# M5 L1

# Cell signalling

As living organisms we are constantly receiving and interpreting signals from our environment. These signals can be light, heat, odours, touch or sound. The cells of our bodies are also constantly receiving signals from other cells. These signals are important to keep cells alive and functioning as well as to stimulate important events such as cell division and differentiation. In animals, rapid responses to the changes in the environment are mediated primarily by the nervous system and by hormones including small peptides, small nonpeptide molecules such as the catecholamines (Dopamine, epinephrine, norepinephrine). We have already studied role of hormones like epinephrine, ACTH and norepinephrine etc in signaling events. We shall discuss downstream process when signaling molecule interacts with receptor. We shall also talk about various secondary messengers involved in signaling process. These signaling molecules are released from the cells and they travel through the blood to their specific target cells as shown in Figure 1. Some molecules are transported long distances by the blood while others have more of local effects. Certain membrane-bound proteins on one cell can directly signal an adjacent cell.

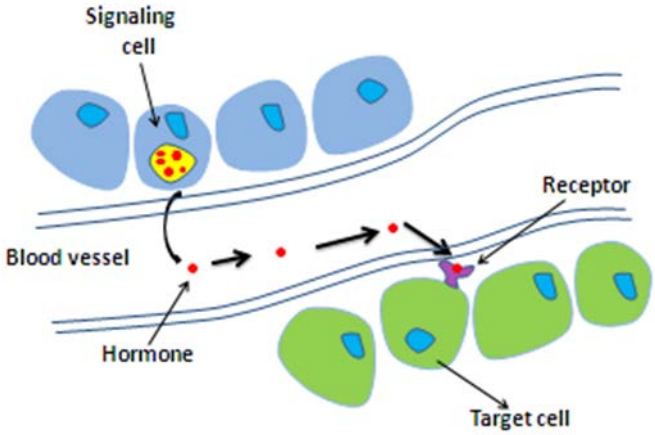


**Figure 1: Signaling molecules released from cell and transported by the blood to the target cell**

Cell signaling can be divided into 3 stages:

**1. Reception:** A cell detects a signaling molecule from the outside of the cell. A signal is detected when the ligand binds to a receptor protein on the surface of the cell or inside the cell.

**2. Transduction:** When the signaling molecule binds to the receptor, it changes the receptor protein. This change initiates the process of transduction. Each relay molecule in the signal transduction pathway changes the next molecule in the pathway.

**3. Response:** Finally, the signal triggers a specific cellular response as shown in Figure 2.

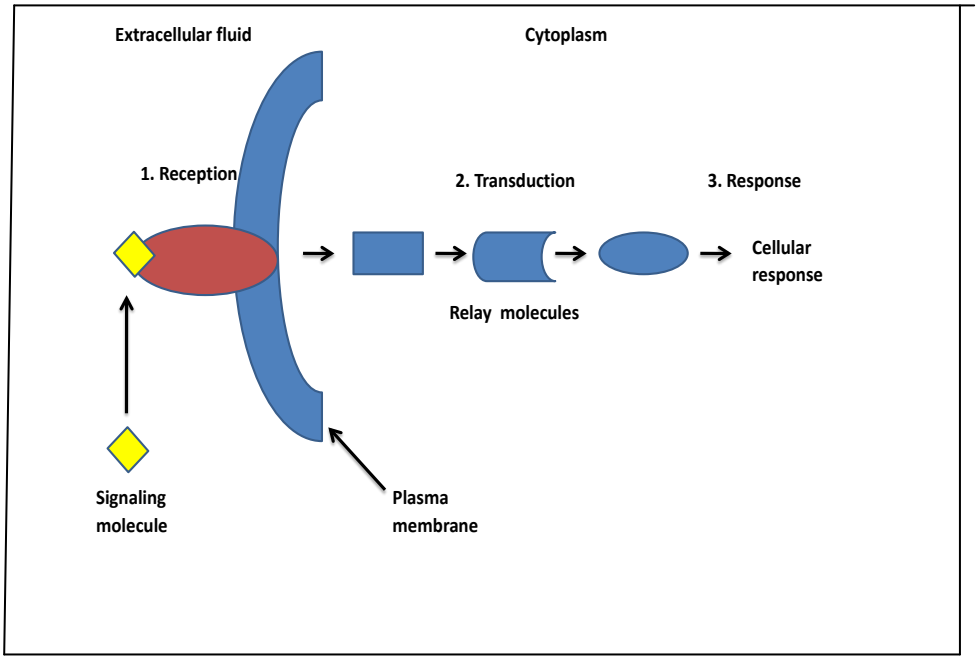


**Figure 2: Cell signalling stages**

**Signal Transduction:**

Signal transduction is phenomenon which involves in the transfer of signal from extracellular to intracellular environment through the cell surface receptor protein that stimulate intracellular target enzymes, which may be either directly linked or indirectly coupled to receptors by G proteins. These intracellular enzymes serve as downstream signalling elements that propagate and amplify the signal initiated by ligand binding. Thus, signal transduction pathway allows cells to respond to extracellular environmental signals. These signals can be physical and chemical such as light, oxygen, nutrient, hormones. Figure 3 represents the signal transduction pathway.

Signal transduction is the combination of following phenomenon:

1. Signal reception
2. Integration
3. Amplification
4. A target that is affected
5. Termination

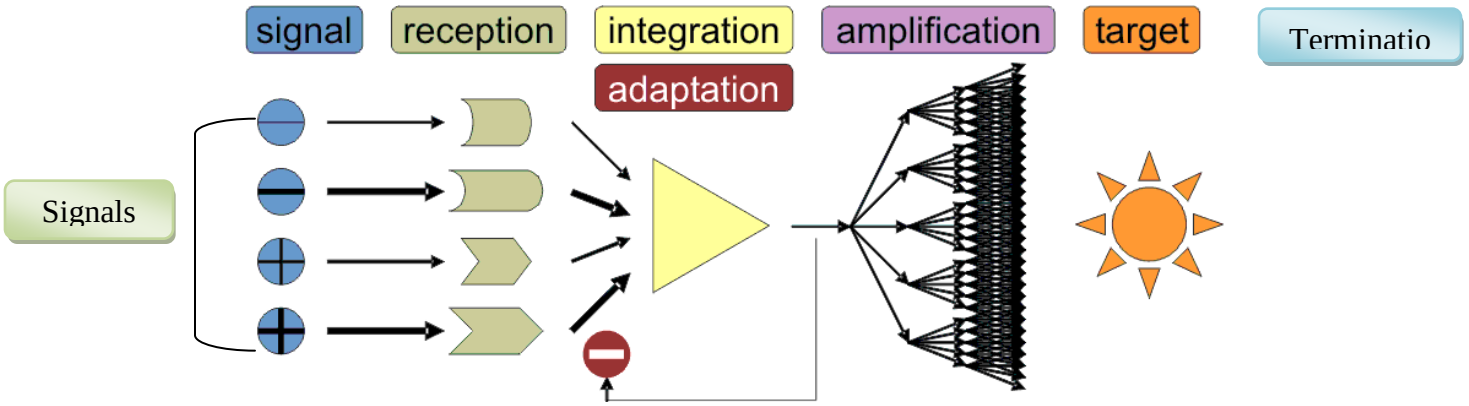


**Figure 3: Representation of Signal Transduction pathway**

Thus signal transduction begins with receiving signal to the cell receptor and end with a change in cellular function. The cell receptor can be of various types- G-protein coupled receptor, tyrosin kinase receptor etc. The transduction process is typically mediated via a cascade of some important second messengers including cAMP, cGMP, calcium ion, inositol 1, 4, 5-trisphosphate, ($IP_3$), and diacylglycerol (DAG). Second messengers are intracellular molecules that change in concentration in response to environmental signals and involve in conveying information inside the cell.

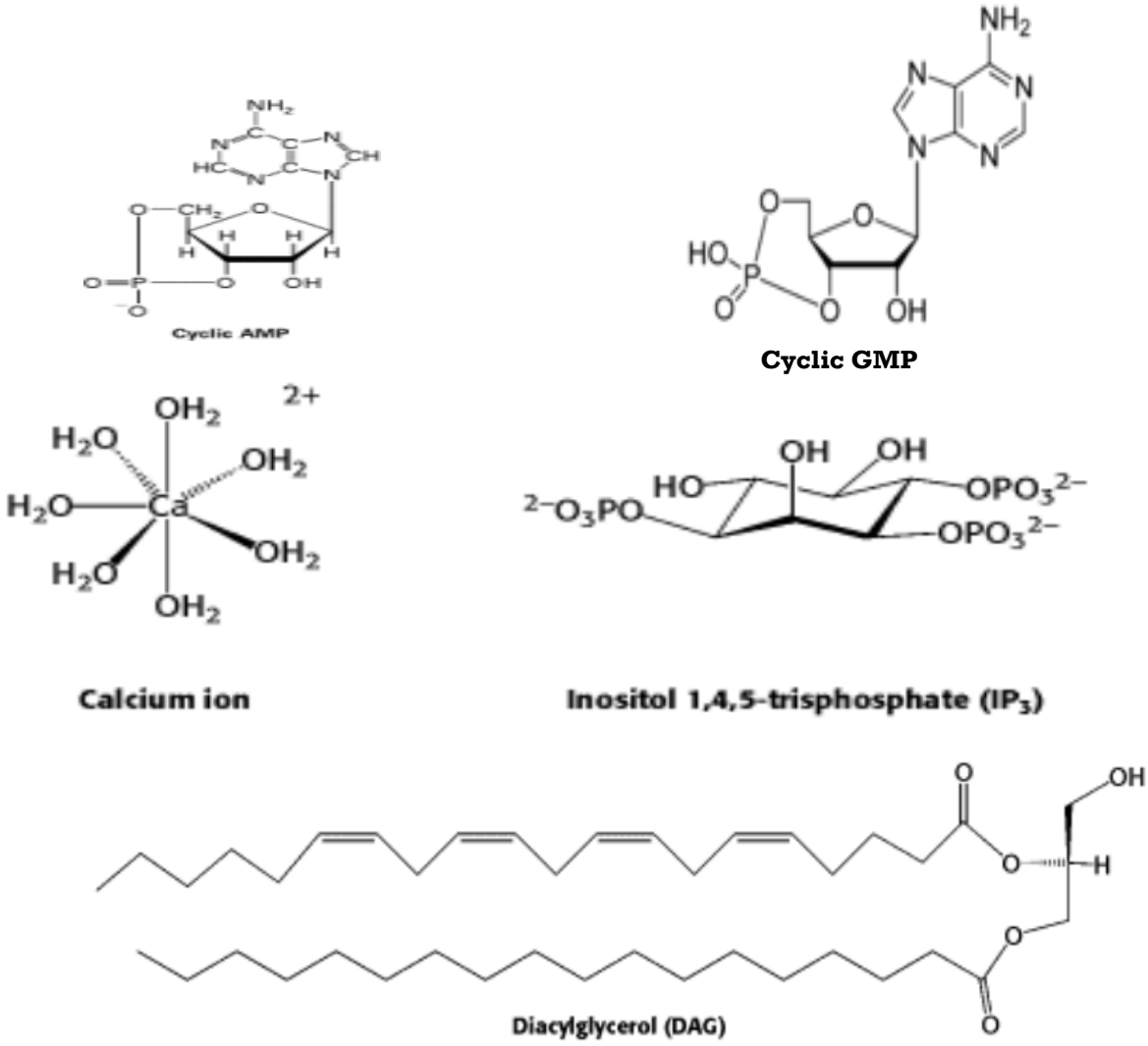


**Figure 4: Common second messengers**

Signal transduction pathways act similar to molecular circuit. This pathway depends on following factors during transformation of signal from extracellular environment to intracellular.

1. Signal reception by cell membrane receptor: Some non polar signaling molecules such as estrogens and other steroid hormones are able to cross the bilipid membrane and hence make entry inside the cell. Once inside the cell, these molecules can bind to proteins that interact directly with DNA and involve in regulation of gene transcription. Thus, a chemical signal enters the cell and directly alters gene-expression patterns. However, most of signalling molecules are too large and too polar so they are unable to cross the membrane, hence there is no appropriate transport system. In this case these signaling

molecules transmit signals through cell surface receptor protein without crossing the cell membrane. We will discuss about cell receptors in upcoming lecture notes.

These receptors are intrinsic membrane protein which consist both extracellular and intracellular domain. A binding site present in extracellular domain specifically recognizes the signaling molecule (i.e. well known as ligand). Such binding sites are analogous to enzyme active sites except that no catalysis takes place within them. When these signal molecules comes and bind to binding site on receptor protein in extracellular region then some conformational change occurs in tertiary and quaternary structure of the receptor which results in the drastic change in the intracellular domain of the receptor. These structural changes are not sufficient to yield an appropriate response, because they are restricted to a small number of receptor molecules in the cell membrane. The information embodied by the presence of the ligand, often called the primary messenger, must be transduced into other forms that can alter the biochemistry of the cell.

2. Second messengers: Second messengers act as the intermediate molecule that relay signals from receptors on cell surface to target molecule inside cells, in cytoplasm or nucleus.

   The use of second messengers has several consequences:

a) The second messengers are able to diffuse frequently into other compartment of the cell such as nucleus where they can influence gene expression and other process.
b) Generation of second messengers leads to amplification of signal. Each signaling molecule is involved in the generation of several second messengers in the cell. Thus, a low concentration of signal in the environment, even as little as a single molecule, can yield a large intracellular signal and response
c) Since common second messengers generate in different signaling pathway, thus the coordination of signal transduction is driven by interaction between these pathways. Multiple signaling pathways create both opportunities and potential problems. Interactions between signaling pathways enables the cell to process and interpret multiple inputs differently in different contexts leading to cross-talk. Cross talk between second messengers cause oscillation of various second messengers and also creates biostability between two steady states. Thus cross talk more precisely involves

in regulation of cell activity than individual independent pathways without cross talk. However, inappropriate cross-talk can cause second messengers to be misinterpreted.

3. Protein phosphorylation: Protein phosphorylation is most common route for transferring information coming through second messenger which involve elicit responses by activating protein kinases. Protein phosphorylation is a post-translational modification of proteins by phosphorylation at serine, threonine or tyrosine residues by a protein kinase by the addition of a covalently bound phosphate group from ATP.

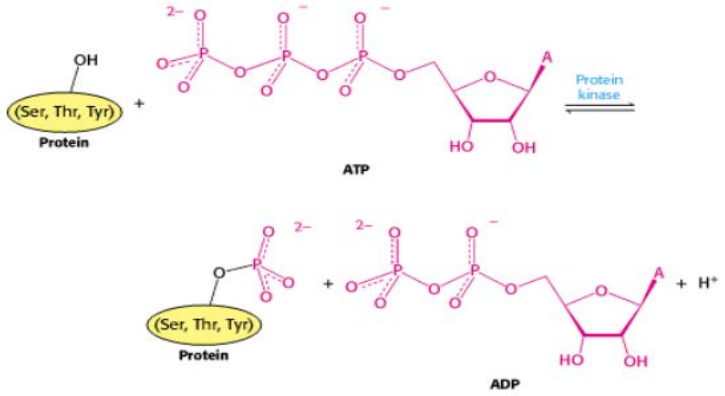


**Figure 5: Action of cAMP-dependent protein kinase**

4. Signal termination by protein phosphatase: Signal termination is final step of signal transduction. The well known route for signal termination is by protein phosphatase enzyme. The signalling process must be terminated after signaling process has been initiated and the information has been transduced to affect other cellular processes, because without such termination cells lose their responsiveness to new signals. Additionally, if termination fails in signaling processes, it may lead to uncontrolled cell growth and thus increases the risk of cancer.

