



APOPTOSIS

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General Pathology Grade – 4**

Lecture: 5

Week - 5

APOPTOSIS

- Apoptosis is a pathway of cell death that is induced by a tightly regulated intracellular programme in which cells destined to die activate enzymes that degrade the cells own nuclear DNA, nuclear & cytoplasmic proteins
- The cells plasma membrane remains intact
- The dead cells rapidly are cleared , before its contents have leaked out & therefore cell death by this pathway does not elicit an inflammatory reaction in the host

APOPTOSIS

- ▶ **Causes of apoptosis**
- ▶ **Physiological**
 - ▶ **Involution of hormone dependent tissues – ovarian follicular atresia in menopause and regression of lactating breast after weaning**
 - ▶ **Elimination of self reactive lymphocytes**
 - ▶ **Death of the host immune cells after acute and chronic inflammation**
- ▶ **Pathological**
 - ▶ **DNA damage by radiation or cytotoxic anticancer drugs**
 - ▶ **Accumulation of misfolded proteins**
 - ▶ **In Viral infections such as HIV**

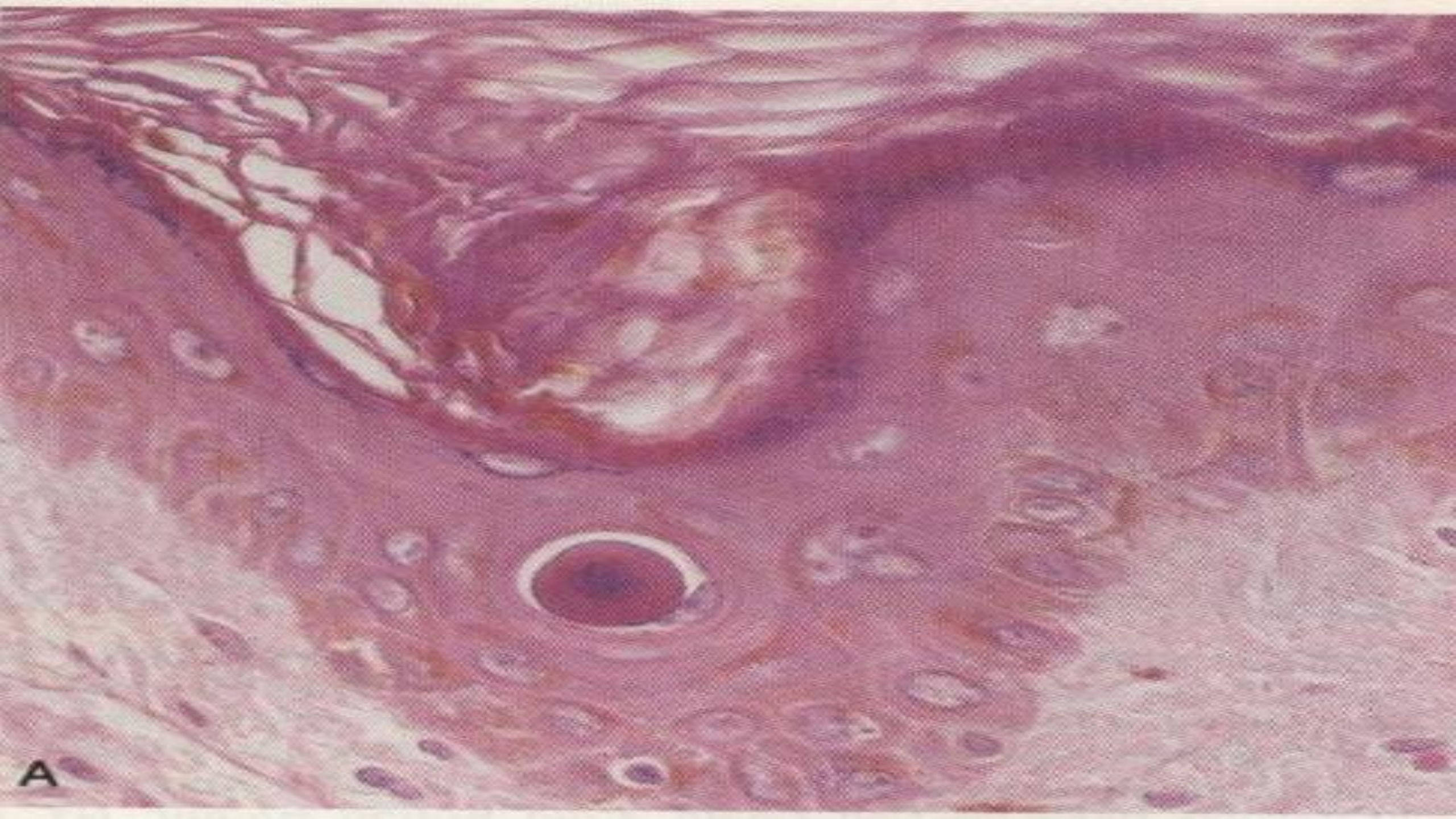
APOPTOSIS

MORPHOLOGY

- Cell shrinkage
- Chromatin condensation
- Formation of nuclear blebs & apoptotic bodies
- Phagocytosis of apoptotic cells or cell bodies usually by macrophages

