no.17

Physiology

Doctor 2019 | Medicine | JU

◉ Sheet ○ Slides

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There are three major classes of surface receptors for signaling :

1. Ion Channels.

2. G protein-coupled receptors (GPRs):

➞ They are the largest family of cell surface receptors and present in all eukaryotes. Examples of this type are: Adrenergic Receptors (Remember that catecholamine bind to those receptors) and opioid receptors.

• General characteristics of these receptors:

(these were fully explained during the last lecture so we'll only focus on the 1st one).

a.7 trans-membrane spanning –helical- domains and a ligand binding site on the extracellular domain while intracellularly it interacts with a group of proteins called the G proteins.

NOTE: The inactive form of a G protein complex is composed of Gα, Gβ and Gγ subunits. In this form Gα is bound to GDP **(guanosine diphosphate)** and both Gβ and Gγ (which are always found together as a complex) are bound to the α subunit, that just happens in inactive state.

Once a hormone is bound to the receptor it will induce conformational changes to the 7 helical transmembrane receptor and this conformational change will activate the receptor and cause it to bind to the α subunit of the G protein complex.

This will activate the α subunit as well (a conformational change also in it) resulting in the replacement of GDP by GTP.

Once the α subunit binds to GTP it will dissociate from both the receptor and **Gβ,γ complex** -which we can think of as an inhibitor for the α subunit-.

Then the α subunit binds to an effector activating it. Also the **Gβ,γ** complex might bind to effectors sometimes changing them.

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

c. large amount of receptor diversity, but common mechanism reaction.

d. Transmit signals to intracellular targets via G proteins.

e. Targets are plasma membrane bound enzymes or ion channels.

Mechanism of Activation of GPRs:

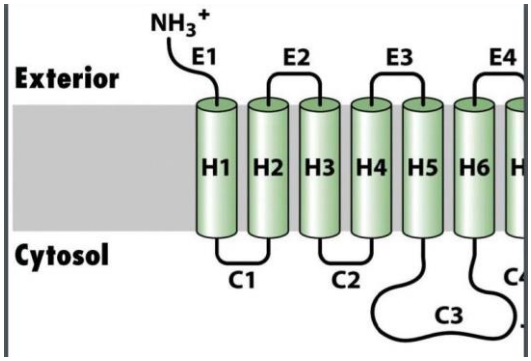
a. Binding of ligand to extracellular domain of GPRs induces conformational change that allows cytosolic domain of the receptor to bind to inactive G protein at inner face of PM.

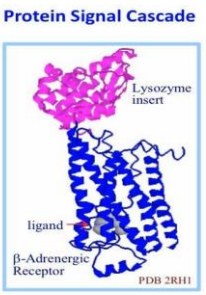
b. This interaction activates the G protein, which dissociates from the receptor.

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.

3. Enzyme -linked receptors.





This is the classical structure of a G-protein coupled receptor. You can see the 7 helical transmembrane domains The N-terminal extracellular domain is the one that binds to specific ligands. Also the C-terminal domain have binding domains with a G-protein complex.

This is an example of a G-protein receptor which is the B-Adrenergic Receptor.

