PROGRAMMED CELL DEATH
(or) APOPTOSIS

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Contant:

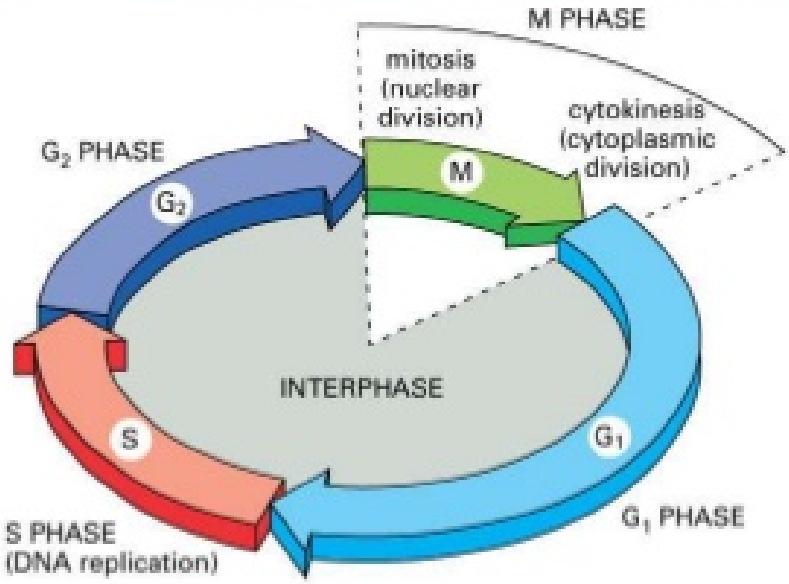
- Cell Cycle
- Cell death definition
- Cell death types
- Necrosis
- Mechanisms and Causes
- Types of necrosis
- Definition of apoptosis
- General characteristics of apoptosis
- Importance of apoptosis

- Morphology of apoptosis
- Biochemical features of apoptosis
- Mechanisms of apoptosis
- **Initiation phase:**
- **Execution phase**
- Role in diseases

Cell Cycle

- Only from existing cells come new cells.
- We are all decedents of the first cells on the planet.
- A cell reproduces by duplicating its contents and then dividing into two
- This cycle of events is known as the cell cycle

Eukaryotic Cell Cycle



Cell cycle regulation

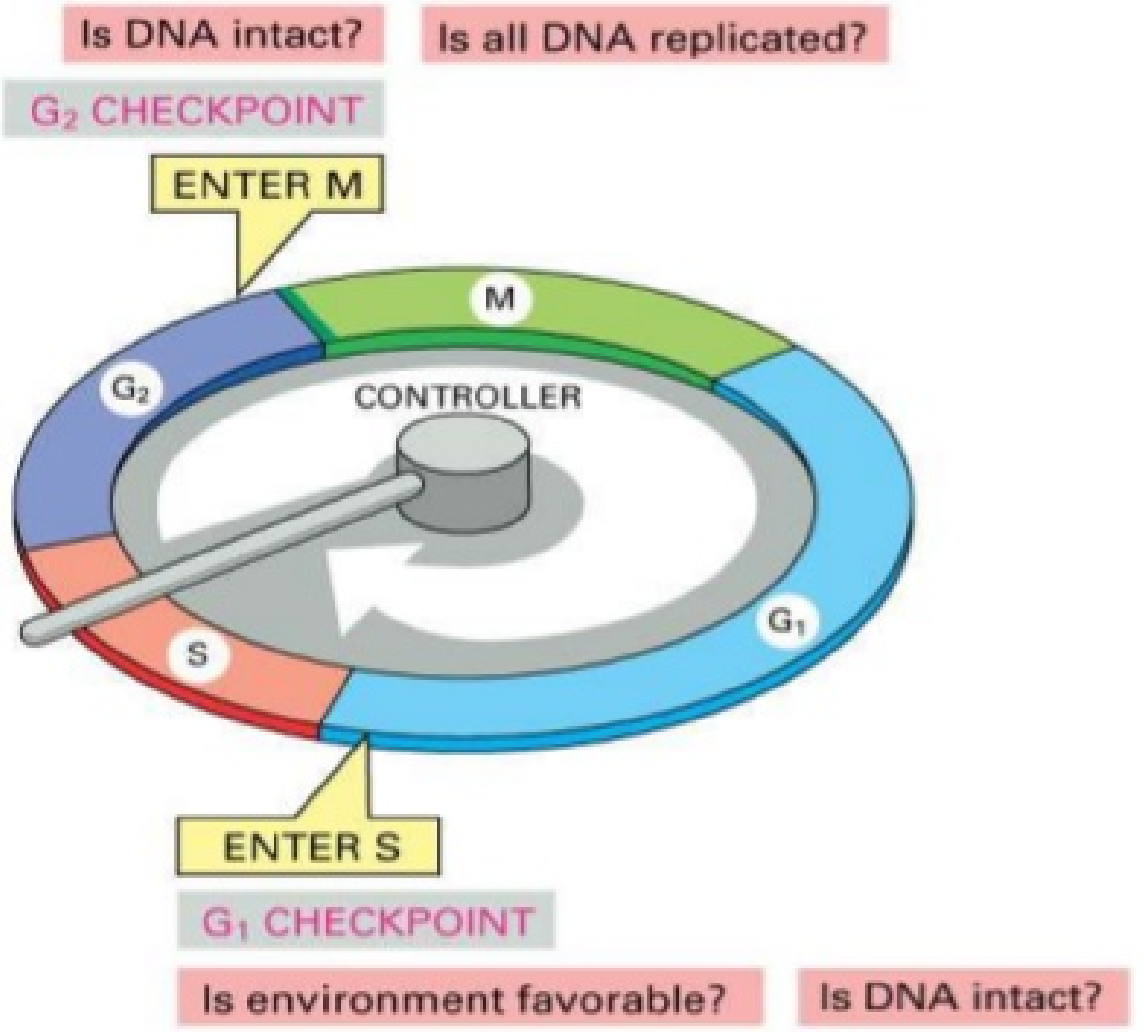
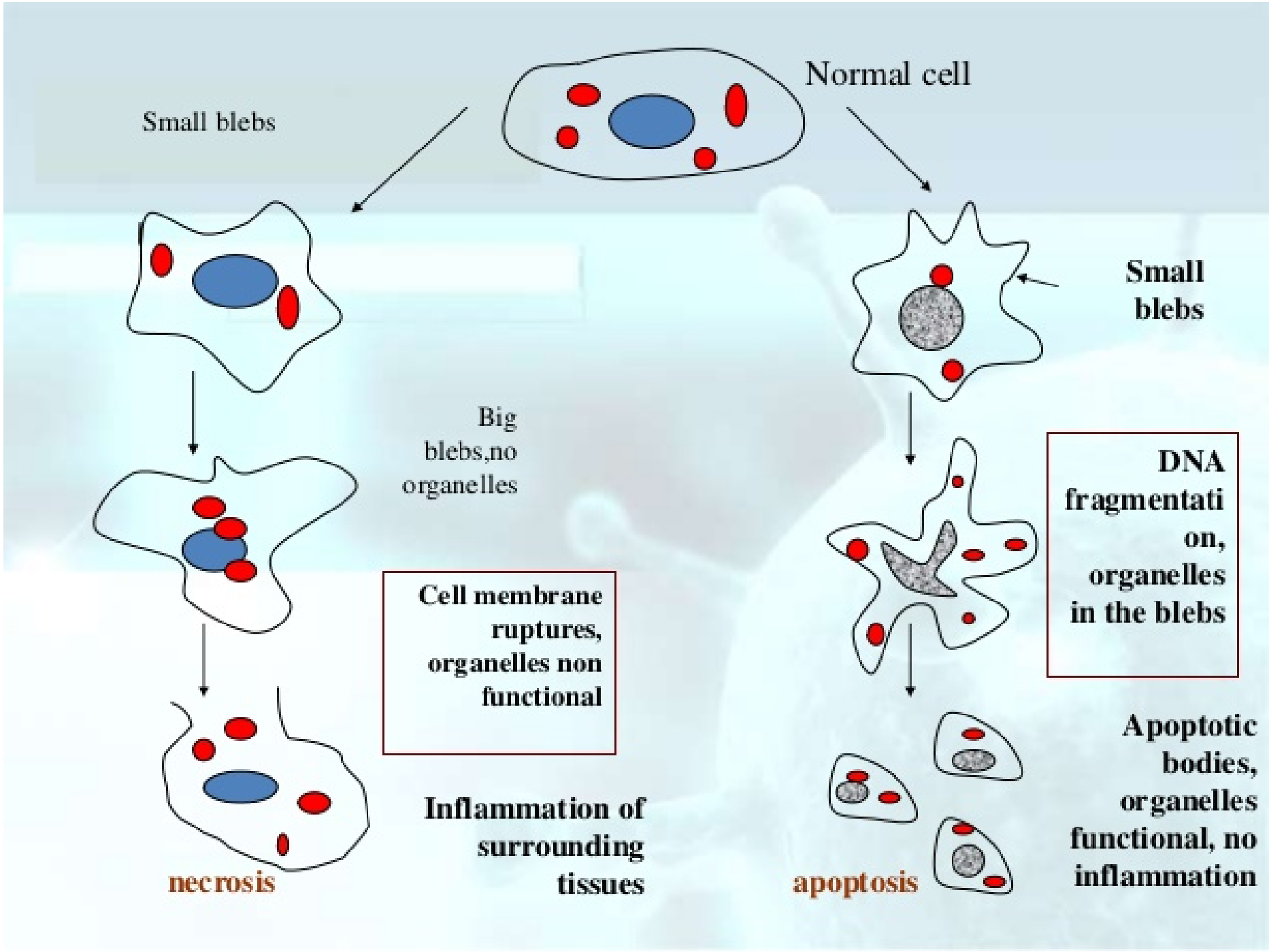


