Detection and generation of cellular response

Transduction of Hormonal Signal

Signal Transduction

- 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
  - Few in number
  - Restricted movement

Second Messengers

- Ability to diffuse to other cellular compartments
- Amplification of the signal
  - Enzyme activation
  - Membrane channels
- Some second messengers are common in multiple signaling pathways (≈ 30 hormones uses cAMP!!!)
  - Permits fine tuning but can pose problems
- Types of 2nd messengers:
  - Small molecules: cAMP, cGMP, Ca^{+2}
  - Phosphorylation through kinases

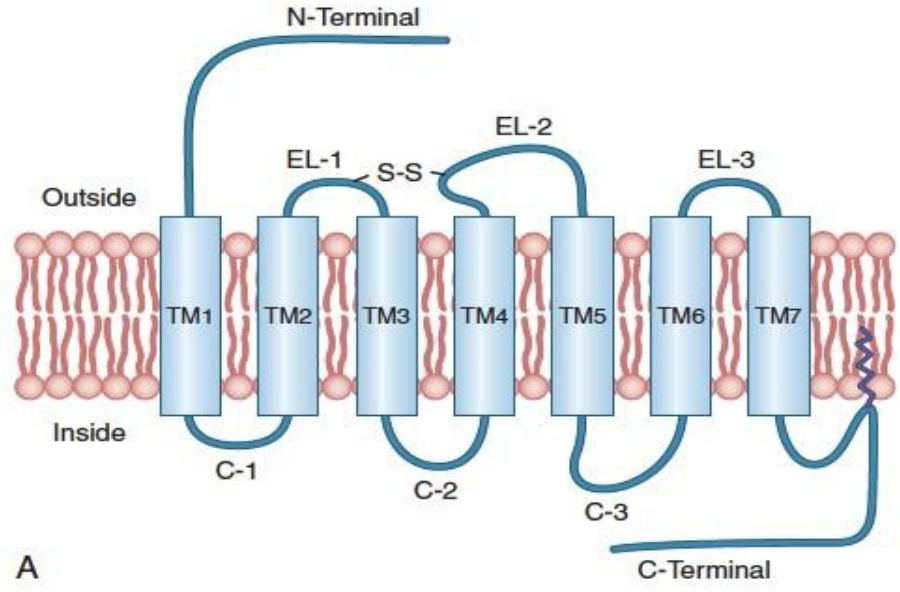
Signal Termination

- Is it important?
  - Keeps cells responsive to new signals
  - Failure of termination may cause problem e.g GH & cancer

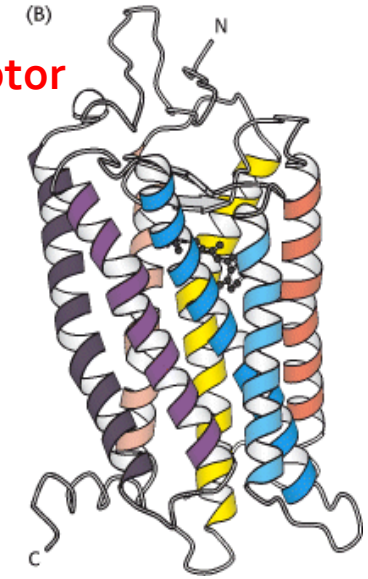
- How it is achieved?
  - Degradation of the second messenger
  - Dephosphorylation by hydrolysis

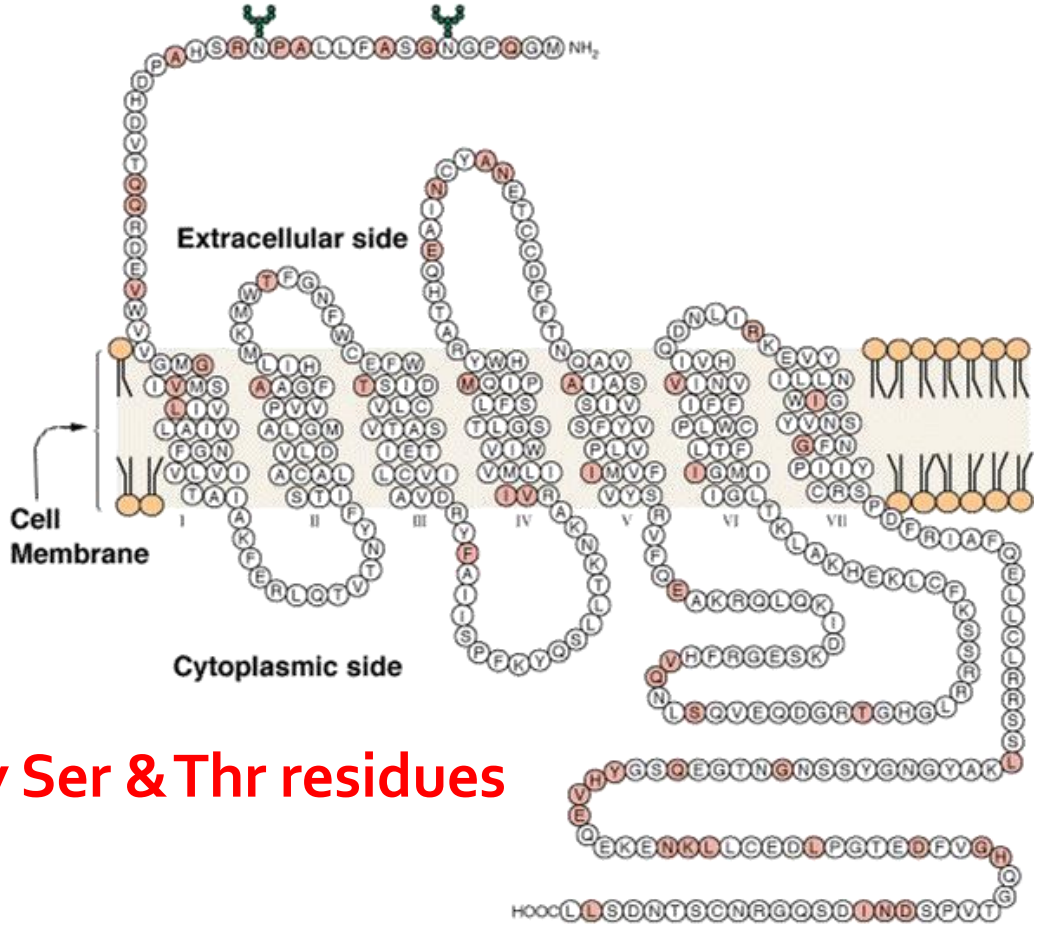
Membrane Associated Receptors
7-Transmembrane Helix Receptors (7TM)

- ✓ 7 α-helices: H-bonding, rigid, hydrophobic
- ✓ Signal induces conformational changes
- ✓ Is it enough?



