Teaching unit 02

MECHANISMS OF CELL DEATH

Apoptosis

- mechanism, role and significance
- the difference between necrosis and apoptosis.
- basic principles of cell death induced by death receptors (external signal)
- basic principles of cell death as a result of loss of survival stimulus (internal signal)

Autophagy

- mechanism, role and significance

Necrosis

- mechanism, role and significance

"There is a time for everything in the world. There is a time to live and a time to die..."

The only certainty of life is his end, which us today's people unaccustomed to living with death, always surprises.

As far as we know today cells die in three ways:

- Necrosis- which is a consequence of significant external damage to the cell by physico-chemical agents (hypoxia, extreme temperatures, action of complement). A cell uses energy to survive.
- Apoptosis- which is a consequence of the activation of the internal program of cellular suicide. A cell uses energy to die.
- Autophagy

APOPTOSIS...

-programmed cell death-

... is a highly regulated process that occurs

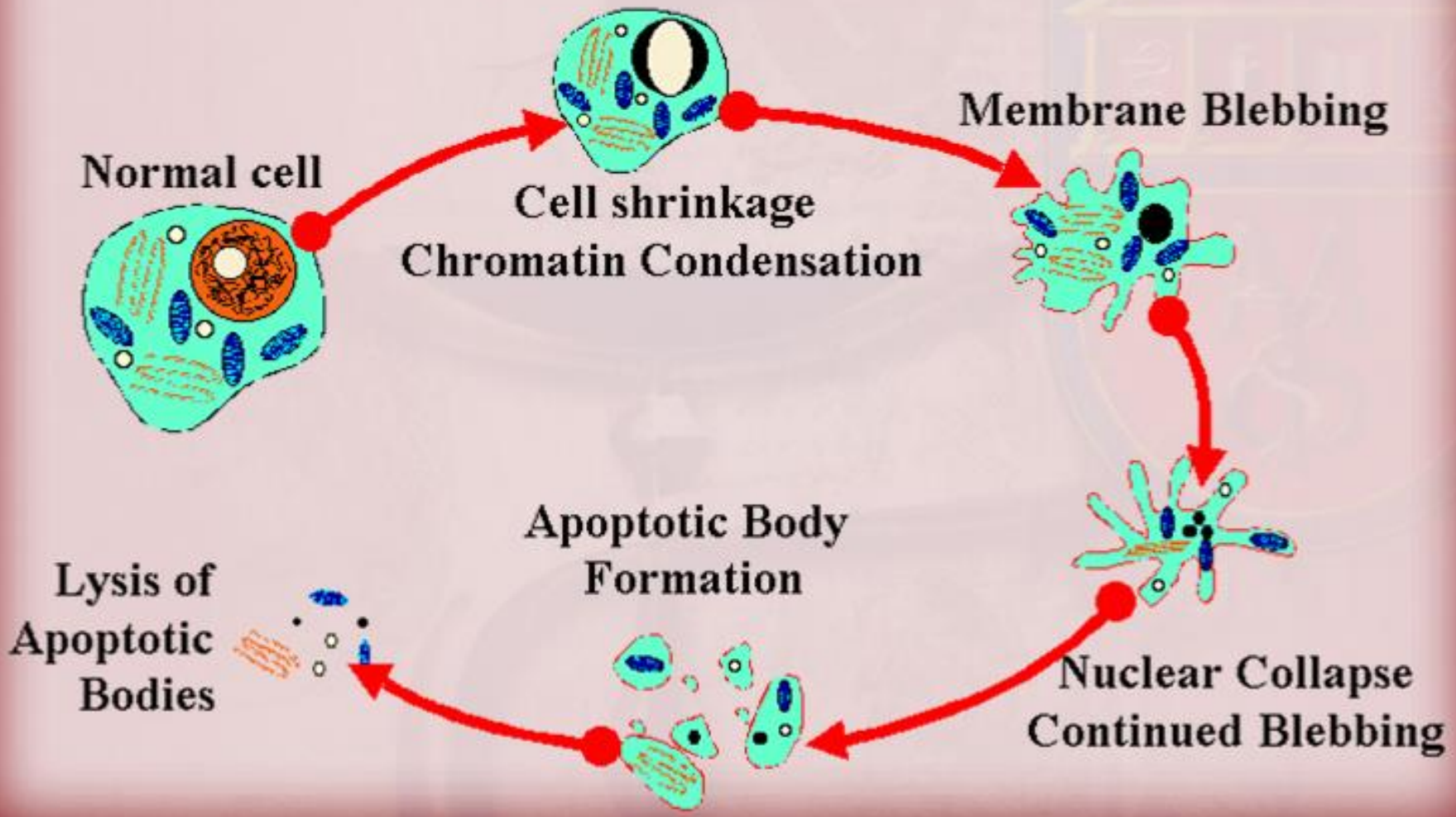
in one cell independently from the surrounding cells.

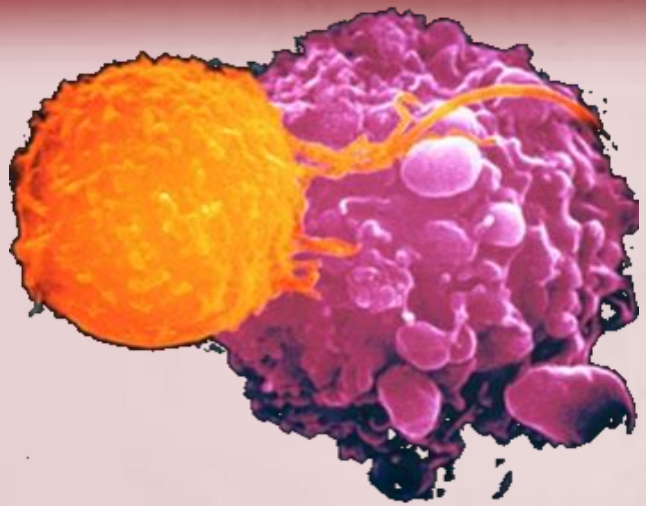
APOPTOSIS

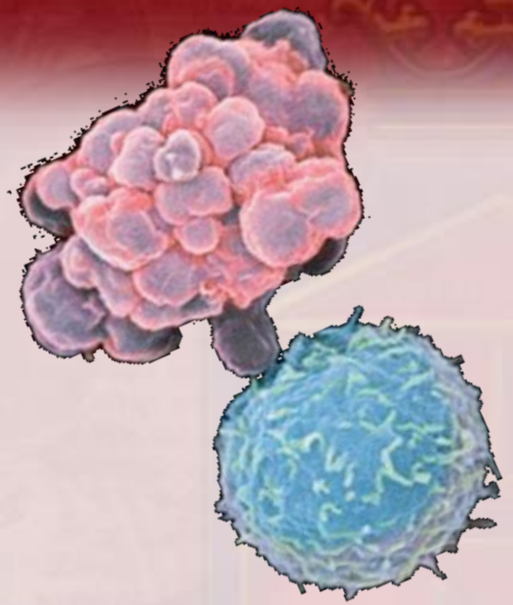
-basics of program morphology-

- chromatin condensation - nuclear pyknosis;
- destruction of laminin and actin filaments - shriveling of the cell;
- thickening of the cytoplasm;
- swelling of the cell membrane - the cell becomes rounded;
- membrane inversion - exposure of phosphatidylserine and calreticulin ("*eat me*" signal);
- destruction of the cell matrix, fragmentation of the nucleus and DNA;
- damage to mitochondria and release of cytochrome-C;
- formation of apoptotic bodies containing cytoplasm and densely packed organelles, as well as nuclear fragments;
- these bodies are phagocytosed by tissue macrophages, which secrete anti-inflammatory cytokines (IL-10, TGF-β).

