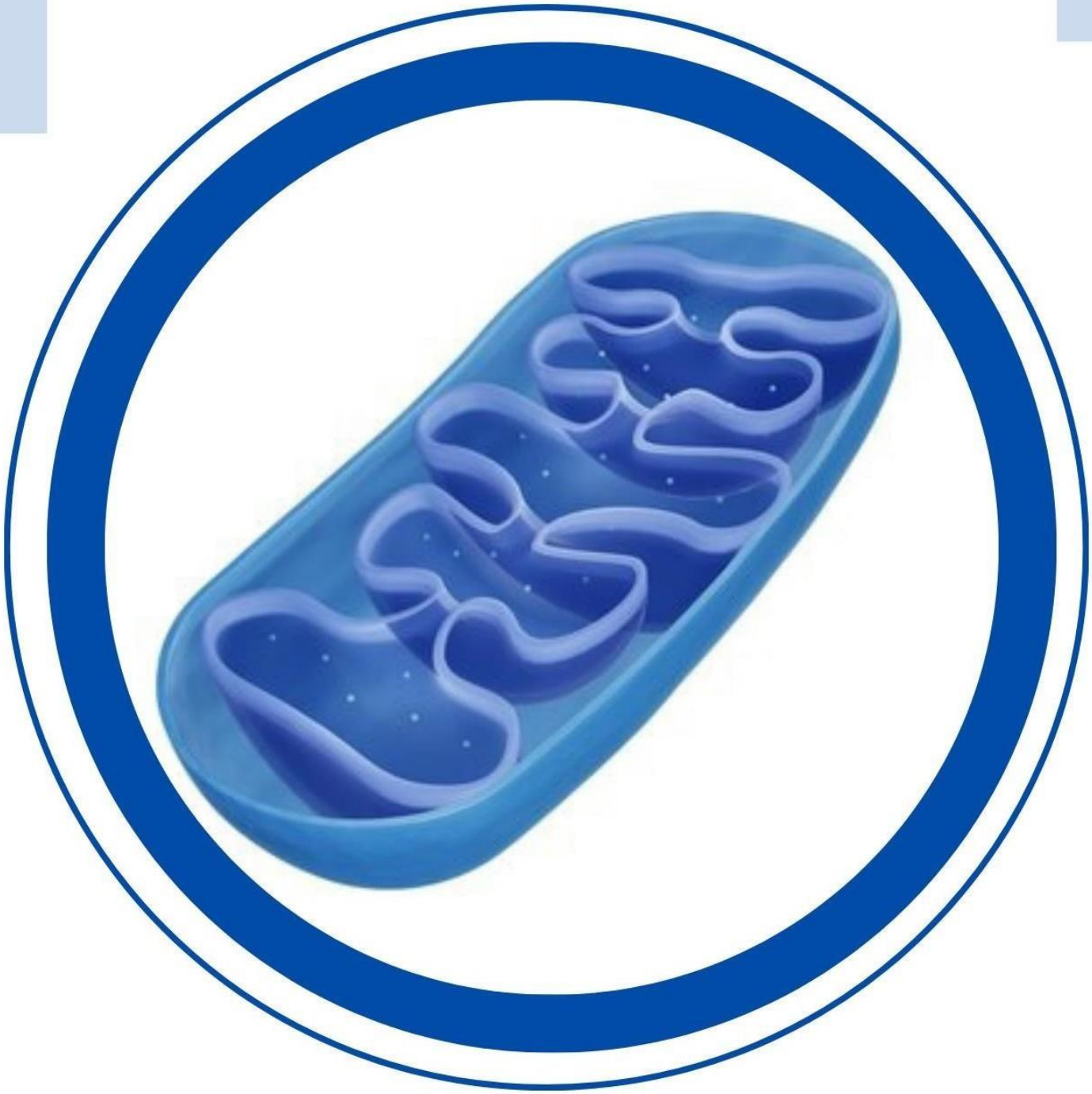
ENDOCRINE

METABOLISM

#3



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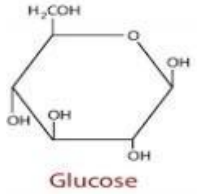
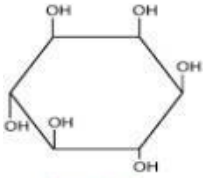
- A few notes before we start:
- 7-transmembrane helix receptors can activate adenylate cyclase but this enzyme is not their only choice (They can activate other enzymes including PLC –it works on a membrane lipid-by Gaq).
- Having a mutation that prevents the hydrolysis of GTP in the alpha subunit of the G protein increases the activity of the enzyme and can lead to cancer.
- The 4 ways that function in switching off the hormone signaling process of the G protein work together because the effect of the signal is huge.



Glucose

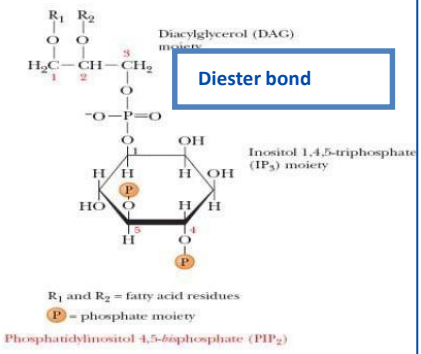
- We all should know the structure of glucose.
- Inositol is also a six-carbon unit molecule, but the ring doesn't contain an oxygen atom, and on every carbon unit there is a hydroxyl group (remember: inositol→ alcohol).



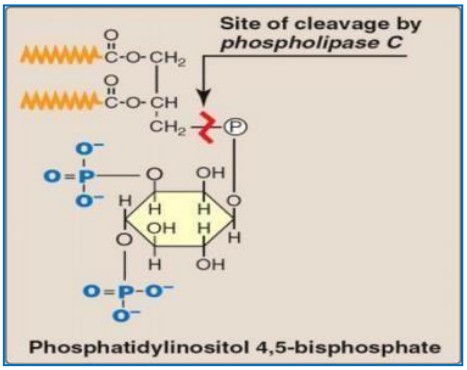
Inositol

- Recall: lipids are two types
  - 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).
  - 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.

