CHAPTER 18

Cell Death

The growth, development, and maintenance of multicellular organisms depend not only on the production of cells but also on mechanisms to destroy them. The maintenance of tissue size, for example, requires that cells die at the same rate as they are produced. During development, carefully orchestrated patterns of cell death help determine the size and shape of limbs and other tissues. Cells also die when they become damaged or infected, ensuring that they are removed before they threaten the health of the organism. In these and most other cases, cell death is not a random process but occurs by a programmed sequence of molecular events, in which the cell systematically destroys itself from within and is then eaten by other cells, leaving no trace. In most cases, this **programmed cell death** occurs by a process called **apoptosis**—from the Greek word meaning "falling off," as leaves from a tree.

Cells dying by apoptosis undergo characteristic morphological changes. They shrink and condense, the cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear chromatin condenses and breaks up into fragments (**Figure 18–1**A). The cell surface often bulges outward and, if the cell is large, it breaks up into membrane-enclosed fragments called *apoptotic bodies*. The surface of the cell or apoptotic bodies becomes chemically altered, so that a neighboring cell or a macrophage (a specialized phagocytic cell, discussed in Chapter 22) rapidly engulfs them, before they can spill their contents (Figure 18–1B). In this way, the cell dies neatly and is rapidly cleared away, without causing a damaging inflammatory response. Because the cells are eaten and digested so quickly, there are usually few dead cells to be seen, even when large numbers of cells have died by apoptosis. This is probably why biologists overlooked apoptosis for many years and still might underestimate its extent.

In contrast to apoptosis, animal cells that die in response to an acute insult, such as trauma or a lack of blood supply, usually do so by a process called *cell necrosis*. Necrotic cells swell and burst, spilling their contents over their neighbors and eliciting an inflammatory response (Figure 18–1C). In most cases, necrosis is likely to be caused by energy depletion, which leads to metabolic defects and loss of the ionic gradients that normally exist across the cell membrane. One form of necrosis, called *necroptosis*, is a form of programmed cell death that is triggered by a specific regulatory signal from other cells, although we are only just beginning to understand the underlying mechanisms.

Some form of programmed cell death occurs in many organisms, but apoptosis is found primarily in animals. This chapter focuses on the major functions of apoptosis, its mechanism and regulation, and how excessive or insufficient apoptosis can contribute to human disease.

Apoptosis Eliminates Unwanted Cells

The amount of apoptotic cell death that occurs in developing and adult animal tissues is astonishing. In the developing vertebrate nervous system, for example, more than half of many types of nerve cells normally die soon after they are formed. It seems remarkably wasteful for so many cells to die, especially as the vast majority are perfectly healthy at the time they kill themselves. What purposes does this massive cell death serve?

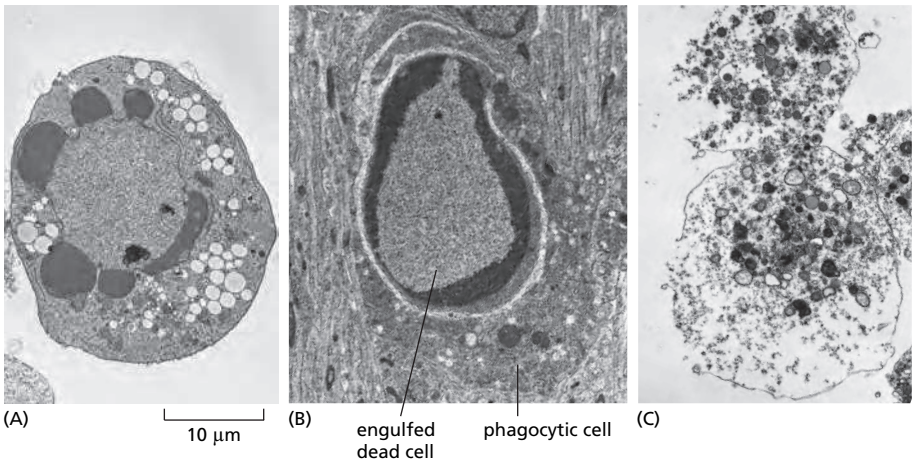


Figure 18–1 Two distinct forms of cell death. These electron micrographs show cells that have died by apoptosis (A and B) or by necrosis (C). The cells in (A) and (C) died in a culture dish, whereas the cell in (B) died in a developing tissue and has been engulfed by a phagocytic cell. Note that the cells in (A) and (B) have condensed but seem relatively intact, whereas the cell in (C) seems to have exploded. The large vacuoles visible in the cytoplasm of the cell in (A) are a variable feature of apoptosis. (Courtesy of Julia Burne.)

In some cases, the answer is clear. Cell death helps sculpt hands and feet during embryonic development: they start out as spade-like structures, and the individual digits separate only as the cells between them die, as illustrated for a mouse paw in **Figure 18–2**. In other cases, cells die when the structure they form is no longer needed. When a tadpole changes into a frog at metamorphosis, the cells in the tail die, and the tail, which is not needed in the frog, disappears. Apoptosis also functions as a quality-control process in development, eliminating cells that are abnormal, misplaced, nonfunctional, or potentially dangerous to the animal. Striking examples occur in the vertebrate adaptive immune system, where apoptosis eliminates developing T and B lymphocytes that either fail to produce potentially useful antigen-specific receptors or produce self-reactive receptors that make the cells potentially dangerous (discussed in Chapter 24); it also eliminates most of the lymphocytes activated by an infection, after they have helped destroy the responsible microbes.

In adult tissues that are neither growing nor shrinking, cell death and cell division must be tightly regulated to ensure that they are exactly in balance. If part of the liver is removed in an adult rat, for example, liver cell proliferation increases to make up the loss. Conversely, if a rat is treated with the drug phenobarbital—which stimulates liver cell division (and thereby liver enlargement)—and then the phenobarbital treatment is stopped, apoptosis in the liver greatly increases until the liver has returned to its original size, usually within a week or so. Thus, the liver is kept at a constant size through the regulation of both the cell death rate and the cell birth rate. The control mechanisms responsible for such regulation are largely unknown.

Animal cells can recognize damage in their various organelles and, if the damage is great enough, they can kill themselves by undergoing apoptosis. An important example is DNA damage, which can produce cancer-promoting mutations if not repaired. Cells have various ways of detecting DNA damage, and undergo apoptosis if they cannot repair it.

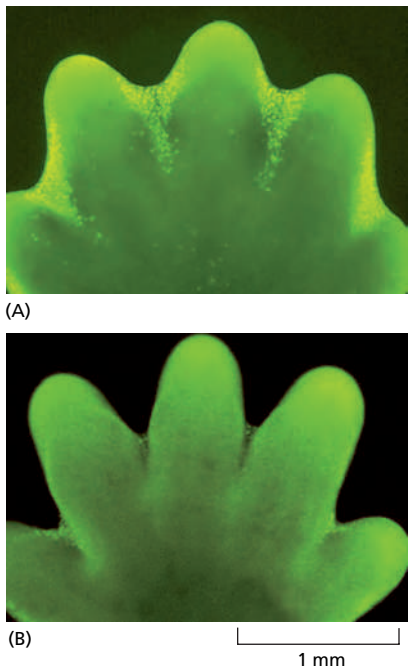


Figure 18–2 Sculpting the digits in the developing mouse paw by apoptosis. (A) The paw in this mouse fetus has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as *bright green dots* between the developing digits. (B) The interdigital cell death has eliminated the tissue between the developing digits, as seen one day later, when there are very few apoptotic cells. (From W. Wood et al., *Development* 127:5245–5252, 2000. With permission from The Company of Biologists.)

Apoptosis Depends on an Intracellular Proteolytic Cascade That Is Mediated by Caspases

Apoptosis is triggered by members of a family of specialized intracellular proteases, which cleave specific sequences in numerous proteins inside the cell, thereby bringing about the dramatic changes that lead to cell death and engulfment. These proteases have a cysteine at their active site and cleave their target proteins at specific aspartic acids; they are therefore called **caspases** (c for cysteine and asp for aspartic acid). Caspases are synthesized in the cell as inactive precursors and are activated only during apoptosis. There are two major classes of apoptotic caspases: *initiator* caspases and *executioner* caspases.

