APOPTOSIS

(Greek Word: Leaves falling from tree)

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Cell Death

Apoptosis

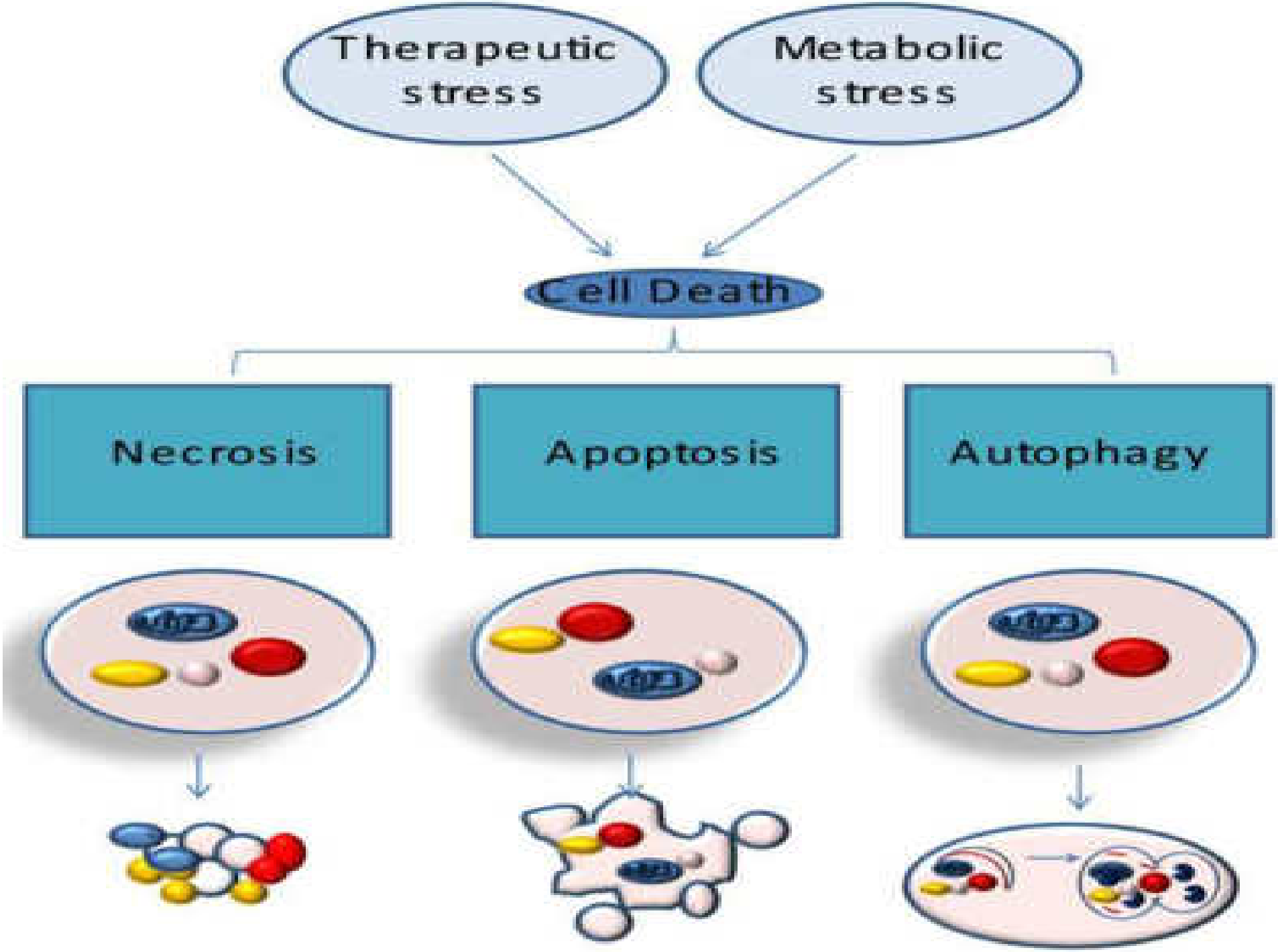
Programmed cell death. Death cycle is **programmed** by the cell itself

Autophagy

'Self-eating' Catabolic process involving lysosomes.

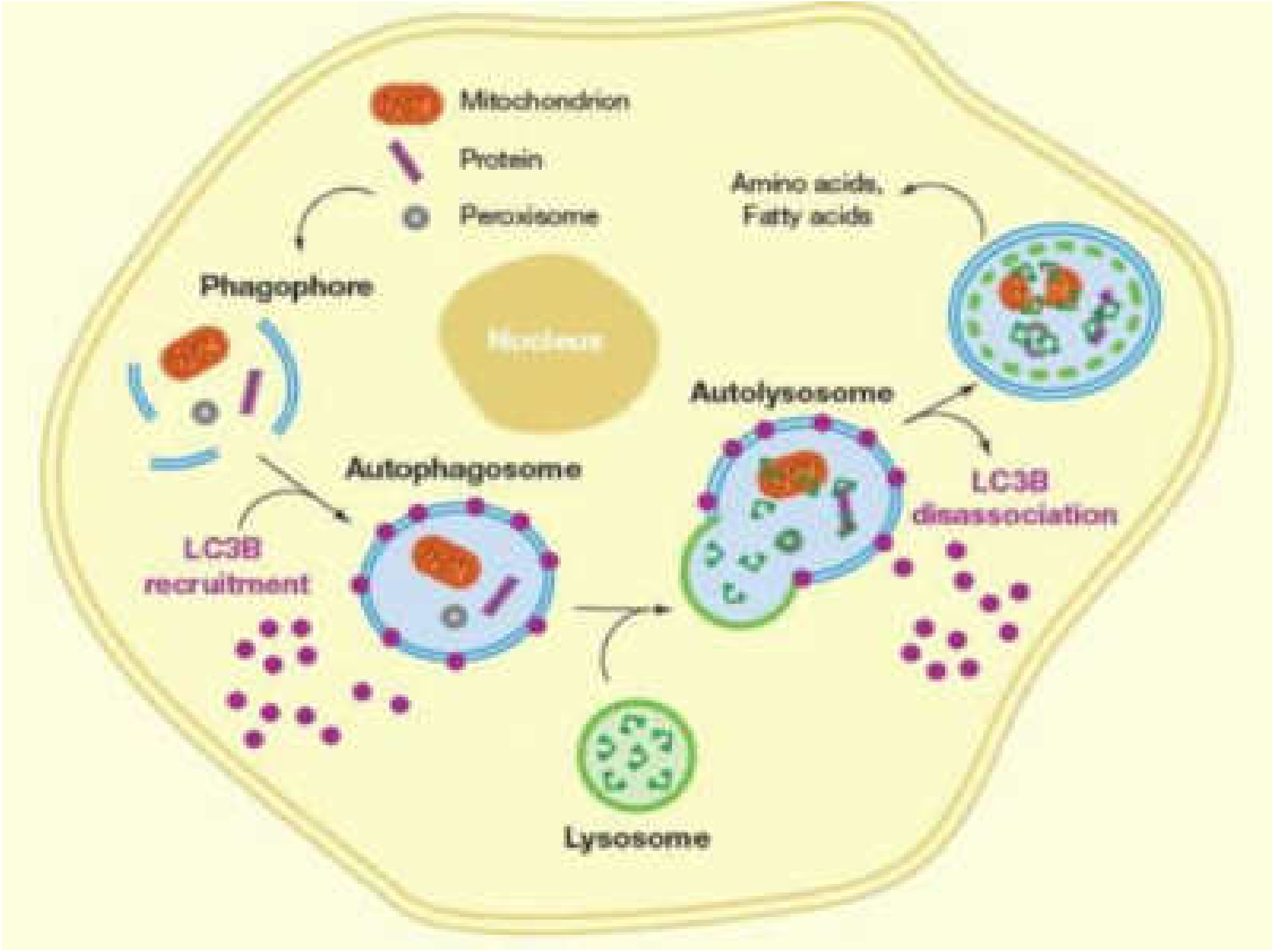
Necrosis

'Death' caused by external factors like trauma or toxins. **Not programmed.**


Therapeutic stress
Metabolic stress
Cell Death
Necrosis
Apoptosis
Autophagy

Necrosis *vs.* Apoptosis

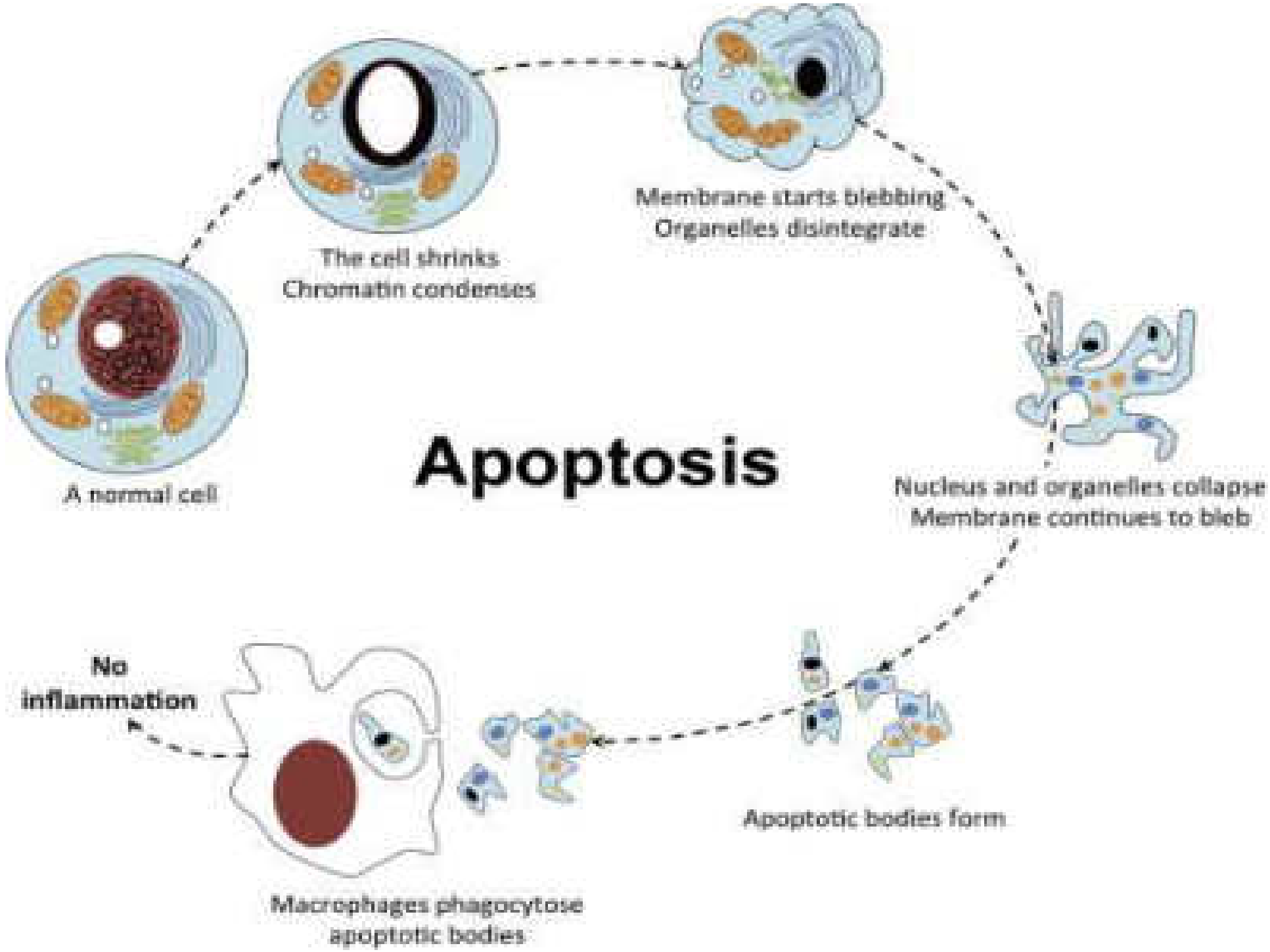
Necrosis	Apoptosis
• Cellular swelling • Membranes are broken • ATP is depleted • Cell lyses, eliciting an inflammatory reaction • DNA fragmentation is random, or smeared • In vivo, whole areas of the tissue are affected	• Cellular condensation • Membranes remain intact • Requires ATP • Cell is phagocytosed, no tissue reaction • Ladder-like DNA fragmentation • In vivo, individual cells appear affected


Mitochondrion
Protein
Peroxisome
Phagophore
Nucleus
Amino acids,
Fatty acids
Autolysosome
Autophagosome
LC3B
recruitment
LC3B
disassociation
Lysosome

- Apoptosis or programmed cell death, is carefully coordinated collapse of cell, protein degradation , DNA fragmentation followed by rapid engulfment of corpses by neighboring cells.

- Essential part of life for every multicellular organism from worms to humans.

- Apoptosis plays a major role from embryonic development to senescence (loss of a cell's power of division and growth).