Figure 18-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

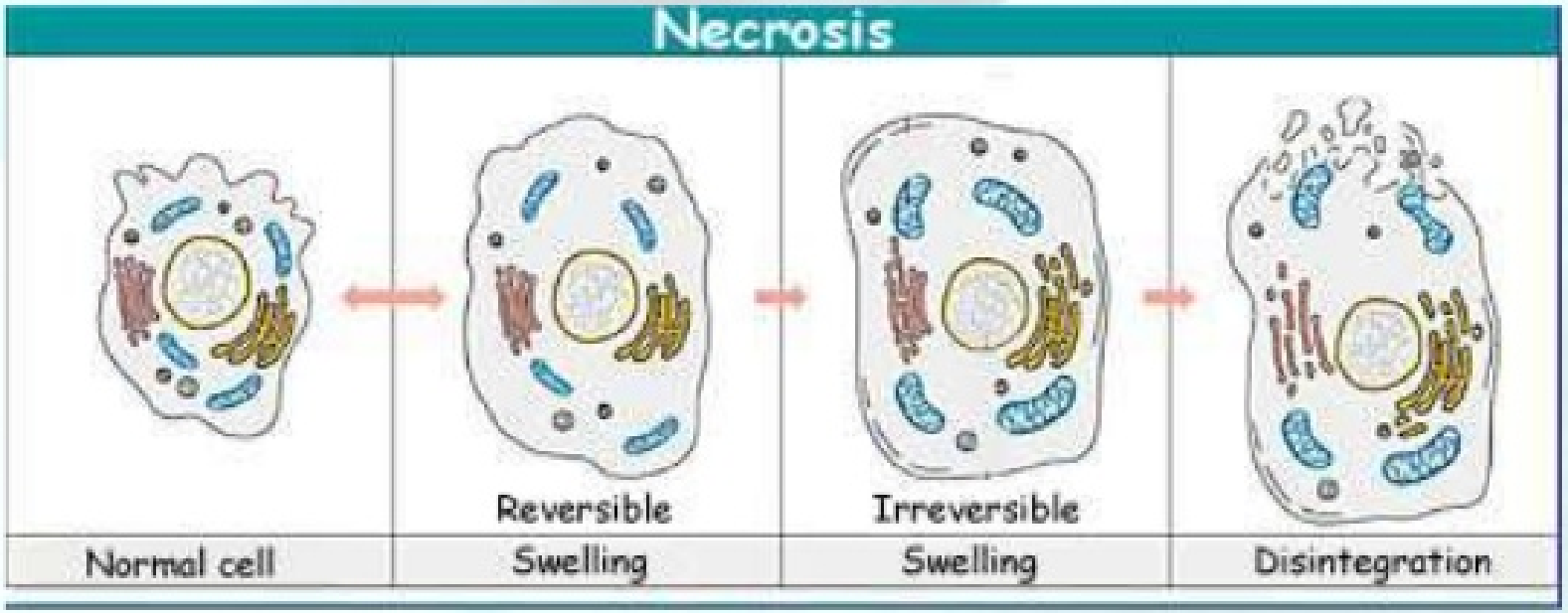
Cell death

- The body is very good at maintaining a constant number of cells. So there has to exist mechanisms for ensuring other cells in the body are removed, when appropriate.
- Two forms
  - Apoptosis - suicide - programmed cell death
  - Necrosis - killing - decay and destruction


Normal cell
Small blebs
Small blebs
Big blebs,no organelles
DNA fragmentation, organelles in the blebs
Cell membrane ruptures, organelles non functional
Inflammation of surrounding tissues
Apoptotic bodies, organelles functional, no inflammation
necrosis
apoptosis

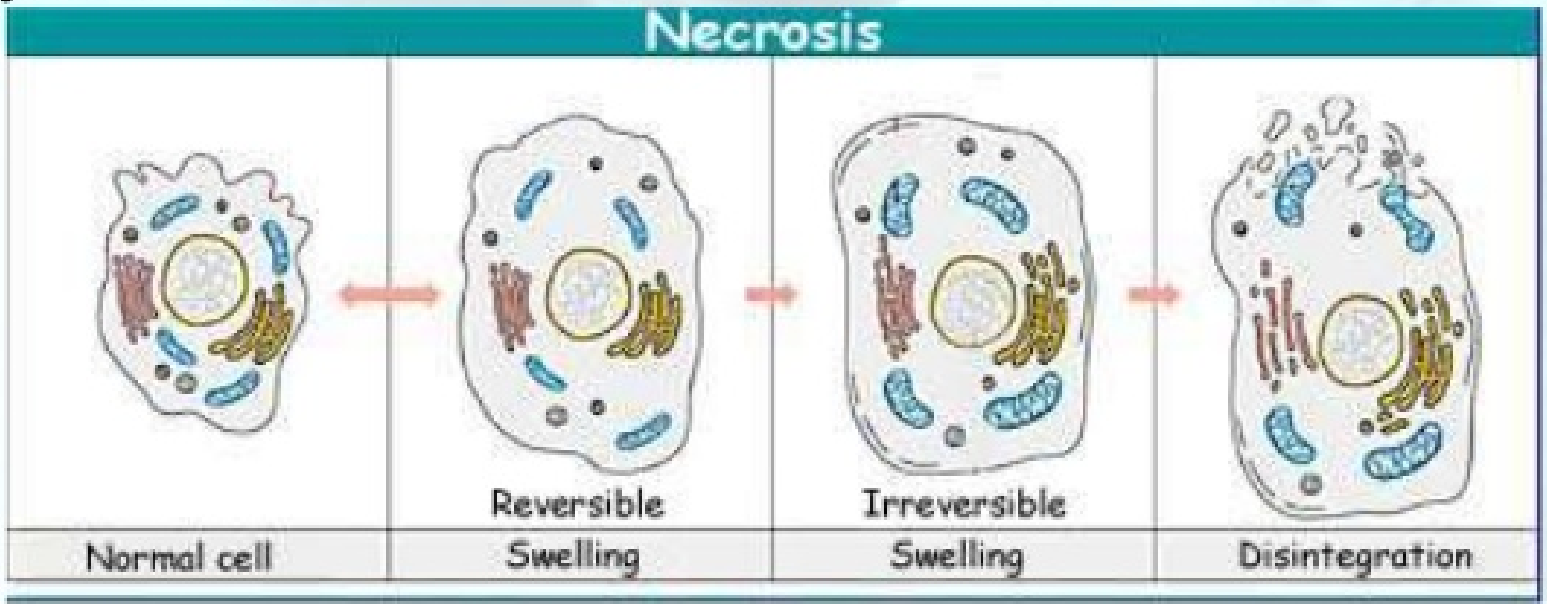
1- Necrosis

- Necrosis is the sum total of morphologic changes that follow cell death in a living tissue or organ
- Dead cells usually show changes in both the cytolasm and in the nucleus.

