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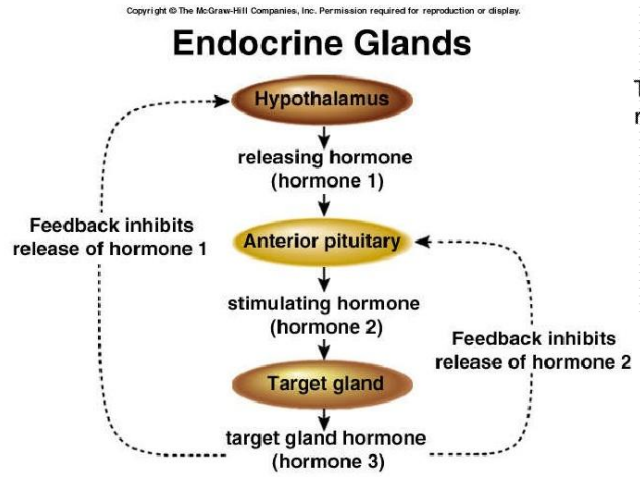
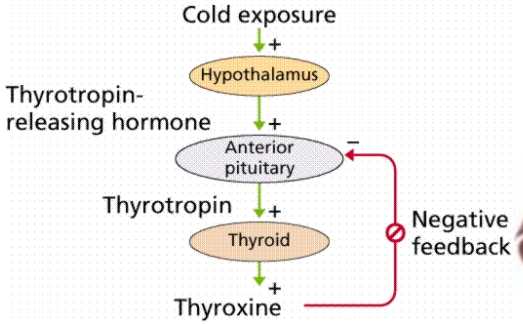
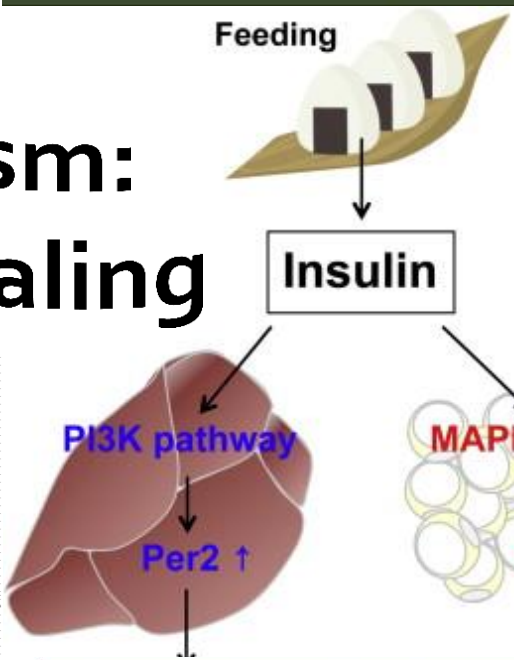
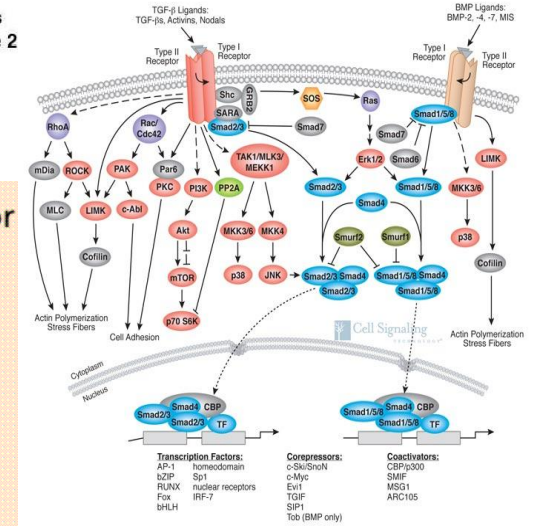
THE UNIVERSITY OF JORDAN

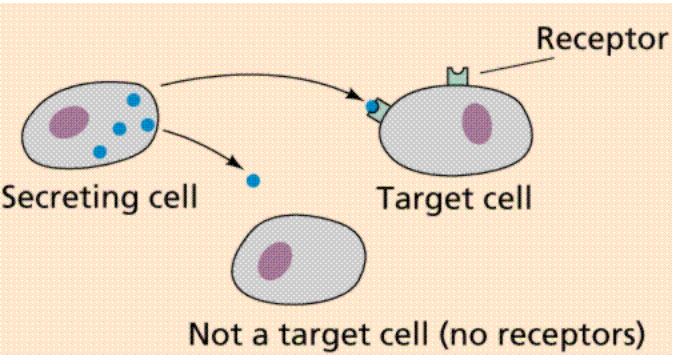
Integration of Metabolism: hormones & Cellular Signaling



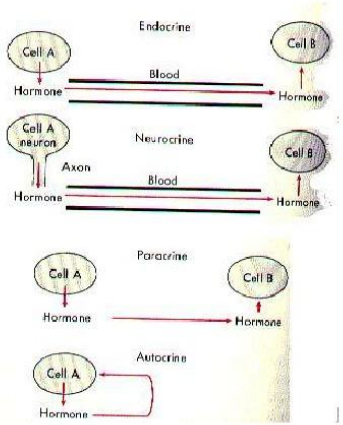








Types of cell-to-cell signaling



Endocrine Hormones: travel via bloodstream to target cells

Neurocrine hormones: released from nerve terminals

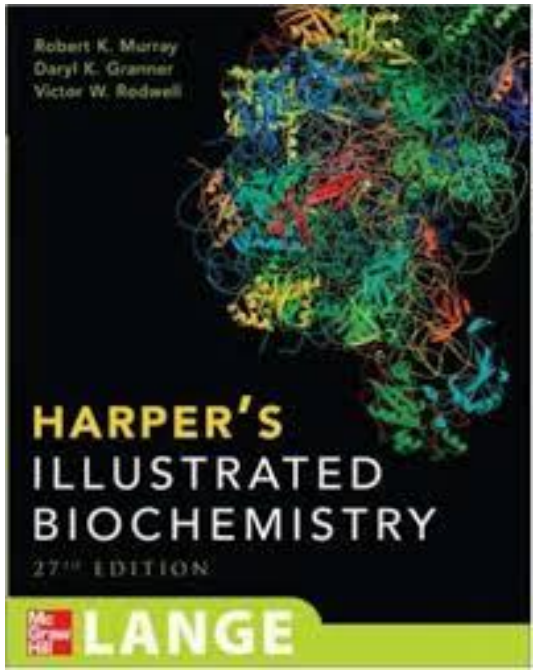
Paracrine hormones: act on adjacent cells

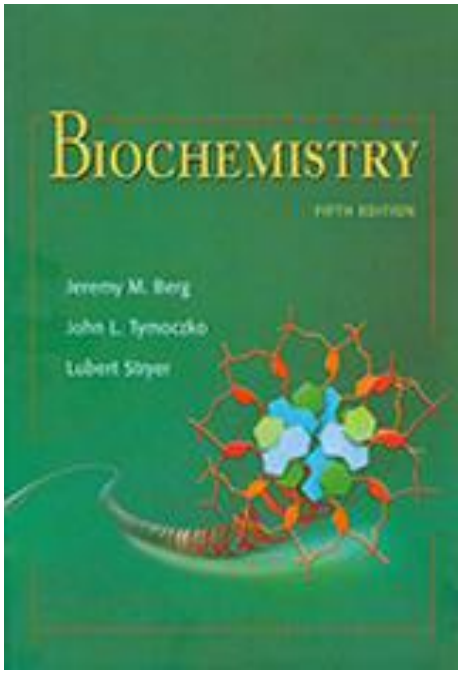
Autocrine hormones: Released and act on the cell that secreted them.

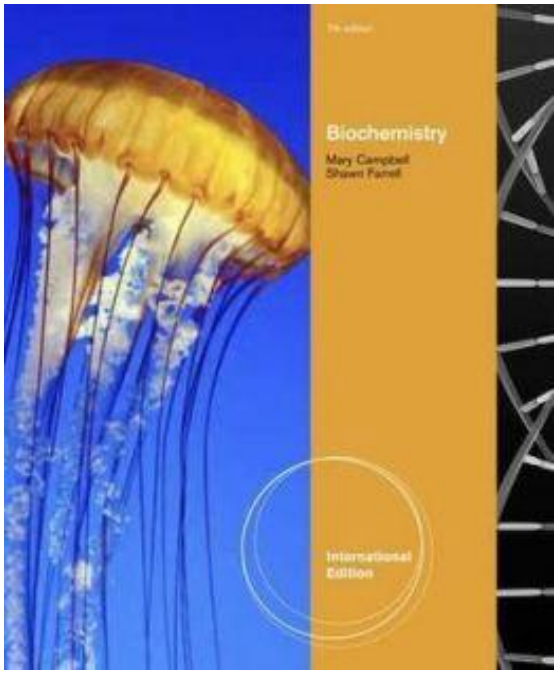
Intracrine Hormones: act within the cell that produces them.

Resources for the 3 lectures

- Harper's Illustrated Biochemistry
- Stryer's Biochemistry
- Campbell's Biochemistry

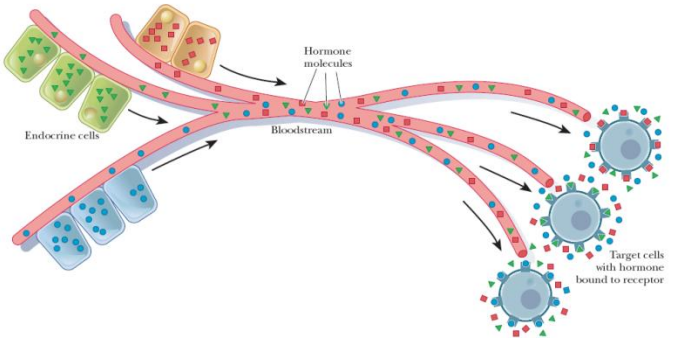




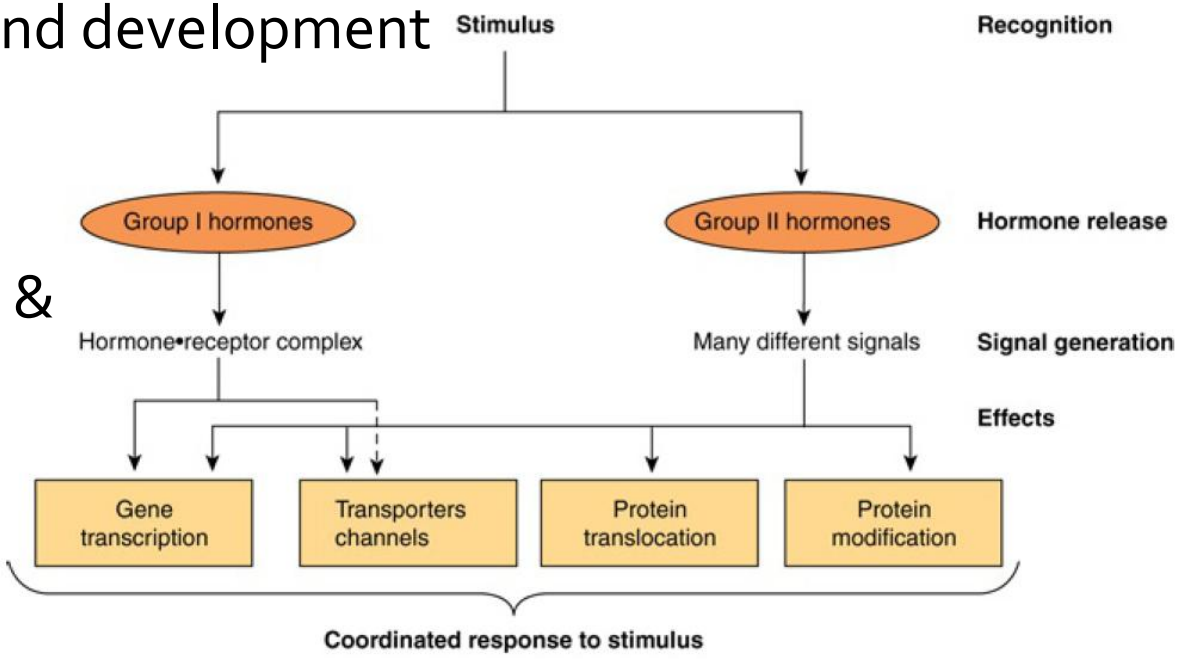


Hormones: The Remote Controllers

- What are hormones? Organic, blood, low amounts, source & target
- Functions:
  - They help maintain homeostasis
  - Mediate responses to external stimuli
  - Play roles in growth and development

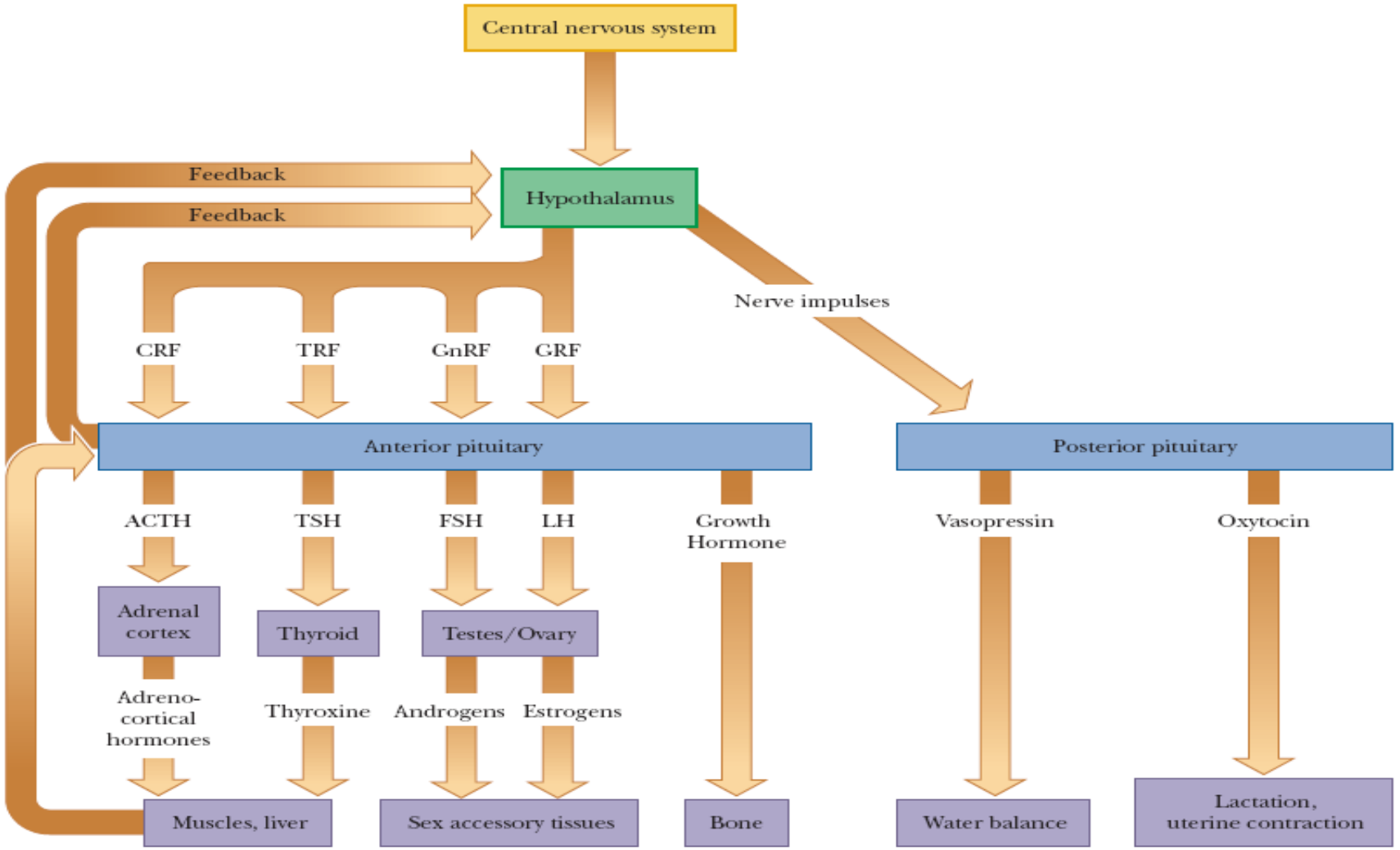


- Classes:
  - Endocrine hormones
    - Distance; stability; & concentration
  - Paracrine hormones
  - Autocrine hormones



Nervous vs./& Endocrine

Two systems act individually and together in regulating the human physiology



THE TARGET CELL CONCEPT

- 200 types of differentiated cells in humans
- Only a few produce hormones! (<50 known hormones)
- All of the 75 trillion cells in a human are targets to one or more

- One hormone → several cell types
- One cell type → several hormones
- One hormone → several effects

- The definition of a target has been expanded to include any cell in which the hormone (ligand) binds to its receptor, regardless of the action

THE TARGET CELL CONCEPT

- Several factors determine the response of a target cell to a hormone:

Factors affect the concentration of the hormone at the target cell
✓ The rate of synthesis and secretion of the hormone
✓ The proximity of the target cell to the hormone source (dilution)
✓ The $\mathbf{K_d}$ of the hormone – receptor complex
✓ The rate of conversion of inactive form to the fully active form
✓ The rate of clearance from the plasma

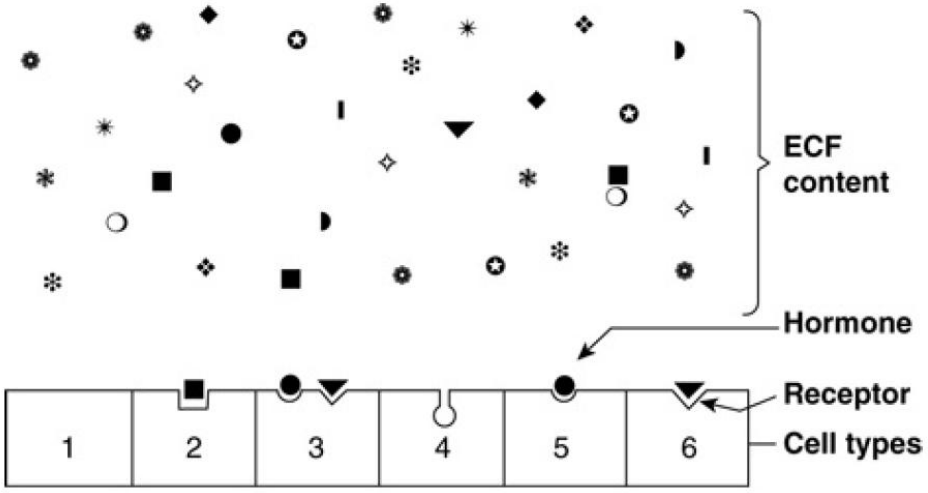
THE TARGET CELL CONCEPT

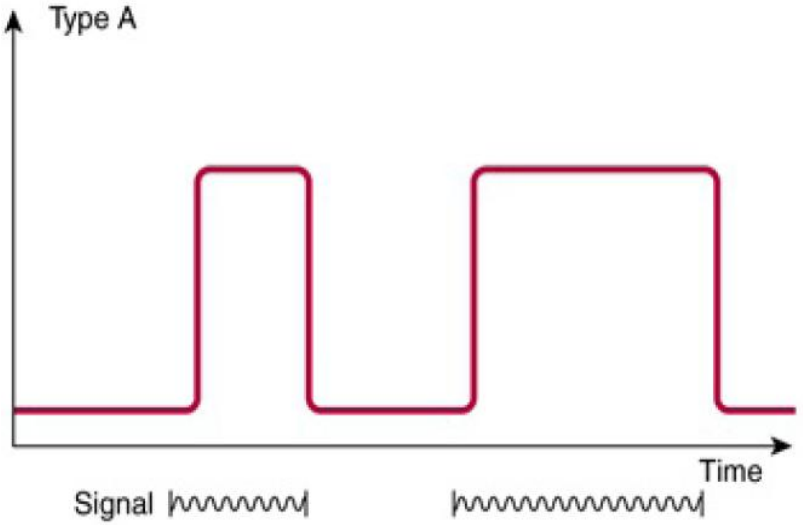
- Several factors determine the response of a target cell to a hormone:

Factors affecting the target cell response
✓ The number, relative activity, and state of occupancy of receptors
✓ The metabolism (activation / inactivation) of the hormone in the target cell
✓ The presence of factors within target cell necessary for the response
✓ Up- or down-regulation of the receptors upon interaction with ligand ✓ Post-receptor desensitization of the cell

Receptors Discriminate Precisely Receptors Follow Type A Response

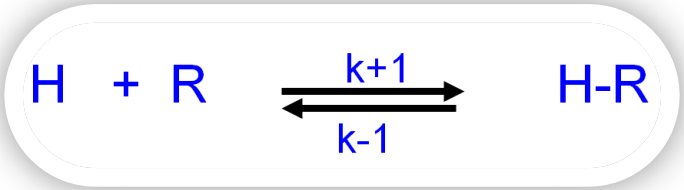
- Major challenge:
  - Atto- to nano-molar range (10^{-15} to 10^{-9} mol/L) vs. Structurally similar molecules (sterols, amino acids, peptides, and proteins): micro- to milli-molar (10^{-6} to 10^{-3} mol/L) range

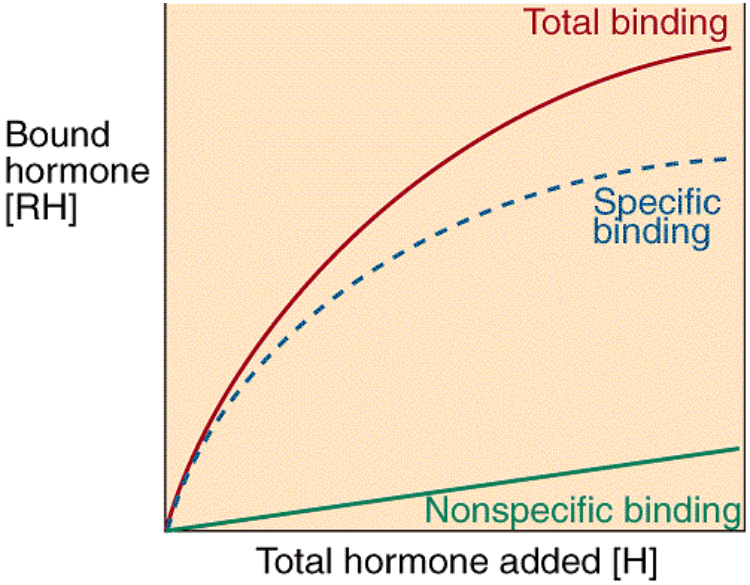




Accordingly; Hormone-Receptor Interactions

- Should be specific: displaceable by agonist or antagonist
- Should be saturable
- Should occur within the concentration range provided



