Apoptosis/Programmed Cell Death

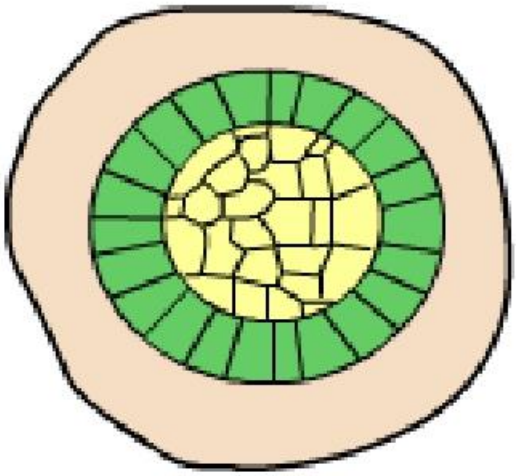
Programmed Cell Death/apoptosis

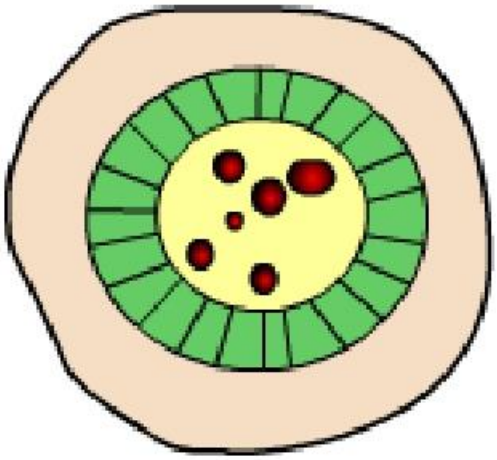
- An organized process in which various cellular components are dismantled systematically
- apoptosis (pronounced apotosis), Greek word meaning ‘‘falling off ’’ of leaves from a tree/
- “dropping off ’’ of petals from flowers
- This term was first used in a classic BJC paper by Kerr, Wyllie, and Currie in 1972
- Human body contains about 10^{14} cells (100 trillion)
- 50 - 70 billion cells/day/person eliminated by apoptosis
- 20 - 30 billion cells/day/child die by apoptosis
- Apoptosis is a form of cell suicide
- Apoptosis is a controlled / coordinated and often energy-dependent process
- Helps the organism in eliminating unwanted cells with minimal damage to surrounding cells
- Apoptosis occurs normally during development and aging
- A homeostatic mechanism to maintain cell populations in tissues
- Apoptosis also occurs as a defense mechanism (immune reactions or when cells are damaged by disease or toxic agents).
- Apoptosis is also necessary to eliminate pathogen-invaded cells and is a vital component of wound healing in that it is involved in the removal of inflammatory cells and the evolution of granulation tissue into scar tissue.

