Molecular basis of hormone action
Hormone receptors

Hormones are the chemically active substances which when released in the general circulation stimulate or inhibit certain functions in the body.

- All the hormones are produced by specialized cells which may be present in the glands and all the hormones have target cells.
- The target cells carry specific receptors on their cell surface or in the cytoplasm to which hormones bind and initiate their responses.
- Based on the nature of the hormone, the receptors vary in structure and the signaling pathways.

The hormone receptors are huge proteins and there are approximately 2000 to 100,000 copies of these receptors on the target cells.

The receptors may be located at different places in a cell like:

A. Receptors on the cell surface : Certain ligands like proteins, peptides and catecholamines hormones have their receptors on the cell surface.
B. Receptors in the cytoplasm: The receptors for steroid hormones are found in cell cytoplasm.
C. Receptors inside the nucleus: Receptors of thyroid hormones are found in the nucleus, closely associated with the chromosomes.

- The number of receptors in a cell varies from time to time.

For example, if the secretion of a hormone is down regulated, the number of receptors will consequently decrease while if the release of hormones is up regulated, the number of receptors will go up.

Properties of Hormone-receptor interactions

The interaction between hormone and its receptor is well regulated and possesses several characteristics like:

1.The hormone-receptor interaction is highly specific.

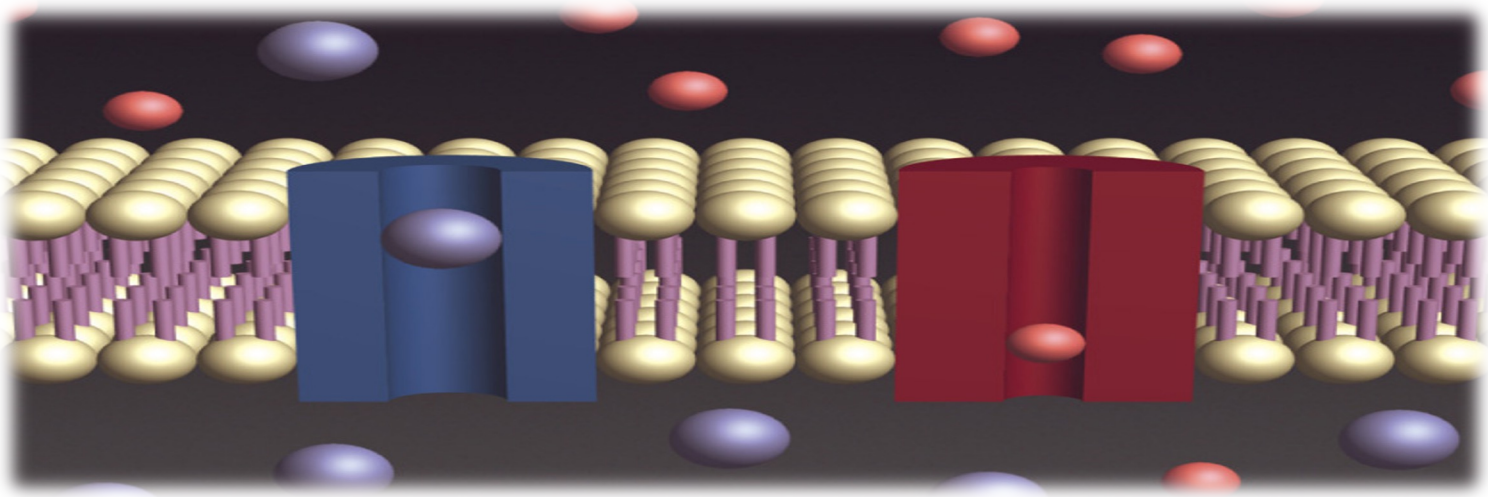
2.The interaction is carried out in a reversible manner i.e. once the binding of hormone to receptor occurs; it stimulates the intracellular signaling pathways. Once the pathways begin, hormone dissociates from the complex and is rendered free.

3.The hormone has a very high affinity for its receptor such that it can bind to its receptor even when the hormone is present in a very low concentration in the plasma.

