Signal Transduction

Lect 3

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- **Membrane receptors**

  Membrane Glycoprotein

- **Intracellular receptors**

  Cytosol or nuclei

  DNA binding protein

- **Receptors superfamilies:**
- Ionotropic receptors (ligand-gated channels)
- Metabotropic receptors (G protein-coupled receptors)
- Tyrosine Kinase

Comparison of Ionotropic and Metabotropic Receptors

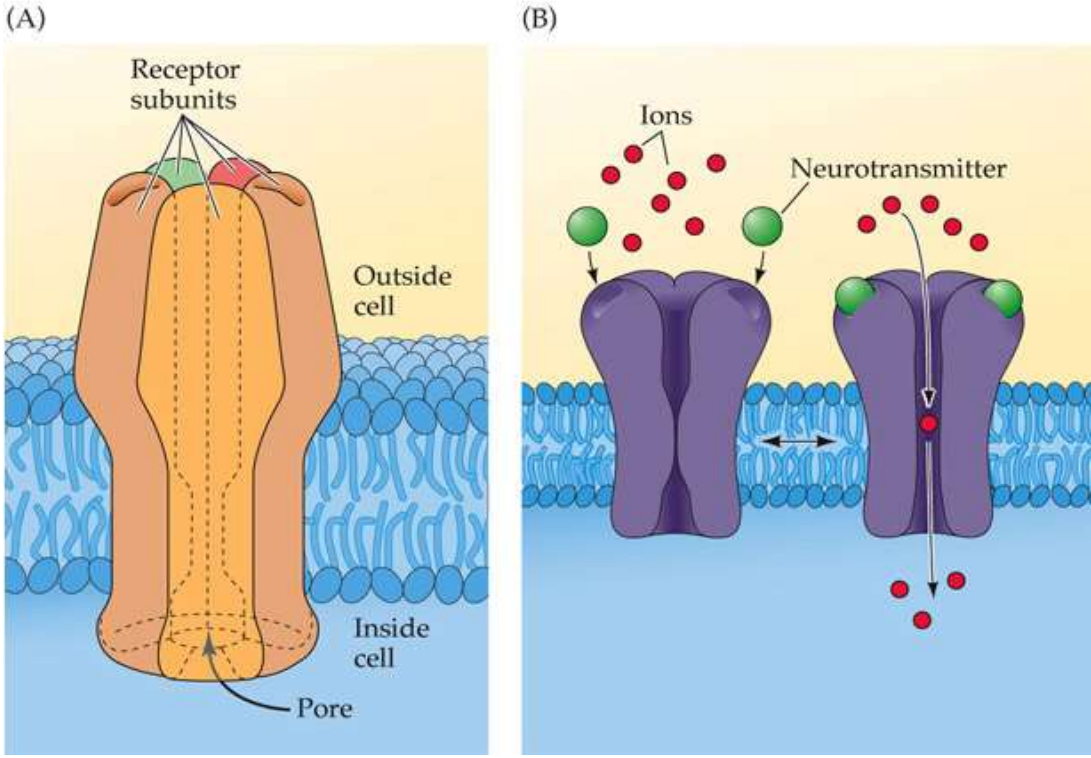
Characteristics	Ionotropic receptors	Metabotropic receptors
Structure	4 or 5 subunits that assemble in the cell membrane	1 subunit
Mechanism of action	Contain an intrinsic ion channel that opens in response to neuro-transmitter or drug binding	Activate G proteins in response to neurotrans-mitter or drug binding
Coupled to second messengers?	No	Yes
Speed of action	Fast	Slower

Almost all neurotransmitters discovered so far have more than one kind of receptor -- called **receptor subtypes.**

Ionotropic Receptors

Work very fast; important role in fast neurotransmission

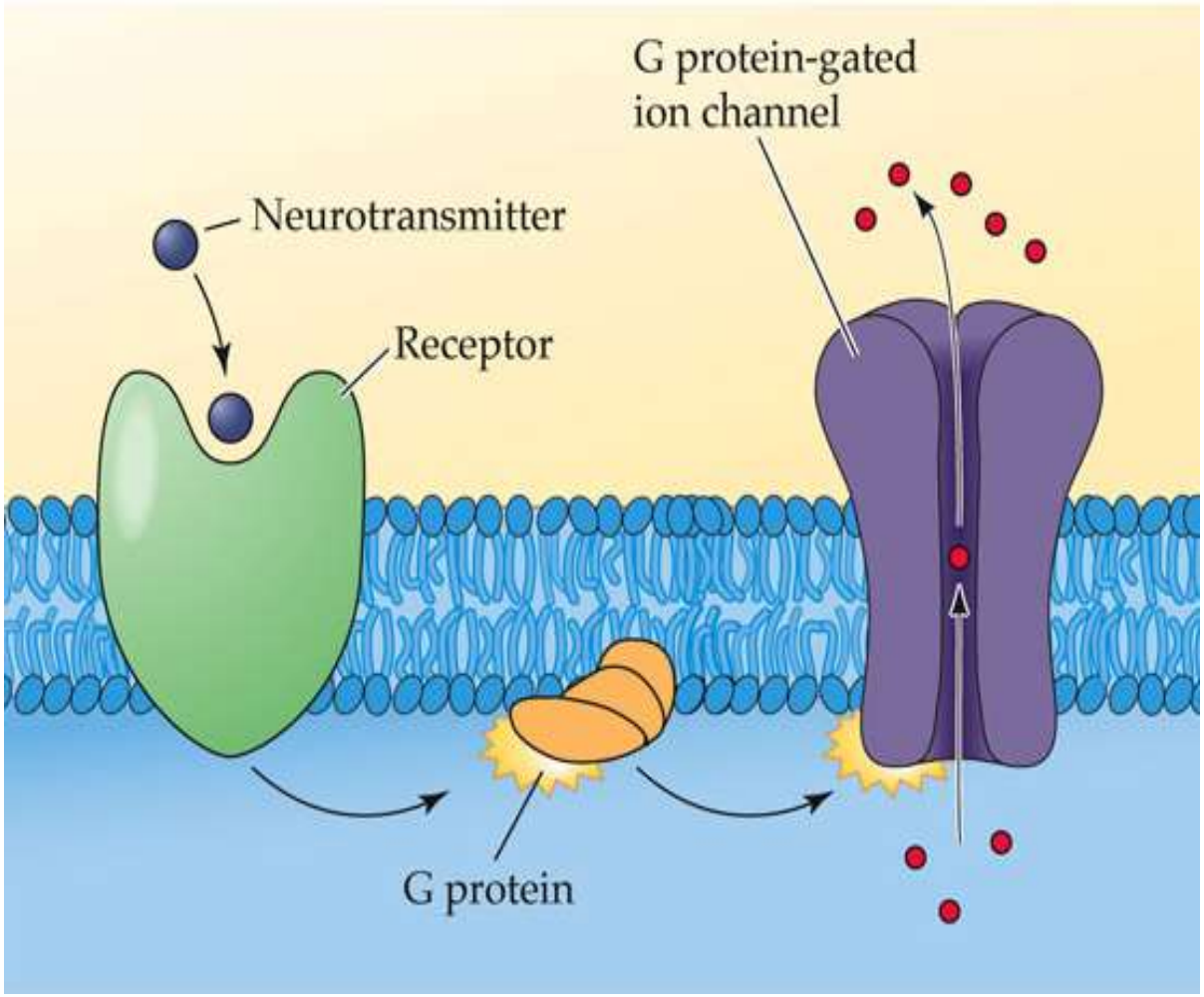
1. Each is made of several subunits (together form the complete receptor)
2. At center of receptors is channel or pore to allow flow of ions
3. At rest - receptor channels are closed
4. When neurotransmitter binds -- channel immediately opens
5. When ligand leaves binding site -- channel quickly closes



