Genetics
& Cell biology

◉ Sheet ○ Slides

Number

13

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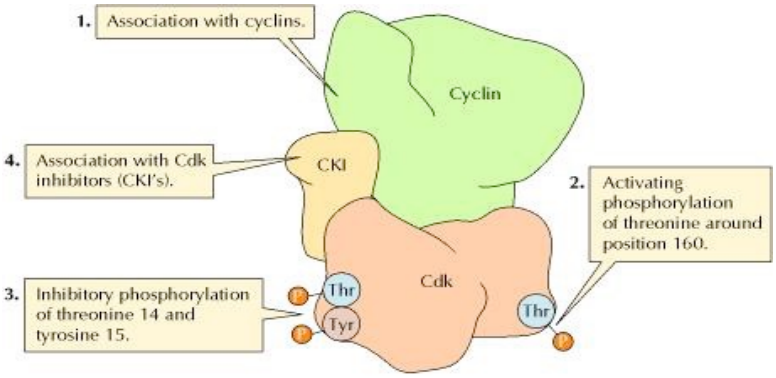
In the last lecture we took a brief introduction about the cell cycle , we mentioned the presence of multiple checkpoints throughout the cycle that are mainly concerned about the DNA damages – was the replication process completed , were there any mistakes in the nucleotides and were they fixed or not , was there equal separation of the chromosomes to the new daughter cells by the spindle fibers – .

Regulators of the cell cycle

- Cyclins are proteins that accumulate throughout the **interphase** and are rapidly degraded toward the end of mitosis.
- Cyclin dependent kinases (CDKs) bind to the cyclins to activate them.
- CDK inhibitors (CKIs) are molecules which inhibit the CDKs.

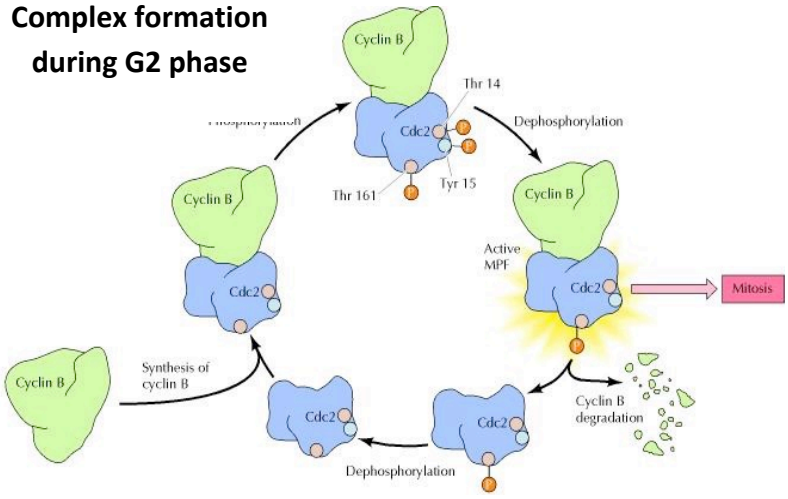
Each phase of the cell cycle has its own cyclin dependent kinase (CDK) which binds a specific cyclin, we also have CDCs which are the same as CDKs but the numbering differs for example CDK1 **isn't** the same as CDC1 .



Mechanism of CDK regulation :

The kinase is regulated by *phosphorylation* of 3 sites: if threonine 14 and tyrosine 15 were **phosphorylated** they will **inhibit** the kinase but if threonine 160 was <u>phosphorylated</u> it will <u>activate</u> the kinase.

