Cell Death-Apoptosis

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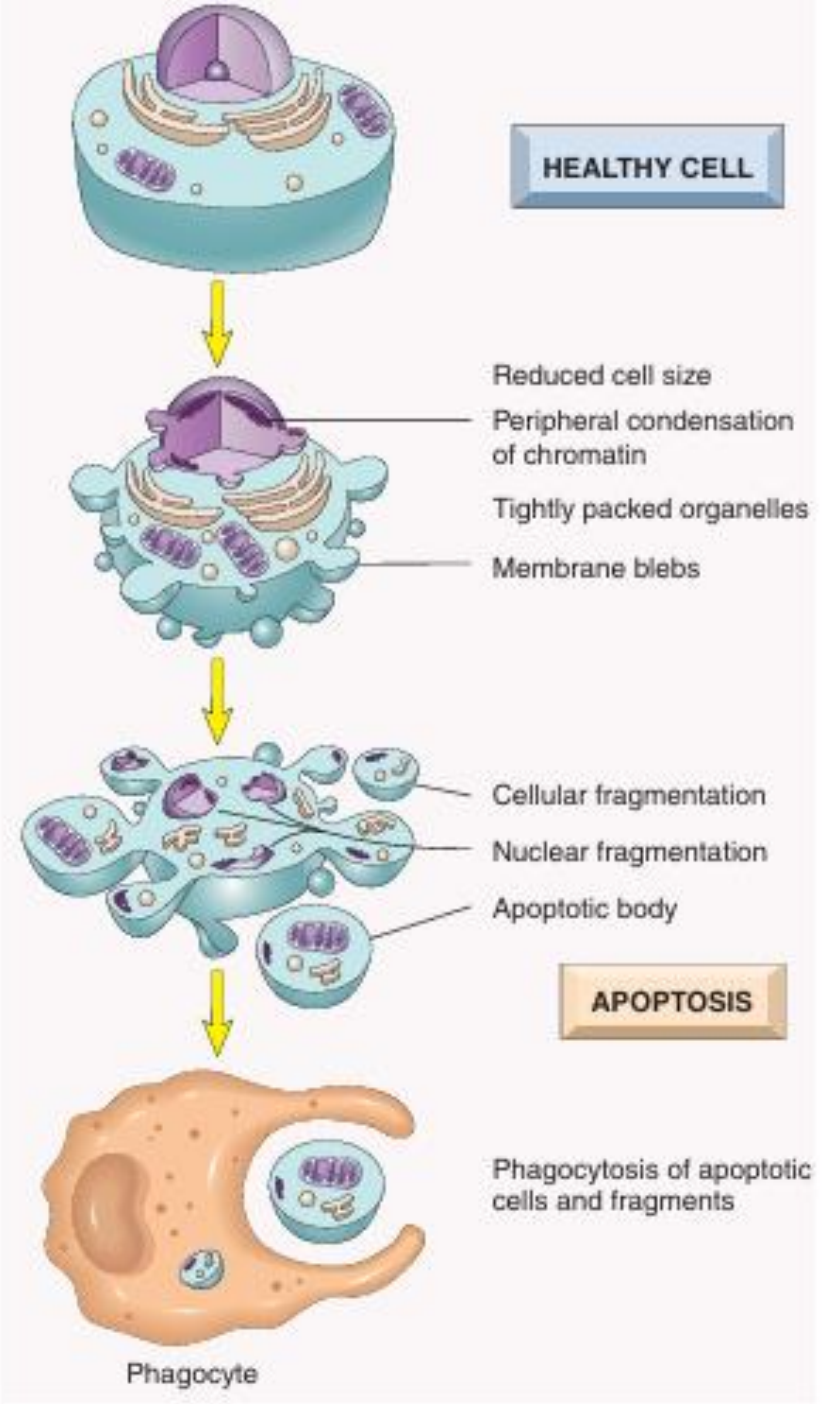
Apoptosis

Apoptosis is a pathway of cell death in which cells activate enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins.

Fragments of the apoptotic cells then break off, giving the appearance that is responsible for the name (apoptosis, "falling off").

The plasma membrane of the apoptotic cell remains intact, but the membrane is altered in such a way that the fragments, called apoptotic bodies, are recognized and rapidly phagocytosed by macrophages.

In contrast to necrosis, the apoptotic cell and its fragments are cleared before cellular contents have leaked out, so apoptotic cell death does not elicit an inflammatory reaction in the host.


HEALTHY CELL
Reduced cell size
Peripheral condensation of chromatin
Tightly packed organelles
Membrane blebs
Cellular fragmentation
Nuclear fragmentation
Apoptotic body
APOPTOSIS
Phagocytosis of apoptotic cells and fragments
Phagocyte

Mechanisms of Apoptosis

Apoptosis is regulated by biochemical pathways that control the balance of death- and survival-inducing signals and ultimately the activation of enzymes called caspases, so named because they are cysteine proteases that cleave proteins after aspartic acid residues.

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

Although these pathways can intersect, they are generally induced under different conditions, involve different molecules, and serve distinct roles in physiology and disease.

The mitochondrial (intrinsic) pathway is responsible for apoptosis in most physiologic and pathologic situations.

Mitochondria contain several proteins that are capable of inducing apoptosis, including **cytochrome c.**

When mitochondrial membranes become permeable, cytochrome c leaks into the cytoplasm, triggering caspase activation and apoptotic death. The permeability of mitochondria is controlled by a family of more than 20 proteins, the prototype of which **is BCL-2.**

In healthy cells, BCL-2 and the related protein BCL-XL are produced in response to growth factors and other stimuli that keep cells viable. These antiapoptotic proteins maintain the integrity of mitochondrial membranes, in large part by holding two **proapoptotic** members of the family, **BAX and BAK,** in check.