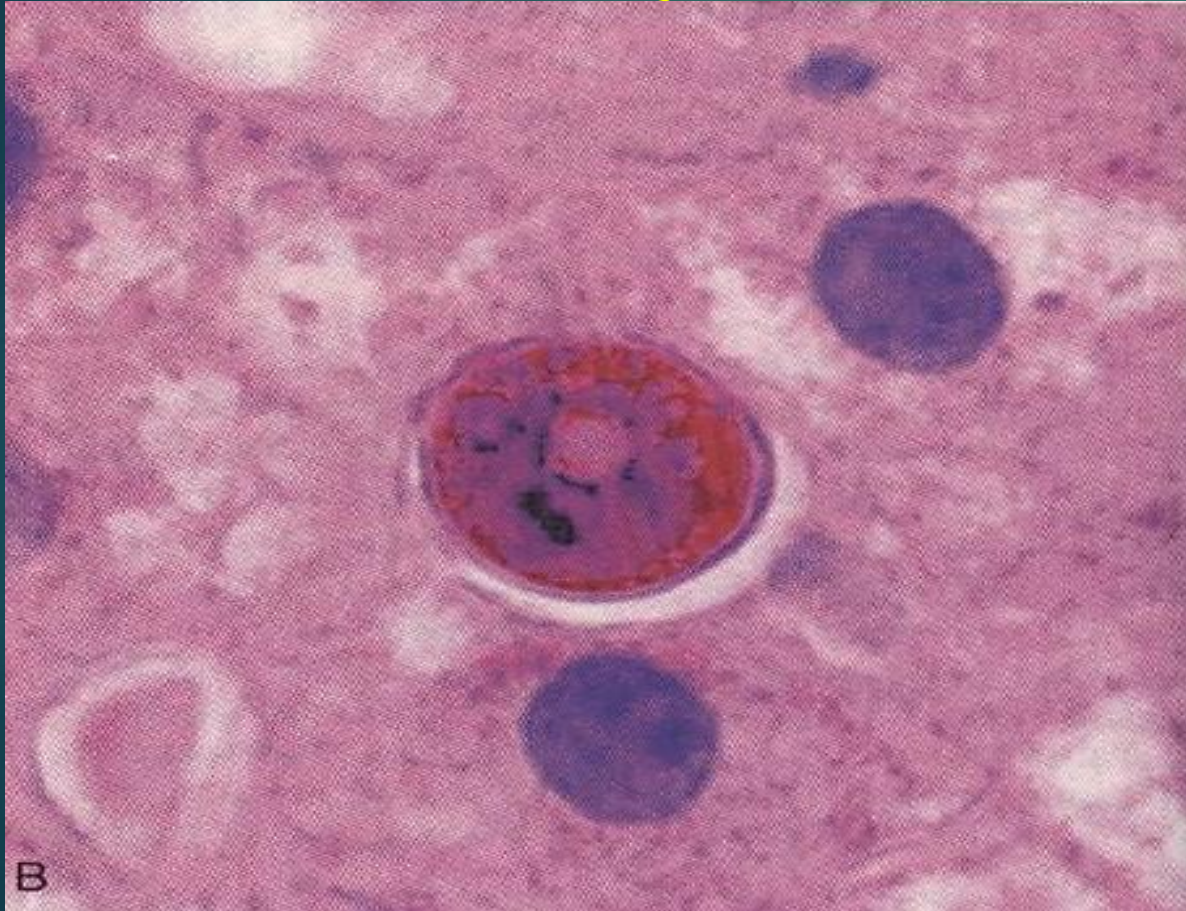
Histologic examination

Tissues stained with H & E show the apoptotic cell as round or oval mass of intensely eosinophilic cytoplasm with dense nuclear chromatin pattern

