Cell Signaling

Receptor enzymes & GPCR

Ms. Kirti Singh
Assistant Professor
Department of Zoology
Udai Pratap College, Varanasi

Need for cell signalling

- All forms of cells rely on communication with their surrounding environment.
- This communication is needed to adjust their internal environment according to the situation outside.

- Examples of such adjustments can be
  1. Movement towards or away from the food.
  2. Movement towards or away from chemical gradient.
  3. Responding to solute concentration changes in the medium.
  4. Responding to growth factors.
  5. Responding to hormones.
  6. Responding to voltage changes.
  7. Responding to insults to the cell.

  And many more such examples.
- Now, the question is how do they respond to the changes in the surrounding medium??
- The answer is cell signalling.

- **Basic outline of how cell signalling works:**

- It has following basic steps in all its forms
  1. An extracellular signal in the form of a chemical molecule, generally called as ligand.

  2. Ligand binds to its receptor on the target cell. Usually, the receptor is a transmembrane protein but sometimes it can be a soluble protein found in cytosol or nucleus.

  3. This receptor-ligand interaction conveys the outer situation inside the cell and initiates a series of reactions.

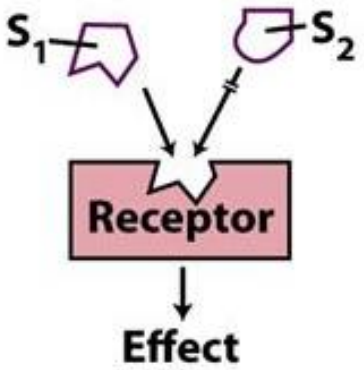
  4. This series of reactions is focussed on two things in most cases
     1. Signal amplification by generating the second messenger.
     2. Activating the genes and proteins that are needed for appropriate response to the signal.

  5. Now cell begins to show its response to the signalling ligand.
     E.g. glycogenesis in response to insulin signalling.

Features of signal transducing systems

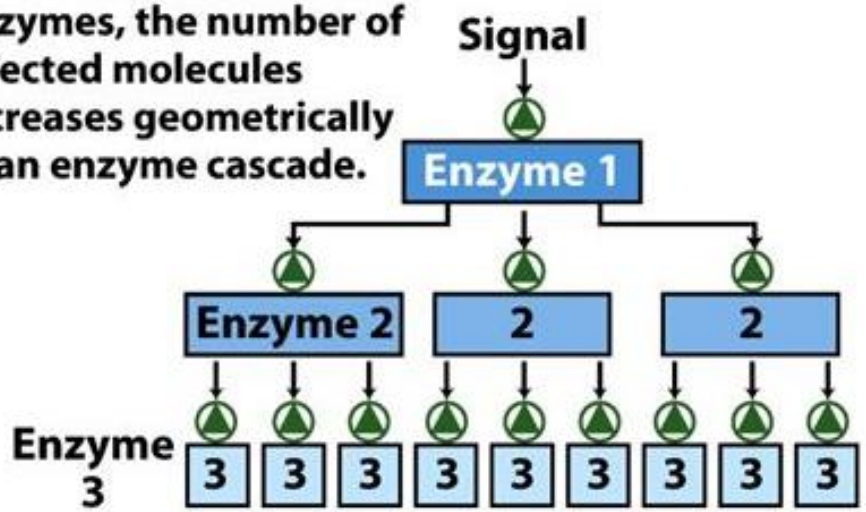
(a) Specificity

Signal molecule fits binding site on its complementary receptor; other signals do not fit.



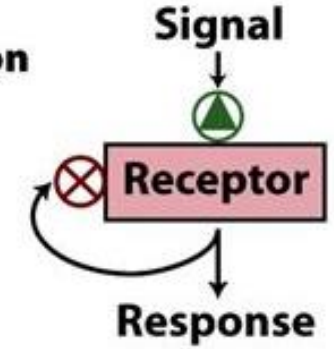
(b) Amplification

When enzymes activate enzymes, the number of affected molecules increases geometrically in an enzyme cascade.



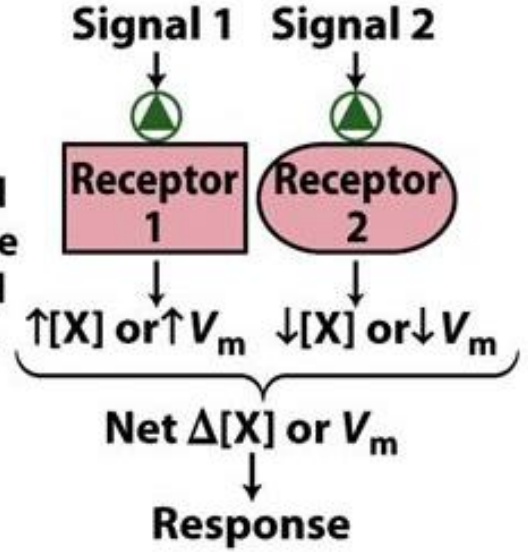
(c) Desensitization/Adaptation

Receptor activation triggers a feedback circuit that shuts off the receptor or removes it from the cell surface.



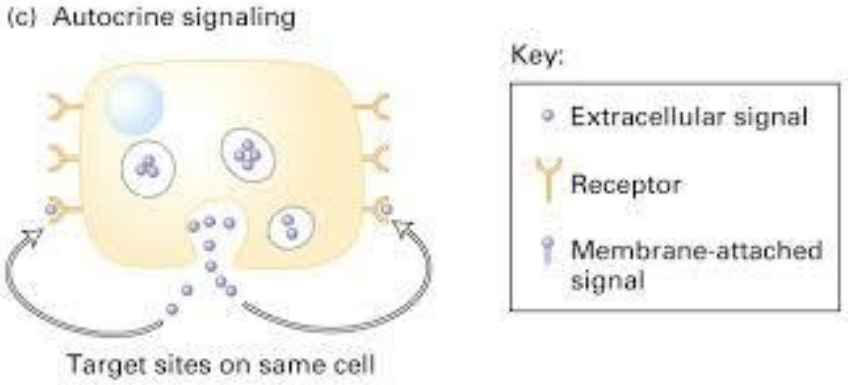
(d) Integration

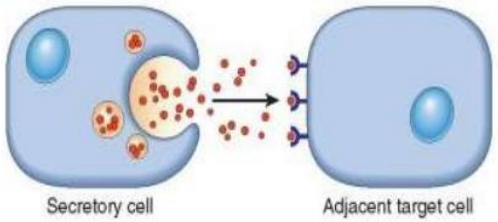
When two signals have opposite effects on a metabolic characteristic such as the concentration of a second messenger X, or the membrane potential V_m, the regulatory outcome results from the integrated input from both receptors.



Types of signalling

- Based on the distance the ligand molecule has to travel signalling is of following types
  1. **Autocrine signalling:** The ligand molecule is diffusible but binds to the same cell that has secreted it. E.g. cytokine signalling in immune system.



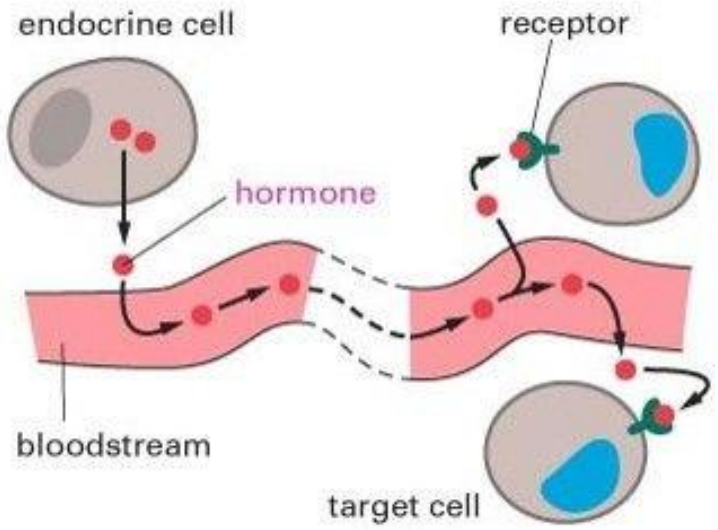


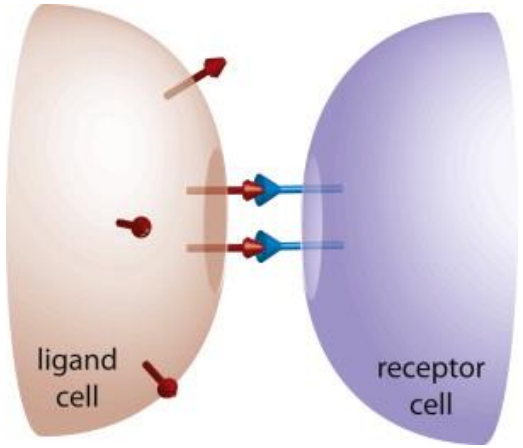
Paracrine Signalling

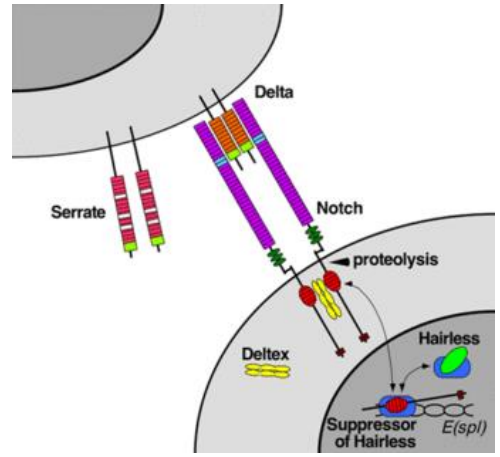
19

2. Paracrine signalling: The ligand molecule is diffusible and binds to the neighbouring cells that are present around the secreting cell. E.g. Prostaglandin signalling during inflammation.