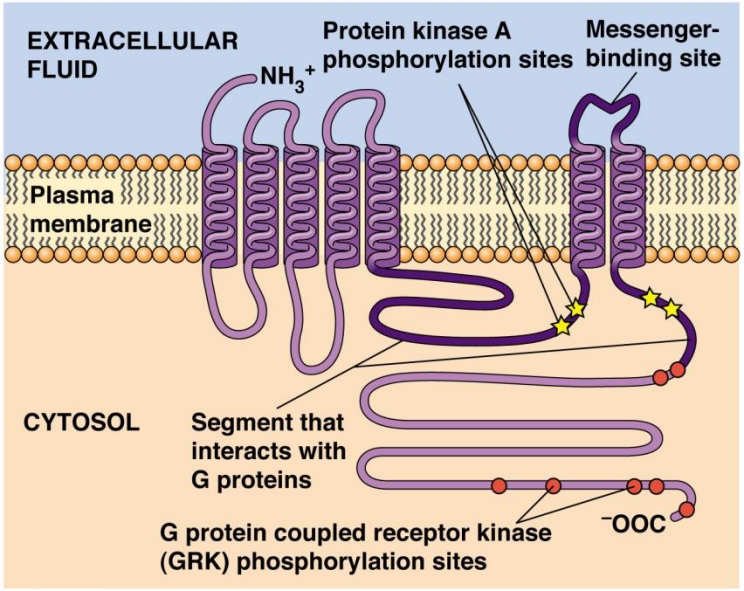


- Association constant K_a
- Dissociation constant K_d

- $K_a = [H\text{-}R] / \{[H] \times [R]\}$
- $K_d = \{[H] \times [R]\} / [H\text{-}R]$

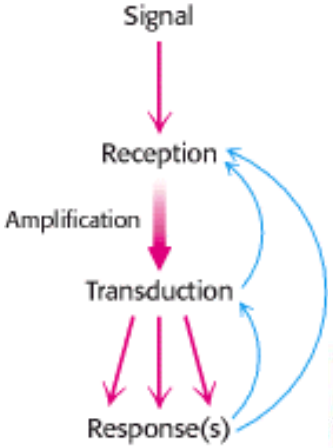
- 20X dissociation constant is enough to saturate the receptor
- $\mathbf{K_d}$ values for many hormone range from 10^{-9} to 10^{-11} M

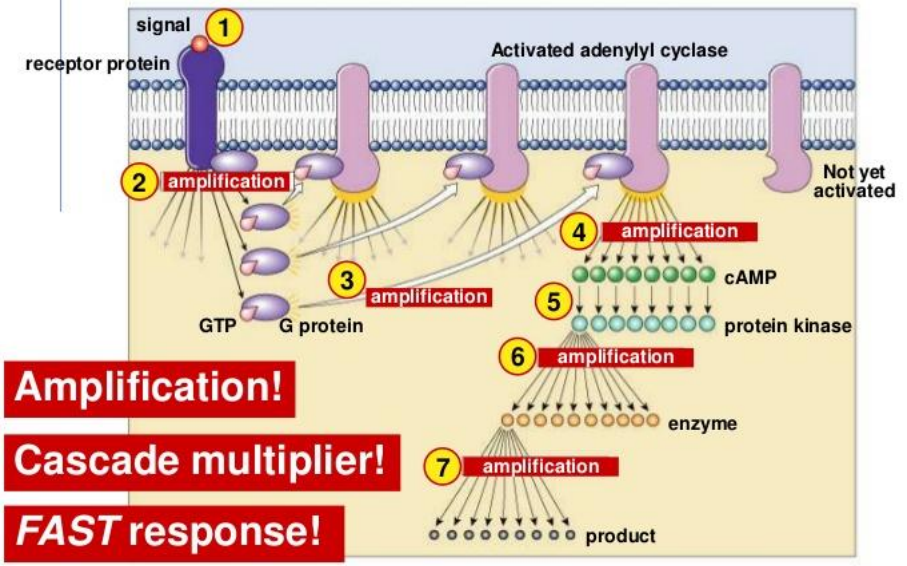
Receptor Domains



- All receptors have at least two functional domains:
  - Recognition domain
  - Coupling or signal transduction domain
- Coupling occurs in two general ways:
  - Changing the activity of an enzyme (Polypeptide & catecholamines, plasma membrane)
  - Direct (steroids, retinoids, and thyroid hormones, intracellular)
- Steroid, thyroid, and retinoid hormone receptors:
  - Hormone binding site ; DNA binding site; co-regulator proteins binding site, cellular trafficking proteins binding site
- Receptor–effector coupling— provides the first step in amplification

Signal Amplification

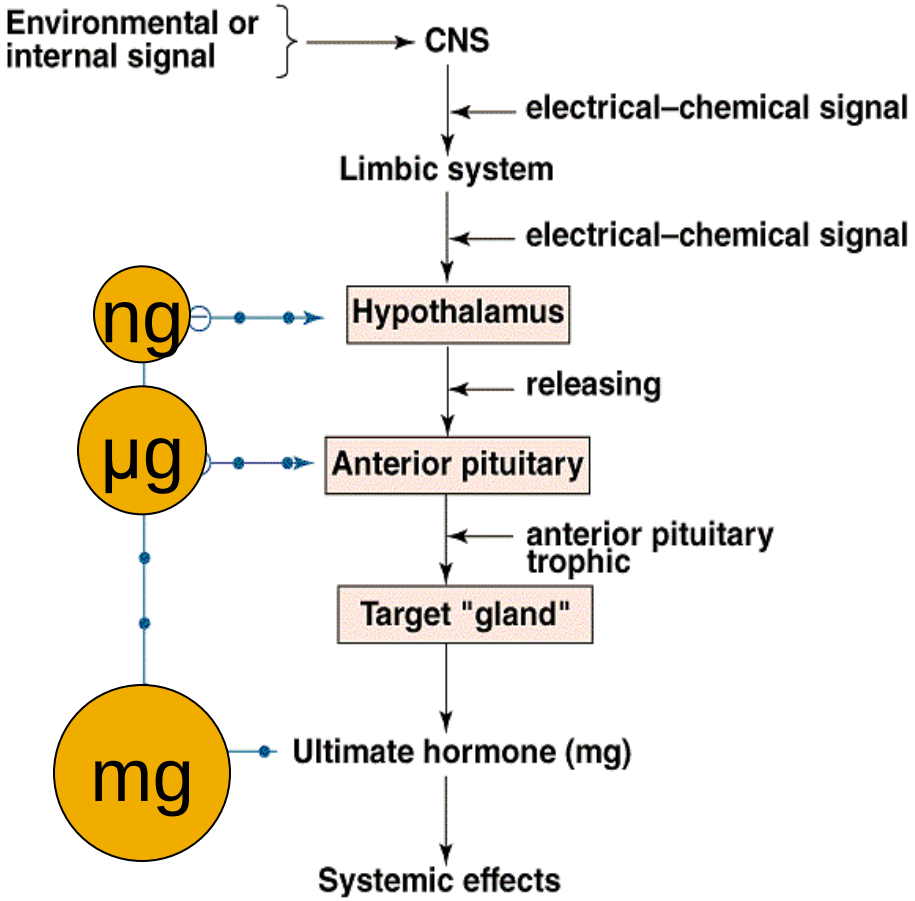


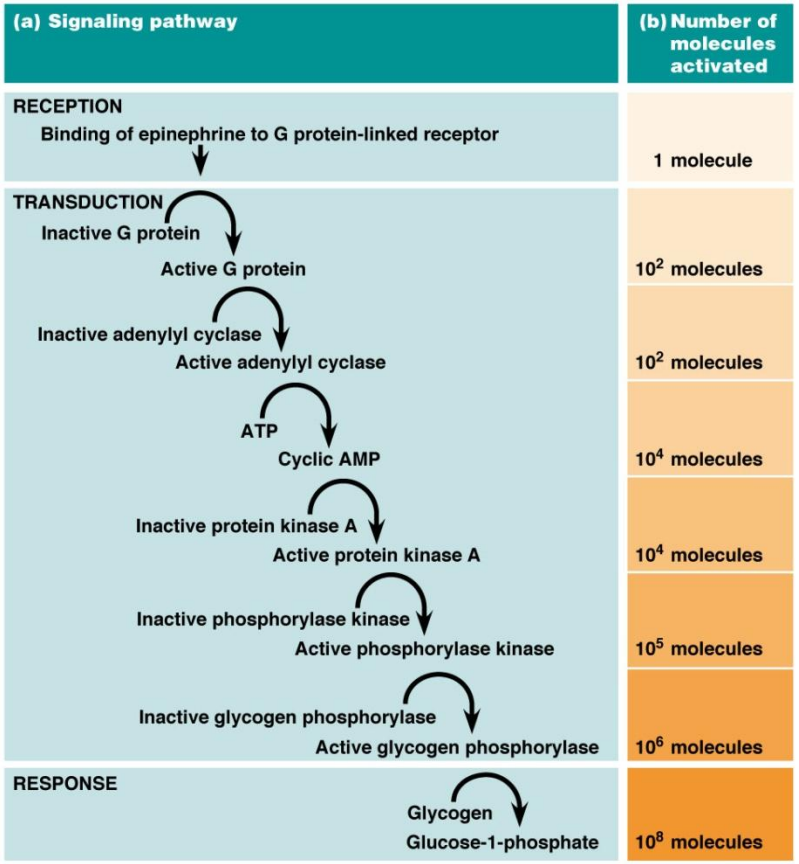


Amplification!

Cascade multiplier!

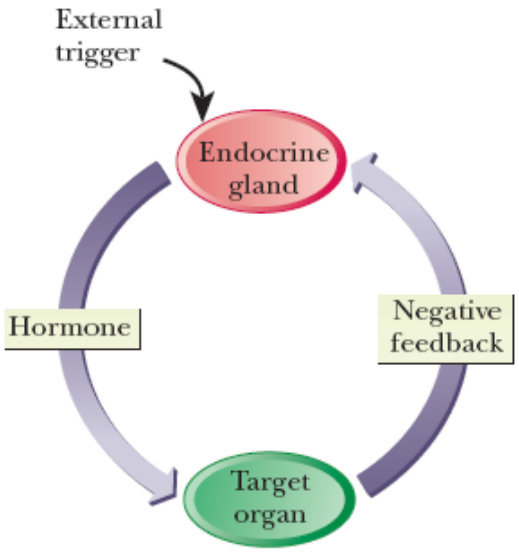
***FAST* response!**

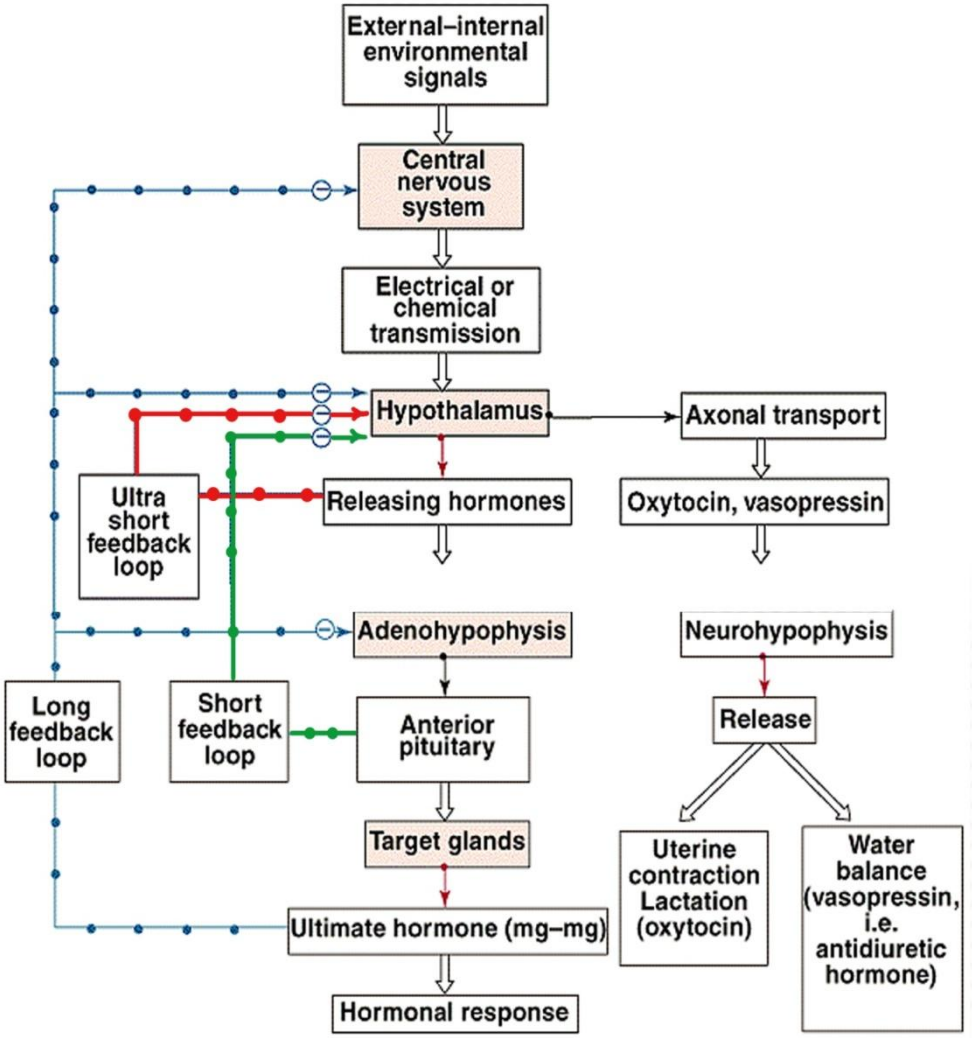




How the release is controlled?

- Feedback inhibition
  - Ultrashort loop
  - Short loop
  - Long loop





Classification of Hormones Chemical Structure

- Chemical composition; solubility; location of receptors; nature of the signal used to mediate hormonal action

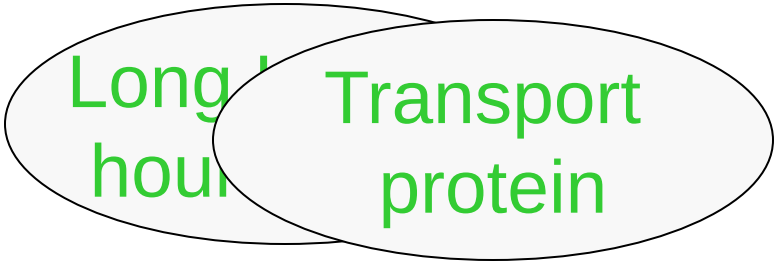
✓ **Polypeptides**: Pituitary hormones; Hypothalamic releasing hormones; Insulin, Growth factors…

✓ **Amino acid derivatives**: Adrenalin, Thyroid hormones

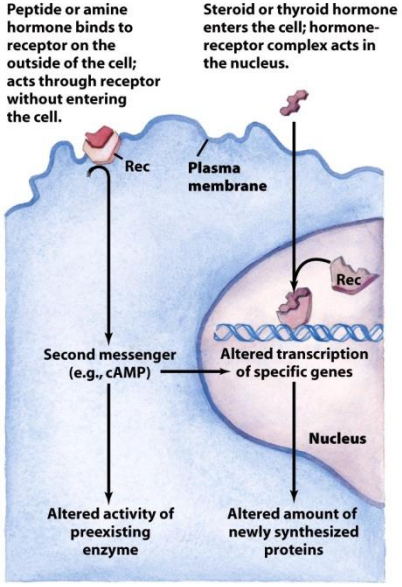
✓ **Steroids**

Classification of Hormones Mechanism of Action

- Hormones that bind to intracellular receptors
  - Steroids
  - Thyroid hormones
  - Calcitriol, retinoic acid

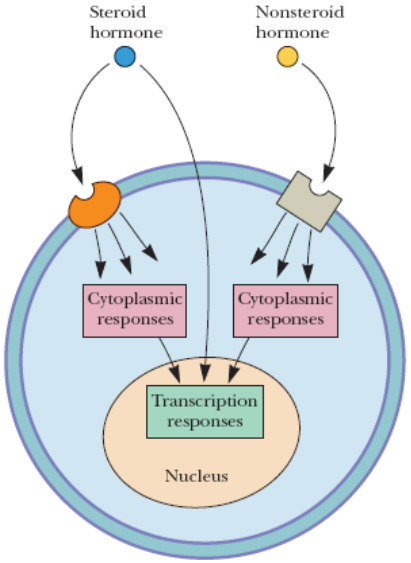


- Hormones that bind to cell surface receptors (According to second messenger):
  - cAMP (β adrenergic factor, glucagon, ACTH)
  - cGMP (atrial natriuretic factor, Nitric oxide)
  - Calcium or phosphatidyl inositol (oxytocin, TRH)
  - Kinase or phosphatase cascade (insulin, GH)



General Features of Hormone Classes

	Group I	Group II
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Action	Slow	Fast
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma $t_{1/2}$	Long (hrs - days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator	Receptor-hormone complex	cAMP, cGMP, Ca^{2+}, kinase cascades, metabolites of phosphoinositols



Hormones Classes
Steroid hormones

A. Sex hormones - are divided into 3 groups
   1. Male sex hormones or Androgens
   2. Female sex hormones or Estrogens
   3. Pregnancy hormones or Progestines

B. Hormones of Adrenal Cortex
   1. Mineralocorticoids: aldosterone. ...
   2. Glucocorticoids: cortisol. ...
   3. Adrenal androgens: male sex hormones mainly dehydroepiandrosterone (DHEA) and testosterone

Hormones Classes
Non steroid hormones

A. Peptide and protein hormones

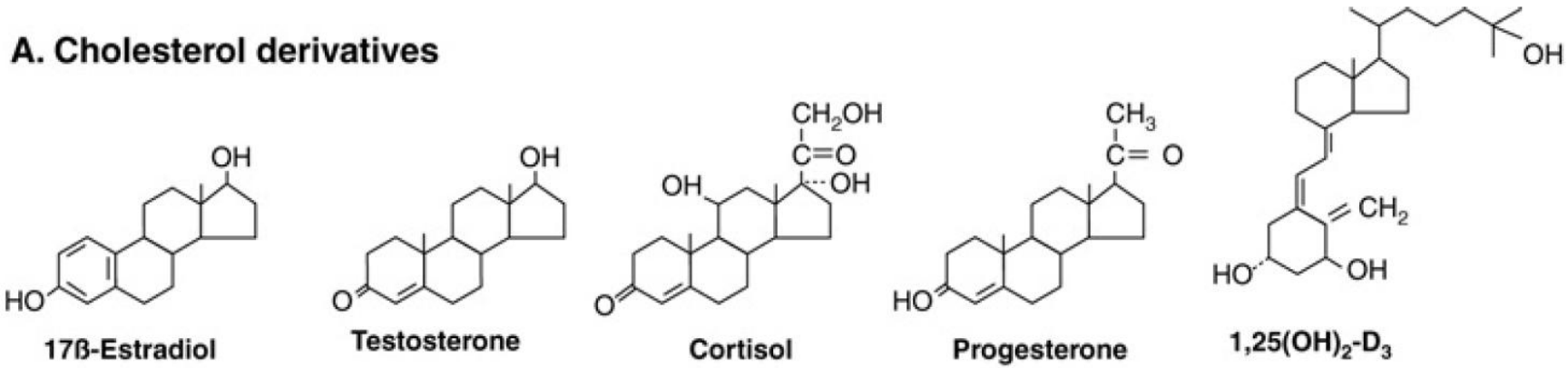
- ✓ All hypothalamic, pituitary, digestive hormones
- ❖ All pituitary hormones are made from single polypeptide chains **EXCEPT**: TSH; FSH; LH (homodimers) – glycoproteins (≈ 25 kDa)

B. Amino acid derivatives

- ✓ Amines - derived from tyrosine or tryptophan
  TH, dopamine, epinephrine, melatonin

Structure of Hormones

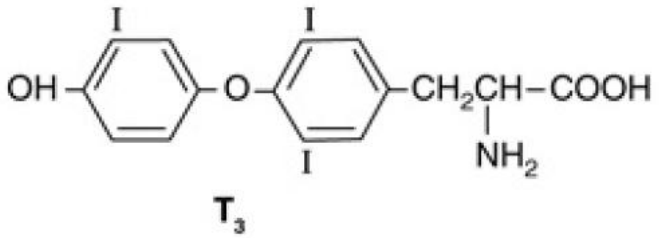
- Lipid – soluble hormones:



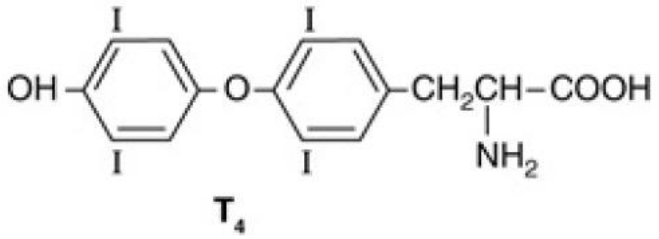
Structure of Hormones

- Amino Acid-Derived Hormones

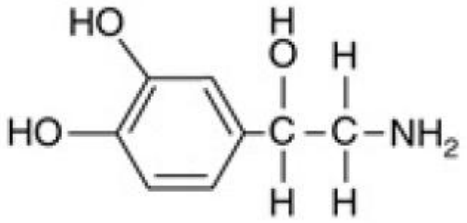
Tyrosine derivatives



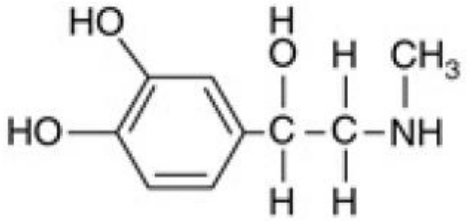
T_3



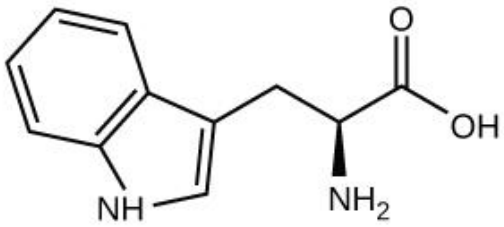
T_4



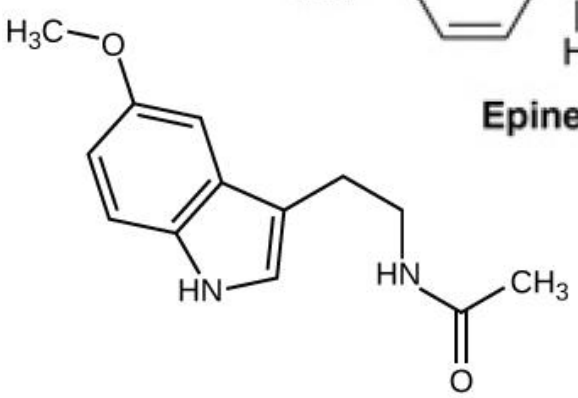
Norepinephrine



Epinephrine



Tryptophan

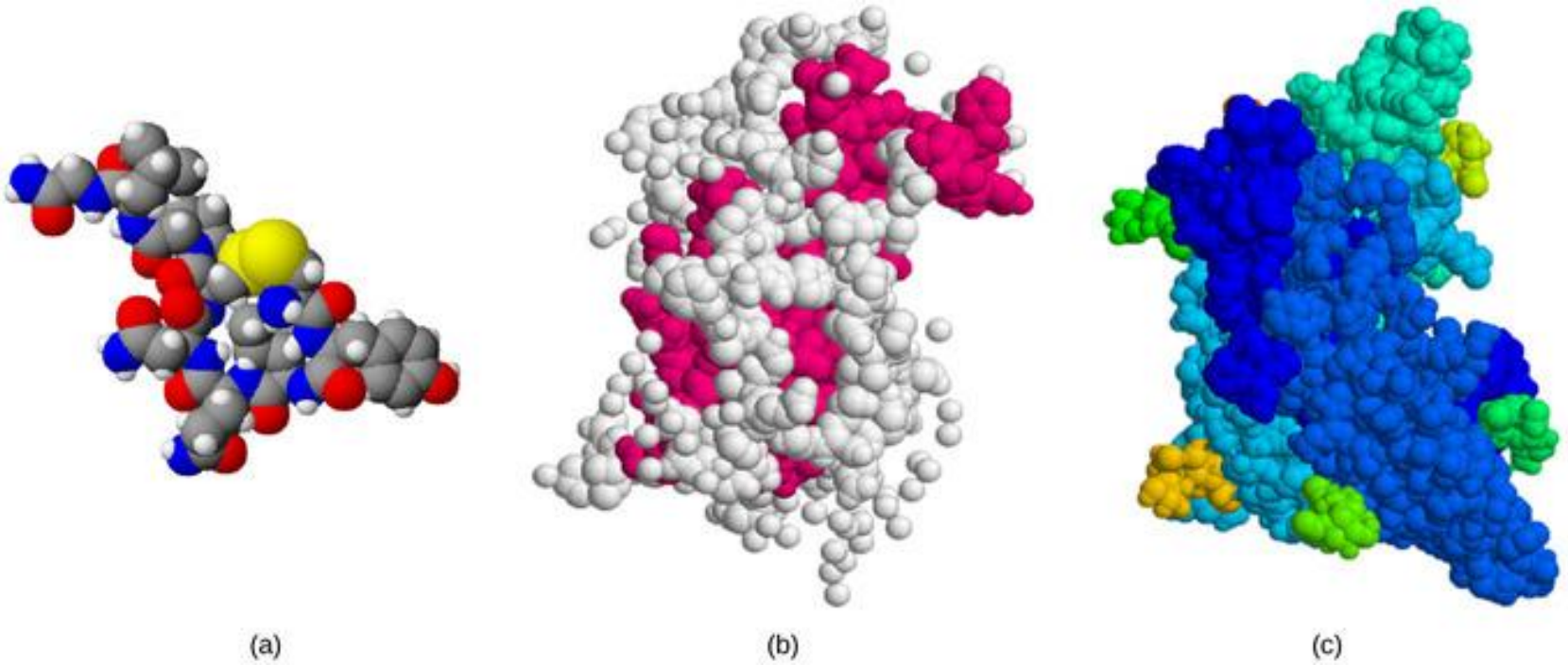


Melatonin

Structure of Hormones

- Peptide & Protein Hormones

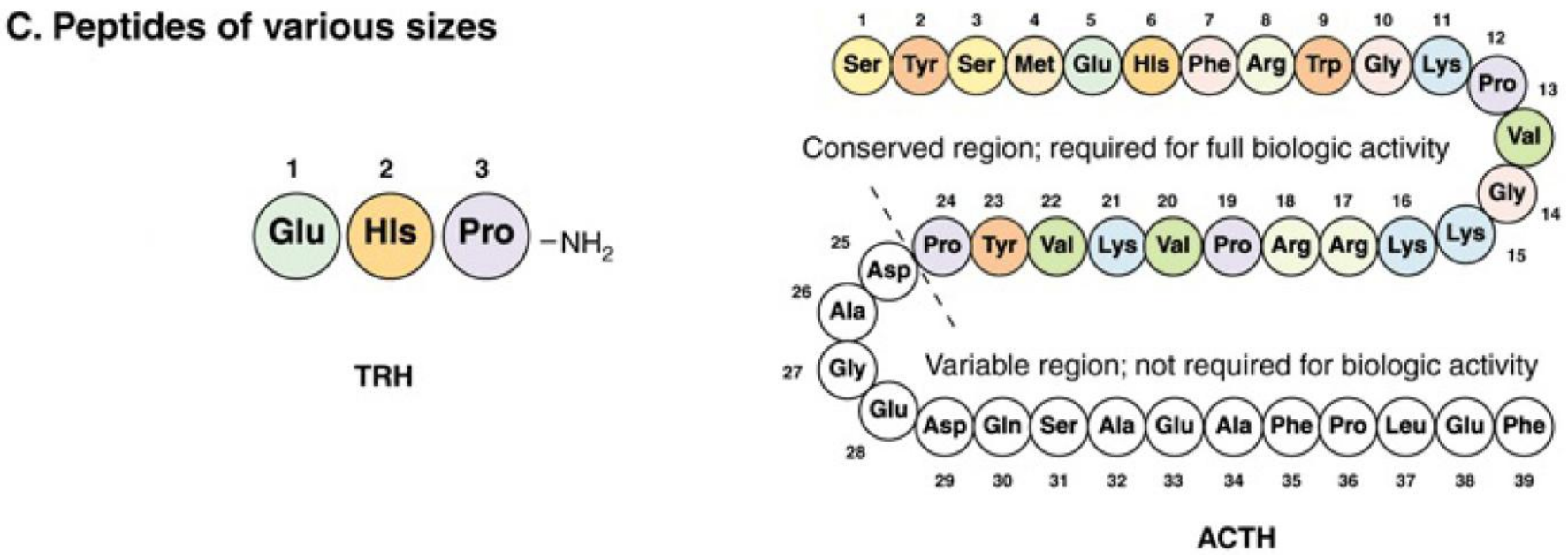
Oxytocin GH FSH



(a) (b) (c)

Structure of Hormones

- Peptide & Protein Hormones



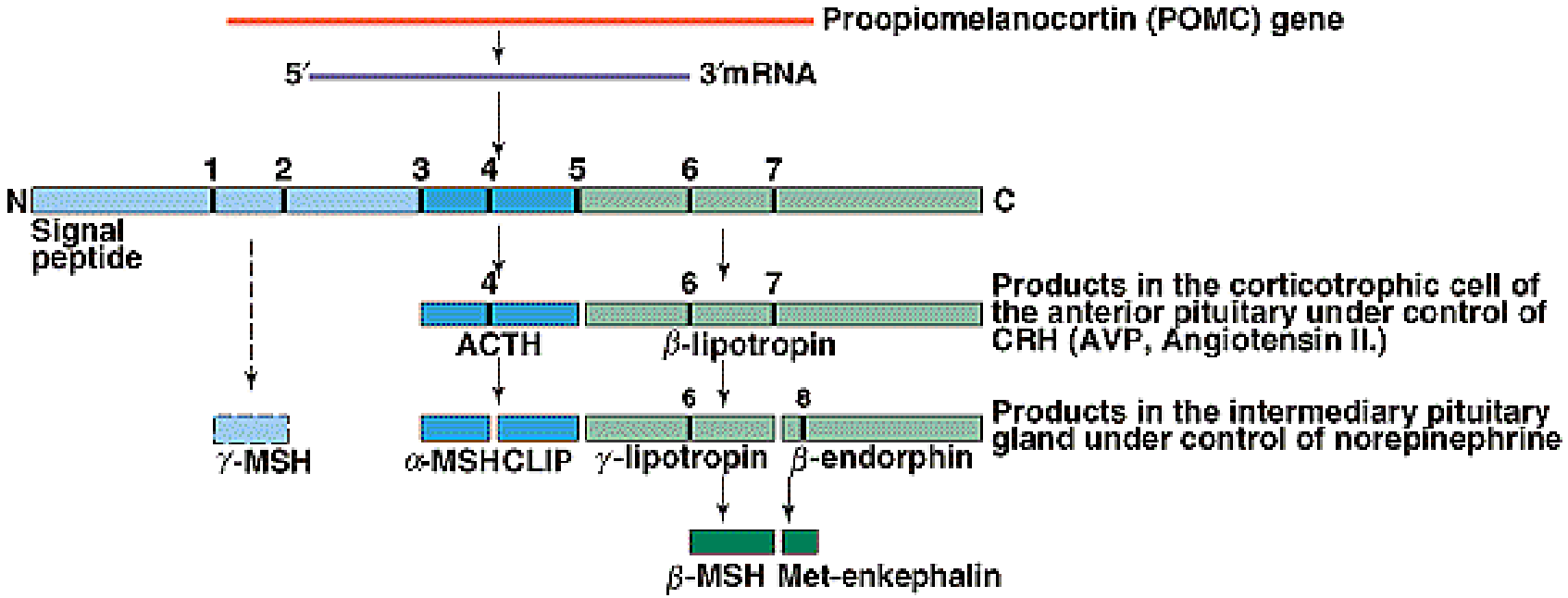
Structure of Hormones

Peptide & Protein Hormones

Hormone	Structure
GHRH	44
TRH	3
GnRH	10
CRH	41
ADH	9
Vasopressin	9
Angiotensin I	10
Angiotensin II	8
Insulin	51
Glucagon	29

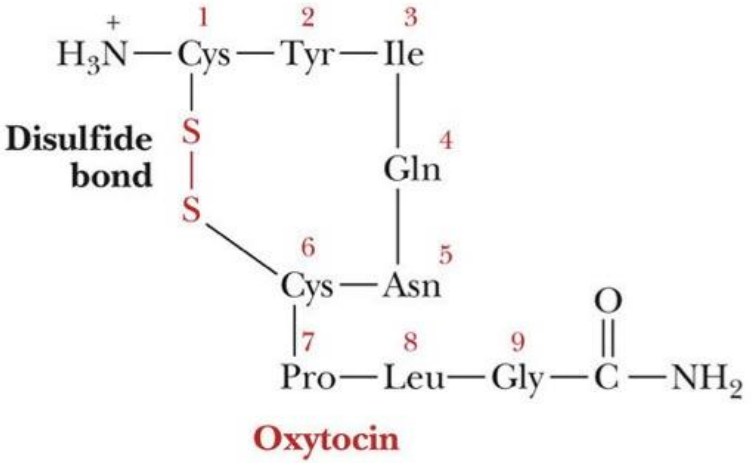
Synthesis of Peptide Hormones

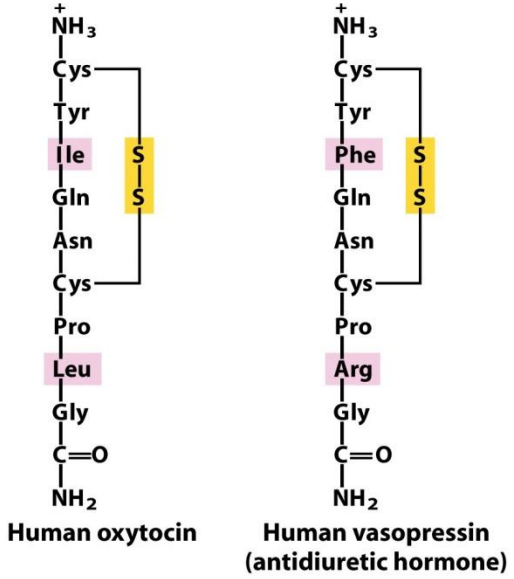
- From precursor polypeptides
  - One gene may code more than one hormone (POMC)
  - The cleavage depends on specific enzymes

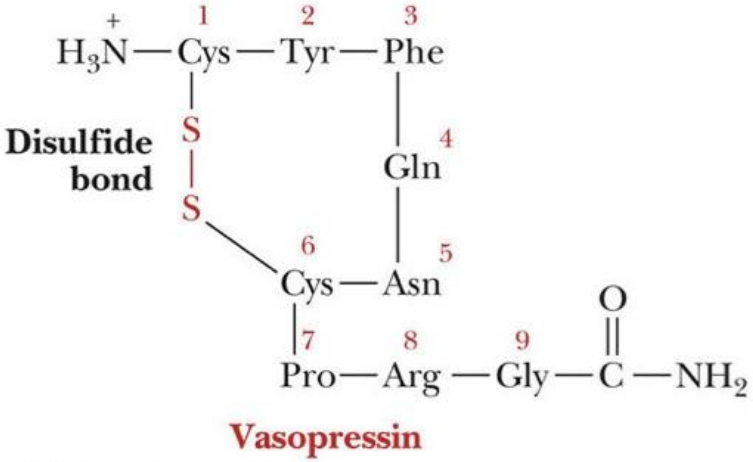


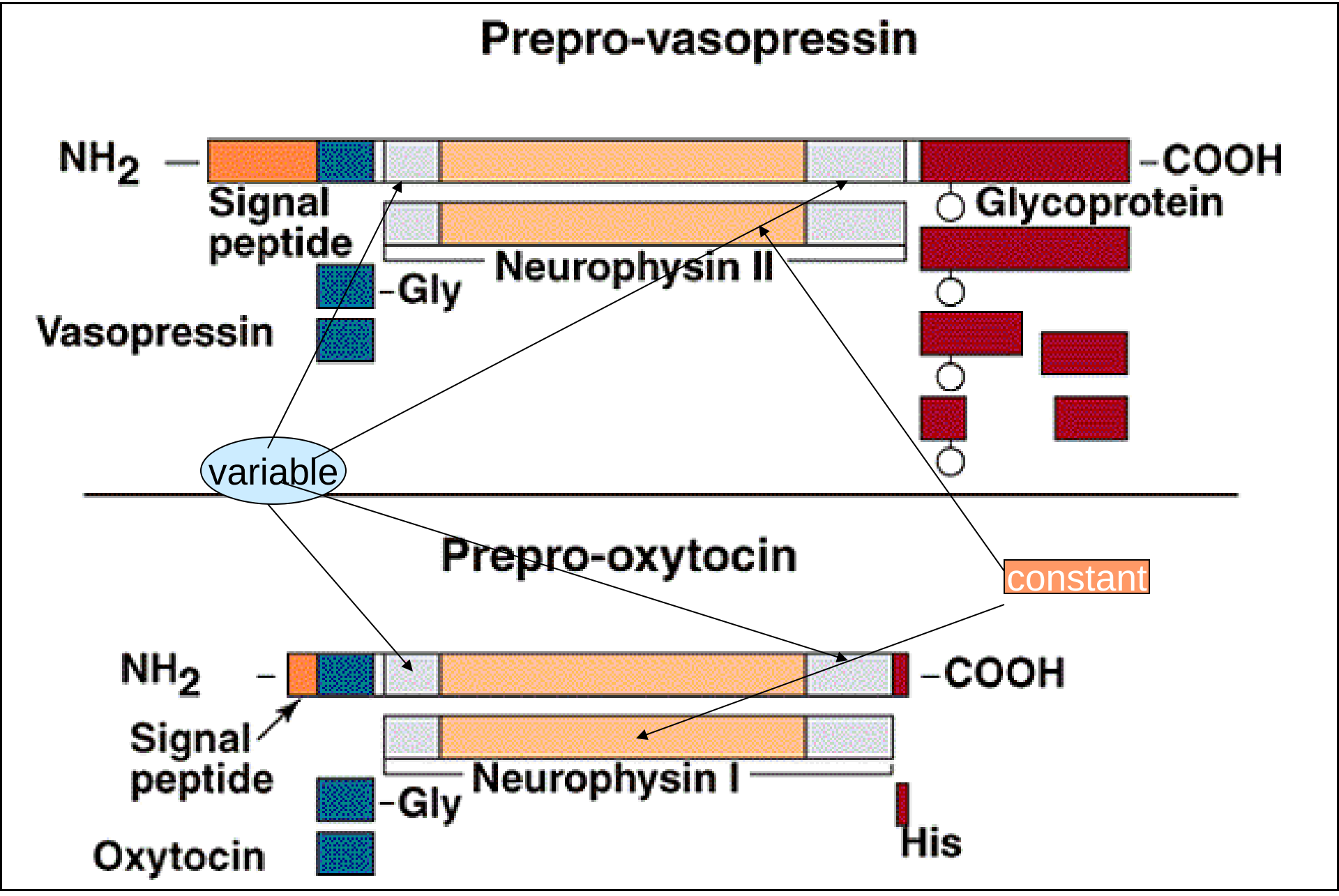
Synthesis of Peptide Hormones

- From precursor polypeptides
  - Vasopressin and oxytocin
  - Synthesis in separate cell bodies of hypothalamic neurons

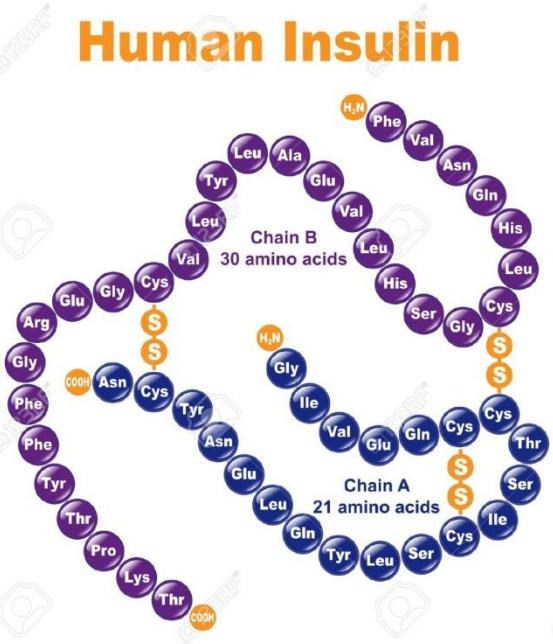







Prepro-vasopressin
NH2
COOH
Signal peptide
Glycoprotein
Neurophysin II
Gly
Vasopressin
variable
Prepro-oxytocin
constant
NH2
COOH
Signal peptide
Neurophysin I
Gly
Oxytocin
His

Synthesis of Peptide Hormones

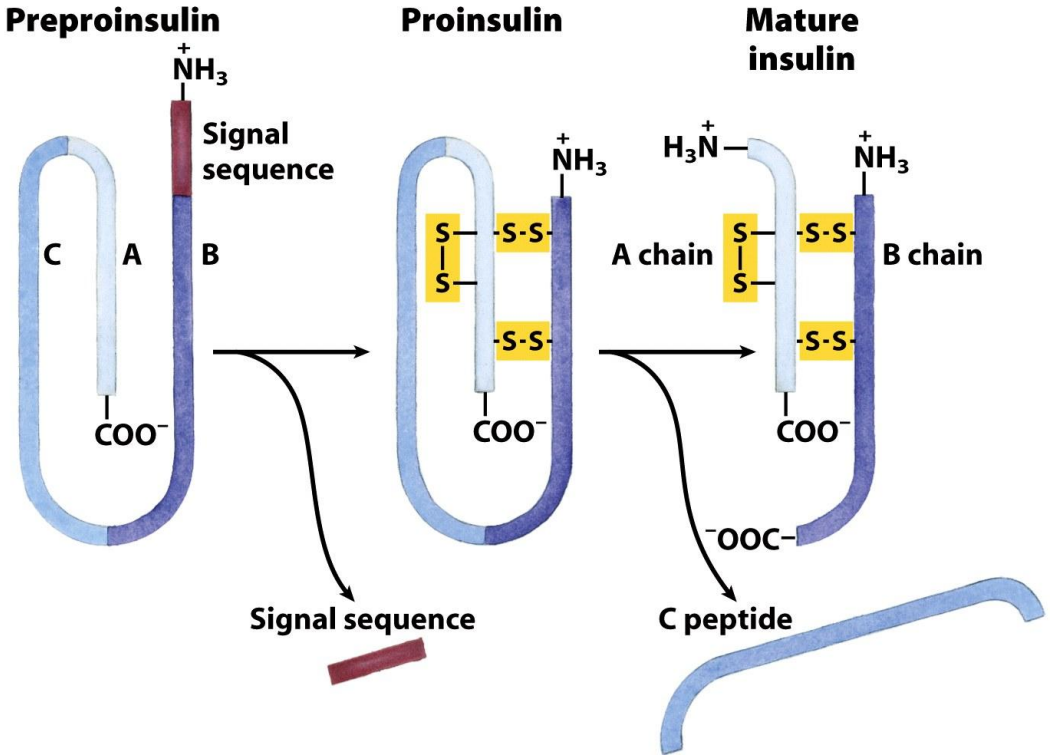


- Peptide & Protein Hormones
- From Pre-pro-hormones

- A larger precursor preproinsulin
  - 23 aa signal sequence
  - 3 disulfide bonds

- Proinsulin
  - Remove the C peptide

- Mature insulin
  - A and B chains



Target cells interactive effects

1. **Permissive effects** – one hormone enhances the effect of a later hormone
   - ✓ **Estrogen up-regulates progesterone receptors** in uterus
   - ✓ **Thyroid hormone increases the effect of epinephrine** on breakdown of triglycerides in adipocytes

2. **Integrative effects –** hormones produce complementary effects on different tissues
   - ✓ **PTH and calcitriol** increase ECF **calcium**

Target cells interactive effects

3. **Synergistic effects:**
   - ✓ Both **FSH and estrogen** necessary for **normal oocyte development**
   - ✓ **FSH and testosterone** together increase **spermatogenesis**

4. **Antagonistic effects:**
   - ✓ **Insulin and glucagon**

Transduction of hormone signal

Detection, and generation of cellular response

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
  - 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

