Biochemistry - ES

Done By :

Waad Barghouthi & Mustafa Ramahi

Corrected By :

Waad Barghouthi

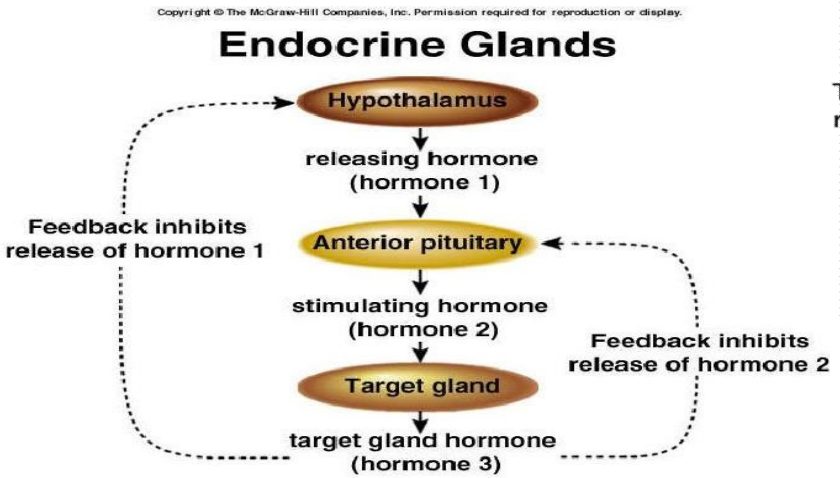
These slides were modified using
015 Sheets

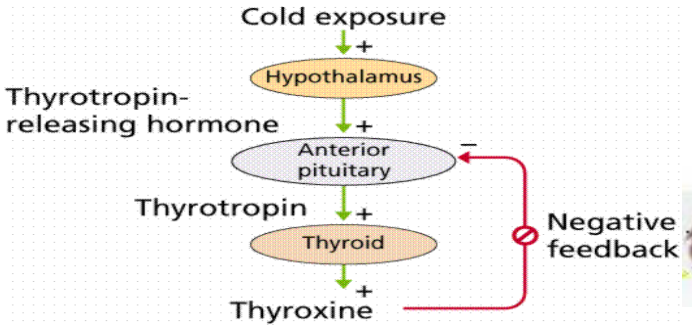
النادي الطلابي
كلية الطب

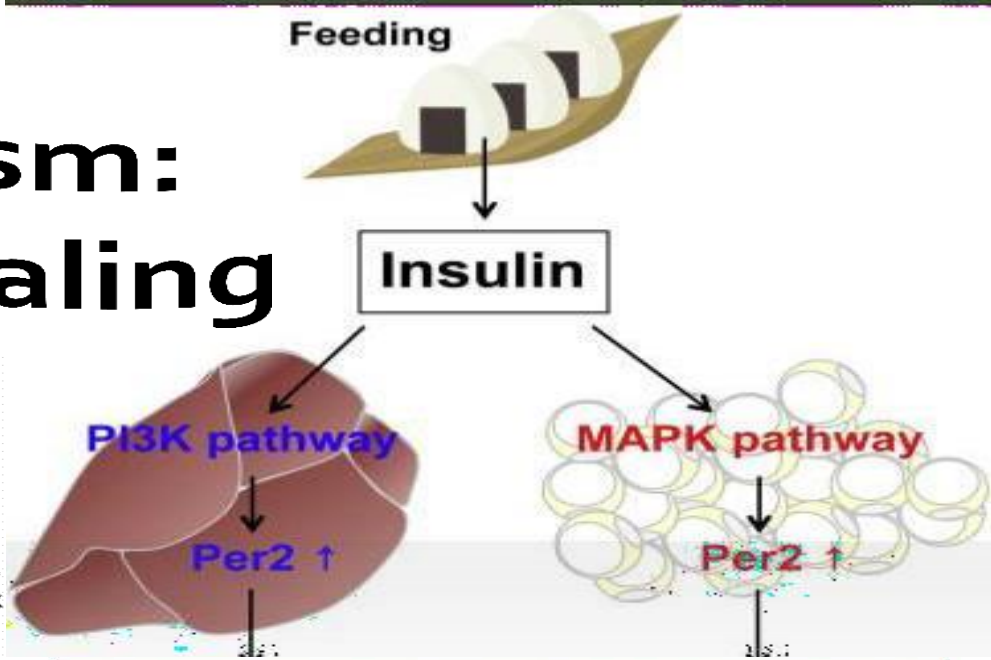
NafithAbuTarboush DDS, MSc, PhD
natarboush@ju.edu.jo
www.facebook.com/natarboush

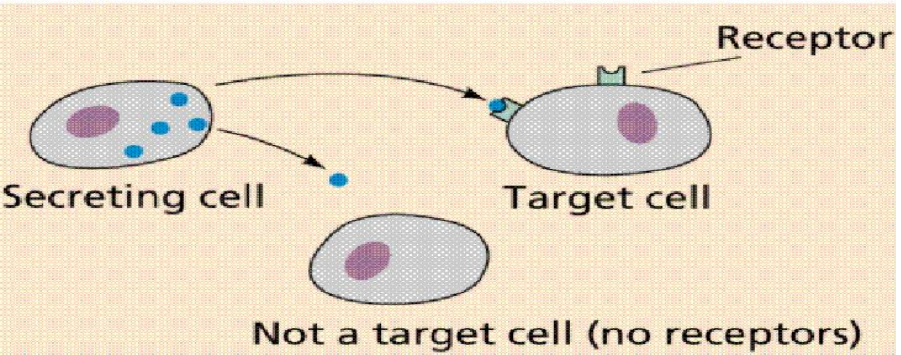
THE UNIVERSITY OF JORDAN

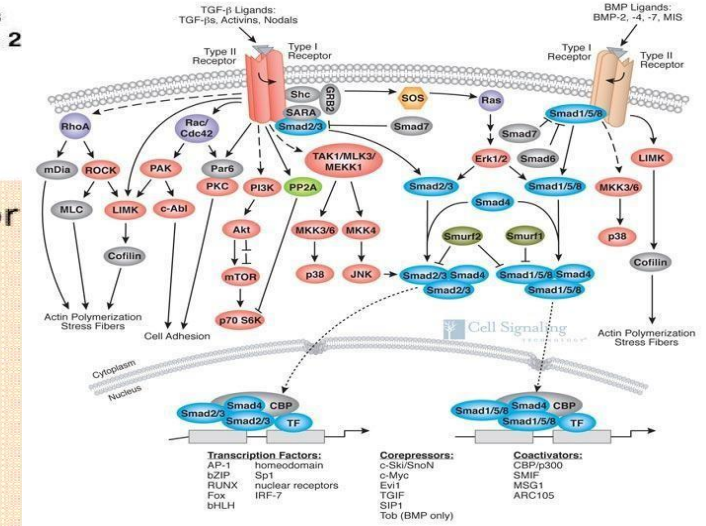
Integration of Metabolism: hormones & Cellular Signaling



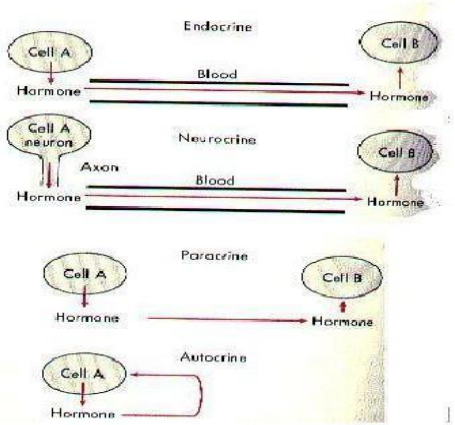








Types of cell-to-cell signaling



Endocrine Hormones: travel via bloodstream to target cells

Neurocrine hormones: released from nerve terminals

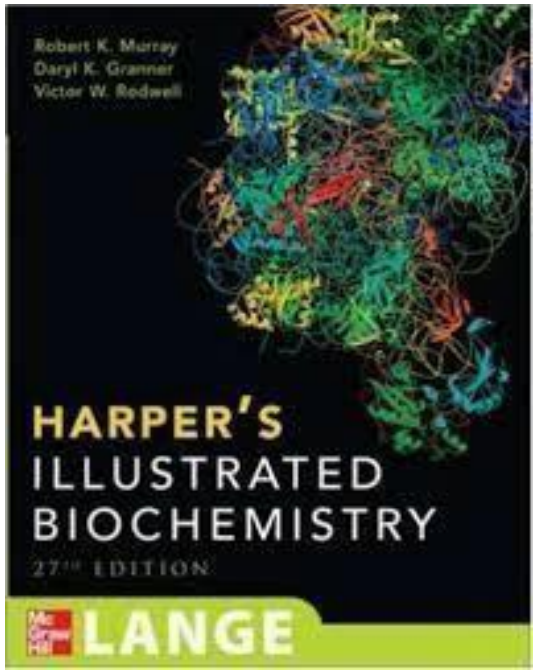
Paracrine hormones: act on adjacent cells

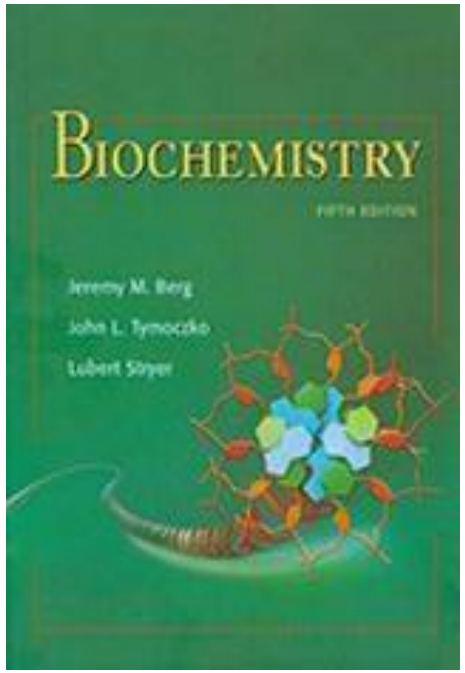
Autocrine hormones: Released and act on the cell that secreted them.

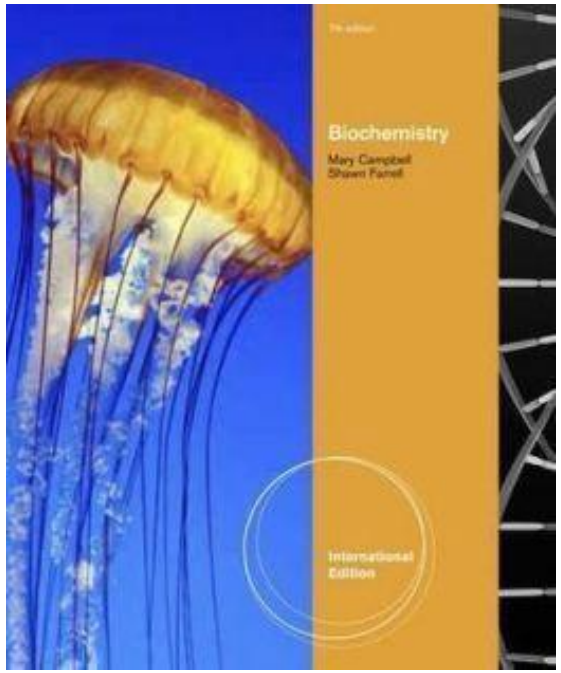
Intracrine Hormones: act within the cell that produces them.