## Signal amplification

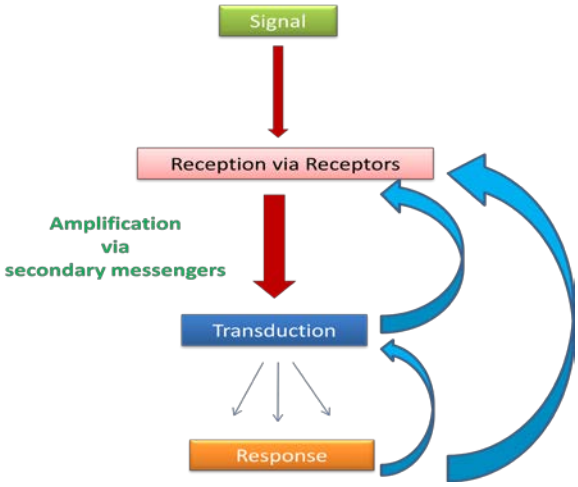


**Figure 6: Signal amplification Pathways**

Signal amplification is phenomenon in which when receptor proteins interact with the signal molecules at the surface of the cell, in most cases signals are relayed to the cytoplasm or the nucleus by second messengers which influences the activity of one or more enzymes or genes inside the cell. However, most signalling molecules are found in such a low concentration that their effect in cytoplasm would be minimal unless the signal was amplified. Therefore, most enzymes linked and G-protein linked receptor use a chain of other protein messenger to amplify the signal as it is being relayed. Thus in case of protein kinase one cell surface receptor activates many G protein molecules. Each G protein activates many adenylyl cyclases. Each cyclic AMP in turn will activate protein kinase which then activates several molecules of a specific enzyme.

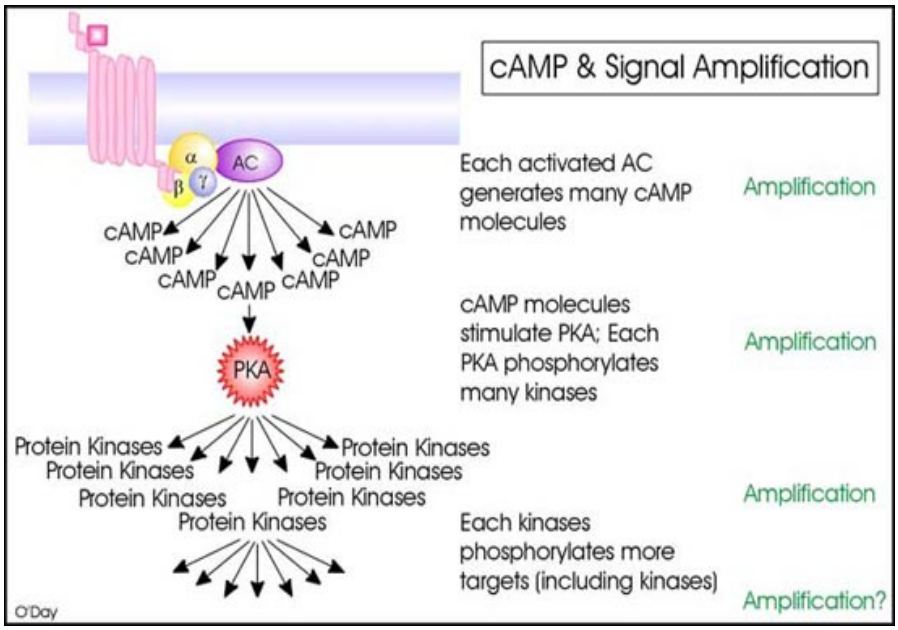


**Figure 7: Signal amplification and cAMP**

For example, the binding of a single molecule (such as glycogen or epinephrine) at the cell surface can activate many effector G proteins and an adenylyl cyclase each of which can produce a large number of cAMP messengers in a short period of time. Thus, the production of a second messenger provides a mechanism to greatly amplify the signal generated from the original message. There are many steps in the reaction cascade, amplification of the signal via cAMP molecules which activate protein kinase K which involve in phosphorylation of Ser, Thr and tyrosine of target protein. PKA is tertameric protein which is made up of two catalytic and two regulatory subunits. Binding of cAMP to the regulatory subunits induces a conformational change that leads to dissociation of the catalytic subunits, which elicit formation of enzymatically active form of protein kinase A, are now able to phosphorylate Ser and Thr residues on their target proteins. In signal amplification, each PKA catalytic subunit phosphorylates a large number of phosphorylase kinase molecules, which in turn phosphorylate an even larger number of glycogen phosphorylase molecules, which in turn can catalyze the formation of a much larger number of glucose phosphates. Thus, what begins as a hardly noticeable stimulus at the cell surface is rapidly transformed into a major mobilization of glucose within the cell.

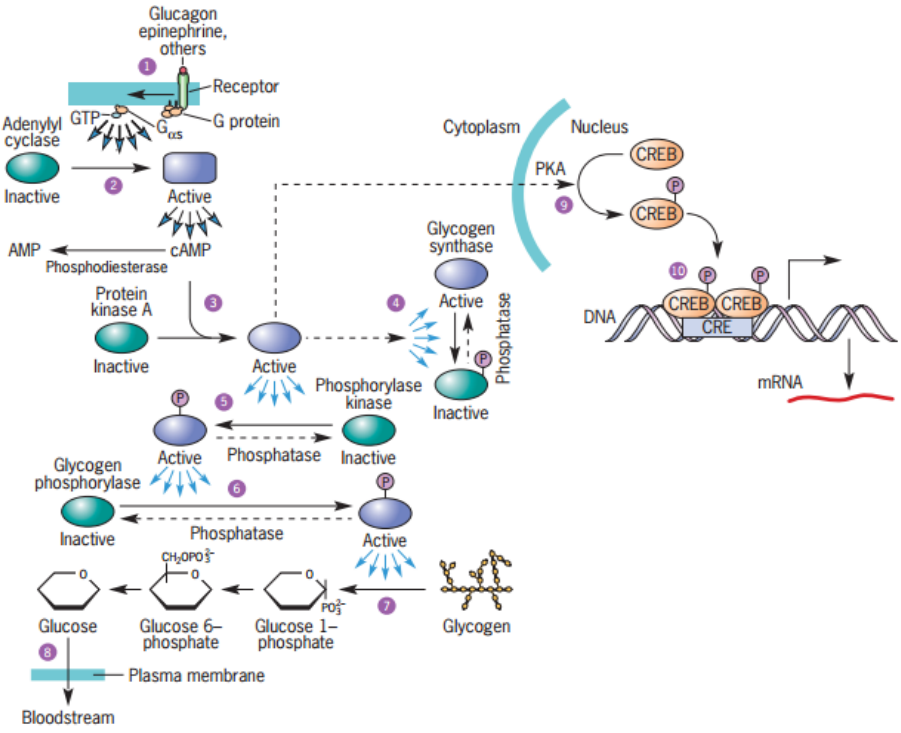


**Figure 8: Signal amplification Pathways for Glycogen degradation**

The different effect of some hormone in different tissues in shown in the following table:

**Table 1: Example of hormone induced response mediated by cAMP**

| Tissue | Hormones | Response |
|---|---|---|
| Liver | Epinephrine and glucagon | Glycogen breakdown, glucose synthesis (glucogenesis), inhibition of glycogen synthesis |
| Skeletal muscle | Epinephrine | Glycogen breakdown, inhibition of glycogen synthesis |
| Cardiac muscle | Epinephrine | Increase contractility |
| Adipose | Epinephrine, ACTH and glucagon | Triacyglycerol catabolism |
| Kidney | Vasopressin(ADH) | Increase permeability of epithelial cells to water |
| Thyroid | TSH | Secretion of thyroid hormones |
| Bone | Parathyroid hormone | Increase calcium resorption |
| Ovary | LH | Increase secretion of steroid hormones |
| Adrenal cortex | ACTH | Increase secretion of glucocorticoids |

In the upcoming chapter we will study about each component of signal transduction in detail.

**Interesting Facts:**

1. Most signaling molecules are found in such low concentration that their effect in cytoplasm would be minimal unless the signal is amplified.
2. Most enzymes linked and G-protein linked receptor use a chain of other protein messenger to amplify the signal as it being relayed.

**Questions:**

1. What is signal amplification?
2. What is Signal transduction? Explain diagrammatically.
3. Which are the possible factors which influence signal amplification?
4. How second messenger take part in the signal amplification pathway?

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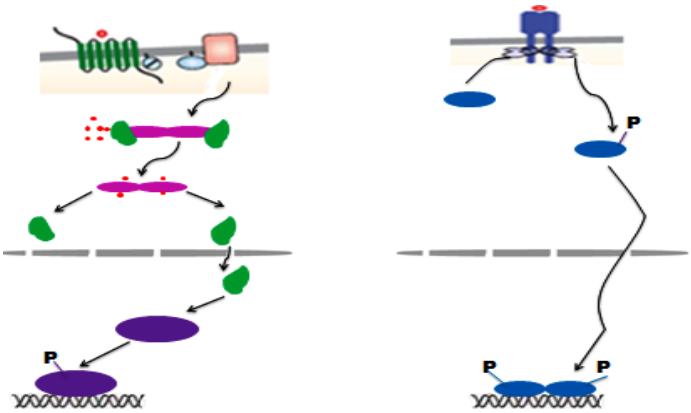
5. Stryer. Biochemistry, $5^{th}$ edition.

# M5 L2

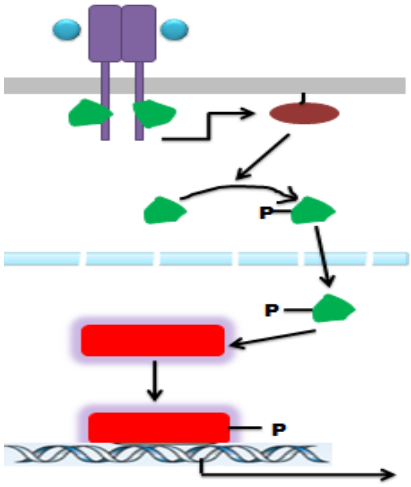
# Cell receptors

**Receptor:** A receptor is a protein molecule found on the surface of a cell which receives chemical signals originating externally from the cell. Binding of specific signalling molecules to a receptor directs a cell to allow certain molecules to enter or exit or directs a cell to divide or die. Cells within multicellular organisms communicate via extracellular mediators: either through diffusible molecules or by direct cell–cell contact. Examples of receptors are G-Protein coupled receptors, Cytokine receptors as shown in Figure 1.

Receptors are located in either the cytoplasm or plasma membrane or nucleus of a cell. A molecule which binds specifically to a receptor is called a ligand. A ligand may be a peptide or other small molecules, such as a hormone, a neurotransmitter, a pharmaceutical drug or a toxin. Each type of receptor recognizes and binds only certain ligand shapes. Binding of a ligand to its receptor causes a conformational change in the cytosolic domain of the receptor which then triggers the subsequent signalling cascade; i.e. it activates or inhibits a specific biochemical pathway. Ligand-induced changes in receptors result in cellular changes which constitute the biological activity of the ligands. Most signalling molecules bind to receptors expressed on the target cell surface but some signalling molecules are able to cross the plasma membrane and bind to intracellular receptors in the cytoplasm or nucleus.




G protein-coupled receptor Cytokine receptor




Receptor tyrosine kinases

**Figure 1: Examples of receptors**

**Receptor types based on cellular location:**

There are mainly three types of receptors present in the cell based on their location in the cell. They are:

1. Cytosolic receptors
2. Nuclear receptors
3. Membrane bound receptors

**1. Cytosolic receptors:** Cytosolic receptors are specialized integral membrane proteins that take part in communication between the cell and the outside world. Extracellular signalling molecules (usually hormones, neurotransmitters, cytokines, growth factors or cell recognition molecules) attach to the receptor, triggering changes in the function of the cell. In this way the receptors play a unique and important role in cellular communications and signal transduction. Most steroid hormones have receptors within the cytoplasm which acts by stimulating the binding of their receptors to the promoter region of steroid-responsive genes. Examples of cytosolic receptors are Estrogen receptors, Glucocorticoid receptors etc.

**Mechanism of action of cytosolic receptors:** Estrogen diffuses across the plasma membrane and binds to its receptor in the nucleus. The estrogen receptor is bound to Hsp 90 chaperones in the absence of the estrogen hormone as shown in Figure 2. The binding of the estrogen induces a conformational change in the receptor, displacing Hsp 90 and then leading to the formation of receptor dimers which binds to DNA, associate with coactivators with histone acetyltransferase (HAT) activity, and stimulate transcription of their target genes. In other cases, the receptor binds the DNA either in the presence or absence of hormone. But hormone binding alters the activity of the receptor as a transcriptional regulatory molecule. For example, in the absence of hormone, thyroid hormone receptor is associated with a corepressor complex and represses transcription of its target genes. Hormone binding induces a conformational change that results in the interaction of the receptor with co activators rather than co repressors, leading to transcriptional activation of thyroid hormone-inducible genes.

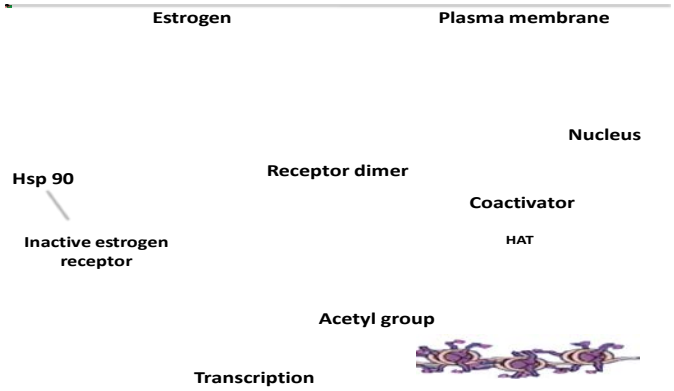



**Figure 2: Estrogen receptor action**

**2. Nuclear receptors:** Nuclear receptors are intracellular proteins expressed in the nucleus of a cell. These receptors are members of a family of proteins known as the nuclear receptor super family. Nuclear receptors constitute a superfamily of dimeric C4 zinc-finger transcription factors that bind lipid-soluble hormones and interact with specific response elements in DNA. Steroid receptors are homodimers of zinc-finger proteins that reside within the nucleus (except for the glucocorticoid receptor which resides in the cytosol until it binds its ligand). Until their ligand finds them, some steroid receptors within the nucleus associate with histone deacetylases (HDACs), keeping gene expression repressed in those regions of the chromosome.

**Structure of nuclear receptors:** Nuclear receptors constitute a superfamily of dimeric C4 zinc-finger transcription factors. They are modular in structure and contain the following structural domains:

**N-terminal regulatory domain (A-B):** The A-B domain is highly variable in sequence between various nuclear receptors. It contains the activation function 1 (AF-1) whose action is independent of the presence of ligand. The transcriptional activation of AF-1 is normally very weak but it synergizes with AF-2 in the E-domain to produce an upregulation of gene expression.

**DNA-binding domain; DBD (C):** It is a highly conserved domain containing two zinc fingers that binds to specific sequences of the DNA called hormone response elements (HRE) as shown in Figure 3.

**Hinge region (D):** It is the flexible domain that connects the DBD with the LBD. It influences subcellular distribution and intracellular trafficking.

**Ligand binding domain LBD (E):** Its sequence is moderately conserved but it is highly conserved in structure between the various nuclear receptors. The structure of the LBD is referred to as an alpha helical sandwich fold in which three anti parallel alpha helices (the sandwich filling) are flanked by two alpha helices on one side and three on the other (the bread). The ligand binding cavity is within the interior of the LBD and just below is present three anti parallel alpha helical sandwich filling. Along with the DBD, the LBD

contributes to the dimerization interface of the receptor. In addition, it binds coactivator and corepressor proteins. The LBD also contains the activation function 2 (AF-2) whose action is dependent on the presence of bound ligand.

**C-terminal domain** (F): It is highly variable in sequence between various nuclear receptors.

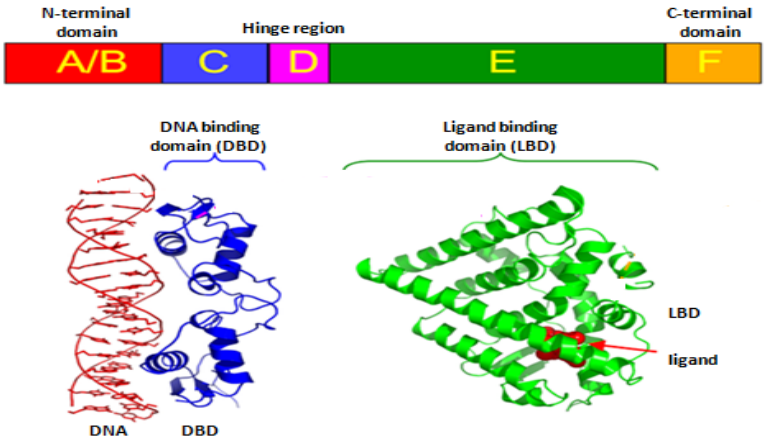



**Figure 3: Structural Organization of Nuclear Receptors (estrogen receptor)**
**Top – Schematic amino acid sequence of a nuclear receptor.**
**Bottom – 3D structures of the DBD (bound to DNA) and LBD (bound to hormone) regions of the nuclear receptor**

**Mechanism of action:** The intracellular nuclear receptors respond to small hydrophobic signaling molecules that diffuse readily across the plasma membrane. These molecules bind to the receptors and a conformational change takes place in the receptor. This is followed by a series of intracellular signal transduction cascade. The steroid hormones like thyroid hormone, vitamin $D_3$ and retinoic acid differ greatly from one another in both chemical structure and function. Once inside the cell, these signaling molecules bind to intracellular receptors that are expressed by the hormonally responsive target cells. These receptors are transcription factors that contain domains for ligand binding, DNA binding and transcriptional activation. Ligand binding regulates their function as activators or repressors of their target genes. So the steroid hormones and related molecules directly regulate gene expression. In response, these receptors work with other proteins to regulate the expression of specific genes, thereby controlling the development, homeostasis, and metabolism of the organism.

