CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

26

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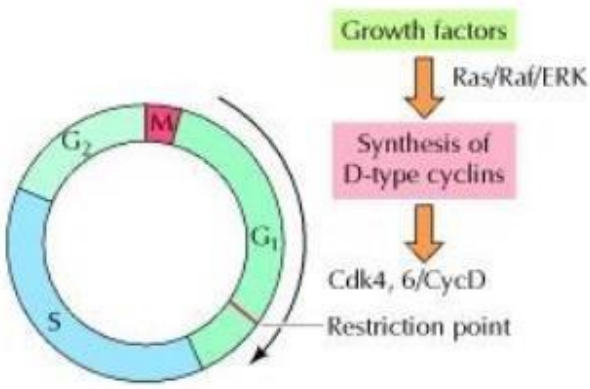
Cell Signaling and the Cell Cycle

The cell cycle is highly regulated to ensure timely (not dividing too quickly) and accurate (no mutated DNA) replication.

Growth Factors



- Function

  Growth factors are what regulate cell cycle progression through the G_1 restriction point.

  ⬇ How?

  Growth factors activate the synthesis of D type cyclins (Cyc D) through the Ras/Raf/ERK signaling pathway.

- What happens if there aren't any growth factors?

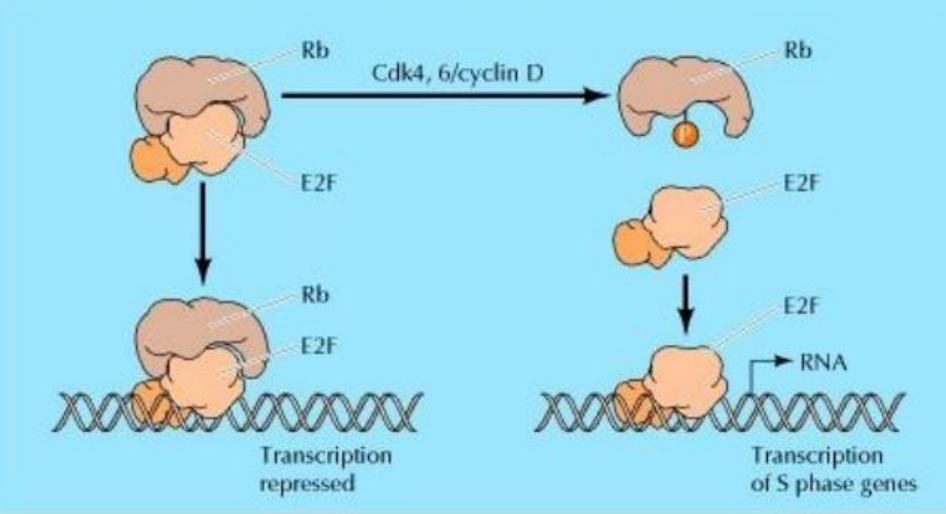
Cyclin D cannot be synthesized without growth factors. Cells will then be unable to pass the restriction checkpoint of G_1 and instead enter G_0 and will be unable to divide. However, they can re-enter the cell cycle should growth factor stimulation occur.

- What if there is a defect in CycD regulation?

If there is continuous, unregulated expression of CycD, the cell will be able to pass the G_1 restriction checkpoint and continues to divide more than it should. This leads to a loss of growth regulation, a characteristic of cancer cells.

Retinoblastoma

- Proteins Involved:
  1. **Rb** – Rb is protein that represses transcription by forming a complex with E2F. Its activity is regulated by changes in phosphorylation.
  2. **E2F** – E2F is a transcription factor that stimulates expression of S phase genes. E2F is always bound to its target sequences, but whether or not the genes are transcribed depends on Rb regulation.



- Case 1: Rb is Unphosphorylated:

When unphosphorylated, Rb can easily bind to E2F and forms an Rb/E2F complex. This complex will then inhibit transcription of E2F regulated genes and the cell will not be able to enter the S phase.

- Case 2: Rb is Phosphorylated:

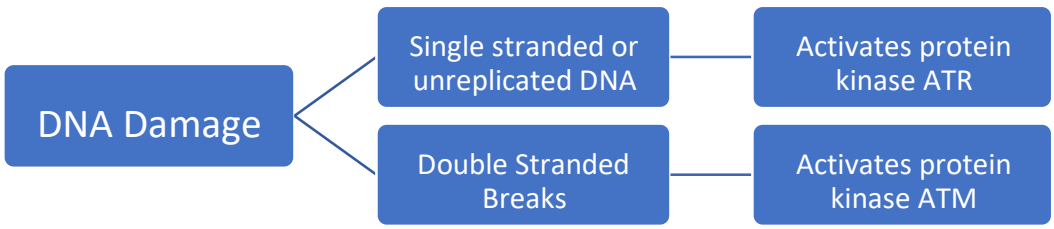
Remember that Cdk4, CycD helps the cell pass the G_1 restriction checkpoint. The cell now needs to produce S phase proteins.

A Cdk4, CycD complex will attach a phosphate group to Rb as the cell passes through the restriction checkpoint in G_1. As you can see in the figure, the phosphate will bind in a way that will force Rb and E2F apart. The free E2F can now activate transcription of S phase genes (you can expect to see proteins related to RNA transcription produced, like polymerases and helicases).

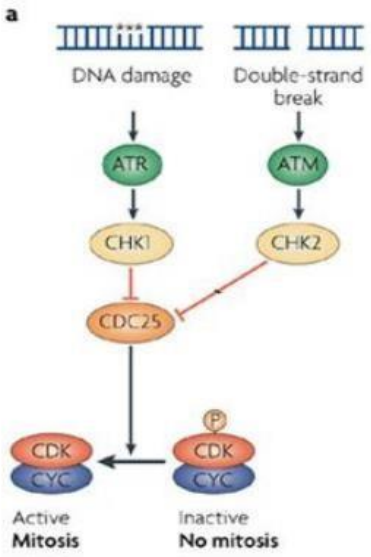
Cell Cycle Arrest due to DNA Damage

If the cell recognizes damaged DNA at checkpoints, it will stop, or "arrest", the cell cycle to give the cell a chance to fix any mistakes in replication.

