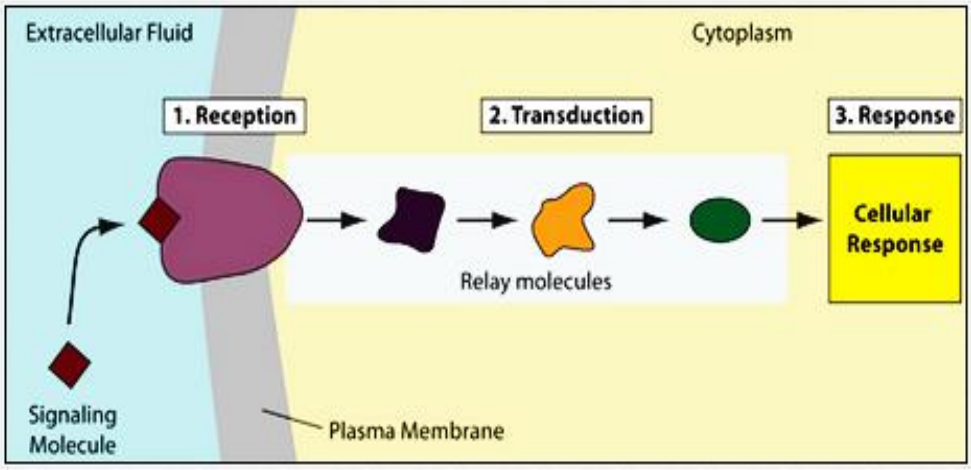
Definition

Cell signaling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. All living organisms are constantly receiving and interpreting signals from their environment in the form of light, heat, odours, touch or sound. These signals are important to keep cells alive and functioning as well as to stimulate important events such as cell division and differentiation, development, tissue repair, immunity as well as normal tissue homeostasis. Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes. Cell signaling pathway involves three main steps:

1. **Reception:**
   Cells communicate by means of extracellular signaling molecules called ***ligands*** that are produced and released by ***signaling cells***, which are detected by a specific protein molecules called ***receptors*** on the surface or inside the ***recipient cell***.
2. **Signal Transduction:**
   The signal is converted in a single step or multiple steps into different molecules called (*relay molecules*) that can bring specific cellular response.
3. **Response:**
   The signal finally triggers specific cellular responses via changing the metabolism of the cell or result in a change in gene expression (transcription) within the nucleus of the cell or both.

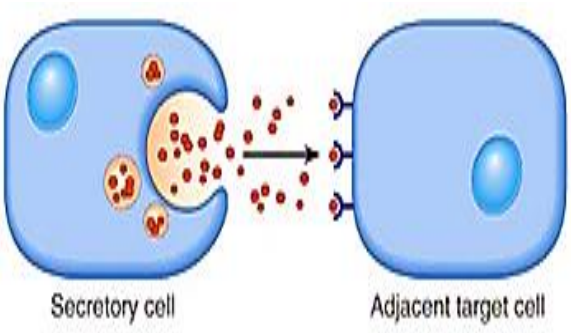


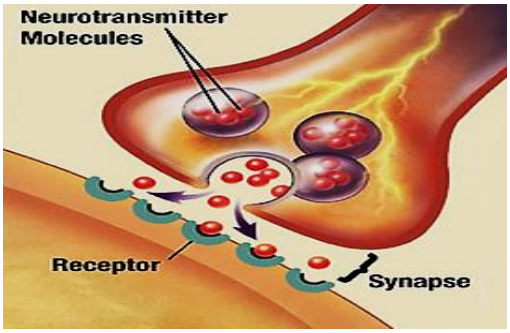
Categories of Cell Signaling:

Depending on the distance that the signaling molecule has to travel, there are five categories of chemical signaling found in multicellular organisms:

A. Paracrine signaling:

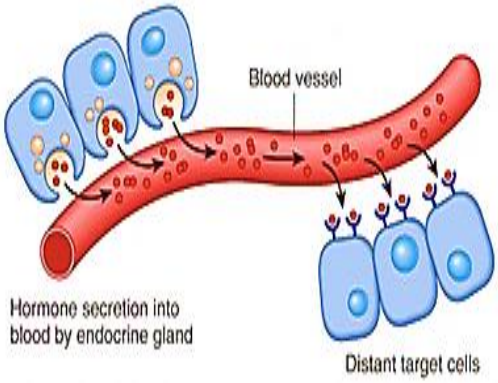
Signals act locally between cells that are close together by diffusion signals through the *extracellular matrix*. Paracrine signaling usually elicit *quick responses* that last for a *short time* because ligand molecules are quickly degraded by enzymes or removed by neighboring cells to reestablish their concentration gradient for allowing them to quickly diffuse through the intracellular space if released again. One example of paracrine signaling is the transfer of signals across synapses between nerve cells. The neurotransmitters are transported across the very small distances between nerve cells, which allows the signal to travel quickly; this enables an immediate response.





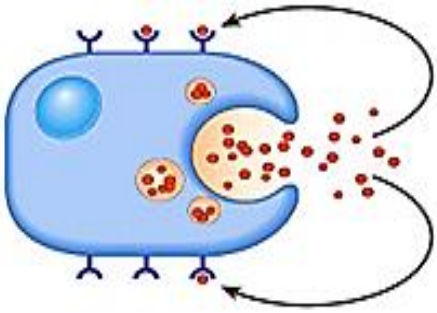
B. *Endocrine signaling*

The signaling molecules (ligands) are called *hormones* that travel a large distances from signaling cells (endocrine gland) via the *bloodstream* until reach their target cells found in other body regions some distance away. Endocrine signaling usually produce a slower response, but have a longer-lasting effect because during transport, hormones get diluted and are present in low concentrations when they act on their target cells. This is different from paracrine signaling in which local concentrations of ligands can be very high.



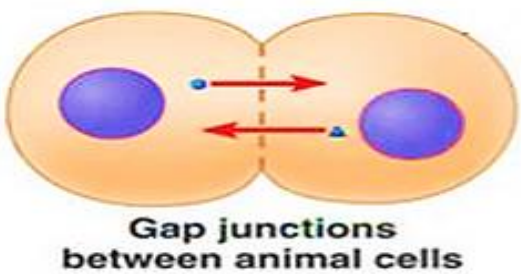
C. *Autocrine signaling*

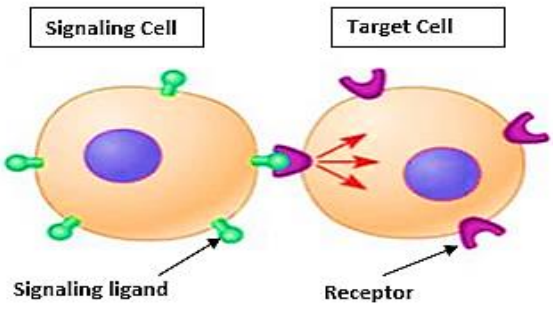
Both ligands and their specific receptors are produced by the same cells. This type of signaling often occurs during the early development of an organism to ensure that cells develop into the correct tissues and take on the proper function, also regulates pain sensation and inflammatory responses. Further, if a cell is infected with a virus, the cell can signal itself to undergo programmed cell death and eliminate virus.



