

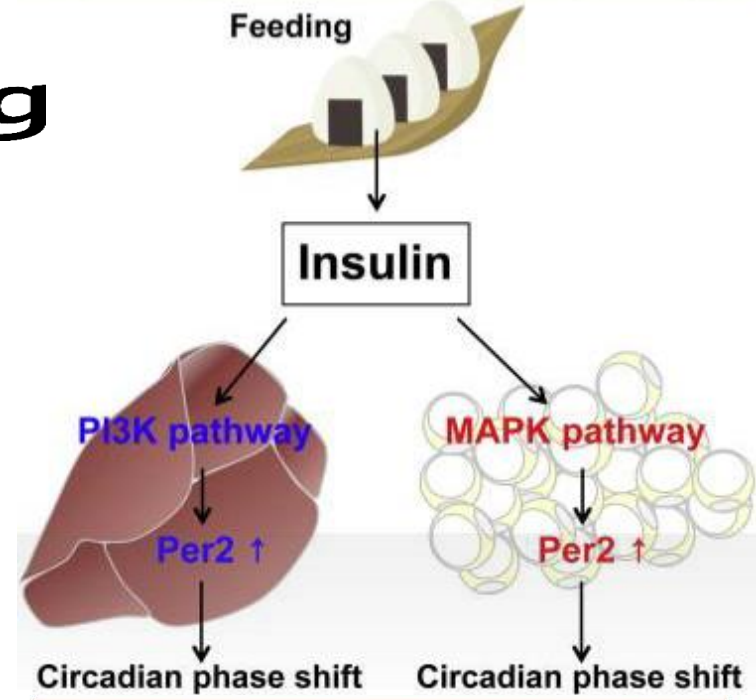
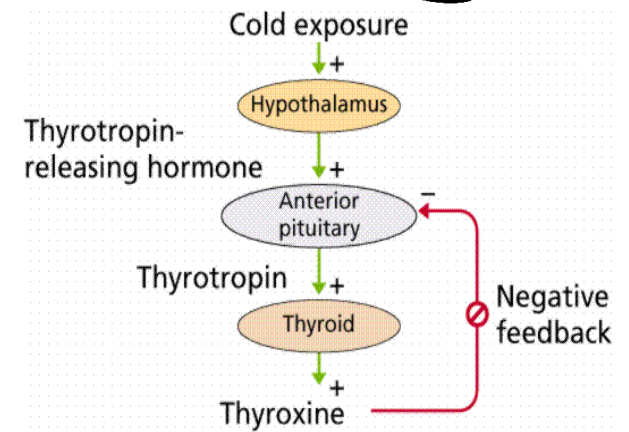
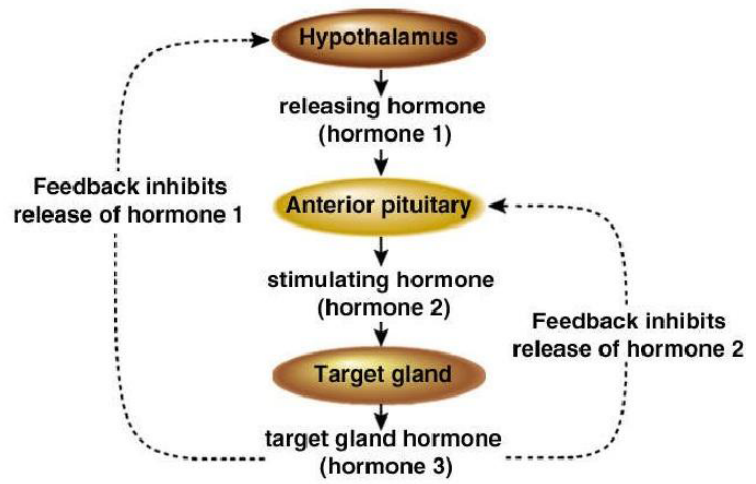


Nafith Abu Tarboush DDS, MSc, PhD
natarboush@ju.edu.jo www.facebook.com/natarboush

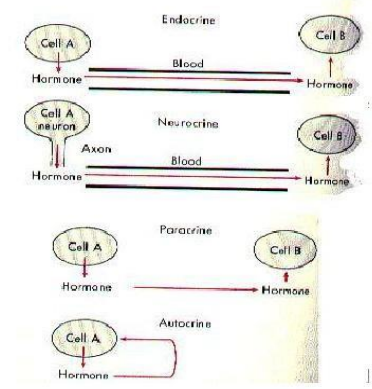
Integration of Metabolism: hormones & Cellular Signaling

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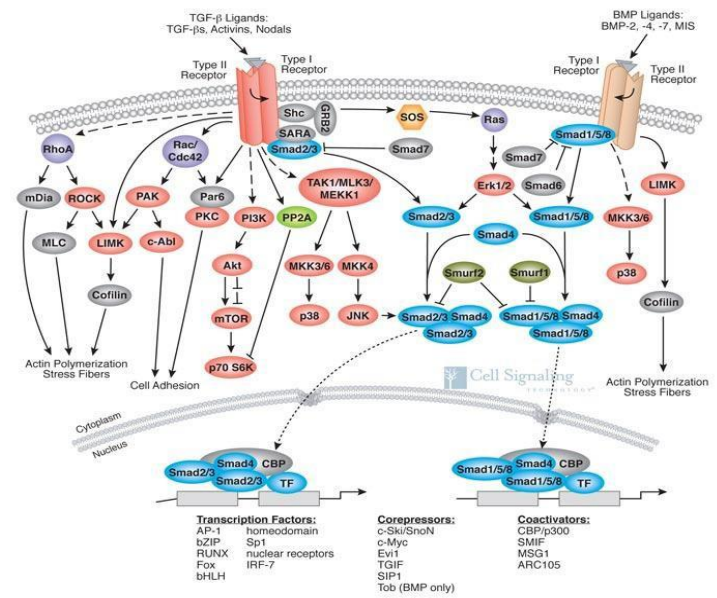
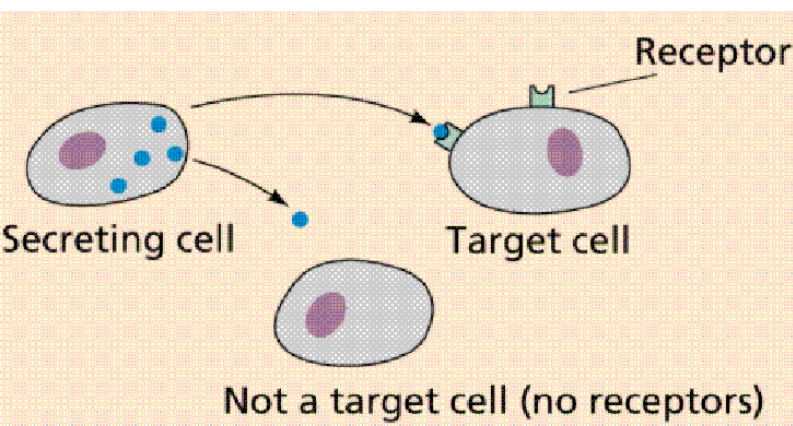
Endocrine Glands



Types of cell-to-cell signaling



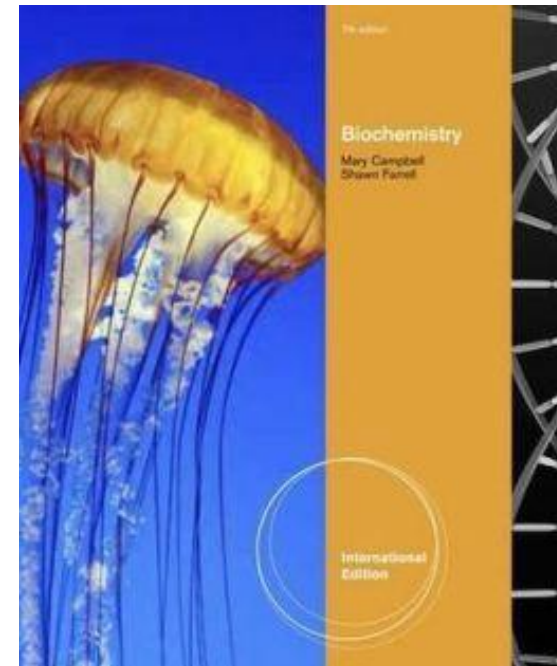
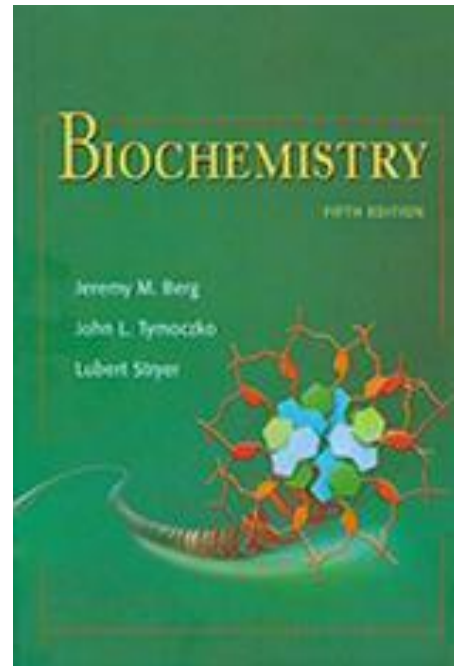
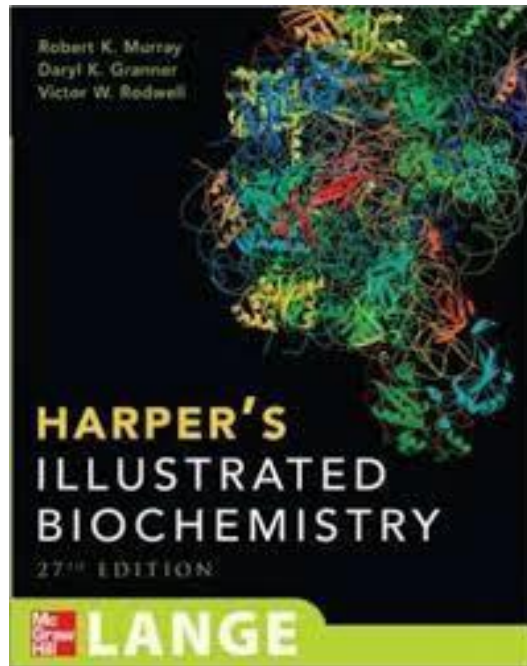
Endocrine Hormones: travel via bloodstream to target cells
Neurocrine hormones: released from nerve terminals
Paracrine hormones: act on adjacent cells
Autocrine hormones: Released and act on the cell that secreted them.
Intracrine Hormones: act within the cell that produces them.





Resources for the lectures

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





Outline

1st lecture

- Definition
- Signaling
- Biochemical issues
- Target cell concept
- Receptor domains
- Amplification
- Regulation
- Classification
- Structure, synthesis, and degradation

2nd lecture

- Continued Structure, synthesis, and degradation
- Signal transduction
- 2nd messengers
- GPCR
- G-proteins
- Adenylate cyclase

3rd lecture

- PI cascade
- Calcium binding proteins
- RTK cascade
- Monomeric G-proteins

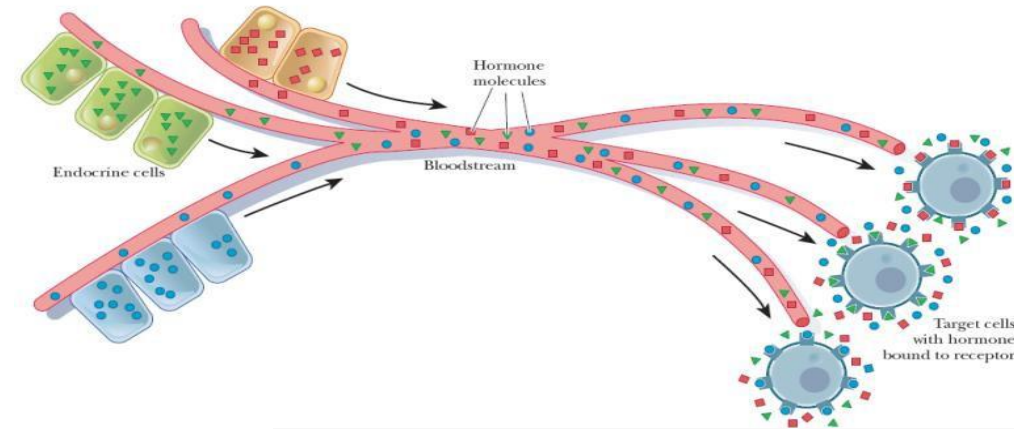
4th lecture (online material)

- Steroidogenesis



Hormones: The Remote Controllers

- **What are hormones?** Organic, blood, low amounts, source & target
- **Classes:**
 - Endocrine hormones
 - Distance; stability; & concentration
 - Paracrine hormones
 - Autocrine hormones

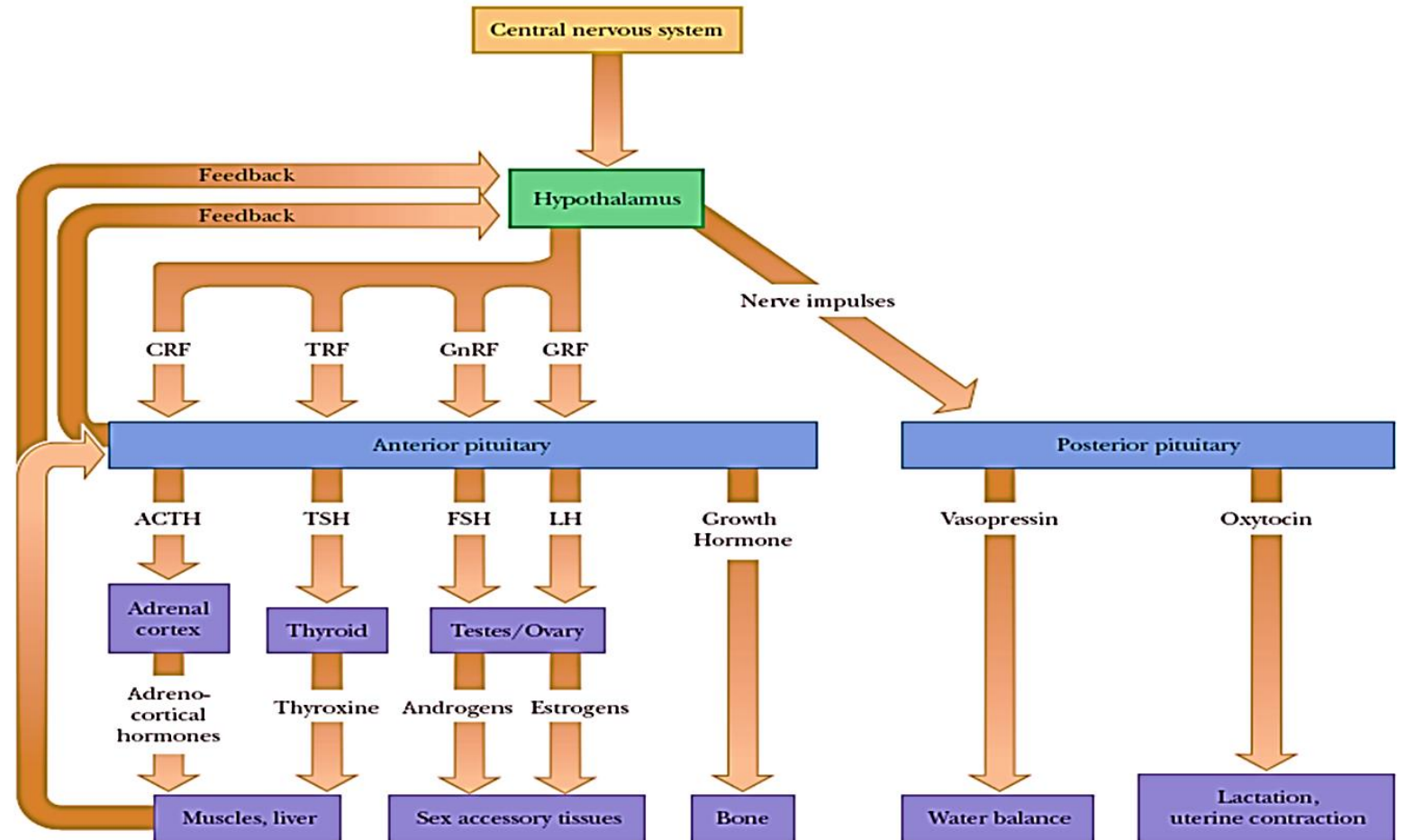


Signaling by Secreted Molecules



Nervous vs. endocrine

- These two systems are functionally interrelated.
- ✓ Both regulate human physiology
- ✓ The endocrine system produces a **slower and more prolonged response** than the nervous system.
- ✓ Both systems may act **simultaneously** on the same target cells and tissues, and **some nerve cells secrete hormones**.





The Target Cell Concept

- 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
- **200 types** of differentiated cells in humans
- Only a few produce hormones! (**<50 known hormones**)
- All of **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 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 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:**

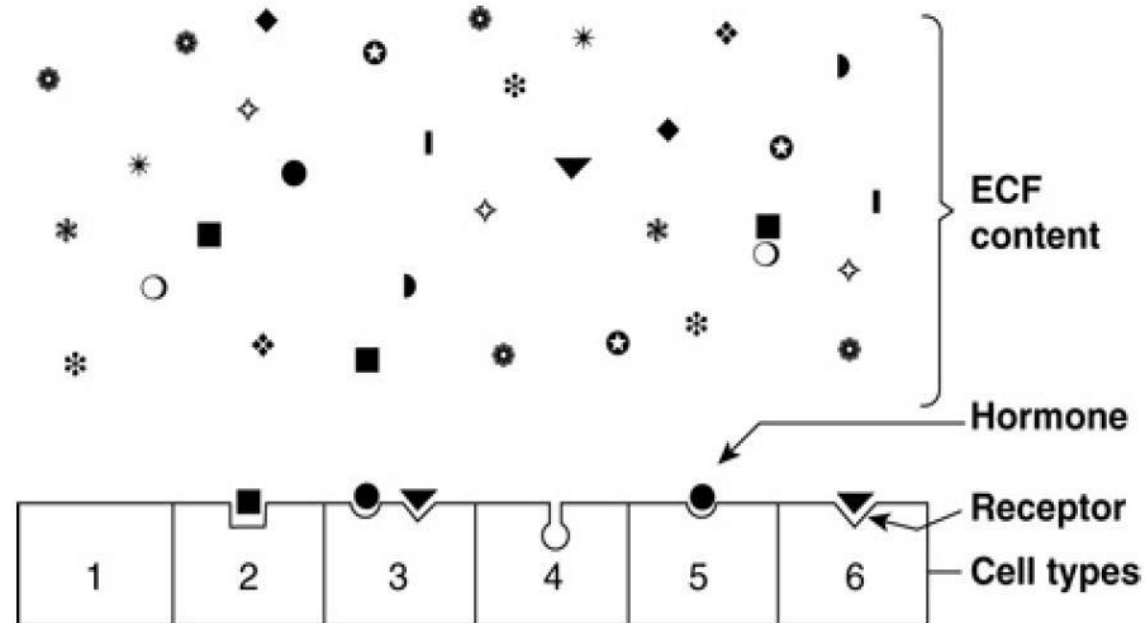
Factors affecting the target cell response

- ✓ 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

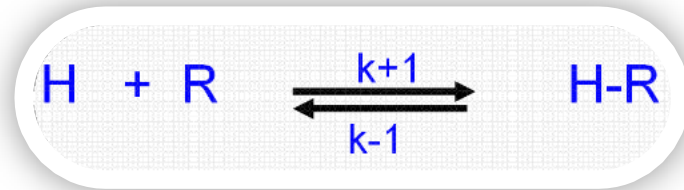
- **Major challenge:**
 - Atto- to nano-molar range (10^{-15} to 10^{-9} mol/L) vs. Structurally similar molecules (sterols, amino acids, peptides, and proteins): micro- to millimolar (10^{-6} to 10^{-3} mol/L) range



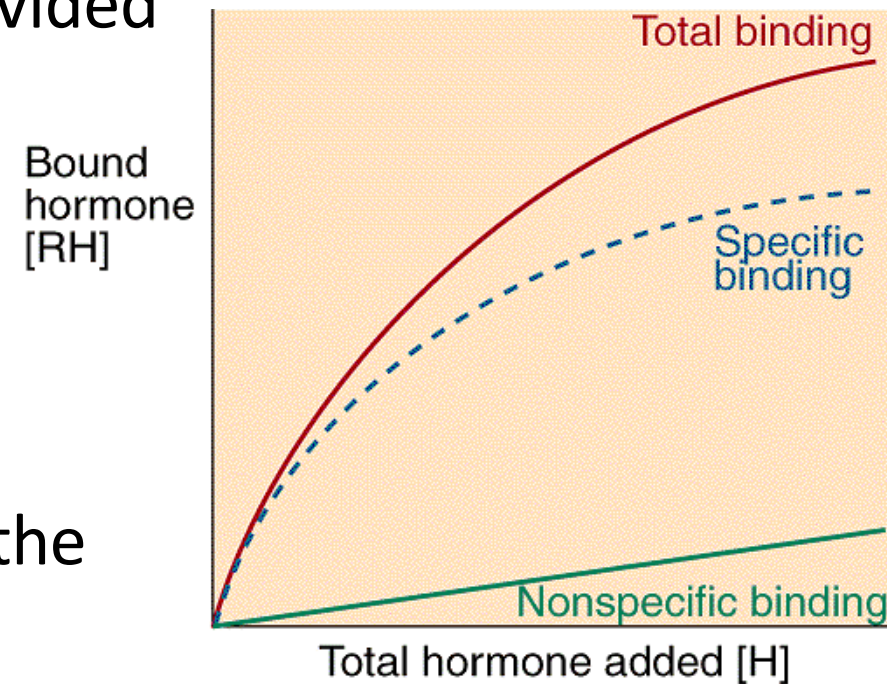


Accordingly; Hormone-Receptor interactions

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



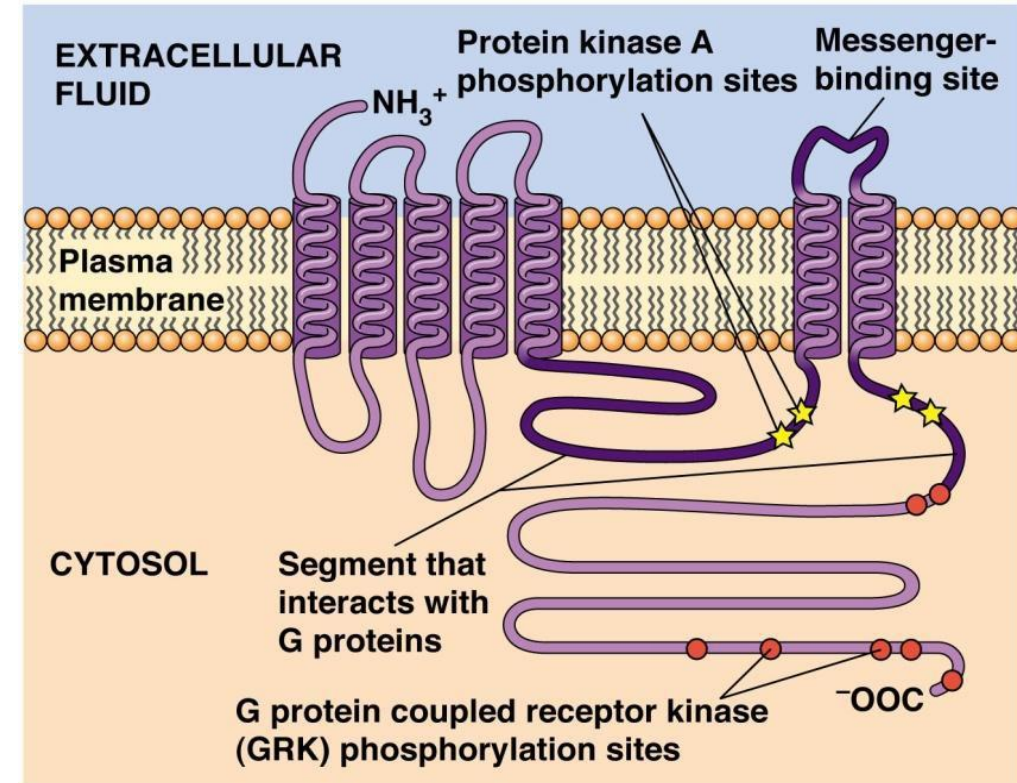
- Dissociation constant K_d
 - $K_d = \{[H] \times [R]\} / [H-R]$
- 20 X dissociation constant is enough to saturate the receptor
- K_d values for many hormone range from 10^{-9} to 10^{-11} M





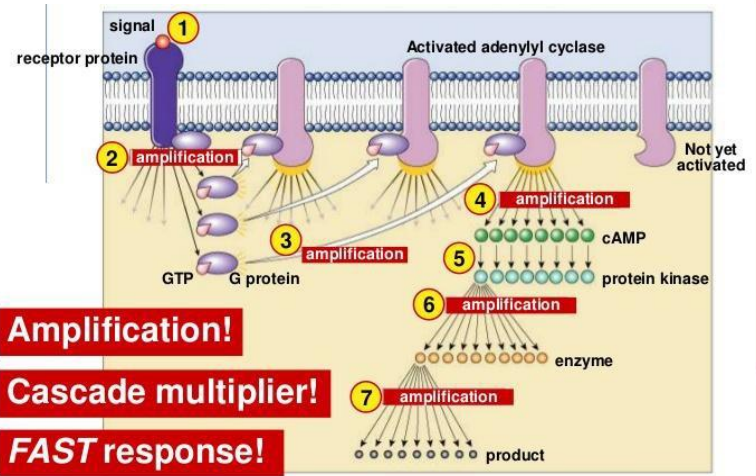
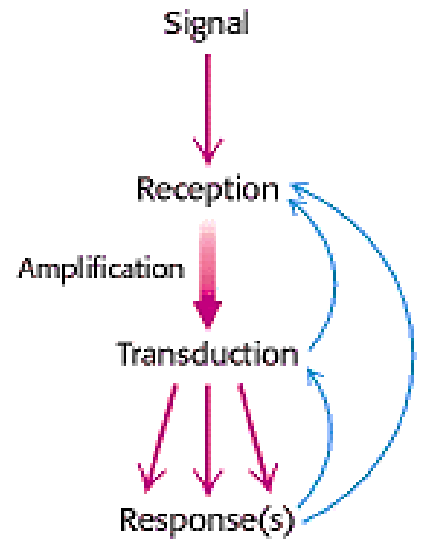
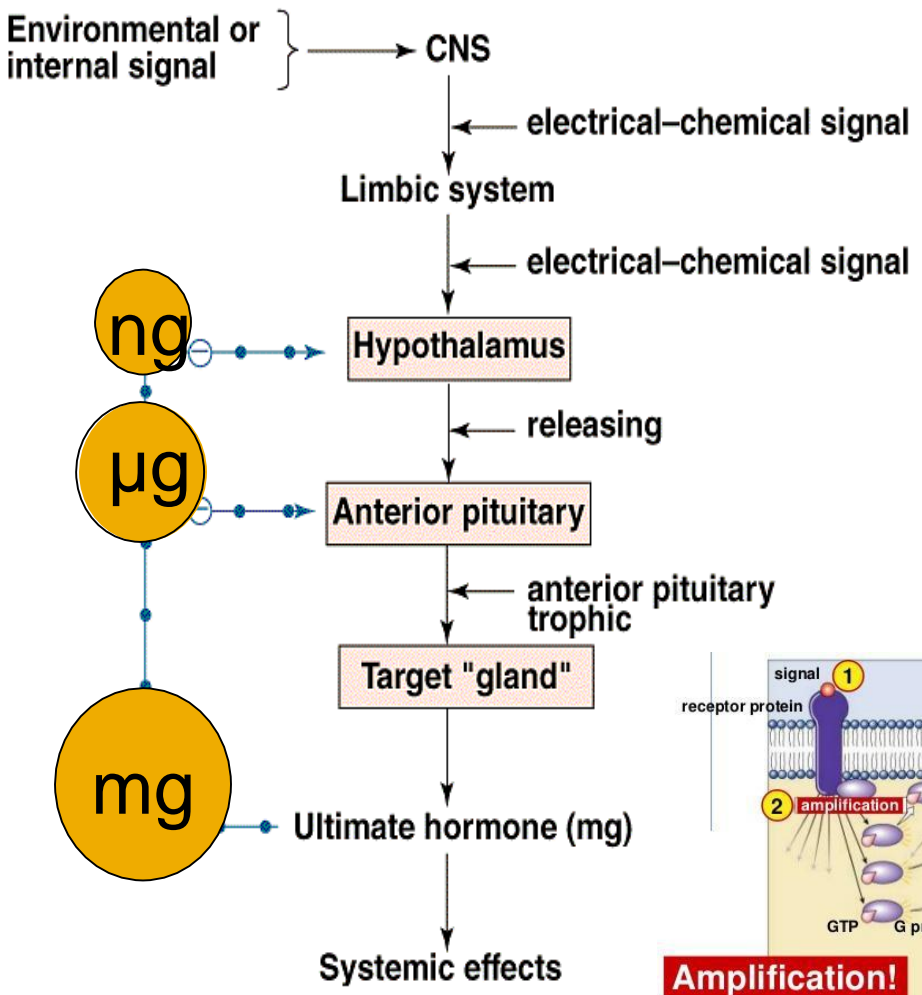
Receptor domains

- 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



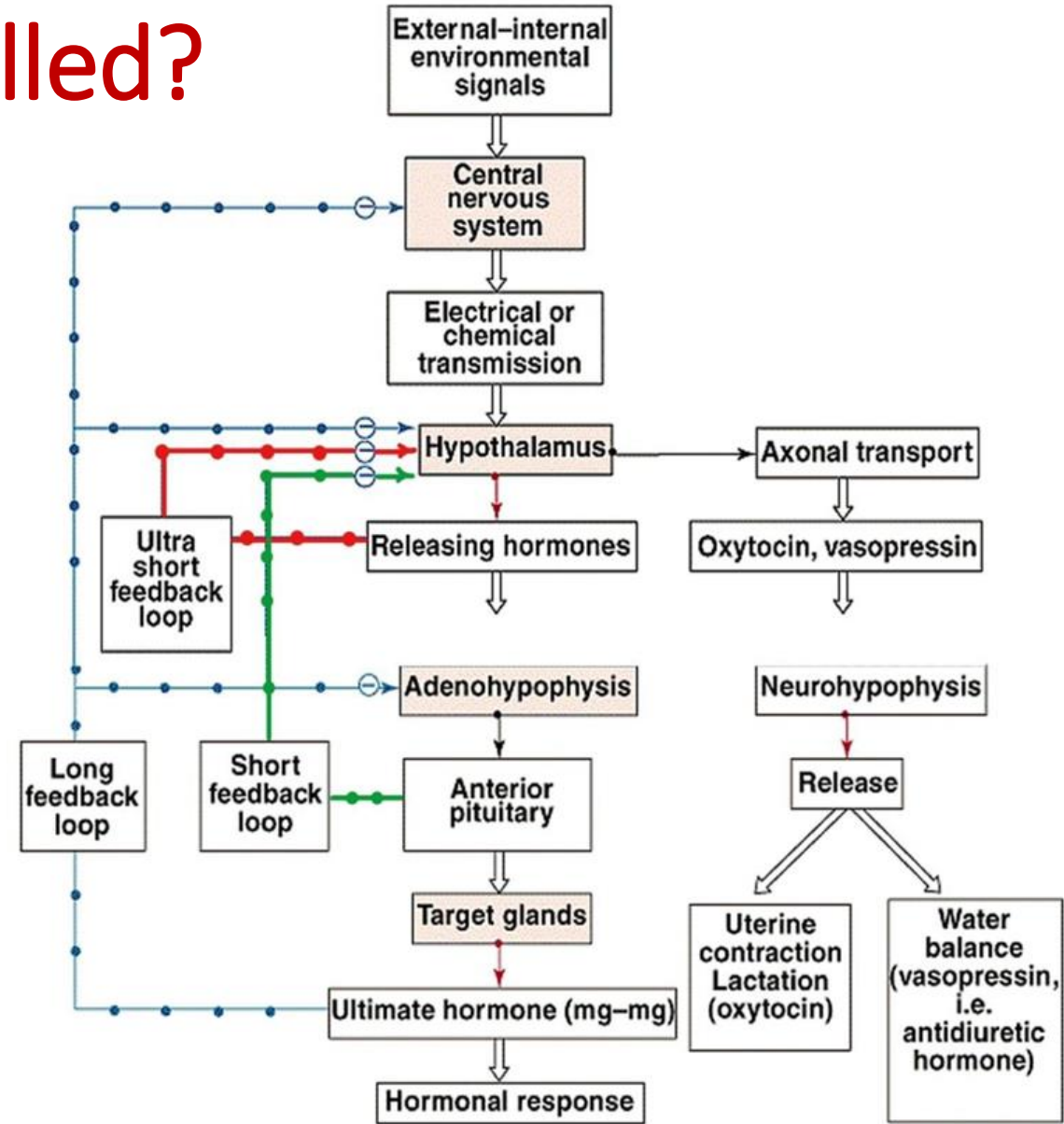
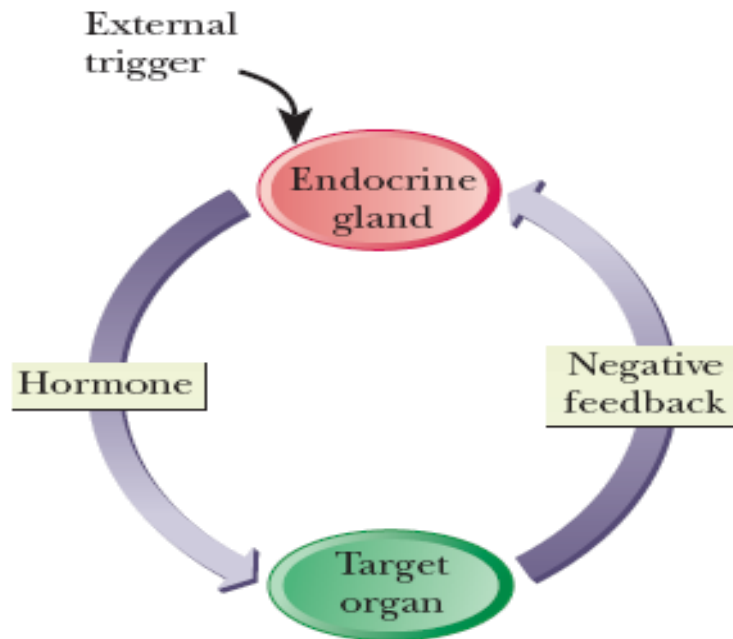
Amplification!
Cascade multiplier!
FAST response!