- Phosphatidyl-inositol 4,5-bisphosphate is a membrane lipid, it is a phosphatidyl-inositol molecule with two phosphate groups at different locations-called bisphosphate- (at carbon no° 4 and no° 5). It is a substrate for phospholipase C.



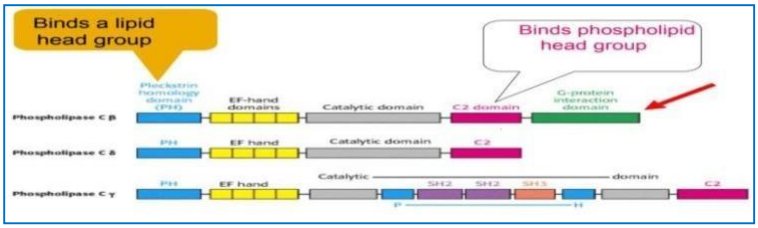
8.3 THE PHOSPHOINOSITIDE CASCADE

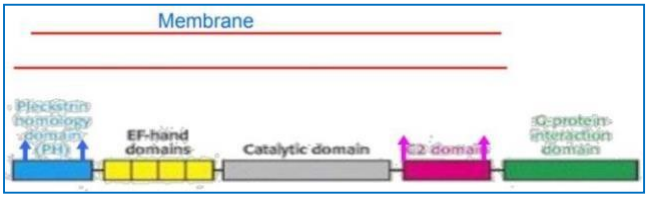


- **Used by many hormones (e.g. ADH)**
- The steps of the reaction:
  - **Binding of a hormone to 7TM receptor**
  - **Activation of G Protein** (Gaq)
  - **Activation of Phospholipase C (many isoforms) – PIP2**, which will cut the Phosphatidylinositol-4,5bisphosphate molecule between the third carbon of glycerol and the phosphate group (note the red line on the picture).
  - **Two messengers are produced**
- **Inositol 1,4,5-trisphosphate** (three phosphate groups on different locations)**, hydrophilic, (**highly **Soluble),** so it is going to function in the cytosol.
  - **IP3 is the actual second messenger**
- **Diacyclglycerol, amphipathic (membrane)** (the remaining glycerol backbone with two fatty acid)

THE DOMAIN STRUCTURE ISOFORMS OF PHOSPHOLIPASE C

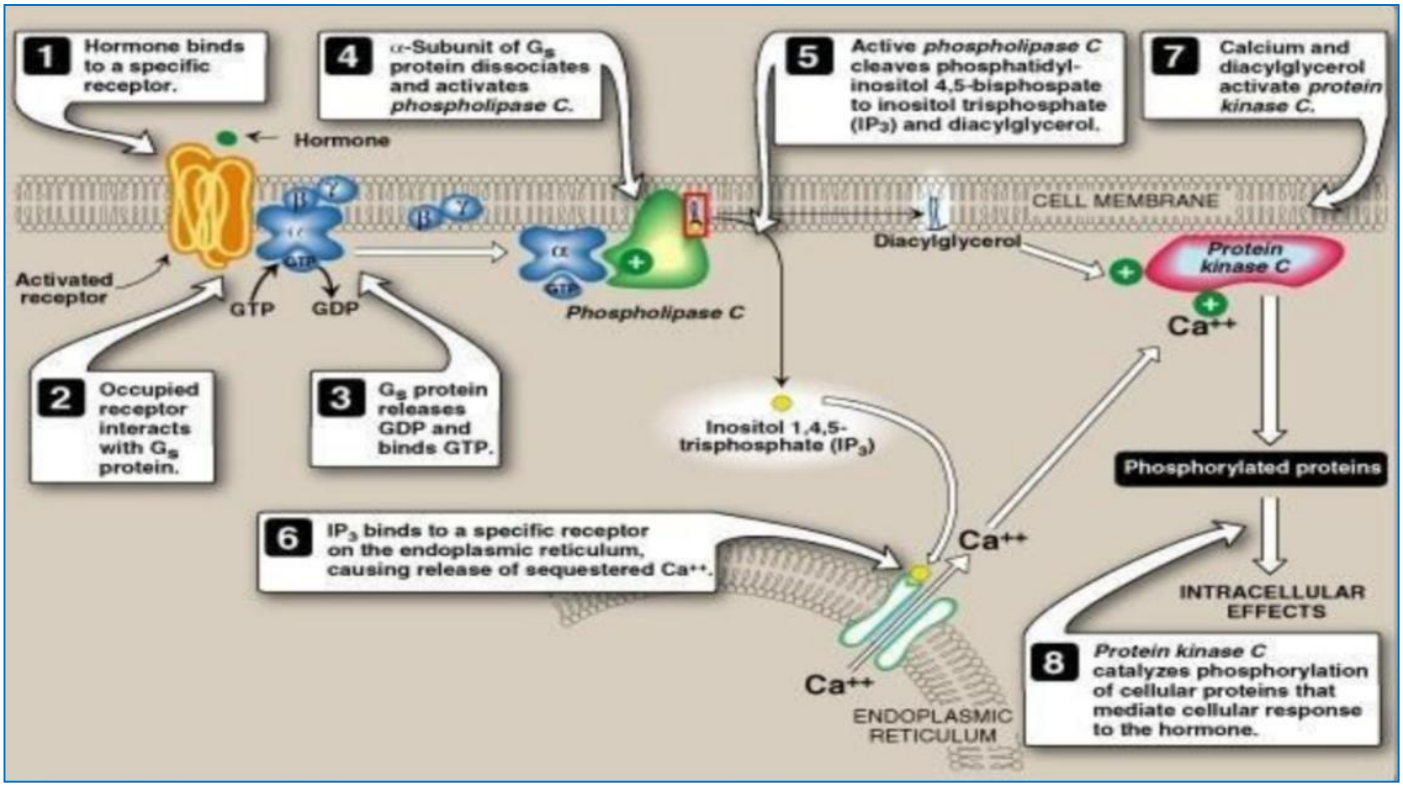
- Phospholipase C has multiple domains; G-protein binding domain (for binding Gaq), catalytic domain (for binding the substrate and performing the reaction), C2 domain (to bind the phospholipids), pleckstrin homology domain which is a membrane binding domain (since it is a membrane associated protein) and Ef domain (we'll get to it later on).
- This enzyme has afferent isozymes. However, we are interested in the β isoform since it is the only one that has a G-protein binding domain.





