MJF COLLEGE OF VETERINARY AND ANIMAL SCIENCE, CHOMU, JAIPUR

DEPARTMENT OF VETERINARY PATHOLOGY

IRREVERSIBLE CELL INJURY AND CELL DEATH

Types of Cell Death

- Two major types:

1. **Necrosis :**

- Cell death by swelling – Oncotic cell death

2. **Apoptosis:**

- Cell death by shrinkage

- It is not always possible to make the distinction between these two types of cell death based on histologic examination, and often both swelling and shrinkage are present.

Necrosis

- **Necrosis refers to a sequence of morphological changes that follow cell death in living tissue or organ**
  - Result of two concurrent processes
    - Enzymatic digestion of cells by
      - Autolysis
      - Heterolysis
    - Denaturation of proteins

Etiology of Necrosis

- Poisons :
  - Chemical, Plant, Animals or Microbe origin
- Disturbances in circulation
  - Ischemia
  - Passive hyperemia
  - General anemia
- Mechanical injuries
- Thermal changes
- Electric currents
- X-ray and nuclear fission substances

Macroscopic appearance

- Necrotic tissue is usually pale, soft and friable, and sharply demarcated from viable tissue by a zone of inflammation/hyperemia
- A sharp line of demarcation between necrotic and viable tissue is often a reliable means to distinguish necrosis from autolysis.

Microscopic appearance

Nuclear changes

- Appear in one of three patterns; All due to nonspecific breakdown of DNA

❶ Karyolysis

- The basophilia of the chromatin may fade
- May be due enzymatic degradation of DNA by endonucleases.

❷ Pyknosis

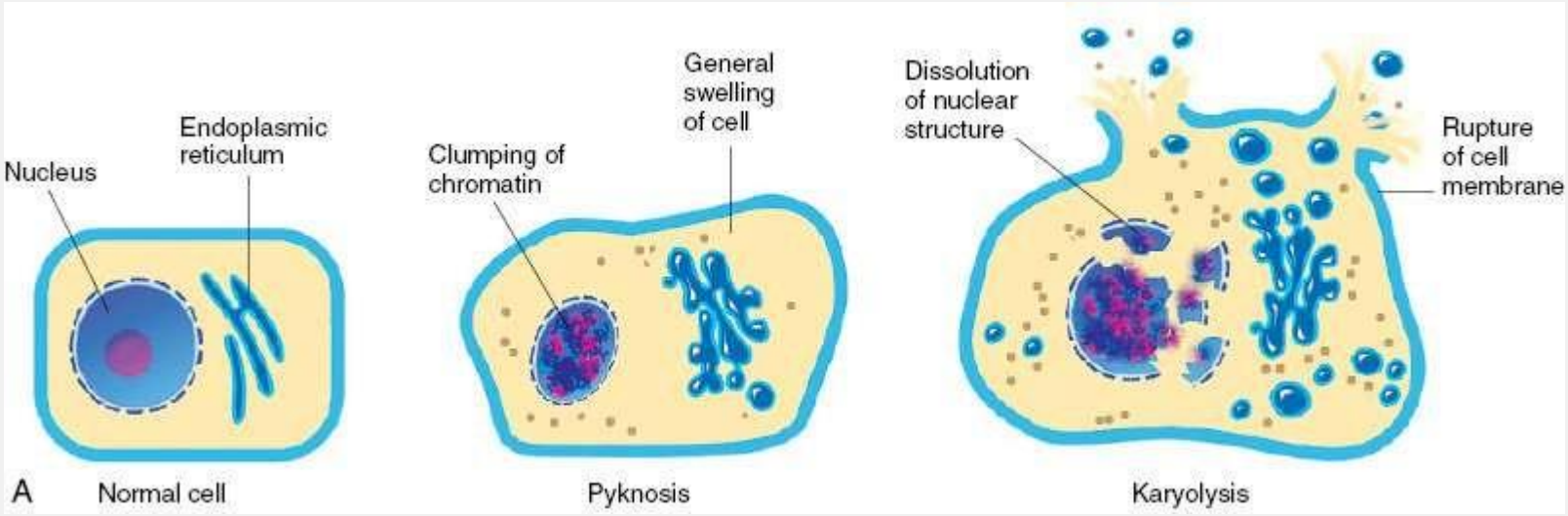
- Characterized by nuclear shrinkage and increased basophilia
- Here the chromatin condenses into a solid, shrunken basophilic mass.

