Molecular basis of hormone action
Hormone receptors
Part 2

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**

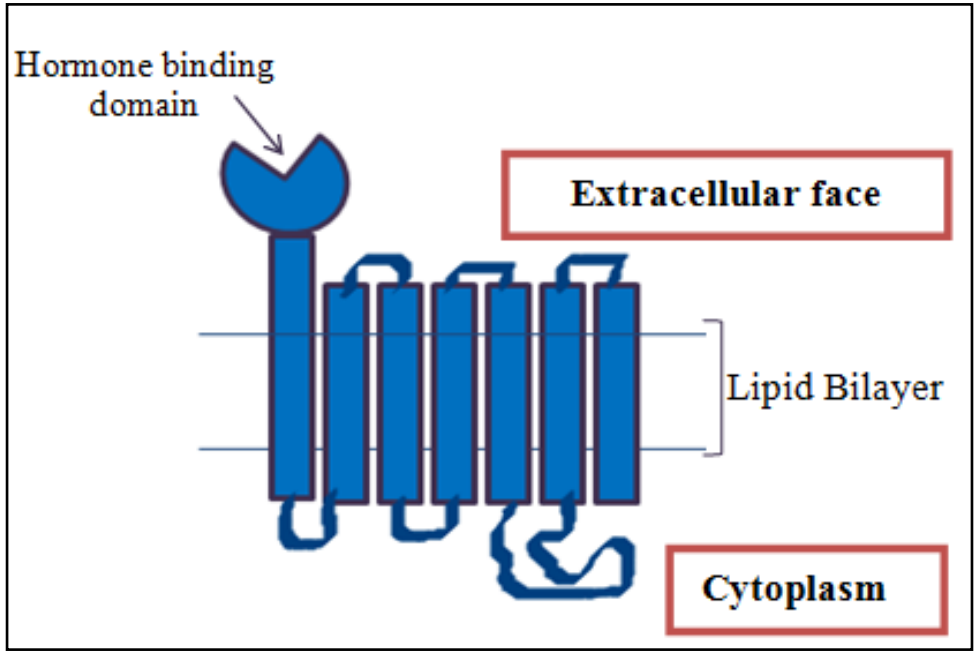
1. G-protein coupled Receptors (GPCRs)

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 **(Guanylate binding protein)**.

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

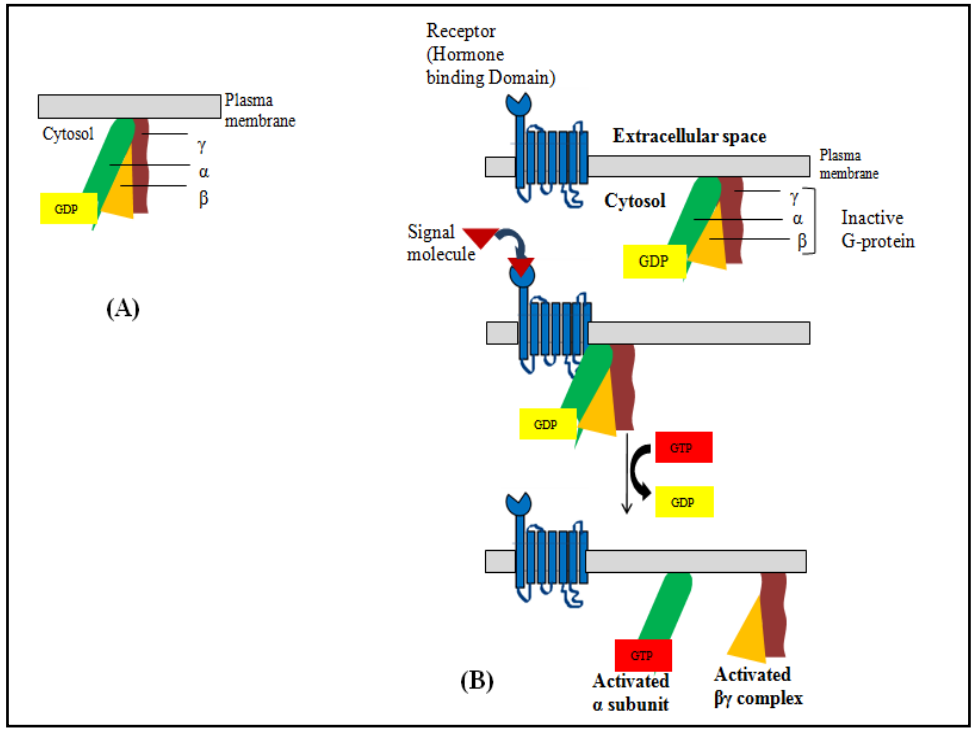


Fig. :(A) Structure of inactive G-protein; (B) Activation of G-protein due to binding of ligand to the receptor

- 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.

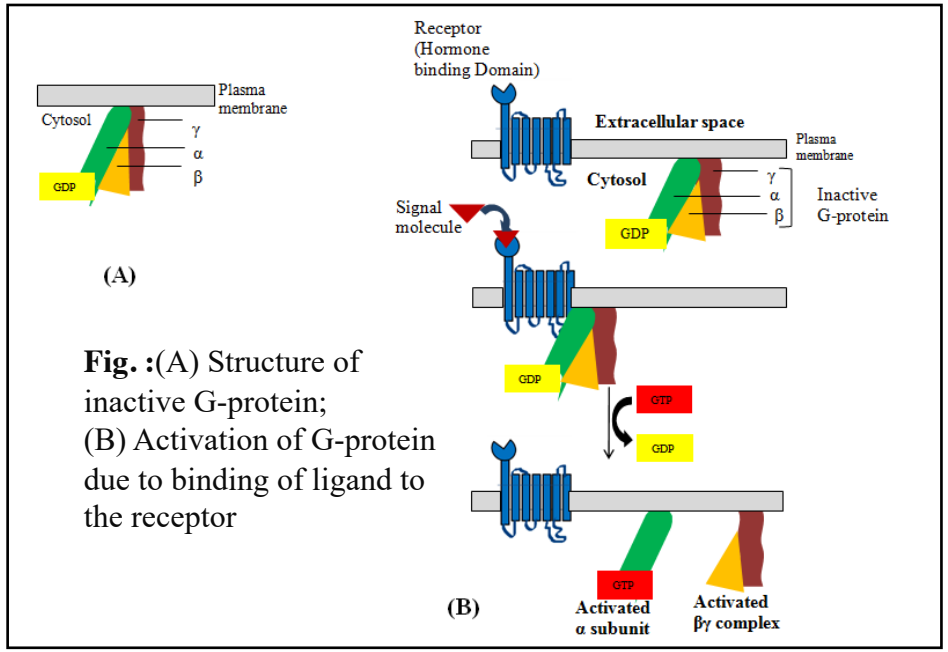
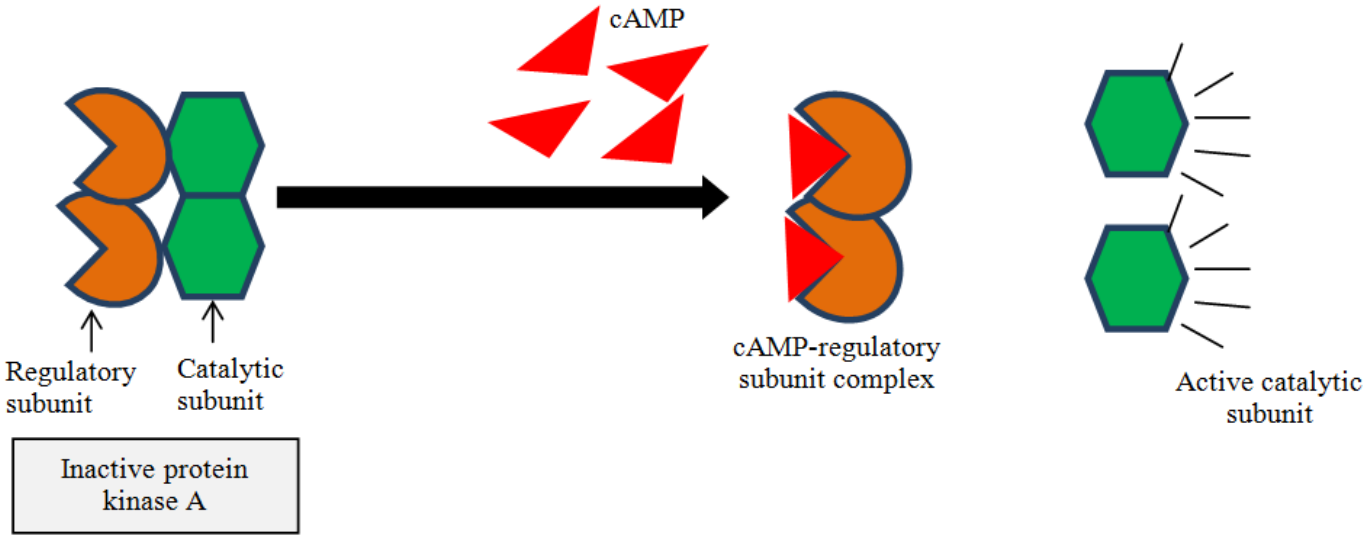


