Dr.Sudipta Chahraborty

# Signal Transduction

A signal represents *information* that is detected by specific receptors and converted to a cellular response, which always involves a *chemical* process. This conversion of information into a chemical change, *signal transduction*, is a universal property of living cells.

## Properties of signaling:

### A. Specificity

It is achieved by precise molecular complementarity between the signal and receptor molecules, mediated by the same kinds of weak (noncovalent) forces that mediate enzyme-substrate and antigen-antibody interactions. Three factors account for the extraordinary sensitivity of signal transducers: the high affinity of receptors for signal molecules, cooperativity in the ligand-receptor interaction, and amplification of the signal by enzyme cascades.

### B. Amplification

It results when an enzyme associated with a signal receptor is activated and, in turn, catalyzes the activation of many molecules of a second enzyme, each of which activates many molecules of a third enzyme, and so on. Such cascades can produce amplifications of several orders of magnitude within milliseconds.

### C. Desensitization/Adaptation

The sensitivity of receptor systems is subject to modification. When a signal is present continuously, desensitization of the receptor system results; when the stimulus falls below a certain threshold, the system again becomes sensitive.

### D. Integration

It is the ability of the system to receive multiple signals and produce a unified response appropriate to the needs of the cell or organism. Different signaling pathways converse with each other at several levels, generating a wealth of interactions that maintain homeostasis in the cell and the organism.

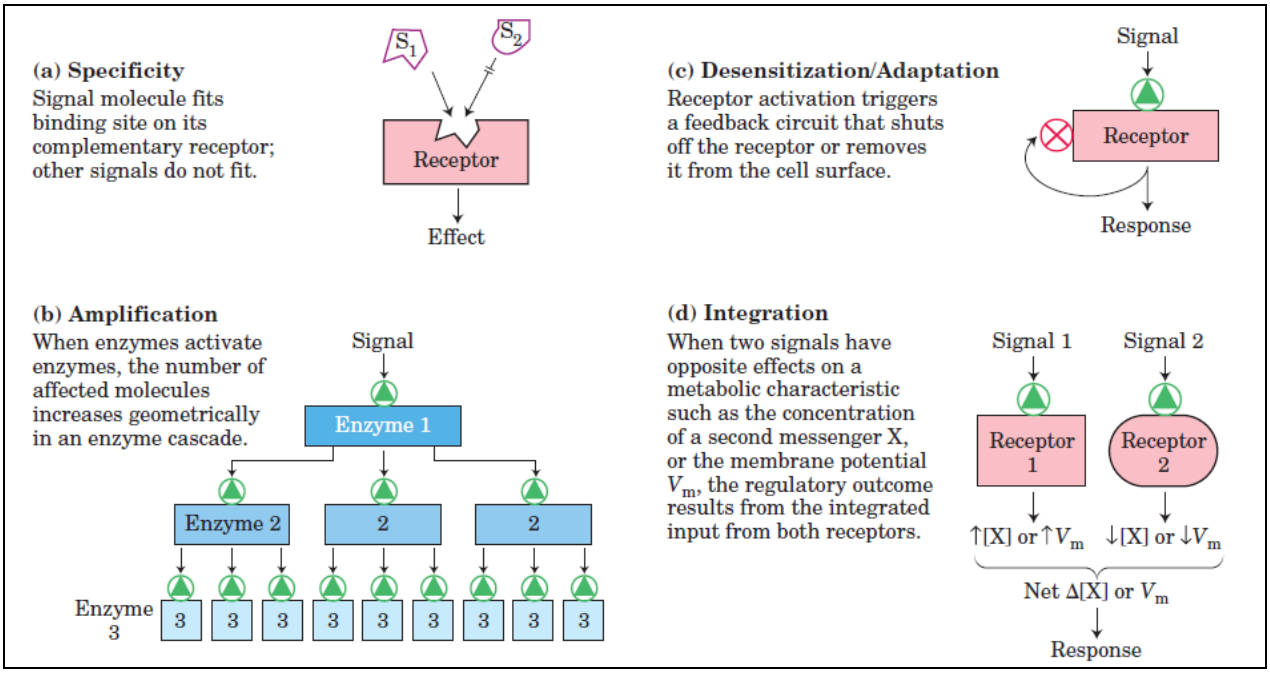



**Figure:** Characteristic properties of signaling

## Classification of signaling pathways:

The trigger for each signal system is different, but the general features of signal transduction are common to all: a signal interacts with a receptor; the activated receptor interacts with cellular machinery, producing a second signal or a change in the activity of a cellular protein; the metabolic activity (broadly defined to include metabolism of RNA, DNA, and protein) of the target cell undergoes a change; and finally, the transduction event ends and the cell returns to its prestimulus state. To demonstrate these general features of signaling systems, six basic signaling mechanisms can be selected:

1. Gated ion channels of the plasma membrane that open and close in response to the binding of chemical ligands or changes in transmembrane potential. These are the simplest signal transducers. The acetylcholine receptor ion channel is an example of this mechanism.
2. Receptor enzymes, plasma membrane receptors that are also enzymes. When one of these receptors is activated by its extracellular ligand, it catalyzes the production of an intracellular second messenger e.g. insulin receptor.
3. Receptor proteins (serpentine receptors) that *indirectly* activate (through GTP-binding proteins, or G proteins) enzymes that generate intracellular second messengers. This is illustrated by the β-adrenergic receptor system that detects epinephrine (adrenaline).
4. Nuclear receptors (steroid receptors) that, when bound to their specific ligand (such as the hormone estrogen), alter the rate at which specific genes are transcribed and translated into cellular proteins. The steroid hormone function is an example.
5. Receptors that lack enzymatic activity but attract and activate cytoplasmic enzymes that act on downstream proteins, either by directly converting them to gene-regulating proteins or by activating a cascade of enzymes that finally activates a gene regulator. The JAK-STAT system exemplifies the first mechanism; and the TLR4 (Toll) signaling system in humans, the second.
6. Receptors (adhesion receptors) that interact with macromolecular components of the extracellular matrix (such as collagen) and convey to the cytoskeletal system instructions on cell migration or adherence to the matrix. Integrins illustrate this general type of transduction mechanism.

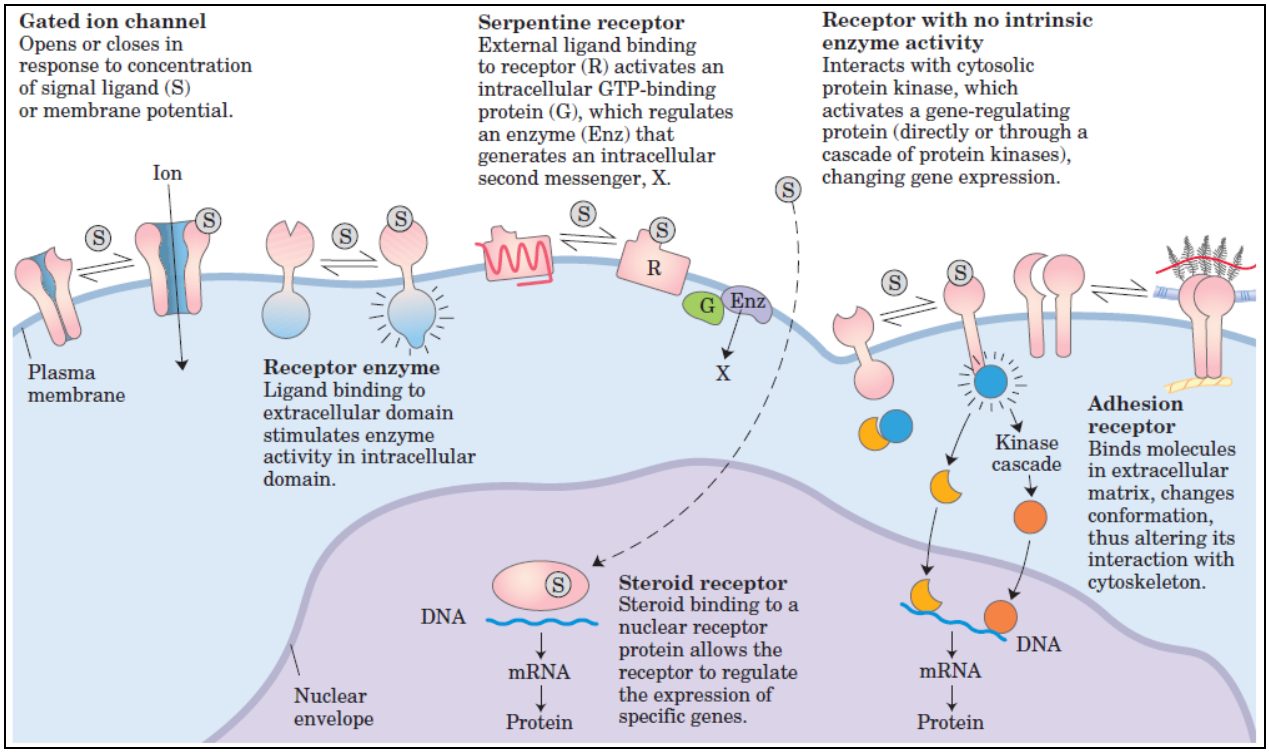



**Figure:** Six general types of signal transducers

**Signaling in acetylcholine receptor**

One of the best-understood examples of a ligand-gated receptor channel is the nicotinic acetylcholine receptor. The receptor channel opens in response to the neurotransmitter acetylcholine (and to nicotine, hence the name). This receptor is found in the postsynaptic membrane of neurons at certain synapses and in muscle fibers (myocytes) at neuromuscular junctions.