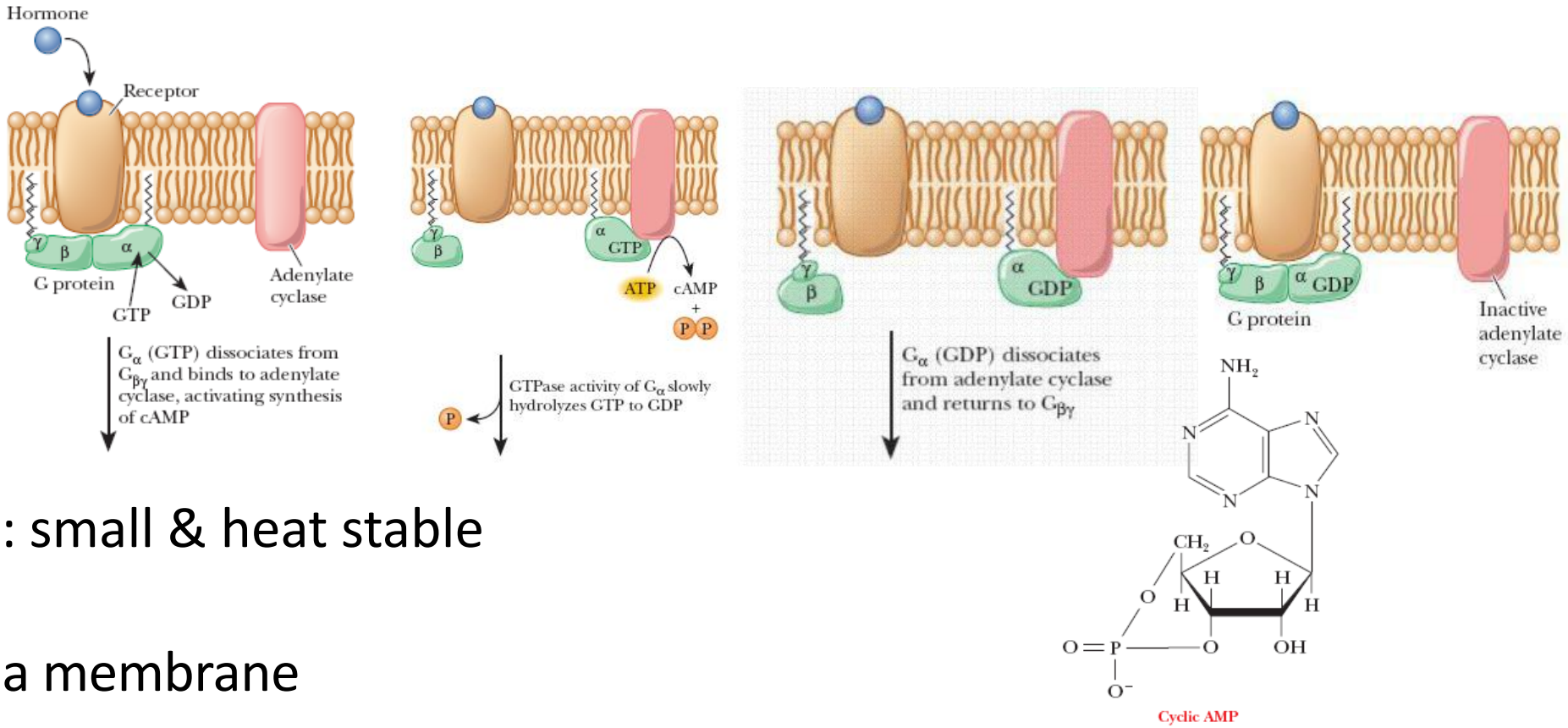
Rhodopsin receptor





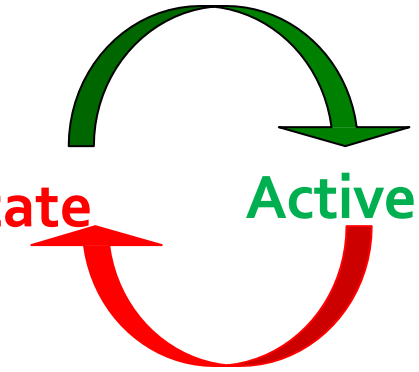
- Many Ser & Thr residues

G-Proteins & cAMP



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

G-proteins 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

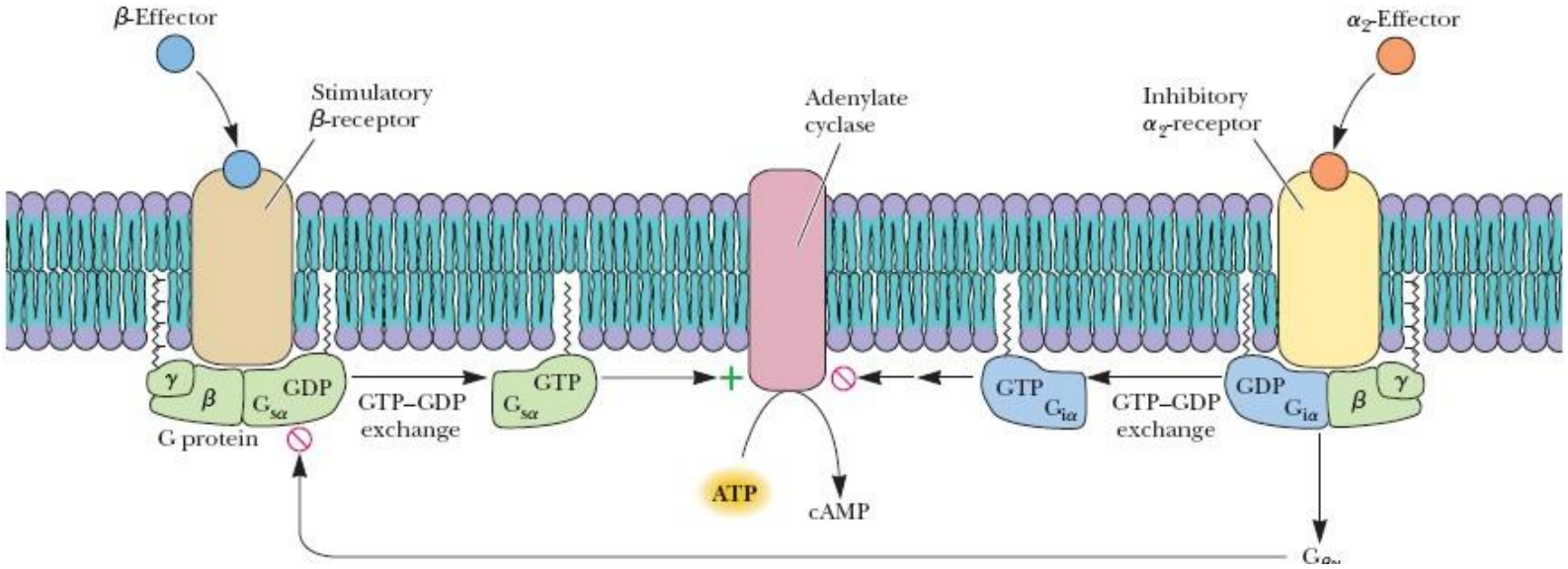
↓

↑ cAMP

↓

Biochemical Response

G-proteins: stimulatory or inhibitory?



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

G-Proteins

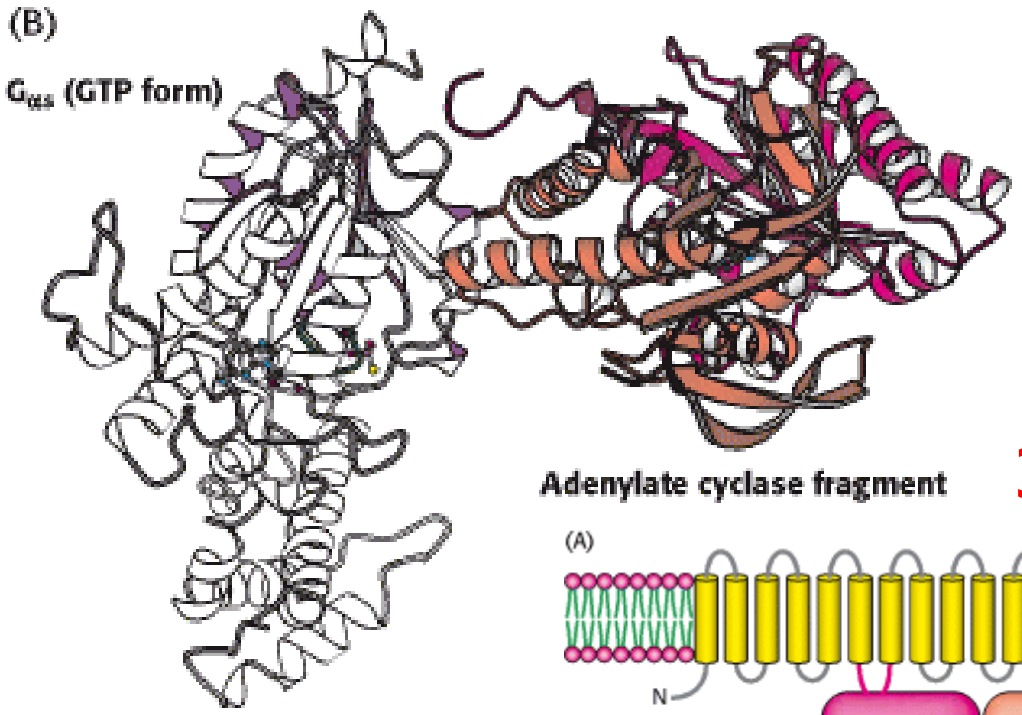
- G proteins:
  - More than 100 known G protein–coupled receptors and more than 20 known G proteins
  - Can be activated by combinations of hormones
    - ✓ Epinephrine & glucagon act via a stimulatory G protein in liver cells
  - Other than cAMP:
    - ✓ Stimulating phospholipase C
    - ✓ Opening or closing membrane ion channels

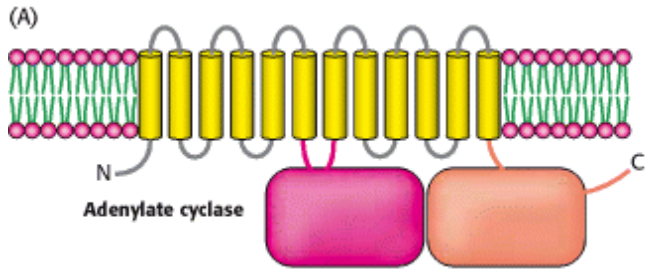
G-Proteins

- α and γ Subunits have covalently attached fatty acid
- 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

Signal Transduction

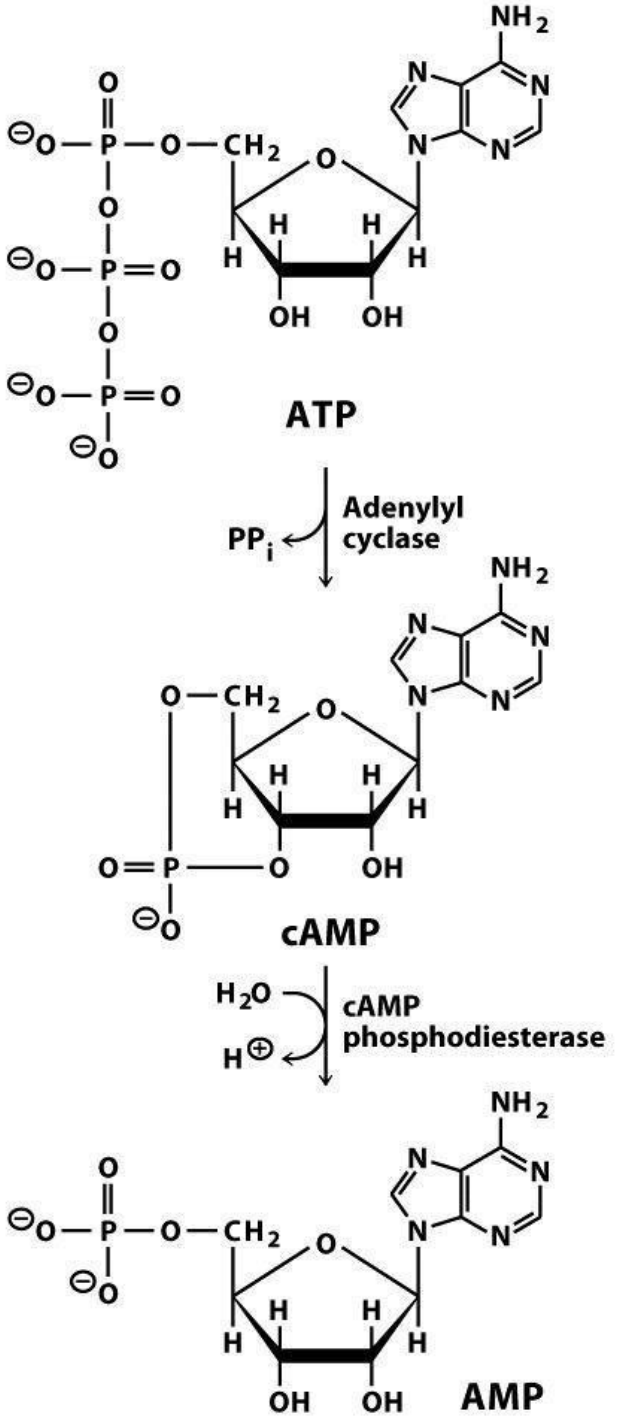
Adenylate Cyclase





3’5’ cAMP

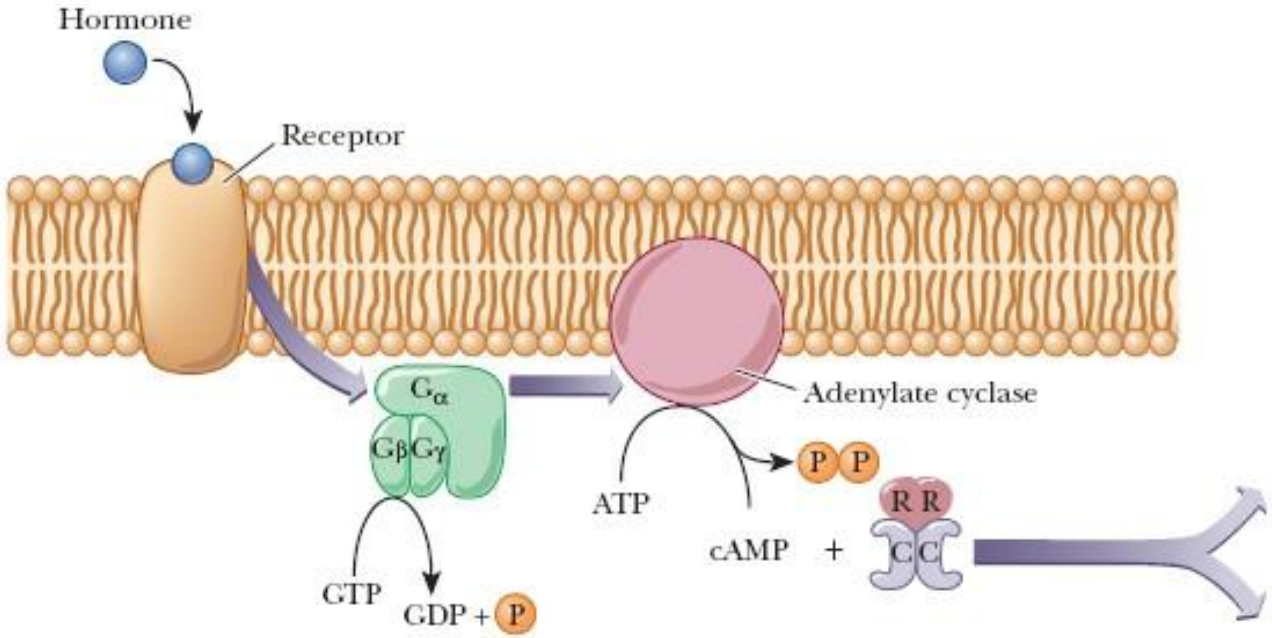
- Membrane protein
- 12 helices
- Two large intracellular domains
- Activated by G protein



cAMP can affect a wide range of cellular processes

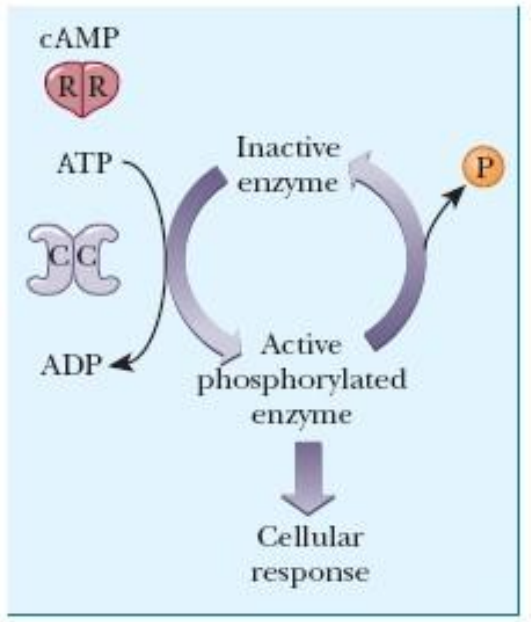
- ↑ degradation of storage fuels
- **↑ secretion of acid by gastric mucosa (caffeine: phosphodiesterase & adenosine)**
- Dispersion of melanin pigment granules
- ↓ aggregation of blood plateletes
- Opening of chloride channels

Then What?



