APOPTOSIS

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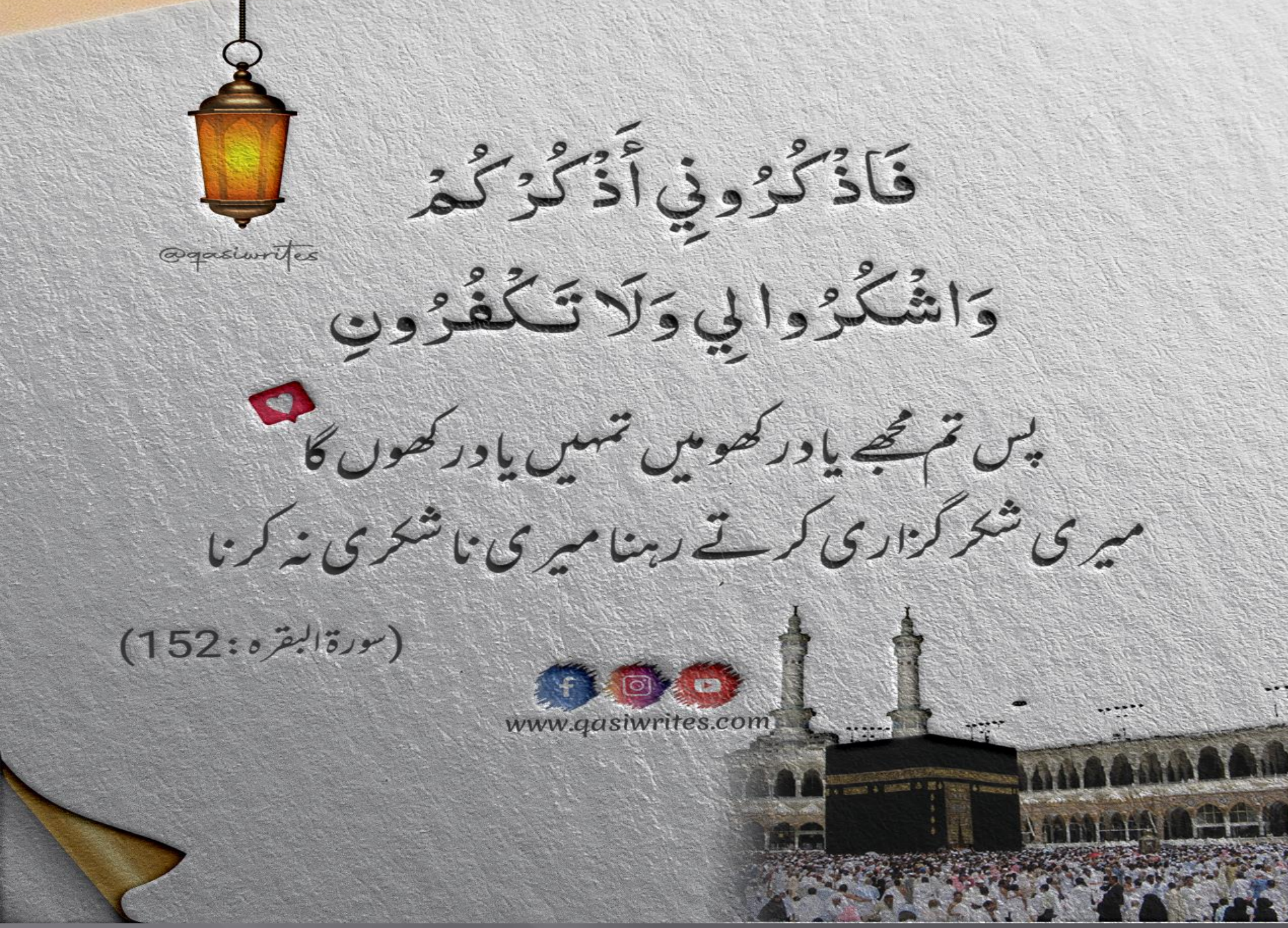
بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ
Bismillāhir-raḥmānir-raḥīm
In the name of God
VectorStock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Learning Objectives

- By the end of this lecture you should be able to:
- Define apoptosis.
- Describe physiological and pathological causes of apoptosis with examples.
- Describe morphology with alterations in cell structure.
- Describe the biochemical features of apoptosis altering the cell structure.
- Describe the intrinsic and extrinsic pathways of apoptosis
- Differentiate between necrosis and apoptosis.

Apoptosis - Definition

- A pathway of cell death induced by a tightly regulated suicidal program, in which the cells destined to die activate enzymes that degrade cells own nuclear DNA and nuclear, cytoplasmic proteins.

- Fragments of the apoptotic cells then break off, giving the appearance that is responsible for the name (apoptosis, “falling off”).
- The plasma membrane of the apoptotic cell remains intact, but the membrane is altered in such a way that the fragments, called apoptotic bodies, become highly "edible,” leading to their rapid consumption by phagocytes.
- The dead cell and its fragments are cleared with little leakage of cellular contents, so apoptotic cell death does not elicit an inflammatory reaction.
- Thus, apoptosis differs in many respects from necrosis

Historical background

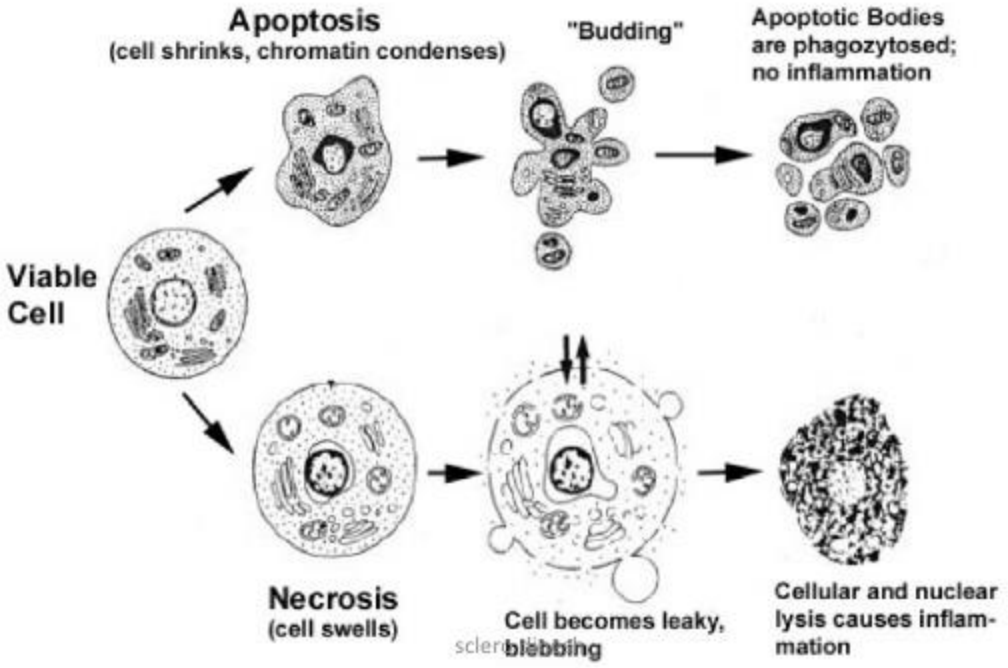
- Walther Flemming – Process of programmed cell death (1845).

- John Foxton Ross Kerr – Distinguish apoptosis from traumatic cell death (1962).

Cell death mechanisms

Death by suicide

Death by injury



Significance of apoptosis

- During development many cells are produced in excess which eventually undergo programmed cell death and thereby contribute to sculpturing many organs and tissues [*Meier, 2000*]
- In human body about one lakh cells are produced every second by mitosis and a similar number die by apoptosis (*Vaux and Korsmayer ,1999, cell*)
- Between 50 and 70 billion cells die each day due to apoptosis in the average human adult. For an average child between the ages of 8 and 14, approximately 20 billion to 30 billion cells die a day. *(Karam, Jose A. (2009). Apoptosis in Carcinogenesis and Chemotherapy. Netherlands: Springer. ISBN 978-1-4020-9597-9)*

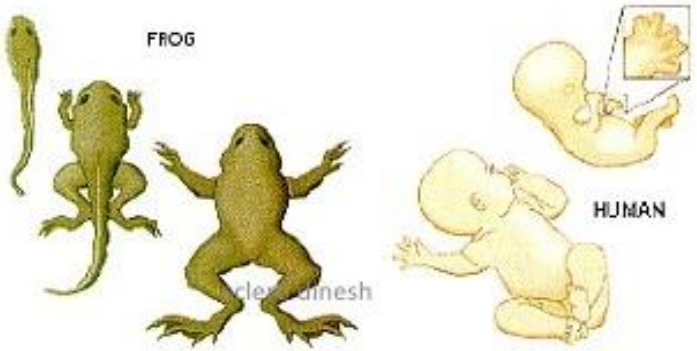
Etiopathogenesis

Why should a cell commit suicide?

- **1. Programmed cell death is as needed for proper normal development as mitosis is.**

Examples:

- The resorption of the tadpole tail in frog .
- The formation of the fingers and toes of the fetus requires the removal, by apoptosis.
- The sloughing off of the endometrium at the start of menstruation.
- The formation of the proper connections (synapses) between neurons in the brain.



