Signal-Transduction Cascades - 2

- The Phosphoinositide Cascade
- Calcium ion as a second messenger
- Tyrosine kinase and receptor dimerization

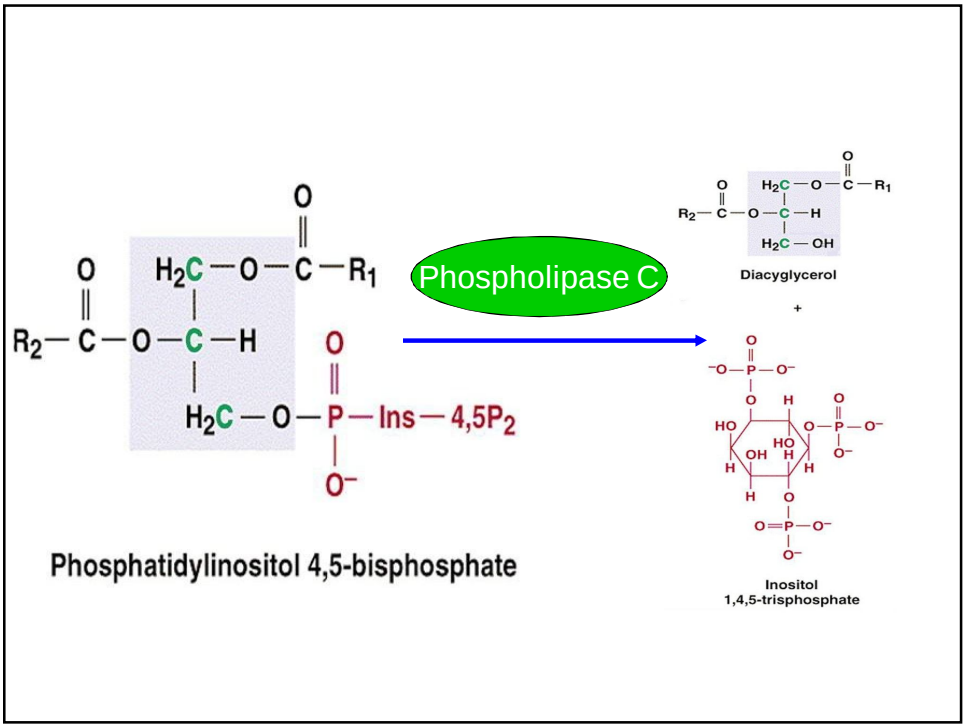
The Phosphoinositide Cascade

- Used by many hormones
- Binding of a hormone to 7TM receptor

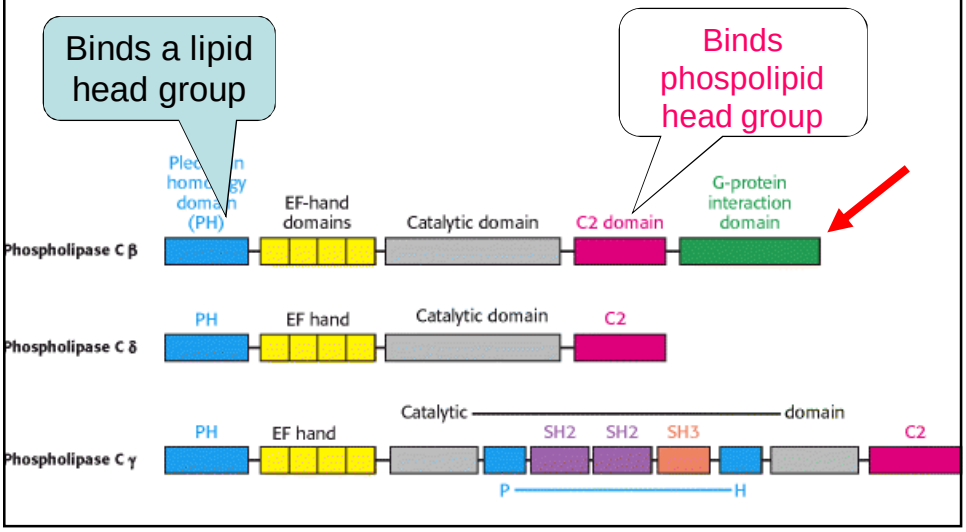
Activation of G Protein

Activation of Phospholipase C

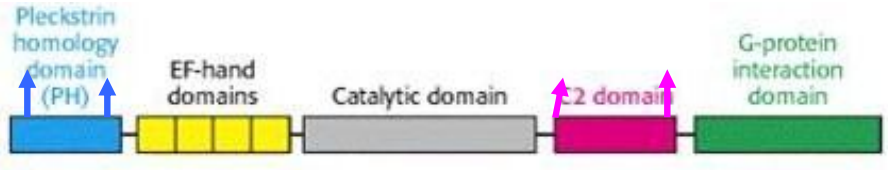
- Two messengers are produced
  - Inositol 1,4,5-trisphosphate (Soluble)
  - Diacyclglycerol (Stays in the membrane)



