CELL SIGNALING

GPCR and role of second messenger (c-AMP)

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CELL SIGNALING

Vital Concepts of Cell Biology

What is cell signaling?

In biology, **cell signaling** is part of any communication process that governs basic activities of cells and coordinates multiple-cell actions.

The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity, as well as normal tissue homeostasis.

Errors in signaling interactions and cellular information processing may cause diseases such as cancer, autoimmunity, and diabetes.

By understanding cell signaling, clinicians may treat diseases more effectively and, theoretically, researchers may develop artificial tissues.

A BRIEF SYNOPSIS OF THE MECHANISM OF CELL SIGNALING

All cells receive and respond to signals from their surroundings. This is accomplished by a variety of signal molecules that are secreted or expressed on the surface of one cell and bind to a receptor expressed by the other cells, thereby integrating and coordinating the function of the many individual cells that make up organisms. Each cell is programmed to respond to specific extracellular signal molecules. Extracellular signaling usually entails the following steps:

- Synthesis and release of the signaling molecule by the signaling cell
- Transport of the signal to the target cell
- Binding of the signal by a specific receptor leading to its activation
- Initiation of signal-transduction pathways.

Classification of Cell Signaling

Parameters		
Between Cells of the same organism	Type of signal transmitted	Between the cells of different organism
➢Intracellular	➢Mechanical	➢Intra-species
➢Intercellular	➢Bio-Chemical	➢Inter-species

SIGNALING BETWEEN CELLS OF SINGLE ORGANISM

Based signaling between cells of single organism, cell signaling is divided into-

- **Intracellular:** In this type of Cell Signaling, the signal specifically called “Intracellular signals” are produced and received by the same cell.

- **Intercellular:** In this type of cell signaling, the signals, specifically called “intracellular signals” are produced and received by distinct cells.

TYPES OF SIGNAL TRANSMITTED

Based upon the type of signal, cell signaling is divided into_

- **Mechanical:** Mechanical signals are the forces exerted on the cell and the forces produced by the cell. These forces can both be sensed and responded to by the cells.

- **Biochemical:** Biochemical signals are biochemical molecules such as proteins, lipids, ions, and gases

FURTHER DIFFERENTIATION OF BIOCHEMICAL SIGNALS

As we know biochemical signals are biochemical molecules such as proteins, lipids, ions, and gases. These signals can be categorized based on the distance between signaling and responder cells. Signaling within, between, and amongst cells is subdivided into the following classifications:

- ***Intracrine:*** These signals are produced by the target cell that stay within the target cell.
- ***Autocrine:*** These signals are produced by the target cell, are secreted, and affect the target cell itself via receptors. Sometimes autocrine cells can target cells close by if they are the same type of cell as the emitting cell. An example of this are immune cells.
- ***Juxtacrine:*** These signals target adjacent (touching) cells. These signals are transmitted along cell membranes via protein or lipid components integral to the membrane and are capable of affecting either the emitting cell or cells immediately adjacent.
- ***Paracrine:*** These signals target cells in the surrounding area of the emitting cell. Neurotransmitters represent an example.
- ***Endocrine:*** These signals target distant cells. Endocrine cells produce hormones that travel through the blood to reach all parts of the body.

BETWEEN THE CELLS OF DIFFERENT ORGANISM

- **Intra-species:** This type of cell signaling occurs between the cell of two or more organisms of the same species.

  Intra-species signaling occurs especially in bacteria, yeast, social insects, but also many vertebrates.

- **Inter-species:** This type of cell-signaling occurs between two or more organisms of different species. .

  In some cases of interspecies signaling, the emitting organism can actually be a host of the receiving organism, or vice versa

Mechanism of Cell Signaling

A fundamental preamble

How do cells recognize signals?

Cells have proteins called **receptors** that bind to signalling molecules and initiate a physiological response. Different receptors are specific for different molecules. Dopamine receptors bind dopamine, insulin receptors bind insulin, nerve growth factor receptors bind nerve growth factor, and so on. In fact, there are hundreds of receptor types found in cells, and varying cell types have different populations of receptors. Receptors can also respond directly to light or pressure, which makes cells sensitive to events in the atmosphere.

A BRIEF DISCUSSION ABOUT RECEPTORS ?