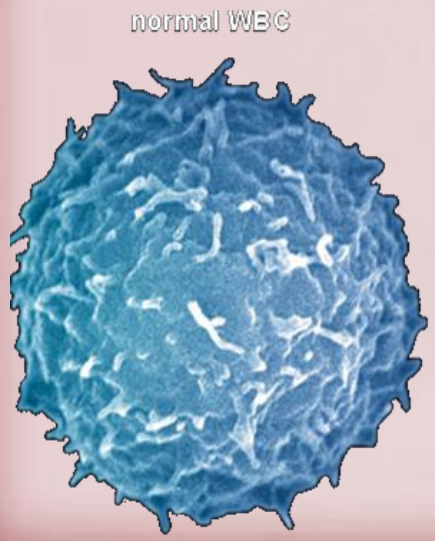
Apoptosis
(Programmed Cell Death)

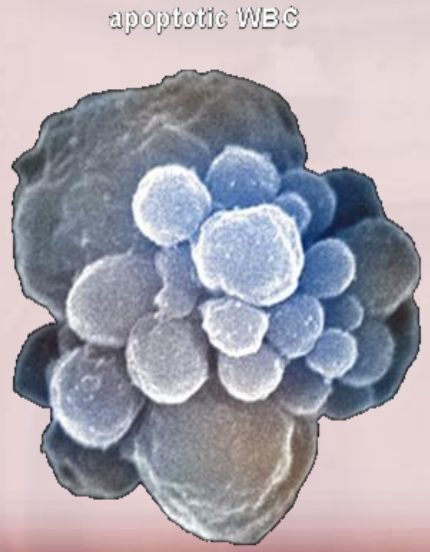


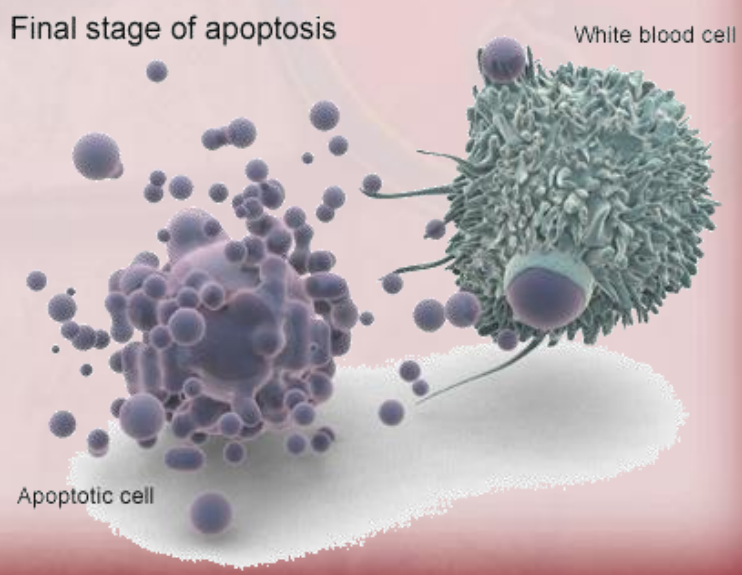


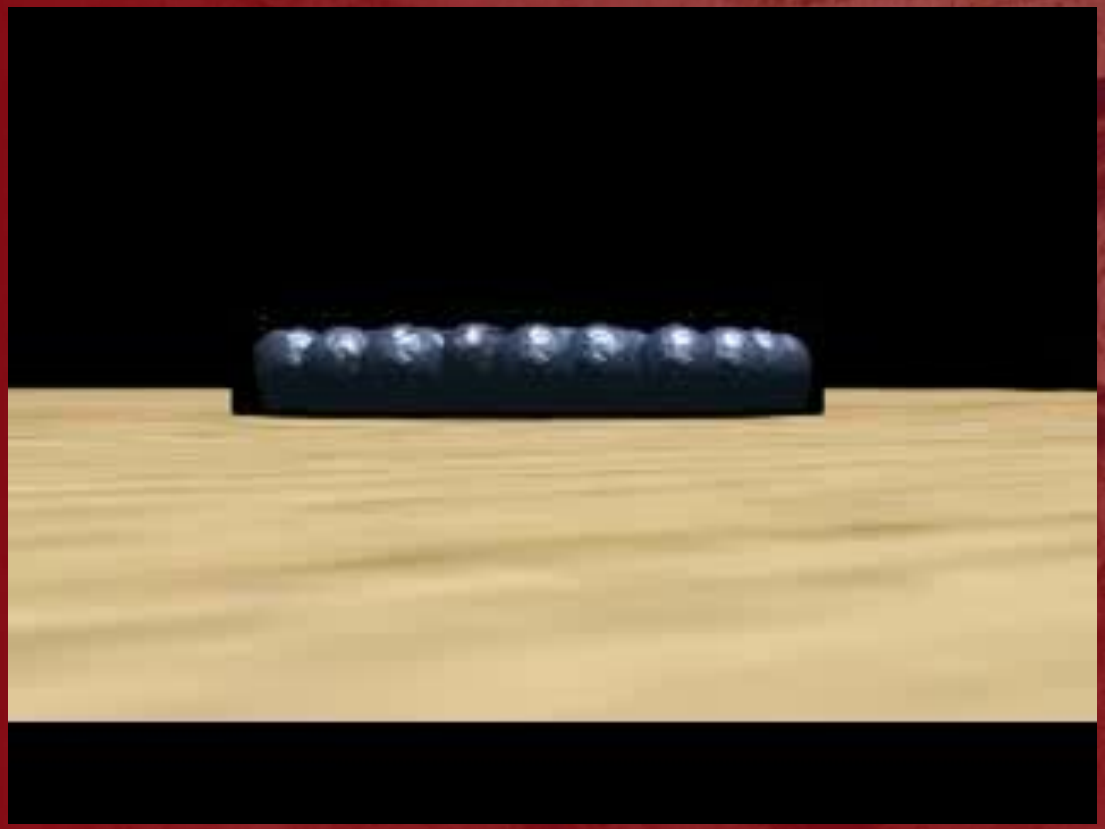


APOPTOTIC CELLS









THE SIGNIFICANCE OF APOPTOSIS

For development of organism, apoptosis is as important as mitosis, because:

Daily, 10 billion cells die this way...

...which is decisive for formation of tissues and organs during intrauterine development and

...formation of neuronal synapses...

...of the lymphocyte repertoire

Reasons sufficient for the life of a cell there are enough reasons for her death.

THE SIGNIFICANCE OF APOPTOSIS

after birth:

- During migration of keratinocytes from deeper parts skin towards the surface - arming;
- hormone-dependent cell death(menstrual cycle, ovarian atrophy during menopause, prostate atrophy after castration);
- Elimination of autoreactive T and B lymphocytes;
- Elimination of virus infected cells, damaged cells or cells with irreparable genetic errors.

HISTORY OF APOPTOSIS

Apoptosis has been discovered many times and forgotten just as many times:

- 1842. Vogt-cell death in amphibians;
- 1885. Flemming- cell death of the rabbit de Graafian follicle (physiological function, death due to lysis of chromosomes);
- 1972 John Kerr, hepatocytes - apoptosis srshrinkage necrosis.

(Kerr JF,et al.Br J Cancer 1972;26:239–57).

CASPASES

induction of apoptosis includes the activation of cytosolic enzymes-**caspase,**

and these are endoproteases that destroy essential structural components including the genetic material of the cell.

Caspases are present in all cells in the form of proenzymes that are activated by a cascade of proteolytic cleavage, and the sequence of their activation depends on the way of initiation of apoptosis

CASPASES

Today, 14 caspases are known, divided into three functional groups:

- initiator caspases (2, 8, 9 and 10);
- effector caspases (3, 6 and 7);
- inflammatory caspases (1, 4 and 6).

MECHANISMS OF APOPTOSIS

two main pathways of inducing apoptosis:

- the outer path (death receptors);
- inner path(mitochondria);

today it is known that two mentioned pathways interconnected and that molecules engaged in one can start other ssignaling pathway

- cytotoxicity(perforins and granzymes A or B).

