



# Genetics

## & Cell biology

☒ Sheet

☐ Slides

Number

12

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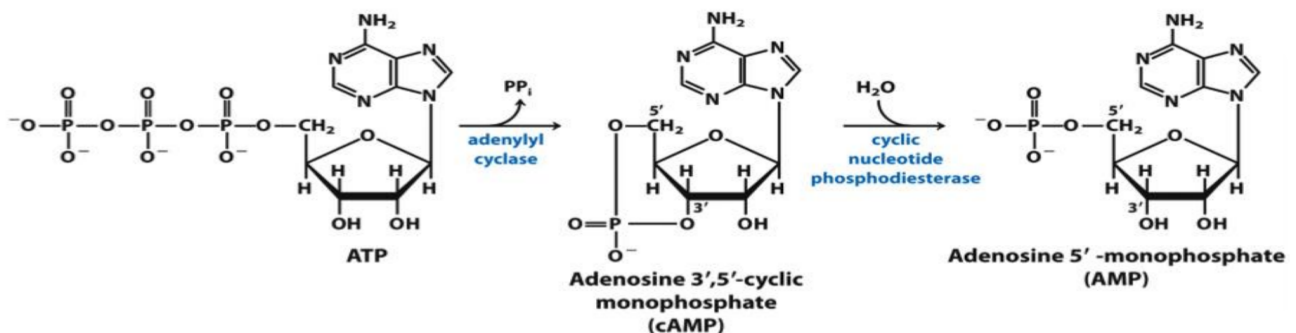
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## Continuation of 2<sup>nd</sup> messengers ...

*Why are they important?*

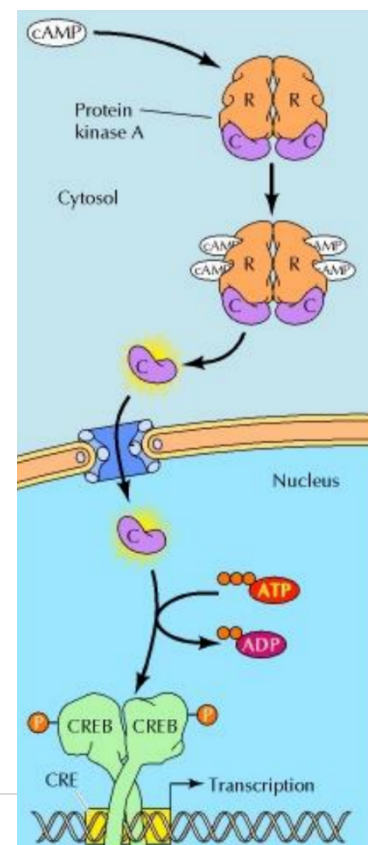
- They are often free to **diffuse to other compartments** of the cell.
- **Signal amplification** by the generation of second messengers.
- Common second messengers in multiple signaling pathways often result in **cross-talk** between different signaling pathways.
- One type of second messenger is cAMP: cAMP is AMP molecule in which its phosphate group forms a ring structure. cAMP is formed from ATP by the enzyme 'adenylyl cyclase' by removing pyrophosphate that leads to cyclization. If we want to convert cAMP back to AMP we hydrolyze it.



How does the cAMP work?

cAMP works under the GPCR (G protein coupled receptor) Binding of the ligand occurs → activation of receptor → activation of G protein → activation of adenylyl cyclase → synthesis of cAMP → cAMP is available now to act on **protein kinase A**. All protein kinases have 2 regulatory and 2 catalytic domains.

Once the cAMP binds to the regulatory domain, the kinase will be activated and a conformational change will take place. This will lead to the release of the catalytic domain. (The active/catalytic site of the kinase is inside (hiding), but once the cAMP binds to the regulatory domain, that site will become exposed. Once it is exposed it will detach from the complex and start to phosphorylate. Now, the catalytic domain is small in