(a) Signaling pathway	(b) Number of molecules activated
RECEPTION Binding of epinephrine to G protein-linked receptor	1 molecule
TRANSDUCTION Inactive G protein → Active G protein	10^2 molecules
Inactive adenylyl cyclase → Active adenylyl cyclase	10^2 molecules
ATP → Cyclic AMP	10^4 molecules
Inactive protein kinase A → Active protein kinase A	10^4 molecules
Inactive phosphorylase kinase → Active phosphorylase kinase	10^5 molecules
Inactive glycogen phosphorylase → Active glycogen phosphorylase	10^6 molecules
RESPONSE Glycogen → Glucose-1-phosphate	10^8 molecules



How the release is controlled?

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





Classification of hormones

- Chemistry, solubility, receptors' location, signaling mediator
 - ✓ **Small peptides, polypeptides and proteins**
 - ✓ **Amino acid derivatives and arachidonic acid analogs**
 - ✓ **Steroids**



Classification of hormones - Chemistry

Steroids

- Cholesterol-derived
- Transported by plasma proteins or specialized carrier (**protection**)

Small peptides, polypeptides, and proteins

- Released and dissolve readily in blood and generally do not require special transport proteins.
- Most if not all polypeptides and proteins have specific carrier proteins

Amino acid derivatives and arachidonic acid analogs

- Catecholamines: released and dissolve readily
- Thyroid: bind transport proteins (prealbumin).



Classification of Hormones

Mechanism of Action

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

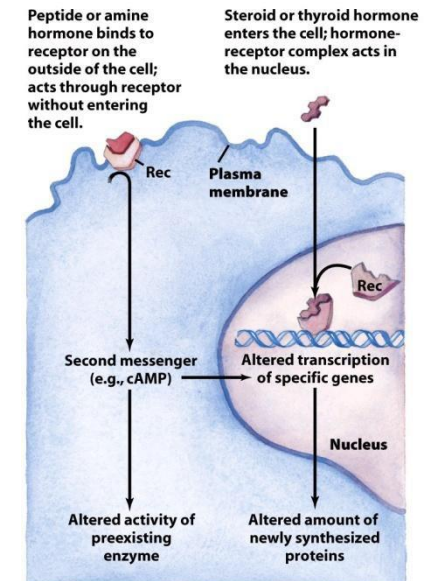
Long Half- life
(hrs-days)

Transport
proteins

KOSH
EDUTECH PVT LTD



compilation of knowledge





Classification of Hormones

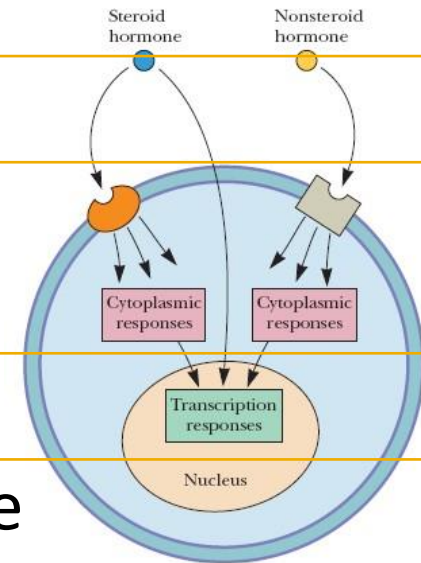
Mechanism of Action

- 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





Genomic responses vs. rapid responses

TABLE 1-1 Nuclear Receptors and Involvement in Genomic and/or Rapid Biological Responses

(Steroid receptor) & ligand	# aa Receptor	Steroid nuclear receptors & gene transcription generate genomic responses (GR)	Steroid membrane receptors generates rapid responses (RR)
(Thyroid β) T_3	461	T_3 binding to its nuclear receptor, in target cells stimulates dissociation of co-repressors, recruitment of co-activators, etc. to complete a GR.	T_3 activates PI3kinases and MAP kinase RR pathways which can result in glucose uptake, Ca^{2+} -ATPase, Na^+/H^+ antiporter.
(Vitamin D receptor) $1\alpha,25(OH)_2$ - vitamin D_3	427	Both intestinal Ca^{2+} absorption & kidney Ca^{2+} reabsorption requires GR for production of new calcium binding proteins (CaBP).	RR opening of Cl^- channels in osteoblasts & keratinocytes in 20 min; insulin secretion from β -cells, MAP kinase activation in NB4 cells.
(Estrogen receptor α) Estradiol	595	ER α GR are required for normal ovarian function.	ER α activates PI3K and then AKt RR stimulates nitric oxide NO.
(Estrogen receptor β) Estradiol	530	ER β GR are required for ovulation & pregnancy.	The cell membrane ER β bound to caveola has been implicated in RR.
(Glucocorticoid receptor) Cortisol	777	Knockout (KO) of the mouse GC receptor is lethal at time of birth.	Cortisol stimulates PI3-kinase/Akt to activate in seconds NO release.
(Mineralocorticoid receptor) Aldosterone	919	MR KO mice die of Na^+ and H_2O deprivation.	Aldosterone activates in 3–15 minutes the RR of Na^+/H^+ exchange in renal cells.
(Progesterone receptor) Progesterone	933	The progesterone receptor participates in GR sexual differentiation determination.	Progesterone stimulates RR within seconds to minutes, the acrosome reaction in spermatozoa.
(Androgen receptor) Testosterone	919	KO of the AR male mouse causes development of female genitalia.	Activation of MAP kinase then activates the ERK pathway via RR.

GR = genomic responses; RR = rapid responses. RR are not dependent on genomic responses. KO = Knock-out renders affected genes inactive.



Structure of hormones by location

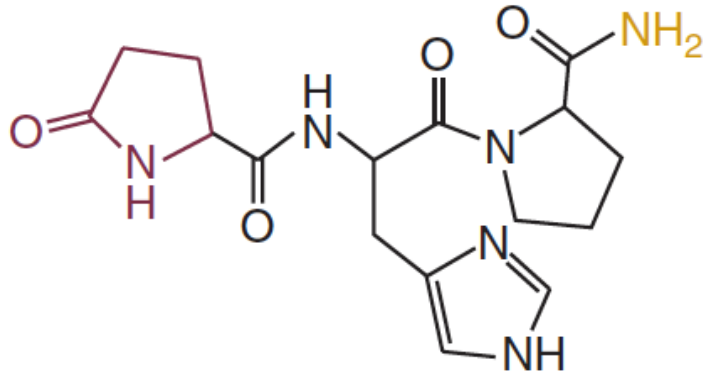


Hypothalamus

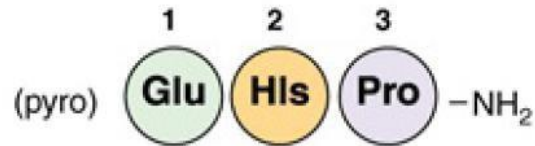
Hormone	Composition
Growth hormone releasing hormone (GHRH)	Two forms: polypeptides (40 & 44 amino acids)
Somatostatin	Two forms: polypeptides (14 & 28 amino acids)
Dopamine	Catecholamine (amino acid derivative)
Corticotropin releasing hormone (CRH)	Polypeptide (41 amino acids)
Gonadotropin releasing hormone (GnRH)	Polypeptide (10 amino acids)
Thyrotropin releasing hormone (TRH)	Polypeptide containing 3 amino acids



Hypothalamus



thyrotropin-releasing hormone, TRH.



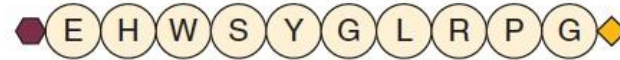
TRH

● (E) = pyroglutamyl

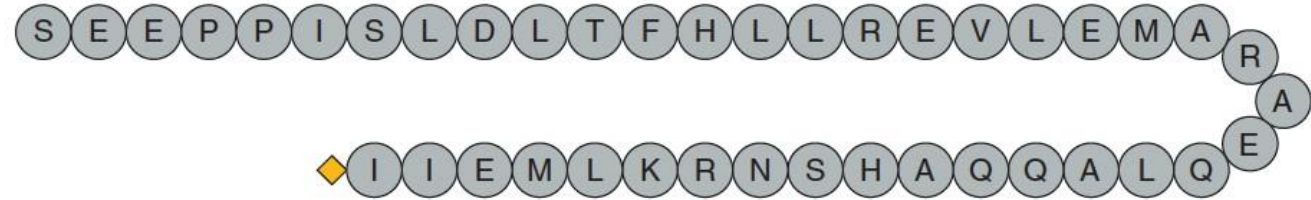
○ ◆ = c-terminal amide: prolinamide (TRH); glycynamide (GnRH); isoleucinamide (CRH); leucinamide (GHRH)



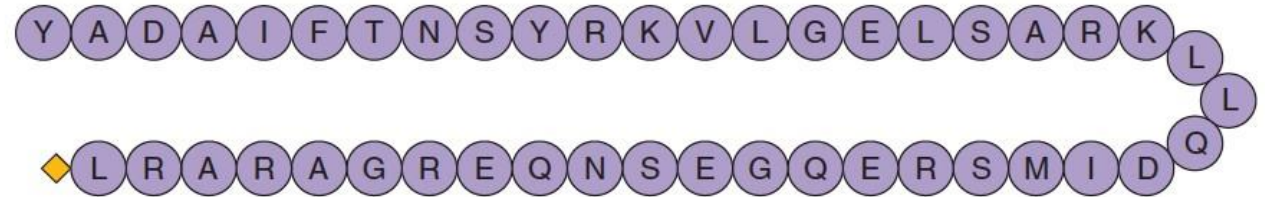
TRH: Thyrotrophin releasing hormone



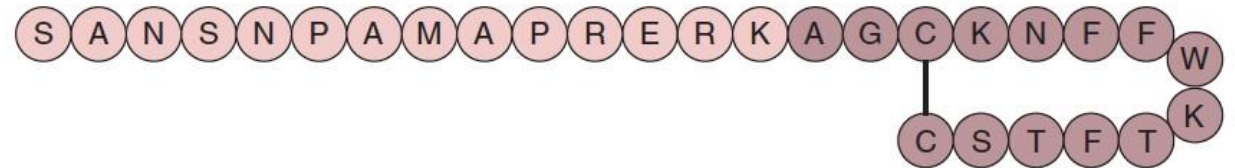
GnRH: Gondaotrophin releasing hormone



CRH: Corticotrophin releasing hormone



GHRH: Growth hormone releasing hormone



ST: Somatostatin



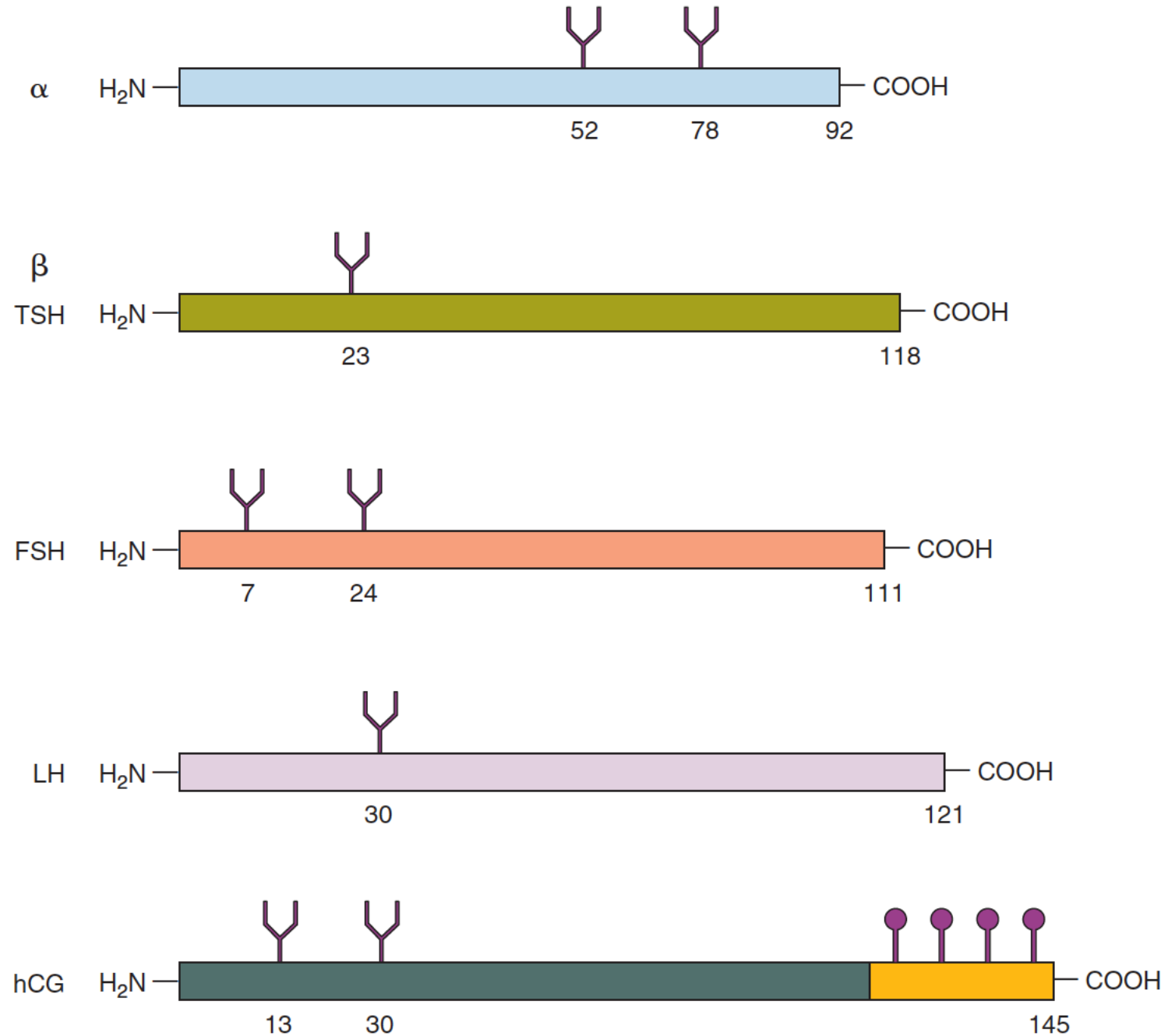
Anterior Pituitary

Hormone	Composition
Growth hormone (somatotropin, GH)	Straight-chain protein: two forms (191 aa) and (176 aa)
Prolactin (PRL)	Straight-chain protein (198 aa)
Adrenocorticotrophic hormone (ACTH)	Small polypeptide (39 aa)
Follicle-stimulating hormone (FSH)	2-chain glycoprotein: (α , 92 aa; β , 111 aa)
Luteinizing hormone (LH)	2-chain glycoprotein: (α , 92 aa; β , 116 aa)
Thyrotropic hormone (TSH)	2-chain glycoprotein: (α , 92 aa; β , 112 aa)



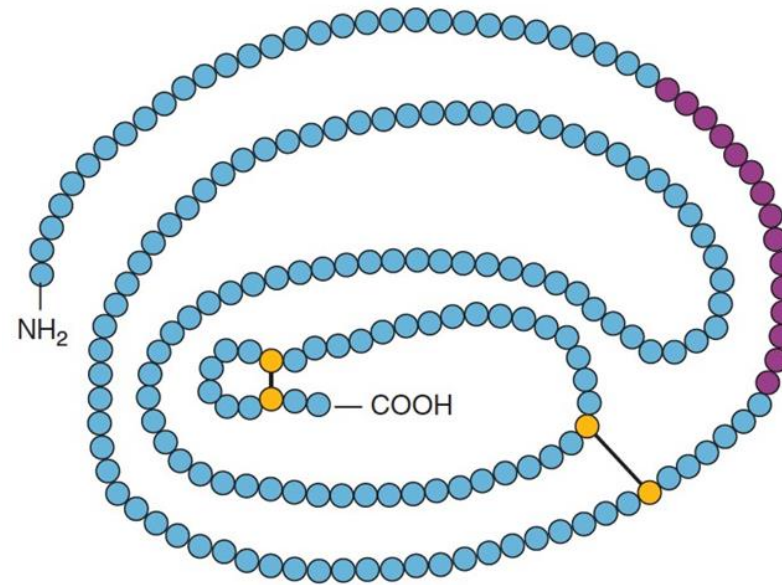
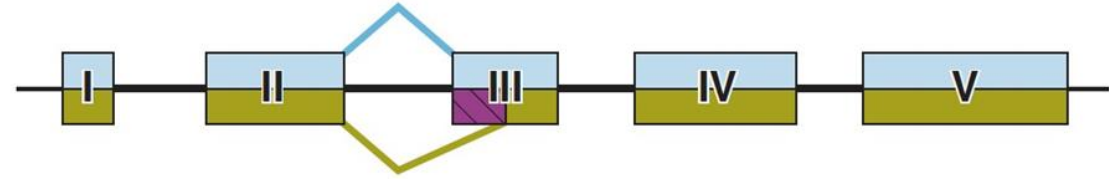
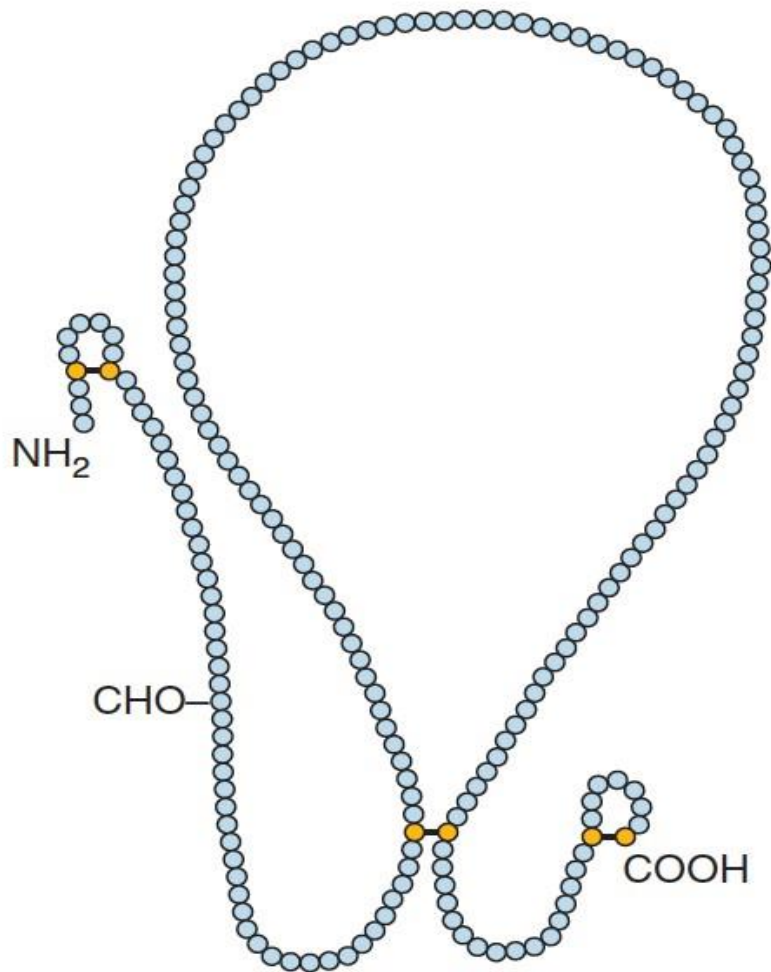
Anterior pituitary

- N-glycosylation (Asn)
vs. O-glycosylation (ser)

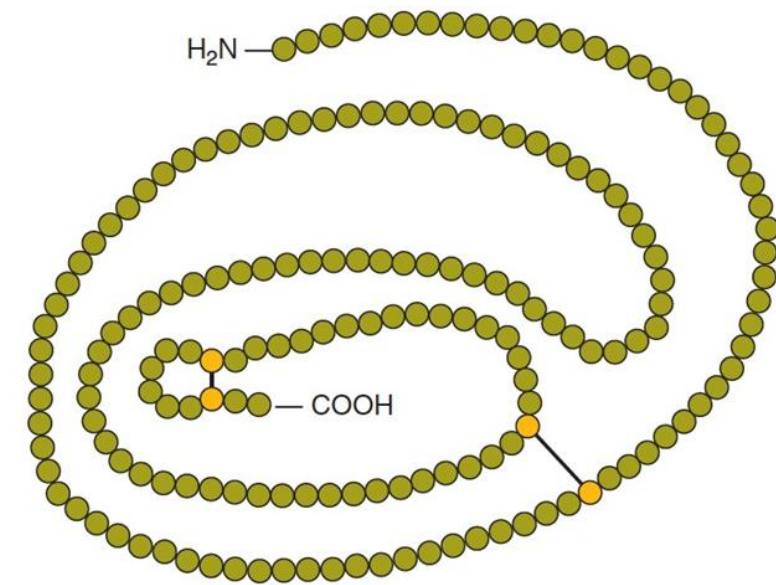




Anterior pituitary – GH & prolactin



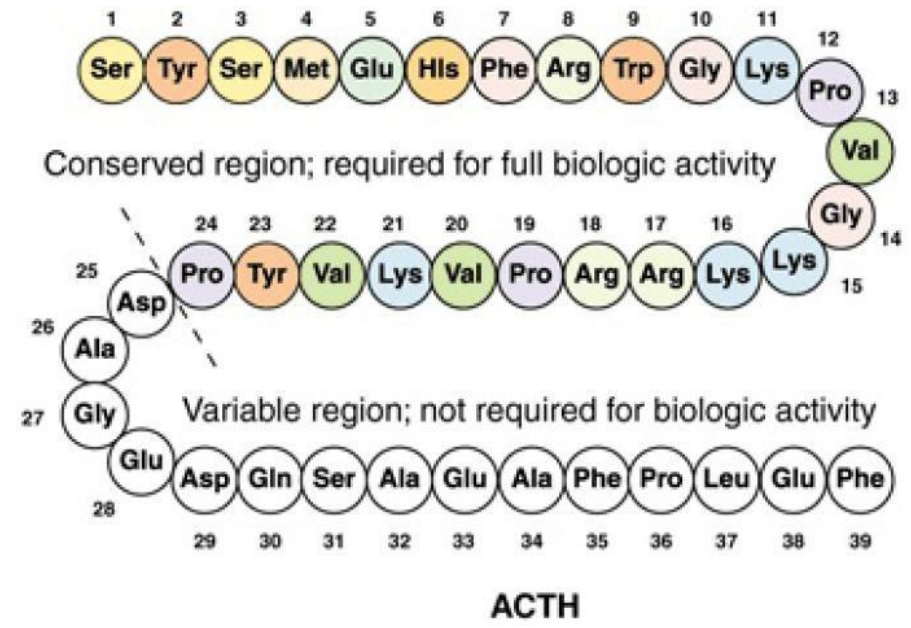
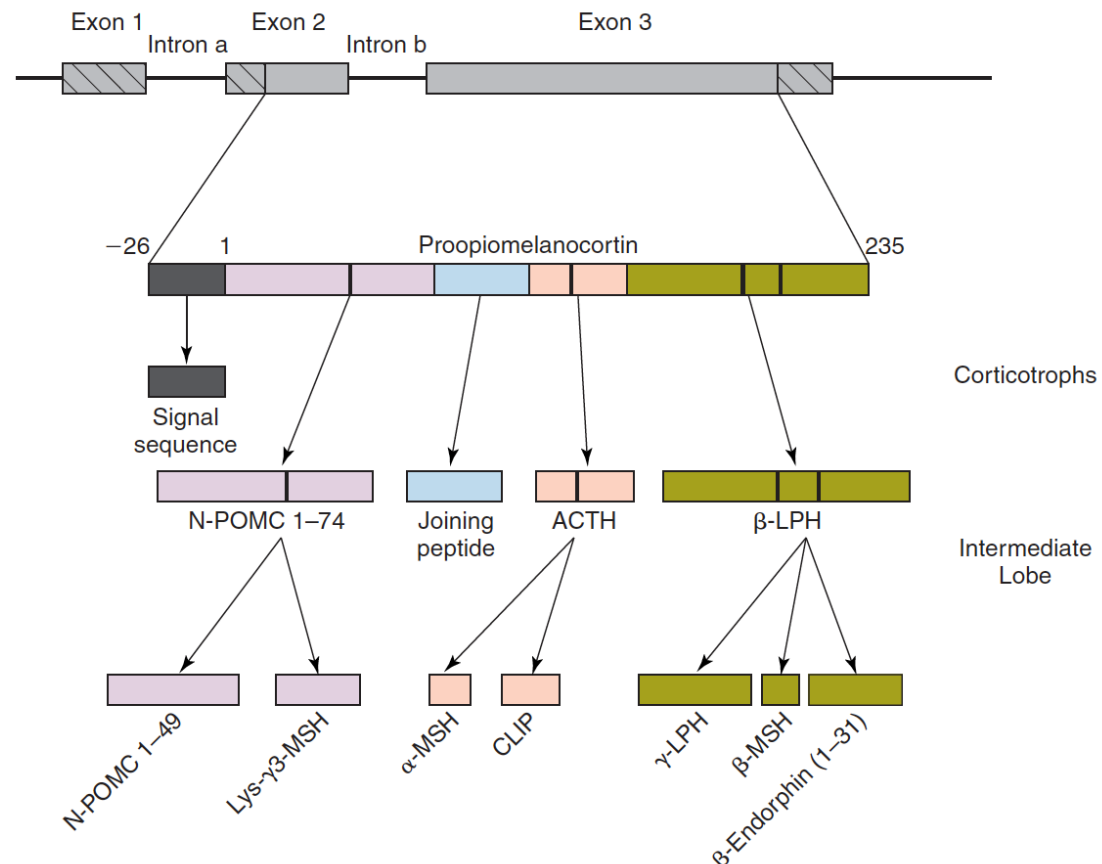
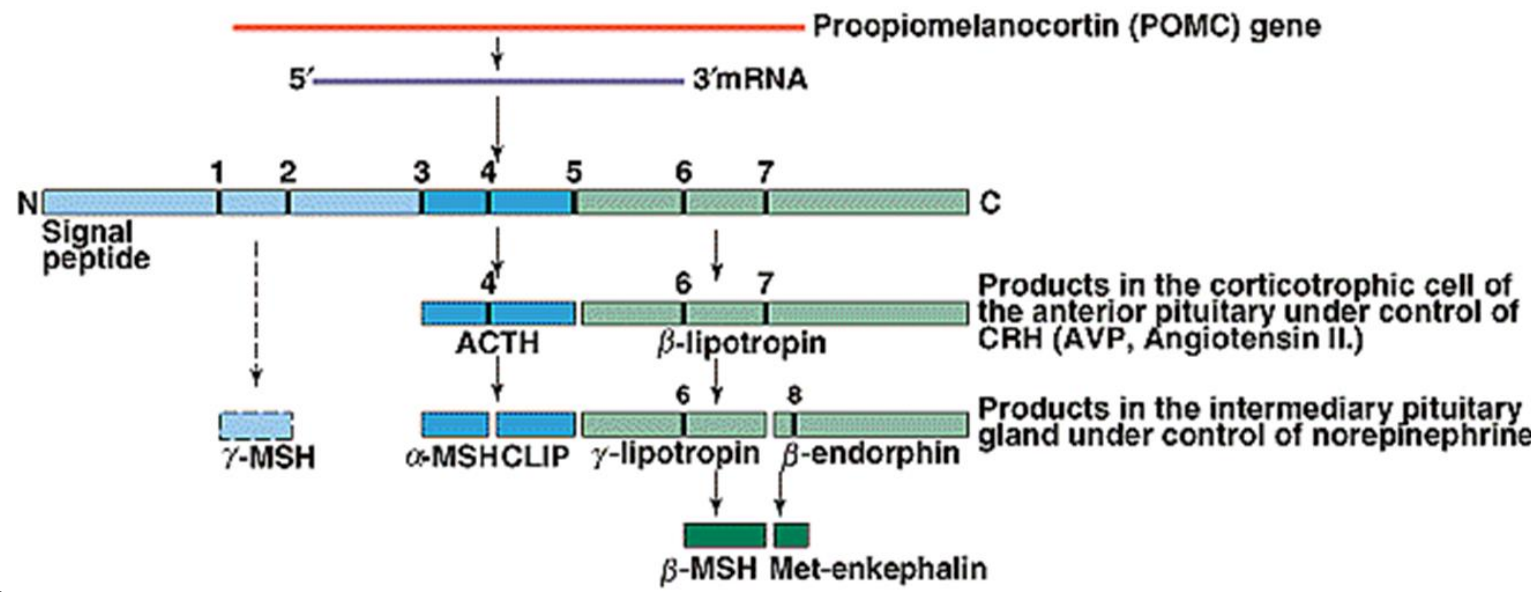
22K-GH
191aa



20K-GH
176aa



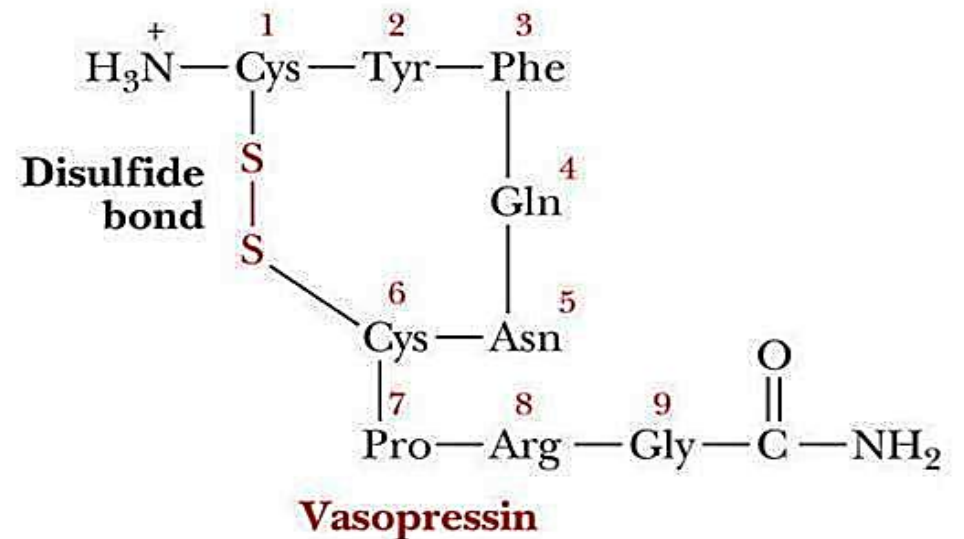
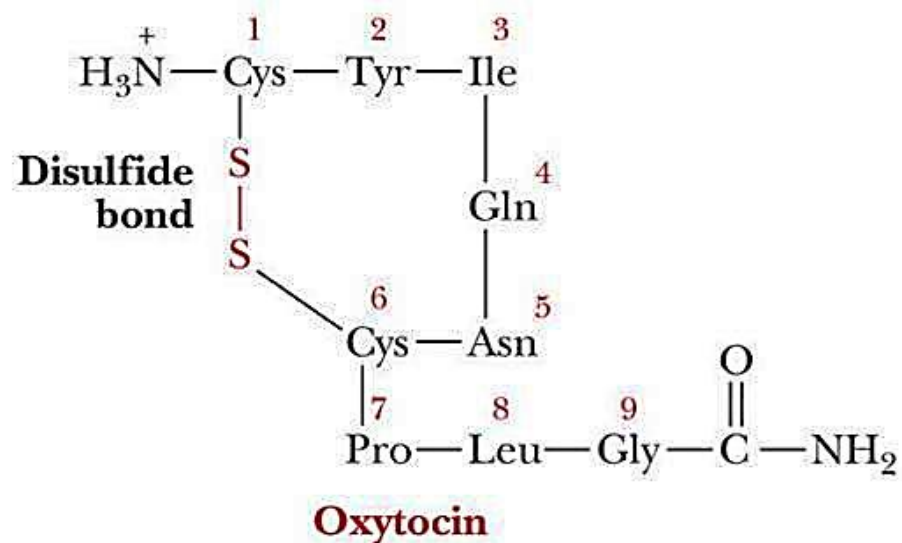
Anterior pituitary – Proopiomelanocortin (POMC)





