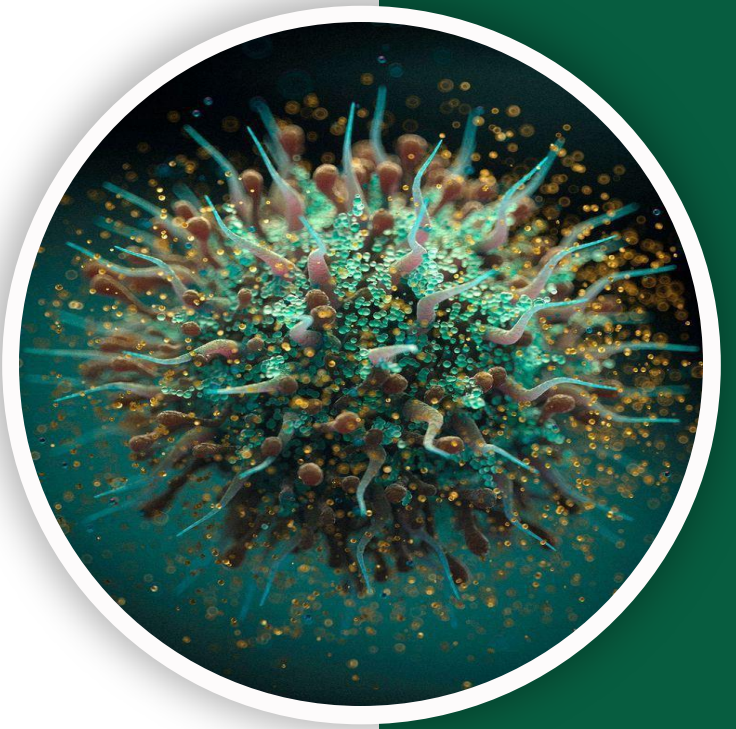
بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Pathology | Lecture 4

Apoptosis & autophagy



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Apoptosis and autophagy

cell injury and adaptations

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Definition

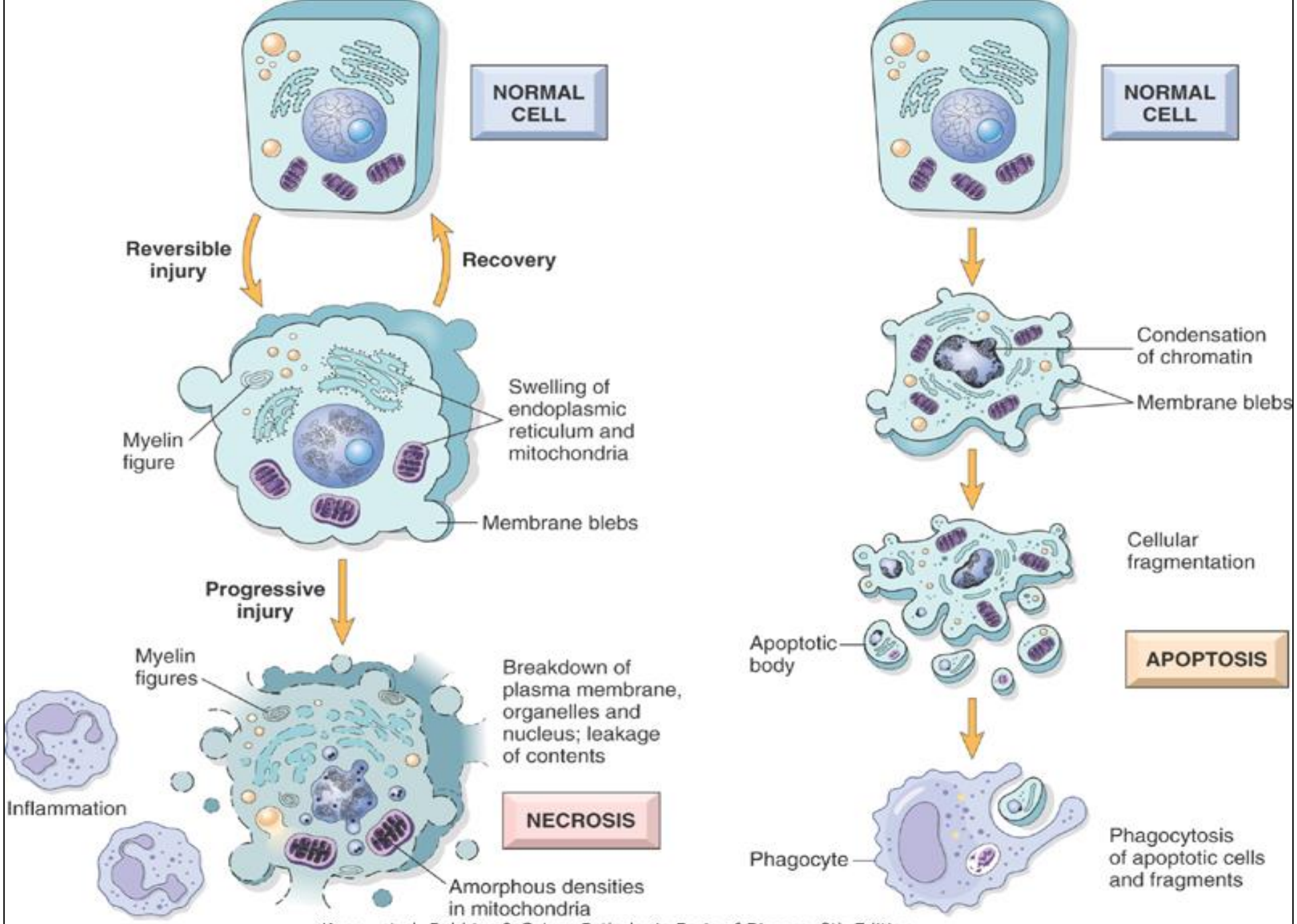
We have two types of cell death: one is called Necrosis and the other is called Apoptosis, and they are different from each other. Apoptosis is considered a programmed, genetically determined process of self-destruction of the cell, and this pathway is highly controlled.

- **"Programmed cell death"**
- "a genetically determined process of cell self-destruction"
- " pathway of cell death in which cells activate enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins."
- The dead cell and its fragments are cleared with little leakage of cellular contents, NO inflammatory reaction.