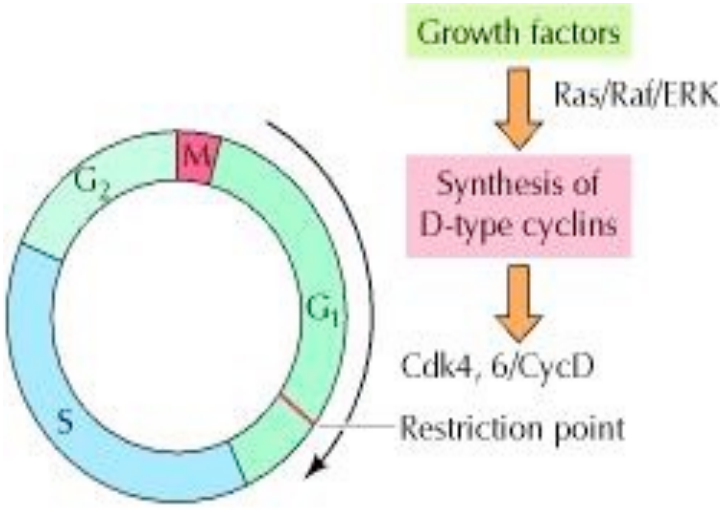
Complex formation during G2 phase



Cyclin B binds to CDK1 = CDC2 which has 3 <u>dephosphorylated</u> sites, so after the formation of this complex *phosphorylation* of the 3 sites happens but still the complex isn't ready to regulate the cell cycle; so **dephosphorylation** of the 14 and 15 sites happens and this **activates** the complex .After the complex finishes its role it has to be <u>inactivated</u> ,so <u>dephosphoryaltion</u> of the threonine 160 site happens and CDK1/CDC2 is recycled and the cyclin is separated from this complex and is targeted for proteasomal degradation .

- Phosphorylation regulates the activation,inactivation and the targeting of these complexes for degradation because the signal and the function of these cyclins has to stop at some point or else it'll lead to a continuous cell cycle which can cause cancer for example.

It is well known that there are signals that induce the cells to either grow or die (apoptosis). Growth factors are like nutrients for the cells and they represent these signals .



Growth factors regulate cell cycle progression through the G1 restriction point by inducing synthesis of cyclins D via the RAS/RAF/ERK signaling pathway.

Defects in cyclin D regulation lead to the loss of growth regulation that is characteristic of cancer cells .

Other factors that regulate the cell cycle :

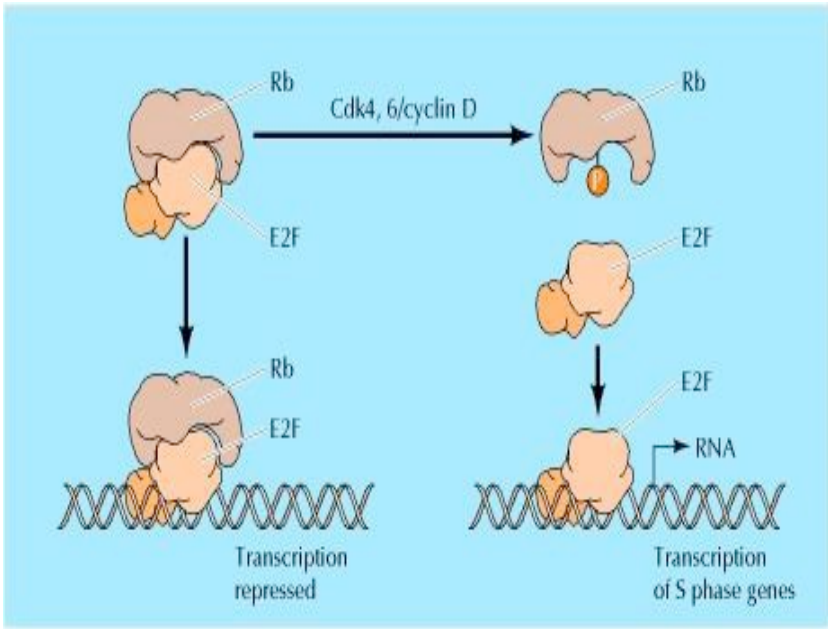
Retinoblastoma (Rb):

It is a Tumor suppresser gene (TSG)

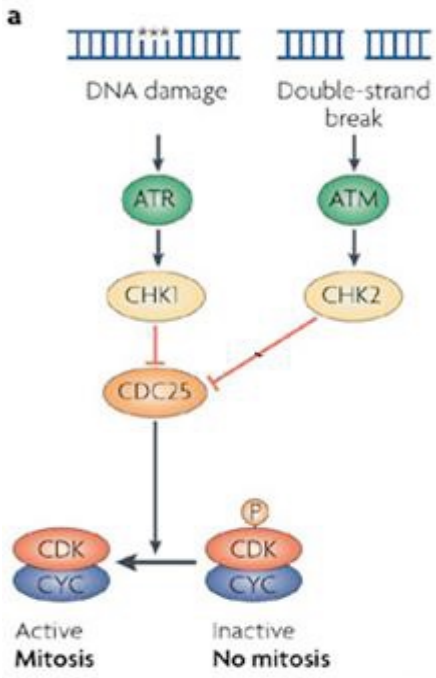
When **unphosphorylated** , Rb binds to E2F proteins and **represses** transcription of E2F-regulated genes .