D. *Juxtacrine signaling*

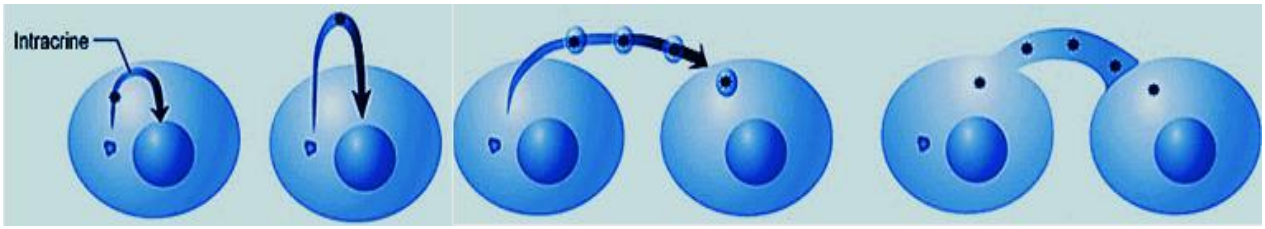
It is a type of cell-cell or cell-extracellular matrix signaling in multicellular organisms that requires close contact, so it is also known as ***contact-dependent signaling.*** Juxtacrine signaling include some growth factors, cytokine and chemokine cellular signals, playing an important role in the immune response. Also direct signaling can occur by transferring signaling molecules across gap junctions between neighbouring cells.





E. Intracrine signaling

If signaling molecules function within the intracellular space, they are termed ***intracrines*** that are associated with a wide variety of peptides/proteins including hormones, growth factors, cytokines, enzymes, and DNA-binding proteins among others. Intracrine factors are involved in the related phenomena of senescence, apoptosis, and regulation of stem cell proliferation and differentiation. There are several types of intracrines; some of them can function in their cells of synthesis without secretion or after secretion and reuptake. While others can function after release in micro-vesicles and internalization by target cells following fusion of the micro-vesicles with the membranes of those cells or can transit between cells via *nanotubes* and function in the recipient cells.



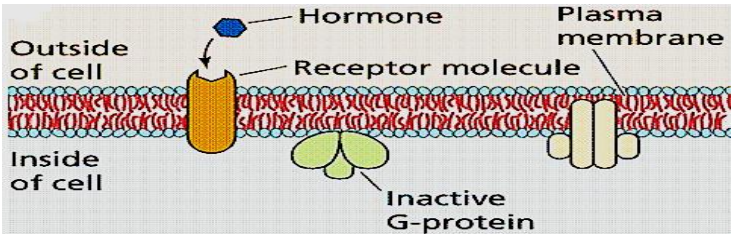
Types of Ligands

Signaling molecules (also known as *first messenger*) can belong to several chemical classes:

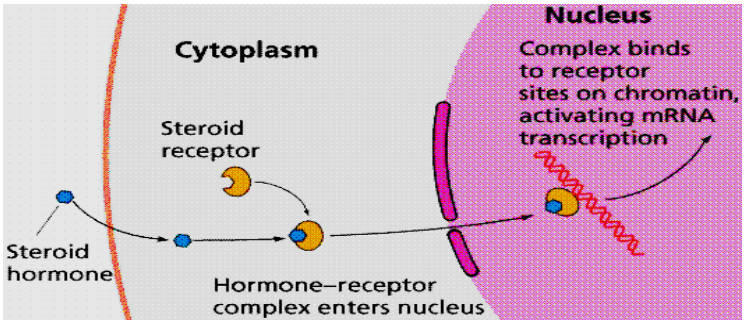
1. ***Hormones:***

They are signaling molecules of the endocrine system and can be grouped into two categories:

a) **Non-steroid hormones** are water soluble (hydrophilic) hormones bind to receptors on the plasma membrane which are either proteins (e.g. insulin, glucagon), or peptides (e.g. vasopressin-ADH), or amino acid derivatives (e.g. histamine, epinephrine). They do not enter the cell but bind to plasma membrane receptors, generating a chemical signal known as *second messenger* inside the target cell that activate other intracellular chemicals to produce the target cell response.

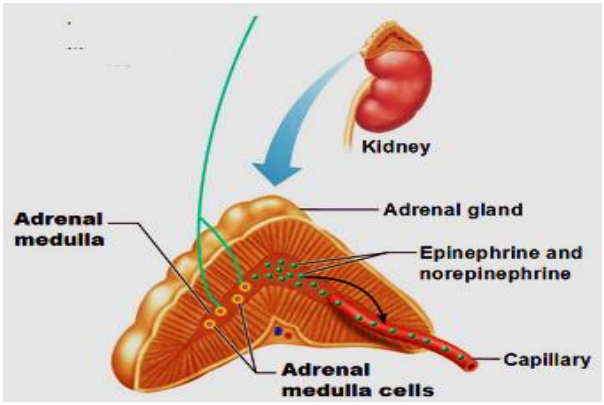


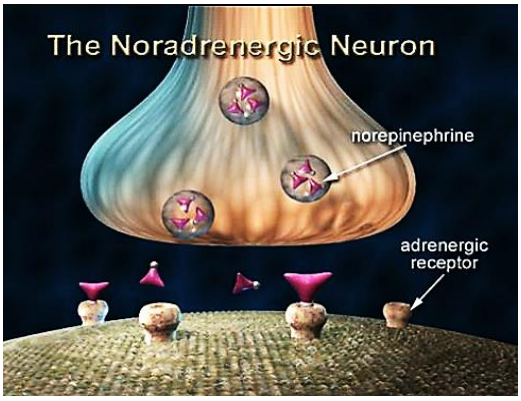
b) **Steroid hormones** are small lipid soluble (lipophilic) hormones can diffuse through the cell membrane to reach cytosolic or nuclear receptors, such as progesterone, estrogen, testosterone, and thyroid hormones, which are generally regulate transcription.



2. ***Neurotransmitters:***

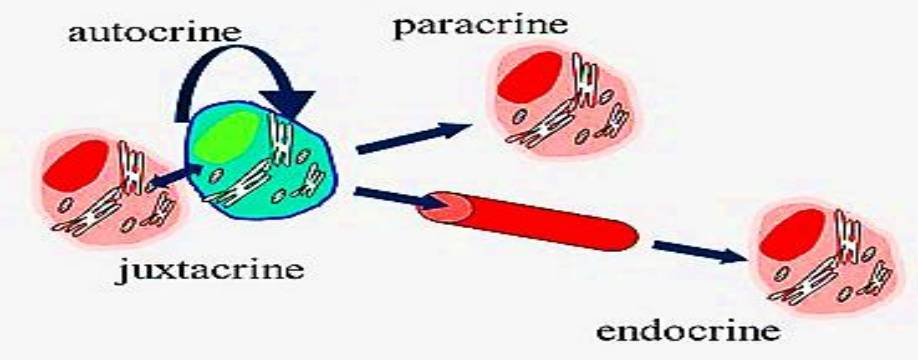
They are signaling molecules of the nervous system, some can function as both a hormone and a neurotransmitter, such as **epinephrine** and **norepinephrine** can function as hormones when released from the adrenal gland into the blood stream, and also be produced by neurons to function as a neurotransmitter.





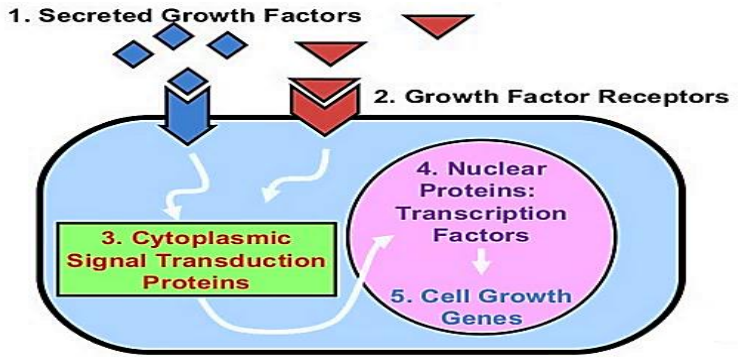
3. ***Cytokines:***

They are signaling molecules of the immune system that function nearly via all categories of cell signaling system, but have a strong presence in the circulation with systemic effect such as altering iron metabolism or body temperature.