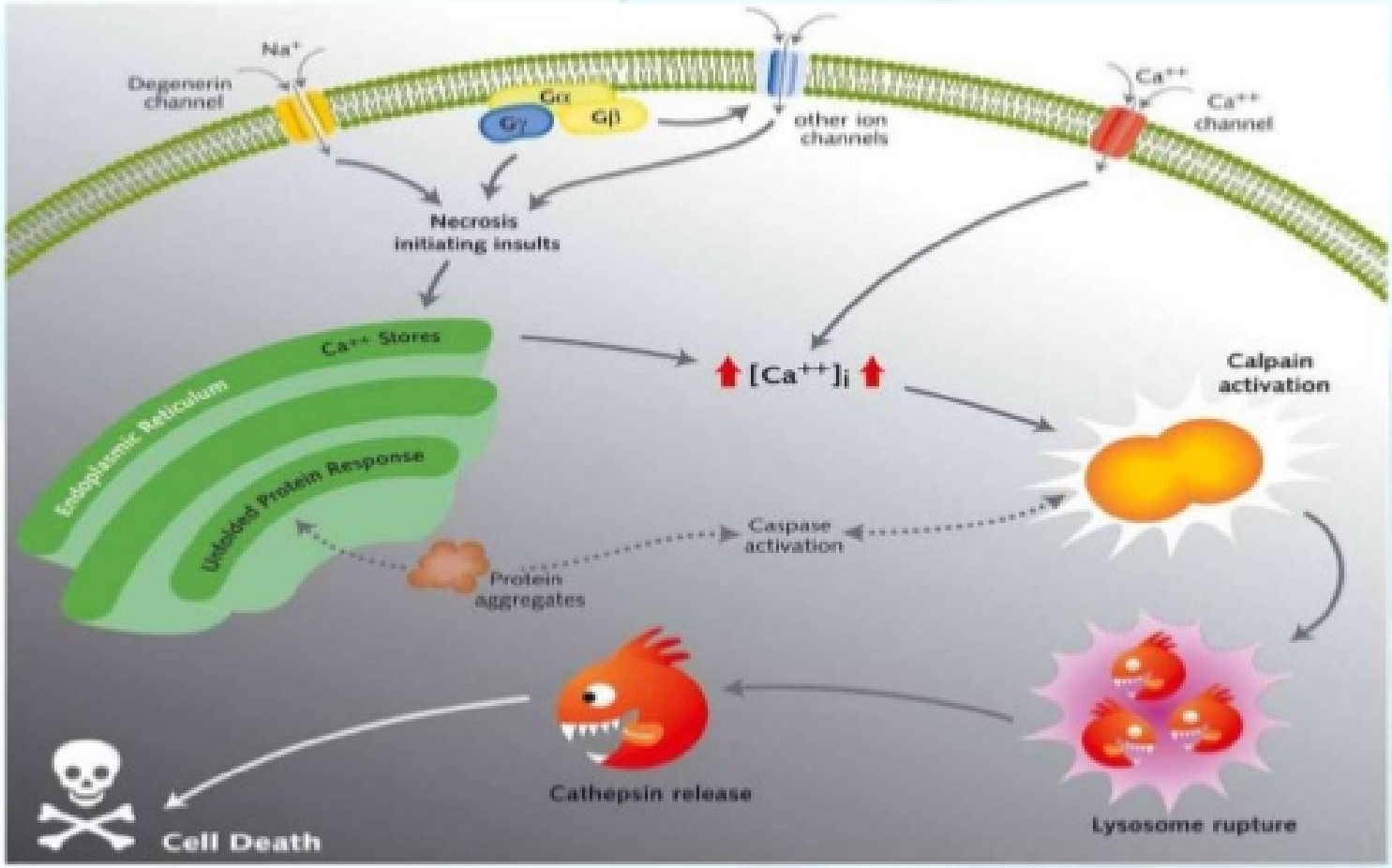


1- Necrosis

- Cytoplasmic changes are: increases eosinohilia, glassy appearance, granular or vacuolated cytolasm, swollen mitochondria, may also show calcification
- Nuclear changes: Karyolysis , Pyknosis and Karyorrhexis.



Necrosis


Na+
Degenerin channel
Gα
Gγ
Gβ
other ion channels
Ca++
Ca++ channel
Necrosis initiating insults
Ca++ Stores
Endoplasmic Reticulum
Unfolded Protein Response
[Ca++]i
Calpain activation
Caspase activation
Protein aggregates
Cathepsin release
Lysosome rupture
Cell Death

Causes

Necrosis may occur due to external or internal factors:

External factors may involve

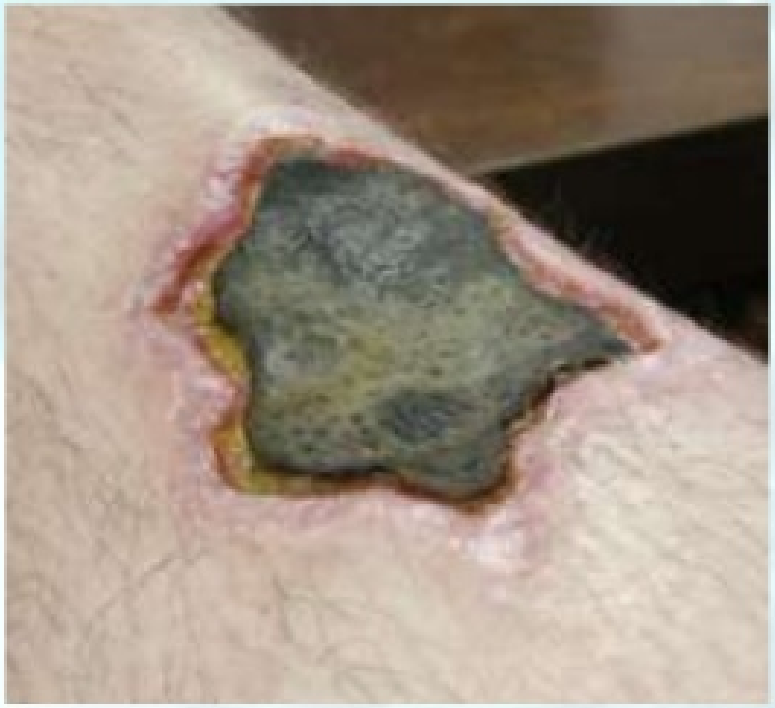
1. **Mechanical trauma** (physical damage to the body that causes cellular breakdown)
2. **Damage to blood vessels** (which may disrupt blood supply to associated tissue),
3. **Thermal effects** (extremely high or low temperature) can result in necrosis due to the disruption of cells.

Causes

Internal factors causing necrosis include

1. **Trophoneurotic disorders**; injury and paralysis of nerve cells.
2. **Pancreatic enzymes** (lipases) are the major cause of fat necrosis.
3. necrosis programs in cells with **immunological barriers** (intestinal mucosa) may alleviate invasion of pathogens through surfaces affected by inflammation
4. **Bacterial toxins;** activated natural killer cells; and peritoneal macrophages.
5. necrosis programs in cells with **immunological barriers** (intestinal mucosa) may alleviate invasion of pathogens through surfaces affected by inflammation
6. **Toxins and pathogens** may cause necrosis; toxins such as snake venoms may inhibit enzymes and cause cell death

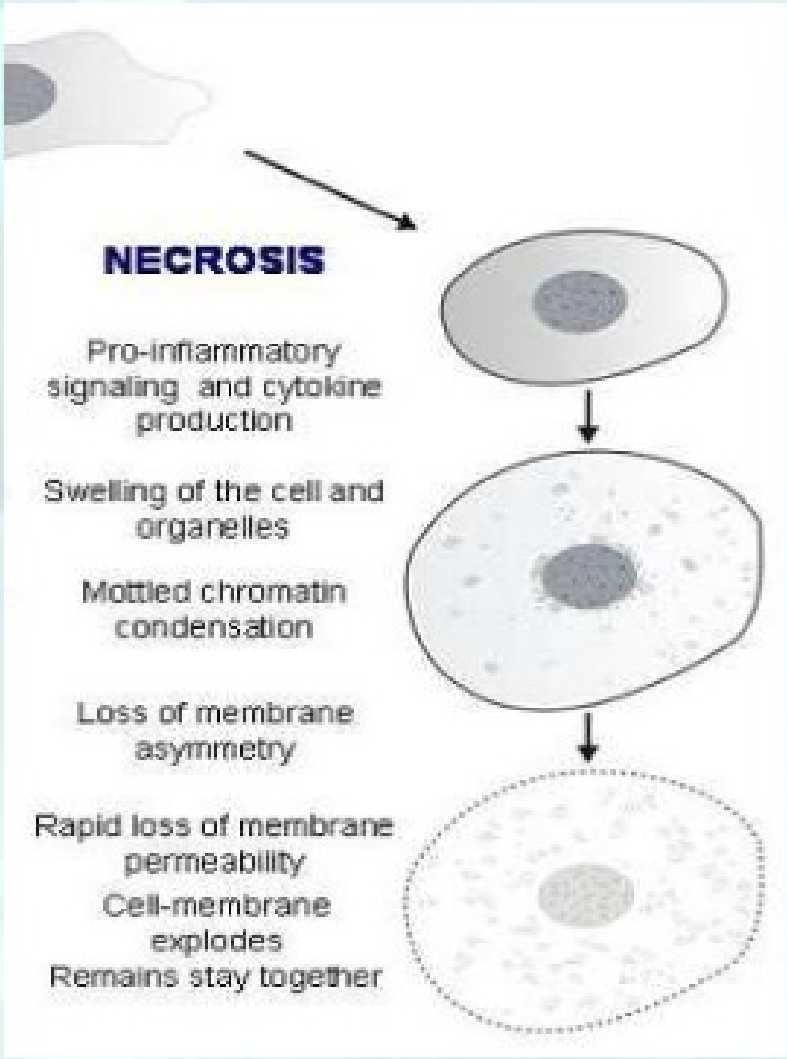
Causes



Mechanisms of Necrosis

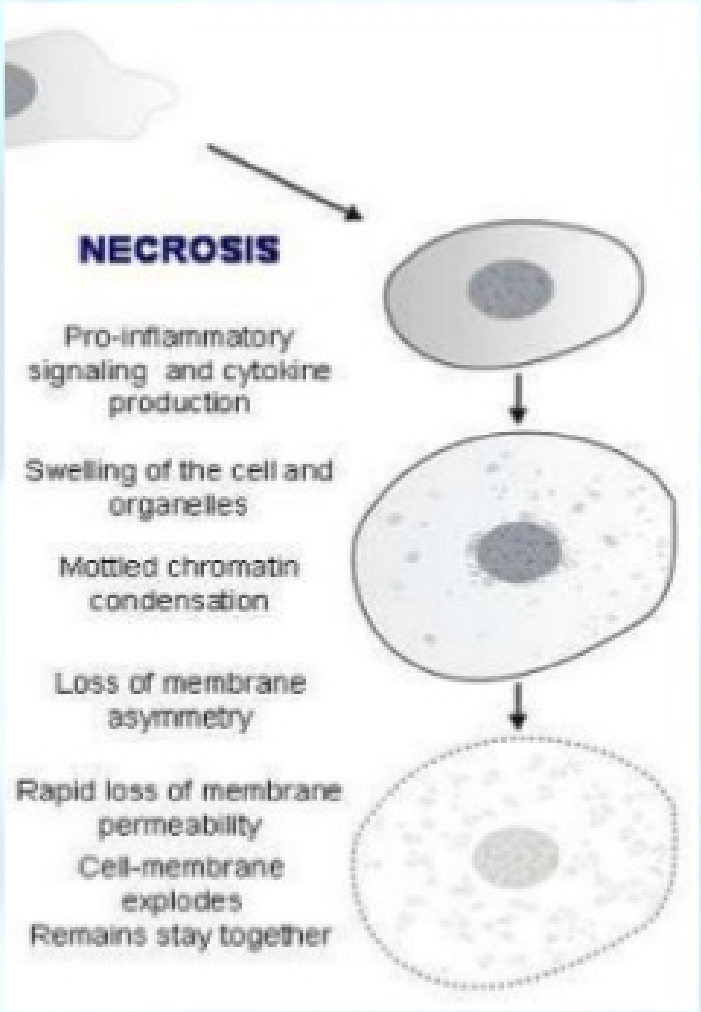
Necrotic Cell Death

- Loss of metabolic functions
- Loss of the integrity of the cell membranes
- Cessation of the production of proteins and ATP.
- Cells organelles swell and become nonfunctional.