Resources for the 3 lectures

- Harper's Illustrated Biochemistry
- Stryer's Biochemistry
- Campbell's Biochemistry





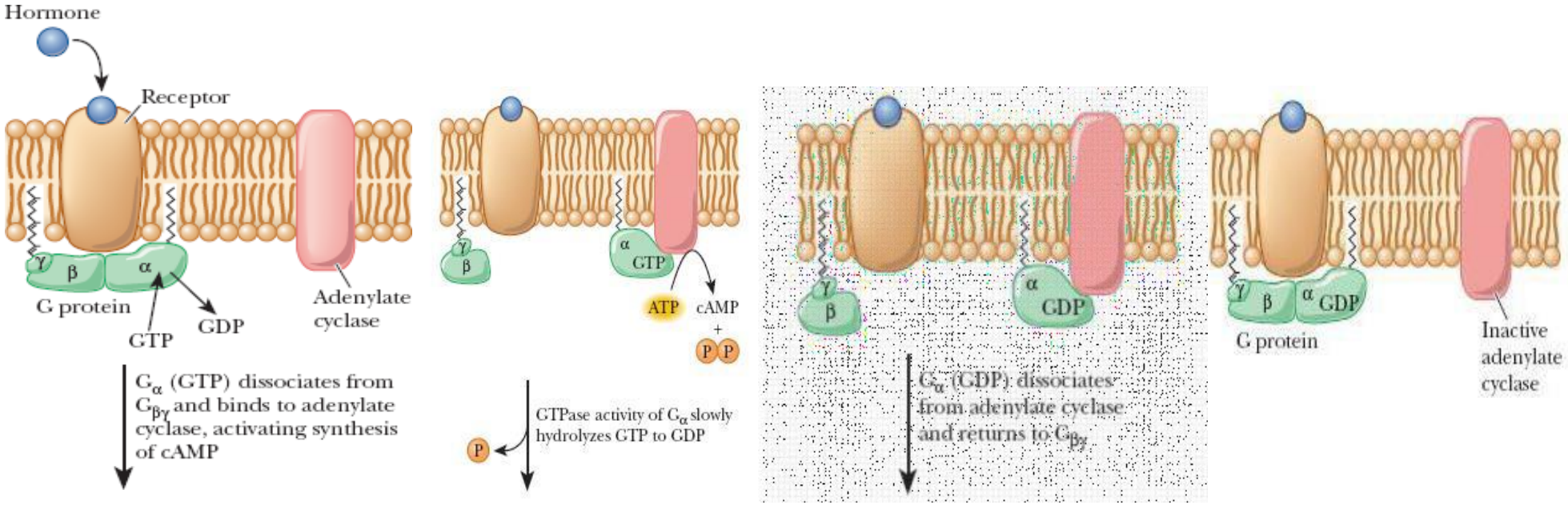


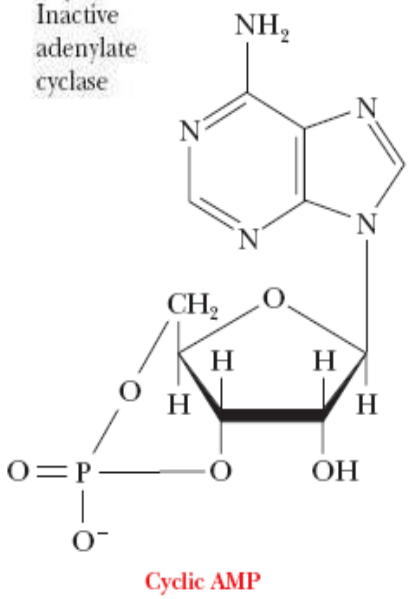
Last lecture we went through the following :

1. definition of hormones
2. classification according to their effect
3. interaction with the nervous system
4. biochemical problems posed on the endocrine system
5. Kd
6. Receptors
7. Domains
8. signal amplification
9. loops to control hormone action
10. chemical structure classification and mechanism of action
11. synthesis of protein hormones
12. hormonal interaction
13. signal transduction , 2nd messengers & signal termination .
14. types of receptors –including seven transmembrane receptor and its functions.

Make sure you understand all these concepts , champion !

G-proteins & cAMP

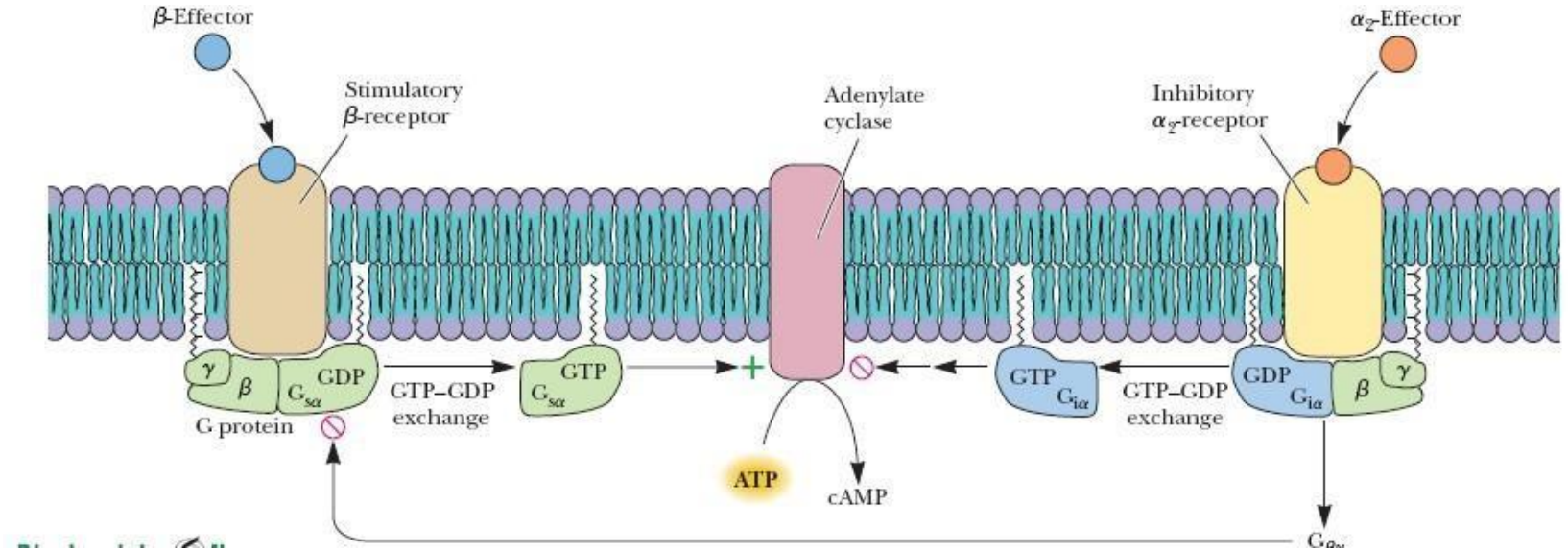




Cyclic AMP

- **cAMP: small & heat stable**
- **Plasma membrane**
- **Hormone → Specific receptor (β1- or β2-adrenergic receptor) → G protein → Adenylate cyclase → cAMP → protein kinase A → phosphorylation**

G protein: stimulatory or inhibitory?



- **Cyclic AMP & G Proteins:**
  - Hormone ⟶ receptor (α2-receptor) ⟶ G protein ⟶ inhibits adenylate cyclase

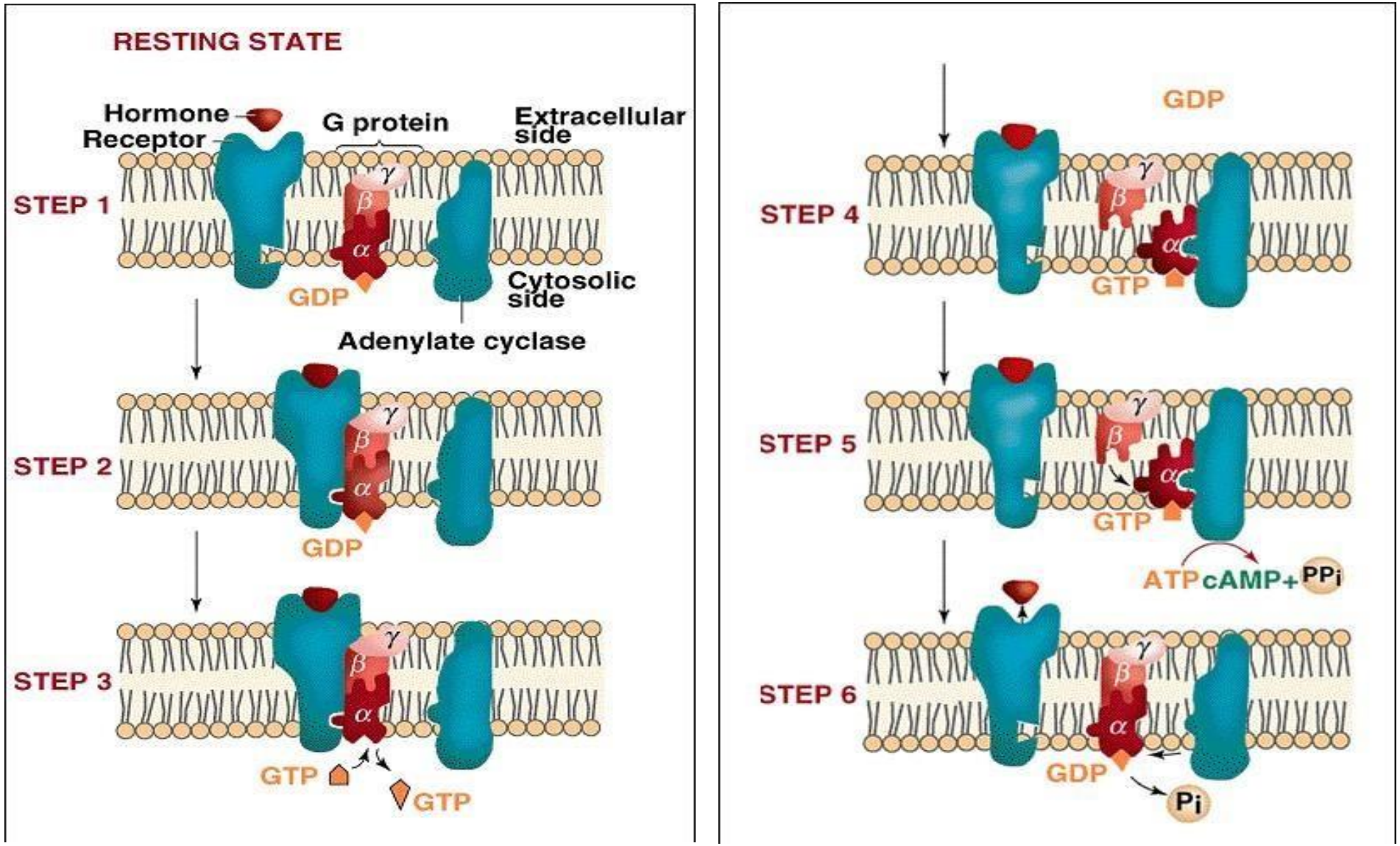
G Proteins

- **G proteins:** Each 7-transmembrane receptor present on the cell membrane is attached to at least one G protein.
  - **More than 100 known G protein–coupled receptors and more than 20 known G proteins** (each consisting of alpha, beta and gamma subunits).
  - **Can be activated by combinations of hormones**
    - **Epinephrine & glucagon act via a stimulatory G protein in liver cells**
  - **Other than cAMP:**
    - **Stimulating phospholipase C**
    - **Opening or closing membrane ion channels**
    - The target of the alpha subunit is not always Adenylate cyclase. It may be the enzyme Phospholipase C, or a membrane ion channel such as chloride or potassium channels ''can open or close the channels''.

G Proteins (cont.)

- **α and γ Subunits have covalently attached fatty acid** Each 7-transmembrane receptor present on the cell membrane is attached to a G protein on the cytosolic side of the membrane. What makes the G protein attached to the membrane and to the receptor in this way is the presence of fatty acids attached with the G protein. Since it's a fatty acid (hydrophobic), it will be attached/embedded in the membrane. The alpha and the gamma subunits each has a fatty acid covalently associated with it, beta doesn't (because beta is associated with gamma subunit making a dimer, they are attached as one unit, while the alpha subunit –which can dissociate from the beta-gamma complex needs its own attachment) .
- **α and βγ can interact with other proteins**
- **All 7TM receptors appear to be coupled to G proteins → GPCRs**

- **Amplification: receptor → 100's of G protein → 100's of adenylate cyclase → 100's X 1000's molecules/sec of cAMP** *(further explanation in slide no.10)



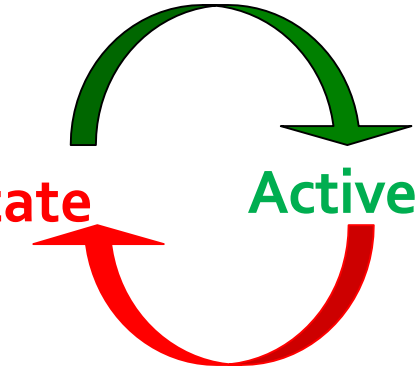
α subunit has GTPase Activity

1. The G protein is attached to the receptor in a way that, when the hormone binds and causes conformational change in the receptor, the G protein alpha subunit will also undergo a conformational change (due to the close proximity to the receptor). Now, the change in the alpha subunit results in low GDP affinity, plus high GTP affinity and the replacement occurs. The alpha subunit loses its already attached GDP to attach to a new GTP molecule. Once the alpha is bound to GTP, the presence of this new phosphate in GTP causes a conformational change in the alpha causing it to detach from the beta-gamma dimer.

