بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Endocrine Biochemistry | Final 4 + 5

Transduction of Hormonal Signals

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The longest Modified Slide in human history

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! → intrinsic means that the receptor it self is an enzyme.
  - Transmembrane
  - Intra- & extracellular domains
- Is that enough? The need for 2nd messenger
  - Few in number
  - Restricted movement

Signal Transduction

- Signal transduction involves the conversion of an **extracellular signal into a specific intracellular response** through a multicomponent system involving:
  - ***Receptors.***
  - ***Second messengers.***
  - ***Effectors.***

- This process enables remarkable **amplification**, allowing the very small amounts of hormones circulating in the blood to generate strong responses because **one molecule generates many intracellular signals**.

- Signal transduction allows the cell to **integrate many signals from multiple hormones**.
- For example, a hepatocyte might have **insulin, glucagon, and epinephrine simultaneously bind to their corresponding receptors and determine the appropriate response.**

- Signal transduction involves the conversion of an **extracellular signal from a chemical messenger to an intracellular biochemical change**.

- **Amplification is a MUST.**

Signal Transduction

- A single molecule can trigger a response that is greater in magnitude than the initial signal.
- The receptors are **integral (Transmembrane) membrane proteins**, with:
  - An **extracellular domain for ligand binding.**
  - **Intracellular signaling domains.**
- When the ligand binds, it induces **conformational changes in the receptor**, allowing downstream interactions.
- The need for a **second messenger** arises from the fact that receptors are restricted to the plasma membrane and are limited in number **(For the need of amplification)**,

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

Second Messengers

- Second messengers are **spatially regulated** → They are not found in the same amount throughout the entire cell, which allows further regulation.
- This occurs because receptors are located in distinct **membrane microdomains**, creating spatially restricted pools.
- Different cell types express different downstream targets.
- There are many other mechanisms that explain the **specificity of each hormone**, even though their corresponding second messengers may be the same.
- Many drugs act by exploiting these mechanisms.
- Second messengers can be small molecules:
  - **cAMP**
  - **cGMP**
  - **Ca^{2+}**
  - **DAG**
  - **IP_3**
- These can **diffuse and amplify signals.**
- Alternatively, **signaling** can occur through **phosphorylation cascades via different kinases**.
- Second-messenger pathways can be targeted therapeutically; for example, **phosphodiesterase inhibitors** inhibit the conversion of cAMP/cGMP to AMP/GMP.

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

Signal Termination

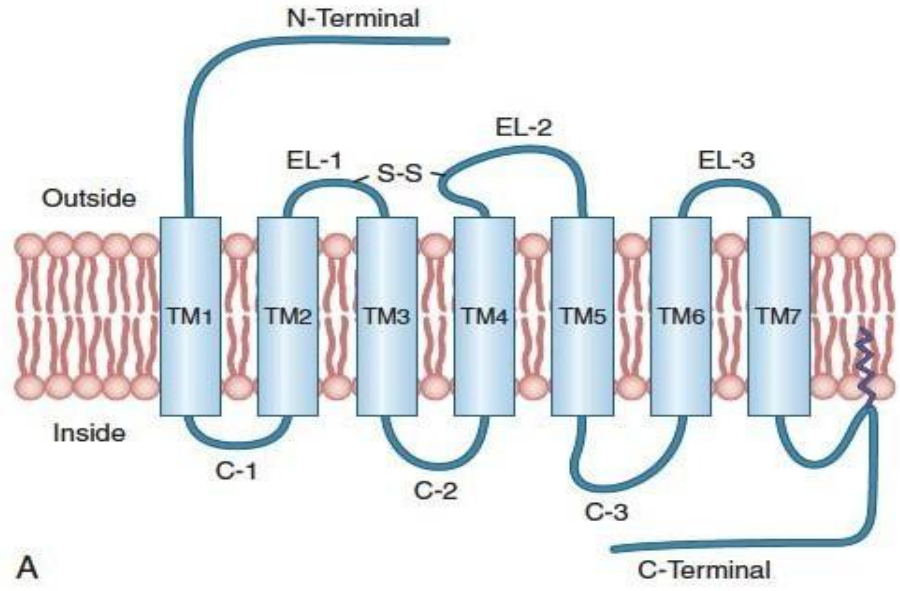
- **Signal termination is as critical as initiation because it:**
  - Prevents pathology
  - Regulates cellular responsiveness
- If signals persist:
  - Cells become **desensitized** and **stop responding to signals**.
  - **Sustained signaling** may contribute to **cancer**.
- Termination occurs through several mechanisms:
  - **Hormone dissociation from the receptor.**
  - **Second-messenger degradation.**
    - Example: **cAMP is hydrolyzed by phosphodiesterase.**
  - **Dephosphorylation by phosphatases.**
  - **Receptor internalization via endocytosis and lysosomal degradation** of the ligand-receptor complex, with receptor recycling or degradation.

Failure of Signal Termination

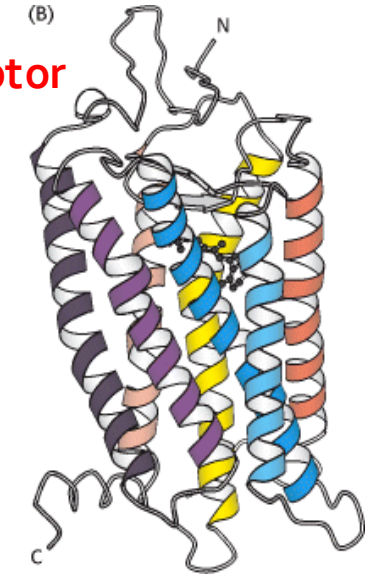
- Failure of termination can occur with:
  - **Mutations** impairing **GTPase activity**.
  - **Cholera toxin**, which **permanently inhibits intrinsic GTPase activity.**
  - **Mutations that impair receptor internalization.**
  - **Phosphatase defects.**
- **Clinically:**
  - **Phosphodiesterase (PDE)** inhibitors **inhibit cGMP breakdown**.
  - This prolongs the **vasodilatory effect (increased cGMP → decrease intercellular Ca+2 → muscle relaxation → vasodilation).**
- **Biased agonists** are engineered to stabilize a specific receptor conformation that activates only a specific subset of downstream signaling pathways.

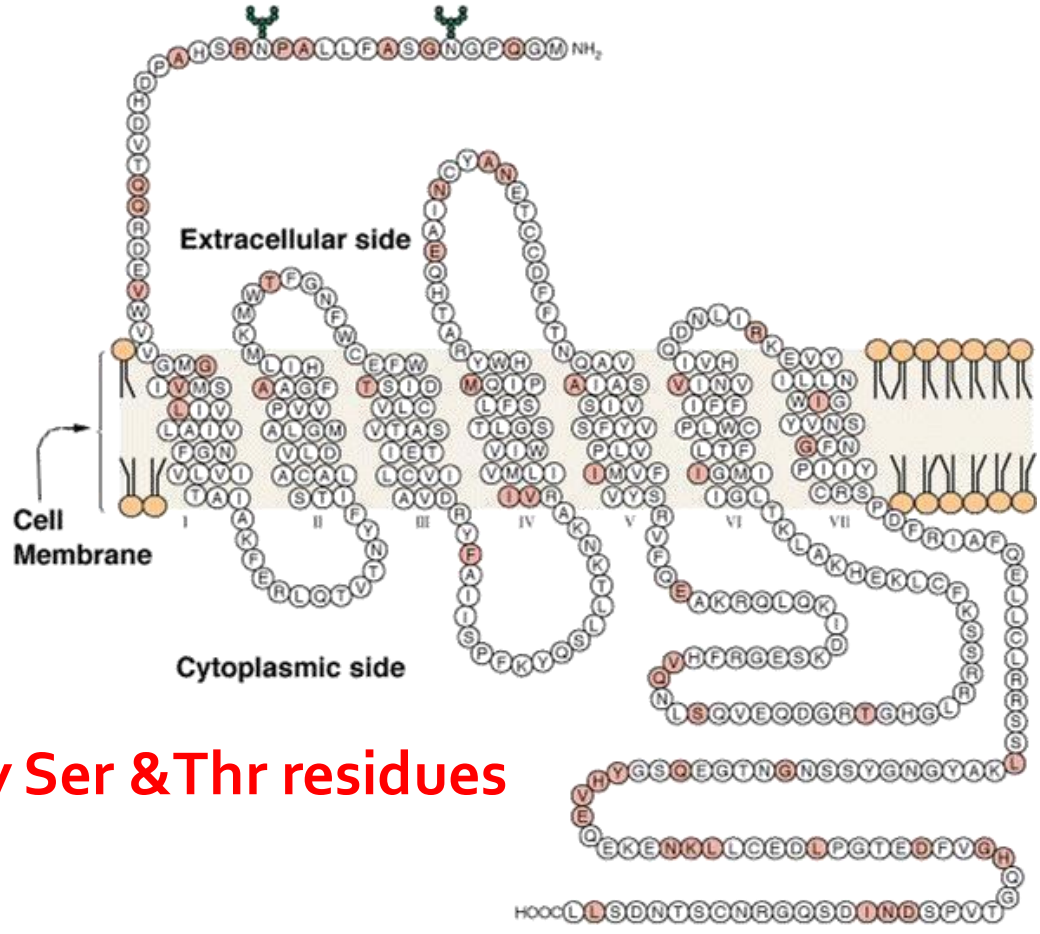
Membrane Associated Receptors
7-Transmembrane Helix Receptors (7TM)

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



Rhodopsin receptor

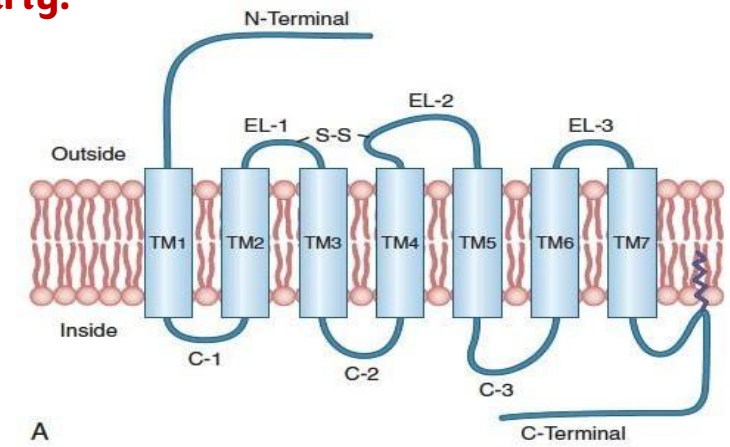


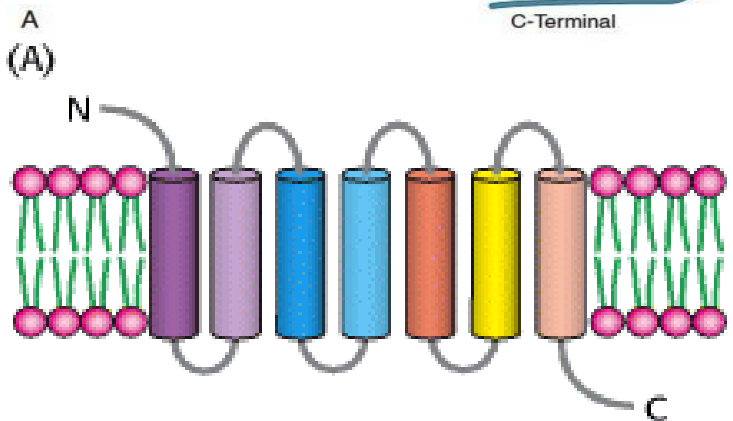


- **Many Ser &Thr residues**

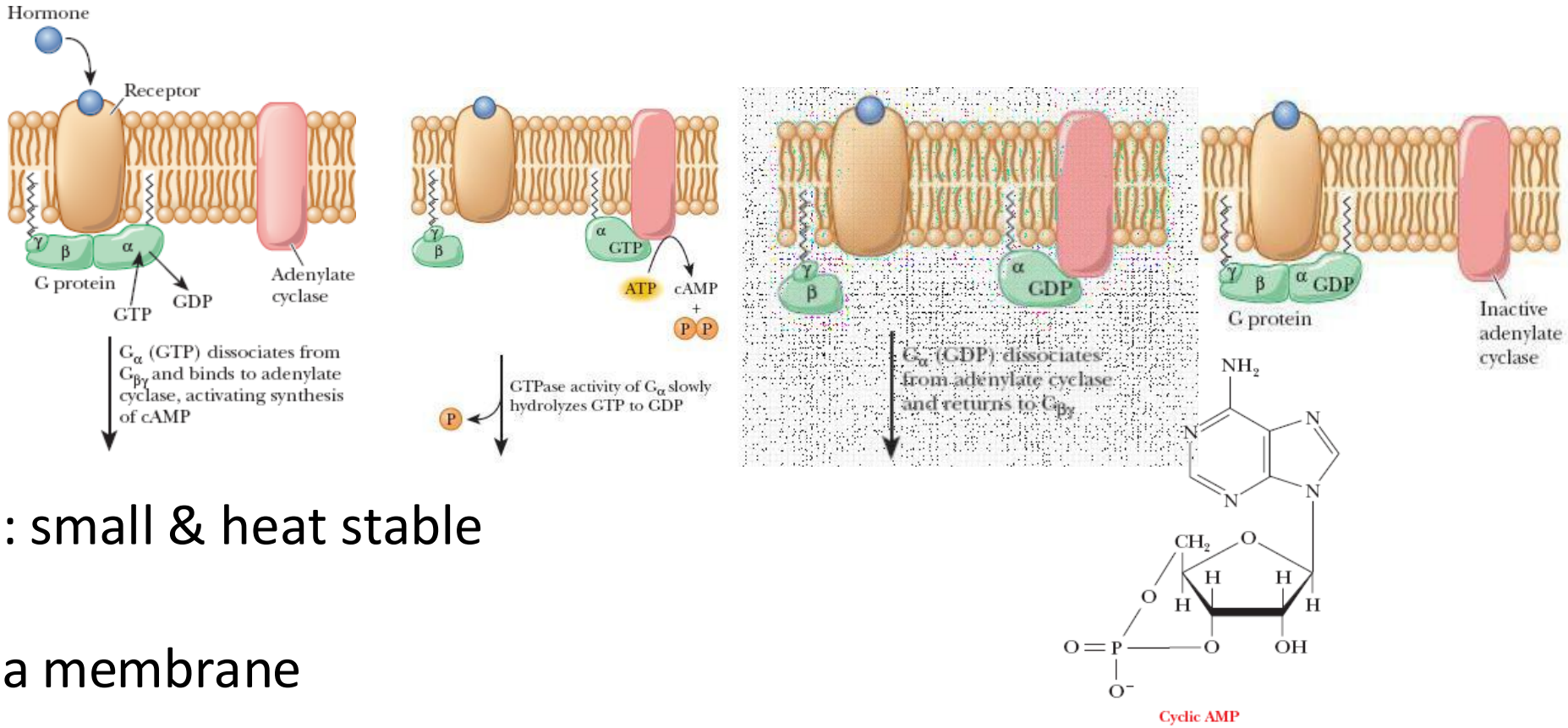
Membrane Associated Receptors (7-Transmembrane Helix Receptors (7TM))

- **GPCRs are the largest receptor family** and are targets of many drugs.
- They have **7 hydrophobic transmembrane domains** that form a rigid structure spanning the membrane.
- **The ligand-binding site is formed by the helices or the extracellular N-terminal domain.** The helices are stabilized by **hydrogen bonds**.
- Ligand binding induces **conformational changes** that are **transmitted intracellularly.**
- This allows interaction with the nearby **G-protein** and **activation of downstream effectors.**
- **Rhodopsin receptors** are located in **rod cells of the retina and are activated by light.**
- These receptors contain many **serine and threonine residues**, which **can be phosphorylated by GPCR kinases.**
- Phosphorylation **creates binding sites** for **β-arrestin**, which:
  - **Inhibits further signaling**
  - **Promotes receptor internalization**
- GPCRs virtually regulate every physiological process.
- There are **over 500 GPCRs**, allowing **signaling specificity.**





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 & cAMP

- **cAMP:**
  - Small.
  - Heat stable.
  - Acts at the **plasma membrane signaling pathway.**

- Pathway:

Hormone → Specific receptor (β1/β2-adrenergic receptor) → G protein → Adenylate cyclase → cAMP → Protein kinase A → Phosphorylation.

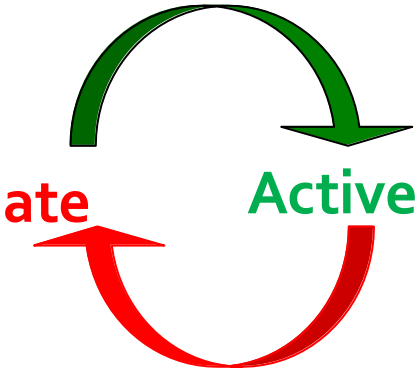
- The cAMP pathway **begins when the hormone binds its receptor.**
- The activated receptor functions as a **GEF (guanine nucleotide exchange factor).** It exchanges **GDP for GTP** on the α-subunit of the heterotrimeric G-protein.

- The α-subunit dissociates from the βγ subunits and activates **adenylyl cyclase** which is a membrane enzyme that converts **ATP → cAMP.**

- cAMP acts as a second messenger and binds to **protein kinase A (PKA)** which phosphorylates **serine or threonine residues** on proteins.

- The pathway **is highly amplified:**
  - **One** receptor activates **many G-proteins.**
  - **These** activate **many adenylyl cyclase molecules.**
  - **Each adenylyl cyclase** catalyzes **many ATP → cAMP conversions.**

G-proteins cycles between two forms



Inactive State — **Active State**

Bound to GDP — **Bound to GTP**

Heterotrimer αβγ — **Dissociated α + βγ dimer**

Activated Receptor

↑ Adenylate Cyclase

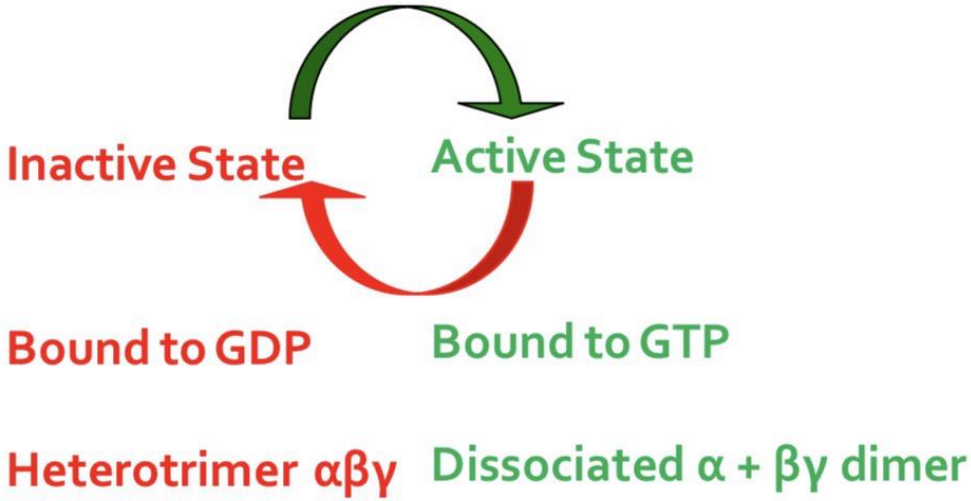
↑ cAMP

Biochemical Response

α subunit has GTPase Activity

G-proteins cycles between two forms

- The **α-subunit** has intrinsic **GTPase activity**.
- It hydrolyzes:
  GTP → GDP
- This **inactivates** the α-subunit and **allows it to reassociate with the βγ subunits**, reforming the **heterotrimeric G-protein**.
- **Phosphodiesterase (PDE)** regulates the pathway by hydrolyzing:
  cAMP → AMP
- **Feedback phosphorylation: GPCR kinases phosphorylate the receptor** to **terminate the signal** and **induce receptor internalization.**
- **Phosphoprotein phosphatases dephosphorylate** proteins, also **contributing to signal termination**.
- **GLP-1 agonists** use a GPCR to:
  - Increase insulin secretion
  - Inhibit glucagon secretion.