Receptors are generally transmembrane proteins, which bind to signaling molecules outside the cell and subsequently transmit the signal through a sequence of molecular switches to internal signaling pathways. {See figure}

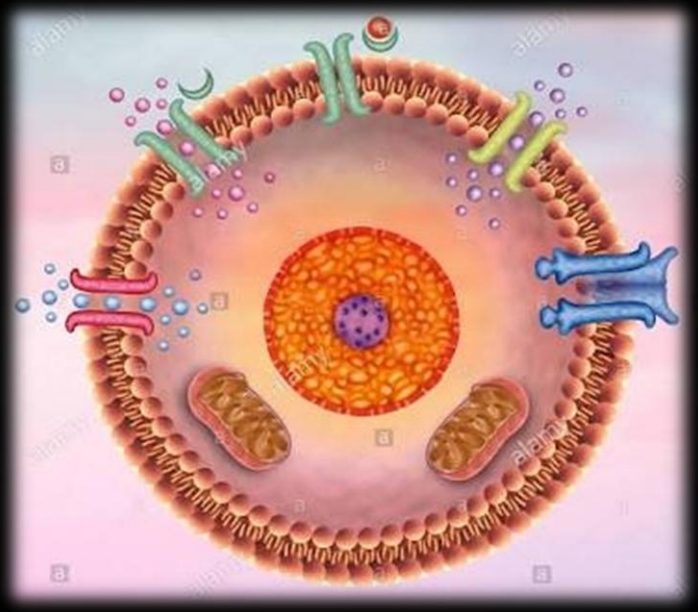


Fig: 1

Classification of Receptors

Membrane receptors fall into three major classes:

- G-protein-coupled receptors,
- Ion channel receptors, and
- Enzyme-linked receptors.

The names of these receptor classes refer to the mechanism by which the receptors transform external signals into internal ones — via protein action, ion channel opening, or enzyme activation, respectively. Because membrane receptors interact with both extracellular signals and molecules within the cell, they permit signaling molecules to affect cell function without actually entering the cell. This is important because most signaling molecules are either too big or too charged to cross a cell's plasma membrane.

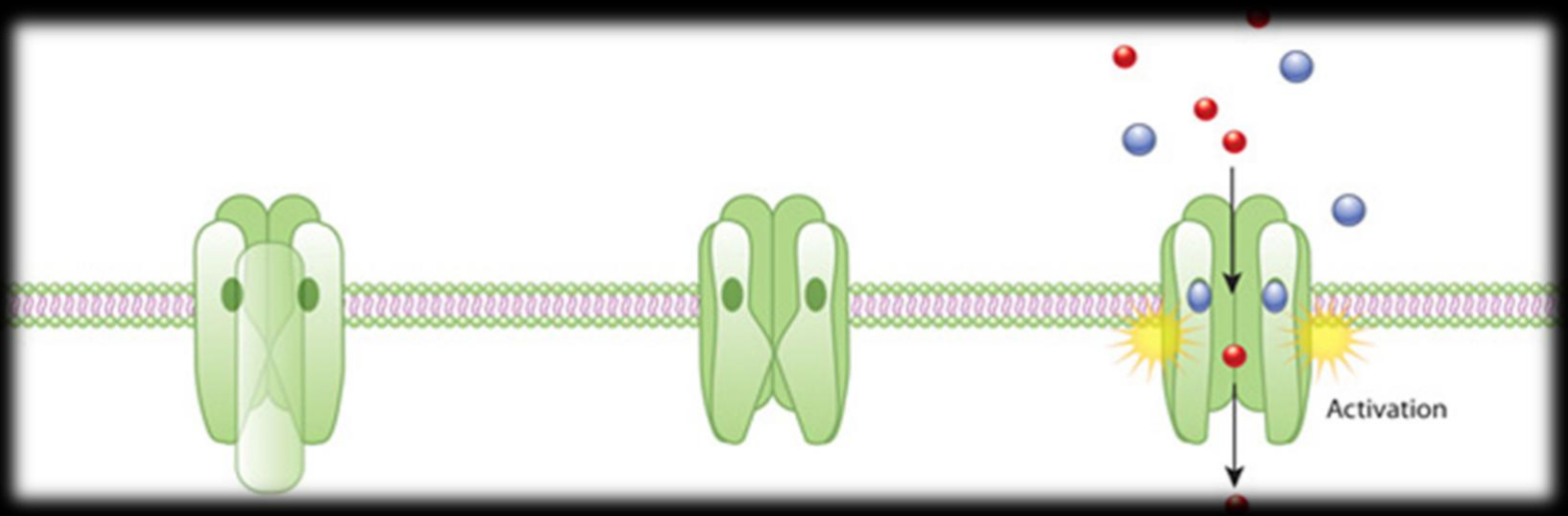


Figure 2: An example of ion channel activation
An acetylcholine receptor (green) forms a gated ion channel in the plasma membrane. This receptor is a membrane protein with an aqueous pore, meaning it allows soluble materials to travel across the plasma membrane when open. When no external signal is present, the pore is closed (center). When acetylcholine molecules (blue) bind to the receptor, this triggers a conformational change that opens the aqueous pore and allows ions (red) to flow into the cell.