Apoptosis
A normal cell
The cell shrinks
Chromatin condenses
Membrane starts blebbing
Organelles disintegrate
Nucleus and organelles collapse
Membrane continues to bleb
Apoptotic bodies form
Macrophages phagocytose
apoptotic bodies
No
inflammation

- **Apoptosis is needed for proper development**

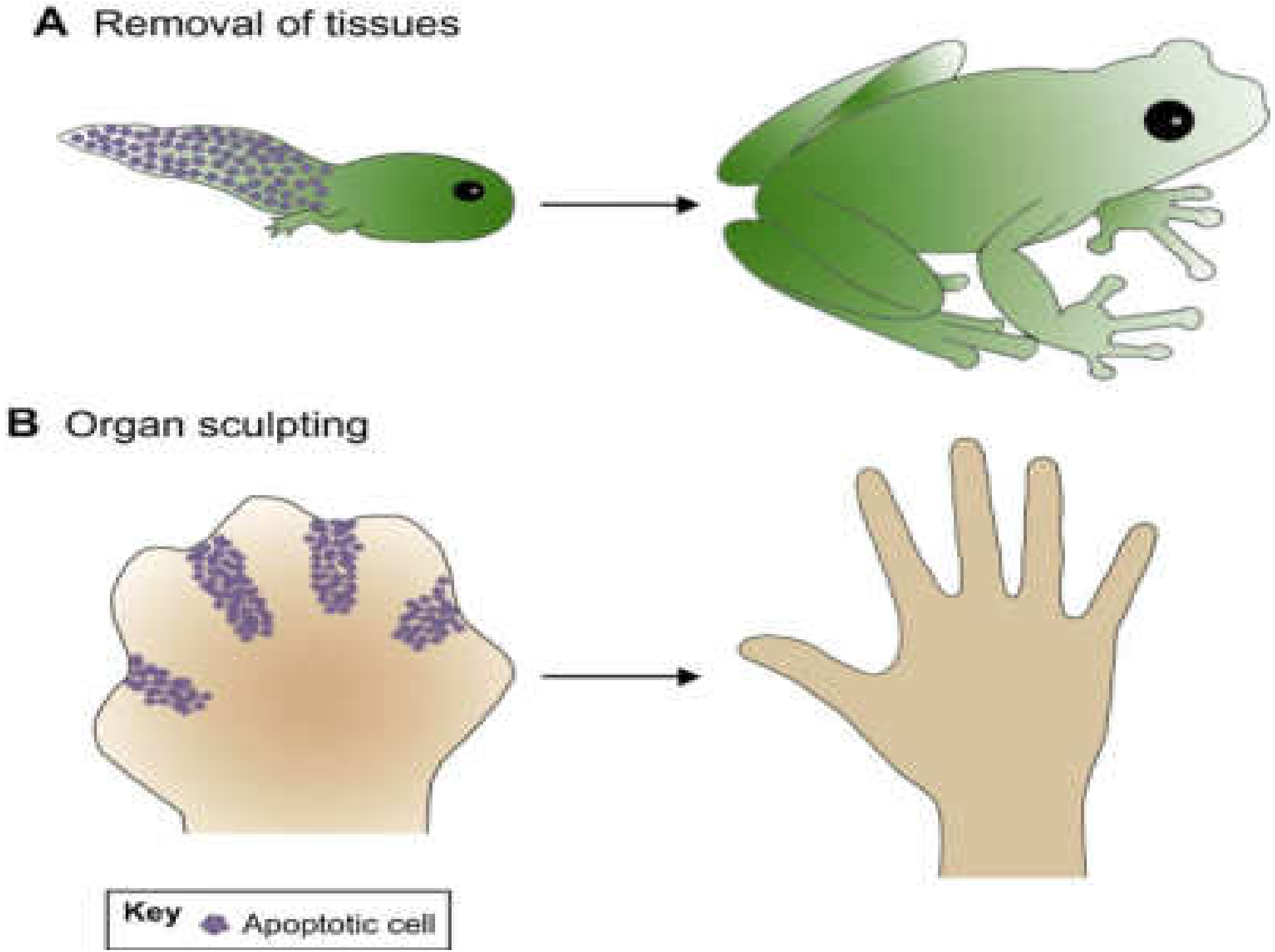
  Examples:

  - **The resorption of the tadpole tail**
  - **The formation of the fingers and toes of the fetus**
  - **The sloughing off of the inner lining of the uterus**
  - **The formation of the proper connections between neurons in the brain.**

- **Apoptosis is needed to destroy cells**

  Examples:

  - **Cells infected with viruses**
  - **Cells of the immune system**
  - **Cells with DNA damage**
  - **Cancer cells**


A Removal of tissues
B Organ sculpting
Key
Apoptotic cell

What makes a cell decide to commit suicide?

- **Withdrawal of positive signals**

  examples :
  - **growth factors for neurons**
  - **Interleukin-2 (IL-2)**

- **Receipt of negative signals**

  examples :
  - **Increased levels of oxidants within the cell**
  - **Damage to DNA by oxidants**
  - **Death activators :**
    - **Tumor necrosis factor alpha (TNF-α)**
    - **Lymphotoxin (TNF-β)**
    - **Fas ligand (FasL)**