α subunit has GTPase Activity

G-proteins cycles between two forms

- G-protein is regulated by cycling between:
  - **Inactive form:** GDP-bound
  - **Active form:** GTP-bound

- The **α-subunit** is **associated** with the **βγ subunits** in the **inactive state**.

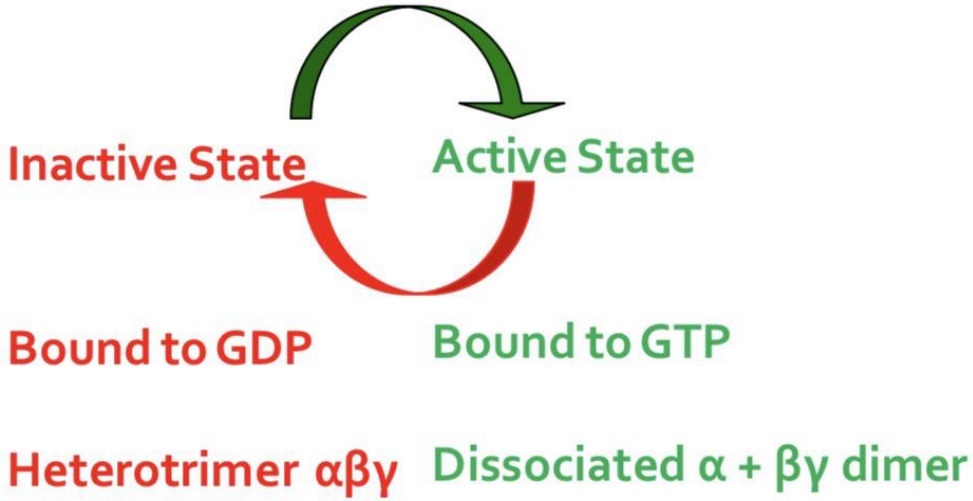
- Upon **activation**, the **α-subunit dissociates** from βγ.

- GTP-bound α-subunit activates downstream effectors such as:
  - **Adenylyl cyclase**
  - **Phospholipase C** through GqPCR.

- The active state terminates through the intrinsic **GTPase activity** of the **α-subunit**:

  GTP → GDP

- This process is accelerated by **GTPase-activating proteins (GAPs)**.
- **GDP-bound α-subunit reassociates** with the **βγ subunits** and **returns to the inactive state**.



α subunit has GTPase Activity

G-proteins: stimulatory or inhibitory?

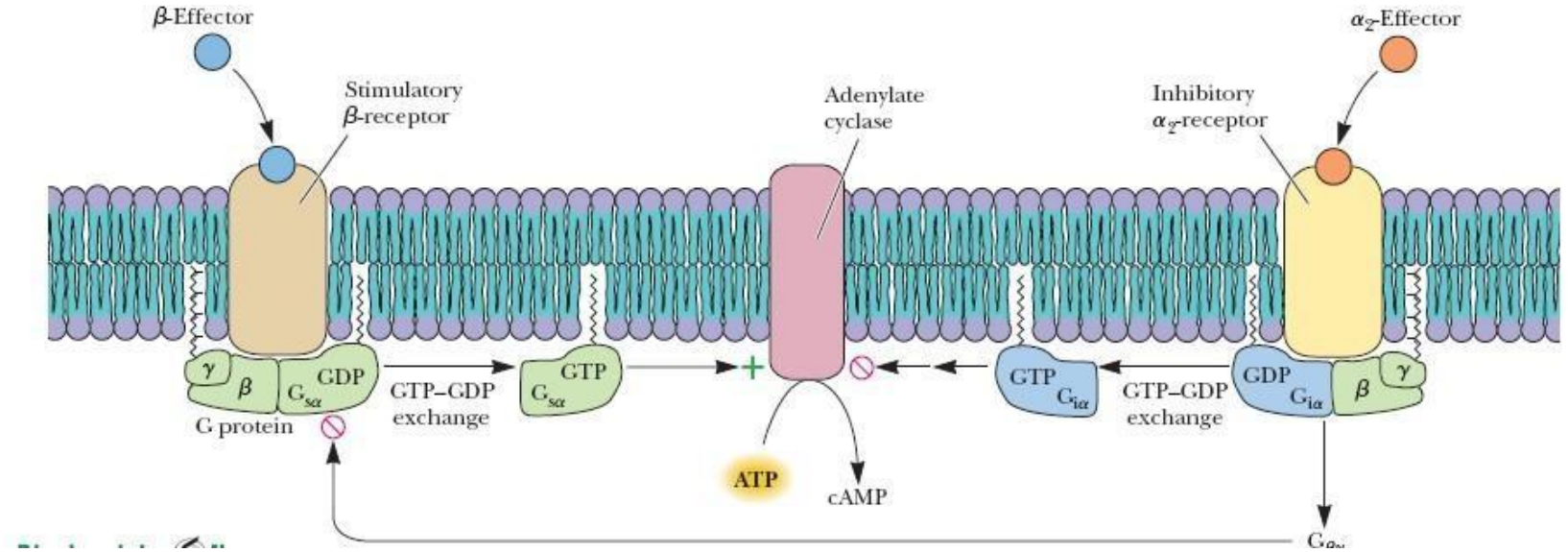
- **Different G-protein subtypes bind to different receptors and produce distinctive effects :**

- **Gs**
  1. Stimulates adenylate cyclase.
  2. ↑ cAMP.
  3. Examples include:
     1. β-adrenergic receptors
     2. Glucagon receptor
     3. TSH receptor
     4. GLP-1 receptor

- **Gi**
  1. Inhibits adenylate cyclase.
  2. ↓ cAMP.
  3. Examples:
     1. α_2-adrenergic receptors
     2. Somatostatin receptor
     3. M_2 muscarinic receptor

- **Gq**
  1. Activates **phospholipase C (PLC)**.
  2. PLC cleaves **PIP_2** into:
     1. DAG
     2. IP_3
  3. DAG activates PKC.
  4. IP_3 binds to ligand-gated Ca^{2+} channels on the **SER**, causing Ca^{2+} release.
  5. Examples:
     1. α_1-adrenergic receptor
     2. Angiotensin II receptor
     3. ADH V_1 receptor.

G-proteins: stimulatory or inhibitory?



- Cyclic AMP & G Proteins:

  - ✓ Hormone → receptor (α2-receptor) → G **inhibitory** protein → inhibits adenylate cyclase

G-Proteins

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

G-Proteins

❑ **G-Protein Specificity:**

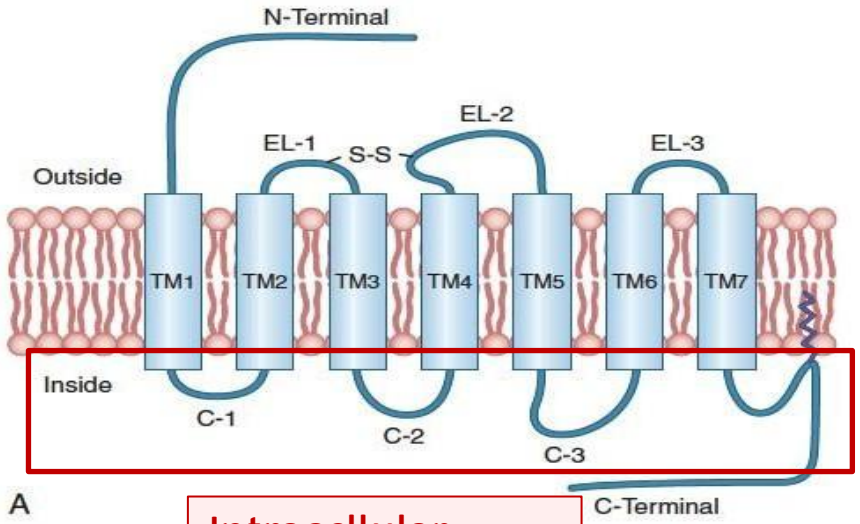
- Receptor–G-protein specificity is determined by **intracellular loops**.
- The intracellular part of the receptor, along with the C-terminal tail, **has a specific amino-acid sequence.**
- This forms a **unique 3D structure complementary to the G-protein α-subunit.**

❑ **Drug selectivity:**

- β1-selective blockers such as **atenolol** target cardiac β1-GPCRs.
- β2 agonists such as **albuterol** target Gs and activate it.

❑ **G-Proteins and Multiple Signaling Pathways**

- More than **100 known GPCRs** and more than **20 known G proteins**.
- G-proteins can be activated by combinations of hormones.
- Example:
  - **Epinephrine + glucagon** act through **stimulatory G-proteins in liver cells**.
- G-proteins can act through pathways other than cAMP:
  - Stimulating phospholipase C.
  - Opening or closing membrane ion channels.
- Each pathway has a distinct **tissue distribution**.



Intracellular loops

G-Proteins

G-Protein Effects Beyond Enzyme Activation

- Even when multiple hormones use the same G-protein, the **effector combination for each hormone can be unique.**
- **Epinephrine and glucagon** both act through **Gs in the liver**, and **their glycogenolytic** effects are **additive**.
- G-proteins **regulate effectors** such as:
  - **Phospholipase C. (PLC)**
  - **Adenylyl cyclase**

- G-proteins also have effects **beyond enzyme activation:**
  - The **βγ subunit** can directly open **GIRK channels** (**G-protein-coupled inwardly rectifying potassium channels**).
  - This causes **K^+ efflux → hyperpolarization → Repolarization → relaxation of the heart.**
  - **Gα** can activate proteins involved in **cytoskeletal remodeling**.

- Therefore, G-proteins can regulate processes beyond simple enzyme activation/inhibition.

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

Each G-Protein will activate one Adenylate Cyclase

- **Amplification: receptor → 100's of G protein → 100's of adenylate cyclase → 100's X 1000's molecules/sec of cAMP** Because of this amplification, small changes in signalling components can lead to profound cellular effects.

G-Proteins

G-Protein Amplification

- G-proteins have lipid modifications that anchor them to the **inner leaflet of the plasma membrane.**
- The α-subunit can be:
  - **Myristoylated**
  - **Palmitoylated**

- The γ-subunit can be:
  - **Farnesylated**
  - **Geranylgeranylate**

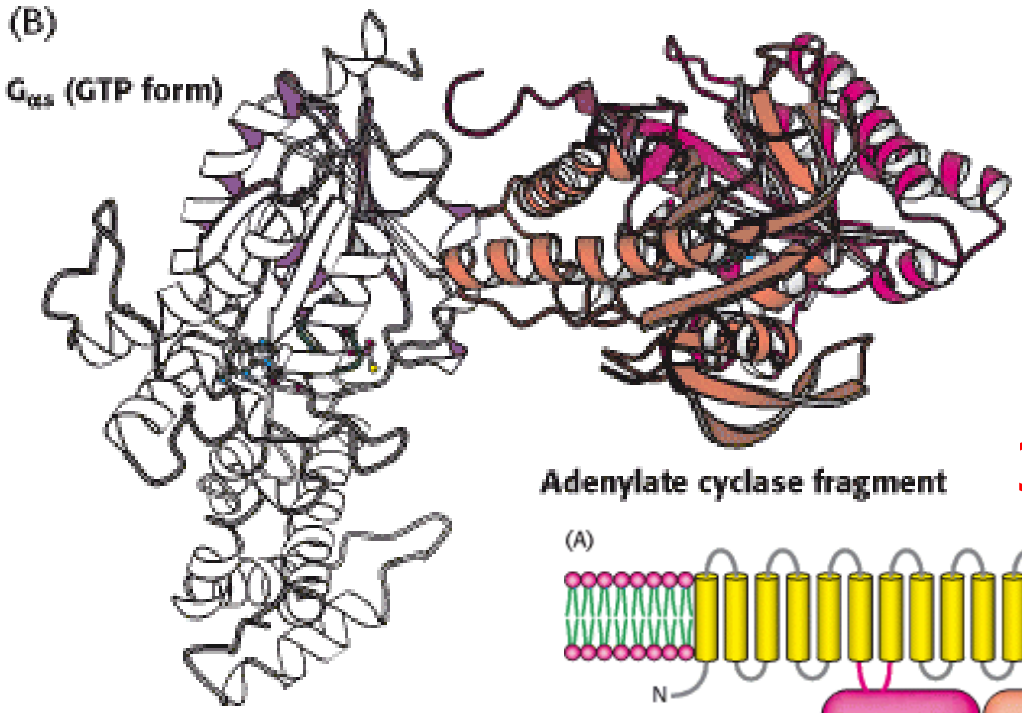
- These modifications are essential for **proper localization of G-proteins.**
- Mutations that prevent these modifications **can disrupt signaling.**

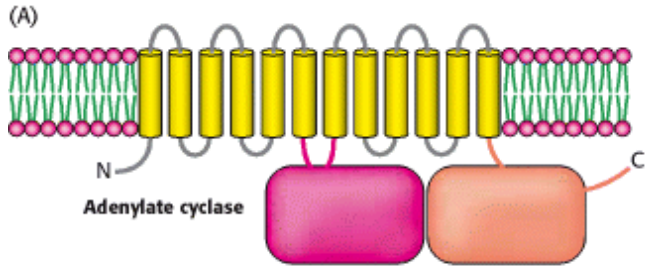
- Amplification:

Receptor → hundreds of G-proteins → hundreds of adenylyl cyclase molecules → hundreds × thousands of cAMP molecules/second.

Signal Transduction

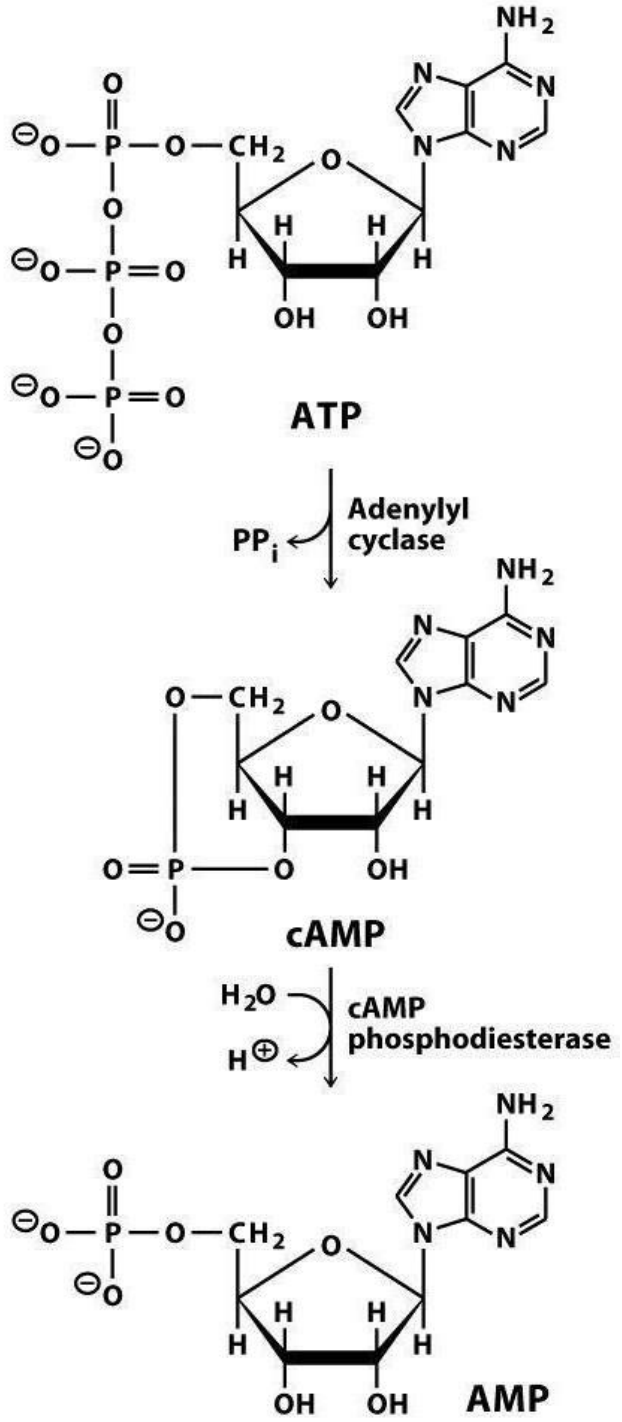
Adenylate Cyclase





3'5' cAMP

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



Adenylate Cyclase

- Adenylyl cyclase is a **large integral membrane enzyme**.
- It has **12 transmembrane helices** anchoring it to the membrane.
- It has **two large intracellular domains** that **form the catalytic site**.
- The **Gα subunit** binds at the **interface** and **induces conformational changes which activates the enzyme**, and converts :: **ATP → cAMP + PP_i**

- There are **9 membrane-bound isoforms** in mammals.
- They have different tissue distributions and kinetics ::

1) Some are activated by **Gs**.
2) Others are inhibited by **Gi**.
3) Some are regulated by **Ca^{2+}**.

- **Gi-GTP** inhibits adenylyl cyclase activity.

- The **Gs/Gi balance** determines cellular cAMP levels.
- In the heart:
  - **β1 receptors → Gs → ↑ cAMP → ↑ heart rate**
  - **M2 receptors → Gi → ↓ cAMP → ↓ heart rate**

- This is the basis for:
  - β-agonists increasing cAMP in **heart failure**.
  - β-blockers decreasing cAMP in **hypertension**.

cAMP can affect a wide range of cellular processes

- ↑ degradation of storage fuels : Gbuconeogensis & Glycogenolysis in the liver, and lipolysis in adipose tissues.
- **↑ secretion of acid by gastric mucosa (caffeine: phosphodiesterase & adenosine)**
- Dispersion of melanin pigment granules
- ↓ aggregation of blood plateletes
- Opening of chloride channels

cAMP : Diverse effects

Caffeine:

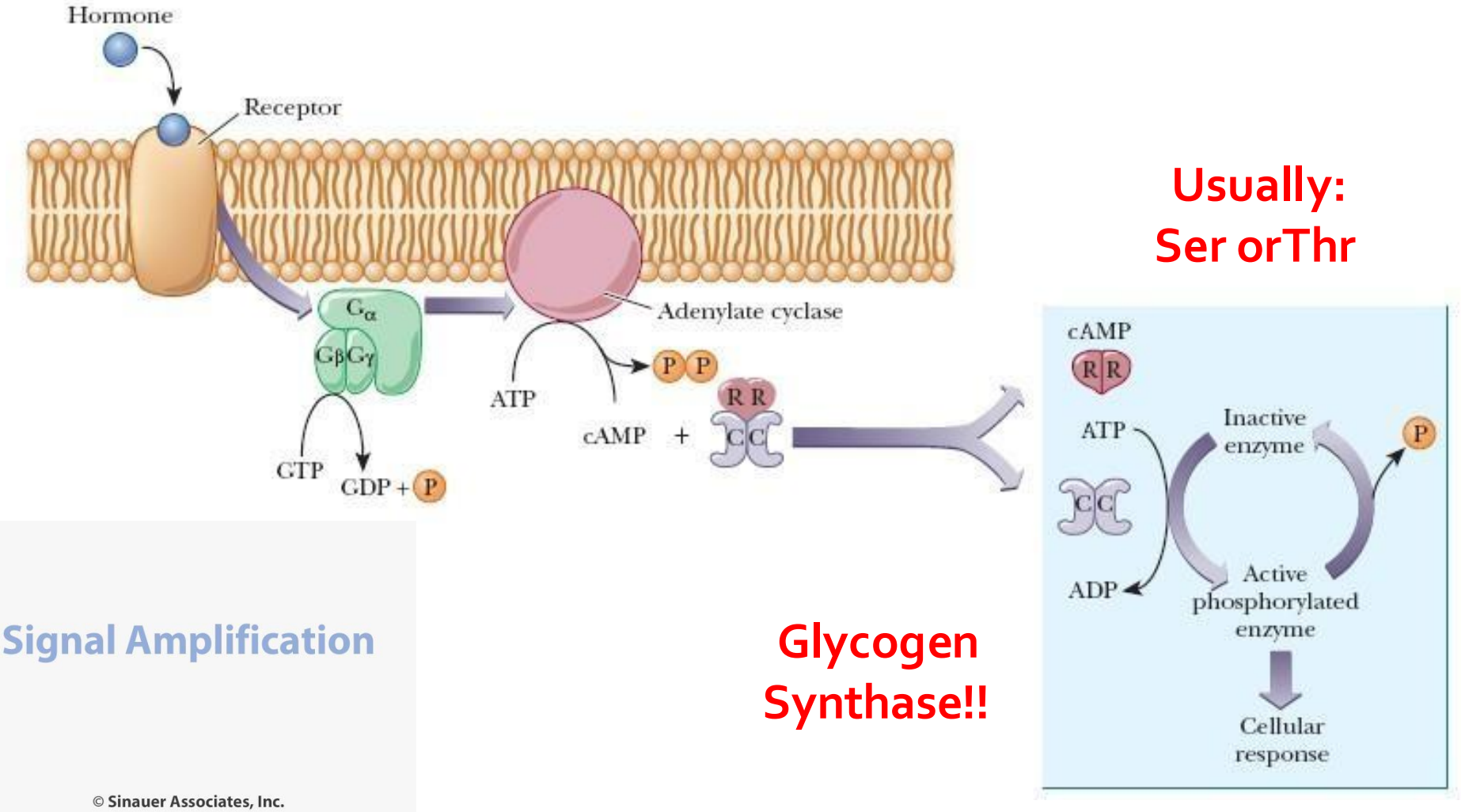
- Inhibits phosphodiesterase.
- Which normally convert cAMP → AMP, results : ↑ cAMP.
- **Can increase gastric acid secretion which may exacerbate ulcers.**

- cAMP has diverse effects on the body, demonstrating its **pleiotropic nature**.
- In platelets:
  - *Prostacyclin* binds to **GsPCR**.
  - This **decreases platelet aggregation**.
- In airway epithelium:
  - **cAMP-dependent PKA phosphorylates CFTR.**
  - **CFTR opens channels and allows Cl^- to leave the cell.**
  - **β2 agonists therefore improve mucus clearance in asthma and cystic fibrosis.**

- In specialized pigment cells of fish and amphibians:
  - ↑ cAMP causes pigment granules to scatter from the center toward the periphery.
  - This makes the animal's skin appear darker.