The nuclear receptor is kept in the cytoplasm by interaction between its ligand-binding domain (LBD) and inhibitor proteins in the absence of a steroid hormone. When hormone is present, it diffuses readily through the plasma membrane and binds to the ligand-binding domain. This causes a conformational change thus releasing the receptor from the inhibitor proteins. The receptor with bound ligand is then translocated into the nucleus, where its DNA-binding domain (DBD) binds to response elements, allowing the ligand-binding domain and an additional activation domain (AD) at the N-terminus to stimulate transcription of target genes as shown in Figure 4.

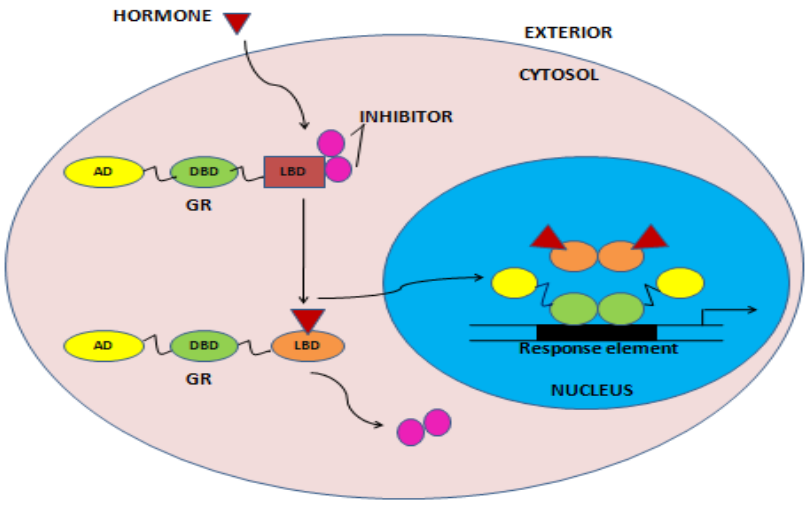



**Figure 4: Model of hormone-dependent gene activation by a homodimeric nuclear receptor.**

**3. Membrane bound receptors:** Membrane bound receptors are proteins that are associated with the cell membrane. They can span across the membrane and can transmit a signal from outside the cell to inside the cell. Outside the cell, a ligand (e.g. Hormone) will bind to the receptor. A few chemical stimuli, including steroid hormones and the gas nitric oxide cross the plasma membrane and bind receptors inside the cell. Thus the receptor undergoes a conformational change. This change in the shape of the receptor is detected inside the cell. It is the shape change that is the transmission of the signal from the outside to the inside. Inside the cell, other proteins can interact with the receptor in its new shape and be turned 'on' to continue the signal pathway.

Selective expression of certain receptors and their associated cytoplasmic transduction machinery allows differentiated cells to respond specifically to particular ligands. Members of each family of receptors share one or more structurally homologous domains. In some families, the members share both ligand binding and signal transducing strategies (seven helix receptors, G-protein coupled receptors, cytokine receptors). Members of other families share either a similar ligand-binding structure (Tumour Necrosis Factor receptor family) or a common signal transducing method (receptor tyrosin kinases).

Membrane receptors are found also in the cis Golgi network which captures the proteins during protein sorting and carries them in transport vesicles back to the ER. Also most cholesterol is transported in the blood bound to protein in the form of particles known as low density lipoproteins or LDL. When a cell needs cholesterol for membrane synthesis, it makes transmembrane receptor for LDL and inserts them into its plasma membrane. Once in the plasma membrane, the LDL receptors diffuse until they associate with clathrin-coated pits as shown in Figure 5.

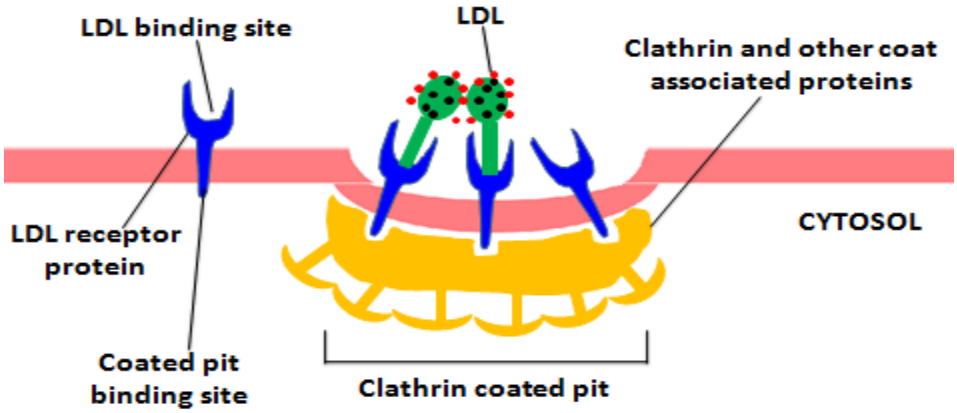



**Figure 5: LDL receptor proteins binding to a coated pit in the plasma membrane of a cell**

The seven major classes of cell surface receptors are:

1. G protein-coupled receptors
2. Cytokine receptors
3. Receptor tyrosine kinases
4. TGFβ receptors
5. Hedgehog receptors
6. Wnt receptors
7. Notch receptor

**Structure of Seven helix receptors:** Membrane bound receptors constitute the members of the largest family of plasma membrane receptors built from a serpentine arrangement of seven transmembrane α helices. G protein–coupled receptors (GPCRs) are examples of seven transmembrane α helices receptors. GPCRs are found in all eukaryotic cells from yeast to man. All G protein–coupled receptors contain seven membrane-spanning regions with their N-terminal segment on the exoplasmic face and their C-terminal segment on the cytosolic face of the plasma membrane.

As shown in Figure 6 (a), all receptors of this type have the same orientation in the membrane and contain seven transmembrane - helical regions (H1–H7) - four extracellular segments (E1–E4), and four cytosolic segments (C1–C4). The carboxyl-terminal segment (C4), the C3 loops and, in some receptors, also the C2 loops are involved in interactions with a coupled trimeric G protein. The long C3 loop between helices 5 and 6 is important for interactions between a receptor and its coupled G protein. This superfamily of seven-pass transmembrane receptor proteins includes rhodopsin, the light-activated protein in the vertebrate eye as shown in Figure 6 (b).

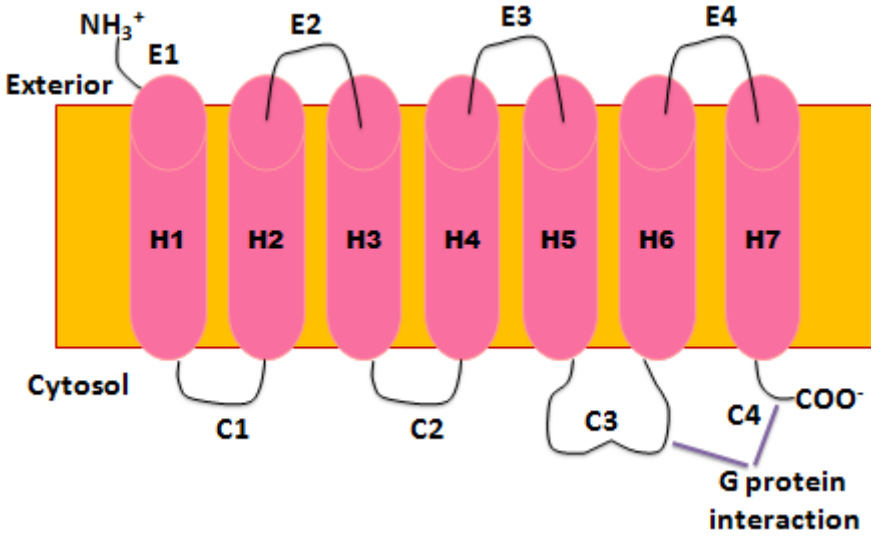



**Figure 6 (a): Schematic diagram of G protein–coupled receptors**

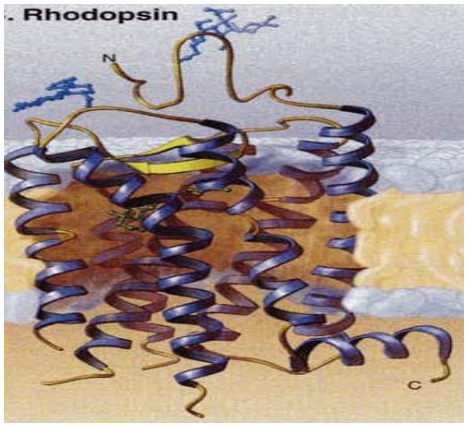



**Figure 6 (b): Atomic structure of seven helix receptor of bovine rhodopsin, the light activated protein in vertebrate eye**

**Mechanism of action of seven helix receptors:** The seven helix receptors use trimeric GTP-binding proteins to relay signals to effector proteins inside the cells. Human genome encodes several thousand GPCR. These include receptors in the visual, olfactory (smell) and gustatory (taste) systems, neurotransmitter receptors and most of the receptors for hormones that control the metabolism of carbohydrate, amino acid and fat. GPCRs are coupled to signal-transducing trimeric G proteins. In mammals, olfactory cells alone use 500 – 1000 different seven-helix receptors to discriminate odorant molecules.

Phosphorylation of the C-terminal tail inactivates many types of seven helix receptors. Two different strategies, sometimes acting on the same receptor, provide negative feedback. One strategy is for the second messengers, which are produced in response to receptor activation to stimulate general protein kinases (including cAMP), protein kinase A (PKA) and protein kinase C (PKC) which phosphorylate the activated receptor. Phosphorylation inhibits the receptor thus allowing for crosstalk between receptors. The second strategy involves a class of protein kinases specific for the receptor themselves. They are called G-protein coupled receptor kinases. These kinases phosphorylate multiple serines or threonines on the C-terminal cytoplasmic tail of active receptors. Phosphorylation promotes binding of a regulatory protein called arrestin which inactivates the receptor by blocking interaction of the receptor with trimeric G-proteins. Arrestin binding to some seven helix receptors promotes their removal from the plasma membrane by endocytosis. G protein–coupled receptors transduce signals from extracellular hormones to associated effector proteins. In the resting state, when no ligand is bound to the receptor, the $G_{\alpha}$ subunit is bound to GDP and complexed with $G_{\beta\gamma}$. As shown in the Figure 6, ligand binding shifts the equilibrium from the resting conformation towards the active conformation. Active receptor promotes dissociation of GDP from α subunit of multiple trimeric G-proteins, allowing GTP to bind. This dissociates $G_{\alpha}$ from $G_{\beta\gamma}$, each of which activate downstream effectors that produce the second messengers cAMP and diacylglycerol (DAG) as shown in Figure 7. cAMP and DAG activates PKA and PKC, which phosphorylate active receptors on their C-terminus. This attracts arrestin, putting the receptor into the inactive adapted state.

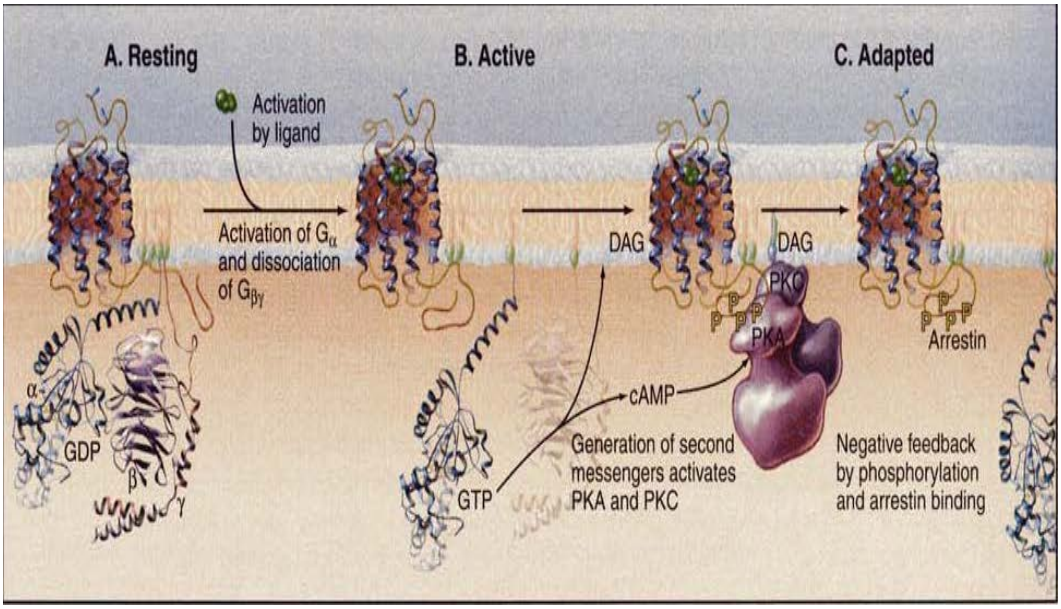



**Figure 7**: **Activation and adaptation of a seven helix receptor: A, resting; B, active; C, adapted.**

Epinephrine Binds to Several Different G Protein–Coupled Receptors. All epinephrine receptors are G protein– coupled receptors, the different types are coupled to different G proteins. These receptors are of interest because they trigger different intracellular signal-transduction pathways. Both subtypes of β-adrenergic receptors, termed β1 and β2, are coupled to a stimulatory G protein (Gs) that activates the membrane-bound enzyme adenylyl cyclase. Once activated, adenylyl cyclase catalyzes synthesis of the second messenger cAMP. That binding of epinephrine to β-adrenergic receptors induces a rise in cAMP.

**Interesting facts:**

- Receptor tyrosine kinase is the first receptor to be discovered.
- G proteins were discovered by Alfred G. Gilman and Martin Rodbell when investigating stimulation of cells by adrenaline.
- Skin contains approximately 640,000 sense receptors scattered unevenly over the body's surface.

**Questions:**

1. What are receptors? Give examples.
2. How many types of receptors are present on the cell?
3. What are cytosolic receptors? Give example.
4. What are nuclear receptors?
5. What is membrane bound receptors? Give example.

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# M5 L3

# Second messengers - cAMP

**Second messengers:** As mentioned din lecture 1 of this module, **s**econd messengers are molecules that relay signals received at receptors on the cell surface such as hormones, growth factors, etc. to appropriate target molecules in the cytosol and/or nucleus. In addition to their job as relay molecules, second messengers serve to amplify the strength of the signal. Binding of a ligand to a single receptor at the cell surface may end up causing massive changes in the biochemical activities within the cell.

There are 3 major classes of second messengers:

1. Cyclic nucleotides (**cAMP** and **cGMP**)
2. Inositol trisphosphate (**$IP_3$**) and diacylglycerol (**DAG**)
3. Calcium ions ($Ca^{2+}$)

We will discuss about all of them in the upcoming lectures.

**Cyclic adenosine monophosphate (cAMP)**

cAMP is an important second messenger involved in a plethora of cellular effects and biological roles by regulating various metabolic process and mediating the effects of many hormones that binds to a specific receptor on the cell membrane of target cells including catecholamines, ACTH, and vasopressin. It also plays imperative role in the transcription of some genes. Earl Sutherland won a Nobel Prize in Physiology or Medicine in 1971 for his discoveries regarding the mechanisms of the action of hormones, especially epinephrine, via second messengers such as cyclic AMP. cAMP is represented by $C_{10}H_{12}N_5O_6P$ and the molecular mass is 329.206.

