Second Messengers

- Diacylglycerol (DAG) & IP_3
  - From membrane lipids
  - DAG → Protein Kinase C (membrane)
  - IP_3 → Ca^{2+} (endoplasmic reticulum) ~

Hormones That Use 2nd Messengers

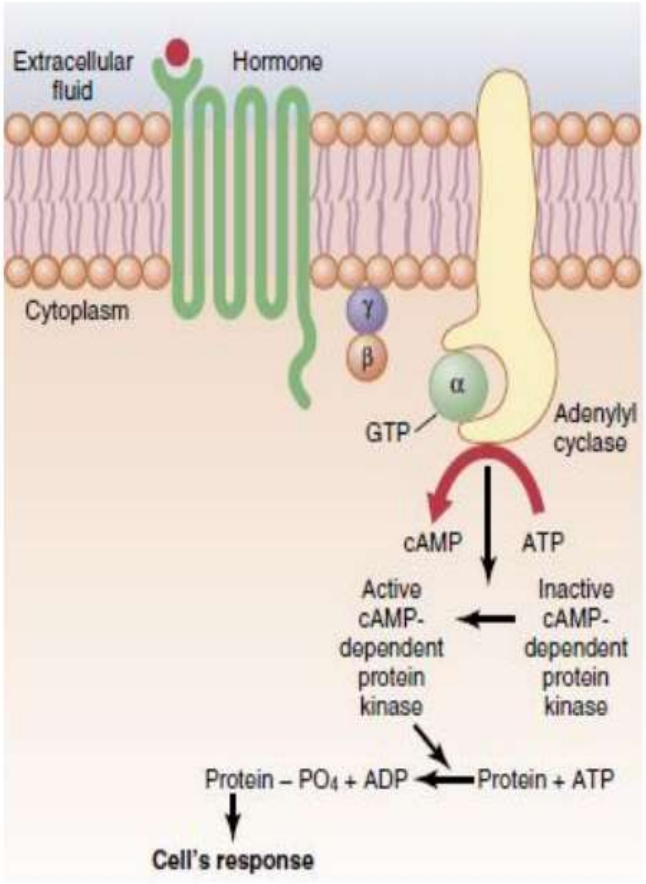
- Hormones cannot pass through plasma membrane use 2nd messengers.
  - Catecholamine, polypeptide, and glycoprotein hormones bind to receptor proteins on the target plasma membrane.
- Actions are mediated by 2nd messengers (signal-transduction mechanisms).
  - Extracellular hormones are transduced into intracellular 2nd messengers.

Adenylate Cyclase-cAMP

- Polypeptide or glycoprotein hormone binds to receptor protein causing dissociation of α subunit of G-protein.
- G-protein subunit binds to and activates adenylate cyclase.
- ATP → cAMP + PP_i
- cAMP attaches to inhibitory subunit of protein kinase.
- Inhibitory subunit dissociates and activates protein kinase.

Adenylyl Cyclase–cAMP Second Messenger System

- Stimulation of adenylyl cyclase, by the Gs protein
- Catalyzes the conversion of a small amount of cytoplasmic *Adenosine triphosphate ATP* into cAMP inside the cell.
- Then activates *cAMP-dependent protein kinase.*
- Phosphorylates specific cell proteins, triggering biochemical reactions that ultimately lead to the cell's response to the hormone.



A. cAMP:

❖ Regulation of adenylate cyclase:

Receptors that cause increase in cAMP do so by activating G_s, a stimulatory protein that activates adenylyl cylcase

Adenylyl cyclase is turned off by G_i, an inhibitory protein.

PKA enters the nucleus and phosphorylates CREB (CRE binding protein), which binds to the cAMP response element (CRE), a regulatory DNA sequence associated with specific genes. This results in activation of transcription of those genes.

B. cGMP:

1. produced from GTP by guanylyl cyclase;
2. activates cGMP-dependent kinases or other targets
3. example: G-prot. Coupled rhodopsin photoreceptor in rod cells of retina

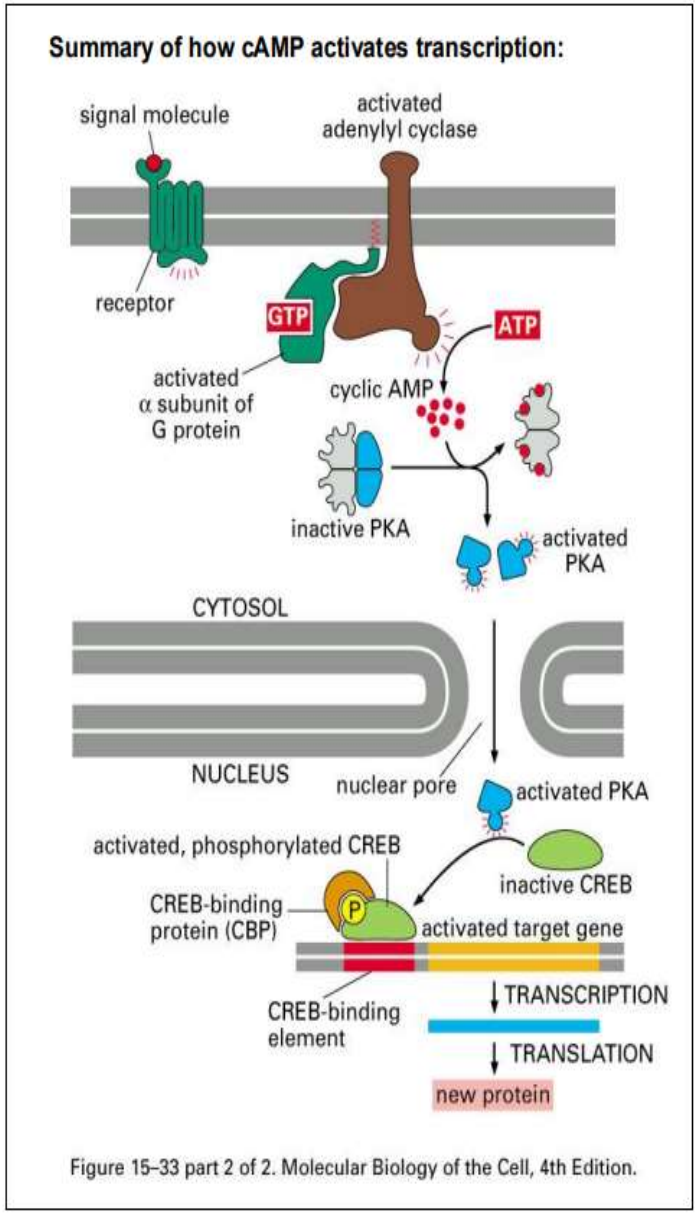
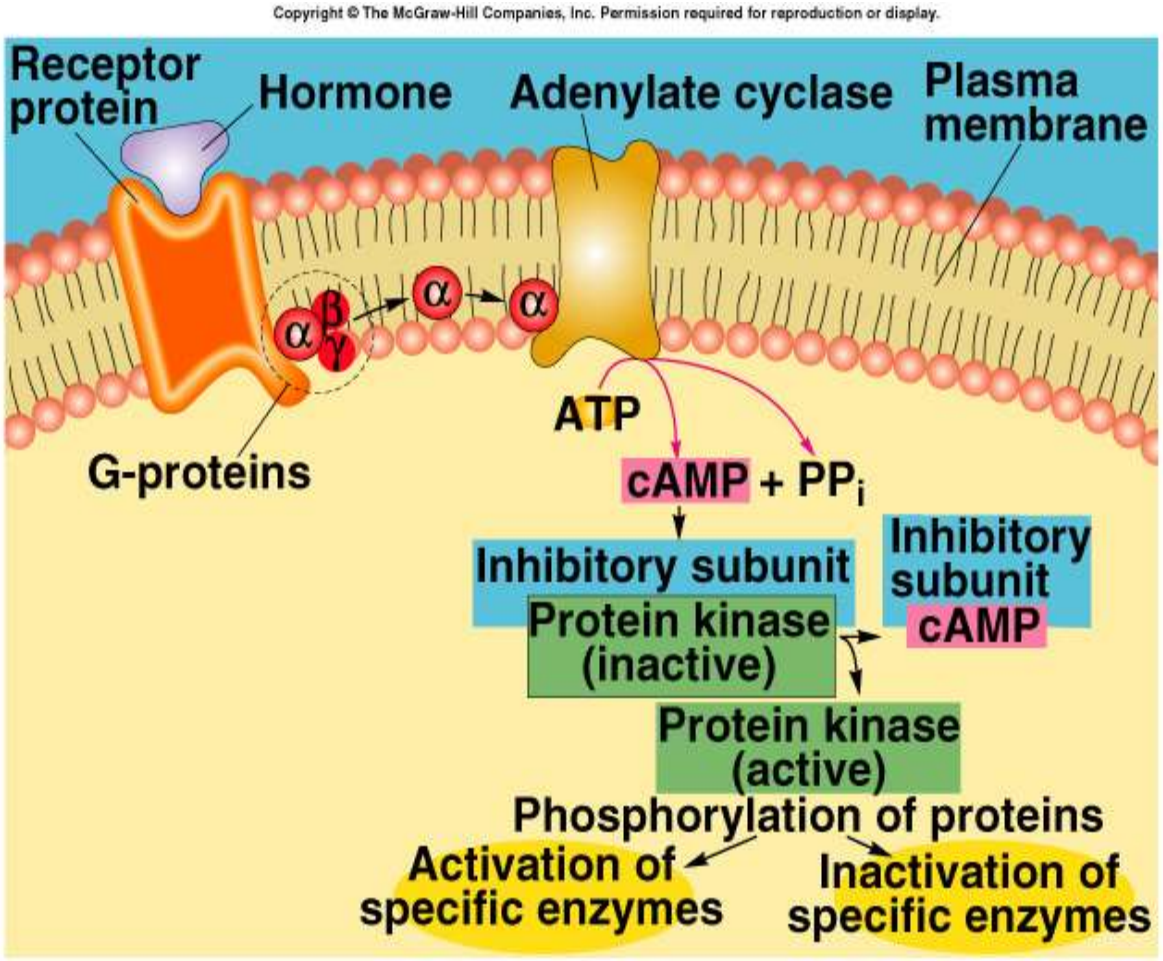
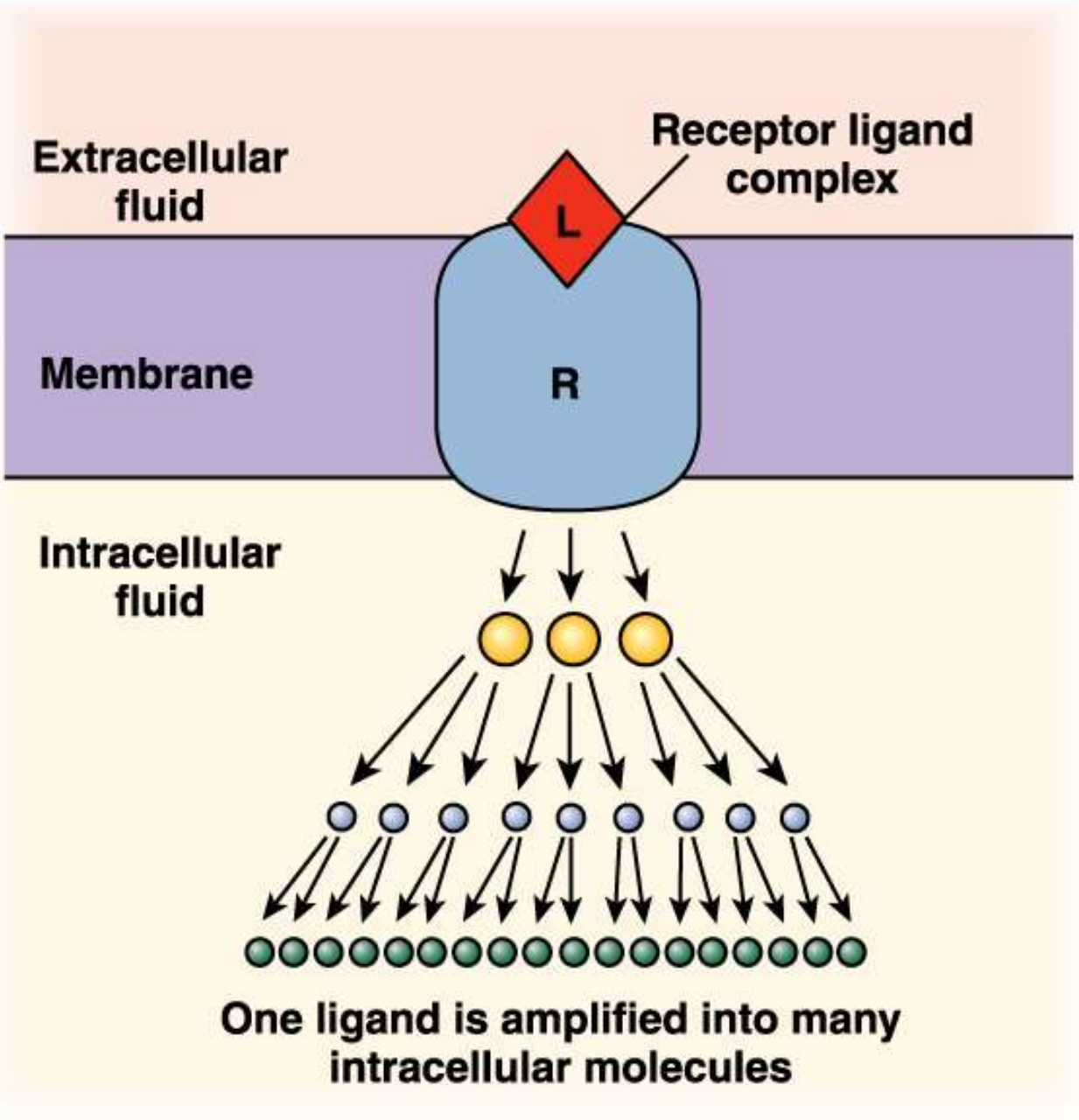


