Alberts • Johnson • Lewis • Morgan • Raff • Roberts • Walter

Molecular Biology of the Cell

Sixth Edition

Chapter 18
Cell Death

Pages: 1021-1034

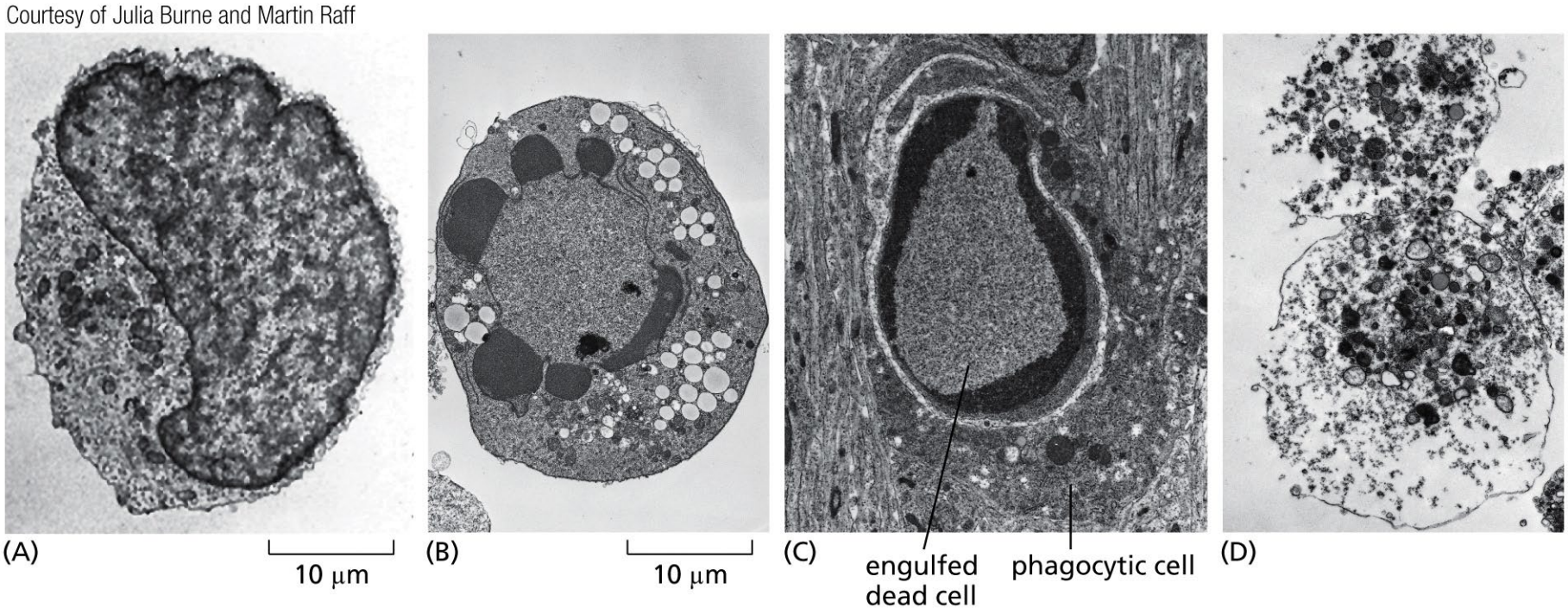
Course overview – Tentative schedule

Date	Lecture		Chapters & Topics	Assignments
25.10.	1	Part 1	*Course overview*, DNA, Chromosomes, Genome, Ch. 4	
27.10.	2 -G		Replication, Repair, Recombination, Ch. 5	
1.11.	3		From DNA to protein, Ch. 6	
3.11.	4		Control of gene expression, Ch. 7	
8.11.	5	Part 2	Membrane structures, Ch. 10 Membrane transport, Ch. 11	**Assignment I (Essay) Draft I** (8.11.)
10.11.	6 -G		Intracellular compartments and protein sorting, Ch. 12	
15.11.	7		Intracellular compartments and protein sorting, Ch. 12 **Susanna Mäkinen, Solar Foods**	**Assignment II – Draft I (15.11.)**
17.11.	8		Membrane Traffic, Ch. 13 **iGEM team 2023**	*+iGEM intro*
22.11.	9	Part 3	Cell signalling, Ch. 15	**Assignment II – Peer review** (22.11.)
24.11.	10 -G		Cell signalling, Ch. 15	**Assignment I (Essay) Draft II** (24.11.)
29.11.	11		Cell cycle, Ch. 17 **Jere Weltner, Folkhälsan**	
1.12.	12		Apoptosis, Ch. 18 + About exam	**Assignment II – final version** (1.12.)
7.12.	EXAM		December 7th	
8.12.	Final version essay		December 8th	**Assignment I (Essay) Final version** (8.12.) Aim at finishing before exam date. Use last days for polishing.

CONTENT

- Apoptosis depends on an intracellular proteolytic cascade mediated by caspases
- Extrinsic and intrinsic pathways of apoptosis
- Bcl2 proteins control the intrinsic pathway of apoptosis
- Extracellular survival factors inhibit apoptosis
- Phagocytosis of apoptotic cells

Apoptosis and necrosis

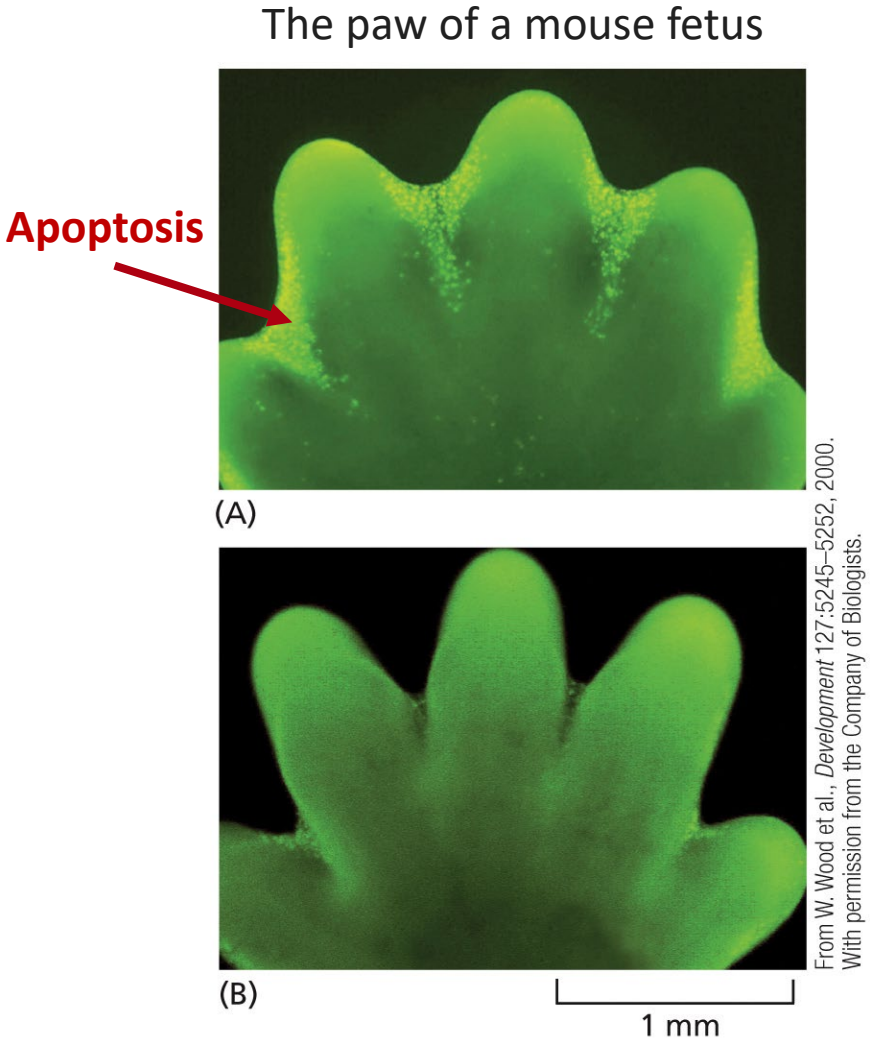


Apoptosis: cells condense and collapse, membrane remains intact, rapidly engulfed by other cells

Necrosis: cells burst their contents around, inflammatory reaction

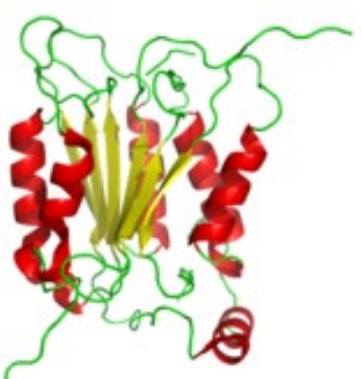
APOPTOSIS

- Eliminates unwanted cells
  - E.g. in development + to maintain renewing of tissues
- Eliminates damaged cells
  - Especially damage in DNA
- In a **controlled manner** that will not damage other cells and will enable reusing components



CASPASES

- **C**ysteine-dependent **asp**artate specific prote**ases**
  - Have a cysteine at the active site
  - Cleave target just after aspartic acid residues
- Substrate specificity is determined by the 4 residues upstream of cleavage site
- Exist in cytosol as single chain proenzymes,
  - Inactive precursors activated in apoptosis (apoptosis signal)
  - Activated by cleavage by other caspases
  - Once activated, cleave other caspases
- Cleave key proteins in the cell, causing the characteristic morphology and biochemistry of apoptosis



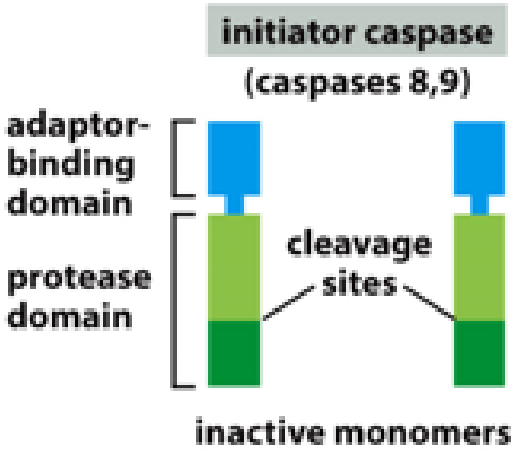
INITIATOR CASPASES

Initiator caspases contain N-terminal adapter binding domain followed by region that forms a 2-subunit catalytic effector domain