This is different from the necrosis pattern of cell death. This process of apoptosis is a highly controlled, predetermined, and genetically determined process. Sometimes, we can call it "cell suicide" or "clean cell death."

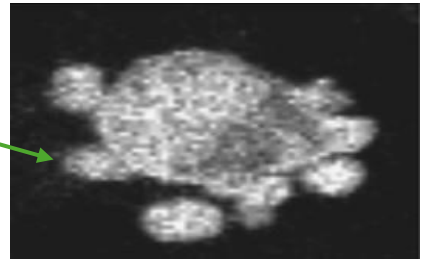
Small hint

- Apoptosis = programmed cell death.
- Necrosis = accidental, unregulated cell death (it will be discussed in a separate lecture) .



Kumar et al: Robbins & Cotran Pathologic Basis of Disease, 8th Edition.

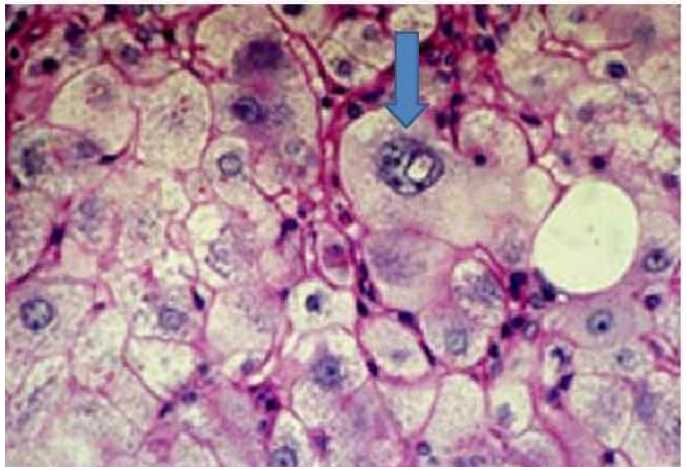
- There are many differences between Apoptosis and Necrosis.
- One of the main differences between them is that, in apoptosis, the cell undergoes shrinkage instead of swelling, and we start to see many blebs forming on the cell membrane.



These two images are for illustration p urpose only

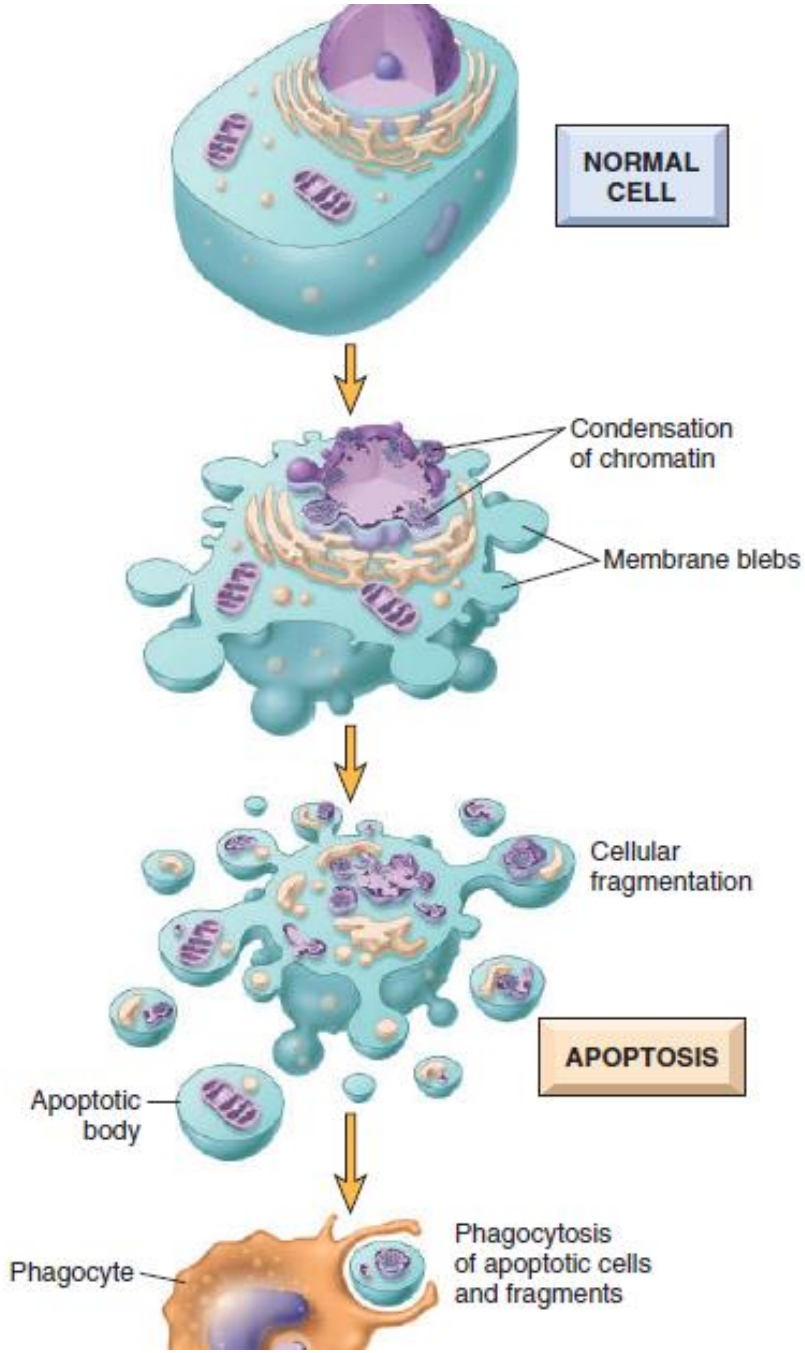
- The nuclear material starts to condense. The chromatin becomes darker under the light microscope, and it starts to fragment, a process known as karyorrhexis.

