

Biochemistry - ES

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These slides were modified using
015 Sheets

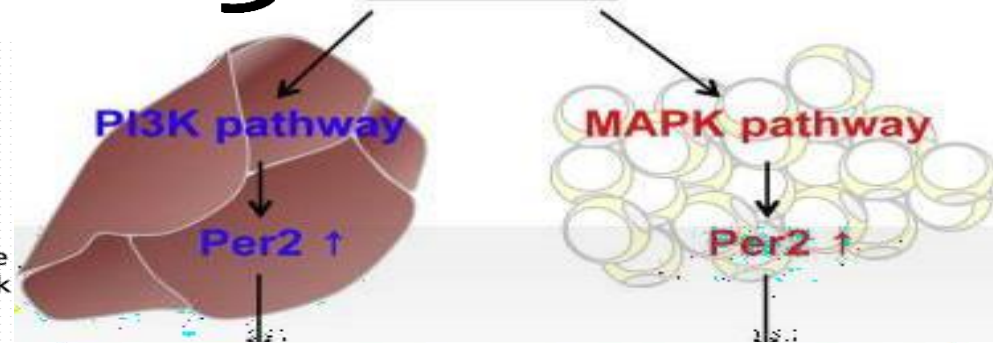
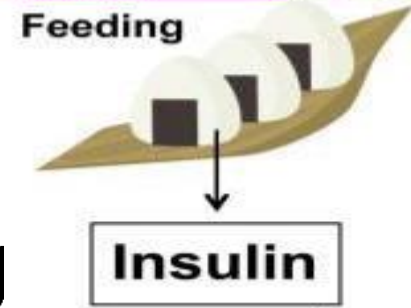


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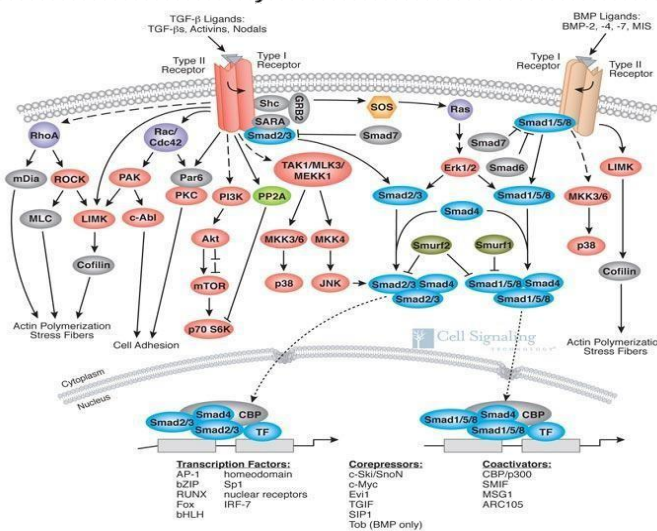
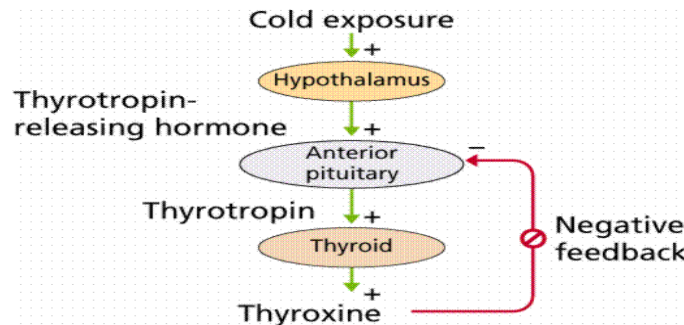
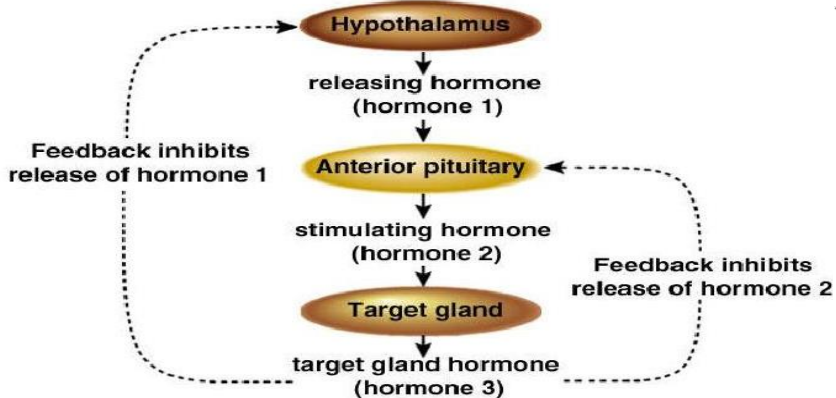
THE UNIVERSITY OF JORDAN

Integration of Metabolism: hormones & Cellular Signaling

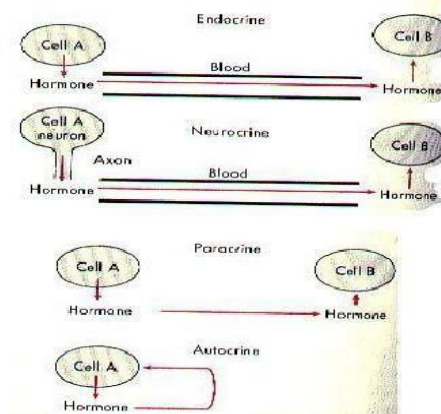


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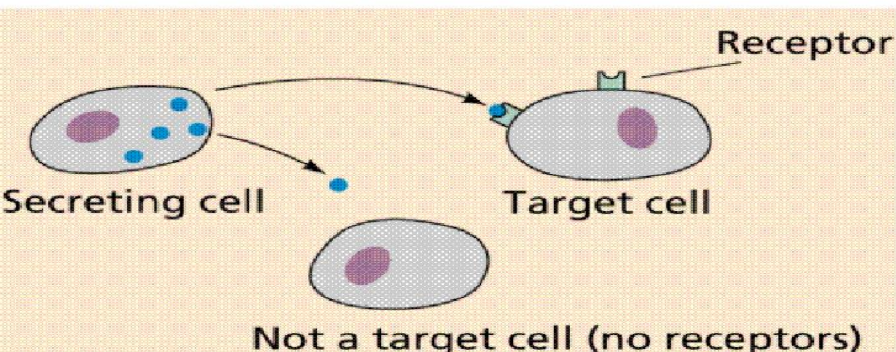
Endocrine Glands



Types of cell-to-cell signaling



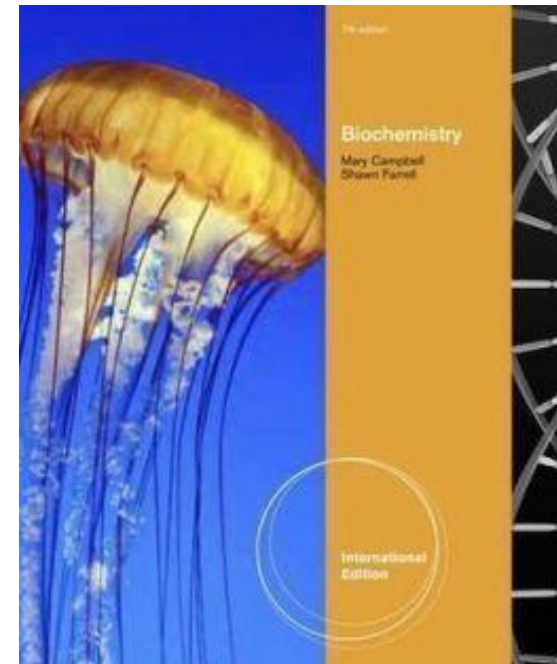
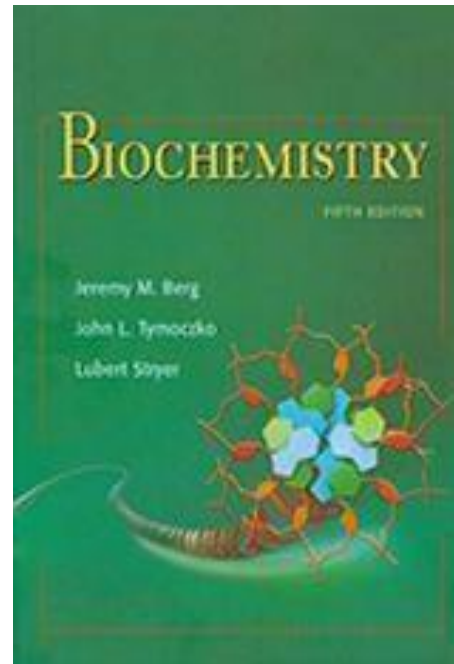
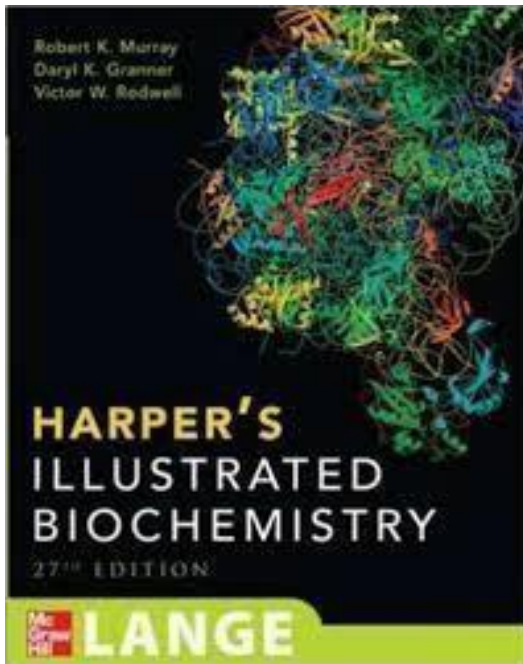
Endocrine Hormones: travel via bloodstream to target cells
Neurocrine hormones: released from nerve terminals
Paracrine hormones: act on adjacent cells
Autocrine hormones: Released and act on the cell that secreted them.
Intracrine Hormones: act within the cell that produces them.





