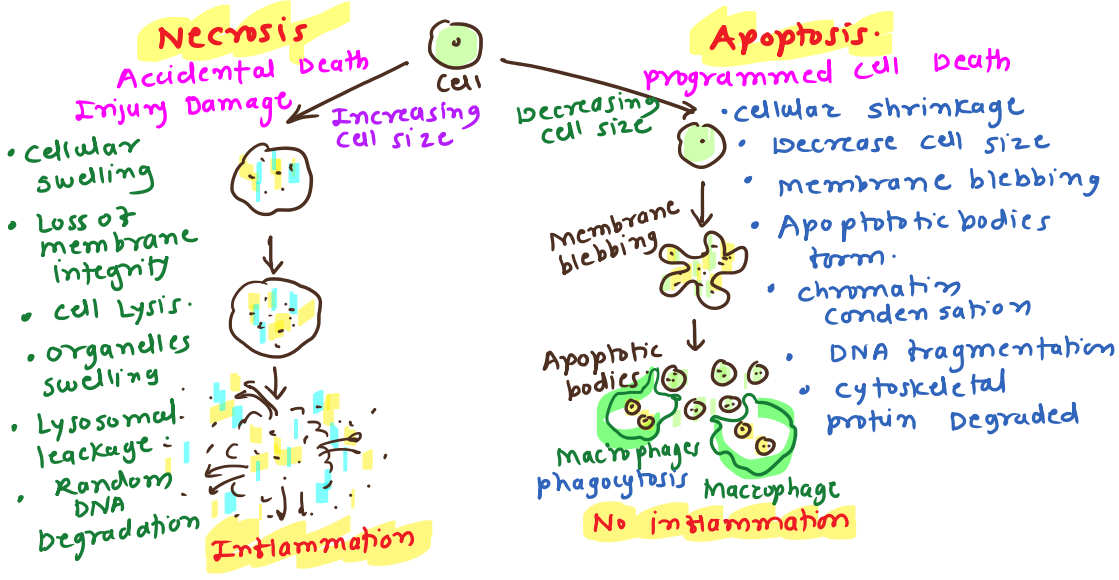


Cell Death

Necrosis
- Accidental cell Death
- Injury
- Damage

Autophagy
- Autophagocytosis
- self cell Distruct Damage organelles.
- Mitochondria
- ER
- peroxisomes.
- Aggregated, mistolded protins in cytosol
- stress condition.

Apoptosis.
- programmed cell Death
- Maintain tissue balance
- Dead cells Removed (Skin, Blood cells)
- metamorphosis.
- Early Development. 50% Neurons eliminated.
- DNA Damage.



Apoptosis...

* Morphological changes occur in Apoptotic cell -.

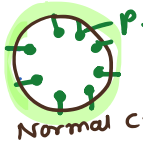
- Decrease cell volume (cell size↓)
- Condensation, shrinking
- Membrane blebbing
- Modification of cytoskeleton
- Degradation of Golgi Matrix proteins.
- [illegible] – smaller

- Degradation of Golgi matrix proteins.
- DNA fragmentation – smaller fragments.
- Apoptotic bodies – Membrane enclosed bodies formation

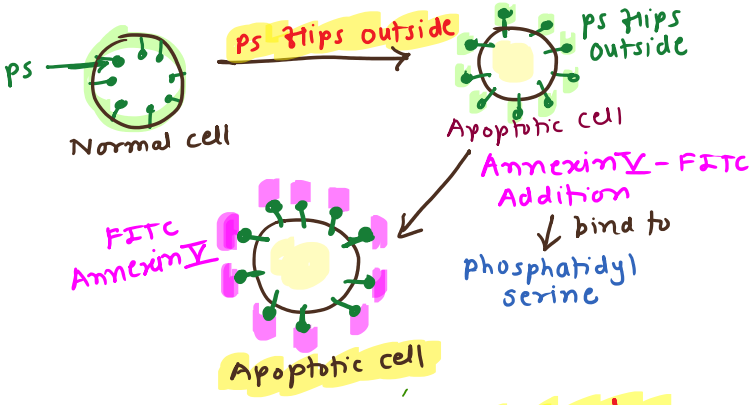
* Detection of Apoptotic cell –

1) phosphatidyl serine (PS)

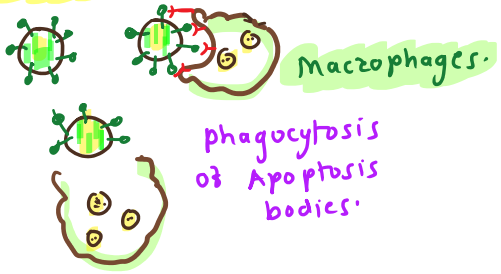
- Negatively charged phospholipid.
- Phosphatidyl serine Located in inner leaflet of Lipid bilayer.



