BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
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Programme: M.Sc., Biomedical science

Course Title : Cancer Biology

Course Code : 18BMS59C16

Unit-IV

TOPIC: Apoptosis Signaling Pathway

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APOPTOSIS

- The word **“apoptosis”** comes from the ancient Greek, meaning the:

  ‘‘falling of petals from a flower’’ or

  ‘‘of leaves from a tree in autumn’’

- The term apoptosis **(a-po-toe-sis)** was first used in a now-classic paper by Kerr et al 1972 to describe a **morphologically** distinct form of cell death.

APOPTOSIS:INTRODUCTION

apoptosis is programmed cell death responsible for balancing cell proliferation & maintaining constant cell number in tissue.

5×10^{ii} RBC cells are eliminated daily in humans by apoptosis balancing their continual production in bone marrow.

PURPOSE OF APOPTOSIS:

It's essential for the proper development and to maintain homeosatsis for the organisms.

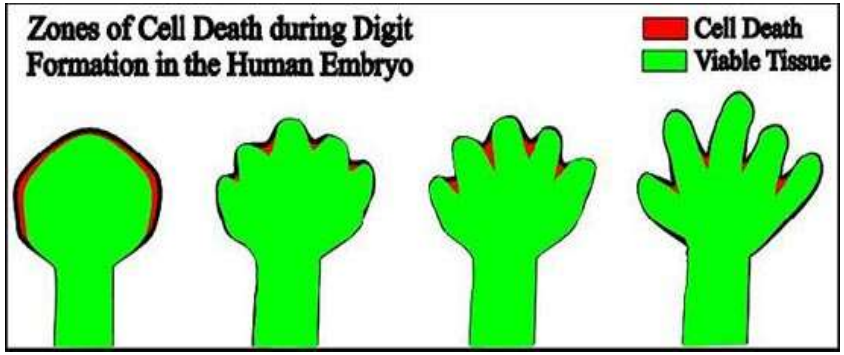
it is defence mechanism in which damaged & dangerrous cells can be eliminated.

Importance of apoptosis

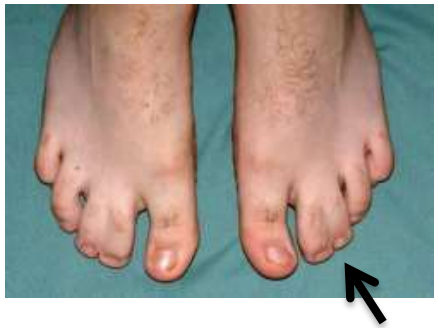
Apoptosis is a beneficial and important phenomenon:

- In embryo

1. During embryonic development, help to digit formation.



- Lack of apoptosis in humans can lead to webbed fingers called " syndactyly ".



- Neurons are produced in excess & up to 50% of developing neurons are eliminated by programmed cell death.

- virus infected cells undergo programmed cell death.

- DNA damaged cells also induce programmed cell death.

HISTORY:

German scientist ” carl vogt” was first to describe the principle of apoptosis in 1842.in 1845, anatamist ”walther flemming” delivered a more precise description of the process of programmed cell death.

the **2002 nobel prize in medicine was awarded to sydney brenner, horvitz and john E.sulston** for their work identifying genes that control apoptosis.

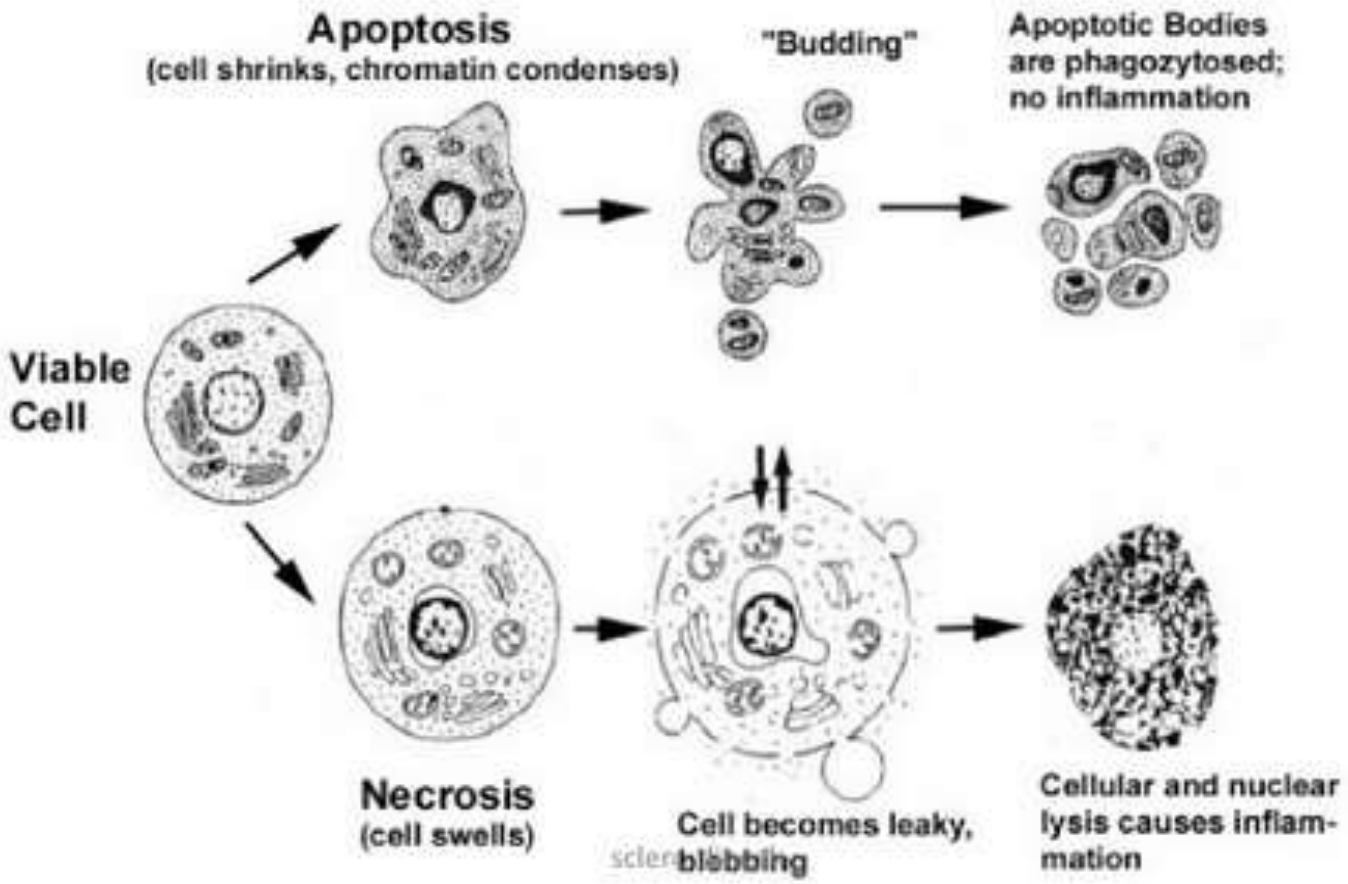
Apoptosis versus Necrosis

- Apoptosis is the physiological cell death which unwanted or useless cells are eliminated during development and other normal biological processes.

- Necrosis is the pathological cell death which occurs when cells are exposed to a serious physical or chemical insult (hypoxia, hyperthermia, ischemia).

Cell death mechanisms

- Death by suicide
- Death by injury



Differences between apoptosis and necrosis

❷ *Biochemical features*

Necrotic cells

- Loss of regulation of ion homeostasis
- No energy requirement (passive process, also occurs at 4 °C)

Apoptotic cells

- Tightly regulated process
- Energy(ATP)- dependent (active process, doesn't occur at 4 °C)
- Release of various factors into cytosol by mitochondria
- Activation of caspase cascade

Differences between apoptosis and necrosis

❸ *Physiological significance*

Necrosis

- Affects groups of contiguous cells
- Evoked by non-physiological disturbances (lytic viruses, hypoxia)
- Phagocytosis by macrophages
- Significant inflammatory response

Apoptosis

- Affects single cells or small clusters of cells
- Induced by physiological stimuli(lack of growth factors, DNA damage)
- Rapidly phagocytized by adjacent epithelial cells or macrophages
- No inflammatory response

Events of apoptosis :-

- During apoptosis chromosomal DNA is usually fragmented as a result of cleavage b/w nucleosomes chromatin condenses & nucleus breaks in to small pieces .