- **2. Programmed cell death is needed to destroy cells that represent a threat to the integrity of the organism.**

- Examples:
  - Cells infected with viruses
  - Cells of the immune system
  - Cells with DNA damage
  - Cancer cells (Uncontrolled proliferated cells)

Apoptosis in physiologic situations

- Programmed destruction during embryogenesis
- Involution of hormone dependent tissues
- Cell loss in proliferating cell populations
- Elimination of harmful self- reactive lymphocytes
- Death of host cells

Apoptosis in bud formation during which many interdigital cells die. They are stained black by a TUNEL method

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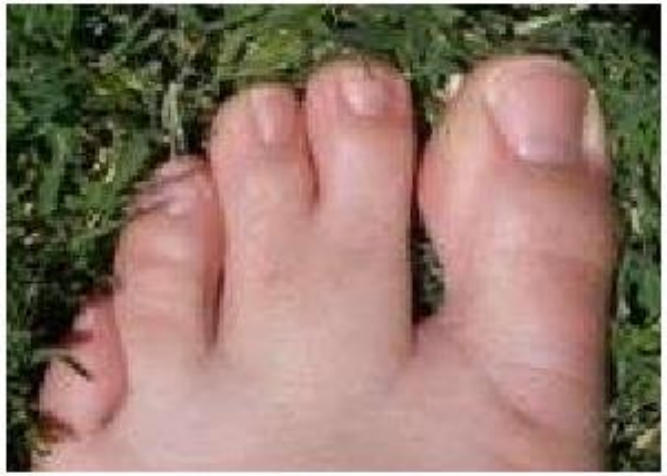
Incomplete differentiation in two toes due to lack of apoptosis



Apoptosis in pathological conditions

- DNA damage

- Accumulation of misfolded proteins

- Cell death in certain infections

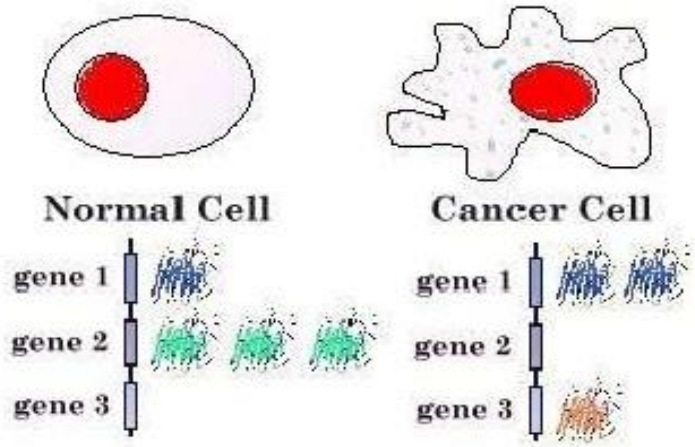
- Pathological atrophy in parenchymal organs

Cells of the immune system

- CTLs induce apoptosis in each other and even in themselves.
- Defects in the apoptotic machinery is associated with autoimmune diseases such as lupus erythematosus and rheumatoid arthritis.

Cells with DNA damage

- Damage to its genome can cause a cell
  - » to disrupt proper embryonic development leading to birth defects
  - » to become cancerous.
- Cells respond to DNA damage by increasing their production of p53. p53 is a potent **inducer** of apoptosis.



– **Cancer cells**

- Radiation and chemicals used in cancer therapy induce apoptosis in some types of cancer cells.

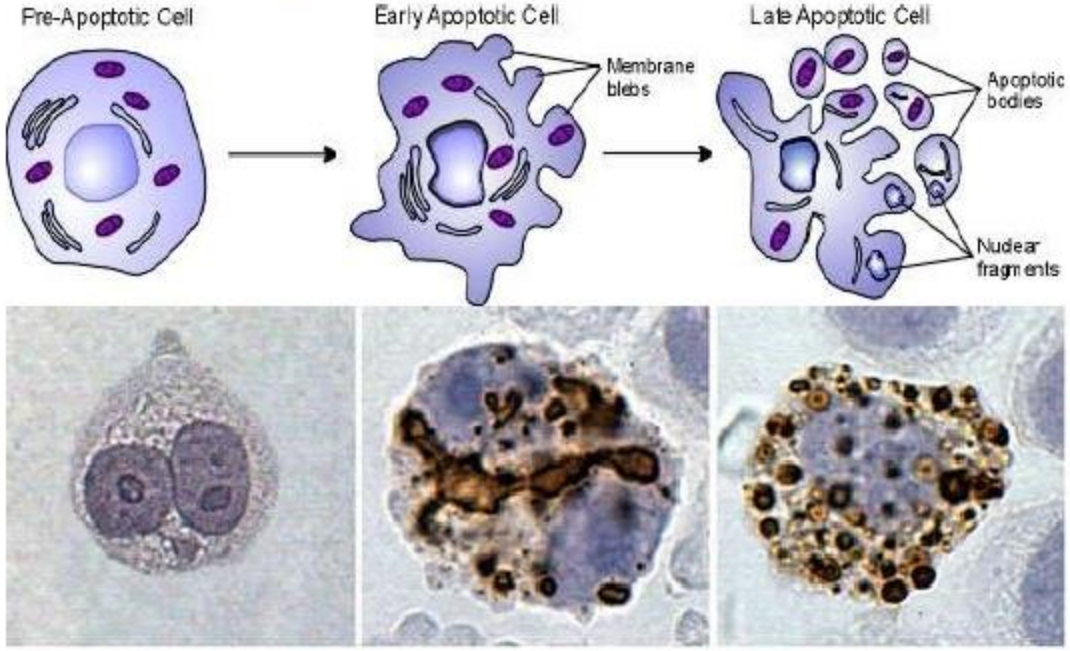


Fig. 1: SC-1 induced apoptosis in stomach carcinoma cells
Left: Before induction
Middle: 24h after induction
Right: 48h after induction

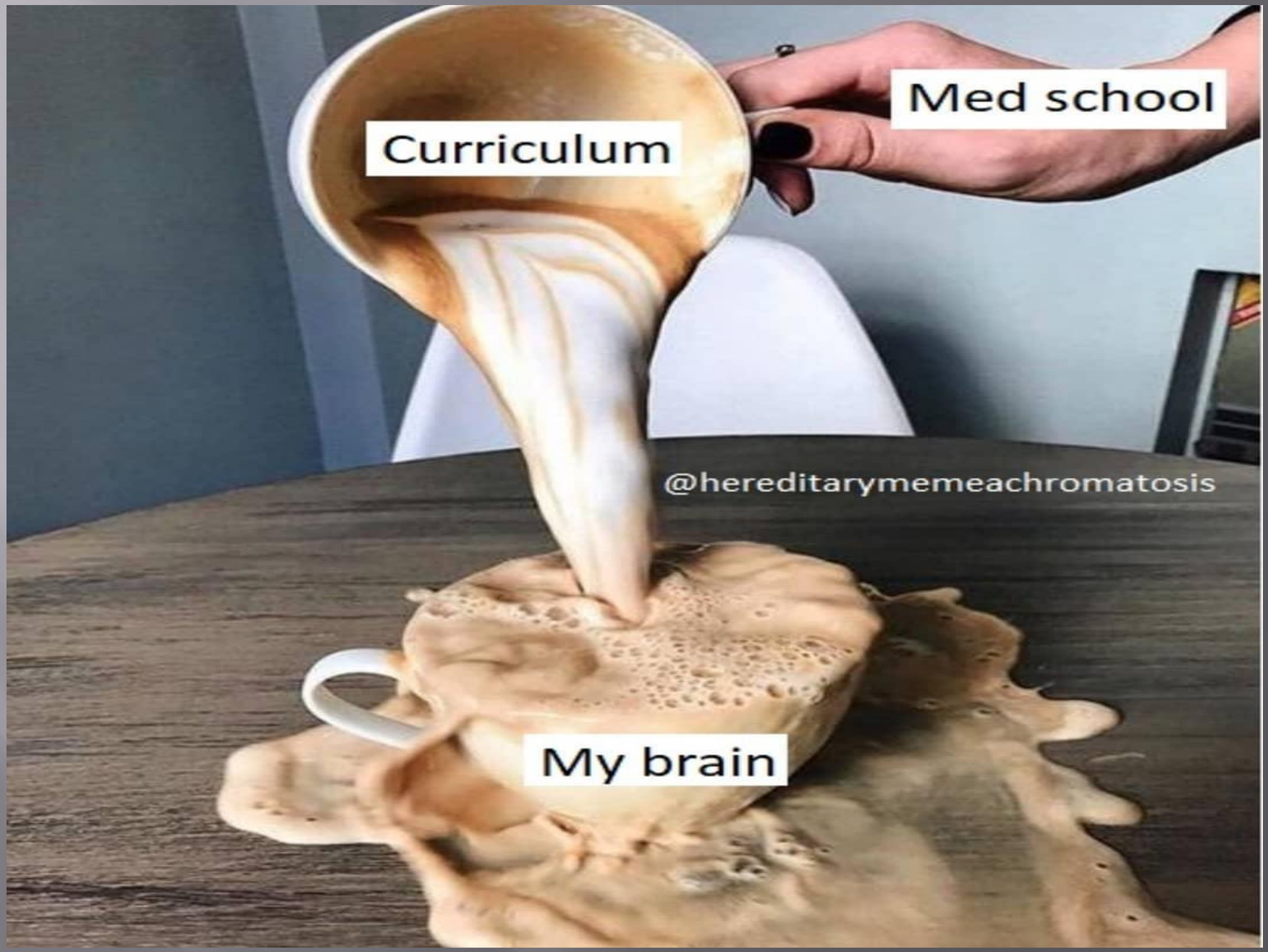
Physiologic

- Embryogenesis (e.g. Involution)
- Hormone withdrawal:
  - Involution of lactating breast
  - Involution of ovarian follicles (menopasuse)
- Keeping cell numbers in steady state e.g. Colonic crypts
- Destruction of harmful self-reactive lymphocytes
- Death of cells having served their purpose e.g. Lymphocytes, neutrophils post inflammation

Pathologic

Diverse conditions drive apoptosis e.g.

