Physiology
Sheet **No.**

16

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G Protein Signal Cascade:

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

1. Initially Gα has bound GDP and α, β, & γ subunits are complexed together. Gβ,γ , the complex of β & γ subunits, inhibits Gα .

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 Gα becomes more accessible to the cytosol, Gα releases GDP & binds GTP (GDP-GTP exchange).

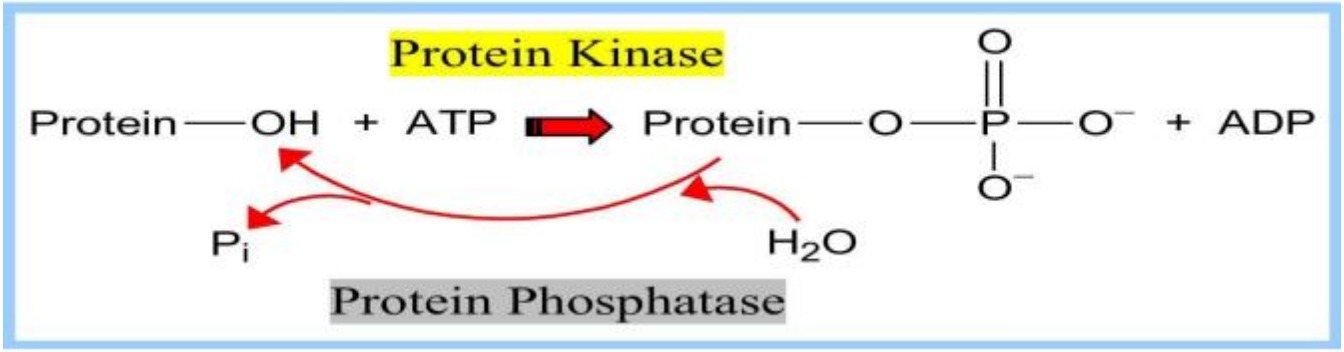
3. Substitution of GTP for GDP causes another conformational change in Gα . Gα -GTP dissociates from the inhibitory βγ complex & can now bind to and activate Adenylate Cyclase to increase synthesis of cAMP.

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

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

ENZYMES THAT INVOLVE IN INTERCELLULAR SIGNALING 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)

*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 memorize 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 catalyze phosphorylation of Ser or Thr on target proteins. PKIs, 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. Gα hydrolyzes GTP to GDP + Pi . (GTPase). The presence of GDP on Gα causes it to rebind to the inhibitory βγ complex. Adenylate Cyclase is no longer activated.

2. Phosphodiesterases catalyze hydrolysis of cAMP to AMP.

Phosphodiesterase enzymes catalyze: cAMP + H2O → AMP(hydrolysis of cAMP).

The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalyzed by Protein Kinase A.

Thus cAMP stimulates its own degradation, leading to rapid turnoff of a cAMP signal. Type of negative feedback

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 β-arrestin.

• β-Arrestin(like tag on the receptor) promotes removal of the receptor from the membrane by clathrin mediated endocytosis.

•ANOTHER way β-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).

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* Different isoforms of Gα have different signal roles. E.g.:

• The stimulatory Gsα , when it binds GTP, activates Adenylate cyclase.

• An inhibitory Giα , when it binds GTP, inhibits Adenylate cyclase.

Different effectors & their receptors induce Giα to exchange GDP for GTP than those that activate Gsα (Giα and Gβ are activated by different effectors).

ω The complex of Gβ,γ that is released when Gα binds GTP is itself an effector that binds to and activates or inhibits several other proteins.

E.g., Gβ,γ 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).

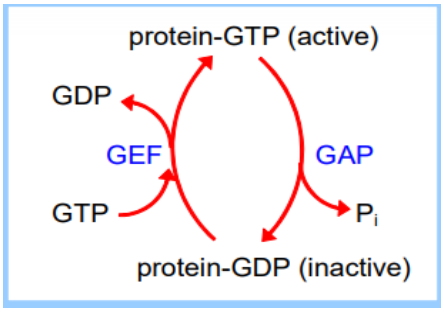
ω **Rab** (vesicle targeting and fusion).

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.



Most GTP-binding proteins depend on helper proteins:

1.**GAPs** 2. **GEF**

1.**GAPs** : **G**TPase **A**ctivating **P**roteins, promote GTP hydrolysis.

