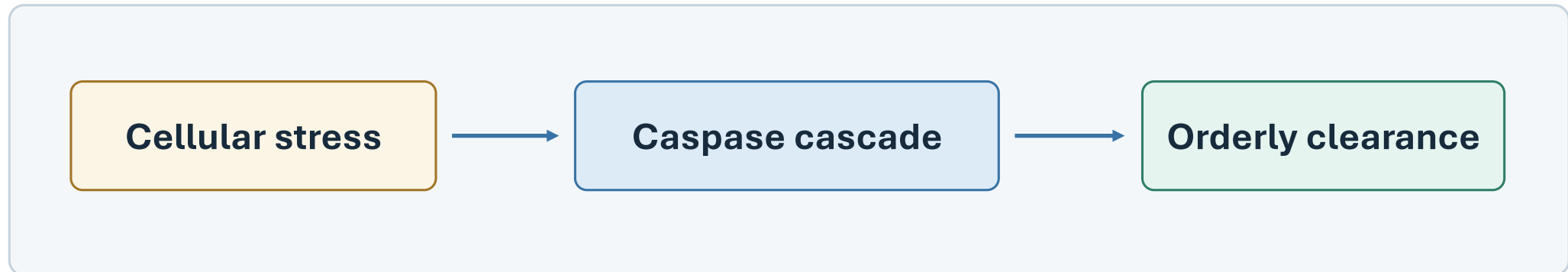


Mechanisms of Apoptosis

Intrinsic pathway • Extrinsic pathway • Caspase execution • Efferocytosis



Dr Abu Suraih Sakhri

MBBS, MD (Pathology)

www.abusuraihsakhri.com

Learning objectives

CORE MECHANISM

After this session, learners should be able to:

Initiation

Distinguish the intrinsic mitochondrial trigger from death-receptor initiation.

Regulation

Classify BCL2-family proteins and identify the main checkpoints.

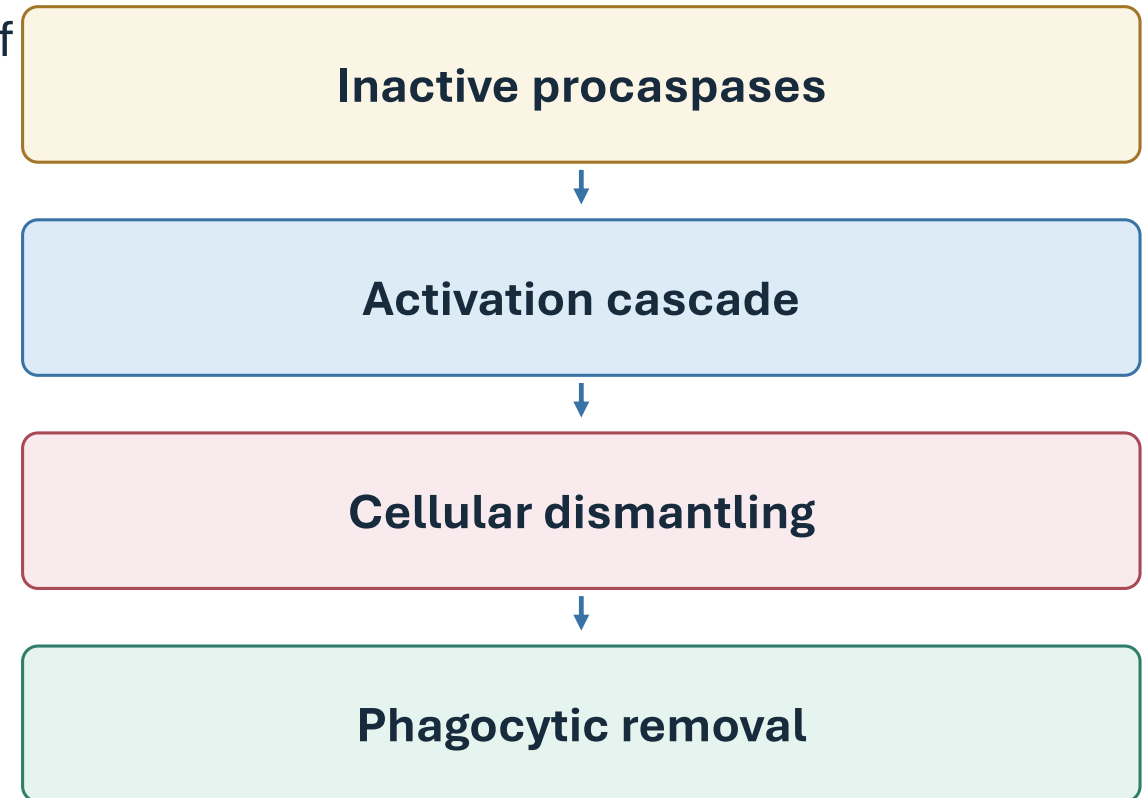
Execution

Trace initiator and executioner caspases to fragmentation and clearance.

Apoptosis is a caspase-dependent program

BIOLOGIC LOGIC

- Caspases are the core proteolytic enzymes of apoptosis.
- Activation is regulated rather than constitutively present.
- Proteolytic events dismantle cells into fragments suitable for clearance.



Two operational phases: Initiation and execution

BIOLOGIC LOGIC

- Initiation: Signal-specific activation of upstream caspases.
- Execution: Downstream proteases act on nuclear and cytoskeletal substrates.
- The phases are sequential, but their molecular triggers differ.

Initiation

Input-specific molecular assemblies generate active initiator caspases.

Execution

Executioner caspases cleave multiple cellular targets.

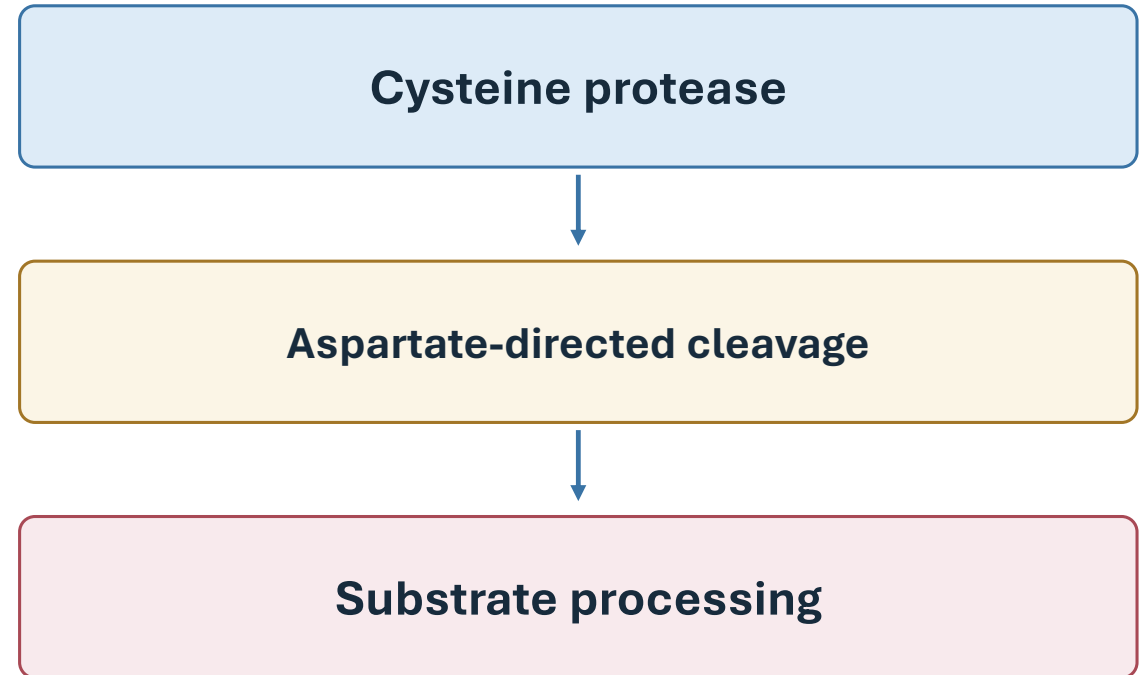
Outcome

Apoptotic fragments are recognized and engulfed.

Why they are called caspases

BIOLOGIC LOGIC

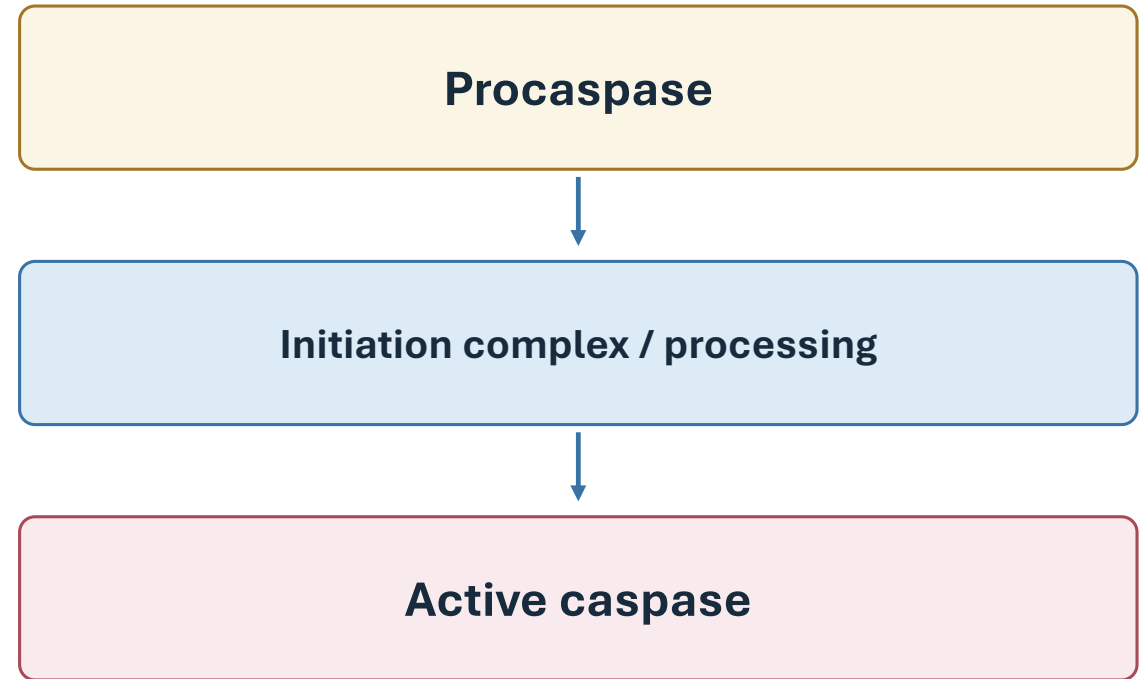
- Proteases with an active-site cysteine residue.
- Their substrates are cleaved following aspartate residues.
- Enzymatic specificity is central to the apoptotic program.



Procaspases are inactive precursors

BIOLOGIC LOGIC

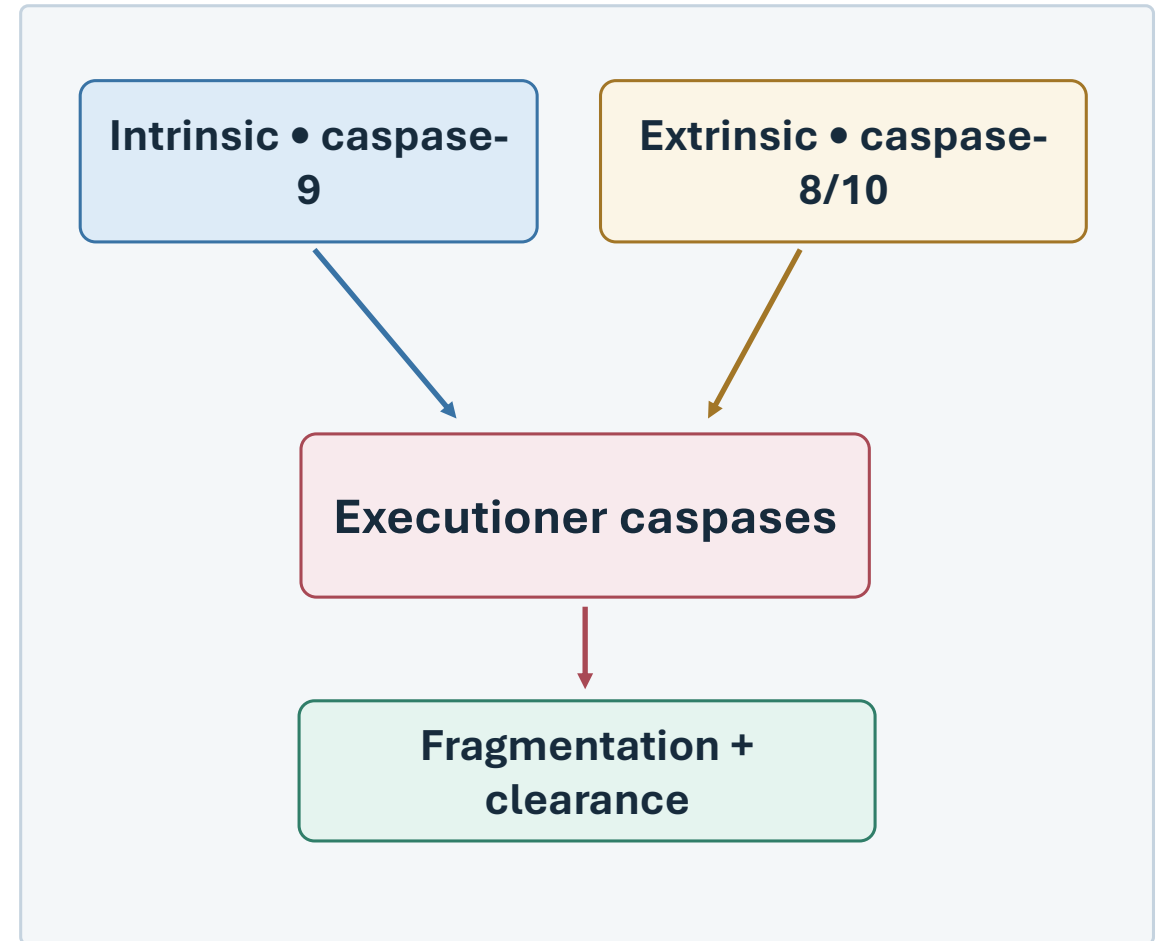
- Caspases are initially synthesized as inactive proenzymes.
- Proteolytic processing or activation within initiation complexes enables activity.
- Active caspases provide evidence of ongoing apoptotic signaling.



Initiator and executioner caspases have different jobs

BIOLOGIC LOGIC

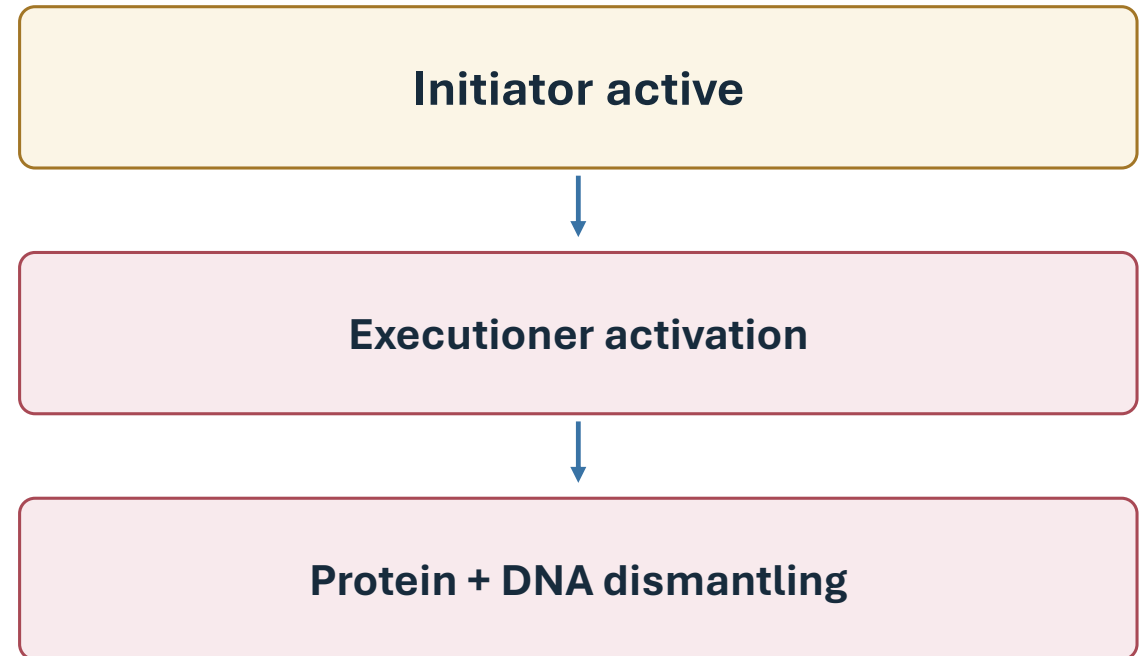
- The intrinsic pathway activates caspase-9.
- The receptor pathway activates caspase-8 (and caspase-10).
- Executioners include caspase-3 and caspase-6 in this Robbins account.



Caspase cascades convert signals into action

BIOLOGIC LOGIC

- Activated initiators can cleave downstream procaspases.
- Sequential activation accelerates the execution phase.
- Multiple substrates can be cleaved after convergence.



Survival requires multiple molecular brakes

BIOLOGIC LOGIC

- Cells express antiapoptotic proteins that prevent initiation.
- Cytosolic inhibitors can restrain active caspases.
- Apoptosis reflects the balance of opposing regulatory signals.

Mitochondrial brake

BCL2, BCL-XL and MCL1 safeguard outer-membrane integrity.

Caspase brake

IAP proteins oppose inappropriate caspase activation.