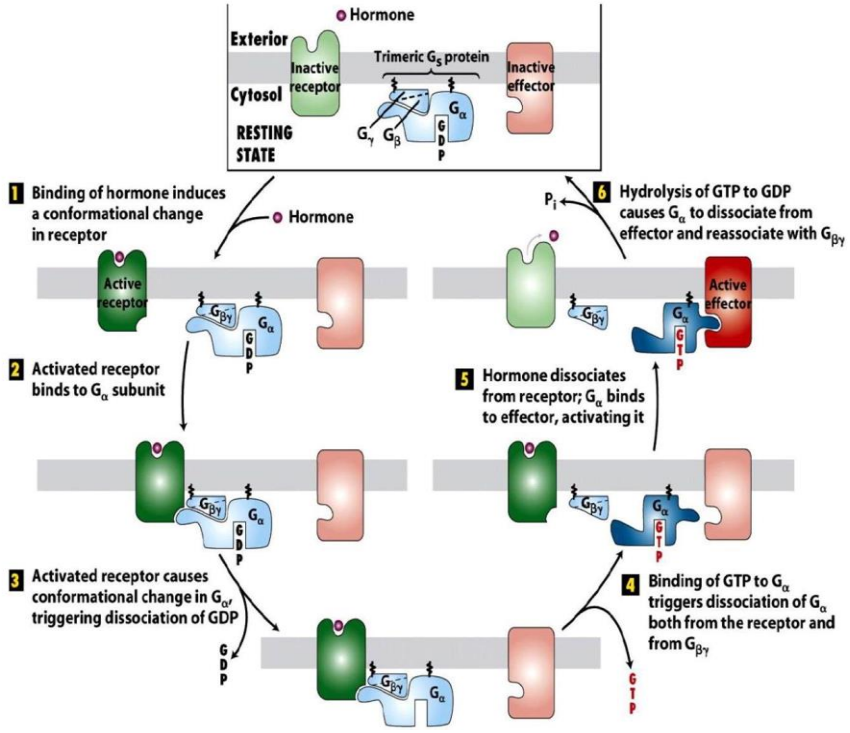
G Protein Signal Cascade

The signal is usually passed from a 7-helix

✓ receptor to an intracellular G-protein.

✓ Seven-helix receptors are thus called GPCR, or G-Protein-Coupled Receptors.

✓Approx. 800 different GPCRs are encoded in the human genome but they all have the same mechanism of actions.



Notes:

♥ Please study the figure above carefully.

-The effector is most likely to be an enzyme spanning the plasma membrane.

-Not all types of α subunits activate the corresponding effector and not all of them have the same effector.

-Immediately after Gα activates the effector, it must become inactivated by the hydrolysis of GTP that is bound to it and thus it is replaced by GDP and it rebinds with the **Gβ,γ** complex, and that complex will anchor to plasma membrane and separate from the receptor until there is signal that will bind to the receptor .

✓ 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 –the first type of G proteins- designated Gs with α subunit Gsα .

-The name of the protein that's activated by the stimulatory α subunit and that converts AMP to cAMP is Adenylate Cyclase.

-The cAMP is considered a 2nd messenger here.

-The receptor in this case is called G-Protein coupled receptor to a Gsα .

- Gs is activated, e.g., by receptors for the hormones epinephrine and glucagon.

-The β-adrenergic receptor is the GPCR for epinephrine- the 1st messenger-.

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

Notes:

♥ Please memorize the ones with a star next to them only.

- In Gαi the (i) means that it is inhibitory.

- Notice that if either epinephrine or norepinephrine binds to α2-Adrenergic Receptor, this leads to the inhibition of Adenylyl cyclase –decreased cAMP-. While if either one binds to β-Adrenergic receptor then this leads to the stimulation of Adenylyl cyclase –increased cAMP- .

Meaning that the same hormone might cause exact opposite actions and signals depending on the receptor it binds to.

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)				

Notes:

<u>You should memorize the first two colomns (IP3 & cAMP columns) FOR NOW. Others will be discussed later on.</u>

- In the first column for example, GnRH binds to a 7 transmembrane receptor called the α2-Adrenergic Receptor, which is bound to Gαq that dissociates from the **Gβ,γ** complex and binds with an effector called Phospholipase C that produces IP3.
- GHRH (Growth hormone releasing hormone) can act via IP3 (Gq) or via cAMP (Gs).

