◉Sheet ○Slide

Number: 33

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So far, we have covered the types of signaling molecules, receptors, and second messengers whilst giving examples on them.

Signaling pathways

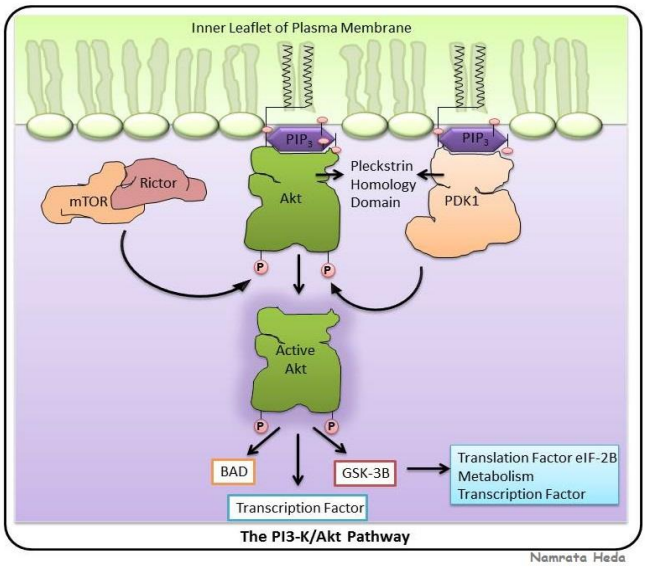
There are abundant signaling pathways, but we will be exploring only a few important ones.

- **Why would two different cells show a different response when both are exposed to the same stimuli?**

1. Cells have distinct receptors: Either different quantities are presented or there is a different combination of receptors embedded in the cell surface.
2. Cells possess a different combination of regulatory proteins that influence cell behavior: Regulators can act as either activators or inhibitors. A different response is dependent upon the concentration of regulatory proteins. For example, in some cells the regulatory protein X (an inhibitor) is absent so the pathway is turned on, while in other cells the inhibitor is present therefore the pathway stops.
3. The final effector (transcription factor) must have access to its DNA-binding site: In the end, it has to be activated to bind to an element on the DNA to activate the gene. In order for this to happen, we need an appropriate chromatin status. If the chromatin is packaged tightly, the complex will not be able to bind DNA and, hence, activate transcription. This explains why, although each cell in our body has the entire genome, it only produces certain proteins.

PI-3 Kinase AKT Pathway:

PI-3 kinase is an enzyme that can add a phosphate group to phosphatidyl inositol diphosphate (PIP2) to form the phospholipid PIP3, which acts as a binding site for AKT. PIP3 phosphorylates AKT and activates it.



Once AKT is activated, it detaches from PIP3 and then functions as a kinase to phosphorylate many target substrates to activate or produce gene products that will lead to cell proliferation, differentiation and survival:

1) **BAD:** Pro-apoptotic protein. *Inhibited* upon phosphorylation, which promotes cell survival.
2) **GSK-3B:** *Inhibited*, which activates metabolism, thus promoting cell survival and proliferation.
3) **mTOR:** The mTOR pathway inhibits autophagy, so AKT will *activate* it to increase cell survival and differentiation

The main point is that AKT as a signaling molecule acts as a "hub", which means it's a point of convergence and divergence >> AKT can be phosphorylated by many molecules, while it also acts on a lot of substrates.

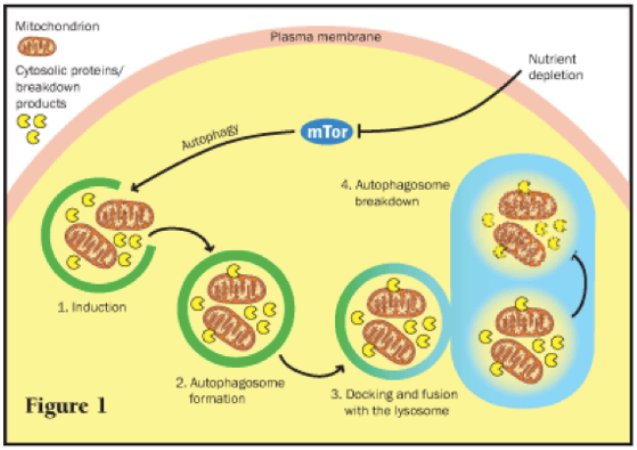
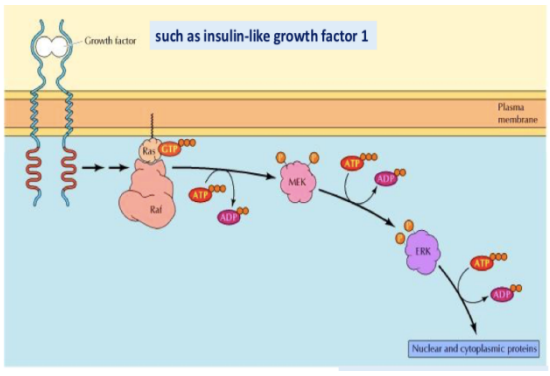


