

# Apoptosis

**Regulated cell death with membrane-contained fragmentation**

Execution requires activation of intracellular proteases and DNA-degrading enzymes.

Fragments are recognized and rapidly engulfed, limiting inflammation.

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# Learning objectives

- Distinguish apoptosis from necrosis using mechanism and morphology.
- Explain physiologic and pathologic triggers for apoptotic cell death.
- Trace intrinsic and extrinsic pathways to executioner caspases.
- Relate apoptotic-body clearance to minimal tissue inflammation.

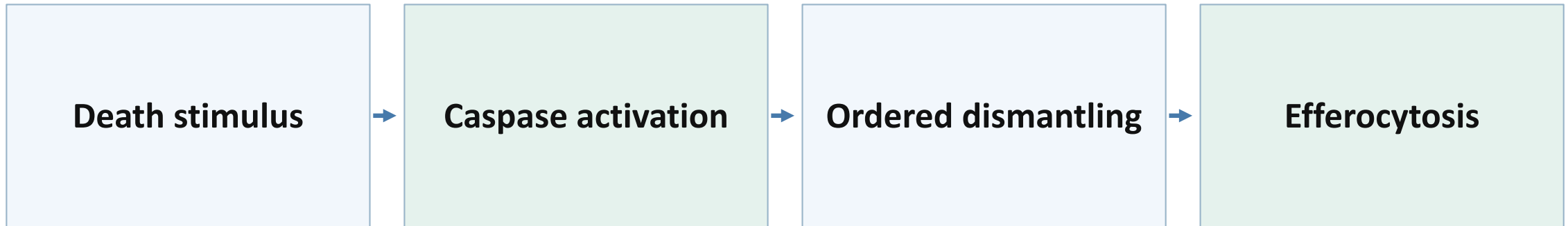
**Mechanism → morphology → clinicopathologic consequences**

# **A cell-directed process of self-destruction**

- Intrinsic enzymes degrade cellular proteins and nuclear DNA.
- Apoptotic bodies retain plasma membrane around cellular fragments.
- Surface signals recruit phagocytes before contents escape.
- The tissue reaction is therefore usually inconspicuous.

**The defining feature is controlled dismantling, not simple cell disappearance.**

# The four linked events in apoptosis



Pathway initiation and execution lead to efficient removal of the dying cell.

# Why apoptosis does not resemble necrosis

## Apoptosis

- Membrane-bound cellular fragments
- Early phagocytosis
- Little surrounding inflammation

## Necrosis

- Loss of membrane integrity
- Leakage of enzymes and contents
- Local inflammatory reaction

# **Programmed cell death: A contextual term**

**Developmental cell removal requires tightly timed elimination**

Apoptosis also occurs in adult tissue homeostasis.

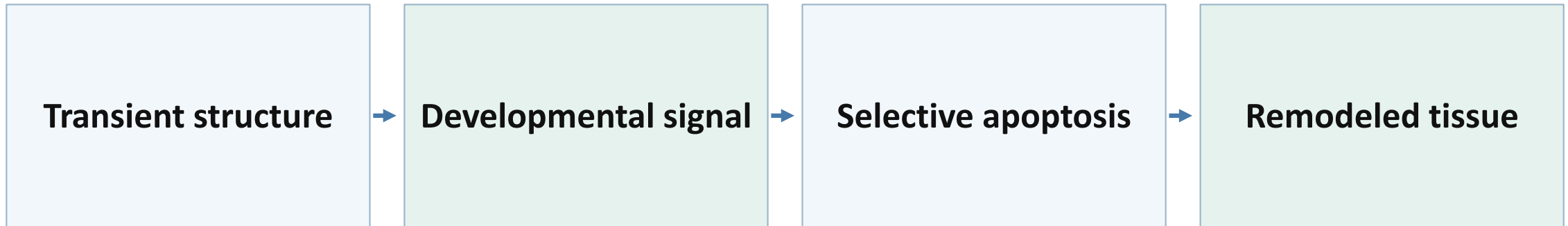
Pathologic apoptosis removes irreparably damaged cells.

# Physiologic apoptosis regulates tissue cell numbers

- Cell deletion matches ongoing production or changing tissue needs.
- Supernumerary cells are removed during organ development.
- Hormone-responsive tissues regress after hormonal withdrawal.
- Immune and epithelial populations are continuously pruned.

**Cell death is integral to normal tissue maintenance.**

# Embryonic remodeling depends on cell removal



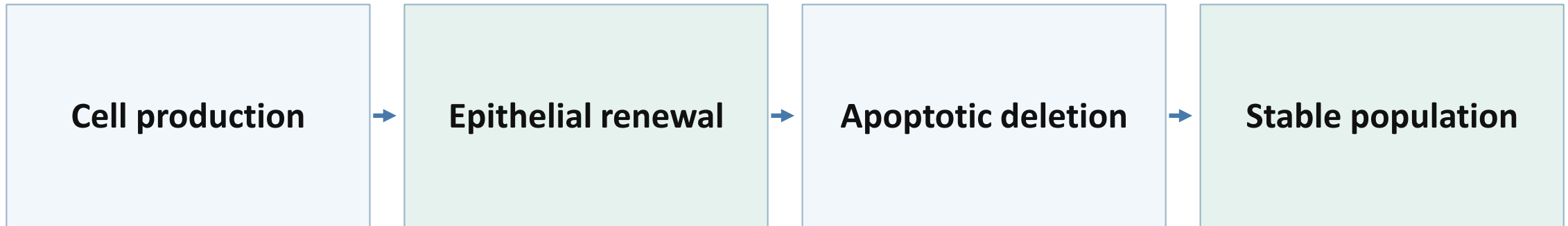
Cells no longer required for the maturing structure are eliminated.

# Hormone withdrawal induces physiologic involution

- Endometrial cells are lost during the menstrual cycle.
- Ovarian follicles undergo atresia when hormonal support changes.
- Lactating mammary tissue regresses after weaning.
- Withdrawal of survival signals permits apoptotic machinery to engage.

**Hormone dependence connects endocrine physiology to controlled cell loss.**

# Epithelial homeostasis balances gain and loss



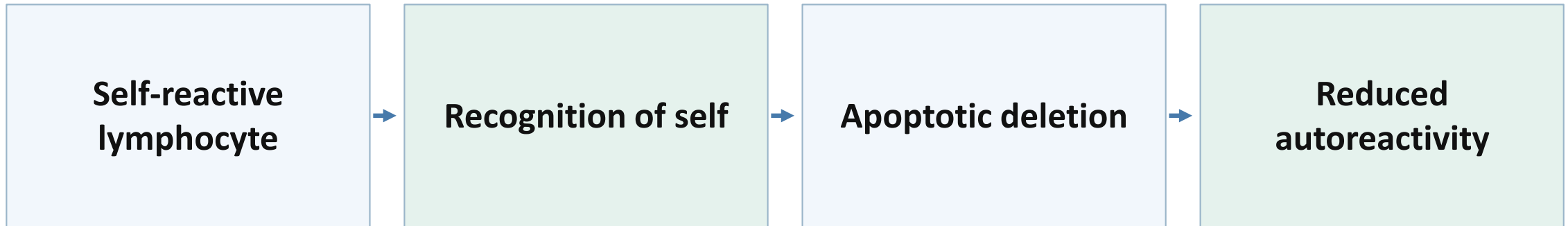
Intestinal crypt epithelium illustrates high-turnover tissue equilibrium.