- Types of DNA Damage



- The Signaling Pathway

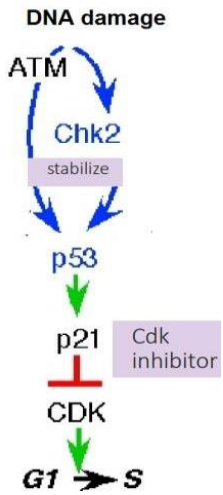


1. ATR and ATM are activated by their specific types of DNA damage.
2. ATR and ATM can then phosphorylate and activate the checkpoint kinases.
   - ATR activates CHK1
   - ATM activates CHK2
3. Chk1 and Chk2 both can then inhibit Cdc25 phosphatase.

- How does this induce cell cycle arrest?

The Cdc25 phosphatase was required to remove inhibitory phosphorylations on Cdks to activate them. Now that Cdc25 phosphatase is inhibited, the cell cycle halts due to the Cdk's remaining inactivated.

Role of p53 in Cell Cycle Arrest



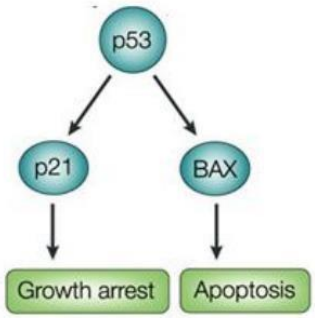
- What is p53?

P53 is a protein that normally stops the cell cycle. It functions as a transcription factor.

- How is p53 stabilized?

ATM and Chk2 can separately target p53 and phosphorylate it. This leads to a rapid increase in p53 levels in response to DNA damage.

***Note:** We say stabilized instead of activated because without phosphorylation, p53 is quickly degraded. So, when we "stabilize" p53, we're preventing it from being destroyed.



- What happens once p53 is stabilized?

Once stabilized, p53 can activate expression of p21, a protein that inhibits a Cdk/Cyclin complex. The cell cycle is now arrested.

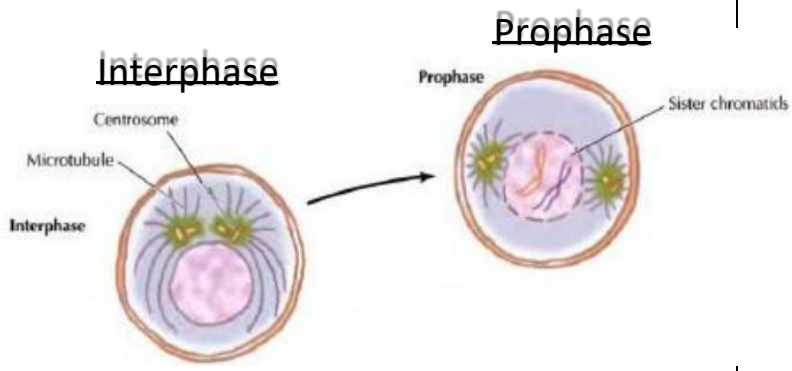
- Can p53 stabilization lead to anything other than cell cycle arrest? Yes! If the DNA damage is too much to the extent the cell can not fix it, p53 can also target BAX, which leads to apoptosis.

Phases of Cell Division

Before we discuss the phases of cell division, we have to differentiate between mitosis and cytokinesis.

- Mitosis is the separation of nuclei (nuclear division).
- Cytokinesis is the separation of two cells (cell division).

Prophase



- Events of Prophase
  - The chromosomes condense and are held in their center by centromeres.
  - The centrosomes (which have duplicated in interphase) will move to opposite sides of the cell to serve as poles of the mitotic spindle.
  - The spindle pole bodies are embedded in the nuclear envelope.
- More on Condensation
  - Before condensation, the chromosomes are not visible. Therefore, we know prophase has begun once we can see the condensed chromosomes
  - Condensation is important for separation of the sister chromatids. When uncondensed, DNA is very long and twisted. By condensing DNA, we prevent the sister chromatids from being broken or twisted around each other while we separate them.
- More on Centromeres
  - A centromere is a non-coding DNA sequence which allows proteins to bind to it to form the kinetochore

Note:

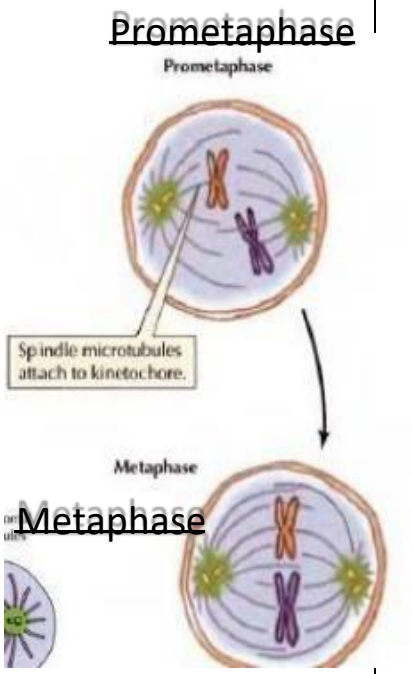
-The kinetochore is the site of spindle attachment

-The metaphase plate is the lining up of chromosomes single file in the center of the cell

Prometaphase and Metaphase

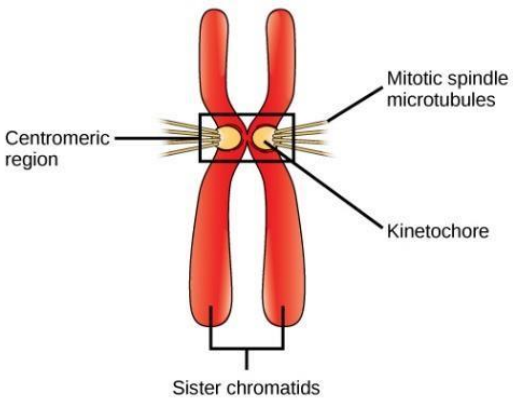
- What are these stages?
  - ***Prometaphase*** is the intermediate step between prophase and metaphase.
  - ***Metaphase*** is the phase in which the chromosomes are aligned on the metaphase plate.

