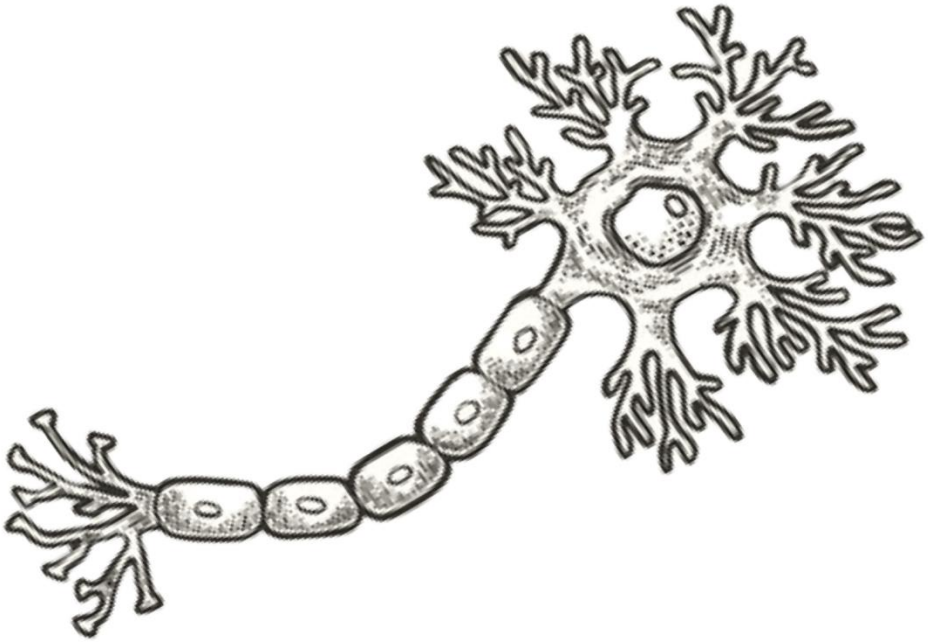
Teejan 2022

PHYSIOLOGY

SHEET NO.

19



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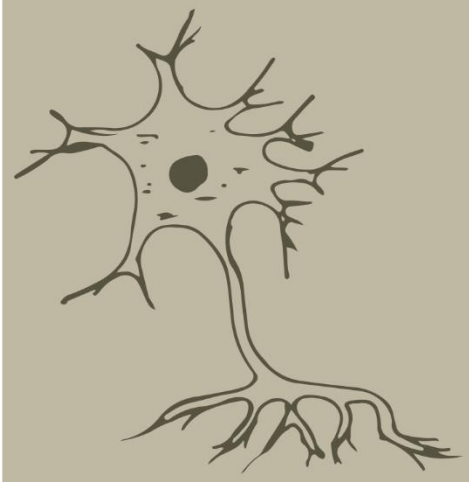


Signal transduction

As we discussed before in the activation of GTP-coupled receptor which will cause the alpha subunit in G protein (**that bound to the receptor**) to replace GDP-bound to be GTP-bound alpha subunit and this process will cause a change on its activity to be activated and it is catalyzed by the receptor, at the same time alpha GTP-bound will deactivate itself (**switching from GTP-bound to GDP-bound**).

Regarding signal molecules they are mostly either GTP/GDP-bound and this binding will effect on their activity, but mostly GTP-bound molecules are in their active state.

As we can see we didn't need any enzyme activity in this type of signaling instead we have small GTP-binding proteins where they differ in conformation depending on whether GDP or GTP is present at their nucleotide binding site.

Small GTP-binding proteins include (roles indicated):

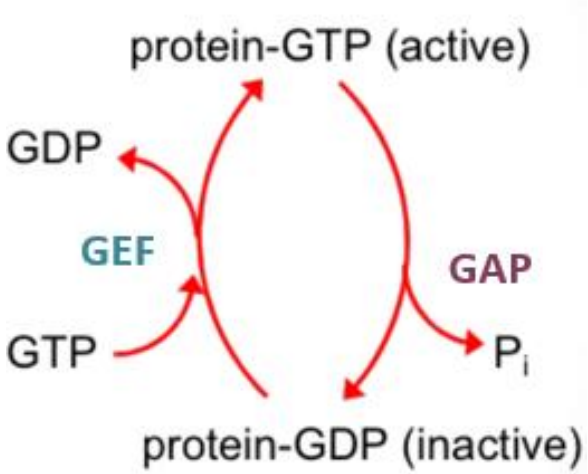
1. **initiation & elongation factors** (protein synthesis).
2. **Ras** (growth factor signal cascades).
3. **Rab** (vesicle targeting and fusion).
4. **ARF** (forming vesicle coatomer coats).
5. **Ran** (transport of proteins into & out of the nucleus).
6. **Rho** (regulation of actin cytoskeleton)

Most GTP-binding proteins depend on **helper proteins** (enzymes to change their conformation):

- **GAPs**, **G**TPase **A**ctivating **P**roteins, promote GTP hydrolysis (will lead from GTP to GDP "**inactivating**").

G_α of a heterotrimeric G protein has innate capability for GTP hydrolysis.

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



- **GEFs**, **G**uanine Nucleotide **E**xchange **F**actors, promote GDP/GTP exchange (will lead from GDP to GTP "**activating**").

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

Now, cellular signaling transduction is a very complicated process, multiple steps at a time as one signal induces a reflect in another signal.