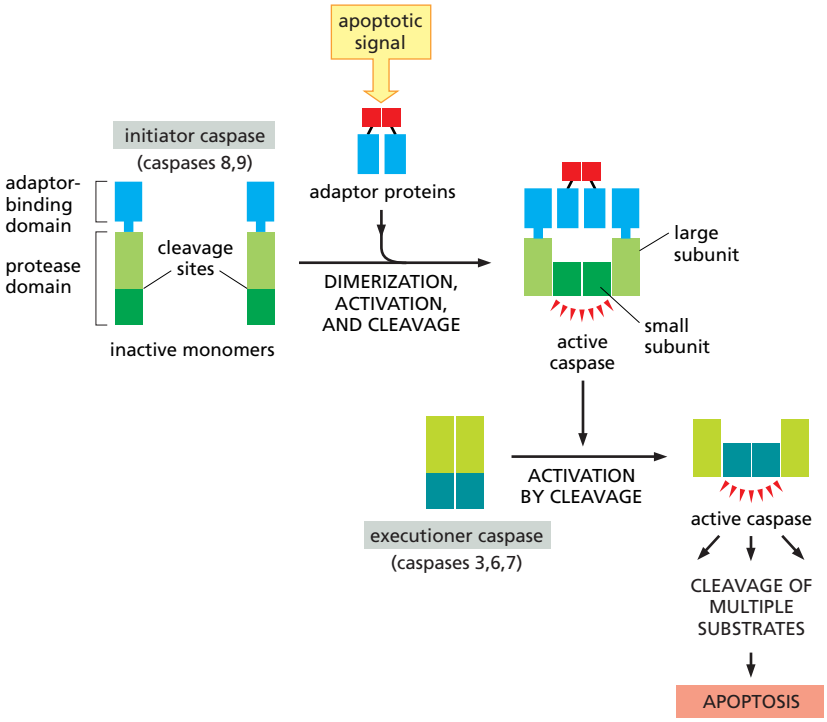


Figure 18–3 Caspase activation during apoptosis. An initiator caspase contains a protease domain in its carboxy-terminal region and a small protein interaction domain near its amino terminus. It is initially made in an inactive, monomeric form, sometimes called procaspase. Apoptotic signals trigger the assembly of adaptor proteins carrying multiple binding sites for the caspase amino-terminal domain. Upon binding to the adaptor proteins, the initiator caspases dimerize and are thereby activated, leading to cleavage of a specific site in their protease domains. Each protease domain is then rearranged into a large and small subunit. In some cases (not shown), the adaptor-binding domain of the initiator caspase is also cleaved (see Figure 18–5). Executioner caspases are initially formed as inactive dimers. Upon cleavage at a site in the protease domain by an initiator caspase, the executioner caspase dimer undergoes an activating conformational change. The executioner caspases then cleave a variety of key proteins, leading to the controlled death of the cell.

Initiator caspases, as their name implies, begin the apoptotic process. They normally exist as inactive, soluble monomers in the cytosol. An apoptotic signal triggers the assembly of large protein platforms that bring multiple initiator caspases together into large complexes. Within these complexes, pairs of caspases associate to form dimers, resulting in protease activation (Figure 18–3). Each caspase in the dimer then cleaves its partner at a specific site in the protease domain, which stabilizes the active complex and is required for the proper function of the enzyme in the cell.

The major function of the initiator caspases is to activate the **executioner caspases**. These normally exist as inactive dimers. When they are cleaved by an initiator caspase at a site in the protease domain, the active site is rearranged from an inactive to an active conformation. One initiator caspase complex can activate many executioner caspases, resulting in an amplifying proteolytic cascade. Once activated, executioner caspases catalyze the widespread protein cleavage events that kill the cell.

Various experimental approaches have led to the identification of over a thousand proteins that are cleaved by caspases during apoptosis. Only a few of these proteins have been studied in any detail. These include the nuclear lamins, the cleavage of which causes the irreversible breakdown of the nuclear lamina (discussed in Chapter 12). Another target is a protein that normally holds a DNA-degrading endonuclease in an inactive form; its cleavage frees the endonuclease to cut up the DNA in the cell nucleus (Figure 18–4). Other target proteins include components of the cytoskeleton and cell–cell adhesion proteins that attach cells to their neighbors; the cleavage of these proteins helps the apoptotic cell to round up and detach from its neighbors, making it easier for a neighboring cell to engulf it, or, in the case of an epithelial cell, for the neighbors to extrude the apoptotic cell from the cell sheet. The caspase cascade is not only destructive and self-amplifying but also irreversible, so that once a cell starts out along the path to destruction, it cannot turn back.

How is the initiator caspase first activated in response to an apoptotic signal? The two best-understood activation mechanisms in mammalian cells are called the *extrinsic pathway* and the *intrinsic*, or *mitochondrial, pathway*. Each uses its own initiator caspase and activation system, as we now discuss.

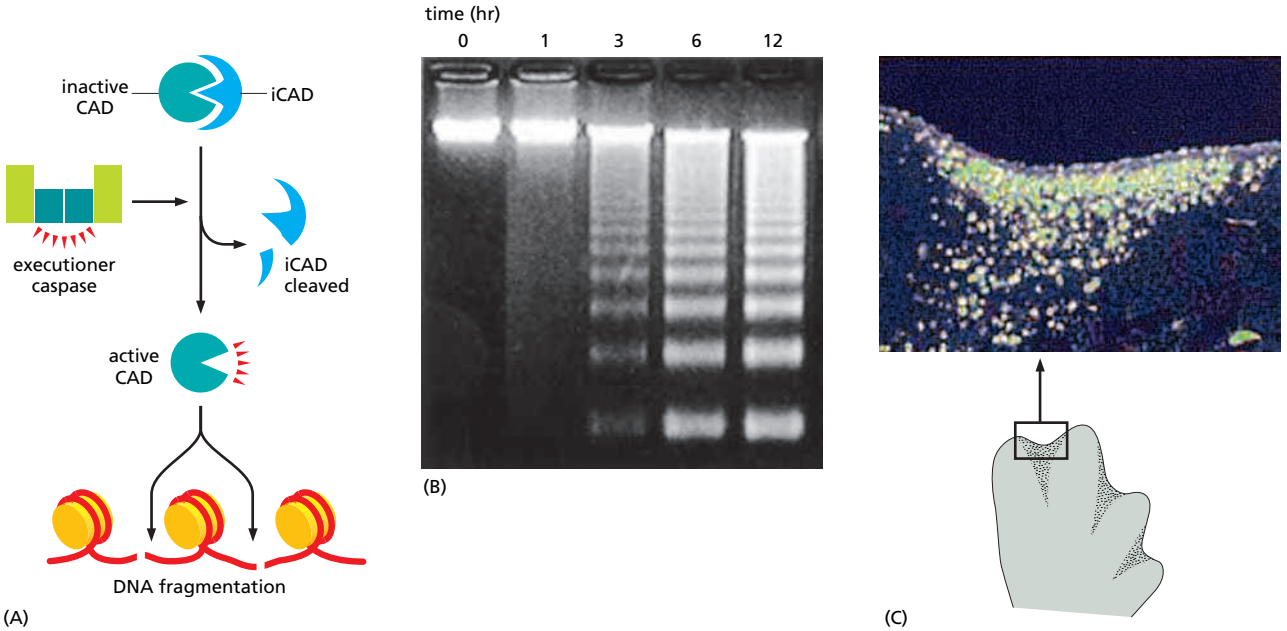
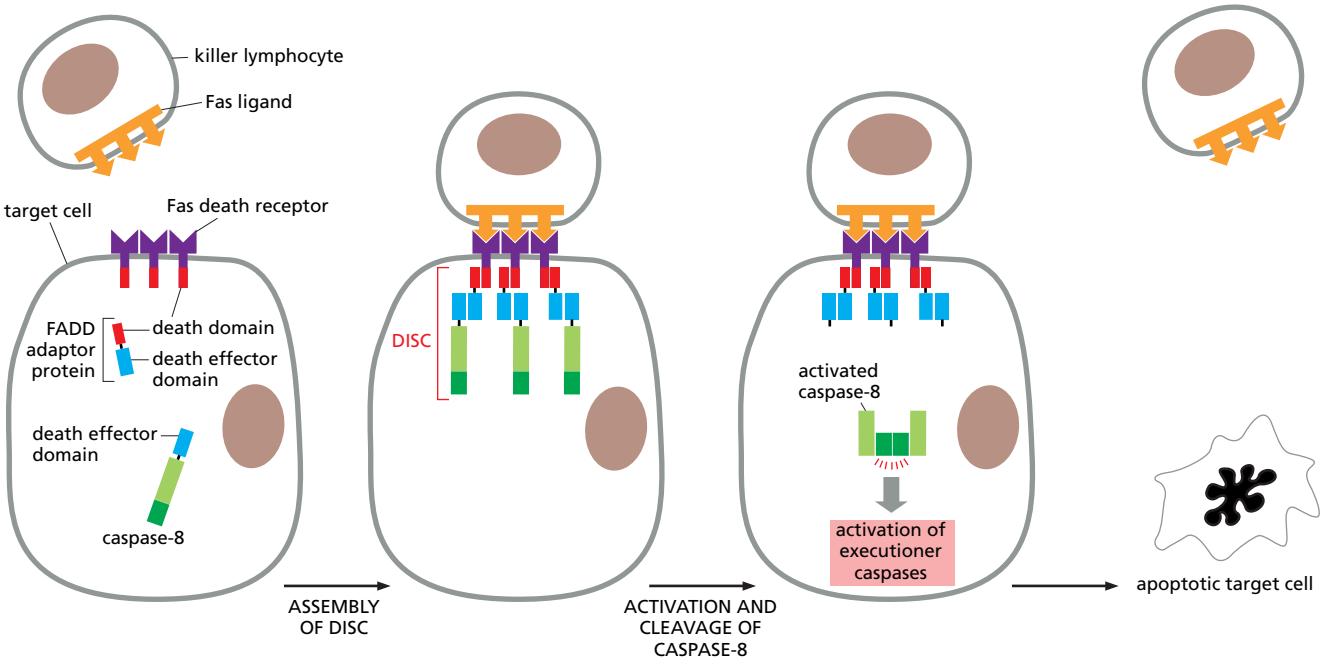