**Mechanism:**

- The acetylcholine receptor is an allosteric protein with two high-affinity binding sites for acetylcholine, about 3.0 nm from the ion gate, on the two α-subunits.
- The binding of acetylcholine causes a change from the closed to the open conformation.
- The process is positively cooperative: binding of acetylcholine to the first site increases the acetylcholine-binding affinity of the second site.
- When the presynaptic cell releases a brief pulse of acetylcholine, both sites on the postsynaptic cell receptor are occupied briefly and the channel opens.
- Either $Na^+$ or $Ca^{2+}$ can now pass, and the inward flux of these ions depolarizes the plasma membrane, initiating subsequent events that vary with the type of tissue.
- In a postsynaptic neuron, depolarization initiates an action potential; at a neuromuscular junction, depolarization of the muscle fiber triggers muscle contraction.
- Normally, the acetylcholine concentration in the synaptic cleft is quickly lowered by the enzyme acetylcholinesterase, present in the cleft.
- When acetylcholine levels remain high for more than a few milliseconds, the receptor is desensitized. The receptor channel is converted to a third conformation in which the channel is closed and the acetylcholine is very tightly bound.
- The slow release (in tens of milliseconds) of acetylcholine from its binding sites eventually allows the receptor to return to its resting state—closed and resensitized to acetylcholine levels.

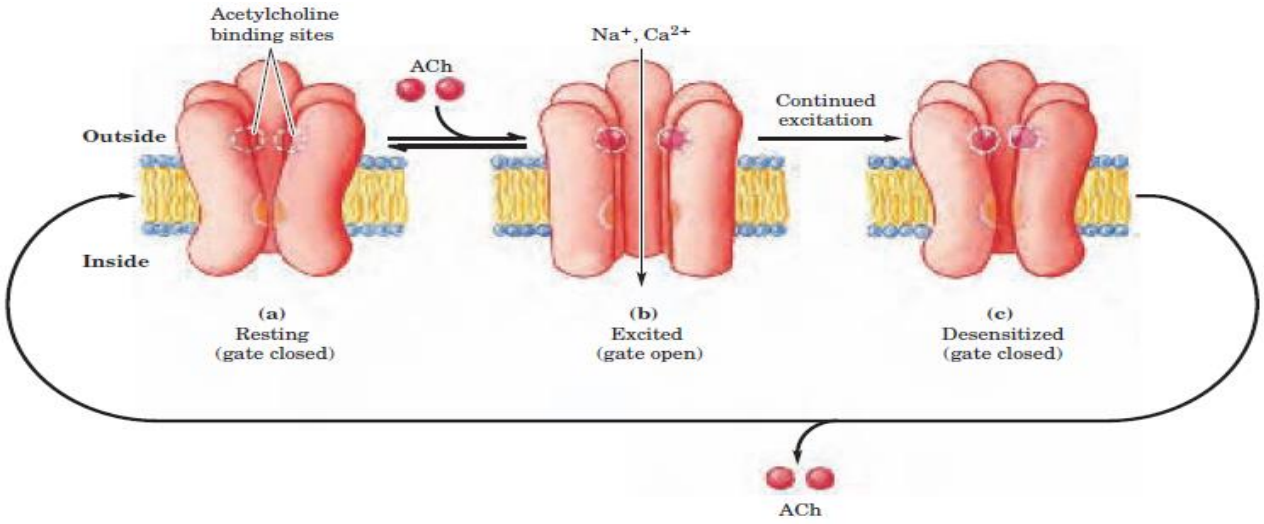



**Figure:** Three states of the acetylcholine receptor: Brief exposure of **(a)** the resting (closed) ion channel to acetylcholine (ACh) produces **(b)** the excited (open) state. Longer exposure leads to **(c)** desensitization and channel closure.

## Voltage-Gated Ion Channels

Signaling in the nervous system is accomplished by networks of neurons, specialized cells that carry an electrical impulse (action potential) from the cell body through the axon. The electrical signal triggers release of neurotransmitter molecules at the synapse, carrying the signal to the next cell in the circuit. Three types of *voltage-gated ion channels* are essential to this signaling mechanism:

1. Voltage-gated $Na^+$ channels,
2. Voltage-gated $K^+$ channels
3. Voltage-gated $Ca^{2+}$ channels.

The gated ion channels convey signals in either of two ways:

- by changing the cytosolic concentration of an ion (such as $Ca^{2+}$) which then serves as an intracellular *second messenger* (hormone or neurotransmitter is the first messenger), or
- by changing $V_m$ and affecting other membrane proteins that are sensitive to $V_m$.

The passage of an electrical signal through one neuron and on to the next illustrates both types of mechanism.