Signal Amplification

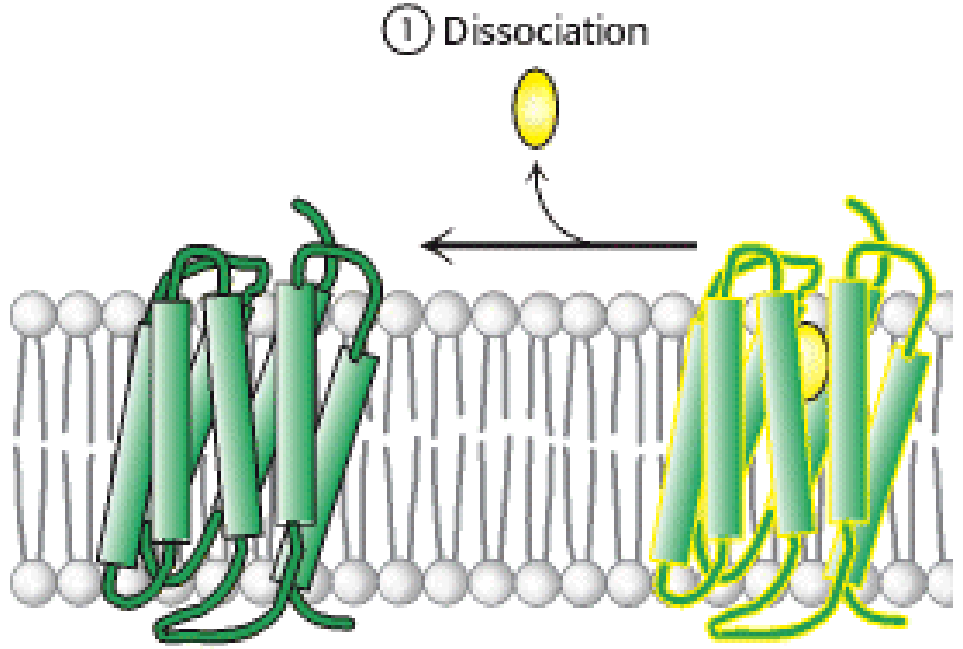
Usually:
Ser or Thr

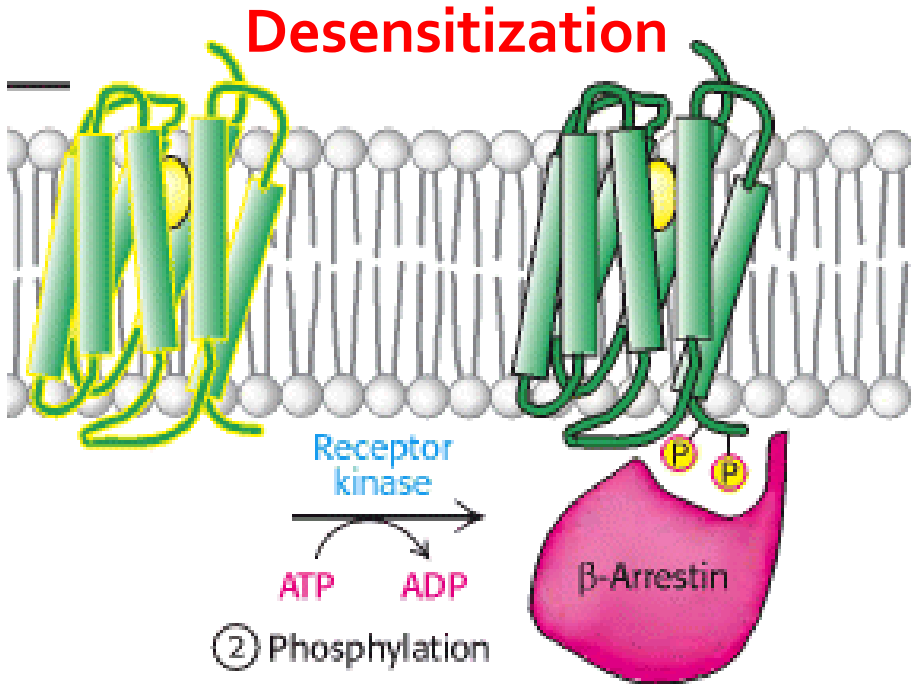


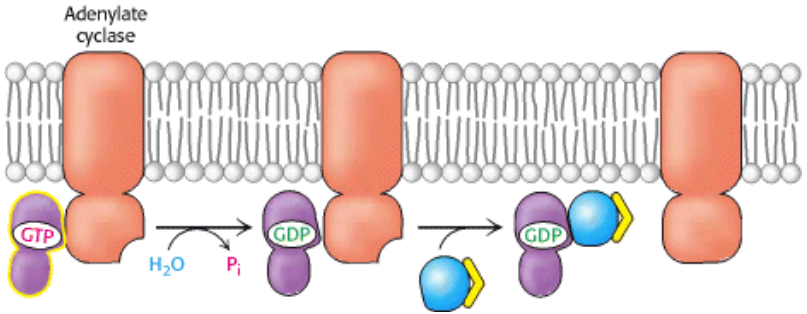
Glycogen Synthase!!

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

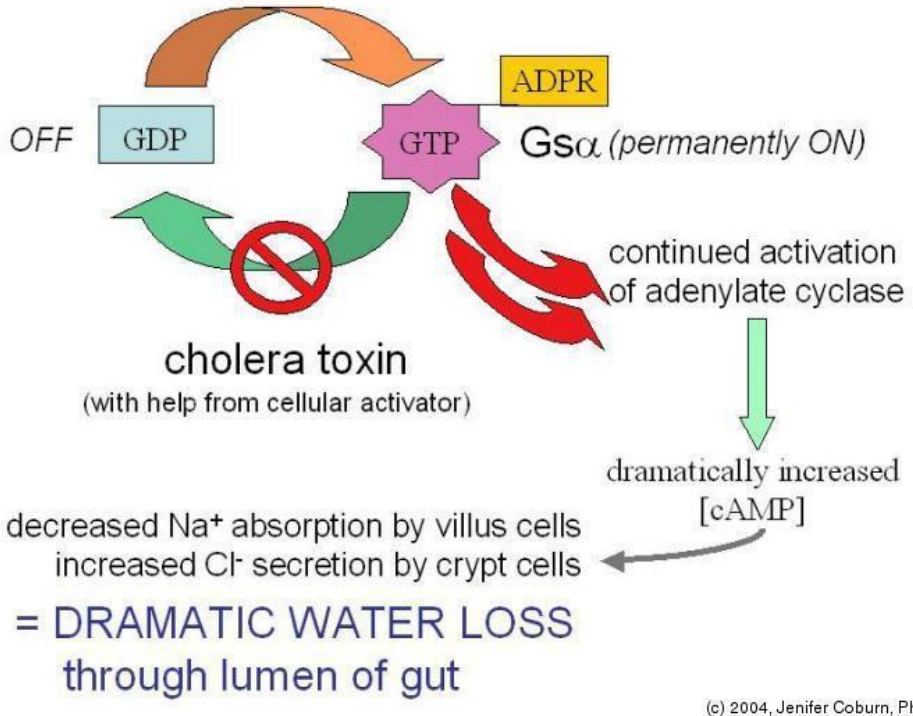


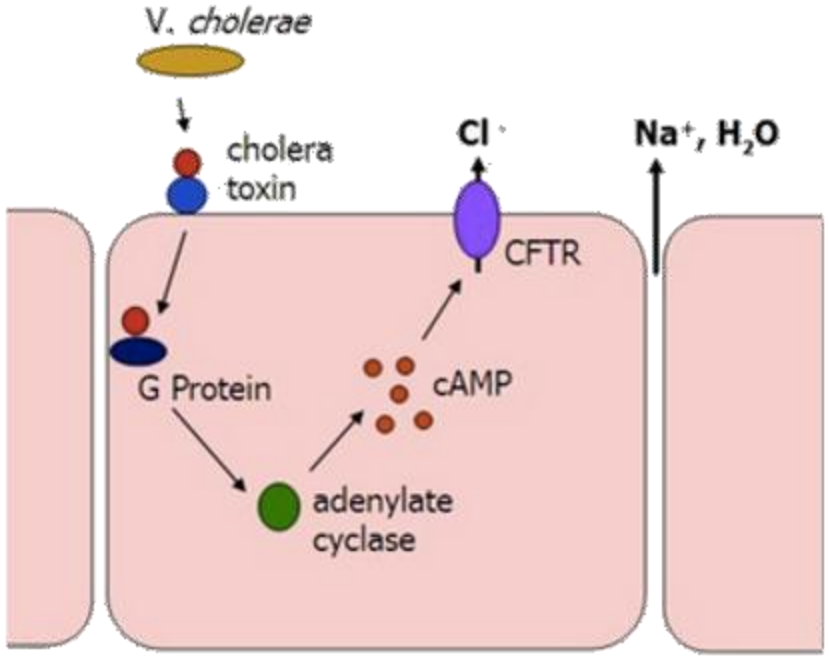




Cholera

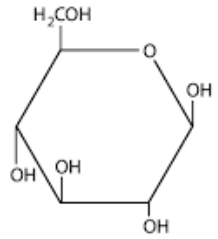
- Cholera toxin → unregulated activity of adenylate cyclase in epithelial cells → Excessive cAMP in epithelial cells stimulates active transport of Na^+ → large flow of Na^+ and water from the mucosa → diarrhea

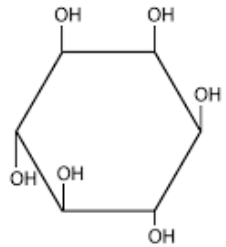




The Phosphoinositide Cascade

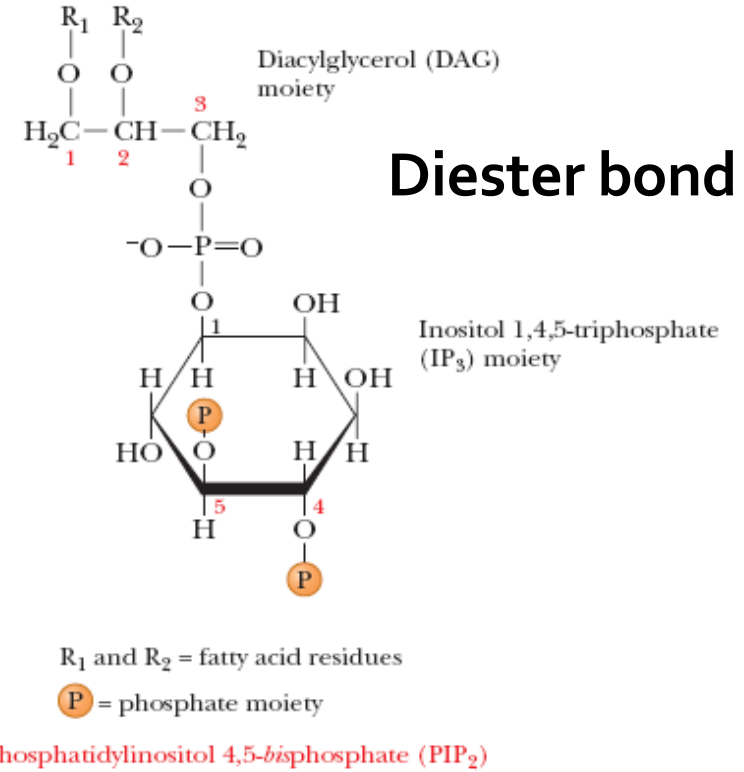
- Used by many hormones (e.g. ADH)
- Binding of a hormone to 7TM receptor