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

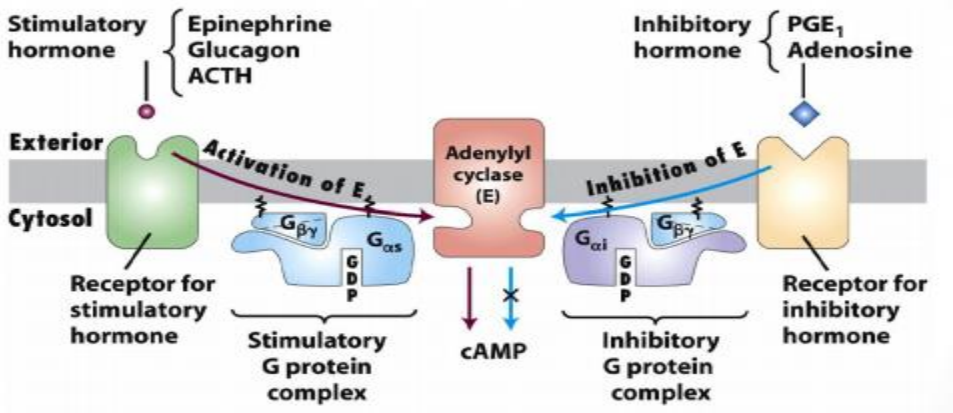
It has the essential arginine residue normally provided by a GAP for small GTP-binding proteins.

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

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

** Alternatively, **AGS** (Activator of G-protein Signaling) proteins may activate some heterotrimeric G-proteins, independent of a receptor.

***Some AGS proteins have GEF activity.



signaling 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.

2- G-protein-coupled receptors (GPCR).

3- Enzyme linked receptors.

"Opportunities present themselves to those who have prepared." Hidemi Tashiro

*** **Enzyme linked receptors:**

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

a) 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.

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

c) 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 .

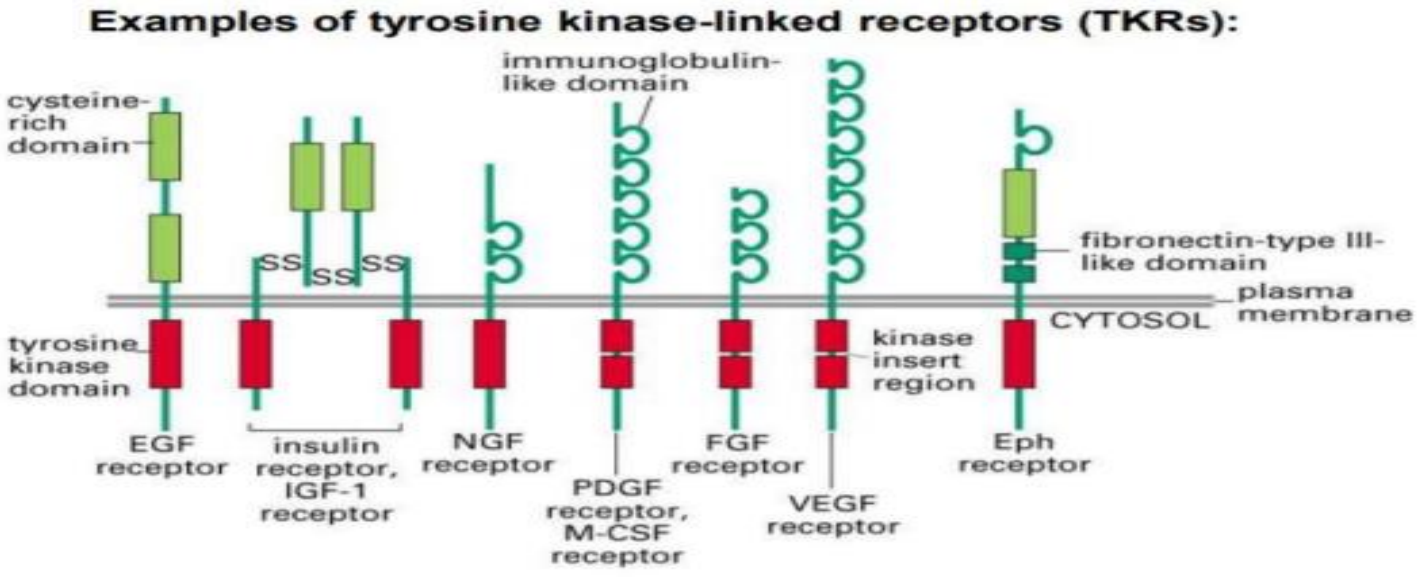
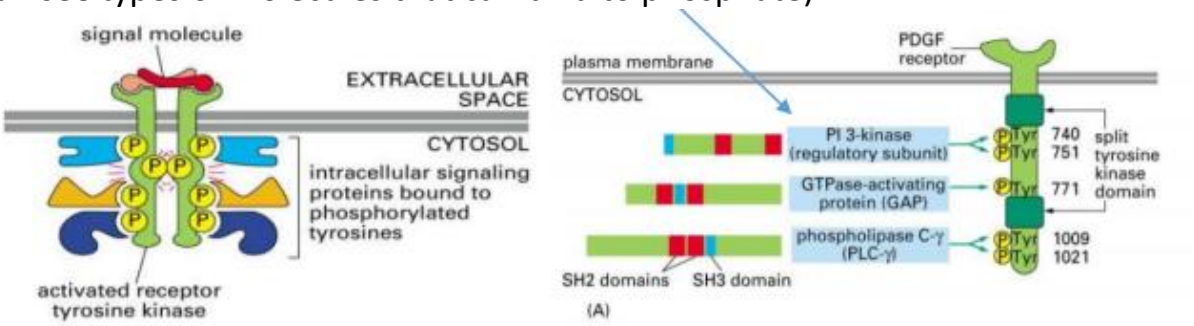