Mechanisms of Necrosis

1. **Depletion of ATP-leads** to breakdown of the cell's ion balance
2. **Reduce oxygen level** (hypoxia)
3. **Oxidative stress** - the presence of excess oxygen radicals



What are the types of necrosis?

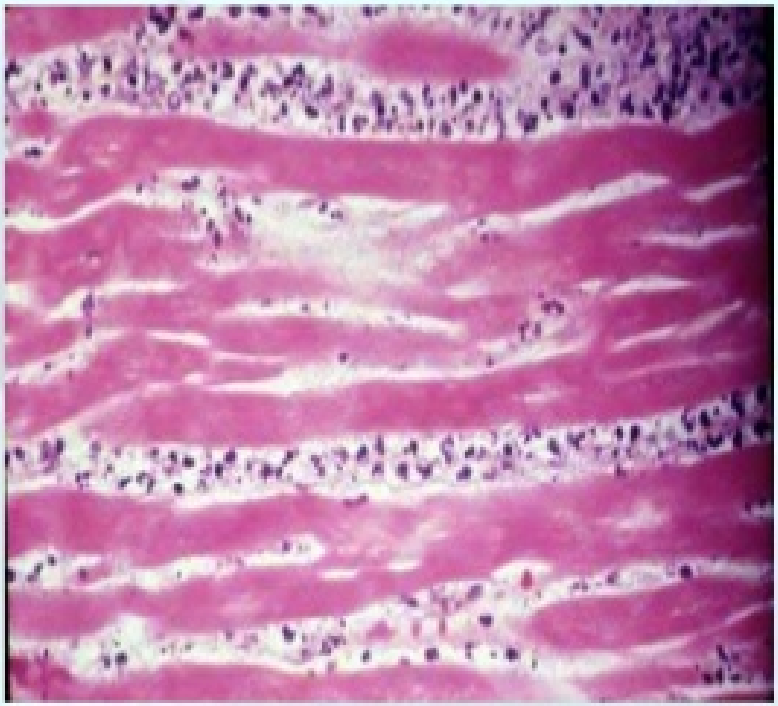
1. Coagulation Necrosis
2. Liquefactive Necrosis
3. Fat Necrosis
4. Caseous Necrosis
5. Gangrenous Necrosis
6. Fibrinoid Necrosis

Necrosis types:

1.Coagulation Necrosis

See this in infarcts in any tissue (except brain)

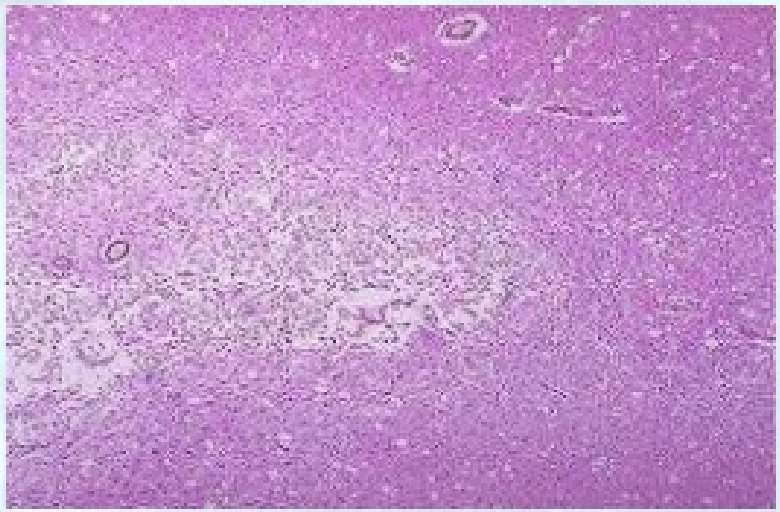
- Due to loss of blood
- Cell outlines are preserved and everything looks red.



Necrosis types

2.Liquefactive Necrosis

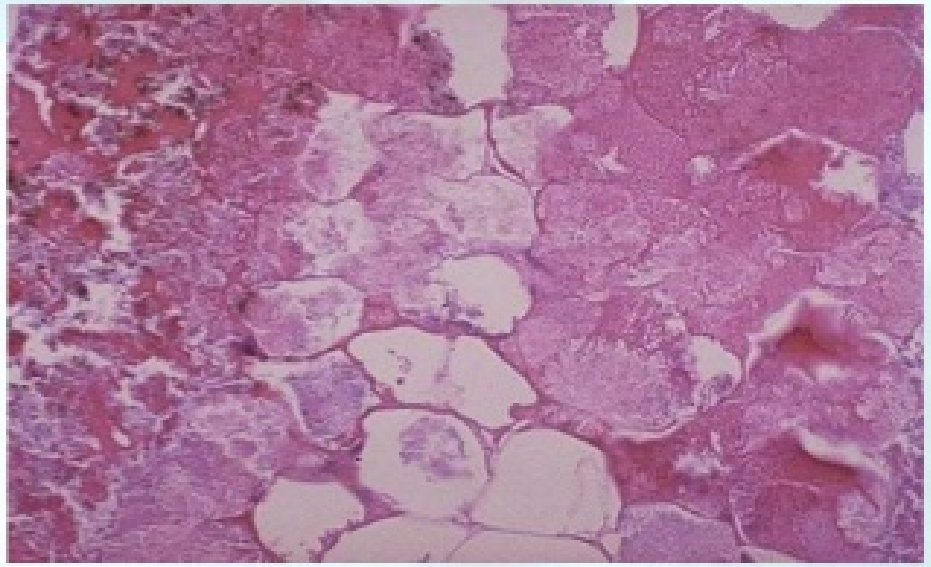
- See this in infections in brain infarcts
- Due to lots of neutrophils around releasing their toxic contents, "liquefying" the tissue.
- tissue is liquidly and creamy yellow (pus)
- lots of neutrophils and cell debris



Necrosis types:

3.Fat Necrosis:

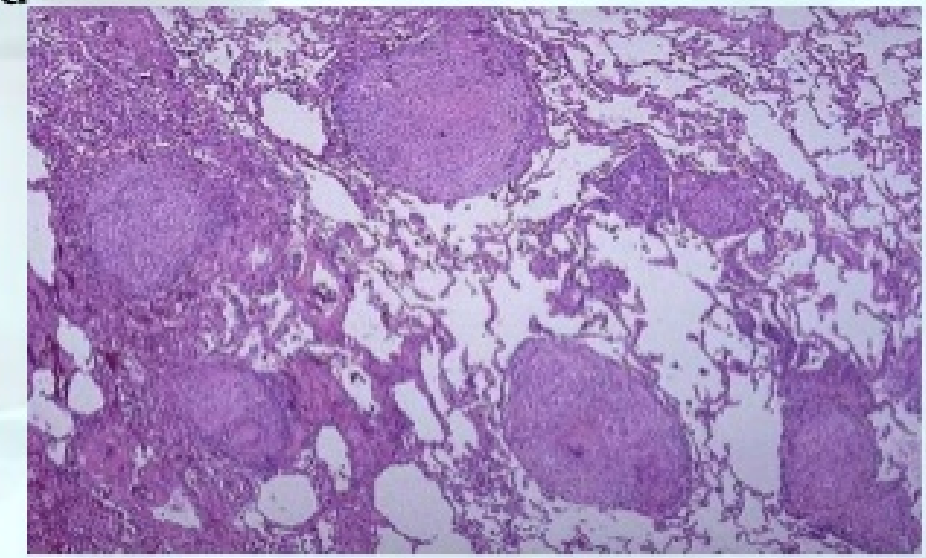
- fat necrosis that in which the neutral fats in adipose tissue are split into fatty acids and glycerol, usually affecting the pancreas
- shadowy outlines of dead fat cells



Necrosis types:

4.CASEOUS NECROSIS-LUNGS

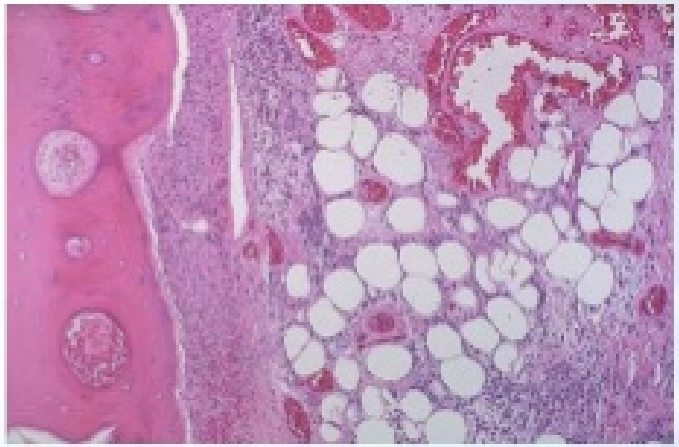
- cheesy necrosis that in which the tissue is soft, dry, and cottage cheese–like; most often seen in tuberculosis and syphilis.