Apoptosis extends of three steps

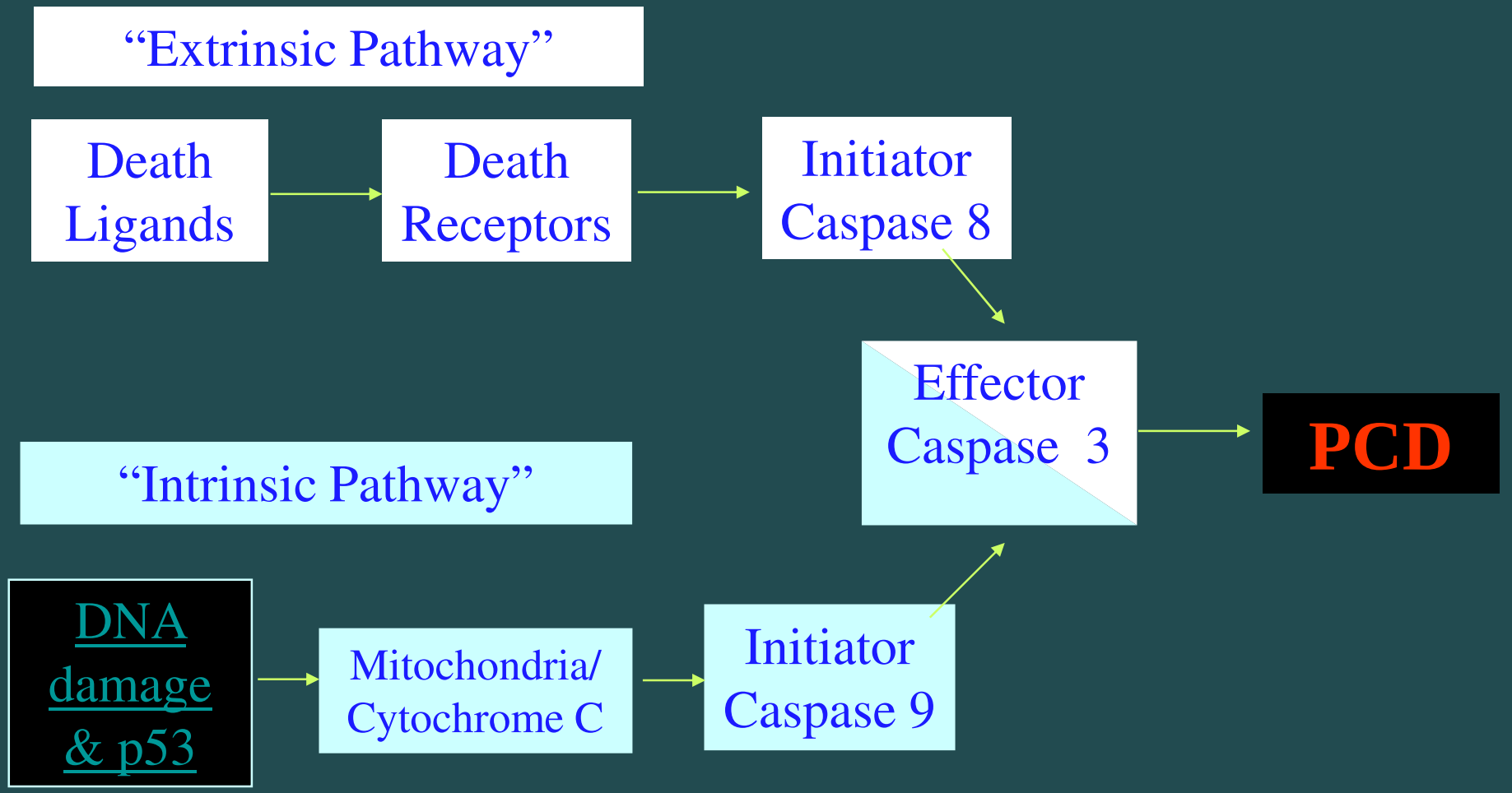
- Initiation
- Execution
- Phagocytosis

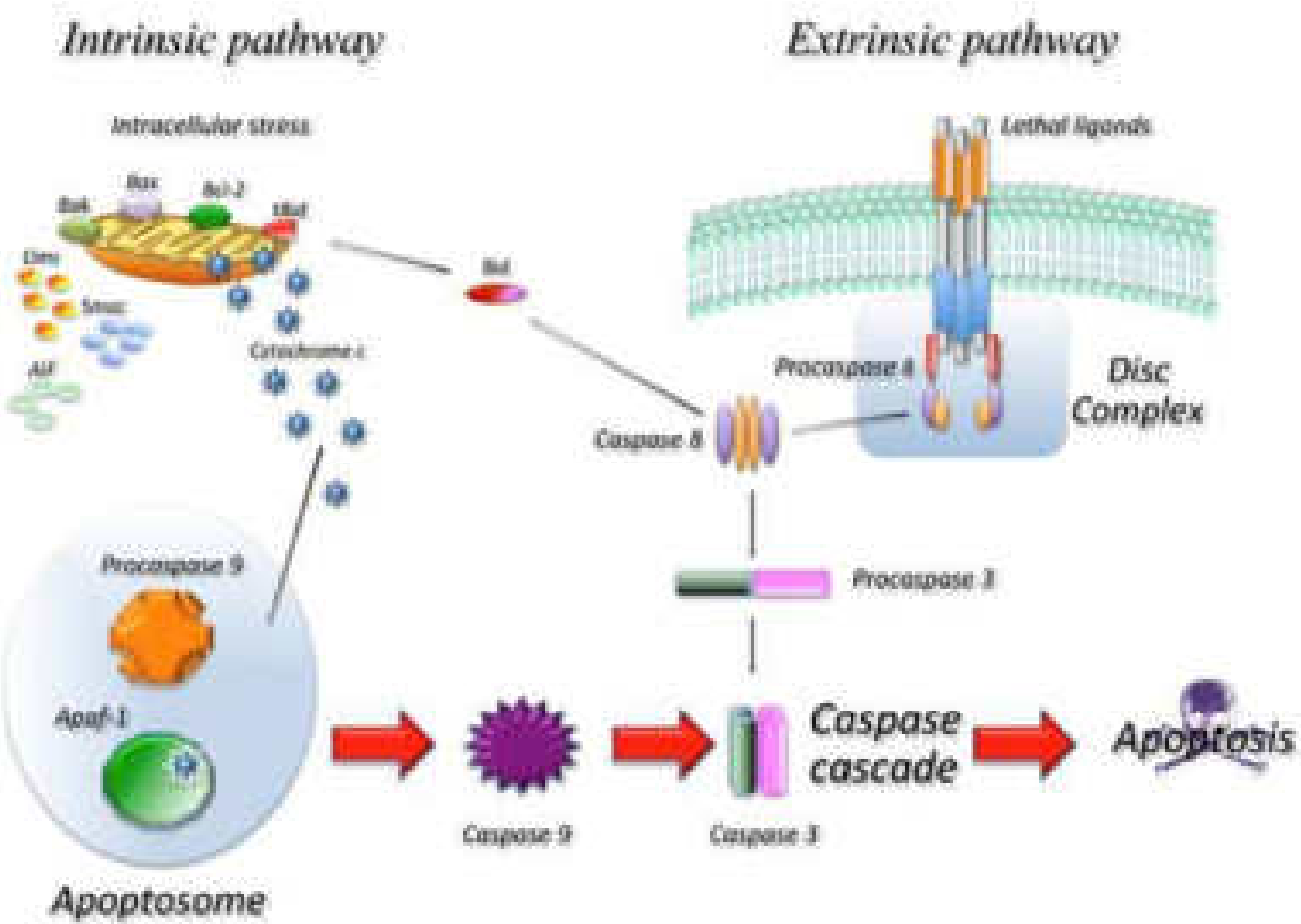
Initiation by two different mechanisms-

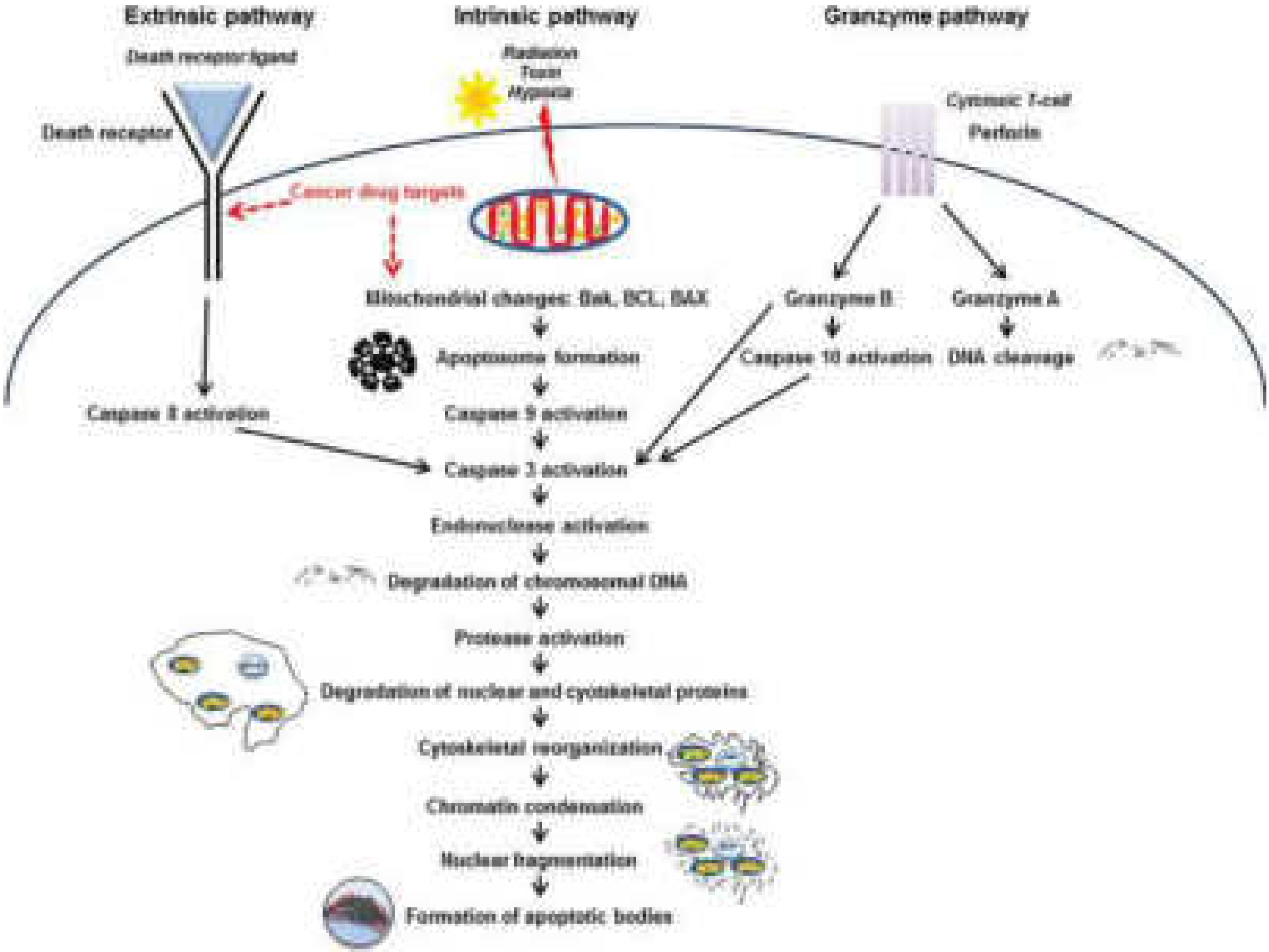
1. Intrinsic Pathway of Initiation

2. Extrinsic Pathway of Initiation

Apoptosis: Pathways



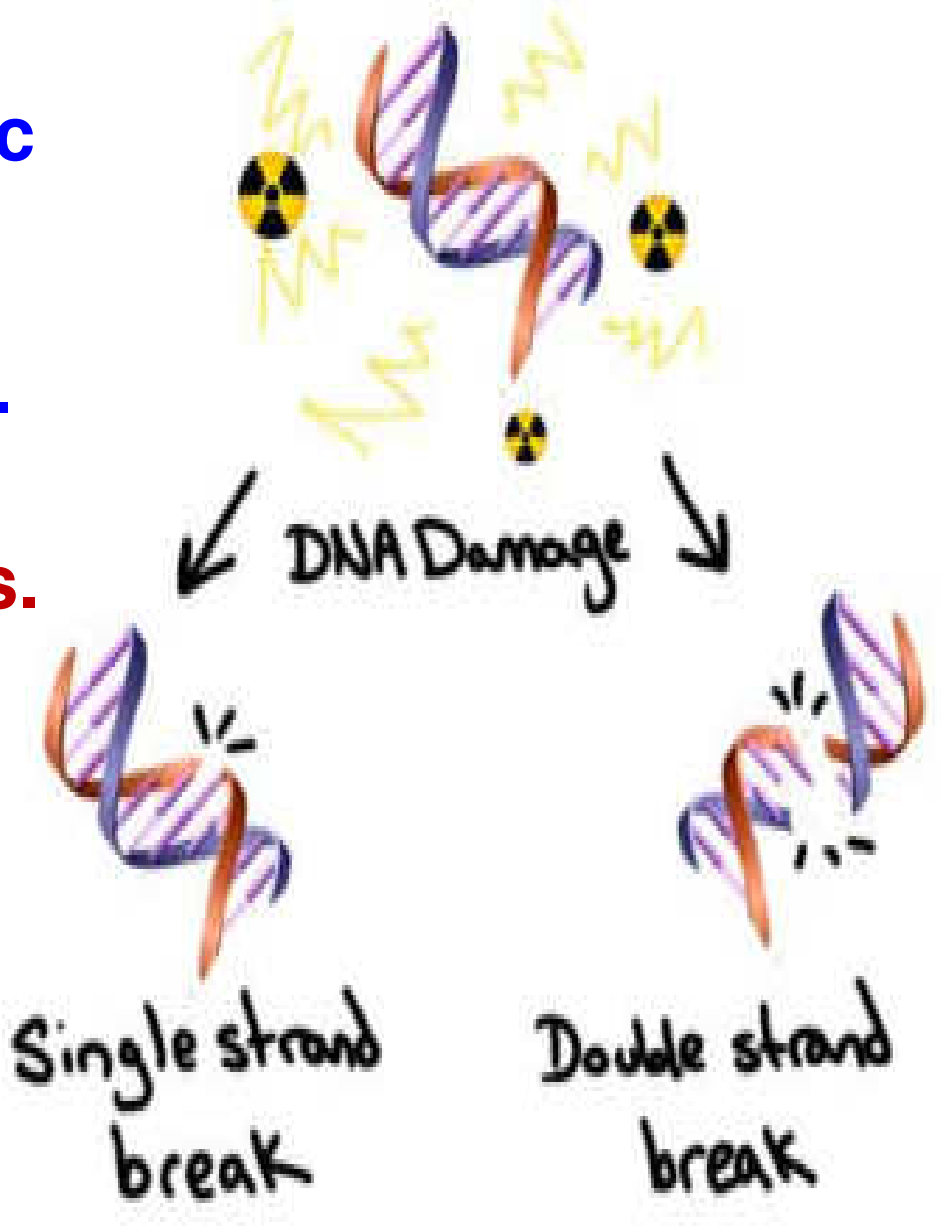

Intrinsic pathway
Extrinsic pathway
Intracellular stress
Bax
Bcl-2
Bak
tBid
Cytochrome c
Bid
Lethal ligands
Procaspase 8
Disc Complex
Caspase 8
Procaspase 3
Procaspase 9
Apaf-1
Caspase cascade
Apoptosis
Caspase 9
Caspase 3
Apoptosome


Extrinsic pathway
Death receptor ligand
Death receptor
Intrinsic pathway
Radiation
Toxin
Hypoxia
Granzyme pathway
Cytotoxic T-cell
Perforin
Cancer drug targets
Mitochondrial changes: Bak, BCL, BAX
Granzyme B
Granzyme A
Apoptosome formation
Caspase 10 activation
DNA cleavage
Caspase 8 activation
Caspase 9 activation
Caspase 3 activation
Endonuclease activation
Degradation of chromosomal DNA
Protease activation
Degradation of nuclear and cytoskeletal proteins
Cytoskeletal reorganization
Chromatin condensation
Nuclear fragmentation
Formation of apoptotic bodies

Intrinsic Pathway of Initiation

The most frequent intrinsic pathway Is initiated by unrepairable and Irreversible lesion of DNA-

