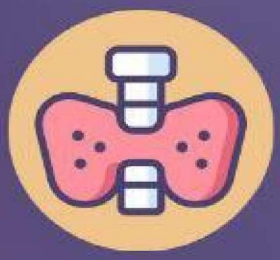
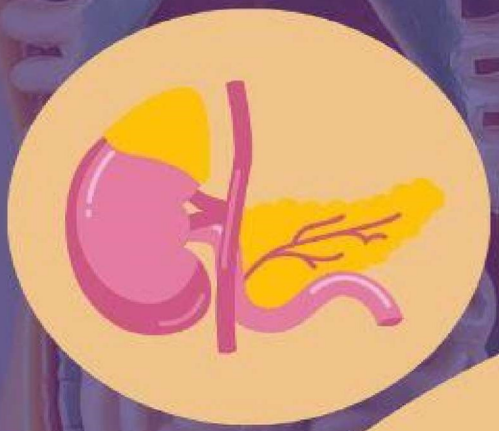
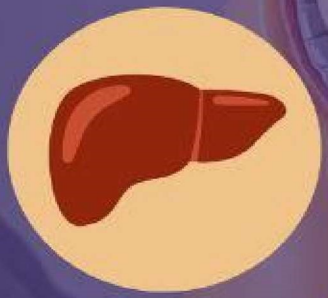
ENDOCRINE

Biochemistry

Writer: Mahmoud Jaradat & Rahaf Al-Barmawi
Corrector: Afnan Abu-Swelem
Doctor: نافذ







Sheet no. 3

First page عنوان الchapter / ملف ال

Normal كلام الدكتور

TITLE العناوين الرئيسية

SLIDES

Extra info كلام خارجي

معلومة

highlight V2,3 … مهمة

التعديلات

TRANSDUCTION OF HORMONAL SIGNAL

SIGNAL TRANSDUCTION

It must exist in order to deliver the message to the cell (that is, for the cell to understand and implement what is required of this signal).

TRANSDUCTION: CONVERSION OF ONE FORM OF A SIGNAL TO ANOTHER SO AS CELLS CAN PRODUCE MANY KINDS OF RESPONSES IN DIFFERENT WAYS

AMPLIFICATION IS A MUST

SIGNAL (POLAR, LARGE) SHOULD BIND RECEPTORS:

- INTRINSIC NOT INTEGRAL!
- TRANSMEMBRANE
- INTRA- & EXTRACELLULAR DOMAINS

IS THAT ENOUGH? THE NEED FOR 2ND MESSENGER (for the following reasons)

- FEW IN NUMBER (hormones are few)
- RESTRICTED MOVEMENT (receptors)

The receptors are outside the cell and are capable of slight lateral movement but not free movement

So, how will the signal reach its target inside the cell?

In order for this signal to arrive, there must be secondary messengers in order to translate the messages outside the cell (by messages we mean the connection of the hormone to the receptor), transmit them, and implement the desired goal inside the cell.

SECOND MESSENGERS

ABILITY TO DIFFUSE TO OTHER CELLULAR COMPARTMENTS.

AMPLIFICATION OF THE SIGNAL.

(Secondary messengers must be amplified due to the small number of both hormones and their receptors)

- ENZYME ACTIVATION
- MEMBRANE CHANNELS

(Then it either stimulates an enzyme or binds to a channel to give its huge effect)

SOME SECOND MESSENGERS ARE COMMON IN MULTIPLE SIGNALING PATHWAYS (APPROX 30 HORMONES USES CAMP!!!)

- PERMITS FINE TUNING BUT CAN POSE PROBLEMS

TYPES OF 2 ^ (ND) MESSENGERS:

- SMALL MOLECULES: CAMP, CGMP, CA+2
- PHOSPHORYLATION THROUGH KINASES

There are different types of receptors, one of them :

MEMBRANE ASSOCIATED RECEPTORS 7TRANSMEMBRANE HELIX RECEPTORS (7TM)

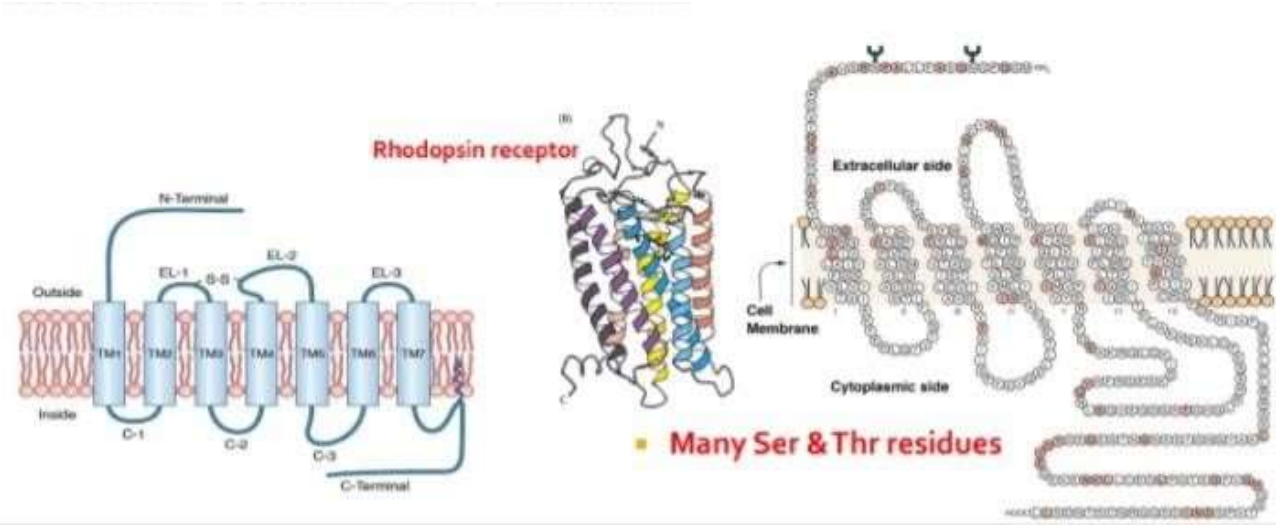
)the most common receptor)

7 A-HELICES: H-BONDING, RIGID, HYDROPHOBIC ✓

SIGNAL INDUCES CONFORMATIONAL CHANGES

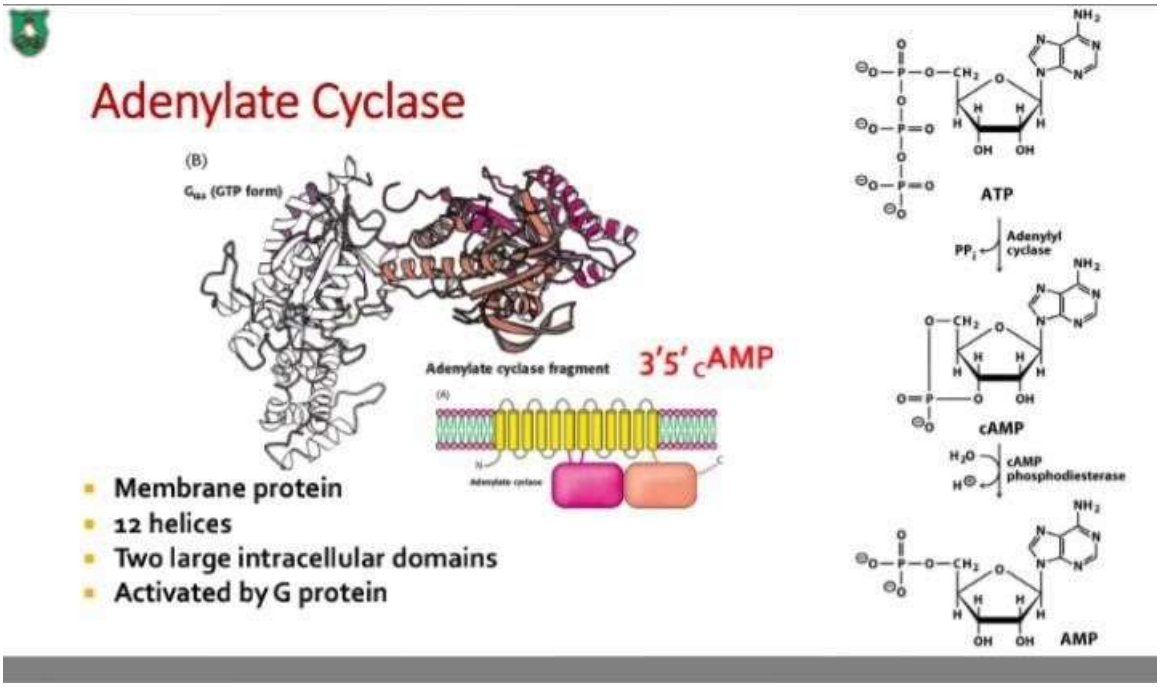
✓ IS IT ENOUGH?

How do we put the alpha helices inside the membrane even though it is hydrophilic? We can change it to become hydrophobic by incorporating more and more of the hydrophobic amino acids، Therefore it can fit within the membrane and the goal is to make a stable receptor inside the membrane

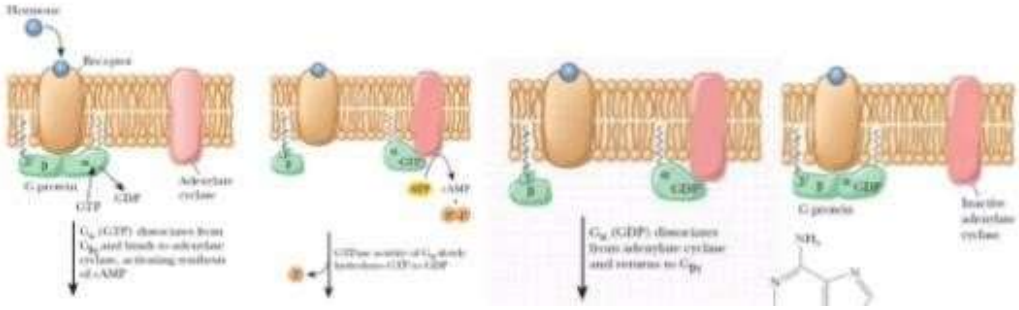


If we look at the figure above, we will find many serein and threonine residue which are the sites of phosphorylation

*Remember this we will need it soon



G-Proteins & cAMP



The receptor is in the cell membrane, which is actually three-dimensional, so it is surrounded from the inside in all directions by a protein, and this protein is G protein.