Metabotropic Receptors…

Work by activating other proteins called **G proteins**

1. Each is made of several transmembrane regions
2. Stimulate or inhibit the opening of ion channels in the cell membrane
3. Work more slowly than ionotrophic receptors but lasts longer

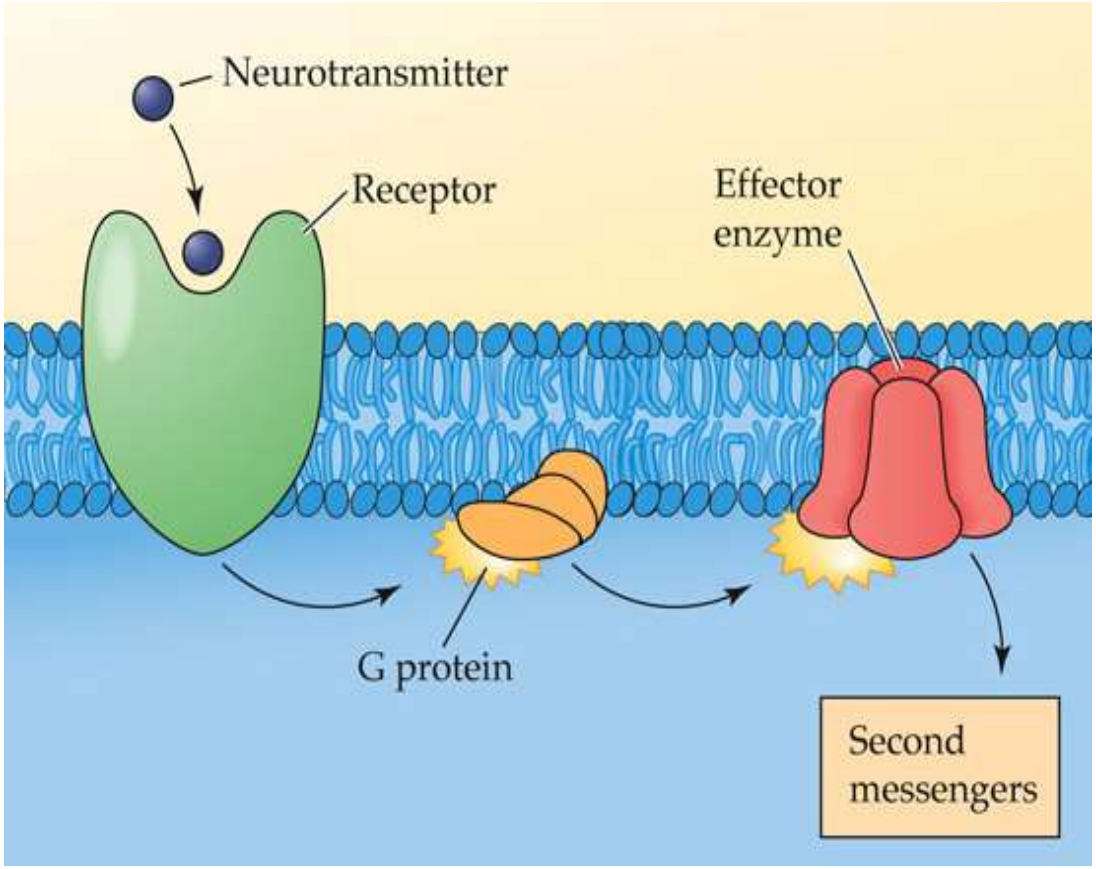


Metabotropic Receptors…