# Immature lymphocyte selection removes failed cells

- Developing lymphocytes must assemble functional antigen receptors.
- Cells that fail this requirement are eliminated by apoptosis.
- Selection operates in bone marrow and thymus.
- Germinal-center B-cell populations also undergo regulated deletion.

**Failed immune-cell development need not produce necrotic injury.**

# Central immune tolerance requires cell deletion



Removal of potentially harmful lymphocytes is one defense against autoimmunity.

# Neutrophil removal helps resolve acute inflammation

- Neutrophils act for a limited interval at inflammatory sites.
- Spent neutrophils subsequently undergo apoptosis.
- Phagocytes clear the fragments without major content spillage.
- Cell removal facilitates the resolution of an inflammatory episode.

**The cessation of inflammation requires active cell clearance.**

# **Immune contraction follows a completed response**

- Expanded effector lymphocyte populations are no longer needed.
- Apoptosis contracts the population after antigen control.
- Cells are removed while surrounding tissue is relatively preserved.
- A failure of effective deletion may allow persistent lymphocytes.

**Physiologic apoptosis limits the duration of immune responses.**

# Survival signals protect against unnecessary death

## Signals that preserve cells

- Growth factors support viability.
- Extracellular-matrix contacts contribute survival cues.
- Anti-apoptotic proteins maintain mitochondrial integrity.

## Events favoring deletion

- Withdrawal of growth support
- Loss of requisite cell contacts
- Acquisition of pro-apoptotic signals

# Cells integrate competing survival and death signals

**Apoptosis depends on the balance of opposing molecular influences**

Survival cues favor anti-apoptotic BCL2-family activity.

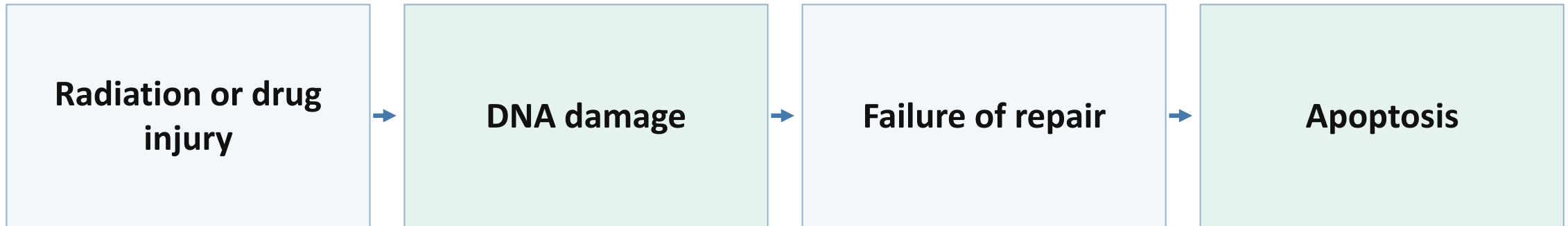
Cell stress activates sensors that favor BAX/BAK-mediated membrane permeabilization.

# Pathologic apoptosis minimizes collateral damage

- Cells injured beyond repair are selectively eliminated.
- DNA lesions or misfolded proteins may initiate the response.
- Viral infection and immune cytotoxicity are further triggers.
- Parenchymal cells may be lost after prolonged duct obstruction.

**Apoptosis is protective in some settings yet contributes to tissue loss in others.**

## Severe DNA damage can trigger deletion



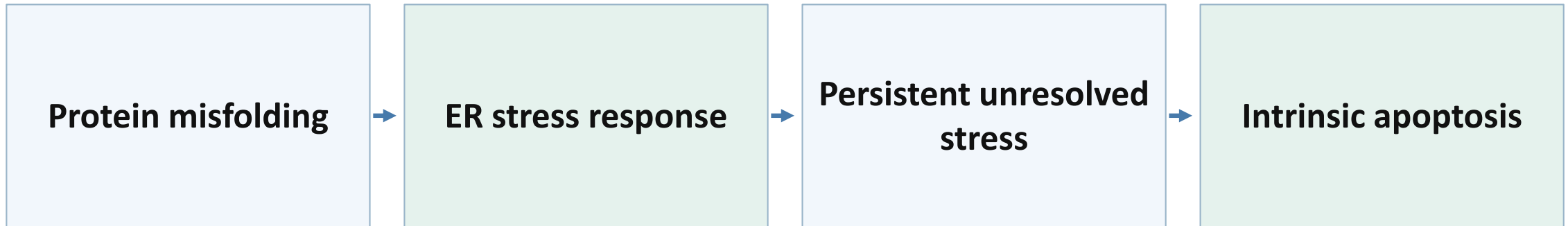
Deletion prevents survival of cells harboring potentially oncogenic mutations.

# Genotoxic insults threaten genomic integrity

- Radiation can damage DNA directly or through reactive species.
- Cytotoxic anticancer drugs can produce DNA lesions.
- Damage-repair mechanisms may restore cellular viability.
- When repair fails, intrinsic apoptotic pathways remove the cell.

**Whether a cell survives depends in part on the ability to repair its DNA.**

# Misfolded proteins couple ER stress to death



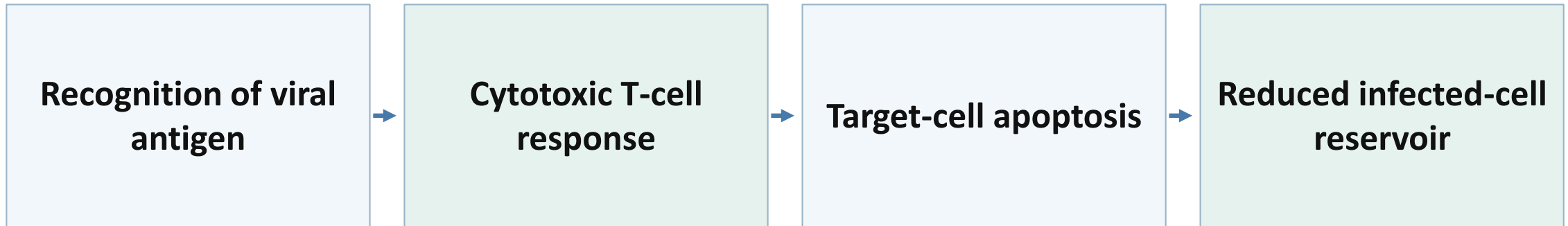
Severe unresolved proteostasis stress can ultimately cause cell loss.