CONTINUED

The activation of adenylyl cyclase can result in the manufacture of hundreds or even thousands of cAMP molecules. These cAMP molecules activate the enzyme **protein kinase A** (PKA), which then **phosphorylates** multiple protein substrates by attaching phosphate groups to them. Each step in the cascade further amplifies the initial signal, and the phosphorylation reactions mediate both short- and long-term responses in the cell (Figure 2). How does cAMP stop signaling? It is degraded by the enzyme phosphodiesterase.

Other examples of second messengers include **diacylglycerol** (DAG) and **inositol 1,4,5-triphosphate** (IP3), which are both produced by the enzyme **phospholipase**, also a membrane protein. IP3 causes the release of Ca^{2+} — yet another second messenger — from intracellular stores. Together, DAG and Ca^{2+} activate another enzyme called **protein kinase C** (PKC).

Figure 3: An example of a signal transduction cascade involving cyclic AMP

The binding of adrenaline to an adrenergic receptor initiates a cascade of reactions inside the cell. The signal transduction cascade begins when adenylyl cyclase, a membrane- bound enzyme, is activated by G-protein molecules associated with the adrenergic receptor. Adenylyl cyclase creates multiple cyclic AMP molecules, which fan out and activate protein kinases (PKA, in this example). Protein kinases can enter the nucleus and affect transcription

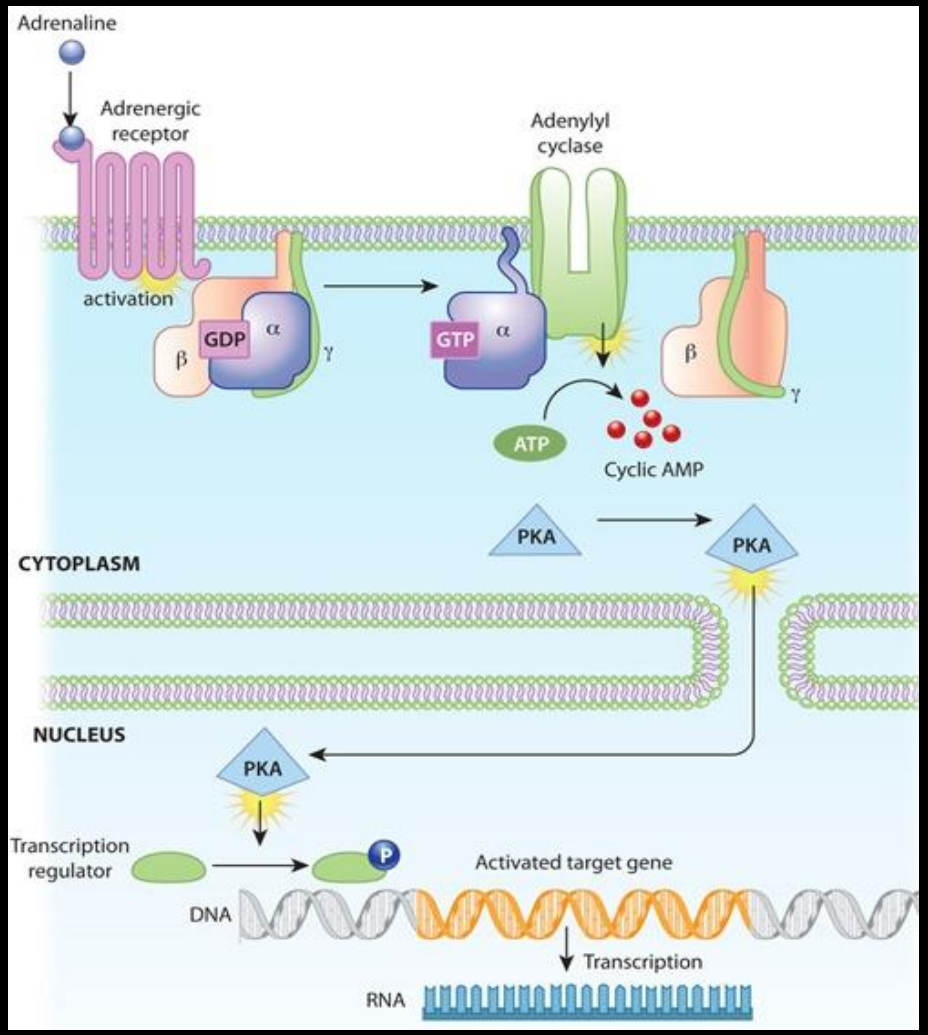


Fig: 3

Effects of Signals Upon Cell Functions

- Protein kinases such as PKA and PKC catalyze the transfer of phosphate groups from ATP molecules to protein molecules. Within proteins, the amino acids serine, threonine, and tyrosine are especially common sites for phosphorylation. These phosphorylation reactions control the activity of many enzymes involved in intracellular signaling pathways. Specifically, the addition of phosphate groups causes a conformational change in the enzymes, which can either activate or inhibit the enzyme activity. Then, when appropriate, protein phosphatases remove the phosphate groups from the enzymes, thereby reversing the effect on enzymatic activity.