The domain structures of three isoforms of phospholipase C



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



Effects of Second Messengers

Inositol trisphosphate

Opens Calcium Channels

Binding to IP_3-gated Channel

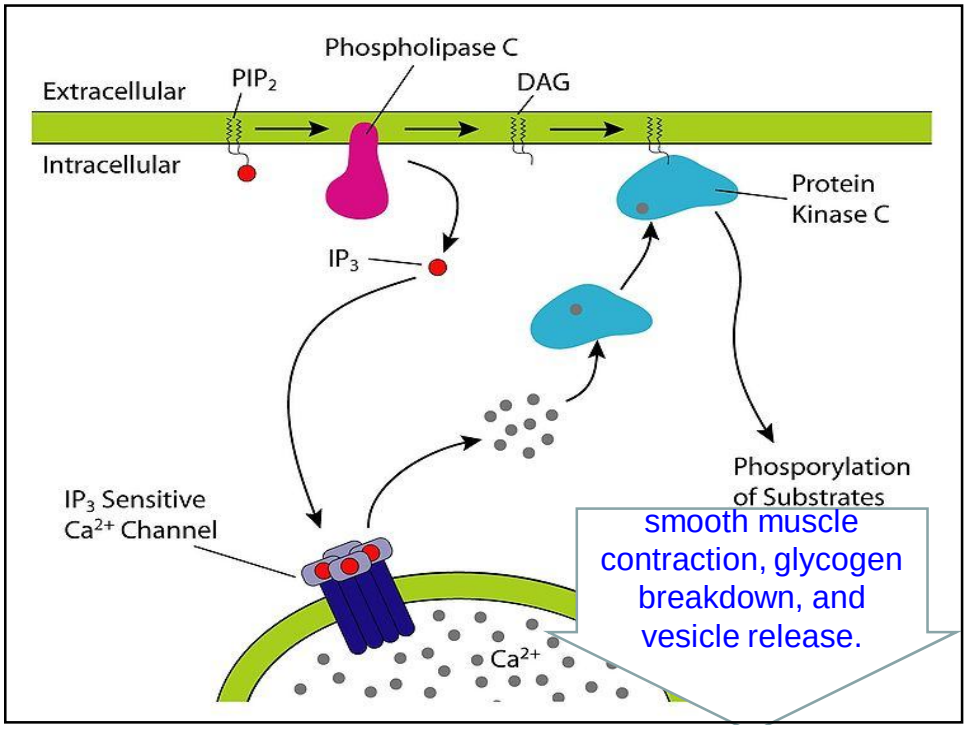
Cooperative binding

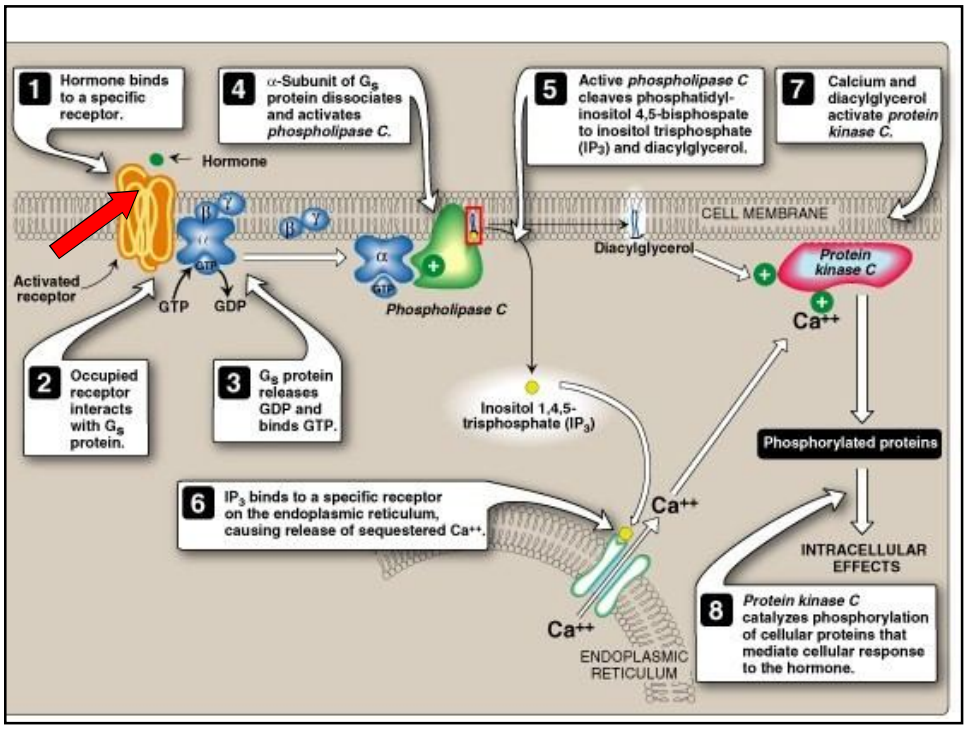
Diacylglycerol

Activates Protein Kinase C

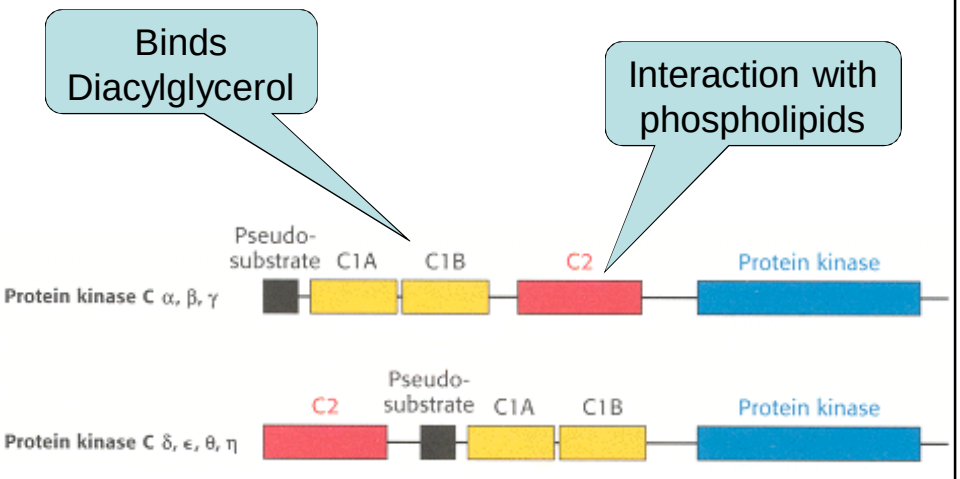
Ca^{2+} is required

Phosphorylation of many target proteins


Phospholipase C
Extracellular
PIP2
DAG
Intracellular
Protein
Kinase C
IP3
Phosporylation
of Substrates
IP3 Sensitive
Ca2+ Channel
Ca2+
smooth muscle contraction, glycogen breakdown, and vesicle release.


1 Hormone binds to a specific receptor.
4 α-Subunit of Gs protein dissociates and activates phospholipase C.
5 Active phospholipase C cleaves phosphatidyl-inositol 4,5-bisphospate to inositol trisphosphate (IP3) and diacylglycerol.
7 Calcium and diacylglycerol activate protein kinase C.
Hormone
CELL MEMBRANE
Activated receptor
GTP
GDP
Phospholipase C
Diacylglycerol
Protein kinase C
Ca++
2 Occupied receptor interacts with Gs protein.
3 Gs protein releases GDP and binds GTP.
Inositol 1,4,5-trisphosphate (IP3)
Phosphorylated proteins
6 IP3 binds to a specific receptor on the endoplasmic reticulum, causing release of sequestered Ca++.
Ca++
INTRACELLULAR EFFECTS
8 Protein kinase C catalyzes phosphorylation of cellular proteins that mediate cellular response to the hormone.
Ca++
ENDOPLASMIC RETICULUM

