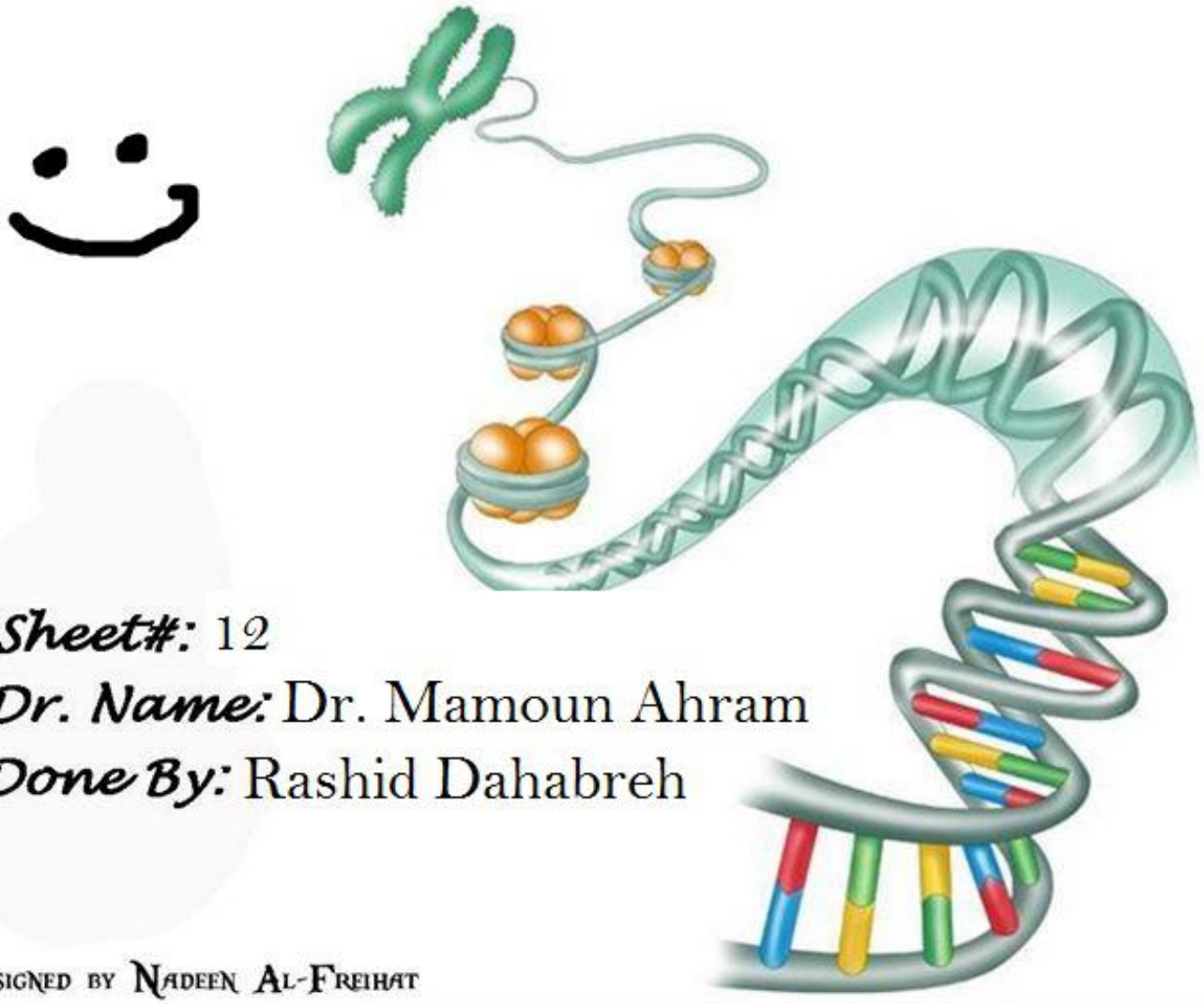
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



Sheet#: 12
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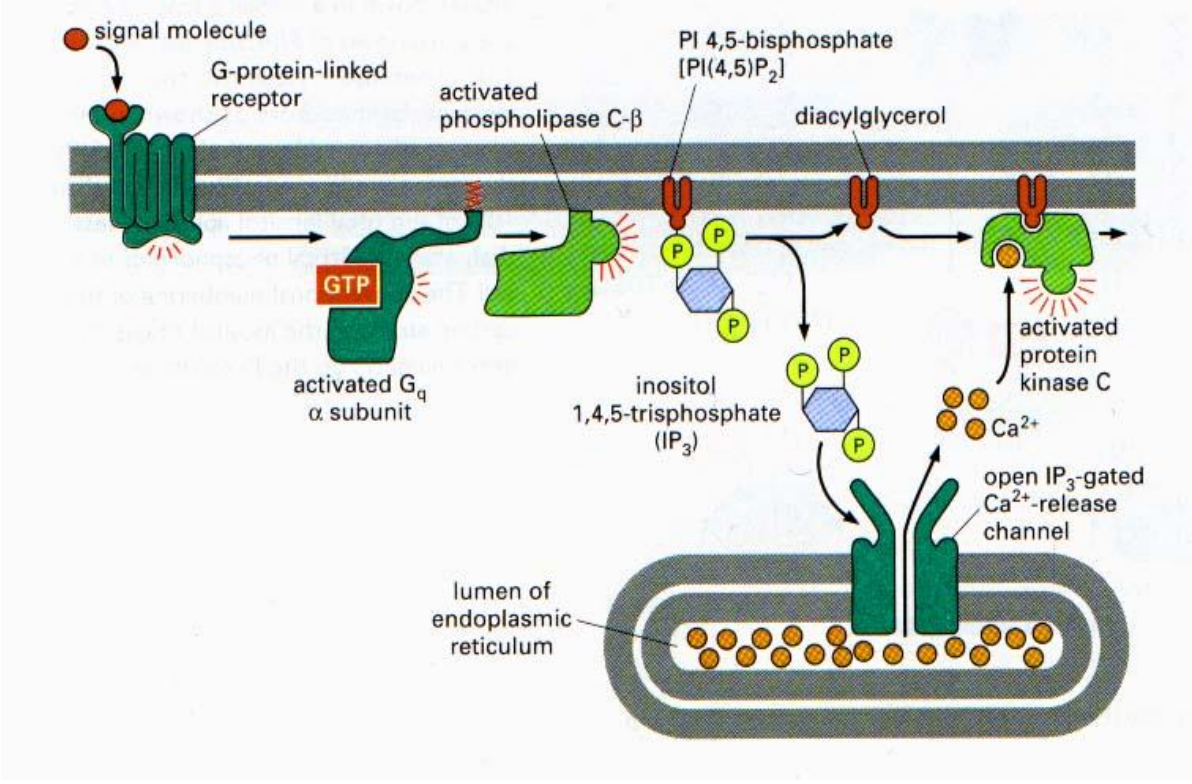
Secondary messengers (2)

