Programmed Cell Death (Apoptosis)

-Dr. Ekta Khare

- The cells of a multicellular organism are members of a highly organized community.
- If cells are no longer needed, they commit suicide by activating an intracellular death program.
- This process is therefore called programmed cell death, although it is more commonly called apoptosis (from a Greek word meaning "falling off," as leaves from a tree).
- In adult tissues, cell death exactly balances cell division.
- Cells that die as a result of acute injury typically swell and burst. They spill their contents all over their neighbors a process called cell necrosis causing a potentially damaging inflammatory response. By contrast, a cell that undergoes apoptosis dies neatly, without damaging its neighbors.

Apoptosis Is Mediated by an Intracellular Proteolytic Cascade

- The cell shrinks and condenses.
- The cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear DNA breaks up into fragments.
- Most importantly, the cell surface is altered, displaying properties that cause the dying cell to be rapidly phagocytosed, either by a neighboring cell or by a macrophage (a specialized phagocytic cell), before any leakage of its contents occurs.
- This not only avoids the damaging consequences of cell necrosis but also allows the organic components of the dead cell to be recycled by the cell that ingests it.
- The intracellular machinery responsible for apoptosis seems to be similar in all animal cells.
- This machinery depends on a family of proteases that have a cysteine at their active site and cleave their target proteins at specific aspartic acids. They are therefore called caspases.
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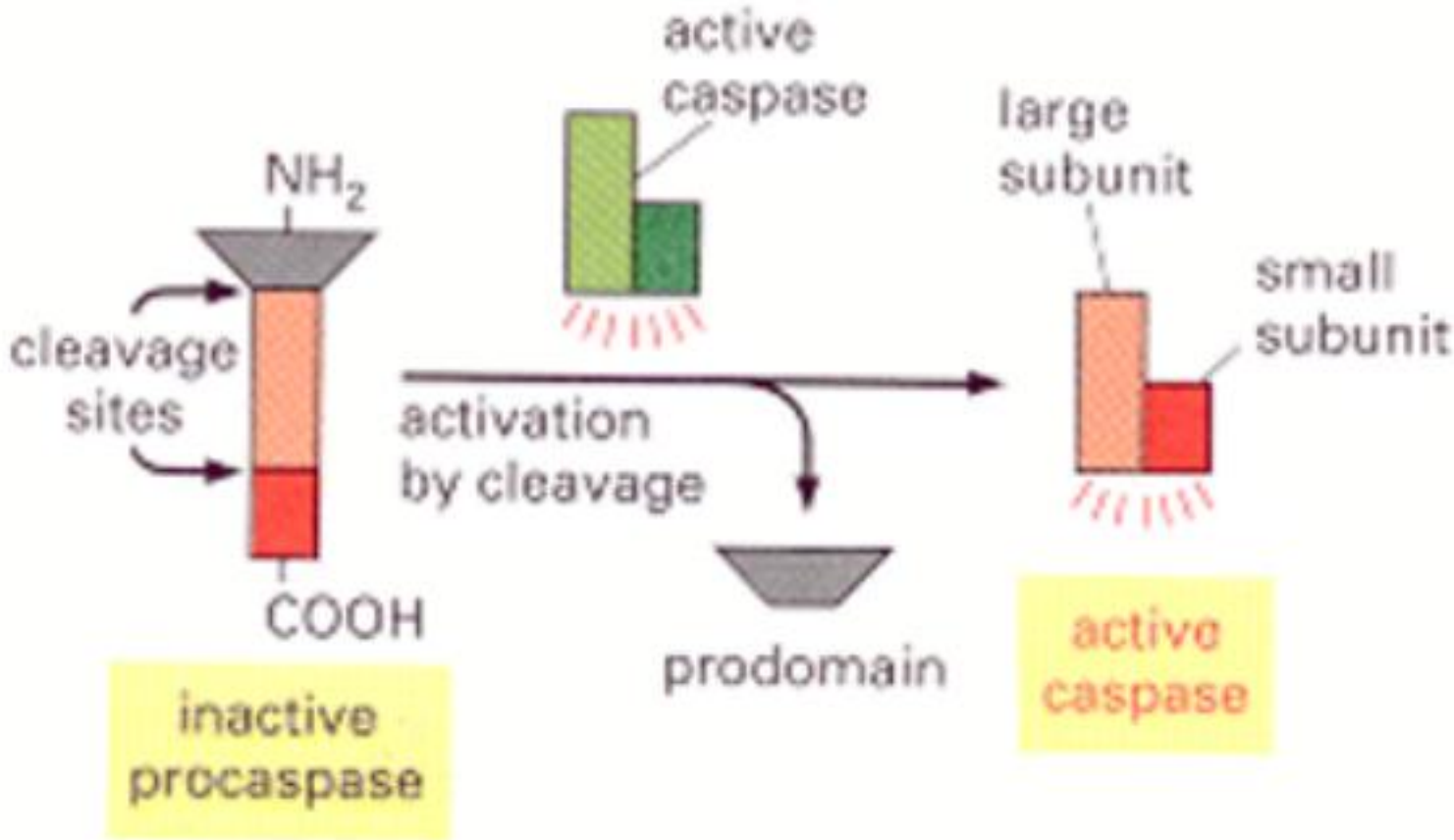
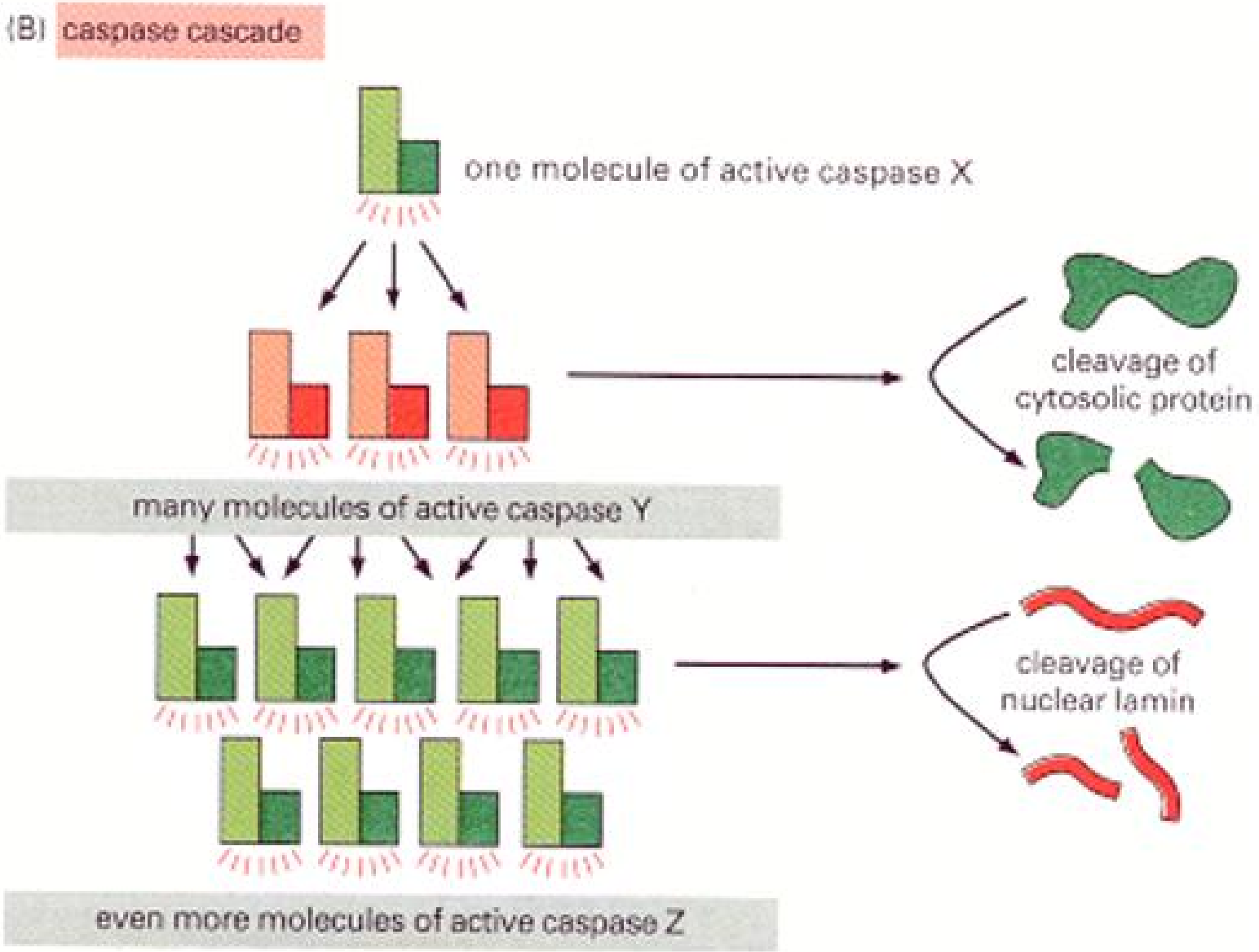
- Once activated, caspases cleave, and thereby activate, other procaspases, resulting in an amplifying proteolytic cascade.
- Some of the activated caspases then cleave other key proteins in the cell.