3. Endocrine signalling: The ligand has to travel a long distance in blood to reach its target cell. E.g. Hormone signalling





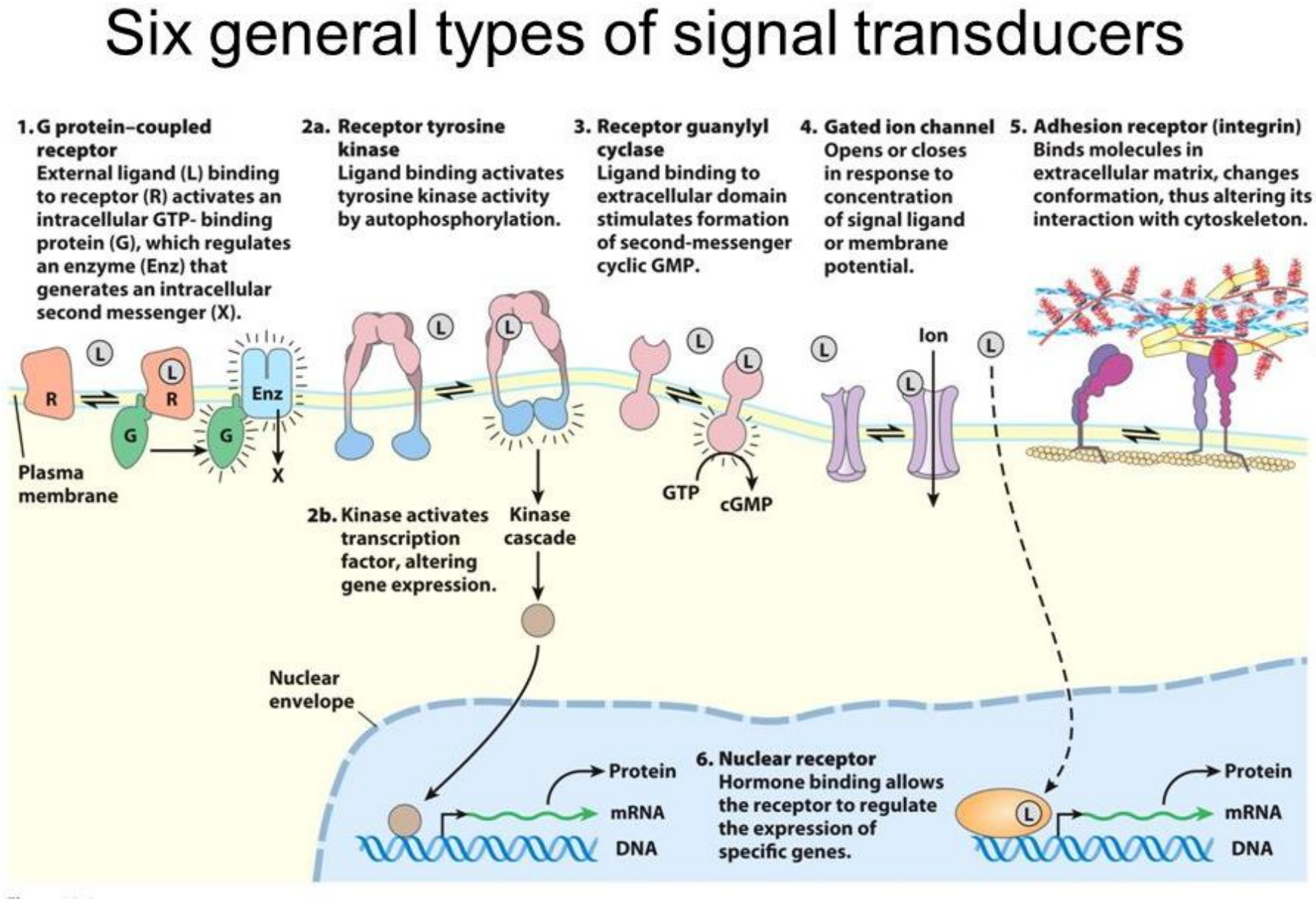


Juxtacrine signalling

4. Juxtacrine signalling: In this ligand is non-diffusible. Thus, receptor and ligand both are fixed transmembrane molecules and found on adjacent cells that are in touch with each other.

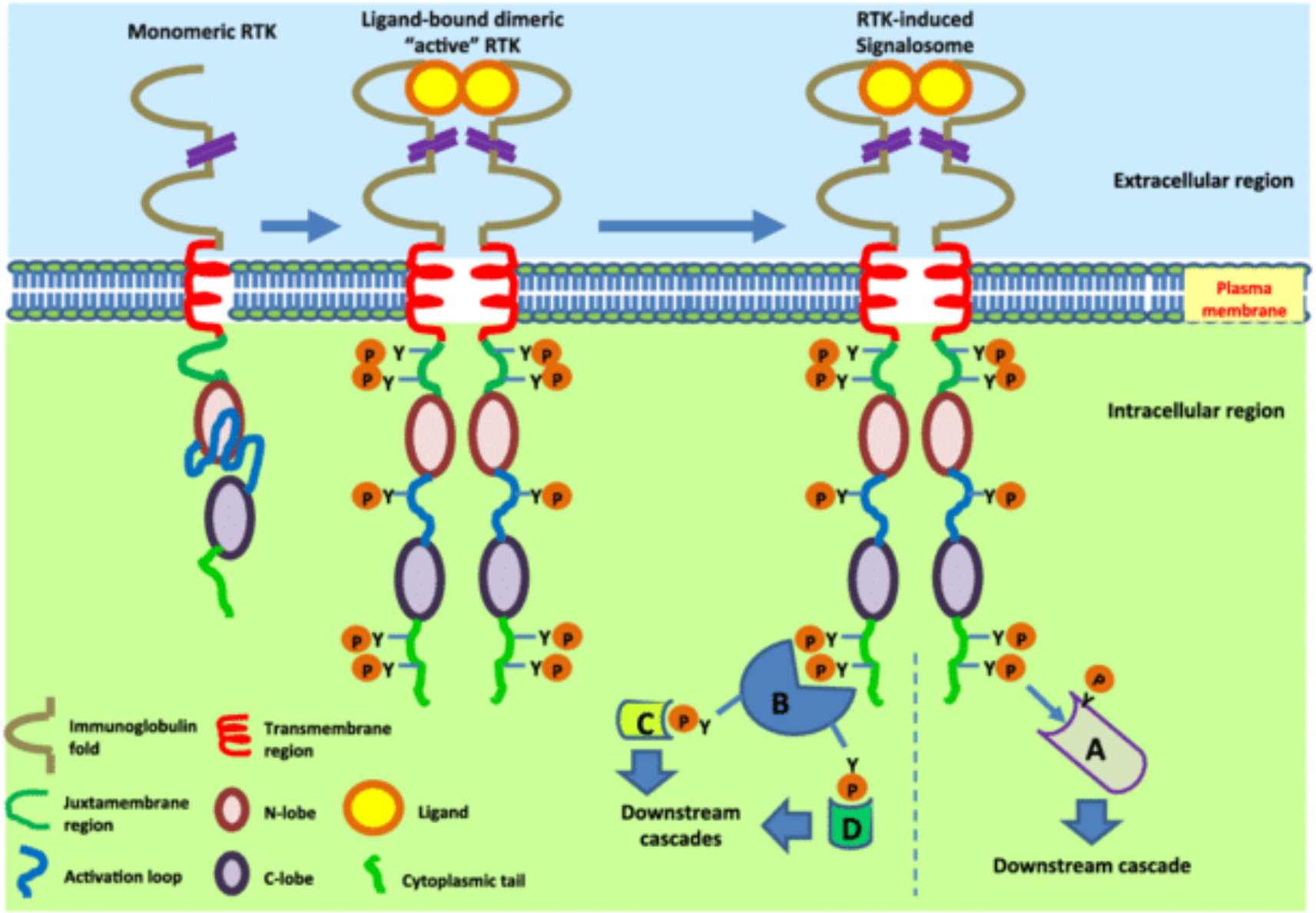
E.g. Delta-notch signalling during embryonic development.

Types of signal transducing systems based on receptors



Receptor enzymes such as insulin receptor

- **General discussion:**These proteins have a ligand-binding domain on the extracellular surface of the plasma membrane and an enzyme active site on the cytosolic side,
- These two domains are connected by a single transmembrane segment.
- Commonly, the receptor enzyme is a protein kinase that phosphorylates Tyr residues in specific target proteins; the insulin receptor is the prototype for this group.
- In plants, the protein kinase of receptors is specific for Ser or Thr residues.
- Other receptor enzymes synthesize the intracellular second messenger cGMP in response to extracellular signals. The receptor for atrial natriuretic factor is typical of this type.



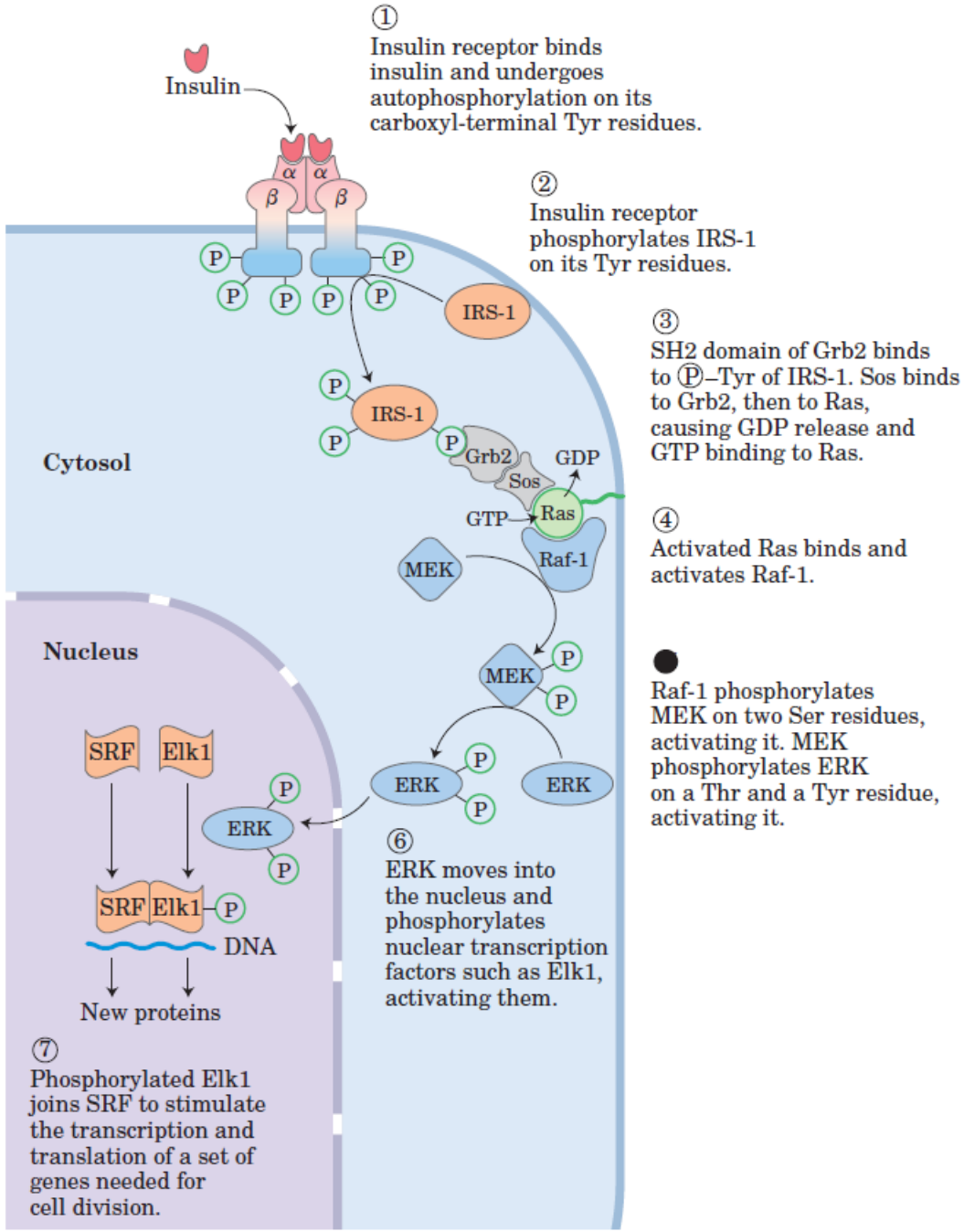
1. The receptor is a monomer in absence of ligand.