Before we start, I'd like to mention that I've mentioned most of the information in the slides in this sheet so I believe there's no need to go back to the slides, only for the figures perhaps.

So ladies and gentlemen, buckle up your seatbelts because we're off to a long, hopefully not too boring, journey.

Let's start, shall we?

Another type of secondary messengers we're going to talk about are phospholipids and calcium ions, and this is how they work as the following:



You have a G protein linked receptor(a seven transmembrane domain receptor), a ligand binds to it, so what's going to happen next? The receptor will activate the G protein by inducing the GDP to GTP exchange in the alpha subunit (Remember that the G protein consists of 3 subunits: α, β, and γ)

In the unstimulated state of G protein; the α subunit has GDP bound to it (inactive state) and when the G protein is activated, the α subunit releases its bound GDP, allowing GTP to bind in its place and notify that this exchange causes the trimer to dissociate into active components, alpha subunit and a Beta-Gama subunit.

After that, there will be an interaction between the α subunit or the β-γ subunit with an enzyme, one of these enzymes is known as phospholipase C (An enzyme exists on the cell surface), when it's activated it will cleave a phospholipid known as **PIP2** (PI 4,5 biphosphate) into two molecules: the first one is called **Diacylglycerol** and the second one is **IP3**(Inositol 1,4,5-triphosphate)

IP3 binds to a channel on the ER known as **IP3-gated Ca^{2+} release channel**, the channel then will open up releasing calcium ions.

Diacylglycerol, on the other hand, binds to an enzyme known as **protein kinase C** (Also known as PKC, which is a protein kinase enzyme). In order for the protein kinase C to be activated, it requires to bind to **both Diacylglycerol and Calcium ions coming from the ER** (in the mechanism we elaborated above).

That been said, the last thing to be mentioned is that protein kinase C is an enzyme (a protein kinase enzyme) and when it's activated, it phosphorylates many other target proteins.

Previously, it was mentioned that protein kinase C (PKC) needs and requires calcium ions and there's also another protein that needs calcium ions known as Ca/Calmodulin

Characteristics of Calmodulin:

The word Calmodulin has two parts, Cal for calcium ions and modluin stands for modulation as in changing or alteration, it changes its structure upon binding to Ca ions.

It can interact with other proteins, like kinases. Example

1)Ca/Calmodulin dependent protein kinases signals actin-myosin contraction (induces muscle contraction)

2) CAM kinases (CAMK- is an abbreviation for the Ca^{2+}/calmodulin-dependent protein kinase class of enzymes) regulates the synthesis and release of Neurotransmitters.

One of the targets of CAM kinase (CAMK) is CREB (CREB is a protein activated by protein kinase A as we said before, and it's also activated by CAM kinase.

Please notify that CREB is a cellular transcription factor that binds to certain DNA sequences called cAMP response elements (CRE) thereby increasing or decreasing the transcription of the downstream genes

Question, why are secondary messengers important?

1)Second messengers are small so they're often free to diffuse to other compartments of the cell(like calcium for example , which can go outside the ER). Not only they can diffuse to outside the dell, they can also transfer from one cell to another via Gap junctions (A specialized intercellular connections that directly connect the cytoplasm of two cells, where small metabolites can pass through them)

2)The signal may be amplified significantly in the generation of second messengers (we don't want the activated enzyme to produce one product, instead, we want to produce thousands of

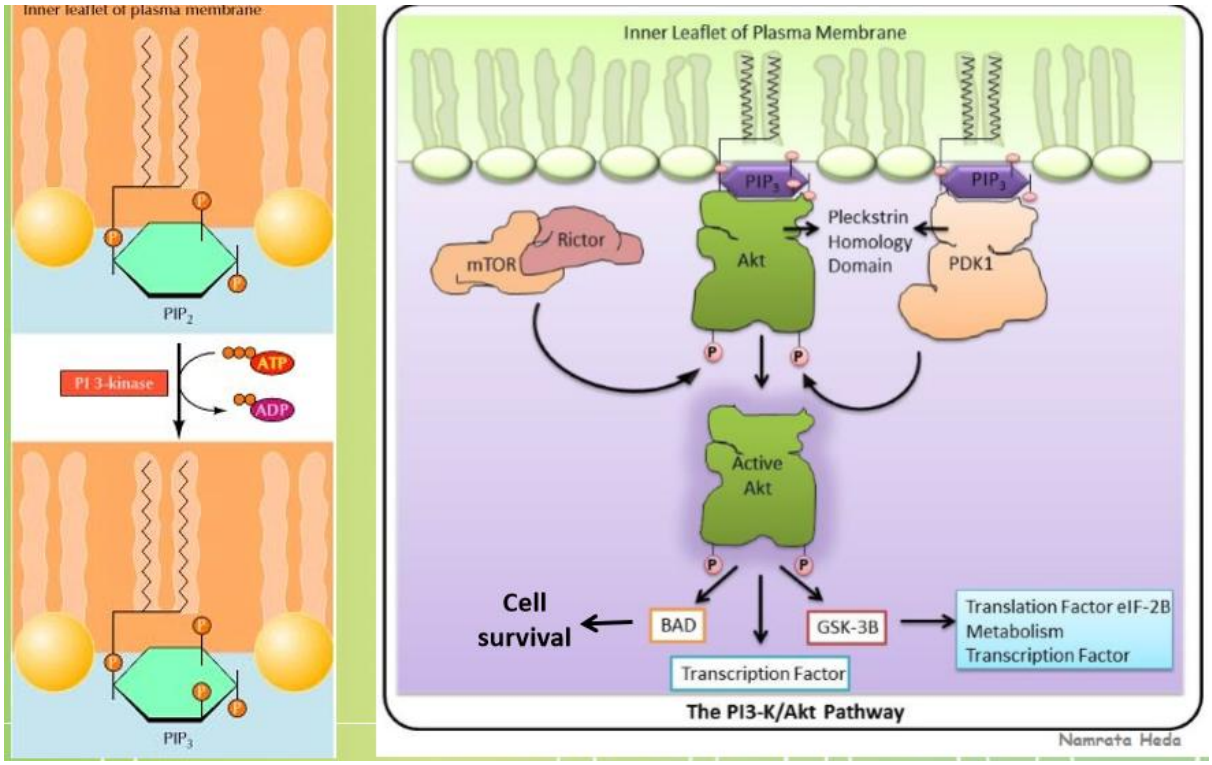
products ,and these products can activate thousands of proteins to have an amplification of a single signal.)

