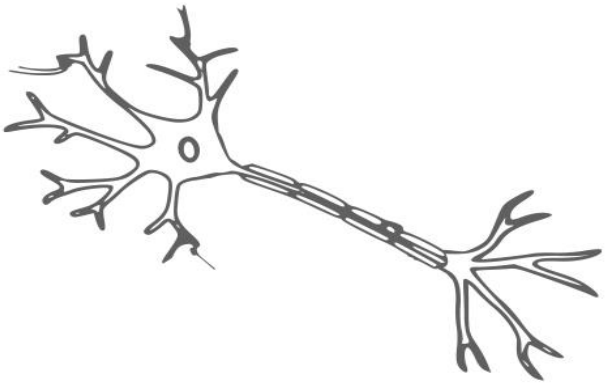
Sheet no.12

Physiology



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-We have talked previously about G protein coupled receptors (largest family of membrane receptors) and it is present in all eukaryotic cells, and there are more than 1000 types of it.

-The mechanism is that we have a receptor wich once it bounds to al ligand it gets activated and the cytosolic domain will activate another protein comlexes that are linked to the receptors, and this activation will results in dissociation of the complex, G(a) now is bound to GTP instead of GDP and is active and ready to go and target another membrane bound target, which is usually an enzyme such as adenylate cyclase.

**There are several subtypes of G(a) proteins subunit, please ponder the table below:

** the outline is required, just the outline, **(cheer up)**□□

TABLE 15-1 Major Classes of Mammalian Trimeric G Proteins and Their Effectors*

G_α CLASS	ASSOCIATED EFFECTOR	2ND MESSENGER	RECEPTOR EXAMPLES
$G_{\alpha s}$	Adenylyl cyclase	cAMP (increased)	β-Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin
$G_{\alpha i}$	Adenylyl cyclase K^+ channel ($G_{\beta\gamma}$ activates effector)	cAMP (decreased) Change in membrane potential	α_2-Adrenergic receptor Muscarinic acetylcholine receptor
$G_{\alpha olf}$	Adenylyl cyclase	cAMP (increased)	Odorant receptors in nose
$G_{\alpha q}$	Phospholipase C	IP_3, DAG (increased)	α_1-Adrenergic receptor
$G_{\alpha o}$	Phospholipase C	IP_3, DAG (increased)	Acetylcholine receptor in endothelial cells
$G_{\alpha t}$	cGMP phosphodiesterase	cGMP (decreased)	Rhodopsin (light receptor) in rod cells

*A given G_α subclass may be associated with more than one effector protein. To date, only one major $G_{\alpha s}$ has been identified, but multiple $G_{\alpha q}$ and $G_{\alpha i}$ proteins have been described. Effector proteins commonly are regulated by G_α but in some cases by $G_{\beta\gamma}$ or the combined action of G_α and $G_{\beta\gamma}$.
IP_3 = inositol 1,4,5-trisphosphate; DAG = 1,2-diacylglycerol.
SOURCES: See L. Birnbaumer, 1992, *Cell* **71**:1069; Z. Farfel et al., 1999, *New Eng. J. Med.* **340**:1012; and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.

