Apoptosis

- Apoptosis is a form of genetically programmed cell death designed to eliminate unwanted cells in the body through activate intrinsic enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins.

- Apoptosis is Cell suicide or (cell kills itself) genetically regulated by genes of mitochondria

- Apoptosis is done according to a series of cellular processes without the release of harmful substances into the surrounding area and induce inflammation

- It occurs in physiological and pathological conditions, and prevent of apoptosis lead to congenital defects in embryogenesis or disease as cancer .

Apoptosis in Physiologic Situations

Any normal cell, when damage or an error occurs inside it, we will find that it has several paths through which it tries to repair that error, but in the event that the cell is unable to repair that damage, it takes a completely different path, which is suicide. This happens if the cell discovers that its genetic material (DNA) is damaged, so it immediately resorts to suicide and disappears from existence in order to preserve the integrity of the surrounding cells and our bodies in general

At the same time that the process of cell death takes place, new cells replace them, and the imbalance may cause the death of old cells and the production of new cells to disturb the balance of these cells and thus the incidence of many diseases such as cancer and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease.

-removes old cells as red blood cells, unnecessary cells, and diseased cells, keeping the body healthy.

- The destruction of cells during embryogenesis, including implantation, organogenesis, , and metamorphosis.

- metamorphosis of frog and In the process of metamorphosis in insects, the cells of the larva die and the nutrients are provided for the formation of the structures of the adult stage.

- the differentiation of fingers and toes in a developing human fetus occurs as a result of cell death between the fingers. The result is a separation of the fingers

- regulation of hormone-dependent tissues upon hormone withdrawal,

such as endometrial cell breakdown during the menstrual cycle, ovarian follicular atresia in menopause, the regression of the lactating breast after weaning, and

prostatic atrophy after castration.

- Apoptosis plays an essential role in the development and maintenance of a healthy immune system. When B and T cells (immune cells)are first produced, they're tested to see if they react against any of the body's own "self" components. Cells that do are eliminated right away by apoptosis. If this process fails, self-reactive cells may be released into the body, where they can attack tissues and cause autoimmune conditions.

- Death of host cells that have served their useful purpose, such as neutrophils in an acute inflammatory response, and lymphocytes at the end of an immune response. In these situations cells undergo apoptosis because they are deprived of necessary survival signals, such as growth factors.

- Cell ageing

Apoptosis in Pathologic Conditions

Apoptosis eliminates cells that are injured beyond repair without eliciting a host reaction, thus limiting collateral tissue damage. Death by apoptosis is responsible for loss of cells in a variety of pathologic states:

- DNA damage. Radiation, cytotoxic anticancer drugs, and hypoxia can damage DNA, either directly or via production of free radicals. If repair mechanisms cannot cope with the injury, the cell triggers intrinsic mechanisms that induce apoptosis. In these situations elimination of the cell may be a better alternative

than risking mutations in the damaged DNA, which may result in malignant transformation.

-to kill the cancer cells because ability of cancer cells avoid or escape the apoptosis is main features to its unlimited growth and the chemical treatment of cancer is used on induction of apoptosis.

-To kill cells that have a defect in their DNA and that develop into malignant tumors, the cells respond to damage in their DNA to secrete the p53 protein, which is a strong stimulus for programmed cell death, and in some other cells, these cells do not interact with the mutations that occur in them, avoiding the process Cell death and cancerous cell production, radiation and chemicals are used to induce the process of cell death in some types of cancer cells

-To destroy cells infected with a virus, but there are some viruses that do not resist this process.An important host response to viruses consists of cytotoxic T lymphocytes specific for viral proteins, which induce apoptosis of infected cells in an attempt to eliminate reservoirs of infection. During this process there can be significant tissue damage. The same T-cell–mediated mechanism is responsible for cell death in tumors and cellular rejection of transplants.

- Pathologic atrophy in parenchymal organs after duct obstruction, such as occurs in the pancreas, parotid gland, and kidney

Disturbance of apoptosis

- **The prevent of apoptosis** will lead To the occurrence (a tumor and hyperplasia), (or an immune disease due to the failure to eliminate lymphocytes that destroy healthy body cells)
- **The prevent of apoptosis during embryogenesis lead to birth defects**
- **The increased of apoptosis** of may lead to diseases :

- Increased of apoptosis of nerve cells lead to neurodegeneration diseases such as Alzheimer's disease, Parkinson's disease
- -increased of apoptosis in red blood cells lead to hypoxia(brain attack, myocardial infarction)
- increased of apoptosis in cd4 T-lymphocyte lead to AIDS

Morphologic and Biochemical Changes in Apoptosis

MORPHOLOGY

-The following morphologic features, some best seen with the electron microscope, characterize cells undergoing apoptosis

1- Cell shrinkage. The cell is smaller in size, the cytoplasm is dense and the organelles, although relatively normal, are more tightly packed. (Recall that in other forms of cell injury, an early feature is cell swelling, not shrinkage.)

2- Chromatin condensation. This is the most characteristic feature of apoptosis. The chromatin aggregates peripherally, under the nuclear membrane, into dense masses of various
-shapes and sizes .The nucleus itself may break up, producing two or more fragments.

3- Formation of cytoplasmic blebs and apoptotic bodies.
The apoptotic cell first shows extensive surface blebbing, then undergoes fragmentation into membrane-bound apoptotic bodies composed of cytoplasm and tightly packed organelles, with or without nuclear fragments .

