بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | Lecture 15

Cell renewal and death

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Lecture 10: Cell renewal and death

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(اللّٰهم إنّي أسألُك فِهمَ النَّبيّين و حِفظَ المُرسَلين و المَلائكة المقرَّبين ، اللّٰهم اجعَل ألسنتنا عامرة بذكرك و قلوبنا بخشيتك و أسرارنا بطاعتك إنكَ على كل شيءٍ قدير)

Programmed cell death

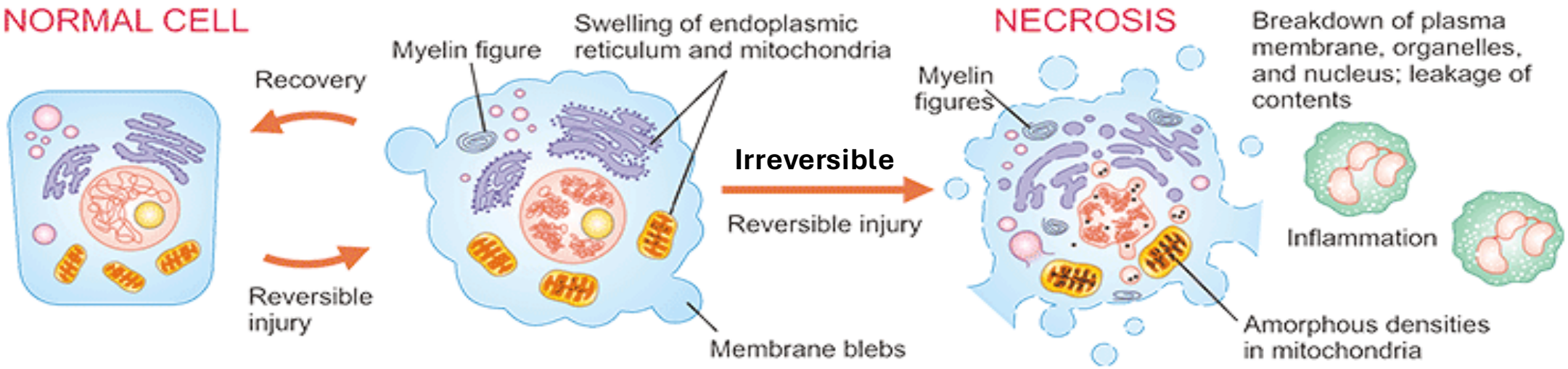
- Cell death occurs due to a harsh injury or by a programmed process called apoptosis, which can be a natural biological reason, due to a signal, or a result of a physical damage to the cell or DNA.
  - Renewal of >100 x 10^9 blood cells a day
  - Elimination of nerve cells with a faulty connection
  - Elimination of damaged cells with DNA damage or viral infection
- Programmed cell death is a normal physiological form of cell death with a distinct process known as apoptosis (“leaves falling down”).

➢ Our body’s cells die and get replaced all the time. For example, we lose many red blood cells every day, and new ones grow . Nerve cells, on the other hand, are removed when they get damaged. Sometimes cells die because they’re damaged by viral infections or DNA problems. This can happen naturally or by force. During growth, some cells die to help shape our body – like forming our fingers.

Necrosis

- The accidental death of cells that results from an acute (harsh) injury.
- Cell necrosis results in membrane damage, enlargement of cells, release of intracellular contents, and inflammation.

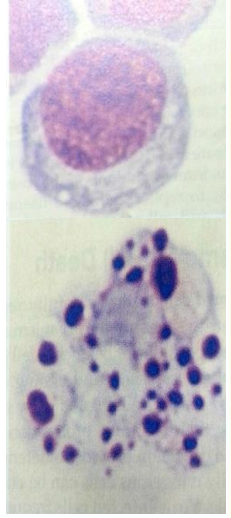
➢ An **inflammatory response** involves the movement of immune and phagocytic cells to the site of inflammation, where they help eliminate pathogens and clean up damaged tissue.

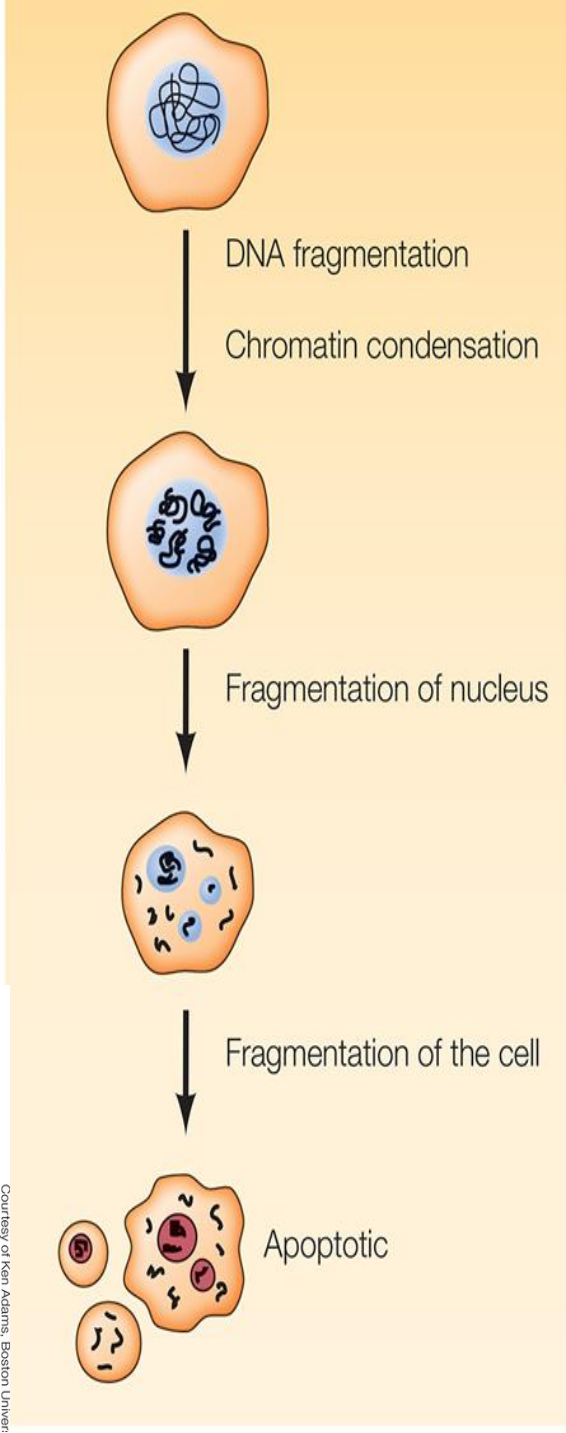


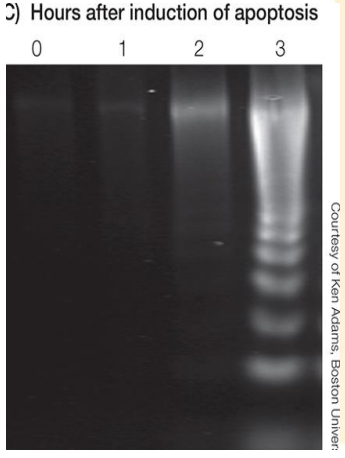
Apoptosis (Programmed cell death)

➢ It's a step wise process, and it goes through steps :

- Fragmentation of chromosomal DNA based on size by electrophoresis (like we do with proteins)
- Chromatin condensation
- Nuclear fragmentation ,the nuclear lamina, the cytoskeleton of nucleus are fragmented
- Cell shrinkage the most important difference between apoptosis and necrosis
- Cell fragmentation (apoptotic bodies)
- Phagocytosis by macrophages and neighboring cells ("eat me" signal) At the end , macrophages will clear up the cell debris (cell fragments).