Resources for the 3 lectures

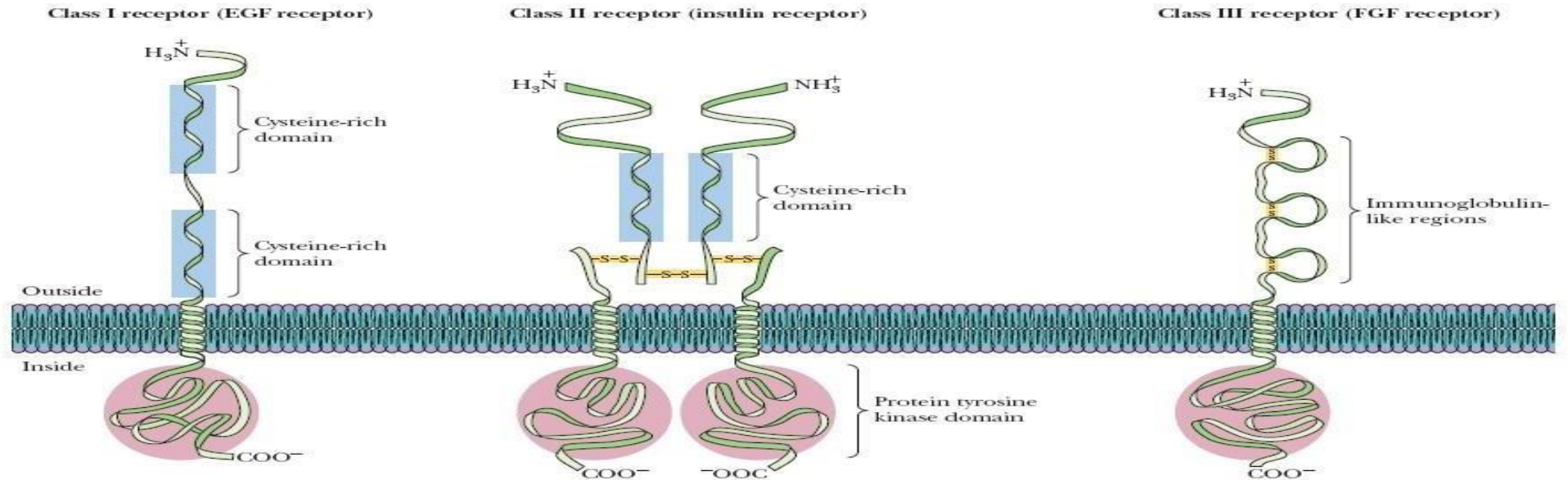
- Harper's Illustrated Biochemistry
- Stryer's Biochemistry
- Campbell's Biochemistry





Receptor Tyrosine Kinases Cascade

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



Review

There are two types of receptors:

1. 7TM receptors ; which are always bound to a G protein "GPCR". This pathway may lead to activation of adenylyl cyclase or phospholipase C.
2. Receptor Tyrosine kinase.

Receptor Tyrosine Kinases Cascade

- ❖ This receptor is a kinase enzyme, and the pathway involves Tyrosine amino acid phosphorylation. This pathway is used by most growth-related hormones (Insulin, GH, growth factors...). There are two classes of this receptor:
 - Monomer, which dimerizes after ligand binding. All receptors of this family are monomers except for insulin receptor.
 - Dimer, and the subunits are bound by disulfide bridges; such as insulin receptor.
- ❖ The receptor spans the membrane, and has several subclasses (class II, Insulin R).

Receptor Domains :

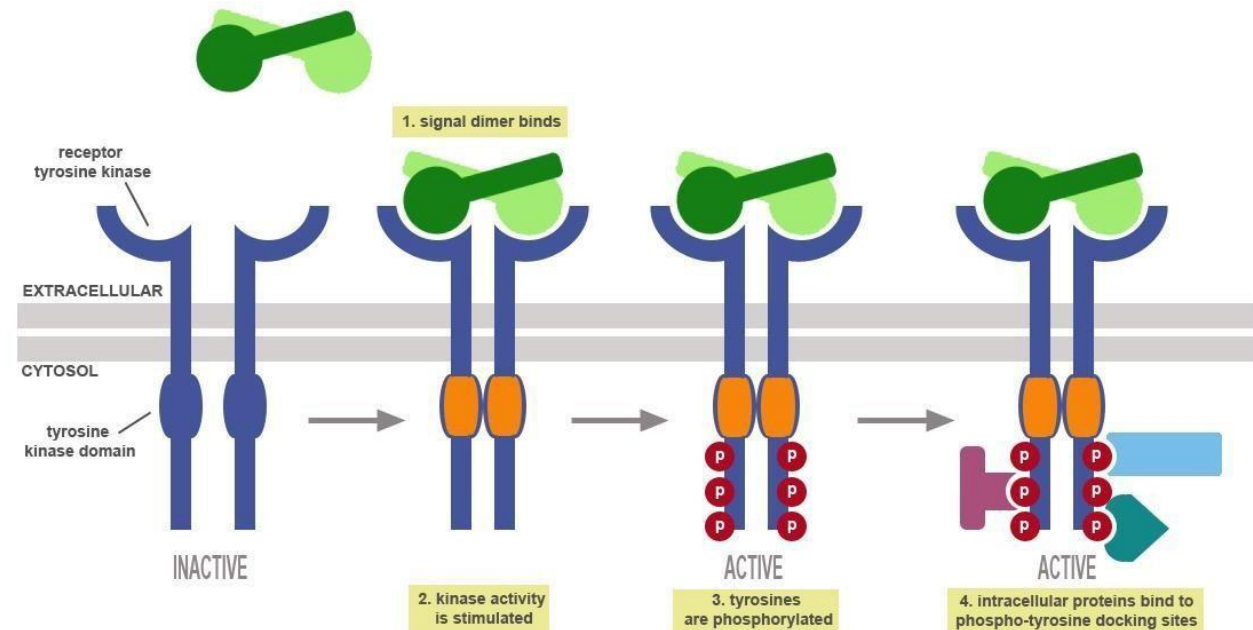
- ❖ The receptor is a hormone receptor that has a Tyrosine kinase portion. The coupling domain of this receptor has Tyrosine residues. Tyrosine is common target for phosphorylation.



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)**
- **PhospholipaseC** is one of the targets
- Insulin-sensitive protein kinase: activates **protein phosphatase 1**





Signal Transduction through Tyrosine Kinase

Growth hormones:

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

Hormone Binding



Dimerization of the receptor



Auto phosphorylation of the receptor



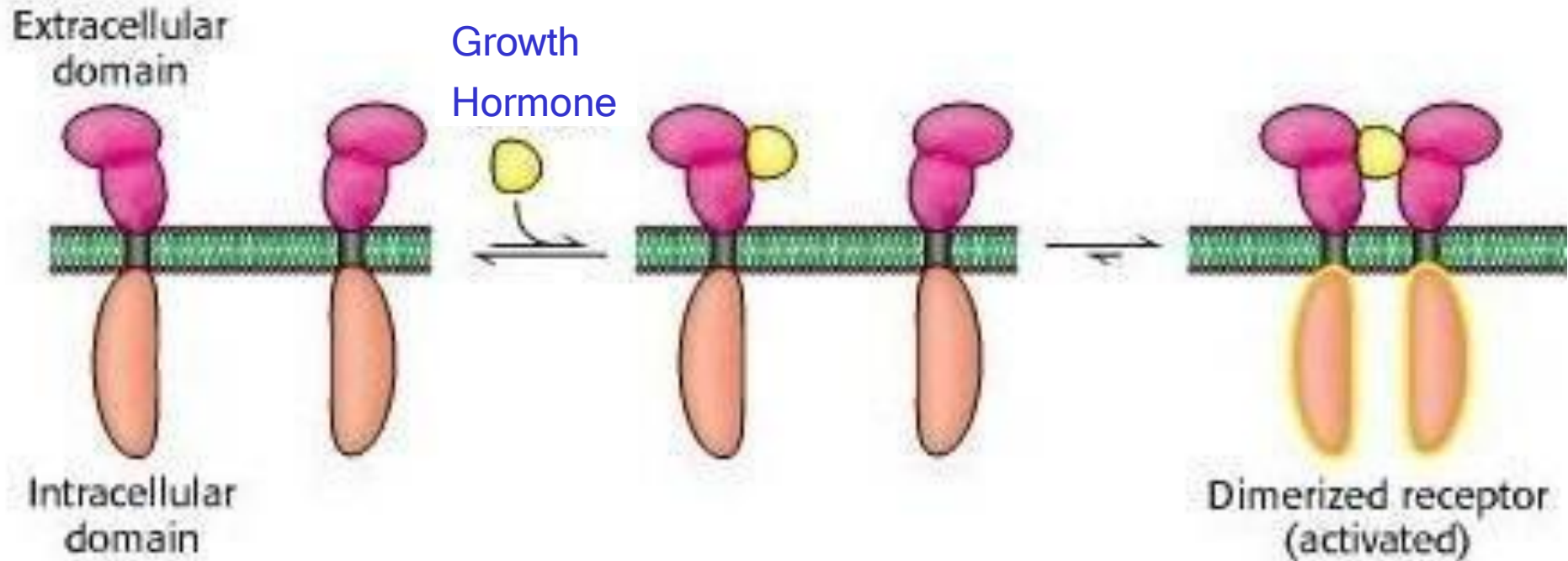
Phosphorylation of the target proteins

Growth Hormone dimerization

Binding of one molecule of growth hormone

Dimerization of the receptor

(B)



- Binding of the ligand leads to conformational change, which results with monomers dimerization. Dimerization is a hallmark of this pathway that can be noted in many levels of the pathway. Binding has two forms:
 1. One ligand binds to a monomer and another binds to another monomer. Conformational changes of the monomers leads to their dimerization. So, 2 ligands bind here.
 2. One ligand binds to a dimer receptor (ex. Insulin receptor).
- Dimerization is not enough for activation. Dimerization induces a conformational change that leads to auto- and cross-phosphorylation of the Tyrosine residues in the coupling domain, and thus fully activating the receptor. Notice that the monomers phosphorylate themselves and each other. Remember that the receptor is itself an enzyme and so performs a kinase activity when activated.
- **VERY IMPORTANT NOTE:** growth hormone is a protein not a peptide , which means it consist of more than 100 amino acid (peptide less than 100). It was a trick in ES midterm exam (015 Batch)

- This receptor is distinct in that it has its second receptor (tyrosine kinase) within itself. So, it does not need a second messenger system.
- Target activities may be 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.
- activation of an originally dimerized receptor (ex. Insulin receptor) is similar to the activation of the monomer receptor, and involves:
Binding – conformational change – activation – tyrosine residues phosphorylation – kinase activity.