- Events of Prometaphase – Attachment of the Mitotic Spindle to the Kinetochores



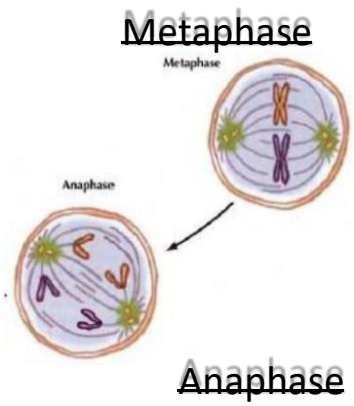
  - We see microtubules begin to grow from the centrosomes to the chromosomes.
    - The (+) end of microtubules faces the center of the cell
    - The (-) end is near the centrosomes (this also helps to prevent depolarization)
  - The microtubules (of the two spindles) attach to the kinetochores of the condensed chromosomes.
  - The kinetochores of the sister chromatids orient in opposite directions to attach to microtubules of the opposite poles (see figure). This will ensure that the chromosomes will be pulled in opposite directions.
  - The chromosomes (with the assistance of motor proteins) will shuffle back and forth until they align on the metaphase plate– ***this is what starts metaphase!***

- How did the spindle fibers form? How do they move chromosomes?



  - Spindle fibers form through the continuous addition of alpha and beta tubulin dimers (subunits of microtubules). Also, the spindle fibers run in opposite directions.
  - Chromosomes move to opposite ends of the cell from the (+) end to the (-) end with the help of dynein

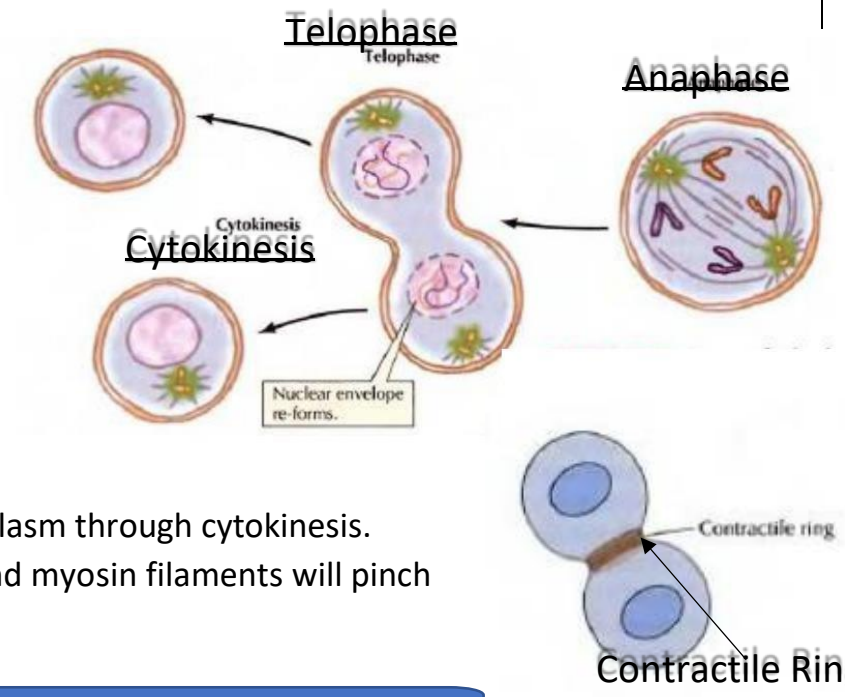
Anaphase



- Once the chromosomes are aligned we can separate them! This is why chromosomes will spend very little time in metaphase.
- Anaphase is triggered by the breakage of the link between sister chromatids. Then they can move to opposite sides of the cell.

Telophase

- The nuclei (and nuclear envelope) reforms.
- The chromosomes decondense.



Cytokinesis

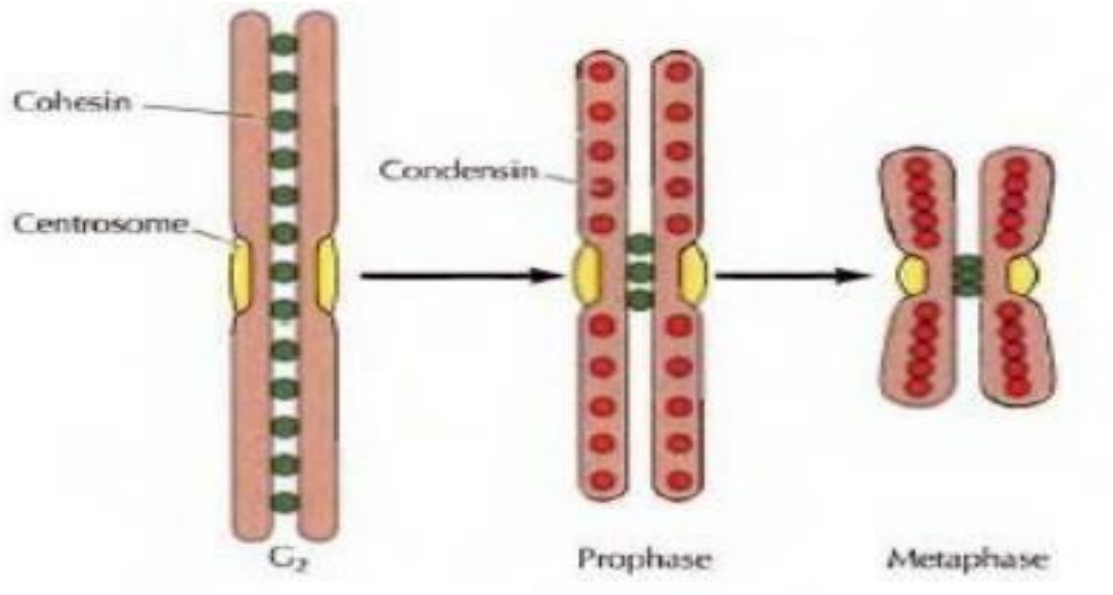
- After the division of nuclei (i.e. chromosomes) we need to divide the cytoplasm through cytokinesis.
- A contractile ring of actin and myosin filaments will pinch the cell into two.

Cell Division is now completed! We have two identical daughter cells as our product.

Additional Details on the Cell Cycle

How Sister Chromatids are Held Together

- Cohesin *(Green Proteins in Figure)*
  - Cohesin (a protein) begins to bind to DNA in the S phase, keeping the two sister chromatids linked together. This is a form of quality control as it ensures replicated chromosomes will stay together with its copy.
  - Cohesin has multiple points of attachment to the chromatids.
  - Cohesin will remain along the entire length of the two chromatids in the S and G_2 phases. Once we enter prophase, most of the cohesins will leave. This is to help the chromatids condense and lessen the attachment between them so we can separate them.
  - However, some cohesin will remain at the centromere because we still want some attachment to keep the chromosomes together until we need to separate them.