Figure 18–4 DNA fragmentation during apoptosis. (A) In healthy cells, the endonuclease CAD associates with its inhibitor, iCAD. Activation of executioner caspases in the cell leads to cleavage of iCAD, which unleashes the nuclease. Activated CAD cuts the chromosomal DNA between nucleosomes, resulting in the production of DNA fragments that form a ladder pattern (see B) upon gel electrophoresis. (B) Mouse thymus lymphocytes were treated with an antibody against the cell-surface death receptor Fas (discussed in the text), inducing the cells to undergo apoptosis. DNA was extracted at the times indicated above the figure, and the fragments were separated by size by electrophoresis in an agarose gel and stained with ethidium bromide. Because the cleavages occur in the linker regions between nucleosomes, the fragments separate into a characteristic ladder pattern on these gels. Note that in gel electrophoresis, smaller molecules are more widely separated in the lower part of the gel, so that removal of a single nucleosome has a greater apparent effect on their gel mobility. (C) Apoptotic nuclei can be detected using a technique that adds a fluorescent label to DNA ends. In the image shown here, this technique was used in a tissue section of a developing chick leg bud; this cross section through the skin and underlying tissue is from a region between two developing digits, as indicated in the underlying drawing. The procedure is called the TUNEL (TdT-mediated dUTP nick end labeling) technique because the enzyme terminal deoxynucleotidyl transferase (TdT) adds chains of labeled deoxynucleotide (dUTP) to the 3′-OH ends of DNA fragments. The presence of large numbers of DNA fragments therefore results in bright fluorescent dots in apoptotic cells. (B, from D. McIlroy et al., *Genes Dev.* 14:549–558, 2000. With permission from Cold Spring Harbor Laboratory Press; C, from V. Zuzarte-Luís and J.M. Hurlé, *Int. J. Dev. Biol.* 46:871–876, 2002. With permission from UBC Press.)

Cell-Surface Death Receptors Activate the Extrinsic Pathway of Apoptosis

Extracellular signal proteins binding to cell-surface **death receptors** trigger the **extrinsic pathway** of apoptosis. Death receptors are transmembrane proteins containing an extracellular ligand-binding domain, a single transmembrane domain, and an intracellular *death domain*, which is required for the receptors to activate the apoptotic program. The receptors are homotrimers and belong to the *tumor necrosis factor (TNF) receptor* family, which includes a receptor for TNF itself and the *Fas* death receptor. The ligands that activate the death receptors are also homotrimers; they are structurally related to one another and belong to the *TNF family* of signal proteins.

A well-understood example of how death receptors trigger the extrinsic pathway of apoptosis is the activation of **Fas** on the surface of a target cell by **Fas ligand** on the surface of a killer (cytotoxic) lymphocyte. When activated by the binding of Fas ligand, the death domains on the cytosolic tails of the Fas death receptors bind intracellular adaptor proteins, which in turn bind initiator caspases (primarily caspase-8), forming a **death-inducing signaling complex** (**DISC**). Once dimerized and activated in the DISC, the initiator caspases cleave their partners and then activate downstream executioner caspases to induce apoptosis (**Figure 18–5**). In some cells, the extrinsic pathway recruits the intrinsic apoptotic pathway to amplify the caspase cascade and kill the cell.



Many cells produce inhibitory proteins that act to restrain the extrinsic pathway. For example, some cells produce the protein *FLIP*, which resembles an initiator caspase but has no protease activity because it lacks the key cysteine in its active site. FLIP dimerizes with caspase-8 in the DISC; although caspase-8 appears to be active in these heterodimers, it is not cleaved at the site required for its stable activation, and the apoptotic signal is blocked. Such inhibitory mechanisms help prevent the inappropriate activation of the extrinsic pathway of apoptosis.

The Intrinsic Pathway of Apoptosis Depends on Mitochondria

Cells can also activate their apoptosis program from inside the cell, often in response to stresses, such as DNA damage, or in response to developmental signals. In vertebrate cells, these responses are governed by the **intrinsic**, or **mitochondrial**, **pathway** of apoptosis, which depends on the release into the cytosol of mitochondrial proteins that normally reside in the intermembrane space of these organelles (see Figure 12–19). Some of the released proteins activate a caspase proteolytic cascade in the cytoplasm, leading to apoptosis.

A key protein in the intrinsic pathway is **cytochrome *c***, a water-soluble component of the mitochondrial electron-transport chain. When released into the cytosol (**Figure 18–6**), it takes on a new function: it binds to an adaptor protein called **Apaf1** (*apoptotic protease activating factor-1*), causing the Apaf1 to oligomerize into a wheel-like heptamer called an **apoptosome**. The Apaf1 proteins in the apoptosome then recruit initiator caspase-9 proteins, which are thought to be activated by proximity in the apoptosome, just as caspase-8 is activated in the DISC. The activated caspase-9 molecules then activate downstream executioner caspases to induce apoptosis (**Figure 18–7**).

Bcl2 Proteins Regulate the Intrinsic Pathway of Apoptosis

The intrinsic pathway of apoptosis is tightly regulated to ensure that cells kill themselves only when it is appropriate. A major class of intracellular regulators of the intrinsic pathway is the **Bcl2 family** of proteins, which, like the caspase family, has been conserved in evolution from worms to humans; a human Bcl2 protein, for example, can suppress apoptosis when expressed in the worm *Caenorhabditis elegans*.

Figure 18–5 The extrinsic pathway of apoptosis activated through Fas death receptors. Trimeric Fas ligands on the surface of a killer lymphocyte interact with trimeric Fas receptors on the surface of the target cell, leading to clustering of several ligand-bound receptor trimers (only one trimer is shown here for clarity). Receptor clustering activates death domains on the receptor tails, which interact with similar domains on the adaptor protein FADD (FADD stands for Fas-associated death domain). Each FADD protein then recruits an initiator caspase (caspase-8) via a death effector domain on both FADD and the caspase, forming a death-inducing signaling complex (DISC). Within the DISC, two adjacent initiator caspases interact and cleave one another to form an activated protease dimer, which then cleaves itself in the region linking the protease to the death effector domain. This stabilizes and releases the active caspase dimer into the cytosol, where it activates executioner caspases by cleaving them.

