# Apoptosis: Programmed cell death

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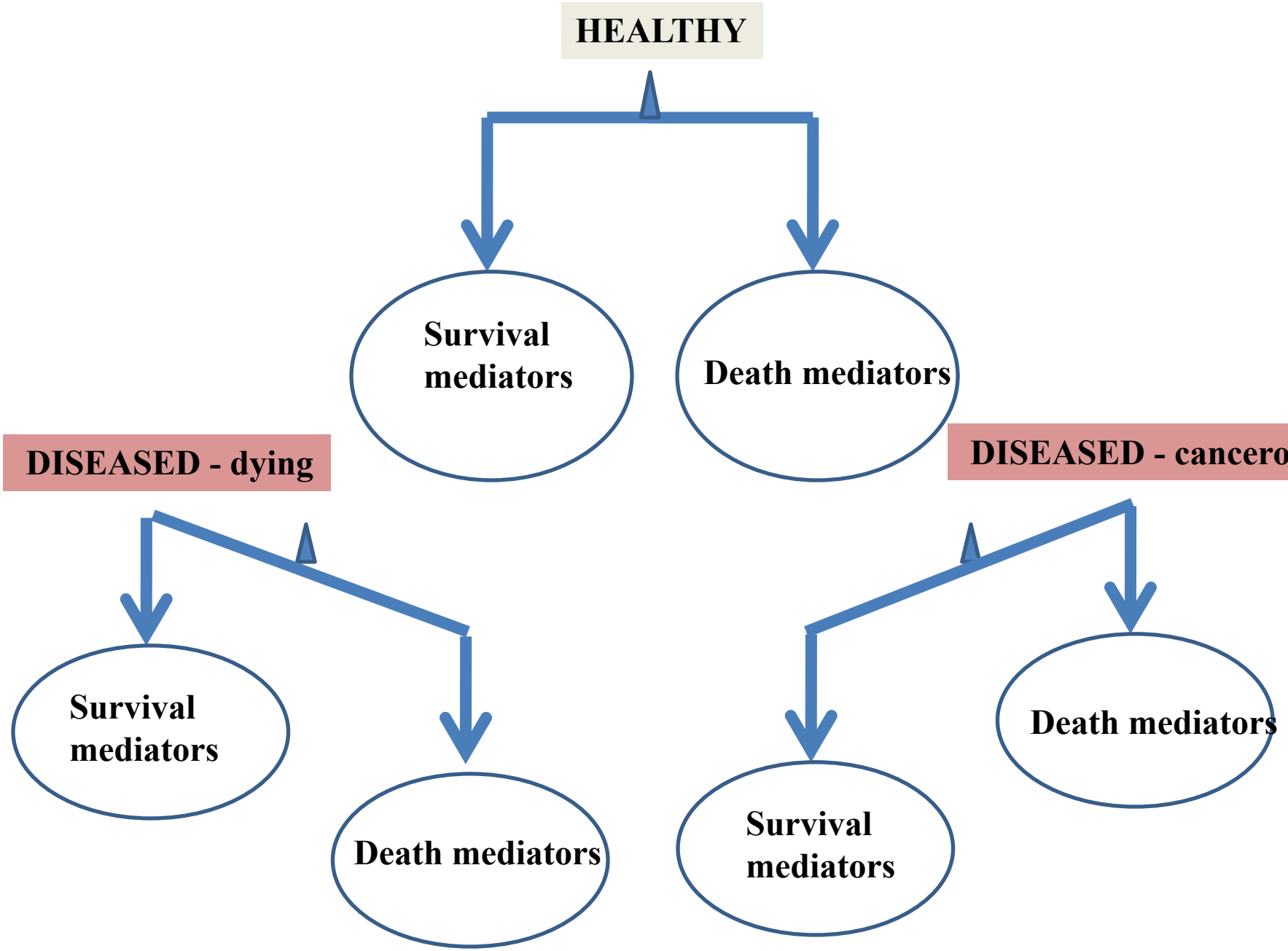
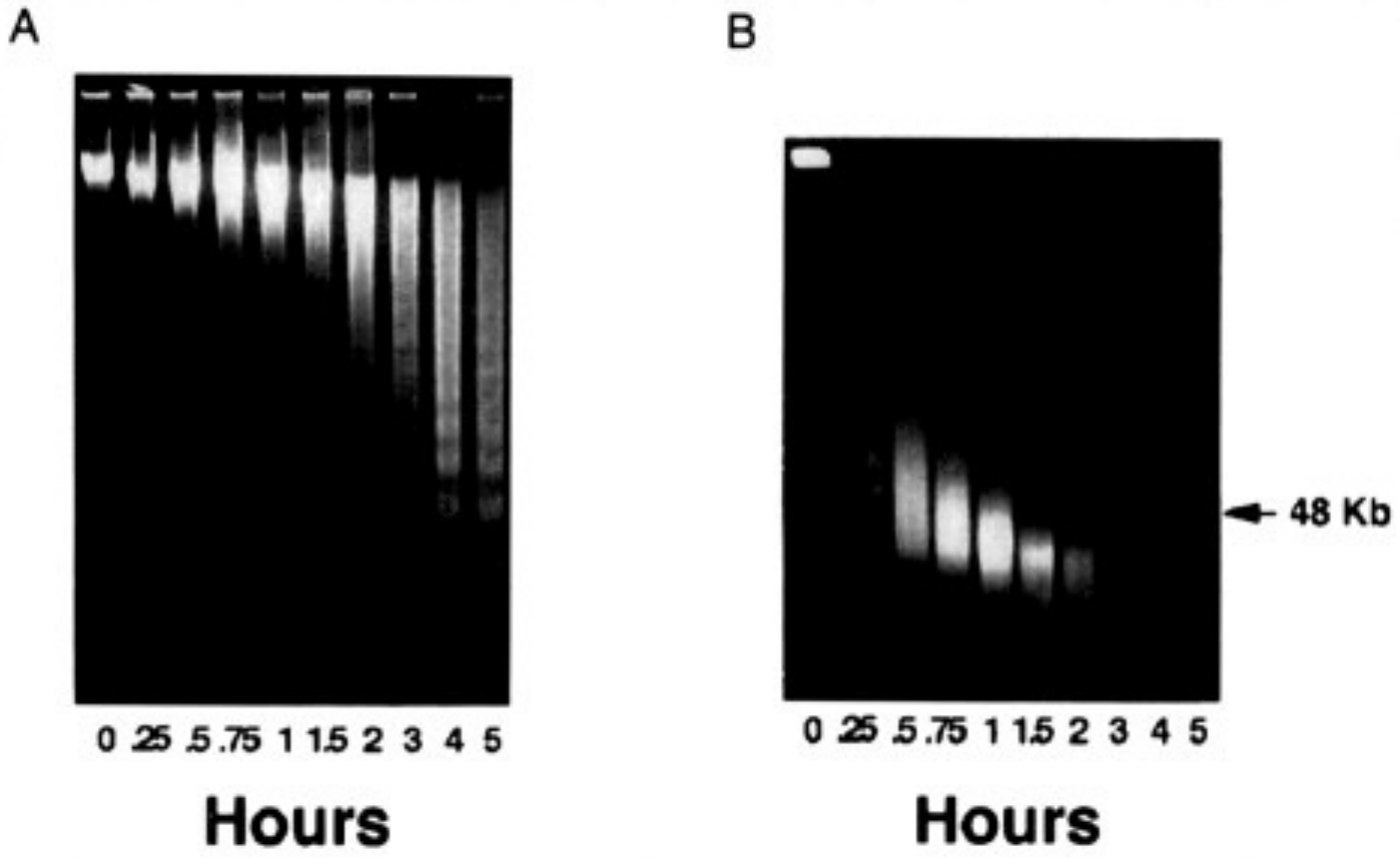