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

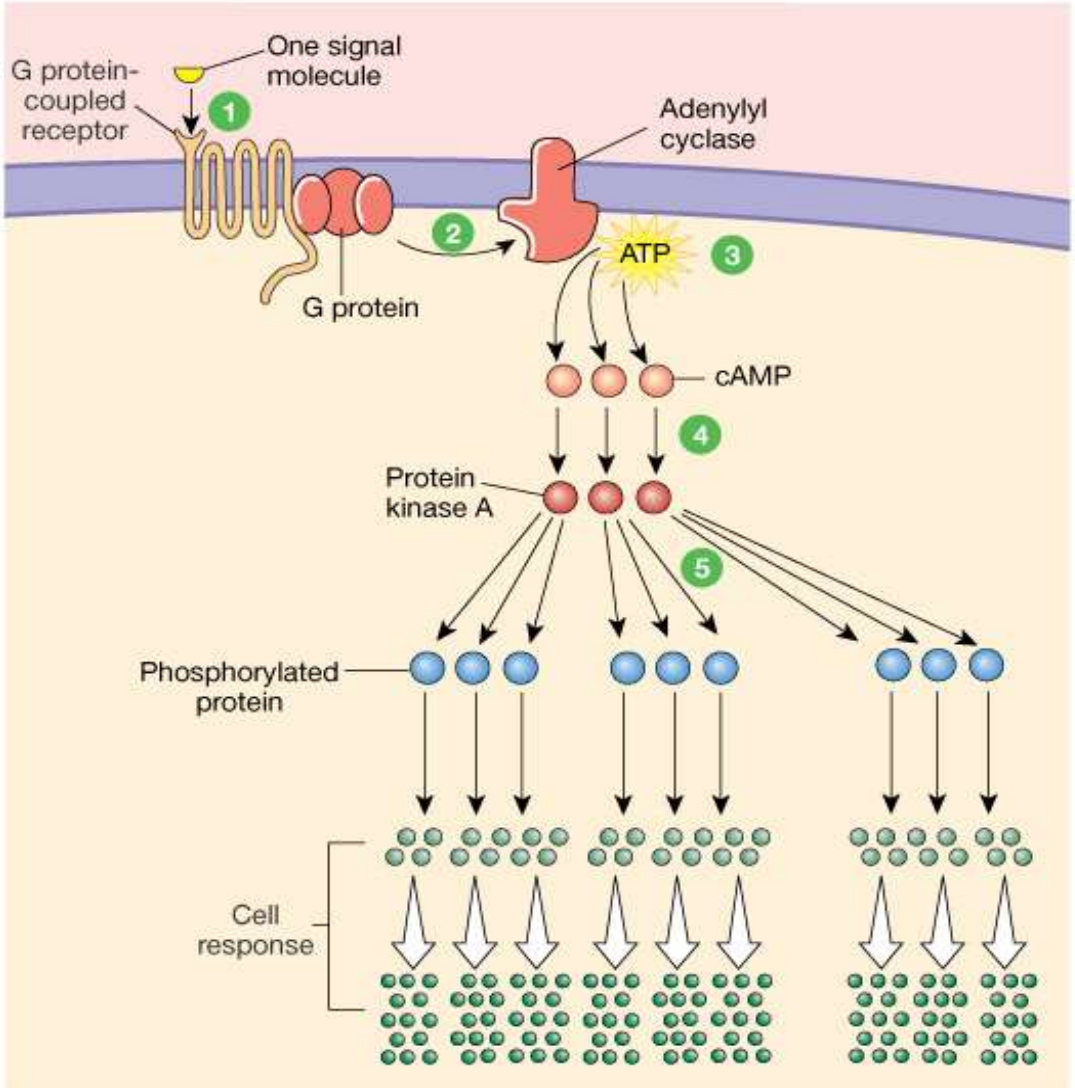
Adenylate Cyclase-cAMP (continued)

- Phosphorylates enzymes within the cell to produce hormone's effects.
- Modulates activity of enzymes present in the cell.
- Alters metabolism of the cell.
- cAMP inactivated by phosphodiesterase.
  - Hydrolyzes cAMP to inactive fragments.




Extracellular fluid
Receptor ligand complex
L
Membrane
R
Intracellular fluid
One ligand is amplified into many intracellular molecules

G-Protein-coupled Receptors

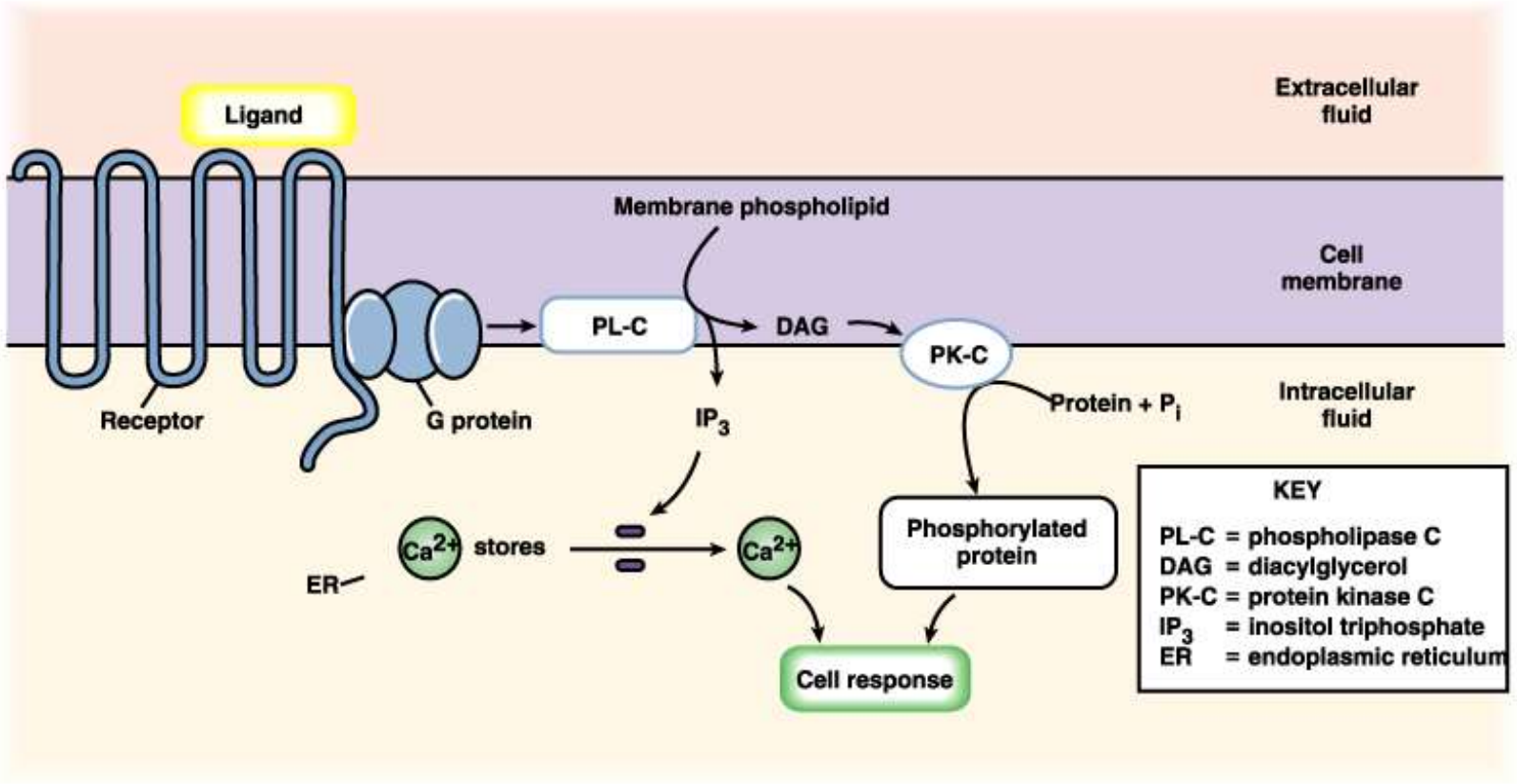


1. Signal molecule binds to G protein-linked receptor, which activates the G protein.
2. G protein turns on adenylyl cyclase, an amplifier enzyme.
3. Adenylyl cyclase converts ATP to cyclic AMP.
4. cAMP activates protein kinase A.
5. Protein kinase A phosphorylates other proteins, leading ultimately to a cellular response.

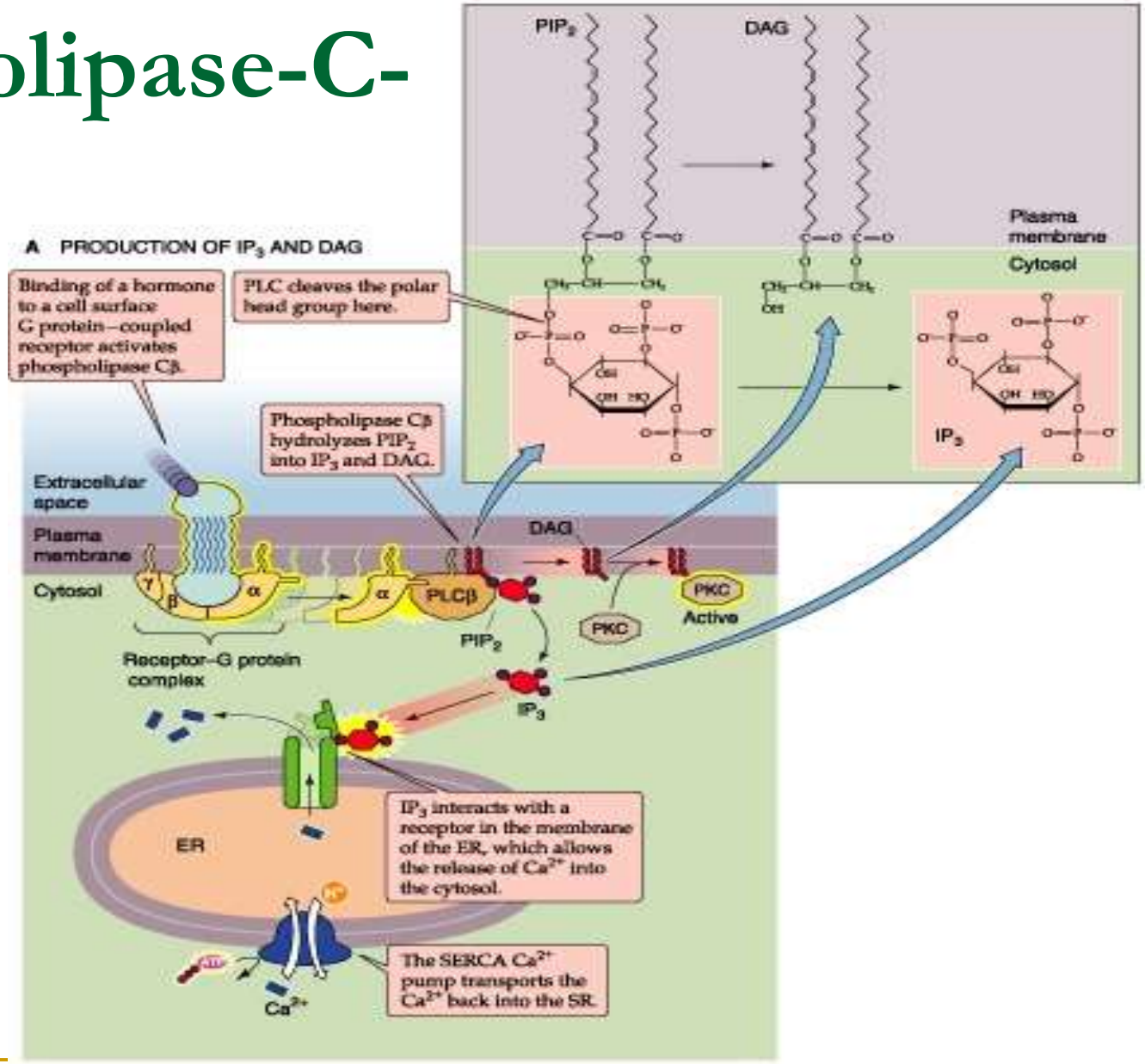
Phospholipase-C-Ca^{2+}

- Binding of Epinephrine to α-adrenergic receptor in plasma membrane activates a G-protein intermediate, phospholipase C.
  - Phospholipase C splits phospholipid into inositol triphosphate (IP_3) and diacyglycerol (DAG).
    - Both derivatives serve as 2^{nd} messengers.
- IP_3 diffuses through cytoplasm to endoplasmic reticulum (ER).
  - Binding of IP_3 to receptor protein in ER causes Ca^{2+} channels to open- releases calcium into the cytosol.