Fig. :(A) Structure of inactive G-protein; (B) Activation of G-protein due to binding of ligand to the receptor

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

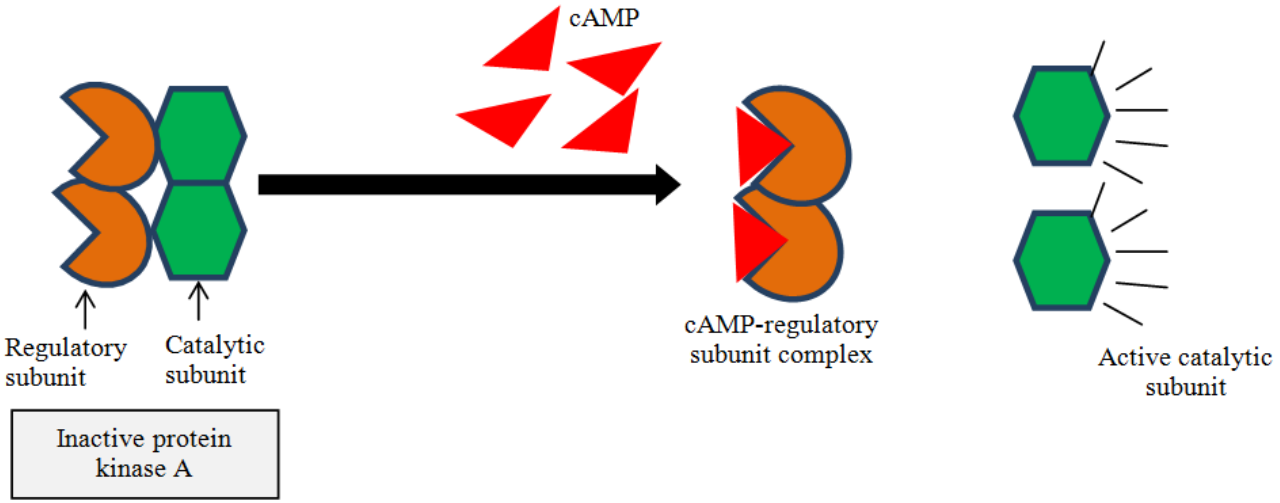
1.Stimulatory G-protein (Gs):

- 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):

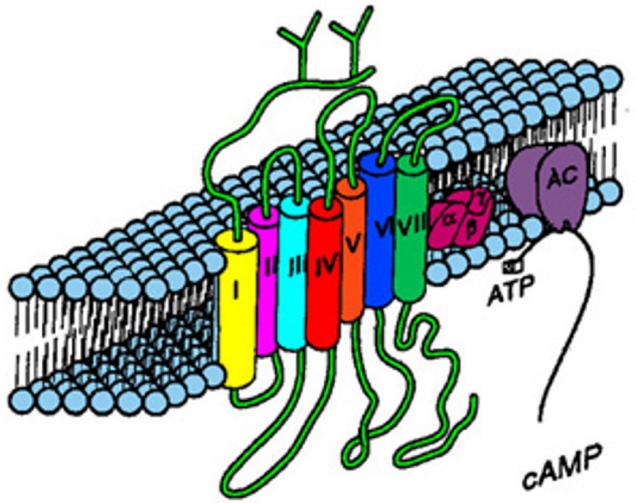
- There is another G-protein called inhibitory G-protein (Gi) which inhibits adenylyl cyclase by regulating ion channels.
- cAMP can directly regulate the ion channels but most of the time it does so by activating cAMP dependent protein kinase A (PKA).



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.



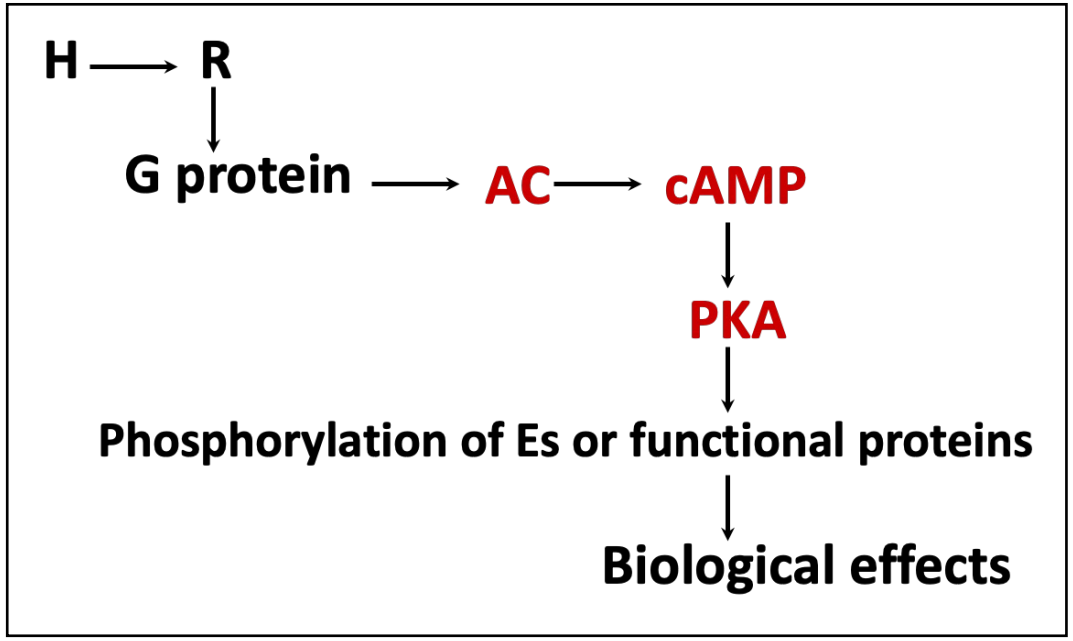
Adenylate Cyclase (Adenylyl Cyclase) catalyzes:

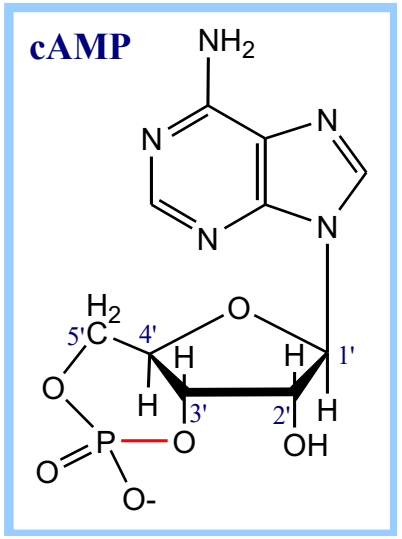
ATP ⟶ cAMP + PP_i

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

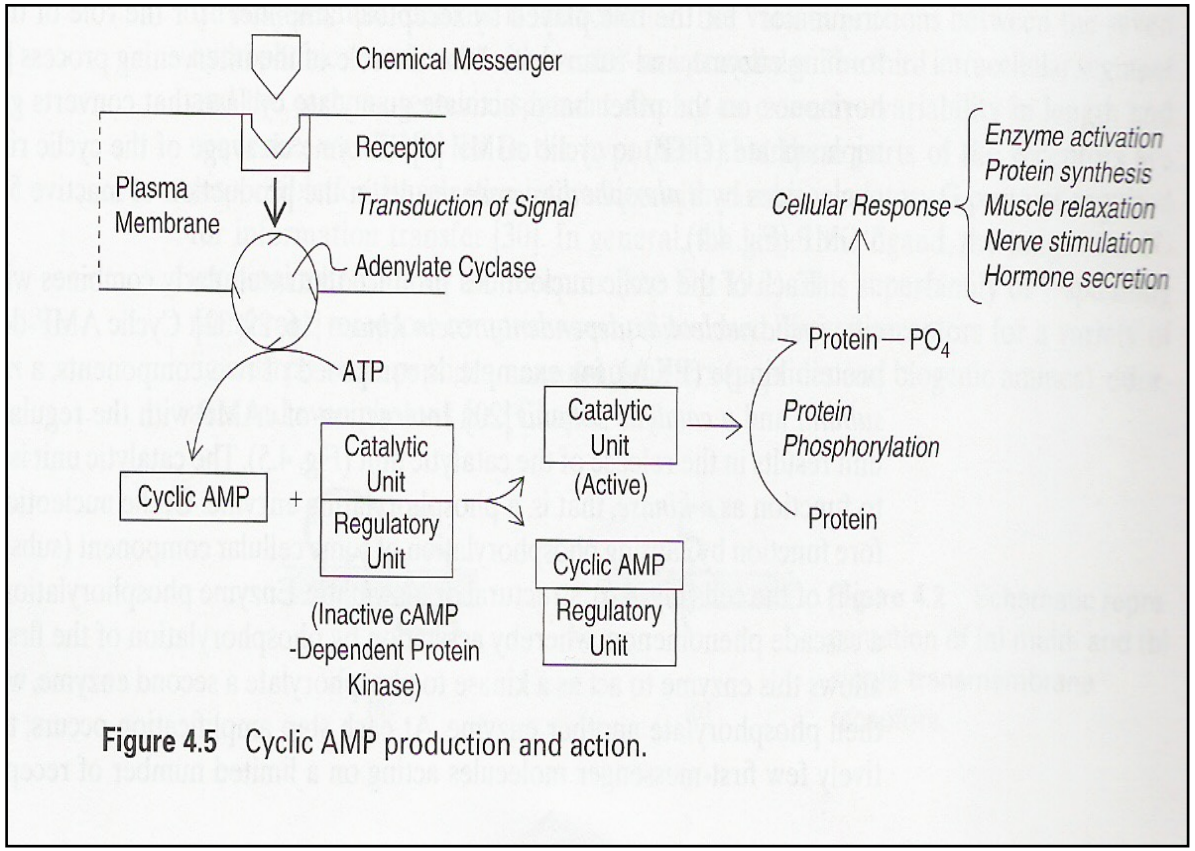


Figure 4.5 Cyclic AMP production and action.

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

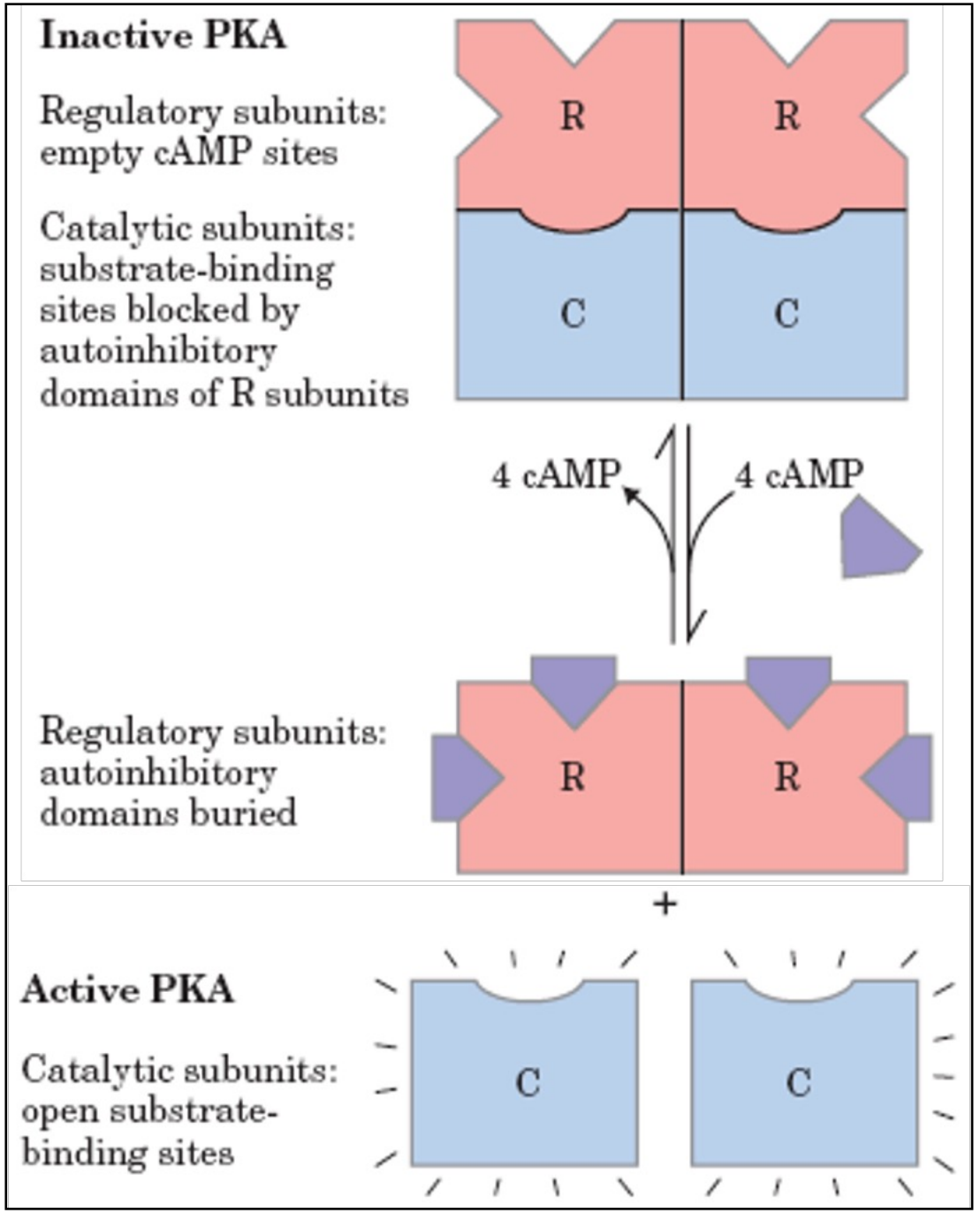
- 2 catalytic subunits (**C**)
- 2 regulatory subunits (**R**).