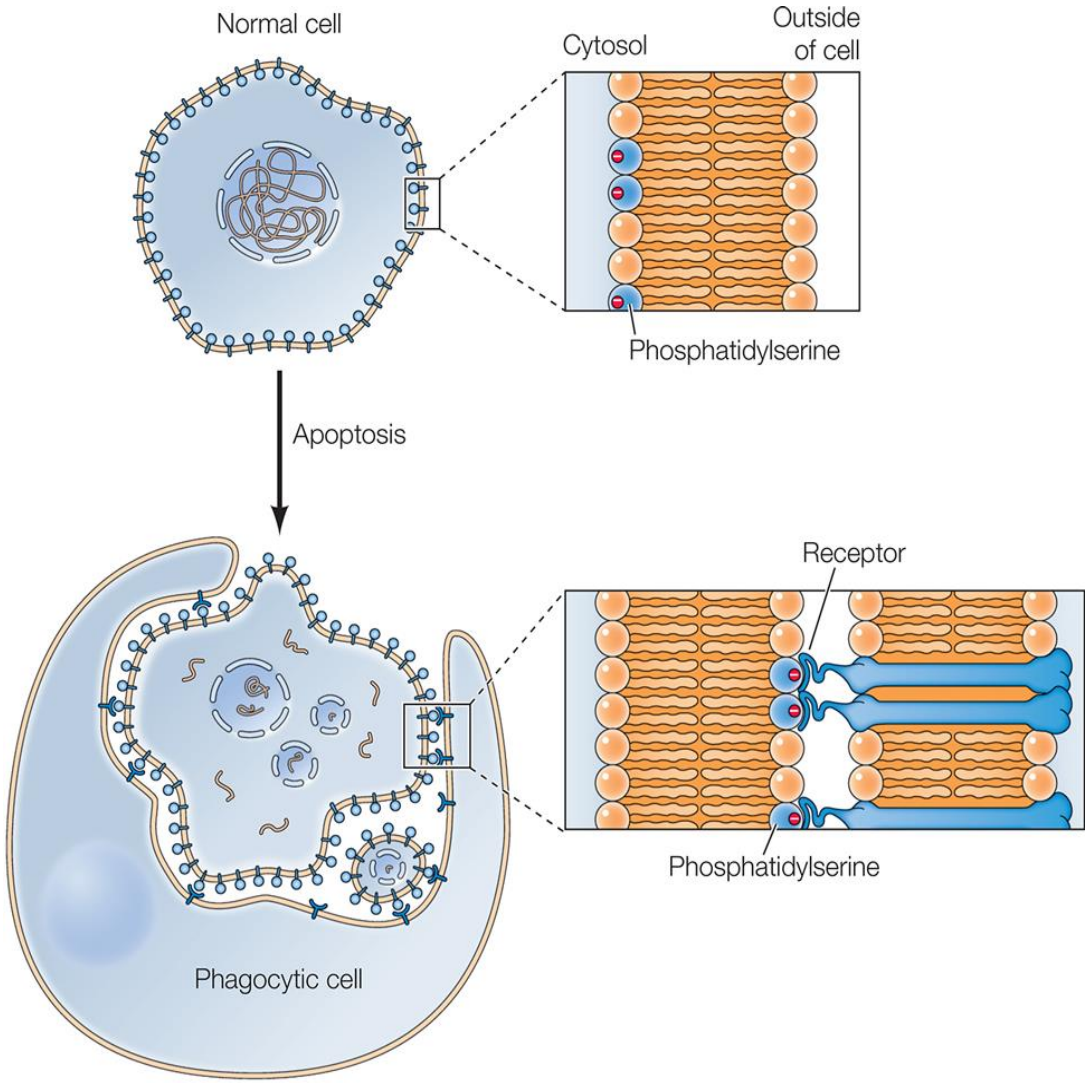






Role of phosphatidylserine

(It's an early marker of apoptosis)

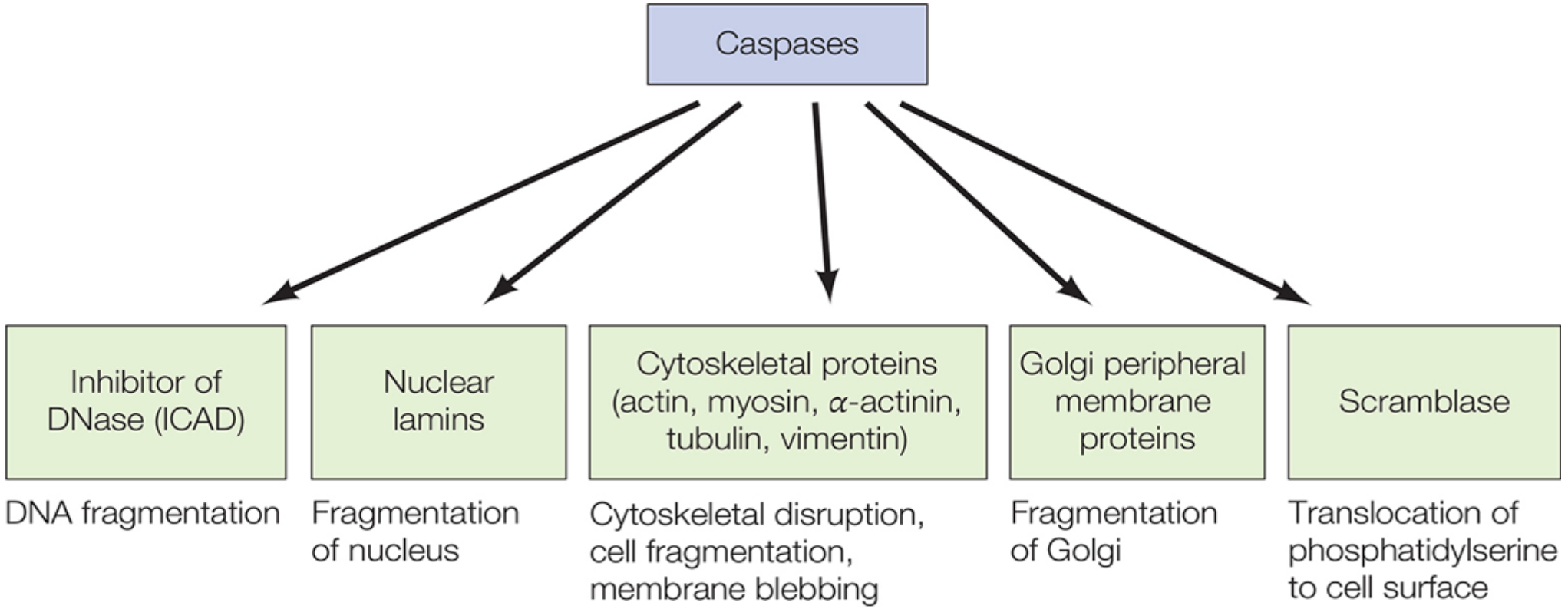


- Normally, PS is expressed on the inner leaflet of cells exposed to the cytosol.
- During the initiation of apoptosis, PS is flipped to the outer leaflet and it gets exposed to the outside (to the inflammatory cells and immune cells)
- It is then recognized by receptor on the membrane surface of phagocytic cells.

➢ So we can identify apoptotic cells by labeling phosphatidylserine , if it's present on the surface, we know that these are dying cell (not dead yet).