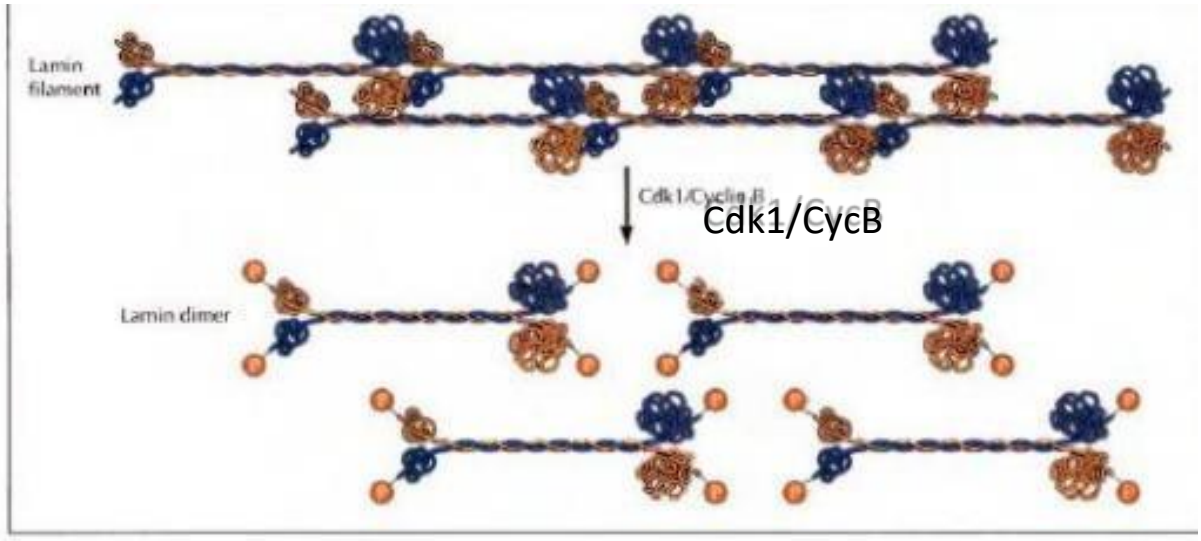


- Condensin *(Red proteins in figure)*
  - As the cell enters the M phase, the cohesins will be replaced by condensins.
  - Condensin will bind *on* the chromosome itself
  - It's activated by Cdk1 (from G2*****), which acts as a kinase to phosphorylate condensins.
  - You can see in the figure that the chromosome is more condensed in metaphase than in prophase. This is because in metaphase we are lining the chromosomes along the metaphase plate, so they will need to be even smaller to be able to fit on a straight line across the center of the cell.

Breakdown of the Nuclear Envelope

- Cdk1/Cyclin B (the last kinase from G2*****) phosphorylates lamin and causes depolymerization of lamin filaments which therefore causes disassembly of the nuclear lamina and weakening of the nuclear structure. Cdk1 also phosphorylates other targets that causes dissociation of nuclear pore complexes. All of this will lead to the breakdown of the nuclear envelope.

***Note**: You may notice that we consistently use Cdk1 (a cyclin dependent kinase) from G2. This is simply because G2 is the last phase before mitosis, so it will have effects that assist in the beginning of mitosis (the stage after G2)



Apoptosis

- What is apoptosis?

  It is a normal physiological process or form of cell death/suicide.

- Examples of Apoptosis
  - Fetal Development – In the fetus, fetal hands start as a web and the fingers are then formed through programmed cell death of the hand's web.

  - Maintenance of Adult Tissues - In adults, it is needed for *the renewal of some cells* - such as red blood cells and intestinal cells - *and to get rid of the damaged and abnormal cells* which can affect other neighboring cells.

Examples of such cells:

1. Nerve cells with faulty connection - some neurons that wrongly synapse or connect with other ones.
2. Damaged and potentially dangerous cells – cells with either DNA damage or those infected by a virus.

- How is apoptosis stimulated?

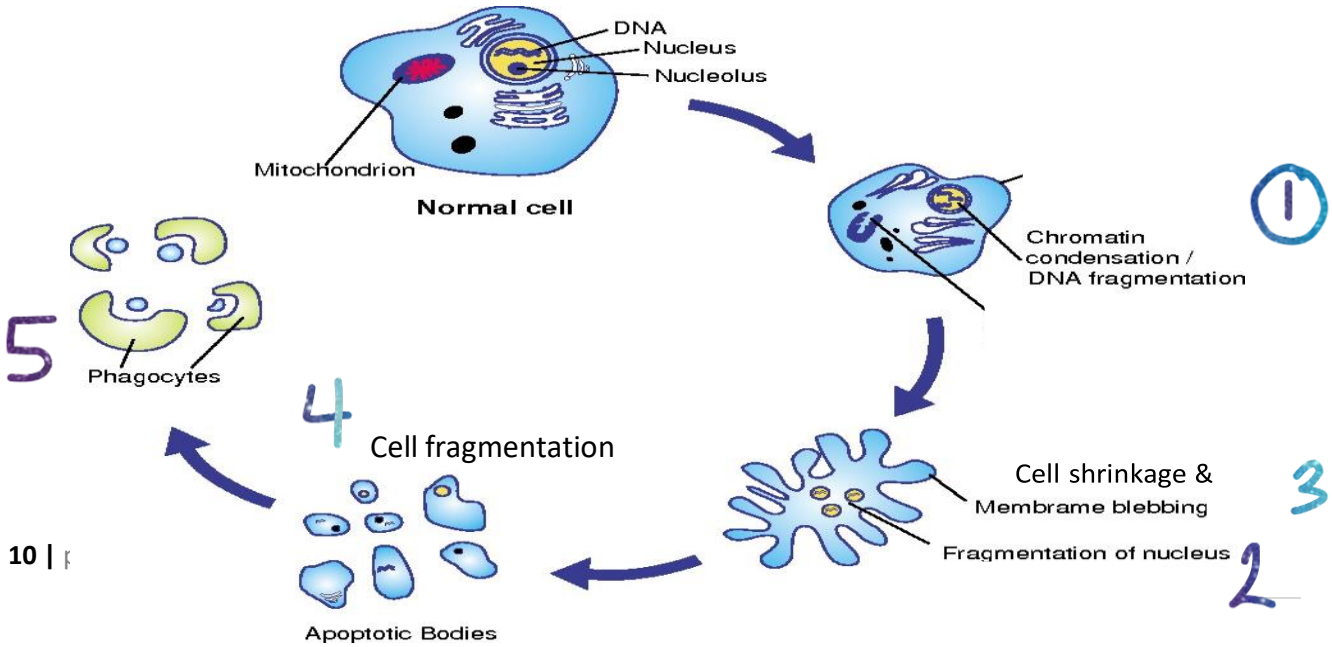
There are two pathways that stimulate and cause apoptosis and each pathway is stimulated by a different factor;

1. Intrinsic pathway (internal pathway); which is stimulated inside the cell due to DNA damage.
2. Extrinsic pathway (external pathway); which is stimulated out of the cell by signal from other cells.