- DNA damage (radiation, chemotherapy, hypoxia)
- Misfolded proteins (genetic/acquired)
- Infections e.g. HIV
- Pathologic atrophy
- Other


Med school
Curriculum
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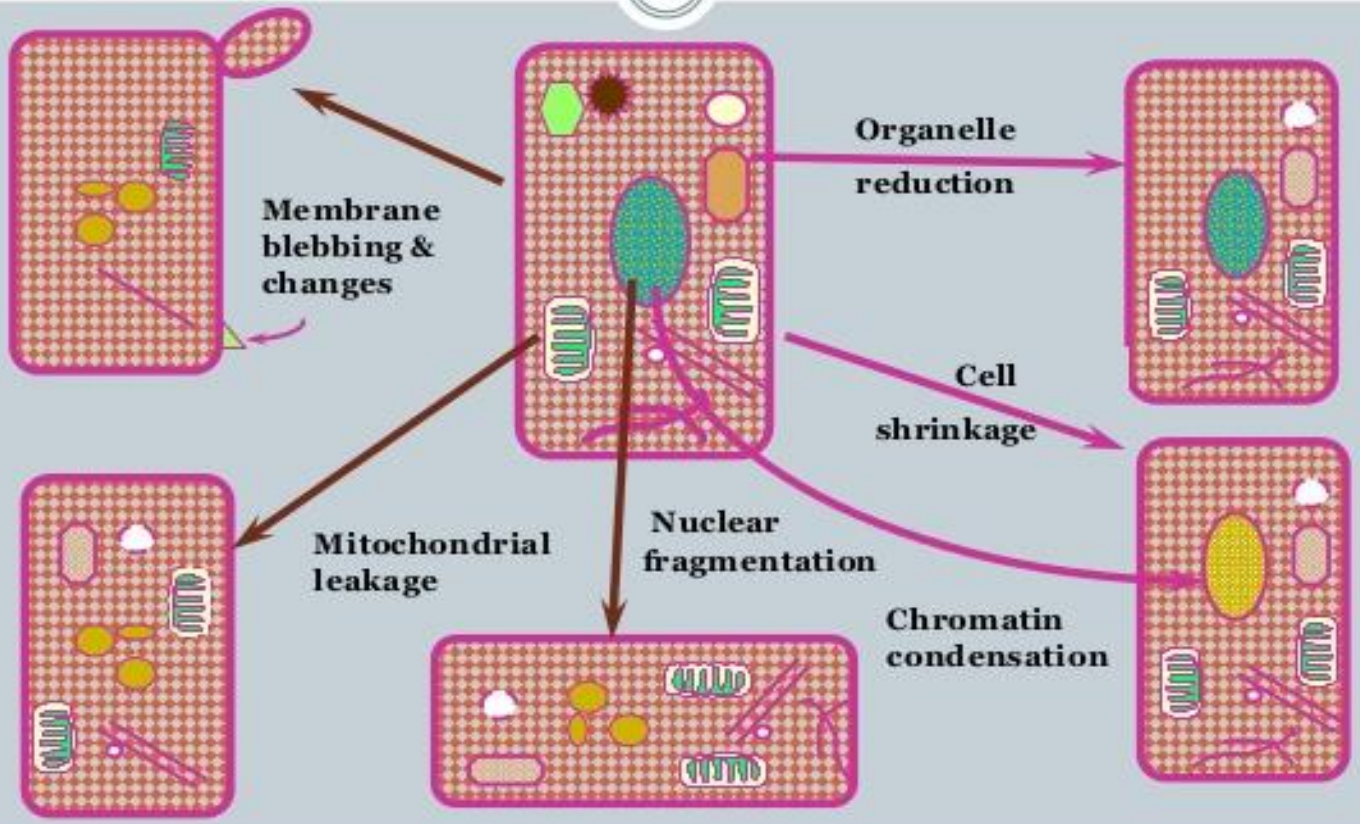
My brain

Morphological & Biochemical changes

Classic changes

- ✓ Cell shrinkage
- ✓ Nuclear fragmentation
- ✓ Chromatin condensation
- ✓ Chromosomal DNA fragmentation
- ✓ Formation of cytoplasmic blebs& apoptotic bodies
- ✓ Phagocytosis

CONT....


Membrane blebbing & changes
Organelle reduction
Cell shrinkage
Mitochondrial leakage
Nuclear fragmentation
Chromatin condensation

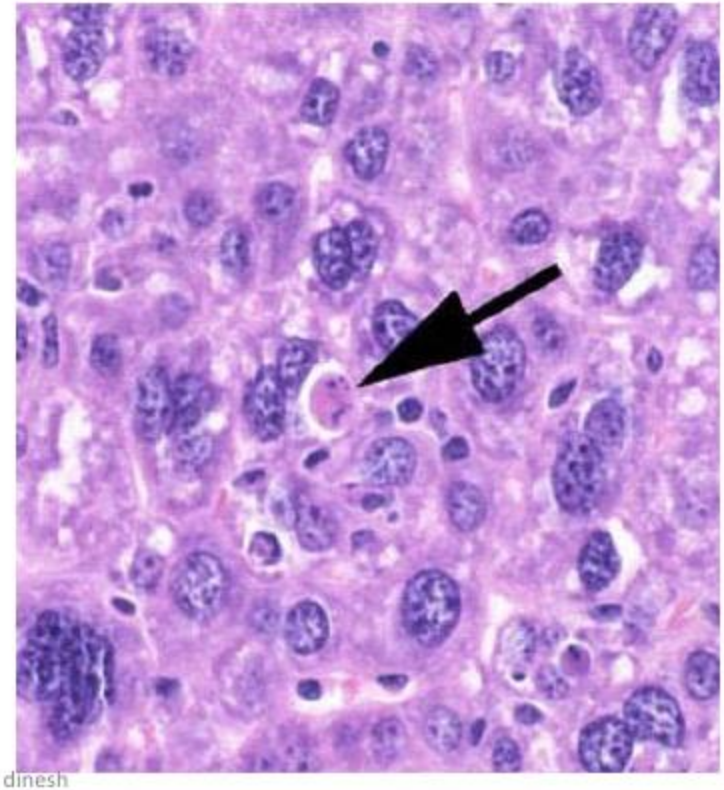
MORPHOLOGY

- In H&E-stained tissue sections, the nuclei of apoptotic cells show various stages of chromatin condensation and aggregation and, ultimately, karyorrhexis ; at the molecular level, this is reflected in the fragmentation of DNA into nucleosome-sized pieces.
- The cells rapidly shrink, form cytoplasmic buds, and fragment into apoptotic bodies that are composed of membrane bound pieces of cytosol and organelles .
- these fragments are quickly extruded and phagocytosed without eliciting an inflammatory response, even significant apoptosis may be histologically undetectable