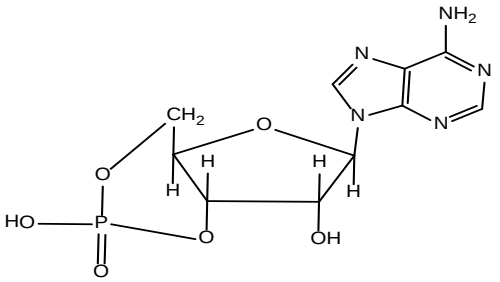


**Figure 1: Cyclic Adenosine monophosphate or 3'-5'-cyclic adenosine monophosphate**

[Compositions of cAMP - Adenine base + Ribose sugar + 3', 5'-cyclic phosphate]
Adenosine in cAMP is a nucleoside composed of the pentose sugar D-ribose and adenine, a base. Cyclic AMP contains an ester linkage between the phosphate and ribose units.
Some of the hormones that achieve their effects through cAMP as a second messenger:

- Adrenaline
- Glucagon
- Luteinizing hormone (LH)

Binding of the hormone to its receptor activates a G protein which, in turn, activates adenylyl cyclase. The resulting rise in cAMP turns on the appropriate response in the cell by either (or both): changing the molecular activities in the cytosol, often using Protein Kinase A (PKA) — a cAMP-dependent protein kinase that phosphorylates target proteins; turning on a new pattern of gene transcription.

**Functions of cAMP**

1. **cAMP as a second messenger:** cAMP is a second messenger, used for intracellular signal transduction. It is involved in transmitting signal from outside the cell to the interior via the process of binding of hormones like glucagon and epinephrine or other signal molecules to cell membrane receptor. It is involved in the activation of protein kinases and regulates the effects of adrenaline and glucagon. cAMP also binds to and regulates the function of ion channels such as the HCN channels and a few other cyclic nucleotide-binding proteins such as Epac1.

**2. Regulation of protein kinase A by cAMP (Phosphorylation of protein kinase):** The most important function of cAMP in animal cell is regulation of protein kinase A activity. Protein kinase A is found primarily in inactive form in the cell in which it consists of two regulatory (R) and two catalytic (C) subunits together. Binding of cAMP to the regulatory subunits induces a conformational change that leads to dissociation of the catalytic subunits, which elicits formation of enzymatically active form of protein kinase A, and are now able to phosphorylate Ser and Thr residues on their target proteins.

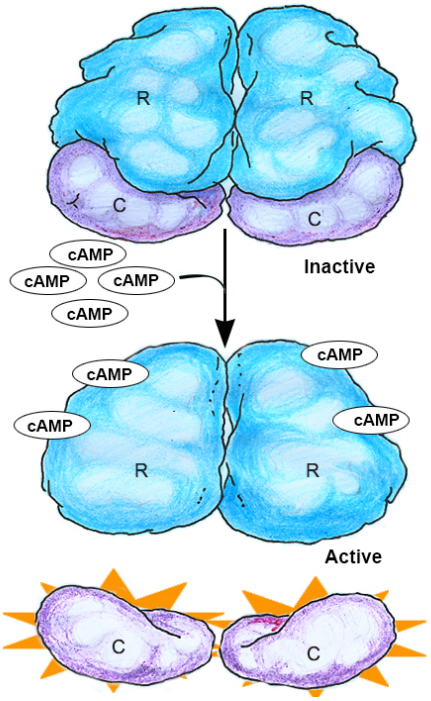


**Figure 2: Regulation of protein kinase A**

**3. Regulation of Cyclic AMP-inducible gene expression via protein kinase A:**

In many animal cells, increase in cAMP activates the transcription of specific target genes that contain a regulatory sequence called the cAMP response element, or CRE. In this case, the signal is passed from the cytoplasm to the nucleus by the catalytic subunit of protein kinase A, which is able to enter the nucleus after its release from the regulatory subunit. Within the nucleus, protein kinase A phosphorylates a transcription factor called CREB (CRE-*b*inding protein), leading to the activation of cAMP-inducible genes. Thus such type of regulation of gene expression by cAMP plays significant role in controlling proliferation, survival, and differentiation of a wide variety of animal cells.

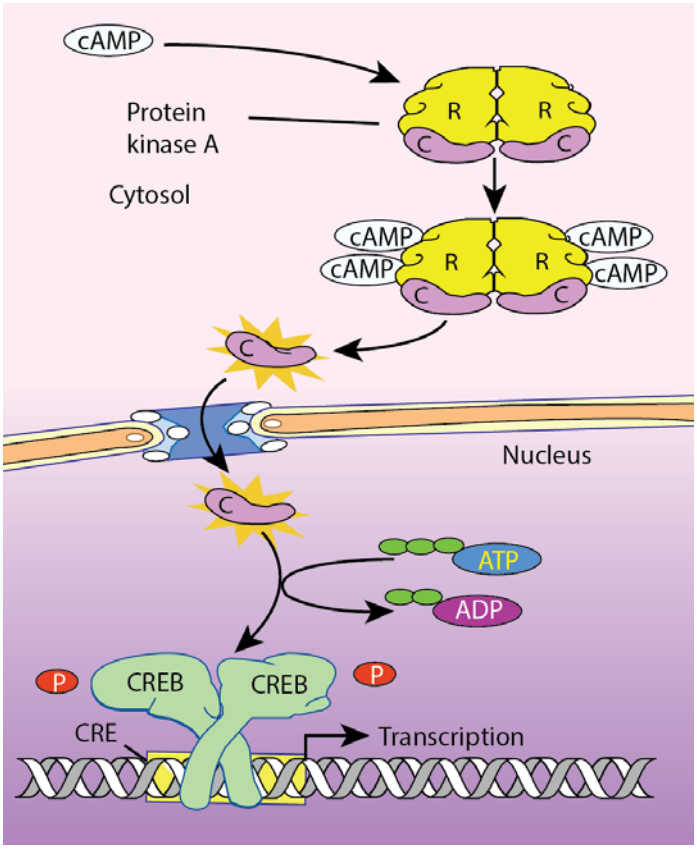


**Figure 3: Cyclic AMP inducible gene expression**

**4. In eukaryotic cells:** cAMP and its associated kinases play key role in numerous biochemical processes, including the regulation of glycogen, sugar, and lipid metabolism. There are several minor PKA-independent functions of cAMP like activation of calcium channels, providing a minor pathway by which growth hormone-releasing hormone causes a release of growth hormone. The GEF (guanine nucleotide exchange factor)

domain is usually covered by the N-terminal region containing the cAMP binding domain. When cAMP binds, the domain dissociates and exposes the active GEF domain, allowing Epac to activate small Ras-like GTPase proteins, such as Rap1.

**5. In bacteria:** In bacteria, cAMP plays a crucial role and its level varies depending on the medium used for growth. In *E.coli*, cAMP involves in the positive regulation of the lac-operon. cAMP is synthesized from ATP by adenylyl cyclase, as a result increase in the level of cAMP causes decrease in glucose concentration which is the carbon source. cAMP then binds to the transcriptional regulatory protein, cAMP receptor protein (CRP) also called catabolic activator protein(CAP). After binding of cAMP to CAP enhance the binding capacity of CAP to its binding site (CAP binding site) on target DNA sequence which in lac operon located 60 nucleotides upstream of transcription start site, making it easier for RNA polymerase to bind to the adjacent promoter to start transcription of the lac-operon, increasing the rate of lac-operon transcription. With a high glucose concentration, the cAMP concentration decreases, and the CRP disengages from the lac-operon.

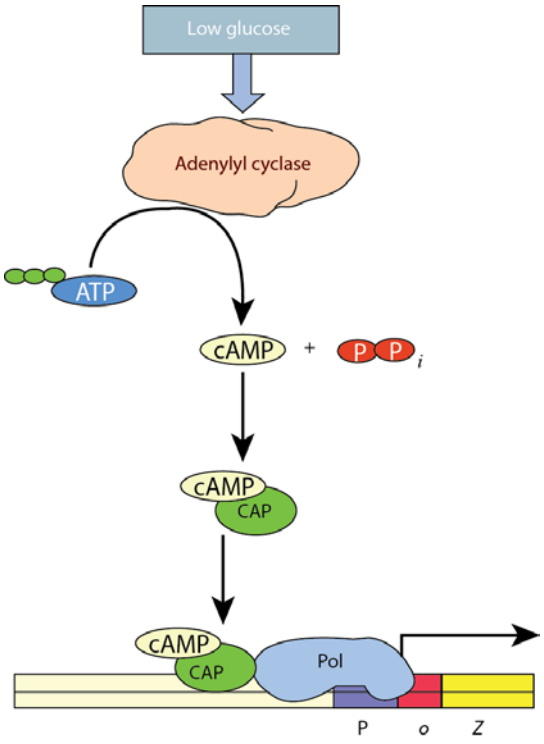


**Figure 4: Positive regulation of lac operon by glucose repression coupled to enhance level of cAMP.**

**6. In some slime moulds:** In some slime mold species such as *Dictyostelium discoideum*, the chemotactic movement of cells is organized by periodic waves of cAMP that propagates through the cell. The waves are the result of a regulated production and secretion of extracellular cAMP and a spontaneous biological oscillator that initiates the waves at centers of territories.

**7. Mitochondrial Biogenesis:** cAMP/PKA has key role in mitochondrial compartment biogenesis. Mitochondria takes part in several vital cellular functions. For example they are involved in ATP production, via the process of oxidative phosphorylation, the ATP production via mitrochondria is 15 times more than glycolysis alone. They also take central part in metabolic regulation and assist diverse cell signalling events. Mitochondria are therefore essential for the maintenance, adaptability and survival of eukaryotic cells. cAMP/PKA signaling balances respiratory activity with mitochondria dependent apoptosis via transcriptional regulation.

**Areas of action of cyclic AMP:**

There are so many application of cAMP for the prevention of several disorders in human beings. Some are shown below:

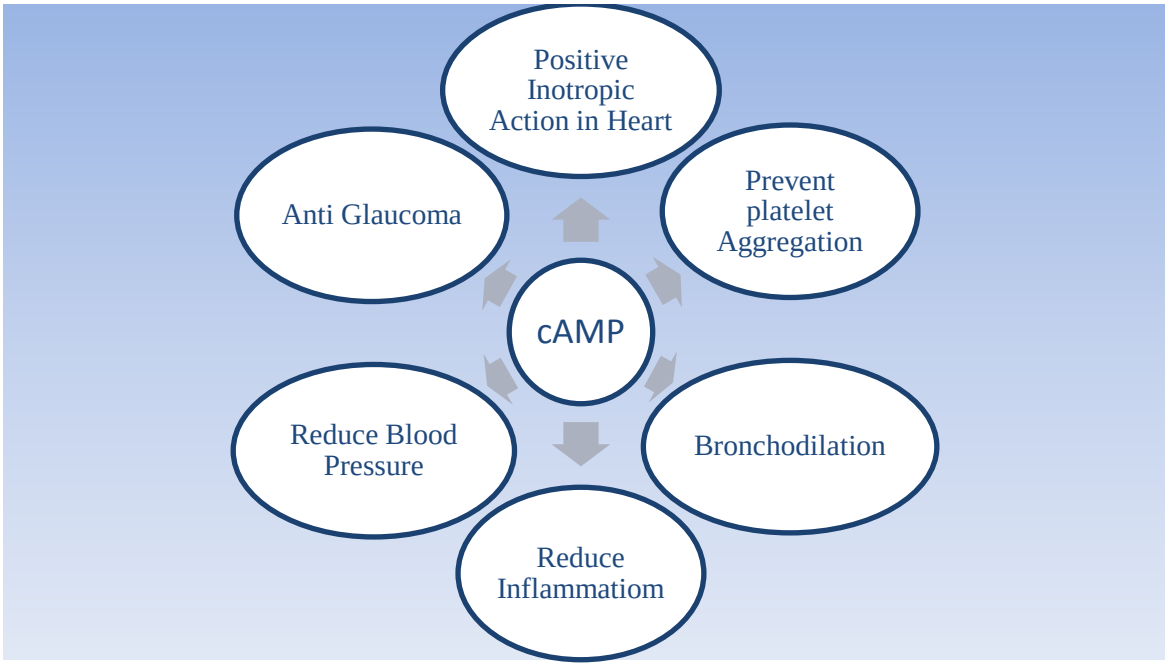


**Figure 5: Action of cAMP in our body**

**Mechanism of Regulation of cAMP:** When stimulatory hormone binds to G-protein coupled receptor some conformational changes occur in the receptor by which its unexposed catalytic part gets exposed due to which it can interact and activate G-protein with exchange of GDP by GTP at $G_\alpha$ subunit. Now $G_\alpha$ subunit goes and activates membrane bound adenylyl cyclase that facilitates the conversion of ATP to cAMP. This cAMP is now ready to activate protein kinase A which is involved in phosphorylation of several proteins that takes part in the further cellular responses. At the same time of cAMP production by adenylyl cyclase, the cAMP level is controlled by other enzyme that is called cAMP phosphodiesterase which converts cAMP into 5'-AMP. On the other hand, when inhibitory hormone binds to G-protein coupled receptor, it counters the effect as shown in Figure 6. In this process, the adenylyl cyclase is inhibited because GPCR activates the G protein that contains inhibitory alpha subunit which binds to enzyme and inhibits cAMP production.

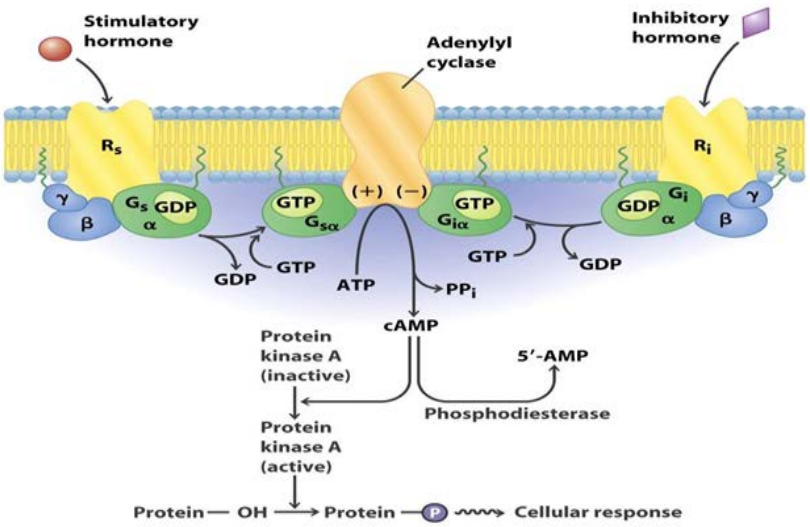


**Figure 6: Synthesis and Regulation of cAMP**

**Synthesis and Degradation of cAMP:** cAMP is a cyclic nucleotide which serves as an intracellular and in some cases extracellular secondary messenger. It is derived from adenosine triphosphate (ATP) by adenylyl cyclase located on inner side of plasma membrane and used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway. The elevated cAMP level is regulated by degradation pathway which takes place by cAMP phosphodiesterase enzyme.

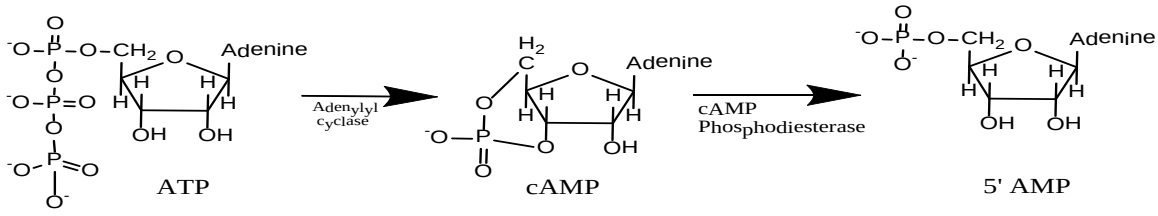


**Figure 7: Synthesis and degradation of cAMP**

## Interesting facts:

1. Cyclic AMP is synthesized from ATP by the action of the enzyme adenylyl cyclase.
2. cAMP decomposition into AMP is catalyzed by the enzyme phosphodiesterase.

3. cAMP and its associated kinases function in several biochemical processes, including the regulation of glycogen, sugar, and lipid metabolism.

**Questions:**

1. Explain the effect of glucose concentration on the cAMP and catabolic activator protein?
2. How cAMP involve in signal transduction pathway?
3. How cAMP synthesizes and regulated in our body?