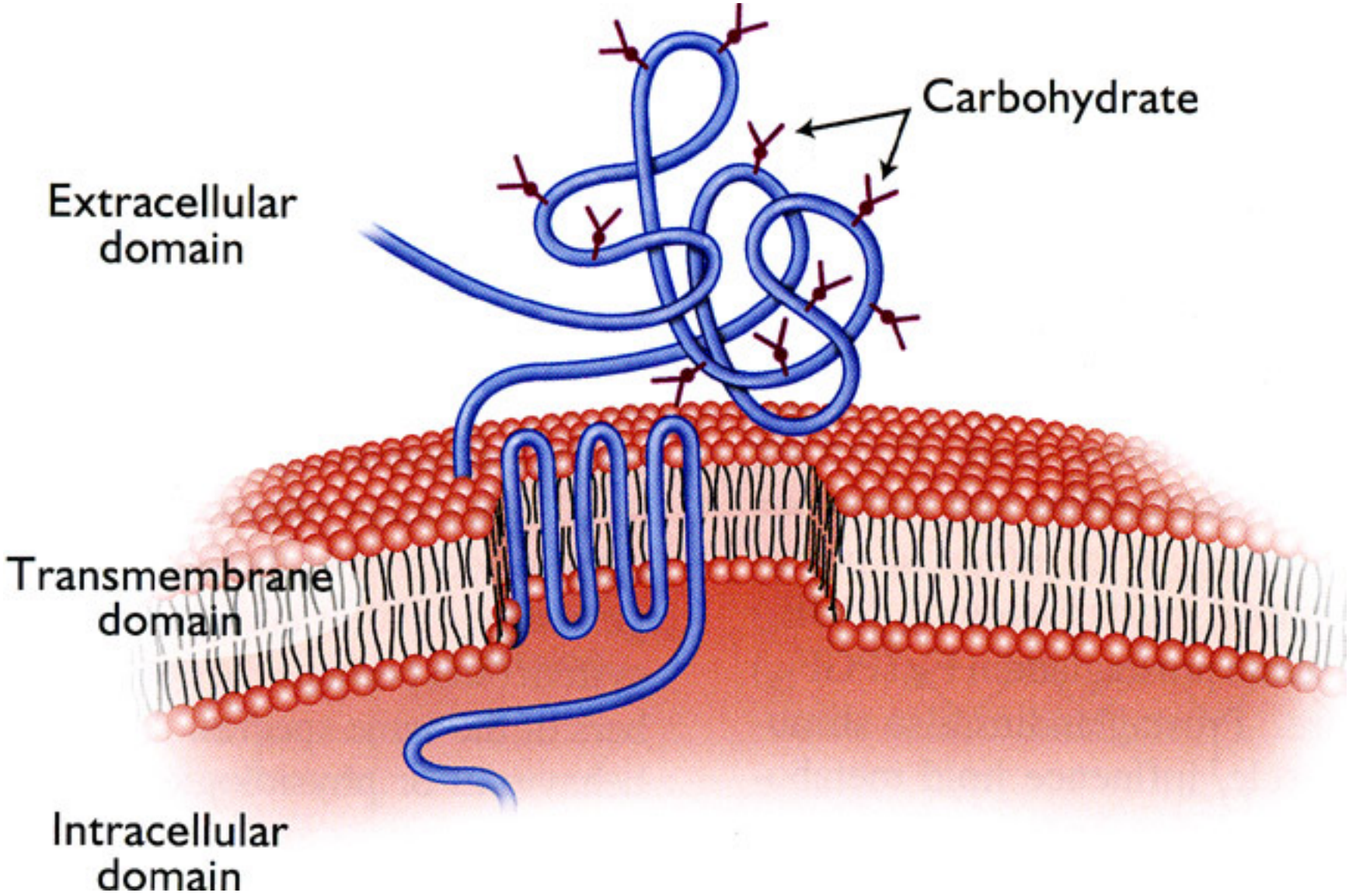
4.The binding of hormone to its receptor occurs only in the sensitive or receptive tissue.

Receptor Structure

- Binding domain rich in cysteine residues to form disulphide bonds
- Hydrophobic domain is rich in uncharged AAs
- Cytoplasmic domain of the C-terminal contains “Catalytic System” that signals the messages of hormones



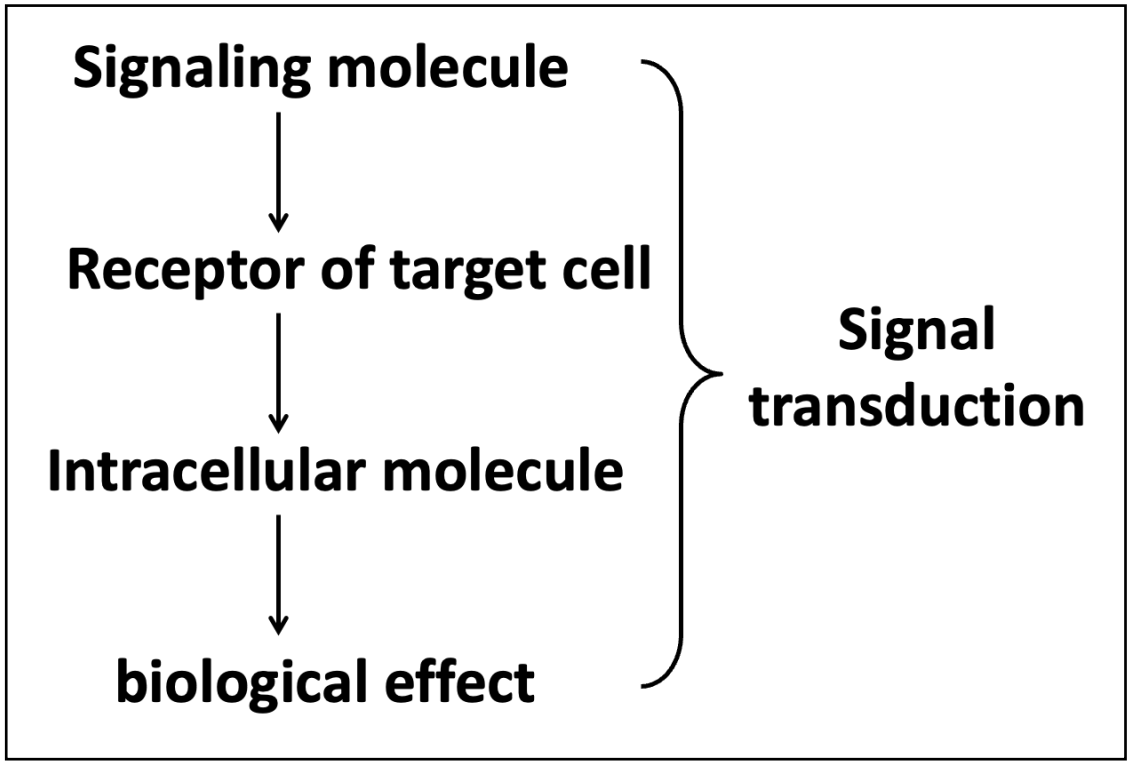
Receptor Structure



Types of receptors

- **Two types**
  - Transmembrane
  - Intracellular/nuclear

  - Proteins regardless of the type
- **Interaction between a hormone and a receptor is an** initial step of hormone action



Coupling (signal transduction) occurs in two general ways:

-Polypeptide and protein hormones and the catecholamines (group II):

bind to receptors located in the plasma membrane and thereby generate a signal that regulates various intracellular functions, often by changing the activity of an enzyme.