APOPTOSIS DUE TO LOSS OF SURVIVAL STIMULUS:
INTERNAL SIGNAL

when the cell is deprived of survival stimuli or if it makes a mistake, apoptosis occurs:

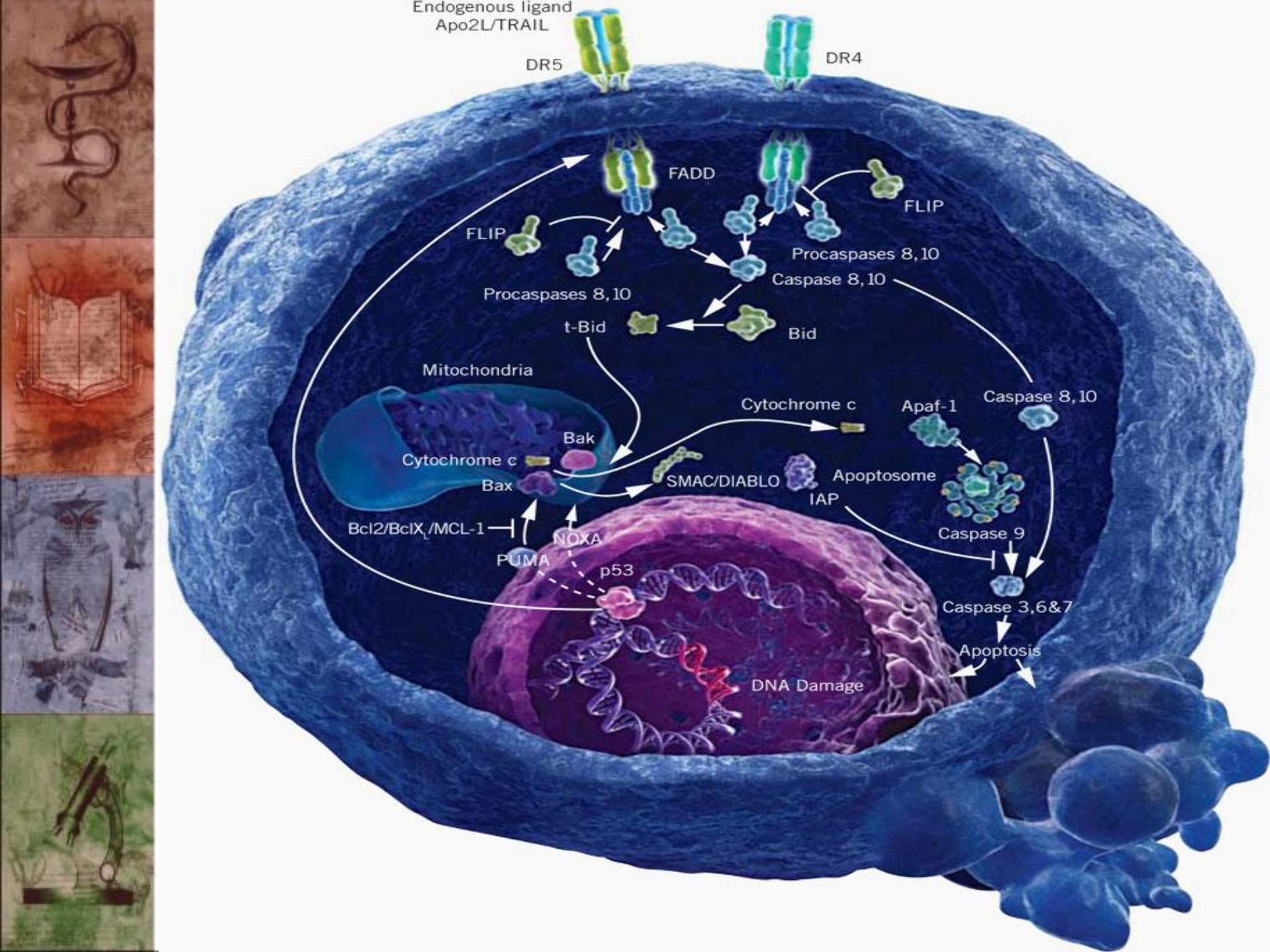
- loss of constant stimulation or adhesion (anoikis);
- the role of cytokines, growth factors and some hormones;
- DNA damage also triggers apoptosis from the cell itself (error in replication, action of ionizing radiation, chemotherapeutics, drugs like nucleoside analogues...)
- reactive oxygen radicals can activate apoptosis - a change in mitochondrial membrane permeability

APOPTOSIS DUE TO LOSS OF SURVIVAL STIMULUS:
INTERNAL SIGNAL

A cell will die by apoptosis if it is not protected by pro-survival stimuli.

The internal pathways of apoptosis are also called ipassivecell death, because this type of apoptosis does not require active signals from death receptors.

This method of apotosis activation is also designated asdeath by neglect.

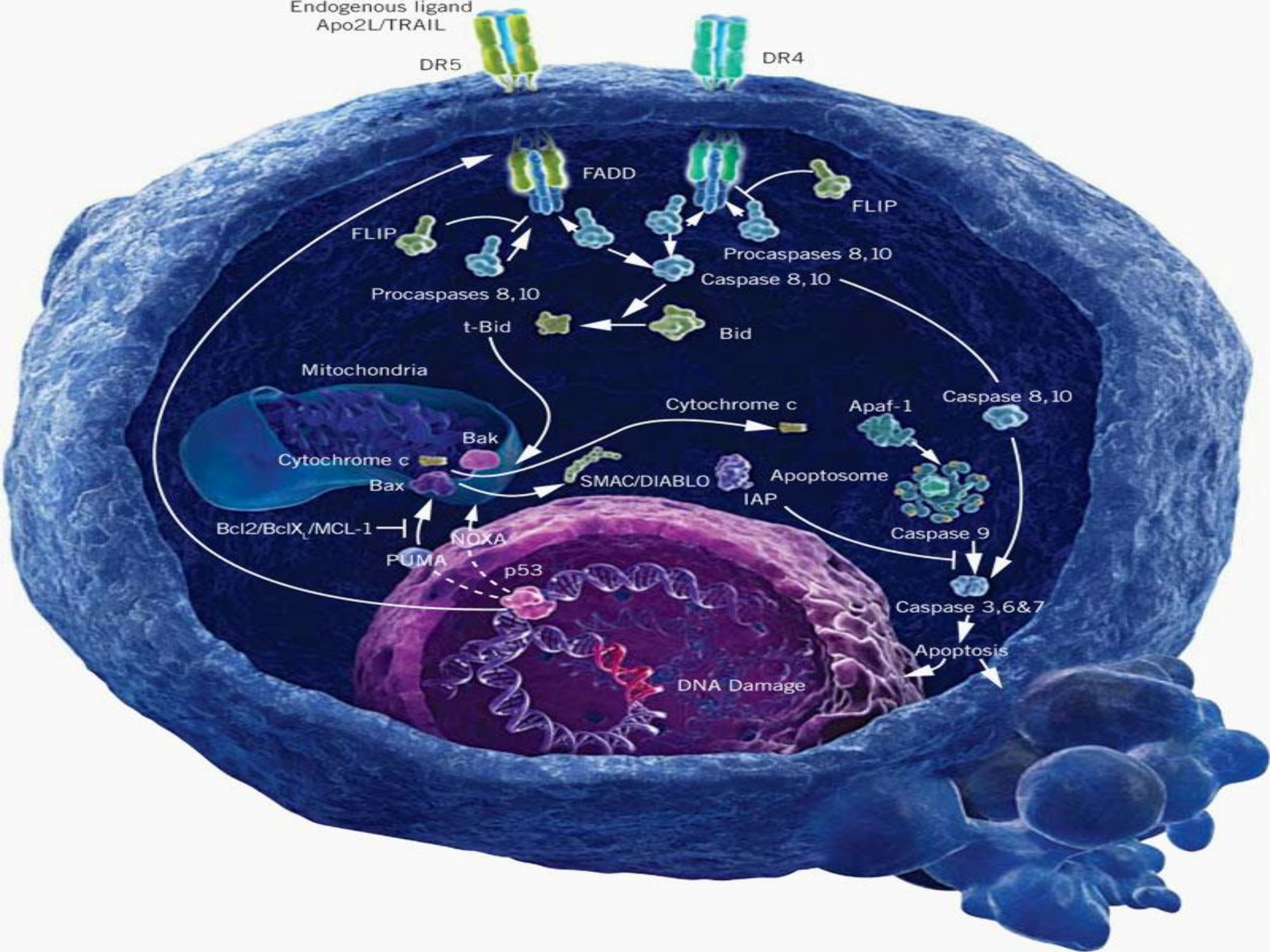

Endogenous ligand
Apo2L/TRAIL
DR5
DR4
FADD
FLIP
FLIP
Procaspases 8,10
Caspase 8,10
Procaspases 8,10
t-Bid
Bid
Mitochondria
Cytochrome c
Apaf-1
Caspase 8,10
Bak
Cytochrome c
Bax
SMAC/DIABLO
Apoptosome
IAP
Bcl2/BclX$_L$/MCL-1
NOXA
PUMA
p53
Caspase 9
Caspase 3,6&7
Apoptosis
DNA Damage