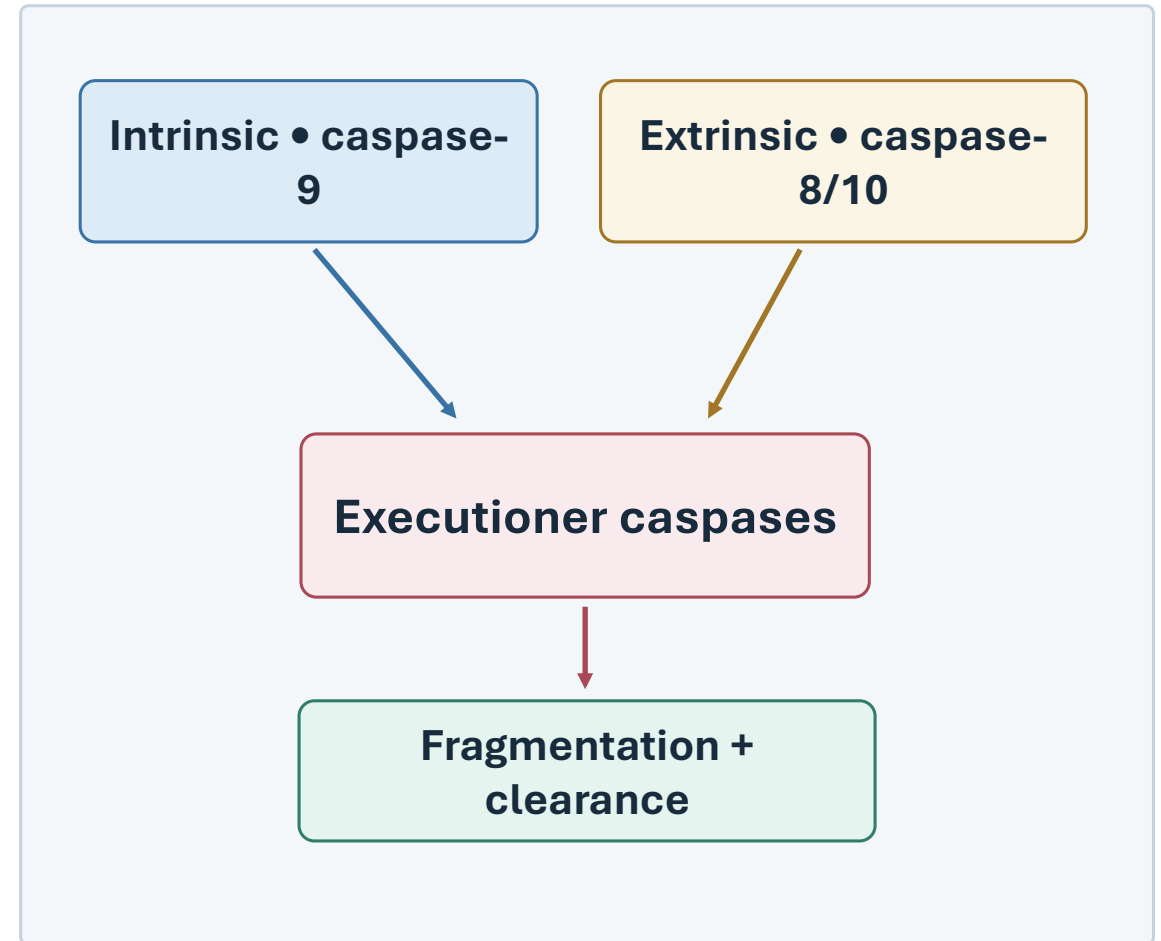
Pro-death drive

BH3-only proteins and BAX/BAK support mitochondrial permeabilization.

Two routes converge on execution

BIOLOGIC LOGIC

- Intrinsic route: Changes at the mitochondrial outer membrane.
- Extrinsic route: Death-receptor signaling at the plasma membrane.
- Both activate executioner caspases; Their regulation is distinct.



Intrinsic and extrinsic initiation are distinct

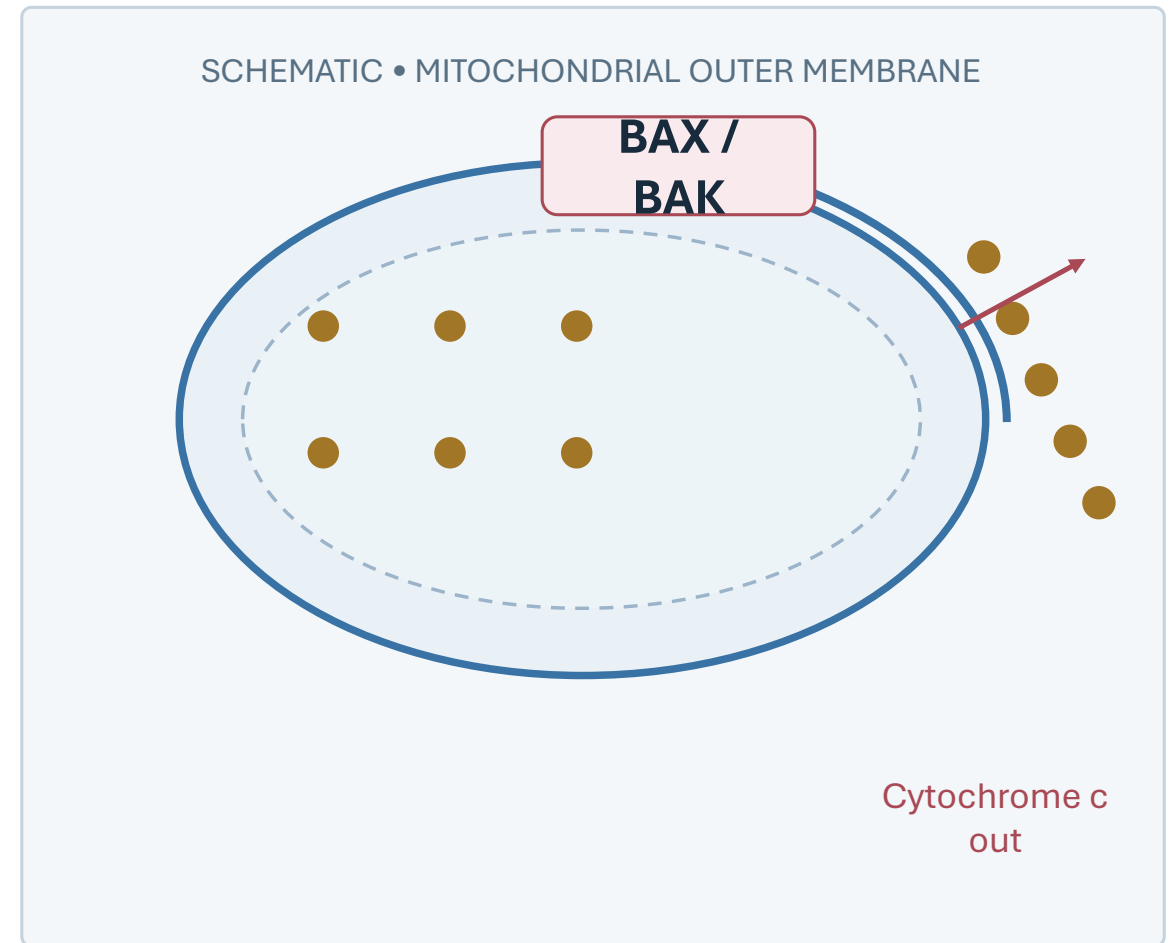
MECHANISTIC COMPARISON

Feature	Intrinsic (mitochondrial)	Extrinsic (death receptor)
Typical inputs	Survival withdrawal; DNA damage; ER stress	Fas ligand or other death-receptor ligation
Proximal regulator	BCL2 family; BAX and BAK	Death domain; Adaptor FADD
Initiator caspase	Caspase-9	Caspase-8 or caspase-10
Final target	Executioner caspases	Executioner caspases

Intrinsic apoptosis: The dominant pathway

BIOLOGIC LOGIC

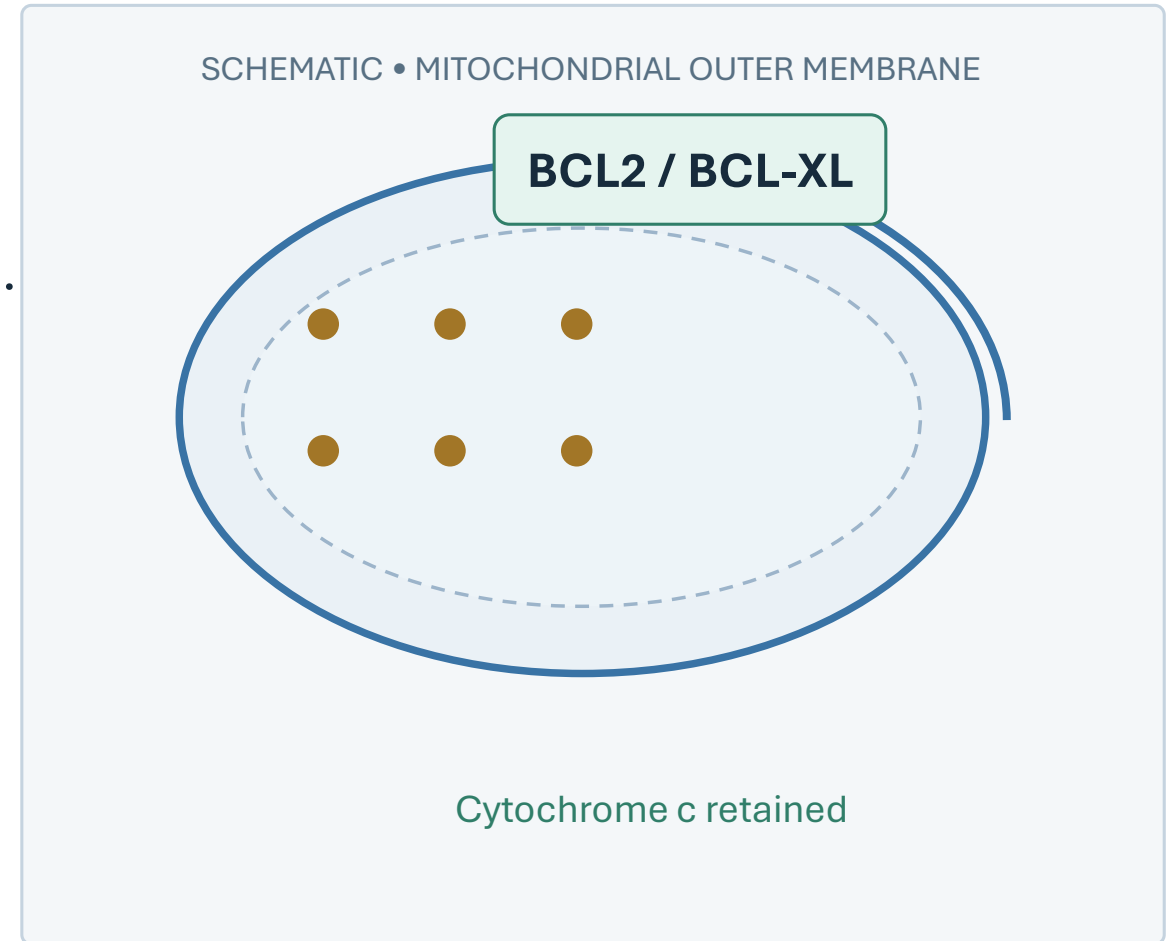
- Most physiologic and pathologic apoptotic scenarios use the mitochondrial pathway.
- The decisive event is increased permeability of the mitochondrial outer membrane.
- Pro-death proteins are thereby released into the cytosol.



The mitochondrial outer membrane is the checkpoint

BIOLOGIC LOGIC

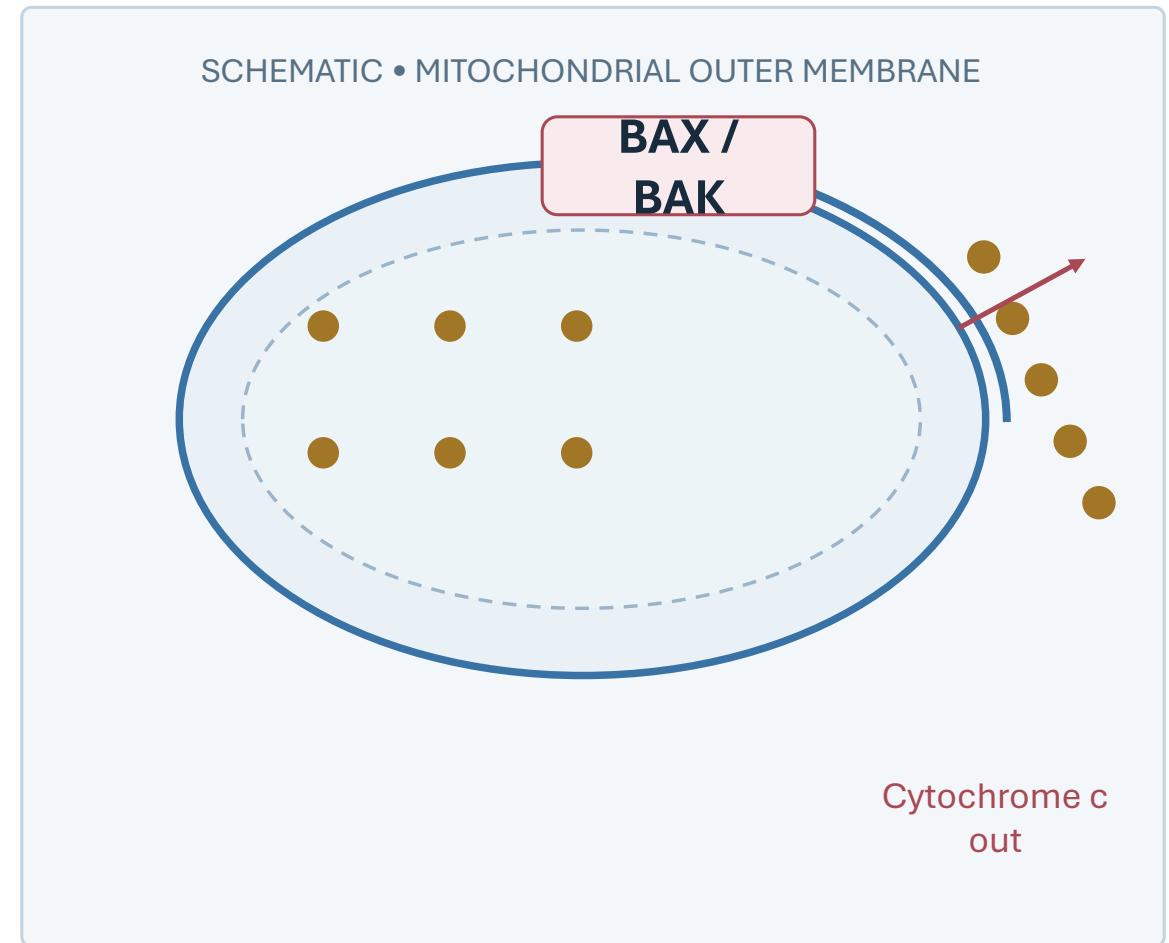
- Intact outer membrane retains death-inducing mitochondrial proteins.
- BCL2-family proteins determine susceptibility to membrane permeabilization.
- Once released, these proteins drive a caspase cascade.



Cytochrome c changes meaning with location

BIOLOGIC LOGIC

- Within mitochondria it participates in energy generation.
- When present in cytosol it can recruit the apoptotic machinery.
- Compartmental release links organellar damage to cell death.



Three functional BCL2-family groups

BIOLOGIC LOGIC

- Antiapoptotic members maintain outer membrane integrity.
- BAX and BAK are membrane-permeabilizing effectors.
- BH3-only proteins act as regulated initiators or sensors.

Antiapoptotic

BCL2 · BCL-XL · MCL1

Effectors

BAX · BAK

BH3-only initiators

BAD · BIM · BID · PUMA · NOXA

BH domains organize the BCL2 family

BIOLOGIC LOGIC

- Antiapoptotic members typically carry BH1–BH4.
- BAX/BAK possess BH1–BH3 classification.
- BH3-only proteins contain the BH3 interaction region.