Figure 15–49. Molecular Biology of the Cell, 4th Edition.

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:

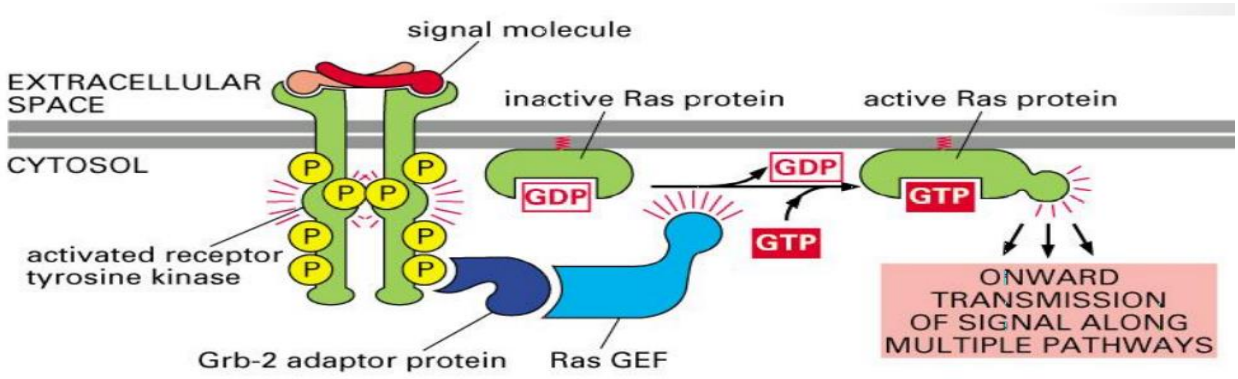
- When Ligand bind to receptor, it induces dimerization (cross linking) of 2 units
- Dimerization lead to autophosphorylation of the enzyme in the receptor (cross phosphorylation)
- Phosphorylation increases kinase activity, it means that there will be phosphate groups in the intracellular domain (TK),(tyrosine becomes phosphorelated) like in the 1st pic below, which create specific binding sites for other signaling proteins, that when binding to the sites, they become activated
- Then phosphate groups can bind to other molecules.(in the 2nd pic below, you can see types of molecules that can bind to phosphate)



How receptor tyrosine kinases work together with monomeric GTPases:

- -gap delete phosphate group from gtp -** GEF adds phosphate group

RAS GEF add phosphate group to gdp .