4. ***Growth Factors:***

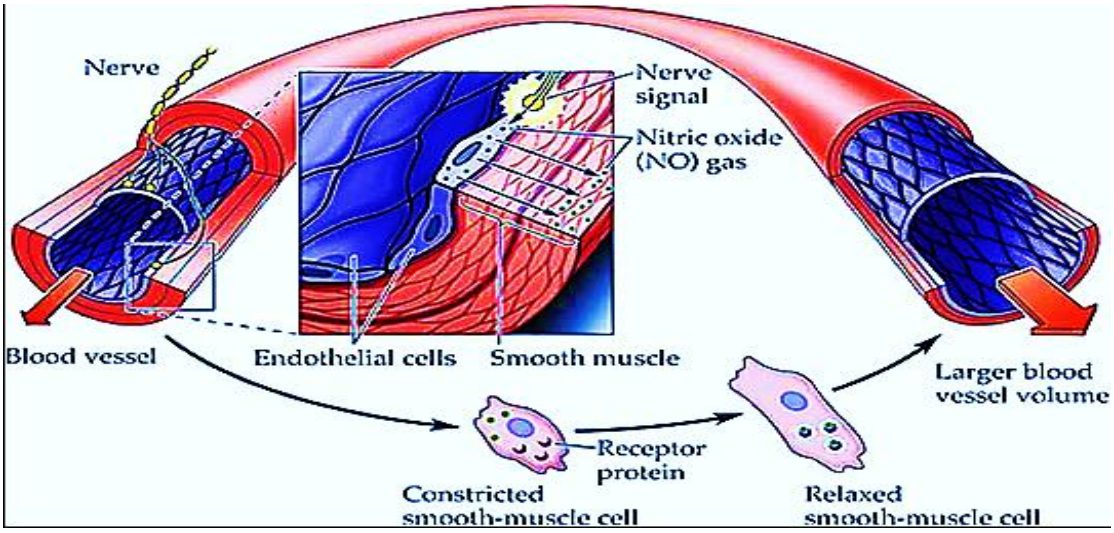
They act as messengers in addition to nutrients because cells often need growth factors to grow, thus these signaling molecules are very important in embryonic & adult development of different tissues such as muscle, cartilage, bone and blood cells. There are several types of growth factors including: Platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF), epidermal growth factor (EGF) and nerve growth factor (NGF).



5. ***Gases:***

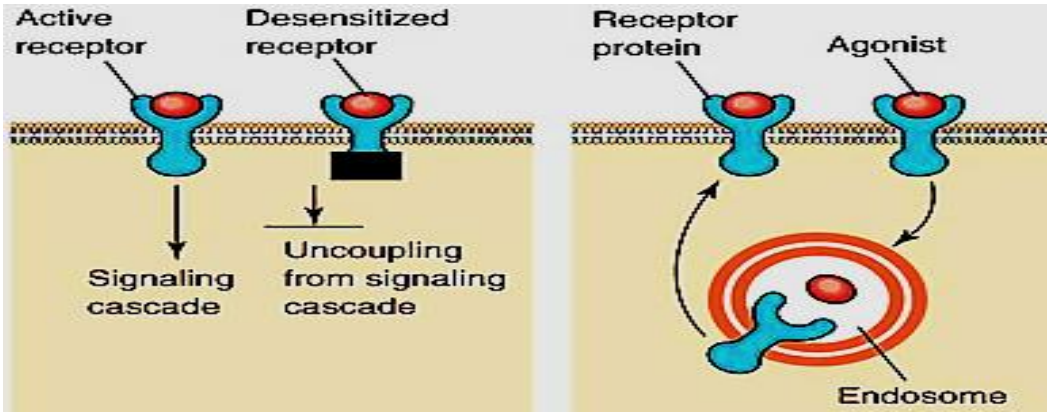
The simple gas nitric oxide (NO) is a major paracrine signaling molecule in the nervous, immune, and circulatory systems. Like the steroid hormones, NO is able to diffuse directly across the plasma membrane of its target cells. The molecular basis of NO action, however, is distinct from that of steroid action; rather than binding to a receptor that regulates transcription, NO alters the activity of intracellular target enzymes.

Another simple gas, carbon monoxide (CO), also functions as a signaling molecule in the nervous system. CO is closely related to NO and appears to act similarly as a neurotransmitter and mediator of blood vessel dilation. The synthesis of CO in brain cells, like that of NO, is stimulated by neurotransmitters.



Types of Receptors

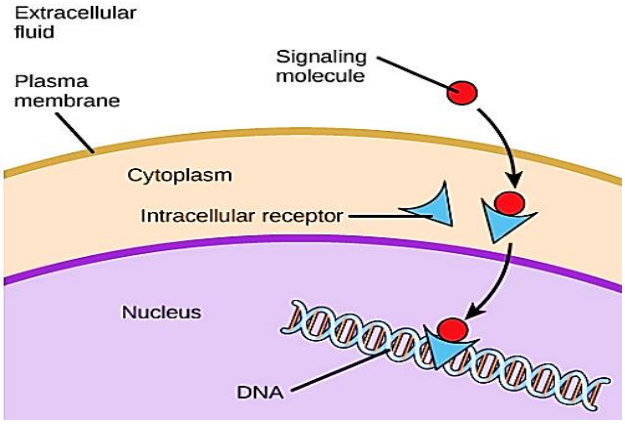
A ligand binds its receptor through weak non-covalent bonds by fitting into a specific binding site or "pocket". Low concentrations of a ligand will result in binding of most of the receptors, because their affinity is high. However, low receptor affinity occurs when a high concentration of the ligand is required for most receptors to be occupied. With prolonged exposure to a ligand (and occupation of the receptor) cells often become desensitized and may lead to tolerance. ***Desensitization*** of the cell to a ligand, depends upon receptor down-regulation by either *Removal of the receptor* from the cell surface (receptor-mediated endocytosis), or by *receptor inactivation* via lowering their affinity to ligand or blocking the subsequent signaling cascade.



According to their location, receptors are classified into two groups:

A. *Intracellular Receptors*

They are protein found in the cytosol or nucleus of target cells. Small or hydrophobic chemical messengers such as steroid and thyroid hormones can readily cross the membrane and activate receptors. An activated hormone-receptor complex can act as a transcription factor, turning on specific genes.