- In Apoptotic cells –
  - PS (phosphatidyl serine) flips outside



- **PS – Act as Eat me signal –**



2) DNA fragmentation –

- Agarose gel electrophoresis.

- In Normal cells –
  - Intact DNA (Normal DNA)

ICAD ⊣ DNAases.

(No DNA Fragmentation)

- In Apoptotic cells –

Caspases ⊣ ICAD.

- DNAases Activation.

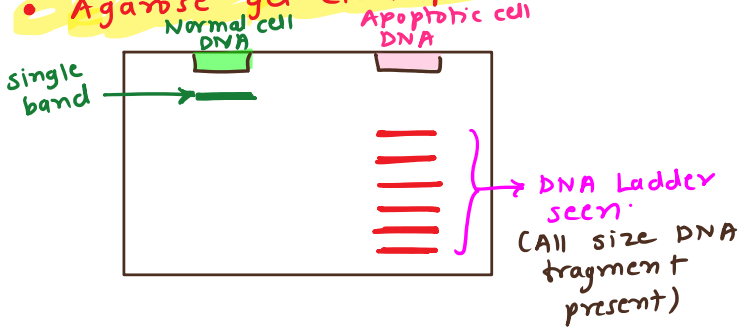
↓

- DNA fragmentation

• cleave DNA at linker Regions.

All size DNA fragment formation

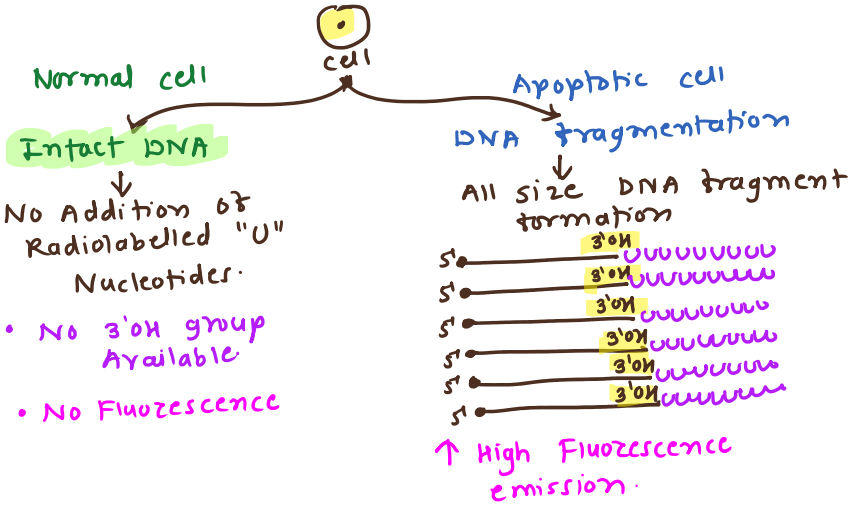
• Agarose gel electrophoresis -



2) TUNEL Technique -

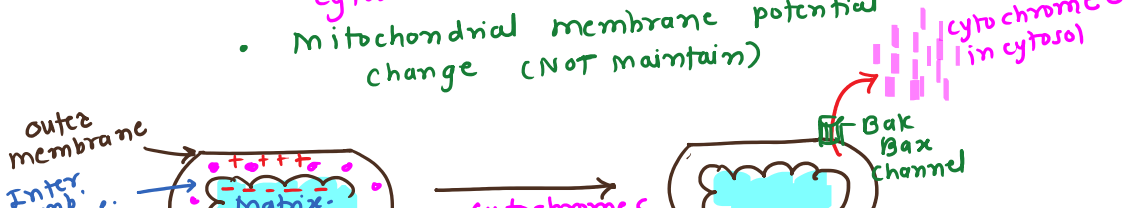
Terminal Deoxy 'U' Nucleotide End Labelling.

