CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

24

Done by:

Abdulrahman Jabr &Ameen Alsaras

Corrected by:

Abdulrahman Jabr &Ameen Alsaras

Doctor

Diala abu hassan

Why are there cell-specific response?

*Each signaling pathway ends with a different response (E.g. cell movement, cell division, or even apoptosis)

How are these responses carried out?

-By activating specific target genes which encode the proteins needed for this cellular change or response. So, we must reach our key player, which is the transcription factor, and give it the access to DNA binding sites in the loose form of DNA as tightly packaged DNA cannot be transcribed

Note that:

*Some transcription factors are already found within the nucleus and get activated because of the signaling pathway, while others are found in the cytosol, so they enter the nucleus after being activating by some specific mechanism.

*Cells have distinct receptors and different combinations of regulatory proteins that affect cell behavior.

Now Let's have a look at some pathways :)

1)AKT pathway.

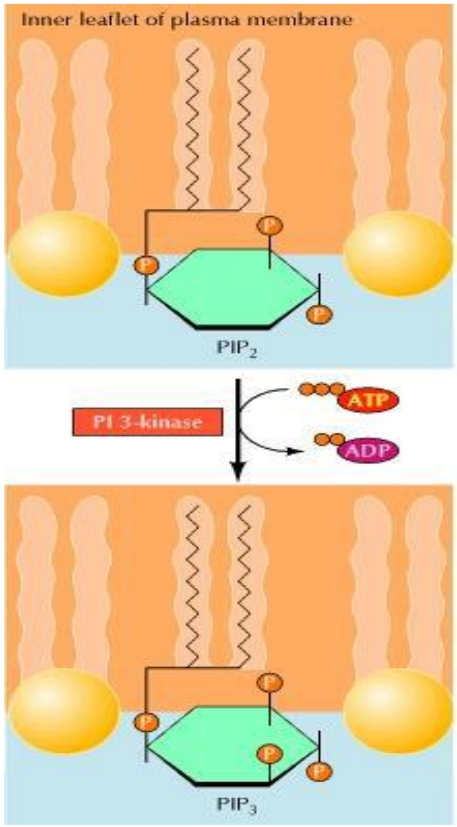
-After activation of RTK (receptor tyrosine kinase), a protein called PI-3 kinase binds to the phosphorylated-outside-kinase domain of RTK.

-As its name implies, PI-3 kinase phosphorylates Phosphatidylinositol that has already two extra phosphates, giving it a third one and changing its name from PIP2 to PIP3. (the numbers indicate no. of extra phosphates)

-**PIP3** attracts a protein (kinase) called **AKT** and binds to it.

-AKT has many types like AKT1, AKT2, etc.

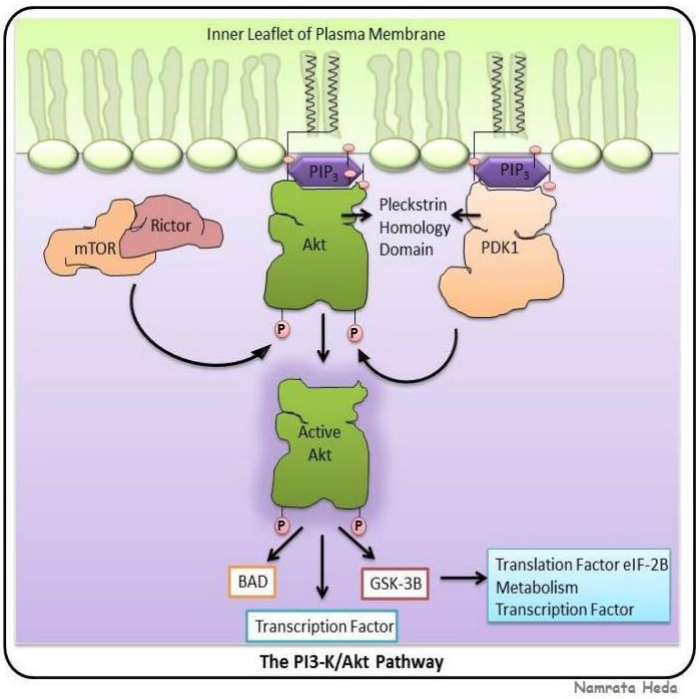
-While it is bound, **AKT** gets phosphorylated by one or more different proteins such as **PDK 1** and **mTOR**.