2. On binding with ligand it undergoes dimerization to form a homodimer.

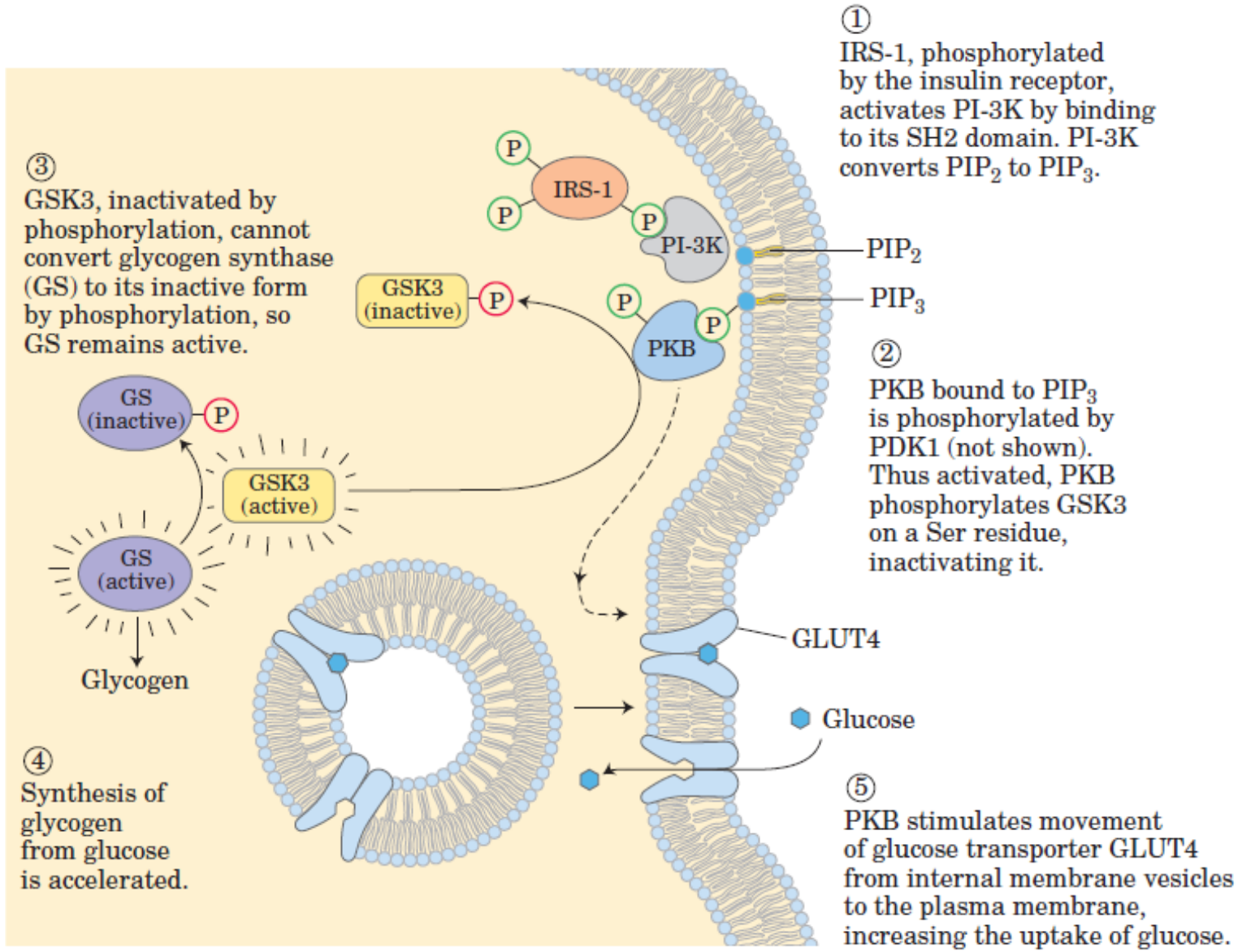
3. The tyrosine kinase activity in the cytosolic domain begins to phosphorylate the tyrosine residue in its partner. This process is called as cross phosphorylation.

4. After this begins the downstream signalling in the form of a cascade.

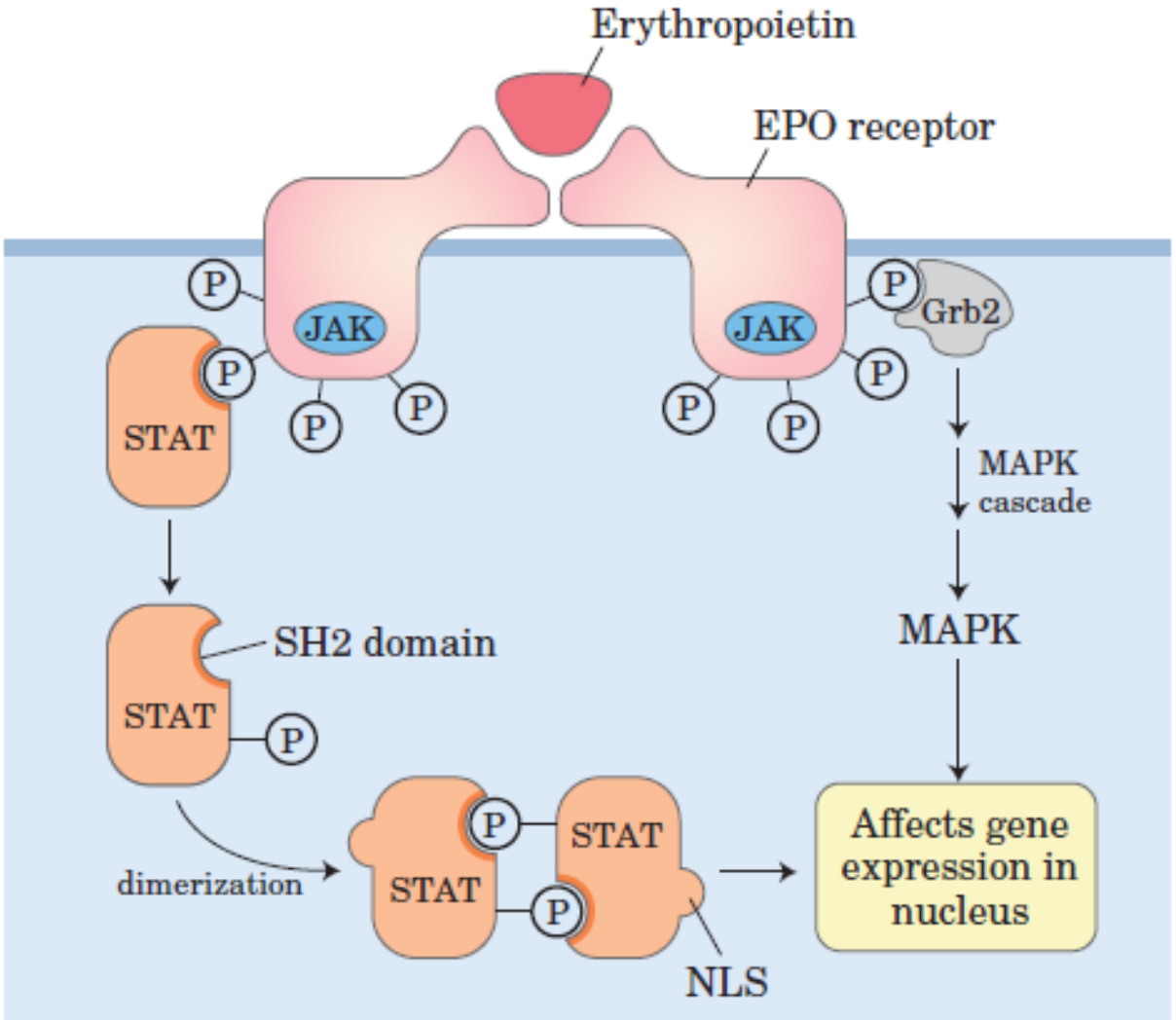
Insulin signalling, its basic steps and effects