1. Stimulate or inhibit certain effector enzymes
2. Most effector enzymes controlled by G proteins are involved in synthesis of second messengers.

   *First messenger: ligand.

   *Second messenger: effector enzyme



Signaling Overview

3. Three major classes of surface receptors for signaling :

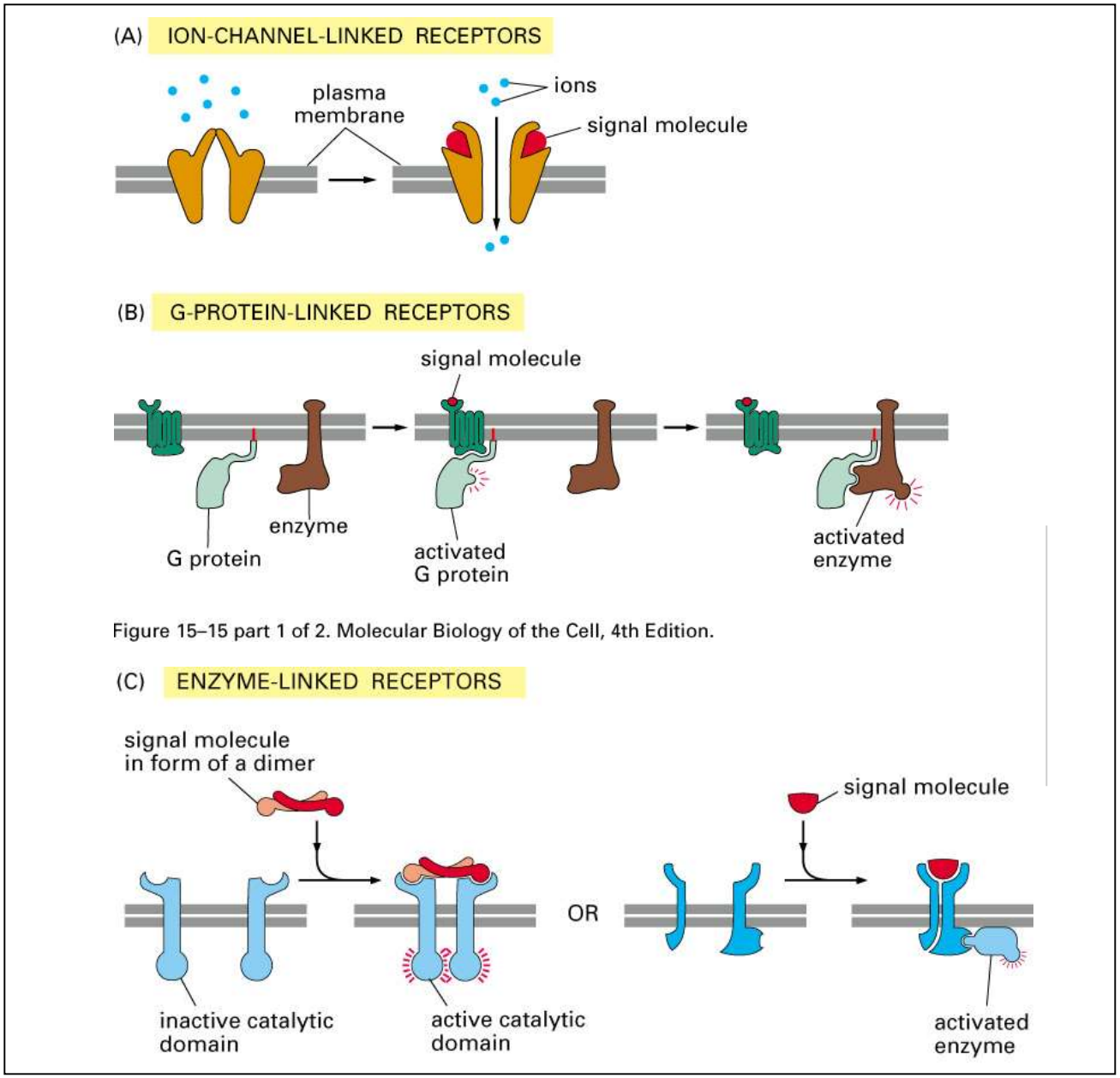
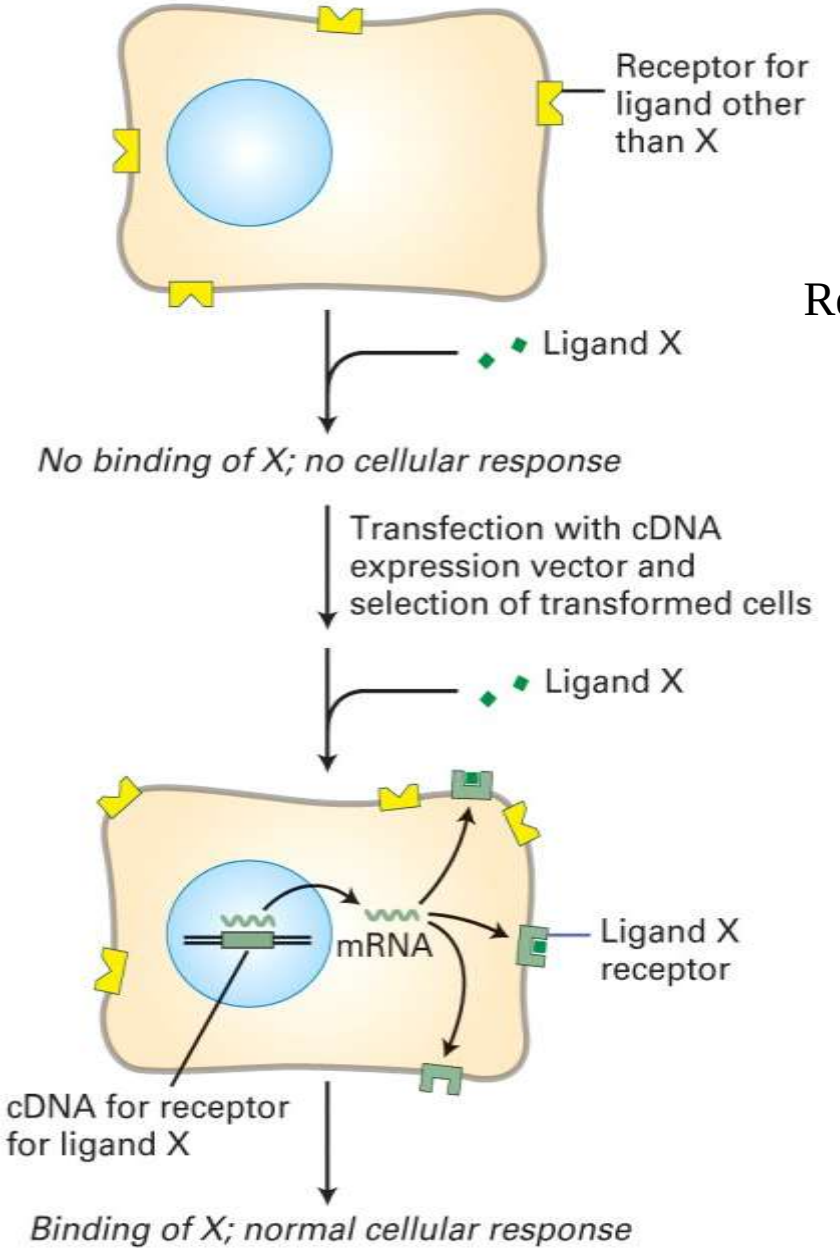


