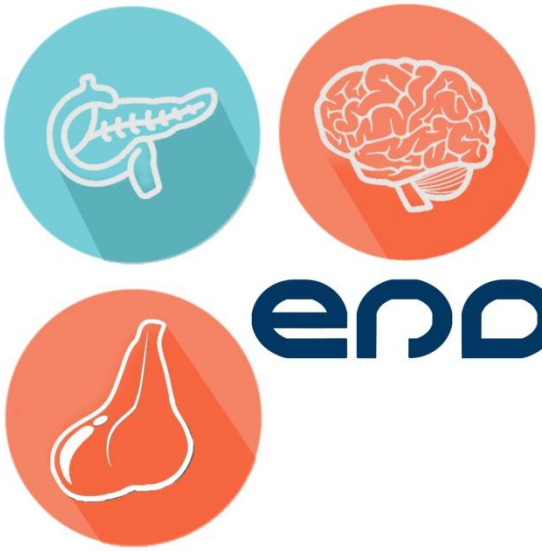


# endocrine system

# Biochemistry

Sheet Slide

number 3

Done by AbdelMuez Siyam & Abdullah Al Zibdeh

Correction

Doctor Nafeth

**At** the end of this sheet, there is a brief summary for the whole lecture. If you don't have time, just go through it.

## Review

There are two types of receptors:

1- 7TM; 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:

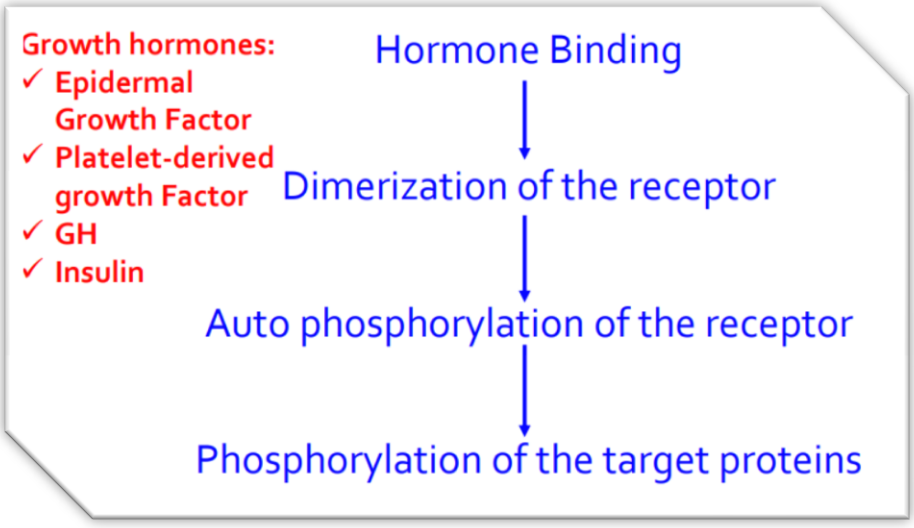
- Monomer, which dimerizes after ligand binding. All receptors of this family are monomers except for insulin receptor.
- Dimer, and the subunits are bound by disulfide bridges; such as insulin receptor.

The receptor spans the membrane, and has several subclasses (class II, Insulin R).

Receptor Domains

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

The Pathway



- 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 perform a kinase activity when activated.
- 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:

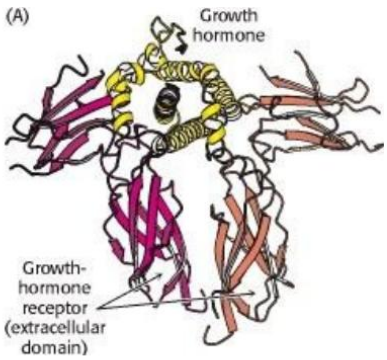
  *Binding – conformational change – activation – tyrosine residues phosphorylation – kinase activity*

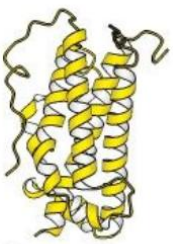
NOTE: although subtle, conformational changes allow the functionality of the proteins to take place.

NOTE: 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.