Microscopic appearance

Nuclear changes

❸Karyorrhexis

- Pyknotic nucleus undergoes fragmentation.
- With the passage of time (a day or two), the nucleus in the necrotic cell totally disappears.



Microscopic appearance

Cytoplasmic changes

- Cytoplasm becomes homogeneous pink in H& E-stained sections
  - Attributable in part to the loss of cytoplasmic RNA (which binds the blue dye, hematoxylin)
  - Denatured cytoplasmic proteins (which bind the red dye, eosin)
- Necrotic cell is pale, ghostlike appearance.
- Necrotic cells become “individualized” and may slough from epithelium

Types of Necrosis

1. Coagulative necrosis
2. Liquefactive necrosis
3. Caseous necrosis
4. Fat necrosis
5. Fibrinoid necrosis
6. Gangrenous necrosis

1 Coagulative necrosis

- Most common pattern of necrosis
- Preservation of the basic structural outline of the coagulated cells or tissue, for at least some days.
- The architectural detail of the area persists, but the cellular detail is lost.
- The denaturation of structural and enzymic proteins of the cytoplasm blocks the proteolysis (dissolution or digestion) of the cell, and thus the tissue architecture is preserved
- Results most commonly from sudden severe ischemia/hypoxia

1 Coagulative necrosis

- Example: **Infraction**
- Gross change
  - The necrotic area is firm and dry in consistency.
  - It has a homogeneous, opaque, cooked appearance, and is grey, white, or tan.
- Microscopic change
  - The architectural outline of the tissue or organ is maintained but the cellular details are lost

2 Liquefactive necrosis

- Usual type of necrosis in the CNS
- Transformation of the tissue into a liquid mass in which **cellular and architectural detail are lost**
- It results from the action of powerful lysosomal enzymes of white blood cells
- Hypoxic death of cells in all tissues produces coagulative necrosis, but in **brain it produces liquefactive necrosis**
- Example:- **Abscess**
- Bacteria attract neutrophils – enzyme released – Heterolysis - focal liquid collection of necrotic neutrophils and tissue debris (pus)

3 Caseous necrosis

- Caseous – cheese-like
- Conversion of dead cells into a granular friable mass grossly resembling cottage cheese
- Absence of both architectural and cellular detail
- Older (chronic) lesion often associated with poorly degradable lipids of bacterial origin
- Example:
  - **Tuberculosis**
  - Corynebacterium infection
- Produced granulomatous inflammation

cottage cheese

3 Caseous necrosis

Gross change

- The area of necrosis is a granular amorphous material resembling cheese
- The mass is dry but creamy in consistency.
- It is soft, friable, and white-grey in colour.
- Calcification commonly occurs in the necrotic areas, especially in sheep and cattle.

Microscopic change

- Amorphous granular debris enclosed within a ring of granulomatous inflammation
- Neither architectural nor cellular detail is present.

4 Fat necrosis

- Four types of fat necrosis

① Enzymatic necrosis of fat

- Also called pancreatic necrosis of fat
- Death of adipose tissue in the vicinity of the pancreas due to the action of lipases
- Most commonly due to acute pancreatitis
- Lipases split the triglyceride and released fatty acids → combine with calcium to produce grossly visible chalky white areas (fat saponification)

4 Fat necrosis

②Nutritional fat necrosis

- Also known as steatitis or yellow fat disease
- Feeding a diet high in unsaturated fatty acids and low in vitamin E or other antioxidants
- Setting the stage for ROS production and lipid peroxidation.
- Yellow fat disease is often seen in carnivores, such as cats or mink, on a fish-based diet.
- Affected adipose tissue is firm, nodular, and yellow-brown.