What is G protein?

It is a trimeric protein that contains alpha, beta, and gamma units beta and gamma are one connected unit and the alpha is alone

The alpha has fatty acid on it that connects it to the membrane, and the gamma on it as well

Thus, the alpha is connected alone, and the beta and gamma are connected together.

There is no covalent bond between alpha and beta-gamma dimer

Signal transmission:

1 When the hormone binds to the receptor,conformational changes occur in this receptor, and it gives a message to G proteins, and from here begins the amplification

2- The G protein is inactive in its form of binding to GDP, and once conformational changes occur , the affinity to GDP becomes very low, and the affinity to GTP increases very much, and an exchange occurs so that the GDP goes and is replaced by GTP.

3- When the G protein binds to GTP, it makes the affinity of the alpha towards the beta-gamma dimer low.

Then the alpha separates from the G protein and goes to its target, which is the adenylate cyclase, which deals with the ATP and cyclizes it, converting the ATP into cAMP.

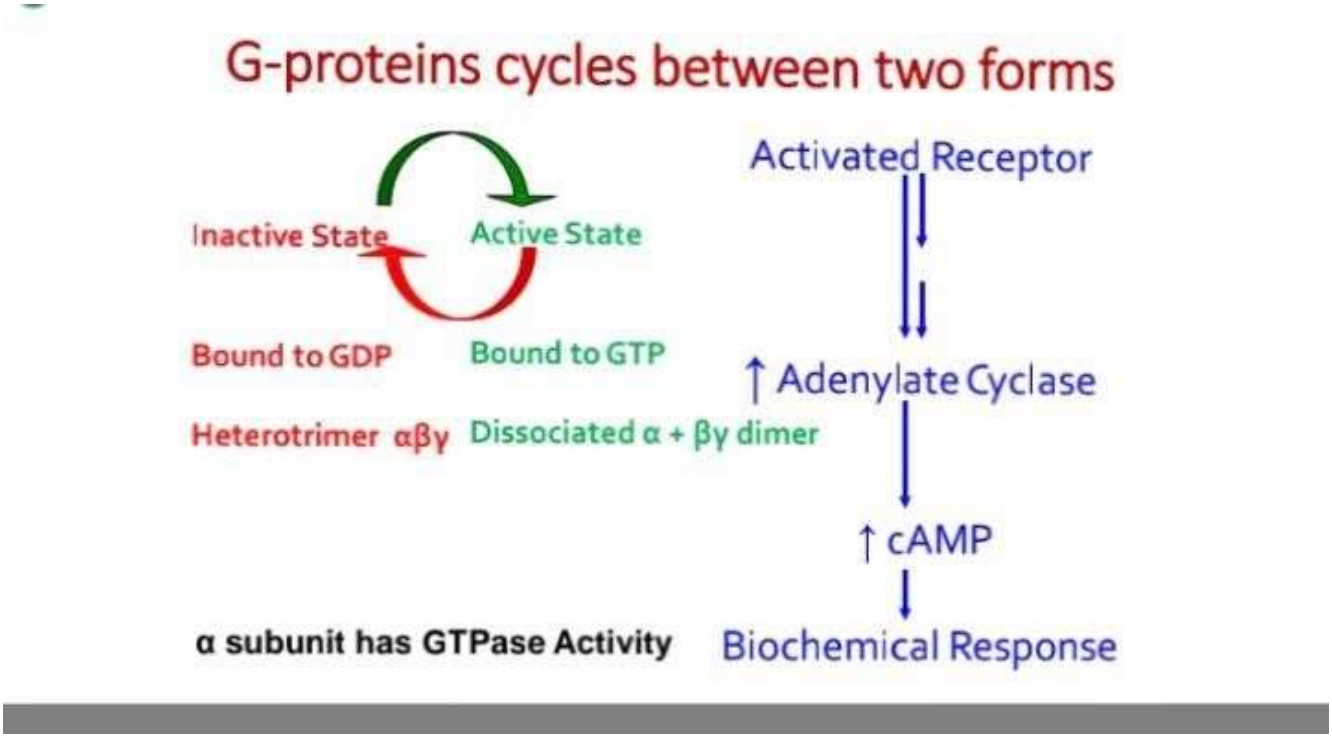
* The cAMP cycle in order to be heat stable

*Let us pause here to review some of the information in the slides and then continue the steps

CAMP: SMALL & HEAT STABLE

PLASMA MEMBRANE

➤ HORMONE → SPECIFIC RECEPTOR (B1- OR B2-ADRENERGIC RECEPTOR) → G PROTEIN → ADENYLATE CYCLASE → CAMP → PROTEIN KINASE A → PHOSPHORYLATION



G-PROTEINS: STIMULATORY OR INHIBITORY?

➤ CYCLIC AMP & G PROTEINS:

✔ HORMONE → RECEPTOR (A2-RECEPTOR) → G PROTEIN → INHIBITS ADENYLATE CYCLASE

Are the receptors excitatory or inhibitory?

This depends on their effect within the cell

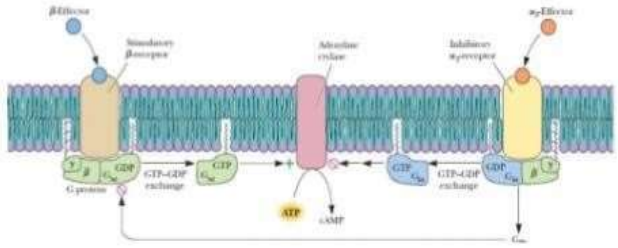
The G proteins have stimulatory and inhibitory subunits, and this also depends on their effect

#conclusion the things that determine whether stimulation or

inhibition occurs

•The receptor (alpha or beta: where alpha acts as inhibitor while beta acts as activator)

• The nature of G protein



■ G PROTEINS:

➤ MORE THAN 100 KNOWN G PROTEIN-COUPLED RECEPTORS AND MORE THAN 20 KNOWN G PROTEINS

➤ 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 CHANNEL

●A AND Y SUBUNITS HAVE COVALENTLY ATTACHED FATTY ACID

●A AND ßY CAN INTERACT WITH OTHER PROTEINS

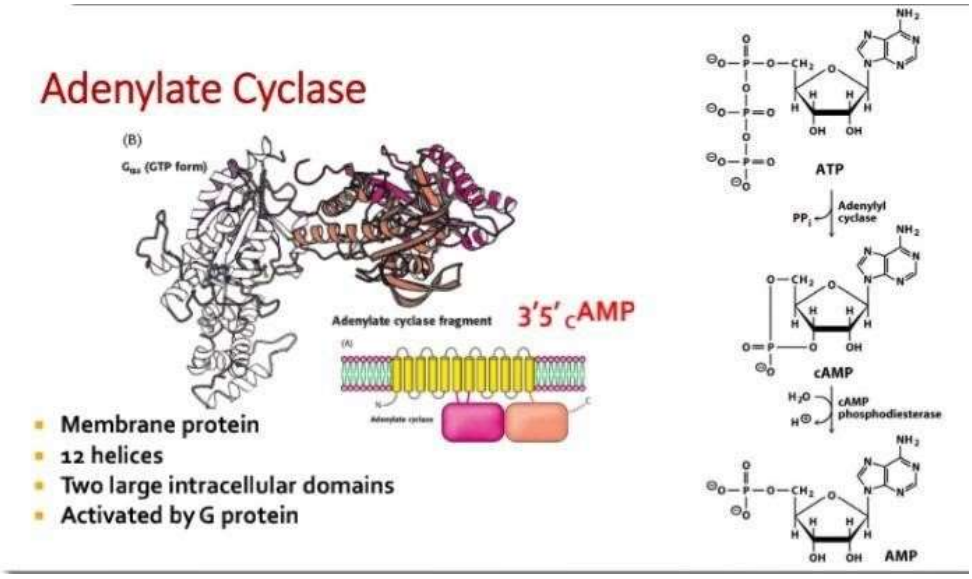
●ALL 7TM RECEPTORS APPEAR TO BE COUPLED TO G PROTEINS GPCRS

• AMPLIFICATION: RECEPTOR 100'S OF G PROTEIN 100'S OF ADENYLATE CYCLASE 100'S X 1000'S MOLECULES/SEC OF CAMP

Let's continue the steps now

We previously mentioned in the third step that alpha targets adenylate cyclase, so what is adenylate cyclase?

It is a protein consisting of 12 membranous alpha helices and has two large intracellular domains (the catalytic domain is toward the cytoplasm)



4- After adenylate cyclase converts ATP into cAMP, the phosphodiesterase enzyme converts AMP into its inactive form (as shown in the figure on the right)

5 - The cAMP goes to protein kinase A (which is a tetrameric protein consisting of 2 cathalytic subunits + 2 regulatory subunits), so the regulatory subunit binds to four cAMPs, and once they bind, they cause a dissociation between the cathalytic and regulatory subunits.

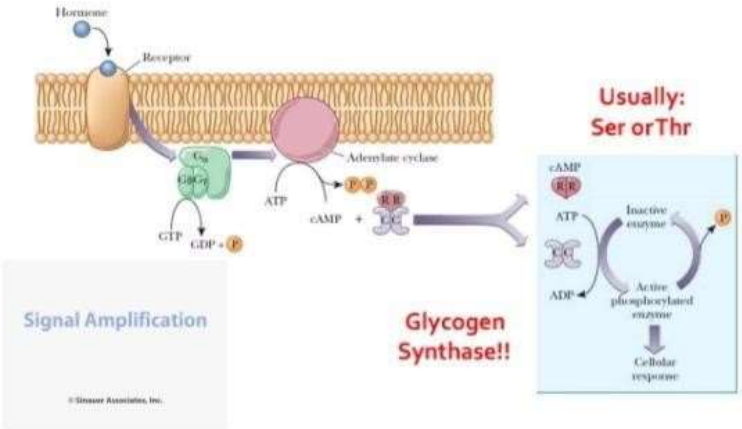
6- Then the catalytic subunit performs phosphorylation of a lot of enzymes and proteins, and the end result is the stimulation or inhibition of these enzymes and proteins.