Figure 15–15 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



Receptors determine response

No receptor - no response

3. Three major classes of surface receptors for signaling, cont.:

A. Ion Channels:

B. G protein-coupled receptors (GPRs): largest family of cell surface receptors; present in all eukaryotes; ex: adrenergic receptors, opioid receptors.

1. Overview:

a. 7 trans-membrane spanning domains

b. Act as receptors for many different ligands including NT, H

c. Large amount of receptor diversity, but common mechanism of action

d. Transmit signals to intracellular targets via G proteins

e. Targets are plasma membrane bound enzymes or ion channels

2. Mechanism of Activation of GPRs:

a. Binding of ligand to extracellular domain of GPRs induces conformational change that allows cytosolic domain of the receptor to bind to inactive G protein at inner face of PM.

b. This interaction activates the G protein, which dissociates from the receptor

c. Activated G protein α subunit can now bind GTP instead of GDP, causing dissociation into activated α vs. βγ subunits. Each of these can go on to activate target proteins.

C. Enzyme-linked receptors:

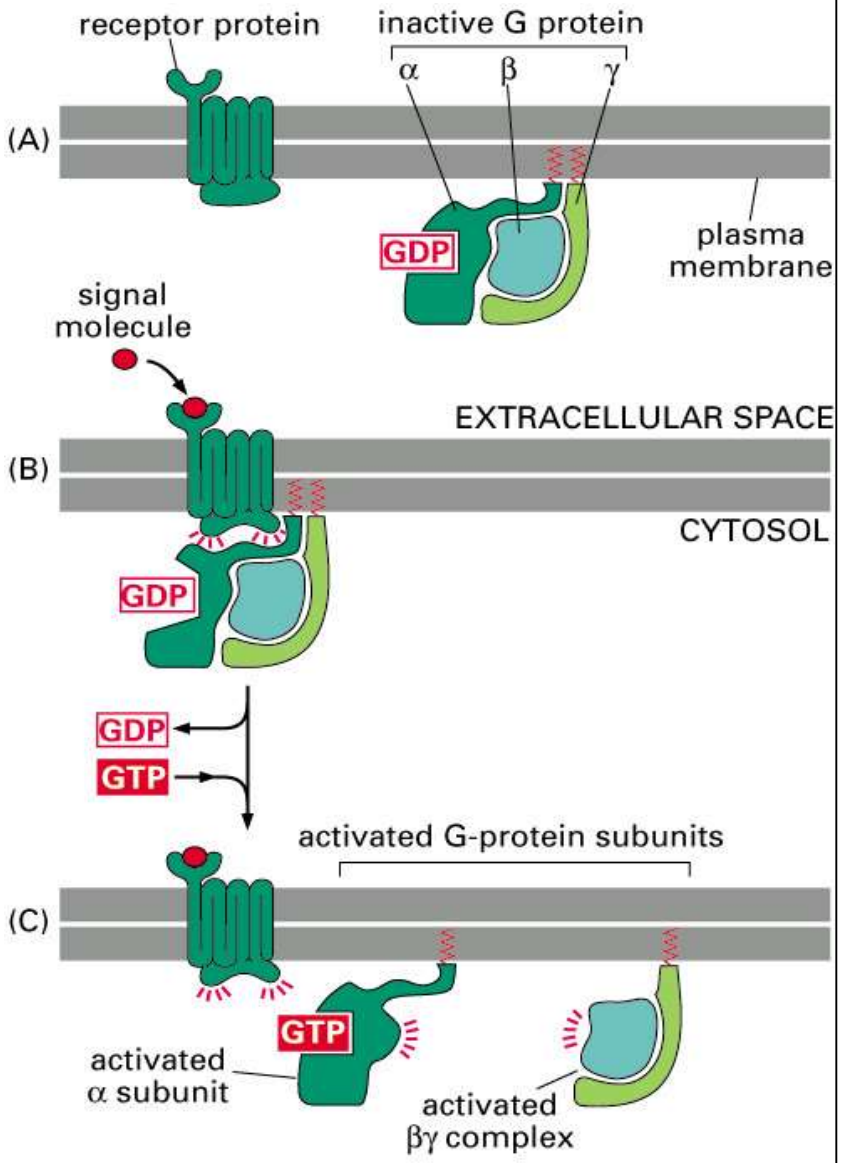
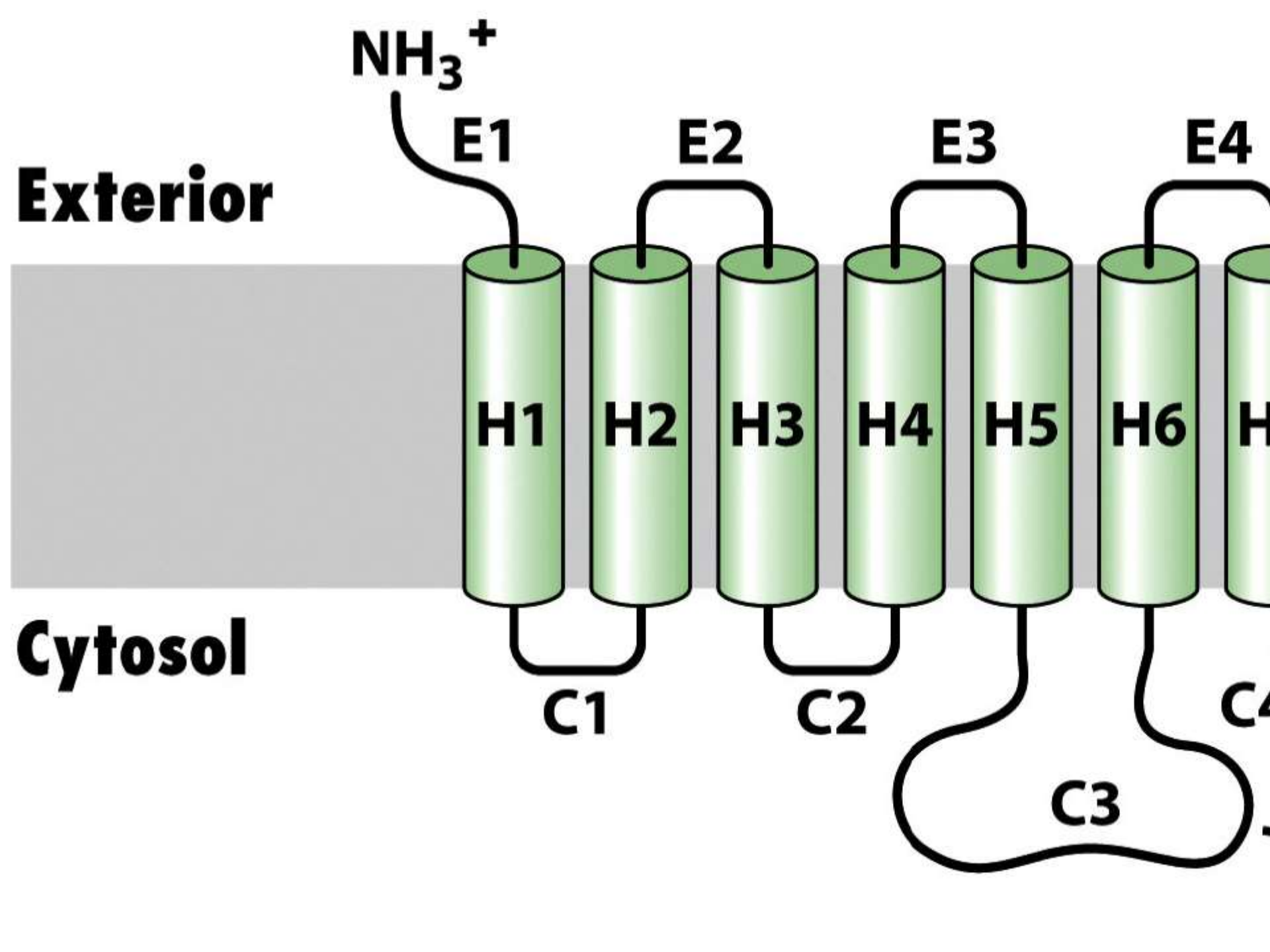
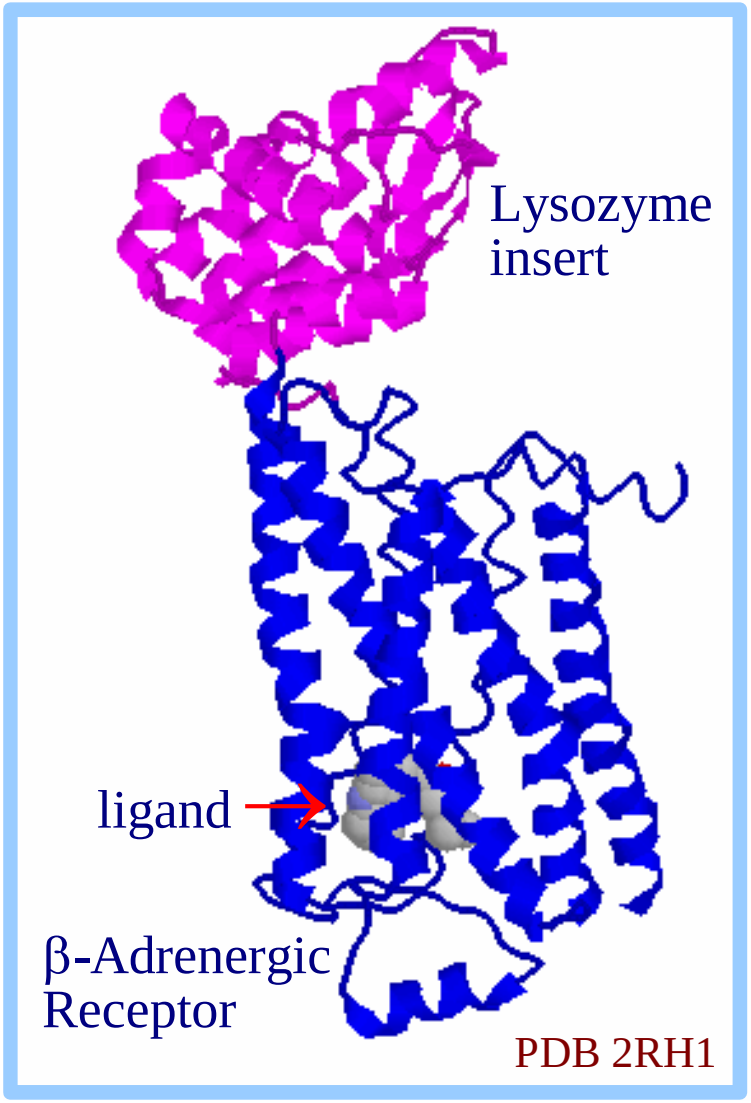


Figure 15–28. Molecular Biology of the Cell, 4th Edition.