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# M5 L4
# Second messengers - cGMP

**Cyclic Guanosine monophosphate:** Cyclic Guanosine monophosphate or cGMP, is a cyclic nucleotide derived from guanosine triphosphate(GTP). cGMP is a multi-functional second messenger molecule, similar in action to cAMP but generally producing opposite effects on cell function. It has molecular formula of $C_{10}H_{12}N_5O_7P$ and has molecular weight of 345.2 g/mol. It has composition of Guanine nucleotide base, ribose sugar and cyclic phosphate between 3' and 5' positions of ribose sugar as shown in figure 1. Cyclic GMP is synthesized from the nucleotide GTP using the enzyme guanylyl cyclise as shown in figure 2.

**Structure:**

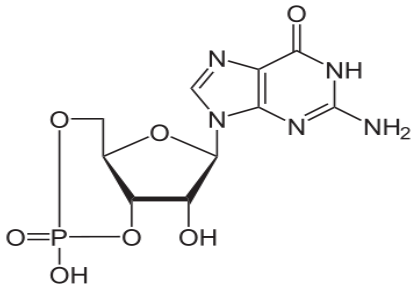


**Figure 1: Guanosine 3',5'-cyclic phosphate**

**Synthesis of cGMP:** cGMP is an important signal molecule that carries different messeges in different tissues. cGMP is generated via two pathways distinguished by the nature of Guanylyl Cyclase (GC) that mediate its conversion from guanosine triphosphate (GTP). The guanyl cyclase is generally found in cell in two forms - soluble form and membrane- bound form. They are generated via two pathways as described below:

1. The soluble pathway, where cGMP is generated via nitric oxide (NO)-activated guanylyl cyclase which is cytosolic protein with tightly associated heme group. NO is sufficiently non-polar which can easily cross the plasma membrane of target cell without any carrier and binds to the heme group of guanylyl cyclase and activates the cGMP production.

2. In the membrane-bound pathway, GCs is transmembrane protein with extracellular ligand binding domain, share some homology with those activated by NO. The ligands for a subset of membrane GCs are members of the Natriuretic Peptide (NP) hormone family including atrial NP hormone, B-type NP hormone and C-type NP hormone.

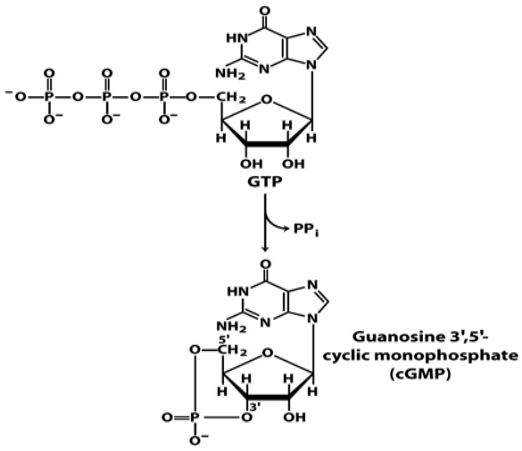


**Figure 2: Conversion of GTP to cGMP**

**Functions of cGMP:**

**1.** cGMP is an important molecule of the cell that takes part in various activities in cellular system. When guanylyl cyclase stimulation leads to elevated levels of cGMP, it then mediates biological responses, such as blood vessel dilation which increases blood flow.

2. The action of cGMP is regularly facilitated by stimulation of cGMP dependent protein kinases, although cGMP is a common regulator of ion channel conductance, glycogenolysis, cellular apoptosis and phosphodiesterases.

3. Another well-known role of cGMP is in the vertebrate eye, where it serves as the second messenger responsible for converting the visual signals received as light to nerve impulses. The photoreceptor in rod cells of the retina is a G protein-coupled receptor called rhodopsin. When light falls on the extracellular side of rhodopsin, then some conformational changes occurs in it by which its bounded chromophore 11-cis retinal is converted to all-trans retinal form, ultimately rhodopsine's unexposed catalytic cytoplasmic side gets exposed which interacts with the G protein transducin and activates them by replacement of GDP by GTP on its α- subunit. The activated $G_\alpha$ then activates cGMP phosphodiesterase 6 which converts all cGMP into 5' GMP. Due to this cGMP level gradually decreases, the cGMP dependent sodium ion-channel becomes closed. This

channel is also entry site of calicium ions so $Ca^{++}$ level also decrease. This critical situation created in the cell is called hyperpolyrisation. But after decreasing $Ca^{++}$ level, guanylyl cyclase is activated and again cGMP synthesis starts. Thus we can summarize the whole phenomenon as, change in cGMP level in retinal rod cells is translated to a nerve impulse by a direct effect of cGMP on ion channels in the plasma membrane.

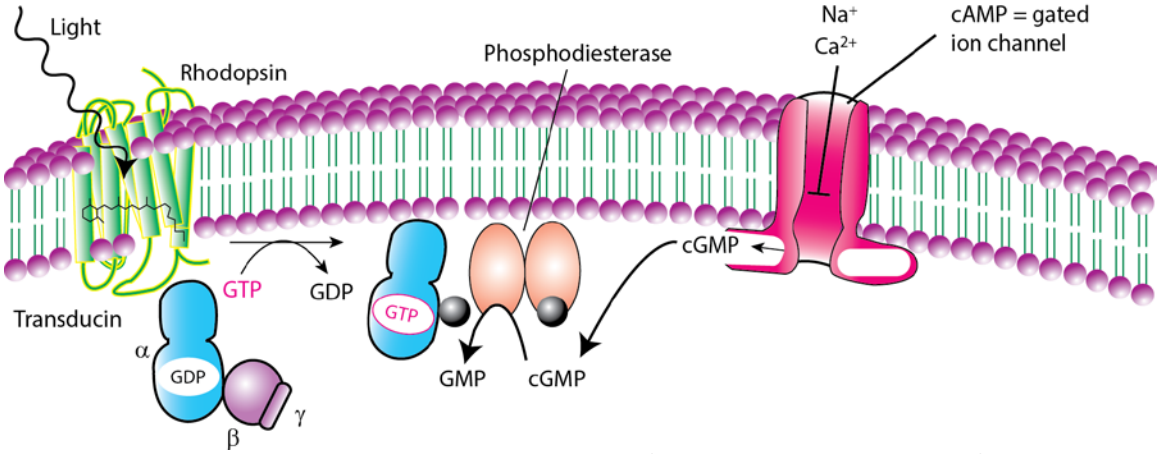


**Figure 3: Visual Signal Transduction. The light-induced activation of rhodopsin leads to the hydrolysis of cGMP, which in turn leads to ion channel closing and the initiation of an action potential.**

4. In kidney, membrane bound guanylyl cyclase is activated by the hormone atrial natriuretic factor (ANF), which is released by cells in atrium of the heart when the heart is stretched by increased blood volume. ANF comes to kidney with blood from heart and activates membrane bound guanylyl cyclase in the cells of collecting ducts. The resulting rise in cGMP level in cells triggers increase renal excretion of $Na^{+}$ and water, driven by change in osmotic pressure. Water loss causes reduction of blood volume which counters the production of ANF. Vascular smooth muscle also contains ANF receptor-guanylyl cyclase which release. On binding to these receptor , ANF causes relaxation of blood vessels which increases the blood flow with decreasing blood pressure.

cGMP, like cAMP get synthesized by receiving odourous input by olfactory receptor. cGMP is produced slowly and has a more sustained life than cAMP. cGMP in the olfactory is synthesized by both membrane guanylylcylcase (mGC) as well as soluble guanylyl cyclase (sGC). cGMP synthesis in the olfactory is due to sGC activation by nitric oxide, a neurotransmitter. cGMP also requires increased intracellular levels of

cAMP and the cross-link between the two second messengers seems to be due to rising intracellular calcium levels.

Vasodilation: NO is an extracellular gaseous second messenger. NO is unusual because it acts both as an extracellular messenger, mediating intercellular communication, and as a second messenger, acting within the cell in which it is generated. NO is synthesized by L-arginine which is catalyzed by nitric oxide synthesae. The NO formed in the endothelial cell diffuses across the plasma membrane and into the adjacent smooth muscle cells, where it binds and stimulates guanylyl cyclase which synthesizes cyclic GMP (cGMP).

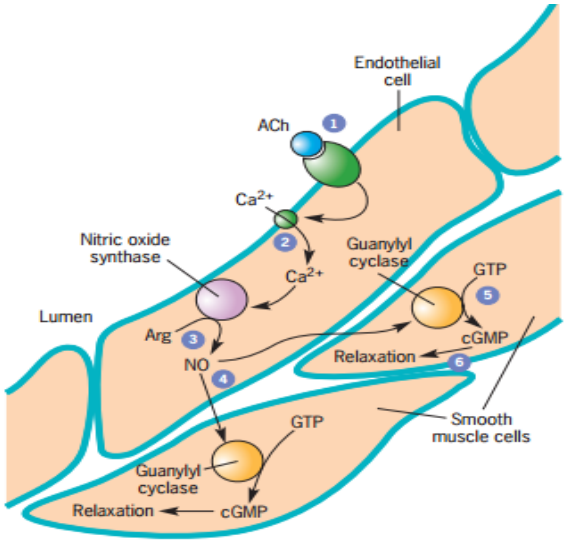


**Figure 4: Signal transduction pathway leads to dilation of blood vessel through NO and cGMP.**

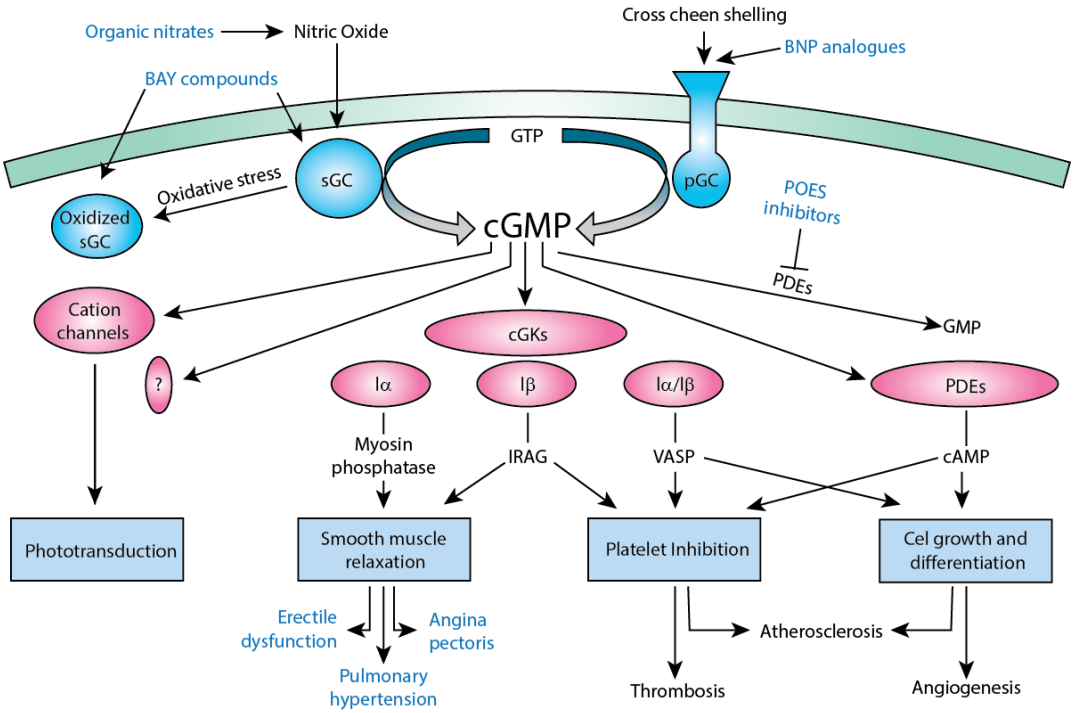


**Figure 5: Pathways of generation of cGMP and several areas of effect**

The conversion mechanism of GTP to cGMP by guanyl cyclase is shown below:

**Regulation Pathway of cGMP:** In animals, most of the actions of cGMP are supposed to be mediated by cGMP-dependent protein kinase, which is also called protein kinase G, abbreviated as PKG. After activation of PKG by cGMP, phosphorylates Ser and Thr residues in target proteins. PKG has catalytic and regulatory domains on a single polypeptide chain (*Mr*, 80,000). A part of regulatory domains fit comfortably in substrate-binding site. After binding of cGMP forces this part of regulatory domain out of the binding site, activating the catalytic domain.

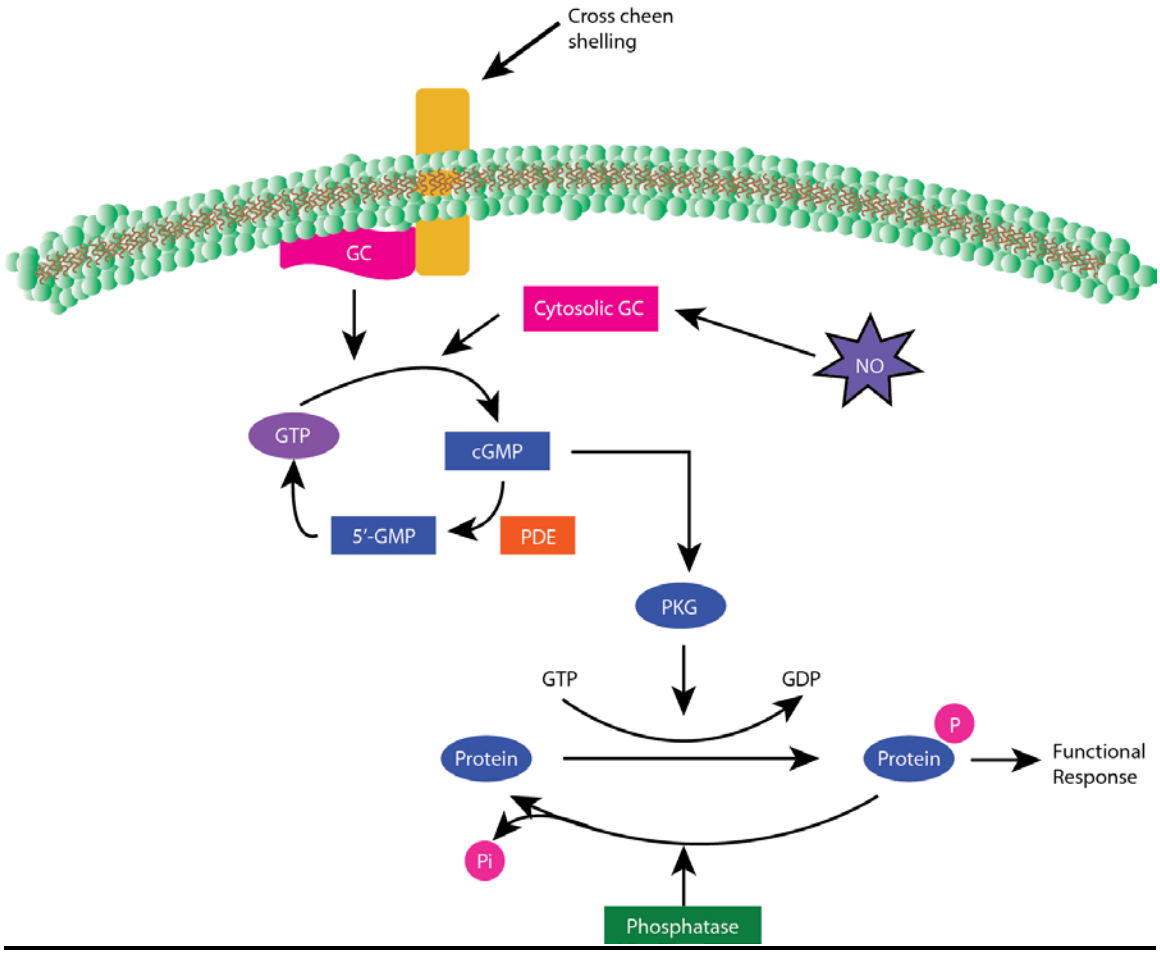


**Figure 6: Regulatory Pathway of cGMP**

**Interesting facts:**

1. Cyclic GMP is synthesized from the nucleotide GTP using the enzyme guanylyl cyclase.

2. Cyclic GMP serves as the second messenger for atrial natriuretic peptide (ANP), nitric oxide (NO), the response of the rods of the retina to light.

3. Some of the effects of cGMP are mediated through Protein Kinase G (PKG) a cGMP-dependent protein kinase that phosphorylates target proteins in the cell.