- **Oxidative Stress.**
- **Radiation.**



Intrinsic Pathway of Initiation

Lesion of DNA

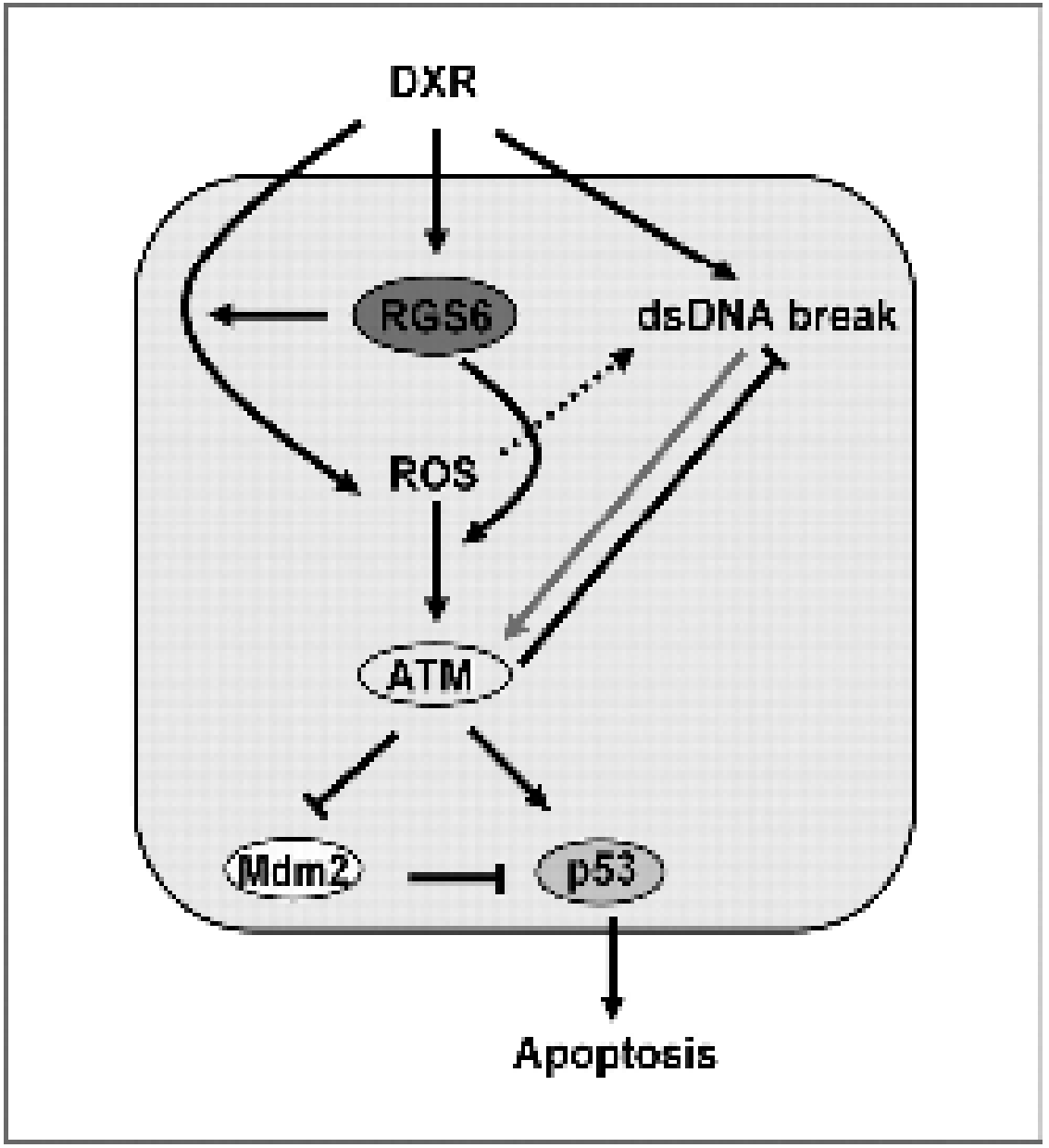
activates the protein

ATM – which in turn

activates tumor

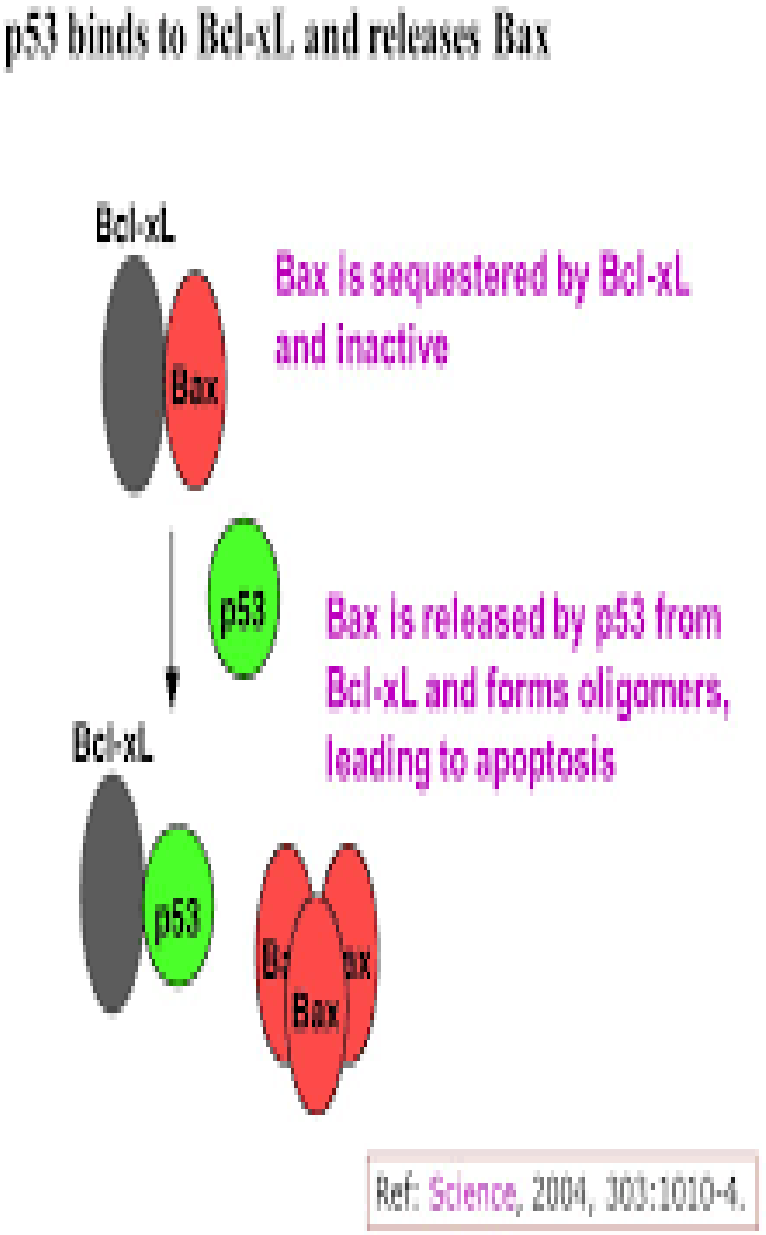
suppressor protein

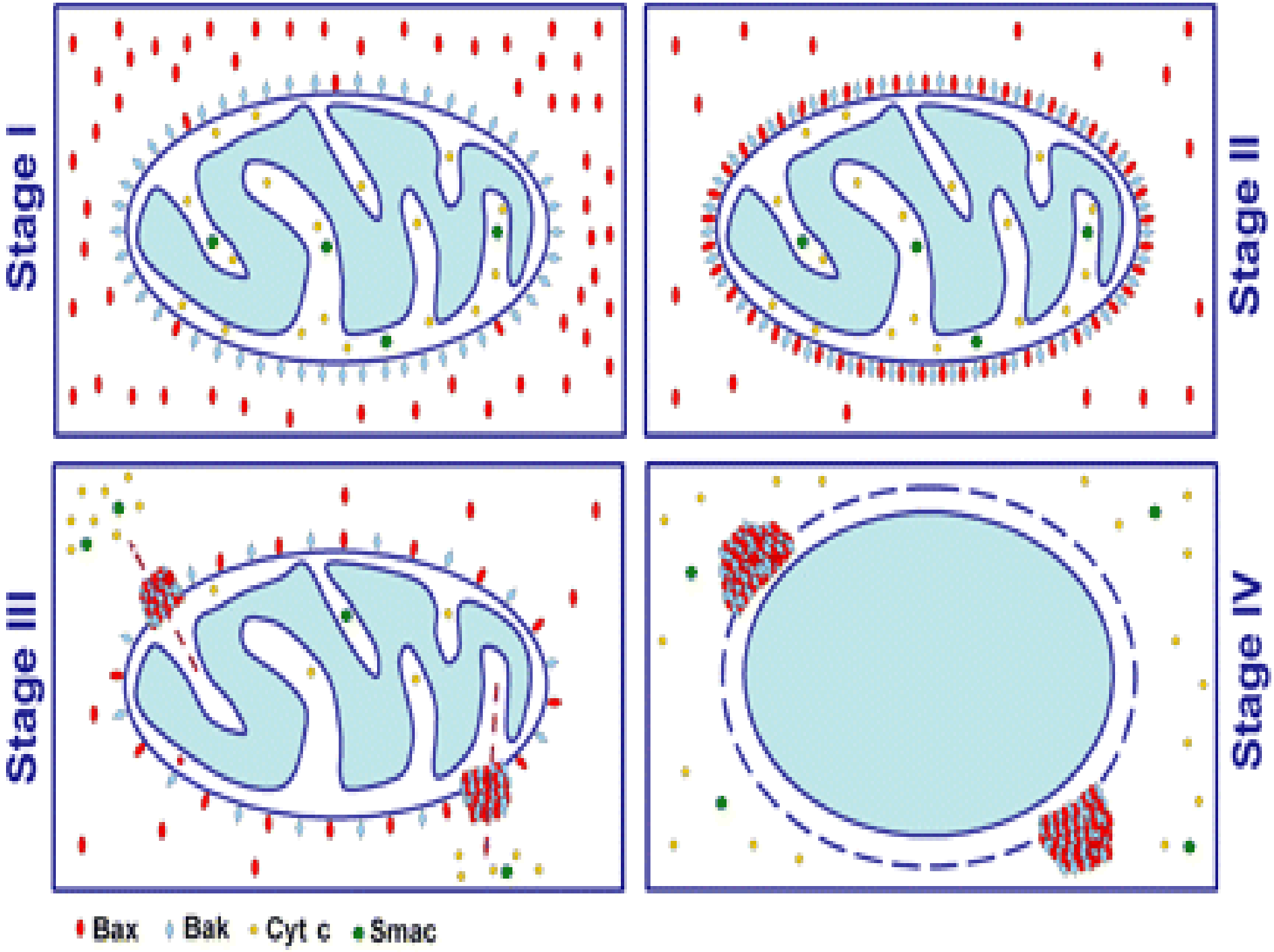
p53.



Intrinsic Pathway of Initiation

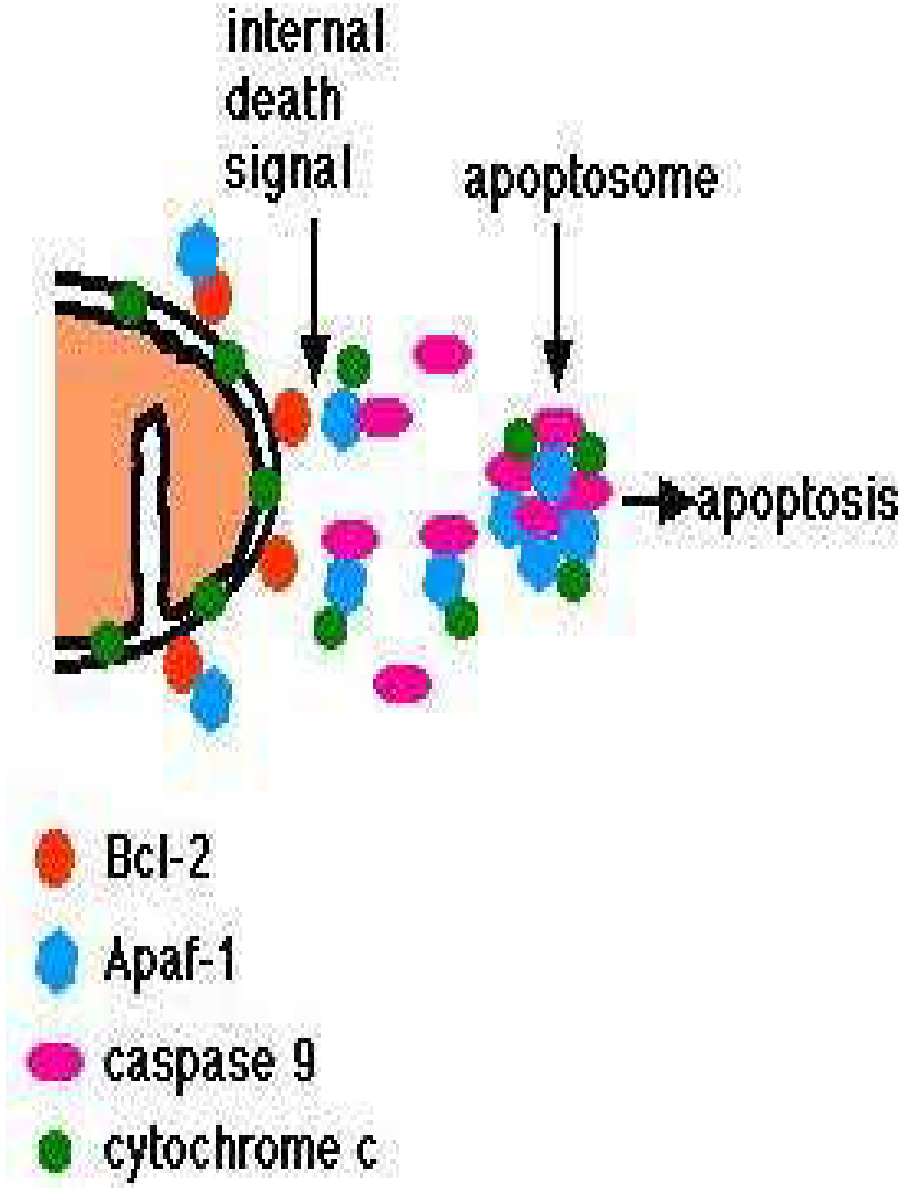
p53 activates the BAX protein – which in turn binds to mitochondria and makes pores in a outer membrane of mitochondria, release of calcium, H^+, cytochrome-C and other substances into the cytoplasm.



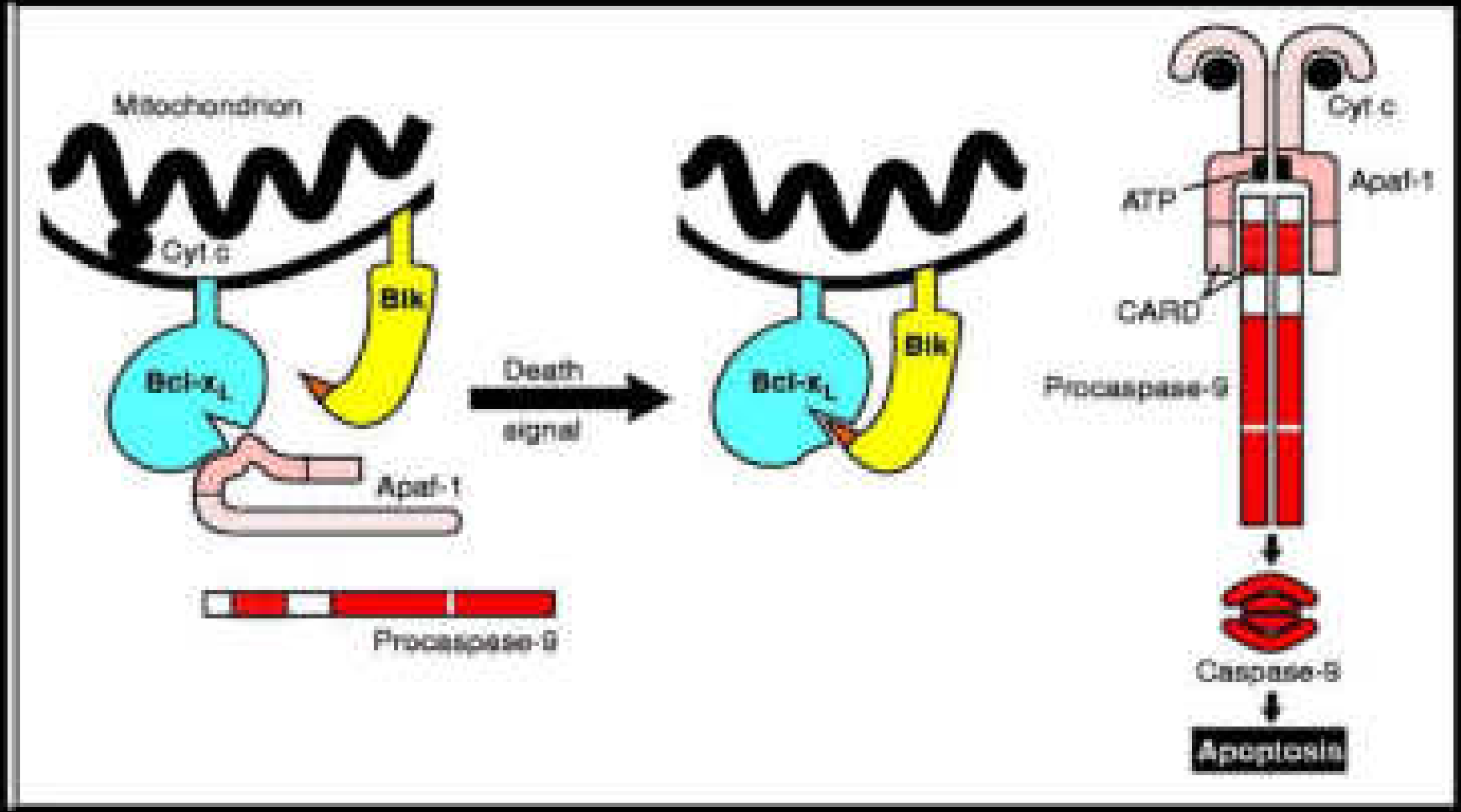

Stage I
Stage II
Stage III
Stage IV
Bax
Bak
Cyt c
Smac

Intrinsic Pathway of Initiation

Cytochrome-C binds to APAF and this Complex binds to Procaspase-9, Inhibiting domain of Procaspase-9 is Hydrolysed.

