APOPTOSIS

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Faculty of Applied Sciences Summer Semester
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

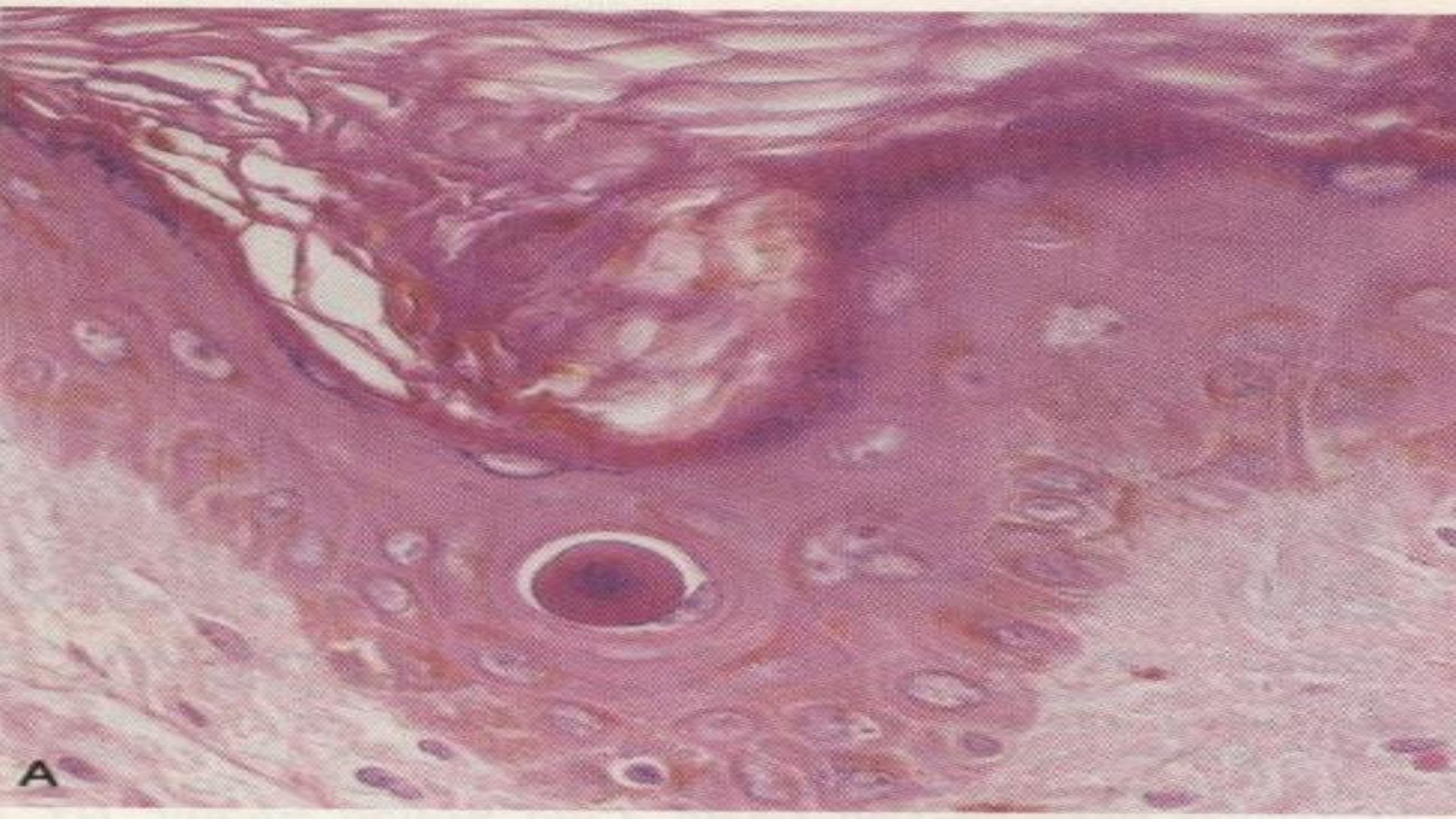
APOPTOSIS

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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