- Features of Apoptosis

A cell that undergoes apoptosis has some special features, such as:

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



More on the Features of Apoptosis

One of the very first changes that apoptotic cells undergo is DNA fragmentation and chromatin condensation which can be seen under a microscope as a very dense darkly colored nucleus of a cell compared to other cells' nuclei. The DNA stains dark because it is highly condensed which allows it to absorb more stain.

After the DNA becomes fragmented and the chromatin condensed, the nucleus starts to break up into small pieces (nuclear fragmentation).

Nuclear fragmentation causes shrinkage of the cell, which is then followed by cell fragmentation during which the cell itself divides into smaller bodies (apoptotic bodies) maintaining the cell membrane intact unlike necrosis. Finally, these small apoptotic bodies are phagocytized by macrophages and other neighboring cells.

What is necrosis?

Necrosis is the death of most or all the cells in an organ or tissue due to **disease**, injury, or failure of the blood supply.
In apoptosis, the cell is phagocytized peacefully and cleanly with the cell membrane intact not leaving any waste products behind, the resulting apoptotic bodies are recycled and then their components get used by other cells. While in necrosis, the diseased cell explodes resulting in;

~ Membrane damage.
~ Enlargement of cells.
~ Release of intracellular contents.
~ Inflammation.

How are the apoptotic bodies phagocytized?

- Normally, the cell membrane contains phosphatidylserine (a glycerophospholipid) with greater amounts on the inner leaflet.
- Once apoptosis is initiated, the phosphatidylserine flips to become highly concentrated on the outer leaflet instead of the inner one.
    - *This flipping process is a very important sign to indicate that this cell is undergoing apoptosis.*

- The flipping of phosphatidylserine (PS) allows the receptors on the phagocytic cells' plasma membrane (eg; macrophages) to bind with the apoptotic cell leading to phagocytosis.
- In fact, the flipping of PS happens after a series of changes that occur inside the cell, such as the DNA fragmentation, chromatin condensation and nuclear fragmentation…etc.

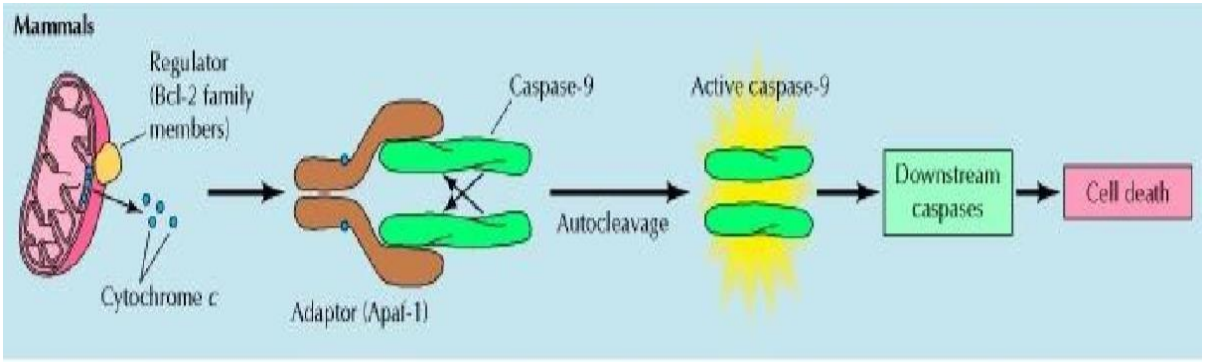
Bcl2 Family Proteins

- One of the most important proteins that activate or mediate apoptosis is actually a group of proteins called the Bcl2 family. These proteins (with their different types) specifically act at the mitochondria.
- <u>Mechanism</u>
  - Regulators of the Bcl2 family proteins will act at the mitochondria leading to the release of a protein called cytochrome c (which is one of the electron transport chain proteins found in the inner mitochondrial membrane).
  - As soon as cytochrome c protein is released from the mitochondria, it binds to an adaptor protein (Apaf-1) and activates it.
  - The activation of the adaptor protein leads to the attachment of a protein called caspase 9 to the adaptor protein.
  - The attachment of the caspase 9 protein to the adaptor protein leads to the activation of caspase 9. This leads to:
    - The digestion of certain molecules.
    - The activation of other caspases (activation of downstream caspases).
    - The activation of other caspases by caspase 9 leads to the degradation of molecules, which finally results in apoptosis "the death of the cell".

- <u>What is a caspase?</u>

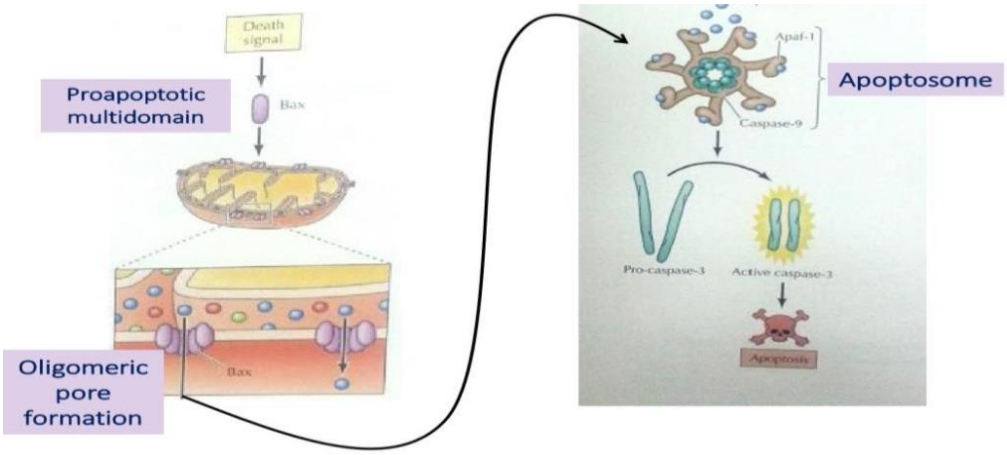
  It is a protein that digests and degrades certain molecules. It can be found either in the cell or in the extra cellular matrix (ECM). Finally, it has many types and each type acts on (degrades) specific molecules.