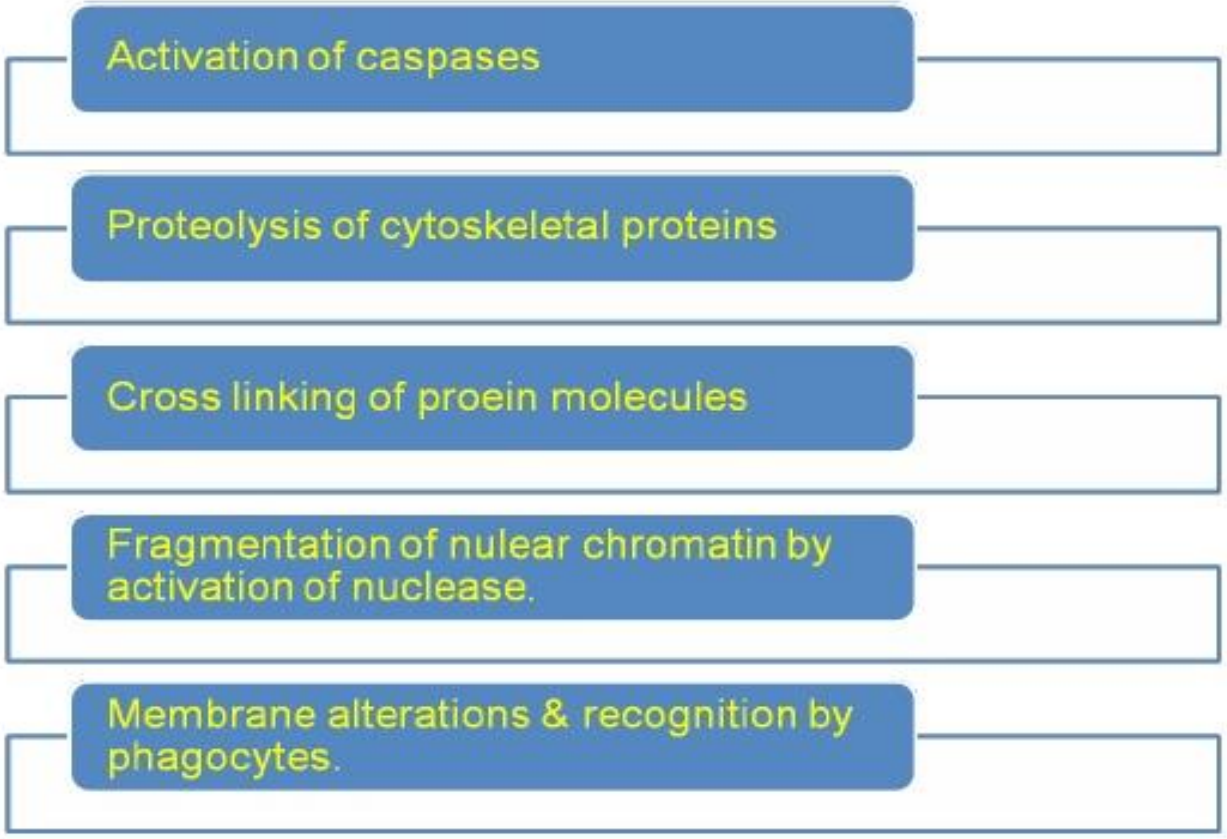
Histology

Apoptotic bodies

- Round oval mass of intensely eosinophillic cytoplasm
- Fragments of dense nuclear chromatin



Bio chemical changes



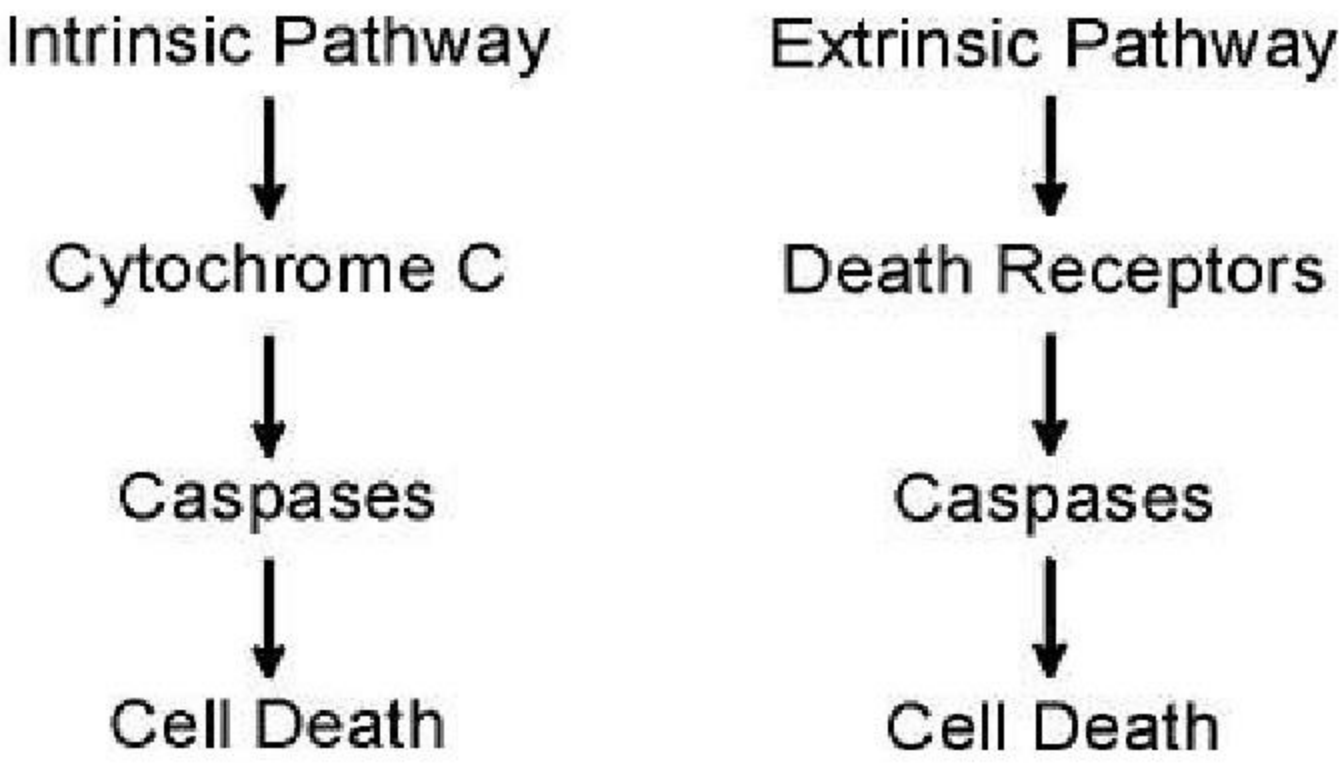
Membrane Changes

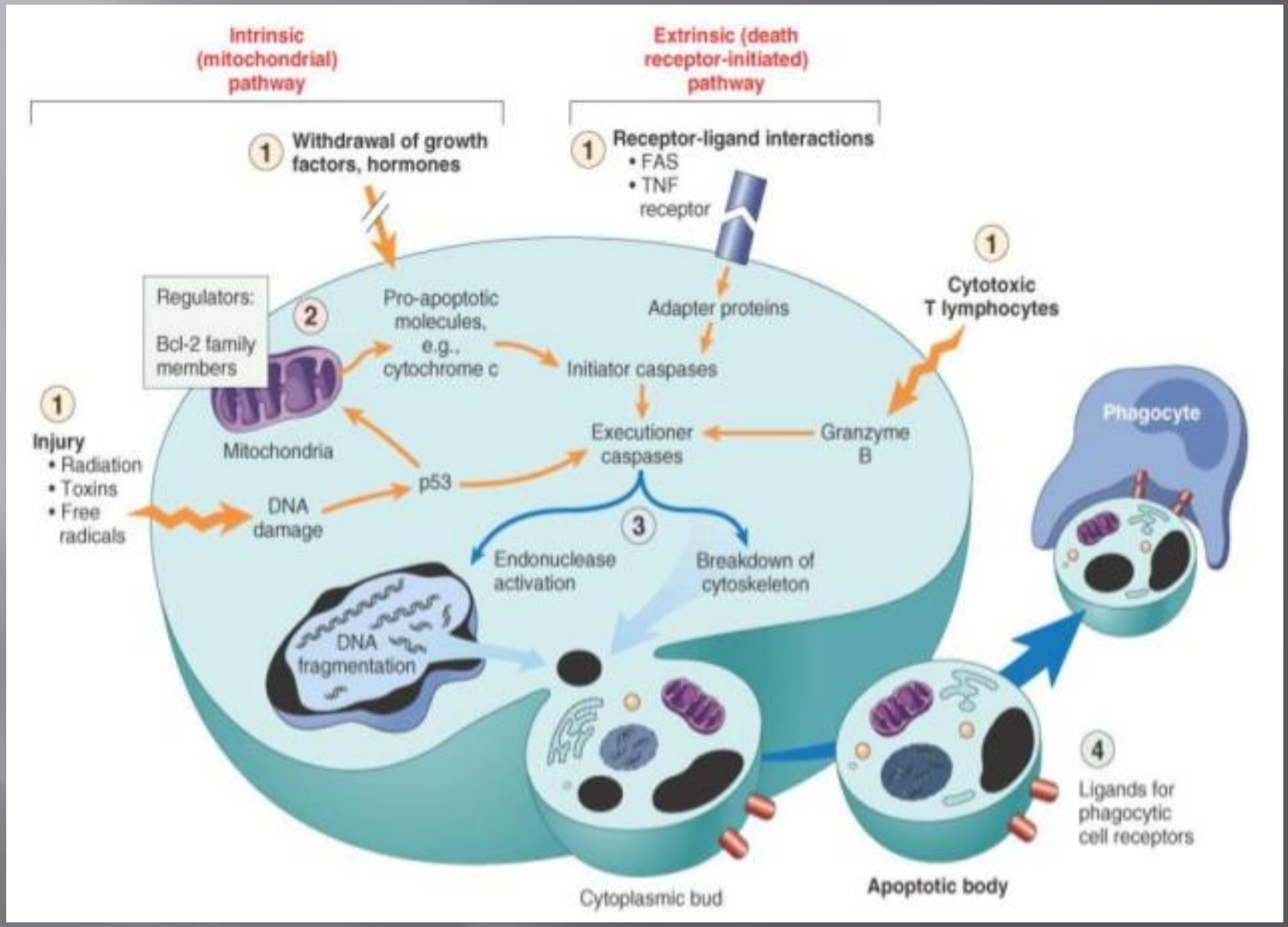
- Phosphatidylserine.
- Secretion of soluble factors by apoptotic cells.
- Thrombospondin coating on apoptotic cells.
- Production of proteins by macrophages.
- Numerous macrophage receptors have been shown to be involved in the binding and engulfment of apoptotic cells
- Coating of apoptotic cells with antibodies and compliment proteins C1q.