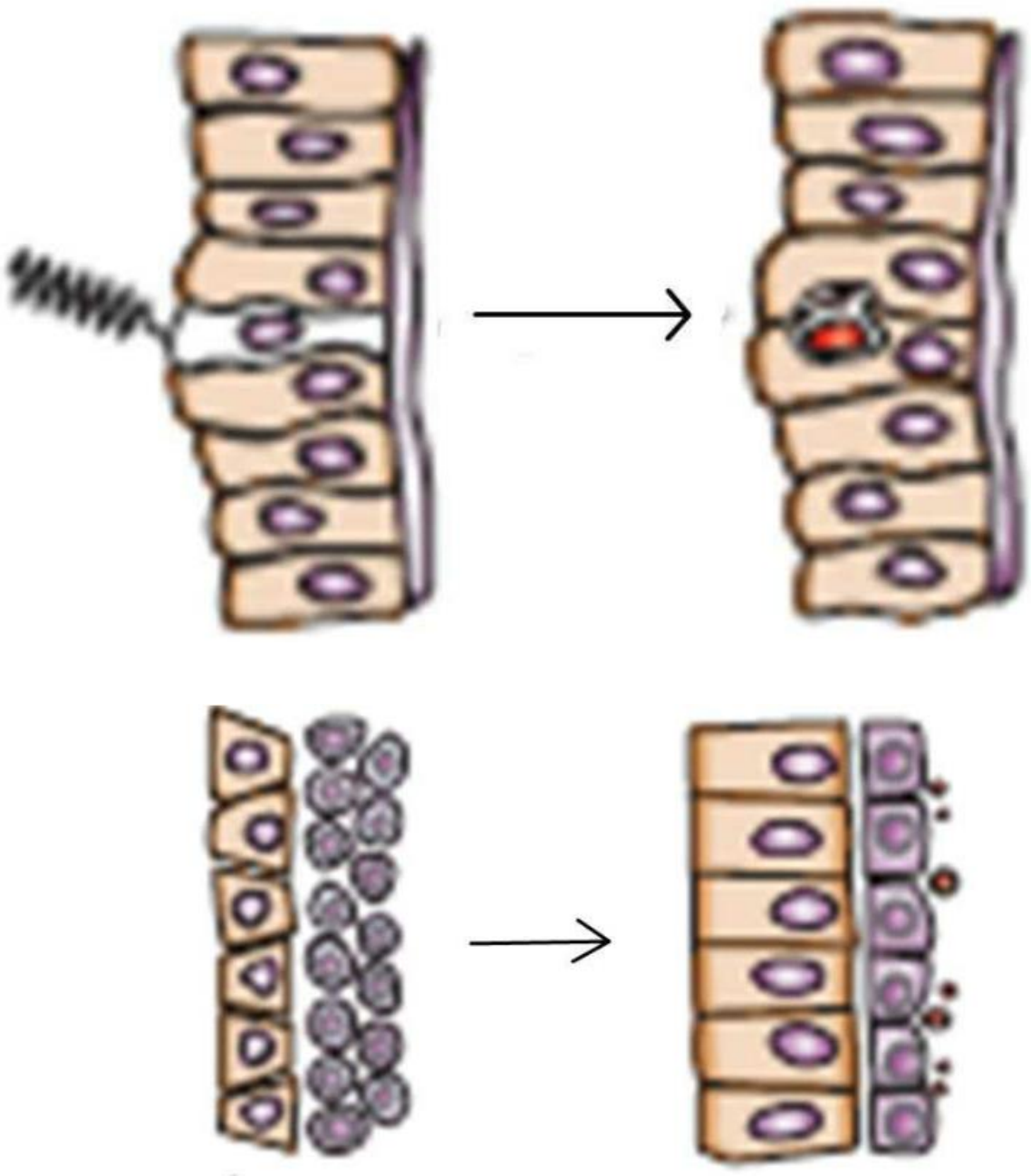
Physiological roles of apoptosis

Before apoptosis

after apoptosis





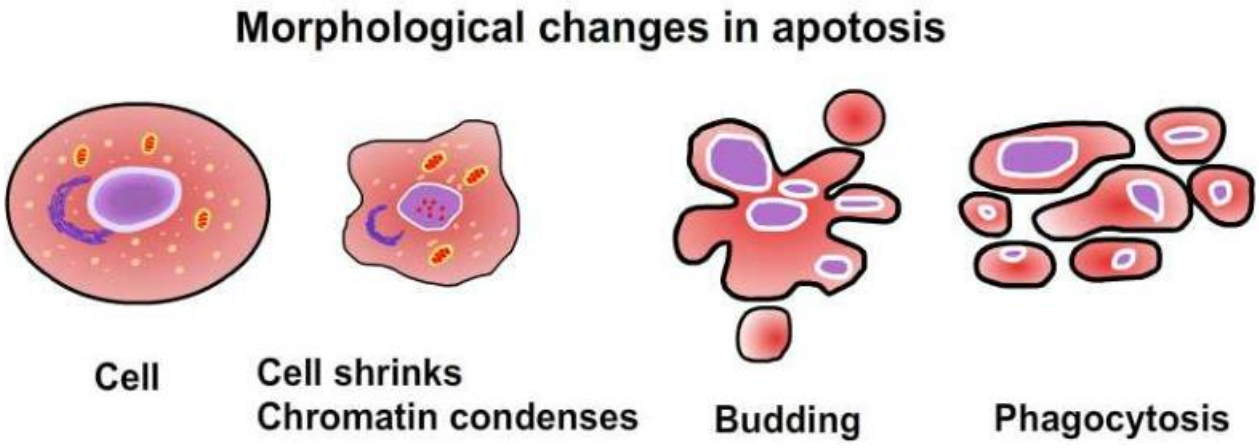


Nobel Prize in Physiology and Medicine 2002

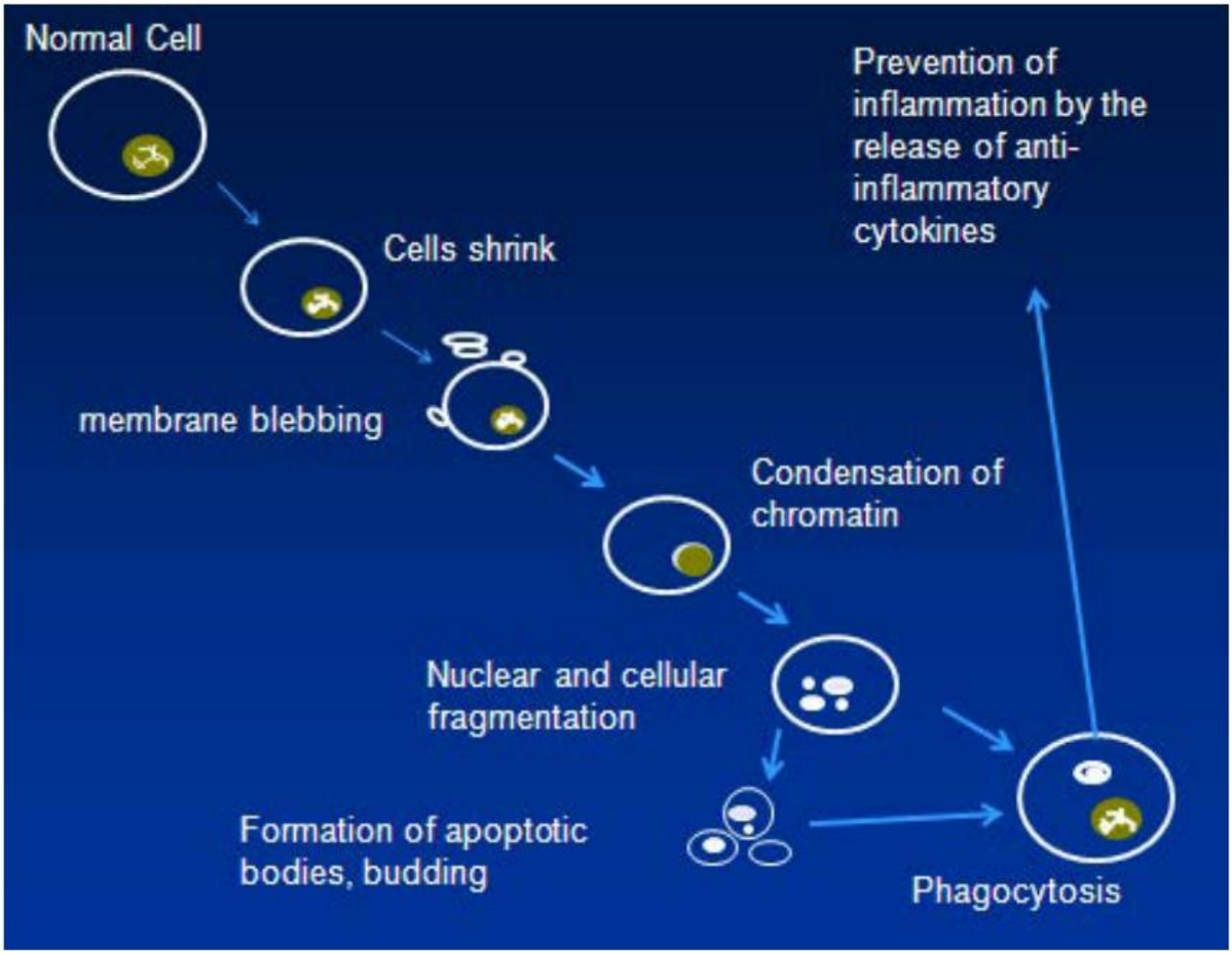
- Sydney Brenner (born 1927), Berkeley, CA, USA, established *C. elegans* as a novel experimental model organism. Brenner's discoveries were carried out in Cambridge, UK.
- Robert Horvitz (born 1947), Cambridge, MA, USA, has discovered and characterized key genes controlling cell death in *C. elegans.* He has shown how these genes interact with each other and that corresponding genes exist in humans.
- John Sulston (born 1942), Cambridge, England. He showed that specific cells undergo programmed cell death as an integral part of the normal differentiation process, and he identified the first mutation of a gene participating in the cell death process.

