Contents

Chapter 1

Molecular mechanisms of cell death

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Summary

Although many stimuli cause death of cells, the mode of cell death typically follows one of two patterns. The first is necrosis, or oncosis. Oncotic necrosis is most often the result of profound metabolic disruption and is characterized by cellular swelling leading to plasma membrane rupture with release of intracellular contents. The second pattern is apoptosis, a form of programmed cell death. Apoptosis causes the orderly resorption of individual cells initiated by well-defined pathways involving activation of proteases called caspases. In contrast to necrotic cell death, which typically occurs from adenosine triphosphate (ATP) depletion, apoptosis is an ATP-requiring process. However, in both modes of cell death, mitochondrial permeabilization and dysfunction typically develop. In some instances, apoptosis and necrosis share signaling pathways as extreme endpoints on a phenotypic continuum of lost cell viability.

Introduction

A common theme in disease is death of cells. In diseases ranging from stroke to congestive heart disease to alcoholic cirrhosis of the liver, death of individual cells leads to irreversible functional loss in whole organs and ultimately mortality. For such diseases, prevention of cell death becomes a basic therapeutic goal. By contrast in neoplasia, the purpose of chemotherapy is to kill proliferating cancer cells. For either therapeutic goal, understanding the mechanisms of cell death becomes paramount.

Modes of cell death

Although many stresses and stimuli cause cell death, the mode of cell death typically follows one of two patterns. The first is necrosis, a pathological term referring to areas of dead cells within a tissue or organ. Necrosis is typically the result of an acute and usually profound metabolic disruption, such as ischemia (loss of blood supply). Since necrosis is an outcome rather than a process, the term oncosis has been introduced to describe the process leading to necrotic cell death, but the term has yet to be widely adopted. Here, the terms oncosis, oncotic necrosis, and necrotic cell death will be used synonymously to refer both to the outcome of cell death and the pathogenic events precipitating cell killing.

The second pattern is programmed cell death, most commonly manifested as apoptosis, a term derived from ancient Greek for the falling of leaves in autumn. In apoptosis, specific stimuli initiate execution of well-defined pathways leading to orderly resorption of individual cells with minimal leakage of cellular components into the extracellular space and little inflammation. Whereas necrotic cell death occurs with abrupt onset after adenosine triphosphate (ATP) depletion, apoptosis may take hours to go to completion and is an ATP-requiring process without a clearly distinguished point of no return. Although apoptosis and necrosis were initially considered separate and independent phenomena, an alternate view is emerging that apoptosis and necrosis can share initiating factors and signaling pathways to become extremes on a phenotypic continuum.

Essential Concepts in Molecular Pathology. DOI: https://doi.org/10.1016/B978-0-12-813257-9.00001-2

Structural features of necrosis and apoptosis

Oncotic necrosis

Cellular changes leading up to onset of necrotic cell death include formation of plasma membrane protrusions called blebs, mitochondrial swelling, dilatation of cisternae of the endoplasmic reticulum (ER), dissociation of polysomes, and cellular swelling leading to rupture with release of intracellular contents (Table 1.1, Fig. 1.1). After necrotic cell death, characteristic histological features of loss of cellular architecture, vacuolization, karyolysis (dissolution of chromatin), and increased eosinophilia soon become evident (Fig. 1.2). Cell lysis evokes an inflammatory response, attracting neutrophils and monocytes to the dead tissue to dispose of the necrotic debris by phagocytosis and defend against infection (Fig. 1.3). In organs like heart and brain with little regenerative capacity, healing occurs with scar formation, namely replacement of necrotic regions with fibroblasts, collagen and other connective tissue components. In organs like the liver that have robust regenerative capacity, cell proliferation can replace areas of necrosis with completely normal tissue within a few days. The healed liver tissue shows little or no residua of the necrotic event, but if regeneration fails, collagen deposition and fibrosis will occur instead to cause cirrhosis.

TABLE 1.1 Comparison of necrosis and apoptosis.

Necrosis	Apoptosis
Accidental cell death	Controlled cell deletion
Contiguous regions of cells	Single cells separating from neighbors
Cell swelling	Cell shrinkage
Plasmalemmal blebs without organelles	Zeiotic blebs containing large organelles
Small chromatin aggregates	Nuclear condensation and lobulation
Random DNA degradation	Internucleosomal DNA degradation
Cell lysis with release of intracellular contents	Fragmentation into apoptotic bodies
Inflammation and scarring	Absence of inflammation and scarring
Mitochondrial swelling and dysfunction	Mitochondrial permeabilization
Phospholipase and protease activation	Caspase activation
ATP depletion and metabolic disruption	ATP and protein synthesis sustained
Cell death precipitated by plasma membrane rupture	Intact plasma membrane

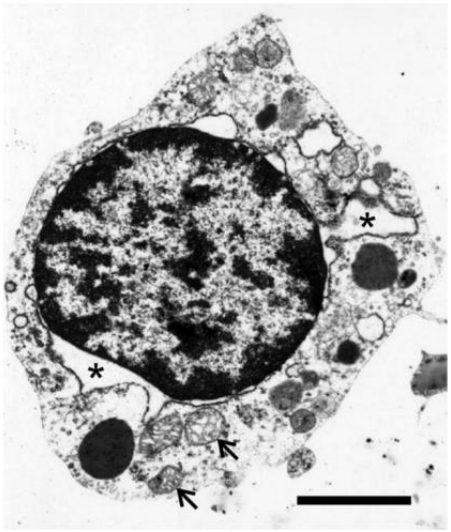

FIGURE 1.1 Electron microscopy of oncotic necrosis in a rat hepatic sinusoidal endothelial cell after ischemia/reperfusion. Note cell rounding, mitochondrial swelling (arrows), rarefaction of cytosol, dilatation of the ER and the space between the nuclear membranes (*), chromatin condensation, and discontinuities in the plasma membrane. Bar is 2 μm.