4-Phagocytosis of apoptotic cells or cell bodies, usually by macrophages. The apoptotic bodies are rapidly ingested by phagocytes and degraded by the phagocyte's lysosomal enzymes.
Plasma membranes are thought to remain intact during apoptosis, until the last stages, when they become permeable to normally retained solutes.
On histologic examination, in tissues stained with hematoxylin and eosin, the apoptotic cell appears as a round or oval mass of intensely eosinophilic cytoplasm with fragments of dense nuclear chromatin .
Because the cell shrinkage and formation of apoptotic bodies are rapid and the pieces are quickly phagocytosed, considerable apoptosis may occur in tissues before it becomes apparent in histologic sections. In addition, apoptosis—in contrast to necrosis—does not elicit inflammation, making it more difficult to detect histologically

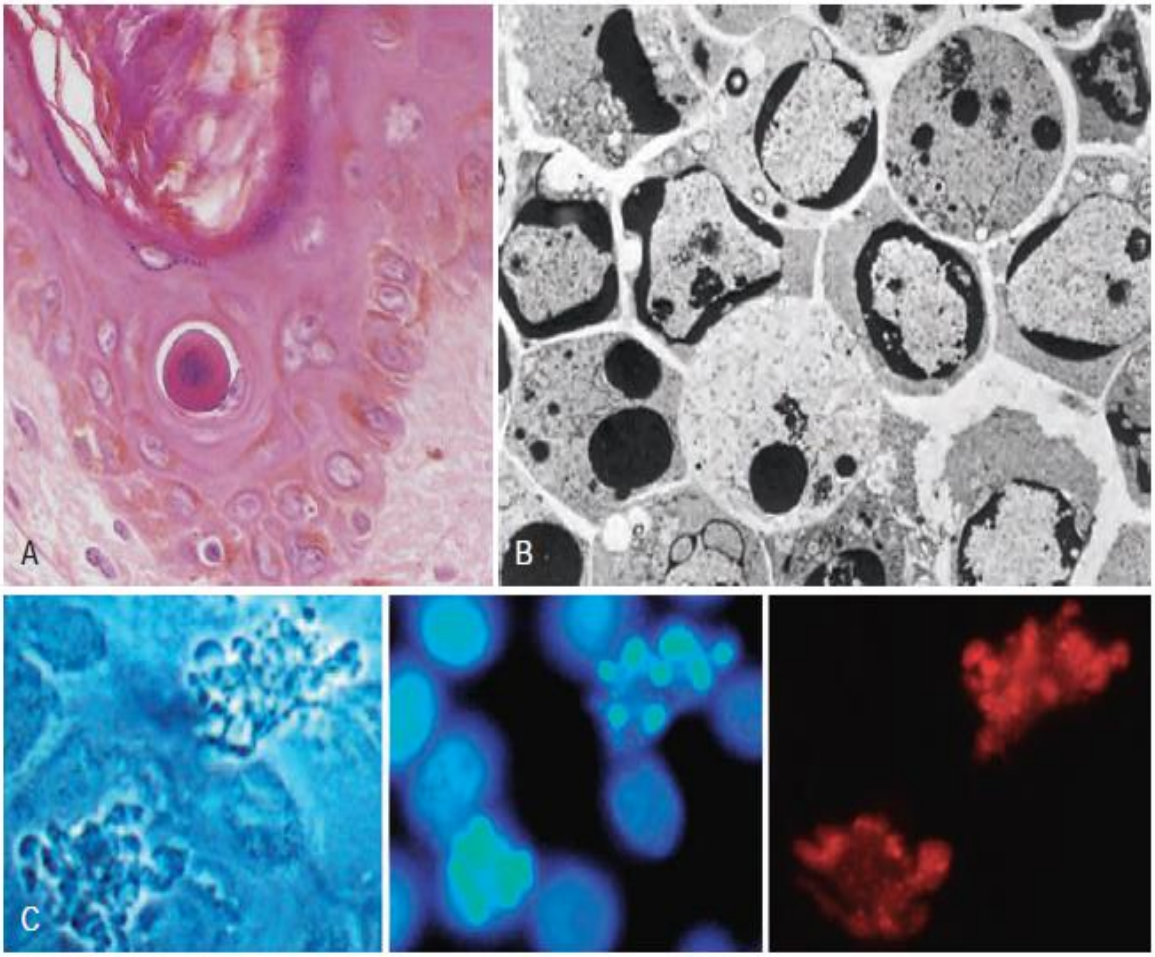


Figure 2-22 Morphologic features of apoptosis. **A,** Apoptosis of an epidermal cell in an immune reaction. The cell is reduced in size and contains brightly eosinophilic cytoplasm and a condensed nucleus. **B,** This electron micrograph of cultured cells undergoing apoptosis shows some nuclei with peripheral crescents of compacted chromatin, and others that are uniformly dense or fragmented. **C,** These images of cultured cells undergoing apoptosis show blebbing and formation of apoptotic bodies (*left panel*, phase contrast micrograph), a stain for DNA showing nuclear fragmentation (*middle panel*), and activation of caspase-3 (*right panel*, immunofluorescence stain with an antibody specific for the active form of caspase-3, revealed as red color). (**B,** From Kerr JFR, Harmon BV: Definition and incidence of apoptosis: a historical perspective. In Tomei LD, Cope FO (eds): Apoptosis: The Molecular Basis of Cell Death. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1991, pp 5-29; **C,** Courtesy Dr. Zheng Dong, Medical College of Georgia, Augusta, Ga.)