3) The use of common second messengers in multiple signaling pathways often results in cross-talk between different signaling pathways. So messengers are shared between different pathways (Ex. cAMP affecting different pathways resulting in a cross talk between them)

Signaling Pathways.

We have many signaling pathways inside the cell and we're going to mention some of them, Keep in mind that for every pathway we're going to discuss, you have to know the end result of each pathway and the most significant proteins that was involved in the pathway.

1)PI-3 Kinase and AKT pathway:



There's an enzyme known as PI-3 kinase (also called phosphatidylinositide 3-kinases) which can convert **PIP2** to another lipid molecule known as **PIP3**. Notify that this reaction takes place in the plasma membrane since PIP2 is found in the inner leaflet of the plasma membrane. So after PIP2 is changed to PIP3, PIP3 will be considered as a docking site as if it's a place where other proteins can bind to.

So once we have PIP3 in the plasma membrane, it can interact with another protein known as AKT (also called protein kinase B). Once AKT exists in the plasma membrane bound to PIP3, it's now very close to other regulatory molecules and kinases. Note that AKT can be activated further by phosphorylation, one of the proteins that can phosphorylate and activate AKT is mTOR, after that the AKT protein can affect the function of other proteins and modulate the status of the cell.

IN ONE LINE: PI3 kinase coverts PIP2 to PIP3, PIP3 interacts with AKT, mTOR further activates AKT, AKT interacts with other proteins and change their activities

Note: the signal of AKT is a pro-survival signal (allows cells to escape death and stay alive).

mTOR pathway and autophagy:

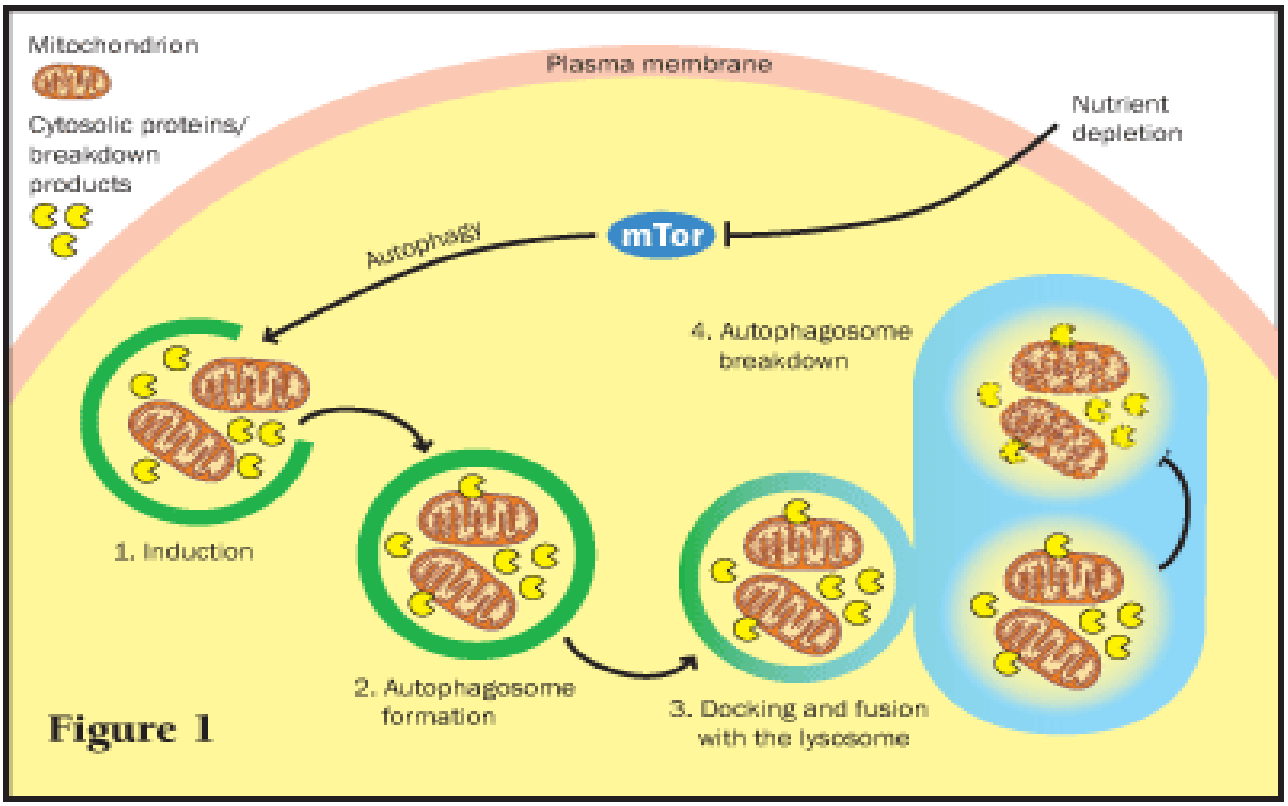


Figure 1

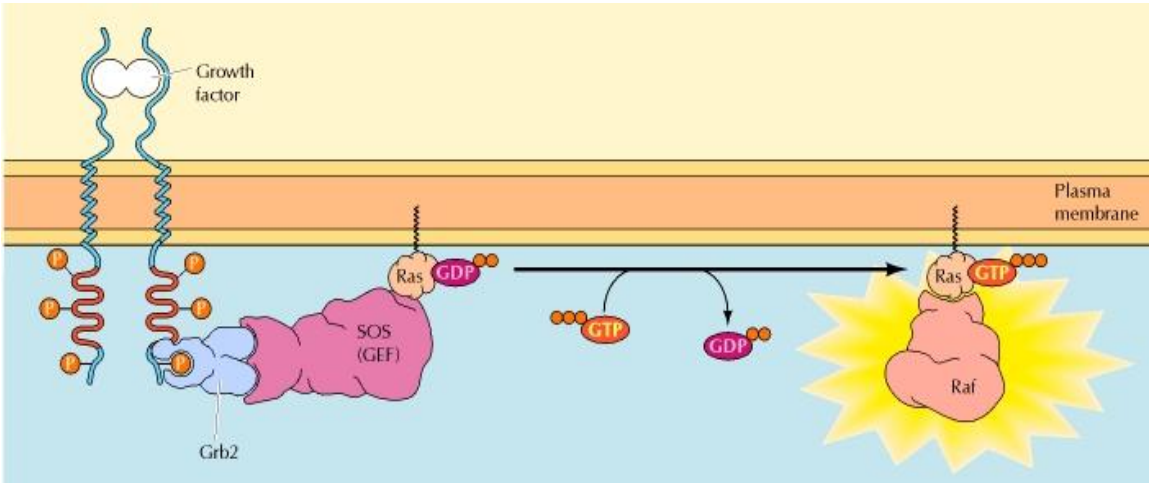
Always keep in your mind that any signaling pathway in the cell has activators and inhibitors. Therefore, mTOR pathway can be inhibited if there's a nutrient depletion and when the cell is healthy and there are nutrients, mTOR pathway is activated.

If there's nutrient depletion, mTOR protein is inhibited, and that leads to activation of autophagy(Self eating process) and that makes perfect sense; There's no nutrients available to the cell so the cell will start eating its extra components and organelles in order to obtain the nutrients and survive

in one line: mTOR is activated, the cell won't die. mTOR is inhibited, the cell will go to autophagy as a last ditch effort to survive.

2)MAP kinase pathway

Ras activation by RTKS (Receptor tyrosine kinases):

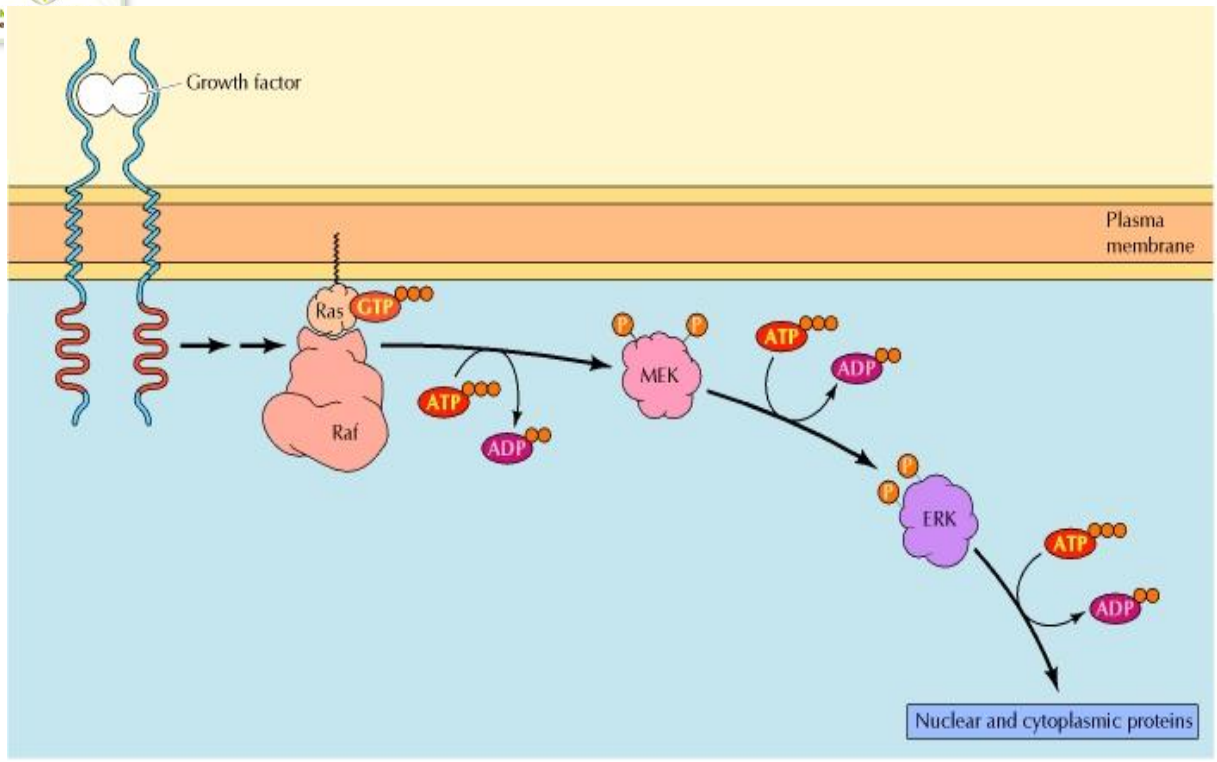


It was mentioned before that Ras is a small GTP-binding protein. We say that it's small to differentiate it from the large G proteins which is a heterotimer.

Since Ras is a GTP-binding protein, you can tell that Ras is activated when it's GTP-bound and inactive when it's GDP-bound.

And as we know, any cycle between changing between binding to GTP or GDP is regulated by two other classes of proteins; GEFs (GTP exchange factors, which are the activators) and GAPs (GTPase activating proteins, which are deactivators).