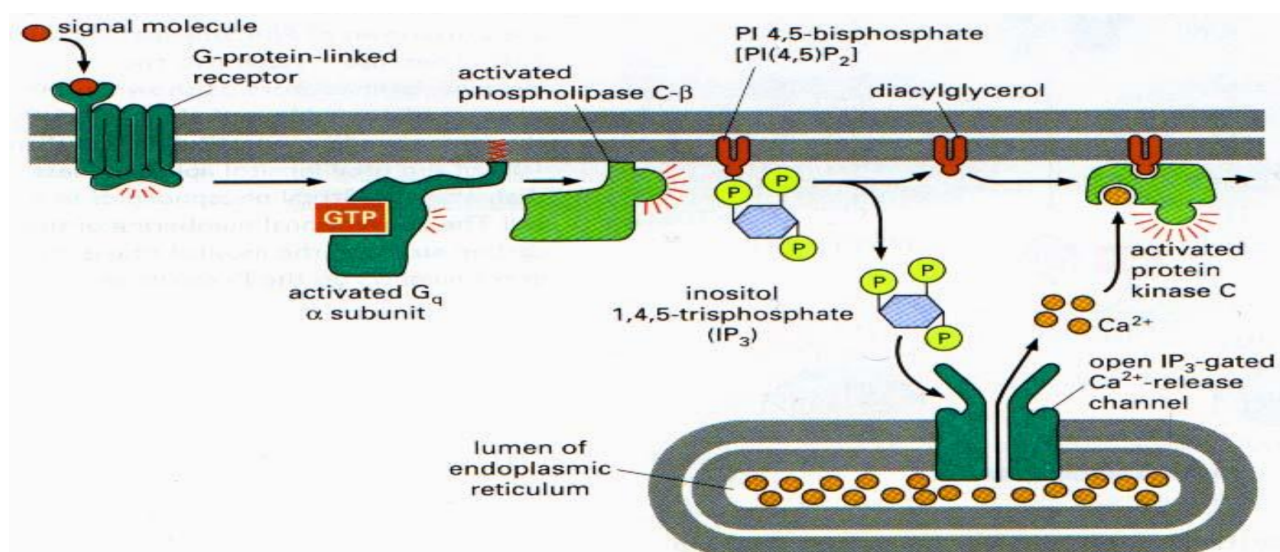
size in comparison with the bigger complex and so is capable of entering the nuclear pore complex to phosphorylate its target which is a transcription factor called **CREB**. CREB is now phosphorylated and will dimerize and bind to a specific location on the DNA and activate gene expression of the target gene leading to expression of **cAMP-inducible genes**.

Activation of kinases either inhibits or activates a certain pathway. At some point we need to stop the action of kinases. We have a lot of kinases in our body that might be over activated (constitutively activated) which means that as long as the conformational shape of the kinase is in the active form, it will continue to trigger 2<sup>nd</sup> messengers in a continuous matter. The kinase might stay in the active form because the ligand is not detaching or sometimes, even with the absence of ligand, the kinase might stay in the active form (like in cancer). At the end, this leads to the amplification of the signal (discussed in the previous lecture).

How can we turn off the kinase? PHOSPHATASE which is an enzyme that works backwardly by removing a phosphate group<sup>1</sup>.

In this example specifically, the phosphorylation of target proteins by protein kinase A is reversed by the action of **protein phosphatase 1**. Please note that here, the phosphorylation process is activating the kinase, and the kinase phosphorylates other proteins; so phosphorylation will take place in order to target proteins. This is why we need to turn it off by dephosphorylation.<sup>2</sup>

*Other types of second messengers: phospholipids and Calcium (which also work under the receptor GPCR)*



<sup>1</sup>removing a phosphate group from a protein can activate or deactivate it.

<sup>2</sup>Sometimes phosphorylating a kinase or a protein inhibits its action (transform it to the inactive form.)

Activation of the receptor by binding of ligand → activation of the G protein → alpha subunit binds to GTP and becomes active. Once it's active, it activates phospholipase C involved in the degradation process of phospholipids) and works specifically on a certain type of phosphatidyl molecules called phosphatidylinositol 4,5-diphosphate (PIP2).

PIP2 structure: inositol linked to glycerol by phosphate group + 2 fatty acids embedded in the membrane

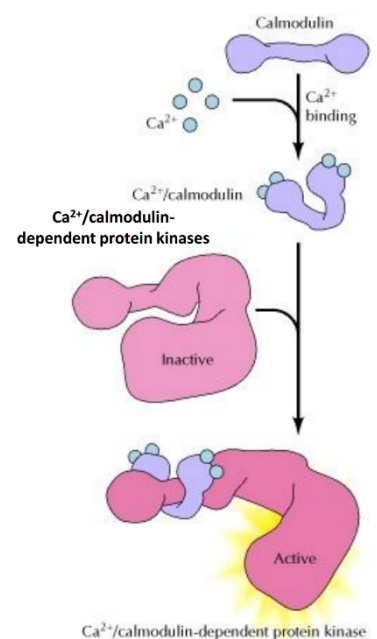
PI will become phosphorylated with two more phosphate groups → Now, we have 3 phosphate groups attached to the inositol in which one of them is attached to glycerol. The phospholipase will break the bond between the phosphate group and glycerol (catalyzes the release of the head group, IP3, from this molecule). Thus, the IP3 will get activated, and it will detach from the membrane → now it can move freely inside the cell. Diacylglycerol stays attached to the membrane.

