

CHAPTER

37

Apoptosis and Necrosis

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OUTLINE

Distinguishing Features of Apoptosis and Necrosis

During embryonic and postnatal development, and throughout adult life, many cells in the nervous system die
Many of the morphological and biochemical changes that occur in cells that die by necrosis are very different from those that occur in apoptosis

Apoptosis

Adaptive apoptosis occurs in the developing and adult nervous system
Apoptosis occurs in acute neurological insults
Apoptosis occurs in neurodegenerative disorders
There are many triggers of apoptosis
Once apoptosis is triggered, a stereotyped sequence of premitochondrial events occurs that executes the cell death process
Several different changes in mitochondria occur during apoptosis
The postmitochondrial events of apoptosis include activation of the caspases

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DISTINGUISHING FEATURES OF APOPTOSIS AND NECROSIS

During embryonic and postnatal development, and throughout adult life, many cells in the nervous system die

Such cell deaths are called 'programmed' because they are normal, being either adaptive or neutral with regard to their impact on the function of the nervous system. One particular form of programmed cell death (PCD) that has been extensively studied is called apoptosis. The details of the molecular cascades and morphological features of apoptosis vary somewhat depending on cell type, death stimulus and other factors

(Yuan et al., 2003). However, there are several morphological and biochemical changes that are generally considered to be defining features of apoptosis (Table 37-1). Morphological changes include cell shrinkage, blebbing of the plasma membrane, and nuclear chromatin condensation and fragmentation with preservation of the structure of mitochondria and the endoplasmic reticulum. Biochemical events that define apoptosis include upregulation and/or mitochondrial translocation of proapoptotic proteins such as Bax and Par-4; formation of mitochondrial permeability transition pores and the release of cytochrome c and/or apoptosis-inducing factor (AIF) from the mitochondria; activation of effector caspases, which control the cell death process by cleaving a range of cytoplasmic, membrane-associated and nuclear protein

TABLE 37-1 Examples of Caspase Substrates

Cytoskeletal and associated proteins	Actin, spectrin, tau, vimentin, β -catenin, gelsolin, kinectin
Nuclear and DNA-associated proteins	Ataxia telangiectasia mutated (ATM), poly(ADP ribose) polymerase (PARP), DNA-dependent protein kinase, DNA replication factor C, DNA topoisomerase I, DNA fragmentation factor (DFF)45, inhibitor of caspase-activated DNase (ICAD), lamins A, B1, and C
Signal transduction proteins	TRAF-1, Raf1, Ras, GAP, GDP dissociation inhibitor of Rho family GTPases, phospholipase A ₂ , Stat1
Kinases and phosphatases	Protein kinase Cd, Akt kinase, calcium/calmodulin-dependent protein kinase IV, mitogen-activated protein kinase kinase (MEKK-1), focal adhesion kinase (FAK), protein phosphatase (PP)2A, calcineurin
Transcription factors	NF- κ B subunits p65 and p50, AP-2 α , forkhead transcription factor FOXO3a, Max
Ion channels	IP3R1 and IP3R2, glutamate (AMPA) receptor subunits GluR1 and GluR4
Neurological-disorder-related proteins	Amyloid precursor protein (APP), presenilin-1, presenilin-2, parkin, tau, huntingtin, ataxin-3

substrates; exposure of phosphatidylserine on the plasma membrane surface, which serves as a signal for recognition; and engulfment of the dying cell by macrophages and microglia (see also Ch. 23). If the preceding events occur *and* if the death of the cells can be prevented by caspase inhibitors and inhibitors of protein synthesis, then the mode of cell death can be considered to be apoptosis.

Many of the morphological and biochemical changes that occur in cells that die by necrosis are very different from those that occur in apoptosis

During necrosis cells swell, mitochondria and endoplasmic reticulum lose their structure and become dysfunctional and the nuclear membrane becomes disrupted (Fig. 37-1). Necrotic death is independent of premitochondrial apoptotic proteins such as Bax, cytochrome c release and caspase activation. Necrosis is further distinguished from apoptosis by the fact that necrosis usually occurs as the result of a traumatic physical injury or stroke and cells die *en masse*, whereas apoptosis typically occurs in individual cells within a population of surviving neighbors.

The information presented in this chapter concerns the mammalian nervous system. However, it should be recognized that similar mechanisms may occur in other organisms and, indeed, many important aspects of the process of apoptosis were initially discovered in studies of the nematode *Caenorhabditis elegans*. The following references provide overviews on these topics: Gleichmann & Mattson, (2010); Mattson, (2000); Mattson et al., (2002); Wilson & Mattson, (2007); Yuan et al., (2003).

APOPTOSIS

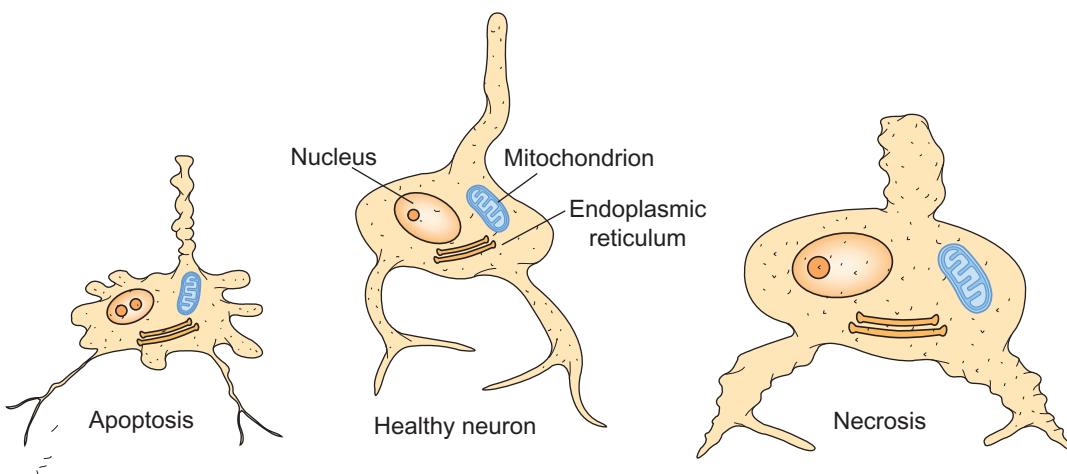
The conditions and signaling pathways that trigger or prevent apoptosis, and the molecular control of the apoptotic process, are topics of intense interest in the field of neuroscience. Knowledge of the cellular and molecular mechanisms of apoptosis is necessary for a full understanding of how the brain and other regions of the nervous system develop. Evidence is increasing that apoptosis and related forms of cell death occur in neurodegenerative disorders including Alzheimer's, Parkinson's and Huntington's diseases; age-related macular degeneration (AMD); amyotrophic lateral sclerosis (ALS) and stroke; there is therefore intense interest in developing approaches directed toward understanding early signaling events that lead to apoptosis as potential therapeutic targets for these diseases (see respective chapters for details related to therapy as well as the following references: (Mattson, 2003; Bazan, 2007; Riess et al., 2003; Vila & Przedborski, 2003; Waldmeier, 2003; Mattson et al., 2008; Mattson, 2010; Texel & Mattson, 2011; Albani et al., 2010; Avila, 2010; Hyman, 2011; Cheng et al., 2011).

Adaptive apoptosis occurs in the developing and adult nervous system

During embryonic and early postnatal development, many cells undergo apoptosis in most regions of mammalian nervous systems. Such developmental apoptosis is believed to be adaptive in that it eliminates cells that are not integrated into neuronal circuits and are therefore unnecessary for the proper function of the nervous system. While initially established in populations of neurons that innervate peripheral targets (dorsal root ganglia, sympathetic ganglia and motor neurons), developmental apoptosis has also been documented in several populations of neurons in the brain. There appear to be two major 'waves' of apoptosis, one occurring early and involving neuronal progenitor cells and another occurring later and involving neurons that are in the process of forming synaptic connections with target cells. While neuronal apoptosis is well established, the extent to which glial cells (oligodendrocytes and astrocytes) undergo apoptosis during development is unclear (see Development in Ch. 28).

Developmental processes continue in at least some locations in the adult nervous system. For example, relatively large numbers of neural stem cells are located in the subventricular zone of the cerebral cortex and in the subgranular layer of the dentate gyrus of the hippocampus (see Stem Cells in Ch. 30). The subventricular zone stem cells give rise to interneurons in the olfactory bulb, while hippocampal stem cells can differentiate into dentate granule neurons. Although some newly generated neurons that arise from stem cells in the adult brain become integrated into neuronal circuits, many of these cells undergo apoptosis. Neurogenesis increases in response to brain injury, which is probably an adaptive mechanism designed to replace damaged neurons (see Regeneration in Ch. 32).