Morphological changes in apoptosis

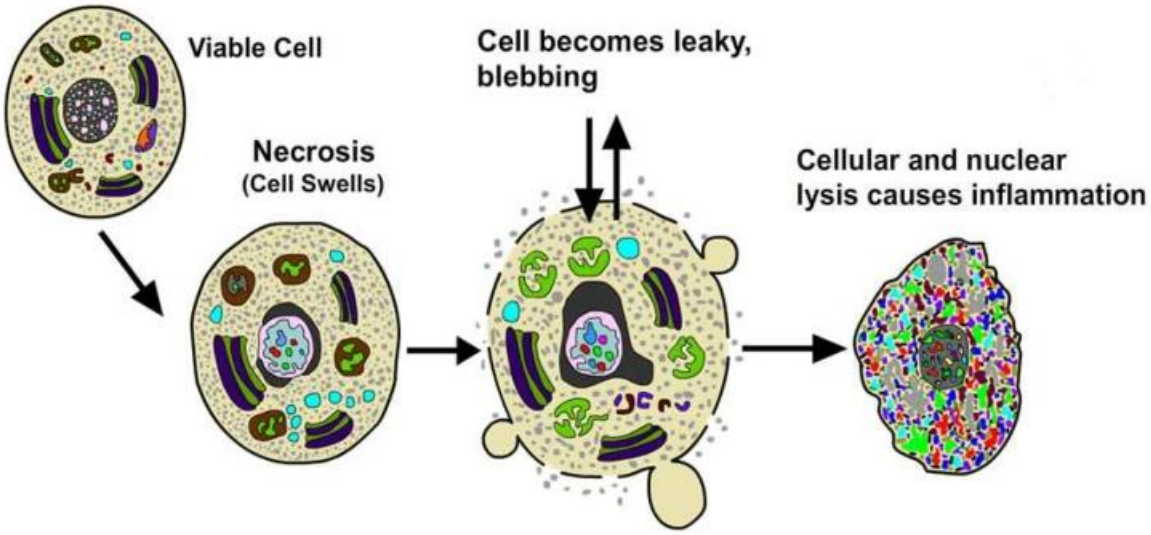
- Light and electron microscopy
- cells shrink, smaller in size, cytoplasm is dense with tightly packed organelles.
- Pyknosis - chromatin condensation - the most characteristic feature
- Cells are round or oval with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments.
- Extensive plasma membrane blebbing occurs



- Separation of cell fragments into apoptotic bodies - “budding.”
- Apoptotic bodies have cytoplasm with tightly packed organelles with or without a nuclear fragment but with an intact membrane.
- Apoptotic cells do not release their cellular constituents into the surrounding interstitial tissue.
- They are quickly phagocytosed by surrounding cells.
- No inflammatory reaction



Necrosis


Viable Cell
Necrosis
(Cell Swells)
Cell becomes leaky,
blebbing
Cellular and nuclear
lysis causes inflammation