BINDING OF A G PROTEIN BRINGS THE ENZYME INTO A CATALYTICALLY ACTIVE FORM

Please, study the following figure and its details carefully.

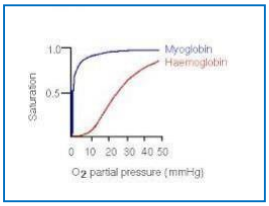


- Few notes on the figure:
  - 1-The hormone binds to a surface (Extracellular) receptor.
  - 4- G-protein is a trimeric protein.
  - 6-Endoplasmic reticulum (or sarcoplasmic reticulum in muscles) has high concentration of calcium. Also, the membrane of endoplasmic reticulum has high number of proteins to transport Calcium ions in and out (actually 80% of the membrane is engaged in this process).
  - Calcium now has two possible destinations: 1- protein Kinase C, 2- Calcium binding proteins (calmodulin is one of them)
  - Kinases are named after the molecules that activate them; PKA→ cAMP, PKC →Calcium.
  - Calcium binding proteins are inactive proteins that are activated by the binding of Calcium which causes conformational changes.

EFFECTS OF SECOND MESSENGERS

➢ INOSITOL TRISPHOSPHATE (IP3)

- **Opens Calcium Channels**
- **Binding to IP3-gated Channel**
- **Cooperative binding (sigmoidal):** Binding of IP3 to its receptors on the membrane is cooperative (like the binding of Oxygen to hemoglobin), a receptor has to bind to 4 IP3 molecules for full activation. If three molecules bind the receptor instead, they will do the same job (enough response). However, if 1 or 2 molecules bind the receptor, there won't be enough release of Calcium as the receptors are not opened enough (same thing for Oxygen and hemoglobin, 2 molecules are less than 50P of the curve -remember the sequential model-).
- P.S: <u>The full opening</u> of the calcium channels isn't achieved unless the channel is bound to 4 IP3 molecules.
- REMEMBER: Cooperative binding occurs if the number of binding sites of a macromolecule that are occupied by a specific type of ligand is a nonlinear function of this ligand's concentration -Google.

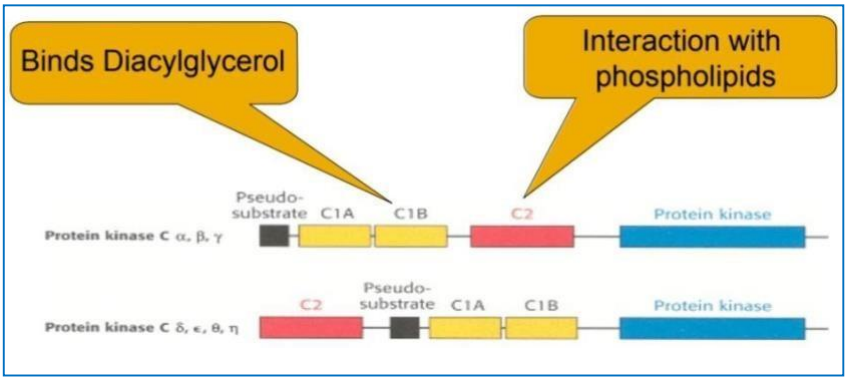


➢ DIACYLGLYCEROL (DAG)

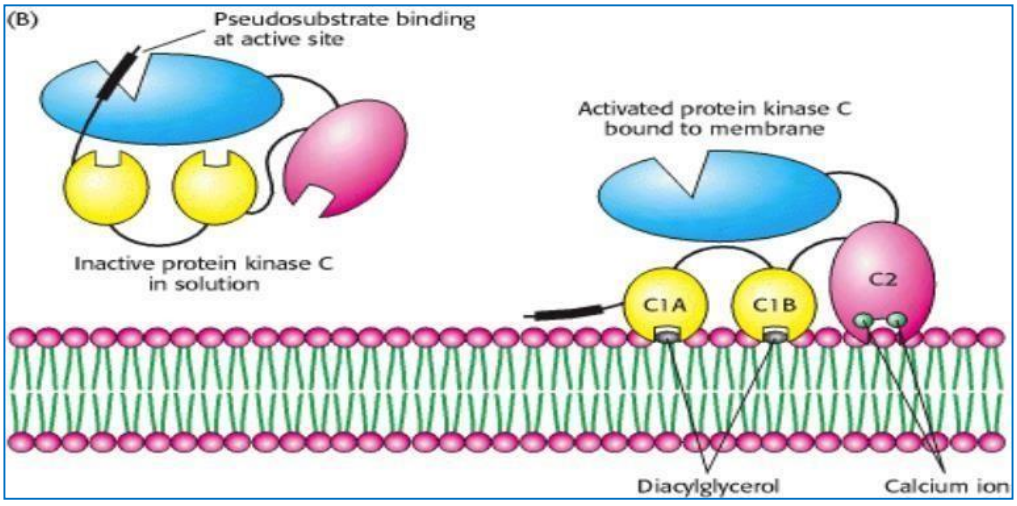
- **Activates Protein Kinase C**
- **Ca+2 is required**
- **Phosphorylation of many target proteins**
- Protein Kinase C is a membrane bound enzyme. To be fully activated, it requires: 1- binding with Diacylglycerol (produced by PLC), 2- binding with calcium.
- Since it is a kinase, it is going to phosphorylate a large number of proteins and enzymes downstream to that signal, activating some of them and inhibiting the others.
- The activated/inhibited proteins may be transcriptional factors; once they are activated, they are going to be translocated to the nucleus causing gene expression.

THE DOMAIN STRUCTURES OF PROTEIN KINASE C ISOFORMS

- PKC domains: catalytic domain, membrane binding domain, DAG binding domain (C1), calcium binding domain.



- How does binding with DAG activate the enzyme?
  - The enzyme has C2 domain that can bind the membrane (has high affinity to it) and Calcium. It is close to the membrane (notice the figure).
  - Once calcium is released, it becomes closer and binds to the membrane.
  - Once DAG (that is produced from PIP2 by PLC) binds to its domain, it causes the flipping of C1A and C1B domains towards the membrane.
  - Flipping of these domains causes the arm that is originally blocking the active site to move away, resulting in the activation of the receptor.