### PIP2 → IP3 + DAG

IP3 will attach to IP3-gated channel on endoplasmic reticulum membrane → channel opens → releasing  $\text{Ca}^{++}$  to the cytosol →  $\text{Ca}^{++}$  works as a second messenger and leave the ER to bind to protein kinase C (in addition to binding to DAG<sup>1</sup>) → activate this kinase that starts to phosphorylate its target protein.

### $\text{Ca}^{++}$ /Calmodulin

Calcium can bind to other proteins such as calmodulin. Notice the figure: Calmodulin is straight in shape. The moment it becomes activated by binding of  $\text{Ca}^{++}$ , it bends from the middle and becomes able to bind to its target;  **$\text{Ca}^{++}$ /Calmodulin dependent kinase** and activates it (opening of the active site to start phosphorylating the target proteins.)



<sup>1</sup>We can count DAG as second messenger.

# Signaling pathways

*General scheme of any pathway:* binding of a ligand to the receptor → activation of the receptor → activation of the second messenger → activation of several effectors during the pathway until we reach the final effector (**transcription factor**) that has a nuclear localization signal to go to nucleus and activate the expression of certain target genes.

*Why are there cell-specific responses?*

- 1- Cells have **distinct receptors**.
- 2- Cells contain a **different combination of regulatory proteins** that influence cell behavior.
- 3- **the final effector (transcription factor) must have access to its DNA-binding site** and if the chromatin is packaged tightly, the complex will not be able to bind DNA and, hence, activate transcription.

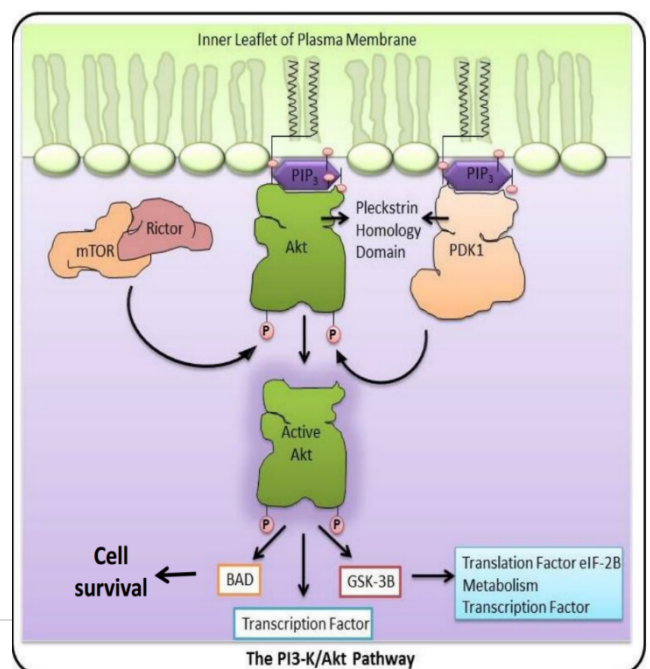
## PI3 kinase AKT pathway

It has a role in the development (formation of bone, skeletal tissues and many others) as well as a role in cancer.

PI3 kinase is not a receptor itself. It is a 2<sup>nd</sup> messenger for a receptor tyrosine kinase that usually responds to growth factors such as Insulin-like growth factors or transforming growth factor (alpha,beta...etc)

After activation of the receptor by a growth factor of any type, protein PI3 kinase gets activated which then phosphorylates PIP2 → PIP3

In the figure, PIP3 now acts as an attraction site for another type of effector molecule called **AKT** (or its called PKB standing for protein kinase B) there are several types of AKT:



-AKT 1(most common) and AKT 2: found in most of the cells

-AKT 3: special for neuronal cells

AKT is now bound to PIP3, so this is a signal for **PDK-1** to resume the activation process of AKT protein. PDK-1 will phosphorylate certain sites on AKT leading to the detachment of AKT from the membrane (as a result of phosphorylation).