Response of Cells to Signals

Once a receptor protein receives a signal, it undergoes a conformational change, which in turn launches a series of biochemical reactions within the cell. These intracellular signaling pathways, also called **signal transduction cascades**, typically amplify the message, producing multiple intracellular signals for every one receptor that is bound.

Activation of receptors can trigger the synthesis of small molecules called **second messengers**, which initiate and coordinate intracellular signaling pathways. For example, **cyclic AMP** (cAMP) is a common second messenger involved in signal transduction cascades. (In fact, it was the first second messenger ever discovered.) cAMP is synthesized from ATP by the enzyme **adenylyl cyclase**, which resides in the cell membrane.

CONTINUED

- Phosphorylation allows for intricate control of protein function. Phosphate groups can be added to multiple sites in a single protein, and a single protein may in turn be the substrate for multiple kinases and phosphatases.
- At any one time, a cell is receiving and responding to numerous signals, and multiple signal transduction pathways are operating in its cytoplasm. Many points of intersection exist among these pathways. For instance, a single second messenger or protein kinase might play a role in more than one pathway. Through this network of signaling pathways, the cell is constantly integrating all the information it receives from its external environment.

CONCLUSION

Cells typically receive signals in chemical form via various signaling molecules. When a signaling molecule joins with an appropriate receptor on a cell surface, this binding triggers a chain of events that not only carries the signal to the cell interior, but amplifies it as well. Cells can also send signaling molecules to other cells. Some of these chemical signals — including neurotransmitters — travel only a short distance, but others must go much farther to reach their targets.

GPCR

A special look at GPCR proteins

INTRODUCTION

- **G-protein-coupled receptors** (GPCRs) are the largest and most diverse group of membrane receptors in eukaryotes. These cell surface receptors act like an inbox for messages in the form of light energy, peptides, lipids, sugars, and proteins. Such messages inform cells about the presence or absence of life-sustaining light or nutrients in their environment, or they convey information sent by other cells.
- GPCRs play a role in an incredible array of functions in the human body, and increased understanding of these receptors has greatly affected modern medicine. In fact, researchers estimate that between one-third and one-half of all marketed drugs act by binding to GPCRs.

APPEARANCE

- GPCRs bind a tremendous variety of signaling molecules, yet they share a common architecture that has been conserved over the course of evolution. Many present-day eukaryotes — including animals, plants, fungi, and protozoa — rely on these receptors to receive information from their environment. For example, simple eukaryotes such as yeast have GPCRs that sense glucose and mating factors. Not surprisingly, GPCRs are involved in considerably more functions in multicellular organisms. Humans alone have nearly 1,000 different GPCRs, and each one is highly specific to a particular signal.
- GPCRs consist of a single polypeptide that is folded into a globular shape and embedded in a cell's plasma membrane. Seven segments of this molecule span the entire width of the membrane — explaining why GPCRs are sometimes called **seven-transmembrane receptors** — and the intervening portions loop both inside and outside the cell. The extracellular loops form part of the pockets at which signaling molecules bind to the GPCR.

ROLE OF GPCR

- As their name implies, GPCRs interact with G proteins in the plasma membrane. When an external signaling molecule binds to a GPCR, it causes a conformational change in the GPCR. This change then triggers the interaction between the GPCR and a nearby G protein.
- **G proteins** are specialized proteins with the ability to bind the nucleotides guanosine triphosphate (GTP) and guanosine diphosphate (GDP). Some G proteins, such as the signaling protein Ras, are small proteins with a single subunit. However, the G proteins that associate with GPCRs are **heterotrimeric**, meaning they have three different subunits: an alpha subunit, a beta subunit, and a gamma subunit. Two of these subunits — alpha and gamma — are attached to the plasma membrane by lipid anchors (Figure 4).