-In contrast, steroid, retinoid, and thyroid hormones (Group I):

interact with intracellular receptors, and it is this ligand-receptor complex that directly provides the signal, generally to specific genes whose rate of transcription is thereby affected.

Table 42–4. General features of hormone classes.

	Group I	Group II
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma half-life	Long (hours to days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator	Receptor-hormone complex	cAMP, cGMP, Ca^{2+}, metabolites of complex phosphoinositols, kinase cascades

GROUP II (PEPTIDE &CATECHOLAMINE) HORMONES HAVE MEMBRANE RECEPTORS & USE INTRACELLULAR MESSENGERS:

- Many hormones are water-soluble, Unable to pass through the plasma membrane

- have no transport proteins (and therefore have a short plasma half-life),

- initiate a response by binding to a receptor located in the plasma membrane (Transmembrane receptors).

- The mechanism of action of this group of hormones can best be discussed in terms of the intracellular signals they generate

These signals(2nd messenger) include:

1- cAMP (cyclic AMP; 3′,5′-adenylic acid; a nucleotide derived from ATP through the action of adenylyl cyclase)

Group II a. e.g epinephrine, glucagon.

2-cGMP, a nucleotide formed from GTP by guanylyl cyclase;

Group II b. e.g Nitic oxide

3- Ca2+; and phosphatidylinositols.

Group II c e.g Oxytocin

4- Kinase or phosphatase cascade.

Group II d. e.g Insulin

1. Transmembrane Receptors

Many hormones are soluble in water like peptide hormones and glycoprotein hormones. These hormones can’t diffuse through the plasma membrane; hence their receptors are located in the plasma membrane. There are two major types of transmembrane receptors:

1) **G-protein coupled receptors (GPCRs)**
2) **Enzyme- linked receptors or kinase receptors**

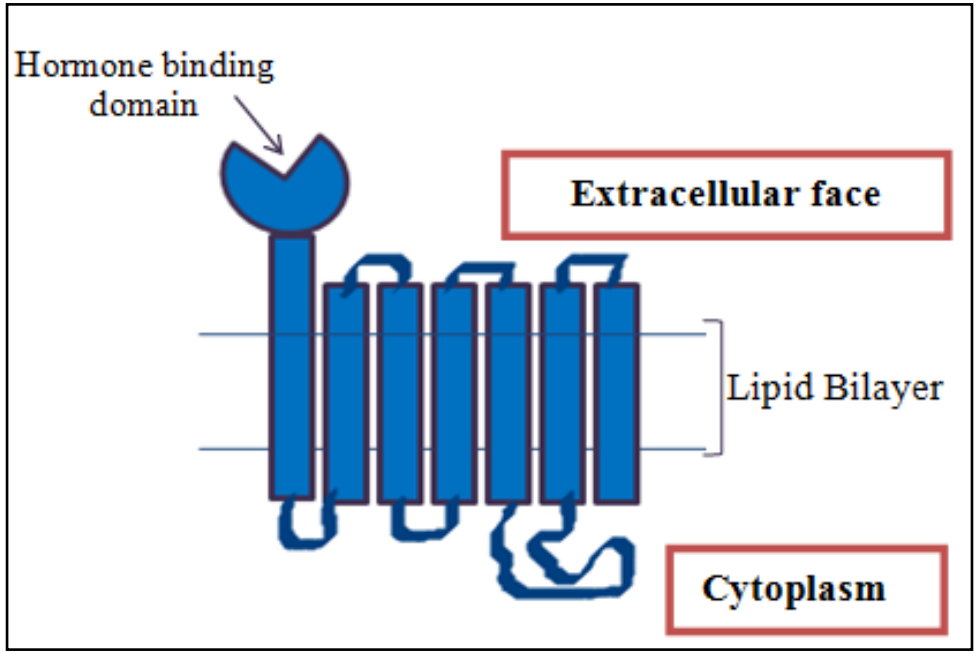
1. G-protein coupled Receptors (GPCRs)

Some hormones like peptide hormones, glycoprotein hormones and amino acid hormones, neurotransmitters and local mediators act by binding to their specific receptors on the target cells, which are coupled with G-proteins.

Structure of G-protein receptors

G-protein coupled receptors, have 7-transmembrane domains and are anchored in the plasma membrane.

The G-protein receptors are a single chain of polypeptide which crosses the lipid bilayer seven times and forms 7-transmembrane domains; hence, G-protein coupled receptors are also called as **serpentine receptors**



The activation of these receptors is that the same ligand can activate several different receptor family members.

Suh as ; adrenaline can activate at least 9 different G-protein linked receptors;

acetylcholine can activate more than 5 different receptors while serotonin can activate at least 15 different G-protein coupled receptors.

For example,

1. there are two receptors for vasopressin (named V1a, V1b and V2).

The V1a and V1b receptors are found in CNS and V1b receptor is highly expressed in pituitary corticotrophs while V2 is exclusively expressed in kidney

2. and one receptor for oxytocin,

all of which are G-protein coupled receptors.