The domain structures of protein kinase C isoforms



Pseudosubstrate Sequence

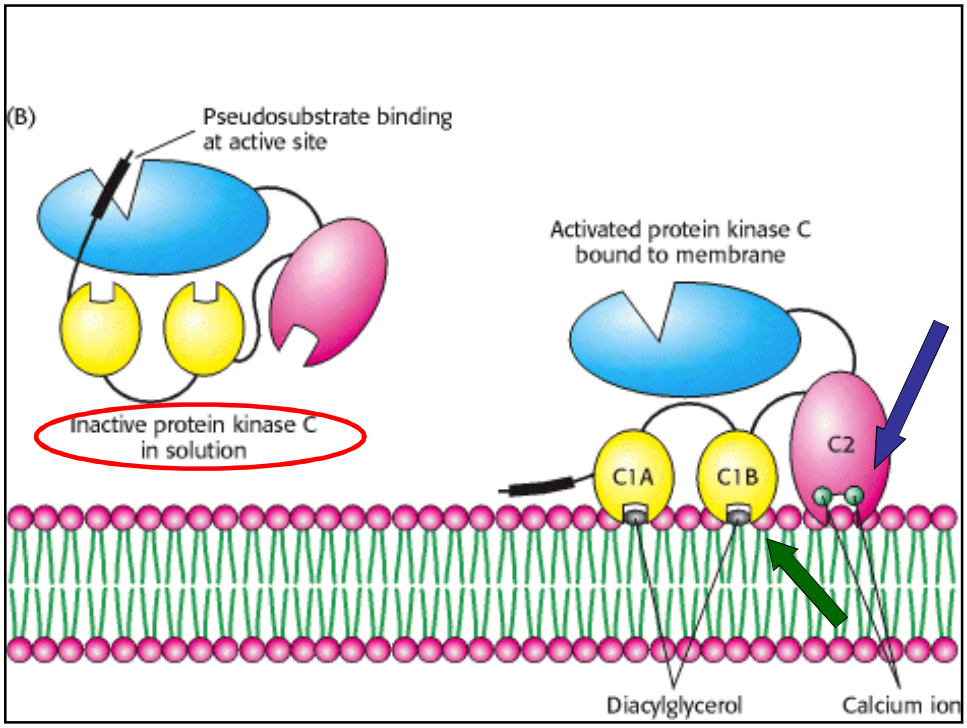
- Resemble the substrate sequence
- Contains

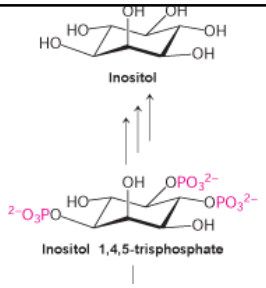
  A-R-K-G-A-L-R-Q-K

  Substrate Sequence

  X-R-X-X-(S,T)-Hyd-R-X

- Binds to the Enzyme's Active Site.