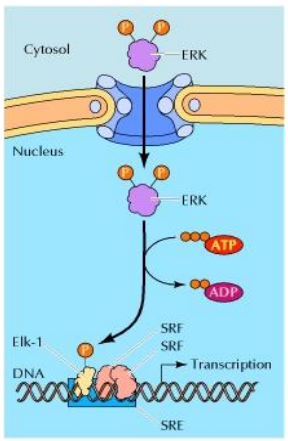
Figure 1

MAP Kinase pathway:

Ras is a GTP binding protein anchored to the membrane by farnesylation. It is activated when the receptor kinase is stimulated by a growth factor, and the response will lead to more cell survival and proliferation.

1. A growth factor binds to the receptor tyrosine kinase >> Dimerization >> Phosphorylation >> we now have an active site for the binding of phosphate groups on the cytosolic side.





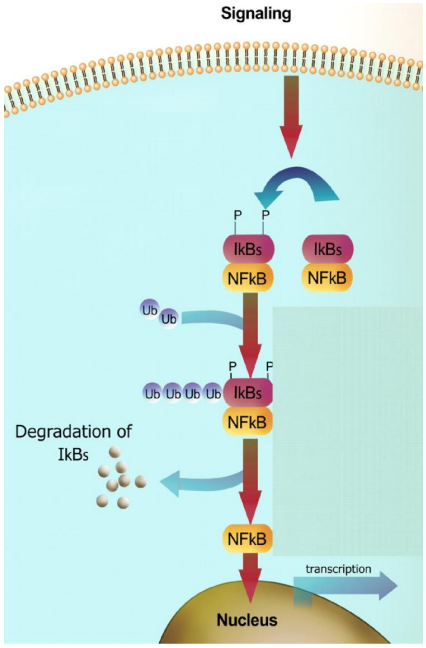
2. The first protein to bind is GRB2, which will activate and bind to SOS **(**a GTP-exchange factor).
3. SOS will remove GDP and add GTP on RAS (GTP-binding protein). Once RAS is bound to GTP, it will become active and will activate downstream molecules including RAF.
4. RAF is a kinase which will phosphorylate MEK. MEK is also a kinase which will phosphorylate a target protein kinase known as ERK. ERK has a nuclear localization signal that allows it to enter the nucleus. ERK will phosphorylate the transcription factor ELK1. Due to phosphorylation, ELK1 will bind to a serum response factor, which will then bind to serum response element and finally activate target genes that lead to cell survival and proliferation.

Summary: GRB2 > SOS > RAS > MEK > ERK > ELK1 > Serum response factor > Serum response element > Activate target genes > Cell survival and Proliferation

NF- κB Signaling Pathway:

This pathway is activated under inflammatory conditions.

1. TNF/ cytokines bind to the receptor
2. A kinase phosphorylates inhibitor (IκBs)– which is in a complex with NF-κB
3. IκBs is assigned for degradation by being ubiquitinated
4. IκBs separates and 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



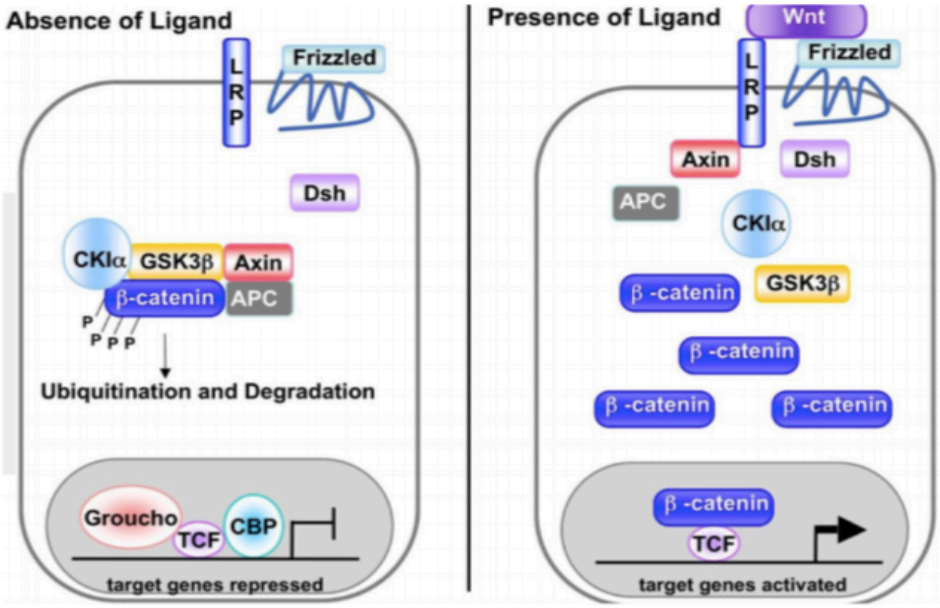
WNT Signaling Pathway:

The WNT protein is a growth factor which will bind to a receptor. Its receptor is characterized by an unconventional surface and is made up of two molecules: the first is the LRP (a membrane protein), and the second has a "frizzled" appearance. The presence of both of these receptors is essential for the WNT molecule to bind and stimulate a response. There are many different types of WNT molecules like Wnt_1, Wnt_3, and Wnt_{5A}.