* One of the things it activates is the phosphodiesterase enzyme, which breaks down the cAMP to stop signal so that it acts as a negative feedback.

cAMP can affect a wide range of cellular processes

- ↑ degradation of storage fuels
- ↑ secretion of acid by gastric mucosa (caffeine: phosphodiesterase & adenosine)
- Dispersion of melanin pigment granules
- ↓ aggregation of blood platelets
- Opening of chloride channels

Then What?



SIGNAL TERMINATION

IS IT IMPORTANT?

- KEEPS CELLS RESPONSIVE TO NEW SIGNALS
- FAILURE OF TERMINATION MAY CAUSE PROBLEM E.G GH & CANCER
- HOW IT IS ACHIEVED?

DEGRADATION OF THE SECOND MESSENGER

- DEPHOSPHORYLATION BY HYDROLYSIS

SWITCHING OFF THE SIGNAL

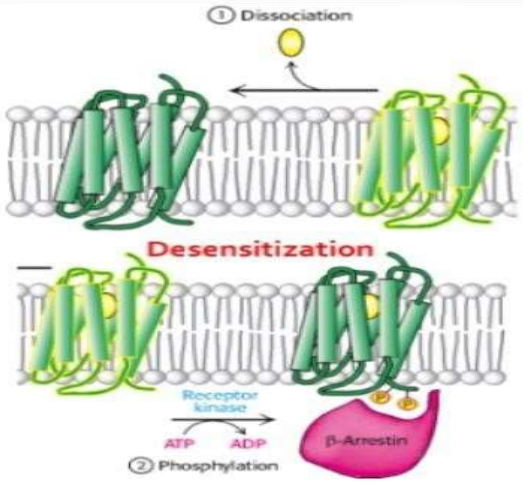
1• DISSOCIATION OF THE HORMONE

2• GTPASE ACTIVITY OF GA SUBUNIT

3• HYDROLYSIS OF CAMP (PHOSPHODIESTERASE)

4• PHOSPHORYLATION OF THE HORMONE BOUND- RECEPTOR FOLLOWED BY BINDING TO ß-ARRESTIN.

(That is, desensitizing the receptor, and this is through serein and threonine residues, which, as we mentioned previously, are phosphorylation sites. When they are phosphorylated, they attract a protein called beta-Arrestin, which binds to the signal transduction domain with high affinity, and is surrounded by it, thus preventing the formation of conformational changes in the G. Protein)



Cholera it happen if signal termination fails

- 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

The phoshoinositide cascade

Used by many hormones (e.g. ADH)

↓ Binding of a hormone to 7TM receptor

↓ Activation of G Protein

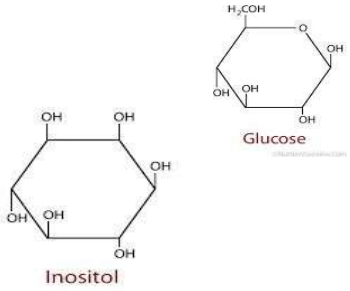
↓ Activation of Phospholipase C (many isoforms) – PIP2

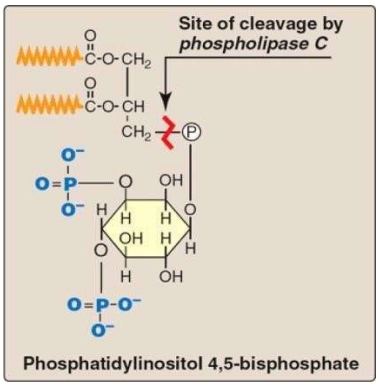
Two messengers are produced:

- Inositol 1,4,5-trisphosphate, hydrophilic, (Soluble)

IP3 is the actual second messenger

- Diacyclglycerol, amphipathic (membrane)





Another signaling pathway, aside from the adenylate cyclase pathway, is the phosphoinositide cascade.

Inositol is an Alcohol in nature(6 member ring molecule) same as glucose, except the glucose has an oxygen in it's s structure and it is an aldehyde .

Every carbon in inositol has a hydroxyl group (OH). Recall: lipids are two types

o Simple lipids: neutral→ have no charge due to their structure and; therefore, they don't participate in any reaction. Considered as stored lipids; they are triacylglycerols (glycerol backbone + three fatty acids).

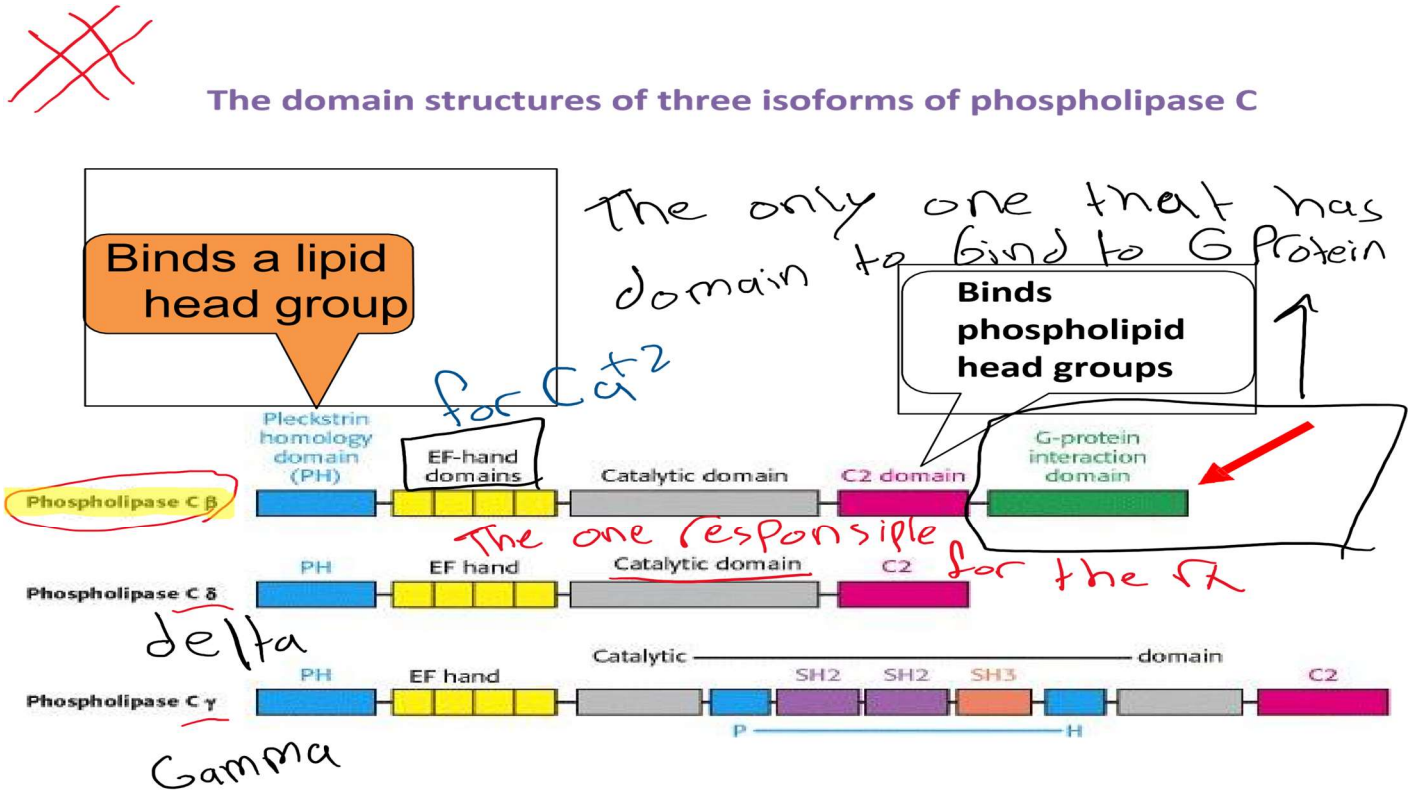
o Complex lipids: phospholipids (either sphingosine based or glycerol based), membrane lipids.