Table 15-1
Molecular Cell Biology, Sixth Edition

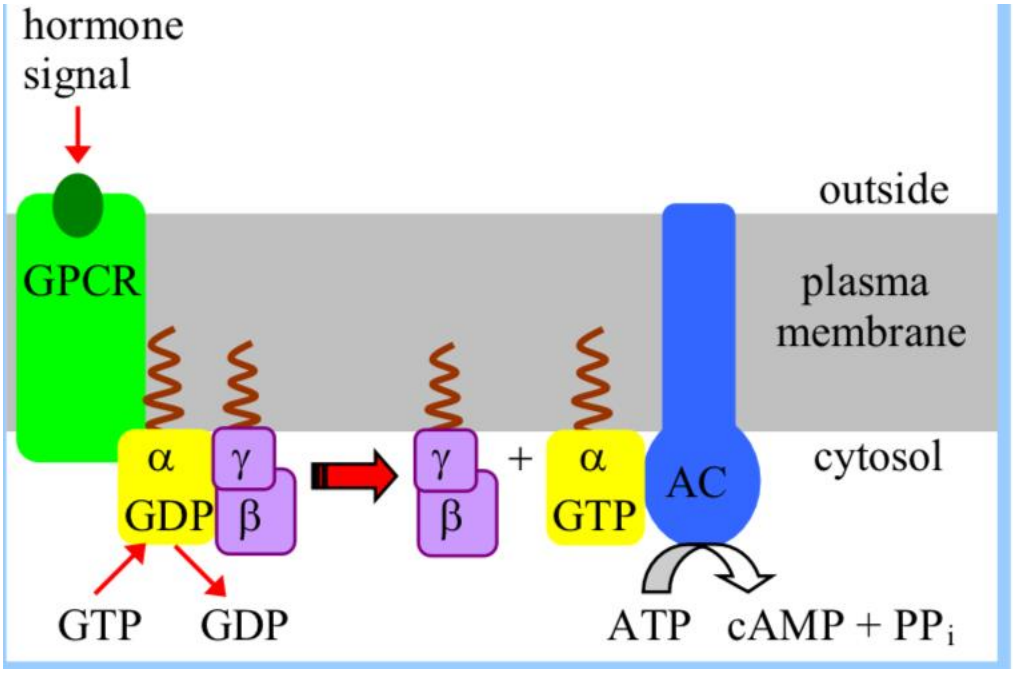
Required are outlined

Notes: the β-adrenergic receptor and α_2-adrenergic receptor and α_1-adrenergic receptor bind to the same ligand (epinephrine,norepinephrine)but induce different effects.

-Sammary of hormones signaling pathways: Will be discussed in the next lecture

IP_3	cAMP	cGMP	Tyrosine kinase - intrinsic	Tyrosine kinase - receptor associated	Steroid
GnRH	FSH	ANP	Insulin	Prolactin	Glucocorticoid
Gastrin	LH	NO (EDRF)	IGF-1	Cytokines (IL-2,6,8)	Estrogen
Oxytocin	ACTH		FGF	GH	Progesterone
TRH	TSH		PDGF		Testosterone
ADH (V_1)	CRH				Aldosterone
Histamine (H_1)	hCG				Vitamin D
Angiotensin II	PTH				T_3/T_4
	Calcitonin				Cortisol
	Glucagon				
	GHRH (can act via IP_3 as well)				

-G protein signal cascade:



-The (a) subunit of a G protein binds GTP, & can hydrolyse it to GPD + Pi.

-(a)& (y) subunits have covalently attached lipid anchors that bind a G protein to the plasma membrane cytosolic surface.

-Adenylate Cyclase (AC) is a transmembrane protein, with cytosolic domains forming the catalytic site.

-Adenylate Cyclase is inhibited by G(ai) and is activated by G(as).

-G protein coupled receptor bind to the hormone causing the activation of the receptor, the G(a) subunit become activated.

G(a) has bound GDP and it replaced by GTP.

-G(a)-GTP dissociates from the inhibitory (B,y) complex & can now bind to and activate Adenylate Cyclase to increase synthesis of cAMP.

-Immediately after G(a) activates the effector, it must become inactivated by the hydrolysis of GTP that is bound to it (happen automatically) and thus it is replaced by GDP and it rebinds with the G(B,y) complex and become inactive complex.

The sequence of events by which a hormone activates cAMP signalling:

1. Initially G(a) has bound GDP and (a), (B), & (y) subunits are complexed together, the complex of (B)& (y) subunits, inhibits G(a).

2. Hormone binding, usually to an extracellular domain of a 7-helix receptor (GPCR), cause a conformational change in the receptor that is transmitted to a G- protein on the cytosolic side of membrane.

-The binding site on G(a) becomes more accessible to the cytosol, G(a) releases GDP & binds GTP (GDP-GTP exchange).

3. Substitution of GTP for GDP causes another conformational change in G(a) .

-G(a)-GTP dissociates from the inhibitory (B,y) complex & can now bind to and activate Adenylate Cyclase to increase synthesis of cAMP.

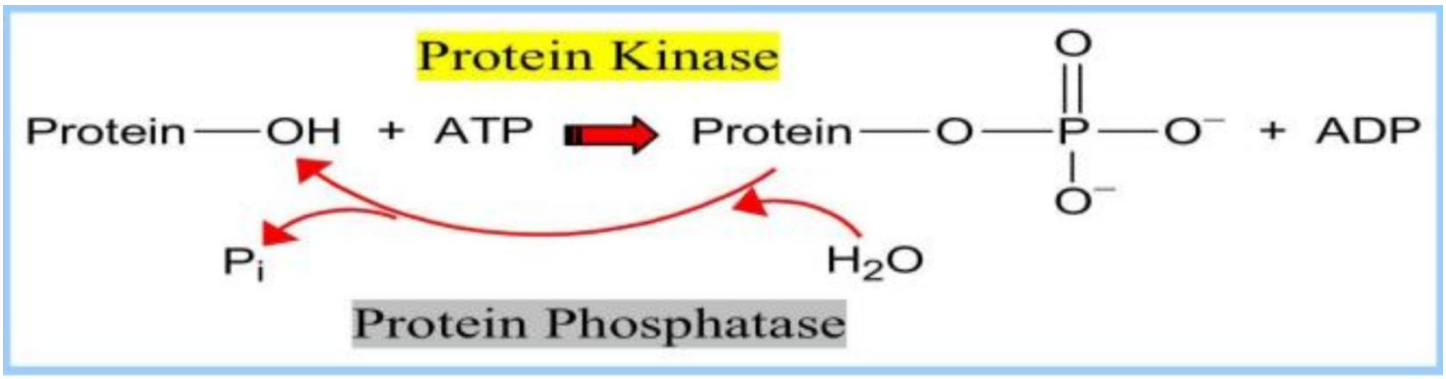
4. Adenylate Cyclase(effector), activated by the stimulatory G(a) -GTP, catalyses synthesis of cAMP(2nd messenger).