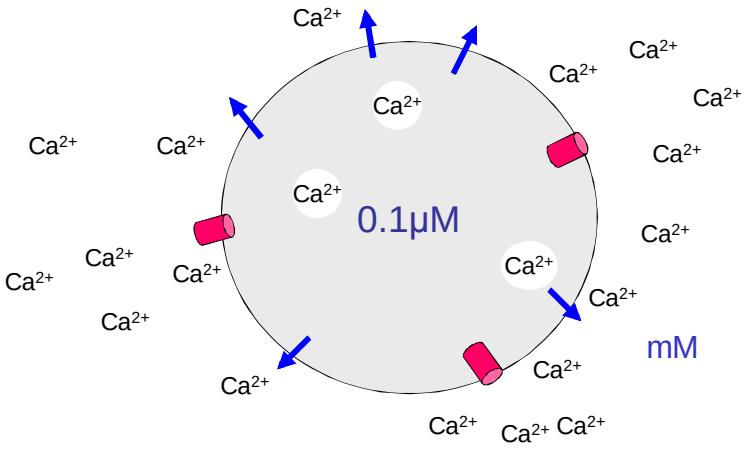
Termination of IP3 Signal

IP3 is a Short-Lived Messenger

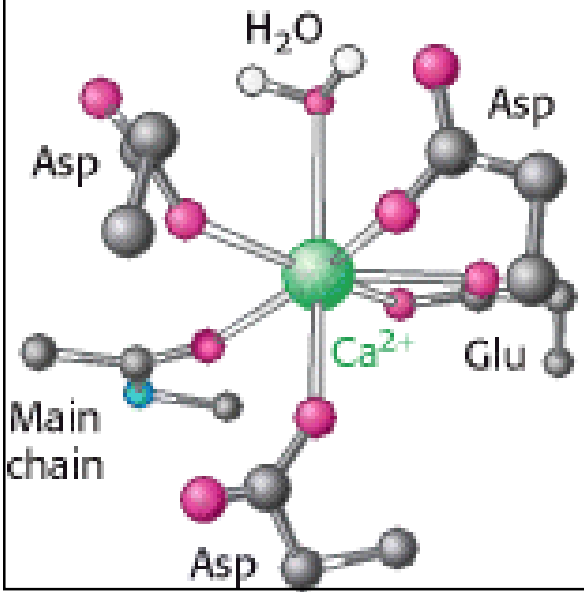
Lithium Ions,
Used to treat some psychological disorders
Inhibits IP_3 recycling

Why Ca^{2+}?

A large difference in concentration



Why Ca^{2+}?



Ability to bind protein tightly

Useful Tools in Studying the role of Calcium

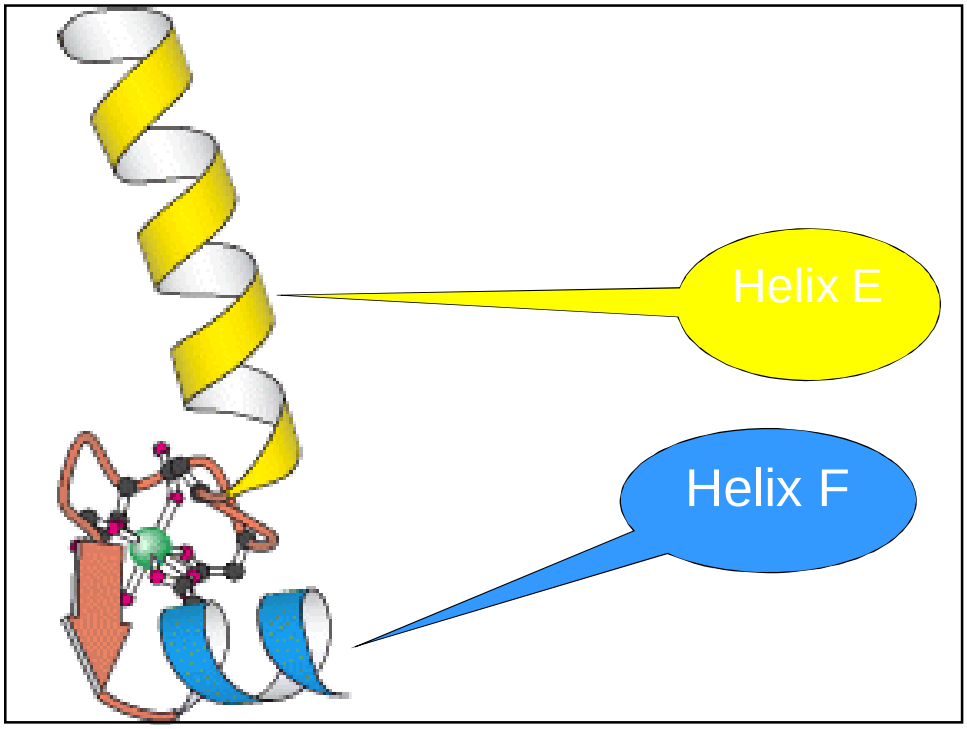
- Ionophores
  - Introducing Calcium into the cell
- Calcium Chelators
  - Decreasing Calcium Concentration
- Fluorescent Chelators
  - Measuring Calcium Concentration