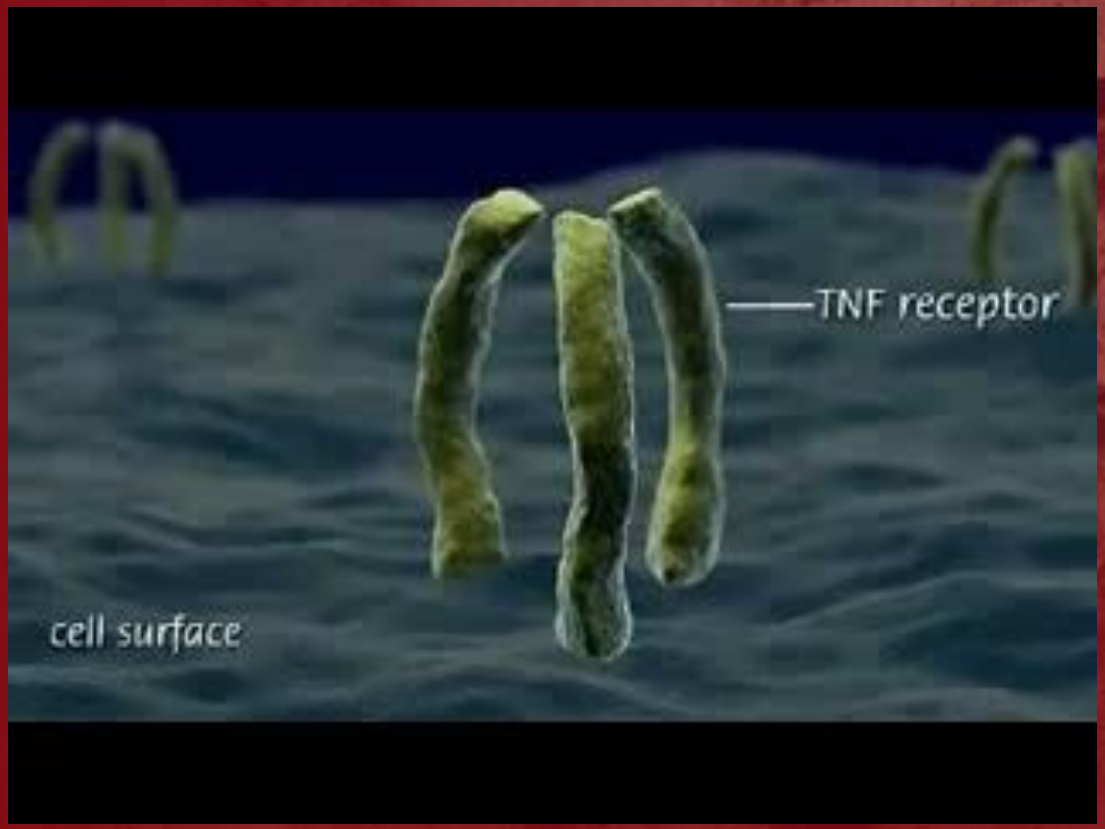
Bcl-2 proteins & apoptosis

DEATH RECEPTOR INDUCED APOPTOSIS: EXTERNAL SIGNAL

apoptosis as a result of the binding of signaling molecules (ligands) to transmembrane death receptors:

- TNFfamily (TNFR,Fas...);
- homotrimeric molecules whose cytoplasmic parts contain death domains;
- Fas ligand, TNF-α, lymphotoxin (TNF-β) and TNF-related apoptosis inducing ligand (TRAIL).


Endogenous ligand
Apo2L/TRAIL
DR5
DR4
FADD
FLIP
FLIP
Procaspases 8,10
Caspase 8,10
Procaspases 8,10
t-Bid
Bid
Mitochondria
Cytochrome c
Apaf-1
Caspase 8,10
Bak
Cytochrome c
Bax
SMAC/DIABLO
Apoptosome
IAP
Caspase 9
Bcl2/BclX$_L$/MCL-1
NOXA
PUMA
p53
Caspase 3,6&7
Apoptosis
DNA Damage


TNF receptor
cell surface

https://www.youtube.com/watch?v=SyvOPXeg4ig

CELL DEATH INDUCED BY PERFORINS AND GRANZYMES

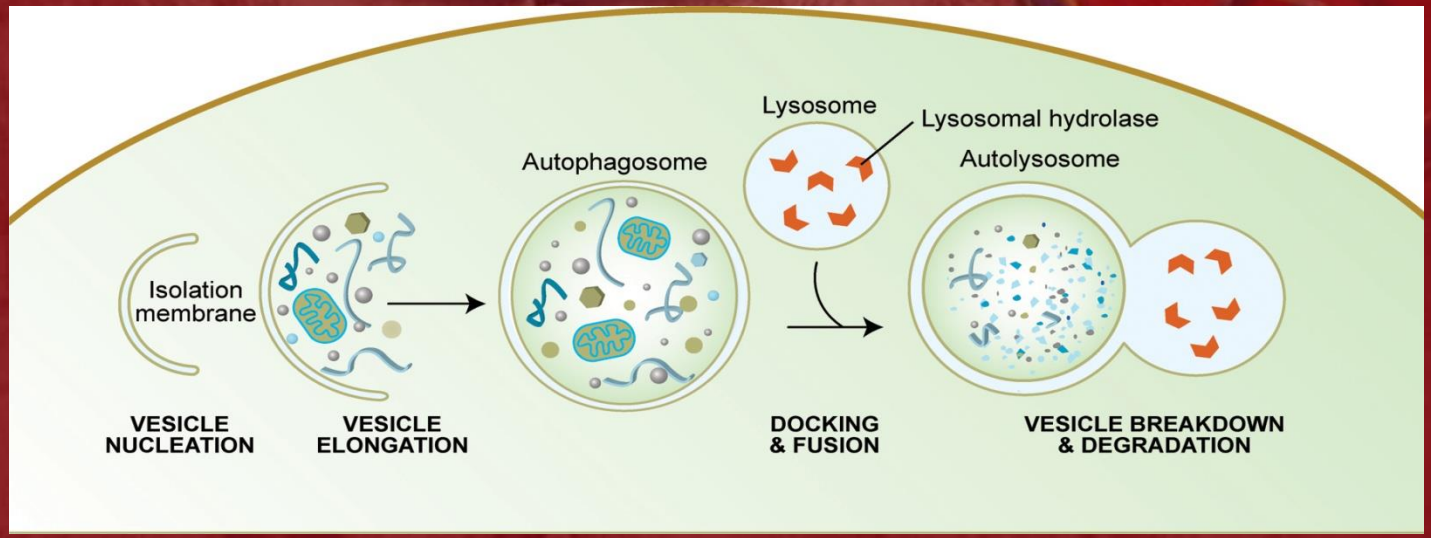
- cytotoxicity – lymphocytes CTL and NK cells
- Perforins: they break down the cell membrane, which allows the entry of granzyme A and B enzymes
- granzyme B:serine esterase
  - directly activates caspases 3, 7, 8 and 10, triggering apoptosis
  - activation of pro-apoptotic Bid protein
- granzyme A: caspase- independent apoptosis

AUTOPHAGY

Autophagy (autophagocytosis) - evolutionarily conserved catabolic process of degradation of cellular components by lysosomal machinery

Formation of autophagosomes, a vacuole with a double membrane, in the cytosol that surrounds and engulfs cell organelles and cytoplasm

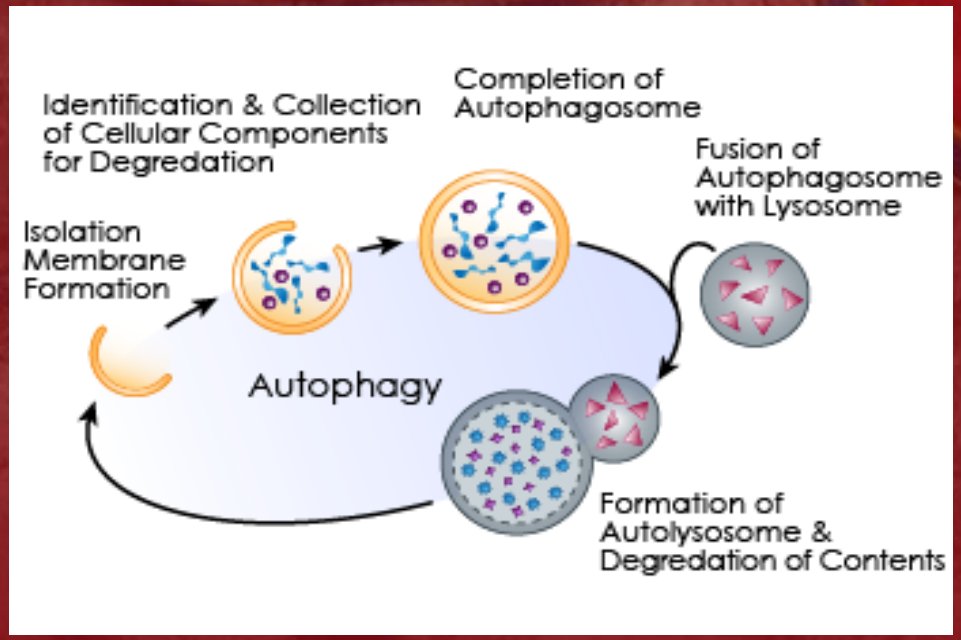
Fusion with lysosomes –autolysosomes in which ingested cellular components are degraded and recycled for protein and ATR synthesis.