Phospholipase-C-Ca^{2+}

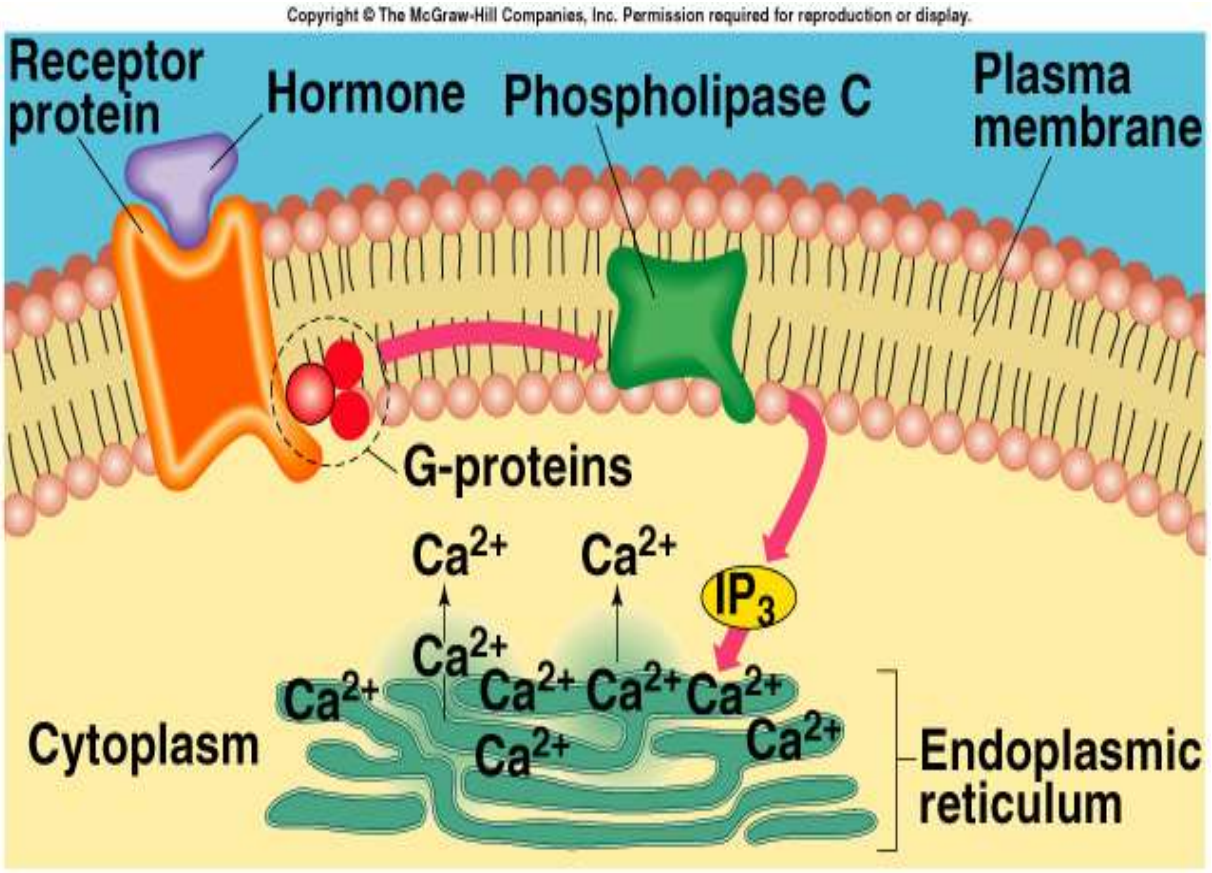


Phospholipase-C-Ca^{2+}

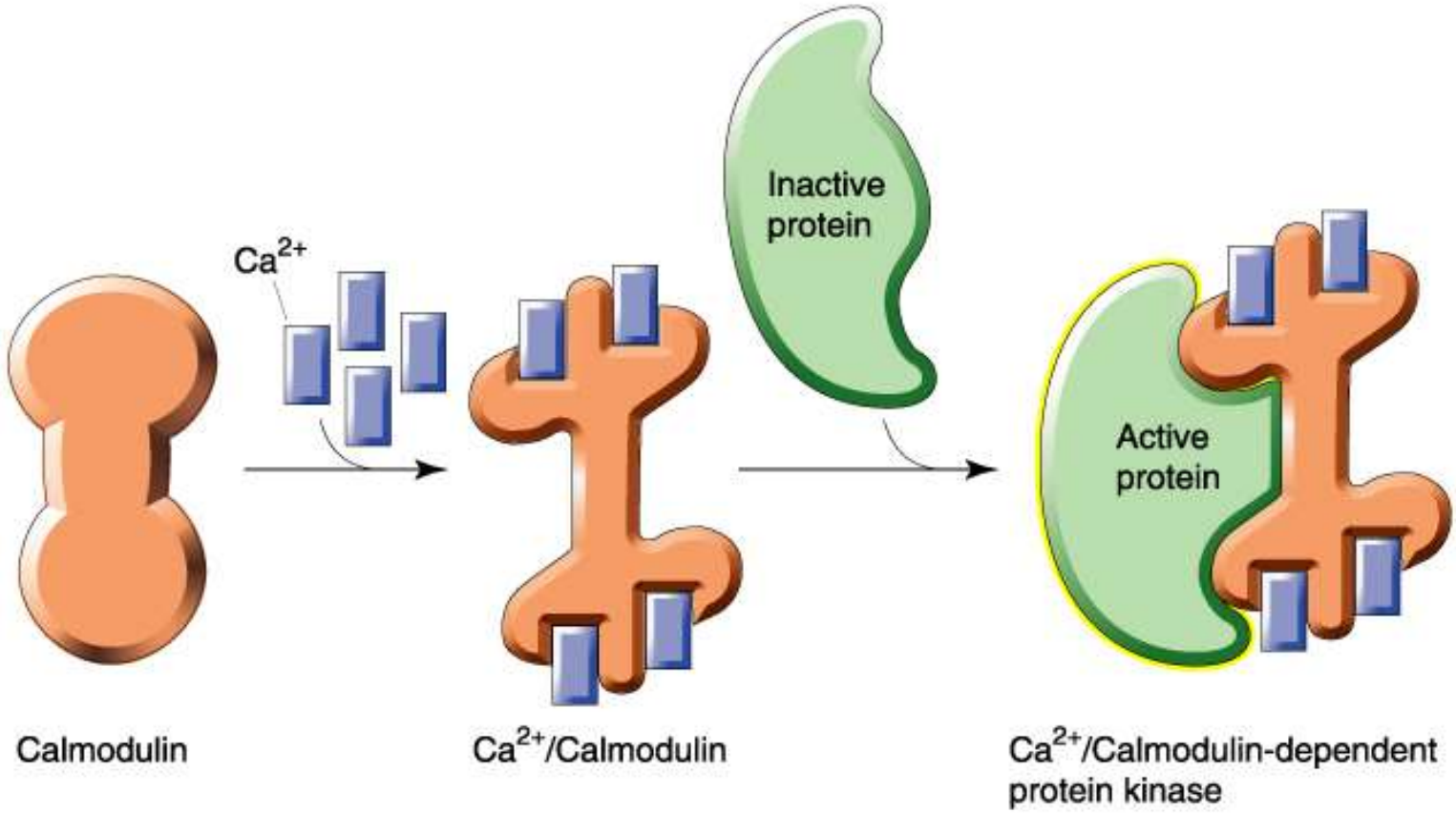


Ca^{2+}- Calmodulin (continued)

- Ca^{2+} diffuses into the cytoplasm.
  - Ca^{2+} binds to calmodulin.
- Calmodulin activates specific protein kinase enzymes.
  - Alters the metabolism of the cell, producing the hormone's effects.

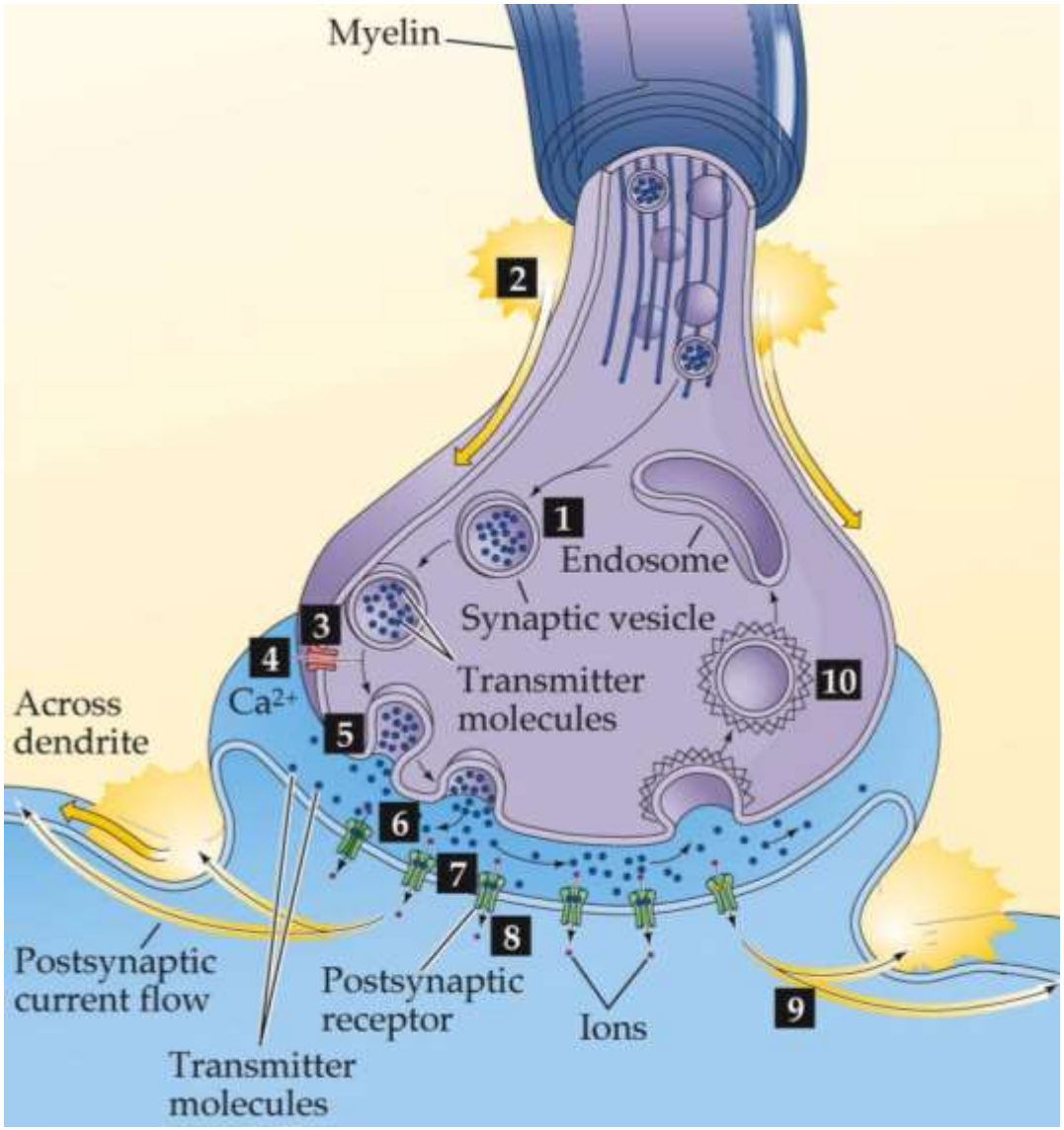


Ca^{2+}- Calmodulin (continued)

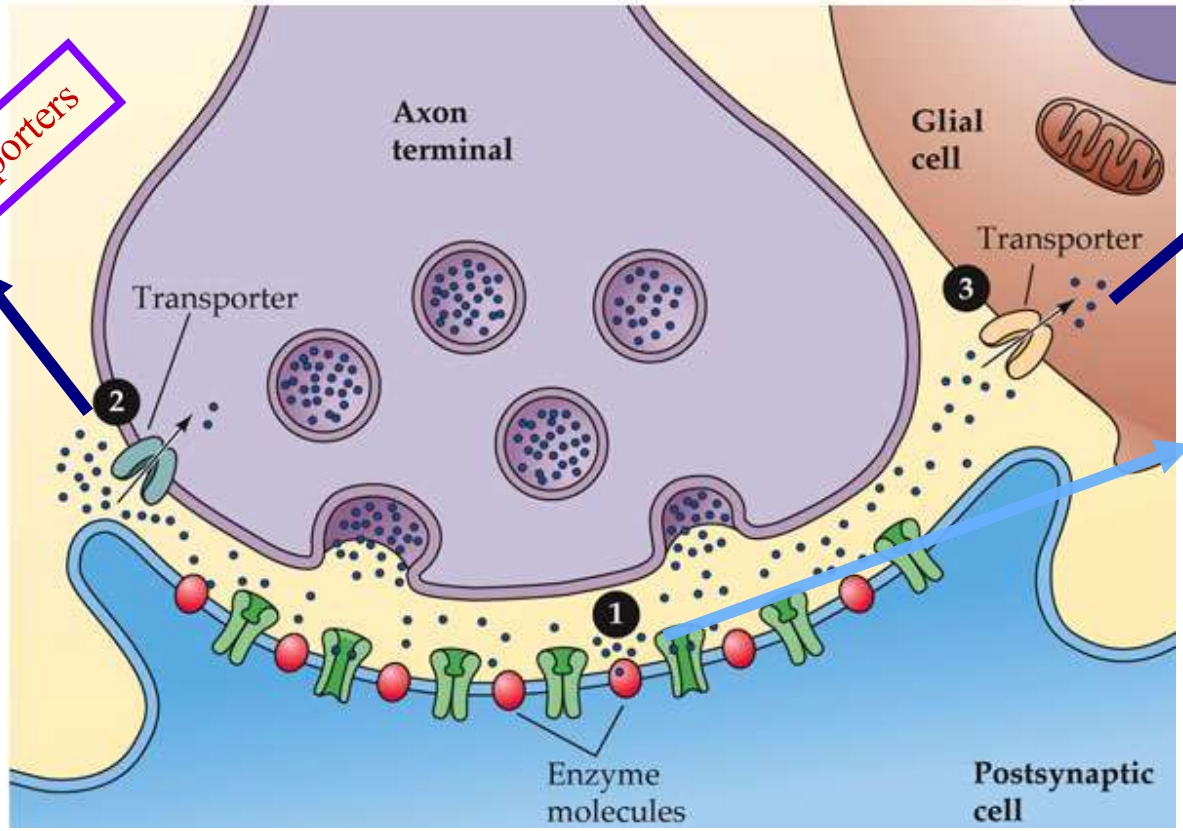


Neurotransmitter Release: exocytosis and endocytosis

1. Transmitter synthesized and stored
2. Action Potential
3. Depolarization: open voltage-gated Ca^{2+} channels
4. Ca^{2+} enter cell
5. Ca^{2+} causes vesicles to fuse with membrane
6. Neurotransmitter released (exocytosis)
7. Neurotransmitter binds to postsynaptic receptors
8. Opening or closing of postsynaptic channels
9. Postsynaptic current excites or inhibits postsynaptic potential to change excitability of cell
10. Retrieval of vesicles from plasma membrane (endocytosis)



Transmitter Inactivation: reuptake and enzymatic breakdown



Reuptake by transporters

Reuptake by transporters (glial cells)

Enzymatic breakdown

Neurotransmitter can be recycled in presynaptic terminal or can be broken down by enzymes within the cell

NT – Receptor Binding

Receptors are large, dynamic proteins that exist along and within the cell membrane.