Caspases: the culprits of apoptotic actions

- The caspases are the ultimate effectors or executioners of apoptosis cleaving more than 100 different target proteins (know specific examples).



Caspases: the culprits of apoptotic actions

- **How fragmentation occurs?**

By enzymes that degrade proteins (caspases) , so they are proteases which have different targets .

- **What caspases do ?**

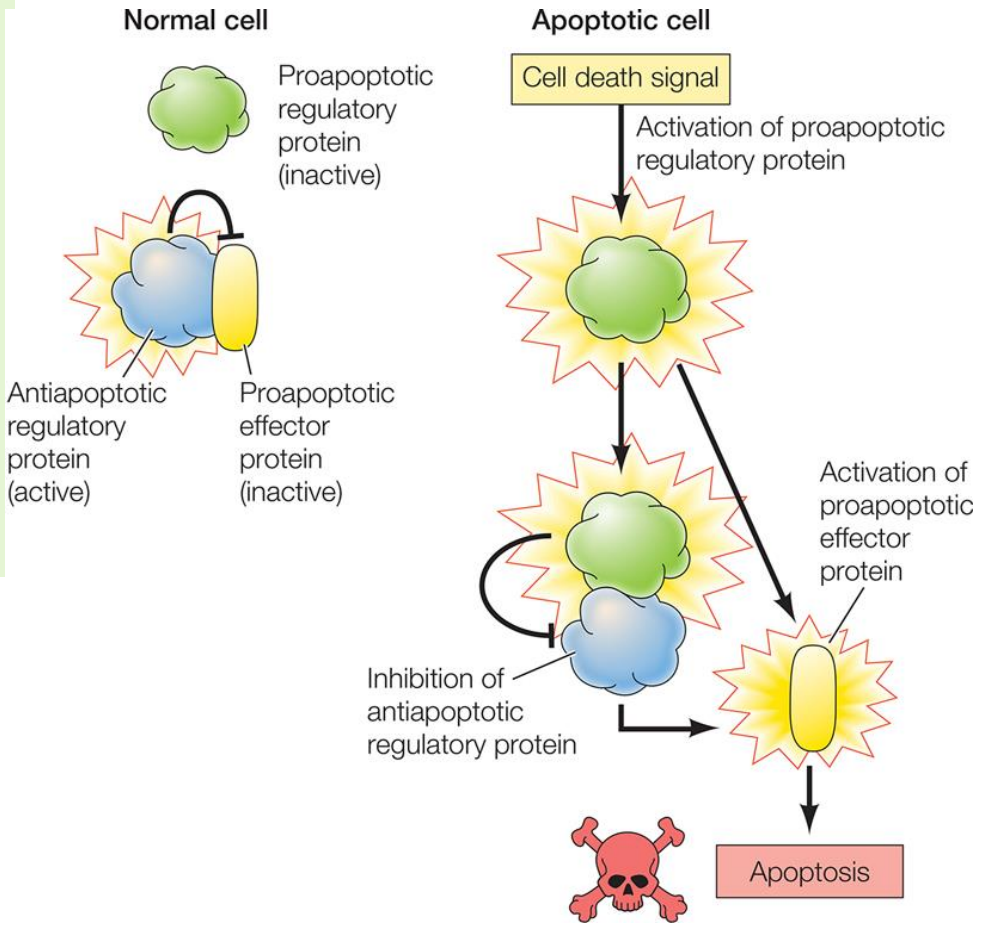
- Degrade an Inhibitor of DNase(it's an enzyme that degrades and fragments DNA)
- Degrade the nuclear lamina . They degrade the actin cytoskeleton and binding proteins.
- Degrade the Golgi apparatus .
- Activate the translocation, leads to the flipping of phosphatidylserine to the outer surface.

اللَّهُمَّ صَلِّ عَلَى سَيِّدِنَا مُحَمَّدٍ ﷺ صَلَاةً تُخْرِجُنَا مِنْ ظُلُمَاتِ الوَهْمِ إِلَى النُّورِ

The regulators

The Bcl-2 family

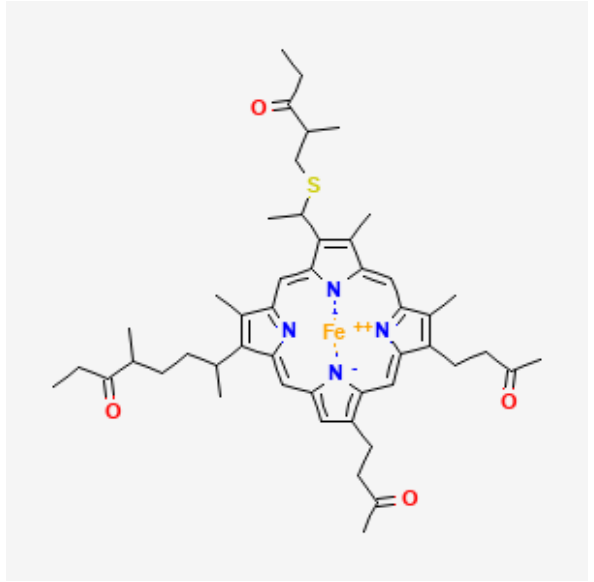
- The antiapoptotic regulatory proteins
  - They inhibit the proapoptotic effector proteins **(Prevent apoptosis from taking place)**
- The proapoptotic effector proteins *directly induce apoptosis*. **(like caspases)**
- The proapoptotic regulatory proteins
  - They bind to and inhibit the antiapoptotic regulatory proteins
  - They allow the release of the proapoptotic effector proteins.



- **Caspases exist in our cells naturally, but they are not active.They are kept inhibited by anti-apoptotic proteins and are synthesized as inactive zymogens (procaspases).These zymogens become active through proteolytic cleavage, carried out by other proteases .**

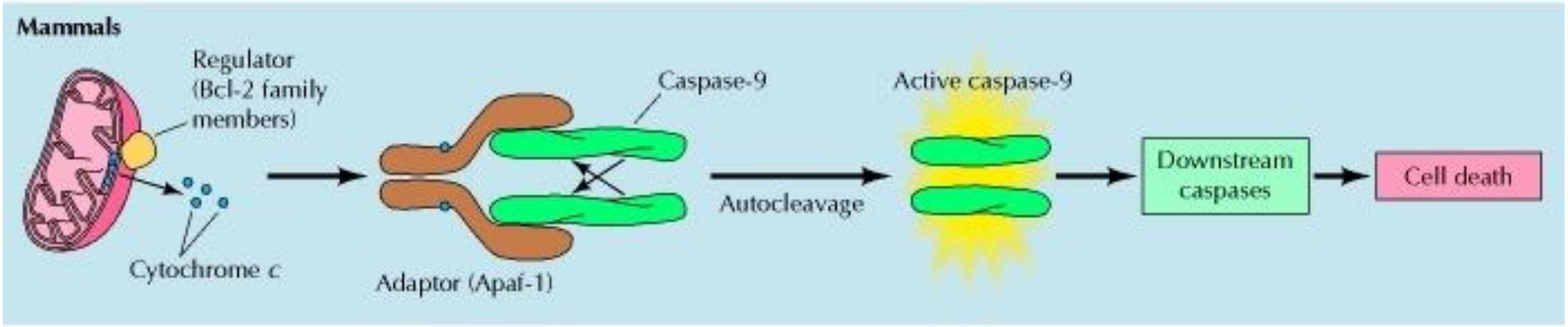
The molecular activation of apoptosis

- Activation of the apoptotic pathway involves stimulation of the proapoptotic effector proteins that oligomerize to form pores in the mitochondrial outer membrane.