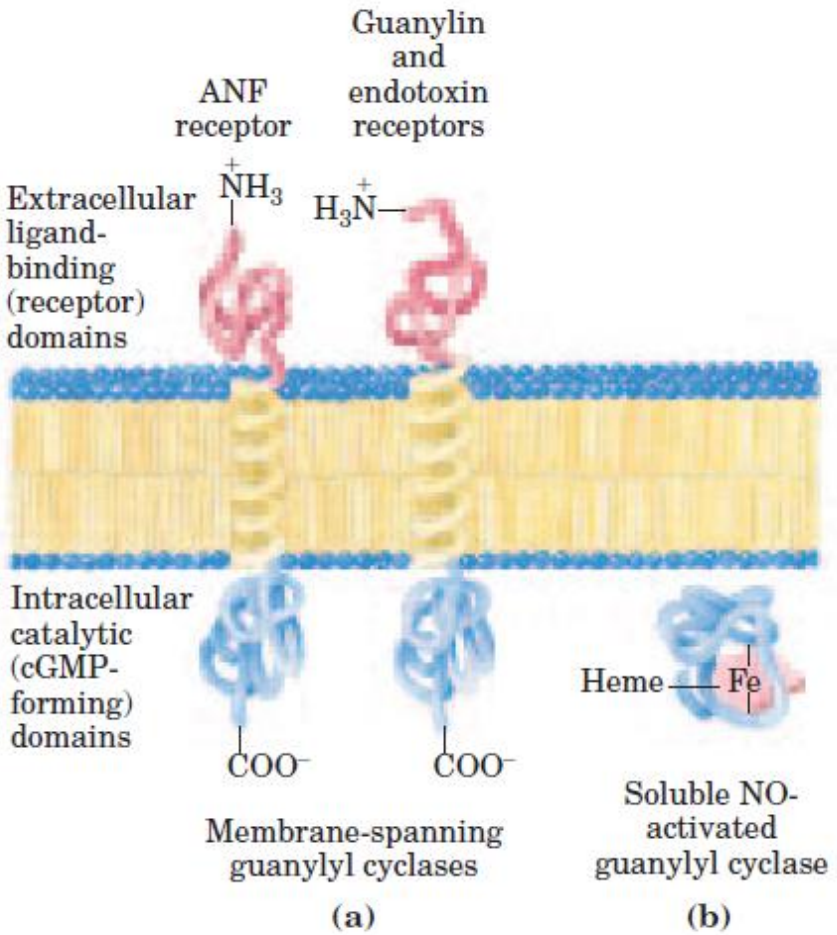
Effect of insulin on muscle cells

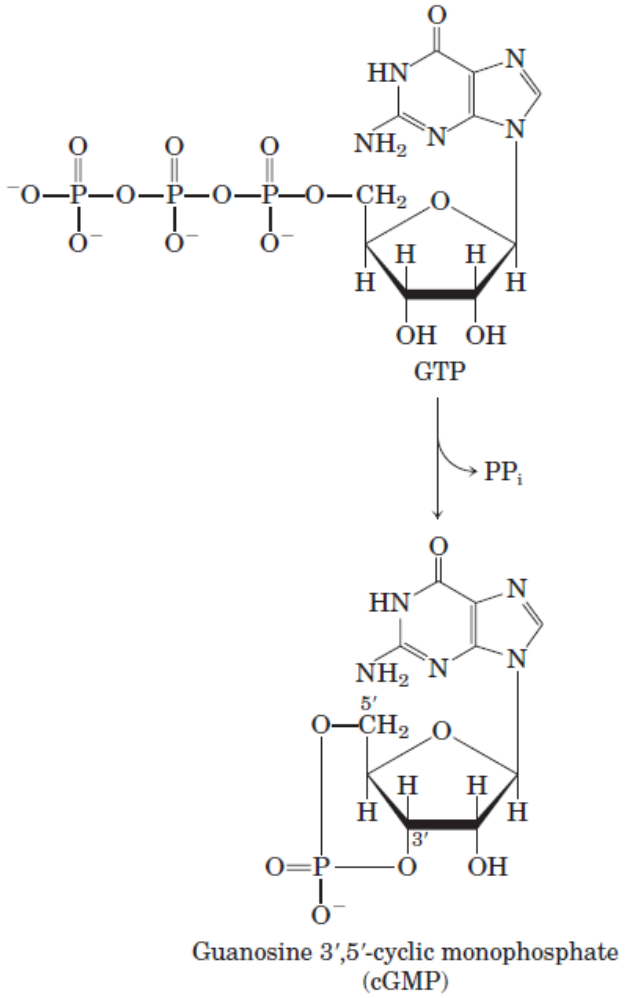


Variation of RTK



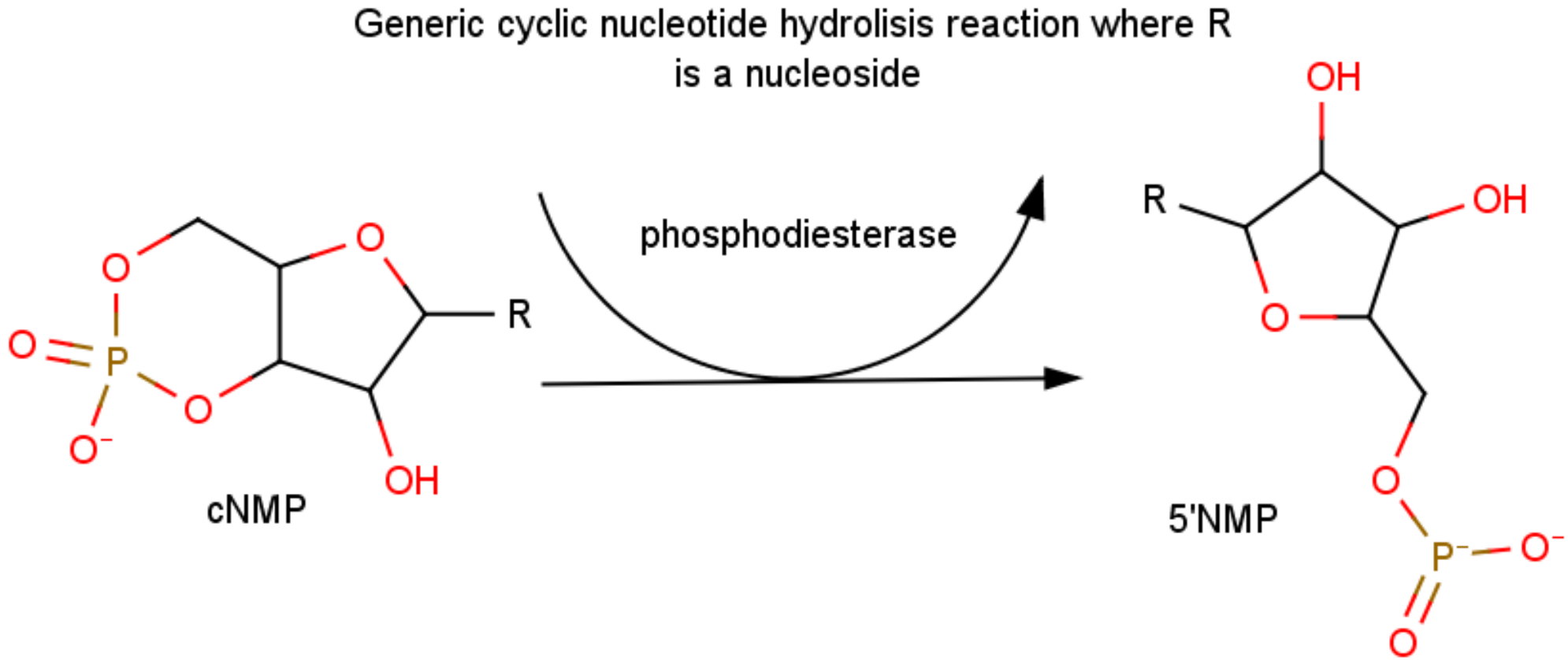
Receptor enzymes with guanylyl cyclase activity





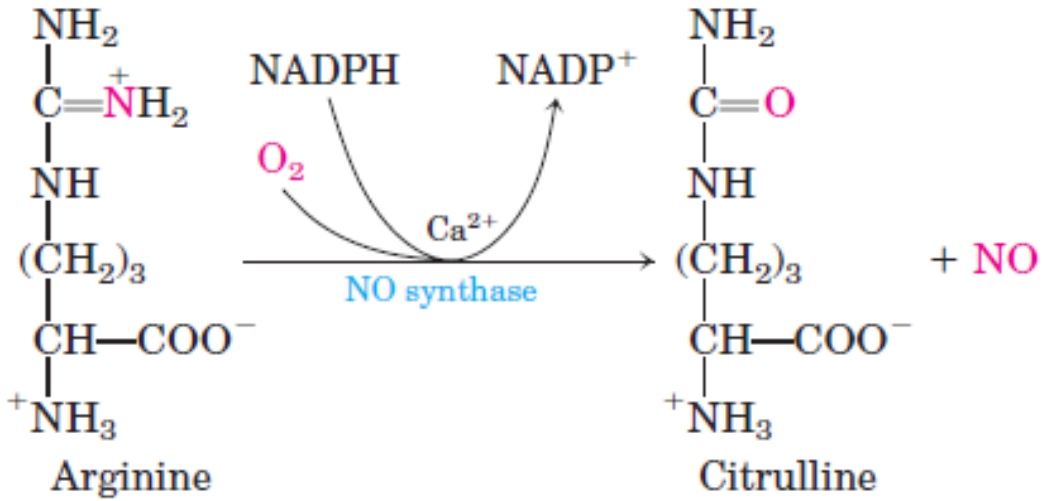
Effects of cGMP are mediated by activating the enzyme Protein kinase G or PKG. It causes phosphorylation of Ser ot Thr.