So this is the flow of series of this pathway:



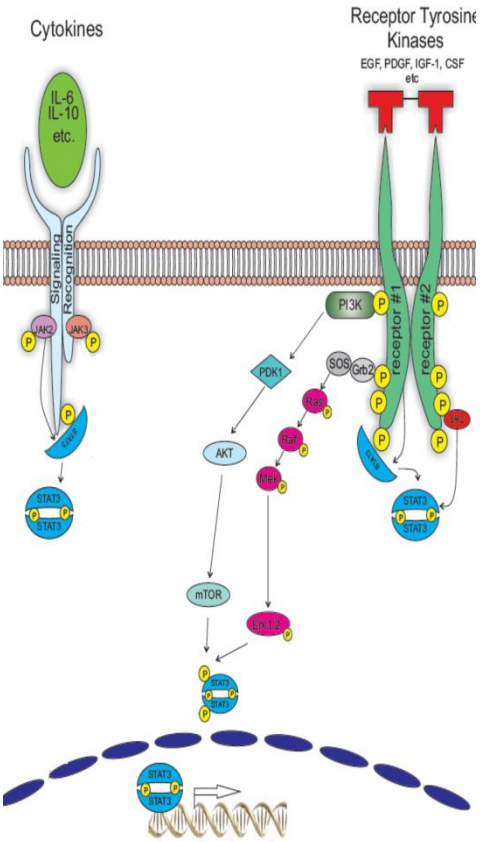
The ligand comes in and binds to a receptor tyrosine kinase, dimerization of the receptors, autophosphorylation of the receptor, it's now active and can bind different proteins and one of these proteins is a well known GEF called son of sevenless (SOS). SOS activates Ras by displacing GTP in GDP.

When Ras is active, it initiates a phosphorylation cascade: involving Raf, MEK, and ERK proteins leading to ERK activation and translocation to the nucleus. Once it's in the nucleus, ERK activates a transcription factor(don't worry about its name) that mediates and induces gene expression.

So as a conclusion of what Ras does, Ras is that it increases cell proliferation and cell growth. (That's why if it's mutated, a cancer will occur).

IMPORTANT NOTE: Ras isn't an enzyme, it's a transducer just like G proteins

3) Regulation of gene expression by STATs



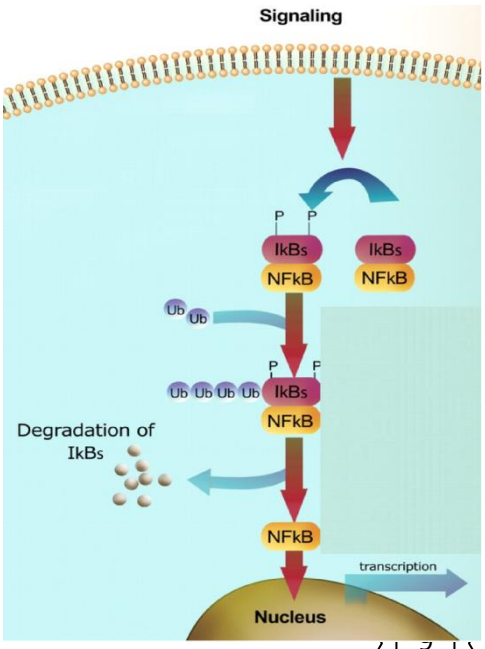
The STAT protein (Signal Transducer and Activator of Transcription) is a transcription factor.

STATs(transcription factors) link non-receptor tyrosine kinase pathways (Like Janus kinase pathway-JAK pathway) to MAP kinase-regulated RTK pathways BECAUSE both pathways activate STATs.

How are STATs activated?

They must be phosphorylated; Phosphorylation of STATs is either by the receptors themselves(in case of RTK) or by receptor-associated kinases (in case of non-receptor associated kinase). Phosphorylation promotes their dimerization and translocation to the nucleus, where they stimulate transcription of their target genes

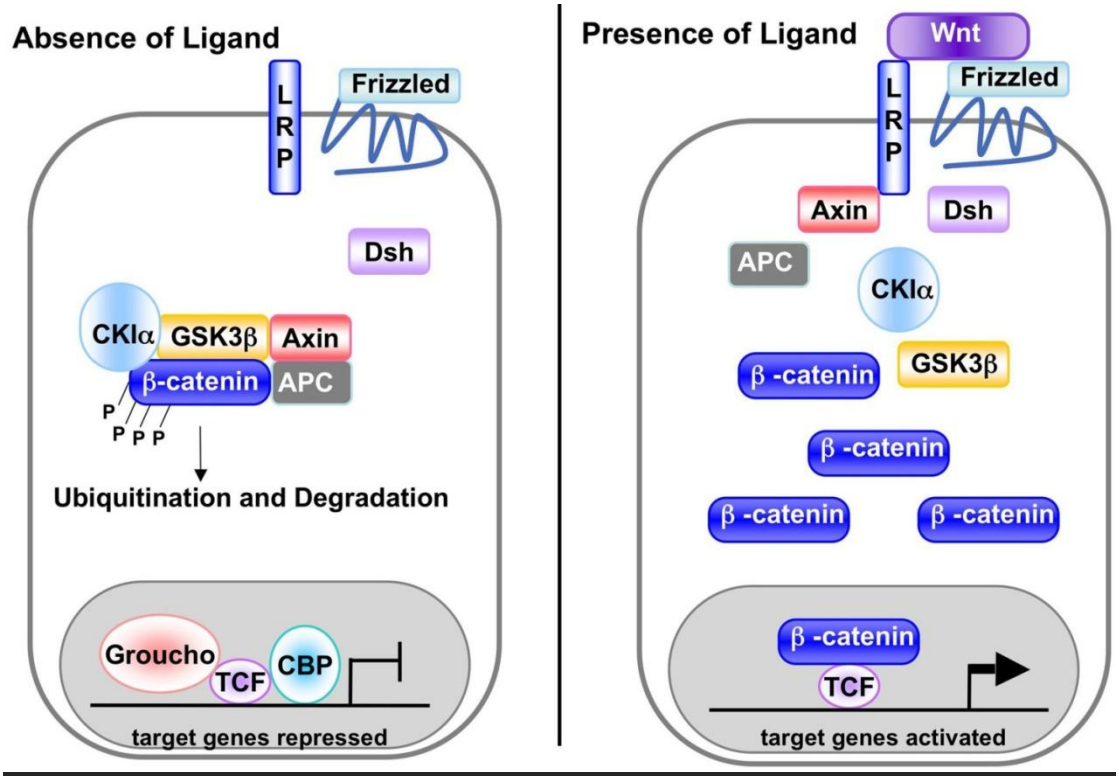
4) Nuclear Factor-KB (NF-KB) signaling:



NF-KB is a transcription factor, it's found in the cytosol bound to Inhibitor of KB (IKB), which prevents it from going into the nucleus.