Bcl2 → cytochrome c → adaptor protein → caspase 9 → other caspases



- How is cytochrome c released from the mitochondria?

1. Regulators of Bcl2 family proteins act at the mitochondria leading to the release of the cytochrome c.
2. Bax protein, which is part of the Bcl2 family of proteins, enters the mitochondria and forms an oligomeric pore complex, leading to the release of cytochrome c through these pores.
   - Soon after its release, cytochrome c binds to an adaptor protein which, as a result, becomes activated and binds to caspase 9 whose activation activates other caspases, such as caspase 3, leading to apoptosis. The complex made of the adaptor protein, cytochrome c and caspase 9 is called an apoptosome.

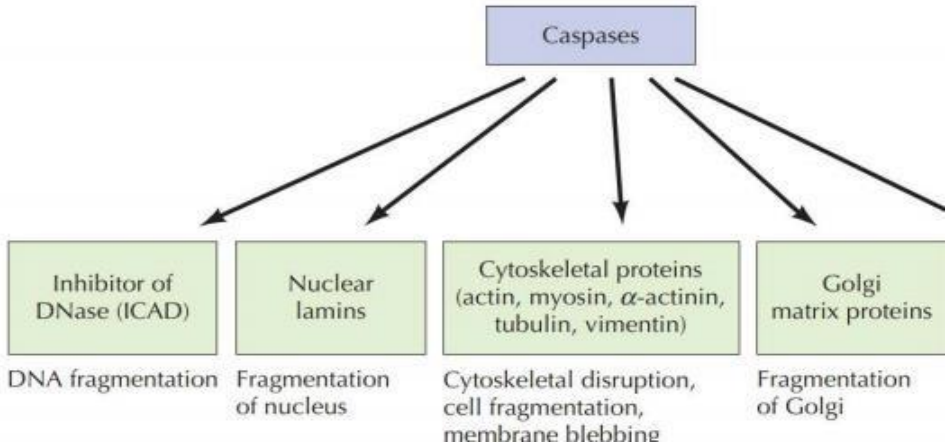


- If caspases degrade molecules, why don't they degrade the cell's components?

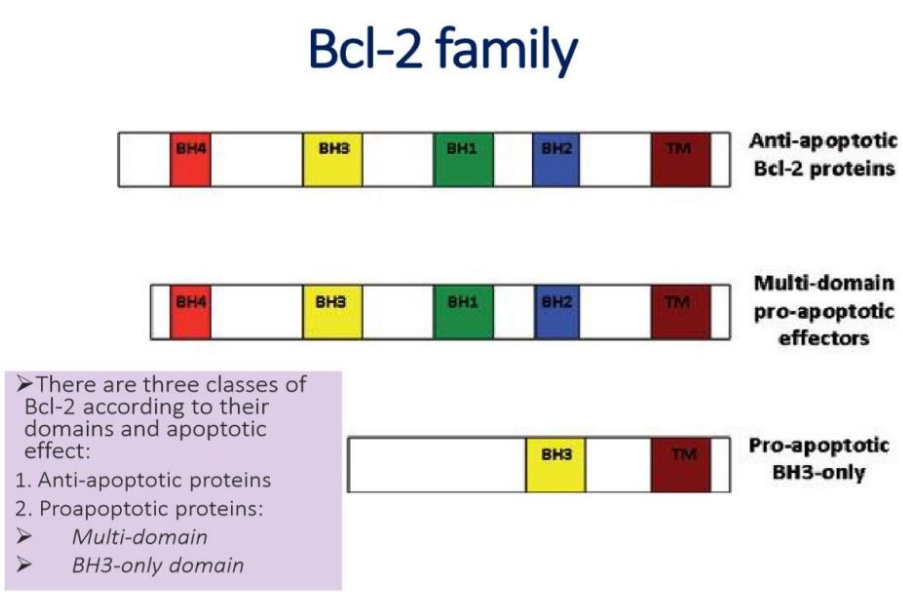
Because all digestive enzymes are always produced in an inactive form, just like the hydrolytic enzymes in the lysosomes, or those found in the stomach.

And so, caspases are produced as procaspases, with the prefix pro- indicating its inactivity, eg: procaspase 3.

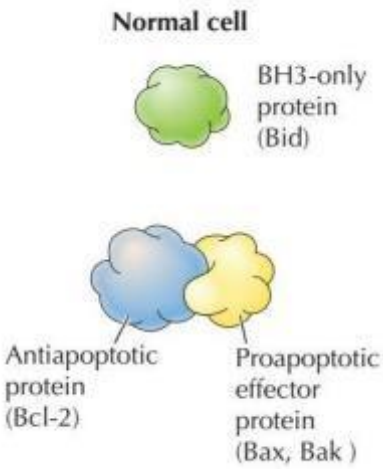
- What does the activation of caspases lead to?
  - DNA fragmentation; Caspases target an inhibitor of DNase, ICAD, and cleave it so that the DNase can function.
  - Nuclear fragmentation; because they degrade the nuclear lamins whose destruction leads to the destruction of the nucleus resulting in its fragmentation.
  - Golgi apparatus fragmentation; because they degrade the Golgi matrix proteins which as a result leads to the fragmentation of Golgi that turns into small vesicles.
  - Disruption of the cytoskeletal structure, membrane blebbing (irregular bulging) and cell fragmentation; because they degrade the proteins of the cytoskeleton.



- What are the Bcl2 family proteins?
  - They are a group of proteins that share certain domains due to sharing some functional aspects.
  - For example, in the figure below, they all have the same yellow domain (i.e. same transmembrane protein).
- Classes of the Bcl2 family according to their domains and apoptotic effect:
  1. Anti-apoptotic proteins; which inactivate apoptosis.
  2. The multi-domain proapoptotic proteins; which activate and prepare apoptosis.
  3. BH3-only domain proapoptotic proteins; which are part of the multidomain proapoptotic proteins and also activates apoptosis.



- How do the Bcl2 family proteins with their different types or classes contribute to the activation and inactivation of apoptosis?



- *Normal Cell (No Death Signal)*

~The BH3-only protein is found inactive in a cell that isn't apoptotic. Which makes sense because BH3 is proapoptotic and it must be inactivated to prevent apoptosis from taking place.

