CELL DEATH PATHWAYS

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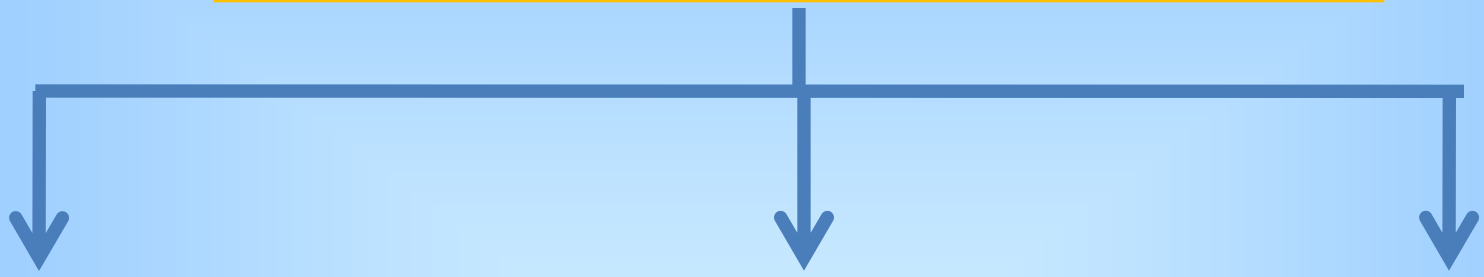
CELL DEATH



PROGRAMMED CELL DEATH (Apoptosis)

ACCIDENTAL CELL DEATH (Necrosis)

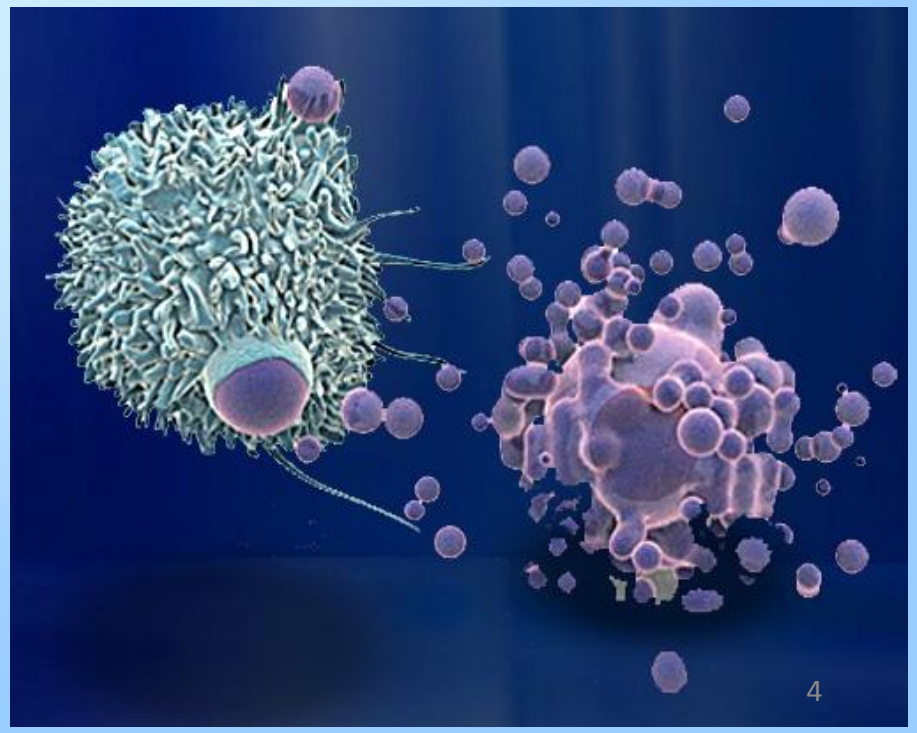
SELF DEMISE (Autophagy)

APOPTOSIS-Programmed cell death

- A term used to describe the morphological changes associated with programmed cell death
- The term ‘programmed cell death’ was introduced in 1964 [Lockshin, 1964]
- The term ‘Apoptosis’ was first introduced by Kerr, Wyllie and Currie [Kerr, 1972]
- Apoptosis is of Greek origin, having the meaning "falling off or dropping off", in analogy to leaves falling off trees or petals dropping off flowers

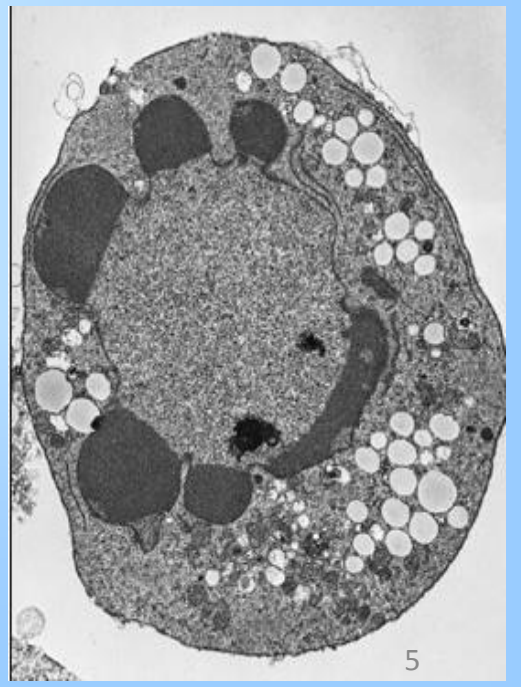
APOPTOSIS-Silent Suicide

- Deliberate elimination of unwanted cells in a morphological distinct manner
- Genetically programmed highly conserved cellular suicide
- Normal physiological form of cell death



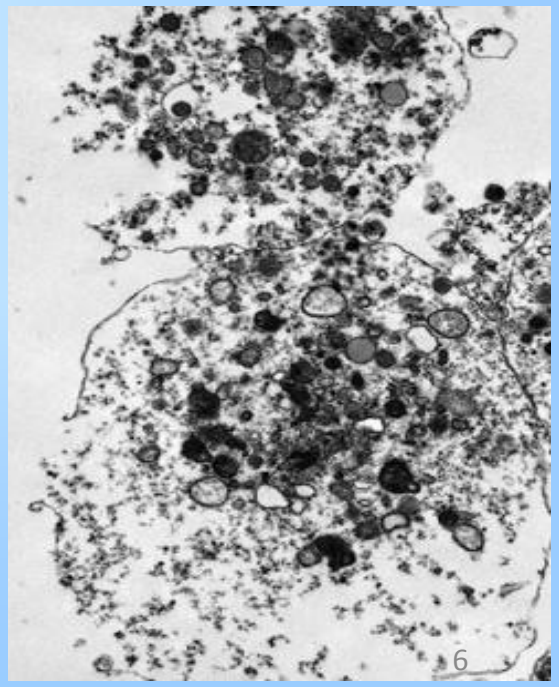
APOPTOSIS-Morphological features

- Active cell death
- Requires energy and RNA and protein synthesis
- DNA cleaved, chromatin condenses
- Cells shrink
- Formation of apoptotic body
- Cleared by phagocytosis
- No inflammation=*no tissue damage*

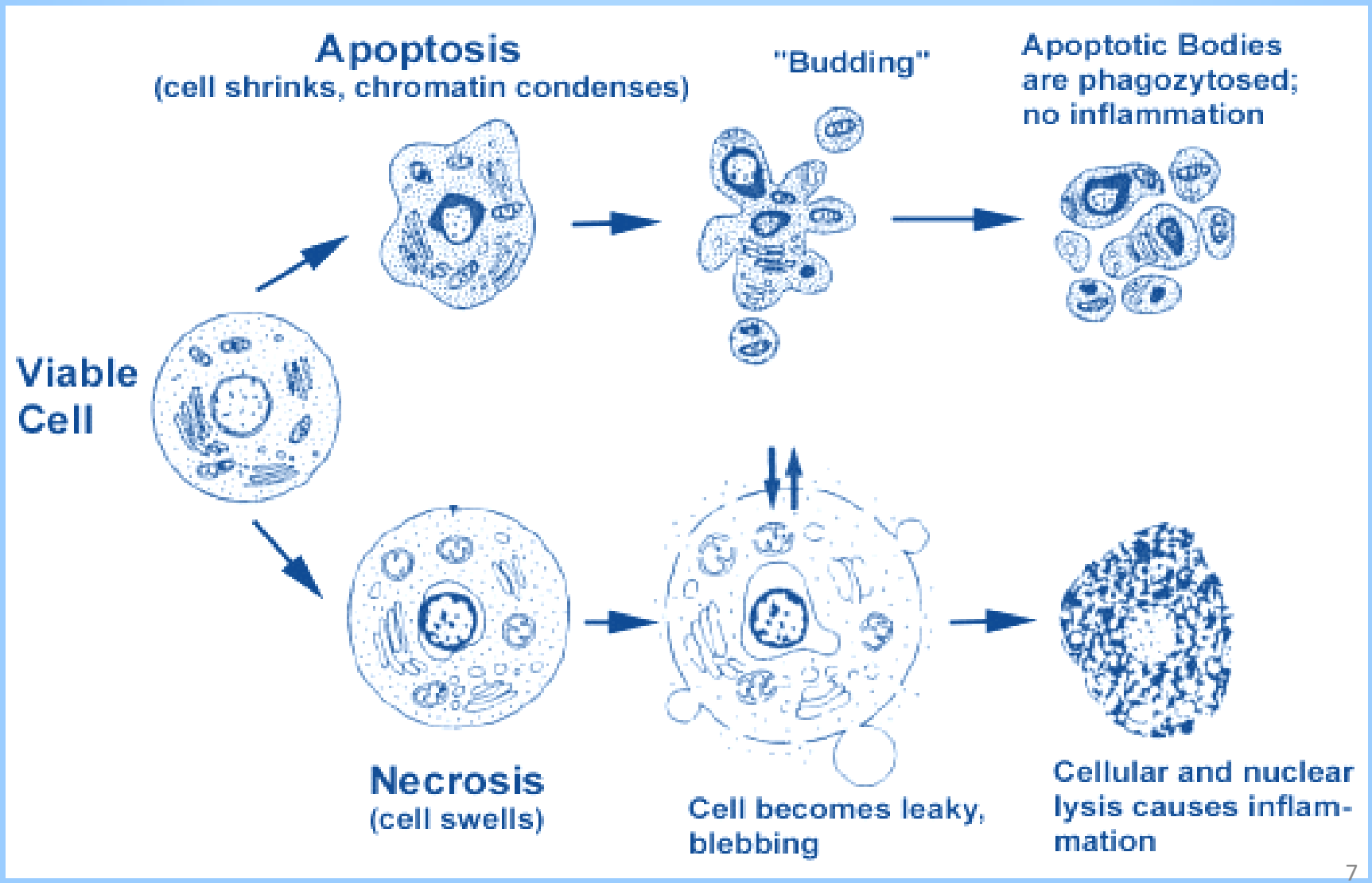


NECROSIS-Morphological features

- *Passive cell death*
- Cells swell up
- Membrane breaks down and cellular contents leak out
- Nucleus disintegrates
- Cell ghosts
- Inflammatory=*tissue damage*



APOPTOSIS vs. NECROSIS



HEALTHY STATE

NECROTIC CELL DEATH

APOPTOTIC CELL DEATH

THE HISTORY OF APOPTOSIS

1858 - Virchow - Cell vanishes and can no longer be seen in its orginal form - **Necrobiosis**

1885 – **Walther Flemming** – Regression of epithilium cells - **Chromatolysis**

1914 – **Ludwig Graper** - First **review** on Chromatolysis

1951 – **Glucksmann** – Chromatosysis in **embryo development**

1960s – **Electron Microscope** – learning @ ultrastructural level

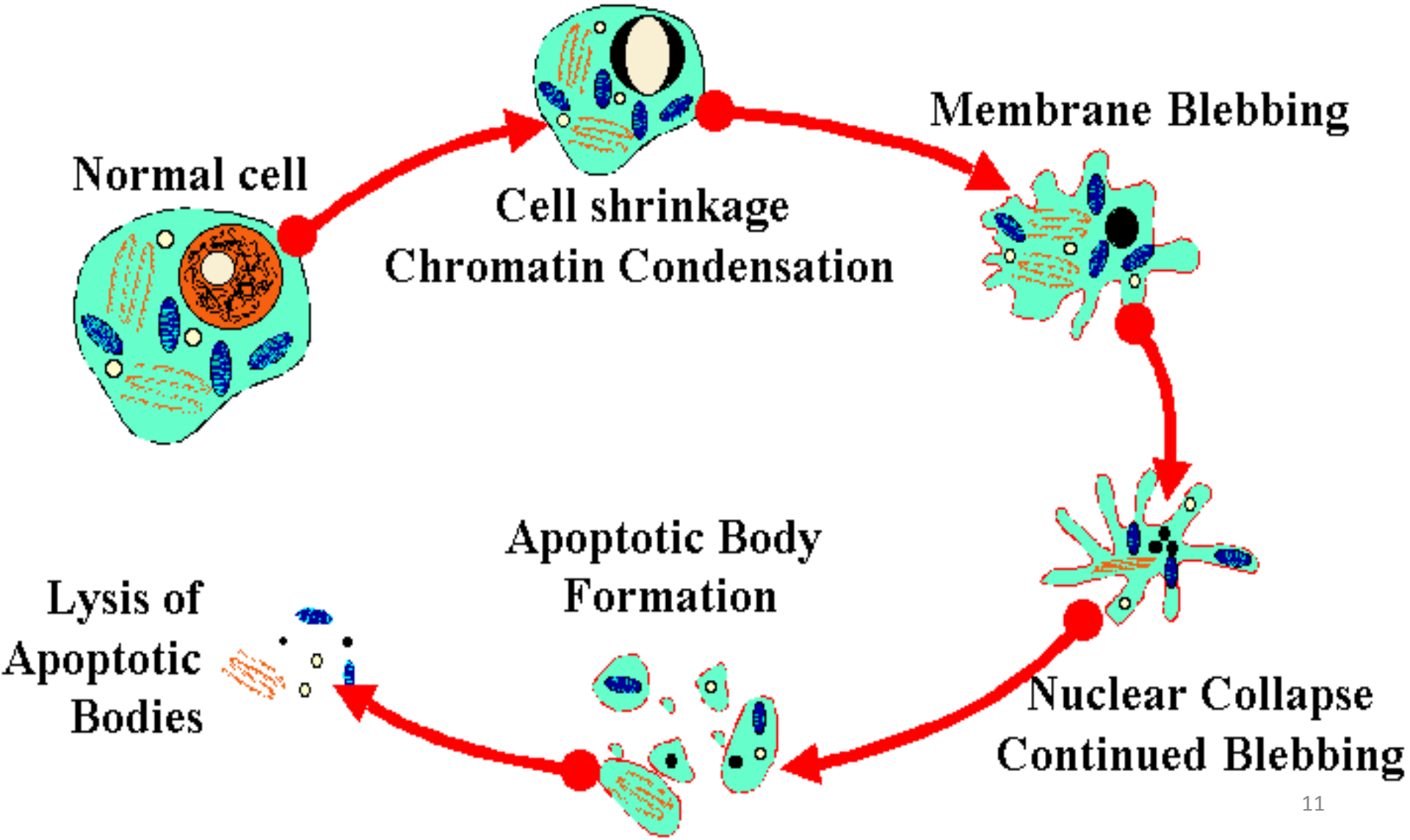
1972 – **Kerr, Wyllie, Curie** – **Apoptosis**

Today > 30000 listed manuscripts on Apoptosis

IMPORTANCE OF APOPTOSIS

- Maintenance of cellular and tissue homeostasis
- Provide defence mechanism
- Development of immune system
- Important in embryonic development
- Regulation of the neuron number
- Regulation of cellular viability by hormones and growth factors
- The body's defence against cancer

Apoptosis (Programmed Cell Death)

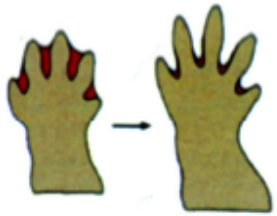


CELLS UNDERGOING APOPTOSIS

- Harmful cells
- Developmentally defective cells
- Excess cells
- Unnecessary cells
- Obsolete cells
- Virus infected cells
- Chemotherapeutic killing of cells

EXAMPLES OF PHYSIOLOGICAL CELL DEATH

In the human body about 100,000 cells are produced every second by mitosis and a similar number die by apoptosis (Vaux and Korsmeyer, 1999, Cell) !

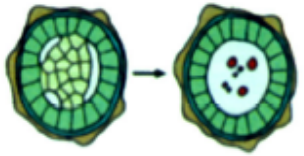


Development and Morphogenesis:

- 131 of the 1,090 somatic cells die during C.elegans development
- during limb formation separate digits evolve by death of interdigital mesenchymal tissue (a)
- ablation of cells no longer needed such as the amphibian tadpole tail during metamorphosis (b)
- demise of cells allows sculpturing of hollow structures (c)
- formation of reproductive organs (d)
  (Müllerian duct → uterus, deleted in males;Wolffian duct → male organs, deleted in females)
- massive cell death occurs during early development of the nervous system (> 50 percent of all neurons die)

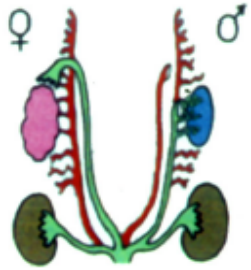
Homeostasis:

- a paradigm for the involvement of apoptosis in homeostasis is the immune system: several millions of B and T cells are generated every day and the majority (> 95 percent) of those die during maturation (death by neglect, negative selection) or by AICD of peripheral immune cells)



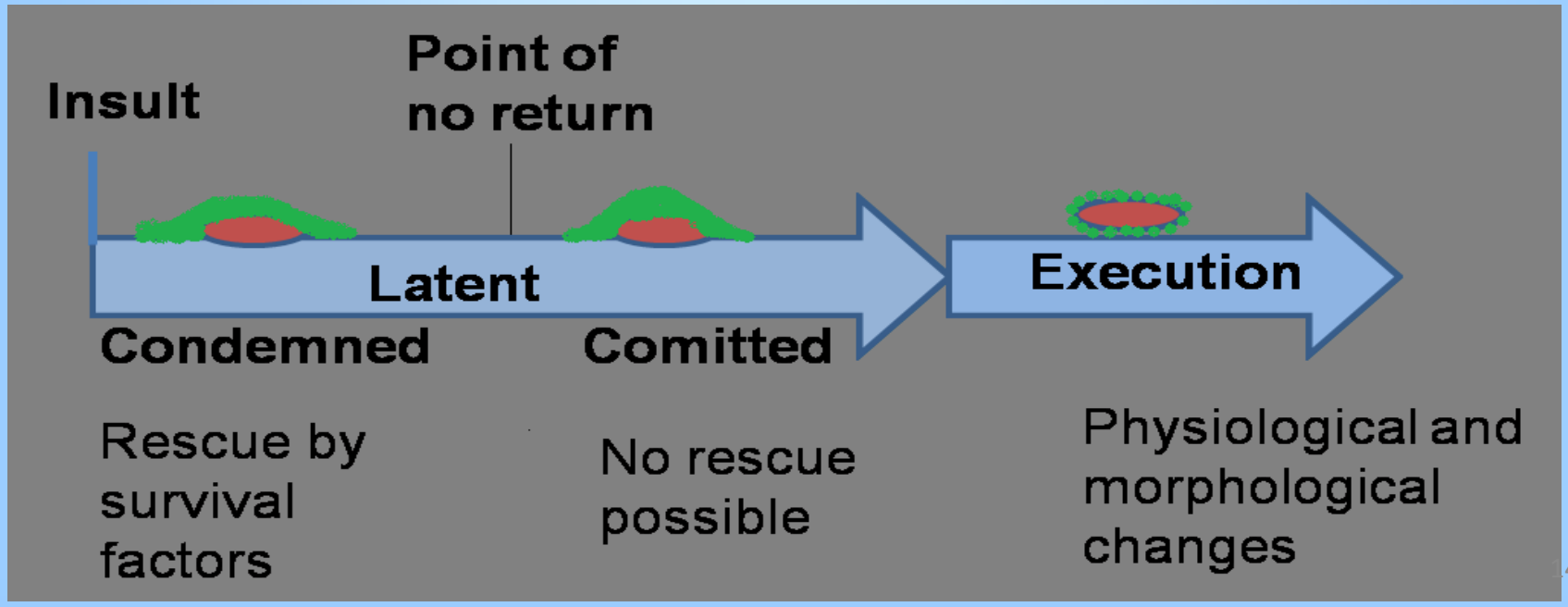
Deletion of damaged and dangerous cells:

- Cells with severely damaged DNA that cannot be repaired appropriately usually are removed by apoptosis
- Inappropriate mitogenic signalling that is in conflict with the environmental or cellular status of the cell usually results in cell cycle arrest or apoptosis
- Autoreactive cells of the immune system are deleted by apoptosis
- Elimination of infected cells