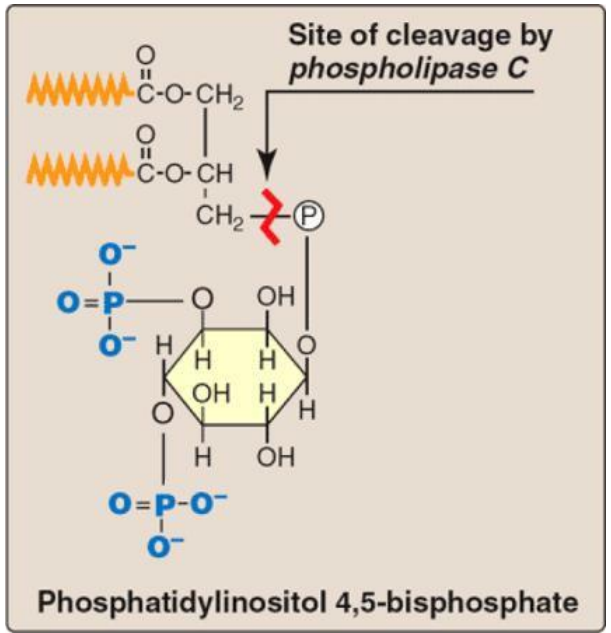


Activation of G Protein

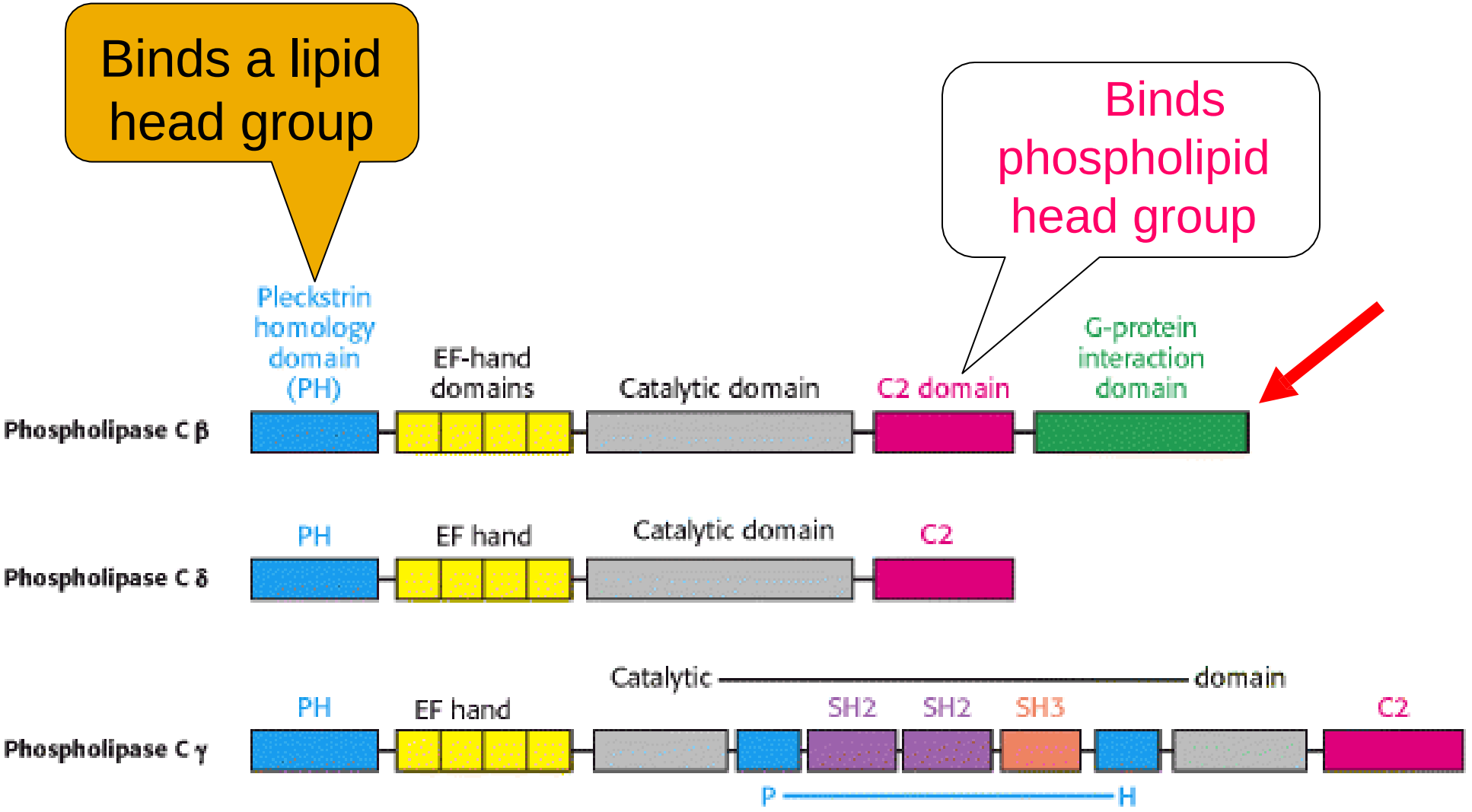
Activation of Phospholipase C (many isoforms) – PIP2



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



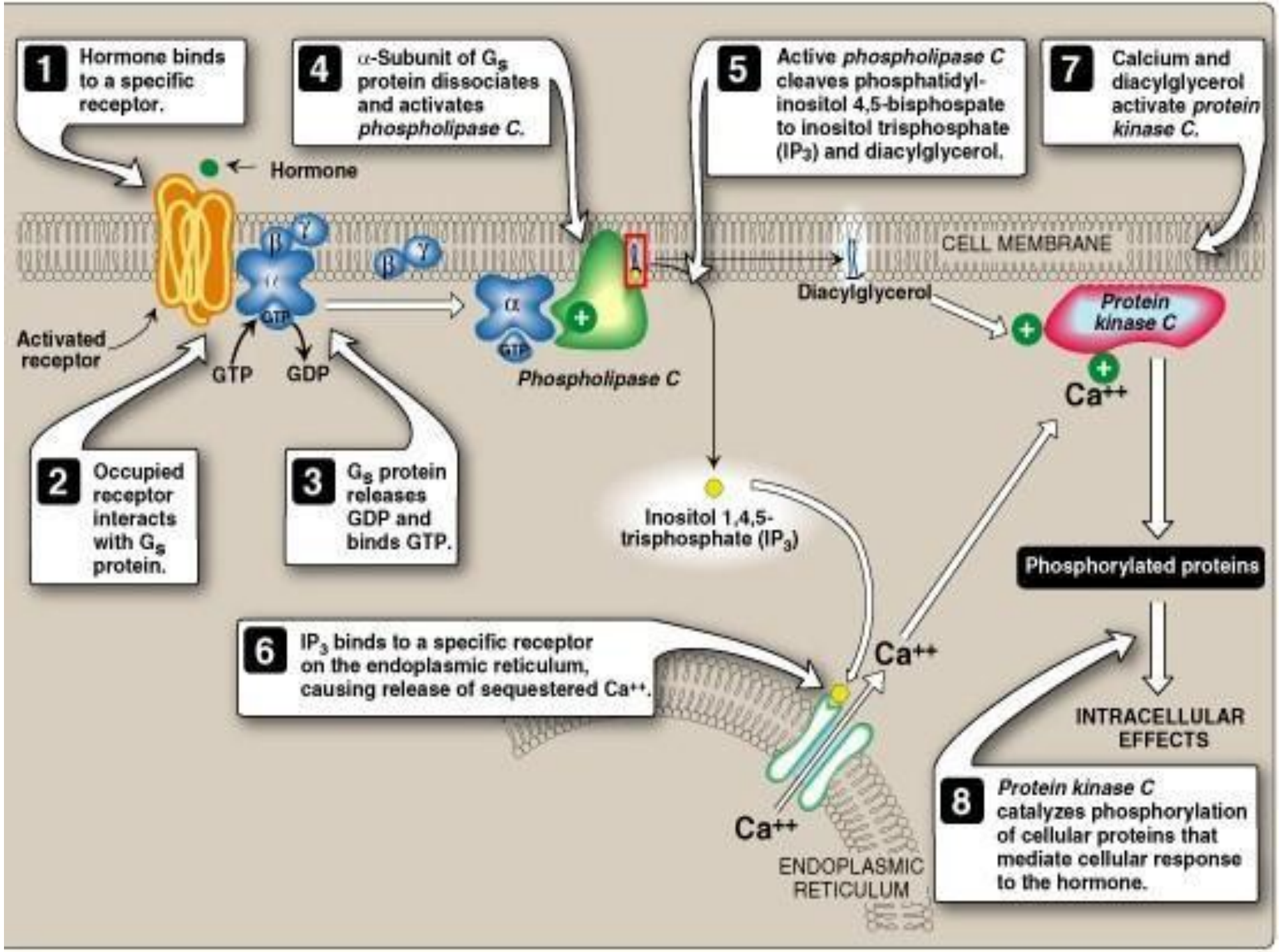
The domain structures of three isoforms of Phospholipase C



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

Membrane

G Protein


1 Hormone binds to a specific receptor.
4 α-Subunit of G_s protein dissociates and activates *phospholipase C.*
5 Active *phospholipase C* cleaves phosphatidyl-inositol 4,5-bisphospate to inositol trisphosphate (IP_3) 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 G_s protein.
3 G_s protein releases GDP and binds GTP.
Inositol 1,4,5-trisphosphate (IP_3)
Phosphorylated proteins
6 IP_3 binds to a specific receptor on the endoplasmic reticulum, causing release of sequestered Ca^{++}.
Ca^{++}
INTRACELLULAR EFFECTS
Ca^{++}
ENDOPLASMIC RETICULUM
8 *Protein kinase C* catalyzes phosphorylation of cellular proteins that mediate cellular response to the hormone.

Effects of Second Messengers

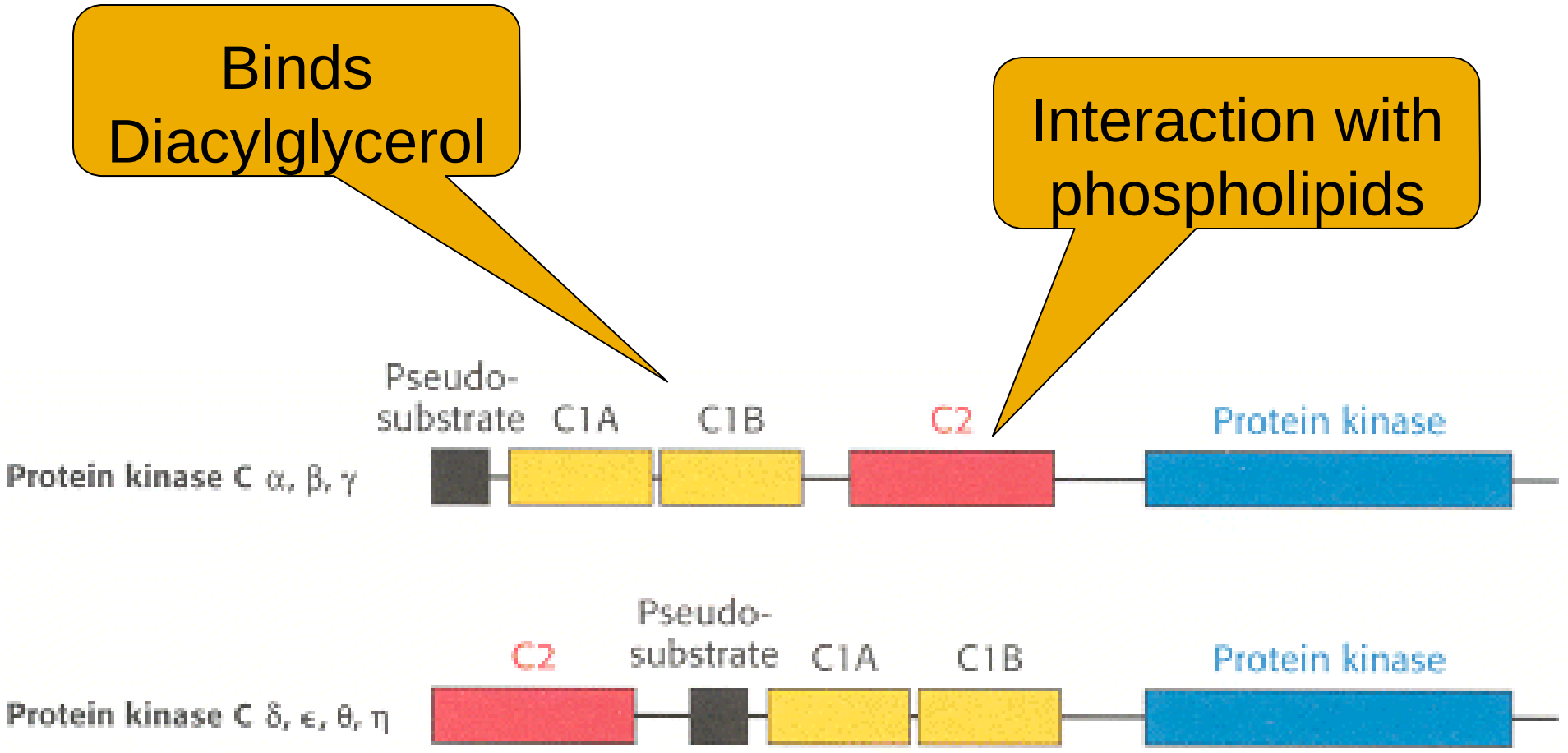
Inositol trisphosphate (IP_3)