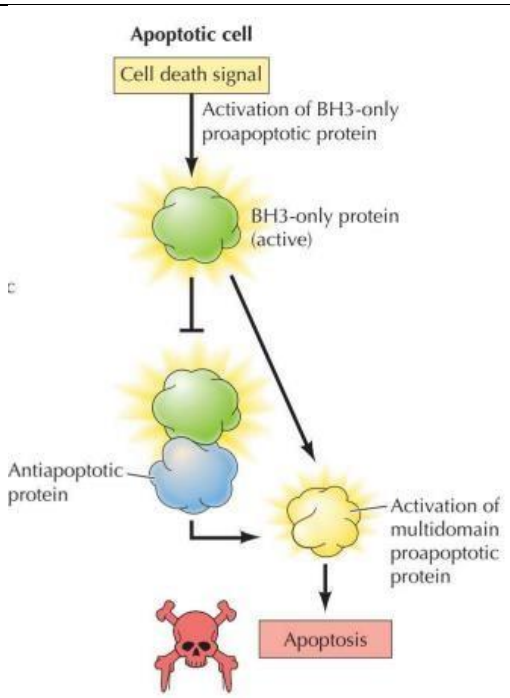
~The anti-apoptotic and multi-domain proapoptotic (can be referred to as "proapoptotic" only) proteins form a complex in which the proapoptotic protein is inactivated by the active anti-apoptotic protein to prevent apoptosis.

Normal cell → ***proapoptotic*** proteins are ***inactive*** & **anti-apoptotic** proteins are **active**

- *Apoptotic Cell*

When a death signal reaches the cell (eg; Tumor Necrosis Factor (TNF) signal), it will activate apoptosis.

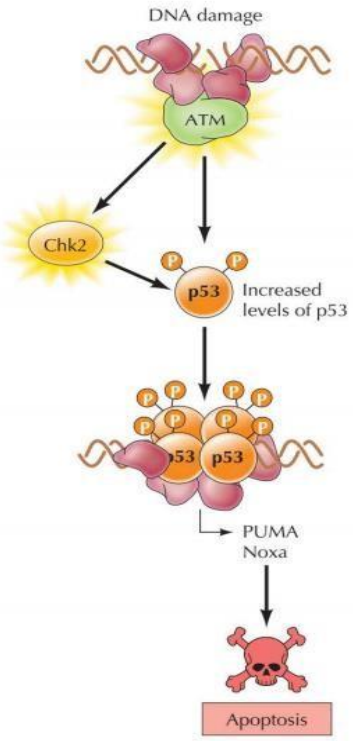
The TNF signal, or death signals in general, activates the BH3-only domain proapoptotic protein. Next, the BH3-only domain can now bind to and inactivate the antiapoptotic protein, resulting in the release and activation of the multi-domain proapoptotic protein. The cell will now enter apoptosis



Apoptotic cell → ***proapoptotic*** proteins are ***active*** & **anti-apoptotic** proteins are **inactive**

As mentioned previously, there are two pathways that stimulate and cause apoptosis and each pathway is stimulated by a different factor.

1. **The internal pathway** - the presence of permanently damaged DNA stimulates the internal pathway. This leads to apoptosis because keeping damaged DNA can lead to dangerous effects (e.g. disease, cancer).



- How is the DNA damage detected?

As we saw earlier, DNA damage is detected by ATM which activates Chk2. Chk2 then activates p53.

Now, the activated P53 will bind to a certain region on the DNA and activate the expression of a group of genes that code for apoptosis, one of which is the gene that codes for the BH3-only domain proapoptotic protein.

So, in an apoptotic cell the P53 protein is activated preventing the progression of the cell cycle leading to apoptosis.

2. The external pathway; the cell receives external signals that can be either survival or death signals.

- What if both survival and death signals were found in the same cell?

The cell can't respond to both signals simultaneously. Therefore, the molecules or proteins supporting cell survival will be inhibited and no conflict occurs.

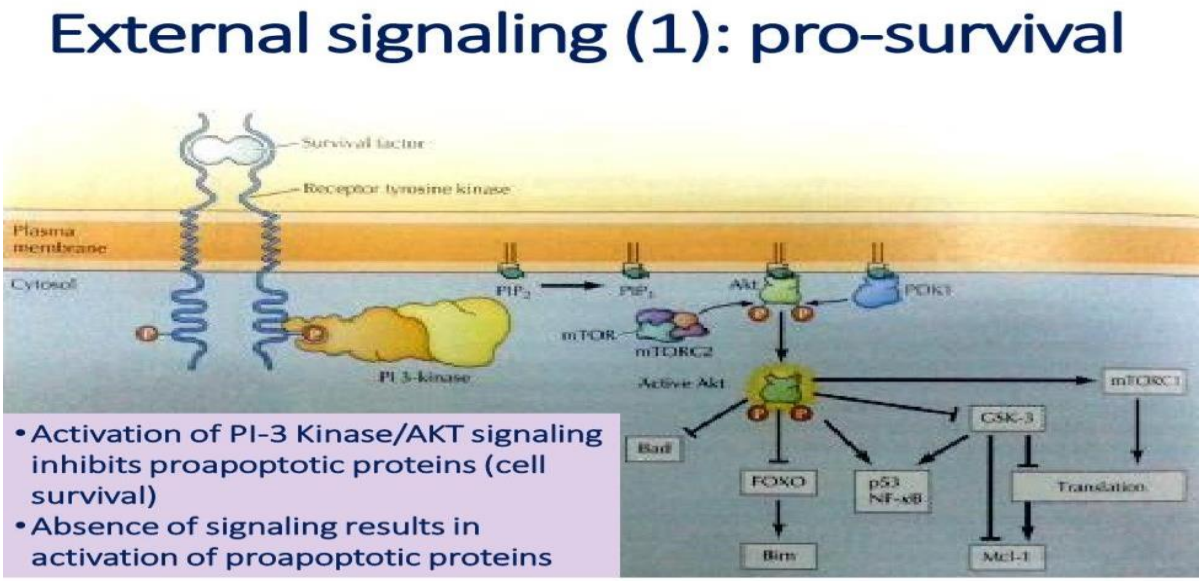
Dr. Diala said "Don't worry about the names of the proteins, just know the main concept".

