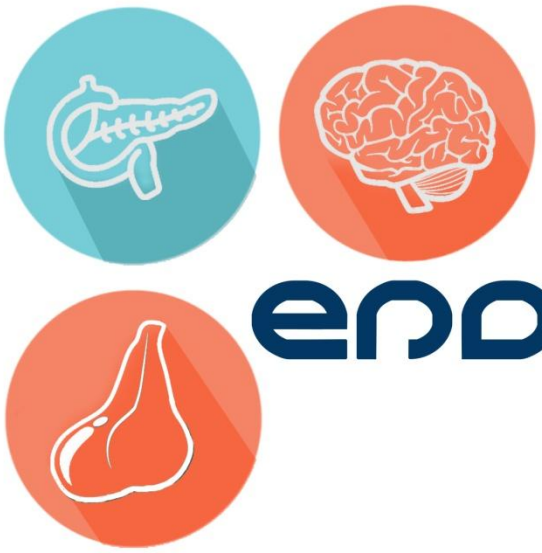


endocrine system

Biochemistry

●Sheet ○Slide

number	2
Done by	AbdelMuez Siyam
Correction	Abdullah AlZibdeh
Doctor	Nafeth

Notes:

1- **At** the end of this sheet, there is a brief summary for the whole lecture. If you don't have time, just go through it.
2- **There** are some points present in the slides but the doctor never gave them that much of a credit (just mentioned them rapidly or didn't say anything). Throughout this sheet, these points will be underlined, put in *italic* and put between double quotation marks. ''*Just like this*''**[Everything is included, no need for slide**]
3- **This** sheet was written based on Section 2 recording and it starts from slide 35.

Good luck

- ✓ Last lecture we went through the definition of hormones, classification according to effect, interaction with the nervous system, biochemical problems posed on the endocrine system, Kd, receptors, domains, signal amplification, loops to control hormone action, chemical structure classification and mechanism of action, synthesis of protein hormones, hormonal interaction, signal transduction and 2nd messengers and signal termination. We finished by talking about the types of receptors –including seven transmembrane receptor and its functions. ***Make sure you are familiar with all of the above mentioned.***

- ✓ Each 7-transmembrane receptor present on the cell membrane is attached to ***at least*** one G protein. The number is variable, and you may find around 100 G proteins (each consisting of alpha, beta and gamma subunits) attached to the same receptor. This type of receptor is also called G protein coupled receptor.

  ******There are around 20 different types of G proteins discovered, and around 100 G protein coupled receptors.

- ✓ Each 7-transmembrane receptor present on the cell membrane is **attached** to 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).

  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

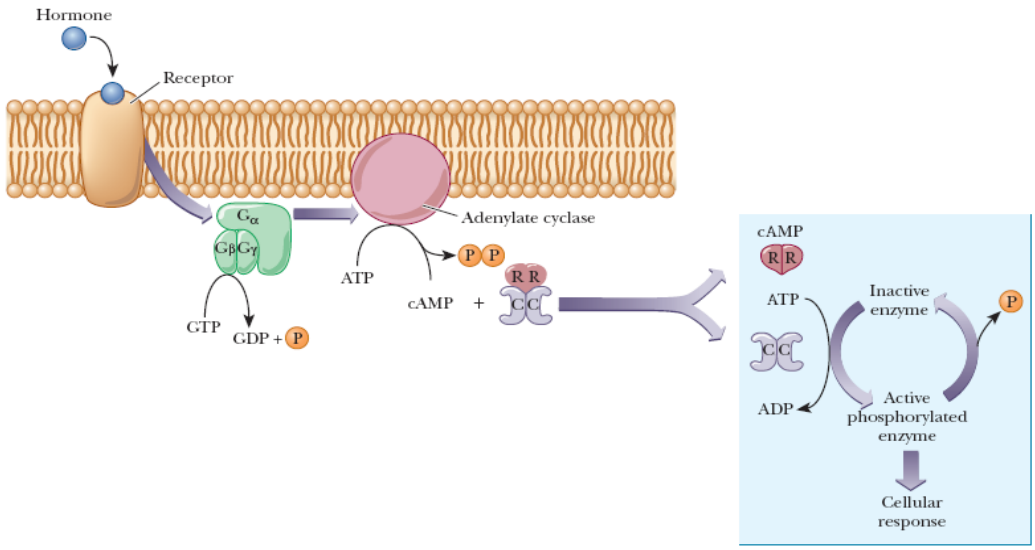
will also undergo a conformational change (due to the close proximity to the receptor). Now, the change in the alpha subunit results inlow GDP affinity, plus high GTP affinity and replacement occurs. The alpha subunit loses its already attached GDP to attach to a new GTP molecule. Once the alpha is bound 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.