-Now AKT is activated so it splits from PIP3 and starts its journey to phosphorylates other molecules including BAD (important for cell cycle as well as cell survival) and GSK-3B (affects metabolism).

*Generally speaking, AKT supports cell survival, proliferation, and differentiation.

*AKT is a signaling hub, it has many targets and it is a target for many molecules in different pathways.

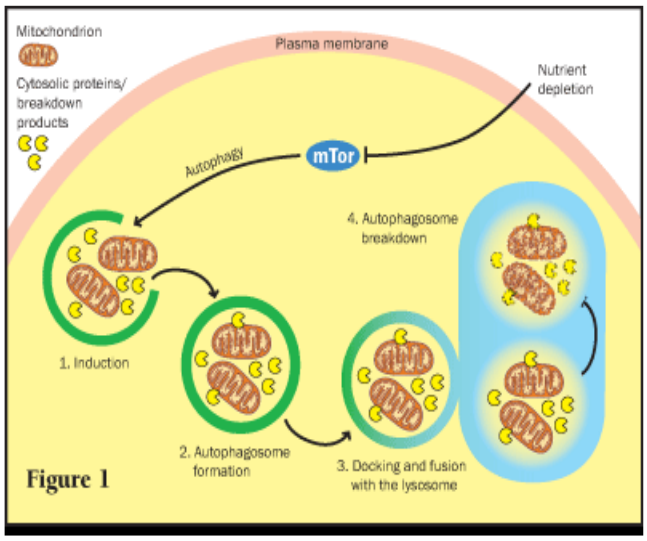


2)mTOR pathway.

-As we've seen earlier, mTOR activates ATK which supports cell survival and proliferation. So, activating it can't have an inhibitory role, right?

-Yes, you're right. When there is a nutrient depletion (low concentration of nutrients), the cell creates a stimulus that inhibits mTOR.

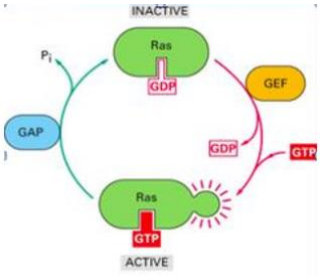
-This leads to an inhibition in the AKT and other cell survival molecules.



-It also activates proteins involved in autophagy.