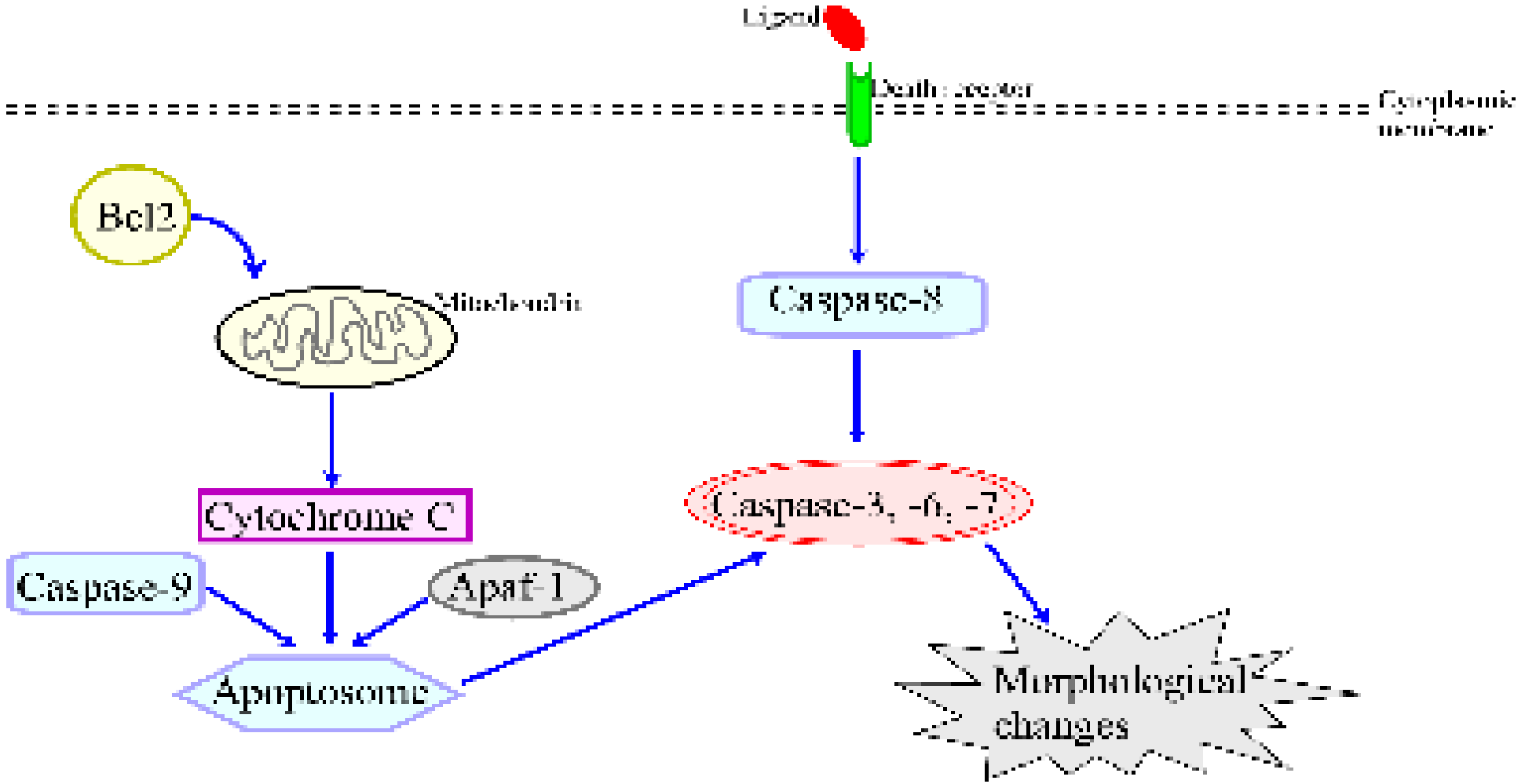


Internal signals



Intrinsic Pathway of Initiation

Caspase-9 activates additional caspases (caspase pathway) and destroy proteins.

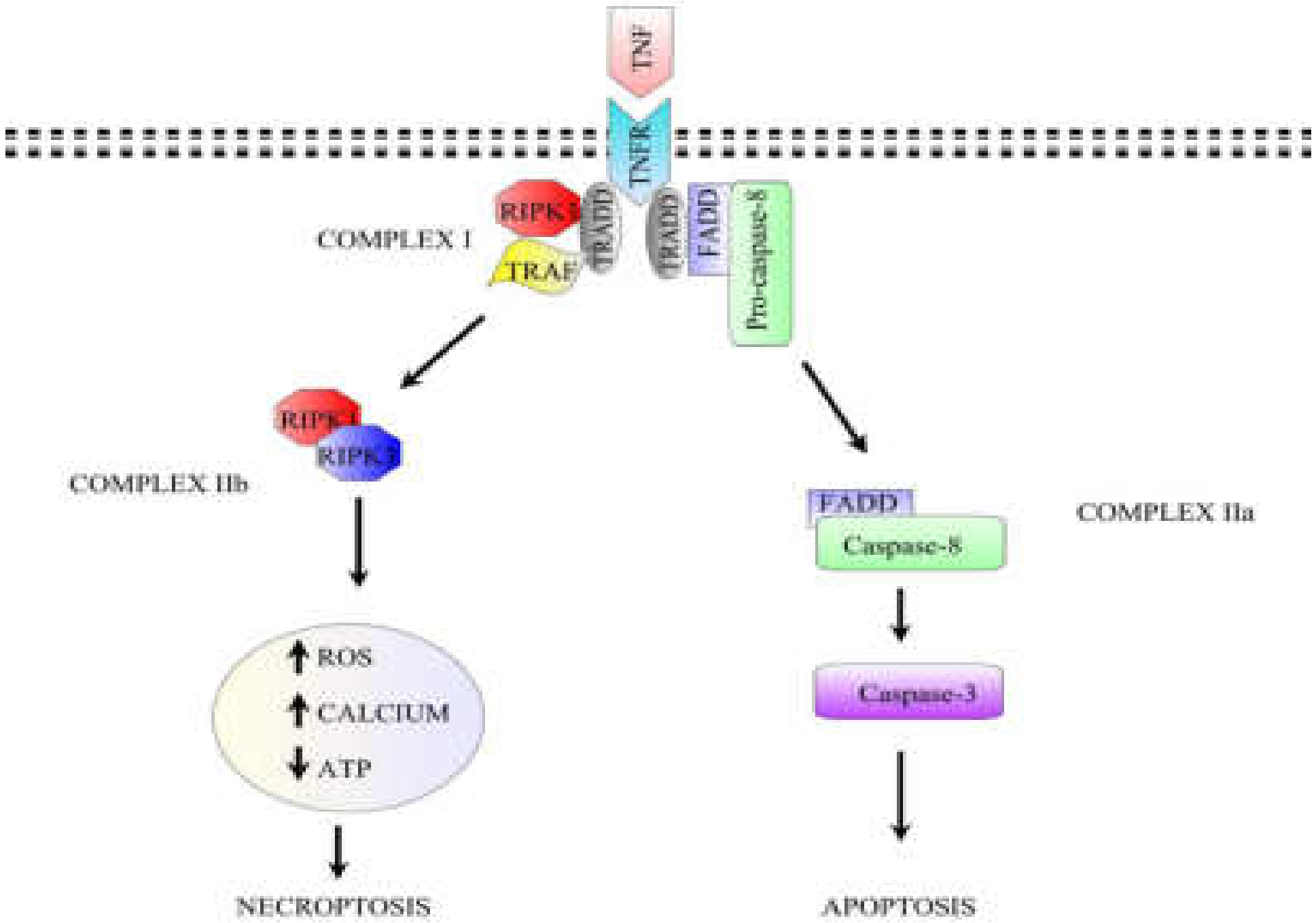


Extrinsic Pathway of Initiation

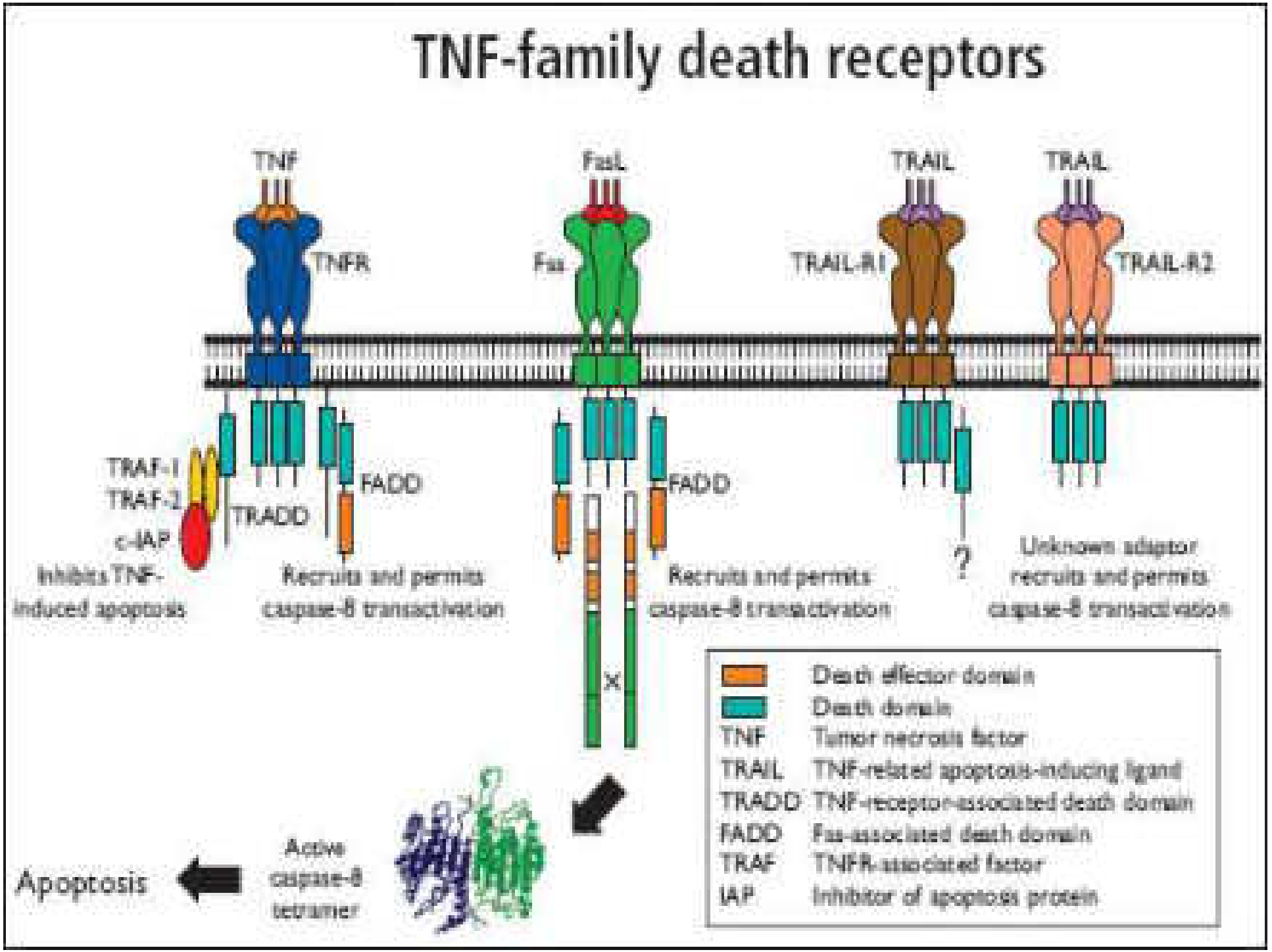
- Cause by extracellular signal.
- Tumor necrosis factor(TNF)-α is the common factor to initiate apoptosis.
- Tumor necrosis factor(TNF)-α is secreted by many cells, like T- Killer cells.
- Tumor necrosis factor(TNF)-α activates death receptors.

Extrinsic Pathway of Initiation

- Death domain activates TRADD (TNF receptor associated protein with death domain).
- TRADD protein complex with the FADD and converts procaspase-8(inactive) to caspase-8 (active).
- Caspase-8 activates caspase cascade pathway, in turn destruction of many proteins.