Differences between Apoptosis and Necrosis

Apoptosis	Necrosis
Controlled	Uncontrolled
Energy dependent	Energy independent
No inflammation	Inflammation
Shrinking of cells	Swelling of cells
Intact membrane	Direct damage to cell membrane
Single cells affected	Groups of cells affected
Condensation of chromatin	Irregular chromatin clumping
Cytoplasm retained in apoptotic bodies	Cytoplasm is released

Inducers of Apoptosis

Physiological Activators	Damage-Related Activators	Therapy-associated Agents
TNF family	Heat Shock	Chemotherapeutic drugs
Fas Ligand	Viral Infection	cisplatin,
TNF	Bacterial Toxins	doxorubicin,
TGF-b	Oncogenes	bleomycin,
Neurotransmitters	myc,rel, E1A	cytosine
Glutamate	tumour suppressors	arabinoside,
Dopamine	P53	nitrogen mustard,
N-methyl-D-aspartate	Cytolytic T cells	methotrexate,
Growth Factor withdrawal	Oxidants	vincristine
Loss of matrix attachment	Free Radicals	Gamma-radiation
Calcium	Nutrient Deprivation	UV-radiation
Glucocorticoids		

Diseases with excessive apoptosis

- AIDS
- Neurodegernative disorders
  - Alzeheimer’s disease,
  - Parkinson’s disease,
  - Amyotrophic lateral sclerosis
  - Retinitis pigmentosa
- Myelodysplastic syndromes
  - Aplastic anaemia
- Ischaemic Injury
  - Myocardial infarction,
  - Stroke,
  - Reperfusion injury
- Toxin-Induced liver disease
  - Alcohol

Diseases with defective apoptosis

- **Cancer**
  - Follicular lymphomas
  - carcinomas with p53 mutations
  - hormone dependent tumours:
  - breast cancer, prostate cancer,
  - ovarian cancer

- **Autoimmune Disorders**
  - Systemic lupus erythematosus
  - Immune-mediated glomerulonephritus
- **Viral Infections**
  - Herpes virus, poxvirus, adenovirus

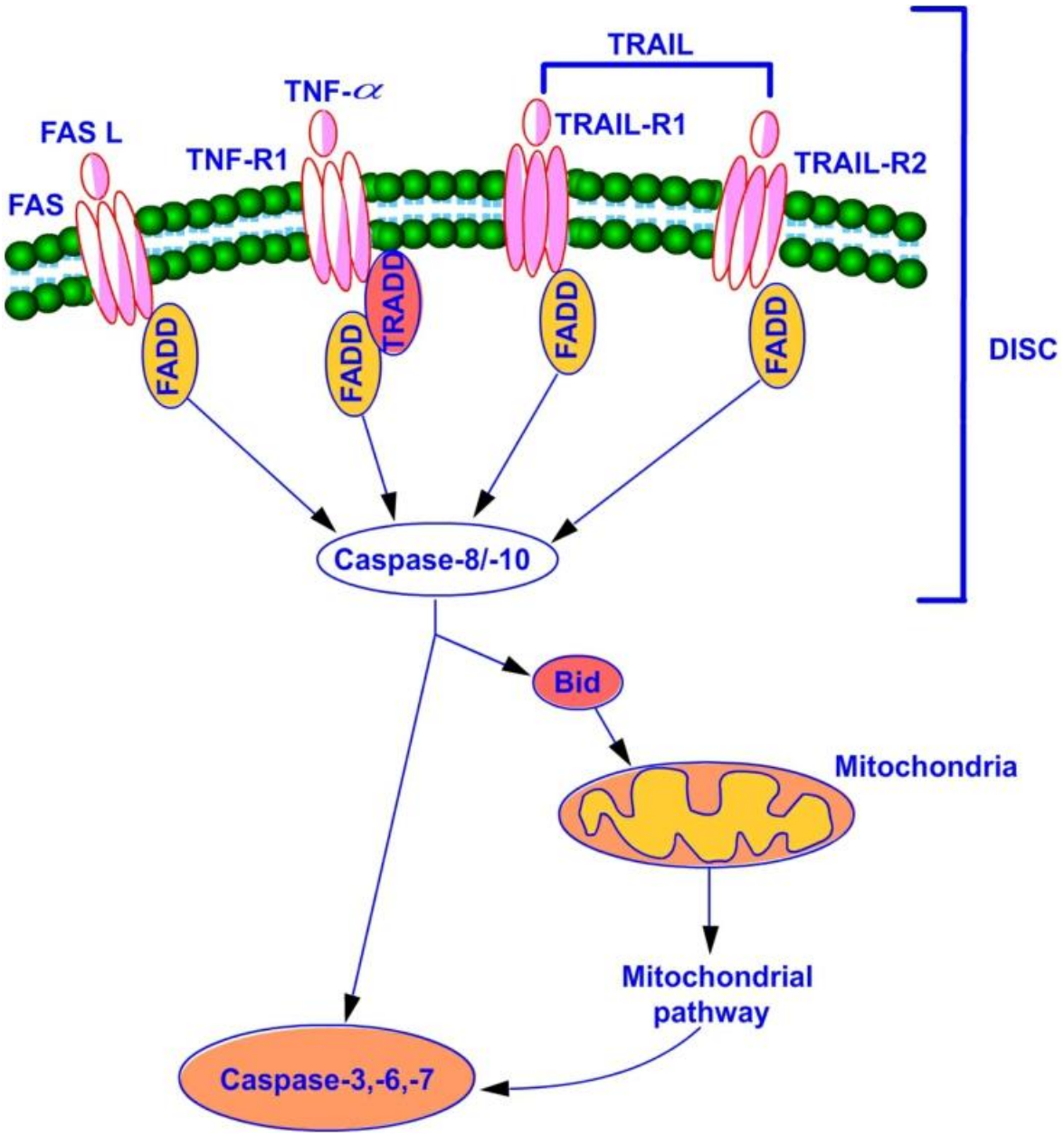
Apoptosis in Cancer

- Every cell in a multicellular organism has the potential to die by apoptosis
- Tumor cells have faulty apoptotic pathways
- These defects not only increase tumor mass, but also render the tumor resistant to therapy