EVENTS OF APOPTOSIS

- Events of apoptosis occur in two phases
  1. Latent phase
  2. Execution phase



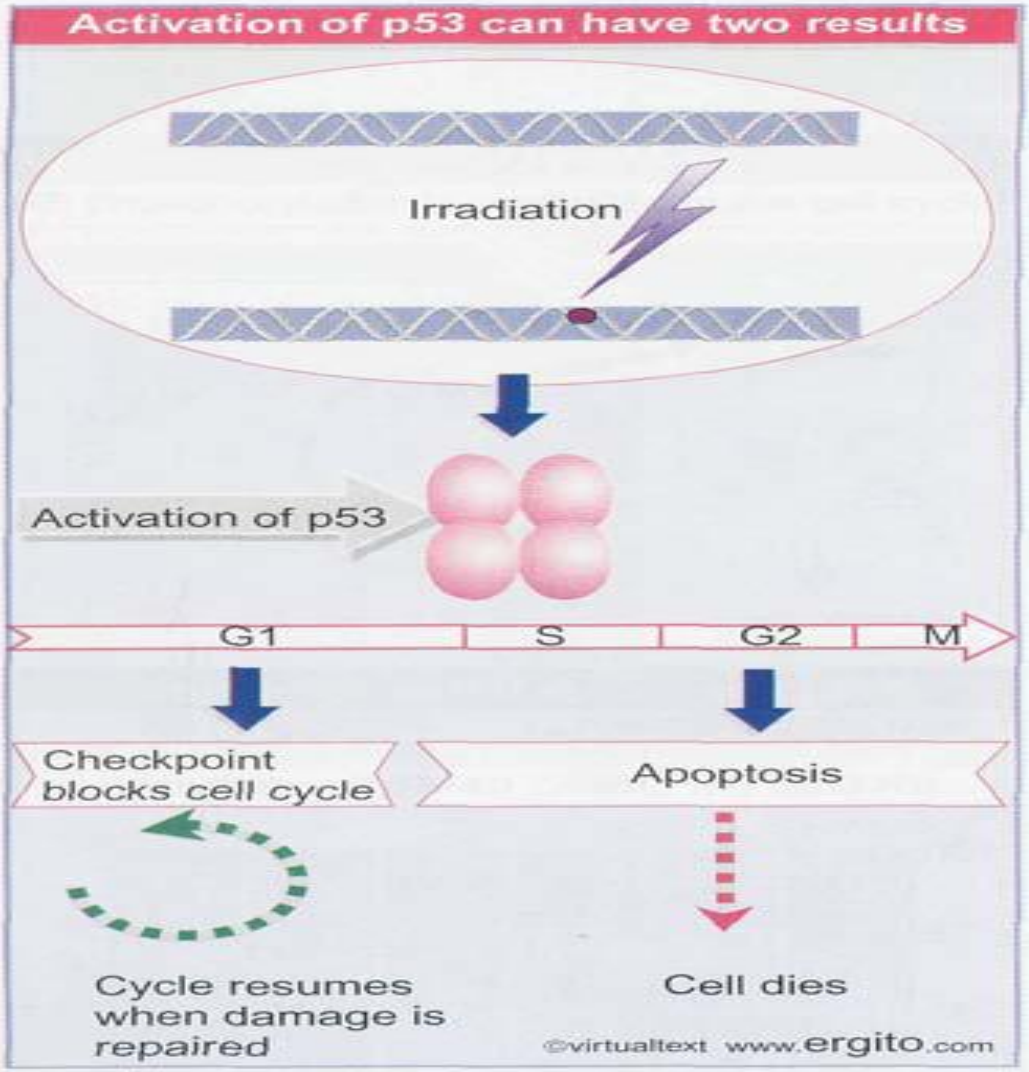
ROLE OF P^{53} IN LATENT PHASE

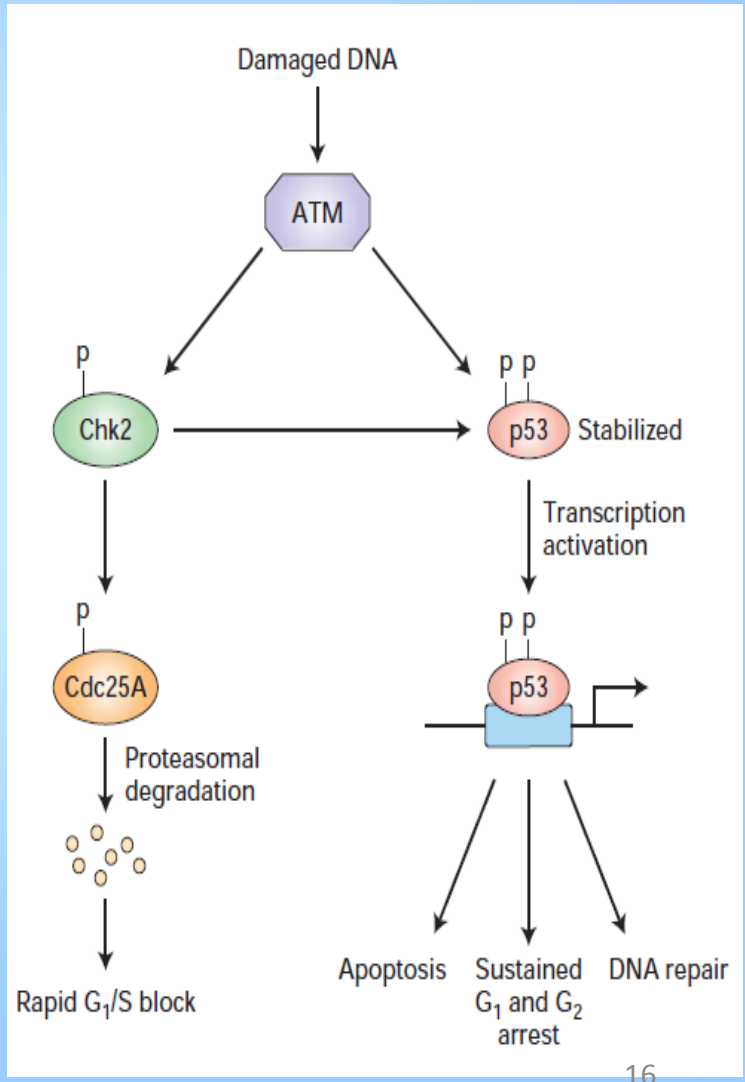
- Regulation of cell cycle progression in response to DNA damage
- Rise in p^{53} level when sense DNA is damaged
- Delay in the entry of cell into S-phase
- Action is important in cancer
- Over expression of this arrested cells in G1/S boundary, where as in cancer derived cells it causes apoptosis

*P^{53} is not involved in all types of apoptosis

E.g.- In knock out mice

P53 and APOPTOSIS

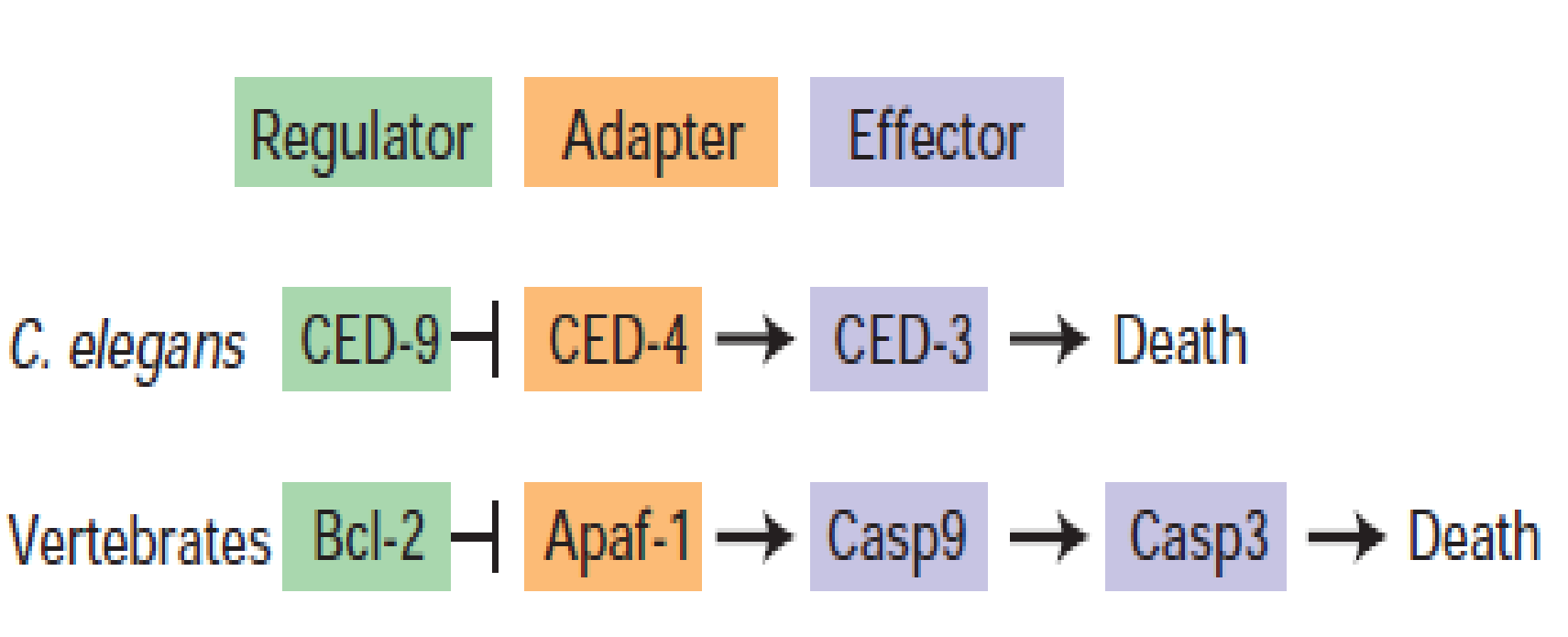




TRIGGERS OF APOPTOSIS

- Programmed cell death in which many more cells are produced than survive (e.g. development of lymphocytes)
- Toxic stimuli (viruses, chemicals, ionizing radiation)
- Extracellular signals (Fas, p^{75} NGF-R, TNF)
- DNA damage (p^{53})

PROTEINS IN APOPTOSIS

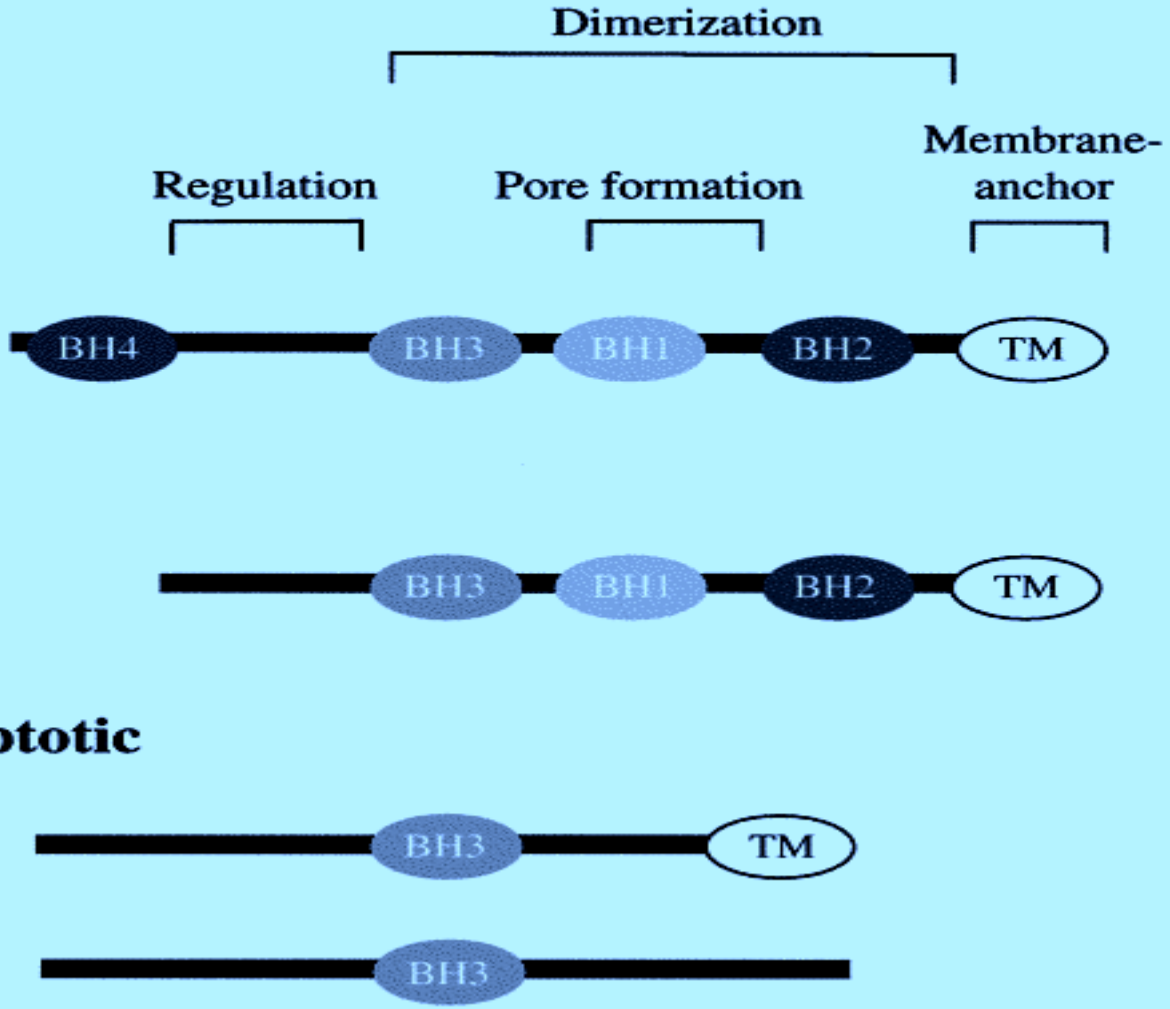


Mammalian Bcl-2 can substitute for Ced-9 in *c. elegans*

BCL-2 FAMILY MEMBERS

- A very large family with 19 members identified
- Bcl-2 (homologous to ced-9) is prototype
- All have the BH3 domain (Bcl-2 Homology)
  - BH-3 is the pro-apoptotic domain exposed on activation
- Act as dimers=either hetero or homodimers
  - Pro-apoptotic dimers (Bax) increase mitochondrial permeability
  - Anti-apoptotic members (Bcl-2, Bcl-XL) form dimers with pro-apoptotic members to inactivate them

THE BCL-2 FAMILY

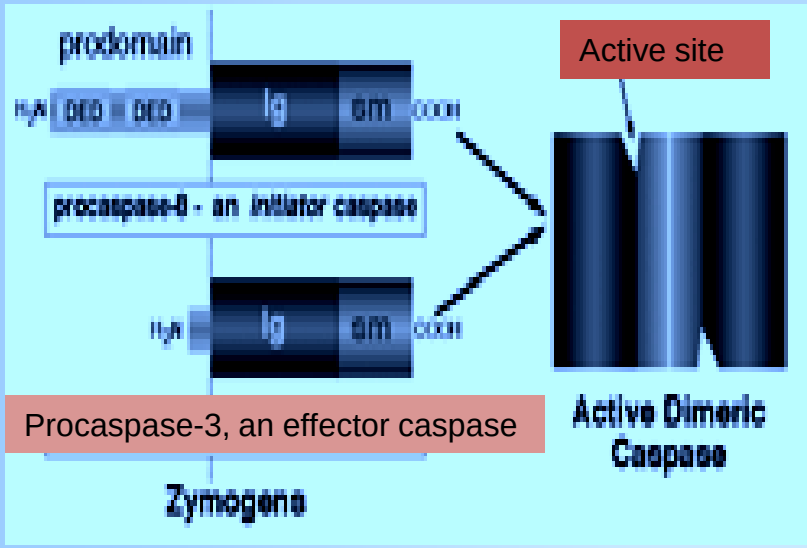


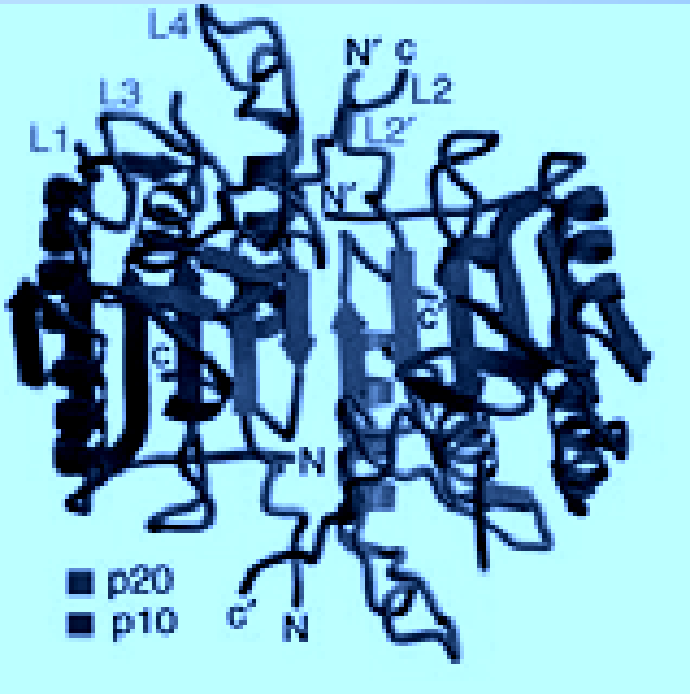
** BH domains=protein-protein interaction domains*

CASPASES-General Features

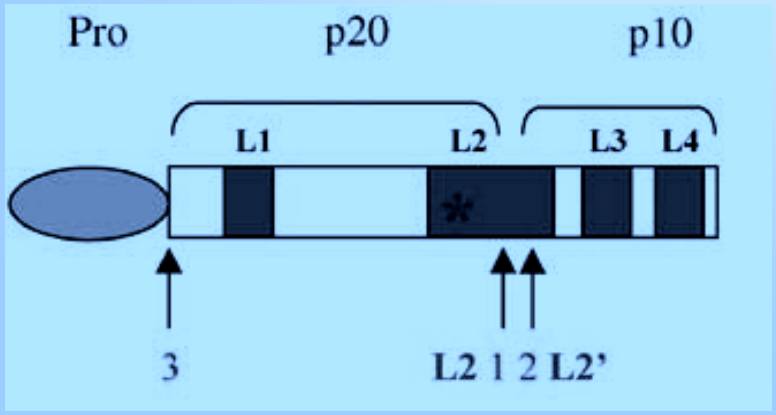
- Caspases are ***Cysteine directed proteases that cleave after ASPartate residues***
- At least 14 family members
- Synthesized as proenzymes with low levels of caspase activity (~1-2 % of active form)
- Activated upon after aggregation or cleavage to mature form
  - Caspases –8 and –9 are “initiator” caspases
  - Caspases –3 is the “effector” caspase
  - Caspase activation requires a stimulus
  - They proteolyze cellular proteins to carry out cell death program