- The effects of cAMP in target cells are largely mediated through activation of **PKA**.
- PKA phosphorylates different substrates in different cells, explaining the diversity of cAMP effects.
- PKA recognizes a consensus phosphorylation sequence: **Arg-Arg-X-Ser/Thr**
- The target proteins vary depending on the cell type.

Then What?



Usually: Ser orThr

Glycogen Synthase!!

Signal Amplification

Glycogen Synthesis

- PKA phosphorylates **glycogen phosphorylase, activating** it → **glycogen breakdown.**

- PKA phosphorylates **glycogen synthase, inactivating** it → **prevents glycogen synthesis.**
- Therefore:

  PKA → ↑ glycogenolysis + ↓ glycogenesis

- This ensures **rapid mobilization of stored glucose.**

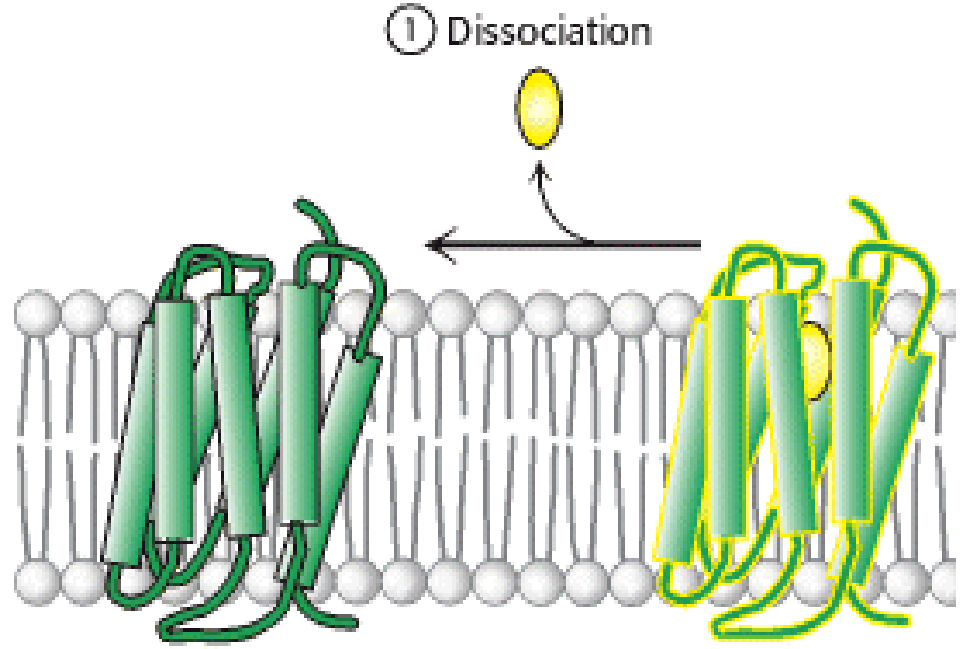
- **PKA** also **phosphorylates transcription factors** such as **CREB (cAMP response element-binding protein), regulating gene expression.**

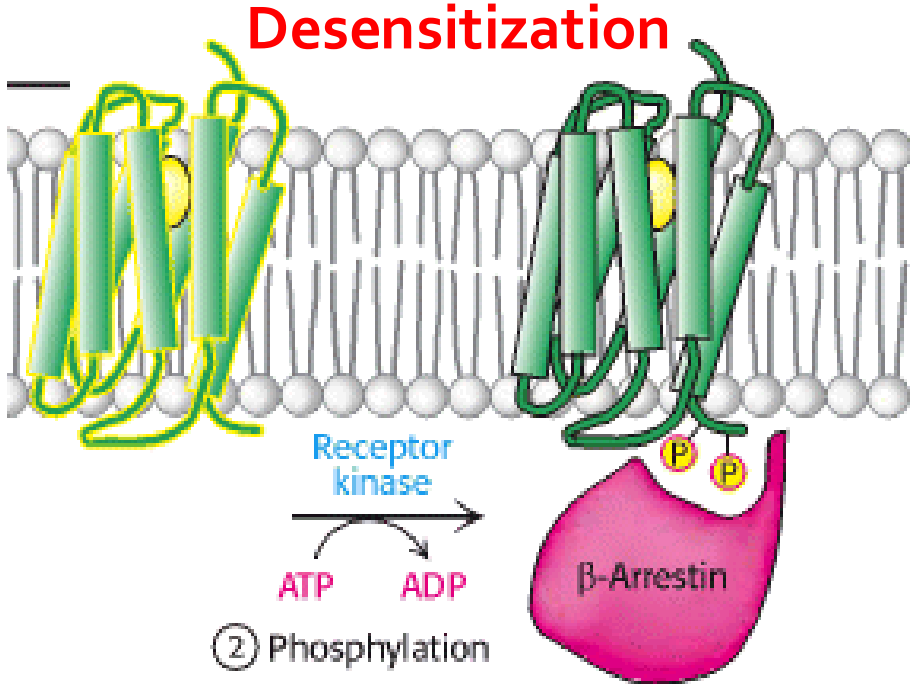
- This explains why cAMP can produce both **rapid and long-term effects.**

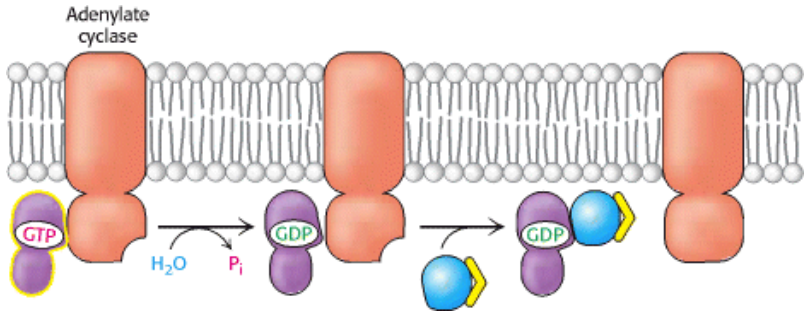
- **Phosphorylation** and **dephosphorylation** are the primary mechanisms of **cAMP signaling regulation.**

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







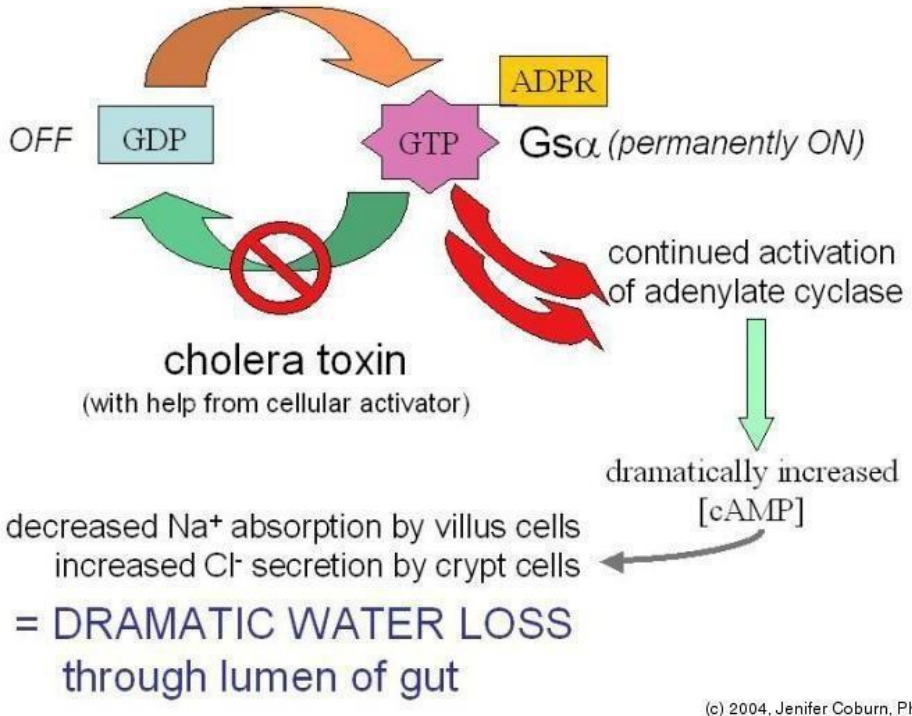
Switching off the signal

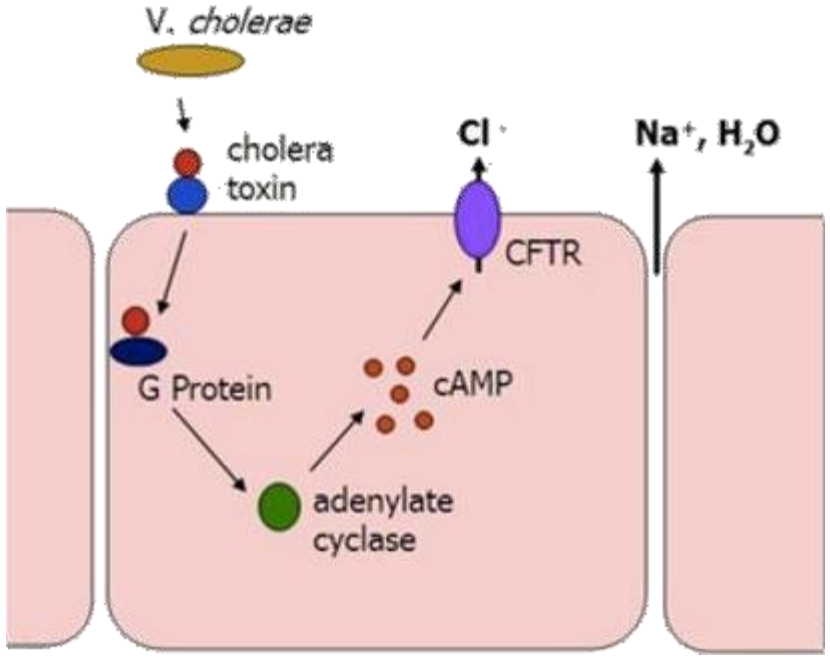
- Termination of the signal happens through different mechanisms:
  - **Dissociation of the hormone from the receptor** when its concentration falls.
  - The **Gα subunit** has **intrinsic GTPase activity → hydrolyzes GTP → GDP, inactivating** the enzyme and allowing **reformation of the G-protein.**
    - This process is accelerated by **GAPs.**
  - **Phosphodiesterases (PDEs)** hydrolyze cAMP to inactive **5′-AMP.**
    - Different PDE isoforms allow tissue-specific regulation.
  - **Receptor desensitization:**
    - **GPCR kinases phosphorylate the active receptor.**
    - This **recruits β-arrestin.**
    - β-arrestin **prevents further G-protein binding** and **promotes receptor internalization.**

- Clinically:
  - PDE inhibitors inhibit cGMP degradation.
  - ↑ cGMP → ↑ vasodilation.
  - **Caffeine and theophylline** are non-selective PDE inhibitors.

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





Cholera

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

- Cholera toxin has **2 subunits:**
  - **B subunit:** binds **to GM1 ganglioside receptor on the surface of epithelial cells.**
  - **A subunit:** has **ADP-ribosyltransferase activity.**

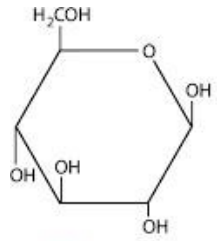
- The **A subunit** transfers an **ADP-ribose group from NAD^+ to Gαs.**
- This prevents the **intrinsic GTPase activity of Gα Subunit.**
- Gα subunit becomes locked in its **active GTP-bound state.**
- **Continuous stimulation of adenylate cyclase → ↑ cAMP.**

- ↑ cAMP causes:
  - **CFTR phosphorylation.**
  - **Massive Cl^- efflux.**
  - **Inhibition of Na^+ absorption,**
  - **Loss of water without reabsorption.**

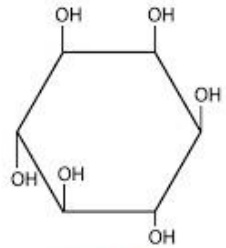
- Clinically treated with **rehydration, since we lose large amount of water in diarrhea.**

The Phosphoinositide Cascade

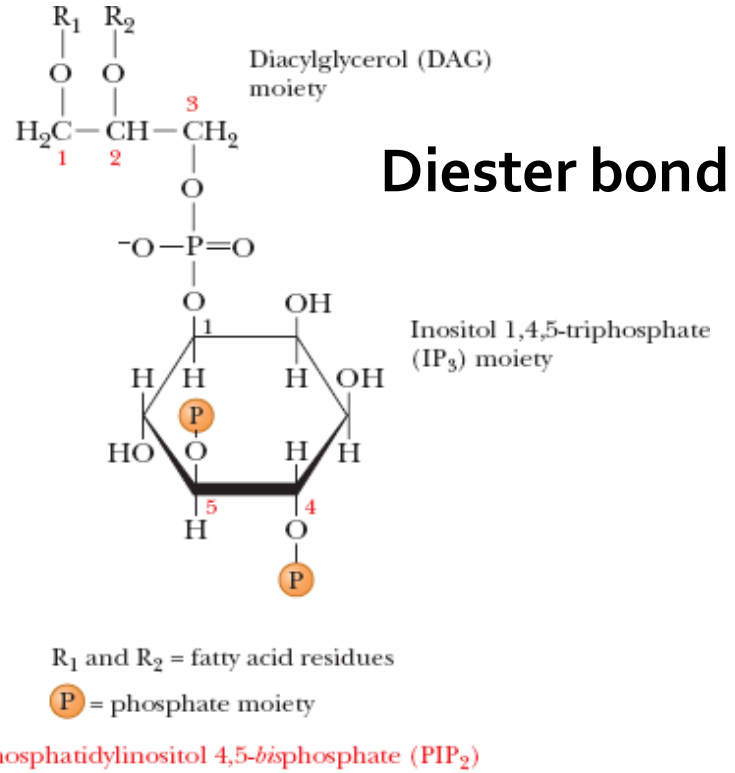
- Used by many hormones (e.g. ADH)
- Binding of a hormone to 7TM receptor



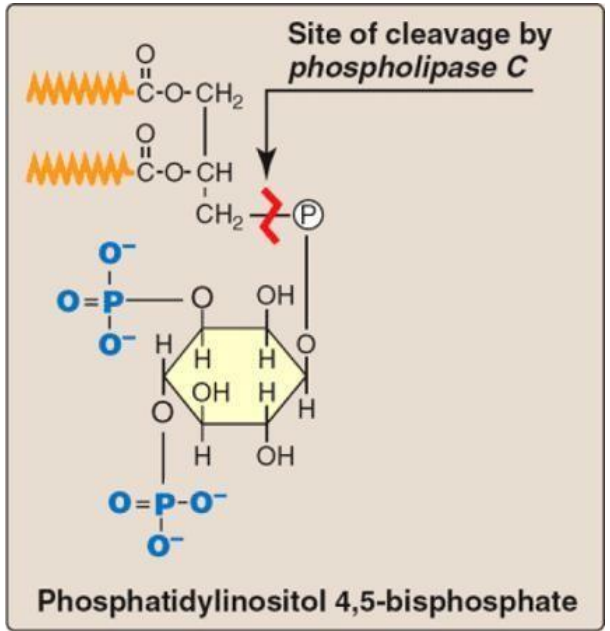
Activation of G Protein



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 Phosphoinositide Cascade

- The **second major GPCR pathway**, activated by **ADH (V1), oxytocin, TRH, Angiotensin II, and α1 adrenergic receptors.**

- Hormone binding to the receptor leads to **conformational change that leads to GEF activity,** converting **GDP with GTP.** The **GTP-bound α** subunit **dissociates and activates phospholipase Cβ (PLCβ).**

- This enzyme **hydrolyzes membrane PIP2** (phosphatidylinositol 4,5-bisphosphate) into 2 second messengers:

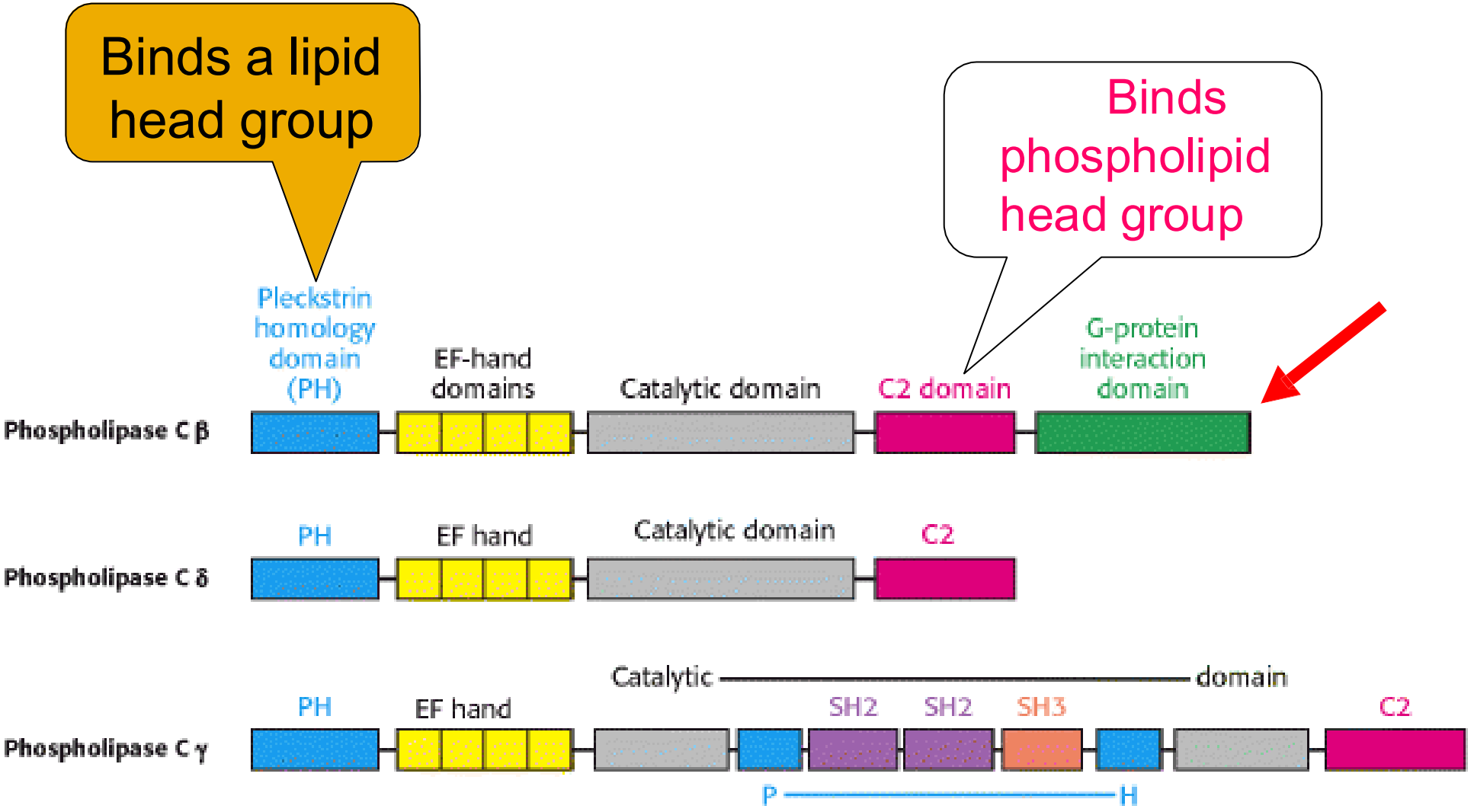
1) **inositol 1,4,5-triphosphate**, which is **soluble** and can diffuse to the cytoplasm and **binds to Ca+2 channels on SER, releasing it**.
2) **DAG** is the 2nd second messenger, and it is **amphipathic** and **remains in the membrane**, where it **activates PKC**.