The signals that determine whether neural stem cells and their progeny survive or undergo apoptosis include growth factors (Ch. 29), cytokines (Chs. 32–34) and cell adhesion



Apoptosis	Necrosis
Cell shrinkage	Cell swelling
Maintenance of organellar integrity	Organellar swelling and damage
Maintenance of ATP levels	Depletion of ATP
Maintenance of ion homeostasis	Loss of ion homeostasis
Membrane surface blebbing	Membrane rupture
Nuclear chromatin condensation/fragmentation	Nuclear lysis
Requires synthesis of death effector proteins	Cessation of protein synthesis
Prevented by blocking steps in the death cascade	Irreversible
Does not adversely affect neighbor cells	Promotes death of neighbor cells

FIGURE 37-1 Distinguishing features of apoptosis and necrosis.

molecules (Ch. 9). The discovery of nerve growth factor (NGF) heralded investigations into the roles of neurotrophic factor signaling in determining whether neurons live or die during development. It was shown by Levi-Montalcini and Hamburger in the 1950s that sensory and sympathetic neurons depend on a substance later identified as nerve growth factor (NGF) for their survival and maintenance. Neurons that do not receive sufficient trophic support undergo apoptosis. Other factors that can determine whether stem cells and/or neurons live or die include basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF) and epidermal growth factor. Examples of other signaling molecules that may regulate developmental apoptosis include nitric oxide, Par-4 and ceramide, PTEN (phosphatase and TENsin homolog) and the transcription factor E2F1.

Apoptosis occurs in acute neurological insults

Traumatic and ischemic injuries to the nervous system are common events that result in considerable morbidity and mortality. Accordingly, there is intense interest in understanding the cellular and molecular mechanisms responsible for the death of neurons in such acute neurodegenerative conditions. Many neurons may undergo apoptosis in response to a traumatic injury to the brain, spinal cord or peripheral nerves,

as indicated by activation of various proapoptotic proteins including p53 and caspases. In particular, neurons adjacent to the necrotic region of severe trauma are prone to apoptosis. Drugs that attenuate apoptosis have been shown to be beneficial in animal models of traumatic brain and spinal cord injury, suggesting a potential for such an approach in human patients.

Ischemic stroke occurs when a blood vessel in the brain becomes occluded, usually as the result of atherosclerosis and clot formation (Ch. 35). Neurons nourished by the affected vessel may undergo apoptosis. Indeed, biochemical, morphological and functional evidence of apoptosis has been documented in animal models of stroke. Triggers of ischemic apoptosis include oxygen free radicals, glutamate receptor activation, calcium influx and release from intracellular stores, and lipid peroxidation. DNA damage and activation of poly (ADP ribose) polymerase (PARP) and p53 are also implicated in stroke-induced neuronal apoptosis. Mediators of ischemic neuronal apoptosis may include the Bcl-2 family of proteins, Par-4, cytochrome c, apoptosis-inducing factor and caspases. Activation of glutamate receptors, particularly the extrasynaptic NMDA receptor, may play an important role (Mattson, 2003).

Phospholipids of cellular membranes in the nervous system are endowed with the highest content of phospholipids of cellular membrane As a result, the high degree of unsaturation

in excitable membranes conforms high-membrane-fluidity domains, where specific proteins (e.g., receptors, transporters, ion channels) perform their functions. Ischemia–reperfusion and oxidative stress target these highly unsaturated phospholipids. Thus, DHA-containing phospholipids are a target for free radical–catalyzed peroxidation (Fig. 37-2) and, as a result, F4-isoprostanes are formed. F2-isoprostanes are also derived from free-radical–catalyzed peroxidation, although from arachidonic acid instead. F4-isoprostanes (neuroprostanes) are found esterified in phospholipids, and it has been reported that their content is increased in the brains of patients with Alzheimer's disease. Excessive accumulation of free-radical-mediated lipid peroxidation products promotes cellular damage and death.

As an example, rhodopsin in photoreceptors is immersed in a lipid environment highly enriched in phospholipids containing DHA, and DHA is essential for rhodopsin function (Bazan, 2007; Mukherjee et al., 2007; Antony et al., 2010). Moreover, retinal DHA, like brain DHA, is very resistant to n-3 fatty acid dietary deprivation. On the other hand, DHA is the precursor of docosanoids, which are enzyme-derived,

stereospecific mediators. The docosanoid neuroprotectin D1 (NPD1; 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid) is a potent inhibitor of brain ischemia–reperfusion–induced polymorphonuclear leukocyte infiltration, as well as ischemia–reperfusion-induced NF- κ B and cyclooxygenase (COX)-2 expression. Moreover, marked attenuation of the stroke volume was observed when NPD1 was infused into the third ventricle during ischemia–reperfusion. The elucidation of this DHA-oxygenation messenger sheds new light on how the brain modulates its response to oxidative stress and inflammatory injury and raises the potential for designing new drugs to treat neurologic disorders, such as stroke, traumatic brain injury, spinal cord injury or Alzheimer's disease. In particular, the very high biological activity of the docosanoid NPD1 marks it as a potential effector of neuroprotection. The newly isolated dihydroxy-containing DHA derivative is called 'neuroprotectin D1' (NPD1) for two reasons: (1) its neuroprotective properties in brain ischemia–reperfusion and in oxidative-stress-challenged retinal pigment epithelial cells (Mukherjee et al., 2004), and (2) its potent ability to target Bcl-2 proteins by inactivating proapoptotic signaling and

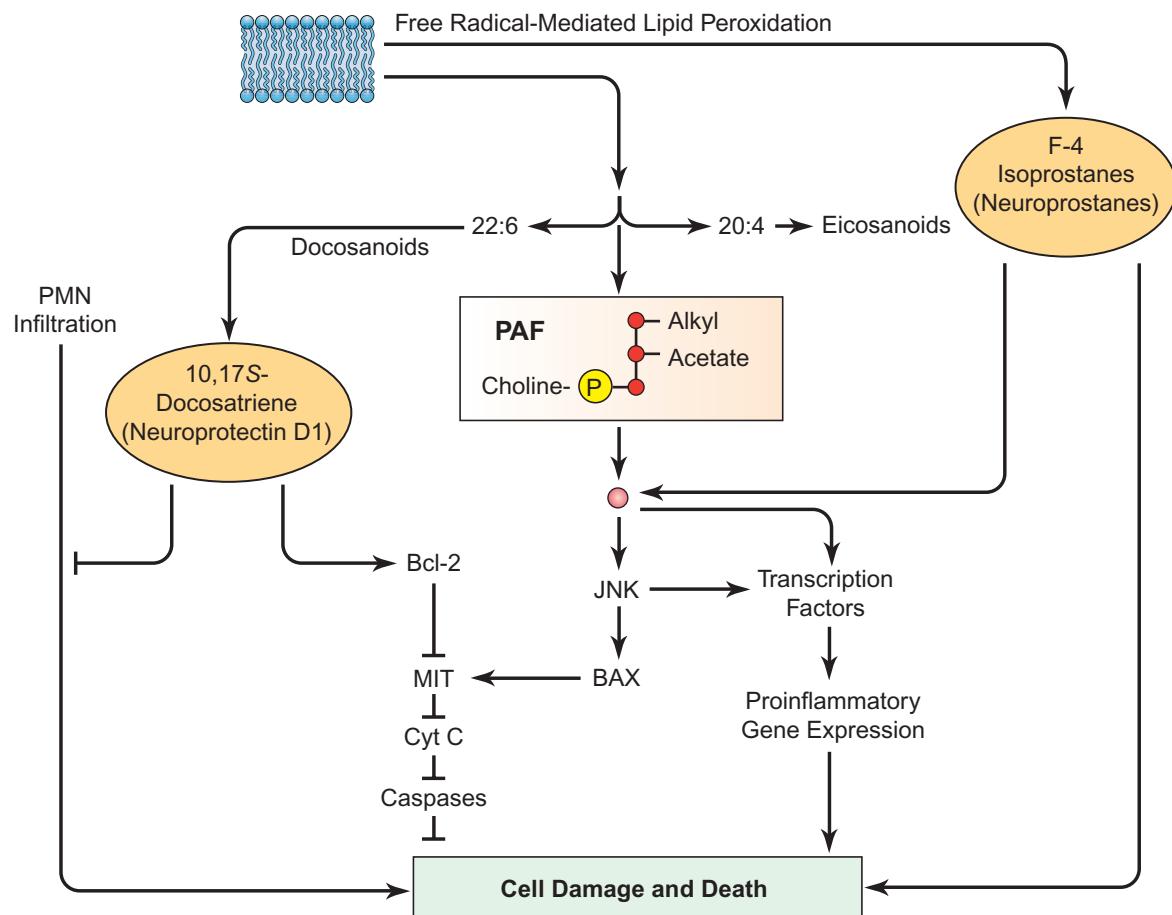


FIGURE 37-2 Phospholipids of cellular membranes can be peroxidized by free-radical–catalyzed reactions. Excessive accumulation of lipid peroxides contributes to cell damage and death. In the nervous system, these phospholipids contain the largest quantities of docosahexaenoic acid (22:6), which was recently demonstrated to be the precursor of the neuroprotective docosanoid 10,17S-docosatriene (neuroprotectin D1, NPD1). NPD1 counteracts proinflammatory cellular signaling and decreases polymorphonuclear leukocyte (PMN) infiltration in ischemic brain, and inactivates proapoptotic signaling and upregulates antiapoptotic signaling in oxidatively stressed retinal pigment epithelial cells (Mukherjee et al., 2004).