- ✓ The alpha is now active. It will head towards a certain target. Note that there are more than one targets, one of them (the most common target) is the enzyme Adenylatecyclase

- ✓ This enzyme is a membrane enzyme, which consists of 12 alpha helices that span the membrane, and two intracellular domains (attached to the membrane). . This enzyme acts on ATP to produce cAMP ''*small and heat stable molecule''*

- ✓ 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 a Adenylatecyclase in the membrane, and each Adenylatecyclase will produce high amounts of cAMP per second, so first we started with one molecule conveying the signal (the hormone molecule) then the number increased (the number of alpha subunits activated) then it further increased (the number of cAMP)→Amplified signal.

- ✓ 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''.*



*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''

Back to G protein, we said that the active alpha subunit targets Adenylatecyclaseenzyme, 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 orβ2 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.***

****

The target of the alpha subunit is not always Adenylatecyclase. It may be the enzyme ***Phospholipase C***, or be a membrane ion channel such as chloride or potassium channels ''*can open or close the channels''*.

"*Functions of cAMP (mainly physiology, should be familiar):*

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

- The full cascade is the following:
  Hormone binding to receptor→ activate G protein→ activate Adenylate cyclase→ produce cAMP→ activation of protein kinase A→ phosphorylation.

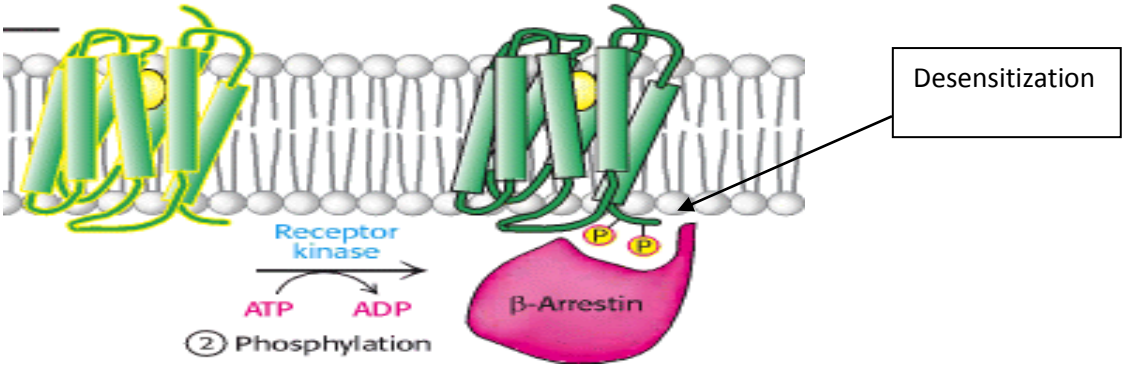
  The ***termination of the signal*** should act on all levels:

  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 alphasubunit 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, which makes the receptor 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 iswhat 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 significance.

- ***Cholera toxin:***
  It 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.

  **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.

****

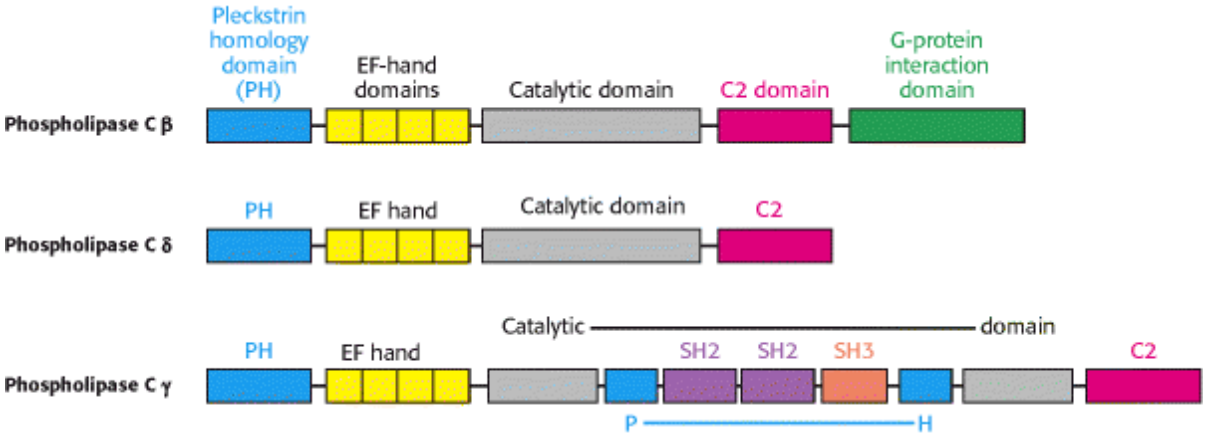
The phosphoinositide Cascade

- ''*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, and delta. Only Phospholipase beta contains the G protein binding domain, so it is the only one involved in this pathway.