Posterior pituitary

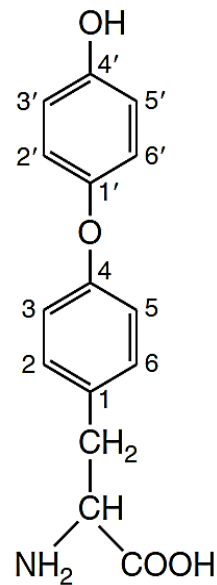
Hormone	Composition
Oxytocin	Polypeptide containing 9 amino acids
Antidiuretic hormone (ADH; vasopressin)	Polypeptide containing 9 amino acids; two forms: arginine-ADH (most common in humans) and lysine-ADH



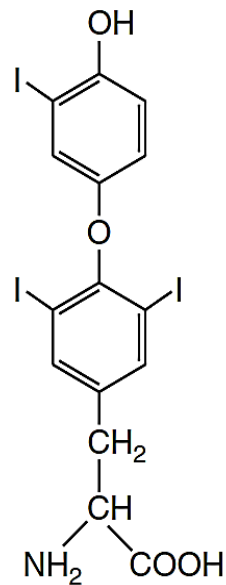


Thyroid

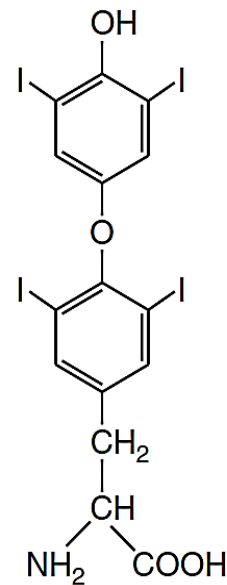
Hormone	Composition
Thyroxine (tetraiodothyronine, T ₄) and triiodothyronine (T ₃)	Amino acid derivative
Calcitonin (thyrocalcitonin)	Polypeptide containing 32 amino acids



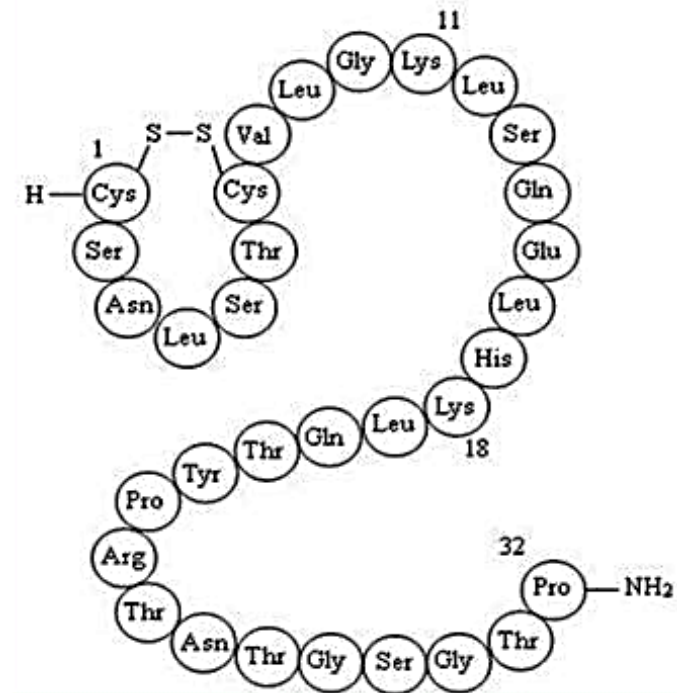
Thyronine



T₃: 3,5,3'-triiodo-
thyronine



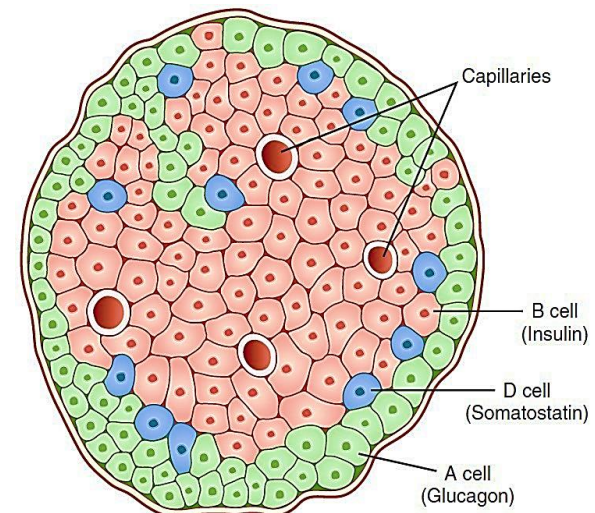
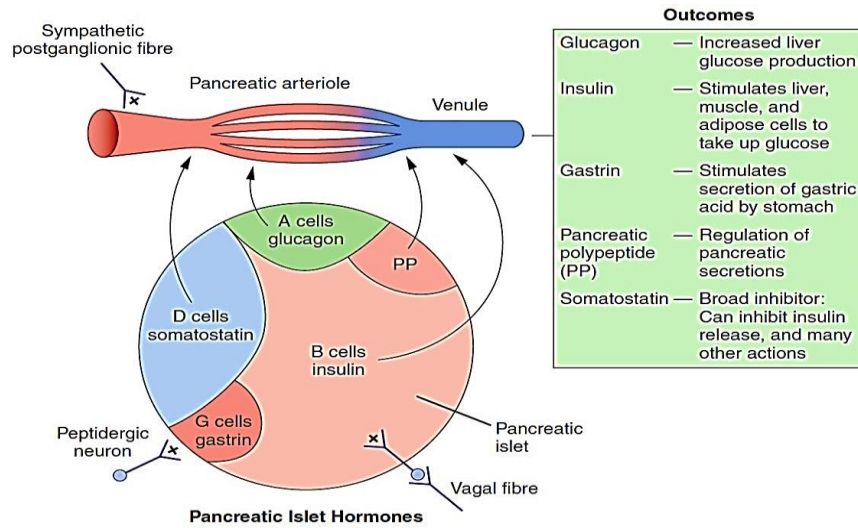
T₄ = Thyroxine:
3,5,3',5'-tetraiodo-
thyronine





Pancreas

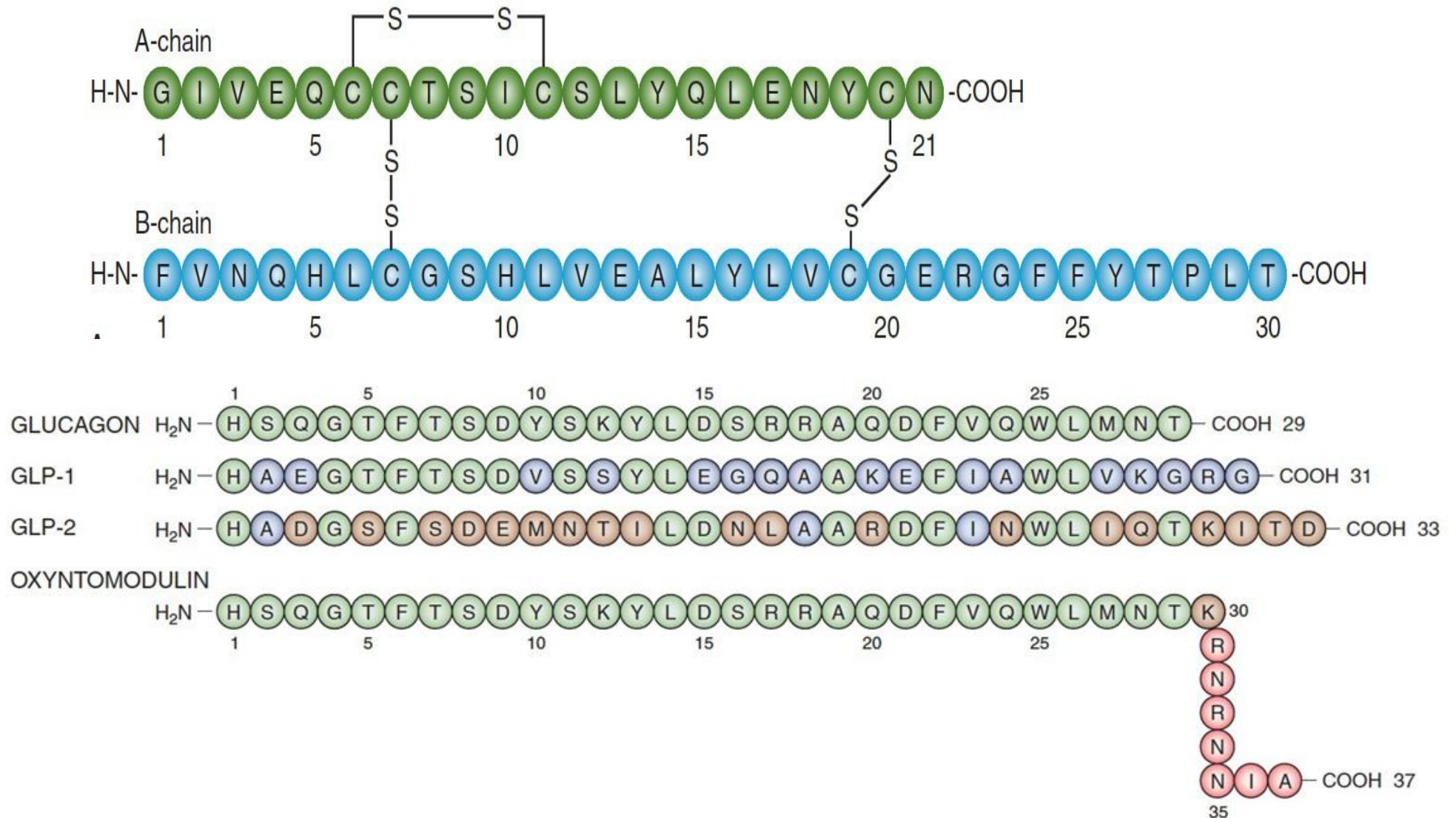
Hormone	Composition (amino acids)
Insulin	51
Glucagon	29
Somatostatin	37
Pancreatic polypeptide (PP)	14
Gastrins	34, 17, 14





Pancreas

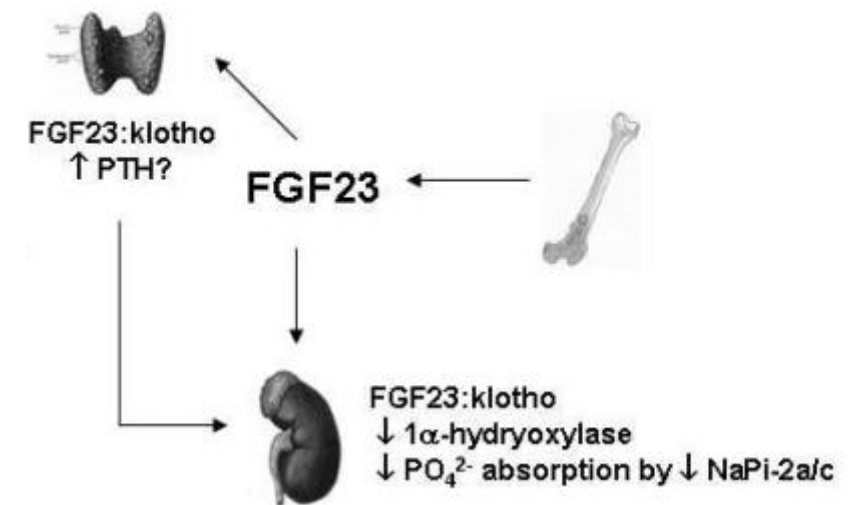
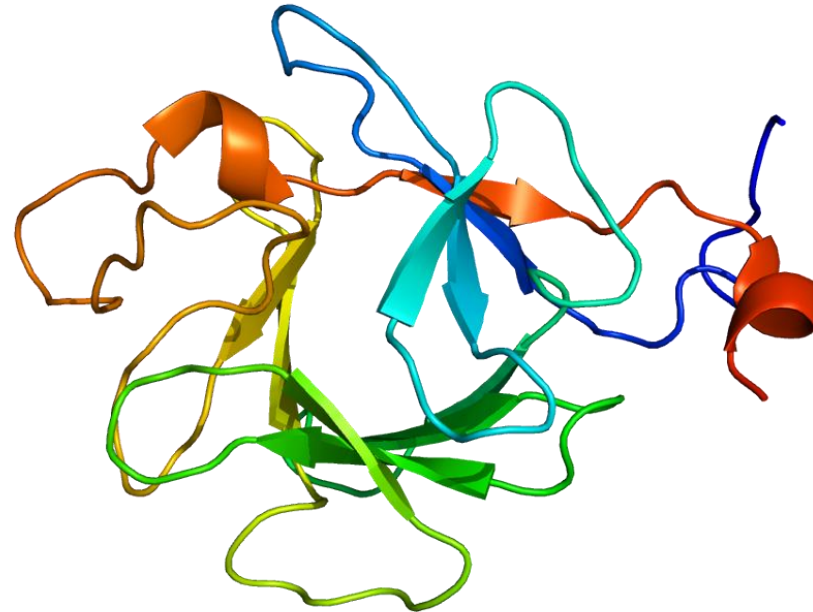
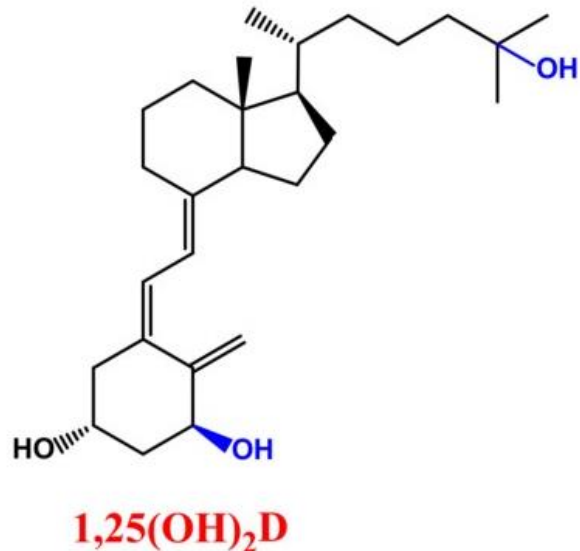
Primary structure of insulin: Amino acid sequence of the A-chain and B-chain of one insulin molecule.





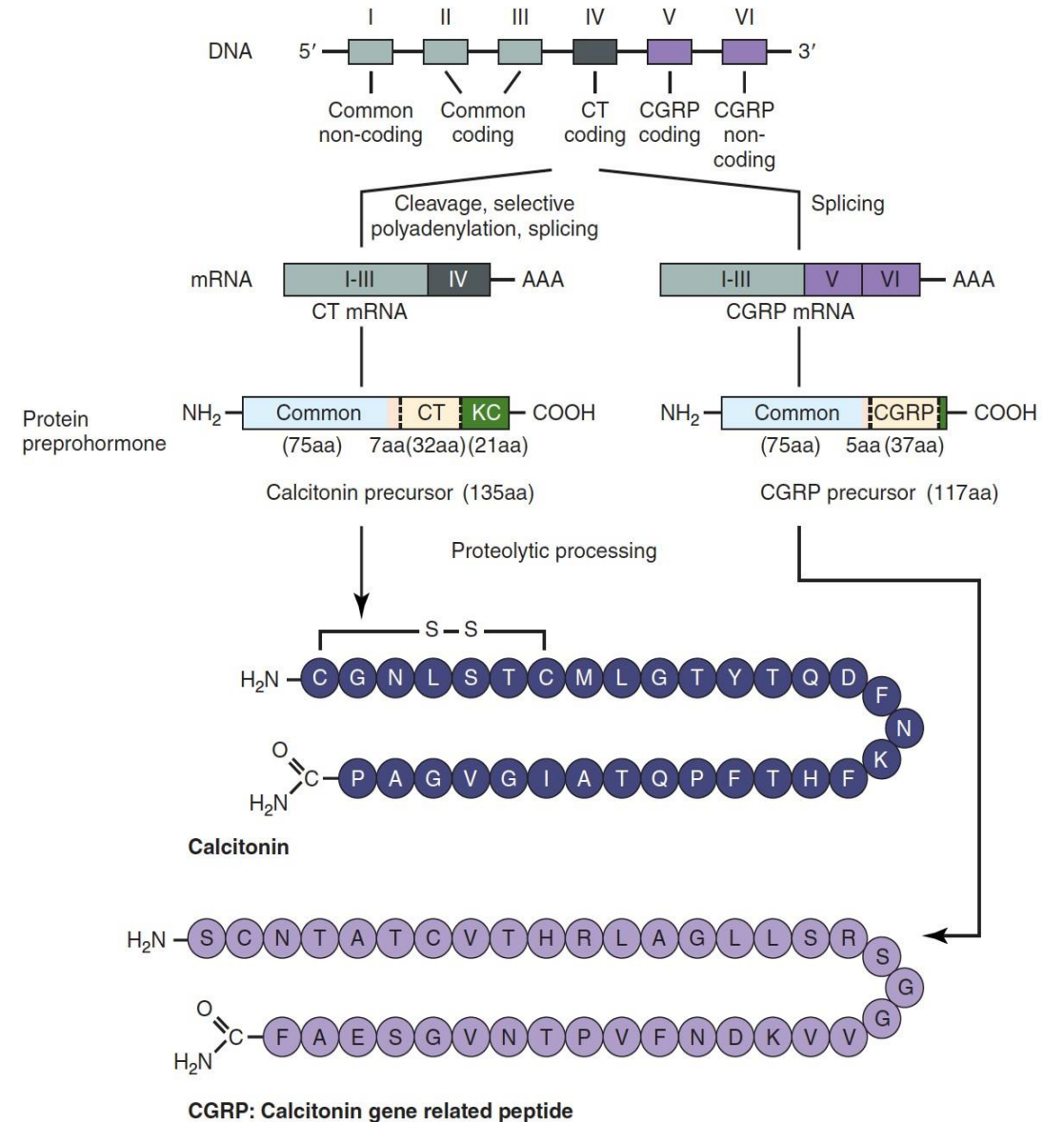
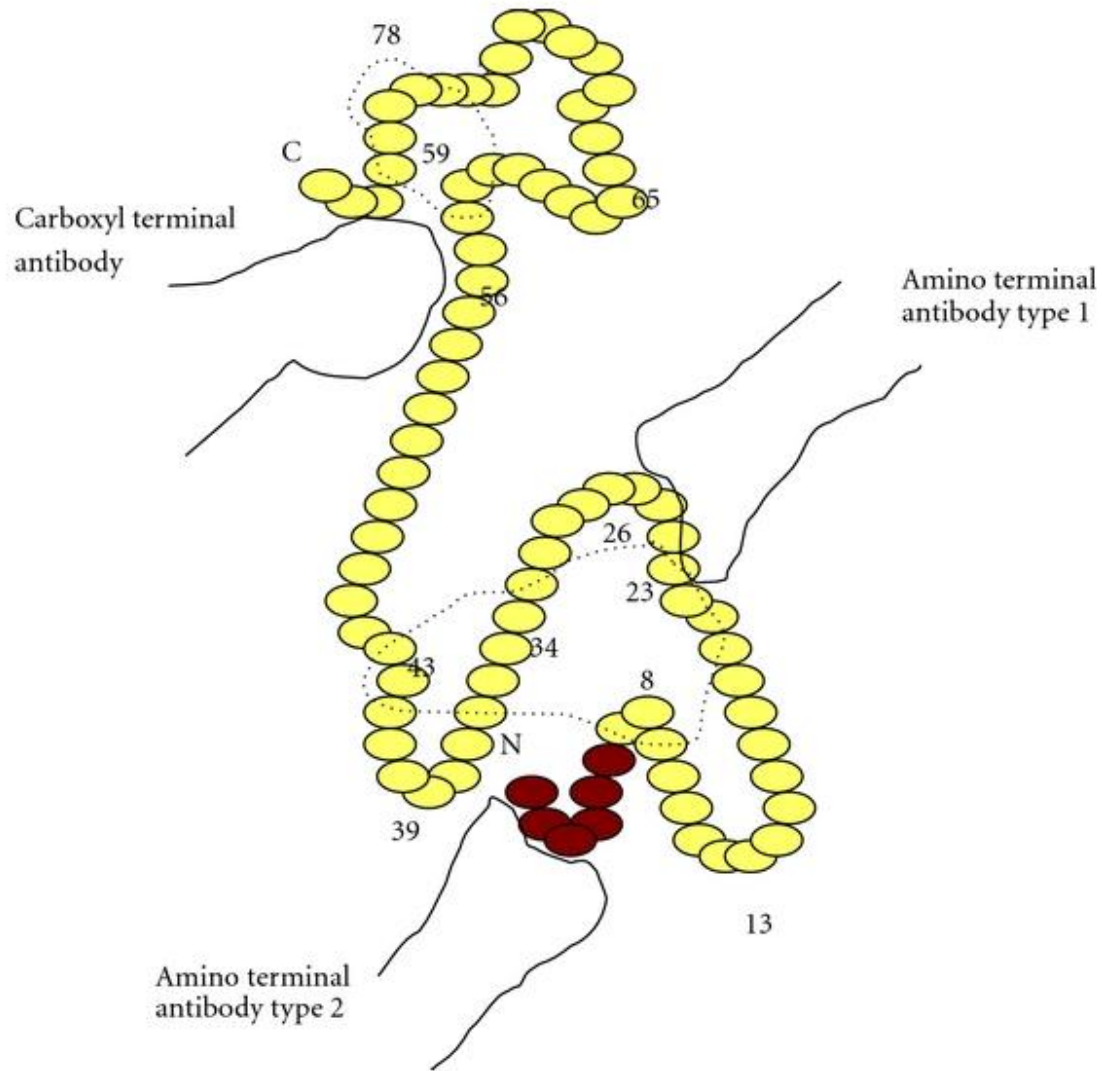
Calcium Regulating Hormones

Hormone	Composition
Parathyroid hormone (PTH)	Polypeptide 84 amino acids
Vitamin D	Steroid
Calcitonin	Polypeptide 32 amino acids
Fibroblast Growth Factor 23	Protein 251 amino acids





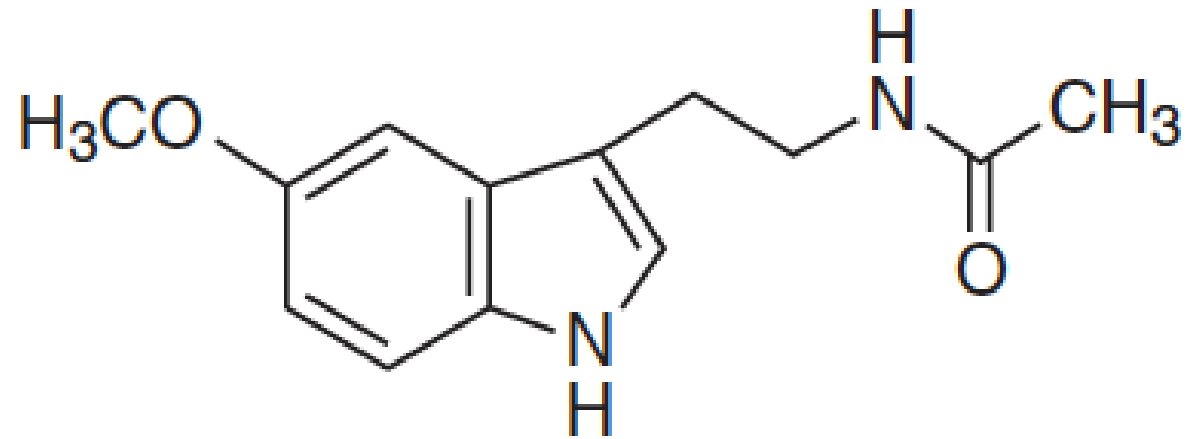
Calcium Regulating Hormones





Pineal Gland Hormones

Hormone	Composition (amino acids)
Melatonin	Indolamine (N-acetyl-5- methoxytryptamine)



Melatonin



Adrenal and Sexual Glands' Hormones

Hormone	Composition (amino acids)
Group of hormones	steroids
Norepinephrine	Amino acid derivative



Synthesis and Degradation of hormones

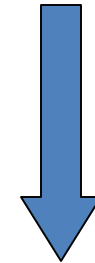
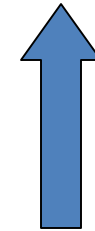


Chemistry of Hormones

- **Steroids**
- Small molecules - NO
- Amino acid derivatives
 - Thyroid hormones

- Catecholamines
- Proteins and peptides
- FA derivatives - eicosanoids

Receptor inside the cell

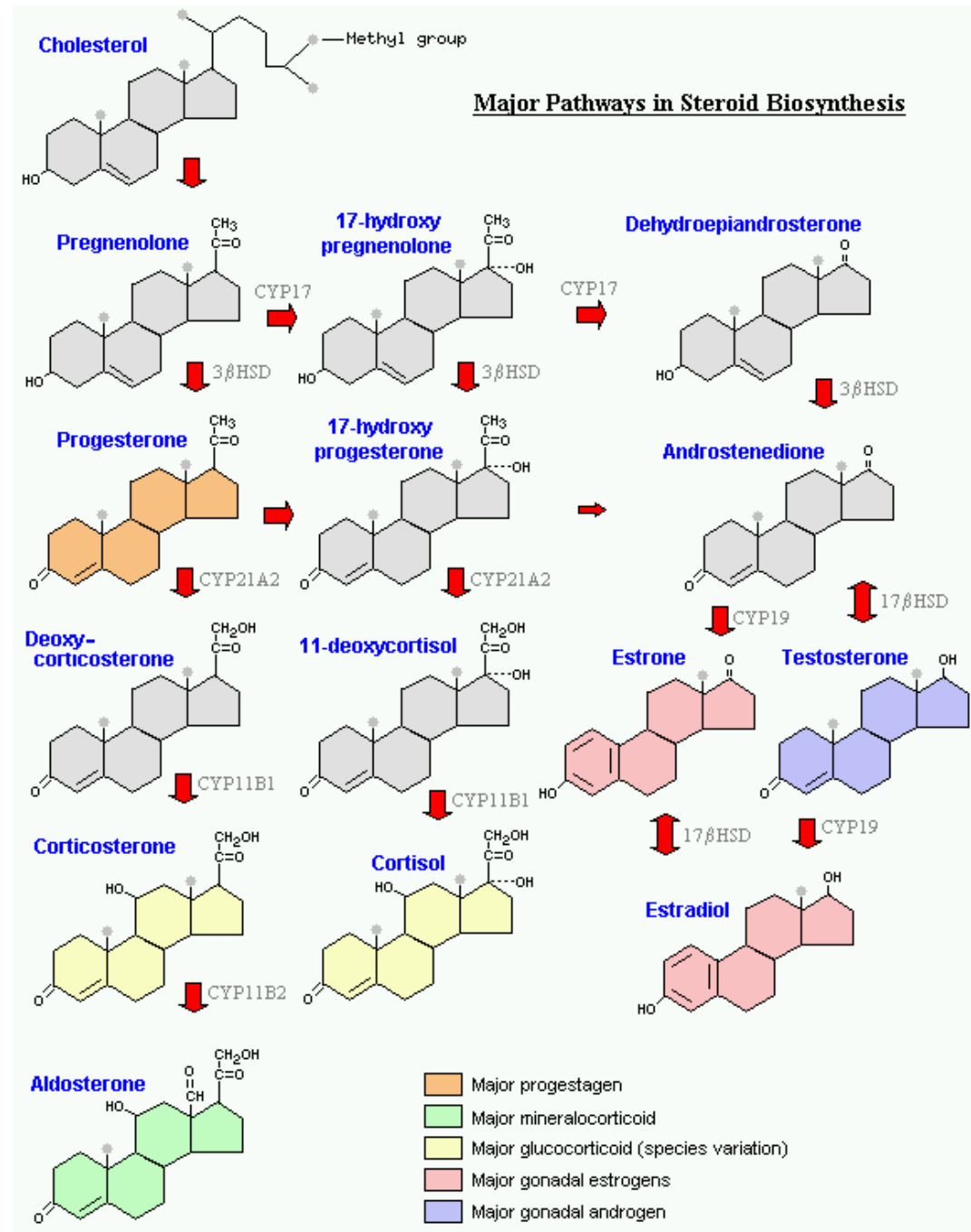


Surface receptor



Steroid hormone synthesis

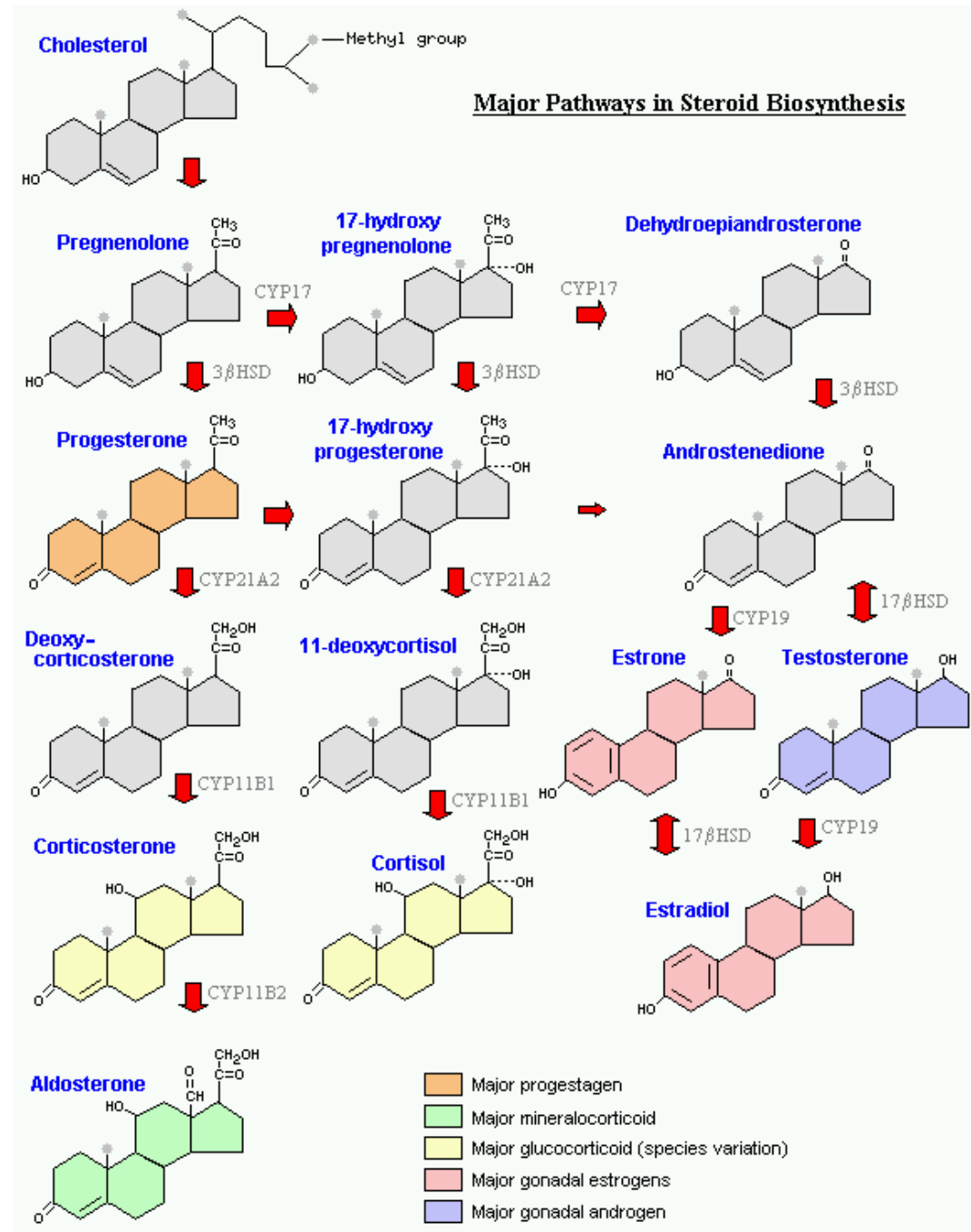
- C21:
 - Progesterone: directly from pregnenolone
 - Cortisol & Aldosterone: from progesterone





Steroid hormone synthesis

- C19
 - Testosterone
 - from progesterone or pregnenolone
 - 2c shortage
- C18 (estrogen):
 - Aromatase
 - Cleaves C18
 - Reduction





Steroid hormone breakdown

- Steran core cannot be cleaved
- In the liver: hydroxylation and conjugation with glucuronides or sulphates
- Urinary excretion:
 - ✓ Of metabolites
 - ✓ Of unchanged hormones