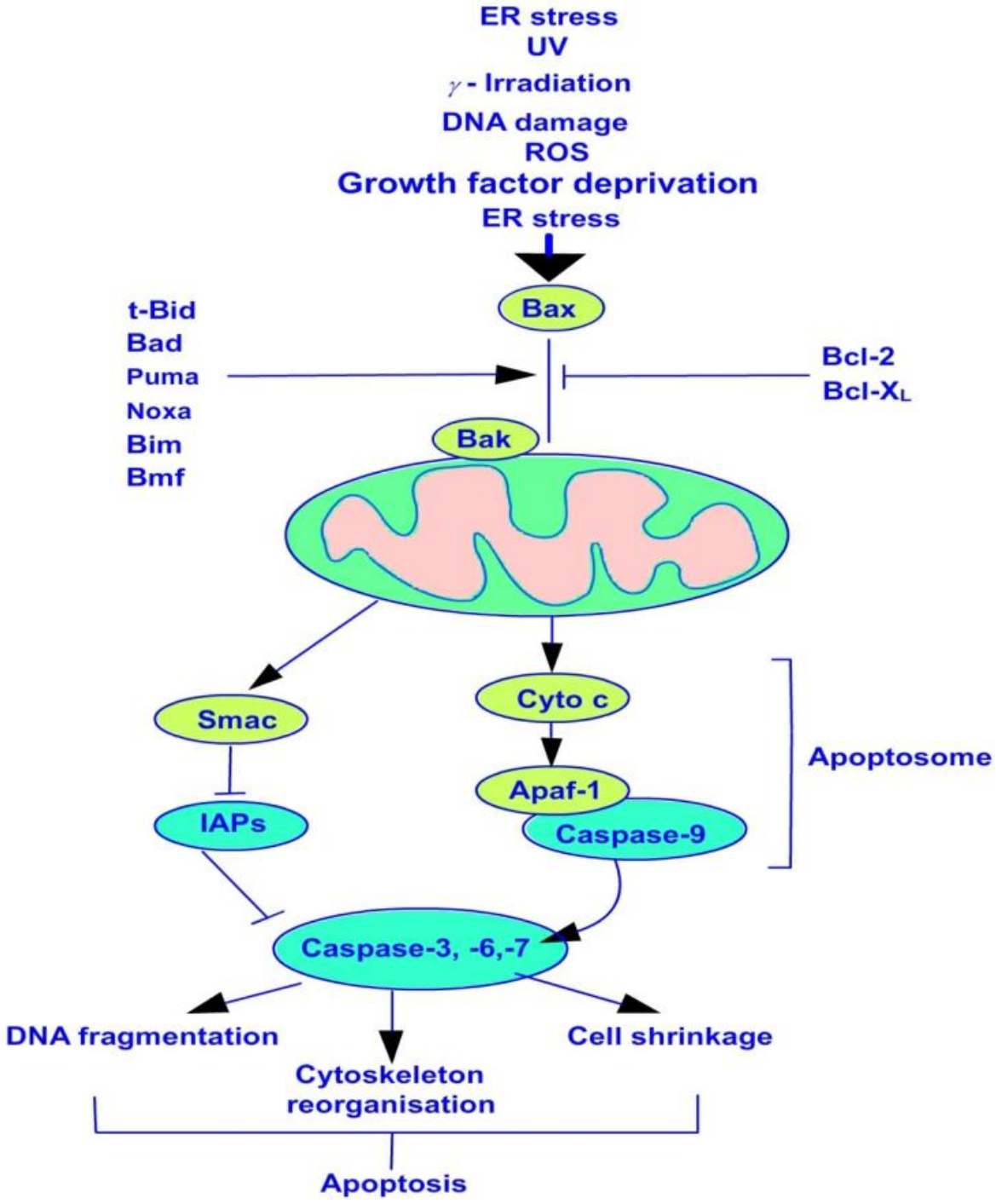
Biochemical changes

- Activation of caspases
  - DNA fragmentation results in a DNA ladder formation
  - DNA breakdown by Ca^{2+}-and Mg^{2+}-dependent endonucleases results in DNA fragments of 180 to 200 base pairs
  - externalization of phosphatidyl serine is a well-known recognition signal for binding with phagocytes on the surface of the apoptotic cell