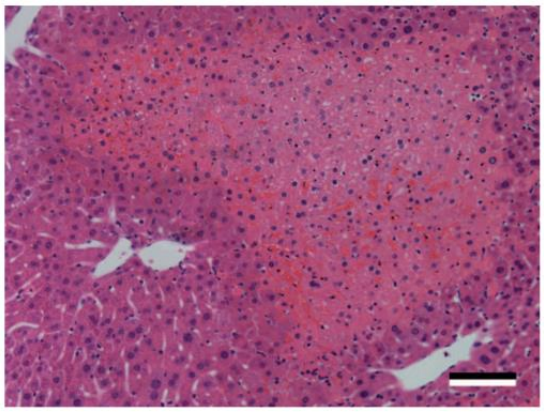

FIGURE 1.2 Histology of necrosis after hepatic ischemia/reperfusion in a mouse. Note increased eosinophilia, loss of cellular architecture, and nuclear pyknosis and karyolysis. Contrast to lower left and right areas that are non-necrotic. Bar is 50 μm.

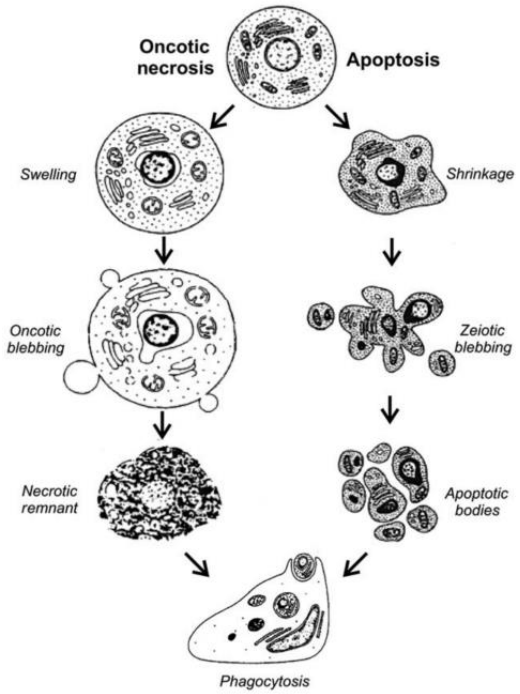

FIGURE 1.3 Scheme of necrosis and apoptosis. In oncotic necrosis, swelling leads to bleb rupture and release of intracellular constituents which attract macrophages that clear the necrotic debris by phagocytosis. In apoptosis, cells shrink and form small zeiotic blebs that are shed as membrane-bound apoptotic bodies. Apoptotic bodies are phagocytosed by macrophages and adjacent cells. *Adapted with permission from Van CS, Van Den BW. Morphological and biochemical aspects of apoptosis, oncosis and necrosis. Anat Histol Embryol 2002;31:214–23.*

Apoptosis

Unlike necrosis, which often occurs in response to an imposed unphysiological stress, apoptosis is a process of physiological cell deletion that has an opposite role to mitosis in the regulation of cell populations. In apoptosis, cell death occurs with little release of intracellular contents, inflammation, and scar formation. Individual cells undergoing

apoptosis separate from their neighbors and shrink rather than swell. Distinctive nuclear and cytoplasmic changes also occur, including chromatin condensation, nuclear lobulation and fragmentation, formation of numerous small cell surface blebs (zeiotic blebbing), and shedding of these blebs as apoptotic bodies that are phagocytosed by adjacent cells and macrophages for lysosomal degradation (Table 1.1, Fig. 1.3). Characteristic biochemical changes also occur, typically activation of a cascade of cysteine-aspartate proteases, called caspases, leakage of pro-apoptotic proteins like cytochrome *c* from mitochondria into the cytosol, internucleosomal deoxyribonucleic acid (DNA) degradation, degradation of poly(ADP-ribose) polymerase (PARP), and movement of phosphatidyl serine to the exterior leaflet of the plasmalemmal lipid bilayer. Thus, apoptosis manifests a very different pattern of cell death than oncotic necrosis (Table 1.1, Fig. 1.3).

Cellular and molecular mechanisms underlying necrotic cell death

Metastable state preceding necrotic cell death

Cellular events culminating in necrotic cell death are somewhat variable from one cell type to another, but certain events occur regularly. As implied by the term oncosis, cellular swelling is a prominent feature of oncotic necrosis. In many cell types, swelling of 30−50% occurs early after ATP depletion associated with formation of blebs on the cell surface (Fig. 1.4). These blebs contain cytosol and ER but exclude larger organelles. Bleb formation is likely due to cytoskeletal alterations after ATP depletion, whereas swelling arises from disruption of cellular ion transport. Mitochondrial swelling and dilatation of cisternae of ER and nuclear membranes accompany bleb formation (see Fig. 1.1). After longer times, a metastable state develops, which is characterized by mitochondrial depolarization, lysosomal breakdown, ion dysregulation, and accelerated bleb formation with more rapid swelling. The metastable state lasts only a few minutes and culminates in rupture of a plasma membrane bleb (Fig. 1.4). At onset of the metastable state, nonspecific pores appear to open, permitting uptake of electrolytes (principally sodium and chloride) and initiating rapid swelling driven by colloid osmotic (oncotic) forces (Fig. 1.5). Bleb rupture leads to loss of metabolic intermediates such as those that reduce tetrazolium dyes, leakage of cytosolic enzymes like lactate dehydrogenase, uptake of dyes like trypan blue, and collapse of all electrical and ion gradients. This all-or-nothing breakdown of the plasma membrane permeability barrier is long-lasting, irreversible, and incompatible with continued life of the cell.

Mitochondrial dysfunction and ATP depletion

Ischemia as occurs in strokes and heart attacks is perhaps the most common cause of necrotic cell killing. In ischemia, oxygen deprivation prevents ATP formation by mitochondrial oxidative phosphorylation, a process providing up to 95% of ATP utilized by highly aerobic tissues. The role of mitochondrial dysfunction in necrotic killing can be assessed experimentally by the ability of glycolytic substrates to rescue cells from lethal cell injury (Fig. 1.6). As an alternative source of ATP, glycolysis partially replaces ATP production lost after mitochondrial dysfunction. Maintenance of as little as 15% or 20% of normal ATP then rescues cells from necrotic death. Glycolysis also protects against toxicity from