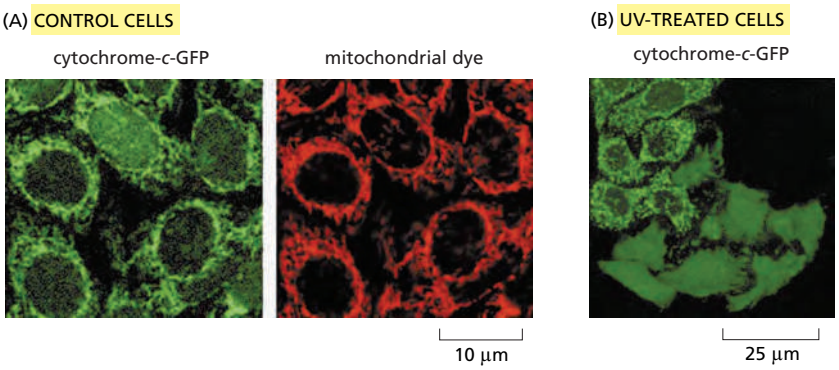


Figure 18–6 Release of cytochrome *c* from mitochondria in the intrinsic pathway of apoptosis. Fluorescence micrographs of human cancer cells in culture. (A) The control cells were transfected with a gene encoding a fusion protein consisting of cytochrome *c* linked to green fluorescent protein (cytochrome-*c*-GFP); they were also treated with a red dye that accumulates in mitochondria. The overlapping distribution of the *green* and *red* indicates that the cytochrome-*c*-GFP is located in mitochondria. (B) Cells expressing cytochrome-*c*-GFP were irradiated with ultraviolet (UV) light to induce the intrinsic pathway of apoptosis and were photographed 5 hours later. The six cells in the bottom half of this micrograph have released their cytochrome *c* from mitochondria into the cytosol, whereas the cells in the upper half of the micrograph have not yet done so (Movie 18.1). (From J.C. Goldstein et al., *Nat. Cell Biol.* 2:156–162, 2000. With permission from Macmillan Publishers Ltd.)

Mammalian Bcl2 family proteins regulate the intrinsic pathway of apoptosis mainly by controlling the release of cytochrome *c* and other intermembrane mitochondrial proteins into the cytosol. Some Bcl2 family proteins are *pro-apoptotic* and promote apoptosis by enhancing the release, whereas others are *anti-apoptotic* and inhibit apoptosis by blocking the release. The pro-apoptotic and anti-apoptotic proteins can bind to each other in various combinations to form heterodimers in which the two proteins inhibit each other's function. The balance between the activities of these two functional classes of Bcl2 family proteins largely determines whether a mammalian cell lives or dies by the intrinsic pathway of apoptosis.

As illustrated in Figure 18–8, the anti-apoptotic Bcl2 family proteins, including *Bcl2* itself (the founding member of the Bcl2 family) and $Bcl X_L$, share four distinctive *Bcl2 homology (BH) domains* (BH1–4). The pro-apoptotic Bcl2 family proteins

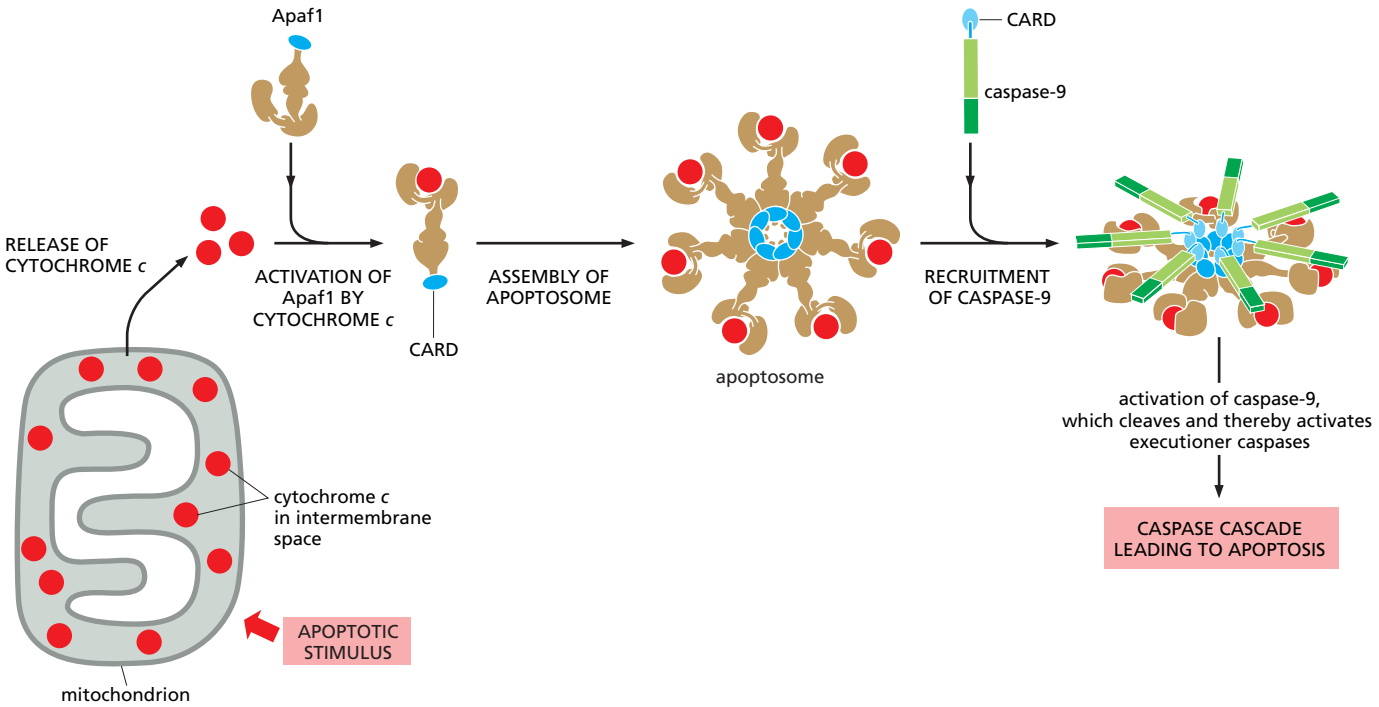


Figure 18–7 The intrinsic pathway of apoptosis. Intracellular apoptotic stimuli cause mitochondria to release cytochrome *c*, which interacts with Apaf1. The binding of cytochrome *c* causes Apaf1 to unfold partly, exposing a domain that interacts with the same domain in other activated Apaf1 molecules. Seven activated Apaf1 proteins form a large ring complex called the apoptosome. Each Apaf1 protein contains a caspase recruitment domain (CARD), and these are clustered above the central hub of the apoptosome. The CARDs bind similar domains in multiple caspase-9 molecules, which are thereby recruited into the apoptosome and activated. The mechanism of caspase-9 activation is not clear: it probably results from dimerization and cleavage of adjacent caspase-9 proteins, but it might also depend on interactions between caspase-9 and Apaf1. Once activated, caspase-9 cleaves and thereby activates downstream executioner caspases. Note that the CARD is related in structure and function to the death effector domain of caspase-8 (see Figure 18–5). Some scientists use the term "apoptosome" to refer to the complex containing caspase-9.

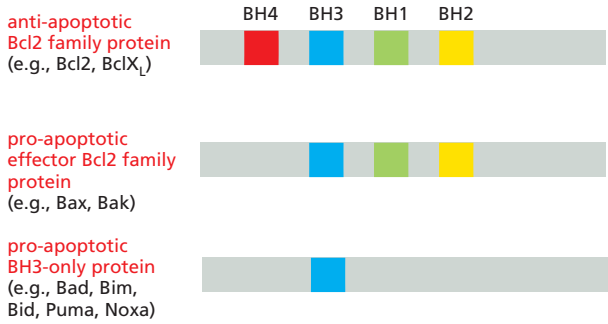


Figure 18–8 The three classes of Bcl2 family proteins. Note that the BH3 domain is the only BH domain shared by all Bcl2 family members; it mediates the direct interactions between pro-apoptotic and anti-apoptotic family members.

consist of two subfamilies—the *effector Bcl2 family proteins* and the *BH3-only proteins*. The main effector proteins are *Bax* and *Bak*, which are structurally similar to Bcl2 but lack the BH4 domain. The BH3-only proteins share sequence homology with Bcl2 in only the BH3 domain.

When an apoptotic stimulus triggers the intrinsic pathway, the pro-apoptotic **effector Bcl2 family proteins** become activated and aggregate to form oligomers in the mitochondrial outer membrane, inducing the release of cytochrome *c* and other intermembrane proteins by an unknown mechanism (**Figure 18–9**). In mammalian cells, **Bax** and **Bak** are the main effector Bcl2 family proteins, and at least one of them is required for the intrinsic pathway of apoptosis to operate: mutant mouse cells that lack both proteins are resistant to all pro-apoptotic signals that normally activate this pathway. Whereas Bak is bound to the mitochondrial outer membrane even in the absence of an apoptotic signal, Bax is mainly located in the cytosol and translocates to the mitochondria only after an apoptotic signal activates it. As we discuss below, the activation of Bax and Bak usually depends on activated pro-apoptotic BH3-only proteins.