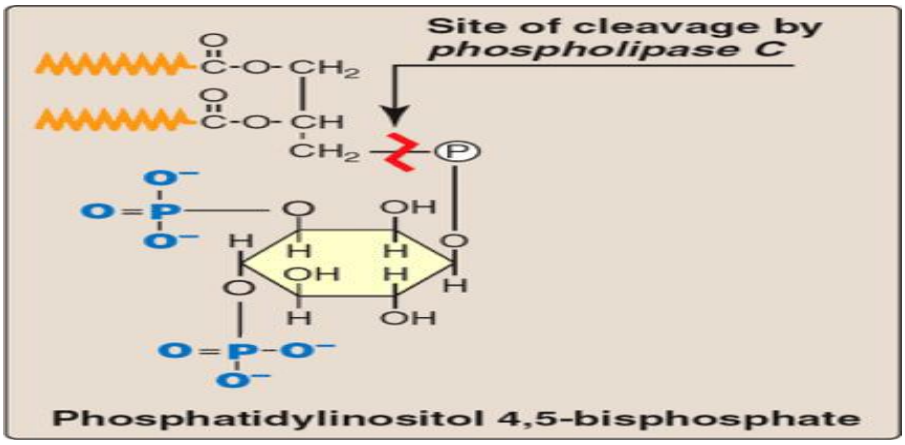
- The PH''*bind lipid head group''*and C2 domains *''bind phospholipid head group''*→membrane attachment.



- 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 (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, activate phospholipase C, breaks down PIP2bond 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 h.philic and h.phobic parts).

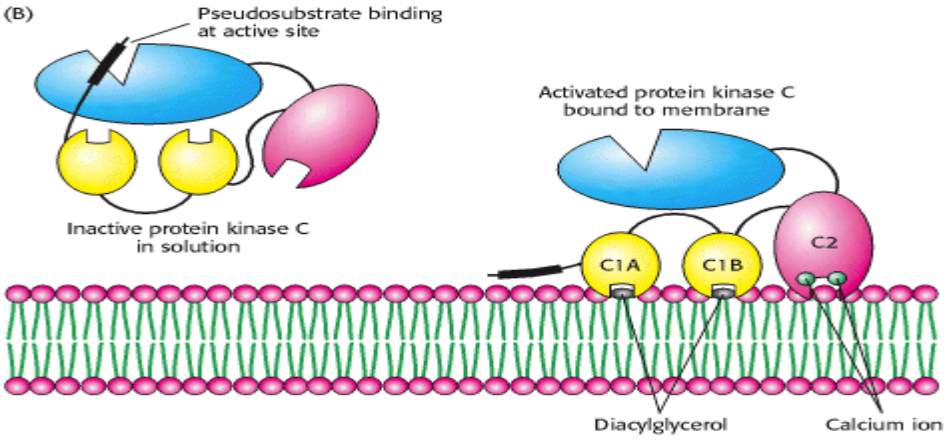
  The ***main*''*actual*''second messenger in this system is the IP3. DAG also works as a 2nd messenger.



- 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→ 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, which makes the 4th binding easier (*recall:* haemoglobin 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 **C**alcium). 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.

  **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 there doesn't exist Ser/Thr, 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.



''*The pseudosubstrate domain acts as a competitive inhibitor''.*

- ***Termination of the signal;*** by two ways, both of them end the activity of the IP3 molecule (inositol 1,4,5trisphosphate), which is a short lived messenger: **either** we remove a phosphate via cellular phosphatases **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, 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 ''*inhibit IP3 recycling''*→IP3 is active→treatment of depression.

- 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 ***hugedifference 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 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→ do 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 on amino acids/water/ negative amino acids…etc. These bonds ensure ***tight binding,*** thus change in its target.