# Infections can engage more than one apoptotic trigger

- Some viruses directly injure infected cells.
- Immune cells can induce apoptosis in infected targets.
- The response limits reservoirs of intracellular pathogens.
- Extensive apoptotic deletion may nevertheless damage tissues.

**Viral hepatitis illustrates host-mediated hepatocyte injury.**

# Cytotoxic T lymphocytes remove infected targets



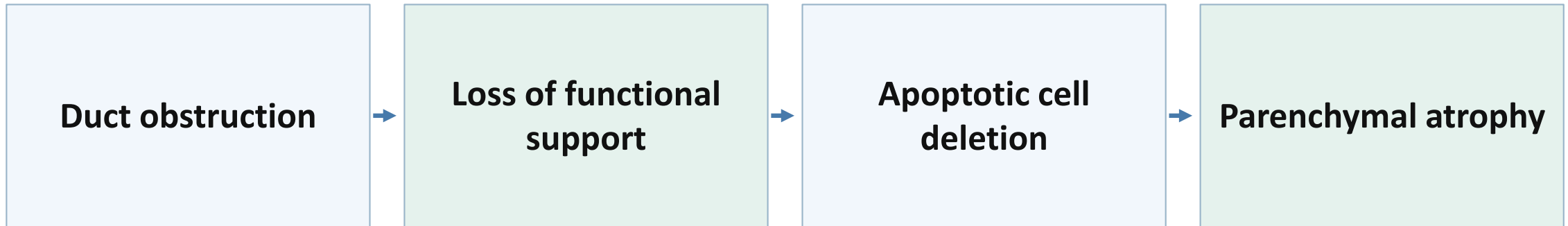
Apoptotic killing protects the host but can contribute to organ injury.

## **Immune cytotoxicity also targets noninfected cells**

- Cytotoxic T lymphocytes may eliminate transformed tumor cells.
- Immune recognition causes cell-death pathways in targets.
- The same cytotoxic capacity underlies cellular allograft rejection.
- Graft-versus-host disease also causes immune-mediated tissue injury.

**One cytotoxic mechanism can be protective or pathologic depending on its target.**

# Duct obstruction can lead to parenchymal atrophy

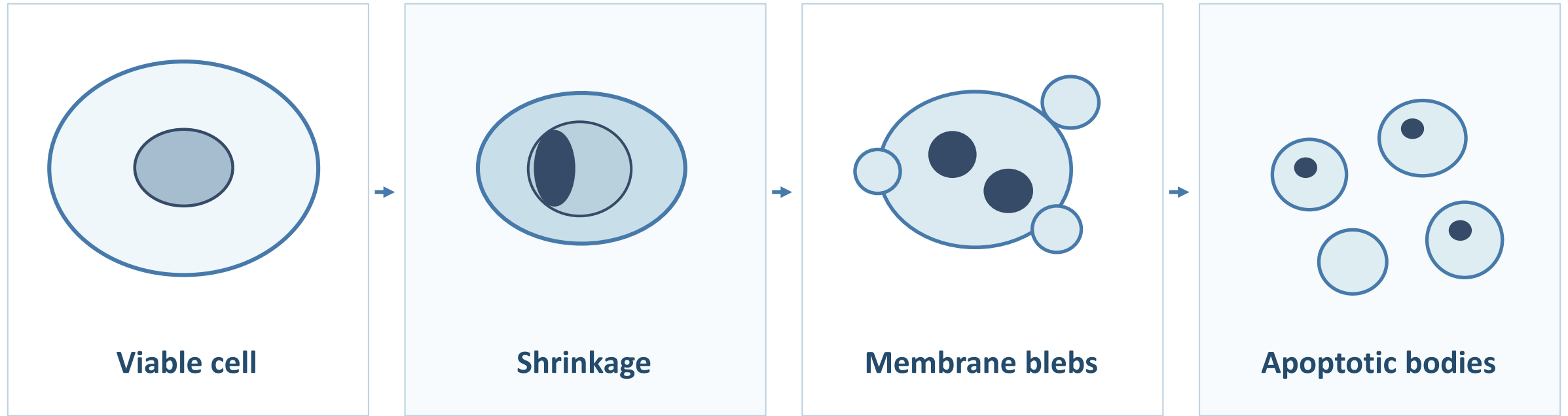


Examples include pancreas, parotid gland, and kidney.

# Physiologic and pathologic contexts

| Physiologic context          | Pathologic context                  |
|------------------------------|-------------------------------------|
| Developmental remodeling     | Radiation-induced DNA injury        |
| Hormone-dependent involution | Persistent misfolded-protein stress |
| Epithelial cell turnover     | Virus-associated tissue damage      |
| Lymphocyte selection         | Immune injury to grafts             |
| Resolution of inflammation   | Obstruction-associated atrophy      |

# Morphologic progression: The cellular overview



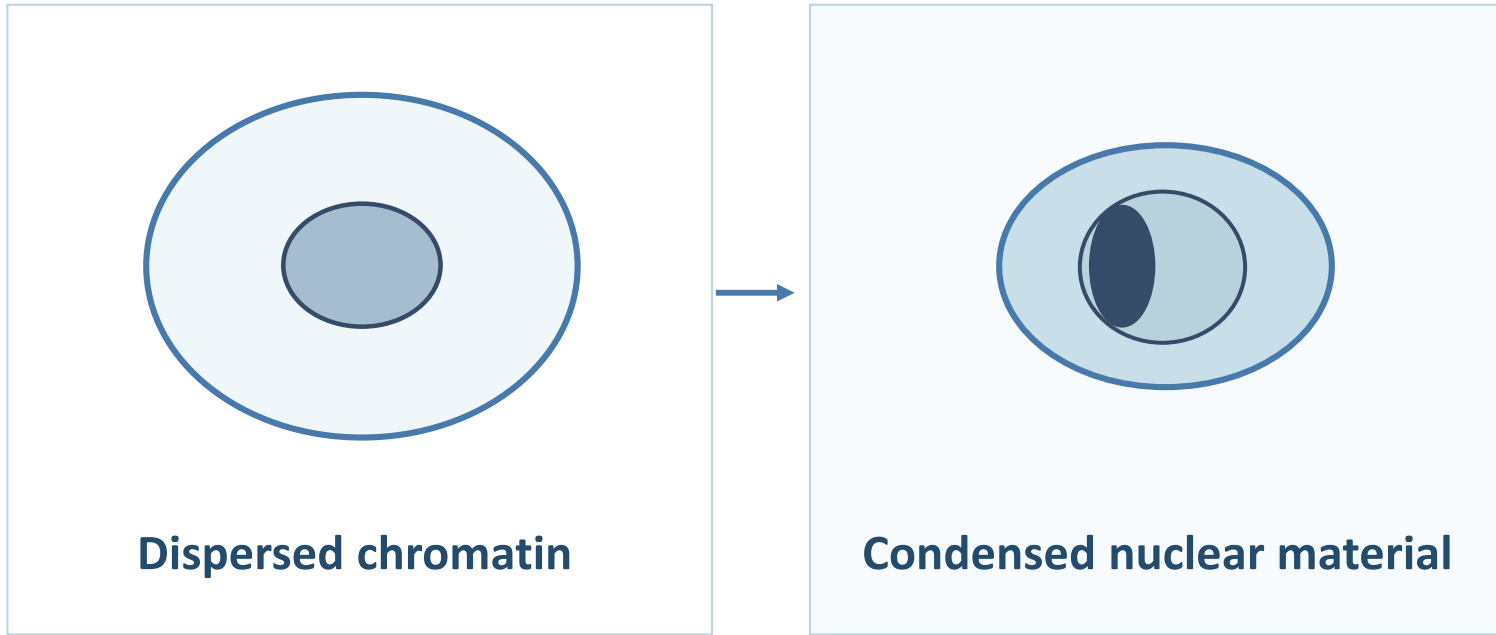
Schematic — Original schematic of successive morphologic changes; Not a histologic specimen.