- When the enzyme is not bound to DAG, this arm causes the deactivation of the enzyme.
- But how can this arm bind to the active site? Because, it looks similar to the substrate, so it has affinity to the binding site.

PSEUDO-SUBSTRATE SEQUENCE

- The arm **resembles the substrate sequence: A-R-K-G-A-L-R-Q-K**
- **Substrate Sequence: A-R-K-G- (S** or **T)-L-R-Q-K**
  - Remember: S and T are the sites for phosphorylation (the activity of the enzyme); so any substrate should contain one of these amino acids to get phosphorylated.

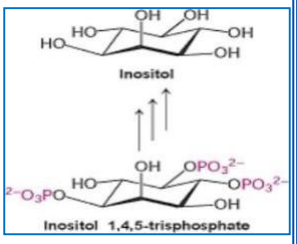
G	Glycine	Gly	P	Proline	Pro
A	Alanine	Ala	V	Valine	Val
L	Leucine	Leu	I	Isoleucine	Ile
M	Methionine	Met	C	Cysteine	Cys
F	Phenylalanine	Phe	Y	Tyrosine	Tyr
W	Tryptophan	Trp	H	Histidine	His
K	Lysine	Lys	R	Arginine	Arg
Q	Glutamine	Gln	N	Asparagine	Asn
E	Glutamic Acid	Glu	D	Aspartic Acid	Asp
S	Serine	Ser	T	Threonine	Thr

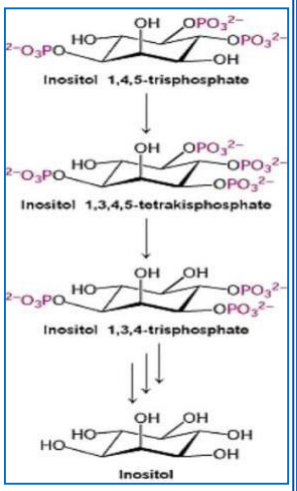
From Google

- Note that the arm has the same sequence of the substrate but with replacement of S and T with alanine. By this, the arm will have the same affinity for the active site, however, the enzyme won't be able to phosphorylate the alanine.
- **Binds to the Enzyme's Active Site**
- **Competitive Inhibitor**

TERMINATION OF IP3 SIGNAL

- Same as adenylate cyclase: the release of the hormone from the receptor, desensitization, GTA hydrolase or by inactivation of IP3.
- It can be inactivated by two mechanisms:
  1. Phosphatases that remove the phosphate groups from IP3 molecule, one phosphate group at a time (from carbon no° 1, 4, 5), producing inositol again. However, this process takes longer time.
  2. Adding another phosphate group on carbon no° 3, producing 1,3,4,5-tetrakisphosphate (which is inactive) to ensure that the molecule will be inactivated immediately. Then, the phosphate groups are removed one by one starting from the original ones (not the one that was added lastly), accordingly, it is not an IP3 molecule anymore and therefore it won't exert any function

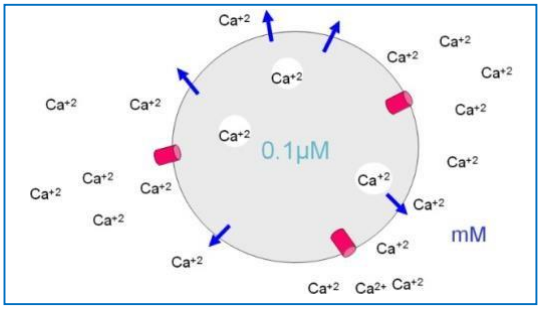




- **IP3 is a Short-Lived Messenger.**
- **Lithium ions, used to treat some psychological disorders** because it **inhibits IP3 recycling** by preventing the release of the phosphate groups.
- It helps to treat depression because higher IP3→ higher metabolism.

WHY CALCIUM

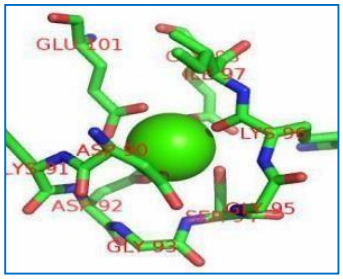
- **A large difference in concentration**

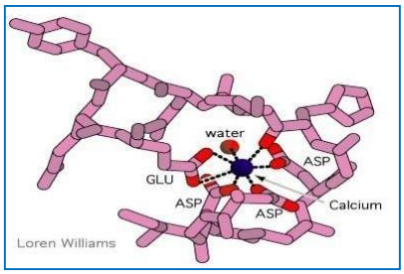


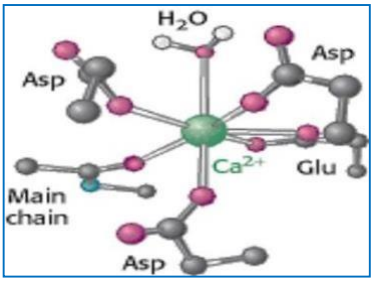
- The concentration difference between inside and outside -with respect to the ER- is huge, so releasing small amounts of calcium is going to cause a great effect.
- similar to protons and mitochondria, the concentration difference between the matrix and the intermembrane space is huge; so when small amounts of protons go back to the matrix, there will be ATP generation due to the high gradient.
- Does this mean that this gradient will fade after calcium is released? No, because there is a very high concentration and therefore not all calcium ions are going to be released.
- This is why huge concentration difference is very effective, we need only small amounts of the atom or molecule to be released in order to cause the required effect.
- 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

- **Ability to bind proteins tightly** since it is positively charged.
- **6-8 bonds with oxygen:** Calcium can make up to eight bonds, either with water, side chains of amino acids or oxygen.
- **Conformational changes:** Calcium by nature is bulky, so when it binds to proteins, it can easily cause conformational changes.