When cells are deprived of growth factors and survival signals, are exposed to agents that damage DNA, or accumulate unacceptable amounts of misfolded proteins, a number of sensors are activated. The most important of these sensors are called **BH3-only proteins** because they contain the third homology domain of the BCL-2 family.

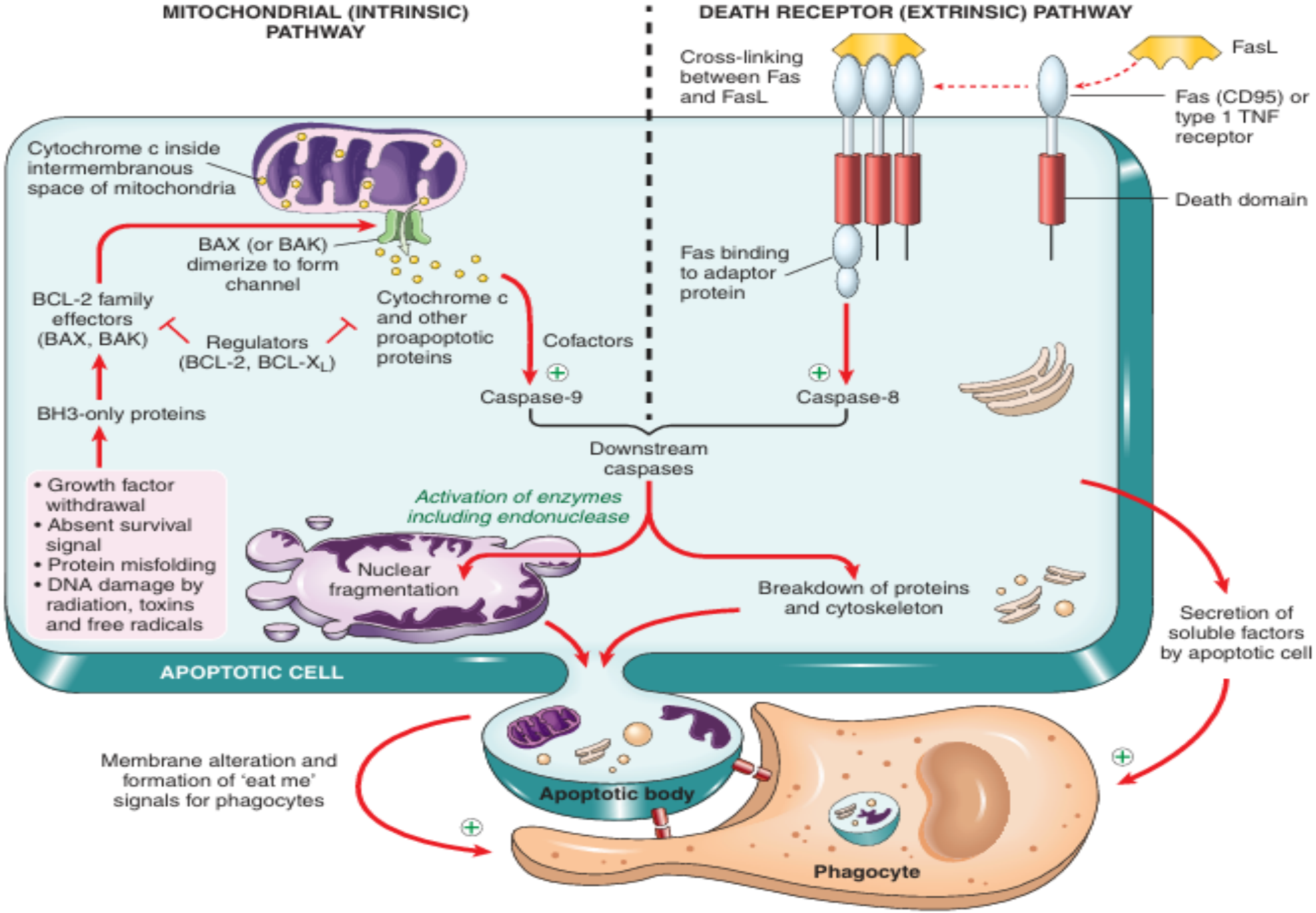
These sensors shift the balance in favor of BAK and BAX, which **dimerize**, insert into the mitochondrial membrane, and form **channels** through which cytochrome c and other mitochondrial proteins escape into the cytosol.

At the same time, the deficiency of survival signals leads to decreased levels of BCL-2 and BCL-XL, further compromising mitochondrial permeability.

Once in the cytosol, cytochrome c interacts with certain cofactors and activates caspase-9, leading to the activation of a caspase cascade.