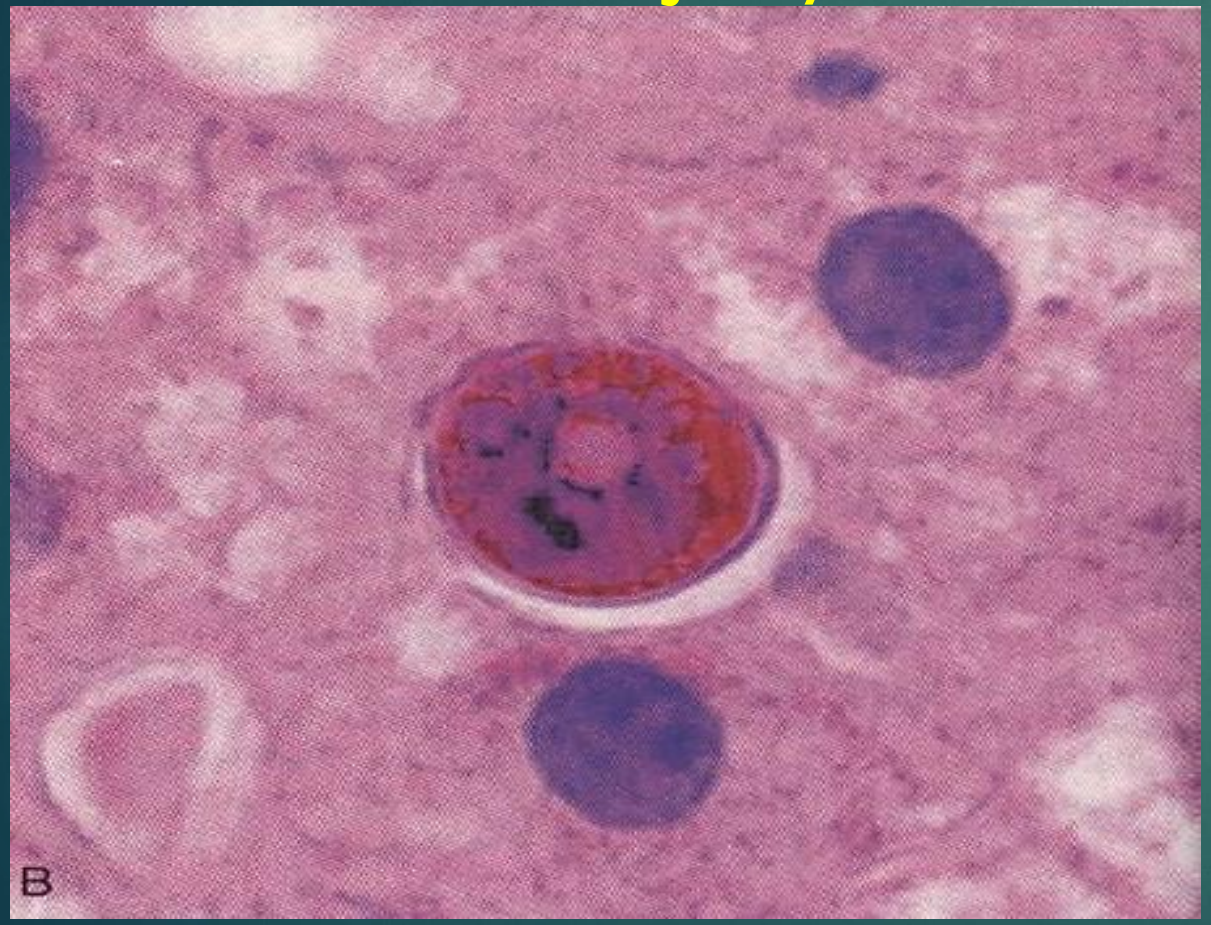
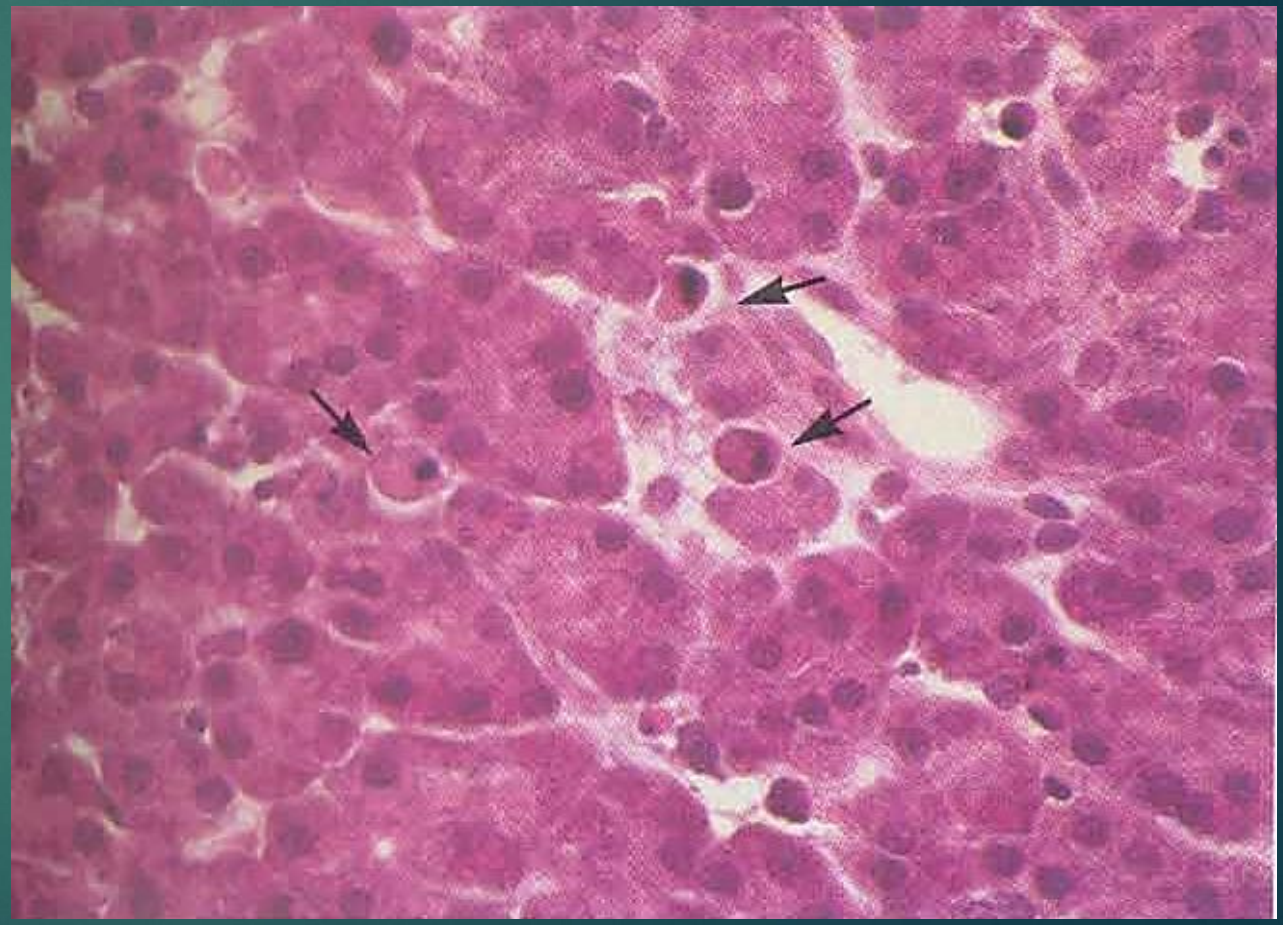
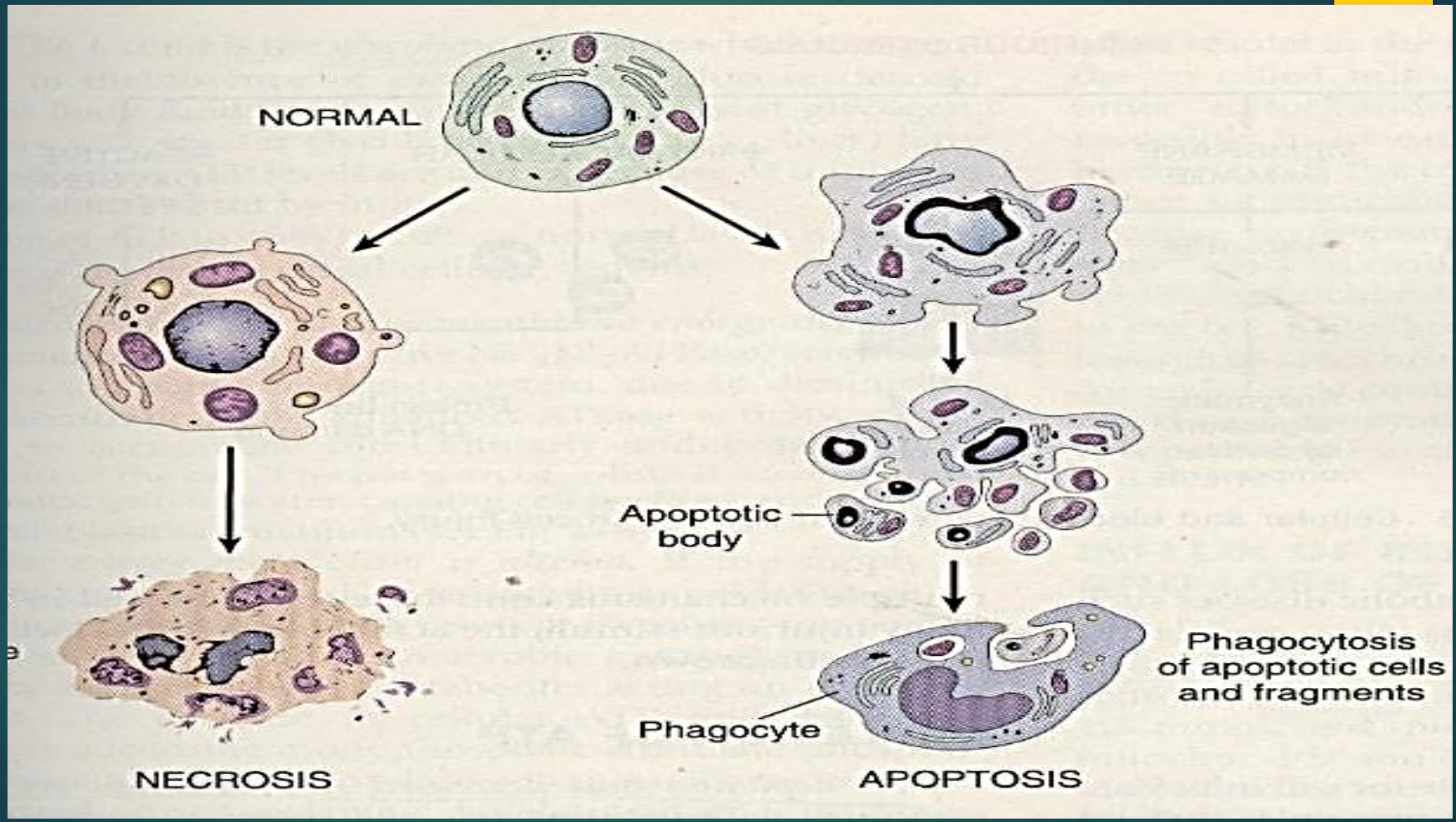
Apoptotic cell in Liver injury