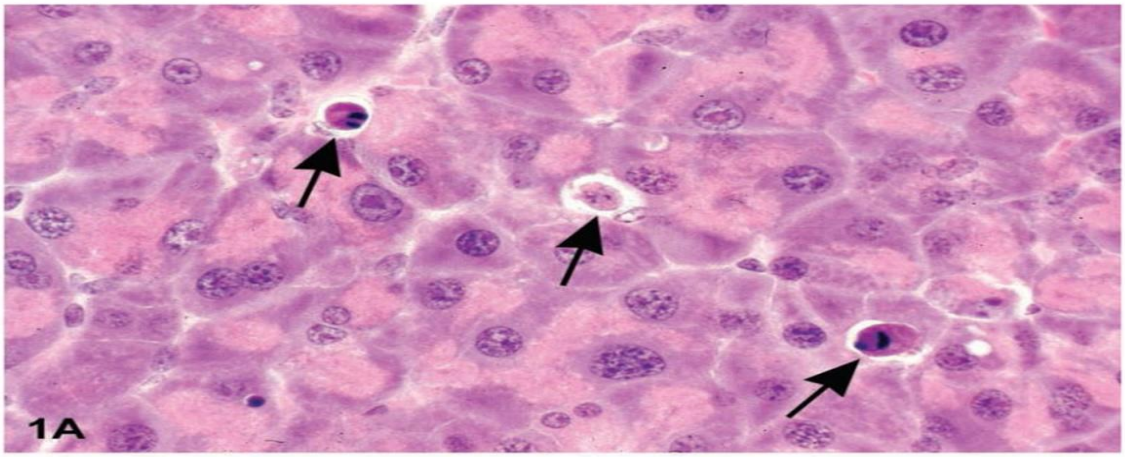


Figure 1A is a photomicrograph of a section of exocrine pancreas from mouse. The arrows indicate apoptotic cells that are shrunken with condensed cytoplasm. The nuclei are pyknotic and fragmented. Note the lack of inflammation

Sequence of Biochemical Events in Apoptosis

1. **Protein cleavage by proteolytic enzymes**

Activation of caspases (family of cysteine proteases having a unique ability to cleave after aspartic acid residues)
Protein hydrolysis
Cleavage or breakup of cytoskeletal proteins
Cleavage or breakup of nuclear scaffold proteins

2. **Protein cross-linkage**

Activation of transglutaminases Cross-linking of cytoplasmic proteins leading to covalently linked shrunken cells Easy breakdown into apoptotic bodies

3. **DNA condensation and breakdown**

DNA breakdown into large pieces (50–300 kb)
Internucleosomal cleavage by endonucleases forming oligonucleosomes (180–200 bp) visualized on agarose gel electrophoresis as DNA ladders

4. **Recognition of dying cells by phagocytes**

Flip-flop of apoptotic cell Phosphatidylserine and thrombospondin flip on the external surface from the inner layers
Easy recognition and phagocytosis of the apoptotic cell

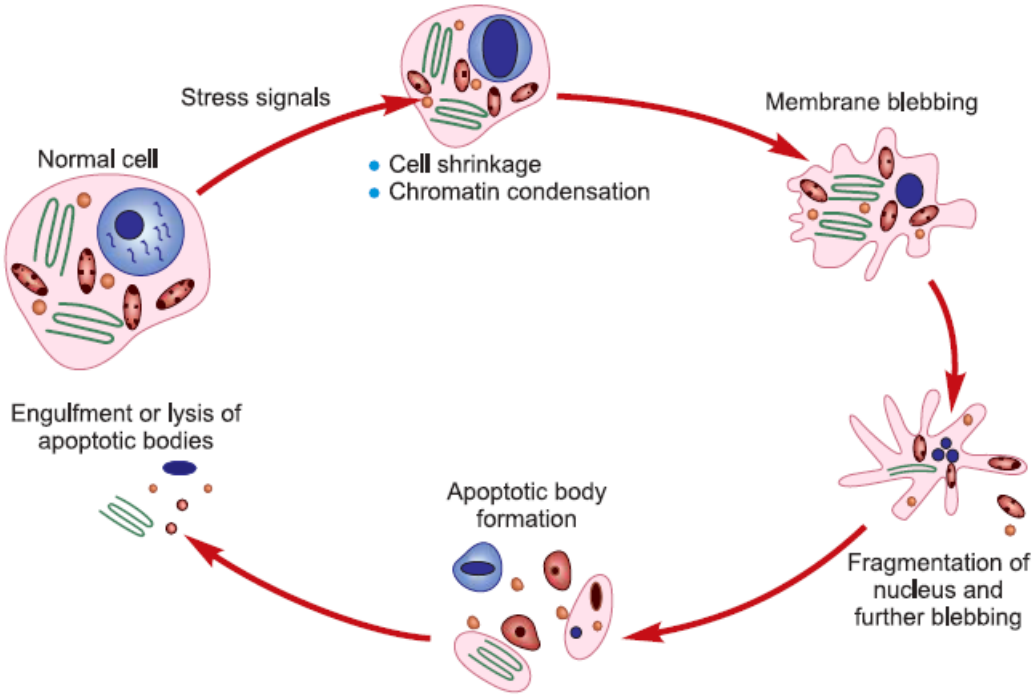
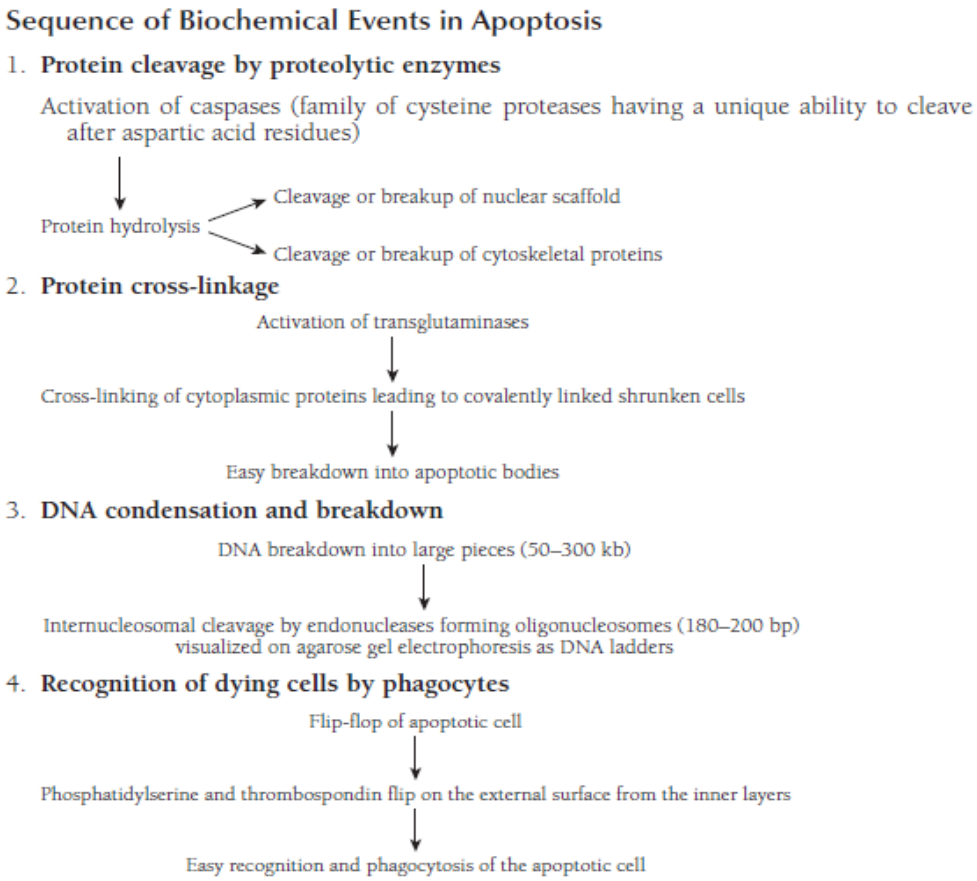