If we want to turn on the cell cycle:

E2F is *freed* when Rb is *phosphorylated* by CDK4,6/cyclin D thus as a transcriptional factor it stimulates cell cycle progression through the restriction point by binding to target sequences which activates target genes thus activating the cell cycle.



How to stop the cell cycle when finding a problem after already starting the cycle ?



There are 2 types of proteins depending on the type of damage detected :

- **ATR** is activated by damage in a **single** strand of DNA.
- **ATM** is activated by damage in **both** strands of DNA.

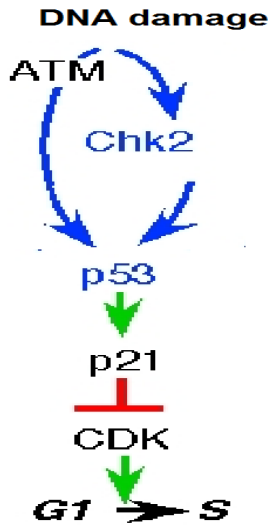
BOTH are protein kinases.

They *activate* other protein kinases (checkpoint kinases)
ATR → Chk1

ATM → Chk2

These Chks inhibit CDC25 which is responsible for the *activation* of *phosphatases* that remove the phosphate groups which are bound to the CDKs and thus *activating* CDKs , but since the CDC25 is inhibited, the phosphate groups remain bound to the CDKs and the cell cycle is arrested (no mitosis)

Role of p53 in cell cycle arrest :



The **ATM → Chk2** complex targets p53 protein for phosphorylation thus *stabilizing* it in the active form .

Activated p53 activates:

- The expression of p21 which is a CDK inhibitor and the cycle becomes arrested.
- BAX protein which activates apoptosis.

Apoptosis (Programmed cell death):

- It is a normal physiological form of cell death (isn't always pathological).
- Has a key role in the maintenance of adult tissues and in embryonic development.
- Renewal of 5×10^{11} blood cells a day, elimination of nerve cells with faulty connection, elimination of damaged and potentially dangerous cells and cells with DNA damage / Virus-infected cells**.**

How is apoptosis stimulated ?

1. **Intrinsic** pathway: simulated by *DNA damage***.**
2. **Extrinsic** pathway: stimulated by *signals from other cells***.**

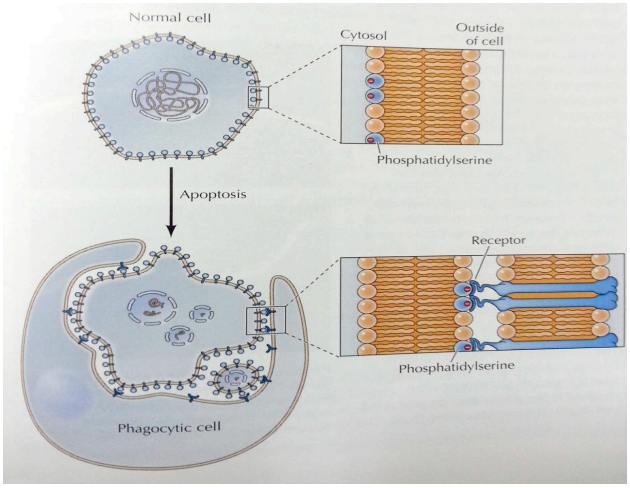
Features of apoptosis :

- Chromatin condensation.
- Fragmentation of chromosomal DNA**.**
- Breaking up the nucleus into small pieces.
- Cell shrinkage.
- Cell fragmentation (apoptotic bodies).
- Phagocytosis by macrophages and neighboring cells.

** In contrast, cell necrosis results in membrane damage, enlargement of cells, release of intracellular contents, and causes inflammation.