HEALTHY
Survival mediators
Death mediators
DISEASED - dying
Survival mediators
Death mediators
DISEASED - cancerous
Survival mediators
Death mediators

# Apoptosis: Programmed cell death

- Pathway of cell death induced by a tightly regulated suicide program, for the removal of damaged or unnecessary cells
- It is controlled by specific genes
- Key events include, **fragmentation of chromosomal DNA** followed by fragmentation of nucleus and then **cellular blebbing** and release of **apoptotic bodies**
- Apoptotic bodies are phagocytized.
- No inflammation is induced

# (i)Fragmentation of Chromosomal DNA



# (ii) Fragmentation of Nucleus

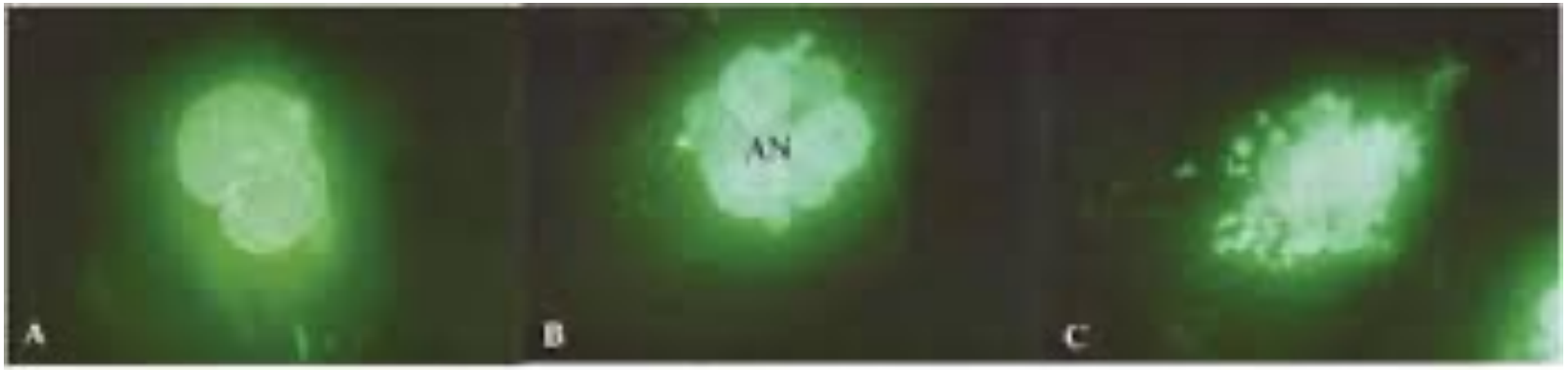
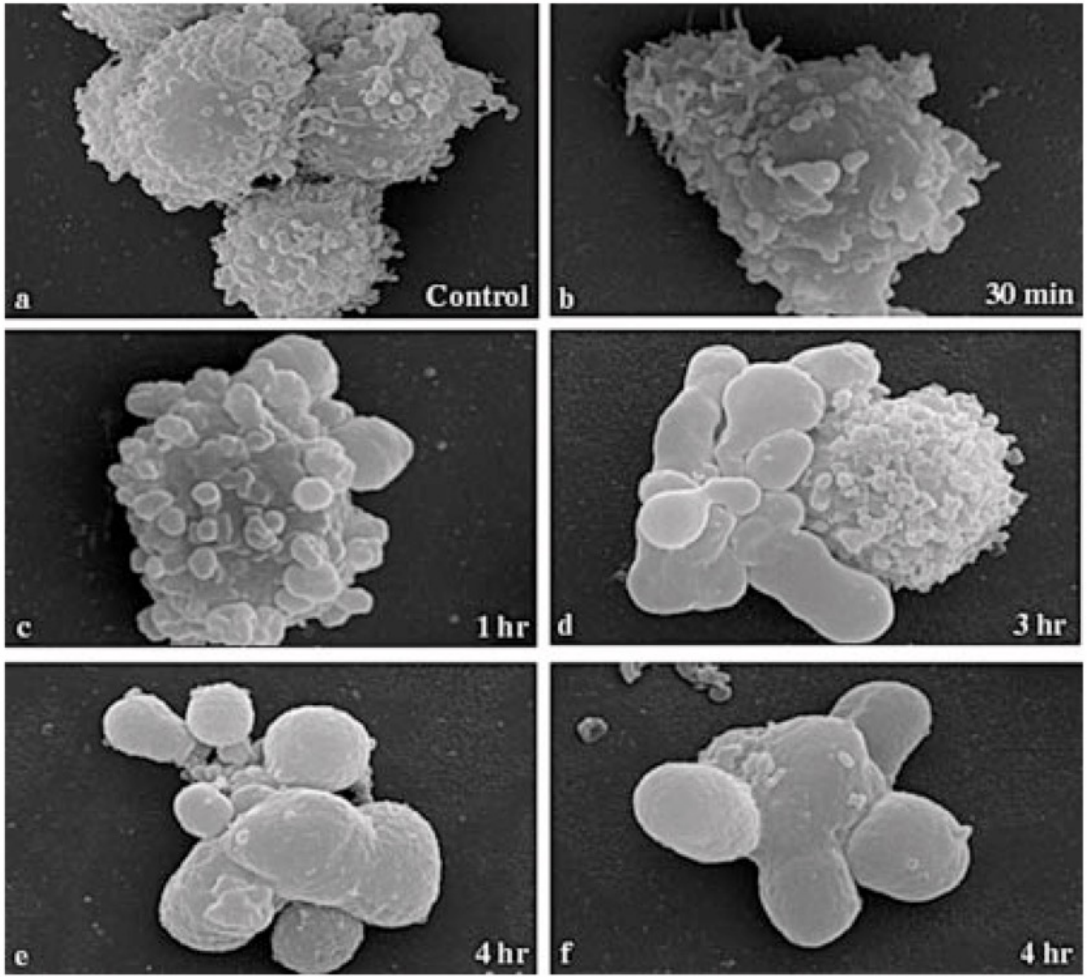
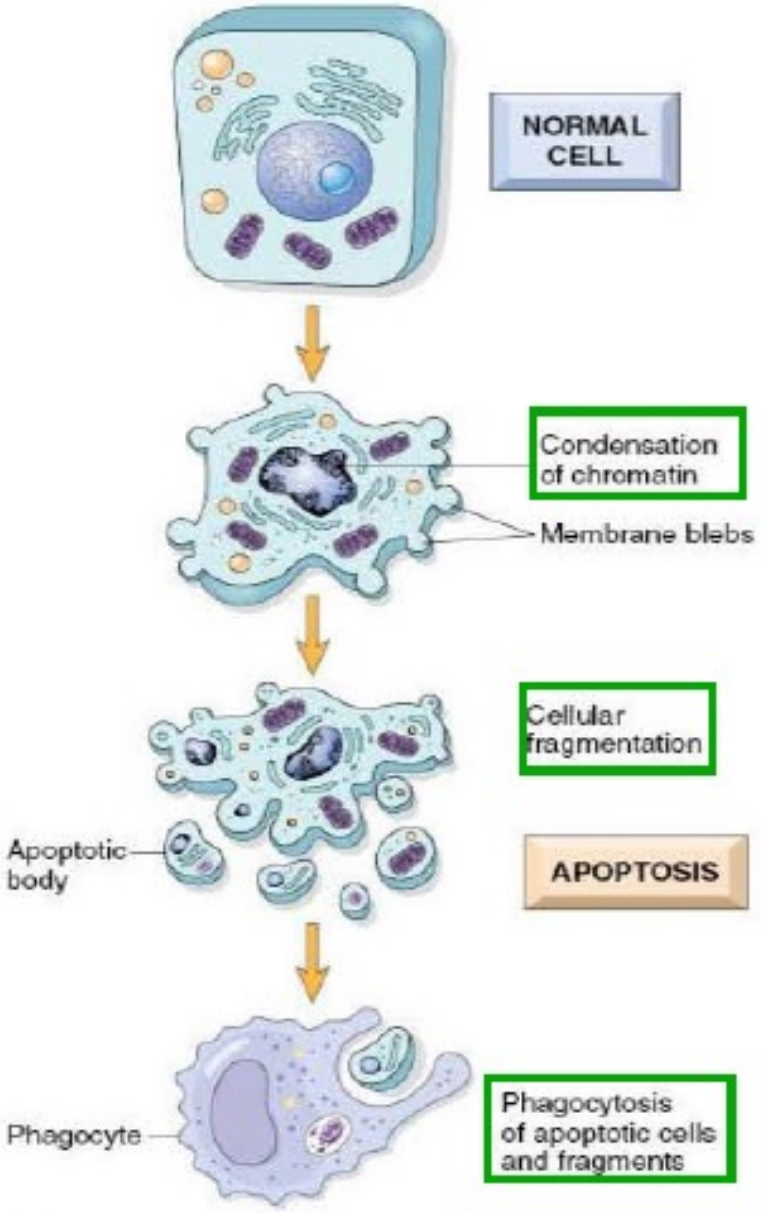



