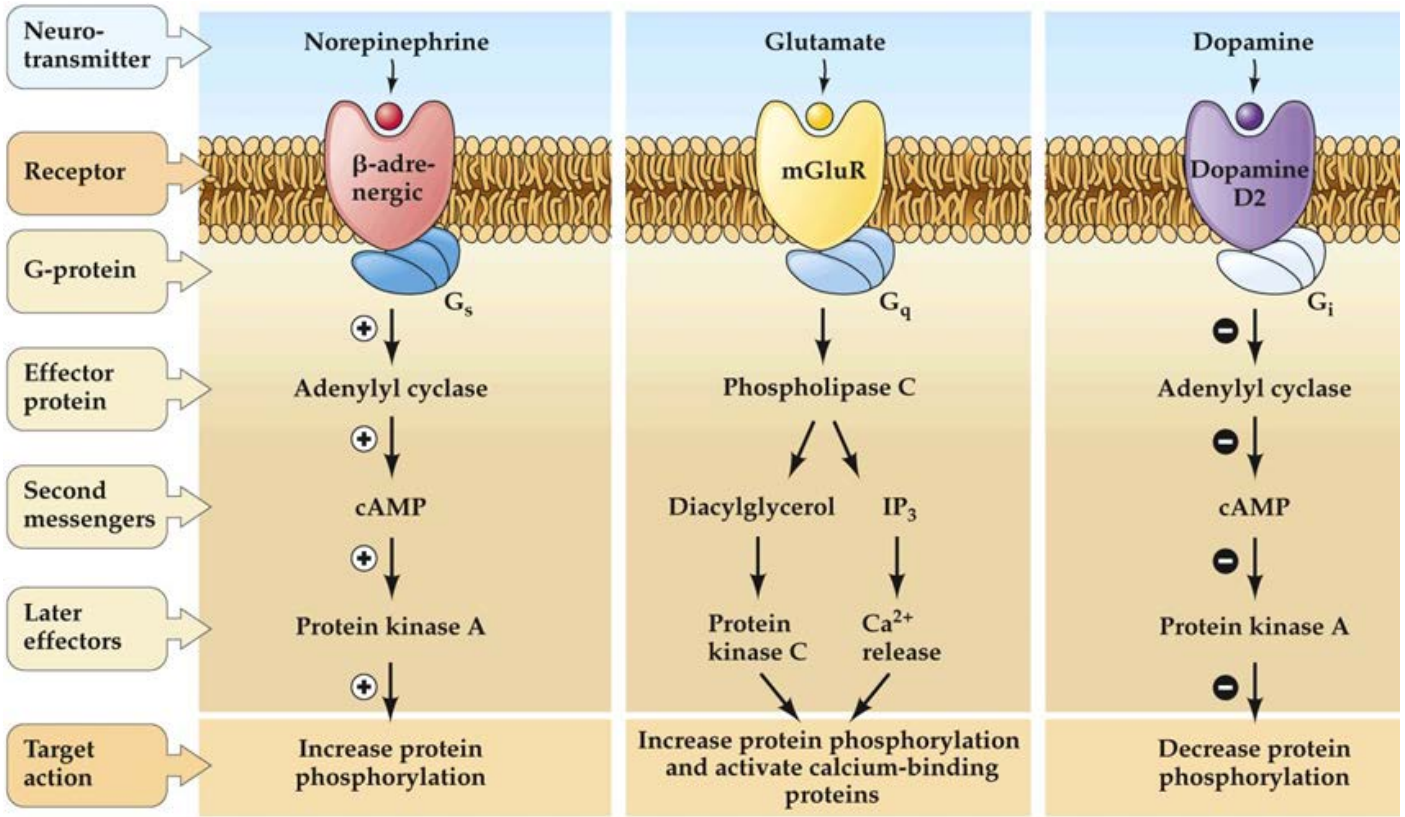
- Termination of signaling by both heterotrimeric and monomeric G-proteins is determined by hydrolysis of GTP to GDP.
- The rate of GTP hydrolysis is an important property of a particular G-protein that can be regulated by other proteins, termed GTPase-activating proteins (GAPs).
- By replacing GTP with GDP, GAPs return G-proteins to their inactive form.
- GAPs were first recognized as regulators of small G-proteins, but recently similar proteins have been found to regulate the α subunits of heterotrimeric G-proteins.



- Hence, monomeric and trimeric G-proteins function as molecular timers that are active in their GTP-bound state, becoming inactive when they have hydrolyzed the bound GTP to GDP.

G-proteins and their molecular targets

- Activated G-proteins alter the function of many downstream effectors.
- Most of these effectors are enzymes that produce intracellular second messengers.
- Effector enzymes include adenylyl cyclase, guanylyl cyclase, phospholipase C, and others.
- Second messengers produced by these enzymes trigger the complex biochemical signaling cascades.



- Because each of these cascades is activated by specific G-protein subunits, the pathways activated by a particular receptor are determined by the specific identity of the G-protein subunits associated with it.

G-proteins and their molecular targets

- G-proteins can also directly bind to and activate ion channels.
- In summary, the binding of chemical signals to their receptors activates cascades of signal transduction events in the cytosol of target cells.
- Within such cascades, G-proteins serve a pivotal function as the molecular transducing elements that couple membrane receptors to their molecular effectors within the cell.
- The diversity of G-proteins and their downstream targets leads to many types of physiological responses.
- By directly regulating the gating of ion channels, G-proteins can influence the membrane potential of target cells.

Second messengers

- Neurons use many different second messengers as intracellular signals.
- These messengers differ in the mechanism by which they are produced and removed, as well as their downstream targets and effects .

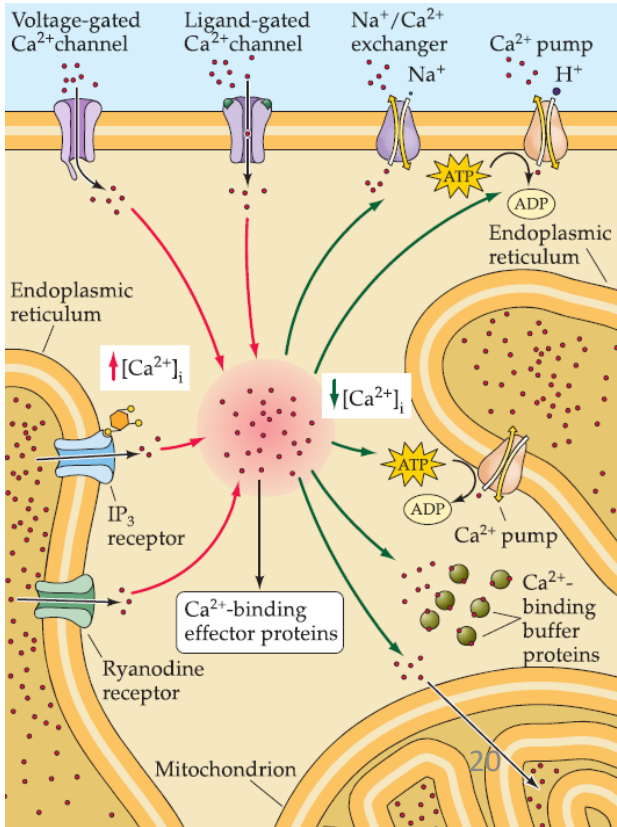
Second messenger	Sources	Intracellular targets	Removal mechanisms
Ca^{2+}	Plasma membrane: Voltage-gated Ca^{2+} channels Various ligand-gated channels Endoplasmic reticulum: IP_3 receptors Ryanodine receptors	Calmodulin Protein kinases Protein phosphatases Ion channels Synaptotagmin Many other Ca^{2+}-binding proteins	Plasma membrane: Na^+/Ca^{2+} exchanger Ca^{2+} pump Endoplasmic reticulum: Ca^{2+} pump Mitochondria
Cyclic AMP	Adenylyl cyclase acts on ATP	Protein kinase A Cyclic nucleotide-gated channels	cAMP phosphodiesterase
Cyclic GMP	Guanylyl cyclase acts on GTP	Protein kinase G Cyclic nucleotide-gated channels	cGMP phosphodiesterase
IP_3	Phospholipase C acts on PIP_2	IP_3 receptors on endoplasmic reticulum	Phosphatases
Diacylglycerol	Phospholipase C acts on PIP_2	Protein kinase C	Various enzymes

Calcium

- The calcium ion (Ca^{2+}) is perhaps the most common intracellular messenger in neurons.
- Information is transmitted by a transient rise in the cytoplasmic calcium concentration, which allows Ca^{2+} to bind to a large number of Ca^{2+}-binding proteins that serve as molecular targets.
- One of the most thoroughly studied targets of Ca^{2+} is **calmodulin**, a Ca^{2+}-binding protein abundant in the cytosol of all cells.
- Binding of Ca^{2+} to calmodulin activates this protein, which then initiates its effects by binding to still other downstream targets, such as protein kinases.