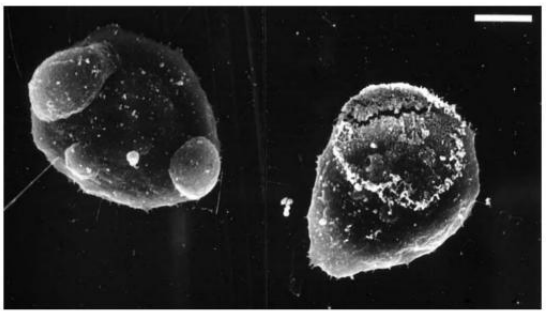

FIGURE 1.4 Bleb rupture at onset of necrotic cell death. After metabolic inhibition with cyanide and iodoacetate, inhibitors of respiration and glycolysis, respectively, a surface bleb of the cultured rat hepatocyte on the right has just burst. Note the discontinuity of the plasma membrane surface in the scanning electron micrograph. The hepatocyte on the left is also blebbed, but the plasma membrane is still intact, and viability has not yet been lost. Bar is 5 μm. *Adapted with permission from Herman B, Nieminen AL, Gores GJ, Lemasters JJ. Irreversible injury in anoxic hepatocytes precipitated by an abrupt increase in plasma membrane permeability. FASEB J 1988;2:146−51.*

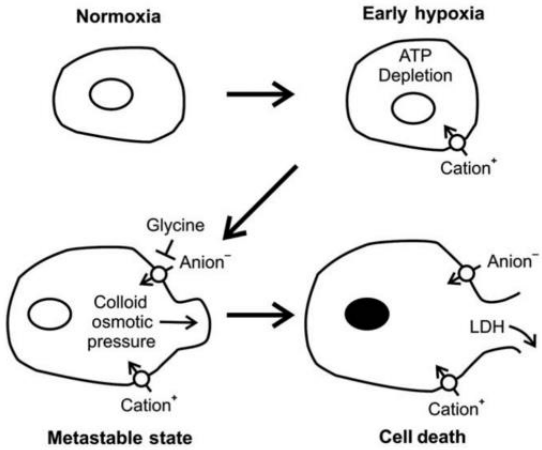

FIGURE 1.5 Plasma membrane permeabilization leading to necrotic cell death. Early after hypoxia and other metabolic stresses, ATP depletion leads to inhibition of the Na,K-ATPase and opening of monovalent cation channels causing cation gradients (Na^+ and K^+) to collapse. Swelling is limited by impermeability to anions. Later, glycine and strychnine-sensitive anion channels open to initiate anion entry and accelerate bleb formation and swelling. Swelling continues until a bleb ruptures. With abrupt and complete loss of the plasma membrane permeability barrier, viability is lost. Supravital dyes like trypan blue and propidium iodide enter the cell to stain the nucleus, and cytosolic enzymes like lactate dehydrogenase (LDH) leak out. *With permission from Lemasters JJ, Qian T, He L, et al. Role of mitochondrial inner membrane permeabilization in necrotic cell death, apoptosis, and autophagy. Antioxid Redox Signal 2002;4:769–81.*

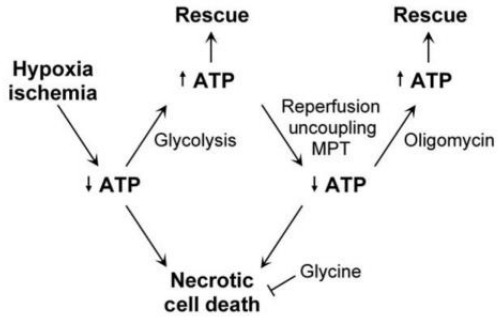

FIGURE 1.6 Progression of mitochondrial injury. Respiratory inhibition inhibits oxidative phosphorylation and leads to ATP depletion and necrotic cell death. Glycine blocks plasma membrane permeabilization causing necrotic cell death downstream of ATP depletion. Glycolysis restores ATP and prevents cell killing. Mitochondrial uncoupling as occurs after reperfusion due to the mitochondrial permeability transition (MPT) activates the mitochondrial ATPase to futilely hydrolyze glycolytic ATP, and protection against necrotic cell death is lost. By inhibiting the mitochondrial ATPase, oligomycin prevents ATP depletion and rescues cells from necrotic cell death if glycolytic substrate is present. *With permission from Lemasters JJ, Qian T, He L, et al. Role of mitochondrial inner membrane permeabilization in necrotic cell death, apoptosis, and autophagy. Antioxid Redox Signal 2002;4:769–81.*

oxidant chemicals, suggesting that mitochondria are also a primary target of cytotoxicity in oxidative stress. However, in pathological settings like ischemia, glycolytic substrates are rapidly exhausted.

Mitochondrial uncoupling in necrotic cell killing

Mitochondrial injury and dysfunction are progressive (Fig. 1.6). Respiratory inhibition as occurs in anoxia causes ATP depletion and ultimately necrotic cell death. Glycolysis can replace this ATP supply, although only partially in highly aerobic cells, to rescue cells from necrotic killing. However, when mitochondrial injury progresses to uncoupling (inner membrane permeability to hydrogen ions), accelerated ATP hydrolysis occurs that is catalyzed by the mitochondrial ATP synthase working in reverse. Since glycolytic ATP production cannot keep pace, ATP levels fall profoundly, and

necrotic cell death ensues. In the progression from respiratory inhibition to uncoupling, mitochondria become active agents promoting ATP depletion and cell death.

Mitochondrial permeability transition

Inner membrane permeability

In oxidative phosphorylation, respiration drives translocation of protons out of mitochondria to create an electrochemical proton gradient composed of a negative inside $\Delta\Psi$ and an alkaline inside pH gradient (ΔpH). ATP synthesis is then linked to protons returning down this electrochemical gradient through the mitochondrial ATP synthase. This chemiosmotic proton circuit requires the mitochondrial inner membrane to be impermeable to ions and charged metabolites.

In some pathophysiological settings, however, the mitochondrial inner membrane abruptly becomes non-selectively permeable to solutes of molecular weight up to about 1500 Da. Ca^{2+}, oxidative stress, and numerous reactive chemicals induce this mitochondrial permeability transition (MPT), whereas cyclosporine A and pH less than 7 inhibit. The MPT causes mitochondrial depolarization, uncoupling, and large amplitude mitochondrial swelling driven by colloid osmotic forces. Opening of highly conductive permeability transition (PT) pores in the mitochondrial inner membrane underlies the MPT. Conductance is so great that opening of a single PT pore may be sufficient to cause mitochondrial depolarization and swelling.