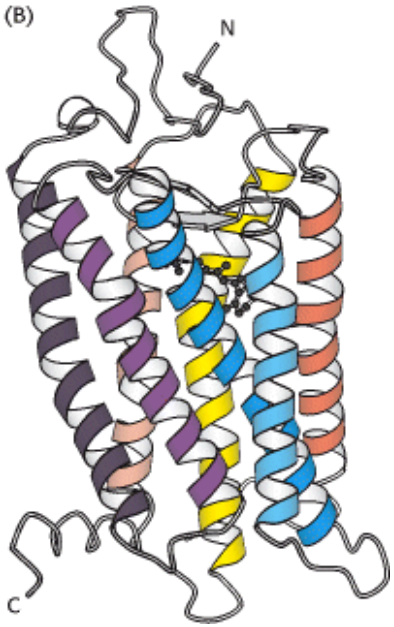
Signal Termination

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

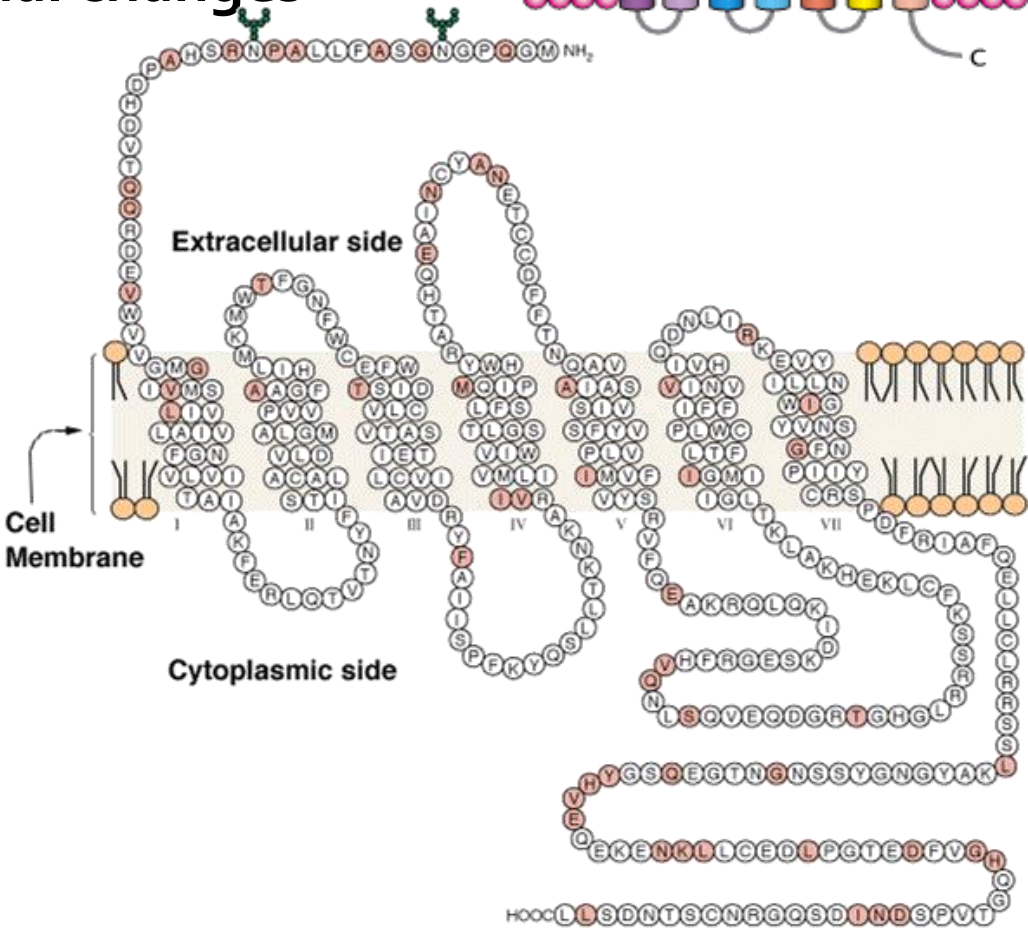
- How it is achieved?
  - Degradation of the second messenger
  - Dephosphorylation by hydrolysis

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

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



Rhodopsin receptor



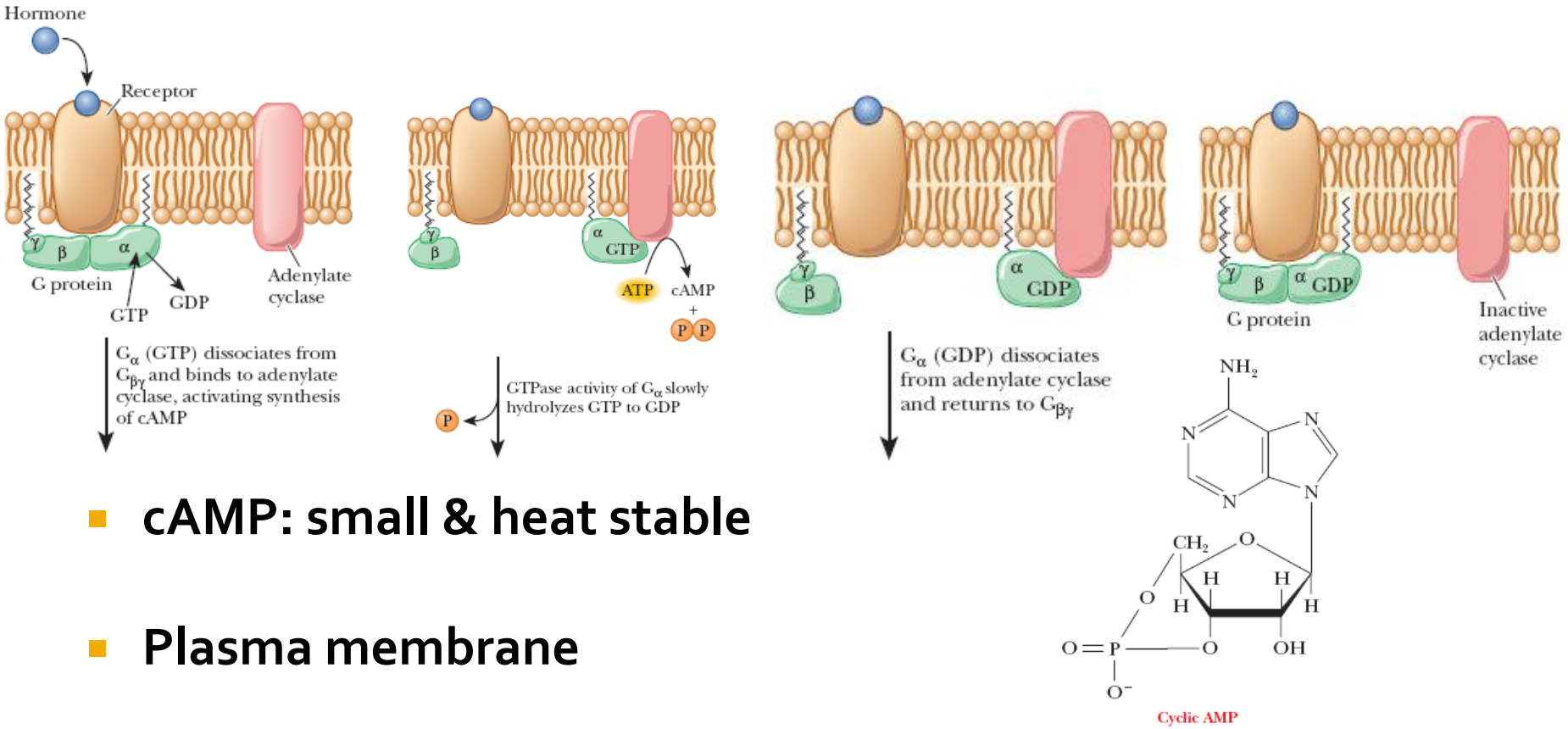
- Many Ser & Thr residues

Biological Functions Mediated by 7TM

- Smell, Taste, Vision
- Neurotransmission
- Hormone Secretion
- Chemotaxis
- Exocytosis
- Cell Growth, Development
- Viral Infection

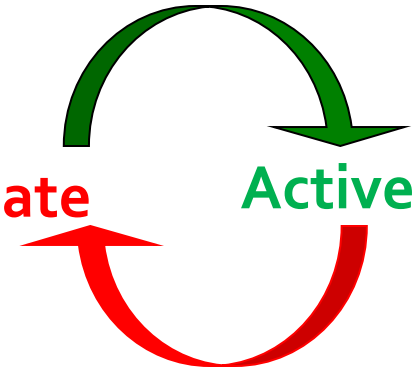
All these receptors share the same basic structure; however, they differ in their specificity and effects

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 Protein cycles between two forms



Inactive State

Active State

Bound to GDP

Bound to GTP

Heterotrimer αβγ

Dissociated α + βγ dimer

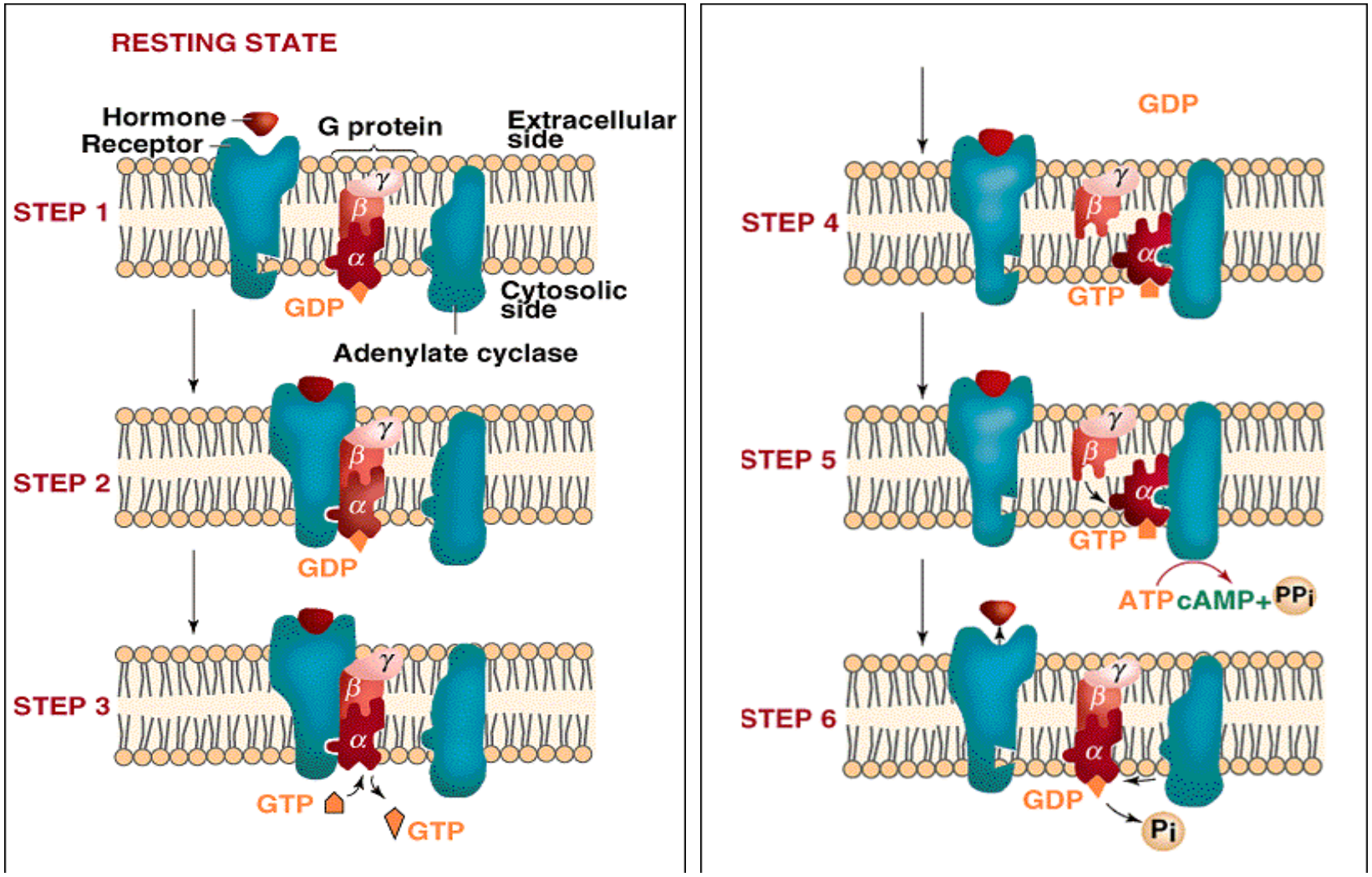
α subunit has GTPase Activity

Activated Receptor

↑ Adenylate Cyclase

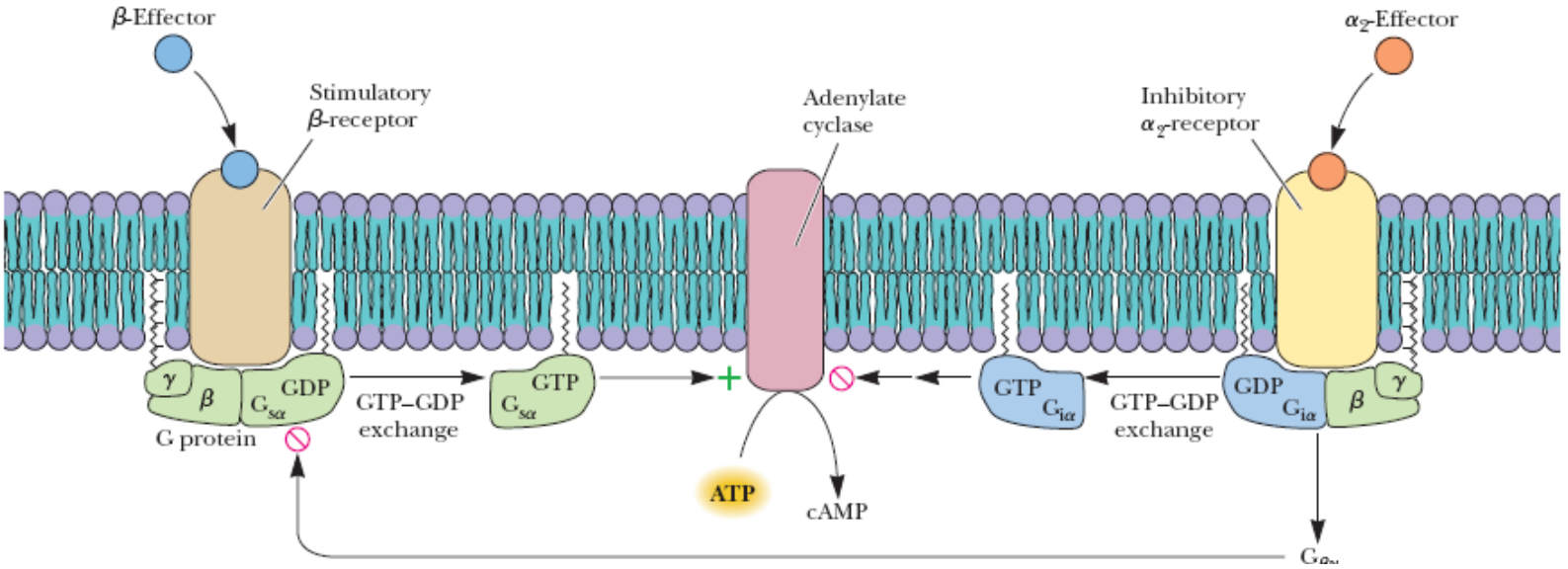
↑ cAMP

Biochemical Response



α subunit has GTPase Activity

G protein: 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_α subunit transduce many activities

G_s	↑ Adenylate Cyclase
G_{olf}	↑ Adenylate Cyclase
Transducin	↑ cGMP Phosphodiesterase
G_i	↓ Adenylate Cyclase
G_o	Ca^{2+} Channels
Gq	↑ Phospholipase C

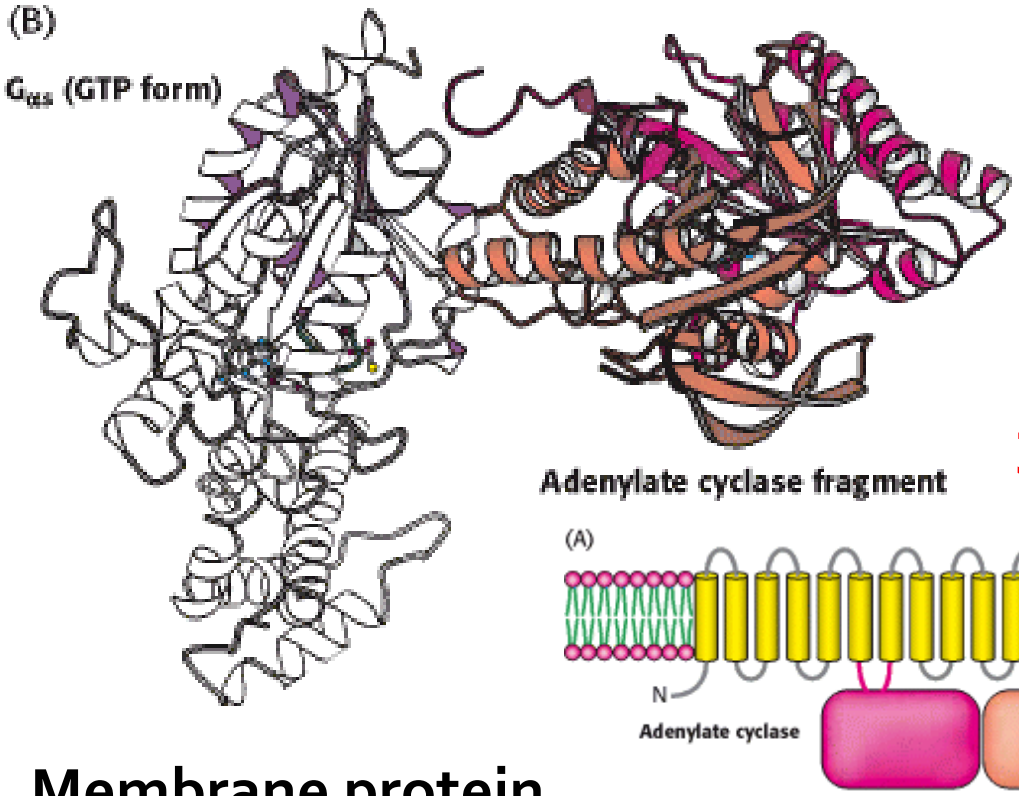
G Proteins (cont.)

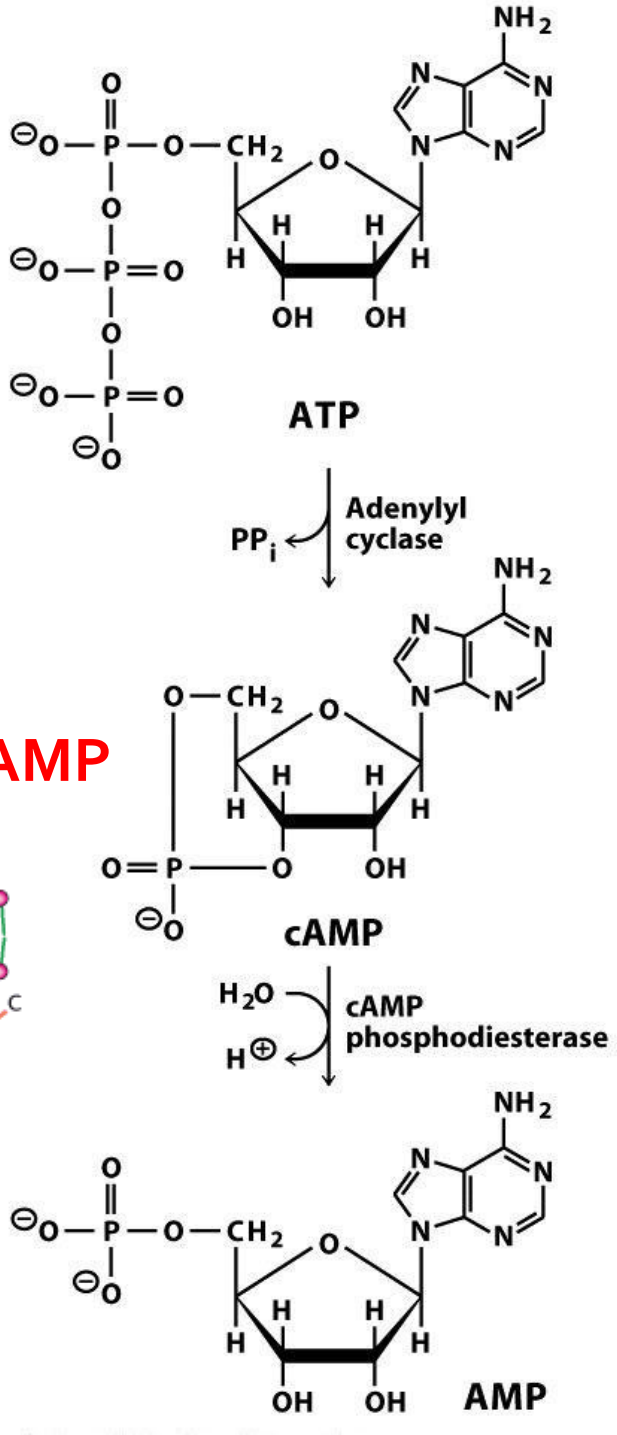
- α and γ Subunits have covalently attached fatty acid
- α and βγ can interact with other proteins
- All 7TM receptors appear to be coupled to G proteins

GPCRs

- Amplification: receptor → 100's of G protein → 100's of adenylate cyclase → 100's X 1000's molecules/sec of cAMP

Adenylate Cyclase



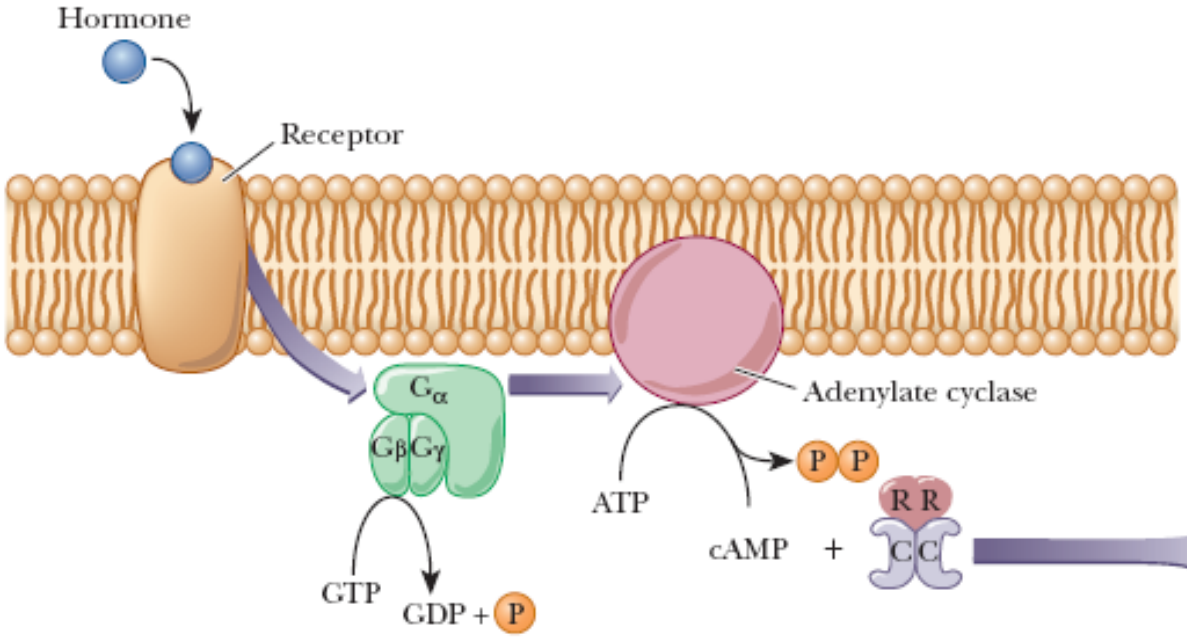


- Membrane protein
- 12 helices
- Two large intracellular domains
- Activated by G protein

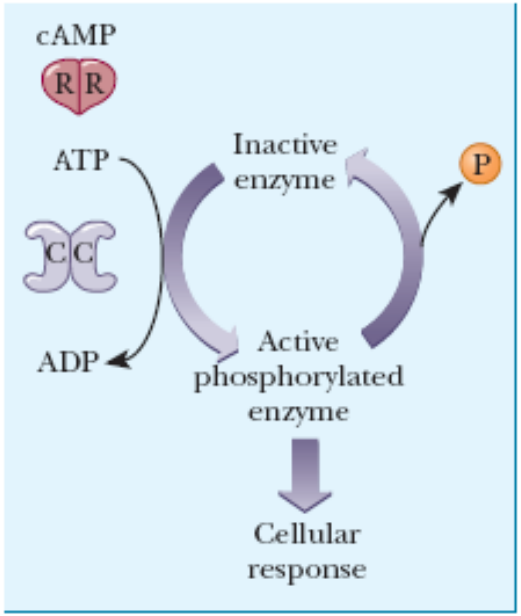
cAMP can affect a wide range of cellular processes

- ↑ degradation of storage fuels
- ↑ secretion of acid by gastric mucosa
- Dispersion of melanin pigment granules
- ↓ aggregation of blood plateletes
- Opening of chloride channels

Then what?