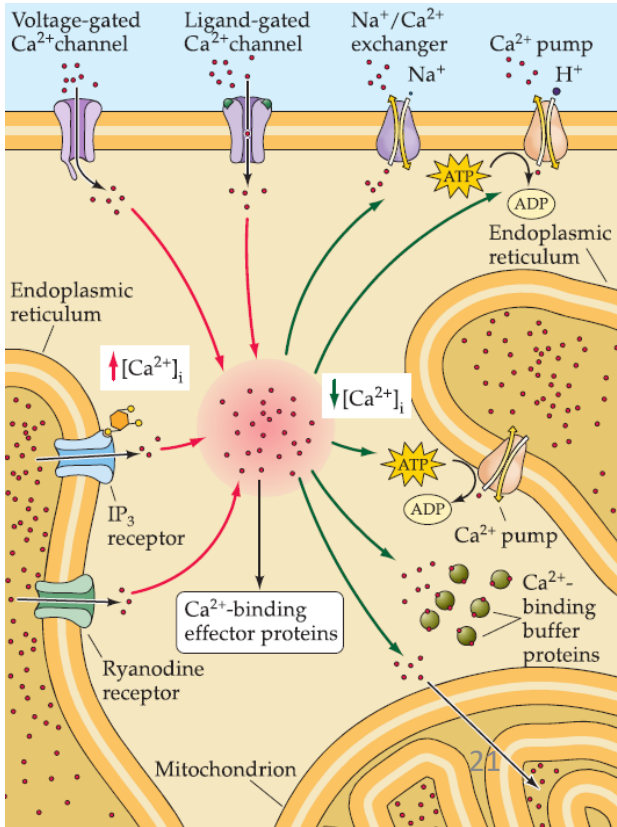
Calcium

- Ordinarily the concentration of Ca^{2+} ions in the cytosol is extremely low, typically 50-100 nanomolar (10^{-9}).
- The concentration of Ca^{2+} ions outside neurons--in the bloodstream or cerebrospinal fluid, for instance--is several orders of magnitude higher, typically several millimolar (10^{-3}).
- This steep Ca^{2+} gradient is maintained by a number of mechanisms.
- Most important in this maintenance are two proteins that translocate Ca^{2+} from the cytosol to the extracellular medium:
  - an ATPase called the **calcium pump**.
  - an **Na^+/Ca^{2+} exchanger**, which is a protein that replaces intracellular Ca^{2+} with extracellular sodium ions.
- Ca^{2+} is also pumped into the endoplasmic reticulum and mitochondria.



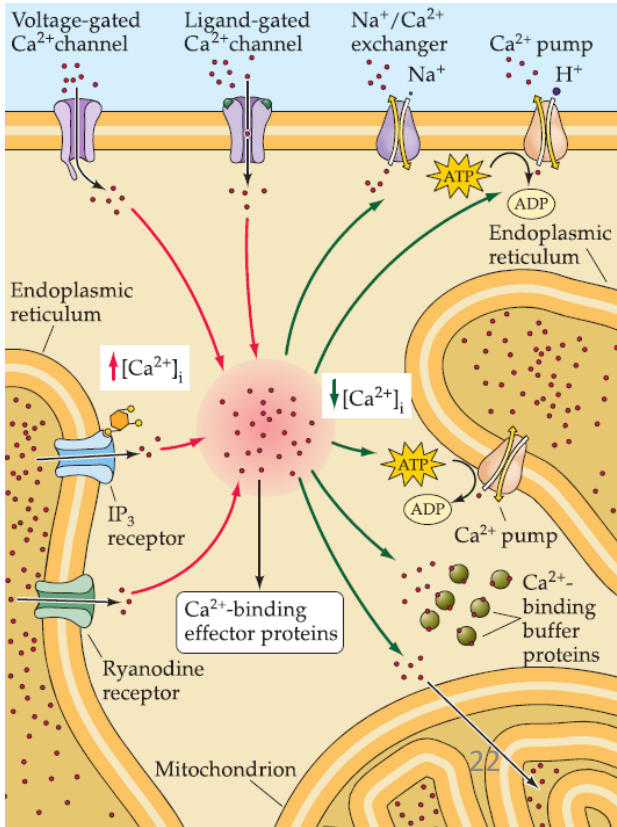
Calcium

- The Ca^{2+} ions that act as intracellular signals enter cytosol by means of one or more types of Ca^{2+}-permeable ion channels.
- These can be voltage-gated Ca^{2+} channels or ligand-gated channels in the plasma membrane, both of which allow Ca^{2+} to flow down the Ca^{2+} gradient and into the cell from the extracellular medium.
- In addition, other channels allow Ca^{2+} to be released from the interior of the endoplasmic reticulum into the cytosol.
  - These intracellular Ca^{2+}-releasing channels are gated, so they can be opened or closed in response to various intracellular signals.
    - One such channel is the inositol trisphosphate (IP_3) receptor, which is regulated by IP_3, a second messenger.
    - A second type of intracellular Ca^{2+}-releasing channel is the ryanodine receptor, named after a drug that binds to and partially opens these receptors.



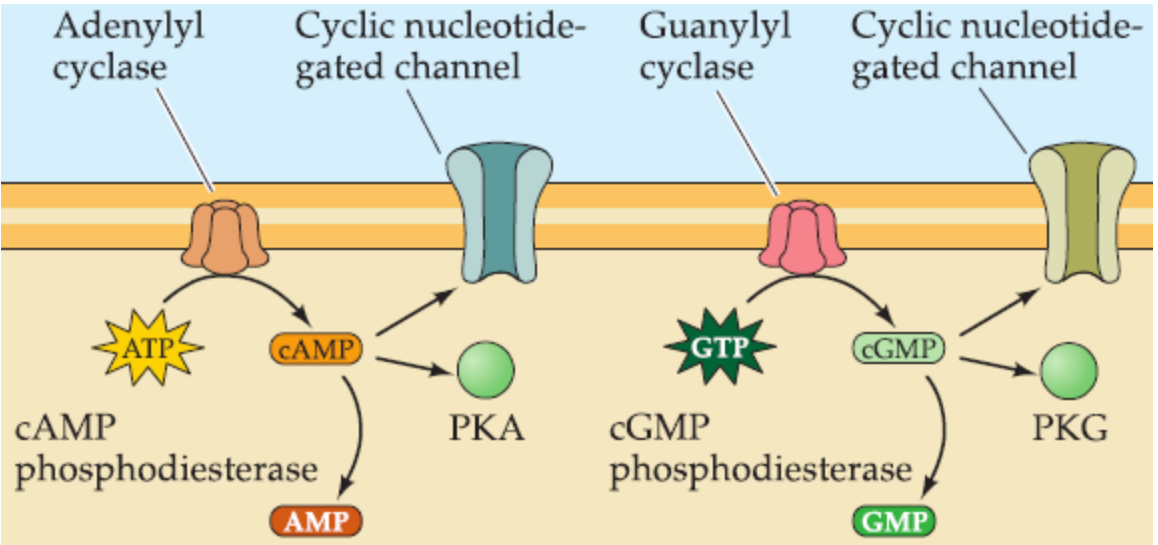
Calcium

- These various mechanisms for elevating and removing Ca^{2+} ions allow precise control of both timing and location of Ca^{2+} signaling within neurons, which in turn permit Ca^{2+} to control many different signaling events.
- For example, voltage-gated Ca^{2+} channels allow Ca^{2+} concentrations to rise very rapidly and locally within presynaptic terminals to trigger neurotransmitter release.
- Slower and more widespread rises in Ca^{2+} concentration regulate a wide variety of other responses, including gene expression in the cell nucleus.