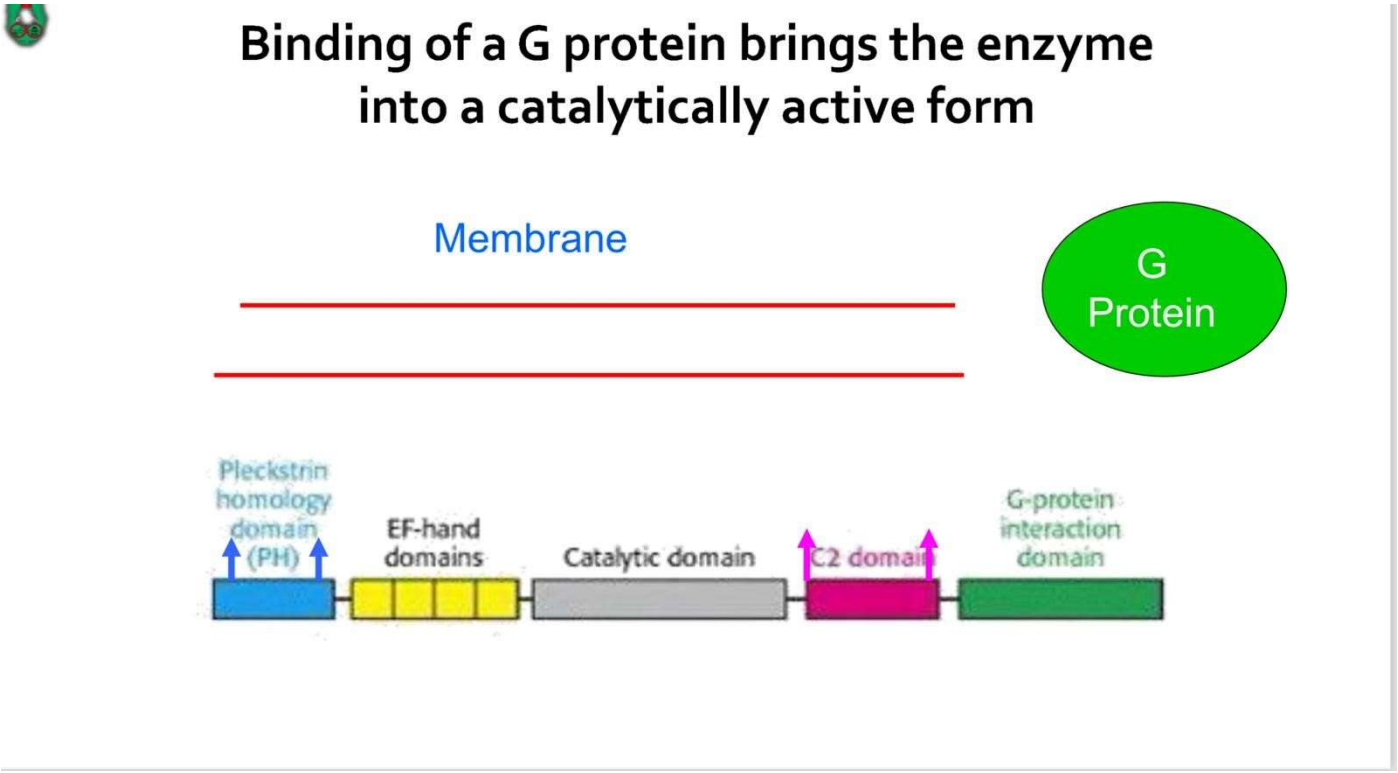
The simplest glycerol phospholipid is phosphatidic acid (phosphatidate), it is composed of glycerol backbone, two fatty acids and one phosphate molecule on carbon number 3.

This phosphate group can be attached to choline forming phosphatidyl-choline, ethanolamine forming phosphatidyl-ethanolamine, or inositol forming phosphatidyl-inositol.

So when the hormone attached to it's receptor it activates G-proteins which activates an enzyme called phospholipase C that breaks down the bond between the C3 and the phosphate with inositol,now inositol has 3 phosphate groups negatively charged (that we call it IP3,is a soluble molecule) and we have glycerol with 2 FA (DAG) which stays in membrane .

Regarding to phospholipase C isoforms in the pic we're just interested in beta isoform because it's the only one with G protein interaction domain





DAG stays in the membrane while **IP3** goes to the cytoplasm to smooth endoplasmic reticulum (which stores Ca+2) to be released.

<u>Look at the pic below carefully it's very important !!!</u>

The Ca+2 channel has 4 binding sites for IP3

To open the channel wide and release the Ca+2 we need at least 3 IP3

1 or 2 of IP3 they don't elicit a good response within the cell.

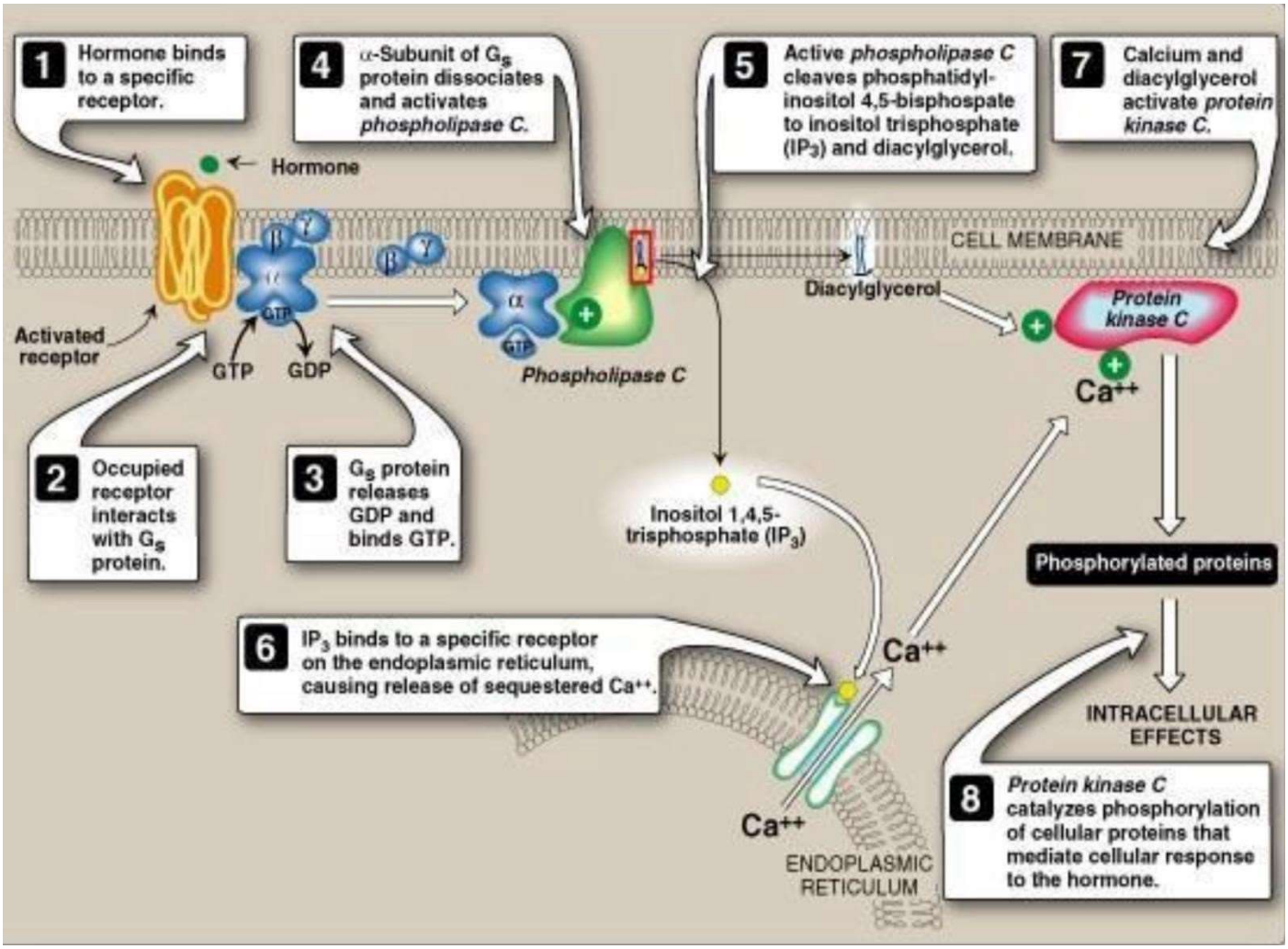
Remember that DAG is a lipid soluble, so it moves freely in the membrane.

DAG and Ca+2 both of them are shared for the activity of protein kinase C.

The active site of the protein kinase C it is covered by an arm of Amino Acids

So when the DAG binds to protein kinase C it pulls up this arm and activates the kinase.

DAG alone can't activate the kinase we need Ca+2 also.



EFFECTS OF SECOND MESSENGERS

INOSITOL TRISPHOSPHATE (IP3):

o Opens Calcium Channels

o Binding to IP3-gated Channel

o Cooperative binding (sigmoidal)

DIACYLGLYCEROL (DAG):

