APOPTOSIS

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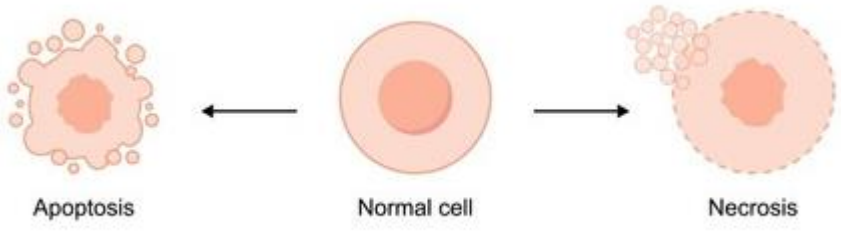
(For B.Sc. Ist Semester, Biotechnology; BT-1)

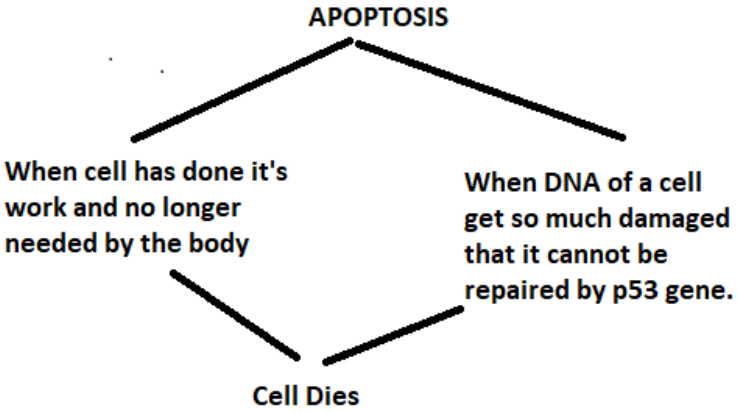
Apoptosis is programmed cell death, a highly regulated process that involves cell shrinkage, DNA fragmentation, and the formation of apoptotic bodies that are then engulfed by other cells. This is distinct from necrosis, where cells swell and burst, causing inflammation. Apoptosis occurs physiologically to remove unwanted cells during development and homeostasis, and pathologically to eliminate damaged or infected cells, and is mediated by caspases.

1. Definition and Key Concepts

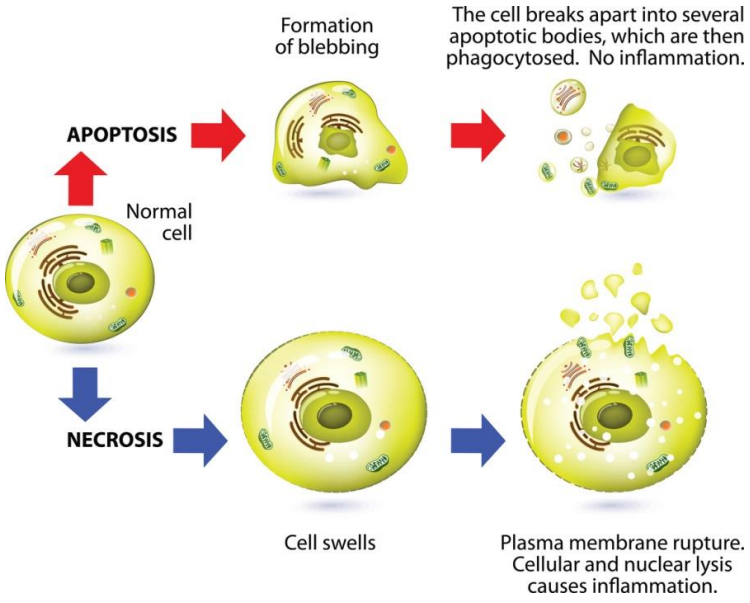
- **Apoptosis** is derived from a Greek word meaning "falling off," like leaves from a tree.
- It is a cell death or cell suicidal pathway.
- It is an active, **genetically controlled** process of "cellular suicide".
- It is distinct from **necrosis**, which is accidental cell death due to acute injury, swelling, and bursting, which causes inflammation in surrounding tissues.
- The primary goal is to maintain **homeostasis** (balance) in the body.