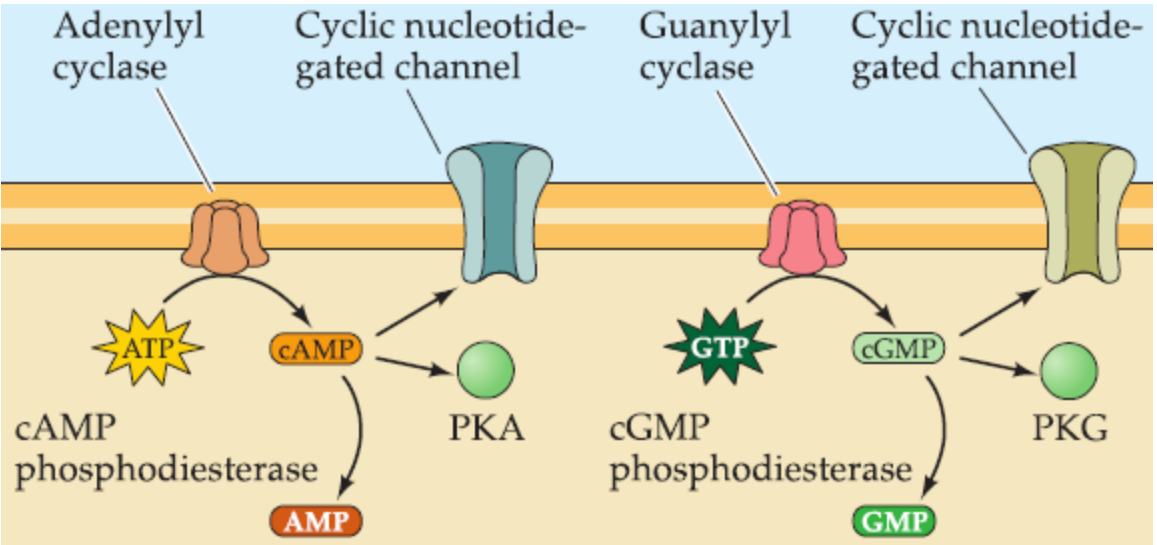
Cyclic nucleotides

- **cyclic adenosine monophosphate (cAMP):**
  - Cyclic AMP is a derivative of the common cellular energy storage molecule ATP, and is produced when G-proteins activate adenylyl cyclase in the plasma membrane.
  - Adenylyl cyclase converts ATP into cAMP by removing two phosphate groups from the ATP.
- **cyclic guanosine monophosphate (cGMP):**
  - Cyclic GMP is similarly produced from GTP by the action of guanylyl cyclase.



Cyclic nucleotides

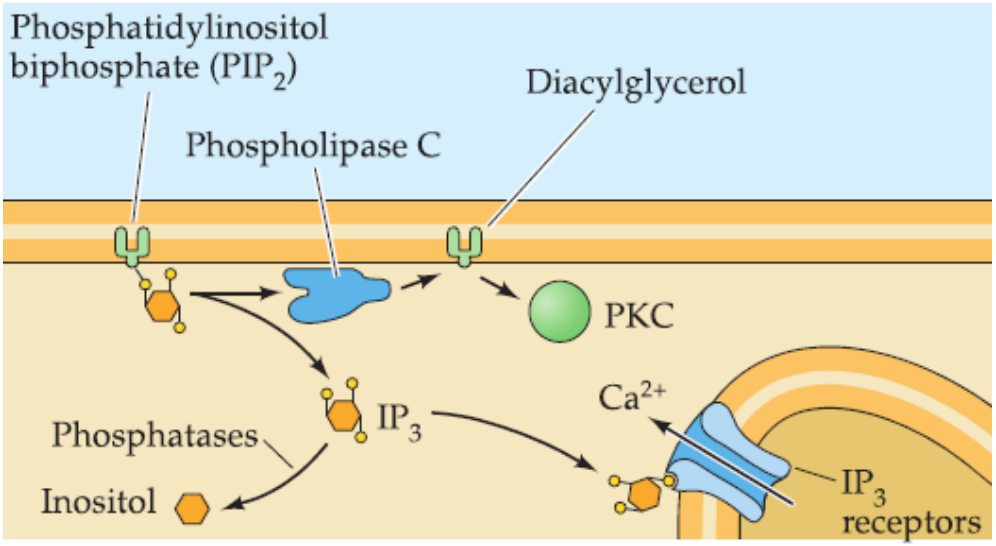
- Once the intracellular concentration of cAMP or cGMP is elevated, these nucleotides can bind to two different classes of targets:
  - The most common targets of cyclic nucleotide action are protein kinases, either the cAMP-dependent protein kinase (PKA) or the cGMP-dependent protein kinase (PKG).
    - These enzymes mediate many physiological responses by phosphorylating target proteins.
  - cAMP and cGMP can bind to certain ligand-gated ion channels, thereby influencing neuronal signaling.
    - These cyclic nucleotide-gated channels are particularly important in phototransduction and other sensory transduction processes, such as olfaction.



- Cyclic nucleotide signals are degraded by phosphodiesterases, enzymes that cleave phosphodiester bonds and convert cAMP into AMP or cGMP into GMP.

Diacylglycerol and IP_3

- Membrane lipids can also be converted into intracellular second messenger.
- The two most important messengers of this type are produced from phosphatidylinositol bisphosphate (PIP_2).
- This lipid component is cleaved by phospholipase C, an enzyme activated by certain G-proteins and by calcium ions.
- Phospholipase C splits the PIP_2 into two smaller molecules, each of which acts as a second messenger:
  - One of these messengers is diacylglycerol (DAG), a molecule that remains within the membrane and activates protein kinase C, which phosphorylates substrate proteins in both the plasma membrane and elsewhere.
  - The other messenger is inositol trisphosphate (IP_3), a molecule that leaves the cell membrane and diffuses within the cytosol.



- IP_3 binds to IP_3 receptors, channels that release calcium from the endoplasmic reticulum.
- The action of IP_3 is to produce yet another second messenger (perhaps a third messenger, in this case!) that triggers a whole spectrum of reactions in the cytosol.

Second messengers

- The intracellular concentration of these second messengers changes dynamically over time, allowing very precise control over their downstream targets.

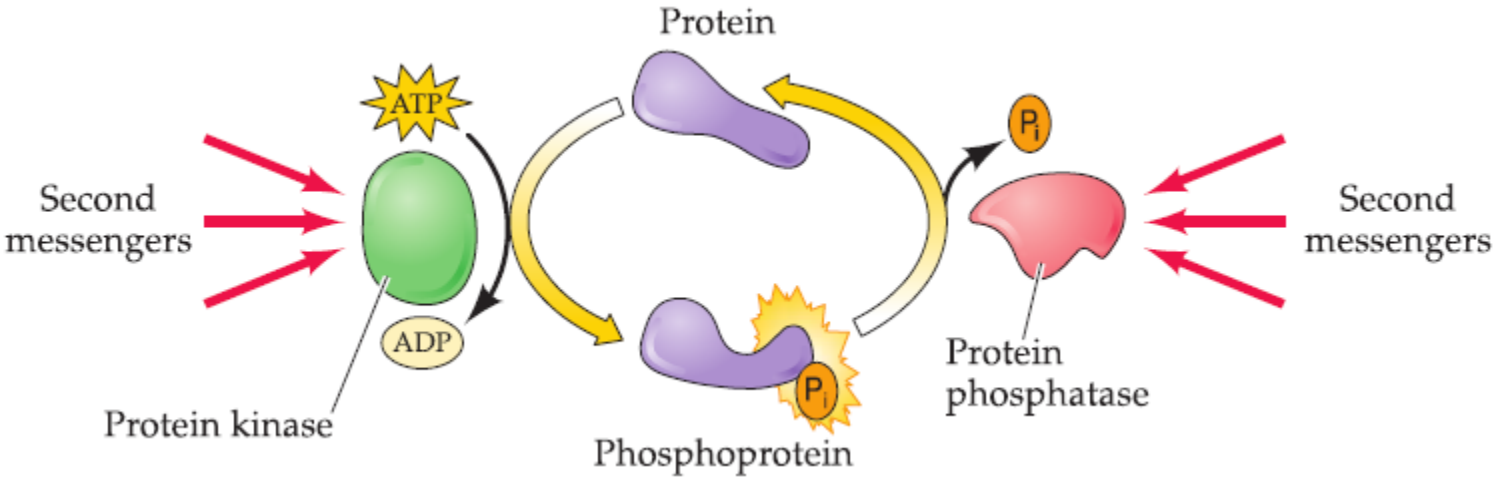
- These signals can also be localized to small compartments within single cells or can spread over great distances, even spreading between cells via gap junctions.