In a given membrane we have different types of receptors like Gαs or Gαi and these receptors can be activated by different ligands like for Gαs can be activated by epinephrine, glucagon, …etc which will lead to activation (**increase**) of cAMP because they are bound to Gαs which in turn is linked to adenylyl cyclase. Same goes for the ligands that activate Gαi but it has a inhibitory activity on adenylyl cyclase (**decrease in cAMP**) and at same time there could be hormones or factors other than ligands. So the net effect will depend on the changes that occur regarding cAMP.

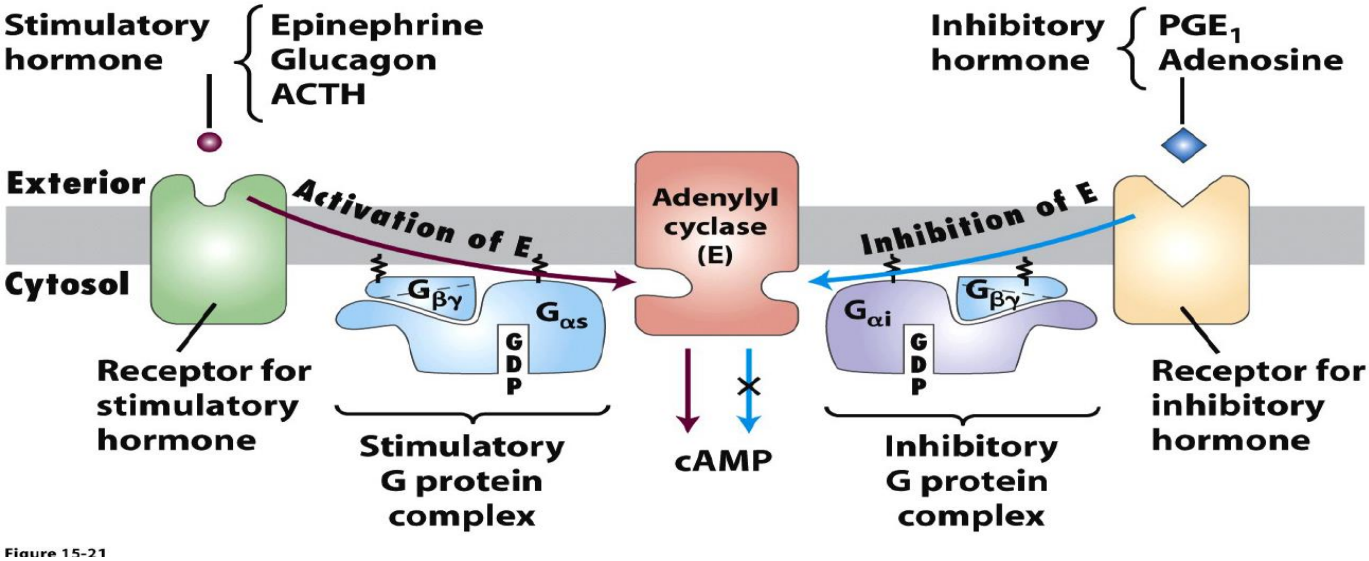


Figure 15-21

Now moving in to the third receptor

Enzyme-linked receptors:

1. **Tyrosine kinase-linked receptors (TKRs/RTKs).**

A. Overview of TKRs/RTKs:

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

2. Includes receptors for most **growth factors** (NGF, EGF, PDGF, FGF, VEGF), insulin (most common), and Src.
3. Common structure: N terminal extracellular ligand-binding domain the (ligand is mostly hormone), single TM domain, cytosolic C-terminal domain with tyrosine kinase activity.
4. Can be single polypeptide or dime

NGF: Neuron growth factor.
EGF: Epidermal growth factor.
PDGF: platelets-derive growth factor.
FGF: Fibroblast growth factor.
VEGF: Vascular endothelial growth factor.