Summary: Nutrient depletion ➔ Inhibition of mTOR ➔ Initiation of Autophagy

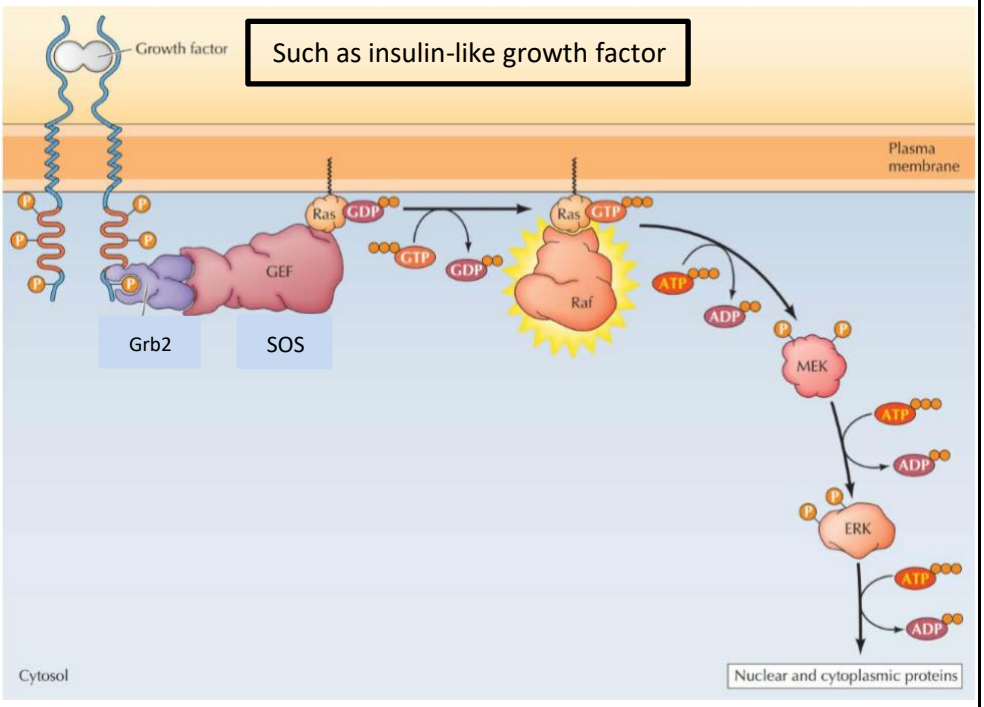
Ras activation by RTKs



➔Ras works underneath RTK which binds to many types of growth factors.

➔Growth factor binding induces receptor dimerization, which results in receptor autophosphorylation as the two polypeptide chains cross-phosphorylate one another.

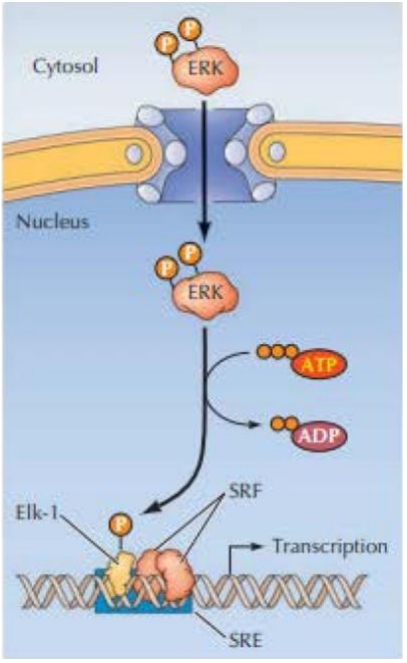
➔Grb2 binds to the phosphate group which is outside the catalytic domain. This binding induces conformational change in Grb2 so the protein SOS (GEF) can bind to it.



➔SOS activates Ras adding GTP instead of its GDP.

➔The activated Ras binds to Raf protein (kinase) which phosphorylates another kinase called MEK.

➔The phosphorylated MEK also phosphorylates another kinase called ERK which has the ability to enter the nucleus through the nuclear pore.



➔ERK translocates to the nucleus and phosphorylates the transcription factor Elk-1.

➔This phosphorylation stimulates Elk-1 allowing it to bind to a specific location on the DNA called serum response element (SRE) in a complex with serum response factor (SRF) to induce expression of immediate-early genes.

➔These genes stimulate expression of secondary response genes.

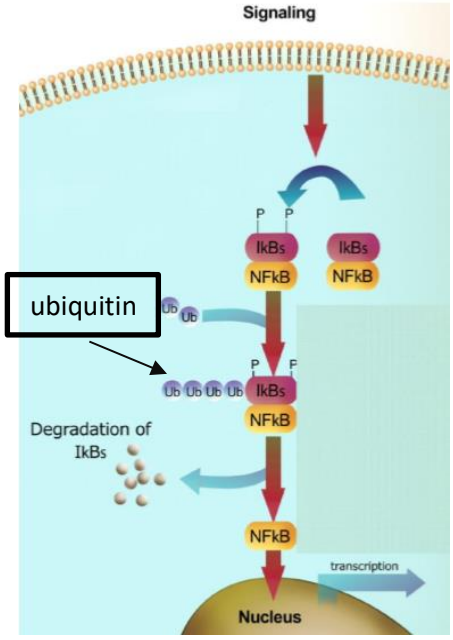
➔The ERK signaling leads to cell proliferation, survival, and differentiation.

NF-ϰB signaling

➔Tumor necrosis factor (TNF) activates its receptor (TNF receptor) and stimulates the phosphorylation and degradation of IκB.

➔ NF-ϰB (transcription factor) is activated as a result of phosphorylation and degradation of IϰB (by ubiquitin), allowing NF-ϰB to translocate to the nucleus and activate transcription of target genes which induce inflammation and cell death.