## **Apoptotic cells become smaller and denser**

- Cell volume decreases, unlike the early swelling of necrosis.
- Cytoplasm becomes densely eosinophilic on H&E.
- Intracellular organelles remain relatively intact initially.
- Organelles become more closely packed as the cell shrinks.

**Cell shrinkage is a characteristic early morphologic cue.**

## Condensed chromatin is a dominant nuclear clue



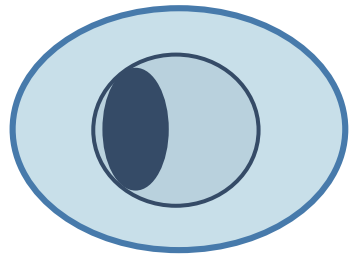
Schematic — In apoptosis, chromatin aggregates into dense peripheral masses near the nuclear envelope.

# **Chromatin condensation precedes nuclear breakdown**

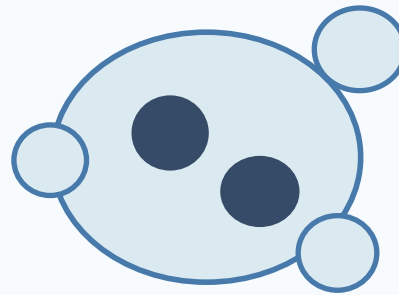
- Condensed chromatin forms dense masses of varying shapes.
- The chromatin often lies beneath the nuclear membrane.
- Later, the nucleus may break into multiple pieces.
- Fragmentation is spatially confined within apoptotic structures.

**Chromatin condensation is emphasized as a particularly characteristic feature.**

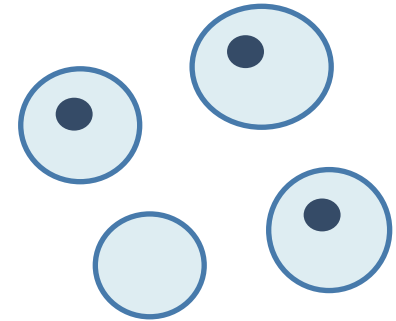
# Membrane blebs precede cell fragmentation



**Cell shrinkage**



**Surface blebbing**



**Discrete fragments**

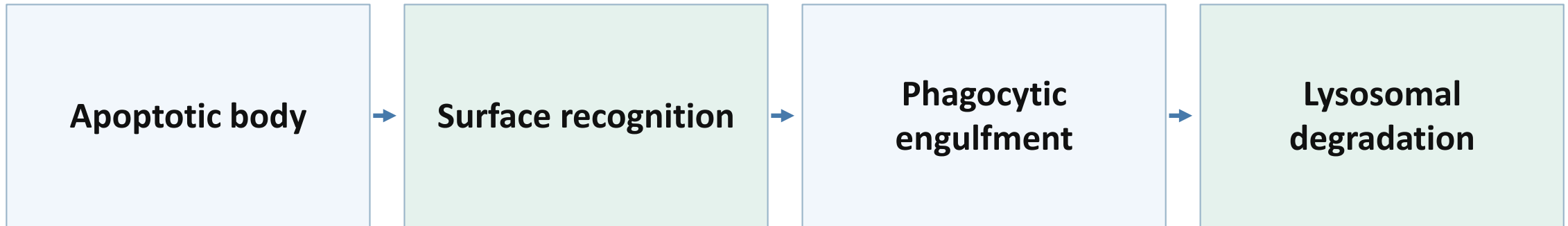
Schematic — The cell surface forms blebs and then separates into membrane-bound bodies.

# **Apoptotic bodies package cellular material**

- Fragments contain cytoplasm and densely packed organelles.
- Some, but not all, bodies also carry fragments of nuclei.
- Each fragment remains bounded by a cellular membrane.
- Membrane confinement reduces uncontrolled tissue exposure.

**An apoptotic body is a fragment of a dead cell, not an intact surviving cell.**

# Phagocytosis completes morphologic removal



Macrophages and other phagocytes efficiently dispose of the remnants.

# What an apoptotic cell looks like on H&E

- A round or oval shrunken cellular profile
- Intensely eosinophilic cytoplasm
- Condensed or fragmented, deeply basophilic chromatin
- Usually little surrounding inflammatory reaction

**Descriptions are; No photomicrograph is reproduced in this deck.**

## Apoptosis versus necrosis: Microscopic clues

| Feature         | Apoptosis               | Necrosis                           |
|-----------------|-------------------------|------------------------------------|
| Cell volume     | Shrinkage               | Swelling                           |
| Plasma membrane | Maintained as fragments | Disrupted                          |
| Nucleus         | Condensation; Fragments | Pyknosis, karyorrhexis, karyolysis |
| Contents        | Membrane-contained      | Leaked into tissue                 |
| Inflammation    | Typically minimal       | Usually present                    |

## **A section may underestimate ongoing apoptosis**

- Apoptotic shrinkage and breakup can occur rapidly.
- Phagocytosis promptly removes the individual fragments.
- Transient events may disappear between sampling and fixation.
- An absence of inflammation offers few secondary clues.

**A low visible apoptotic-body count does not establish absent apoptotic activity.**

# **Caspases execute controlled cell disassembly**

**Caspases are cysteine proteases that cleave substrates after aspartate residues**

They are produced as inactive precursors.

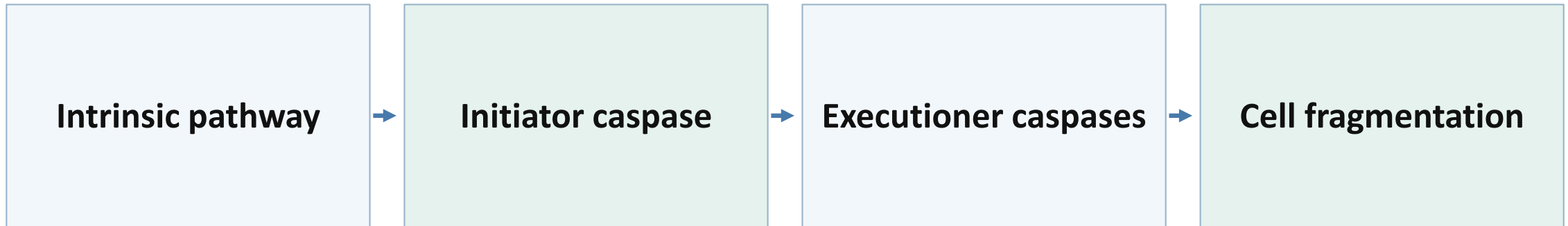
Proteolytic activation initiates an amplifying enzymatic cascade.