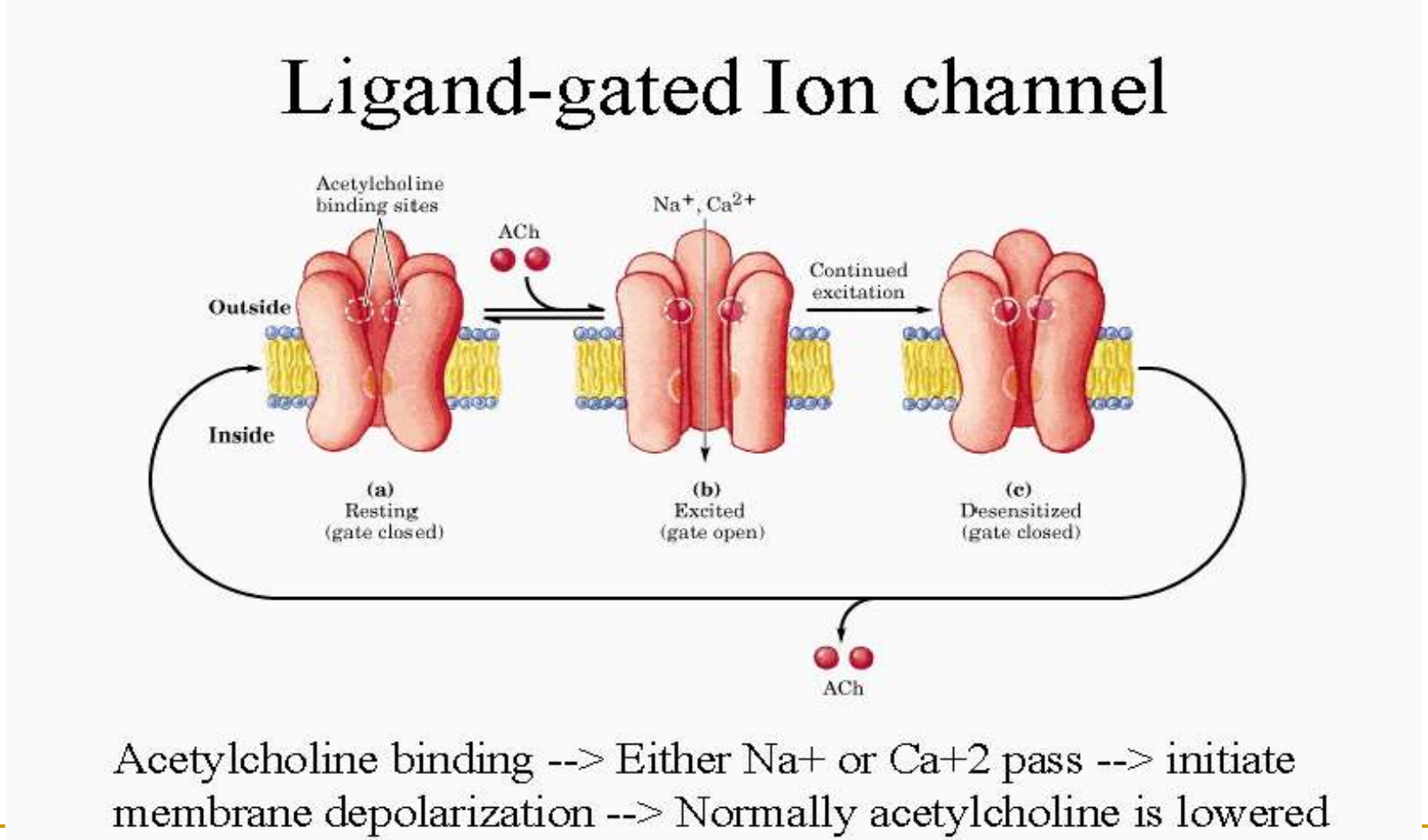
Dynamic – they can increase in number and avidity for their neurotransmitter according to circumstances.

Two Types of Post synaptic Receptors:

Ionotropic receptors: NT binding results in direct opening or closure of specific ion channels

Metabotropic receptors: binding of NT initiates a sequence of internal molecular events which in turn open specific ion channels

NT binding -> Membrane Potential Response

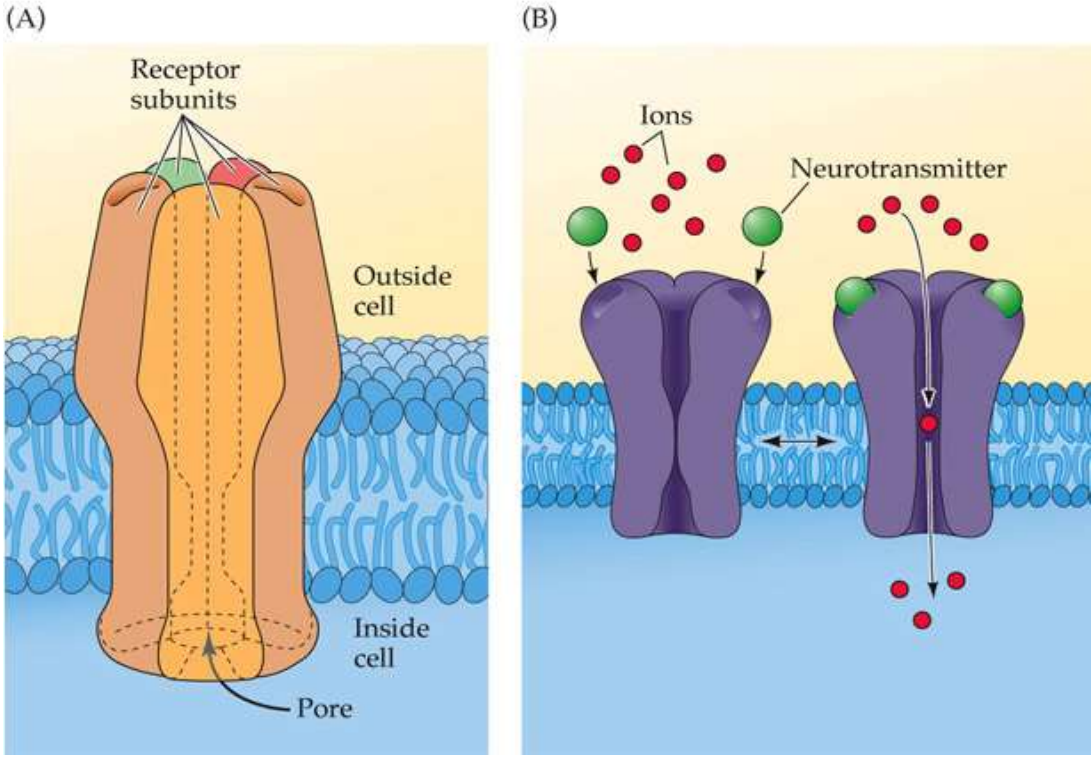


Acetylcholine binding --> Either Na+ or Ca+2 pass --> initiate membrane depolarization --> Normally acetylcholine is lowered

Ionotropic Receptors

Work very fast; important role in fast neurotransmission

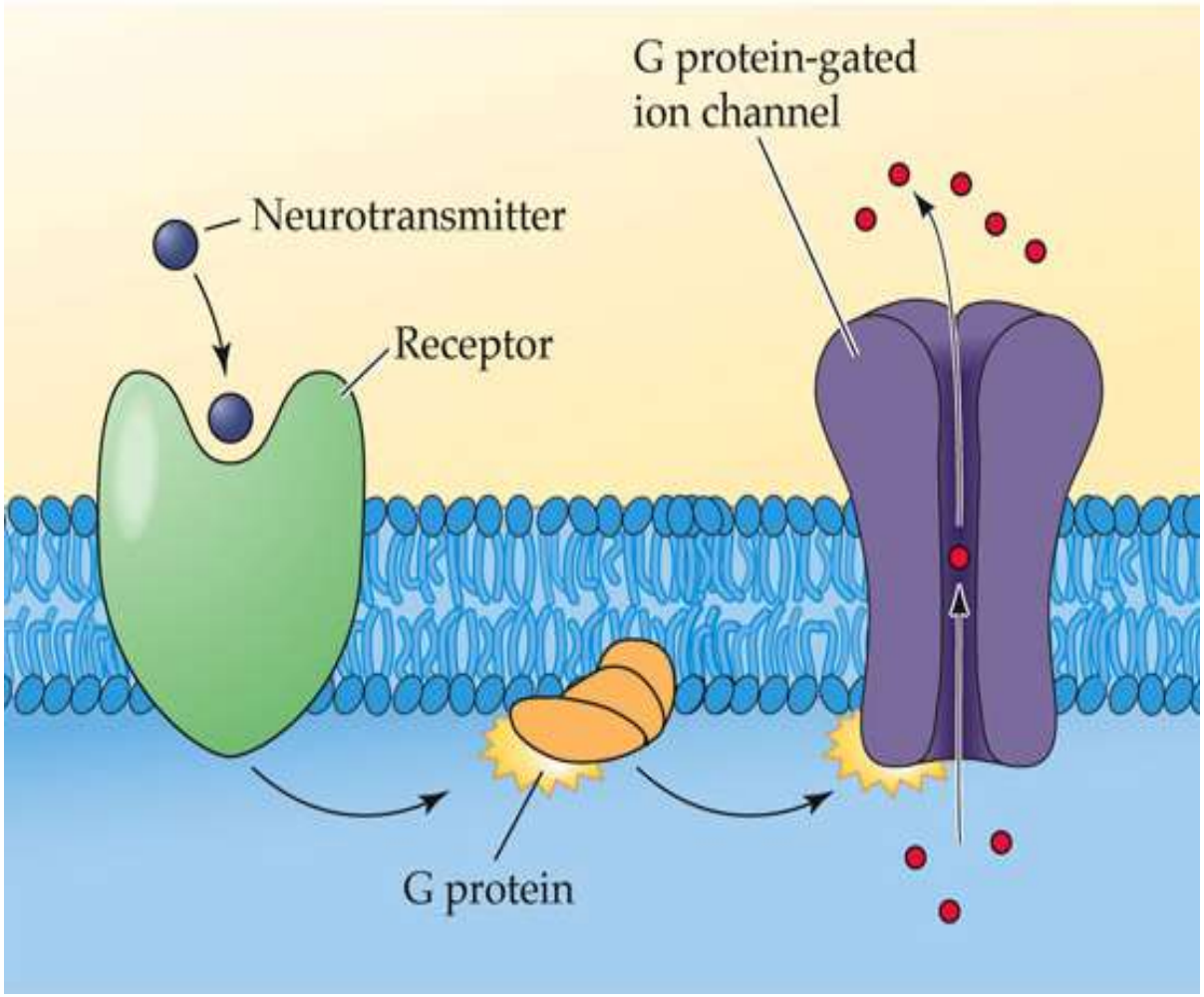
1. Each is made of several subunits (together form the complete receptor)
2. At center of receptors is channel or pore to allow flow of ions
3. At rest - receptor channels are closed
4. When neurotransmitter binds -- channel immediately opens
5. When ligand leaves binding site -- channel quickly closes



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



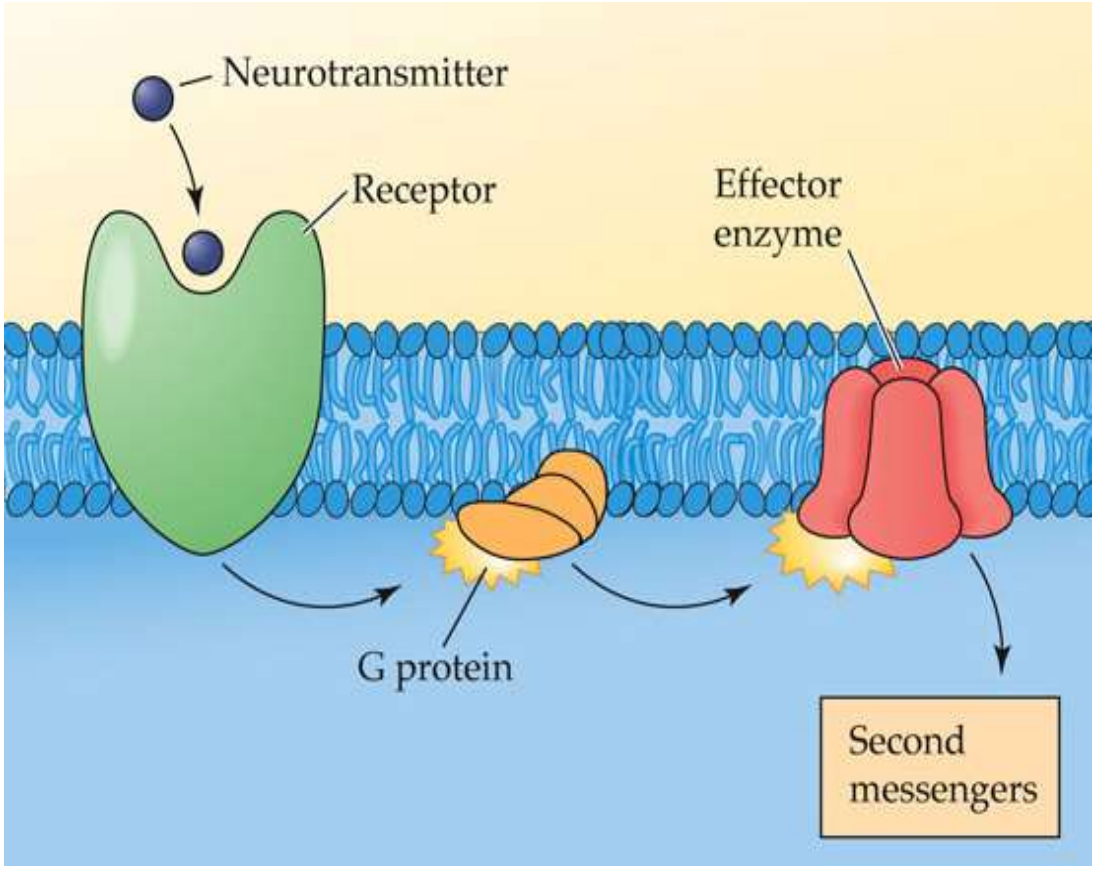
Metabotropic Receptors…

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.