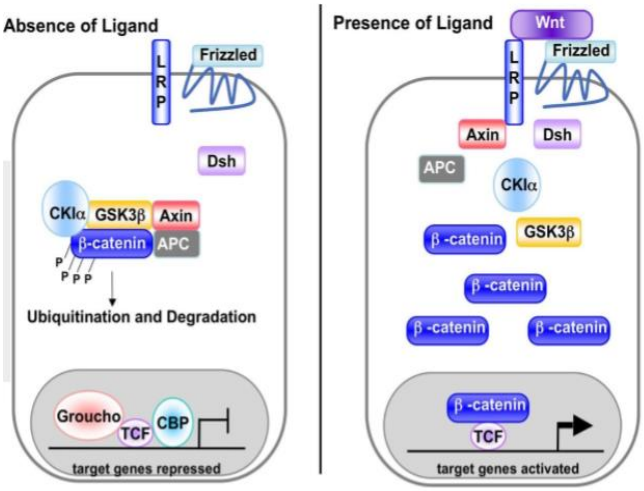
➔ NF-ϰB was inactive because of its binding with its inhibitory protein IϰB and when IϰB is phosphorylated and degraded, the function of NF-ϰB can be done.



Wnt signaling

➔With the absence of Wnt (inactive state), the downstream molecules are condensed in a complex in the cytosol and *B*-catenin is phosphorylated and inactive by ubiquitin as in IϰB.

➔ Wnt proteins are growth factors that bind to the Frizzled receptors (consists of LRP and frizzled) and block *B*-catenin degradation by the binding of the receptor with axin (one of the downstream molecules) which result in the separating of the complex.



➔*B*-catenin then is activated and can translocate into nucleus and activate gene expression by its binding to Tcf (a specific location in the DNA).

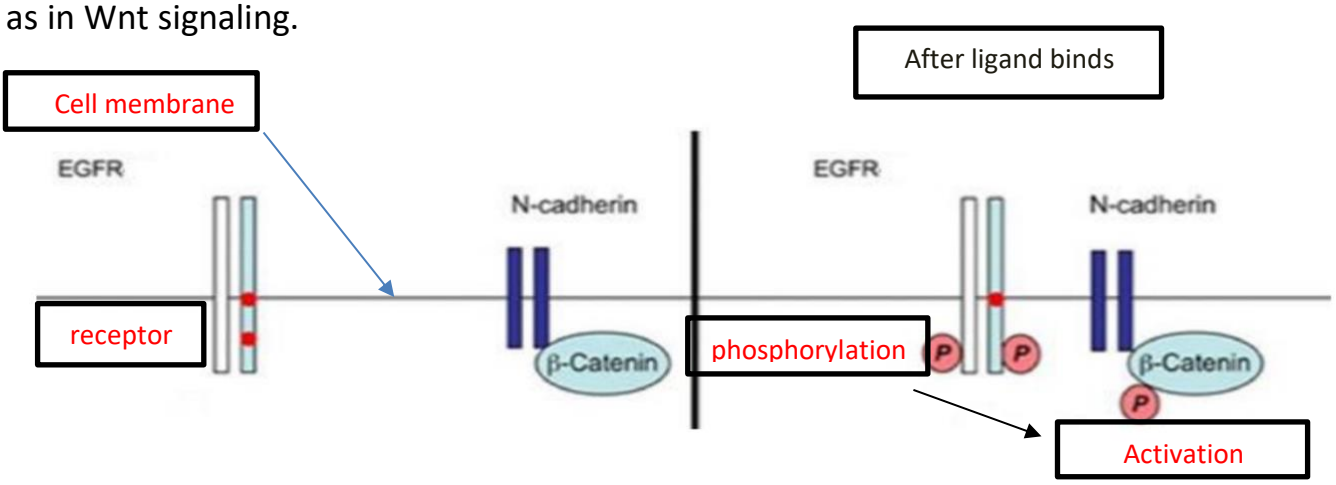
➔Remember: *B*-catenin links cadherins to actin in adherens junctions. But here it is a transcription factor.