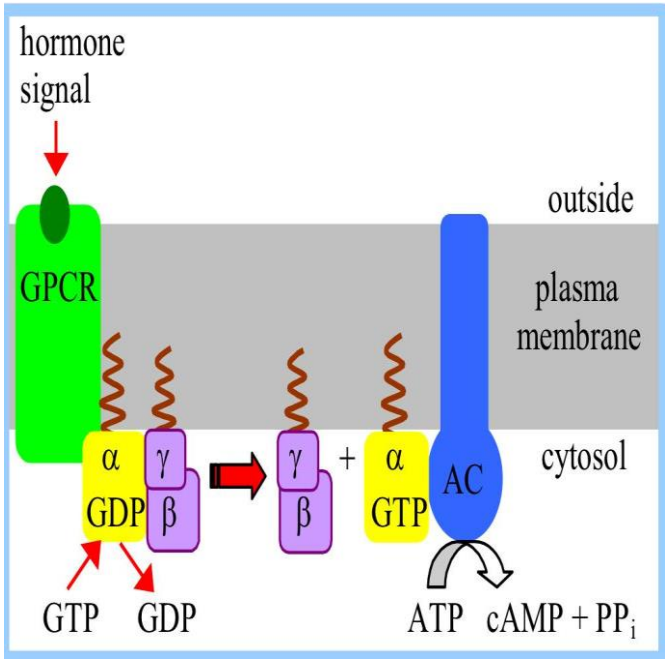
G Protein Signal Cascade

-the α subunit of a G protein binds GTP, & can hydrolyze it to GPD + Pi.

-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 is inhibited by Gαi and is activated by Gαs.



G Protein Signal Cascade:

The sequence of events by which a hormone activates cAMP signaling:
(study the following steps with the previous figure).

1. Initially Gα has bound GDP and it replaced by GTP when it activated, and α, β, & γ subunits are complexed together. Gβ,γ , the complex of β & γ subunits, inhibits Gα .
2. **Hormone binding,** usually to an extracellular domain of a 7-helix receptor (GPCR), cause a **conformational change** in the receptor that is transmitted to a **G-protein** on the cytosolic side of membrane.

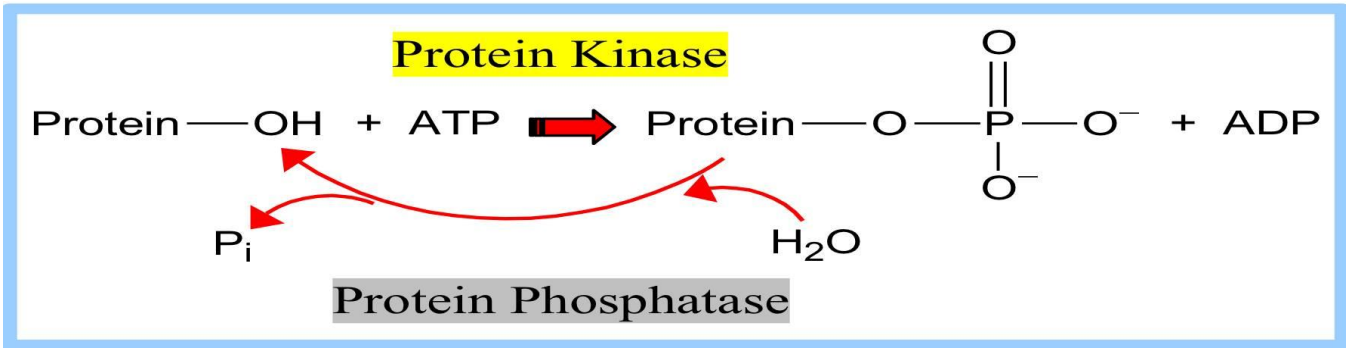
The nucleotide-binding site on Gα becomes more accessible to the cytosol, where [GTP] --> [GDP].

Gα releases GDP & binds GTP (GDP-GTP exchange).

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

Gα -GTP dissociates from the inhibitory βγ complex & can now bind to and activate Adenylate Cyclase to increase synthesis of cAMP.

4. **Adenylate Cyclase**, activated by the stimulatory Gα -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 protein phosphatases do the exact opposite thing, as protein kinases phosphorylate the protein using ATP (note the phosphate group that is added to the protein in the products).However, protein phosphatases remove the phosphate groups from proteins.