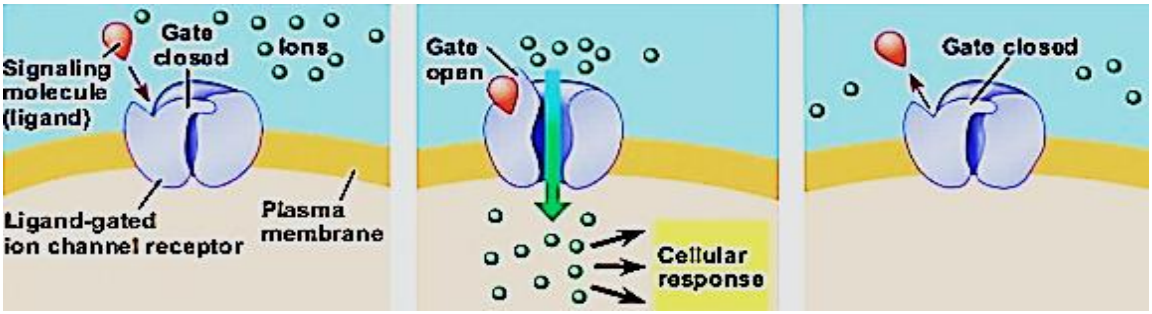


B. *Cell surface receptors*

These receptors bind signal molecules, including neurotransmitters and signal proteins, on their surface. This binding of a ligand is then converted into intracellular signals to alter the behavior of the target cell. There are three main classes of cell surface receptors:

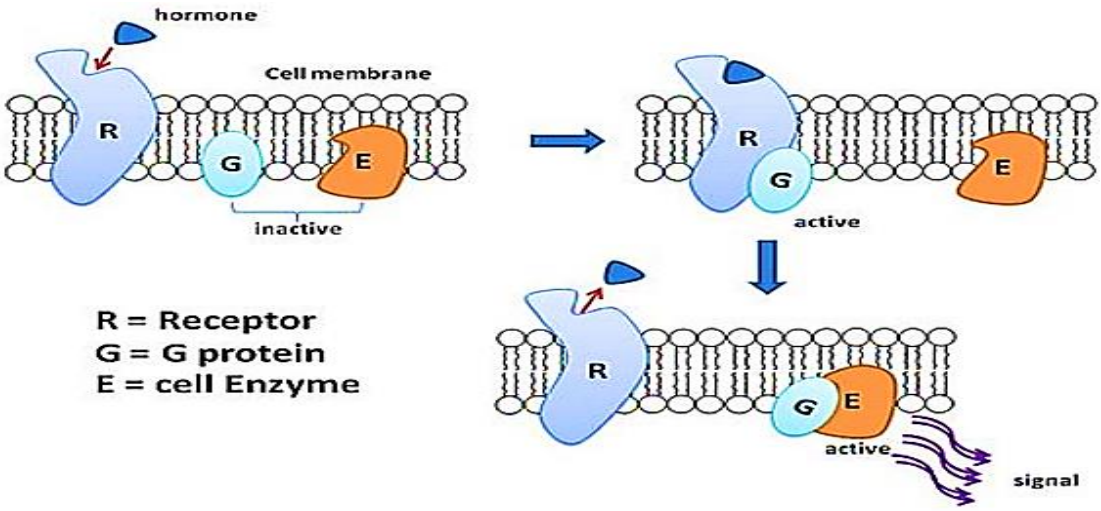
1) *Ion-channel-linked receptors*

Ion-channel-linked receptors also called (*ligand-gated channels* or *fast ionotropic receptors*) are cell membrane bound receptors act through synaptic signaling on electrically excitable cells and convert chemical signals (ligand) to electrical ones that is essential in neuronal activities. The molecules that pass through these receptors are often ions, such as (Na^+) or (K^+). The ion channels are opened only for a short time, after which the ligand dissociates from the receptor and the receptor is available once again for a new ligand to bind.



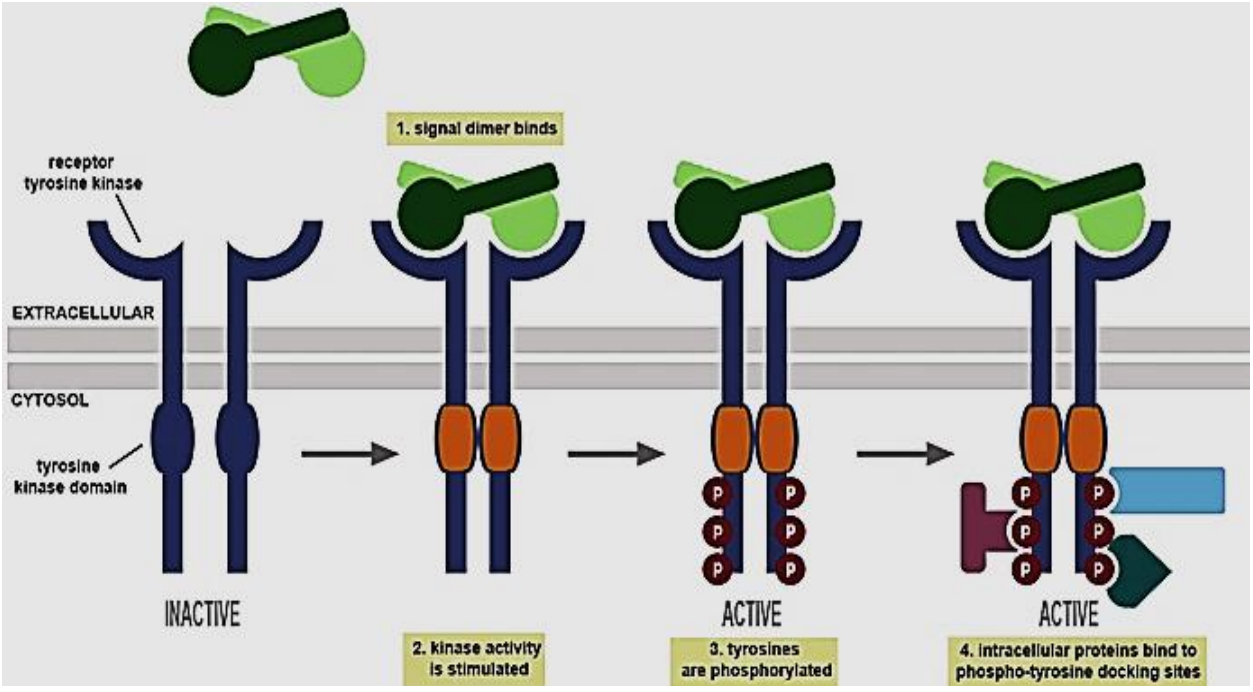
2) *G protein- coupled receptor (GPCR)*

Many different mammalian cell-surface receptors are coupled to a Heterotrimeric G protein, covalently linked to a lipid in the membrane. All G protein-coupled receptors (GPCRs) are ligand specific and differ in their extracellular surface and involved in a range of signaling pathways, including detection of light, odor, certain hormones and neurotransmitters.



3) Enzyme-linked receptor:

They are integral membrane proteins that have two important domains, an extra-cellular ligand binding domain and an intracellular domain, which has a Catalytic function. The signaling molecule binds to the receptor outside of the cell and causes a conformational change on the Catalytic function located on the receptor inside of the cell. These receptors fall into two subgroups: *a)* ***R****eceptor* ***T****yrosine* ***K****inases* ***(RTKs)*** with high-affinity for many polypeptide growth factors, cytokines, and hormones, that act as key regulators of normal cellular processes and also play a critical role in the development and progression of many types of cancer; *b)* ***R****eceptor* ***S****erine/****T****hreonine* ***K****inases* ***(RS/TK)*** that play a role in the regulation of cell proliferation, programmed cell death (apoptosis), cell differentiation, and embryonic development.