FIGURE 1.12. Sequence of morphological changes in apoptosis.



Mechanisms of Apoptosis

- Apoptosis results from the activation of enzymes called caspases Like many proteases, caspases exist as inactive proenzymes, or zymogens, and must undergo enzymatic cleavage to become active.
- The presence of cleaved, active caspases is a marker for cells undergoing apoptosis .
- The process of apoptosis may be divided into an:
- 1- **initiation phase**, during which some caspases become catalytically active
- 2- **execution phase**, during which other caspases trigger the degradation of critical cellular components.
- The activation of caspases depends on a finely tuned balance between production of pro-apoptotic and anti-apoptotic proteins.

First : initiation phase

Two distinct pathways converge on caspase activation: the mitochondrial pathway and the death receptor pathway

1-The Intrinsic (Mitochondrial) Pathway of Apoptosis

- The mitochondrial pathway is the major mechanism of apoptosis in all mammalian cells.
- It results from increased permeability of the mitochondrial outer membrane with consequent release of death-inducing (pro-apoptotic)molecules from the mitochondrial intermembrane space into the cytoplasm .
- Mitochondria are contain proteins such as cytochrome c that are essential for life, but some of the same proteins, in particular cytochrome c, when released into the cytoplasm (an indication that the cell is not healthy), initiate the suicide program of apoptosis. The release of mitochondrial
- pro-apoptotic proteins is tightly controlled by the BCL2 family of proteins This family is named *BCL2*, which is frequently overexpressed due to chromosomal translocations and resulting rearrangements in certain B cell lymphomas.
- There are more than 20 members of the *BCL* family, which can be divided into three groups based on their pro-apoptotic or antiapoptotic function and the BCL2 homology (BH) domains they possess.

A-• *Anti-apoptotic.* BCL2, BCL-XL, and MCL1 are the principal members of this group; These proteins reside in the outer mitochondrial membranes as well as the cytosol and ER membranes. By keeping the mitochondrial outer membrane impermeable they prevent leakage of cytochrome c and other death-inducing proteins into the cytosol .

B-• *Pro-apoptotic.* BAX and BAK are the two prototypic members of this group.. Upon activation, BAX and BAK oligomerize within the outer mitochondrial protein and promote mitochondrial outer membrane permeability, they form a channel in the outer mitochondrial membrane, allowing leakage of cytochrome c from the intermembranous space.

C-• *Sensors.* Members of this group, including BAD, BIM, BID, Puma, and Noxa, , and hence are sometimes called BH3-only proteins. BH3-only proteins act as sensors of cellular stress and damage, and regulate the balance between the other two groups, thus acting as arbiters of apoptosis.

- Growth factors and other survival signals stimulate the production of anti-apoptotic proteins such as BCL2, thus preventing the leakage of death-inducing proteins from the outer mitochondrial membrane.