Figure 3: Visualization of nucleus of CHO-K1 cells after *C. d. terrificus* crude venom treatment. (A) Control cells. (B) Cells treated with 10µg/ml of *C. d. terrificus* crude venom for 1 hour. Apoptotic nucleus (AN). (C) Cells treated with 100µg/ml of crude venom for 24 hours. Chromatin condensation and nucleus fragmentation. X 2,500.

# (iii) Cellular Blebbing and Release of Apoptotic Bodies




NORMAL CELL
Condensation of chromatin
Membrane blebs
Cellular fragmentation
Apoptotic body
APOPTOSIS
Phagocyte
Phagocytosis of apoptotic cells and fragments

# Mediators of Apoptosis: Caspases

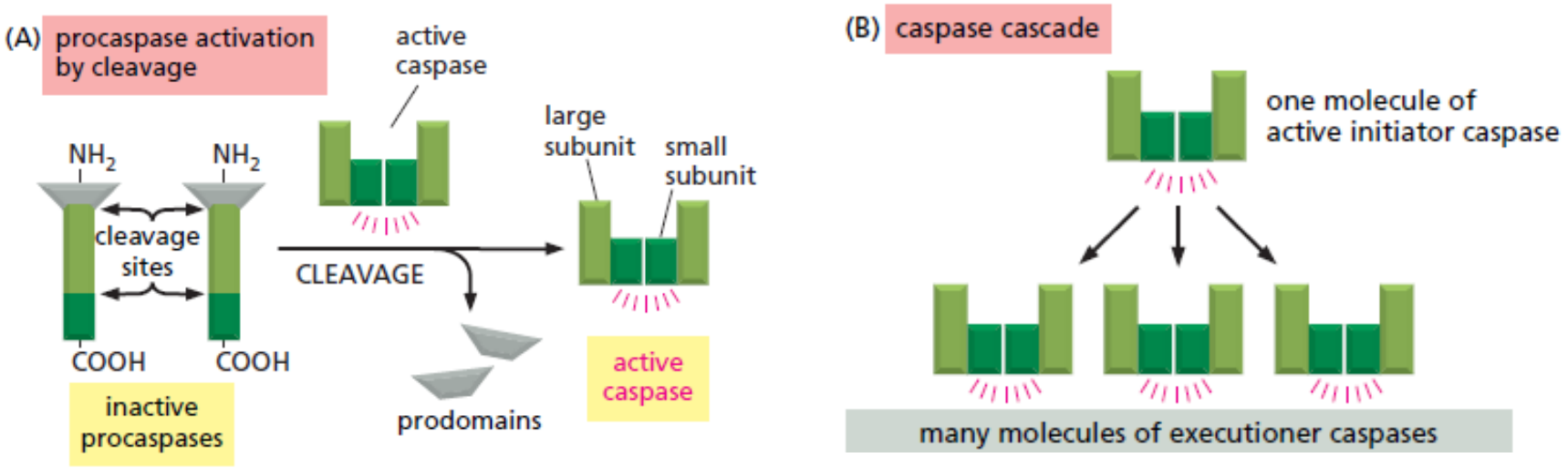
i. The **intracellular mediators** consists of a family of proteases that have a cysteine at their active site and cleave their target proteins at specific aspartic acids, hence called **caspases (c for cysteine and asp for aspartic acid).**

ii. Caspases are synthesized in the cell as inactive precursors, or **procaspases*, which are typically activated*** by proteolytic cleavage.

iii. Procaspase cleavage is catalyzed by other (already active) caspases. **Once activated,** caspases cleave, and thereby activate, other procaspases, resulting in an amplifying proteolytic cascade.

iv. Initiator procaspases when activated, they cleave and activate downstream executioner procaspases, which, then cleave and activate other executioner procaspases, as well as specific target proteins in the cell.