Adaptor binding domain binds to upstream regulators and effector proteins

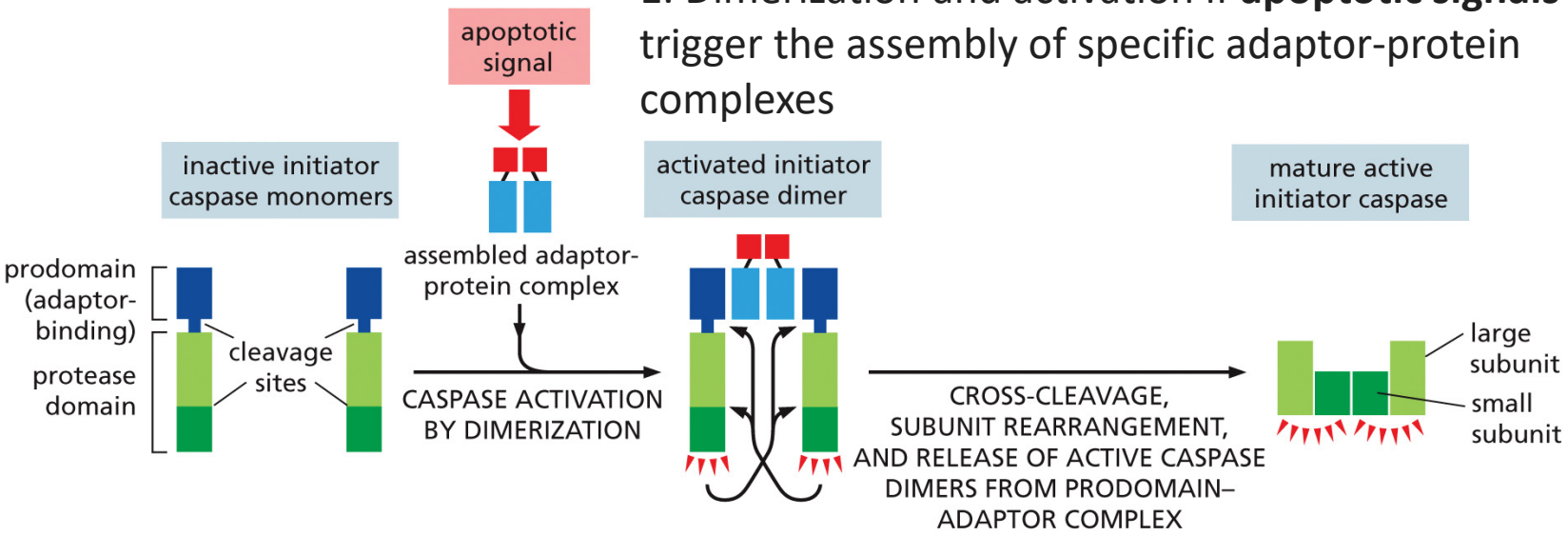
- DED – death effector domain (e.g. Caspase 8)
- CARD – caspase activation and recruitment domain (e.g. Caspase 9)

Initiator caspase is also called procaspase



INITIATOR CASPASES AND EXECUTIONER CASPASES

1. Dimerization and activation if **apoptotic signals** trigger the assembly of specific adaptor-protein complexes

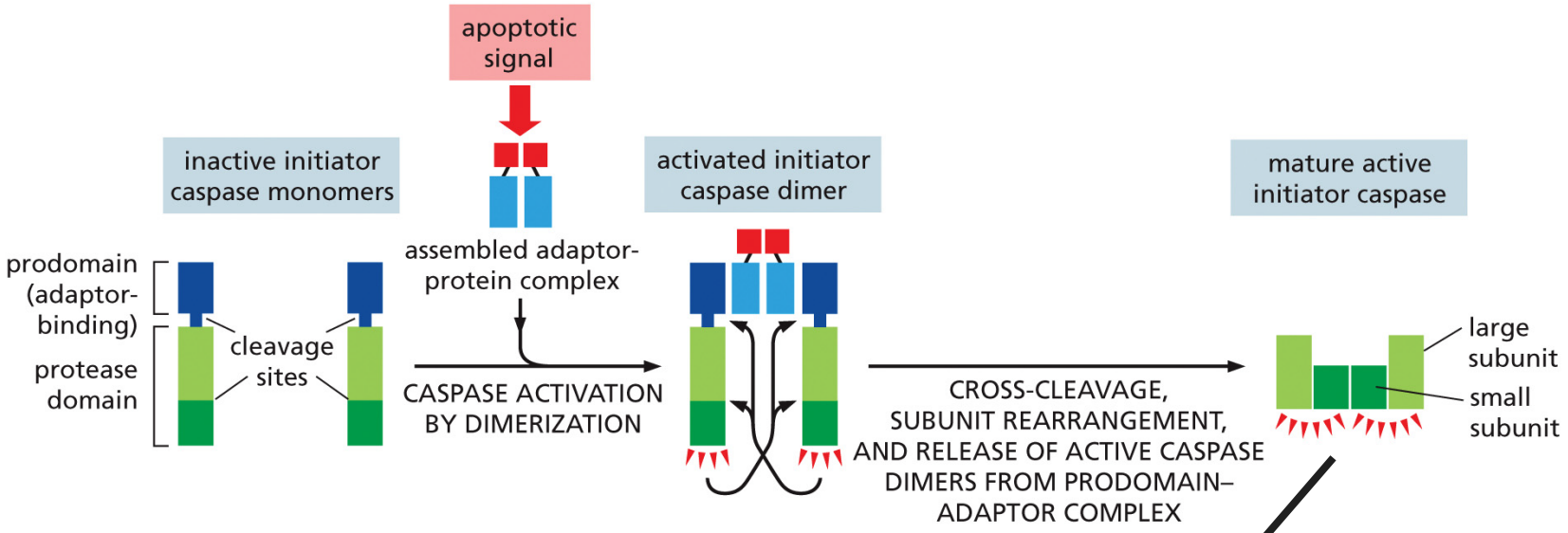


2. **Inactive monomer activated by dimerization**

3. The cleavage in the protease domain enables the *large and small caspase subunits to rearrange.*

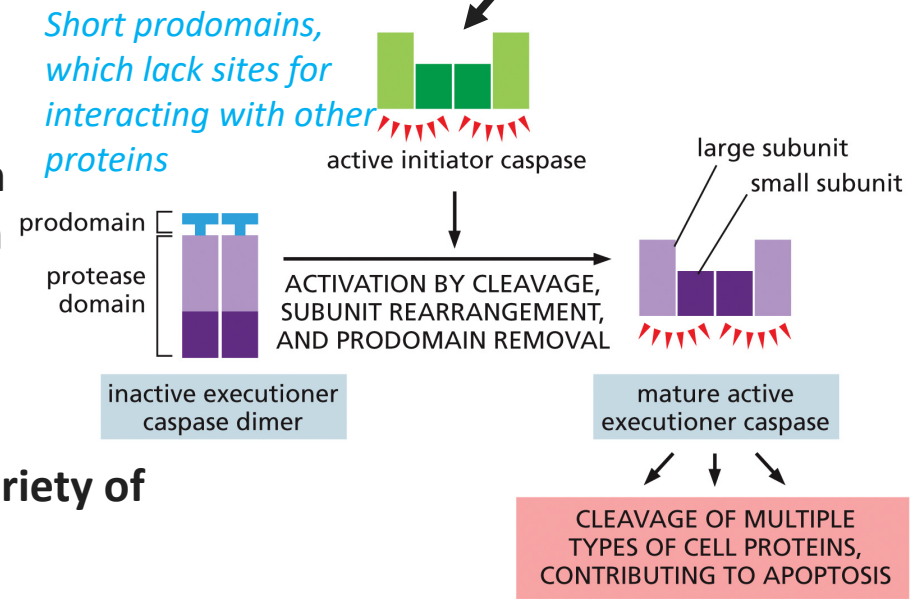
4. Cleavage between the large protease domain and the *prodomain releases the mature, active caspase dimer from the prodomain–adaptor complex*.