Karyorrhexis

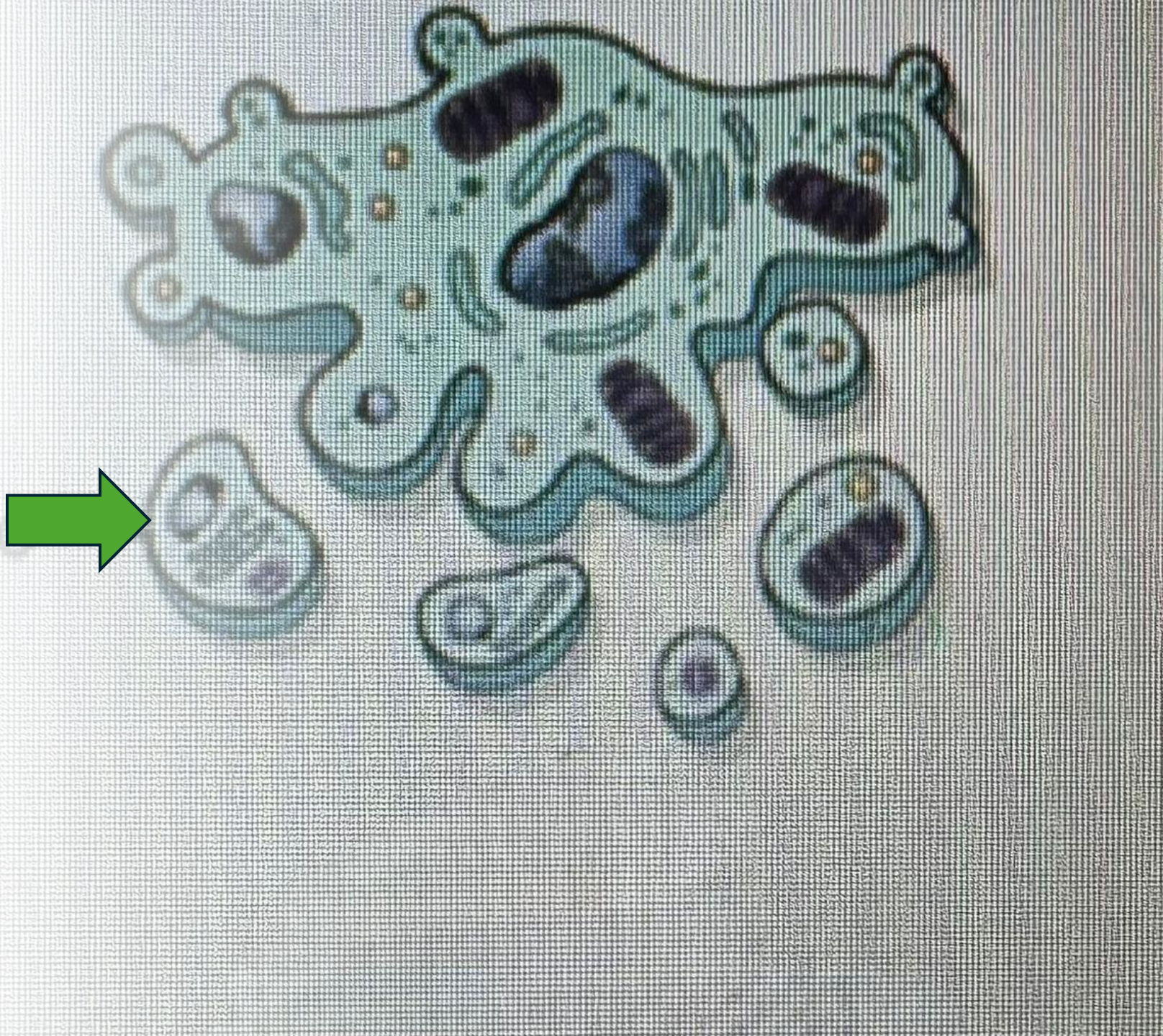


- The cell membrane stays intact and the cell starts to fall off that's why it is called "Apoptosis"

Apoptosis means falling off like(عنقود العنب)


NORMAL CELL
Condensation of chromatin
Membrane blebs
Cellular fragmentation
APOPTOSIS
Apoptotic body
Phagocyte
Phagocytosis of apoptotic cells and fragments

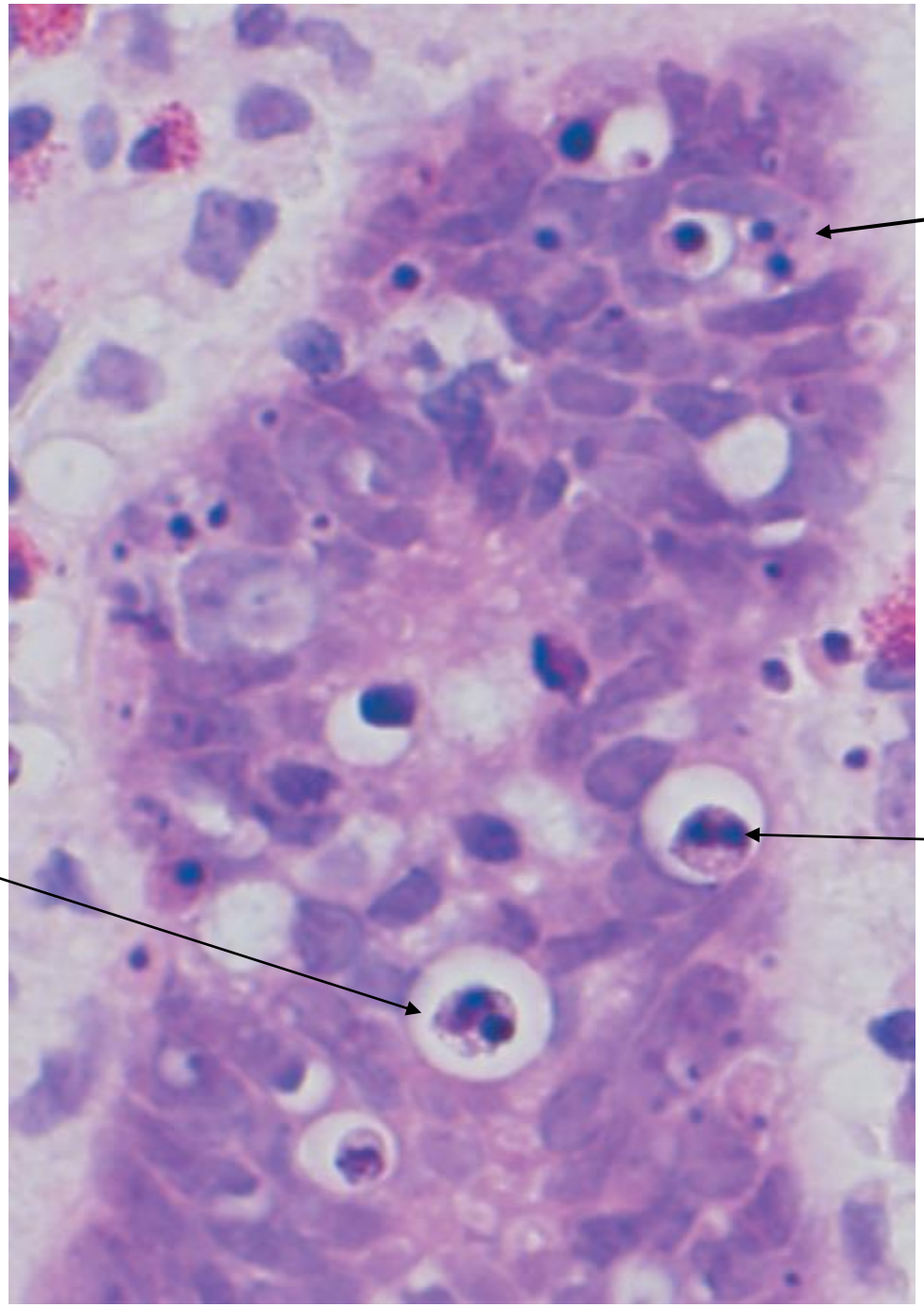
- Each part of the cell membrane encloses some organelles and cytoplasmic proteins, fragmented nucleus , DNA material separated from the main cell. These fragments are called apoptotic bodies. They give specific signals to phagocytes, which come to engulf and remove them without eliciting any inflammatory reaction. That’s why we call apoptosis "the clean cell death"