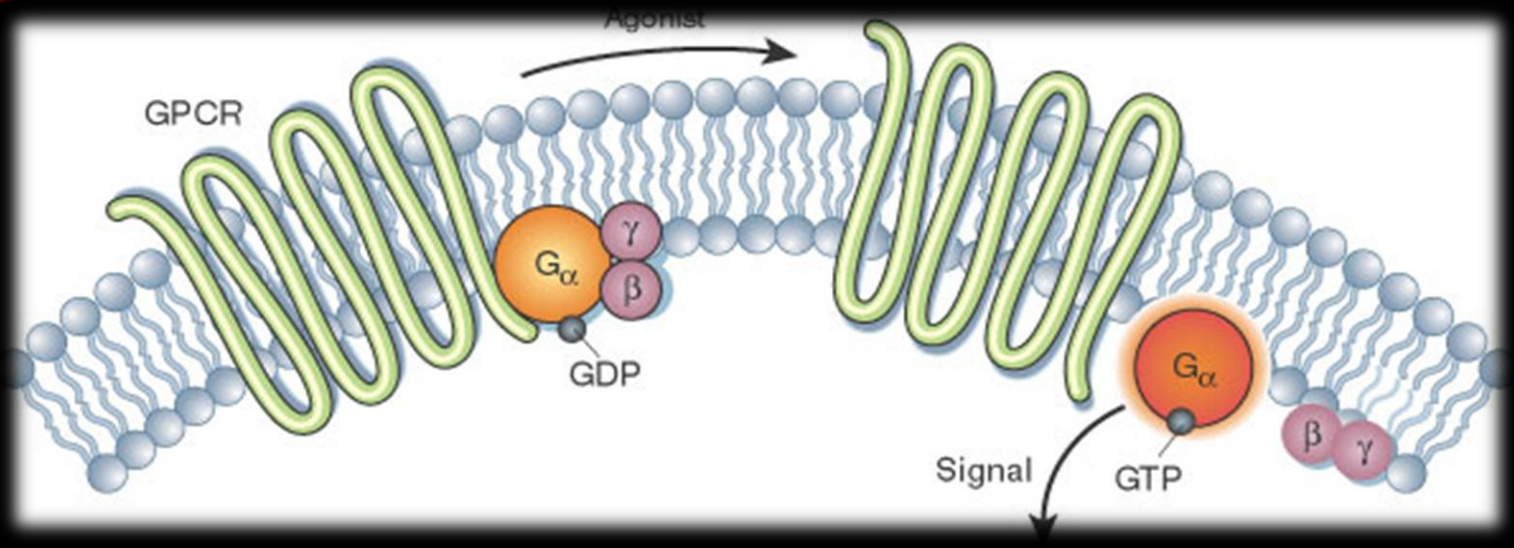
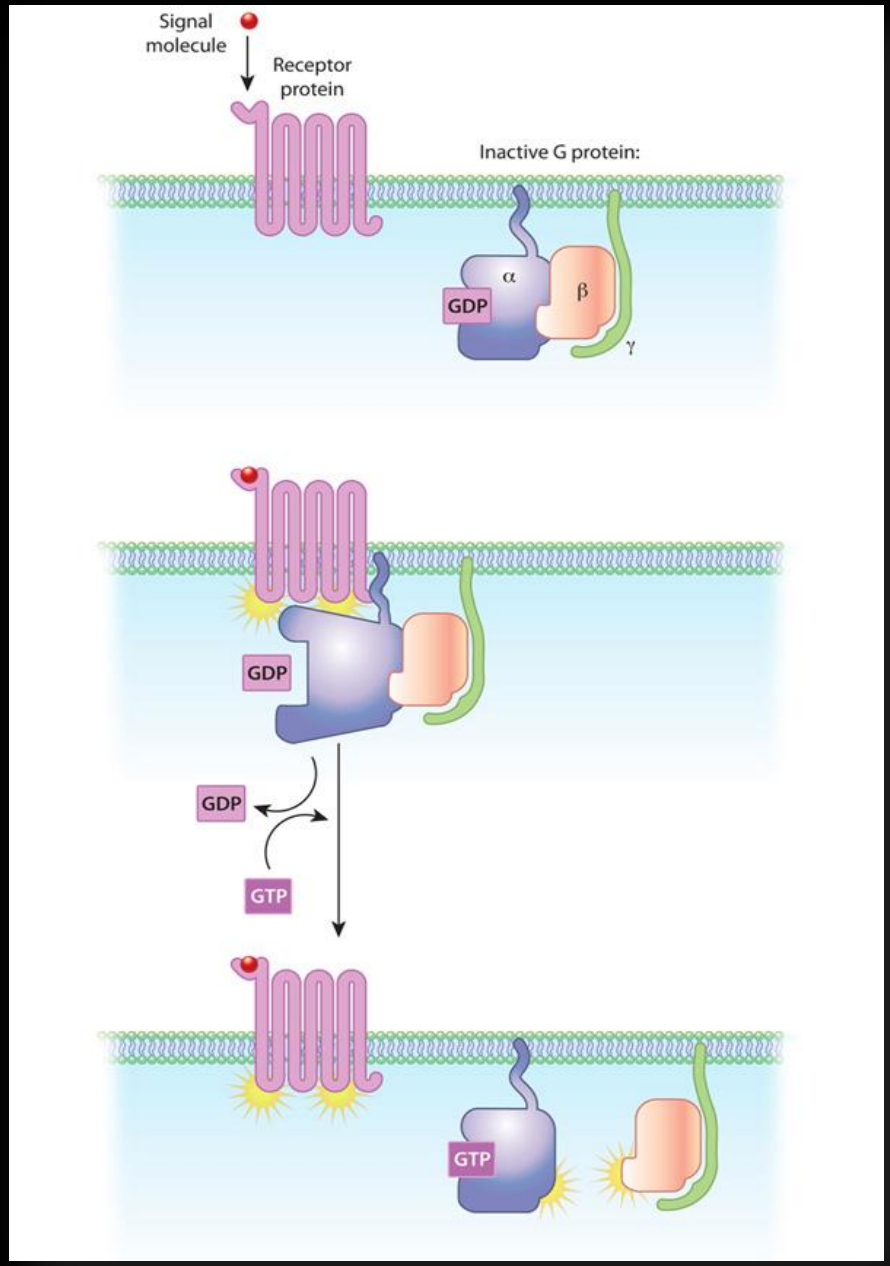