Tyrosine Kinase

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

** – Insulin binds to ligand-binding site

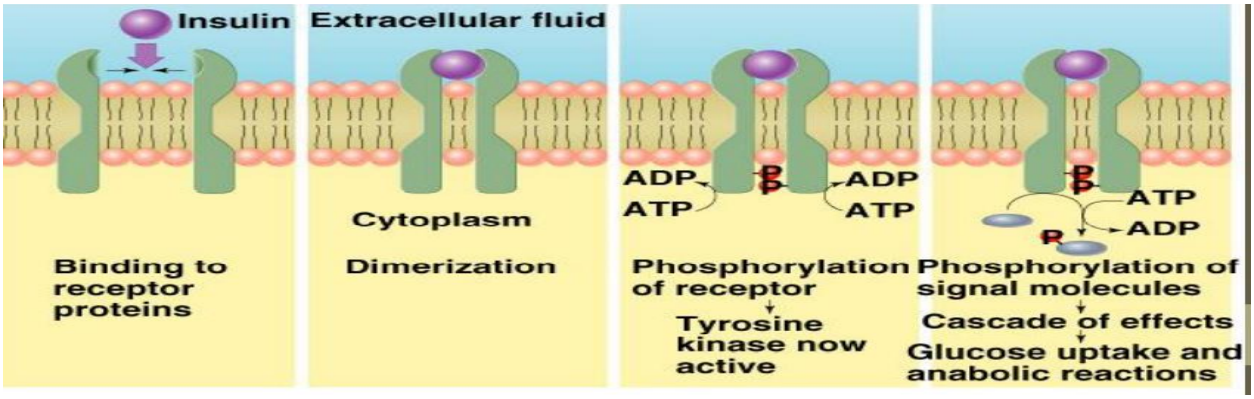
- binding site on plasma membrane, activating enzymatic site in the cytoplasm (intracellular domain)

B. Autophosphorylation occurs, increasing tyrosine kinase activity.

C. Activates signaling molecules.

– Stimulate glycogen, fat and protein synthesis.

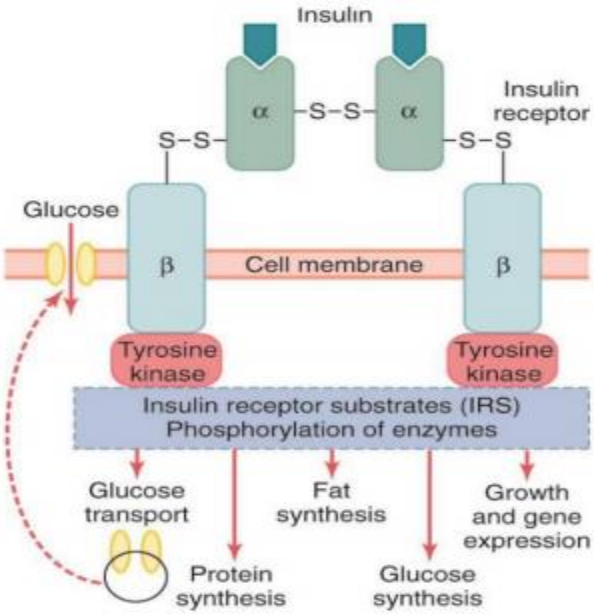
– Stimulate insertion of GLUT-4 carrier proteins, to facilitate entrance of glucose into the cell



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 above

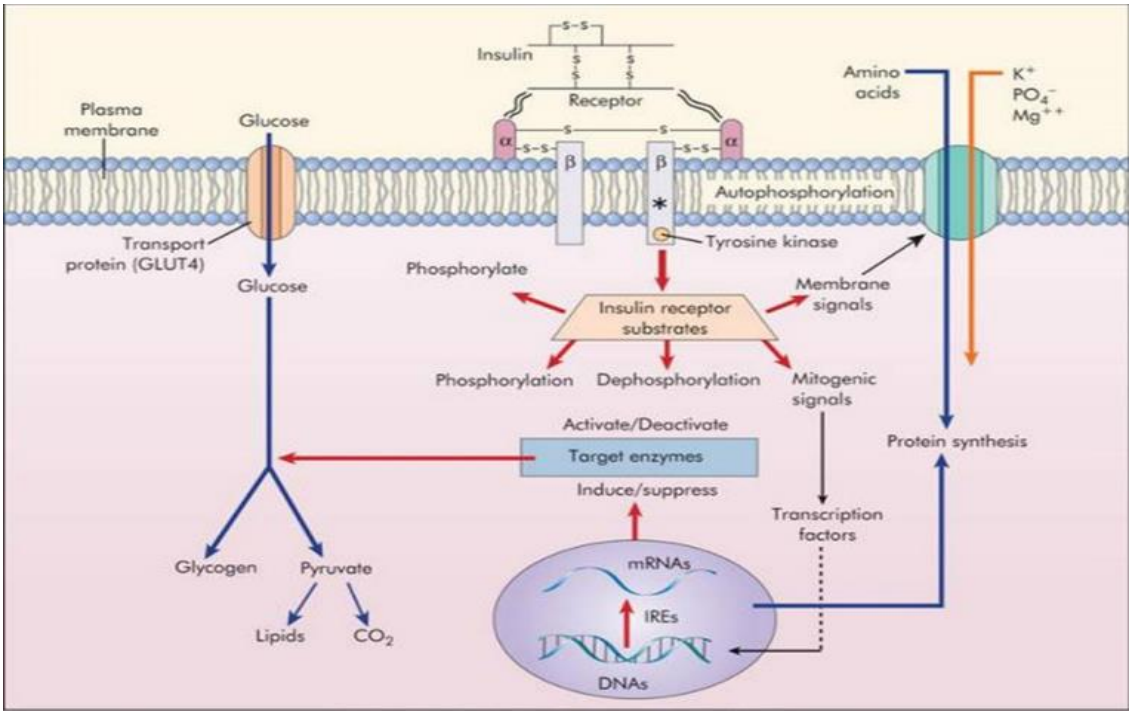
Note: Insulin has many downstream targets such:

✓ Opens transporter of glucose.

✓ Increases the cell metabolic activity.

✓ Stimulates glycogen, fat and protein synthesis.

✓ Stimulates insertion of GLUT-4 carrier protein



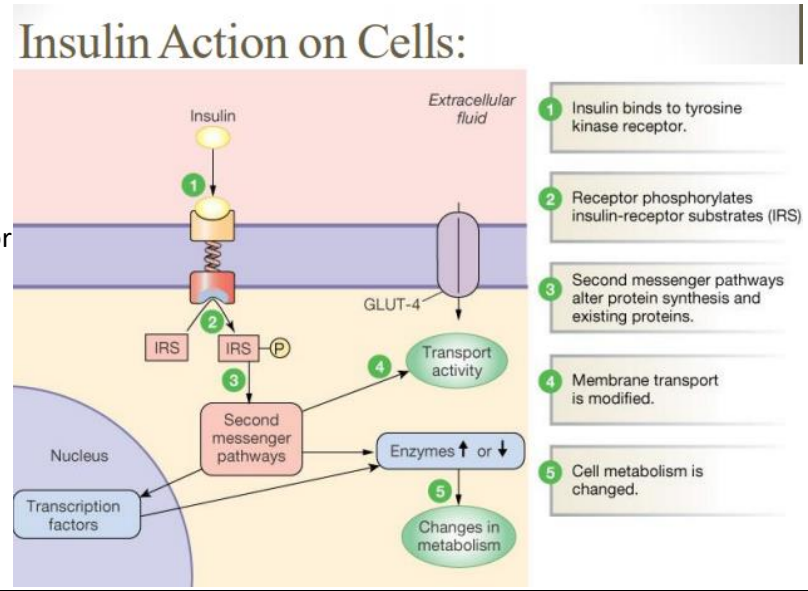
THE slide above shows the general function of insulin.

Step no.3 : signaling molecules become activated to endues diverse changes,

it could be transport of glucose, increase

or decrease the activity of different enzymes, or

stimulates certain transcription factors)



-Insulin function: Glucose uptake and anabolic reaction(anabolic functions)

IRS: Insulin receptor substrate Once the IRS is activated ,the effects are very wide

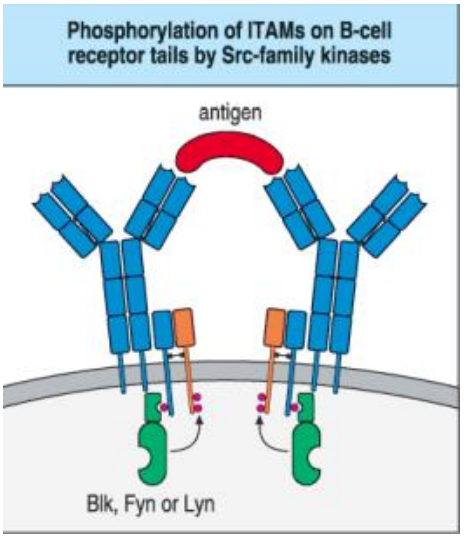
Mitogenic signals: signals to the nucleus.

-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 conjuction with a non-receptor tyrosine kinase that is activated as a result of ligand binding.