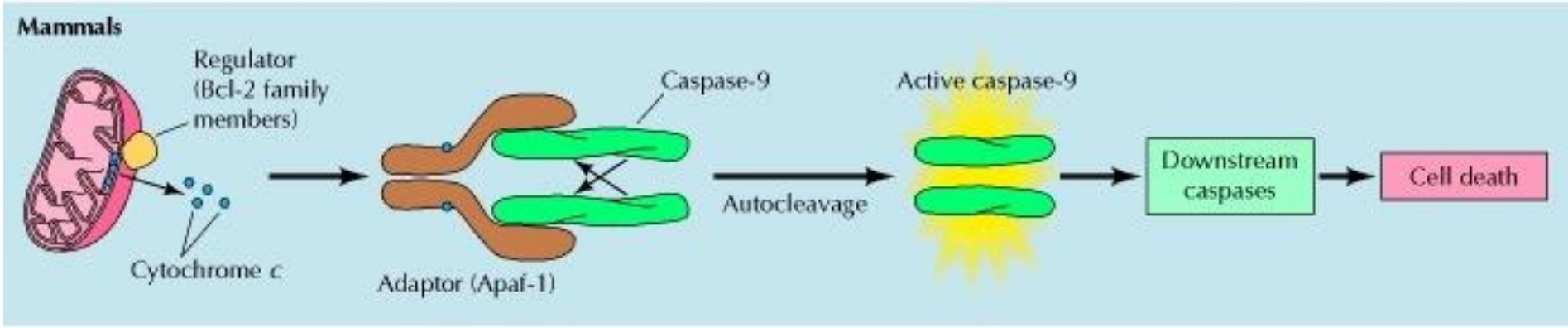


- The pores allow the release of cytochrome c from the intermembrane space.
- Cytosolic cytochrome c forms the **apoptosome complex** with caspase 9 and Apaf-1.
- Caspase 9 activates caspase 3 (a downstream effector).

oligomerize: n. the process by which several molecules (monomers) join together to form a multi-part complex called an oligomer.



The molecular activation of apoptosis



1. Proapoptotic effector proteins form a pore (channel) in the outer mitochondrial membrane.
2. This lead to Cytochrome C leakage to cytosol.
3. Cytochrome C binds to Apaf-1 and then it can complex with Caspase-9, forming Apoptosome complex.
4. In this complex the activated Caspase-9 work in different Protein targets including caspase 3 (final target).
5. The modification on these proteins facilitate the cell death.

Pathways of apoptosis

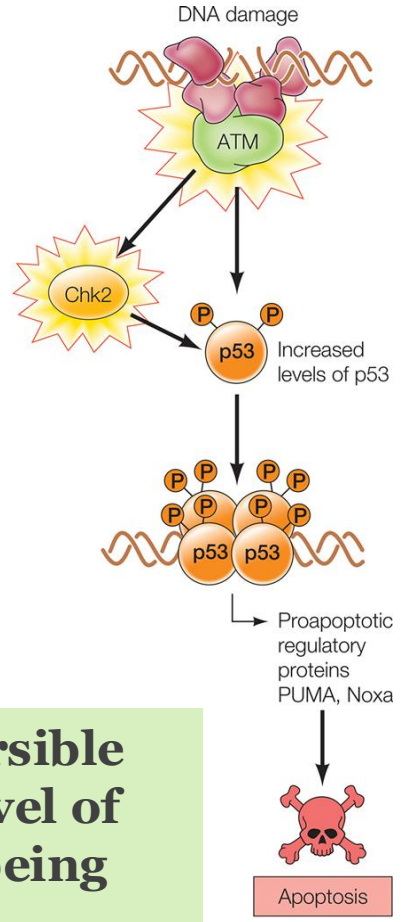
- **Intrinsic pathway** (starts within the cell) is simulated by DNA damage, viral infection, and cell stress such as growth factor deprivation.
- It involves the regulation of the proapoptotic regulatory members of the Bcl-2 family.

- **Extrinsic pathway** (starts from outside the cell) is stimulated by ligands or by receptors of other cells such as immune cells they release cytokines which activate the signal that leads to apoptosis.

They differ in the involvement of Bcl-2 family proteins and in the identity of the caspase that initiates cell death

Intrinsic pathway
DNA damage

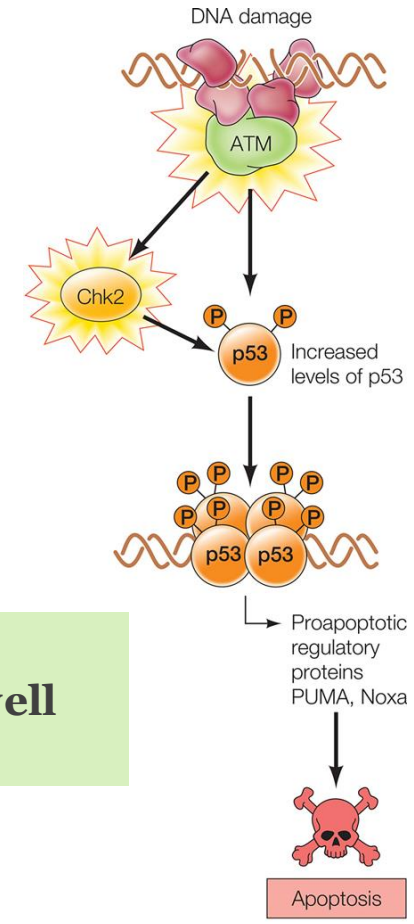
- DNA damage leads to activation of the ATM and Chk2 protein kinases, which lead to the phosphorylation, stabilization, and increased levels of p53.
- p53 then activates the transcription of genes encoding:
  - (1) proapoptotic regulatory proteins that drive cell death by releasing cytochrome c
  - 2) p21, which inhibits Cdk2/cyclin E complexes.



Note: Whether DNA damage in a given cell leads to apoptosis or reversible cell cycle arrest depends on the extent of damage and the resulting level of p53 induction, as well as on the influence of other life/death signals being received by the cell.

Intrinsic pathway DNA damage

- When DNA damage occurs (for example, a double-strand DNA break). **This damage activates the ATM** (act as sensors that detect the damage) , then **it binds to Chk2 protein kinase** . These kinases **phosphorylate p53 protein** , and it becomes stable , it increases in levels, otherwise it will undergo degradation when it is not phosphorylated .**High levels of p53** lead to the activation (transcription) of specific genes that produce:
- Pro-apoptotic regulatory proteins .
- p21 protein, which inhibits the Cdk2/cyclin E complex.



Note: Whether DNA damage in a given cell leads to apoptosis or reversible cell cycle arrest depends on the extent of damage and the resulting level of p53 induction, as well as on the influence of other life/death signals being received by the cell.

<u>If the DNA damage is minor (it can be fixed):</u>
The level of p53 increases but not too much , it blocks the cell cycle until the cell DNA is repaired.