Protein kinases and phosphatases are themselves regulated (switched on and off) 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 Pi from ATP to OH of a Ser or Thr in a particular 5-amino acid sequence.
(domains and subunits and the way it works are for your general knowledge, you don't have to memorize them ☺).

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

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

R2C2 : 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:

(so that the cell can be receptive for another stimulus after the first signal causes the required action.)

this could be achieved using two ways:

1. **Gα** hydrolyzes GTP to GDP + Pi . (**GTPase**). The presence of **GDP** on Gα causes it to rebind to the inhibitory βγ complex. Adenylate Cyclase is no longer activated.
2. **Phosphodiesterases** catalyze hydrolysis of **cAMP to AMP**.

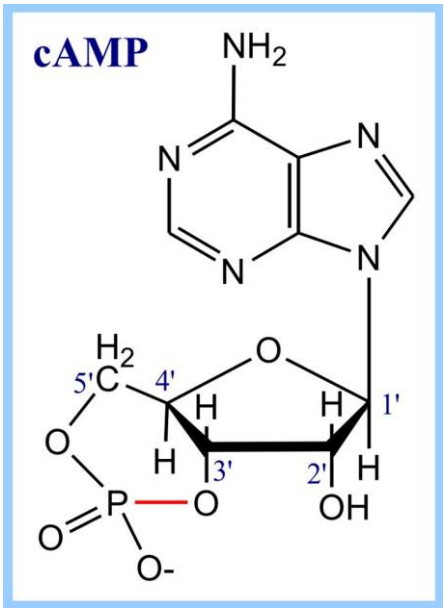
Phosphodiesterase enzymes catalyze:

cAMP + H2O ➞ AMP.

(hydrolysis of cAMP).

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. (if the enzyme was activated it inhibits it).

- **Different** isoforms of **Gα** have different signal roles. E.g.:

• The **stimulatory Gsα** , when it binds GTP**, activates** Adenylate cyclase.

• An **inhibitory Giα** , when it binds GTP, **inhibits** Adenylate cyclase.

-The complex of **Gβ,γ** that is released when Gα binds GTP is itself an effector that binds to and **activates or inhibits** several other proteins.

E.g., **Gβ,γ** inhibits one of several isoforms of **Adenylate Cyclase**, contributing to rapid signal turn off in cells that express that enzyme.

Signaling Overview

As important as turning signaling ON is turning signaling OFF:

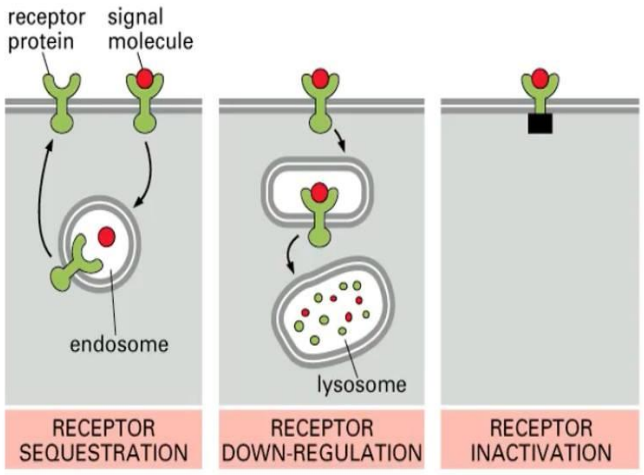


Figure 15–25 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

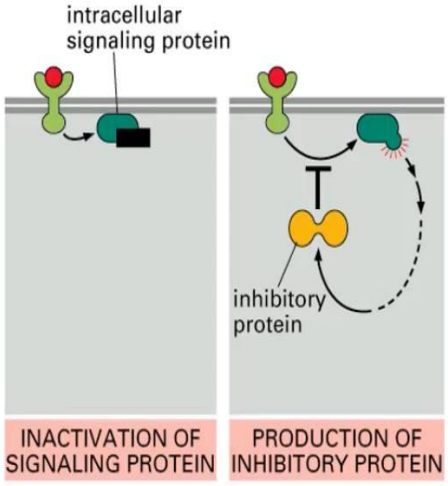


Figure 15–25 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

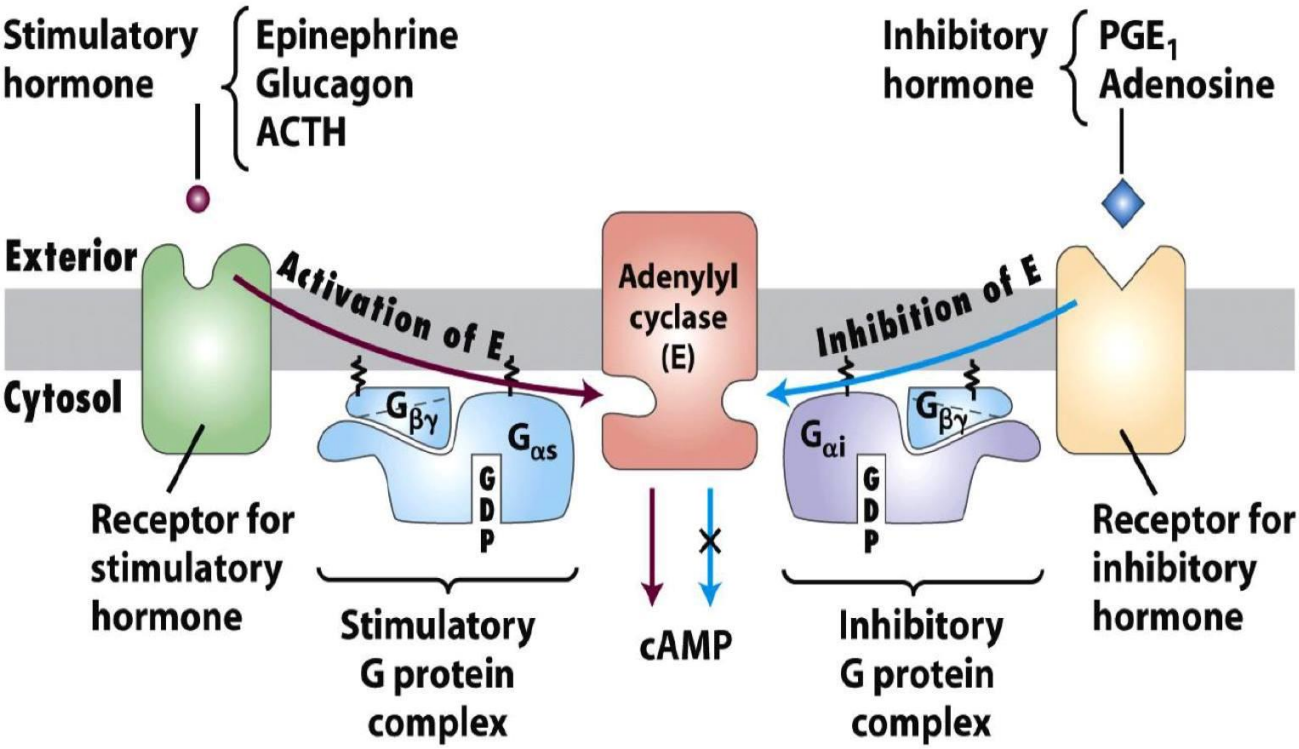


Figure 15-21

*This illustration shows us how signaling is complex.

This complexity lies in fact that we have different receptors that may have different effects at the same time!

We can see GPCR that are bound to Gαs and at the same time we have GPCR that is bound to Gαi.

Now different ligands or hormones might bind to the receptor with the Gαs such as Epinephrine, glucagon and ACTH to activate Gαs and activate A.C to increase cAMP. At the same time we can have inhibitory hormones acting on the GαI such as PGE1 and Adenosine thus reducing production of cAMP.

And we will have a net effect at the end which decides whether there is an increase or decrease in the cAMP conc. .

"However difficult life may seem, there is always something you can do and succeed at. It matters that you don't just give up."

-Stephen Hawking.

GOOD LUCK♥