Chemistry of Hormones

- Steroids
- **Small molecules - NO**
- Amino acid derivatives
 - Thyroid hormones

- Catecholamines
- Proteins and peptides
- FA derivatives - eicosanoids

Receptor inside the cell

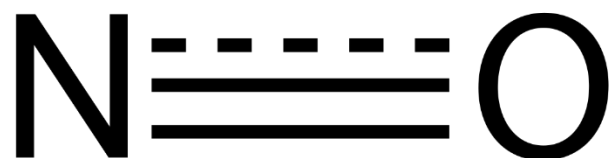
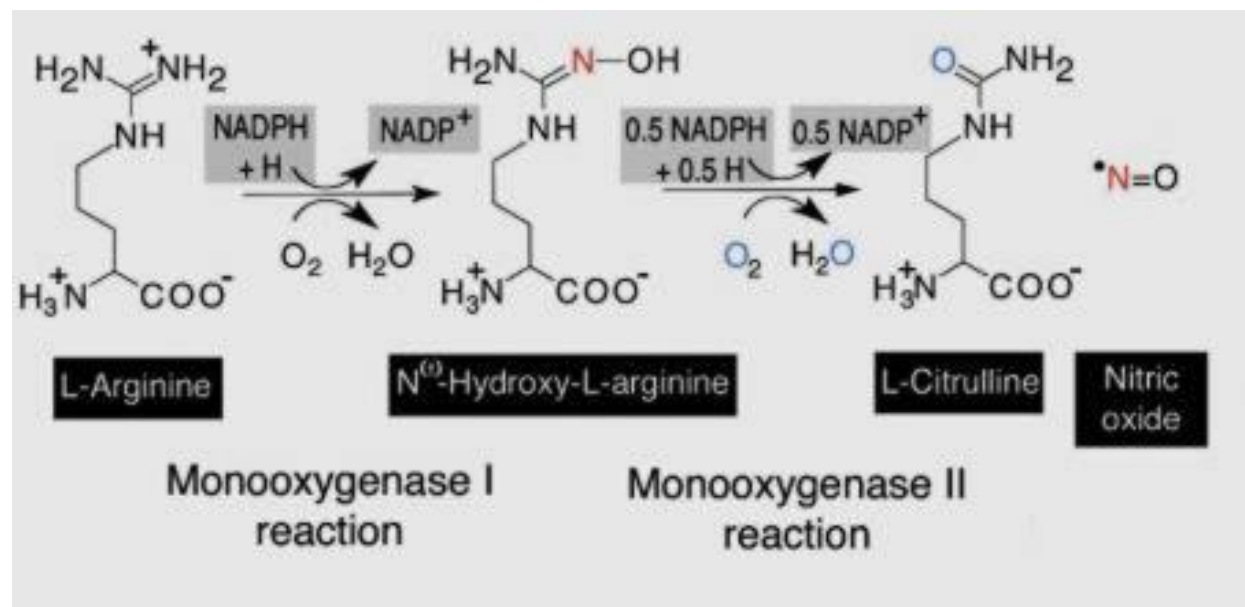
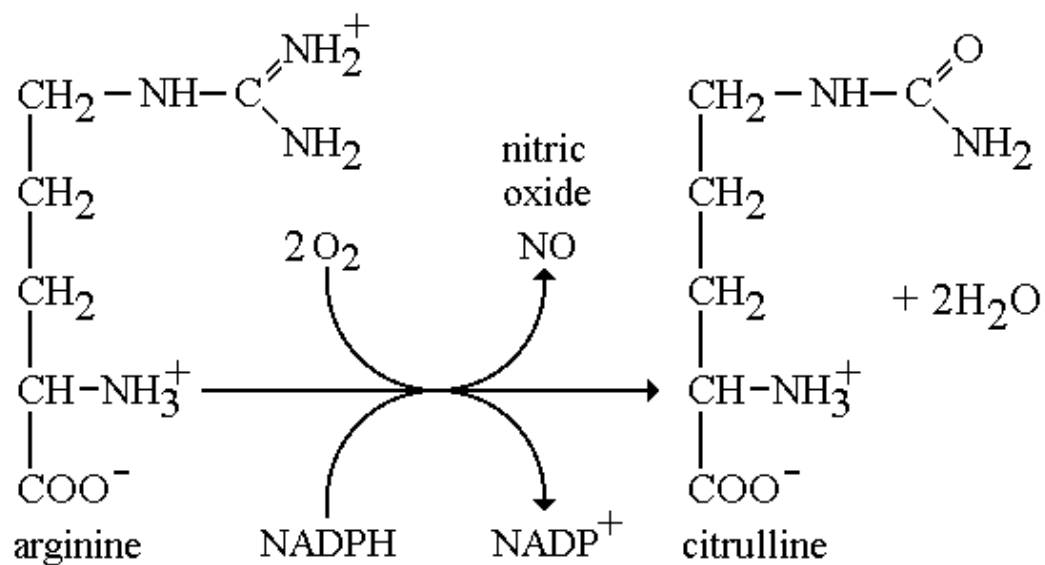


Surface receptor



Nitric oxide (NO)

➤ NO: synthesized by NO-synthase





Nitric oxide synthase isozymes

- NO-synthase (NOS)
 - ✓ In neurons (NOS-I): neurotransmission
 - ✓ In macrophages (NOS-II): kills bacteria
 - ✓ Endothelial (NOS-III): smooth muscle → **cGMP** → vasodilation

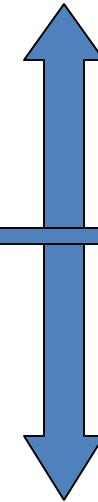
- Clinical correlation:
 - ✓ Nitrates in the treatment of angina
 - ✓ Refractory hypotension during septic shock



Chemistry of Hormones

- Steroids
- Small molecules - NO
- **Amino acid derivatives**
 - Thyroid hormones
 - Catecholamines
- Proteins and peptides
- FA derivatives - eicosanoids

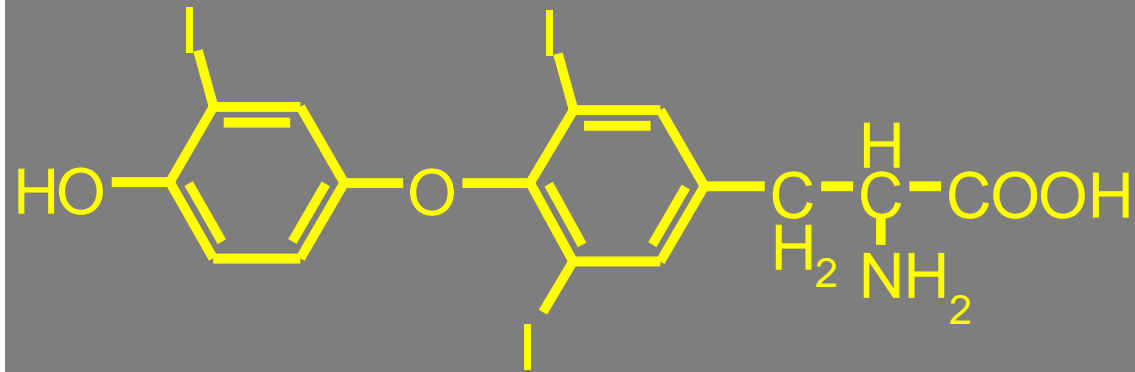
Receptor inside the cell



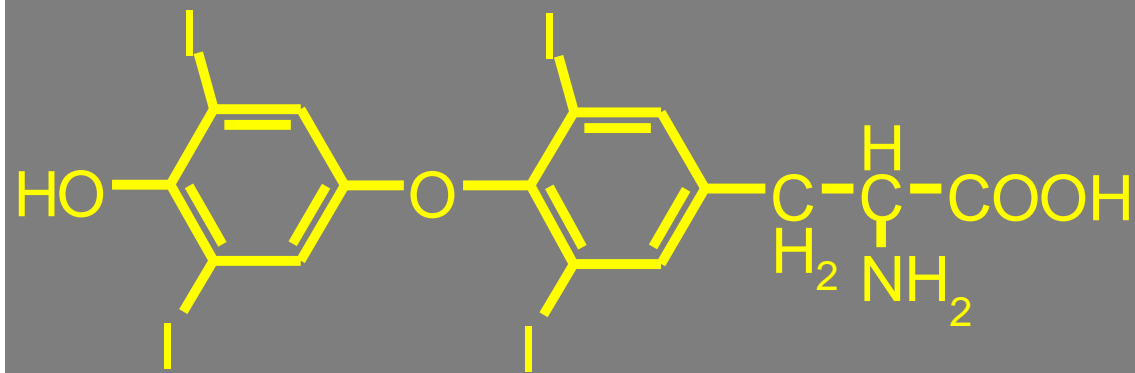
Surface receptor



Thyroid hormones



Triiodothyronine (T3)

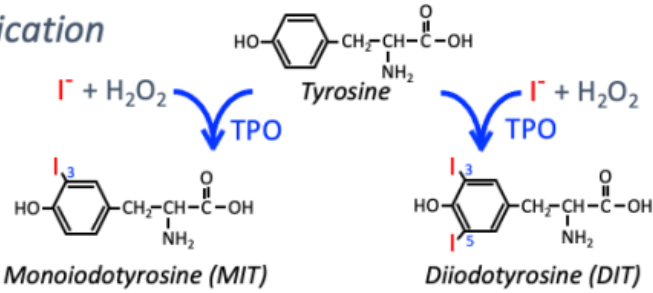


Thyroxine (T4)

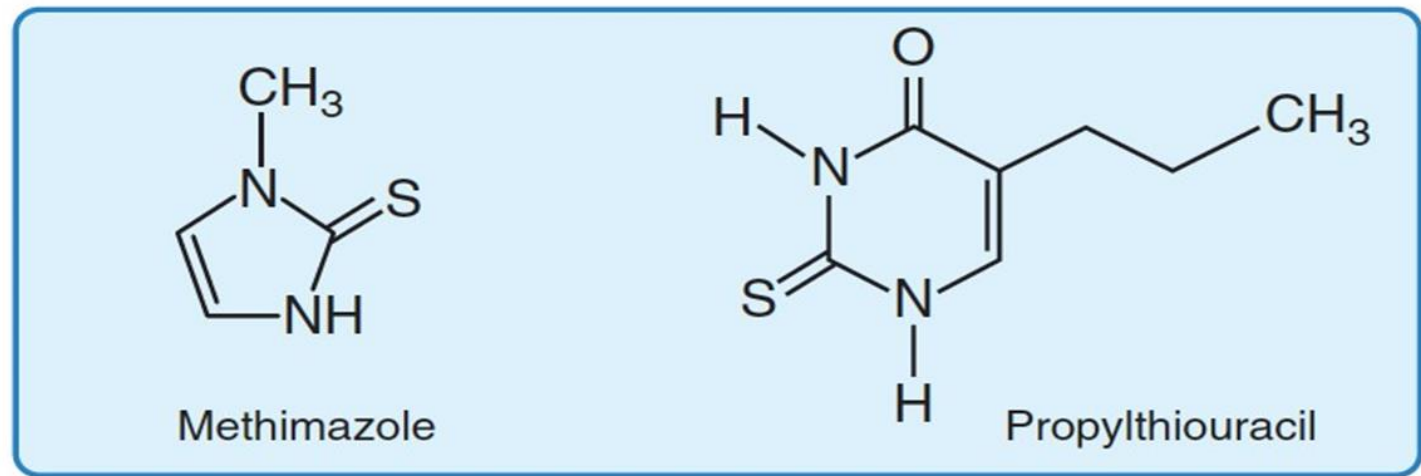


Thyroid hormones

Organification

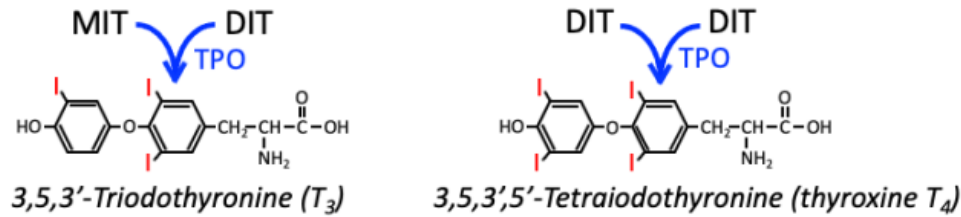


Inhibitors of TPO
Methimazole
PTU

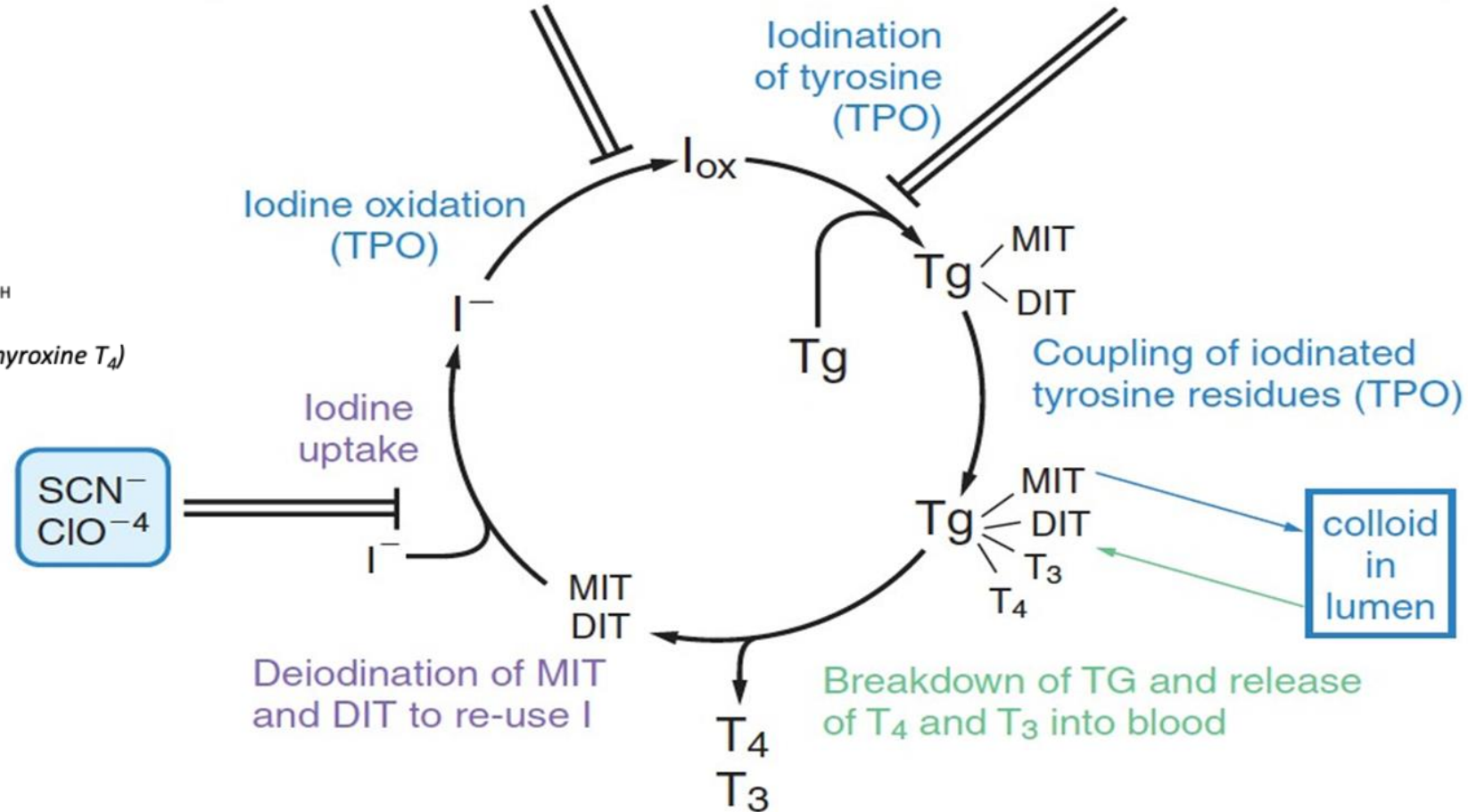


Coupling

(phenolic coupling)

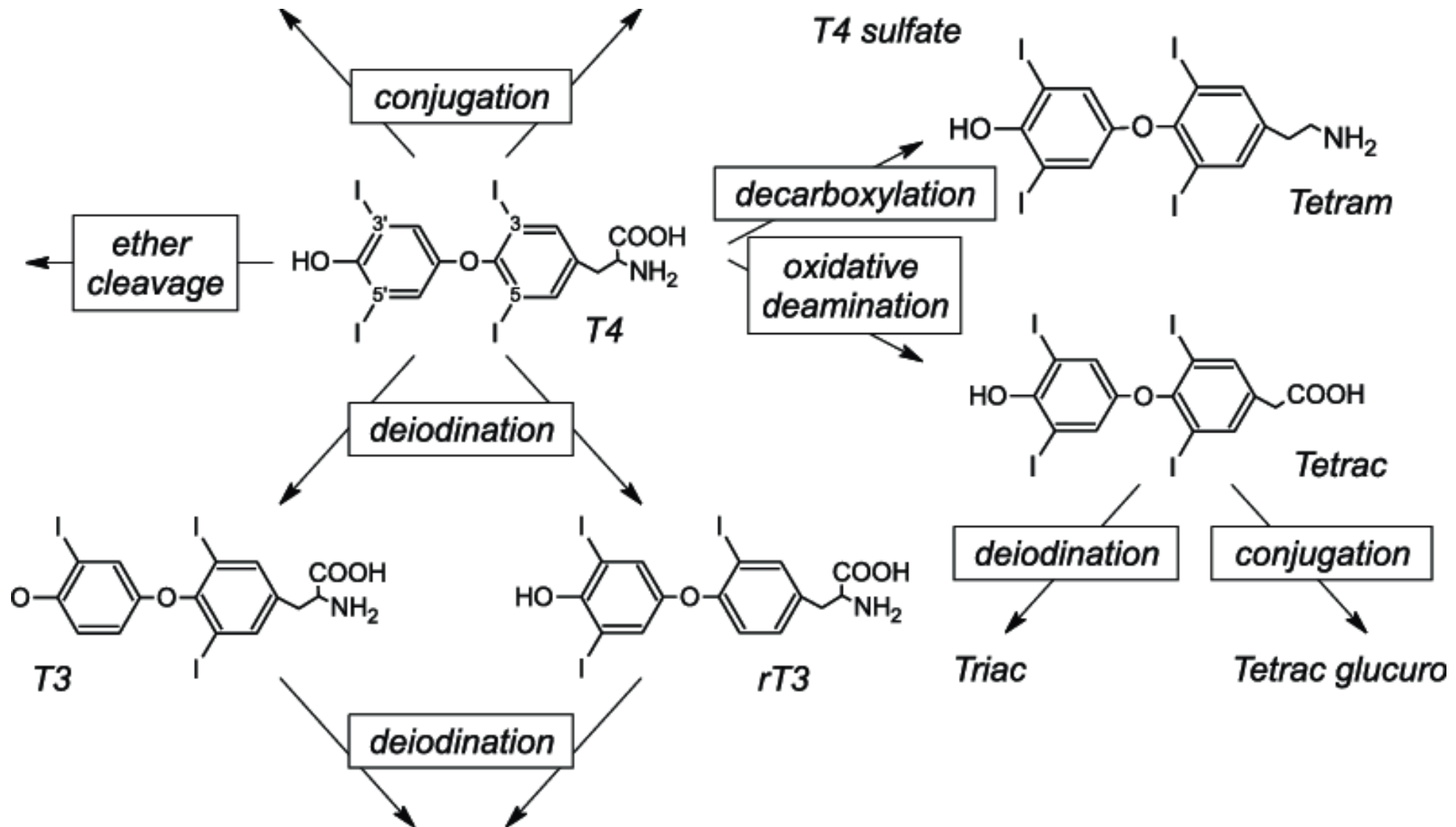


- ✓ TPO, thyroid peroxidase;
- ✓ Tg, thyroglobulin;
- ✓ MIT, monoiodotyrosine;
- ✓ DIT, diiodotyrosine;
- ✓ SCN⁻, thiocyanate;
- ✓ ClO⁻⁴, perchlorate.





Thyroid hormones degradation

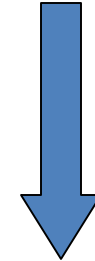




Chemistry of Hormones

- Steroids
 - Small molecules - NO
 - Amino acid derivatives
 - Thyroid hormones
-
- **Catecholamines**
 - Proteins and peptides
 - FA derivatives - eicosanoids

Receptor inside the cell



Surface receptor

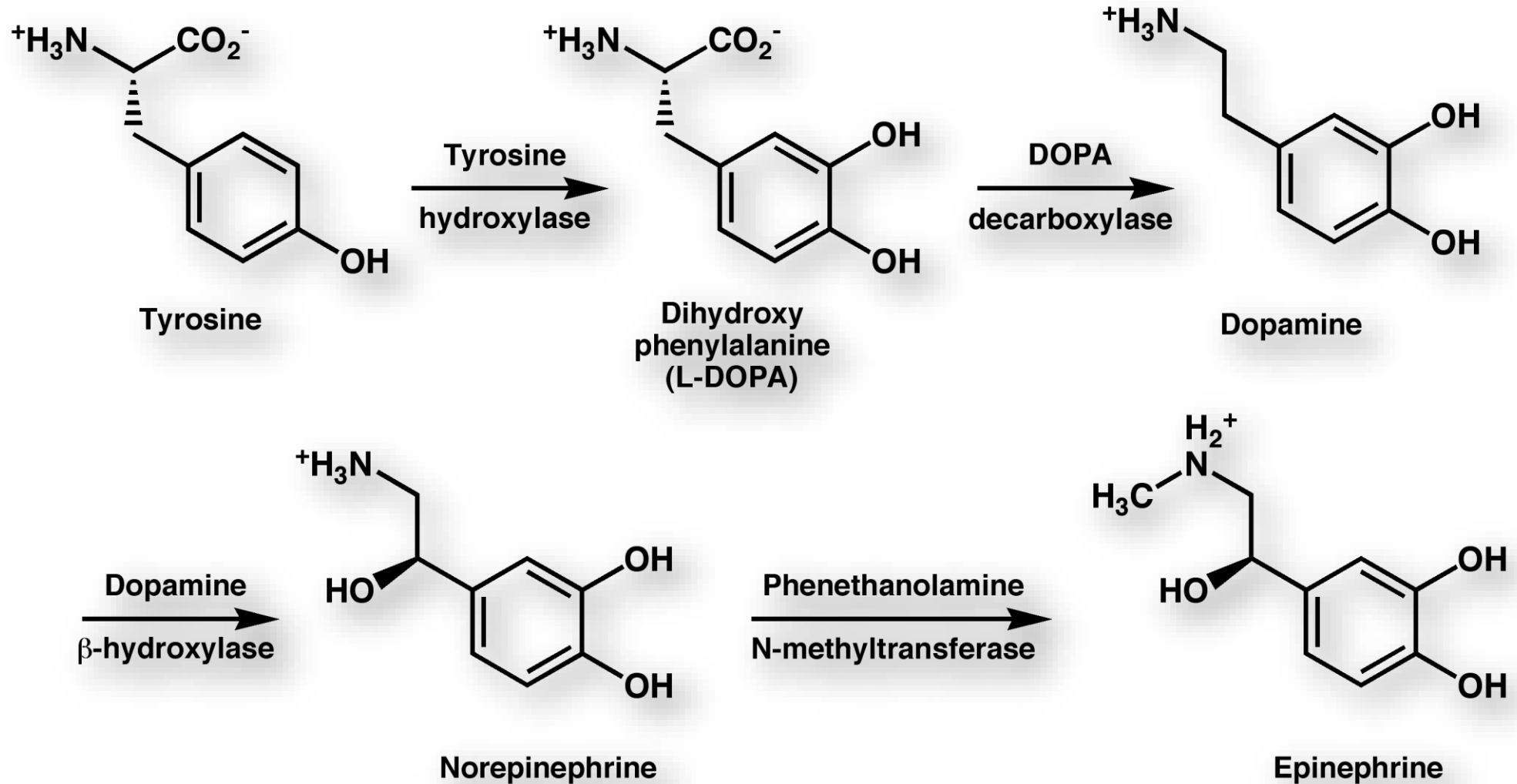


Catecholamine synthesis

- Substrate = Phe or Tyr
- Synthesis located in: adrenal medulla, nerve tissue
- Products:
 - Dopamine, adrenaline (hormones)
 - Noradrenaline (neurotransmitter)

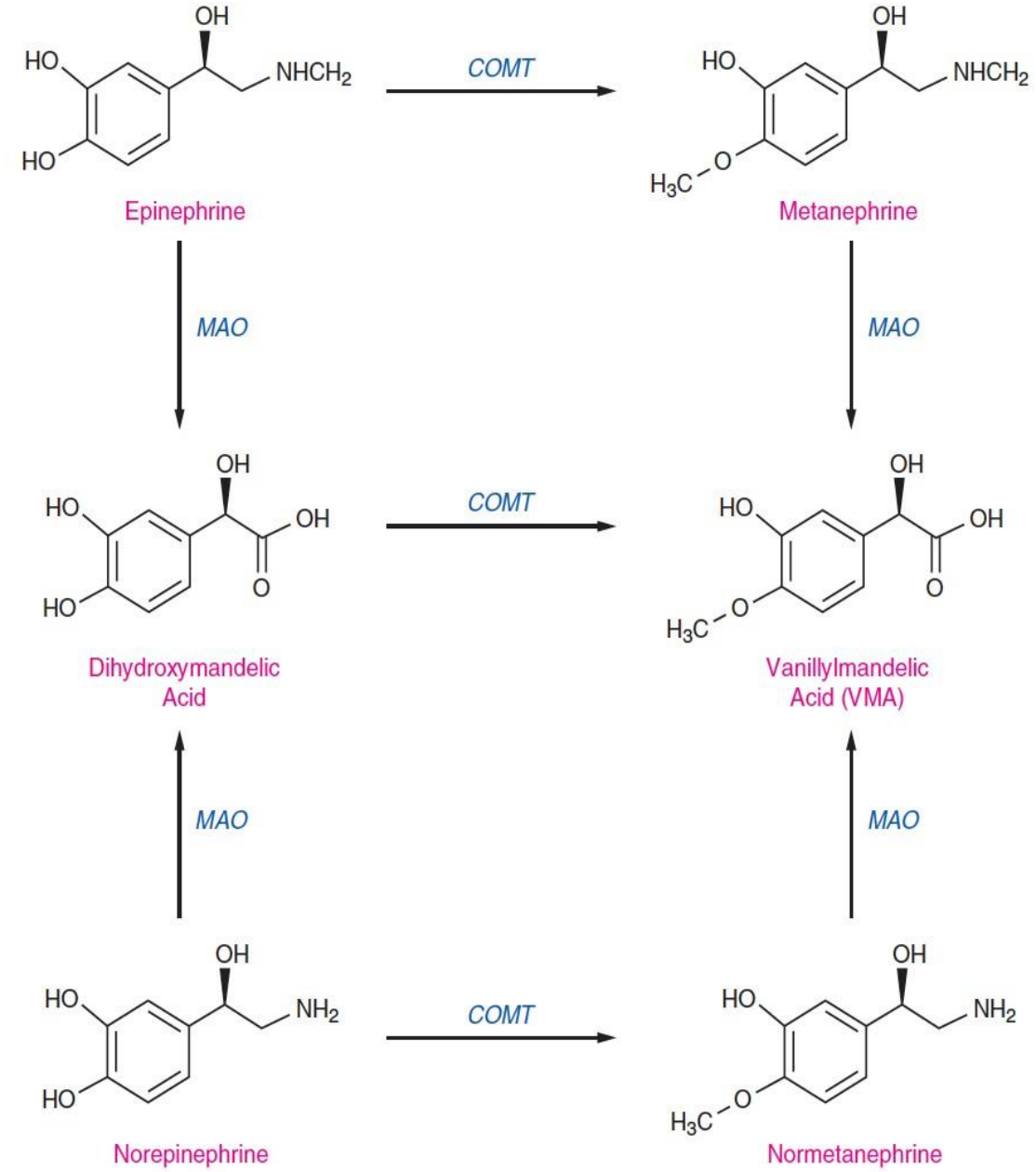
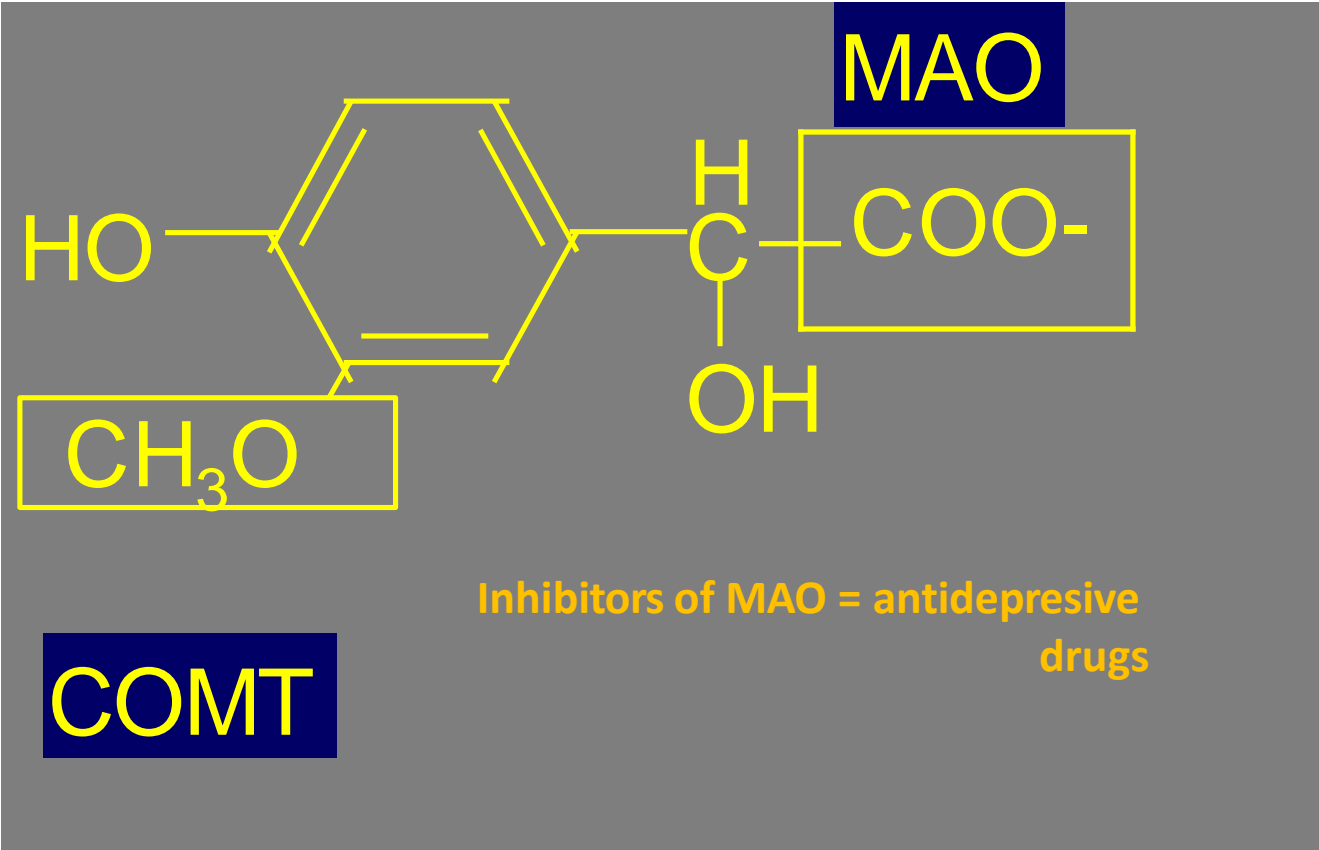