Termination of cGMP signalling

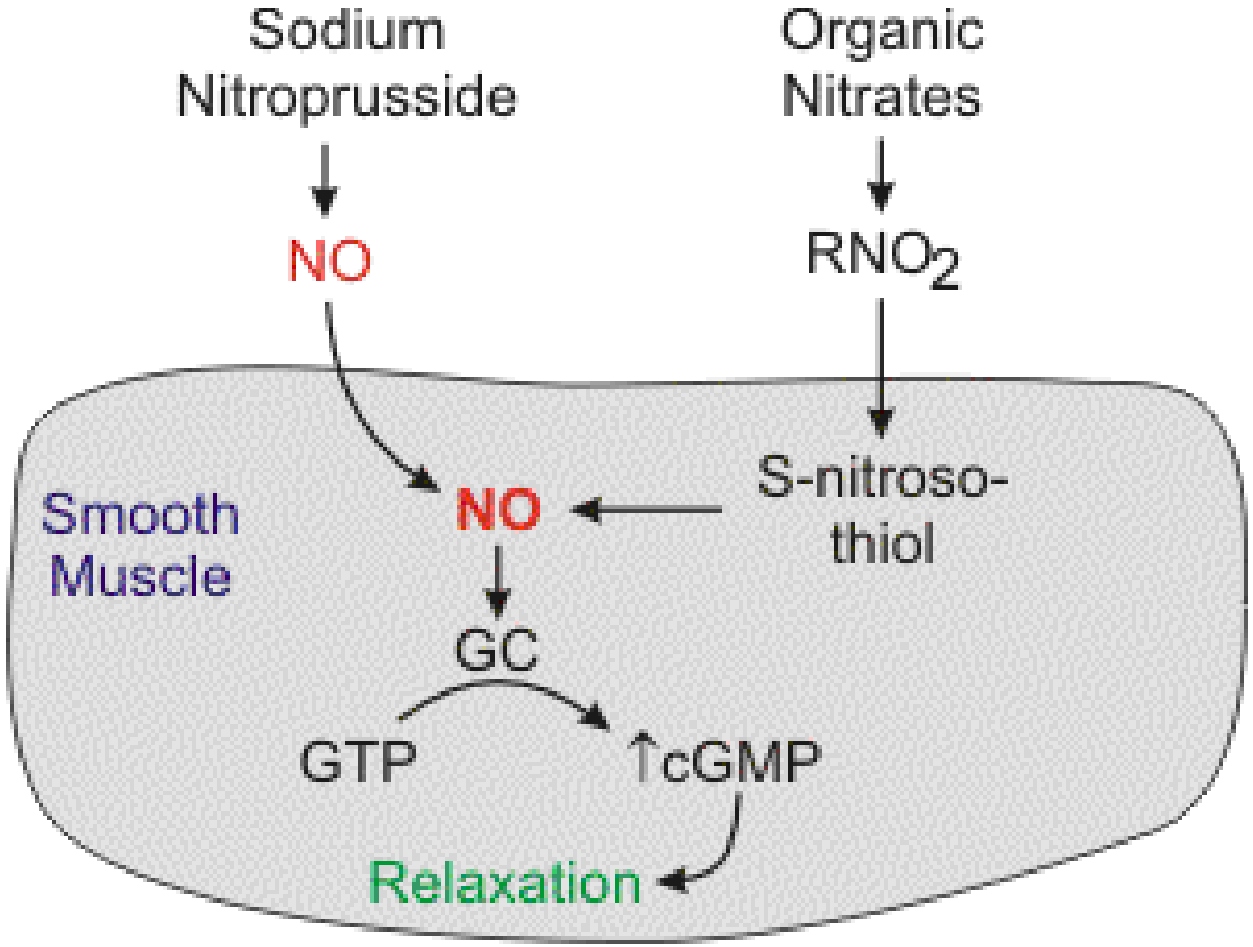


Nitric oxide signalling and its effects

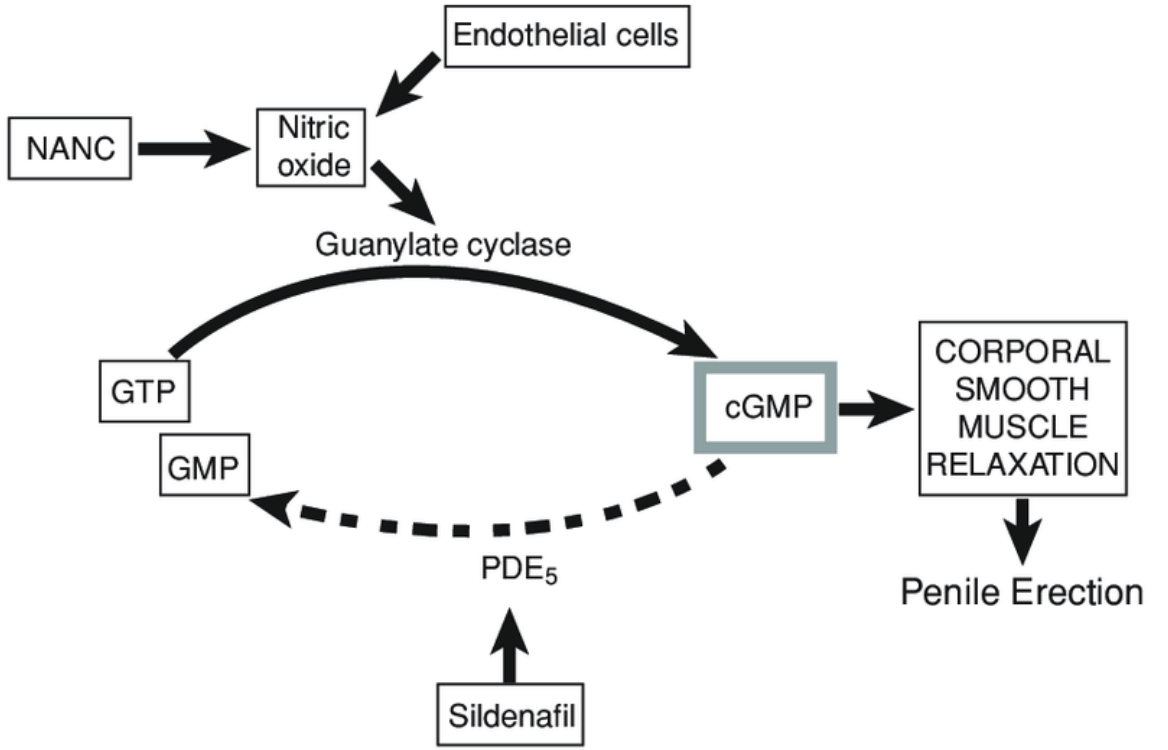
Nitric oxide production



How nitroglycerine works??



Nitric oxide, Sildenafil citrate and Nobel prize in medicine

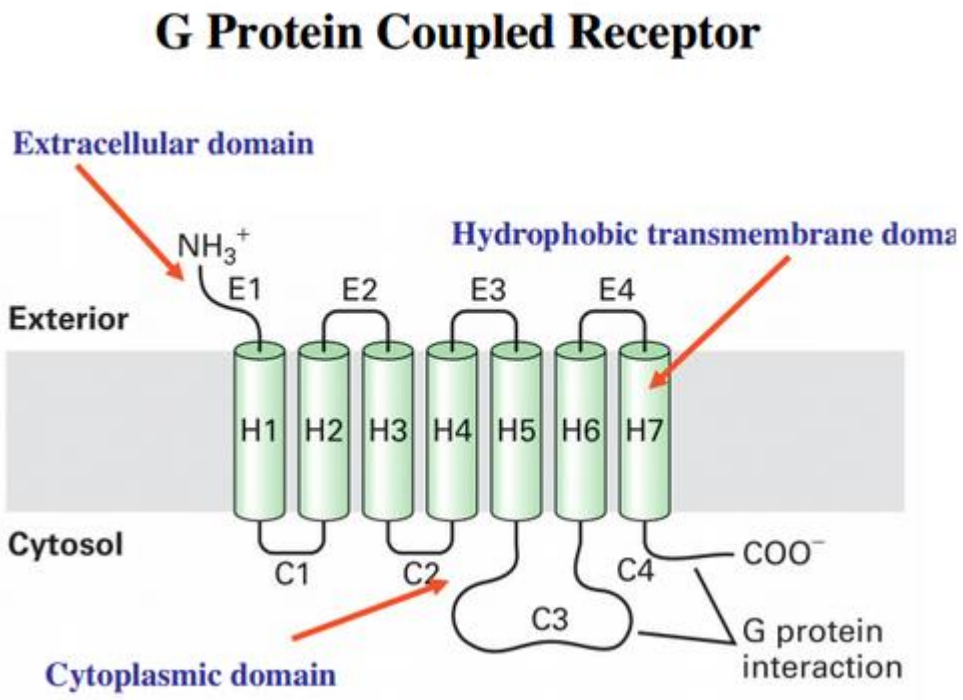


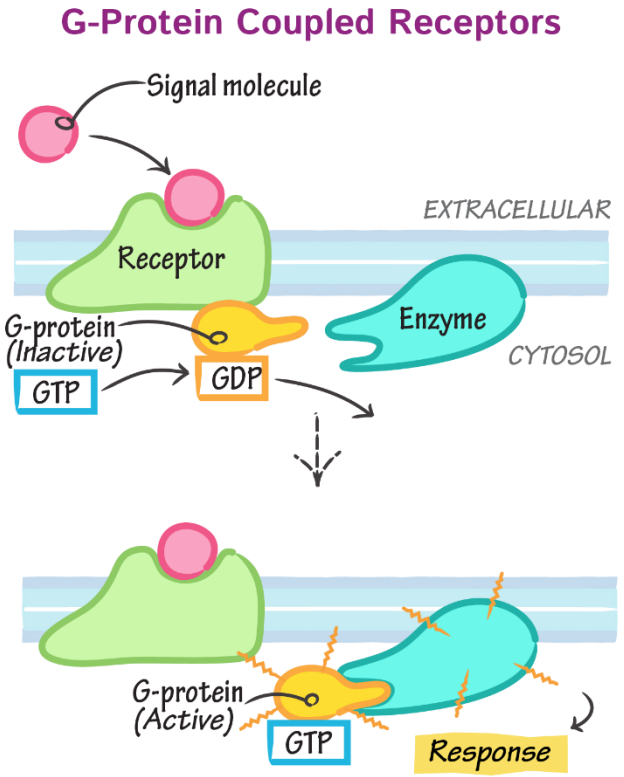
NANC =Non-adrenergic, noncholinergic nerve endings; GTP = guanylate triphosphate; cGMP = guanylate monophosphate; PDE_5 = phosphodiesterase

G-protein coupled receptors(GPCR) and their second messenger

- They are called as serpentine receptor because they have 7 transmembrane domains that resemble the twists of snake.

- They are called as G-protein coupled because they have a cytosolic region that is found associated with a guanosine-nucleotide binding protein or G-protein.





- Epinephrine receptors are called as adrenergic receptors and are of 4 general types viz α_1, α_2, β_1, β_2.
- The β family of receptors are found in the muscles, liver and adipose tissues.

Epinephrine signalling

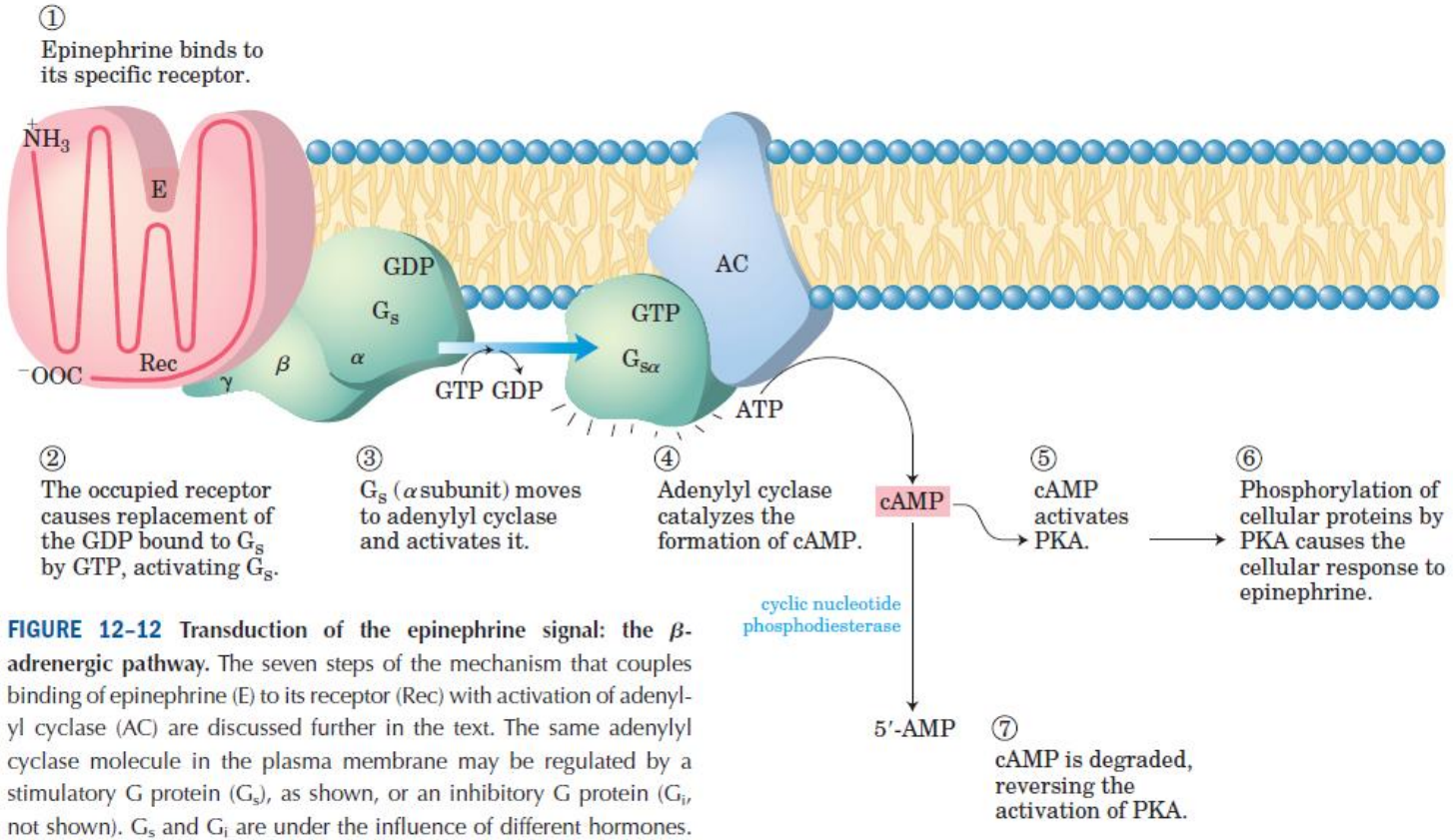
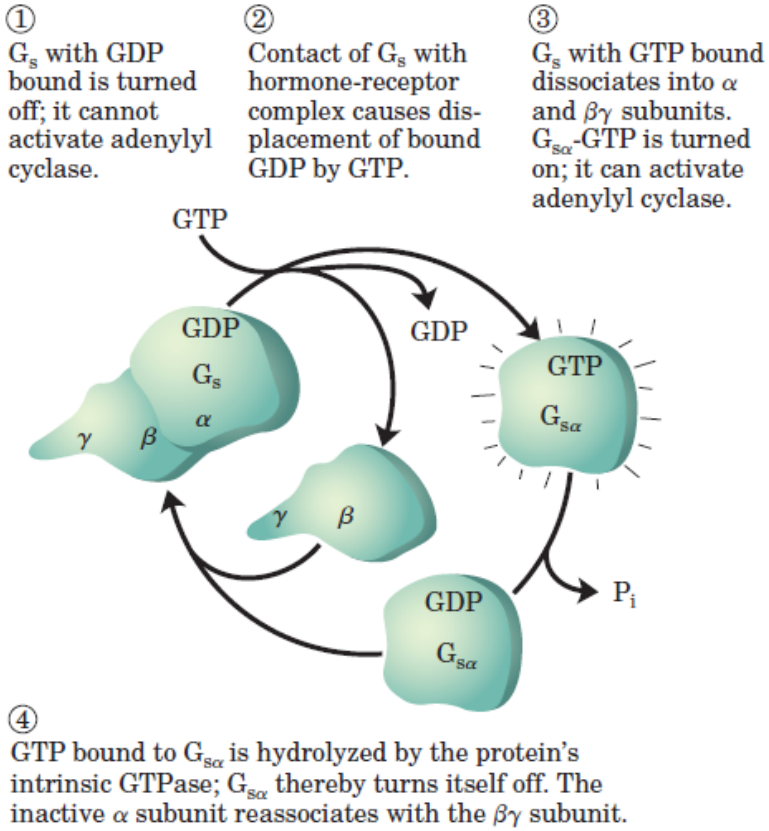
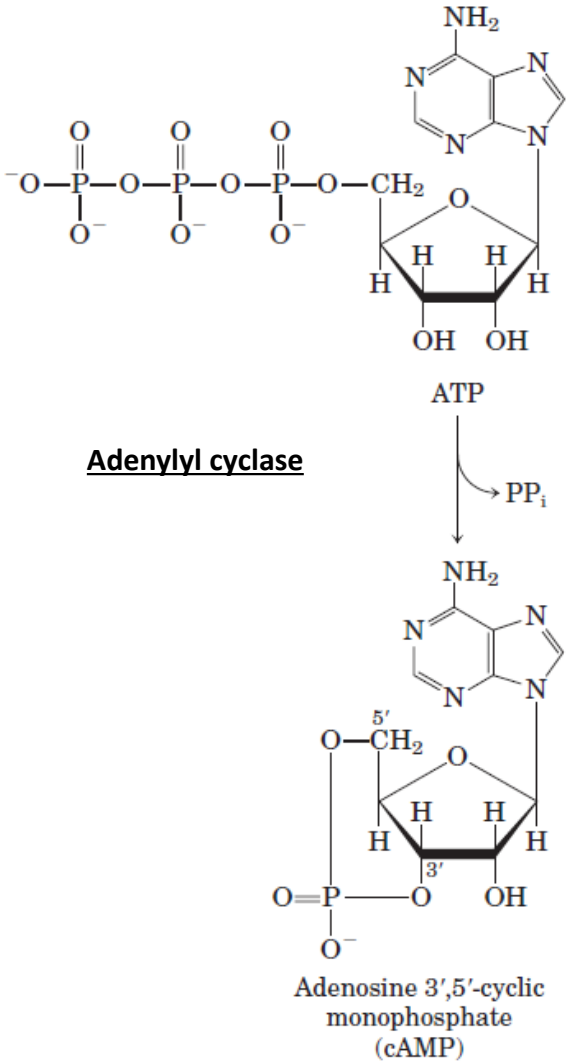