Mechanisms of apoptosis

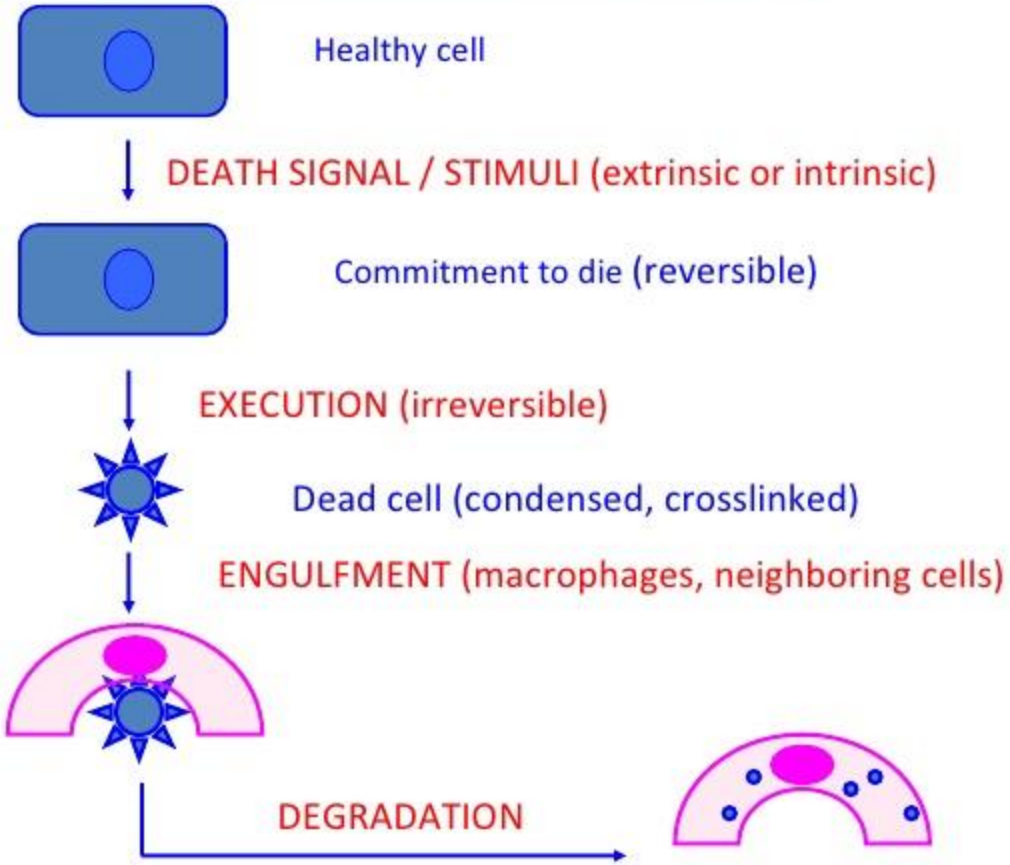
- Apoptosis is regulated by biochemical pathways that control the balance of death- and survival-inducing signals and ultimately the activation of enzymes called caspases.
- Caspases were so named because they are cysteine proteases that cleave proteins after aspartic acid residues.
- Two distinct pathways converge on caspase activation: the mitochondrial pathway and the death receptor pathway .
- Although these pathways can intersect, they are generally induced under different conditions, involve different molecules, and serve distinct roles in physiology and disease.
- The end result of apoptotic cell death is the clearance of apoptotic bodies by phagocytes

APOPTOSIS




Intrinsic (mitochondrial) pathway
Extrinsic (death receptor-initiated) pathway
1 Withdrawal of growth factors, hormones
1 Receptor-ligand interactions
• FAS
• TNF receptor
1 Cytotoxic T lymphocytes
Regulators:
Bcl-2 family members
2
Pro-apoptotic molecules, e.g., cytochrome c
Adapter proteins
Initiator caspases
1 Injury
• Radiation
• Toxins
• Free radicals
Mitochondria
Executioner caspases
Granzyme B
Phagocyte
DNA damage
p53
3
Endonuclease activation
Breakdown of cytoskeleton
DNA fragmentation
4 Ligands for phagocytic cell receptors
Cytoplasmic bud
Apoptotic body

STAGES OF CLASSIC APOPTOSIS



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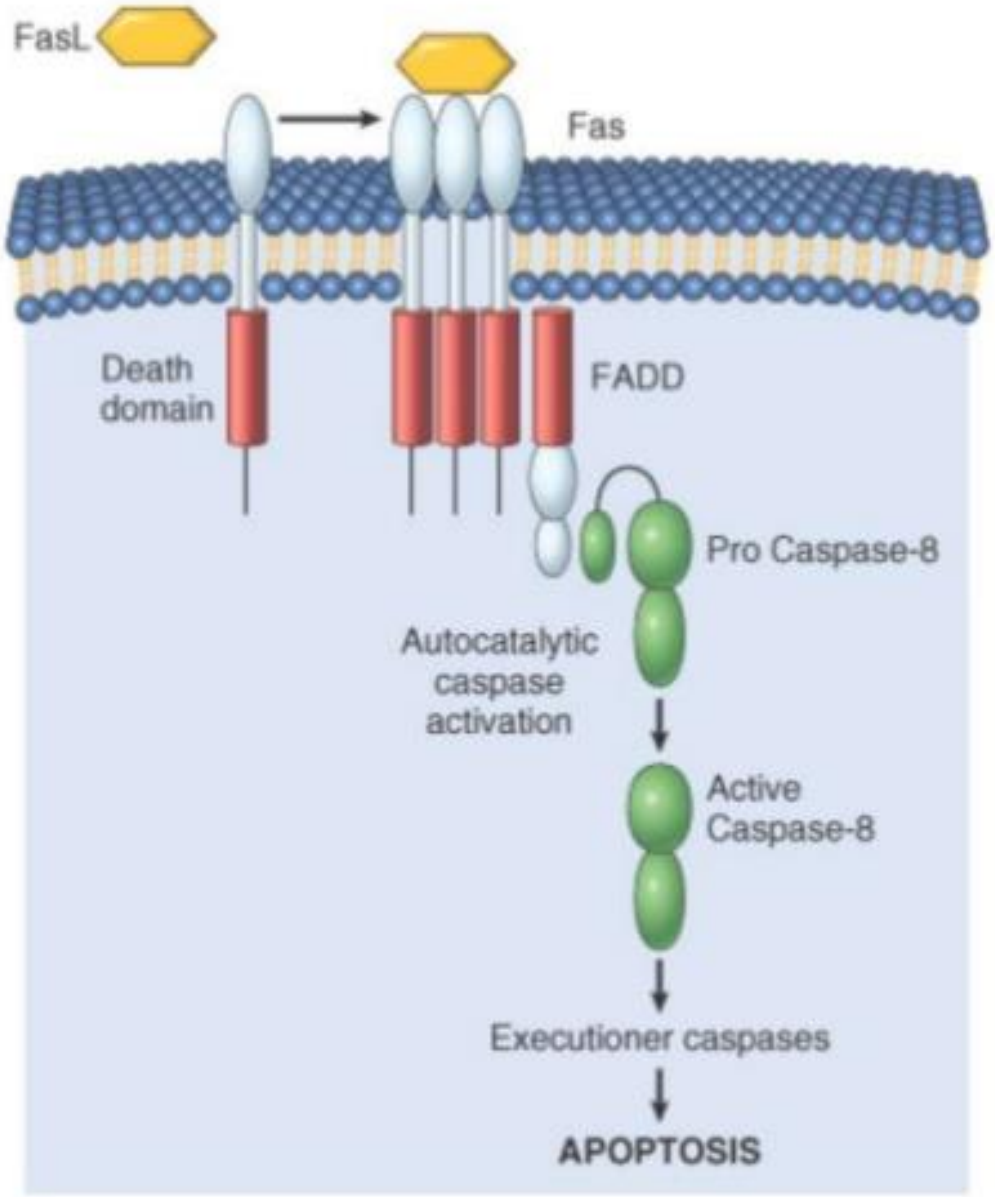
Death receptor (extrinsic) pathway of apoptosis

- Many cells express surface molecules, called death receptors, that trigger apoptosis.
- Most of these are members of the tumor necrosis factor (TNF) receptor family, which contain in their cytoplasmic regions a conserved "death domain," so named because it mediates interaction with other proteins involved in cell death.
- The prototypic death receptors are the type I TNF receptor and Fas (CD95).
- Fas ligand (FasL) is a membrane protein expressed mainly on activated T lymphocytes.