NH3+
E1
E2
E3
E4
Exterior
H1
H2
H3
H4
H5
H6
Cytosol
C1
C2
C3

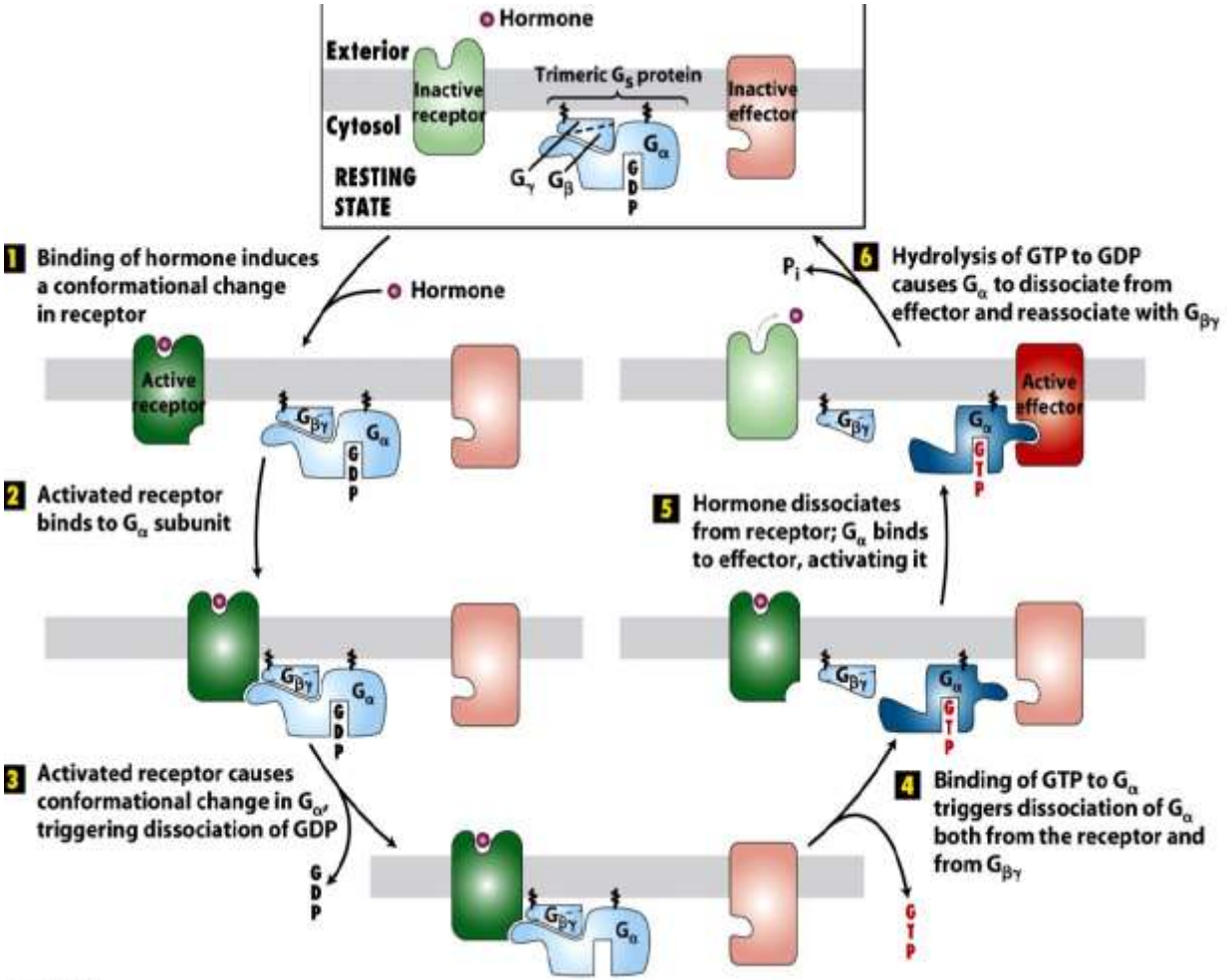
G Protein Signal Cascade



G Protein Signal Cascade

The **signal** is usually passed from a **7-helix receptor** to an intracellular **G-protein**.

- Seven-helix receptors are thus called **GPCR**, or **G-P**rotein-**C**oupled **R**eceptors.
- Approx. 800 different GPCRs are encoded in the human genome.


Hormone
Exterior
Cytosol
RESTING STATE
Inactive receptor
Trimeric G_s protein
Inactive effector
G_γ
G_β
G_α
GDP
1 Binding of hormone induces a conformational change in receptor
Hormone
Active receptor
$G_{\beta\gamma}$
2 Activated receptor binds to G_α subunit
3 Activated receptor causes conformational change in G_α, triggering dissociation of GDP
4 Binding of GTP to G_α triggers dissociation of G_α both from the receptor and from $G_{\beta\gamma}$
GTP
5 Hormone dissociates from receptor; G_α binds to effector, activating it
Active effector
6 Hydrolysis of GTP to GDP causes G_α to dissociate from effector and reassociate with $G_{\beta\gamma}$
P_i

igure 15-13

G Protein Signal Cascade

- **G-proteins** are **heterotrimeric**, with 3 subunits α, β, γ.
- A G-protein that activates cyclic-AMP formation within a cell is called a **stimulatory G-protein**, designated $\mathbf{G_s}$ with alpha subunit $\mathbf{G_{s\alpha}}$.
- $\mathbf{G_s}$ is activated, e.g., by receptors for the hormones **epinephrine** and **glucagon**.

  The **β-adrenergic receptor** is the **GPCR** for epinephrine.

TABLE 15-1 Major Classes of Mammalian Trimeric G Proteins and Their Effectors*

G_α CLASS	ASSOCIATED EFFECTOR	2ND MESSENGER	RECEPTOR EXAMPLES
$G_{\alpha s}$	Adenylyl cyclase	cAMP (increased)	β-Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin
$G_{\alpha i}$	Adenylyl cyclase K^+ channel ($G_{\beta\gamma}$ activates effector)	cAMP (decreased) Change in membrane potential	α_2-Adrenergic receptor Muscarinic acetylcholine receptor
$G_{\alpha olf}$	Adenylyl cyclase	cAMP (increased)	Odorant receptors in nose
$G_{\alpha q}$	Phospholipase C	IP_3, DAG (increased)	α_1-Adrenergic receptor
$G_{\alpha o}$	Phospholipase C	IP_3, DAG (increased)	Acetylcholine receptor in endothelial cells
$G_{\alpha t}$	cGMP phosphodiesterase	cGMP (decreased)	Rhodopsin (light receptor) in rod cells

*A given G_α subclass may be associated with more than one effector protein. To date, only one major $G_{\alpha s}$ has been identified, but multiple $G_{\alpha q}$ and $G_{\alpha i}$ proteins have been described. Effector proteins commonly are regulated by G_α but in some cases by $G_{\beta\gamma}$ or the combined action of G_α and $G_{\beta\gamma}$.
IP_3 = inositol 1,4,5-trisphosphate; DAG = 1,2-diacylglycerol.
SOURCES: See L. Birnbaumer, 1992, *Cell* **71**:1069; Z. Farfel et al., 1999, *New Eng. J. Med.* **340**:1012; and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.

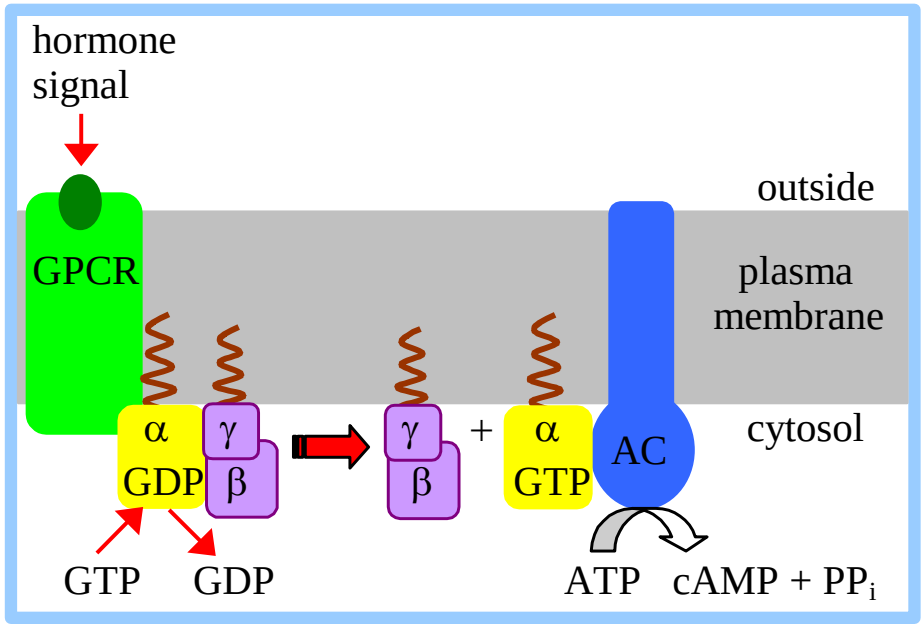
Table 15-1
Molecular Cell Biology, Sixth Edition

Summary of Hormones signaling pathways

IP_3	**cAMP**	**cGMP**	**Tyrosine kinase - intrinsic**	**Tyrosine kinase - receptor associated**	**Steroid**
GnRH	FSH	ANP	Insulin	Prolactin	Glucocorticoid
Gastrin	LH	NO (EDRF)	IGF-1	Cytokines (IL-2,6,8)	Estrogen
Oxytocin	ACTH		FGF	GH	Progesterone
TRH	TSH		PDGF		Testosterone
ADH (V_1)	CRH				Aldosterone
Histamine (H_1)	hCG				Vitamin D
Angiotensin II	PTH				T_3/T_4
	Calcitonin				Cortisol
	Glucagon				
	GHRH (can act via IP_3 as well)				

G Protein Signal Cascade

- The α subunit of a G-protein (G_α) binds **GTP**, & can hydrolyze it to GDP + P_i.