Apoptotic bodies in pancreas



DIFFERENCES BETWEEN NECROSIS AND APOPTOSIS



	APOPTOSIS	NECROSIS
	Apoptosis if 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

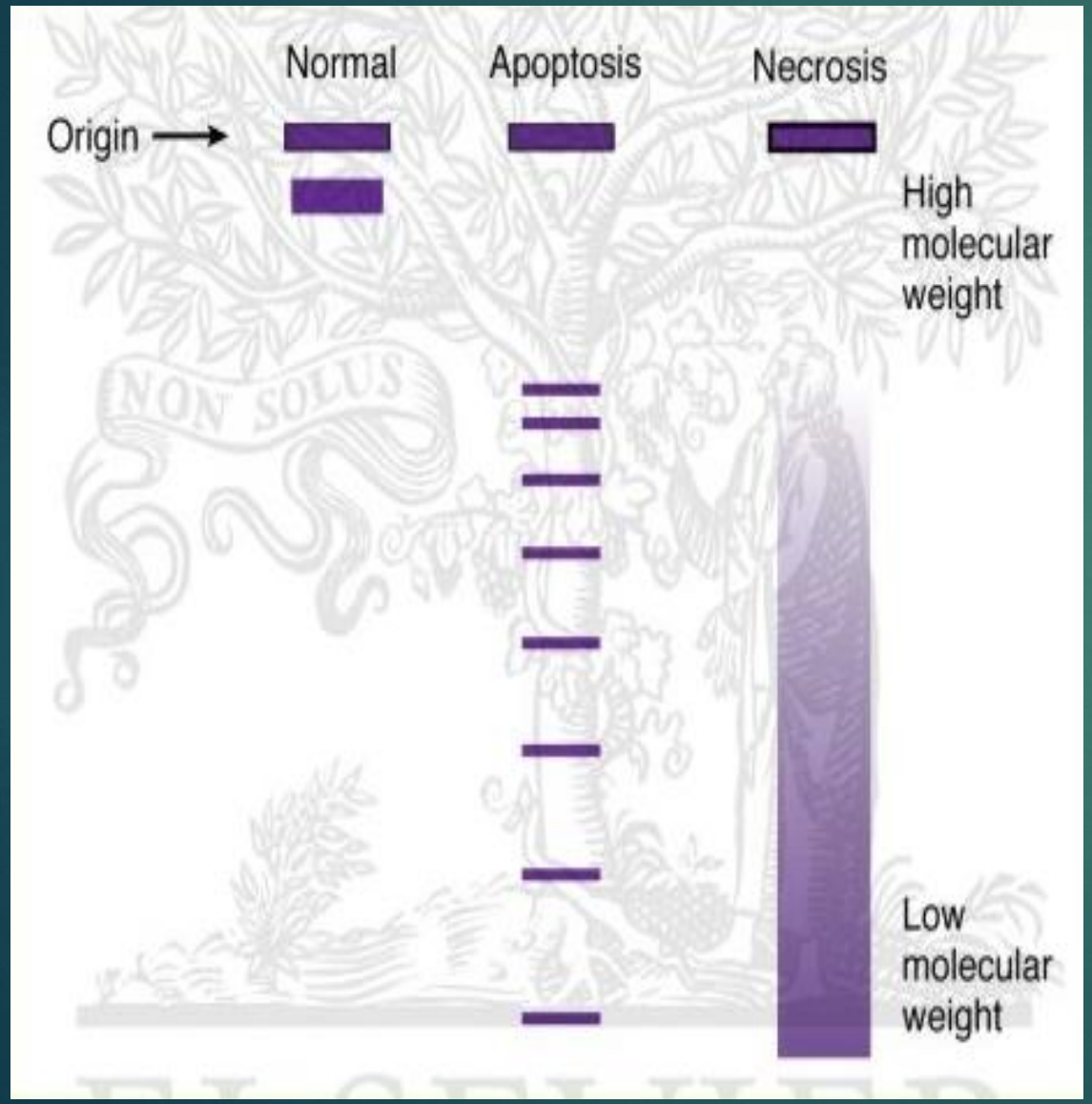
- Extensive protein cross linking is another characteristic of apoptotic cell and is achieved through the expression and activation of tissue transglutaminase