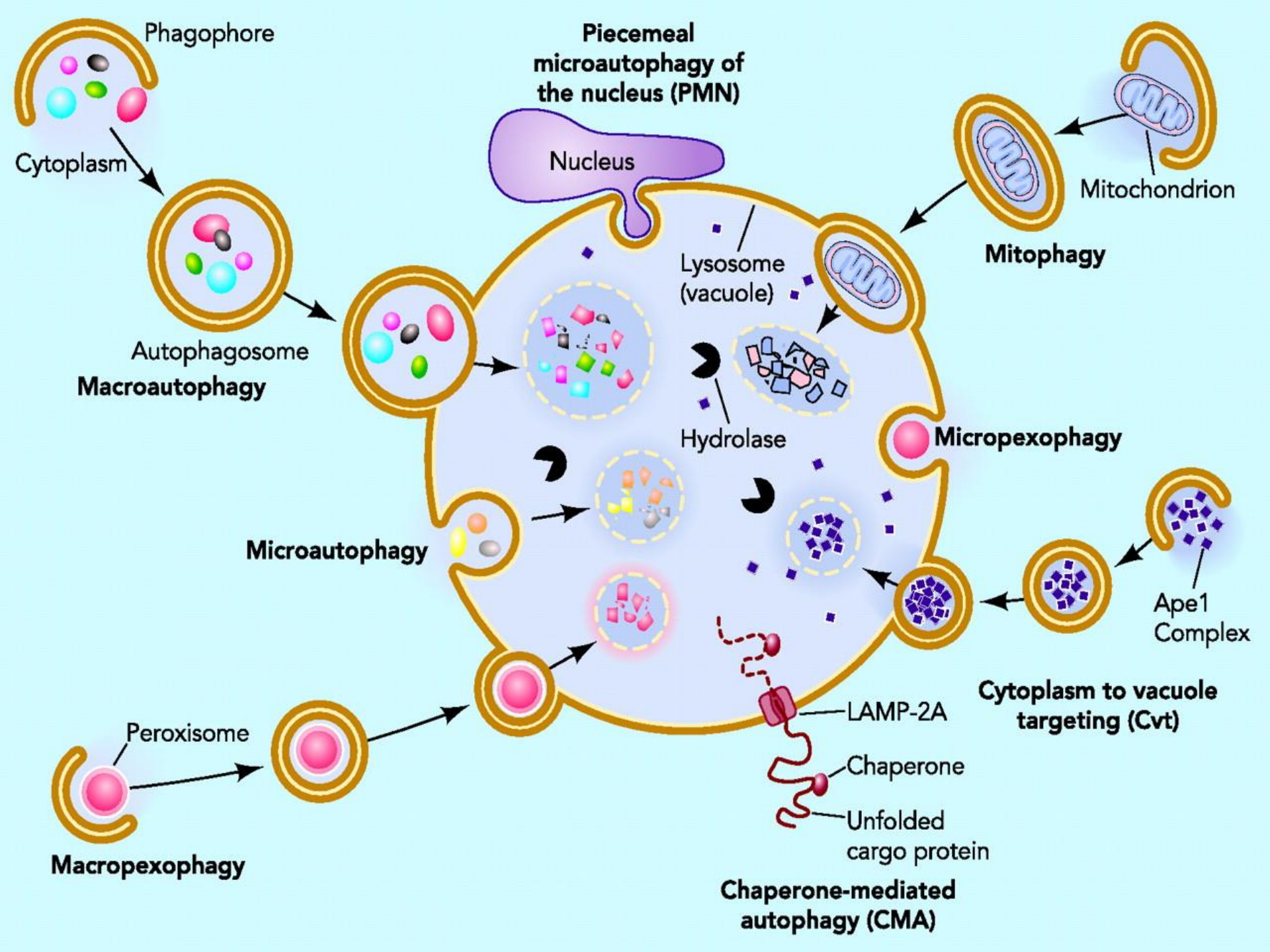


https://www.youtube.com/watch?v=R9svlQU4ruY

AUTOPHAGY

Microautophagy - cytosolic structures are directly taken over through invaginations on the lysosomal membrane
Macroautophagy - formation of vacuole and then subsequent fusion with lysosome in a structure called autolysosome
"Schaperones-mediated"autophagy requires presence of Hsc-70 protein.

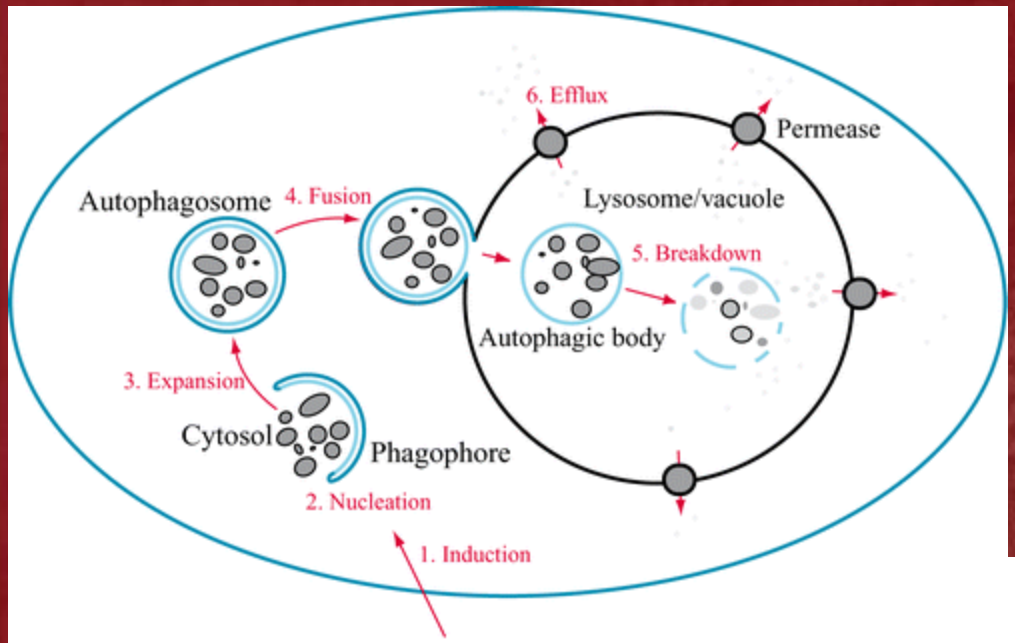



Phagophore
Cytoplasm
Autophagosome
Macroautophagy
Piecemeal microautophagy of the nucleus (PMN)
Nucleus
Lysosome (vacuole)
Hydrolase
Mitochondrion
Mitophagy
Micropexophagy
Microautophagy
Ape1 Complex
Cytoplasm to vacuole targeting (Cvt)
Peroxisome
Macropexophagy
LAMP-2A
Chaperone
Unfolded cargo protein
Chaperone-mediated autophagy (CMA)