- α & γ subunits have covalently attached **lipid anchors** that bind a G-protein to the plasma membrane cytosolic surface.
- **Adenylate Cyclase** (AC) is a transmembrane protein, with cytosolic domains forming the catalytic site.

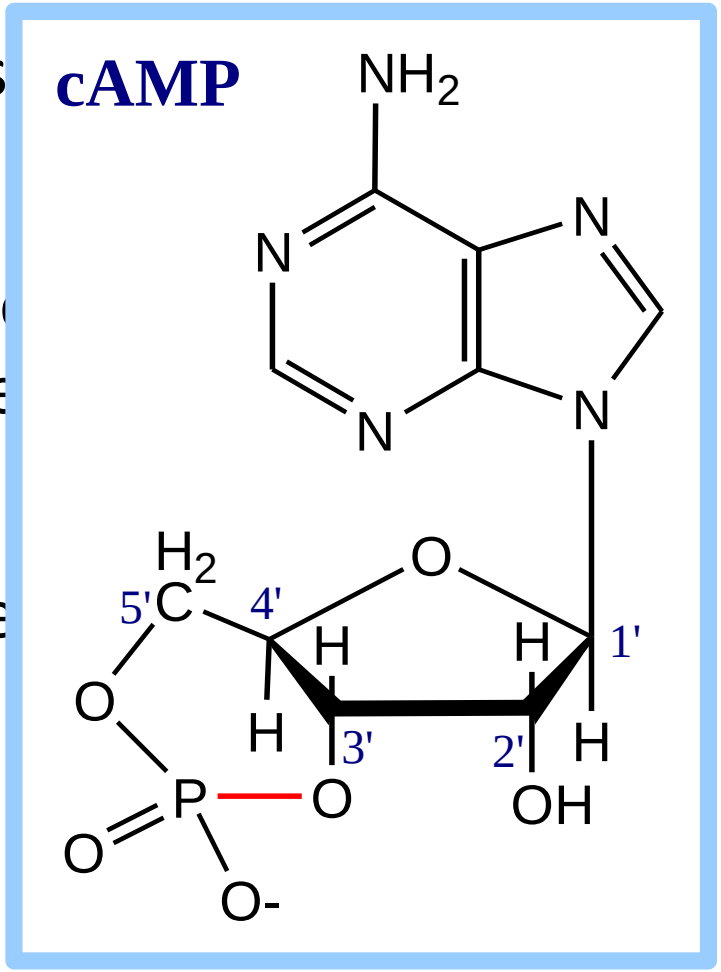
Adenylate Cyclase

Adenylate Cyclase (Adenylyl Cyclas

ATP → cAMP + PP_i

Binding of certain **hormones** (e.g.,
outer surface of a cell activates Ade
form cAMP within the cell.

Cyclic AMP is thus considered to be
messenger.

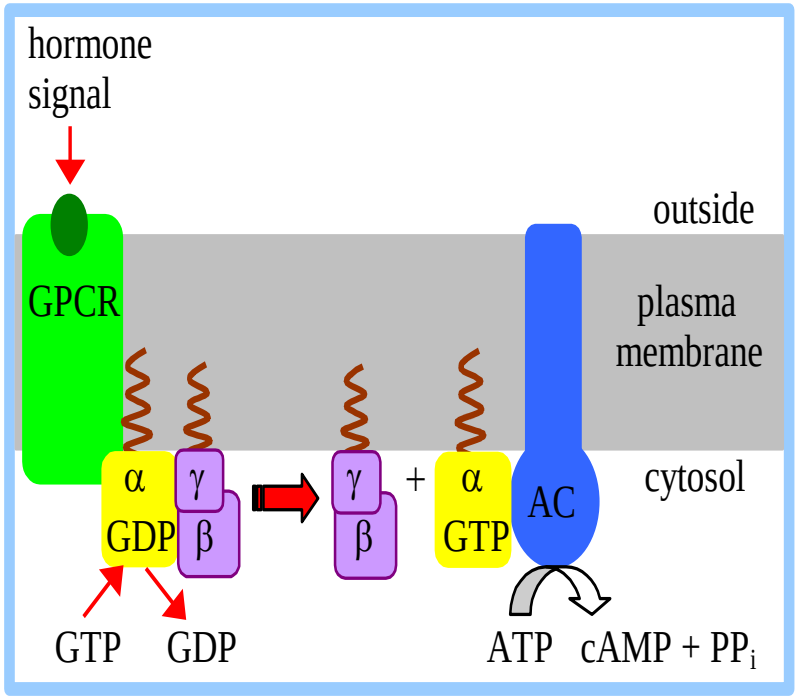


G Protein Signal Cascade

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

1. Initially **G_α** has bound **GDP**, and **α**, **β**, & **γ** subunits are complexed together.

$G_{\beta,\gamma}$, the complex of β & γ subunits, **inhibits G_α**.

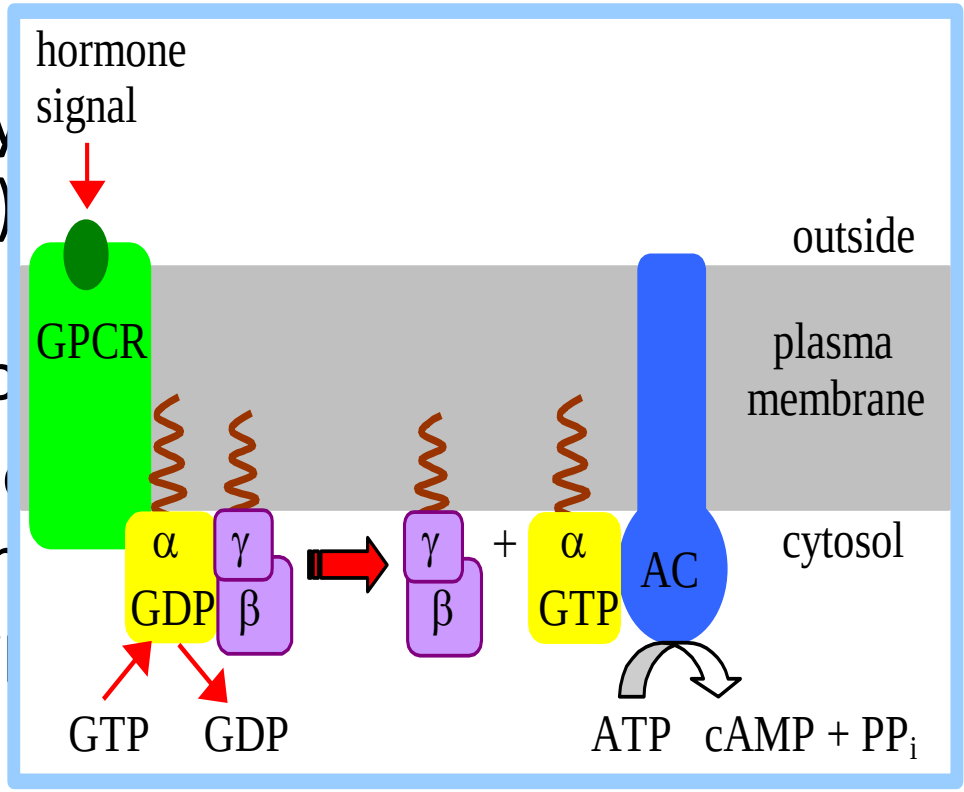


G Protein Signal Cascade

2. **Hormone binding**, usually
of a 7-helix receptor (GPCR)
change in the receptor that
protein on the cytosc