Feature	necrosis	Apoptosis
Cell size	Enlarged(swelling)	Reduced(shrinkage)
Nucleus	Pyknosis, Karyorrhexis, karyolysis	Fragmentation into nucleosome- size fragments separates into apoptotic bodies, which are then cleaned up by phagocytes.
Plasma membrane	**Disrupted**	**Intact**, altered structure, especially orientation of lipids
Cellular content	Enzymatic digestion, may leak out of cell	Intact, may be released in apoptotic bodies.
Adjacent inflammation	**Frequent**	**No**
Physiologic or pathologic role	Invariably pathologic Affects many cells	often physiologic and may be pathologic Affect few cells

In other words, the nucleus in apoptosis undergoes Pyknosis, Karyorrhexis then these karyorrhetic debris will separate into apoptotic bodies

However, apoptosis and necrosis sometimes coexist, and apoptosis induced by some pathologic stimuli may progress to necrosis, like in ischemia.



Died cells

Empty lacuna around the cell

nuclear material is fragmented, condensed, very dark in color

- In the previous picture, it’s an H& E stained section under the light microscope. You can see these cells they are shrunken. There is an empty lacuna around the cell and the nuclear material is fragmented, condensed, very dark in color, and there are a few cells that die by this pattern. As you can see also, there are fragments of the DNA material, and you don’t see an inflammatory reaction.

Causes of apoptosis

- Physiologic apoptosis
- Apoptosis in pathologic conditions

Causes of Apoptosis

- **Physiologic** → normal scenarios in which apoptotic cell death takes place as a part of normal body processes.

- **Examples about physiologic causes of apoptosis:**
- During embryogenesis
- Involution of tissues upon hormone deprivation (endometrium, lactating breast)
- Steady state population (Gut, Skin)
- End of function/life (neutrophils at end of inflammation)
- Self reacting lymphocytes

Here is the explanation of each example :

- **During embryogenesis:**

During embryonic development, new cells are formed and old cells die by apoptosis to shape the tissues.

.Involution of tissues upon hormone deprivation :

Another example is at the end of the menstrual cycle, when hormone levels drop and the endometrial glands break down. The uterine lining sheds as menses, and this process also happens through apoptosis. Another example, In the lactating breast, after lactation stops, the hormone levels decrease, and the new glands that appeared during lactation will shrink and die by apoptosis. This happens because of the loss of hormonal stimulation.

- **Steady state population :**

In certain organs like in the gut, in the GI tract, and in the skin. As you know, these organs have a rapid turnover, so new cells are made all the time and the old cells die by apoptosis in a rapid regenerative capacity process.

- **End of function/life:**

Sometimes cells perform their function and there is no need for them anymore, so they should die by apoptosis. An example of that is the neutrophils at the end of the acute inflammatory reaction. These neutrophils have left the blood vascular space into the tissue. When they perform their function, they will die by apoptosis. They will not return back to the blood vessels or to the bloodstream another time; they will perform their function in the inflammatory action and then die by apoptosis.

- **Self reacting lymphocytes**

During the development of lymphocytes in the bone marrow and other lymphoid organs, the body produces many new immune cells. Some of these new lymphocytes may react against self-antigens, meaning they attack the body's own cells instead of foreign invaders. If these self-reactive lymphocytes remain in the body, they can cause autoimmune diseases such as lupus or rheumatoid arthritis. To prevent this, the body removes these harmful cells through apoptosis, which occurs during the development or maturation of lymphocytes. This process is a natural protective mechanism that prevents autoimmune diseases by eliminating self-reactive cells before they spread in the body.

Pathologic: (damaged cells beyond repair)

The body can't repair the damage that happened, so the cell is targeted and dies by apoptosis.

- DNA damage (Rx, chemoTx, tempreture, UV, hypoxia)
- Accumulation of misfolded proteins

This will be discussed in the mechanisms of cell death lecture.

- Some infections (adenovirus, HIV, hepatitis viruses)

some infections, especially viral infections like infection by adenovirus, HIV/AIDs, or hepatitis viruses (التهاب الكبد الوبائي) can cause cell death either by necrosis or by apoptosis.

All of these scenarios can lead to DNA damage. And if this DNA damage is not repaired, the cell will be directed to apoptosis, because if a DNA-damaged cell continues to replicate, we will have cancer at the end.