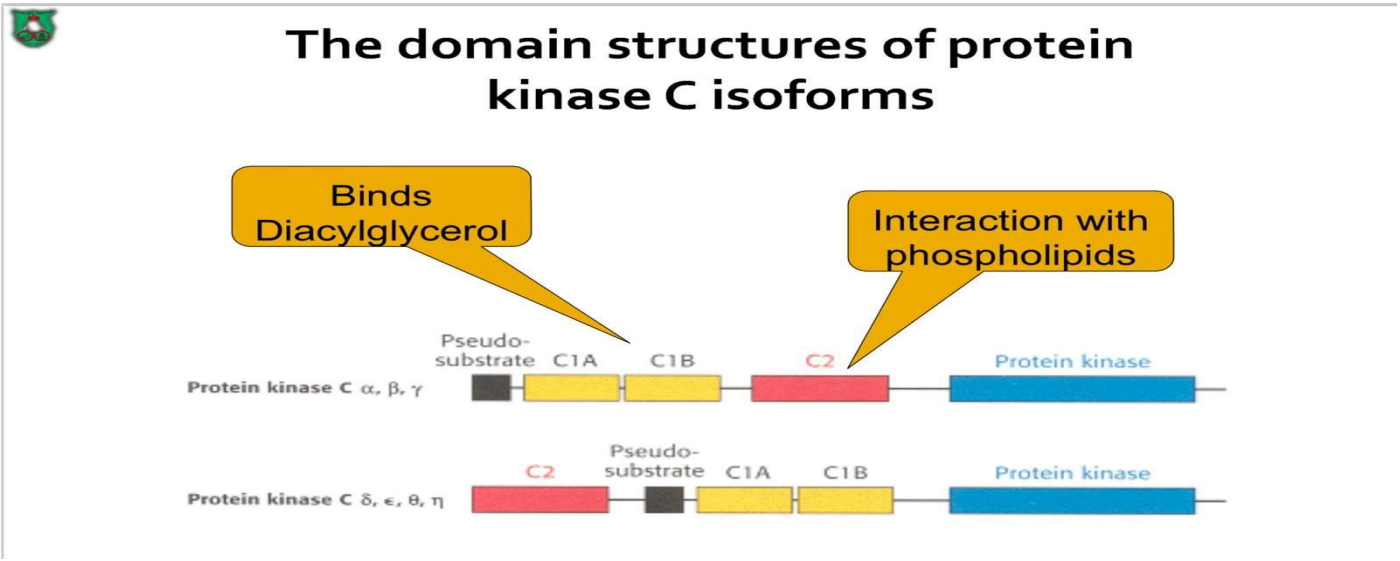
o Activates Protein Kinase C

o Ca+2 is required

o Phosphorylation of many

target proteins

THE DOMAIN STRUCTURES OF PROTEIN KINASE C ISOFORMS



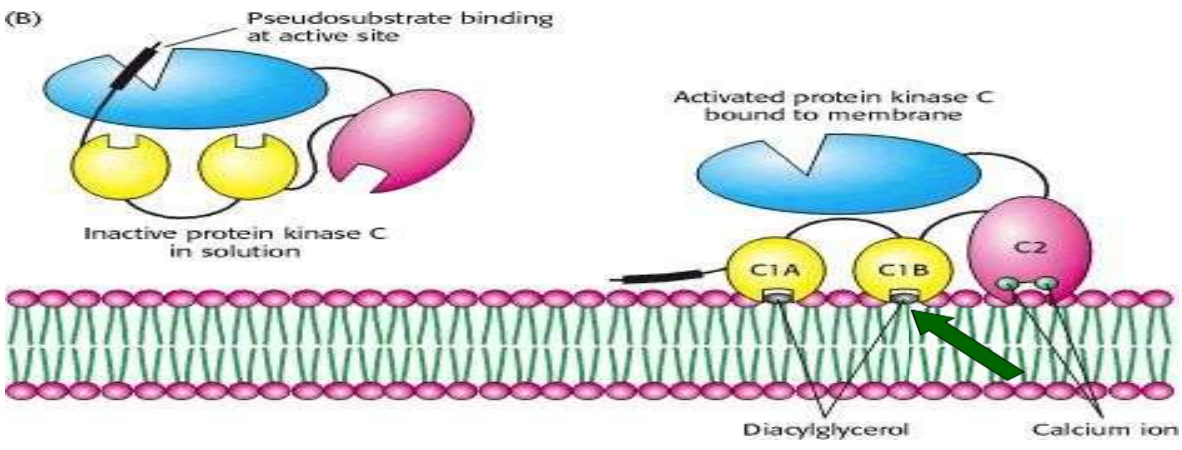
Protein kinase phosphorylate substrates (proteins and enzymes) in serine and threonine.

Pseudo-substrate is a competitive inhibitor for protein kinase C.

Note that the arm of pseudo-substrate has the same sequence of the substrate but with replacement of S and T with alanine. When the protein kinase enzyme bind to pseudo-substrate it can't phosphorylate the alanine, so the active site will be closed & inhibited.

Pseudo substrate sequence

Competitive inhibitor

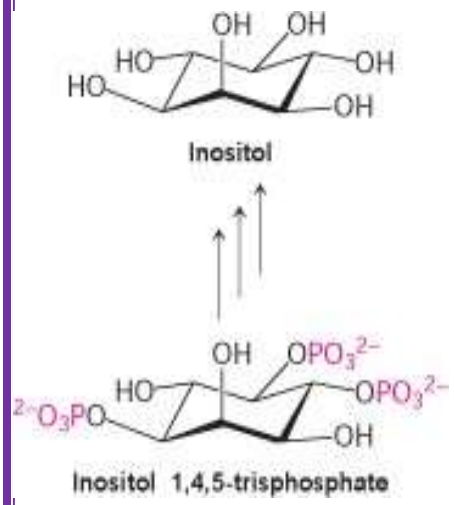


- Resembles pseudo-substrate sequence: A-R-K-G-A-L-R-Q-K
- Substrate Sequence: (S,T)
- Binds to the Enzyme's Active Site

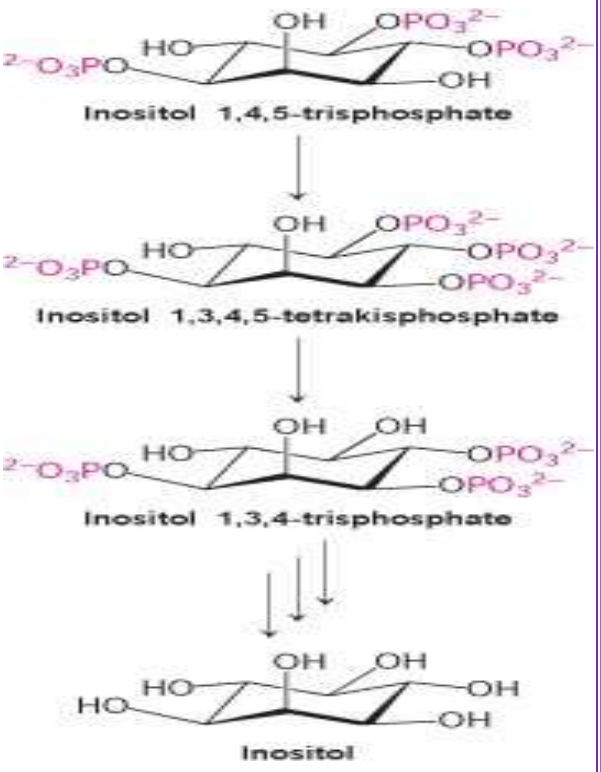
The protein kinase C should have these domains :catalytic domain, domain to bind to the membrane ,domain to bind to DAG and domain to bind to Ca+2

Termination of signal IP3

IP3 is a short - lived messenger



Lithium ions, used to treat. some psychological disorders Inhibits IP3 recycling



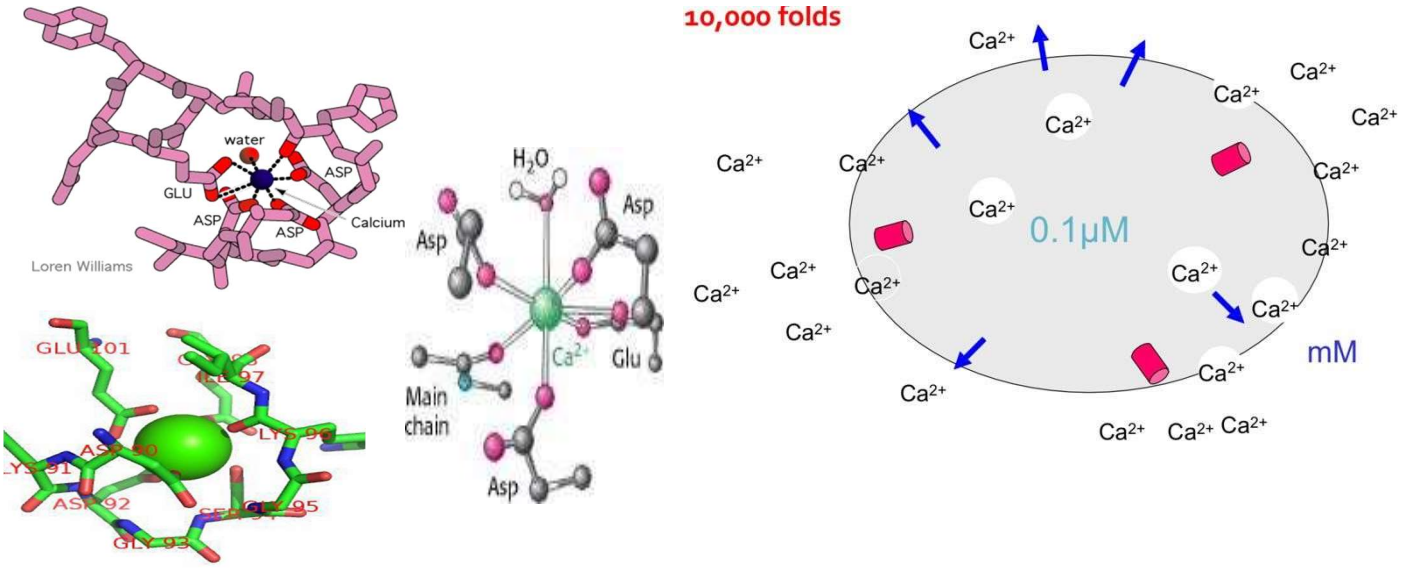
IP3 is the main signaling molecule in this system, it is terminated by removing phosphate groups one by one, but it is physiologically longer.so it does not terminate on the spot.

The simple way is by adding phosphate group on C3 so it becomes IP4 which is inactive Then, the phosphate groups are removed one by one starting from the original ones (not the last one that was added).

Lithium ions, used to treat certain psychiatric disorders because it

inhibits IP3 recycling by preventing the degradation of the phosphate groups, so IP3 is active.

WHY CALCIUM?? A large difference in concentration



Ca+2 is one of the main thing in the cascade why?? Because there is difference in concentration (the Ca+2 stored in sarcoplasmic reticulum with regard to cytoplasm which is low) so releasing small amounts of calcium is going to cause an effect, because the concentration gradient doesn't change.

similar to protons and mitochondria, the concentration difference between the matrix and the intermembrane space is huge; so when small amounts of protons go back to the matrix, there will be ATP generation due to the high gradient.

Meanwhile, pumping Calcium back into the ER is a very expensive process (we need 1 ATP for every 2 Calcium ions pumped back); this is why calcium isn't released in high amounts.

WHY CALCIUM again

Ability to bind proteins tightly since it is positively charged so it binds to negatively charged and polar molecules.

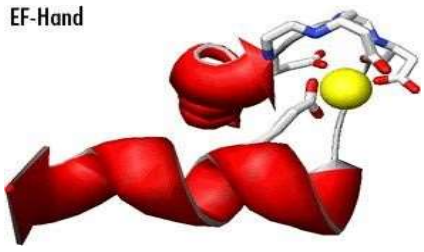
6-8 bonds with oxygen: Calcium can make up to eight bonds.

Conformational changes: Calcium by nature is bulky, so when it binds

to molecules , it induces conformational changes all the time and this the goal because it can make many bond with molecules .

Another destination for the Ca+2 is calcium binding proteins.

CALCIUM BINDING PROTEINS



Mediate the effects of Calcium (Ca+2).