Mechanism of action of GPCRs

- When the hormone binds to the receptor at the extracellular domain, there is conformational change in the receptor, which activates the cytoplasmic trimeric GTP binding protein (G-protein).
- This result in functional coupling between receptors and enzymes or ion channels associated with the plasma membrane.
- The G-proteins in the GPCRs consist of three subunits: α, β and γ.
- In unstimulated condition of the cell, the α-subunit remains bound to GDP and G-protein remains inactivated.
- When the G-protein is activated due to binding of ligand to receptor, the α-subunit releases GDP and replaces it with GTP. Due to this switch, the G-protein is then divided into two active components i.e. the α-subunit and βγ complex

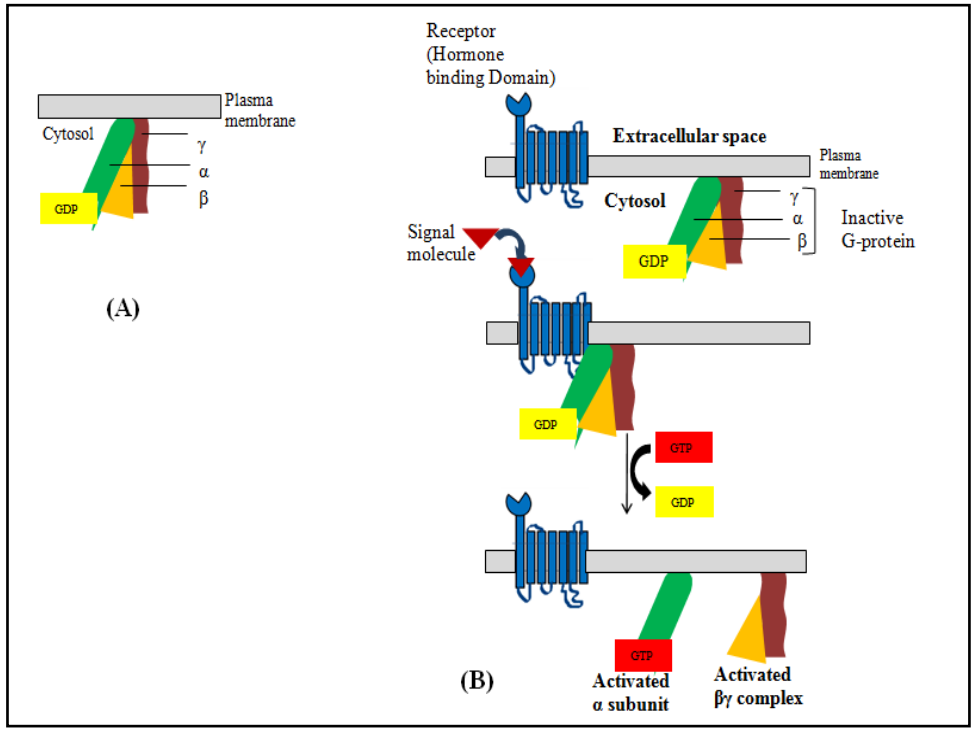


Fig. :(A) Structure of inactive G-protein; (B) Activation of G-protein due to binding of ligand to the receptor

- The binding of GTP to α-subunit causes a conformational change in the α-subunit such that it releases the βγ complex and the α-subunit interacts with the target protein.
- However, the conformation of the βγ complex does not change.
- Actually, in inactive state, βγ complex is masked by α-subunit, but in activated state it is exposed and free to interact with other set of target proteins.
- The targets of the dissociated G-protein components are either enzymes or ion channels in the plasma membrane.
- The α-subunit of G-protein is a GTPase and when it hydrolyzes GTP, bound to it, it converts GTP into GDP.
- In this form i.e. α-GDP, it again binds to the βγ complex and forms the inactive G-protein.
- The duration of the switching of GTP to GDP is very short and it determines the efficiency of the signal transferred.