Condition	Mechanism of Apoptosis
Physiologic	
During embryogenesis	Loss of growth factor signaling (presumed mechanism)
Turnover of proliferative tissues (e.g., intestinal epithelium, lymphocytes in bone marrow, and thymus)	Loss of growth factor signaling (presumed mechanism)
Involution of hormone-dependent tissues (e.g., endometrium)	Decreased hormone levels lead to reduced survival signals
Decline of leukocyte numbers at the end of immune and inflammatory responses	Loss of survival signals as stimulus for leukocyte activation is eliminated
Elimination of potentially harmful self-reactive lymphocytes	Strong recognition of self antigens induces apoptosis by both the mitochondrial and death receptor pathways
Pathologic	
DNA damage	Activation of proapoptotic proteins by BH3-only sensors
Accumulation of misfolded proteins	Activation of proapoptotic proteins by BH3-only sensors, possibly direct activation of caspases
Infections, especially certain viral infections	Activation of the mitochondrial pathway by viral proteins Killing of infected cells by cytotoxic T lymphocytes, which activate caspases

Mechanisms of Apoptosis

- **Activation of enzymes called caspases**
- Two distinct pathways can lead to caspase activation:
- 1) The mitochondrial pathway called intrinsic pathway because it starts at the level mitochondria inside the cell.
- 2) The death receptor pathway.

➢ These two pathways will overlap at the end by activation of executive caspases but each pathway has it's own distinct properties.

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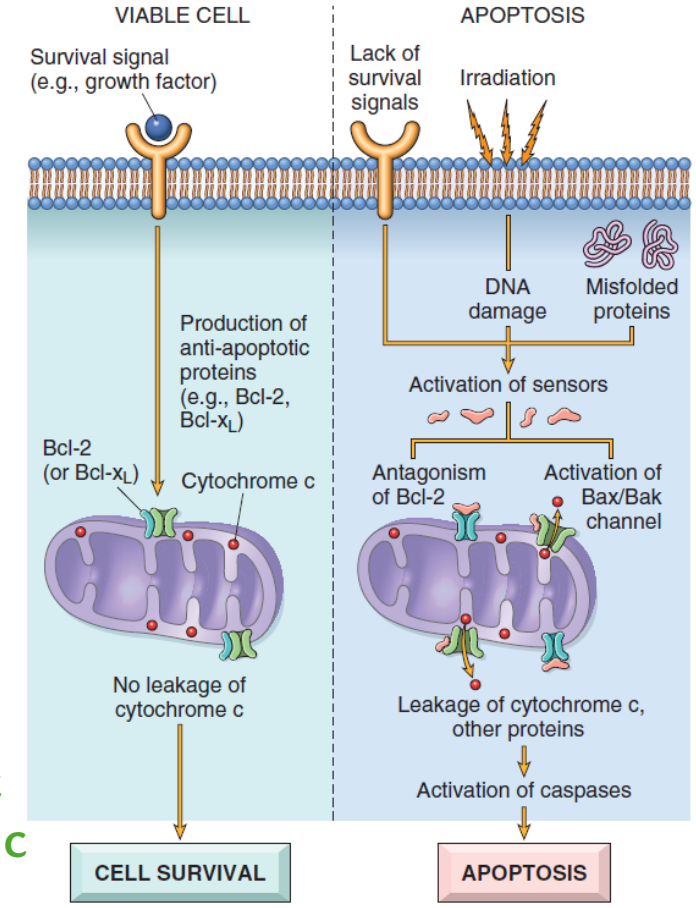
Mitochondrial (intrinsic)

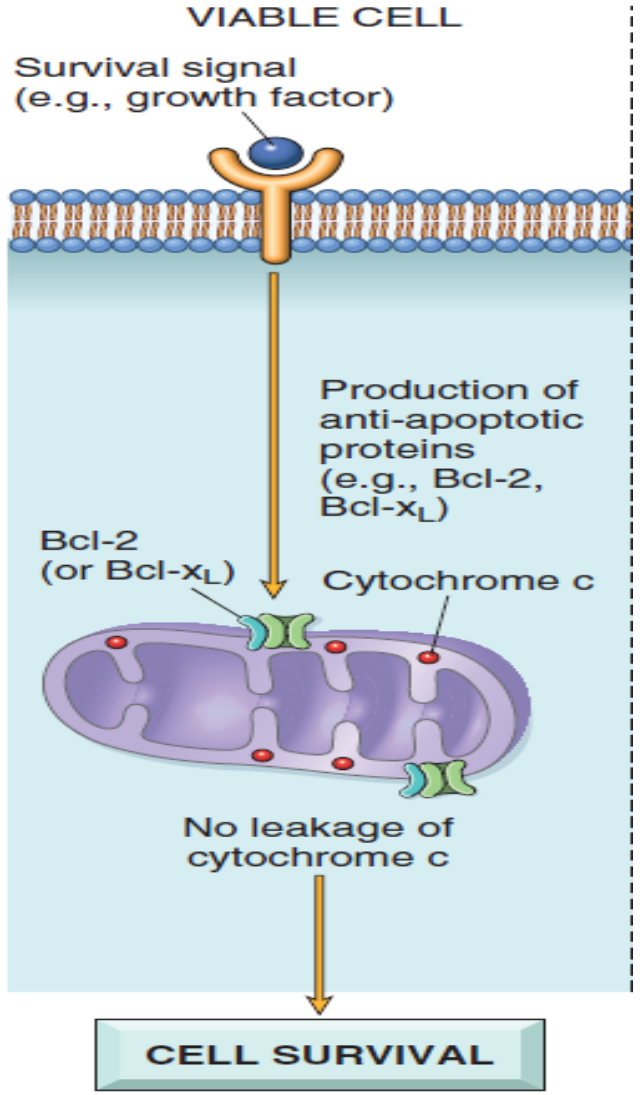
- ✓ Responsible for apoptosis in most physiologic and pathologic situations

- ✓ Bcl2 family of proteins control mitochondrial membrane permeability
- ✓ Bcl2 antiapoptotic
- ✓ Bax/Bak proapoptotic
- ✓ BH3 sensors
- ✓ Cytochrome c activates caspase-9