INITIATOR CASPASES AND EXECUTIONER CASPASES



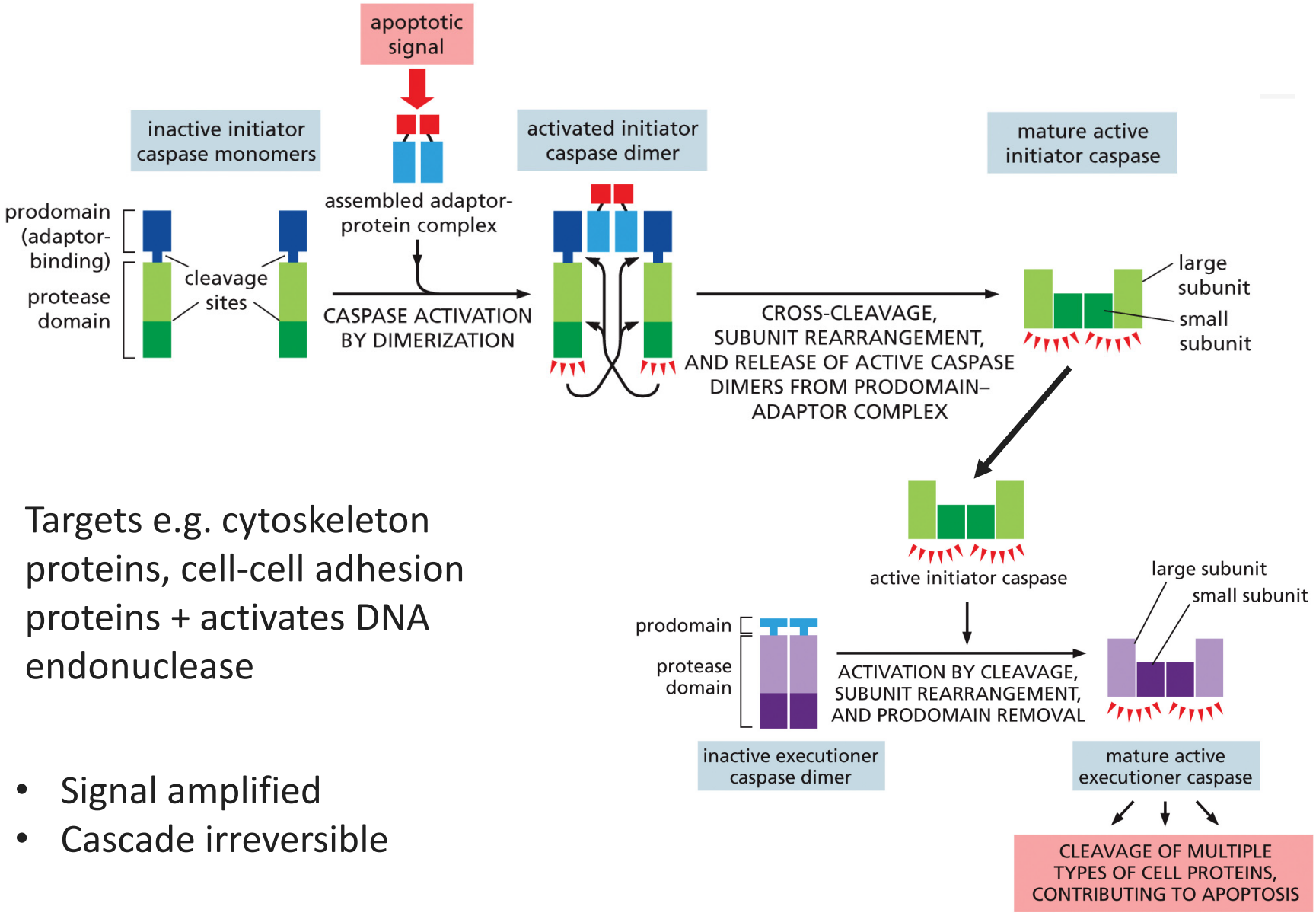
1. **Inactive dimers are activated by cleavage** at a site in each protease domain.

2. *Large and small subunits rearrange* to form **two active protease sites**, each of which then **cleaves off the prodomain of its partner monomer to produce the mature, active executioner caspase**



3. The **mature activated caspase cleaves a variety of cell target proteins**, leading to the controlled apoptotic death of the cell.

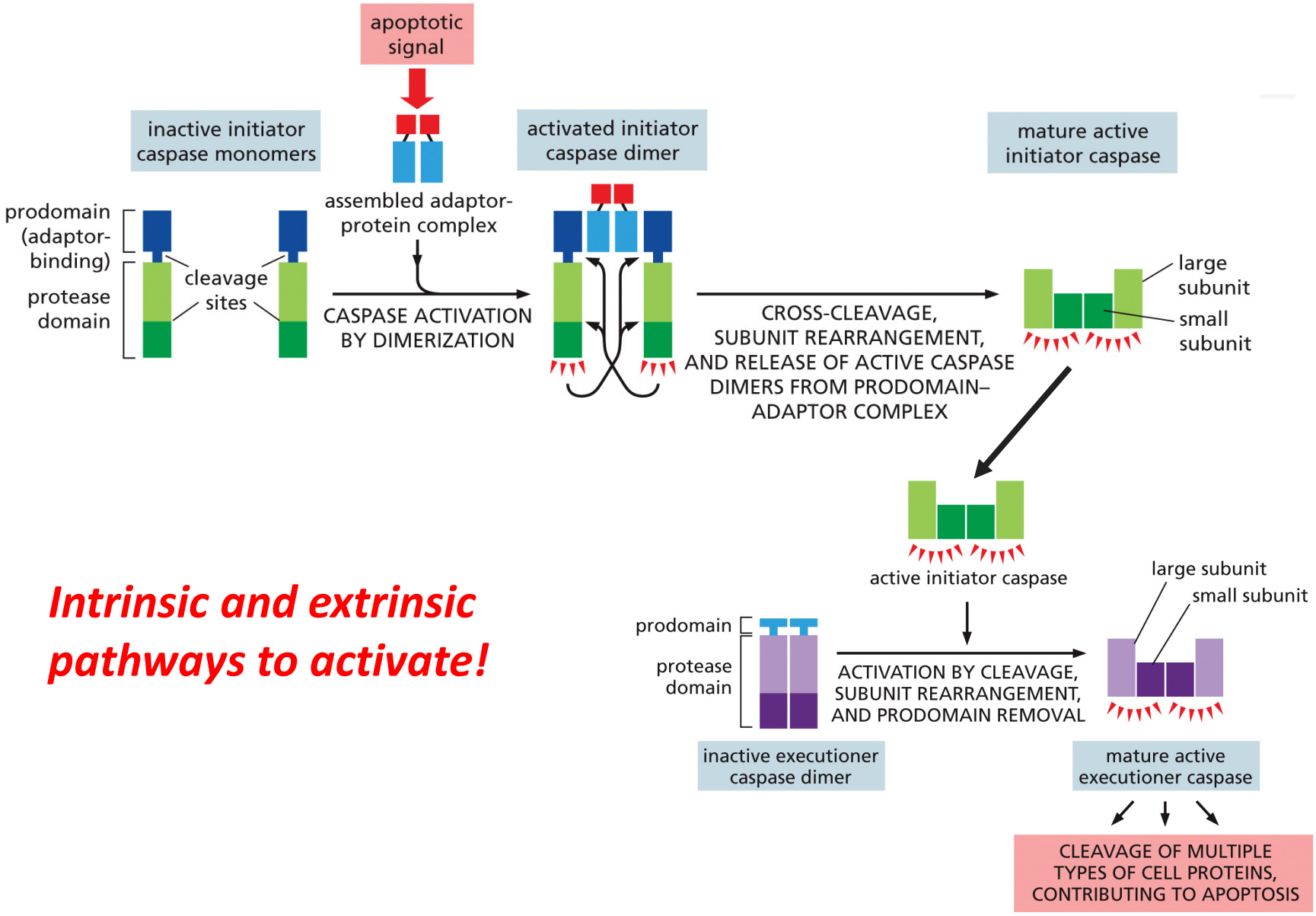
INITIATOR CASPASES AND EXECUTIONER CASPASES



Targets e.g. cytoskeleton proteins, cell-cell adhesion proteins + activates DNA endonuclease

- Signal amplified
- Cascade irreversible

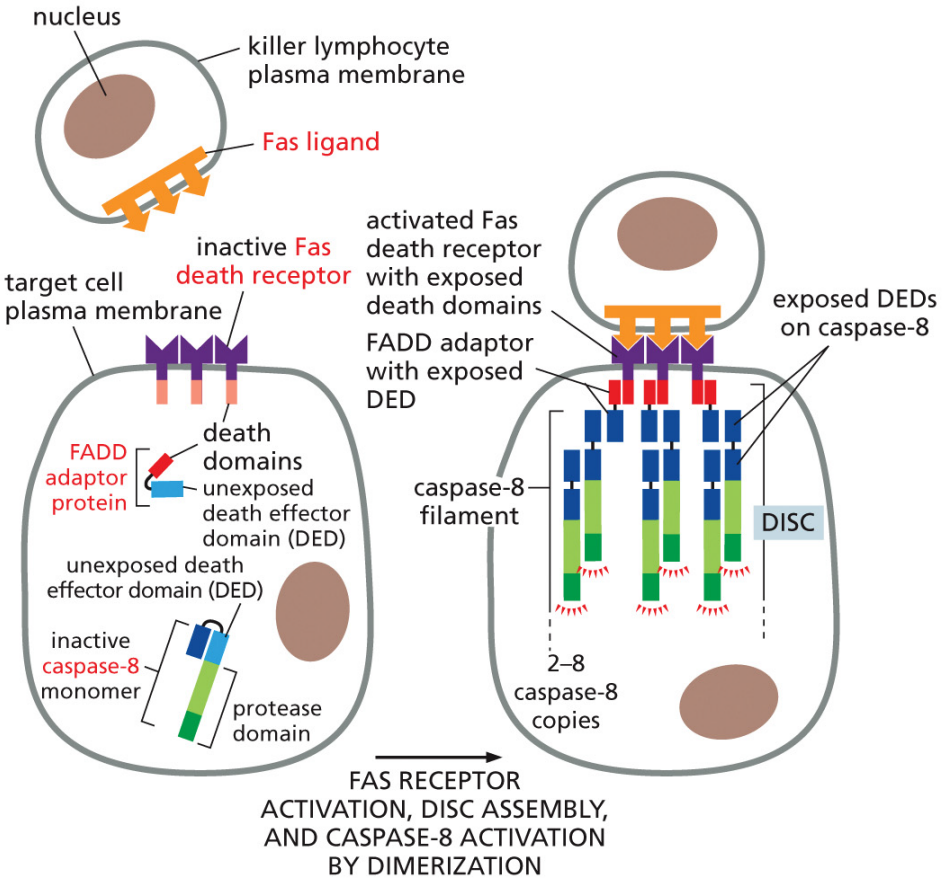
INITIATOR CASPASES AND EXECUTIONER CASPASES



Intrinsic and extrinsic pathways to activate!