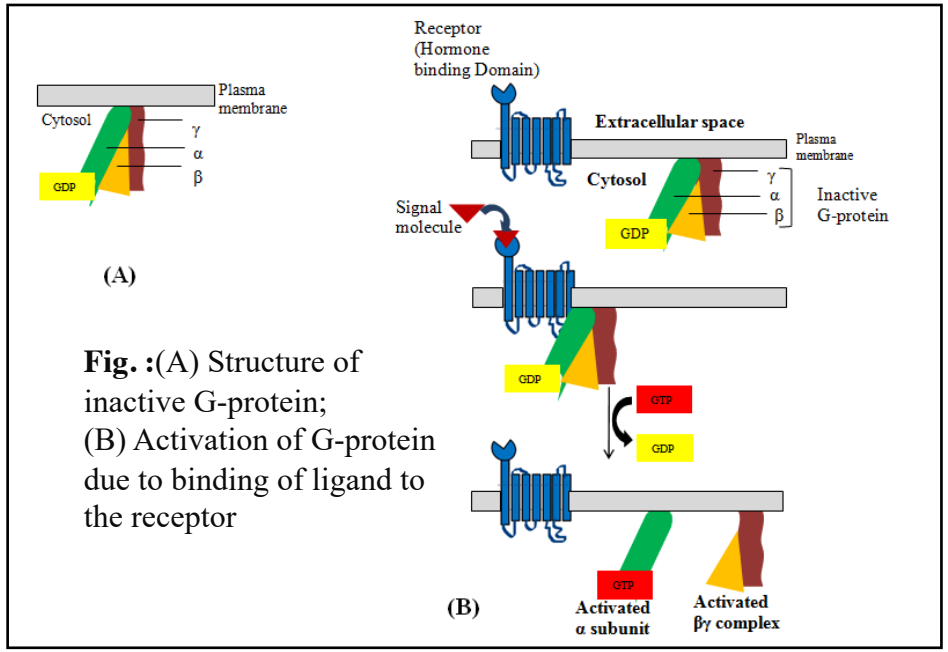
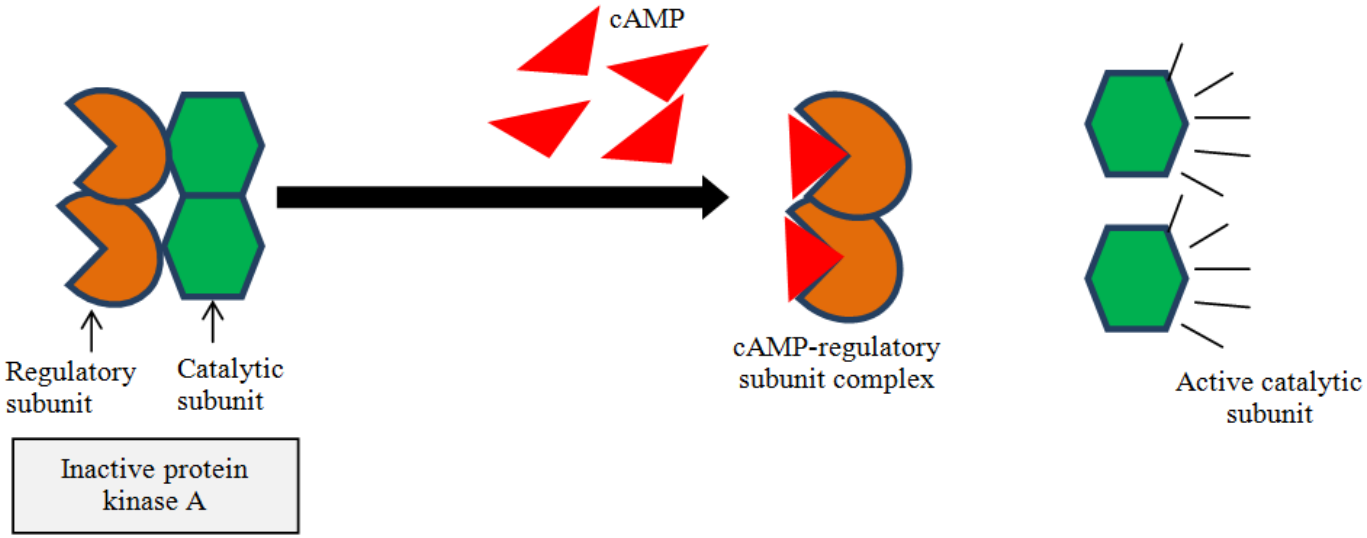


Fig. :(A) Structure of inactive G-protein; (B) Activation of G-protein due to binding of ligand to the receptor

The G-proteins are of two types, based on their function:

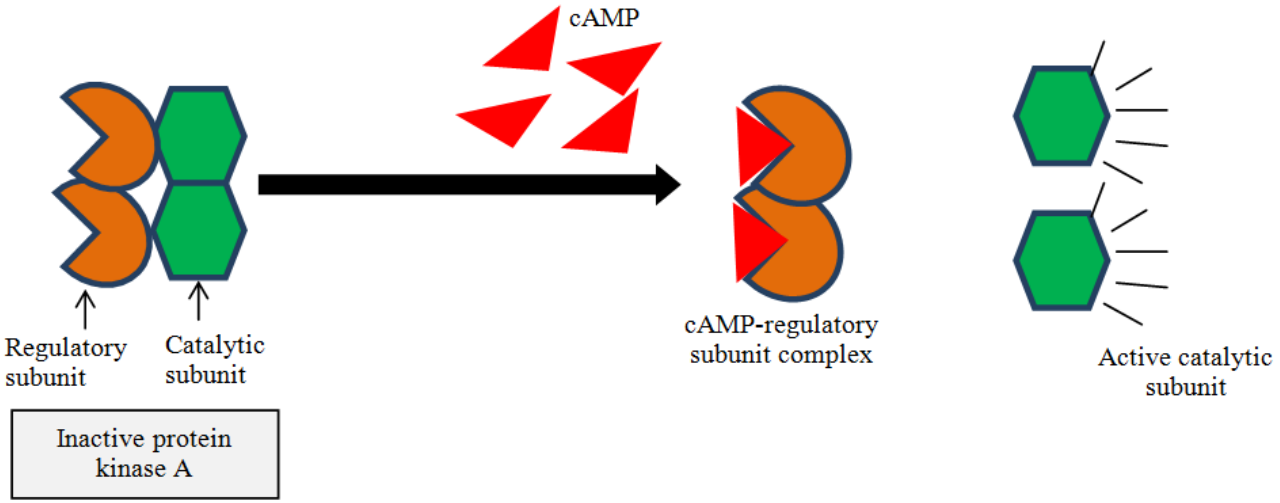
1.Stimulatory G-protein (Gs):

- The ligands bind to the receptors and the receptors which use cyclic AMP (cAMP) as second messenger are coupled to stimulatory G-protein (Gs).
- The Gs in turn activates adenylyl cyclase which produces more cAMP by acting on ATP.
- Adenylyl cyclase has two catalytic domains towards the cytoplasmic face of the membrane and two structural domains, each of which contains six transmembrane α helices.