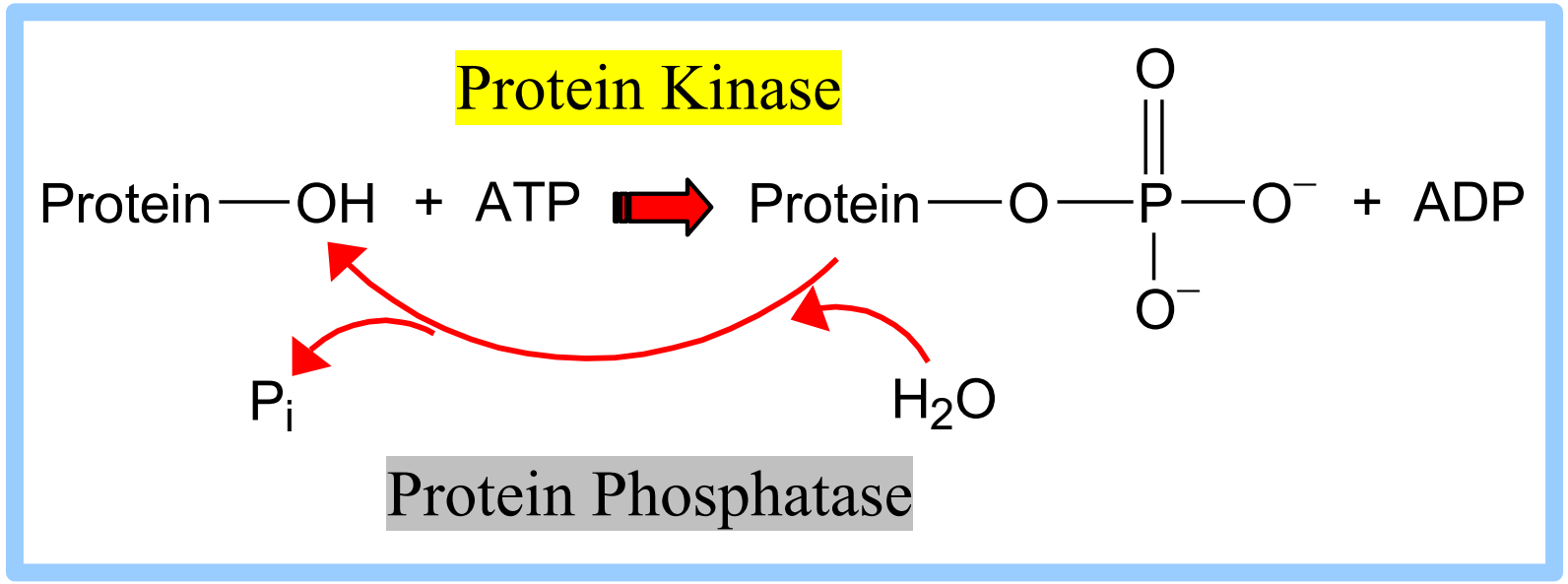
R_2C_2

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

Turn off of the signal:

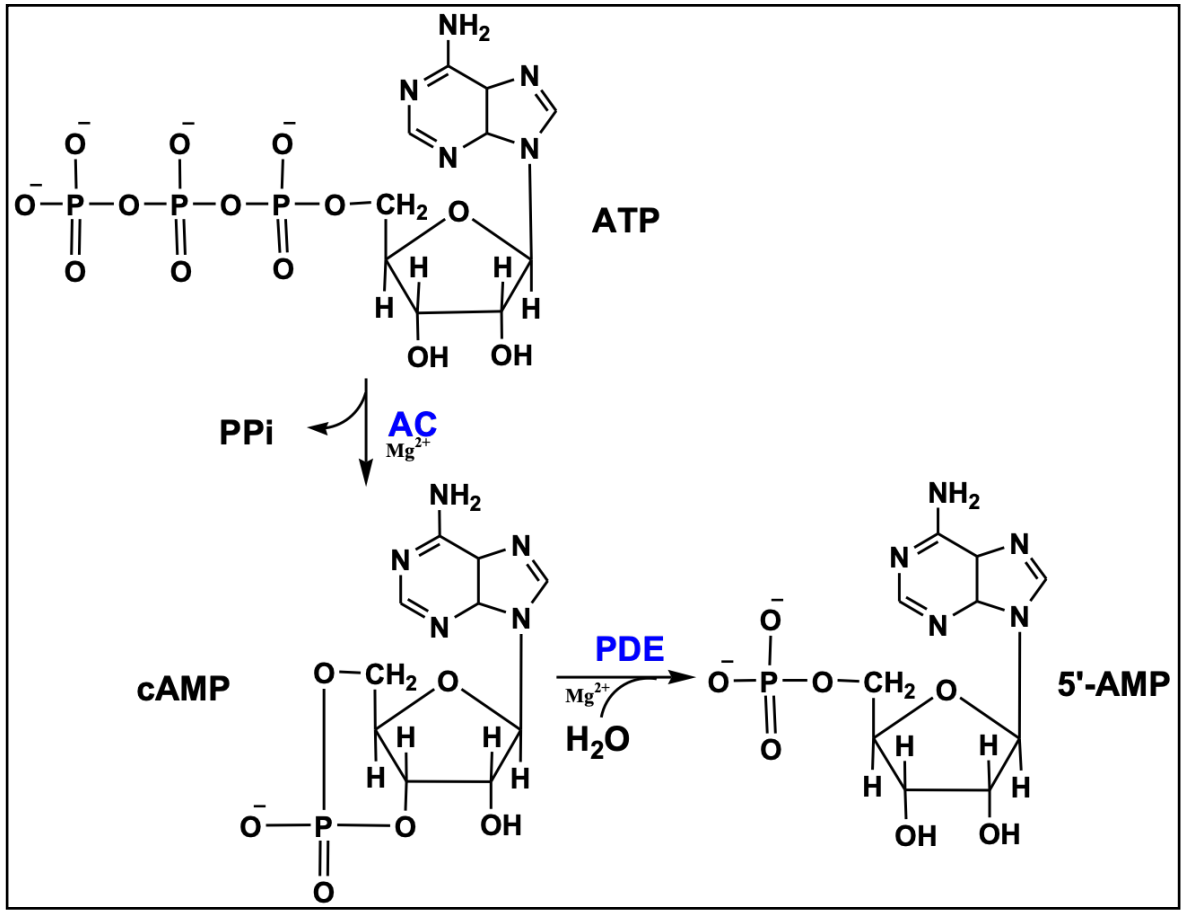
1. **G_a** hydrolyzes GTP to GDP + P_i. (**GTPase**).

   The presence of **GDP** on G_a causes it to rebind to the inhibitory **bg** complex.

   Adenylate Cyclase is no longer activated.

2. **Phosphodiesterases** catalyze hydrolysis of

   cAMP → AMP.



PDE : Phosphodiesterase

AC : Adenylate cyclase

- Receptors not interact directly with AC but interact with G-protein **(Guanylate binding protein)** that activates the AC enzyme.