Figure 4: Activation of the G alpha subunit of a G-protein-coupled receptor

In unstimulated cells, the state of G alpha (orange circles) is defined by its interaction with GDP, G beta-gamma (purple circles), and a G-protein-coupled receptor (GPCR; light green loops). Upon receptor stimulation by a ligand called an agonist, the state of the receptor changes. G alpha dissociates from the receptor and G beta-gamma, and GTP is exchanged for the bound GDP, which leads to G alpha activation. G alpha then goes on to activate other molecules in the cell.

Figure 5: The relationships of G proteins to the plasma membrane

In this diagram of G-protein-coupled receptor activation, the alpha, beta, and gamma subunits are shown with distinct relationships to the plasma membrane. After exchange of GDP with GTP on the alpha subunit, both the alpha subunit and the beta-gamma complex may interact with other molecules to promote signaling cascades. Note that both the alpha subunit and the beta-gamma complex remain tethered to the plasma membrane while they are activated. These activated subunits can act on ion channels in the cell membrane, as well as cellular enzymes and second messenger molecules that travel around the cell.



What second messengers do GPCR signals trigger in cells ?

- Activation of a single G protein can affect the production of hundreds or even thousands of second messenger molecules (Figure 3). (Recall that second messengers — such as cyclic AMP [cAMP], diacylglycerol [DAG], and inositol 1, 4, 5-triphosphate [IP3] — are small molecules that initiate and coordinate intracellular signaling pathways.) One especially common target of activated G proteins is adenylyl cyclase, a membrane-associated enzyme that, when activated by the GTP-bound alpha subunit, catalyzes synthesis of the second messenger cAMP from molecules of ATP. In humans, cAMP is involved in responses to sensory input, hormones, and nerve transmission, among others.
- Phospholipase C is another common target of activated G proteins. This membrane-associated enzyme catalyzes the synthesis of not one, but two second messengers — DAG and IP3 — from the membrane lipid phosphatidyl inositol. This particular pathway is critical to a wide variety of human bodily processes. For instance, thrombin receptors in platelets use this pathway to promote blood clotting.