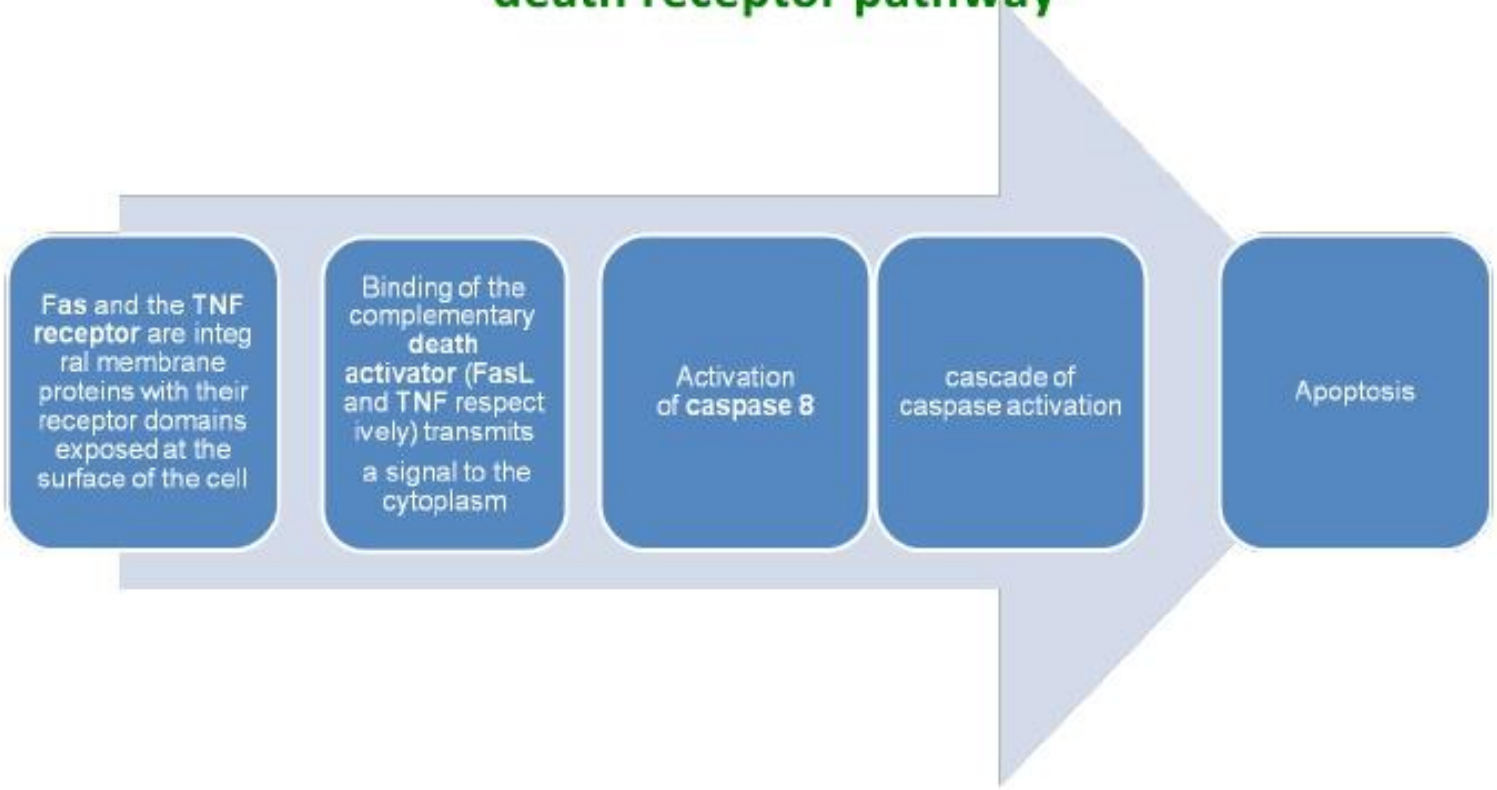
- When these T cells recognize Fas-expressing targets, Fas molecules are crosslinked by FasL and bind adaptor proteins via the death domain.
- These then recruit and activate caspase-8, which, in turn, activates downstream caspases.
- The death receptor pathway is involved in the elimination of self-reactive lymphocytes
- and in the killing of target cells by some cytotoxic T lymphocytes (CTLs) that express FasL

- Extrinsic pathway

The death receptor pathway



Apoptosis triggered by external signals: the extrinsic or death receptor pathway



Mitochondrial (intrinsic) pathway

- The mitochondrial (intrinsic) pathway seems to be responsible for apoptosis in most physiologic and pathologic situations.

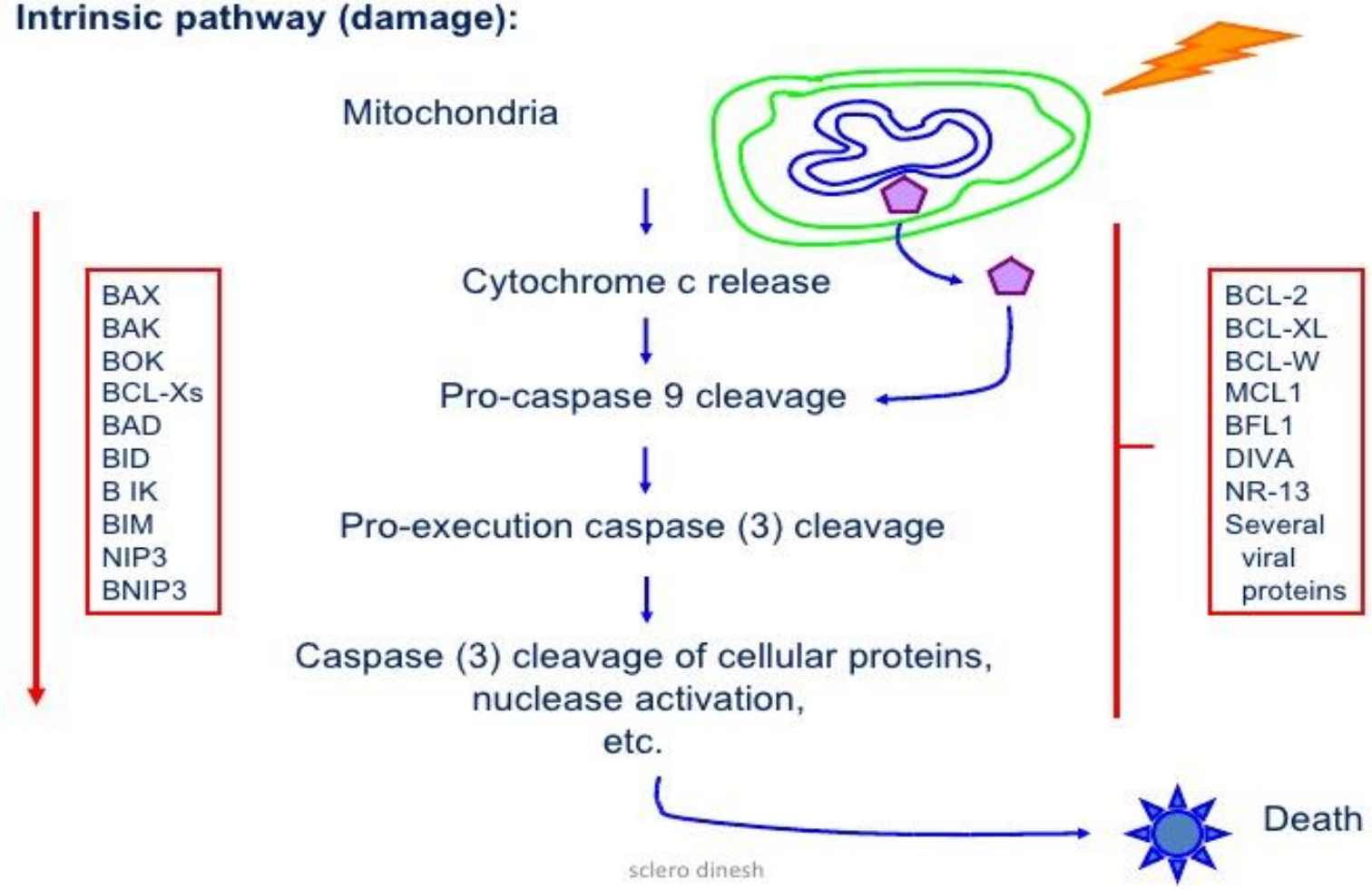
- Mitochondria contain several proteins that are capable of inducing apoptosis, including cytochrome c.

- A family of more than 20 proteins, the prototype of which is Bcl-2, controls the permeability of mitochondria.
- In healthy cells, Bcl-2 and the related protein Bcl-xL, which are produced in response to growth factors and other stimuli, maintain the integrity of mitochondrial membranes, in large part by holding two proapoptotic members of the family, Bax and Bak, in check

- When cells are deprived of growth factors and survival signals, or are exposed to agents that damage DNA, or accumulate unacceptable amounts of misfolded proteins, a number of sensors are activated.
- These sensors are called BH3 proteins.
- They in turn shift this delicate, life-sustaining balance in favor of pro-apoptotic Bak and Bax.