✓ Each one produces distinct but coordinated effects.

- This pathway is **essential for smooth muscle contraction (vasoconstriction), secretion, and cell proliferation.**

- Dysregulation is seen in cancer and CVD, which is why pharmaceutical agents target it. PKC inhibitors and Ca+2 channel blockers are targeted.

The domain structures of three isoforms of Phospholipase C



The domain structures of three isoforms of Phospholipase C

- PLC *exists* in **3 major isoforms**:
  - **PLCβ**
    - Activated by the α-subunit of G-proteins, especially **Gq**.
  - **PLCγ**
    - Activated by **receptor tyrosine kinase-mediated tyrosine phosphorylation**.
  - **PLCδ**
    - Activated by **Ca^{2+}**.

- All share conserved domains:
  - **PH (Pleckstrin Homology) domain**
    - Membrane localization.
  - **EF-hand domain**
    - Ca^{2+} binding.
    - Ca^{2+} induces a conformational change that helps fully activate the enzyme.
  - **Catalytic domain**
    - Responsible for PIP_2 hydrolysis.

Unique PLC Domains

- Each PLC isoform has unique domains:

The domain structures of three isoforms of Phospholipase C

Unique PLC Domains

- Each PLC isoform has unique domains:
  - **PLCγ**
    - Has SH2 and SH3 domains.
    - These bind phosphorylated tyrosines on receptor tyrosine kinases.
    - Important for RTK signaling.
  - **PLCβ**
    - Has a **G-protein interaction domain** at its C-terminus.
  - **PLCδ**
    - Lacks specific extra domains.

- **Dysregulation of PLC contributes to:**
  - Cancer
  - Inflammation

- Many PLC inhibitors are being investigated as therapeutic agents.

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

Membrane

G Protein

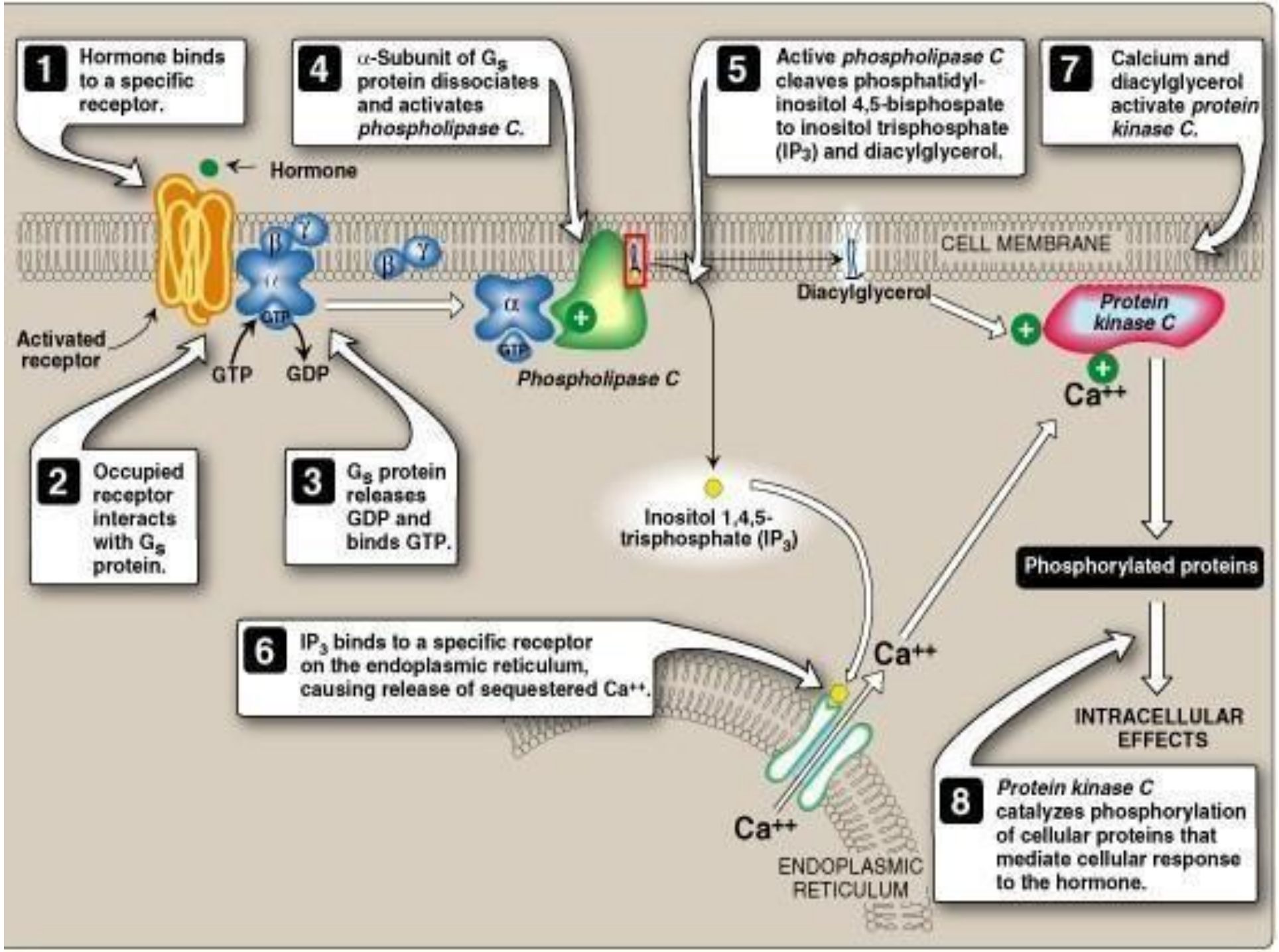
Binding of a G protein brings the enzyme into a catalytically active form

- PLCβ activation by Gαq involves a precise molecular mechanism
- .
- At rest, PLCβ is **autoinhibited**.
- Its catalytic domain is blocked by intramolecular interactions.

- Gαq-GTP binds directly to the **C-terminal region**.
- This induces conformational changes that remove autoinhibition.
- The catalytic domain becomes exposed to PIP_2.
- PIP_2 is hydrolyzed to : **$PIP_2 \rightarrow IP_3 + DAG$**

- The intrinsic GTPase activity of Gαq, together with regulatory mechanisms that phosphorylate PLCβ, ensures tight regulation of the pathway.

These Modifieds won't be free anymore ←


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.

GPCR → PLCβ → IP_3/DAG → PKC Pathway

1. Hormone-Receptor Binding

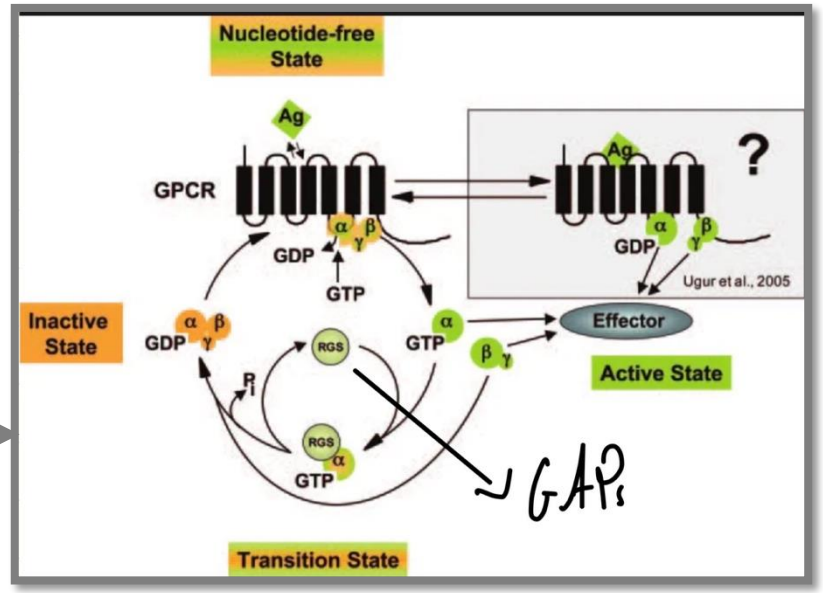
- The hormone binds to its specific receptor.
- Binding-pocket specificity is determined by the receptor's **3D structure**.
- **The same hormone can produce different effects through different receptors:**
  - ADH → **V1: Gq**
  - ADH → **V2: Gs**

Keep an eye on the previous slide picture while reading the explanation since I'm not going to put it, in every slide
(خلص صرت كبير يا غالي)

2. GPCR Activation

- Ligand binding induces a **conformational change** in the receptor.
- This conformational change is **transmitted intracellularly, enabling interaction between the receptor and the G-protein.**
- The associated G-protein is determined by the **intracellular loop sequence** of the receptor.
- The activated receptor acts as a **GEF (guanine nucleotide exchange factor):**
  - Binds to the **α-subunit**.
  - Opens the nucleotide-binding pocket.
  - Causes **GDP release**.
  - Because the cell has a high **GTP/GDP ratio**, GTP binds to the α-subunit.
  - The **GTP-bound α-subunit dissociates from the βγ subunit**.

- **Look at the Digram →** GPCR activation cycle showing **Nucleotide-free State → Inactive State → Transition State → Active State**, with **GDP/GTP exchange, RGS, GAP**, and effector activation. **Clinically:** The receptor-binding step is targeted by many pharmaceutical agents:
  - **Agonists:** bind to and activate the receptor by mimicking hormone action.
  - **Antagonists:** inhibit hormone binding/action.



GPCR → PLCβ → IP_3/DAG → PKC Pathway

3. PLCβ Activation

- The activated **α-subunit** activates membrane-bound **PLCβ**.
- PLCβ cleaves **PIP2** into:
  - **DAG**
  - **IP_3**

4. IP_3 and DAG

- **IP_3 triggers Ca^{2+} release** by binding to **ligand-gated Ca^{2+} channels** on the **SER**.
- These receptors are **tetrameric**, with each subunit containing an **IP_3-binding site**.
- **Binding is cooperative: binding of one molecule makes it easier for subsequent molecules to bind**, producing a **sigmoidal curve**.
- **DAG remains membrane-bound.**
- The increase in intracellular **Ca^{2+} promotes PKC movement toward the membrane**, where **DAG activates PKC**.

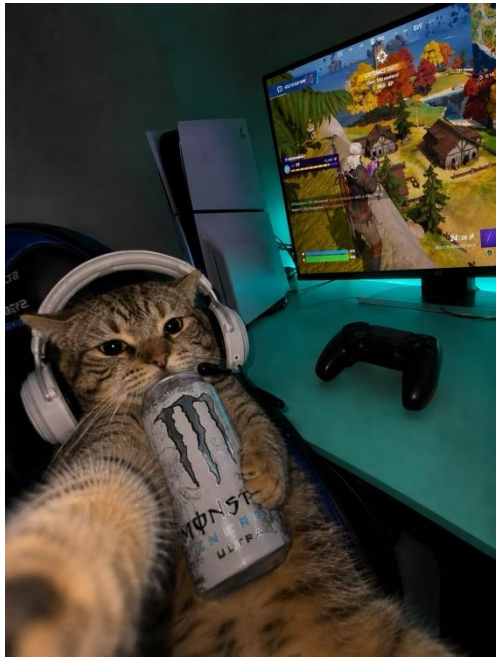
GPCR → PLCβ → IP_3/DAG → PKC Pathway

5. PKC Activation

- **PKC** is a family of **serine/threonine kinases.**
- In the resting state, PKC is **autoinhibited** by a **pseudosubstrate sequence:**
  - A sequence that **closely resembles the enzyme's normal substrates.**
  - It **occupies** the **active site** and **prevents substrate phosphorylation.**
- **Ca^{2+}** binds to the **C2 domain** of PKC.
- **DAG** binds to the **C1 domain** of PKC.
- These interactions **displace the pseudosubstrate from the active site,** enabling **substrate phosphorylation.**

PKC targets include:

- Ion channels
- Receptors
- Enzymes
- Transcription factors



Effects of Second Messengers

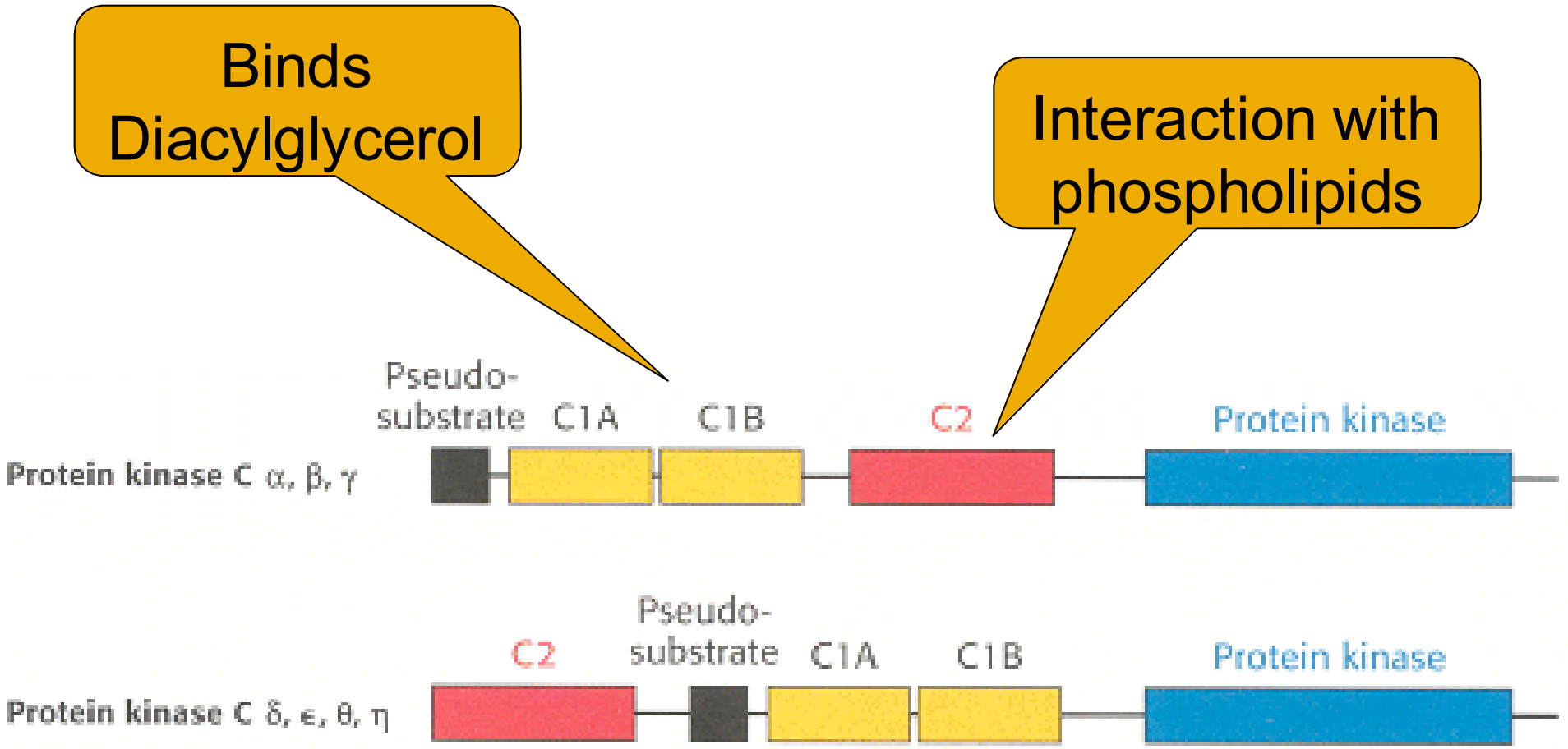
Inositol trisphosphate (IP_3)

- ✓ 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



Tissue Specific Regulation of PKC

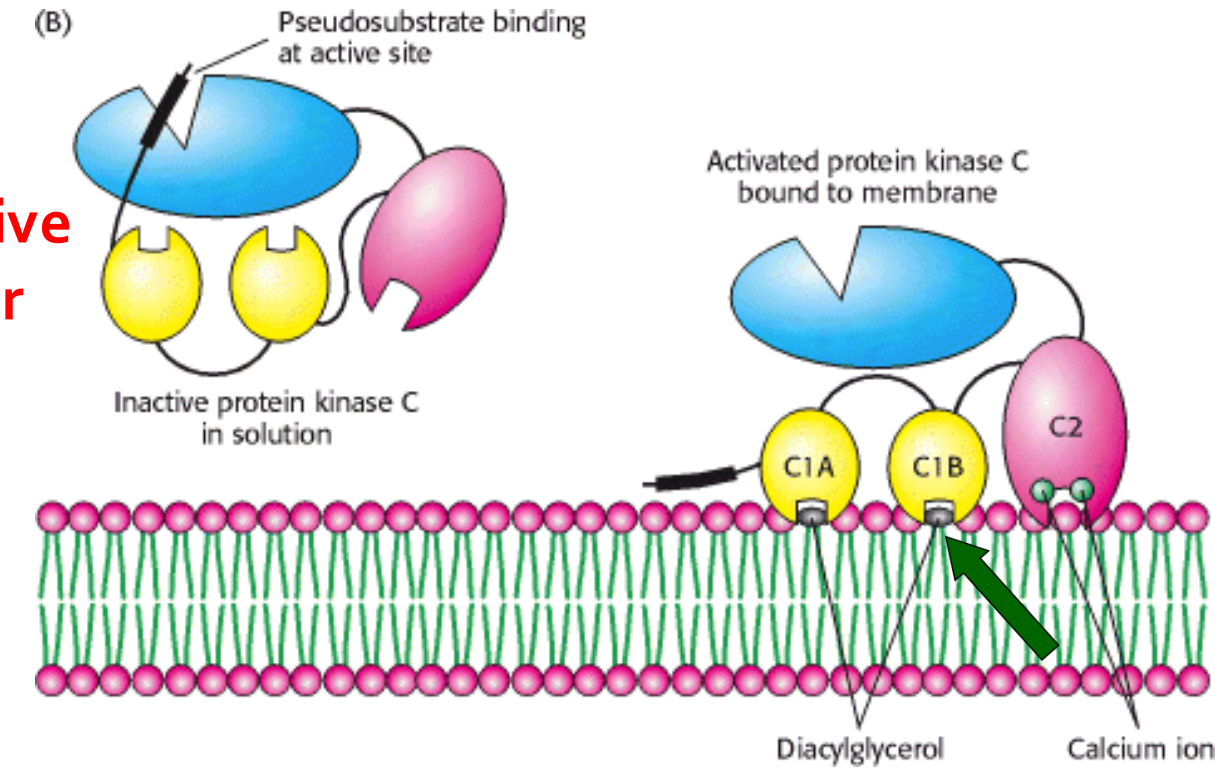
- PKC isoforms allow **tissue-specific regulation**.
- Some PKC isoforms are highly expressed in the **brain**.