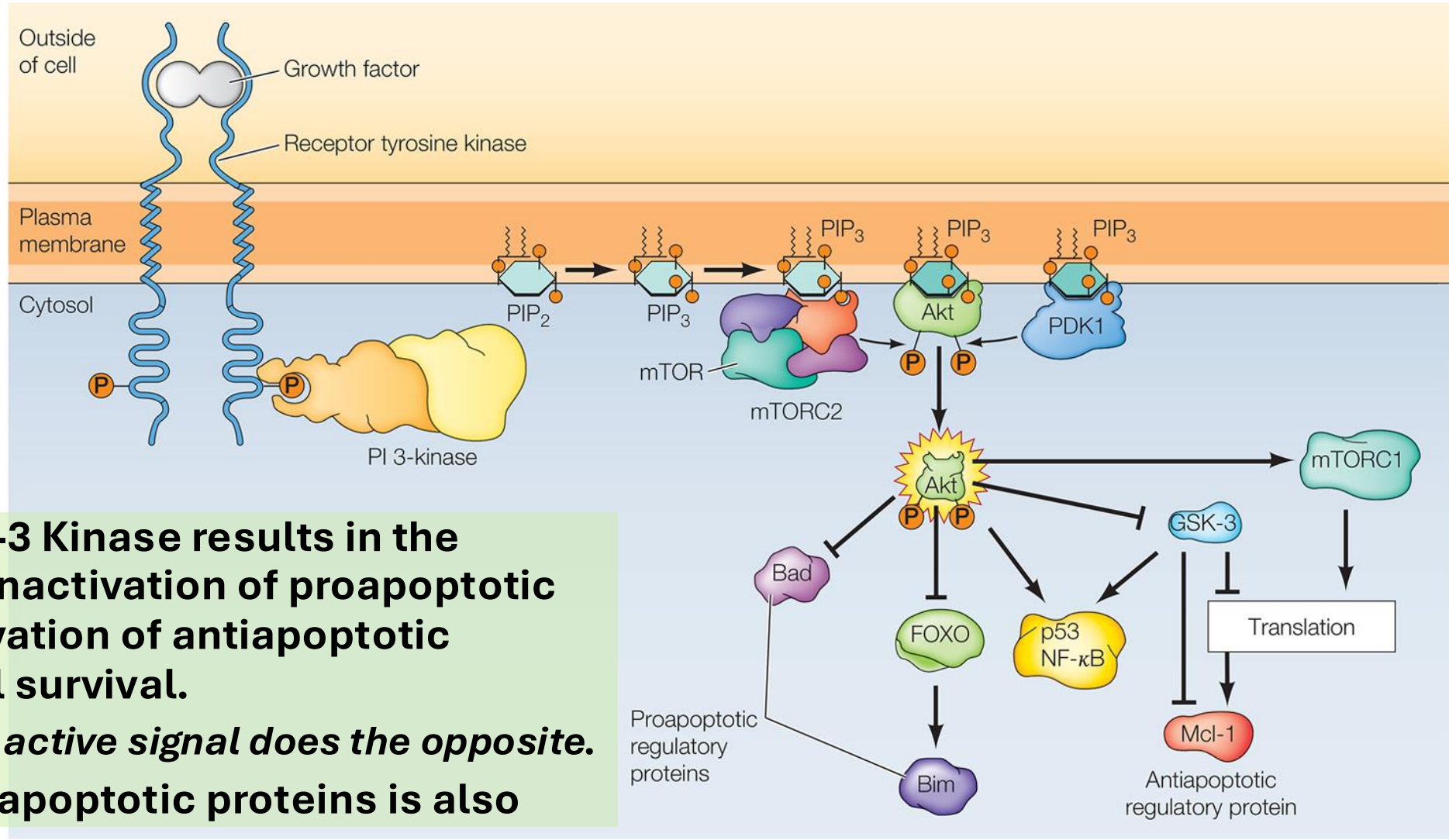
<u>If the DNA damage is extensive :</u>
the high level of p53 triggers apoptosis .

"وَمَن يَتَّقِ اللَّهَ يَجْعَل لَهُ مَخْرَجًا"

Intrinsic pathway
Growth factors

No stimulation of PI-3 kinase pathway IS the intrinsic pathway of apoptosis

The PI-3 kinase pathway was explained in cell-signaling. We call this pathway the "pro-survival" pathway as it must be activated for cells to survive



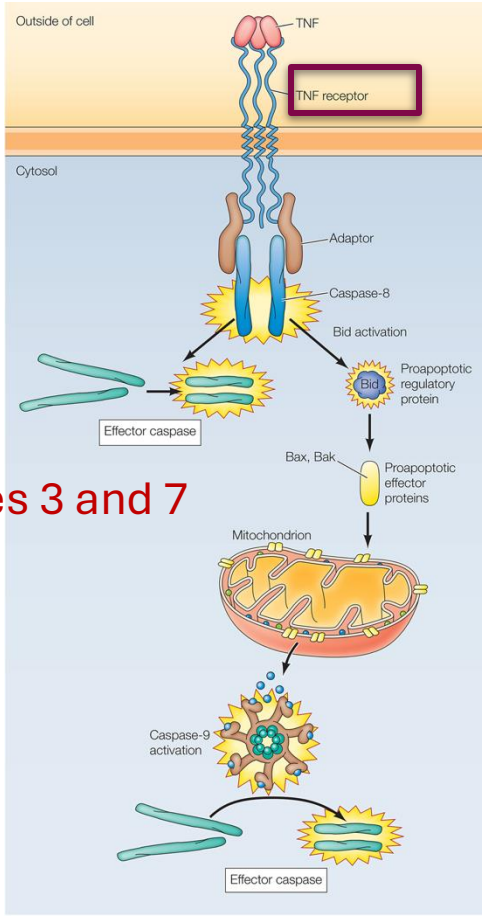
- **Activation of Akt by PI-3 Kinase results in the phosphorylation and inactivation of proapoptotic proteins, and the activation of antiapoptotic proteins, inducing cell survival.**
  - ***The absence of an active signal does the opposite.***
- **The expression of antiapoptotic proteins is also targeted.**

Extrinsic pathway

The tumor necrosis factor (TNF) signaling

Immune cells release cytokines; cytokines then bind to their receptors on target cells inducing cell death. An example on these cytokines is a ligand called TNF (it kills tumors)

- TNF binds to TNF receptors such as Fas, inducing apoptosis in a variety of cell types, like:
  - Apoptosis induced by activation of Fas is responsible for killing target cells of the immune system, such as cancer cells or virus-infected cells.
- Receptor activation leads to the activation of caspase 8 (an initiator protein), which either activates:
  - Proapoptotic effector molecules (caspases 3 and 7)
  - Proapoptotic proteins that, ultimately, activate caspase 9 (for signal amplification purposes).



Caspases 3 and 7

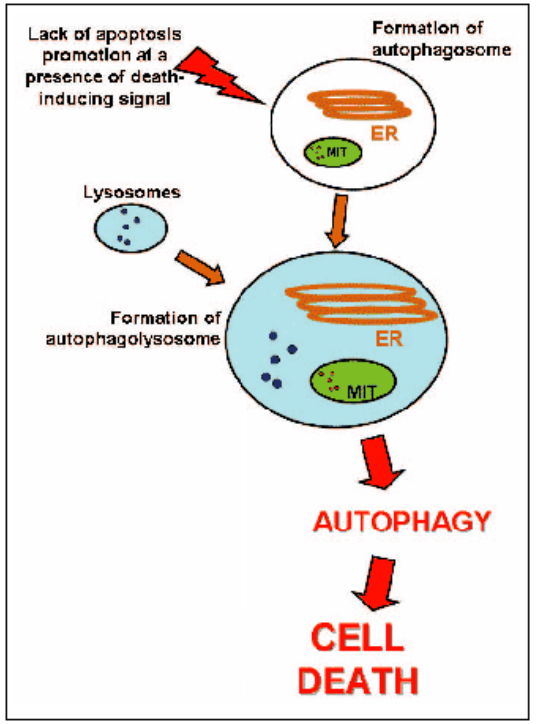
- This pathway results in the activation of Caspase 3 either directly or indirectly (through the mitochondrial/ molecular pathway of apoptosis)
- Why do we have two pathways? For signal amplification