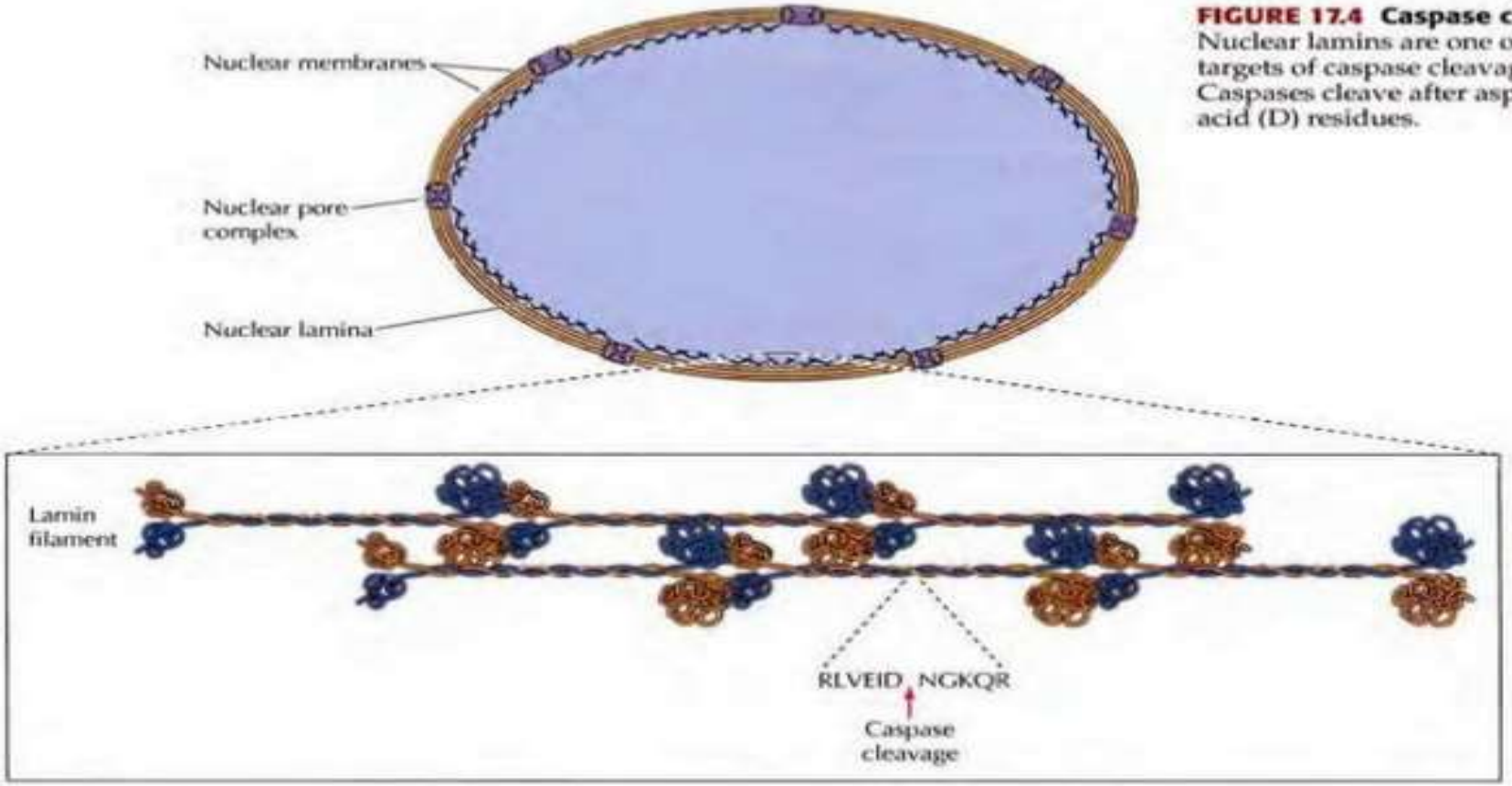
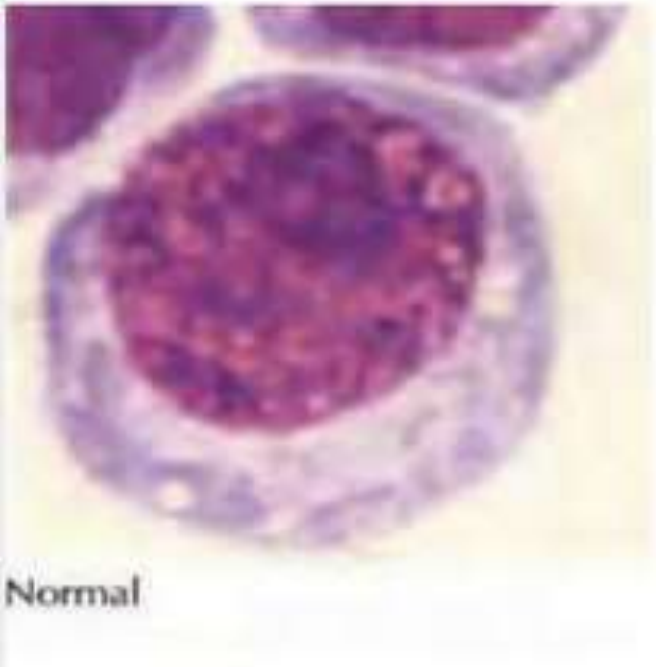
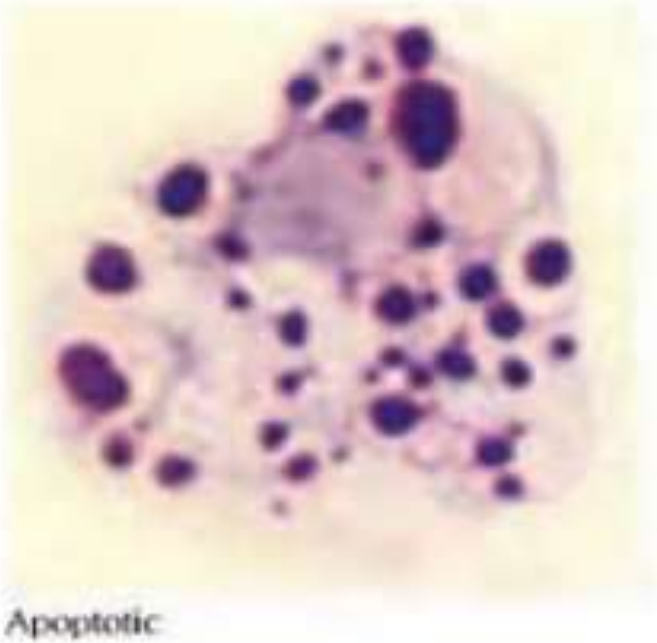
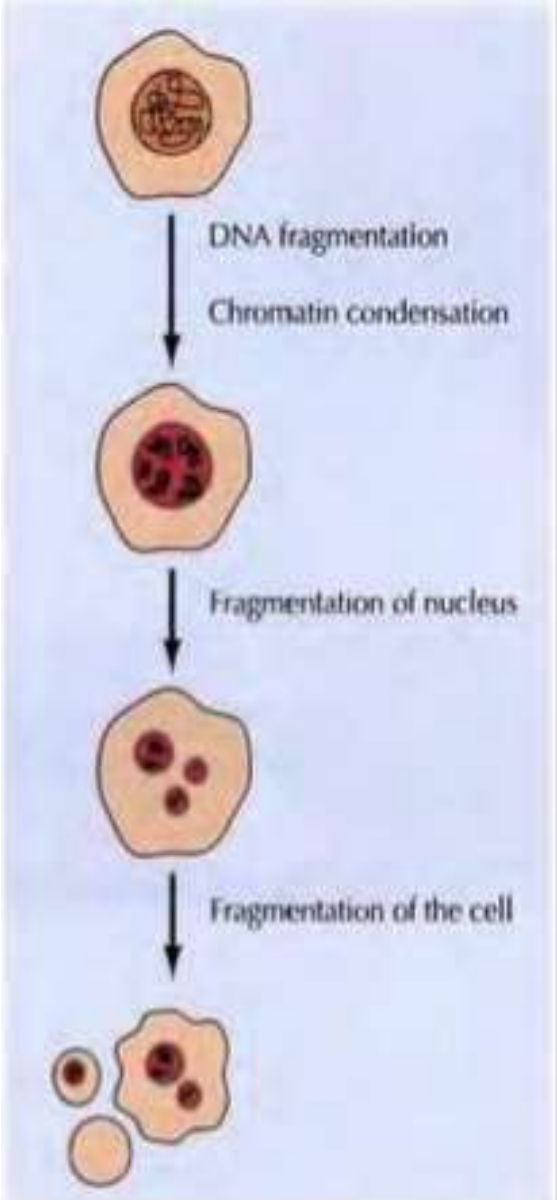


FIGURE 17.4 Caspase cleavage Nuclear lamins are one of the targets of caspase cleavage. Caspases cleave after aspartic acid (D) residues.