## 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)

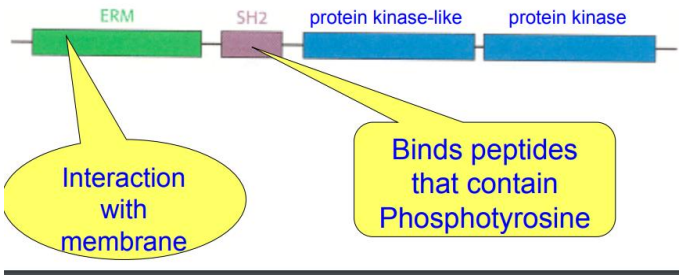




Human growth hormone

## Janus Kinase

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




JAK kinase domains include:

- Membrane binding domain (to be close to the receptor)
- 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 with their activation. Activated JAK kinases phosphorylate target molecules, and STAT, or *SignalTransducer&Activators ofTranscription*, is the most common one. STAT leads to transcription activation, having the DNA as its final target.

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

STAT is phosphorylated on a Tyrosine residue nearthe carboxyl terminus. Phosphorylated Tyrosine binds to SH2 domain of anotherSTAT 5 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 tgat follow the binding of a ligand to a receptor tyrosine kinase.

Examples on Receptor Tyrosine Kinases

Epidermal Growth Factor Receptor :♣Monomeric (inactive) ♣ EGF binding

⎩Dimerization ⎩Cross Phosphorylation ⎩Activation

Insulin Receptor:♣ Tetramer (2 α ; 2 β ), dimer (2 αβ pairs) ♣ Disulfide bridges

Insulin Binding →Activation of the Kinase

## RAS Protein

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 – RAS conformational change – GDP for GTP replacement – activation – 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: Loss of ability to hydrolyze GTPRas is locked in "ON" position. Continuous stimulation of growth.

- In mammals, three known types of RAS proteins are known, which are known to be mutated. If RAS (regardless of 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.

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***Eicosanoids***

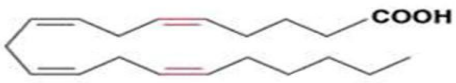
- ✓ They are examples of paracrine and autocrine hormones. They are small molecules which are derived of fatty acids in their nature. They do not go through the blood to far destinations (act locally). They are very potent, have short half lives and are not stored.

- ✓ Mainly include: Prostaglandins (PGs), Thromboxanes (TXs), Leukotrienes (LTs). All these are derived from one parent molecule called Arachidonic acid (fatty acid).

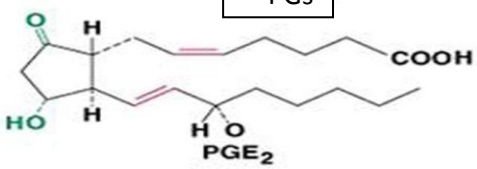
- ✓ This fatty acid is present in the plasma membrane's phospholipids. It is always bound to carbon number 2 of glycerol, and is released by the action of Phospholipase A2, which breaks it from glycerol. Then, it undergoes modifications in several reactions to produce either one of the above classes.

- ✓ Eicosanoids (Eicosa=20): group of molecules, each consists of 20 carbon unit. How to differentiate between them?

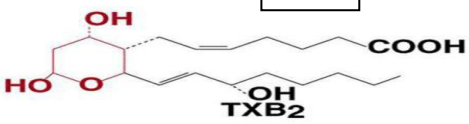
Arachidonic acid



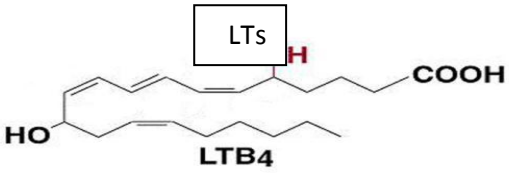

PGs




TXs




LTs




→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 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.

- ✓ Some of their functions (mainly physiology):

**PGI2, PGE2, PGD2 :**
**Increase Vasodilation, cAMP**
**Decrease**
- **▪Platelet Agregation**
- **▪Lymphocyte Migration**
- **▪Leucocyte Aggregation**

**PGF2α Increases**
***Vasoconstriction**
***Bronchoconstriction**
***Smooth Muscle Contraction**

**Thromboxane Increases**
***Vasoconstriction**
*** Platelet Aggregation**
***Lymphocyte Proliferation**
***Bronchoconstriction**

- ✓ 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.