4 Fat necrosis

③ Traumatic necrosis of fat

- When adipose tissue is crushed.
- It occurs in fat adjacent to the pelvic canal of heifers after dystocia and in subcutaneous tissue that has been injured

④ Idiopathic necrosis of fat

- By large masses of necrotic fat in the mesentery, omentum, and retroperitoneally
- The cause is unknown and may not be detected until necropsy.

5 Fibrinoid necrosis

- Special form of necrosis, visible by light microscopy
- Immune reactions in which complexes of antigens and antibodies are deposited in the walls of arteries.
- The deposited immune complexes, together with fibrin that has leaked out of vessels, produce a bright pink and amorphous appearance on H&E preparations called fibrinoid (fibrin-like) necrosis

APOPTOSIS

Apoptosis

Definition

- **Apoptosis is a pathway of cell death that is induced by a tightly regulated suicide program in which cells activate enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins**
- Because it is genetically regulated, apoptosis is sometimes referred to as **programmed cell death**.
- Apoptosis - a falling away from' (G. apo = away from; ptosis = falling).
- Apoptosis (pronounced with a silent 'p', as 'apotosis')

Causes of Apoptosis

Apoptosis in Physiologic Situations

- Apoptosis of cells during embryogenesis
- Involution of hormone-dependent tissues upon hormone deprivation
    - Regression of the lactating breast after weaning
- Cell loss in proliferating cell populations
    - Intestinal crypt epithelia - maintain a constant number
- Elimination of cells that have served their useful purpose
    - Neutrophils in an acute inflammatory response
- Elimination of potentially harmful self-reactive lymphocytes

Causes of Apoptosis

Apoptosis in Pathologic Conditions

- **DNA damage**
  - If repair mechanisms cannot cope with the injury → Apoptosis
  - Radiation, free radical, cytotoxic anticancer drugs etc.,
- **Accumulation of misfolded proteins**
  - Gene mutations → misfolded proteins → accumulation in ER → ER stress → apoptosis
- **Viral infections** :- Adenovirus and HIV
- **Pathologic atrophy** in parenchymal organs after duct obstruction, such as occurs in the pancreas, parotid gland, and kidney

Morphology

- Cell shrinkage
- Condensed chromatin and cytoplasm
- Fragments of chromatin and cytoplasm are often found in adjacent or phagocytic cells as **apoptotic bodies**
- Because single cells are dead, gross changes are usually not obvious.
- In addition, because the cell fragments into membrane-bound particles – No inflammation

Mechanisms of Apoptosis

- Apoptosis results from the activation of enzymes called **caspases**
  - so named because they are cysteine proteases that cleave proteins after aspartic acid residues
  - **C**ysteine-dependent **ASP**artyl-specific prote**ASE**
  - **CASPASE**
- Caspases exist as inactive proenzymes, or zymogens, and must undergo enzymatic cleavage to become active.

Mechanisms of Apoptosis

- **Initiator caspases**
  - Caspase 8
  - Caspase 9
  - Caspase 10
- **Executioner caspase**
  - Caspase 3
  - Caspase 6
  - Caspase 7

Mechanisms of Apoptosis

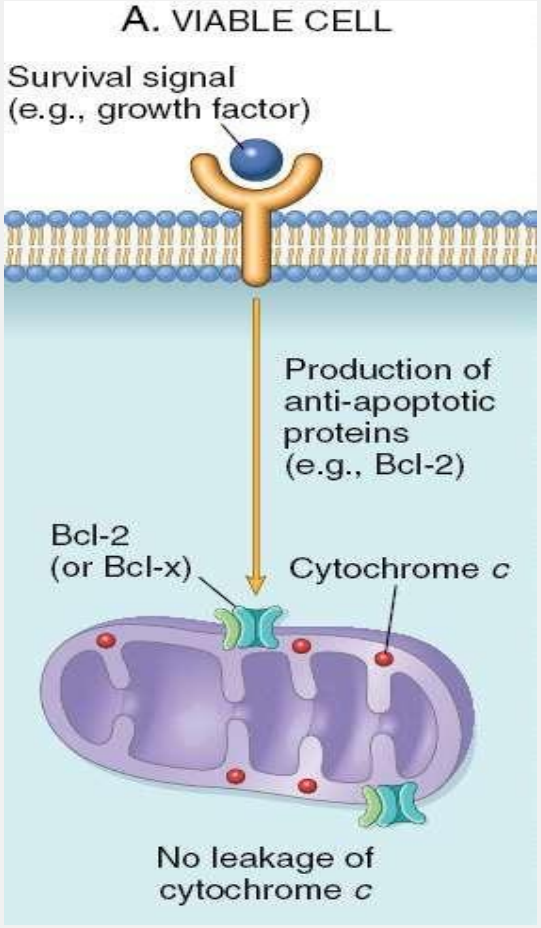
- Apoptosis may be divided into two phase
  - **Initiation phase**
    - Activation of initiator caspases
    - Two distinct but convergent pathways
      - The Intrinsic (Mitochondrial) Pathway
      - The Extrinsic (Death Receptor-Initiated) Pathway
  - **Execution phase**
    - Activated initiator caspases activate executioner caspase to cause cell death