The **anti-apoptotic Bcl2 family proteins** such as **Bcl2** itself and **BclX$_L$** are also located on the cytosolic surface of the outer mitochondrial membrane, where they help prevent inappropriate release of intermembrane proteins. The anti-apoptotic Bcl2 family proteins inhibit apoptosis mainly by binding to and inhibiting pro-apoptotic Bcl2 family proteins—either on the mitochondrial membrane or in the cytosol. On the outer mitochondrial membrane, for example, they bind to Bak and prevent it from oligomerizing, thereby inhibiting the release of cytochrome *c* and other intermembrane proteins. There are at least five mammalian anti-apoptotic Bcl2 family proteins, and every mammalian cell requires at least one to survive. Moreover, a number of these proteins must be inhibited for the intrinsic pathway to induce apoptosis; the BH3-only proteins mediate the inhibition.

The **BH3-only proteins** are the largest subclass of Bcl2 family proteins. The cell either produces or activates them in response to an apoptotic stimulus, and they are thought to promote apoptosis mainly by inhibiting anti-apoptotic Bcl2 family

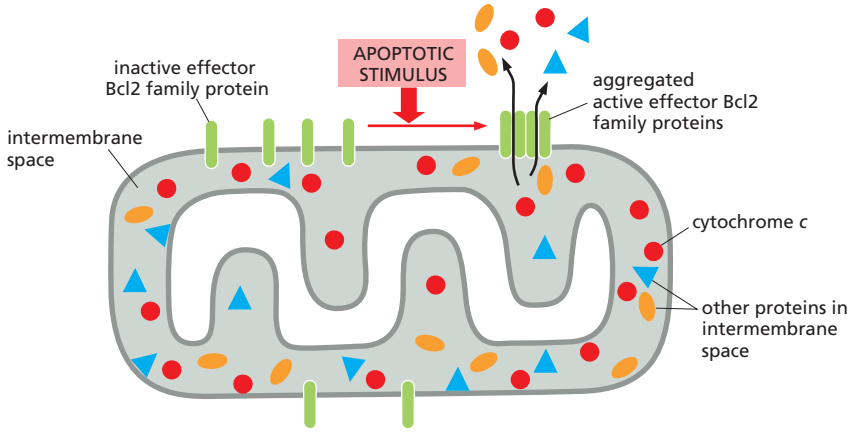


Figure 18–9 The role of pro-apoptotic effector Bcl2 family proteins (mainly Bax and Bak) in the release of mitochondrial intermembrane proteins in the intrinsic pathway of apoptosis. When activated by an apoptotic stimulus, the effector Bcl2 family proteins aggregate on the outer mitochondrial membrane and release cytochrome *c* and other proteins from the intermembrane space into the cytosol by an unknown mechanism.

proteins. Their BH3 domain binds to a long hydrophobic groove on anti-apoptotic Bcl2 family proteins, neutralizing their activity. This binding and inhibition enables the aggregation of Bax and Bak on the surface of mitochondria, which triggers the release of the intermembrane mitochondrial proteins that induce apoptosis (Figure 18–10). Some BH3-only proteins may bind directly to Bax and Bak to help stimulate their aggregation.

BH3-only proteins provide the crucial link between apoptotic stimuli and the intrinsic pathway of apoptosis, with different stimuli activating different BH3-only proteins. Some extracellular survival signals, for example, block apoptosis by inhibiting the synthesis or activity of certain BH3-only proteins (see Figure 18–12B). Similarly, in response to DNA damage that cannot be repaired, the tumor suppressor protein **p53** accumulates (discussed in Chapters 17 and 20) and activates the transcription of genes that encode the BH3-only proteins *Puma* and *Noxa*. These BH3-only proteins then trigger the intrinsic pathway, thereby eliminating a potentially dangerous cell that could otherwise become cancerous.

As mentioned earlier, in some cells the extrinsic apoptotic pathway recruits the intrinsic pathway to amplify the caspase cascade to kill the cell. The BH3-only protein *Bid* is the link between the two pathways. Bid is normally inactive. However, when death receptors activate the extrinsic pathway in some cells, the initiator caspase, caspase-8, cleaves Bid, producing an active form of Bid that translocates to the outer mitochondrial membrane and inhibits anti-apoptotic Bcl2 family proteins, thereby amplifying the death signal.

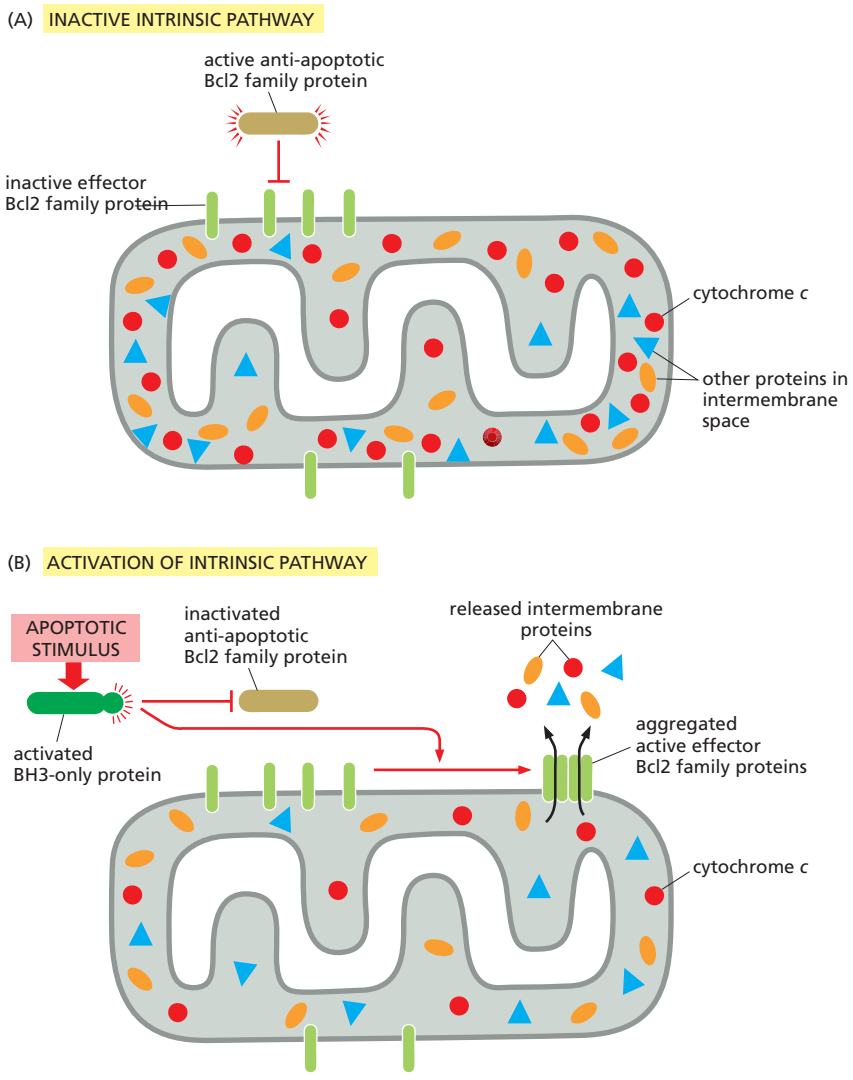


Figure 18–10 How pro-apoptotic BH3-only and anti-apoptotic Bcl2 family proteins regulate the intrinsic pathway of apoptosis. (A) In the absence of an apoptotic stimulus, anti-apoptotic Bcl2 family proteins bind to and inhibit the effector Bcl2 family proteins on the mitochondrial outer membrane (and in the cytosol—not shown). (B) In the presence of an apoptotic stimulus, BH3-only proteins are activated and bind to the anti-apoptotic Bcl2 family proteins so that they can no longer inhibit the effector Bcl2 family proteins; the latter then become activated, aggregate in the outer mitochondrial membrane, and promote the release of intermembrane mitochondrial proteins into the cytosol. Some activated BH3-only proteins may stimulate mitochondrial protein release more directly by binding to and activating the effector Bcl2 family proteins. Although not shown, the anti-apoptotic Bcl2 family proteins are bound to the mitochondrial surface.