CASPASE-Structures

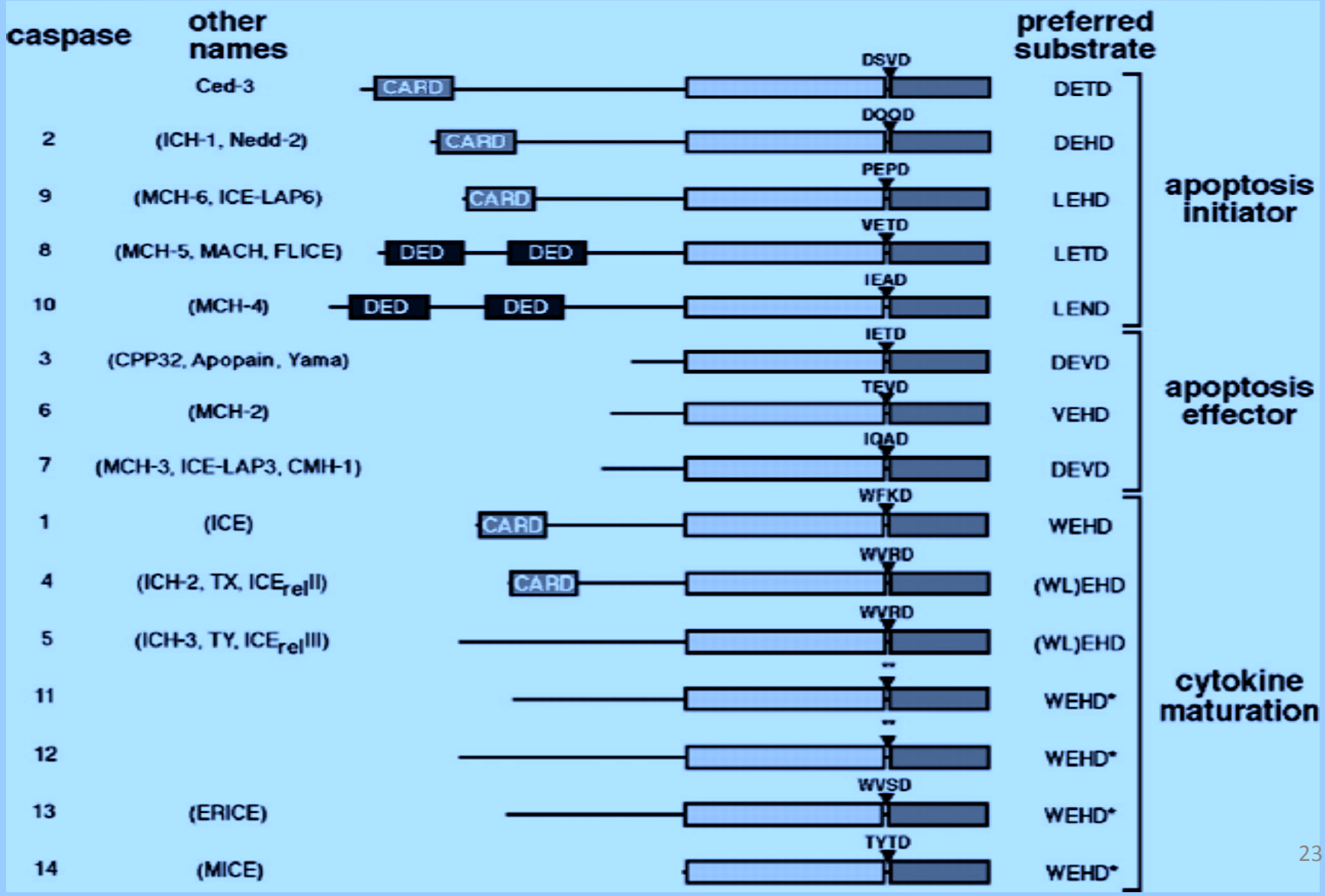




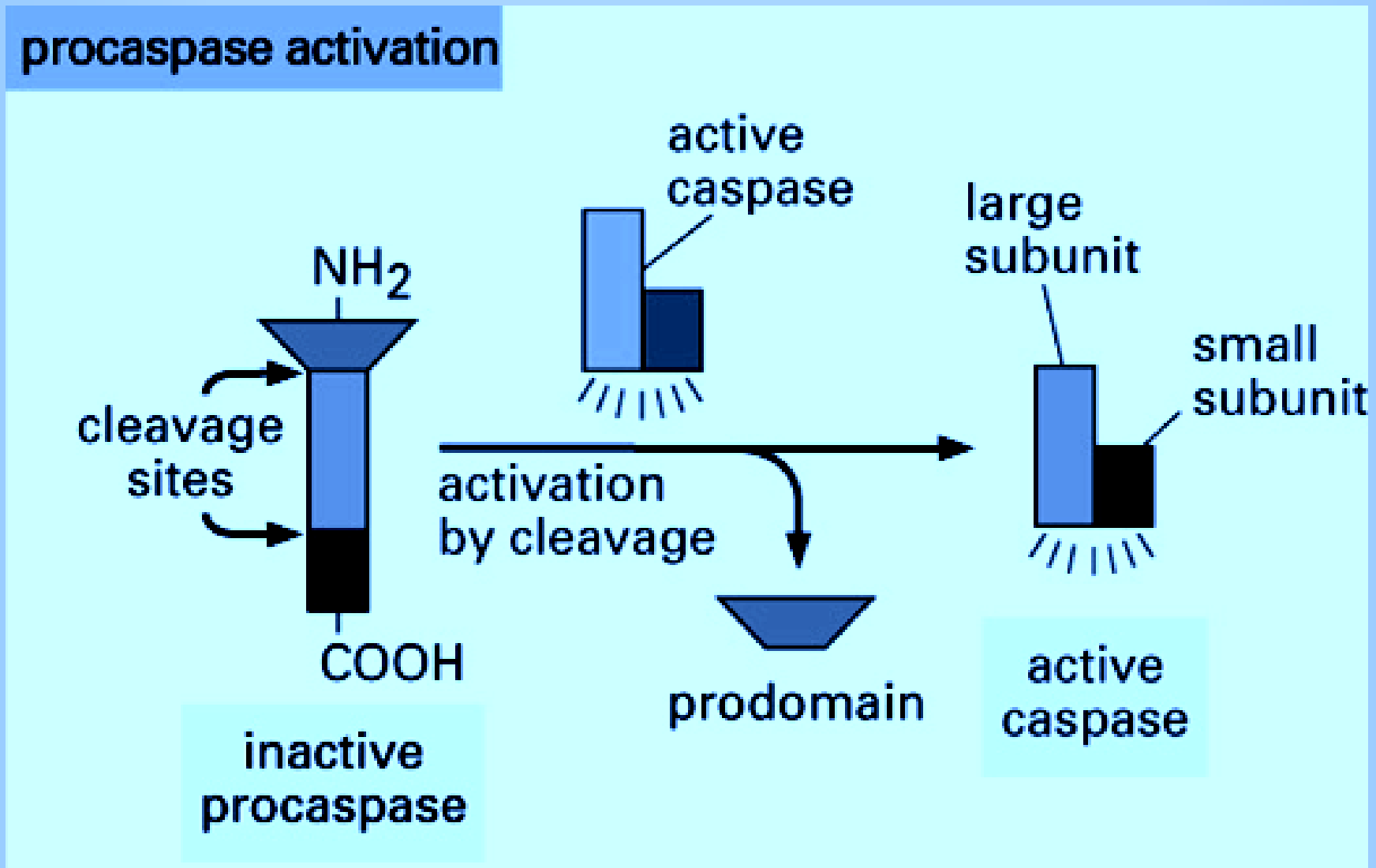
Caspase-3



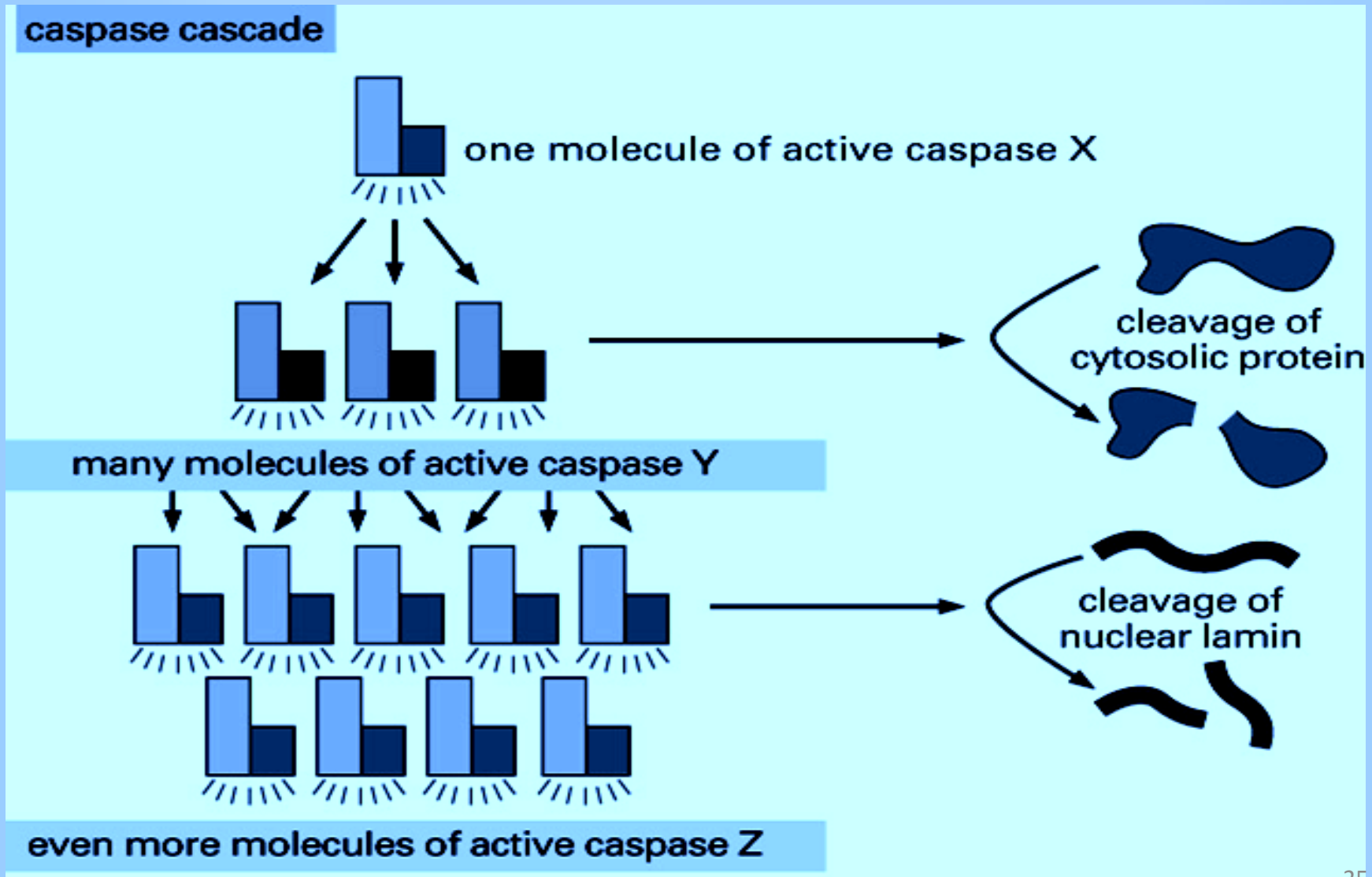
THE CASPASE FAMILY

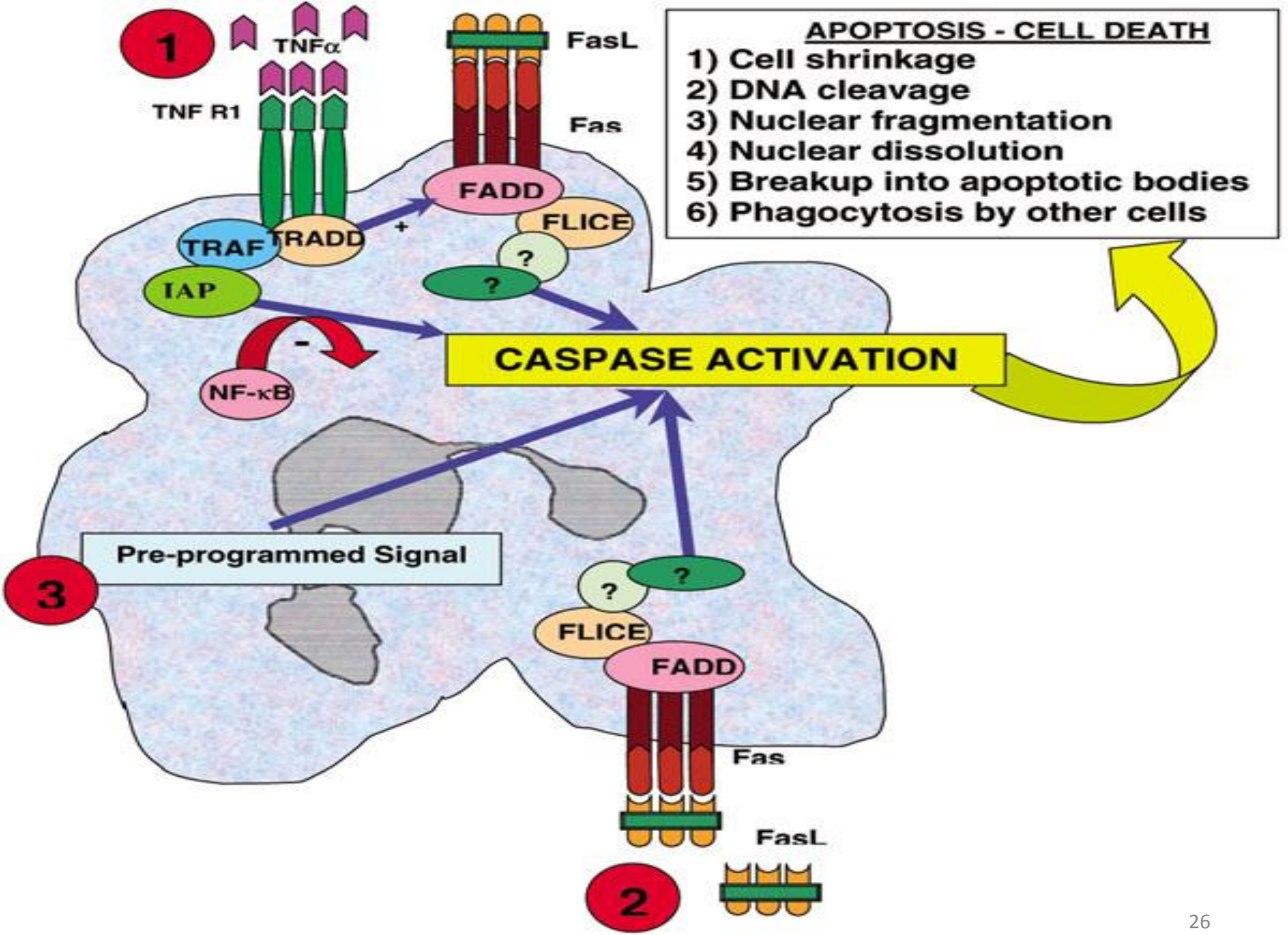


PROCASPASE ACTIVATION



PROCASPASE CASCADE

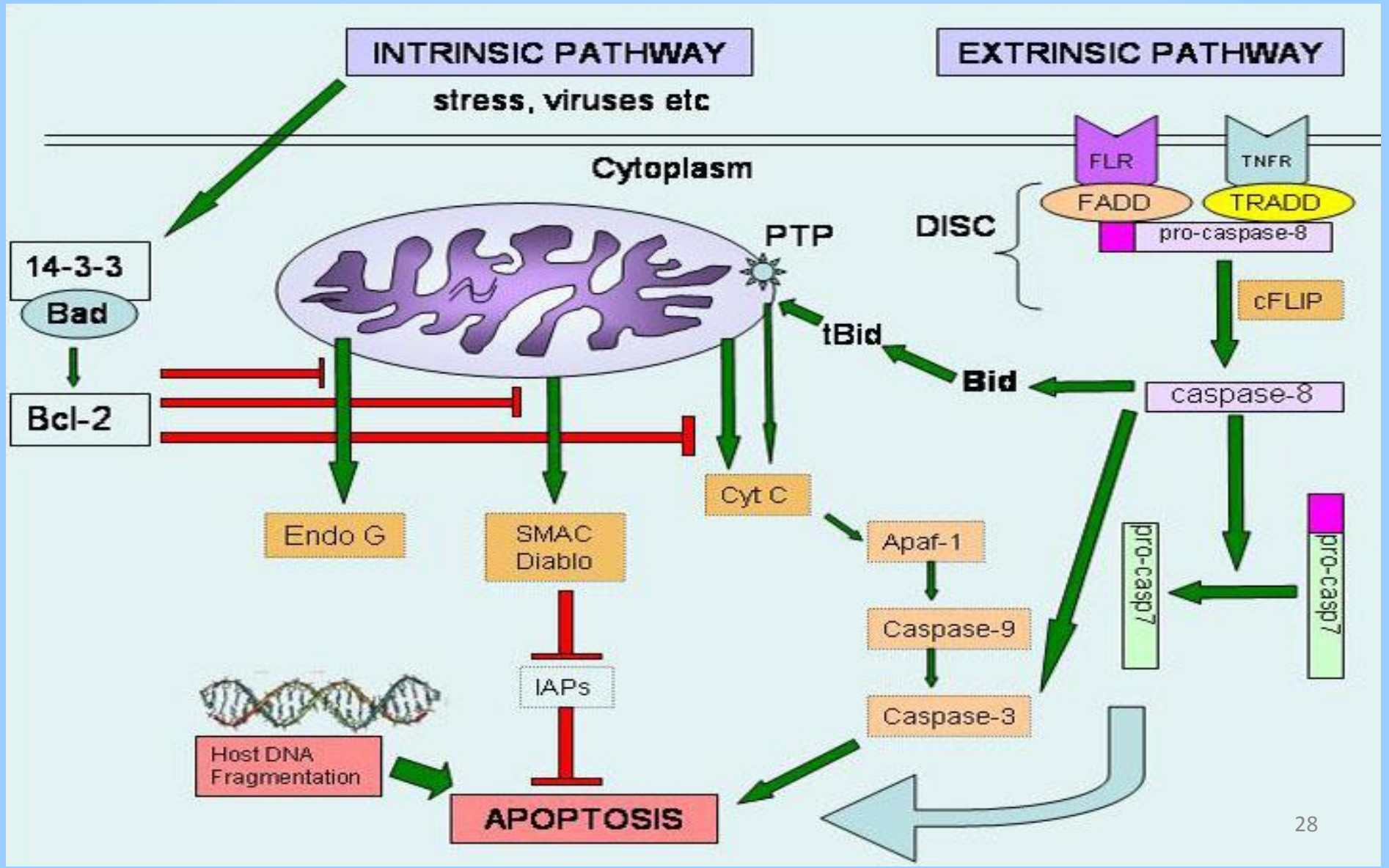



APOPTOSIS - CELL DEATH
1) Cell shrinkage
2) DNA cleavage
3) Nuclear fragmentation
4) Nuclear dissolution
5) Breakup into apoptotic bodies
6) Phagocytosis by other cells
1
TNFα
TNF R1
FasL
Fas
FADD
FLICE
?
?
TRAF
TRADD
+
IAP
-
NF-κB
CASPASE ACTIVATION
Pre-programmed Signal
3
?
?
FLICE
FADD
Fas
FasL
2

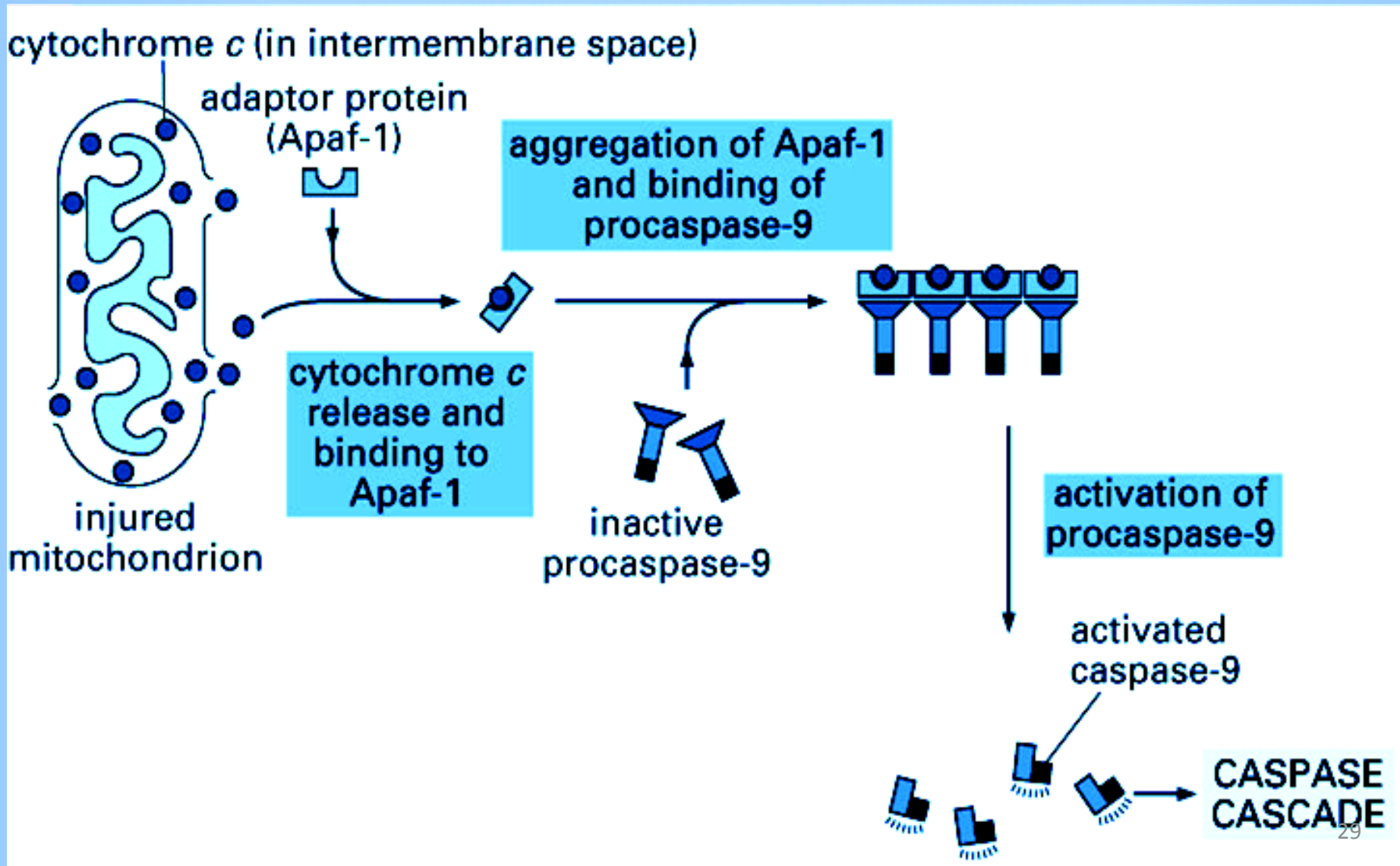
PROTEOLYTIC TARGETS OF CASPASES

- Cytoskeletal regulatory proteins
  - Actin
- Nuclear Lamins
- Poly(ADP-ribose) polymerase (PARP)
  - PARP activity depletes ATP, thus cleavage of PARP may maintain store of ATP to drive apoptosis
- DNA-fragmentation factor (DFF)