# Activation of Procaspases




Mammalian caspases can be subdivided into three functional groups, ***apoptotic initiator caspases*** (Caspase-2, -8, -9, -10), ***apoptotic effector caspases*** (Caspase-3, -6, -7), and caspases involved in ***inflammatory cytokine processing*** (Caspase-1, -4, -5, 11, and -12L/12S).

# Targets of Effector Caspases

i. Among the many target proteins cleaved by ***executioner caspases*** are ***the nuclear lamins***, the cleavage of which causes the irreversible breakdown of the nuclear membrane.

ii. Another target is a protein that normally holds the ***DNA-degrading enzyme*** (an endonuclease) in an inactive form; its cleavage frees the endonuclease to cut up the DNA in the cell nucleus.

iii. Other target proteins include components of the ***cytoskeleton and cell–cell adhesion proteins*** that attach cells to their neighbors; the cleavage of these proteins helps the apoptotic cell to round up and detach from its neighbors, making it easier for a healthy neighboring cell to engulf it, or, in the case of an epithelial cell, for the neighbors to extrude the apoptotic cell from the cell sheet.

iv. The caspase cascade is not only destructive and self-amplifying but also irreversible, so that once a cell reaches a critical point along the path to destruction, it cannot turn back.

# Apoptosis: Extrinsic Pathway

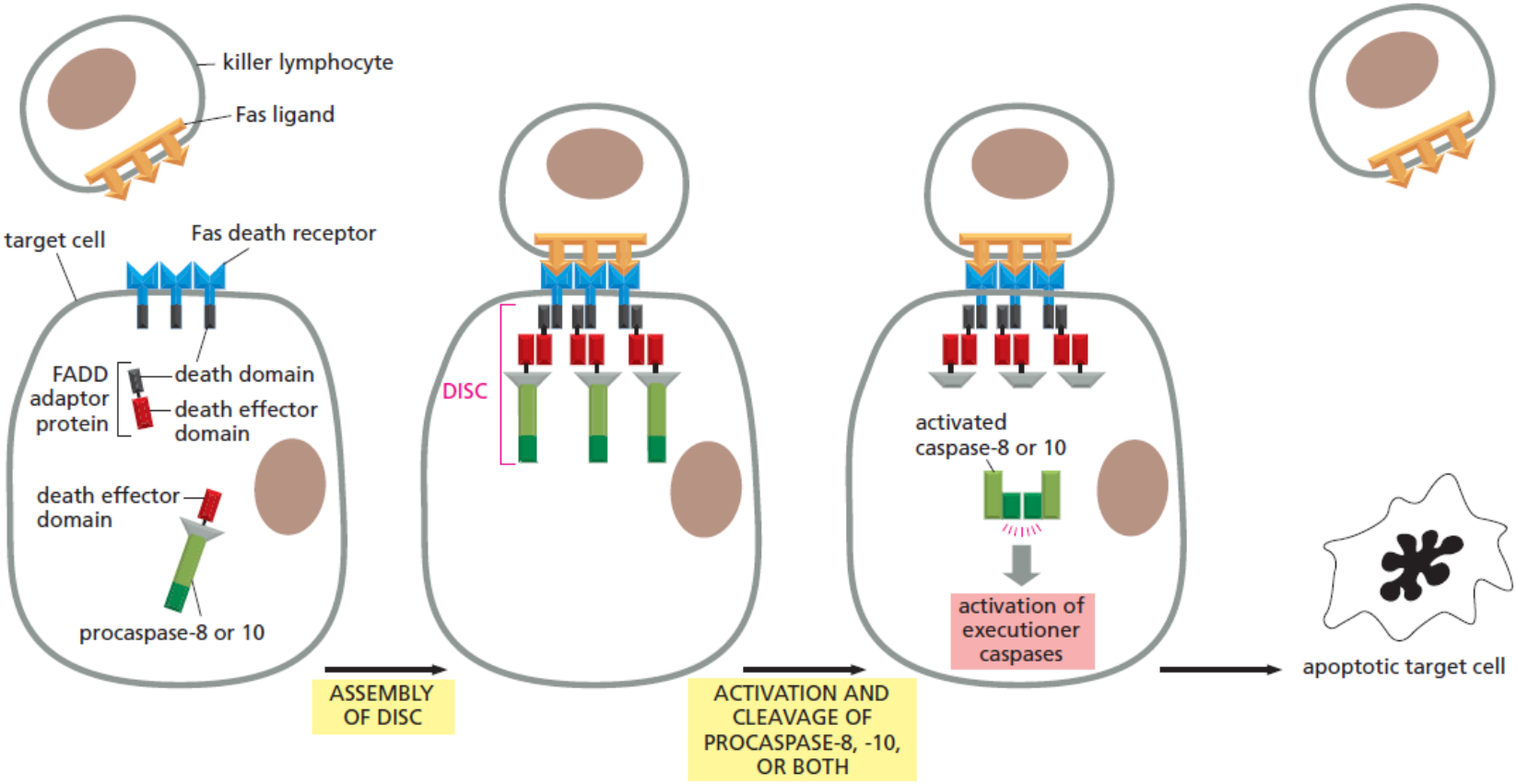
i. Extracellular signal proteins binding to cell-surface death receptors trigger the extrinsic pathway of apoptosis. Death receptors are transmembrane proteins containing an ***extracellular ligand-binding domain***, ***a single transmembrane domain***, and ***an intracellular death domain****, which is required for the receptors* to activate the apoptotic program.