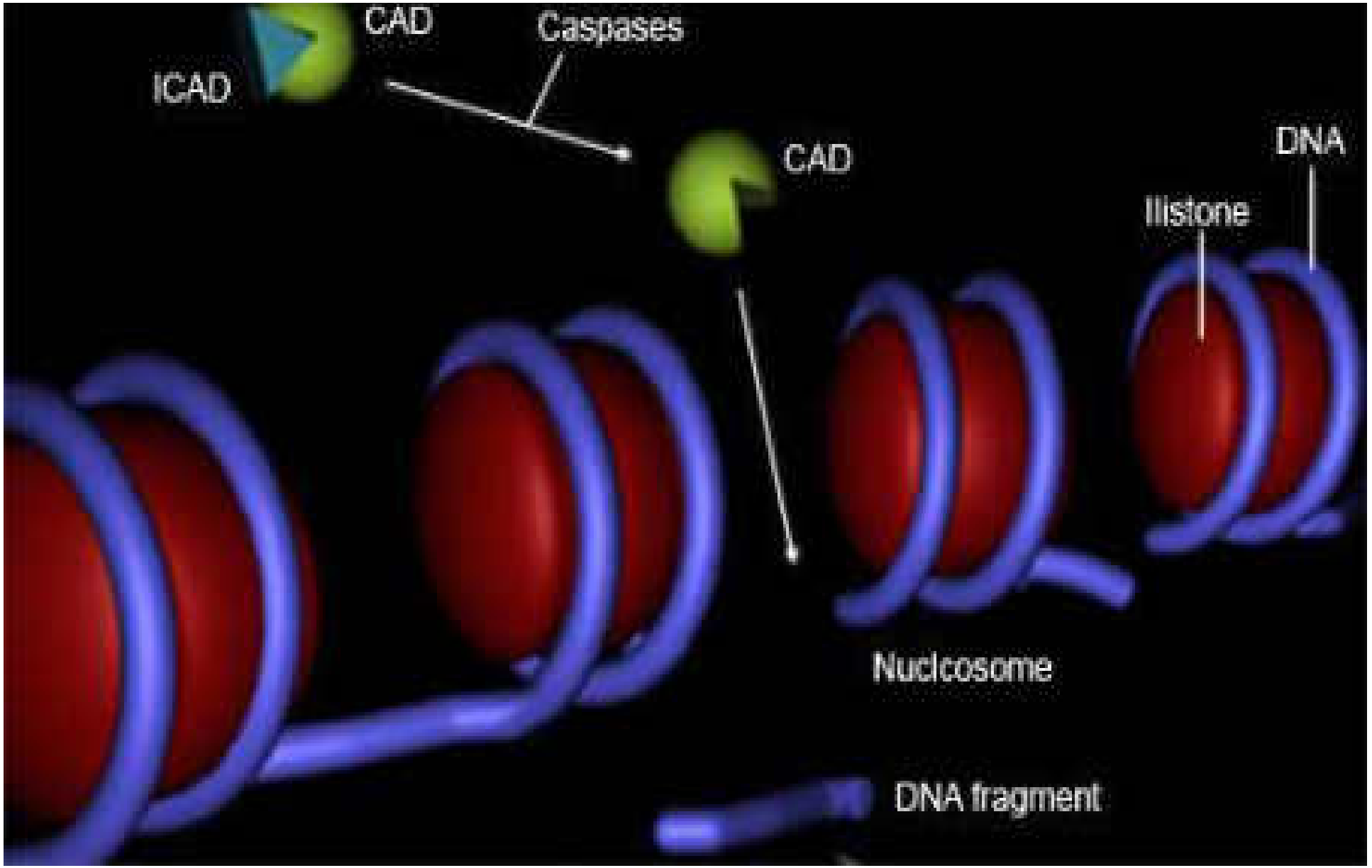

TNF
TNFR
COMPLEX I
RIPK1
TRAF
TRADD
TRADD
FADD
Pro-caspase-8
RIPK1
RIPK3
COMPLEX IIb
ROS
CALCIUM
ATP
NECROPTOSIS
FADD
Caspase-8
COMPLEX IIa
Caspase-3
APOPTOSIS

TNF-family death receptors

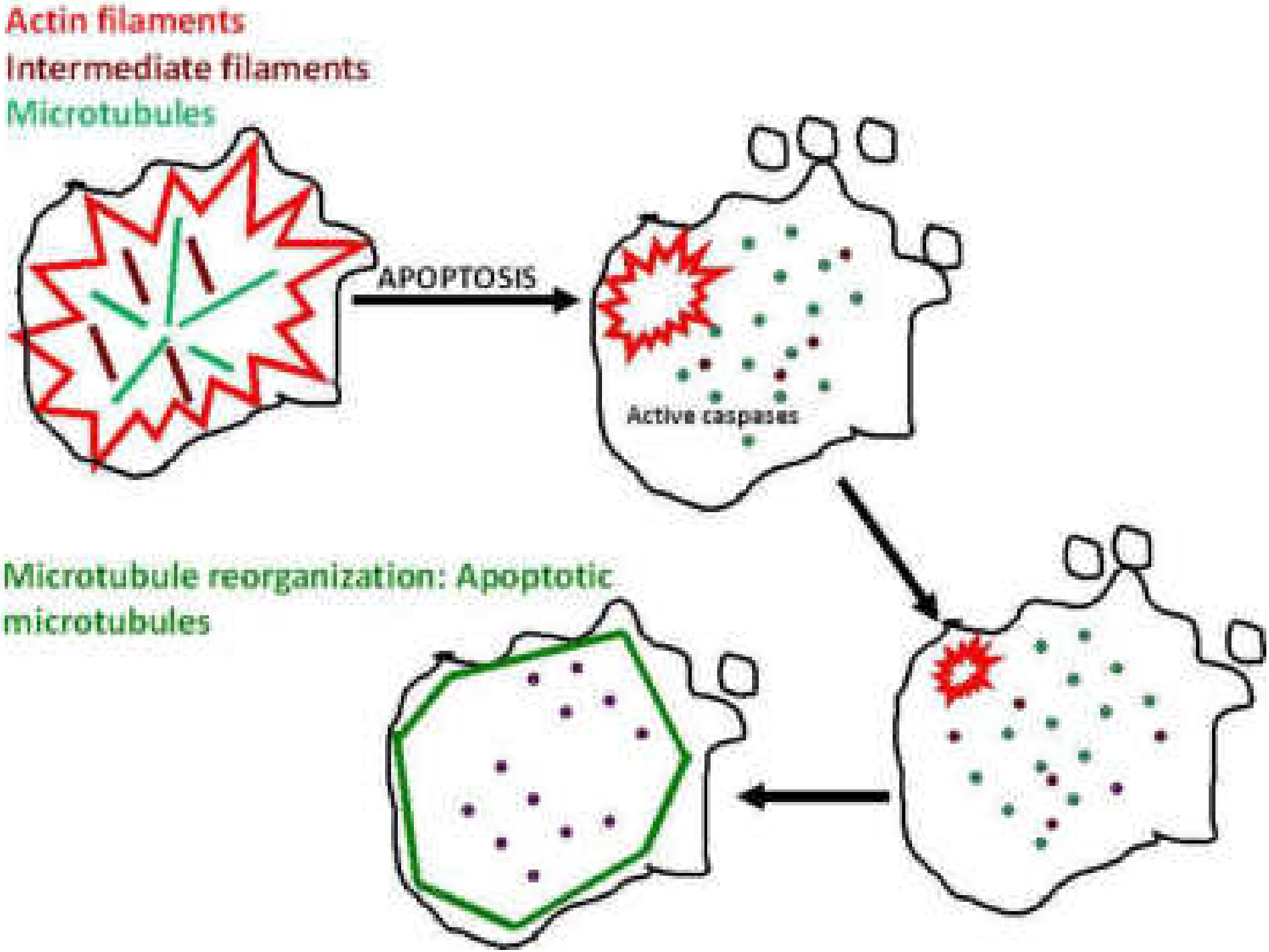


Execution- Cleavage of DNA

- The important step of cleavage of DNA is the cleavage of cytoskeleton.
- In a normal cell DNase is complex with inhibitor and inactive.
- Active caspase-3 able to cleave the -
  - Inhibitor of DNase
  - cleavage cytoskeleton.
- Active DNase cleave the DNA, cleavage site located at an regular interval of 200 base pairs.
- Other proteins makes the cell into apoptotic vesicle (contains mainly mitochondria and nuclear material.

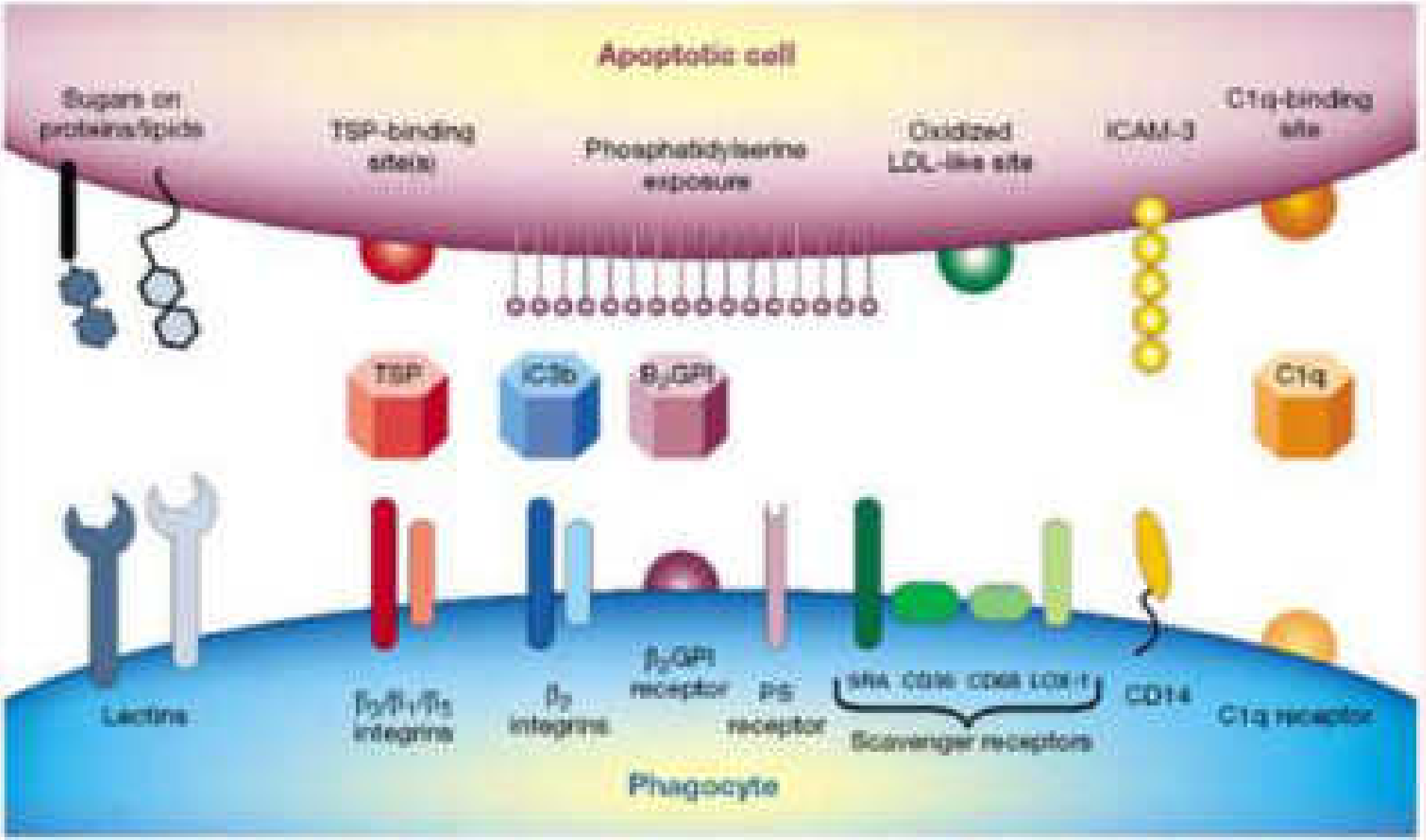


DNA winds around histone to form nucleosome. Apoptosis induces cleavage of naked DNA between nucleosomes with caspase-activated DNase (CAD)