upregulating anti-apoptotic signals. References on lipid mediators and their involvement in neuroinflammation can be found in Chapters 34 and 36, respectively, as well as in the following references: (Bazan, 2007; Mukherjee et al., 2007; Antony et al., 2010; Mukherjee et al., 2004; Zhao et al., 2011; Belayev et al., 2011; Bazan et al., 2010)

Apoptosis occurs in neurodegenerative disorders

Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS) are four prominent fatal neurodegenerative disorders that involve the death of specific populations of neurons (see details in respective chapters). Studies of patients and animal and culture models have provided considerable insight in the cellular and molecular mechanisms responsible for synaptic dysfunction and neuronal degeneration in each disorder (Mattson, 2000). In Alzheimer's disease, abnormalities in proteolytic processing of the amyloid precursor protein, due to gene mutations

(in the amyloid precursor protein or in the presenilins) or age-related factors, result in accumulation of neurotoxic forms of amyloid beta peptide (Gleichmann & Mattson, 2010) (Fig. 37-3). In Parkinson's disease, age-related increases in oxidative stress, and possibly environmental neurotoxins, cause selective degeneration of dopaminergic neurons in the substantia nigra. In Huntington's disease, inheritance of a mutant huntingtin gene that encodes a huntingtin protein with increased numbers of polyglutamine repeats causes degeneration of striatal neurons. ALS, which is characterized by selective degeneration of motor neurons, can result from mutations in Cu/Zn-superoxide dismutase or from other unknown genetic and environmental causes. While the causes of these neurodegenerative disorders may differ, they each involve increased oxidative stress, metabolic compromise and disruption of cellular calcium ion homeostasis as important contributors to the cell death process (Mattson, 2000).

There is considerable evidence that many of the neurons that die in Alzheimer's disease, Parkinson's disease,

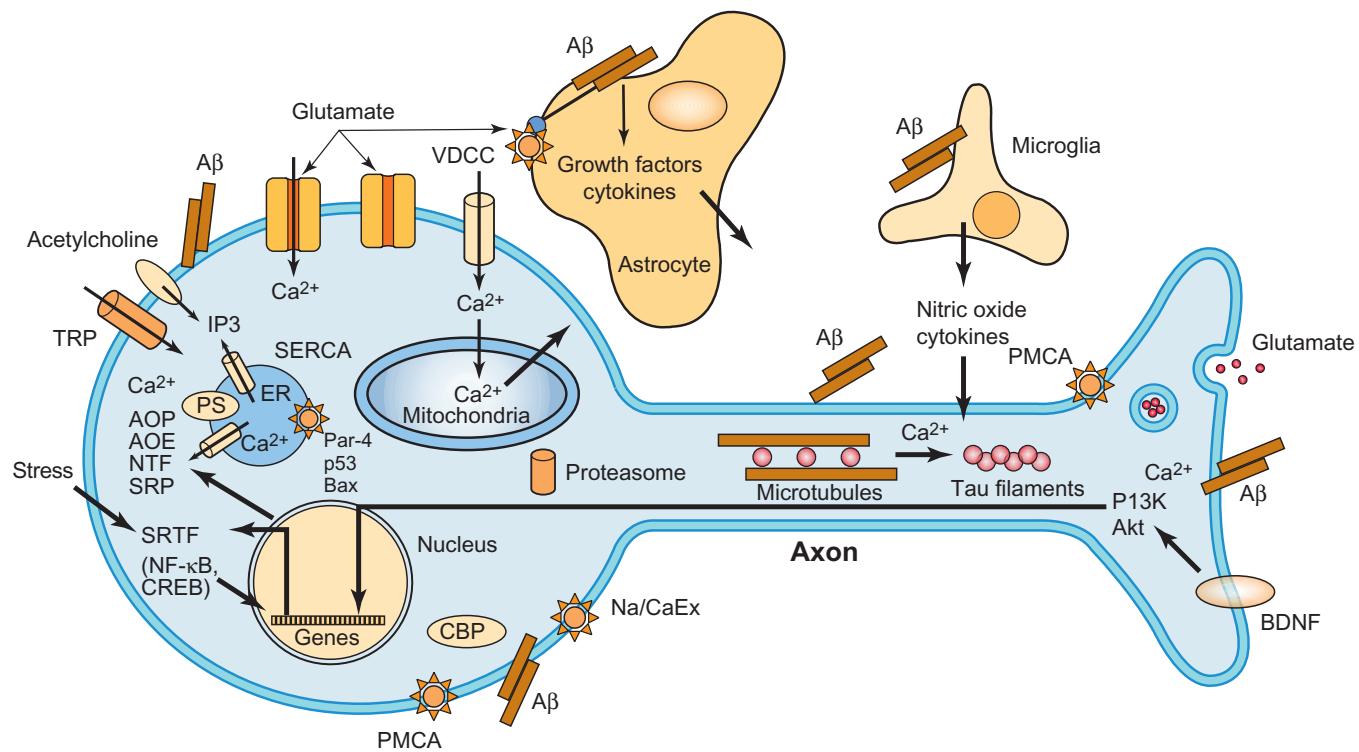


FIGURE 37-3 Examples of inter- and intracellular signaling mechanisms and biochemical cascades that can determine whether a neuron lives or dies in physiological and pathological settings. Neurons respond to a wide array of extracellular signals that can either prevent or promote cell death; examples include neuroprotective growth factors and neurotoxic amyloid β -peptide ($A\beta$). The calcium ion (Ca^{2+}) often plays important roles in determining whether neurons live or die. Excessive influx of Ca^{2+} through glutamate receptor channels, voltage-dependent Ca^{2+} channels and capacitative Ca^{2+} entry channels (TRP) in the plasma membrane, and/or excessive release of Ca^{2+} from endoplasmic reticulum (ER) and mitochondrial stores, can trigger apoptosis. On the other hand, effective removal of Ca^{2+} from the cytoplasm via the activities of plasma membrane (PMCA) and ER (SERCA) Ca^{2+} -ATPases, the plasma membrane Na^+/Ca^{2+} exchanger ($Na/CaEx$) or sequestration by Ca^{2+} -binding proteins (CBP) can prevent apoptosis. Cell death may also be triggered by oxidative stress, insufficient trophic factor support and abnormalities in the cytoskeleton, such as occur, for example, in Alzheimer's disease where microtubules depolymerize and the microtubule-associated protein tau forms abnormal aggregates. By activating transcription factors that induce the expression of cytoprotective genes, some signaling pathways can prevent cell death. For example, stress-responsive transcription factors such as NF- κ B and CREB induce the expression of antiapoptotic proteins (AOP; Bcl-2 and IAPs, for example), antioxidant enzymes (AOE), neurotrophic factors (NTF) and stress resistance proteins (SRP; HSP-70 and GRP-78, for example). Microglia may facilitate neuronal death by producing neurotoxic substances such as nitric oxide and proinflammatory cytokines.