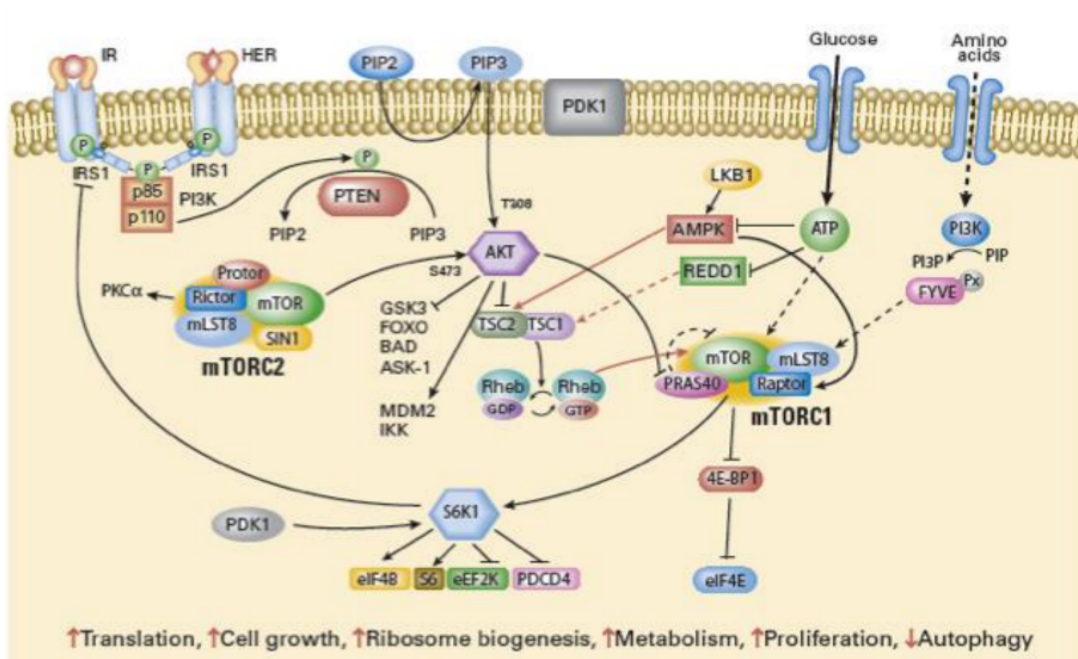
AKT is now in its active state and since it's a kinase itself, it will start to phosphorylate (since we said it is also called PKB protein **kinase B**). So, it phosphorylates an enormous amount of target proteins. You can notice the diversion of this signaling pathway and the amplification process until the final effector (which is a transcription factor) is reached.

PI3 kinase specifically is an upstream kinase and is a drug target so you can notice a lot of drug designers that are designing PI3 kinase inhibitors.

In order for AKT to go back to its inactive state we need to dephosphorylate it so there is a special enzyme called PTEN: phosphatase and tensin homolog (**it works as a tumor suppressor**) and AKT works as an oncogene if it becomes over-activated.

AKT in scientific terminology is often named 'hub' which means it's a convergent point and a divergent point.

AKT pathway is a very complex one, notice the figure (of course not for memorizing)

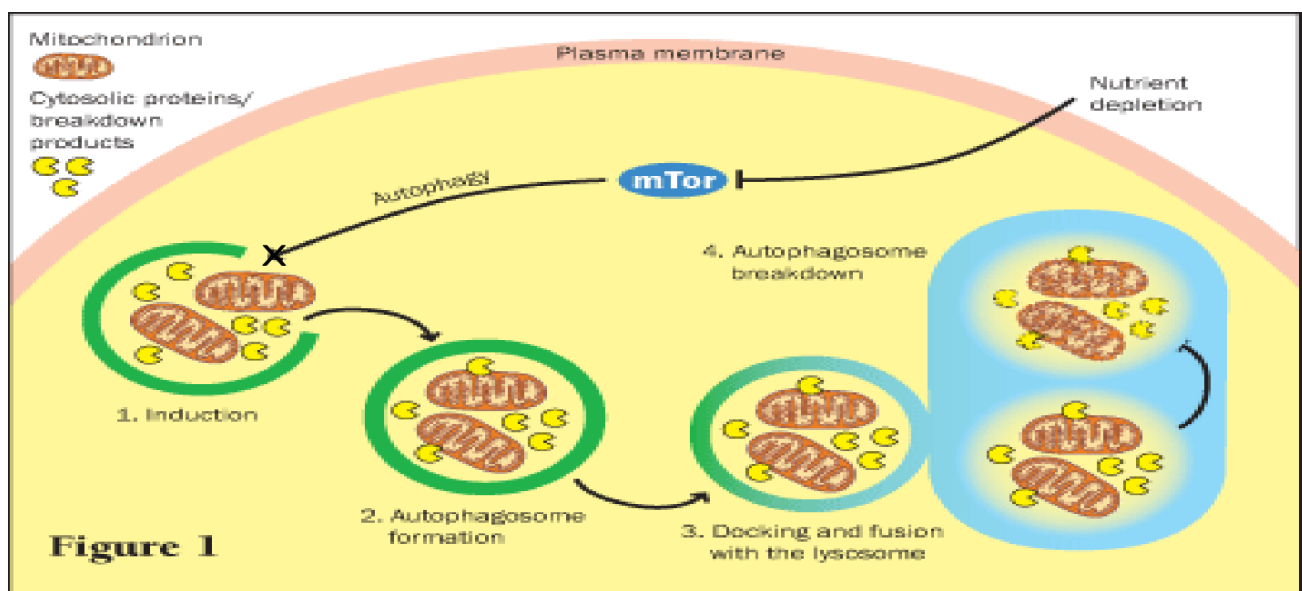


## mTOR PATHWAY and AUTOPHAGY

Which is downstream of AKT pathway in the figure above (note: it can also be activated individually meaning: its not necessarily for it to be a downstream AKT or it might be affected by different signals other than AKT)