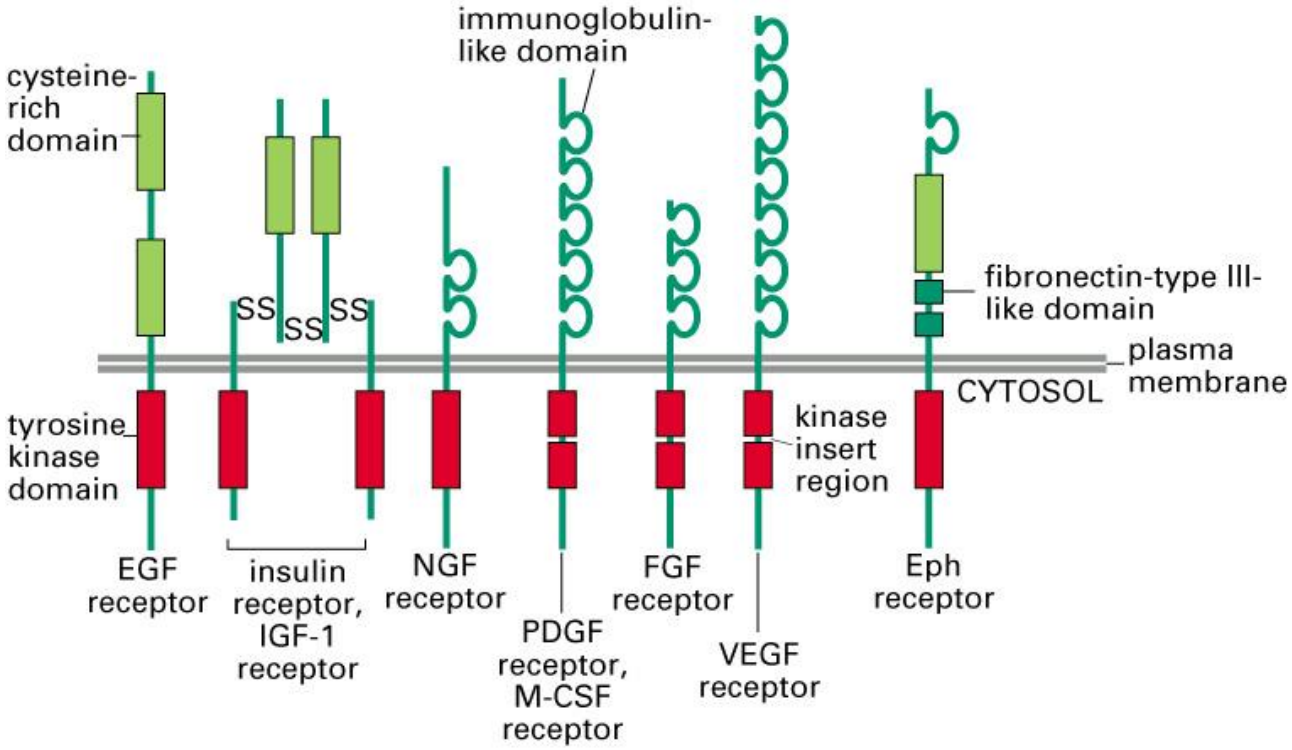


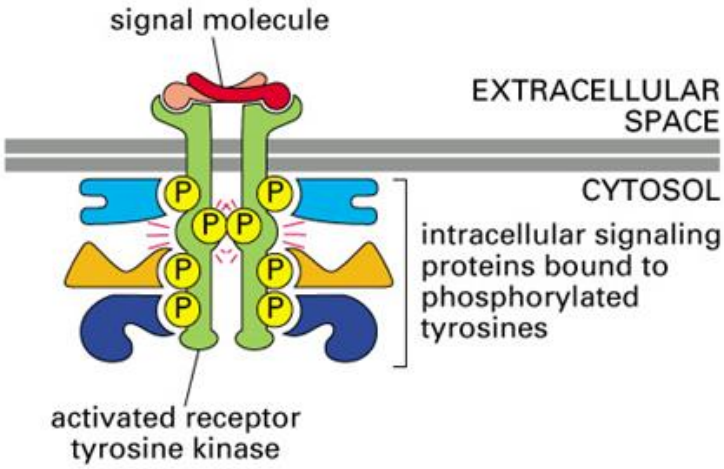
Figure 15–49. Molecular Biology of the Cell, 4th Edition.

B. Mechanism of activation of TKRs:

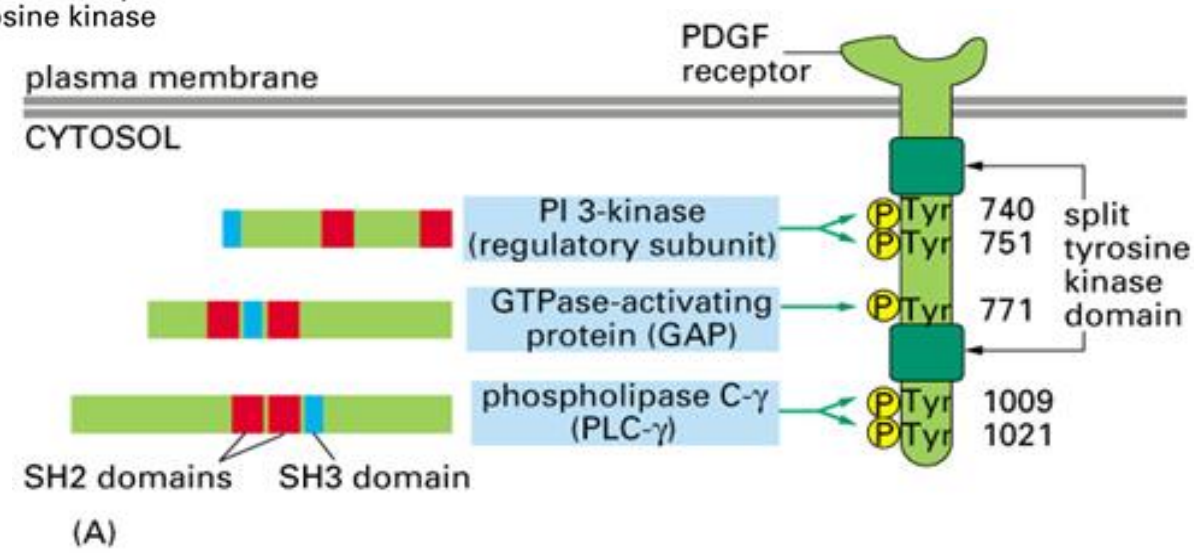
1. ligand binding induces receptor dimerization (receptor crosslinking).
2. dimerization leads to autophosphorylation of the receptor (cross-phosphorylation).
3. phosphorylation increases kinase activity (the more kinase is phosphorylated the more increase in its activity and will lead to phosphorylate other substrate that binds to it, like **IP3** binds to the activated or phosphorylated kinase and get phosphorylated/activated) & also creates specific new binding sites.

- The activation of the substrates is induced by either two ways the **1st** phosphate groups where they form a booking sites on the receptor bind to a protein **linking/anchoring**, the **2nd** way is by phosphorylation by kinase enzyme, either way the addition of phosphate group will induce changes in their activity leading to changes the activity of the cell).