Apoptosis takes place due to two reasons –



- The whole process is mediated by a pathway called as CASPASES ENZYMATIC PATHWAY.
- These caspases enzymes normally remain inactive in the cell.
- But they can be activated by either INTRINSIC PATHWAY or EXTRINSIC PATHWAY.
- These pathways degrade Nucleus, cytoplasmic proteins, cell membrane, organelles etc.
- Multiple fragments of nucleus and other parts are formed surrounded by a vesicle like membrane.
- These vesicles are called as APOPTOTIC BODIES.
- Ultimately Phagocytosis of these apoptotic bodies occurs
- No leakage/ No inflammation occurs (Hallmark of Apoptosis).
- Apoptosis generally occurs in one cell at a time manner, whereas Necrosis occurs in group of cells.
- Apoptosis requires ATP for action of caspase pathway.



2. Morphological Changes (Steps)

Apoptosis follows a highly ordered sequence of events:

1. **Cell Shrinkage:** The cell loses volume and detaches from neighboring cells.
2. **Chromatin Condensation (Pyknosis):** The nuclear material condenses into dense, dark-staining masses, typically at the periphery of the nucleus.
3. **Nuclear Fragmentation (Karyorrhexis):** The nucleus breaks down into several smaller fragments.
4. **Membrane Blebbing:** The cell surface develops bubble-like protrusions (blebs).
5. **Apoptotic Body Formation:** The cell fragments into small, membrane-enclosed vesicles called apoptotic bodies, which contain tightly packed organelles and nuclear fragments.
6. **Phagocytosis:** Specialized immune cells, such as macrophages, rapidly engulf and degrade the apoptotic bodies, preventing the release of cellular contents and inflammation.

3. Biochemical Features

- **Energy-dependent:** Apoptosis requires ATP (energy) to proceed in an orderly fashion.
- **Caspase Activation:** The process is orchestrated by a family of cysteine proteases called caspases (cysteine-aspartic proteases). They are synthesized as inactive precursors (procaspases) and activated by cleavage in a cascade.

Biochemical Events

The core of apoptosis is the activation of a family of cysteine proteases called **caspases**.

- **Initiator Caspases** (e.g., caspase-8, caspase-9, caspase-10) are activated first by specific signaling complexes.
- **Executioner Caspases** (e.g., caspase-3, caspase-6, caspase-7) are then cleaved and activated by initiator caspases. These executioners dismantle the cell by cleaving vital proteins, including nuclear lamins, cytoskeletal proteins, and an enzyme (CAD) that fragments DNA.
- **DNA Fragmentation:** Executioner caspases activate an enzyme (Caspase-Activated DNase, or CAD) that chops up genomic DNA into characteristic fragments of about 180-200 base pairs (visible as a "DNA ladder" on gel electrophoresis).
- **Phagocytosis Signals::** A lipid called phosphatidylserine, normally on the inner cell membrane leaflet, flips to the outer surface, acting as an "eat-me" signal for phagocytes.

4. Pathways of Apoptosis

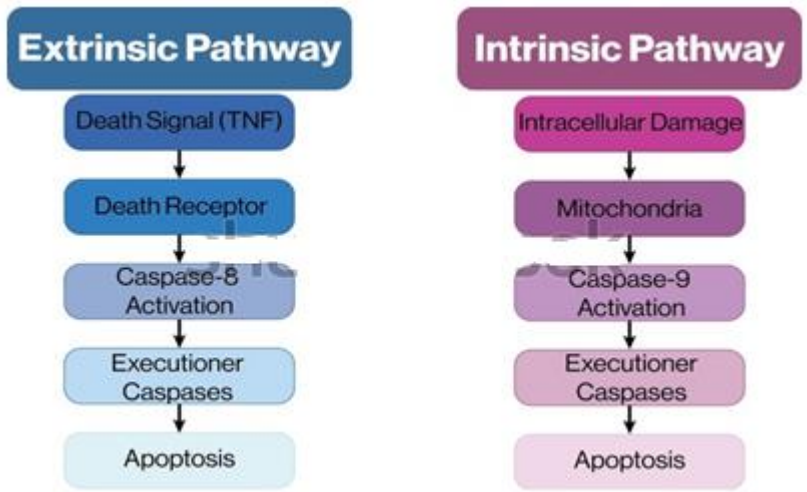
Apoptosis can be triggered by two main signaling pathways, both of which activate the caspase cascade:

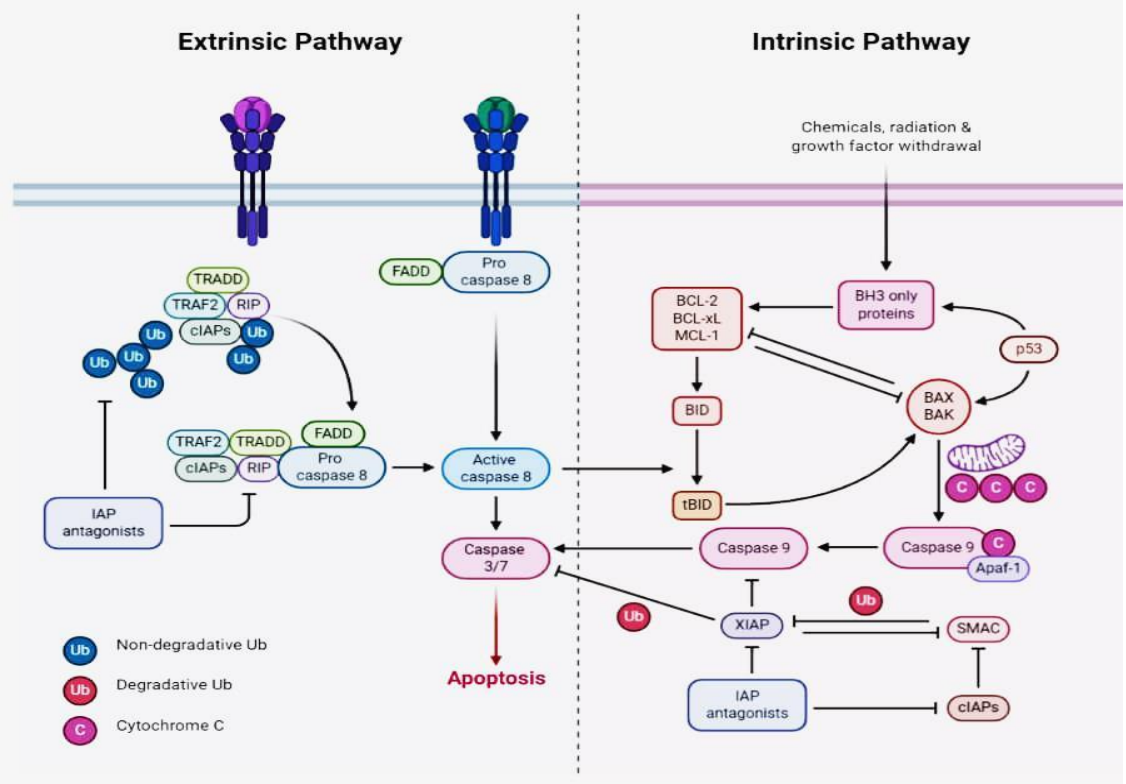
A. Extrinsic Pathway (Death Receptor Pathway)

- **Triggered by external signals** from other cells.
- **Death ligands** (e.g., Fas ligand, TNF-alpha) bind to **death receptors** (e.g., FasR, TNFR1) on the cell surface.
- This binding leads to the formation of a **Death-Inducing Signaling Complex (DISC)**, which activates the initiator caspase-8.
- Activated caspase-8 then activates executioner caspases (caspase-3, -7).

B. Intrinsic Pathway (Mitochondrial Pathway)

- **Triggered by internal cellular stress** (e.g., severe DNA damage, oxidative stress, lack of growth factors, hypoxia).
- Stress signals lead to the release of pro-apoptotic proteins (like **cytochrome c**) from the mitochondria into the cytoplasm.
- Cytochrome c binds to Apaf-1 and ATP to form the **apoptosome** complex.
- The apoptosome activates the initiator caspase-9, which in turn activates executioner caspases.
- This pathway is regulated by the **Bcl-2 family of proteins**, which can be pro-apoptotic (Bax, Bak) or anti-apoptotic (Bcl-2, Bcl-xL). The balance between these determines the cell's fate.





Apoptosis

Triggered by intracellular pathway; downstream of p53

Extrinsic (receptor mediated)

TNF or FAS

TRADD FADD

Induce DISC

procaspase 8/10*

Caspase 8/10**

Intrinsic (mito mediated)

DNA damage or ER stress

Caspase 2

A permeability of mito membrane

Increase Bad/Bax = Increase →
Increase Bcl2 = Decrease →

Release of Cytochrome C

+ Procaspase 9*

Caspase 9** (apoptosome)

* = initiator caspase

** = executioner or effector caspase

Caspase 3/6/7 → APOPTOSIS

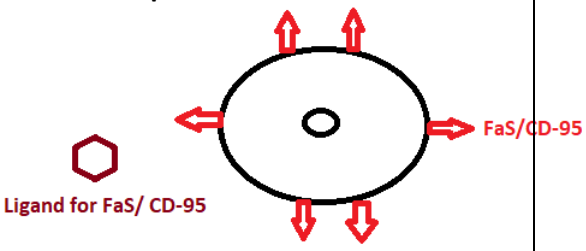
5. Physiological and Pathological Significance