- **G protein refers to any protein which binds to GDP or GTP and act as signal transduction.**

- **G proteins consist of three different subunits (α, β, γ-subunit) heterotrimeric.**

Types of G-protein complex :Gα subunit

Gs (stimulatory Gα): **G_s→ α_s→AC→cAMP↑**

Gi (inhibitory Gα) **: G_i→ α_i→AC→cAMP↓**

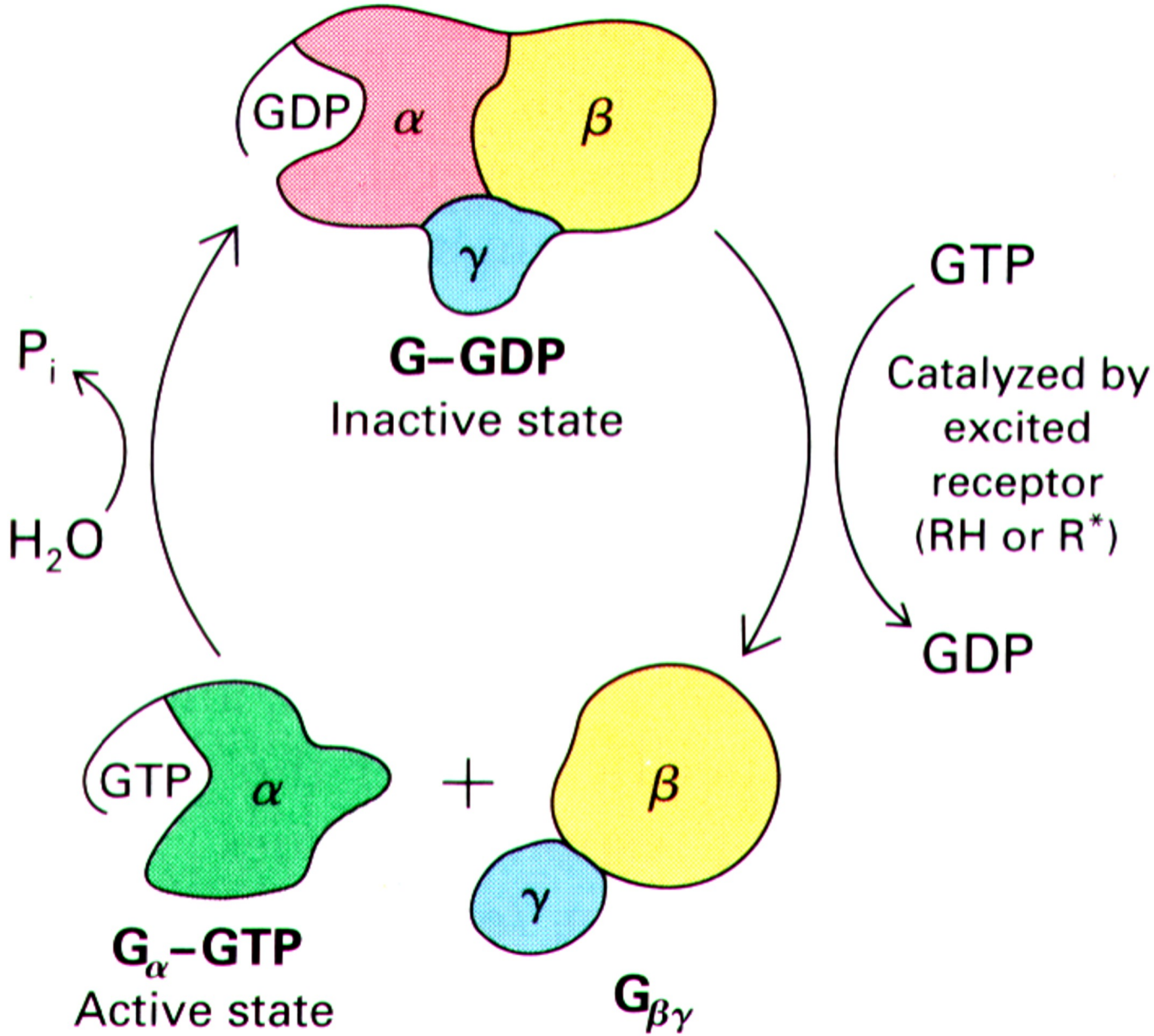
- **α-subunit carries GTPase activity, binding and hydrolysis of GTP.**

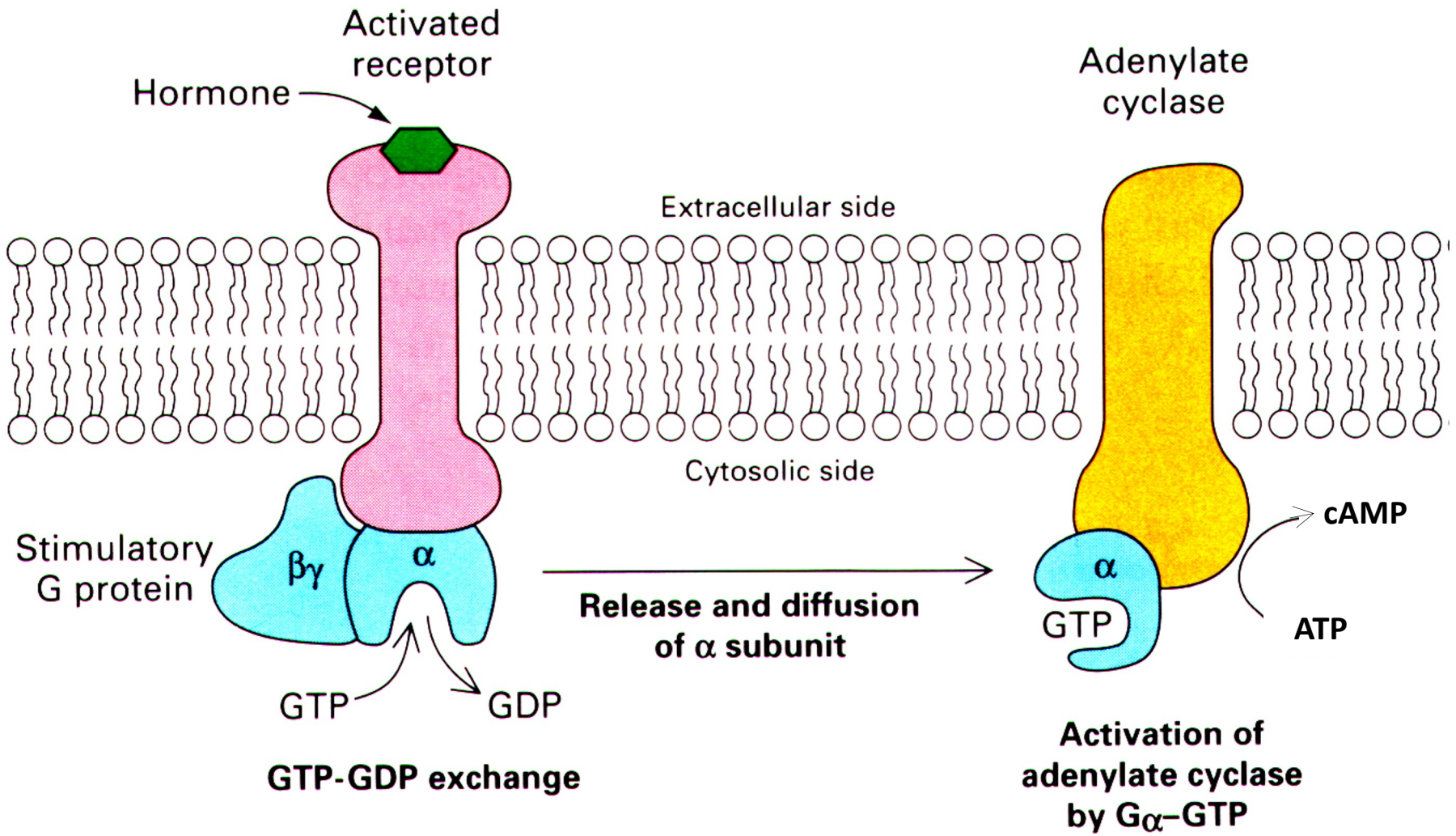
- The **sequence of events** by which a hormone activates cAMP signaling:

1. Initially **G_a** has bound **GDP**, and **a**, **b**, & **g** subunits are complexed together. **$G_{b,g}$**, the complex of b & g subunits, **inhibits G_a**.