NOTE 1 : although subtle, conformational changes allow the functionality of the proteins to take place.

NOTE 2: the monomers before dimerization are not so moving, they rather stand facing each other, and when they bind a ligand, the resultant conformational change allows them to get dimerized.

Janus

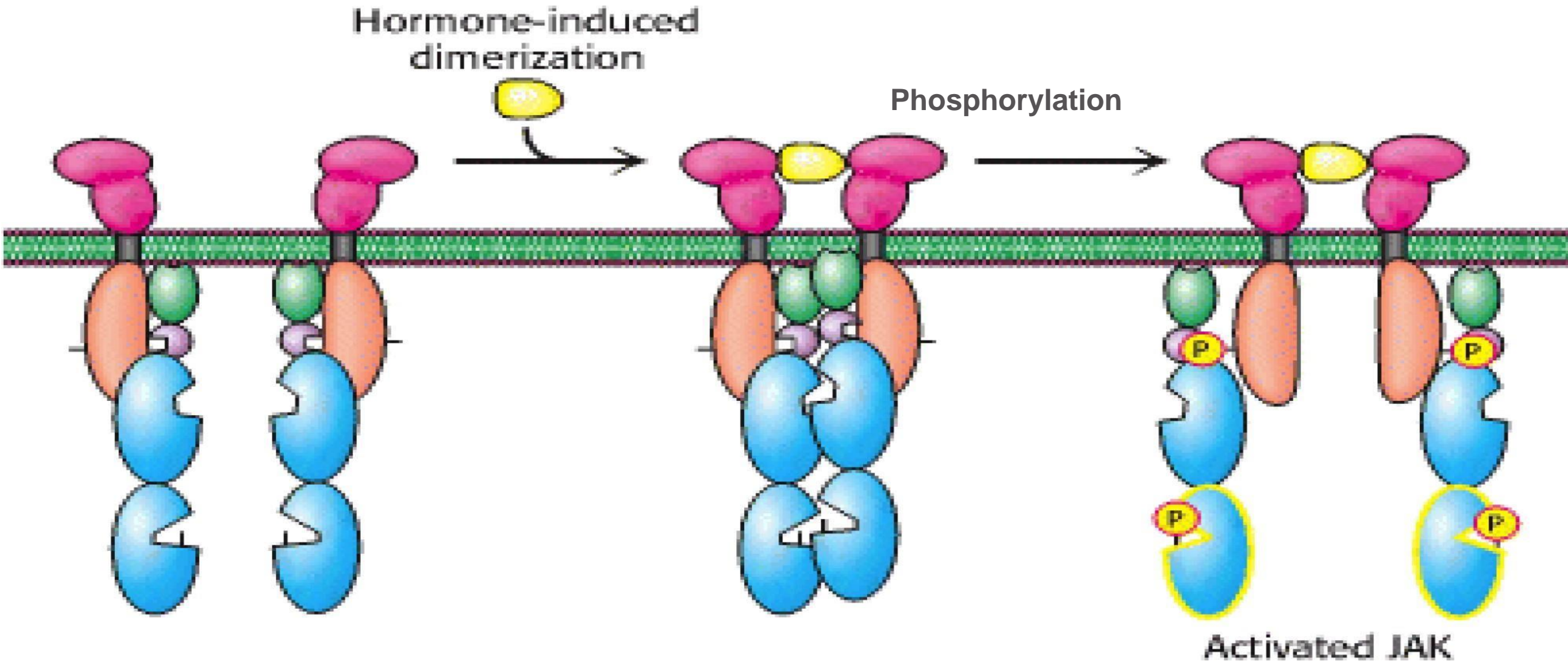
Each Intracellular Domain is associated with a protein kinase called Janus Kinase



Interaction with membrane

Binds peptides that contain Phosphotyrosine

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



- With each monomer, a Janus kinase, or JAK, is bound. Janus is a Greek god that has two identical faces, and this is how JAK is bound to the monomers that get dimerized. JAK also has Tyrosine residues.
- **JAK kinase domains include:**
 1. **Membrane binding domain (to be close to the receptor)**
 2. **Kinase domain - SH2 domain (discussed below)**
- Dimerization of the receptor monomers allows JAK kinases of each monomer to get closer. This will lead to a conformational change. After that, auto- and cross-phosphorylation occurs between the two JAK kinases, resulting in their activation.
- Activated JAK kinases phosphorylate target molecules, and STAT, or Signal Transducer & Activators of Transcription, is the most common one. STAT leads to transcription activation, having the DNA as its final target.

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 phosphorylation leads to dimerization of STAT molecules. **How is that?**

STAT is phosphorylated on a Tyrosine residue near the carboxyl terminus.

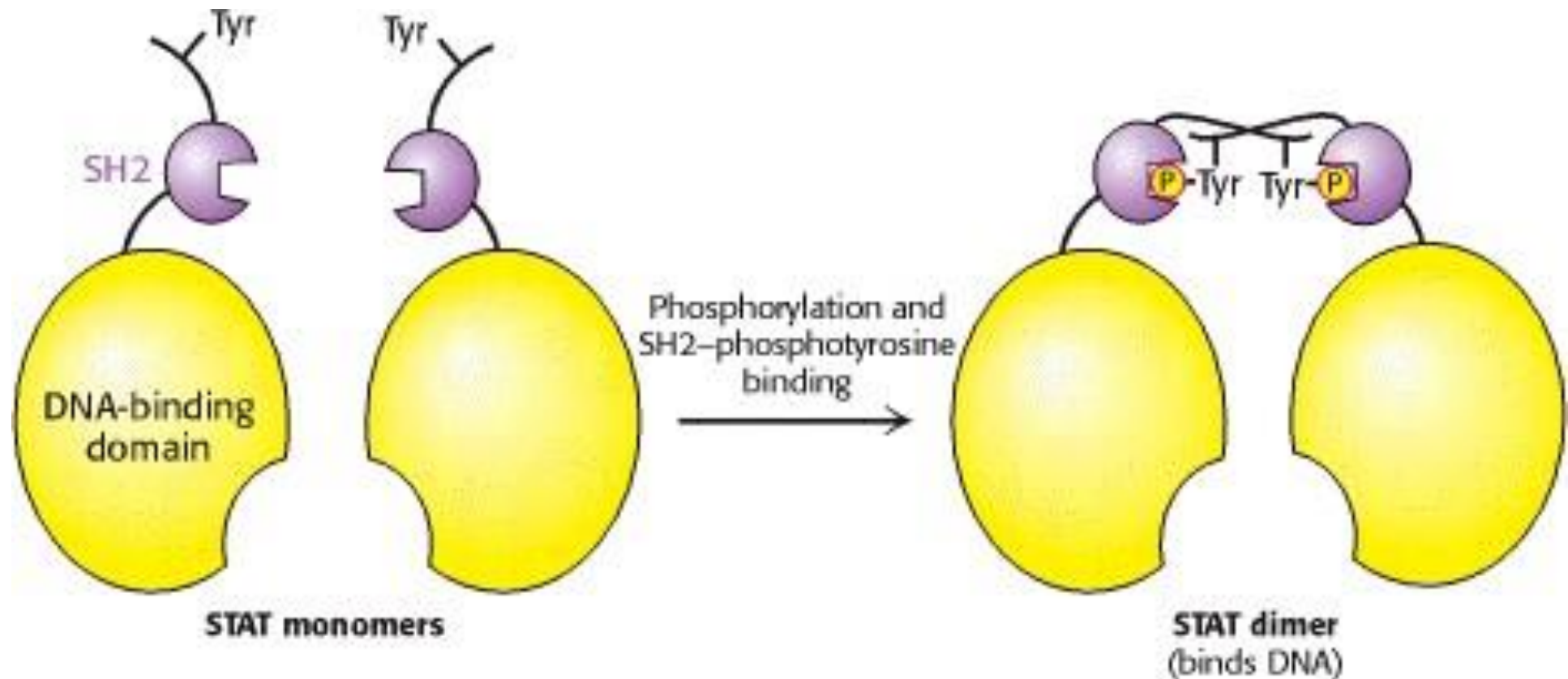
Phosphorylated Tyrosine binds to SH2 domain of another STAT molecule. **But what is SH2 domain already?**

Src Homology 2; SH2:

- SH2 domain is a phosphorylated Tyrosine binding domain. It is present in JAK kinase as well as STAT molecules.
- After that, the resultant STAT dimer heads towards its final target, the DNA, to activate transcription.
- Note that JAK/STAT pathway is an example of the pathways that follow the binding of a ligand to a receptor tyrosine kinase.

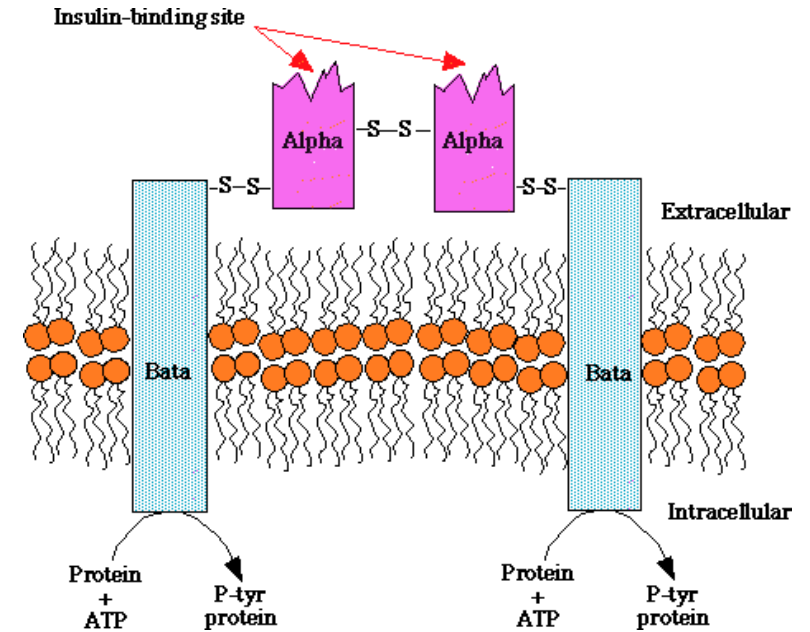
STAT is phosphorylated on a tyrosine residue near the carboxyl terminus