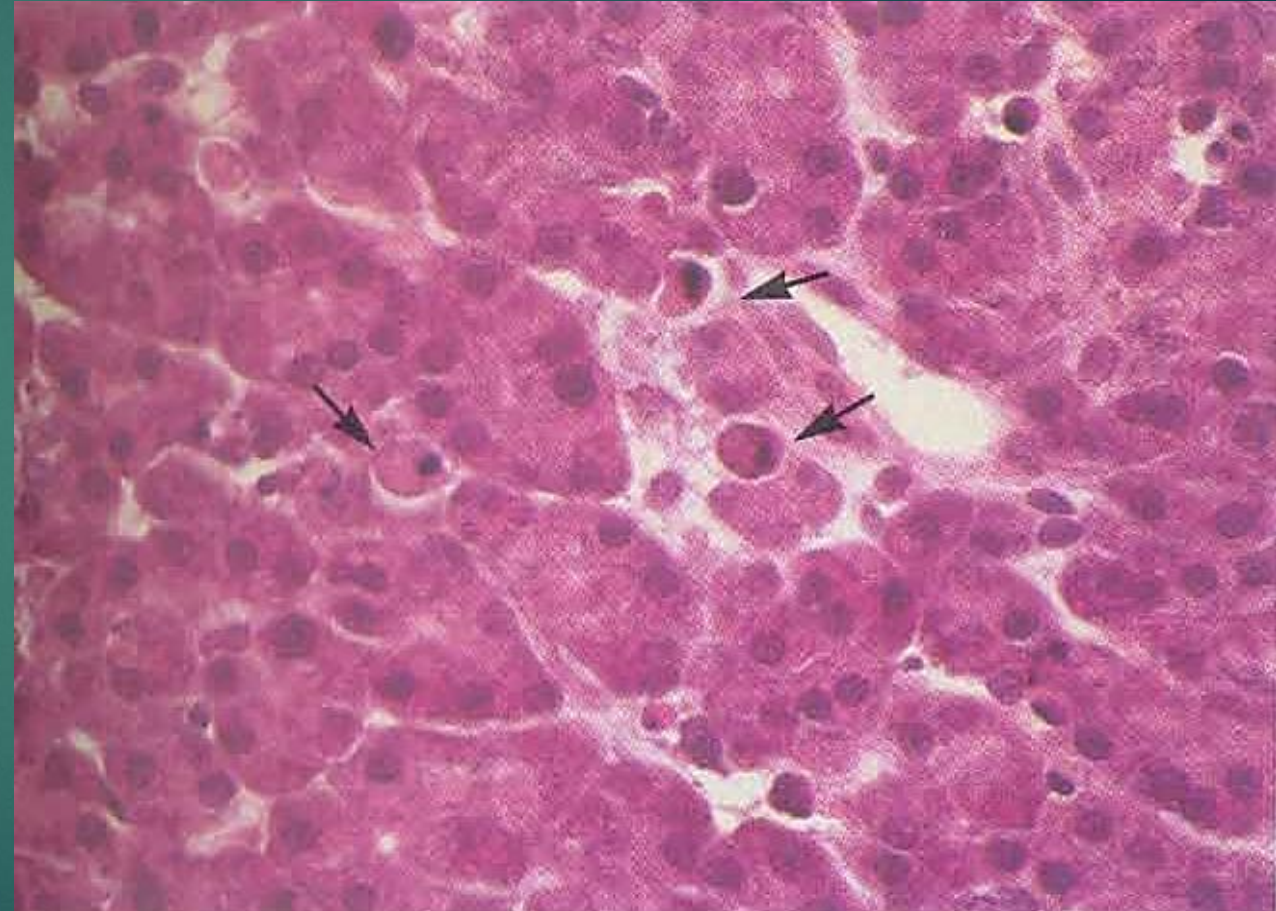


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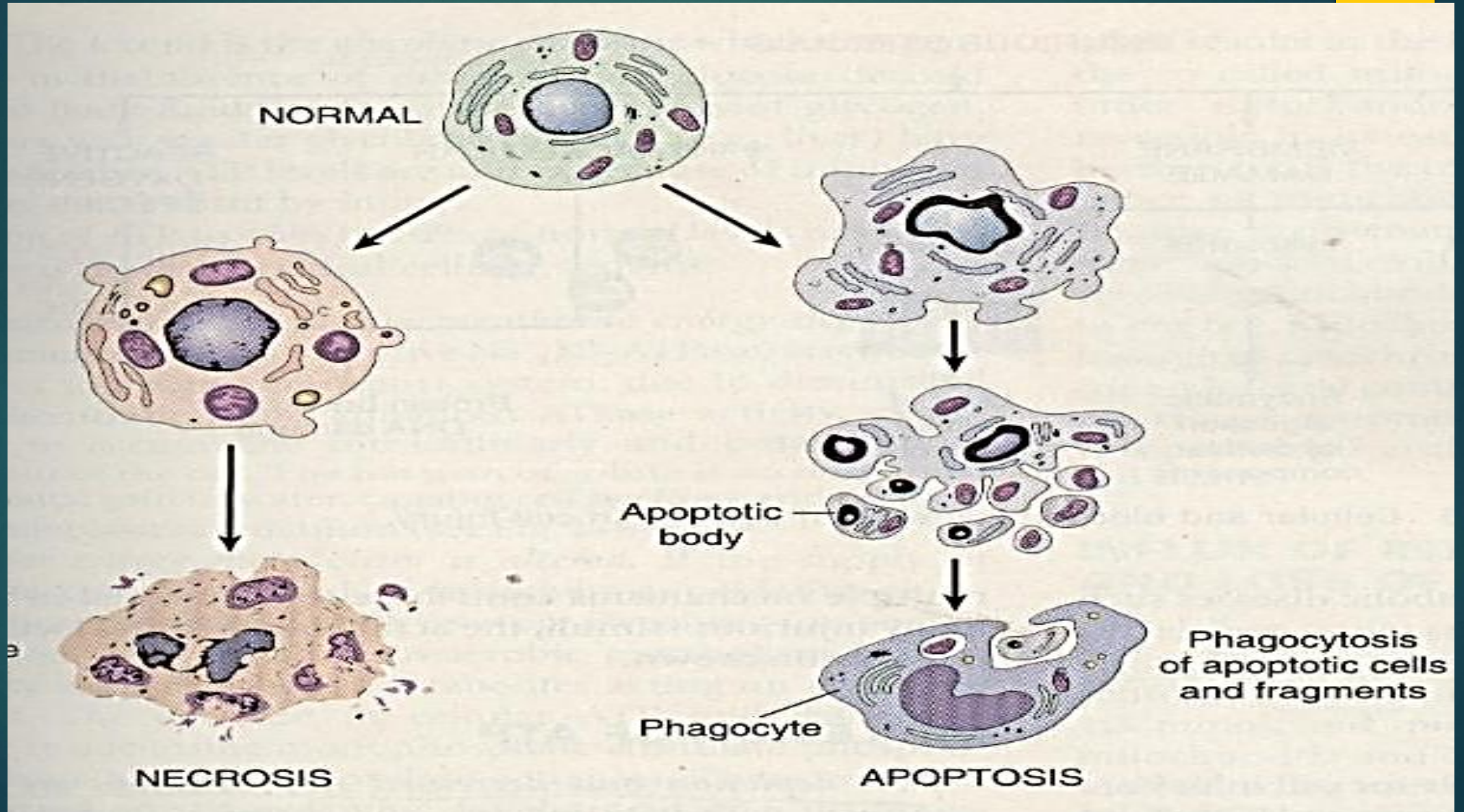
Apoptotic cell in Liver injury



Apoptotic bodies in pancreas



DIFFERENCES BETWEEN NECROSIS AND APOPTOSIS



| | APOPTOSIS | NECROSIS |
|---------------------------------------|--|---|
| | Apoptosis is programmed cell death | Necrosis is premature cell death |
| Mechanism | Activation of caspases, endonucleases and proteases | Depletion of ATP, membrane injury and free radical injury |
| Causative agents | Mostly Physiological (can be pathological) | Pathological caused by toxins or hypoxia causing cell injury |
| Number of cells involved | Individual cells | Group of contagious cells |
| Size of the cell | Cell shrinkage | Cell swelling |
| Nucleus | Chromatin condensation | Nuclear disruption |
| Cell membrane | No rupture | Rupture of cell membrane |
| Formation of apoptotic bodies | Present | Absent |
| Inflammatory reaction | Absent | Present |
| Cell organelles like lysosomes | Intact | Break down with liberation of hydrolytic enzymes |