BCL2 HOMOLGY (BH) DOMAINS • CONCEPTUAL

Antiapoptotic

BH1

BH2

BH3

BH4

BAX / BAK

BH1

BH2

BH3

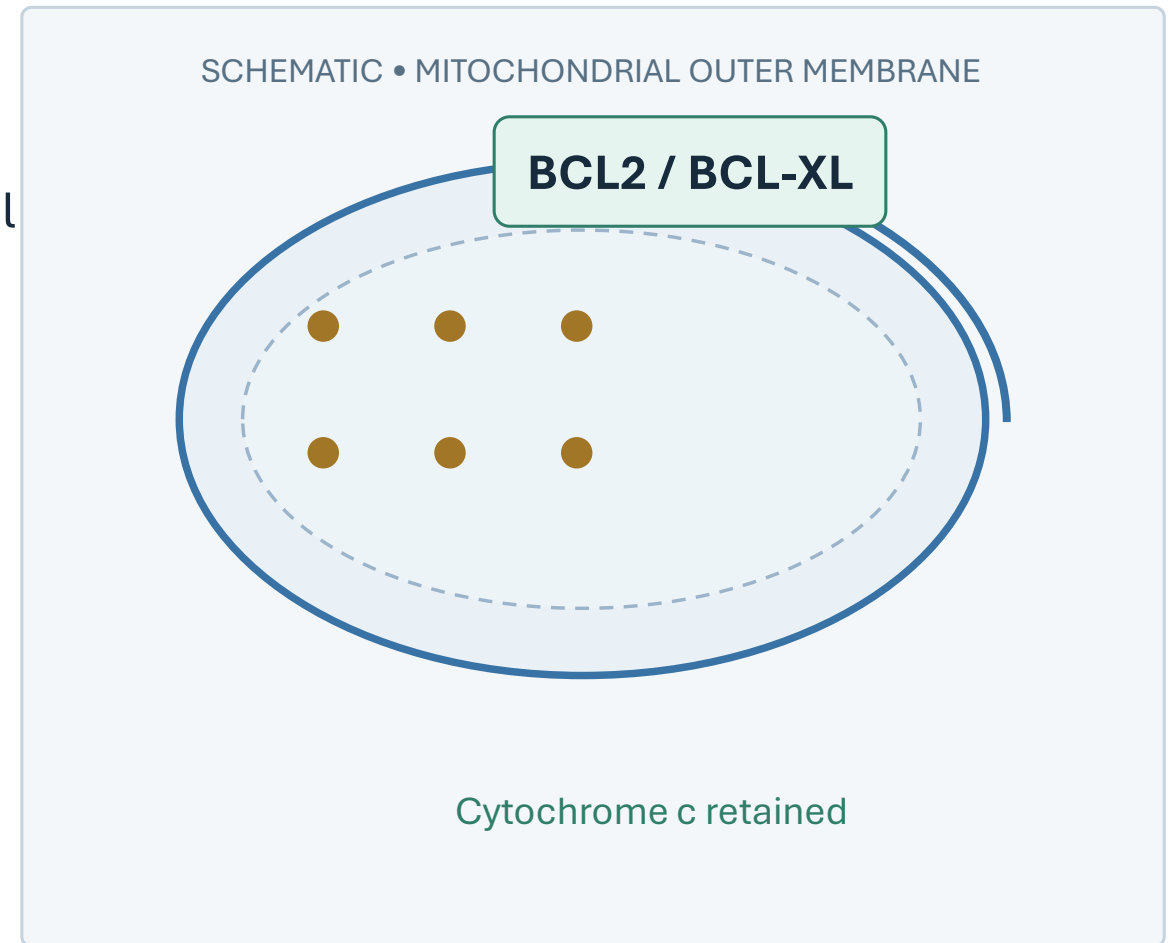
BH3-only

BH3

Antiapoptotic BCL2-family proteins

BIOLOGIC LOGIC

- BCL2, BCL-XL and MCL1 are key protective proteins.
- They preserve an impermeable mitochondrial outer membrane.
- Their influence prevents cytochrome c leakage and downstream apoptosis.



BCL2-family protection is not only mitochondrial

BIOLOGIC LOGIC

- Antiapoptotic members occur in outer mitochondrial membranes.
- They can also be present in the cytosol and ER membranes.
- The main pathway outcome remains suppression of mitochondrial protein release.

Mitochondrion

Outer-membrane protection preserves cytochrome c compartmentalization.

Cytosol

BCL2-family protein distribution extends beyond mitochondria.

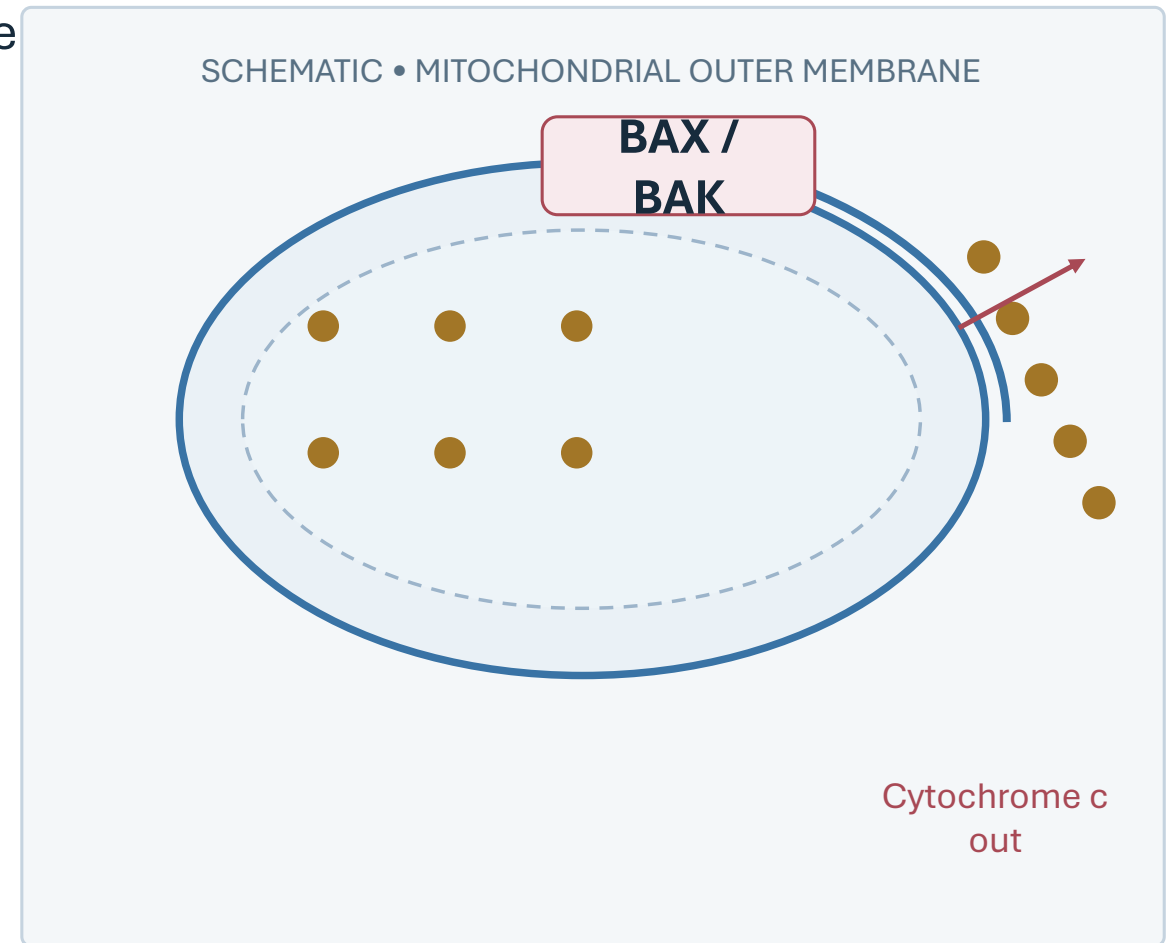
Endoplasmic reticulum

Some protective BCL2-family members also reside at ER membranes.

BAX and BAK are the membrane effectors

BIOLOGIC LOGIC

- Activation permits their oligomerization at the outer membrane.
- BAX/BAK assemblies increase membrane permeability.
- This is the immediate link between BH3 stress sensors and protein release.



How BAX/BAK permeabilize membranes remains debated

BIOLOGIC LOGIC

- A pore or channel model is commonly used in pathway schematics.
- The precise mechanism is not fully settled.
- Diagrams should describe oligomerization and permeabilization without asserting a definitive pore structure.

Established

BAX/BAK oligomerization increases outer-membrane permeability.

Working model

Oligomers may provide routes for cytochrome c escape.

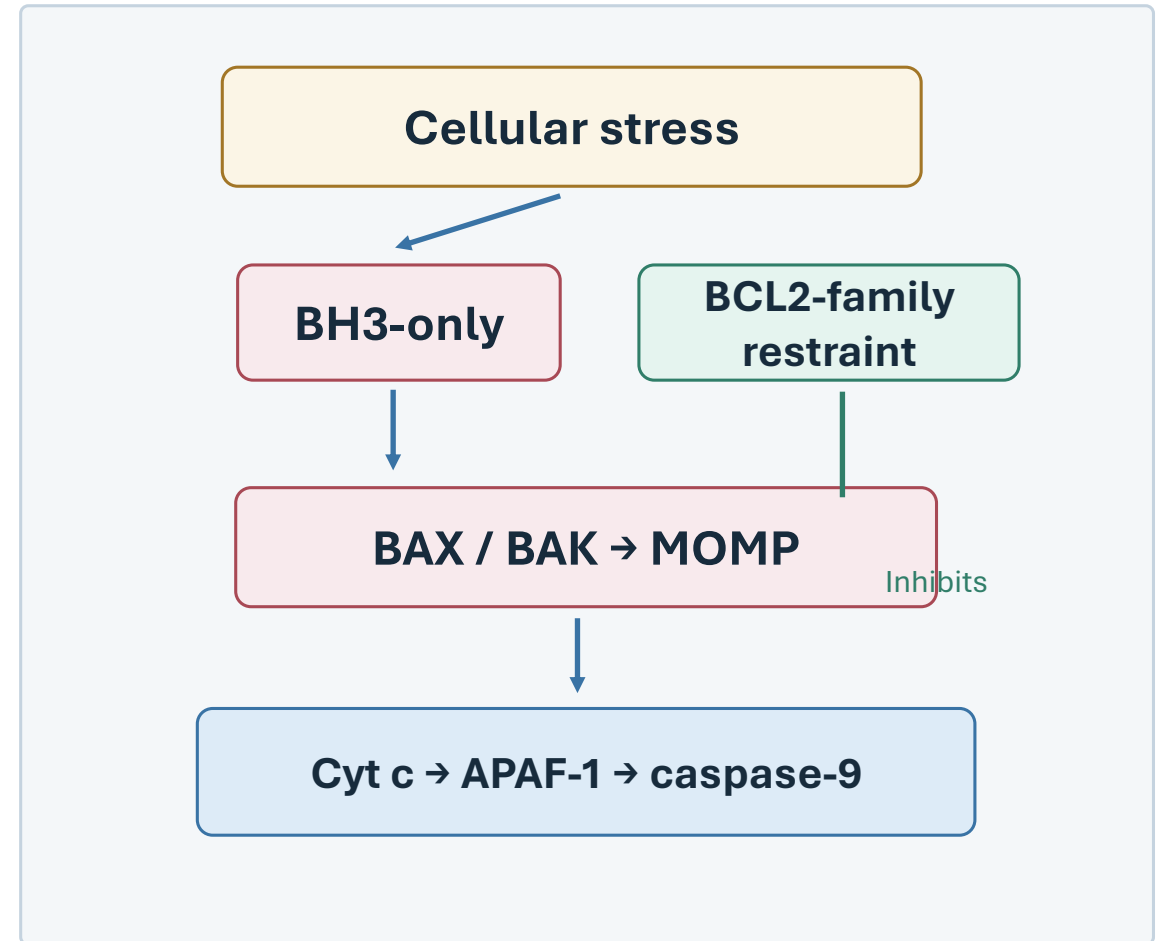
Limit of certainty

The full structural mechanism remains unresolved.

BH3-only proteins connect injury to death

BIOLOGIC LOGIC

- BH3-only proteins respond to cell stress and damage.
- Their activity changes via expression and posttranslational regulation.
- They initiate apoptosis by acting on BAX/BAK or on antiapoptotic proteins.



The BH3-only initiator repertoire

BIOLOGIC LOGIC

- Representative members include BAD and BIM.
- BID can transmit signals from extrinsic to intrinsic apoptosis.
- PUMA and NOXA are additional BH3-only regulators.

BAD · BIM

BH3-only members supporting the proapoptotic response.

BID

BH3-only connector that can be cleaved by active caspase-8.

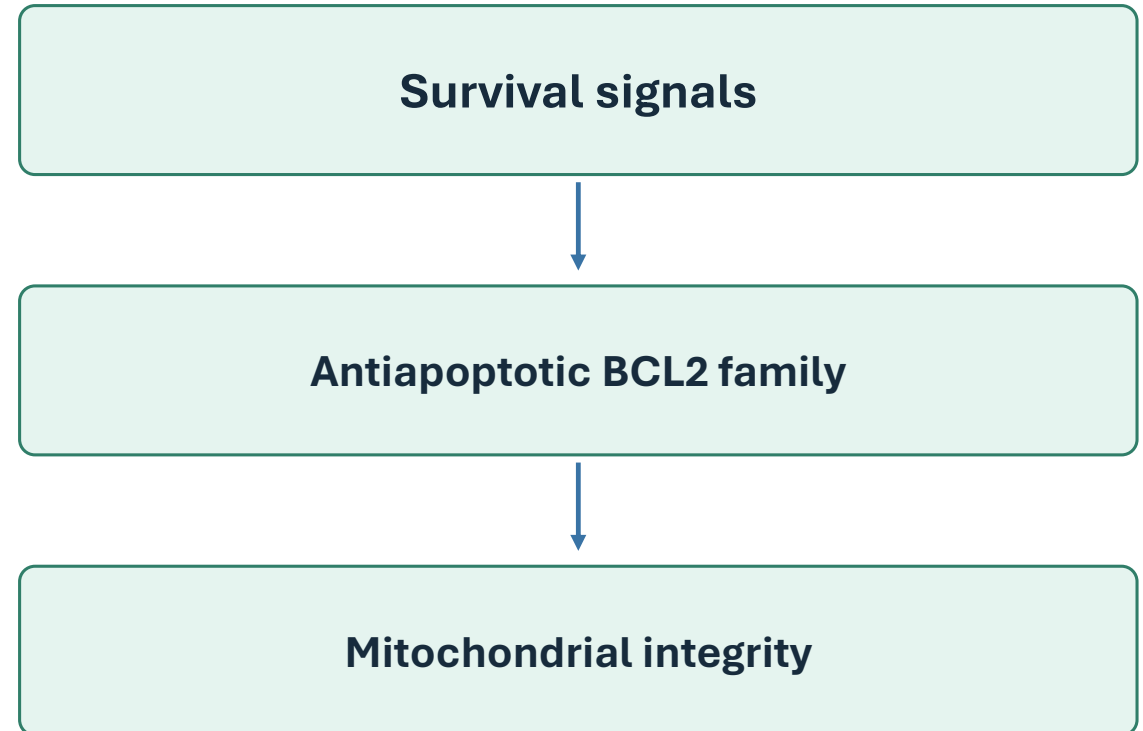
PUMA · NOXA

Additional BH3-only members upregulated during stress responses.

Growth-factor signals promote survival

BIOLOGIC LOGIC

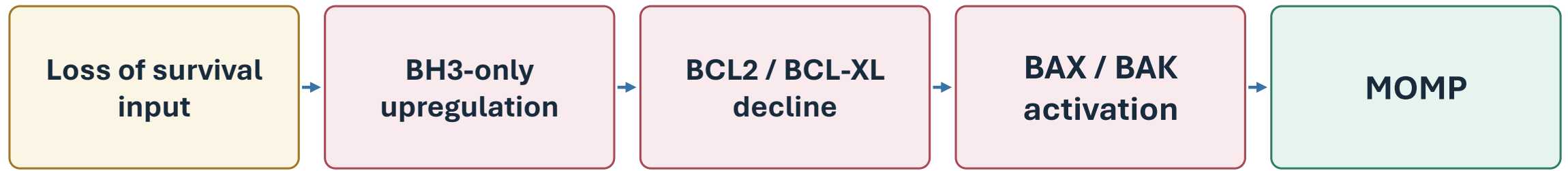
- Growth factors and related signals support antiapoptotic protein expression.
- BCL2-family restraints help keep cytochrome c inside mitochondria.
- Withdrawal shifts the balance toward BH3 activation and MOMP.



Withdrawal of survival signals removes restraint

SIGNAL TRANSDUCTION

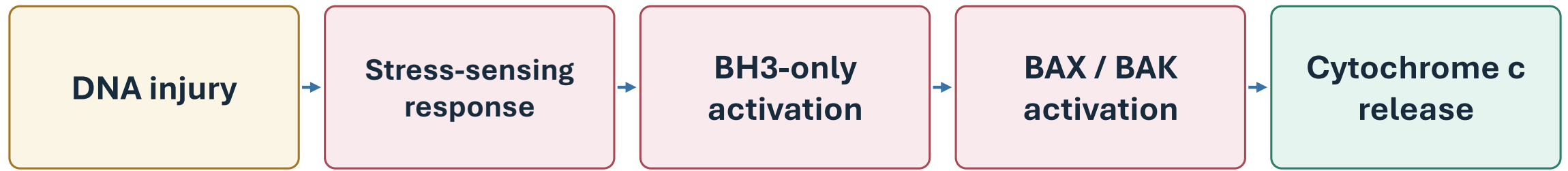
A survival-dependent cell can undergo intrinsic apoptosis when trophic input falls.



DNA damage can feed the mitochondrial pathway

SIGNAL TRANSDUCTION

DNA damage as a major initiating insult for intrinsic apoptosis.