The death receptor (extrinsic) pathway of apoptosis

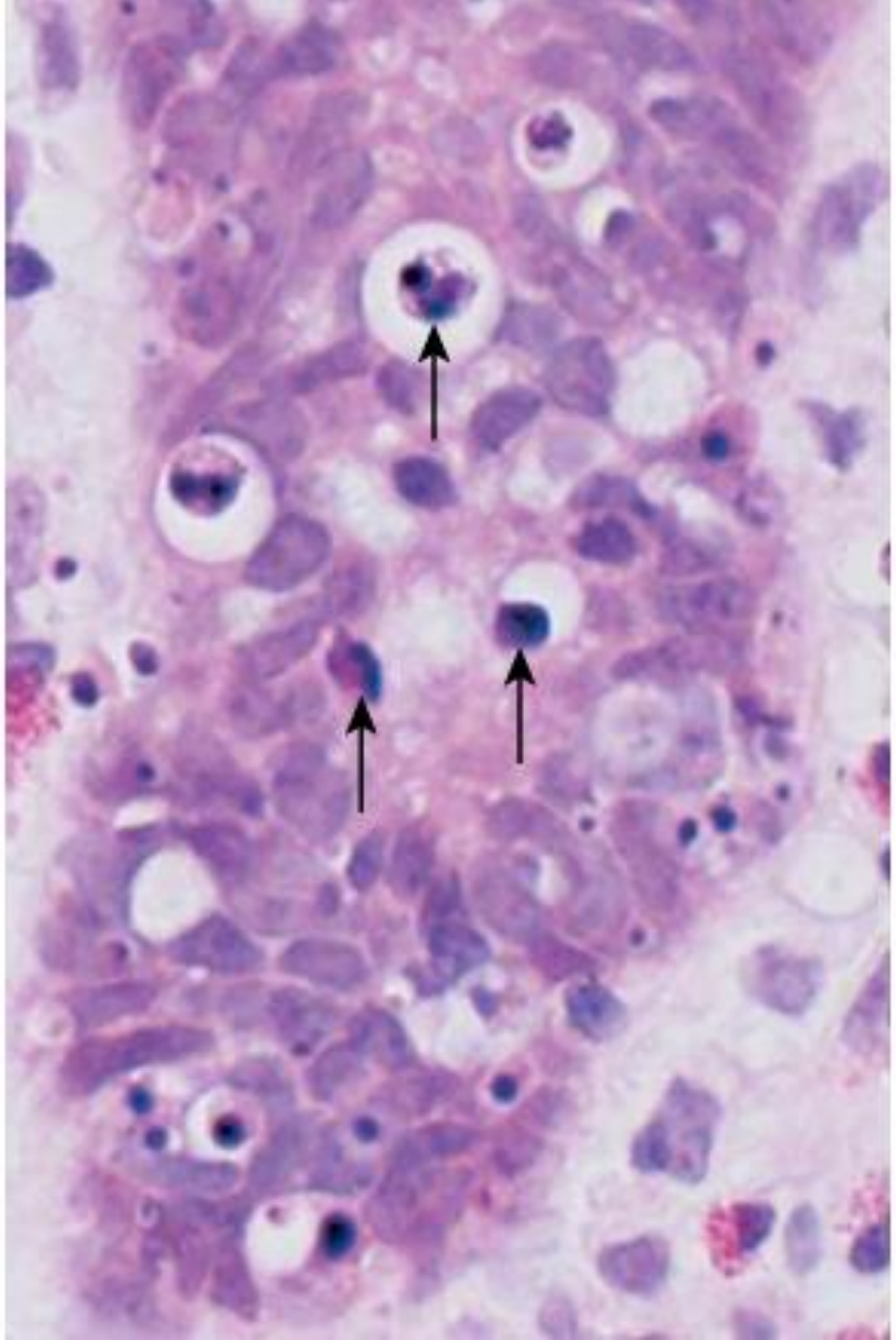
- Many cells express surface molecules, called death receptors, which trigger apoptosis.
- Most of these are members of the tumor necrosis factor (TNF) receptor family, which contain in their cytoplasmic regions a conserved “death domain,” so named because it mediates interaction with other proteins involved in cell death.
- The prototypic death receptors are the type I TNF receptor and Fas (CD95).
- Fas ligand (FasL) is a membrane protein expressed mainly on activated T lymphocytes. When these T cells recognize Fas-expressing targets, Fas molecules are cross linked by FasL and bind adaptor proteins via the death domain.
- These recruit and activate caspase-8, which in turn activates downstream caspases.
- The death receptor pathway is involved in elimination of self-reactive lymphocytes and in killing of target cells by some cytotoxic T lymphocytes that express FasL.


MITOCHONDRIAL (INTRINSIC) PATHWAY
DEATH RECEPTOR (EXTRINSIC) PATHWAY
Cross-linking between Fas and FasL
FasL
Fas (CD95) or type 1 TNF receptor
Death domain
Cytochrome c inside intermembranous space of mitochondria
BAX (or BAK) dimerize to form channel
Fas binding to adaptor protein
BCL-2 family effectors (BAX, BAK)
Regulators (BCL-2, BCL-XL)
Cytochrome c and other proapoptotic proteins
Cofactors
Caspase-9
Caspase-8
BH3-only proteins
Downstream caspases
• Growth factor withdrawal
• Absent survival signal
• Protein misfolding
• DNA damage by radiation, toxins and free radicals
Activation of enzymes including endonuclease
Nuclear fragmentation
Breakdown of proteins and cytoskeleton
Secretion of soluble factors by apoptotic cell
APOPTOTIC CELL
Membrane alteration and formation of 'eat me' signals for phagocytes
Apoptotic body
Phagocyte

Terminal phase of apoptosis.

Activated caspase-8 and caspase-9 act through a final common series of reactions that first involve the activation of additional caspases, which through numerous substrates ultimately activate enzymes that degrade the cell's proteins and nucleus. The end result is the characteristic cellular fragmentation of apoptosis.