Role of adhesion molecules in signaling

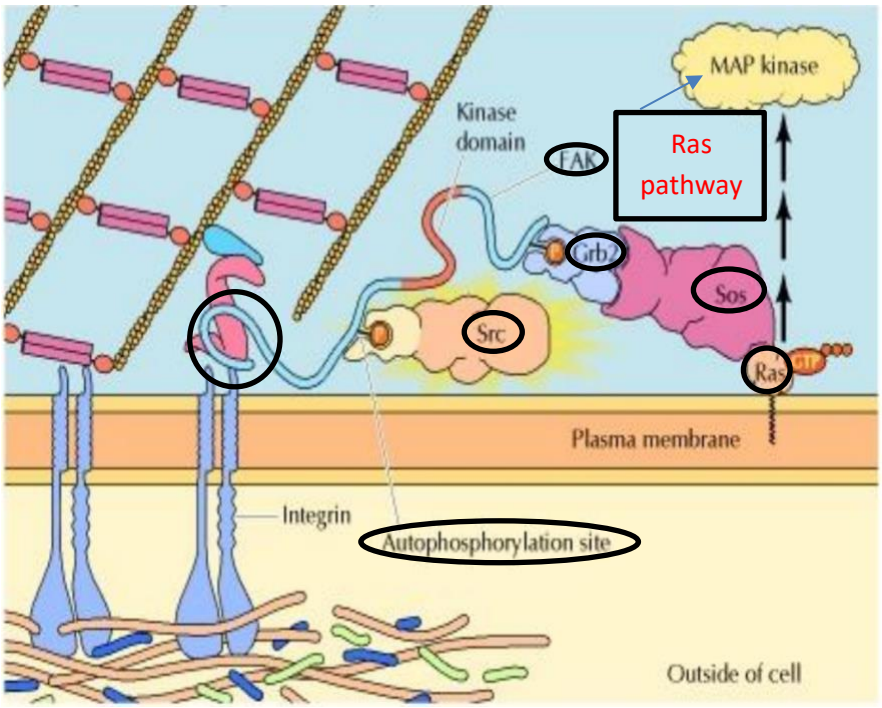
Remember: β-catenin is involved in the cellular junctions.

*Interaction of cadherins with cell surface receptors result in dual regulation and signaling and promotion of cell survival.

Note: The phosphorylation of β-catenin here doesn't happen on the same amino acid as in Wnt signaling.



Another example on cell signaling through cellular junctions:



Integrin signaling in focal adhesions:

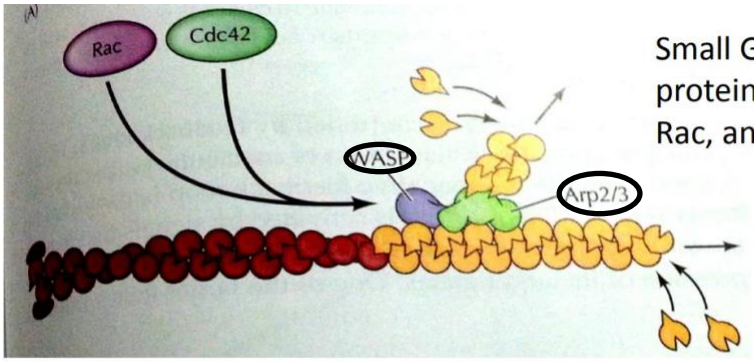
➔ Integrins serve as sensors for specific changes in the ECM and make responses.

➔ Binding of integrins to the ECM induces Src binding to focal adhesion kinase (FAK) and its tyrosine phosphorylation.