ii. The cytosolic tail of Fas (death receptor) then recruits the intracellular adaptor protein FADD via the death domain on each protein (FADD stands for Fas-associated death domain), *which in turn recruit initiator procaspases (procaspase-8, procaspase-10, or both), forming a death-inducing signaling complex (DISC).*

iii. Within the DISC, the initiator procaspase molecules are brought into close proximity, which activates them; the activated procaspases then cleave one another to stabilize the activated caspase. Activated caspase-8 and caspase-10 then cleave and activate executioner procaspases, producing a caspase cascade, which leads to apoptosis.

**iv. In some cells the extrinsic** pathway must recruit the intrinsic apoptotic pathway to amplify the caspase cascade in order to kill the cell.

# Apoptosis: Extrinsic Pathway/Death receptor pathway

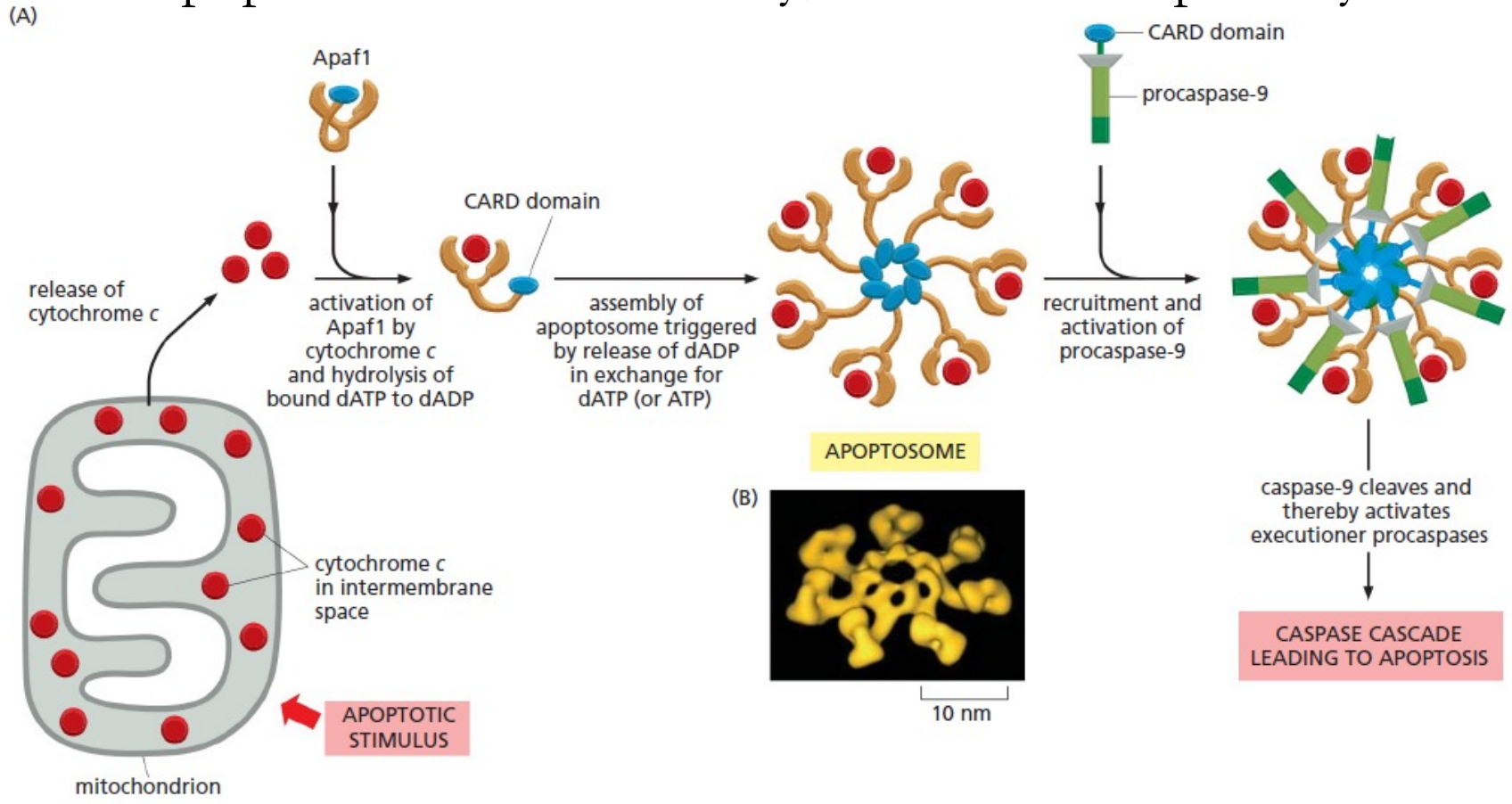



Fas ligand on the surface of a killer lymphocyte activates Fas death receptors on the surface of the target cell.

# Apoptosis: Intrinsic Pathway

i. Cells can also activate their apoptosis program from inside the cell, usually in response to injury or other stresses, such as DNA damage or lack of oxygen, nutrients, or extracellular survival signals