IAPs Help Control Caspases

Because activation of a caspase cascade leads to certain death, the cell employs multiple robust mechanisms to ensure that these proteases are activated only when appropriate. One line of defense is provided by a family of proteins called **inhibitors of apoptosis** (**IAPs**). These proteins were first identified in certain insect viruses (baculoviruses), which encode IAP proteins to prevent a host cell that is infected by the virus from killing itself by apoptosis. It is now known that most animal cells also make IAP proteins.

All IAPs have one or more BIR (baculovirus IAP repeat) domains, which enable them to bind to and inhibit activated caspases. Some IAPs also polyubiquitylate caspases, marking the caspases for destruction by proteasomes. In this way, the IAPs set an inhibitory threshold that caspases must overcome to trigger apoptosis.

In *Drosophila* at least, the inhibitory barrier provided by IAPs can be neutralized by **anti-IAP** proteins, which are produced in response to various apoptotic stimuli. There are numerous anti-IAPs in flies, including *Reaper*, *Grim*, and *Hid*, and their only structural similarity is their short, N-terminal, IAP-binding motif, which binds to the BIR domain of IAPs, preventing the domain from binding to a caspase. Deletion of the three genes encoding Reaper, Grim, and Hid blocks apoptosis in flies. Conversely, inactivation of one of the two genes that encode IAPs in *Drosophila* causes all of the cells in the developing fly embryo to undergo apoptosis. Clearly, the balance between IAPs and anti-IAPs is tightly regulated and is crucial for controlling apoptosis in the fly.

The role of mammalian IAP and anti-IAP proteins in apoptosis is less clear. Anti-IAPs are released from the mitochondrial intermembrane space when the intrinsic pathway of apoptosis is activated, blocking IAPs in the cytosol and thereby promoting apoptosis. However, mice appear to develop normally if they are missing either the major mammalian IAP (called XIAP) or the two known mammalian anti-IAPs (called Smac/Diablo and Omi). Worms do not even contain a caspase-inhibiting IAP protein. Apparently, the tight control of caspase activity is achieved by different mechanisms in different animals.

Extracellular Survival Factors Inhibit Apoptosis in Various Ways

Intercellular signals regulate most activities of animal cells, including apoptosis. These extracellular signals are part of the normal "social" controls that ensure that individual cells behave for the good of the organism as a whole—in this case, by surviving when they are needed and killing themselves when they are not. Some extracellular signal molecules stimulate apoptosis, whereas others inhibit it. We have discussed signal proteins such as Fas ligand that activate death receptors and thereby trigger the extrinsic pathway of apoptosis. Other extracellular signal molecules that stimulate apoptosis are especially important during vertebrate development: a surge of thyroid hormone in the bloodstream, for example, signals cells in the tadpole tail to undergo apoptosis at metamorphosis. In mice, locally produced signal proteins stimulate cells between developing fingers and toes to kill themselves (see Figure 18–2). Here, however, we focus on extracellular signal molecules that inhibit apoptosis, which are collectively called **survival factors**.

Most animal cells require continuous signaling from other cells to avoid apoptosis. This surprising arrangement apparently helps ensure that cells survive only when and where they are needed. Nerve cells, for example, are produced in excess in the developing nervous system and then compete for limited amounts of survival factors that are secreted by the target cells that they normally connect to (see Figure 21–81). Nerve cells that receive enough survival signals live, while the others die. In this way, the number of surviving neurons is automatically adjusted so that it is appropriate for the number of target cells they connect with (**Figure 18–11**). A similar competition for limited amounts of survival factors produced by neighboring cells is thought to control cell numbers in other tissues, both during development and in adulthood.

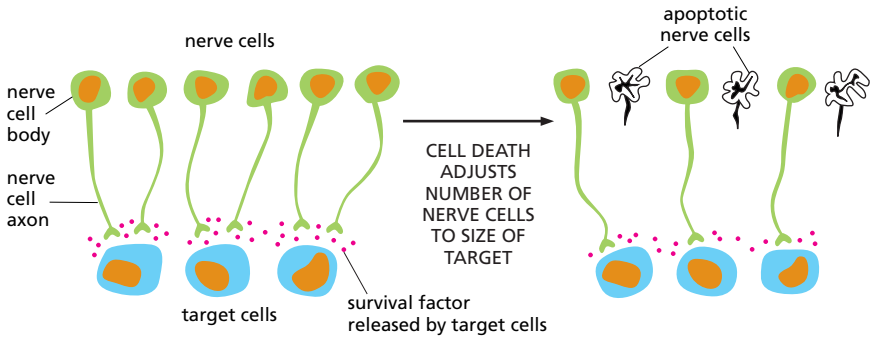


Figure 18–11 The role of survival factors and cell death in adjusting the number of developing nerve cells to the amount of target tissue. More nerve cells are produced than can be supported by the limited amount of survival factors released by the target cells. Therefore, some nerve cells receive an insufficient amount of survival factors to avoid apoptosis. This strategy of overproduction followed by culling helps ensure that all target cells are contacted by nerve cells and that the extra nerve cells are automatically eliminated.

Survival factors usually bind to cell-surface receptors, which activate intracellular signaling pathways that suppress the apoptotic program, often by regulating members of the Bcl2 family of proteins. Some survival factors, for example, stimulate the synthesis of anti-apoptotic Bcl2 family proteins such as Bcl2 itself or $BclX_L$ (**Figure 18–12**A). Others act by inhibiting the function of pro-apoptotic BH3-only proteins such as *Bad* (Figure M18–12B). In *Drosophila*, some survival factors act by phosphorylating and inactivating anti-IAP proteins such as Hid, thereby enabling IAP proteins to suppress apoptosis (Figure 18–12C). Some developing neurons, like those illustrated in Figure 18–11, use an ingenious alternative approach: survival-factor receptors stimulate apoptosis—by an unknown mechanism—when they are not occupied, and then stop promoting death when survival factor binds. The end result in all these cases is the same: cell survival depends on survival factor binding.

Phagocytes Remove the Apoptotic Cell

Apoptotic cell death is a remarkably tidy process: the apoptotic cell and its fragments do not break open and release their contents, but instead remain intact as they are efficiently eaten—or *phagocytosed*—by neighboring cells, leaving no trace and therefore triggering no inflammatory response (see Figure 18–1B and Movie 13.5). This engulfment process depends on chemical changes on the surface of the apoptotic cell, which displays signals that recruit phagocytic cells. An especially important change occurs in the distribution of the negatively charged phospholipid *phosphatidylserine* on the cell surface. This phospholipid is normally located exclusively in the inner leaflet of the lipid bilayer of the plasma membrane (see Figure 10–15), but it flips to the outer leaflet in apoptotic cells. The

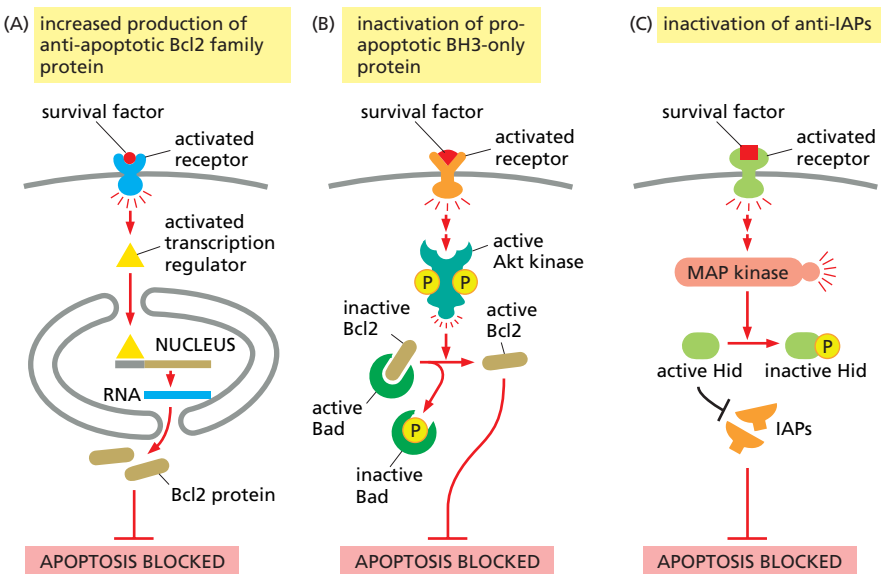


Figure 18–12 Three ways that extracellular survival factors can inhibit apoptosis. (A) Some survival factors suppress apoptosis by stimulating the transcription of genes that encode anti-apoptotic Bcl2 family proteins such as Bcl2 itself or $BclX_L$. (B) Many others activate the serine/threonine protein kinase Akt, which, among many other targets, phosphorylates and inactivates the pro-apoptotic BH3-only protein Bad (see Figure 15–53). When not phosphorylated, Bad promotes apoptosis by binding to and inhibiting Bcl2; once phosphorylated, Bad dissociates, freeing Bcl2 to suppress apoptosis. Akt also suppresses apoptosis by phosphorylating and inactivating transcription regulatory proteins that stimulate the transcription of genes encoding proteins that promote apoptosis (not shown). (C) In *Drosophila*, some survival factors inhibit apoptosis by stimulating the phosphorylation of the anti-IAP protein Hid. When not phosphorylated, Hid promotes cell death by inhibiting IAPs. Once phosphorylated, Hid no longer inhibits IAPs, which become active and block apoptosis. MAP kinase, mitogen-activated protein kinase.