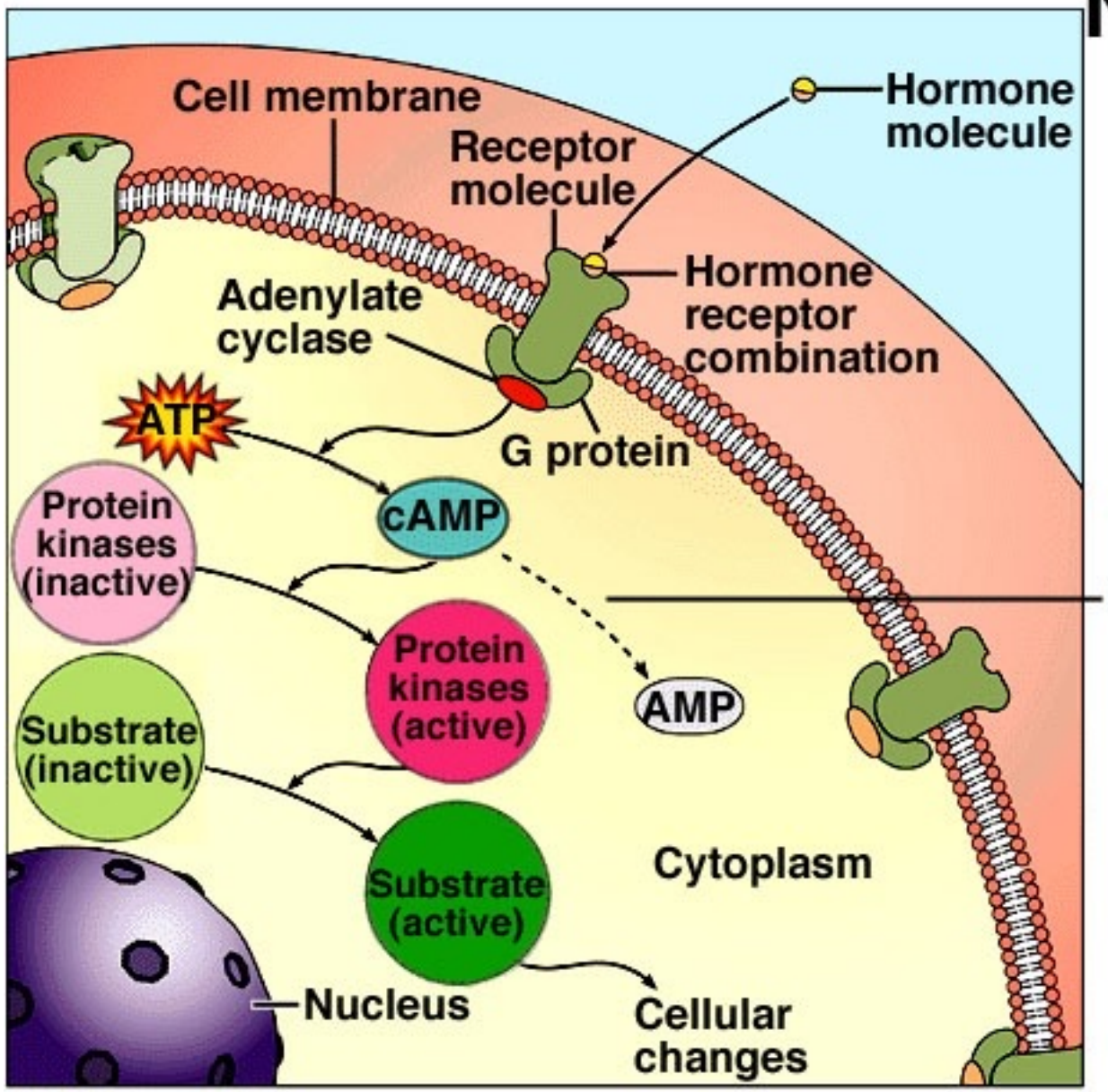
2.

2. **Hormone binding**, **G_a** releases GDP & binds GTP (**GDP-GTP exchange**).
3. **G_a-GTP** dissociates from the inhibitory **bg** complex & can now bind to and activate Adenylate Cyclase.
4. **Adenylate Cyclase**, activated by the stimulatory G_a-GTP, catalyzes synthesis of **cAMP**
5. **Protein Kinase A** (cAMP Dependent Protein Kinase) catalyzes transfer of phosphate from ATP to serine or threonine residues of various cellular proteins, altering their activity.


GDP
α
β
γ
G–GDP
Inactive state
GTP
Catalyzed by
excited
receptor
(RH or R*)
GDP
Pi
H2O
GTP
α
β
γ
Gα–GTP
Active state
Gβγ


Activated receptor
Hormone
Adenylate cyclase
Extracellular side
Cytosolic side
Stimulatory G protein
βγ
α
GTP
GDP
GTP-GDP exchange
Release and diffusion of α subunit
α
GTP
cAMP
ATP
Activation of adenylate cyclase by Gα–GTP