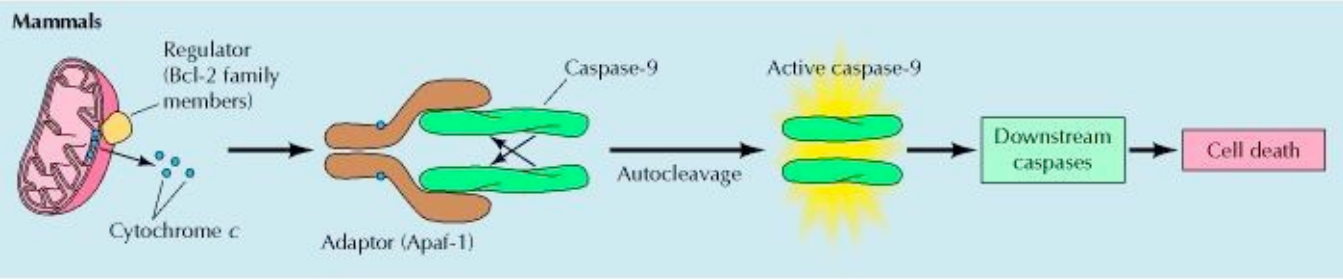
NOTE: The cell tries to save itself by autophagy and decreasing energy demand if these fail it resorts to Apoptosis but how can we detect autophagy under the microscope ? by the presence of a lot of lysosomes in the cytoplasm.

Role of phosphatidylserine (PS):



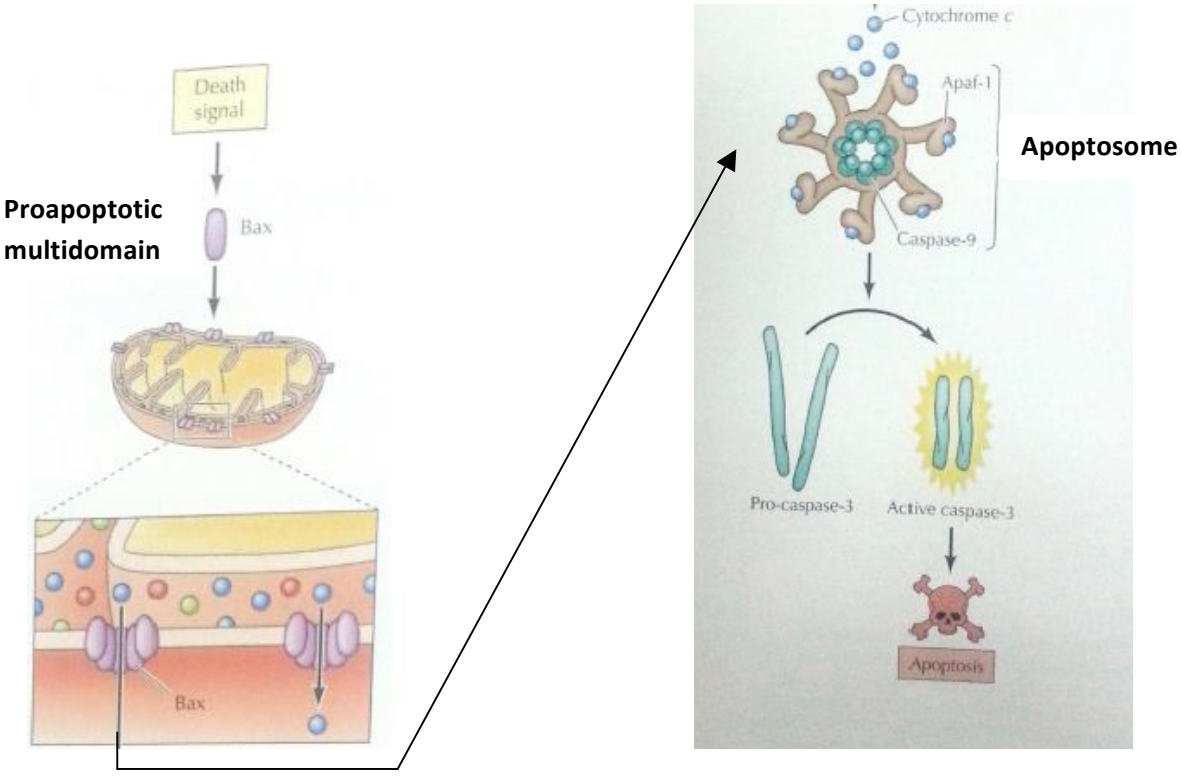
- ✓ Normally, PS is expressed on the **inner** leaflet of cell membrane.
- ✓ During the initiation of apoptosis, PS is **flipped** to the **outer leaflet**.
- ✓ It is then recognized by receptors on the membranes of phagocytic cells which leads to engulfement and phagocytosis.