The Mitochondrial (Intrinsic) Pathway

- Mitochondria contain proteins such as cytochrome c

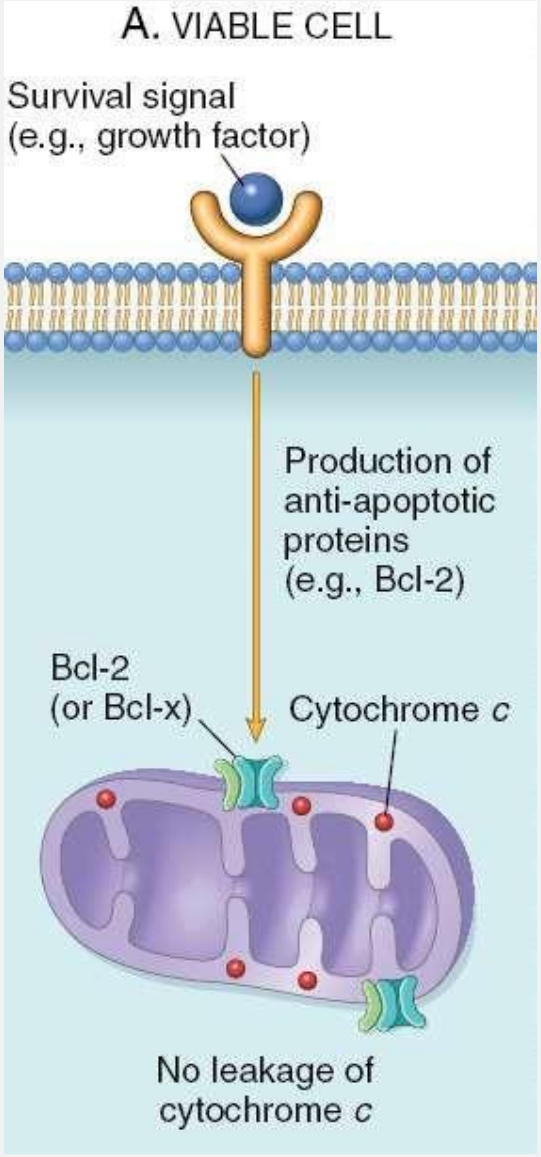
Cytochrome c

- When cell is healthy remains in mitochondria
- When cell is not healthy → releases in to cytoplasm
- This activate caspase enzyme → induced apoptosis



The Mitochondrial (Intrinsic) Pathway

- Release of mitochondrial cytochrome c is tightly controlled by the **BCL2 family of proteins**
- There are more than 20 members of the BCL family
- Can be divided into **three groups** based on their pro-apoptotic or antiapoptotic functions

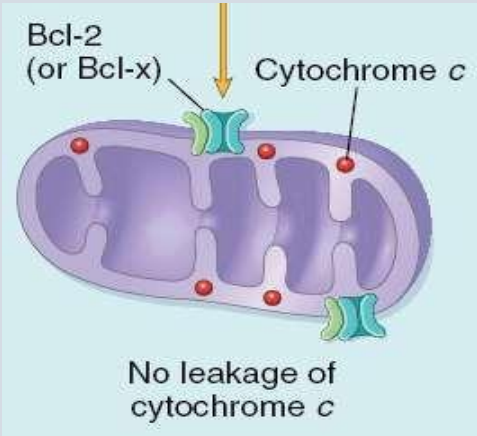


The Mitochondrial (Intrinsic) Pathway

BCL2 family of proteins

❶ Anti-apoptotic proteins (Prevent apoptosis)