…Apoptosis Is Mediated by an Intracellular Proteolytic Cascade

- Some cleave the nuclear lamins, for example, causing the irreversible breakdown of the nuclear lamina; another cleaves a protein that normally holds a DNA-degrading enzyme (a DNAse) in an inactive form, freeing the DNAse to cut up the DNA in the cell nucleus.
- In this way, the cell dismantles itself quickly and neatly, and its corpse is rapidly taken up and digested by another cell.
- The protease 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.

(A) procaspase activation



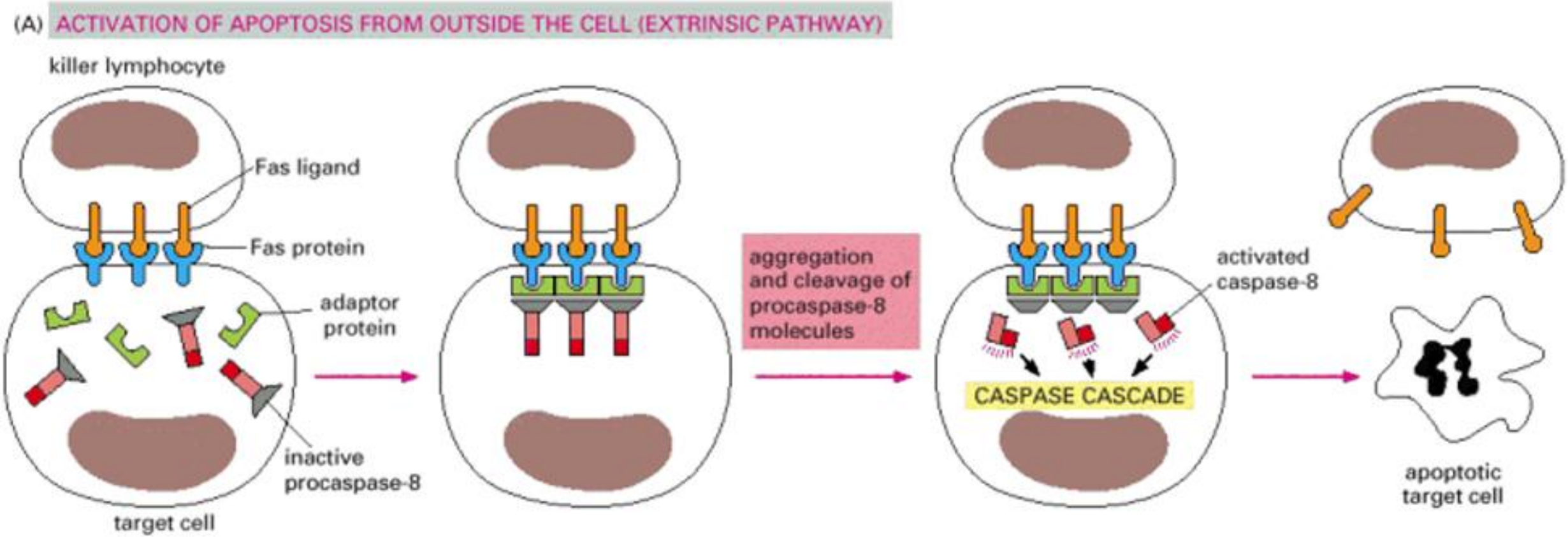

(B) caspase cascade
one molecule of active caspase X
cleavage of cytosolic protein
many molecules of active caspase Y
cleavage of nuclear lamin
even more molecules of active caspase Z

Procaspases Are Activated by Binding to Adaptor Proteins