The molecular activation of apoptosis:



Caspases are proteases **- Caspase 9** which is very important in the process of apoptosis needs an **adaptor protein Apaf-1** to activate it **-** regulators of the Bcl-2 family act on the mitochondria to *control the release of cytochrome C* ,which is required for the active binding of caspase-9 to the adaptor Apaf-1.Once this complex forms Autocleavage occurs thus *activating* caspase 9 and a downstream of caspases which induces apoptosis.

How is cytochrome C released from mitochondria?

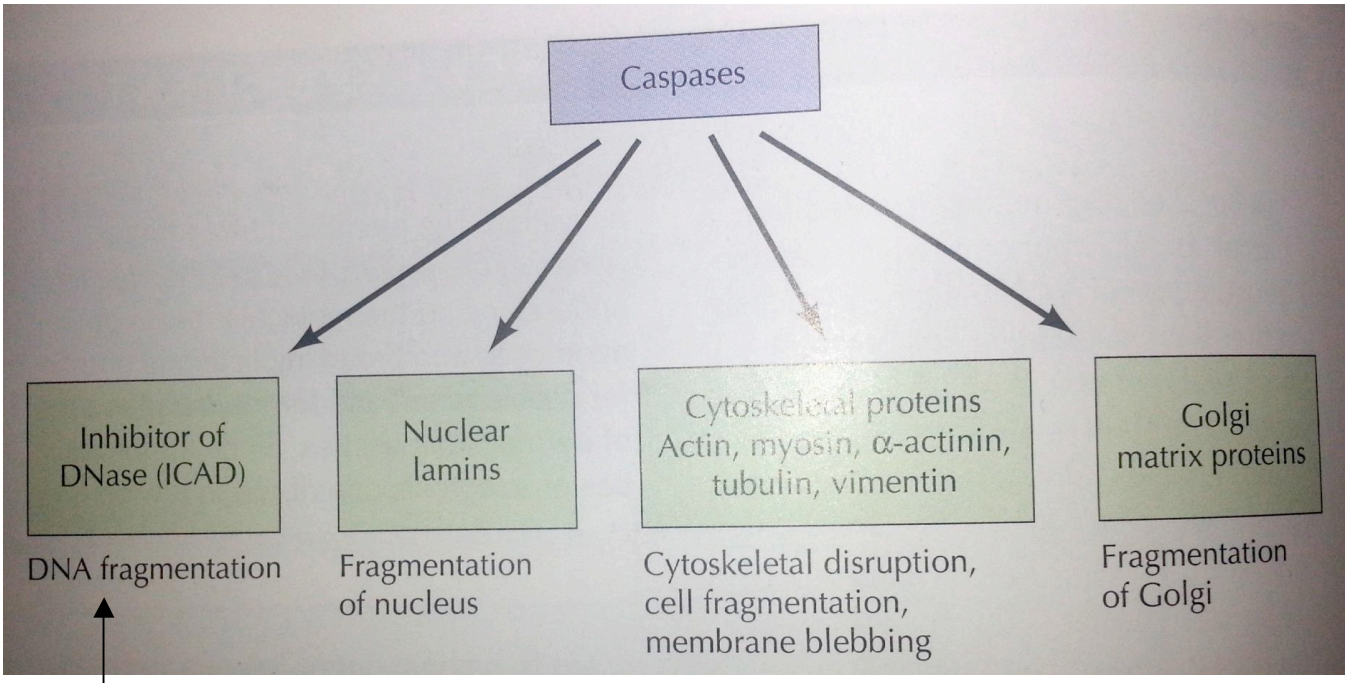


Proapoptotic multidomain

Oligomeric pore formation

Bax and Bak proteins form an **oligomer** in the membrane, thus forming a channel so the *cytochrome C* can move from the mitochondria to the cytosol .

The roles of the Caspases : (what do they target?)



NOTE:

- ✓ *DNase* breaks down the DNA so we <u>inhibit its inhibitors</u>, which results in its **activation**.
- ✓ Nuclear lamina are intermediate filaments which support the nuclear envelope.

Bcl-2 family:

A group of proteins that contribute to apoptosis.

There are three classes of Bcl-2 according to their domains and apoptotic effect:

1. Anti-apoptotic protein →

<u>Inhibition</u> of apoptosis.