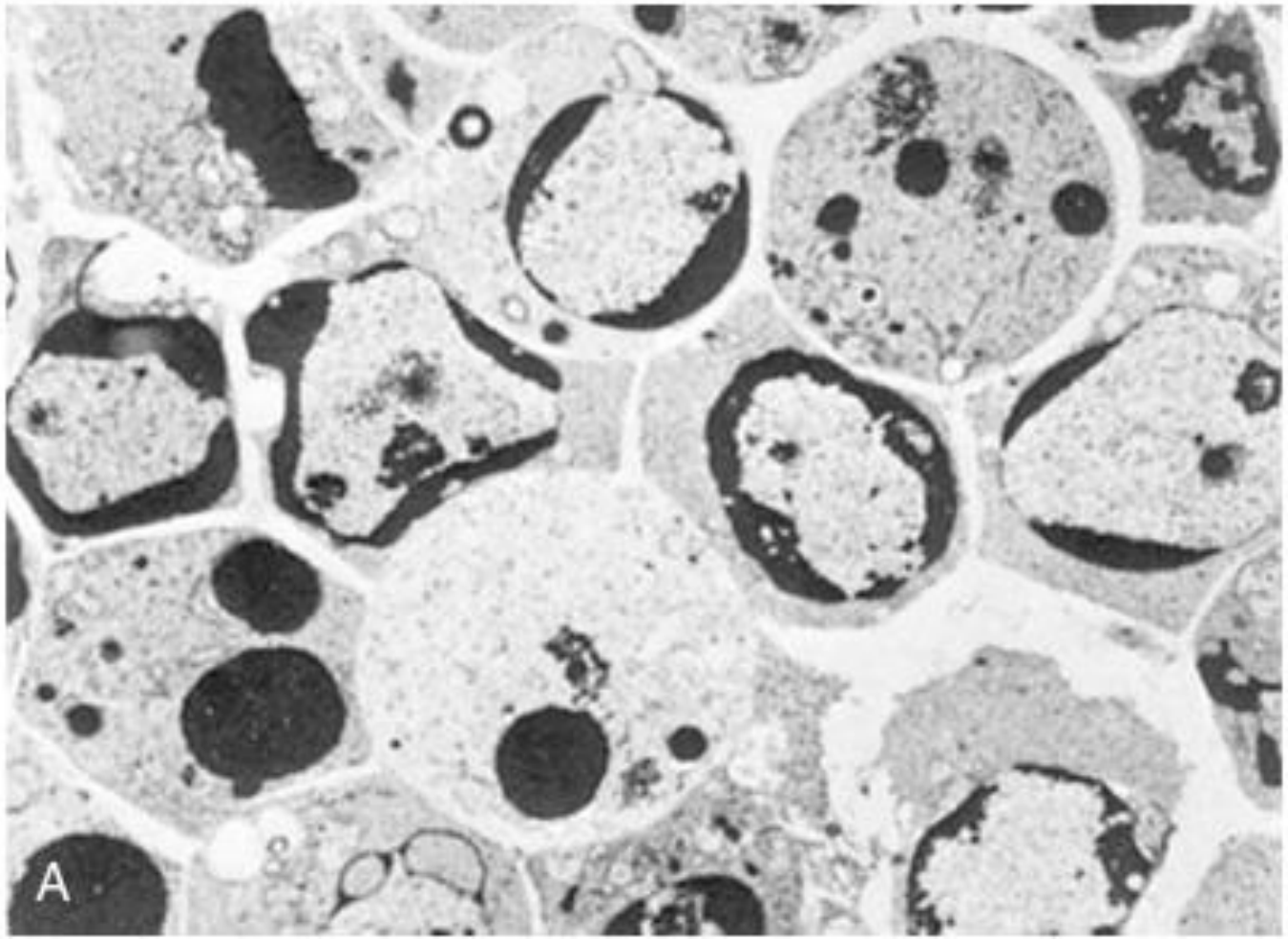
• Clearance of apoptotic cells. Apoptotic cells and their fragments entice phagocytes by producing a number of "eat-me" signals. Numerous macro phage receptors are involved in the binding and engulfment of apoptotic cells. This process is so efficient that the dead cells disappear without leaving a trace, and there is no accompanying inflammation.



MORPHOLOGY

The nuclei of apoptotic cells show various stages of chromatin condensation, aggregation, and, ultimately, karyorrhexis.

Morphologic appearance of apoptotic cells. Apoptotic cells (some indicated by arrows) in a normal crypt in the colonic epithelium are shown. (The preparative regimen for colonoscopy frequently induces apoptosis in epithelial cells, which explains the abundance of dead cells in this normal tissue.) Note the fragmented nuclei with condensed chromatin and the shrunken cell bodies, some with pieces falling off.



At the molecular level this is reflected in fragmentation of DNA into nucleosome-sized pieces. The cells rapidly shrink, form cytoplasmic buds, and fragment into apoptotic bodies that are composed of membrane-bound pieces of cytosol and organelles.

Physiologic and Pathologic Conditions Associated With Apoptosis

Condition	Mechanism of Apoptosis
Physiologic	
During embryogenesis	Loss of growth factor signaling (presumed mechanism)
Turnover of proliferative tissues (e.g., intestinal epithelium, lymphocytes in lymph nodes and thymus)	Loss of growth factor signaling or survival signals (presumed mechanism)
Involution of hormone-dependent tissues (e.g., endometrium)	Decreased hormone levels lead to reduced survival signals
Decline of leukocyte numbers at the end of immune and inflammatory responses	Loss of survival signals as stimulus for leukocyte activation is eliminated
Elimination of potentially harmful self-reactive lymphocytes	Strong recognition of self antigens induces apoptosis by both the mitochondrial and death receptor pathways
Pathologic	
DNA damage	Activation of proapoptotic BH3-only proteins
Accumulation of misfolded proteins	Activation of proapoptotic BH3-only proteins, possibly direct activation of caspases
Infections, especially certain viral infections	Activation of proapoptotic proteins or caspases by viral proteins; killing of infected cells by cytotoxic T lymphocytes (CTLs), which activate caspases

Necroptosis

- Other pathways of cell death, in addition to necrosis and apoptosis, have been described. Necroptosis is a form of cell death caused by the cytokine tumor necrosis factor (TNF) that shows features of both necrosis and apoptosis, hence its name.