BIOCHEMICAL FEATURES APOPTOSIS

Biochemical modifications seen in apoptotic cells are –

- ▶ Protein cleavage
- ▶ Protein cross linking
- ▶ DNA break down
- ▶ Phagocytic recognition

BIOCHEMICAL FEATURES APOPTOSIS

PROTEIN CLEAVAGE

- ▶ Protein cleavage occurs mainly by enzymes called “Caspases”
- ▶ Caspases are present as proenzyme form which when activated activates other caspases and initiates protease cascade.
- ▶ Some procaspases can aggregate and gets autoactivated
- ▶ Activated caspases activate other caspases amplifying the apoptotic signaling pathway and leads to rapid cell death.

BIOCHEMICAL FEATURES APOPTOSIS

PROTEIN CLEAVAGE

CASPASES

- ▶ Caspase belong to the family of cysteine proteases
- ▶ Term caspase is based on two properties of this family of enzymes
- ▶ The “C” refers to a cysteine protease i,e an enzyme with cysteine in its active site and “aspase” refers to the unique ability of these enzymes to cleave after aspartic acid residues

BIOCHEMICAL FEATURES APOPTOSIS

PROTEIN CLEAVAGE

CASPASES

- **10 major caspases have been identified and are broadly categorized into**
 - **Initiators – caspases 2, 8, 9,10**
 - **Effectors or executioners – caspases 3, 6, 7**