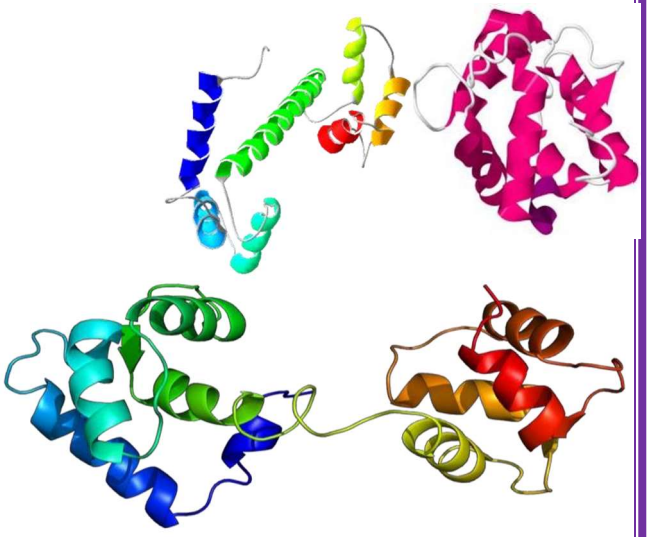
Many proteins

Examples: Calmodulin, Troponin C, Parvalbumin.

Parvalbumin the first one was discovered as a structure, it has alpha helices " A ,B ,C ,D ,E and F " the calcium binds between E and F helix (it is called EF-hand),so any protein containing Ca+ 2 has EF-domain. Similar structures o Rich in Asp and Glu (charged AA) o Gln, Asn, Ser (polar AA) o Several alpha helical segments o Binding site is formed by:

Helix Loop Helix

Super-secondary structure



CALMODULIN ≈ 17 KD

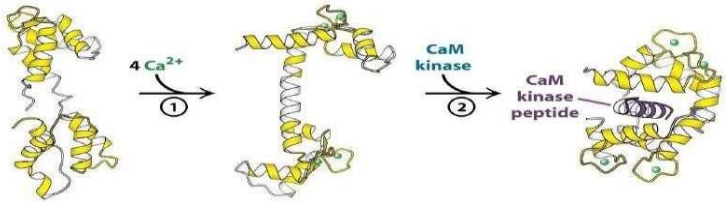
Calcium-modulated protein

The underlined letters refer to where the name came from.

Found in almost all eukaryotes.

Consists of two globular regions:

Connected by flexible region



Each contains 2 EF hands in each region therefore 4 Ca+2 binding sites.

Calcium-Calmodulin complex can bind to a large number of target

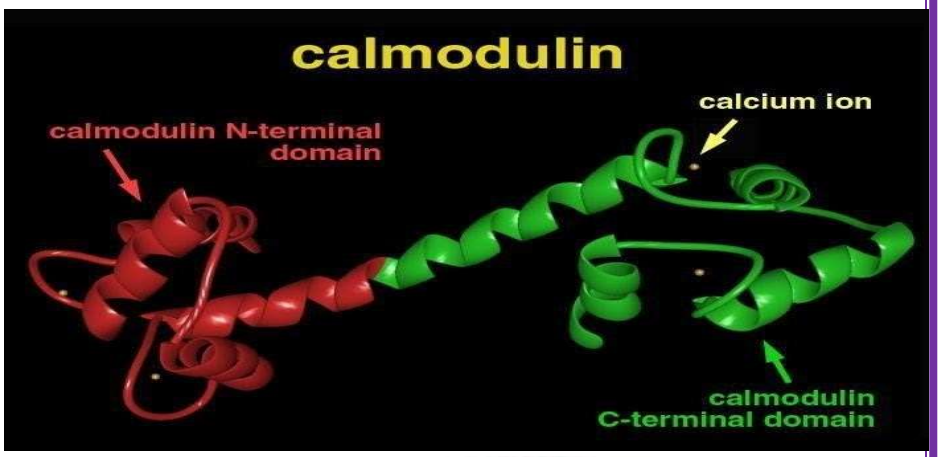
proteins including: Calmodulin-dependent protein kinase &

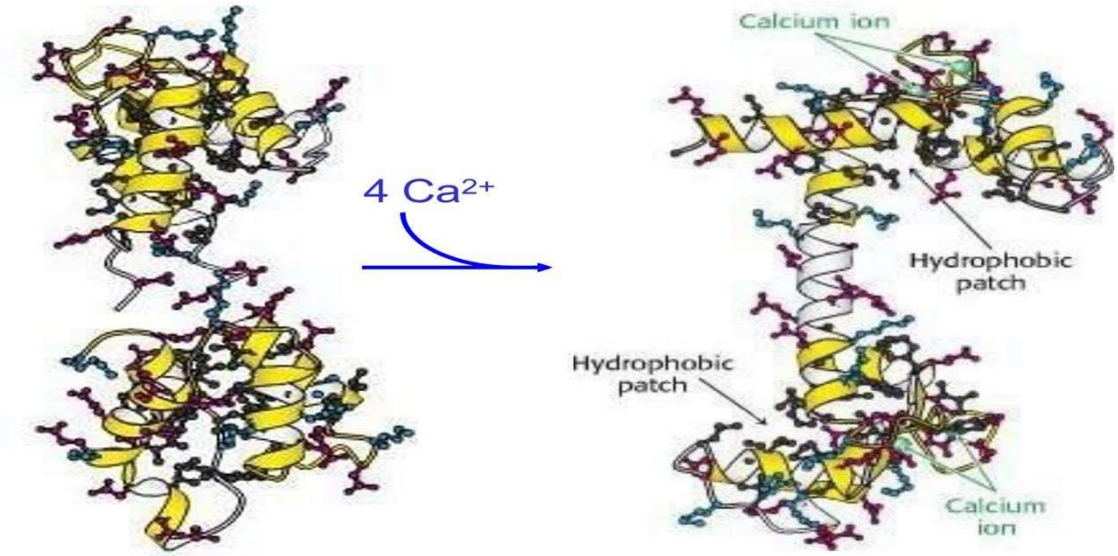
Ca2+ ATPase Pump (which is present on SER membrane)

The Ca+2 bind to the calmodulin and cause conformational changes (now it's effective)

Calmodulin-dependant Protein Kinase. (An enzyme)

Ca2+ A TP'ase Pump.(a protein that utilizes ATP to pump Ca+2 against it's concentration). 149 amino acids





Calmodulin binds to Ca+2 which results in change in conformation (Moving some hydrophobic residues from the inside to the outside of the domains).

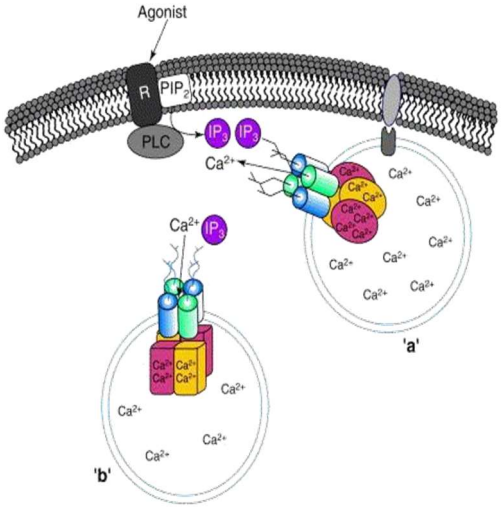
CALCIUM TRANSPORTER

The main function of sarcoplasmic reticulum is to store calcium.
in sarcoplasmic reticulum o 80% of the membrane proteins. o
10 membrane spanning helices.

Ca+2 move against a large concentration gradient.

2 Ca+2 / ATP (high, energy-expensive process) and this is two much in terms of energy o Depletion of ATP leads to tetany,
Rigor mortis.

Ca+2 is required for muscle contraction.

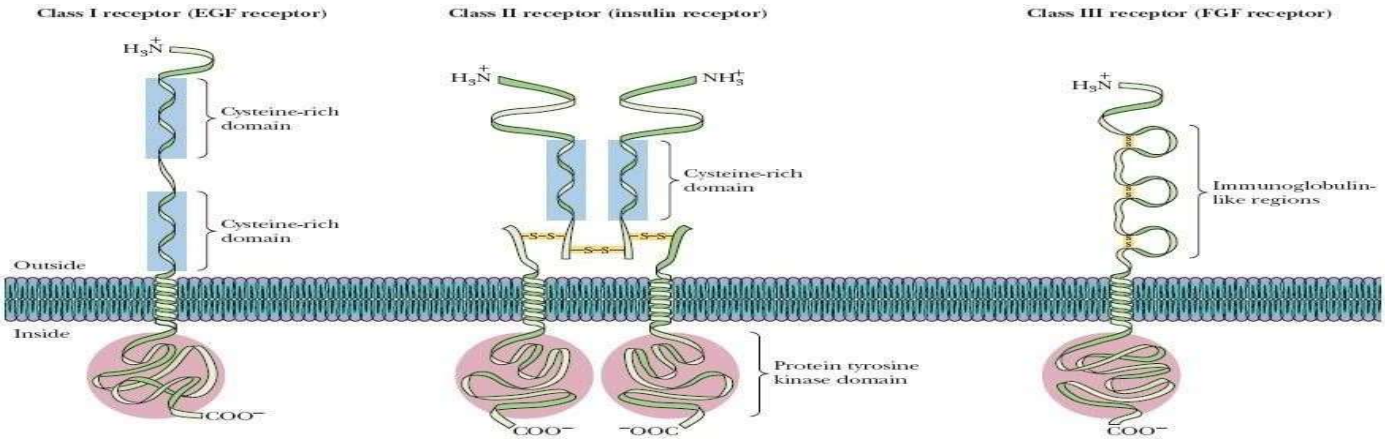


RECEPTOR TYROSINE KINASES CASCADE

- **Second Messengers**
- **Span the membrane, several subclasses (class II, Insulin R), hormone receptor & tyrosine kinase portion**