- Novel PKCs are expressed in many tissues and are implicated in:
  - **Inflammation**
  - **Cancer**

- Therefore, **isoform-selective PKC inhibitors** are being developed as potential:
  - Anticancer agents
  - Anti-inflammatory agents

Pseudosubstrate Sequence

Competitive Inhibitor



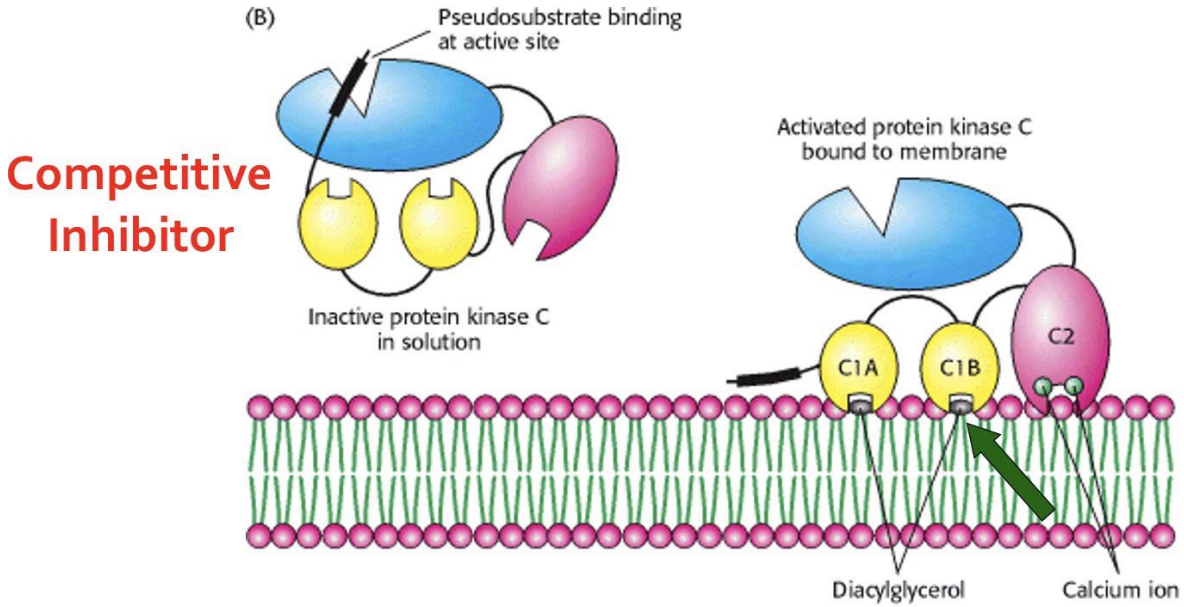
- Resembles the substrate sequence: A-R-K-G-**A**-L-R-Q-K
- Substrate Sequence: (**S, T**)
- Binds to the Enzyme's Active Site

Pseudosubstrate Sequence

- The **pseudosubstrate** sequence is the **main inhibitory mechanism** of PKC.

- It is a **short regulatory-domain peptide** that **mimics the substrate sequence**.

- However, it contains a **non-phosphorylatable amino acid**:
  - **Alanine instead of serine/threonine.**
- **It binds to the enzyme's active site** and **blocks** access of the real substrate.
- Therefore, it acts similarly to a **competitive antagonist/inhibitor**.

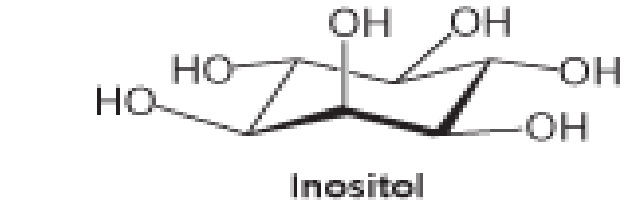


- Activation by **DAG + Ca^{2+}** induces a conformational change.
- This displaces the pseudosubstrate and allows the real substrate to bind.
- Mutations preventing pseudosubstrate binding can cause **constitutive enzyme activation**, which has been implicated in some cancers.

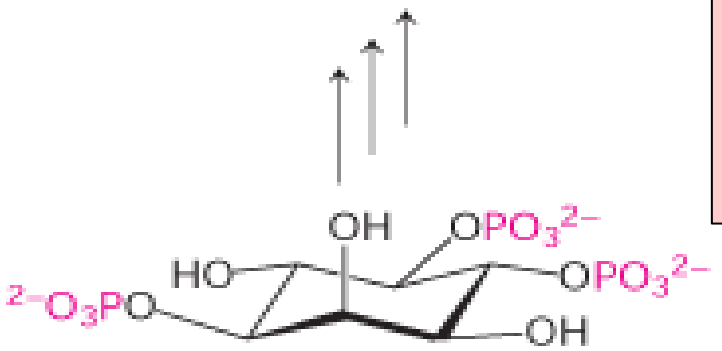
- Some pharmacological inhibitors are designed to **mimic the pseudosubstrate**.

Termination of IP3 Signal

IP3 is a Short-Lived Messenger

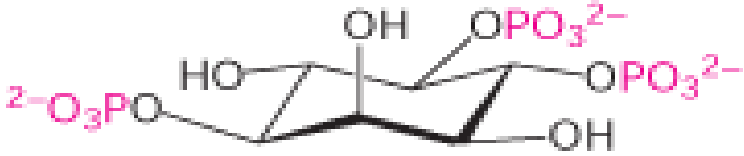


Inositol

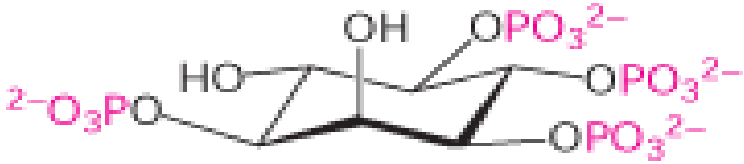


Inositol 1,4,5-trisphosphate

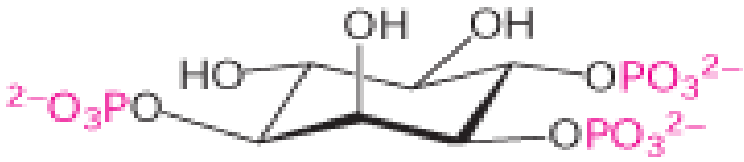
Lithium ions, used to treat some psychological disorders Inhibits IP_3 recycling



Inositol 1,4,5-trisphosphate



Inositol 1,3,4,5-tetrakisphosphate



Inositol 1,3,4-trisphosphate

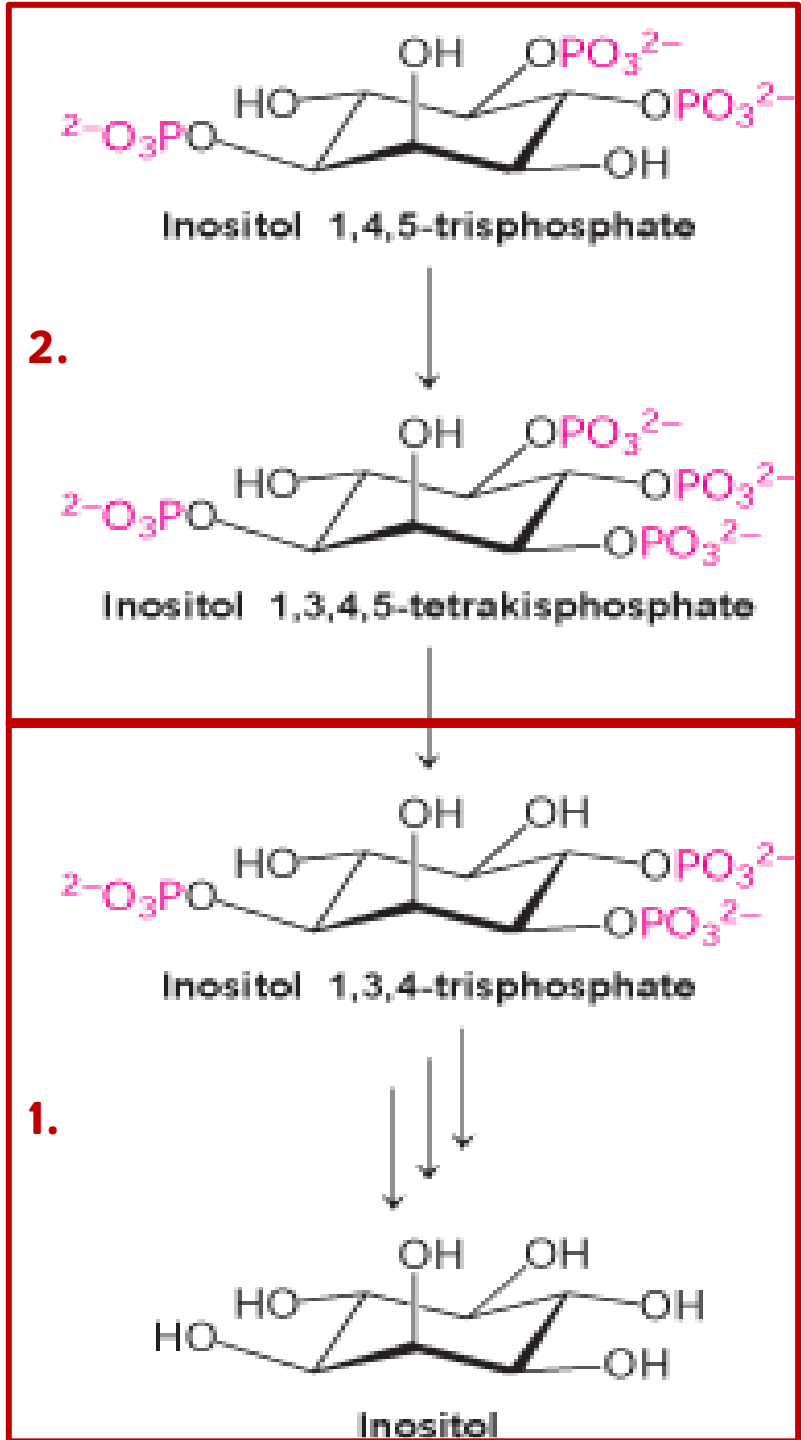
Inositol

Termination of IP3 Signal

- **IP3 is short-lived (seconds to mins)**, and this's because **rapid degradation** is essential for **Ca+2 homeostasis and prevents toxic, sustained elevation.**
- There're **2 main pathways of degradation:**

1. The main, most common pathway is the **sequential dephosphorylation of IP3. IP3 ⟶ IP2 ⟶ IP ⟶ free inositol.**
2. The alternative pathway is **phosphorylation of IP3 to IP4 (Inositol 1,3,4,5-tetrakisphosphate) (on O-number 3).** This IP4 is **inactive** for **Ca+2 release**, and this **IP4 is eventually degraded to free inositol.**

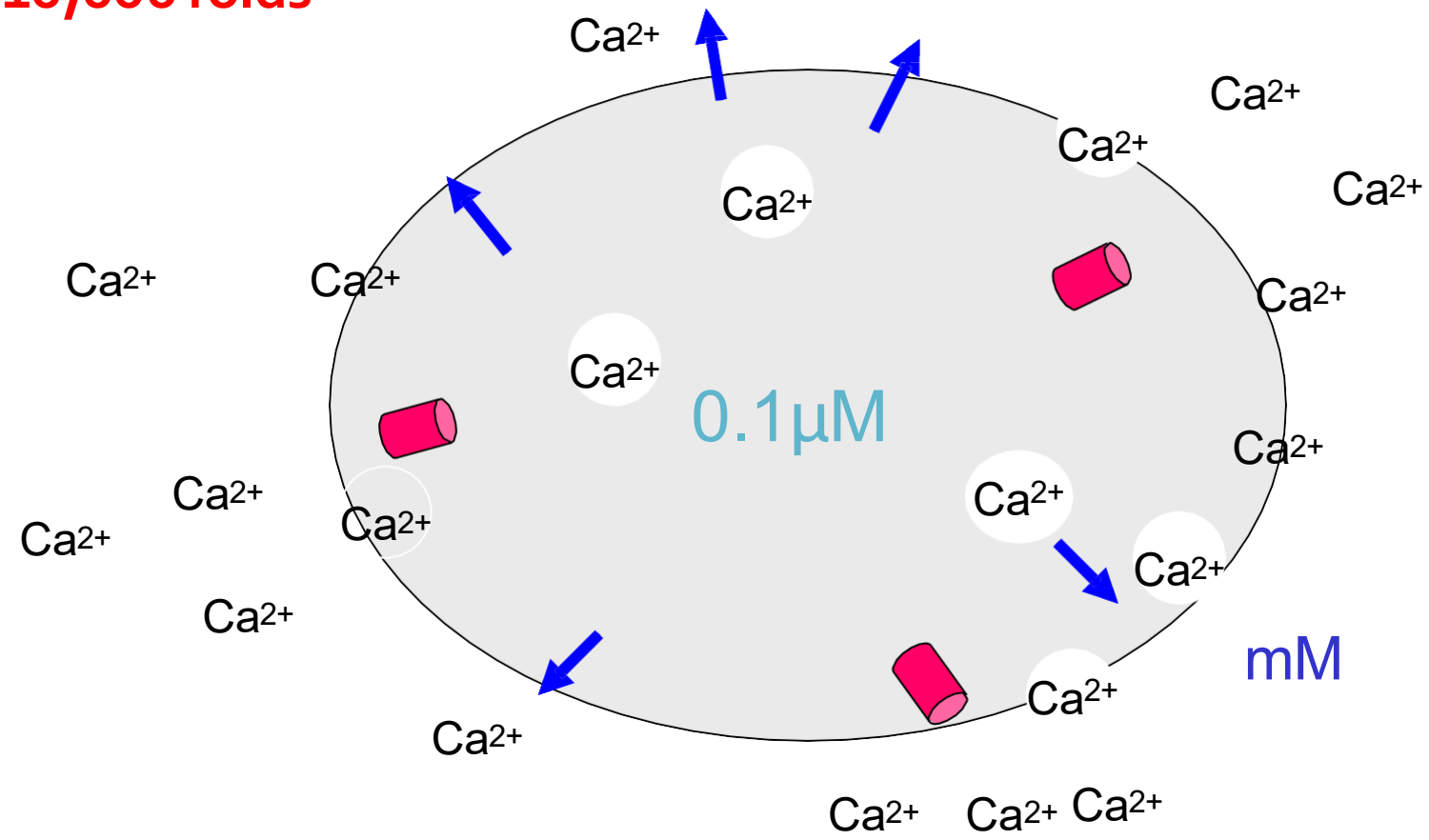
The free inositol is recycled **to Phosphatidylinositol (PI)**, and then **PIP2 to replenish membrane PIP2.**



Why Ca^{2+}?

A large difference in concentration

10,000 folds



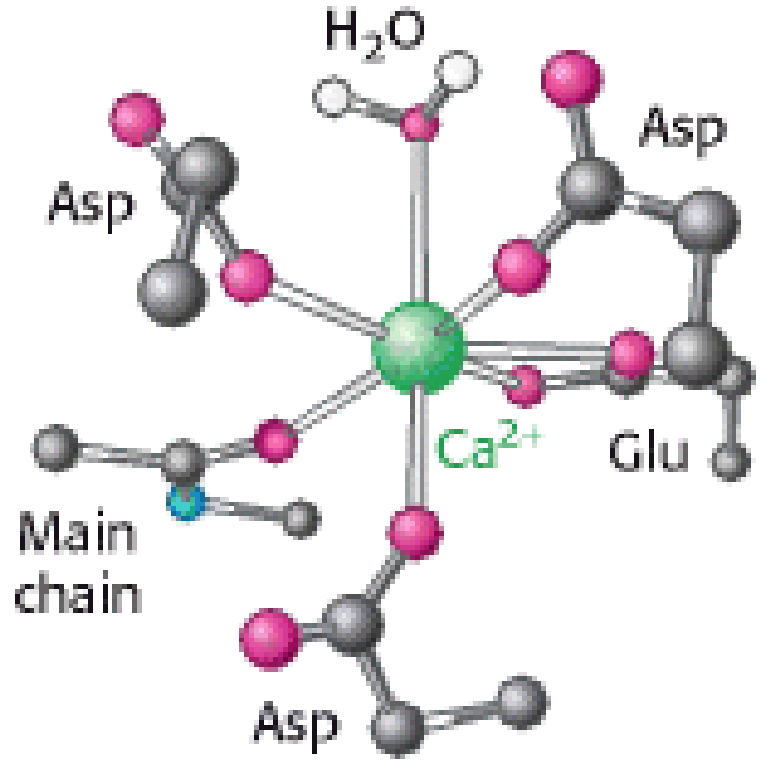
Ca+2 Removal

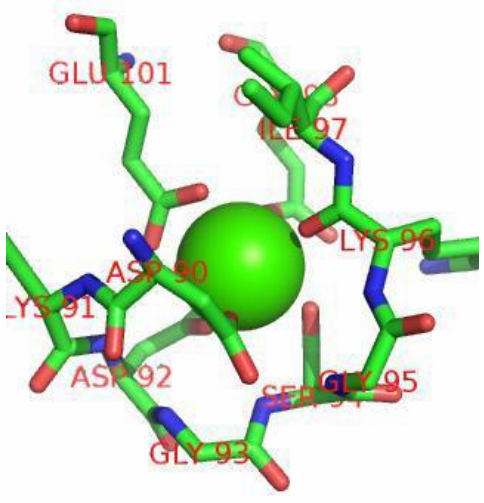
- Ca^{2+} is rapidly removed from the cytosol by:
  - **SERCA**
    - ***Sarco/endoplasmic reticulum Ca^{2+}-ATPase.***
    - Uses ATP to actively pump Ca^{2+} back into the ER/SER.
  - **PMCA**
    - ***Plasma membrane Ca^{2+}-ATPase.***
    - Pumps Ca^{2+} out of the cell into the extracellular space.