- Understanding of the complex temporal and spatial dynamics of these second messenger signals has been greatly aided by the development of imaging techniques that visualize second messengers and other molecular signals within cells.

Second messenger targets:

Protein kinases and phosphatases

- Second messengers typically regulate neuronal functions by modulating the phosphorylation state of intracellular proteins.



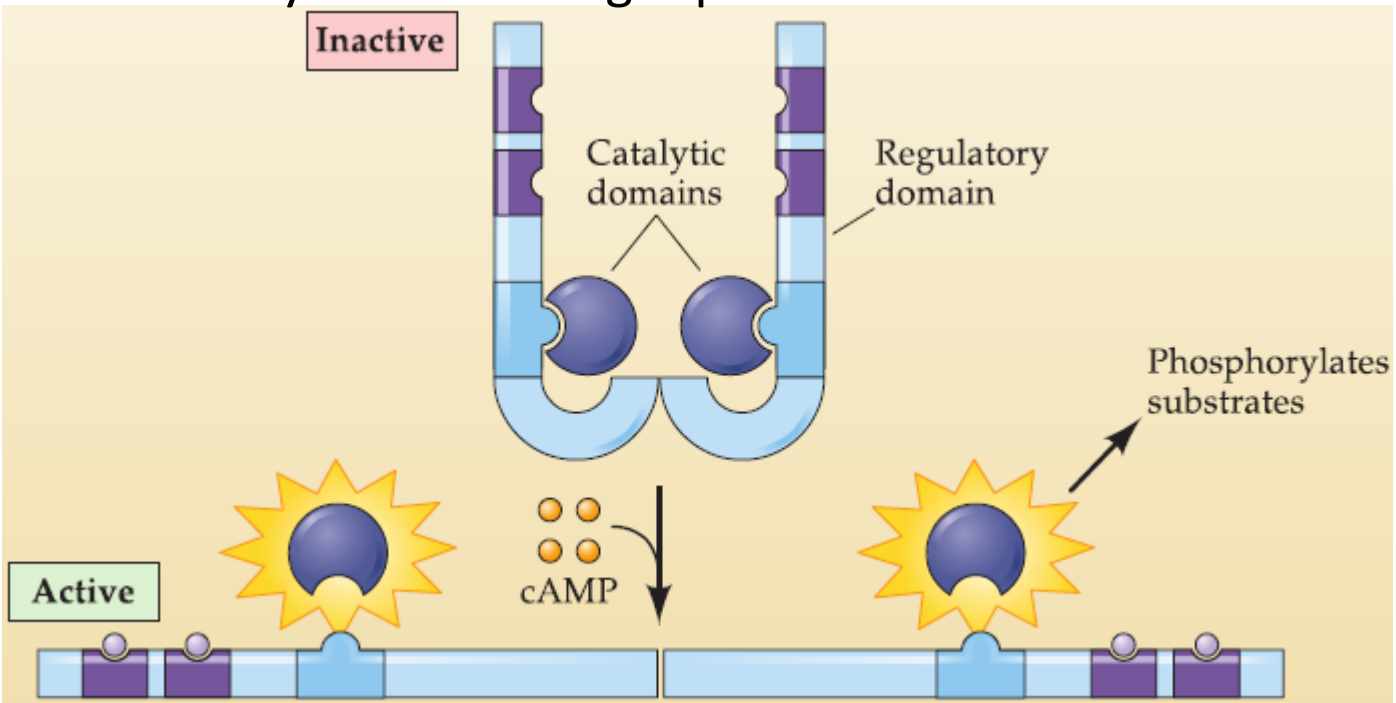
- Phosphorylation (the addition of phosphate groups) rapidly and reversibly changes protein function.
- Proteins are phosphorylated by a wide variety of **protein kinases**; phosphate groups are removed by other enzymes called **protein phosphatases**.
- The degree of phosphorylation of a target protein reflects a balance between the competing actions of protein kinases and phosphatases, thus integrating a host of cellular signaling pathways.

Protein kinases and phosphatases

- The substrates of protein kinases and phosphatases include enzymes, neurotransmitter receptors, ion channels, and structural proteins.
- Protein kinases and phosphatases typically act either on the serine and threonine residues (Ser/Thr kinases or phosphatases) or on the tyrosine residues (Tyr kinases or phosphatases) of their substrates.
- Some of these enzymes act specifically on only one or a handful of protein targets, while others are multifunctional and have a broad range of substrate proteins.
- The activity of protein kinases and phosphatases can be regulated either by second messengers, such as cAMP or Ca^{2+}, or by extracellular chemical signals such as growth factors.
- Typically, second messengers activate Ser/Thr kinases, whereas extracellular signals activate Tyr kinases.

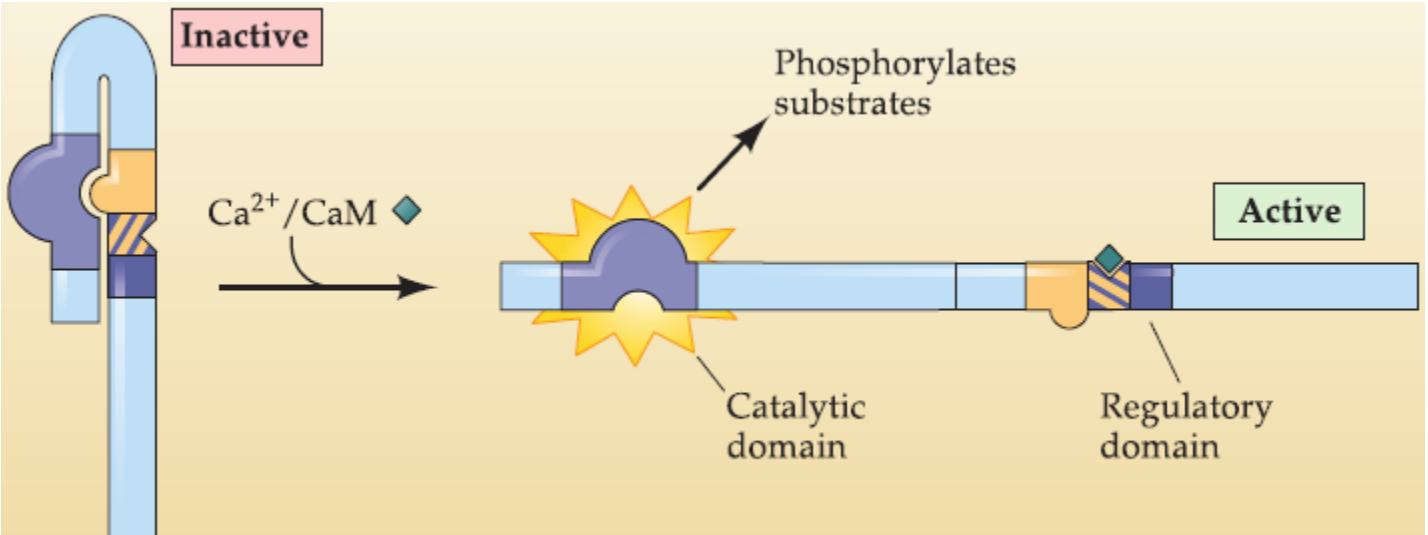
cAMP-dependent protein kinase (PKA)

- The primary effector of cAMP is the cAMP-dependent protein kinase (PKA).
- PKA is a tetrameric complex of two catalytic subunits and two inhibitory (regulatory) subunits.
- cAMP activates PKA by binding to the regulatory subunits and causing them to release active catalytic subunits.
- Such displacement of inhibitory domains is a general mechanism for activation of several protein kinases by second messengers.
- The catalytic subunit of PKA phosphorylates serine and threonine residues of many different target proteins.