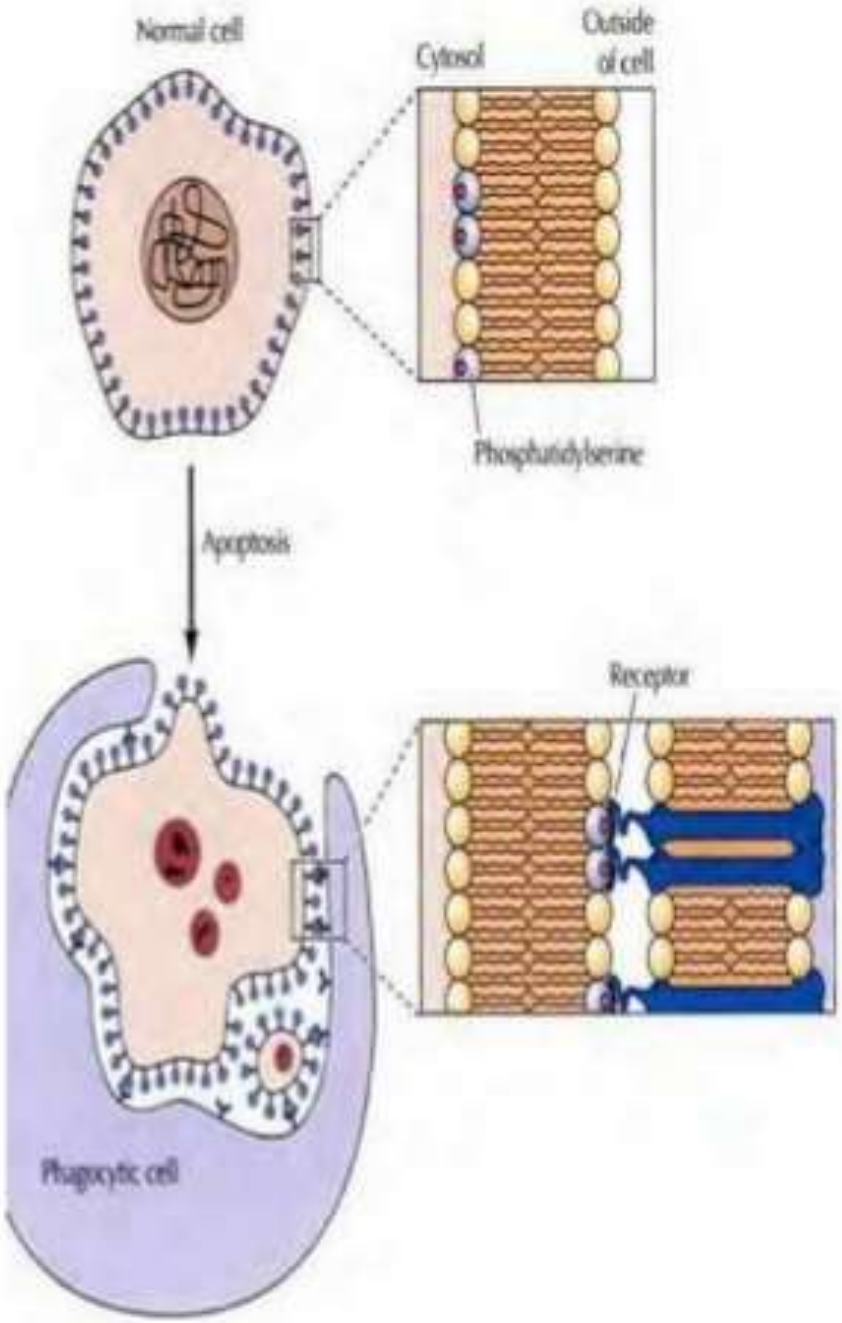
- Finally the cell it self shrinks & Breaks up in to membrane enclosed Fragments called apoptotic bodies .
- Apoptotic cells are usually Recognized by both macrophages & Neighboring cells so they removed From tissues .



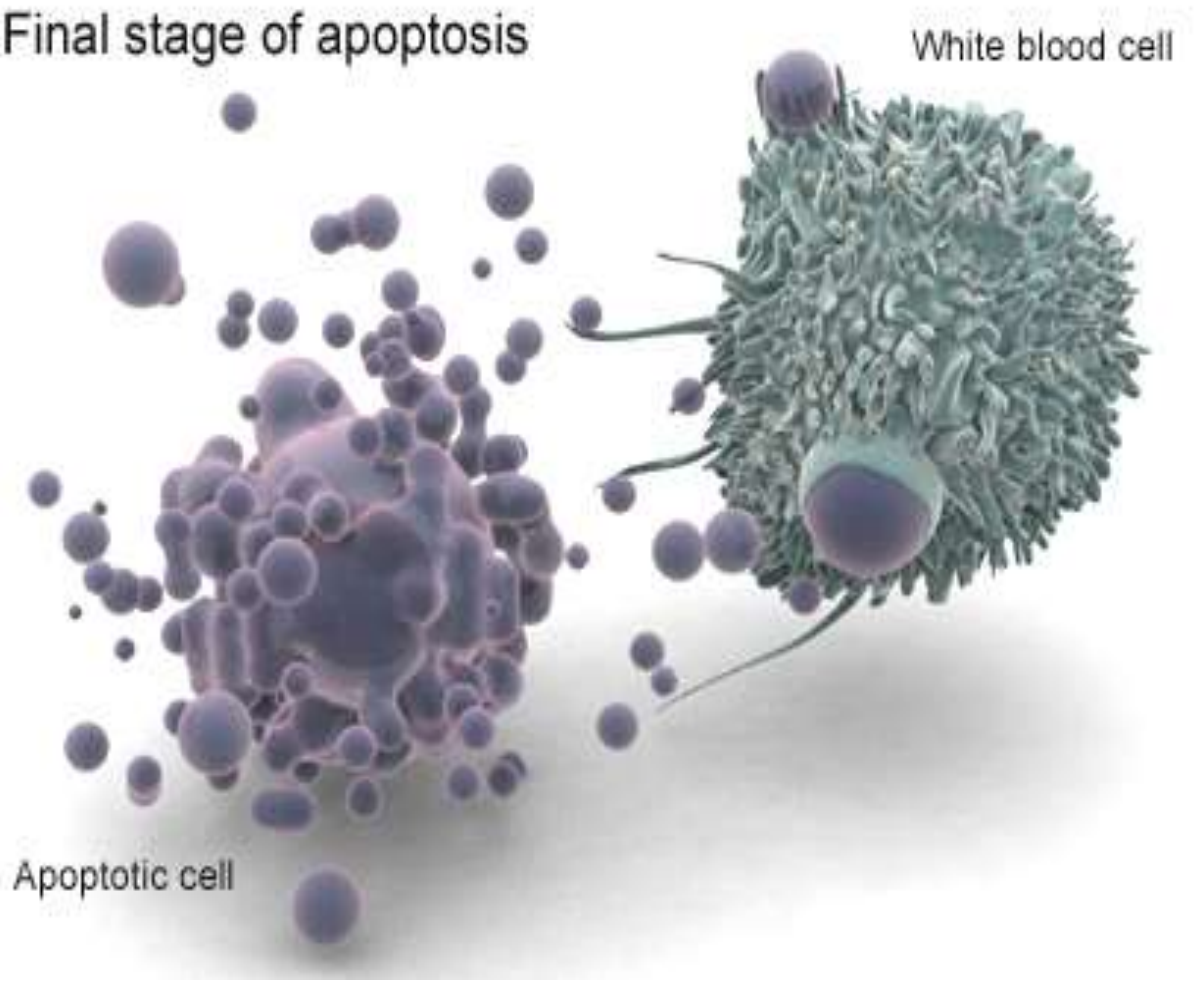




- The removal of apoptotic Cell is mediated by signals Induce phosphatidyl serine Which is restricted to inner Plasma membrane .
- During apoptosis phosphatidyl serine expressed on cell Surface where it is Recog. by Receptor expressed on phagocytic cells .