- When cells are deprived of survival signals or their DNA is damaged, or misfolded proteins induce ER stress, the BH3-only proteins "sense" such damage and are activated.
- These sensors in turn activate the two critical (pro-apoptotic) effectors, BAX and BAK, which form oligomers that insert into the mitochondrial membrane and allow proteins(cytochrome C and other proapoptotic factors from the inner mitochondrial membrane to leak out into the cytoplasm.
- Cytochrome C binds to apoptosis activating factor (Apaf-1) and formation
- Cytochrome C- Apaf-1 complex (apoptosome)
- BH3-only proteins may also bind to and block the function of BCL2 and BCL-XL.
- At the same time, the synthesis of BCL2 and BCL-XL may decline because of the relative deficiency of survival signals.
- Other mitochondrial proteins, enter the cytoplasm, where they bind to and neutralize cytoplasmic proteins that function as physiologic inhibitors of apoptosis (called IAPs). The normal function of the IAPs is to block the activation of caspases, including executioners like caspase-3, and keep cells alive. Thus, the neutralization of these IAPs permits the initiation of a caspase cascade.

2-The Extrinsic (Death Receptor-Initiated) Pathway of Apoptosis

- This pathway is initiated by engagement of plasma membrane death receptors on a variety of cells Death receptors are members of the TNF receptor family that contain a cytoplasmic domain involved in protein-protein interactions that is called the *death domain* because it is essential for delivering apoptotic signals.
- (Some TNF receptor family members do not contain cytoplasmic death domains; their function is to activate inflammatory cascades ,and their role in triggering apoptosis is much less established.)
- The best known death receptors are the type 1 TNF receptor (TNFR1) and a related protein called Fas (CD95), but several others have been described. The mechanism of apoptosis induced by these death receptors is well illustrated by Fas, a death receptor expressed on many cell types .
- The ligand for Fas is called Fas ligand(FasL). FasL is expressed on T cells that recognize self antigens (and functions to eliminate self-reactive lymphocytes), and on some cytotoxic T lymphocytes (which kill virus-infected and tumor cells).
- When FasL binds to Fas, three or more molecules of Fas are brought together, and their cytoplasmic death domains form a binding site for an adaptor protein that also contains a death domain and is called FADD (*F*as-*a*ssociated *d*eath *d*omain).

- FADD that is attached to the death receptors in turn binds an inactive form of caspase-8 (and, in humans, caspase-10), again via a death domain. Multiple pro-caspase-8 molecules are thus brought into proximity, and they cleave one another to generate active caspase-8.
- This pathway of apoptosis can be inhibited by a protein called FLIP, which binds to pro-caspase-8 but cannot cleave and activate the caspase because it lacks a protease domain.
- Some viruses and normal cells produce FLIP and use this inhibitor to protect themselves from Fas-mediated apoptosis.

Second: Execution Phase

- The mitochondrial pathway leads to activation of the initiator caspase-9, and the death receptor pathway to the initiator caspases-8 and -10.
- After an initiator caspase is cleaved to generate its active form, the enzymatic death program is get involved by rapid and sequential activation of the executioner caspases.
- Executioner caspases, such as caspase-3 and -6, act on many cellular components. For instance, these caspases, once activated, cleave an inhibitor of a cytoplasmic DNase and thus make the DNase enzymatically active; this enzyme induces cleavage of DNA.
- Caspases also degrade structural components of the nuclear matrix and thus promote fragmentation of nuclei.

Removal of Dead Cells

- The formation of apoptotic bodies breaks cells up into fragments that are edible for phagocytes.
- Apoptotic cells and their fragments also undergo several changes in their membranes that actively promote their phagocytosis .
- In healthy cells, phosphatidylserine is present on the inner layer of the plasma membrane, but in apoptotic cells this phospholipid on the outer layer of the membrane, where it is recognized by several macrophage receptors.
- Cells that are dying by apoptosis secrete soluble factors that recruit phagocytes. Some apoptotic bodies are coated by thrombospondin, an adhesive glycoprotein that is recognized by phagocytes, and macrophages themselves may produce proteins that bind to apoptotic cells (but not to live cells) and thus target the dead cells for engulfment.
- Apoptotic bodies may also become coated with natural antibodies and proteins of the complement system, notably C1q, which are recognized by phagocytes. Thus, numerous receptors on phagocytes and ligands induced on

apoptotic cells serve as “eat me” signals and are involved in the binding and engulfment of these cells.

- This process of phagocytosis of apoptotic cells is so without leaving a trace, and inflammation is absent even in the face of extensive apoptosis.