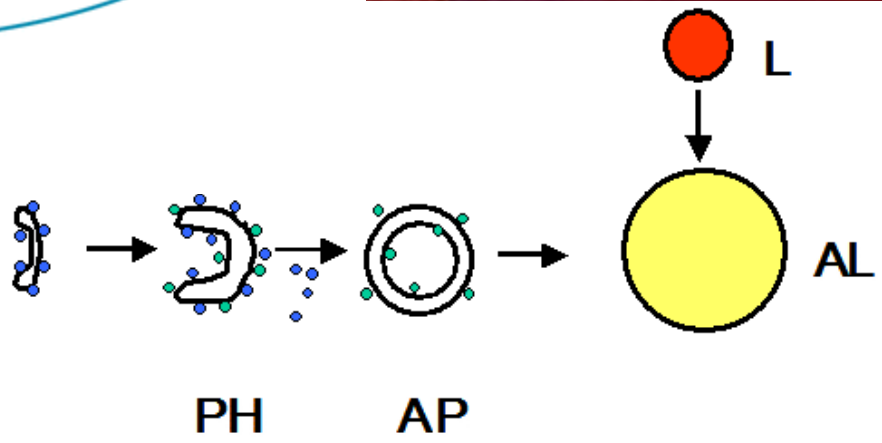
AUTOPHAGY

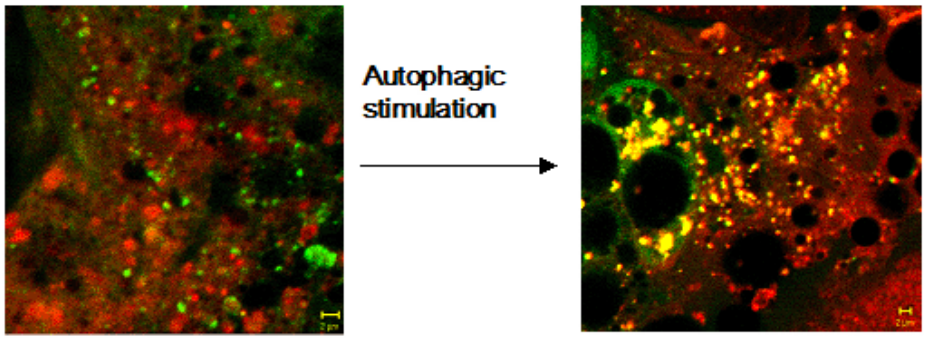
Autophagy is regulated via the RI3-kinase/AKT signaling pathway, which is a link between the availability of nutrients to the cell and cellular metabolism. In the absence of nutrients in normal cells, the RI3-kinase/AKT signaling pathway is activated, which stops protein synthesis and at the same time activates the catabolic processes of autophagy.

Both normal and tumor cells “use” autophagy to survive metabolic stress (lack of nutrients and building materials).



Mizushima N, Klionsky DJ. 2007.
Annu. Rev. Nutr. 27:19–40



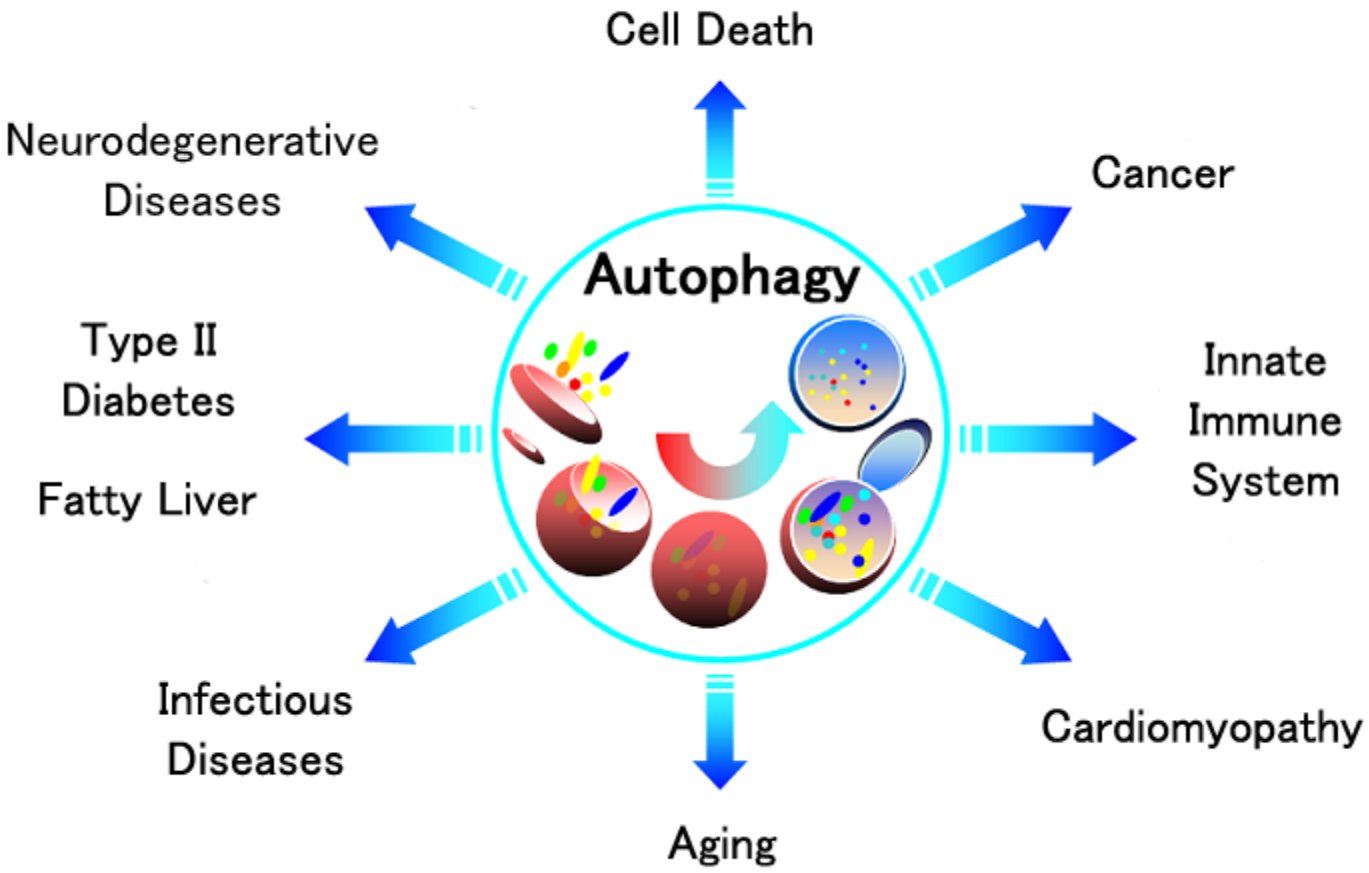


Cell death type II, cytoplasmic cell death

Autophagy is also a form of cell death, when most cellular components in the cell are broken down and programmed cell death is induced in cells that cannot "enter apoptosis".

It is not always clear whether autophagy is involved in the initiation or effector stages of cell death or simply represents a failed survival case of a cell deprived of nutrients/building blocks.