Ca^{2+}/calmodulin-dependent protein kinase type II (CaMKII)

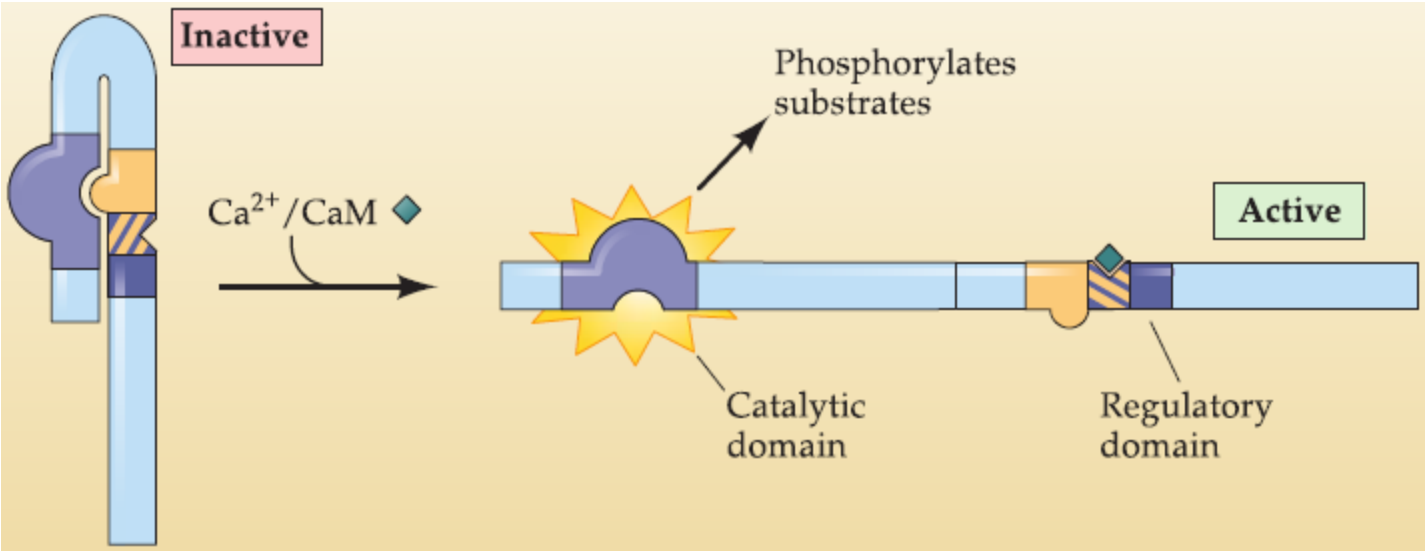
- Ca^{2+} ions binding to calmodulin can regulate protein phosphorylation/dephosphorylation.
- In neurons, the most abundant Ca^{2+}/calmodulin-dependent protein kinase is CaMKII, a multifunctional Ser/Thr protein kinase.
- CaMKII is composed of approximately 14 subunits, which in the brain are the α and β types.
- Each subunit contains a catalytic domain and a regulatory domain, as well as other domains that allow the enzyme to oligomerize and target to the proper region within the cell.



Ca^{2+}/calmodulin-dependent protein kinase type II (CaMKII)

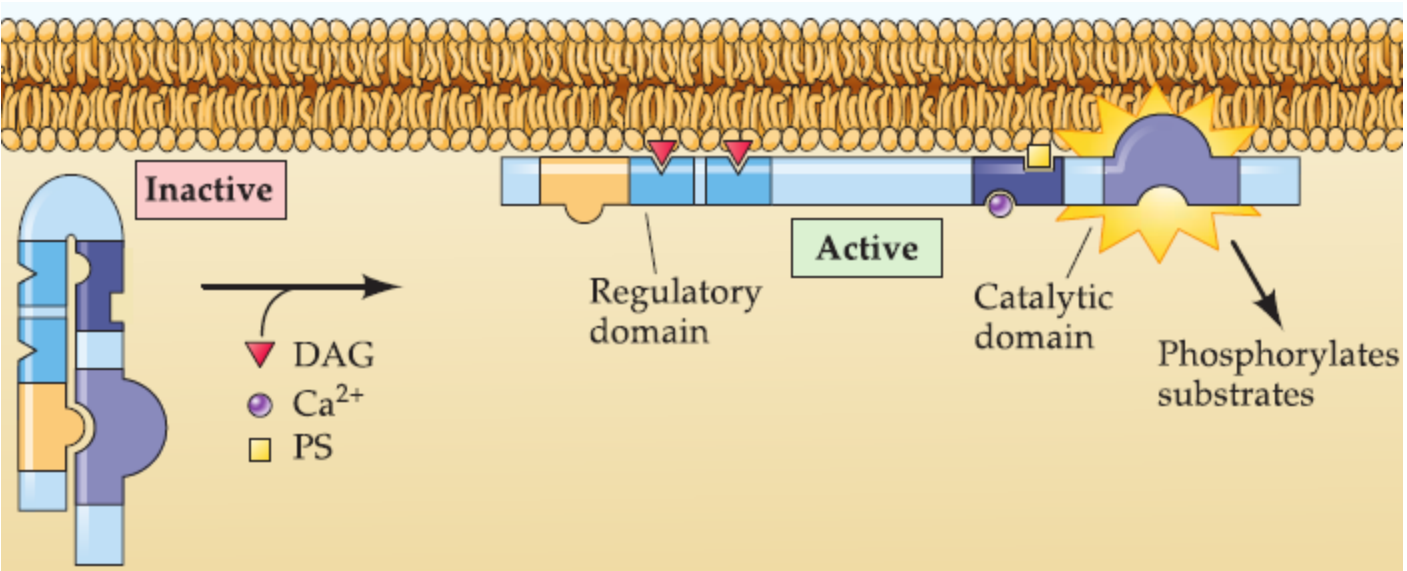
- Ca^{2+}/calmodulin activates CaMKII by displacing the inhibitory domain from the catalytic site.

- CaMKll phosphorylates a large number of substrates, including ion channels and other proteins involved in intracellular signal transduction.



Protein kinase C (PKC)

- PKCs are diverse monomeric kinases activated by the second messengers DAG and Ca^{2+}.
- DAG causes PKC to move from the cytosol to the plasma membrane, where it also binds Ca^{2+} and phosphatidylserine, a membrane phospholipid.
- These events relieve autoinhibition and cause PKC to phosphorylate various protein substrates.
- PKC also diffuses to sites other than the plasma membrane--such as the cytoskeleton, perinuclear sites, and the nucleus--where it phosphorylates still other substrate proteins.



Protein tyrosine kinases

- Two classes of protein kinases transfer phosphate groups to tyrosine residues on substrate proteins:
  - Receptor tyrosine kinases are transmembrane proteins with an extracellular domain that binds to protein ligands (growth factors, neurotrophic factors, or cytokines) and an intracellular catalytic domain that phosphorylates the relevant substrate proteins.
  - Nonreceptor tyrosine kinases are cytoplasmic or membrane-associated enzymes that are indirectly activated by extracellular signals.
- Tyrosine kinases are particularly important for cell growth and differentiation.