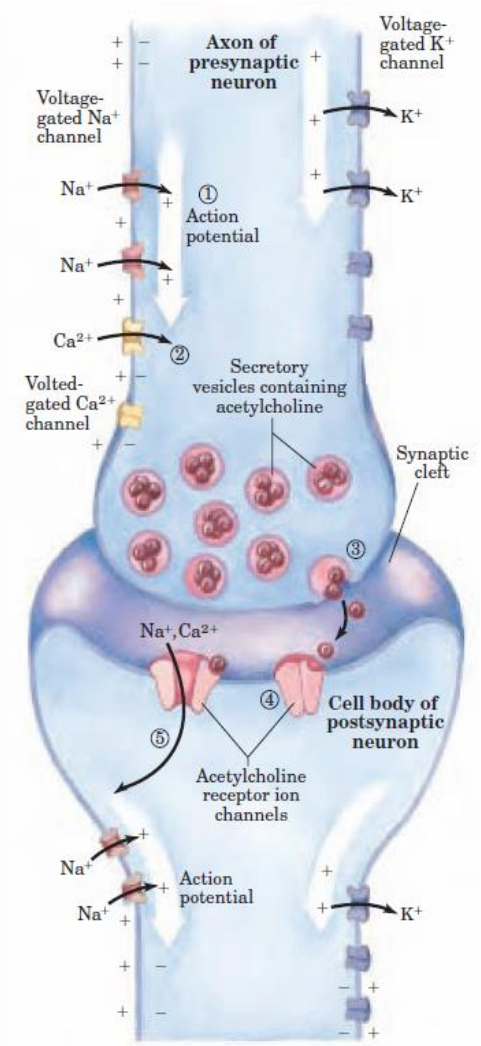



**Figure:** Role of voltage-gated and ligand-gated ion channels in neural transmission

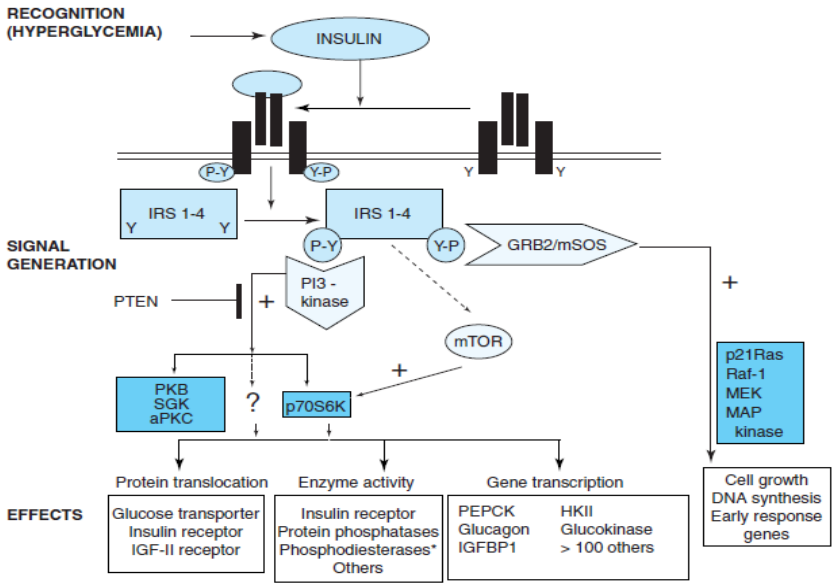
Initially, the plasma membrane of the presynaptic neuron is polarized (inside -ve) through the action of the electrogenic $Na^+$-$K^+$-ATPase, which pumps 3 $Na^+$ out for every 2 $K^+$ pumped into the neuron.

1. A stimulus to this neuron causes an action potential to move along the axon (white arrow), away from the cell body. The opening of one voltage-gated $Na^+$ channel allows $Na^+$ entry, and the resulting local depolarization causes the adjacent $Na^+$ channel to open, and so on. The directionality of movement of the action potential is ensured by the brief refractory period that follows the opening of each voltage-gated $Na^+$ channel.
2. When the wave of depolarization reaches the axon tip, voltage gated $Ca^{2+}$ channels open, allowing $Ca^{2+}$ entry into the presynaptic neuron.
3. The resulting increase in internal [$Ca^{2+}$] triggers exocytic release of the neurotransmitter acetylcholine into the synaptic cleft.
4. Acetylcholine binds to a receptor on the postsynaptic neuron, causing its ligand-gated ion channel to open.
5. Extracellular $Na^+$ and $Ca^{2+}$ enter through this channel, depolarizing the postsynaptic cell. The electrical signal has thus passed to the cell body of the postsynaptic neuron and will move along its axon to a third neuron by this same sequence of events.

**Insulin signaling pathways**:

The insulin signaling pathways provide an excellent example of the "recognition →hormone release →signal generation →effects" paradigm.