5. cAMP will activate protein kinase A (ENZYME) catalyses transfer of phosphate from ATP to serine or threonine residues of various cellular proteins, altering their activity (PHOSPOLYRATES THEM).

-ENZYMES THAT INVOLVE IN INTERCELLULAR SIGNALLING CAN BE KINASES OR PHOSPATESES:

Protein kinases and protein phosphatases do the exact opposite thing, as protein kinases phosphorylate the protein using ATP (note the phosphate group that is added to the protein in the products).

-However, protein phosphatases remove the phosphate groups from proteins. DEPHOSPLYERATED PROTIEN BY HYDROLYSIS REACTION.



-Protein kinases and phosphatases are themselves regulated (switched on and off) by complex signal cascades.

LIKE : *Protein Kinase A is activated by cyclic-AMP (cAMP).

For clarification and understanding (NOT required), **cheer up**

*Some protein kinases are activated by Ca++ -calmodulin.

-Protein Kinase A (cAMP-Dependent Protein Kinase) transfers Pi from ATP to OH of a Ser or Thr in a particular 5-amino acid sequence. (domains and subunits and the way it works are for your general knowledge, you don't have to memorise them also). Protein Kinase A in the resting state is a complex of:

• 2 catalytic subunits (C)

• 2 regulatory subunits (R).

-R2C2 : When each (R) binds 2 cAMP, a conformational change causes (R) to release (C).

-The catalytic subunits can then catalyse phosphorylation of Ser or Thr on target proteins.

-PKI and Protein Kinase Inhibitors, modulate activity of the catalytic subunits (C).

……

Turn off of the signal:

The signal should be turned off to avoid over regulation (so that the cell can be receptive for another stimulus after the first signal causes the required action).

It could be happen by different ways :

1. G(a) hydrolyses GTP to GDP + Pi . (GTPase). The presence of GDP on G(a) causes it to rebind to the inhibitory (B,y) complex.

-Adenylate Cyclase is no longer activated.

2. Phosphodiesterases catalyse hydrolysis of cAMP to AMP.

-Phosphodiesterase enzymes catalyse: cAMP + H_2O ⟶AMP(hydrolysis of cAMP).

-The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalysed by Protein Kinase A, Thus cAMP stimulates its own degradation, leading to rapid turn off of a cAMP signal. Don't panic (It is just The negative feedback thing)..

3. Receptor desensitization varies with the hormone.

• In some cases the activated receptor is phosphorylated via a G-protein Receptor Kinase.

• The phosphorylated receptor then may bind to a protein (B)-arrestin.

• (B)-Arrestin, (like tags on the receptor) promotes removal of the receptor from the membrane by clathrin mediated endocytosis.

•ANOTHER way (B)-Arrestin may also bind a cytosolic Phosphodiesterase, bringing this enzyme close to where cAMP is being produced, contributing to signal turnoff.

4. Protein Phosphatase catalyzes removal by hydrolysis of phosphates that were attached to proteins via Protein Kinase A. (if the enzyme was activated it inhibits it).

……..

* Different isoforms of G(a) have different signal roles. E.g.:

• The stimulatory Gs(a) , when it binds GTP, activates Adenylate cyclase

• An inhibitory Gi(a) , when it binds GTP, inhibits Adenylate cyclase.

-Different effectors & their receptors induce Gi(a) to exchange GDP for GTP than those that activate Gs(a) (Gi(a) and G(B) are activated by different effectors).

-The complex of G(B,y) that is released when G(a) binds GTP is itself an effector that binds to and activates or inhibits several other proteins.

-E.g., G(B,,y) inhibits one of several isoforms of Adenylate Cyclase, contributing to rapid signal turnoff in cells that express that enzyme.

Small GTP-binding proteins include (roles indicated):

ω initiation & elongation factors (protein synthesis).

ω Ras (growth factor signal cascades).

ω Rab (vesicle targeting and fusion).

ω ARF (forming vesicle coatomer coats).

ω Ran (transport of proteins into & out of the nucleus).

ω Rho (regulation of actin cytoskeleton)

**All GTP-binding proteins differ in conformation depending on whether GDP or GTP is present at their nucleotide binding site. Generally, GTP binding induces the active state.

NOTE: the doctor said you don't have to memorize their function (written in black)

In general u need to know there are GTP binding molecules that function like G α they function in signalling pathways and change their conformation depending on the GTP or GDP

And they transmit signals to other molecules or activate other molecules.