# Initiator and executioner caspases have different roles

- Initiator caspases are activated by upstream signaling assemblies.
- Caspase-9 is central to the intrinsic pathway.
- Caspase-8 or -10 is activated by death receptor signaling.
- Executioner caspases such as caspase-3 and -6 cleave cellular substrates.

**Initiation differs; The final execution program converges.**

## Two routes converge on a common execution program



Extrinsic signaling reaches the same terminal proteolytic machinery.

# The initiation phase and the execution phase

## Initiation

- Distinct cellular death signals
- Formation of pathway-specific complexes
- Activation of initiator caspases

## Execution

- Rapid activation of downstream caspases
- Protein and nuclear component degradation
- Fragmentation and clearance

# Common triggers of intrinsic apoptosis

- Withdrawal of essential survival factors
- DNA damage that cannot be adequately repaired
- Accumulation of misfolded proteins and ER stress
- Other cellular injuries sufficient to activate BH3-only sensors

**Mitochondrial apoptosis operates in many physiologic and pathologic situations.**

# **Mitochondrial outer membrane permeabilization is pivotal**

**Permeabilization releases normally sequestered death-promoting molecules**

Cytochrome c functions in mitochondrial energy metabolism.

In the cytosol, released cytochrome c contributes to caspase activation.

## The BCL2 family: Three functional groups

| Group             | Representative members    | Principal function                         |
|-------------------|---------------------------|--------------------------------------------|
| Anti-apoptotic    | BCL2, BCL-XL, MCL1        | Preserve mitochondrial integrity           |
| Effector proteins | BAX, BAK                  | Promote membrane permeabilization          |
| BH3-only sensors  | BAD, BIM, BID, PUMA, NOXA | Respond to death signals; Engage effectors |

# **BCL2-family proteins govern the life–death threshold**

- Family members are distinguished by BCL2 homology domains.
- Pro-survival proteins oppose mitochondrial membrane permeabilization.
- BAX and BAK are pro-apoptotic effector molecules.
- Stress-responsive BH3-only proteins shift the balance toward death.

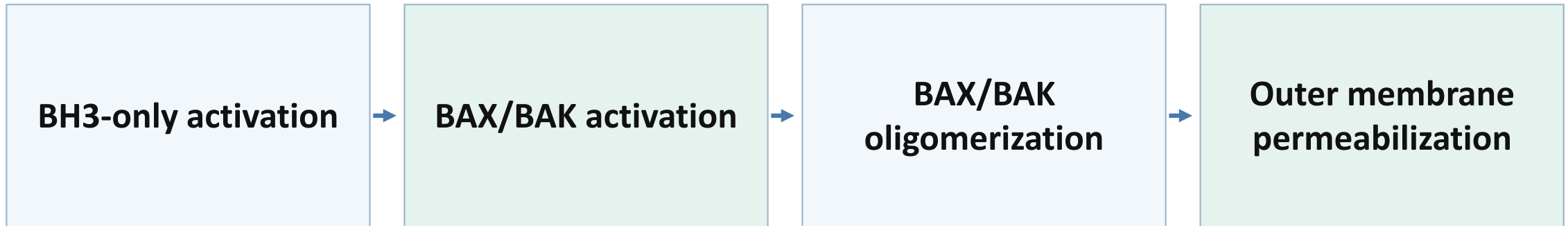
**The relevant output is permeability of the outer mitochondrial membrane.**

## **BCL2, BCL-XL, and MCL1 protect cells**

- These proteins are major anti-apoptotic BCL2-family members.
- They help prevent release of cytochrome c and related factors.
- They occur at outer mitochondrial membranes and other sites.
- Survival signals can support anti-apoptotic protein expression.

**Protection is functional rather than a simple fixed on/off gene state.**

# **BAX and BAK produce the mitochondrial commitment step**



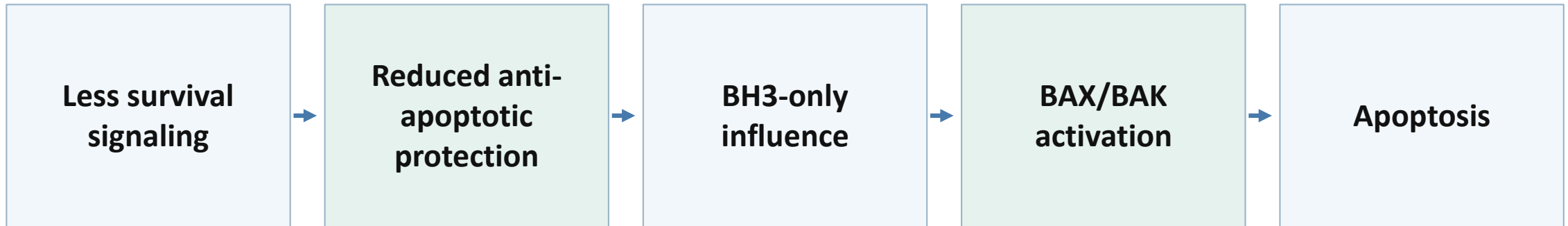
The exact biophysical pore-forming mechanism is not completely settled.

## **BH3-only proteins sense cellular stress**

- Textbook examples include BAD, BIM, BID, PUMA, and NOXA.
- Stress can increase their abundance or activity.
- They can activate BAX/BAK and oppose BCL2/BCL-XL.
- Their combined actions favor cytochrome c release.

**BH3-only proteins link diverse injuries to the common mitochondrial response.**

# Growth factor withdrawal can activate apoptosis



Loss of trophic signals removes protection that previously held the pathway in check.