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Synthesis and degradation of Hormones

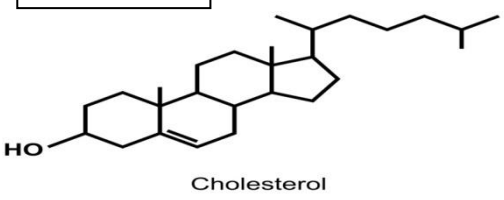
- Hormones are classified according to mechanism of action into 2 groups: some bind on receptors outside the cell (membrane), some bind intracellular receptors.

  **Hormones binding intracellular receptors:

- **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.

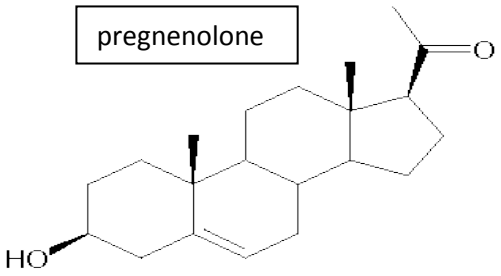
  **You must know these 18,19,21 carbon steroids. To count quickly, it is a given that the rings have 17 carbons, just count what is extra.

Cholesterol




Cholesterol

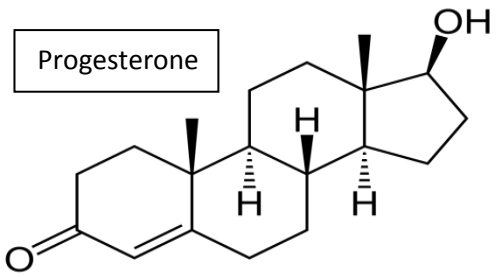
pregnenolone



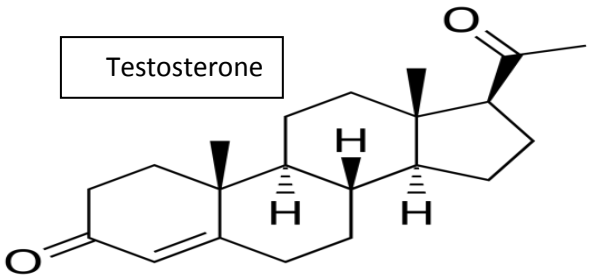

→**Pregnenolone**, 21-carbon steroid, is a parent molecule of sex hormones.

By further modification on it, like desaturation, we get the 21-carbon steroid of **Progesterone** (parent for others like Aldosterone and cortisol, both have 21Carbons)

Progesterone

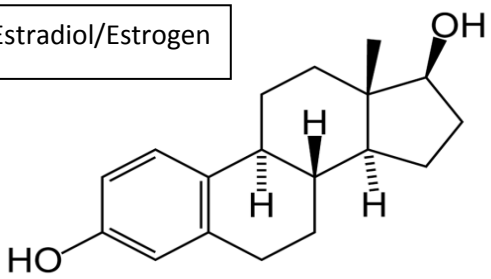



Testosterone




Removal of 2 carbons from progesterone (the acetyl group) produces the 19-carbon steroid **Testosterone.** If testosterone loses one carbon, Estrogen is produced (18 carbons). This step (Testosterone→Estrogen) is catalyzed by Aromatase.

Estradiol/Estrogen



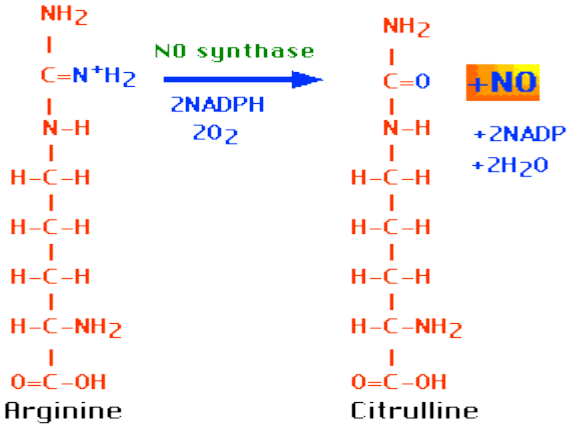

Side Note: Aromatase enzyme is affected by some pesticides. This may pose a problem for farmers by affecting male/female characteristics, and may also affect the ability to have children

- **Small molecules – Nitric oxide (NO):** it works locally (paracrine hormone). NO is made by Nitric Oxide Synthase (NOS), which has different isozymes in different tissues: it is present in neurons (neurotransmission), macrophages (kills bacteria), and most importantly in smooth muscles of blood vessels. NO is a local vasodilator.