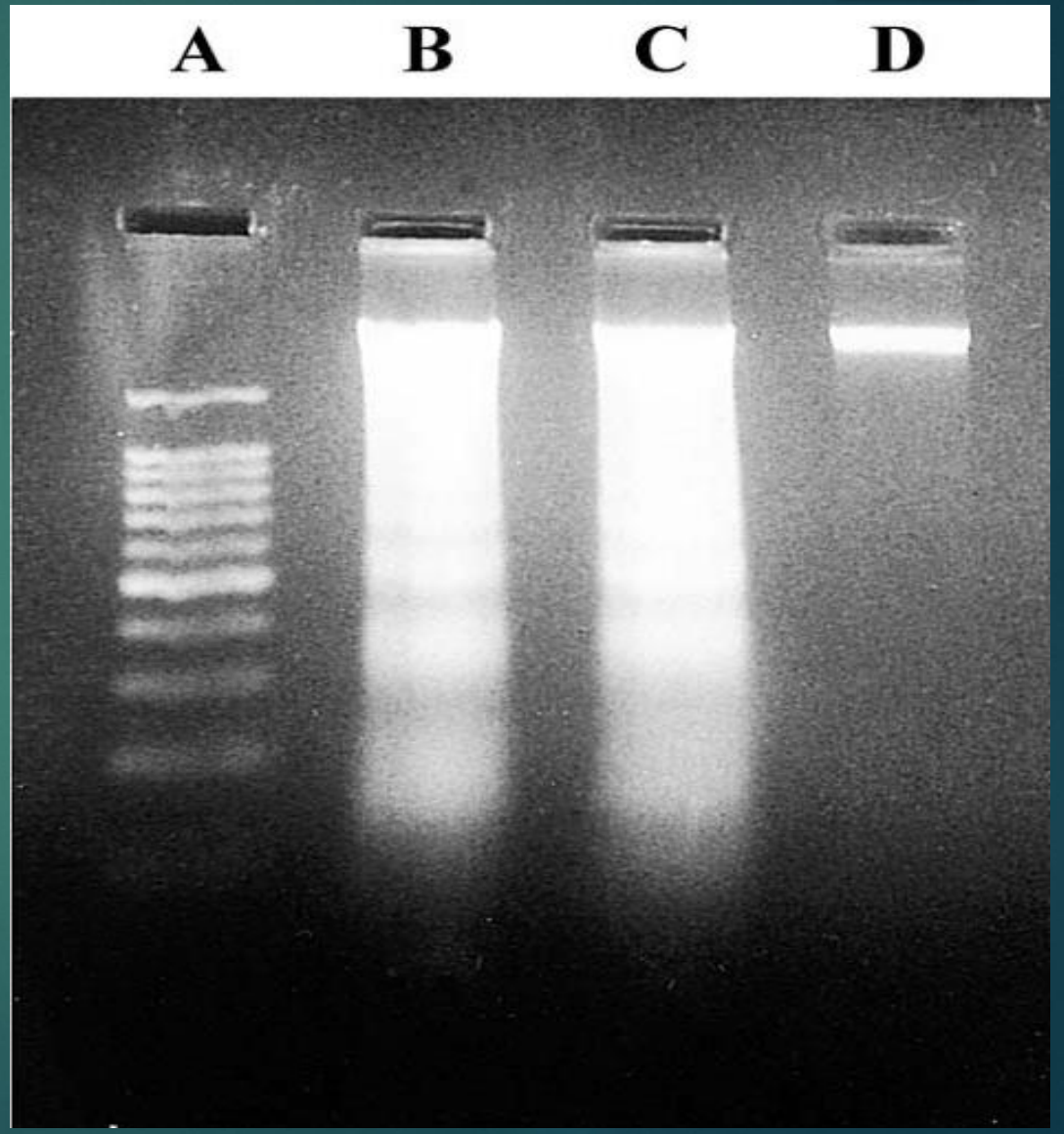
BIOCHEMICAL FEATURES APOPTOSIS

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 phosphotidyl 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 pathway