- Prevent leakage of cytochrome c into the cytosol by keeping the mitochondrial outer membrane impermeable → Prevent apoptosis
- **BCL2, BCL-XL, and MCL1**
- Present in outer mitochondrial membranes as well as the cytosol and ER membranes

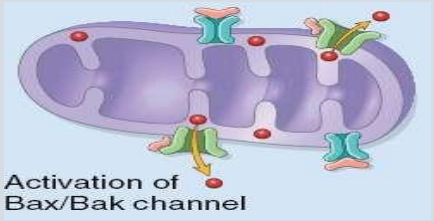


The Mitochondrial (Intrinsic) Pathway

BCL2 family of proteins

❷ Pro-apoptotic proteins (Induced apoptosis)

- Form a channel in the outer mitochondrial membrane
  → Leakage of cytochrome c in to cytoplasm
  → Activation of caspase → Induced apoptosis
- **BAX and BAK**

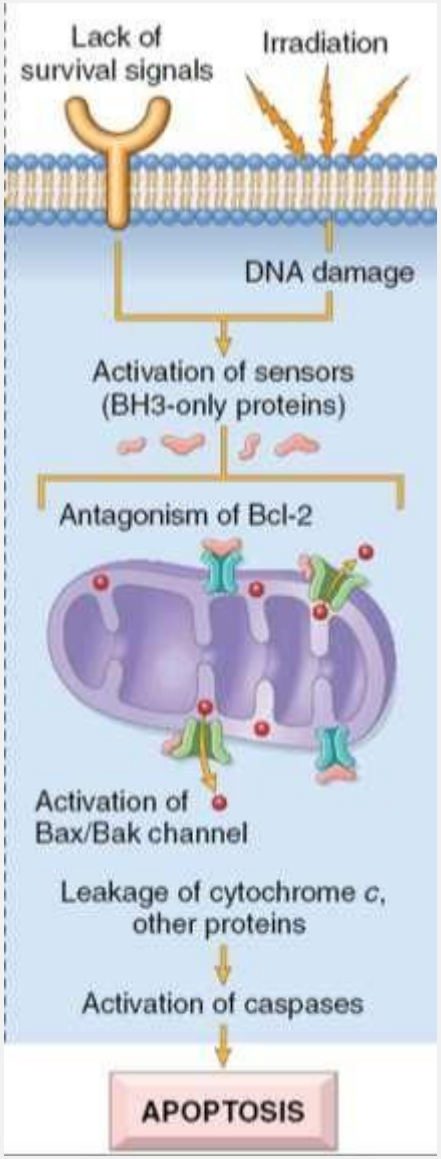


The Mitochondrial (Intrinsic) Pathway

BCL2 family of proteins

❸ Sensors (Arbiters of apoptosis)

- Sometimes called BH3-only proteins
- Sense the cellular stress and damage
- Regulate the balance between the other anti and pro apoptotic proteins
- **BAD, BIM, BID, Puma, and Noxa**