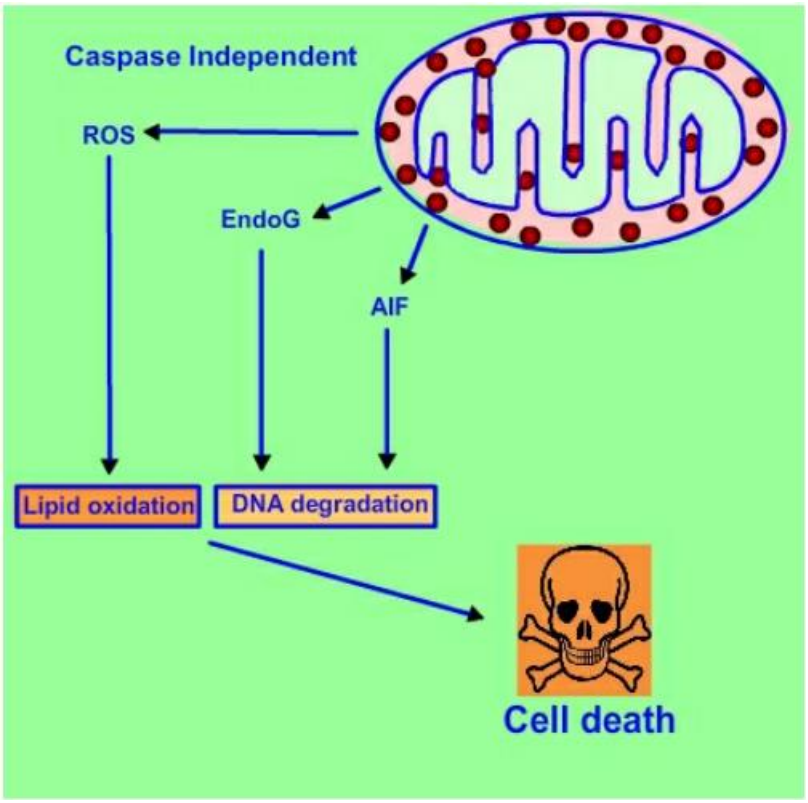
Extrinsic pathway



Intrinsic pathway



Caspase-independent pathways



Caspase – cysteine aspartic acid protease

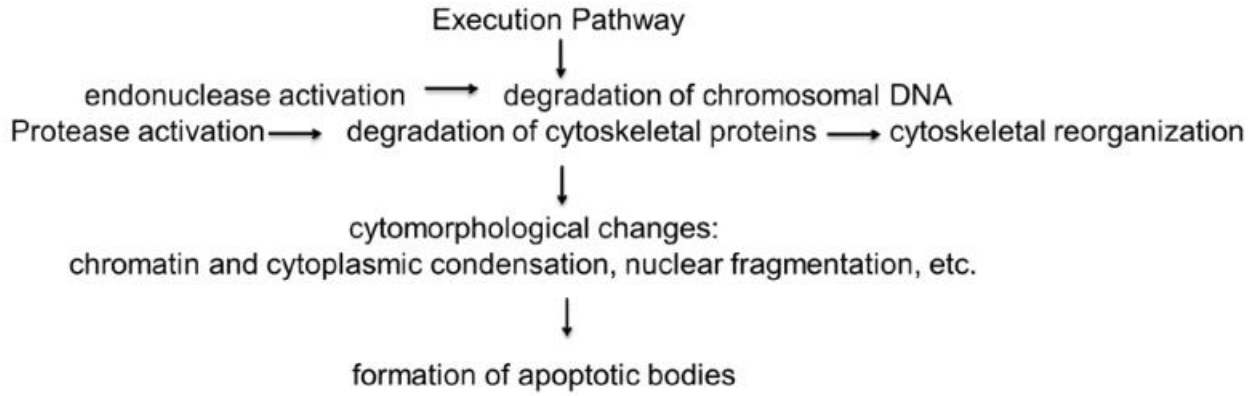
14 caspases in mammalian cells

- **Initiator/Apical caspases**
  - Caspase – 2
  - Caspase – 8
  - Caspase – 9
  - Caspase – 10
- **Effector/eecutioner caspases**
  - Caspase – 3
  - Caspase – 6
  - Caspase – 7
- **Inflammatory caspases**
  - 1, 4, 5, 11, 12, 13, 14

Caspases

- Cysteine proteases cleave Asp in P1 position of tetrameric recognition sequence
- Synthesised as inactive zymogens
- Activated by Asp cleavage
  - removes N-terminal prodomain
  - active tetramer formed
- Activated by 3 mechanisms:
  - autoactivation (caspase 8)
  - trans-activation (caspase 3, 6 and 7)
  - conformational change (caspase 9)
- **Proapoptotic Factors**
  - Caspases, Cytochrome c, Smac, Bax, Bid, AIF, CAD, DFF-45
- **Antiapoptotic Factors**
  - NF-Kappa B, Bcl-2, Bcl-XL, IAP, HSPs
- There exists a balance between pro and anti-apoptotic factors
- Anti-cancer agents shift this balance towards apoptosis