Phosphorylated tyr binds to SH2 domain of another STAT molecule



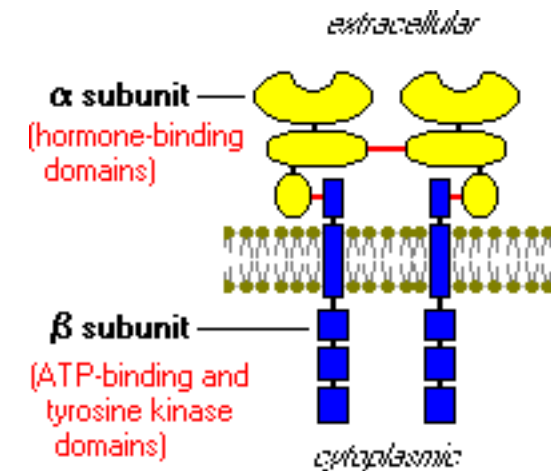
Tyrosine Kinase & other Hormones

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



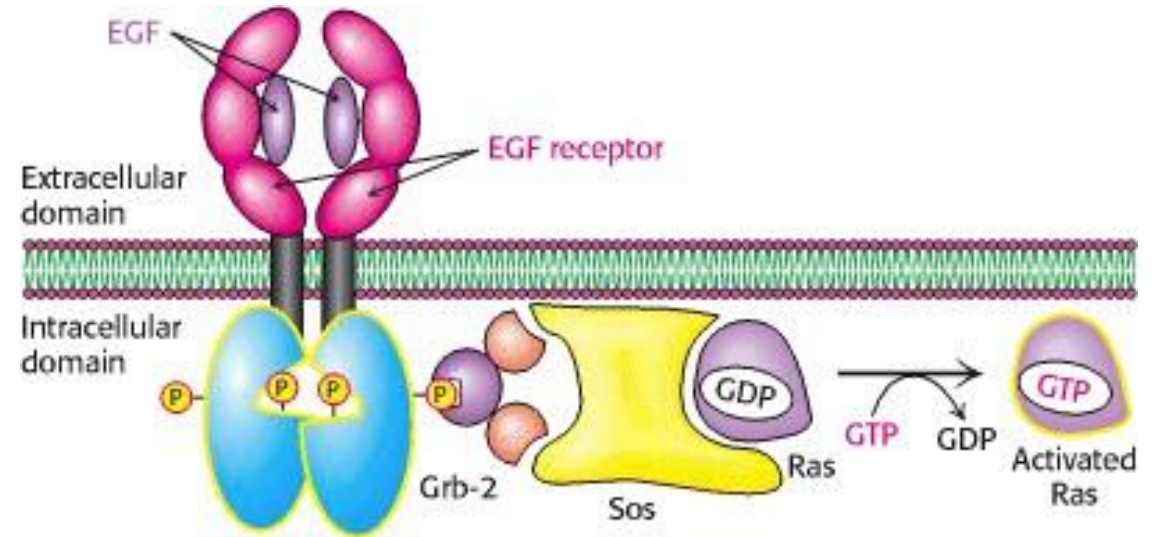
Examples on Receptor Tyrosine Kinases:

1. Epidermal Growth Factor Receptor : Monomeric (inactive) → EGF binding → Dimerization → Cross Phosphorylation → Activation .
2. Insulin Receptor: Tetramer (2α ; 2β), 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 \leftrightarrow 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 different Ras proteins

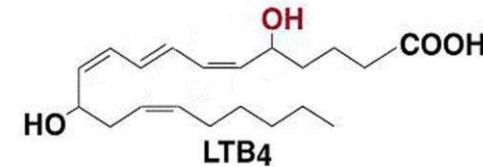
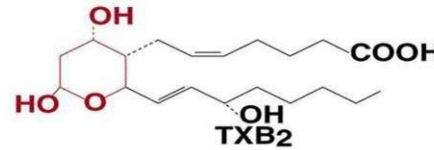
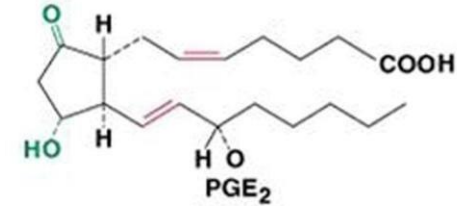
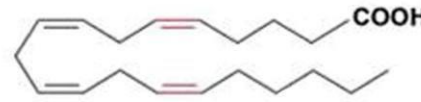
Mutation → Loss of ability to hydrolyze GTP → Ras is locked in “ON” position → continuous growth stimulation

- RAS protein is a monomeric G protein. It works in a similar manner to α -subunit of G proteins.
- So, its activation includes:
Ligand binding leads to receptor activation \rightarrow RAS conformational change \rightarrow GDP for GTP replacement \rightarrow activation \rightarrow activates another effector protein Like G protein α -subunit .
- RAS protein also has a slow GTPase activity that leads to GTP for GDP replacement and signal termination.
- RAS includes several groups or subfamilies.
- RAS has a major role in growth, differentiation, cellular transport, motility etc..
- Mammalian cells contain 3 Ras proteins.
- Mutation of RAS GTPase domain \rightarrow Loss of ability to hydrolyze GTP \rightarrow Ras is locked in “ON” position \rightarrow Continuous stimulation of growth.
- In mammals, three known types of RAS proteins are known, which are known to be mutated. If RAS (regardless of the type) is mutated, this might affect its GTPase activity, so RAS is locked in GTP-bound form and it remains active. This might lead to cancer.

The following 4 slides are explained, check slides 25,26

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?

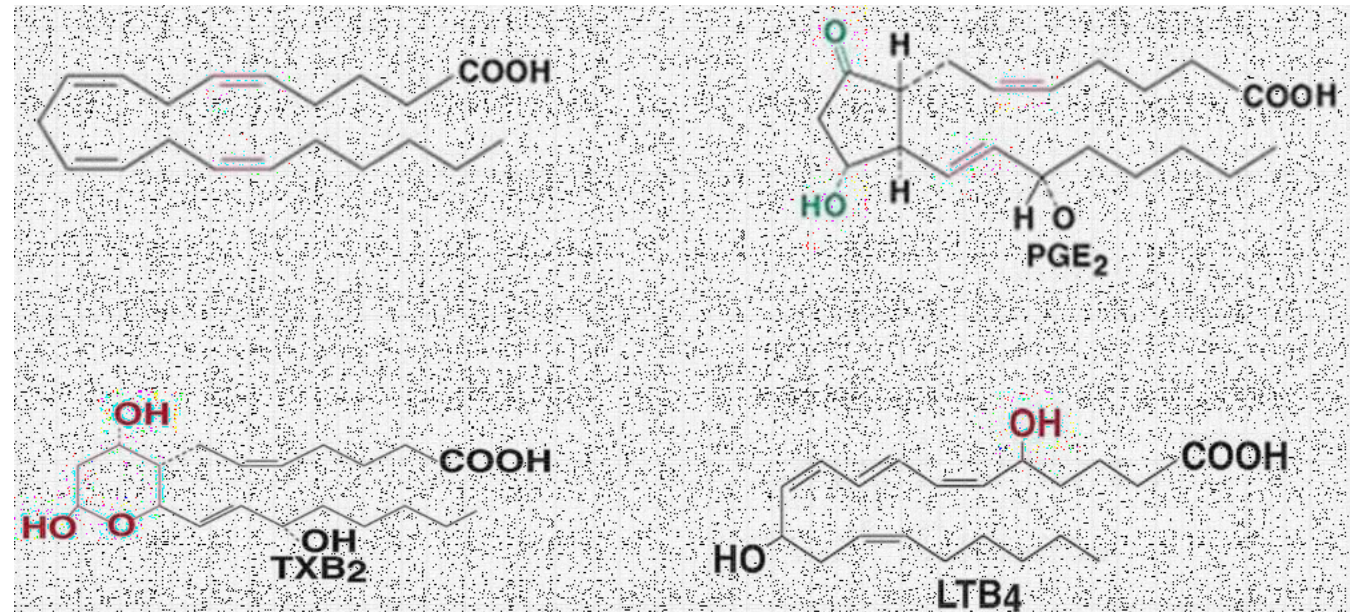
- PGI₂, PGE₂, PGD₂
 - Increase
 - Vasodilation, cAMP
 - Decrease
 - Platelet Aggregation
 - Lymphocyte Migration
 - Leucocyte Aggregation

- PGF₂α Increases
 - Vasoconstriction
 - Bronchoconstriction
 - Smooth Muscle Contraction

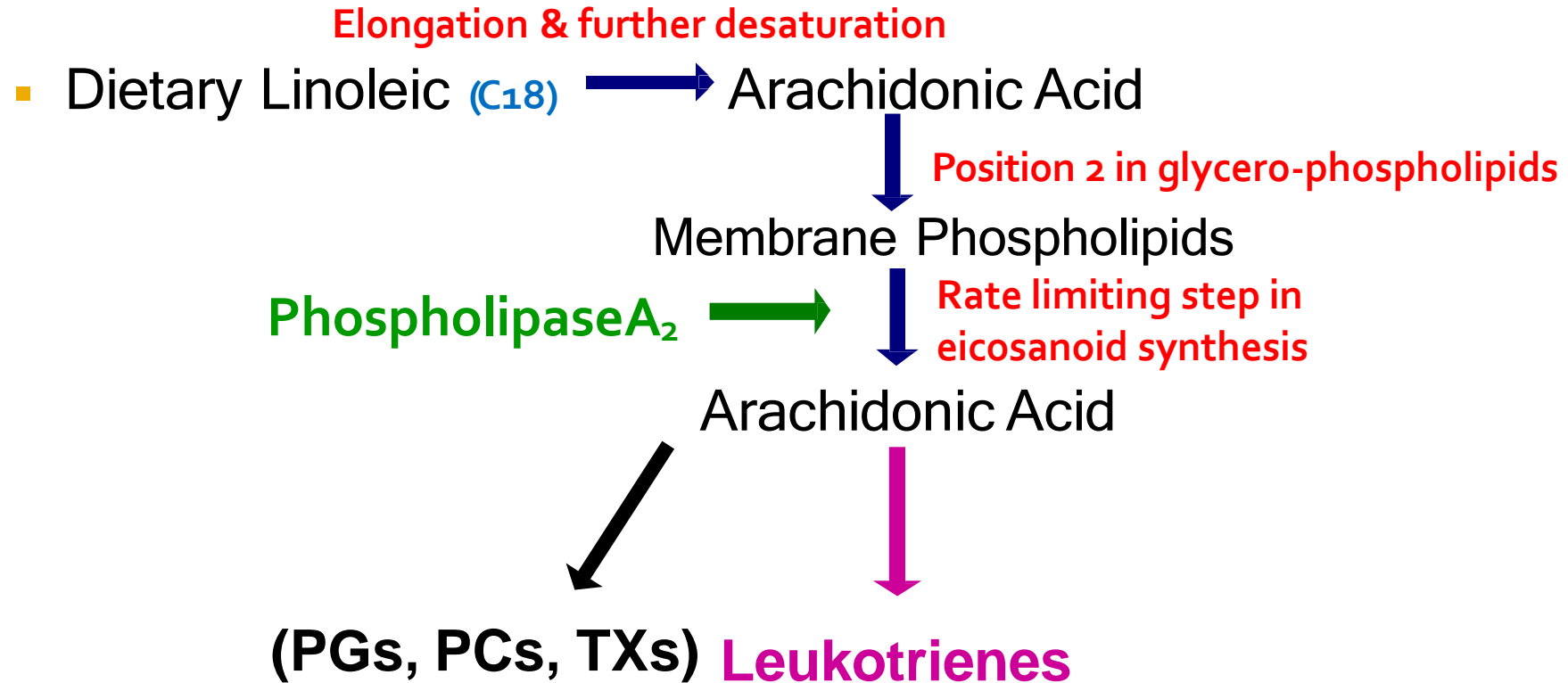
- Thromboxanes Increases
 - Vasoconstriction
 - Platelet Aggregation
 - Lymphocyte Proliferation
 - Bronchoconstriction