Mitogen-activated protein kinase (MAPK)

- In addition to protein kinases that are directly activated by second messengers, some of these molecules can be activated by other signals, such as phosphorylation by another protein kinase.
- Important examples of such protein kinases are the mitogen-activated protein kinases (MAPKs), also called extracellular signal-regulated kinases (ERKs).
- MAPKs were first identified as participants in the control of cell growth and are now known to have many other signaling functions.
- MAPKs are normally inactive in neurons but become activated when they are phosphorylated by other kinases.
- In fact, MAPKs are part of a kinase cascade in which one protein kinase phosphorylates and activates the next protein kinase in the cascade.
- The extracellular signals that trigger these kinase cascades are often extracellular growth factors that bind to receptor tyrosine kinases that, in turn, activate monomeric G-proteins such as ras.
- Once activated, MAPKs can phosphorylate transcription factors, various enzymes including other protein kinases, and cytoskeletal proteins.

Phosphatase

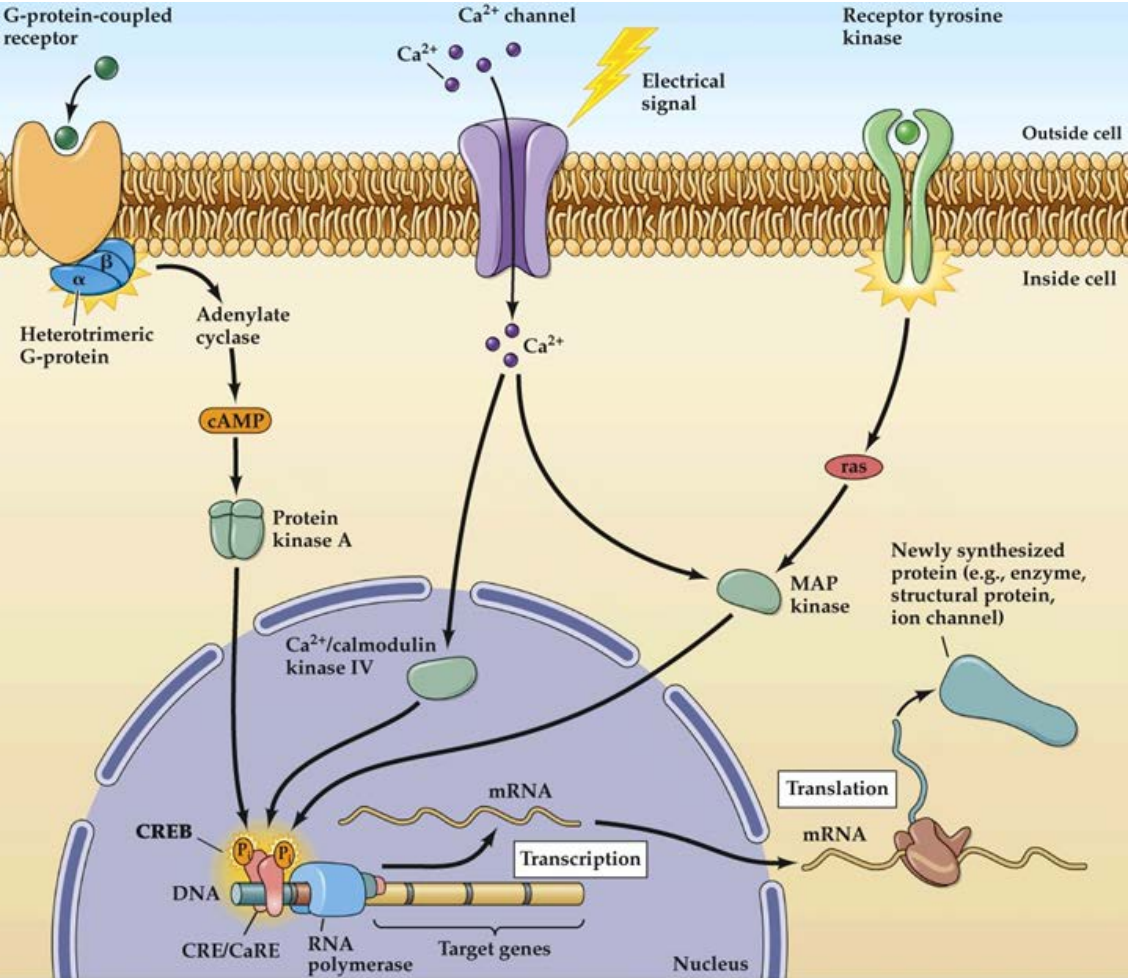
- The best-characterized protein phosphatases are the Ser/Thr phosphatases PP1, PP2A, and PP2B (also called calcineurin).
- In general, protein phosphatases display less substrate specificity than protein kinases.
- Their limited specificity may arise from the fact that the catalytic subunits of the three major protein phosphatases are highly homologous though each still associates with specific targeting or regulatory subunits.
- PP1 dephosphorylates a wide array of substrate proteins and is probably the most prevalent Ser/Thr protein phosphatase in mammalian cells.
  - PP1 activity is regulated by several inhibitory proteins expressed in neurons.
- PP2A is a multisubunit enzyme with a broad range of substrates that overlap with PP1.
- PP2B, or calcineurin, is present at high levels in neurons.
  - A distinctive feature of this phosphatase is its activation by Ca^{2+}/calmodulin.
  - PP2B is composed of a catalytic and a regulatory subunit.
  - Ca^{2+}/calmodulin activates PP2B primarily by binding to the catalytic subunit and displacing the inhibitory regulatory domain.
  - PP2B generally does not have the same molecular targets as CaMKII, even though both enzymes are activated by Ca^{2+}/calmodulin.

Nuclear signaling

- Second messengers elicit prolonged changes in neuronal function by promoting the synthesis of new RNA and protein.
- The resulting accumulation of new proteins requires at least 30-60 minutes, a time frame that is orders of magnitude slower than the responses mediated by ion fluxes or phosphorylation.
- Intracellular signal transduction cascades regulate gene expression by converting transcription factors from an inactive state to an active state in which they are able to bind to DNA .

CREB

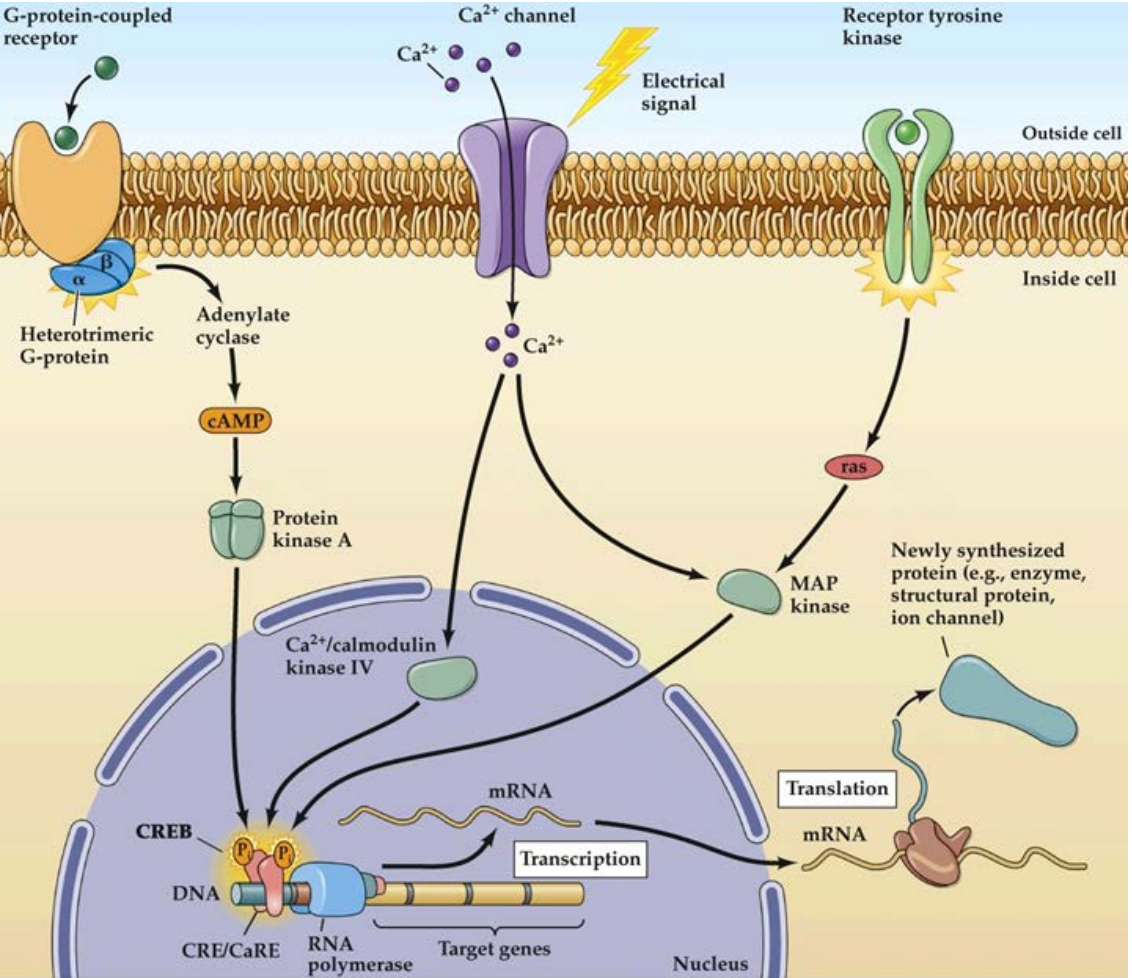
- The *c*AMP *r*esponse *e*lement *b*inding protein, usually abbreviated **CREB**, is a ubiquitous transcriptional activator.
- CREB is normally bound to its binding site on DNA (called the cAMP response element, or CRE), either as a homodimer or bound to another, closely related transcription factor.