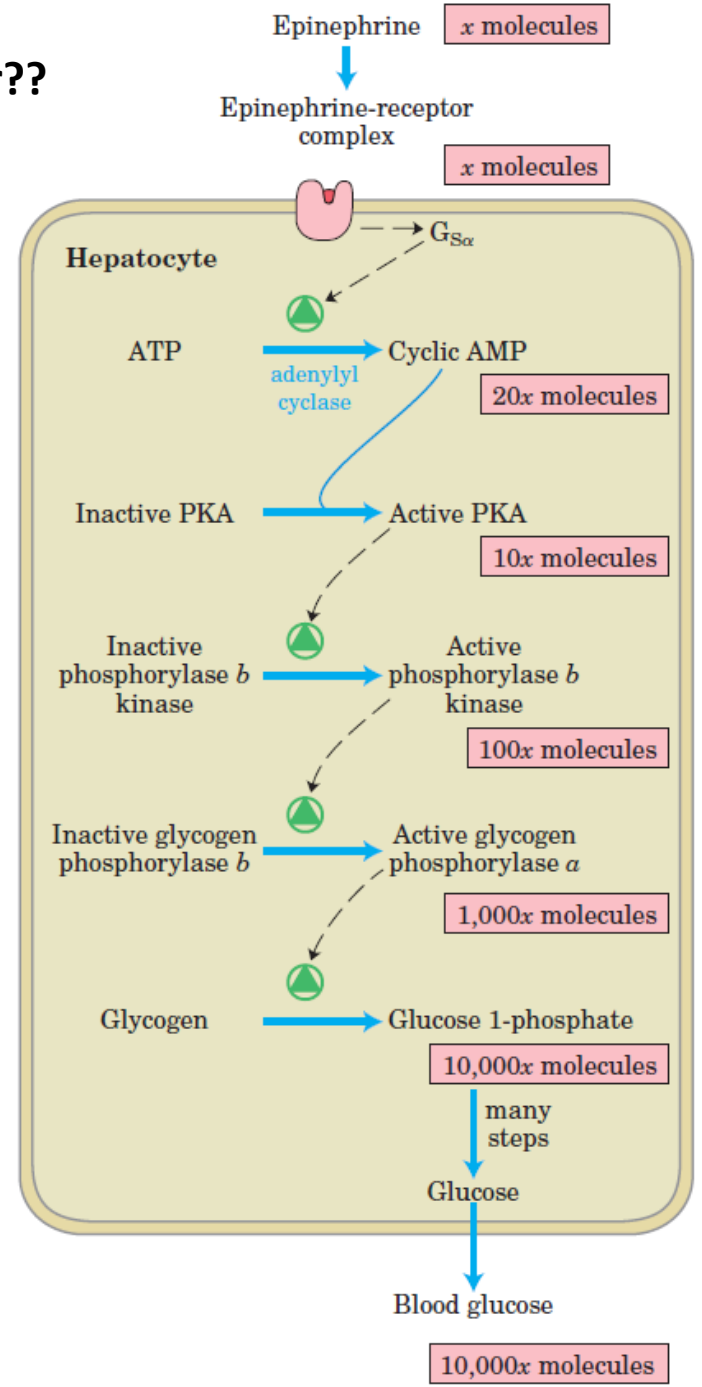
FIGURE 12–12 Transduction of the epinephrine signal: the β-adrenergic pathway. The seven steps of the mechanism that couples binding of epinephrine (E) to its receptor (Rec) with activation of adenylyl cyclase (AC) are discussed further in the text. The same adenylyl cyclase molecule in the plasma membrane may be regulated by a stimulatory G protein (G_s), as shown, or an inhibitory G protein (G_i, not shown). G_s and G_i are under the influence of different hormones. Hormones that induce GTP binding to G_i cause *inhibition* of adenylyl cyclase, resulting in lower cellular [cAMP].

G-protein cycle

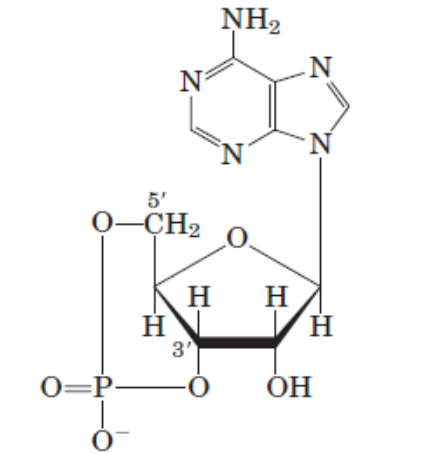


Why we call cAMP as second messenger??





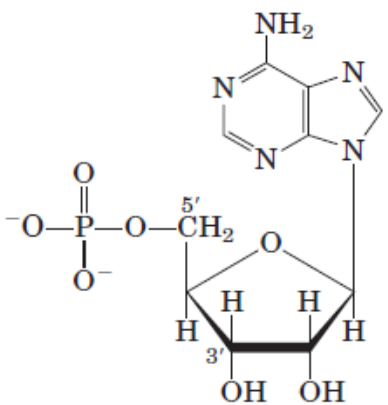
Termination of epinephrine signalling



Cyclic AMP

Cyclic nucleotide PDE

H_2O



Adenosine 5′-monophosphate (AMP)