Huntington's disease and ALS undergo apoptosis or a related form of PCD. The spatial and temporal patterns of neuronal death in these neurodegenerative disorders are consistent with apoptosis in that neurons in vulnerable brain regions do not die in unison; instead, individual neurons die on a progressive basis. Examinations of the brains of Alzheimer's disease, Parkinson's disease and Huntington's disease patients and of the spinal cords of ALS patients have revealed evidence for activation of caspases and upregulation of apoptotic proteins such as Bax, p53 and Par-4. Perhaps the strongest evidence that apoptosis is a major mode of cell death comes from studies in which mutant genes that cause disease (amyloid precursor protein and presenilin mutations in Alzheimer's disease, alpha-synuclein mutations in Parkinson's disease, huntingtin mutations in Huntington's disease and Cu/Zn-SOD mutations in ALS) have been shown to increase the vulnerability of neurons to apoptosis (Riess et al., 2003; Vila & Przedborski, 2003). Dietary modifications such as dietary restriction and folate supplementation, and/or drugs that block apoptotic pathways, have proved to be effective in reducing neuronal degeneration and improving functional outcome in animal models of Alzheimer's disease, Parkinson's disease, Huntington's disease and ALS. When taken together with epidemiological studies that have identified risk factors for Alzheimer's disease and Parkinson's disease, findings from animal studies support a role for apoptosis in these disorders. For example, dietary restriction can protect hippocampal and dopaminergic neurons in models of Alzheimer's disease and Parkinson's disease by a mechanism involving upregulation of BDNF and stress resistance proteins, and epidemiological data suggest that individuals with low calorie intakes have a reduced risk of Alzheimer's disease and Parkinson's disease (Mattson et al., 2002). Further details on these topics can be found in the following references: (Wilson & Mattson, 2007; Avila, 2010; Zhao et al., 2011; Lukiw et al., 2005).

There are many triggers of apoptosis

Hundreds of factors that can trigger apoptosis of neural cells have been described, and there are surely many others that remain to be discovered. Examples of five prominent types of apoptotic trigger are described below.

Insufficient trophic support

When developing neurons are deprived of neurotrophic support, either by withdrawal of the neurotrophic factor in cell culture or by removal of the target cells for the neurons *in vivo*, they undergo apoptosis. For example, when sympathetic neurons in culture are deprived of NGF they upregulate Bax, release cytochrome c from their mitochondria, activate caspases and exhibit cell body shrinkage and nuclear chromatin condensation and fragmentation. Depletion of neurotrophic factors may also contribute to the deaths of neurons that occur during aging and in various neurodegenerative conditions. Indeed, there is evidence that this is the case in Alzheimer's disease, Parkinson's disease and Huntington's disease.

Death receptor activation

Several different ligands can induce apoptosis of neural cells including certain cytokines (Fas ligand and

interleukin-1 β), the neurotransmitter glutamate and thrombin. Like tumor necrosis factor (TNF) receptors, Fas is coupled to downstream death effector proteins that ultimately induce caspase activation (Ch. 22). Fas and TNF receptors recruit proteins called FADD and TRADD respectively; FADD and TRADD then activate caspase-8, which, in turn, activates caspase-3 (Fig. 37-4). Calcium ion influx mediates neuronal apoptosis induced by glutamate receptor activation; calcium induces mitochondrial membrane permeability transition pore opening, release of cytochrome c and caspase activation. Interestingly, in the absence of neurotrophic factors some neurotrophic factor receptors can activate apoptotic cascades, the low-affinity NGF receptor being one example of such a death receptor mechanism.

DNA damage

Considerable evidence suggests that DNA damage may be a pivotal trigger of apoptosis in both physiological and pathological settings. Neurons undergoing developmental apoptosis exhibit DNA damage and upregulation of DNA-damage-responsive proteins. The causes of DNA damage during development have not been clearly established but may include increased oxyradical production, reduced trophic support and impaired DNA repair mechanisms. DNA damage can induce the upregulation of proapoptotic proteins including ATM kinase, p53 and PARP. Damage to telomeric DNA (located at the ends of chromosomes) may be a particularly potent trigger of apoptosis in neural progenitor cells and newly generated neurons. DNA damage has been documented in vulnerable neuronal populations in patients suffering from traumatic brain injury, stroke, Alzheimer's disease, Parkinson's disease, Huntington's disease and ALS. An increase in the amounts of oxidatively modified DNA bases in these disorders suggests a major role for oxidative stress. In addition, abnormalities in one-carbon metabolism that may be induced by homocysteine, for example, may contribute to accumulation of damaged DNA during aging and in neurodegenerative disorders.

Oxidative and metabolic stress

Studies of cell culture and animal models have clearly shown that oxidative stress and impaired energy metabolism can trigger neuronal apoptosis. START HERE For example, exposure of cultured neurons to hydrogen peroxide, Fe²⁺ or amyloid β -peptide induces membrane-associated oxidative stress and activates a mitochondrial-dependent apoptotic cascade. Oxidative stress may trigger apoptosis by activating membrane-associated apoptotic signalling cascades; for example, ceramide generated from membrane sphingomyelin in response to oxidative stress can be a potent inducer of apoptosis. Oxidative damage to various membrane transporters, mitochondrial proteins and membranes and nuclear DNA can also trigger apoptosis. The ability of metabolic compromise to induce apoptosis is evident from the fact that various mitochondrial toxins (e.g., rotenone, MPP⁺, cyanide and 3-nitropropionic acid) can trigger apoptosis. While impaired energy metabolism clearly plays a role in many neurodegenerative disorders, its role in physiological cell death remains to be established.