ii. A crucial protein released from mitochondria in the intrinsic pathway is **cytochrome c**, a water-soluble component of the mitochondrial electron-transport chain. When released into the cytosol, it has an entirely different function: it binds to a procaspase-activating adaptor protein called **Apaf1** *(apoptotic protease activating factor-1), causing the Apaf1 to oligomerize into a* wheel-like heptamer called an **apoptosome.**
iii. The binding of cytochrome c causes the Apaf1 to hydrolyze its bound dATP to dADP. The replacement of the dADP with dATP or ATP then induces the complex of Apaf1 and cytochrome c to aggregate to form a large, heptameric apoptosome.
iv. Apoptosome then recruits procaspase-9 through a caspase recruitment domain (CARD) in each protein. The procaspase-9 molecules are activated within the apoptosome and are now able to cleave and activate downstream executioner procaspases, which leads to the cleavage and activation of these molecules in a caspase cascade.

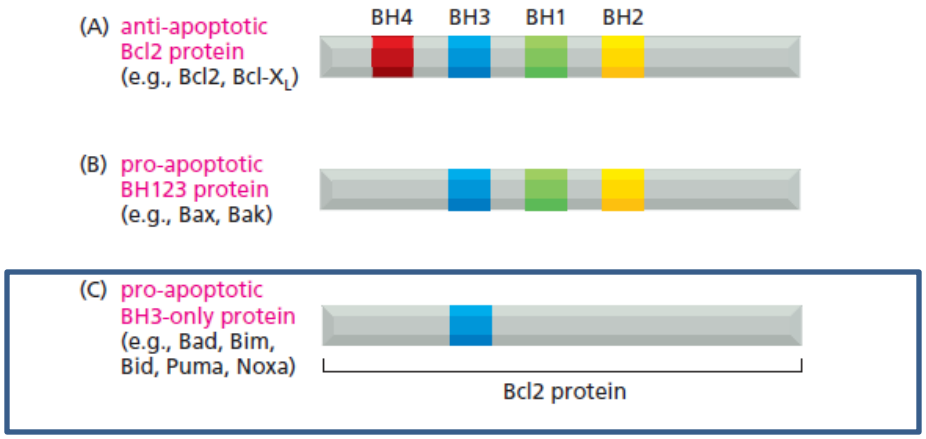
# Apoptosis: Intrinsic Pathway/Mitochondrial pathway




The ***binding of cytochrome c*** causes the ***Apaf1 to hydrolyze its bound dATP to dADP.*** The replacement of the dADP with dATP or ATP then induces the complex of Apaf1 and cytochrome c to aggregate to form a large, ***heptameric apoptosome, which then recruits procaspase-9 through a caspase recruitment domain*** (CARD) in each protein. The procaspase-9 molecules are activated within the apoptosome and are now able to cleave and activate downstream executioner procaspases, which leads to the cleavage and activation of these molecules in a caspase cascade.