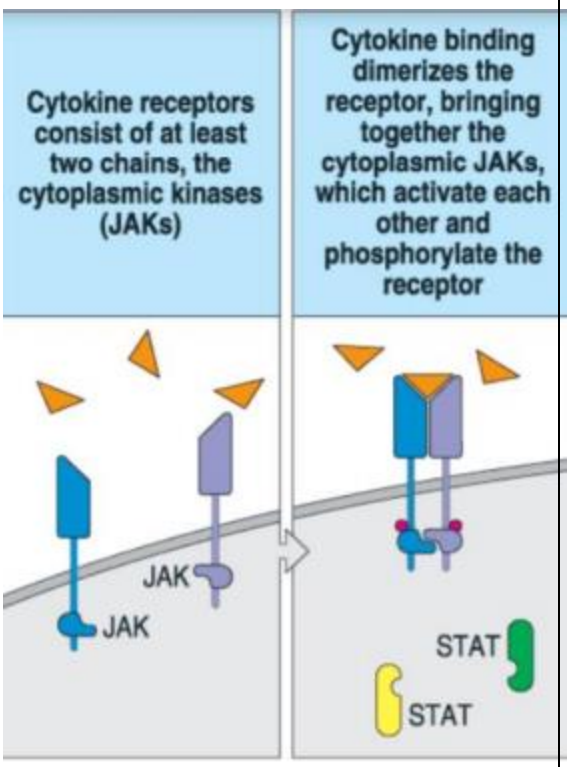
4. Activation is similar to that of RTKs: ligand binding causes cross phosphorylation of associated tyrosine kinases that phosphorylate the receptor, providing phosphotyrosine binding sites for recruitment of proteins with SH2 domains

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

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

Protein-tyrosine phosphatases

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

Serine/ threonine kinases

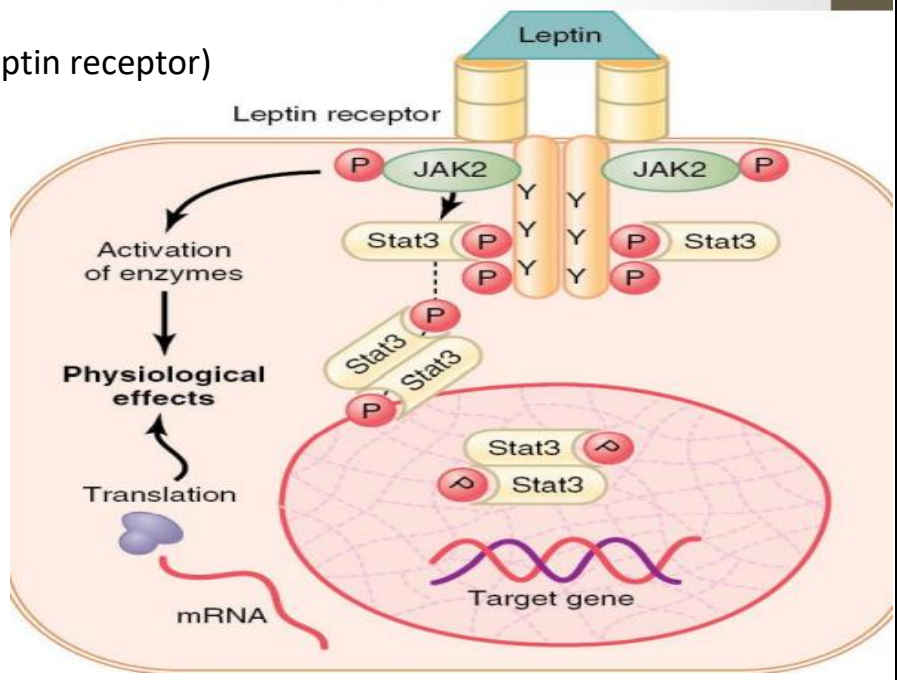
i.e. TGF-β (required example) مهم

Guanylyl cyclases :(required example)

Enzyme-linked Receptor (the Leptin receptor)