- In unstimulated cells, CREB is not phosphorylated and has little or no transcriptional activity.
- However, phosphorylation of CREB greatly potentiates transcription.

CREB

- Both PKA and the ras pathway can phosphorylate CREB.
- CREB can also be phosphorylated in response to increased intracellular calcium, in which case the CRE site is also called the CaRE (*ca*lcium *r*esponse *e*lement) site.



- The calcium-dependent phosphorylation of CREB is primarily caused by Ca^{2+}/calmodulin kinase IV (a relative of CaMKII) and by MAP kinase, which leads to prolonged CREB phosphorylation.
- CREB is thus an example of the convergence of multiple signaling pathways onto a single transcriptional activator.

CREB

- Many genes whose transcription is regulated by CREB have been identified.
- CREB-sensitive genes include the immediate early gene, c-fos, the neurotrophin BDNF, the enzyme tyrosine hydroxylase (which is important for synthesis of catecholamine neurotransmitters), and many neuropeptides (including somatostatin, enkephalin, and corticotropin-releasing hormone).
- CREB also is thought to mediate long lasting changes in brain function, including spatial learning, behavioral sensitization, long-term memory of odorant-conditioned behavior, and long-term synaptic plasticity.

Nuclear receptors

- Nuclear receptors for membrane-permeant ligands also are transcriptional activators.
  - The receptor for glucocorticoid hormones illustrates one mode of action of such receptors.
    - In the absence of glucocorticoid hormones, the receptors are located in the cytoplasm.
    - Binding of glucocorticoids causes the receptor to unfold and move to the nucleus, where it binds a specific recognition site on the DNA.
    - This DNA binding activates the relevant RNA polymerase complex to initiate transcription and subsequent gene expression.
  - The receptor for thyroid hormone (TH) illustrates a second mode of regulation.
    - In the absence of TH, the receptor is bound to DNA and serves as a potent repressor of transcription.
    - Upon binding TH, the receptor undergoes a conformational change that ultimately opens the promoter for polymerase binding.
    - Hence, TH binding switches the receptor from being a repressor to being an activator of transcription.

c-fos

- In resting cells, c-fos is present at a very low concentration.
- Stimulation of the target cell causes c-fos to be synthesized, and the amount of this protein rises dramatically over 30-60 minutes.
- Therefore, c-fos is considered to be an **immediate early gene** because its synthesis is directly triggered by the stimulus.
- Once synthesized, c-fos protein can act as a transcriptional activator to induce synthesis of second-order genes, which are termed **delayed response genes**.

c-fos

- Multiple signals converge on c-fos, activating different transcription factors that bind to at least three distinct sites in the promoter region of the gene.

1. The regulatory region of the c-fos gene contains a binding site that mediates transcriptional induction by cytokines and ciliary neurotropic factor.

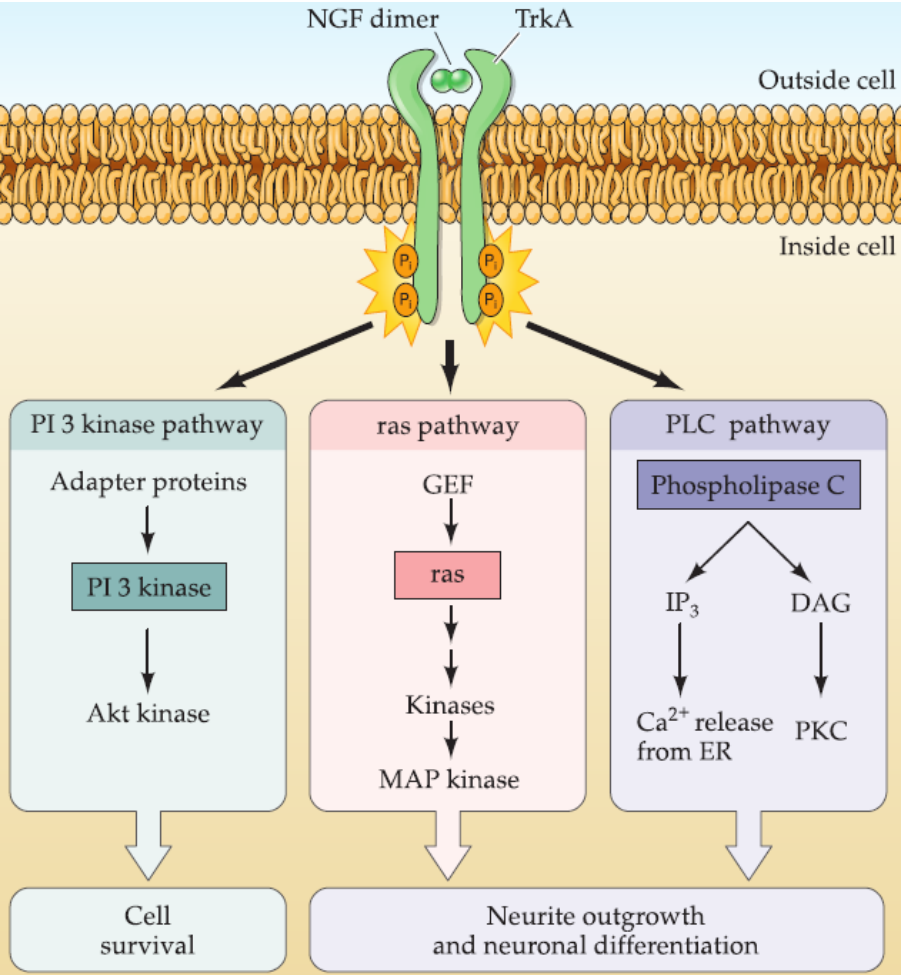
2. Another site is targeted by growth factors such as neurotrophins through ras and protein kinase C.

3. A CRE/CaRE that can bind to CREB and thereby respond to cAMP or calcium entry resulting from electrical activity.

Examples of Neuronal Signal Transduction

NGF/TrkA

- **Nerve growth factor** (**NGF**) is a member of the neurotrophin growth factor family and is required for the differentiation, survival and synaptic connectivity of sympathetic and sensory neurons.
  - NGF works by binding to a high-affinity tyrosine kinase receptor, TrkA, found on the plasma membrane of these target cells.