Most GTP-binding proteins depend on helper proteins:

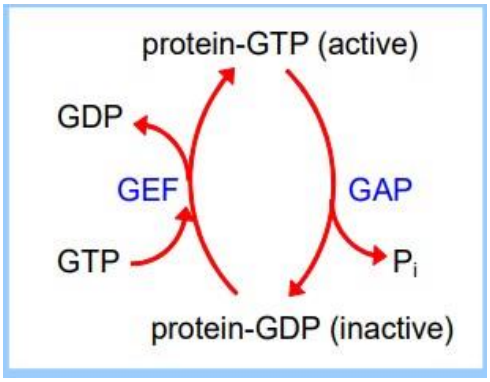
1.GAPs 2. GEF

1.GAPs: GTPase Activating Proteins, promote GTP hydrolysis.

So when the enzyme hydrolyses the GTP the protein becomes inactive

2. **GEF : G**uanine Nucleotide **E**xchange **F**actors, promote GDP/GTP exchange.

It transforms inactive GDP to active GTP protein.

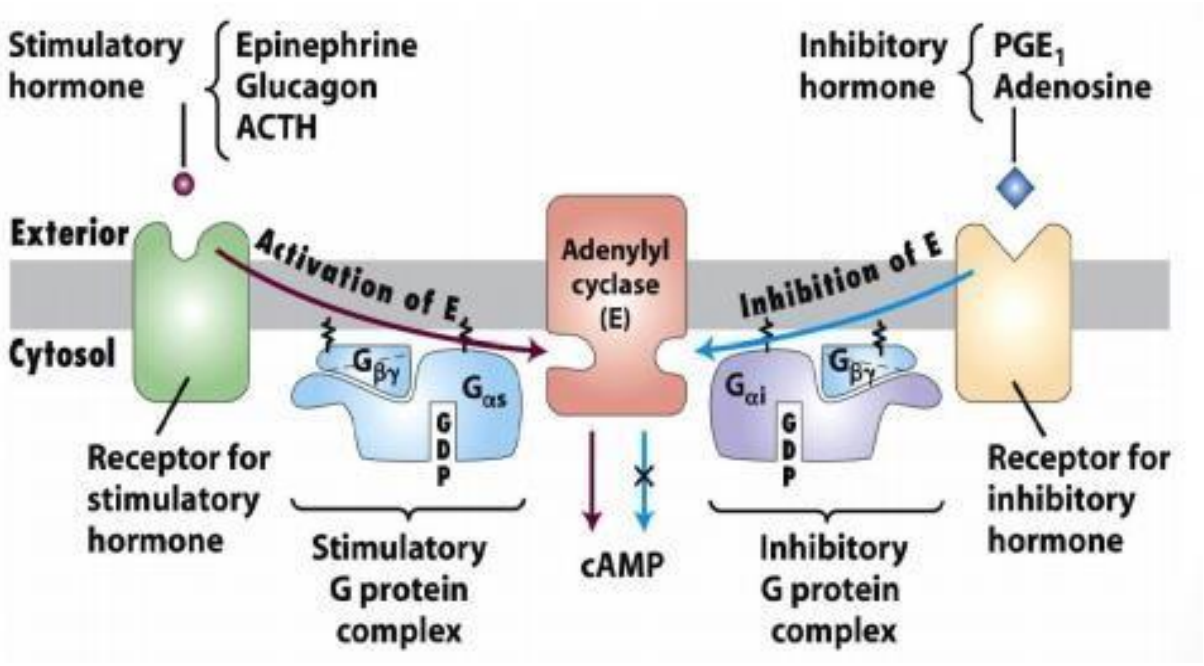


Gα of a heterotrimeric G protein has innate capability for GTP hydrolysis.it doesn't need GAPs

It has the essential arginine residue normally provided by a GAP for small GTP-binding proteins.

an activated receptor(GPCR) normally serves as GEF for a heterotrimeric G-protein.

the receptor transforms the Gα from GDP to GTP(activates it) so it does the work of GEF



signalling is complex.

This complexity lies in fact that we have different receptors that may have different effects at the same time!

We can see GPCR that are bound to Gαs and at the same time we have GPCR that is bound to Gαi.

Now different ligands or hormones might bind to the receptor with the Gαs such as Epinephrine, glucagon and ACTH to activate Gαs and activate Adenylyl Cyclase to increase cAMP.

At the same time, we can have inhibitory hormones acting on the GαI such as PGE1 and Adenosine thus reducing production of cAMP.

And we will have a net effect at the end which decides whether there is an increase or decrease in the cAMP concentration.

NOTE the names are for understanding you are required to understand the big picture only.

There are three types of surface receptors:

1-Ion-channel-linked receptors. (explained in the previous sheet).