BIOCHEMICAL FEATURES APOPTOSIS

PROTEIN CROSS LINKING

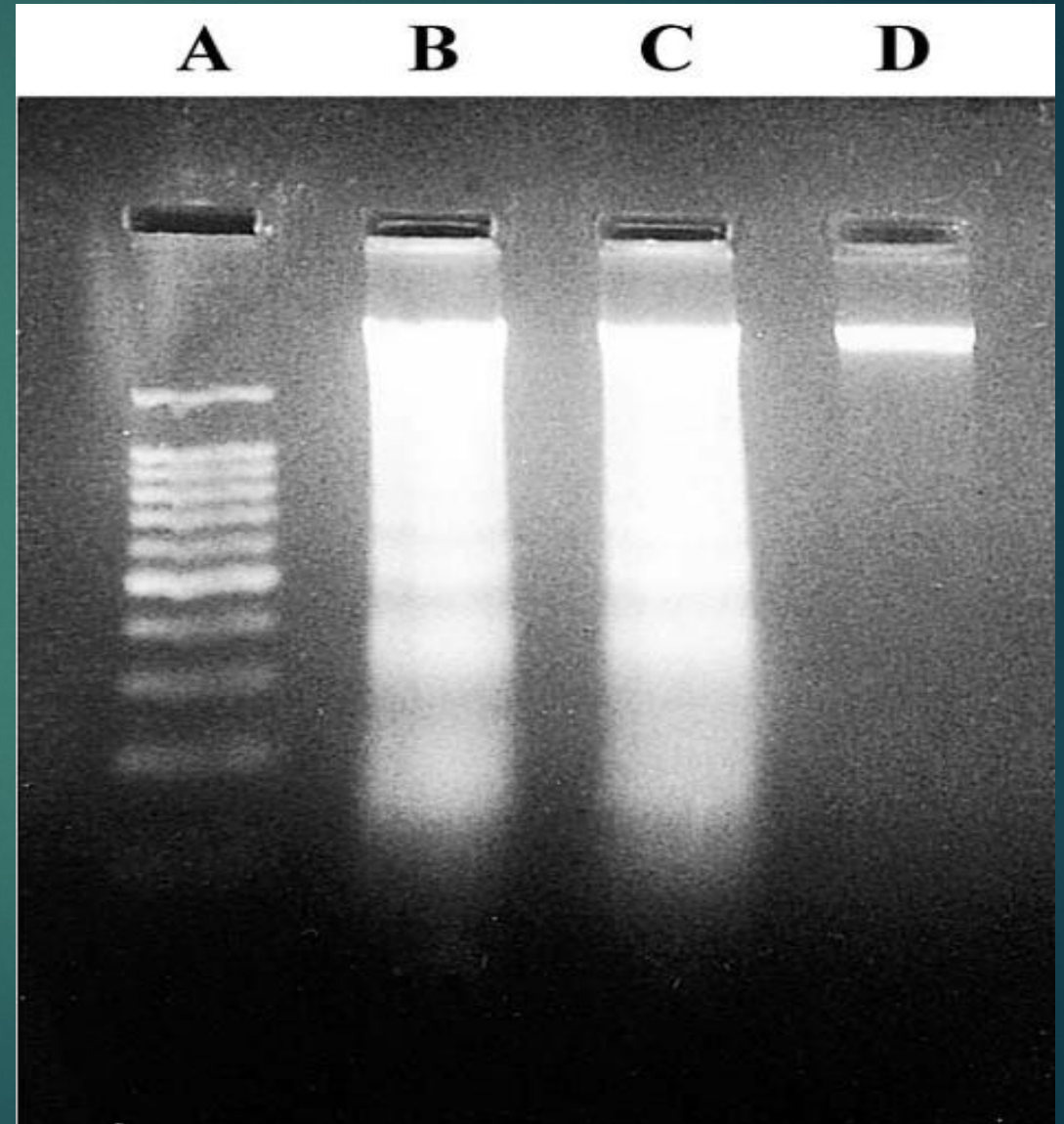
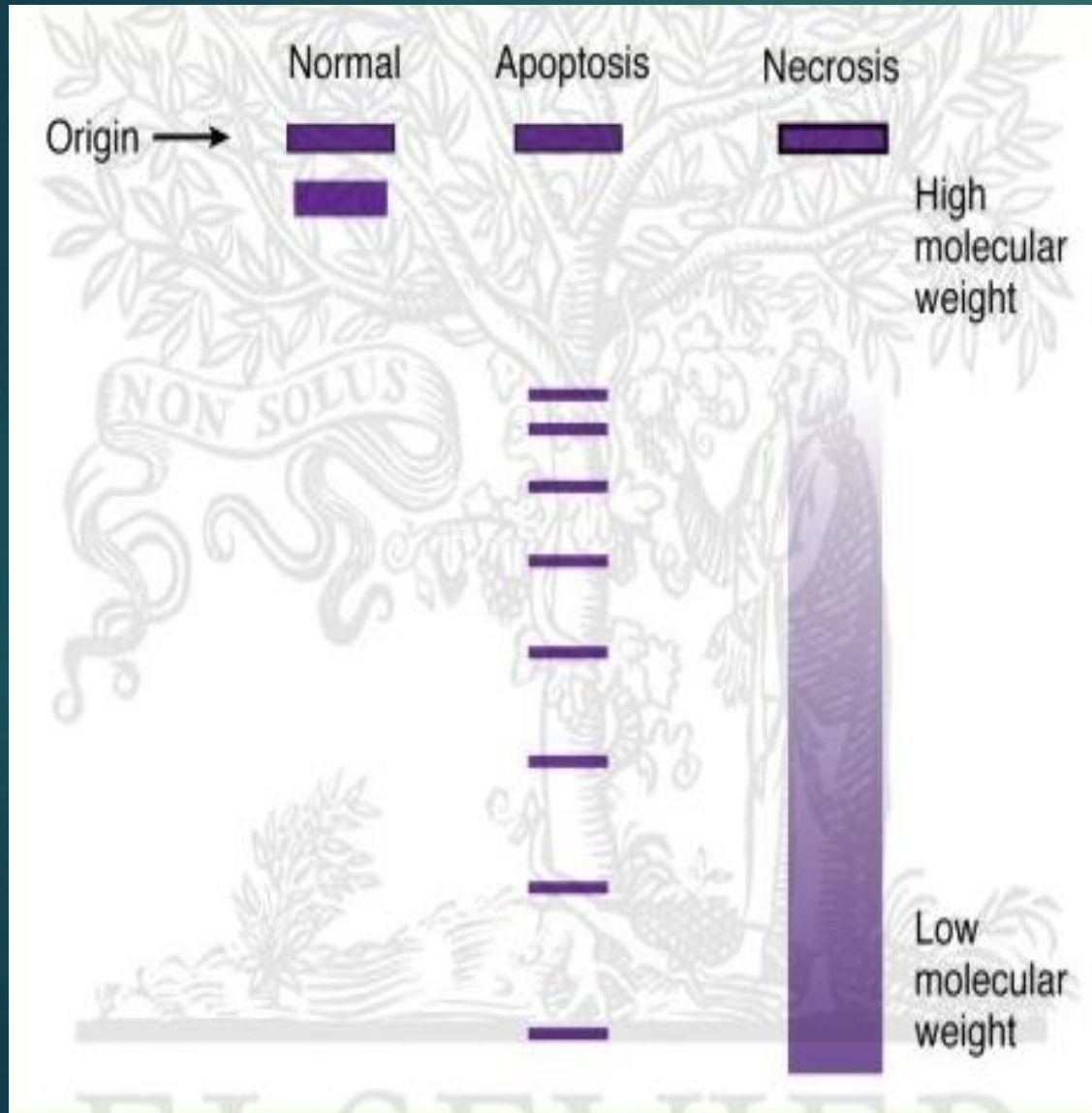
- ▶ Extensive protein cross linking is another characteristic of apoptotic cell and is achieved through the expression and activation of **tissue transglutaminase**

BIOCHEMICAL FEATURES APOPTOSIS

DNA BREAKDOWN

- DNA breakdown occurs by Ca and Mg dependent **endonucleases** resulting in DNA fragments of 180 to 200 base pairs
- The fragments may be visualized by electrophoresis as DNA **“ladders”** with an ethidium bromide stain and ultraviolet illumination
- A **“smeared”** pattern of DNA fragmentation is thought to be indicative of necrosis.

DNA ELECTROPHORESIS - APOPTOSIS



BIOCHEMICAL FEATURES OF APOPTOSIS

MEMBRANE ALTERATION

- Apoptotic cells are identified by the phagocytes due to expression of cell surface markers
- This is achieved by the movement of normal inward-facing **phosphatidyl serine** of the cells lipid bilayer to expression on the outer layers of the plasma membrane
- Though phosphatidyl serine is well known recognition ligand, other cell surface markers are **thrombospondin, Annexin 1 and Calreticulin**

MORPHOLOGY OF CELL INJURY

APOPTOSIS

Mechanism of Apoptosis

The process of Apoptosis can be divided into

- Initiation phase – during which Caspases become catalytically active*
- Execution phase – during which enzymes act to cause cell death*