Calcium Binding Proteins

- Mediate the effects of Calcium
- Many proteins

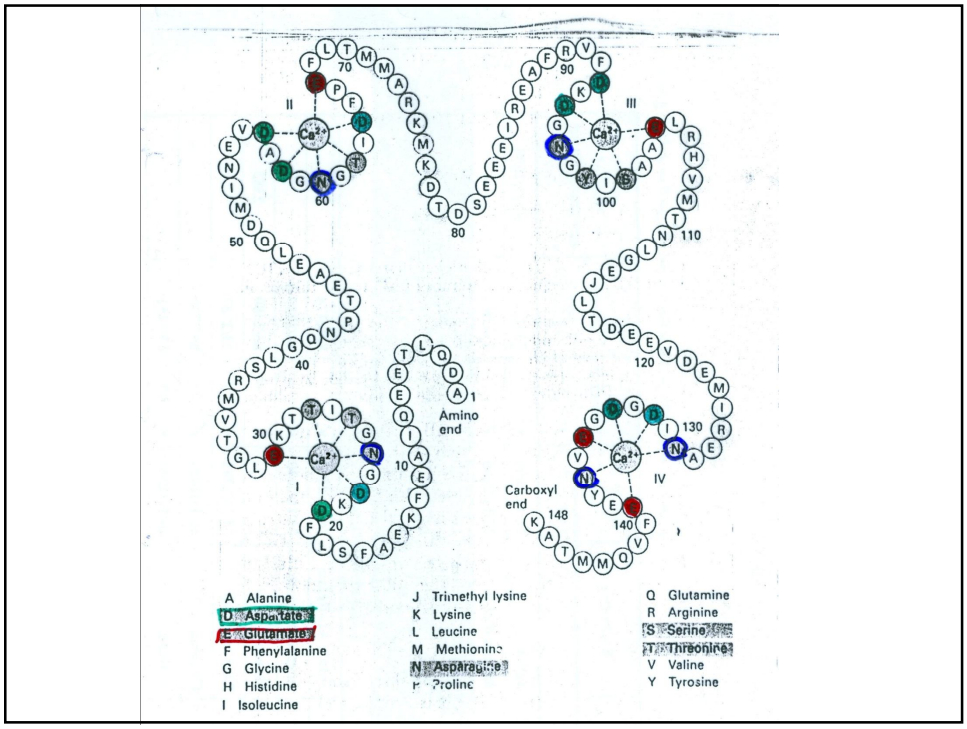
  Calmodulin, Troponin C, Parvalbumin
- Similar structures
  - Rich in Asp and Glu
  - Several α helical segments
  - Binding site is formed by

    Helix Loop Helix

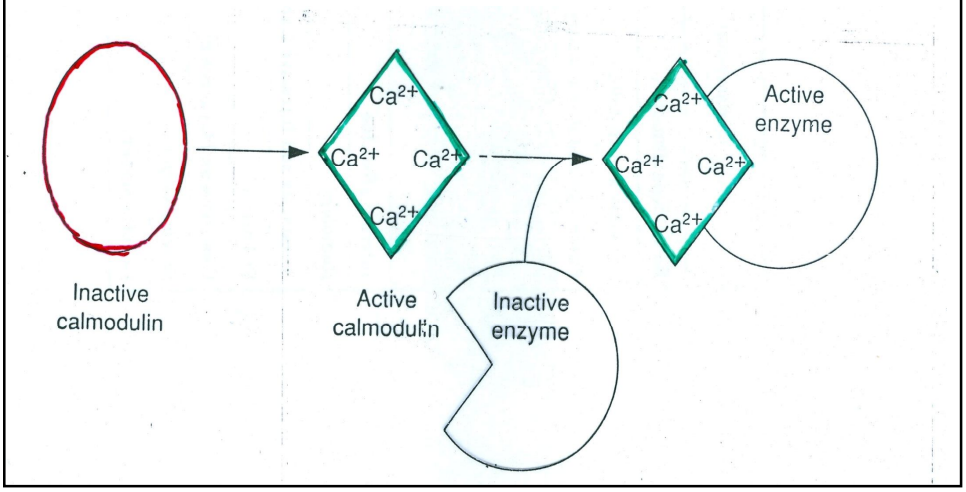


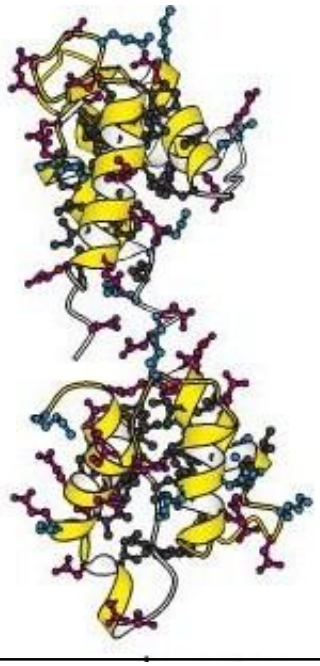
Calmodulin

- Found in almost all eukaryotic cells
- Consists of two globular regions
  - Connected by flexible region
  - Each contains 2 EF hands
  - Four Ca^{2+} binding sites.