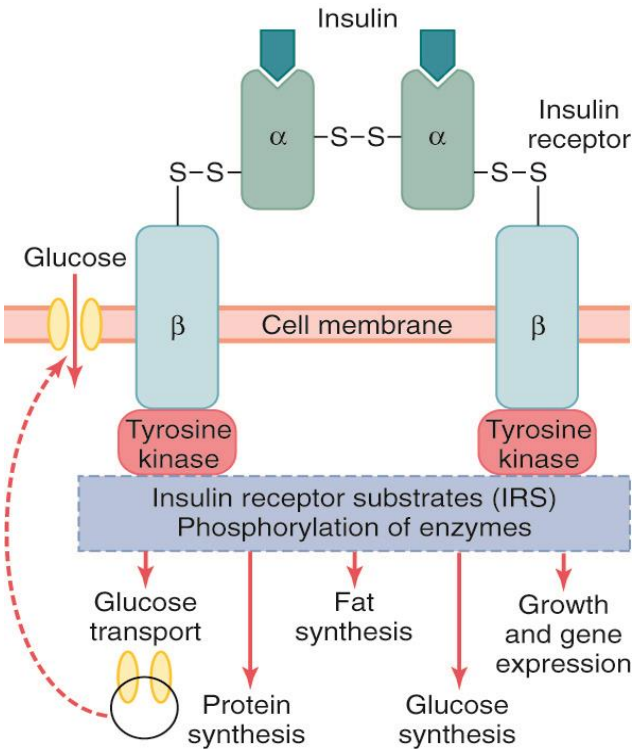
4. proteins that bind to these new binding sites transmit intracellular signals.

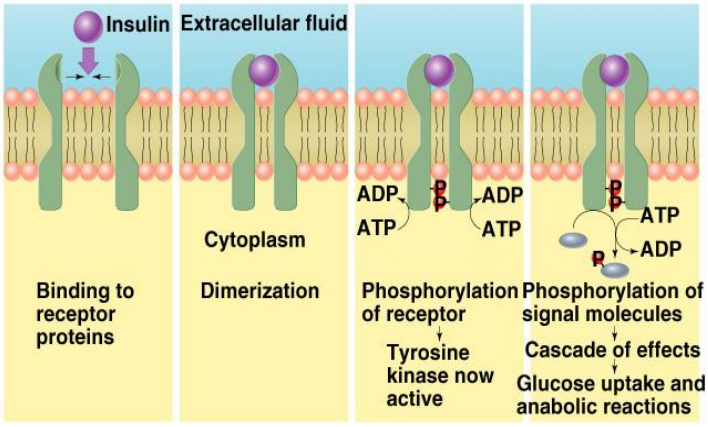


It could be a second messenger or phosphorylated proteins that transmit intercellular signals.

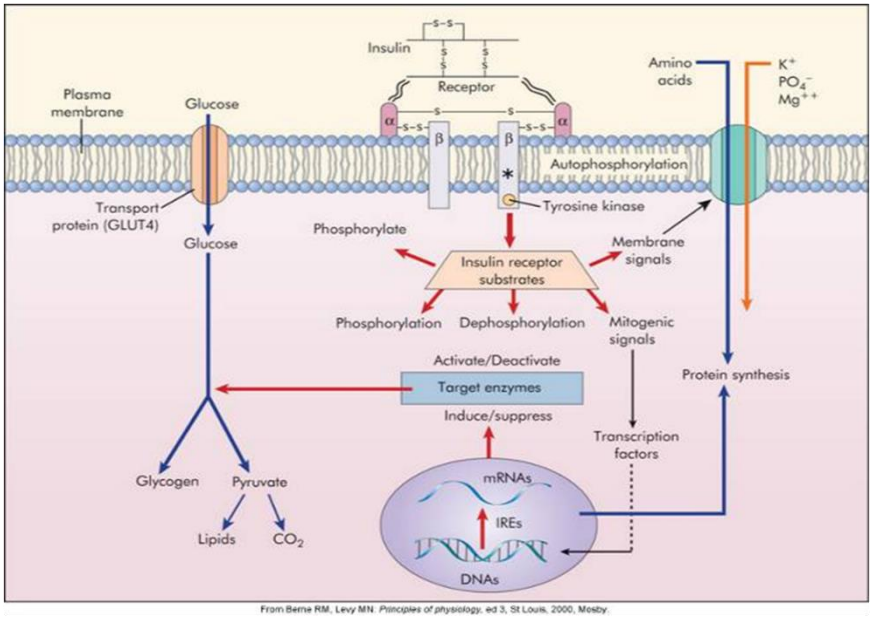


Insulin receptor **(as we said before it's the most common and it's also considered as one of the GF)**:
Insulin receptor consists of 2 units that dimerize when they bind with insulin. Insulin binds to ligand–binding site on plasma membrane, activating enzymatic site in the cytoplasm (**Tyrosine-kinase**). Cross/Autophosphorylation occurs, increasing tyrosine kinase activity which leads in activating other signaling molecules. The Results of insulin signaling have very varied effects, stimulation of glycogen, fat and protein synthesis (**revolving metabolism**) and stimulate insertion of GLUT-4 carrier proteins and increase of the glucose uptake.