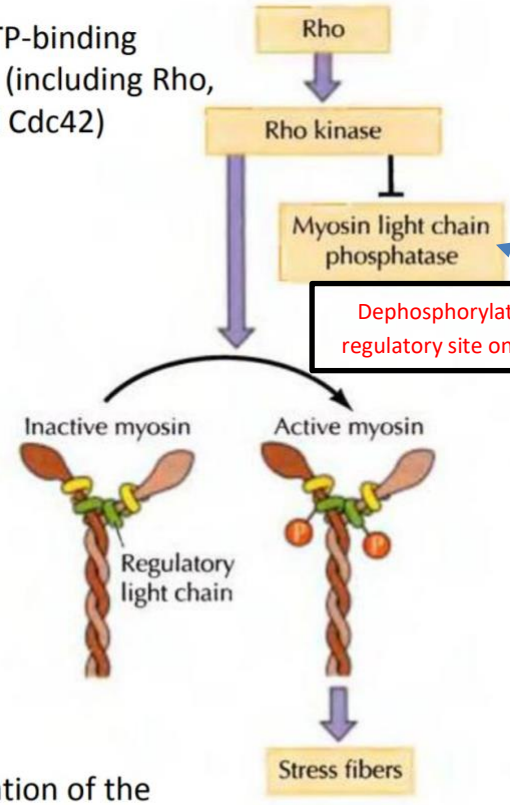
➔ These phosphotyrosines serve as binding sites for the Grb2-Sos complex, leading to activation of Ras and the MAP kinase cascade, as well as for additional downstream signaling molecules, including PI 3-kinase.

➔ This may affect the structure of focal adhesions and the cytoskeleton.

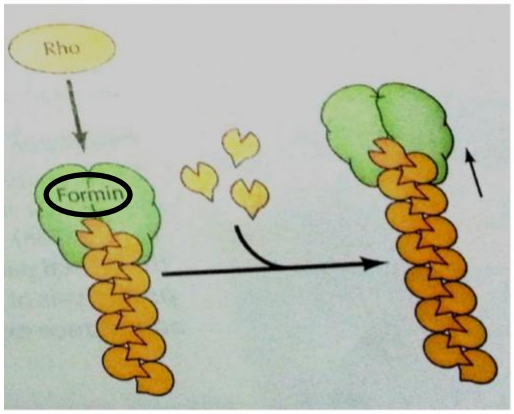
The Rho subfamily- Mechanisms of action



Small GTP-binding proteins (including Rho, Rac, and Cdc42)



Dephosphorylates the regulatory site on myosin



- Members of the Rho subfamily regulate the organization of the actin cytoskeleton (cell motility, cell adhesion, and cytokinesis).

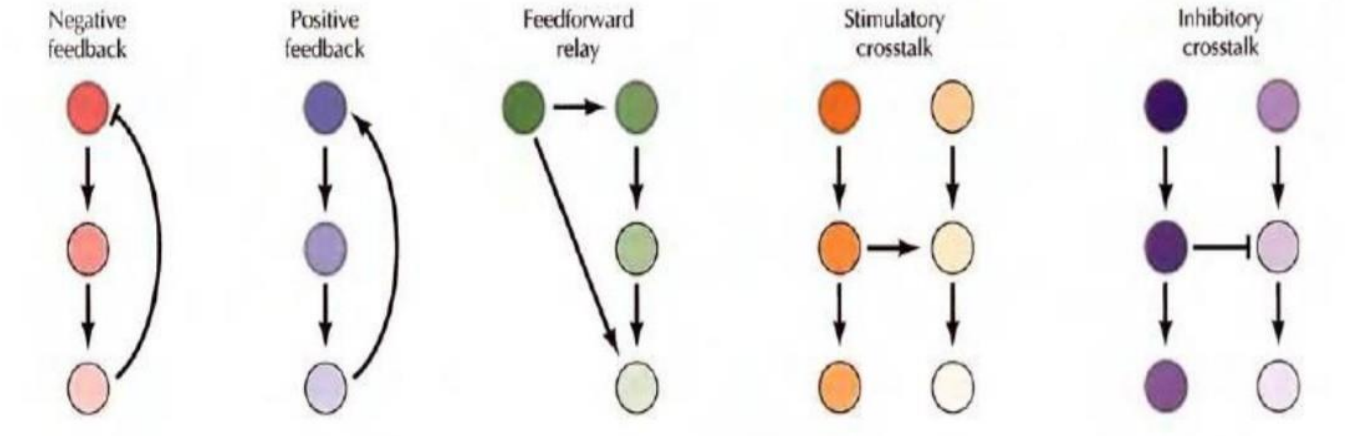
Rho, Rac and cdc42 are members of Rho subfamily

By binding, they activate Rho kinase.

Cytokinesis = part of cells division where the cytoplasm of new daughter cells is separated. Remember that the contractile ring that separates the two cells from each other is made of actin filaments.