The nucleotide-binding site
accessible to the cytosol, wh

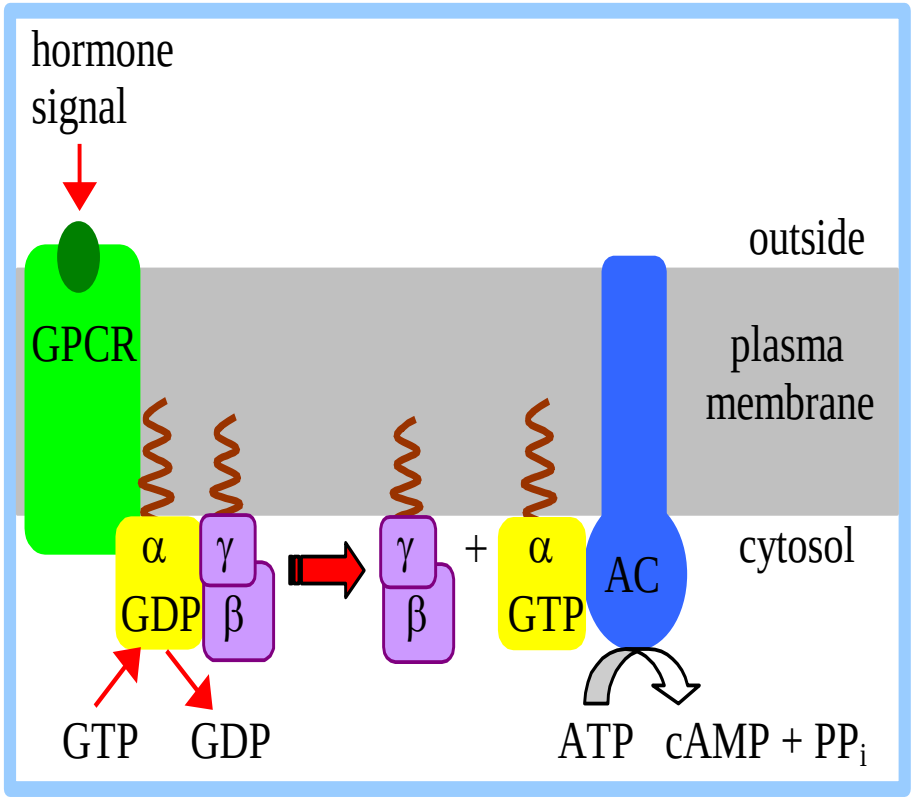
G_α releases GDP & binds GT



G Protein Signal Cascade

3. Substitution of **GTP** for GDP causes another conformational change in **G_α**.

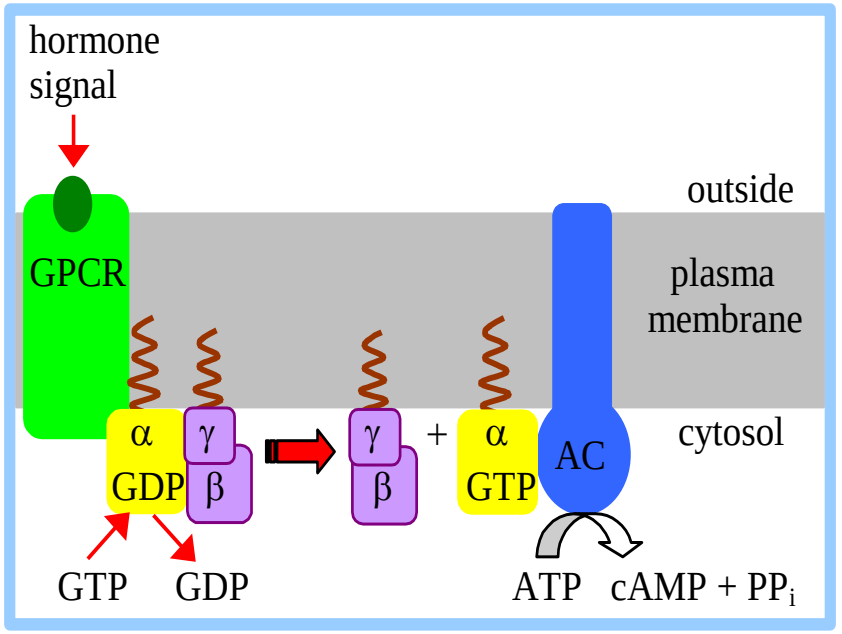
G_α-GTP dissociates from the inhibitory **βγ** complex & can now bind to and activate **Adenylate Cyclase**.

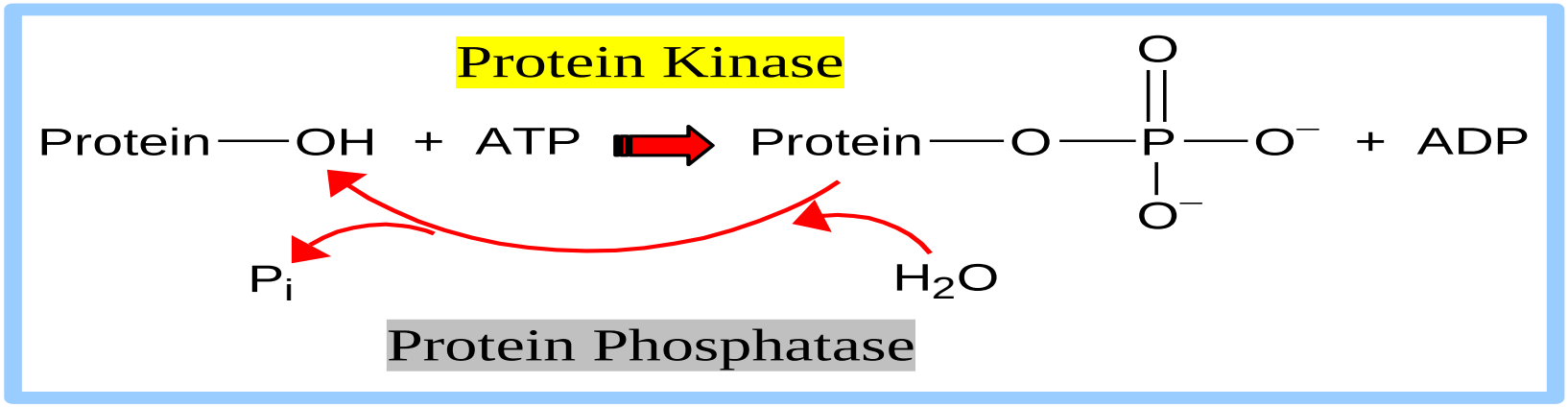


G Protein Signal Cascade

4. **Adenylate Cyclase**, activated by the stimulatory G_{α}-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.





Protein kinases and phosphatases are themselves regulated by complex signal cascades. For example:

- Some protein kinases are activated by **Ca^{++}-calmodulin**.
- **Protein Kinase A** is activated by **cyclic-AMP** (cAMP).

Protein Kinase A (cAMP-Dependent Protein Kinase) transfers P_i from ATP to OH of a Ser or Thr in a particular 5-amino acid sequence.

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

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

R_2C_2 : 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.

PKIs, Protein Kinase Inhibitors, modulate activity of the catalytic subunits (**C**).

Turn off of the signal:

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

   The presence of **GDP** on G_α causes it to rebind to the inhibitory **$\beta\gamma$** complex.

   Adenylate Cyclase is no longer activated.