THE EXTRINSIC PATHWAY OF APOPTOSIS

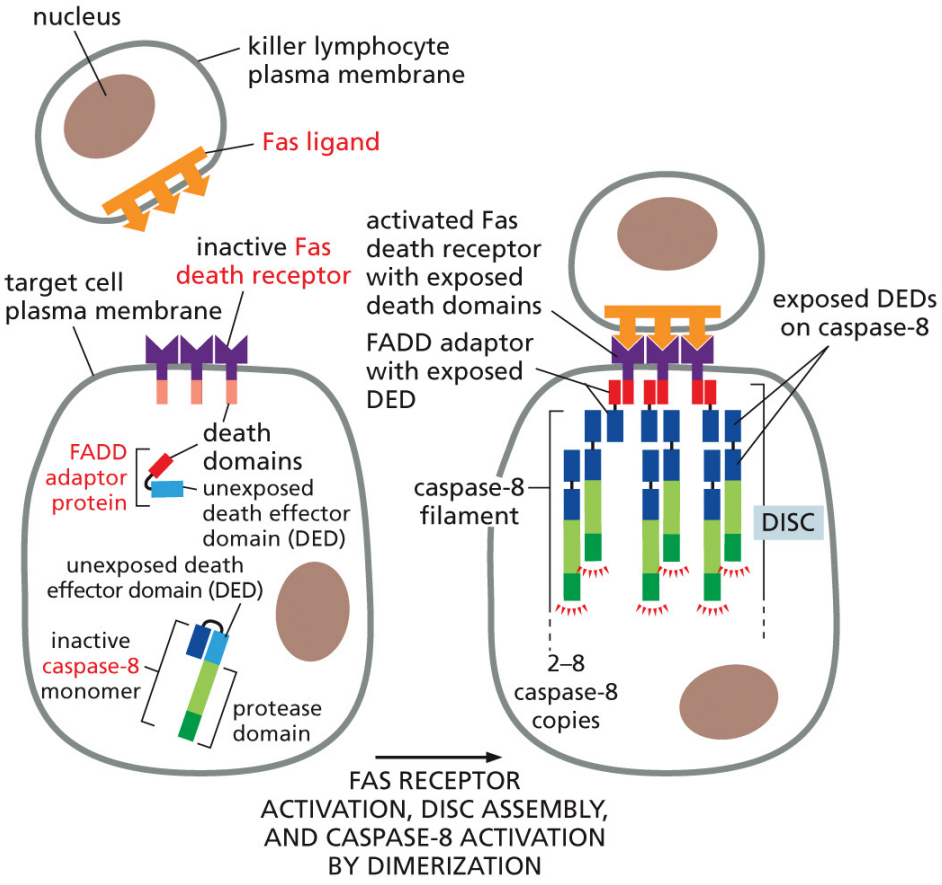
- Activation of **cell-surface death receptors** initiates the extrinsic pathway of apoptosis



- Tumor necrosis factor (TNF) family of receptors include TNF and Fas
- Single transmembrane domain homotrimers
- Extracellular domains bind ligand
- Cytosolic domains bind adaptor proteins, which bind caspases

THE EXTRINSIC PATHWAY OF APOPTOSIS

- Activation of **cell-surface death receptors** initiates the extrinsic pathway of apoptosis



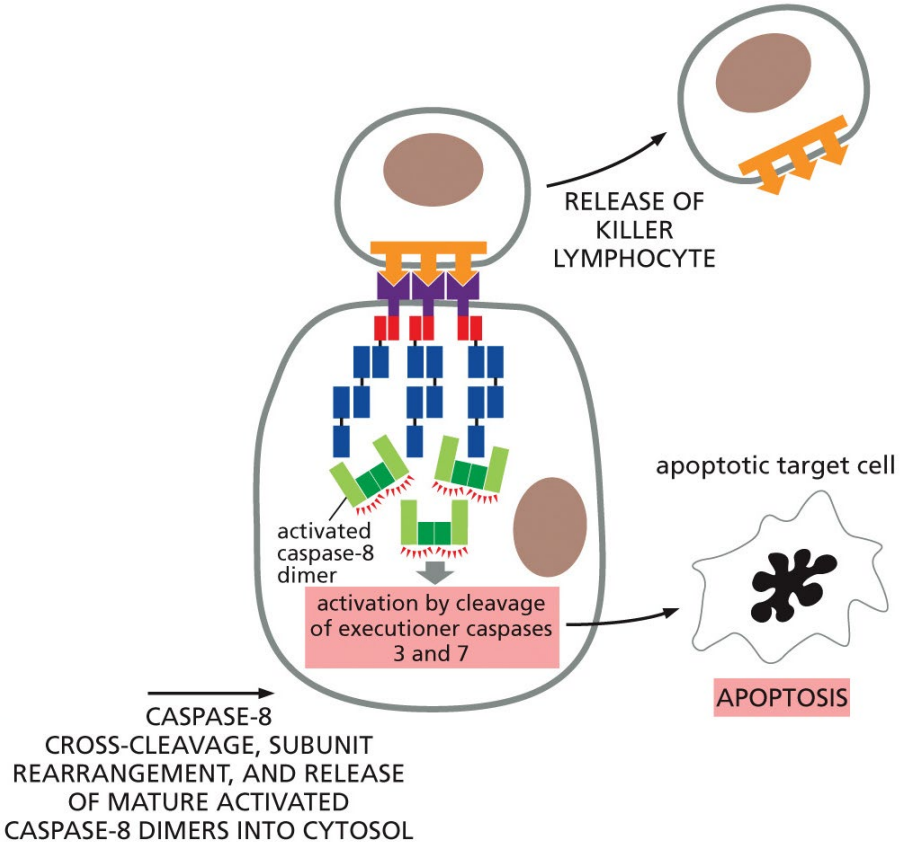
- Bound FADD protein exposes a *death effector domain* (DED), enabling FADD to recruit an inactive, monomeric initiator caspase by binding to an exposed DED on the prodomain of the caspase.
- Each caspase-8 monomer has two DEDs
- When one binds to an exposed DED, the other becomes exposed and can recruit another caspase-8 monomer
- -> chain reaction in which the caspase-8 monomers oligomerize into a three-dimensional helical filament (not shown), with each FADD protein attached to up to eight caspase-8 molecules.

-> Assembly of a large death-inducing signaling complex (DISC) composed of multiple copies of Fas, FADD, and caspase-8.

THE EXTRINSIC PATHWAY OF APOPTOSIS

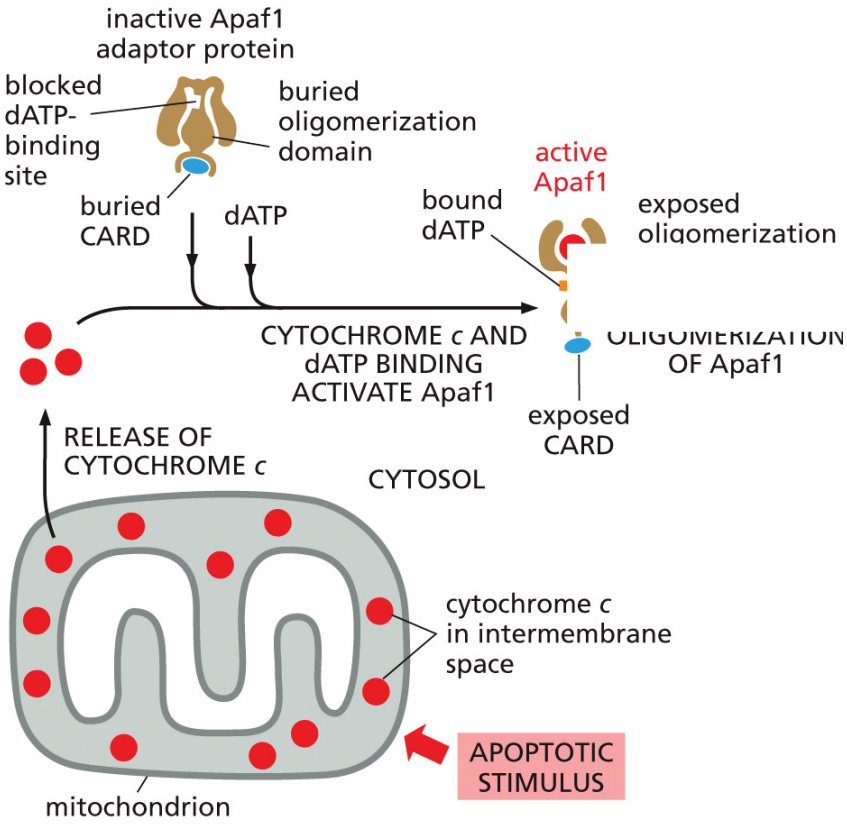
Neighboring caspase-8 monomers interact to form activated dimers → cross-cleave their partner monomers → cleave off the prodomain → mature activated dimers

Active dimers released into the cytosol → cleave and activate executioner caspases to induce apoptosis



THE INTRINSIC PATHWAY OF APOPTOSIS

- Usually initiated by *cellular stress* (UV, cytotoxic drugs, etc) causing alterations in mitochondria membrane potential (MMP)
- The intrinsic pathway of apoptosis depends on proteins released from mitochondria



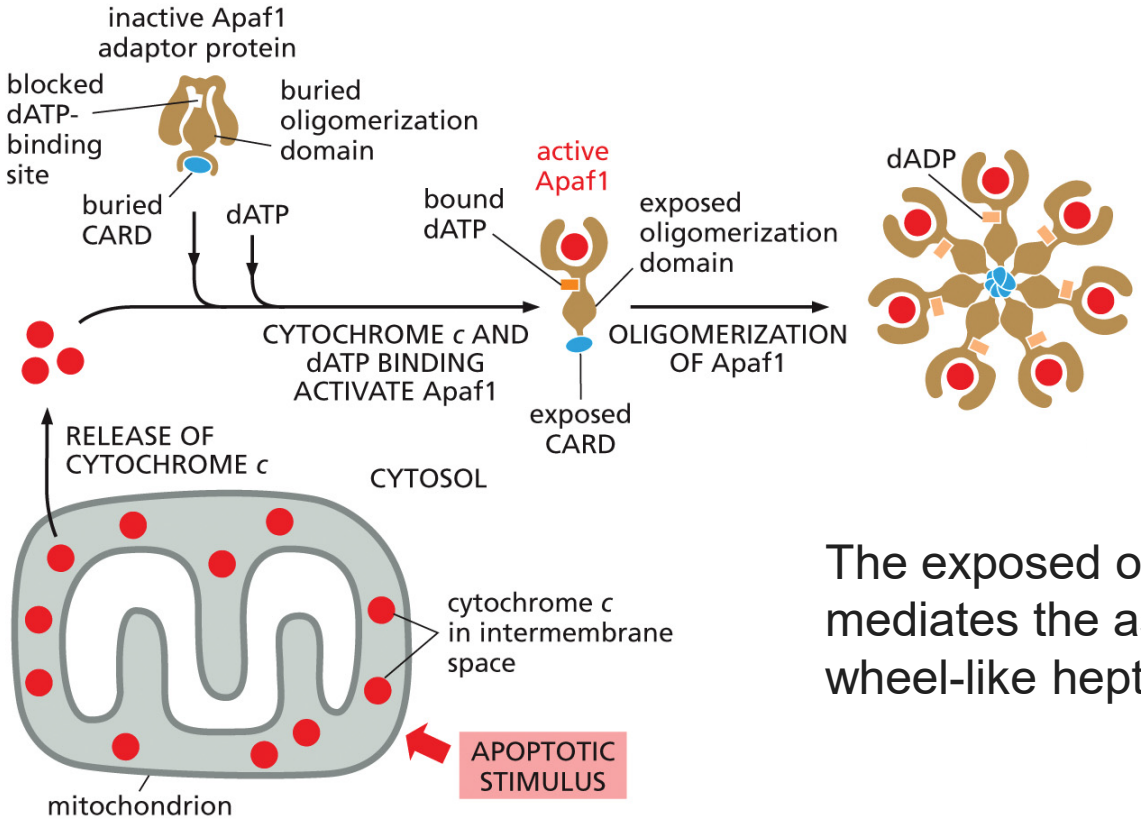
1. Cytochrome *c* binds to the cytosolic adaptor protein Apaf1
2. Conformational change in Apaf1