The composition of PT pores is uncertain. In one model, PT pores are formed by the adenine nucleotide transporter (ANT) from the inner membrane, the voltage dependent anion channel (VDAC) from the outer membrane, the cyclosporine A binding protein cyclophilin D (CypD) from the matrix, and possibly other proteins (Fig. 1.7A). Although once widely accepted, the validity of this model has been challenged by genetic knockout studies showing that the MPT still occurs in mitochondria that are deficient in ANT, VDAC and CypD. More recently, PT pores are proposed to form in association with the F_1F_O-ATP synthase (Fig. 1.7B); with spastic paraplegia 7 (SPG7), a mitochondrial AAA-type membrane protease; or with the inorganic phosphate carrier of the inner membrane. An alternative model for the PT pore is that oxidative and other stresses damage membrane proteins that then misfold and aggregate to form PT pores in association with CypD and other molecular chaperones (Fig. 1.7C).

pH-dependent ischemia/reperfusion injury

Ischemia is an interruption of blood flow and hence oxygen supply. In ischemic tissue, anaerobic metabolism causes tissue pH to decrease by a unit or more. The naturally occurring acidosis protects against necrotic cell death during ischemia and also after various toxic stresses. After reperfusion, the protection of acidotic pH is lost, and onset of necrotic cell death occurs. Much of reperfusion injury is attributable to recovery of pH, since reoxygenation at low pH prevents cell killing entirely, whereas restoration of normal pH without reoxygenation produces similar cell killing as restoration of pH with reoxygenation, a so-called pH paradox (Fig. 1.8). Cell killing in the pH paradox is linked specifically to intracellular pH and occurs independently of changes of cytosolic and extracellular free Na^+ and Ca^{2+}.

Role of the mitochondrial permeability transition in pH-dependent reperfusion injury

pH below 7 inhibits PT pores, and recovery of intracellular pH to 7 or greater after reperfusion induces the MPT (Fig. 1.8). Mitochondria depolarize during ischemia, but after reperfusion at normal pH, mitochondria repolarize initially and in parallel with recovery of intracellular pH to neutrality, the MPT occurs (Fig. 1.8 and Fig. 1.9). ATP depletion and necrotic cell death then follow. Reperfusion in the presence of PT pore blockers (*e.g.*, cyclosporine A and its derivatives) prevents mitochondrial inner membrane permeabilization, depolarization and cell killing. Notably, cyclosporine A protects when added only during the reperfusion phase. Thus, the MPT is the proximate cause of pH-dependent cell killing in ischemia/reperfusion injury.

Oxidative stress

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxide, hydrogen peroxide, hydroxyl radical, and peroxynitrite, have long been implicated in cell injury leading to necrosis (Fig. 1.10). Reperfusion after ischemia stimulates intramitochondrial ROS formation, MPT onset and cell death (Fig. 1.9). In neurons, excitotoxic stress with glutamate and N-methyl-D-aspartate (NMDA) receptor agonists also stimulates mitochondrial ROS formation, leading to the MPT and excitotoxic injury.

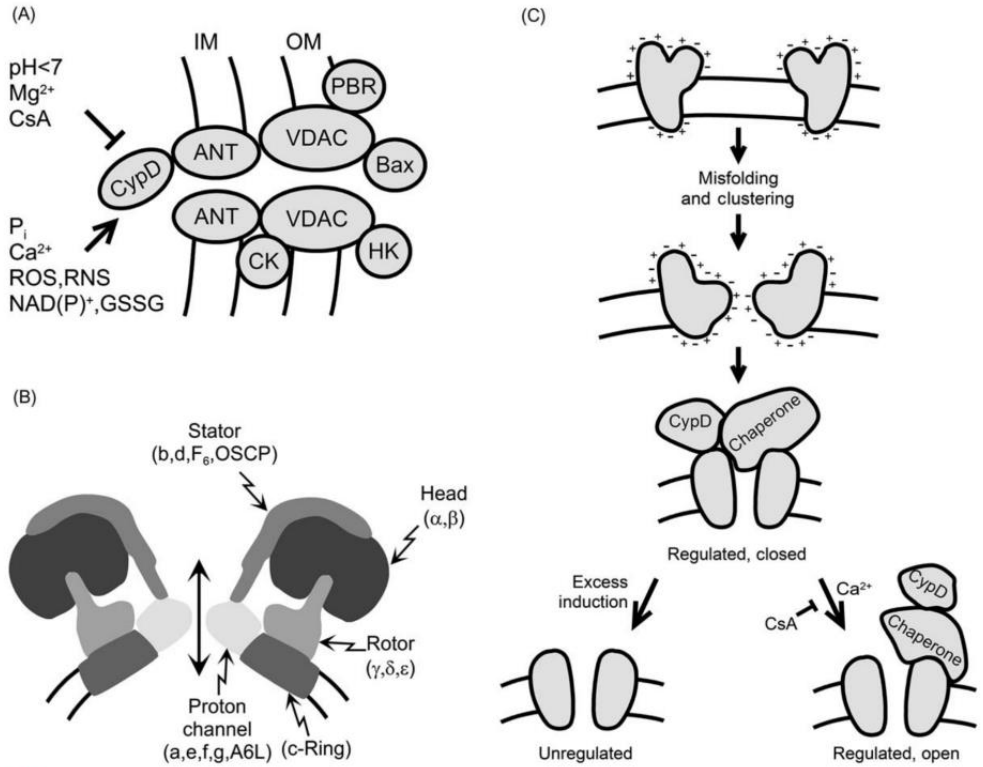

FIGURE 1.7 Models of mitochondrial permeability transition pores. In one model (A), PT pores are composed of the adenine nucleotide translocator (ANT) from the inner membrane (IM), cyclophilin D (CypD) from the matrix and the voltage-dependent anion channel (VDAC) from the outer membrane (OM). Other proteins, such as the peripheral benzodiazepine receptor (PBR), hexokinase (HK), creatine kinase (CK), and Bax may also contribute. PT pore openers include Ca^{2+}, inorganic phosphate (Pi), reactive oxygen and nitrogen species (ROS, RNS), and oxidized pyridine nucleotides ($NAD(P)^+$) and glutathione (GSSG). A newer model (B) has PT pores forming in F_1F_O-ATP synthase dimers at the interface between monomers (or possibly in association with c-rings). OSCP (oligomycin sensitivity-conferring protein), a, b, c, d, e, f, g, α, β, γ, δ, ε, A6L and F8 are subunits of the synthase. An alternative proposal (C) suggests that oxidative and other damage to integral inner membrane proteins leads to misfolding. These misfolded proteins aggregate at hydrophilic surfaces facing the hydrophobic bilayer to form aqueous channels. CypD and other chaperones block conductance of solutes through these nascent PT pores. High matrix Ca^{2+} acting through CypD leads to PT pore opening, an effect blocked by cyclosporine A (CsA). As misfolded protein clusters exceed the number of chaperones to regulate them, constitutively open channels form. Such unregulated PT pores are not dependent on Ca^{2+} for opening and are not inhibited by CsA. *Adapted with permission from Kim JS, He L, Qian T, Lemasters JJ. Role of the mitochondrial permeability transition in apoptotic and necrotic death after ischemia/reperfusion injury to hepatocytes. Curr Mol Med 2003;3:527–35.*