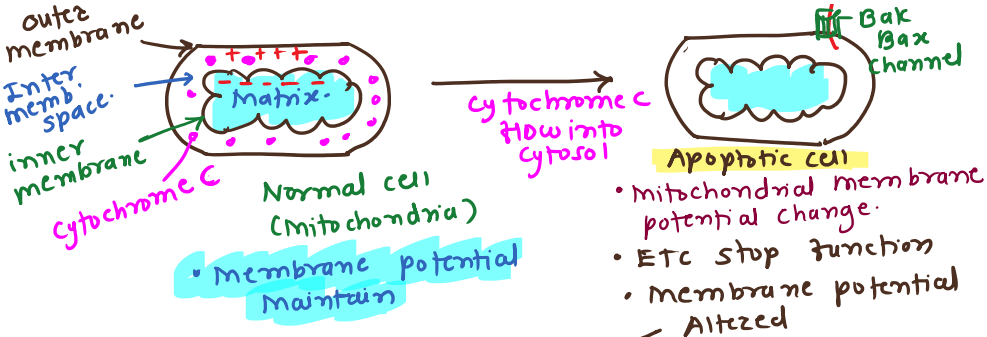
- Use of TdT – Terminal deoxytransferase
- Addition of Nucleotides in 3'OH group in Template independent manner.
- Addition of Labelled 'U' Nucleotides.



* Mitochondrial membrane potential

- In Normal cell –
  - Normal ETC function
  - Mitochondrial membrane potential maintain.
- In Apoptotic cell –
  - ETC stop functioning.
  - cytochrome c flow outside into cytosol.
  - Mitochondrial membrane potential change (NOT maintain)





Detection of Altered membrane potential

- Use of positively charged Dye.
- Use of Voltage sensitive Dye.

* FACS – Fluorescent Activated cell sorting –

- Use of flow cytometry

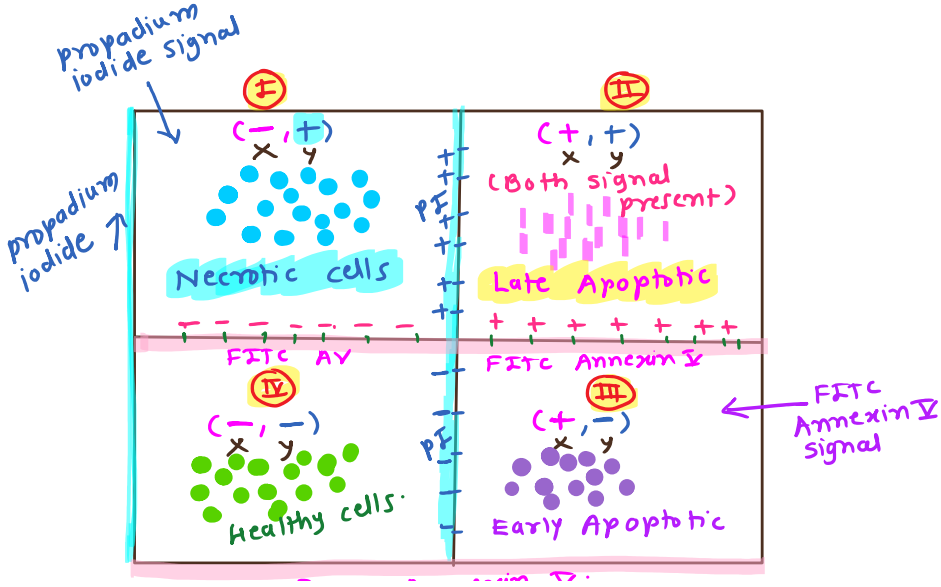


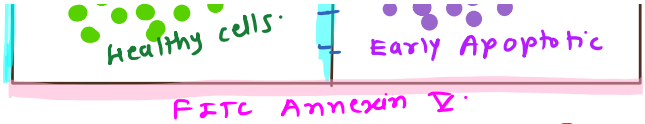
FACS
Flow cytometry
- Identification
- separation
- Detection

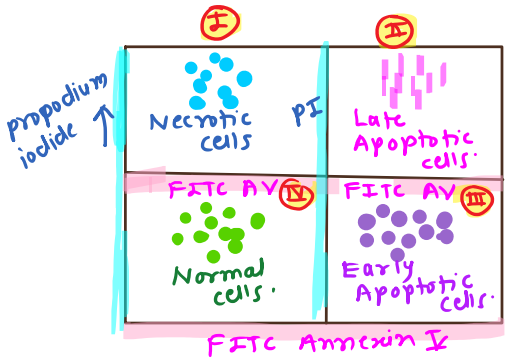
- Normal cells
- Necrotic cells
- Early Apoptotic cells.
- Late Apoptotic cells.

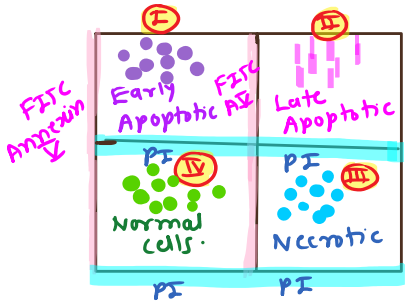
* Labelling of cells →

- Normal Healthy cells → Not Labelled. (No signal)
- Necrotic cells → Emit propadium iodide signal.
- Early Apoptotic cells → Emit FITC-Annexin V signal
- Late Apoptotic cells → Emit FITC-Annexin V
  Emit propadium iodide signal.