Necrosis types:

5.Gangrenous Necrosis

- See this when an entire limb loses blood supply and dies
- skin looks black and dead; underlying tissue is in varying stages of decomposition
- initially there is coagulative necrosis from the loss of blood supply (this stage is called "dry gangrene"); if bacterial infection is superimposed, there is liquefactive necrosis (this stage is called "wet gangrene").



Necrosis types

6.Fibrinoid necrosis

- See this in immune reactions in vessels
- Complexes of antigens and antibodies (immune complexes) combine with fibrin
- changes too small to see grossly
- vessel walls are thickened and pinkish-red (called "fibrinoid"

Necrosis types

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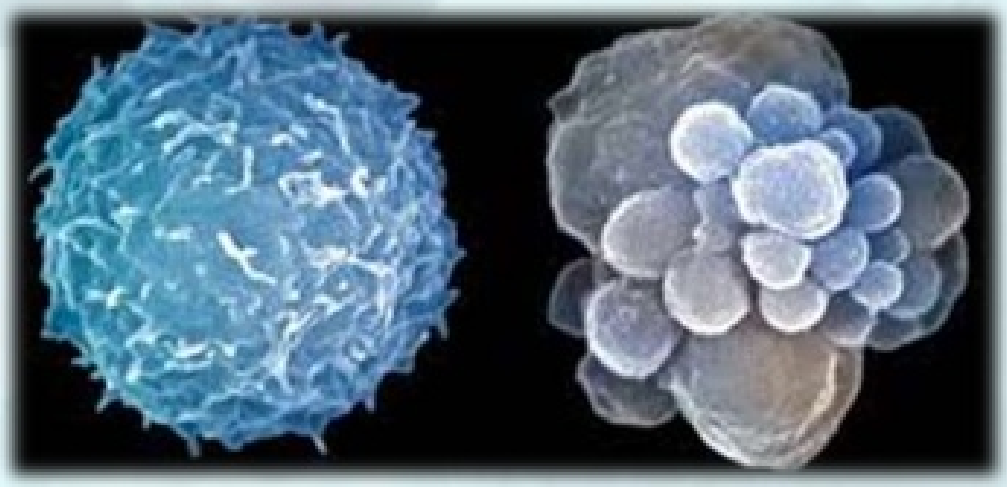
2 apoptosis
programmed cell death

- In human body, cells were produced every second by mitosis there is a similar number die by apoptosis.

- Between 50_ 70 billion cells die each day in adult.
- Between 20_ 30 billion cells die each day in child.

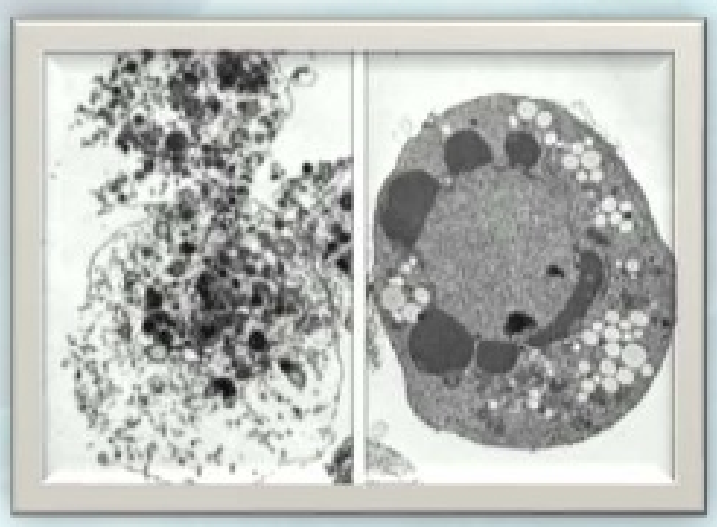
Apoptosis
programmed cell death

- Form of cell death, in which a 'suicide' program is activated within the cell, leading to:
- fragmentation of the DNA.
- shrinkage of the cytoplasm.
- membrane changes and cell death without lysis or damage to neighboring cells.



General characteristics of apoptosis

- It is a normal phenomenon, occurring frequently in a **multicellular organism**.
- a cell that undergoes apoptosis dies neatly, without damaging its neighbors. The cell shrinks and condenses.
- There is no inflammation in apoptosis.

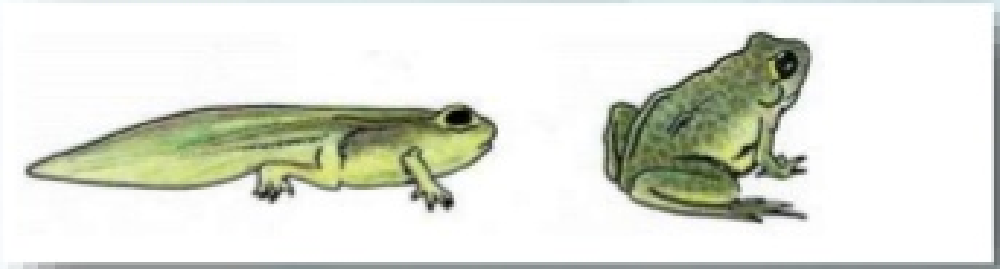


Importance of apoptosis

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

Examples: –

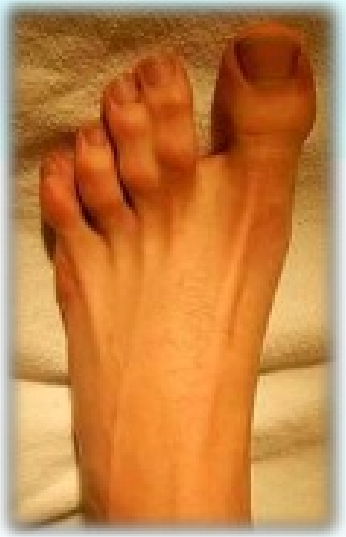
- The moldiness of the tadpole tail in frog .
- The formation of the fingers and toes of the fetus requires the removal, by apoptosis.
- The sloughing off of the endometrium at the start of menstruation.

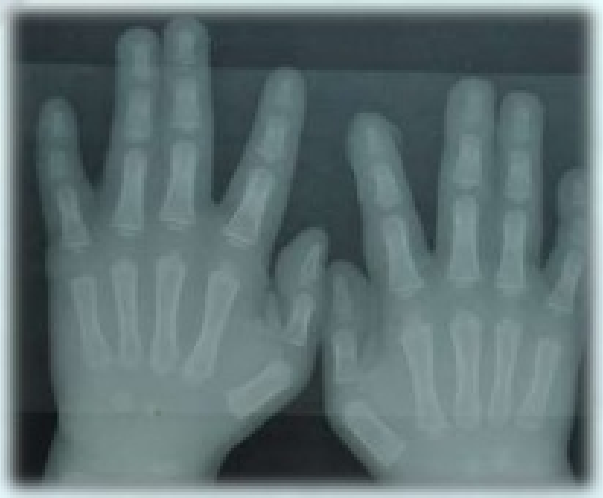


Importance of apoptosis (cont..)

- **Example:**

Incomplete differentiation in two toes due to lack of apoptosis





Morphology of apoptosis

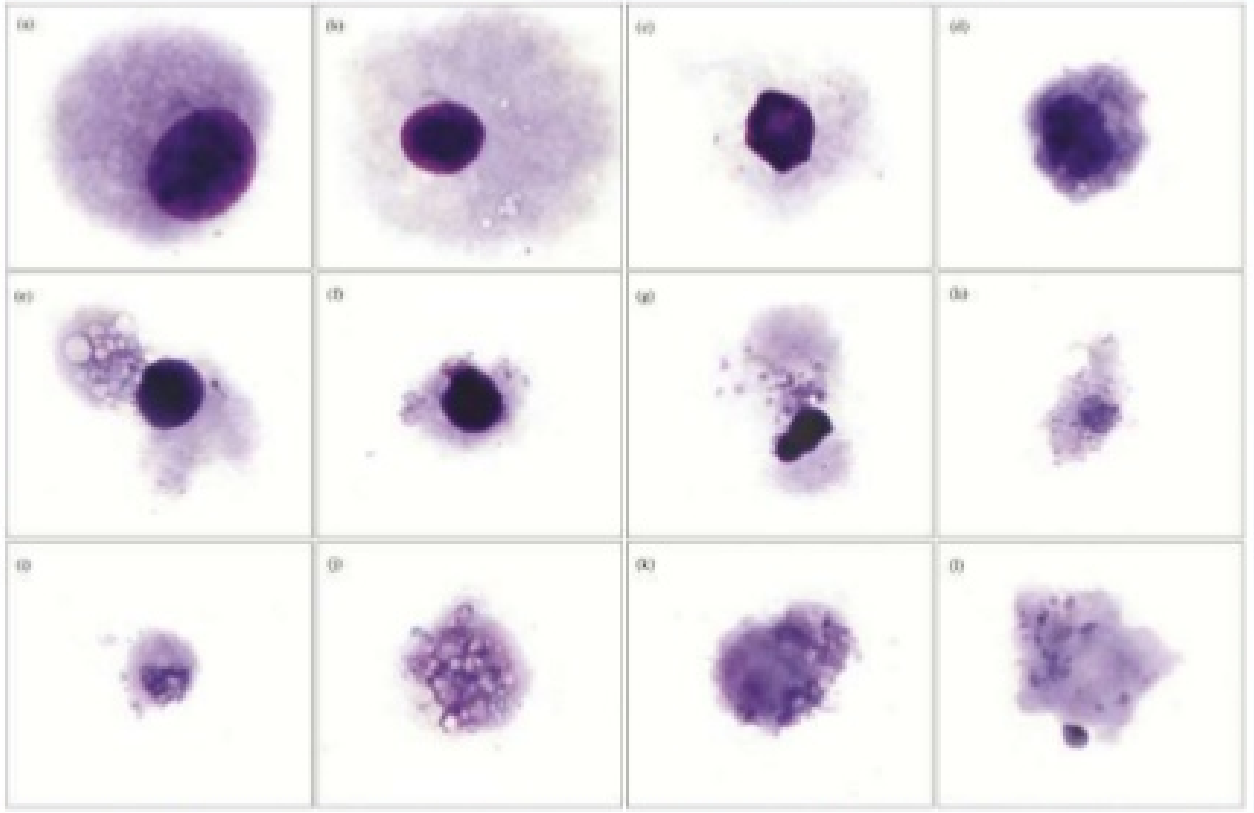


Figure 4. Cells in different stages of apoptosis in treated cultures are easily distinguishable. Most cells had normal morphology (a). Nuclear condensation is evident in cells (dark, condensed and rounded nuclei), as well as vacuolated cytoplasm (b-e). Degradation of nuclei and cytoplasm is also present (f-k). Membrane blebbing and apoptotic bodies are also evident.