As you can see once some sort of substrates get phosphorylated, they will induce these effects which are signifying the insulin signaling and those receptors are called Insulin receptor substrates (IRS)



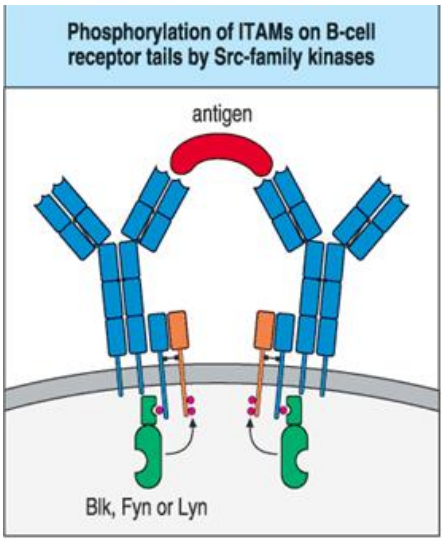
You aren't asked to know the details of the above process just to know the type of the receptor and how the activation of that receptor effects on the cell function, by explaining the changes of state of the substrate phosphorylation or dephosphorylation so either activation or inactivation and that what induce changes on the cell function. Like promoting the transporters when there is insertion, glycogen/lipids cell synthesis or gene expression through activation of transcription factors.

2. TKs **non-covalently** (**not considered as a part of the complex**) **associated** with receptor (includes cytokine receptors, T & B cell receptors "**Immune receptors**") = NRTKs:

Cytokine receptors, as well as T and B cell receptors, stimulate tyrosine kinases that are non-covalently associated with receptor once the ligand (mostly antigen) is bounded.

A. Overview

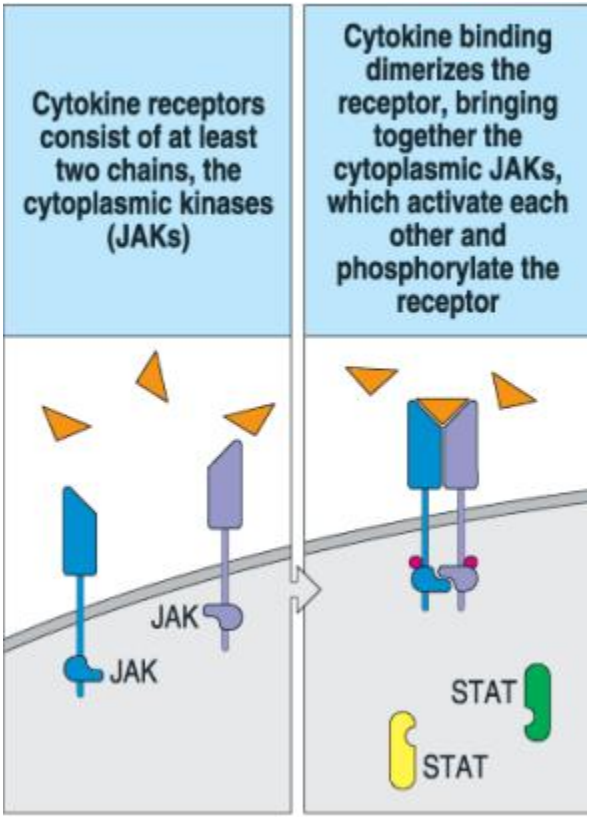
1. N-term. extracellular. ligand-binding domain, transmembrane a helix, C-term. cytosolic domain
2. Cytosolic domain has no catalytic (kinase) activity.
3. Acts in conjunction with a non-receptor tyrosine kinase that is activated as a result of ligand binding.
4. Activation is similar to that of RTKs: ligand binding causes cross phosphorylation of associated tyrosine kinases that phosphorylate the receptor, providing phosphotyrosine binding sites for recruitment of proteins with SH2 domains.



B. Two kinds of kinases associate with NRTKs:

1. Src family protein kinases - important for B and T cell signaling. (**The details are NOT REQUIRED**)

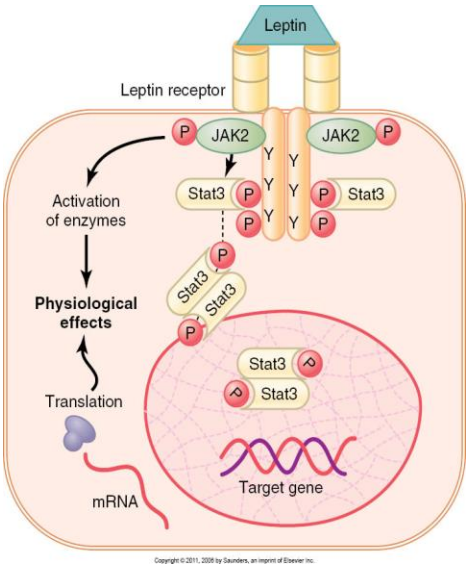
2. Janus kinases (JAK)- universally required for signaling from cytokine receptors (local factors) cytokine-like hormone leptin which work as the cytokine receptor (a NRTK receptor that is associated with Janus kinase **not intrinsic** that it is a tyrosine kinase).



C. Receptors can be linked to or associated with other enzymes, **besides** TKs, i.e.

Protein-tyrosine phosphatases (remove phosphates, thereby terminate signals initiated by protein-tyrosine kinases), serine/ threonine kinases and Guanylyl cyclases and there's TGF-β receptors.

❖ Enzyme-linked Receptor (the Leptin hormone which derived from fat cells e torn sensity the one that makes you feel full after eating) JAK= Janus Kinase STAT= Signal Transducer and Activator of Transcription.