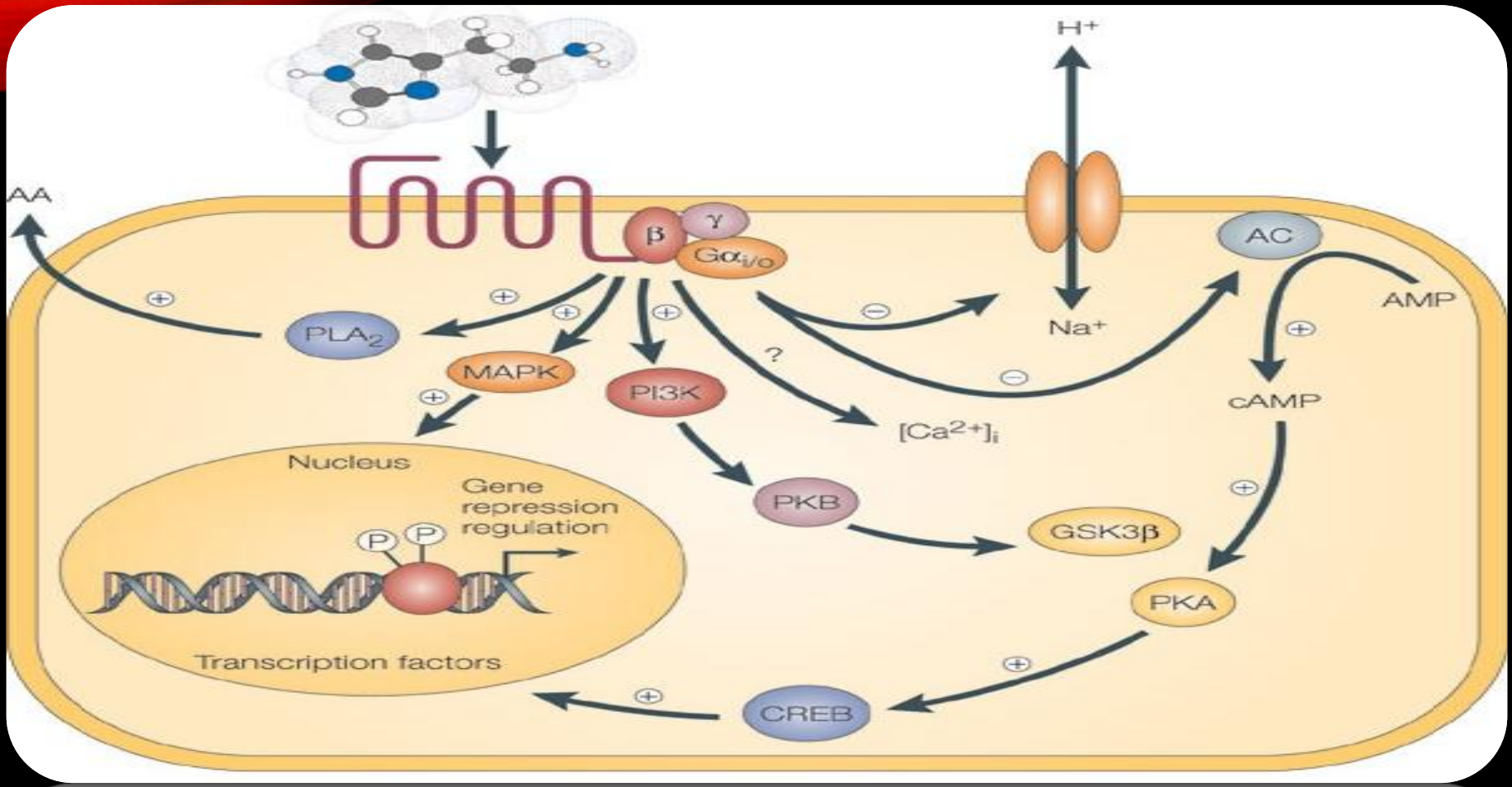


Figure 7: Signaling cascades within a cell can interact to affect multiple molecules in the cell, leading to secretion of substances from the cell, ion channel opening, and transcription.

CONCLUSION

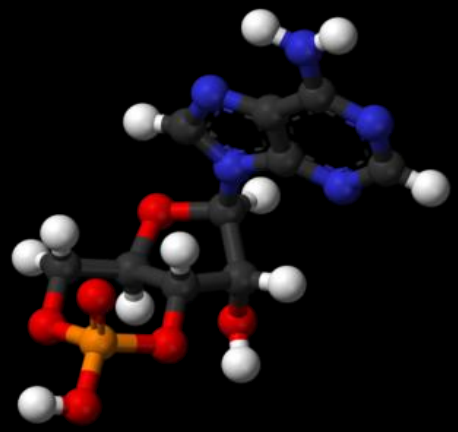
GPCRs are a large family of cell surface receptors that respond to a variety of external signals. Binding of a signaling molecule to a GPCR results in G protein activation, which in turn triggers the production of any number of second messengers. Through this sequence of events, GPCRs help regulate an incredible range of bodily functions, from sensation to growth to hormone responses.

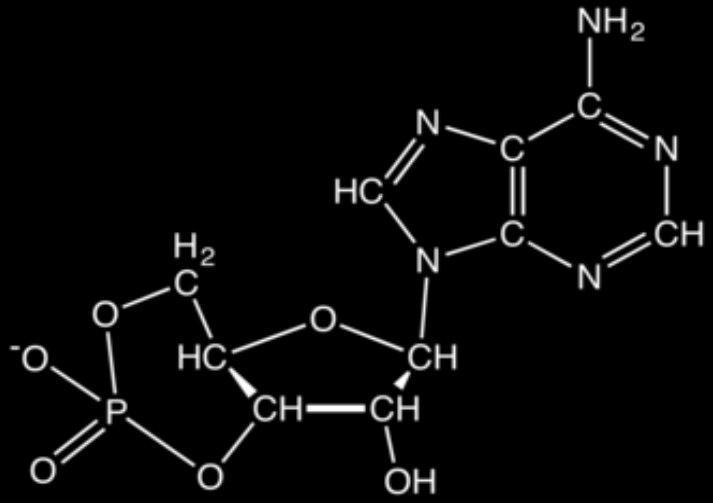
c-AMP

A special look at **Adenylyl Cyclase Pathway**

INTRODUCTION

- **Cyclic adenosine monophosphate** (**cAMP**, **cyclic AMP**, or **3',5'-cyclic adenosine monophosphate**) is a second messenger important in many biological processes. cAMP is a derivative of adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway. It should not be confused with **5'**-AMP-activated protein kinase (AMP-activated protein kinase).