- Insulin is released in response to hyperglycemia. Binding of insulin to a target cell-specific plasma membrane receptor results in a cascade of intracellular events.
- Stimulation of the intrinsic tyrosine kinase activity of the insulin receptor marks the initial event, resulting in increased tyrosine (Y) phosphorylation (Y →Y-P) of the receptor and then one or more of the insulin receptor substrate molecules (IRS 1–4).
- This increase in phosphotyrosine stimulates the activity of many intracellular molecules such as GTPases, protein kinases, and lipid kinases, all of which play a role in certain metabolic actions of insulin.
- The two best-described pathways are :
  - First, phosphorylation of an IRS molecule (probably IRS-2) results in docking and activation of the lipid kinase, PI-3 kinase, which generates novel inositol lipids that may act as "second messenger" molecules. These, in turn, activate PDK1 and then a variety of downstream signaling molecules, including protein kinase B (PKB or akt), SGK, and aPKC. An alternative pathway involves the activation of p70S6K and perhaps other as yet unidentified kinases.
  - Second, phosphorylation of IRS (probably IRS-1) results in docking of GRB2/mSOS and activation of the small GTPase, p21RAS, which initiates a protein kinase cascade that activates Raf-1, MEK, and the p42/p44 MAP kinase isoforms. These protein kinases are important in the regulation of proliferation and differentiation of several cell types.
- The mTOR pathway provides an alternative way of activating p70S6K and appears to be involved in nutrient signaling as well as insulin action. Each of these cascades may influence different physiologic processes and each of the phosphorylation events is reversible through the action of specific phosphatases. For example, the lipid phosphatase PTEN dephosphorylates the product of the PI-3 kinase reaction, thereby antagonizing the pathway and terminating the signal.




- The asterisk after phosphodiesterase indicates that insulin indirectly affects the activity of many enzymes by activating phosphodiesterases and reducing intracellular cAMP levels. (IGFBP, insulin-like growth factor binding protein; IRS 1–4, insulin receptor substrate isoforms 1–4); PI-3 kinase, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PKD1, phosphoinositide- dependent kinase; PKB, protein kinase B; SGK, serum and glucocorticoid-regulated kinase; aPKC, atypical protein kinase C; p70S6K, p70 ribosomal protein S6 kinase; mTOR, mammalian target of rapamycin; GRB2, growth factor receptor binding protein 2; mSOS, mammalian son of sevenless; MEK, MAP kinase kinase and ERK kinase; MAP kinase, mitogen-activated protein kinase.)

## Signaling in Olfaction

Olfactory neurons have a number of long thin cilia extending from one end of the cell into a mucous layer that overlays the cell. These cilia present a large surface area for interaction with olfactory signals. The receptors for olfactory stimuli are ciliary membrane proteins with the familiar serpentine structure of seven transmembrane α-helices. The olfactory signal can be any one of the many volatile compounds for which there are specific receptor proteins. Our ability to discriminate odors stems from hundreds of different olfactory receptors in the tongue and nasal passages and from the brain's ability to integrate input from different types of olfactory receptors to recognize a "hybrid" pattern, extending our range of discrimination far beyond the number of receptors.

**Mechanism:**

- The olfactory stimulus arrives at the sensory cells by diffusion through the air. In the mucous layer overlaying the olfactory neurons, the odorant molecule binds directly to an olfactory receptor or to a specific binding protein that carries the odorant to a receptor.
- Interaction between odorant and receptor triggers a change in receptor conformation that results in the replacement of bound GDP by GTP on a G protein, $G_{olf}$, analogous to transducin and to Gs of the β-adrenergic system.
- The activated $G_{olf}$ then activates adenylyl cyclase of the ciliary membrane, which synthesizes cAMP from ATP, raising the local [cAMP].
- The cAMP-gated $Na^+$ and $Ca^{2+}$ channels of the ciliary membrane open, and the influx of $Na^+$ and $Ca^{2+}$ produces a small depolarization called the *receptor potential*.
- If a sufficient number of odorant molecules encounter receptors, the receptor potential is strong enough to cause the neuron to fire an action potential.
- This is relayed to the brain in several stages and registers as a specific smell. All these events occur within 100 to 200 ms.