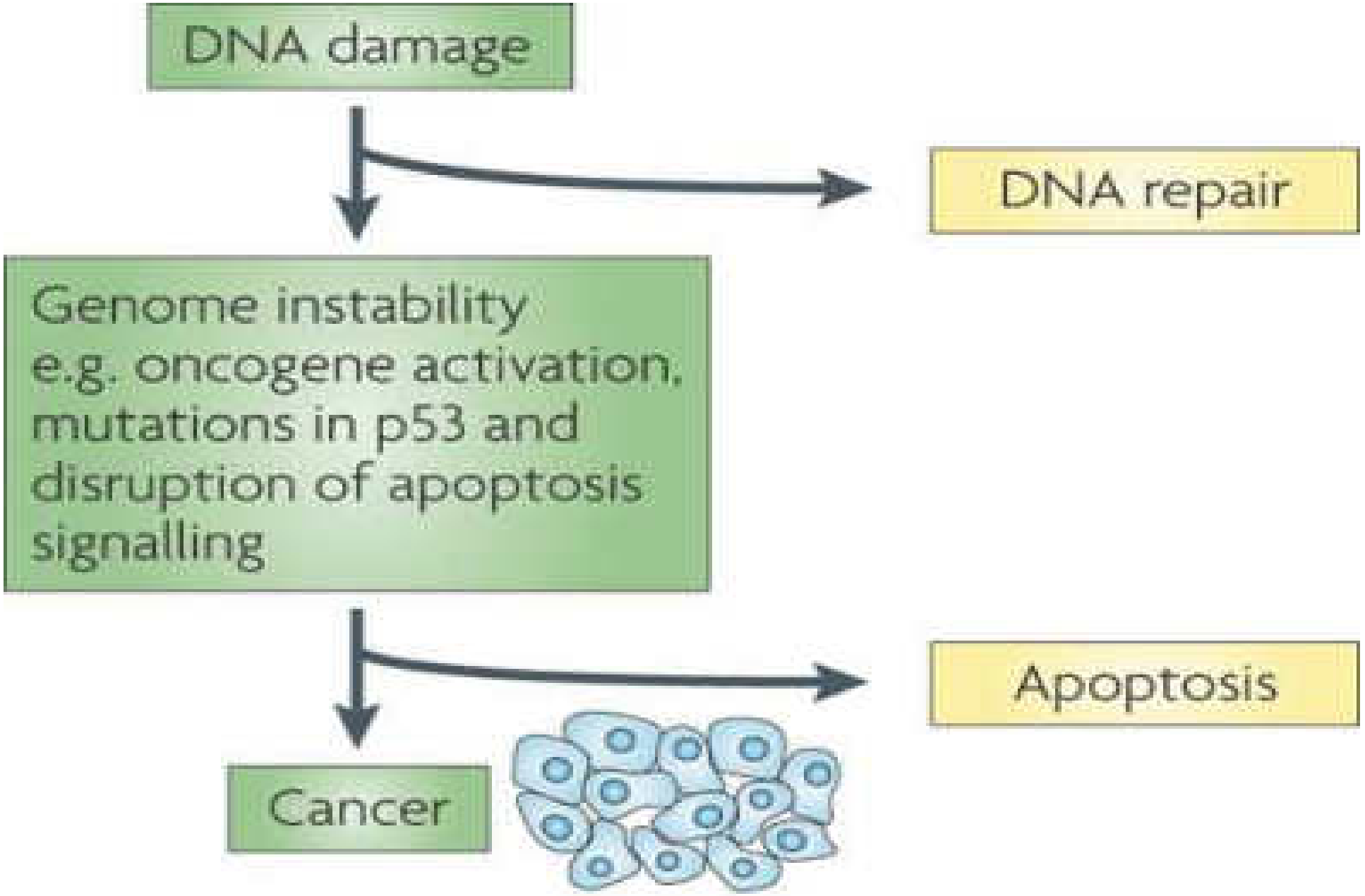

Actin filaments
Intermediate filaments
Microtubules
APOPTOSIS
Active caspases
Microtubule reorganization: Apoptotic microtubules

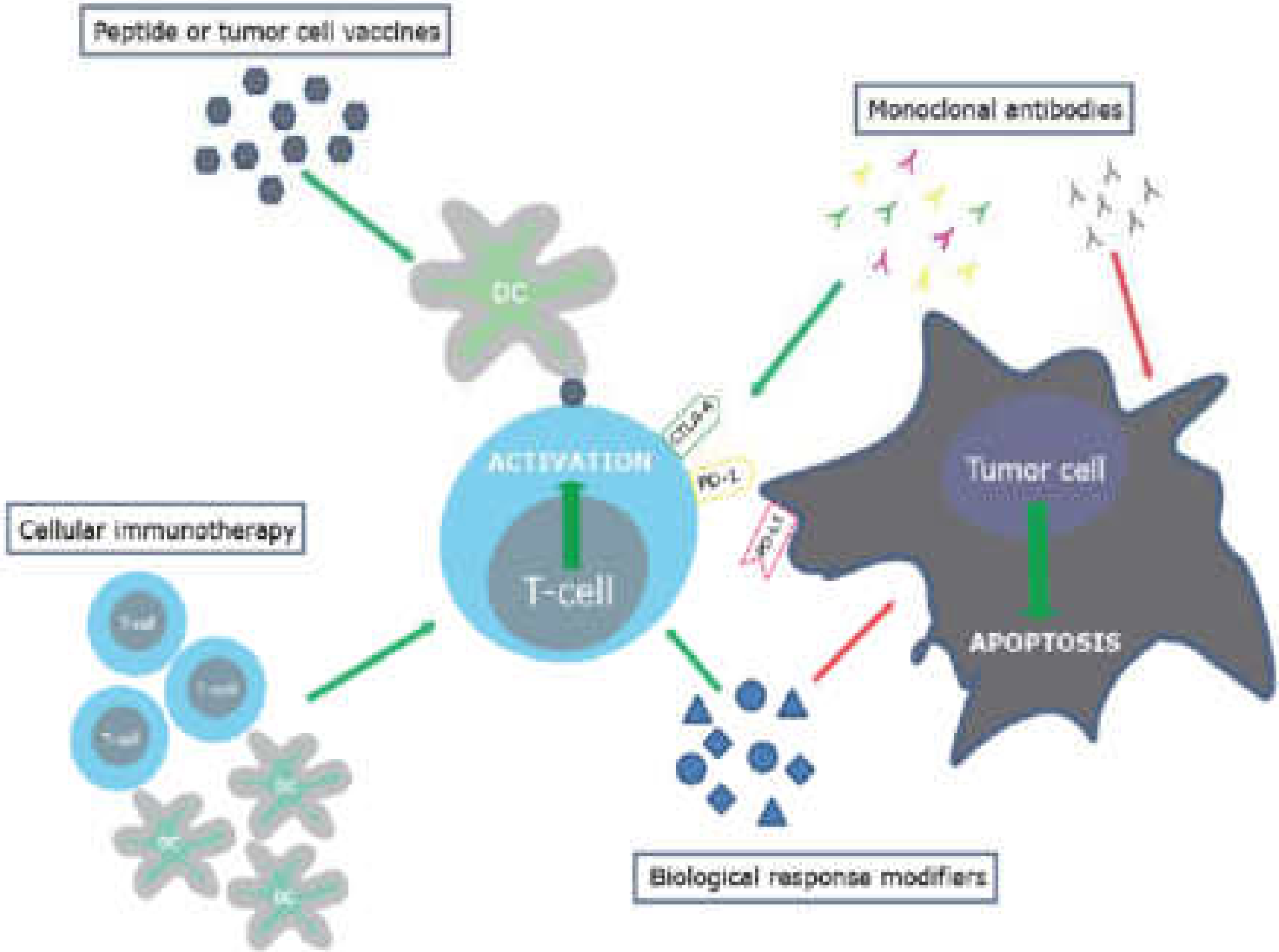
PHAGOCYTOSIS

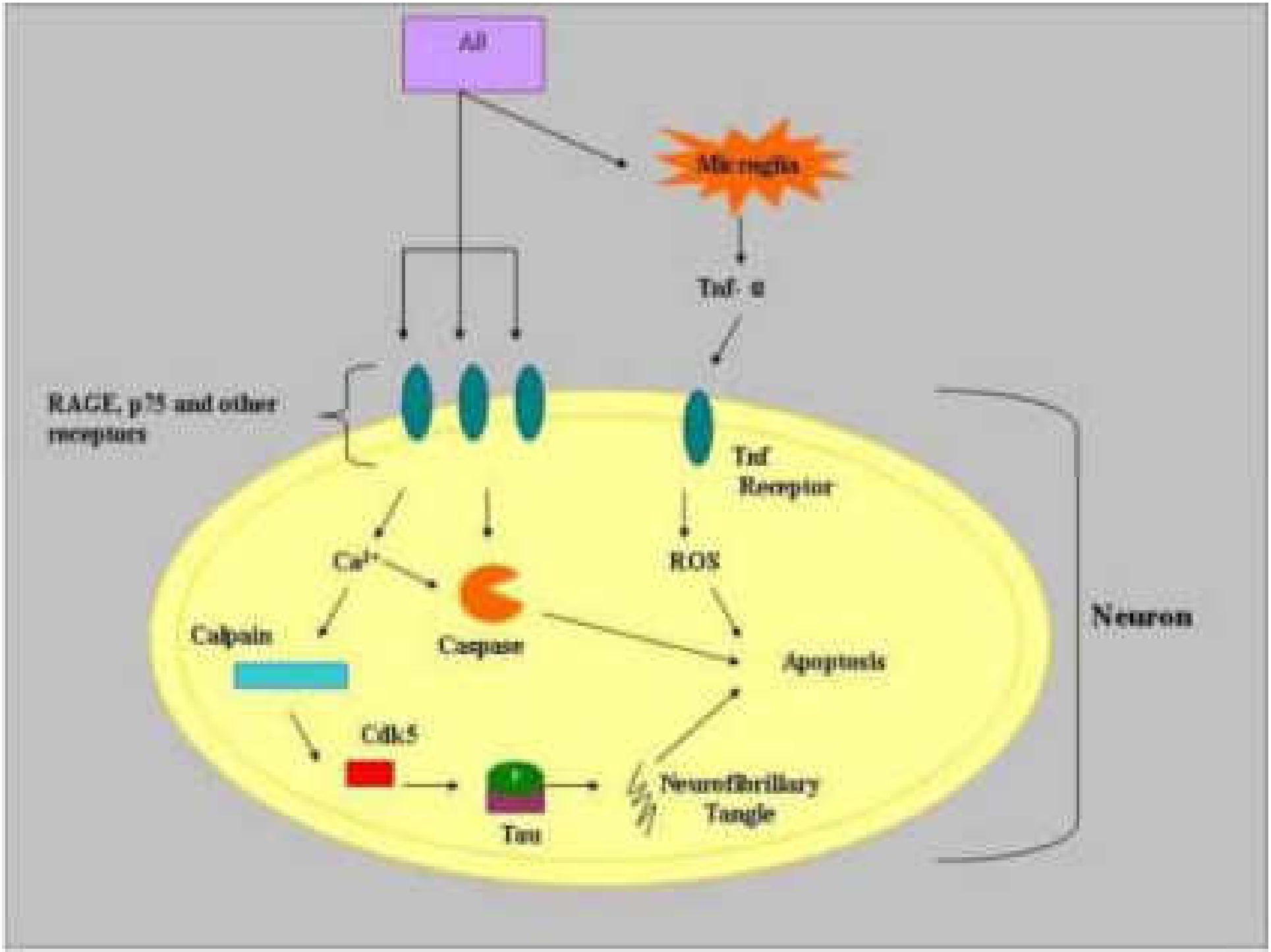
Apoptotic cells are discriminated from live cells and cleared up by phagocytes such as macrophages.