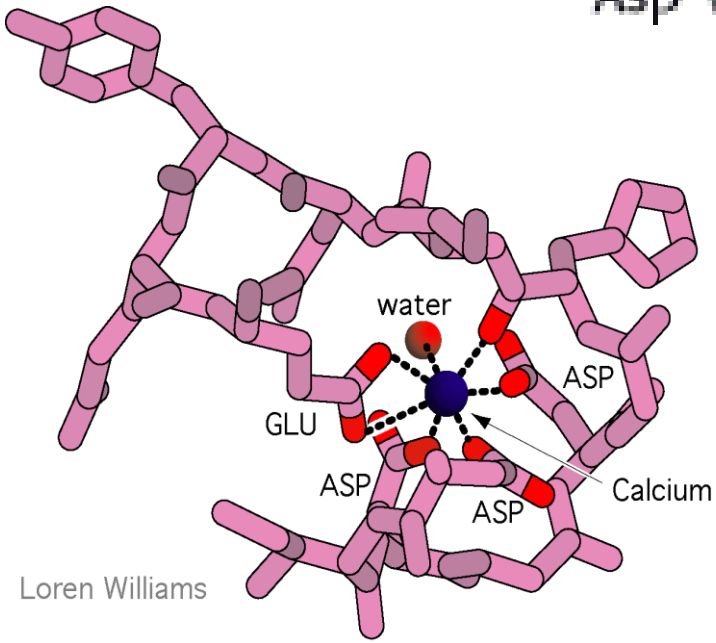
- Rapid removal is essential because **Ca^{2+} signaling** depends on **transient spikes, not sustained elevations.**
- Ca^{2+} overload can lead to **cell death**.

Why Ca^{2+}?

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

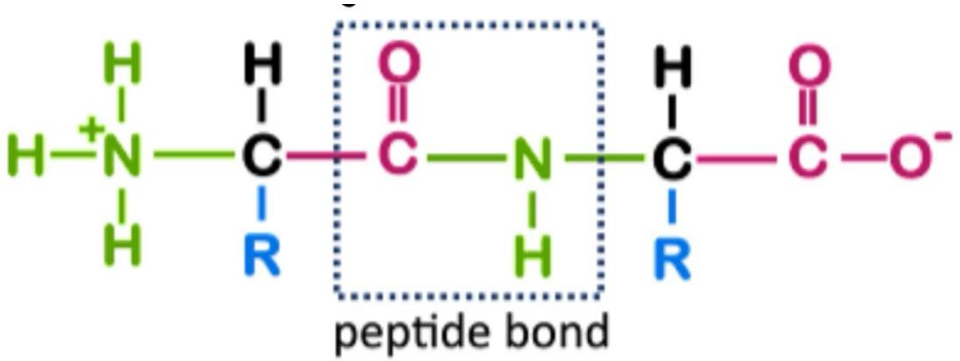






Why Ca^{2+}?

- **Ca^{2+} is an ideal second messenger**, because it binds proteins with high affinity and specificity. In order to do so, it requires 6–8 oxygen coordinating bonds with glutamate and aspartate (Their side chains (carboxyl groups)), **alongside peptide carbonyl oxygen atoms**, which allow tight but reversible binding.



- **Ca^{2+} binding induces conformational changes**, because coordination with O_2 favors specific 3D structures, exposing hydrophobic patches (which usually face inward), while hydrophilic groups face outward to keep the protein soluble, or changing charge positions.

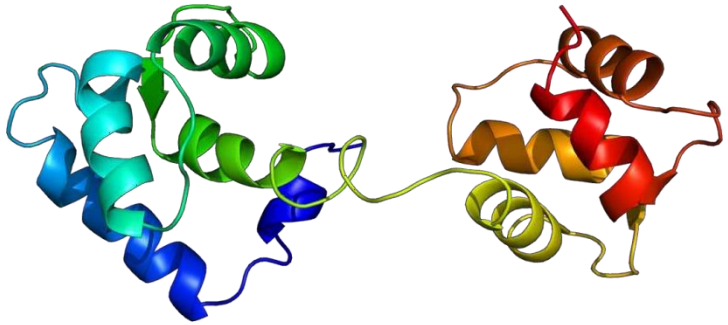
- **The combination of** :
  ① tight binding,
  ② conformational change,
  ③ rapid spike in concentration with subsequent rapid termination (as a result of steep gradient),

✓ makes Ca^{2+} perfect for rapid, transient signals underlying muscle contraction, NT release, and gene expression.

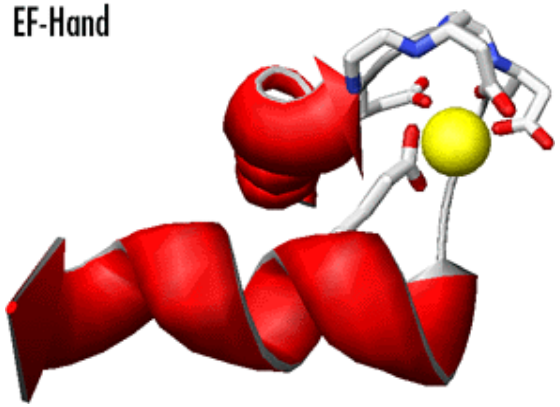
Calcium Binding Proteins

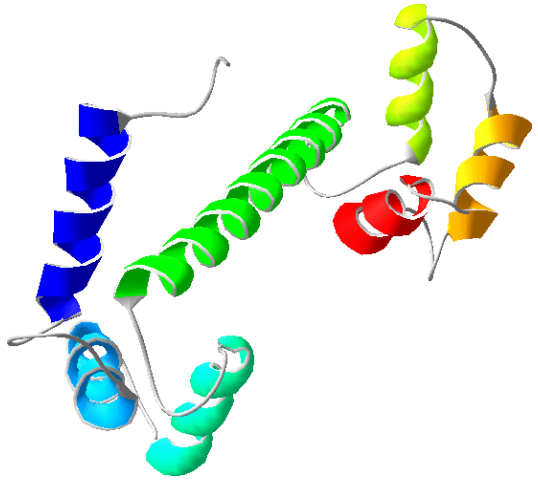
- Mediate the effects of Calcium (Ca^{+2})
- Many proteins

  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





Calcium Binding Proteins

❑ **Calcium binding proteins modulate (Ca^{2+}) effects:**

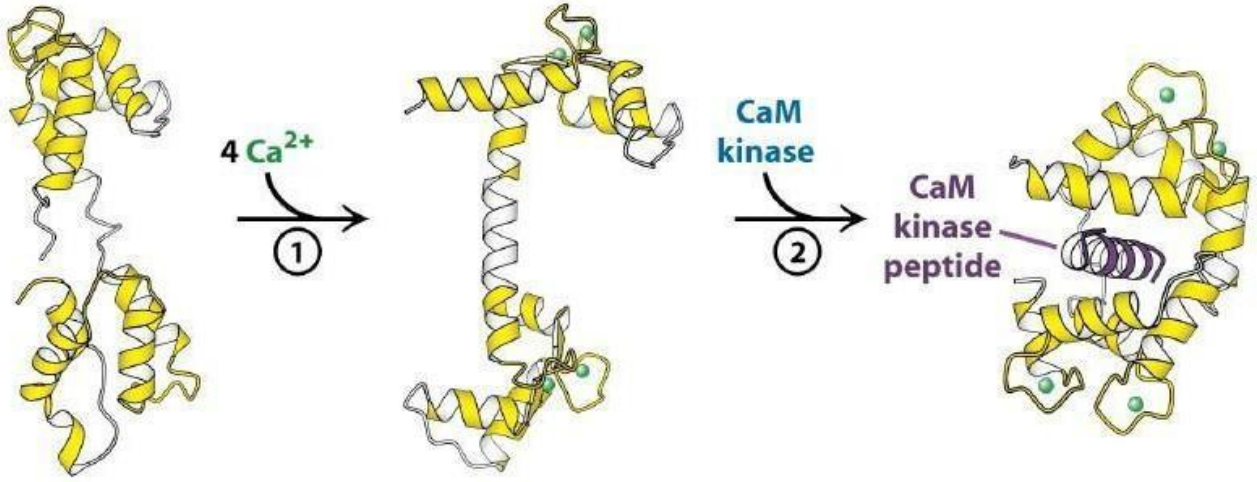
- They share the **EF-hand motif,** which is a **helix-loop-helix structure, with 2 α-helices connected by a flexible loop of 12 a.a,** which is packed with **oxygen-rich amino acids (mainly Asp and Glu, with Ser, Asn, Gln).**
- **This loop is where Ca^{2+} forms the 6-8 coordination bonds with O_2.**

- When ***Ca^{2+} binds,*** **it causes conformational changes which lead to movement of nearby α-helices, exposing the hydrophobic patches, which binds to the target protein.**

- In the case of **Calmodulin**, this **allows binding of Calmodulin-dependent protein kinases (CaMK II) and their activation**, (calneurin [calcineurin] phosphatase), ion channels opening, and certain Adenylyl Cyclase isoforms.

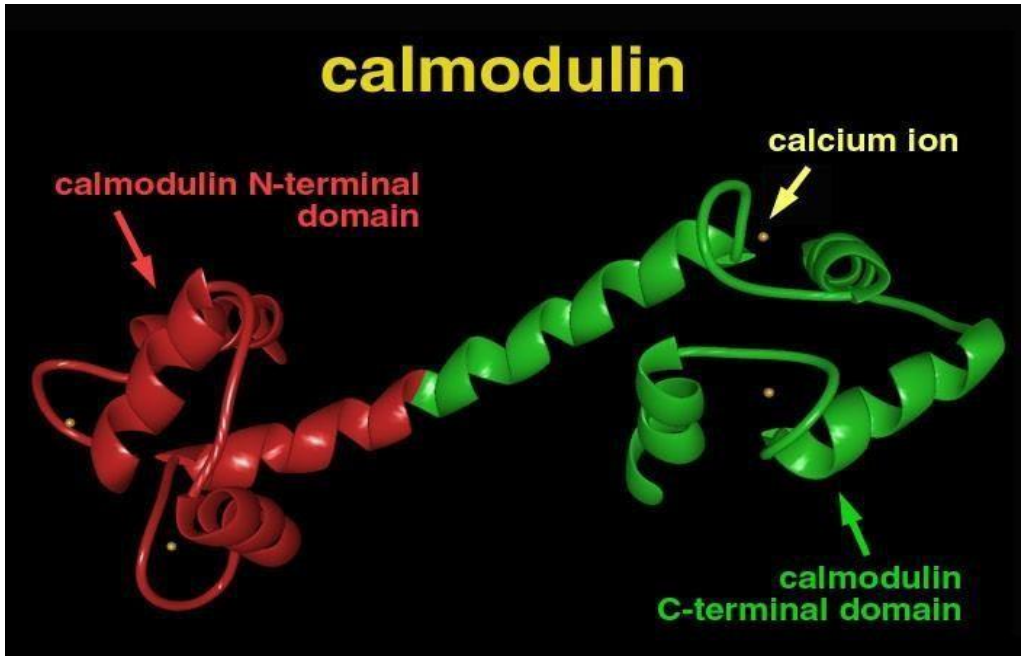
Calmodulin (≈17 kD)

Calcium-modulated protein



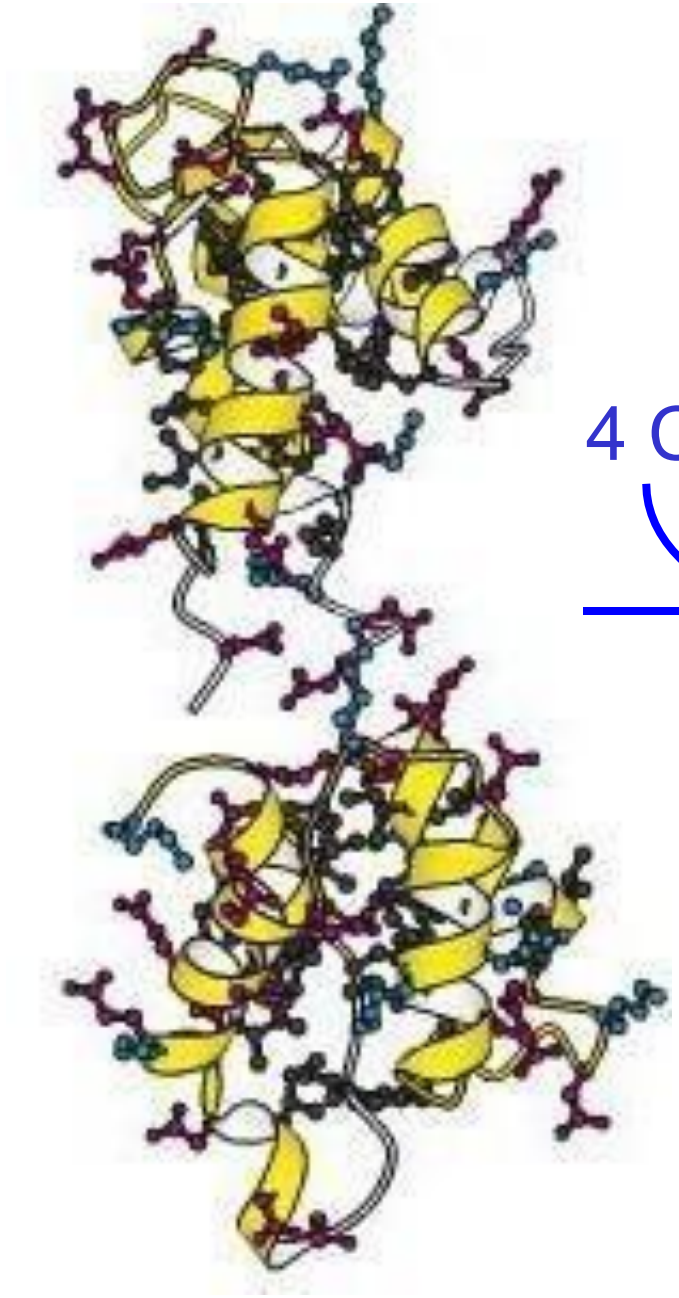
- Found in almost all eukaryotes
- Consists of two globular regions
  - Connected by flexible region
  - Each contains 2 EF hands
  - Four Ca^{2+} binding sites
- Calcium-Calmodulin complex can bind to a large number of target proteins including:

149 amino acids



Calmodulin-dependant Protein Kinase

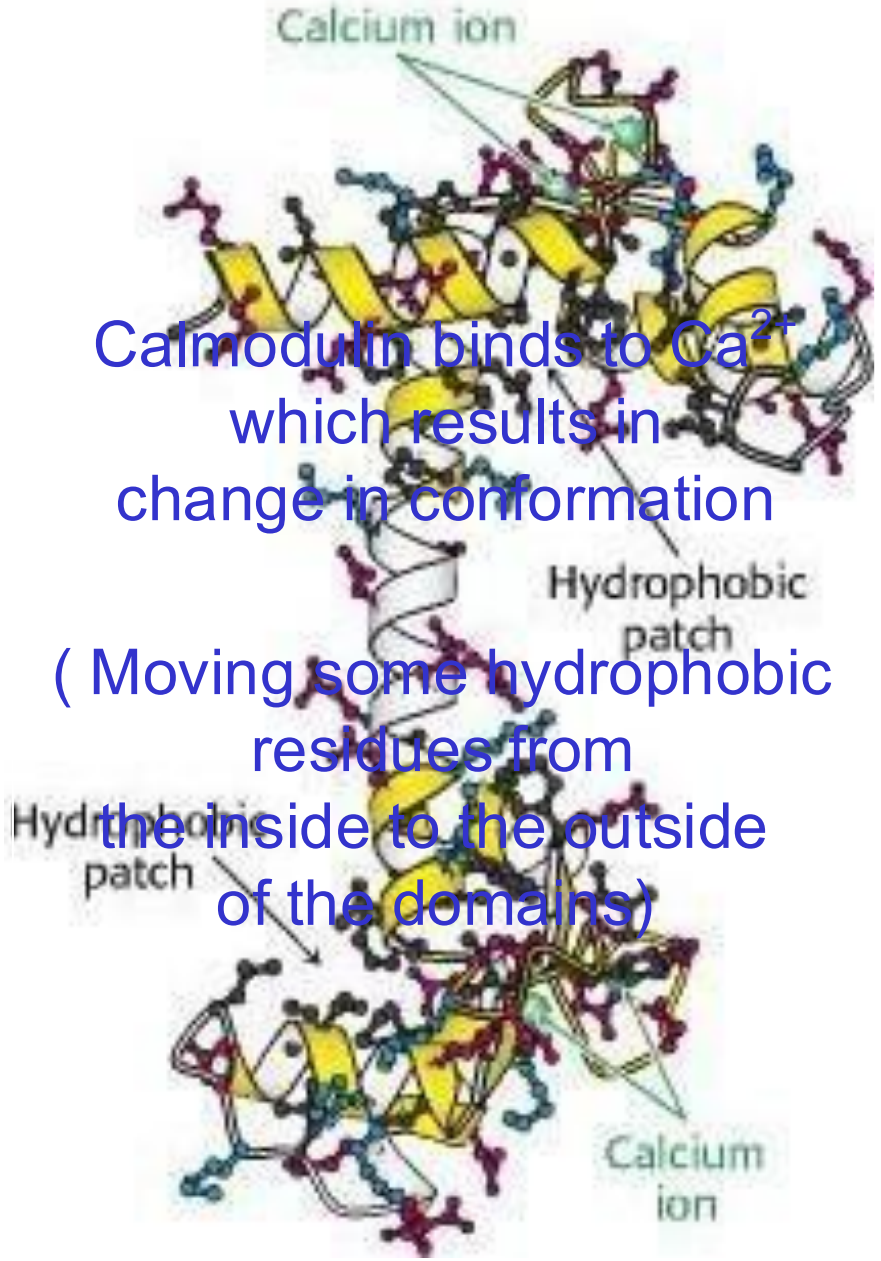
Ca^{2+} ATP'ase Pump



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)

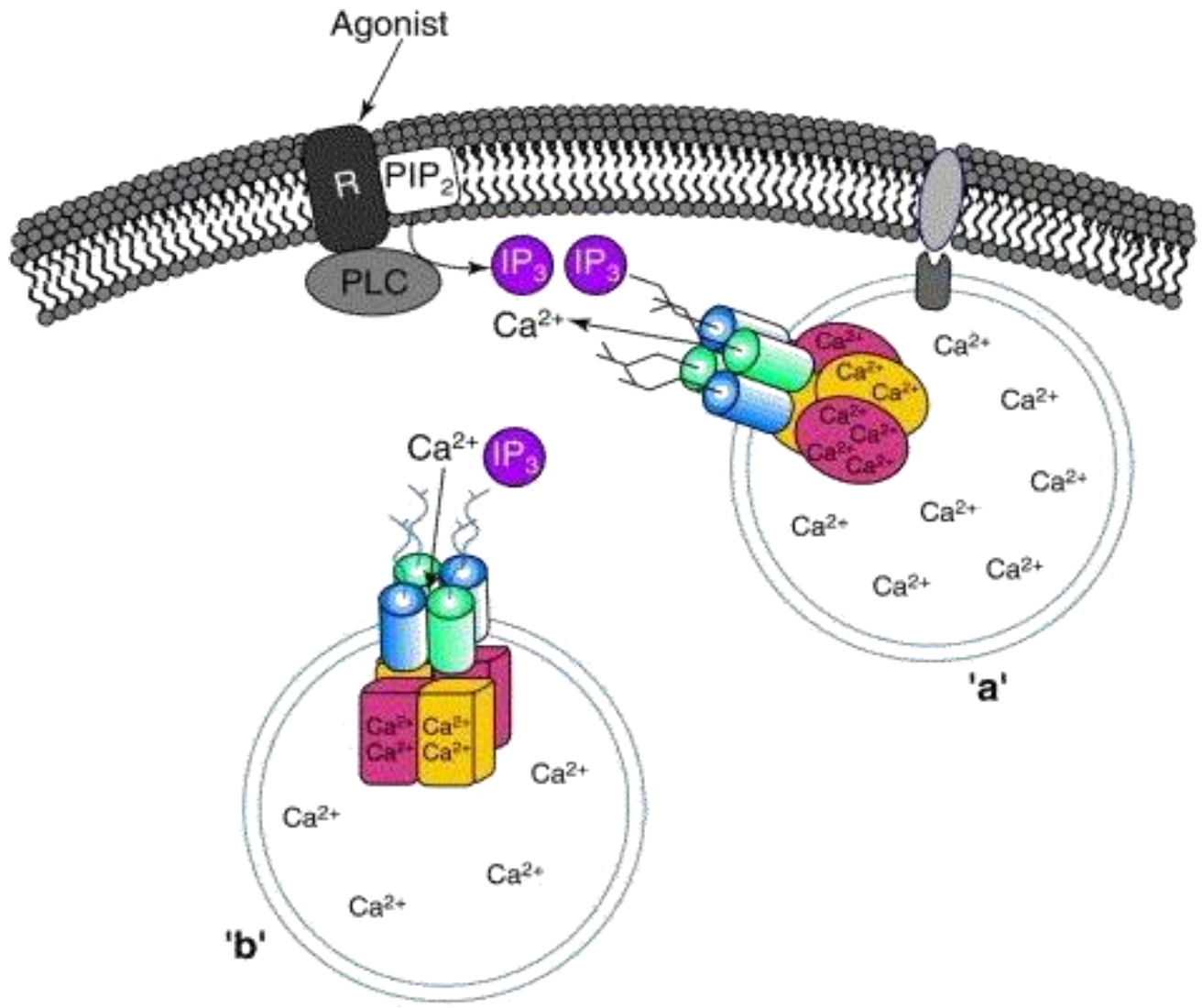


Calmodulin

- Calmodulin is the most ubiquitous Ca^{+2} binding protein, and it is highly conserved across species. It is a small protein made of 149 a.a, and consists of 2 globular domains connected together by a flexible α-helix. Each domain contains 2 EF-motifs, which is why it can bind 4 Ca^{+2} atoms.
- **Ca^{+2} induces exposure of hydrophobic patches for target proteins, including: Calmodulin-dependent protein kinases (CaMKII), Calcineurin, Ca^{+2}-ATPase pumps, certain isoforms of Adenylyl Cyclase and ion channels.**
- Binding is often **cooperative, and it is regulated by Ca^{+2} concentration.**
- CaMKII is activated when Ca^{+2}-calmodulin binds its regulatory domain, releasing the pseudosubstrate sequence.
- Calmodulin is the primary mediator of Ca^{+2} signal.
- Its dysregulations are implicated in cardiac arrhythmias and neuronal disorders.
- Ca^{+2}-induced conformational change is the mechanism by which Ca^{+2} regulates cellular processes. Without it, the EF-hand motif's α-helices have their hydrophobic residues buried, but Ca^{+2} binding to the loop, by 6-8 coordination atoms, leads to loop rotation which induces helix movement, exposing the buried hydrophobic residues. ⟶ Calcium binding leads to exposure of binding sites, which turned it from an inactive (closed), to an active (open) conformation. This is reversible, removing Ca^{+2} leads to return to the closed state, reburying the hydrophobic patch. This mechanism is used by Calmodulin, Troponin C, and Parvalbumin.