→ Apf1 activated

- a binding site for deoxy-ATP (dATP),
- an oligomerization domain,
- and a caspase recruitment domain (CARD) exposed.

THE INTRINSIC PATHWAY OF APOPTOSIS

- The intrinsic pathway of apoptosis depends on proteins released from mitochondria

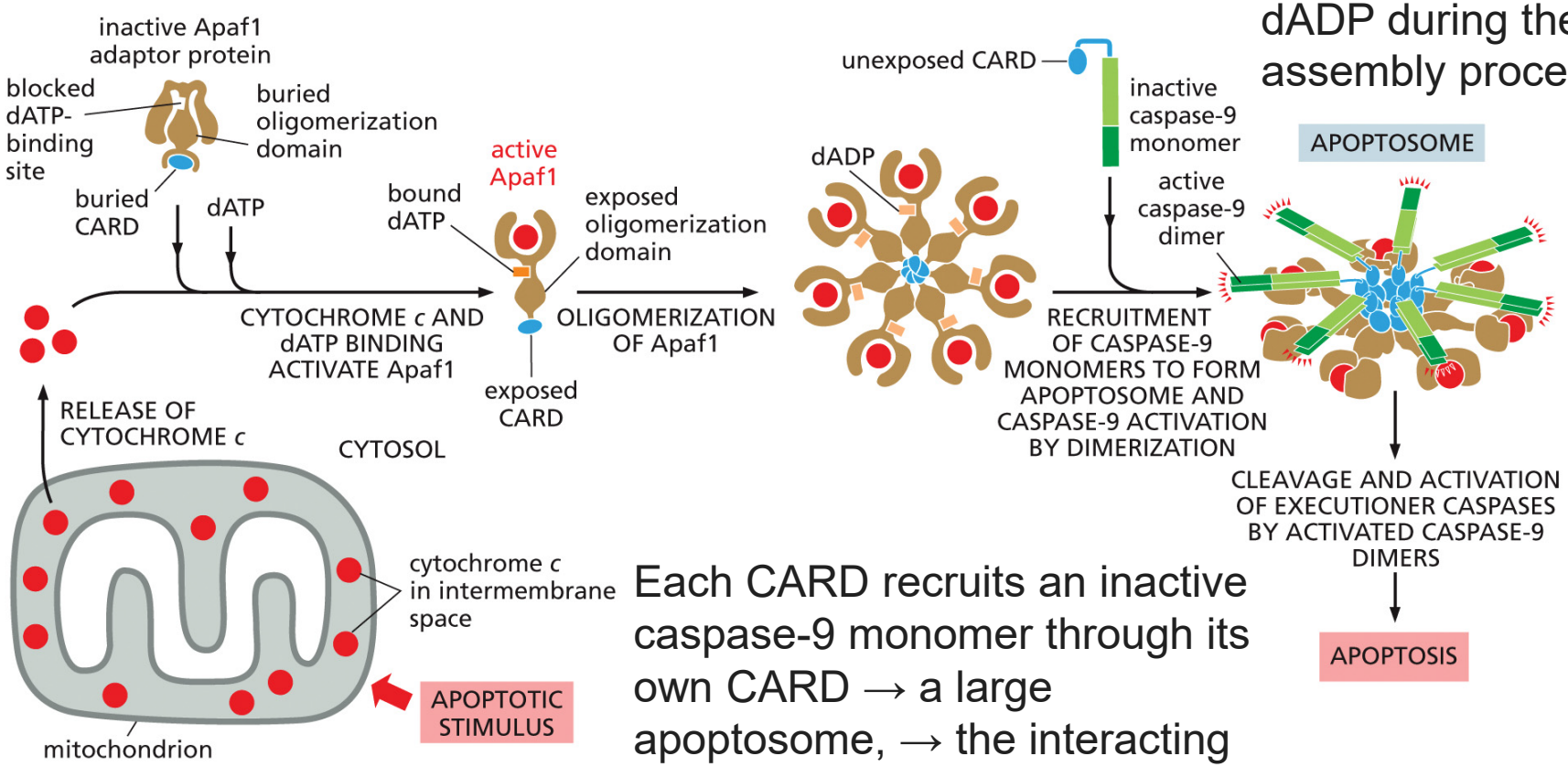


The exposed oligomerization domain mediates the assembly of Apaf1 into a wheel-like heptamer.

THE INTRINSIC PATHWAY OF APOPTOSIS

- The intrinsic pathway of apoptosis depends on proteins released from mitochondria

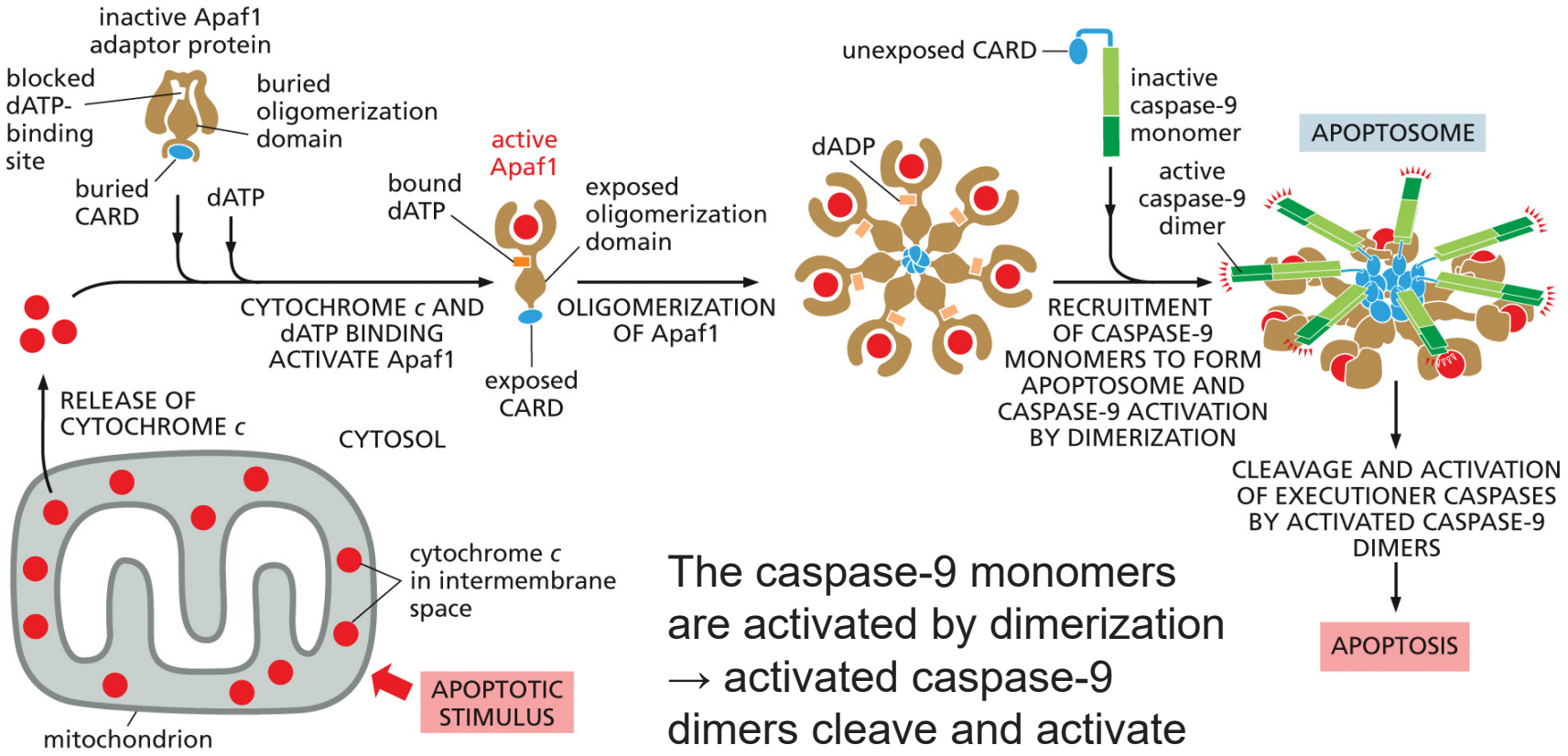
dATP is hydrolyzed to dADP during the assembly process.



Each CARD recruits an inactive caspase-9 monomer through its own CARD → a large apoptosome, → the interacting CARDs clustered above the central hub of the apoptosome.