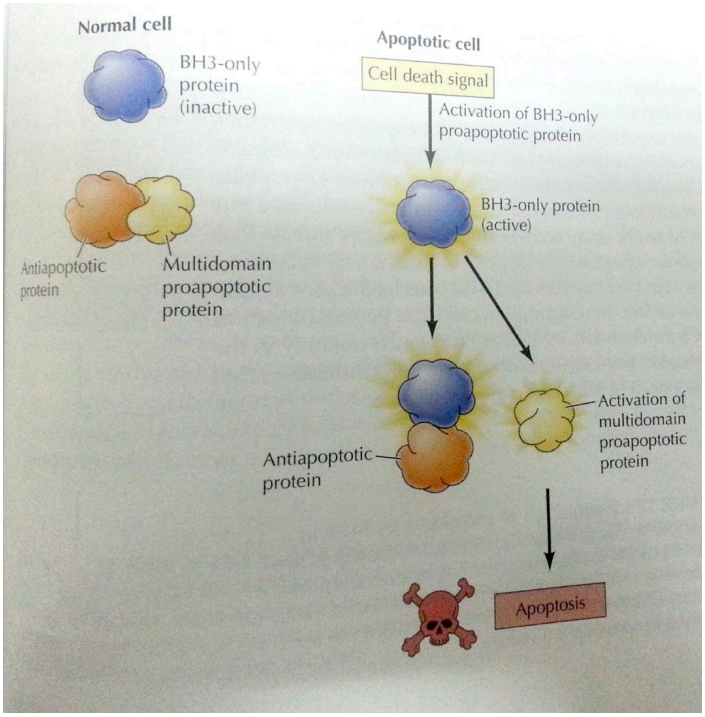
2. Proapoptotic proteins: <u>activate</u> apoptosis.

A) <u>Multi-domain</u> →

B) <u>BH3-only domain</u> →

NOTE: TM (brown in the figure) is the transmembrane domain which is shared between them all ,it <u>doesn't</u> contribute to the function but only to the structure.

How is apoptosis activated upstream?



Normally (to the left of the figure)

BH3-only protein is inactive.

The antiapoptotic protein **binds** the multidomain proapoptotic protein thus inactivating it.

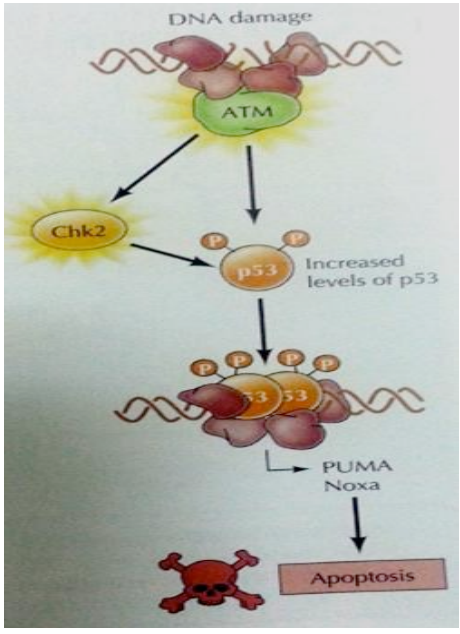
(to the right of the figure)

Death signals activate the BH3-only protein, which inactivates the antiapoptotic proteins resulting in the release and activation of the multi-domain proapoptotic protein.

Keep in mind that direct interaction between the proteins isn't necessary for their activation and inhibition of each other .