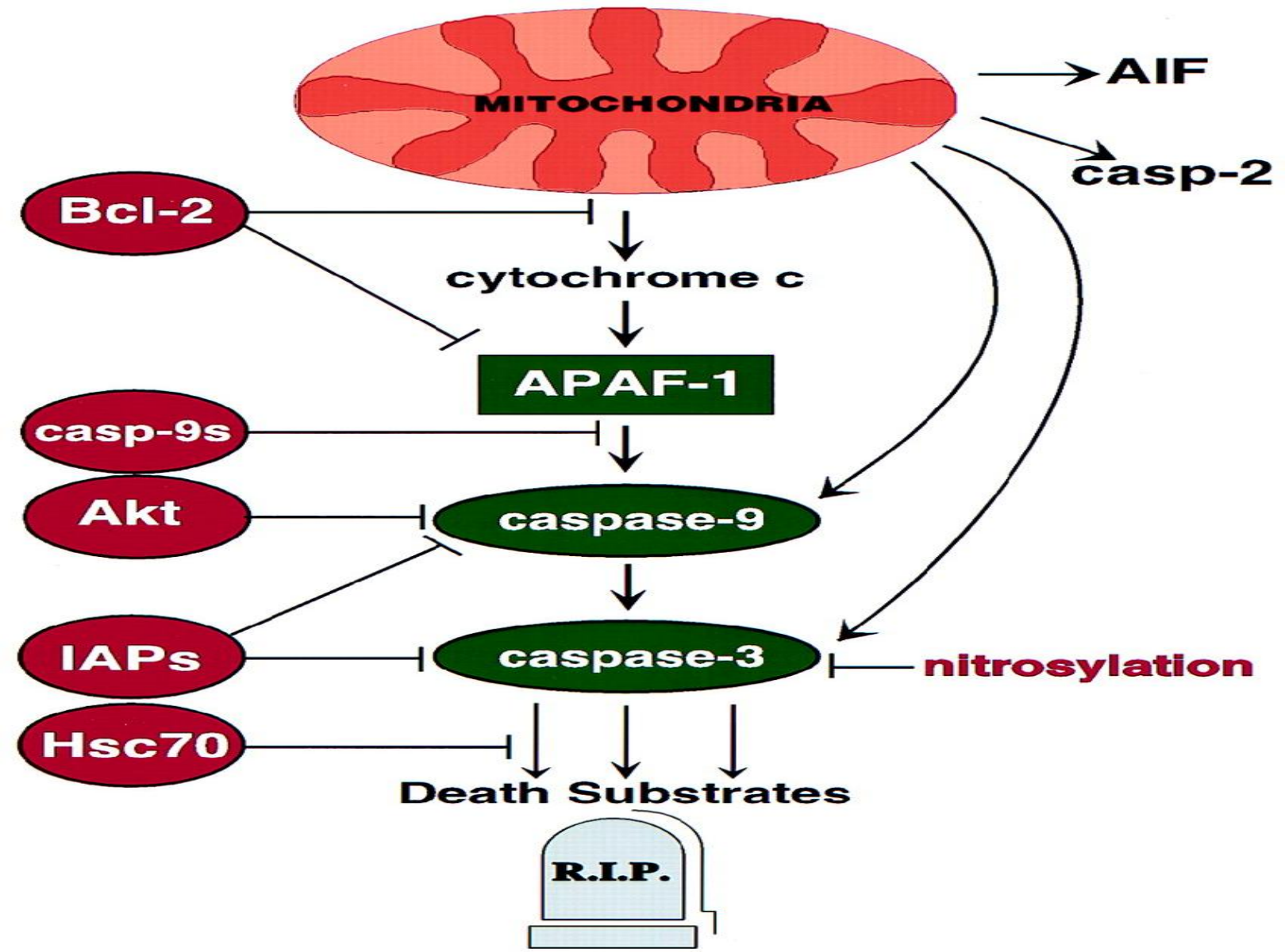
TWO PATHWAYS OF APOPTOSIS



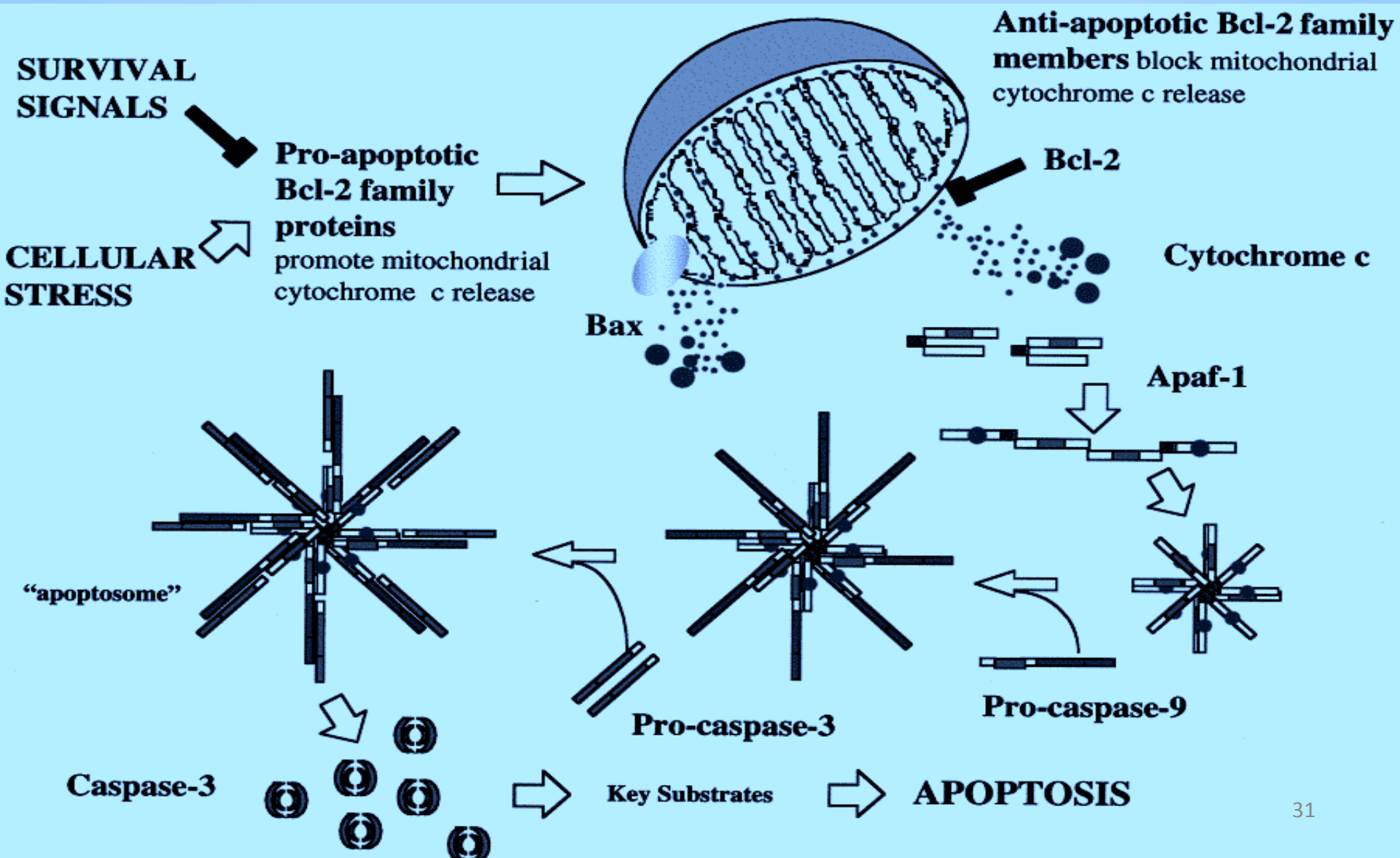
INTRINSIC PATHWAYS



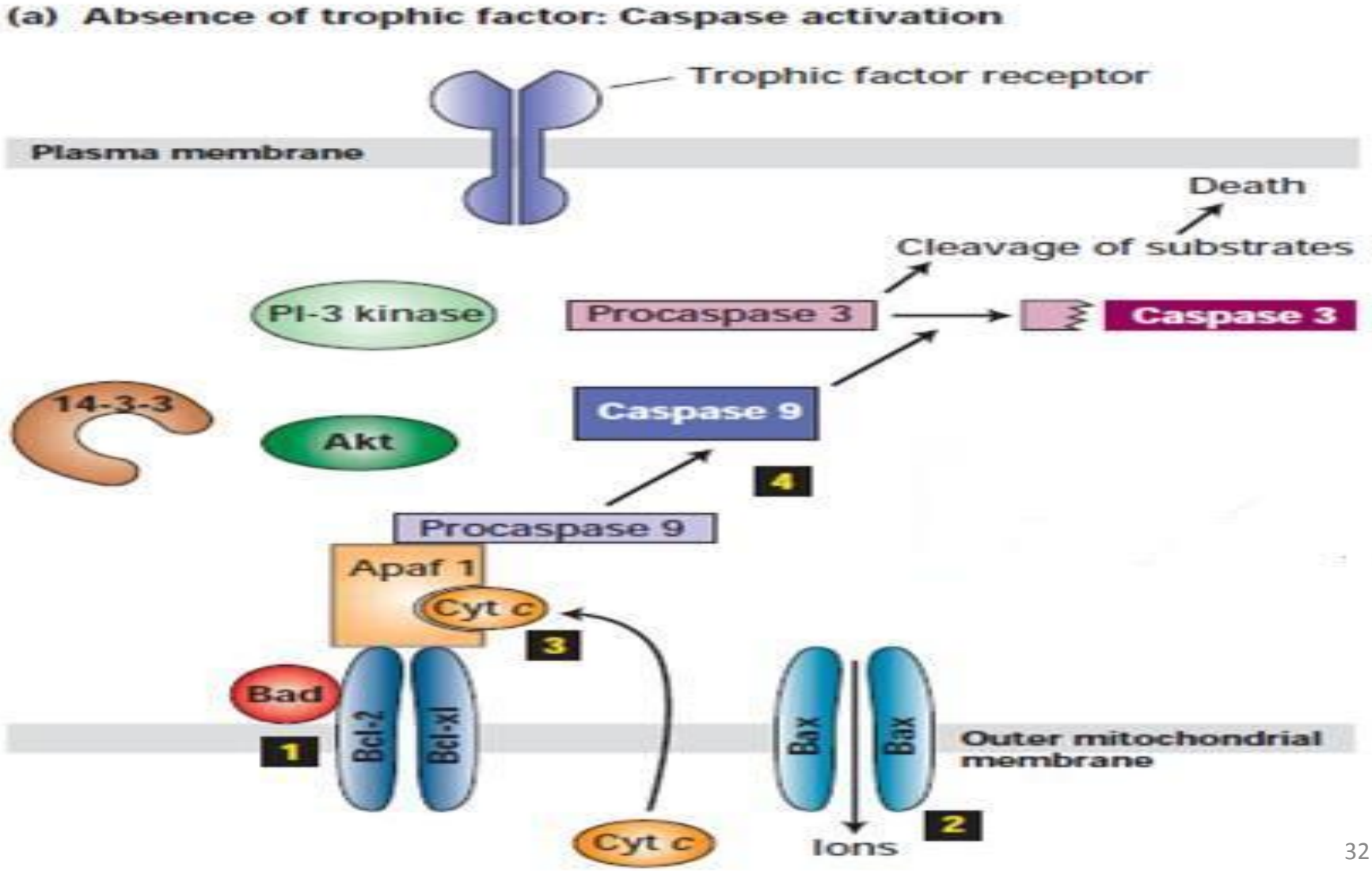
MITOCHONDRIAL AND POSTMITOCHONDRIAL REGULATION OF APOPTOSIS



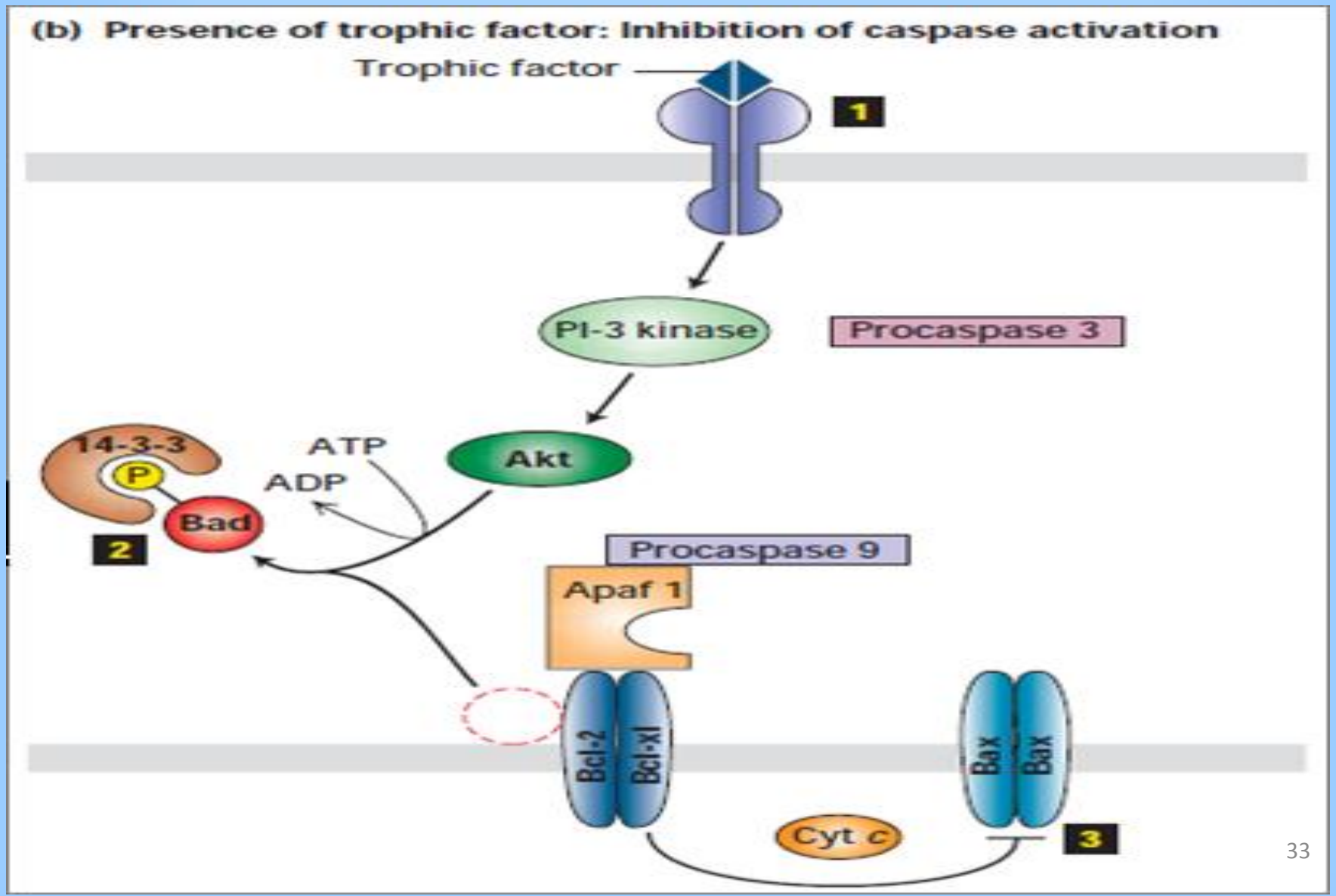
Apaf-1 INDUCED PATHWAYS



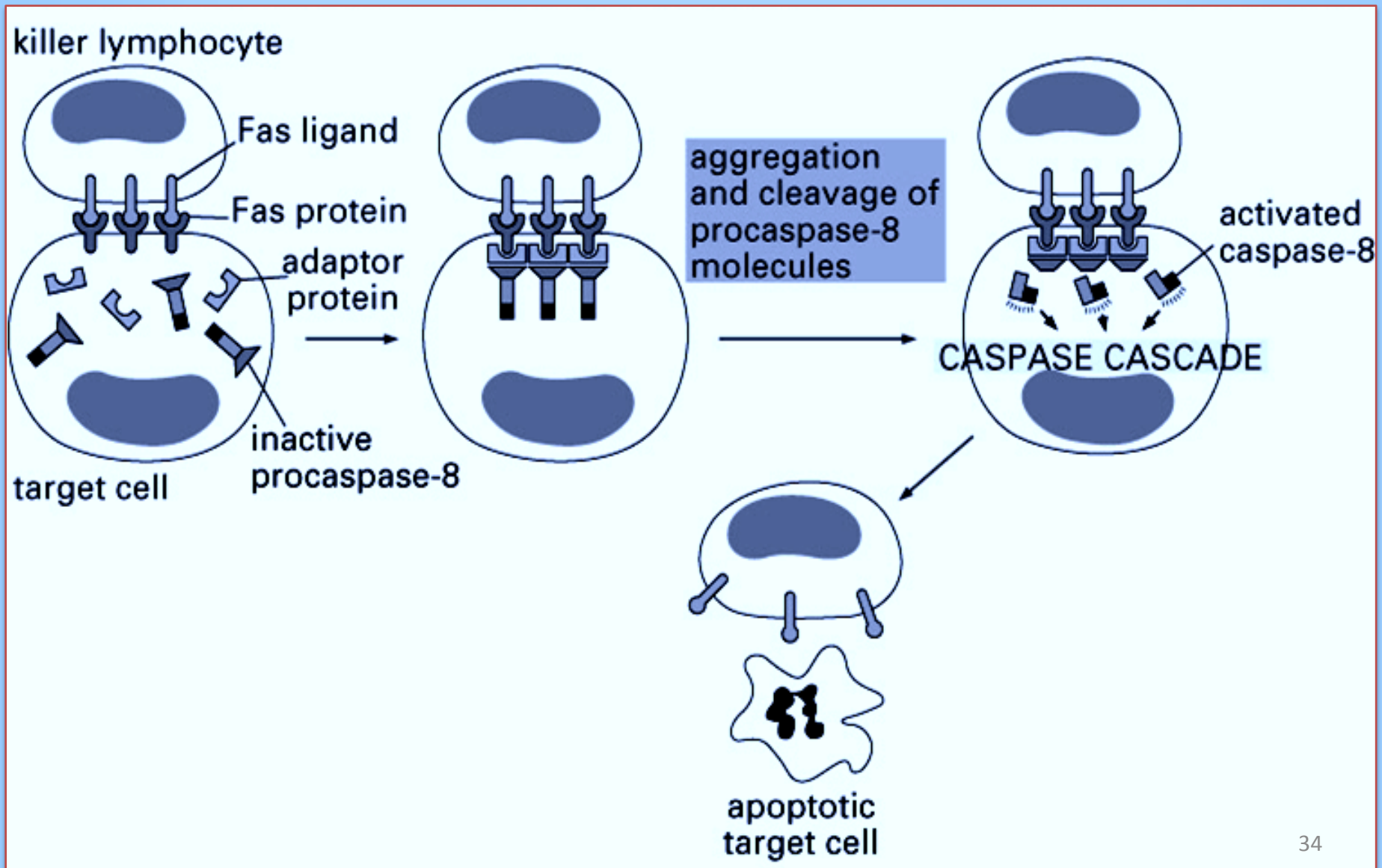
TROPHIC FACTOR and APOPTOSIS