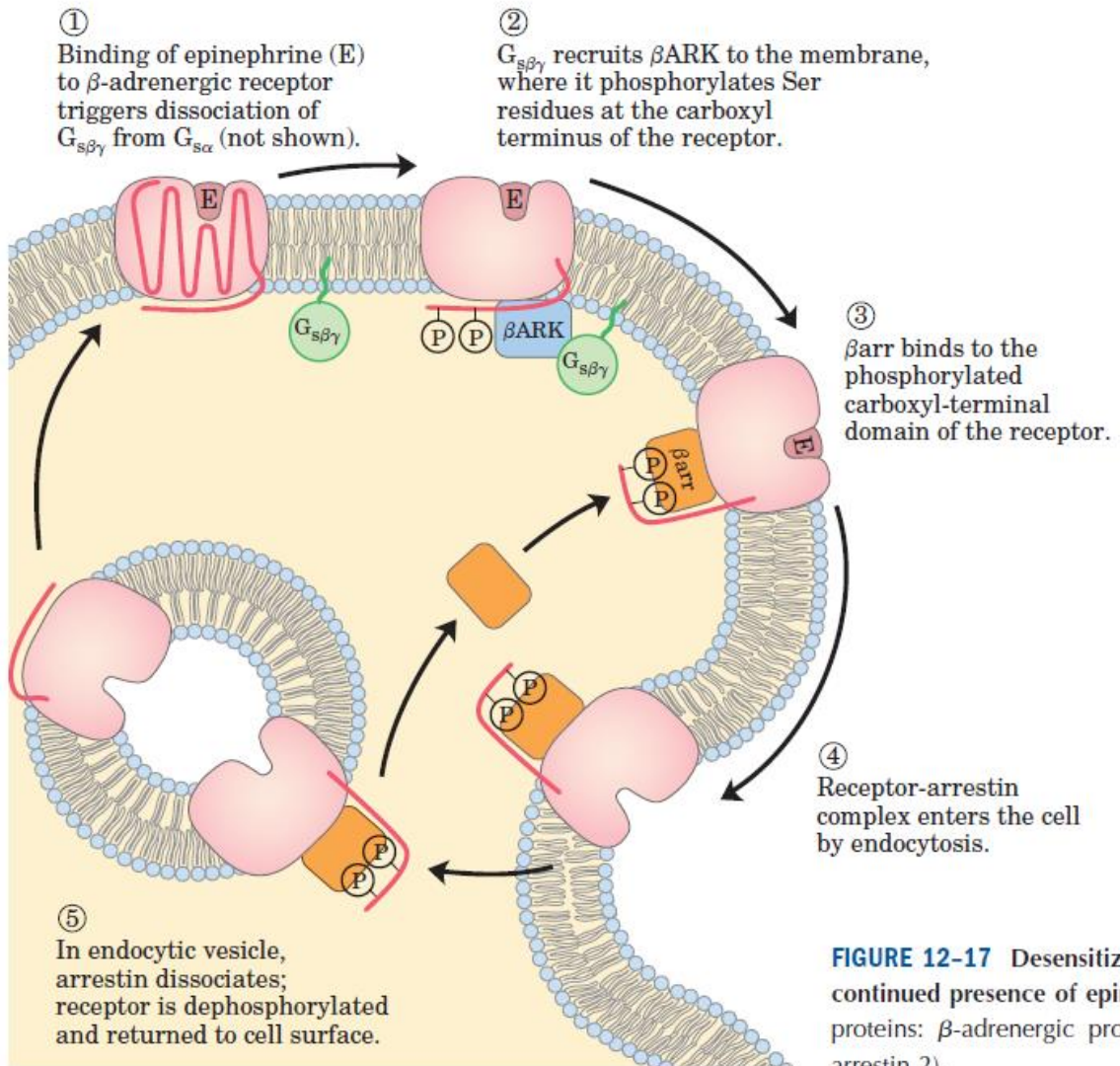


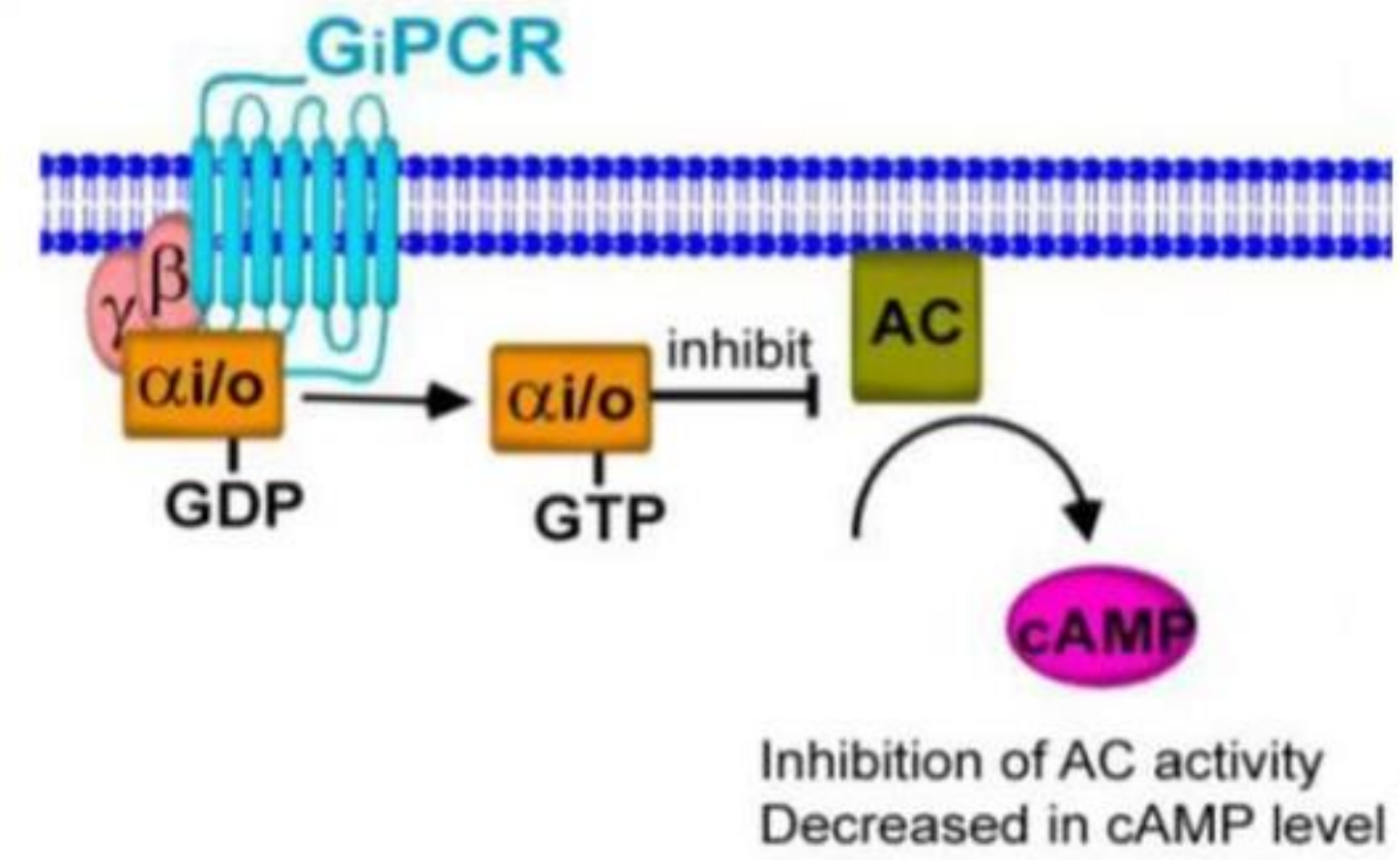
FIGURE 12–17 Desensitization of the β-adrenergic receptor in the continued presence of epinephrine. This process is mediated by two proteins: β-adrenergic protein kinase (βARK) and β-arrestin (βarr; arrestin 2).

Modulations of cAMP during signal transduction

- Not just epinephrine but many other hormones use cAMP as their second messenger such as glucagon, ACTH etc.
- All of them act to temporarily increase the concentration of cAMP in the cell. However, it is not the only modulation possible on cAMP.
- Some molecules act to decrease the levels of cAMP in the cell.
- They do so by inhibition of the enzyme adenylyl cyclase.
- Examples of such molecules include epinephrine acting through acting through α_2 receptors, somatostatins and some prostaglandins.

G_i cAMP Dependent Pathway

- Receptors for these proteins are linked with inhibitory G-proteins or G_i.
- They are structural homologs of G_s proteins.
- Once activated these proteins bind to GTP and act to inhibit the enzyme adenylyl cyclase.



Disruption of G-protein signalling and toxins

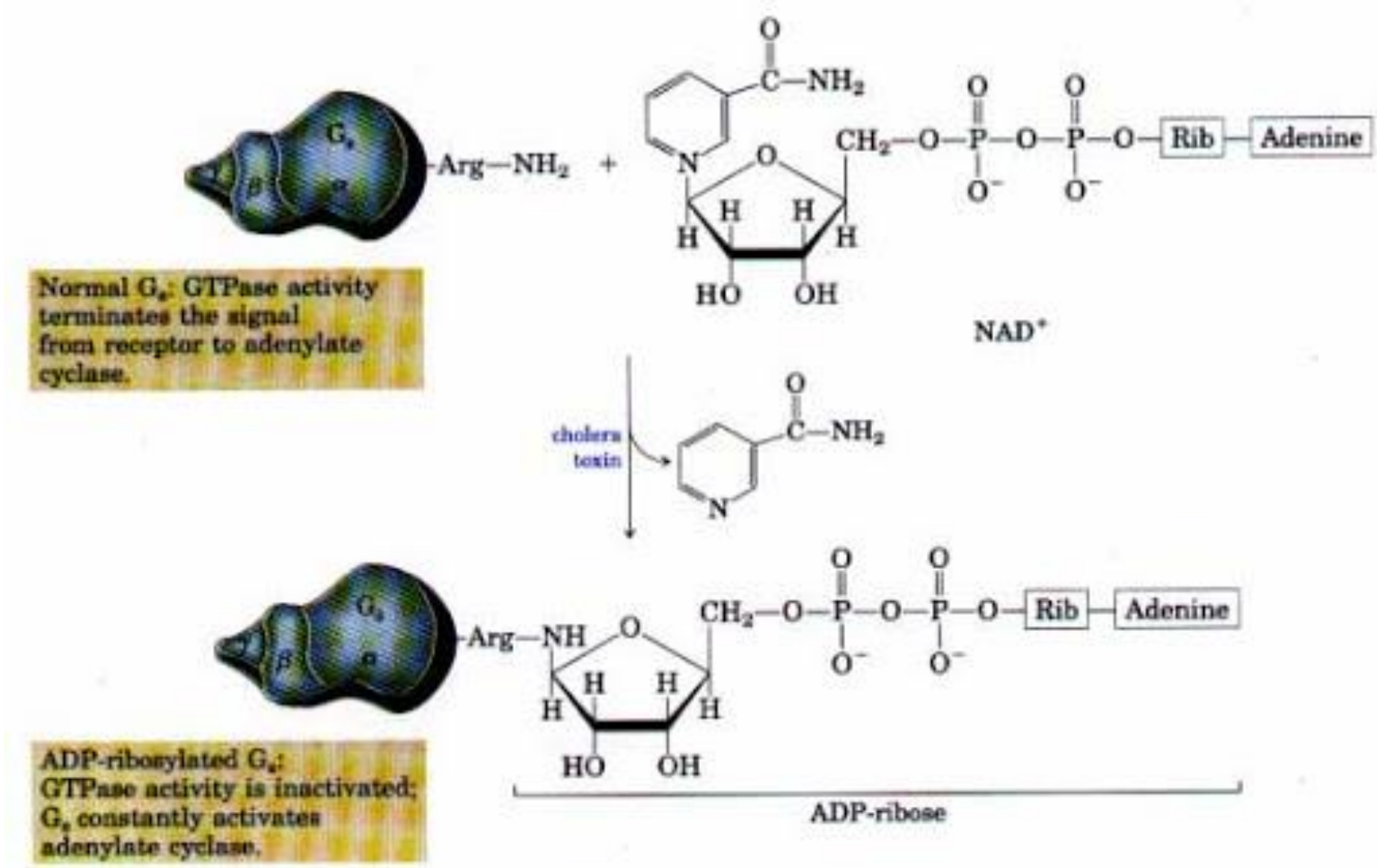
- **Cholera toxin:** It is responsible for the symptoms of cholera and is an enzyme.

- Once inside the cell in small intestine, it causes ADP-ribosylation of the α-subunit of the G_s protein.
- The ADP-ribose is taken from NAD^+ .