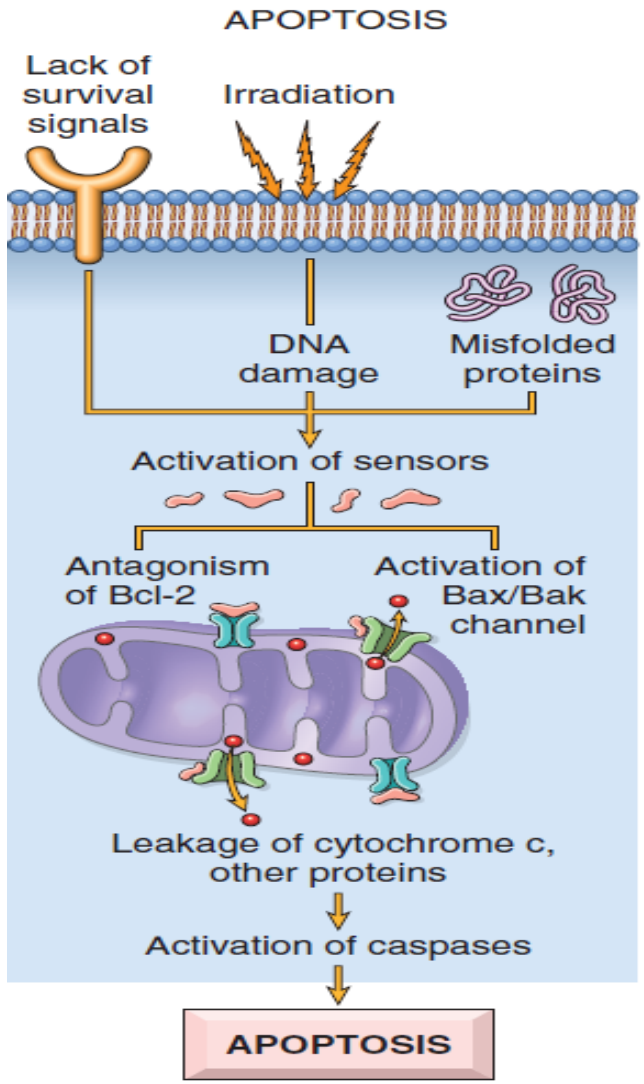
- ➢ BCL-2 is an anti-apoptotic protein that is encoded by an anti-apoptotic gene.The BCL-2 family of proteins prevents apoptosis by controlling mitochondrial membrane permeability.Inside the mitochondria, there are certain enzymes and molecules that if they leak into the cytoplasm, can trigger the activation of caspases .When the mitochondrial membrane permeability increases, these molecules can leak out and start the apoptotic process.The most important molecule of them is **cytochrome c**.Cytochrome c normally stays inside the mitochondria, away from the cytoplasm.However, if mitochondrial permeability increases, cytochrome c will leak into the cytoplasm and cause **activation of caspases**, leading to **apoptosis**.



In normal conditions, cytochrome C must remain inside the mitochondria so that the cell can survive.The BCL-2 family of proteins regulates this process.This family includes both anti-apoptotic proteins (such as BCL-2 and BCL-XL) and pro-apoptotic proteins (such as Bax and Bak).All of these proteins are located in the mitochondrial membrane.There are also BH3 sensor proteins in the cytoplasm.These sensors detect any changes or stress signals that could lead to apoptosis . **In a viable cell**, survival signals (such as growth factors and hormones) continuously reach the cell.These signals activate **anti-apoptotic proteins**, such as BCL-2, which then inhibit the Bax/Bak channels in the mitochondrial membrane .By blocking these channels, BCL-2 prevents **cytochrome C** from leaking out of the mitochondria into the cytoplasm.As a result, the cell remains alive.This mechanism represents the cell survival signal.



Once the cell is exposed to stress such as lack of survival signals (e.g. growth factors or hormones), exposure to radiation, DNA damage, or accumulation of misfolded proteins, these conditions lead to the activation of sensors in the cytoplasm (BH3 sensors).When these sensors are activated, they **inhibit the anti-apoptotic proteins** of the BCL-2 family. This inhibition allows the **pro-apoptotic proteins (BAX/BAK) to become active**. Activated BAX and BAK form channels in the mitochondrial membrane, leading to the **release of cytochrome c from the mitochondria into the cytoplasm**. This molecule then activates a group of enzymes called caspases, beginning with **caspase-9**, which in turn activates other caspases leading to Apoptosis.