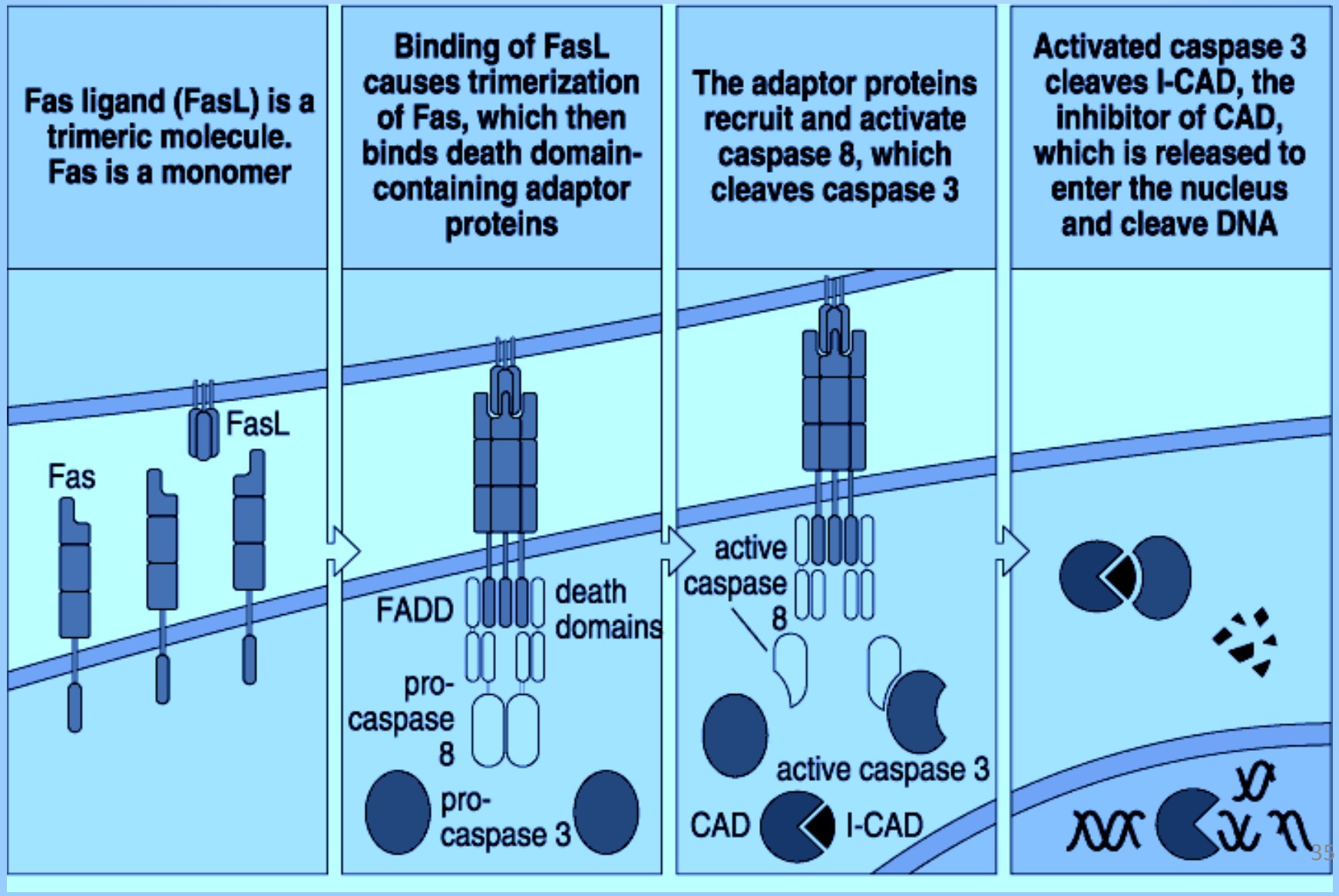
TROPHIC FACTOR and APOPTOSIS



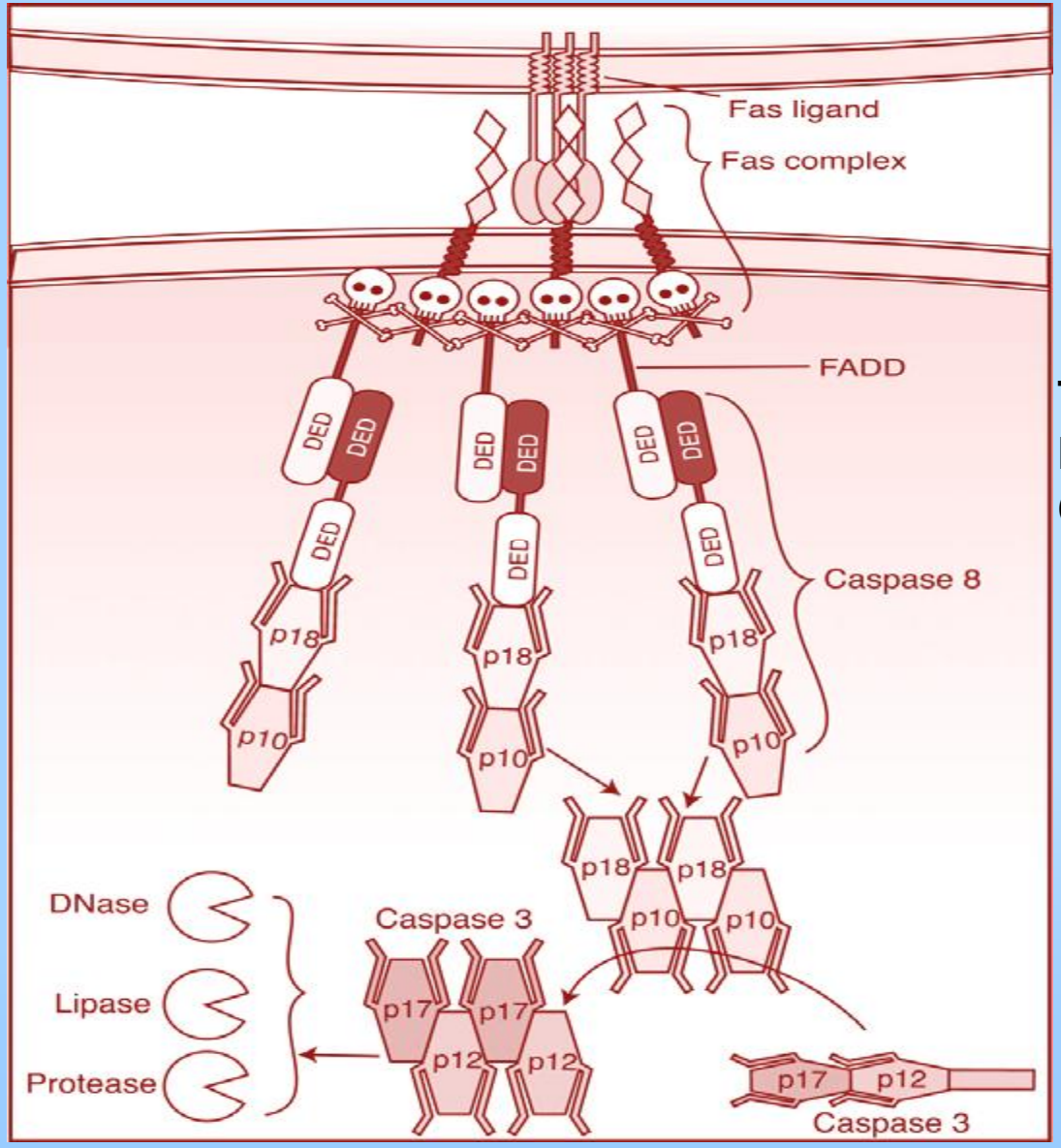
EXTRINSIC PATHWAY



Fas LIGAND AND APOPTOSIS

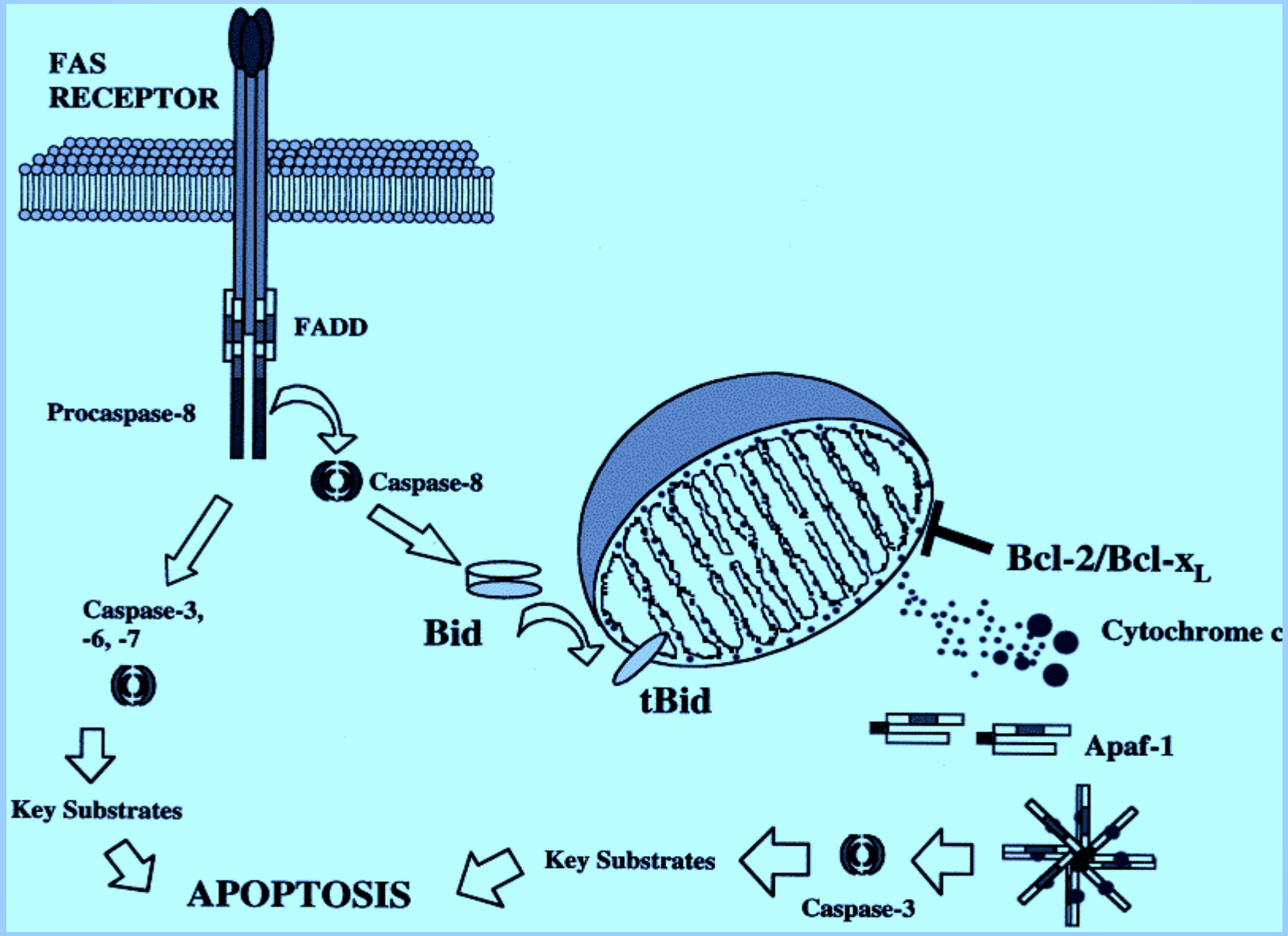


Fas INDUCED APOPTOSIS

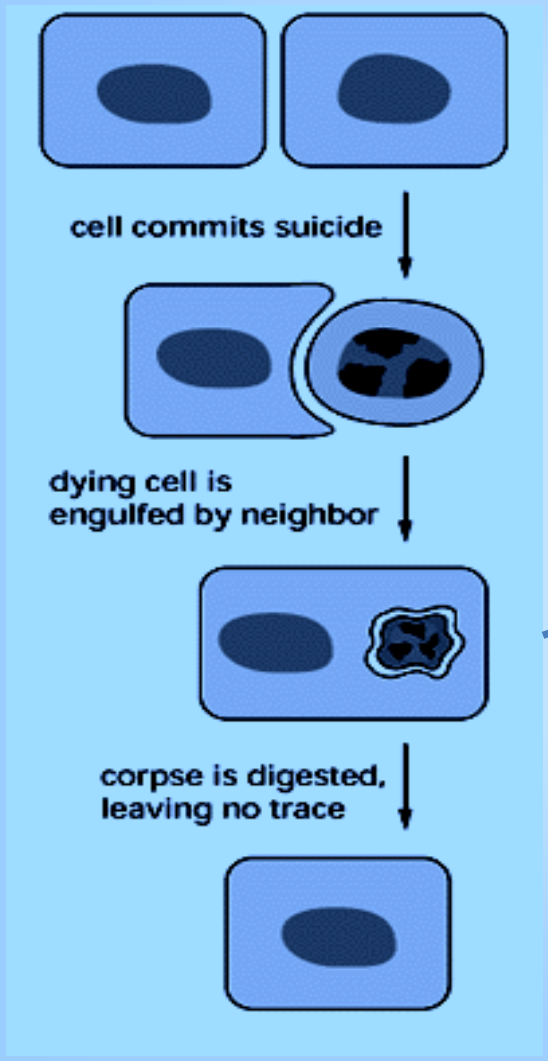


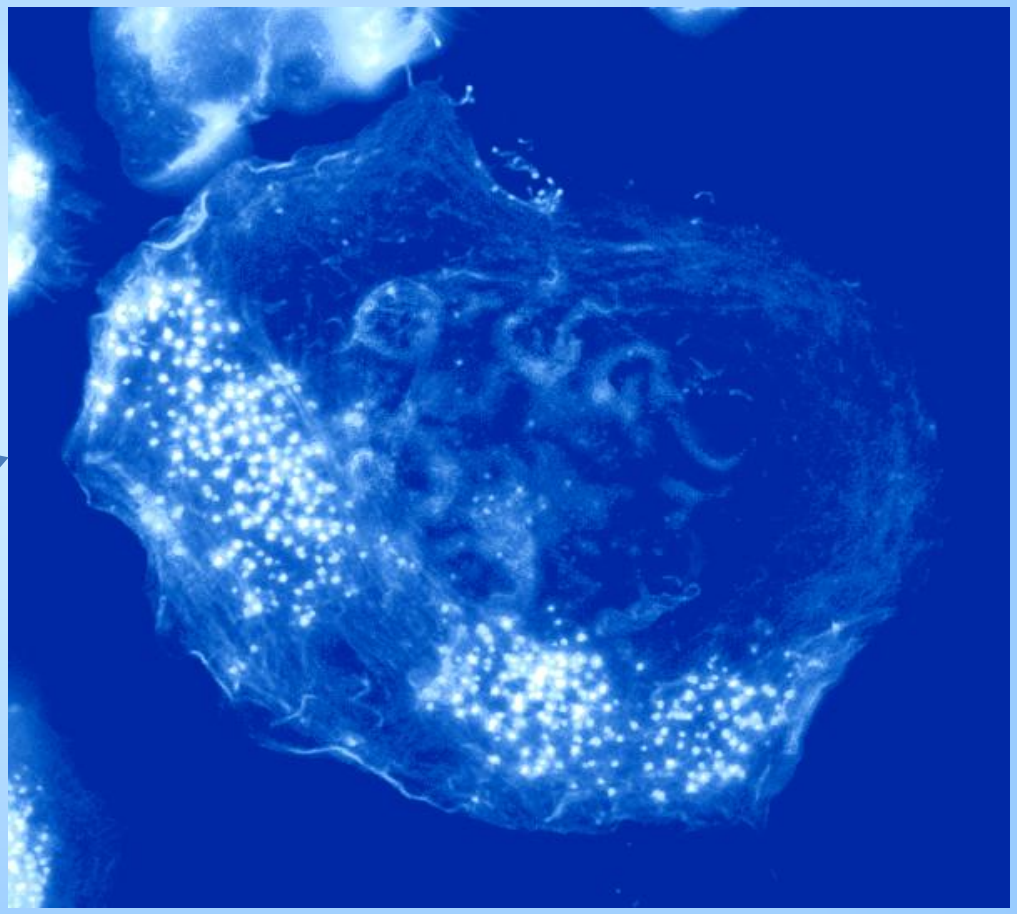
The Formation of the Death Initiating Signal Complex (DISC)