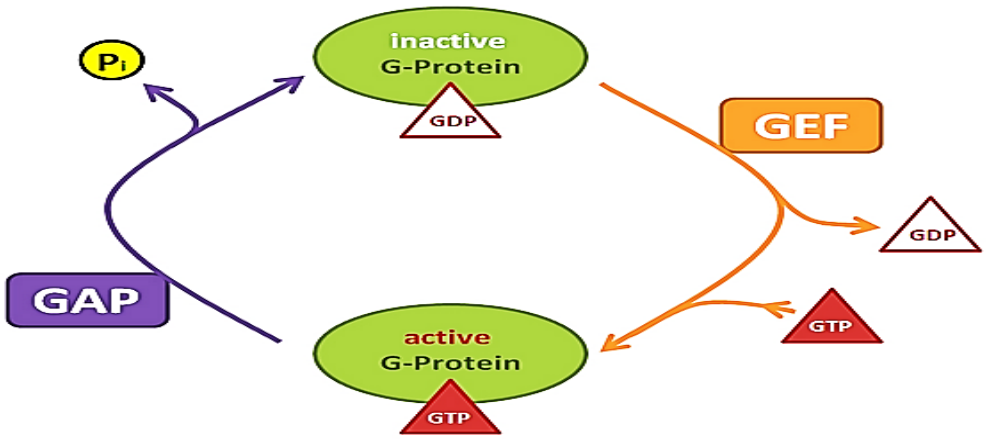


Signal Transduction

Ligand-receptor complex activates series of events known as signal transduction that relays the signal to the interior of the cell. Many intracellular signaling proteins behave as molecular switches and fall into two main classes:

1. G proteins:

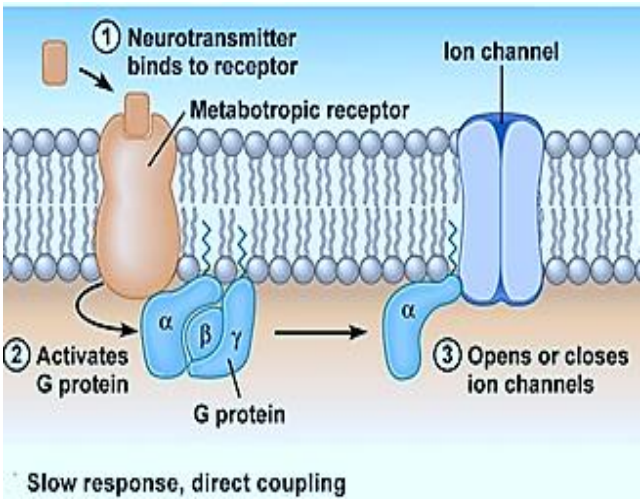
The main feature of G proteins is that they are regulated to either turn (**On** or **off)** based on their binding to guanosine diphosphate (GDP) or guanosine triphosphate (GTP). Firstly, inactive G protein is normally bound to GDP and waiting for the reception of a signal on the surface of the cell, which will cause the G protein to exchange GDP for GTP and become active. Once this occurs the G protein will begin interacting with other signaling components to transmit a signal. Both features of a G protein (i.e. their ability to exchange GDP for GTP and also degrade GTP to GDP) require the use of numerous other signaling proteins. These additional signaling proteins help the cell keep tight control over when a G protein switches on or off. These associated proteins include GTPases-activating proteins **(GAPs)**, guanine exchange factors **(GEFs)** and guanine-dissociation inhibitors **(GDIs).**

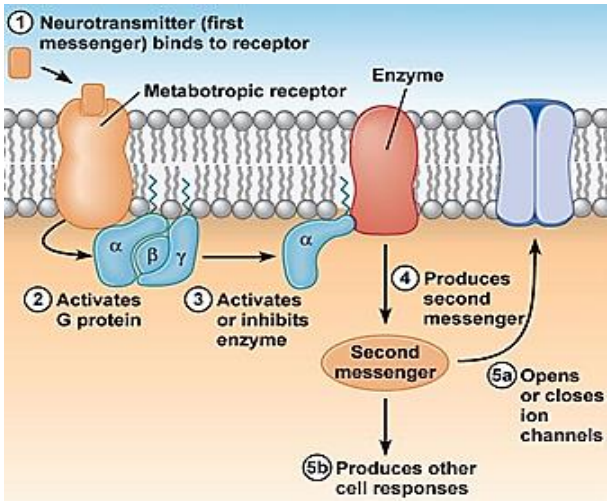


All G proteins fall into two major groups:

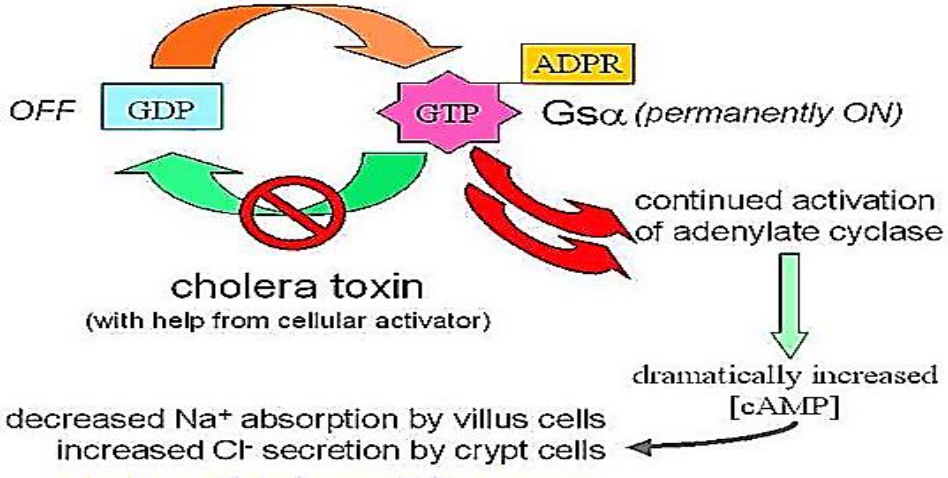
A. Heterotrimeric G proteins

These proteins are composed of three subunits; an alpha (α) subunit that is the activator portion of the G-protein, and beta (β) and gamma (γ) subunits that are attached to the alpha component and also to the inside of the cell membrane adjacent to the receptor protein. When no stimulus is present, α subunit is bound to GDP and complexes to the β/γ subunits. A signal binding to the receptor promotes the dissociation of bound GDP from α subunit of the G protein and stimulates the association of GTP and the alpha subunit of the G-protein separates from the beta and gamma portions and may either be directly coupled to an ion channel or may trigger the activation or inhibition through a ***secondary messenger system***. Both α and βγ subunits may then interact with other proteins to transmit the signal. The ending of the response occurs when GTP is hydrolyzed the Gα subunit rebinds to Gβγ, returning the complex to its inactivated state. In a cell, this all occurs within a few seconds.





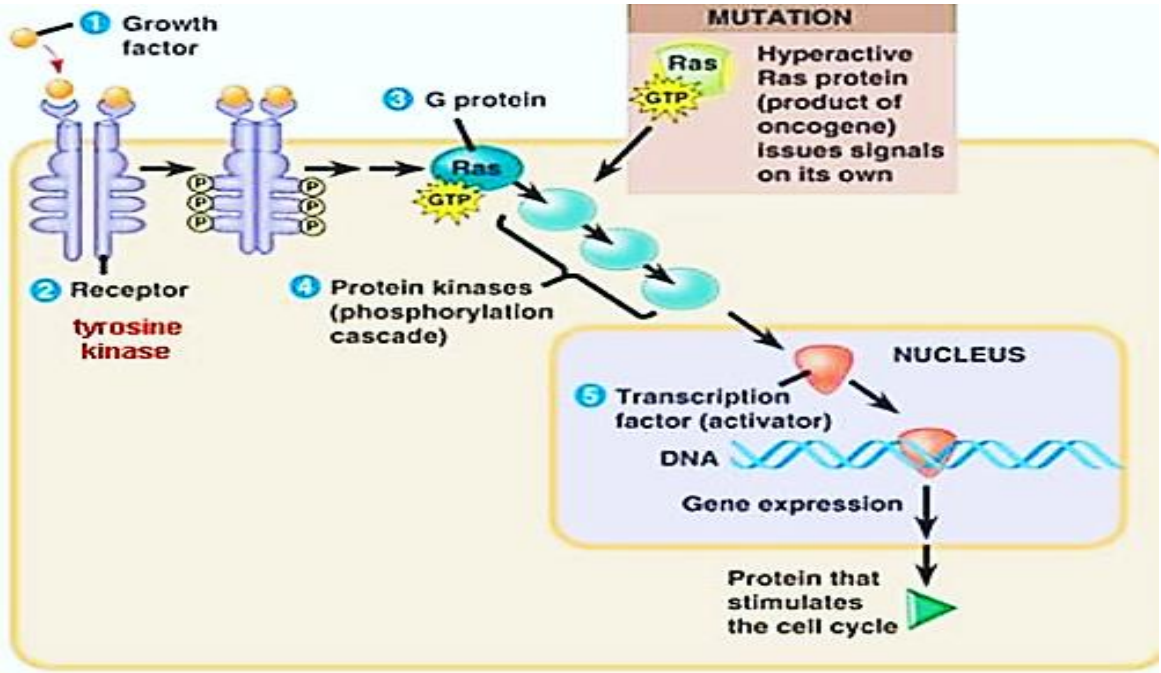
Some times this system become unregulated as in certain diseases like Cholera which is caused by a bacterium that multiples in the intestine, where it produces a protein called ***cholera toxin***. This protein enters the cells lining the intestine and modifies α subunit of a G protein so that it is no longer able to convert GTP back to GDP. And thus once activated, this G protein cannot be turn off and continues to transmit its message to the cellular machinery. In this case, adenylate cyclase is dramatically increased and causes the intestinal cells to continually expel sodium and water into the gut, resulting in diarrhea and dehydration. This can result in death if steps are not taken to replace water and salts.