• *Let's first discuss the pro-survival external signals*

A survival factor or signal reaches the cell and binds to a receptor tyrosine kinase which phosphorylates PI-3 kinase leading to its activation. Once activated, the PI-3 kinase phosphorylates other cell survival proteins such as the AKT protein (which is also phosphorylated by the PDK 1 protein). Once AKT is phosphorylated, it becomes activated and released into the cytoplasm. There, it inhibits proteins supporting apoptosis, such as P53, Bad, NF KB (Necrosis Factor Kapa B), etc. So AKT blocks such proteins so that they don't contradict its function in cell survival.

What if AKT is inhibited? It then can't function and so it can't inhibit the proapoptotic proteins leading to their activation and as a result apoptosis occurs.

- Some of the cell survival proteins' targets are proapoptotic proteins which are normally inhibited by the cell survival proteins. And so, when the cell survival proteins are inhibited, the proapoptotic proteins become activated resulting in apoptosis.

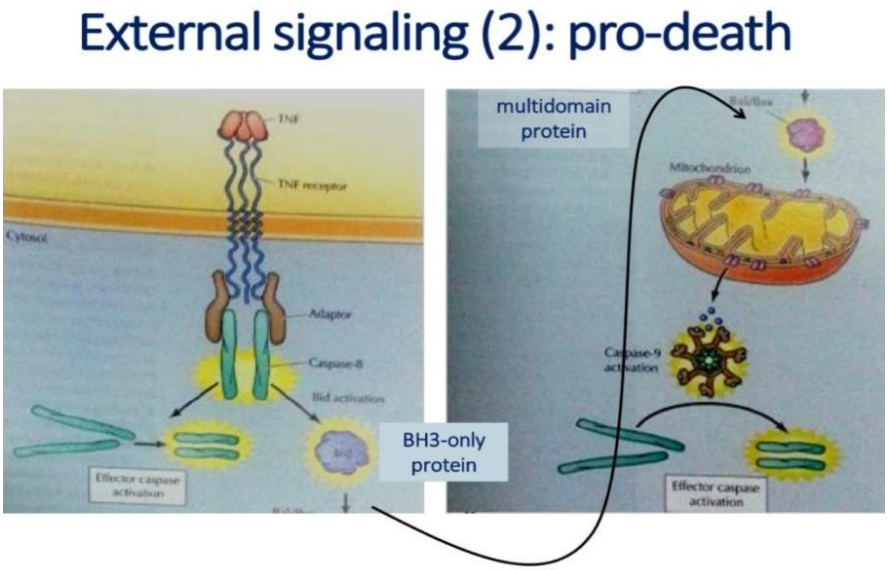


• *And now let's discuss the pro-death external signals*

The Tumor Necrosis Factor (TNF) is an external death signal that reaches the cell and can either;

~ directly react with caspases and activate them (such as caspase 9).

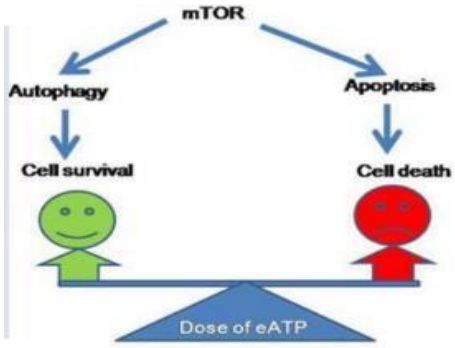
~ bind to a TNF receptor which then binds to an adaptor protein that activates a caspase. The activated caspase then activates a protein called Bid (which is a BH3-only protein agonist, i.e. it performs the same function as BH3-only) which then activates the Bax protein (a multi-domain proapoptotic protein, member of the BCl2 family proteins) which enters the mitochondria releasing the cytochrome c. Then the cytochrome c binds to an adaptor protein which then binds to caspase 9 and it activates caspase 3 resulting in apoptosis.



Autophagy

- What is Autophagy?
  - Autophagy is a rescuing process that acts as a final attempt for correction and repair before the cell loses hope and decides to kill itself.
- What are the advantages of autophagy?
  - When the cell lacks molecular machinery (ex: proteins) of apoptosis; autophagy gives the cell the needed time for preparation of what's required for apoptosis.
  - It gives the cell a chance to repair the damage in it before it undergoes apoptosis.

- The balance between autophagy and apoptosis:
  - Autophagy might take place and then be followed by apoptosis
  - Or, autophagy can occur alone and get rid of the damage in the cell without having to be followed by apoptosis. This depends on:
    - The situation
    - The different types of signals, either death signals (TNF) or survival signals (AKT signaling).



To sum up, different changes occur to maintain the balance in the cell. It may choose cell survival and save itself through autophagy or it may choose to do apoptosis.

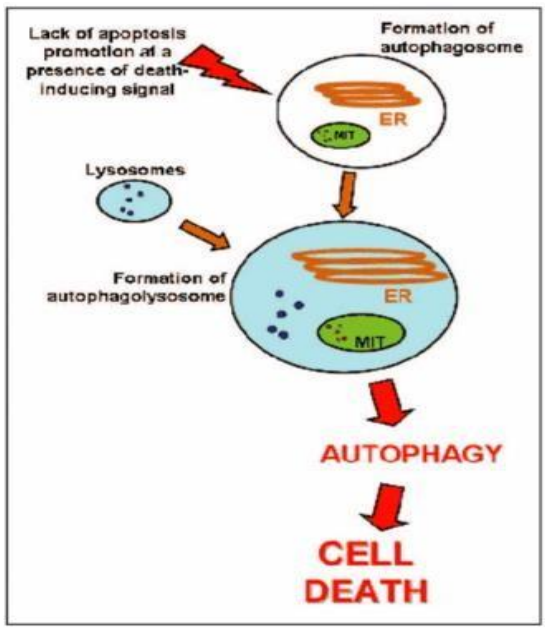
- Apoptosis can be caspase-independent (and function without capsases). In this case, it will be mediated by autophagy through mTOR signaling.

- What is mTOR?

mTOR is a member of the phosphatidylinositol 3 kinase-related kinase family of protein kinases.

- Mechanism

The lack of apoptosis promotion at the presence of a death-inducing signal leads to the formation of an autophagosome (a vesicle containing old or damaged organelles). This autophagosome then fuses with the lysosomes that accumulated during autophagy forming an autophagolysosome. Finally, this leads to the degradation of the vesicle's contents.



So, in autophagy, the cell doesn't change shape or fragment. Instead, lysosomes accumulate within it.

Best of luck ☺