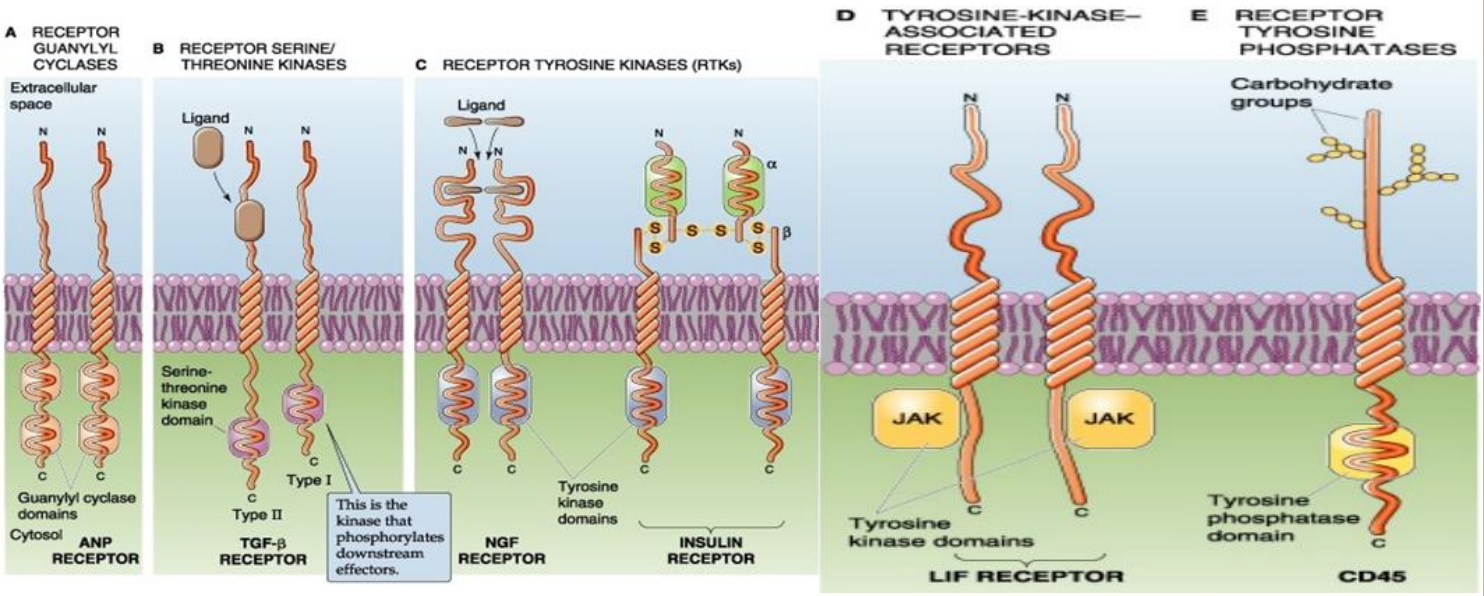
- As we can see cytokine-like hormone leptin receptor it dimerizes once the ligand (leptin) binds to it which will cause the auto/cross phosphorylation of the **associated** tyrosine kinase which in this case the JAK (Janus kinase) will lead in increasing their activity and forming as we called them before booking sites, so we can phosphorylate/activate other substrates.

In this example the substrate is STAT which has a binding site for the phosphate group then after activation the dimerization takes place with other STAT substrate then the two dimer will enter the nucleus and bind to the target gene, changes expression and translation and that will induces effects on the cell.

From the slides:

- The receptor exists as a homodimer (two identical parts)
- Leptin binds to the extracellular part of the receptor.
- This causes activation of the intracellular associated Janus kinas 2
- This causes phosphorylation of signal transducer and activator of transcription (STAT) proteins.
- This then activates the transcription of target genes and synthesis of proteins.
- JAK 2 phosphorylation also activates several other enzyme systems that mediate some of the more rapid effects of leptin.

❖ Examples of Enzyme-linked receptors:



ANP receptor (atrial natriuretic peptide) which has within inside it **(not associated)** Guanylyl cyclase enzyme domain and produces cGMP which a second messenger and has a similar effect of the AMP hormone.

TGF-β receptor (Transforming growth factor) a local factor it relates to normal cell function but if there's upregulation or we have excess of it, will make a promotion of fibrosis and it has a role in healing of the skin. It binds to serine-threonine kinase (enzyme linked receptor), that's mean it adds phosphate to serine & threonine while TK adds phosphate to tyrosine, that's the difference between them.

NGF & INSULIN RECEPTORS (both are RTK)