Catecholamine synthesis

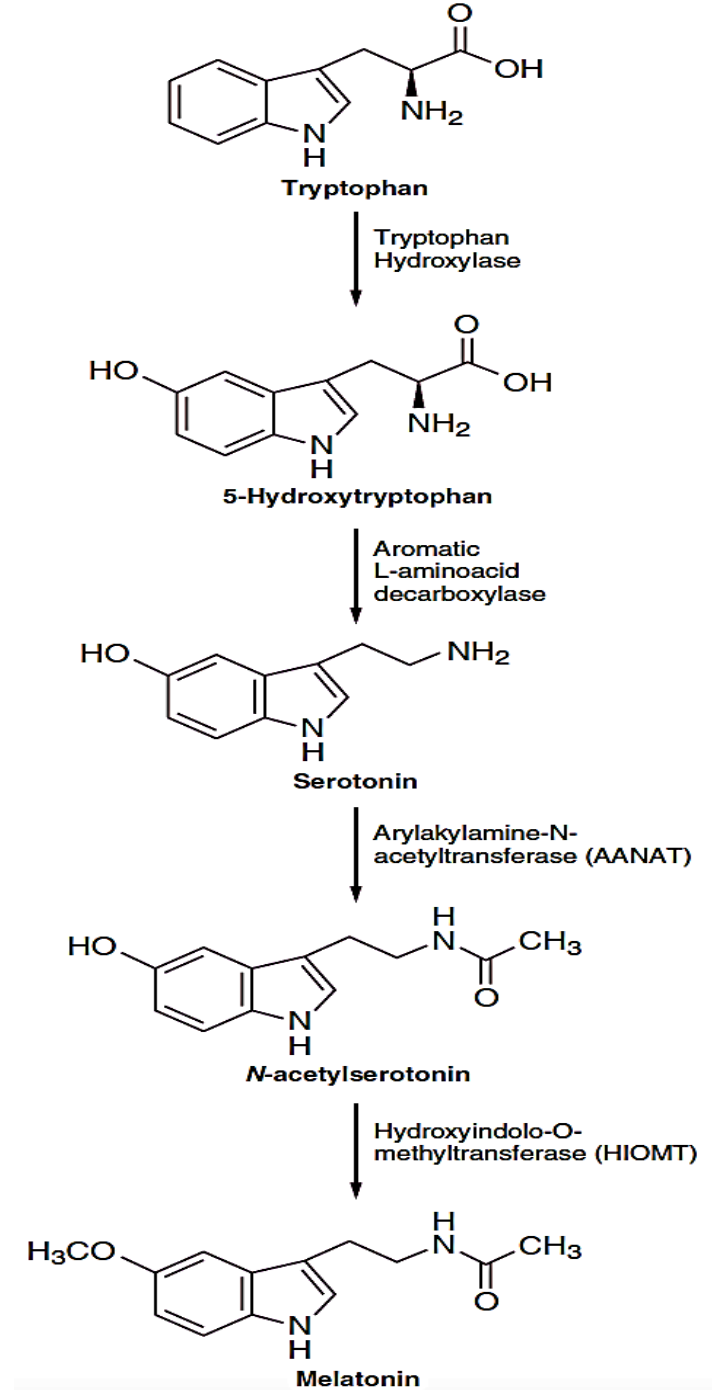
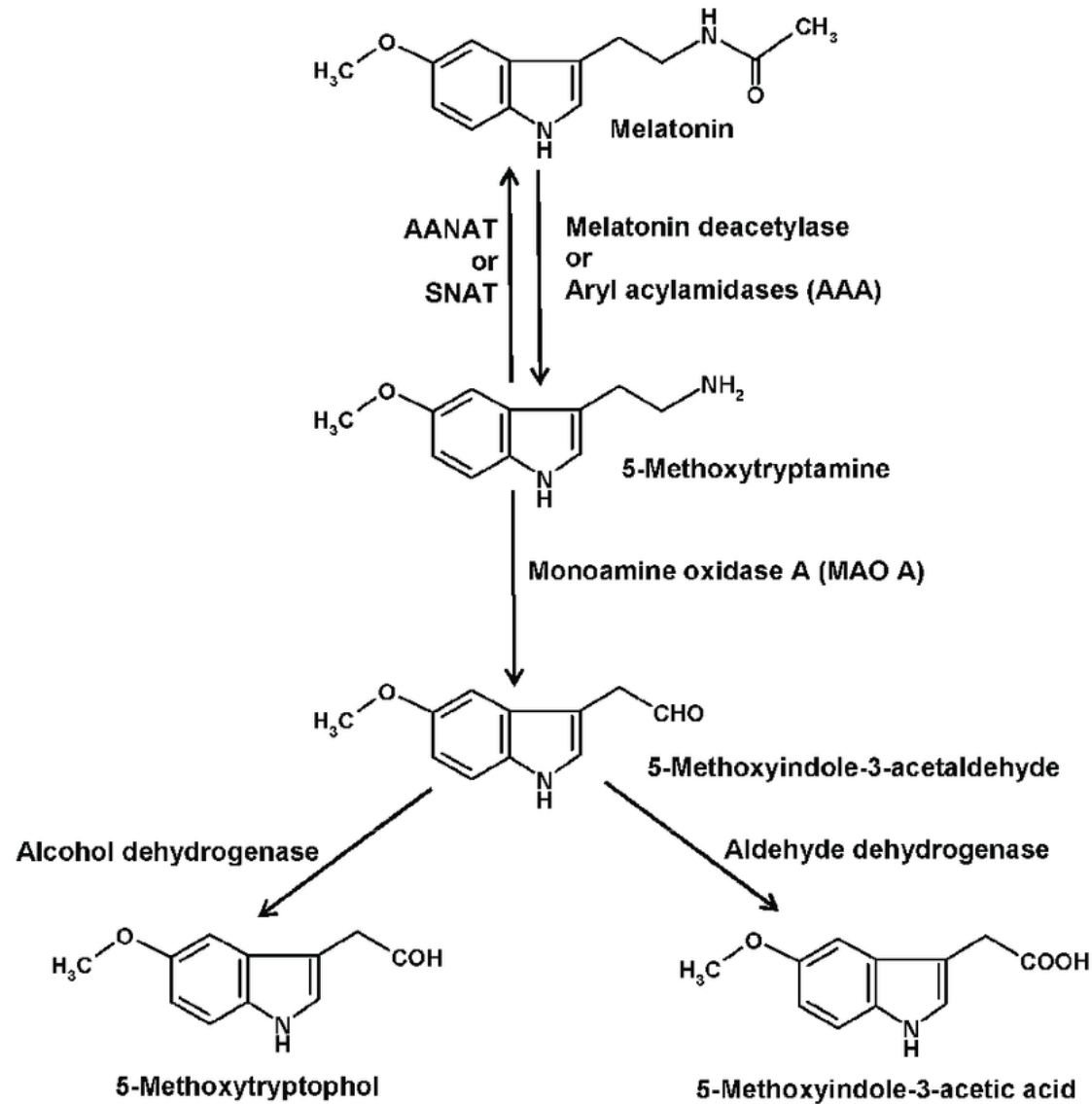




Catecholamine breakdown



Melatonin



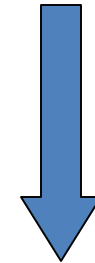


Chemistry of Hormones

- Steroids
- Small molecules - NO
- Amino acid derivatives
 - Thyroid hormones

- Catecholamines
- **Proteins and peptides**
- FA derivatives - eicosanoids

Receptor inside the cell



Surface receptor



Protein and peptide hormones

- CNS mediators: neuropeptides, opioids
- Hypothalamic releasing hormones and pituitary peptides
- Insulin and glucagone
- Growth factors: IGF, CSF, EPO

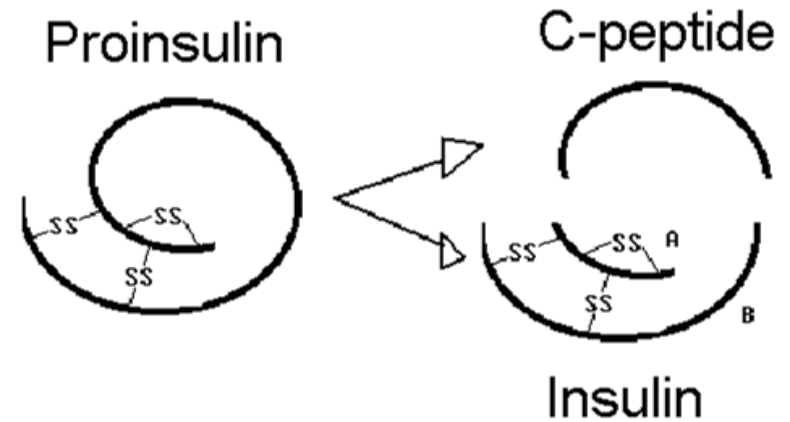
...and many others



General steps of peptide synthesis

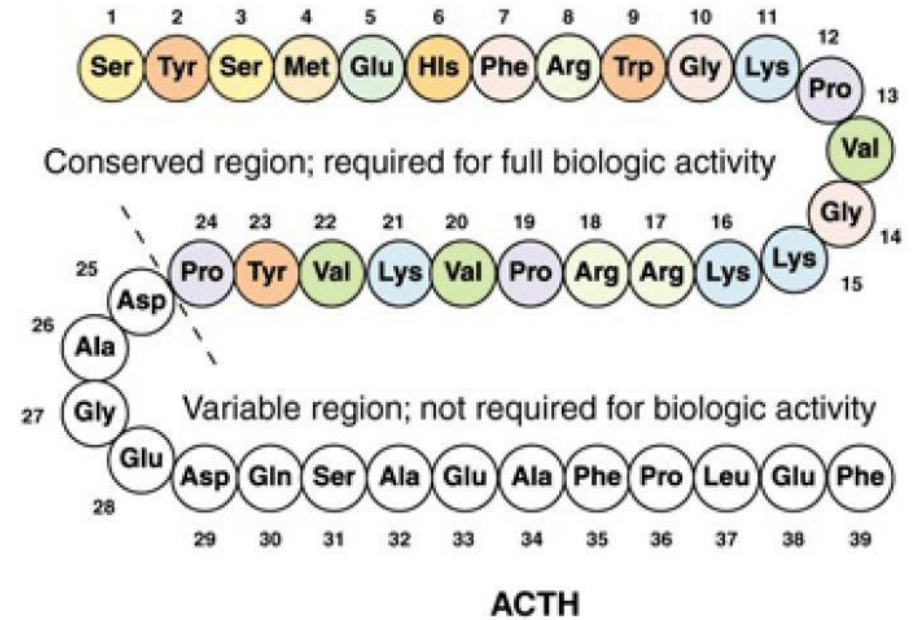
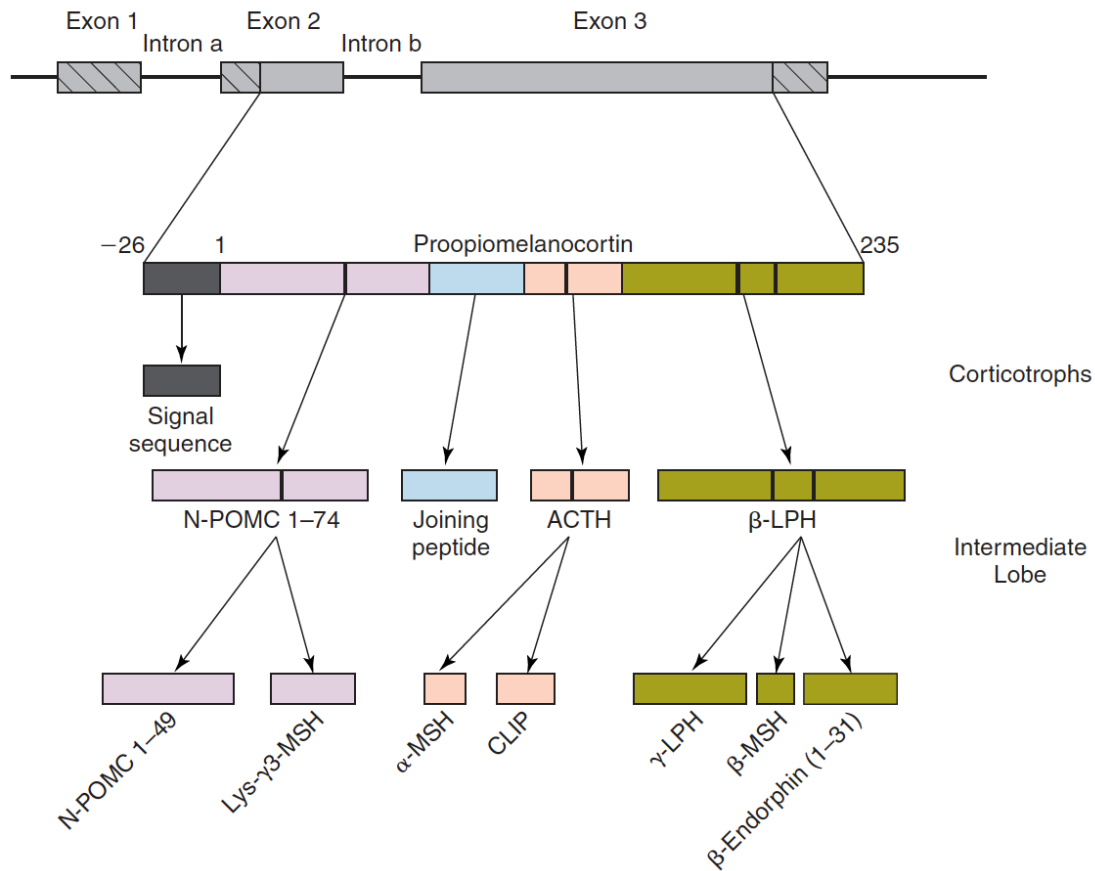
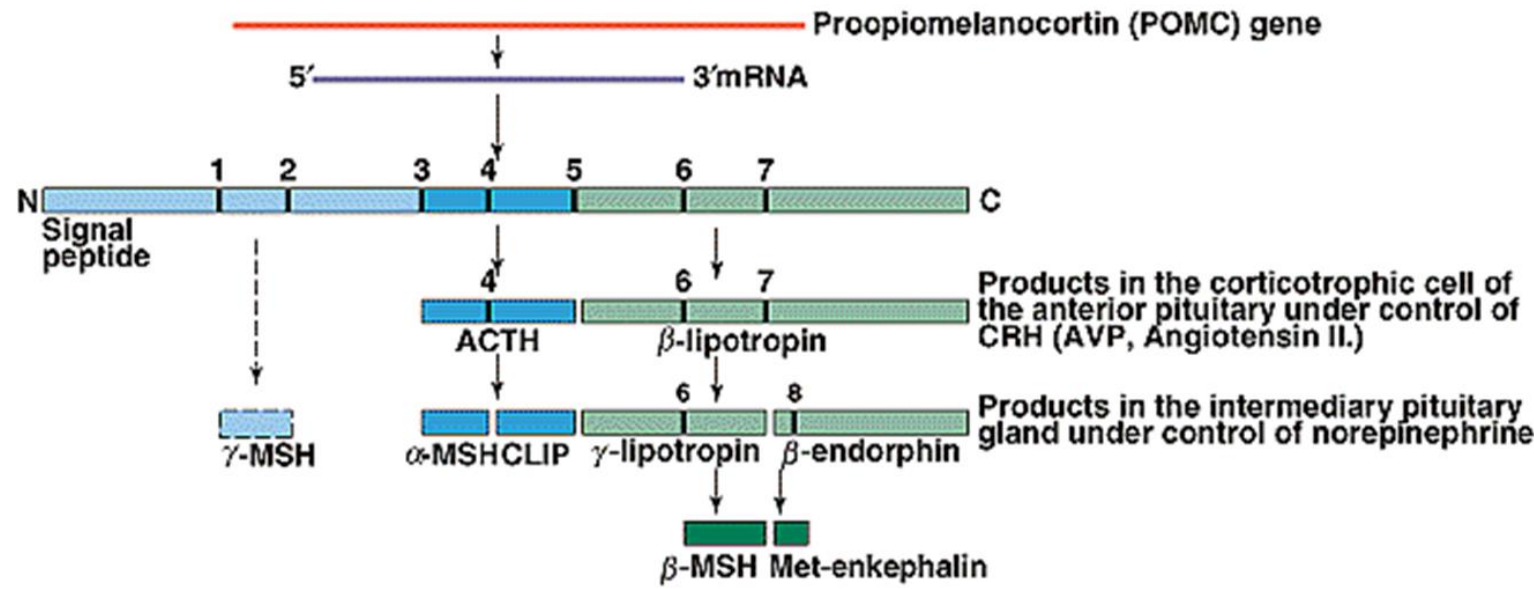
“Precursor Polypeptides”

- Expression of “pre-pro” protein
- Transport to ER
- Splitting the signaling sequence
- Cleavage to definite peptide(s) and final modification in Golgi
 - Proinsulin to insulin
 - Proopiomelanocortine (POMC) to MSH and ACTH





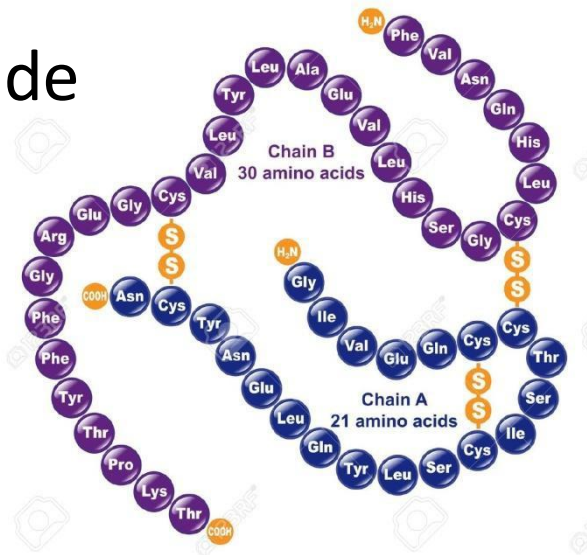
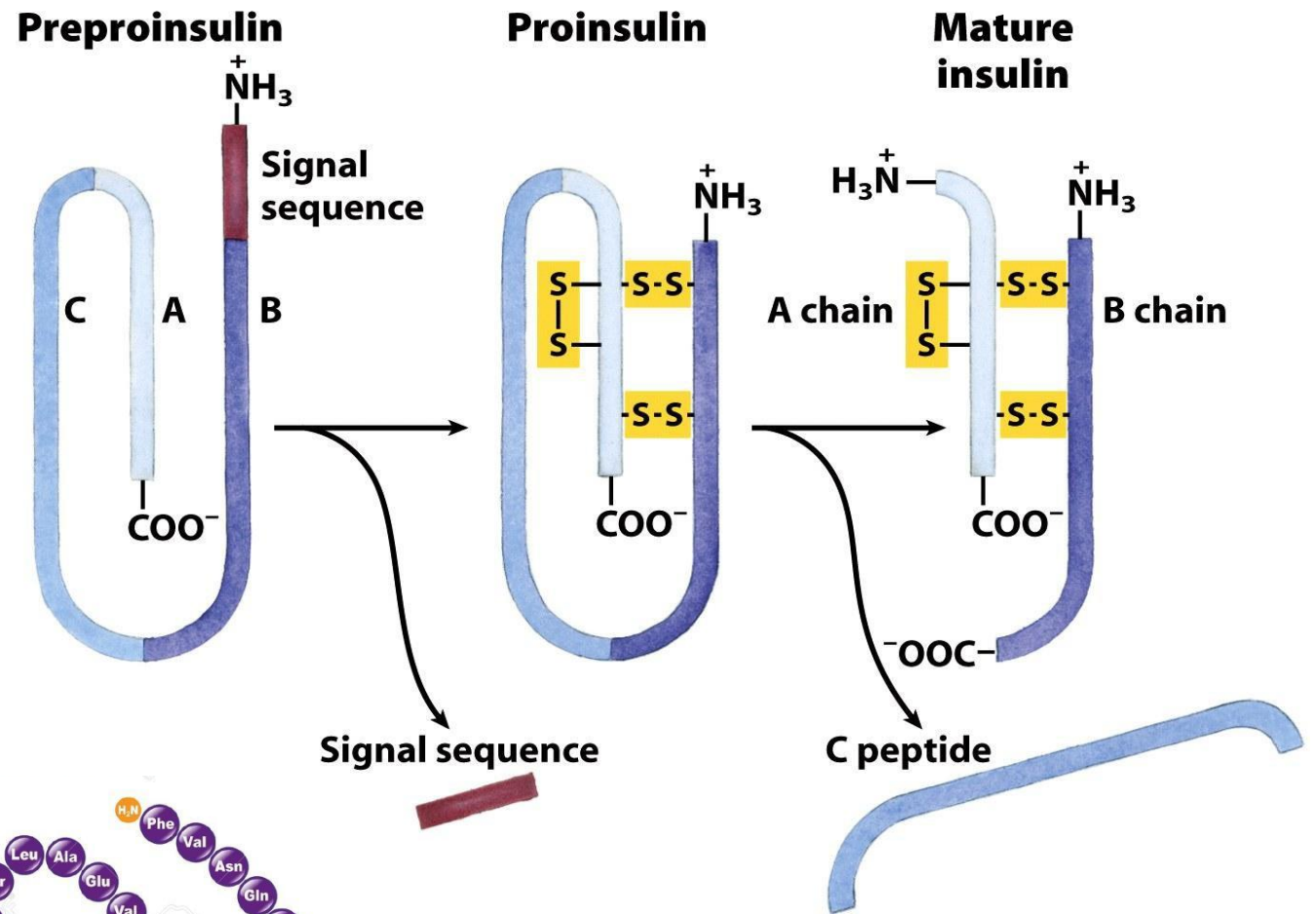
Proopiomelanocortin (POMC)





Insulin

- A larger precursor preproinsulin
 - ✓ 23 aa signal sequence
 - ✓ 3 disulfide bonds
- Proinsulin
 - ✓ Removal of the C peptide
- Mature insulin
 - ✓ A and B chains





Degradation of peptide hormones

- Lysosomal after endocytosis of complex hormone-receptor
- Chemical modification (liver): rearrangement of S-S bridges, cleavage
- Renal excretion of small peptides



Target cell interactive effects

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



Target cell interactive effects

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

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



Detection and generation of cellular response

Transduction of Hormonal Signal



Signal Transduction

- Transduction: conversion of one form of a signal to another so as cells can produce many kinds of responses in different ways
- Amplification is a **MUST**
- Signal (polar, large) should bind receptors:
 - Intrinsic not integral!
 - Transmembrane
 - Intra- & extracellular domains
- Is that enough? The need for 2nd messenger
 - **Few in number**
 - **Restricted movement**



Second Messengers

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



Signal Termination

- **Is it important?**
 - Keeps cells responsive to new signals
 - Failure of termination may cause problem e.g GH & cancer
- How it is achieved?
 - Degradation of the second messenger
 - Dephosphorylation by hydrolysis

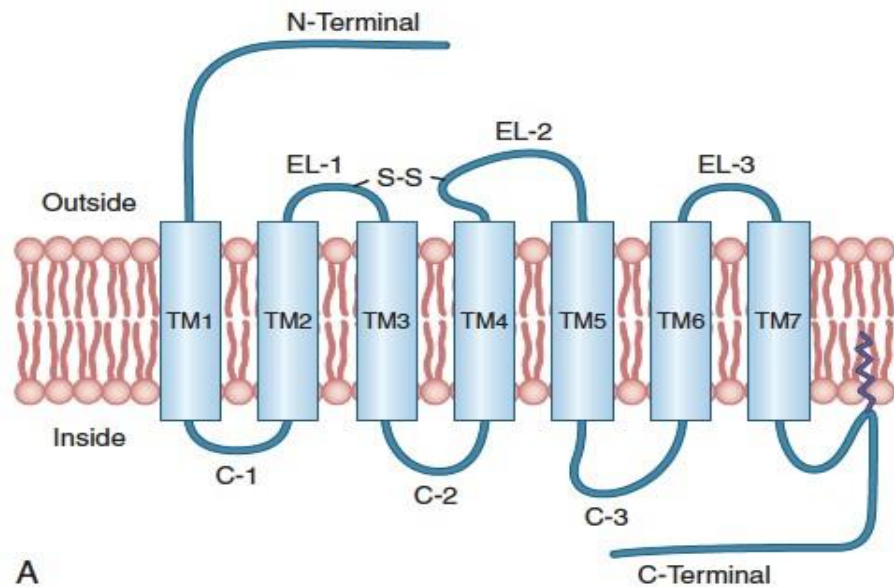


Membrane Associated Receptors

7-Transmembrane Helix Receptors (7TM)

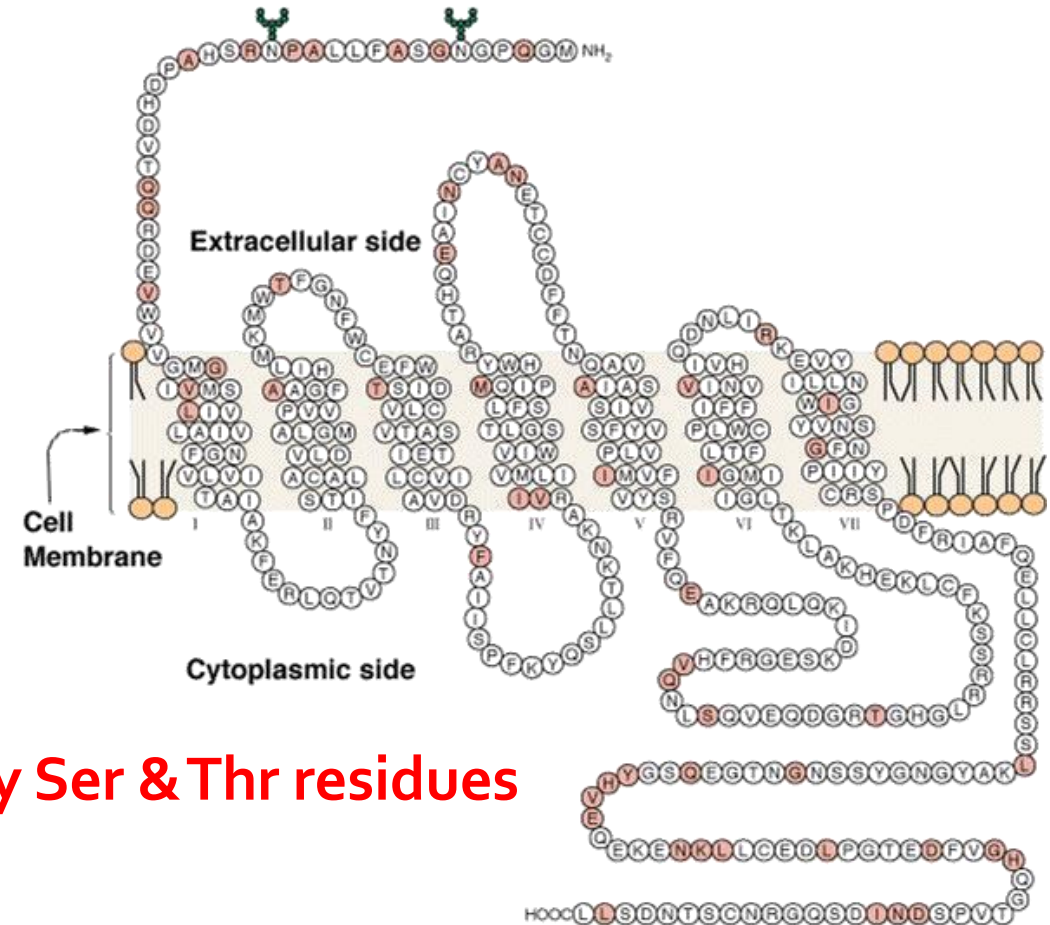


- ✓ 7 α -helices: H-bonding, rigid, hydrophobic
- ✓ Signal induces **conformational changes**
- ✓ Is it enough?



A

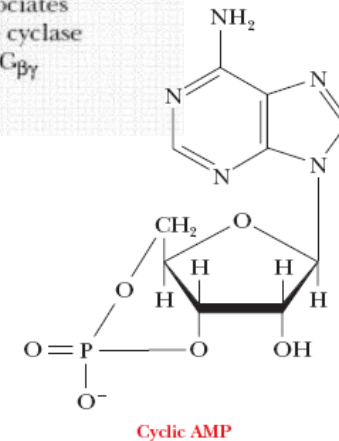
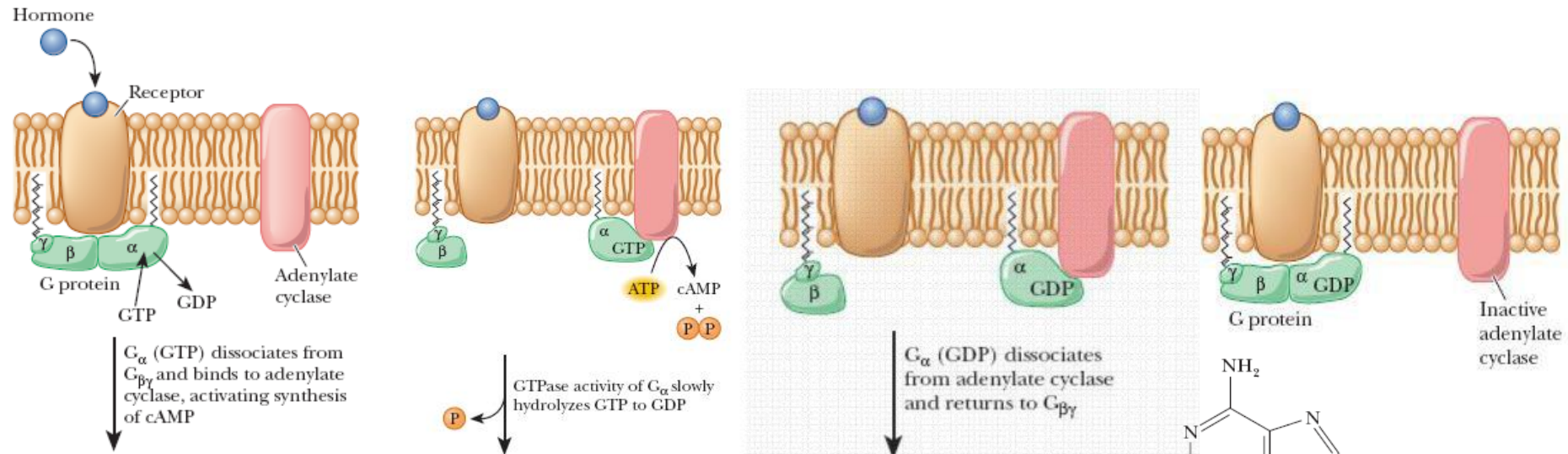
Rhodopsin receptor



■ Many Ser & Thr residues



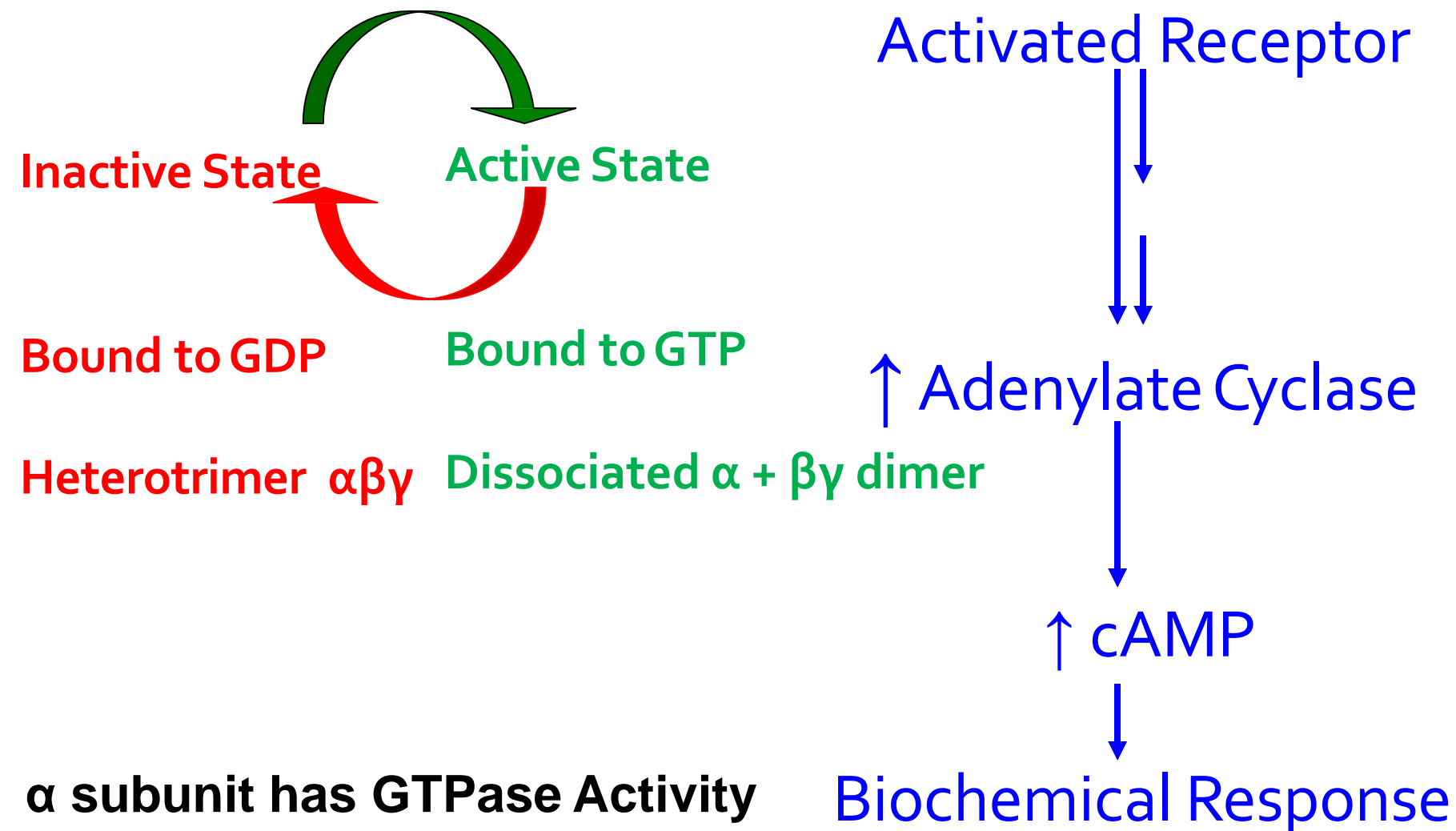
G-Proteins & cAMP



- cAMP: small & heat stable
- Plasma membrane
- Hormone → Specific receptor (β 1- or β 2-adrenergic receptor) → G protein → Adenylate cyclase → cAMP → protein kinase A → phosphorylation

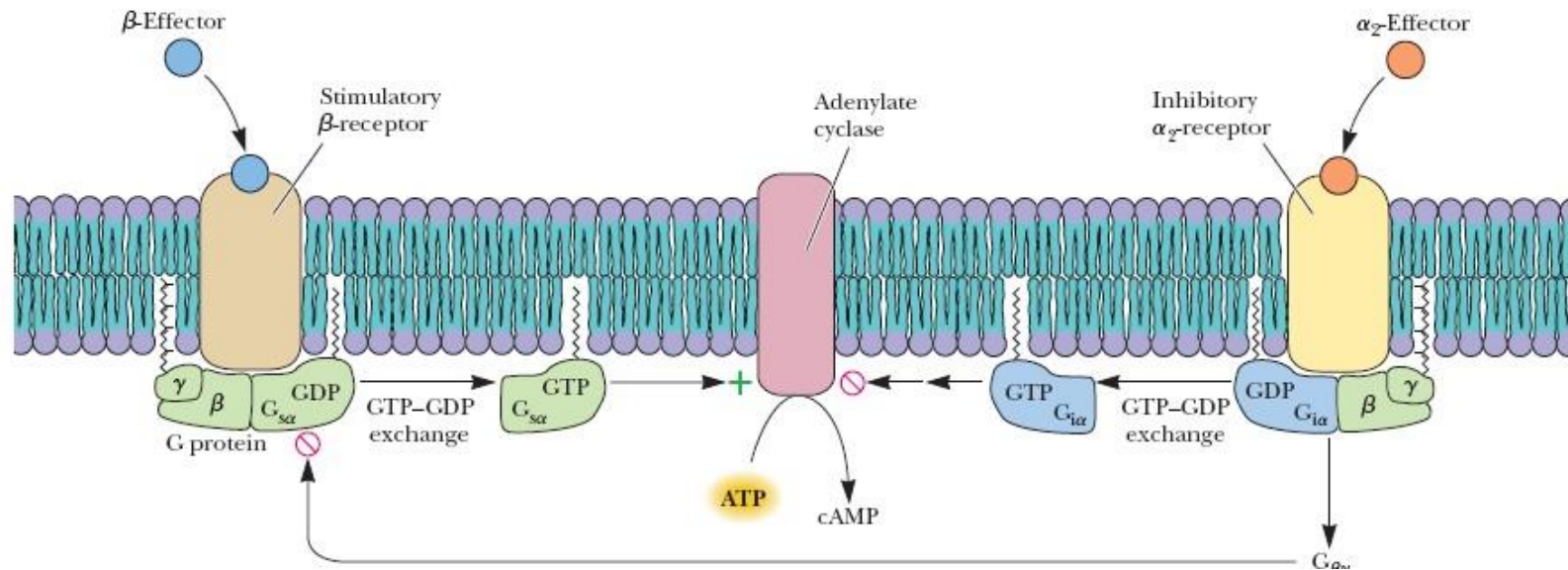


G-proteins cycles between two forms





G-proteins: stimulatory or inhibitory?