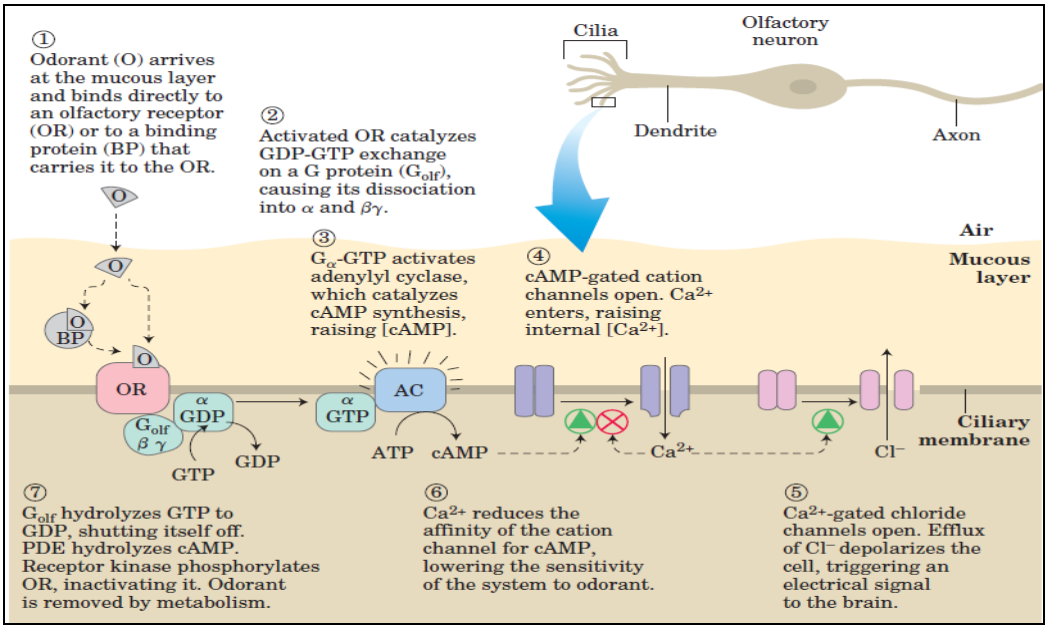



**Figure: The molecular events of olfaction**

**Remark:**

When the stimulus is no longer present, the transducing machinery shuts itself off in several ways. A cAMP phosphodiesterase returns [cAMP] to the prestimulus level. $G_{olf}$ hydrolyzes its bound GTP to GDP, thereby inactivating itself. Phosphorylation of the receptor by a specific kinase prevents its interaction with $G_{olf}$, by a mechanism analogous to that used to desensitize the β-adrenergic receptor and rhodopsin. Lastly, some odorants are enzymatically destroyed by oxidases.

## Epinephrine signaling

Epinephrine action begins when the hormone binds to a protein receptor in the plasma membrane of a hormone-sensitive cell. *Adrenergic receptors* ("adrenergic" reflects the alternative name for epinephrine, adrenaline) are of four general types, α1, α2, β1, and β2, defined by subtle differences in their affinities and responses to a group of agonists and antagonists. *Agonists* are structural analogs that bind to a receptor and mimic the effects of its natural ligand; *antagonists* are analogs that bind without triggering the normal effect and thereby block the effects of agonists. In some cases, the affinity of the synthetic agonist or antagonist for the receptor is greater than that of the natural agonist.

- The four types of adrenergic receptors are found in different target tissues and mediate different responses to epinephrine. Adrenergic receptors of the β1 and β2 subtypes act through the same mechanism.
- The β -adrenergic receptor is an integral protein with seven hydrophobic regions of 20 to 28 amino acid residues that "snake" back and forth across the plasma membrane seven times.
- This protein is a member of a very large family of receptors, all with seven transmembrane helices that are commonly called *serpentine receptors, G protein–coupled receptors (GPCR), or 7 transmembrane segment (7tm) receptors.*
- The binding of epinephrine to a site on the receptor deep within the membrane promotes a conformational change in the receptor's intracellular domain that affects its interaction with the second protein in the signal-transduction pathway, a heterotrimeric GTP-binding *stimulatory G protein*, or **$G_s$,** on the cytosolic side of the plasma membrane.
- When the nucleotide-binding site of Gs (on the α-subunit) is occupied by GTP, Gs is active and can activate adenylyl cyclase (AC); with GDP bound to the site, Gs is inactive. Binding of epinephrine enables the receptor to catalyze displacement of bound GDP by GTP, converting Gs to its active form.
- As this occurs, the β and γ subunits of Gs dissociate from the α-subunit, and $G_{s\alpha}$, with its bound GTP, moves in the plane of the membrane from the receptor to a nearby molecule of AC . The $G_{s\alpha}$ is held to the membrane by a covalently attached palmitoyl group.