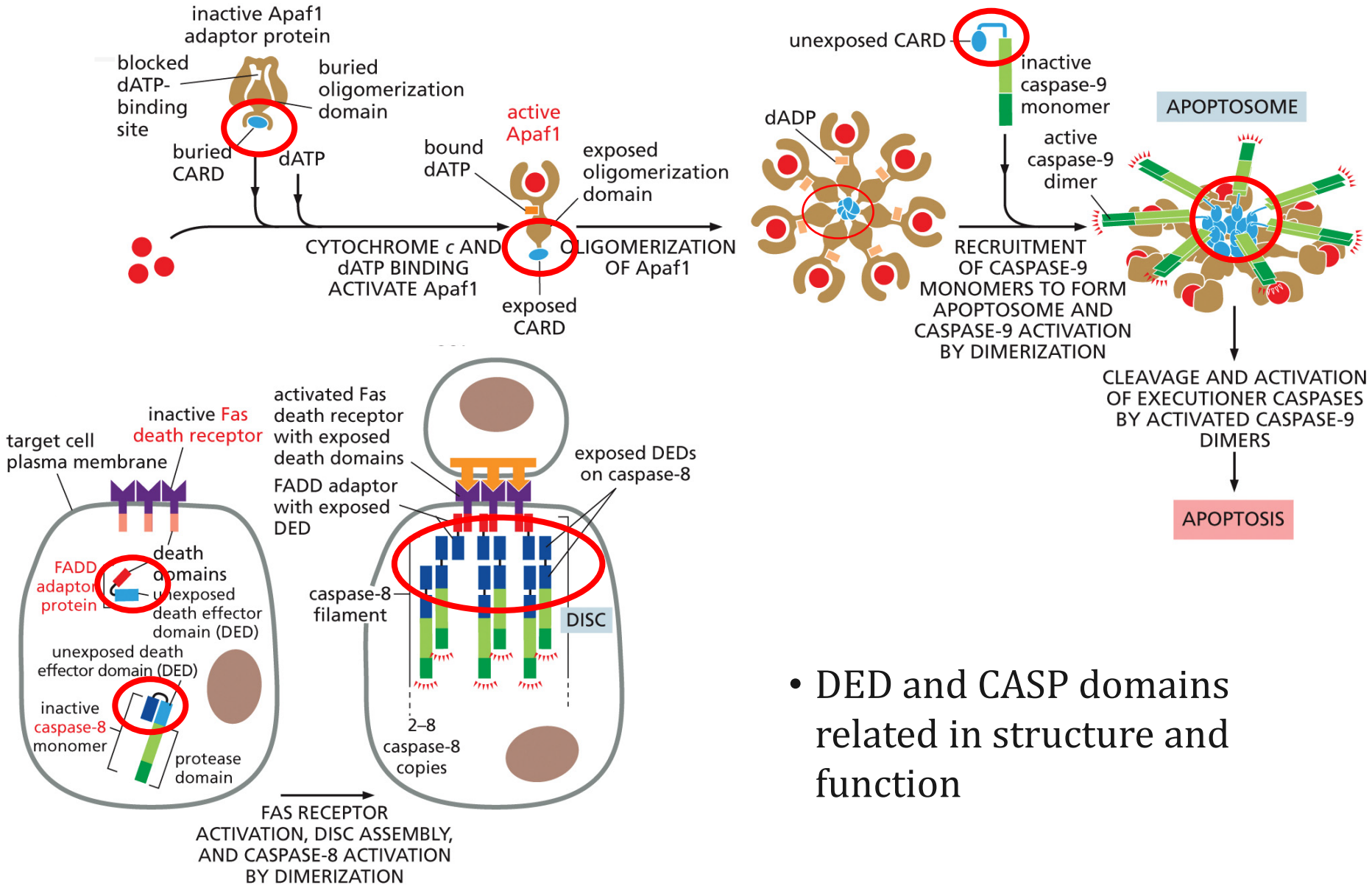
THE INTRINSIC PATHWAY OF APOPTOSIS

- The intrinsic pathway of apoptosis depends on proteins released from mitochondria



The caspase-9 monomers are activated by dimerization → activated caspase-9 dimers cleave and activate downstream executioner caspases → apoptosis.

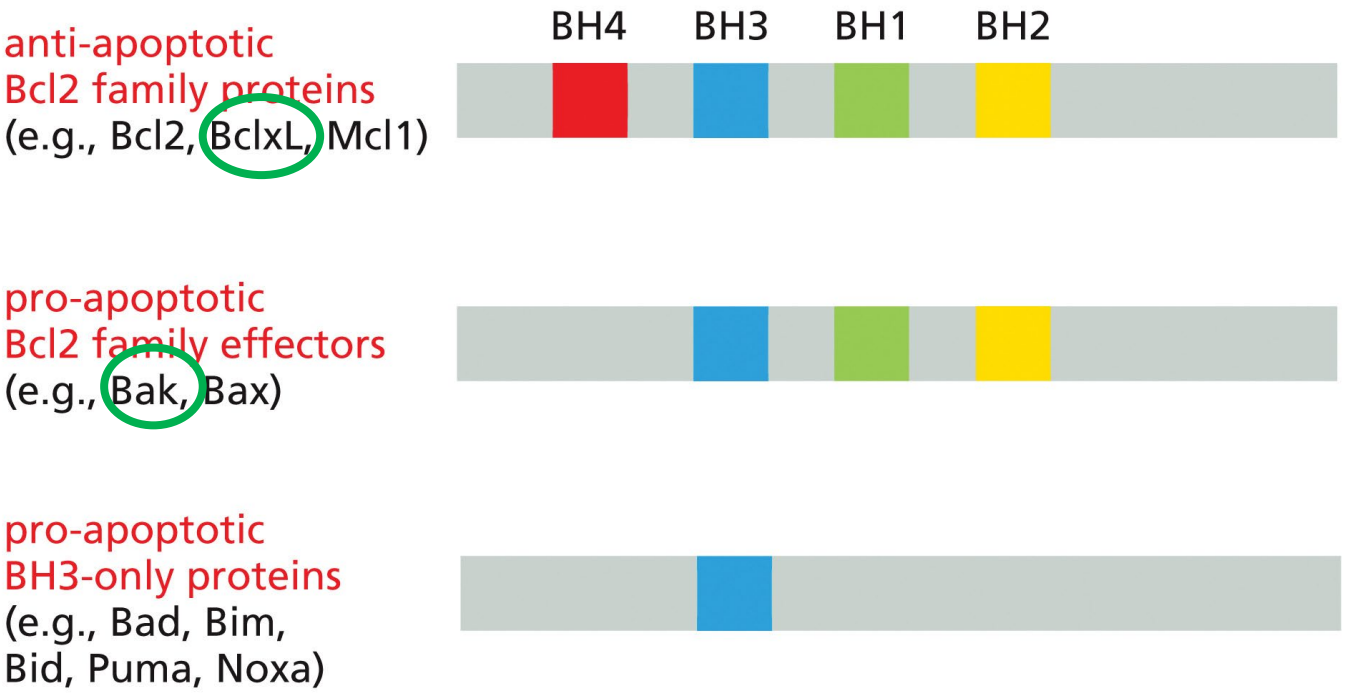
DED AND CARD DOMAINS



- DED and CASP domains related in structure and function

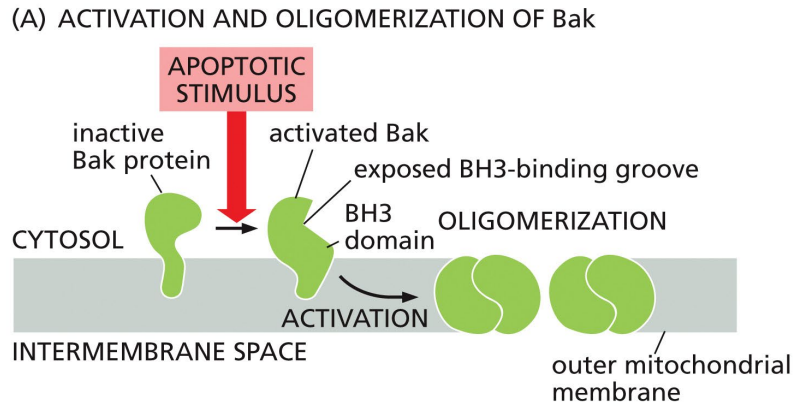
BCL2 PROTEINS CONTROL THE INTRINSIC PATHWAY

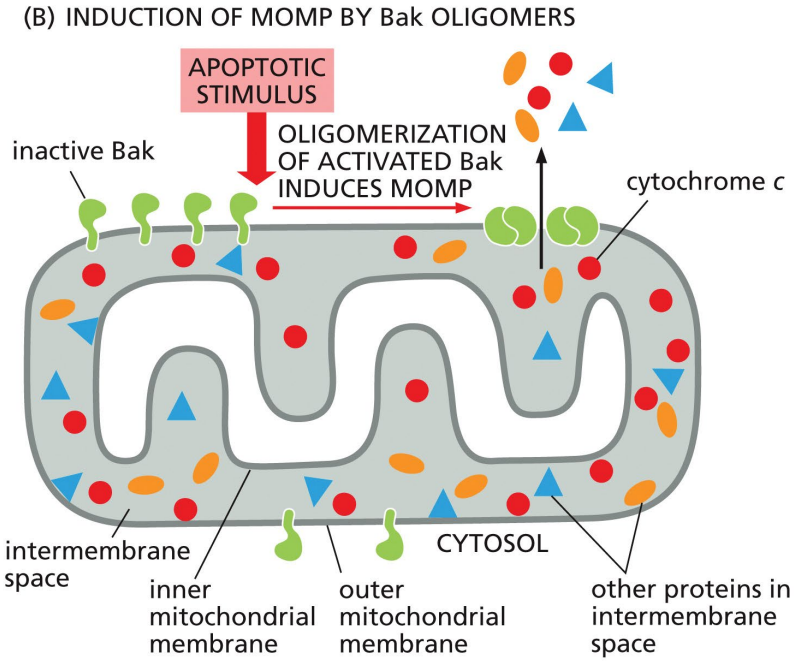
- **Bcl2 proteins** are the critical controllers of the intrinsic pathway of apoptosis
- Control e.g. the release of cytochrome c
- Both **pro-apoptotic** and **anti-apoptotic** members