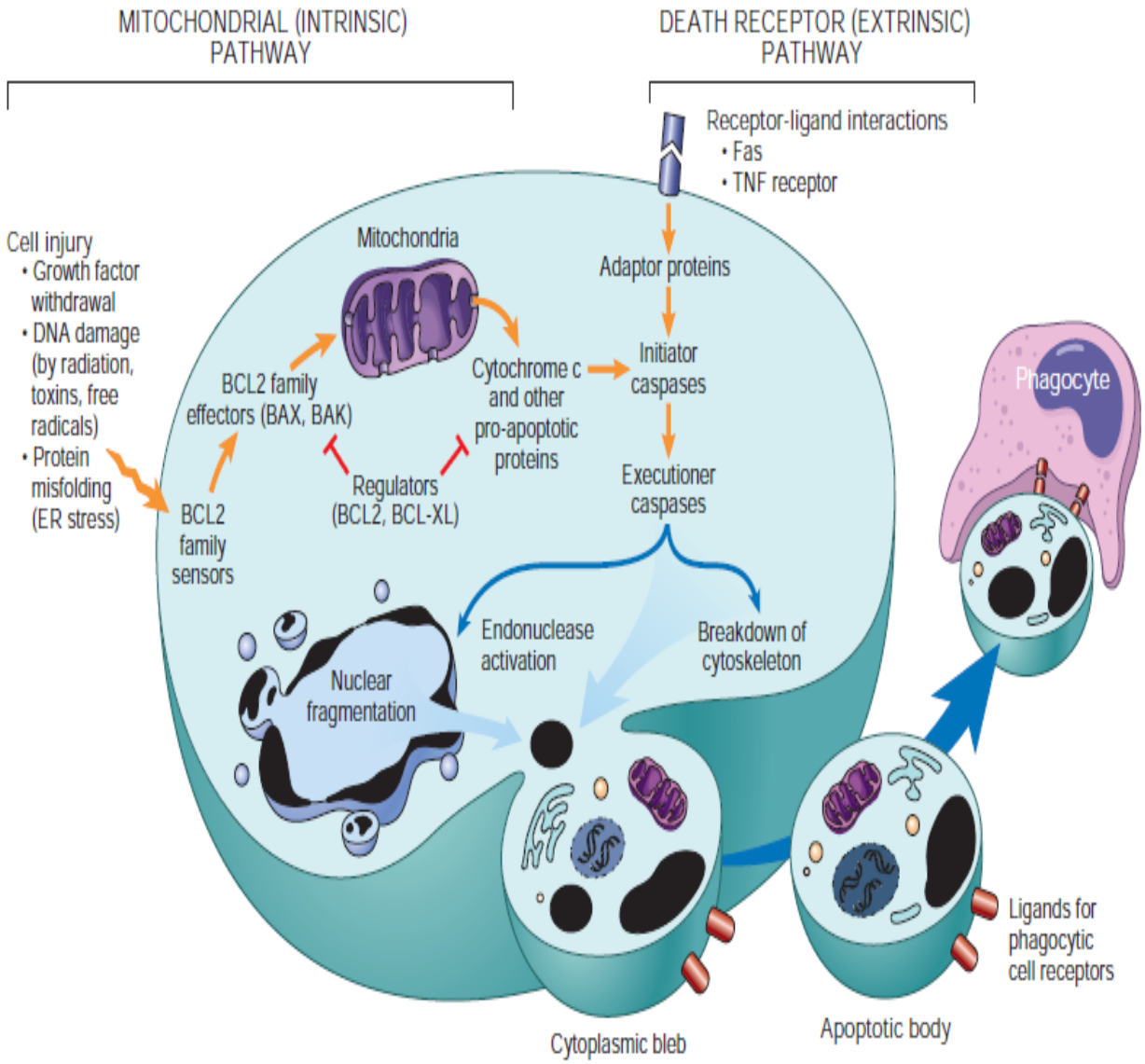


Figure 2-23 Mechanisms of apoptosis. The two pathways of apoptosis differ in their induction and regulation, and both culminate in the activation of caspases. In the mitochondrial pathway, proteins of the BCL2 family, which regulate mitochondrial permeability, become imbalanced and leakage of various substances from mitochondria leads to caspase activation. In death receptor pathway, signals from plasma membrane receptors lead to the assembly of adaptor proteins into a “death-including signaling complex,” which activates caspases, and the end result is the same.

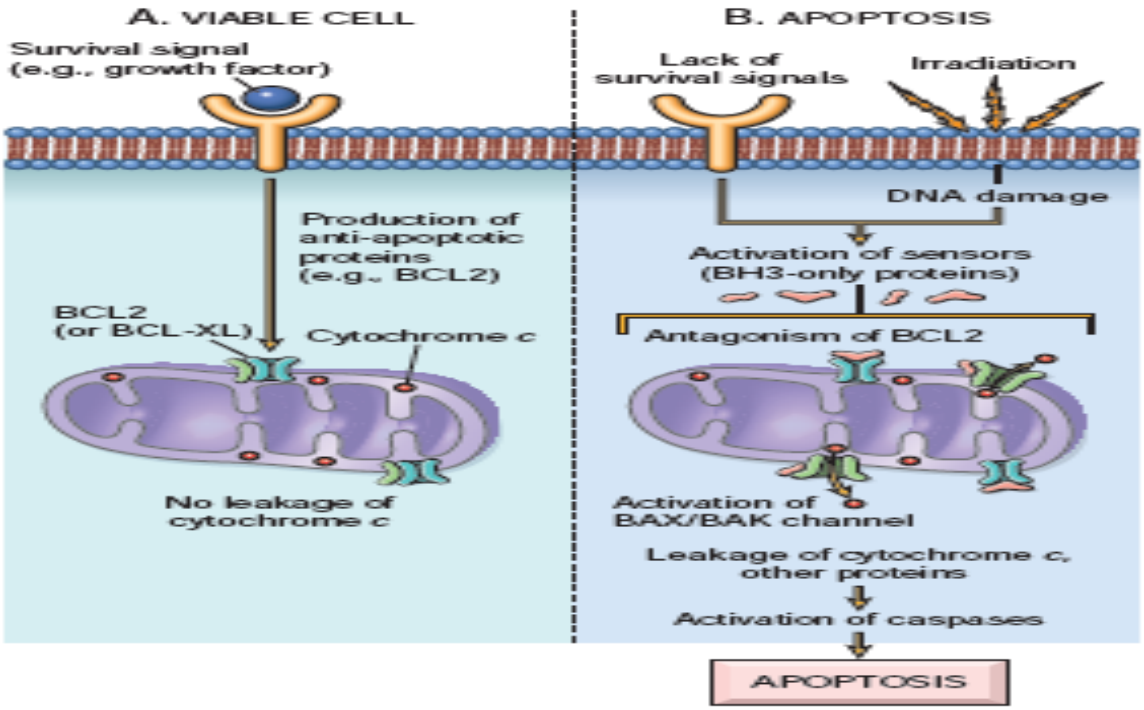


Figure 2-24 The intrinsic (mitochondrial) pathway of apoptosis. **A,** Cell viability is maintained by the induction of anti-apoptotic proteins such as BCL2 by survival signals. These proteins maintain the integrity of mitochondrial membranes and prevent leakage of mitochondrial proteins. **B,** Loss of survival signals, DNA damage, and other insults activate sensors that antagonize the anti-apoptotic proteins and activate the pro-apoptotic proteins BAX and BAK, which form channels in the mitochondrial membrane. The subsequent leakage of cytochrome c (and other proteins, not shown) leads to caspase activation and apoptosis.