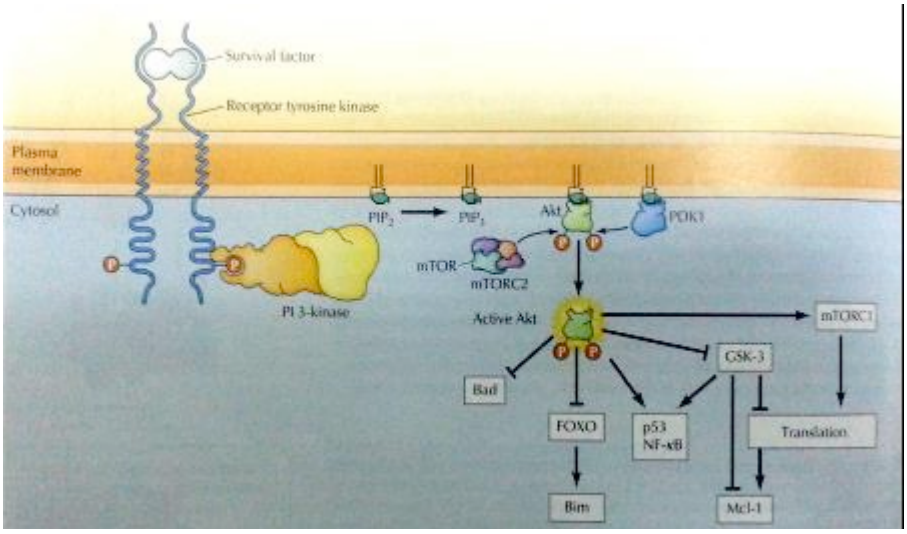
Internal pathway

Actually this pathway was discussed throughout this lecture so **to sum up** : if we want to activate such a pathway, damage must be detected for example: DNA damage detected by ATM which will activate Chk2 thus specifically *activating* p53 which will *induce* the expression of BH3- only proteins and BAX proteins, it also activates p21 which arrests the cell cycle.



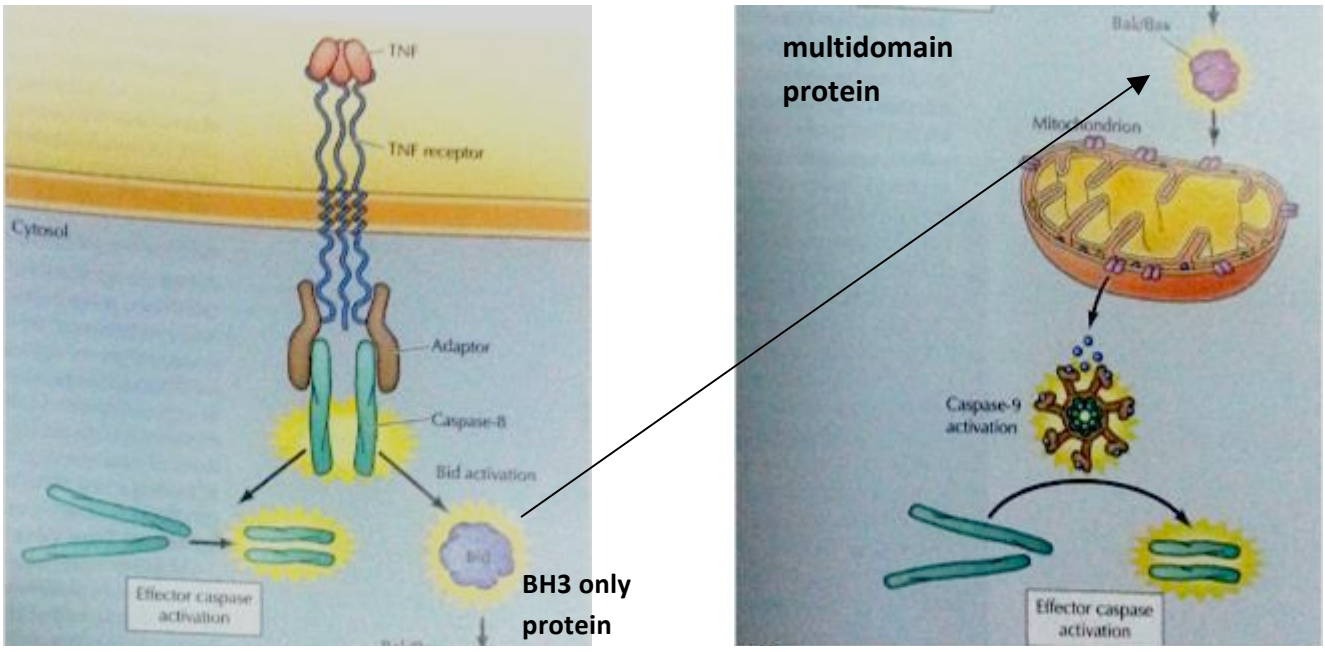
The external signaling pathway is composed of 2 types:

(1) Pro-survival



Activation of PI-3 Kinase/AKT (tyrosine kinase receptor) signaling pathway **inhibits** proapoptotic proteins (which leads to cell survival) so if this signaling is *absent* this will result in **activation** of proapoptotic proteins .