Fas INDUCED APOPTOSIS

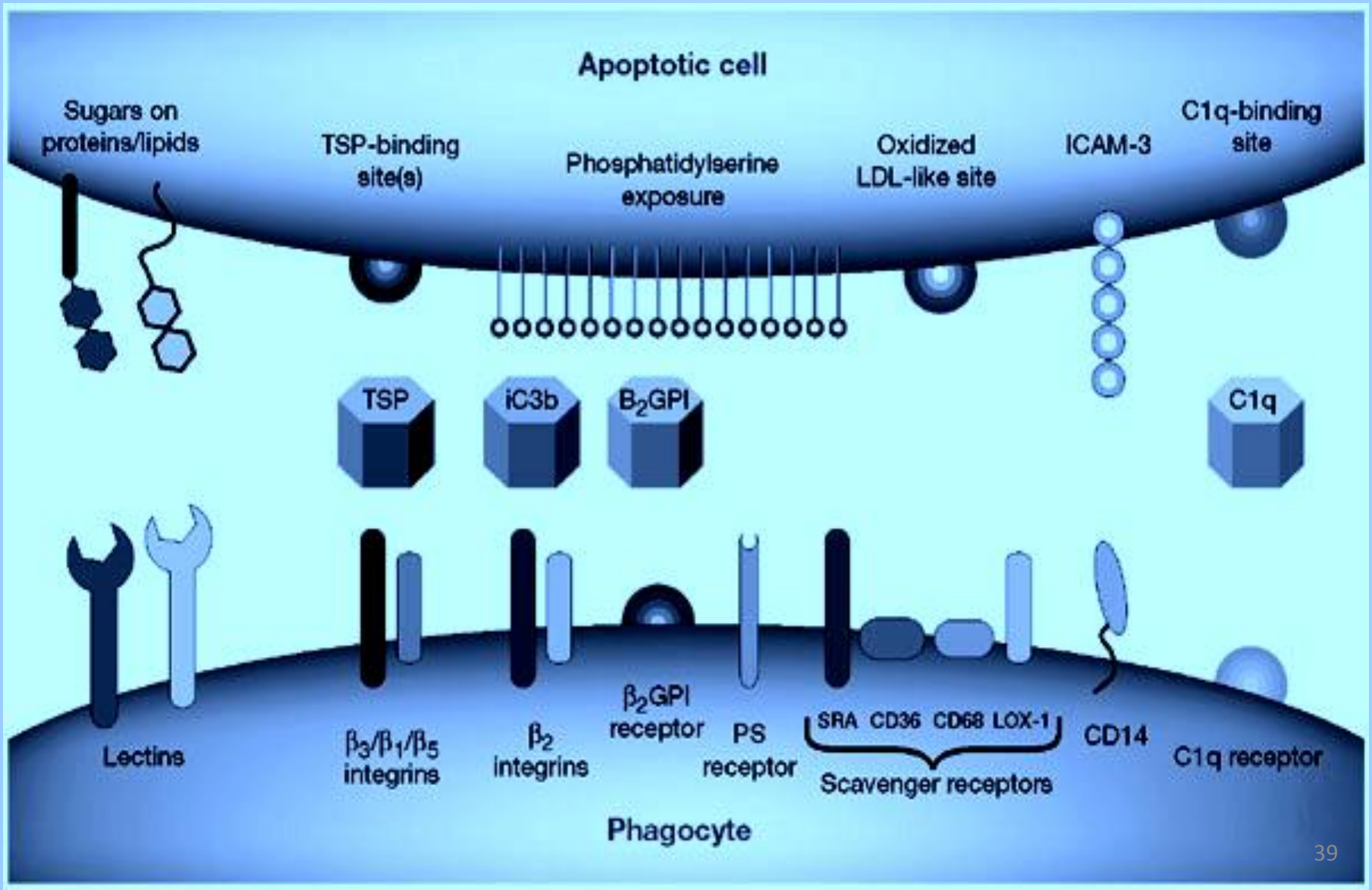


APOPTOSIS AND PHAGOCYTOSIS

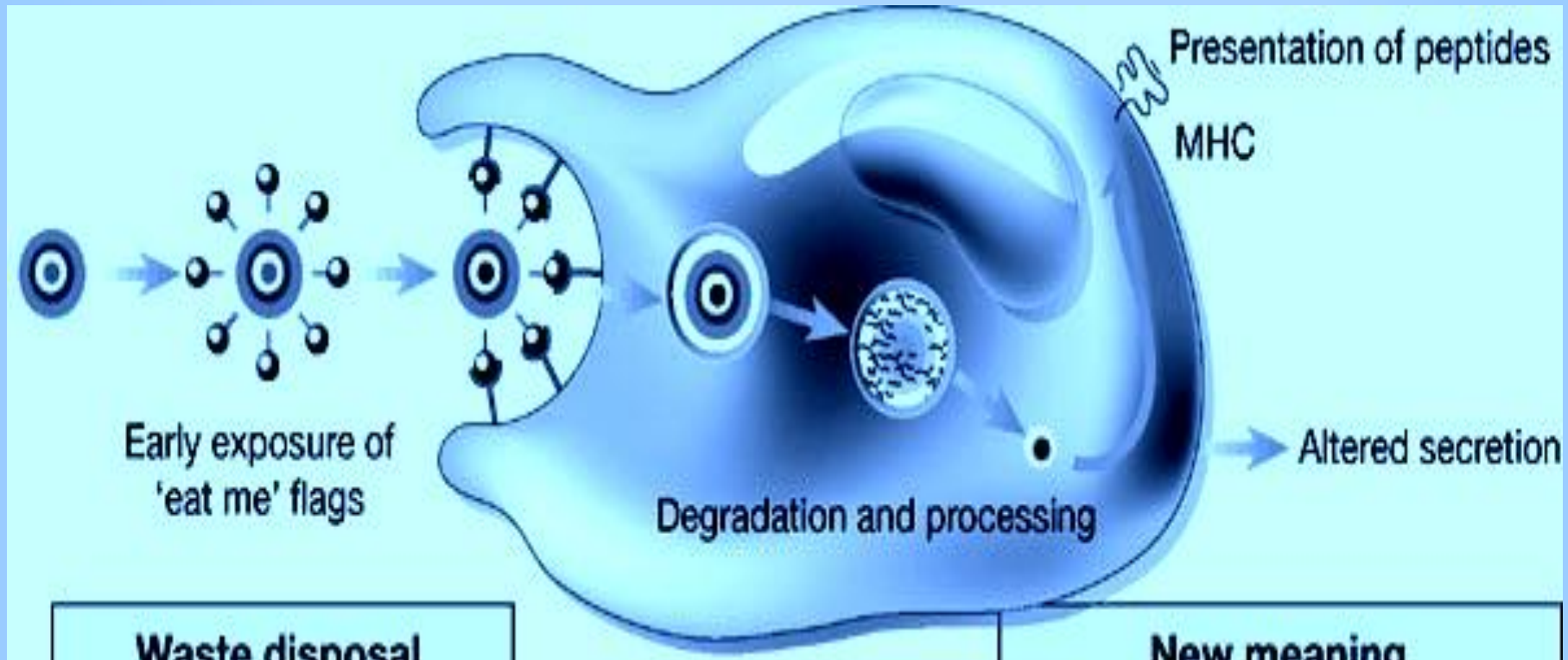




PHAGOCYTOTIC TAGS AND RECEPTORS



REMOVAL OF CELL CORPSES



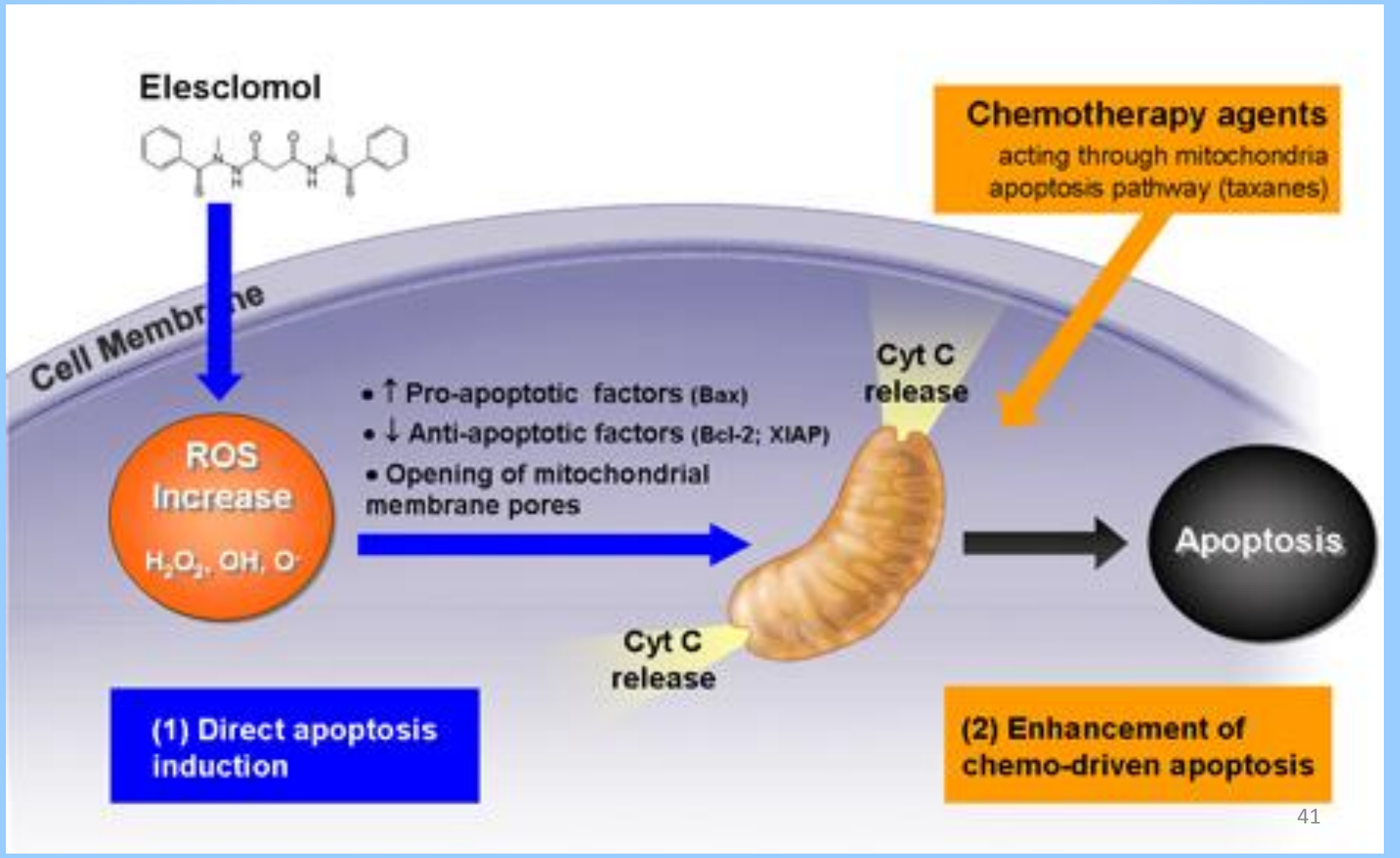
Waste disposal

- Removal of cell corpses
- Prevention of leakage of contents from dying cells

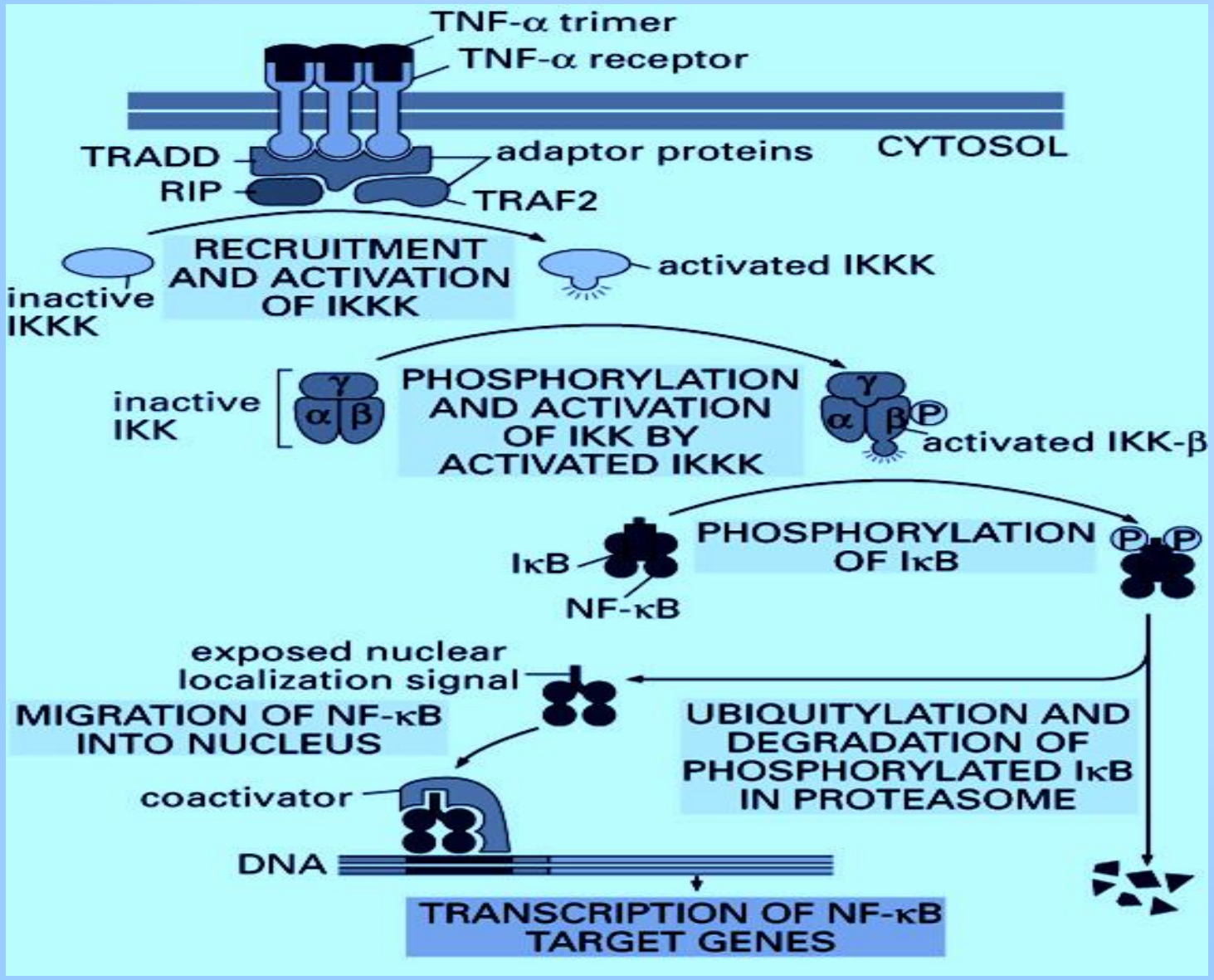
New meaning

- Suppression of inflammation (TGF-β1↑, PGE_2↑, TNF-α↓)
- Modulation of cell killing (NO↓, CD95L↑)

ROS AND APOPTOSIS



TNF-α AND APOPTOSIS



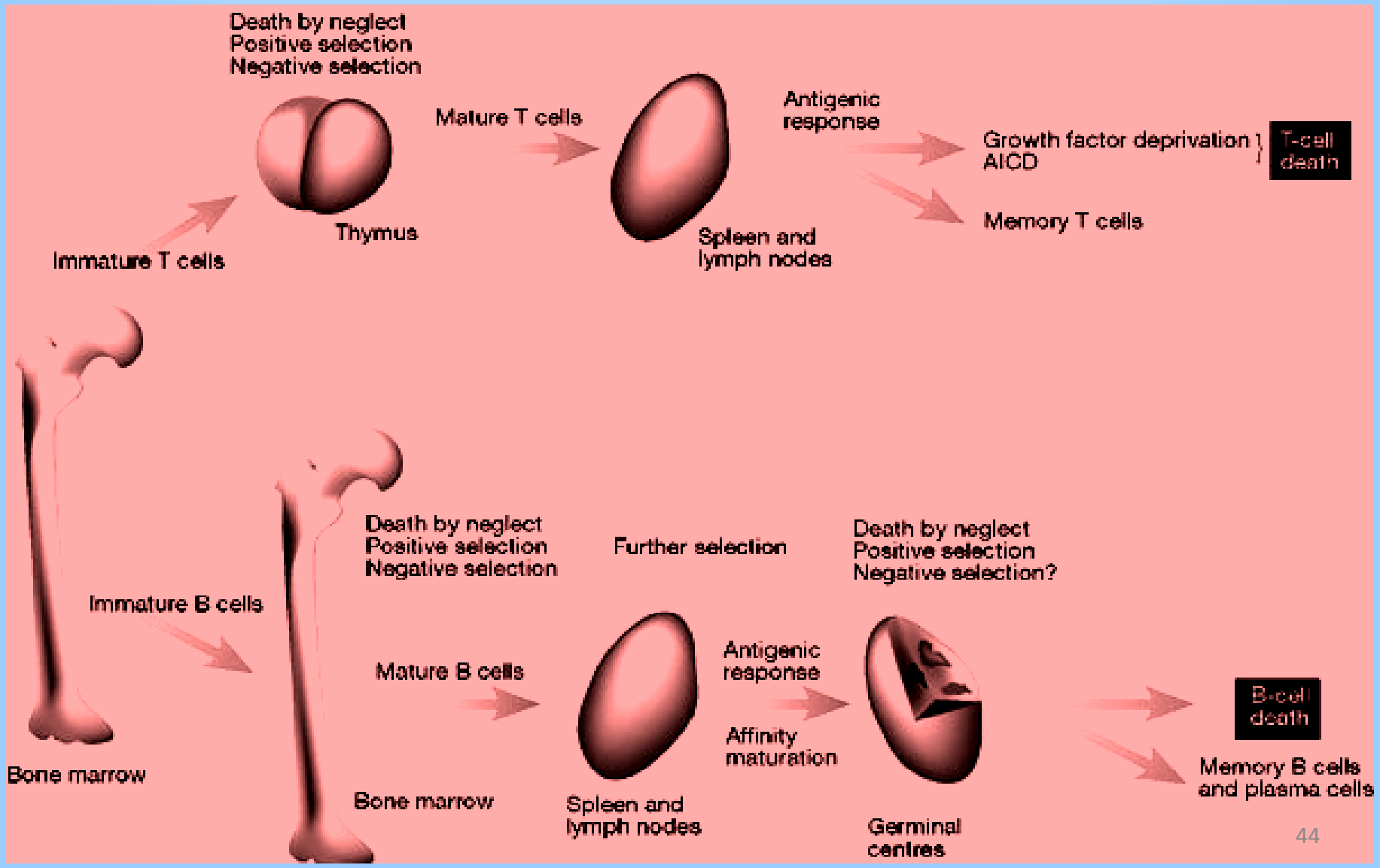
IKKK=IkB Kinase kinase

NFkB activates transcription of several anti-apoptotic proteins including IAPs and Bcl-2.

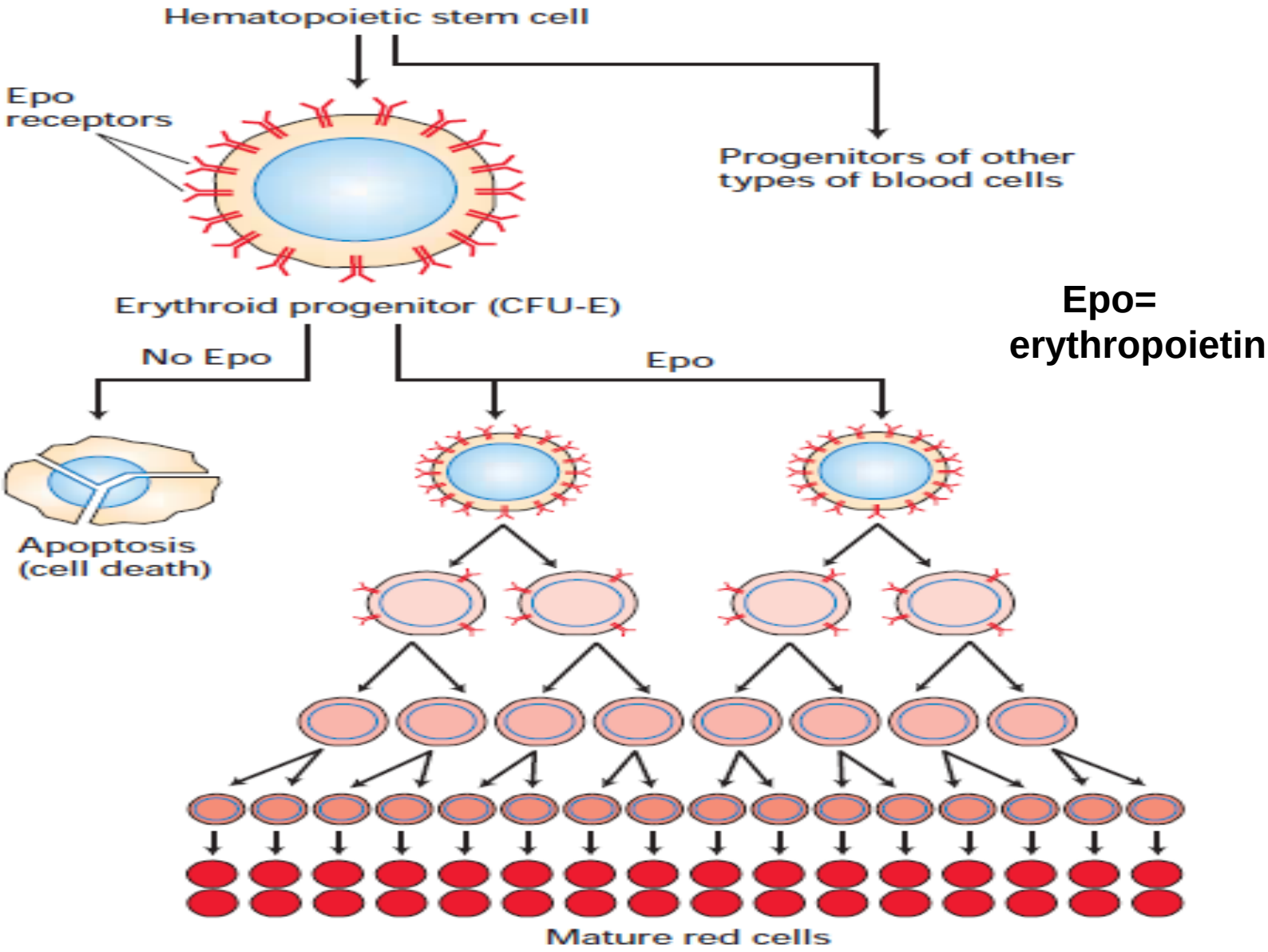
IAPs: INHIBITORS OF APOPTOSIS

- **Bind procaspases to prevent activation**
  - initial description of bacculovirus product (p35)
  - contain metal-binding (~80aa) BIR repeats
  - number and sequence of BIR's determine specificity of procaspase inhibition
  - many contain RING fingers
- **Survivin**
  - expressed in many fetal tissues
  - expressed in many colon cancers
- **IAP modulators**
  - inhibitors of IAPs
  - Smac/Diablo

APOPTOSIS IN IMMUNE FUNCTION

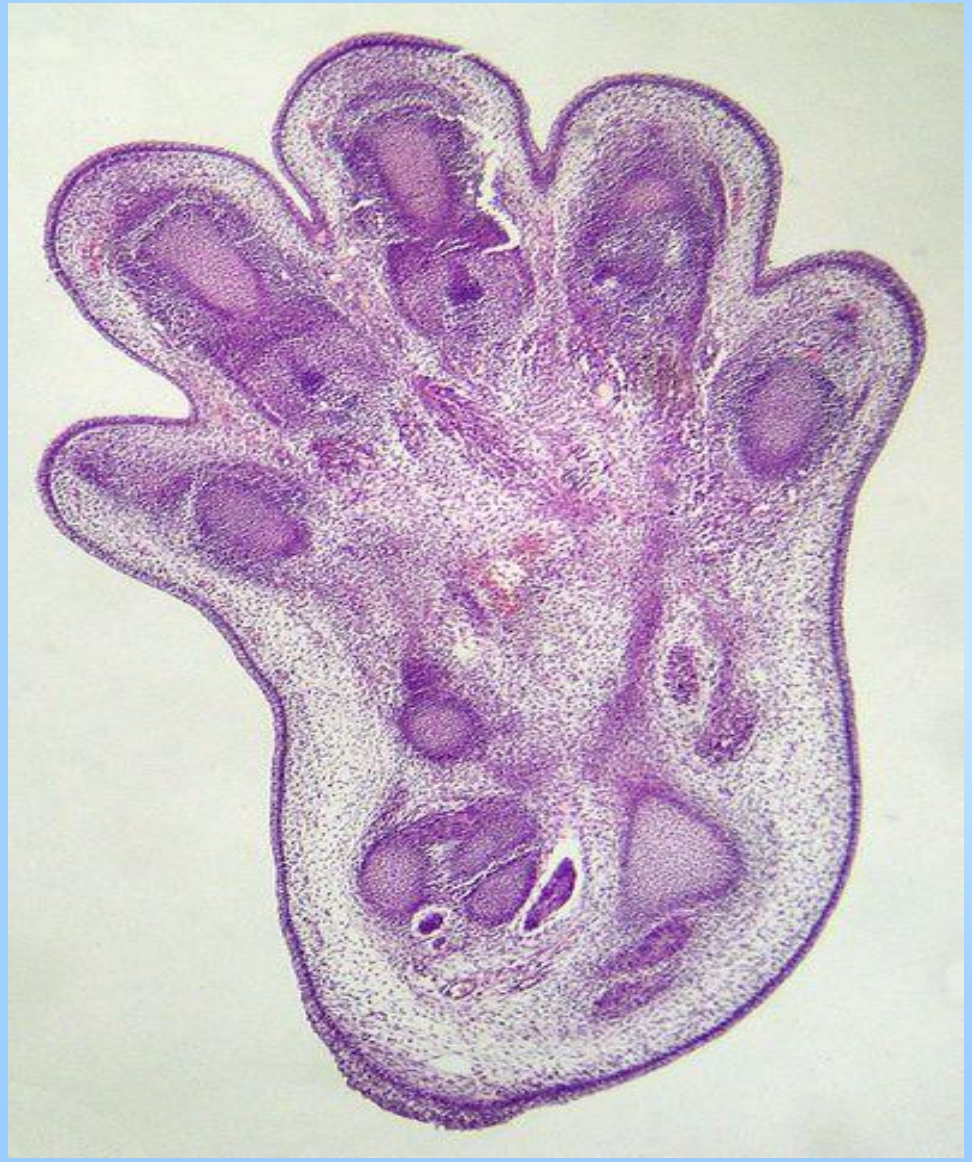


APOPTOSIS IN RBC DEVELOPMENT



Epo= erythropoietin

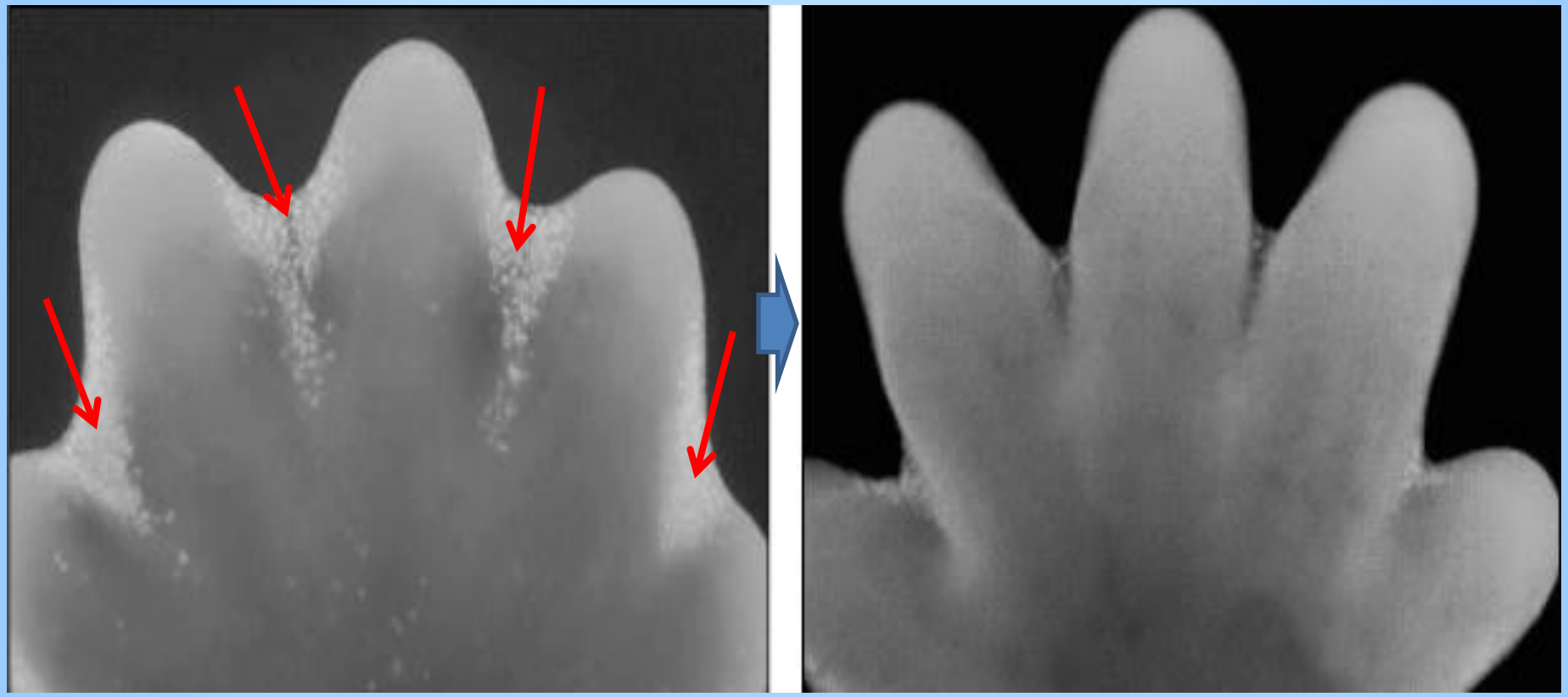
APOPTOSIS IN ANIMAL LIFE



Histologic cross section of embryonic foot of mouse (*Mus musculus*) in 15.5 day of its development

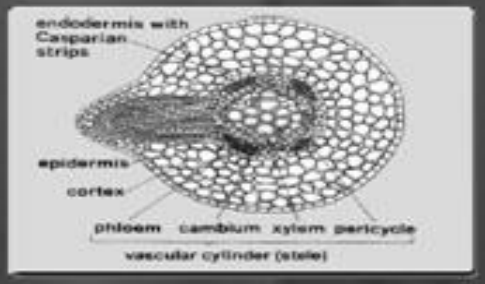
APOPTOSIS IN ANIMAL LIFE

Cell Death and the mouse's paw



Indicated portions show apoptotic cells.