- ✓ Opens Calcium Channels
- ✓ Binding to IP_3-gated Channel
- ✓ Cooperative binding (sigmoidal)

Diacylglycerol (DAG)

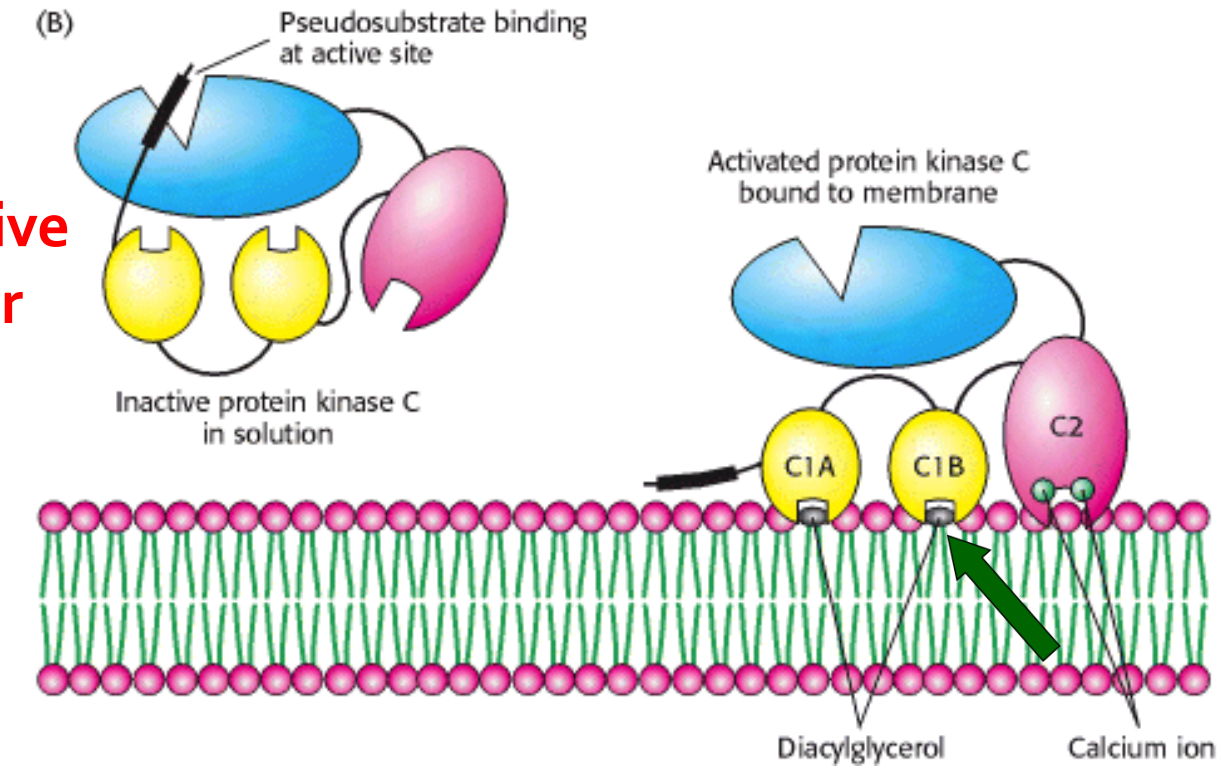
- ✓ Activates Protein Kinase C
- ✓ Ca^{2+} is required
- ✓ Phosphorylation of many target proteins

The domain structures of protein kinase C isoforms



Pseudosubstrate Sequence

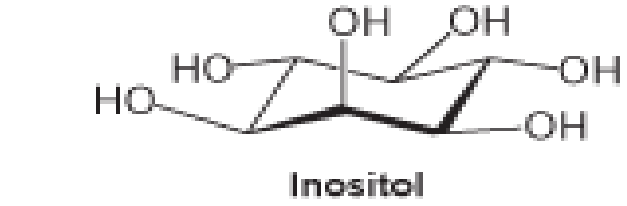
Competitive Inhibitor



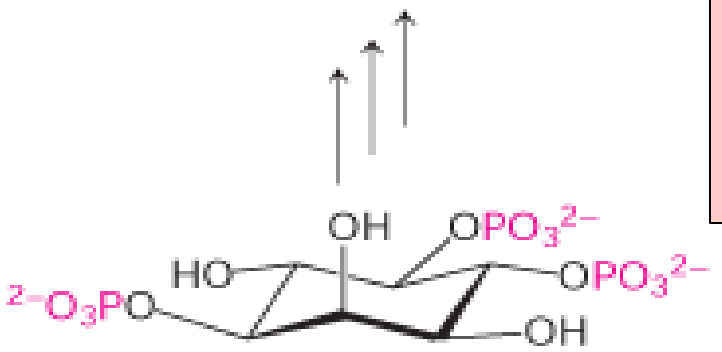
- Resembles the substrate sequence: A-R-K-G-A-L-R-Q-K
- Substrate Sequence: (S,T)
- Binds to the Enzyme's Active Site

Termination of IP3 Signal

IP3 is a Short-Lived Messenger

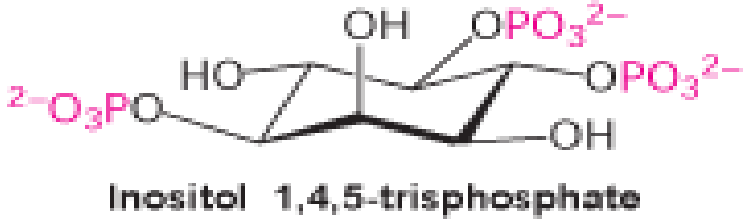


Inositol

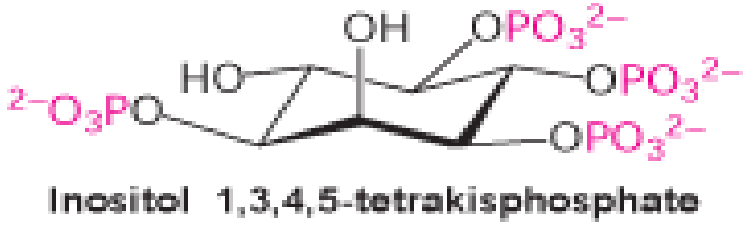


Inositol 1,4,5-trisphosphate

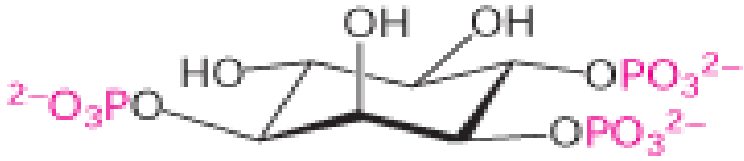
Lithium ions, used to treat some psychological disorders Inhibits IP_3 recycling



Inositol 1,4,5-trisphosphate



Inositol 1,3,4,5-tetrakisphosphate



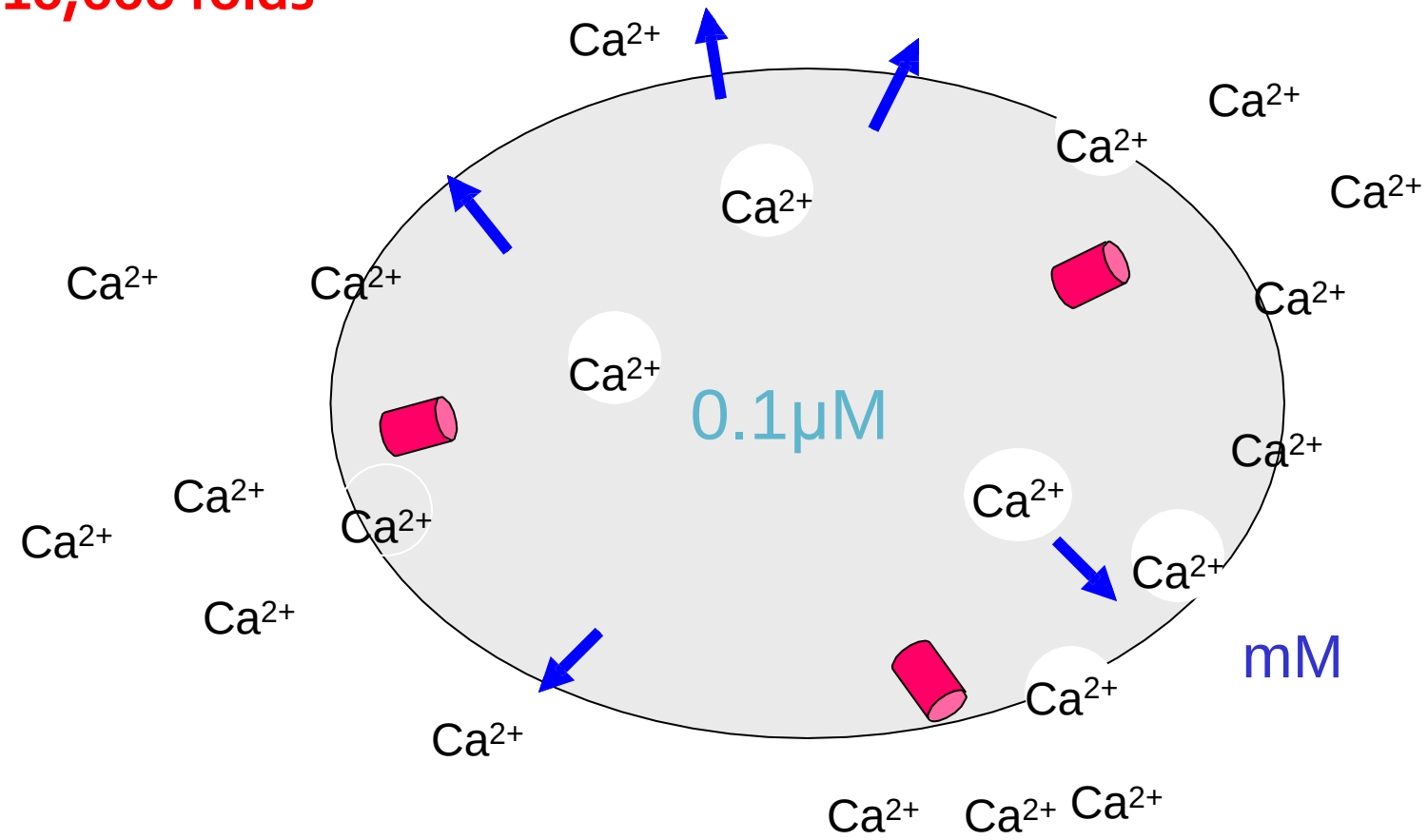
Inositol 1,3,4-trisphosphate

Inositol

Why Ca^{2+}?