2-G-protein-coupled receptors (GPCR). (explained in the previous sheet).

3-Enzyme linked receptors. (will be explained now).

Enzyme linked receptors: The 3rd type of cell membrane receptors is classified into:

1-Tyrosine Kinase-Linked receptors (TKRs). these receptors have enzyme called "Tyrosine kinase" in the same receptor, the enzyme is part of the receptor, it is located in the intracellular part.

2-Tyrosine Kinase non-covalently associated with receptor (NRTKs). TK is associated to these receptors, it's not part of the receptors.

3-Receptors associated with other types of enzymes.

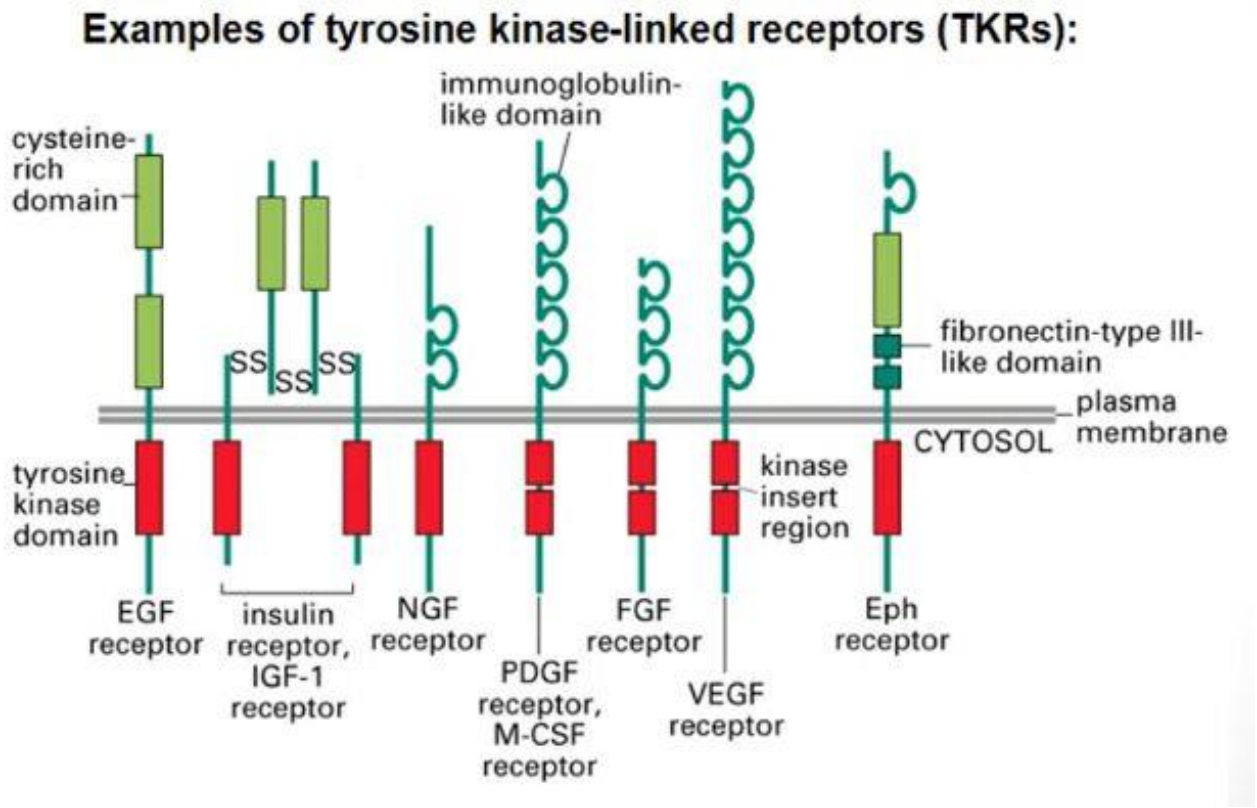
-Tyrosine Kinase-Linked receptors (TKRs) Overview about TKRs:

1. Cell surface receptors that are directly linked to intracellular enzymes (kinases).

2. Includes receptors for most growth factors (NGF, EGF. PDGF), insulin, and Src (Insulin receptor is the most famous).

3. Common structure: N terminal extracellular ligand-binding domain, single TM domain, cytosolic C-terminal domain with tyrosine kinase activity.

4. Can be single polypeptide or dimer " receptor can consist of one or more unit; Ex. Insulin is a dimer.



In the figure above you can see a group of cell surface receptor that are directly linked to intracellular enzyme (kinase), they are intrinsic enzyme in the receptors.