Calmodulin changes conformation upon binding to Calcium





4 Ca^{2+}

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-Calmodulin Complex can Bind to a large Number of Enzymes,Pumps and Target proteins including

Calmodulin-dependant Protein Kinase

Ca^{2+} ATP'ase Pump

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

Signal Transduction through Tyrosine Kinase

Hormone Binding

↓

Dimerization of the receptor

↓

Auto phosphorylation of the receptor

↓

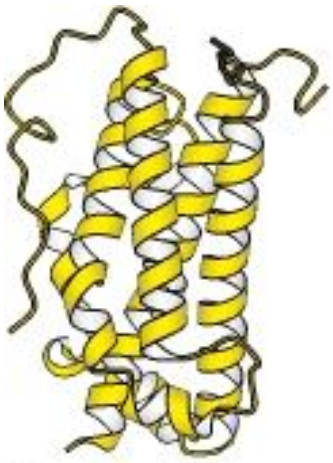
Phosphorylation of the target proteins

Some Hormones that use Tyrosine Kinase

- Growth Hormone
- Insulin
- Epidermal Growth Factor
- Platelet-derived growth Factor

Growth Hormone

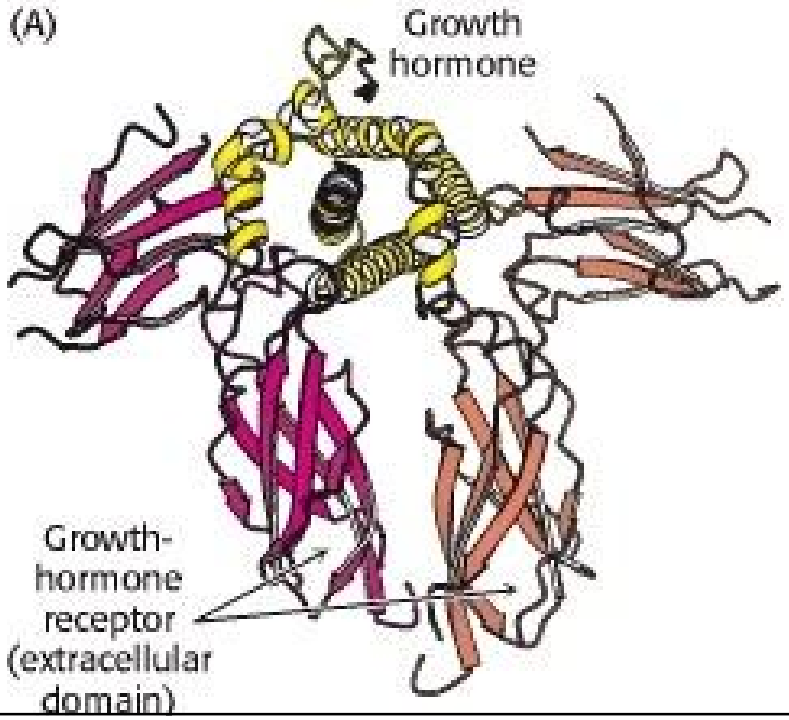
- Monomeric Protein
- 217 Amino Acids
- Compact Four-helix Bundle



Human growth hormone

Growth Hormone Receptor

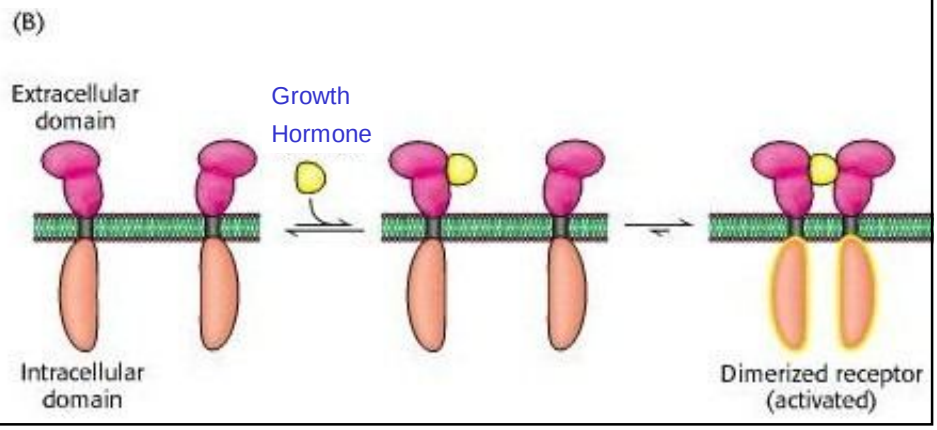
- 638 A.Acid
  - Membrane Spanning Protein
  - Extracellular Domain ≈250 A.A
  - Single Membrane-Spanning Helix
  - Intracellular Domain 350 A.A
- Monomeric when not bound to hormone
- Dimeric when bound to hormone