Caspase 3 activation

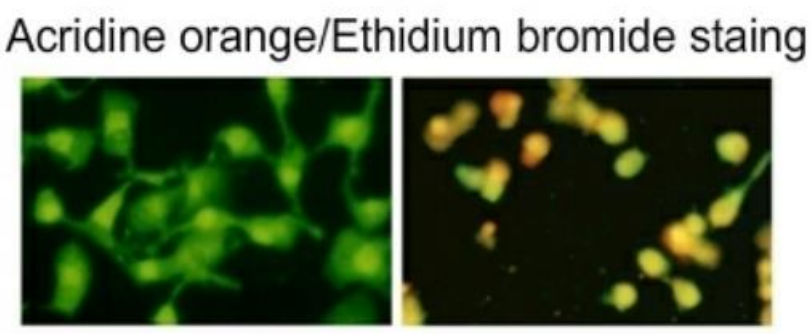


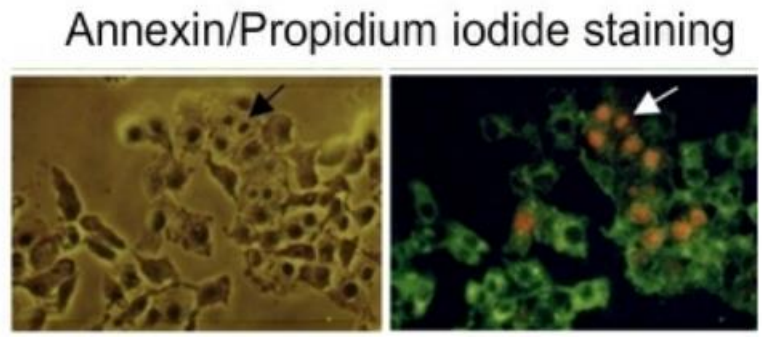
Apoptosis Assays

Light and electron microscopy

- A simple hematoxylin and eosin-stained tissue sections with light microscopy doesallow the visualization of apoptotic cells.
- However, this method does not detect the early events of apoptosis Confirmation with other methods is generally needed.
- Transmission electron microscopy (TEM) is the best to confirm apoptosisbecause the ultrastructural morphological characteristics can be visualized with clarity.
- Disadvantages of TEM are the cost, time, expenditure, and the ability to only assay a small region at a time