Wingless is a counterpart signaling molecule, which was found in the drosophila fly. When a mutation took place, the fly would be wingless and hence where the name came from. However, humans only have the WNT protein.

Wnt signaling includes a canonical (classical) and a non-canonical pathway. We will only discuss the canonical pathway.

Canonical WNT Pathway (Classical):

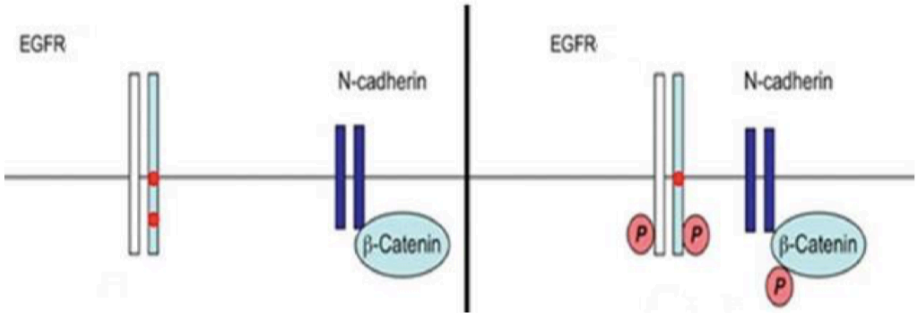


In the absence of the Wnt ligand on the receptor, a group of proteins that include GSK3β, axin, β-catenin, and APC are present in the form of an inactive complex in the cytosol. The β-catenin in this case will be phosphorylated, ubiquitinated, and degraded.

When Wnt binds to the receptor, it induces a conformational change which encourages the binding of some proteins from the complex including the axin to the receptor. When the axin binds, the rest of the complex in the cytosol will disassemble. β-catenin will thus remain in the dephosphorylated form (its active form). It can go to the nucleus to act as a transcription factor and activate target genes through its binding.

The β-catenin in this case differs from the other one that we discussed previously, which was bound to adherens junctions. Here it acts as a signaling molecule in the Wnt pathway.

- How do we differentiate between the two forms of β-catenin (the one in adherens junctions or the one that is the signaling molecule)?

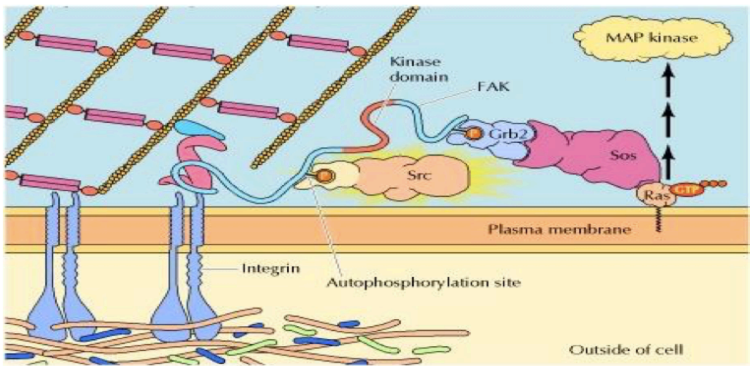


The β-catenin in the adherens junctions is bound to a N-cadherin, and an actin cytoskeleton on the cell membrane. A growth factor receptor may also be present like EGFR (epidermal growth factor receptor). In this case the inactive form of the β-catenin is the dephosphorylated state. When the growth factor binds to the receptor, dimerization, phosphorylation and activation will take place including that of kinases that will work on β-catenin. Here, the phosphorylation is not a marker for the degradation of the molecule. Instead, it induces activation so that it will become a transcription factor.

Therefore, the β-catenin in the adherens junctions is activated by phosphorylation, while the signaling form is inactivated by it. This is due to the different positions in which the phosphate group was added to both molecules.

Integrin signaling:

Integrin is a part of the focal adhesion. When integrin is attached to the ECM it's found in the activated state, but when it detaches it changes its conformation and becomes inactive.