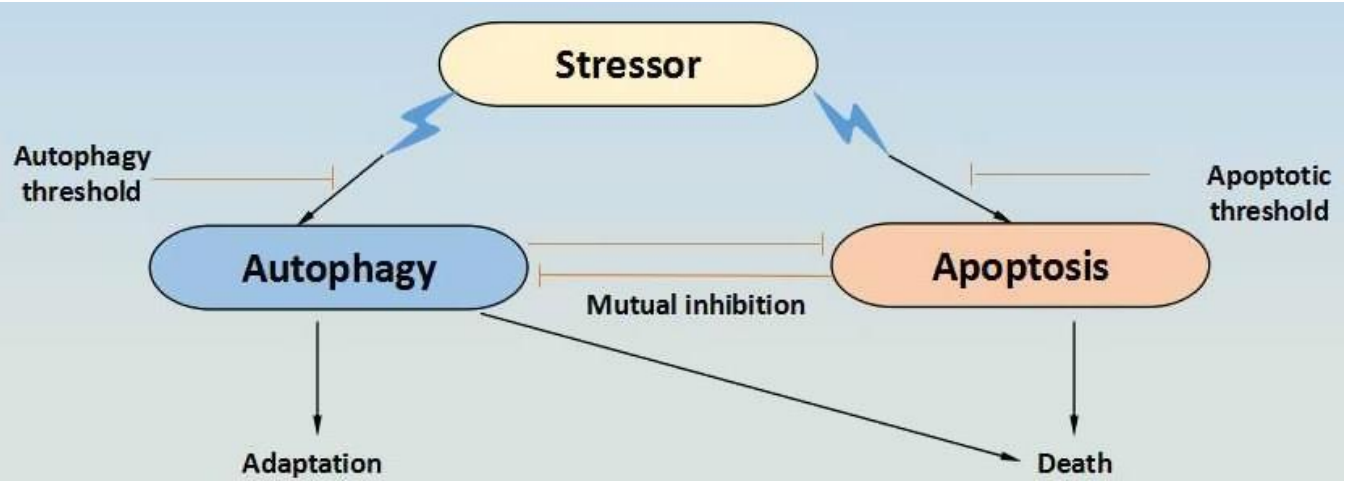
Another type of programmed cell death is:

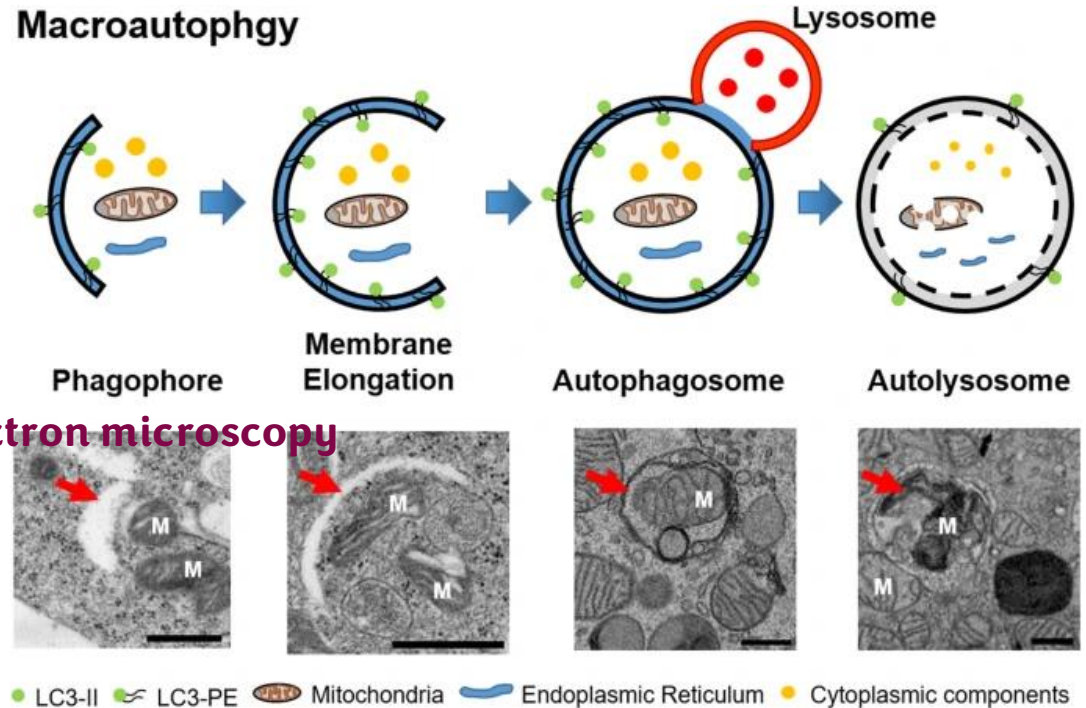
Autophagy (cell self-eating)

An alternative mechanism of apoptosis

- Autophagy is caspase-independent and is mediated by mTOR signaling.
- The dying cell has accumulating lysosomes.
- Autophagy and apoptosis inhibit each other.







Autophagy

purpose

- The cell begins degrading its own organelles, such as mitochondria, when there is a lack of nutrients – the cell is “hungry” and needs energy (ATP).

mechanism

- Damaged organelles are enclosed by a membrane to form cytosolic autophagosomes.
- Autophagosomes fuse with lysosomes, forming autolysosomes / autophagolysosomes.
- Inside these vesicles, degradation of organelles occurs.

Autophagy and apoptosis

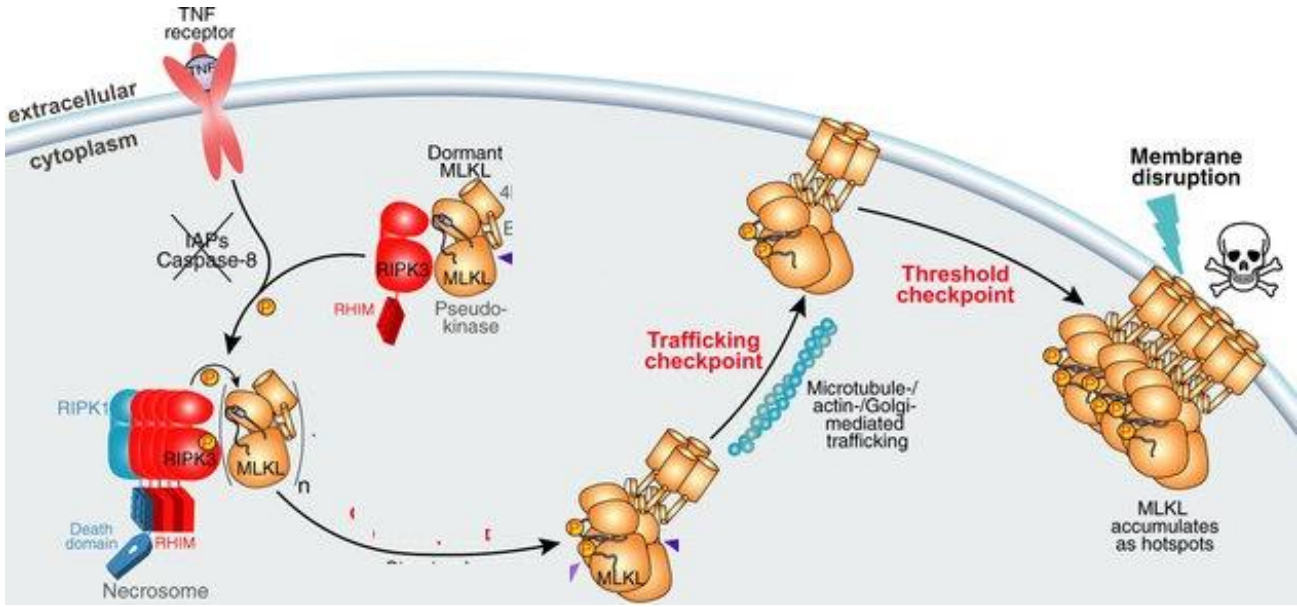
Apoptosis and autophagy don't happen together. At first, apoptosis stops to give the cell a chance to survive by activating autophagy and producing energy. If the cell can recover, apoptosis stays off. If it cannot, apoptosis restarts and the cell undergoes programmed death.