- Caspase activity is tightly regulated inside the cell to ensure that the death program is held in check until needed.
- The adaptor proteins bring multiple copies of specific procaspases, known as initiator procaspases, close together in a complex or aggregate.
- In some cases, the initiator procaspases have a small amount of protease activity, and forcing them together into a complex causes them to cleave each other, triggering their mutual activation.
- In other cases, the aggregation is thought to cause a conformational change that activates the procaspase.
- Within moments, the activated caspase at the top of the cascade cleaves downstream procaspases to amplify the death signal and spread it throughout the cell.

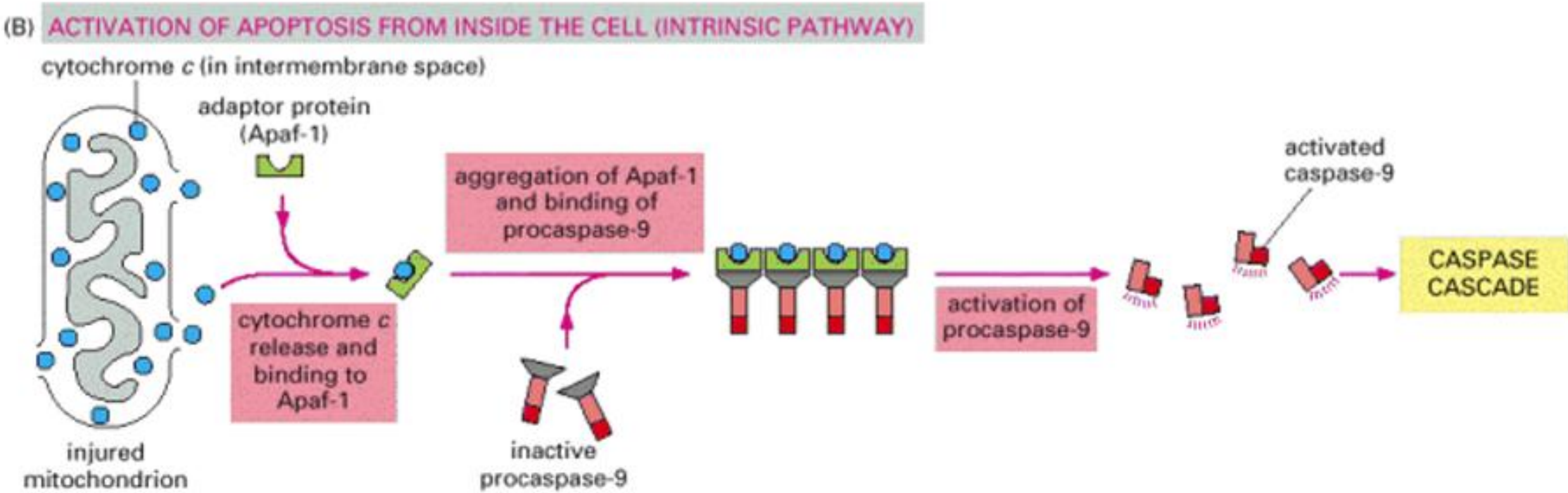
…Procaspases Are Activated by Binding to Adaptor Proteins