➤ Cyclic AMP & G Proteins:

- ✓ Hormone \rightarrow receptor (α_2 -receptor) \rightarrow G protein \rightarrow inhibits adenylate cyclase



G-Proteins

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



G-Proteins

- α and γ Subunits have covalently attached fatty acid
- α and $\beta\gamma$ can interact with other proteins
- All 7TM receptors appear to be coupled to G proteins
 - GPCRs
- Amplification: receptor \rightarrow 100's of G protein \rightarrow 100's of adenylate cyclase \rightarrow 100's X 1000's molecules/sec of cAMP

Signal Transduction

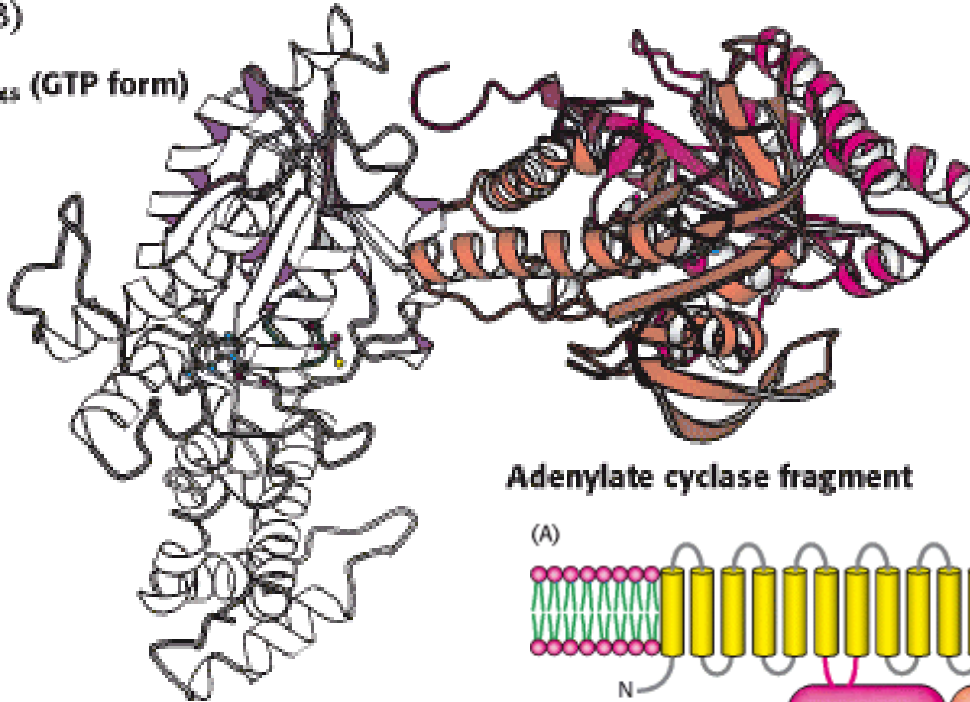
© Sinauer Associates, Inc.



Adenylate Cyclase

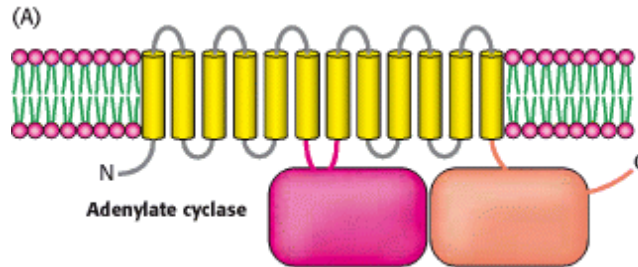
(B)

$G_{\alpha s}$ (GTP form)

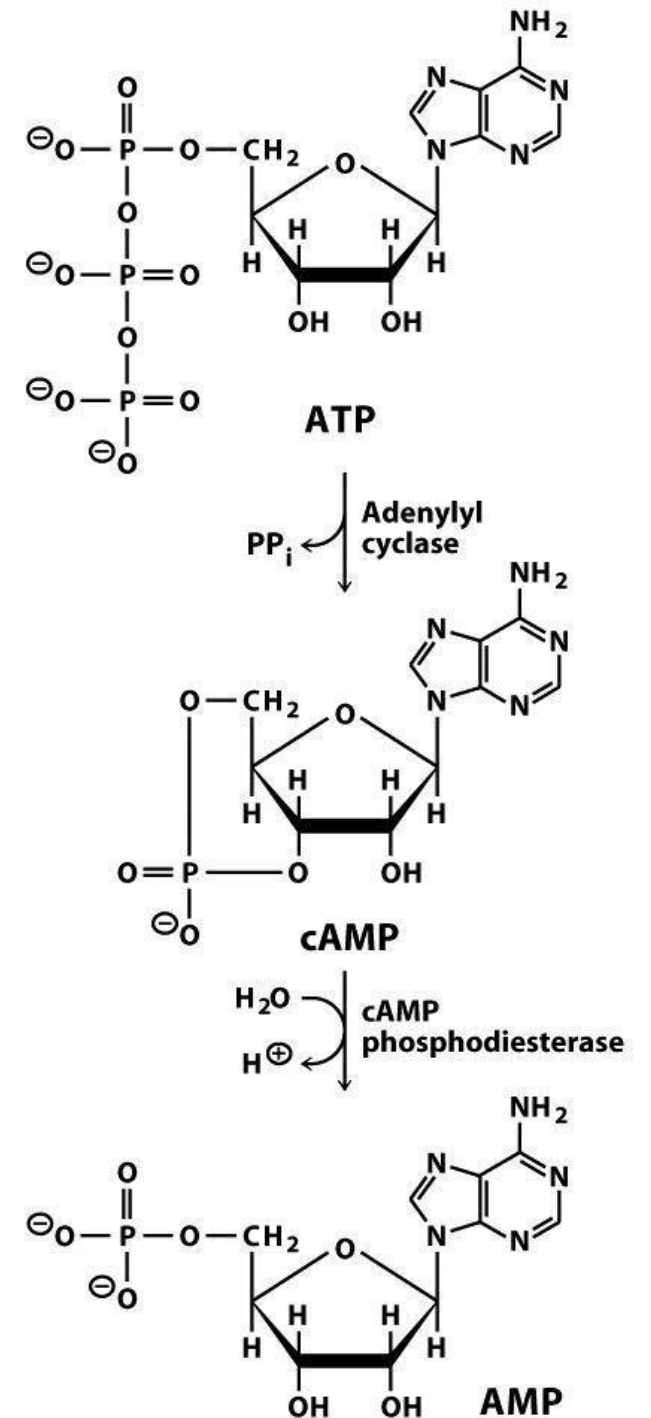


Adenylate cyclase fragment

3'5' cAMP



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



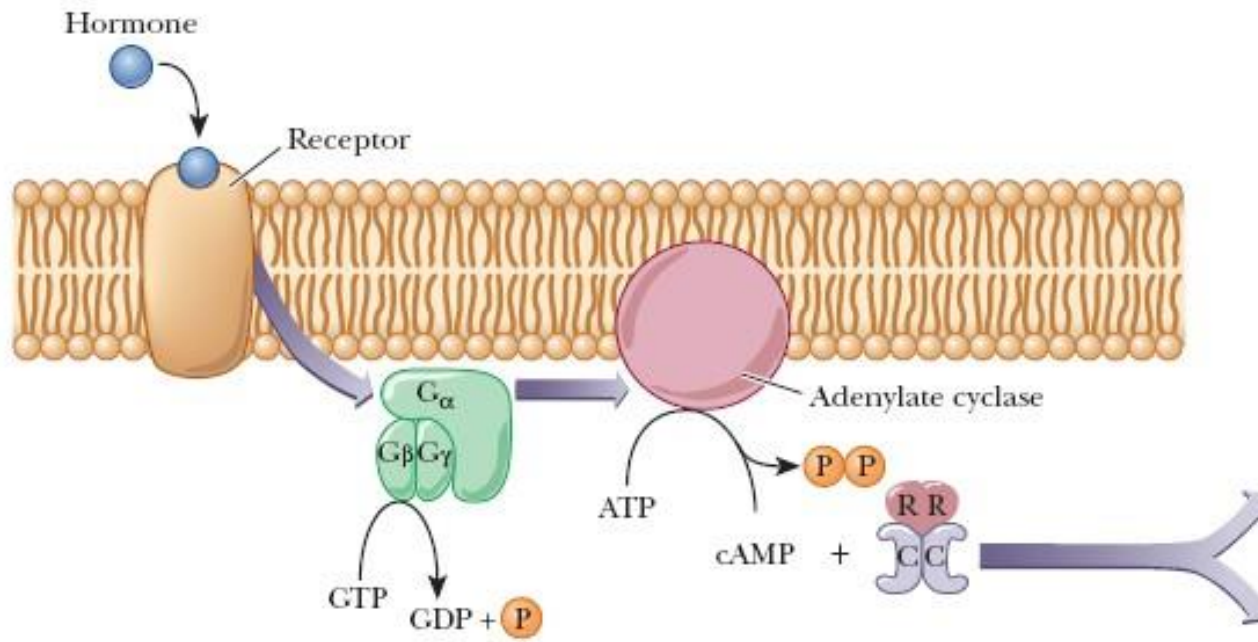


cAMP can affect a wide range of cellular processes

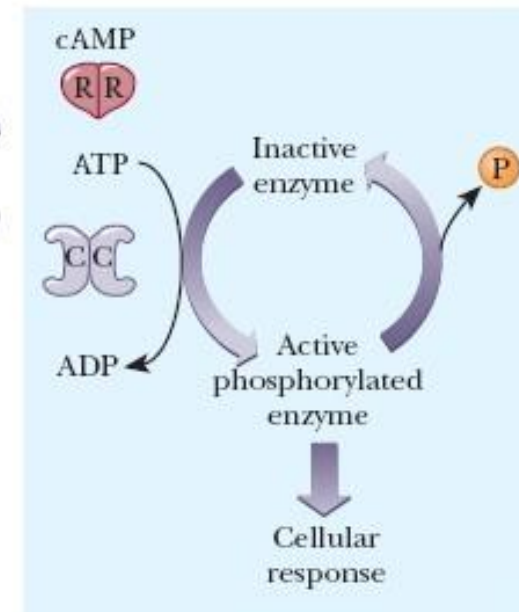
- ↑ degradation of storage fuels
- ↑ **secretion of acid by gastric mucosa (caffeine: phosphodiesterase & adenosine)**
- Dispersion of melanin pigment granules
- ↓ aggregation of blood platelets
- Opening of chloride channels



Then What?



Usually:
Ser or Thr



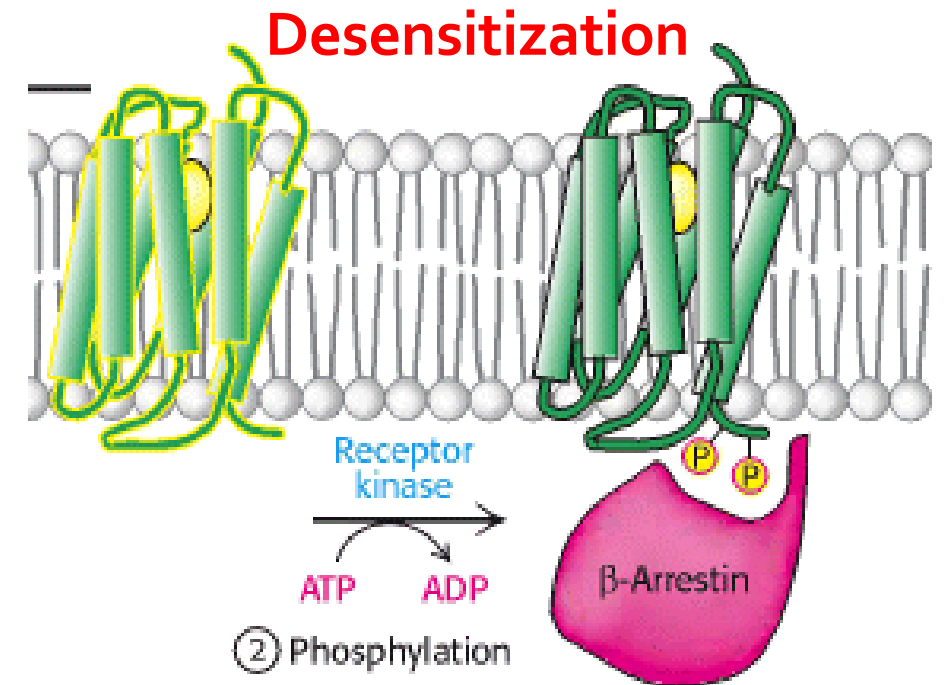
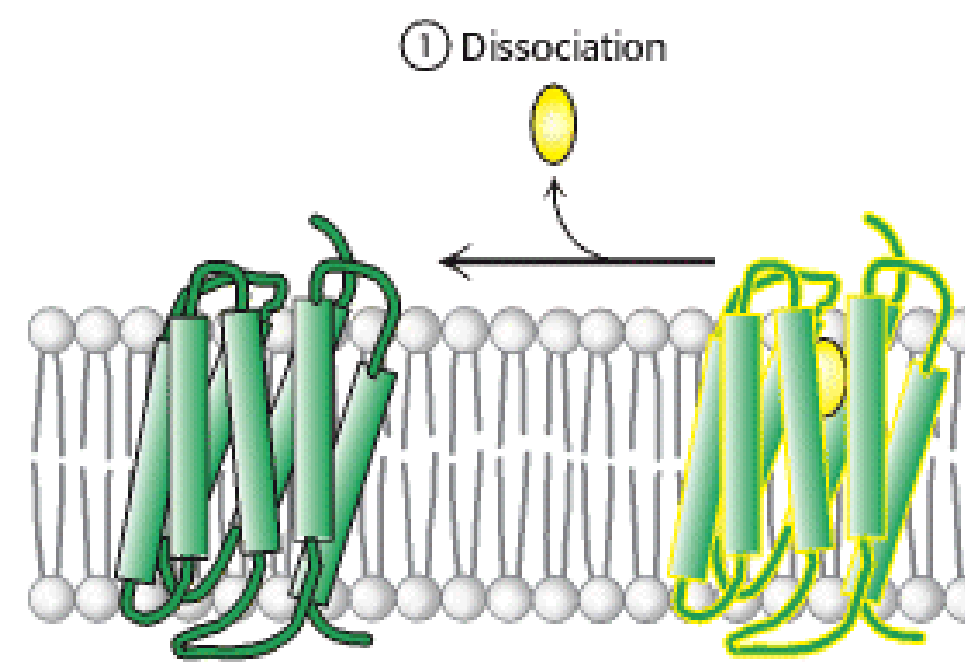
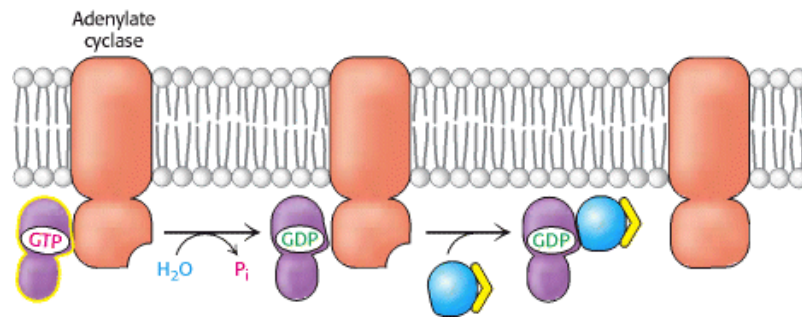
Signal Amplification

Glycogen
Synthase!!



Switching off the signal

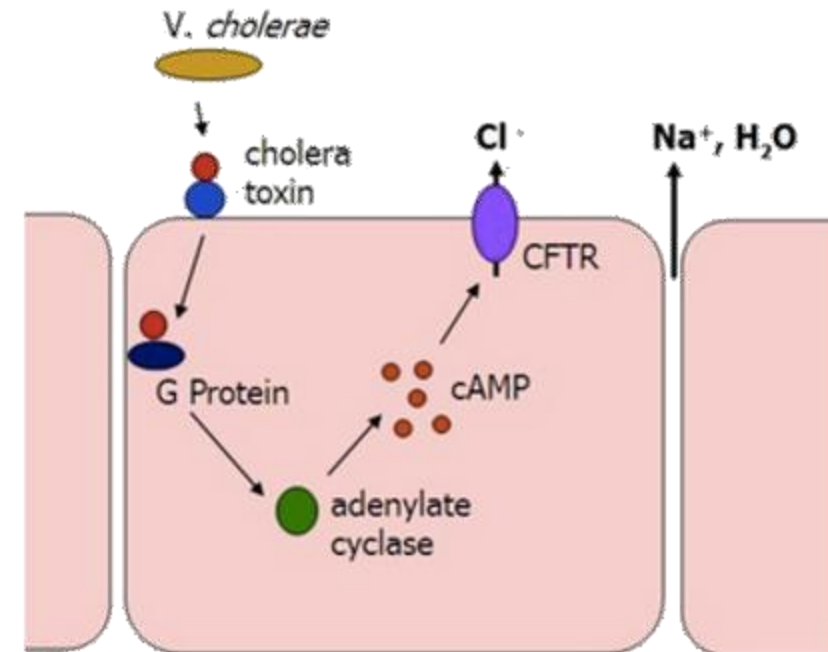
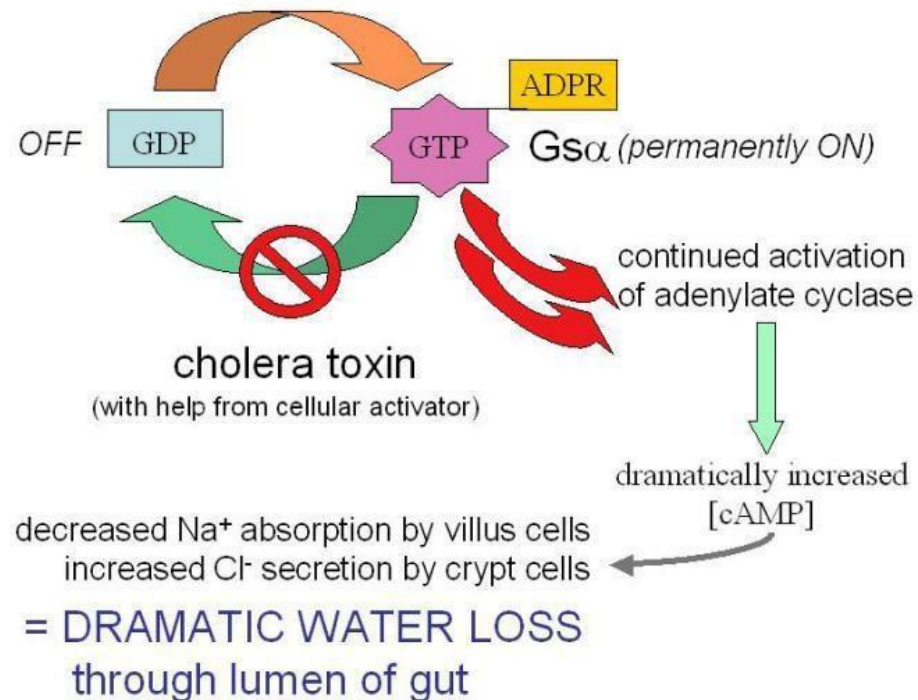
- **Dissociation** of the hormone
- **GTPase** activity of $G\alpha$ subunit
- **Hydrolysis** of cAMP (phosphodiesterase)
- Phosphorylation of the hormone bound-receptor followed by binding to **β -Arrestin**





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

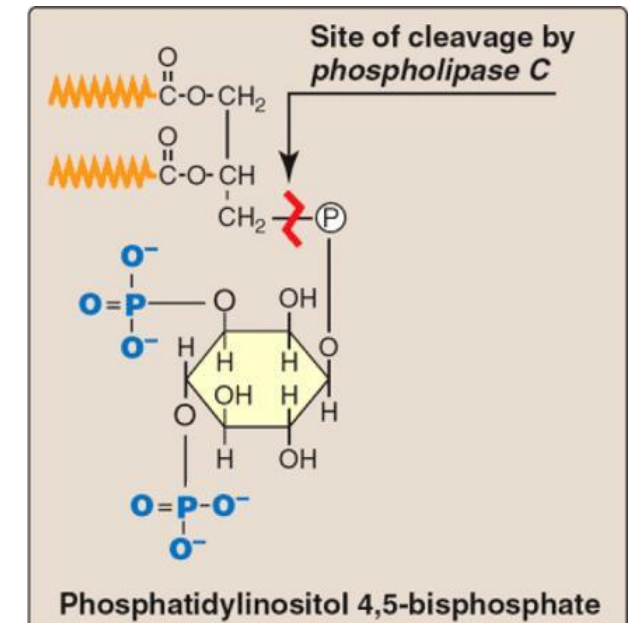
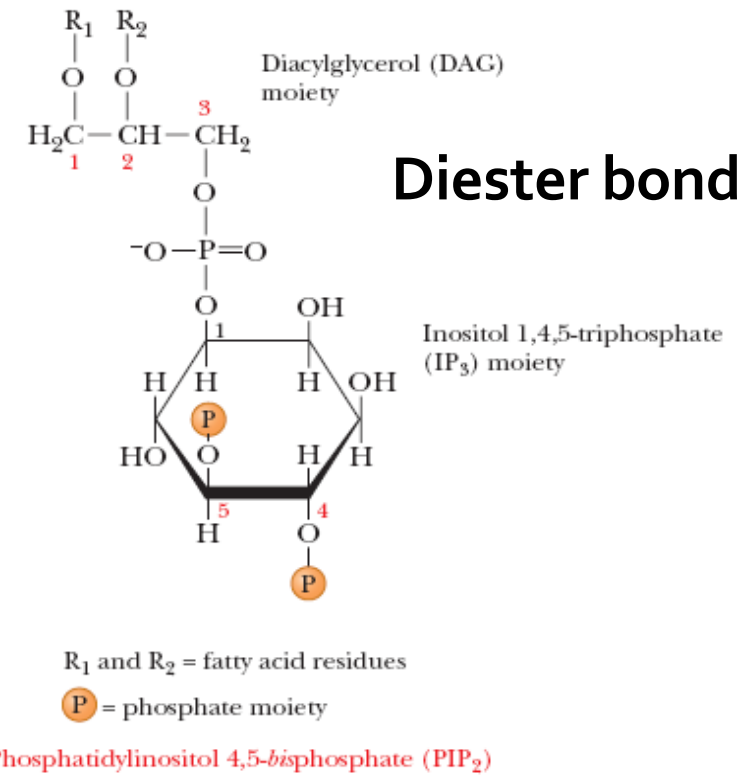
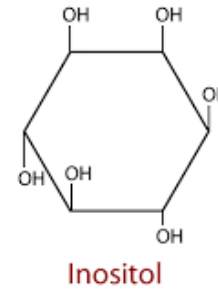
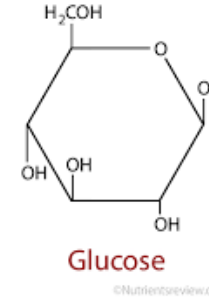


Activation of G Protein



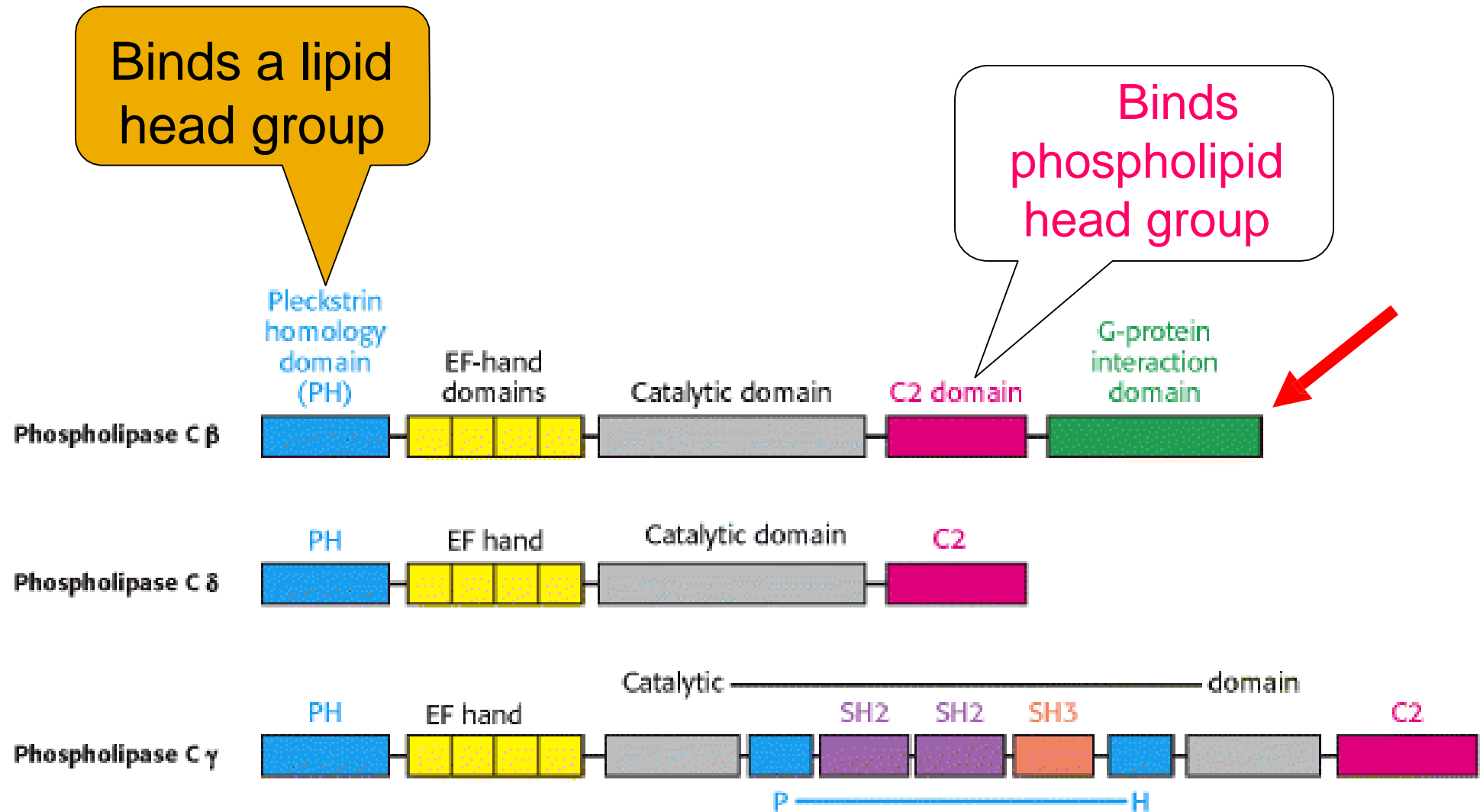
Activation of Phospholipase C (many isoforms)
– PIP₂

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





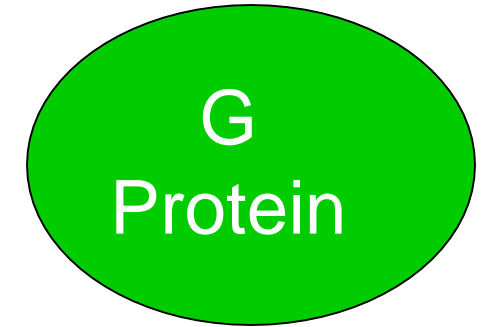
The domain structures of three isoforms of Phospholipase C

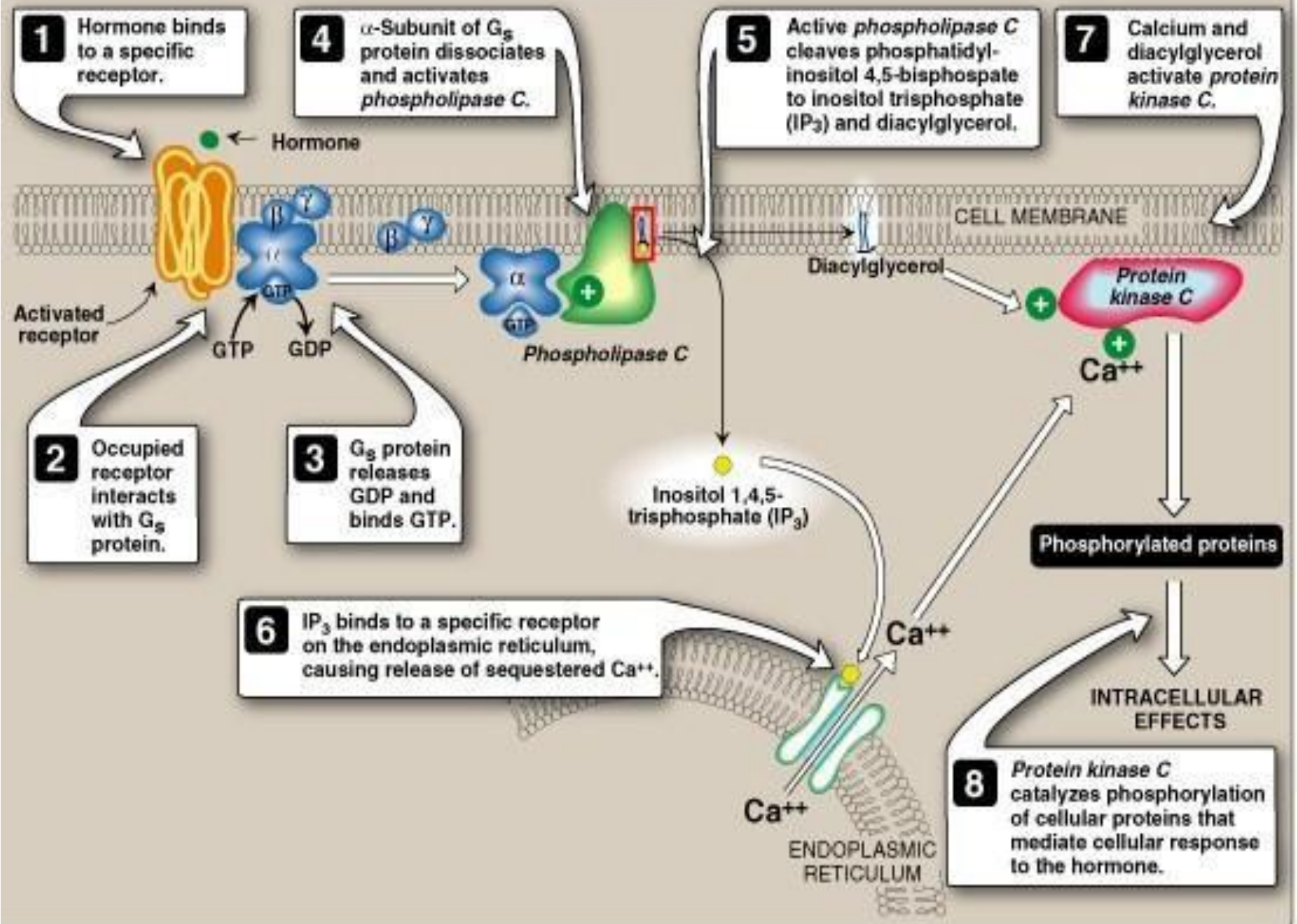




Binding of a G protein brings the enzyme into a catalytically active form

Membrane







Effects of Second Messengers

Inositol trisphosphate (IP₃)