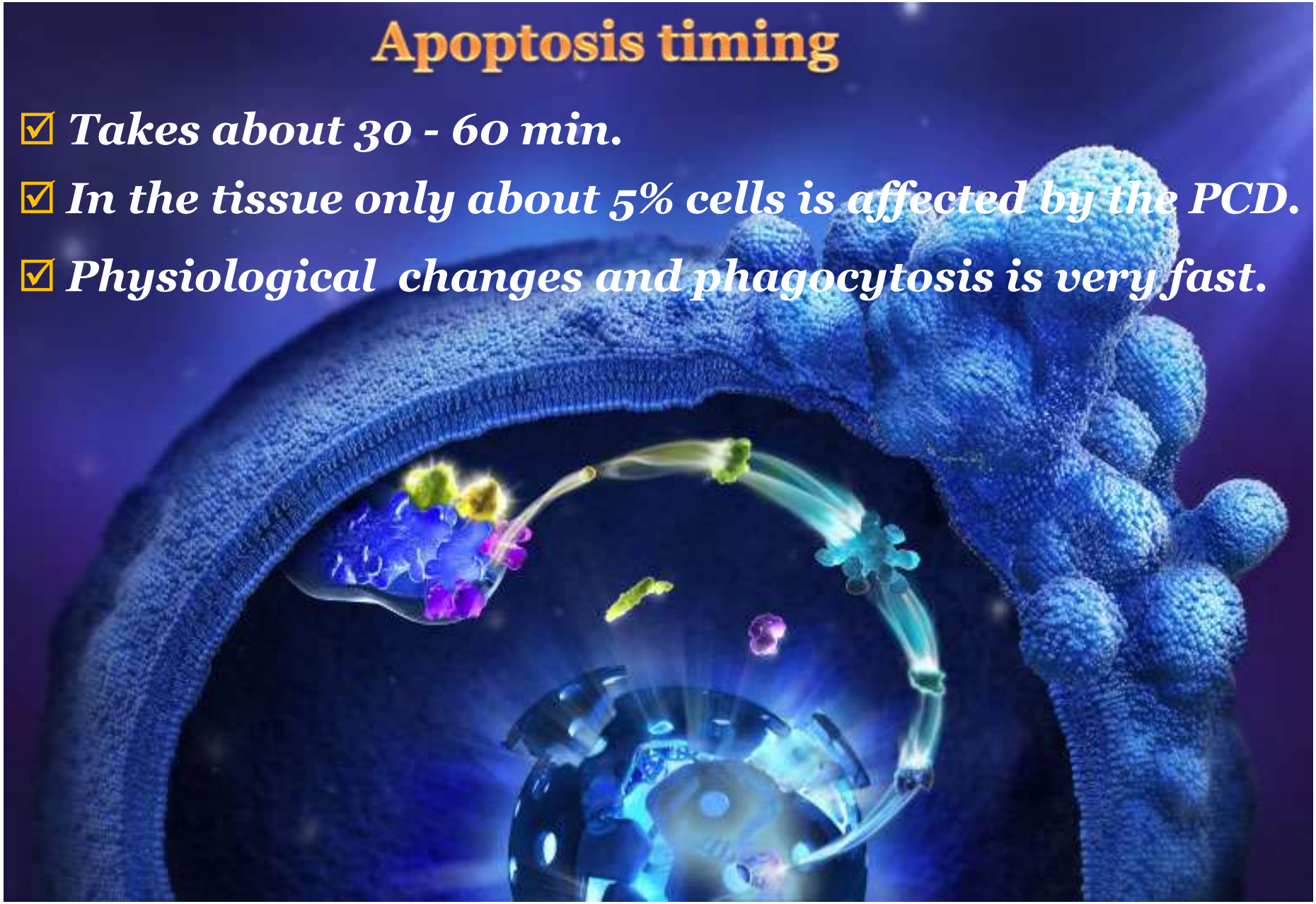


Apoptotic cells



Apoptosis timing

- ☑ *Takes about 30 - 60 min.*
- ☑ *In the tissue only about 5% cells is affected by the PCD.*
- ☑ *Physiological changes and phagocytosis is very fast.*



PROTEIN PARTICIPATE IN APOPTOSIS:

Central regulators in apoptosis :-

- Ced 9 gene in c.elegans was closely related to a mammalian gene Bcl – 2 .
- Bcl – 2 & ced9 was thus similar in function .
- In addition both Bcl – 2 & Ced 9 contain a single trans membrane domain in the outer mitochondrial membrane .

Mammals encode approximately 20 related apoptotic proteins which were divided into 3 functional groups

1. **Antiapoptotic family :-**

   Functions as an inhibitor of apoptosis .

   Eg:- Bcl – 2 & Bcl Xl .

This family contains 4 bcl-2 homology domains

2. Proapoptotic multi domain :-

Induce apoptosis .

Eg:- BAX , BAK .

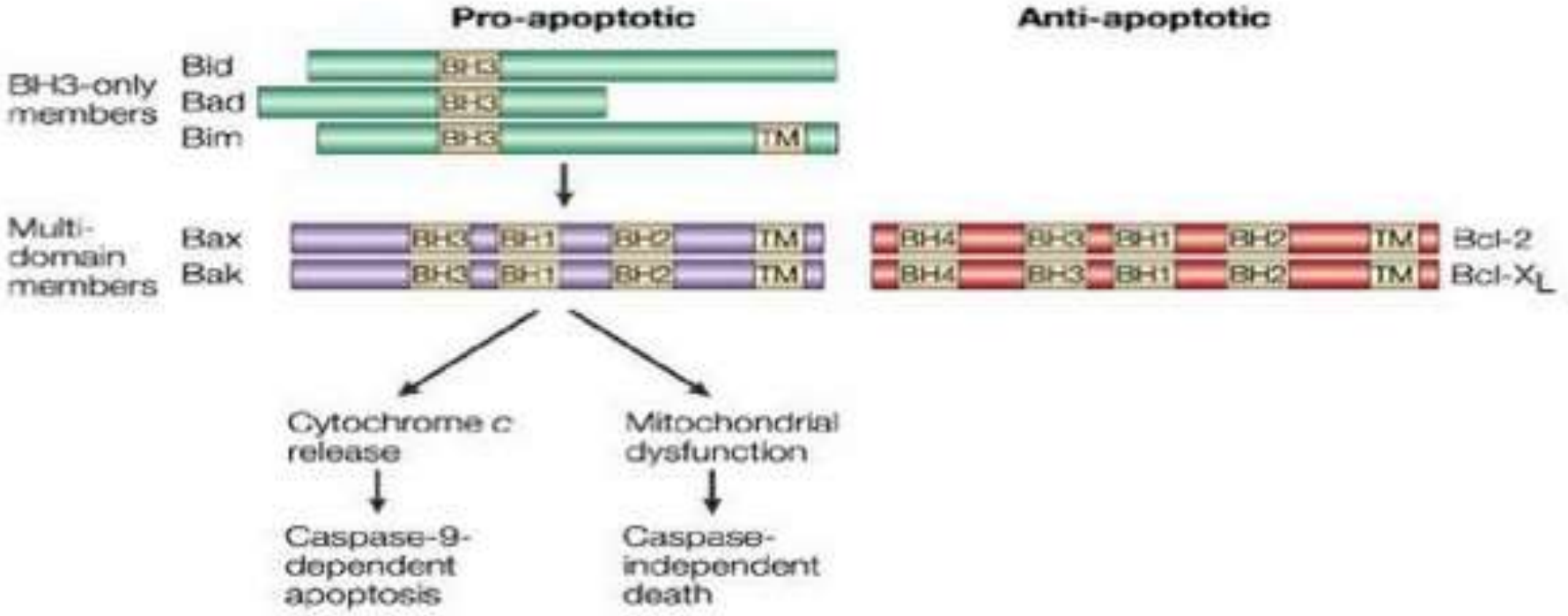
Contains 3 homologous domains

3. Pro apoptotic BH – 3 only :-

Induce apoptosis.

Eg:-Bid , Bad , Noxa , PUMA , Bin .

Contains only one bcl –3homology domain .