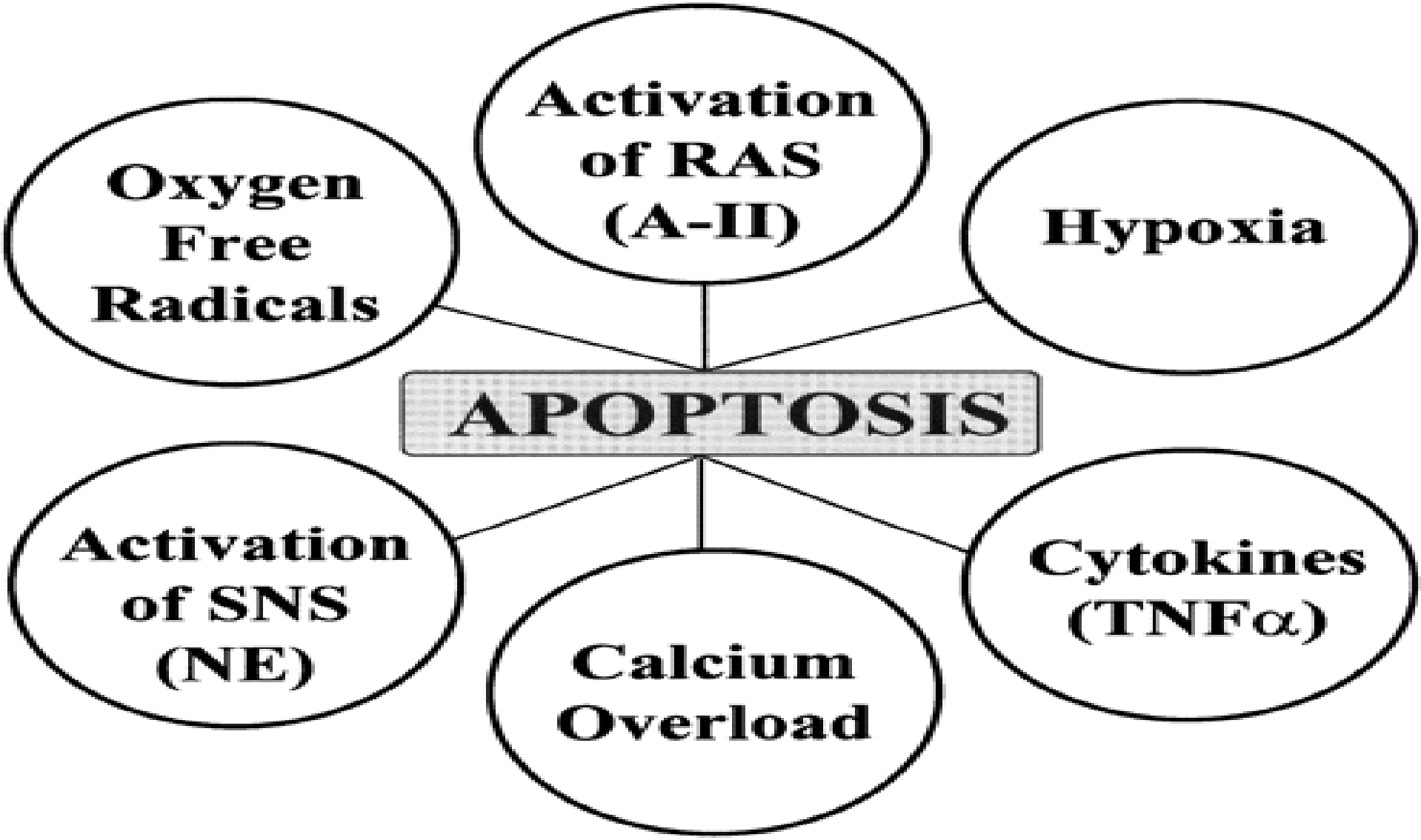


Pathophysiological Role Apoptosis

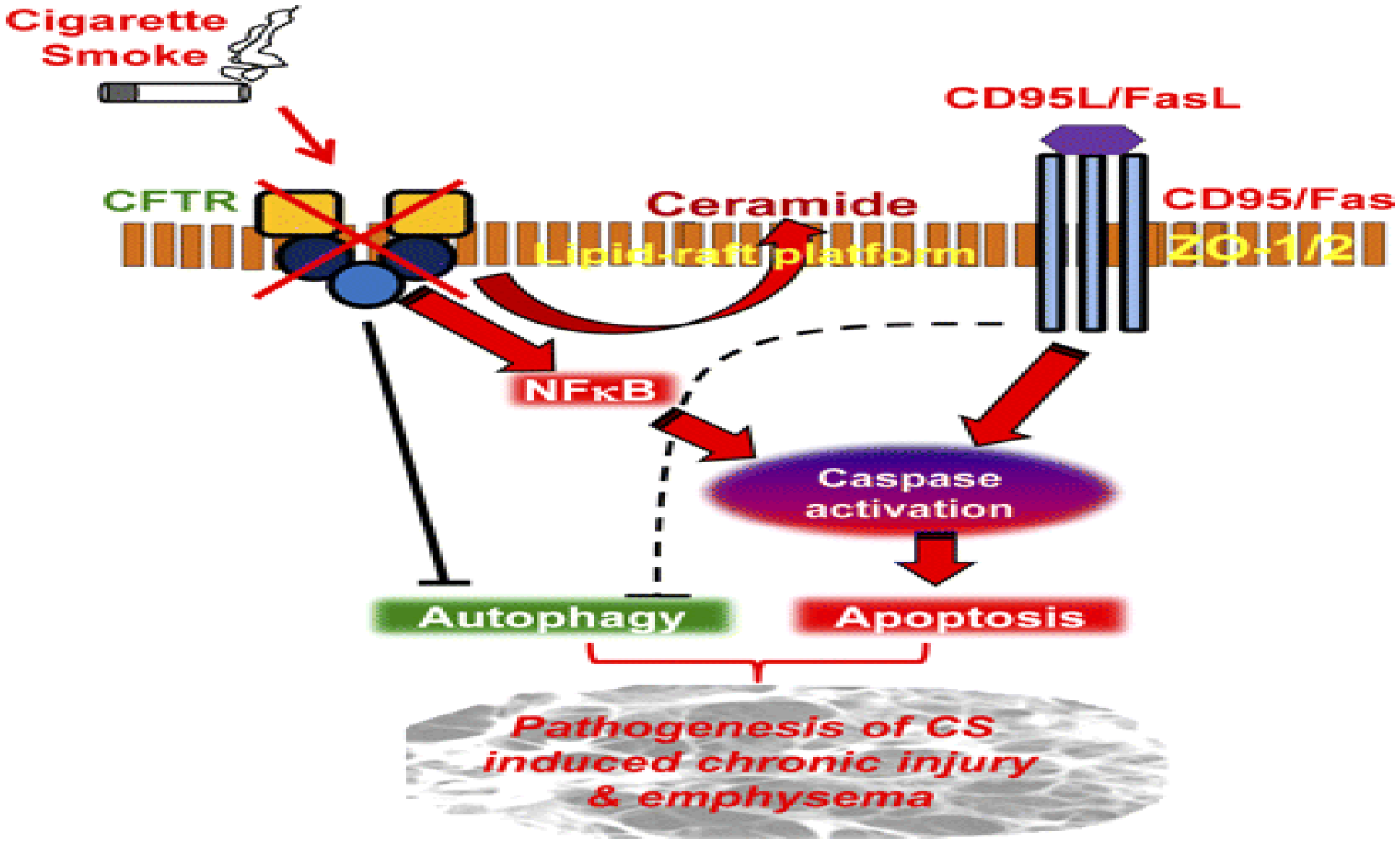

DNA damage
DNA repair
Genome instability
e.g. oncogene activation,
mutations in p53 and
disruption of apoptosis
signalling
Apoptosis
Cancer


Peptide or tumor cell vaccines
Monoclonal antibodies
DC
ACTIVATION
CTLA-4
PD-1
PD-L1
Tumor cell
T-cell
APOPTOSIS
Cellular immunotherapy
Biological response modifiers

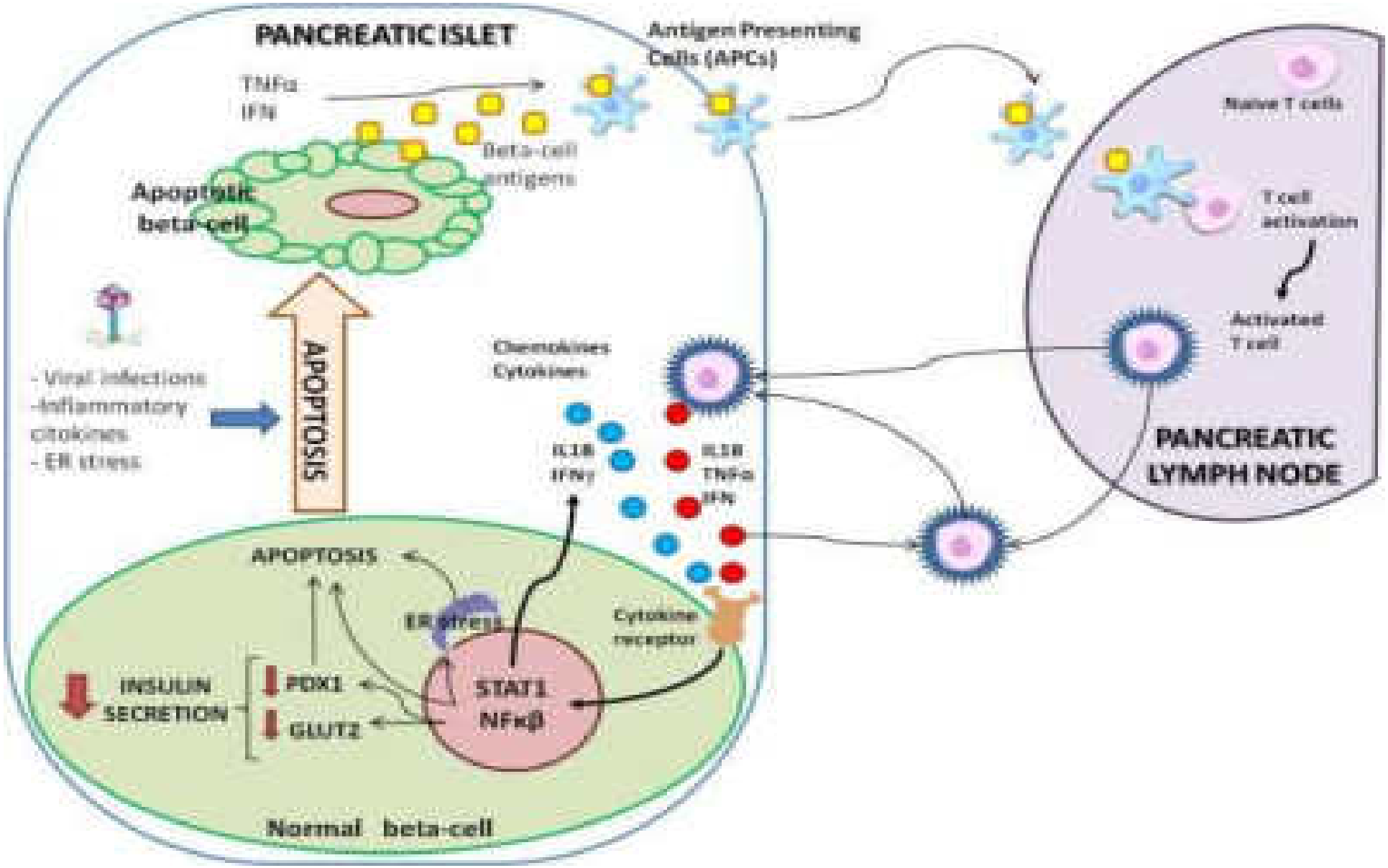

AII
Microglia
TnF- α
RAGE, p75 and other receptors
TnF Receptor
Ca2+
ROS
Calpain
Caspase
Apoptosis
Cdk5
P
Tau
Neurofibrillary Tangle
Neuron



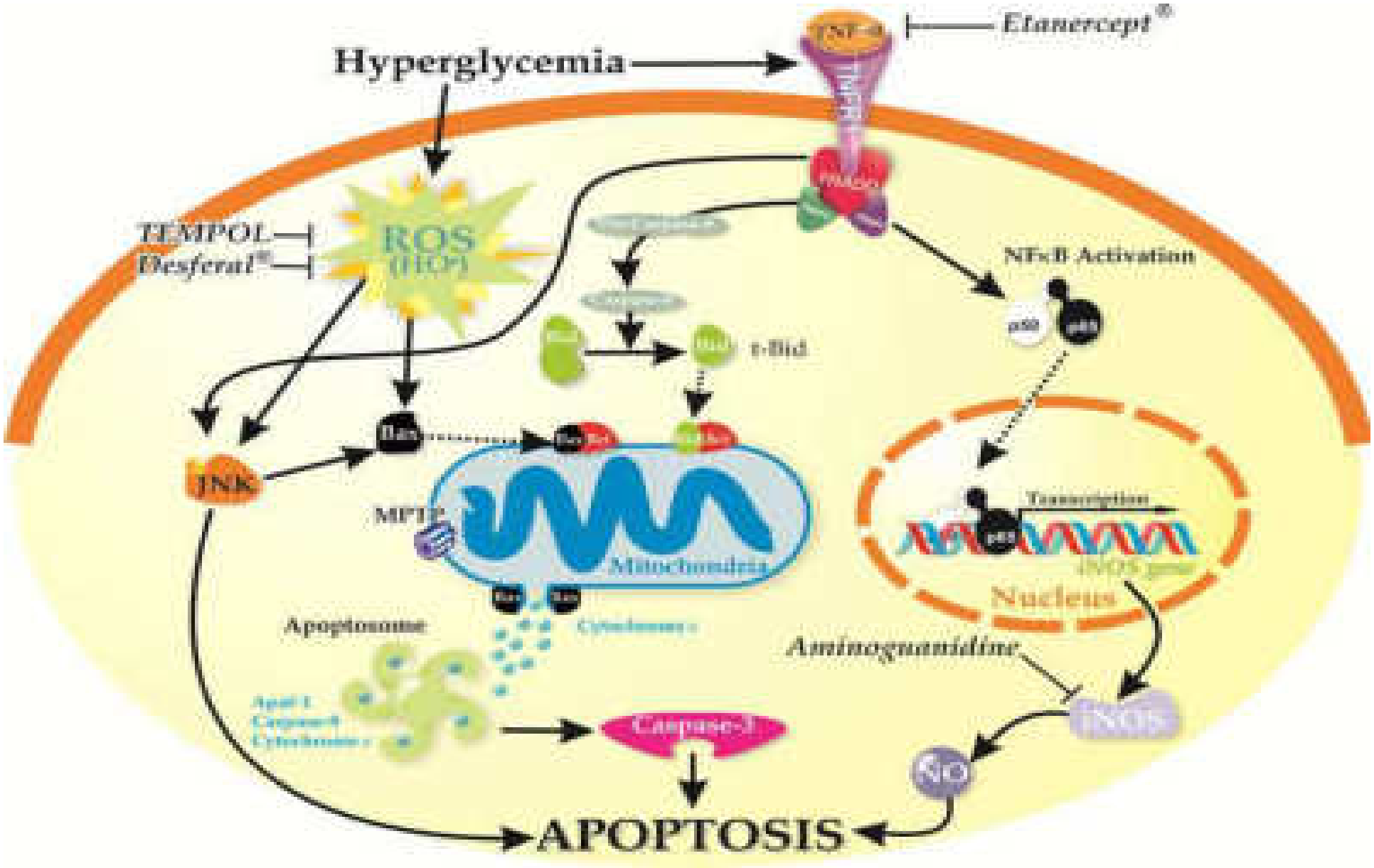
Pathophysiologic factors implicated in cardiomyocyte apoptosis of the failing heart.



Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is a membrane protein, mutations of the CFTR gene affecting chloride ion channel function lead to dysregulation of epithelial fluid transport in the lung, pancreas and other organs, resulting in cystic fibrosis



Induction and progression of insulitis. Viral infections or inflammatory processes may lead to beta-cell apoptosis.



Proposed scheme for the mechanism involved in the hyperglycemia-induced apoptosis that occurs in type 1 diabetes-induced liver disease.

Thank you