ER stress can initiate apoptosis

SIGNAL TRANSDUCTION

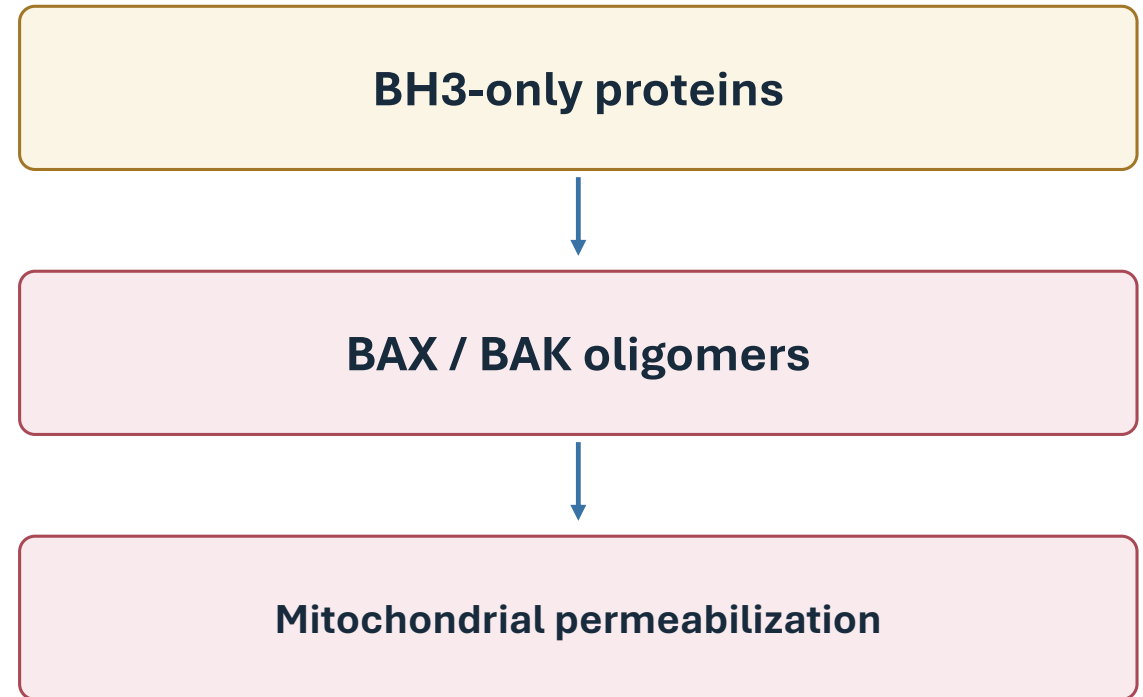
Persistent protein-folding stress contributes to the proapoptotic balance.



BH3-only proteins can activate BAX/BAK

BIOLOGIC LOGIC

- Stress-responsive BH3-only proteins can directly facilitate BAX/BAK activation.
- Activated BAX and BAK oligomerize in the outer mitochondrial membrane.
- Membrane permeabilization permits cytochrome c redistribution.



BH3-only proteins also neutralize protection

BIOLOGIC LOGIC

- Certain BH3-only members can bind antiapoptotic BCL2-family proteins.
- Blockade of BCL2 or BCL-XL weakens mitochondrial restraint.
- This works alongside BAX/BAK activation to drive permeabilization.

Functional antagonism

BH3-only protein binds a protective BCL2-family member.

Loss of restraint

BCL2 / BCL-XL antiapoptotic influence declines.

Consequence

BAX/BAK effectors more readily drive MOMP.

Intrinsic commitment reflects opposing forces

BIOLOGIC LOGIC

- Pro-death input: BH3-only activation and BAX/BAK effectors.
- Pro-survival input: BCL2, BCL-XL and MCL1 restrain membrane changes.
- The combined balance determines cytochrome c release.

Pro-death

BH3-only sensors → BAX / BAK

Pro-survival

BCL2 · BCL-XL · MCL1

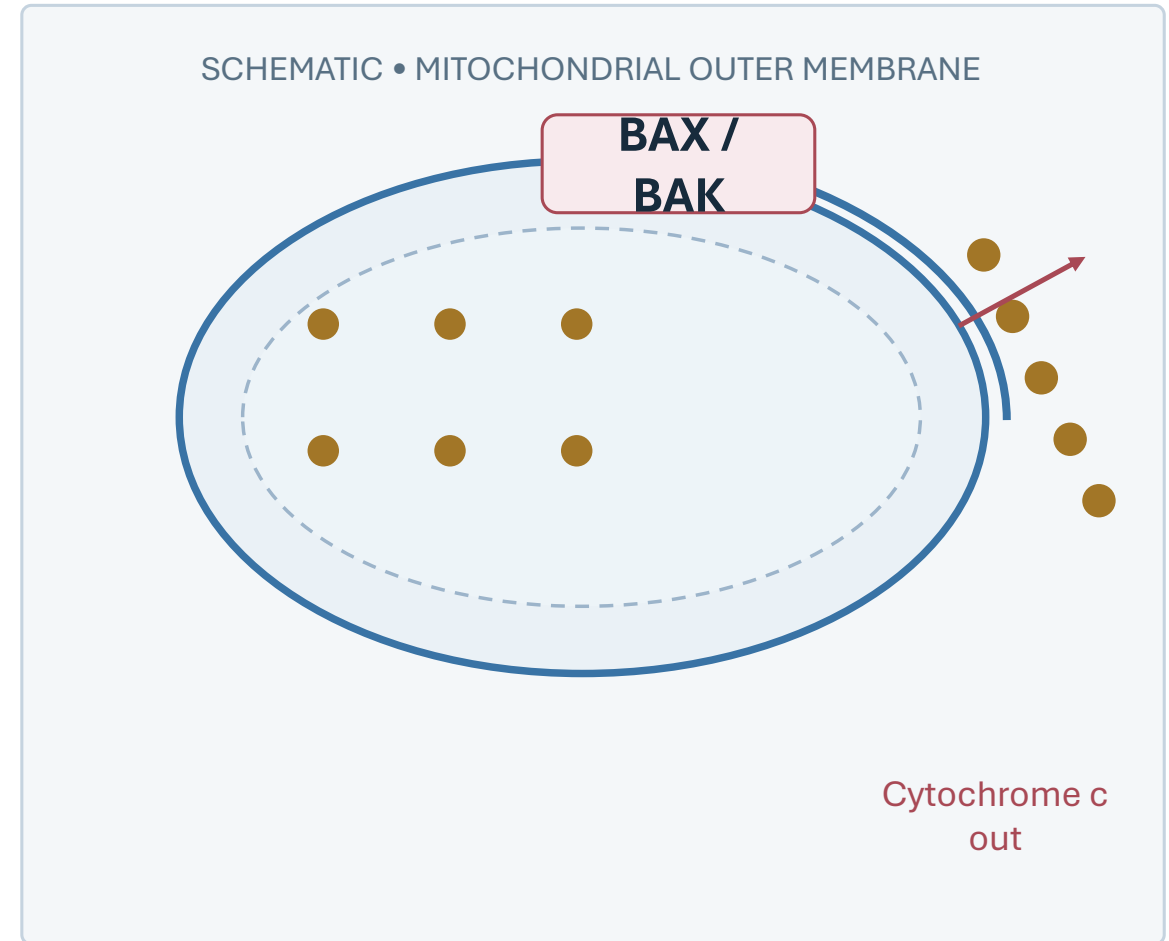
Decision

Outer-membrane permeabilization or preservation

MOMP releases mitochondrial death signals

BIOLOGIC LOGIC

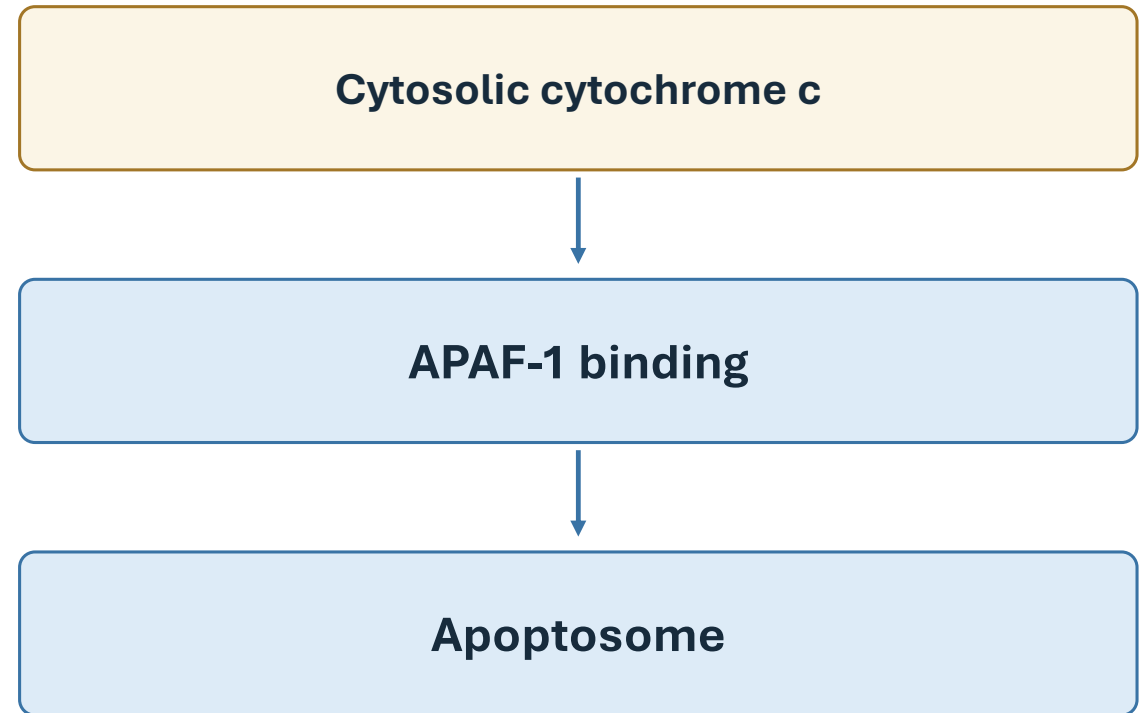
- Outer-membrane permeabilization allows cytochrome c into cytosol.
- Other mitochondrial factors, including Smac/DIABLO, may also emerge.
- Together these favor caspase activation and apoptotic execution.



Cytochrome c recruits APAF-1 in the cytosol

BIOLOGIC LOGIC

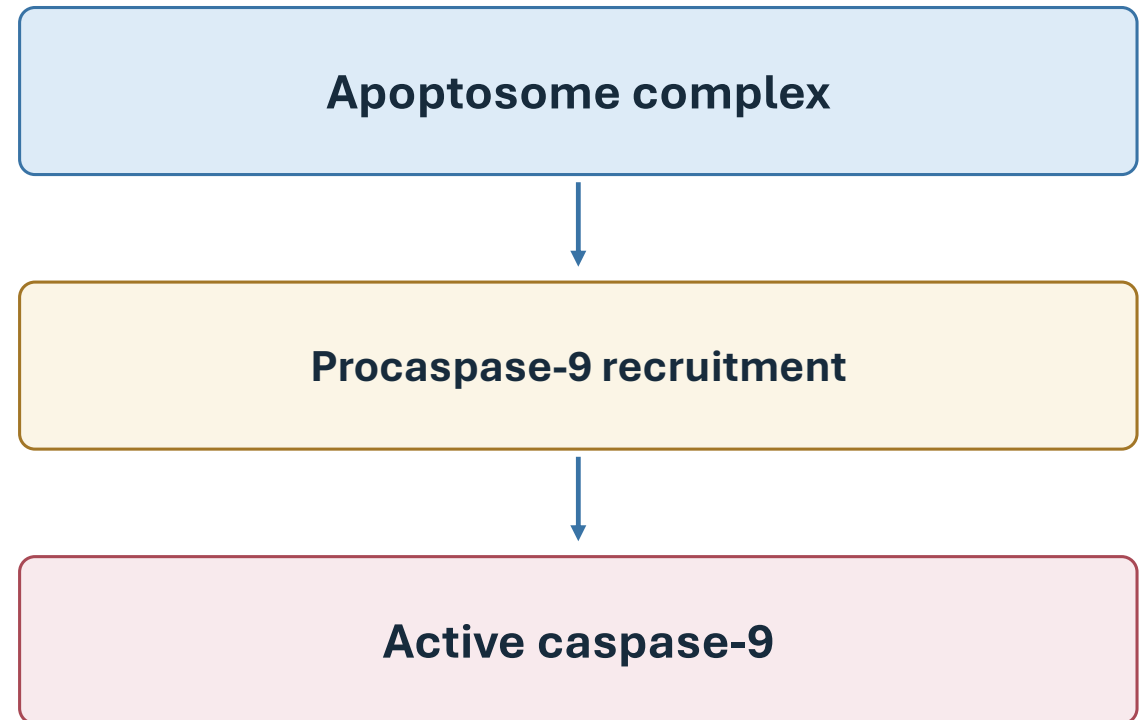
- Released cytochrome c binds apoptosis-activating factor-1 (APAF-1).
- The complex assembles into a multimeric apoptosome.
- The apoptosome is the intrinsic pathway platform for caspase-9 activation.



The apoptosome activates caspase-9

BIOLOGIC LOGIC

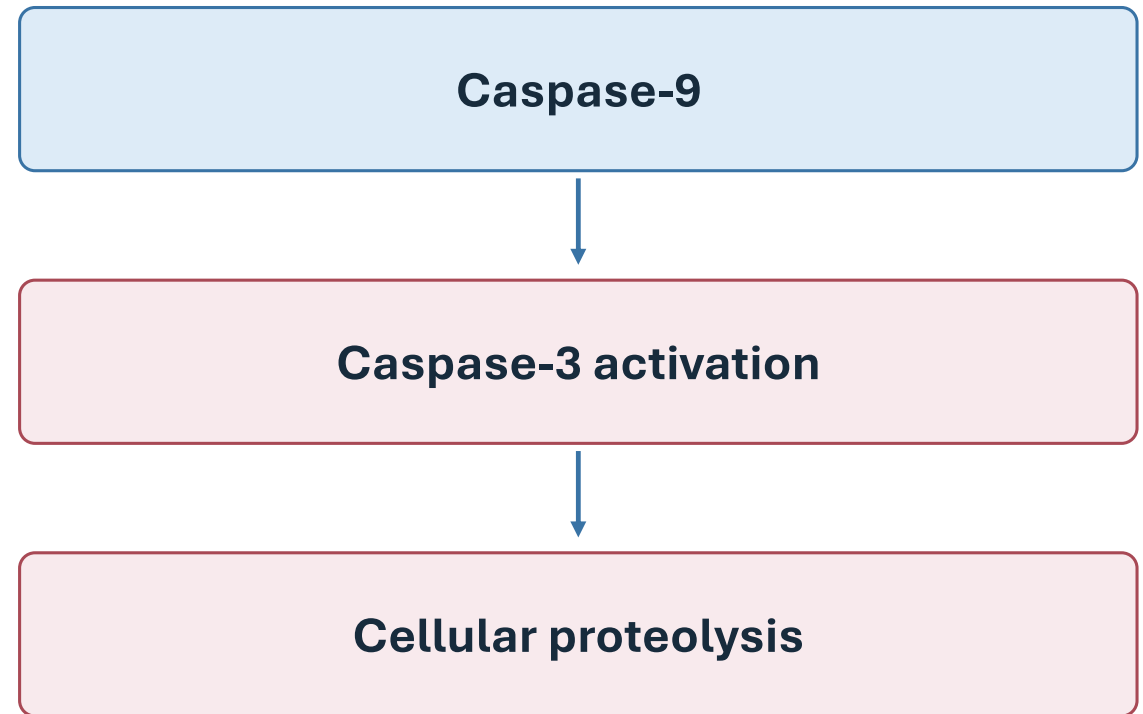
- The multimeric apoptosome recruits procaspase-9.
- Assembly promotes activation of caspase-9.
- Caspase-9 provides the upstream proteolytic drive for executioner activation.



Caspase-9 links initiation to execution

BIOLOGIC LOGIC

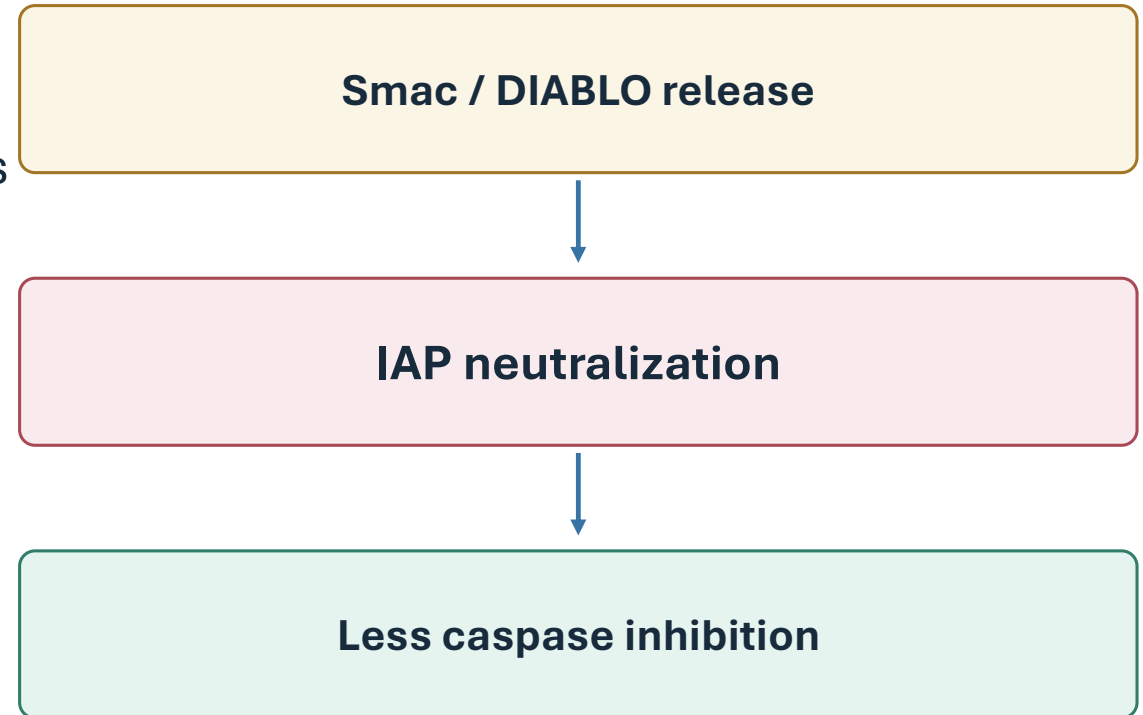
- Activated caspase-9 cleaves downstream procaspases.
- Caspase-3 is a major executioner target.
- Multiple cellular substrates are then dismantled.