BH domain mediates the direct interactions between pro-apoptotic and anti-apoptotic family members

PRO-APOPTOTIC BAK





Bak attached to the outer mitochondrial membrane

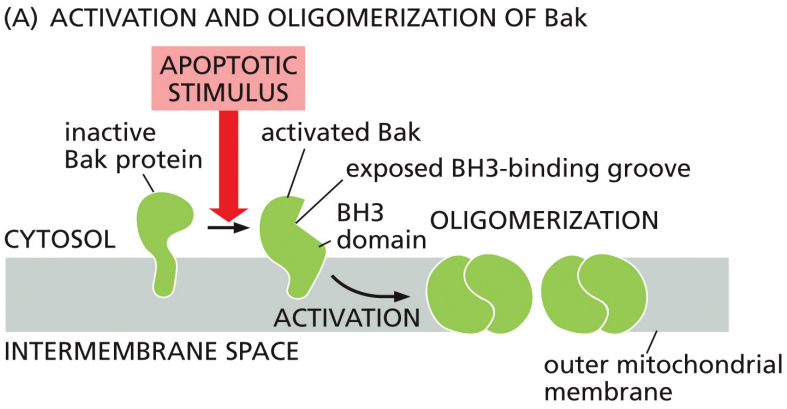
1. Apoptotic stimulus → Bak undergoes a conformational change → exposes a BH3 domain and creates a BH3-binding groove → Bak–Bak oligomerization in the outer membrane.

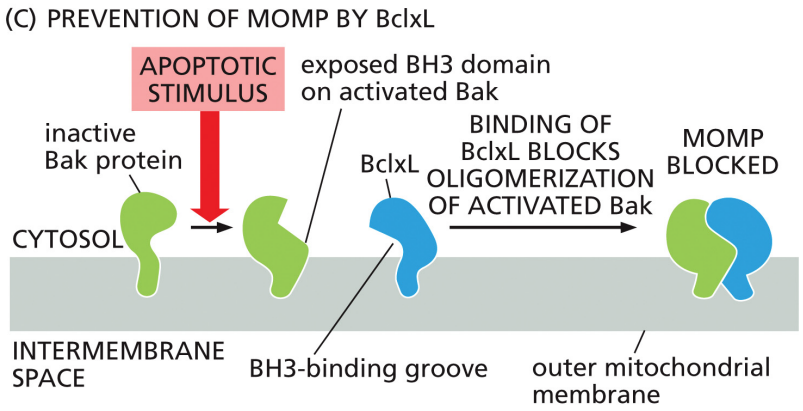
2. The Bak oligomers induce MOMP by creating openings in the outer membrane(by forming large membrane structures?) → cytochrome *c* and other soluble proteins in the intermembrane space diffuse into the

3. Cytochrome *c* in cytosol stimulates the assembly of apoptosomes

Mitochondrial outer membrane permeabilization (*MOMP*)

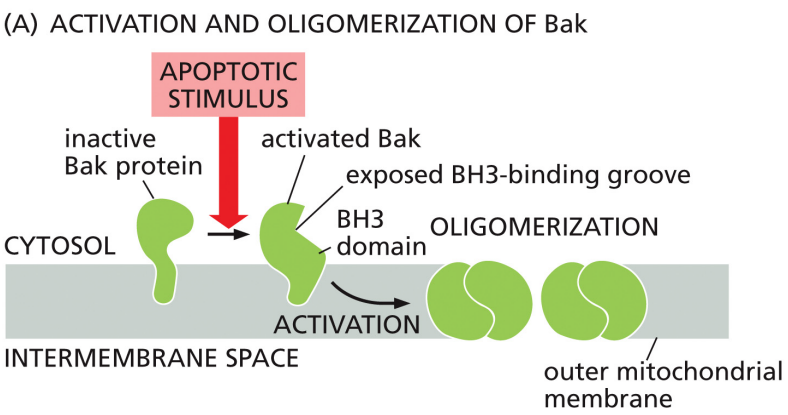
ANTI-APOPTOTIC BCLXL

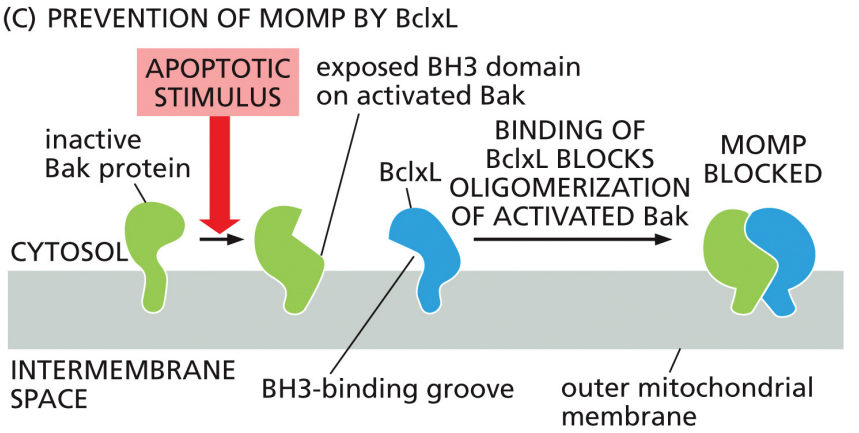


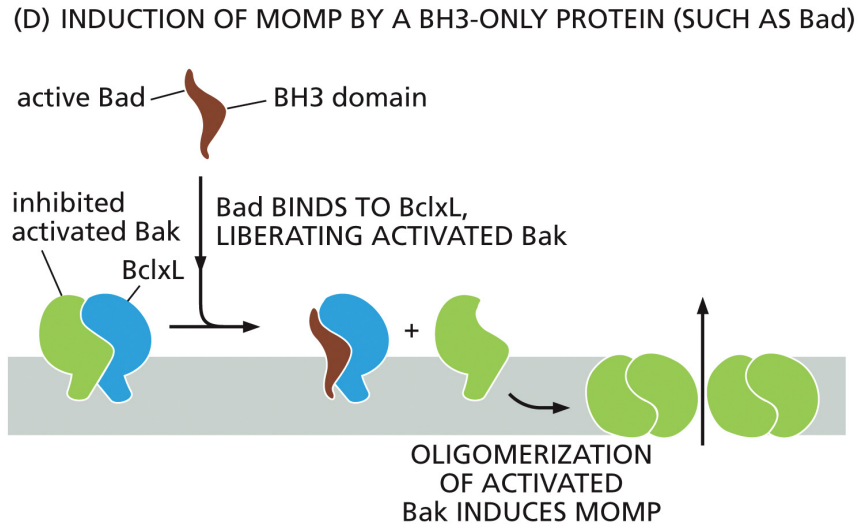


- BclxL bound to the outer mitochondrial membrane
- Interacts via its BH3-binding groove to the exposed BH3 domain on activated Bak → blocking Bak–Bak oligomerization, MOMP, and apoptosis.

ANTI-APOPTOTIC BCLXL



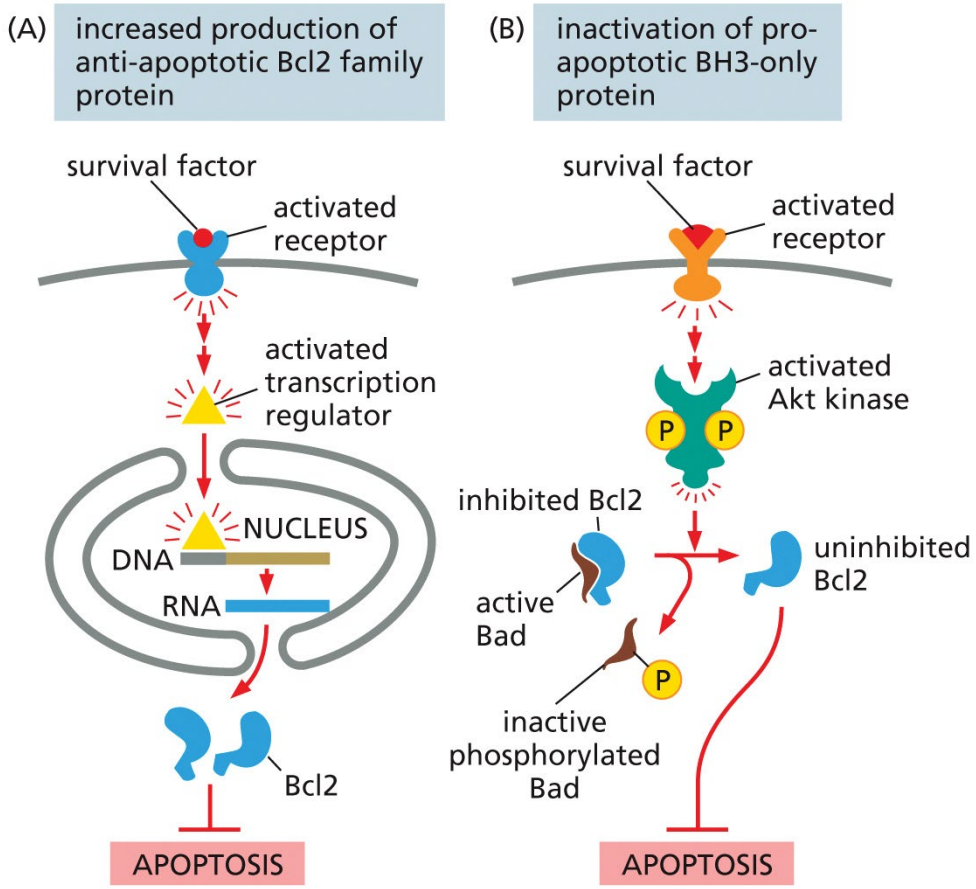




One way BH3-only proteins such as Bad are thought to indirectly induce MOMP and apoptosis is by inhibiting certain anti-apoptotic Bcl2 family proteins such as BclxL.