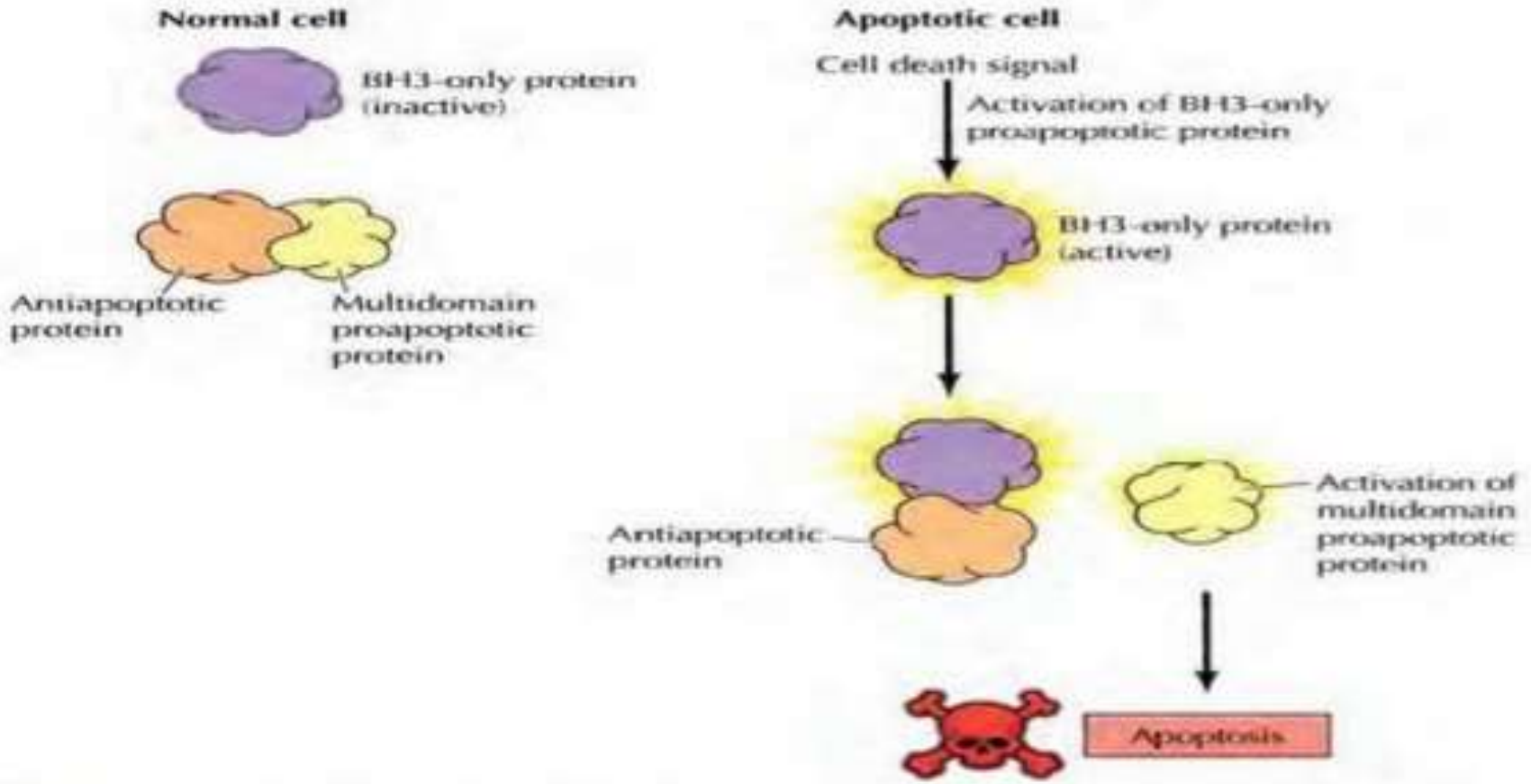
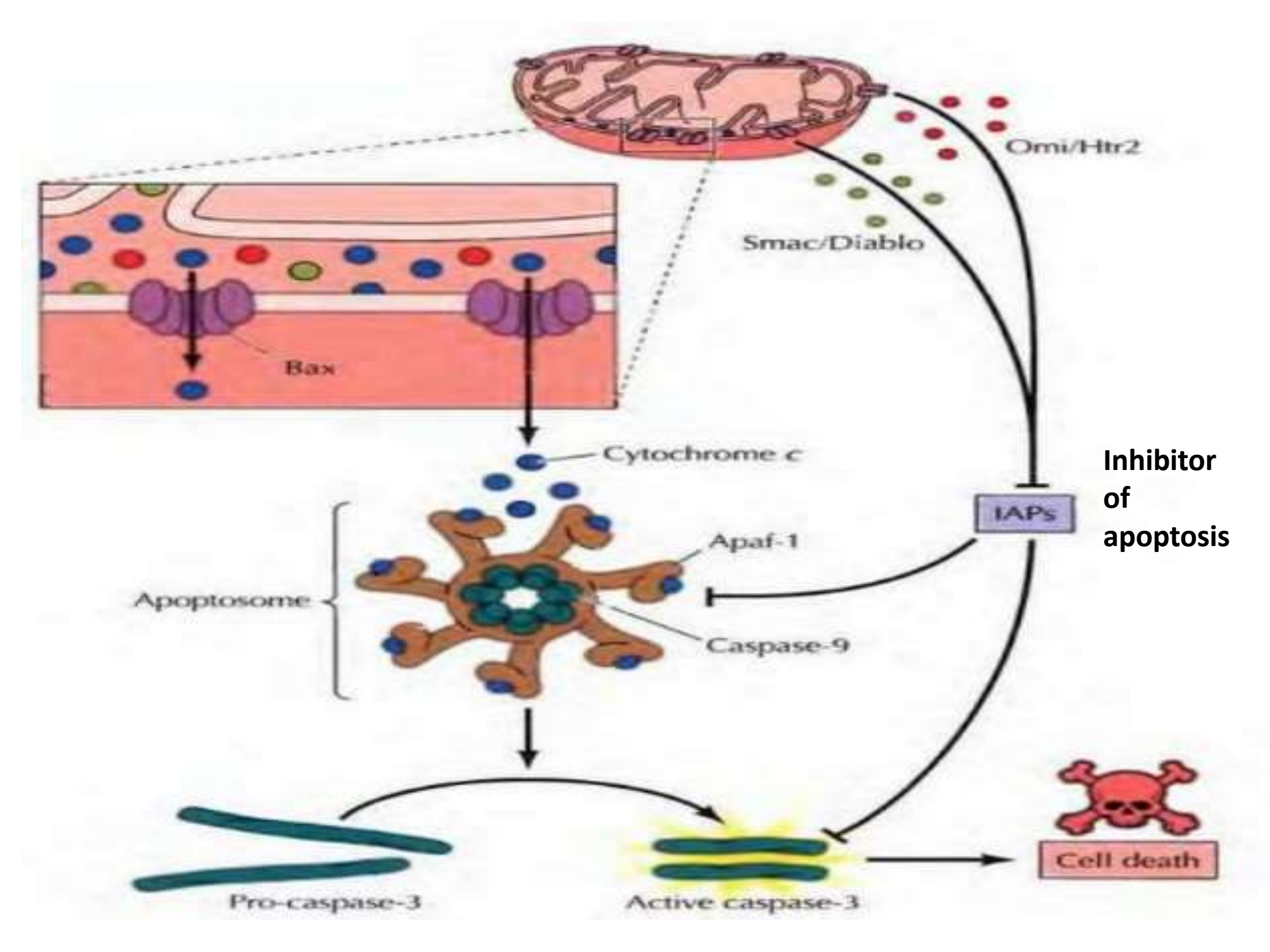


FIGURE 17.7 Regulatory interactions between Bcl-2 family members In normal cells, the BH3-only proapoptotic proteins are inactive, and the multidomain proapoptotic proteins are inhibited by interaction with antiapoptotic proteins. Cell death signals activate the BH3-only proteins, which then interact with the antiapoptotic proteins, leading to activation of the multidomain proapoptotic proteins and cell death.



Inhibitor of apoptosis

Bcl2 family protein:

Role in disease:

Damage to the Bcl-2 gene has been identified as a cause of a number of cancers, including melanoma, breast, prostate, chronic lymphocytic leukemia, and lung cancer, and a possible cause of schizophrenia and autoimmunity. It is also a cause of resistance to cancer treatments.

Cancer occurs as the result of a disturbance in the homeostatic balance between cell growth and cell death. Over-expression of anti-apoptotic genes, and under-expression of pro-apoptotic genes, can result in the lack of cell death that is characteristic of cancer. An example can be seen in lymphomas.

Caspases

*Caspases= Cysteinyl aspartate specific proteases*Single chain of pro-enzymes.

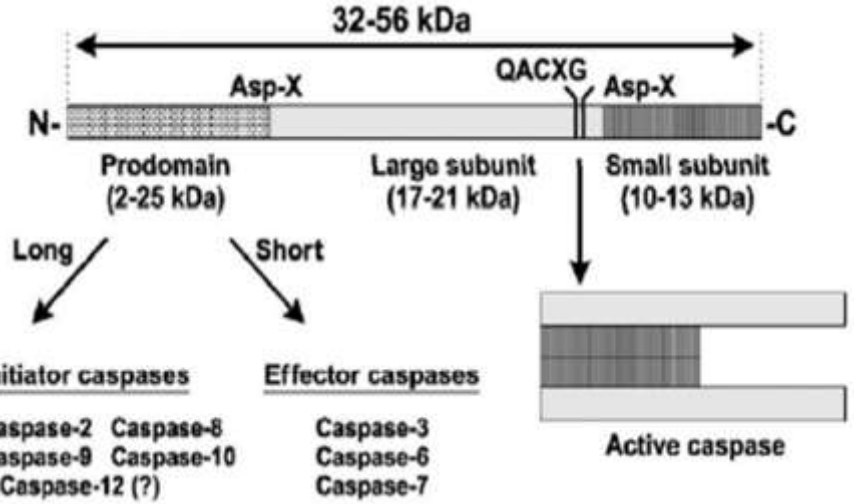
Contains an N-terminal domain, a small subunit and a large subunit (similar to a ribosome)

Apoptotic stimulus ➔Activation ➔Substrate Cleavage ➔Enzyme

- A family of intracellular cysteine proteases that play a pivotal role in the initiation and execution of apoptosis.
- At least 14 different members of caspases in mammalian cells have been identified
- All are synthesized as inactive proenzymes (zymogen) with 32-56 kDa

Caspase subgroups

- To date, ten major caspases have been identified and broadly categorized into:
  ① Signaling or Initiator caspases (2, 8, 9,
    – Long N-terminal domain
    – Interact with effector caspases
  ② Effector or Executioner caspases (3, 6
    – Little to no N-terminal domain
    – Initiate cell death
  ③ Inflammatory caspases (1, 4, 5)



Caspase structure

- The other caspases that have been identified include:
  Caspases 11, 12, 13, 14

- Central role in cascade of apoptotic events is played by caspase 3 (CPP32)