B. ***Monomeric G proteins***:

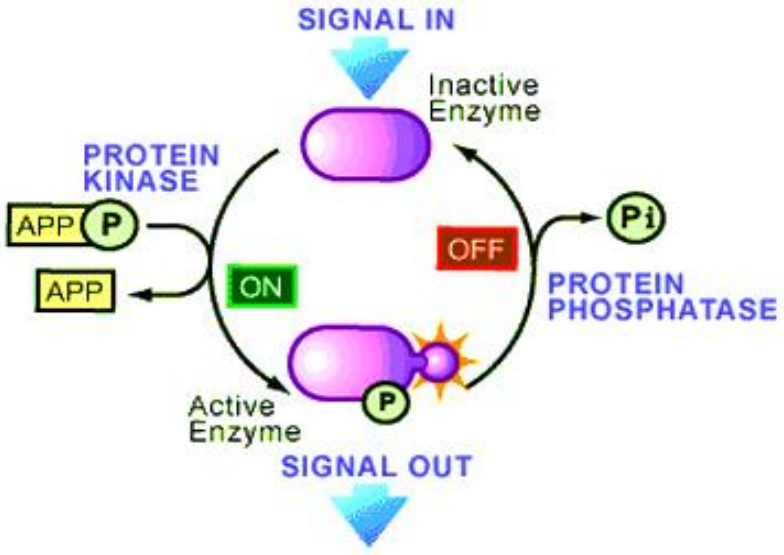
Monomeric G-protein comprised of a single unit and binds GTP and at the same time has GTPase activity (often called GTPases) to distinguish them from heterotrimeric G proteins. They are resemble α subunit of heterotrimeric G proteins and function as a molecular switch in a similar way. Monomeric G-proteins play important role in regulation of growth, morphogenesis, cell motility, and cytokinesis. Normally they are bound to GDP and inactive, upon the reception of a signal, they exchange GDP for GTP and become active. Once in the active state, the GTPase will initiate a cascade in which a series of proteins will phosphorylate each other to transmit the signal. They differ in their mode of activation by not being directly associated with the receptor. Instead, GTPases are several steps away from the receptor (often termed an enzyme-linked

receptor) that ultimately cause activation a variety of adaptor proteins. These proteins include 5 families; Ras, Rho, Arf, Rab, and Ran proteins which are anchored to lipid membrane. One of the most famous small monomeric G proteins is Ras, which was first discovered as a cellular copy of a viral gene that causes cancer in mammals (the name 'Ras' is an abbreviation of Rat sarcoma). It was later discovered that Ras genes are proto-oncogenes, thus mutation in these genes induce pathologic proliferation and anti-apoptosis. About 30% of all human tumors involve cells expressing mutated Ras oncogene.

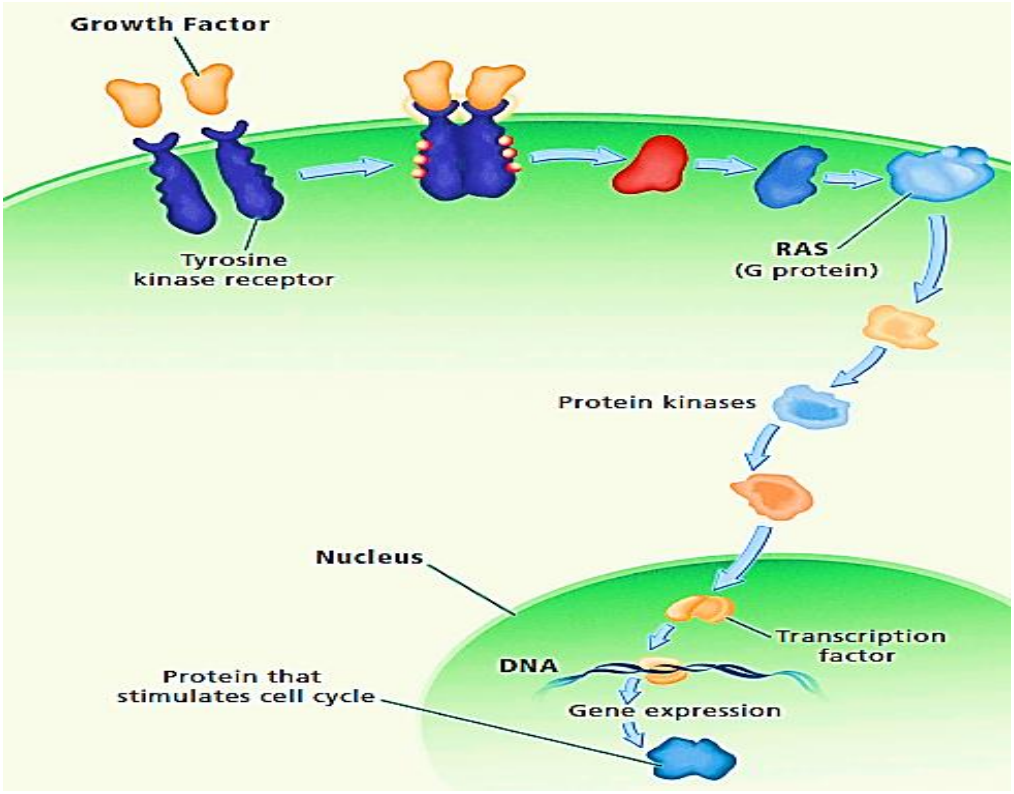


2. Protein Kinases

Protein kinases are enzymes that modify their target proteins by transferring phosphate groups from ATP (phosphorylation) resulting in a signaling cascade. A cascade can be stopped by removal of phosphate groups by protein ***phosphatases***, which can remove phosphate groups from kinase substrates. As many as 30% of all human proteins are modified by phosphorylation, making kinases extremely important in cellular regulation.