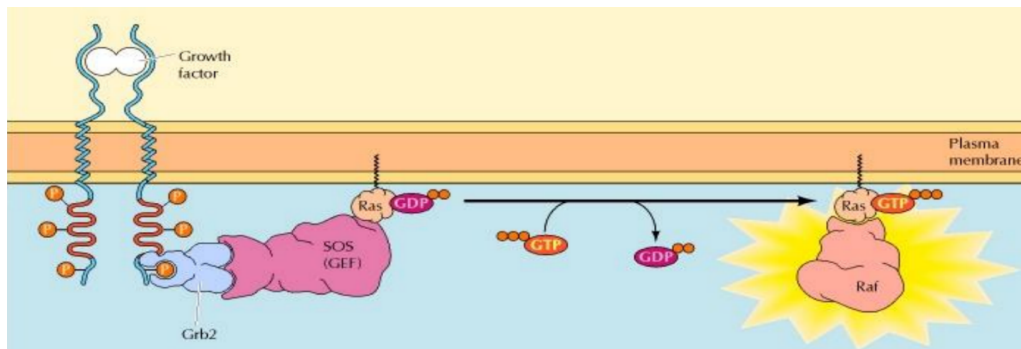
When is the cell in need of performing autophagy? In stress situations (before apoptosis) → In case of nutrient depletion, the cell needs to lower its energy consumption by getting rid of some organs.

Nutrient depletion will basically **inactivate mTOR pathway** which will → **ACTIVATE AUTOPHAGY**. So, the normal function of mTOR is to inhibit autophagy. Notice the steps mentioned in the figure below:



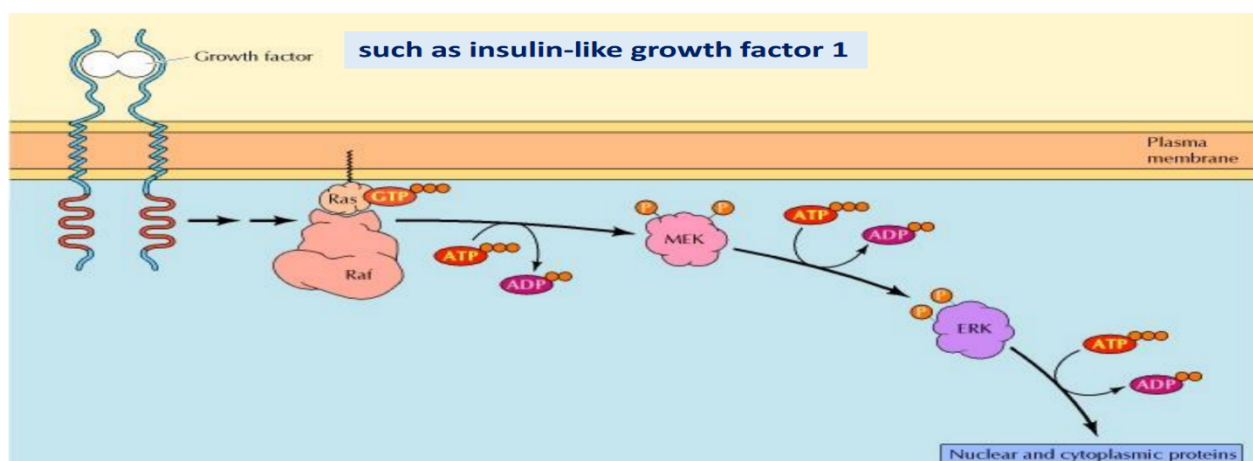
## RAS activation by RTKs

(Also important in cancer) RAS is a small GTP-binding protein that works on the receptor tyrosine kinases.



Ligand binds to growth factor → dimerization → phosphorylation → active sites will start binding to different proteins with compatible shapes and with special type of domains called **SH2**. Those domains will recognize the phosphorylated tyrosine. An example of these proteins is **Grb2**. Once the Grb2 binds to the receptor, it will get activated and activates another protein called **SOS** (GEF: GTP-Exchange-Factor) which will work on **RAS** (anchored to the Plasma membrane). RAS will exchange of GDP for GTP. Now RAS is bonded to GTP, so it is active and attracts other molecules such as **RAF**.

Since RAF it is a kinase it will start phosphorylating the next protein → **MEK**. MEK is also a kinase, so it will phosphorylate its target protein called **ERK** which is also a kinase that will phosphorylate its target protein until it reaches the final effector that will go to the nucleus. What type of genes? Genes that are responsible for proliferation and cell survival, and sometimes the genes of the regulators of cell cycle.