Necroptosis

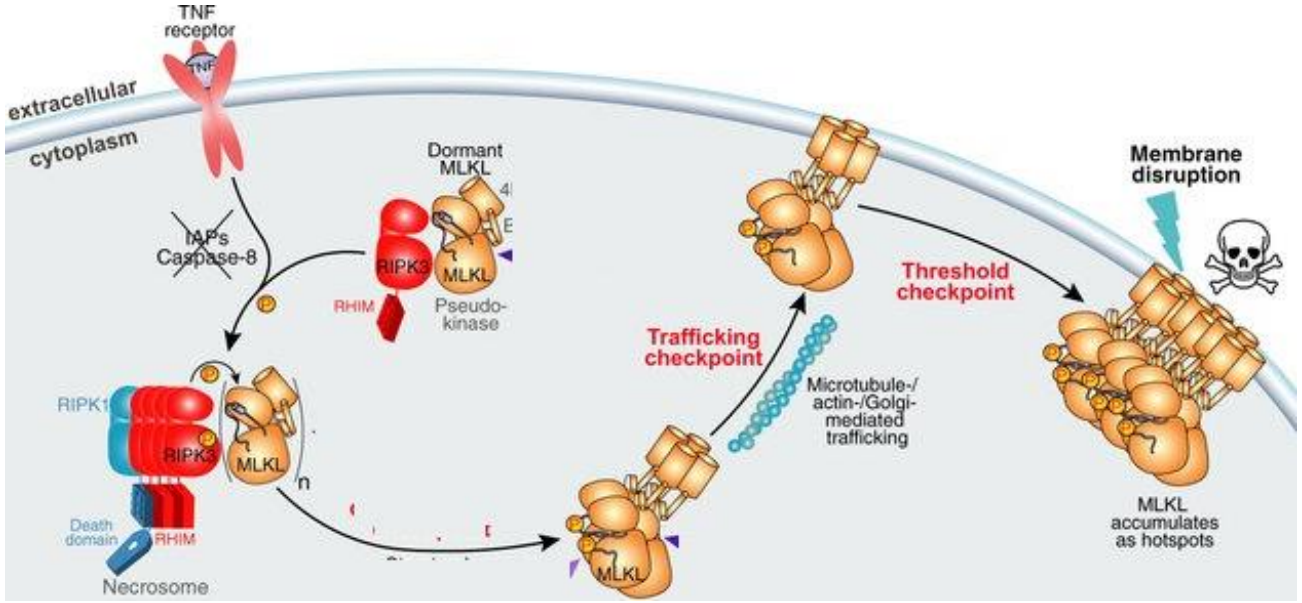
- Inflammatory response
- Enlargement of cell size
- Caspase independent

- Necroptosis (like necrosis, but not necrosis) results in the extracellular release of intracellular substances triggering an immune response.
- Unlike necrosis, necroptosis is:
  - triggered by specific stimuli such as bacterial infection, DNA damage, or TNF signaling
  - executed by a specific molecular mechanism

- The protein MLKL assembles into an oligomeric pore in the plasma membrane allowing for a rapid flux of ions into and out of the cell, causing cell swelling and rupture.
- The immune response facilitates the attack.



Necroptosis



All you need to know (from the diagram) is :

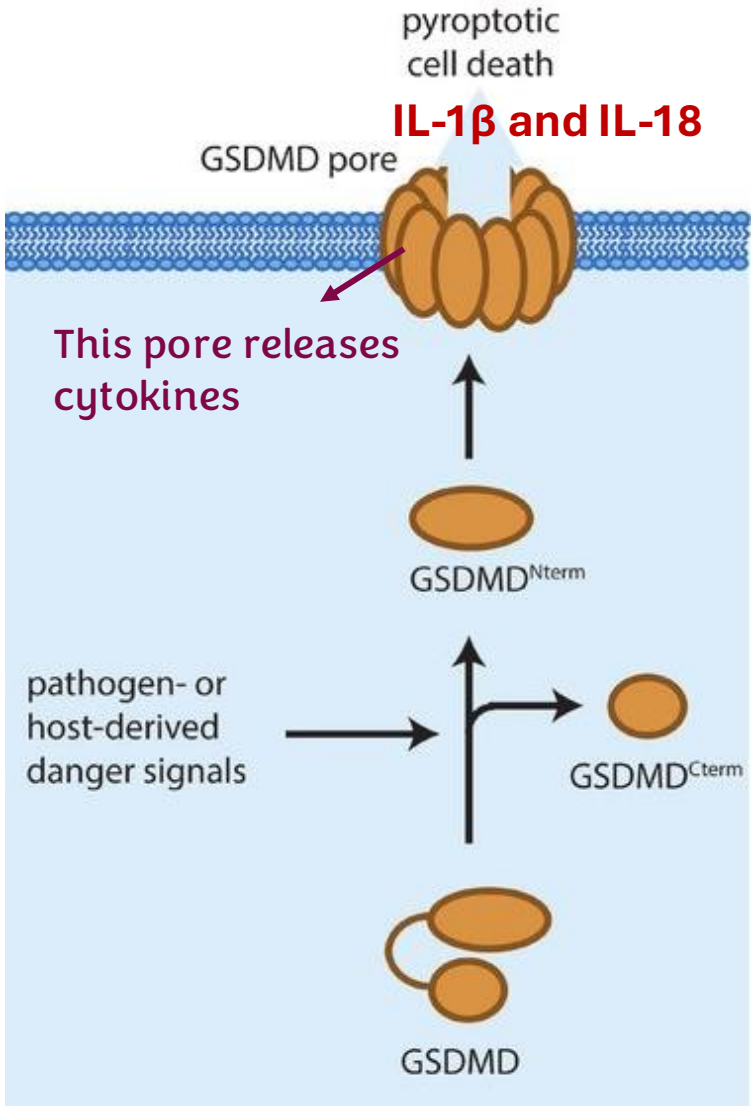
After protein MLKL gets activated by signaling, it assembles into a channel, this channel gets localized in the plasma membrane allowing for a rapid flux of ions which triggers an immune response.