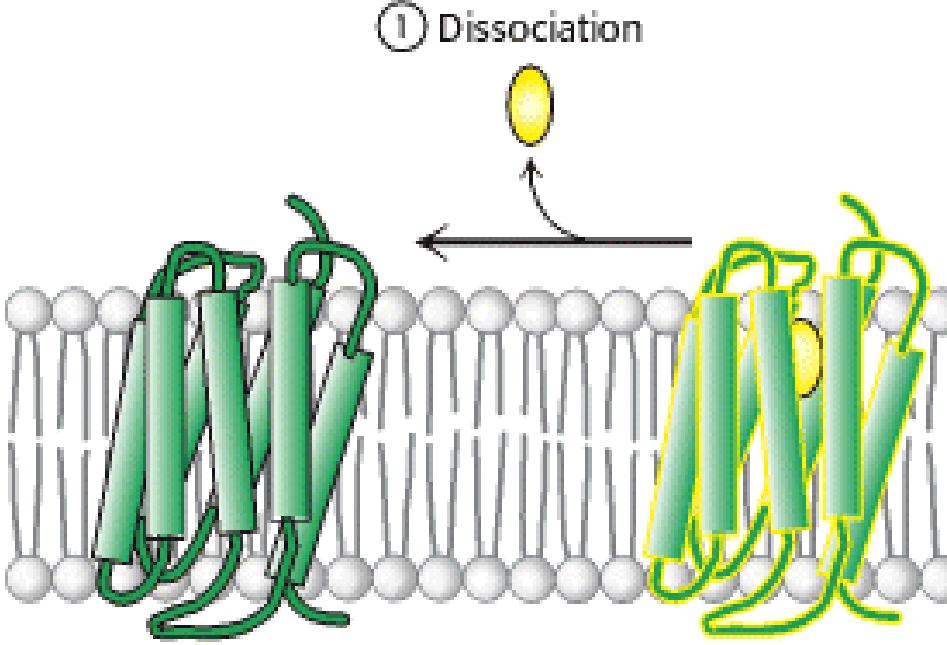
Usually:
Ser or Thr

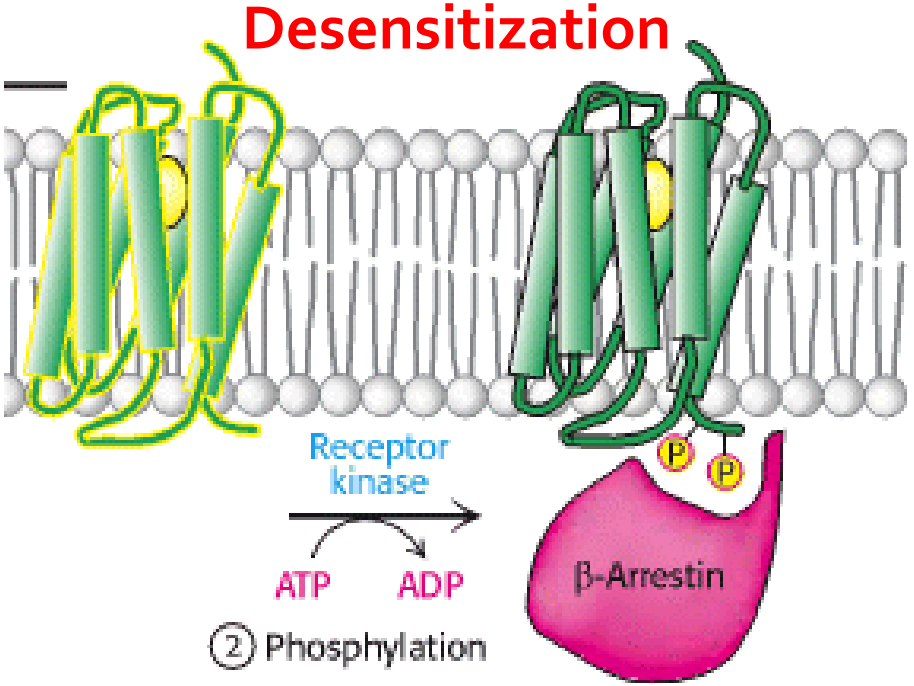


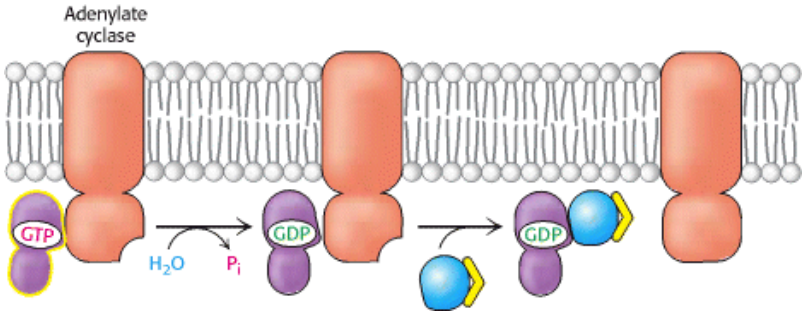
Glycogen Synthase!!

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

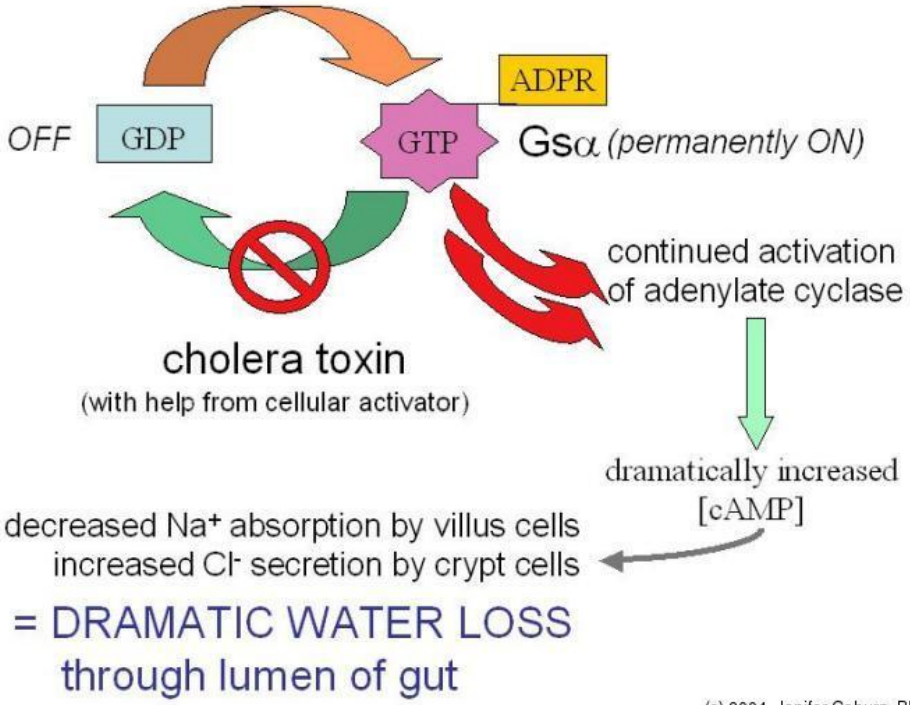


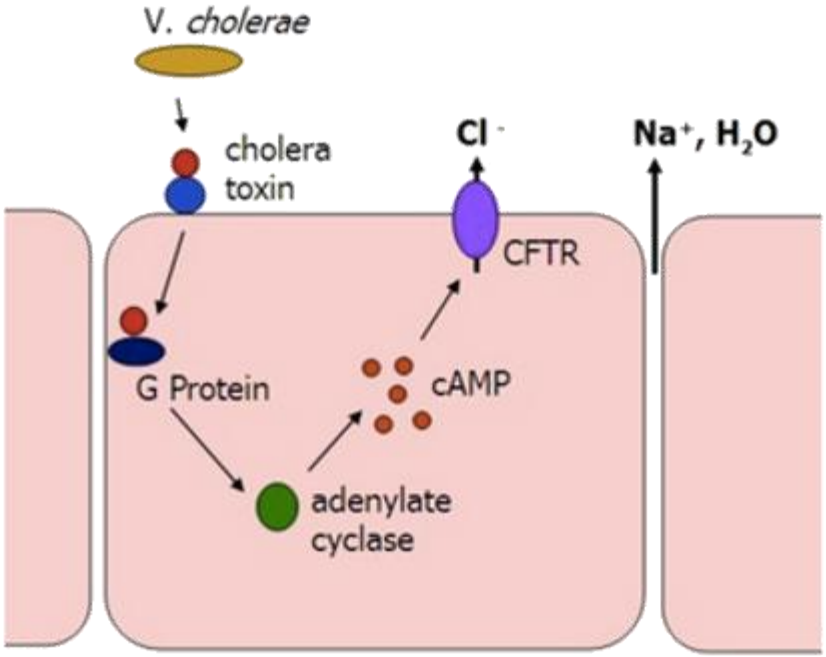




Cholera

- **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

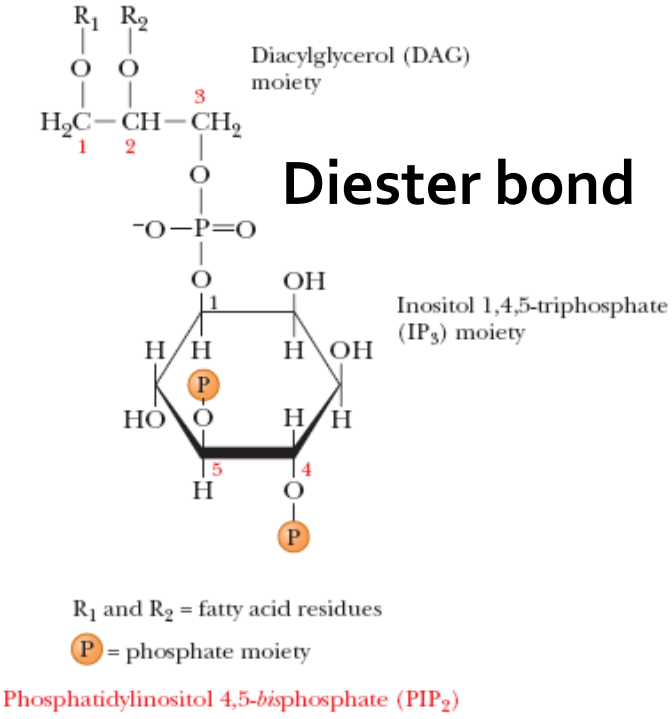
↓

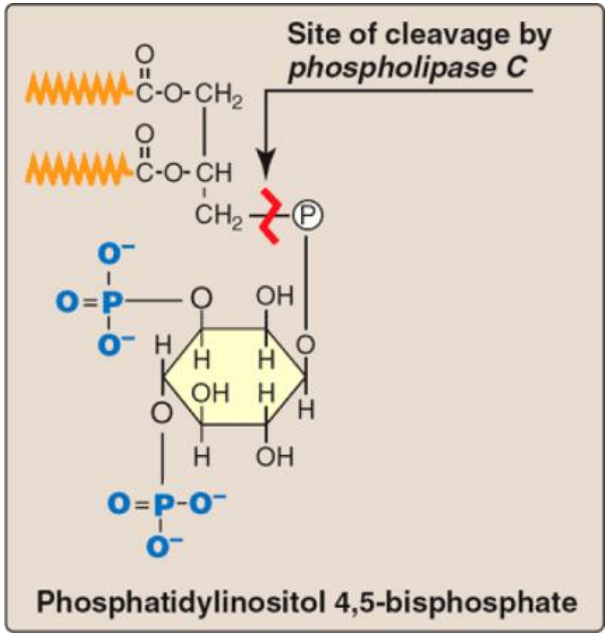
Activation of G Protein

↓

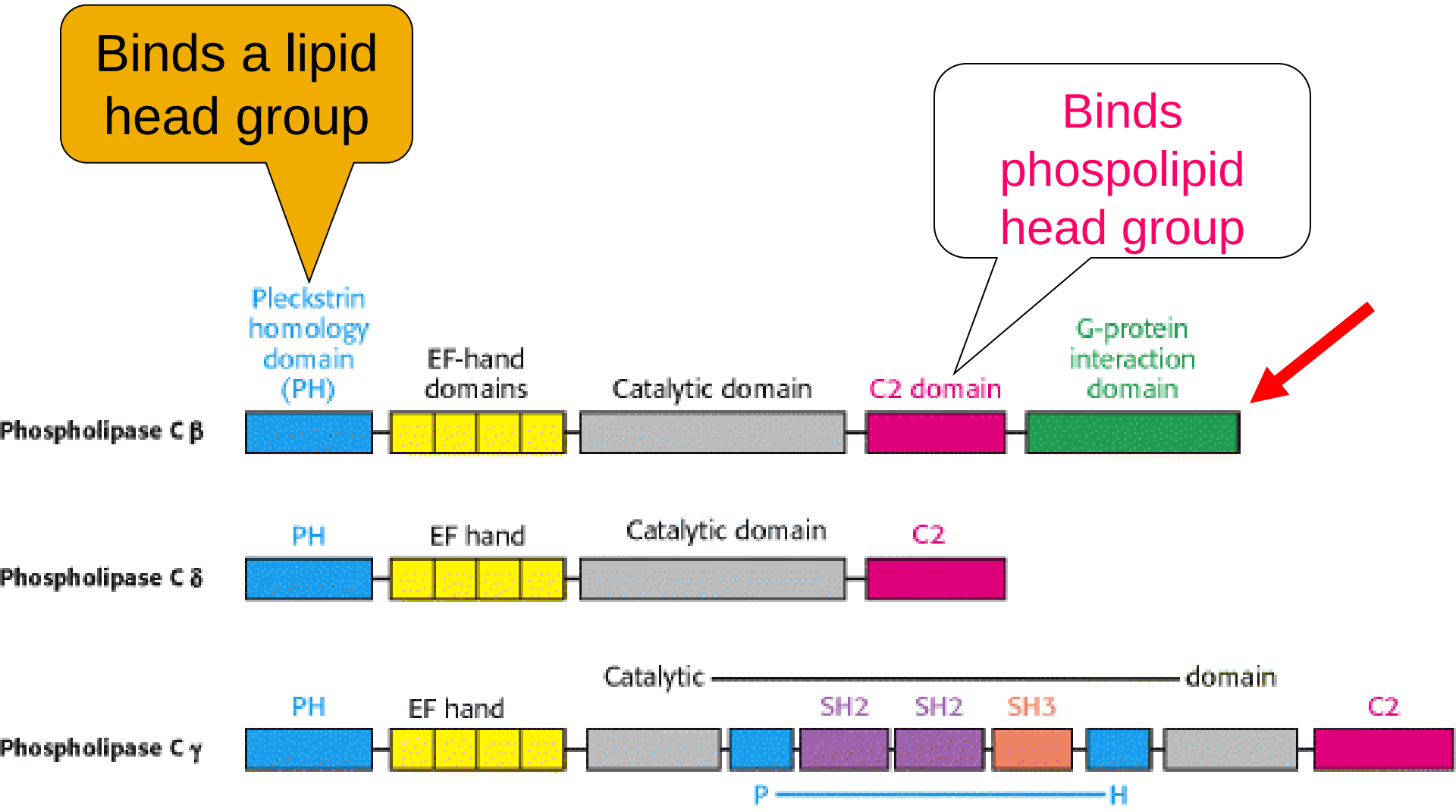
Activation of Phospholipase C (many isoforms) – PIP2

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





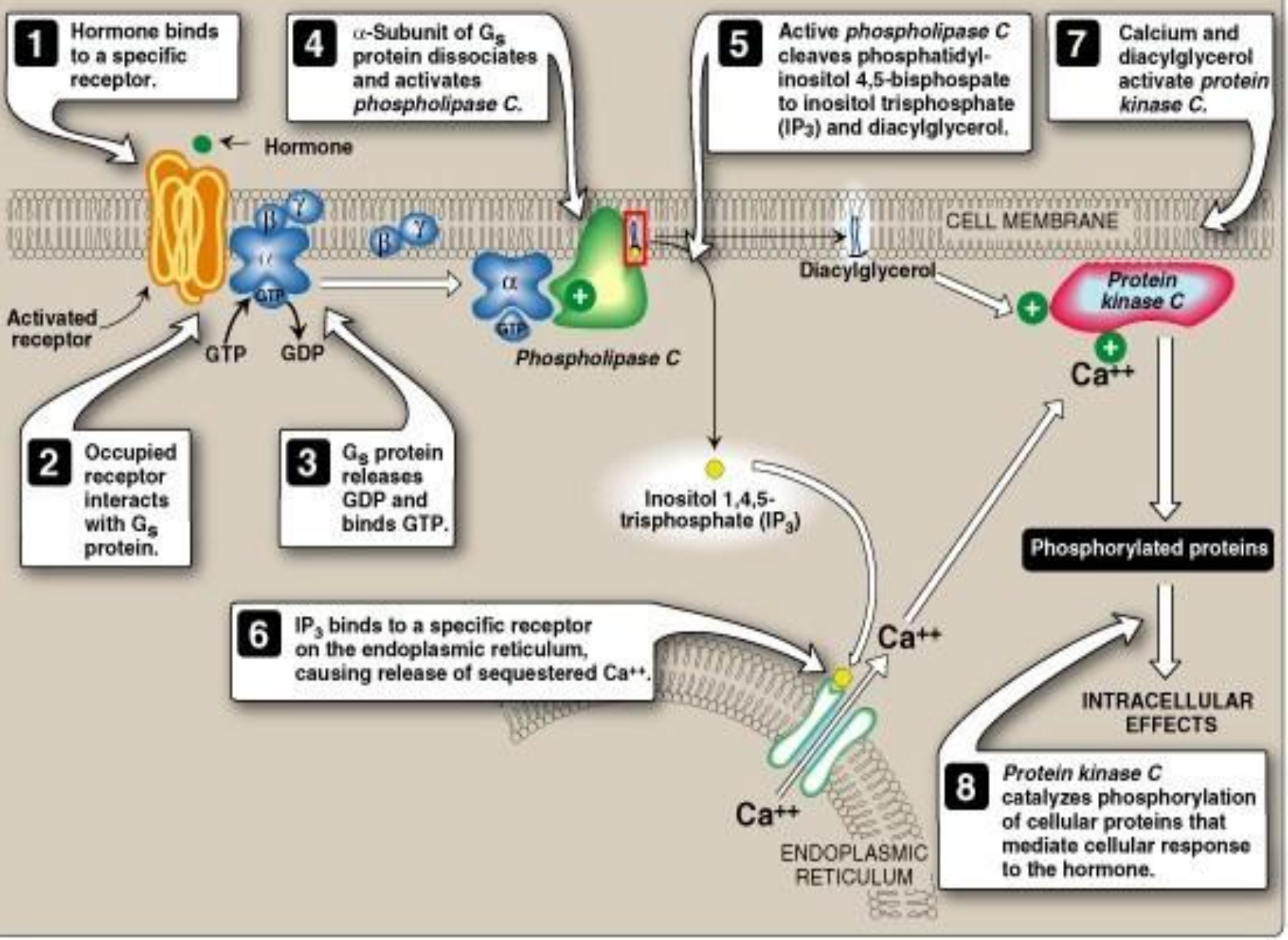
The domain structures of three isoforms of Phospholipase C



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

Membrane

G Protein


1 Hormone binds to a specific receptor.
Hormone
Activated receptor
2 Occupied receptor interacts with G_s protein.
GTP
GDP
3 G_s protein releases GDP and binds GTP.
4 α-Subunit of G_s protein dissociates and activates *phospholipase C*.
Phospholipase C
5 Active *phospholipase C* cleaves phosphatidyl-inositol 4,5-bisphospate to inositol trisphosphate (IP_3) and diacylglycerol.
CELL MEMBRANE
Diacylglycerol
7 Calcium and diacylglycerol activate *protein kinase C*.
Protein kinase C
Ca^{++}
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^{++}
Ca^{++}
ENDOPLASMIC RETICULUM
INTRACELLULAR EFFECTS
8 *Protein kinase C* catalyzes phosphorylation of cellular proteins that mediate cellular response to the hormone.