Initiation of apoptosis occurs by two convergent pathways

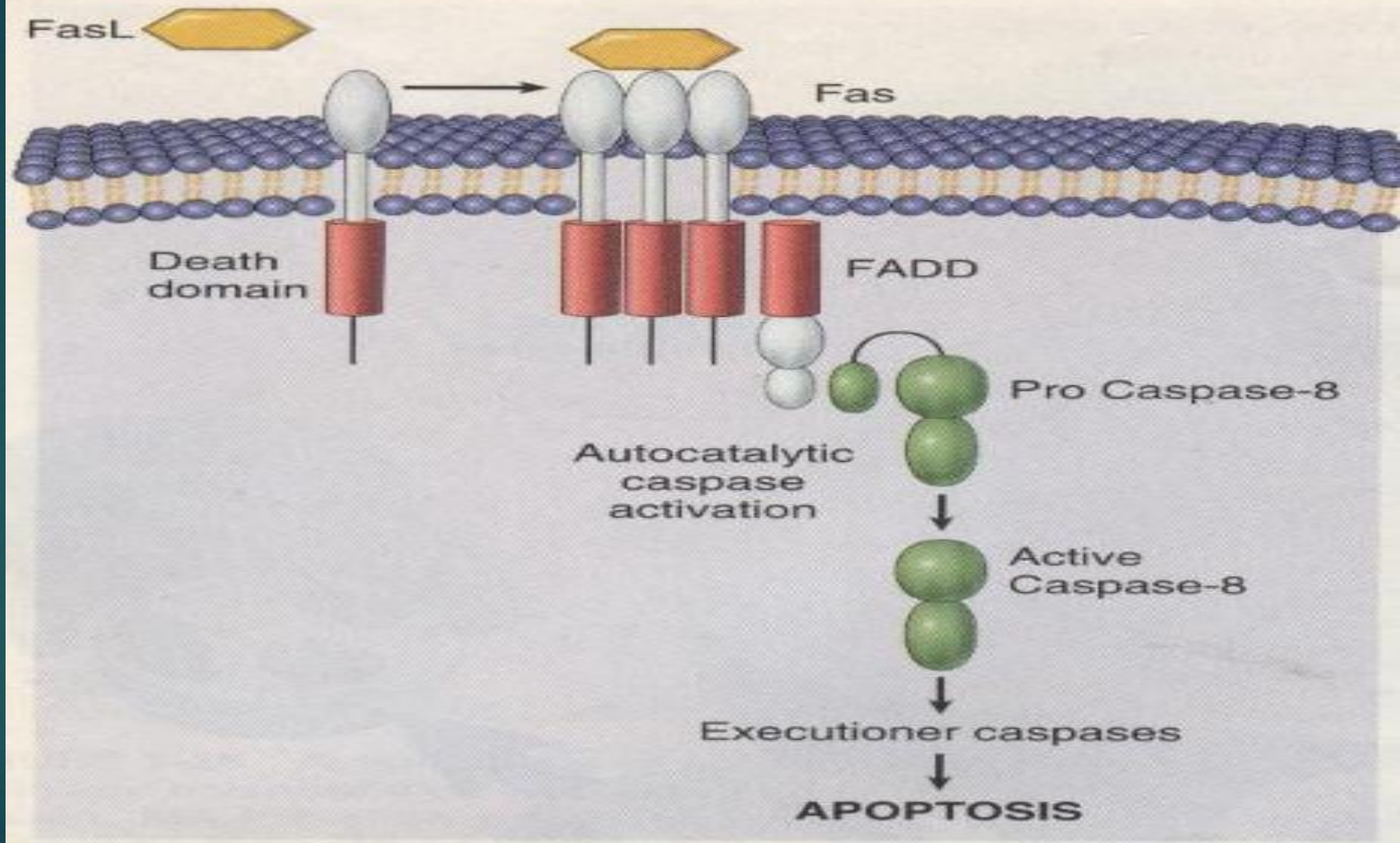
- the extrinsic receptor initiated pathway*
- the intrinsic , or mitochondrial pathway*

MECHANISM OF APOPTOSIS

EXTRINSIC (DEATH RECEPTOR INITIATED) PATHWAY

- ▶ *When the Fas is cross linked to its ligand i,e Fas L, 3 or more molecules of Fas come together and their cytoplasmic death domains form binding site for an adapter protein FADD and it also has death domain.*
- ▶ *FADD in turn binds to inactive form of Caspase 8 via dimerization of the death effector domain*
- ▶ *Formation of this death inducing signaling complex (DISC) results in autocatalytic activation of procaspase-8*
- ▶ *Once caspase -8 is activated, the execution phase of apoptosis is triggered*