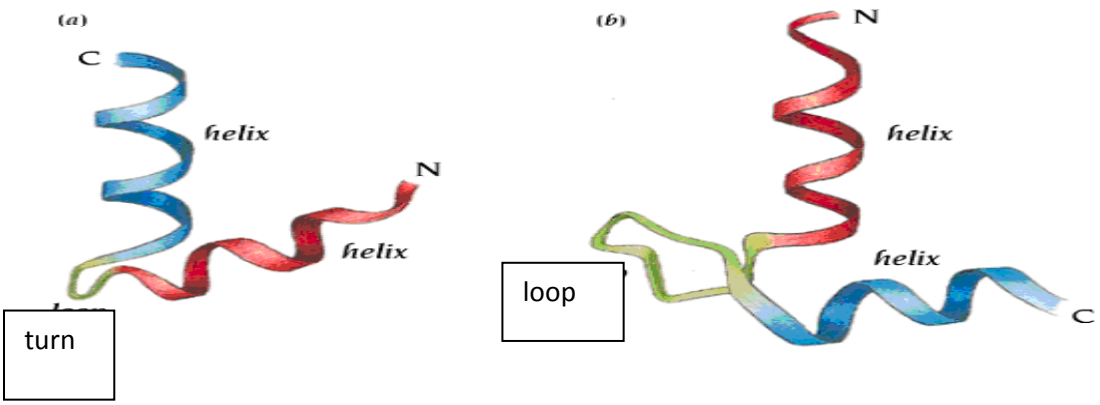
4- Ca is ***bulky***, so when it binds to the protein it produces the desired effect, which is the conformational change in that protein.

- 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:
  A-Calmodulin
  B-Troponin C
  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?

Ans: 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…….DNA binding proteins
Helix-loop-Helix…….Ca binding proteins.

- Ca binding proteins characteristics:

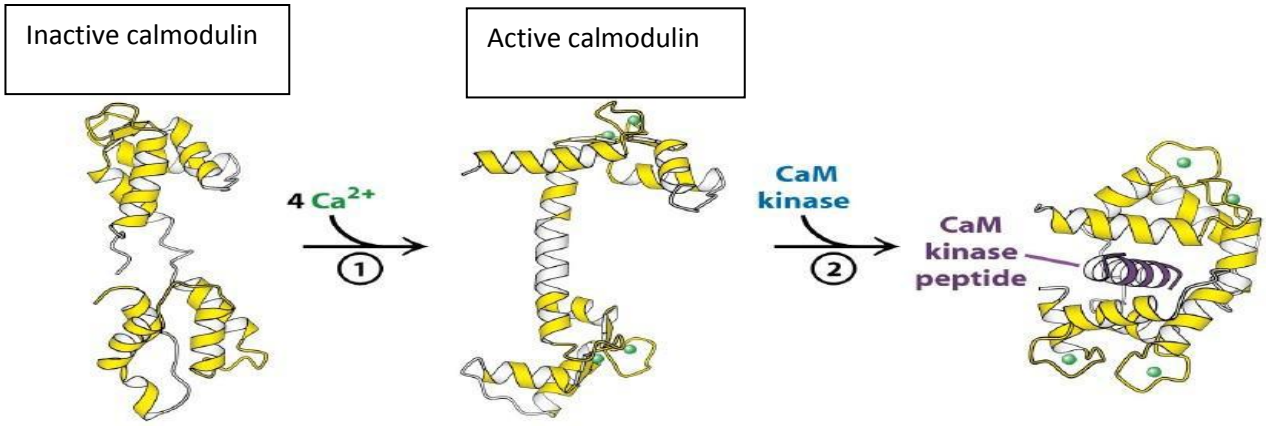
  1- Contain EF hand.

  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''

- A common example is ***Calmodulin*** (17kD), 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 Caions). 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.



- When Ca is bound to calmodulin, calmodulin underwent a tremendous conformational change. Also some hydrophobic amino acids were 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 ATPase pump***: 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 hydrolysed. 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''*

Good luck

The summary

*G protein is attached to the membrane because it is covalently bound to fatty acids (one on alpha, one on gamma subunit).

*Hormone binding→conformational change in receptor→conf. change in G protein→ alpha subunit loses GDP and takes GTP to get activated→ detach from βɣ dimer, and go for several targets: phospholipase C, ion channels (open or close them), or more commonly: Adenylatecyclase.

*Aden. Cyclase is a membrane enzyme, consists of 12 membrane spanning α helices, 2 intracellular domains. It converts ATP→cAMP.