APOPTOSIS IN PLANT LIFE



Xylogenesis

The parenchymatous cells differentialtes into *phloem* and xylem cells, where apoptosis plays a vital role in *preferential* elimination of cells

Reproduction

In maize, sex determination involves the selective killing of the female reproductive primordia in order that the male floral structures (the stamens) can develop in the tassel.

Senescence

An important process for defence mechanism, where *apoptosis* plays a vital role

Also involved in fruit ripening [ehylene biosynthesis]

APOPTOSIS IN PLANT LIFE

Pathogenesis

Plants can recognize certain pathogens and activate defence (called the resistance response) that result in the limitation of pathogen growth at the site of infection.

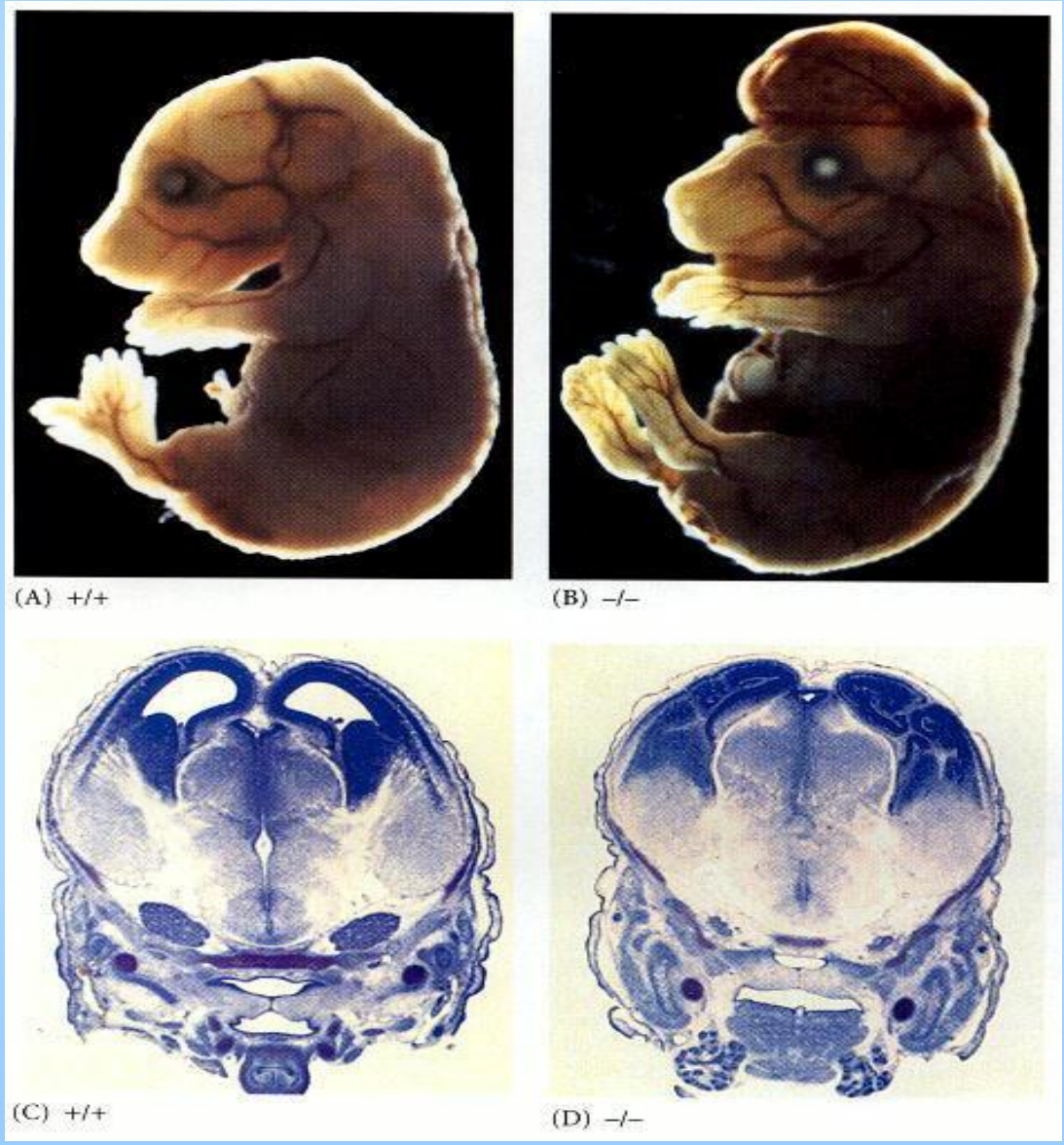
A Ubiquitous Process in Plants?

In addition to the examples given above, cell death also occurs predictably at specific sites and times throughout the life history of flowering plants.

- Embryo development
- Root cap growth and disintegration
- Determining leaf shape
- Tapetum layer degeneration during pollen development
- Meagsporogenesis
- Selective death of certain organs during development
- etc...!

FAILURE OF APOPTOSIS

FAILURE OF APOPTOSIS



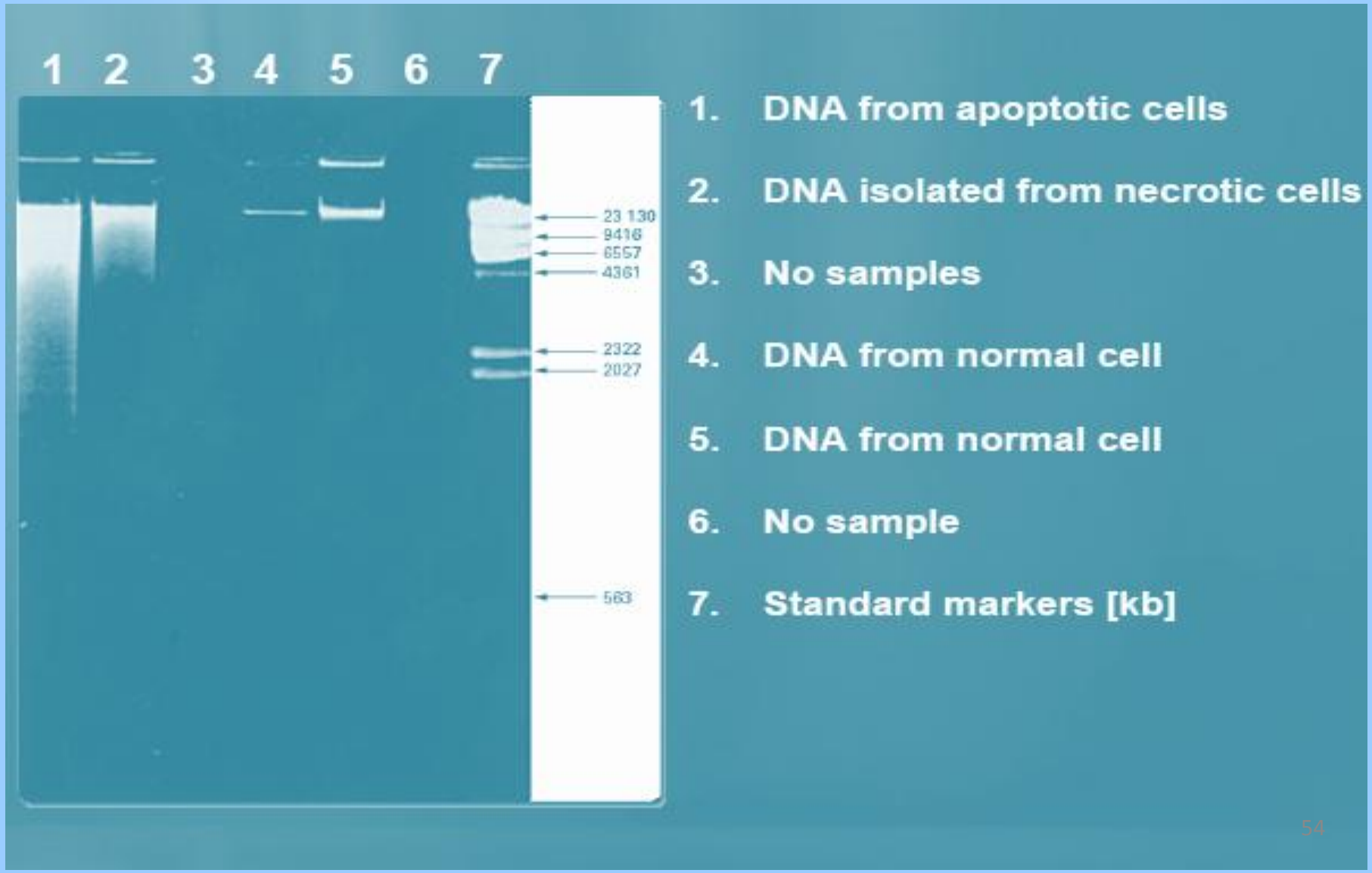
DISEASES DUE TO FAILURE OF APOPTOSIS

- Alzheimer's diseases
- Parkinson's disease
- Huntington's disease
- Amyotrophic lateral sclerosis
- Cancer
- Autoimmunity
- Acquired immune deficiency syndrome (AIDS)
- Ischemia (smoke, myocardial infarction)

DETECTION OF APOPTOSIS

- **Microscopy**
  – Cells have classic features (e.g. small darkly stained nuclei)
  – Detection of free 3’ ends of DNA by TUNEL assay (terminal deoxytransferase-mediated dUTP-biotin nick end labelling)
- **Gel electrophoresis**
  – Detect DNA ladder of 180 bp intervals caused by internucleosomal DNA cleavage
- **Flow cytometry**
  – Measure externalization of phosphatidylserine (PS) with fluorescently labeled Annexin-V
  – Measure DNA fragmentation with propidium iodide fluorescence

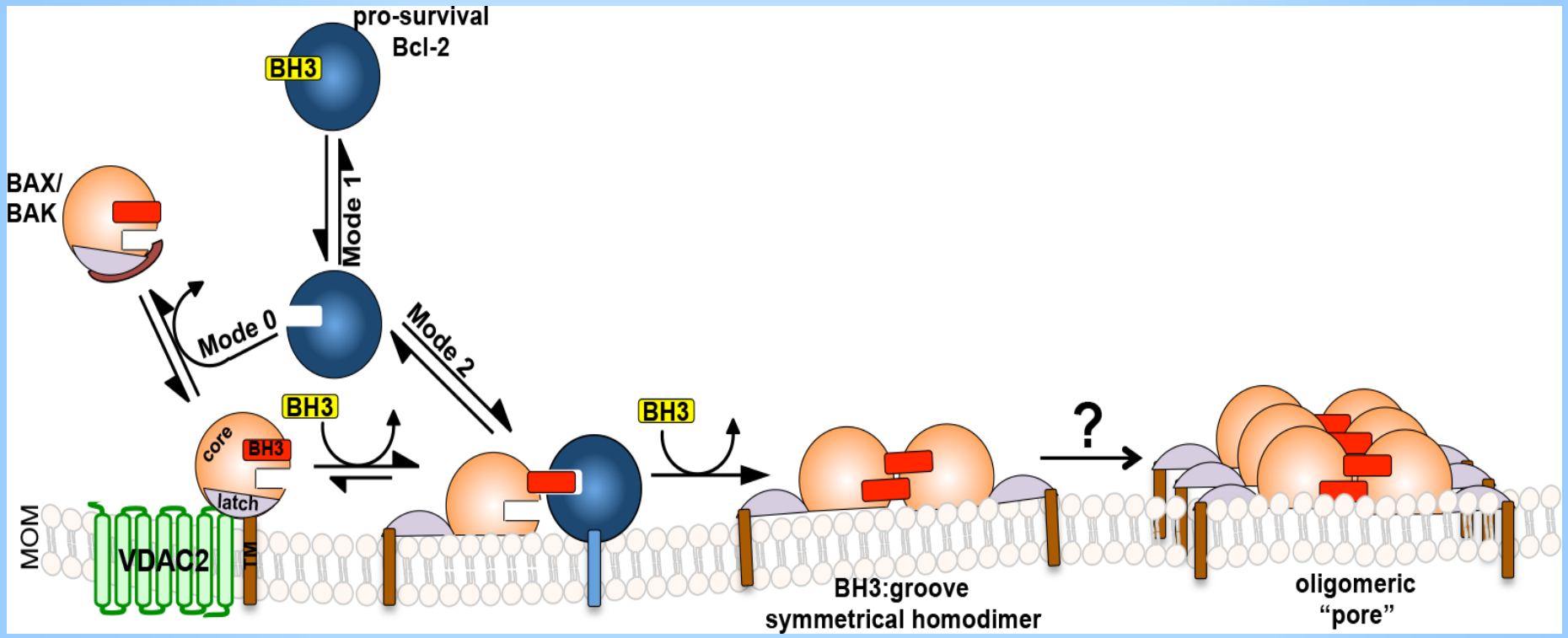
MOLECULAR DETECTION OF APOPTOSIS

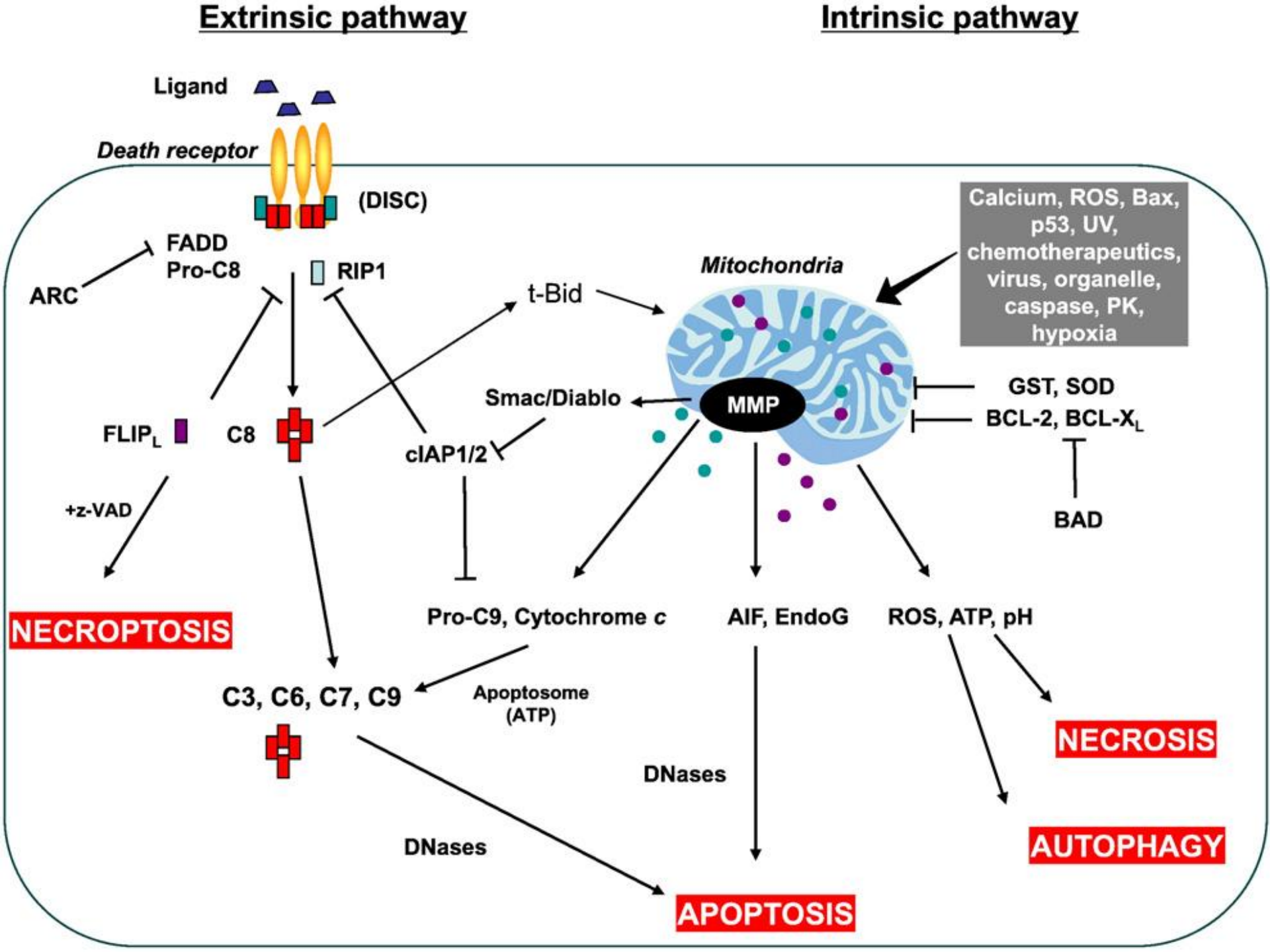


“BY THE TIME I WAS BORN, MORE OF ME HAD DIED THAN SURVIVED. IT WAS NO WONDER, I CAN NOT REMEMBER; DURING THAT TIME I WENT THROUGH BRAIN AFTER BRAIN FOR NINE MONTHES……. FINALLY CONTRIVING THE ONE MODEL THAT COULD BE HUMAN EQUIPPED FOR LANGUAGE.”