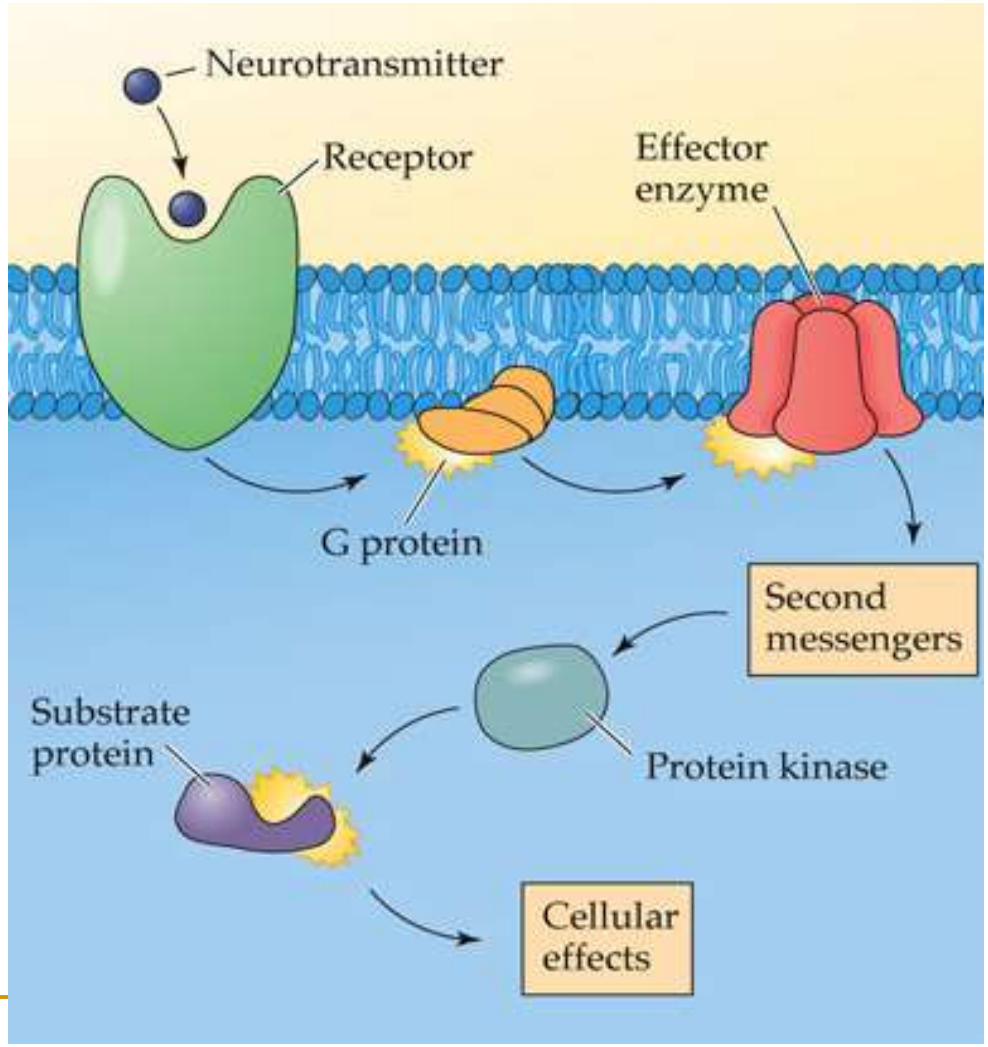
   *Second messenger:

   effector enzyme



Second messengers: Activate Protein Kinases

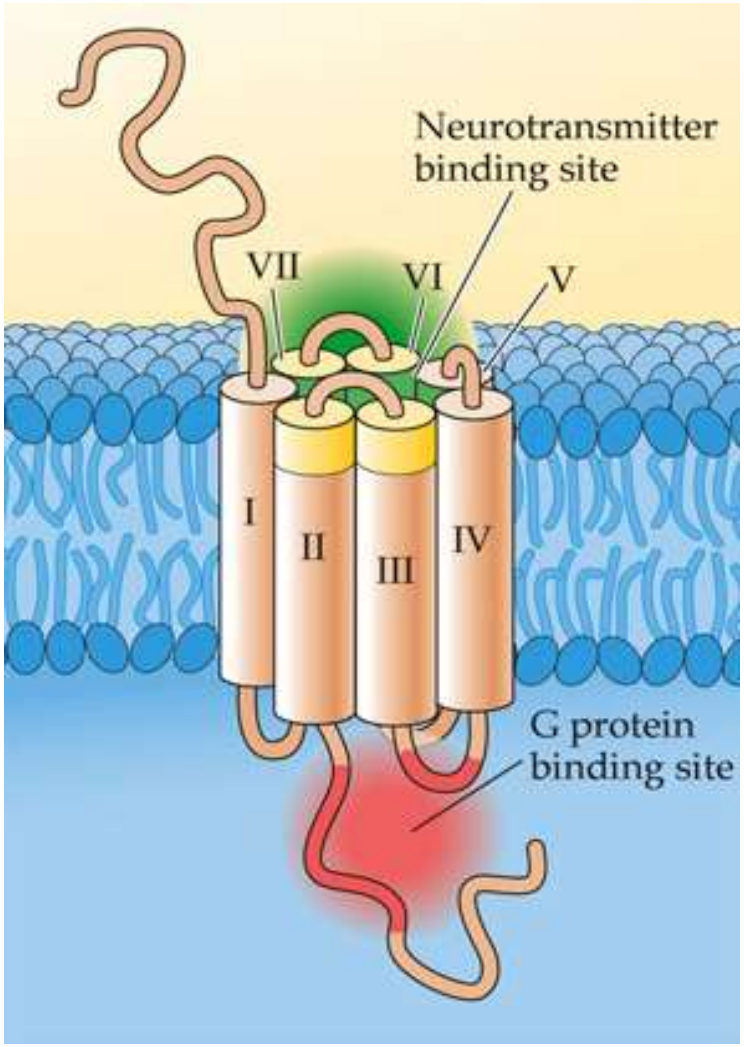
Can work by affecting:
NT production, no. synapses formed, sensitivity of receptors, or expression of genes (long term effects).
Can result in amplification - interconnections.