Smac/DIABLO supports apoptosis by releasing a brake

BIOLOGIC LOGIC

- Smac/DIABLO can enter the cytoplasm after mitochondrial permeabilization.
- It binds and neutralizes inhibitor-of-apoptosis proteins (IAPs).
- This disinhibits the caspase cascade.



IAPs prevent inappropriate caspase activity

BIOLOGIC LOGIC

- IAPs act as physiological inhibitors of apoptosis.
- They restrain the activity of caspases, including executioners.
- Smac/DIABLO removes some of that inhibitory pressure.

Baseline restraint

IAPs keep inappropriate caspase activity in check.

Mitochondrial input

Smac/DIABLO binds selected IAPs.

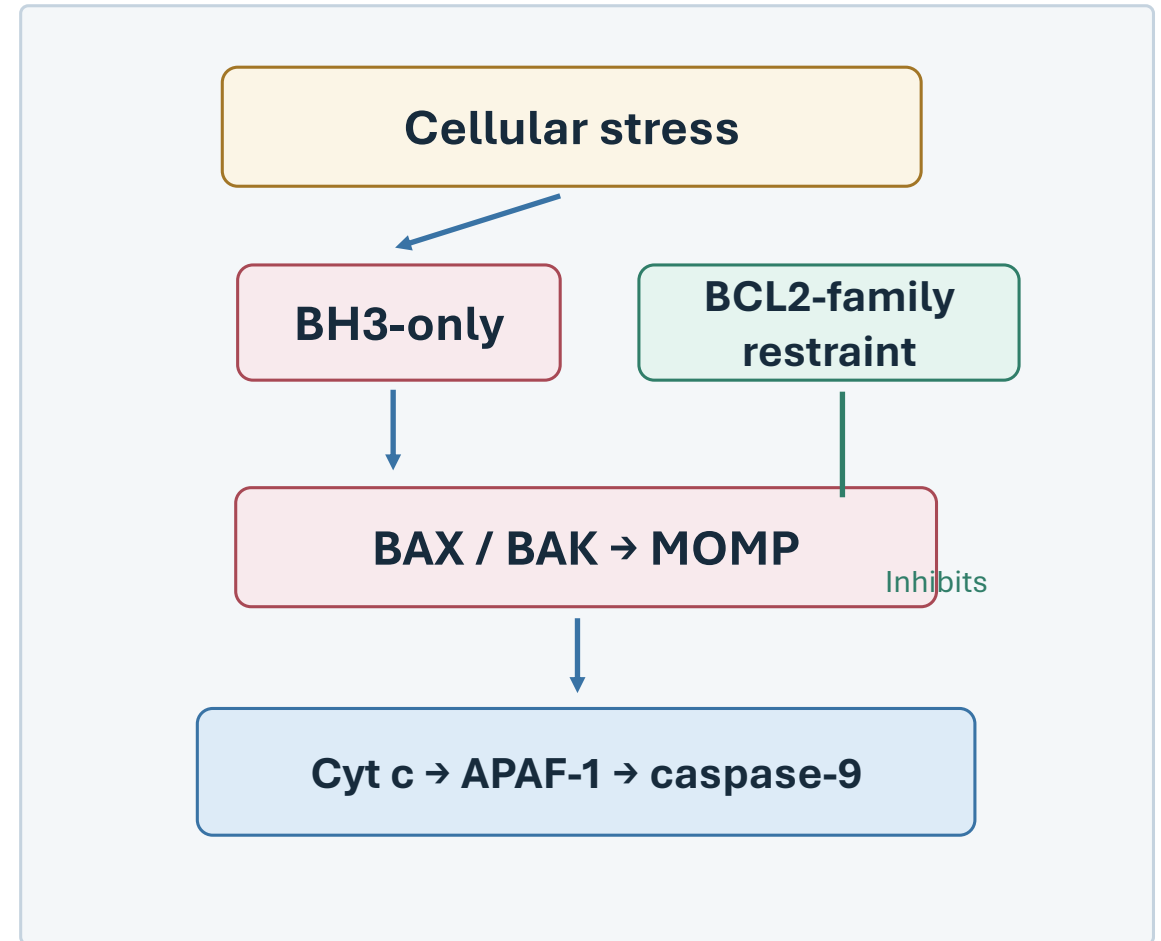
Downstream effect

Permits the apoptotic cascade to proceed.

Intrinsic cascade: One integrated map

BIOLOGIC LOGIC

- Survival withdrawal, DNA injury or ER stress increases pro-death signals.
- BH3-only proteins shift BCL2-family balance and favor BAX/BAK.
- MOMP → cytosolic cytochrome c → apoptosome → caspase-9.



BCL2 dysregulation illustrates disease relevance

BIOLOGIC LOGIC

- The BCL2 gene is frequently overexpressed in certain B-cell lymphomas.
- Increased protective activity makes mitochondrial permeabilization less likely.
- The mechanistic consequence is impaired apoptotic elimination.

Molecular change

Excess BCL2 is a recognized feature of some B-cell lymphomas.

Cellular effect

Enhanced survival despite signals favoring deletion.

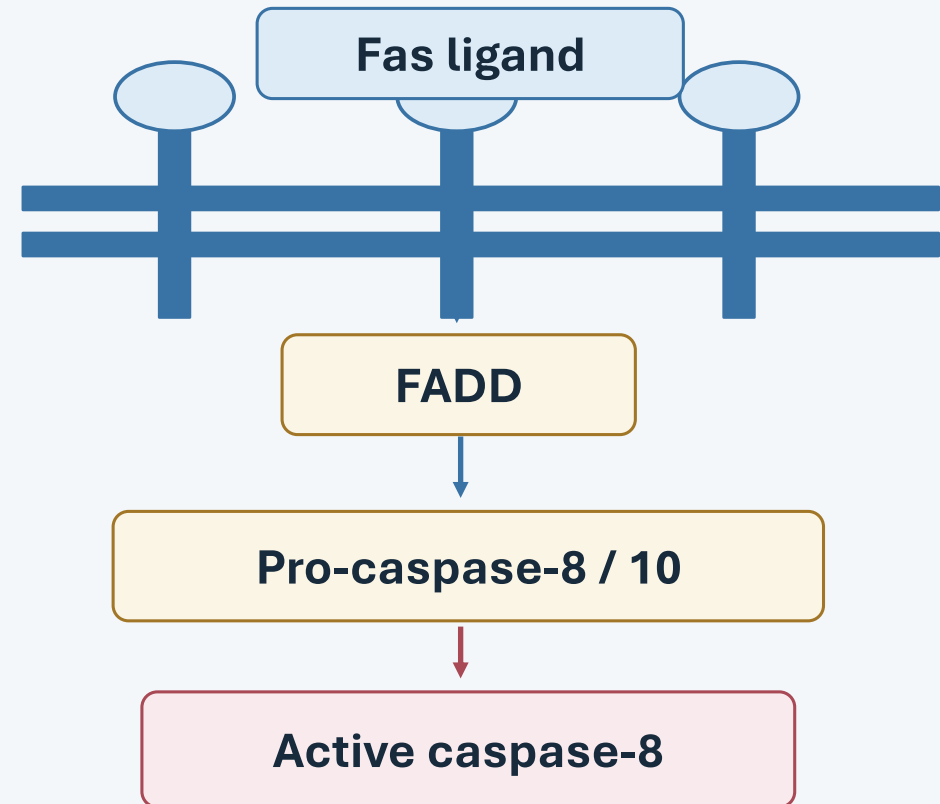
Teaching point

Resistance to apoptosis can aid cellular accumulation.

Extrinsic apoptosis begins at surface death receptors

BIOLOGIC LOGIC

- Death receptors belong to the TNF-receptor superfamily.
- Their intracellular death domains recruit specific signaling adaptors.
- Fas (CD95) is a canonical example; TNFR1 is another.



SCHEMATIC • CELL MEMBRANE RECEPTOR

The death domain is the key receptor element

BIOLOGIC LOGIC

- A cytoplasmic death domain supports interactions with adaptor proteins.
- Not every TNF-receptor family member contains such a domain.
- Therefore ligand binding to any TNF-family receptor does not imply apoptosis.

Death domain present

Can support assembly of apoptotic signaling complexes.

Absent / different receptor

Some related TNF receptors principally activate inflammatory pathways.

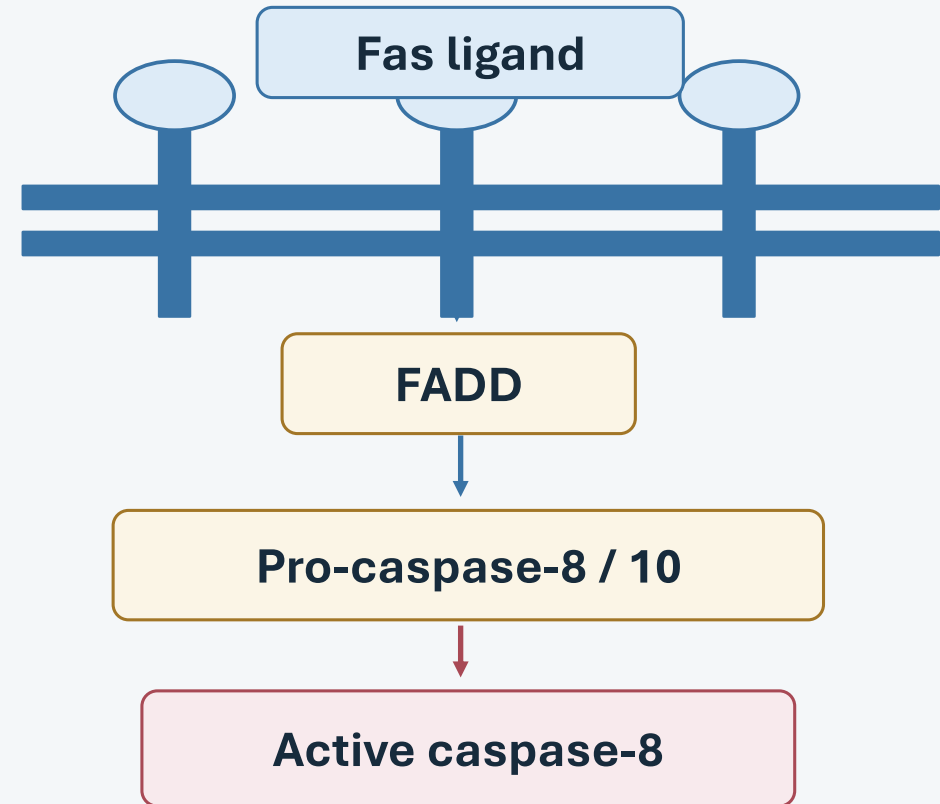
Interpretive rule

Receptor structure and downstream context matter.

Fas (CD95) and Fas ligand (FasL)

BIOLOGIC LOGIC

- Fas can be expressed on many cell types.
- FasL is present on some T lymphocytes, including cytotoxic T cells.
- Ligand-receptor contact initiates a receptor-clustering signal.

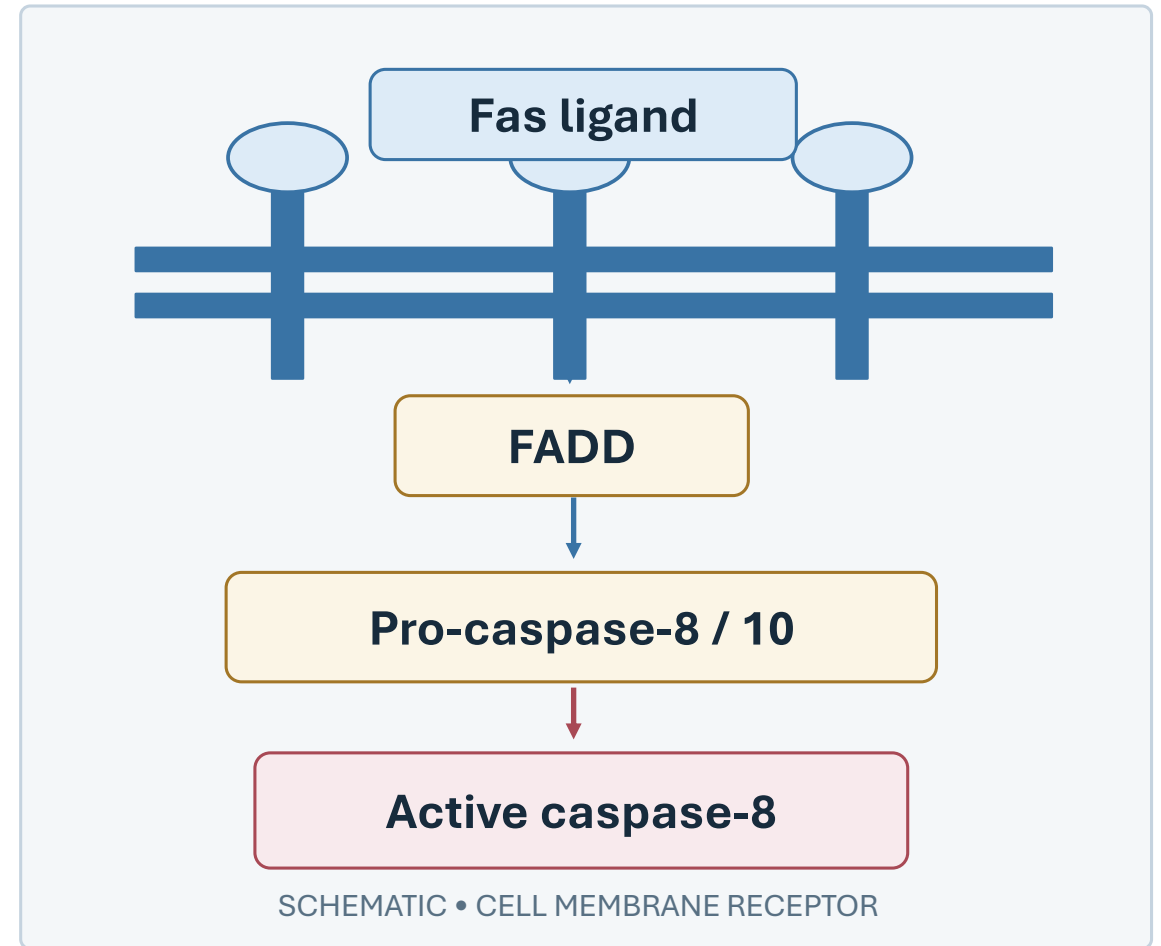


SCHEMATIC • CELL MEMBRANE RECEPTOR

Ligand binding brings Fas receptors together

BIOLOGIC LOGIC

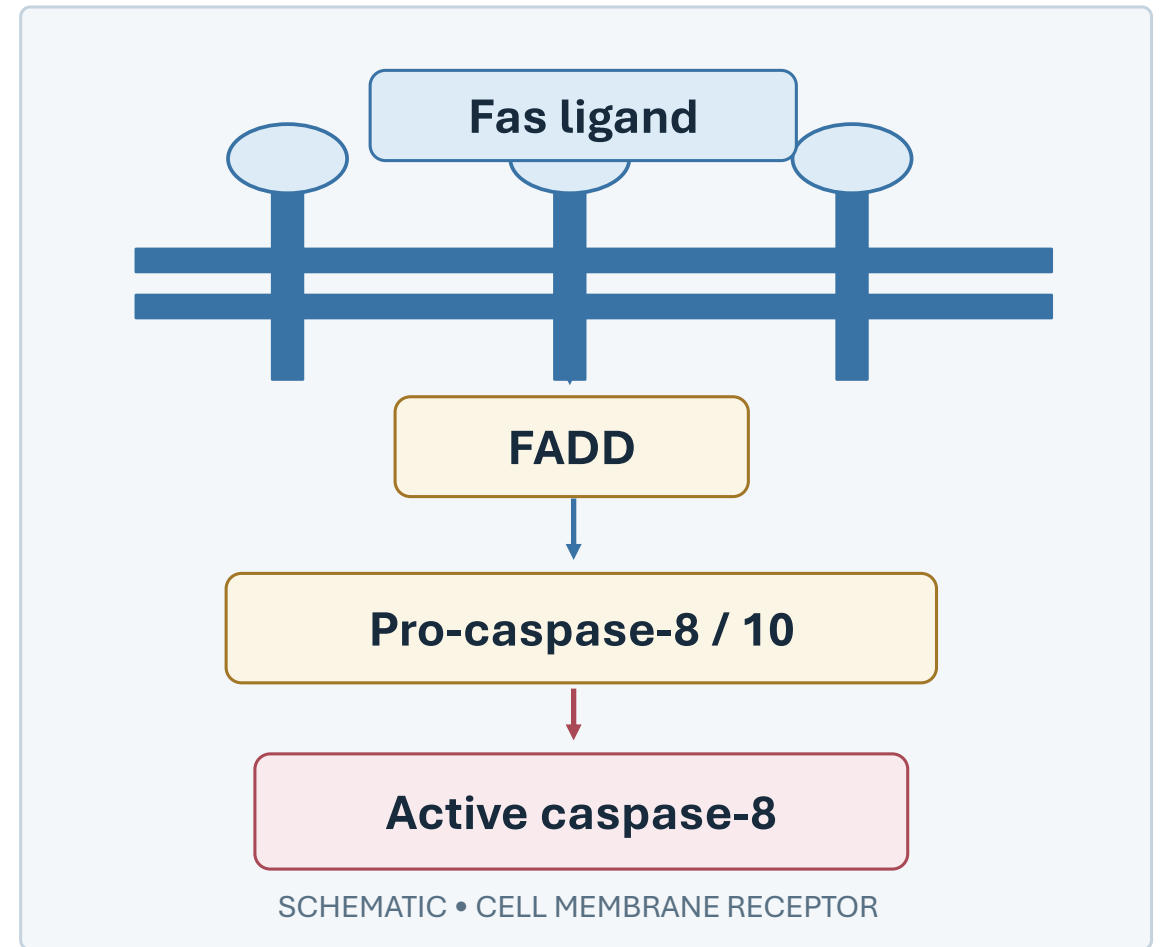
- FasL engagement clusters several Fas molecules.
- The clustered intracellular death domains create an adaptor-binding platform.
- This precedes recruitment of caspase precursors.