2. The alpha is now active. It will head towards a certain target. Note that there are more than one target , one of them (the most common target) is the enzyme **Adenylate cyclase**

Further explanation:
Since many G proteins can be attached to the same receptor, this causes signal amplification. Each activated alpha subunit (of the various G proteins attached to the same receptor) will target an Adenylate cyclase in the membrane, and each Adenylate cyclase will produce high amounts of cAMP per second, so first we start with one molecule conveying the signal (the hormone molecule) then the number increases (the number of alpha subunits activated) then it further increases (the number of cAMP) → **Amplified signal**

G Protein cycles between two forms



Inactive State — Active State

Bound to GDP — Bound to GTP

Heterotrimer αβγ — Dissociated α + βγ dimer

α subunit has GTPase Activity

Activated Receptor

↓

↑ Adenylate Cyclase

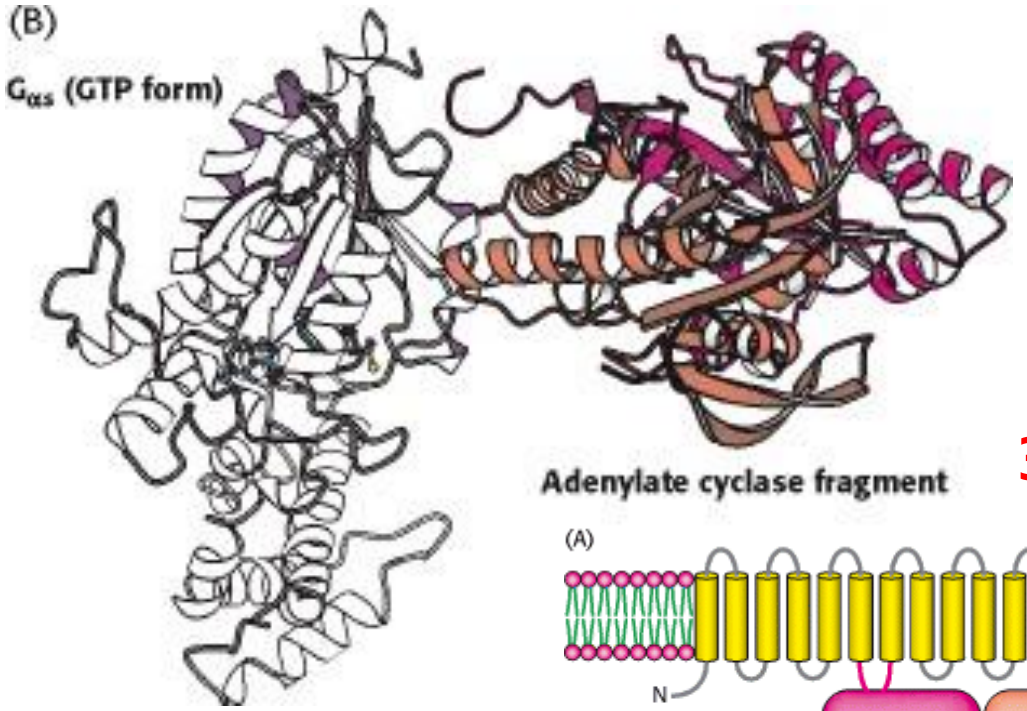
↓

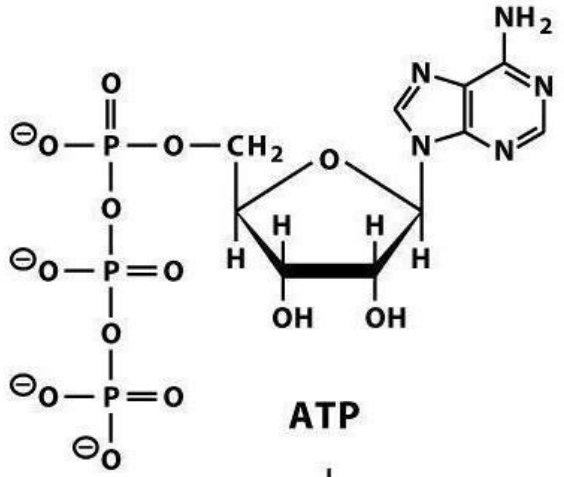
↑ cAMP

↓

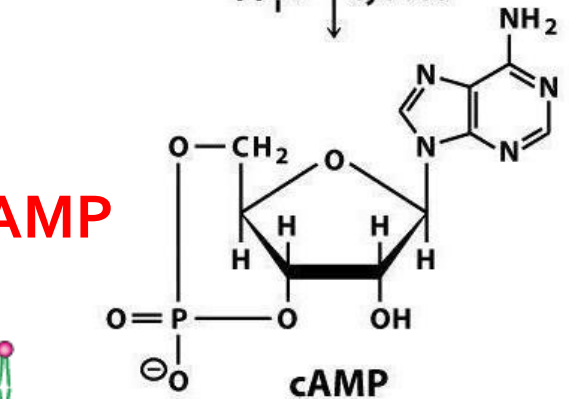
Biochemical Response

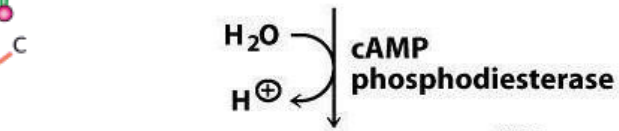
Adenylate Cyclase

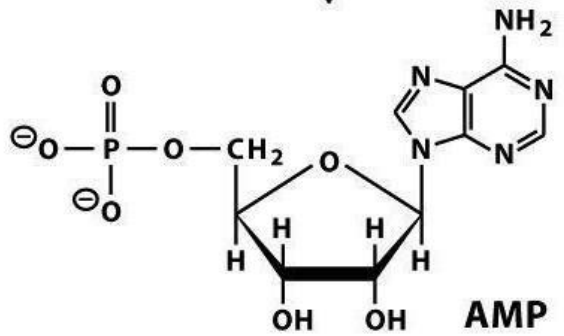




3'5' CAMP





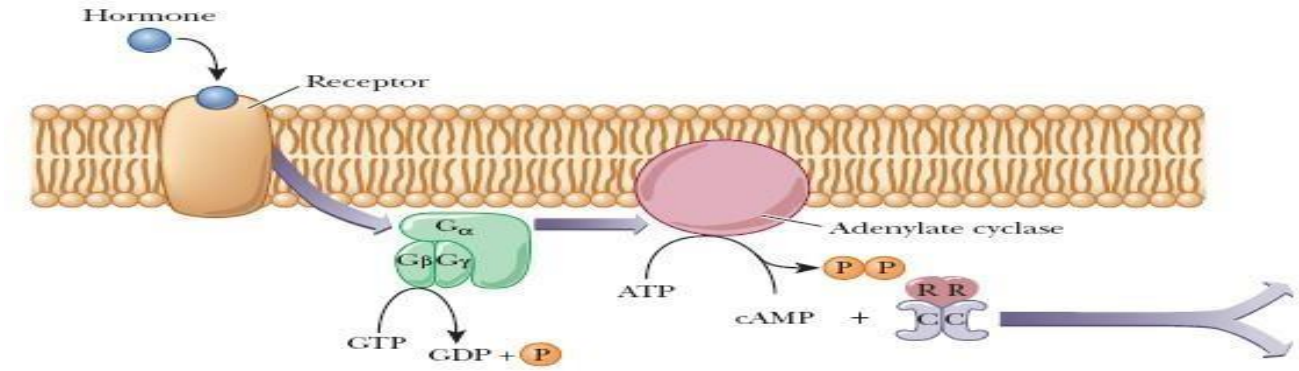


- Membrane protein
- 12 helices
- Two large intracellular domains
- Activated by G protein

cAMP can affect a wide range of cellular processes

- ↑ degradation of storage fuels
- **↑ secretion of acid by gastric mucosa**
- Dispersion of melanin pigment granules
- ↓ aggregation of blood plateletes
- Opening of chloride channels

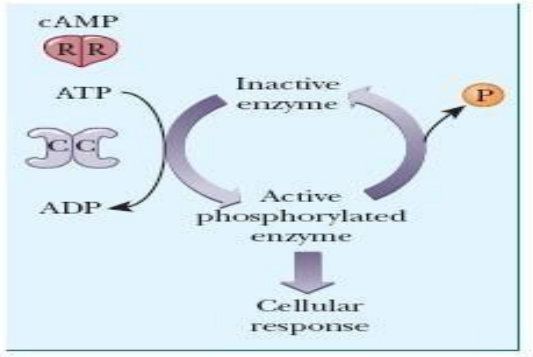
Then what?



Signal Amplification

© Sinauer Associates, Inc.

Usually:
Ser orThr



Glycogen Synthase!!

3. cAMP then targets Protein kinase A [kinase= it phosphorylates other proteins, A= because it is activated by cyclic Adenosine monophosphate]. It is composed of two catalytic subunits and two regulatory subunits, which contain 4 binding sites to cAMP. When 4 cAMP molecules bind, the regulatory subunits detach from the catalytic ones, which are now able to phosphorylate other proteins. "Usually, it happens on Ser/Thr residues".

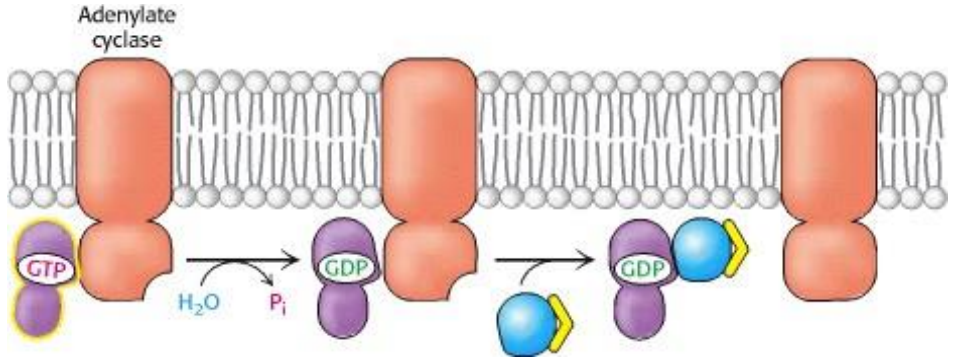
Continuation:

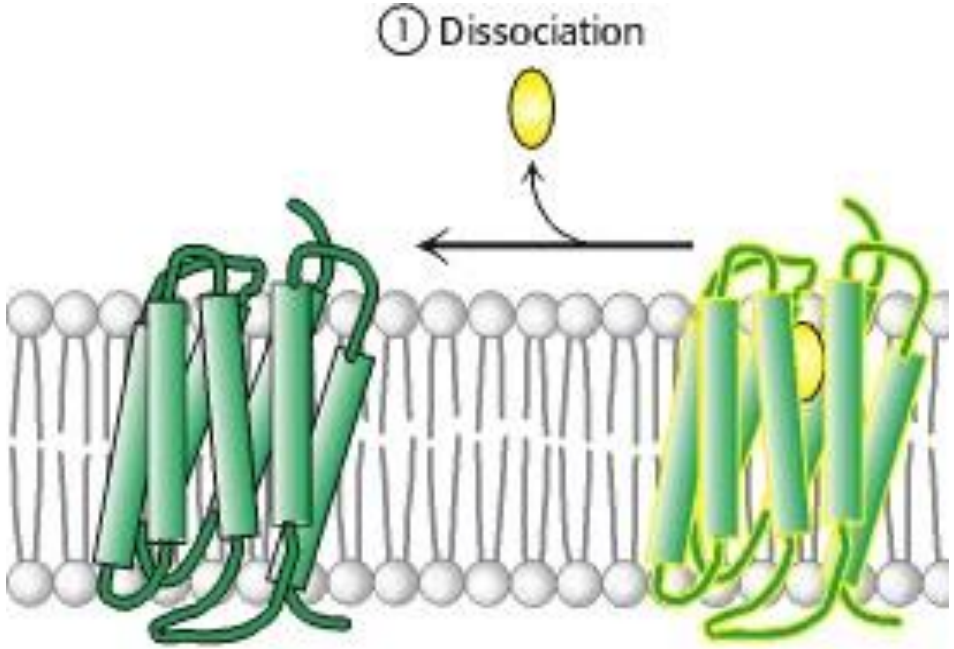
- Phosphorylation does NOT always lead to activation of the phosphorylated protein. A famous example is the enzyme Glycogen Synthase, which gets inhibited by phosphorylation (the signal here is due to Glucagon hormone or Epinephrine, both are secreted to increase blood glucose level, so it is not the right time to build glycogen, so it's only logical to inhibit such enzyme).

- G proteins can be activated by combinations of hormones. For example, Glucagon and epinephrine act via a stimulatory G protein in liver cells.

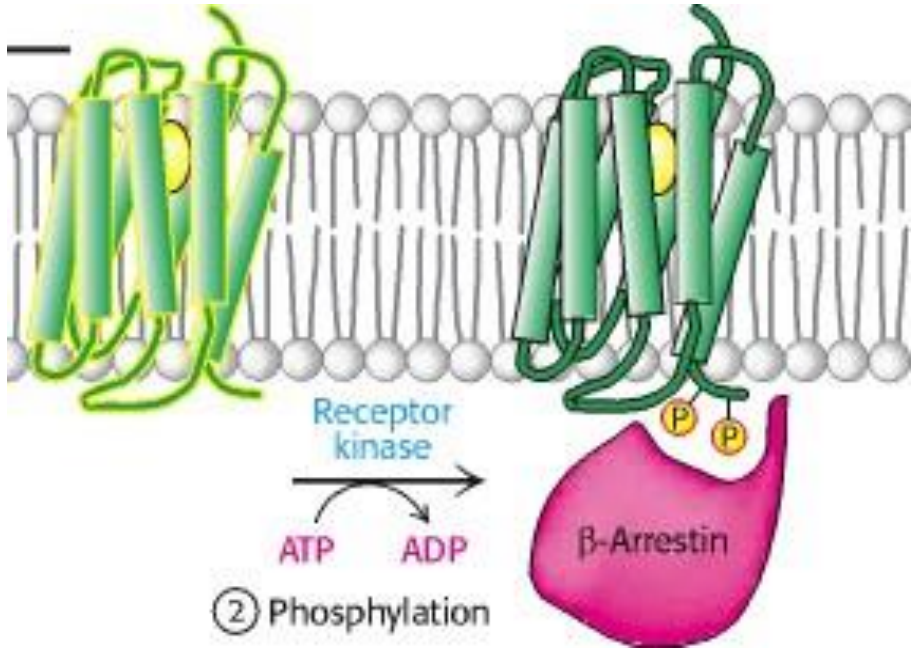
Switching off the signal

- **Dissociation** of the hormone
- **GTPase** activity of Gα subunit
- **Hydrolysis** of cAMP (phosphodiesterase)
- Phosphorylation of the hormone bound-receptor followed by binding to **β-Arrestin**





Desensitization



Further explanation:

Back to G protein, we said that the active alpha subunit targets Adenylate cyclase enzyme, but does it always have to involve activation of this enzyme?
NO, the type of the pathway (stimulatory or inhibitory in its nature) depends on the nature of the alpha subunit itself (some alpha subunits are stimulatory by nature, some are inhibitory and are called Gαi or Gi) and it also depends on the receptor itself. There are stimulatory receptors (such as β1 or β2 receptors) and there are inhibitory receptors (such as α2 receptors) . So there are different types .