ERK (the phosphorylated form) is capable of entering the nucleus. Since it is also a kinase, it will phosphorylate certain types of transcription factors. Phosphorylation stimulates **ELK-1** allowing it to bind to the 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.

*Quick summary of the classic pathway: RTK → GRB2 → SOS → RAS → RAF → MEK → ERK*

## NF-κB pathway

Usually activated during inflammation not in normal cell survival or proliferation conditions.

NF-κB is present inside the cell in its inhibitory state (bound to an inhibitor ikBs)

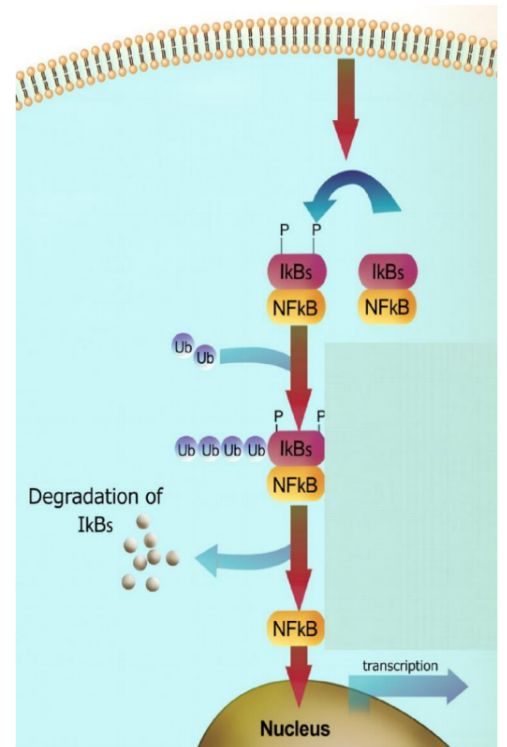
One of the receptors that trigger this pathway: *TNF receptors*

- Tumor necrosis factor (TNF) activates its receptor (TNF receptor). TNF induces inflammation and cell death via activation of the transcription factor NF-κB by stimulating the phosphorylation and degradation of IκB.

### The process in depth:

TNF binds to its receptor → phosphorylation of the inhibitor (ikBs) → separation of ikBs from NF-κB (which are bonded to each other in the normal state) → ikBs assigned for degradation by the proteasome system to perform ubiquitination (so technically we are not just separating them but also getting rid of the inhibitor by degradation) → NF-κB is now free to enter the nucleus and act as a transcriptional factor to act on **target genes** which can be:

- Proteins related to the inflammation process
- ikB itself (in order to increase its production and stop the signal.)



## WNT PATHWAY

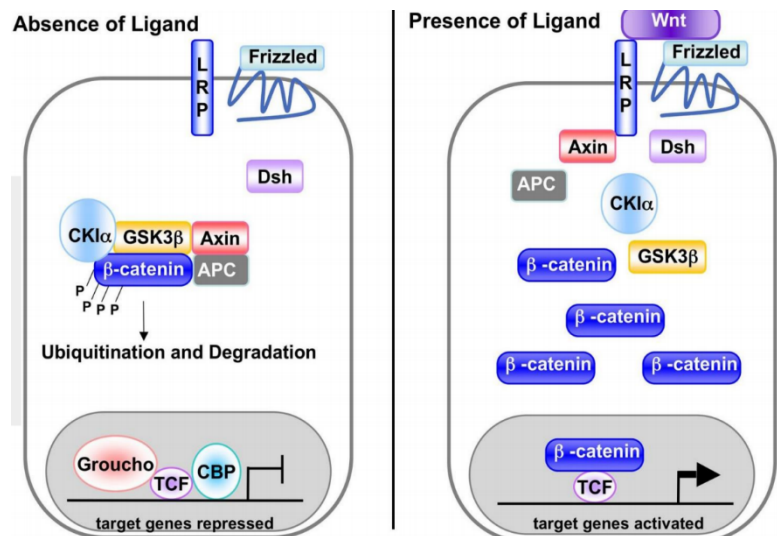
WNT pathway is one of the most important pathways during differentiation and development. Wnt proteins are growth factors that bind to the Frizzled receptors and block β-catenin degradation. β-catenin can then translocate into nucleus and activate gene expression by Tcf. (Remember: β-catenin links cadherins to actin in adherens junctions)

There are two ways of activation of WNT: canonical (classical) and non-canonical (we will not discuss this)

### CLASSICAL

The WNT **receptor** is a bit complicated it's made up of more than one protein component (notice the figure): **LRP (membrane protein) and FRIZZLED**

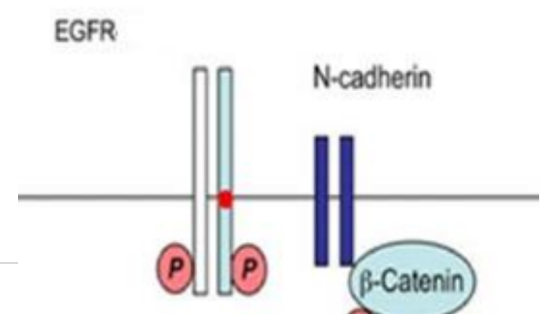
In the *absence of a ligand*: The second messengers are gathered up as a large complex inside the cytosol; inactive state.