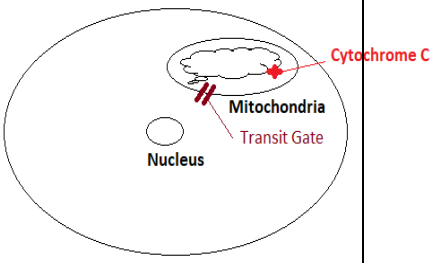
Apoptosis is crucial for normal development and health:

- **Embryonic Development:** Sculpting body parts, like separating fingers and toes from webbed tissue.
- **Tissue Homeostasis:** Removing old, worn-out cells to make way for new ones (e.g., in the gut lining and immune system).
- **Immune System:** Eliminating self-reactive immune cells and cells infected by viruses.
- **Disease Prevention:** Destroying cells with irreparable DNA damage to prevent cancer.

Dysregulation of apoptosis is involved in many diseases:

- **Insufficient apoptosis:** Leads to cancer and autoimmune disorders.
- **Excessive apoptosis:** Linked to neurodegenerative diseases (e.g., Alzheimer's, Parkinson's, Huntington's) and ischemic damage.

<table>
<tr><th colspan="4">APOPTOSIS PATHWAYS</th></tr>
<tr><th colspan="2">Extrinsic Pathway</th><th colspan="2">Intrinsic Pathway</th></tr>
<tr><th>Initiation</th><th>Execution</th><th>Initiation</th><th>Execution</th></tr>
<tr><td>Separate for intrinsic pathway</td><td>Common for both intrinsic and extrinsic pathways</td><td>Separate for extrinsic pathway</td><td>Common for both intrinsic and extrinsic pathways</td></tr>
<tr><td>Specific Receptors called as DEATH RECEPTORS -
1. FaS Protein (CD-95)
2. TNF Receptors
 

1. When ligand attaches with FaS/ CD-95 receptor, the Aggregation of Fas receptors occurs and then it is called as FADD (FaS Associated Domain)</td><td></td><td>• Non Receptor Mediated Stimuli
(DNA damage)
• Mitochondria initiated events.
 

1. The Mitochondrial Transit Gate is Gated by GUARD ANTIAPOPTOTIC PROTIENS that keeps the gate closed to prevent escaping of cytochrome C protein from inner mitochondrial membrane in to the cytoplasm.
2. These Guard anti-</td><td></td></tr>
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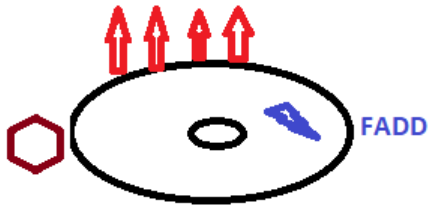
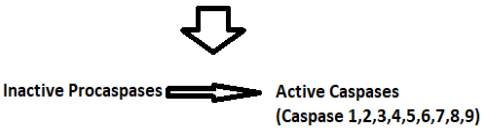
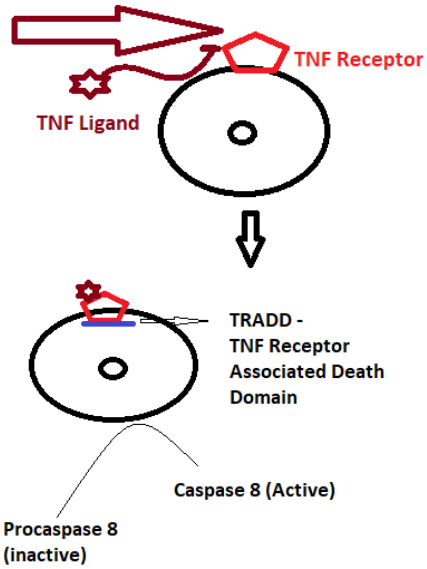


Here multipile Death Receptors comes close to each other and FADD domain in the cytoplasm is formed
Which is called as Death Domain.



The firs procaspase (inactive) that becomes active is Caspase 8

2. **TNF Receptors**



apoptotic proteins are named as Bcl2, Bclx, Mcl, FLIP.