Biochemical features of apoptosis

- DNA break down in apoptosis.
- protein cleavage
- phagocytic.

Mechanisms of apoptosis

- **Apoptosis occur in two phases:**

1. **Initiation phase:**

It happen when apoptotic enzymes are getting activated.

1. **Execution phase:**

Activating enzymes are causing cell death.

Mechanisms of apoptosis

- **Initiation phase:**

1. Extrinsic pathway.
2. Intrinsic pathway.

1) Extrinsic pathway

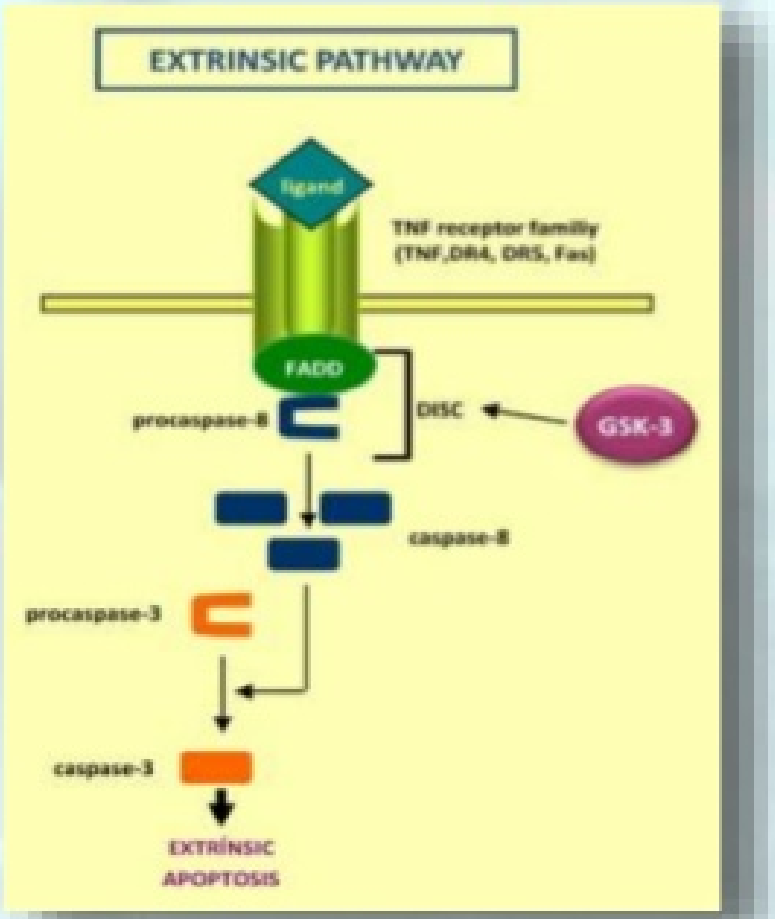
- Called **death receptor pathway**, mediated by:

death receptors.

- **TNF** family protein(tumor necrosis factor) .e.g. TNF R1, TNF fas.
- **Caspase** are (Cysteine- Aspartic acid) specific proteases that mediates the events that are associated with programmed cell death.

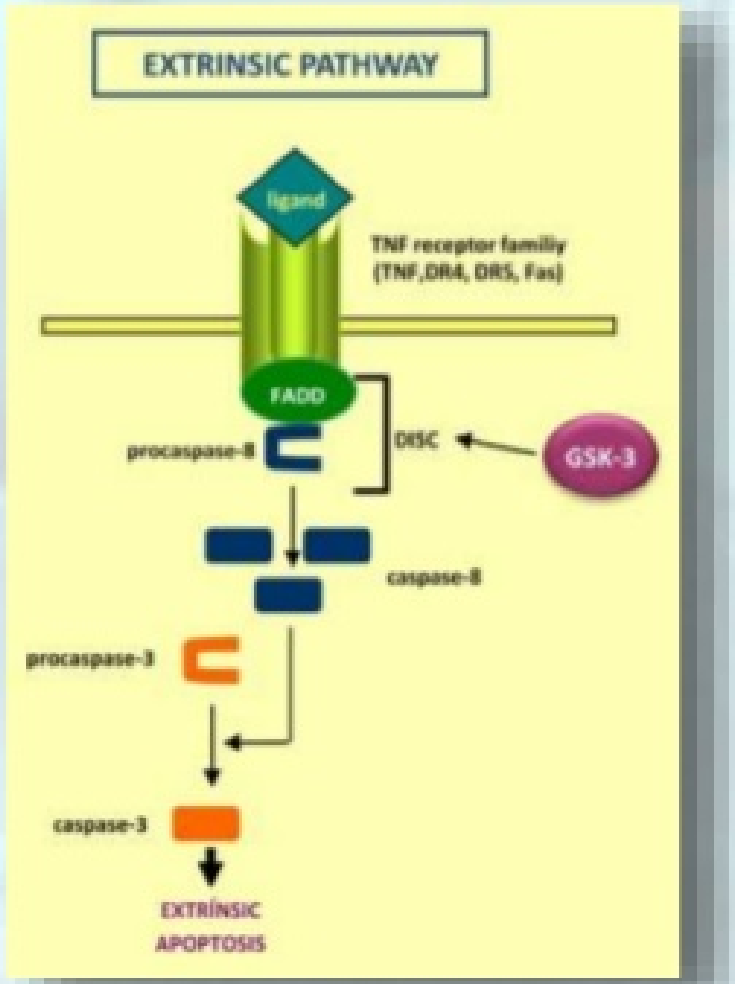
1) Extrinsic pathway

- Apoptosis initiated by extrinsic pathway.
- In cytoplasmic side death receptor has death domains.



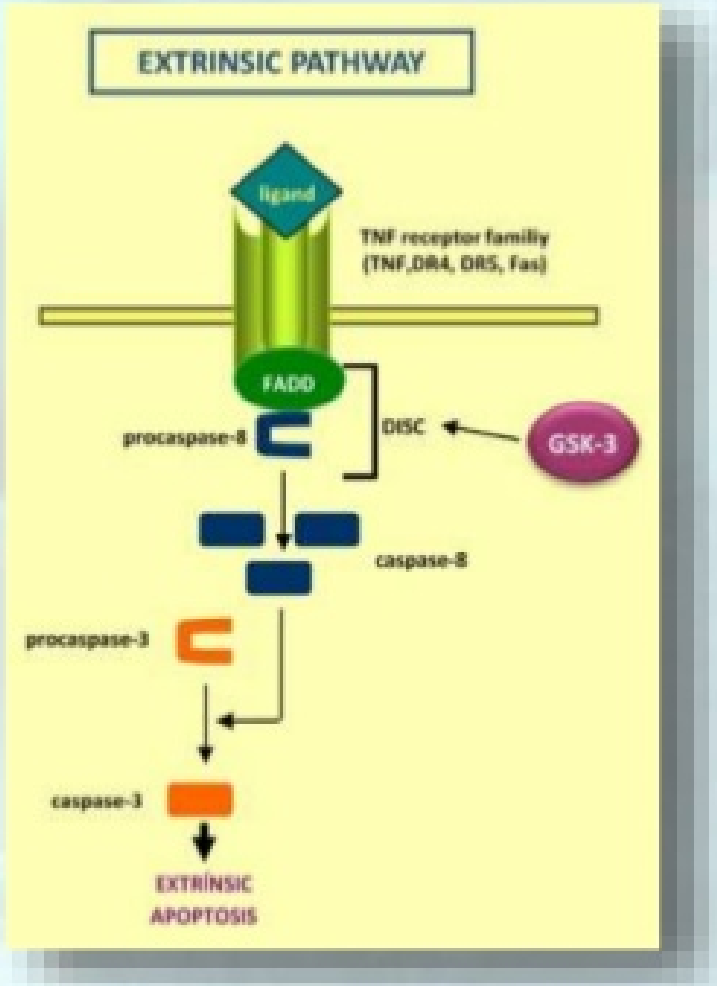
1) Extrinsic pathway

- Fas L(fas ligand) bind to fas receptor, and form cross link between three or more fas receptor then they can form binding site for adaptor molecule called **death domain,** which attract inactivated pro_ caspases and FADD(fas associated death domain).



1) Extrinsic pathway

- **Caspases 8** start to cleave other pro_ caspases to result active form of caspases.
- These caspases go to execution phase and causing apoptosis.