Signaling pathway interaction

-Signal transduction networks



-Some of the most important regulation pathways are <u>positive and negative feedback</u> (the last product of the pathway would activate or inhibit the first molecule in the pathway <u>respectively</u>).

-Feedforward relay: the first molecule can either go through the pathway directly or through multiple steps (We have more than one option which reach the same result)

*The cell can do its job by more than one way, so we can say that this is a mechanism of defense.

-Crosstalk: a molecule inside the pathway (not the receptor) can activate or inhibit a molecule in another pathway (the will affect all steps after the target molecule).

*If the target molecule is activated, the process is called **stimulatory** crosstalk.

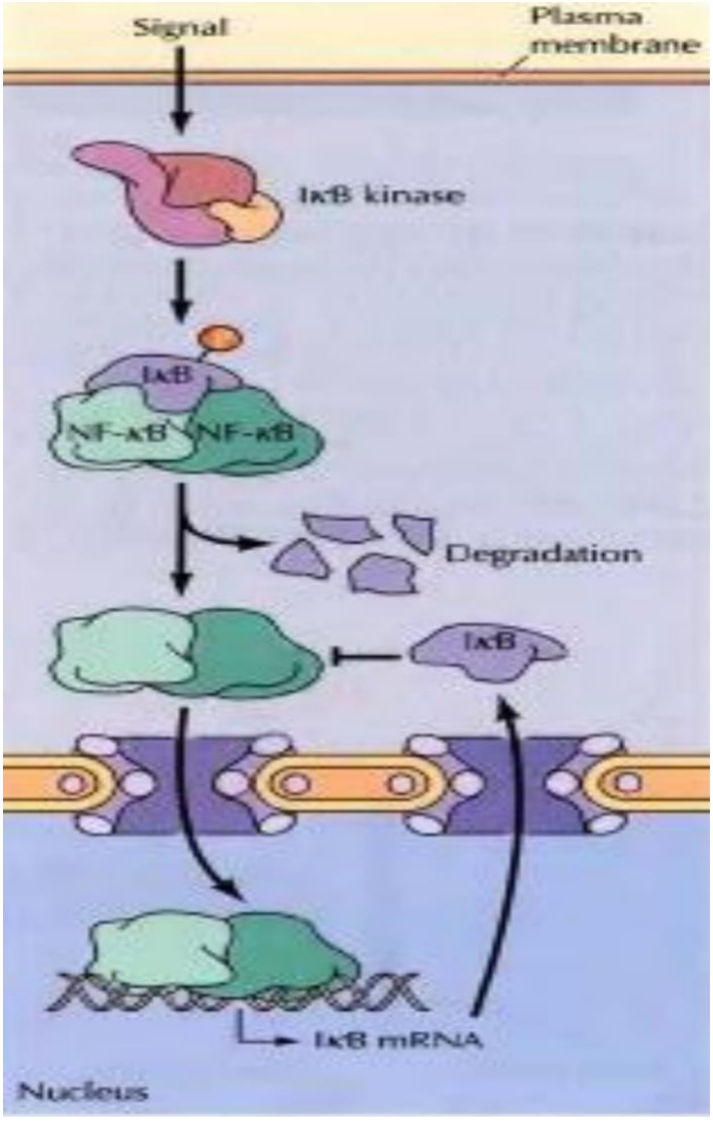
* If the target molecule is inhibited, the process is called **inhibitory** crosstalk.

Signaling networks and regulation

-Activation of one pathways leads to the expression of its inhibitors.

NF-κB is activated as a result of phosphorylation and degradation of IκB, allowing NF-κB to translocate to the nucleus and activate transcription of target genes.

One of the genes activated by NF-κB encodes IκB. generating a negative feedback loop that inhibits NF-κB. activity.



Crosstalk

➔ The interaction of one signaling pathway with another.

➔ Examples:

*cAMP and ERK.

*Cell adhesion molecules and receptor tyrosine kinases.

*ERK and PI-3 kinase.

*** The pictures are for better understanding and you don't have to remember the details.

-Other examples about GPCR & RCT pathways are below.

*ركّز على اللي محدّدهم(ذكرتهم الدكتورة) كأمثلة فهم مش حفظ ويعطيك العافية ^^