Effects of Second Messengers

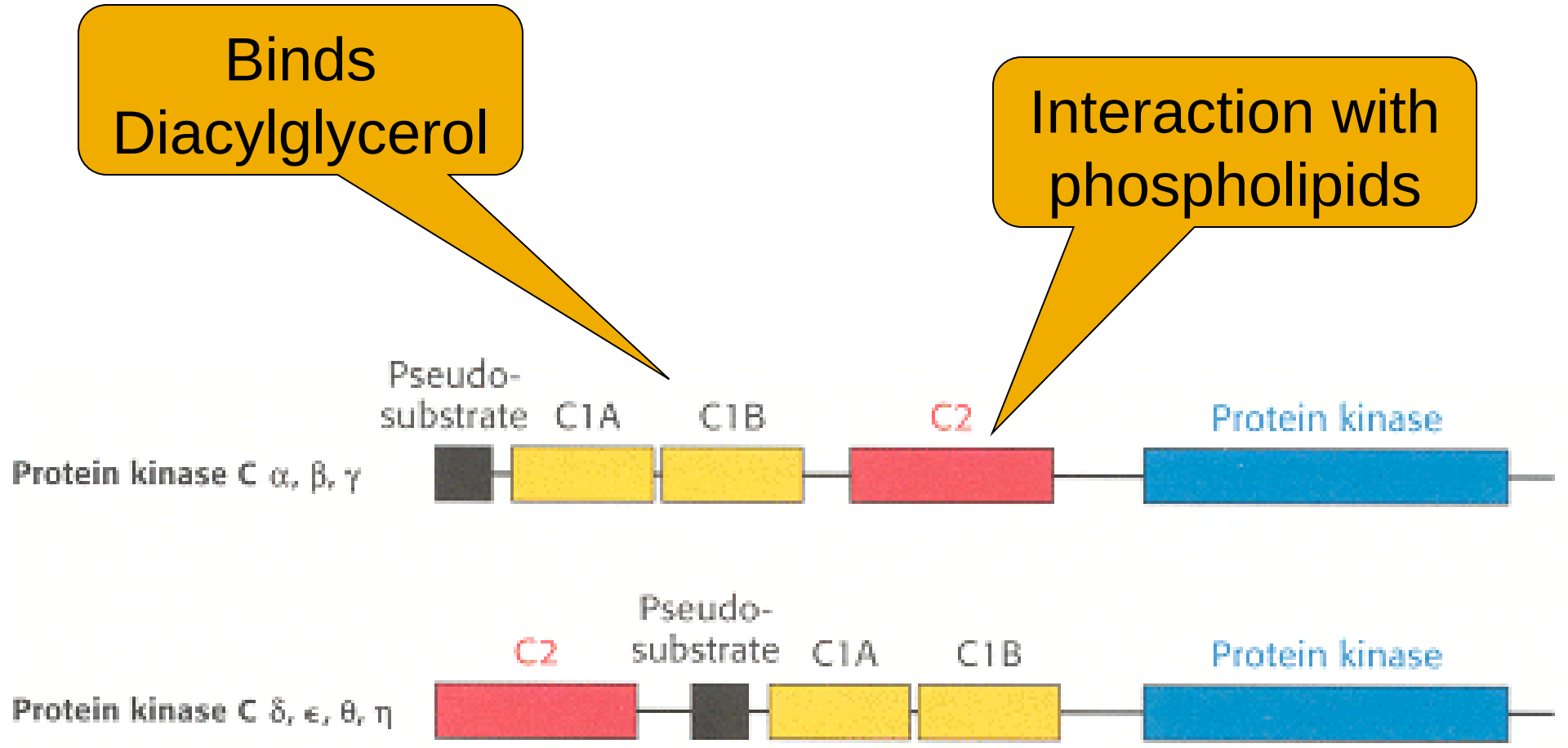
Inositol trisphosphate (IP_3)

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

Diacylglycerol (DAG)

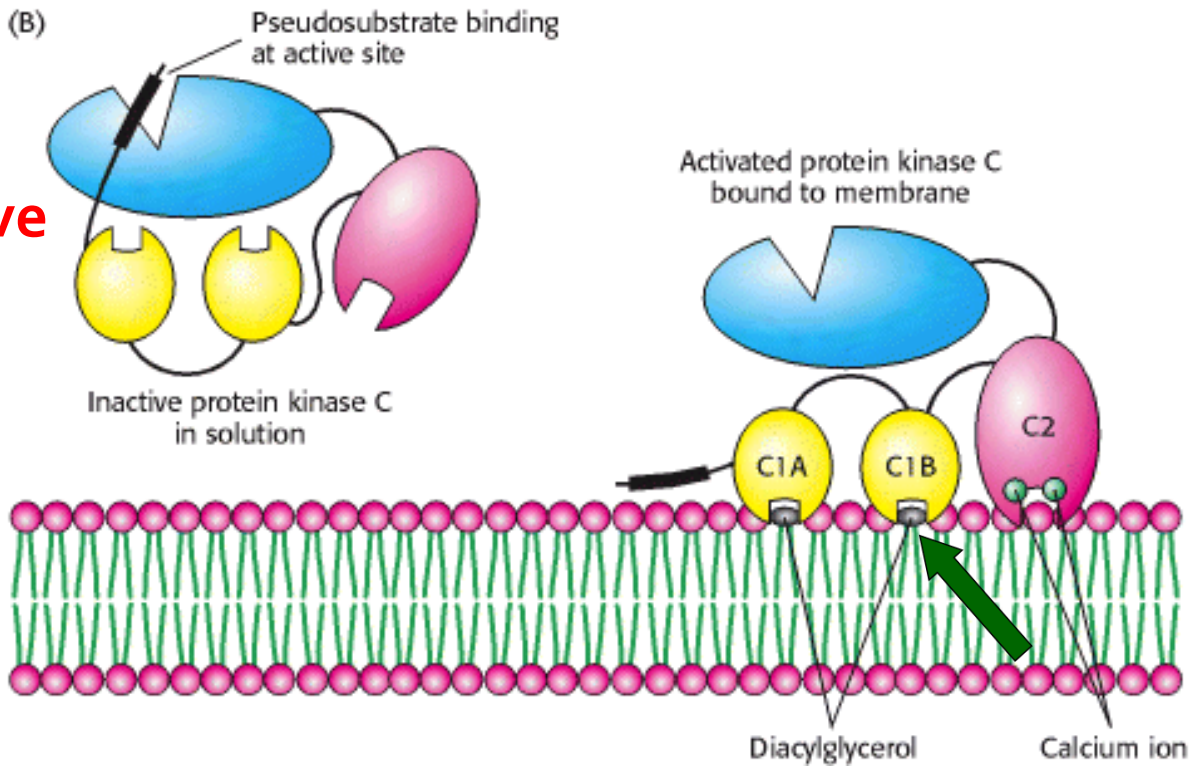
- ✓ Activates Protein Kinase C
- ✓ Ca^{2+} is required
- ✓ Phosphorylation of many target proteins

The domain structures of protein kinase C isoforms



Pseudosubstrate Sequence

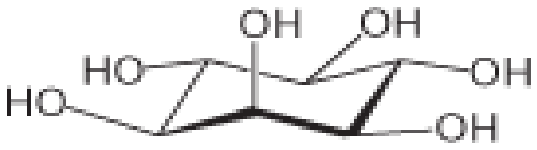
Competitive Inhibitor



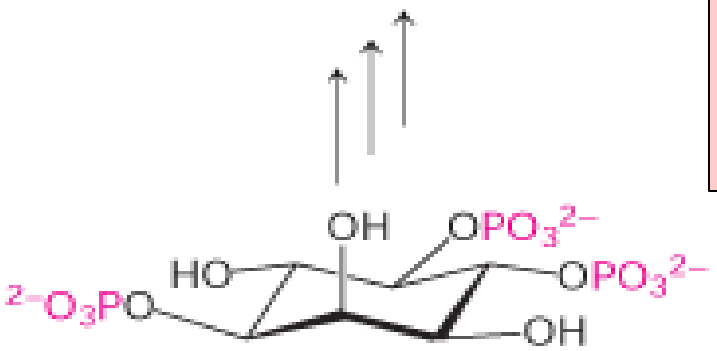
- **Resembles the substrate sequence: A-R-K-G-A-L-R-Q-K**
- **Substrate Sequence: X-R-X-X-(S,T)-Hyd-R-X**
- **Binds to the Enzyme's Active Site**

Termination of IP3 Signal

IP3 is a Short-Lived Messenger



Inositol

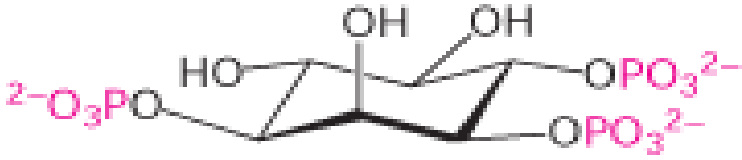


Inositol 1,4,5-trisphosphate

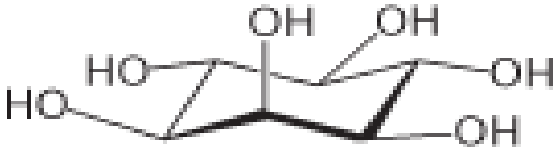
Lithium Ions, Used to treat some psychological disorders Inhibits IP_3 recycling

Inositol 1,4,5-trisphosphate

Inositol 1,3,4,5-tetrakisphosphate



Inositol 1,3,4-trisphosphate

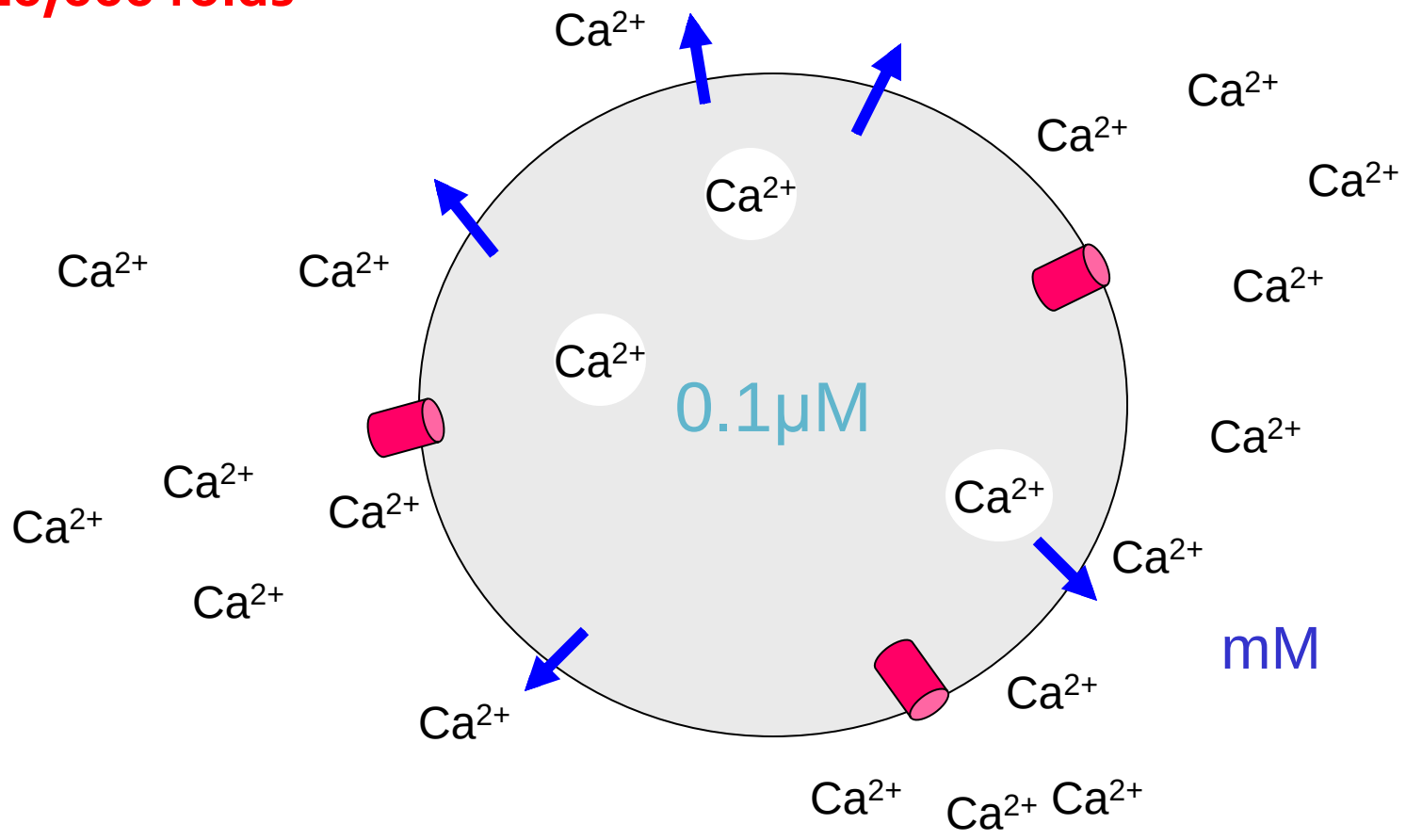


Inositol

Why Ca^{2+}?

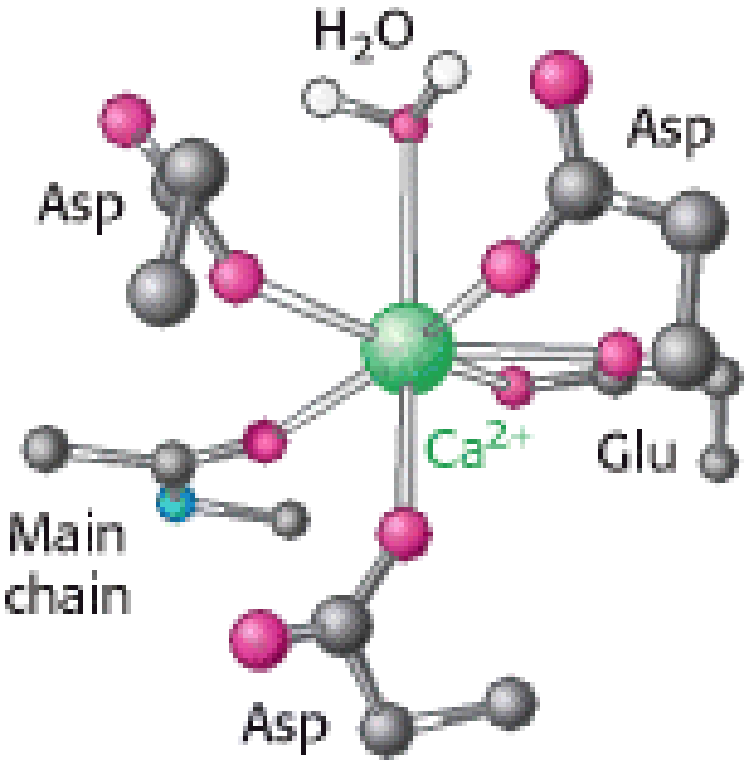
A large difference in concentration

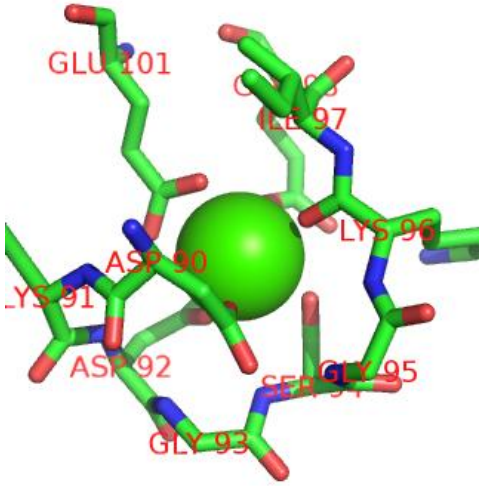
10,000 folds

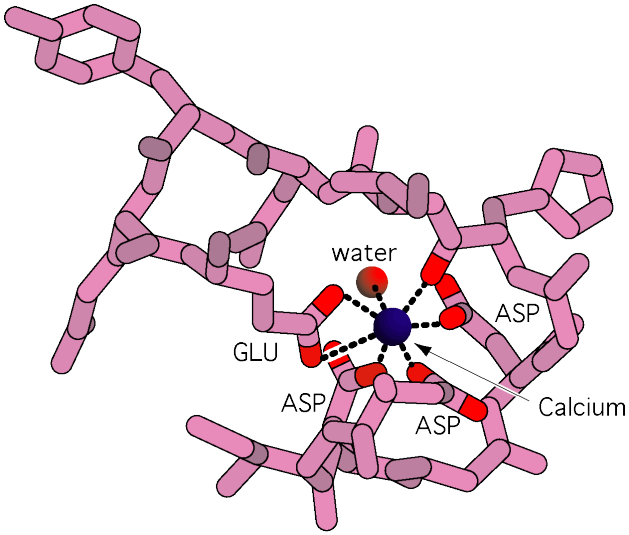


Why Ca^{2+}?

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



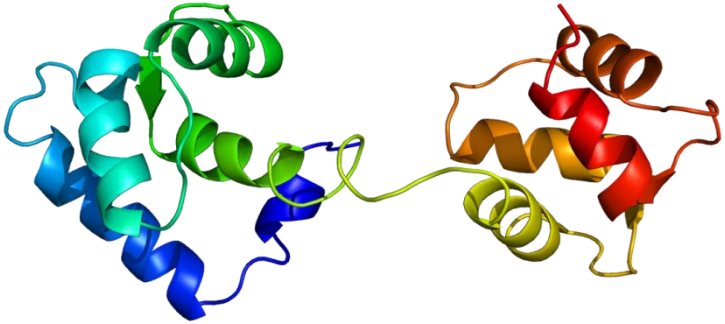




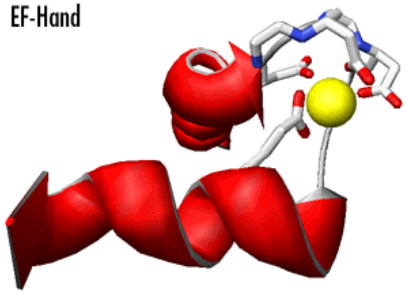
Calcium Binding Proteins

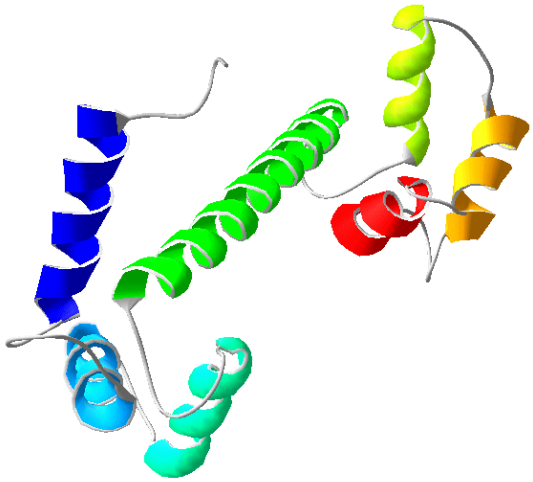
- Mediate the effects of Calcium (Ca^{+2})
- Many proteins

  Calmodulin, Troponin C, Parvalbumin

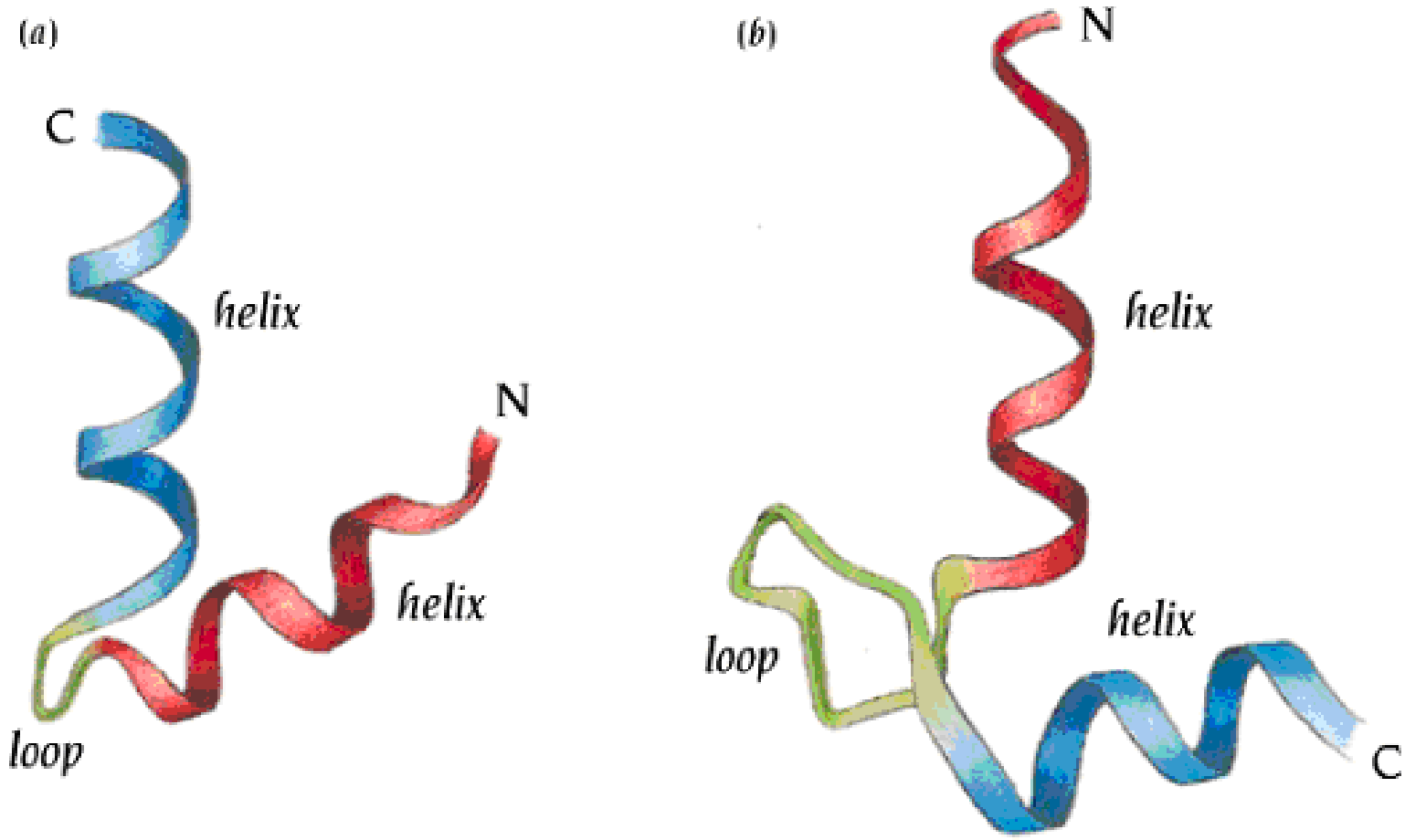


- Similar structures
  - Rich in Asp and Glu
    - Gln, Asn, Ser
  - Several α helical segments
  - Binding site is formed by
    - Helix Loop Helix
      - Super-secondary structure