Once WNT binds  $\rightarrow$  it will induce changes in conformation of the receptor (the two subunits are now separate)  $\rightarrow$  it will attract some components of the second messenger complex, one of these components is B-catenin, a *transcription factor*. Unlike its function in the adherens junctions, here B-catenin is a signaling molecule. B-catenin will be separated from the complex and will enter the nucleus and bind to a certain sequence activating the target gene.

### Role of adhesion molecules in signaling.

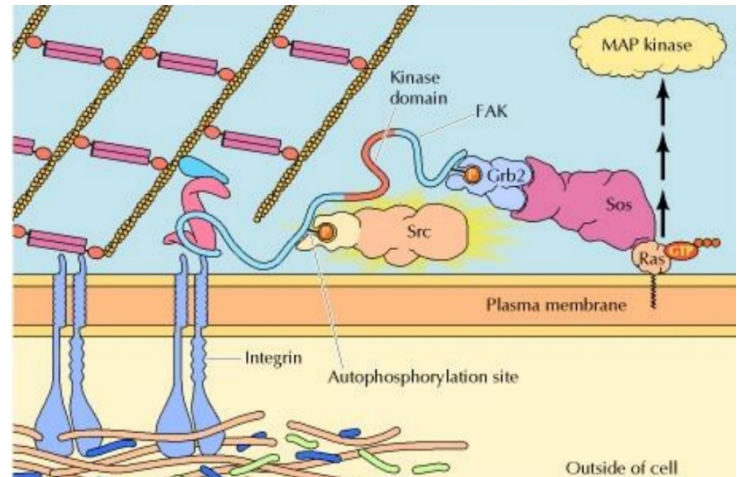
Because B-catenin is part of the adherens junctions and since the junctions contain membrane components and receptors that are present on the membrane, there is a chance for the interactions between cell-cell junctions and receptors (that trigger signaling pathways) to take place. How can this happen? EGF (epidermal growth factor) will bind to EGFR and the classical pathway of phosphorylation and dimerization takes place. As a sequence B-catenin gets activated and thus becomes a transcription factor. So, B-catenin can directly get activated when it's a part of the cellular adhesion, or indirectly as part of the 2<sup>nd</sup> messenger complex present in the cytosol.



## Integrin Signaling

Integrin is part of the cell junctions, and it also can function as part of the signaling pathway (in similar ways to the RAS pathway).

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.



Sometimes we need to transfer a signal from the extracellular matrix to the cytosol, this would affect the structure of the integrin (it must reflect as a change in conformation of integrin in order to know that a change will take place.)

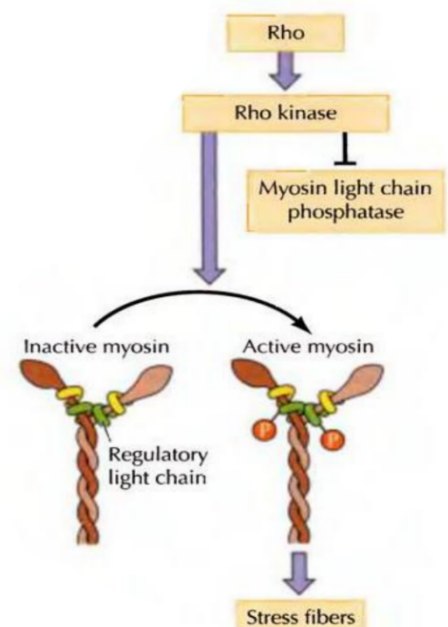
Eventually integrin will trigger FAK (focal adhesion kinase) → FAK is now activated due to the conformational change of integrin → FAK will phosphorylate Src and activates it → Src will activate the downstream effector of Grb2 which will interact with SOS

Activating the downstream of SOS: → RAS → RAF → MEC → ERK. This is called MAPK pathway. Please note that that we can start in a certain pathway then continue in another (cross-talk).

## RHO subfamily:

It is important for regulation and organization of actin cytoskeleton.

Small GTP-binding proteins (*including **RHO**, Rac, and Cdc42*) will activate **RHO kinase**. RHO kinase will *inhibit the myosin light chain phosphatase* (Notice the figure). This myosin becomes phosphorylated and active. Myosin can now form structures that are important in



regulation and organization of cytoskeleton such as **stress fibers** that support the cell.