FADD couples receptor clustering to caspases

BIOLOGIC LOGIC

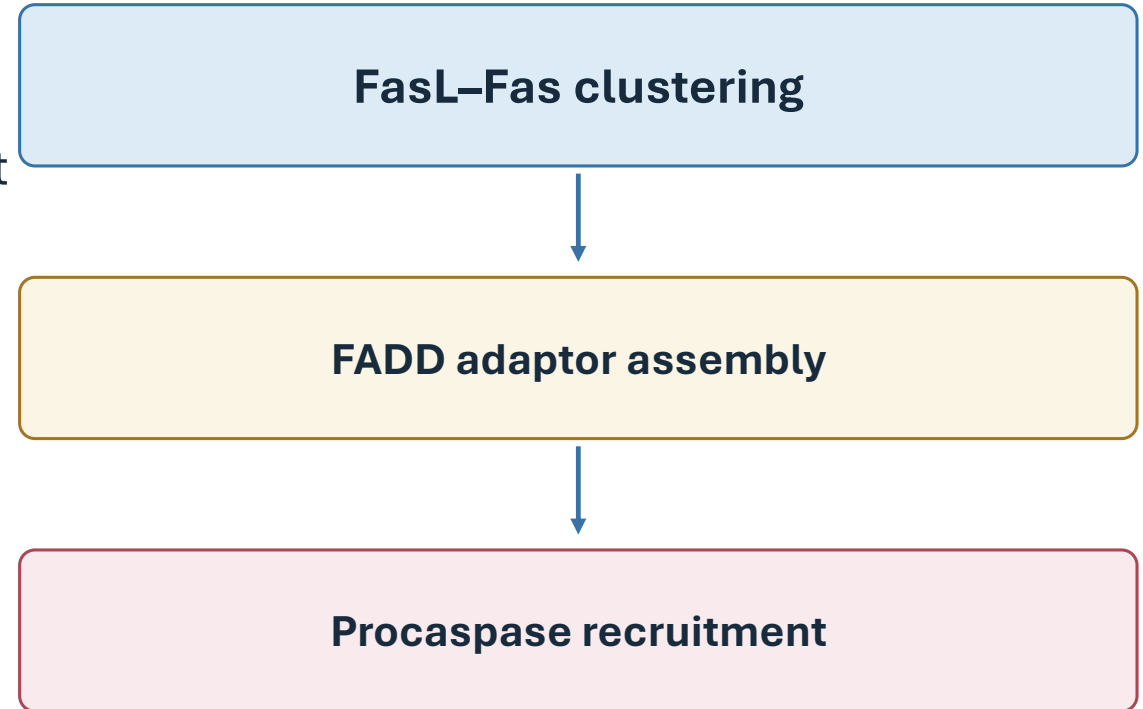
- FADD stands for Fas-associated death domain.
- It binds activated receptor complexes through death-domain interactions.
- It organizes the recruitment of inactive initiator caspases.



The death-inducing signaling complex forms

BIOLOGIC LOGIC

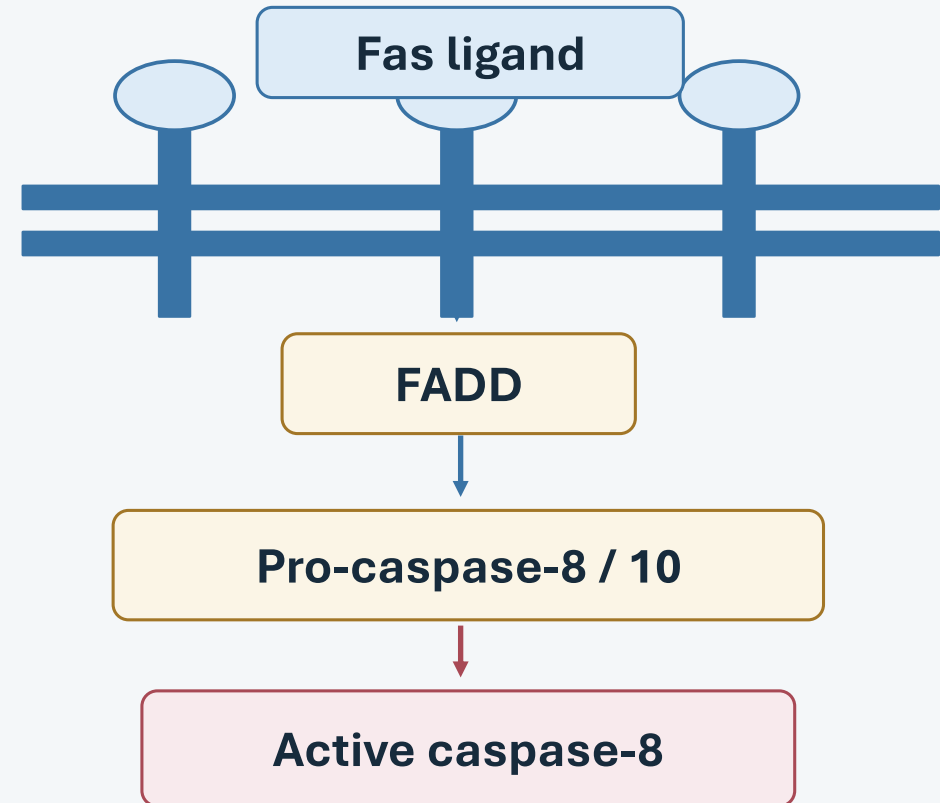
- Ligated Fas recruits FADD and procaspases into a local complex.
- Multiple caspase-8/10 molecules are brought into proximity.
- This assembly enables upstream caspase activation.



Initiator caspase-8 or -10 is recruited

BIOLOGIC LOGIC

- FADD interacts with inactive caspase-8 or caspase-10.
- Local concentration facilitates autocatalytic processing and activation.
- Caspase-8 is the best illustrated initiator in the Fas pathway.

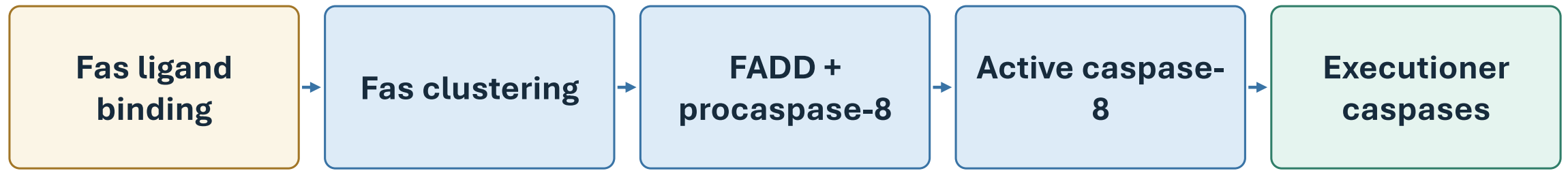


SCHEMATIC • CELL MEMBRANE RECEPTOR

Fas signaling activates the execution arm

SIGNAL TRANSDUCTION

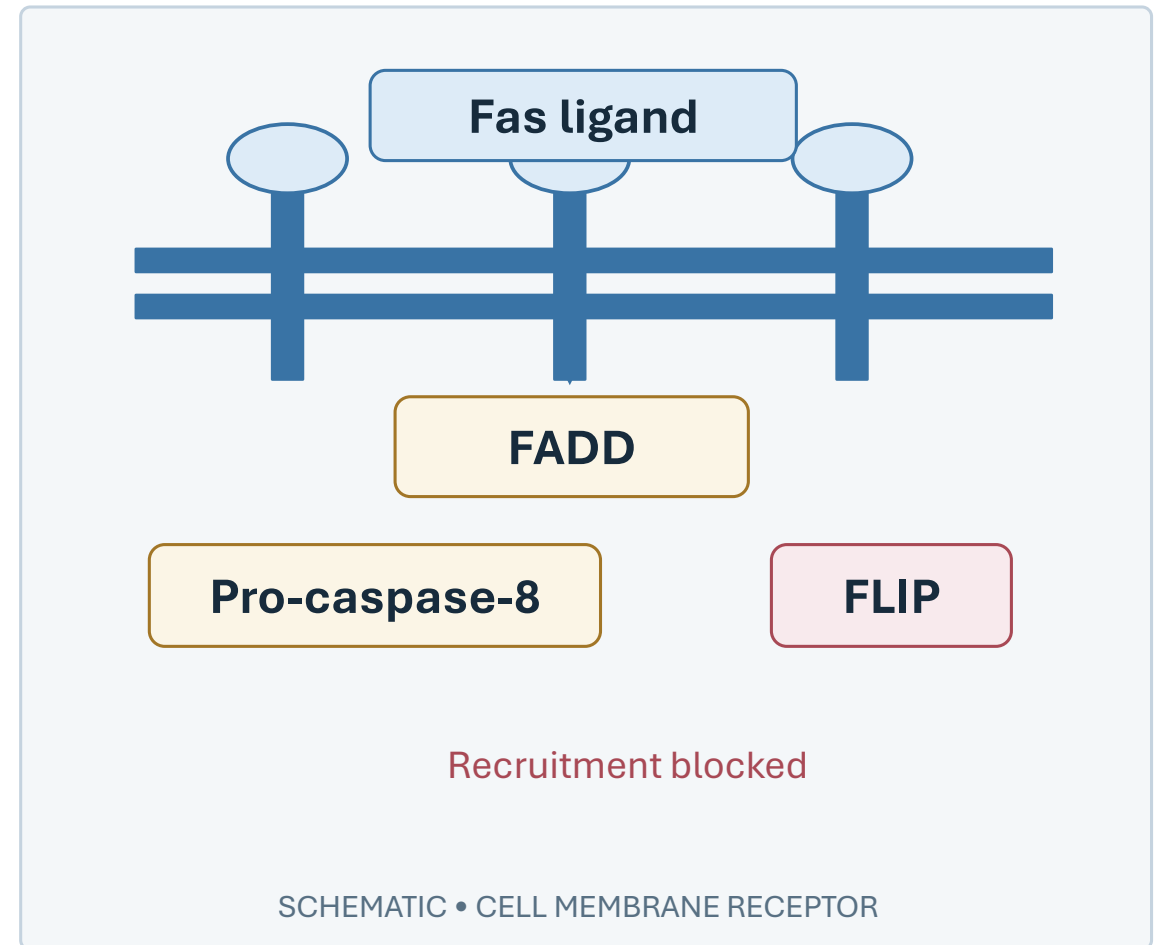
Death-receptor initiation proceeds through assembly and processing at the receptor.



FLIP inhibits death-receptor caspase recruitment

BIOLOGIC LOGIC

- FLIP interferes with procaspase-8 recruitment or activation at the complex.
- This can prevent Fas-mediated apoptosis despite receptor engagement.
- The inhibition is pathway-specific rather than an absolute block of all apoptosis.



FLIP may protect normal or virus-infected cells

BIOLOGIC LOGIC

- Normal cells can express FLIP as a survival safeguard.
- Some viruses use related inhibition to evade Fas-mediated cell death.
- Pathway interference can occur before executioner caspases activate.

Host regulation

FLIP can reduce susceptibility to Fas-induced death.

Viral survival

Some viruses encode or induce interference with caspase-8 activation.

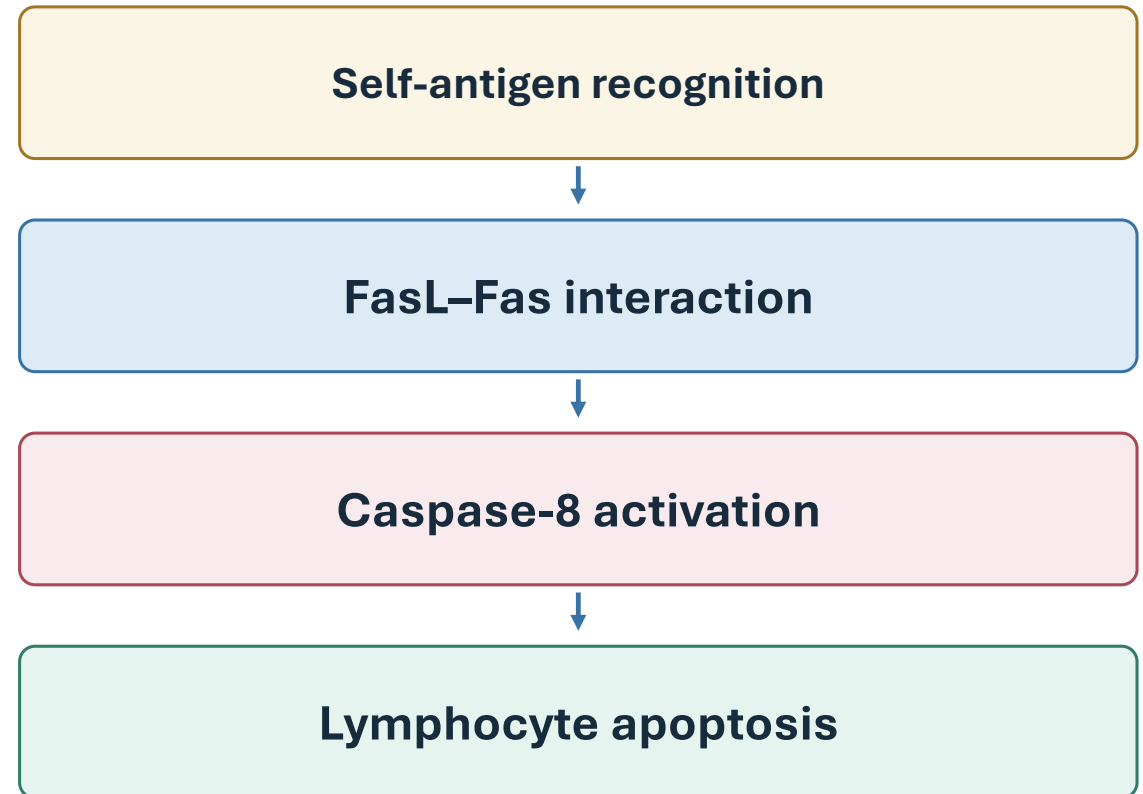
Mechanistic location

Proximal death-receptor complex—not at mitochondrial cytochrome c release.

Fas contributes to eliminating self-reactive lymphocytes

BIOLOGIC LOGIC

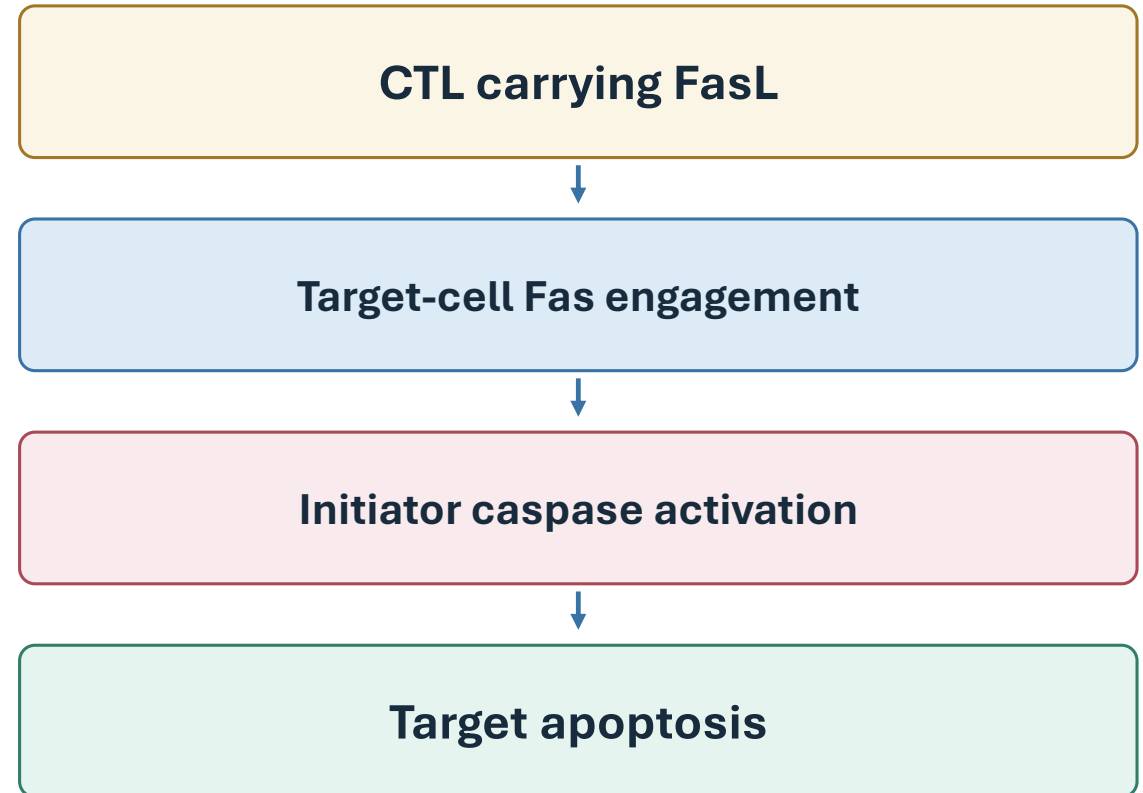
- Fas ligand may be expressed by T cells recognizing self antigens.
- Engagement with Fas on susceptible self-reactive lymphocytes induces deletion.
- This is one mechanism preserving immune-cell homeostasis.