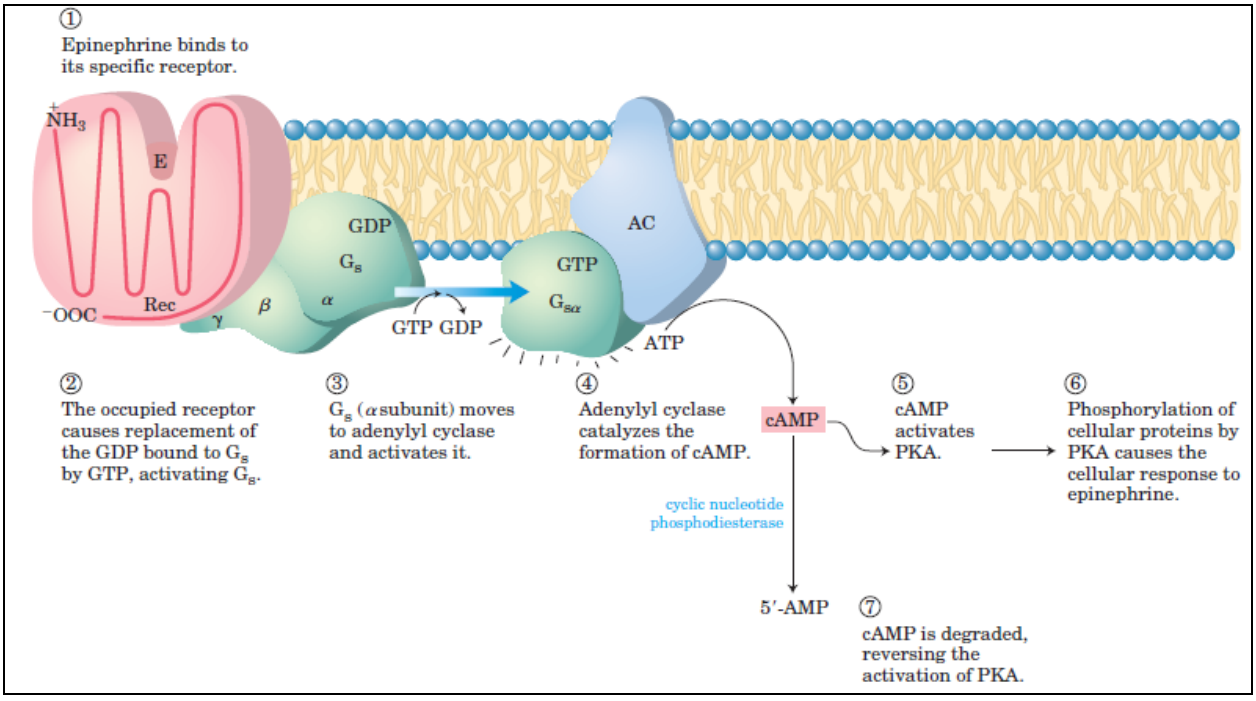



Figure: Transduction of the epinephrine signal: the *β*-adrenergic pathway

- Adenylyl cyclase is an integral protein of the plasma membrane, with its active site on the cytosolic face. It catalyzes the synthesis of cAMP from ATP.
- The association of active $G_{s\alpha}$ with adenylyl cyclase stimulates the cyclase to catalyze cAMP synthesis, raising the cytosolic [cAMP].
- This stimulation by $G_{s\alpha}$ is self-limiting; $G_{s\alpha}$ is a GTPase that turns itself off by converting its bound GTP to GDP.
- The now inactive $G_{s\alpha}$ dissociates from adenylyl cyclase, rendering the cyclase inactive.
- After $G_{s\alpha}$ reassociates with the β and γ subunits ($G_{s\beta\gamma}$), Gs is again available to interact with a hormone-bound receptor.

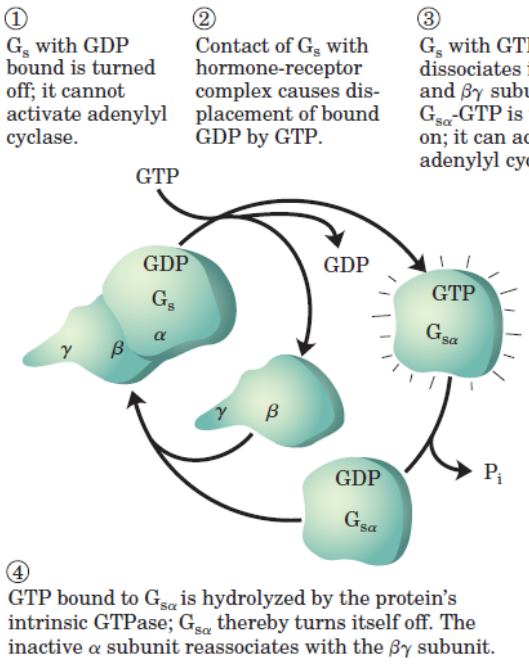



**Figure:** Self-inactivation of Gs

- One downstream effect of epinephrine is to activate glycogen phosphorylase *b*. This conversion is promoted by the enzyme phosphorylase *b* kinase, which catalyzes the phosphorylation of two specific Ser residues in phosphorylase *b*, converting it to phosphorylase *a* .
- Cyclic AMP does not affect phosphorylase *b* kinase directly. Rather, cAMP-dependent protein kinase, also called *protein kinase A* or PKA**,** which is allosterically activated by cAMP, catalyzes the phosphorylation of inactive phosphorylase *b* kinase to yield the active form.
- The inactive form of PKA contains two catalytic subunits (C) and two regulatory subunits (R), which are similar in sequence to the catalytic and regulatorydomains of PKG (cGMP-dependent protein kinase).
- The tetrameric $R_2C_2$ complex is catalytically inactive, because an autoinhibitory domain of each R subunit occupies the substrate-binding site of each C subunit.
- When cAMP binds to two sites on each R subunit, the R subunits undergo a conformational change and the $R_2C_2$ complex dissociates to yield two free, catalytically active C subunits.
- This same basic mechanism—displacement of an autoinhibitory domain—mediates the allosteric activation of many types of protein kinases by their second messengers.
- PKA regulates a number of enzymes; although the proteins regulated by cAMP-dependent phosphorylation have diverse functions, they share a region of sequence similarity around the Ser or Thr residue that undergoes phosphorylation, a sequence that marks them for regulation by PKA.
- The catalytic site of PKA interacts with several residues near the Thr or Ser residue in the target protein, and these interactions define the substrate specificity.
- Comparison of the sequences of a number of protein substrates for PKA has yielded the consensus sequence—the specific neighboring residues needed to mark a Ser or Thr residue for phosphorylation.
- Signal transduction by adenylyl cyclase entails several steps that amplify the original hormone signal
- First, the binding of one hormone molecule to one receptor catalytically activates several Gs molecules.