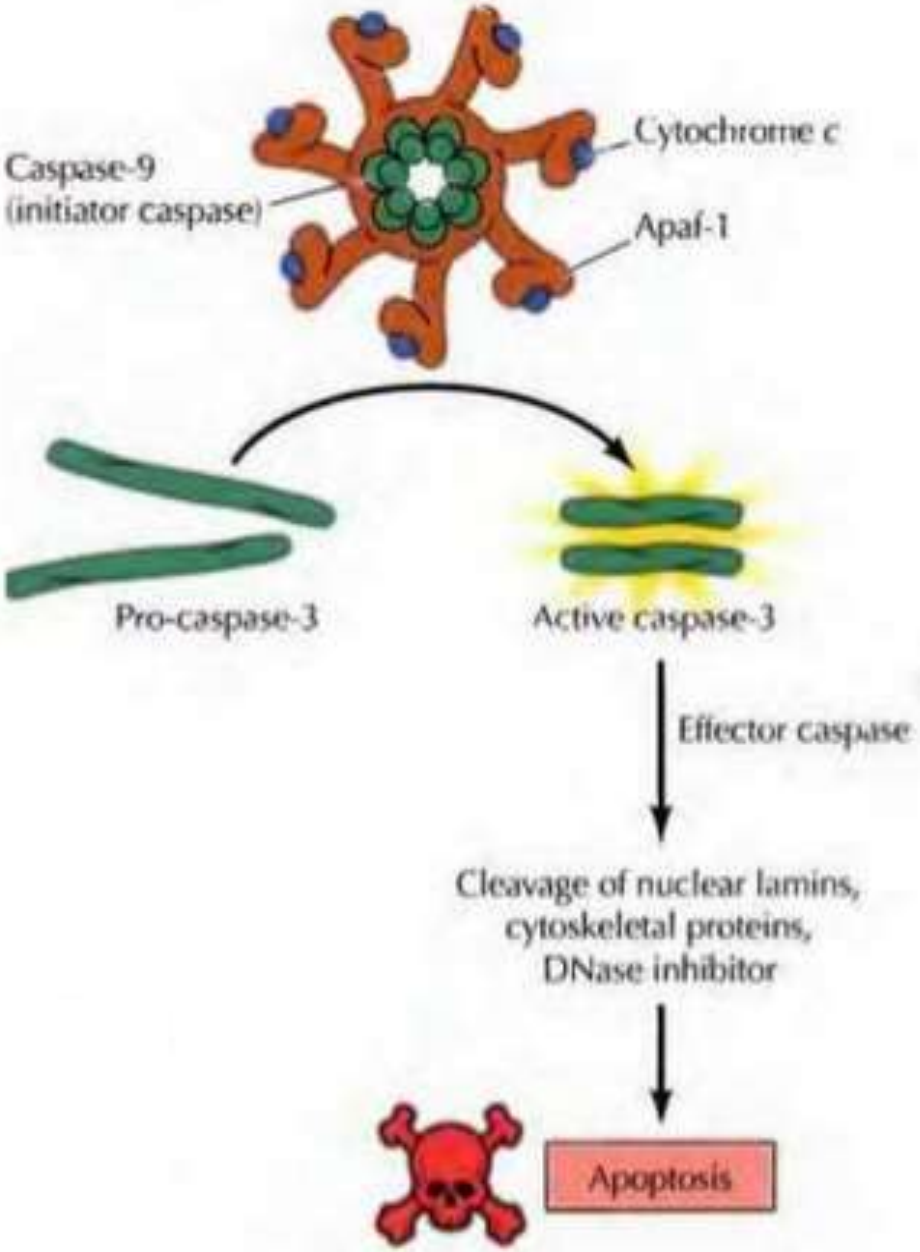
- The Caspases are named because they contain cysteine residue in the catalytic site & cleaves proteins at C terminal to aspartic residues .
- Caspases are the ultimate effectors of the apoptosis cleaving nearly 100 cell target proteins .
- ***Key targets of caspases include***

  1.fragments of nuclear DNA

  2.cleavage of nuclear lamins

  3.cytoskletal proteins

- Ced 3 is only caspases in c . Elegans.
- All caspases are synthesized as inactive precursor (procaspases) that can be converted to active forms by proteolytic cleavage catalyzed by caspases .
- Caspases 9 activated as complex with apaf -1 & cyt c in the apoptosome .
- Caspases – 9 that cleaves & activates effector caspases , such as caspases 3 & 7 .
- The effector caspases cleave variety of cell , cell protein , including nuclear lamins , cytoskeletal proteins & an inhibitor of DNAse , leading to cell death .


Caspase-9
(initiator caspase)
Cytochrome c
Apaf-1
Pro-caspase-3
Active caspase-3
Effector caspase
Cleavage of nuclear lamins,
cytoskeletal proteins,
DNase inhibitor
Apoptosis

Apoptosis pathways

1. Extrinsic pathway (death receptor- mediated events)

2. Intrinsic pathway (mitochondria- mediated events)

Extrinsic Pathway

Components:

- Death Receptors
- Death Ligands
- Adaptor Proteins
- Caspaseses

Extrinsic Pathway

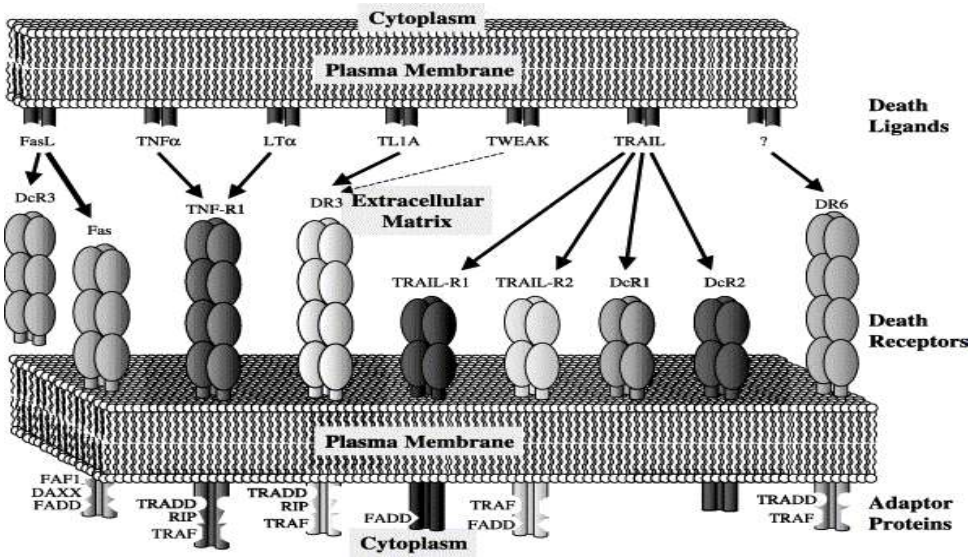
- "Death receptors" that are members of the tumor necrosis factor (TNF) receptor superfamily.
- Death receptors have a cytoplasmic domain of about 80 amino acids called the "death domain".
- This death domain plays a critical role in transmitting the death signal from the cell surface to the intracellular signaling pathways.

Death Receptors & their ligands

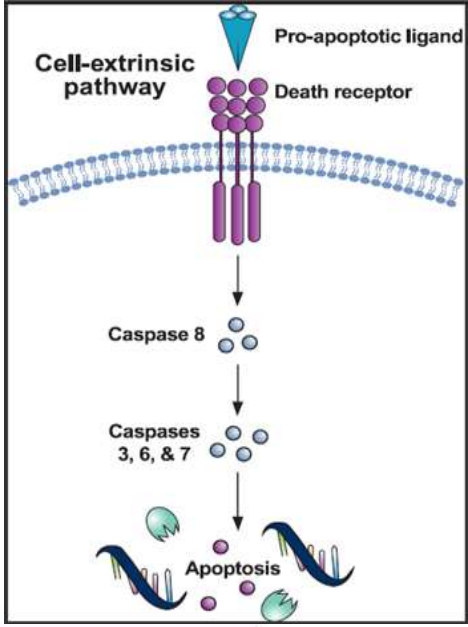
- The best characterized receptors & ligands corresponding

death include:

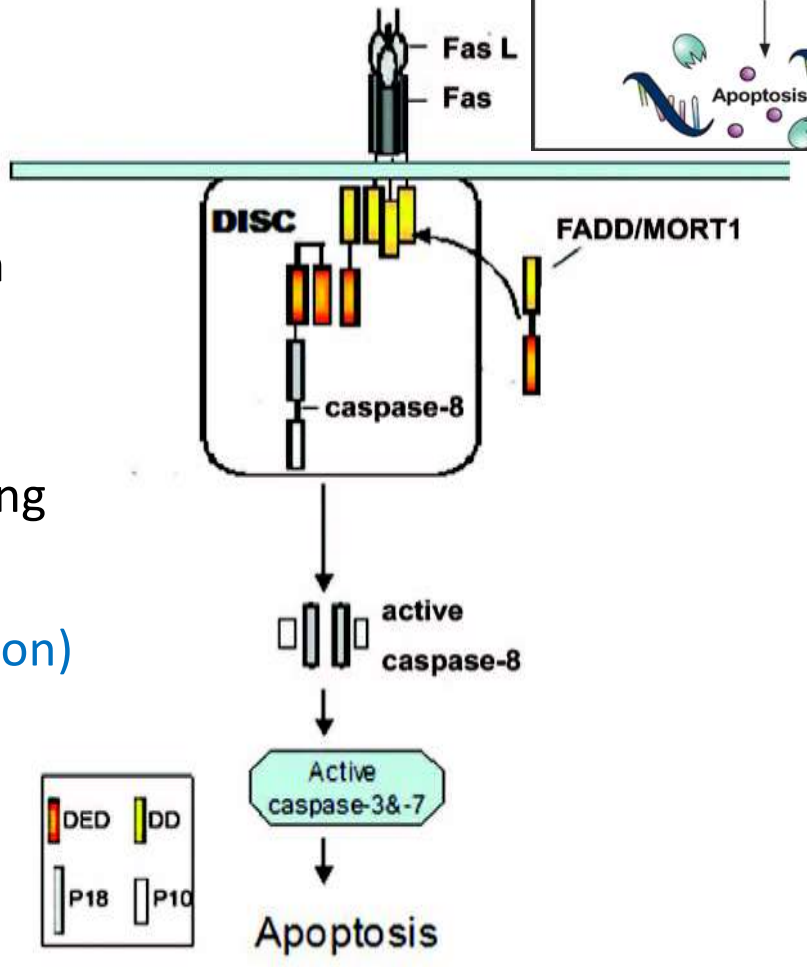
Receptors	Ligands
FasR (CD95/APO1)	FasL
DR3	Apo3L
DR4 (TRAIL-R1)	Apo2L
DR5 (TRAIL-R2)	Apo2L
TNFR1	TNF-α
TNFR2	TNF-ß