3. When DNA of a cell get damaged beyond capacity of p53 gene repair system, then the cell itself sends a signal to it's Mitochondria to send the cell in the Apoptotic Pathway.
4. During this process, Anti-apoptotic proteins (Bcl2, Bclx, Mcl, FLIP) are replaced by other three guard proteins called PRO-APOPTOTIC PROTEINS.
5. These includes –
   - Bax
   - Bak
   - Bim
   - P53
   - Apaf-1
   - CytC
6. Bax, Bak & Bim activated first and opens the Transit Gate.
7. As the gates opens, the cytochrome C escapes from mitochondria in to the cytoplasm.
8. As cytochrome reaches cytoplasm, it first converts inactive Pro-caspase -9 to Active CASPASE-9 (by removing Apaf-1 inhibitory protein from Procaspase-9)
9. **Cell enters Apoptotic pathway.**

Execution Phase

(Common to both extrinsic and Intrinsic Pathways)

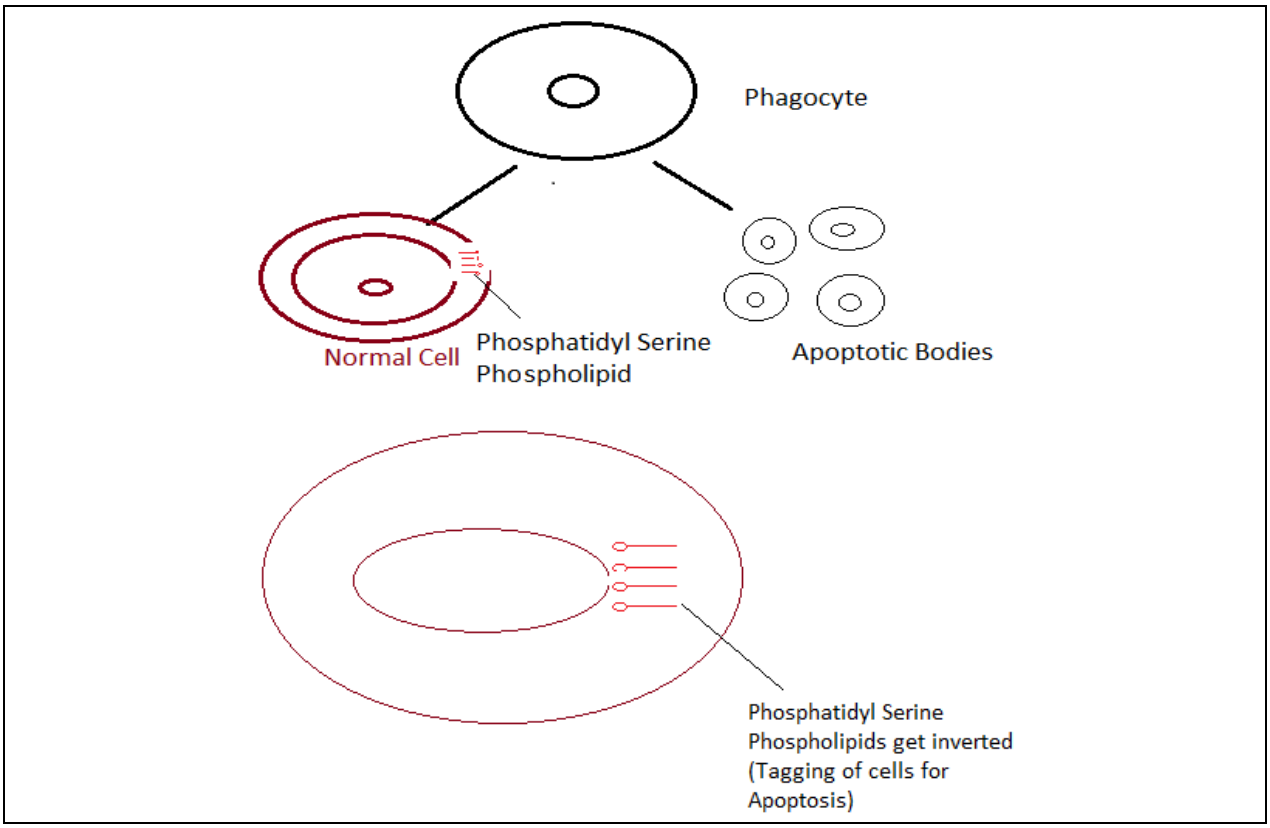
1.Active caspase 8 from Extrinsic Pathway and in a separate case Caspase 9 from Intrinsic pathways Activates caspase 3&7

2. Sequentially activates all other caspases.

3. Caspases cleaves cytoskeletal & Nuclear Matrix Proteins

4. Cell gets degenerated into Apoptotic Bodies.

5. Phagocytosis Occurs by Phagocytic cells.


Phagocyte
Normal Cell
Phosphatidyl Serine
Phospholipid
Apoptotic Bodies
Phosphatidyl Serine
Phospholipids get inverted
(Tagging of cells for
Apoptosis)