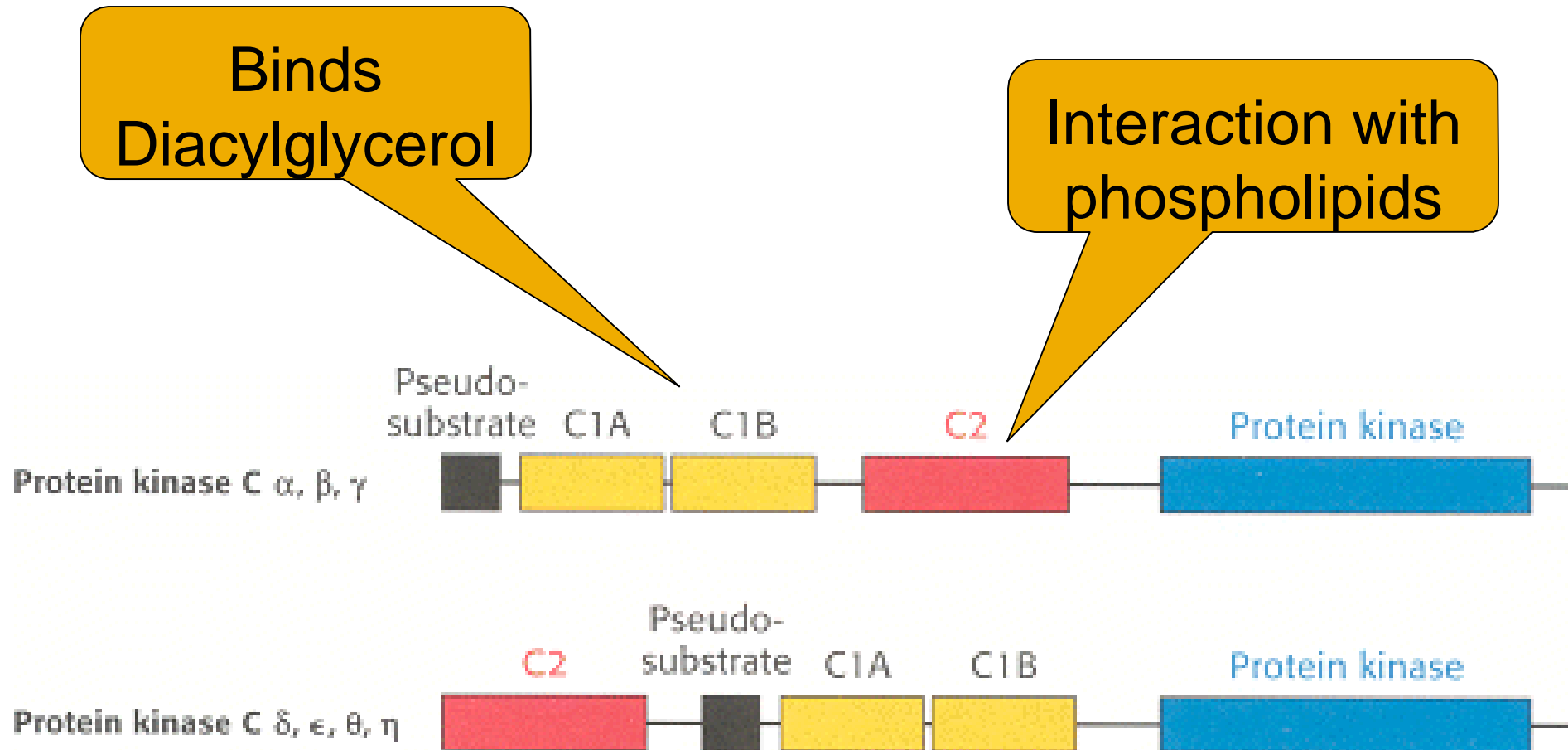
- ✓ Opens Calcium Channels
- ✓ Binding to IP₃-gated Channel
- ✓ Cooperative binding (sigmoidal)

Diacylglycerol (DAG)

- ✓ Activates Protein Kinase C
- ✓ Ca²⁺ is required
- ✓ Phosphorylation of many target proteins

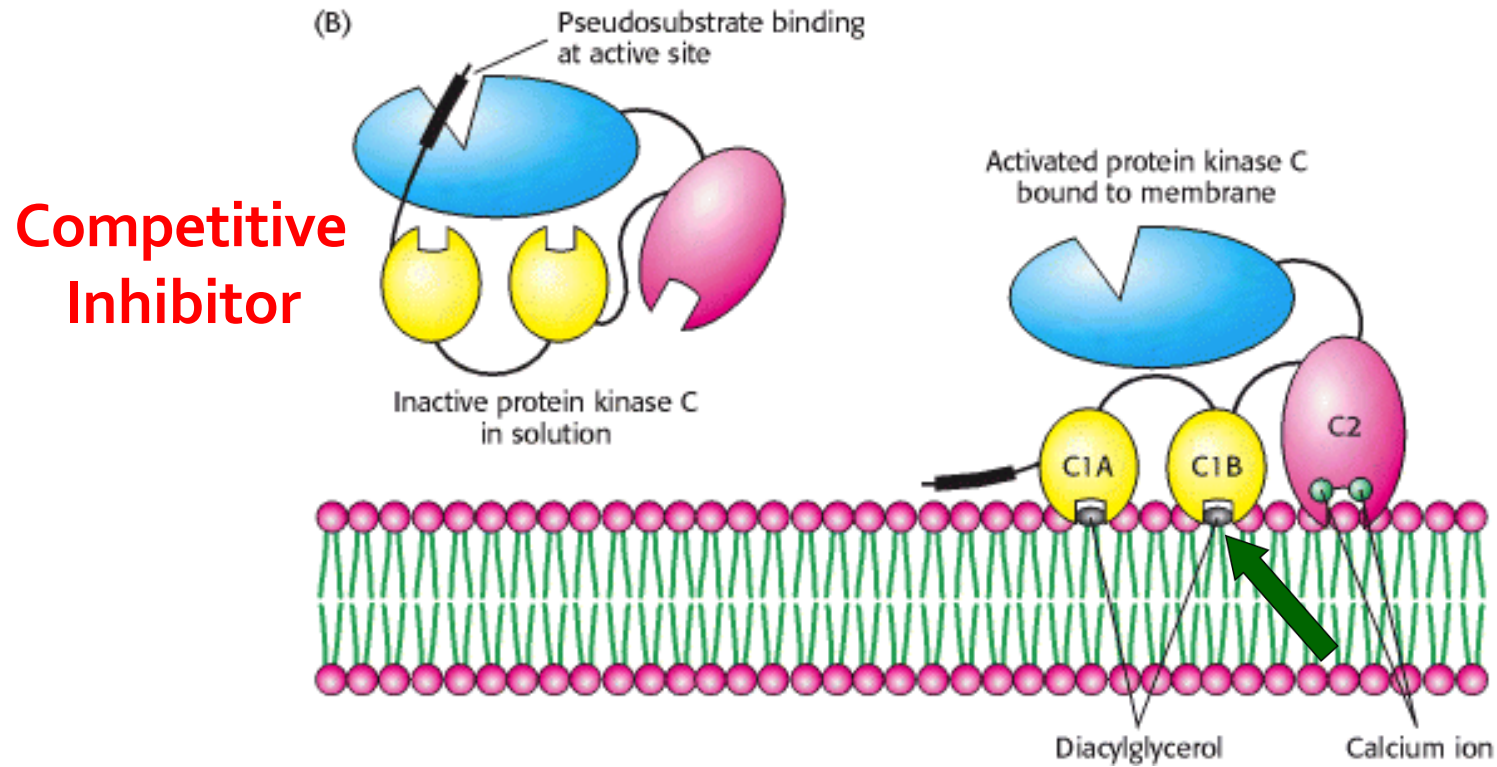


The domain structures of protein kinase C isoforms





Pseudosubstrate Sequence

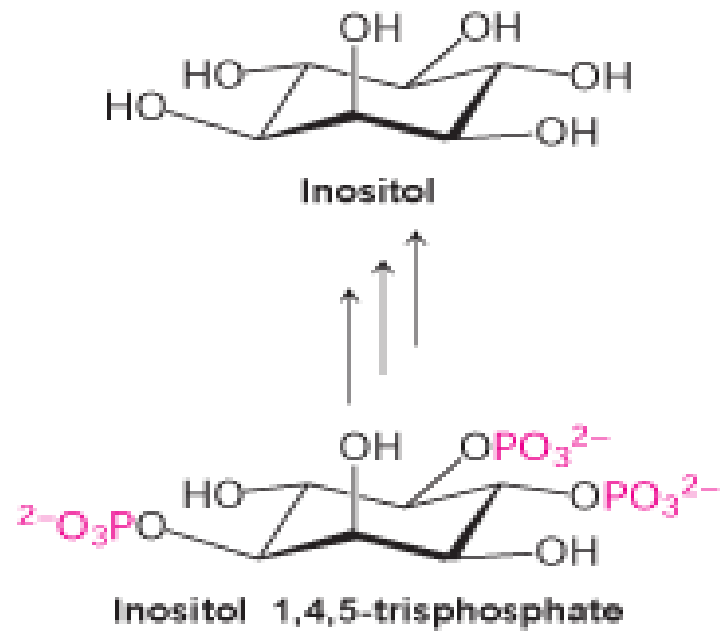


- Resembles the substrate sequence: A-R-K-G-A-L-R-Q-K
- Substrate Sequence: (S,T)
- Binds to the Enzyme's Active Site

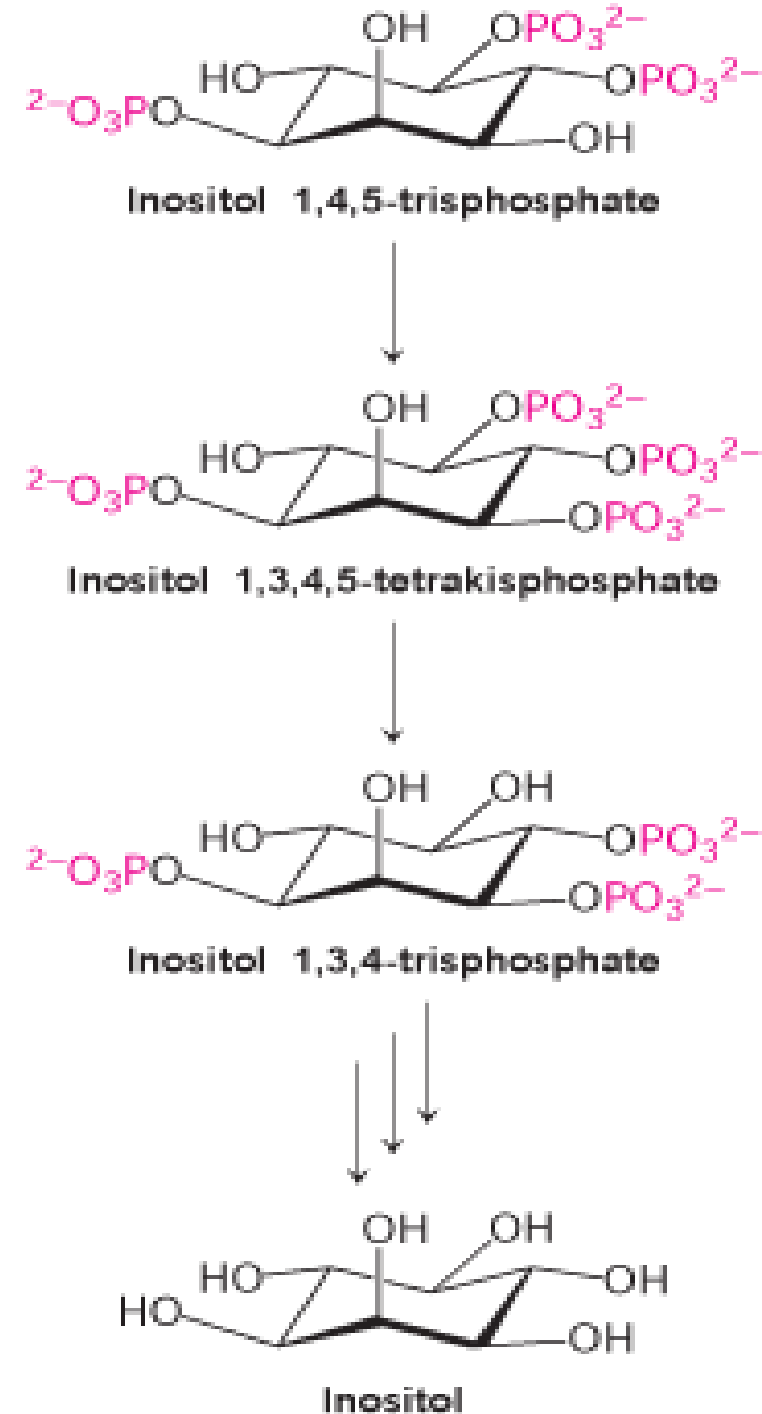


Termination of IP₃ Signal

IP₃ is a Short-Lived Messenger



Lithium ions,
used to treat
some
psychological
disorders
Inhibits IP₃
recycling

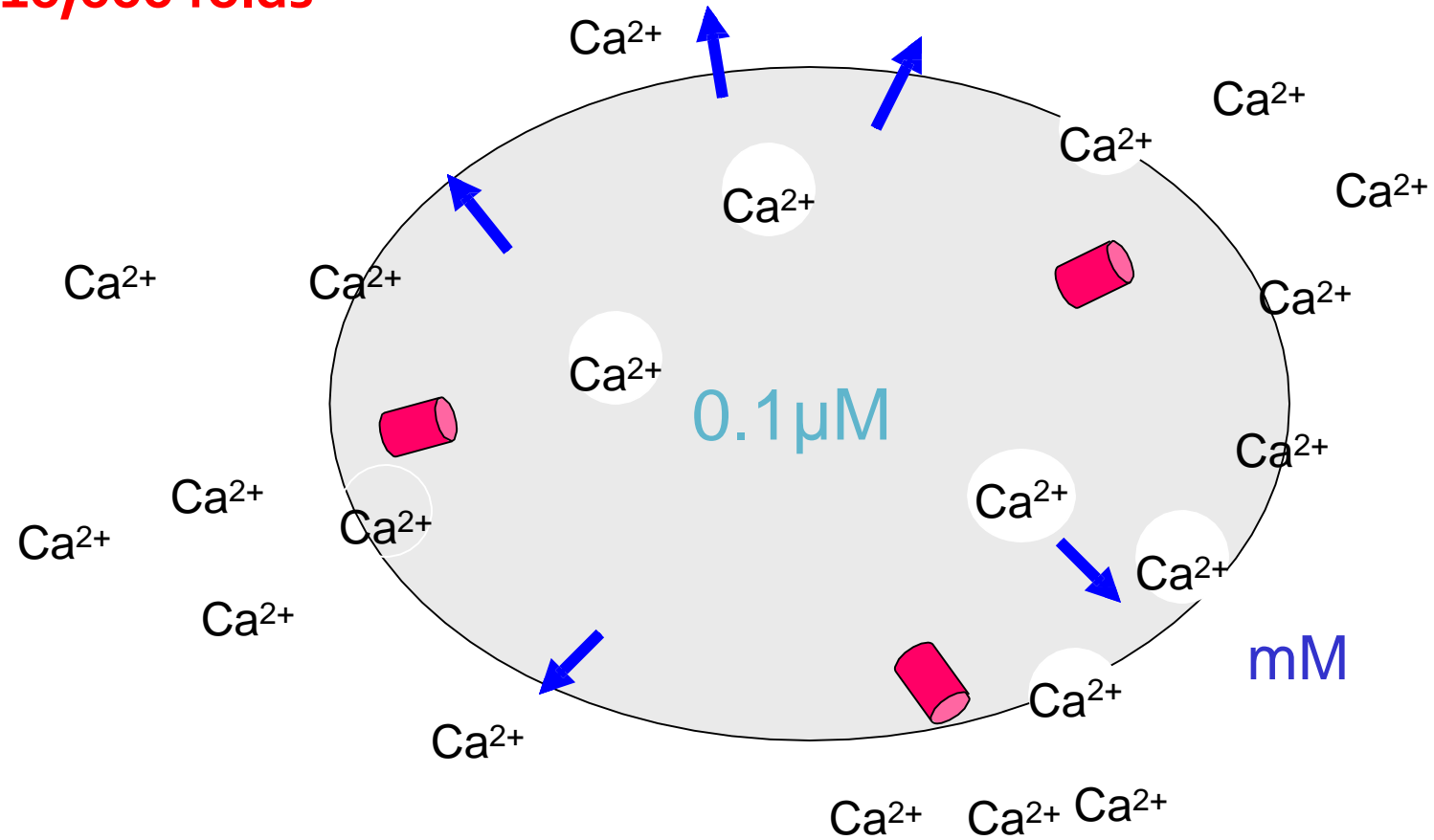




Why Ca^{2+} ?

A large difference in concentration

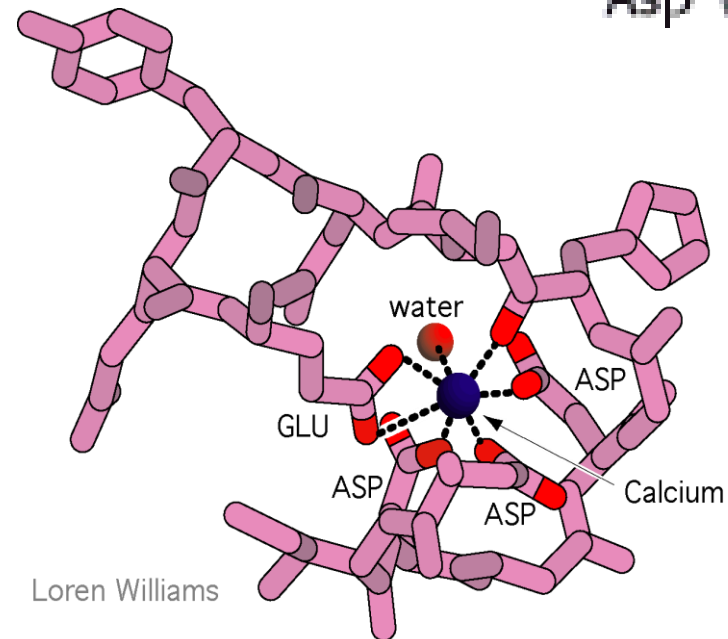
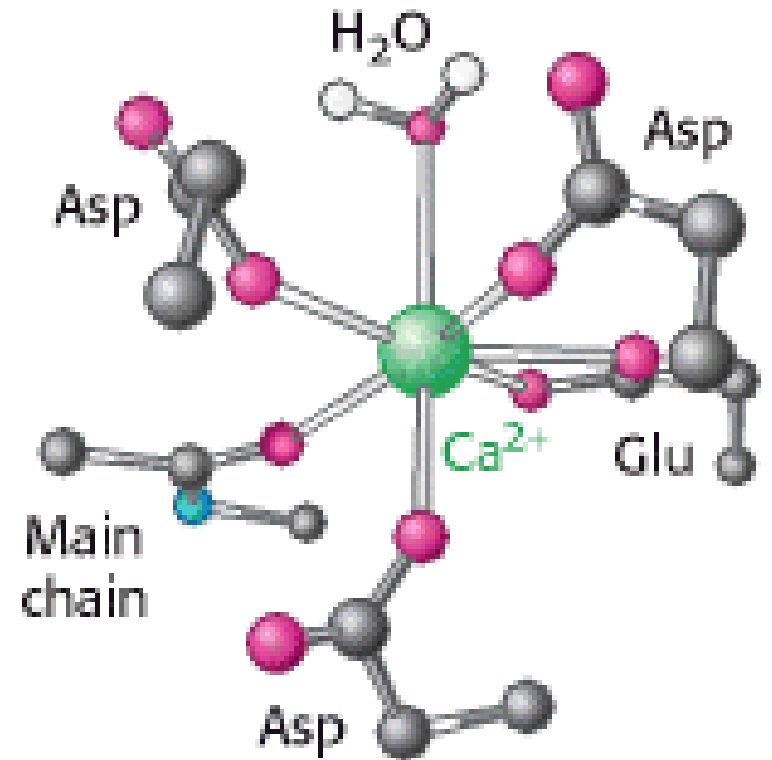
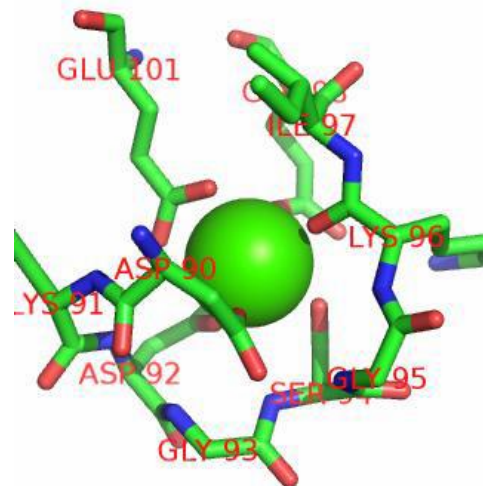
10,000 folds





Why Ca^{2+} ?

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



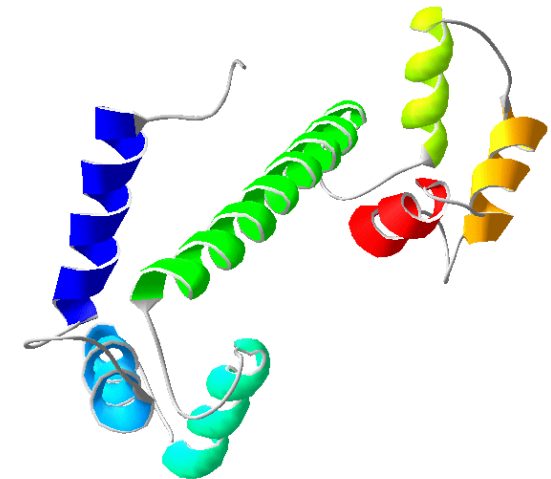
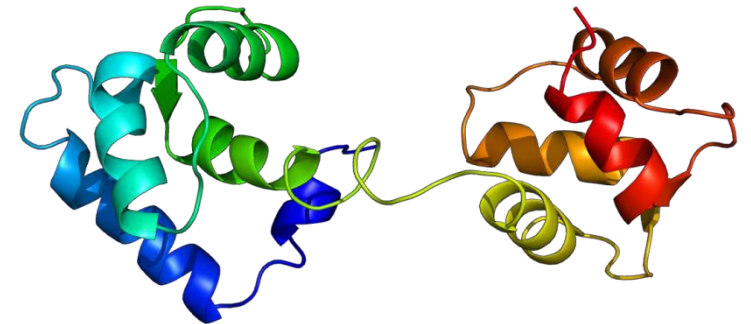
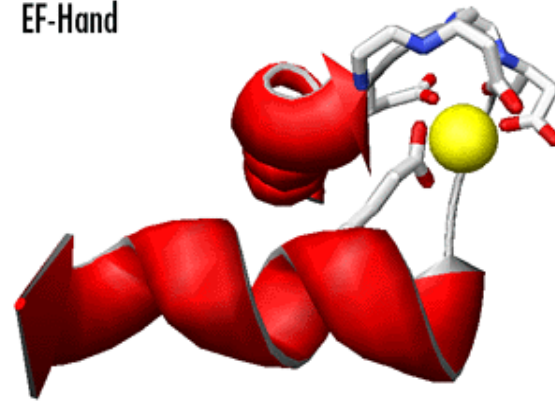
Loren Williams



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

EF-Hand



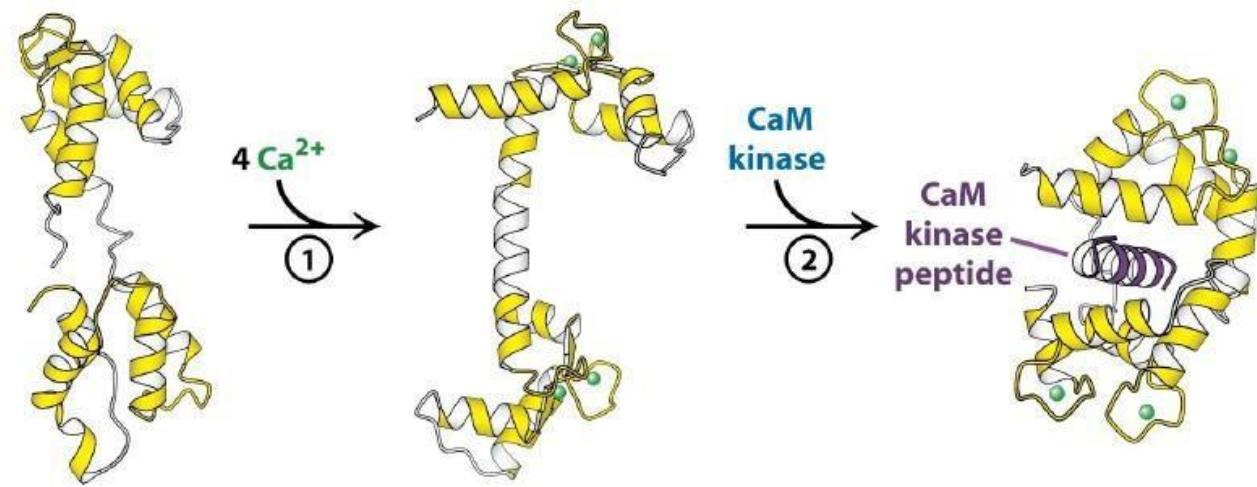


Calmodulin (≈ 17 kD)

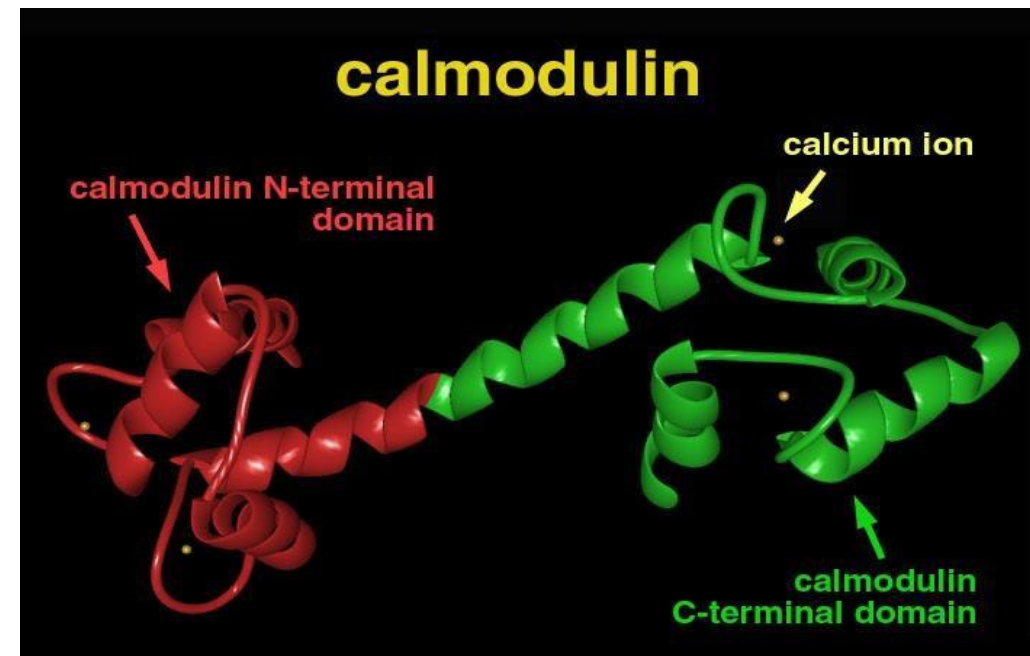
Calcium-modulated protein

- 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:

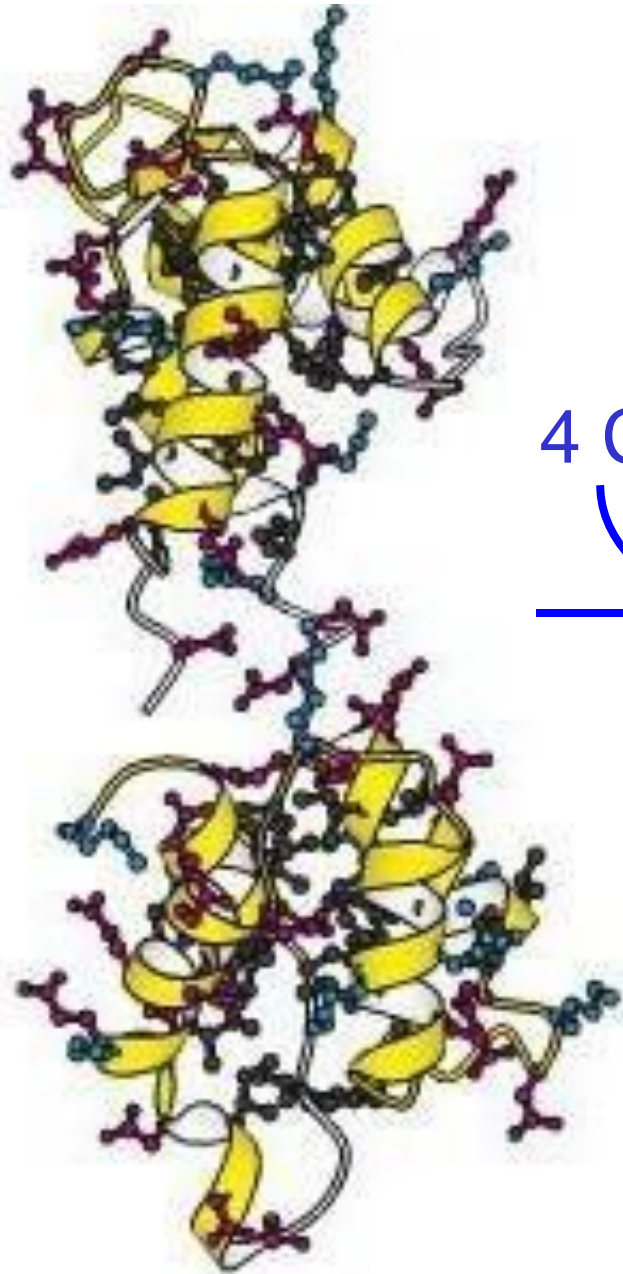
Calmodulin-dependant Protein Kinase



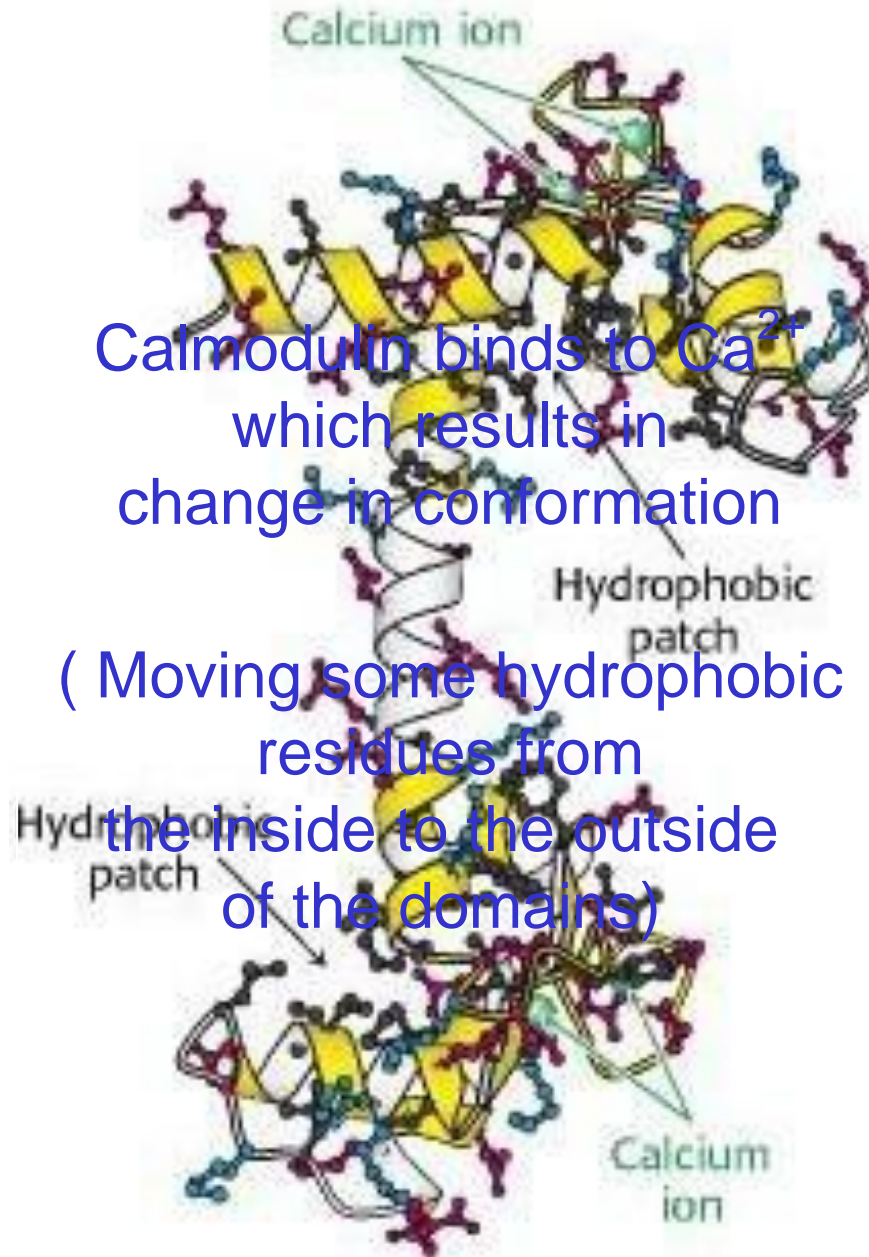
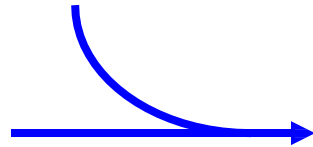
149 amino acids