Iron potentiates injury in a variety of diseases and is an important catalyst for hydroxyl radical formation from superoxide and hydrogen peroxide (Fig. 1.10). During oxidative stress, acetaminophen hepatotoxicity and hypoxia/ischemia, lysosomes release chelatable (loosely bound) iron with consequent pro-oxidant cell damage. This iron is taken up into mitochondria by the mitochondrial calcium uniporter and helps catalyze mitochondrial ROS generation. Iron chelation with desferal prevents mitochondrial ROS formation and decreases cell death.

Other stress mechanisms inducing necrotic cell death

Poly (ADP-ribose) polymerase

Single strand breaks induced by ultraviolet (UV) light, ionizing radiation, and ROS (particularly hydroxyl radical and peroxynitrite) activate PARP. PARP transfers ADP-ribose from NAD^+ to the strand breaks and elongates ADP-ribose polymers attached to the DNA. Excess consumption of NAD^+ in this fashion leads to NAD^+ depletion, disruption of

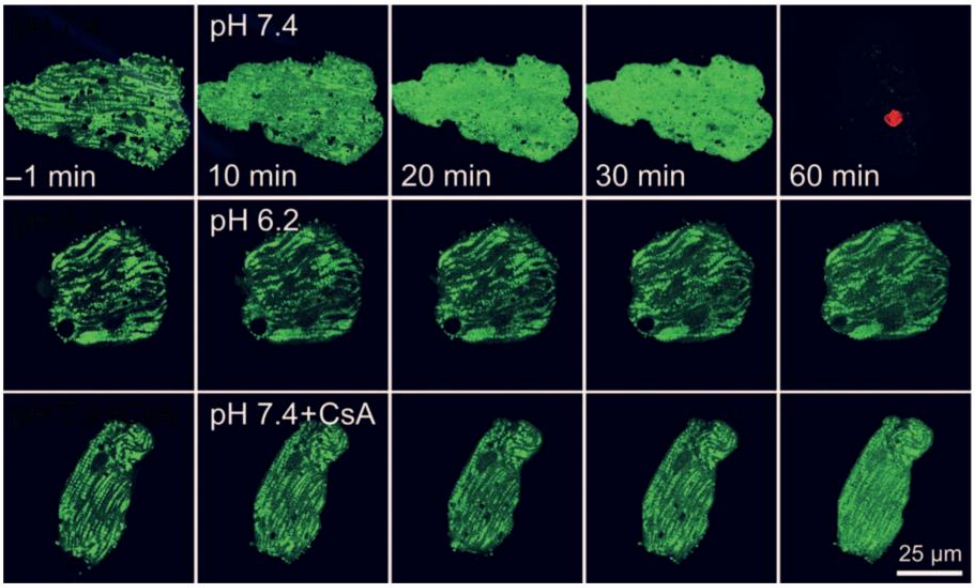

FIGURE 1.8 Mitochondrial inner membrane permeabilization in adult rat cardiac myocytes after ischemia and reperfusion. After loading mitochondria of cardiac myocytes with calcein, cells were subjected to 3 h of anoxia at pH 6.2 (ischemia) followed by reoxygenation at pH 7.4 (A), pH 6.2 (B), or pH 7.4 with 1 μM CsA (C). Red-fluorescing propidium iodide was present to detect loss of cell viability. Note that green calcein fluorescence was retained by mitochondria at the end of ischemia (1 min before reperfusion), indicating that PT pores had not opened. After reperfusion at pH 7.4, mitochondria progressively released calcein over 30 min at which time calcein was nearly evenly distributed throughout cytosol. After 60 min, all cellular calcein was lost, and the nucleus stained with PI, indicating loss of viability. After reperfusion at pH 6.2 (B) or at pH 7.4 in the presence of CsA (C), calcein was retained and cell death did not occur. Thus, reperfusion at pH 7.4 induced onset of the MPT and necrotic cell death that were blocked with CsA and acidotic pH. *Adapted with permission from Kim JS, Jin Y, Lemasters JJ. Reactive oxygen species, but not Ca^{2+} overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia-reperfusion. Am J Physiol Heart Circ Physiol 2006;290:H2024–34.*

ATP-generation, and ATP depletion-dependent cell death. PARP-dependent necrosis is an example of programmed necrosis, since PARP actively promotes a cell death-inducing pathway that otherwise would not occur. Necrotic cell death also frequently occurs when apoptosis is interrupted, as by caspase inhibition. Such caspase-independent cell death is the consequence of mitochondrial dysfunction or other metabolic disturbance.

Plasma membrane injury

An intact plasma membrane is essential for cell viability. Detergents and pore-forming agents like mastoparan from wasp venom defeat the barrier function of the plasma membrane and cause immediate cell death. Immune-mediated cell killing can act similarly. In particular, complement mediates formation of a membrane attack complex that in conjunction with antibody lyses cells. Complement component 9, an amphipathic molecule, inserts through the cell membrane, polymerizes, and forms a tubular channel visible by electron microscopy. Indeed, a single membrane attack complex is sufficient to cause lysis of an individual erythrocyte.