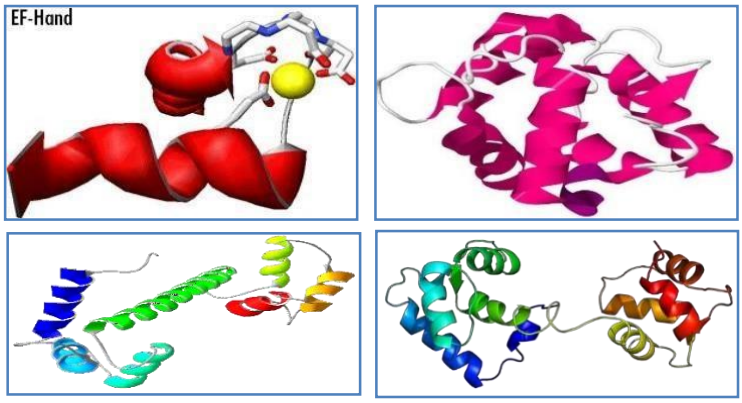






CALCIUM BINDING PROTEINS

- **Mediate the effects of Calcium (Ca^{+2})**
- **Many proteins** (get affected by calcium and undergo change in conformation)
- Examples: **Calmodulin, Troponin C, Parvalbumin**.
  - Parvalbumin (1st one discovered): a single subunit protein, composed of a helices, labeled A through F, between helix E and F there is high calcium binding affinity. Helix E and F are perpendicular to each other, and calcium sits in between them (in the loop region which connects the 2 helices together).

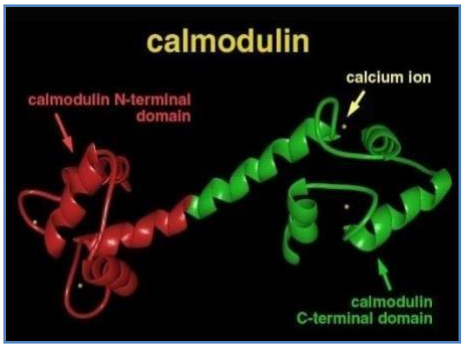


- **Similar structures**
  - **Rich in Asp and Glu** (charged AA)
  - **Gln, Asn, Ser** (polar AA)
  - **Several a helical segments**
  - **-=Binding site is formed by:**
    - **Helix Loop Helix** (AKA EF-hand; Ca-binding site).
    - **Super-secondary structure**

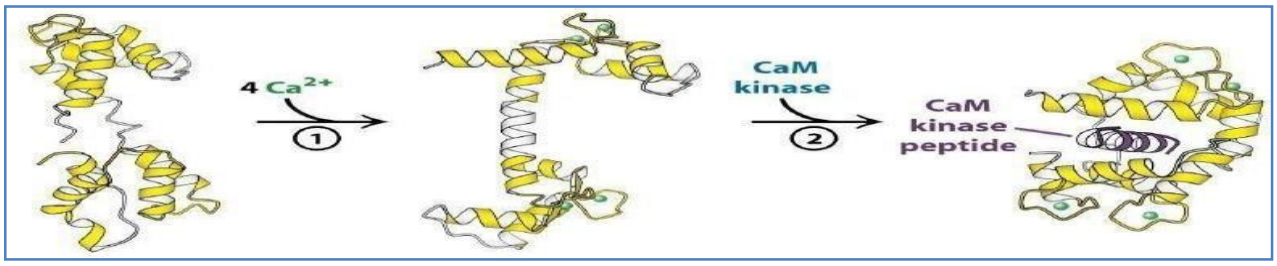
CALMODULIN ≈ 17 KD

149 amino acids

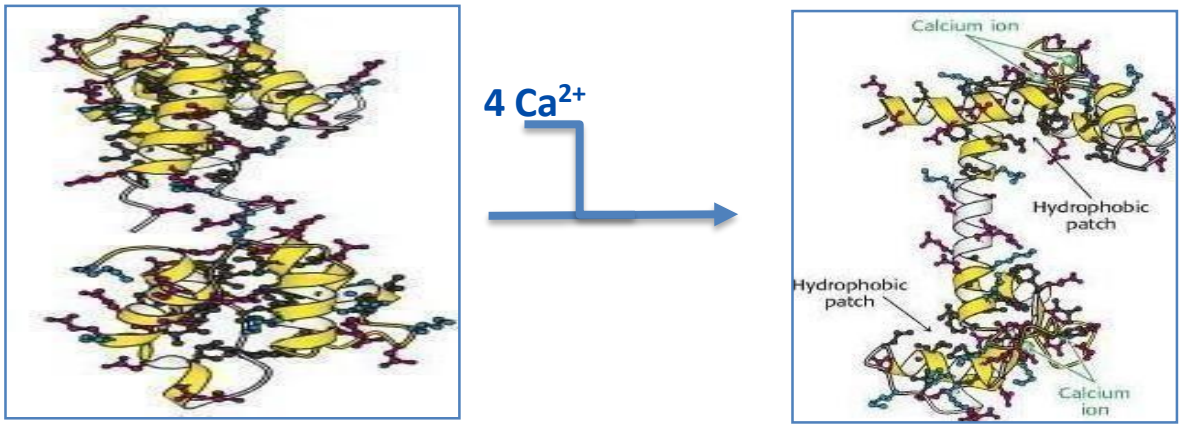
- **Found in almost all eukaryotes.**
- **Consists of two globular regions**
- **Connected by flexible region**
- **Each contains 2 EF hands, hence, four Ca^{+2} binding sites.**



- **Calcium-Calmodulin complex can bind to a large number of target proteins including: Calmodulin-dependent protein kinase, Ca^{2+} ATP'ase Pump** (which is present on SER membrane)

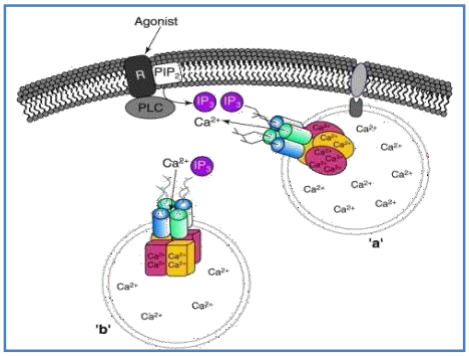


- Calcium activates calmodulin which activates ATPase pump to return Ca^{2+} to SER and stop signaling.
- **Calmodulin binds to Ca^{2+} which results in change in conformation (Moving some hydrophobic residues from the inside to the outside of the domains).**