  →Clinical application: 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 nitroglycerin 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).

  →Septic shock: due to presence of bacterial toxins in the circulation. These toxins interfere with this pathway, causing huge NO synthesis, and extreme vasodilation→ severe hypotension, might cause coma.

*NOS:




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

**Hormones with receptors outside the cell:**

- **Catecholamines:** 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.

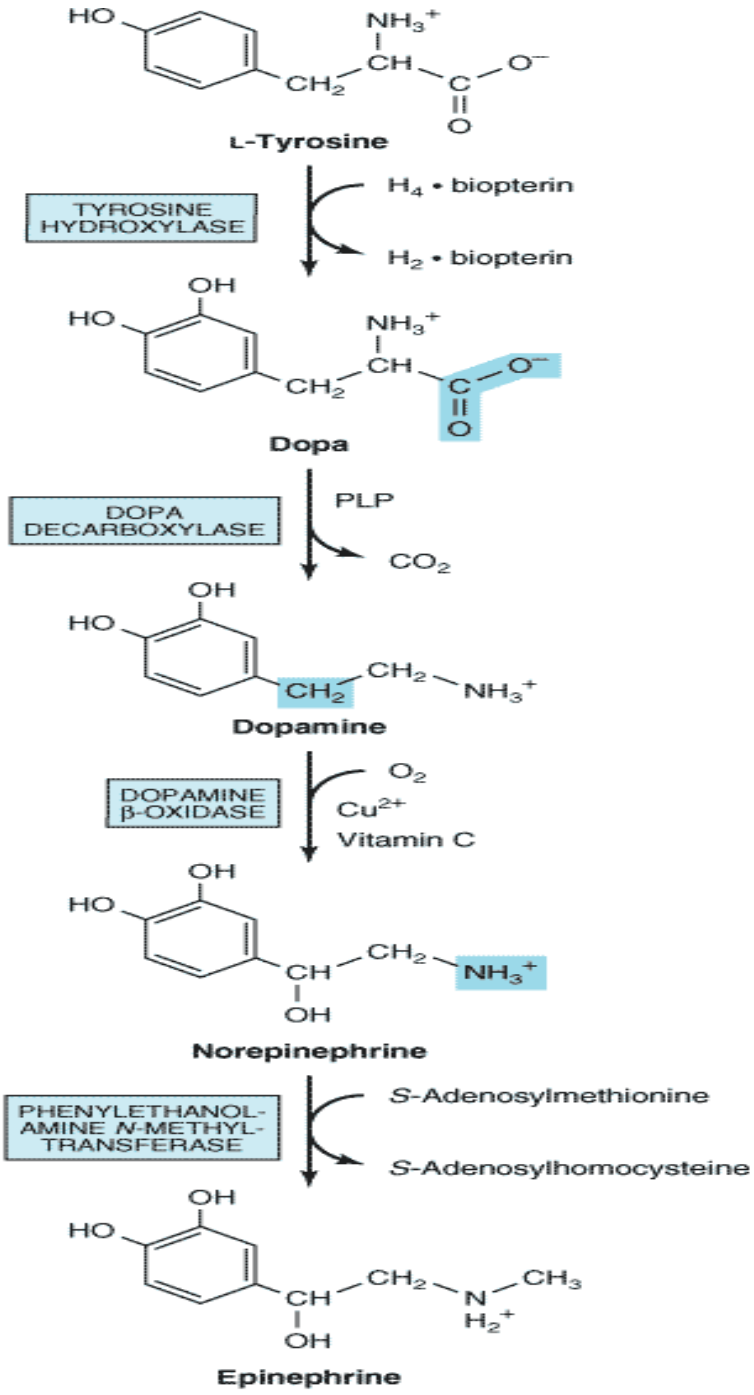
  *Their synthesis is important and required:

  1. First we have Phenylalanine (essential amino acid which is the base for synthesis of all catecholamines). This amino acid is hydroxylated by Phenyalanine hydroxylase, the deficiency of which causes Phenylketonuria disease (PKU), and this step yields Tyrosine.