Gα → some stimulatory and some inhibitory .

Gs → ↑ AdenylateCyclase

Golf → ↑ AdenylateCyclase

Transducin → ↑ cGMPPhosphodiesterase

Gi → ↓ AdenylateCyclase

Go → Ca2+ Channels

Gq → ↑ Phospholipase C

After cAMP does its function, it is broken down by the enzyme Phosphodiesterase.

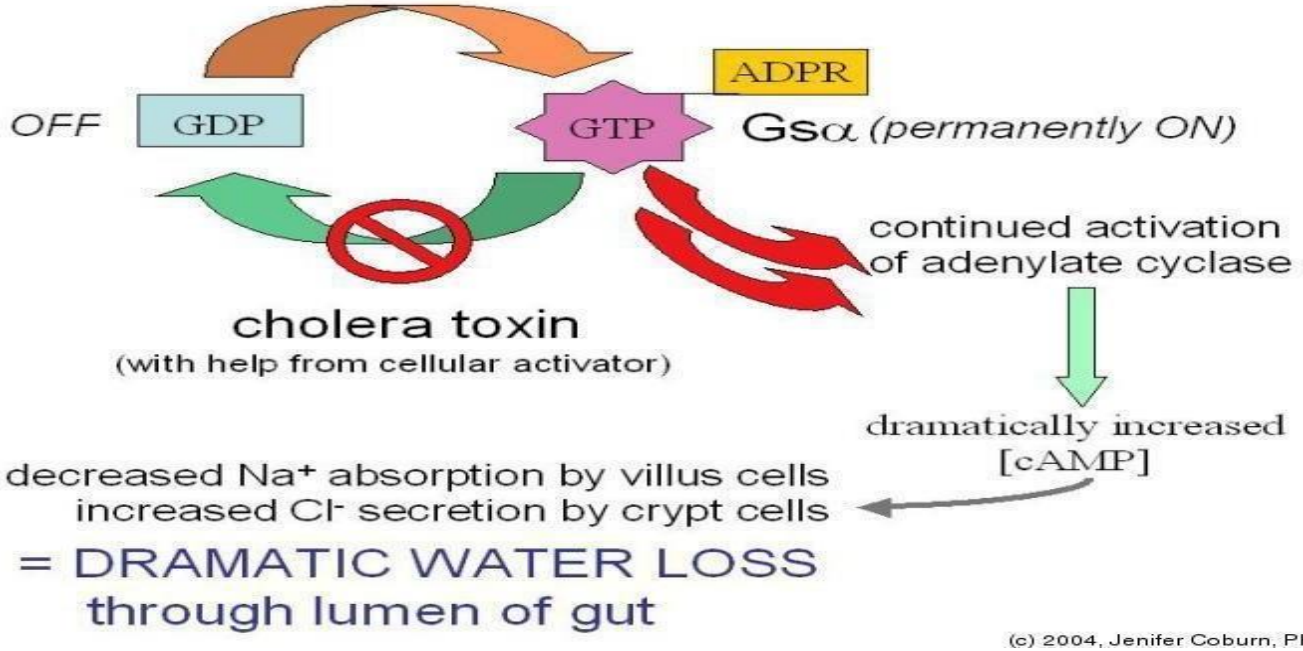
Further explanation:

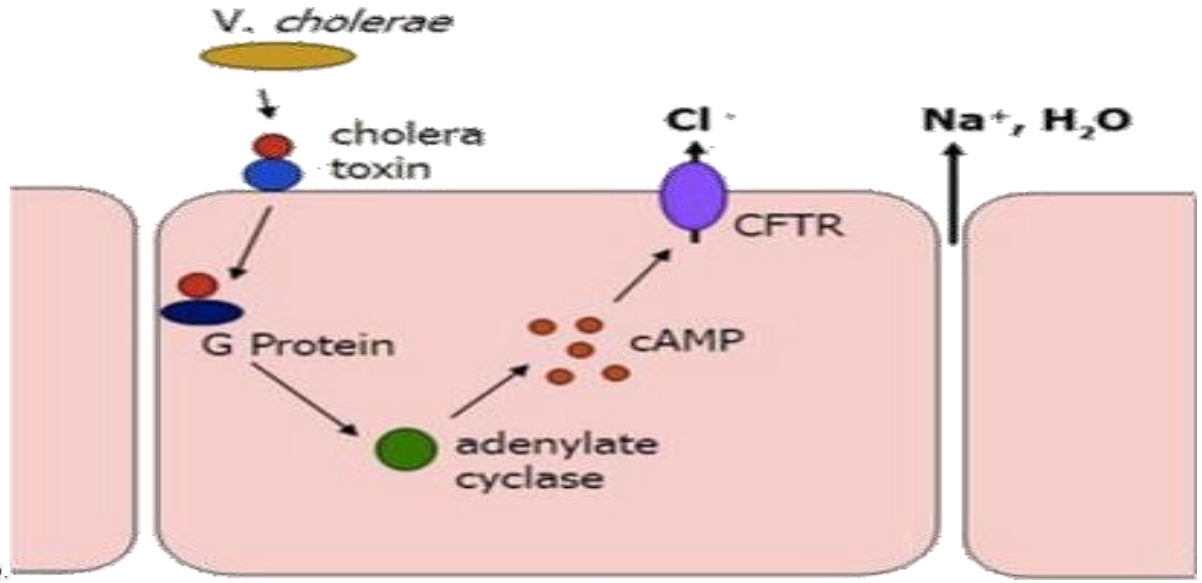
- Hormone dissociation from the receptor. The mode of hormone-receptor binding is non-covalent interactions, so that at the end, dissociation occurs. (If the binding was covalent, then the ligand is a toxin).
- Active alpha subunit becoming inactive again through the slow GTPase activity, which hydrolyses the GTP with the active alpha subunit to GDP. Now it is inactive and it re-associates with the beta-gamma dimer.
- Breaking cAMP by Phosphodiesterase enzyme.
- The receptor itself contains many Ser/Thr residues in the Cytoplasmic part, which constitute a site for phosphorylation. After the hormone has done its work (by conveying the signal inside the cell and changing the cell's metabolism), these Ser/Thr residues get phosphorylated by receptor kinase, which leads to a conformational change in the receptor. This allows the receptor to have high affinity to a protein called β-Arrestin. This protein binds to the intracellular side of the receptor (the coupling domain), and now that this domain is masked/covered by β-Arrestin, even if the hormone is binding the receptor, the G protein will NOT get activated (the conformational change in the receptor due to hormone binding cannot affect the G protein because β-Arrestin lies between them, preventing direct contact between the receptor and the G protein). → This is what we call Desensitization of the receptor, meaning that even when the hormone is bound to the receptor, there is NO signal being transduced/propagated/conveyed in the cell.
- Note: Another way is by the action of phosphatases, to remove the effect of PKA, but since PKA phosphorylates many proteins, this effect is not of that big significance.

Cholera

- **Cholera toxin → unregulated activity of adenylate cyclase in epithelial cells → Excessive cAMP in epithelial cells stimulates active transport of Na^+ → large flow of Na^+ and water from the mucosa → diarrhea.**

- Explanation → Cholera toxin is a toxin produced by Vibrio cholerae, which is transmitted by contaminated water. If one ingests it and it reaches the intestines, it binds to a 7-transmembrane receptor, activates G protein, activates Adenylate cyclase, and produces many cAMP molecules. Due to extreme binding affinity between the receptor and the toxin, huge amount of cAMP is produced, which affects membrane channels; it causes Cl- release , and pumping of Na+ out, which causes increased osmolarity and water getting out of cells as well. All of this leads to excessive and uncontrolled diarrhoea which may be fatal.



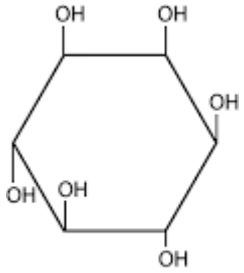


Extra note

I asked the doctor whether cholera toxin produces its action via inhibition of GTPase activity in the active alpha subunit (which will also lead to huge production of cAMP) and he said that this activity wouldn't be of such importance without the presence of extreme binding affinity between the toxin and the receptor, meaning that even if it really inhibits it but the toxin can dissociate, the effect would be temporary, but because of the high affinity, there is constant and persistent cAMP production.

The Phosphoinositide Cascade

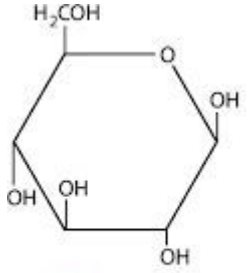
- Used by many hormones (e.g. ADH)
- Binding of a hormone to 7TM receptor



Inositol

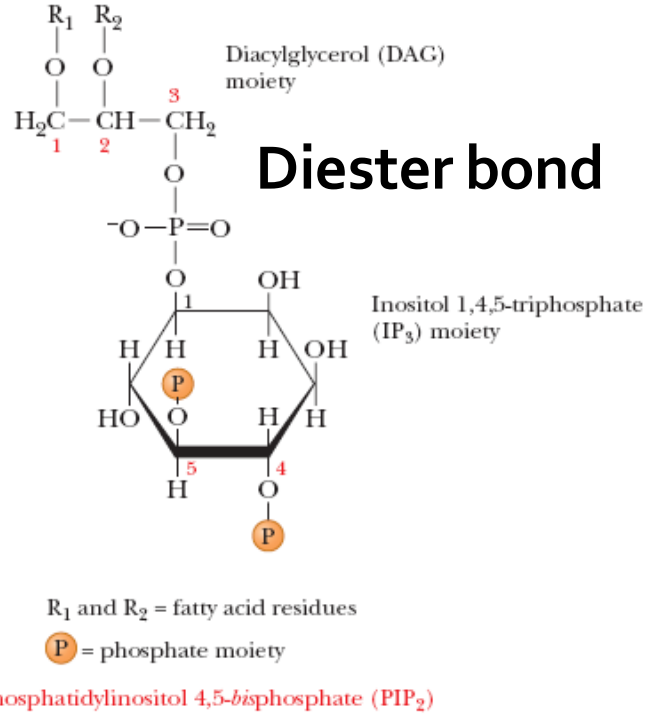
Activation of G Protein

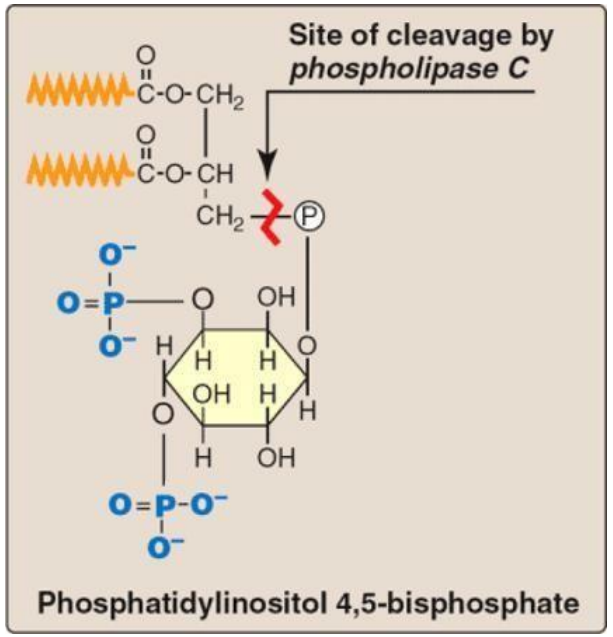
Activation of Phospholipase C (many isoforms) – PIP2



Glucose

- Two messengers are produced
  - Inositol 1,4,5-trisphosphate, hydrophilic, (Soluble)
    - IP3 is the actual second messenger
  - Diacyclglycerol, amphipathic (membrane)