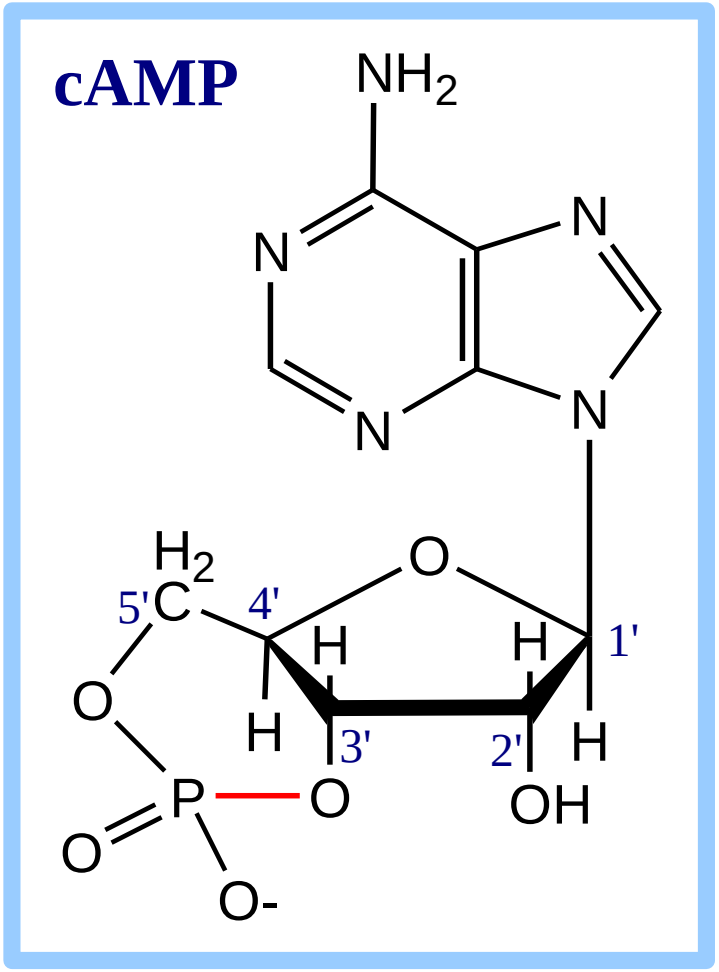
2. **Phosphodiesterases** catalyze hydrolysis of **cAMP → AMP**.

Phosphodiesterase enzymes catalyze:

cAMP + H_2O → AMP

The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalyzed by Protein Kinase A.

Thus **cAMP stimulates its own degradation**, leading to rapid turnoff of a cAMP signal.



3. **Receptor desensitization** varies with the hormone.

- In some cases the **activated receptor** is **phosphorylated** via a G-protein Receptor Kinase.
- The phosphorylated receptor then may bind to a protein **β-arrestin.**
- **β-Arrestin** promotes **removal of the receptor** from the membrane by clathrin-mediated endocytosis.
- **β-Arrestin** may also bind a cytosolic **Phosphodiesterase**, bringing this enzyme close to where cAMP is being produced, contributing to signal turnoff.

4. **Protein Phosphatase** catalyzes removal by hydrolysis of phosphates that were attached to proteins via Protein Kinase A.

- **Different** isoforms of **G_α** have different signal roles. E.g.:
  - The **stimulatory $G_{s\alpha}$**, when it binds GTP, **activates** Adenylate cyclase.
  - An **inhibitory $G_{i\alpha}$**, when it binds GTP, **inhibits** Adenylate cyclase.

    .

- The complex of **$G_{\beta,\gamma}$** that is released when G_α binds GTP is itself an effector that binds to and **activates or inhibits** several other proteins.

  E.g., **$G_{\beta,\gamma}$ inhibits** one of several isoforms of **Adenylate Cyclase**, contributing to rapid signal turnoff in cells that express that enzyme.

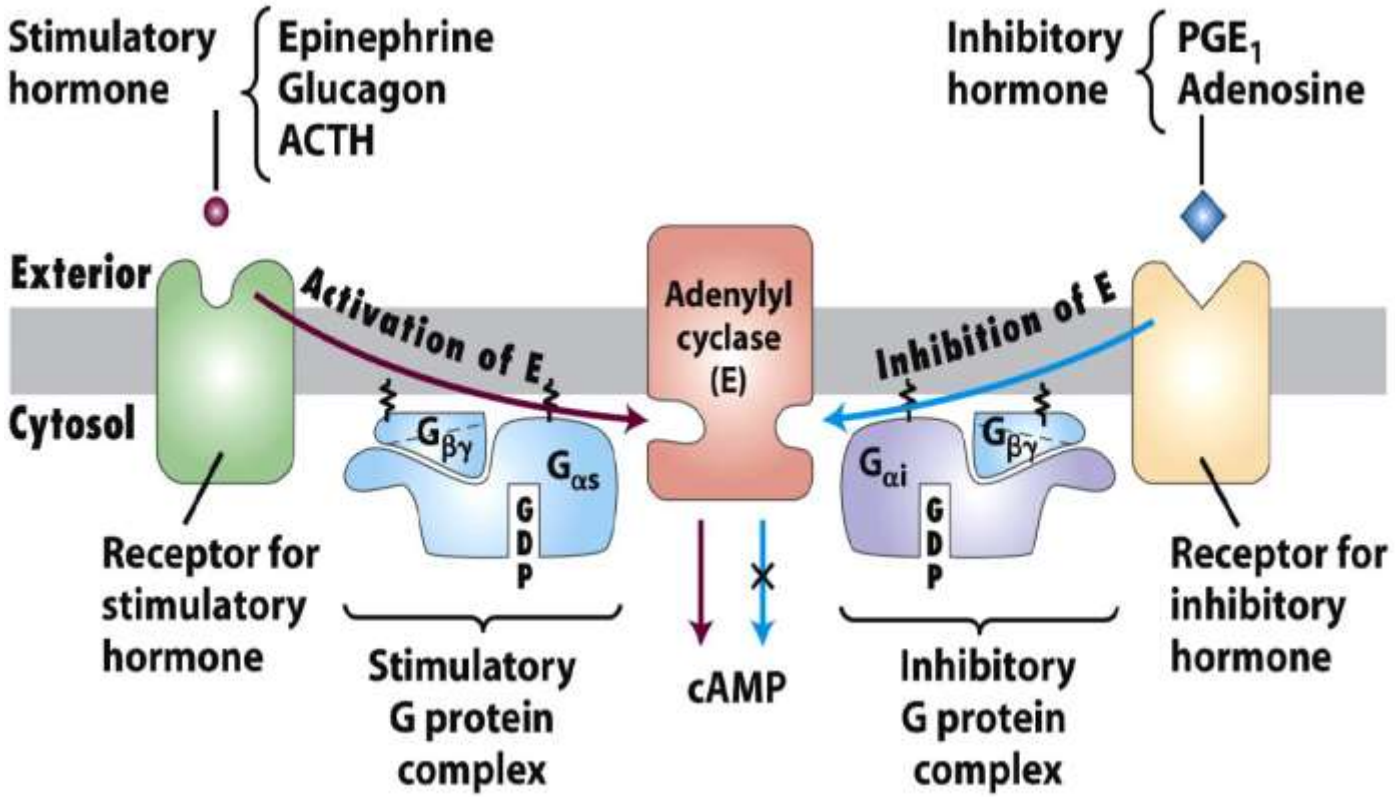

Stimulatory hormone
Epinephrine
Glucagon
ACTH
Inhibitory hormone
PGE_1
Adenosine
Exterior
Cytosol
Activation of E
Inhibition of E
Adenylyl cyclase (E)
$G_{\beta\gamma}$
$G_{\alpha s}$
$G_{\alpha i}$
G D P
Receptor for stimulatory hormone
Receptor for inhibitory hormone
Stimulatory G protein complex
cAMP
Inhibitory G protein complex

Figure 15-21