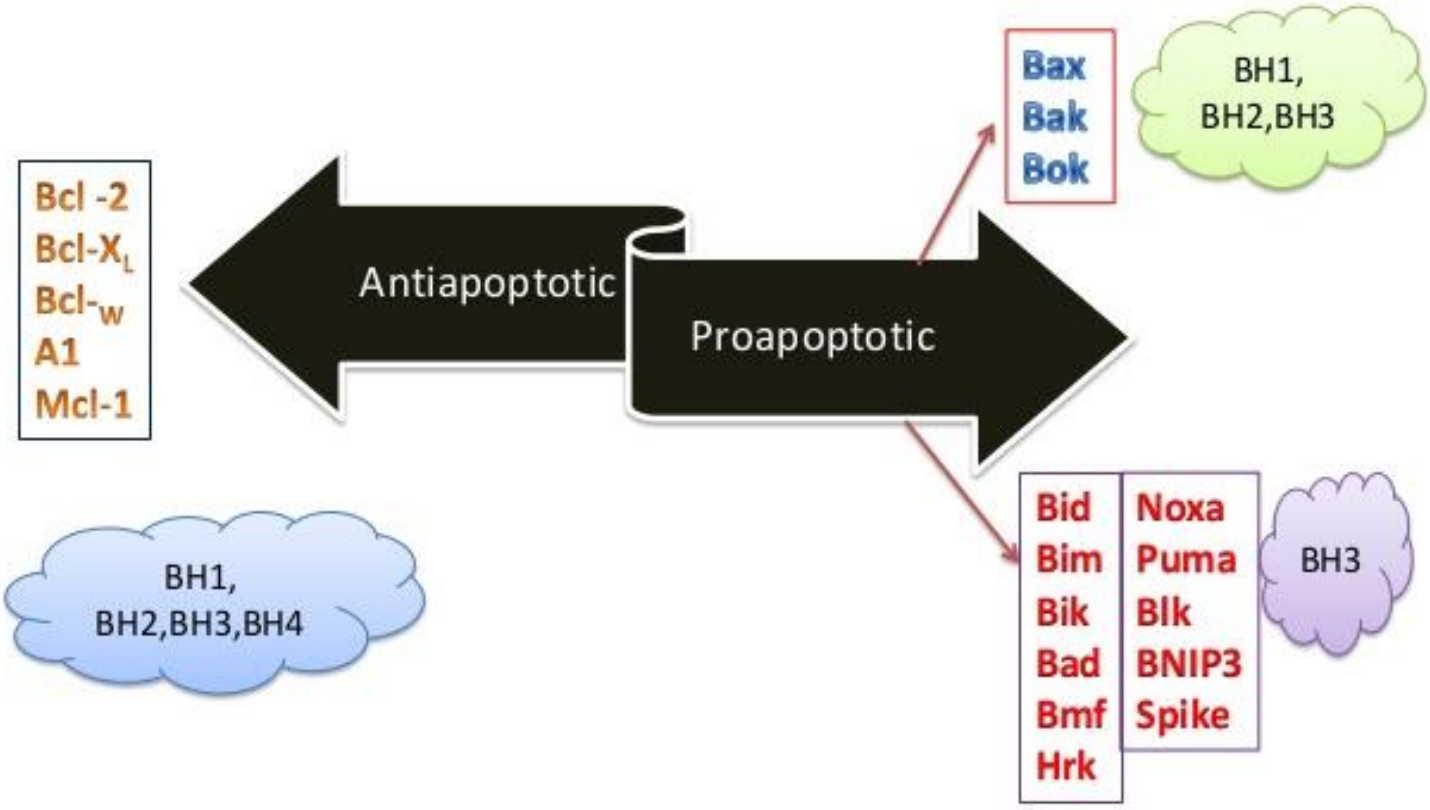
- Bak and Bax dimerize, insert into the mitochondrial membrane, and form channels through which cytochrome c and other mitochondrial proteins escape into the cytosol.
- After cytochrome c enters the cytosol, it, together with certain cofactors, activates caspase-9.
- The net result is the activation of a caspase cascade, ultimately leading to nuclear fragmentation and formation of apoptotic bodies

Intrinsic pathway (damage):



Bcl-2 family members

A very large family with 30 members identified and belongs to both:



Intracellular signals

Oxidative damage from free radicals, Radiation, Virus infection, Nutrient deprivation, Pro-apoptotic Factors

↓

Damage to the mitochondrial membrane increasing permeability

↓

Entry of Cytochrome C into the cytoplasm

↓

Cytochrome C binds to Apaf-1 forming an apoptosome

↓

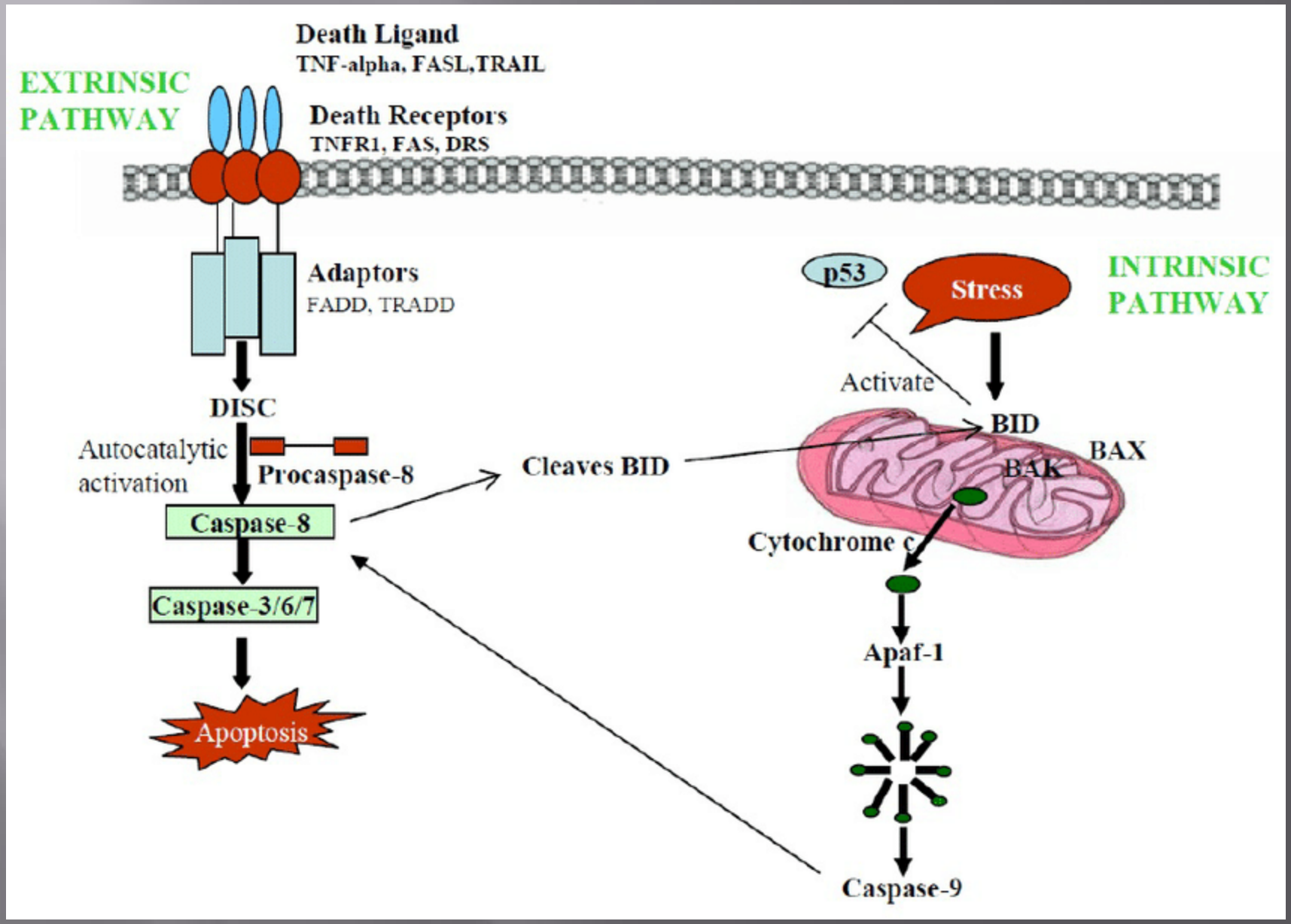
Apoptosome activates procaspase-9 to caspase-9

↓

Caspase-9 cleaves and activates caspase-3 and caspase-7.

↓

This executioner caspases activate a cascade of proteolytic activity that leads to: Chromatin condensation, DNA fragmentation, Protein cleavage, Membrane permeability


Death Ligand
TNF-alpha, FASL,TRAIL
EXTRINSIC PATHWAY
Death Receptors
TNFR1, FAS, DRS
Adaptors
FADD, TRADD
DISC
Autocatalytic activation
Procaspase-8
Caspase-8
Caspase-3/6/7
Apoptosis
Cleaves BID
p53
Stress
INTRINSIC PATHWAY
Activate
BID
BAX
BAK
Cytochrome c
Apaf-1
Caspase-9

Apoptosis: Role in Disease

TOO MUCH: Tissue atrophy

Neurodegeneration
Thin skin
etc

TOO LITTLE: Hyperplasia

Cancer
Athersclerosis
etc

Apoptosis: Role in Disease

Cancer

- Apoptosis eliminates damaged cells (damage => mutations => cancer

- Tumor suppressor p53 controls senescence and apoptosis responses to damage.