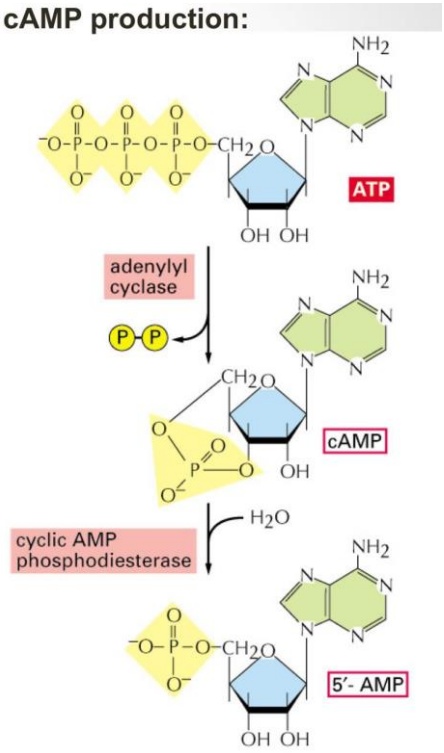
LIF RECEPTOR & CD45 ARE NOT REQUIRED

Second Messengers: for Hormones that can't cross PM

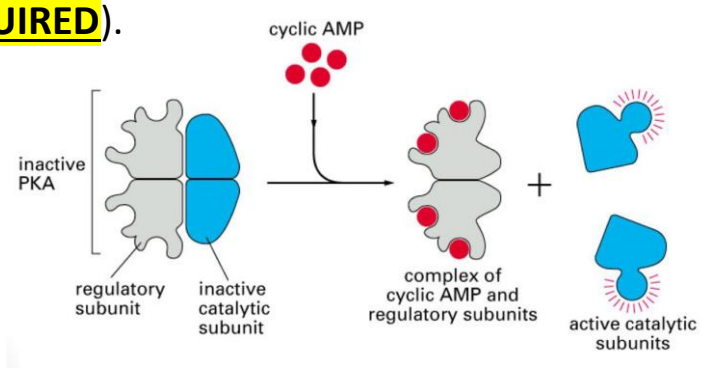
A. cAMP:

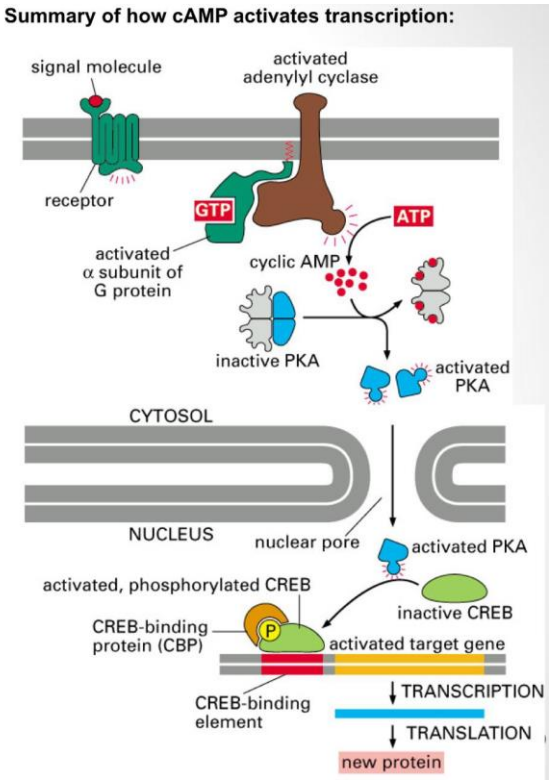
1. Production: ATP converted to cAMP by adenylate cyclase (a large multipass TM protein) Degraded by cAMP phosphodiesterase.

2. Action:



a. Phosphorylate/activate cAMP-dependent protein kinase (protein kinase A (PKA)). PKA is a tetramer of catalytic and regulatory subunits. cAMP binding leads to dissociation of regulatory subunits and release of catalytic subunits which then phosphorylate target proteins in cytoplasm. (**The activation is NOT REQUIRED**).

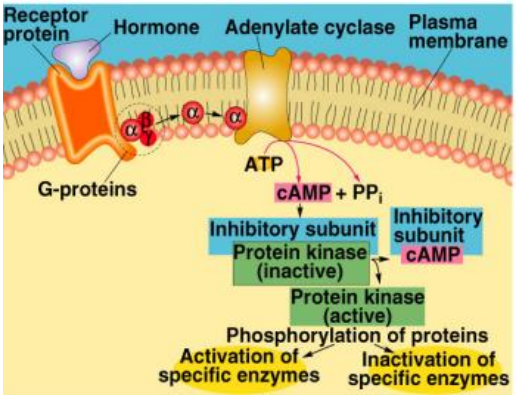




Now other function of the PKA **(that got phosphorylated/activated by cAMP, which got produced by activation of the adenylyl cyclase, which again got activated by Gα pairing GTP coming from the G-protein tetramer complex that bind to a receptor especially Gαs)**, other than phosphorylating target proteins in cytoplasm, it can enter the nucleus from the cytosol to phosphorylate a protein named CREB (cAMP response element binding-protein) when get phosphorylated/activated a protein binds to the cAMP response element (CRE), that protein promote/activate gene activation then we get expression and produce other proteins which gonna change the way the cell work/function. So, the cAMP functions in changing the activity of other enzyme and it has a function in gene expression in the nucleus by activating the PKA (Alter metabolism of the cell).

The cAMP get inactivated by phosphodiesterase (hydrolyzes cAMP to inactive fragments) and the PKA get inactivated by phosphatases.

❖ Regulation of adenylate cyclase:



- Receptors that cause an increase in **cAMP** do so by activating G_S, a stimulatory protein that activates adenylyl cyclase.
- Adenylyl cyclase is turned off by G_i, an inhibitory protein.

❖ Pathogens alter cAMP production:

Cholera toxin active subunit catalyzes transfer of ADP ribose from intracellular NAD to the α subunit of G_S, causing it to be continuously active, stimulating adenylyl cyclase indefinitely. This causes ion channels that export chloride to produce a net efflux of Cl^- and water, leading to severe diarrhea characteristic of cholera.

B. cGMP:

Produced from GTP by guanylyl cyclase, activates phosphorylates cGMP-dependent kinases or other targets.