Pathways to apoptosis

Roles of apoptosis in biology

Apoptosis is an essential event in both the normal life of organisms and in pathobiology. In development, apoptosis sculpts and remodels tissues and organs, for example, by creating clefts in limb buds to form fingers and toes. Apoptosis is also responsible for reversion of hypertrophy to atrophy and immune surveillance-induced killing of pre-neoplastic and virally infected cells. Each of several organelles can give rise to signals initiating apoptosis. Often these signals converge on mitochondria as a common pathway to apoptotic cell death. In most apoptotic signaling, activation of caspases 3 or 7

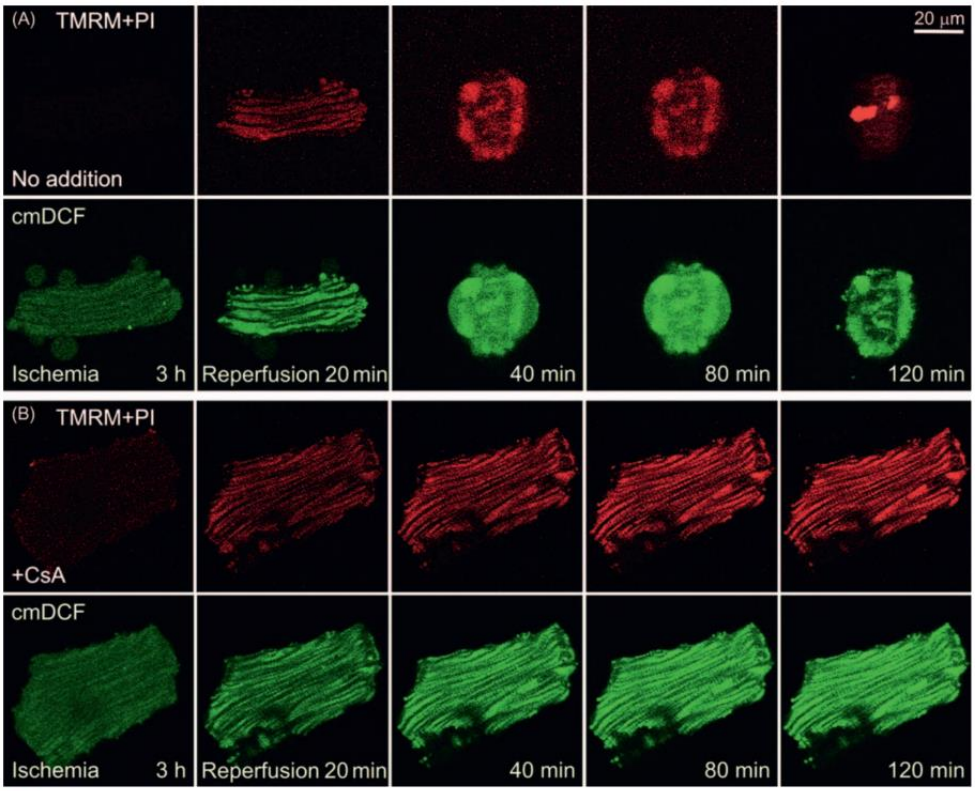

FIGURE 1.9 Mitochondrial ROS formation after reperfusion. Myocytes were co-loaded with red-fluorescing tetramethylrhodamine methyester (TMRM) and green-fluorescing chloromethyldichlorofluorescin (cmDCF) to monitor mitochondrial membrane potential and ROS formation, respectively. At the end of 3 h of ischemia, mitochondria were depolarized (lack of red TMRM fluorescence). After 20 min of reperfusion, mitochondria took up TMRM, indicating repolarization, and cmDCF fluorescence increased progressively inside mitochondria (A). Subsequently, hypercontraction and depolarization occurred after 40 min, and viability was lost within 120 min, as indicated by nuclear labeling with red-fluorescing propidium iodide. When cyclosporine A was added at reperfusion (B), mitochondria underwent sustained repolarization, and hypercontracture and cell death did not occur. Nonetheless, mitochondrial cmDCF fluorescence still increased. By contrast, reperfusion with antioxidants prevented ROS generation and MPT onset with subsequent cell death (data not shown). Thus, mitochondrial ROS generation induces the MPT and cell death after ischemia/reperfusion. *Adapted with permission from Kim JS, Jin Y, Lemasters JJ. Reactive oxygen species, but not Ca^{2+} overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia-reperfusion. Am J Physiol Heart Circ Physiol 2006;290:H2024–34.*

from a family of caspases (Table 1.2) begins execution of the final and committed phase of apoptotic cell death. Caspase 3/7 has many targets. Degradation of the nuclear lamina and cytokeratins contributes to nuclear remodeling, chromatin condensation, and cell rounding. Endonuclease activation leads to internucleosomal DNA cleavage. The resulting DNA fragments have lengths in multiples of 190 base pairs, the nucleosome to nucleosome repeat distance. In starch gel electrophoresis, these fragments produce a characteristic ladder pattern. DNA strand breaks are also recognized in tissue sections by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. Additionally, caspase activation leads to cell shrinkage, phosphatidyl serine externalization on the plasma membrane, and formation of numerous small surface blebs (zeiosis). Unlike necrotic blebs, zeiotic blebs contain membranous organelles. However, not all apoptotic changes depend on caspase 3/7 activation. For example, release of apoptosis-inducing factor (AIF) from mitochondria and its translocation to the nucleus promotes DNA degradation in a caspase 3-independent fashion.

Pathways leading to activation of caspase 3 and related effector caspases like caspase 7 are complex and variable between cells and specific apoptosis-instigating stimuli, and each major cellular structure can originate its own set of unique signals to induce apoptosis (Fig. 1.11). Pro-apoptotic signals are often associated with specific damage or perturbation to the organelle involved. Consequently, cells choose death by apoptosis rather than life with organelle damage.