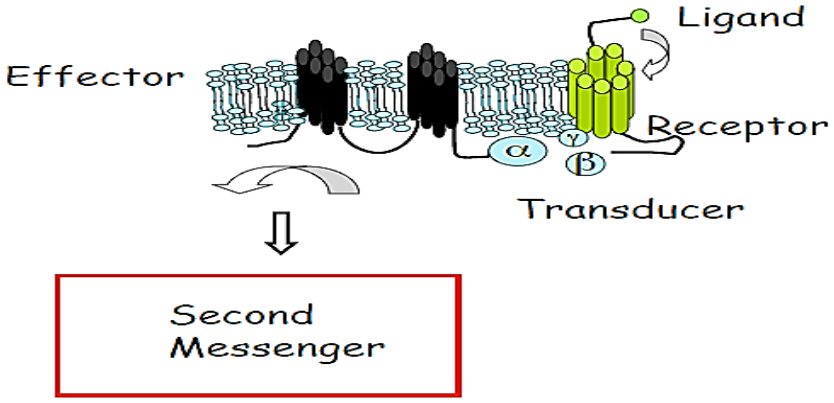


Protein kinases can be broadly divided into two groups based on the amino acids to which they add phosphate groups: serine/threonine (Ser / Thr) kinases that involved in regulation of metabolic and cytoskeletal activity; and tyrosine (Tyr) kinases that act primarily in downstream signaling from growth factors. When a signal (growth factor) arrives, binds to two receptors, causes the two tyrosine kinase domains to come into contact with one another. Each kinase phosphorylates each other on multiple tyrosine sites. Several other proteins may be involved in the cascade, ultimately activating one or more transcription factors. The activated transcription factors enter the nucleus where they stimulate the expression of the genes that are under the control of that factor. This is an example of the *RAS* pathway, which results in cell division. Abnormal activity of protein kinases is a frequent cause of disease, particularly cancer, therefore, protein kinases have emerged as one of the most frequently targeted families of proteins in drug discovery. The development of small-molecule inhibitors that have the potency and selectivity necessary to be effective cancer drugs has gained notable successes over the past decades.



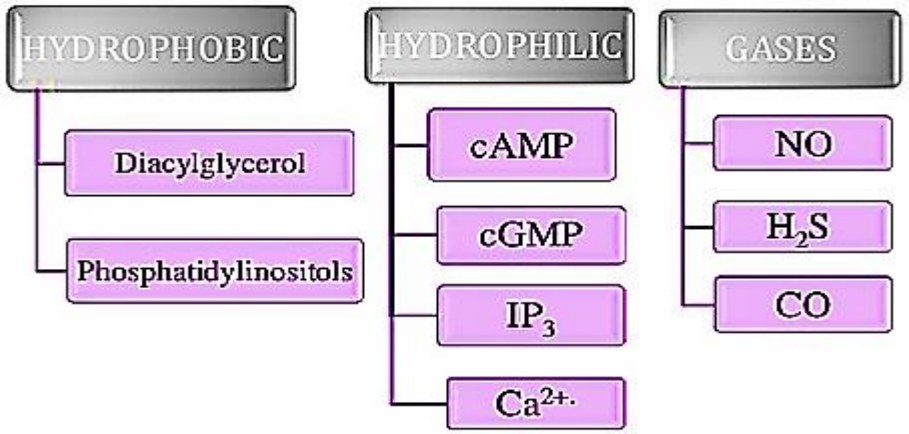
Second Messengers

Second messengers are molecules that relay signals from receptors on the cell surface to target molecules inside the cell (cytoplasm or nucleus). Because the signal from an extracellular molecule (***first messenger***, e.g., a hormone, neurotransmitter or drug) does not cross the cell membrane to enter the cytoplasm, they bound to the cell's surface receptor which in turn activate another molecules called ***effectors*** which bind to specific receptors to initiate the pathways for the production of ***'second messengers*** to continue the control function inside the cell. The binding of this effector to receptor is often- mediated by a protein, whose activity depends on GTP/GDP binding or kinases proteins. Therefore, secondary messengers are a component of signal transduction cascades that relay and greatly amplify the strength of the signal via binding and activating protein kinases, ion channels, and other proteins, thus continuing the signaling cascade.



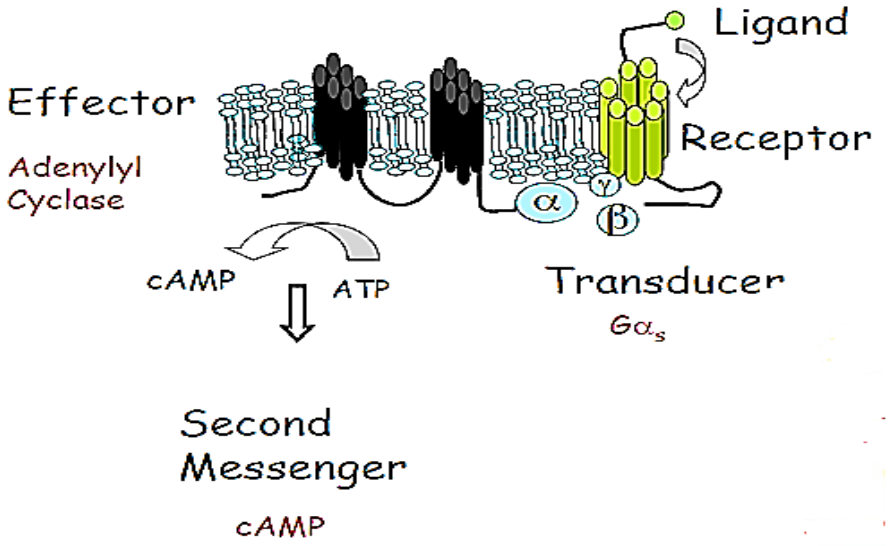
There are three basic types of 2nd messengers which are chemically diverse:

1. **Hydrophobic molecules:** They are water-insoluble molecules, like diacylglycerol (DAG), and phosphatidylinositol (PI), which are membrane-associated and diffuse from the plasma membrane into the inter membrane space where they can reach and regulate membrane-associated effector proteins. They reach different targets in the cell depending on whether they are more soluble in the lipid bilayer or in water.
2. **Hydrophilic molecules:** They are water-soluble molecules, like cyclic nucleotides (cAMP & cGMP), inositol triphosphate (IP_3), and calcium ions that are located within the cytosol and act globally because they diffuse rapidly throughout the cytoplasm.
3. **Gases**: They include nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H_2S) which can diffuse both through cytosol and across cellular membranes.



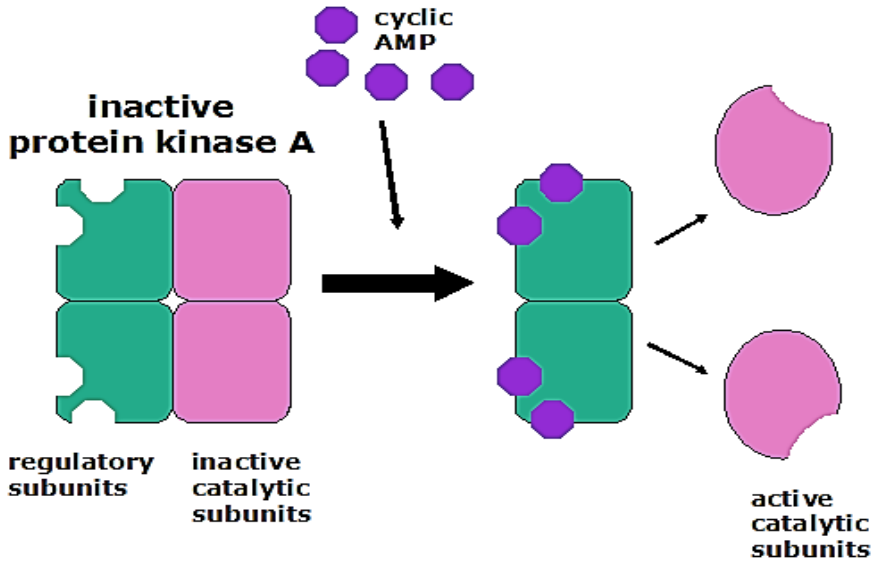
Cyclic AMP (cAMP):