Pyroptosis

If a drug is antipyretic = it reduces fever

pyro (fire/fever) and ptosis (to-sis, falling)

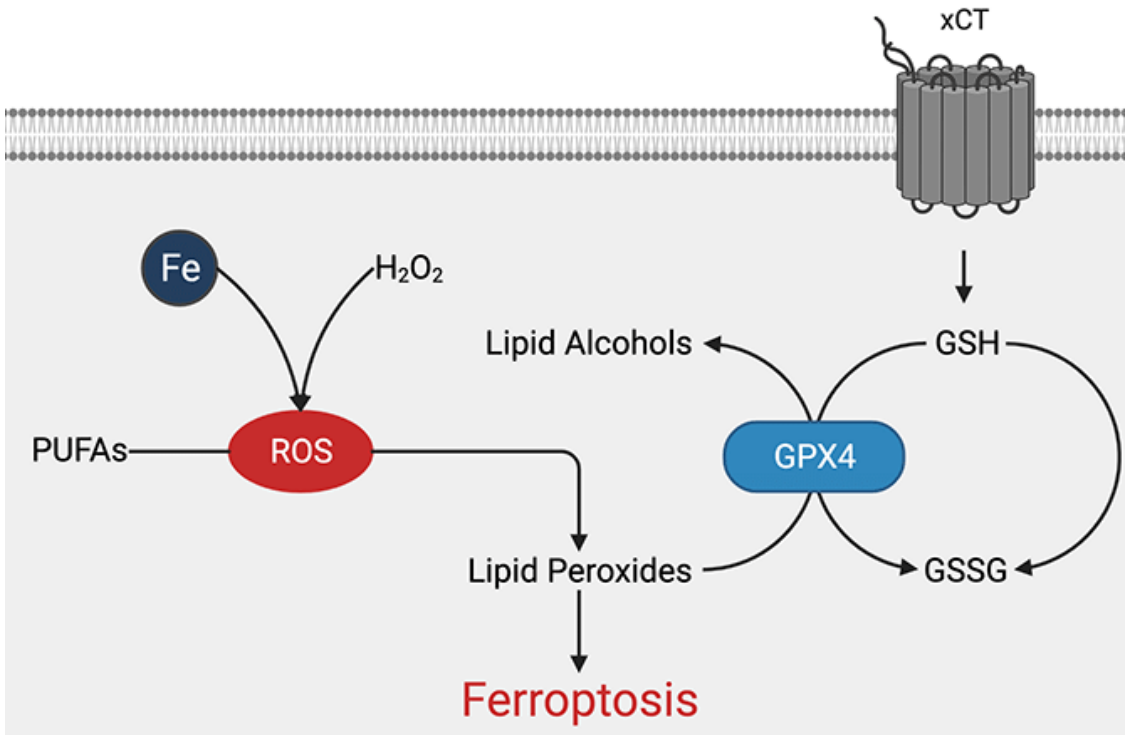
- Pyroptosis is a form of cell death that is triggered by proinflammatory signals and associated with inflammation.
- It is seen primarily in inflammatory cells such as macrophages.
- It is induced by specific stimuli such as microbial infection, executed by specific pyroptotic machinery, and involves activation and oligomerization of a protein—**gasdermin**—into a pore complex at the plasma membrane.
- A feature of pyroptosis is the activation and release of pro-inflammatory cytokines IL-1β and IL-18 through the gasdermin pore, which triggers an active immune response.



Ferroptosis

- Ferroptosis is an iron-dependent and oxidative damage-induced cell death that results from iron accumulation and lipid peroxidation, and loss of selective permeability of the plasma membrane.
- It involves depletion in the protective antioxidant enzymes, particularly glutathione peroxidase.

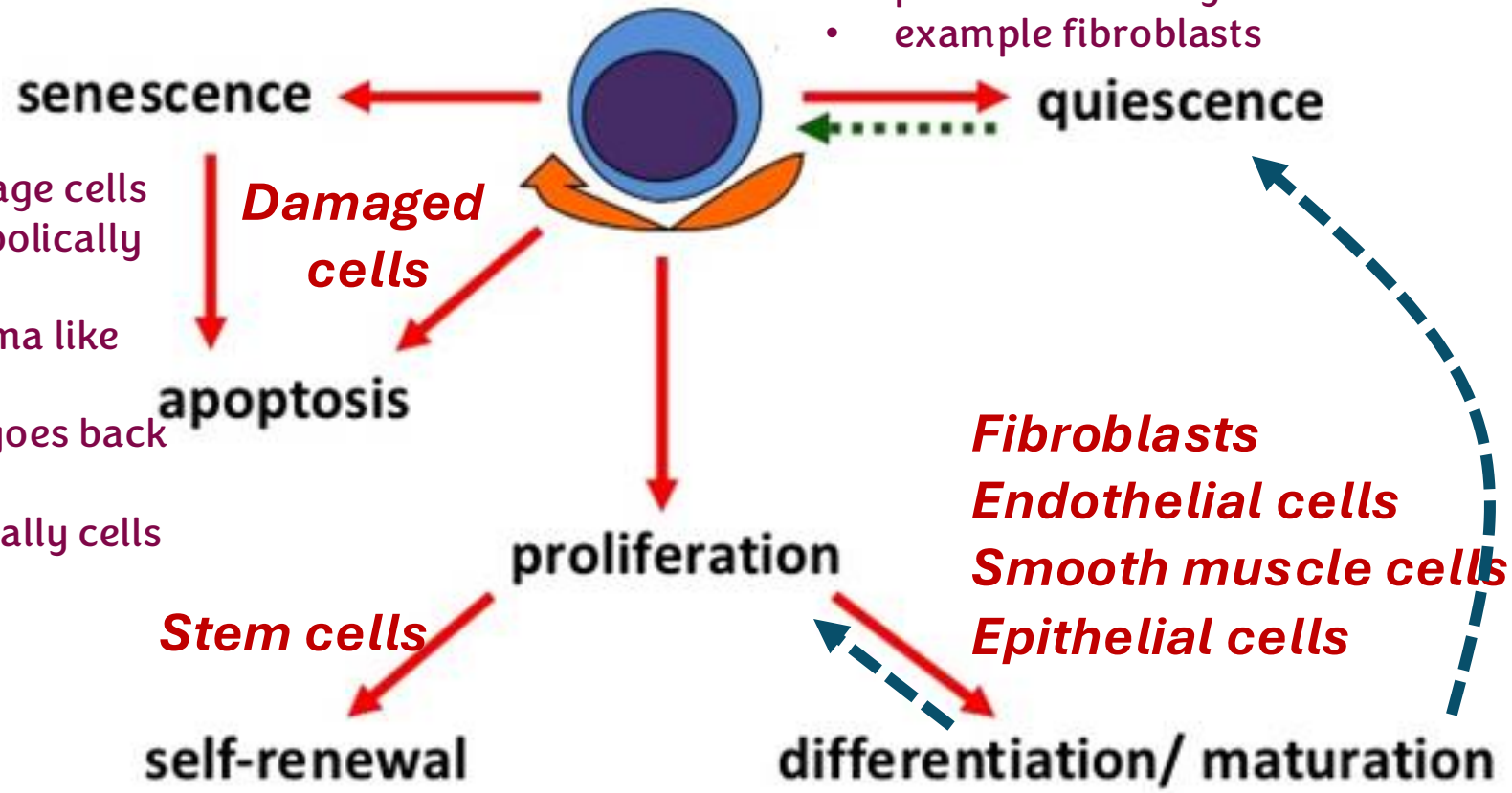
Iron is a toxic metal, and when its levels increase, it leads to lipid peroxidation. Since lipids are major components of the cell membrane, their damage results in membrane damage. Reactive oxygen species (ROS) attack these lipids, causing the membrane to become more permeable. Normally, glutathione peroxidase regenerates glutathione, which acts as an antioxidant protecting the cell from ROS. However, in ferroptosis there is depletion in glutathione peroxidase (this depletion is a marker of ferroptosis).



(Different cell stages)

Cell fate

- In the quiescence stage cells are alive/ metabolically active/ healthy/ non-dividing
- Reversible = can receive a signal to enter proliferation stage
- example fibroblasts



- In the senescence stage cells are damaged/ metabolically active with very low metabolism/ in a coma like state
- Irreversible = never goes back to proliferation, eventually cells will die

رسالة من الفريق العلمي:

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			