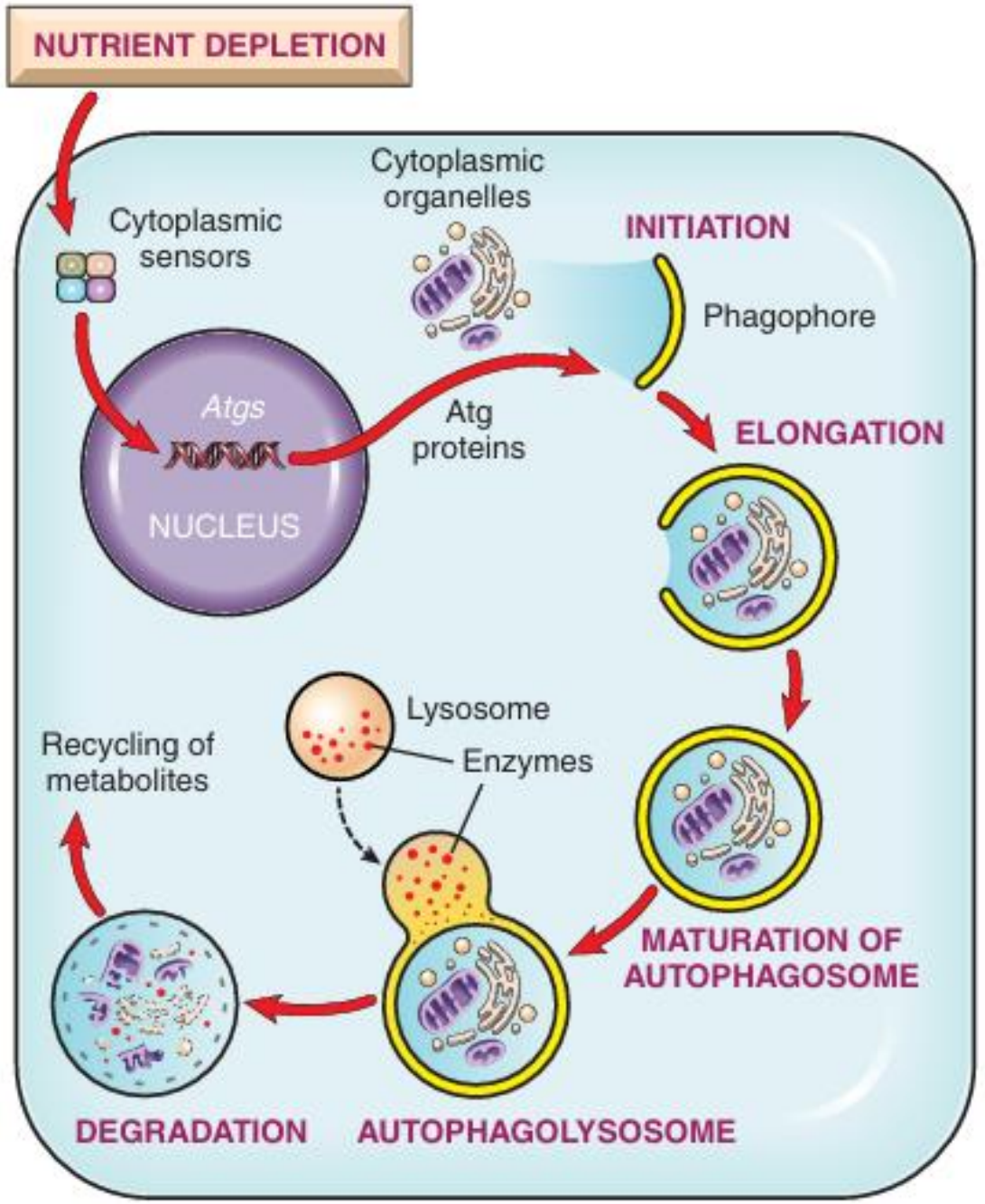
- Pyroptosis (pyro, fever) is induced by activation of inflammasomes , which releases the cytokine interleukin-1 (IL-1), which cause inflammation and fever.

- Ferroptosis depends on levels of cellular iron.

- The roles of these mechanisms of cell death in normal physiology and pathologic states are not clearly established and remain topics of investigation.

Autophagy (“self-eating”)

- Refers to lysosomal digestion of the cell’s own components.
- It is a survival mechanism in times of nutrient deprivation that enables the starved cell to live by eating its own contents and recycling these contents to provide nutrients and energy.
- In this process, intracellular organelles and portions of cytosol are first sequestered within an ER-derived double membrane (phagophore), which matures into an autophagic vacuole.
- The formation of this auto phagosome is initiated by cytosolic proteins that sense nutrient deprivation
- The vacuole fuses with lysosomes to form an autophagolysosome, and lysosomal enzymes digest the cellular components.


NUTRIENT DEPLETION
Cytoplasmic sensors
Cytoplasmic organelles
INITIATION
Phagophore
Atgs
NUCLEUS
Atg proteins
ELONGATION
Lysosome
Enzymes
Recycling of metabolites
MATURATION OF AUTOPHAGOSOME
DEGRADATION
AUTOPHAGOLYSOSOME

Associated with,,,,,

- In some circumstances, autophagy may be associated with **atrophy** of tissues and represent an adaptation that helps cells survive lean times. If, however, the starved cell can no longer cope by devouring its contents, autophagy may also signal cell death by apoptosis.
- Extensive autophagy is seen in ischemic injury and some types of myopathies.
- Autophagic vacuoles may also form around microbes in infected cells, leading to destruction of these infectious pathogens.
- Cancer cells acquire the ability to survive even in times of stress without autophagy.