Nonsteroid Hormone Action

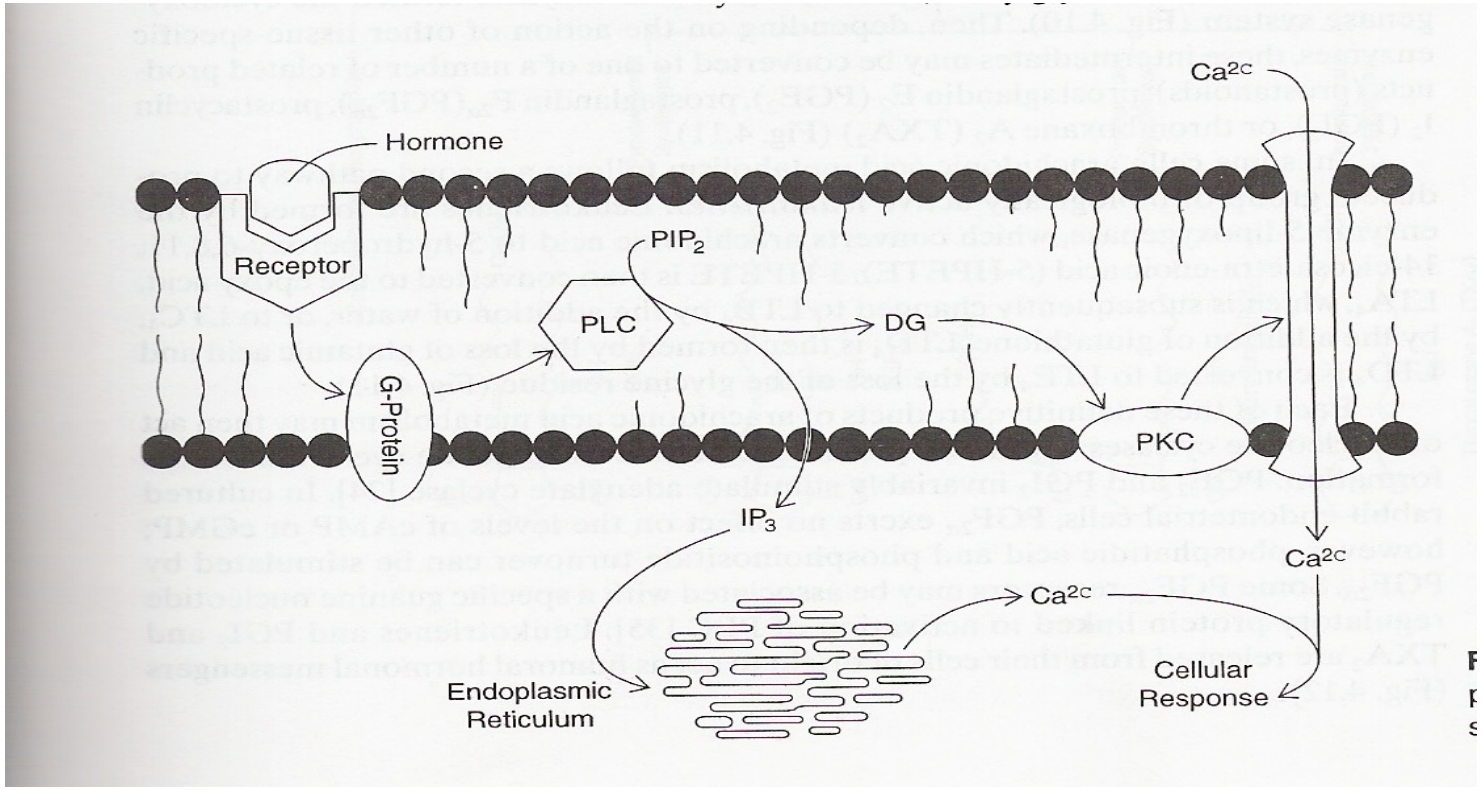


Secondary messengers

- Amplification of hormonal signals
  - Binding of hormone to the receptor
  - Activation of adenyl cyclase by activated Ga
  - Activation of protein kinase A by cAMP
- Rapid clearance and inactivation
  - Phosphodiesterases
  - Inhibited by methylxanthines (caffeine, theophylline, and theobromine) may be due to blockade of adenosine receptors and/or inhibition of phosphodiesterase
  - Phosphoprotein phosphatases

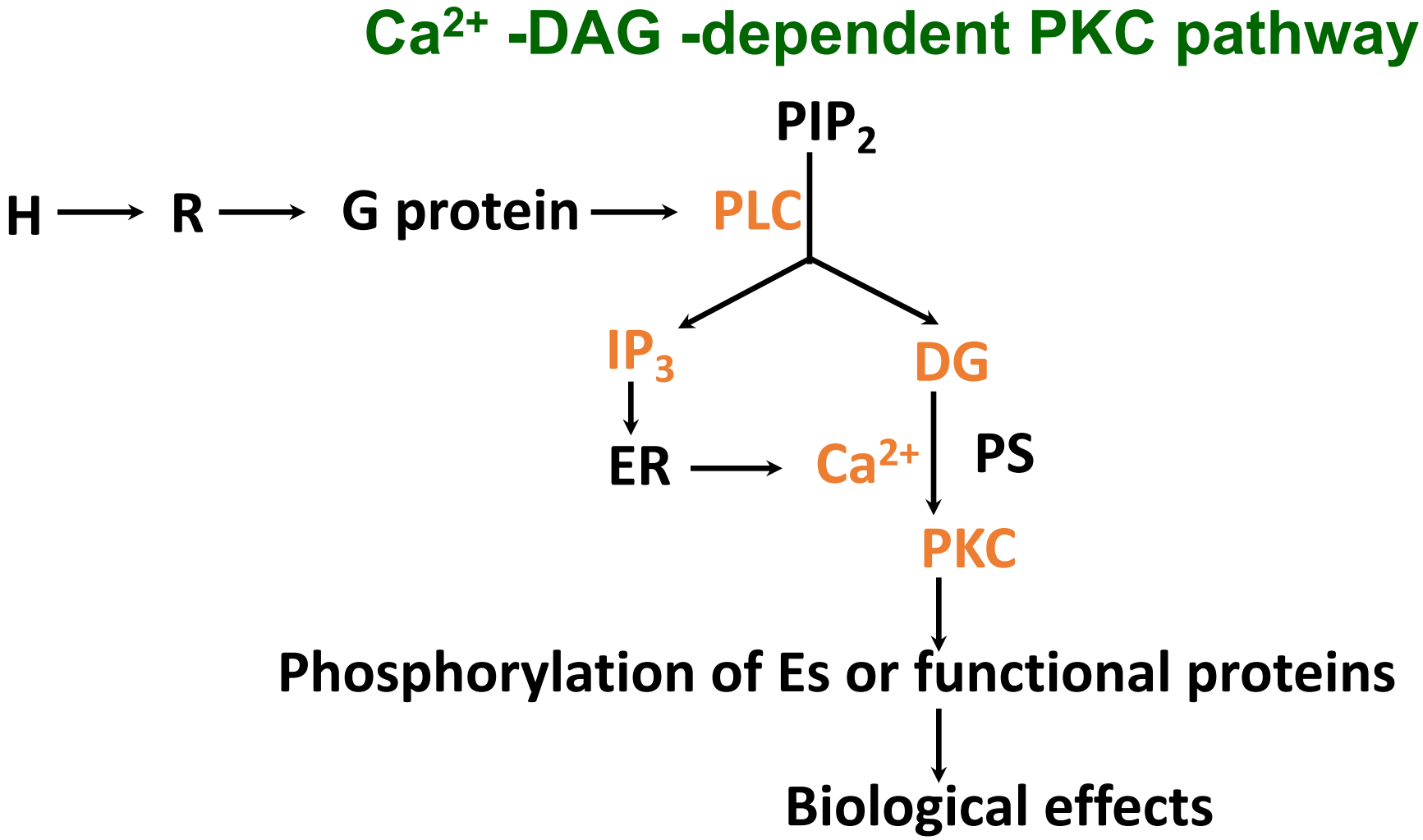
2-G-protein complex coupled with secondary messenger system other than cyclic nucleotides (group II c acts through phospholipase C (PLC)

- Certain hormone-receptor interactions result in the activation of phospholipase C(PLC) by activated specific G protein (Ga).
- PLC catalyses hydrolysis **of phosphtidyl inositol bisphosphate (PIP2)** to Diacylglycerol **(DAG)** and Inositol triphosphate **(IP_3)** (which are the second messengers generated through phospholipid metabolism)



- IP3
  - Water-soluble
    - Stimulate release of stored intracellular Ca2+ from endoplasmic reticulum (ER) and mitochondria
- DAG
  - In the presence of calcium activates protein kinase C (PKC) that phosphorylates various proteins to increase or decrease their activity.

Ca^{2+} dependent PK pathway



- Calmodulin (CaM)i s a calcium-dependent regulatory protein , Calmodulin has four Ca2+ binding sites.
- The Ca2+-calmodulin complex can activate specific kinases.
- Calmodulin kinase (CaM kinase) phosphorylation of substrates, and this leads to altered physiologic responses.
- Ca2+ can enter cells through voltage- or ligand gated Ca2+ channels.