**Questions:**

1. How cGMP is involved in vasodilation or smooth muscle relaxation?
2. What is the visual pathway for light transduction in brain?
3. How cGMP regulation take place and what are the enzyme involve in its regulation?

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# M5 L5
# Calcium ion flux and its role in cell signalling

**Calcium ion flux:** Calcium ions are also important intracellular messengers. In fact, calcium ions are probably the most widely used intracellular messengers. Calcium ($Ca^{2+}$) plays an essential role in the physiology and biochemistry of organisms and the cell. It plays role in common signalling mechanism because once it enters the cytoplasm it exerts allosteric regulatory affects on many enzymes and proteins. Calcium is a second messenger produced by indirect signal transduction pathways such as G-protein coupled receptors. Calcium ions ($Ca^{2+}$) impact nearly every aspect of cellular life. The principles of $Ca^{2+}$ signaling, from changes in protein conformations driven by $Ca^{2+}$ to the mechanisms that control $Ca^{2+}$ levels in the cytoplasm and organelles. The highly localized nature of $Ca^{2+}$-mediated signal transduction and its specific roles in excitability, exocytosis, motility, apoptosis, and transcription. Normally, cytosolic calcium [$Ca^{2+}$] ions is kept very low ($10^{-7}$ M) by the action of $Ca^{2+}$ pumps in the ER, mitochondria and plasma membrane. Hormonal, neural, or other stimuli cause either an influx of $Ca^{2+}$ into the cell through specific $Ca^{2+}$ channels in the plasma membrane or the release of sequestered $Ca^{2+}$ from the ER or mitochondria, in either case raising the cytosolic [$Ca^{2+}$] and triggering a cellular response. This phenomenon is called Calcium ion flux.

**Role of Calcium in cell signalling:**

In response to many different signals, a rise in the concentration of $Ca^{2+}$ in the cytosol triggers many types of events such as:

- Muscle contraction
- Exocytosis

  a) Release of neurotransmitters at synapses (and essential for the long-term synaptic changes that produce Long-Term Potentiation (LTP) and Long-Term Depression (LTD)

  b) Secretion of hormones like insulin
- Activation of T cells and B cells when they bind antigen with their antigen receptors (TCRs and BCRs respectively)

- Adhesion of cells to the extracellular matrix (ECM)
- Apoptosis
- A variety of biochemical changes mediated by Protein Kinase C (PKC)

The concentration of calcium ions in a particular cellular compartment is guarded by the regulated activity of $Ca^{2+}$ pumps, $Ca^{2+}$ exchangers, and/or $Ca^{2+}$ ion channels located within the membranes that surrounds the compartment. There are two major types of signaling receptors- G-protein coupled receptor (GPCRs) and receptor protein-tyrosine kinases (RTKs). One of the most important pathways of intracellular signalling is based on the use of second messengers derived from the membrane phospholipids phosphatidylinositol 4, 5-bisphosphate ($PIP_2$). $PIP_2$ is a minor component of the plasma membrane, localized to the inner leaflet of the phospholipids bilayer. Hydrolysis of $PIP_2$ by phospholipase C-β is stimulated by variety of hormones and neurotransmitters. After hydrolysis $PIP_2$ is cleaved into two components- Diacyl glycerol (DAG) and inositol l,4,5- triphosphate ($IP_3$), both are also the secondary messengers of cell. Diacylglycerol and $IP_3$ stimulate distinct down-stream signalling pathways (protein kinase C and $Ca^{2+}$ mobilization, respectively), so $PIP_2$ hydrolysis triggers a two-armed cascade of intracellular signalling.

The first secondary messenger diacylglycerol produced by hydrolysis of $PIP_2$ remains associated with the plasma membrane and activates protein-serine/threonine kinases belonging to the protein kinase C family, many of which play important roles in the control of cell growth and differentiation.

The other second messenger produced by $PIP_2$ cleavage, $IP_3$, is a small polar molecule that is released into the cytosol, where it acts to signal the release of $Ca^{2+}$ from intracellular stores.

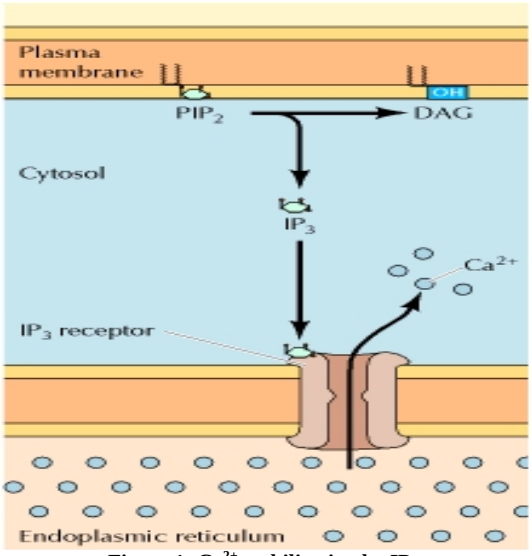


**Figure 1: $Ca^{2+}$ mobilization by $IP_3$**

Whereas the other important pathway in which extracellular messengers that signal through RTKs can trigger a similar response as in GPCR signal pathway. The major difference is that RTKs activate members of the phospholipase C-γ subfamily, which possess an $SH_2$ domain that allows them to bind to the activated, phosphorylated RTK.

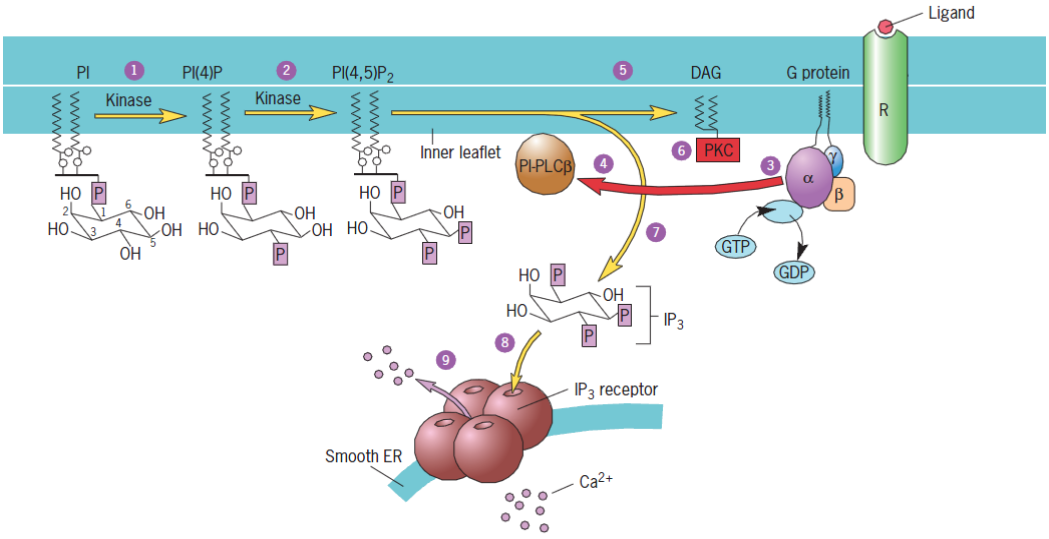


**Figure 2: Comparative pathway of GPCR and RTKs for release of calcium from ER**

There are numerous other PLC isoforms. For example, PLC δ is activated by $Ca^{2+}$ ions, and PLCε is activated by Ras-GTP. All PLC isoforms carry out the same reaction,

producing $IP_3$ and linking a multitude of cell surface receptors to an increase in cytoplasmic $Ca^{2+}$. There is another major route leading to elevation of cytosolic [$Ca^{2+}$] which involve in synaptic transmission. In this case, a nerve impulse leads to a depolarization of the plasma membrane, which triggers the opening of voltage-gated calcium channels in the plasma membrane, allowing the influx of $Ca^{2+}$ ions from the extracellular medium.

**Properties of Calcium Ion ($Ca^{2+}$)**

There are two major properties that allow Calcium ($Ca^{2+}$) ion to work effectively as a signaling mechanism:

- $Ca^{2+}$ levels inside the cell are readily detectable. This is because the levels of $Ca^{2+}$ are highly regulated by transport systems that expel $Ca^{2+}$ from the cell. The level of $Ca^{2+}$ in the cytoplasm is approximately 100nM, which are several orders of magnitude lower than outside the cell as a result of $Ca^{2+}$ pumps that actively export $Ca^{2+}$ from the cell. $Ca^{2+}$ is pumped not only across the plasma membrane but also into the endoplasmic reticulum, which therefore serves as an intracellular $Ca^{2+}$ store. $IP_3$ acts to release $Ca^{2+}$ from the endoplasmic reticulum by binding to receptors that are ligand-gated $Ca^{2+}$ channels. As a result, cytosolic $Ca^{2+}$ levels increase to about 1 $\mu M$, which affects the activities of a variety of target proteins, including protein kinases and phosphatases.

- $Ca^{2+}$ can readily bind to proteins and cause conformational changes. $Ca^{2+}$ is attracted to the negatively charged oxygen atoms in the side chains of glutamate and asparagines, and the uncharged oxygen in both the side chains and main chains of glutamine and asparagine. $Ca^{2+}$ is readily able to cause large conformational changes due to the fact that it can form ligand with up to eight oxygen atoms. This can lead to cross linking of amino acids in a protein that did not exist before $Ca^{2+}$ was introduced.

**Function of $Ca^{2+}$ in cell:**

- Protein function is governed by shape and charge. $Ca^{2+}$ binding triggers changes in protein shape and charge. Similarly, phosphorylation imparts a negative charge, altering protein conformations and their interactions. Protein kinases, comprising ~2% of eukaryotic genomes, remove phosphate from ATP and covalently attach it to the free hydroxyl groups of serine, threonine, or tyrosine residues. The abilities of Ca2+ and phosphate ions to alter local electrostatic fields and protein conformations are the two universal tools of signal transduction.
- $Ca^{2+}$ had a new and totally unexpected function: it carried the information necessary for the contraction of heart.
- Changes in intracellular [$Ca^{2+}$] are detected by $Ca^{2+}$-binding proteins that regulate a variety of $Ca^{2+}$-dependent enzymes.

Example of $Ca^{2+}$-dependent enzymes**:**

- **Calmodulin (CaM)** (*M*r 17,000) is an acidic protein with four high-affinity $Ca^{2+}$-binding sites. When intracellular [$Ca^{2+}$] rises to about $10^{-6}$ M (1 $\mu$M), the binding of $Ca^{2+}$ to calmodulin drives a conformational change in the protein. Calmodulin associates with a variety of proteins and, in its $Ca^{2+}$-bound state, modulates their activities. Calmodulin is a member of a family of $Ca^{2+}$-binding proteins that also includes troponin, which triggers skeletal muscle contraction in response to increased [$Ca^{2+}$]. This family shares a characteristic $Ca^{2+}$-binding structure.
- **C**a*l*modulin is also an integral subunit of a family of enzyme[s,] the $Ca^{2+}$/calmodulin-dependent protein [k]inases (CaM kinases [I]–IV). *W*hen intracellular [$Ca^{2+}$] increases in response to some stimulus, calmodulin binds $Ca^{2+}$, undergoes a change in conformation, and activates the CaM ki[na]se. The kinase then phosphorylates a number of target enzymes, regulating their ac[ti]vities. Calmodulin is also a regulatory subunit of phosphorylase *b* kinase of muscle, which is activated by $Ca^{2+}$. Thus $^{Ca2+}$ triggers ATP-requiring muscle contract[io]ns while also activating glycogen breakdown, providing fuel for ATP synthesis. Many other enzymes are also known to be modulated by $Ca^{2+}$ through calmodulin.

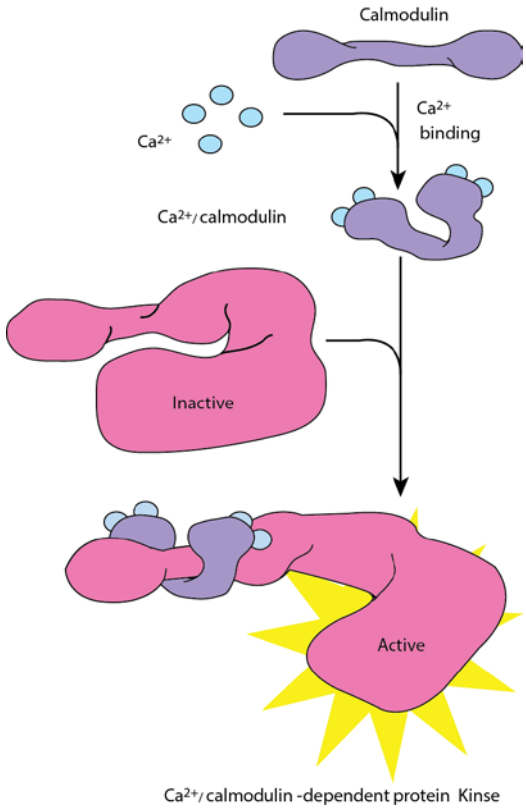


**Figure 3: Calcium ion dependent enzyme- calmodulin and Ca2+/calmodulin-dependent protein kinases**

- The rise of intracellular free $Ca^{2+}$ ($[Ca^{2+}]_i$) is an crucial triggering signal for T-cell activation by antigen and other stimuli that cross-link the T cell antigen receptor (TCR).
- The downstream consequences of $Ca^{2+}$ signalling, also involve in gene expression on the influence of signal complexity.

**Some Proteins Regulated by $Ca^{2+}$ and Calmodulin:**

- Adenylyl cyclase (brain)
- $Ca^{2+}$/calmodulin-dependent protein kinases (CaM kinases I to IV)
- $Ca^{2+}$-dependent $Na^{+}$ channel (*Paramecium*)
- $Ca^{2+}$_-release channel of sarcoplasmic reticulum
- Calcineurin (phosphoprotein phosphatase 2B)
- cAMP phosphodiesterase
- cAMP-gated olfactory channel
- cGMP-gated $Na^{+}$, $Ca^{2+}$ channels (rod and cone cells)
- Glutamate decarboxylase
- Myosin light chain kinases
- $NAD^{+}$ kinase
- Nitric oxide synthase
- Phosphoinositide 3-kinase
- Plasma membrane $Ca^{2+}$ ATPase ($Ca^{2+}$ pump)
- RNA helicase (p68)

**Interesting facts:**

1. Calcium ions are probably the most widely used intracellular messengers.

2. Normally, the level of calcium in the cell is very low (~100 nM).

3. Getting $Ca^{2+}$ into (and out of) the cytosol is via Voltage-gated channels.

**Questions:**

1. $Ca^{2+}$, $IP_3$ and cAMP have all been described as second messengers. In what ways are their mechanisms of action similar? In what ways are they different?

2. Describe the function of $Ca^{2+}$ as a second messenger.

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# M5 L6
# G proteins in signal transduction

**G- Protein:** G proteins (guanine nucleotide-binding proteins) are a family of proteins involved in transmitting chemical signals outside the cell, and causing changes inside the cell. They communicate signals from many hormones, neurotransmitters, and other signaling factors.

**Type of G protein:**

G protein can refer to two distinct families of proteins.

- **Heterotrimeric G proteins:** sometimes also known as the large G proteins that are activated by G protein-coupled receptors and made up of alpha (α), beta (β), and gamma (γ) subunits.

- **Small G proteins:** They are proteins of 20-25kDa that belong to the Ras superfamily of small GTPases. These proteins are homologous to the alpha (α) subunit found in heterotrimers, and are in fact monomeric. However, they also bind GTP and GDP and are involved in signal transduction.

**Heterotrimeric G-protein**

Heterotrimeric G proteins are more complex protein which were first characterized by Martin Rodbellare. It consists of three different subunits- α, β, and γ having molecular weight of these are 45, 37, and 9 kD respectively, among these α subunit binds to GDP in unactive state or GTP in active state, hence heterotrimeric G-protein is known as a member of the G protein superfamily. Heterotrimeric G proteins are held at the plasma membrane by lipid chains that are covalently attached to the α and γ subunits.