THE EXTRINSIC PATHWAY



MECHANISM OF APOPTOSIS

INTRINSIC (DEATH RECEPTOR INITIATED) PATHWAY

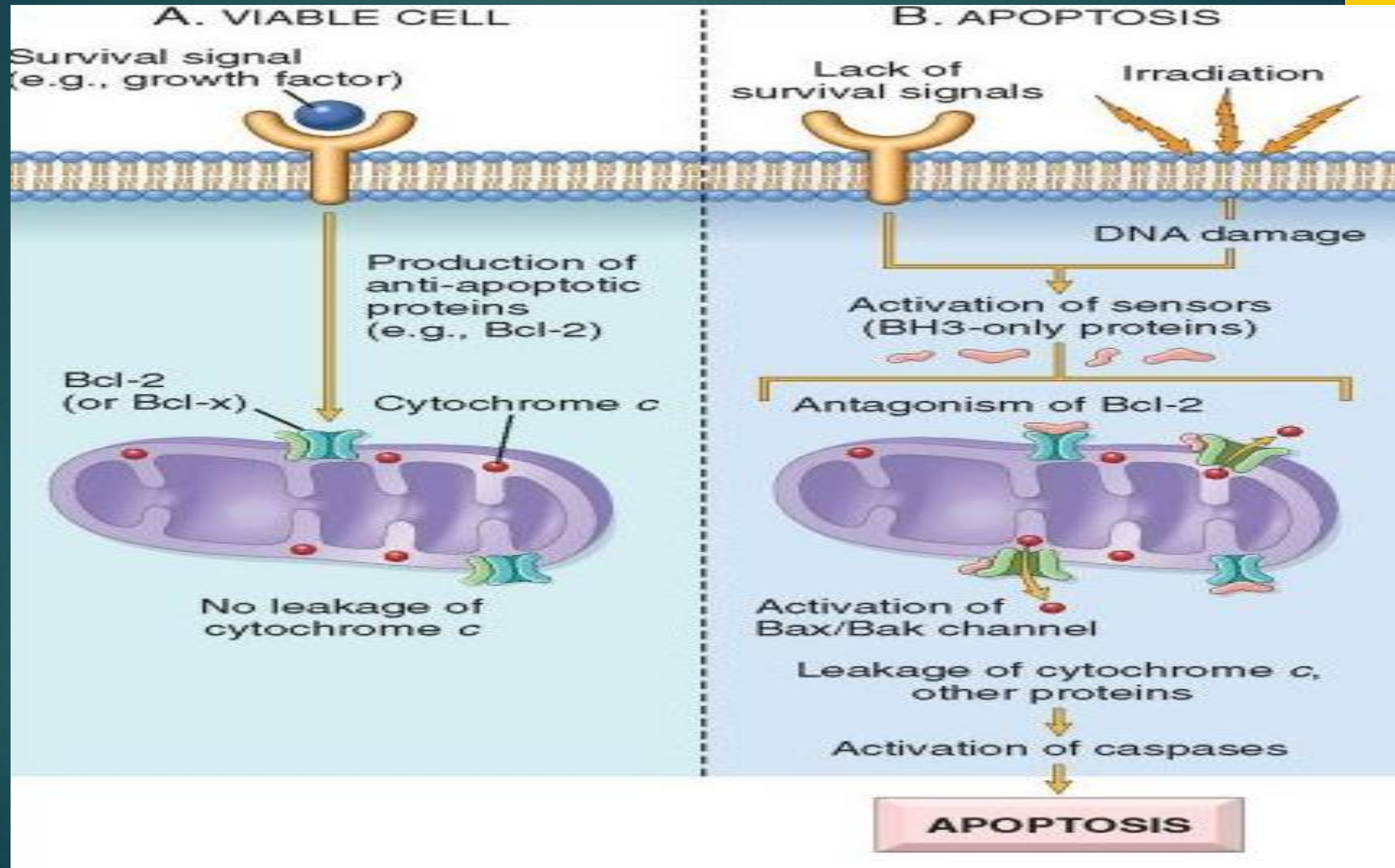
- ▶ *All these stimuli cause changes in the inner mitochondrial membrane resulting in*
 - ▶ *Opening of the mitochondrial permeability transition (MPT) pore*
 - ▶ *Loss of the mitochondrial transmembrane potential*
 - ▶ *Release of two main groups of normal sequestered proapoptotic proteins from the intermembrane space into the cytosol*