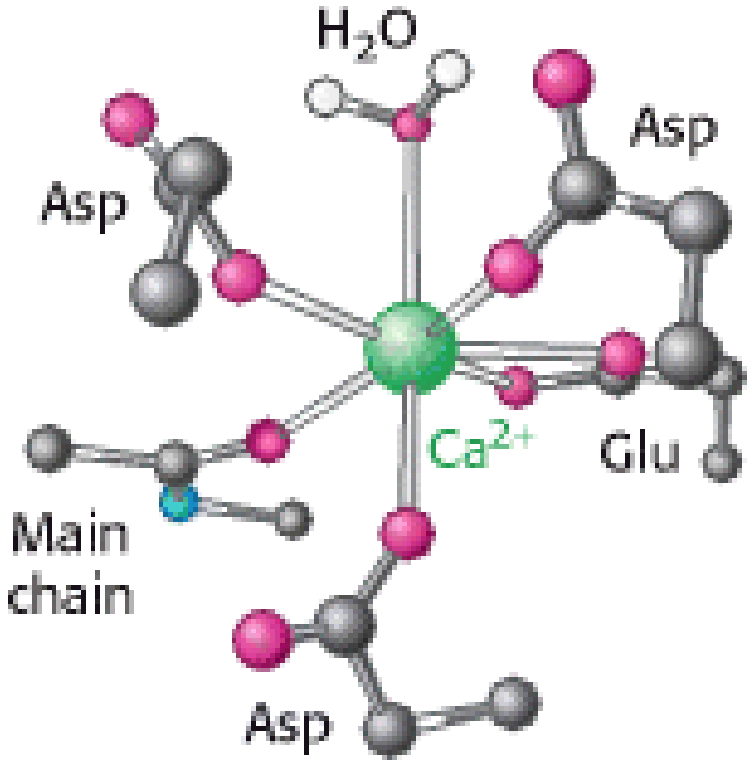
A large difference in concentration

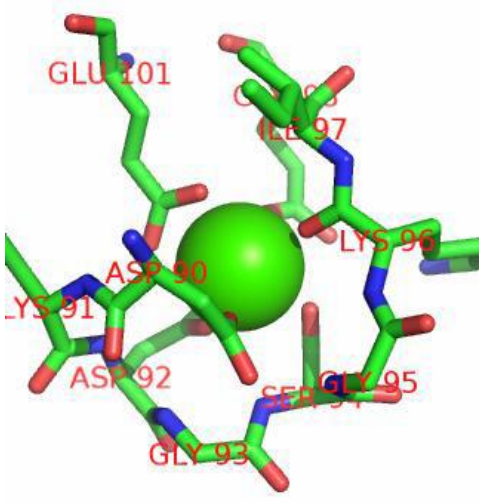
10,000 folds

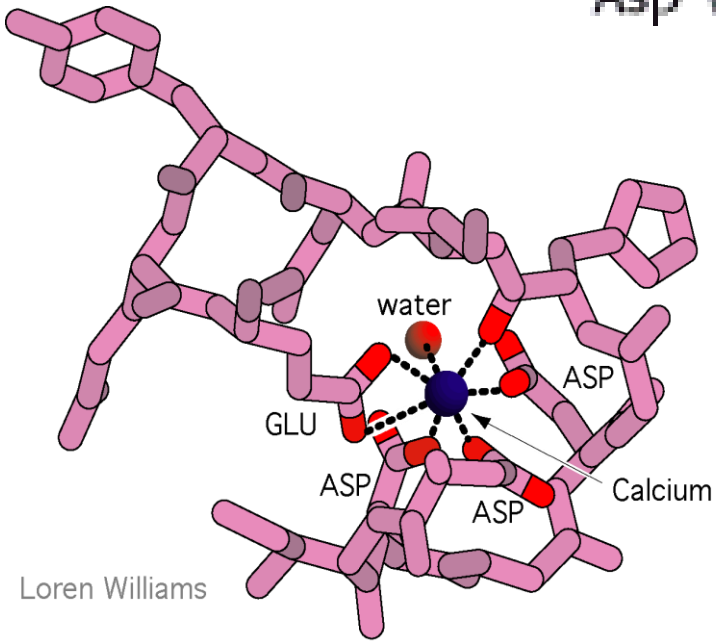


Why Ca^{2+}?

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



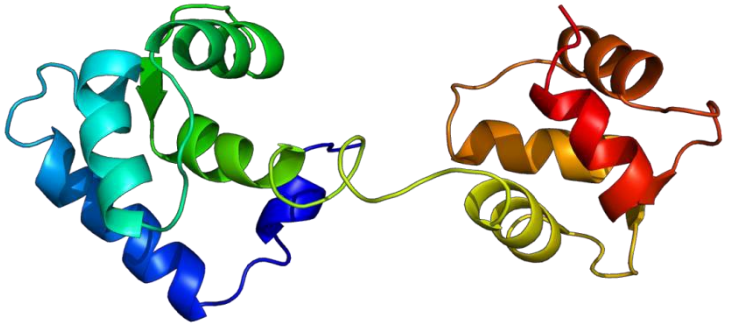




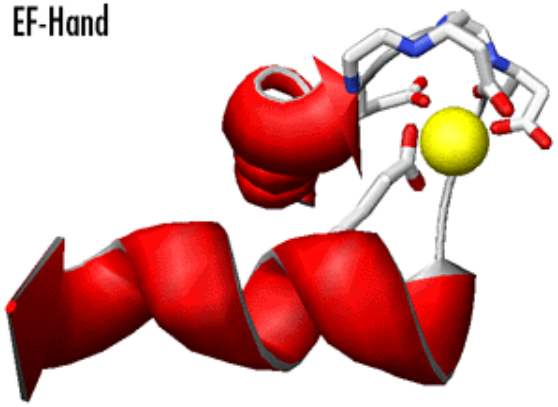
Calcium Binding Proteins

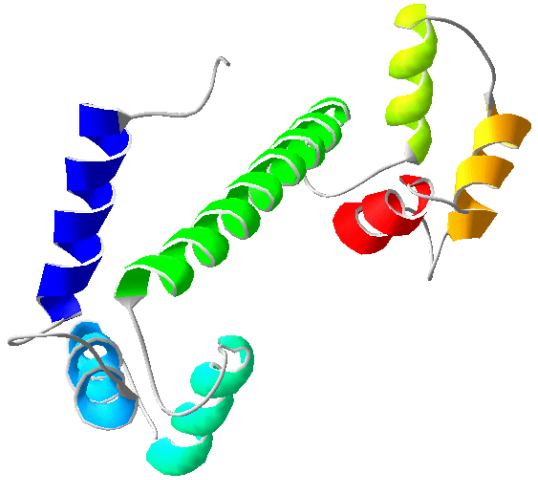
- Mediate the effects of Calcium (Ca^{+2})
- Many proteins

  Calmodulin, Troponin C, Parvalbumin



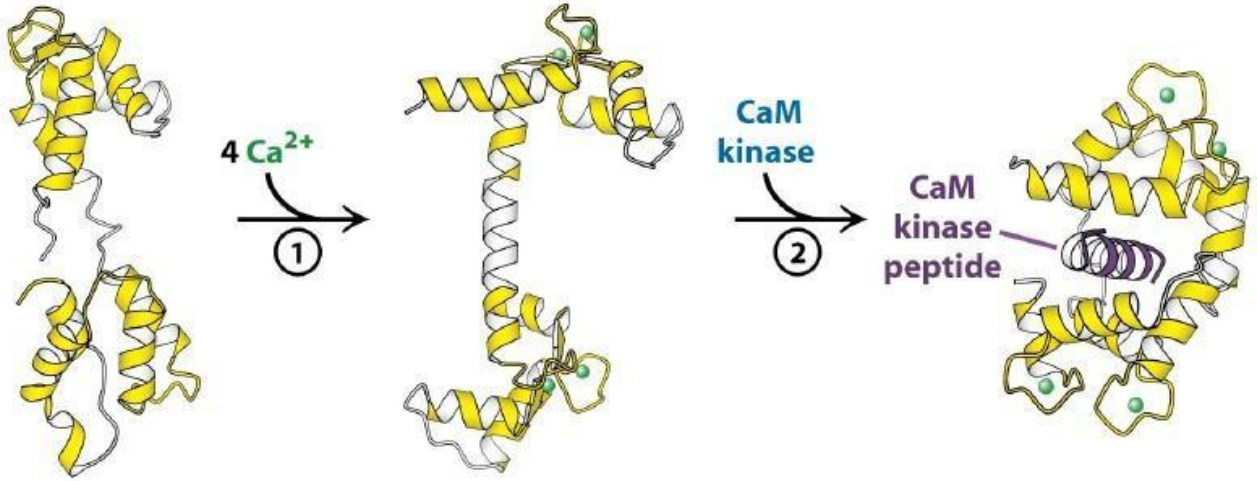
- Similar structures
  - Rich in Asp and Glu
    - Gln, Asn, Ser
  - Several α helical segments
  - Binding site is formed by
    - Helix Loop Helix
      - Super-secondary structure





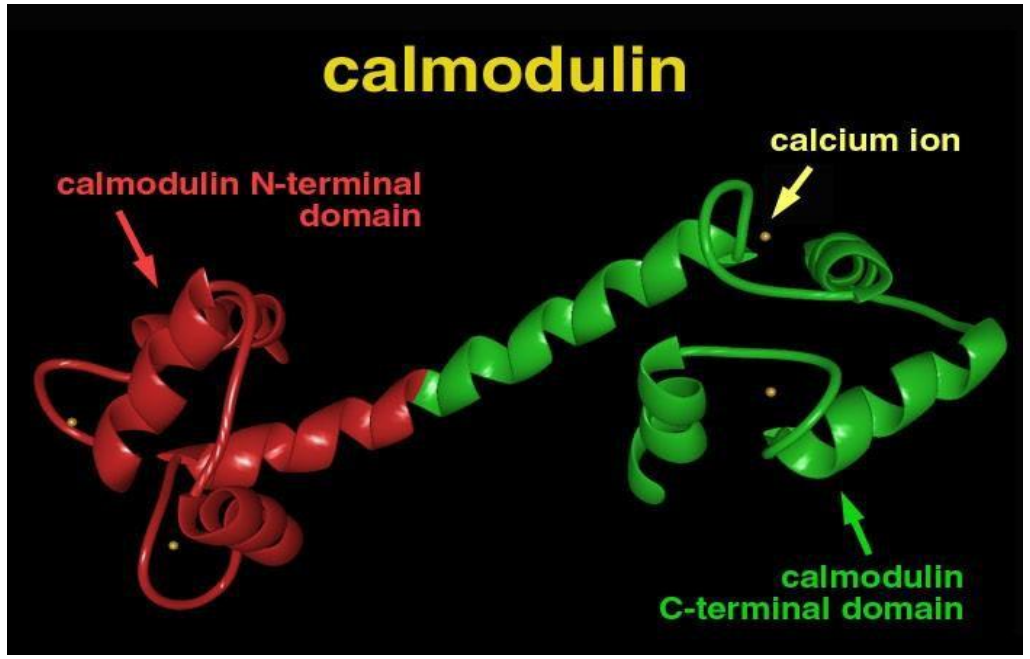
Calmodulin (≈17 kD)