- Next, by activating a molecule of adenylyl cyclase, each active Gsα molecule stimulates the catalytic synthesis of many molecules of cAMP.
- The second messenger cAMP now activates PKA, each molecule of which catalyzes the phosphorylation of many molecules of the target protein—phosphorylase *b* kinase.
- This kinase activates glycogen phosphorylase *b*, which leads to the rapid mobilization of glucose from glycogen.
- The net effect of the cascade is amplification of the hormonal signal by several orders of magnitude, which accounts for the very low concentration of epinephrine (or any other hormone) required for hormone activity.
- Cyclic AMP, the intracellular second messenger in this system, is short-lived. It is quickly degraded by *cyclic nucleotide phosphodiesterase* to 5′-AMP, which is not active as a second messenger. The intracellular signal therefore persists only as long as the hormone receptor remains occupied by epinephrine.

**Remark:**

Methyl xanthines such as caffeine and theophylline (a component of tea) inhibit the phosphodiesterase, increasing the half-life of cAMP and thereby potentiating agents that act by stimulating adenylyl cyclase.

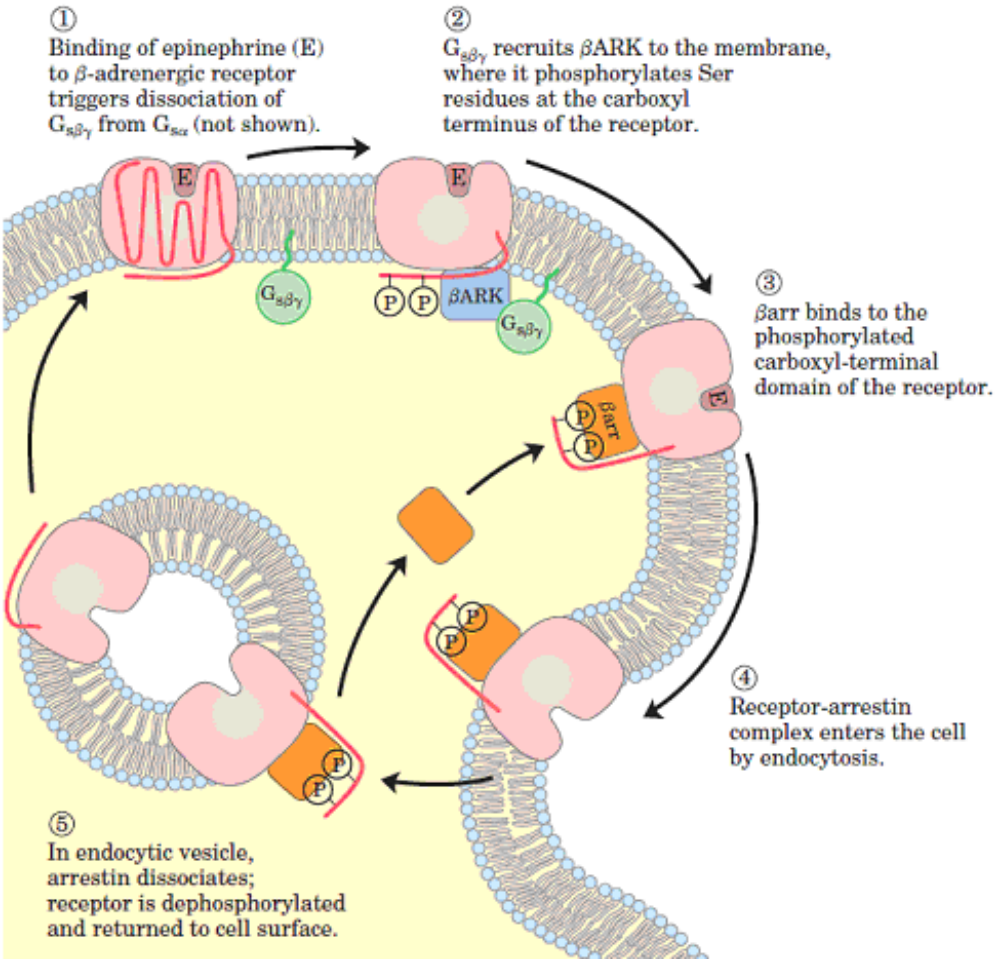



**Figure**: Desensitization of the β -adrenergic receptor in the continued presence of epinephrine. This process is mediated by two proteins: β -adrenergic protein kinase (β ARK) and β-arrestin (β arr; arrestin 2).