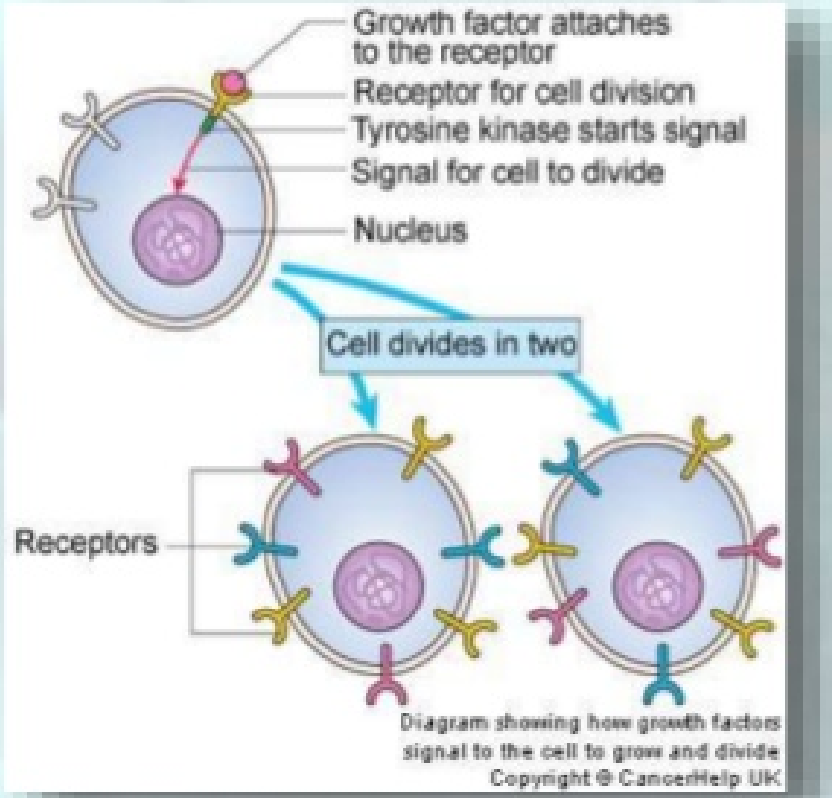


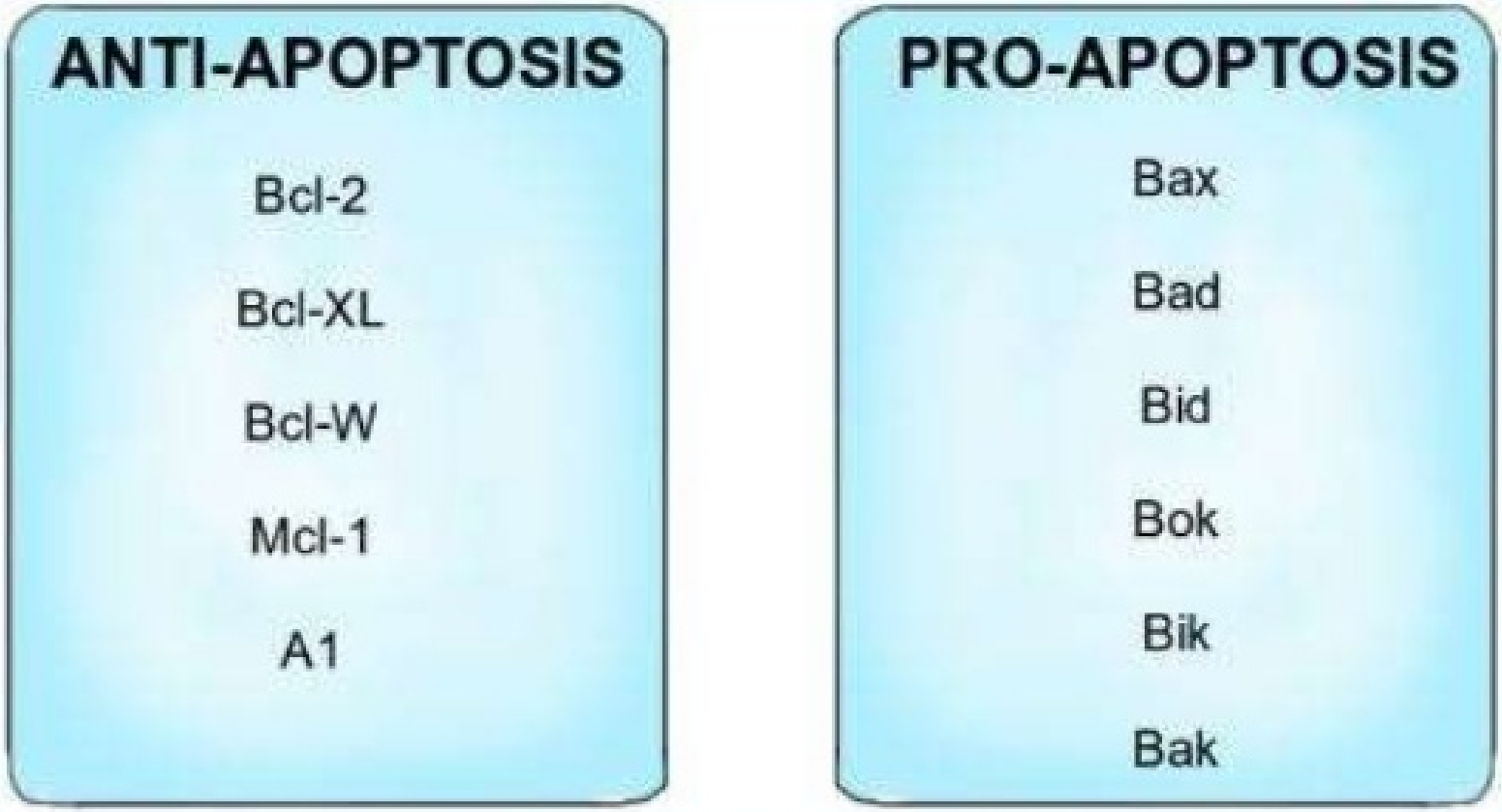
2) Intrinsic pathway

- Known as mitochondrial pathway.
- Apoptosis occur due to increase permeability of mitochondria.

2) Intrinsic pathway

- **In normal situation,**
- growth factor bind to growth receptor in plasma membrane which causing formation of some **anti_ apoptotic protein** .e.g. **Bcl2, Bcl X** in mitochondria membrane.
- Those will prevent leakage of pro_ apoptotic molecule.

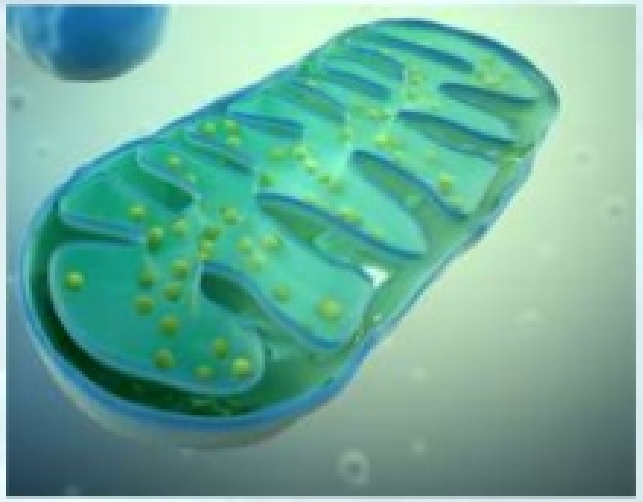



ANTI-APOPTOSIS
Bcl-2
Bcl-XL
Bcl-W
Mcl-1
A1
PRO-APOPTOSIS
Bax
Bad
Bid
Bok
Bik
Bak

2) Intrinsic pathway

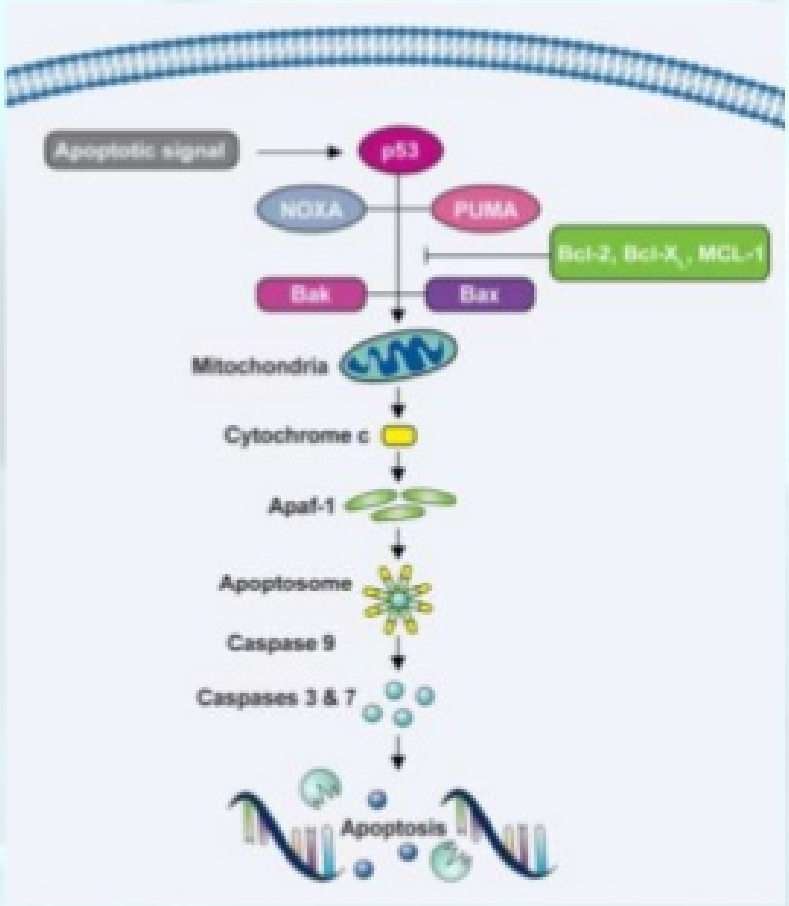
- Radiation and other factors assist to remove these signal e.g. Bcl 2, and BclX via other type of **pro_ apoptotic proteins** such as **Bak, and Bax.**

- Increasing the permeability of mitochondria resulting leakage of pro_ apoptotic molecules from mitochondria to cytoplasm.