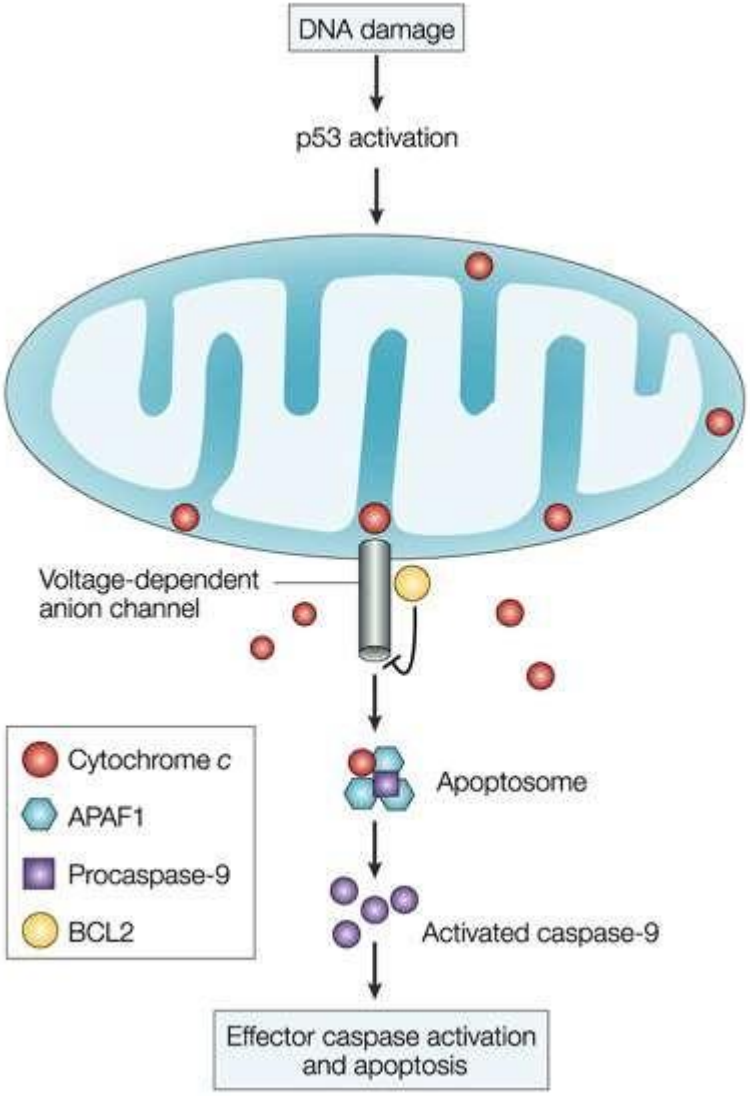
The Mitochondrial (Intrinsic) Pathway

Cytochrome C in cytoplasm

Cytochrome c binds to a protein APAF-1 (apoptosis-activating factor-1)