Ca^{2+} ATP'ase Pump



4 Ca^{2+}



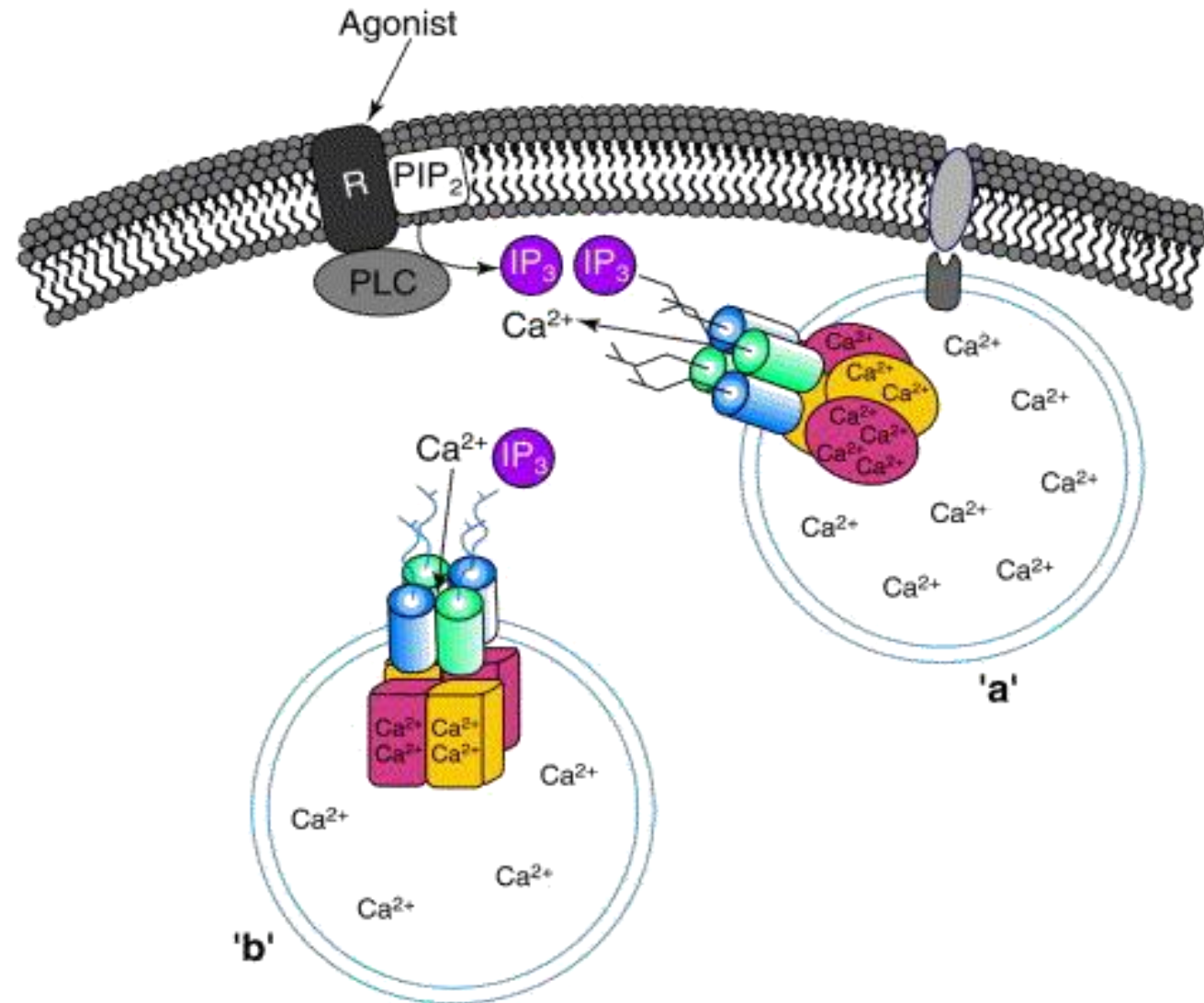
Calmodulin binds to Ca^{2+}
which results in
change in conformation

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



Ca²⁺ Transporter

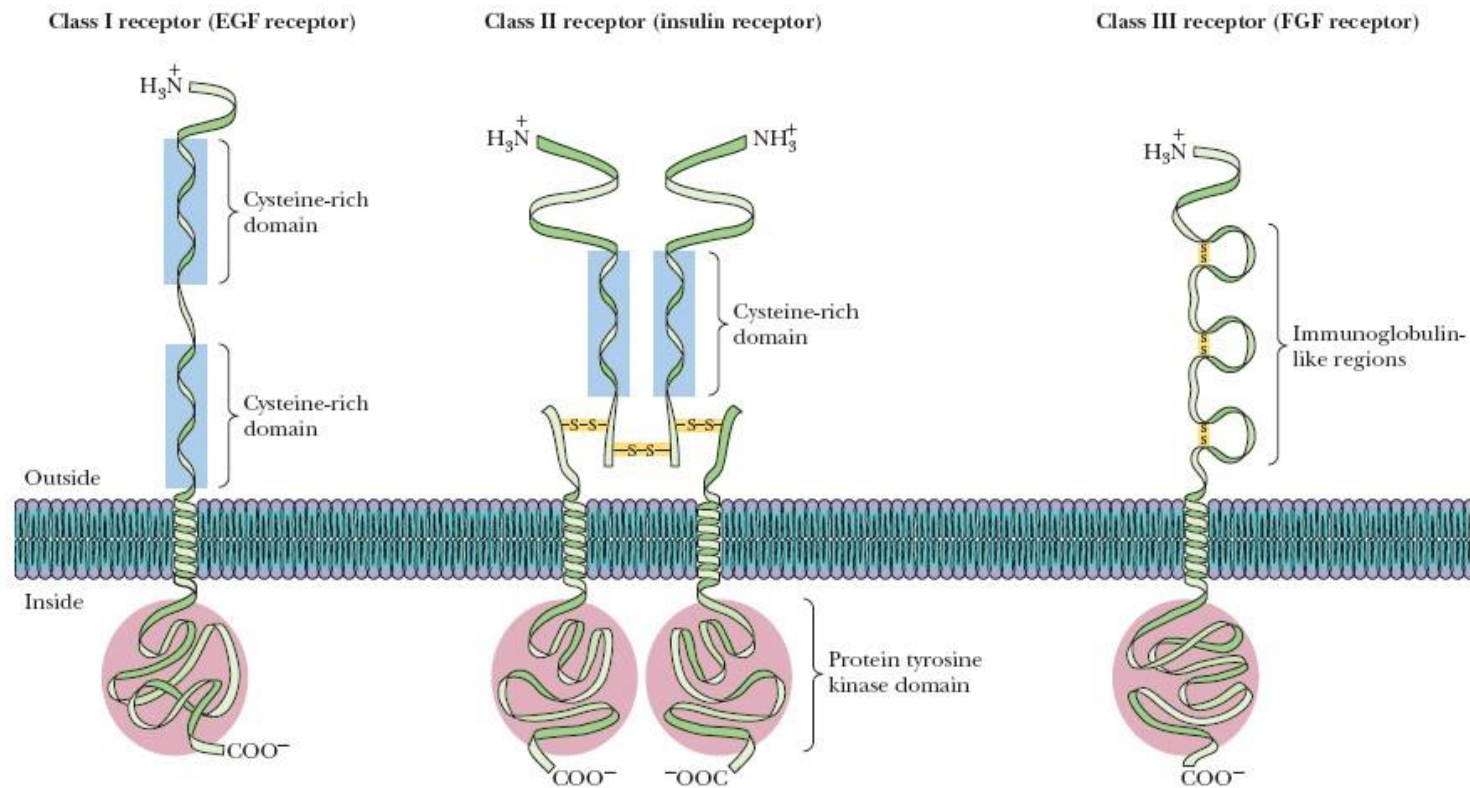
- In sarcoplasmic reticulum
- 80% of the membrane proteins
- 10 membrane spanning helices
- Ca²⁺ move against a large concentration gradient
- 2 Ca²⁺ / ATP (high)
 - Depletion of ATP leads to tetany, Rigor mortis





Receptor Tyrosine Kinases Cascade

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



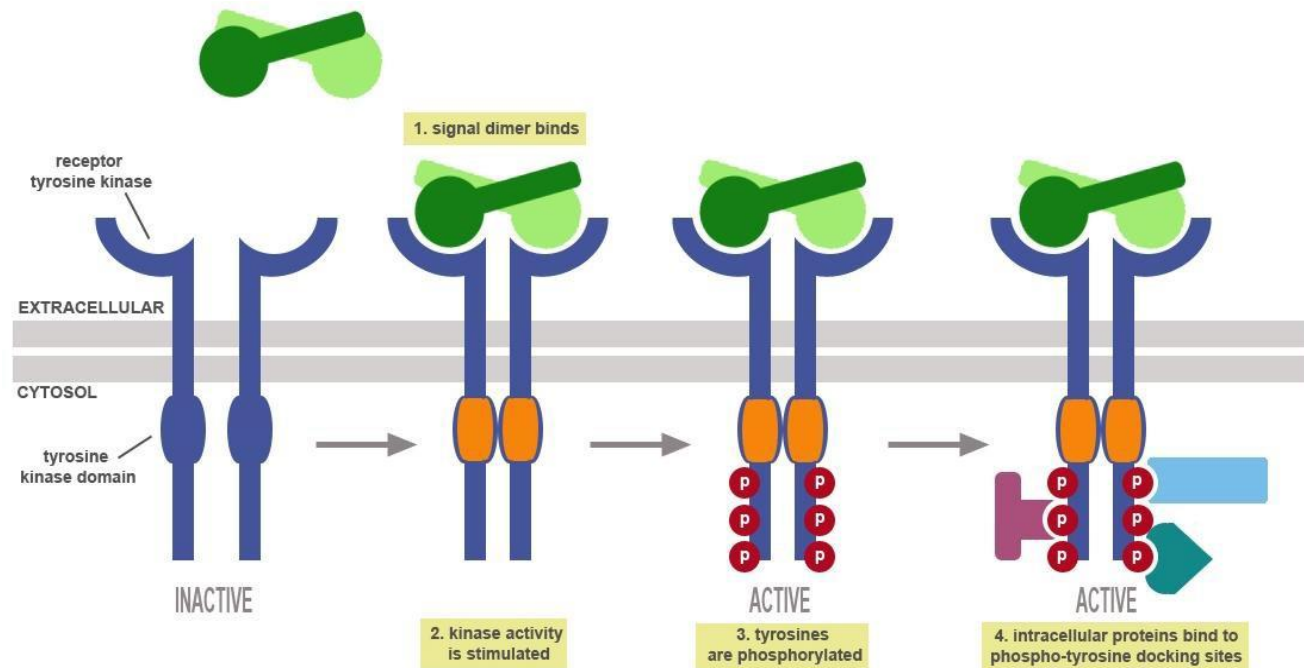


Second Messengers

Receptor Tyrosine Kinases

- When activated (**dimer**) → tyrosines on target proteins:
 - Alterations in membrane transport of ions & amino acids & the transcription of certain genes
 - **Dimerization is necessary but not sufficient for activation (kinase activity)**

- **Phospholipase C** is one of the targets
- Insulin-sensitive protein kinase: activates **protein phosphatase 1**

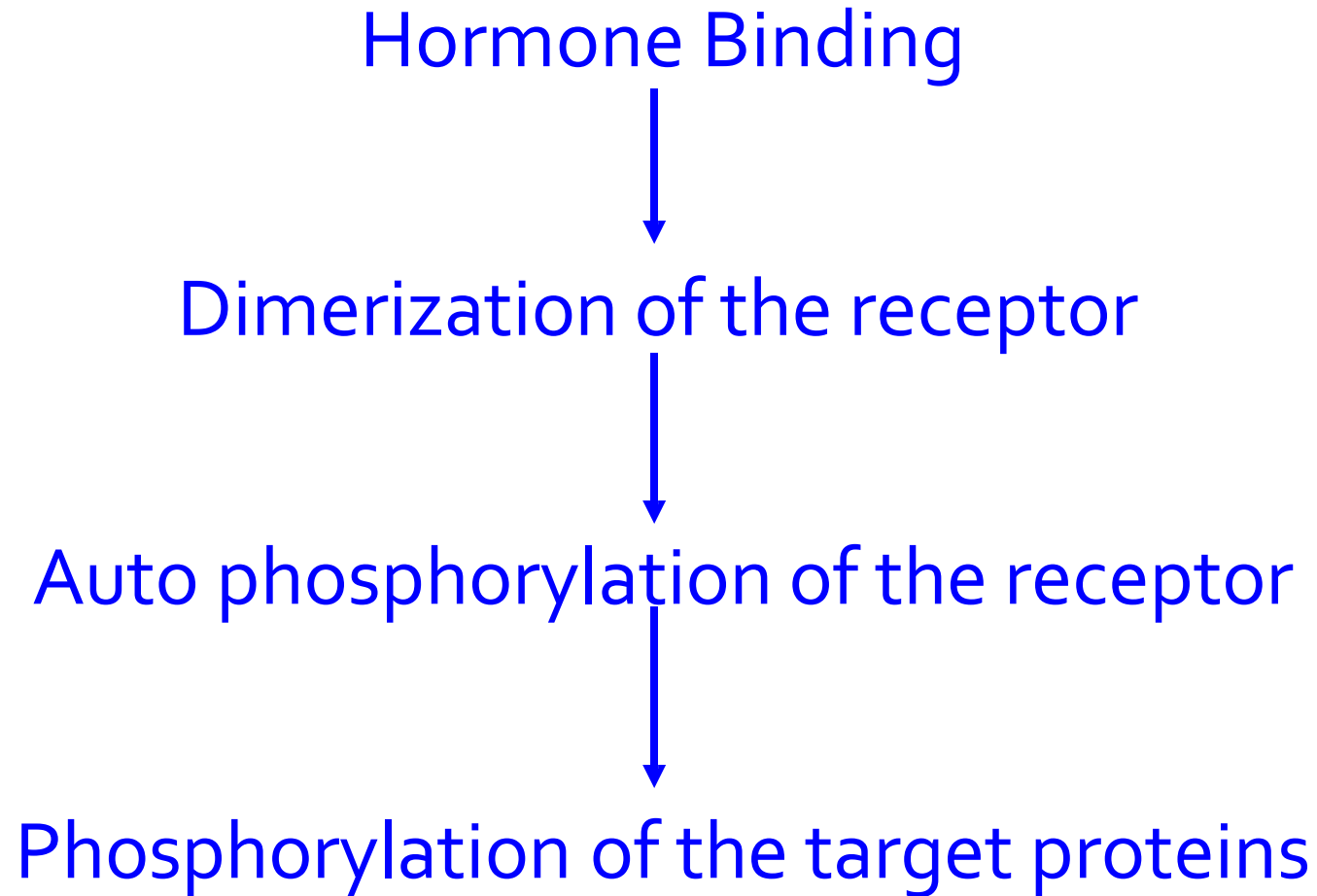




Signal Transduction through Tyrosine Kinase

Growth hormones:

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





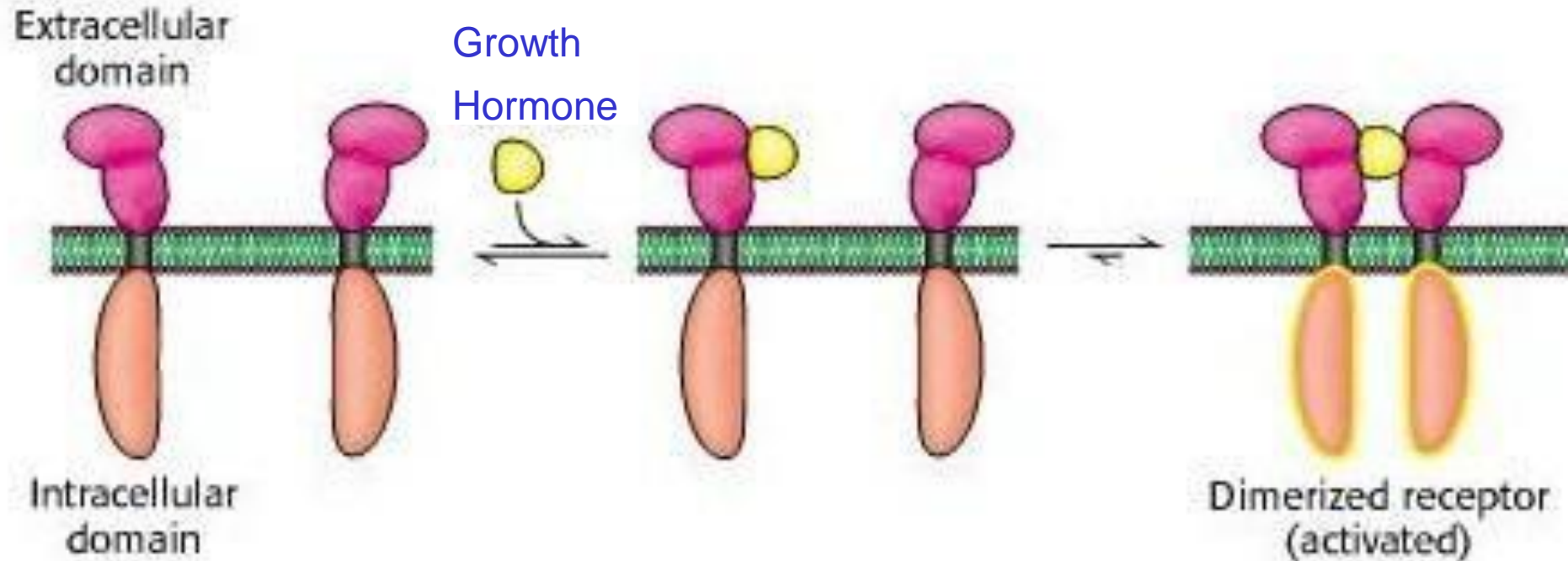
Growth Hormone dimerization

Binding of one molecule of growth hormone



Dimerization of the receptor

(B)





Janus

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

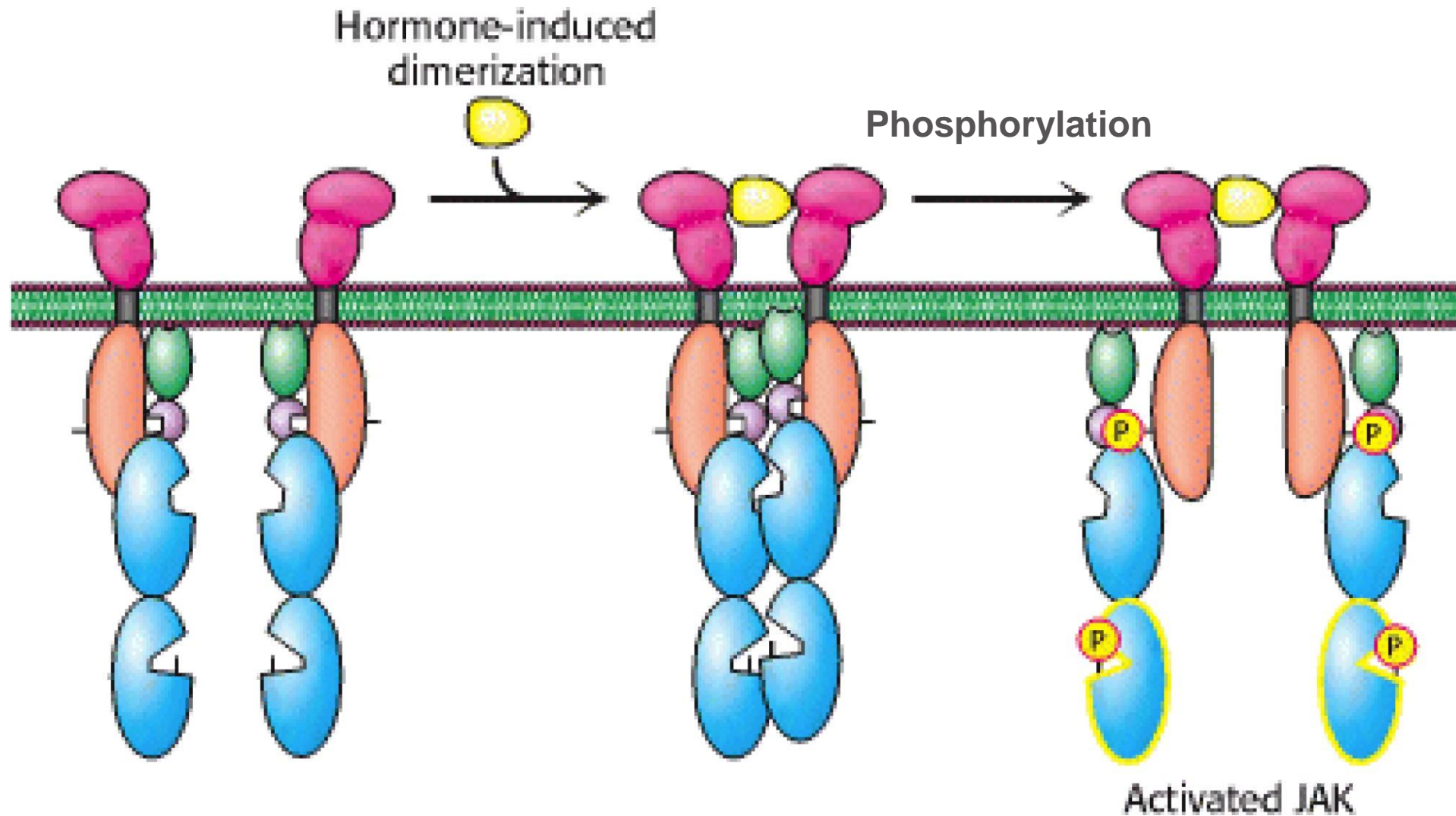


Interaction with membrane

Binds peptides that contain Phosphotyrosine



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





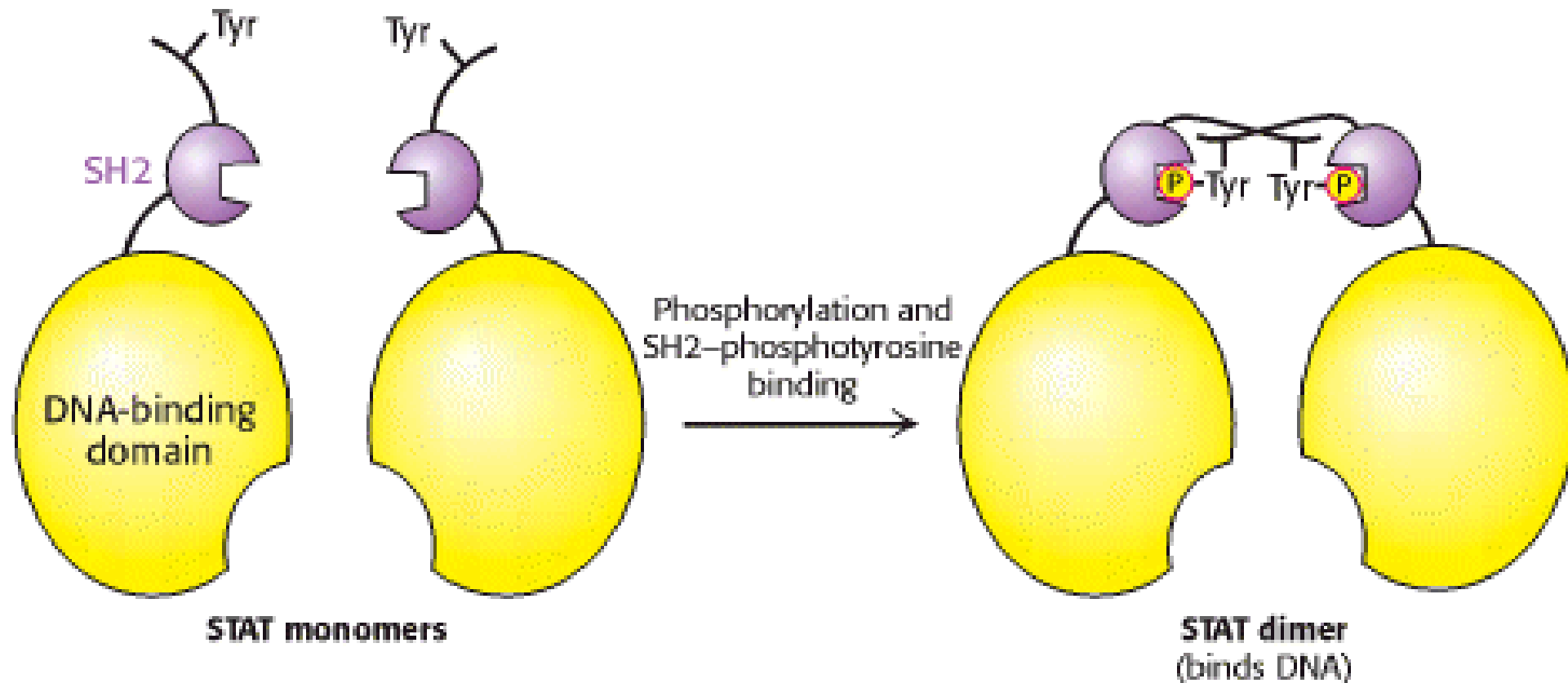
Activated JAK 2 can Phosphorylate other substrates

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



STAT is phosphorylated on a tyrosine residue near the carboxyl terminus

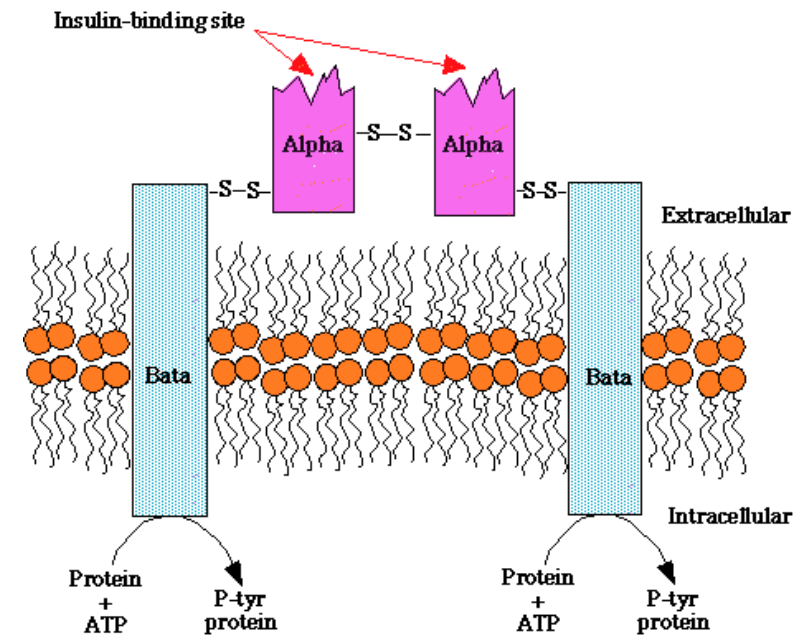
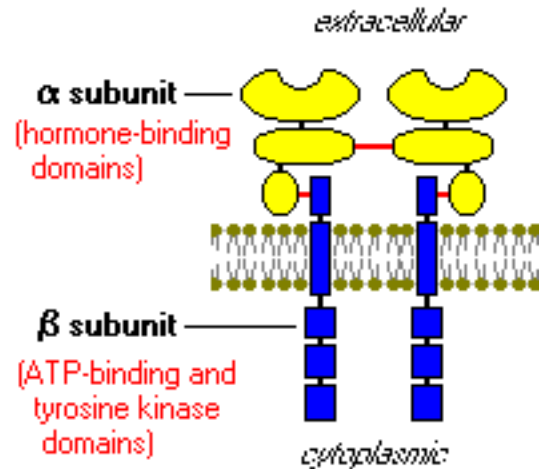
Phosphorylated tyr binds to SH2 domain of another STAT molecule





Tyrosine Kinase & other Hormones

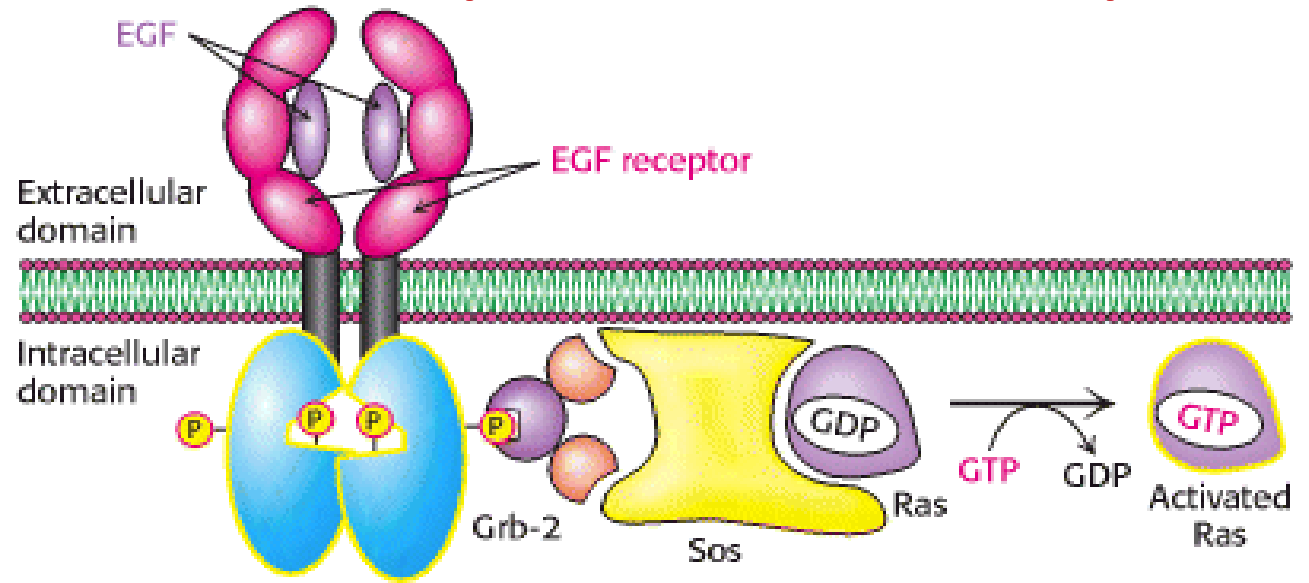
- Insulin Receptor
- Tetramer (2^{α} ; 2^{β}), dimer ($2^{\alpha\beta}$ pairs)
- Disulfide bridges
- Insulin Binding \rightarrow Activation of the Kinase





Ras is a member of small G proteins family

- Monomeric
- 2 forms: $\text{GDP} \leftrightarrow \text{GTP}$
- Smaller (1 subunit)
- GTPase activity
- Many similarities in structure and mechanism with $\text{G}\alpha$
- 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