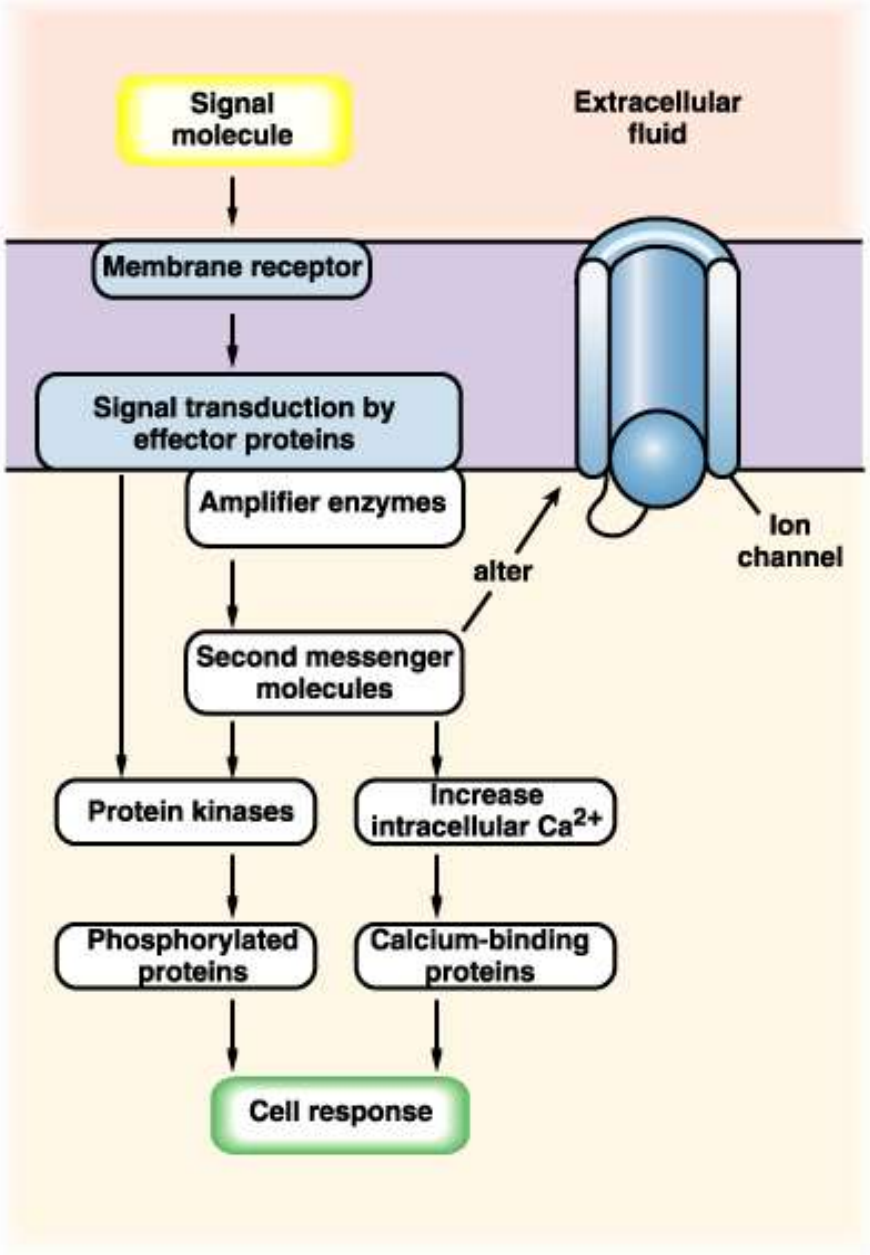


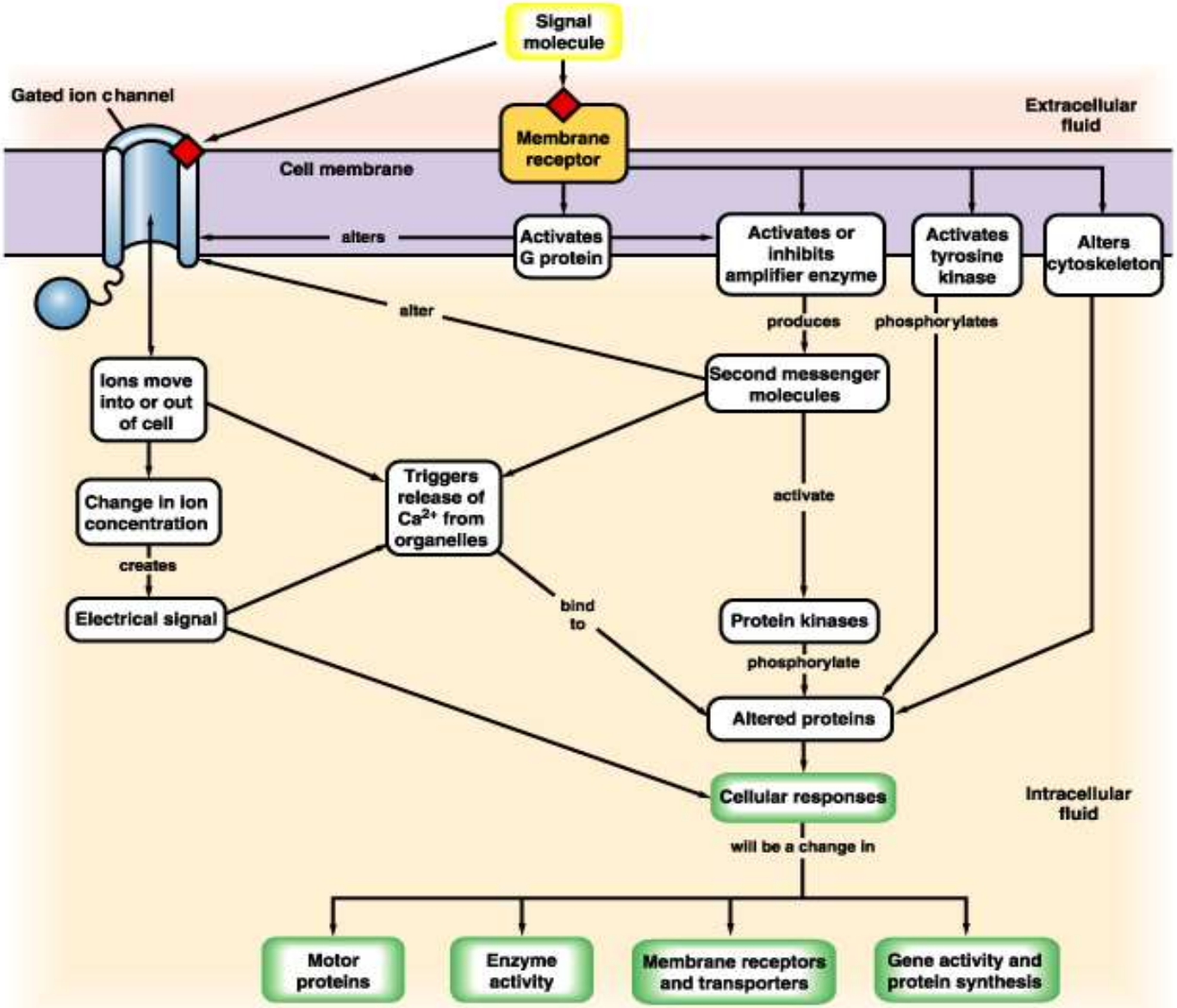
Other Metabotropic Receptors

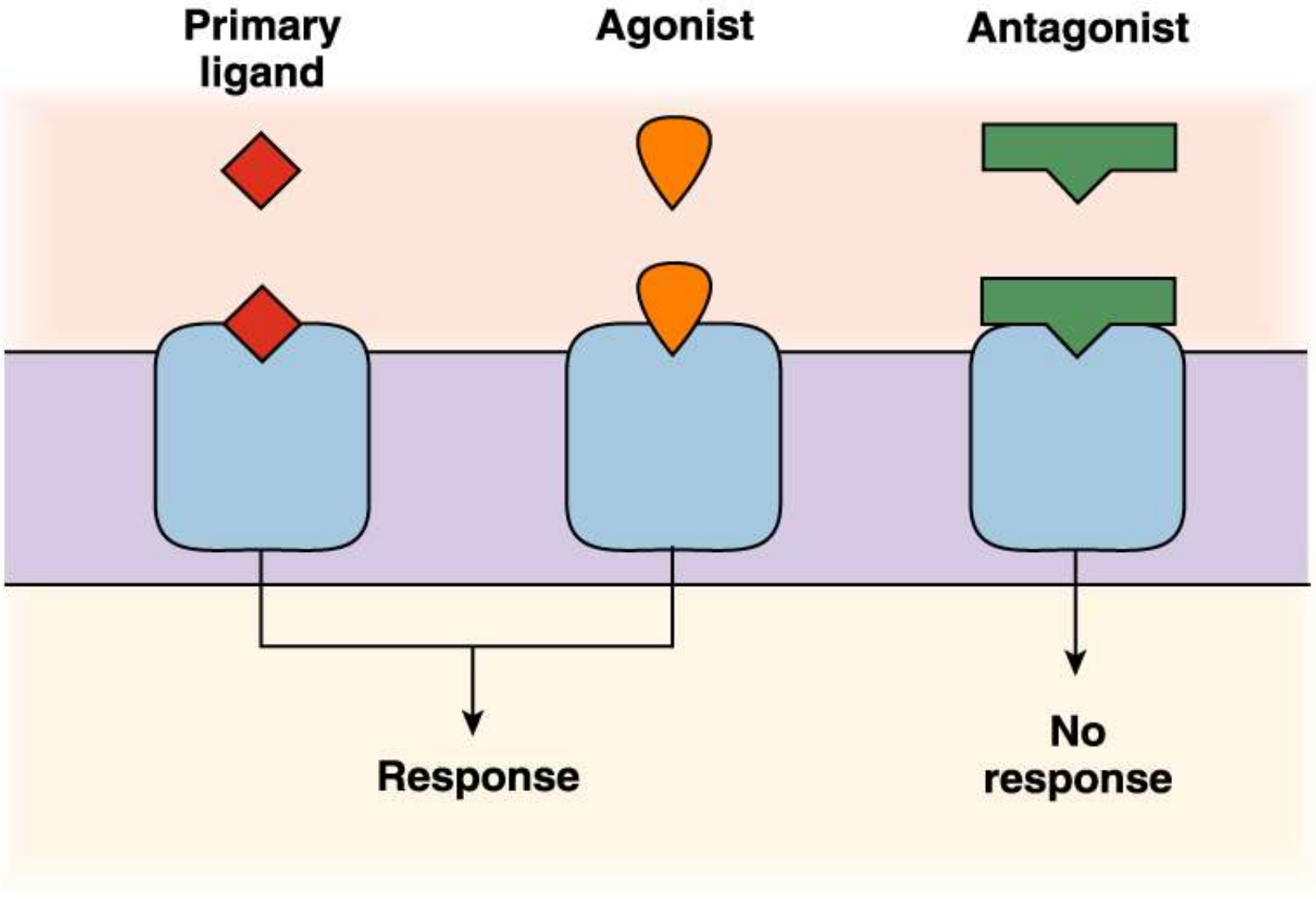
Work more slowly than ionotropic receptors

1. Though it takes longer for postsynapic cell to respond, response is somewhat longer-lasting
2. Comprise a single protein subunit, winding back-and-forth through cell membrane seven times (**transmembrane domains**)
3. They do not possess a channel or pore

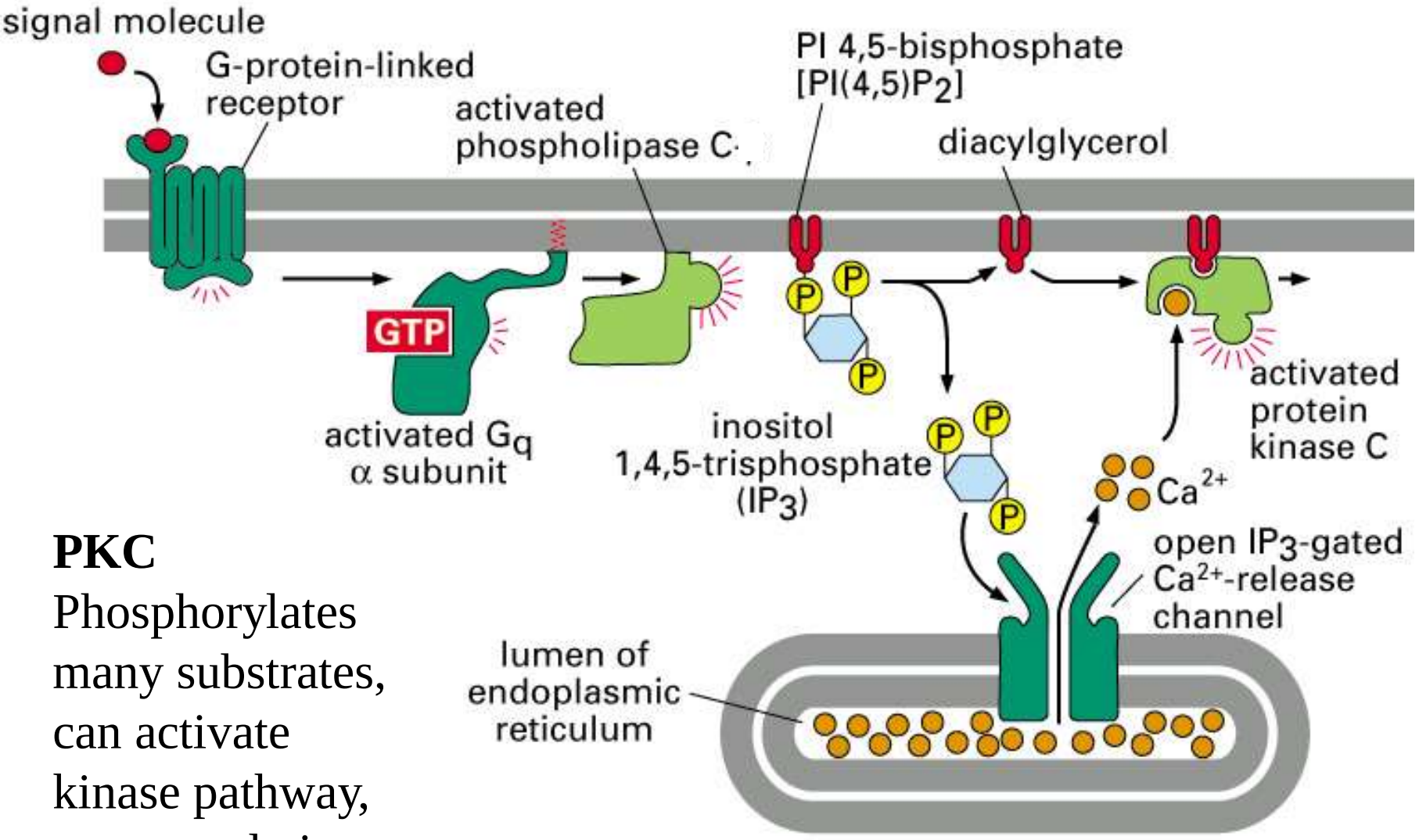



Signal molecule
Extracellular fluid
Membrane receptor
Signal transduction by effector proteins
Amplifier enzymes
Ion channel
alter
Second messenger molecules
Protein kinases
Increase intracellular Ca^{2+}
Phosphorylated proteins
Calcium-binding proteins
Cell response


Signal molecule
Gated ion channel
Extracellular fluid
Membrane receptor
Cell membrane
alters
Activates G protein
Activates or inhibits amplifier enzyme
Activates tyrosine kinase
Alters cytoskeleton
alter
produces
phosphorylates
Ions move into or out of cell
Second messenger molecules
Triggers release of Ca^{2+} from organelles
activate
Change in ion concentration
creates
Electrical signal
bind to
Protein kinases
phosphorylate
Altered proteins
Cellular responses
Intracellular fluid
will be a change in
Motor proteins
Enzyme activity
Membrane receptors and transporters
Gene activity and protein synthesis


Primary ligand
Agonist
Antagonist
Response
No response

PLC- signaling pathway



PKC

Phosphorylates many substrates, can activate kinase pathway, gene regulation


THANK YOU

Receptors Functions and Signal Transduction- L4- L5

Faisal I. Mohammed, MD, PhD

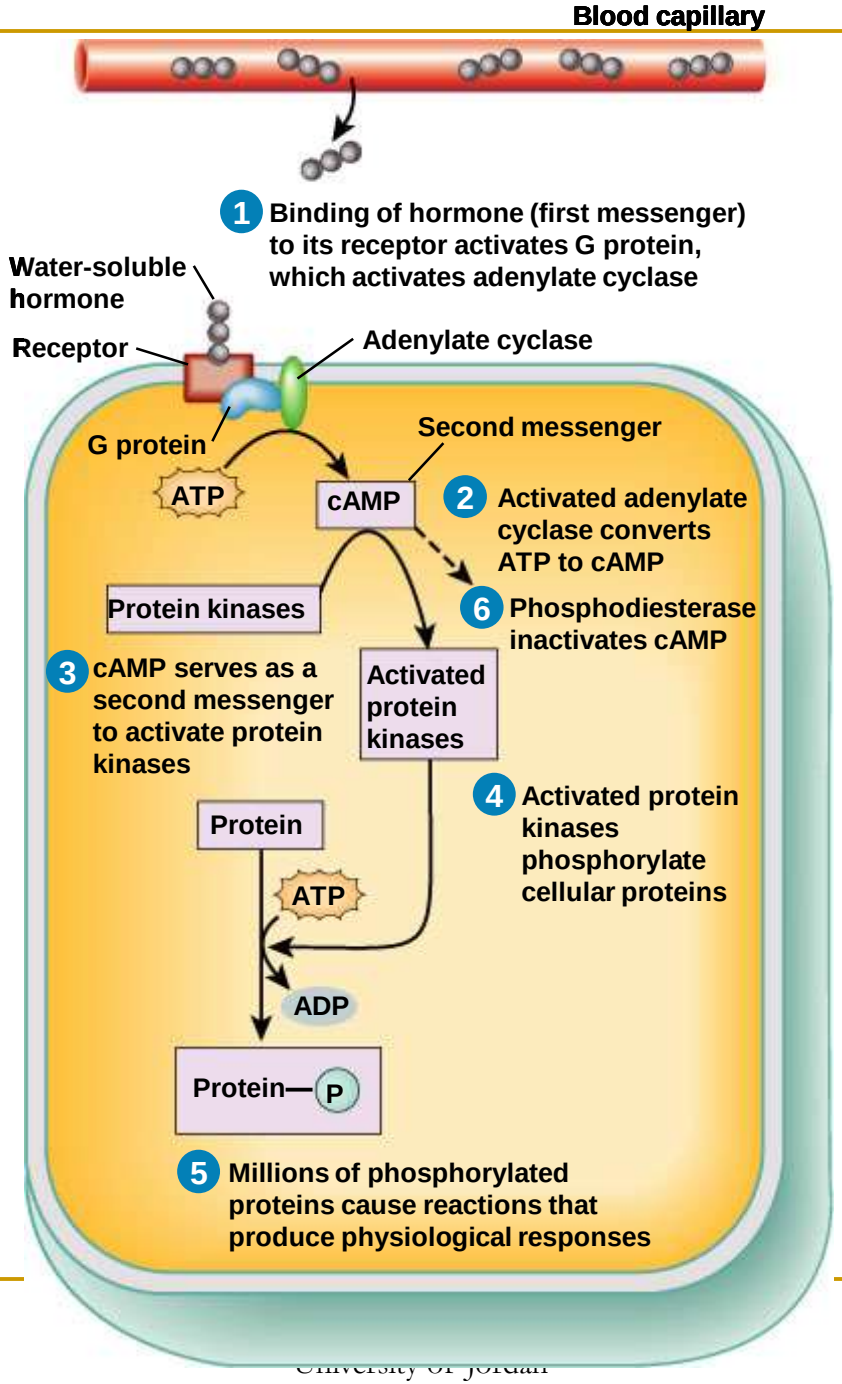
- **Receptors superfamilies:**
- Ionotropic receptors (ligand-gated channels)
- Metabotropic receptors (G protein-coupled receptors)
- Tyrosine Kinase

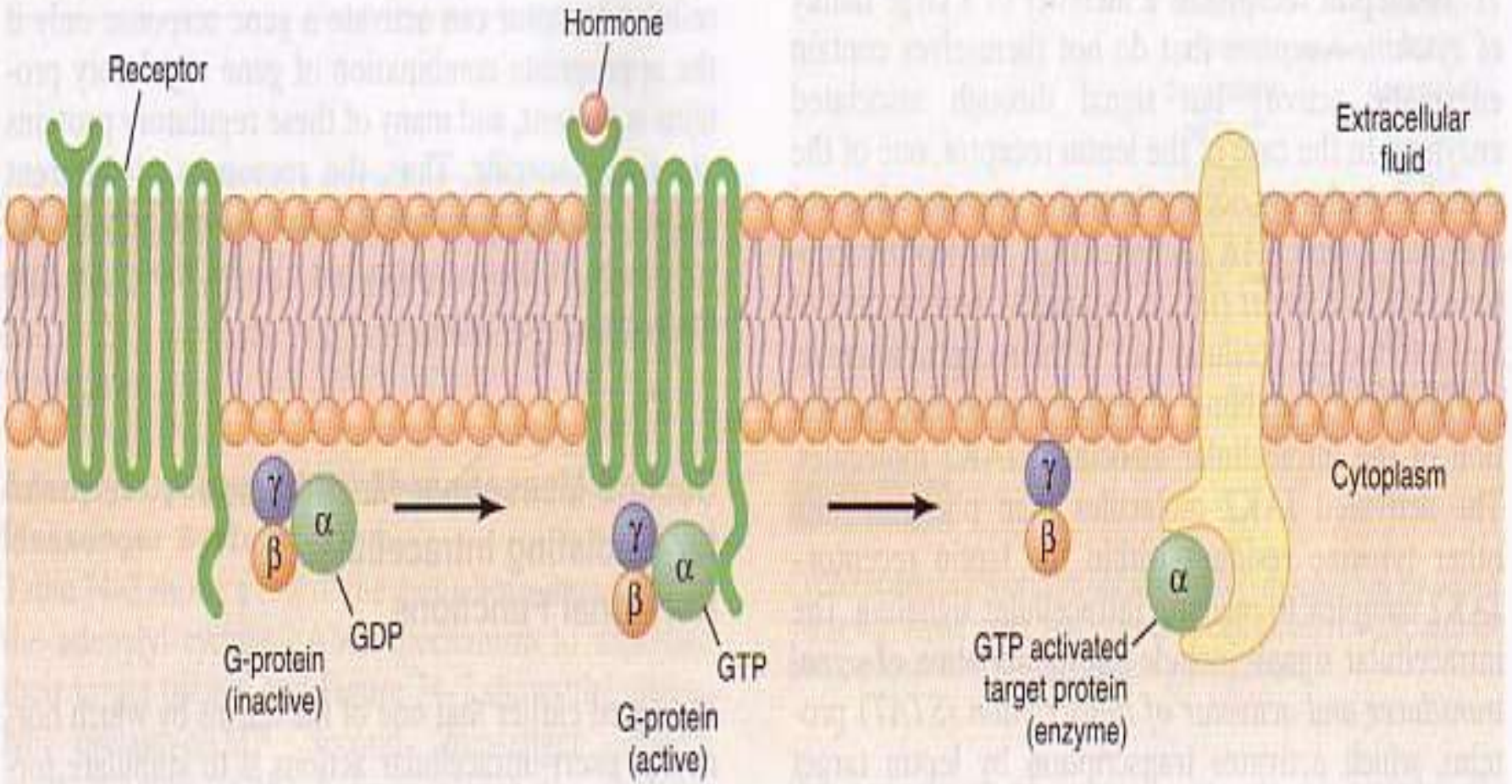
Comparison of Ionotropic and Metabotropic Receptors