Cytotoxic T cells also use death-receptor killing

BIOLOGIC LOGIC

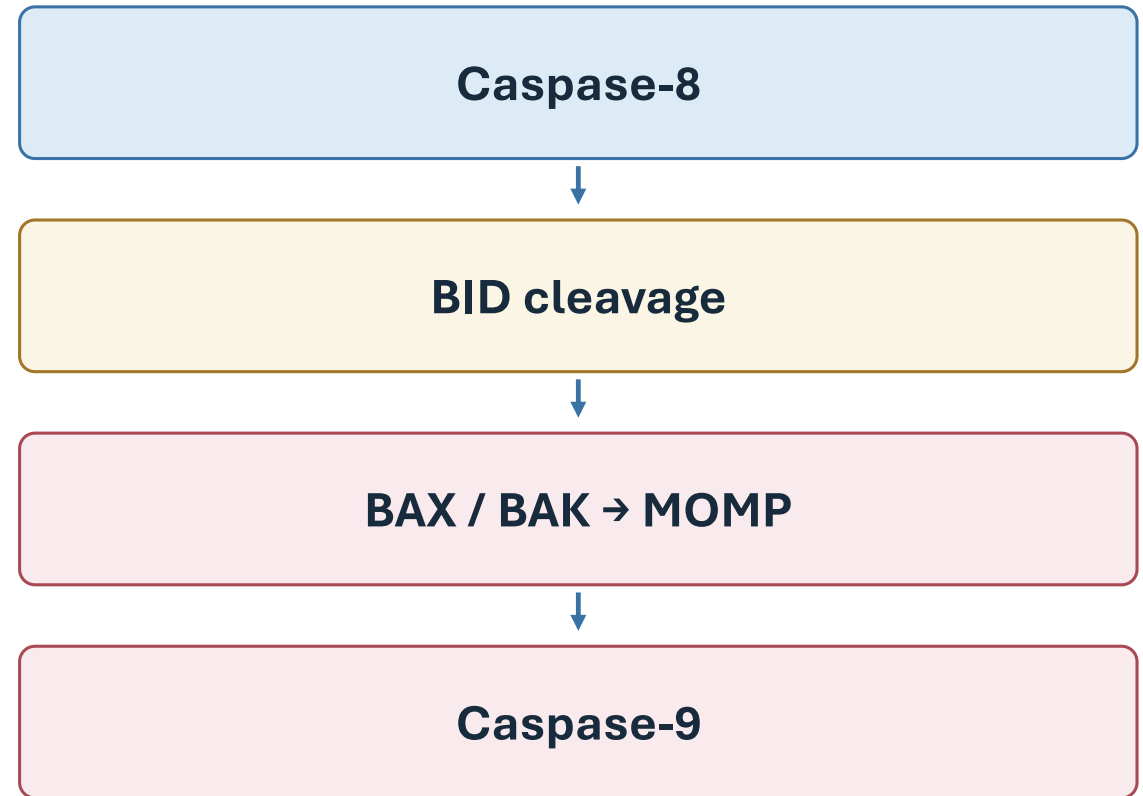
- Some cytotoxic T lymphocytes express FasL.
- Target cells expressing a responsive Fas pathway can undergo apoptosis.
- Examples include virus-infected or tumor cells.



BID links the receptor and mitochondrial routes

BIOLOGIC LOGIC

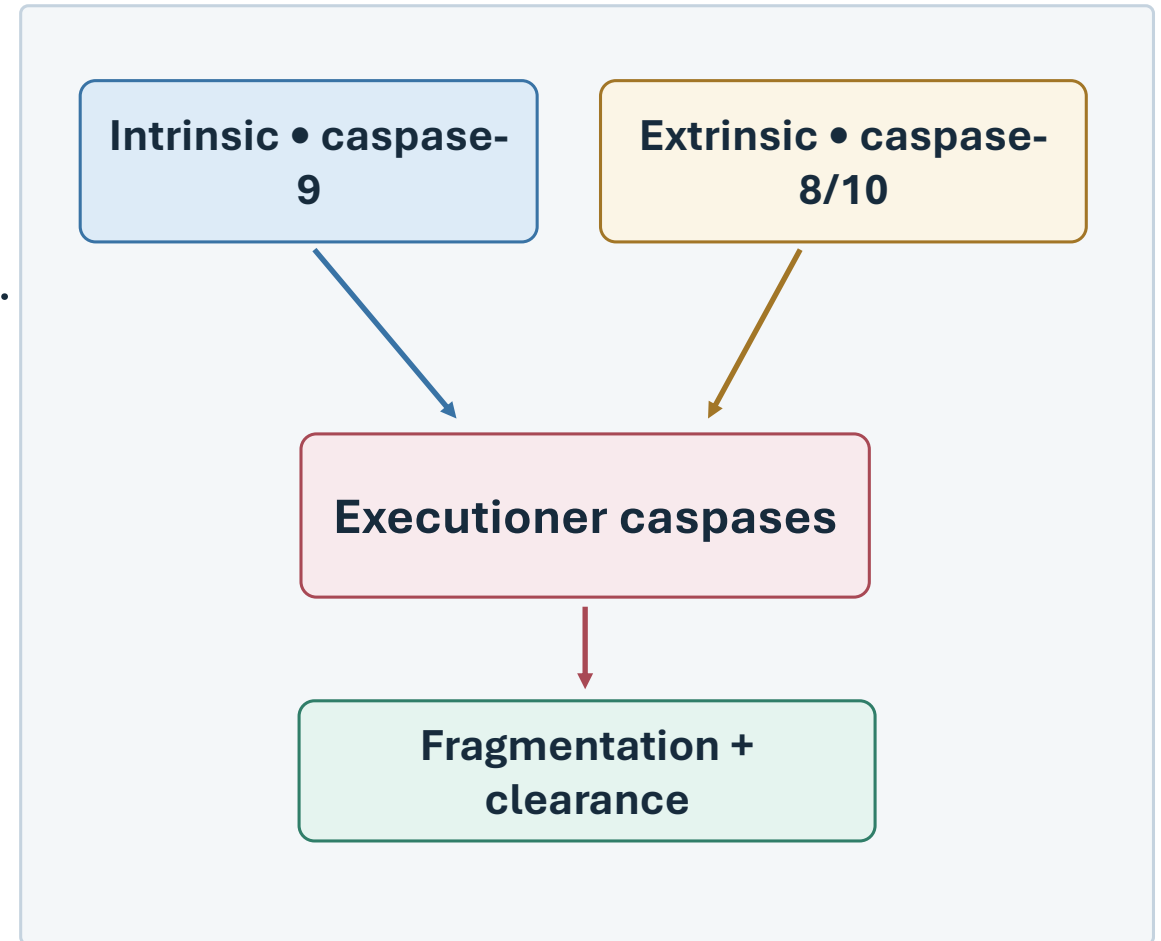
- In selected cell types, caspase-8 can cleave BID.
- Activated BID engages the mitochondrial proapoptotic machinery.
- Fas signaling may therefore be amplified through cytochrome c release.



Crosstalk varies by cell type

BIOLOGIC LOGIC

- Hepatocytes and pancreatic β cells as settings for BID-mediated amplification.
- The extrinsic route can stimulate intrinsic machinery instead of remaining independent.
- This example cautions against treating the pathways as isolated boxes.



Both initiation pathways reach a shared endpoint

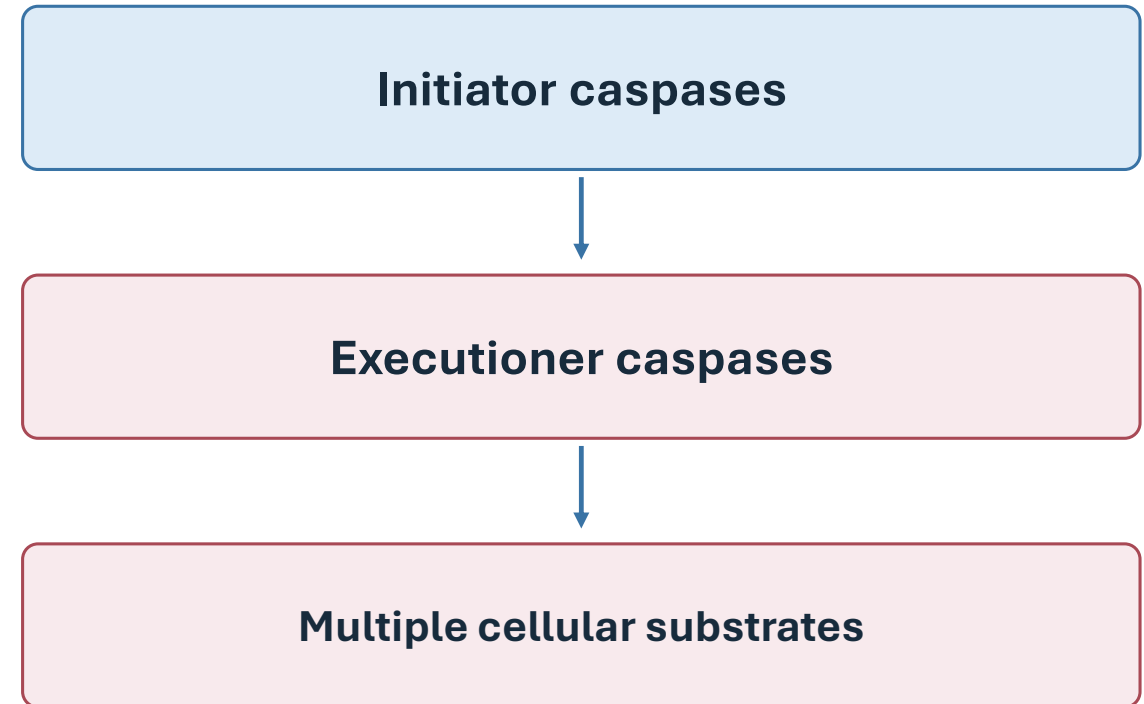
MECHANISTIC COMPARISON

Element	Intrinsic	Extrinsic
Trigger	Internal stress or loss of survival	External receptor ligand
Proximal gate	BCL2 family / BAX–BAK	Fas–FADD complex; FLIP
Caspase entry	Caspase-9	Caspase-8 / -10
Crosstalk	Can be engaged by cleaved BID	Can recruit mitochondrial machinery
Common output	Executioner caspases	Executioner caspases

Executioner caspases dismantle the cell

BIOLOGIC LOGIC

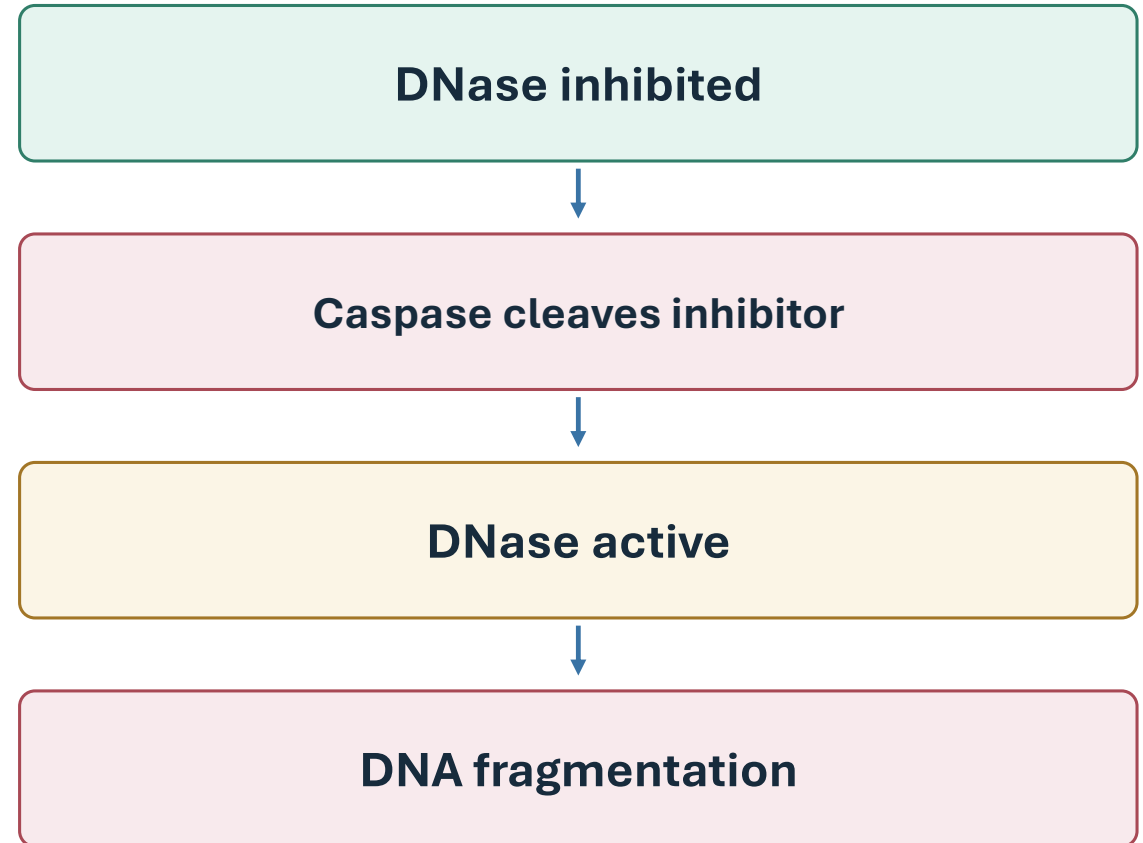
- The two initiation pathways ultimately activate common executioner proteases.
- Caspase-3 and caspase-6 are emphasized mechanism.
- Their targets include DNA-regulatory, nuclear and cytoskeletal proteins.



A DNase is activated by removing its inhibitor

BIOLOGIC LOGIC

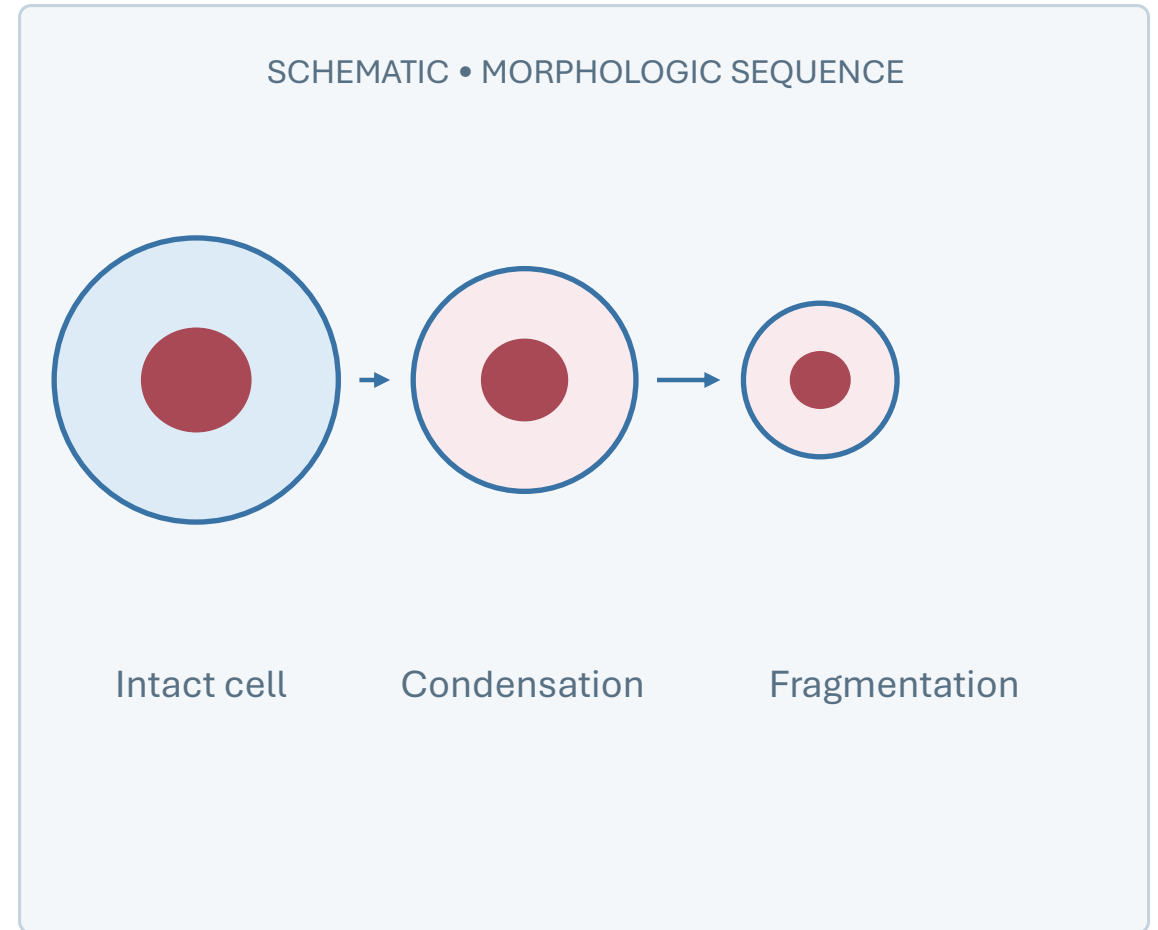
- Executioner caspases can cleave an inhibitory protein bound to a DNase.
- The released DNase becomes active and degrades nuclear DNA.
- The nucleus undergoes characteristic fragmentation.



Nuclear-matrix cleavage contributes to fragmentation

BIOLOGIC LOGIC

- Caspases proteolyze structural components of the nuclear matrix.
- Structural collapse complements DNA cleavage.
- Nuclear fragmentation is thus a biochemical consequence of execution.



Not every morphologic step is fully explained

BIOLOGIC LOGIC

- Chromatin and nuclear damage follow defined proteolytic events.
- The precise molecular formation of membrane blebs and apoptotic bodies remains less well characterized.
- Avoid assigning a definitive single effector to every visible feature.