JAK= Janus Kinase

STAT= Signal Transducer and Activator of Transcription

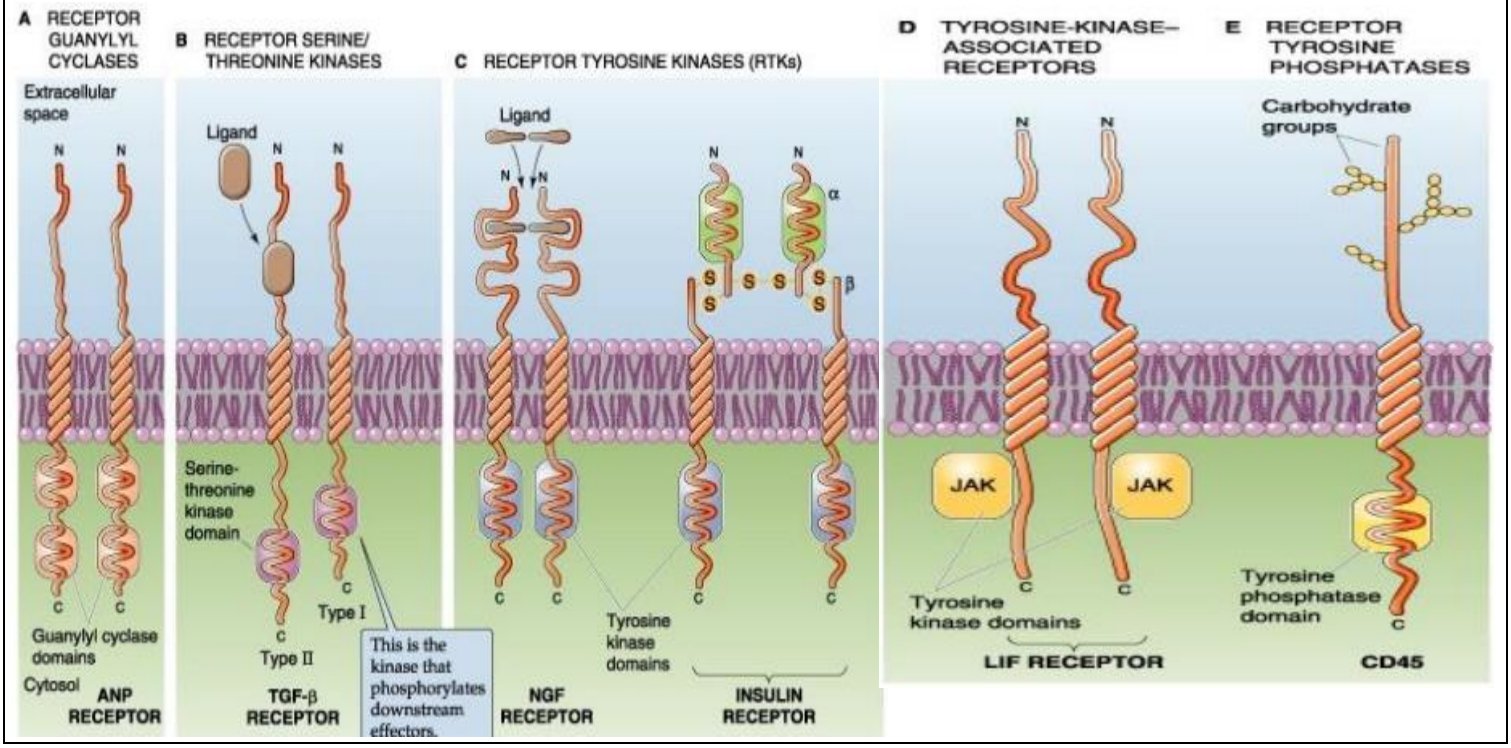


(the picture above),is an example of enzyme-linked receptor non-covalently linked to the receptor → **LEPTIN RECEPTOR**

-Once the liptin receptor is activated it induces phosphorylation of JAK, this will activate downstream enzymes, or the phosphorylation of Stat3 molecules, Stat3 (signal transducers and activator of transcription),so it activate the transcription Usually, Stat3 dimerise and enter the nucleus to induce transcriptions of certain targets.

- 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 kinase 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

(leptin is an important hormone of satiaty ,and lipid-tissue metabolism



A- Receptor guanylyl cyclase is an enzyme-linked receptor and the guanylyl cyclase is a part of it →(ANP receptors),c GMP is 2nd messenger

B- Receptor serine/threonine kinase, the receptor contain serine-threonine kinase EX. TGF-B

C- RTK ,contains tyrosine kinase domains. (INSULIN RECEPTOR)

D- Tyrosine-Kinase-Associated Receptors (non-covalently) -JAK

E- Receptor Tyrosine Phosphatases

^^و إياك أن تقول إنك لن تستطيع ؛فإنه من قال لا استطيع فلن يستطيع^^

“Talent is something you make bloom. Instinct is something you polish.” Oikawa Tooru