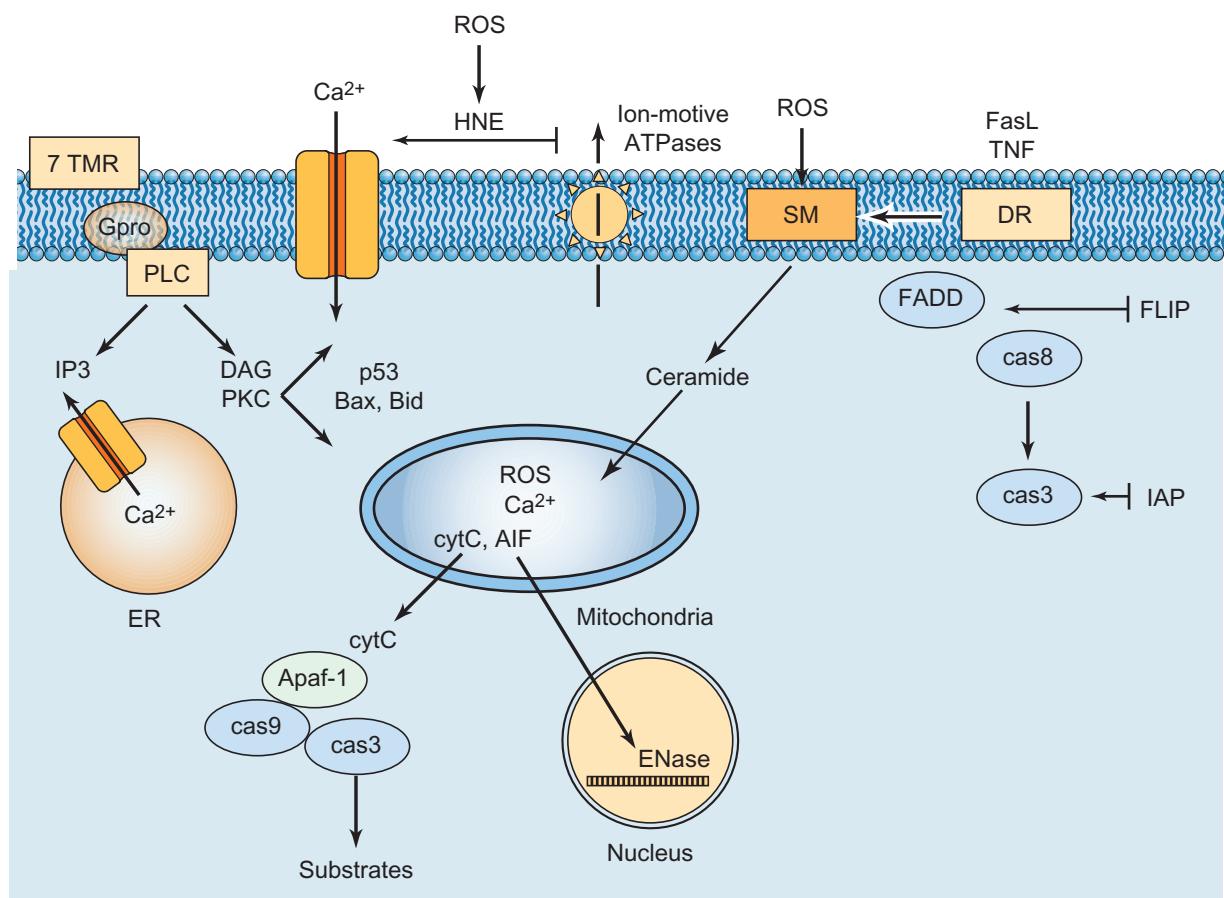


FIGURE 37-4 Examples of plasma-membrane-initiated cell death cascades. Many cells, including neurons, express so-called death receptors (DR). In the example shown, a receptor for Fas ligand (FasL) or tumor necrosis factor (TNF) binds a protein called FADD (Fas-associated death domain), which then recruits and activates caspase 8 (cas8). Caspase 8 then activates caspase 3, which plays a major role in executing the cell death process. The latter death receptor pathway can be blocked by the activities of FLIP (FLICE inhibitory protein) and IAPs (inhibitor of apoptosis proteins). Cell death can also be triggered by reactive oxygen species (ROS) that induce membrane-associated oxidative stress. Membrane lipid peroxidation generates the aldehyde 4-hydroxynonenal (HNE) which can induce apoptosis by covalently modifying various membrane proteins including ion-motive ATPases and calcium channel proteins. Membrane oxidative stress also activates sphingomyelinases, which cleave sphingomyelin (SM), resulting in the production of ceramide, which can trigger apoptosis by inducing mitochondrial membrane permeability transition. Receptors with seven transmembrane domains (7TMR) coupled to GTP-binding proteins (Gpro) and activation of phospholipase C (PLC) can trigger cell death by inducing calcium release from IP₃-sensitive ER stores. Once initiated, such cell death cascades often involve proapoptotic proteins acting at mitochondrial membranes (p53, Bax and Bid, for example), proteins released from mitochondria (cytochrome C and AIF), caspases and endonucleases (ENase).

Once apoptosis is triggered, a stereotyped sequence of premitochondrial events occurs that executes the cell death process

In many cases proteins and/or lipid mediators that induce changes in mitochondrial membrane permeability and calcium regulation are produced or activated. For example, the pro-apoptotic Bcl-2 family members Bax, Bad and Bid may associate with the mitochondrial membrane and modify its permeability. Membrane-derived lipid mediators such as ceramide and 4-hydroxynonenal can also induce mitochondrial membrane alterations that are critical for the execution of apoptosis.

Numerous mechanisms have been described that are involved in early premitochondrial steps in apoptosis (Figs. 37-3, 37-4). For example, the polymerization state of actin

filaments and microtubules can determine whether or not apoptosis is triggered by glutamate because these cytoskeletal proteins modulate the activity of ionotropic glutamate receptors and voltage-dependent calcium ion channels (Mattson, 2003). Similarly, the presence and amounts of calcium-binding proteins and antioxidants such as glutathione and vitamin E can shift the threshold for activation of the cell death cascade by different apoptotic triggers. Events occurring in the endoplasmic reticulum (ER) have been shown to induce, prevent or modify apoptotic cascades at a premitochondrial step; calcium ion release and uptake by the ER appears to be particularly important in this regard (see Chs. 3 and 24).

Apoptosis requires macromolecular synthesis. In this regard, several transcription factors and target genes have been identified as playing pivotal roles in apoptosis. For example, stress-activated protein kinases (SAPK) and c-Jun

N-terminal kinase (JNK) mediate apoptotic responses to various triggers and affect cell death by activating the transcription factors AP-1 and GADD153. Another prominent example is the transcription factor p53, which is activated in response to DNA damage and which induces the transcription of proapoptotic genes including those encoding Bax and p21. Another protein whose upregulation is required for at least some cases of apoptosis is Par-4, which can be induced at the translational level.

Several different changes in mitochondria occur during apoptosis

These include a change in membrane potential (usually depolarization), increased production of reactive oxygen species, potassium channel activation, calcium ion uptake, increased membrane permeability and release of cytochrome c and apoptosis inducing factor (AIF). Increased permeability of the mitochondrial membranes is a pivotal event in apoptosis and appears to result from the formation of pores in the membrane; the proteins that form such permeability transition pores (PTP) may include a voltage-dependent anion channel (VDAC), the adenine nucleotide translocator cyclophilin D, the peripheral benzodiazepine receptor, hexokinase and creatine kinase (Fig. 37-5). Pro-apoptotic Bcl-2 family members, such as Bax and Bid, promote PTP formation. Permeabilization of the mitochondrial membranes provides a route for the release of cytochrome c into the cytoplasm, where it binds to a protein called Apaf-1. The synchronous release of cytochrome c from all mitochondria in a cell is thought to be orchestrated by calcium ion release from IP₃-sensitive ER stores. Smac/DIABLO and Omi/HtrA2 are two other mitochondrial intermembrane proteins that are released from mitochondria during apoptosis; they then bind to and neutralize cytosolic inhibitors of apoptosis proteins (IAPs). Because IAPs normally bind and inhibit caspases 9 and 3, the binding of IAPs by Smac/DIABLO and Omi/HtrA2 enhance the activation of these caspases. Two DNA-degrading enzymes (AIF and endonuclease G) are also released from mitochondria and then translocate to the nucleus where they cleave DNA into nucleosomal fragments. AIF is a flavoprotein with an oxidoreductase domain and may, in addition to its proapoptotic function, modulate mitochondrial redox state.

The postmitochondrial events of apoptosis include activation of the caspases

Cytochrome c binds to the protein Apaf-1 in the cytosol, resulting in the recruitment and activation of caspase-9, which in turn activates caspase-3 (Figs. 37-4, 37-5). Fourteen different mammalian caspases have been identified and each may play a key role in apoptosis depending upon the cell type and the nature of the specific cell death stimulus. For example, caspases 8 and 10 can be activated by death receptor engagement, independently of mitochondria, and caspase-12 is activated in response to ER stress. Numerous caspase substrates have been identified (Table 37-1), and in several cases the consequences of cleavage of the substrate in the context of the cell death process are known. Cleavage of a protein by a caspase

may disable the protein or, in some cases, may activate the protein. Indeed, effector caspases themselves (caspases 3, 6 and 7) can be activated by cleavage of their procaspase forms by initiator caspases (caspases 8, 9 and 10). Cellular processes modified by caspase-mediated proteolysis include inactivation of antiapoptotic proteins, impairment of homeostatic and repair processes (DNA repair enzymes, for example), disruption of the cell cytoskeleton, nuclear and plasma membrane morphological changes, and marking of cells for phagocytosis.

A widely used criterion for identifying a cell as ‘apoptotic’ is nuclear chromatin condensation and fragmentation

The nature of the molecular alterations that occur in the nucleus and the proteins responsible for inducing these changes are in the process of being identified. Some caspases (caspase-6, for example) enter the nucleus and cleave nuclear matrix proteins including lamins and NuMA. Other caspases that cleave nuclear proteins involved in regulating DNA replication and repair processes include topoisomerases, PARP, DNA-dependent protein kinase and Rad51. Caspases may also activate endonucleases such as ICAD and DNases I and II, resulting in DNA fragmentation. Caspase-3 can cleave acinus resulting in chromatin condensation. Granzyme B, cathepsin B and histone-associated protease also cleave nuclear substrates during apoptosis. Other apoptotic proteins, such as DEDD and Daxx, which act in the nucleus, may influence the apoptotic process by altering gene transcription.

Dramatic morphological changes in the plasma membrane occur during apoptosis; as the cell shrinks, membrane blebs form. Individual apoptotic cells are removed from tissues via phagocytosis by macrophages/microglia. The phagocytic cells recognize the apoptotic cell because during the process of apoptosis, phosphatidylserine, which is normally present in the inner leaflet of the plasma membrane bilayer, translocates to the cell surface (see Chs. 2 and 23). The phosphatidylserine binds to a specific phosphatidylserine receptor (PSR) on the surface of the phagocytic cells. In this way, apoptotic cells are removed when their membrane is still intact, thereby preventing release of potentially damaging intracellular contents. Indeed, mice lacking the PSR accumulate dead cells, particularly in the brain and lungs. This process results in damage to adjacent living cells, and ultimately these animals cannot survive.

Cells in the nervous system possess different mechanisms to prevent apoptosis

These include intercellular signals and the pathways activated by those signals, intrinsic antiapoptotic proteins, antioxidants, protein chaperones and systems that regulate ion homeostasis (Fig. 37-6).