underlying mechanism is poorly understood, but the external exposure of phosphatidylserine is likely to depend on caspase cleavage of some protein involved in phospholipid distribution in the membrane. A variety of soluble "bridging" proteins interact with the exposed phosphatidylserine on the apoptotic cell. These bridging proteins also interact with specific receptors on the surface of a neighboring cell or macrophage, triggering cytoskeletal and other changes that initiate the engulfment process.

Macrophages do not phagocytose healthy cells in the animal—despite the fact that healthy cells normally expose some phosphatidylserine on their surfaces. Healthy cells express signal proteins on their surface that interact with inhibitory receptors on macrophages that block phagocytosis. Thus, in addition to expressing cell-surface signals such as phosphatidylserine that stimulate phagocytosis, apoptotic cells must lose or inactivate these "don't eat me" signals that block phagocytosis.

Either Excessive or Insufficient Apoptosis Can Contribute to Disease

There are many human disorders in which excessive numbers of cells undergo apoptosis and thereby contribute to tissue damage. Among the most dramatic examples are heart attacks and strokes. In these acute conditions, many cells die by necrosis as a result of ischemia (inadequate blood supply), but some of the less affected cells die by apoptosis. It is hoped that, in the future, drugs that block apoptosis—such as specific caspase inhibitors—will prove useful in saving such cells.

There are other conditions where too few cells die by apoptosis. Mutations in mice and humans, for example, that inactivate the genes that encode the Fas death receptor or the Fas ligand prevent the normal death of some lymphocytes, causing these cells to accumulate in excessive numbers in the spleen and lymph glands. In many cases, this leads to autoimmune disease, in which the lymphocytes react against the individual's own tissues.

Decreased apoptosis also makes an important contribution to many tumors, as cancer cells often regulate their apoptotic program abnormally. The *Bcl2* gene, for example, was first identified in a common form of lymphocyte cancer in humans, where a chromosome translocation causes excessive production of the Bcl2 protein; indeed, Bcl2 gets its name from this *B cell lymphoma*. The high level of Bcl2 protein in the lymphocytes that carry the translocation promotes the development of cancer by inhibiting apoptosis, thereby prolonging lymphocyte survival and increasing their number; it also decreases the cells' sensitivity to anticancer drugs, which commonly work by causing cancer cells to undergo apoptosis.

Similarly, the gene encoding the tumor suppressor protein p53 is mutated in about 50% of human cancers so that it no longer promotes apoptosis or cell-cycle arrest in response to DNA damage. The lack of p53 function therefore enables the cancer cells to survive and proliferate even when their DNA is damaged; in this way, the cells accumulate more mutations, some of which make the cancer more malignant (discussed in Chapter 20). As many anticancer drugs induce apoptosis (and cell-cycle arrest) by a p53-dependent mechanism (discussed in Chapters 17 and 20), the loss of p53 function also makes cancer cells less sensitive to these drugs.

If decreased apoptosis contributes to many cancers, then we might be able to treat those cancers with drugs that stimulate apoptosis. This line of thinking has recently led to the development of small chemicals that interfere with the function of anti-apoptotic Bcl2 family proteins such as Bcl2 and $Bcl X_L$. These chemicals bind with high affinity to the hydrophobic groove on anti-apoptotic Bcl2 family proteins, blocking their function in essentially the same way that BH3-only proteins do (**Figure 18–13**). The intrinsic pathway of apoptosis is thereby stimulated, which in certain tumors increases the amount of cell death.

Most human cancers arise in epithelial tissues such as those in the lung, intestinal tract, breast, and prostate. Such cancer cells display many abnormalities in

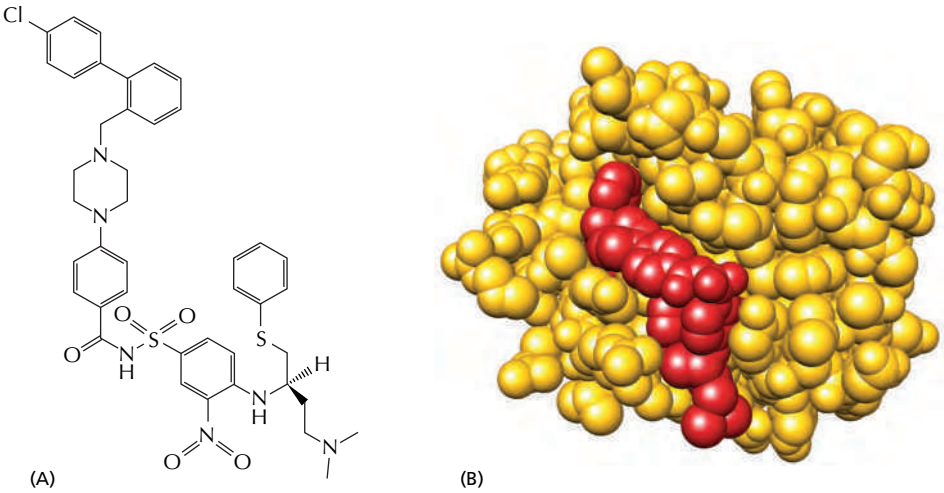


Figure 18–13 How the chemical ABT-737 inhibits anti-apoptotic Bcl2 family proteins. As shown in Figure 18–10B, an apoptotic signal results in activation of BH3-only proteins, which interact with a long hydrophobic groove in anti-apoptotic Bcl2 family proteins, thereby preventing them from blocking apoptosis. Using the crystal structure of the groove, the drug shown in (A), called ABT-737, was designed and synthesized to bind tightly in the groove, as shown for the anti-apoptotic Bcl2 family protein, $Bcl{X_L}$, in (B). By inhibiting the activity of these proteins, the drug promotes apoptosis in any cell that depends on them for survival. (PDB code: 2YXJ.)

their behavior, including a decreased ability to adhere to the extracellular matrix and to one another at specialized cell–cell junctions. In the next chapter, we discuss the remarkable structures and functions of the extracellular matrix and cell junctions.

Summary

Animal cells can activate an intracellular death program and kill themselves in a controlled way when they are irreversibly damaged, no longer needed, or are a threat to the organism. In most cases, these deaths occur by apoptosis: the cells shrink, condense, and frequently fragment, and neighboring cells or macrophages rapidly phagocytose the cells or fragments before there is any leakage of cytoplasmic contents. Apoptosis is mediated by proteolytic enzymes called caspases, which cleave specific intracellular proteins to help kill the cell. Caspases are present in all nucleated animal cells as inactive precursors. Initiator caspases are activated when brought into proximity in activation complexes: once activated, they cleave and thereby activate downstream executioner caspases, which then cleave various target proteins in the cell, producing an amplifying, irreversible proteolytic cascade.

Cells use at least two distinct pathways to activate initiator caspases and trigger a caspase cascade leading to apoptosis: the extrinsic pathway is activated by extracellular ligands binding to cell-surface death receptors; the intrinsic pathway is activated by intracellular signals generated when cells are stressed. Each pathway uses its own initiator caspases, which are activated in distinct activation complexes: in the extrinsic pathway, the death receptors recruit caspase-8 via adaptor proteins to form the DISC; in the intrinsic pathway, cytochrome c *released from the intermembrane space of mitochondria activates Apaf1, which assembles into an apoptosome and recruits and activates caspase-9.*

Intracellular Bcl2 family proteins and IAP proteins tightly regulate the apoptotic program to ensure that cells kill themselves only when it benefits the animal. Both anti-apoptotic and pro-apoptotic Bcl2 family proteins regulate the intrinsic pathway by controlling the release of mitochondrial intermembrane proteins, while IAP proteins inhibit activated caspases and promote their degradation.