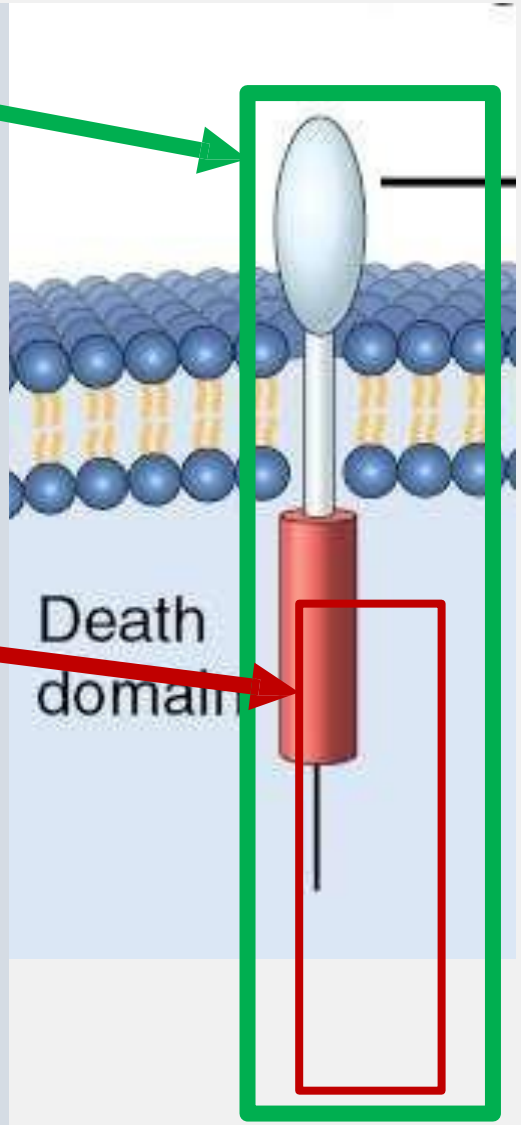
Formation of apoptosome

Bind and activate caspase-9



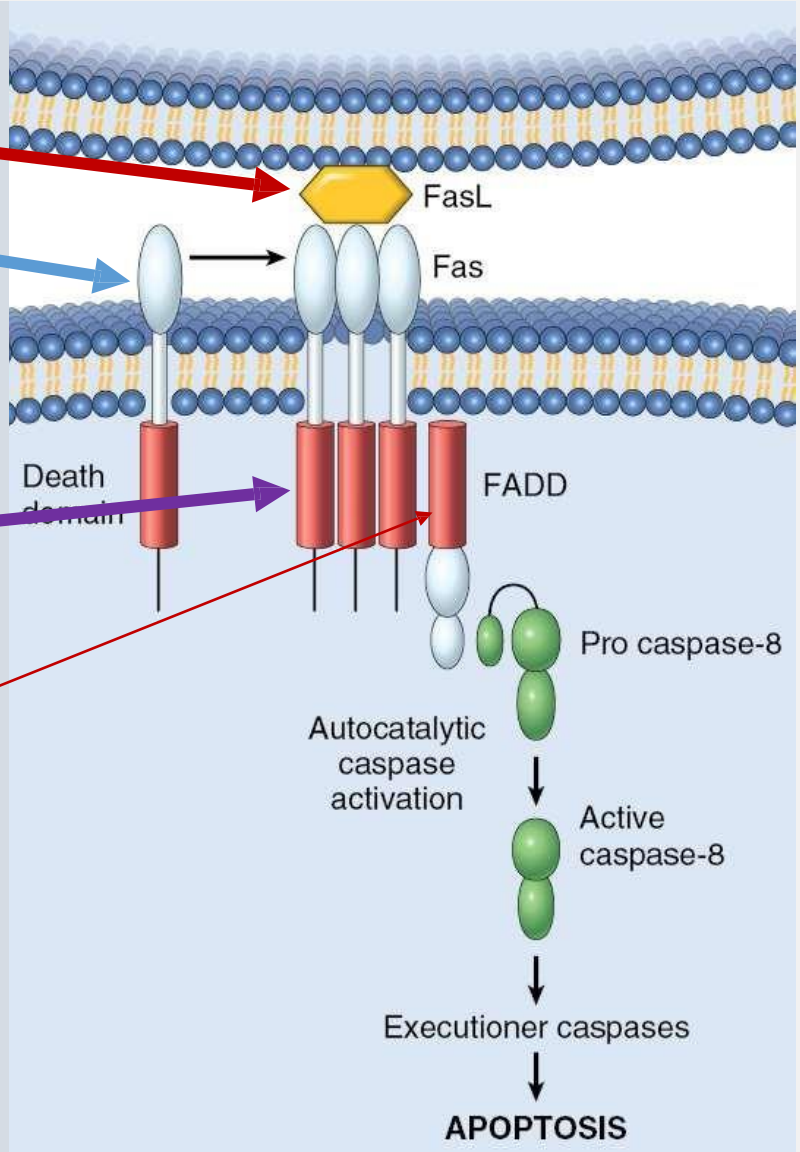
Extrinsic (Death Receptor-Initiated) Pathway

- Variety of cells have **death receptors** in cell membrane
- Belongs TNF receptor family
- Cytoplasmic domain delivering apoptotic signals – hence called as **death domain**
- The best known death receptors are the type 1 TNF receptor (**TNFR1**) and a related protein called **Fas**
- Eliminate self-reactive lymphocytes



Extrinsic (Death Receptor-Initiated) Pathway

- Fas ligand (FasL) expressed on T cells membrane
- FasL binds to Fas receptor
- Three or more molecules of Fas are brought together
- Death domain of Fas receptors comes nearer to each other
- form a binding site for adaptor protein called FADD (Fas-associated death domain)
- FADD binds to multiple inactive caspase-8
- Cleave one another to generate active caspase-8.



Execution phase

Activated initiator caspase 8, 9, 10

Act on executioner caspases 3, 6,

7 Activated executioner

Activate DNase

Cleavage of DNA & nucleoproteins

Degrade components of the nuclear matrix and cytoskeleton

Fragmentation of cells

Phagocytosis by macrophages and

Thank you