Neurotrophic factors, cytokines and cell adhesion molecules

A remarkable number of growth factors and cytokines have been identified that can prevent neuronal apoptosis. A short list includes NGF, bFGF, BDNF, neurotrophin-4/5, glial-cell-line-derived neurotrophic factor (GDNF), ciliary neurotrophic

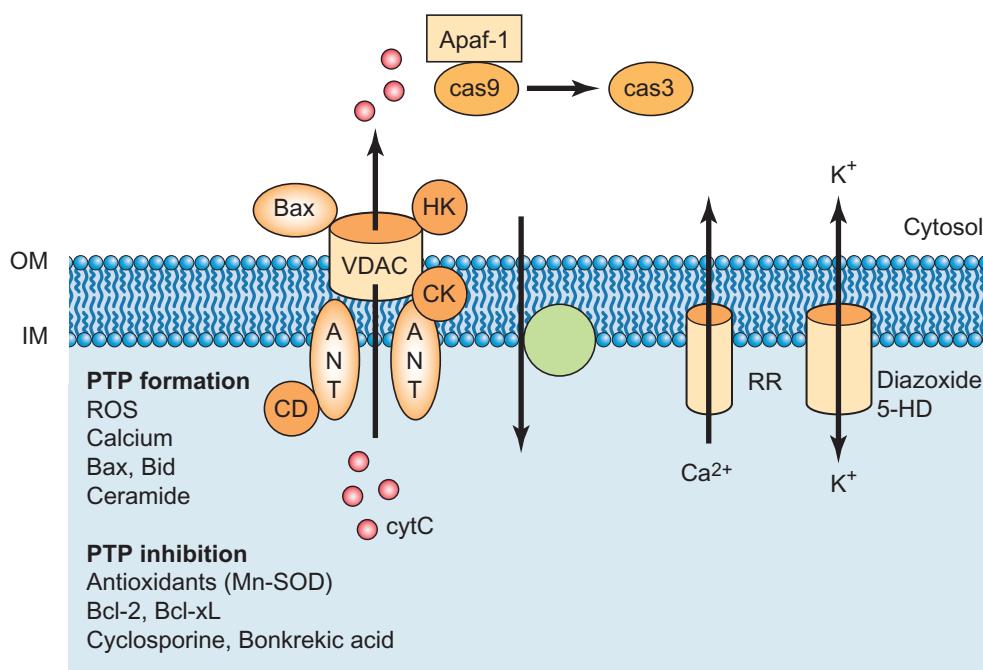


FIGURE 37-5 The presumptive mitochondrial permeability transition pore complex and its regulation by Ca^{2+} and K^+ fluxes. The membrane permeability transition pore is believed to consist of VDAC (voltage-dependent anion channel) and ANT (adenine nucleotide translocator) and associated proteins that modulate its opening including hexokinase (HK), creatine kinase (CK), cyclophilin D (CD) and Bax. Formation and opening of the channel results in the release of cytochrome C (*cytC*), which then binds to Apaf-1, resulting in the sequential activation of caspases 9 and 3. Mitochondrial membrane permeability pore formation is subject to regulation by fluxes of Ca^{2+} and K^+ . Agents that suppress Ca^{2+} flux (RR, ruthenium red) and K^+ flux (5-HD, 5-hydroxydecanoate) can prevent pore formation. Interestingly, activation of mitochondrial K^+ channels with diazoxide can also prevent apoptosis by inducing a preconditioning response.

factor (CNTF), insulin-like growth factor (IGF)-1, transforming growth factor (TGF) β , tumor necrosis factor (TNF) α , interleukin-6 and secreted forms of amyloid precursor protein. In addition to such soluble ligands, extracellular matrix (laminin, fibronectin, etc.) or cell-surface-bound ligands (NCAM, cadherins) bind to receptors that are involved in cell-substratum adhesion. The latter receptors are coupled to intracellular signaling pathways similar to those employed by growth factors. For example, engagement of integrins by laminin activates the PI3-kinase-Akt pathway, a signaling pathway also activated by BDNF and IGF-1. In general, signaling by growth factors and cell adhesion molecules results in activation of transcription factors, which activate genes that encode antiapoptotic proteins such as Bcl-2, IAPs, antioxidant enzymes and calcium-regulating proteins. However, these signaling pathways can also antagonize apoptosis by modifying the phosphorylation state of apoptosis-related proteins such as Bcl-2 family members.

Antiapoptotic proteins

There are many different intracellular proteins that can prevent apoptosis by inhibiting specific steps in the cell death process. These include Bcl-2 family members such as Bcl-2 and Bcl-xL, which can stabilize membranes (mitochondrial, ER and plasma). Bcl-2 may also have intrinsic antioxidant activity. Other proteins, IAPs such as XIAP (X-linked) and NIAP (neuronal), can directly inhibit caspases. Additional examples of antiapoptotic proteins include protease inhibitors such as calpastatin, and protein chaperones such as GRP-78 and heat shock protein (HSP)-70.

Hormesis-based mechanisms

When cells are exposed to sublethal levels of stress (heat, oxidative stress, metabolic stress or other stresses) they often become resistant to being killed by more severe stress. This process, which is a form of hormesis or 'preconditioning,' typically involves the activation of stress signaling pathways that activate transcription factors that, in turn, induce the expression of genes encoding cytoprotective proteins. Examples of antiapoptotic proteins upregulated in response to a preconditioning stress are neurotrophic factors such as BDNF and bFGF, protein chaperones such as HSP-70 and glucose-regulated protein-78, Bcl-2 and antioxidant enzymes such as Mn-superoxide dismutase (Mn-SOD). Interestingly, hormesis appears to be a major mechanism whereby mild stresses such as dietary calorie restriction, physical exercise and environmental enrichment (mental gymnastics) protect neurons against apoptosis (Mattson, 2008; Mattson, 2008; Calabrese et al., 2010).

Antioxidants and calcium-stabilizing proteins

Oxidative stress and perturbed cellular calcium homeostasis have been shown to play pivotal roles in the apoptosis of neural cells in physiological and pathological settings. Accordingly, cells possess multiple mechanisms to reduce levels of oxidative stress and to maintain and restore calcium homeostasis. Oxidative stress is suppressed by antioxidant enzymes (Cu/Zn-SOD, Mn-SOD, catalase, glutathione peroxidase) and molecules with intrinsic radical-scavenging ability

such as glutathione, bilirubin, vitamins E and C, uric acid and creatine. Proteins that stabilize calcium homeostasis include calcium-binding proteins (calbindin, calreticulin and parvalbumin, for example); calcium pumps in the plasma and ER membranes; and sodium/calcium exchangers. Interestingly, antiapoptotic proteins such as Bcl-2 may act, in part, by suppressing -oxidative stress and enhancing calcium homeostasis.

The morphological and biochemical characteristics of apoptosis are not always manifest in cells undergoing programmed cell death (PCD)

Studies have clearly shown that there are several, and perhaps many, variant forms of PCD and that there can be a continuum of cell death cascades that is related to the specific cell death trigger involved and its intensity and duration. In some cases cells exhibit characteristics of both apoptosis and necrosis (Yuan et al., 2003). A form of PCD involving autophagy occurs in many instances, including developmental apoptosis of neurons in sympathetic and isthmo-optic nuclei. Autophagy is a lysosome-mediated mechanism by which cells degrade and recycle damaged proteins and organelles (see Lysosomes in Ch. 43). Autophagy is characterized by the formation of cytoplasmic vacuoles with double membranes that enclose fragments of mitochondria and ER and contain high concentrations of lysosomal hydrolases. Autophagic cell death is often a variant of apoptosis in which caspase activation plays a pivotal role. Proteins that may play a role in autophagic PCD include Beclin1, PI3 kinase, TOR (target of rapamycin), p70S6 kinase, AMPA receptors and Bcl-2. There is evidence that at least some neurons undergo autophagic cell death in Parkinson's, Huntington's and Alzheimer's diseases.

Neuronal PCD with features of necrosis was reported in studies of naturally occurring motor neuron death in which the cells exhibited dilation of ER, Golgi and nuclear membranes but not condensation of chromatin. A similar form of necrotic PCD may occur in response to Fas activation; such cell deaths involve swelling of ER and mitochondria but cytochrome c is not released. Perturbed ER calcium homeostasis, sustained increases in cytoplasmic calcium ion concentrations and activation of calpains can trigger necrotic PCD in some types of cells.

Apoptotic cascades can be triggered, and pre- and postmitochondrial events can occur, without the cell dying

For example, studies have shown that apoptotic cascades involving Par-4, p53, mitochondrial membrane permeabilization and caspase activation can be induced locally in synaptosomes and in growth cones and neurites of cultured neurons. Such cascades can be triggered locally by activation of glutamate receptors and can occur in a reversible manner. Interestingly, the cleavage of certain synaptic substrate proteins by caspases (AMPA receptor subunits and cytoskeletal proteins, for example) may play roles in synaptic plasticity (learning and memory) and perhaps in remodeling of synaptic structure and the regulation of growth cone motility (Fig. 37-7).