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

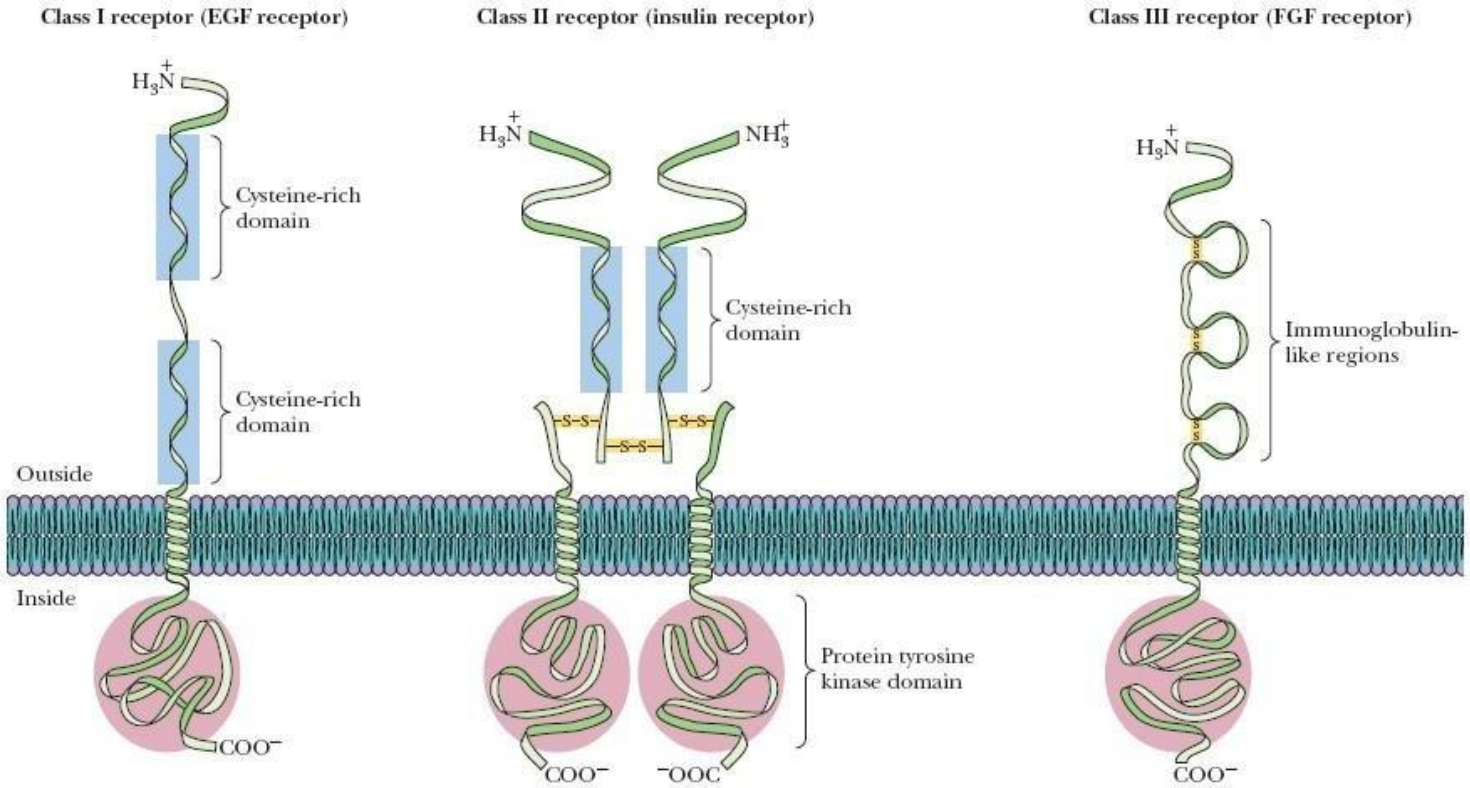


Ca^{2+} Transporter

- **SERCA (Sarco/Endoplasmic reticulum Calcium ATPase)** is the **primary pump removing Ca+2** from the cytosol.
- It does so by pumping it into the **Sarcoplasmic reticulum SER**. It's a **P-type ATPase (P stands for phosphorylation, because they autophosphorylate a residue of their own while pumping).**
- This integral membrane protein has **10 Transmembrane helices,** and it is **the most abundant SR membrane protein**. It pumps **2 Ca+2 ions into the SR/ER for each ATP** it hydrolyzes, **creating a steep (very larg) Ca+2 gradient.**
- This pump is critical for **muscle relaxation after contraction**. **ATP depletion (ischemia or death), leads to sustained contraction** \longrightarrow **tetany** (not true tetany but this is how it's written) and **rigor mortis (in death).**
- SERCA is targeted by Thapsigargine, which is a potent inhibitor of SERCA used experimentally.
- SERCA modulators are being investigated for heart failure and skeletal muscle disorders.

Receptor Tyrosine Kinases Cascade

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



RTKs

Recap: Enzyme-Linked Receptos

1. Main Types
Enzyme-linked receptors are divided into:

- **Receptor Tyrosine Kinases (RTKs):**
  - The receptor has **intrinsic enzymatic activity**.
  - The receptor itself contains a **tyrosine kinase domain**.

- **Non-RTKs:**
  - The receptor has **no intrinsic enzymatic activity**.
  - It is **closely associated with a separate tyrosine kinase** that carries **out the phosphorylation**.

2. Receptor Tyrosine Kinases (RTKs)
RTKs are a major receptor class associated with:
- **Growth factors**
- **Cytokines**
- **Insulin**

1. They are **single-pass transmembrane proteins** consisting of: **Extracellular ligand-binding domain**
   1. Binds the ligand.
2. **Single transmembrane helix**
   1. Anchors the receptor in the cell membrane.
3. **Intracellular tyrosine kinase domain**
   1. Has intrinsic enzymatic activity.
   2. **Transfers a phosphate group from ATP → tyrosine residue on target proteins.**

RTKs

3. RTK Subclasses
Several RTK subclasses exist.

- **Class II = Insulin receptor family**

✓ Unlike most RTKs, the **insulin receptor exists as a preformed heterotetramer**: **$\alpha_2\beta_2$**

- **2 α-subunits**
  - Extracellular.
  - Mainly involved in **insulin binding.**

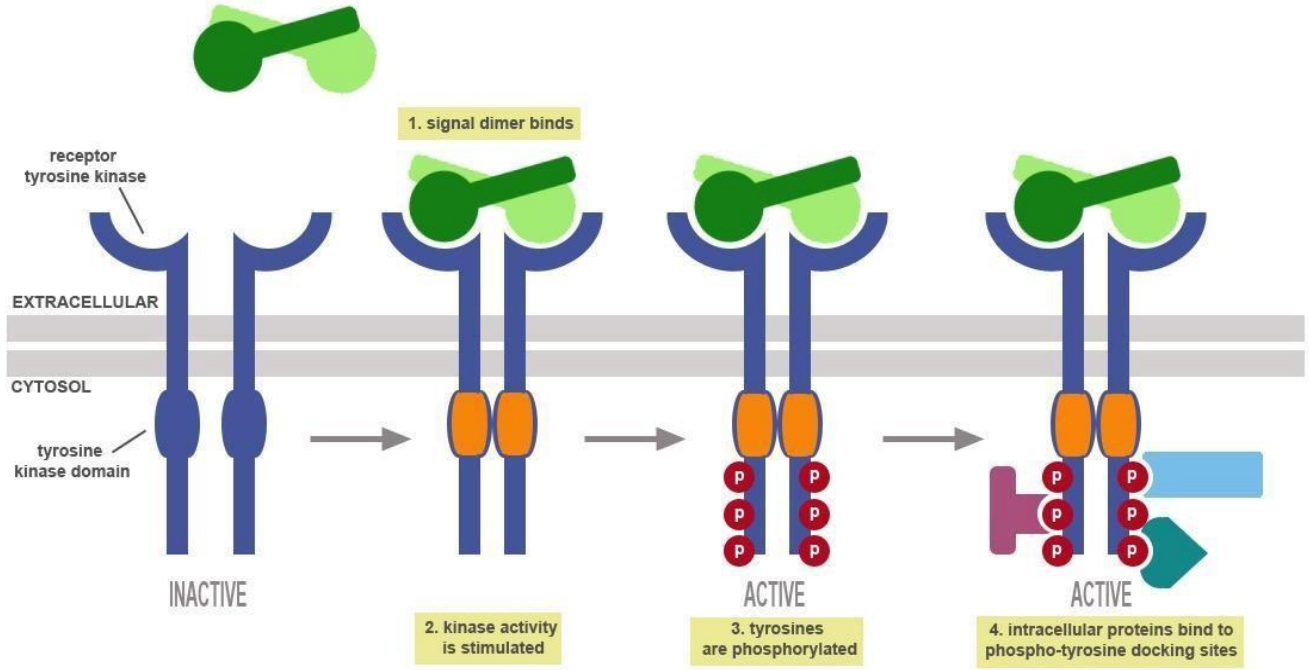
- **2 β-subunits**
  - Have a **membrane-bound portion.**
  - Their intracellular portions contain the **tyrosine kinase domains.**

High-yield point

Most RTKs:
Ligand binding → **dimerization** → activation
Insulin receptor:
Already exists as **$\alpha_2\beta_2$ heterotetramer** → insulin binding causes **activation**, not receptor dimerization.

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**



Second messenger, receptor tyrosine kinase

- Signaling is highly conserved.

- When the **ligand binds, it leads to dimerization of the receptor**, **except for insulin which exists as a preformed heterotetramer.**

- This dimerization **brings the cytoplasmic tyrosine kinase domains closer together** for **transphosphorylation (a specific type of autophosphorylation)**. These phosphorylated **tyrosine residues act as docking sites for 2 main domains :**

1. The SH2 domain.
2. The PTB (Phosphotyrosine-binding) domain

✓ They are contained in proteins, that activates **MAPK pathway, or PI3K/Akt cascades**.

- RTKs (Receptor Tyrosine Kinase) regulate **proliferation, differentiation, migration and metabolism, and their dysregulation is implicated in many cancers,** making tyrosine kinase inhibitors important in chemotherapy.

- RTKs activate Ras/MAPK pathway;
- GRB2 (Growth factor receptor-bound protein 2 is an adaptor protein that doesn't have any enzymatic activity, but it rather connects the activated receptor to SOS (Son of sevenless – Guanine nucleotide exchange factor that activates Ras)) docks to the phosphorylated tyrosine residues and recruit SOS, which activates the Ras/MAPK pathway, leading to proliferation.

- Important note: The SH2 domain is a highly conserved domain involved in protein-protein interactions. The PI3K/Akt pathway regulates cell survival and metabolism.

Second messenger, receptor tyrosine kinase

❑ In insulin signaling,

- **The phosphorylated insulin receptor phosphorylates insulin receptor substrates (IRS)**, which **activates the PI3K/Akt pathway.**

- This leads to **GSK3 inactivation (Glycogen Synthase Kinase**, which **phosphorylates glycogen synthase and inactivates it**) and **protein phosphatase 1 activation** (to remove the **phosphate residues on Glycogen Synthase and activates it**). This leads to **glycogen synthesis.**

✓ **Glucagon** works to inhibit **glycogenesis** through **phosphorylation, while insulin promotes it by dephosphorylation.**

Signal Transduction through Tyrosine Kinase

Growth hormones:

- ✓ Epidermal Growth Factor (EGF)
- ✓ Platelet-derived growth Factor – PDGF which is important in wound healing
- ✓ GH – Non- RTK
- ✓ Insulin

Hormone Binding

↓

Dimerization of the receptor

↓

Auto phosphorylation of the receptor

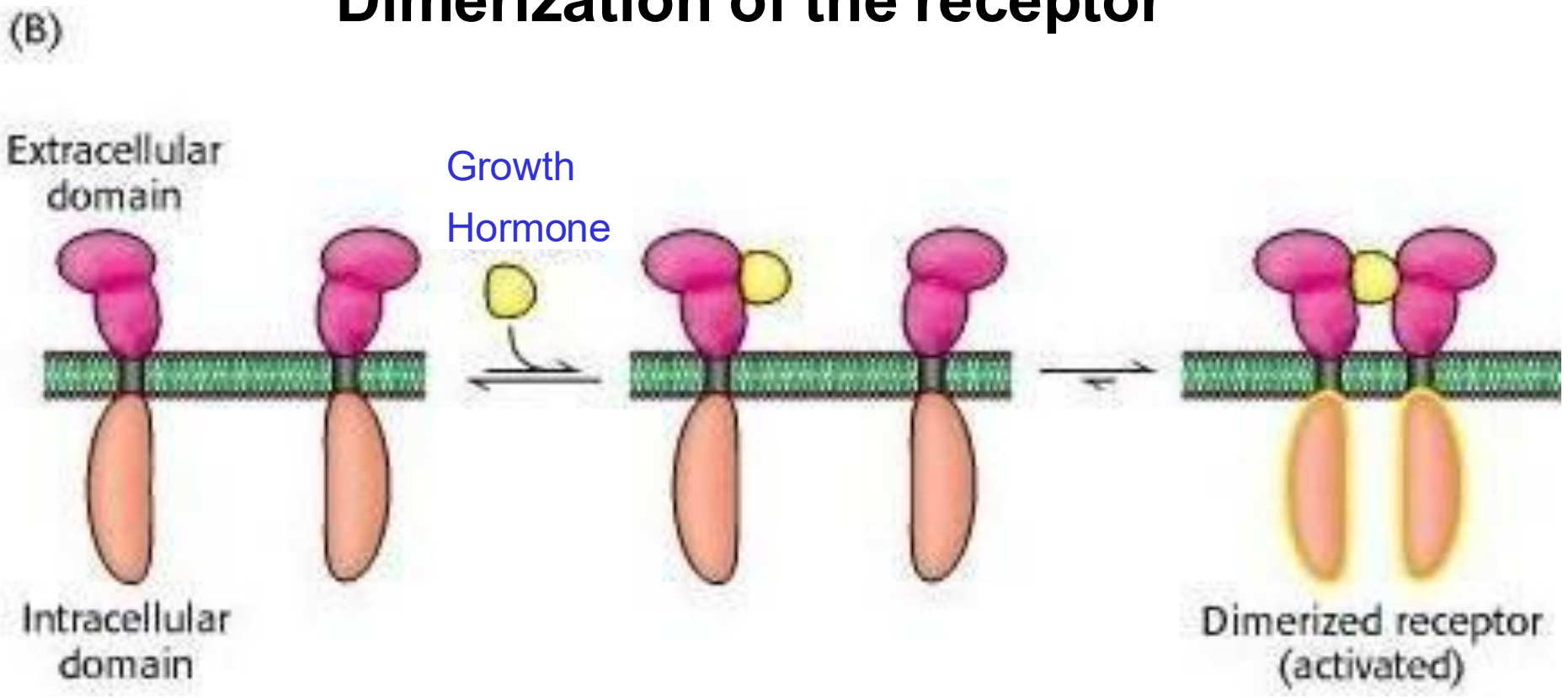
↓

Phosphorylation of the target proteins

Growth Hormone dimerization

Binding of one molecule of growth hormone

Dimerization of the receptor



Janus

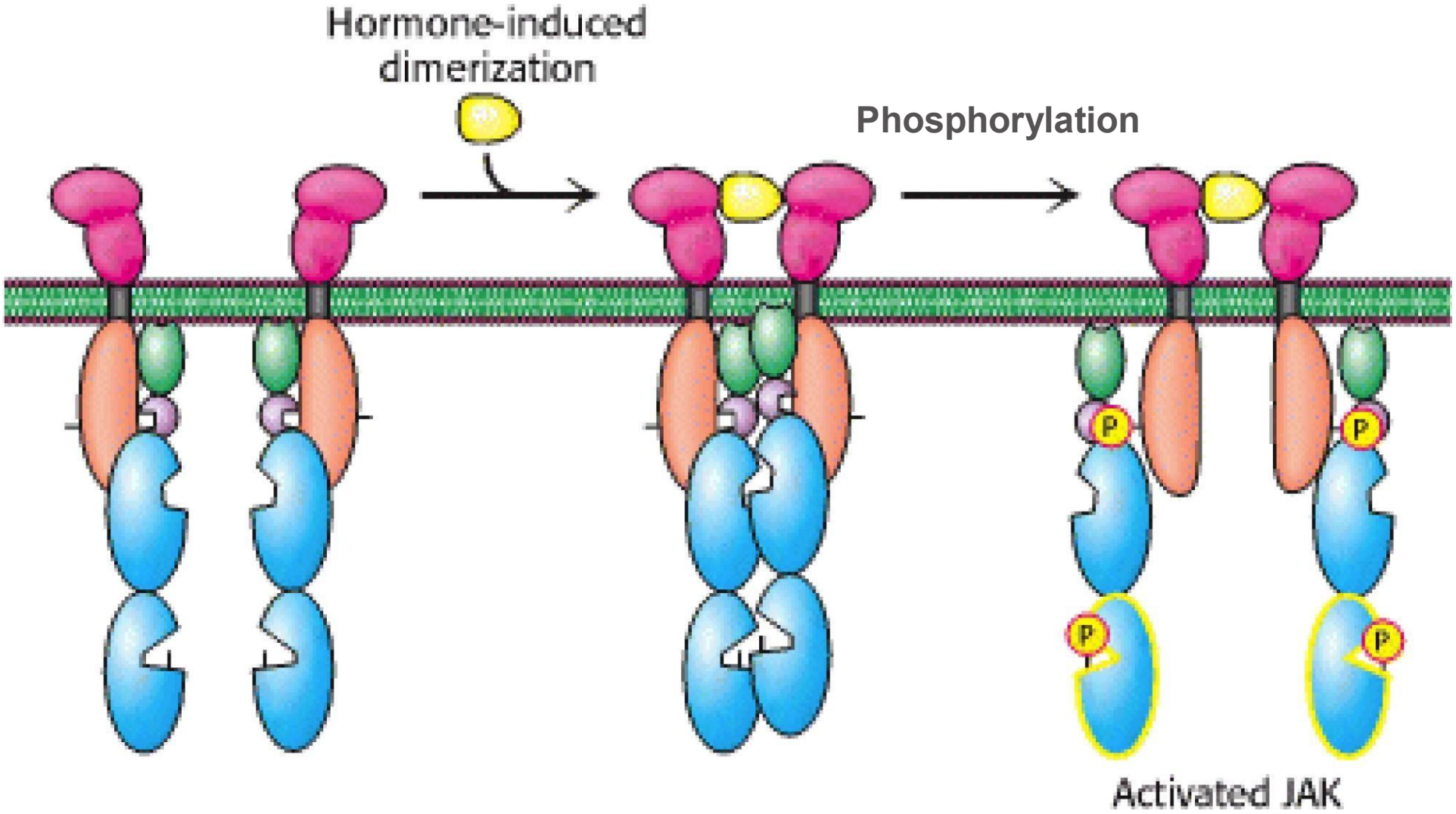
Each Intracellular Domain is associated with a protein kinase called Janus Kinase 2

Interaction with membrane

Binds peptides that contain Phosphotyrosine

- The GH receptor belongs to the **Cytokine receptor superfamily,** which **lacks an intrinsic tyrosine kinase activity**, but it is closely associated with cytoplasmic protein tyrosine kinases; **The Janus kinase 2 (non-receptor tyrosine kinase)**. The receptor exists as a **monomer**, but ***ligand binding induces dimerization.***

Receptor dimerization brings two JAKs together Each Phosphorylates key residues on the other



JAK Receptor

- The receptor is attached to **JAK2**. When GH binds to the receptor, **dimerization** brings the JAK2 (Also associated with **Erythropoietin and Prolactin**). close enough to cause **transphosphorylation and activation on the JH1 domain**. **ONLY one molecule of GH is needed for dimerization**, since it binds 2 receptor molecules, unlike in RTKs which requires one ligand for each receptor.