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 are examples of paracrine and autocrine hormones. They are small molecules which are derived from fatty acids in their nature. They do not go through the blood to far destinations (they act locally). They are very potent & they have short half lives and are not stored .
- ✓ Mainly include: Prostaglandins (PGs), Thromboxanes (TXs), Leukotrienes (LTs). All of these are derived from one parent molecule called Arachidonic acid (fatty acid).
- ✓ This fatty acid is present in the plasma membrane's phospholipids. It is always bound to carbon number 2 of glycerol, and is released by the action of Phospholipase A2, which breaks it from glycerol. Then, it undergoes modifications in several reactions to produce either one of the above classes.
- ✓ **Eicosanoids (Eicosa=20): group of molecules, each consists of 20 carbon unit. How to differentiate between them?**

- Arachidonic acid is a 20 carbon unit molecule, doesn't contain rings, has 4 double bonds.
- Prostaglandins, 20 carbon unit molecules, all have five-membered ring .
- Thromboxanes, 20 carbon unit molecules, all have six-membered ring.
- Leukotrienes, 20 carbon unit molecules, have at least 3 conjugated double bonds. Meaning that they alternate: double—single—double—single—double—single, don't have ring

- ✓ There are many Prostaglandins, Thromboxanes, and Leukotrienes. They have diverse functions and may be opposite to one another. Some of them promote platelet aggregation; some inhibit platelet aggregation, some cause vasodilation while others cause vasoconstriction, some cause bronchodilation while others promote bronchoconstriction but all act locally.
- ✓ The dominating function is determined by the signal coming to that area and according to what is being secreted, the effect appears.
- ✓ The rate limiting step in Eicosanoid synthesis is the release of Arachidonic acid from glycerophospholipids by Phospholipase A2. Now, cyclooxygenases convert it to PGs and TXs, while lipoxygenases convert it to LTs.

Synthesis and Degradation of Hormones

Chemistry of Hormones

Hormones are classified according to their mechanism of action into 2 groups some bind on receptors outside the cell (cell membrane), others bind intracellular receptors.

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

- Catecholamines
- Proteins and peptides
- FA derivatives - eicosanoids

Receptor inside the cell



Surface receptor

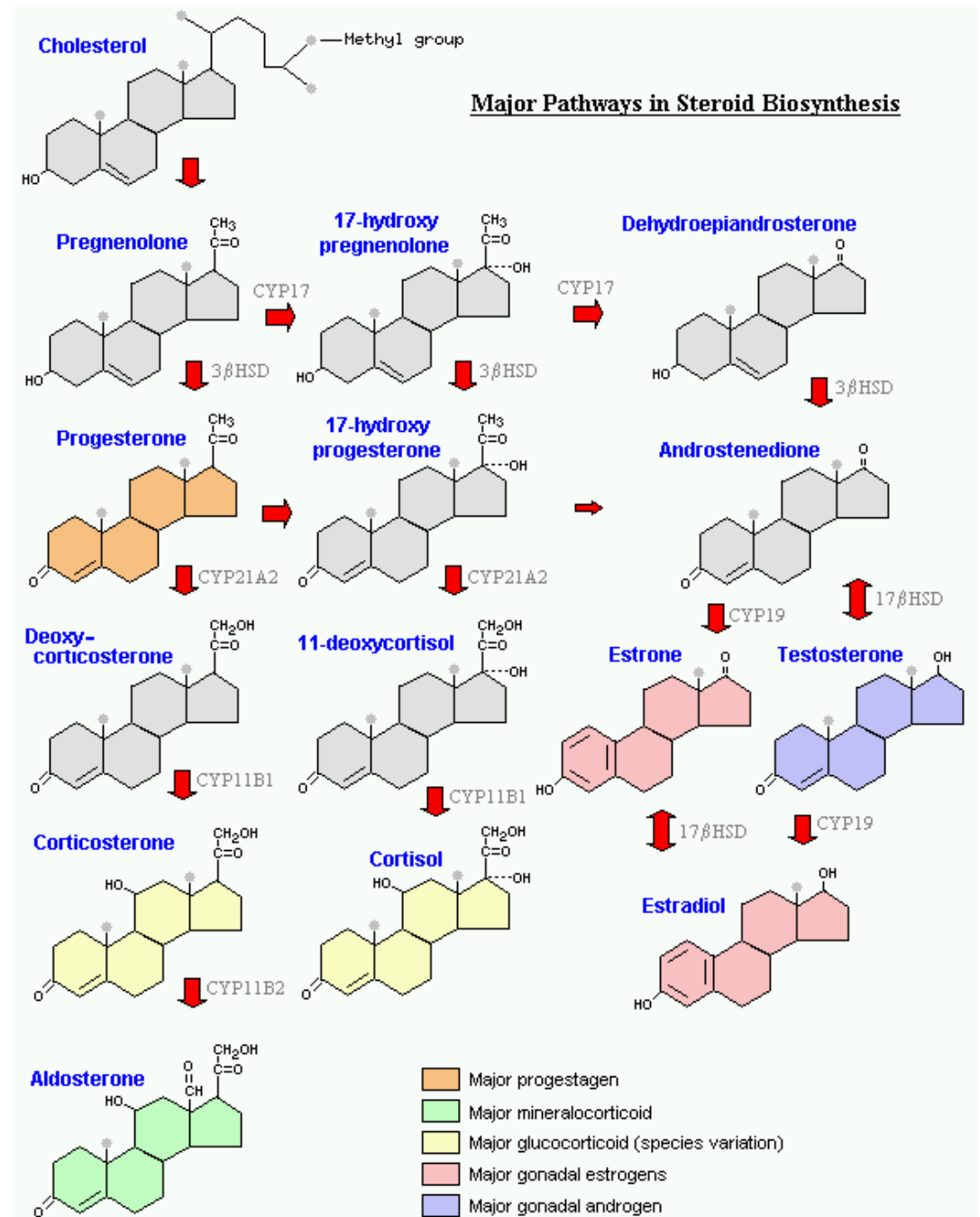
Steroids

- All steroids are synthesized from cholesterol, which contains 27 carbons.
- All steroids have the four sterane rings, accounting for 17 carbons. These 17-carbon rings are not metabolized/cleaved in the human body. Rather, the rings are conjugated to something else for excretion (mainly with bile products, small amount in the urine). What we can actually metabolize is what is attached to the ring.
- We can increase or decrease the number of carbons to produce different steroids → We have 18-carbon unit steroids, 19-carbon unit steroids, 21-carbon unit steroids....until we reach the parent (cholesterol) with 27-carbons.

You must know these 18,19,21 carbon steroids. To count quickly, it is given that the rings have 17 carbons, just count what is extra.

Steroid hormone synthesis

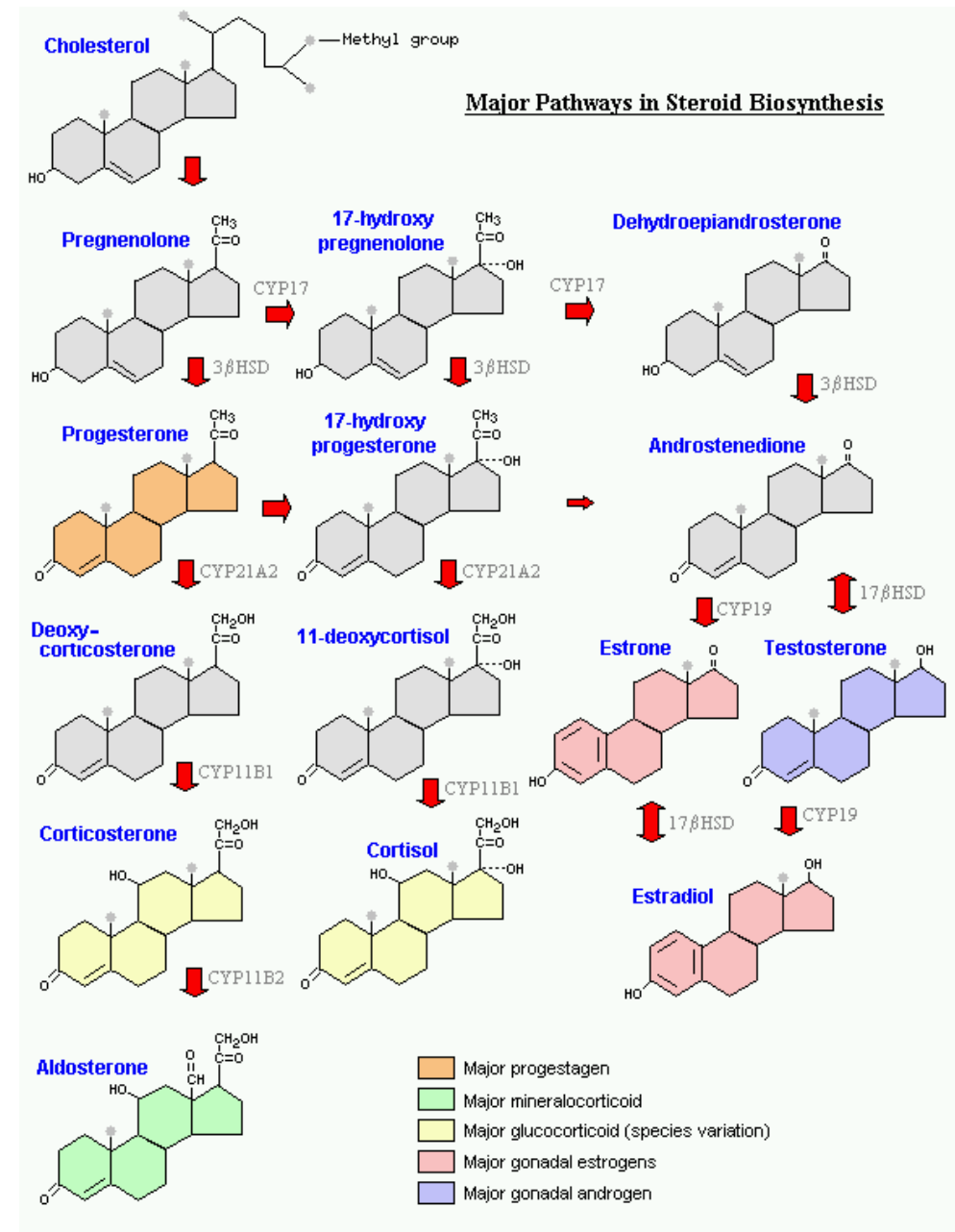
- **C21: Pregnenolone, 21-carbon steroid, is a parent molecule of sex hormones.**
- ✓ Progesterone: directly from pregnenolone, it's produced by further modification on pregnenolone (desaturation)
- ✓ Cortisol & Aldosterone: from progesterone