# Survival and death influences at mitochondria

## Survival bias

- Growth-factor signaling present
- BCL2-family protection maintained
- Cytochrome c retained in mitochondria

## Death bias

- DNA injury or ER stress persists
- BH3-only activity increases
- BAX/BAK permits cytochrome c escape

# Cytochrome c initiates apoptosome assembly



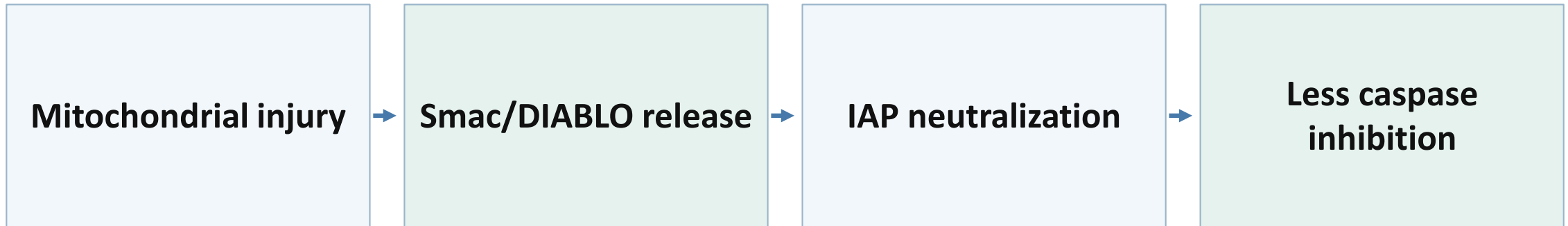
The apoptosome is a multiprotein platform for initiation of caspase signaling.

## **Caspase-9 launches the intrinsic caspase cascade**

- Cytochrome c–APAF-1 complexes recruit procaspase-9.
- Proximity promotes caspase-9 activation.
- Active caspase-9 activates downstream caspases.
- Caspase-3 is one of the key executioner enzymes.

**The cytosolic apoptosome connects mitochondrial injury to proteolysis.**

# Mitochondrial proteins also release caspase brakes



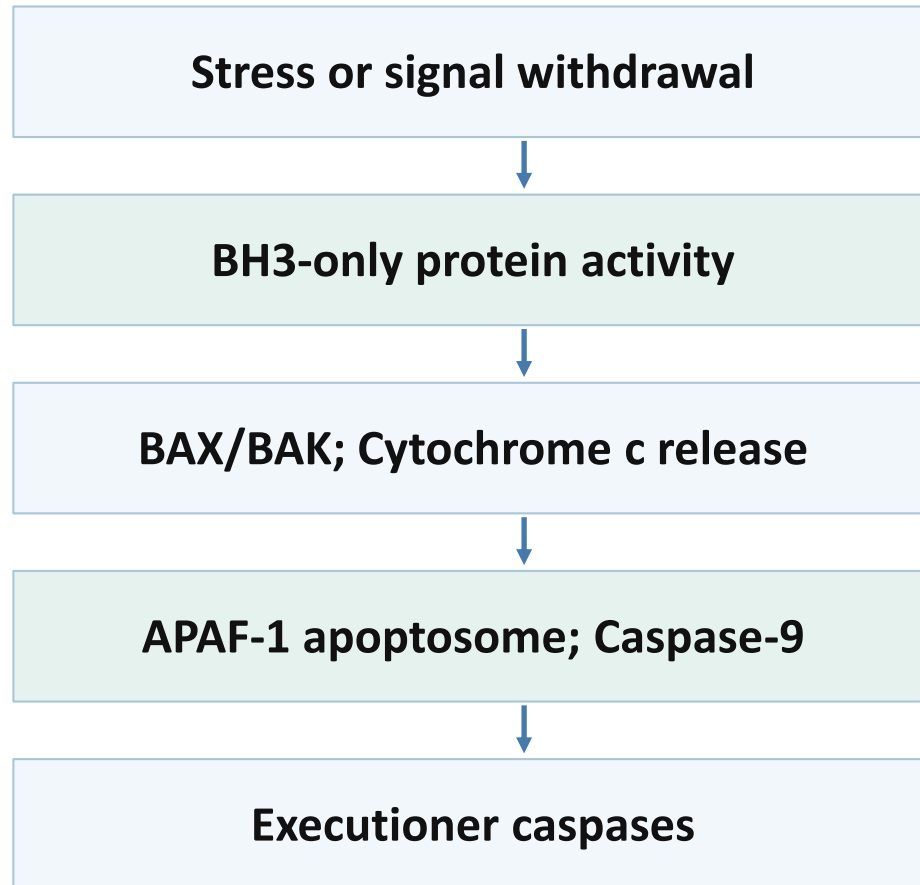
IAPs physiologically restrain unwanted caspase activation.

# Inhibitor-of-apoptosis proteins restrain death

- IAPs oppose inappropriate caspase activity in viable cells.
- Mitochondrial Smac/DIABLO can antagonize IAP function.
- Removing the inhibitory influence facilitates executioner action.
- Apoptotic commitment reflects activating and inhibitory mechanisms.

**The caspase cascade is regulated by more than its initiator complexes.**

# Intrinsic pathway from injury to execution



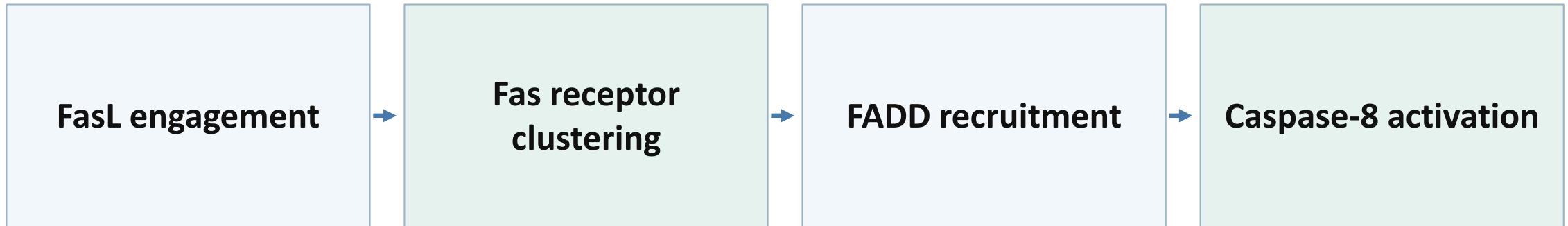
Key logic: Internal stress changes mitochondrial outer-membrane permeability, allowing cytochrome c to activate the APAF-1/caspase-9 platform. Anti-apoptotic BCL2 proteins oppose this transition.

# Death receptors initiate an extracellularly triggered route

- Death receptors are members of the TNF receptor superfamily.
- A cytoplasmic death domain recruits signaling adaptors.
- Fas (CD95) and TNFR1 are major examples.
- Receptor engagement can trigger caspase activation.

**Death-receptor signaling is not identical to all TNF-family receptor signaling.**

# Fas ligand initiates death receptor signaling



The cytoplasmic death domain transmits the receptor-associated death signal.

# **Fas-mediated lymphocyte deletion supports tolerance**

- Fas is present on multiple cell types.
- FasL is expressed by certain activated T cells.
- Engagement can help eliminate self-reactive lymphocytes.
- The same system contributes to CTL killing of targets.