- The receptor extracellular domain undergoes **conformational changes** that are transmitted intracellularly.
- This mechanism demonstrates how **Cytokine Signaling** causes downstream **tyrosine phosphorylation** even **with no intrinsic tyrosine kinase activity**, which is critical for **growth, metabolism and immune function**.
- **JAKs are non-receptor tyrosine kinases** that mediate **NRTK receptor signaling** (GH, Cytokines, Prolactin…). There are **4 family members: JAK1, JAK2, JAK3, and TYK2** (Tyrosine Kinase 2).

- They are relatively large, characterized by a **kinase domain (JH1)** and **pseudo-kinase domain (JH2)**. The latter is catalytically inactive, and its primary role is **autoinhibition**. The transphosphorylation removes the autoinhibition and **fully activates the kinases**.
- Activated JAKs **phosphorylate the receptor**, creating **SH2 docking sites** for downstream proteins, particularly **STATs** (Signal Transducers and Activators of Transcription).
- **JAK3** is primarily limited to **hematopoietic cells**.
- **JAK inhibitors** treat **Myeloproliferative neoplasms** (blood cancers) and **autoimmune diseases**.
- **JAK activation parallels the RTK transphosphorylation**, but the kinase is **not part of the receptor**.
- The **JAK/STAT pathway is direct and rapid**, skipping the intermediates seen in RTKs, which is why it's a **preferred target for drugs**.
- **JAK2 mutations** that lead to its **constitutive activation** are seen in **Polycythemia vera (V617F mutation)** and other **myeloproliferative neoplasms**.

Activated JAK 2 can Phosphorylate other substrates

- STAT – Latent transcription factor activated by JAKs
  - Signal Transducers & Activators of Transcription
- Regulator of transcription
  - STAT Phosphorylation
    - ➔ **Dimerization**
      - ➔ Binding to specific DNA sites
- If JAK2 remains active it will produce **Cancer**

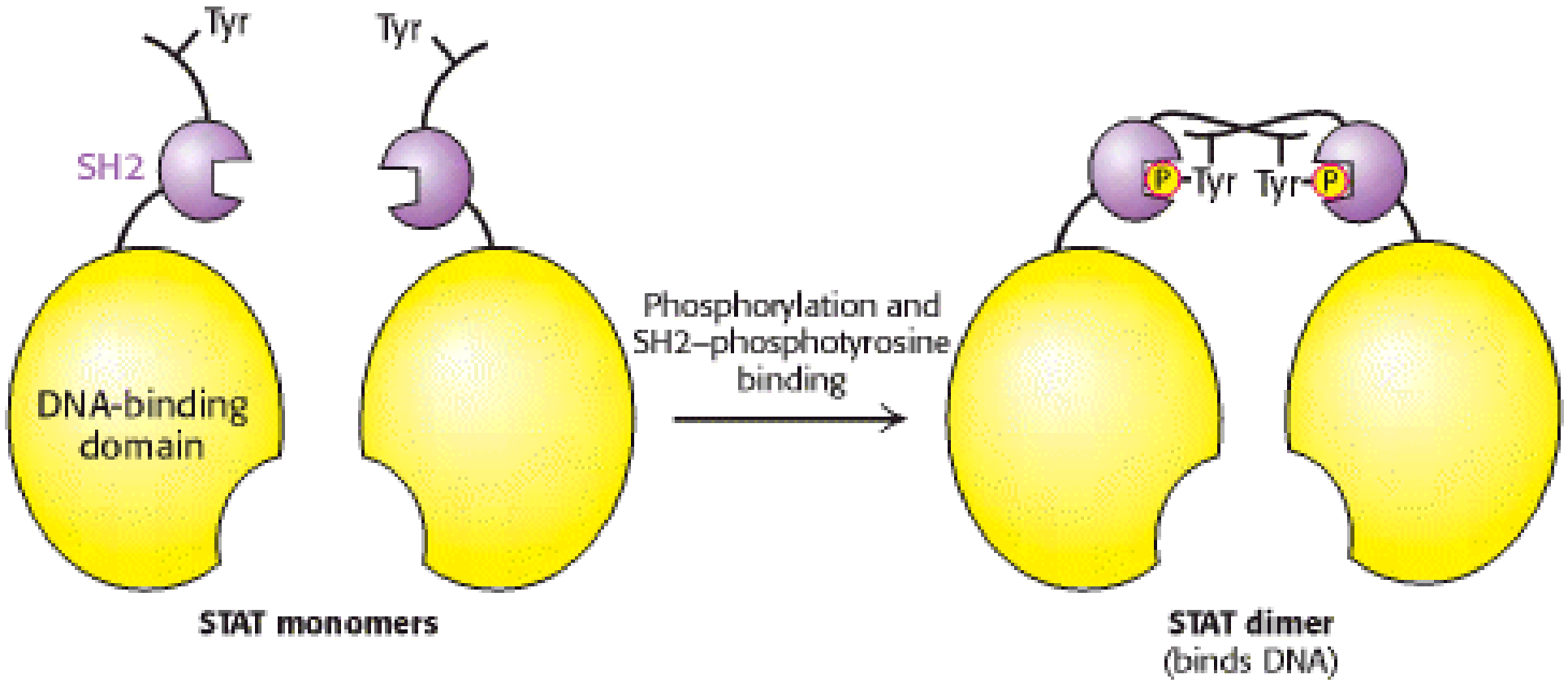
STAT Isoforms

- **7 isoforms of STAT exist: STAT1-STAT6**, with **2 forms of STAT5**.

- Receptor **phosphorylated tyrosine residues** act as **docking sites for STATs** via their **SH2 domains**.
- Once bound, STATs are **phosphorylated by JAKs on a single C-terminal tyrosine**, which then **dissociate** and form **dimers** via the **phosphotyrosine binding to the SH2 domain of the other STAT and vice versa**.
- The dimers **translocate to the nucleus** and bind to specific DNA elements called **GAS elements**, to activate transcription of target genes involved in **proliferation, survival, immune responses, and differentiation**.

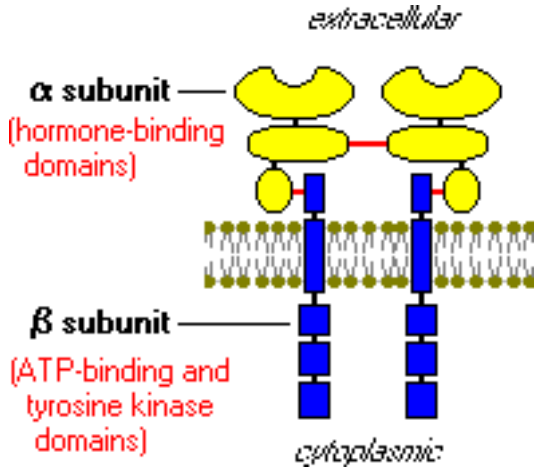
STAT is phosphorylated on a tyrosine residue near the carboxyl terminus

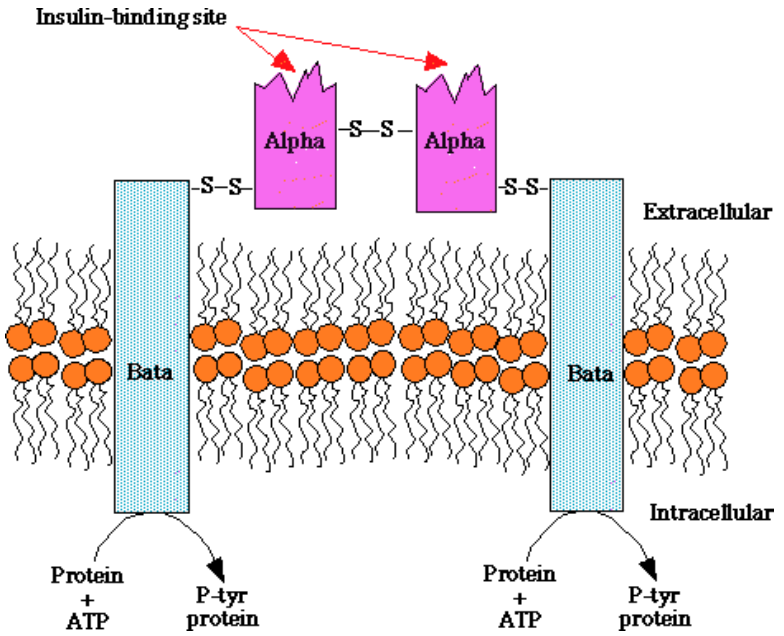
Phosphorylated tyr binds to SH2 domain of another STAT molecule



Tyrosine Kinase & other Hormones

- Insulin Receptor
- Tetramer (2^{α}; 2^{β}), dimer ($2^{\alpha\beta}$ pairs)
- Disulfide bridges
- Insulin Binding → Activation of the Kinase





Insulin receptor

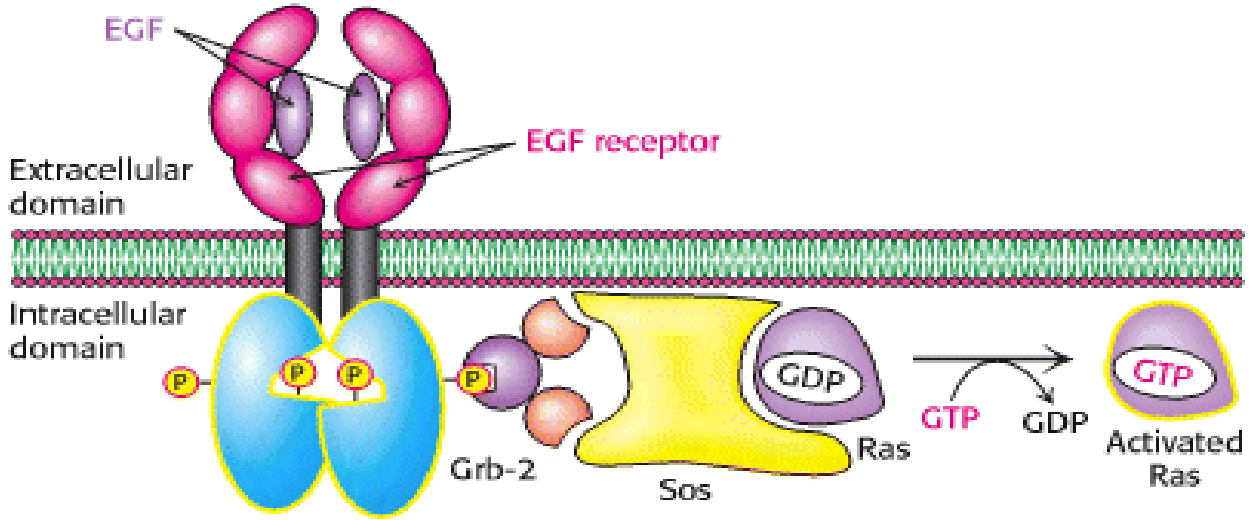
- The **insulin receptor is an RTK**. It is a **tetramer of 2 α subunits and 2 β subunits**:
  - **2 α subunits:** extracellular, responsible for **ligand binding**.
  - **2 β subunits: transmembrane and intracellular**, containing the **tyrosine kinase domain**.

- The **α and β subunits are linked by disulfide bonds**, and the **2 α subunits are also linked by a disulfide bond**.
- The receptor exists as a **preformed dimer**, so it **does not require ligand-induced dimerization**.
- **Insulin binding** leads to **conformational changes** that are transmitted across the membrane to the **β-subunits**, activating the **kinase domain**.

- This leads to **β-subunit transphosphorylation** and **full activation of the kinase activity**.
- The activated receptors phosphorylate **IRSs (Insulin Receptor Substrates)** on **tyrosine residues**, which then bind to **SH2 domain-containing proteins**, including **PI3K and GRB2**.
- **PI3K → PI3K/Akt pathway**
- **GRB2 → Ras/MAPK pathway**
- **T2DM** involves **defective receptor signaling at multiple levels**.

Ras is a member of small G proteins family

- Monomeric
- 2 forms: GDP ⟷ GTP (GEFs: GDP → GTP; GAPs: GTP → GDP)
- Smaller (1 subunit)



- GTPase activity – Slower than G-alpha intrinsic activity.
- Many similarities in structure and mechanism with Gα
- Include several groups or subfamilies
- Major role in growth, differentiation, cellular transport, motility etc…

- **RAS activates many downstream effectors**, including:
  - **RAF → MEK → ERK (MAPK pathway)**
  - **PI3K**

Impaired GTP_{ase} activity can lead to cancer in human

RAS is the most commonly muted oncogene.

- Mammalian cells contain 3 Ras proteins

1. H-Ras
2. K-Ras : mostly muted in Pancreatic, colorectal, Lung Cancer.
3. N-Ras :

Mutation ➔

Loss of ability to hydrolyze GTP ➔

Demonstrates how impaired signal limitation can lead to cancer

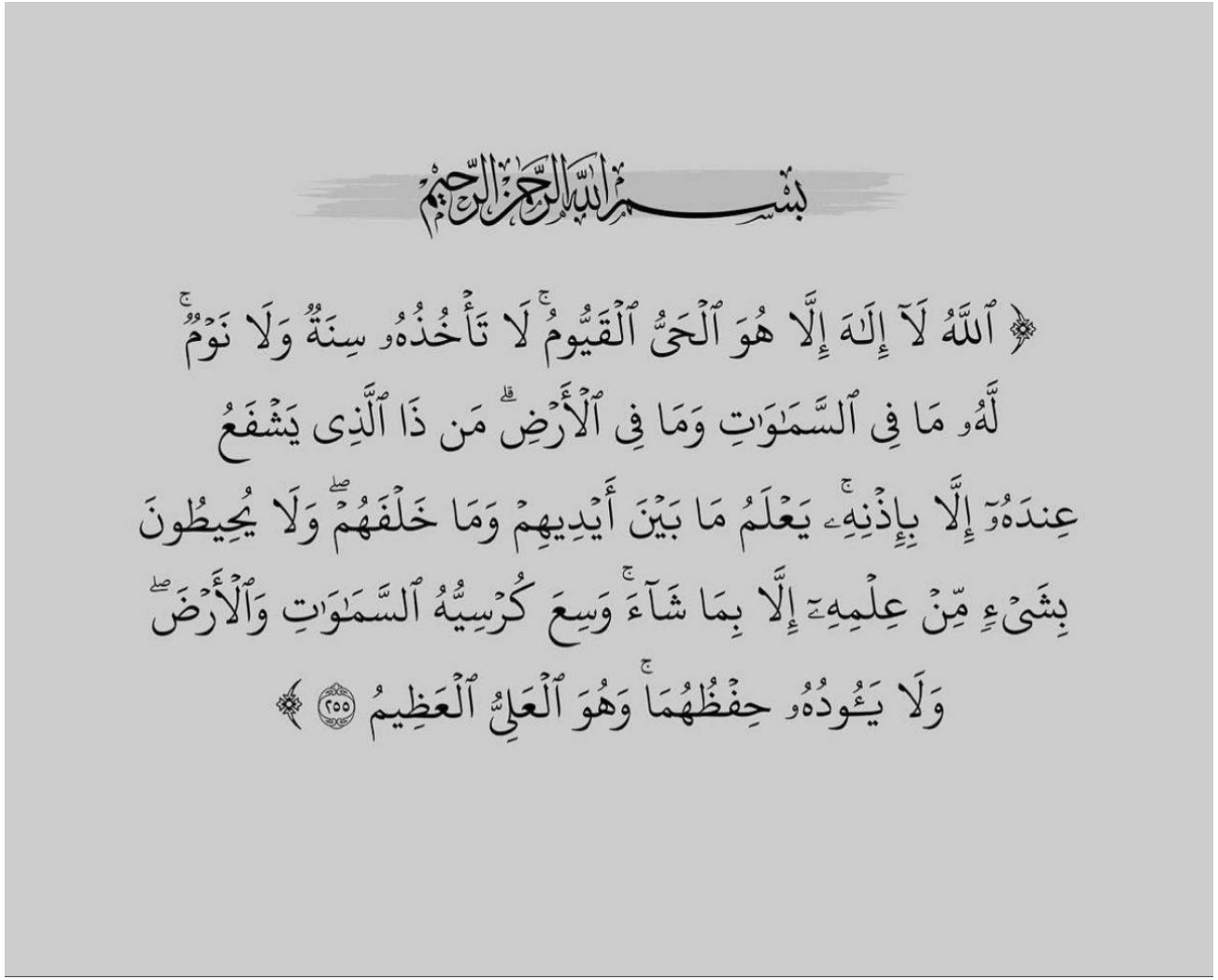
← Ras is locked in "ON" position ➔ continuous stimulation of growth

Impaired GTP_{ase} activity can lead to cancer in human

- Mutations that **impair GAP interaction** or **impair Ras GTPase activity** lock Ras in its **active form**, leading to continuous activation of:
  - **MAPK pathway → proliferation**
  - **PI3K pathway → survival**

- The high frequency of these mutations has made **RAS a drug target**.
- An example is **G12C inhibitors**, which bind the **cysteine introduced by the G12C mutation**, locking RAS in an **inactive form**.

رسالة من الفريق العلمي:



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			