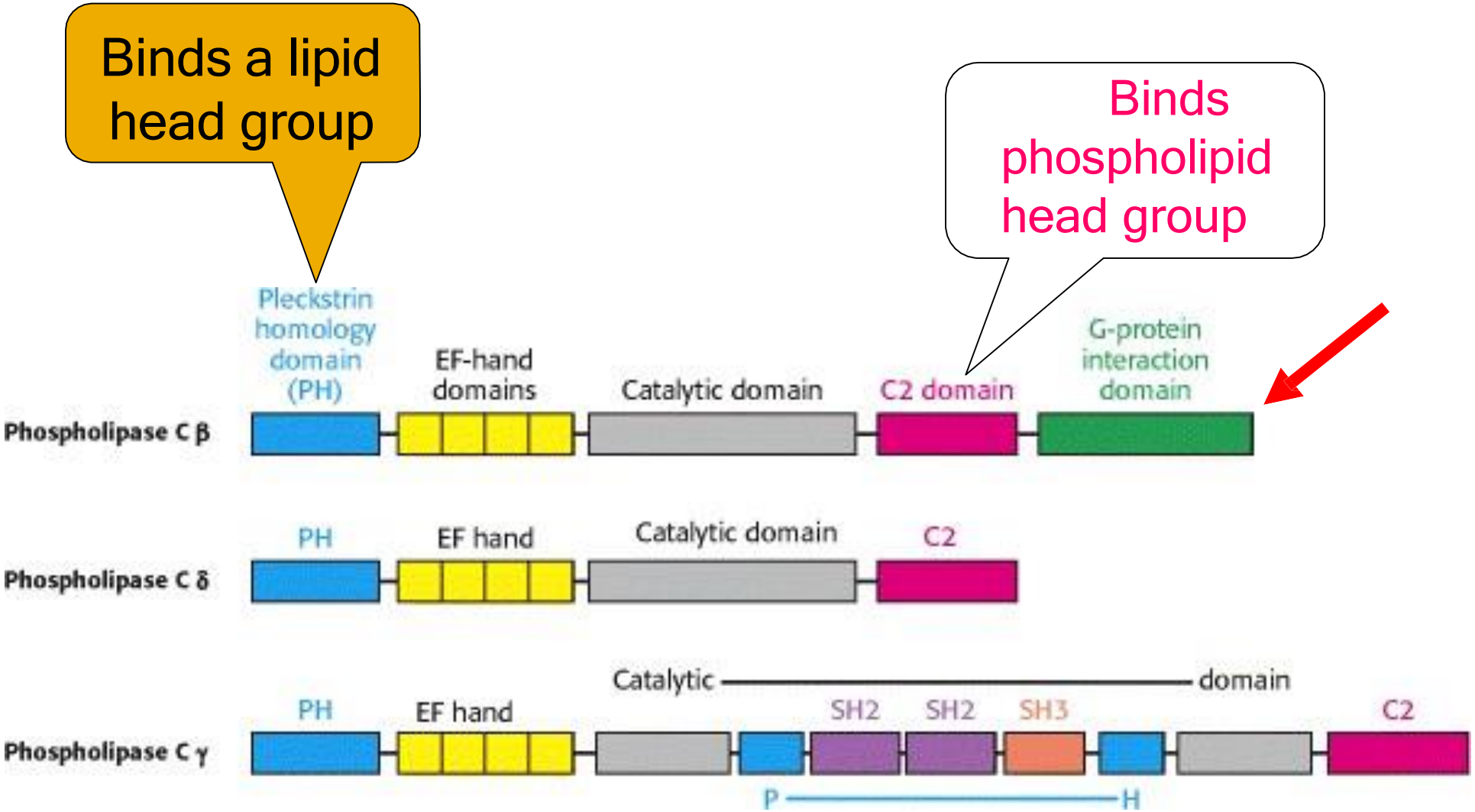
The Phosphoinositide Cascade

- This pathway is used by many hormones, like ADH .The active alpha subunit can target another enzyme, called Phospholipase C. It is an enzyme that is attached to the cell membrane. Since it's a protein, it contains domains. The major ones include:
    1. Catalytic domain (does the catalysis of the reaction).
    2. Domain to bind the cell membrane
    3. Domain to receive/bind the active G protein alpha subunit.

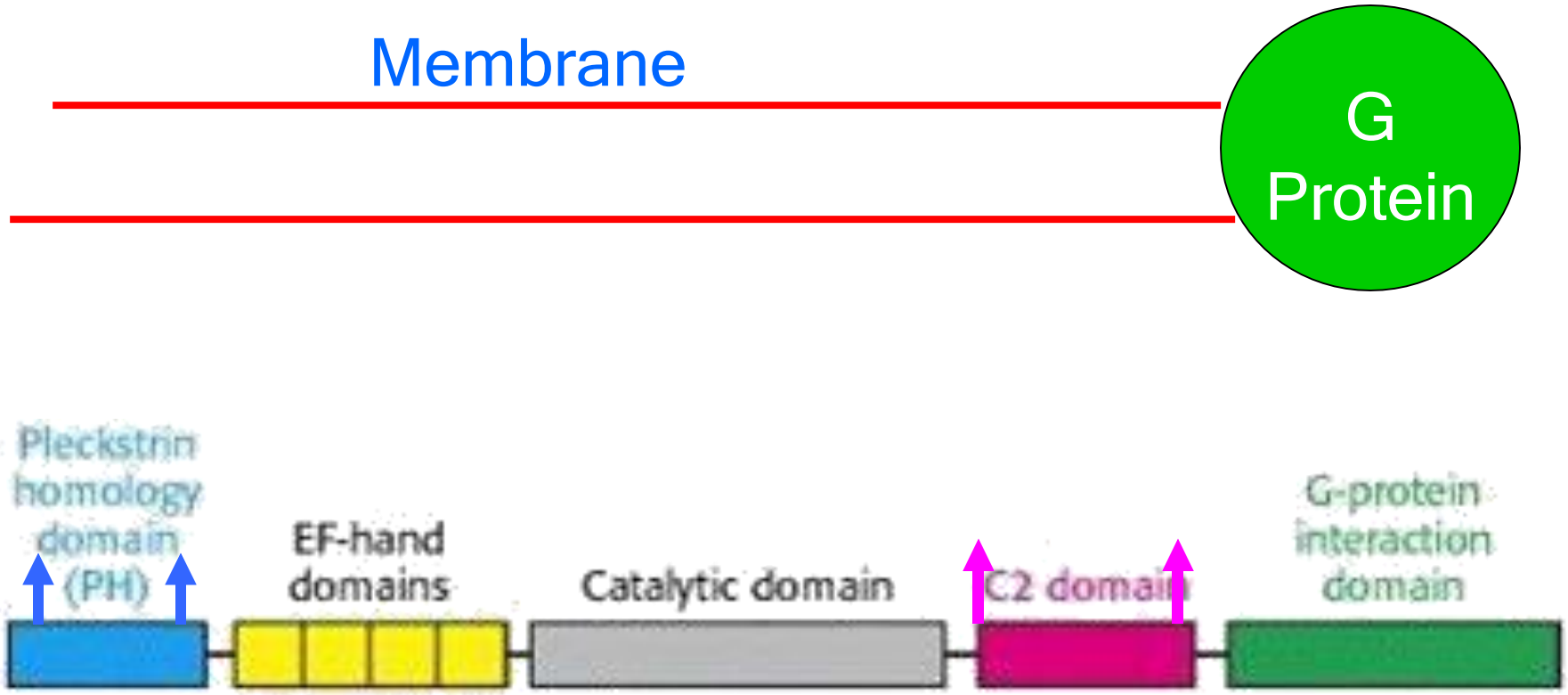
- This enzyme has isozymes (multiple forms of the enzyme, each with certain tissue localization). There are Phospholipases beta, gamma & delta(check next slide). Only Phospholipase beta contains the G protein binding domain, so it is the only one involved in this pathway.

- The PH (binds a lipid head group) and C2 domains (binds a phospholipid head group) → membrane attachment.

The domain structures of three isoforms of Phospholipase C



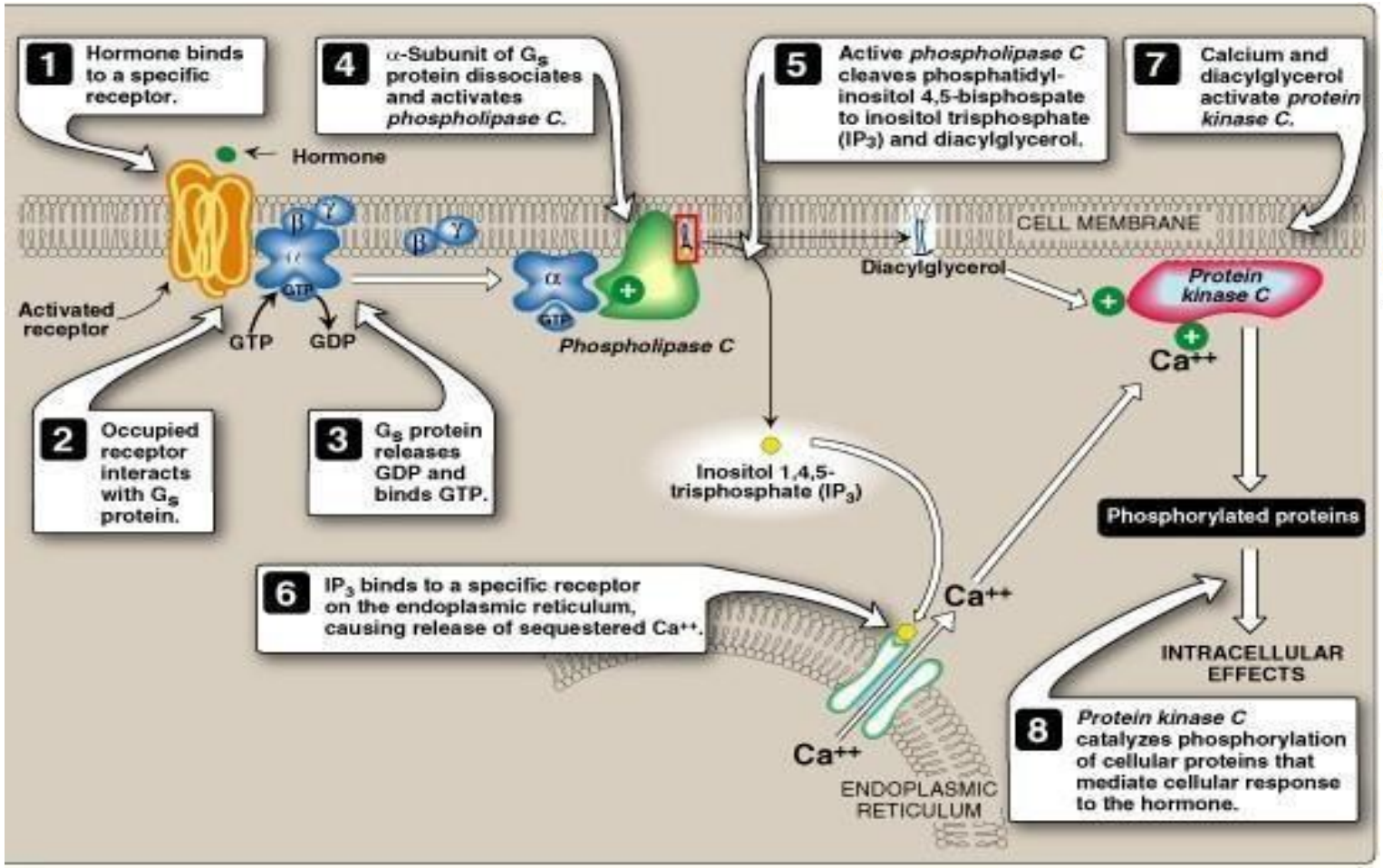
Binding of a G protein brings the enzyme into a catalytically active form

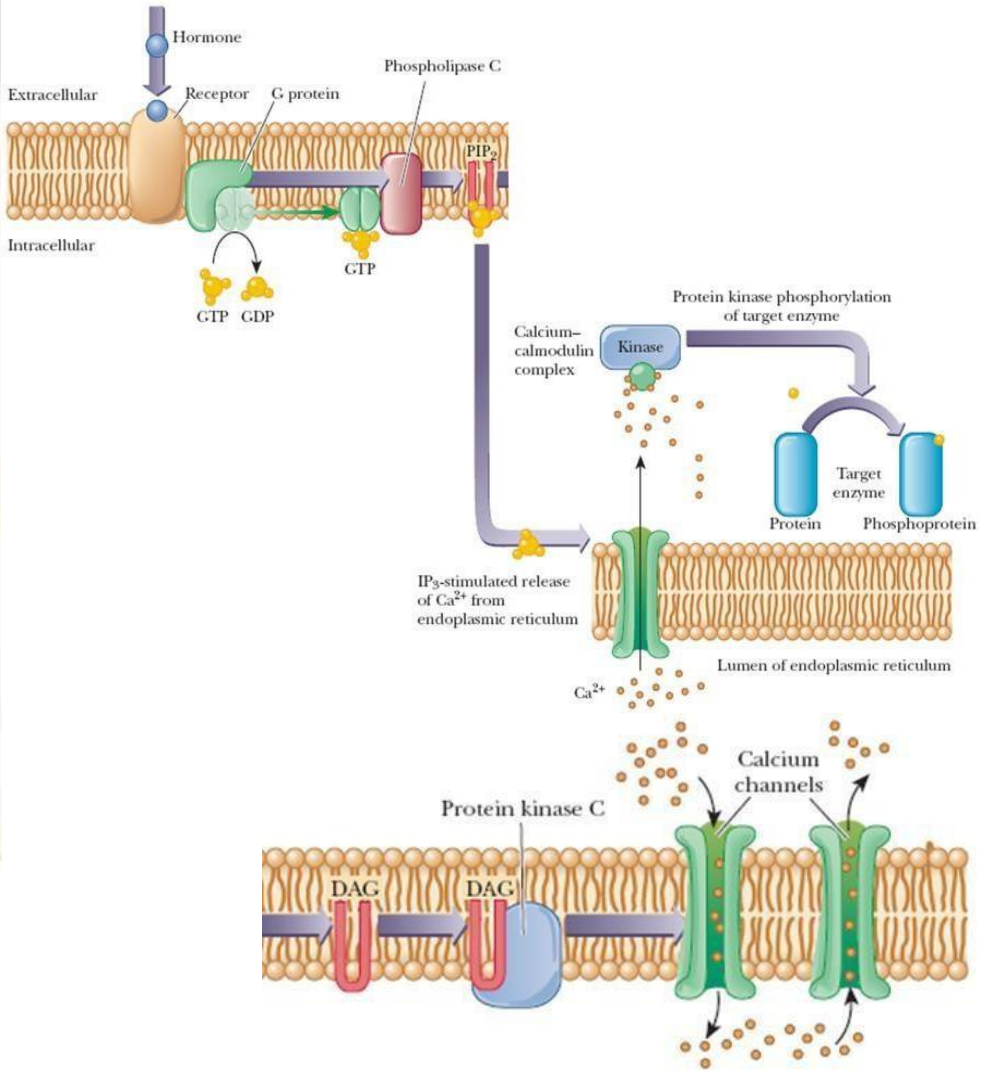


What is Next?