Pathological and Physiological Functions of Autophagy



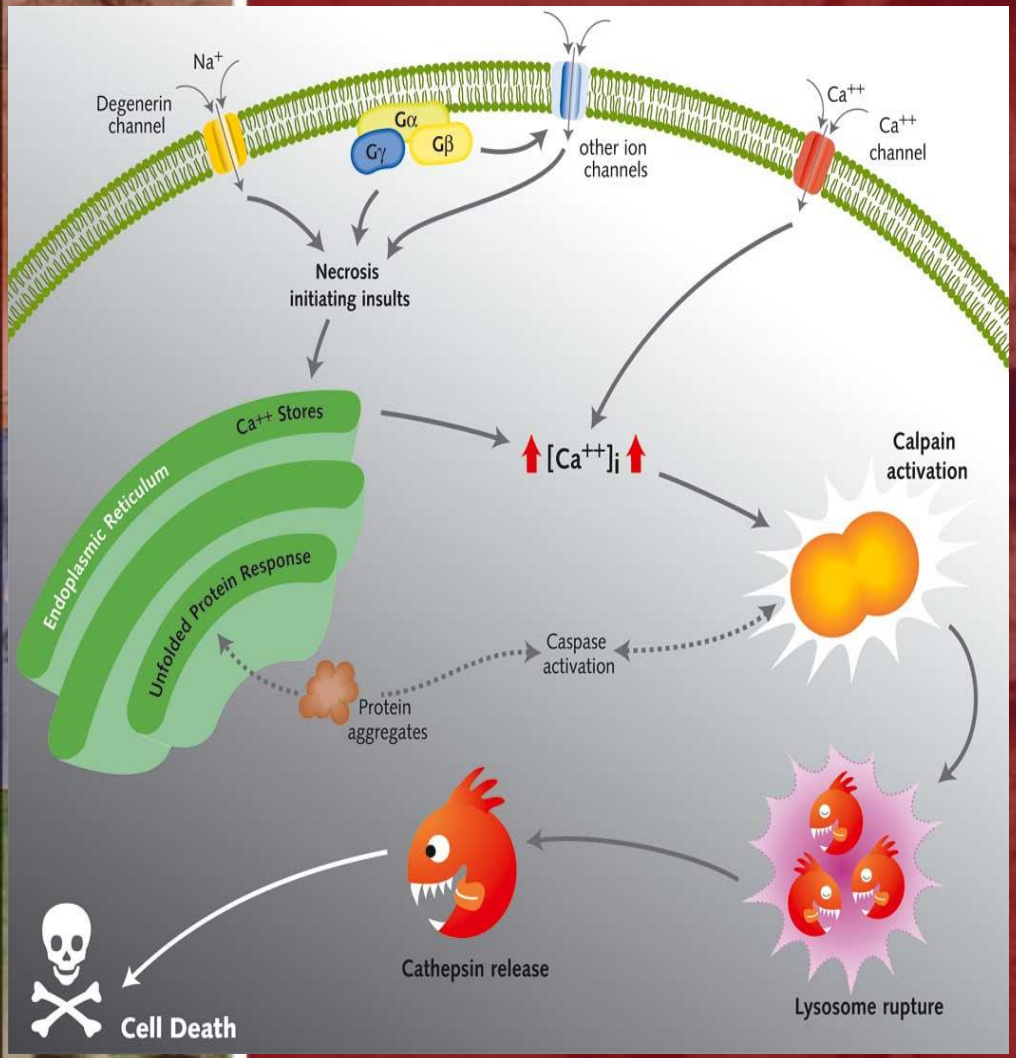
NECROSIS

Greek word necros – corpse

Unregulated/unprogrammed cell death

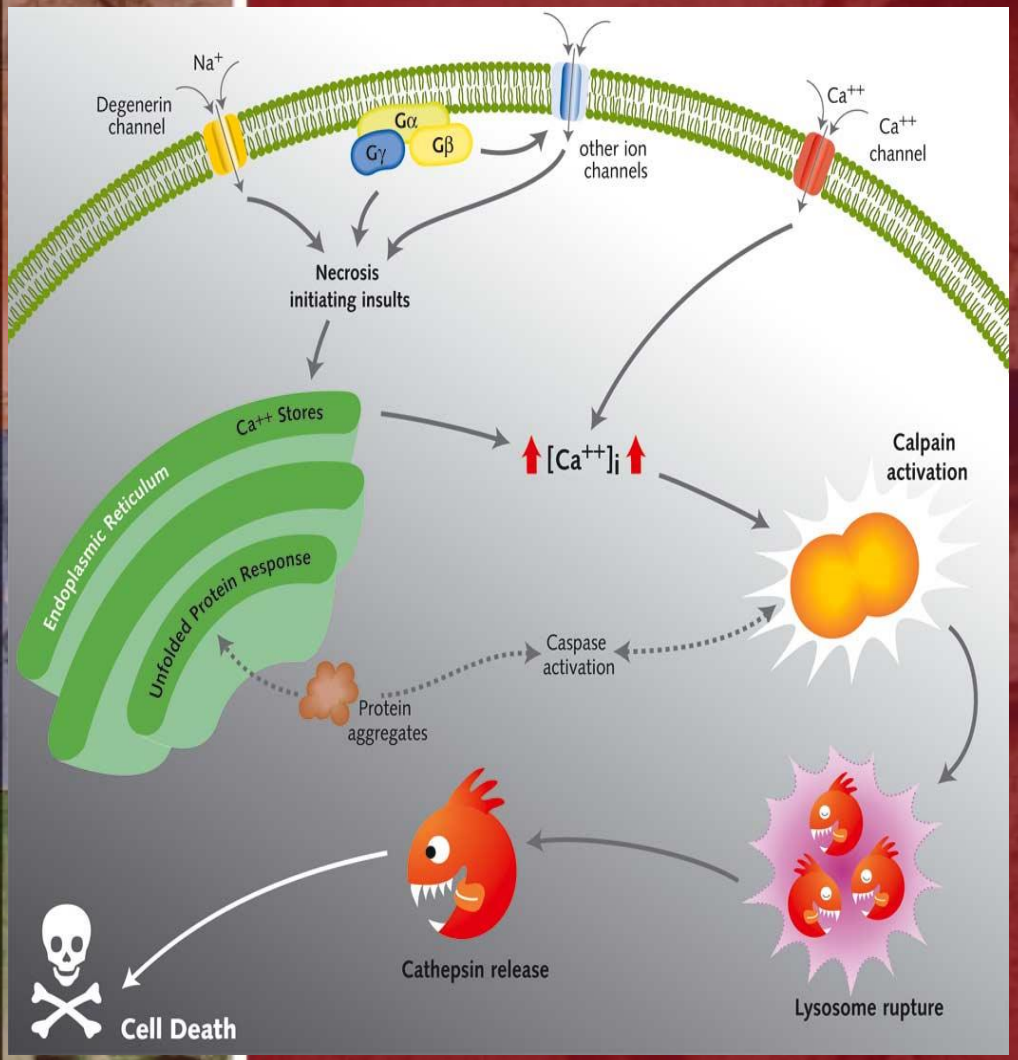
Rapid cell swelling, release of cell contents, acute inflammatory reaction

NECROSIS



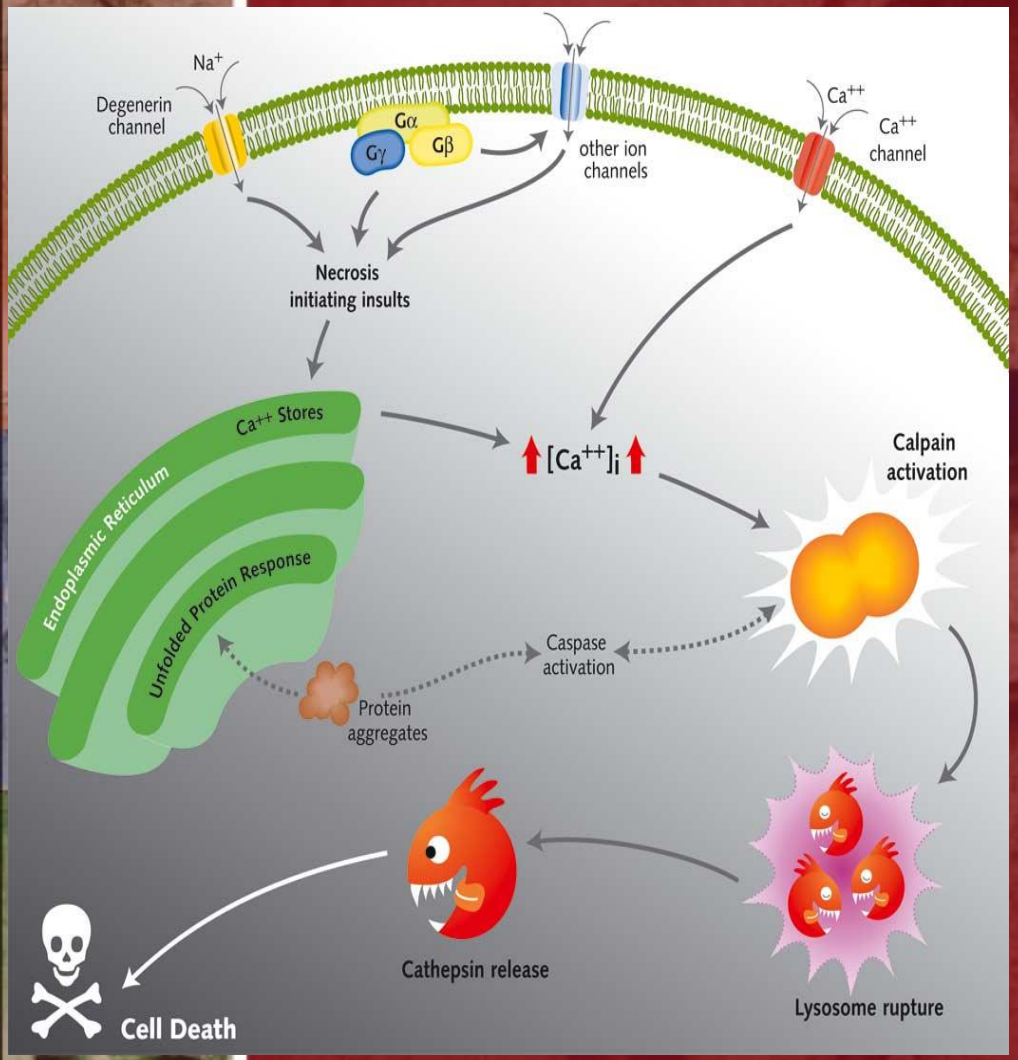
- Consequence of significant cell damage caused by various physical and chemical agents (hypoxia, extreme temperatures, action of complement).
- Rupture of the cell membrane, which results in cell swelling (osmotic pressure).

NECROSIS



- A severely damaged cell cannot maintain fluid and electrolyte balance (Na and K ions enter the cell uncontrollably).
- Random destruction of cellular structures. Loss of cell membrane integrity results in the release of cytoplasmic contents into the surrounding tissue.