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.**

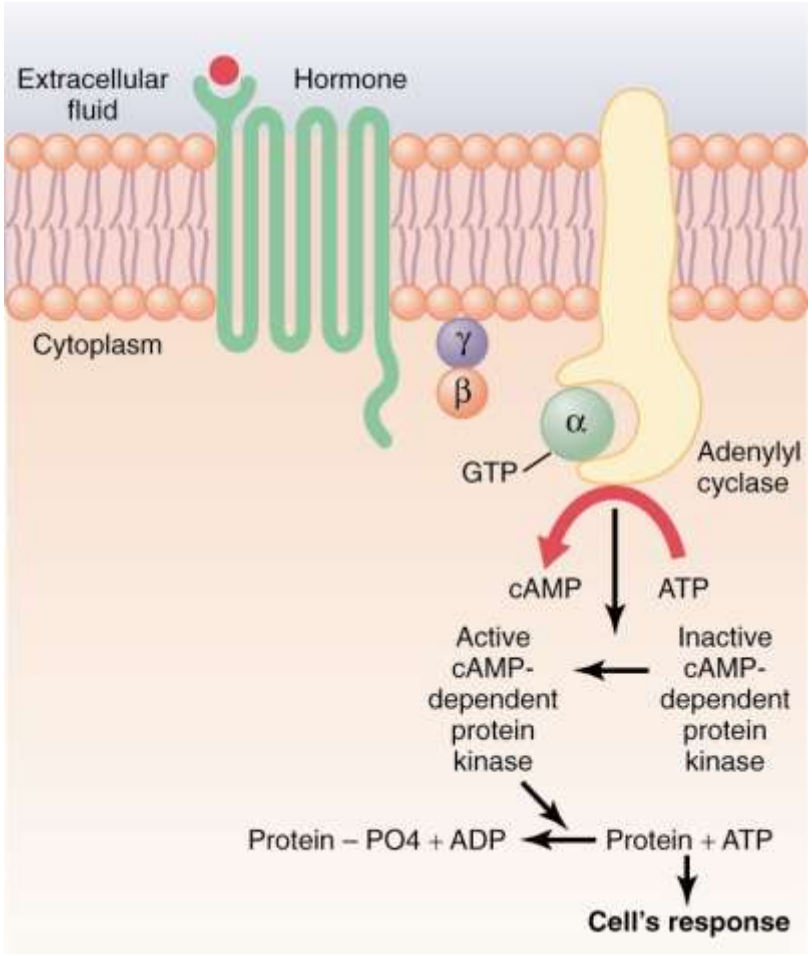
Water-soluble Hormones



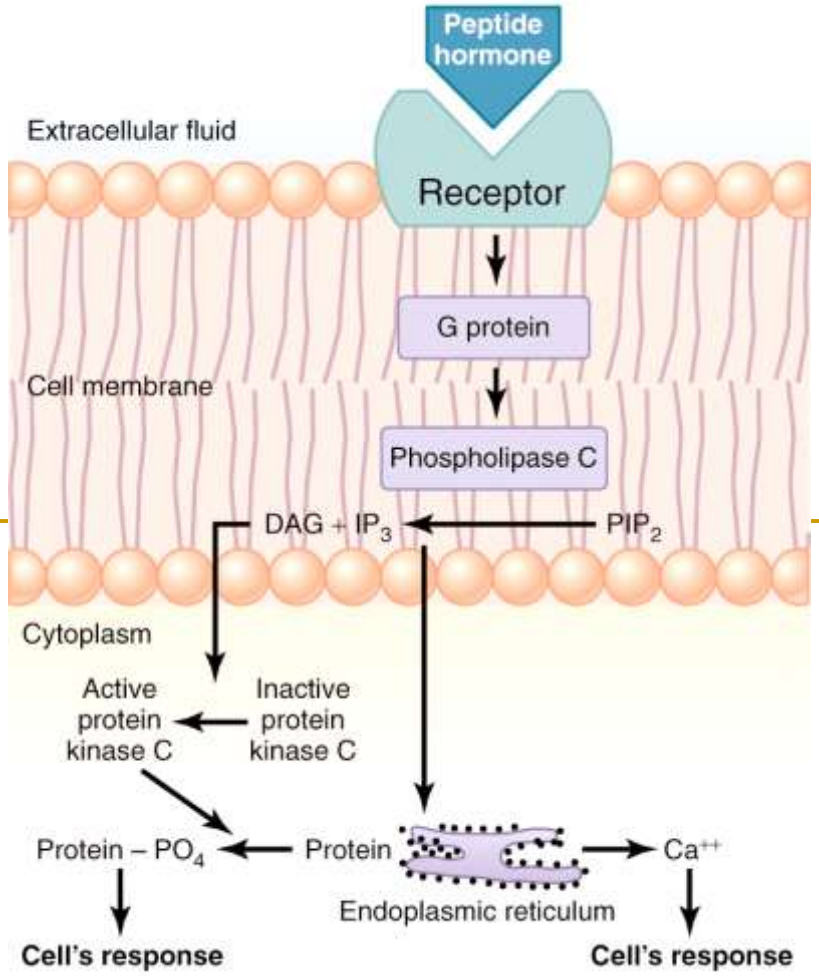


Mechanism of activation of a G protein–coupled receptor. When the hormone activates the receptor, the inactive α, β, and γ G protein complex associates with the receptor and is activated, with an exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP). This causes the α subunit (to which the GTP is bound) to dissociate from the β and γ subunits of the G protein and to interact with membrane-bound target proteins (enzymes) that initiate intracellular signals.

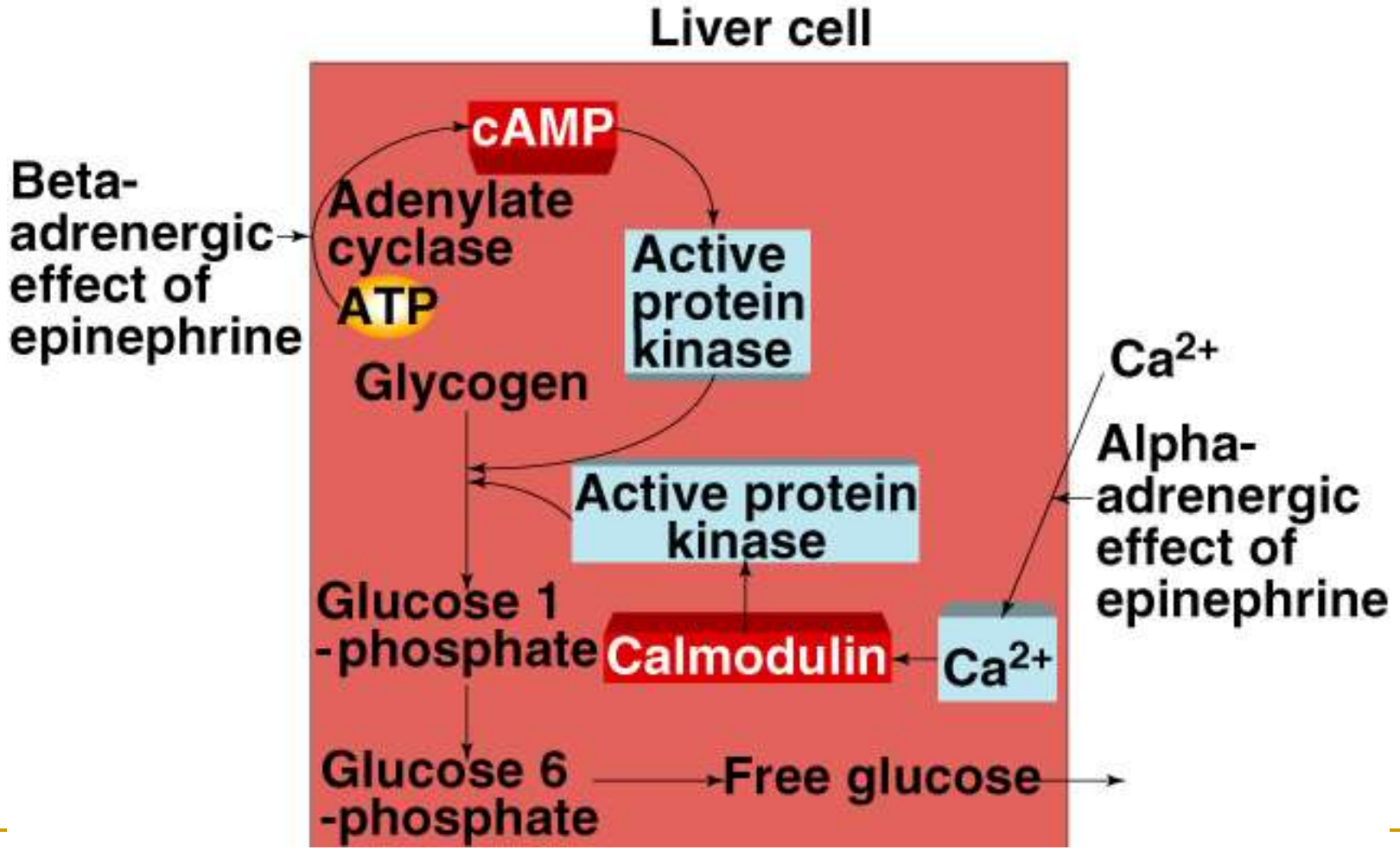
Cyclic Monophasphate (cAMP) Second Messenger Mechanism