2. Inhibitory G-protein (Gi):

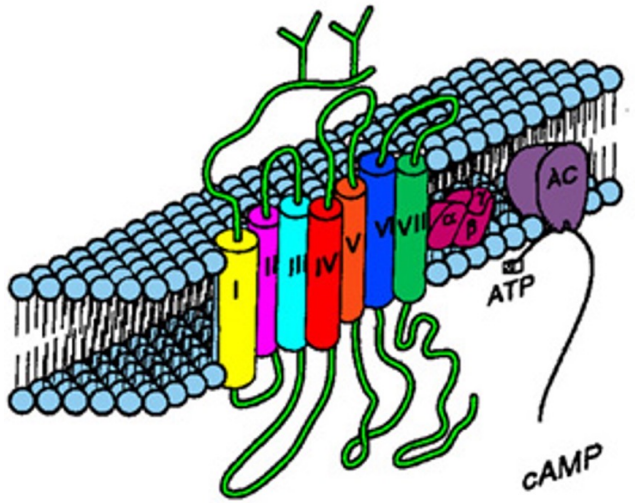
- There is another G-protein called inhibitory G-protein (Gi) which inhibits adenylyl cyclase by regulating ion channels.
- cAMP can directly regulate the ion channels but most of the time it does so by activating cAMP dependent protein kinase A (PKA).



Second messengers

1- Cyclic nucleotides (cAMP) (group II a)

- cAMP (cyclic adenosine monophosphate)
  - Widely used secondary messenger
  - Generated by adenyl cyclase (AC)
- Binding of hormone to the receptor leads to activation of adenyl cyclase by activated G-protein (Ga)
- Activated AC produces cAMP from ATP.
- cAMP leads to activation of cAMP- dependent protein kinases. (Protein kinase A) (PK A)
- PK A phosphorylates critical proteins that causes physiologic effects.



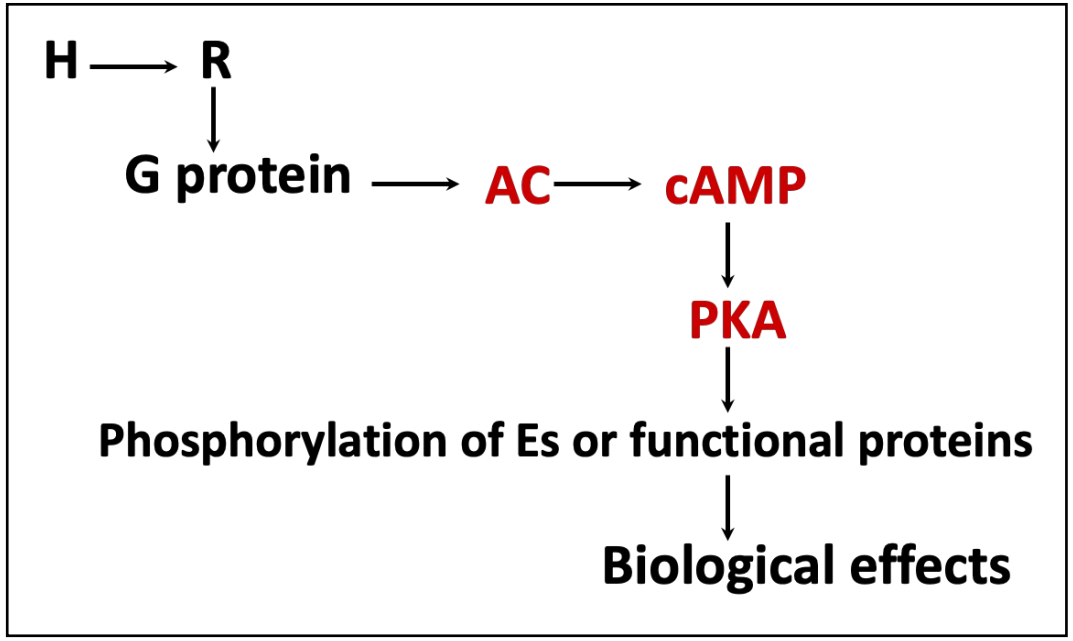
Adenylate Cyclase (Adenylyl Cyclase) catalyzes:

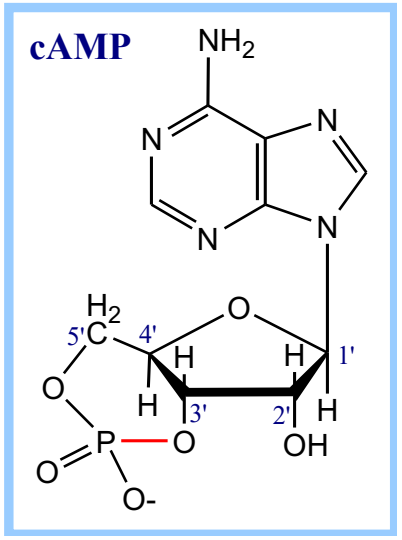
ATP $\longrightarrow$ cAMP + PP_i

Binding of certain **hormones** (e.g., epinephrine) to the outer surface of a cell activates Adenylate Cyclase to form cAMP within the cell.

Cyclic AMP is thus considered to be a **second messenger**.

cAMP dependent-protein kinase A pathway





Mechanism of cAMP effect

Activate cAMP-dependent protein kinase (PKA).

- cAMP dependent protein kinase A (PKA) enzymes transfers phosphate groups from ATP to specific serine or threonine residues in target proteins and thus regulate their activity.