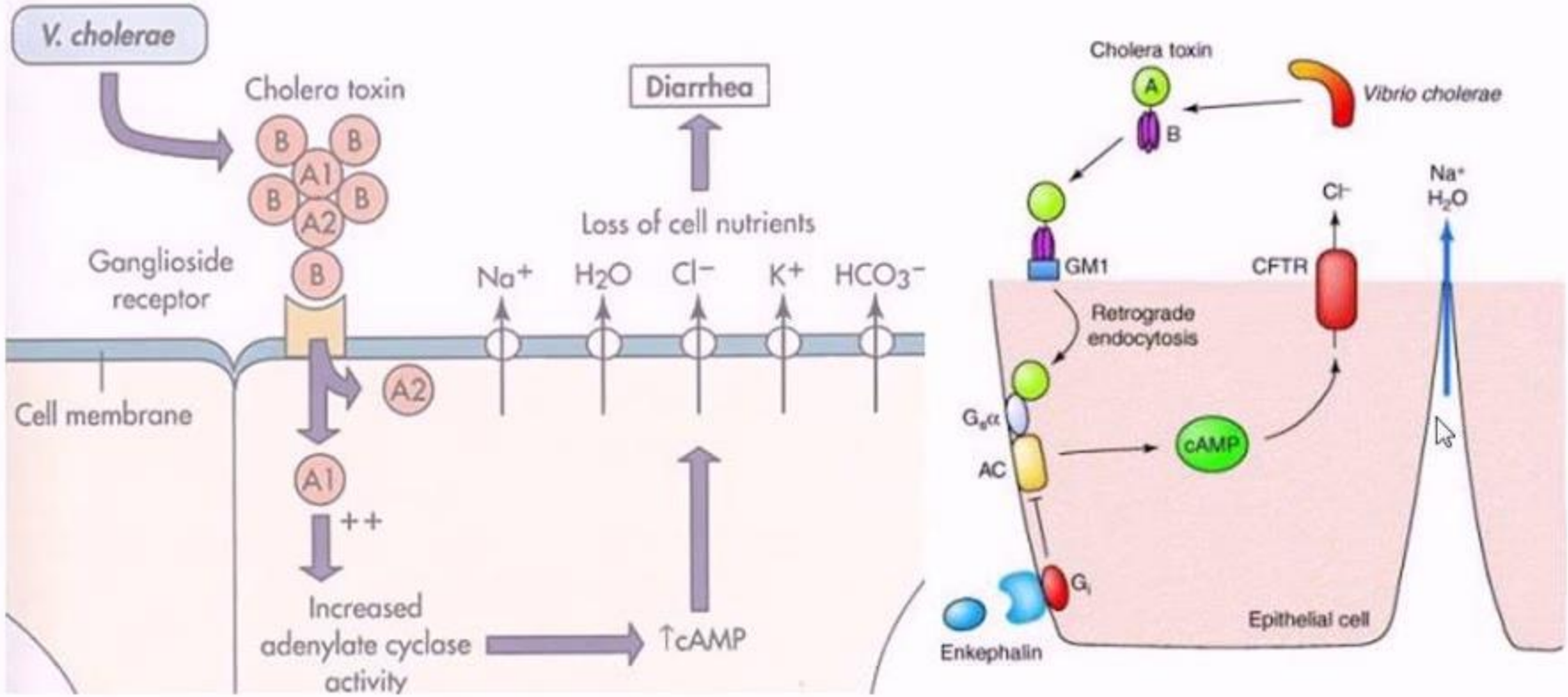
- This reaction blocks the GTPase activity of the G_α protein.

- Thus, adenylyl cyclase is permanently on and continues to produce cAMP at high levels.

- As a result, chloride channels are left open and this leads to loss of electrolytes.


Normal G_s: GTPase activity terminates the signal from receptor to adenylate cyclase.
Arg—NH_2
+
NAD+
cholera toxin
ADP-ribosylated G_s: GTPase activity is inactivated; G_s constantly activates adenylate cyclase.
Arg—NH
ADP-ribose
Rib
Adenine

Cholera toxin : Mode of action

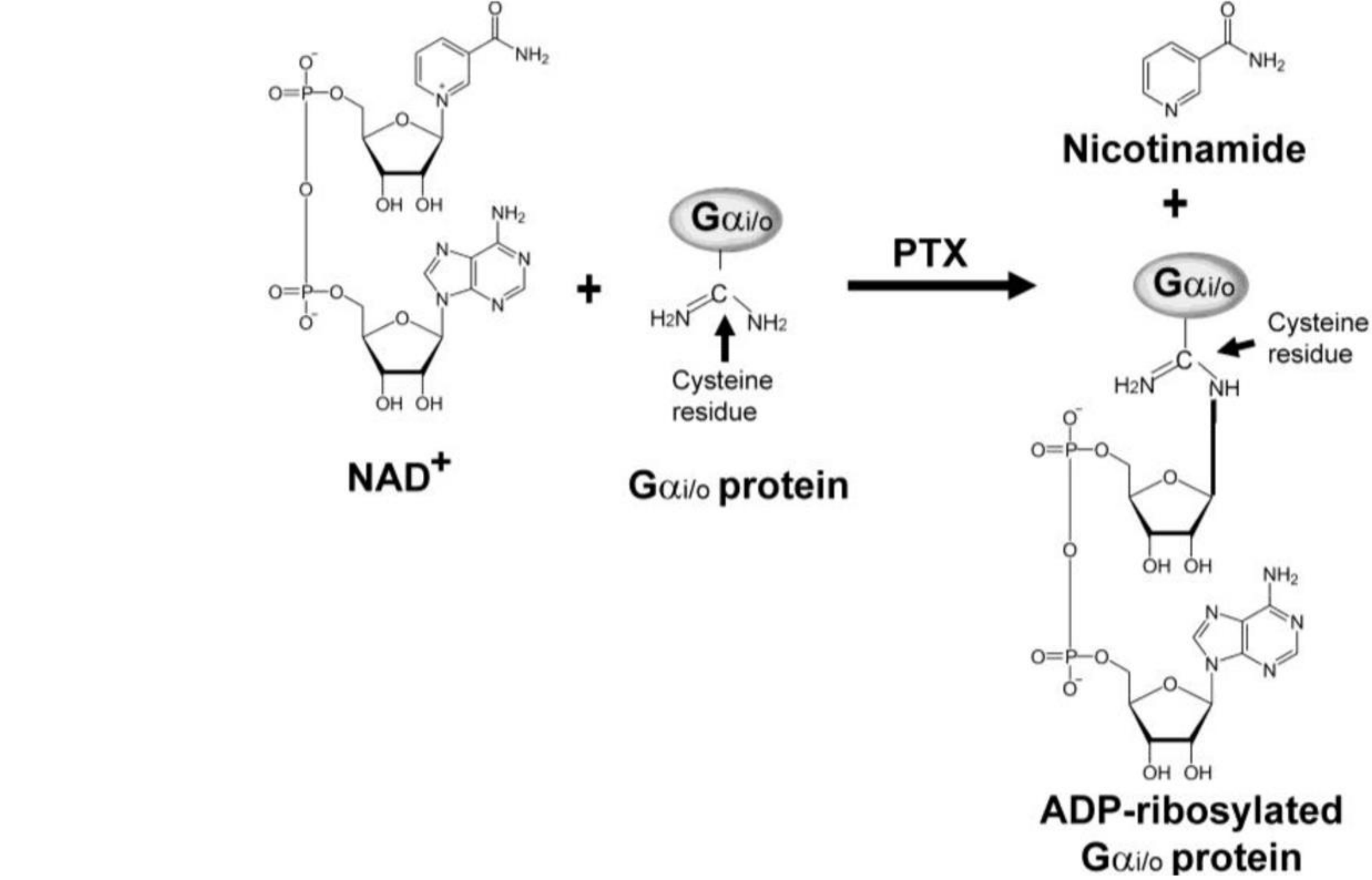


- **<u>Pertussis toxin:</u>** It is responsible for symptoms of whooping cough and is an enzyme like the cholera toxin.
- It also acts by causing the ADP-ribosylation reaction by transferring the ADP-ribose moiety from NAD^+.

- However, it does so on the α-subunit of the G_i protein.

- This reactions blocks the displacement of GDP by GTP.

- Thus, it fails to inhibit the adenylyl cyclase enzyme and cAMP production continues.


NAD+
Gαi/o protein
Cysteine residue
PTX
Nicotinamide
+
Gαi/o
Cysteine residue
ADP-ribosylated Gαi/o protein

Other secondary messengers

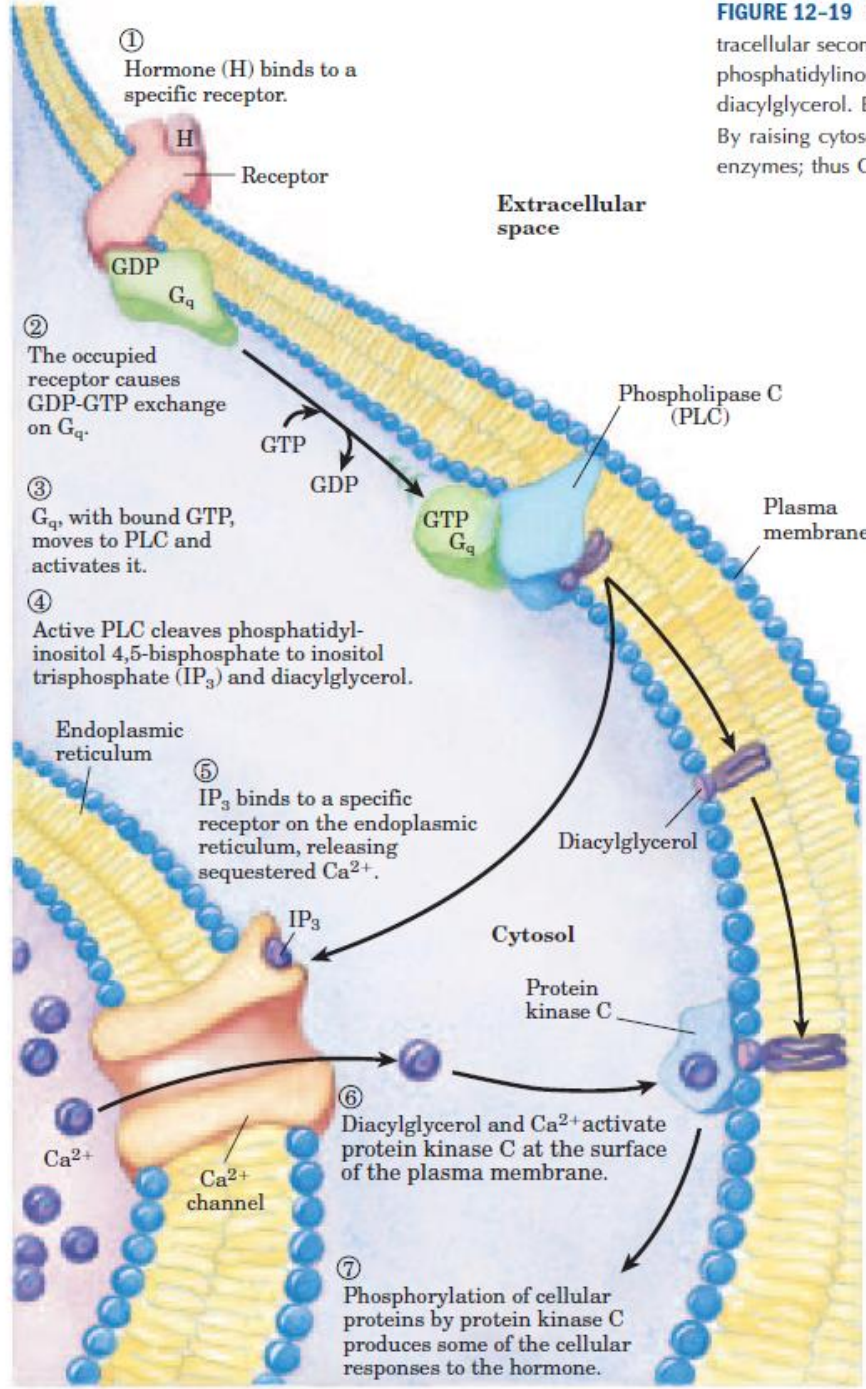


FIGURE 12–19 Hormone-activated phospholipase C and IP_3. Two intracellular second messengers are produced in the hormone-sensitive phosphatidylinositol system: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. Both contribute to the activation of protein kinase C. By raising cytosolic [Ca^{2+}], IP_3 also activates other Ca^{2+}-dependent enzymes; thus Ca^{2+} also acts as a second messenger.

Thank you