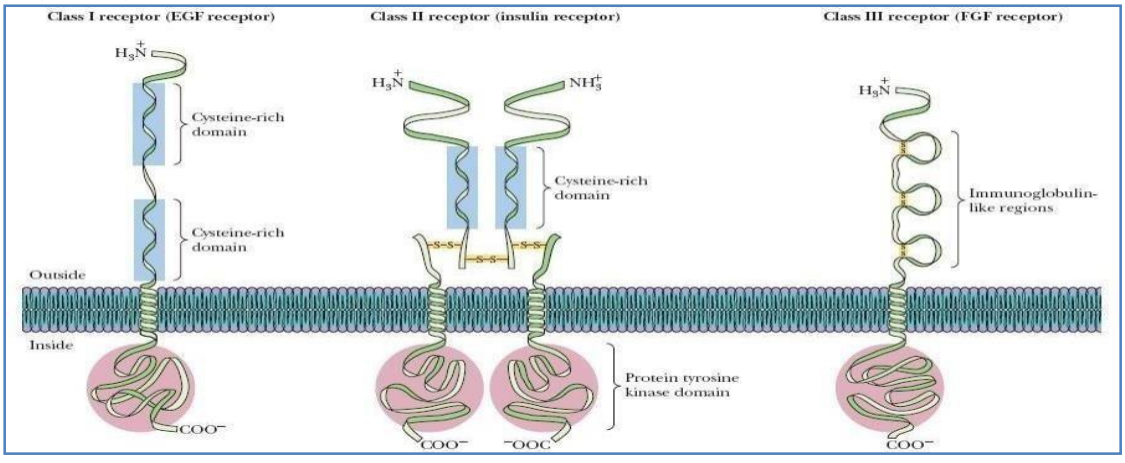
CALCIUM TRANSPORTER

- The main function of sarcoplasmic reticulum is to store calcium. You don't need exorbitant amounts of calcium in the cytoplasm for cellular functions to occur sufficiently; calcium is already present in the cytoplasm in small amounts. Moreover, returning extravagant amounts of calcium back to the SER is energy expensive (2 Ca^{+2} / ATP).
- **Thus, 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,** energy-expensive process**)**
  - **Depletion of ATP leads to tetany, Rigor mortis.**

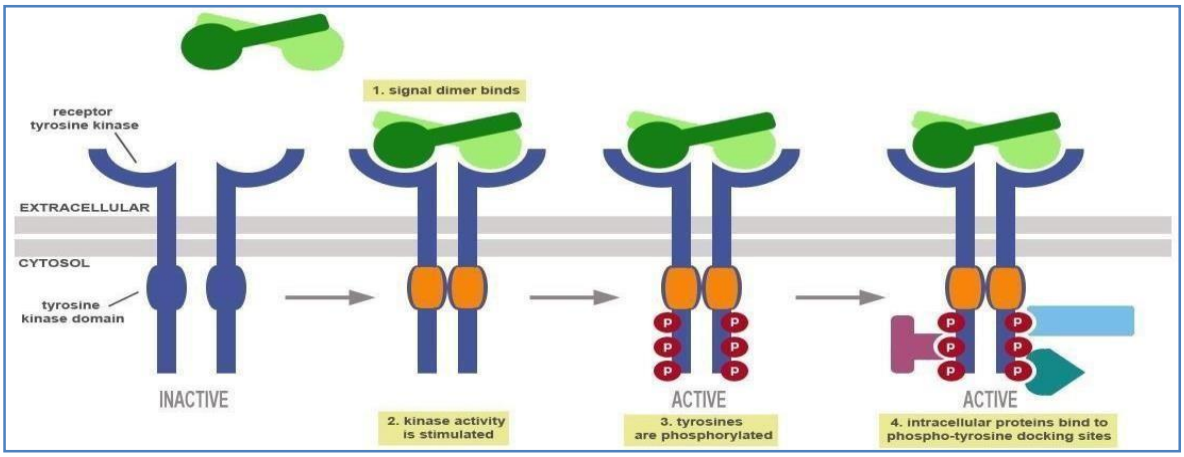


8.4 RECEPTOR TYROSINE KINASES CASCADE

- Previously, we've talked about hormones that bind to the 7 transmembrane helices receptor and perform their function through second messengers. Some hormones don't bind to the 7 transmembrane helices receptor but rather to receptors which work as enzymes-receptor tyrosine kinases- those receptors are kinases by nature and have the ability of phosphorylating other proteins, enzymes, etc. From its name, you can conclude that it contains tyrosine residues.
- Receptor: a protein, tyrosine kinases: the action of the kinase enzyme is on tyrosine residues.
- **Second Messengers** (no coupled protein; the receptor itself has an enzymatic activity)**.**
- **Span the membrane, several subclasses.**
- Have a cytoplasmic domain, a recognition domain and a spanning domain.
- There must be dimerization in the process; therefore, if you are working with a receptor that is a monomer by nature, you need another one just like it (which is also a monomer) to perform dimerization. Classes such as I & III are found as monomers while II (insulin receptor) is found as a dimer.
- The receptor is either found as a dimer and waiting for the hormone or as a monomer and after binding of the hormone it dimerizes with another monomer.
- Activation of the receptors requires both dimerization and binding of the hormone. As a result, the conformation of the enzyme changed (now active) and the monomers containing tyrosine have become close to each other now. The enzyme is a kinase and tyrosine is a site of phosphorylation.



- By activation we mean the activation of the kinase function and phosphorylation of tyrosine residues. Both Auto-phosphorylation and cross-phosphorylation happen (Auto-phosphorylation: same chain, cross-phosphorylation of the dimer chain).
- Source of phosphate is ATP. ATP then leaves as ADP.
- Phosphotyrosine binds a specific domain which is the SH2 domain (Src homology 2) of other proteins in the tyrosine kinase cascade.
- Tyrosine that has phosphate is a kinase which is going to phosphorylate other enzymes or molecules downstream of the signaling process.
  -Couldn't find the same video, enjoy this one instead :)
- **When activated (dimer)→ tyrosines on target proteins:**
  - **Alterations in membrane transport of ions & amino acids & the transcription of certain genes.**
  - **Dimerization is necessary but not sufficient for activation (kinase activity).**
  - **Phospholipase C is one of the targets.**
  - **Insulin-sensitive protein kinase: activates protein phosphatase 1.**