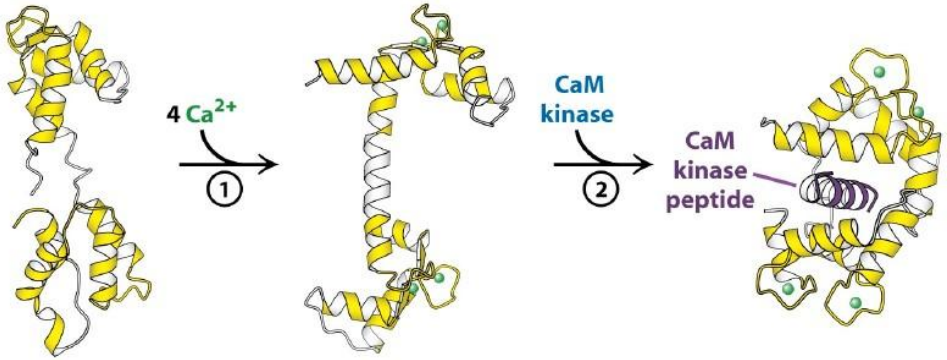


Calcium Binding Proteins



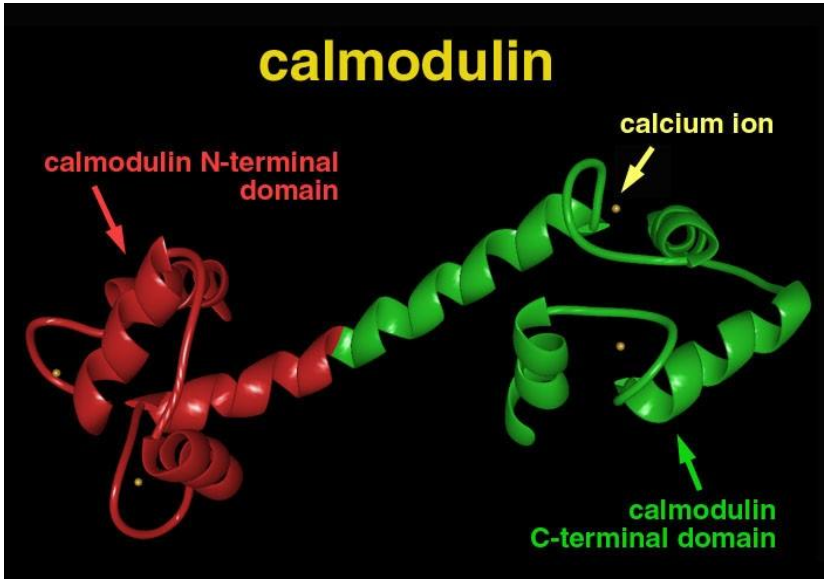
Calmodulin (≈17 kD)

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:

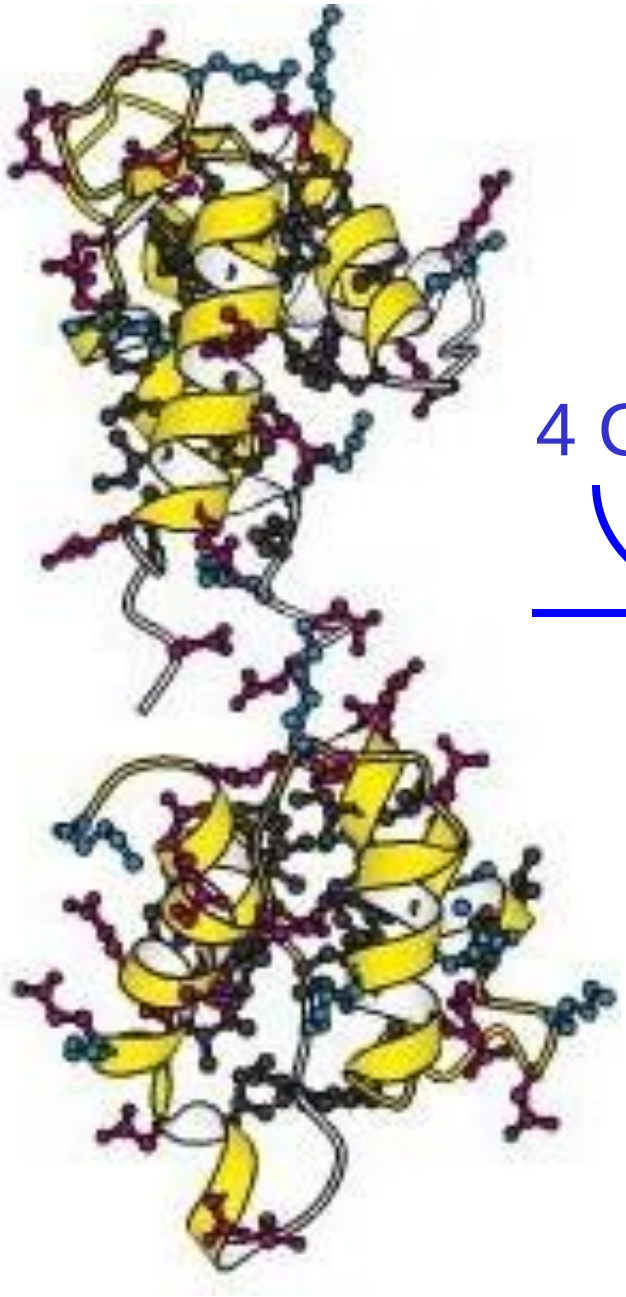
149 amino acids



Calmodulin-dependant Protein Kinase

Sort of memory

Ca^{2+} ATP'ase Pump



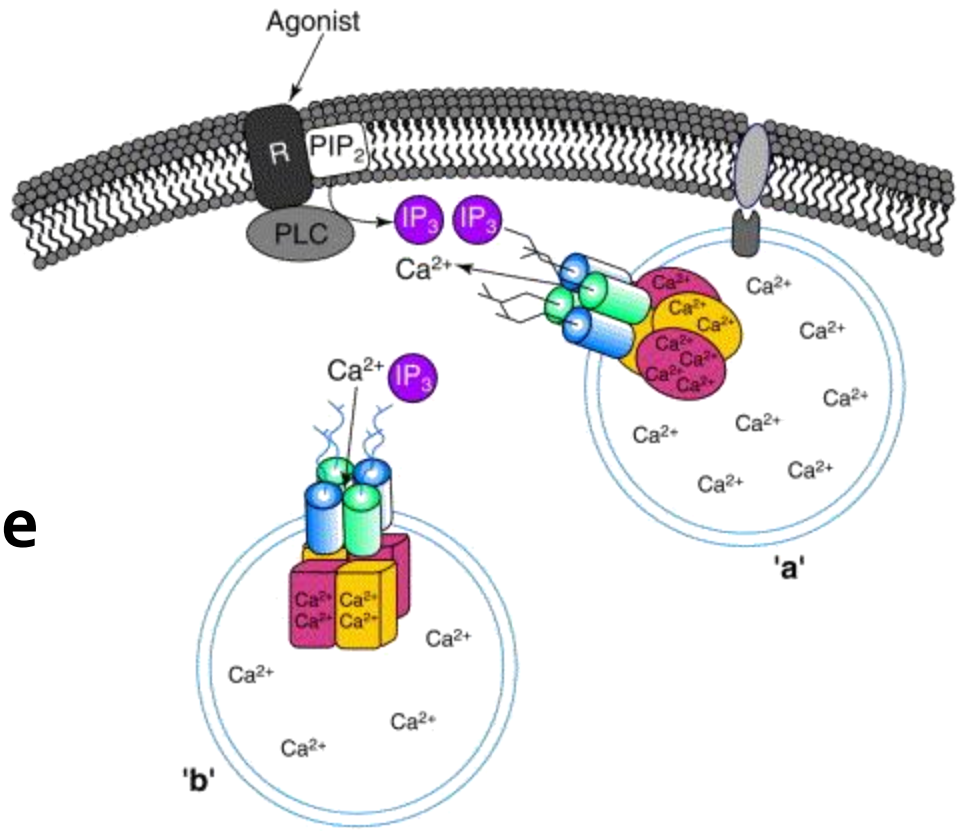
4 Ca^{2+}

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

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

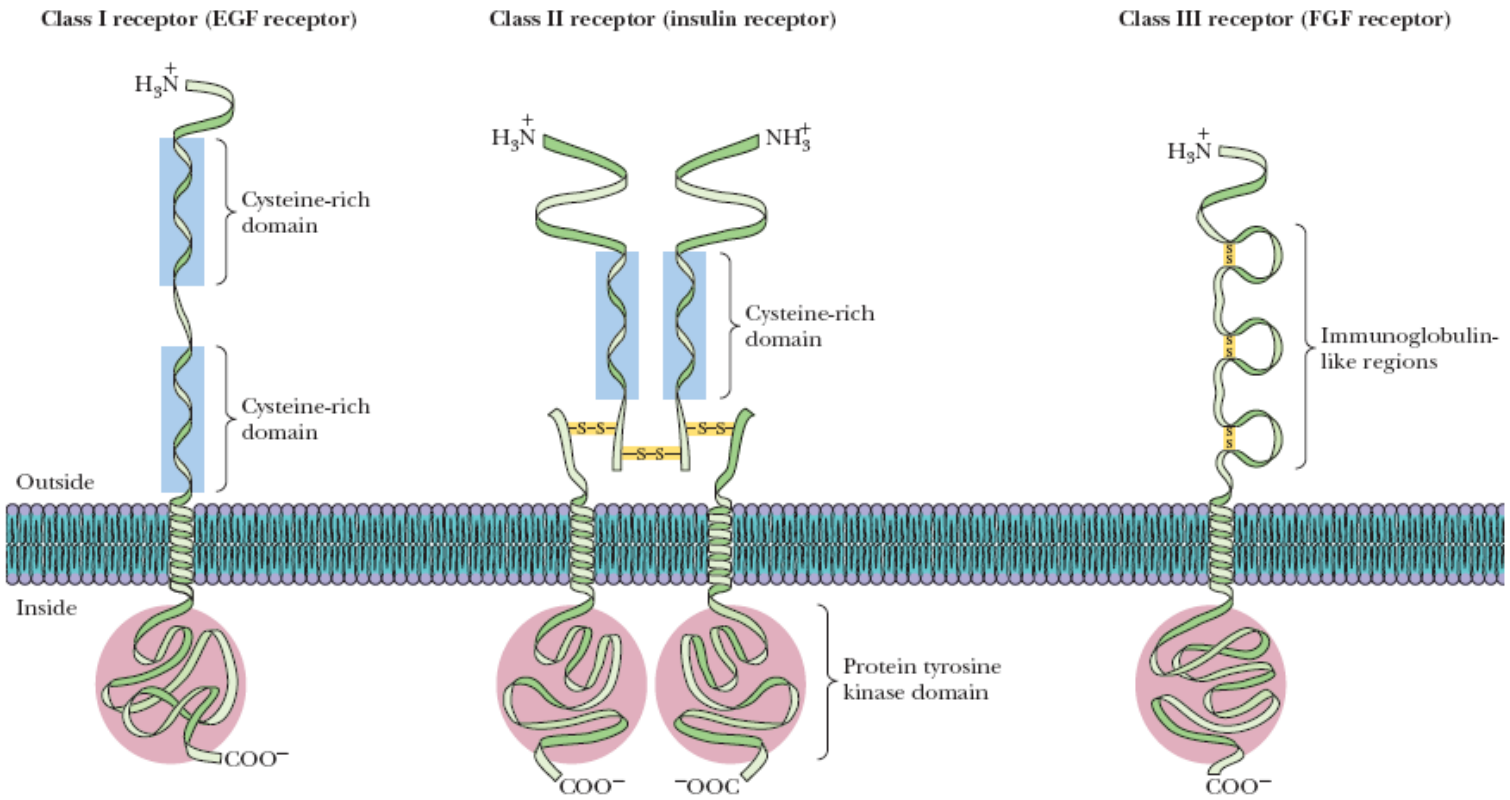
Ca^{2+} Transporter

- 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

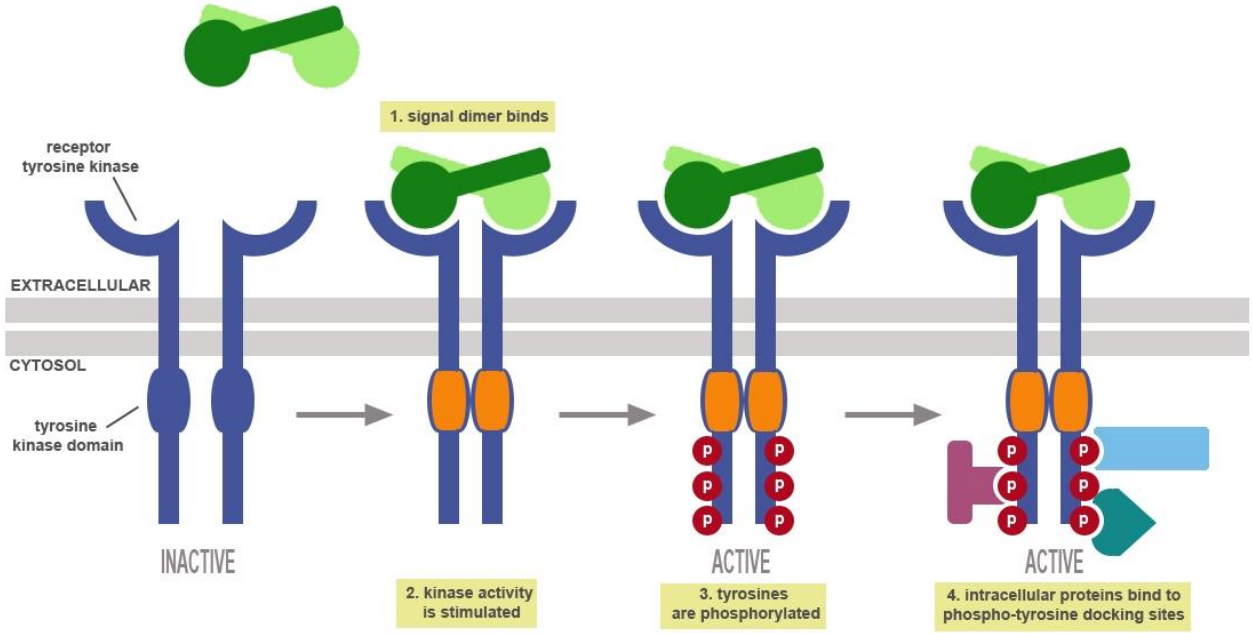
- Second Messengers
- Span the membrane, several subclasses (class II, Insulin R), hormone receptor & tyrosine kinase portion



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

  - **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

↓

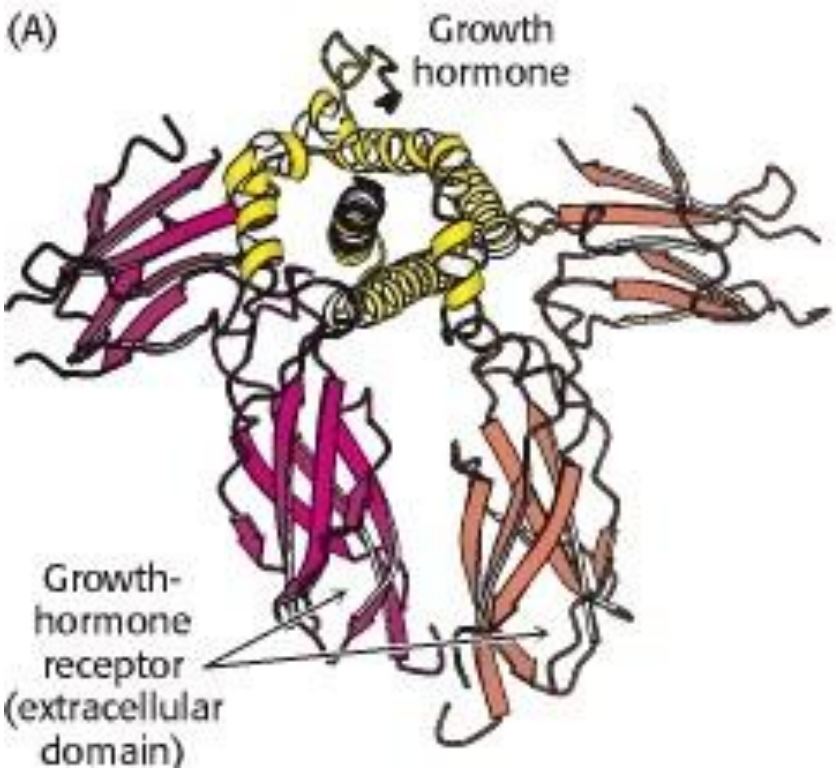
Auto phosphorylation of the receptor

↓

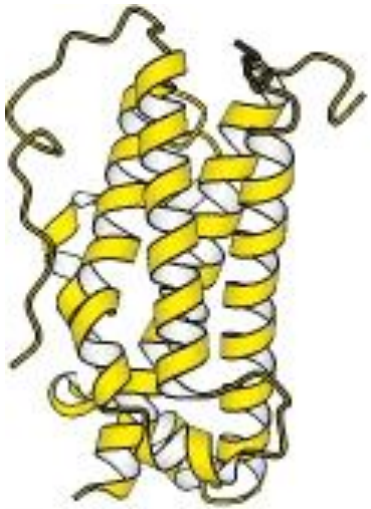
Phosphorylation of the target proteins

Growth Hormone & GH receptor

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



- GH receptor (cooperative binding)
  - 638 A.Acid
  - Extracellular Domain (≈250 A.A) & Intracellular Domain (≈350 A.A)
  - Single Membrane-Spanning Helix
  - Monomeric (free) vs. Dimeric (bound)