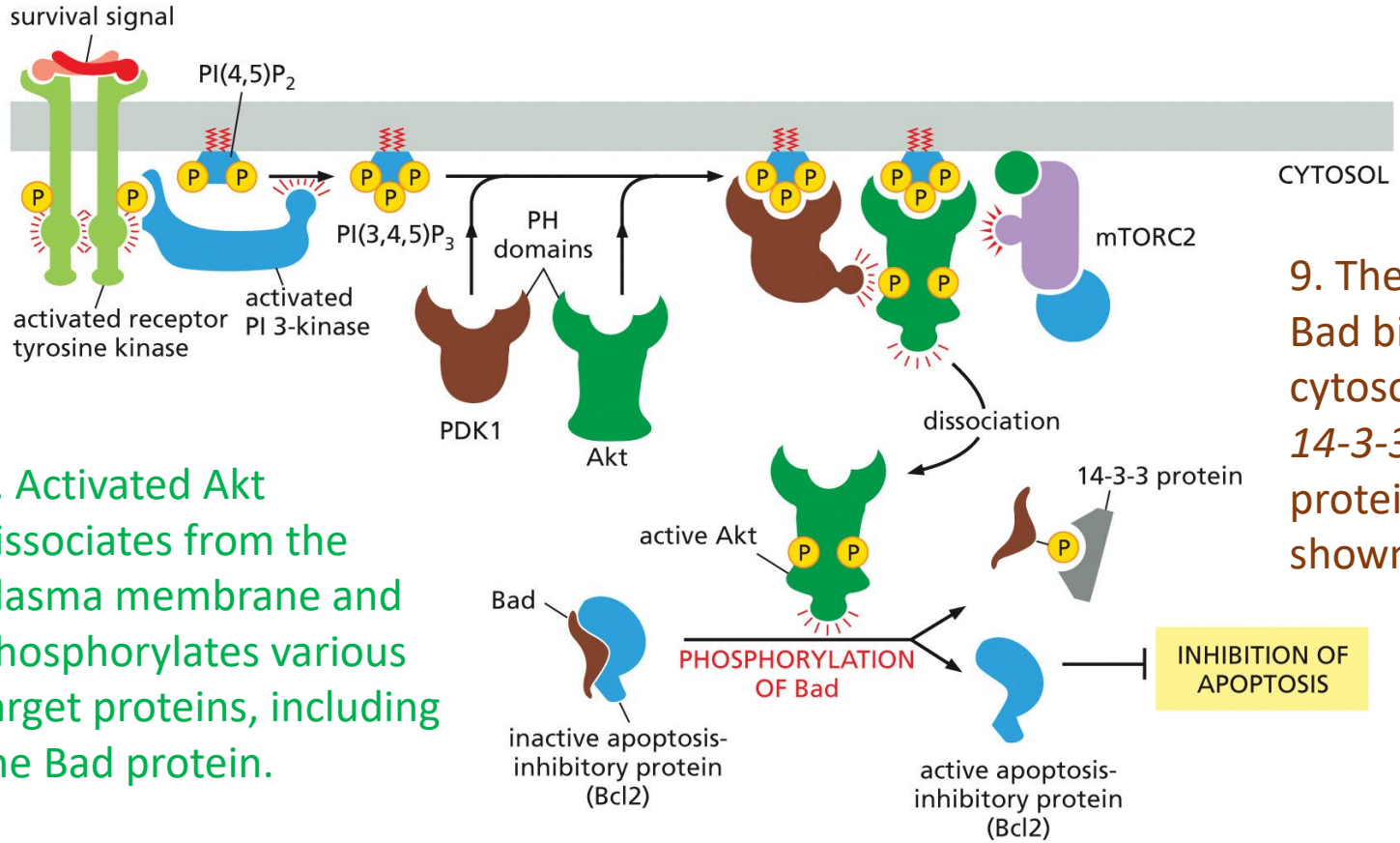
EXTRACELLULAR SURVIVAL FACTORS INHIBIT APOPTOSIS IN VARIOUS WAYS

A. Some survival factors suppress apoptosis by stimulating the transcription of genes that encode anti-apoptotic Bcl2 family proteins such as Bcl2 (BclxL)
B. Many others activate the serine/threonine protein kinase Akt → phosphorylates and inactivates the pro-apoptotic BH3-only protein Bad. Once phosphorylated, Bad dissociates from Bcl2 → Bcl2 to suppresses



PI-3-KINASE–AKT SIGNALING PATHWAY

- The PI-3-kinase–Akt signaling pathway stimulates animal cells to **survive** and grow



7. Activated Akt dissociates from the plasma membrane and phosphorylates various target proteins, including the Bad protein.

9. The phosphorylated Bad binds to a ubiquitous cytosolic protein called *14-3-3*, which keeps the protein out of action, as shown.

8. Unphosphorylated Bad holds the apoptosis-inhibitory Bcl2 in an inactive state (lecture 12). Phosphorylated, Bad releases Bcl2, which now can block apoptosis and thereby promote cell survival.

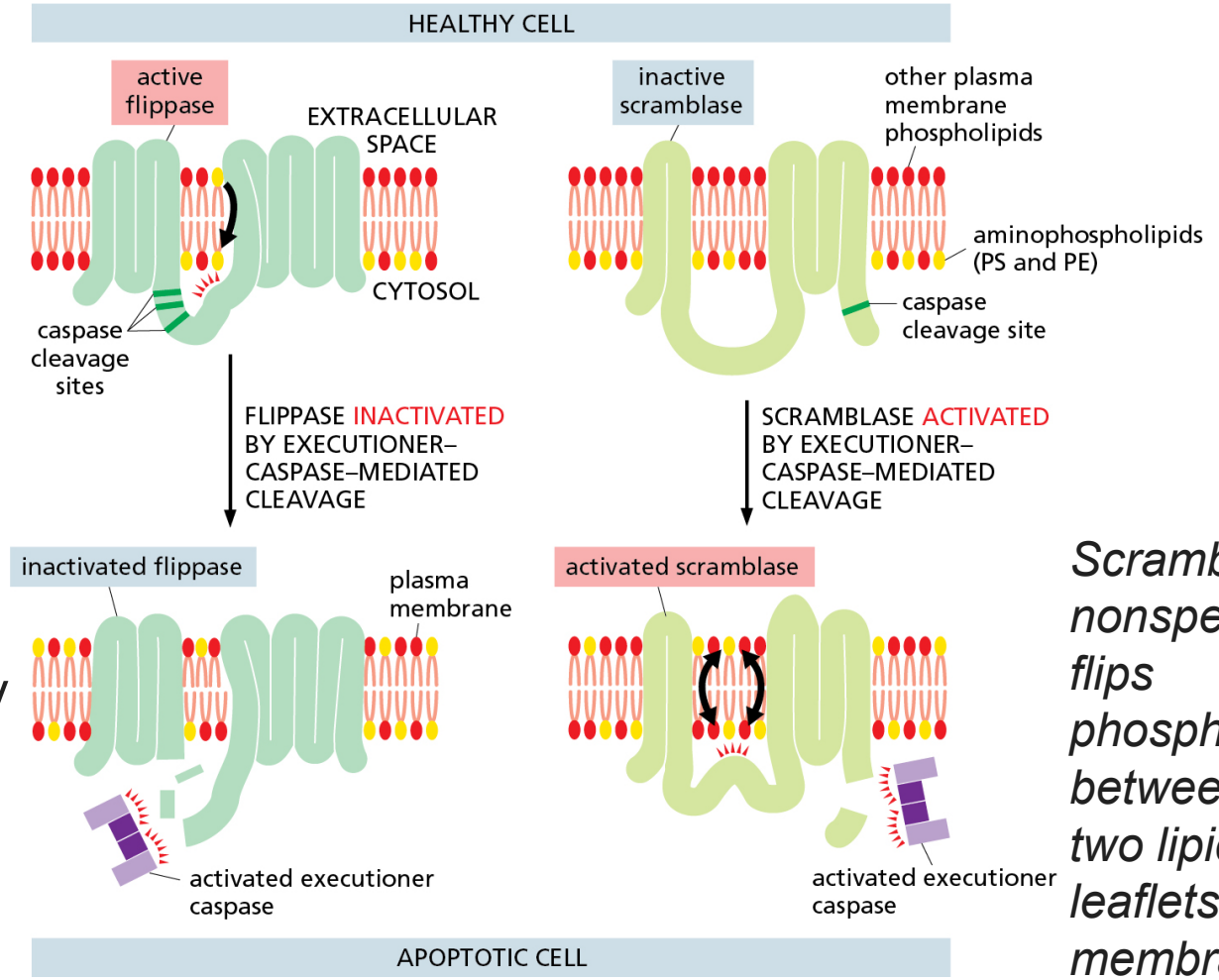
CLEARING THE DYING CELL

- A defining point of apoptosis is to clear the dying cell before it can release inflammatory molecules
- Cells undergoing apoptosis show changes in surface of plasma membrane
  - Exposure of phosphatidyl serine (PS)
  - Change in cell surface sugars – detected by lectins on phagocyte cell
- Macrophages ingesting apoptotic cells release anti-inflammatory and immunosuppressive cytokine transforming growth factor-beta1 (tgf-β1)

PHAGOCYTOSIS OF APOPTOTIC CELLS

Flippase actively flips phosphatidylserine (PS) and phosphatidylethanolamine (PE) from the outer to the inner leaflet of the plasma membrane lipid bilayer

- Healthy neighbors phagocytose and digest apoptotic cells
- Phosphatidylserine (PS) and phosphatidylethanol amine (PE) in the outer surface of apoptotic cells a key “eat me” -signal



Scramblase nonspecifically flips phospholipids between the two lipid leaflets of the membrane

SUMMARY

- Apoptosis eliminates unwanted cells
- Apoptosis depends on an intracellular proteolytic cascade mediated by caspases
- Activation of cell-surface death receptors initiates the extrinsic pathway of apoptosis
- The intrinsic pathway of apoptosis depends on proteins released from mitochondria
- Bcl2 proteins are the critical controllers of the intrinsic pathway of apoptosis
- Extracellular survival factors inhibit apoptosis in various ways
- Healthy neighbors phagocytose and digest apoptotic cells