Calcium-modulated protein



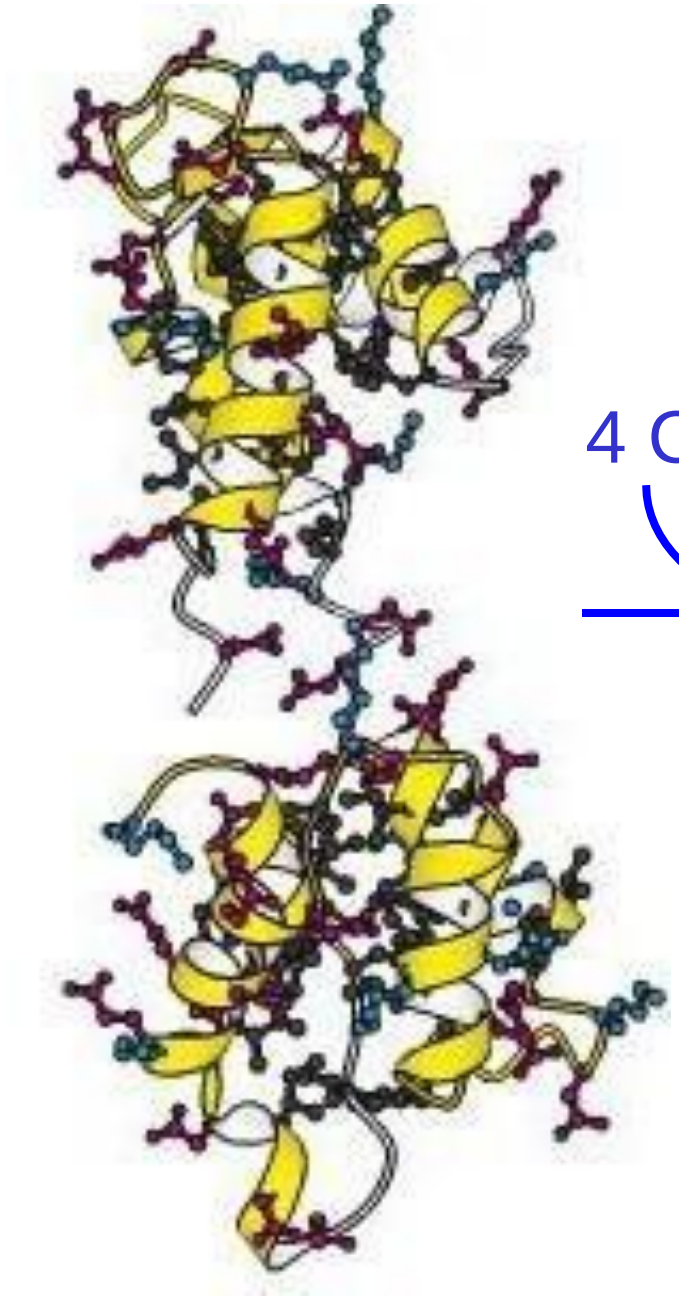
- Found in almost all eukaryotes
- Consists of two globular regions
  - Connected by flexible region
  - Each contains 2 EF hands
  - Four Ca^{2+} binding sites
- Calcium-Calmodulin complex can bind to a large number of target proteins including:

149 amino acids



Calmodulin-dependant Protein Kinase

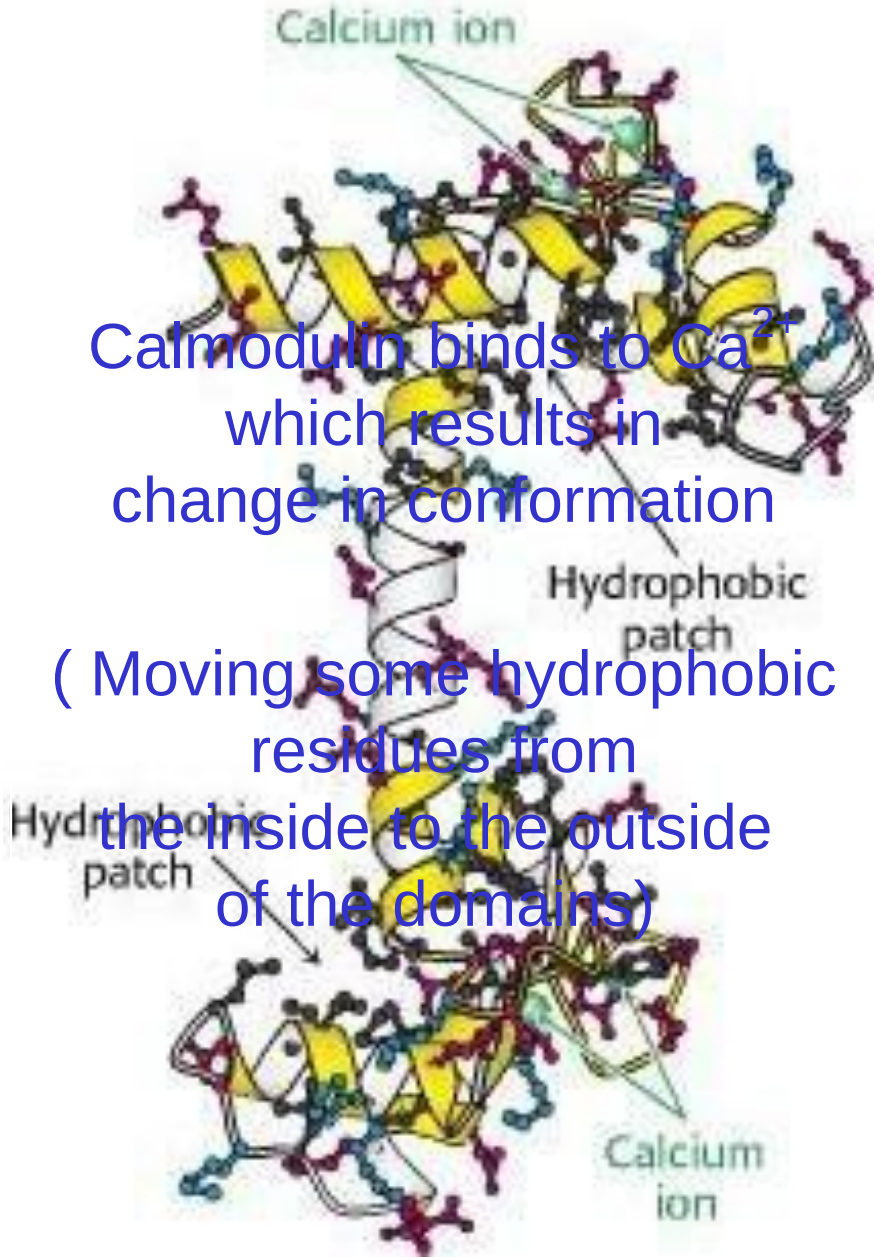
Ca^{2+} ATP'ase Pump



4 Ca^{2+}

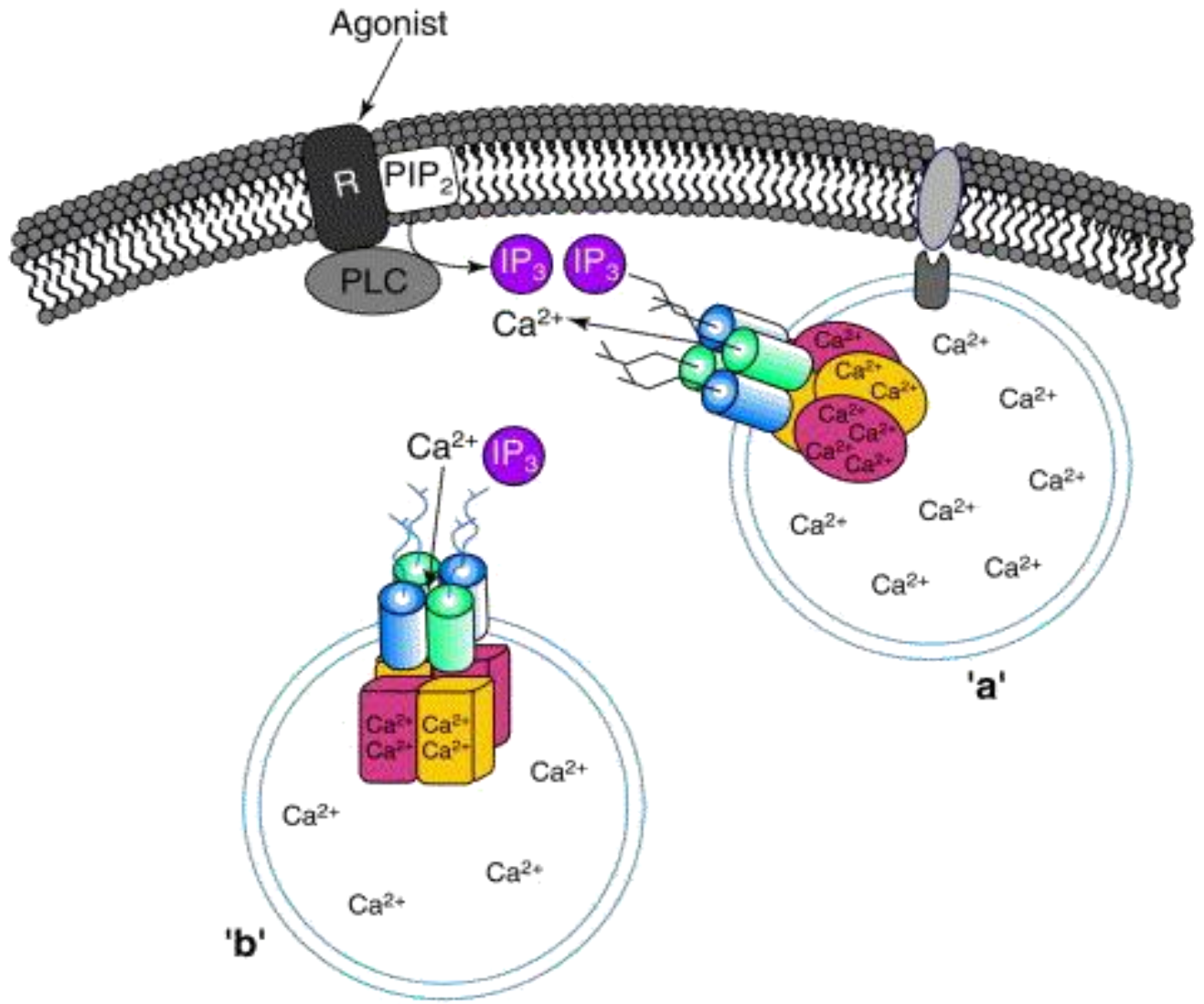
Calmodulin binds to Ca^{2+} which results in change in conformation

(Moving some hydrophobic residues from the inside to the outside of the domains)