MECHANISMS OF CELL INJURY AND CELL DEATH

General principles should be emphasized:

- The cellular response to injurious stimuli depends on the type of injury and its duration and severity.
- The consequences of an injurious stimulus also depend on the type of cell and its metabolic state, adaptability, and genetic makeup.
  - For instance, skeletal muscle in the leg can survive complete ischemia for 2 to 3 hours, whereas more metabolically active cardiac muscle dies after only 20 to 30 minutes.
  - Genetically determined diversity in metabolic pathways can contribute to differences in responses to injurious stimuli. For instance, when exposed to the same dose of a toxin, individuals who inherit variants in genes encoding cytochrome P-450 may catabolize the toxin at different rates, leading to different outcomes.
- Cell injury usually results from functional and biochemical abnormalities in one or more essential cellular components

1-Mitochondrial Dysfunction and Damage

- Failure of oxidative phosphorylation, leading to decreased ATP generation and depletion of ATP in cells.

➢ Reduced activity of plasma membrane ATP-dependent sodium pumps results in intracellular accumulation of sodium and efflux of potassium. The net gain of solute is accompanied by osmotic gain of water, causing cell swelling and dilation of the ER.

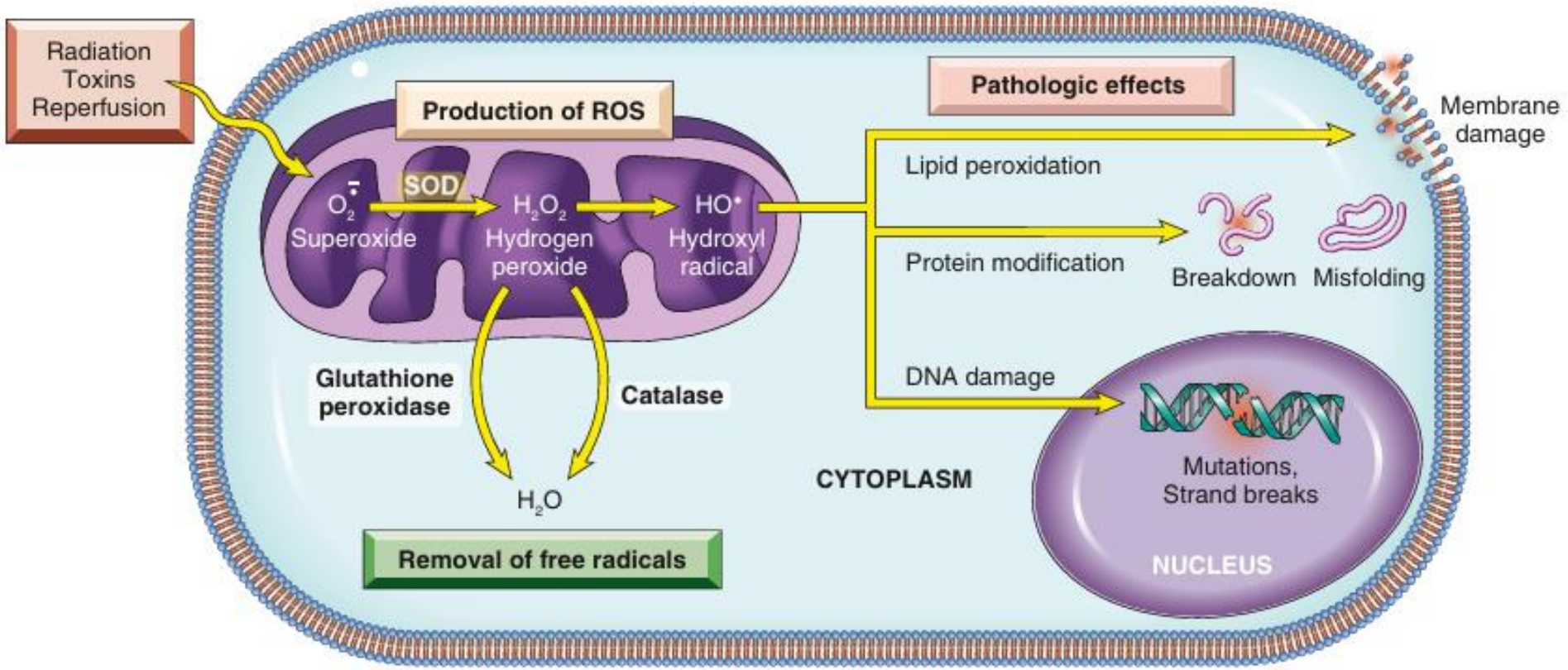
➢ The compensatory increase in anaerobic glycolysis leads to lactic acid accumulation, decreased intracellular pH, and decreased activity of many cellular enzymes.

➢ Prolonged or worsening depletion of ATP causes structural disruption of the protein synthetic apparatus, manifested as detachment of ribosomes from the rough ER and dissociation of polysomes, with a consequent reduction in protein synthesis. Ultimately, the cell undergoes necrosis

➢ Damage to mitochondria is often associated with the formation of a high-conductance channel in the mitochondrial membrane, called the mitochondrial permeability transition pore----→ activate apoptosis.

2- Oxidative Stress

- Abnormal oxidative phosphorylation also leads to the formation of reactive oxygen species
- Oxidative Stress Oxidative stress refers to cellular damage induced by the accumulation of reactive oxygen species (ROS), a form of free radical.
- Cell injury in many circumstances involves damage by free radicals; these situations include chemical and radiation injury, hypoxia, cellular aging, tissue injury caused by inflammatory cells, and ischemia reperfusion injury.
- Free radicals are chemical species with a single unpaired electron in an outer orbital, such chemical species are extremely unstable and readily react with inorganic and organic compounds, such as nucleic acids, proteins, and lipids. During this reaction, the molecules that are “attacked” by free radicals are often themselves converted into other types of free radicals, thereby propagating the chain of damage.