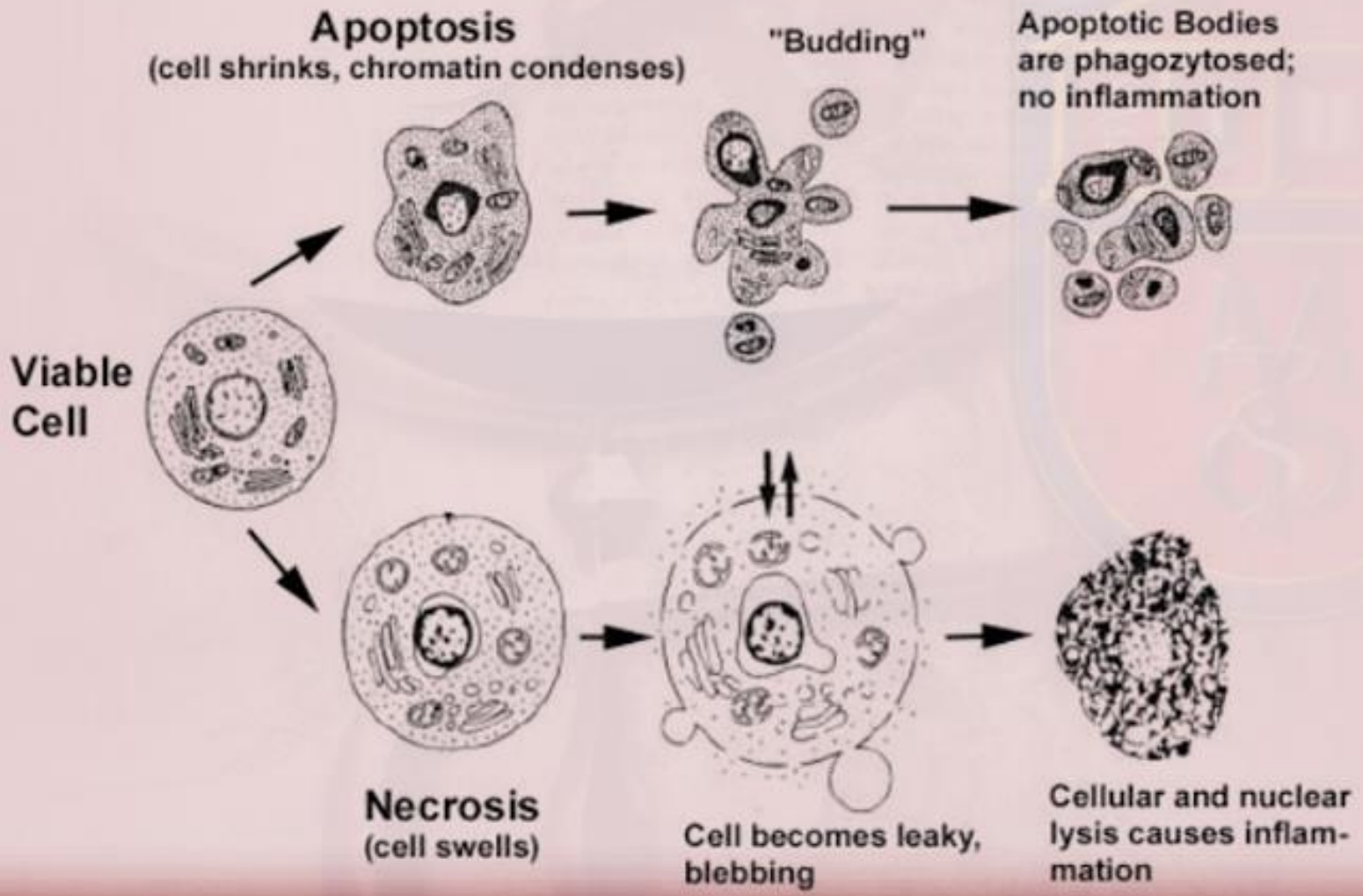
NECROSIS



- Cellular proteins (enzymes) that are released and reach the intercellular space, trigger an inflammatory reaction.
- Inflammation makes it possible to limit possible infection, but also damage to the surrounding tissue.
- Necrosis usually involves a large number of cells.

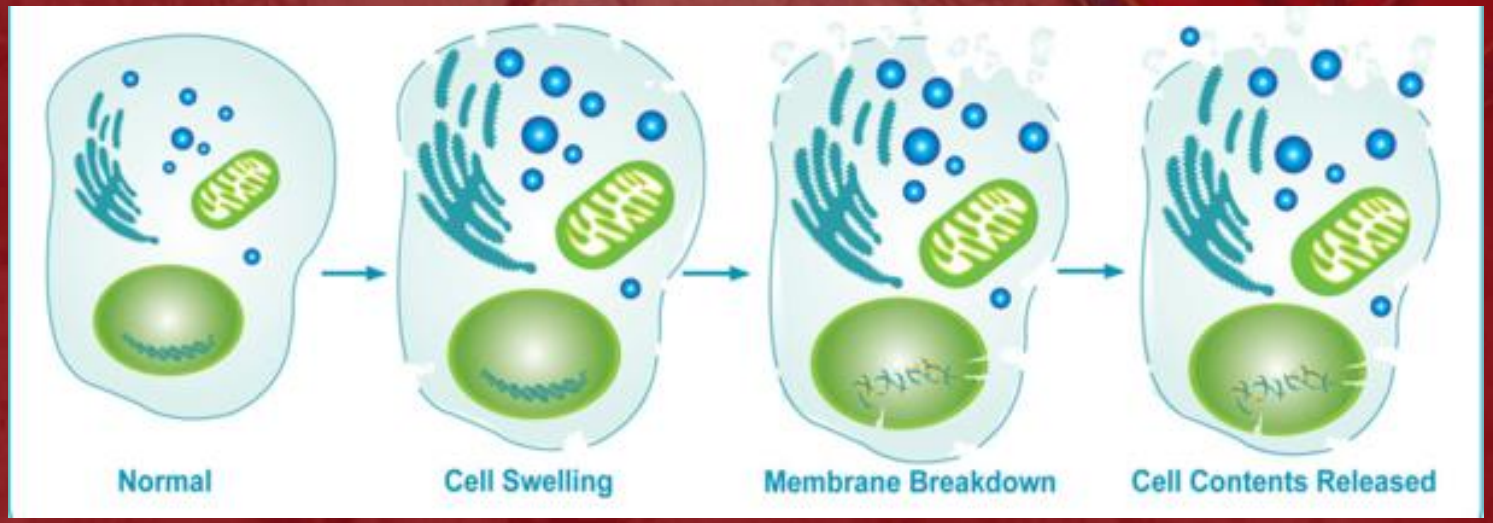
https://www.youtube.com/watch?v=7WRkY8q_F3k

APOPTOSISVS. NECROSIS



NECROPTOSIS

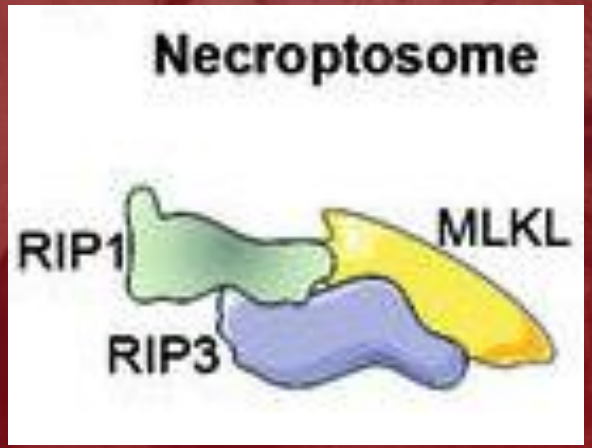
- Necroptosis is a form of programmed necrosis
- A mechanism to prevent infection with viruses that inhibit apoptosis
- It results in a change in the permeability of the plasma membrane and the release of cell contents and molecules - molecular patterns associated with cell damage



NECROPTOSIS

- RIPkinases (eng. Receptor-interacting protein kinase, RIP), serine-threonine kinasee

- In the active form, they build necrosome, the initiation of necroptosis
- When caspases are active, necrosome does not form because caspase 8 cleaves active RIP kinases



https://www.youtube.com/watch?v=CwpKS9jd68I

NECROPTOSIS

- Embryonic growth and development
- Defense mechanism against intracellular pathogens
- Myocardial infarction
- Stroke
- Atherosclerosis
- Ischemia-reperfusion injury
- Pancreatitis
- Inflammatory bowel diseases