Steroid hormone synthesis

- C19
 - Testosterone (Removal of 2 carbons from progesterone (the acetyl group) produces the 19-carbon steroid Testosterone)
 - from progesterone or pregnenolone
 - 2c shortage
- C18 (estrogen):
 - If testosterone loses one carbon, Estrogen is produced (18 carbons).
 - Aromatase catalyses the conversion of testosterone into Estrogen .
 - Cleaves C18
 - Reduction

Extra Note : Aromatase enzyme is affected by some pesticides. This may pose a problem for farmers by affecting male/female characteristics, and may also affect the ability to have children.

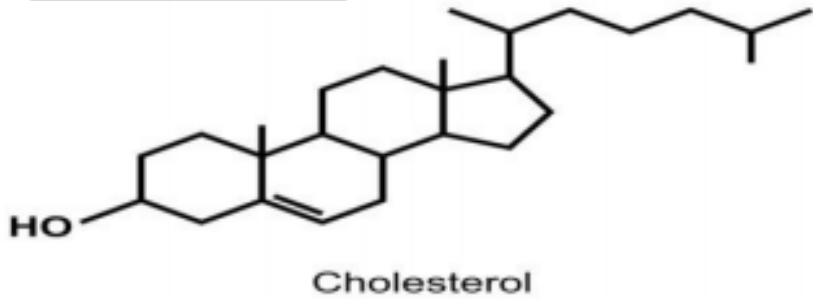


Steroid hormone breakdown

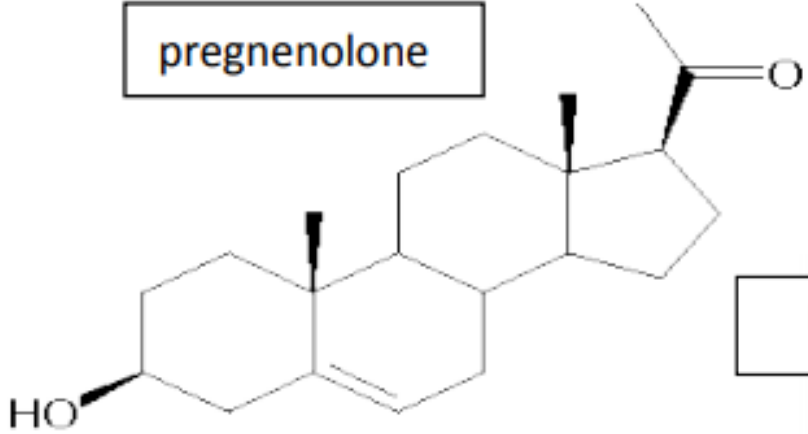
- Steran core cannot be cleaved
- In the liver: hydroxylation and conjugation with glucuronides or sulphates
- Urinary excretion:
 - Of metabolites
 - Of unchanged hormones