- Procaspase activation can be triggered from outside the cell by the activation of death receptors on the cell surface.
- Killer lymphocytes, for example, can induce apoptosis by producing a protein called Fas ligand, which binds to the death receptor protein Fas on the surface of the target cell.
- The clustered Fas proteins then recruit intracellular adaptor proteins that bind and aggregate procaspase-8 molecules, which cleave and activate one another.
- The activated caspase-8 molecules then activate downstream procaspases to induce apoptosis.
- Some stressed or damaged cells kill themselves by producing both the Fas ligand and the Fas protein, thereby triggering an intracellular caspase cascade.


(A) ACTIVATION OF APOPTOSIS FROM OUTSIDE THE CELL (EXTRINSIC PATHWAY)
killer lymphocyte
Fas ligand
Fas protein
adaptor protein
inactive procaspase-8
target cell
aggregation and cleavage of procaspase-8 molecules
activated caspase-8
CASPASE CASCADE
apoptotic target cell

…Procaspases Are Activated by Binding to Adaptor Proteins

- **When cells are damaged or stressed, they can also kill themselves** by triggering procaspase aggregation and activation from within the cell.
- In the best understood pathway, mitochondria are induced to release the electron carrier protein cytochrome c into the cytosol, where it binds and activates an adaptor protein called Apaf-1.
- This mitochondrial pathway of procaspase activation is recruited in most forms of apoptosis to initiate or to accelerate and amplify the caspase cascade.
- DNA damage, for example, as discussed earlier, can trigger apoptosis.
- This response usually requires p53, which can activate the transcription of genes that encode proteins that promote the release of cytochrome c from mitochondria.
- These proteins belong to the Bcl-2 family.


(B) ACTIVATION OF APOPTOSIS FROM INSIDE THE CELL (INTRINSIC PATHWAY)
cytochrome c (in intermembrane space)
adaptor protein (Apaf-1)
cytochrome c release and binding to Apaf-1
aggregation of Apaf-1 and binding of procaspase-9
activation of procaspase-9
activated caspase-9
CASPASE CASCADE
injured mitochondrion
inactive procaspase-9

Bcl-2 Family Proteins and IAP Proteins Are the Main Intracellular Regulators of the Cell Death Program

- The Bcl-2 family of intracellular proteins helps regulate the activation of procaspases.
- Some members of this family, like Bcl-2 itself or Bcl-XL, inhibit apoptosis, at least partly by blocking the release of cytochrome c from mitochondria.
- Other members of the Bcl-2 family like *Bax, Bak* are not death inhibitors, but instead promote procaspase activation and cell death.
- Bax and Bak are themselves activated by other apoptosis promoting members of the Bcl-2 family such as Bid.

Inhibitor of apoptosis (IAP) family

- Another important family of intracellular apoptosis regulators is the IAP (inhibitor of apoptosis) family.
- These proteins are thought to inhibit apoptosis in two ways: they bind to some procaspases to prevent their activation, and they bind to caspases to inhibit their activity.
- IAP proteins were originally discovered as proteins produced by certain insect viruses, which use them to prevent the infected cell from killing itself before the virus has had time to replicate.
- When mitochondria release cytochrome c to activate Apaf-1, they also release a protein that blocks IAPs, thereby greatly increasing the efficiency of the death activation process.
- The intracellular cell death program is also regulated by extracellular signals, which can either activate apoptosis or inhibit it.
- These signal molecules mainly act by regulating the levels or activity of members of the Bcl-2 and IAP families.