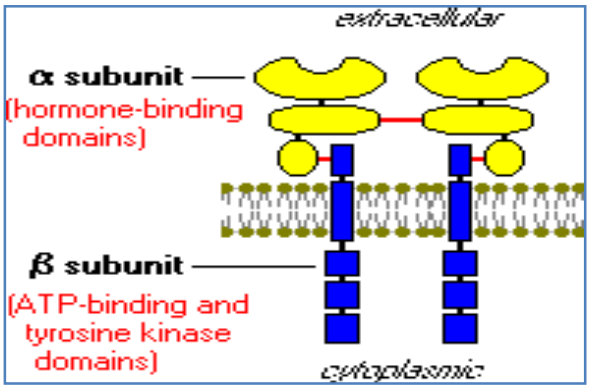
SIGNAL TRANSDUCTION THROUGH TYROSINE KINASES

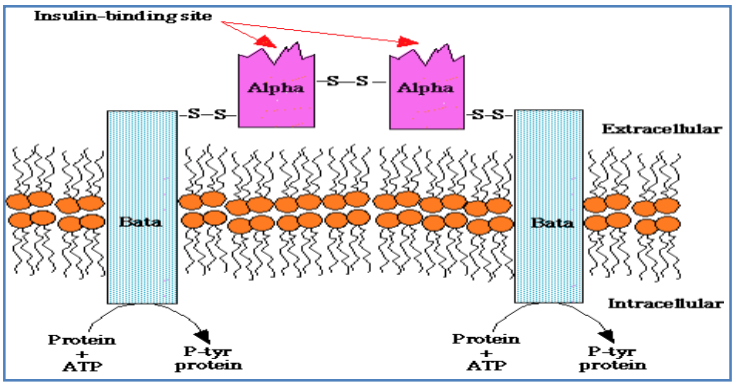
- **Growth hormones:**
  - **Epidermal Growth Factor**
  - **Platelet-derived growth Factor**
  - **GH**
  - **Insulin**

- **So, hormone binding→ dimerization→ auto-phosphorylation of the receptor** and cross phosphorylation**→ phosphorylation of target proteins.**

INSULIN RECEPTOR (CLASS II RECEPTOR)

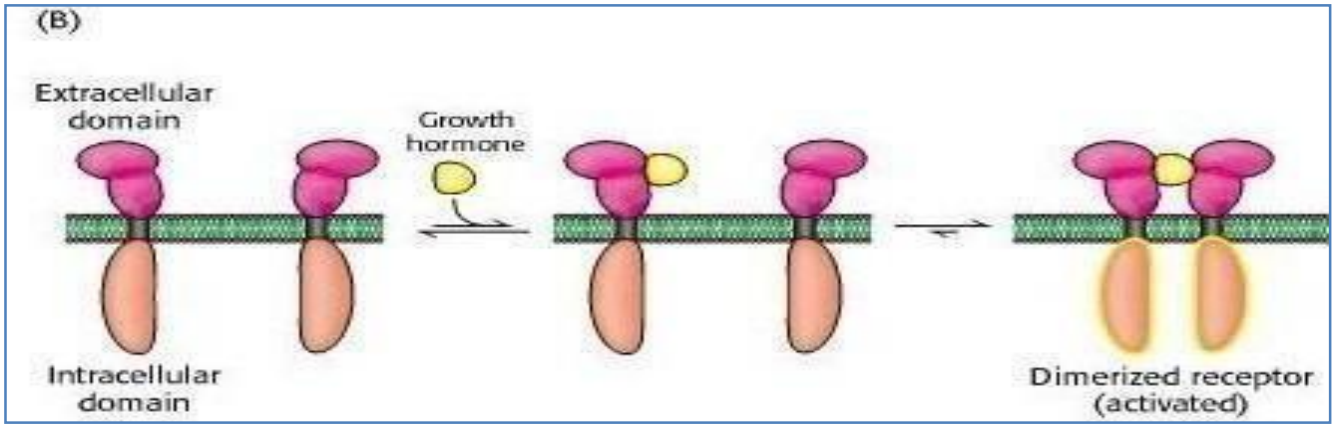
- **Tetramer (2a; 2β), dimer (2aβpairs)**
- **Disulfide bridges**
- **Insulin Binding→ Activation of the Kinase**