Upon an induction of signaling like the binding of the ligand Tumor Necrosis Factor (TNF), which is produced by killer T cells, to its receptor (TNF receptor), the signal induced then shall stimulate the phosphorylation and degradation of the Inhibitor of KB (IKB), the NK-KB can then go into the nucleus and change the expression of certain genes.

5)WNT signaling pathway:



In resting state, when there isn't a WNT ligand, B-catenin forms a macromolecular complex containing the APC protein (Adenomatous polyposis coli) which is a transcription factor and a tumor suppressor gene. The complex is known as destruction complex and it leads to phosphorylation, ubiquitination, and destruction of B-Catenin.

When there are WNT ligands (they're growth factors), they'll bind to their receptors which are known as Frizzled receptors (another family of G-protein coupled receptors) and deactivates the destruction complex and blocks B-Catenin degradation;

B-Catenin can then transolcate into nucleus and activate gene expression by TCF.

Note: remember that B-Catenin is the protein that links E-cadherins to actin filaments in adherens junctions.

E-Cadherins can interact with other receptors like receptor tyrosine kinases and they can regulate each other, example: you could have cadherins interacting with Epidermal growth factor receptor (EGFR) and then the receptor will phosphorylate the B-Catenin and regulate its function, also the E-cadherin can change its activity when it interacts with the receptor. So this is a link between cell adhesion and signaling as well.

Role of adhesion molecules in signaling:

-Interaction of Cadherins with cell surface receptors result in dual regulation and signaling and promotion of cell survival.

-Recall how integrins are activated?

(Note: The following paragraph is found in the book but not in the slides)

You know that integrins link the cell to the ECM , These integrins bind to matrix proteins that activate them and lead to the recruitment of other integrin binding proteins , these proteins are phosphorylated and activated.

one of the most important proteins that is activated is Src, which is a kinase, this Src will phosphorylate other proteins that can interact with and activate SOS that will activate Ras (

a transducer) which will initiate a signaling pathway as we mentioned before, ending in cell proliferation and growth. So there is a link between signaling and the binding of the cell to the ECM via integrins

6) The Rho subfamily signaling pathway:

-Rho subfamily of proteins includes 3 proteins (Rho, Rac, and Cdc42)

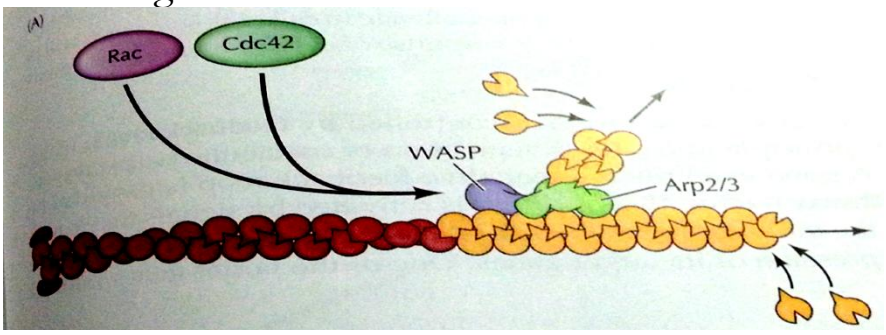
-these proteins are small GTP-binding proteins (just like RAS, RAN, ARF, and RAB.

-This family is responsible for regulating and changing the organization of actin cytoskeleton (Cell motility, cell adhesion, and cytokinesis).

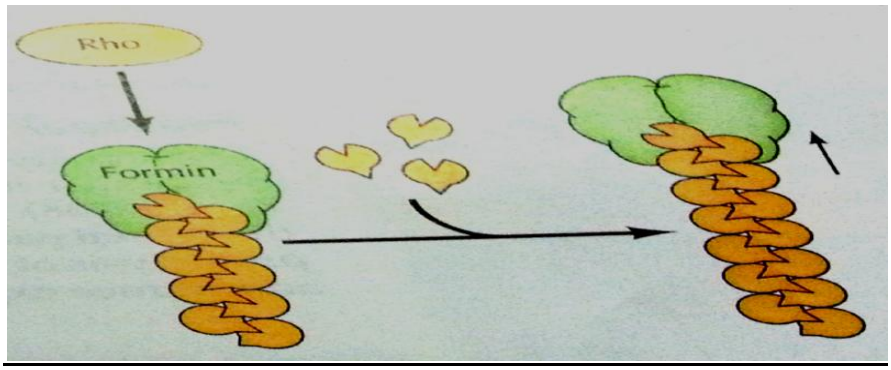
-Biological effects: Rho activation induces formation of stress fibers (actin bundles) / Rac activation induces formation of laminopeudia/ Cdc42 activation induces formation of filopeudia.

-Mechanisms of action:

1)Rac and Cdc42 activates WASP protein, and WASP activates Arp2/3 (which is the protein responsible for growing and branching of the actin filament



2)Rho activates the protein Formin, which is responsible for the formation of the acti bundles (stress fibers).



Signaling Networks and regulation:

Recall the NK-KB and its inhibitor (IKB); a signal comes in and phosphorylates and degrades IKB allowing NK-KB to go inside the nucleus and induces the transcription of certain proteins. Ironically, one of these proteins is the inhibitor itself so it shall terminate the signal again.

Conclusion: An activation of one pathway leads to expression of its inhibitors. (i.e. a transcription factor signal can be terminated when this factor induces the expression of its own inhibitor , and this is a way of regulating that signals)

CrossTalk.