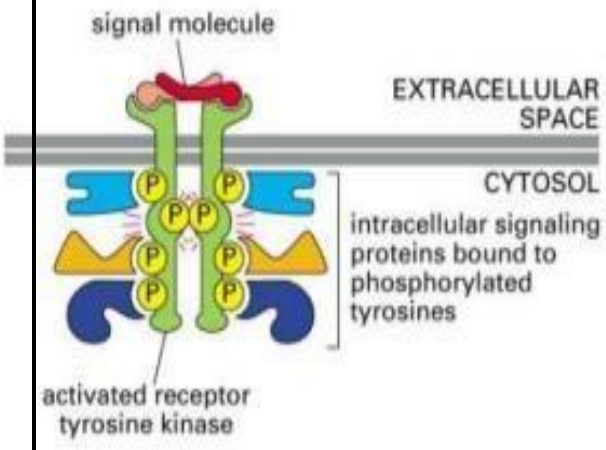
Mechanism of activation of TKRs:

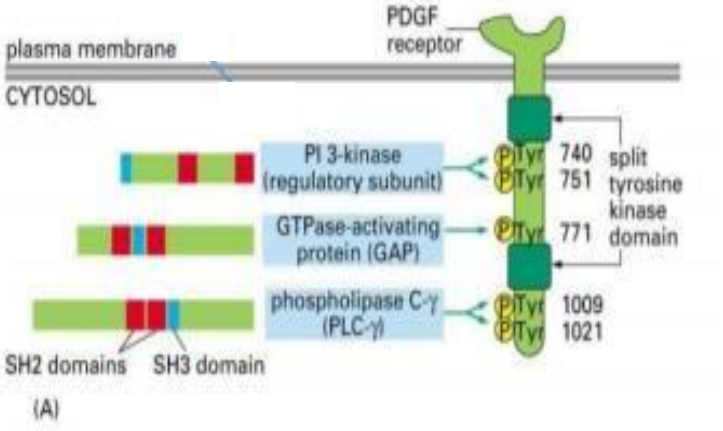
When Ligand bind to receptor, it induces dimerization (cross linking) of 2 units

Dimerization lead to auto phosphorylation of the enzyme in the receptor (cross phosphorylation)

Phosphorylation increases kinase activity, it means that there will be phosphate groups in the intracellular domain (TK),(tyrosine becomes phosphorylated) like in the 1st pic below, which create specific binding sites for other signalling proteins, proteins that bind to these binding sites transmit intracellular signals.

Then phosphate groups can bind to other molecules. (in the 2nd pic below, you can see types of molecules that can bind to phosphate).





Tyrosine Kinase

A.Insulin receptor consists of 2 units that dimerize when they bind with insulin.

** – Insulin binds to ligand-binding site

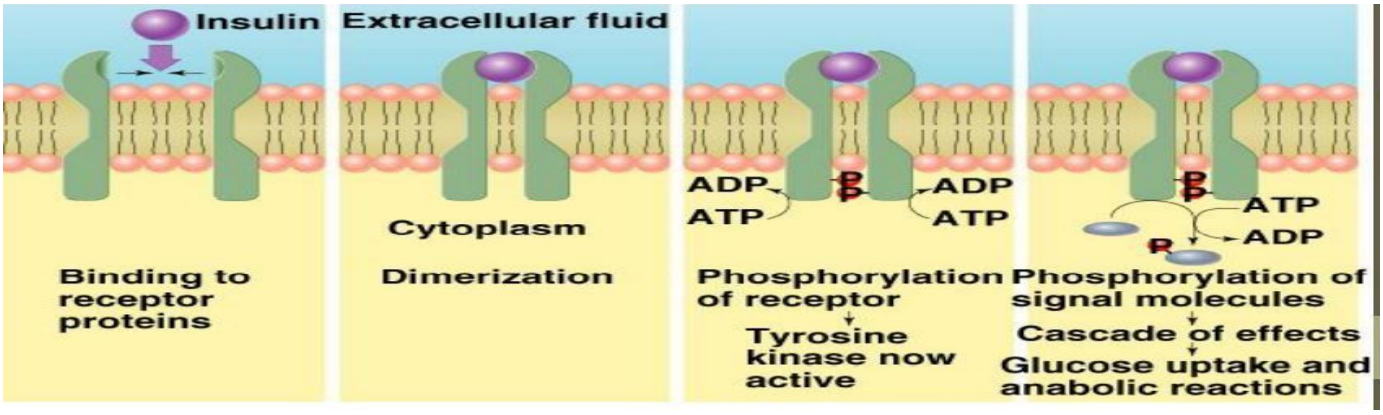
- binding site on plasma membrane, activating enzymatic site in the cytoplasm (intracellular domain)

B. Auto phosphorylation occurs, increasing tyrosine kinase activity.

C. Activates signalling molecules.

– Stimulate glycogen, fat and protein synthesis.