Enjoy

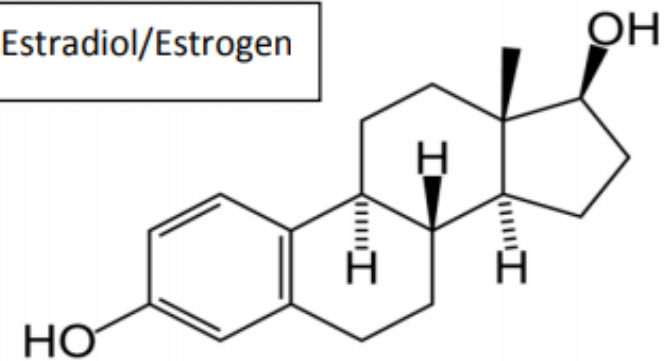
Cholesterol



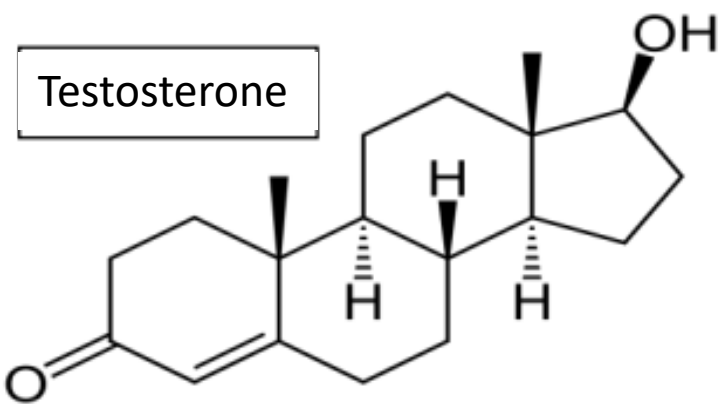
pregnenolone



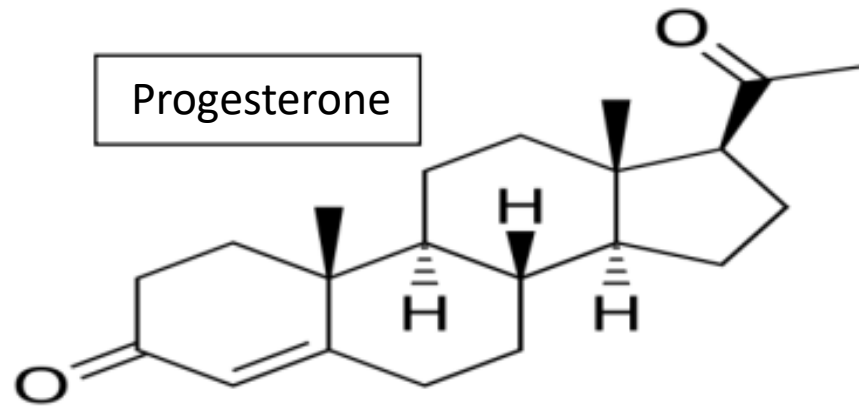
Estradiol/Estrogen



Testosterone



Progesterone



Chemistry of Hormones

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

- Catecholamines
- Proteins and peptides
- FA derivatives - eicosanoids

Receptor inside the cell

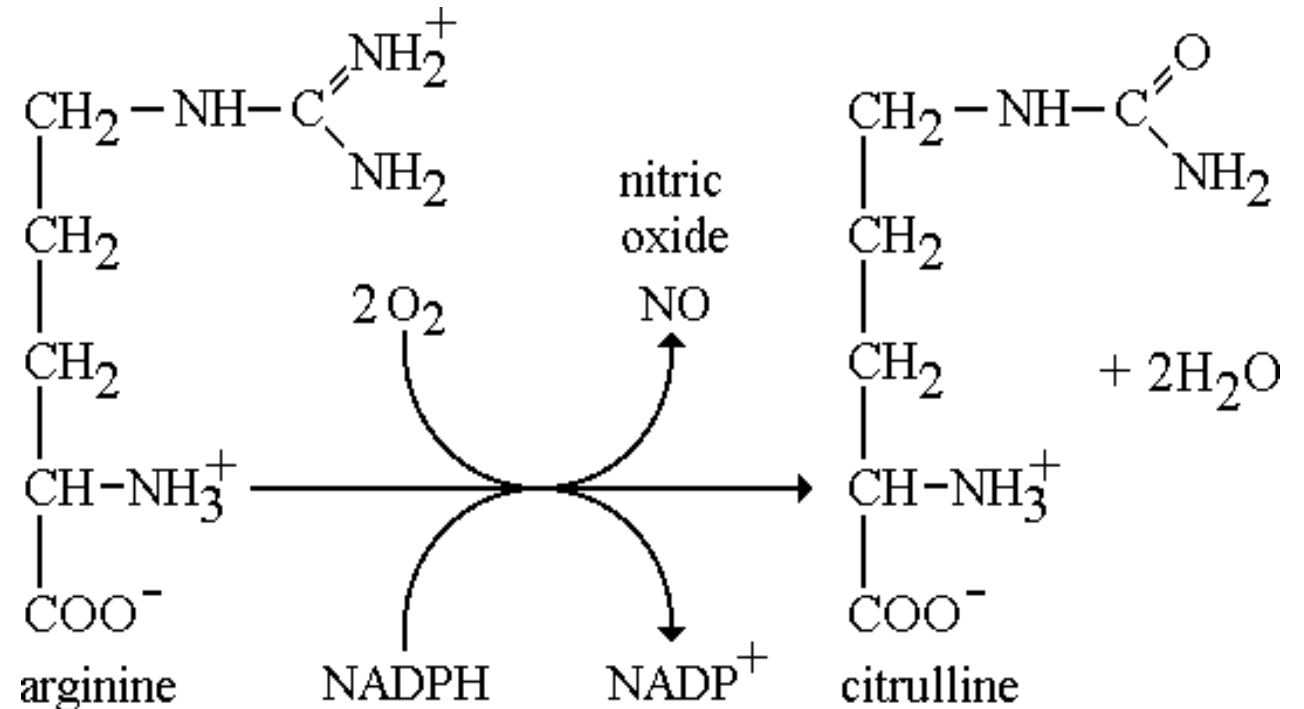
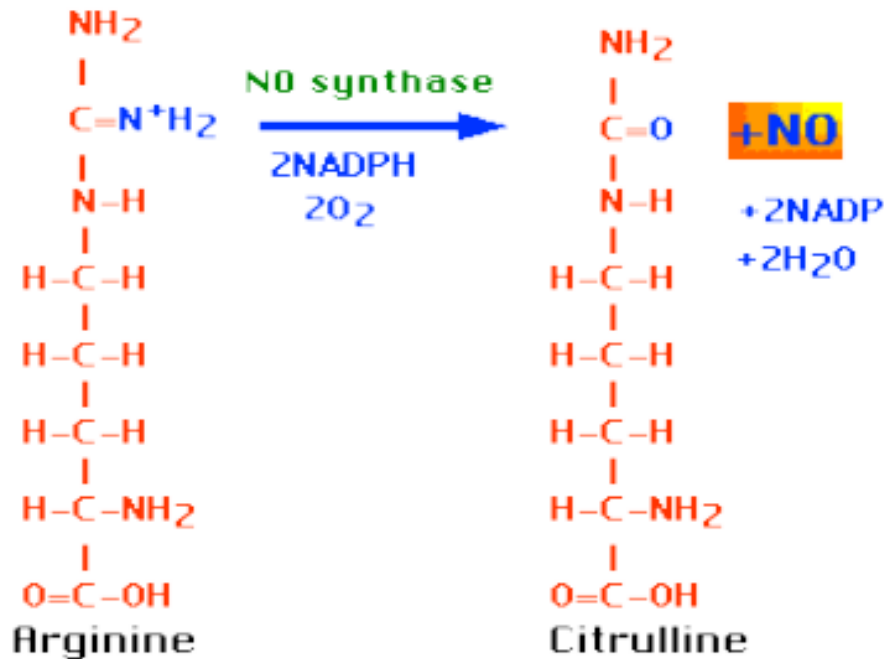


Surface receptor

Nitric oxide (NO)

- NO: synthesized by NO-synthase
- it works locally (paracrine hormone). NO is made by Nitric Oxide Synthase (NOS), which has different isozymes in different tissues.
- NO is a local vasodilator.

*NOS:



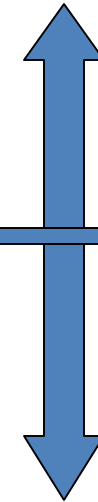
Nitric oxide synthase isozymes

- 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 . Nitrates, due to their vasodilatory action, are used to treat conditions resulting from vasoconstriction (leading to decreased blood flow to organs and damage). A famous example is the use of nitro-glycerine pills (sublingual pills) to treat Angina Pectoris (Chest-pain causing disease, due to decreased blood flow to the heart, major cause is obstruction/constriction of coronary vessels).
 - Refractory hypotension during septic shock . due to the presence of bacterial toxins in the circulation, septic shock may occur . These toxins interfere with this pathway, causing huge NO synthesis, and extreme vasodilation → severe hypotension, might cause coma.

Chemistry of Hormones

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- **Amino acid derivatives**
 - Thyroid hormones
 - Catecholamines
- Proteins and peptides
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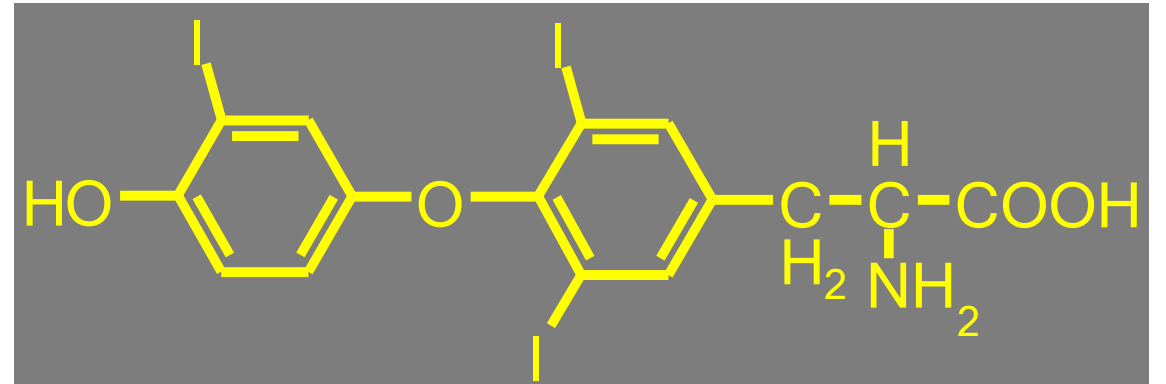
Receptor inside the cell



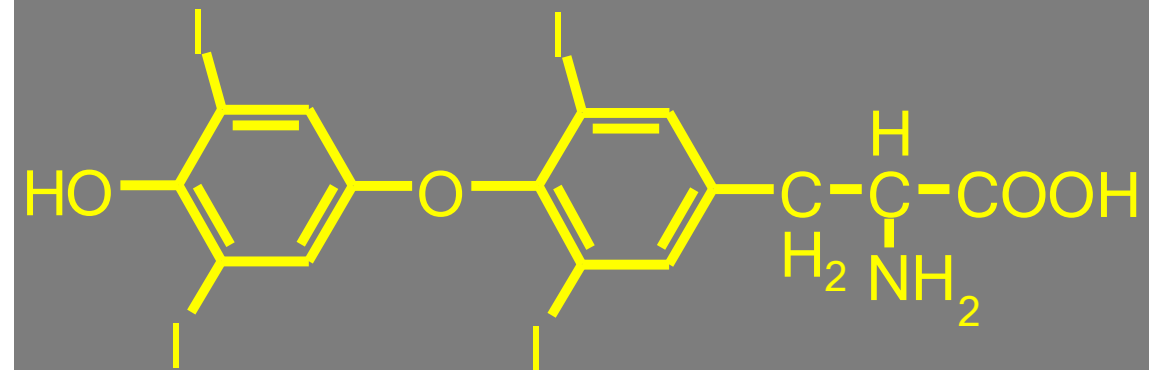
Surface receptor

Thyroid hormones

Thyroid hormones are basically a tyrosine molecule attached to a phenol (benzene ring and OH). Depending on how many iodines are added, we get T3 or T4.



Triiodothyronine (T3)



Thyroxine (T4)