Another way of how signal transduction pathways are regulated is something called **Crosstalk**, which is the interaction of one signaling pathway with another.

Examples on crosstalk:

1) cAMP and ERK

2)Cell adhesion molecules and receptor tyrosine kinases

3) ERK and PI-3 kinases

One of the slides shows you how different pathways are interconnected and the beauty of the cross talk. It's a perfectly made network of interactions that we need to only appreciate how complicated it is without memorizing the details for sure (the doctor won't ask about them).

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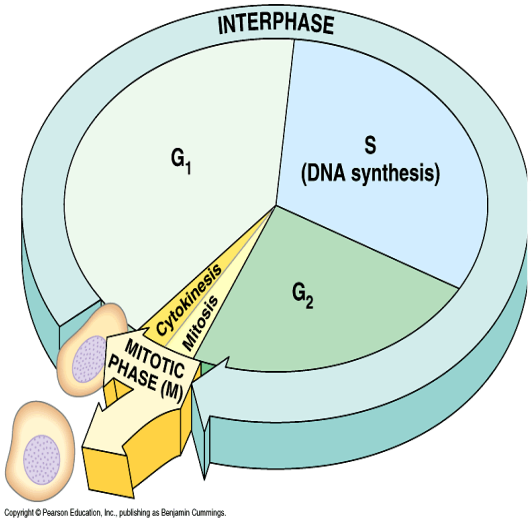
The beginning of a new topic, cell cycle.

Cell cycle

The Cell cycle, a way by which cells can undergo different phases, It's the series of events that take place in a cell leading to its division and duplication (replication) that produces two daughter cells. Note: The whole cell cycle takes about 24 hours in a typical eukaryotic cell.



-Phases of the cell cycle (4 phases): Interphase (G1, S, G2) and mitotic phase (M phase)

The very last phase, M phase (Mitotic phase), includes cell division and it takes only 1 hour, the other 23 hours (That include the first 3 phases of the cell cycle) are important for protein and DNA synthesis.

Multiple Notes:

1) Cell cycle of a yeast cell takes about 90 minutes, a bacterial cell takes about 20 minutes to divide.

2) A zygot (The earliest developmental stage of the embryo) has no G1 or G2, but it has a rapid S and M phases. Why? Because you'll have rapid cell division without cell growth and that what makes cells different from each other where every cell would have a different phenotype. (A cell responsible for development of the head, another one responsible for development of the leg, etc..)

3) Some cells (like nerve cells) enter a phase known as a G0 phase, which is also called a quiescent stage. Cells in this phase would be metabolically active but they're not growing. That been said, it has been noticed that some cells can re-enter the G1 phase from the G0 phase.

Phases of the cell cycle in a table:

State	Abbreviation	What happens?
Quiescent	G0	A resting phase where the cell has left the cycle and has stopped dividing.
	G1	increased metabolism and cell growth; cells are dipoloid (2n)
Interphase	S	DNA replication; cells are 2-4n
	G2	metabolism and cell growth; cells are 4n
Cell division	M	chromosomal segregation, nuclear and cell division(4n

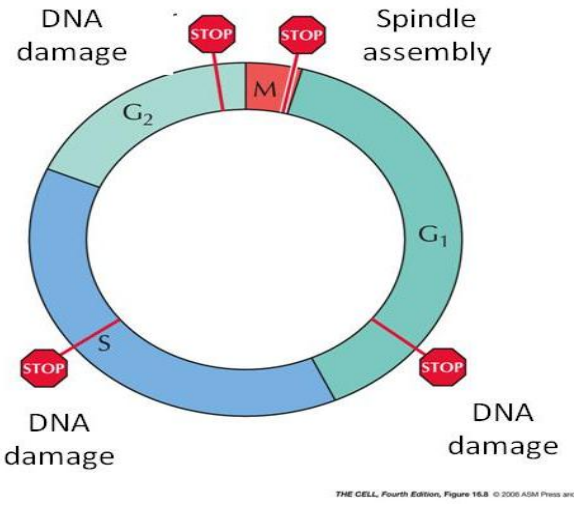
Regulation of a cell cycle:

The cell cycle is highly regulated and it's regulated at different stages and one of these stages is called the restriction point, it exists in the G1 phase right before the cells enter the S phase. In the restriction phase, the cells make sure that they have sufficient amount of nutrient because if they don't then they can't grow, they'd stop at this restriction point and enter the Go phase instead of growing.

Cell Cycle Checkpoints:

We have four checkpoints in the cell cycle where the cell gets checked to make sure that everything is going according to the plan.

3 of them are DNA damage checkpoints (to ensure that incomplete or damaged DNA is not replicated and passed on to daughter cells); the first one is in the G1 phase at the restriction phase, the second one is during S phase during replication, the third one is right before mitotic phase



and the last check point is a spindle assembly checkpoint (monitors the alignment of chromosomes on the mitotic spindle) and it's during the M phase where the cell makes sure that microtubules are fine for the separation of chromosomes.

Regulators of Cell cycle:

We have 3 classes of molecules that regulate the cell cycle:

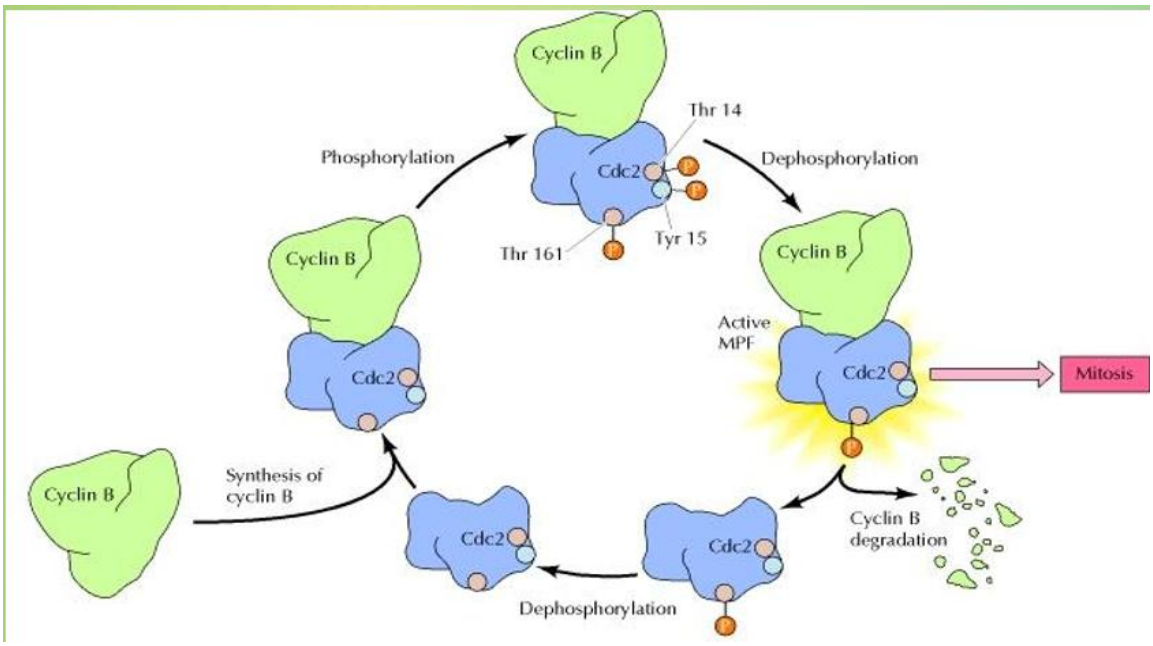
1)Cyclins: During interphone their expression increases(up regulation) , and in mitosis they're all degraded(down regulation). So their name is cyclins because they cycle between synthesis and degradation

2)Cyclin dependent kinases (CDKs): activated when they bind to cyclins

3) Cyclin dependent kinases inhibitors (CDKIs): they bind to the Cyclin-Cyclin dependent kinase complex and inhibits them.

Note: Cyclins are kinases are known as maturation promotion factors (PMF

Here's an example of regulation of cell cycle progression:



We have an increased synthesis of cyclin B here, it bind to cyclin dependent kinase, this kinase undergoes now

phosphorylation and afterwards dephosphorylation, After it's dephosorylated it becomes active and cell go then into mitosis; when the cell goes into mitosis the cyclin would get degraded and the kinase won't be active anymore until cyclin B is synthesized again.

So there are 4 levels of regulation for kinases:

1) They have to bind to cyclins.
2) They have to be phosphorylated
3) They have to be Dephosphorylated eventually
4) They bind to an inhibitor molecule and this inhibitor has to be released from the complex in order for the cyclins and CDKs to be active.

What is the link between the cell cycle and cell signaling?

Growth factors induce signaling pathways and the most important one is the Ras pathway (remember the pathway). During Ras activation pathway (Ras, Raf, and ERK pathway). We said that Ras induces a phosphrylation cascade that will eventually lead to activation of ERK and transolcate it into the nucleus where it will induce the expression of certain genes.

One of these expressed genes is responsible for synthesizing cyclin D, Cyclin D will bind to kinase and you'll have an activation of the cell cycle by going through the restriction point. What's the restriction point again? The point where the cell doesn't do DNA synthesis until there's enough nutrients So there are growth factors that have bounded to their receptor (RTK), so it's a signal that there are enough nutrients to push the cell to progress through the cell cycle.

Assume there's a mutation in cyclin D, what's going to happen?

That means that cyclin D would always be active and won't be degraded, that will cause cells to proliferate over and over again, so there's a loss of growth regulation and that's indeed a characteristic of cancer cells.

Retinoblastoma :

One of regulatory proteins of the cell cycle is called Reintoblastoma (RB-The governor of the cell cycle). It's given this name because it was first discovered in a type of retinal cancer (eye cancer) that was also called retinoblastoma.

Quick reminder:

Cyclin E is needed to initiate the S phase and Cyclin E transcription is dependent on E2F transcription factor.

When RB is hypophosphroylated it binds to E2F and prevents the transcription of cyclin E. When RB is phosphorylated, it releases E2f and cyclin E is transcribed and cell cycle progresses then.

Now, returning to the cell cycle; when the cell wants to pass through the restriction point, which is in the G1 phase, cyclin D has been up regulated, so it will bind to the cyclin dependent kinases , the whole complex is going to phosphorylate RB, when RB is phosphorylated, it releases E2f, so cyclin E is transcribed and cell cycle progresses then to the next step .

Cell cycle arrest by DNA damage.

The cell cycle can arrest and stop at a certain point if the DNA is damaged.

there are different proteins that are activated when there's DNA damage depending on what the damage is. An example of these proteins are:

1)ATR (activated by ssDNA damage)

2) ATM(activated by dsDNA damage).

What do these protein do? ATR and ATM activate the checkpoint kinases, these kinases inhibit a phosphatase, the Phosphatase cannot activate Cdk's . (If You Remember that for Cdks to be active ,they must be dephosphorylated , and this step needs phosphatases , which are inactivated by the checkpoint kinases that where activated by ATR &ATM) . So now the Cdks are inactive causing cell arrest and the cell would stop until the DNA damage is fixed.

Role of p53 in cell cycle arrest.

P53 (Guardian of the genome) is given this name because it weights 53 Kda

When it was first discovered, it was called the molecule of the year by a science magazine for what its role in fixing DNA damage.

When it's activated by being phosphorylated, it inhibits the cell cycle progression by increasing the expression of p21 which is a protein that inhibits CDK/cyclin complex stopping the cell at the restriction point before it goes into the S phase.

References for this sheet.

1) Robbins Basic Pathology (9th edition)/Chapter 5- Neoplasia
2) The Cell: A Molecular Approach (4th edition)
3) Wikipedia
4) Doctor's slides And of course, the record.

End of the sheet.

You may wonder why I used so many references! Well It depends, do you want to understand science or to get a high grade and that's it? Because I want both.

☺بالتوفيق للجميع وأتمنى صيام مبارك لإخوتي المسيحيين في الزمن الأربعيني.

Shout out to my friends: Qusai Masad, sura Dya'a, Muna Husban, Zakria Shkoukani, abu zghoul, and another big shout out to my frienemie, you know who you are. ;)

Written by, Rashid Dahabreh.

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