Extrinsic Pathway

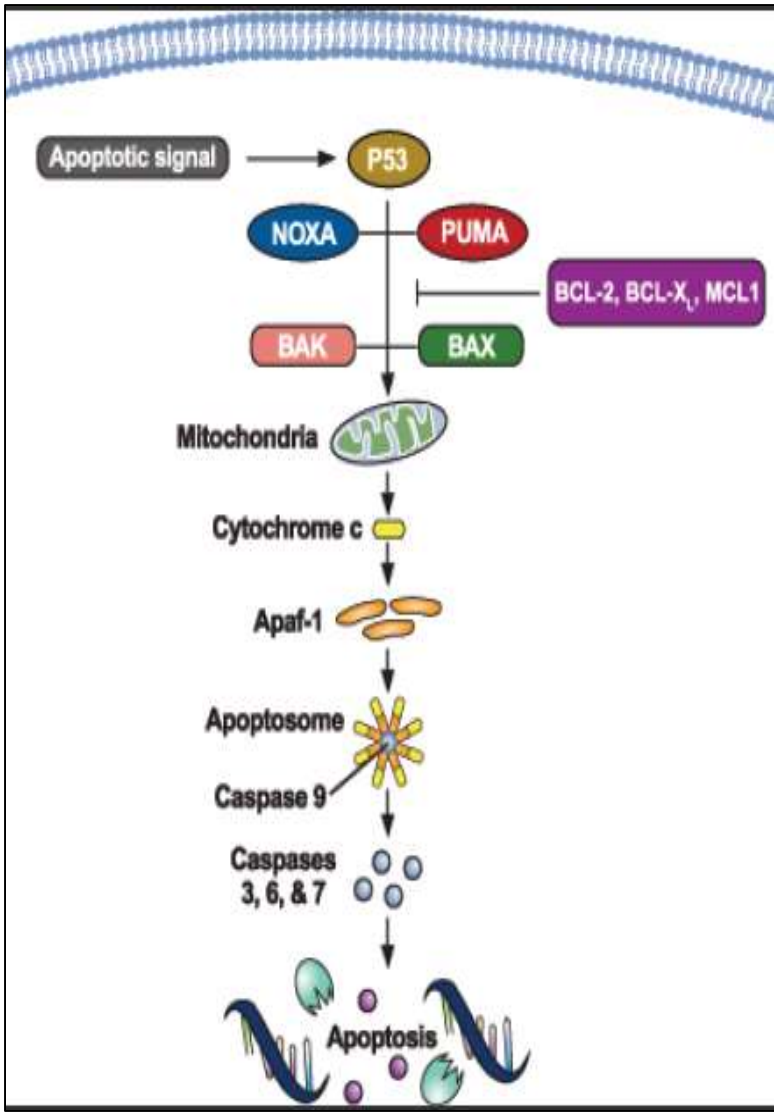


- → Binding of trimeric FasL to Fas
- → Trimerization and clustering of Fas
- → Recruitment of Fas-associated death domain (FADD) to Fas
- → Recruitment of caspase-8 to FADD
- → Formation of Death-Inducing Signaling Complex (DISC)
- → Activation of caspase-8 (autoactivation)
- → Activation of effector caspases
- → Apoptosis



Intrinsic Pathway

- The stimuli that initiate the intrinsic pathway produce intracellular signals such as radiation (DNA damage), absence of certain growth factors, hormones and cytokines.
- All of these stimuli cause changes in the mitochondrial outer membrane permeabilization (MOMP)
- Release of pro-apoptotic proteins such as cytochrome c, Smac/DIABLO, AIF, endonuclease G and CAD from the inter-membrane space into the cytosol.
- Cytochrome *c* binds and activates Apaf-1 as well as procaspase-9, forming an **"apoptosome".**
- Caspase-9 activation, subsequent caspase-3 activation and cell death.



Intrinsic Pathway & Bcl2 family

- The control & regulation of apoptotic mitochondrial events occurs through members of the Bcl-2 family of proteins

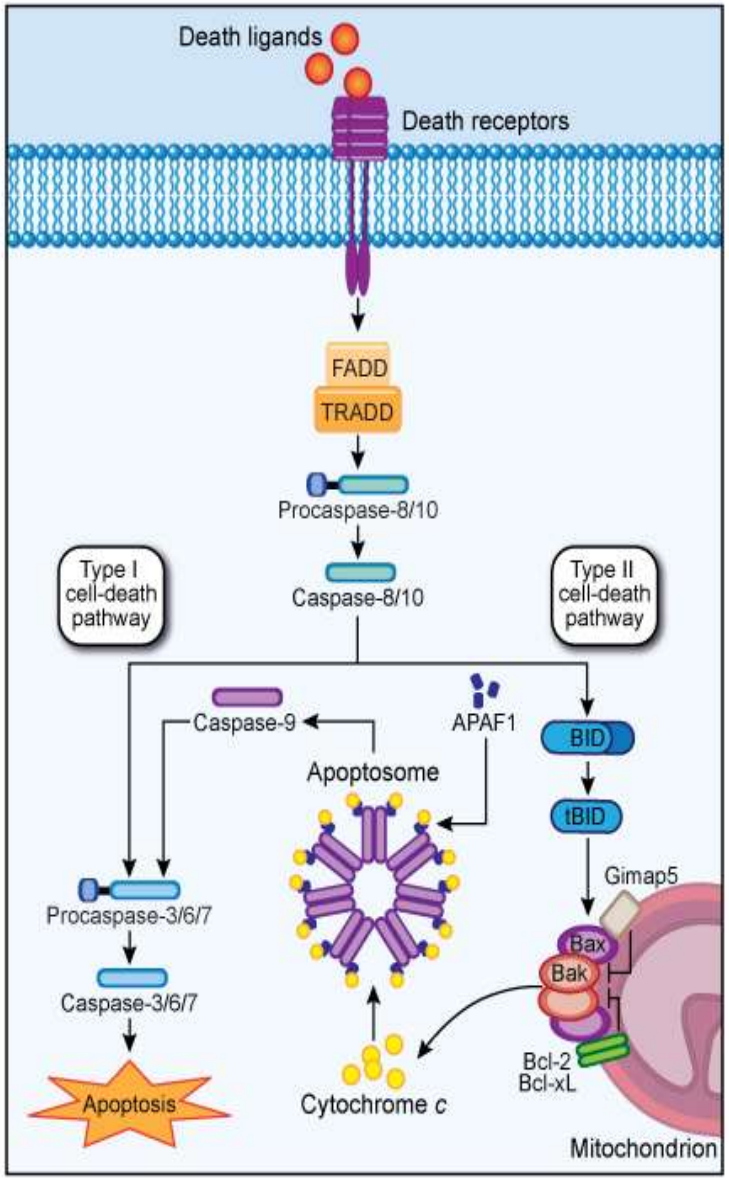
❶ Anti-apoptotic proteins include ,

Bcl-2, Bcl-x, Bcl-XL, Bcl-w

❷ Pro-apoptotic proteins include.

Bax, Bak, Bid, Bad, Bim, Bik

➢ The main mechanism of action of the Bcl-2 family of proteins is the regulation of cytochrome c release from the mitochondria via alteration of mitochondrial membrane permeability.



Apoptosis assay methods

- Apoptosis assays, based on methodology, can be classified into six major groups:

1. Cytomorphological alterations
2. DNA fragmentation
3. Detection of caspases, cleaved substrates, regulators and inhibitors
4. Membrane alterations
5. Detection of apoptosis in whole mounts
6. Mitochondrial assays

Cytomorphological alterations

- The evaluation of hematoxylin and eosin-stained tissue sections with light microscopy does allow the visualization of apoptotic cells.
- This method detects the later events of apoptosis.
- TEM is considered the gold standard to confirm apoptosis:

  (1) electron-dense nucleus

  (2) nuclear fragmentation

  (3) intact cell membrane

  (4) disorganized cytoplasmic organelles

  (5) large clear Vacuoles

  (6) blebs at the cell surface

DNA fragmentation

- DNA fragmentation occurs in the later phase of apoptosis.
- TUNEL (Terminal dUTP Nick End-Labeling) assay quantifies the incorporation of deoxyuridine triphosphate (dUTP) at single and double stranded DNA breaks in a reaction catalyzed by the template-independent enzyme, terminal deoxynucleotidyl transferase (TdT).
- Incorporated dUTP is labeled such that breaks can be quantified either by flowcytometry, fluorescent microscopy, or light microscopy.