- PKA consists of four sub units: two catalytic subunits and two regulatory subunits. When cAMP binds to regulatory subunits, the conformation of PKA changes such that the catalytic subunits get released from the complex and get activated to phosphorylate the target proteins i.e. transcription factors followed by transcription of the target gene

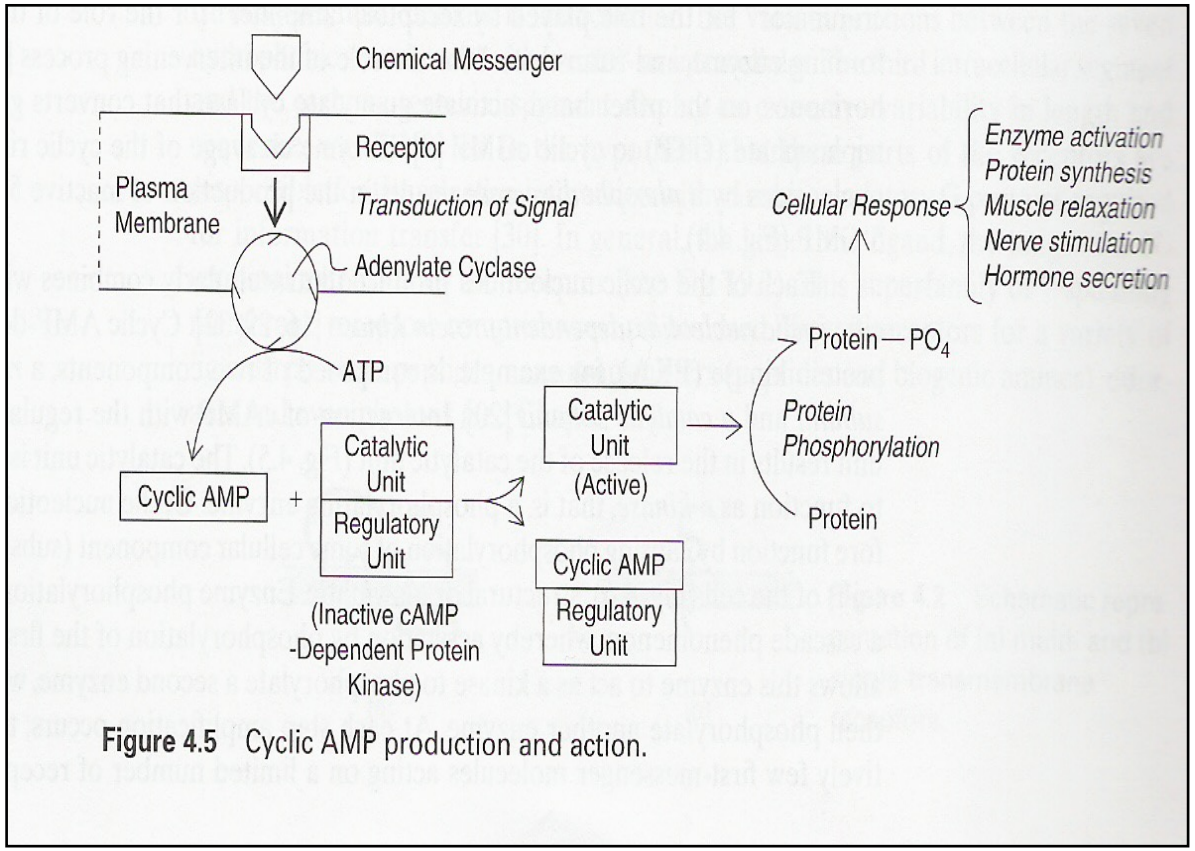


Figure 4.5 Cyclic AMP production and action.

Protein Kinase A in the resting state is a complex of:

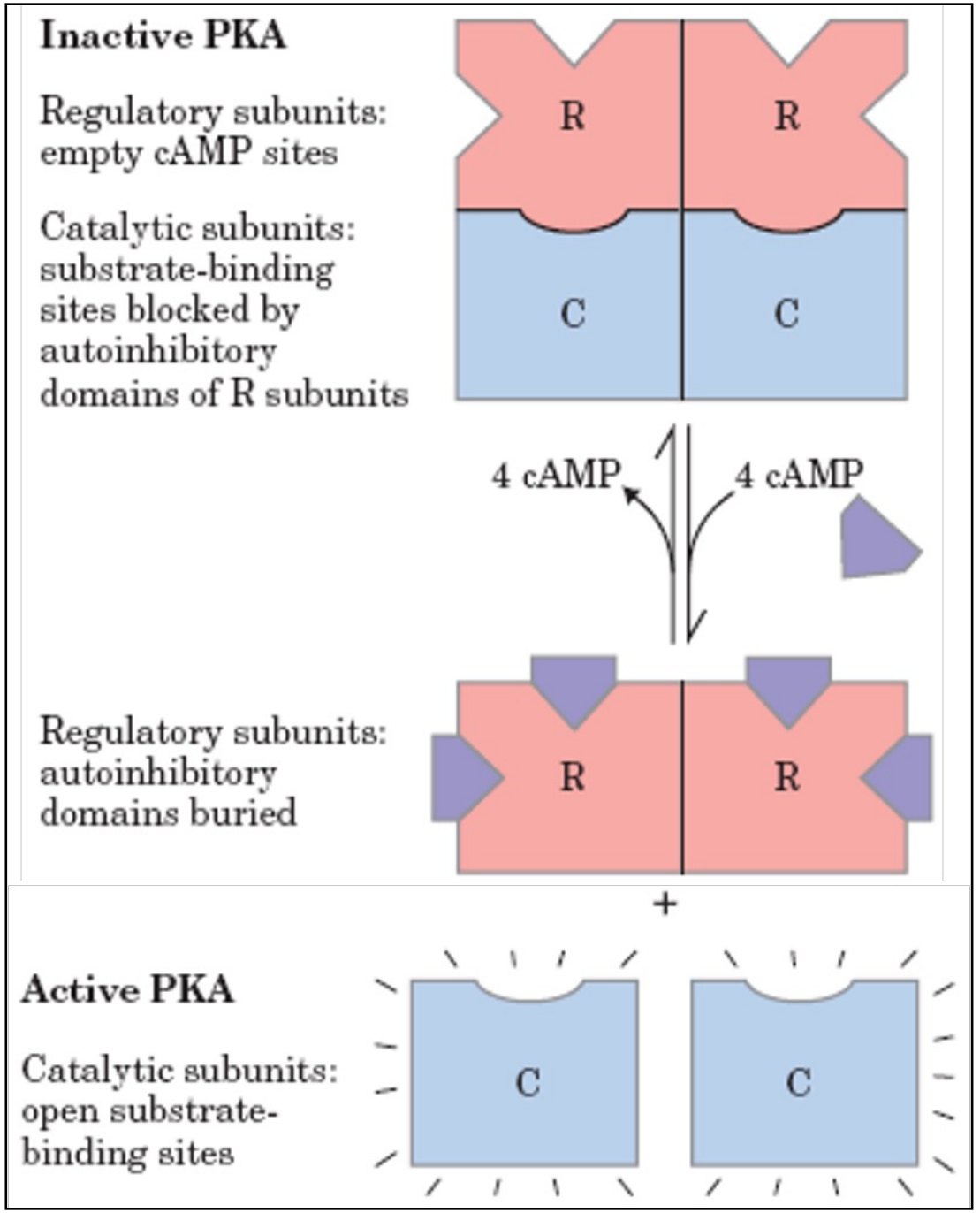
- 2 catalytic subunits (**C**)
- 2 regulatory subunits (**R**).