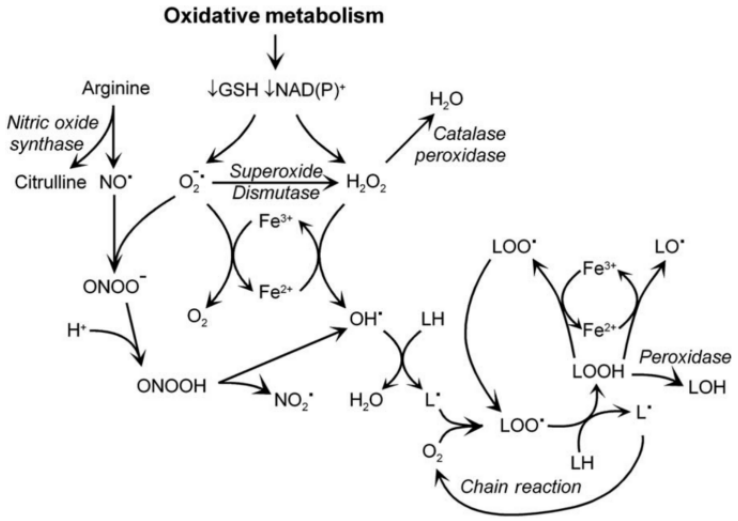

FIGURE 1.10 Iron-catalyzed free radical generation. Oxidative stress causes oxidation of GSH and NAD(P)H, important reductants in antioxidant defenses, promoting increased net formation of superoxide ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2). Superoxide dismutase converts superoxide to hydrogen peroxide, which is further detoxified to water by catalase and peroxidases. In the iron-catalyzed Haber Weiss reaction (or Fenton reaction), superoxide reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), which reacts with hydrogen peroxide to form the highly reactive hydroxyl radical ($OH^{\bullet}$). Hydroxyl radical reacts with lipids to form alkyl radicals ($L^{\bullet}$) that initiate an oxygen-dependent chain reaction generating peroxyl radicals ($LOO^{\bullet}$) and lipid peroxides (LOOH). Iron also catalyzes a chain reaction generating alkoxyl radicals ($LO^{\bullet}$) and more peroxyl radicals. Nitric oxide synthase catalyzes formation of nitric oxide (NO) from arginine. Nitric oxide reacts rapidly with superoxide to form unstable peroxynitrite anion ($ONOO^{-}$), which decomposes to nitrogen dioxide and hydroxyl radicals. In addition to attacking lipids, these radicals also attack proteins and nucleic acids.

Plasma membrane

The plasma membrane is the target of many receptor-mediated signals. In particular, death ligands (*e.g.*, tumor necrosis factor α, or TNFα; Fas ligand or FasL; tumor necrosis factor-related apoptosis-inducing ligand, or TRAIL) acting through their corresponding receptors (TNF receptor 1, or TNFR1; Fas; death receptor 4 and 5, or DR4/5) initiate activation of apoptotic pathways. Binding of ligands like TNFα leads to receptor trimerization and formation of a complex with adapter proteins (*e.g.*, TNF receptor-associated death domain protein, or TRADD). After receptor dissociation, a death-inducing signaling complex (DISC) forms through association with Fas-associated protein with death domain (FADD) and pro-caspase 8, which are internalized. Pro-caspase 8 becomes activated and in turn proteolytically activates other downstream effectors (Fig. 1.12). In Type I signaling, caspase 8 activates caspase 3 directly, whereas in Type II signaling, caspase 3 cleaves Bid (novel BH3 domain-only death agonist) to truncated Bid (*t*Bid) to activate a mitochondrial pathway to apoptosis. Similar signaling occurs after association of FasL with Fas (also called CD95) and TRAIL with DR4/5.

Many events modulate death receptor signaling in the plasma membrane. For example, the extent of gene and surface expression of death receptors is an important determinant in cellular sensitivity to death ligands. Stimuli like hydrophobic bile acids can recruit death receptors to the cell surface and sensitize cells to death-inducing stimuli. Surface recruitment of death receptors may also lead to self-activation even in the absence of ligand. Death receptors localize to lipid rafts containing cholesterol and sphingomyelin. After death receptor activation, ceramide forms from sphingomyelin hydrolysis, which promotes raft coalescence and formation of molecular platforms that cluster components of DISC. Glycosphingolipids, such as ganglioside GD3, also integrate into DISCs to promote apoptosis.

Mitochondria

Cytochrome *c* release

Bid is a BH3 only domain member of the B-cell lymphoma-2 (Bcl2) family that includes both pro- and anti-apoptotic proteins (Fig. 1.13). *t*Bid formed after caspase 8 activation translocates to mitochondria where it interacts with either

TABLE 1.2 Mammalian caspases.

Initiator caspases	Molecular weight of proenzyme (kDa)	Active subunits (kDa)	Prodomain	Amino acid target sequence for proteolysis
Caspase 2	51	19/12	Long with CARD	VDVAD
Caspase-8	55	18/11	Long with two DED	(L/V/D)E(T/V/I)D
Caspase-9	45	17/10	Long with CARD	(L/V/I)EHD
Caspase-10	55	17/12	Long with two DED	(I/V/L)EXD
Caspase-12	50	20/10	Long with CARD	ATAD
Effector caspases				
Caspase-3	32	17/12	Short	DE(V/I)D
Caspase-6	34	18/11	Short	(T/V/I)E(H/V/I)D
Caspase-7	35	20/12	Short	DE(V/I)D
Inflammatory caspases				
Caspase 1	45	20/10	Long with CARD	(W/Y/F)EHD
Caspase 4	43	20/10	Long with CARD	(W/L)EHD
Caspase 5	48	20/10	Long with CARD	(W/L/F)EHD
Caspase 11	42	20/10	Long with CARD	(V/I/P/L)EHD
Other caspases				
Caspase 14	42	20/10	Short	(W/I)E(T/H)D

Caspases are evolutionarily conserved aspartate specific cysteine-dependent proteases that function in apoptotic and inflammatory signaling. Initiator caspase are involved in the initiation and propagation of apoptotic signaling, whereas effector caspases act on a wide variety of proteolytic substrates to induce the final and committed phase of apoptosis. Initiator and inflammatory caspases have large prodomains containing oligomerization motifs such as the caspase recruitment domain (CARD) and the death effector domain. Effector caspases have short pro-domains and are proteolytically activated by large pro-domain caspases and other proteases. Proteolytic cleavage of pro-caspase precursors forms separate large and small subunits that assemble into active enzymes consisting of two large and two small subunits. Caspase activation occurs in multimeric complexes that typically consist of a platform protein that recruits pro-caspases either directly or by means of adaptors. Such caspase complexes include the apoptosome and the death-inducing signaling complex (DISC). Caspase 14 plays a role in terminal keratinocyte differentiation in cornified epithelium.

Bak (Bcl2 homologous antagonist/killer) or Bax (a conserved homolog that heterodimerizes with Bcl2), two other pro-apoptotic Bcl2 family members, to induce cytochrome *c* release through the outer membrane into the cytosol. Cytochrome *c* in the cytosol interacts with apoptotic protease activating factor-1 (Apaf-1) and pro-caspase 9 to assemble haptomeric apoptosomes and an ATP (or deoxyadenosine triphosphate, or dATP)-dependent cascade of caspase 9 and caspase 3 activation.