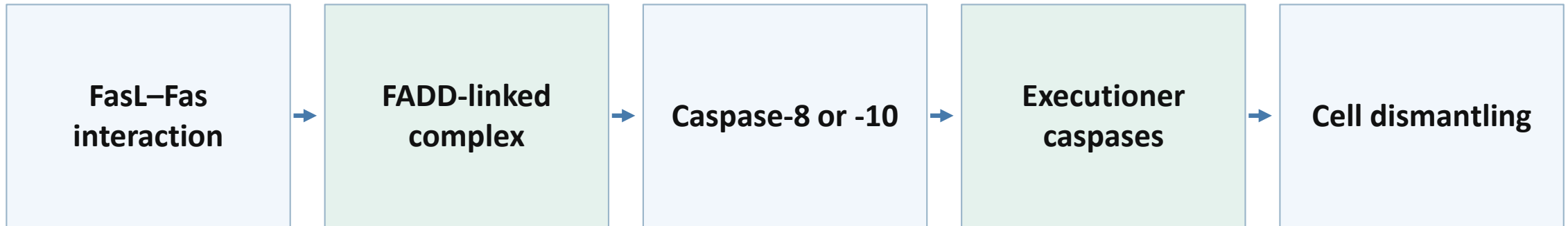
**Extrinsic apoptosis has a prominent immune-system role.**

## **FADD organizes the death-inducing complex**

- Fas receptor clustering juxtaposes intracellular death domains.
- FADD binds these receptor-associated domains.
- FADD recruits procaspase-8 or procaspase-10 molecules.
- Proximity allows processing into active initiator caspases.

**The death-inducing signaling complex is a protein assembly, not a membrane pore.**

## The extrinsic route reaches the same executioners



Death receptor engagement changes the initiation machinery but not the broad end point.

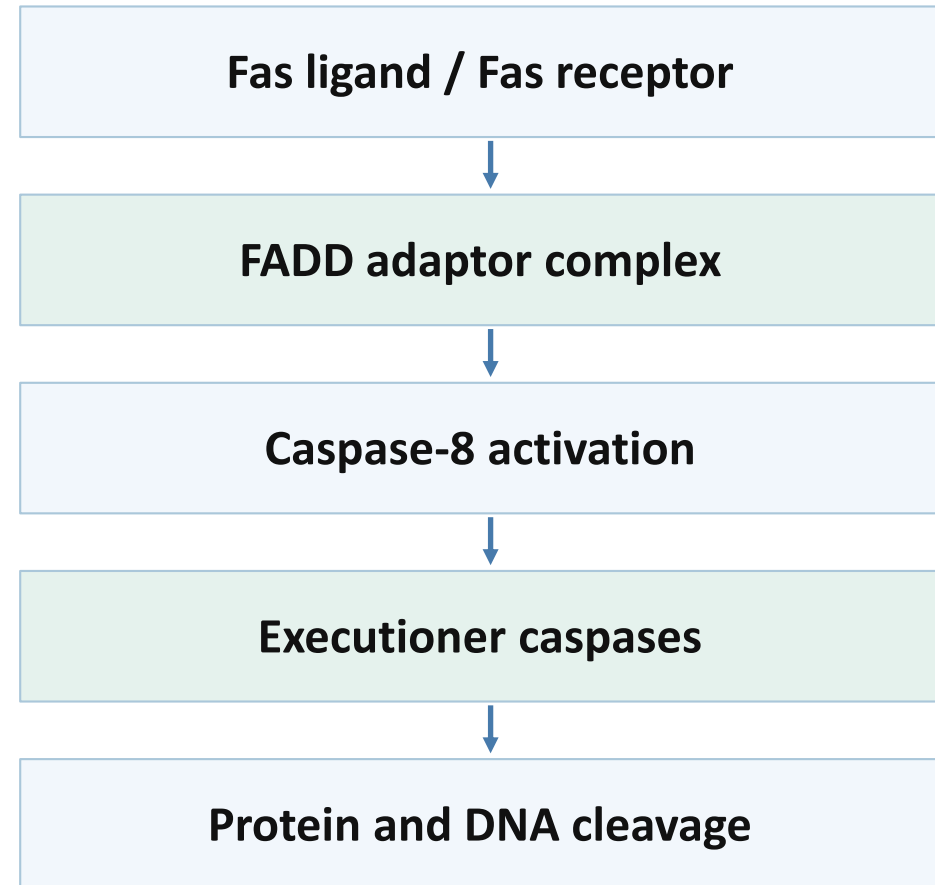
## **FLIP can inhibit death-receptor apoptosis**

- FLIP interacts with the procaspase-8 activation machinery.
- It can interfere with assembly of productive FADD–caspase complexes.
- Some normal cells use FLIP as a protective mechanism.
- Certain viruses also express molecules that antagonize Fas killing.

**Receptor engagement alone does not always guarantee target-cell apoptosis.**

# Fas signaling, from membrane to nucleus

At the surface, receptor aggregation recruits FADD; Initiator caspase processing then drives a downstream caspase cascade. This is distinct from cytochrome c–APAF-1 signaling.



## **BID provides cross-talk to mitochondria**



Hepatocytes and pancreatic  $\beta$ -cells as examples of this linkage.

# Intrinsic and extrinsic apoptosis compared

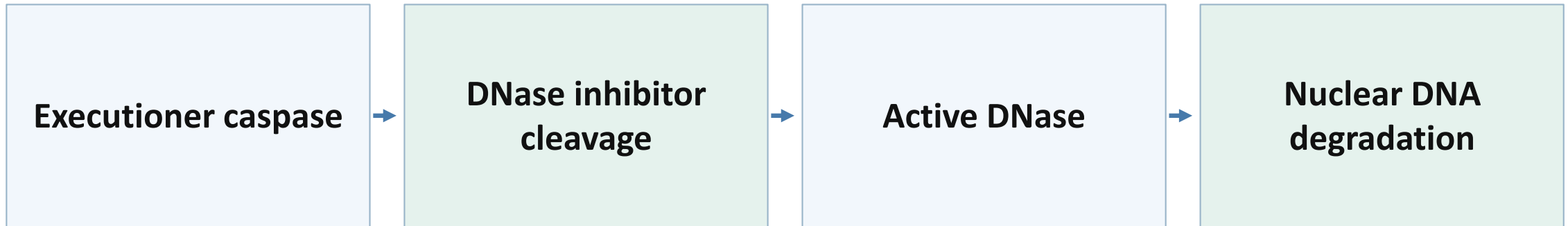
| Parameter                   | Intrinsic                           | Extrinsic                       |
|-----------------------------|-------------------------------------|---------------------------------|
| Usual starting signal       | Internal stress / signal withdrawal | Death ligand / receptor binding |
| Main initiating platform    | Cytochrome c–APAF-1 apoptosome      | FADD-dependent receptor complex |
| Initiator caspase           | Caspase-9                           | Caspase-8 or -10                |
| Central regulatory proteins | BCL2 family; IAPs                   | Fas, FADD, FLIP                 |
| Common endpoint             | Executioner caspases                | Executioner caspases            |

## **Executioner caspases target the cell interior**

- Activated caspase-3 and -6 cleave multiple cellular proteins.
- Nuclear scaffolding proteins are dismantled.
- An inhibitor of DNase is cleaved, enabling DNA fragmentation.
- Proteolysis and nucleolysis cooperate in cell disassembly.