NECROSIS

Necrosis is a dramatic and very rapid form of cell death in which essentially every compartment of the cell disintegrates

Necrosis is characterized by marked dysregulation of ion homeostasis resulting in cell swelling, dilation of mitochondria and the ER and the formation of vacuoles in the cytoplasm. Proteases play important roles in the degradation of cells during necrosis. In contrast to apoptosis, where caspases are the key death proteases, calpains and lysosomal proteases (cathepsins B and D, in particular) are major players in necrosis. Caspases may be activated in response to mitochondrial damage and cytochrome c release during necrosis but appear not to be essential for cell death. During the cell death process the chromatin clumps and the nuclear membrane is disrupted. Finally, the cell lyses, releasing its contents into the extracellular compartment, where the contents may damage neighboring cells and induce an inflammatory response. Transcription of genes and protein synthesis stop and ATP is rapidly depleted in cells undergoing necrosis.

There are few cell death triggers that are only capable of inducing either apoptosis or necrosis

Instead, whether a cell undergoes apoptosis or necrosis is usually determined by the intensity and/or duration of the death-inducing stimulus. In general, severe and/or sustained insults trigger necrosis whereas less severe transient stresses induce apoptosis. For example, moderate over-activation of glutamate receptors may trigger apoptosis while more intense and sustained activation of glutamate receptors induces excitotoxic necrosis (Chs. 17 and 35). The environment of the cell at the time it is subjected to the death stimulus can also determine the mode of death. Thus, a certain level of glutamate receptor activation may induce apoptosis in a cell receiving normal amounts of oxygen and glucose but may cause necrosis when that same cell is subjected to ischemia.

Trauma

Acute trauma as in head injury (e.g., automobile accidents, sports injuries) can induce necrosis of the tissue at and surrounding the site of the trauma. Physical damage to cellular membranes may induce necrosis in the traumatized tissue, while disruption of ion homeostasis and energy depletion may trigger necrosis in cells adjacent to the directly damaged cells. Traumatized cells release the contents of their organelles into the extracellular space, resulting in exposure of adjacent cells to various lysosomal proteases and to acidosis. Marked changes in pH can also trigger necrosis; interestingly, intracellular acidification may contribute to necrotic cell death induced by extensive DNA damage.

Energy failure/ischemia

Cellular energy failure is one defining feature of necrosis, and severe reduction in glucose and/or oxygen availability is sufficient to trigger necrosis. Mitochondrial toxins can trigger

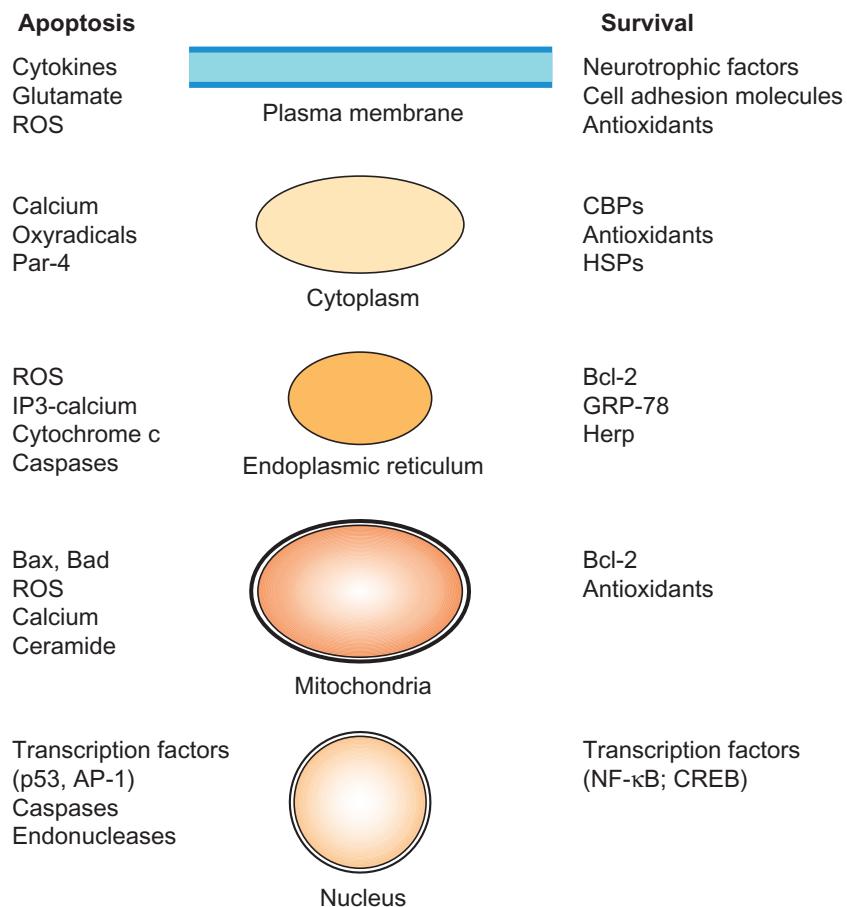


FIGURE 37-6 Examples of apoptotic and antiapoptotic mechanisms that act on or within different subcellular compartments. CBP, Ca^{2+} binding protein; CREB, cyclic AMP response element binding protein; HSP, heat shock protein; IP3, inositol 1,4,5-trisphosphate; ROS, reactive oxygen species.

apoptosis at low levels but at higher levels they induce necrosis as the result of severe depletion of ATP.

Excitotoxicity

Overactivation of glutamate receptors, particularly when neurons are subjected to metabolic and oxidative stress, can trigger excitotoxic necrosis. Sodium ion influx through AMPA/kainate receptors and voltage-dependent sodium channels induces cell swelling, and calcium ion influx through NMDA receptors and voltage-dependent calcium channels activates various proteases (calpains, for example) that degrade various structural and metabolic proteins.

Calcium ion release from the ER and the accumulation of misfolded proteins in the ER can trigger necrosis, and agents that inhibit such calcium release can protect cells against necrosis.

the preventative and therapeutic potential of antiapoptotic approaches (Mattson, 2000; Waldmeier, 2003). The most effective means of preventing apoptosis is to abolish the triggering event. Examples include dietary and drug treatments that suppress atherosclerosis and blood clot formation to prevent a stroke from occurring; eliminating exposure to neurotoxins that may cause Parkinson's disease; blocking the production of amyloid β -peptide to prevent Alzheimer's disease; and wearing a safety helmet when riding a motorcycle to prevent traumatic brain injury. Once the initiating events in the neurodegenerative process have been triggered, specific early events in the apoptotic cascade can be targeted. Examples include antagonists of glutamate receptor or calcium channels to prevent excitotoxic apoptosis; drugs that block production of, or receptors for, apoptotic cytokines; antioxidants such as vitamin E and glutathione; and agents such as PARP and p53 inhibitors that block DNA damage response pathways. Drugs that modulate the cytoskeleton, such as cytochalasins and taxol, have also proved to be effective in experimental models of neuronal apoptosis. Targets further downstream of the triggering events have also been successfully blocked to prevent apoptosis. Among such agents are cyclosporin, which blocks mitochondrial membrane permeability transition pores; dantrolene, which blocks ER calcium channels; and caspase inhibitors.

TARGETING APOPTOSIS AND NECROSIS IN NEUROLOGICAL DISORDERS

Studies of cell culture and animal models of neurodegenerative conditions that involve apoptosis have established