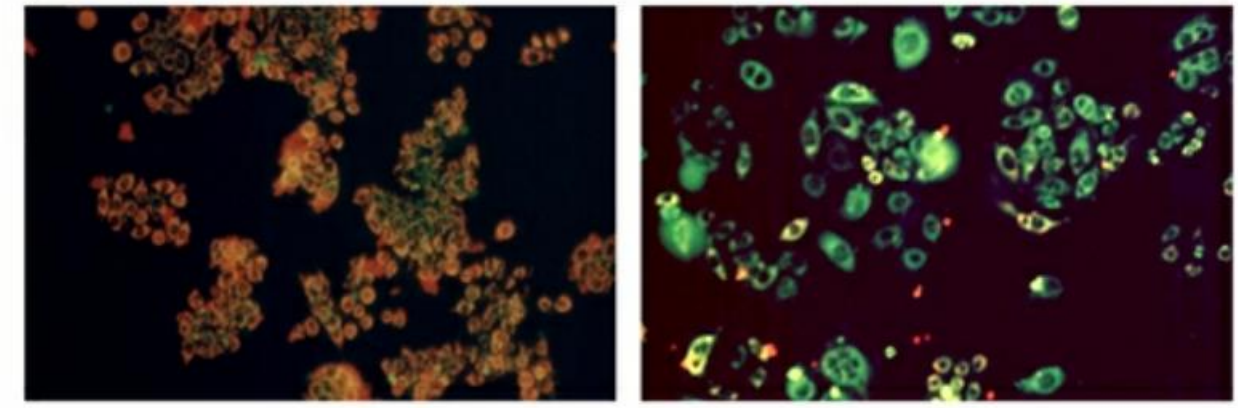
Apoptosis

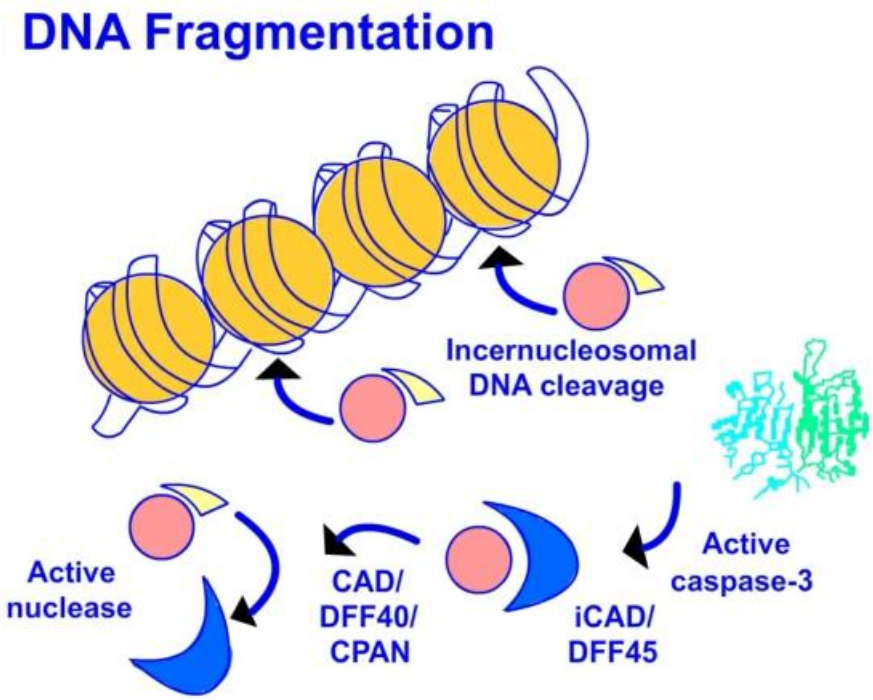




Mitochondrial Membrane Potential Detection

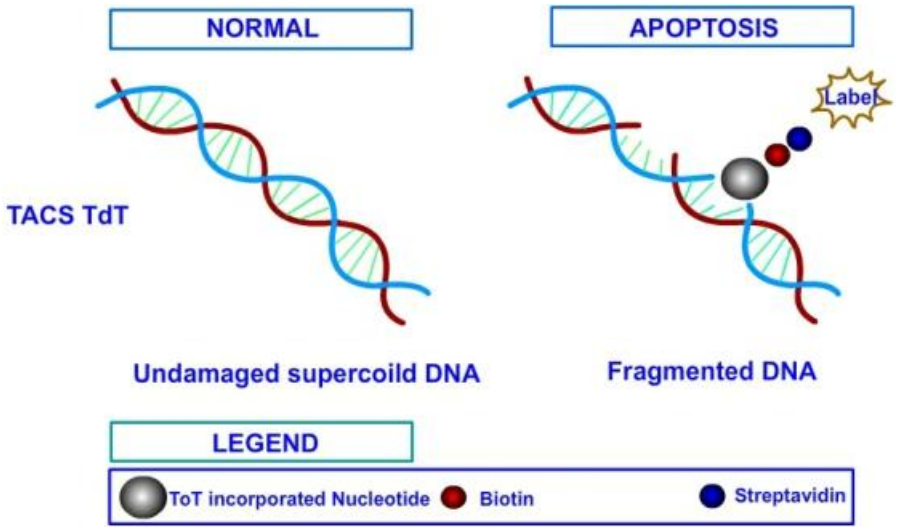
Non-apoptotic cells **Apoptotic cells**



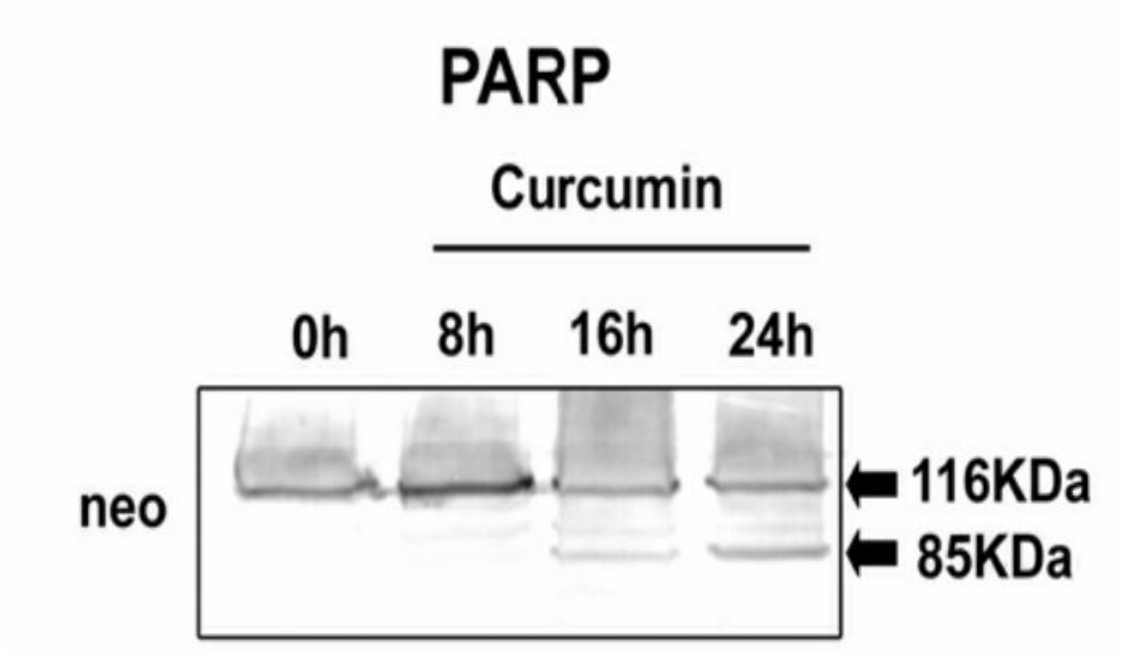


- Cytochrome *c* release from the mitochondria can also be assayed using fluorescence and electron microscopy in living or fixed cells.
- Apoptotic or anti-apoptotic regulator proteins such as Bax, Bid, and Bcl-2 can also be detected using fluorescence and confocal microscopy

- However, the fluorescent protein tag may alter the interaction of the native protein with other proteins.
- Therefore, other apoptosis assays should be used to confirm the results.
- The TUNEL (Terminal dUTP Nick End-Labeling) method
- Endonuclease cleavage products are assayed by enzymatically end-labeling the DNA strand breaks
- Terminal transferase is used to add labeled UTP to the 3′-end of the DNA fragments.
- The dUTP can then be labeled with a variety of probes to allow detection by light microscopy, fluorescence microscopy or flow cytometry.
- This method is also subject to false positives from necrotic cells and cells in the process of DNA repair and gene transcription. For these reasons,it should be paired with another assay.



PARP (poly [ADP-ribose] polymerase)



- Apoptosis PCR microarray is a relatively new methodology that uses real-time PCR to profile the expression of at least 112 genes involved in apoptosis.
- These PCR microarrays are designed to determine the expression profile of genes that encode key ligands, receptors, intracellular modulators, and transcription factors involved in the regulation of programmed cell death.
- Genes involved in anti-apoptosis can also be assessed with this methodology. Comparison of gene expression in cells or tissues can be performed between test samples and controls.