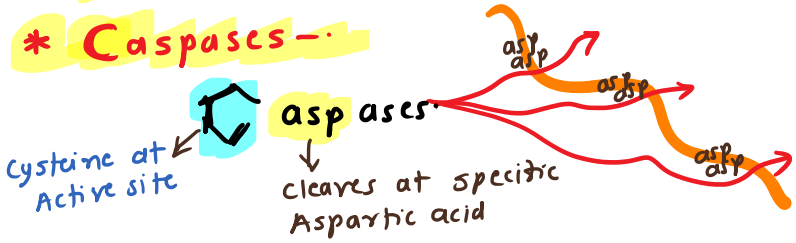




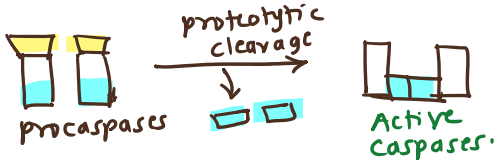


Apoptosis...

* Caspases -.

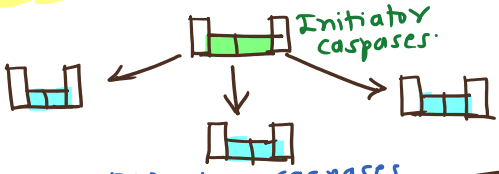


- caspases are cysteine proteases.
- synthesize in inactive form.



Zymogens examples.
- caspase
- complement protein
- Digestive enzymes

* Caspase Cascade -.



- Effector caspases
- Executioner caspase.

Inhibition of ICAD → DNA fragmentation

Nuclear Lamins Degraded → Fragmentation of Nucleus

Cytoskeleton protein Degradation → Membrane blebbing Apoptotic bodies Formation

Golgi Matrix protein Degradation → Golgi Fragmentation

Scramblase flippase Inactive → PS flips outside

* Caspases involved in Apoptosis -.

caspase 2, 8, 9, 10.

* Caspases involved in Apoptosis -

- Initiator caspases → caspase 2, 8, 9, 10.
- Effector caspases → caspase 3, 6, 7,
- caspase 8/10 → Initiator caspase → Extrinsic pathway
- caspase 2/9 → Initiator caspase → Intrinsic pathway

* NoT all Caspases involved in Apoptosis —

- caspase 1 → ICE (Interleukin-1 converting enzyme)
  - Human protein
  - Involved in inflammation
- Caspases involved in Inflammation -.
  caspase 1, caspase 4, caspase 5.

* C. elegans Apoptosis —.

Caenorhabditis elegans



1090 somatic cells.
131 · Apoptosis
959 cells Adult. (survive)

* In 131 Apoptotic cells →

- two genes are expressed.

↑ CED4 ↑CED3 → Apoptosis.

- CED4 — Gain of function
- CED3 — Loss of function

} No Apoptosis

CED4 → CED3 → Apoptosis.

- CED4 — Loss of function
- CED3 — Gain of function

} Apoptosis occur.

CED4 → CED3 → Apoptosis.
Adaptor protein — Effector caspase.

* In 959 cells — survive because -.

CED9 ⊣ CED4 → CED3 → No Apoptosis.

- CED9 — Loss of function → Apoptosis occur
- CED9 — Gain of function → No Apoptosis occur

CED9 ⊣ CED4 → CED3 → No Apoptosis.

CED9 ⊣ CED4 → CED3 → No Apoptosis.

Anti-Apoptotic protein

* In Death signal – In C.elegans-

Egl I ⊣ CED9 CED4 → CED3 → Apoptosis.

(pro-Apoptotic protein)

* Apoptosis pathway in C. elegans –

Egl I ⊣ CED9 ⊣ CED4 → CED3 → Apoptosis

(pro-Apoptotic protein) Anti-apoptotic Adaptor protein Effector caspase.

* Apoptosis protins Highly conserve –

	Pro-Apoptotic	Anti-Apoptotic	Adaptor protin	Effector caspase
C.elegans	Egl I	CED9	CED4	CED 3
Mammals.	BH3 only. Bad, Bid Bim, puma Noxa, Bik.	Bcl2 Bcl-XL Mcl-1 Bcl-xs.	Apaf-1 (Apoptotic protease Activating factor-1)	caspase 3.