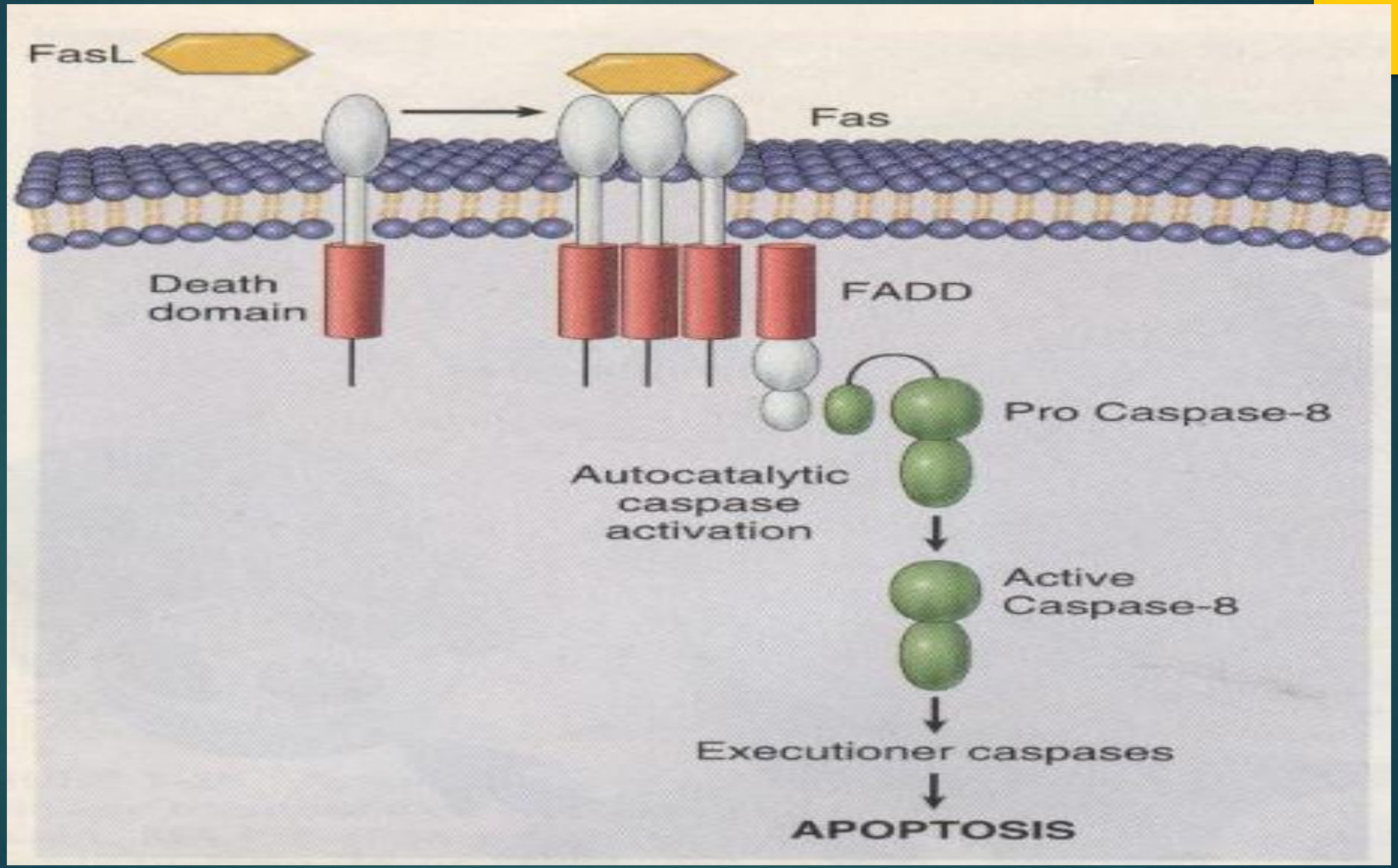
- ***the extrinsic receptor initiated pathway***
- ***the intrinsic , or mitochondrial pathway***

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

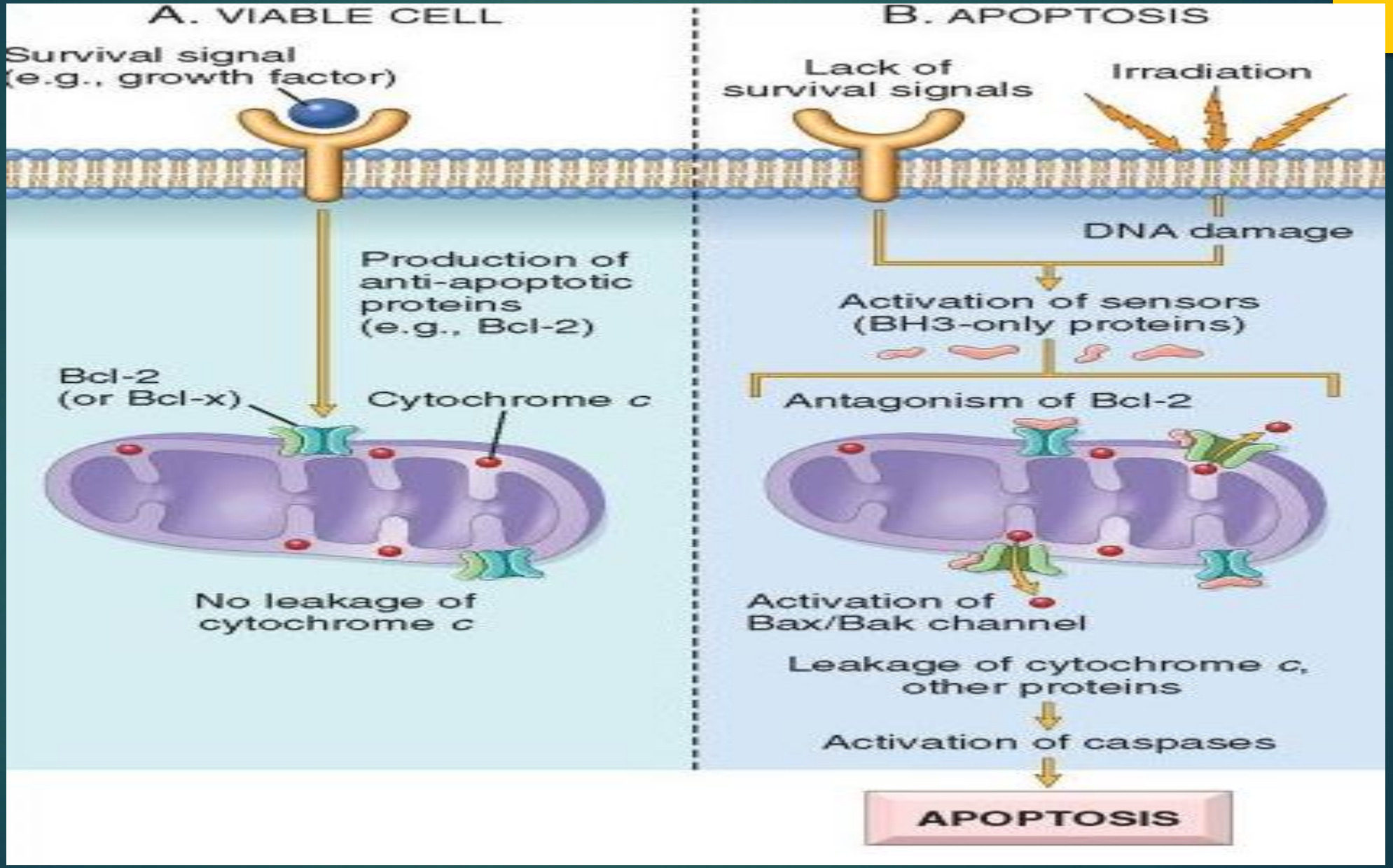
- *All these stimuli cause changes in the inner mitochondrial membrane resulting in*
  - *Opening of the mitochondrial permeability transition (MPT) pore*
  - *Loss of the mitochondrial transmembrane potential*
  - *Release of two main groups of normal sequestered proapoptotic proteins from the intermembrane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