The generation, removal, and role of reactive oxygen species (ROS) in cell injury. The production of ROS is increased by many injurious stimuli. These free radicals are removed by spontaneous decay and by specialized enzymatic systems. Excessive production or inadequate removal leads to accumulation of free radicals in cells, which may damage lipids (by peroxidation), proteins, and DNA, resulting in cell injury. *SOD,* Superoxide dismutase.

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Principal Free Radicals Involved in Cell Injury

Free Radical	Mechanisms of Production	Mechanisms of Removal	Pathologic Effects
Superoxide ($O_2^{\cdot-}$)	Incomplete reduction of O_2 during mitochondrial oxidative phosphorylation; by phagocyte oxidase in leukocytes	Conversion to H_2O_2 and O_2 by superoxide dismutase	Direct damaging effects on lipids (peroxidation), proteins, and DNA
Hydrogen peroxide (H_2O_2)	Mostly from superoxide by action of SOD	Conversion to H_2O and O_2 by catalase, glutathione peroxidase	Can be converted to •OH and ClO^-, which destroy microbes and cells
Hydroxyl radical (•OH)	Produced from H_2O, H_2O_2, and $O_2^{\cdot-}$ by various chemical reactions	Conversion to H_2O by glutathione peroxidase	Direct damaging effects on lipids, proteins, and DNA
Peroxynitrite ($ONOO^-$)	Interaction of $O_2^{\cdot-}$ and NO mediated by NO synthase	Conversion to nitrite by enzymes in mitochondria and cytosol	Direct damaging effects on lipids, proteins, and DNA

ClO^-, Hypochlorite; *NO*, nitric oxide; *SOD*, superoxide dismutase.

Ischemia-Reperfusion Injury

- Under certain circumstances, the restoration of blood flow to ischemic but viable tissues results, paradoxically, in increased cell injury and necrosis. This is the reverse of the expected outcome of restoration of blood flow, which should result in recovery of reversibly injured cells.

This so-called ischemia-reperfusion injury is a clinically important process that may contribute significantly to tissue damage, especially after myocardial and cerebral ischemia.

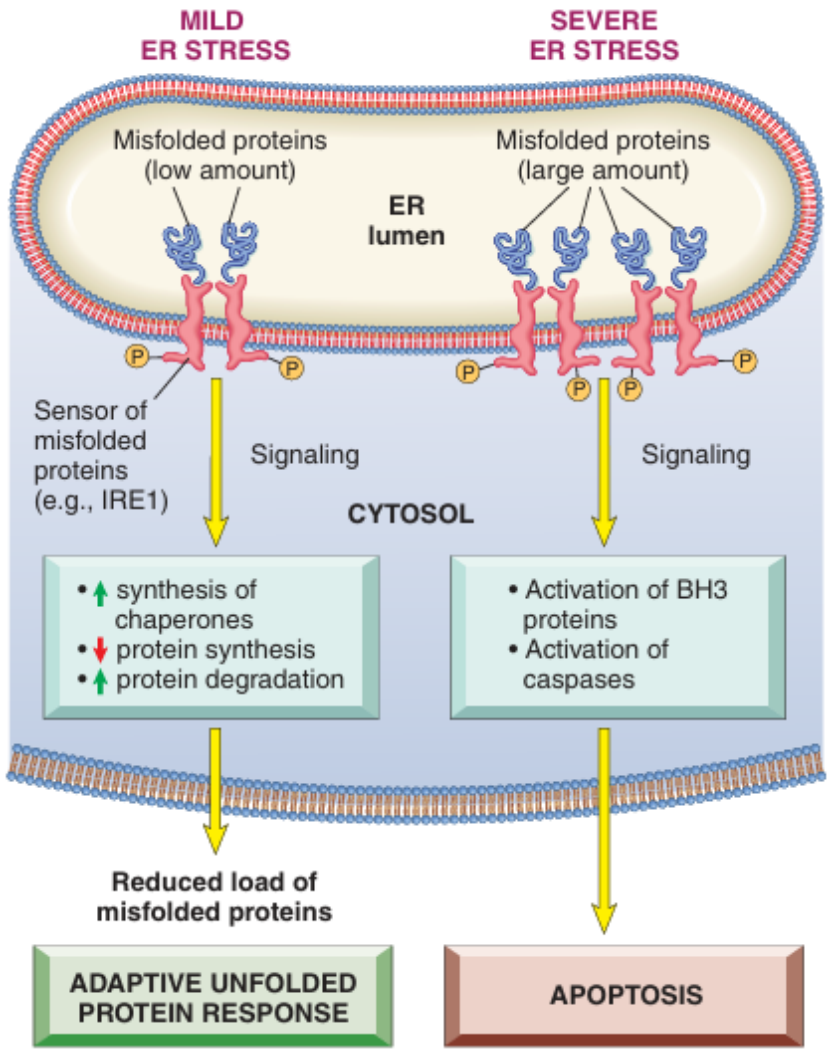
- Several mechanisms may account for the exacerbation of cell injury by reperfusion of ischemic tissues:
  - Increased ROS production may occur during reoxygenation.
  - Influx of calcium may cause injury.

- The inflammation that is induced by ischemic injury increases with reperfusion because of increased influx and activation of leukocytes, the products of which cause additional tissue injury.

3- **Disturbance in Calcium Homeostasis:** Excessive intracellular Ca2þ may cause cell injury by activating various enzymes, e.g., proteases and phospholipases, that damage cellular components.

4- **Membrane Damage**: Most forms of cell injury that culminate in necrosis are characterized by increased membrane permeability that ultimately leads to overt membrane damage.

5- **DNA Damage**: Exposure of cells to radiation or chemotherapeutic agents, intra cellular generation of ROS, and acquisition of mutations may all induce DNA damage.