- Most cancer cells are defective in apoptotic response(damaged, mutant cells survive)

- High levels of anti-apoptotic proteins
  or
- Low levels of pro-apoptotic proteins ===> CANCER

Apoptosis: Role in Disease

Cancer

Virus associated cancer

- Several human papilloma viruses (**HPV**) have been implicated in causing cervical cancer. One of them produces a protein (E6) that binds and inactivates the apoptosis promoter **p53**.

- **Epstein-Barr Virus** (**EBV**), the cause of mononucleosis and associated with some lymphomas
  - produces a protein similar to Bcl-2
  - produces another protein that causes the cell to increase its own production of Bcl-2. Both these actions make the cell more resistant to apoptosis (thus enabling a cancer cell to continue to proliferate).

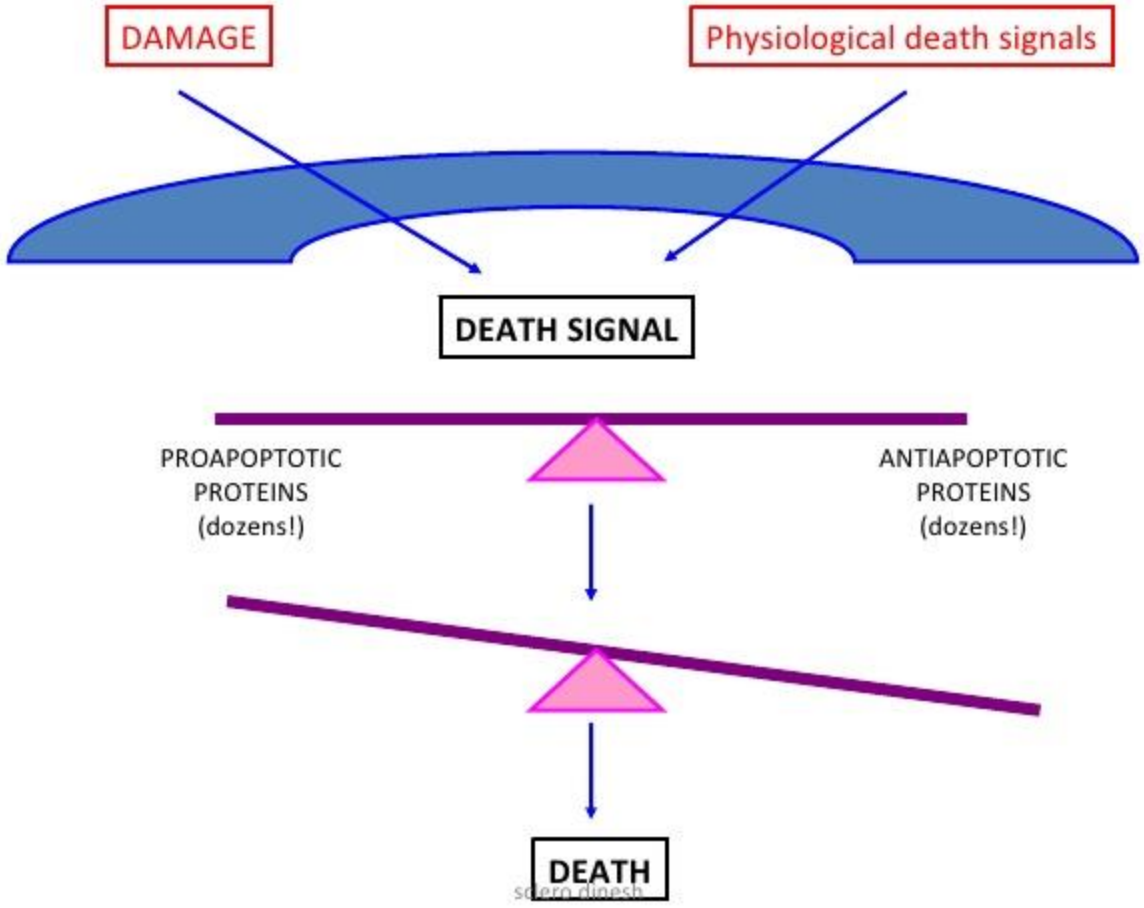
Apoptosis: Role in Disease
Cancer

- Some **B-cell leukemia** and lymphomas express high levels of **Bcl-2**, thus blocking apoptotic signals they may receive. The high levels result from a translocation of the *BCL-2* gene into an **enhancer** region for antibody production.

- **Melanoma** (the most dangerous type of skin cancer) cells avoid apoptosis by inhibiting the expression of the gene encoding **Apaf-1**.

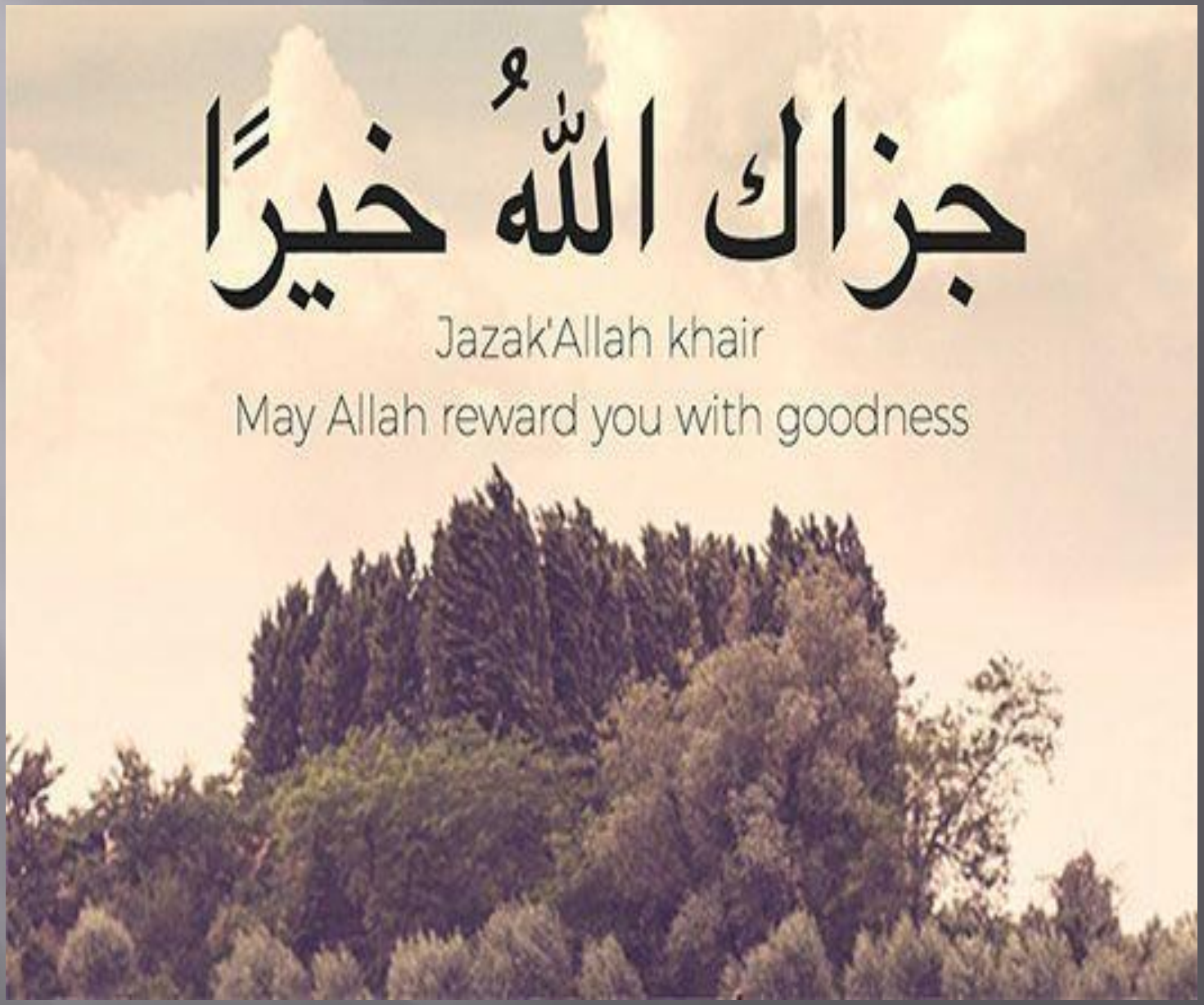
Conclusion

Cells are balanced between life and death



	APOPTOSIS	NECROSIS
NATURAL	YES	NO
EFFECTS	BENEFICIAL	DETRIMENTAL
	Physiological or pathological	Always pathological
	Single cells	Sheets of cells
	Energy dependent	Energy independent
	Cell shrinkage	Cell swelling
	Membrane integrity maintained	Membrane integrity lost

APOPTOSIS	NECROSIS
Role for mitochondria and cytochrome C	No role for mitochondria
No leak of lysosomal enzymes	Leak of lysosomal enzymes
Characteristic nuclear changes	Nuclei lost
Apoptotic bodies form	Do not form
DNA cleavage	No DNA cleavage
Activation of specific proteases	No activation
Regulatable process	Not regulated
Evolutionarily conserved	Not conserved
Dead cells ingested by neighboring cells	Dead cells ingested by neutrophils and macrophages


جزاك اللهُ خيرًا
Jazak'Allah khair
May Allah reward you with goodness