Membrane alterations

Fluorescein isothyocyanate (FITC) conjugated Annexin V

- During the process of apoptosis, one of the earliest events is Externalization of Phosphatidyl serine (PS) from the inner to the outer plasma membrane of apoptotic cells.
- These cells can be demonstrated by bound with FITC-labeled Annexin V and detected with fluorescent microscopy.
- Positive cell in the Annexin V assay beginning of the apoptotic process

Membrane alterations

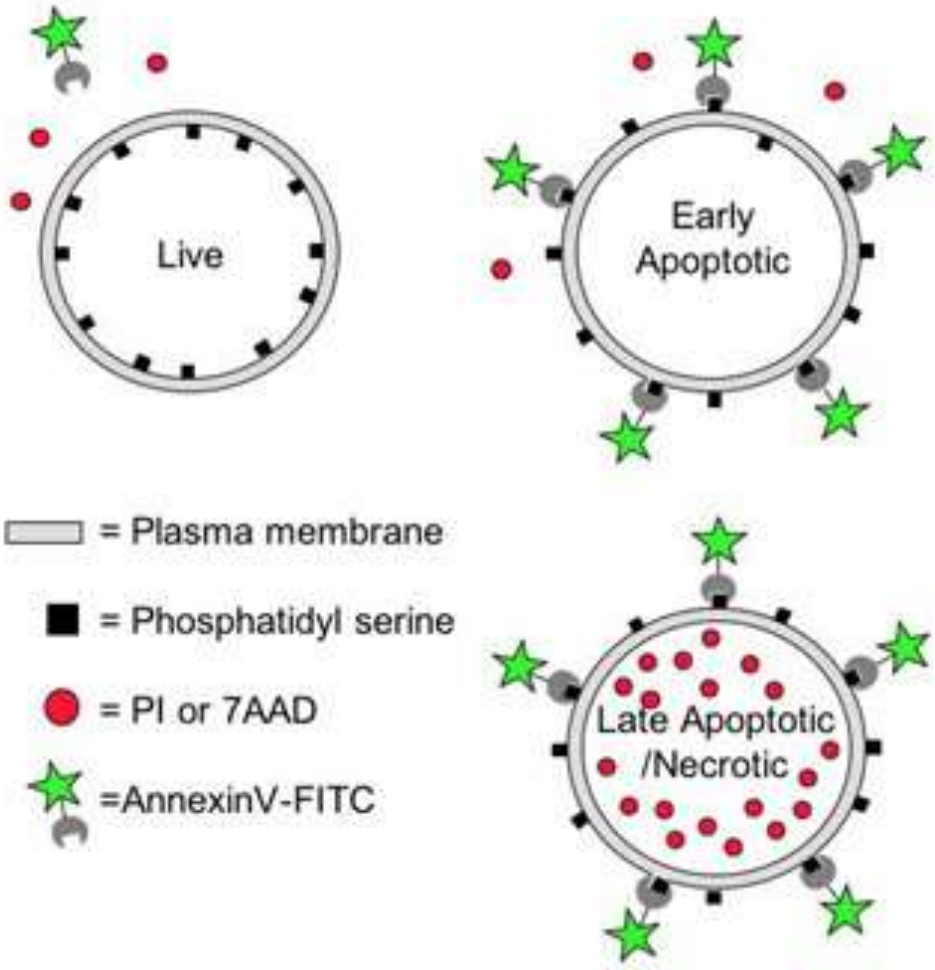
Fluorescein isothyocyanate (FITC) conjugated Annexin V + PI

- The membranes of necrotic cells are labeled with annexin V. Since loss of membrane integrity is a pathognomonic feature of necrotic cell death, necrotic cells will stain with specific membrane-impermeant nucleic acid dyes such as propidium iodide and trypan blue.

- The membrane integrity of apoptotic cells can be demonstrated by the exclusion of these dyes.

- Annexin V negative - PI negative populations are healthy cells. Annexin V positive - PI negative populations represent cells in early apoptosis.

FITC conjugated Annexin V + PI



Detection of Apoptosis in Whole Mounts

- Apoptosis can also be visualized in whole mounts of embryos or tissues using dyes such as acridine orange (AO), Nile blue sulfate (NBS), and neutral red (NR).

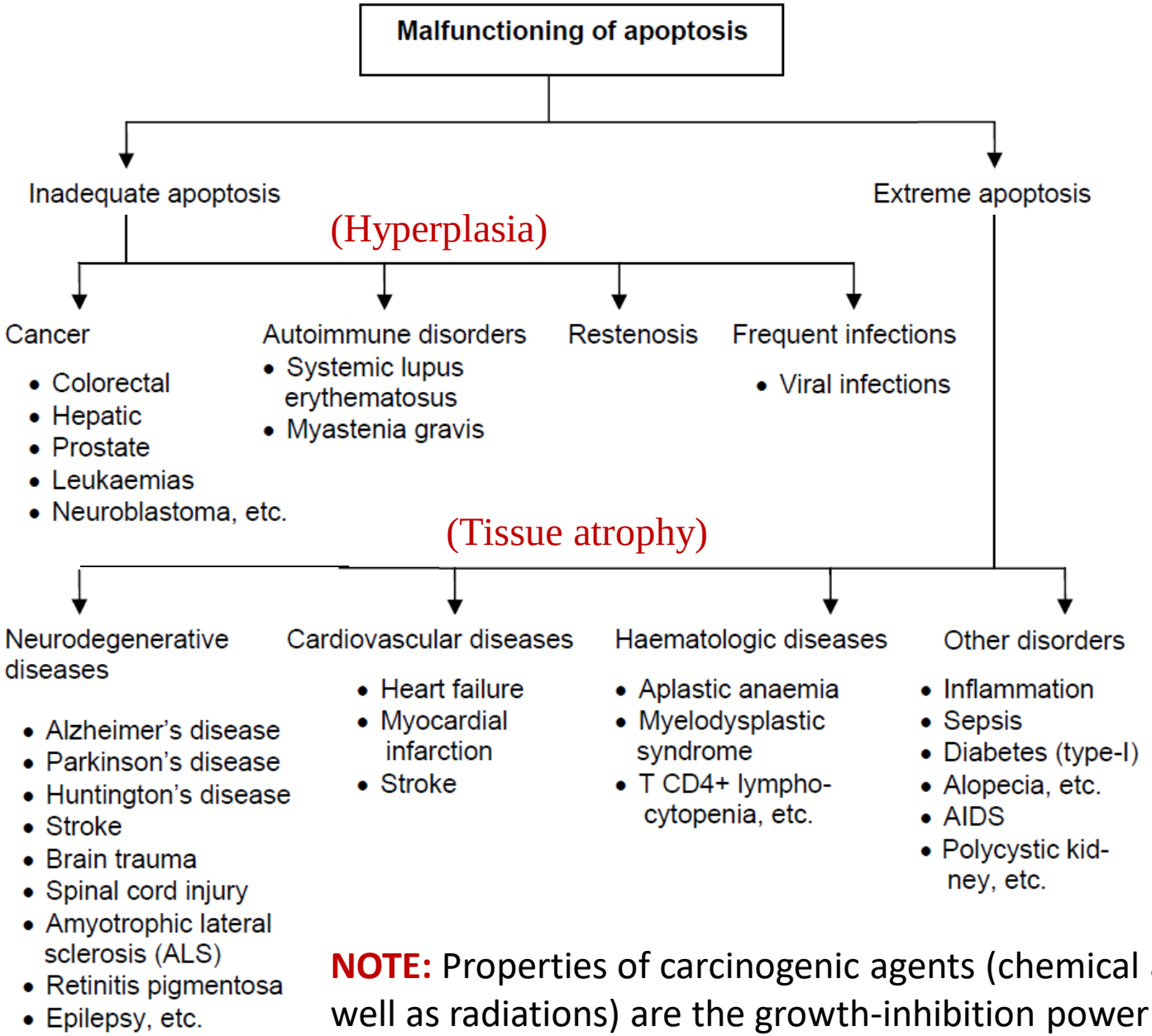
- Since these dyes are acidophilic, they are concentrated in areas of high lysosomal and phagocytotic activity.

Mitochondrial Assays

- Mitochondrial assays and cytochrome c release allow the detection of changes in the early phase of the intrinsic pathway.
- The mitochondrial outer membrane (MOM) collapses during apoptosis, allowing detection with afluorescent cationic dye.

- Cytochrome c release from the mitochondria can also be assayed using fluorescence and electron microscopy in living or fixed cells.

- Apoptotic or anti-apoptotic regulator proteins such as Bax, Bid, and Bcl-2 can also be detected using fluorescence and confocal microscopy



NOTE: Properties of carcinogenic agents (chemical agents as well as radiations) are the growth-inhibition power and the ability to induce cell death. These properties are widely used in anticancer chemo- and radiotherapies

Thanks for your Attention

Acknowledgement

- The presentation is being used for educational and non-commercial purposes.
- Thanks are due to all the original contributors and entities whose pictures were used in the creation of this presentation.