**Structure of Heterotrimeric G-protein:**

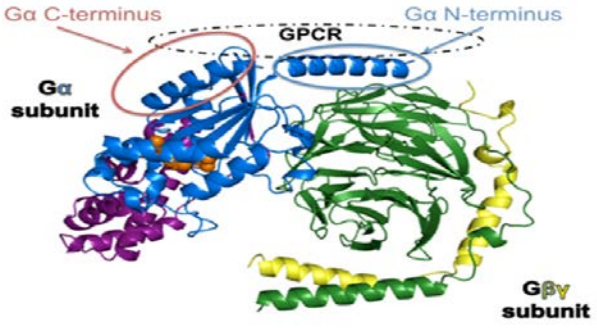


**Figure 1: Structure of Heterotrimeric G-protein**

The alpha subunit has two domains - the transducing insertion domain fold whereas the other is a P-loop containing nucleoside triphosphate hydrolase fold. P-loop or a phosphate-binding loop is an ATP or GTP- binding site motif found in many nucleotide-binding proteins. It is a glycine-rich loop led by a beta sheet and followed by an alpha helix. It interacts with the nucleotide phosphate groups and with the $Mg^{2+}$ ion that coordinates the β- and γ-phosphates in GTP. Upon nucleotide hydrolysis the P-loop does not significantly change conformation, but stays bound to the remaining phosphate groups. β- and γ- subunits are usually anchored to the membrane by covalently attached fatty acids. $G_{\beta\gamma}$ can also directly participate in signal transduction. It activates a wide variety of signaling proteins including several isoforms of Adenylate Cyclase.

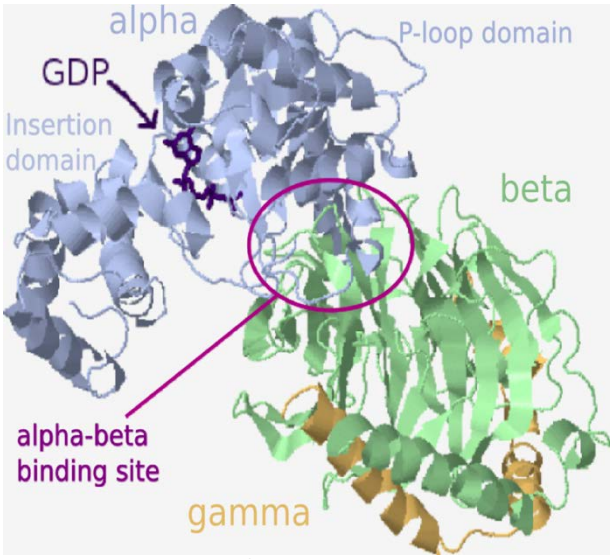


**Figure 2: Close view of G-protein with loops and subunits**

**Small G protein**

These proteins belong to a large superfamily referred to as small G proteins based on their low $M_r$ of 20,000 to 35,000. The small G proteins, like the heterotrimeric G proteins, bind guanine nucleotides, possess intrinsic GTPase activity and cycle through GDP- and GTP-bound forms. One unifying feature of the various classes of G protein is that the binding of GTP versus GDP dramatically alters the affinity of the protein for some target molecule, apparently by inducing a large conformational change. Small G proteins appear to function as molecular switches that control several cellular processes.

Examples of small G-protein: These are the following cellular actions which are performed by small G-protein.

**Table 1: Classification of small G-proteins on the basis of their functions**

| Sl. no. | Class | Proposed cellular function |
|---|---|---|
| 1. | Ras | Signal transduction (control of growth factor and MAP- kinase pathway) |
| 2. | Rac, CDC42 | Signal transduction (control of cellular stress responses and MAP-kinase pathways) |
| 3. | Rab | Localized to synaptic vesicle, where it regulates vesicle trafficking and exocytosis |
| 4. | Rho | Assembly of cytoskeleton structures (e.g. actin microfilament) |
| 5. | ARF | ADP-ribosylation |
| 6. | EFTU | Associated with ribosomes where it regulates protein synthesis |
| 7. | Ran | Nuclear-cytoplasmic trafficking of RNA and protein |

**Conformational changes occur in G protein during nucleotide exchange:**

When G- protein couple receptor comes in contact of ligand then some conformational changes occurs in GPCR; then the activated GPCR interact with G-protein, causes conformational change in alpha subunit of G-protein. Due to conformational change, the G-protein that bound to GDP prior to activation get exchange by GTP through which the three switch regions on the α- subunit close to the nucleoside triphosphate, generating the active conformation.

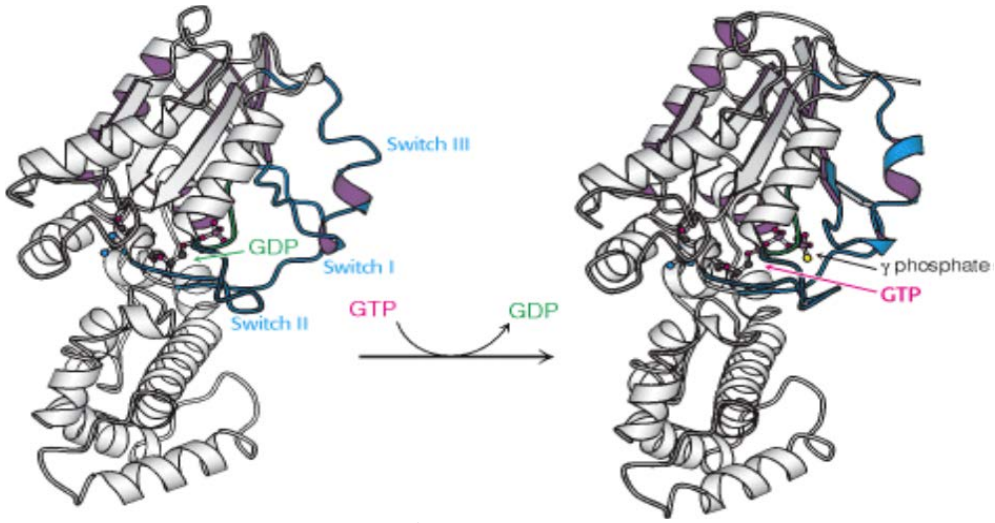


**Figure 3: Conformational change in G-protein**

**Nature of G protein:** G-Protein generally found in two states- *active form and unactive form.*

In unactivated state, the guanylyl nucleotide bound to the G-Protein is GDP. In this form, the G-protein exists as a heterotrimer consisting of α, β and γ subunits in which the α subunit ($G_\alpha$) binds to nucleotide. The role of the hormone- bound receptor is to catalyze the exchange of GTP for GDP. Thus, inactive G-protein converted to active form and $G_\alpha$ subunit has no much affinity for $G_{\beta\gamma}$ subunits, hence α-subunit dissociated from βγ-subunits. Now this is called activated state of G-protein. In activated state, $G_\alpha$ subunit stimulate effector protein such as adenylyl cyclase that lead to production of second messenger cAMP which may activate one or more signalling molecules. Other effectors are cGMP phosphodiesterase, phospholipase C-β. After dissociation from the $G_\alpha$ subunit, the βγ complex also has a signaling function and it can couple to at least four different types of effectors: PLC-β, $K^+$ ion channels, adenylyl cyclase, and PI 3-kinase.

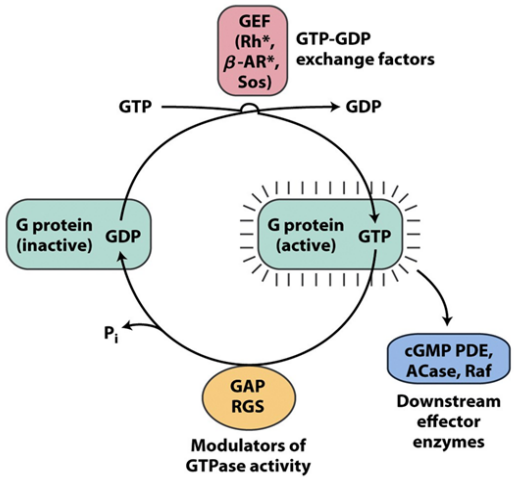


**Figure 4: Regulation of G- protein activation and inactivation**

After certain amount of time passed , the $G_\alpha$ turn themselves off by hydrolysis of bound GTP to GDP and inorganic phosphate (Pi). This results in conformational change caused decrease in affinity for effector and increase in affinity for βγ complex, thus $G_\alpha$ subunit dissociate from effector and associate with βγ subunit for the reformation of inactive form of G-protein.

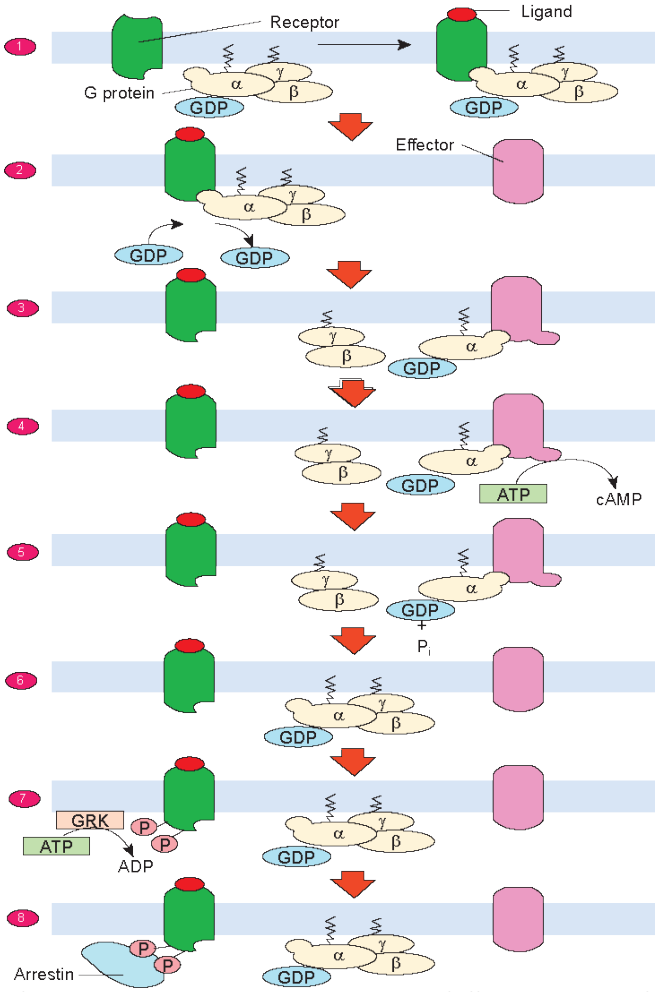


**Figure 5: The mechanism of receptor-mediated activation (or inhibition) of effectors by means of heterotrimeric G proteins**

Heterotrimeric G proteins come in five flavors, $G_s$, $G_q$, $G_{t\alpha}$, $G_i$, and $G_{12/13}$. This classification is based on the $G_\alpha$ subunits and the effectors to which they couple. The particular response produced by an activated GPCR depends on the type of G protein with which it interacts, although some GPCRs can interact with different G proteins and trigger more than one physiologic response.

1. **$G_s$** family members couple receptors to adenylyl cyclase. Adenylyl cyclase is activated by GTP-bound $G_s$ subunits.

2. **$G_{q\alpha}$,** Gq family members contain $G_\alpha$ subunits that activate PLC-β. PLC-β hydrolyzes phosphatidylinositol bisphosphate, producing inositol trisphosphate and diacylglycerol.
3. **Transducin ($G_{t\alpha}$)**, a variant of $G_{i\alpha}$, which transduces visual stimuli by coupling the light-induced conformational change of rhodopsin to the activation of a specific phosphodiesterase, which then hydrolyzes cGMP to GMP. This cGMP-phosphodiesterase (cGMP-PDE) is an $\alpha\beta\gamma_2$ heterotetramer that is activated by the displacement of its inhibitory subunits (PDE) by their tighter binding to $G_{t\alpha}$ . GTP. A cation-specific transmembrane channel that is held open by the binding of cGMP closes on the resulting reduction in [cGMP], thereby triggering a nerve impulse indicating that light has been detected.
4. **$G_i$** , Activated $G_i$ subunits function by inhibiting adenylyl cyclase
5. **$G_{olf}$,** a variant of $G_{s\alpha}$, which is expressed only in olfactory sensory neurons and participates in odorant signal transduction.
6. **$G_{12\alpha}$**and **$G_{13\alpha}$,** $G_{12/13}$ members are less well characterized than the other G protein families although their inappropriate activation has been associated with excessive cell proliferation and malignant transformation.

Thus heterogeneity in G proteins occurs in the β and γ subunits as well as in the α subunits. In fact, 21 different α subunits, 6 different β subunits, and 12 different γ subunits have been identified in humans, some of which appear to be ubiquitously expressed whereas others are expressed only in specific cells.Thus, a cell may contain several closely related G proteins of a given type that interact with varying specificities with receptors and effectors.This complex signalling system presumably permits cells to respond in a graded manner to a variety of stimuli.

**Role of G protein in signal transduction**

Since, organs or cells in order to do their function properly within an organism must have capacity to respond signals from distant cells as well as from its local environment. Thus the process in which information carried by extracellular messenger molecules is translated into changes that occur inside a cell is referred to as **signal transduction**. In this process:

- Cells in different organs communicate with one another through extracellular signalling molecules released by one set of cells and received by the other.
- Not all molecules can pass through the lipid bilayer of a Cell, and so signal transduction systems are used in order to transmit an external signal to the cell interior.

G-proteins play key role in signal transduction with the help of G- protein couple receptor which abbreviated as GPCR.

**G- protein couple receptor:** GPCR comprise a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways and ultimately, cellular responses. G protein-coupled receptors are found only in eukaryotes, including yeast, choanoflagellates and animals. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, and vary in size from small molecules to peptides to large proteins. G protein-coupled receptors are involved in many diseases, and are also the target of approximately 40% of all modern medicinal drugs.

**Classification of GPCR:** The exact size of the GPCR superfamily is unknown but nearly 800 different human genes (or ≈4% of the entire protein-coding genome) have been predicted from genome sequence analysis. Although numerous classification schemes have been proposed, the superfamily is classically divided into three main classes (A, B, and C) with no detectable shared sequence homology between classes. The largest class so far is class A, which accounts for nearly 85% of the GPCR genes. Of class A GPCRs, over half of these are predicted to encode olfactory receptors while the remaining receptors are liganded by known endogenous compounds or are classified as orphan receptors. Despite the lack of sequence homology between classes, all GPCRs share a common structure and mechanism of signal transduction.

In general, GPCRs can be classified into 5 classes based on sequence homology and functional similarity:

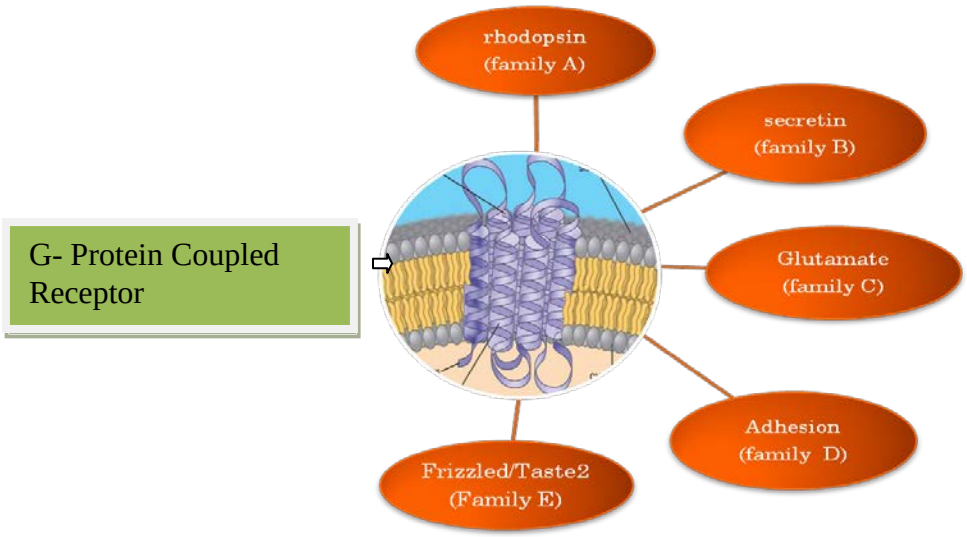


**Figure 6: Classification of G-protein**

**Role of G- protein coupled receptor:** GPCRs are involved in a wide variety of physiological processes. Some examples of their physiological roles are as follows:

1. The visual sense: Rhodopsine which is complex of opsins and chromophore 11-cis retinal, use a photoisomerization reaction to translate electromagnetic radiation into cellular signals due to the conversion of *11-cis*-retinal to *all-trans*-retinal.
2. The sense of smell: receptors of the olfactory epithelium bind odorants (olfactory receptors) and pheromones (vomeronasal receptors)
3. Behavioral and mood regulation: receptors in the mammalian brain bind several different neurotransmitters, including serotonin, dopamine, GABA, and glutamate
4. Regulation of immune system activity and inflammation: Chemokine receptors bind ligands that mediate intercellular communication between cells of the immune system; receptors such as histamine receptors bind inflammatory mediators and engage target cell types in the inflammatory response
5. Autonomic nervous system transmission: Both the sympathetic and parasympathetic nervous systems are regulated by GPCR pathways, responsible for control of many automatic functions of the body such as blood pressure, heart rate, and digestive processes
6. Cell density sensing: A novel GPCR role in regulating cell density sensing.
7. Homeostasis modulation (water balance).