- IP_3 → cytosol → ER → Ca^{2+} → calmodulin-Ca^{2+} → protein kinase → phosphorylation
- DAG: non-polar (membrane) → protein kinase C → 2nd messenger

Check next slide for further explanation





- This enzyme breaks down phospholipids, particularly PIP2 (phosphatidyl-inositol 4,5bisphosphate). It consists of glycerol (3 carbons), two of which are connected to fatty acyl chains; the 3rd is connected to a phosphate group (so far this is the structure of phosphatidic acid). This phosphate is connected to inositol (a sugar that forms a hexagonal ring, each carbon has an OH group (hydrophilic)). On carbons number 4 and 5 of the inositol ring, there exists two phosphate groups, so the result is PIP2 .

- This structure (PIP2) is present in the cell membrane and the enzyme as well. When a signal comes (hormone), the G protein gets activated, activates phospholipase C, breaks down PIP2 bond between the phosphate and the carbon of the glycerol, producing inositol 1,4,5trisphosphate (IP3) and diacylglycerol (DAG). IP3 is totally hydrophilic, so as soon as it is formed, it leaves the membrane directly towards the cytoplasm, while DAG contains two fatty acids, so it can still hang in the membrane. It is an amphipathic structure (having both hydrophilic & hydrophobic parts).

- The main (actual) second messenger in this system is the IP3. DAG also works as a 2nd messenger.

Effects of Second Messengers

Inositol trisphosphate (IP_3)	Diacylglycerol (DAG)
✓ Opens Calcium Channels	✓ Activates Protein Kinase C
✓ Binding to IP_3-gated Channel	✓ Ca^{2+} is required
✓ Cooperative binding (sigmoidal)	✓ Phosphorylation of many target proteins

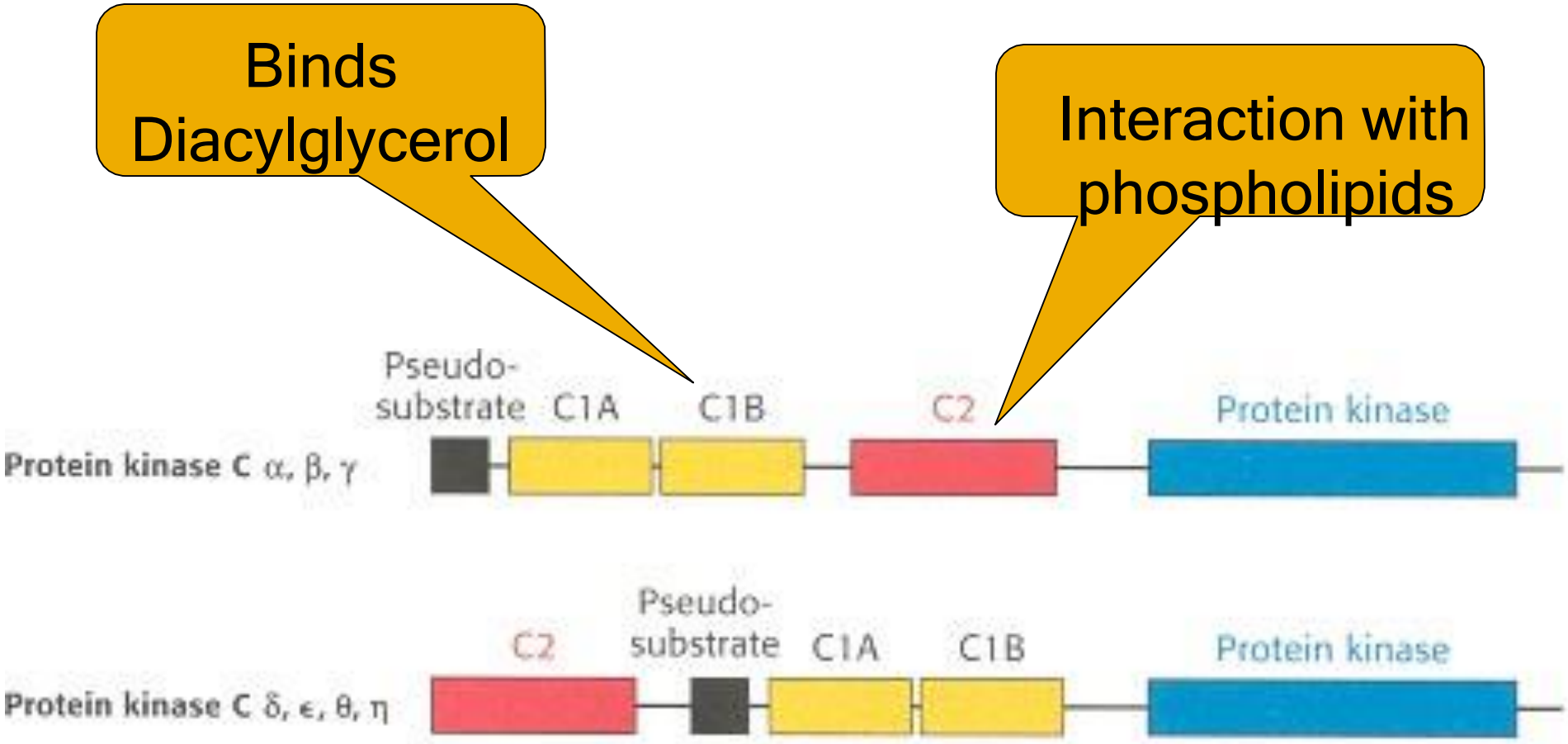
Explanation of the previous slide

- IP3 destination is the sarcoplasmic reticulum (smooth ER), which is a reservoir of Ca. IP3 binds to Ca protein channels on sER to cause Ca release. Each channel binds four IP3 molecules to fully open, and if (at least) three IP3 molecules bind they cause considerable opening of the channel (but not full). So, three IP3 ➡ can do the job, four ➡ even better. Note that the IP3 binding to the channel is cooperative, meaning that binding of the first IP3 makes it easier for the second IP3 to bind, which makes the 3rd binding easier, making the 4th binding even much easier (recall: hemoglobin and oxygen).

- Ca release into the cytoplasm occurs. Since Ca is positively charged, it binds with negatively charged proteins (not one, they are a group), which are called Calcium binding proteins. Once bound to calcium, they get activated. Another target of Ca is protein kinase C (maybe considered as a Ca binding protein, and the reason for calling it Protein kinase C is that it gets activated by Calcium). This PKC is a membrane enzyme, and gets partially activated when bound to Ca, now it is able to bind to what is left of PIP2 in the membrane (DAG) and get fully activated.

The domain structures of protein kinase C isoforms

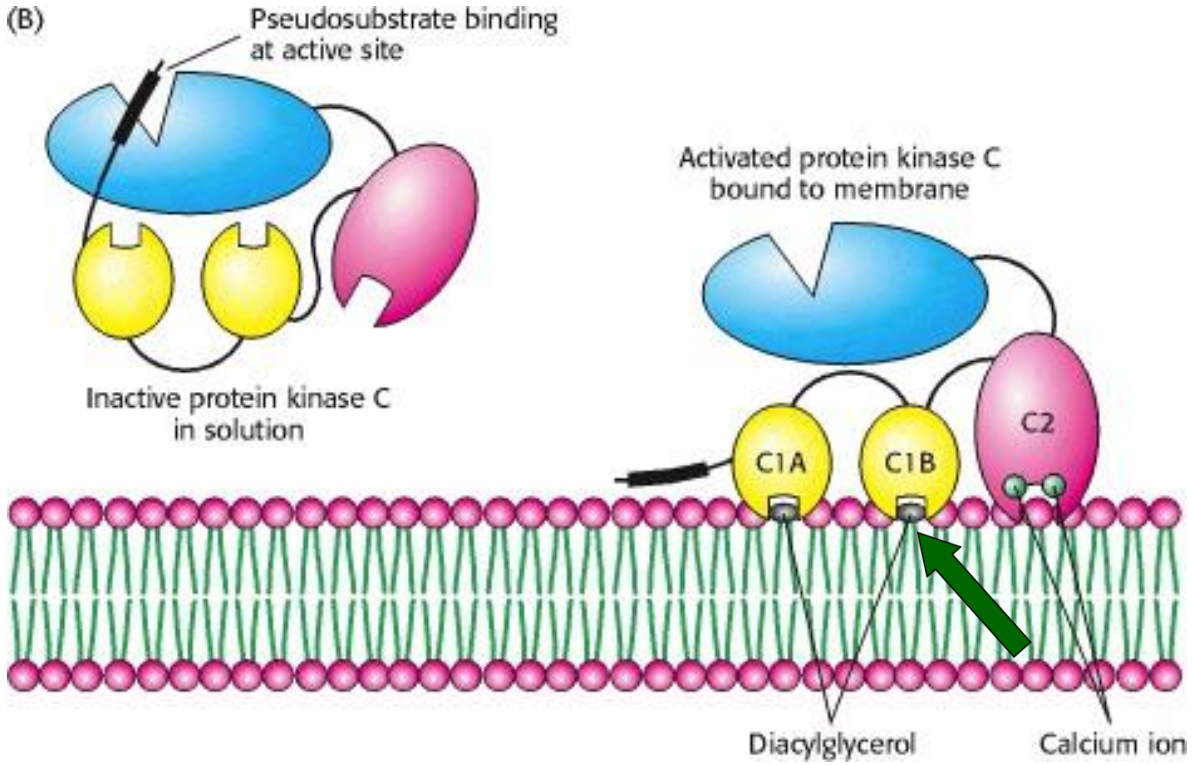
Check next slide



- PKC which is a protein attached to the membrane, contains these domains:
  1. Catalytic domain (protein kinase domain).
  2. Membrane binding domain (C2) , which must consist of hydrophobic amino acids and it may also contain fatty acids, in order to attach to the membrane.
  3. DAG binding domain (C1A-C1B)
  4. Ca binding domain.
  5. Pseudosubstrate domain
     - PKC is an enzyme that phosphorylates proteins; it contains this domain, which resembles/looks like the substrates of this enzyme. This domain looks like the sequence that enters the active site to be phosphorylated, but instead of having Ser/Thr, it contains hydrophobic amino acids, such as Alanine.
     - This domain (since it resembles the substrate) can fit in the active site, but because Ser/Thr don't exist there , no phosphorylation occurs.
     - Since the active site is occupied/full, the enzyme is inactive. Before Ca binding, it is not closely related to the membrane (only C2 domain faces the membrane and attach phospholipids), but when Ca binds → conformational change → DAG domains flip to face the membrane and be able to interact with DAG present within the membrane. In addition, when they flip, they draw/pull the Pseudosubstrate domain with them, exposing the active site. Now, the enzyme is active and can act on other proteins.