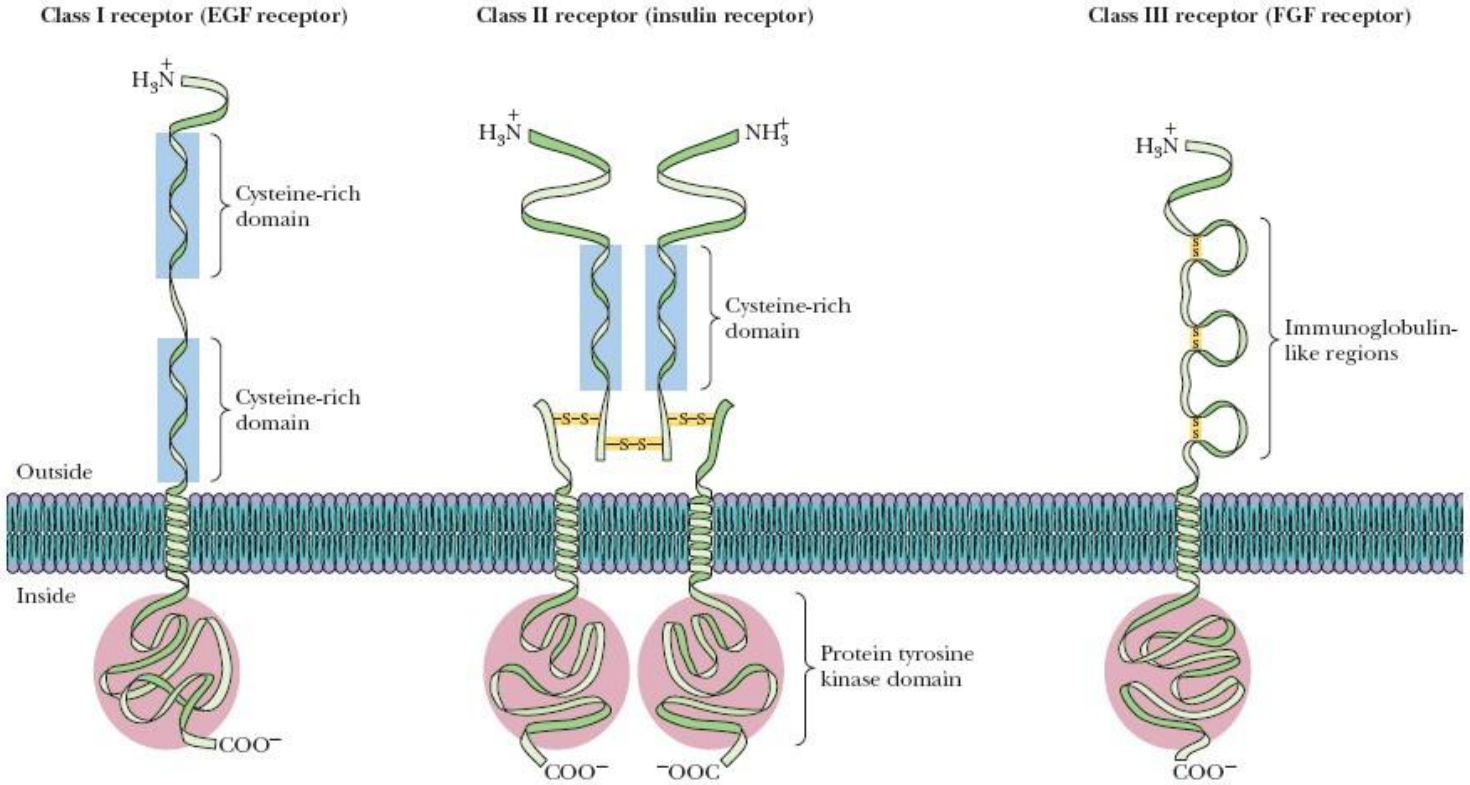
Ca^{2+} Transporter

- 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



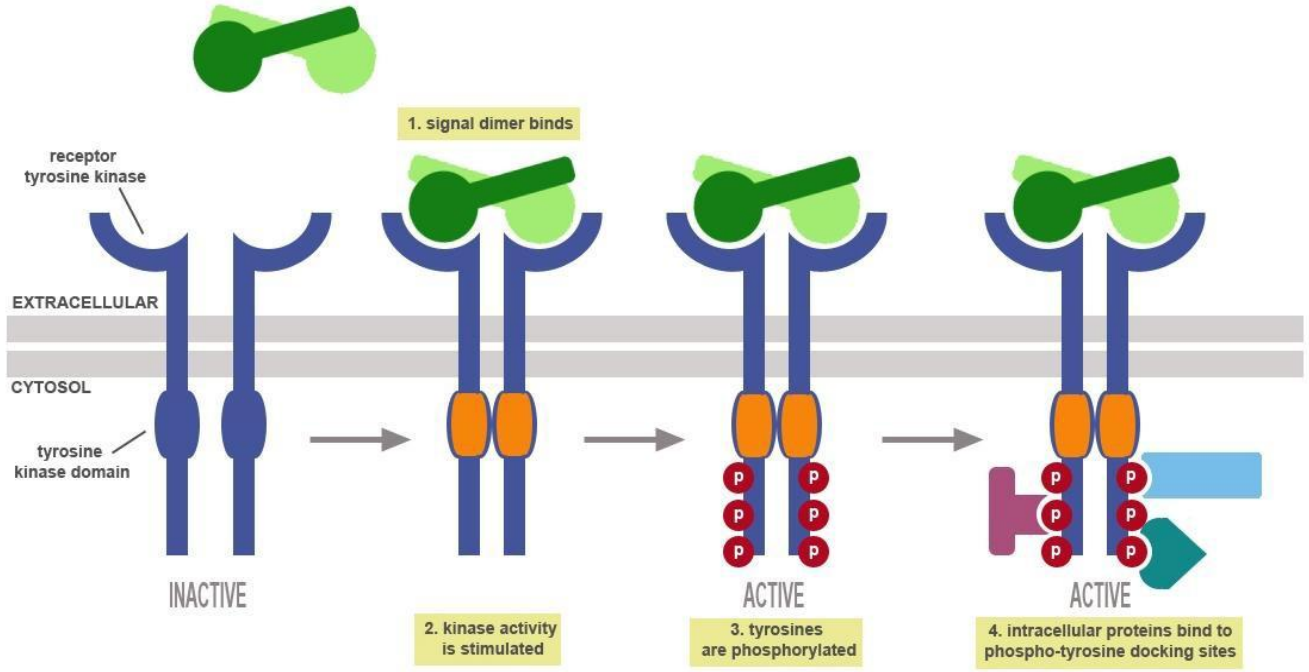
Receptor Tyrosine Kinases Cascade

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



Second Messengers
Receptor Tyrosine Kinases

- 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 Kinase

Growth hormones:

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

Hormone Binding

↓

Dimerization of the receptor

↓

Auto phosphorylation of the receptor

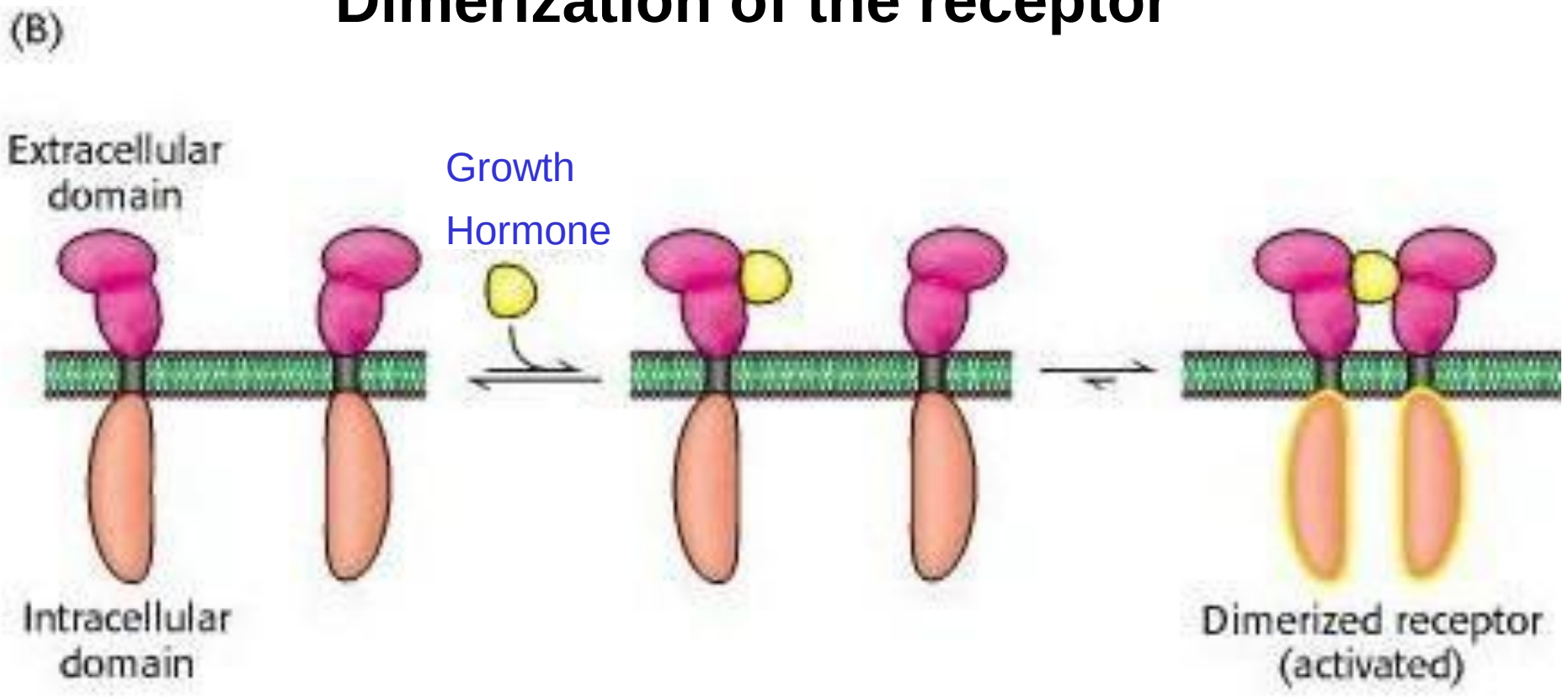
↓

Phosphorylation of the target proteins

Growth Hormone dimerization

Binding of one molecule of growth hormone

Dimerization of the receptor



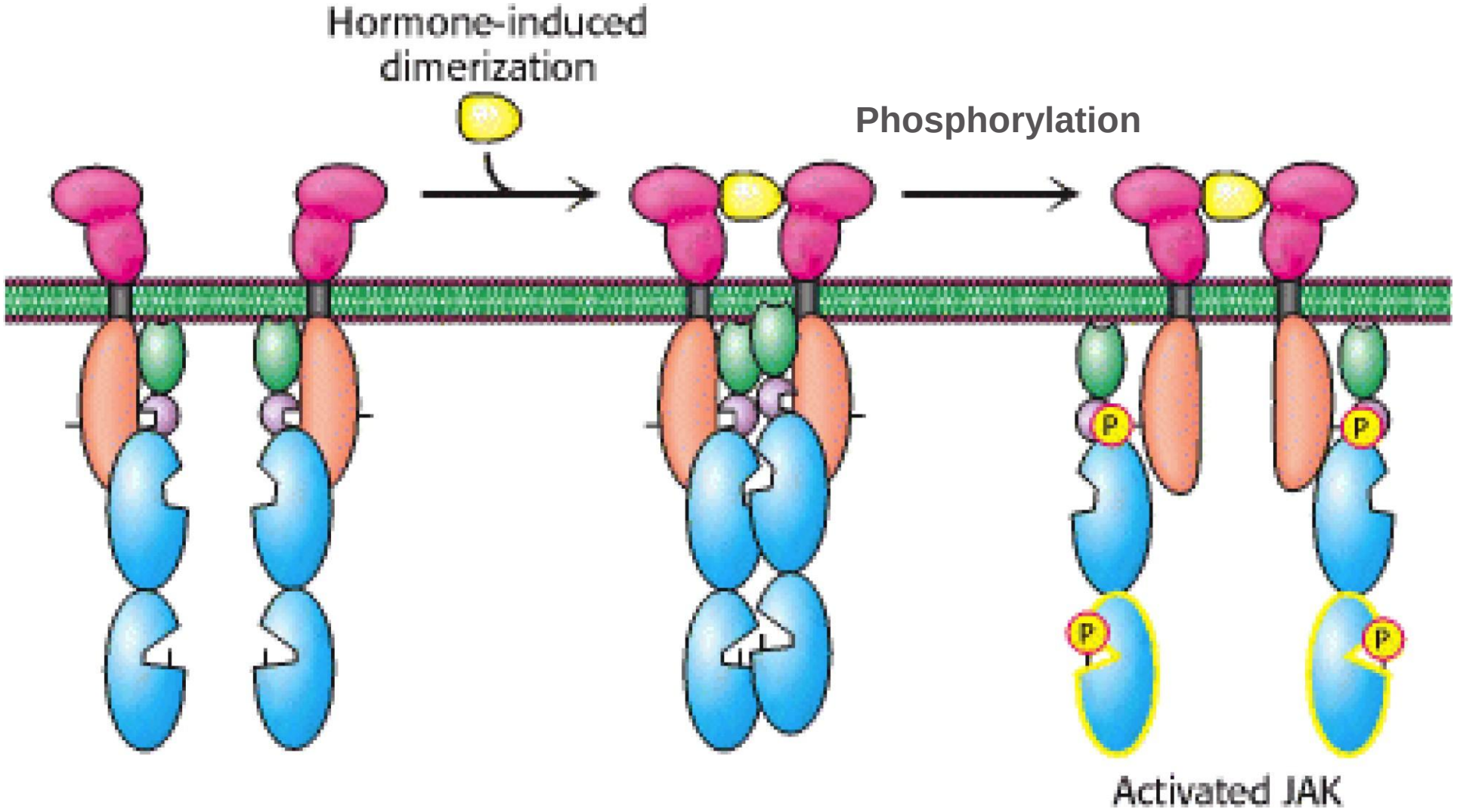
Janus

Each Intracellular Domain is associated with 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
  - Signal Transducers & Activators of Transcription
- Regulator of transcription
- STAT Phosphorylation

  ➔ **Dimerization**

  ➔ Binding to specific DNA sites
- If JAK2 remains active it will produce **Cancer**

STAT is phosphorylated on a tyrosine residue near the carboxyl terminus

Phosphorylated tyr binds to SH2 domain of another STAT molecule