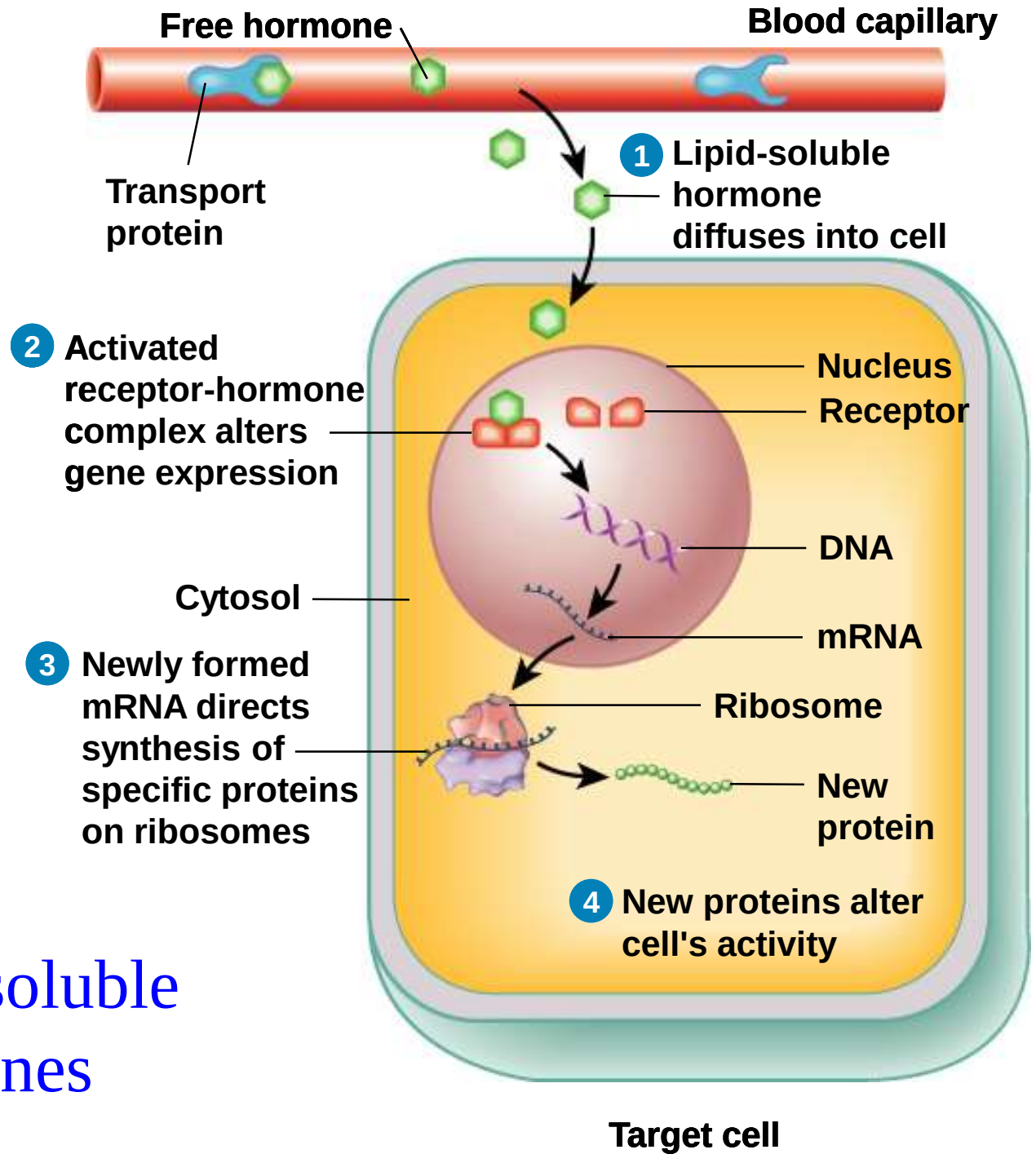


Cell Membrane Phospholipid Second Messenger System



Epinephrine Can Act Through Two 2nd Messenger Systems

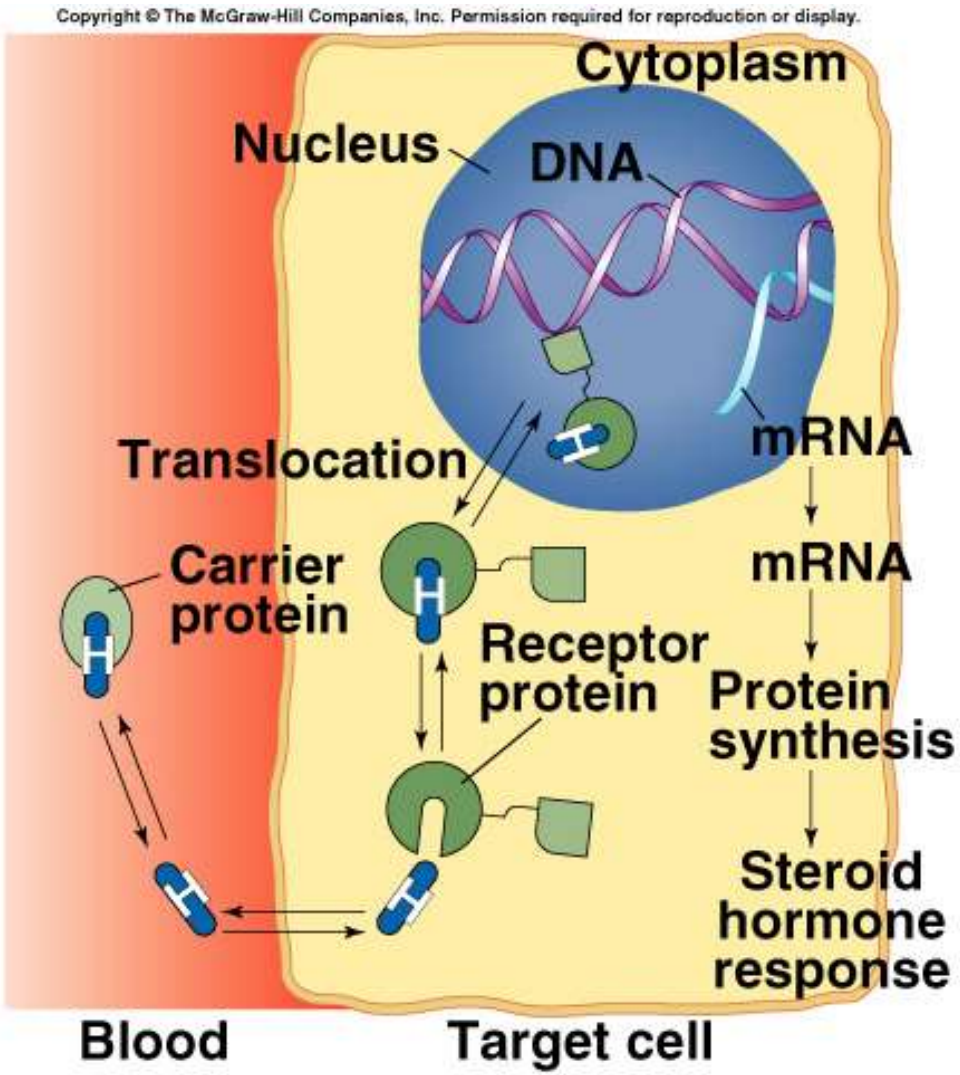




Lipid-soluble Hormones

Hormones That Bind to Nuclear Receptor Proteins

- Lipophilic steroid and thyroid hormones are attached to plasma carrier proteins.
  - Hormones dissociate from carrier proteins to pass through lipid component of the target plasma membrane.
- Receptors for the lipophilic hormones are known as nuclear hormone receptors.

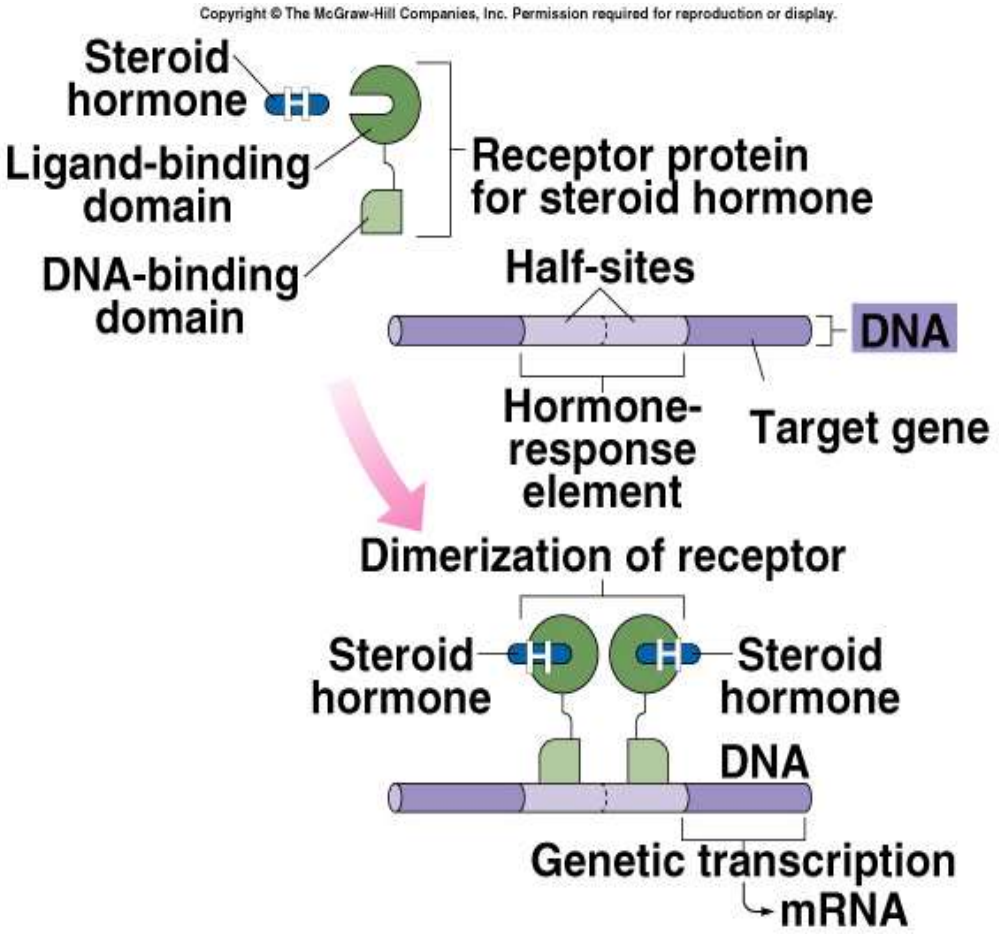


Nuclear Hormone Receptors

- Steroid receptors are located in cytoplasm and in the nucleus.
- Function within cell to activate genetic transcription.
  - Messenger RNA directs synthesis of specific enzyme proteins that change metabolism.
- Each nuclear hormone receptor has 2 regions:
  - A ligand (hormone)-binding domain.
  - DNA-binding domain.
- Receptor must be activated by binding to hormone before binding to specific region of DNA called HRE (hormone responsive element).
  - Located adjacent to gene that will be transcribed.

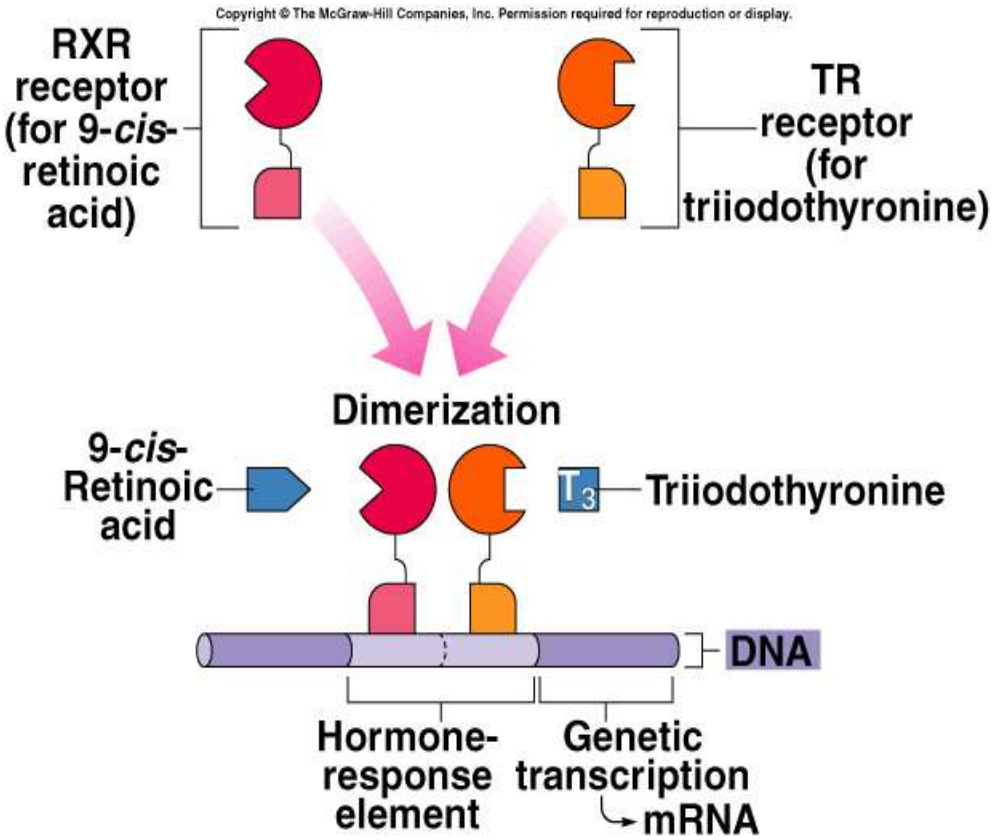
Mechanisms of Steroid Hormone Action

- Cytoplasmic receptor binds to steroid hormone.
- Translocates to nucleus.
- DNA-binding domain binds to specific HRE of the DNA.
- Dimerization occurs.
  - Process of 2 receptor units coming together at the 2 half-sites.
- Stimulates transcription of particular genes.

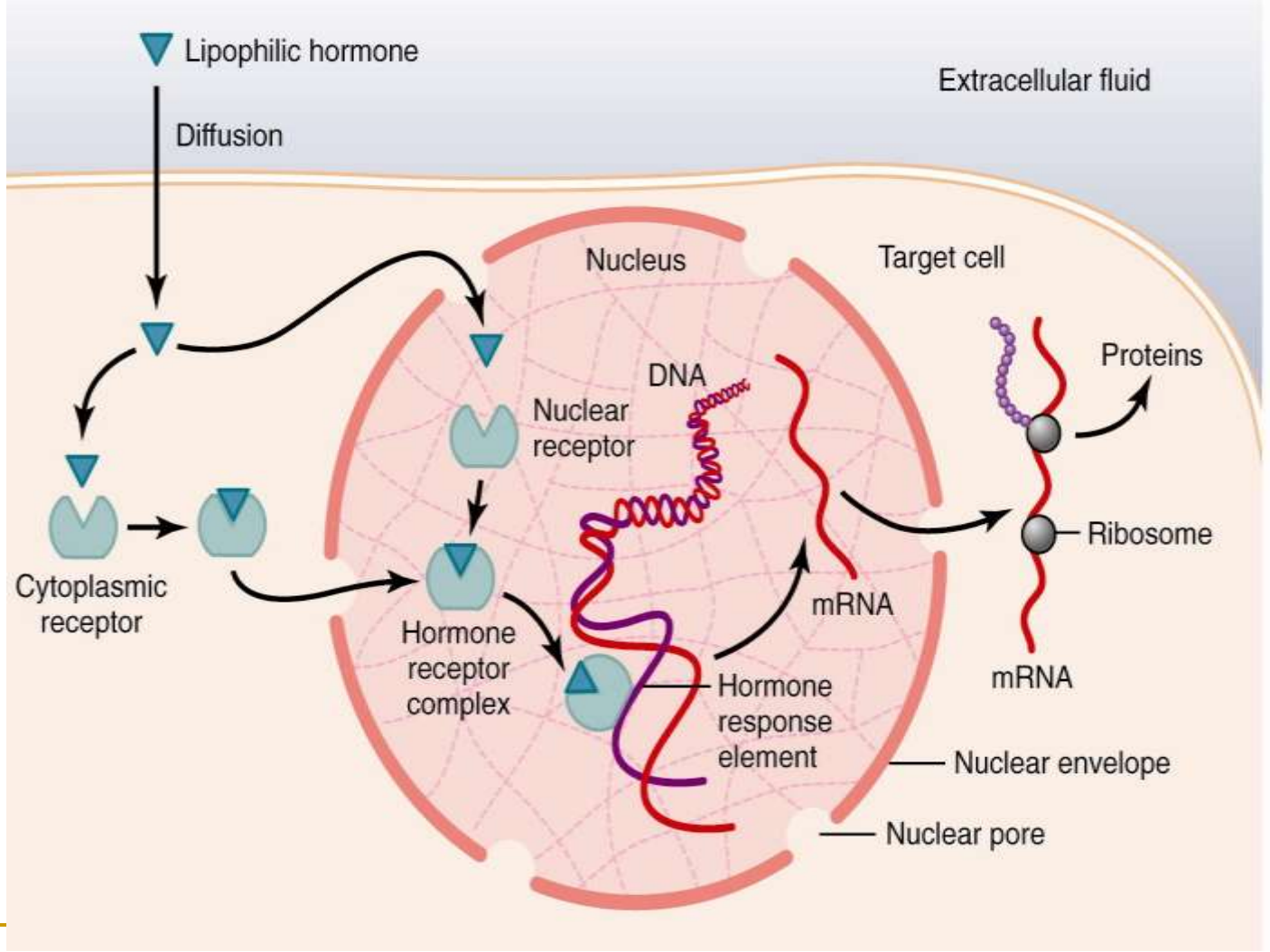


Mechanism of Thyroid Hormone Action

- T_4 passes into cytoplasm and is converted to T_3.
- Receptor proteins located in nucleus.
  - T_3 binds to ligand-binding domain.
  - Other half-site is vitamin A derivative (9-cis-retinoic) acid.
    - DNA-binding domain can then bind to the half-site of the HRE.
  - Two partners can bind to the DNA to activate HRE.
    - Stimulate transcription of genes.



Steroid & Thyroid Hormones - Mechanism of Action



Actions of Thyroid Hormones