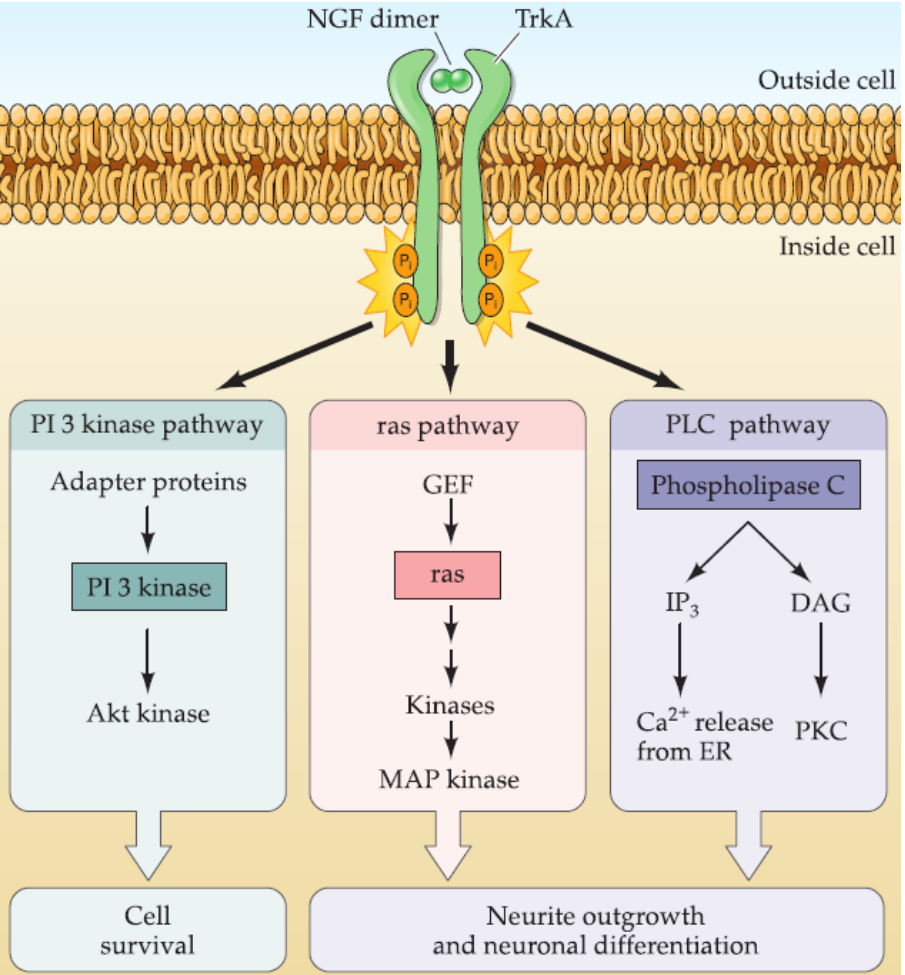


- NGF binding causes TrkA receptors to dimerize, and the intrinsic tyrosine kinase activity of each receptor then phosphorylates its partner receptor.

1. Phosphorylated TrkA receptors trigger the ras cascade, resulting in the activation of multiple protein kinases.
   - Some of these kinases translocate to the nucleus to activate transcriptional activators, such as CREB.
   - This ras-based component of the NGF pathway is primarily responsible for inducing and maintaining differentiation of NGF-sensitive neurons.

NGF/TrkA

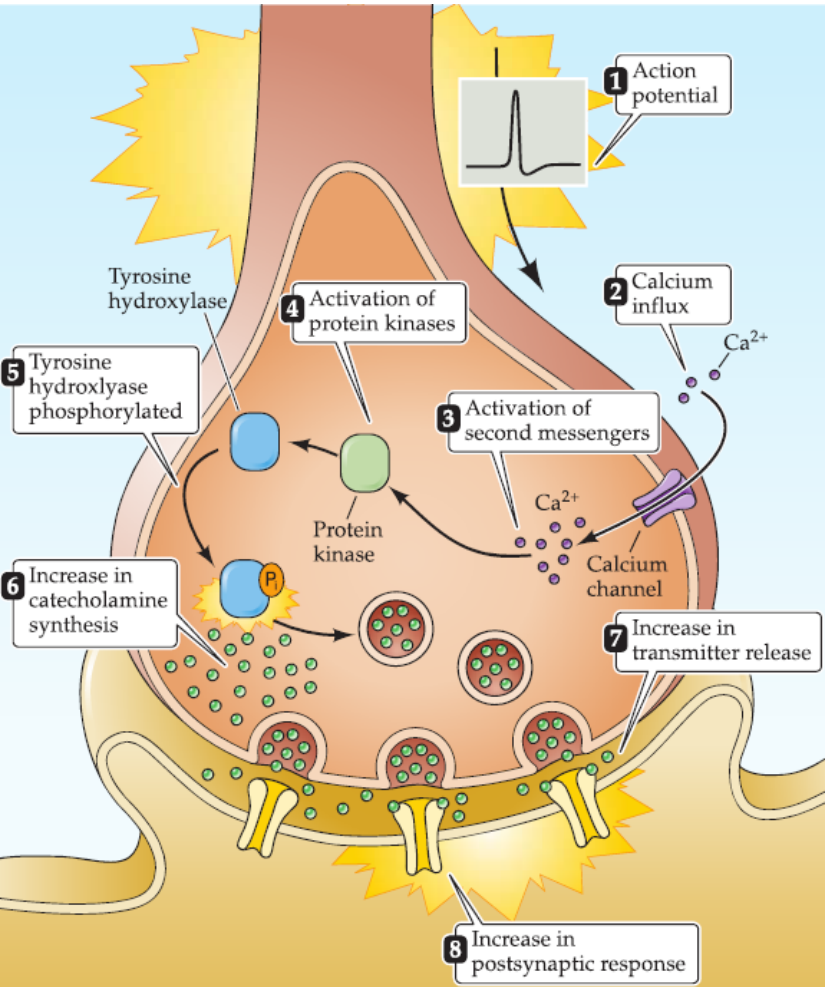
2. Phosphorylation of TrkA also causes this receptor to stimulate the activity of phospholipase C, which increases production of IP_3 and DAG.
   - IP_3 induces release of Ca^{2+} from the endoplasmic reticulum, and diacylglycerol activates PKC.
   - These two second messengers appear to target many of the same downstream effectors as ras.



3. Activation of TrkA receptors also causes activation of other protein kinases (such as Akt kinase) that inhibit cell death.
   - This pathway primarily mediates the NGF-dependent survival of sympathetic and sensory neurons.

Phosphorylation of tyrosine hydroxylase

- Tyrosine hydroxylase governs the synthesis of the catecholamine neurotransmitters: dopamine, norepinephrine, and epinephrine.
  - A number of signals, including electrical activity, other neurotransmitters, and NGF, increase the rate of catecholamine synthesis by increasing the catalytic activity of tyrosine hydroxylase.



- The rapid increase of tyrosine hydroxylase activity is largely due to phosphorylation of this enzyme.
- Tyrosine hydroxylase is a substrate for several protein kinases, including PKA, CaMKII, MAP kinase, and PKC.
- Phosphorylation causes conformational changes that increase catalytic activity of tyrosine hydroxylase.
- Stimuli that elevate cAMP, Ca^{2+}, or DAG can all increase tyrosine hydroxylase activity and thus increase the rate of catecholamine biosynthesis.

Summary

- Diverse signal transduction pathways exist within all neurons.
- Activation of these pathways typically is initiated by chemical signals such as neurotransmitters and hormones, which bind to receptors that include ligand-gated ion channels, G-protein-coupled receptors and tyrosine kinase receptors.
- Many of these receptors activate either heterotrimeric or monomeric G-proteins that regulate intracellular enzyme cascades and/or ion channels.
- A common outcome of the activation of these receptors is the production of second messengers, such as cAMP, Ca^{2+}, IP_3, that bind to effector enzymes.
- Particularly important effectors are protein kinases and phosphatases that regulate the phosphorylation state of their substrates, and thus their function.
- These substrates can be metabolic enzymes or other signal transduction molecules such as ion channels, protein kinases, or transcription factors that regulate gene expression, such as CREB and c-fos.