  2. Tyrosine is also hydroxylated by Tyrosine hydroxylase (also 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 –COOH from the amino acid backbone to yield Dopamine.

  4. Hydroxylation of dopamine to yield Norepinephrine. Lastly, methylation of norepinephrine to produce epinephrine.




****The degradation of Catecholamines***: It is done through 2 pathways: either we start with the ring, 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-depressent, by preventing degradation of Catecholamines. The other pathway (start with the catechol ring) is by transferring a methyl group to one of the OHs present on the ring. This step is done by Catechol-O-methyl tranferases (COMT). Thus, catecholamines lose their activity. COMT inhibitors maybe used therapeutically.

- **Proteins and peptide hormones:** 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 cleave to get mature hormone (preproinsulin→proinsulin→insulin).

  3-Parent gene like neurophysin present in posterior pituitary gland, to which certain codons are attached, which make Oxytocin in one place and Vasopressin in another.

  **Degradation of protein hormones: Proteins generally are degraded as follows:

  I. 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)

  II. 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***.
  **Some protein hormones are excreted in the urine or broken down in blood.

***Good luck***

***The summary***

## Receptor Tyrosine Kinases Cascade

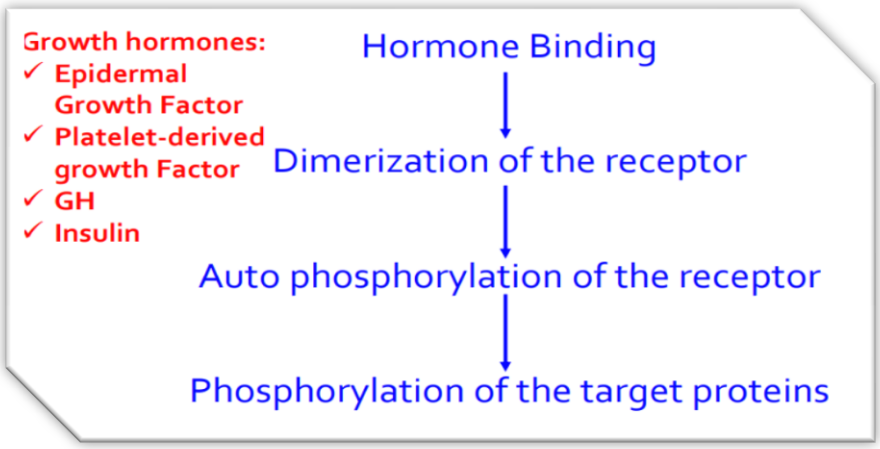
This receptor is a kinase enzyme, and the pathway involves Tyrosine amino acid phosphorylation. This pathway is used by most growth-related hormones (Insulin, GH, growth factors…). There are two classes of this receptor:

- 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.

Receptor Domains

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

The Pathway




- Binding of the ligand leads to conformational change, which results with monomers dimerization. Dimerization is a hallmark of this pathway. Binding has two forms:
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- 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.
- This receptor 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.
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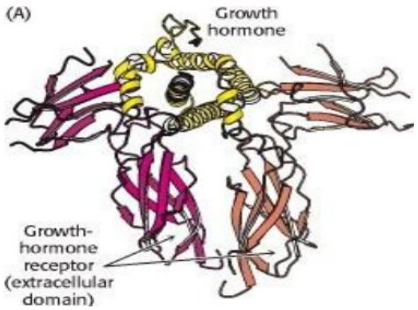
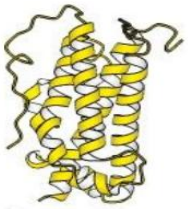
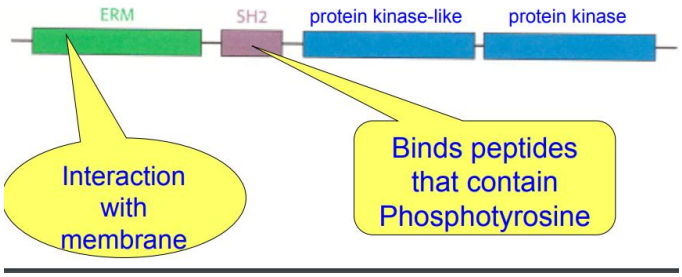




Human growth hormone

## Janus Kinase

JAK is bound to the monomers that get dimerized. JAK also has Tyrosine residues.