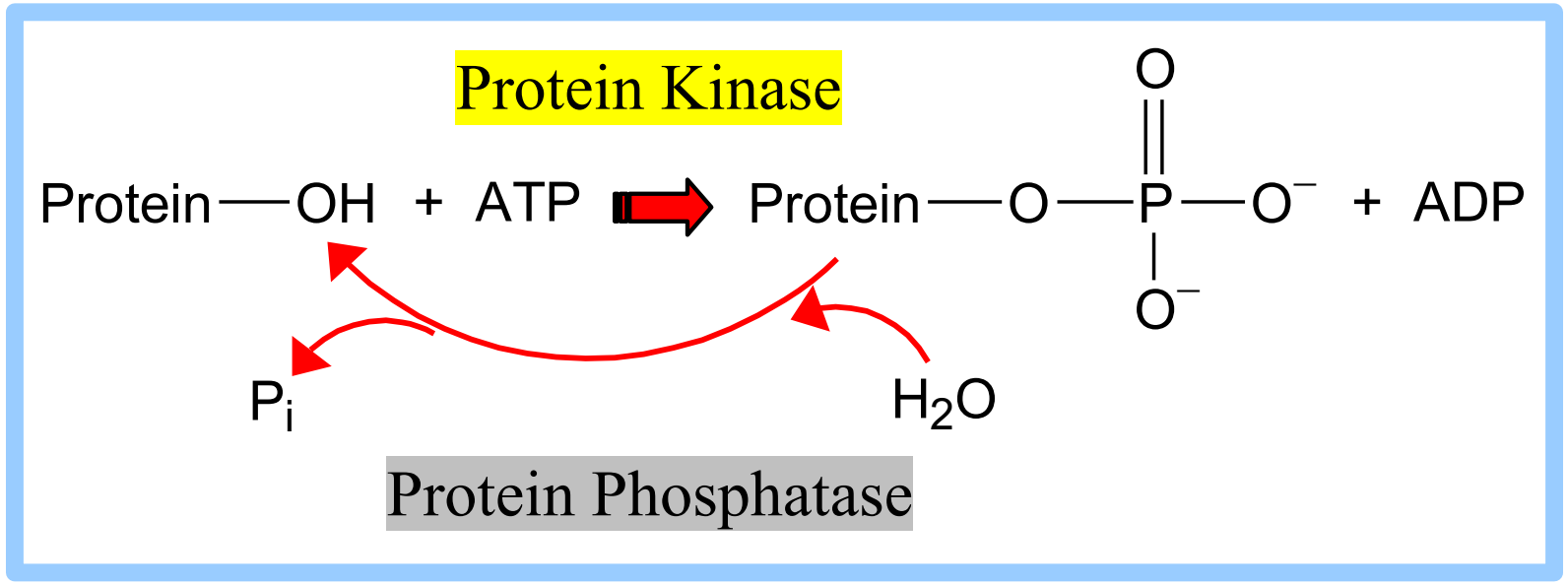
R_2C_2

R_2C_2 + 4 cAMP → R_2cAMP_4 + 2 C

When each (**R**) binds 2 cAMP, a conformational change causes (**R**) to release (**C**).

The catalytic subunits can then catalyze phosphorylation of Ser or Thr on target proteins.





- **Protein Kinase A**(cAMP-Dependent Protein Kinase) transfers the terminal phosphate (Pi) from ATP to a hydroxyl group (OH) of a Ser or Thr on a protein.
- A **protein phosphatase** catalyzes removal of the P_i by hydrolysis.

cAMP metabolism

PDE : Phosphodiesterase

AC : Adenylate cyclase