Well characterized

Caspase-mediated protein processing and DNA degradation.

Less completely resolved

Detailed mechanism of bleb and apoptotic-body formation.

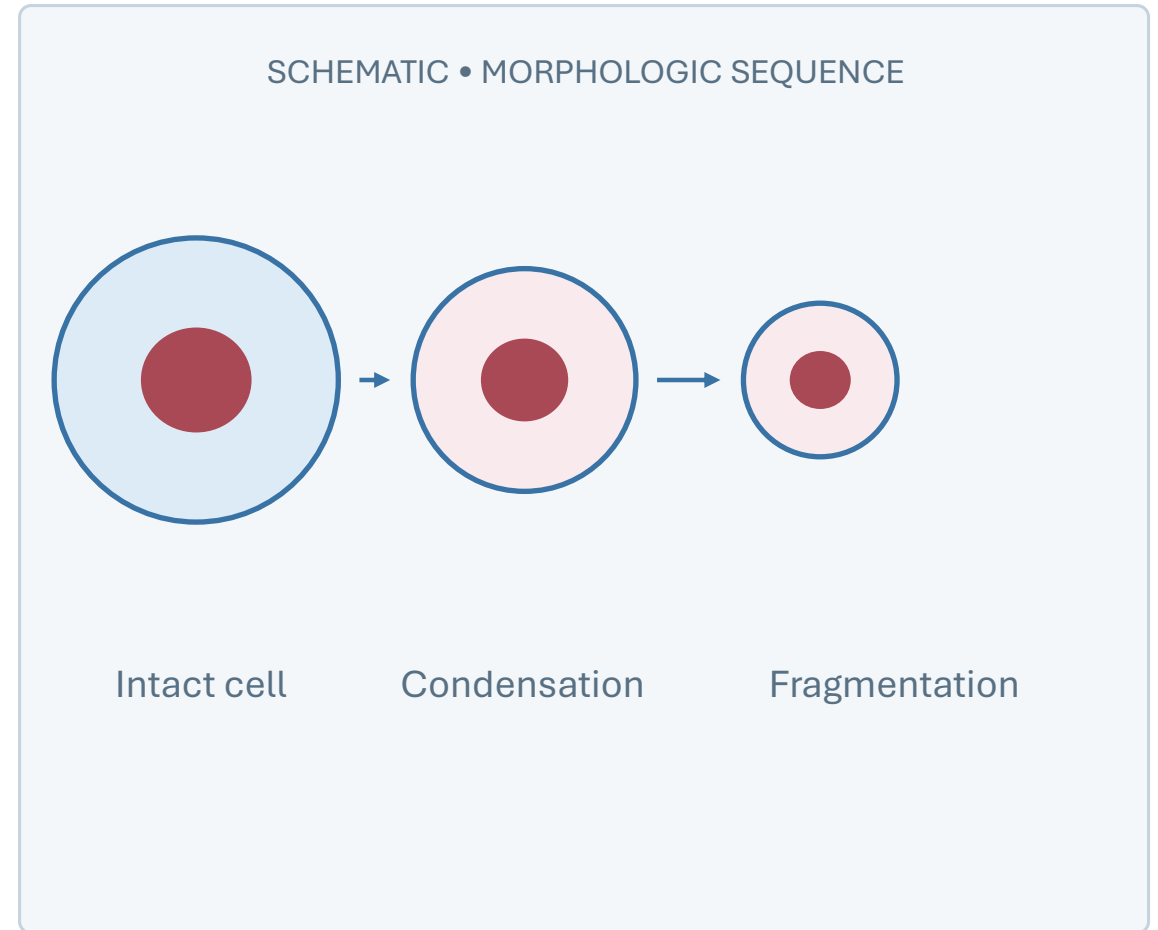
Teaching distinction

Visible morphology need not imply a fully solved molecular sequence.

Apoptotic bodies package cellular fragments

BIOLOGIC LOGIC

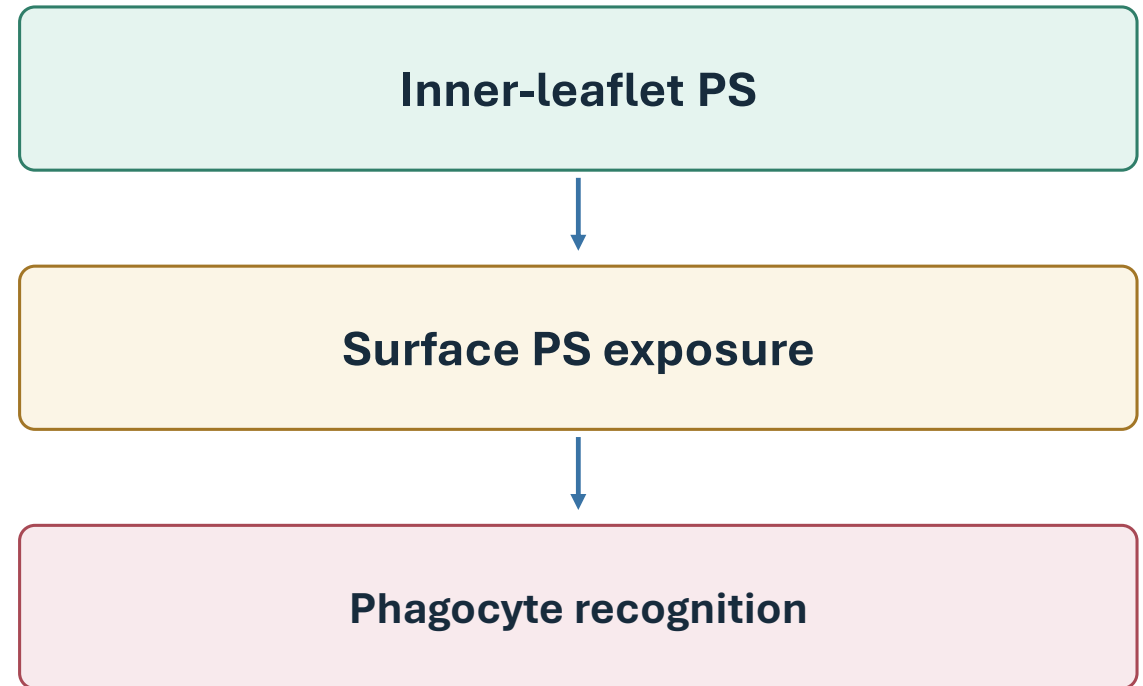
- Apoptosis generates discrete membrane-enclosed fragments.
- The fragments can be ingested without widespread cellular lysis.
- Fragmentation prepares dying cells for rapid clearance.



Phosphatidylserine becomes an eat-me signal

BIOLOGIC LOGIC

- In healthy cells, phosphatidylserine is predominantly on the inner membrane leaflet.
- During apoptosis it becomes exposed on the outer surface.
- Phagocytes recognize this altered surface as a clearance cue.



Dying cells signal for phagocyte recruitment

BIOLOGIC LOGIC

- Apoptotic cells can release soluble factors attracting phagocytes.
- Macrophages can supply bridging proteins that preferentially recognize dying cells.
- Recruitment and recognition improve the efficiency of clearance.

Recruitment

Soluble signals draw phagocytes toward apoptotic material.

Recognition

Macrophage-derived bridging proteins link apoptotic surfaces to receptors.

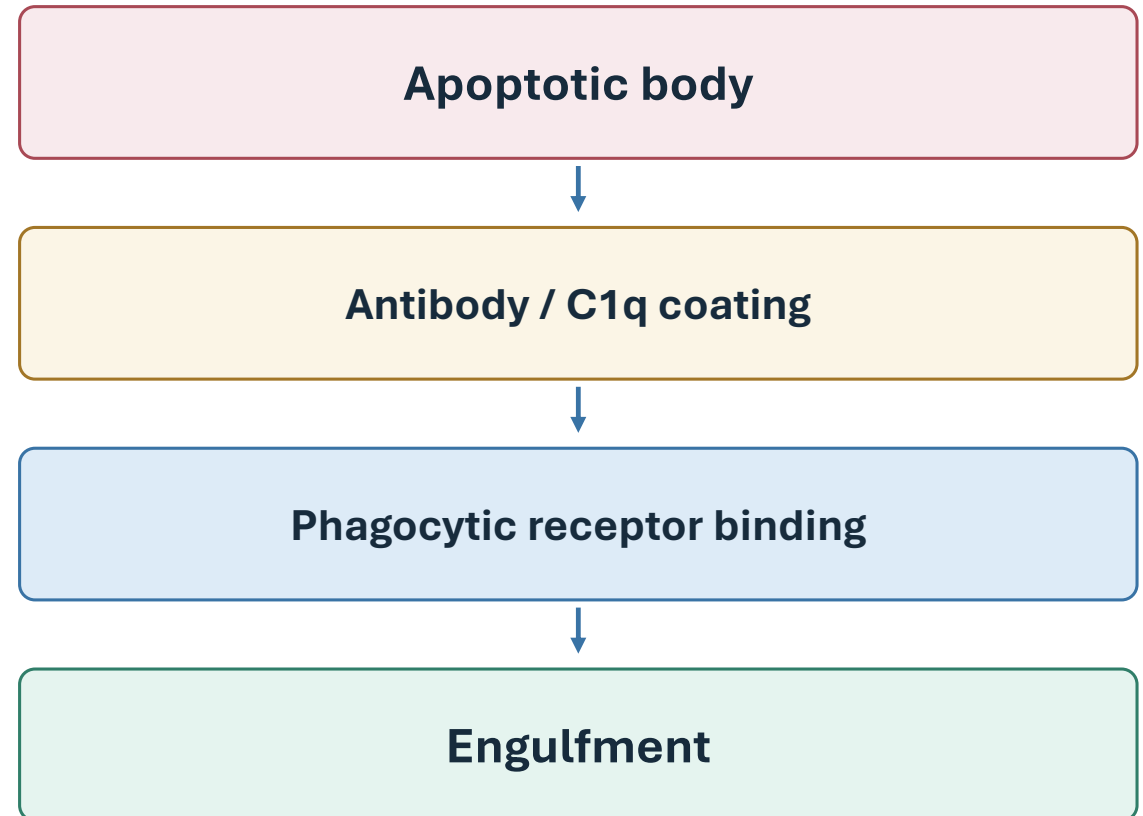
Outcome

Efficient engulfment of apoptotic fragments.

C1q and natural antibodies can aid recognition

BIOLOGIC LOGIC

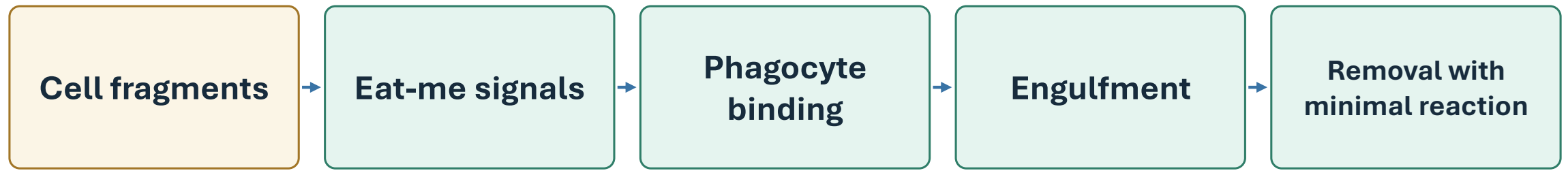
- Apoptotic bodies may acquire surface-bound natural antibodies.
- Complement proteins, particularly C1q, can also decorate fragments.
- These opsonins facilitate binding and engulfment by phagocytes.



Efferocytosis clears apoptotic cells

SIGNAL TRANSDUCTION

Efferocytosis is the phagocytic disposal of apoptotic cells and bodies.



Efficient clearance limits tissue inflammation

BIOLOGIC LOGIC

- Apoptotic material is usually engulfed before membrane integrity is lost.
- Ingesting macrophages reduce proinflammatory cytokine output.
- The combined effect is little inflammatory response, even with extensive apoptosis.

Timing

Rapid removal reduces release of intracellular contents.

Immune response

Post-engulfment macrophages reduce inflammatory cytokine production.

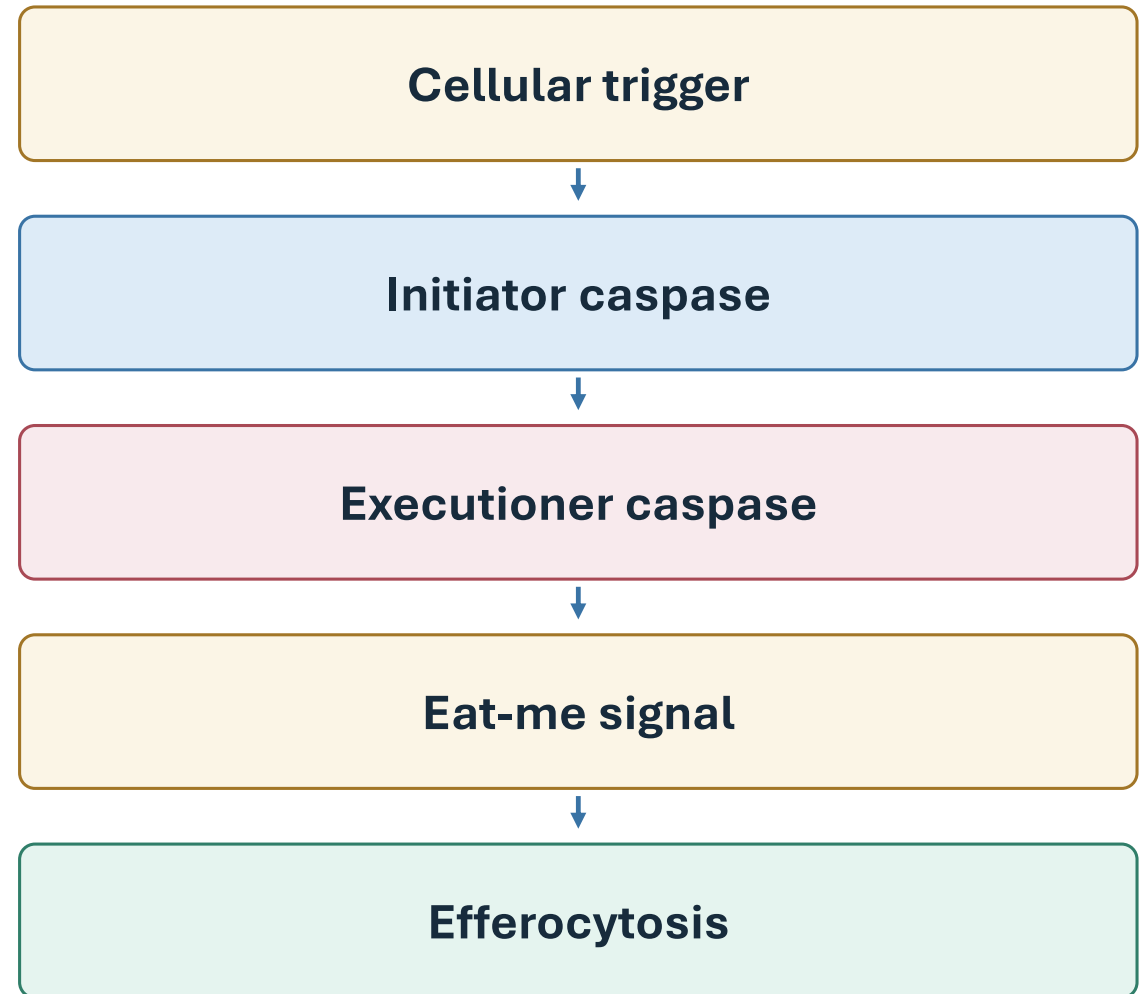
Tissue consequence

Apoptosis is typically a low-inflammatory death process.

Link the molecular pathway to the tissue outcome

BIOLOGIC LOGIC

- Initiation determines whether the program starts.
- Executioner caspases fragment nuclear and cellular components.
- Surface recognition and efferocytosis clear remnants before leakage.



Application: Increased BCL2 expression

APPLY THE PATHWAY

A B-cell clone shows strong antiapoptotic BCL2 activity. Which immediate checkpoint is most protected?

A. Fas clustering

B. Mitochondrial outer membrane

C. DNase activation

D. Macrophage engulfment

Discuss the mechanism before revealing the answer.

Application: A defective FADD interaction

APPLY THE PATHWAY

Fas ligand binds its receptor, but FADD cannot recruit initiator caspases. Which step is impaired?

A. Apoptosome formation

B. Caspase-8 initiation

C. Phosphatidylserine exposure as a direct receptor step

D. Cytochrome c respiratory role

Discuss the mechanism before revealing the answer.

Application: Mitochondrial pathway amplification

APPLY THE PATHWAY

After Fas ligation, caspase-8 cleaves BID. What is the expected cross-pathway consequence?

A. Activation of BAX / BAK

B. Direct C1q activation

C. Inactivation of APAF-1

D. Suppression of cytochrome c release

Discuss the mechanism before revealing the answer.

Mechanistic synthesis: One cellular endpoint

CORE MECHANISM

The route to cell death matters, even when the morphology is similar.

Inputs

Survival loss, DNA damage, ER stress, or death-receptor ligation.

Signal processors

BCL2 family, apoptosome and FADD-containing receptor complexes.

Terminal biology

Executioner caspases, membrane recognition and efferocytosis.

Textbook reference and boundaries

SOURCES

Robbins, Cotran & Kumar: Pathologic Basis of Disease

11th edition (2026), Chapter 2: Cell Injury, Cell Death, and Adaptations

Primary section: “Mechanisms of Apoptosis,” printed pp. 42–44

Original editable scientific schematics; no synthetic histologic or clinical images.