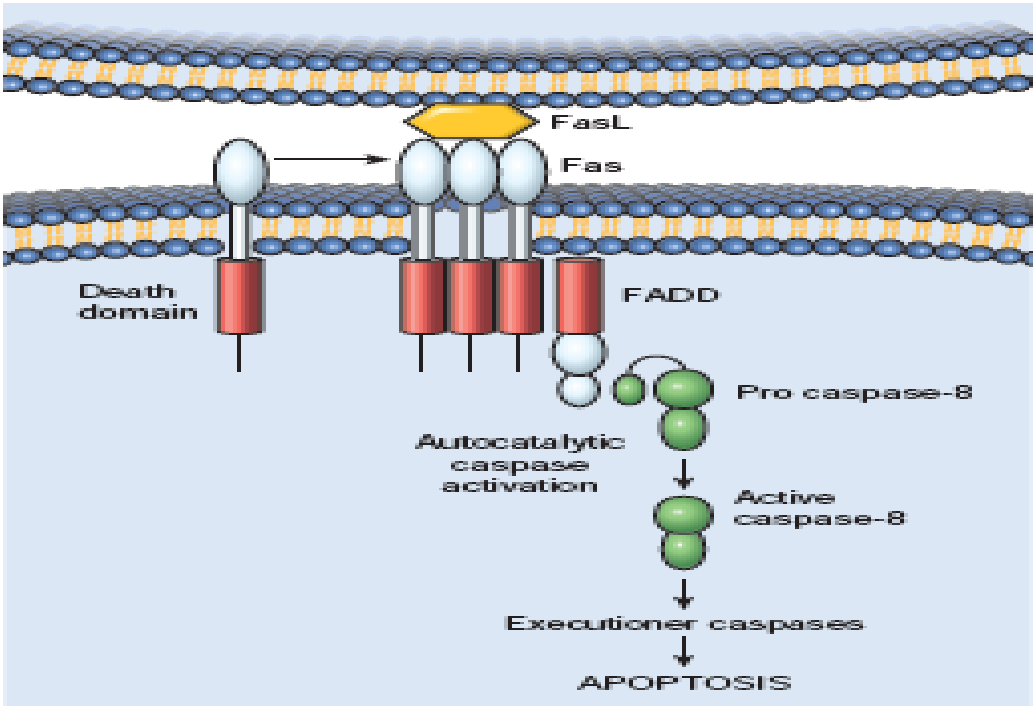
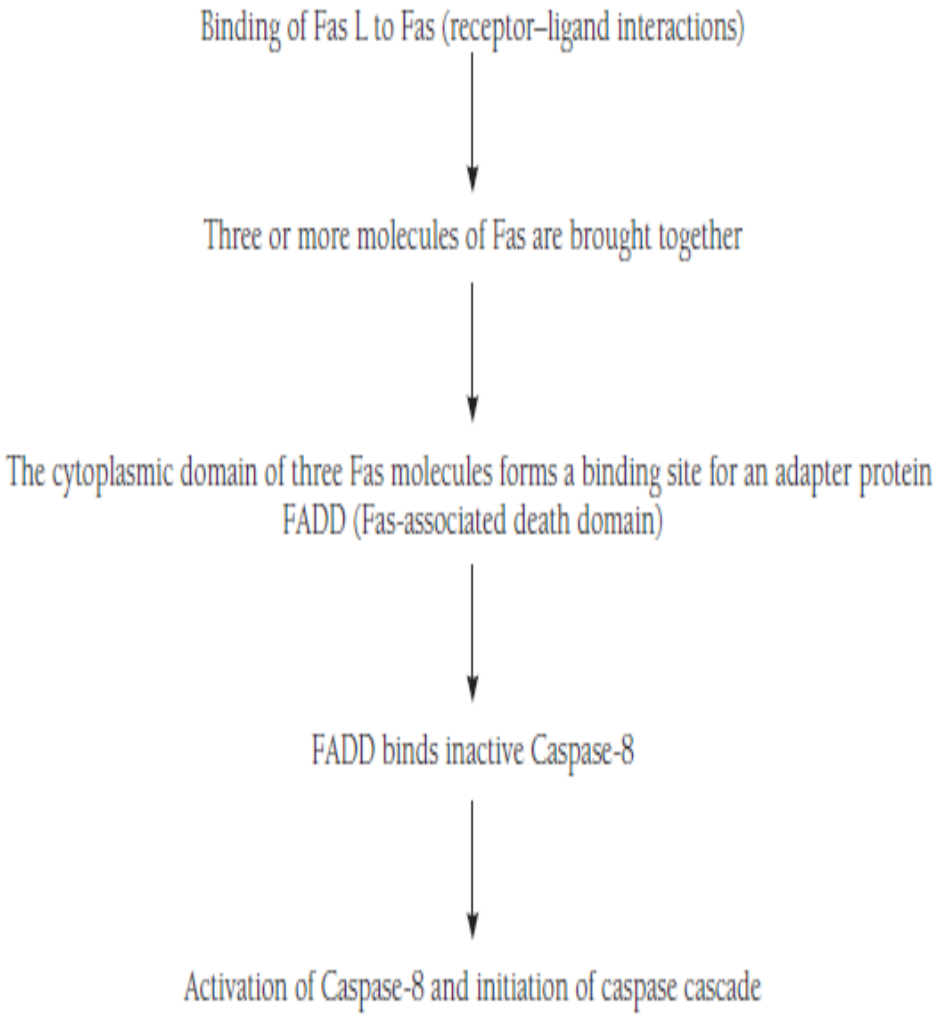