* Each receptor is bound to at least 1 G protein, but it may be bound to more (like 100 G proteins with 1 receptor).So 1 hormone binds→100 G protein activated→100 aden. Cyclase activated→ much more cAMP produced. (**SIGNAL AMPLIFICATION**).

*Sometimes, Aden. Cyclase is inhibited by Gα. This depends on the nature of the receptor (some receptors are inhibitory) and the nature of the Gα subunit itself.

*cAMP targets protein kinase A (PKA). It is an enzyme, consists of 2 catalytic subunits and 2 regulatory subunits, which bind 4 cAMP molecules to detach from the catalytic, thus activating the kinase activity.

*PKA phosphorylates target proteins. This can either lead to activation or inhibition (famous example: Glycogen synthase gets inactivated if phosphorylated).

*Termination of the signal occurs by : 1-Hormone dissociating from the receptor (because they are bound non-covalently).
2-Slow GTPase activity in α subunit (GTP→GDP, so Gα is inactivated).
3-Phosphodiesterase breaking cAMP.
4-Phosphorylation of the receptor, making it attract/bind a protein called β-Arrestin, so it masks the receptor. So even if the hormone is bound, the receptor cannot activate G protein because it's masked (hormone bound + no response…this is desensitization).

*Cholera toxin: toxin that binds GPCR in intestine→ HUGE production of cAMP→release of Cl, pumping of Na, water out by osmosis→excessive and life-threatening diarrhoea.
****
*Active Gα targeting Phospholipase C (PLase C) : a membrane protein, consists of a Catalytic domain, a Membrane binding domain(PH and C2), and a G protein binding domain (to get activated).

*This enzyme has isozymes (multiple forms in different tissues). Only PLase C β has the G protein binding domain, and it is the one involved in this pathway.

*PLase C breaks phosphatidyl inositol 4,5-bisphosphate (PIP2) to inositol 1,4,5 trisphosphate (IP3-the main 2nd messenger) and diacylglycerol (DAG). IP3 is h.philic, it leaves the membrane and binds calcium channels on sarcoplasmic reticulum to cause Ca release. Four IP3s are required for full opening of the channel, but 3 can do the work (considerable opening). Released Ca (positively charged) targets negatively charged Calcium binding proteins, and Protein kinase C (PKC; membrane enzyme), that gets fully activated by binding of both Ca and DAG to it.

*Binding of IP3 to the channel is cooperative; meaning the binding of the first IP3 makes the binding of the 2nd easier, which makes the 3rd easier, which makes the 4th easier.

*PKC domains: 1-Membrane binding domain 2-Catalytic domain
3-DAG binding domain
4-Calcium binding domain
5-Pseudosubstrate domain: it fits in the active site of PKC (like the substrate), but it contains Ala instead of Ser/Thr, so it's not phosphorylated. It keeps the enzyme inactive (by blocking/closing the active site, by working as a competitive inhibitor). When Ca and DAG bind, this domain is displaced, exposing the active site and activating the enzyme.

*Termination is by removing IP3 phosphates or by addition of new phosphate producing IP4 (faster), both ways make IP3 inactive. And when the cell has the time, it removes IP4 phosphates, but the last added phosphate must not be removed first, because this will produce active IP3 and keep the signal running.

*Clinical hint: Lithium based drugs prevent IP3 recycling in the CNS→ IP3 remains active → treat depression.

*Characteristics of Ca:
1- Positively charged, so it binds Ca binding proteins (contain negative charges).
2-Huge difference between sER and cytosol (10000 times), the release has huge impact
3- It can make 6-8 bonds (ligations) with oxygen of amino acids. So it's tightly bound.
4-It is bulky, so when bound to protein, it causes the desired conformational change.

*Ca binding proteins:
1-Parvalbumin: the 1st one discovered, has 6 α helices (A-E), Ca binds in a loop between helices E&F. This Ca binding structure (helix-loop-helix) is called an EF hand, and is present in other proteins.
Note: helix-loop-helix: bind Ca, while helix-turn-helix: smaller, bind DNA.
2-Calmodulin: 2 globular regions, each has 2 EF hands, so it can bind 4 Ca ions. When Ca binds→conf. change→active→activate other proteins like Calmodulin dependent protein kinase and **Ca ATPase pump→to pump Ca back and terminate the signal.**

*Ca pump: 80% of proteins on sER surface, energy expensive (2Ca pumped: 1ATP), directly –fast- activated after Ca channels opening, 10 helices,↓ATP→tetany&R.mortis.