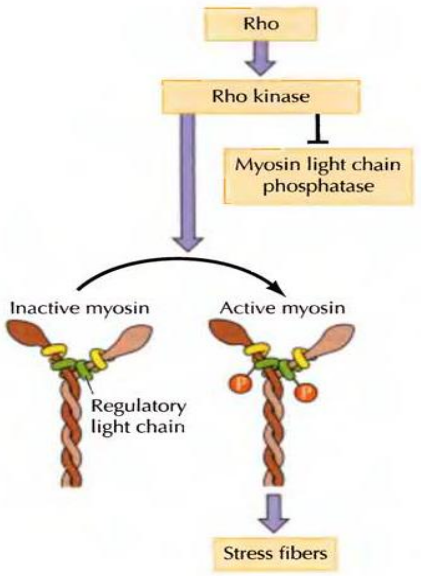
Certain changes in the conditions of the extracellular matrix will signal to the integrin in turn changing its structure, resulting in the Autophosphorylation and activation of

FAK (Focal Adhesion Kinase - the blue rope in the picture). Autophosphorylation is facilitated by the Src protein, specifically at the tyrosine residues of FAK. Once FAK is activated, the phosphotyrosines bind to Grb2-SOS complex, activating the Ras Pathway.

The Rho Subfamily Signaling Pathway:

The members of this subfamily are Rho, Rac and Cdc42, which are small GTP binding proteins. They have a role in organization and regulation of the actin cytoskeleton. These molecules will activate:

1) Rho kinase, which phosphorylates and activates myosin while inhibiting the myosin light chain phosphatase
2) Proteins that polymerize the actin cytoskeleton (ex.WASP, formins, profilin, and Arp2/3).



These series of steps are needed when forming contractile rings, lamellipodium during cell movement, or cell and focal adhesions that attach to the actin cytoskeleton.

Regulation of pathways

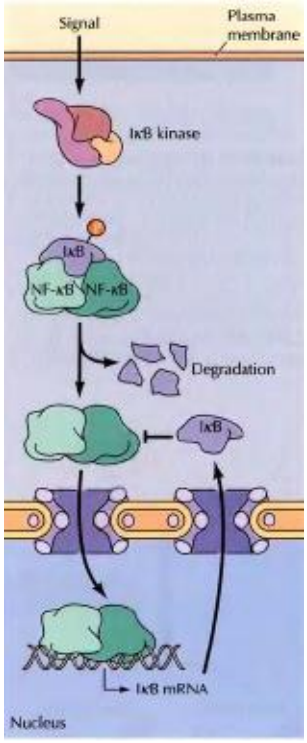
It is a very complex procedure that involves feedback mechanisms whether be it negative or positive.

How does regulation of the pathway happen?

1. Self-regulation by expression of the pathway's <u>inhibitors</u> (ex. IκBs in the NF- κB Signaling Pathway)

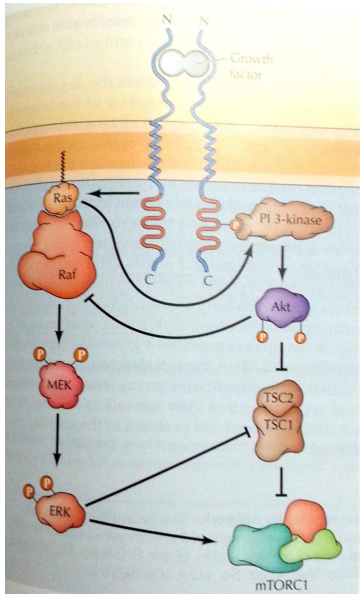
2. <u>Crosstalk</u>**:** interactions between separate signaling pathways.

   Benefit: In a theoretical pathway X, a mutation happens in the enzyme of one step. Usually, this will lead to the disruption of the proceeding steps. However, due to crosstalk, a protein in a step previous to the mutation can interact with another pathway and stimulate it activating its target genes. In some



cases, this can have a compensatory effect for the mutation that happened in the first pathway.

Examples (No need to memorize the details):



1) Tyrosine kinases receptor: PI 3-kinase can either activate the Akt or alternatively the Ras in another pathway. Akt can inhibit the TSC2 and TSC1 complex, which will inhibit the mTORC1. Independently from the AKT, ERK in the Ras pathway can activate mTORC1 and inhibit the TSC2 and TSC1 complex. Also, AKT can also inhibit the Raf earlier on in the pathway. These are examples of how two different pathways can interact with each other.

2) G protein coupled receptors with the tyrosine kinase: Phospholipase C can be activated from two pathways from the tyrosine kinase receptor and the G-protein linked receptors. Also, Ca^{2+} from the G protein linked receptor pathway can activate the protein kinase C which is a part of the tyrosine kinase one.

Sometimes, target genes can be common between multiple pathways or absent in others. For example, pathway X can work on the target genes 1, 2, and 3, while pathway Y works on gene 3 only.

THE END