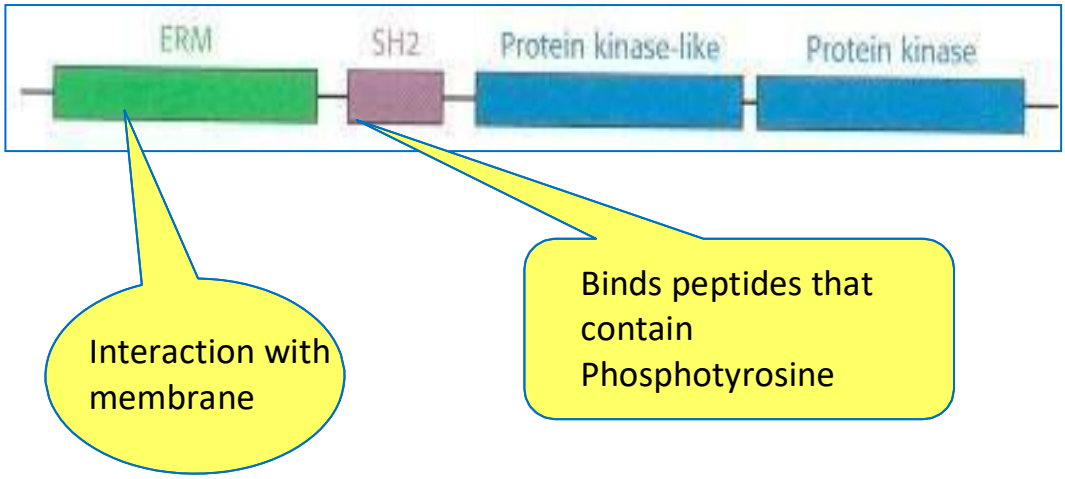
GROWTH HORMONE DIMERIZATION

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



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

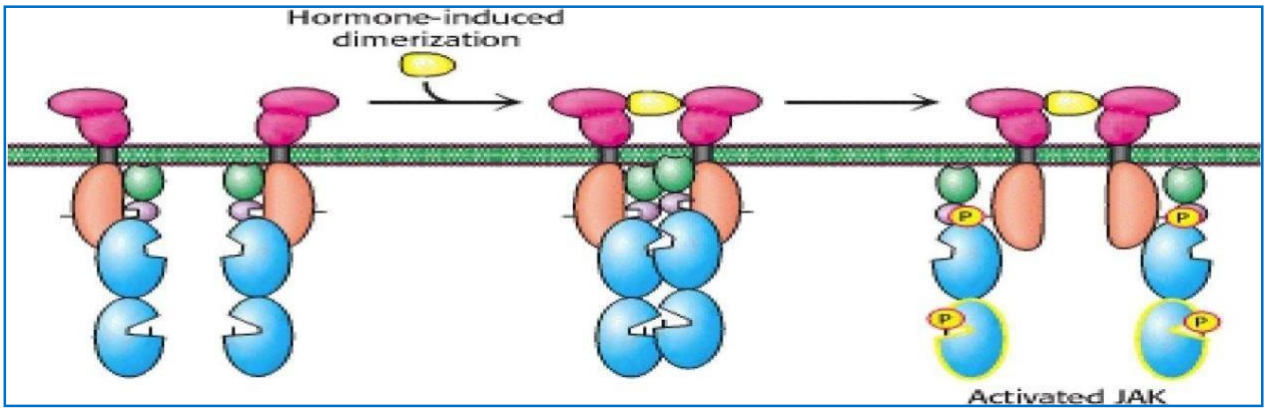
janus



- As expected, it has a catalytic domain (like any enzyme), SH2 domain (phosphotyrosine- binding domain) and membrane-associated domain (to be close to the receptor).

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.

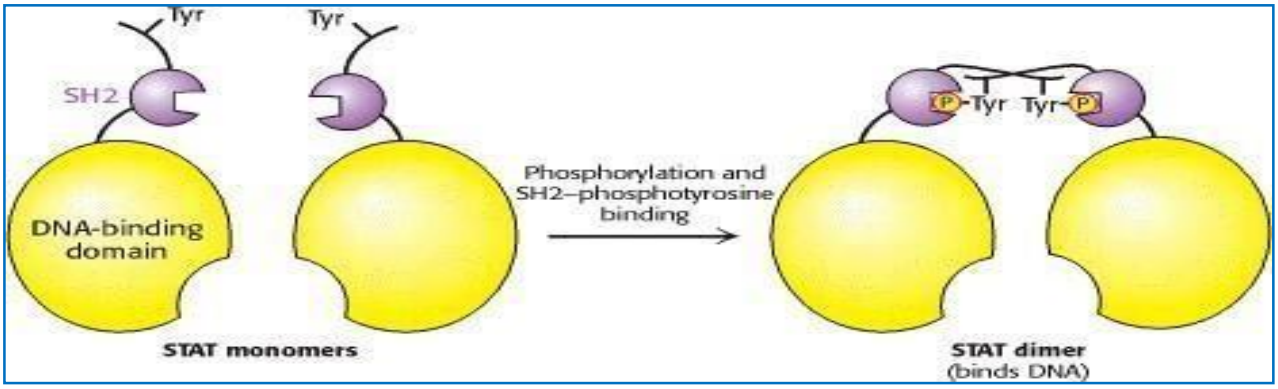


ACTIVATED JAK2 CAN PHOSPHORYLATE OTHER SUBSTRATES

- **STAT (Signal Transducers & Activators of Transcription).**
  - Where does transcription happen? Nucleus, obviously!
  - Transcriptional factors also work through a dimerization process.
- **Regulator of transcription.**
- **STAT Phosphorylation.** (one of the main JAK phosphorylation targets)
  - STAT has tyrosine residues (phosphorylation), SH2 domain (JAK-binding) and DNA-binding domain; once they are present as a

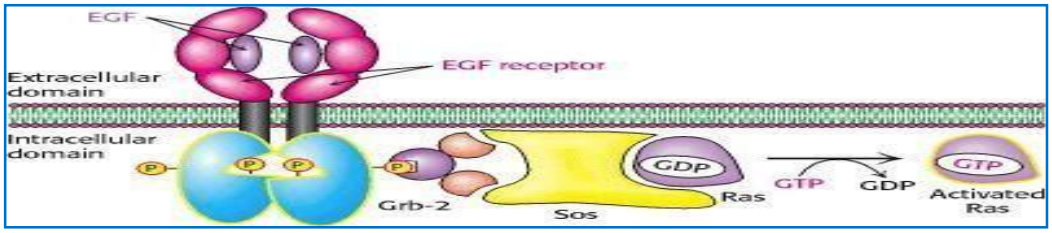
dimer, they can sit on DNA and work on increasing synthesis of certain mRNAs.

- **Dimerization.**
- **Binding to specific DNA sites.**
- **If JAK2 remains active, it will produce Cancer.**
- Hormone binding→ Dimerization → Auto-phosphorylation of the receptor and cross phosphorylation→ Activation of other molecules in the cell.
- The JAK-STAT pathway is a common pathway in many cells.



RAS IS A MEMBER OF SMALL G PROTEINS FAMILY

- Not all activation happens through G proteins. 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…**



IMPAIRED GTP_{ASE} 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** (which can result in cancer).

PAST PAPERS:

1. **All of the following are correct regarding Protein Kinase C except:**
   a. When activated, it phosphorylates specific tyrosine residues.
   b. Binds to membrane phospholipids when activated.
   c. Activated by DAG.
   d. Activated by Ca +2.
   e. Has a pseudo-substrate sequence.

   Answer: A

2. **IP3 "inositol 1,4,5- triphosphate":**
   a. is generated by phosphorylating inositol diphosphate
   b. activates protein Kinase C
   c. its activity can be augmented by phosphorylating it into inositoltetraphosphate.
   d. produced by G protein-activated enzyme **Answer: D**

3. **Tyrosine Kinase Termination is done through**:
   a. Inhibition of binding of the ligand
   b. Endocytosis of receptors by lysosomes
   c. SOS proteins
   d. Phosphatases

   Answer: D

Good luck :)

V2

Highlighted

2 ATP for each calcium X

1 ATP for every 2 Ca ions.

-Link fixed.