– Stimulate insertion of GLUT-4 carrier proteins, to facilitate entrance of glucose into the cell

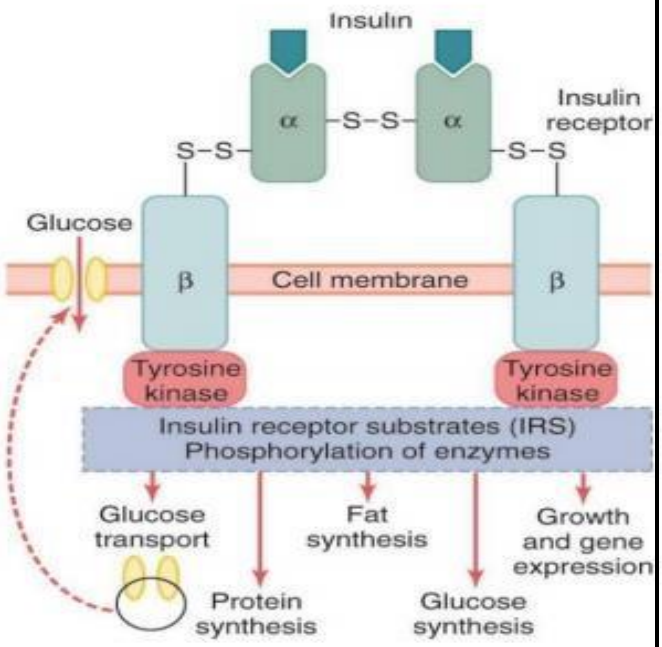


The pic shows:

1. how the insulin receptor has been dimerized after binding with insulin

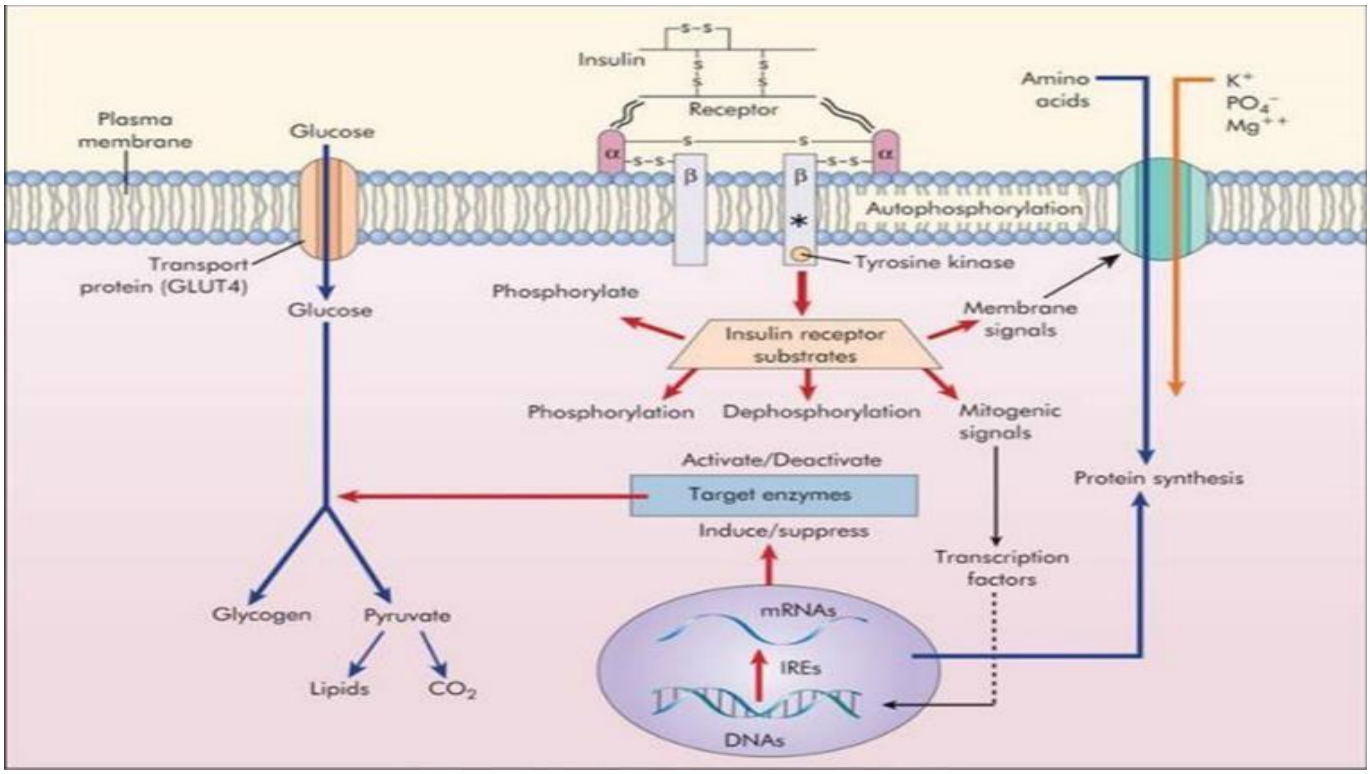
2. tyrosine kinase will become activated and then bind to IRS (there is a lot of substrate for insulin receptors)

3. a lot of enzymes that involves in insulin functions will be phosphorylation "" look at the function in the pic



Note: Insulin has many downstream targets such:

✓ Opens transporter of glucose. ✓ Increases the cell metabolic activity. ✓ Stimulates glycogen, fat and protein synthesis. ✓ Stimulates insertion of GLUT-4 carrier protein



This figure shows the general function of insulin.