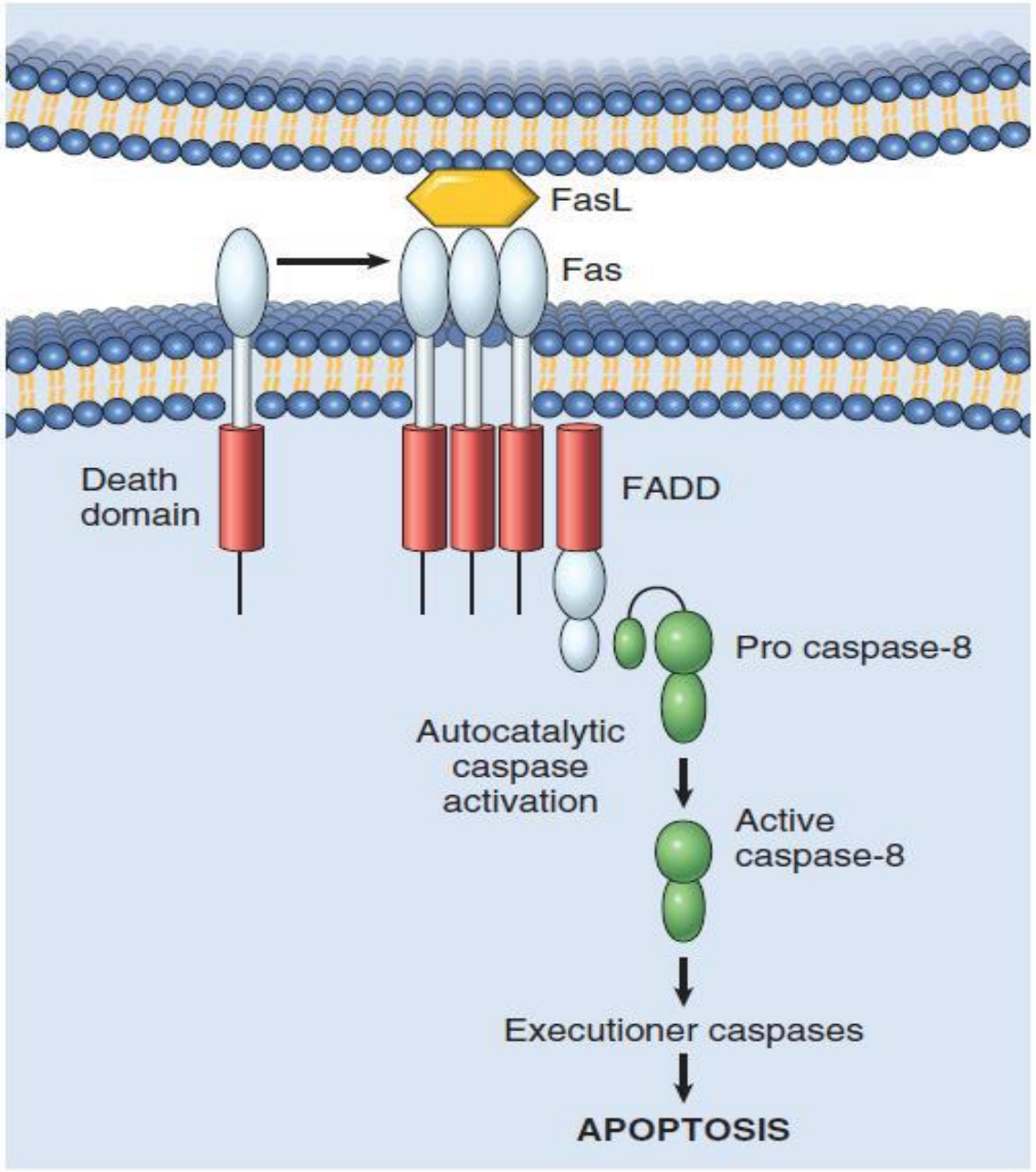


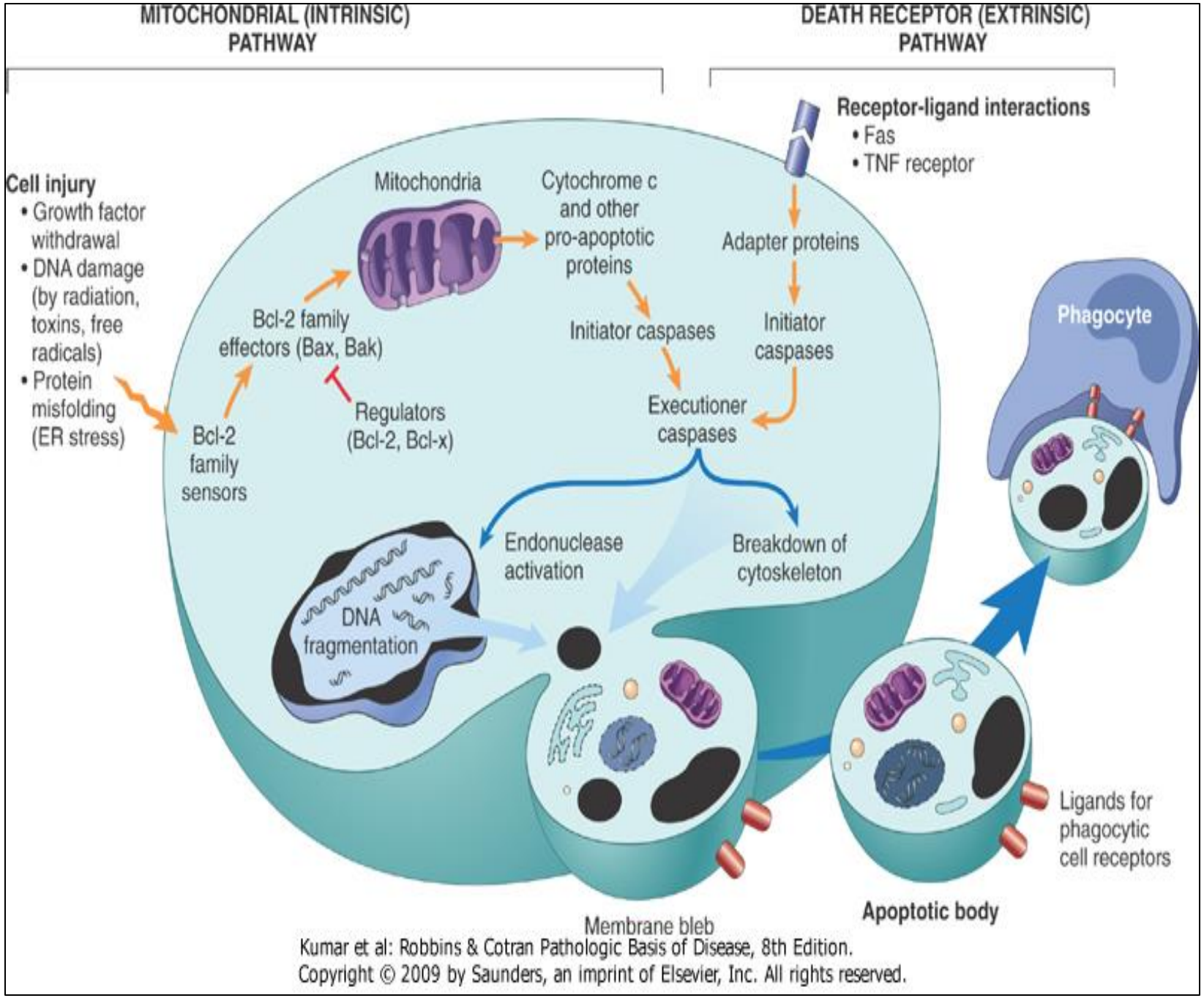
Death receptor (extrinsic) ⟹ It's a receptor located on the cell membrane and it has a domain in the cytoplasm which is called the death domain, this domain interacts with proteins inside the cell leading to activation of caspases.