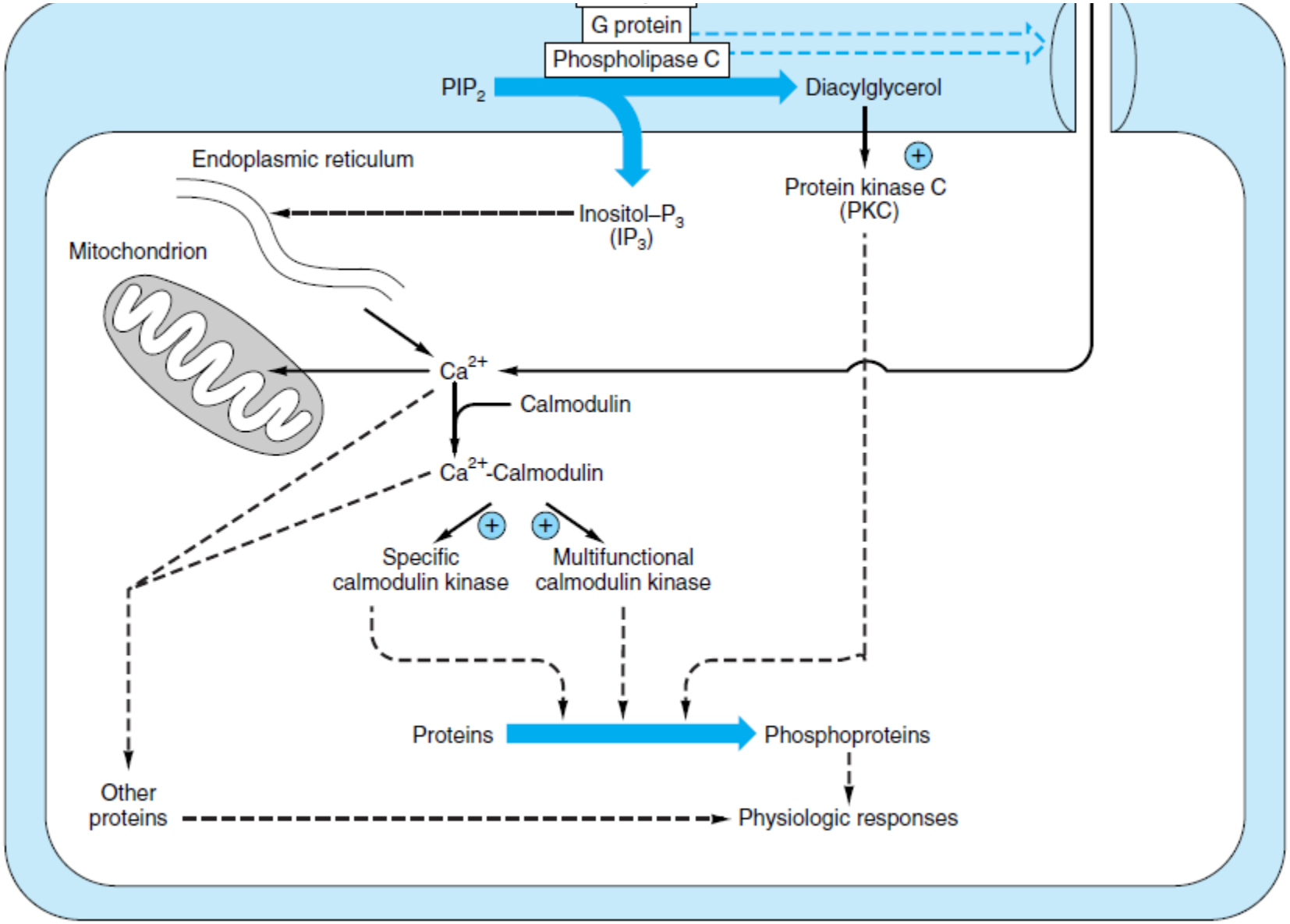
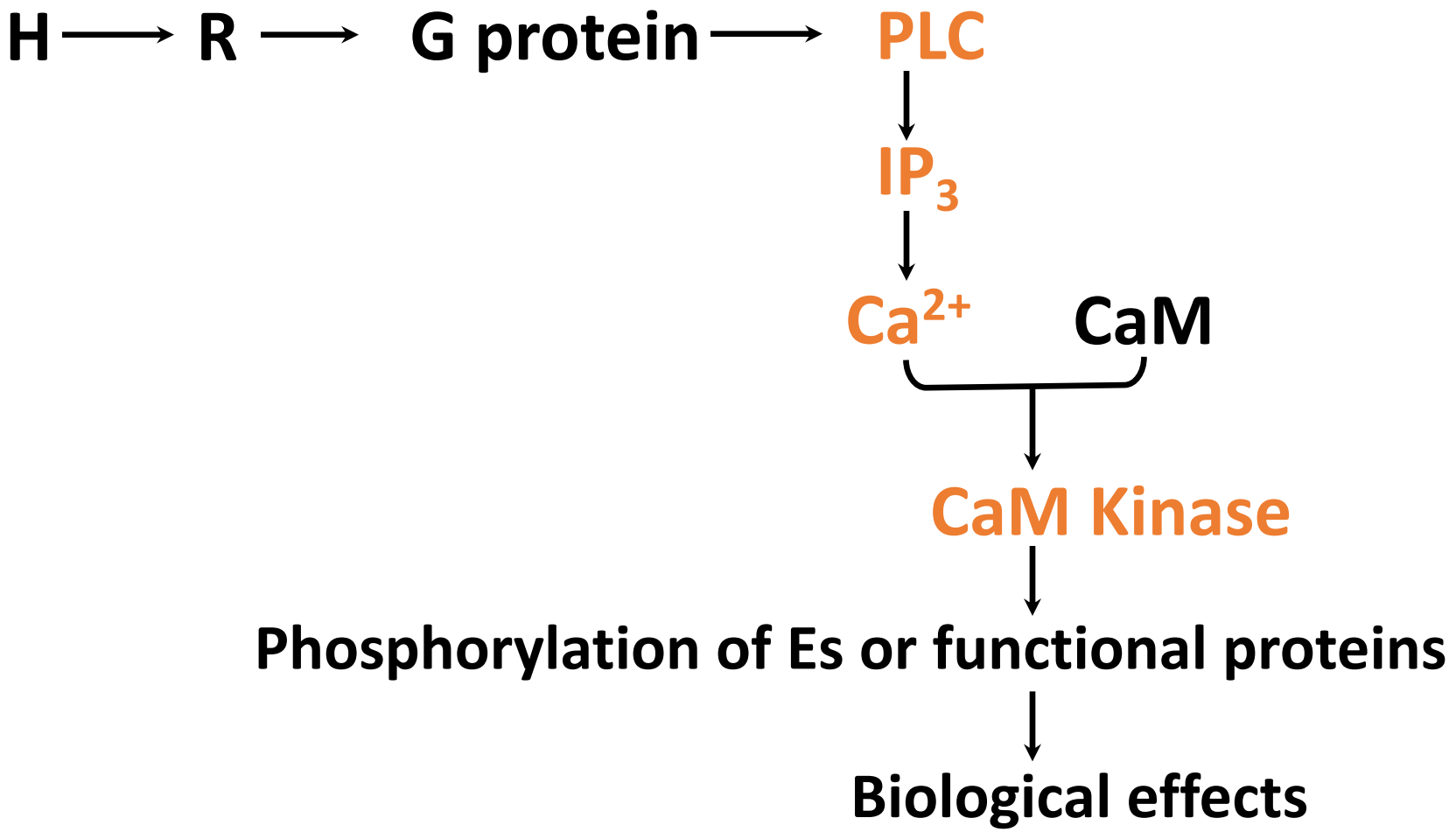


Figure 43–6. Certain hormone-receptor interactions result in the activation of phospholipase C. This ap-

Ca^{2+}-CaM dependent protein kinase pathway



Some Hormones Act Through a Protein Kinase Cascade:

Single protein kinases such as PKA, PKC, and Ca2+- calmodulin (CaM)-kinases, which result in the phosphorylation of serine and threonine residues in target proteins, play a very important role in hormone action.

Enzyme-linked receptors or Kinase Receptors

The kinase receptors are single membrane spanning receptors and contain associated kinase enzymes in the cytoplasmic domain. They are of further following types:

1.Tyrosine kinase receptors (such as: Insulin, epidermal growth factor)

2.Serine / Threonine kinase receptors (such as: Mullerian inhibitory substance, inhibin)

3.Guanylate cyclase receptors

4.Cytokine receptor family (such as: Prolactin)

Growth Hormone Binding protein (GHBP):

- It is soluble form of protein and is the extracellular domain of the full-length receptor.
- The growth hormone has two binding sites for growth hormone receptor, so when one molecule of GH binds to a receptor, the binding activates the nearby receptor to bind the second domain. Thus, one molecule of GH binds two growth hormone receptors resulting in a homodimer. This dimerization of receptors activates the intracellular signaling pathways .
- GH has multitude of functions in the body and thus uses several signaling pathways to exert its effects for example JAK2- STAT pathway

JAK2 is a tyrosine kinase and belongs to Janus family of cytoplasmic tyrosine kinases. STATs (Signal Transducers and Activators of Transcription) are cytoplasmic transcription factors. When STATs are phosphorylated by JAK kinases, they move to the nucleus and activate GH regulated genes.