MECHANISM OF APOPTOSIS

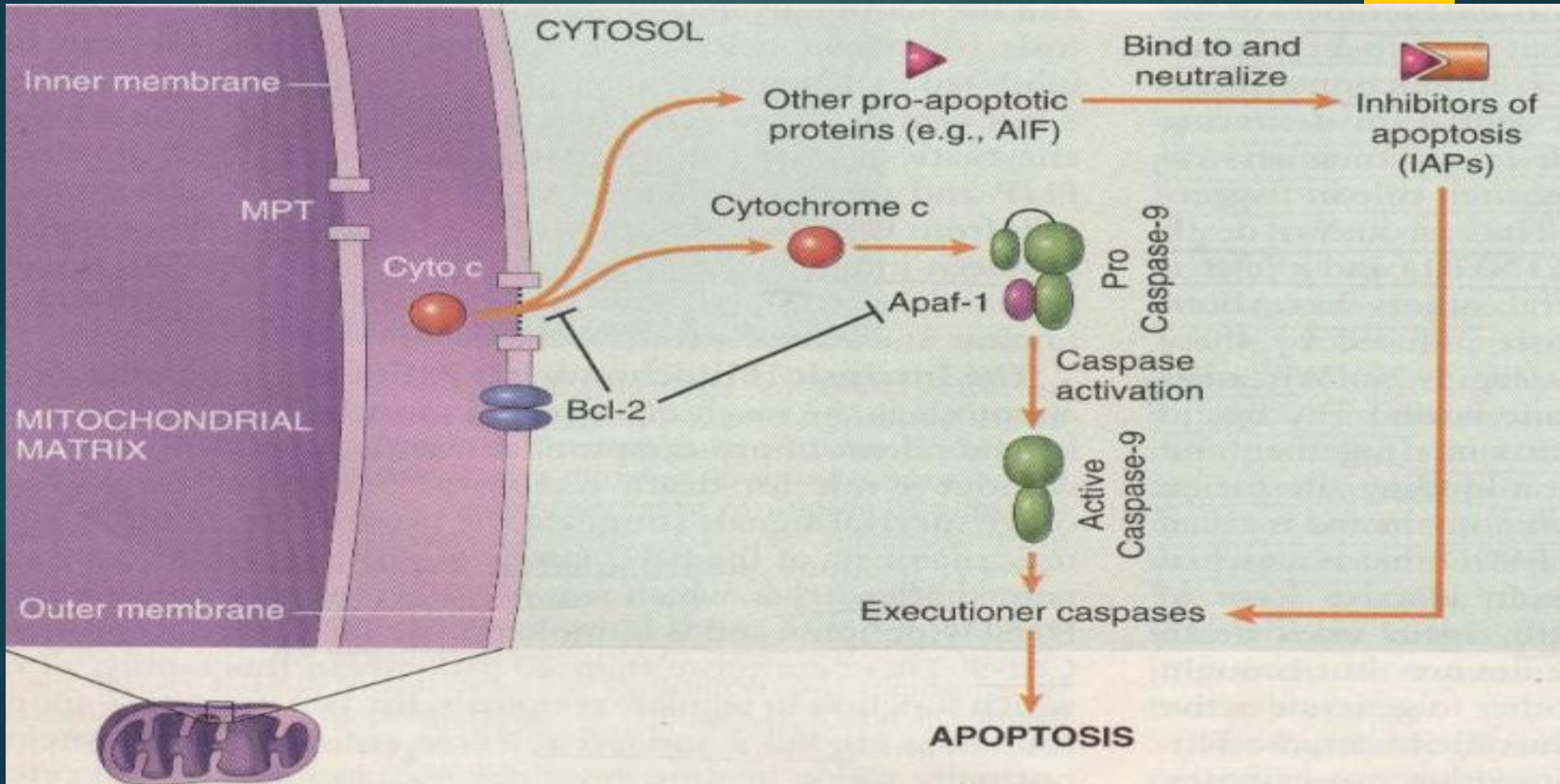
INTRINSIC (DEATH RECEPTOR INITIATED) PATHWAY

- ▶ *Control and regulation of these apoptotic mitochondrial events occurs through the members of the Bcl-2 family of proteins*
- ▶ *Tumor suppressor protein p53 has a critical role in regulation of the Bcl-2 family . They can be either anti-apoptotic or pro-apoptotic.*
- ▶ *Anti apoptotic proteins include – Bcl-2, Bcl-x, Bcl-XL, Bcl-XS, Bcl-W, BAG*
- ▶ *Pro apoptotic proteins include – Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, Blk.*

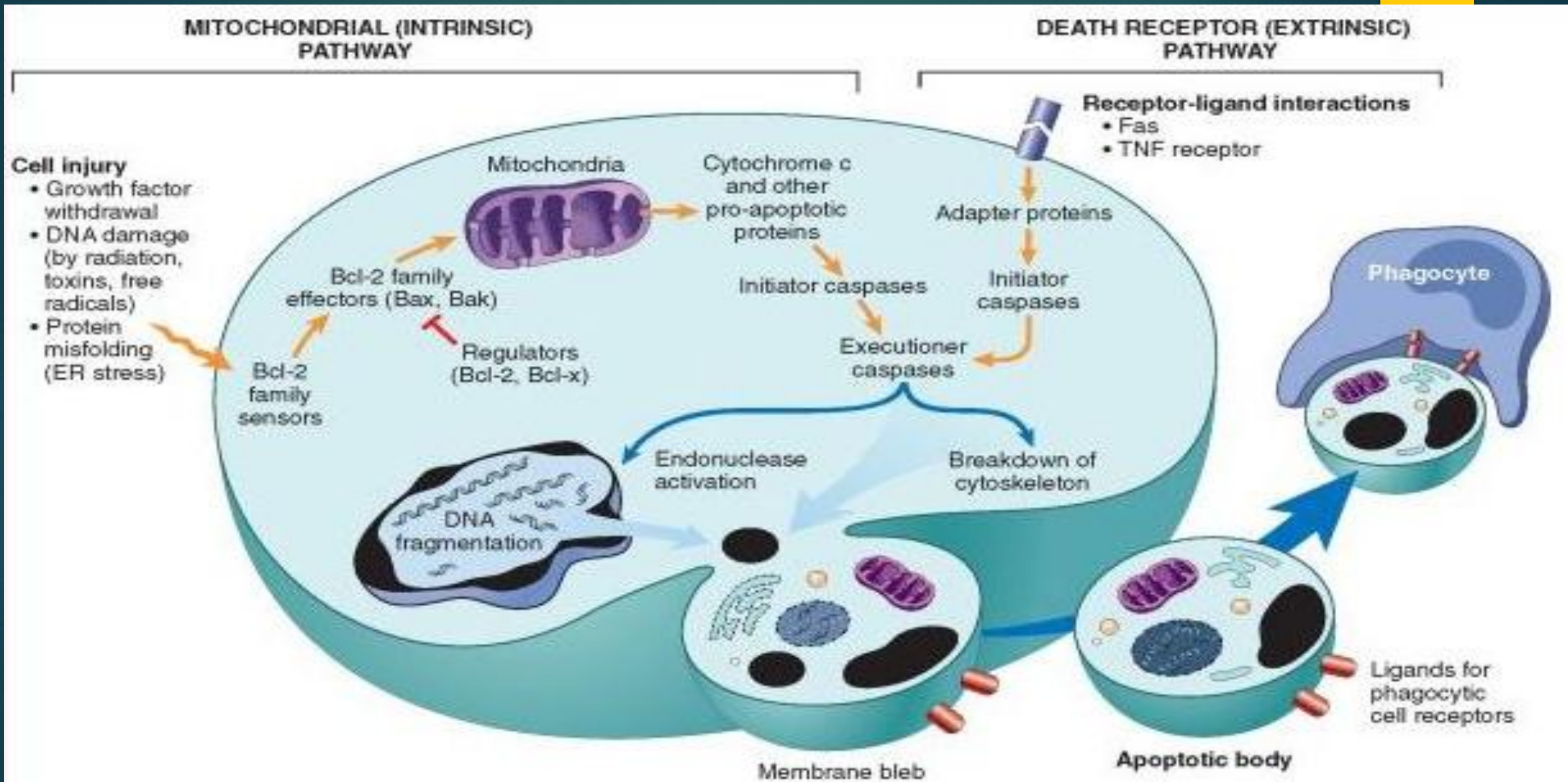
INTRINSIC PATHWAY



THE INTRINSIC PATHWAY



APOPTOSIS



THANK YOU