JAK kinase domains

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STAT is phosphorylated by JAK kinase on a Tyrosine residue nearthe carboxyl terminus. Phosphorylated Tyrosine binds to SH2 domain of anotherSTAT 5 molecule. SH2 domain is a phosphorylated Tyrosine binding domain. (SH2 domain 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 tgat follow the binding of a ligand to a receptor tyrosine kinase.

Note: If JAK2 remains active it will produce Cancer

Examples on Receptor Tyrosine Kinases

Epidermal Growth Factor Receptor :♣Monomeric (inactive) ♣ EGF binding

⎩Dimerization ⎩Cross Phosphorylation ⎩Activation

Insulin Receptor:♣ Tetramer (2 α ; 2 β ), dimer (2 αβ pairs) ♣ Disulfide bridges

Insulin Binding →Activation of the Kinase

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RAS protein is a monomeric G protein. It works in a similar manner to α-subunit of G proteins. So, its activation includes:

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Mammalian cells contain 3 Ras proteins.

Mutation of RAS GTPase domain: Loss of ability to hydrolyze GTP. Ras is locked in "ON" position. Continuous stimulation of growth.

***Eicosanoids** act locally (autocrine and paracrine), all members have 20 carbons, they have short half life and are very potent. They include Prostaglandins(PGs), Thromboxanes(TXs), and Leukotrienes (LTs).

*All eicosanoids are made from Arachidonic acid, which is attached to carbon 2 of glycerol in membrane lipids. Phospholipase A2 breaks that bond and release AA. This is the rate limiting step in eicosanoid synthesis. Then AA is modified by cyclooxygenases to give PGs/TXs, or lipoxygenase to give LTs.

*Differences in structure: AA: no rings, 4 double bonds
PGs: 5 membered ring……..TXs: 6 membered ring
LTs: no rings, at least 3 conjugated double bonds (double-single-double-single-double-single)

*Eicosanoids have diverse and sometimes opposing actions. Some promote v.dilation, some v.constriction.

***Steroids** are made of cholesterol (27 carbons). All have 4 steran rings (17 carbons), which cannot be cleaved by the body; it's conjugated and released with bile (small amount with urine).

*What differs between steroids is what is attached to the rings. There are 18/19/21 carbon steroids.

21 carbon: Pregnenolone, progesterone, Aldosterone, cortisol.
19 carbon: testosterone
18 carbon: Estrogen (testosterone is converted to estrogen by Aromatase)

*Nitric Oxide (NO): local action, made by nitric oxide synthase NOS (Arginine +O2→citrulline +NO).

*NOS has many isoforms in different tissues, such as neurons, macrophages and smooth muscles of blood vessels, where it produces NO (local vasodilator).

*Treatment of Angina Pectoris (less blood to the heart) is by nitrates (due to v.dilatory action).
*Septic shock is when bacterial toxins cause huge NO synthesis→v.dilation→severe hypotension.

*thyroid hormones: [tyrosine with a phenol (benzene ring and OH)]+ 3 iodines (T3) **or** 4 iodines (T4).

*Catecholamines are synthesized as follows:
1-Phenylalanine is hydroxylated to tyrosine. 2-Tyrosine is hydroxylated to DOPA
3-DOPA is decarboxylated to dopamine. 4- dopamine is hydroxylated to norepinephrine
5-Norepinephrine is methylated to epinephrine

Note: If step 1 is deficient→phenylketonuria. If step 2 is deficient→Albinism.

*they are degraded by monoamine oxidases (MAO, remove amine group from backbone) or Catechol-o-methyl transferase (COMT, add methyl to catechol ring). If we inhibit MAO→Active catecholamines→treat depression.

*Protein hormones: synthesis is by
1-One long protein, cleaved to many hormones (POMC→MSH,ACTH,endorphins)
2-Immature protein, cleaved to mature one (preproinsulin→proinsulin→insulin)
3-parent gene, surrounded by codons that make it express one hormone in one place and another hormone in another place (Neurophysin→Oxytocin or vasopressin).

*Protein hormones can be excreted in urine or degraded in plasma, but mainly they are degraded by endocytosis of the hormone, then the vesicle fuses with a lysosome (energy independent process).

*Intracellular proteins (not for protein hormones), they are degraded by the Ubiquitin-proteasomal pathway (energy dependent).