6- The accumulation of **misfolded proteins** in a cell can stress compensatory pathways in the ER and lead to cell death by apoptosis. Intracellular accumulation of misfolded proteins may be caused by abnormalities that increase the production of misfolded proteins or reduce the ability to eliminate them.

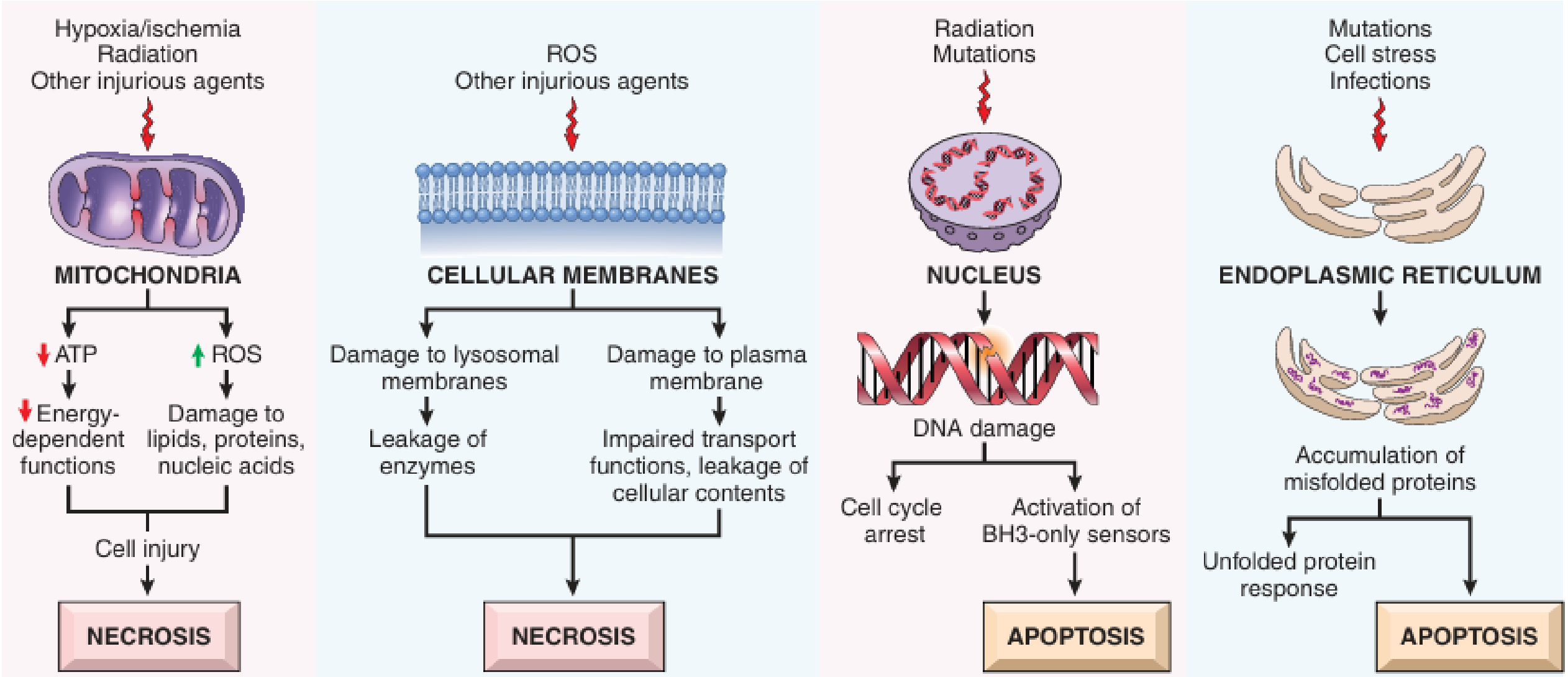
Diseases Caused by Misfolded Proteins

Disease	Affected Protein	Pathogenesis
Diseases Caused by Mutant Proteins That Are Degraded, Leading to Their Deficiency		
Cystic fibrosis[a]	Cystic fibrosis transmembrane conductance regulator (CFTR)	Loss of CFTR leads to defects in ion transport
Familial hypercholesterolemia[a]	LDL receptor	Loss of LDL receptor leading to hypercholesterolemia
Tay-Sachs disease[a]	Hexosaminidase α-subunit	Lack of the lysosomal enzyme leads to storage of GM_2 gangliosides in neurons
Diseases Caused by Misfolded Proteins That Result in ER Stress-Induced Cell Loss		
Retinitis pigmentosa[a]	Rhodopsin	Abnormal folding of rhodopsin causes photoreceptor loss and blindness
Creutzfeldt-Jakob disease	Prions	Abnormal folding of PrP^{sc} causes neuronal cell death
Diseases Caused by Misfolded Proteins That Result From Both ER Stress-Induced Cell Loss and Functional Deficiency of the Protein		
α-1-antitrypsin deficiency	α-1 antitrypsin	Storage of nonfunctional protein in hepatocytes causes apoptosis; absence of enzymatic activity in lungs causes destruction of elastic tissue, giving rise to emphysema

[a]Misfolding is responsible for protein dysfunction and cellular injury in a subset of molecular subtypes.

Shown are selected illustrative examples of diseases in which protein misfolding is a mechanism of functional derangement or cell or tissue injury.

CFTR, Cystic fibrosis transporter; *LDL*, low density lipoprotein; *PrP*, prion protein.



The principal biochemical mechanisms and sites of damage in cell injury. Note that causes and mechanisms of cell death by necrosis and apoptosis are shown as being independent but there may be overlap; for instance, both may occur as a result of ischemia, oxidative stress, and radiation-induced cell death. *ATP*, Adenosine triphosphate; *ROS*, reactive oxygen species.