Note the doctor said:

انا ما بركز كثير على ال Functions انا بهمني تعرفو شو ال Signaling mechanism

-second sub-group of enzyme-linked receptor:

B- Tyrosine Kinase non-covalently associated with receptor (NRTKs): (Examples: cytokine receptors, T & B cell receptors) = NRTKs

Cytokine receptors (famous example), as well as T and B cell receptors, stimulate tyrosine kinases that are non-covalently associated with receptor.

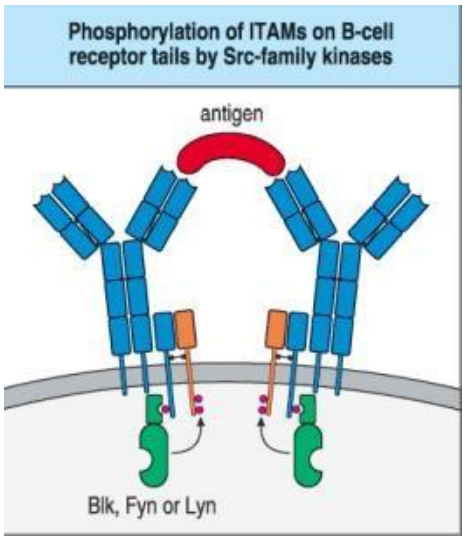
**Overview of Activation

1.N-terminus , extracellular ligand-binding domain, transmembrane a-helix, C-term. cytosolic domain

2. Cytosolic domain has no catalytic (kinase) activity(The difference between NRTKs and RTKs)

3. Acts in conjunction with a non-receptor tyrosine kinase that is activated as a result of ligand binding.

4. Activation is similar to that of RTKs: ligand binding causes cross phosphorylation of associated tyrosine kinases that phosphorylate the receptor, providing phosphotyrosine binding sites for recruitment of proteins with SH2 domains



(Ligand binding→dimerization→phosphorylation of the enzyme not to the receptor→ proteins (SH2) bind to the active site…(نفس القصة الحزينة) :

Two kinds of kinases associate with NRTKs:

1. Src family protein kinases - important for B and T cell signaling (not required)
2. Janus kinases (JAK) universally required for signaling from cytokine receptors. (Leptin) (required example)

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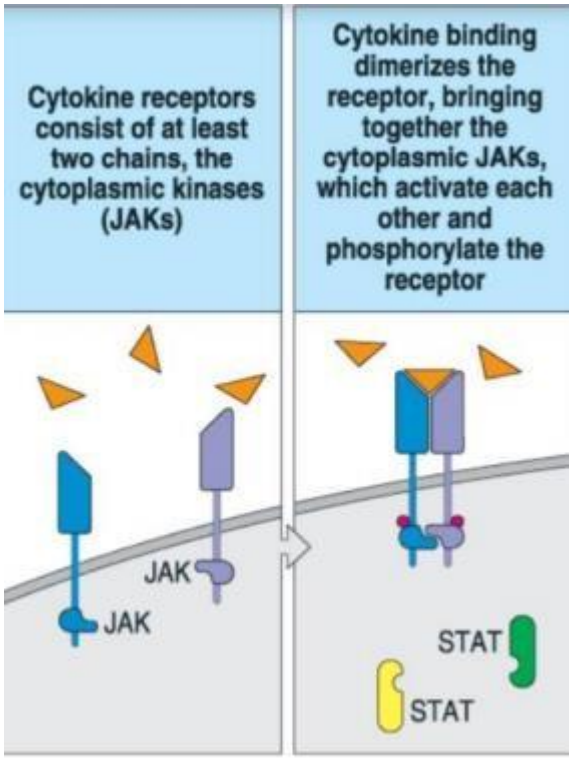

-Third sub-group of enzyme-linked receptor:

Receptors can be linked to or associated with other enzymes, besides TKs, i.e. *they can be linked (intrinsic)or associated*.

Protein-tyrosine phosphatases Remove phosphates, instead of adding phosphates, thereby terminate signals initiated by protein- tyrosine kinases.

Serine/ threonine kinases

i.e. TGF-β .

Guanylyl cyclases .

MASTER THE ART OF TIMING

Become a detective of the right moment; sniff out the spirit of time.

Learn to stand back when the time is not yet ripe, and to strike fiercely when it reaches fruition.