Pseudosubstrate Sequence

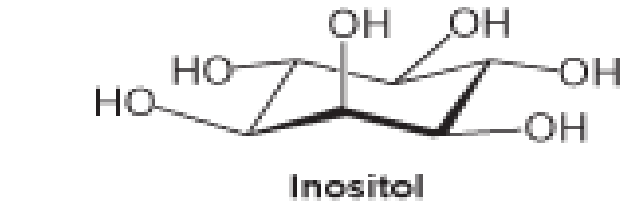
Competitive Inhibitor:
The Pseudosubstrate domain acts as a competitive inhibitor



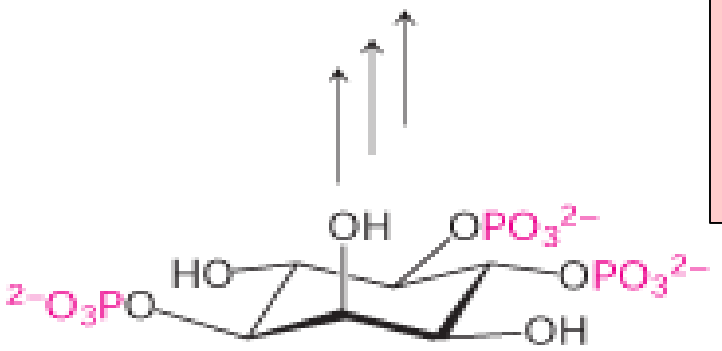
- Resembles the substrate sequence: A-R-K-G-A-L-R-Q-K
- Substrate Sequence: (S,T)
- Binds to the Enzyme's Active Site

Termination of IP3 Signal

IP3 is a Short-Lived Messenger

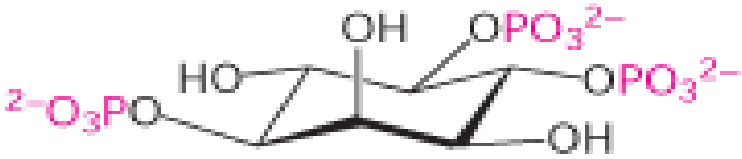


Inositol



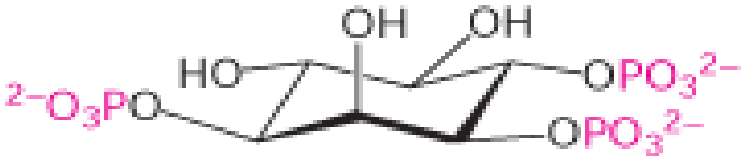
Inositol 1,4,5-trisphosphate

Lithium ions, used to treat some psychological disorders Inhibits IP_3 recycling

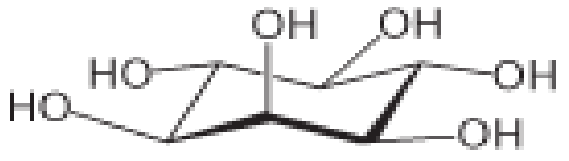


Inositol 1,4,5-trisphosphate

Inositol 1,3,4,5-tetrakisphosphate



Inositol 1,3,4-trisphosphate



Inositol

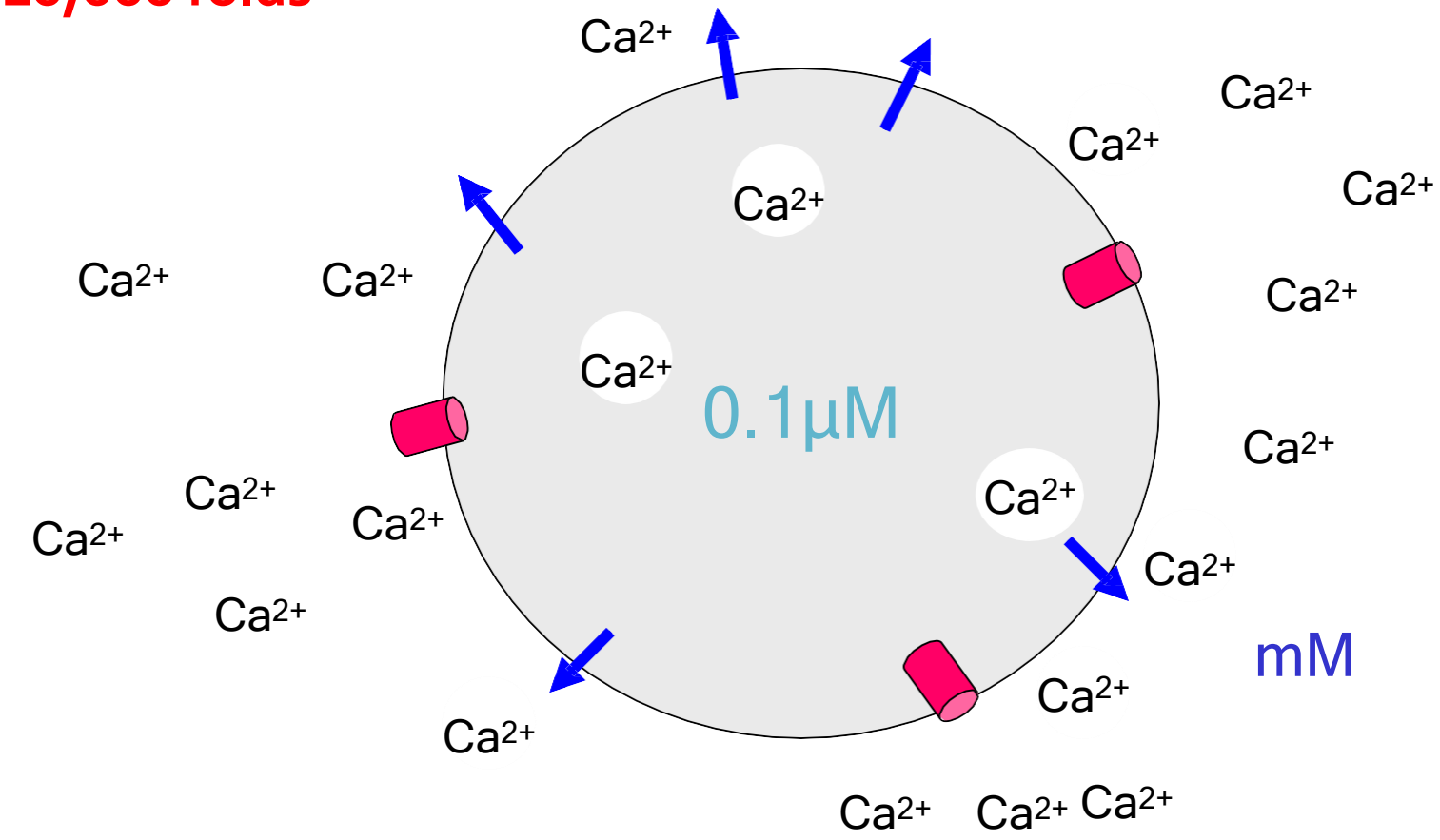
- Termination of the signal; occurs by two ways, both of them end the activity of the IP3 molecule (inositol 1,4,5trisphosphate), which is a short-lived messenger.
    1. We either remove a phosphate via cellular phosphatases .
    2. Or we add a phosphate producing IP4 (inositol 1,3,4,5 tetrakisphosphate) and this is a faster way as an initial solution .
    - then when the cell has the time, it starts removing the phosphates out of the IP4 molecule. And when you remove the phosphates, you do not remove the last phosphate added first, meaning that if you remove the last phosphate added –which is on carbon number 3- the molecule will return to active inositol 1,4,5trisphosphate, but if you remove any other phosphate, you will have the bonds (1,3,4 or 1,3,5 or 3,4,5) which are all inactive and the signal is terminated.

➢ Clinical hint: Lithium based drugs (psychiatric medicine, used for depression). Lithium is a heavy metal, which inhibits enzymes in the CNS, such as phosphatases. Now, IP3 cannot be broken (inhibiting IP3 recycling) → IP3 is active → treatment of depression.

Why Ca^{2+}?

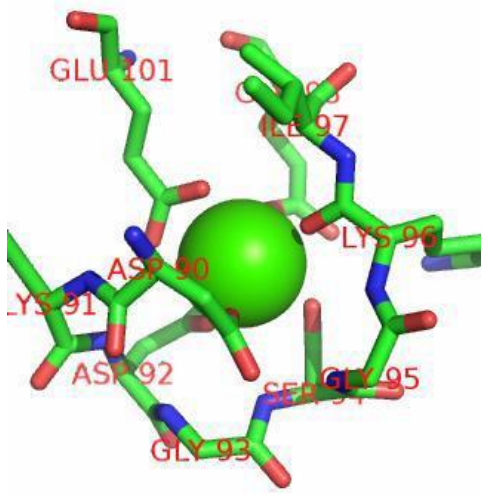
A large difference in concentration

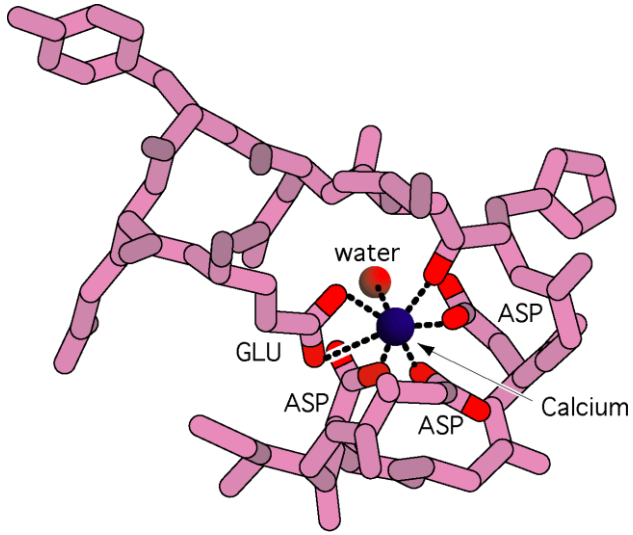
10,000 folds



Why Ca^{2+}?

- Ability to bind protein tightly
- 6-8 bonds with oxygen
- Conformational changes





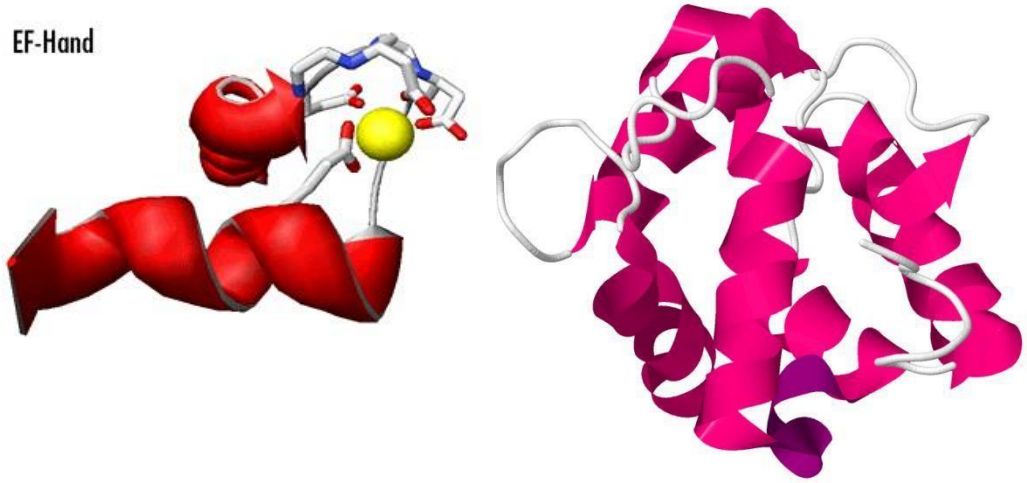
- All this pathway is based on Ca release, so why Ca?? What characteristics Ca has that make it suitable for this pathway's function?

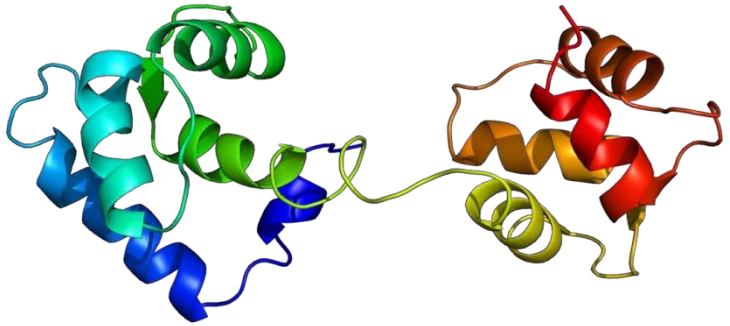
1. Ca is positively charged (+2), it has the ability to bind negatively charged structures (including proteins with negative charges).
2. Concentration: there is a very huge difference in the concentration of Ca between the cytoplasm and sER (around 10000 times) and between the cytoplasm and outside the cell is also 10000. This difference is not present for other molecules/ions. This difference produces huge impact when Ca channels open. Note that when Ca channels open, if we just want to wait until Ca is released and then pump it back, large amounts of Ca will be released due to the high difference which acts as a driving force and after that the driving force is over, so this must not be allowed. What happens is that as soon as Ca is being released (just a small amount), Ca pumps start pumping it back to the sER, in order to maintain this large difference between the cytoplasm and the sER, and thus maintain the driving force. So small release → does the desired function → maintain large difference.
3. It can make up to 8 bonds (called ligations), so it can ligate up to 6-8 bonds with polar charges on oxygen , amino acids , water , negative amino acids…etc. These bonds ensure tight binding, thus change in its target.
4. Ca is bulky, so when it binds the protein it produces the desired effect, which is the conformational change in that protein.