Binding of one molecule of growth hormone

↓

Dimerization of the receptor



Each Intracellular Domain is associated with Janus a protein kinase called

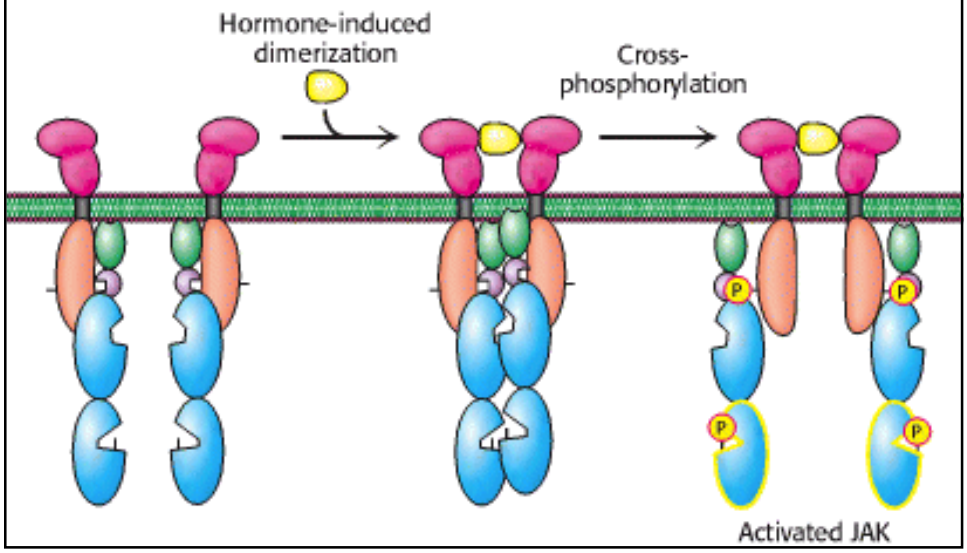
Janus Kinase 2

Interaction with membrane

Binds peptides that contain Phosphotyrosine

Receptor dimerization brings two JAKs together

Each Phosphorylates key residues on the other



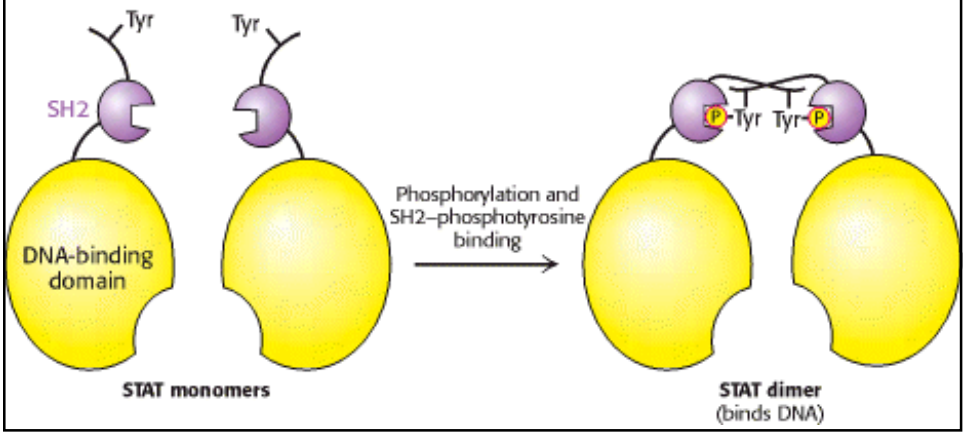
Activated JAK 2 can Phosphorylate other substrates

- STAT 5
  - Signal transducer and activators of transcription
- Regulator of transcription

- STAT5 Phosphorylation
  - ➔ Dimerization
    - ➔ Binding to specific DNA sites

STAT is phosphorylated on a tyrosine residue near the carboxyl terminus

Phosphorylated tyr binds to SH2 domain of another STAT 5 molecule



Activated JAK 2 can Phosphorylate other substrates (cont.)

- Phosphorylation of the Receptor
  - Association with JAK 2
  - Association with other proteins in the signal transduction pathway