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

There are several ways to stop the signaling pathways such as:

- 1- PTEN, which is a phosphatase that dephosphorylates the AKT in the AKT pathway.
- 2- Activation of one pathway leads to the expression of its inhibitor. This is seen in the NF- $\kappa$ B pathway; NF- $\kappa$ B regulates itself by including the inhibitory  $\kappa$ B as part of its target genes the genes.

## Cross talk

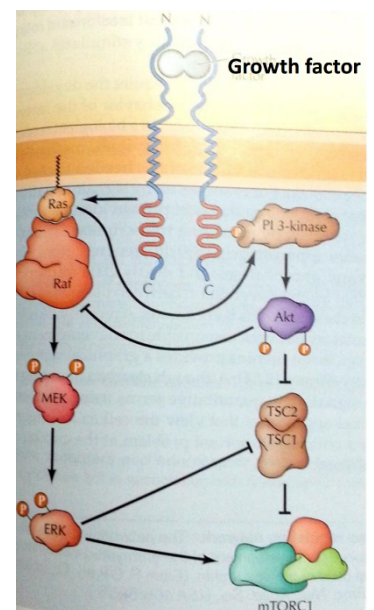
- The interaction of one signaling pathway with another and it can occur at early or late stages and it is EXTREMELY COMPLICATED.
- Examples: cAMP and ERK , Cell adhesion molecules and receptor tyrosine kinases, ERK and PI-3 kinases

## New subject: Cell Cycle (slide 10)

- A typical eukaryotic cell cycle divides ~every 24 hours.
- Mitosis and cytokinesis = ~1 hour
- Interphase: cell growth and DNA replication occur in an orderly manner in preparation for cell division.
- Zygote: no G1 or G2, but rapid S and M phases
- Some cells (nerve cells) enter a quiescent stage (G0 phase)

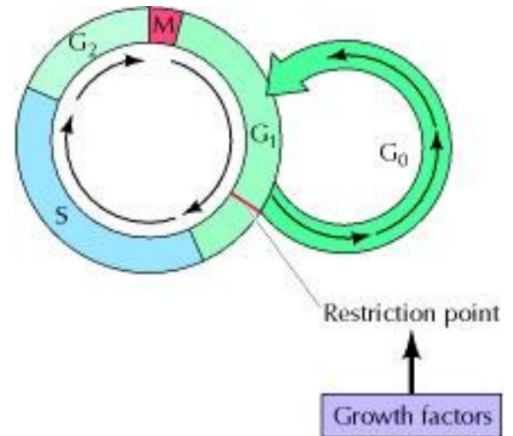
### Phases of cell cycle

1. G1: ( $2n$ ; diploid) Increased metabolism and cell growth. After G1 the cell might enter S or G0 if no division necessary)
2. S: DNA replication ( $2n \rightarrow 4n$ )
3. G2: prepare for mitosis  $\rightarrow$  metabolism and cell growth ( $4n$ )
4. M: chromosomal segregation, nuclear and cell division ( $2n$ )



## Regulation of cell cycle

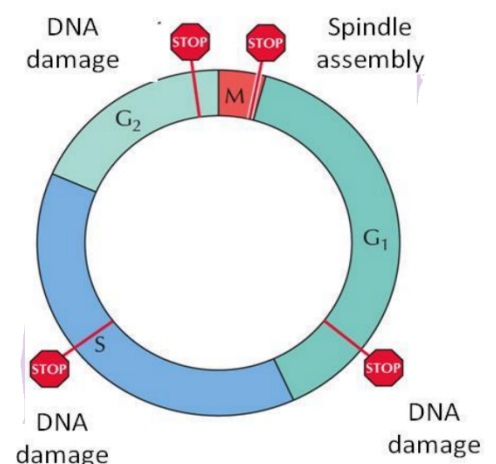
Restriction point decides whether you will enter the S or G<sub>0</sub> (very sensitive point): it is a decision point in late G<sub>1</sub> regulated by the extracellular growth factors rather than the availability of nutrients. If not there, cells enter G<sub>0</sub> phase where they are metabolically active without growth.



## Checkpoints

We have three major checkpoints during cell cycle regulated mainly by DNA damage:

- 1- **DNA damage checkpoints** ensure that incomplete or damaged DNA is not replicated and passed on to daughter cells. If it senses a damaged DNA → it will stop the proliferation process. (S and G<sub>1</sub> phase)
- 2- **Restricting DNA replication to once per cell cycle by helicase complexes** → (G<sub>2</sub> phase)
- 3- **Spindle assembly checkpoints** monitor the alignment of chromosomes on the mitotic spindle to ensure complete and accurate distribution of chromosomes. (**M phase**)



The end

*“Be realistic: Plan for a miracle”*

□ Osho