- Mediate the effects of Calcium (Ca^{+2})
- Many proteins

  Calmodulin, Troponin C, Parvalbumin

- Similar structures
  - Rich in Asp and Glu
    - Gln, Asn, Ser
  - Several α helical segments
  - Binding site is formed by
    - Helix Loop Helix
      - Super-secondary structure





- Ca target is Ca binding proteins, which are a group of proteins that get activated when bound to Ca, thus changing cell metabolism. Examples include:
  1. Calmodulin
  2. Troponin C

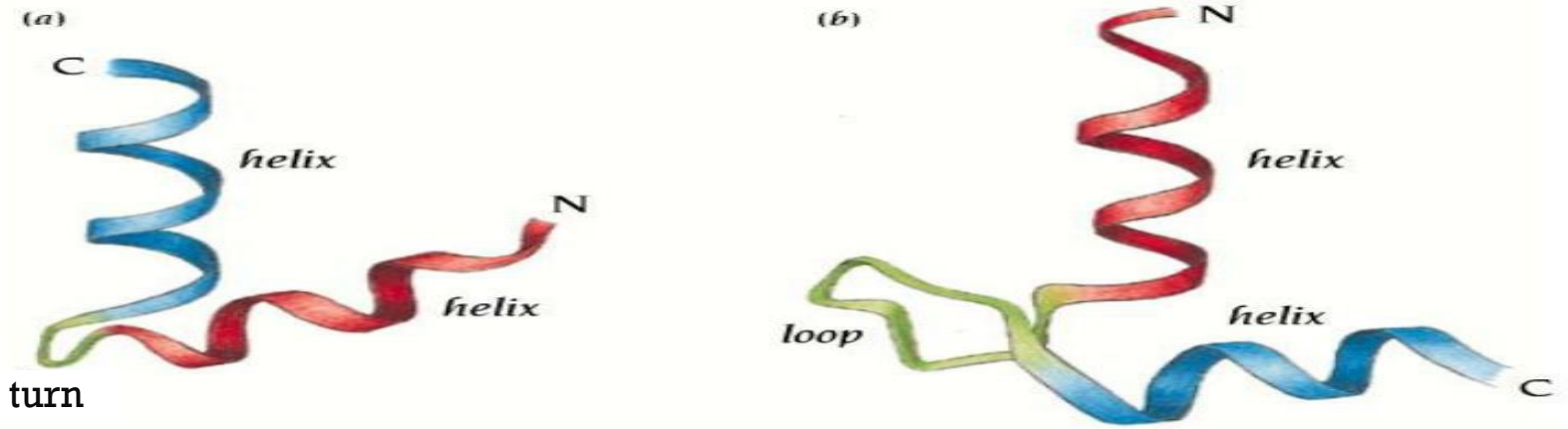
  Parvalbumin (the first discovered, it has 6 alpha helices, called: A-B-C-D-E-F) . This proteins binds Ca, the site of Ca binding is a loop between helix E and helix F. If you look at the structure: helix E-Ca binding loop-helix F (Helix-loop-Helix), this is a domain, and wherever you see this domain (in other proteins), most probably it is a Ca binding domain). This domain is called an EF hand.

- So, EF hand is a Ca binding domain that consists of helix-loop-helix, first discovered in parvalbumin protein but is present in other proteins.

- **Q/ Why a loop, not a turn? What is the difference between a loop and a turn?**

  A turn is very small; it consists of 4 amino acids only, and it makes a sharp edge, so it is hard for Ca to fit there. A loop is not a regular structure, more amino acids so they can move to accommodate Ca binding.

Helix-Turn-Helix Motif



- Two α helices that are connected by a short loop region in a specific geometric arrangement constitute a helix-turn-helix motif. (a) the DNA-binding motif and (b) the calcium-binding motif, which are present in many proteins whose function is regulated by calcium.

Helix – turn - helix → DNA binding proteins
Helix – loop - Helix → Ca binding proteins

❖ Ca binding proteins characteristics:

1. Contain EF hand.
2. Contain negatively charged amino acids, to bind the Ca.

❖ Ca binding proteins have similar structures:

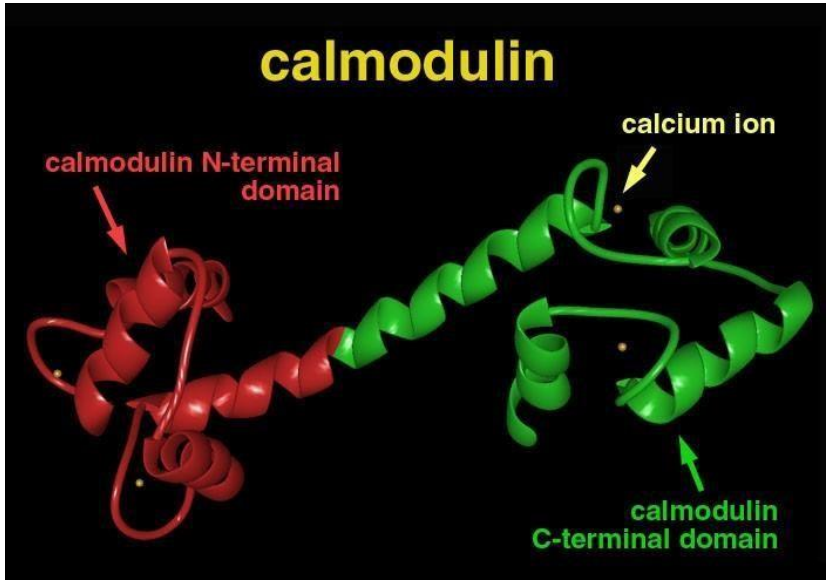
- Rich in Asp and Glu
- Gln, Asn, Ser → Several α helical segments →binding site is formed by Helix Loop Helix, which is a Super-secondary structure.

Calmodulin (≈17 kD)

Calcium-modulated protein

149 amino acids

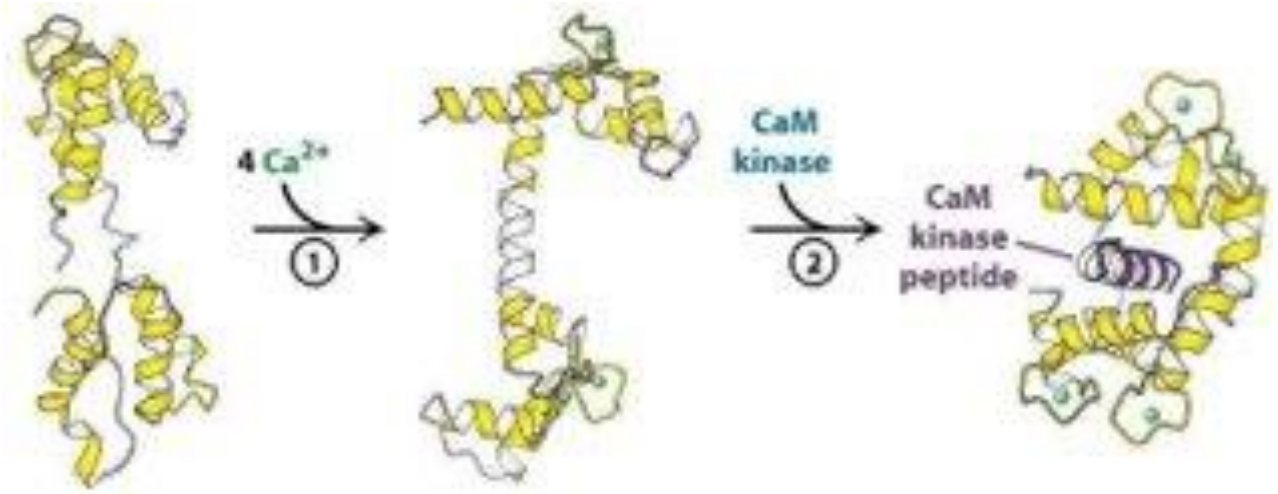
- Found in almost all eukaryotes
- Consists of two globular regions
  - Connected by flexible region
  - Each contains 2 EF hands
  - Four Ca^{2+} binding sites
- Calcium-Calmodulin complex can bind to a large number of target proteins including:



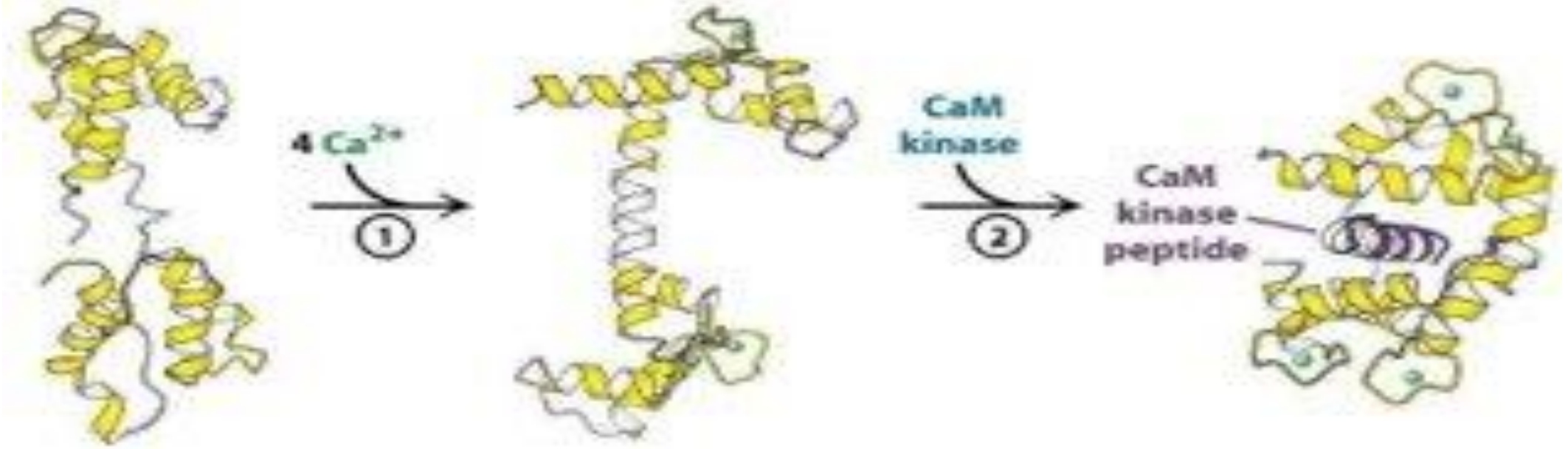
Calmodulin-dependant Protein Kinase

Ca^{2+} ATP'ase Pump

A common example is **Calmodulin (17kD)**, it consists of two globular domains, each one has two EF hands. Since each EF hand binds one Ca, in total, calmodulin molecule can bind four Ca molecules (each globular domain binds 2 Ca ions). Following Ca binding, it becomes active, and activates other proteins, including Calmodulin-dependent protein kinase. Also, it activates Ca ATPase pump, in order to return Ca inside and terminate the signal.



Inactive calmodulin Active calmodulin

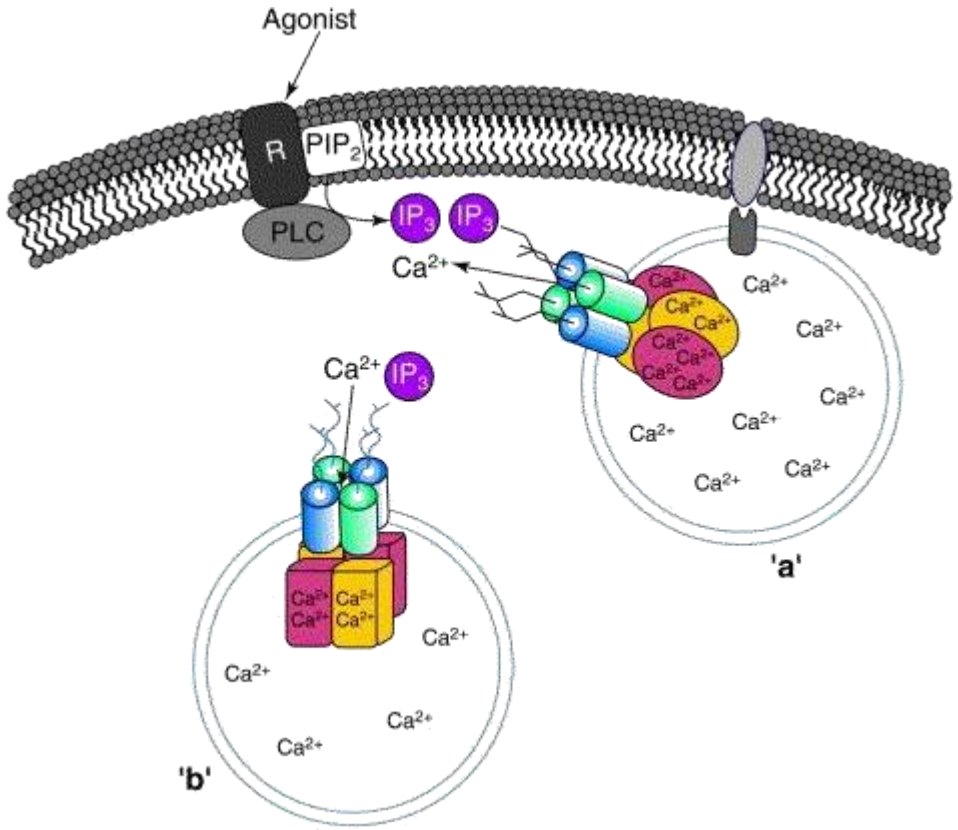


- When Ca is bound to calmodulin, calmodulin undergoes a tremendous conformational change. Also some hydrophobic amino acids are exposed, which means that they will interact with other hydrophobic regions of other proteins, causing activation of these proteins.

- Calmodulin structure: 17kD, consists of 149 amino acids, comprised of 2 globular regions connected via a flexible region, contains 2 EF hands, 4 Ca binding sites.

Ca^{2+} Transporter

- In sarcoplasmic reticulum
  - 80% of the membrane proteins
  - 10 membrane spanning helices
  - Ca^{2+} move against a large concentration gradient
  - 2 Ca^{2+} / ATP (high)
    - Depletion of ATP leads to tetany, Rigor mortis



- Ca ATPase pump is activated directly after Ca release (fast activation). It will pump Ca against its large concentration gradient (from the cytoplasm to the sER), so it is energy expensive, for each 2 Ca ions pumped, 1 ATP is hydrolyzed. This pump is present in large amounts on the surface of the sER; it constitutes around 80% of all proteins present on the surface. It consists of 10 membrane spanning helices. This pump is highly ATP expensive, and depletion of ATP leads to tetany & Rigor mortis .

The end :)