Cyclic adenosine monophosphate (cAMP) was the first second messenger to be identified and plays fundamental roles in cellular responses to many hormones and neurotransmitters. It is derived from adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms. Ligands such as hormones and neurotransmitters require at least four components to regulate the intracellular cAMP concentration: (1) GPCR, (2) heterotrimeric G protein, (3) adenylyl cyclase (AC), and (4) phosphodiesterase (PDE). The intracellular levels of cAMP are regulated primarily by the balance between the activities of two enzymes; (AC) and (PDE). The cAMP is synthesized from ATP by *adenylyl cyclase* which is large transmembrane proteins. However, cAMP decomposition into AMP is catalyzed by the enzyme *phosphodiesterase.*



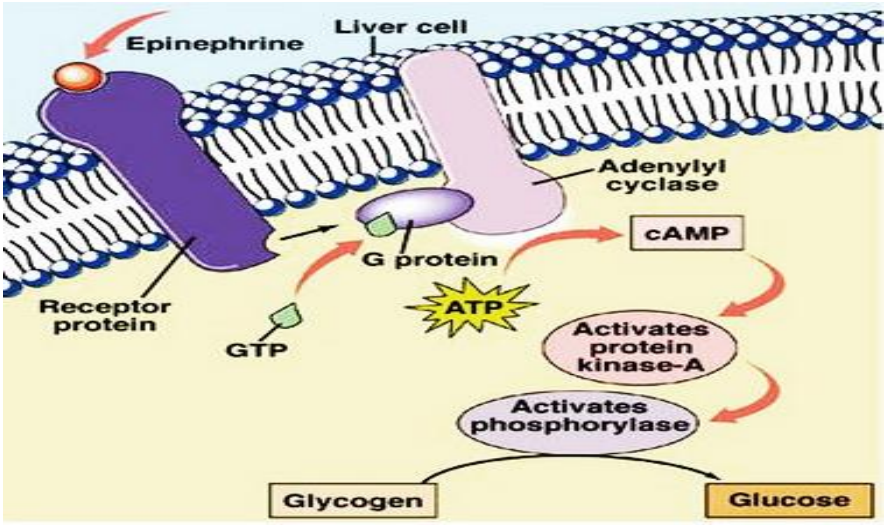
Effectors of cAMP

The cAMP has been shown to be a universal intracellular messenger that mediates a wide variety of biological responses in almost all tissues in mammals. An elevation of cAMP activates several effectors, the best known is the ***protein kinase A (PKA).*** Not all protein kinases respond to cAMP, for example protein kinase C is not cAMP-dependent. In eukaryotes, cyclic AMP works by activating PKA which is normally inactive as a tetrameric holoenzyme, consisting of two catalytic and two regulatory units (C_2R_2), with the regulatory units blocking the catalytic centers of the catalytic units. Cyclic AMP binds to specific locations on the regulatory units and causes dissociation between the regulatory and catalytic subunits, thus enabling those catalytic units to phosphorylate substrate proteins which may either directly act on the ion channels, or activated / inhibited enzymes, or bind to promoter regions of DNA causing increased expression of specific genes.



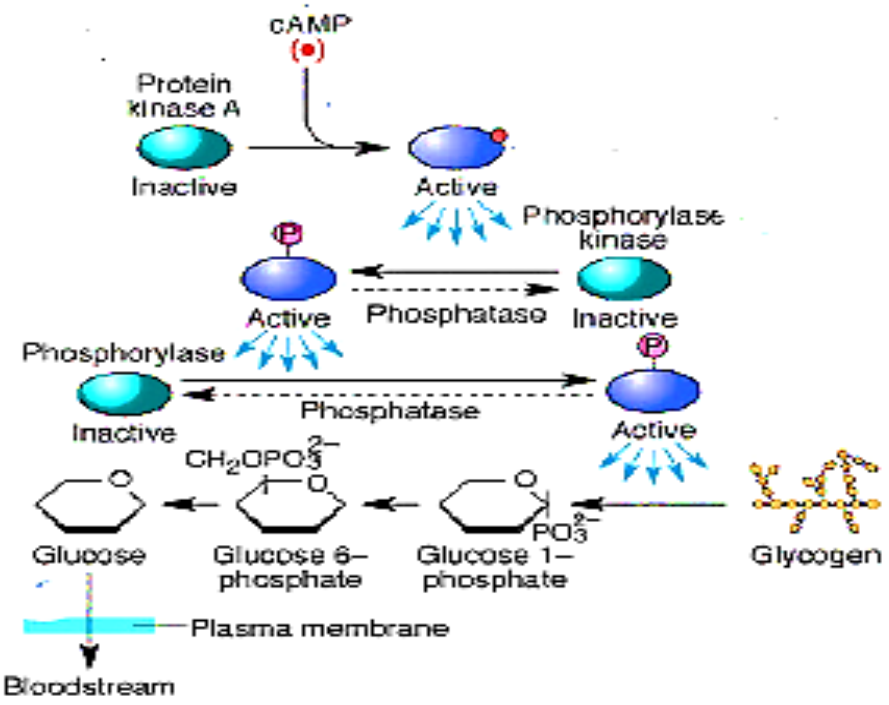
Example (Glucose Mobilization):

Glucose is stored in animal cells as an insoluble polymer (glycogen). Liver adenylyl cyclase responds more strongly to glucagon, and muscle adenylyl cyclase responds more strongly to adrenaline. The enzyme ***phosphorylase*** catalyzes glycogen breakdown, while glycogen ***synthase*** catalyzes polymerization. Regulation is achieved by several hormones, such as ***glucagon*** (from the pancreas) and ***epinephrine*** (from the adrenal medulla). Binding of epinephrine or glucagon to their receptors activates adenylyl cyclase. The cAMP binds to the regulatory subunit of protein kinase A (PKA) causing the release of the catalytic subunit which acts on several targets leading to conversion of glycogen into glucose and release it into blood circulation.

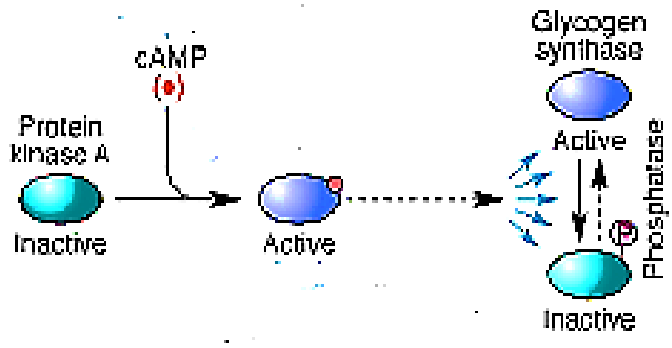


PKA can do that by acting on three different targets:

1) PKA phosphorylates and activates phosphorylase kinase, which activates phosphorylase to breakdown glycogen into glucose-1-phosphate, then glucose-6-phosphate, and finally to glucose molecules that enters blood circulation.



2) PKA phosphorylates and inhibits glycogen synthase.



3) PKA also translocates to the nucleus where it phosphorylates the transcription factor **CREB** (cAMP response element-binding protein), that binds to certain DNA sequences called *cAMP response elements* (CRE), thereby increasing or decreasing the transcription of the downstream genes involved in gluconeogenesis. CREB was first described in 1987 as a cAMP-responsive transcription factor regulating the somatostatin gene.