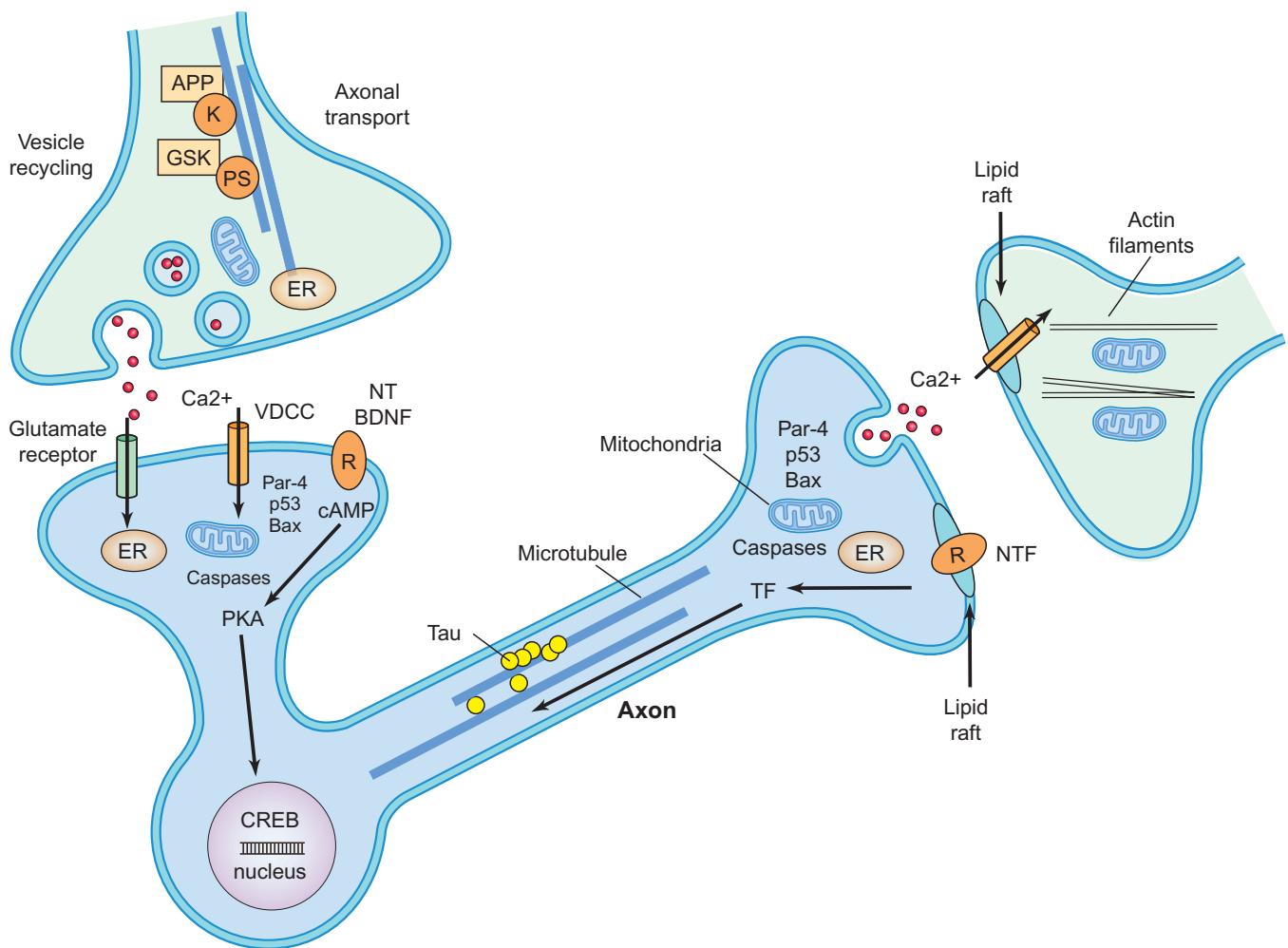


FIGURE 37-7 Apoptotic and anti-apoptotic synaptic signaling mechanisms. Synapses are sites where various signal transduction pathways are activated including those of neurotransmitters (NT; glutamate receptors linked to calcium influx and receptors coupled to cAMP production are shown), and neurotrophic factors (NTF). Synapses contain all the major organelles (except the nucleus) and proteins involved in apoptosis including *Bcl-2* family members, p53, Par-4, mitochondria and ER, and caspases. Alterations in axonal transport may also trigger apoptosis. APP, amyloid precursor protein; CREB, cyclic AMP response element binding protein; ER, endoplasmic reticulum; GSK, glycogen synthase kinase; K, kinesin; PKA, protein kinase A; PS, presenilin; R, receptor; TF, transcription factor; VDCC, voltage-dependent calcium channel.

Additional evolving mechanisms in life-and-death decisions in the nervous system include autophagy; micro RNA as therapeutic targets; endoplasmic reticulum stress; intercellular transfer of proteins (e.g., α -synuclein, prions); and microglial cell death (Nijholt et al., 2011; Steiner et al., 2011; Yun et al., 2011; Xilouri & Stefanis, 2010; Banerjee et al., 2010; Alvarez-Erviti et al., 2010; Heng et al., 2010; Santos et al., 2010; Bishop et al., 2010; Hutchison et al., 2009). Moreover, *in vivo* imaging of some of these events, such as autophagy in experimental stroke (Tian et al., 2010), are opening a new avenue of exploration for understanding and harnessing the potential of targeting apoptotic signaling as possible therapeutic strategies.

An alternative (or complementary) strategy for preventing apoptosis is to activate antiapoptotic pathways. This might be accomplished by administering a growth factor (bFGF, BDNF, GDNF or others) that activates a signaling pathway, which in turn induces the expression of *Bcl-2*, antioxidant enzymes or IAPs, for example. Mild preconditioning stimuli

that upregulate stress resistance proteins and neurotrophic factor signaling can prevent neuronal apoptosis in models of Alzheimer's disease, Parkinson's disease, Huntington's disease and stroke. Examples of such hormetic stimuli include dietary calorie restriction, environmental enrichment and physical exercise (Mattson et al., 2002).

In regards to necrosis, it is clear that the old adage 'an ounce of prevention is worth a pound of cure' applies. Agents that stabilize ion homeostasis have proved to be effective in preventing necrosis in cell culture studies. For example, drugs that activate plasma membrane potassium ion channels or chloride ion channels can prevent membrane depolarization and so inhibit sodium and calcium ion influx. Agents that prevent large sustained increases in intracellular free calcium levels can also prevent neuronal necrosis; examples include the calcium chelator BAPTA and dantrolene, which block calcium release from the ER. Calpain inhibitors can prevent necrosis, as can inhibitors of lysosomal proteases. Treatments that promote maintenance of

LAST STOP, CELL DEATH: CASPASES AND SYNAPTIC PLASTICITY

Scott T. Brady

Discussions of cell death in the nervous system, whether the cell death occurs as part of neuronal development, trauma, or a neurodegenerative disease, naturally focus on the final stages of somal death. Through these observations, we learn that the final common steps in cell death pathways (i.e., nuclear fragmentation, etc.) are generally shared between neuronal and nonneuronal cells (D'Amelio et al., 2010). Depending on the triggering event, both extrinsic and mitochondrial-mediated apoptotic cell death may occur (Mattson & Bazan 2006). Activation of cell death pathways in the cell soma initiates a standard apoptotic sequence, including nuclear fragmentation and disruption of translational machinery (see main text). However, the size and shape of neurons means that pre- and postsynaptic specializations may be located at some distance from the cell soma. Neuronal cell death may take considerably longer to complete than apoptosis. Final stages of apoptotic cell death are similarly rapid in both neuronal and nonneuronal cells, but if the first steps leading to cell death begin in the distal axon and presynaptic terminals, months or even years may elapse between the first decrements in neuronal function and initiation of a final apoptotic cascade (Brady & Morfini, 2010).

The ways in which cell death pathways are managed in neurons differ from nonneuronal cells. The complex architecture of neurons adds layers of complexity that are seen both during development and in pathological states. In development, programmed cell death plays a critical role in establishing functional connections in the nervous system by assuring that appropriate matches exist between neurons and target cells (Raff et al., 2002). Activation of cell death signaling components during development is typically initiated in synaptic and axonal compartments. If these steps are limited in scope and extent, synaptic and axonal activation of apoptotic signaling leads to pruning of nonproductive synaptic contacts or axonal branches (Raff et al., 2002). Atrophy of an axonal branch follows loss of synaptic function and degeneration of the presynaptic terminal. However, loss of too many connections triggers apoptosis in the cell body. An analogous loss of synaptic function and subsequent axonopathy is seen in many adult-onset neurodegenerative diseases, producing a classic dying-back neuropathy (Morfini et al., 2009).

The distance of synaptic specializations from the cell soma allows caspases and other components of cell death signaling

pathways to play roles in neurons unrelated to apoptosis (D'Amelio et al., 2010). If an executioner caspase is activated in a pre- or postsynaptic compartment, apoptosis does not follow as a matter of course. Instead, caspases may regulate aspects of axon growth, synaptic function and plasticity. Activation of caspase may occur in the growth cones of developing axons (McLaughlin, 2004) and during remodeling of synapses associated with learning and memory (D'Amelio et al., 2010). The separation between distal axon and soma prevents the interaction of caspases with key downstream targets required for the normal progression of apoptosis. Instead, the caspases may act on cytoskeletal proteins, kinases and other signaling molecules (Chan & Mattson, 1999; Morfini et al., 2009). As a result, activation of neuronal caspases affects hippocampal synaptic plasticity and long-term potentiation and song response habituation in zebra finches, as well as neuronal apoptosis (D'Amelio et al., 2010).

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cellular ATP levels, such as pyruvate and uridine, and creatine can also prevent necrosis of neurons under some conditions.

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