**Biochemical degradation explains the appearance of nuclear fragments.**

## Caspases activate DNA fragmentation



The execution phase converts a death decision into irreversible structural changes.

# How the nucleus fragments during apoptosis

- Executioner enzymes degrade nuclear structural elements.
- Activated nucleases cleave nuclear DNA.
- Chromatin compacts and breaks into discrete fragments.
- Fragments may become enclosed within apoptotic bodies.

**Morphologic nuclear segmentation reflects a coordinated biochemical process.**

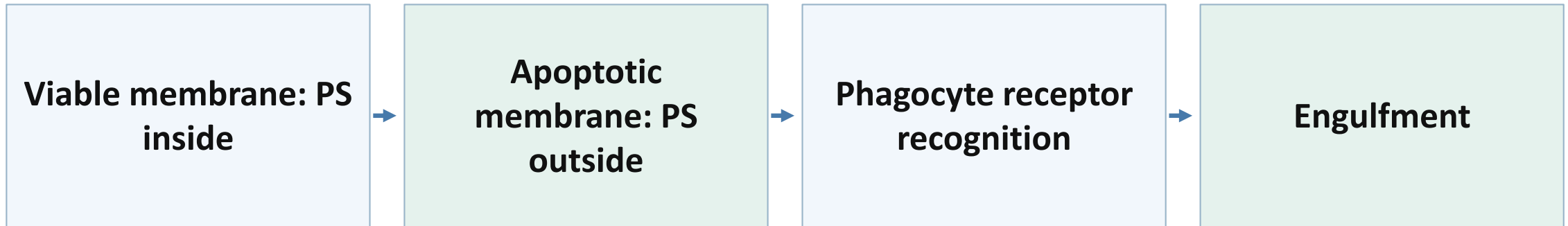
# **Membrane blebbing remains incompletely explained**

**Not every morphologic step is mechanistically resolved**

Caspases clearly degrade many structural proteins.

The precise formation of blebs and apoptotic bodies is less well defined.

# Phosphatidylserine externalization signals disposal



Phosphatidylserine on the outer leaflet is a central “eat-me” signal.

# Apoptotic cells attract and identify themselves to phagocytes

- Dying cells can release soluble phagocyte-recruiting factors.
- Exposed phosphatidylserine promotes target recognition.
- Macrophage-associated bridging proteins may aid adhesion.
- Multiple recognition signals make clearance efficient.

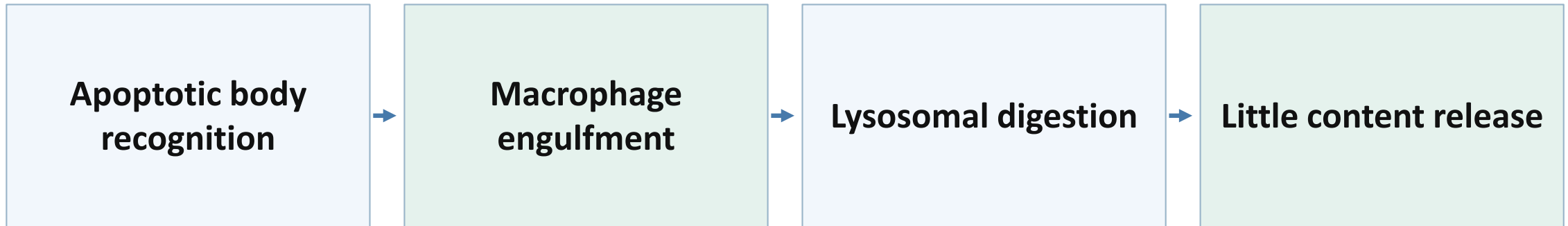
**“Find-me” and “eat-me” are distinct but cooperating functions.**

## **Opsonins can aid apoptotic-body recognition**

- Natural antibodies may coat apoptotic fragments.
- Complement proteins including C1q can associate with these bodies.
- Phagocyte receptors recognize the coating molecules.
- This adds redundant pathways for prompt engulfment.

**The receptor–ligand repertoire extends beyond phosphatidylserine alone.**

# Efferocytosis completes apoptosis without spillage



Rapid efferocytosis explains why cell loss can be histologically inconspicuous.

## **Clearance also limits inflammatory cytokine production**

- Macrophages ingest apoptotic cells before membrane rupture.
- Engulfment is associated with reduced proinflammatory cytokines.
- This suppressive milieu helps prevent collateral tissue injury.
- Efficient clearance is therefore an active anti-inflammatory process.

**Limited inflammation is a combined consequence of containment and clearance.**

## **Application: Radiation-induced cell loss**

After radiation exposure, a cell accumulates DNA damage that cannot be repaired, yet its plasma membrane has not ruptured.

**Which major apoptotic pathway is expected to dominate?**

**Intrinsic (mitochondrial) pathway.**

DNA injury engages stress sensors that favor BAX/BAK and cytochrome c release.

## **Application: Self-reactive T-cell deletion**

An activated autoreactive lymphocyte expresses Fas and interacts with a T cell expressing Fas ligand.

**Which initiator caspase is associated with the receptor complex?**

**Caspase-8 (or caspase-10).**

FADD recruits the procaspases to a death-inducing signaling complex.

## **Application: An eosinophilic shrunken cell**

A tissue section shows an isolated small round cell with intensely eosinophilic cytoplasm and dense fragmented chromatin, with no surrounding neutrophil influx.

**What mechanism best fits these observations?**

**Apoptosis.**

Shrinkage, chromatin condensation, and absent substantial inflammation support this mechanism.

## **Application: Why tissue inflammation is absent**

A biopsy contains isolated apoptotic bodies and relatively preserved adjacent parenchyma.

**Which event best explains the limited tissue reaction?**

**Timely recognition and efferocytosis.**

Membrane-contained bodies are swallowed before releasing their contents.

# Interpretation pitfalls when recognizing apoptosis

| Observation                   | Appropriate interpretation                                  |
|-------------------------------|-------------------------------------------------------------|
| Few visible apoptotic figures | Rapid clearance may underestimate cumulative death          |
| No inflammation               | Consistent with apoptosis, but not independently diagnostic |
| Fragmented nuclear chromatin  | Assess cell shrinkage and membrane-bound bodies             |
| Caspase activation            | Supports active execution machinery                         |
| Large necrotic zone           | Consider membrane breakdown and necrosis instead            |

## Five mechanisms worth remembering

- Intrinsic injury acts through BH3-only proteins and BAX/BAK.
- Cytosolic cytochrome c and APAF-1 activate caspase-9.
- Fas/FADD signaling typically engages caspase-8 or -10.
- Executioner caspases degrade proteins and enable DNA cleavage.

**Both pathways end in membrane-contained fragments that phagocytes remove.**

# Core references and source boundaries

## **Robbins, Cotran & Kumar: Pathologic Basis of Disease**

11th edition (2026) · Elsevier

Sections covered: apoptosis, its causes, morphology, signaling pathways, and removal of dead cells.