- TNF receptor family (tumor necrosis factor receptors), cytoplasmic death domain
- Prototypes: Type 1 TNF receptor and Fas
- Fas ligand on activated T lymphocytes
- Fas –FasL interaction activates death domain which in turn activates caspase 8. (cytoplasmic enzyme)

- **Used in:**
- Elimination of self-reactive lymphocytes
- killing of target cells by some cytotoxic T lymphocytes (CTLs) ⟶ Target cells could be virally infected cells , tumor cells that are recognized by cytotoxic T lymphocytes.

➢TNF receptors has two types: Type 1 TNF and Fas . When the **Fas receptor binds to its ligand FasL** (present on the surface of T lymphocytes) , they interact like a lock and key mechanism.This binding activates the death domain of the Fas receptor, which in turn activates a cytoplasmic enzyme called **Caspase-8**. Activated **Caspase-8** then triggers a cascade of other caspases, eventually leading to **Apoptosis**.





Both pathways (intrinsic and extrinsic) eventually link or converge at the stage of executioner caspases activation. These caspases break down the cytoskeleton, activate endonucleases, and cause fragmentation of the DNA. At the end of the process, apoptotic bodies are formed. These apoptotic bodies will attract phagocytes with certain signal mechanisms to engulf and remove them from the tissue.

Autophagy ➡ Related to apoptosis and adaptive mechanism (atrophy)

- ✓Self-eating
- ✓Lysosomal digestion of the cells own components
- ✓Survival mechanism in times of nutrient deprivation.
- ✓Recycling cells contents to provide nutrients and energy in times of starvation .
- ✓ER-derived autophagic vacuole
- ✓Vacuole fuses with lysosome >>>autophagolysosome
- ✓May lead to atrophy.
- ✓Failure of adaptation >>>apoptosis

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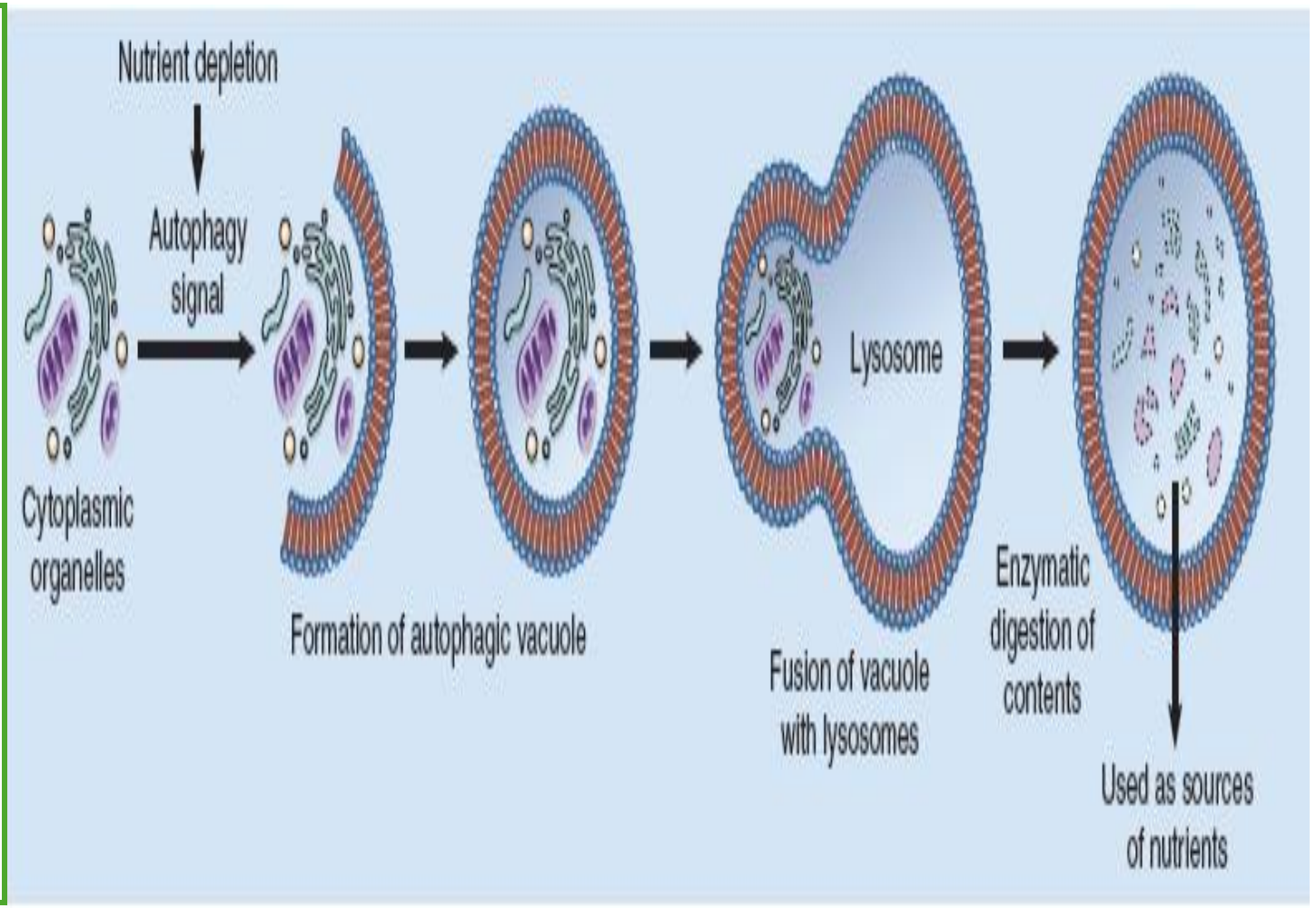
During autophagy, membrane-bound vacuoles form inside the cell.
These vacuoles are derived from ER membrane , they are called **autophagic vacuoles.**
These vacuoles will fuse with lysosomes to form **autophagolysosomes**, then the lysosomal enzymes will start the process of digestion . The end result will be shrinkage and atrophy of the cell , this is sometimes considered an adaptive mechanism. If the damage or stress continues and the cell can no longer adapt, it will eventually undergo apoptosis.



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