Figure 2-25 The extrinsic (death receptor initiated) pathway of apoptosis, illustrated by the events following Fas engagement. FAAD, Fas-associated death domain; FasL, Fas ligand.



FLOWCHART 1.11. cont'd

(b) Intrinsic/mitochondrial pathway (the major mechanism of apoptosis; Flowchart 1.12):

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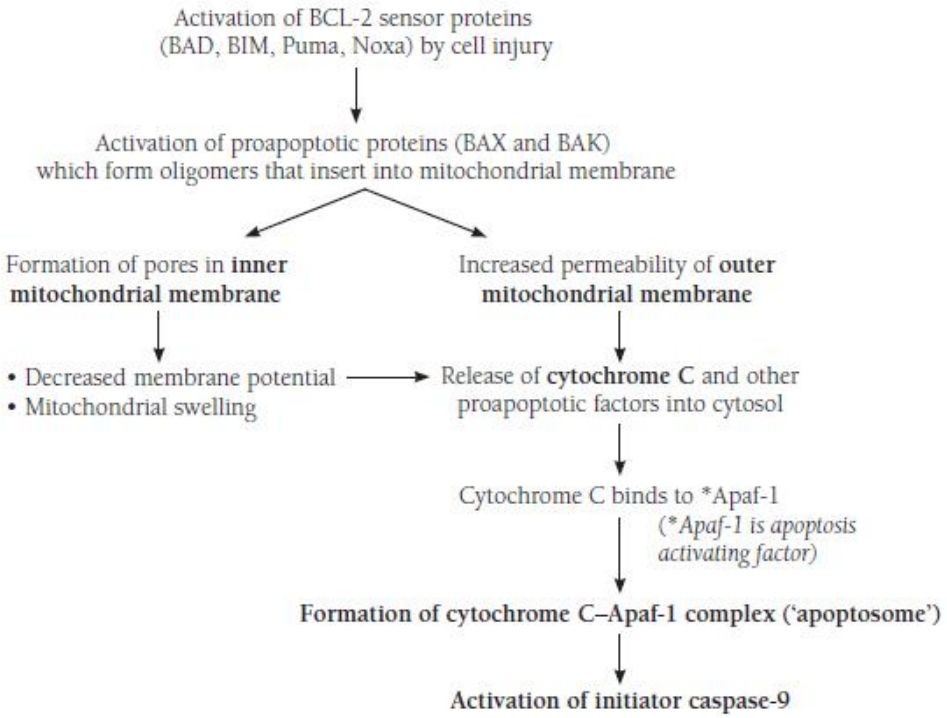

FLOWCHART 1.12. Intrinsic/mitochondrial pathway.

Methods for distinguishing apoptotic from necrotic cells

-Label-free live cell imaging, time-lapse microscopy, flow fluorocytometry, and transmission electron microscopy can be used to compare apoptotic and necrotic cells.

-There are also various biochemical techniques for analysis of cell surface markers (phosphatidylserine exposure versus cell permeability by flow cytometry), cellular markers such as DNA fragmentation (flow cytometry), caspase activation, Bid cleavage, and cytochrome c release (Western blotting).

-Supernatant screening for caspases, HMGB1, and cytokeratin 18 release can identify primary from secondary necrotic cells. However, no distinct surface or biochemical markers of necrotic cell death have been identified yet, and only negative markers are available. These include absence of apoptotic markers (caspase activation, cytochrome c release, and oligonucleosomal DNA

fragmentation) and differential kinetics of cell death markers (phosphatidylserine exposure and cell membrane permeabilization).

TABLE 1.5: Differences between apoptosis and necrosis

Features	*Apoptosis*	*Necrosis*
Cause	Often physiological, means of eliminating unwanted cells; may also be pathological	Invariably pathological
Biochemical events	Energy-dependent fragmentation of DNA by endogenous endonucleases	Impairment or cessation of ion homeostasis
Lysosomes	Intact	Leak lytic enzymes
Morphology		
Extent	Single or small cluster of cells	Involves group of cells
Cell size	Cell reduced (shrinkage) and fragmentation to form apoptotic bodies with dense chromatin	Cell enlarged (swelling) and undergo lysis
Integrity of cell membrane	Maintained	Disrupted/lost
Nucleus	Fragmentation into nucleosome-size fragments	Pyknosis, karyorrhexis, karyolysis
Cellular contents	Intact; may be released in apoptotic bodies	Enzymatic digestion; may leak out of cell
Adjacent Inflammatory response	None	Usual
Fate of dead cells	Ingested (phagocytosed) by neighboring cells	Ingested (phagocytosed) by neutrophil polymorphs and macrophages
DNA electrophoresis	DNA laddering is seen	Shows smearing effect
TUNEL staining	Positive	Negative

Apoptosis : No inflammatory response from adjacent tissue.

Leakage of proteins from the necrotic cells into the circulation is useful for identifying the necrosis using blood and serum samples.