* Higher Animals Apoptosis –

BH3 only. Bad, Bid Bim, puma, Noxa, Bik. (pro-Apoptotic) ⊣ Bcl2 Bcl-xl Mcl-1 Bcl-xs (Anti-Apoptotic) ⊣ BH123 Bak Bax (pro-Apoptotic) (cytosol) → Apaf-1 (Apoptotic protease Activating factor-1) + cytochrome C → caspase 9 Active (Initiator caspase) → caspase 3 Active (Effector caspase) → Apoptosis

Apoptosis

Extrinsic Apoptosis

Intrinsic Apoptosis path.

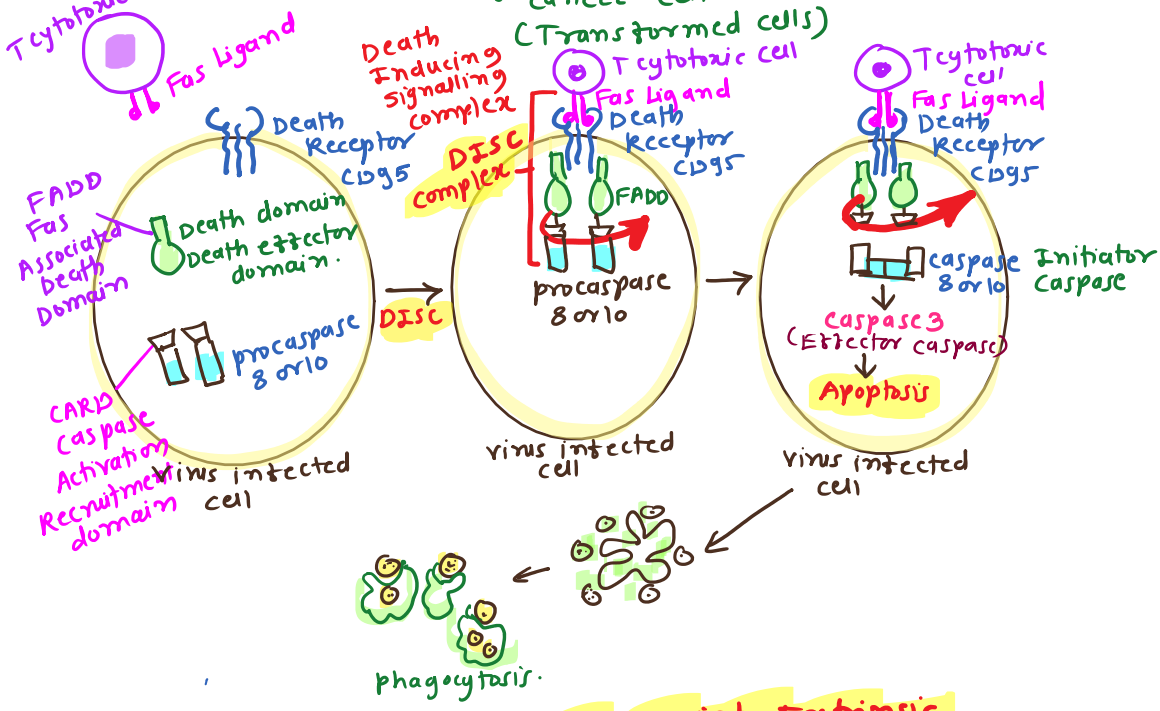
Extrinsic Apoptosis path

Intrinsic Apoptosis path.

* Extrinsic pathway of Apoptosis-.

- Extracellular signal protuins.
- signal → • TNF- Tumor Necrosis factor (macrophage)
  - Fas Ligand. (T cytotoxic cells)

• Target cells → • Virus infected cells.
• cancer cell (Transformed cells)



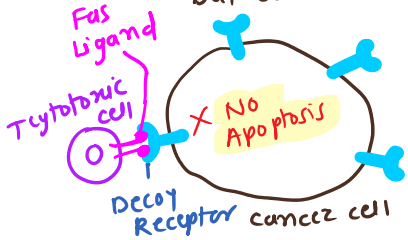
* Some Cancer cells Resist Extrinsic Apoptosis path-.

- Decoy Receptor express -.
  - False Receptor
  - Resemble Death Receptor



Having Ligand binding domain but NOT Death domain

They can bind to Fas Ligand but cannot Activate Apoptosis



2) C- FLIP proteins -.

- C- FLIP – Intracellular blocking proteins.

- Resemble initiator caspase
- It Lacks – proteolytic domain
- It compete with Initiator caspase & bind to FADD.

↓

No Apoptosis occur