Cytochrome *c* release from the space between the mitochondrial inner and outer membranes appears to occur via formation of specific pores in the mitochondrial outer membrane. Except for the requirement for either Bak or Bax, the molecular composition and properties of cytochrome *c* release channels remain incompletely understood. Alternatively, cytochrome c release can occur as a consequence of the MPT due to mitochondrial swelling and rupture of the outer membrane.

After the MPT, progression to apoptosis or necrosis depends on other factors. If the MPT occurs rapidly and affects most mitochondria of a cell, as happens after severe oxidative stress and ischemia/reperfusion, a precipitous fall of ATP (and dATP) will occur that actually blocks apoptotic signaling by inhibiting (d)ATP-requiring caspase 9/3 activation. With ATP depletion, oncotic necrosis ensues. However, when alternative sources for ATP generation are present (*e.g.*, glycolysis), then necrosis is prevented and caspase 9/3 becomes activated, and caspase-dependent apoptosis occurs instead (Fig. 1.14). Crosstalk between apoptosis and necrosis also occurs in other ways. For example, after TNFα binding to TNRR1, recruitment of receptor-interacting serine/threonine-protein kinase 1 (RIPK1) can activate NADPH oxidase leading to superoxide generation and sustained activation of c-Jun nuclear kinase (JNK), resulting in oncotic necrosis rather than apoptosis.

Regulation of the mitochondrial pathway to apoptosis

Mitochondrial pathways to apoptosis vary depending on expression of pro-caspases, Apaf-1, and other proteins. Some terminally differentiated cells, particularly neurons, do not respond to cytochrome *c* with caspase activation and

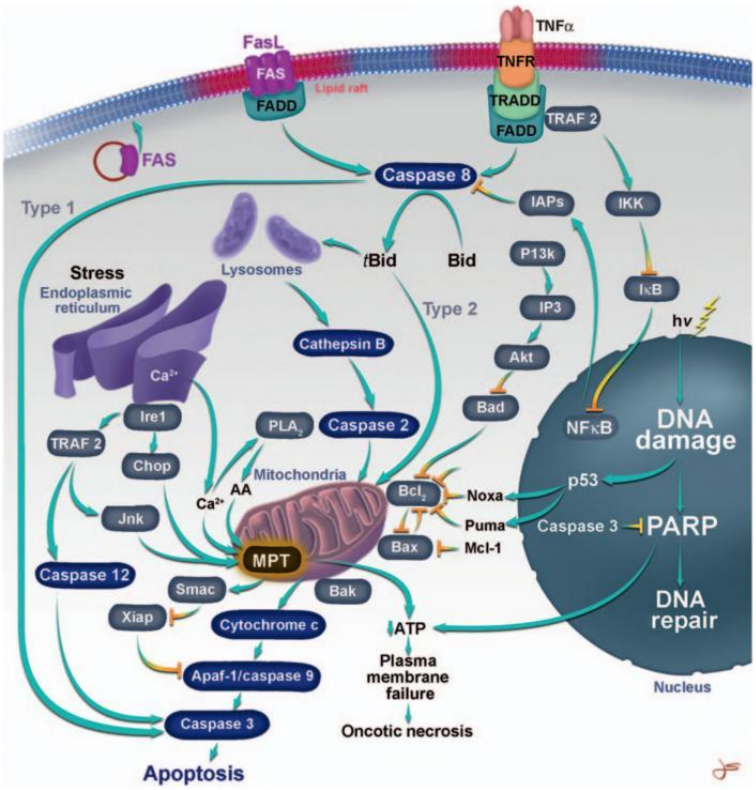

FIGURE 1.11 Scheme of apoptotic signaling from organelles. See text for details. *Adapted with permission from Lemasters JJ. Dying a thousand deaths: redundant pathways from different organelles to apoptosis and necrosis. Gastroenterology 2005;129:351–60.*

apoptosis, which may be linked to lack of Apaf-1 expression. Anti-apoptotic Bcl2 proteins, like Bcl2, Bcl extra long (Bcl-xL), and myeloid cell leukemia sequence 1 (Mcl-1), block apoptosis and are frequently overexpressed in cancer cells (Fig. 1.13). Anti-apoptotic Bcl2 family members form heterodimers with pro-apoptotic family members like Bax and Bak, to prevent the latter from oligomerizing into cytochrome *c* release channels.

Inhibitor of apoptosis proteins (IAPs), including X-linked inhibitor of apoptosis protein (XIAP), cellular IAP1 and 2 (cIAP1/2), and survivin, oppose apoptotic signaling by inhibiting caspase activation. Many IAPs recruit E2 ubiquitin-conjugating enzymes to promote ubiquitination of target proteins and subsequent proteosomal degradation. Some IAPs inhibit apoptotic pathways upstream of mitochondria at caspase 8, whereas others like XIAP inhibit caspase 9/3 activation downstream of mitochondrial cytochrome *c* release. Additional proteins like Smac suppress the action of IAPs, providing an "inhibitor of the inhibitor" effect promoting apoptosis. Smac is a mitochondrial intermembrane protein that is released with cytochrome *c*. Smac inhibits XIAP and promotes apoptotic signaling after mitochondrial signaling. Thus, high Smac to XIAP ratios favor caspase 3 activation after cytochrome *c* release. Other pro-apoptotic proteins released from the mitochondrial intermembrane space during apoptotic signaling include AIF, endonuclease G, and high temperature requirement A2 (HtrA2/Omi, a serine protease that degrades IAPs).

Disruption of mitochondrial function induces fragmentation of larger filamentous mitochondria into smaller more spherical structures. Such changes are also often prominent in apoptosis. Mitochondrial fission is mediated by dynamin-like protein type 1 (Drp1), a large cytosolic GTPase mechanoenzyme, and fission-1 (Fis1) in the outer membrane. Drp1 forms complexes with pro-apoptotic Bcl2 family members like Bax to promote cytochrome *c* release during apoptosis. Mitochondrial fusion depends on optic atrophy-1 (Opa1) in the inner membrane, which is mutated in dominant optic atrophy, and mitofusin 1 and 2 (Mfn1/2), two proteins in the outer membrane. Fission events in mitochondria seem to promote apoptotic signaling, since dynamin-like protein type 1 (Drp-1) overexpression promotes apoptosis, whereas Mfn1/2 overexpression retards apoptosis.