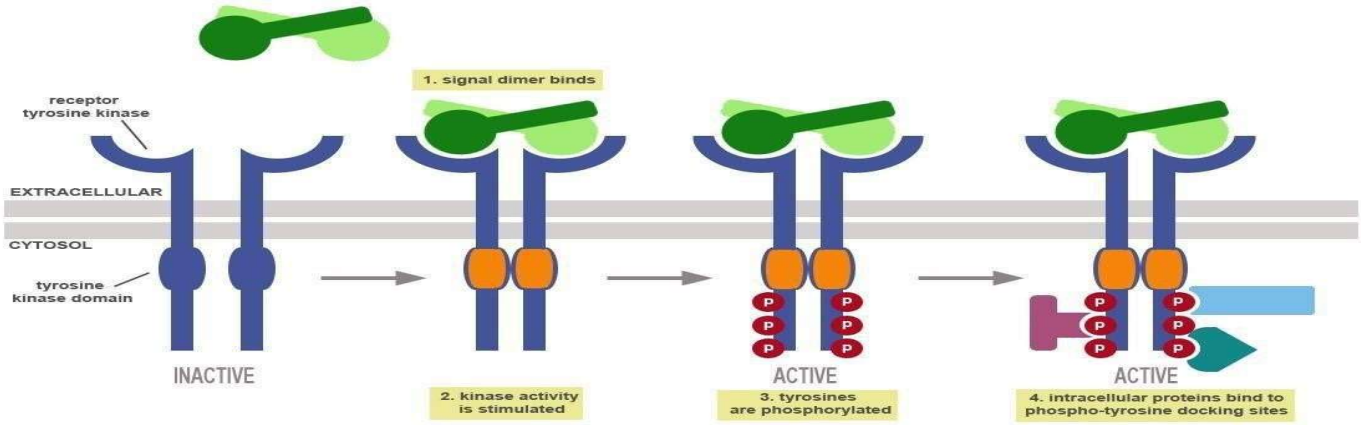
Previously, we've talked about receptors like 7 transmembrane helix that binds to G protein then the signaling pathway either goes to adynelate kinase or phospholipase . Another receptor that works as a a second messenger that is called tyrosine kinases

Tyrosine kinases involve dimerization. They function as dimers. The receptors differ from each other as they belong to different classes: either dimers, like the insulin receptor (which has a disulfide bond), or monomer receptors, where the hormone binds to its receptor, causing conformational changes that result in two receptors attaching to each other with the hormone between them.



Second messengers receptor tyrosine kinases When activated (dimer)→ tyrosines on target proteins:

- o Alterations in membrane transport of ions & amino acids & the transcription of certain genes.
- o **Dimerization is necessary but not sufficient for activation (kinase activity). o Phospholipase C is one of the targets. o Insulin-sensitive protein kinase: activates protein phosphatase 1.**



SIGNAL TRANSDUCTION THROUGH TYROSINE KINASES

Growth hormones:

- o **Epidermal Growth Factor**
- o **Platelet-derived growth Factor**
- o **GH**
- o **Insulin**

Hormone Binding

↓

Dimerization of the receptor

↓

Auto phosphorylation of the receptor

↓

Phosphorylation of the target proteins

Dimerization is required but it won't give you full activity,

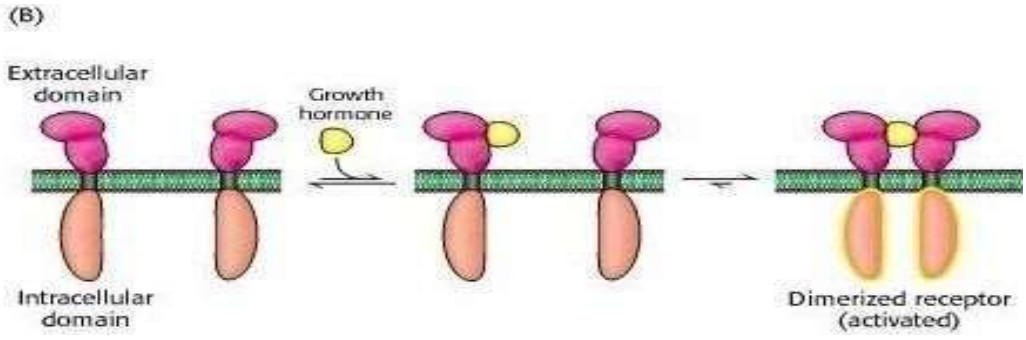
Receptor tyrosine kinase which causes phosphorylation of the tyrosine.

To make Dimerization the two monomer should be close to each other and phosphorylate each other on specific tyrosine residues then it becomes active.

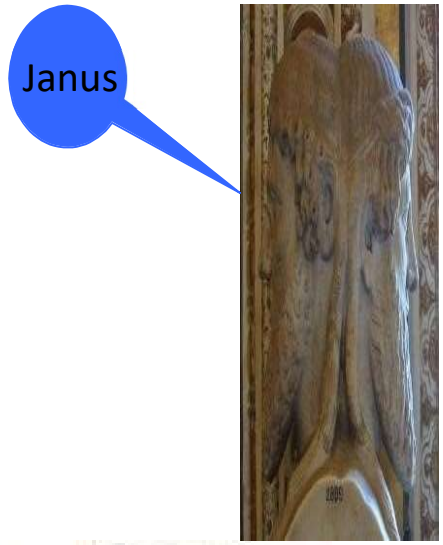
In tyrosine dimer the hormone causes conformational changes that allows this action to happen

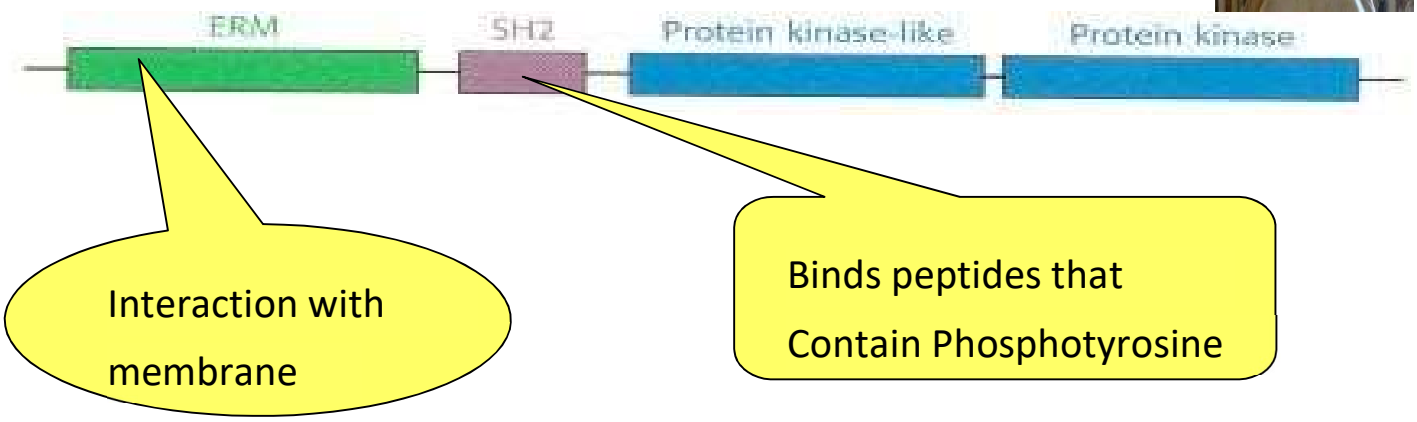
GROWTH HORMONE DIMERIZATION

Binding of one molecule of growth hormone→ Dimerization of the receptor.



Each Intracellular Domain is associated with a protein kinase called Janus Kinase 2 "JAK"(works as a dimer).

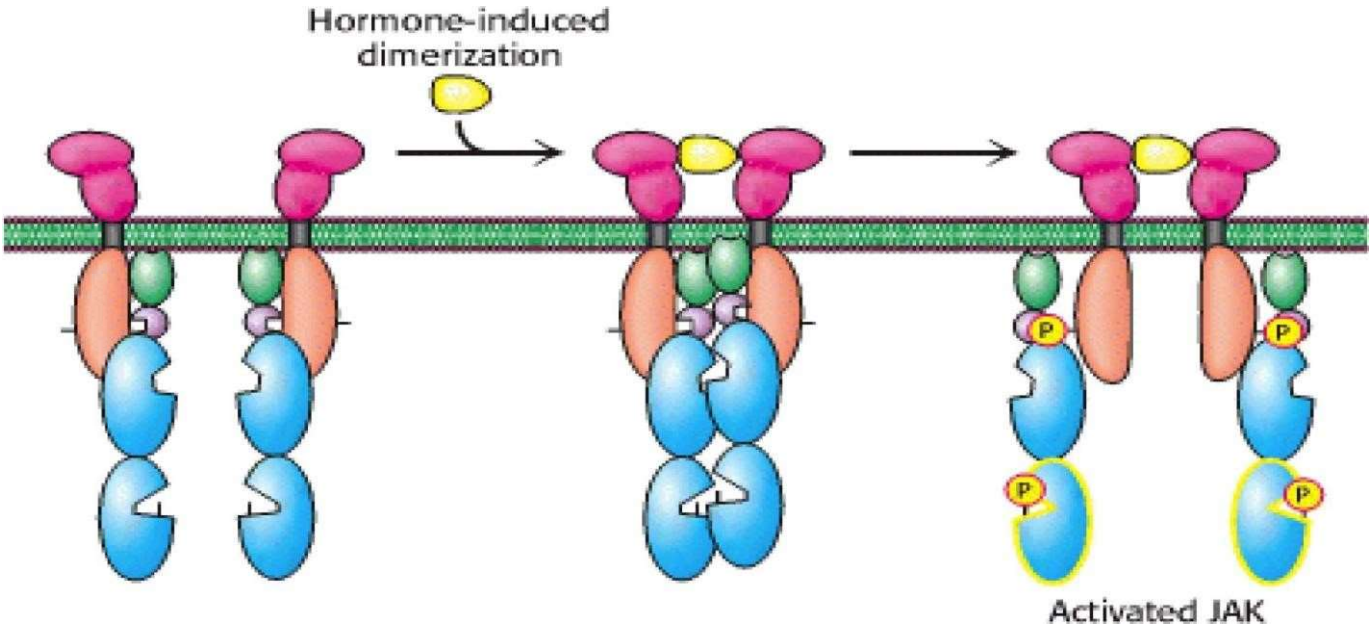




RECEPTOR DIMERIZATION BRINGS TWO JAKS TOGETHER EACH PHOSPHORYLATES KEY RESIDUES ON THE OTHER

Note the structure (JAK in blue).