**Mechanism of action:** When a ligand binds to the GPCR it causes a conformational change in the GPCR, which allows it to act as a guanine nucleotide exchange factor (GEF). The GPCR then activate an associated G-protein by exchanging its bound GDP for a GTP. The G-protein's α subunit, together with the bound GTP then dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the α subunit type ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$).

There are two principal signal transduction pathways followed by the G protein-coupled receptors:

- the cAMP signal pathway
- the Phosphatidylinositol signal pathway.

**Adenylate Cyclase regulated by $G_s$ and $G_i$ type of $G_\alpha$**

The adenylate cyclase pathway regulated by both stimulatory and inhibitory subunits of G protein $G_s$ and $G_i$ respectively.

In case of stimulatory subunit, $G_\alpha$ have intrinsic GTPase activity, which is used to hydrolyze bound GTP to GDP and $P_i$. This hydrolysis reaction is slow, however, requiring from second to minutes. Thus the GTP form of $G_\alpha$ is able to activate downstream components of signal transduction pathway before GTP hydrolysis that deactivates the subunit. In essence, the bound GTP acts as a built in clock that spontaneously resets the $G_\alpha$ subunit after a short time period. After GTP hydrolysis and release of $P_i$, the GDP –bound form of $G_\alpha$ then reassociates with $G_{\beta\gamma}$ to re-form the inactive heterotrimeric protein. Since $G_\alpha$ hydrolyzes its bound GTP at a characteristic rate, it functions as a molecular clock that limits the length of time that both $G_\alpha$. GTP and $G_{\beta\gamma}$ can interact with their effectors.

On the other hands, $G_{\beta\gamma}$ can also directly participate in signal transduction by activation of wide variety of signaling proteins including several isoforms of AC, certain $Na^+$, $K^+$ and $Ca^{2+}$-specific ion channels, various protein tyrosine kinases and phospholipase C- β (PLC-β; a component of the phospho-inositide signaling system; Section. $G_{\beta\gamma}$ thereby provides an important source of cross talk between signaling systems.

“Several types of ligand–GPCR complexes may activate the same G protein.This occurs, for example, in liver cells in response to the binding of the corresponding hormones to

glucagon receptors and to β-adrenergic receptors. In such cases, the amount of cAMP produced is the sum of that induced by the individual hormones."

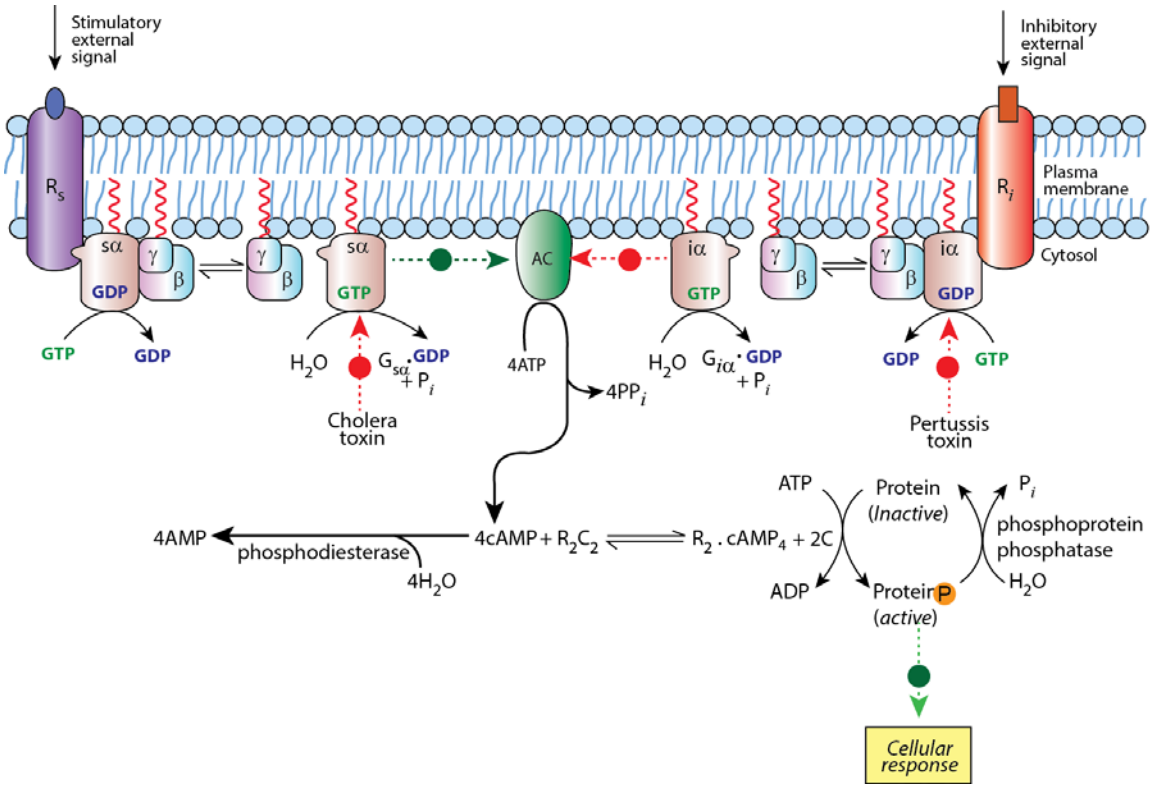


**Figure 6: Mechanism of receptor-mediated activation/inhibition of Adenylate Cyclase.**

In case of inhibitory type of α-subunit, some ligand–GPCR complexes inhibit rather than activate AC. These include the *$\alpha_2$-adrenergic receptor* and receptors for *somatostatin and opioids*. The inhibitory effect is mediated by "inhibitory" G protein, **$G_i$**, which may have the same β and γ subunits as does "stimulatory" G protein, $G_s$, but has a different α subunit,$G_{iα}$ (41 kD). $G_i$ acts analogously to $G_s$ in that on binding to its corresponding ligand–GPCR complex, its $G_{iα}$ subunit exchanges bound GDP for GTP and dissociates from G. However, $G_{iα}$inhibits rather than activates AC, through direct interactions and possibly because the liberated $G_{βγ}$ binds to and sequesters **$G_{sα}$**. The latter mechanism is supported by the observation that liver cell membranes contain far more $G_i$ than $G_s$. The activation of $G_i$ in such cells would therefore release enough $G_{iα}$ to bind than available $G_s$.

**Cholera Toxin Stimulates Adenylate Cyclase by Permanently Activating $G_{s\alpha}$:**

The major symptom of cholera, an intestinal disorder caused by the bacterium *Vibrio cholerae*, is massive diarrhea that, if untreated, frequently results in death from dehydration. This dreaded disease is not an infection in the usual sense since the vibrio neither invades nor damages tissues but merely colonizes the intestine, much like E. coli. The catastrophic fluid loss that cholera induces (often over 6 liters per hour!) occurs in response to a bacterial toxin. Indeed, merely replacing cholera victims' lost water and salts enables them to survive the few days necessary to immunologically eliminate the bacterial infestation.

Cholera toxin (CT; also known as choleragen) is an 87-kD protein of subunit composition AB5 in which the B subunits (103 residues each) form a pentagonal ring to which the A subunit (240 residues) is bound its A subunit is cleaved at a single site by a bacterial protease to yield two fragments, A1 (the N-terminal ~195 residues) and A2 (the C-terminal ~45 residues), that remain joined by a disulfide bond. In the cytoplasm, A1 catalyzes the irreversible transfer of the ADP–ribose unit from NAD to a specific Arg side chain of Gs. This reaction is greatly accelerated by the interaction of A1 with the small Ras-like G protein ADP- ribosylation factor (ARF) in complex with GTP, which normally functions to prime the formation of clathrin-coated vesicles.

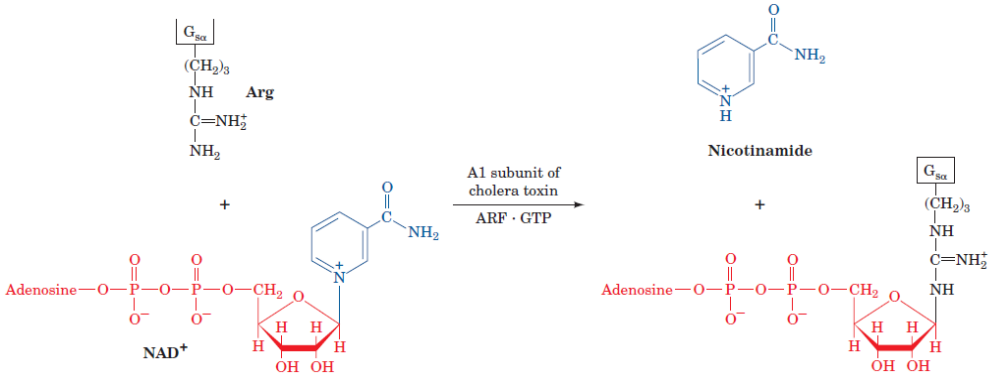

**Figure 7: Mechanism of action of cholera toxin. The cholera toxin's A1 fragment in complex with ARF. GTP catalyzes the ADP- ribosylation of a specific Arg residue on $G_{s\alpha}$ by $NAD^+$, thereby rendering this subunit incapable of hydrolysing GTP.**

ADP-ribosylated $G_{s\alpha}$ GTP can activate AC but is incapable of hydrolyzing its bound GTP. As a consequence, the AC remains "locked" in its active state. The epithelial cells of the small intestine normally secrete digestive fluid (an – $HCO_3^-$ rich salt solution) in response to small increases in [cAMP] that activate intestinal $Na^+$ pumps through their phosphorylation by PKA. The ~100-fold rise in intracellular [cAMP] induced by CT causes these epithelial cells to pour out enormous quantities of digestive fluid, thereby producing the symptoms of cholera. $G_{s\alpha}$ and $G_{i\alpha}$ are members of a family of related proteins, many of which have downstream effectors other than AC.

**Termination of GPCR and interrupt binding with G protein**

The hormone-bound activated receptor must be reset as well to prevent the continuous activation of G proteins. This resetting is accomplished by two processes.

First, the hormone dissociates, returning the receptor to its initial, unactivated states. The likelihood that the receptor remains in its unbound states depends on the concentration of hormone.

Second, the hormone- receptor complex is deactivated by the phosphorylation of serine and threonine residues in the carboxyl-terminal tail of the receptor kinase (also called G protein receptor kinase 2, GRK 2) phosphorylates the carboxyl terminal tail of hormones-receptor complex but not the unoccupied receptor. Finally, the molecule β-arrestin binds to the phosphorylated receptor and further diminish its ability to activate G-protein.

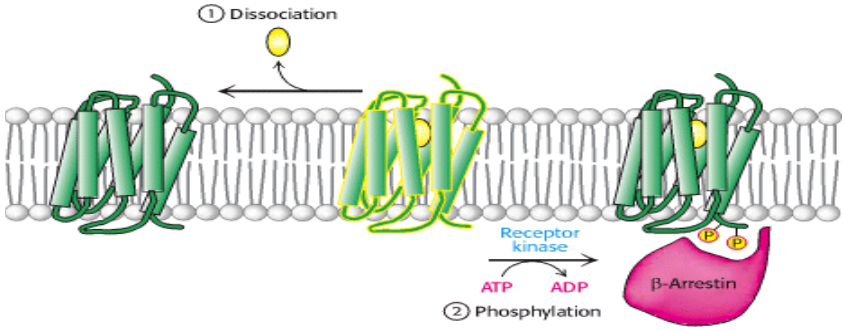


**Figure-8: Signal termination**

**Human Diseases Linked to the G Protein Pathway**

There are so many human diseases are related to disfunction of G-protein and G-protein coupled receptor which may be due to mutation in the genes that encode for these proteins. Some of them are given in the following table:

**Table 2: Disease due defective G-protein**

| Sl. No. | Disease | Defective G-protein |
|---|---|---|
| 1. | Albright's hereditary osteodystrophy and pseudohypathyroidism | $G_{S\alpha}$ |
| 2. | McCune-Albright syndrome | $G_{s\alpha}$ |
| 3. | Pituitary, thyroid tumors(gsp oncogene) | $G_{s\alpha}$ |
| 4. | Adernocortical, ovarian tumors (*gip* oncogene) | $G_{i\alpha}$ |
| 5. | Combined precocious puberty and pseudohypoparathyroidism | $G_{s\alpha}$ |

**Table 3: Disease due to defective G-protein couple receptor**

| Sl. No. | Disease | Defective G- protein couple receptor |
|---|---|---|
| 1. | Family hypocalciuric hypercalcemia | Human analogue of BoPCAR1 receptor |
| 2. | Neonatal severe hyperparathyroidism (thyroid adenomas) | Human analogue of BoPCAR1 receptor |
| 3. | hyperparathyroidism (thyroid adenomas) | Thyrotrophin receptor |
| 4. | Familial male precocious puberty | Luteinizing Hormone receptor |
| 5. | X-linked nephrogenic diabetes inspidus | V2 vasopressin recept |
| 6. | Retinitis pigmentosa | Rhodopsine receptor |
| 7. | Color blindness, spectral sensitivity variations | Cone opsin receptor |
| 8. | Familial glucocorticoid deficiency and isolated glucocorticoid deficiency | Adernocorticotrophic hormone (ACTH) receptor |

The mechanism for transmitting signals across the plasma membrane by G proteins is of ancient evolutionary origin and is highly conserved.

**G Proteins often Require Accessory Proteins to Function**

The proper physiological functioning of a G protein often requires the participation of several other types of proteins which are described below:

1. A GTPase-activating protein (GAP), which as its name implies, stimulates its corresponding G protein to hydrolyze its bound GTP. This rate enhancement can be > 2000-fold. The downstream effectors of $G_{t\alpha}$ and $G_{qt}$, cGMP-PDE and PLC-β respectively, exhibit GAP activities toward $G_{t\alpha}$ and $G_{q\alpha}$ (which otherwise would hydrolyze GTP at physiologically insignificant rates), but AC does not exhibit GAP activity toward either $G_{s\alpha}$ or $G_{i\alpha}$. However, in humans, a diverse family of 37 RGS proteins (for regulators of G protein signaling) function as GAPs for $G_{\alpha}$ subunits by binding most avidly to them when they are in the transition state conformation for hydrolyzing GTP.

2. A guanine nucleotide exchange factor [GEF; alternatively guanine nucleotide releasing factor (GRF)], which induces its corresponding G protein to release its bound GDP. The G protein subsequently binds another guanine nucleotide (GTP or GDP, which most G proteins bind with approximately equal affinities), but since cells maintain a GTP concentration that is 10-fold higher than that of GDP, this, in effect, exchanges the bound GDP for GTP. For heterotrimeric G proteins, the agonist–GPCR complexes function as GEFs.

3. A guanine nucleotide-dissociation inhibitor (GDI). A $G_{\beta\gamma}$ may be regarded as its associated $G_{\alpha}$'s GDI because GDP dissociates slowly from isolated $G_{\alpha}$ subunits but is essentially irreversibly bound by heterotrimers.

**Interesting facts:**

1. G proteins are molecular switches that use GDP to control their signaling cycle. G protein is inactive when GDP bounds. To activate the protein, the GDP is replaced with GTP, and then G protein will deliver its signal.
2. The G protein system plays a central role in many signalling tasks, so it became sensitive target for many drugs and toxins.

3. The diversity of GPCRs is observed not only by the multiplicity of stimuli to which they respond, but also by the variety of intracellular signalling pathways they activate

**Questions:**

1. How G-protein activated when light fall on eye? What is the role of G-protein in signalling in eye?
2. What are the effect of cholera toxin in cell signaling?
3. Which are the enzyme dependent on G-protein activation? How G-protein involve in signal transduction?

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