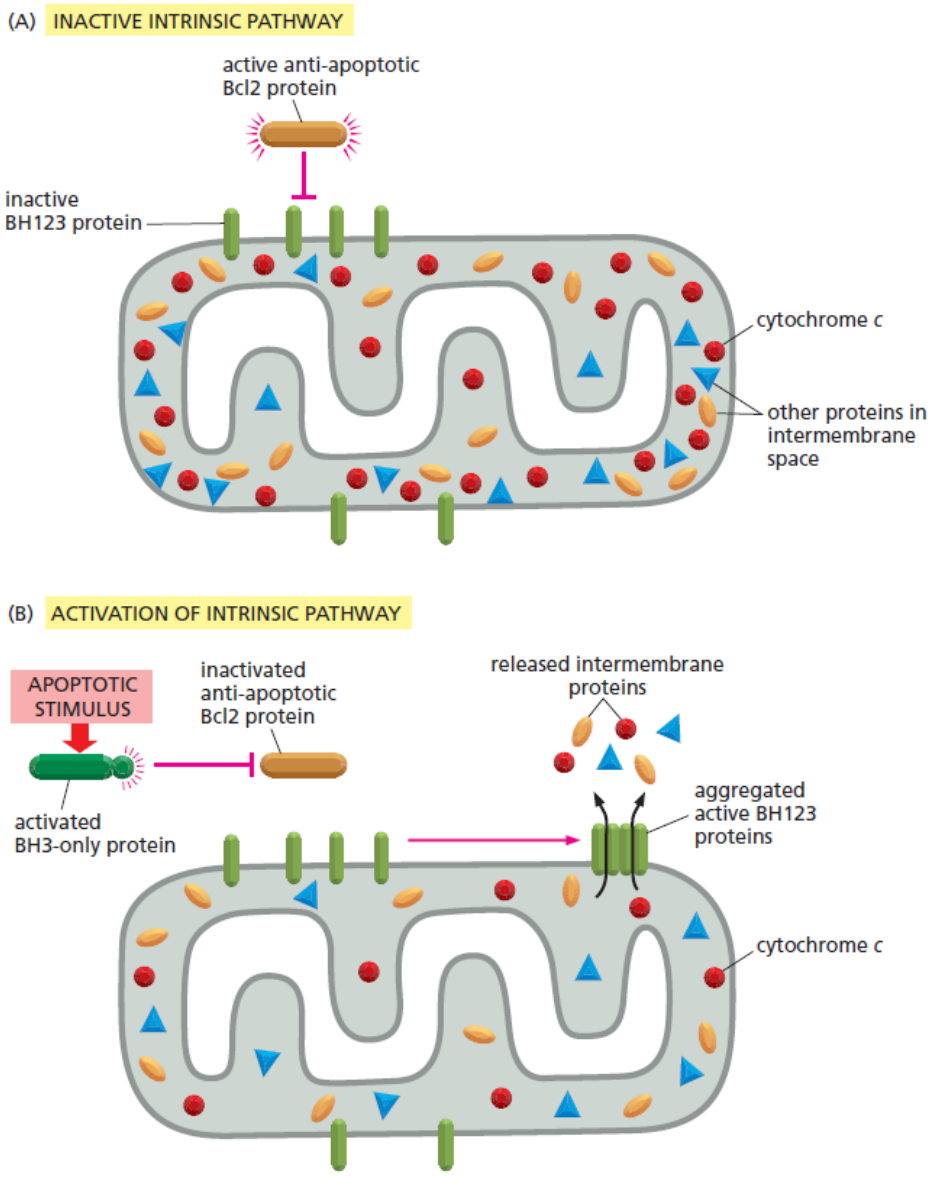
# Regulators of Apoptosis: Bcl2 Family of proteins

| Pro-apoptotic Bcl2 family | Anti-apoptotic Bcl2 family |
|---|---|
| Enhance ***cytochrome c*** release | Prevent ***cytochrome c*** release |
| Pro-apoptotic Bcl2 proteins consist of two subfamilies—Bcl2 homology (BH) domains ***BH123 proteins and the BH3-only proteins***. **BH3-only proteins are the largest subclass of Bcl2 family proteins** | Anti-apoptotic Bcl2 proteins, including Bcl2 and Bcl-XL, share four distinctive ***Bcl2 homology (BH) domains (BH1–4)*** |




The BH3-only proteins ***Bid, Bim, and Puma*** can ***inhibit all of the anti-apoptotic Bcl2*** proteins, whereas the other BH3-only proteins can inhibit only a small subset of the anti-apoptotic proteins. Thus, Bid, Bim, and Puma are the most potent activators of apoptosis in the BH3-only subfamily of Bcl2 proteins.

# Regulators of Apoptosis: Bcl2 Family of proteins




(A) In the absence of an apoptotic stimulus, anti-apoptotic Bcl2 proteins bind to and inhibit the BH123 proteins on the mitochondrial outer membrane. (B) In the presence of an apoptotic stimulus, BH3-only proteins are activated and bind to the anti-apoptotic Bcl2 proteins so that they can no longer inhibit the BH123 proteins, which now become activated and aggregate in the outer mitochondrial membrane and promote the release of intermembrane mitochondrial proteins into the cytosol.

# Regulators of Apoptosis: Extracellular Survival Factors

i. Most animal cells require continuous signaling from other cells to avoid apoptosis. This surprising arrangement apparently helps ensure that ***cells survive only when and where they are needed***.

***ii.*** ***Survival factors*** usually bind to cell-surface receptors, which activate intracellular signaling pathways that ***suppress the apoptotic program***, often ***by regulating members of the Bcl2 family of proteins***.

iii. Some survival factors, for example, stimulate ***an increased production of anti-apoptotic*** Bcl2 proteins such as Bcl2 itself or Bcl-XL**.** Others act by ***inhibiting the function of BH3-only pro-apoptotic Bcl2 proteins*** such as *Bad.*