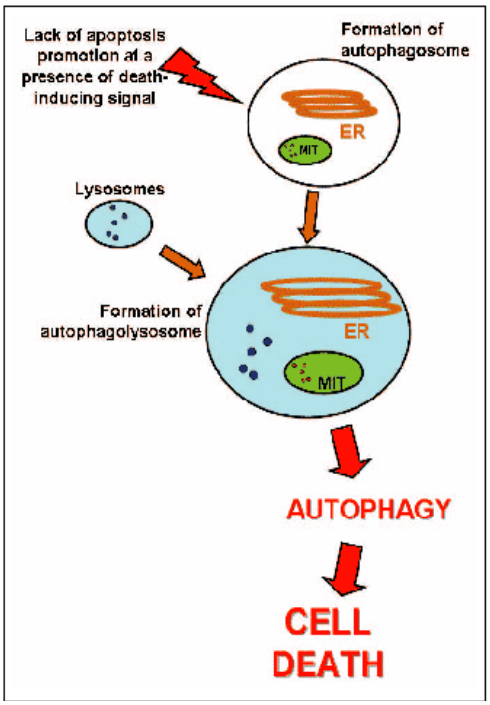
(2) Pro-death

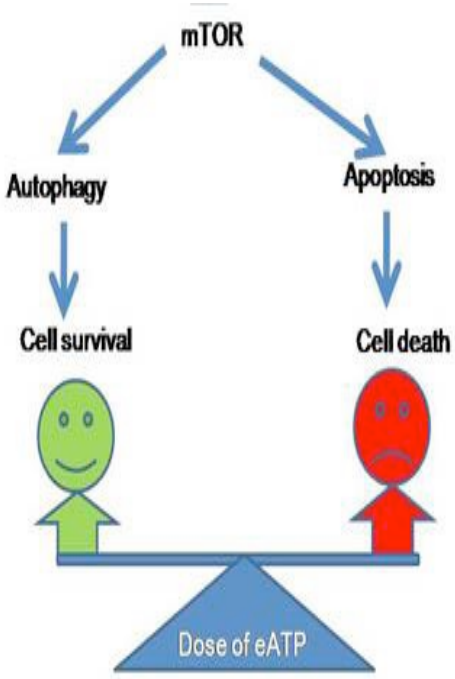


An example on this type is the binding of **TNF** to its receptor (non-tyrosine kinase) thus activating the adaptor Apaf-1 which binds to the cytochrome C and activates caspase 9 leading to a cascade of caspases activation as we already discussed.

Autophagy:

In previous lectures we discussed autophagy and its relation to vesicular transport and a lot of other things … concerning apoptosis :



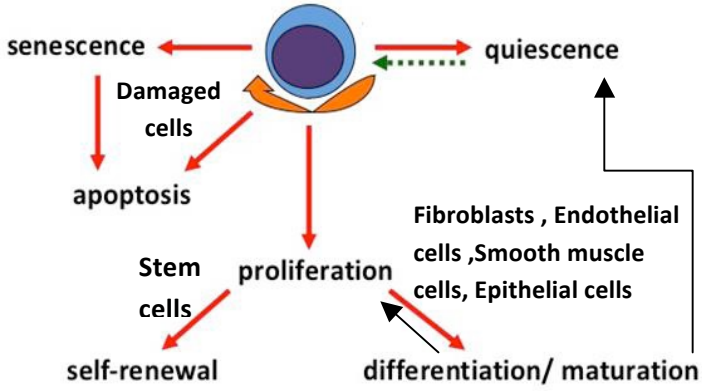


Apoptosis can be *caspase-independent*, but mediated by autophagy through **mTOR** signaling ; the mTOR when *inhibited* , results in the *activation* of autophagy. The dying cell does not go through the same morphological features, but accumulates lysosomes.

Advantages:

When cells lack molecular machinery of apoptosis it provides cells with an opportunity to repair the damage prior to death.

Cell fate



This topic will be further discussed in the CNS course.

Fetal stem cells have the ability to proliferate for self-renewal and maintaining their reservoir or they can differentiate to other types of cells but keep in mind that they have different capacities for differentiation and that their environment (niche) affects the type of resulting cells.

Adult stem cells may go through senescence due to a deficiency in their functions which will lead to apoptosis. Normal cells may also die by apoptosis due to damages .

" It is our choices, Harry, that show what we truly are, far more than our abilities. "

-J.K. Rowling