Chemistry of Hormones

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

Receptor inside the cell

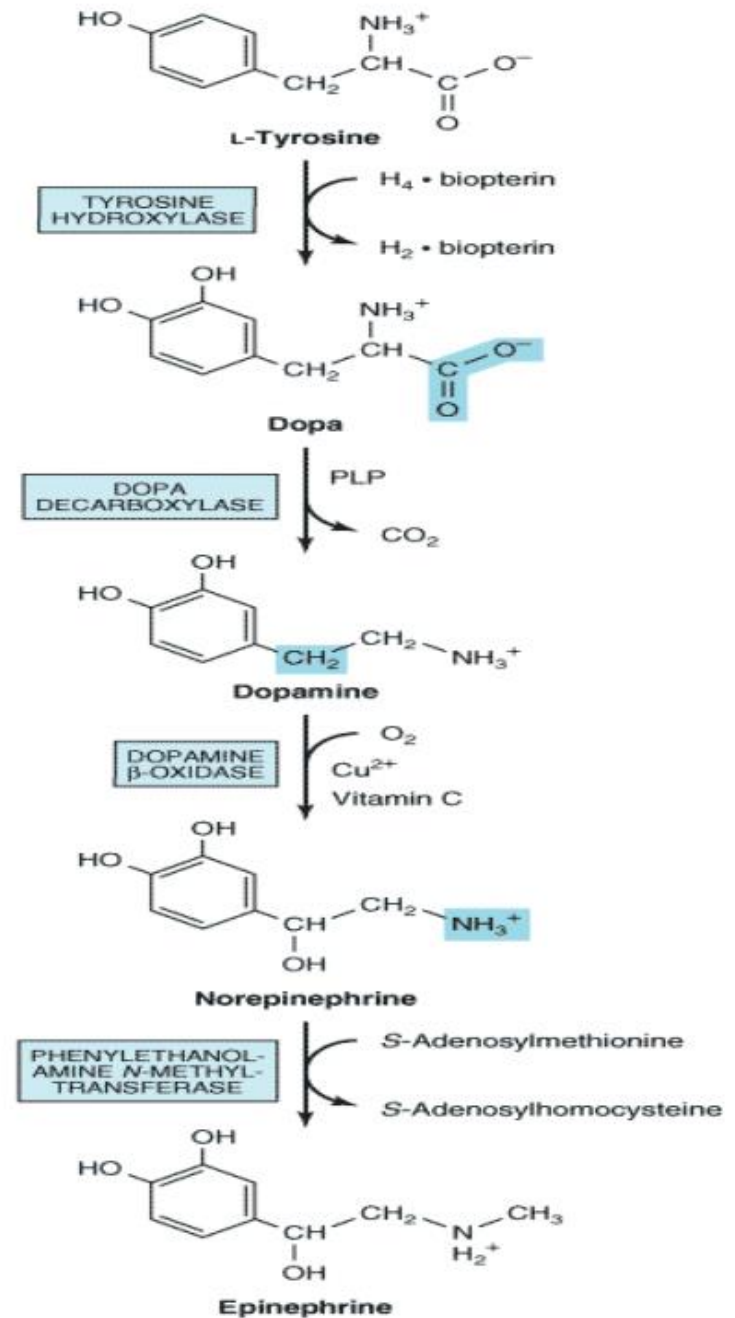
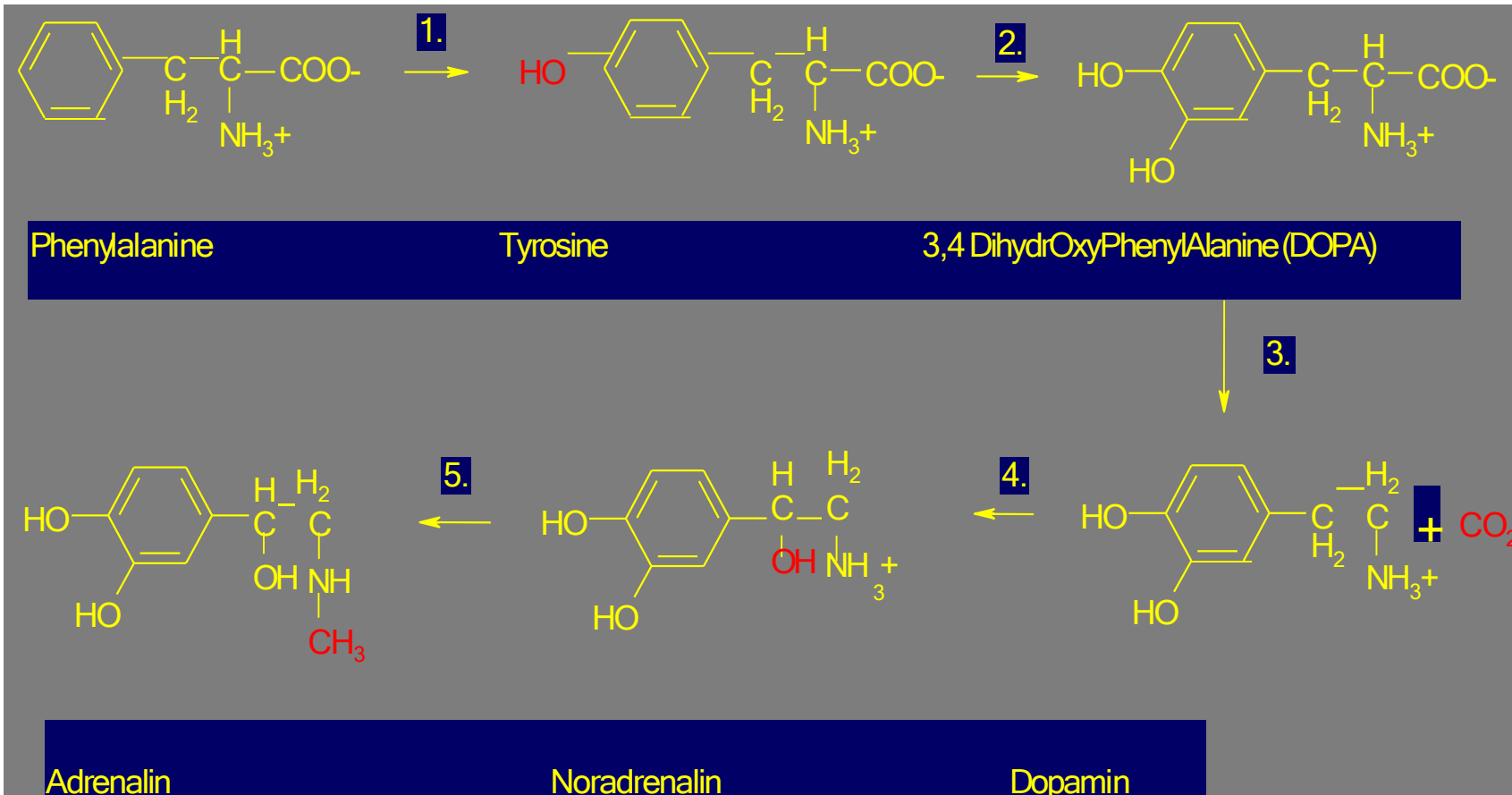


Surface receptor

Catecholamine synthesis

- Catecholamines are a group of molecules, all contain a catechol ring present on Tyrosine, they also have an amino group in their backbone. They include epinephrine, norepinephrine and dopamine.
- Substrate = Phe or Tyr
- Synthesis located in : adrenal medulla, nerve tissue.
- Products:
 - Dopamine, adrenaline (hormones)
 - Noradrenaline (neurotransmitter)

Catecholamine synthesis



Catecholamine synthesis

Very Important

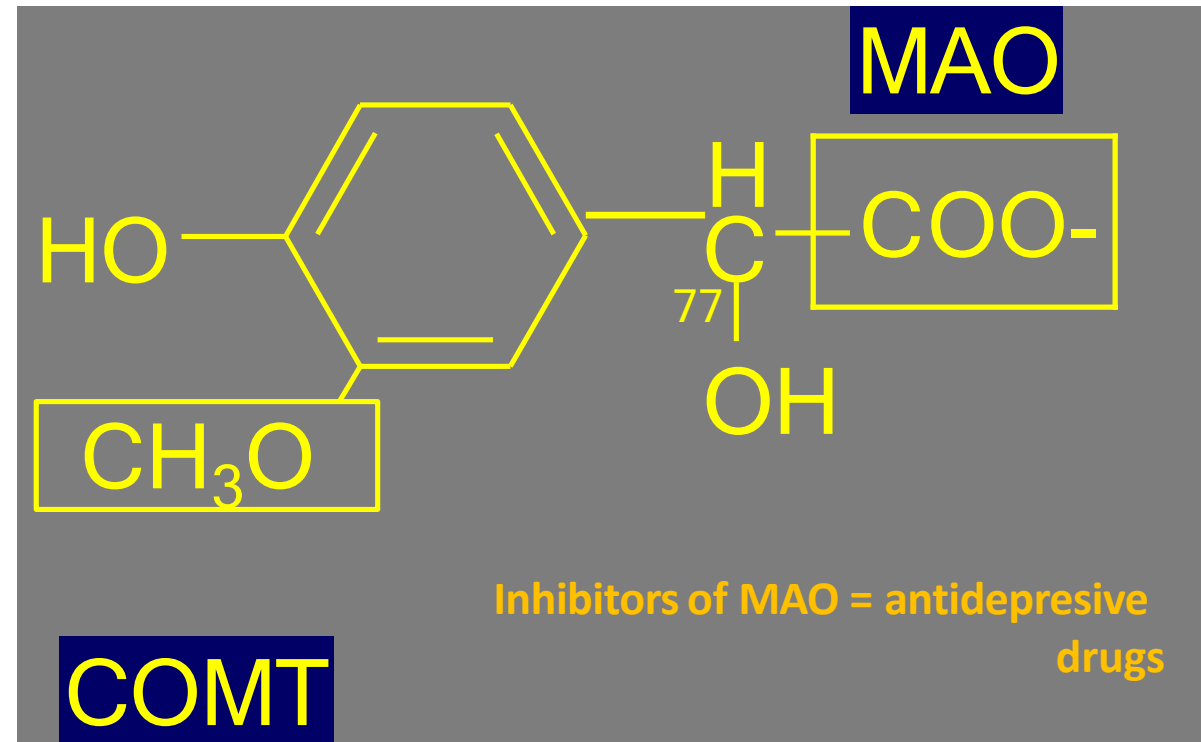
1. First we have Phenylalanine (essential amino acid which is the base for synthesis of all catecholamines). This amino acid is hydroxylated by Phenylalanine hydroxylase, the deficiency of which causes **Phenylketonuria disease** (PKU), and this step yields Tyrosine.
2. Tyrosine is also hydroxylated by Tyrosine hydroxylase (called Tyrosinase) to yield DihydroxyPhenylAlanine (DOPA). This enzyme deficiency results in variable degrees of **Albinism**, because it is involved in Melanin biosynthesis.
3. DOPA is decarboxylated by removing the $-\text{COOH}$ from the amino acid backbone to yield Dopamine.
4. Hydroxylation of dopamine to yield Norepinephrine. Lastly, methylation of norepinephrine to produce epinephrine.

Catecholamine breakdown

The degradation of Catecholamines:

It is done through 2 pathways :

1. We either start with the ring by transferring a methyl group to one of the OHs present on the ring. This step is done by Catechol-O-methyl transferases (COMT). Thus, catecholamines lose their activity. COMT inhibitors may be used therapeutically.
2. Or we start with the backbone. If we start with the backbone, we remove the amino group from the backbone through an oxidation process, and the enzymes which remove the amino group oxidatively are called monoamine oxidases (MAO). MAO inhibitors are used in psychiatric medicine as Anti-depressant, by preventing degradation of Catecholamines.



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

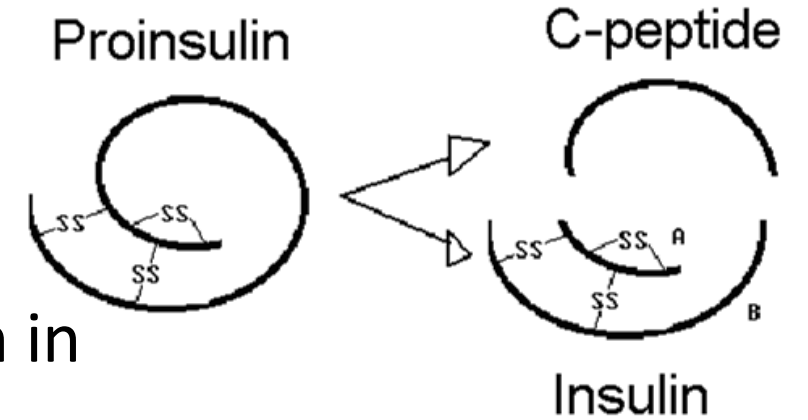
we previously discussed their synthesis. We have more than one way:

1. Synthesis of one major very long polypeptide chain, then fractionate it to more than one hormone (POMC → ACTH, MSH, Endorphines).
2. Synthesis of one big immature protein, then cleaving it to get mature hormone (preproinsulin → proinsulin → insulin).
3. Parent gene like neurophysin present in posterior pituitary gland, to which certain codons are attached, which makes Oxytocin in one place and Vasopressin in another.

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



Degradation of peptide hormones

- Lysosomal after endocytosis of complex hormone-receptor. If the protein is outside the cell (this applies to protein hormones), it undergoes endocytosis → vesicle → fuse with lysosomes → degradation. This is the energy-independent pathway (no need for energy).
- Chemical modification (liver): rearrangement of S-S bridges, cleavage. If the protein is inside the cell, it's degraded by the energy dependent Ubiquitin proteasomal pathway. But this does not apply to protein hormones.
- Renal excretion of small peptides, might also be broken down in blood.