MECHANISM OF APOPTOSIS

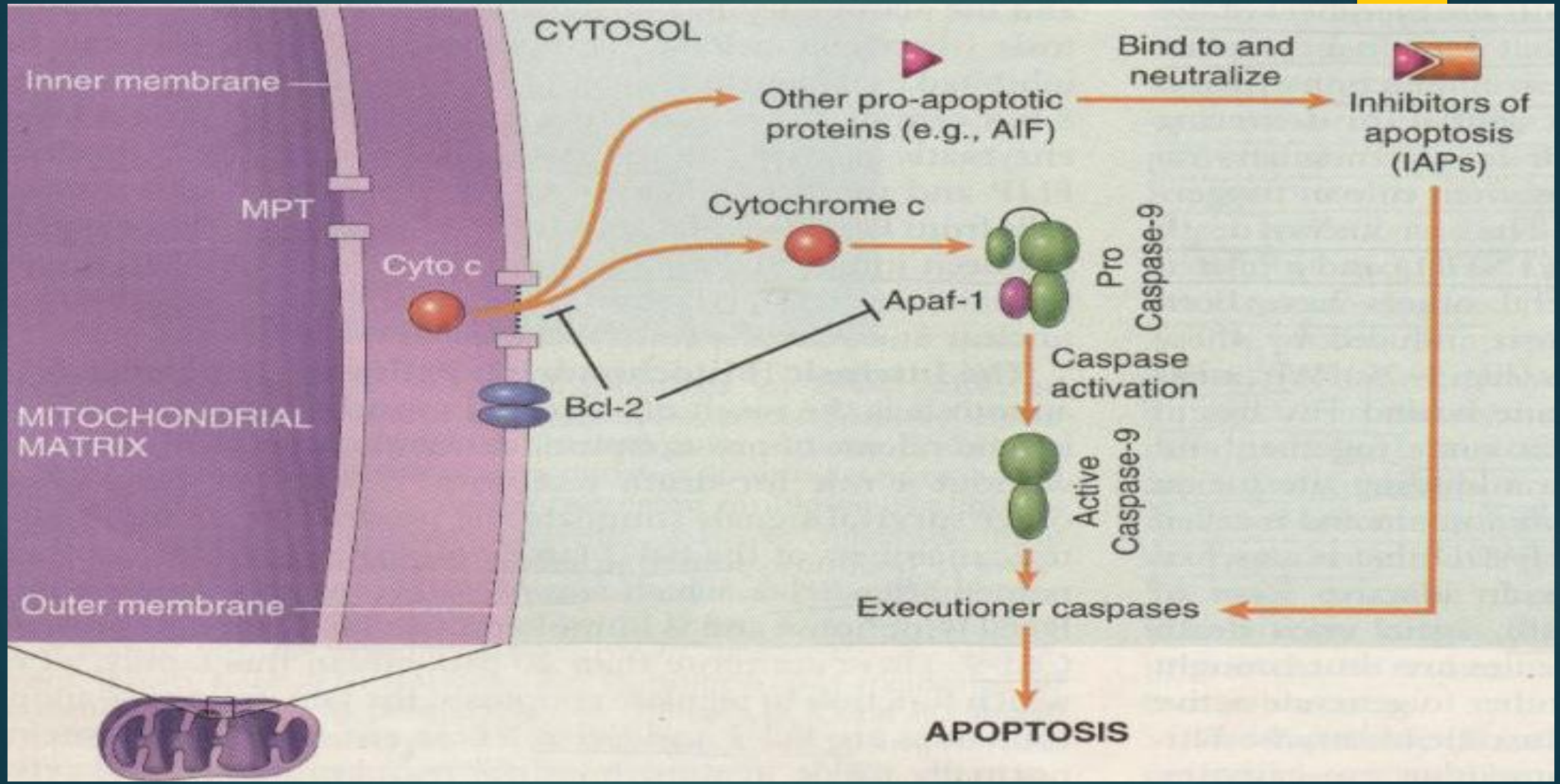
INTRINSIC (DEATH RECEPTOR INITIATED) PATHWAY

- *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.*

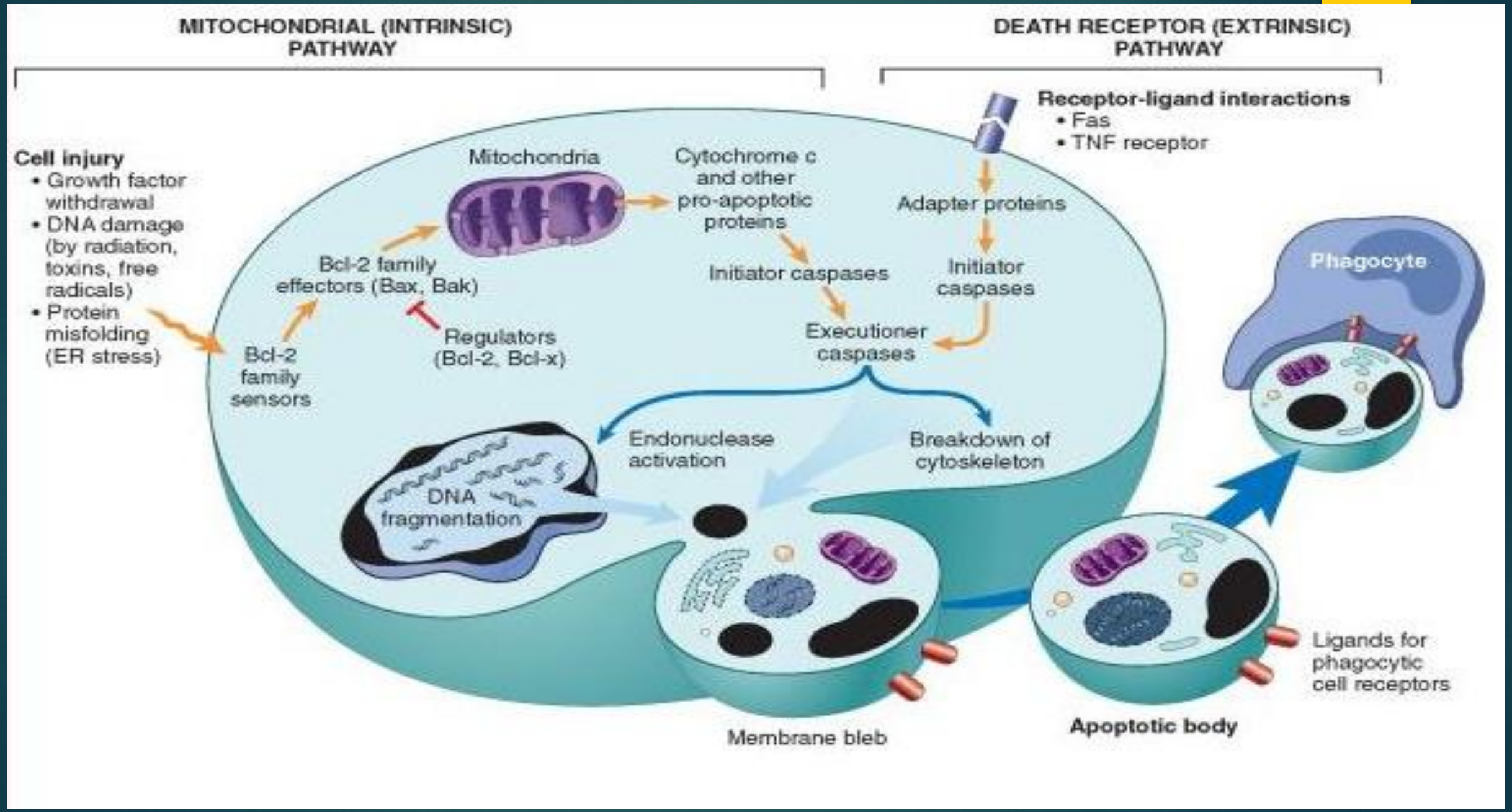
INTRINSIC PATHWAY



THE INTRINSIC PATHWAY



APOPTOSIS


MITOCHONDRIAL (INTRINSIC) PATHWAY
DEATH RECEPTOR (EXTRINSIC) PATHWAY
Cell injury
• Growth factor withdrawal
• DNA damage (by radiation, toxins, free radicals)
• Protein misfolding (ER stress)
Bcl-2 family sensors
Bcl-2 family effectors (Bax, Bak)
Regulators (Bcl-2, Bcl-x)
Mitochondria
Cytochrome c and other pro-apoptotic proteins
Initiator caspases
Executioner caspases
Receptor-ligand interactions
• Fas
• TNF receptor
Adapter proteins
Initiator caspases
Endonuclease activation
Breakdown of cytoskeleton
DNA fragmentation
Membrane bleb
Apoptotic body
Phagocyte
Ligands for phagocytic cell receptors

THANK YOU