- JAK hangs on receptor tyrosine kinase.
- Green and purple ones are associated with JAK.
- When the two receptors become close to each other they cause auto and cross phosphorylation for themselves which induces a message also for the JAK to become phosphorylated then the STAT molecules attach to JAK



ACTIVATED JAK2 CAN PHOSPHORYLATE OTHER SUBSTRATES

- - STAT (Signal Transducers and Activators of Transcription) proteins. Their destination is the genes, where they cause transcription. o

Where does transcription happen? Nucleus, obviously!

o Transcriptional factors also work through a dimerization process. ○ Regulator of transcription. ○ STAT Phosphorylation. (one of the main JAK phosphorylation targets)

o STAT has tyrosine residues (phosphorylation), SH2 domain (JAK-binding) and DNA-binding domain; once they are present as a dimer, they can sit on DNA and work on increasing synthesis of certain mRNAs. ○ Dimerization.

o Binding to specific DNA sites.

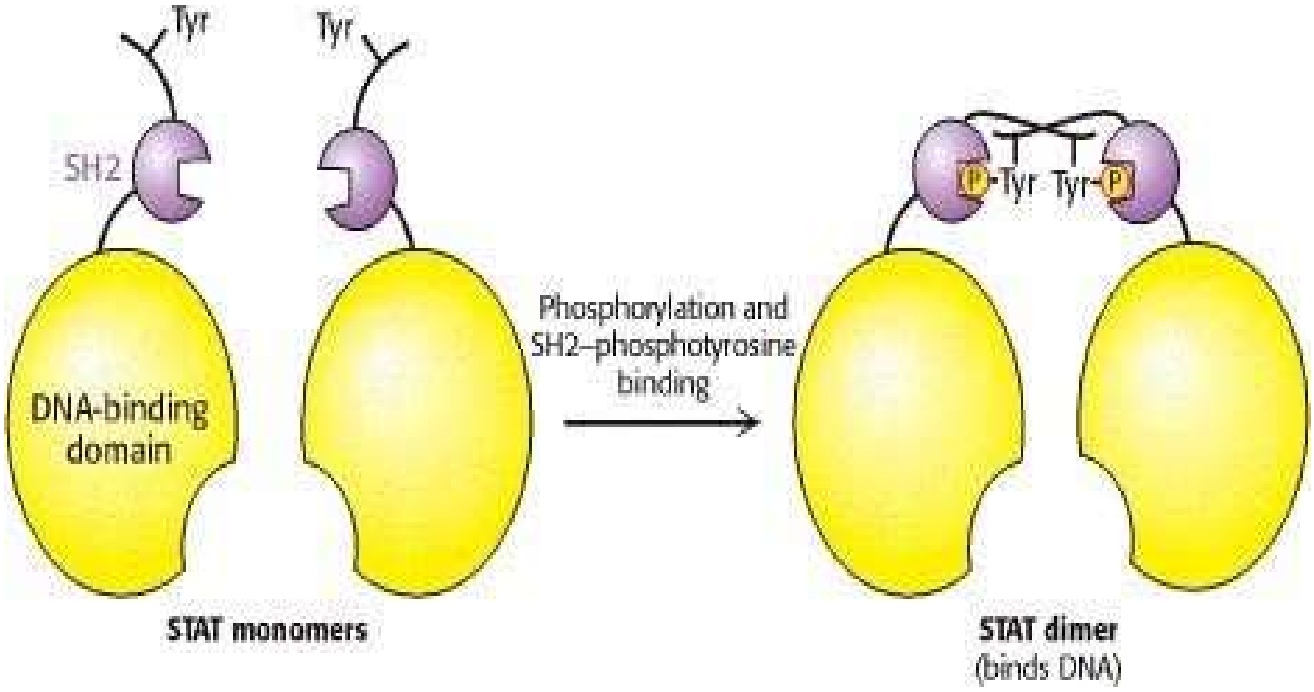
If JAK2 remains active, it will produce Cancer.

The JAK-STAT pathway is a common pathway in many cells.

Wherever there is tyrosine to be phosphorylated, it must contain an SH2 domain (Src homology 2 domain), which is present in receptors, JAKs, and STATs.

STAT is phosphorylated on a tyrosine residue
near the carboxyl terminus.

Phosphorylated Tyr binds to SH2 domain of another STAT molecule.



Look at the structure carefully!!

Once Tyr is phosphorylated they fit their places They

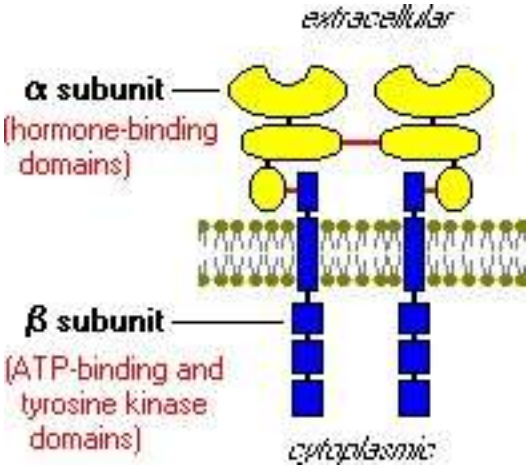
works only in dimer form.

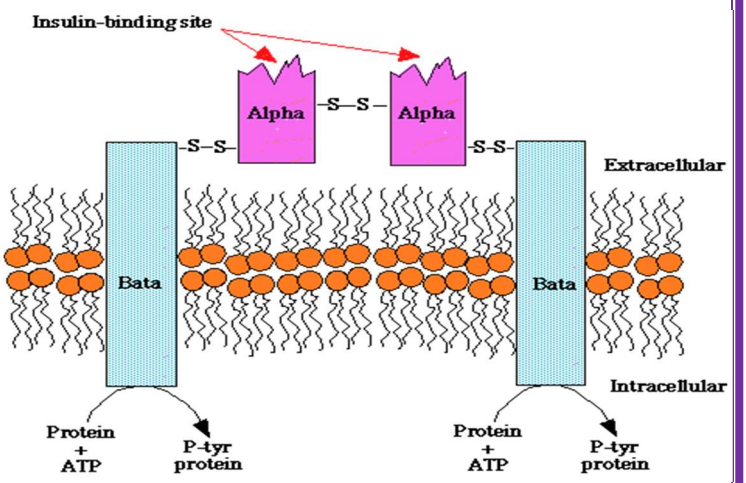
The DNA binding domain responsible for transcription

Tyr kinase and other hormones

INSULIN RECEPTOR (CLASS II RECEPTOR)

- Tetramer (2a; 2β), dimer (2aβpairs)
- Disulfide bridges
- Insulin Binding→ Activation of the Kinase

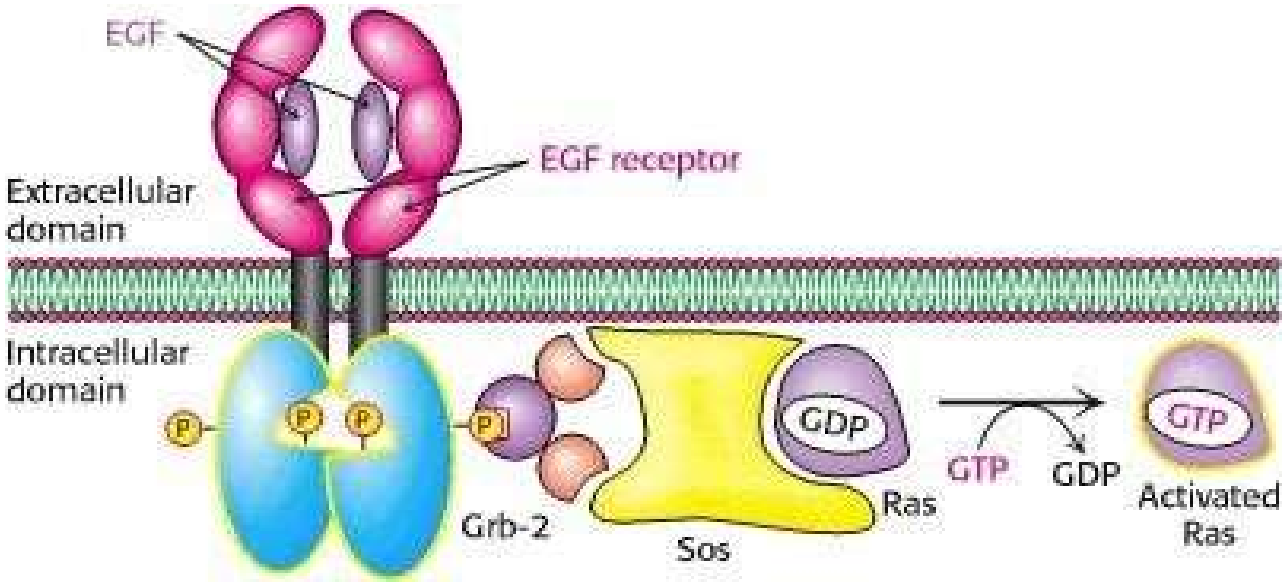




RAS IS A MEMBER OF SMALL G PROTEINS FAMILY

There are monomeric G proteins such as RAS which function just like alpha subunit in G proteins.

- Monomeric
- 2 forms: GDP↔ GTP
- Smaller (1 subunit, unlike other G-proteins)
- GTPase activity
- Many similarities in structure and mechanism with Ga
- Include several groups or subfamilies
- Major role in growth, differentiation, cellular transport, motility etc…
- It works(it has an effect)cytoplasmic or nuclear at the end
- The trimeric G proteins they bind to the membrane while the monomeric the move freely inside the cell



Impaired GTPase activity can lead to cancer in human

Mammalian cells contain 3 Ras proteins

Mutation →

Loss of ability to hydrolyze GTP →

Ras is locked in "ON" position →

continuous stimulation of growth