LEWIS THOMAS, 1992

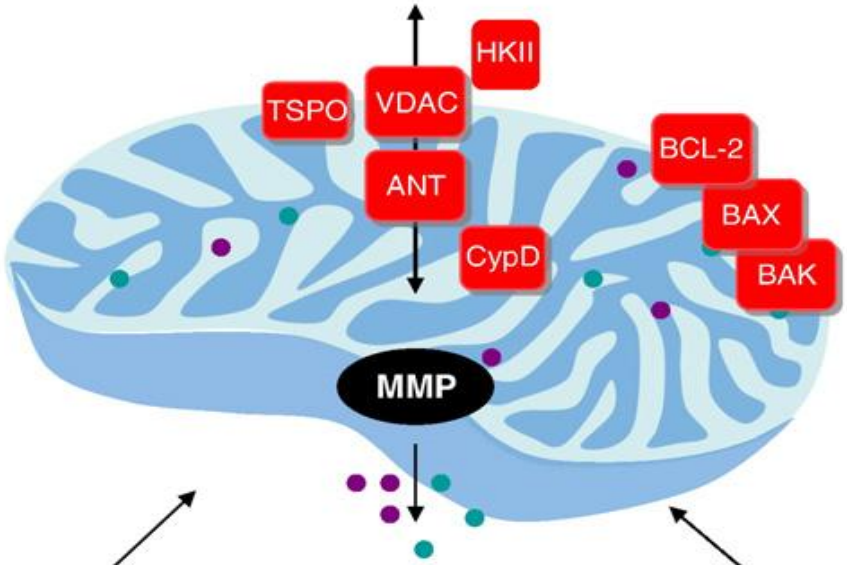
Role of Bax and BAK in mitochondrial activity during apoptosis




Extrinsic pathway
Intrinsic pathway
Ligand
Death receptor
(DISC)
FADD
Pro-C8
RIP1
ARC
t-Bid
Mitochondria
Calcium, ROS, Bax, p53, UV, chemotherapeutics, virus, organelle, caspase, PK, hypoxia
Smac/Diablo
MMP
GST, SOD
BCL-2, BCL-XL
FLIPL
C8
cIAP1/2
BAD
+z-VAD
NECROPTOSIS
Pro-C9, Cytochrome c
AIF, EndoG
ROS, ATP, pH
C3, C6, C7, C9
Apoptosome (ATP)
NECROSIS
DNases
DNases
AUTOPHAGY
APOPTOSIS

Direct targeting

ER	Nox	OXPHOS	Cancer	Virus	Small molecules
Calcium, ROS	ROS	ROS	BAX, BH3	Vpr, Vmia, PB1-F2	ABT-737, LND, MT-21, Ars, jasmonate, lamellarin, Verteporfin, PPIX

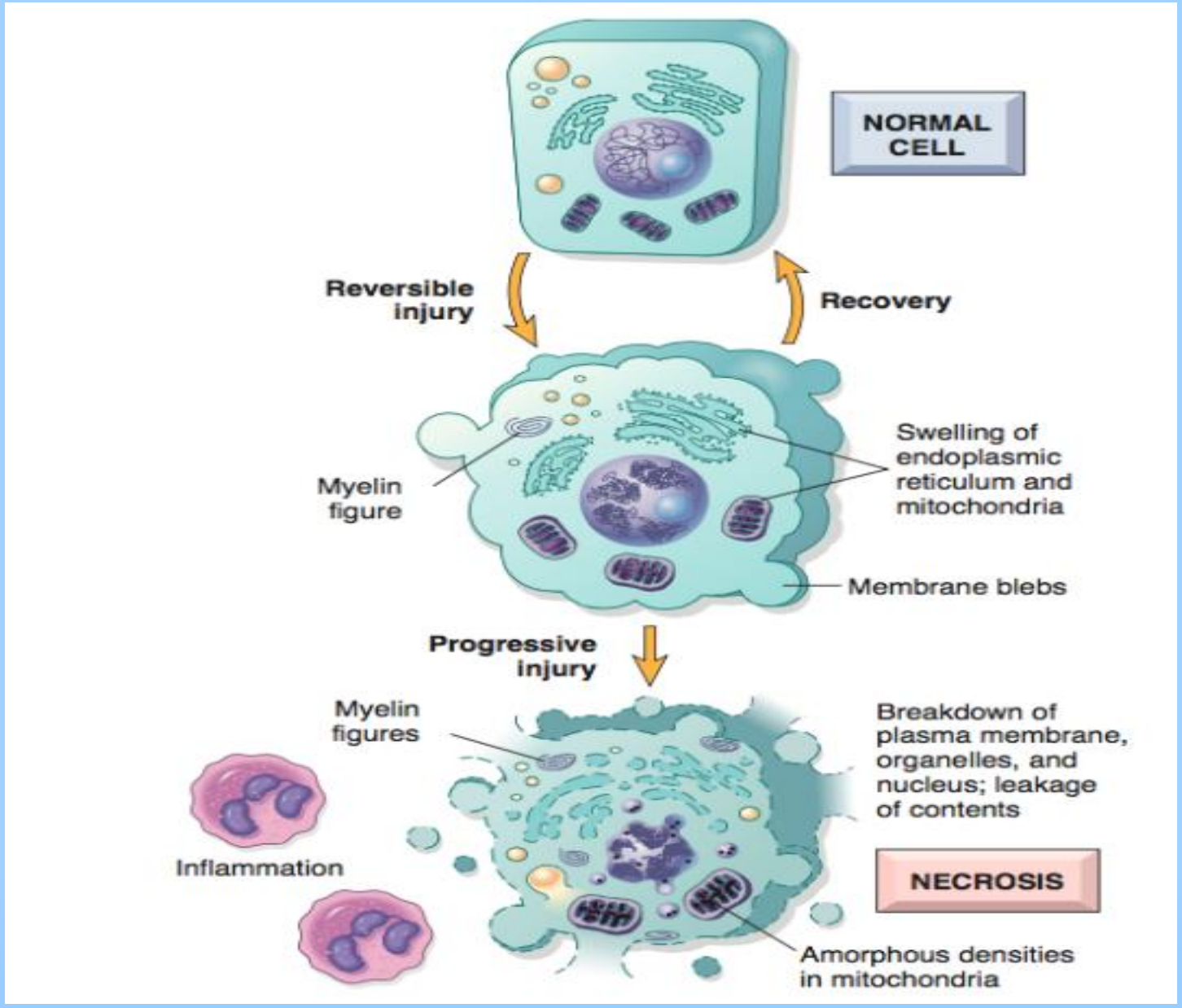


Gene expression modulation

Metabolism reprogramming

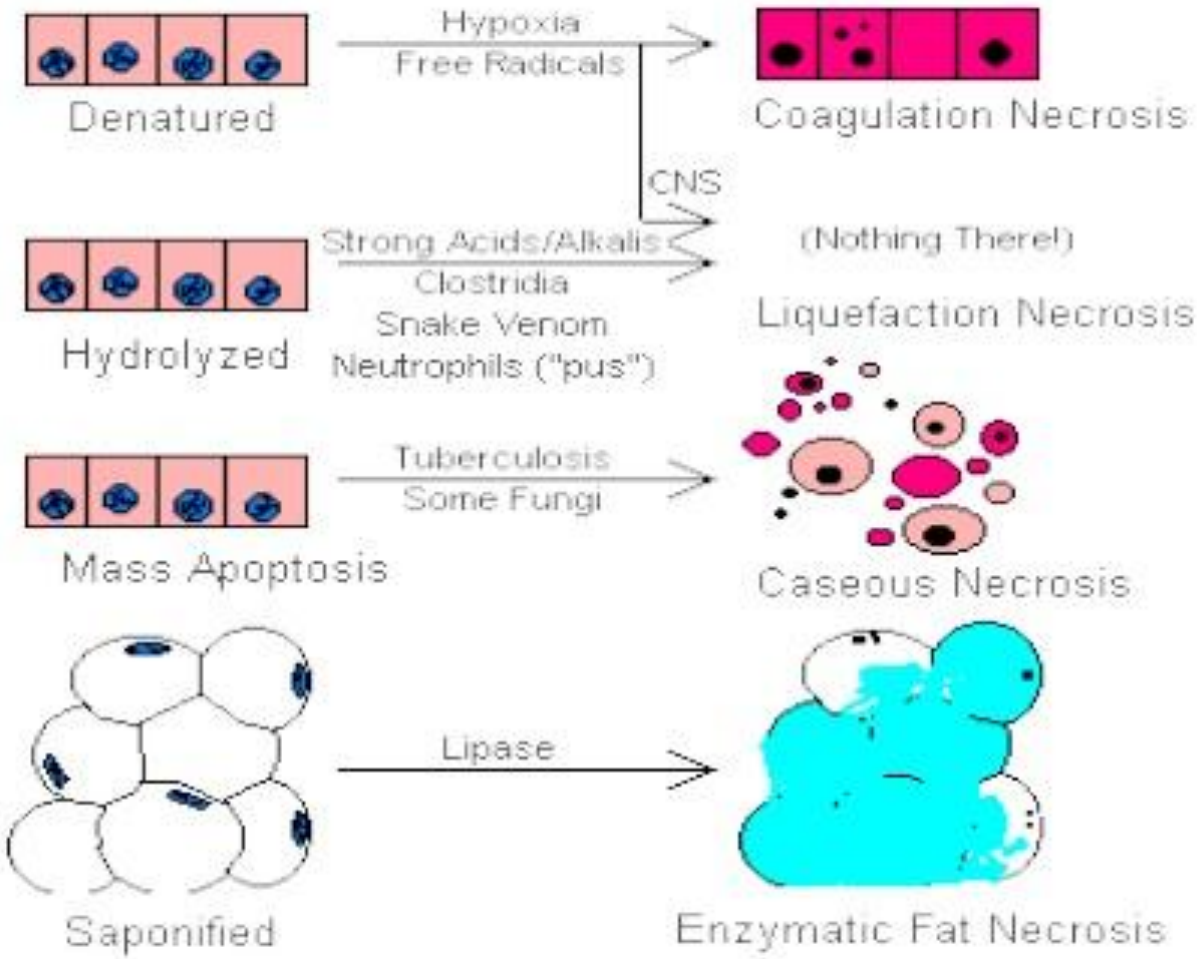
Indirect targeting

NECROSIS


NORMAL CELL
Reversible injury
Recovery
Myelin figure
Swelling of endoplasmic reticulum and mitochondria
Membrane blebs
Progressive injury
Myelin figures
Breakdown of plasma membrane, organelles, and nucleus; leakage of contents
Inflammation
NECROSIS
Amorphous densities in mitochondria

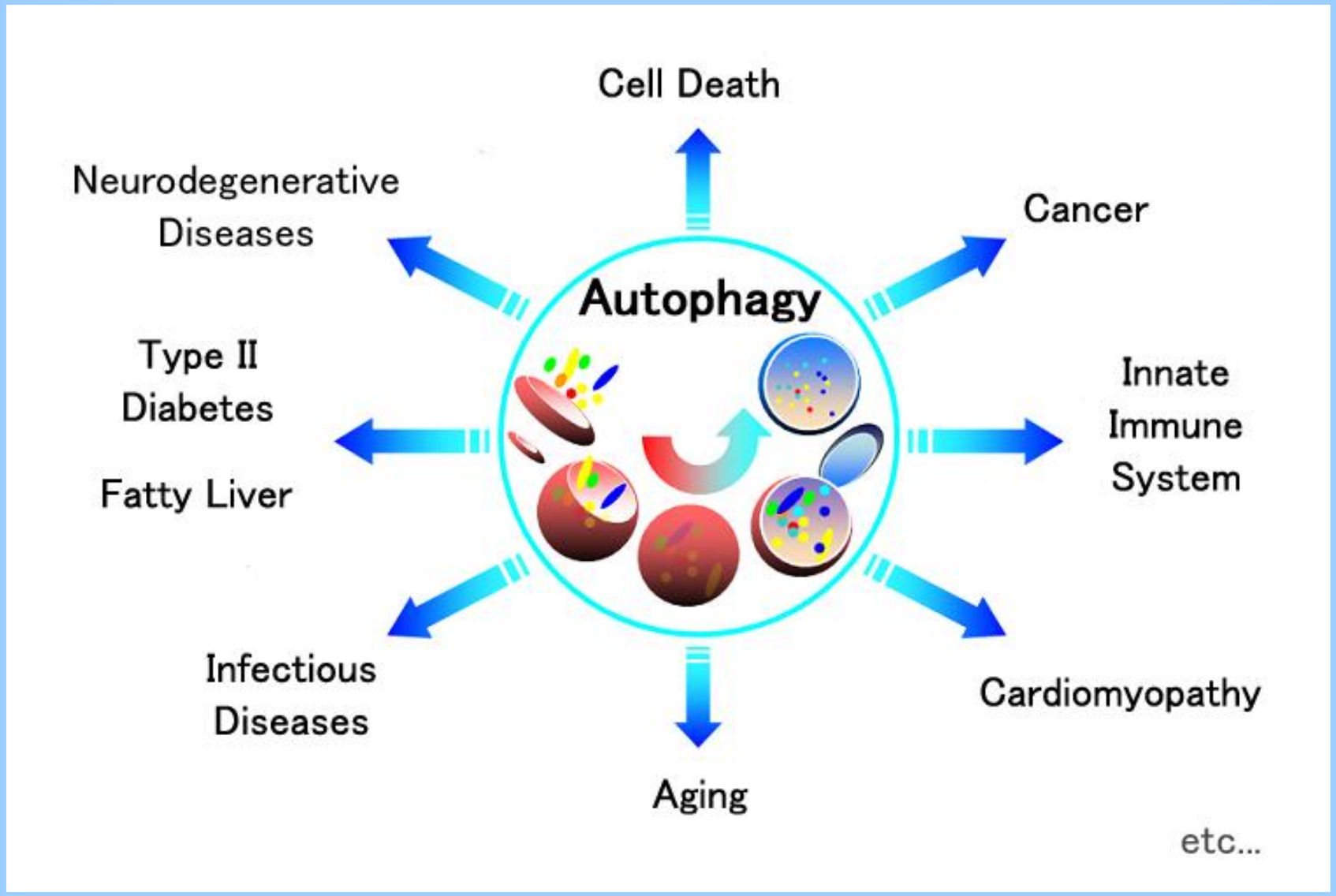
TYPES OF NECROSIS

The cytoplasm tell you HOW cells have died.

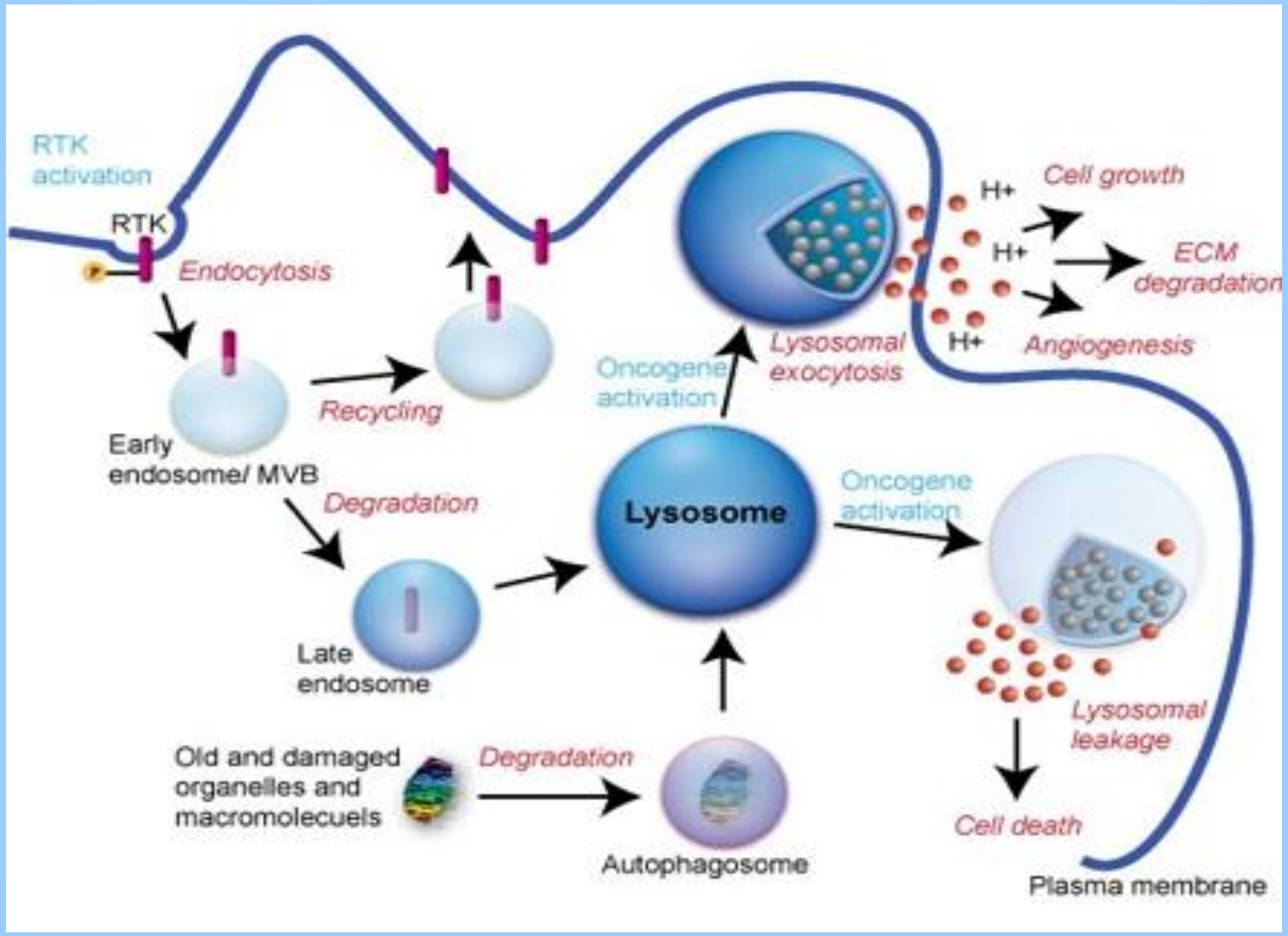


Severe cases of Necrosis

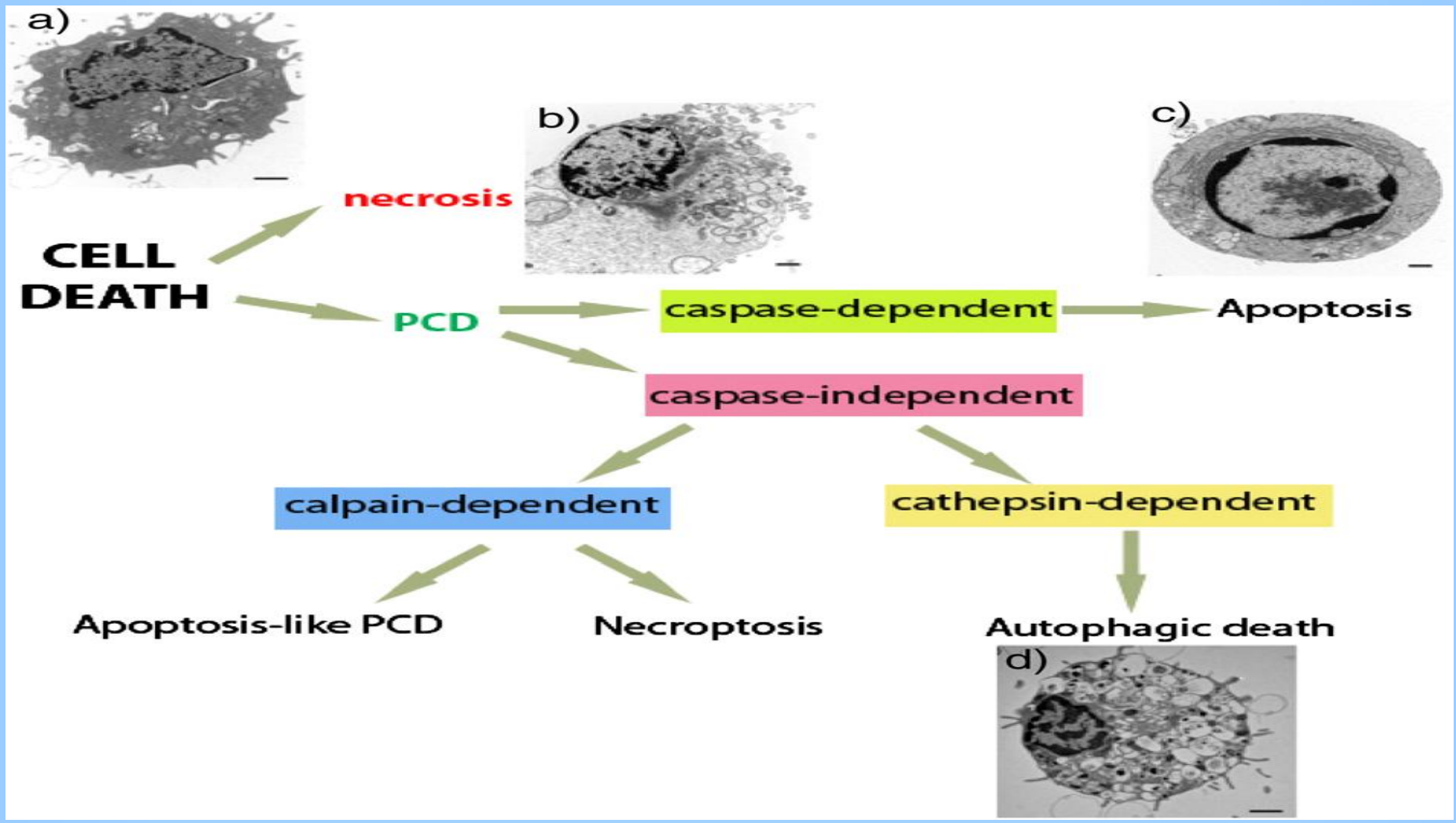
AUTOPHAGY
Pathological and physiological functions



Events in Autophagy



CROSSTALK


a)
b)
c)
necrosis
CELL DEATH
PCD
caspase-dependent
Apoptosis
caspase-independent
calpain-dependent
cathepsin-dependent
Apoptosis-like PCD
Necroptosis
Autophagic death
d)

Thank you
for your attention