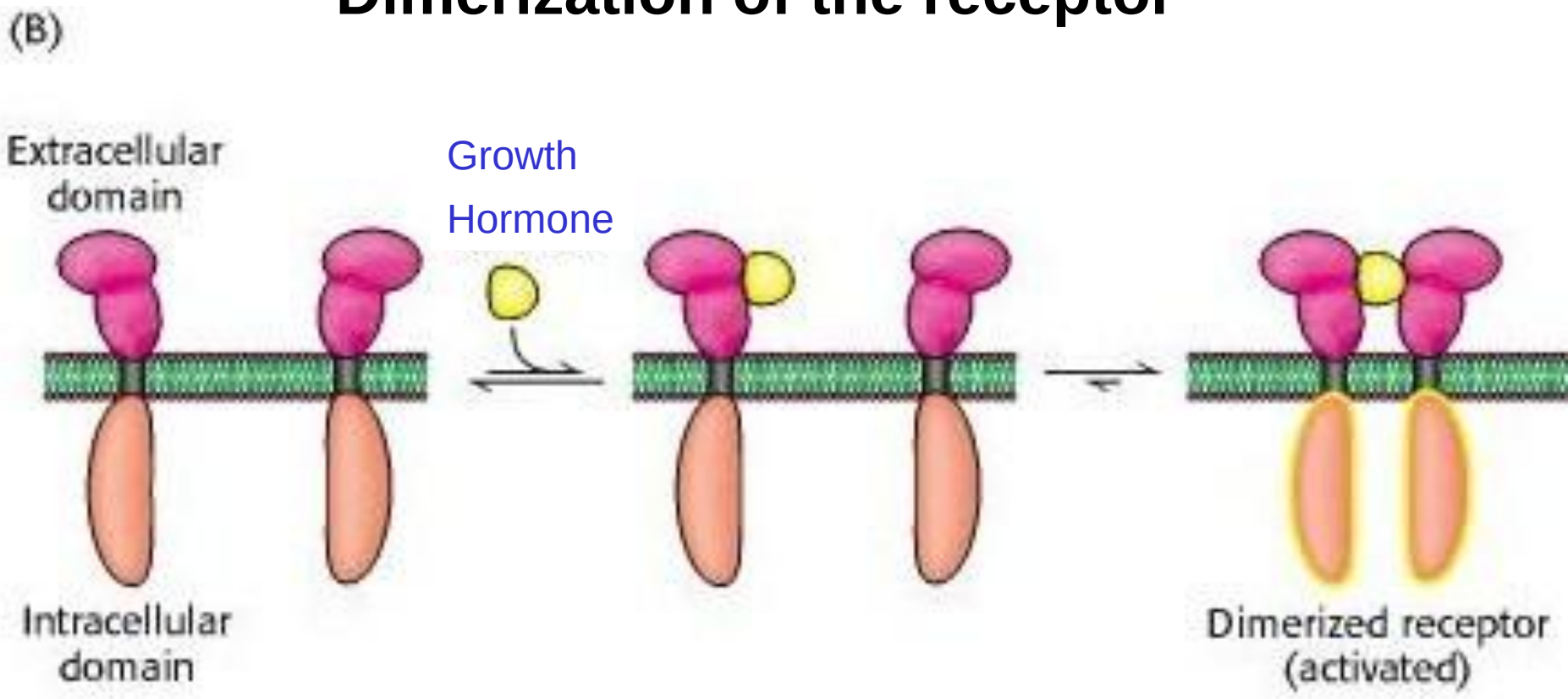

Human growth hormone

Growth Hormone dimerization

Binding of one molecule of growth hormone

↓

Dimerization of the receptor



Each Intracellular Dom ith

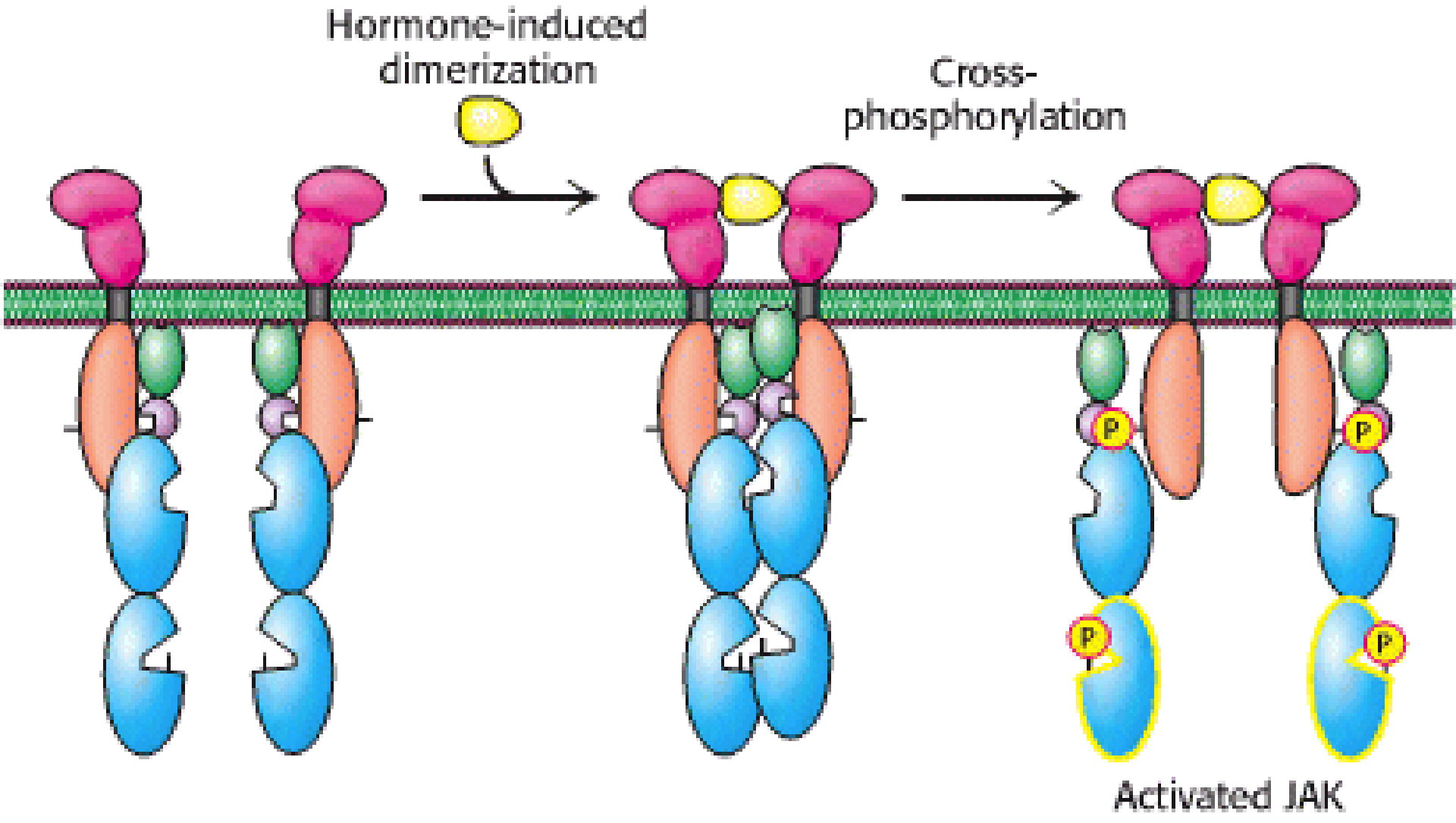
Janus a protein kin

Janus K

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

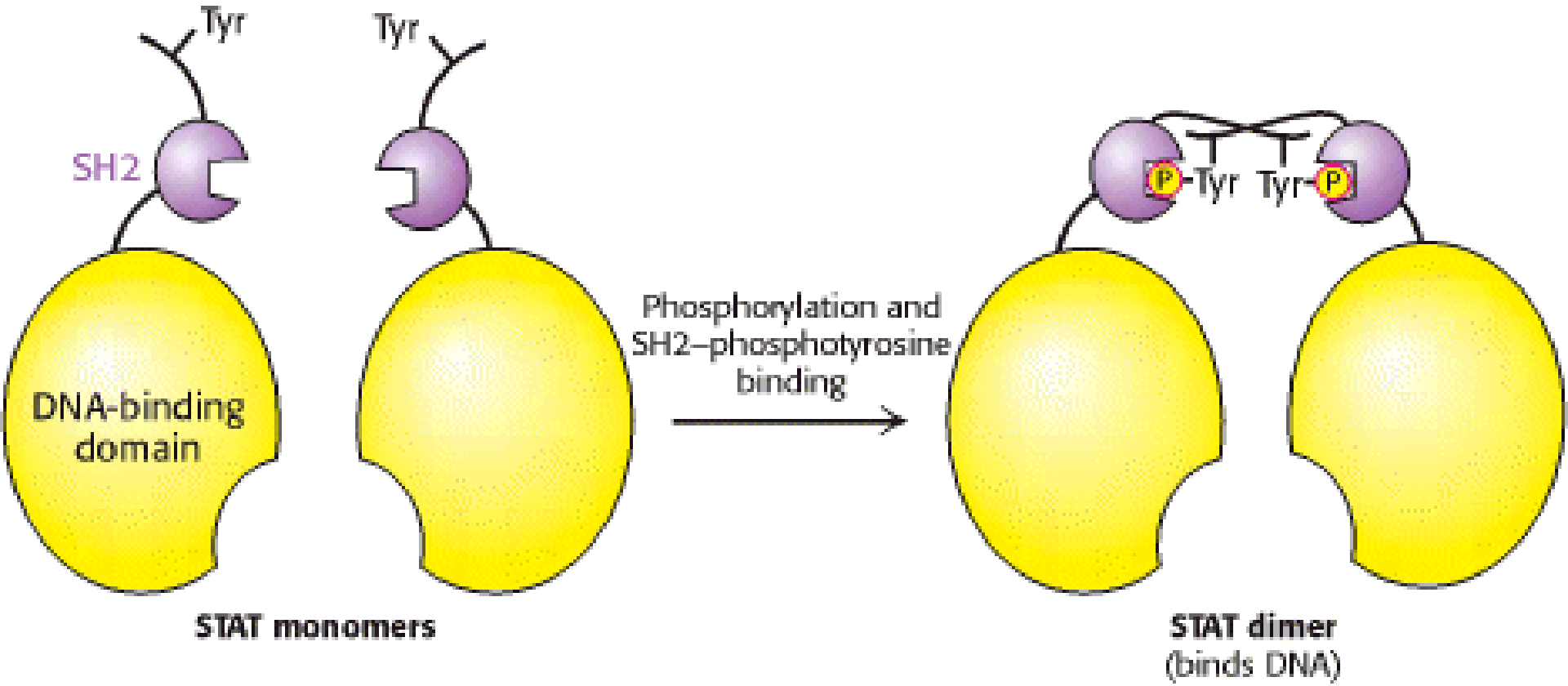
- STAT
  - Signal Transducer & Activators of Transcription
- Regulator of transcription
- STAT Phosphorylation

  → **Dimerization**

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

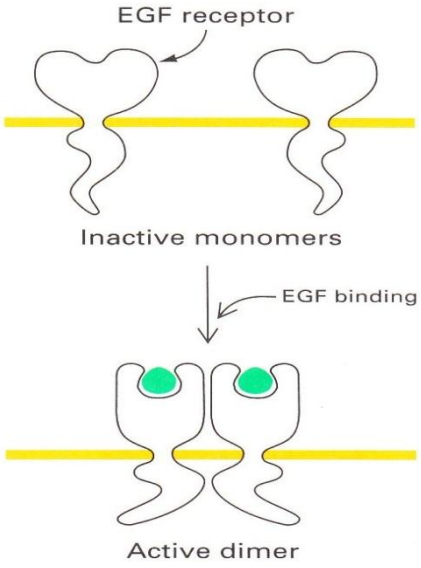
STAT is phosphorylated on a tyrosine residue near the carboxyl terminus

Phosphorylated tyr binds to SH2 domain of another STAT 5 molecule



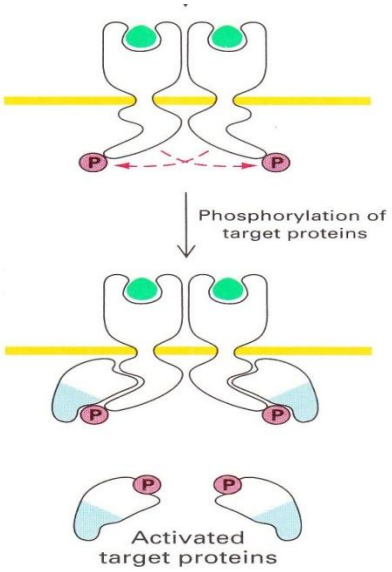
Tyrosine Kinase & other Hormones
EGF

- Epidermal Growth Factor Receptor
  - Monomeric (inactive)
  - EGF binding ➔Dimerization ➔ Cross Phosphorylation ➔ Activation



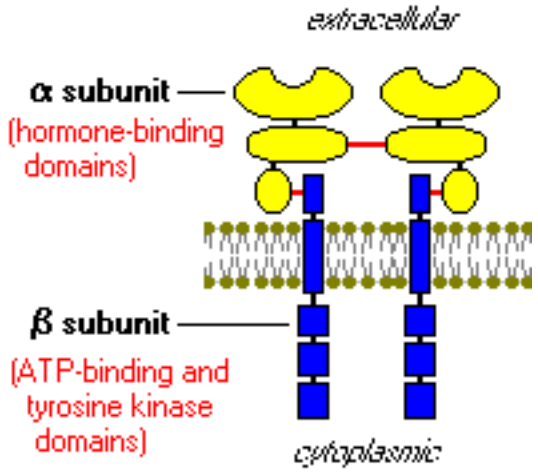
Autophosphorylation

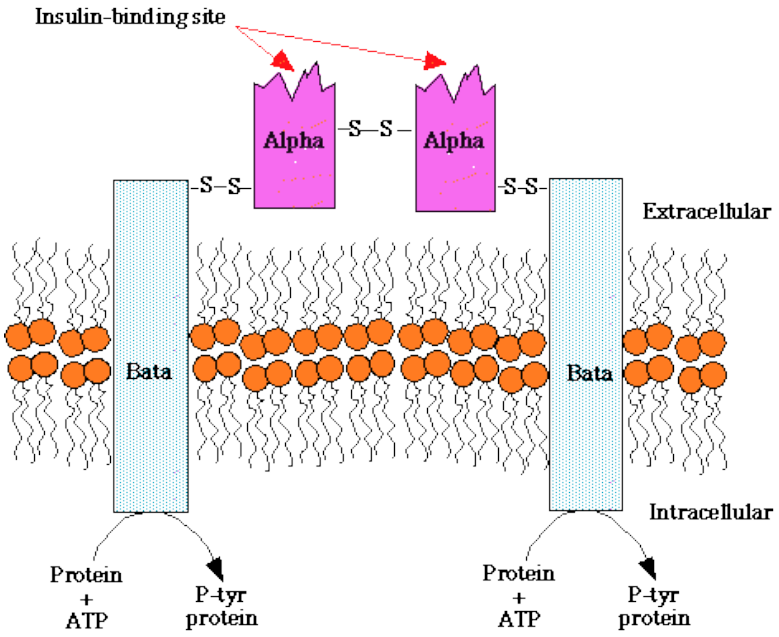
Dimerization is necessary but not sufficient for activation (kinase activity)



Tyrosine Kinase & other Hormones
Insulin

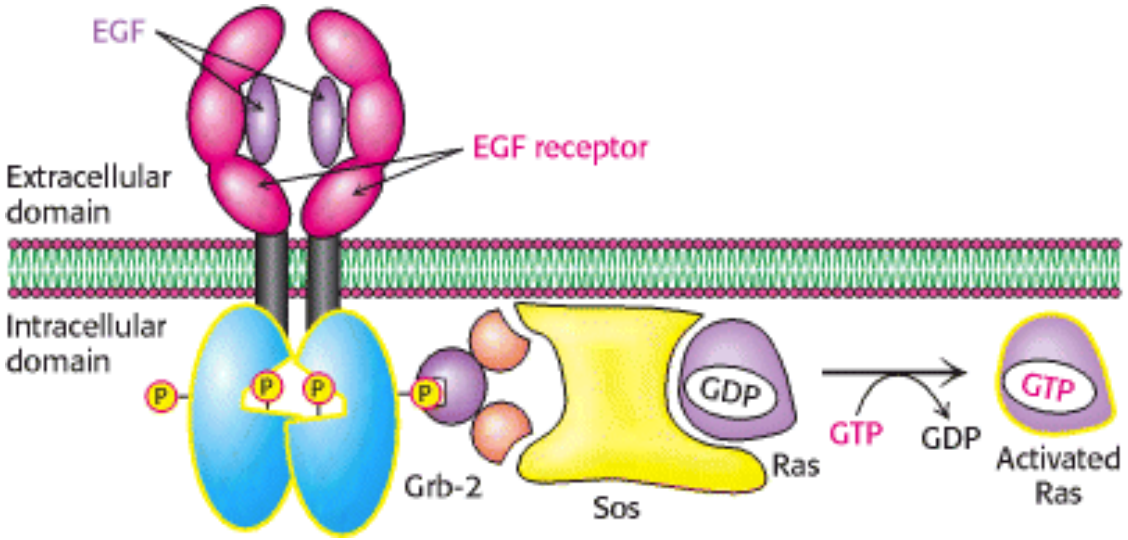
- Insulin Receptor
- Tetramer (2^{α}; 2^{β}), dimer ($2^{\alpha\beta}$ pairs)
- Disulfide bridges
- Insulin Binding → Activation of the Kinase





Ras is a member of small G proteins family

- Monomeric
- 2 forms: GDP ↔ GTP
- Smaller (1 subunit)
- GTPase activity
- Many similarities in structure and mechanism with G_{α}



- 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 ➔

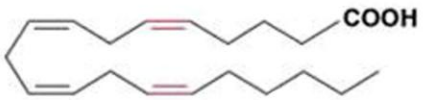
Loss of ability to hydrolyze GTP ➔

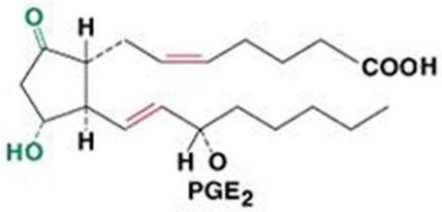
Ras is locked in "ON" position ➔

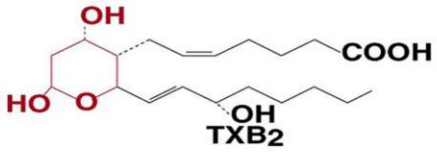
continuous stimulation of growth

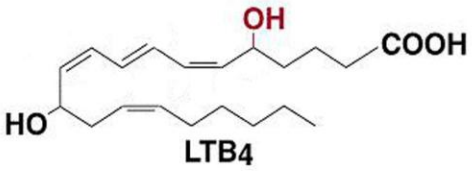
Eicosanoids

- 20 carbon signaling molecules
- Several Classes:
  - Prostaglandins
  - Thromboxanes
  - Leukotrienes









- Very Potent (very low conc.)
- Short Half Life
- Not Stored

- Produced In Almost all Tissues
- Wide Range of Responses
- Local Hormones (autocrine & paracrine)

Some Functions of the Prostaglandins and Thromboxanes

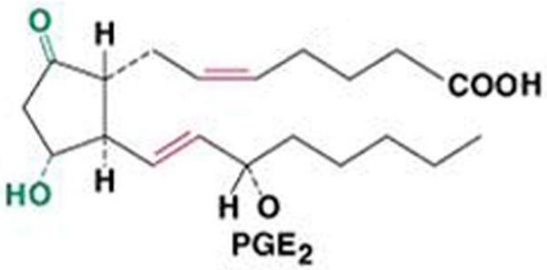
- What 2 stands for?

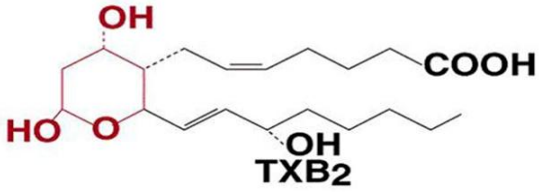
- PGI_2, PGE_2, PGD_2
  - Increase
    - Vasodilation, cAMP
  - Decrease
    - Platelet Agregation
    - Lymphocyte Migration
    - Leucocyte Aggregation

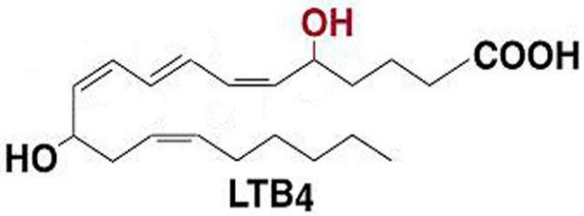
- $PGF_{2\alpha}$ Increses
  - Vasoconstriction
  - Bronchoconstriction
  - Smooth Muscle Contraction

- Thromboxane Increases
  - Vasoconstriction
  - Platelet Agregation
  - Lymphocyte Proliferation
  - Bronchoconstriction

Eicosanoids Structure

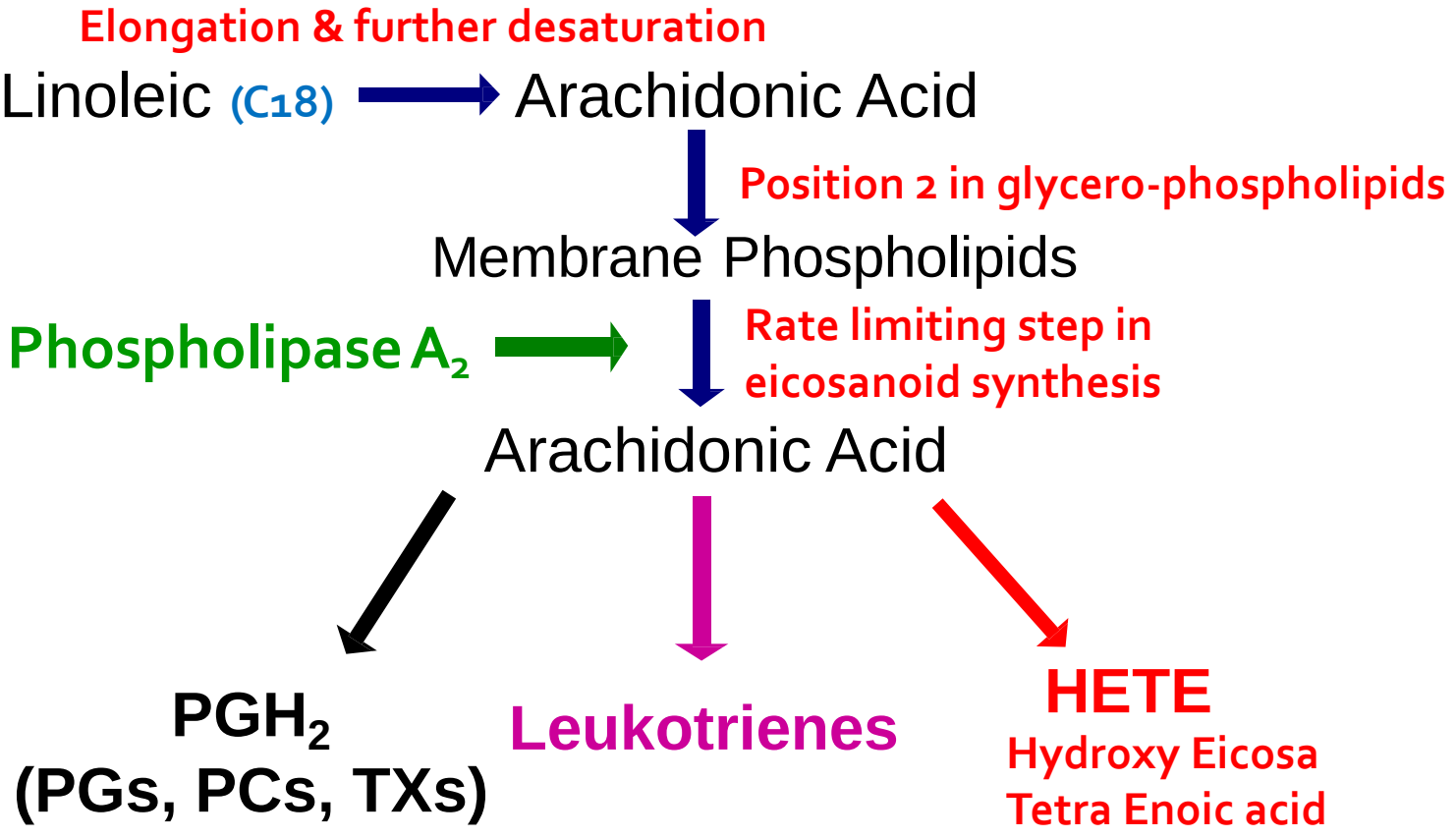






- Arachidonic acid (20, 4, no ring)
- Prostaglandins (20, 2, 5-ring)
- Thromboxanes (20, 2, 6-ring, oxygen)
- leukotrienes (20, 3 conjugated, no ring)

Eicosanoids Synthesis



Eicosanoids Can be Synthesized from other Polyunsaturated Fatty Acids

- **Fatty acids of 20 carbons with:**
  - **3 double bonds like Eicosatrienoic acid (omega-6)**
    - 1 double bonds, **PGE1 (3 → 1)**
  - **4 double bonds as Eicosatetraenoic acid (arachidonic acid)**
    - 2 double bonds, **PGE2, PGF2, TXB2 (4 → 2)**
  - **5 double bonds (Eicosapentaneoic acid : (omega-3)**
    - 3 double bonds, **PGE3, TXB3 (5 → 3)**
- **Which is more healthy? Less MI**
  - **Omega-3: TxB3 → inhibits platelet aggregation**
  - **Omega-6: TxB2 → stimulates platelet aggregation**