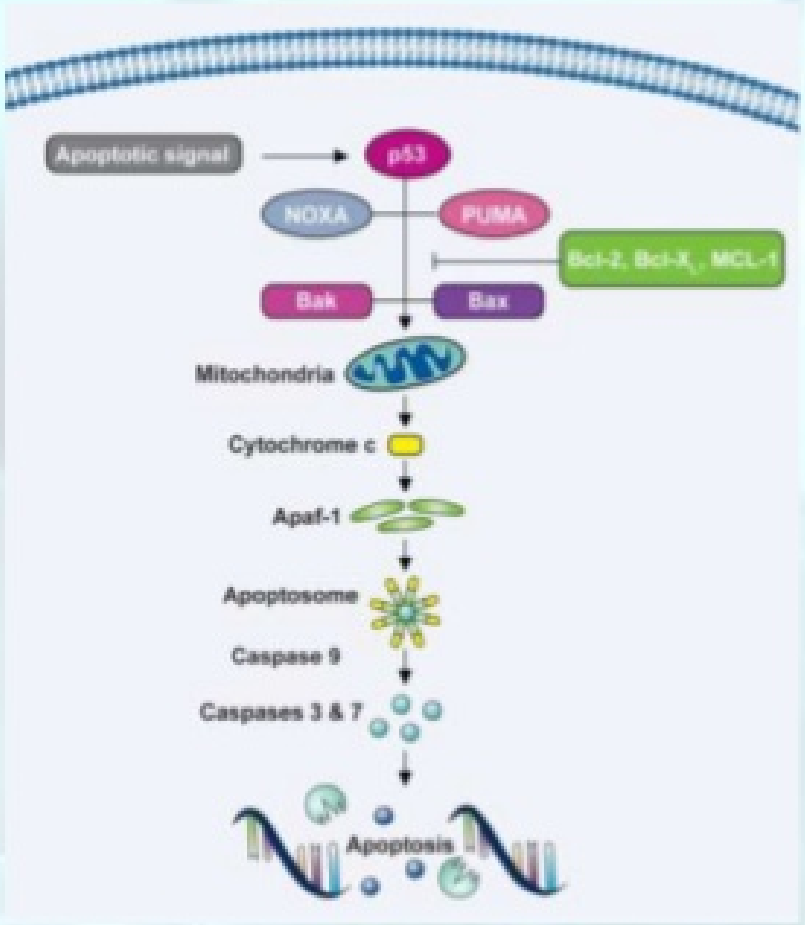
2) Intrinsic pathway

- **In cytosol,**
- Pro-apoptotic Factors Damage to the mitochondrial membrane increasing permeability Entry of **Cytochrome C** into the cytoplasm
- Cytochrome c will bind to another molecule known as **ApaF1** (apoptosis activity factor 1) which activate **caspases 9** resulting apoptosome .



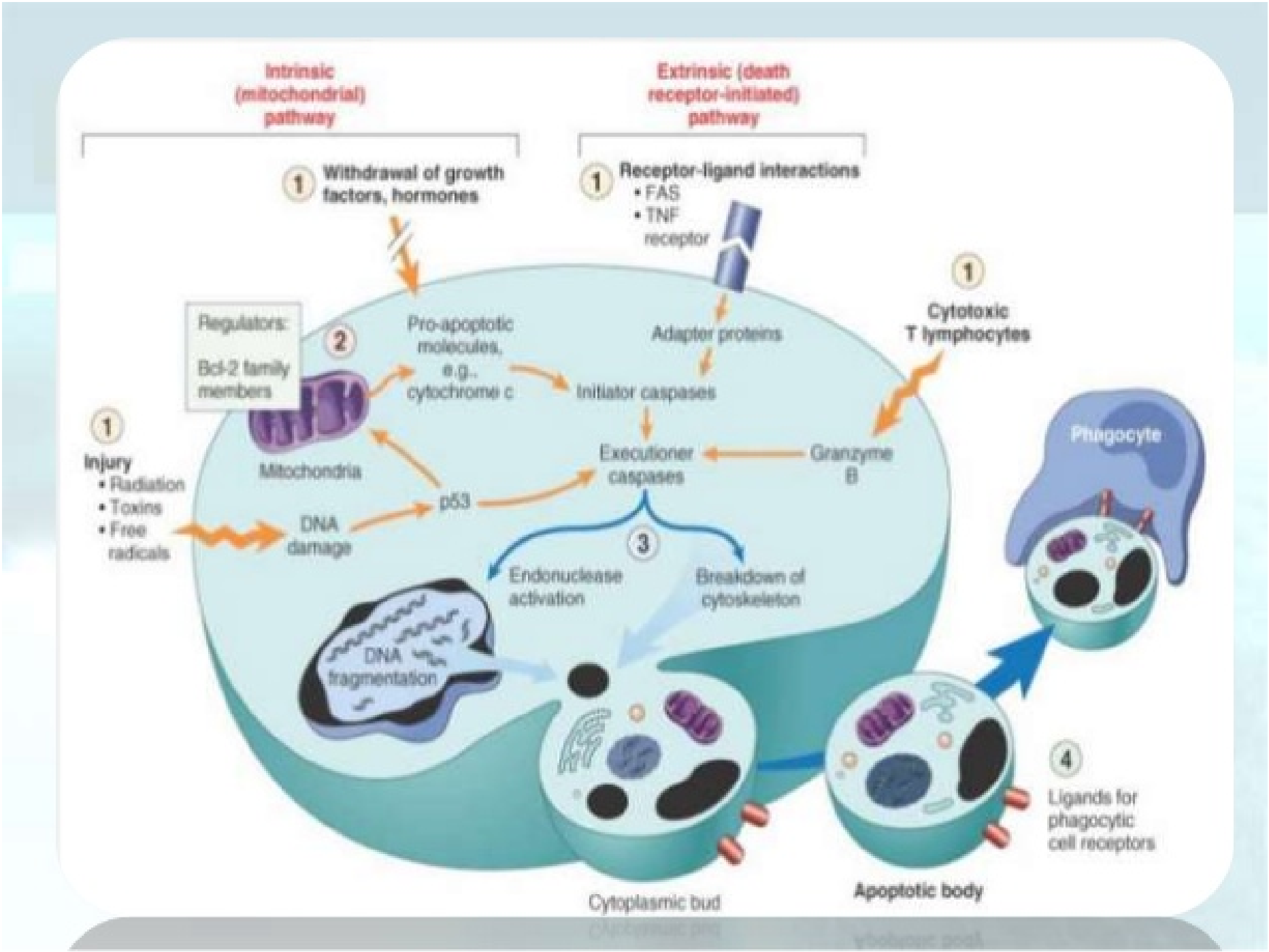
2) Intrinsic pathway

- Apoptosome activates procaspase-9 to caspase-9
- Caspase-9 cleaves and activates **pro_ caspase-3** and **pro_ caspase-7**.
- This executioner caspases activate a cascade of proteolytic activity that leads to apoptosis.



2) Intrinsic pathway

- Other factor releasing form mitochondria is **apoptosis inducing factor** that neutralize anti_ apoptosis factor and promote 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
DNA damage
p53
3
Endonuclease activation
Breakdown of cytoskeleton
DNA fragmentation
Phagocyte
4 Ligands for phagocytic cell receptors
Cytoplasmic bud
Apoptotic body

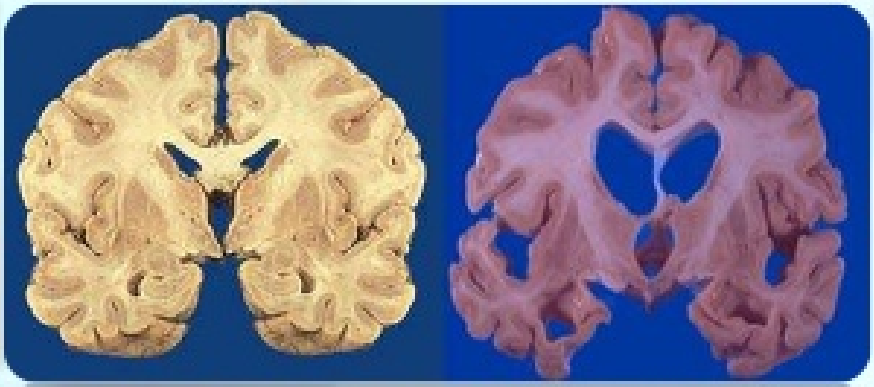
Execution phase

- It is mediated by **caspase 3 and caspase 6** , recall caspase 8 and caspase 9(initiation caspases).
- When it's activated, they form sequence chain of reaction that can activate Caspase 3 and 6.
- They **break down cytoskeleton protein**, and **nuclear matrix protein** that resulting **breaking the nucleus**.

Role in diseases

- **Too much apoptosis:**

  Tissue atrophy.



- **Too little apoptosis:**

Cancer, Atherosclerosis.

APOPTOSIS & NECROSIS

	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

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

APOPTOSIS & NECROSIS