SYNTHESIS

- Cyclic AMP is synthesized from ATP by adenylate cyclase located on the inner side of the plasma membrane and anchored at various locations in the interior of the cell. Adenylate cyclase is *activated* by a range of signaling molecules through the activation of adenylate cyclase stimulatory G (G_s)-protein-coupled receptors. Adenylate cyclase is *inhibited* by agonists of adenylate cyclase inhibitory G (G_i)-protein-coupled receptors. Liver adenylate cyclase responds more strongly to glucagon, and muscle adenylate cyclase responds more strongly to adrenaline.
- cAMP decomposition into AMP is catalyzed by the enzyme phosphodiesterase.

ITS FUNCTIONS

- cAMP is a second messenger, used for intracellular signal transduction, such as transferring into cells the effects of hormones like glucagon and adrenaline, which cannot pass through the plasma membrane. It is also involved in the activation of protein kinases. In addition, cAMP 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 and RAPGEF2.

c-AMP DEPENDENT PATHWAY

MECHANISM

- G protein-coupled receptors (GPCRs) are a large family of integral membrane proteins that respond to a variety of extracellular stimuli. Each GPCR binds to and is activated by a specific ligand stimulus that ranges in size from small molecule catecholamines, lipids, or neurotransmitters to large protein hormones. When a GPCR is activated by its extracellular ligand, a conformational change is induced in the receptor that is transmitted to an attached intracellular heterotrimeric G protein complex.
  The G_s alpha subunit of the stimulated G protein complex exchanges GDP for GTP and is released from the complex.

CONTINUED

- In a cAMP-dependent pathway, the activated G_s alpha subunit binds to and activates an enzyme called adenylyl cyclase, which, in turn, catalyzes the conversion of ATP into cyclic adenosine monophosphate (cAMP). Increases in concentration of the second messenger cAMP may lead to the activation of-
- Cyclic nucleotide-gated ion channels
- Exchange proteins activated by cAMP (EPAC) such as RAPGEF3
- Popeye domain containing proteins (Popdc)
- An enzyme called protein kinase A (PKA).

CONTINUED

- The PKA enzyme is also known as cAMP-dependent enzyme because it gets activated only if cAMP is present. Once PKA is activated, it phosphorylates a number of other proteins including:
- enzymes that convert glycogen into glucose
- enzymes that promote muscle contraction in the heart leading to an increase in heart rate
- transcription factors, which regulate gene expression
- also phosphorylate AMPA receptors
- Specificity of signaling between a GPCR and its ultimate molecular target through a cAMP-dependent pathway may be achieved through formation of a multiprotein complex that includes the GPCR, adenylyl cyclase, and the effector protein.

ITS IMPORTANCE

- In humans, cAMP works by activating protein kinase A (PKA, cAMP-dependent protein kinase), one of the first few kinases discovered. It has four sub-units two catalytic and two regulatory. cAMP binds to the regulatory sub-units. It causes them to break apart from the catalytic sub-units. The Catalytic sub-units make their way in to the nucleus to influence transcription.Further effects mainly depend on cAMP-dependent protein kinase, which vary based on the type of cell.

CONTINUED

- cAMP-dependent pathway is necessary for many living organisms and life processes. Many different cell responses are mediated by cAMP; these include increase in heart rate, cortisol secretion, and breakdown of glycogen and fat. cAMP is essential for the maintenance of memory in the brain, relaxation in the heart, and water absorbed in the kidney. This pathway can activate enzymes and regulate gene expression. The activation of preexisting enzymes is a much faster process, whereas regulation of gene expression is much longer and can take up to hours. The cAMP pathway is studied through loss of function (inhibition) and gain of function (increase) of cAMP.
- If cAMP-dependent pathway is not controlled, it can ultimately lead to hyper-proliferation, which may contribute to the development and/or progression of cancer.

SUMMARY

- In biology, **cell signaling** is part of any communication process that governs basic activities of cells and coordinates multiple-cell actions.
- The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity, as well as normal tissue homeostasis.
- All cells receive and respond to signals from their surroundings.
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- Classification of cell signaling-
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    2. Bio-Chemical
  - Between the cells of different organism
    1. Intra-species
    2. Inter-species

- . Further differentiation of biochemical signals
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  - Þ. *Autocrine*
  - Þ. *Juxtacrine*
  - Þ. *Paracrine*
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- Membrane receptors fall into three major classes:
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  - ❑. Enzyme-linked receptors.
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- GPCRs consist of a single polypeptide that is folded into a globular shape and embedded in a cell's plasma membrane.
- As their name implies, GPCRs interact with G proteins in the plasma membrane.
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- Cyclic AMP is synthesized from ATP by adenylate cyclase located on the inner side of the plasma membrane and anchored at various locations in the interior of the cell.
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THANK YOU.