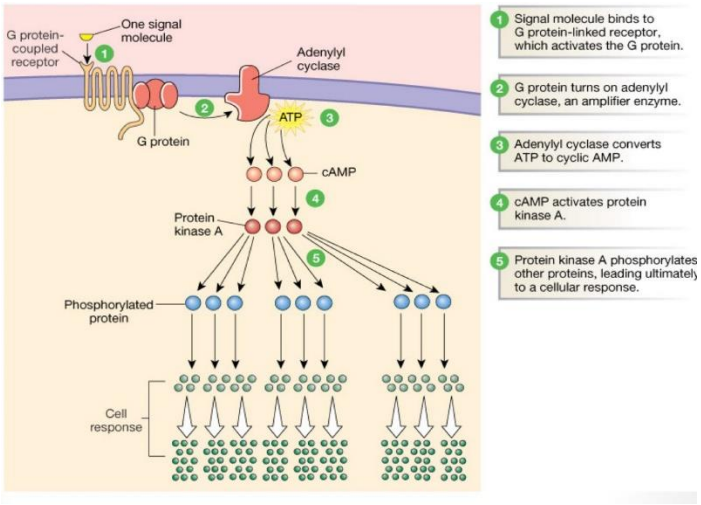
Examples: G-protein coupled receptors that have cGMP as a second messenger rhodopsin photoreceptor in rod cells of retina, nitrogen oxide and ANP.

- If we have a rise in cAMP regardless of the hormone that caused it we would find a variation of responses by different types of cell even if the hormone that caused it is a common one, that's due different enzymes and the cAMP will have different targets. **(You don't have to memorize this table)**

TABLE 20-3 Metabolic Responses to Hormone-Induced Rise in cAMP in Various Tissues

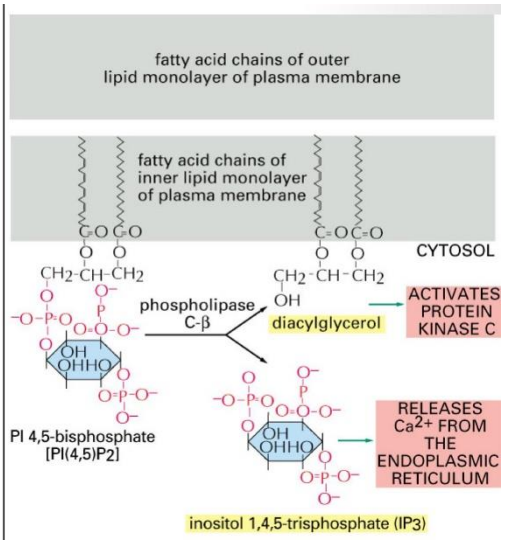
Tissue	Hormone Inducing Rise in cAMP	Metabolic Response
Adipose	Epinephrine; ACTH; glucagon	Increase in hydrolysis of triglyceride; decrease in amino acid uptake
Liver	Epinephrine; norepinephrine; glucagon	Increase in conversion of glycogen to glucose; inhibition of synthesis of glycogen; increase in amino acid uptake; increase in gluconeogenesis (synthesis of glucose from amino acids)
Ovarian follicle	FSH; LH	Increase in synthesis of estrogen, progesterone
Adrenal cortex	ACTH	Increase in synthesis of aldosterone, cortisol
Cardiac muscle cells	Epinephrine	Increase in contraction rate
Thyroid	TSH	Secretion of thyroxine
Bone cells	Parathyroid hormone	Increase in resorption of calcium from bone
Skeletal muscle	Epinephrine	Conversion of glycogen to glucose
Intestine	Epinephrine	Fluid secretion
Kidney	Vasopressin	Resorption of water
Blood platelets	Prostaglandin I	Inhibition of aggregation and secretion

- **Amplification of signal at each step of signaling pathway - characteristic feature of signal transduction.**



C. IP3 and DAG:

Overview: Phosphatidylinositol 4,5 bisphosphate (PIP2) triggers a 2-armed signaling pathway



- PIP2 is a minor PL in inner leaflet of PM bilayer that is produced by phosphorylation of phosphatidyl-inositol and is involved in signaling Ligand binding to certain receptors stimulates PIP2 hydrolysis by phospholipase C (PLC)
- This produces diacylglycerol (DAG)((remains bound to the membrane and activates PKC) and inositol 1,4,5-phosphate (IP3) (causes the release of Ca^{++} from ER, a third 2nd messenger), both of which are 2nd messengers.
- PIP2 hydrolysis is activated by both **GPRs** and **TKRs** via different forms of PLC.
- PLC-β (isoform of the PLC) is stimulated by **$G\alpha_q$** proteins while PLC-γ (isoform of the PLC) has SH2 domains that allow binding to activated tyrosine kinases (enzyme-linked receptor) both PLC- β/ γ produce IP3 and DAG.

The END of SHEET #19

(اعْلَمْ أَنَّ الْعِلْمَ أَشْرَفُ مَا رَغَّبَ فِيهِ الرَّاغِبُ، وَأَفْضَلُ مَا طَلَبَ وَجَدَّ فِيهِ الطَّالِبُ، وَأَنْفَعُ مَا كَسَبَهُ وَاقْتَنَاهُ الْكَاسِبُ؛ لِأَنَّ شَرَفَهُ يُثْمِرُ عَلَى صَاحِبِهِ، وَفَضْلَهُ يُنْمِي عَلَى طَالِبِهِ)