WHAT WE DON'T KNOW

- How many forms of programmed cell death exist? What are the underlying mechanisms and benefits of each?
- Thousands of caspase substrates have been identified. Which ones are the critical proteins that must be cleaved to trigger the major cell remodeling events underlying apoptosis?
- How did the intrinsic pathway of apoptosis evolve, and what is the advantage of having mitochondria play such a central role in regulating apoptosis?
- How are "don't eat me" signals eliminated or inactivated during apoptosis to allow the cells to be phagocytosed?

PROBLEMS

Which statements are true? Explain why or why not.

18–1 In normal adult tissues, cell death usually balances cell division.

18–2 Mammalian cells that do not have cytochrome *c* should be resistant to apoptosis induced by DNA damage.

Discuss the following problems.

18–3 One important role of Fas and Fas ligand is to mediate the elimination of tumor cells by killer lymphocytes. In a study of 35 primary lung and colon tumors, half the tumors were found to have amplified and overexpressed a gene for a secreted protein that binds to Fas ligand. How do you suppose that overexpression of this protein might contribute to the survival of these tumor cells? Explain your reasoning.

18–4 Development of the nematode *Caenorhabditis elegans* generates exactly 959 somatic cells; it also produces an additional 131 cells that are later eliminated by apoptosis. Classical genetic experiments in *C. elegans* isolated mutants that led to the identification of the first genes involved in apoptosis. Of the many mutations affecting apoptosis in the nematode, none have ever been found in the gene for cytochrome *c*. Why do you suppose that such a central effector molecule in apoptosis was not found in the many genetic screens for "death" genes that have been carried out in *C. elegans*?

18–5 Imagine that you could microinject cytochrome *c* into the cytosol of wild-type mammalian cells and of cells that were doubly defective for Bax and Bak. Would you expect one, both, or neither type of cell to undergo apoptosis? Explain your reasoning.

18–6 In contrast to their similar brain abnormalities, newborn mice deficient in Apaf1 or caspase-9 have distinctive abnormalities in their paws. Apaf1-deficient mice fail to eliminate the webs between their developing digits, whereas caspase-9-deficient mice have normally formed digits (**Figure Q18–1**). If Apaf1 and caspase-9 function in the same apoptotic pathway, how is it possible for these deficient mice to differ in web-cell apoptosis?

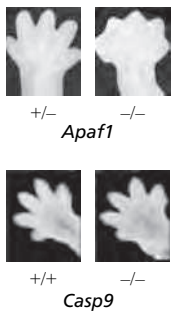


Figure Q18–1 Appearance of paws in *Apaf1*$^{-/-}$ and *Casp9*$^{-/-}$ newborn mice relative to normal newborn mice (Problem 18–6). (From H. Yoshida et al., *Cell* 94:739–750, 1998. With permission from Elsevier.)

18–7 When human cancer cells are exposed to ultraviolet (UV) light at 90 mJ/cm^2, most of the cells undergo apoptosis within 24 hours. Release of cytochrome *c* from mitochondria can be detected as early as 6 hours after exposure of a population of cells to UV light, and it continues to increase for more than 10 hours thereafter. Does this mean that individual cells slowly release their cytochrome *c* over this time period? Or, alternatively, do individual cells release their cytochrome *c* rapidly but with different cells being triggered over the longer time period?

To answer this fundamental question, you have fused the gene for green fluorescent protein (GFP) to the gene for cytochrome *c*, so that you can observe the behavior of individual cells by confocal fluorescence microscopy. In cells that are expressing the cytochrome *c*–GFP fusion, fluorescence shows the punctate pattern typical of mitochondrial proteins. You then irradiate these cells with UV light and observe individual cells for changes in the punctate pattern. Two such cells (outlined in white) are shown in **Figure Q18–2**A and B. Release of cytochrome *c*–GFP is detected as a change from a punctate to a diffuse pattern of fluorescence. Times after UV exposure are indicated as hours:minutes below the individual panels.
Which model for cytochrome *c* release do these observations support? Explain your reasoning.

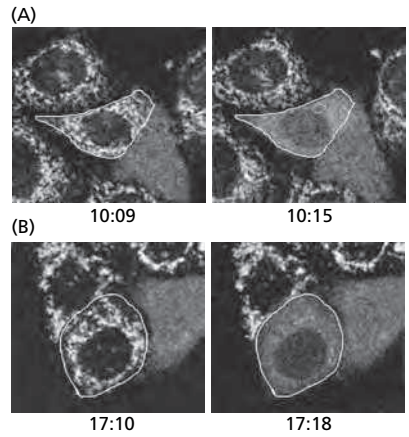


Figure Q18–2 Time-lapse video fluorescence microscopic analysis of cytochrome *c*–GFP release from mitochondria of individual cells (Problem 18–7). (A) Cells observed for 6 minutes, 10 hours after UV irradiation. (B) Cells observed for 8 minutes, 17 hours after UV irradiation. One cell in (A) and one in (B), each *outlined in white*, have released their cytochrome *c*–GFP during the time frame of the observation, which is shown as hours:minutes below each panel. (From J.C. Goldstein et al., *Nat. Cell Biol.* 2:156–162, 2000. With permission from Macmillan Publishers Ltd.)

18–8 Fas ligand is a trimeric, extracellular protein that binds to its receptor, Fas, which is composed of three identical transmembrane subunits (**Figure Q18–3**). The binding of Fas ligand alters the conformation of Fas so that it binds an adaptor protein, which then recruits and activates caspase-8, triggering a caspase cascade that leads to cell death. In humans, the autoimmune lymphoproliferative syndrome (ALPS) is associated with dominant mutations in Fas that include point mutations and C-terminal

truncations. In individuals that are heterozygous for such mutations, lymphocytes do not die at their normal rate and accumulate in abnormally large numbers, causing a variety of clinical problems. In contrast to these patients, individuals that are heterozygous for mutations that eliminate Fas expression entirely have no clinical symptoms.

A. Assuming that the normal and dominant forms of Fas are expressed to the same level and bind Fas ligand equally, what fraction of Fas–Fas ligand complexes on a lymphocyte from a heterozygous ALPS patient would be expected to be composed entirely of normal Fas subunits?

B. In an individual heterozygous for a mutation that eliminates Fas expression, what fraction of Fas–Fas ligand complexes would be expected to be composed entirely of normal Fas subunits?

C. Why are the Fas mutations that are associated with ALPS dominant, while those that eliminate expression of Fas are recessive?

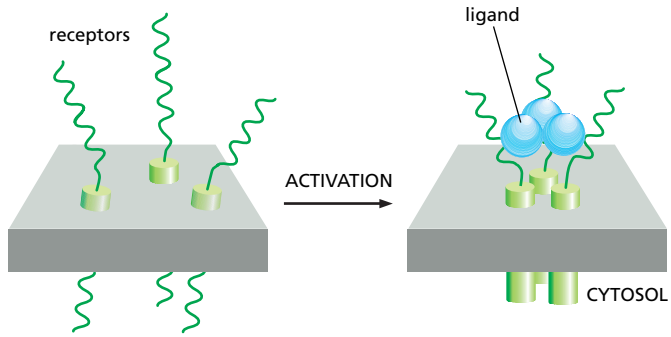


Figure Q18–3 The binding of trimeric Fas ligand to Fas (Problem 18–8).

REFERENCES

Crawford ED & Wells JA (2011) Caspase substrates and cellular remodeling. *Annu. Rev. Biochem.* 80, 1055–1087.

Czabotar PE, Lessene G, Strasser A et al. (2014) Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat. Rev. Mol. Cell Biol.* 15, 49–63.

Danial NN & Korsmeyer SJ (2004) Cell death: critical control points. *Cell* 116, 205–219.

Elliott MR & Ravichandran KS (2010) Clearance of apoptotic cells: implications in health and disease. *J. Cell Biol.* 189, 1059–1070.

Ellis RE, Yuan JY & Horvitz RA (1991) Mechanisms and functions of cell death. *Annu. Rev. Cell Biol.* 7, 663–698.

Fadok VA & Henson PM (2003) Apoptosis: giving phosphatidylserine recognition an assist—with a twist. *Curr. Biol.* 13, R655–R657.

Green DR (2011) Means to an End: Apoptosis and Other Cell Death Mechanisms. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Jacobson MD, Weil M & Raff MC (1997) Programmed cell death in animal development. *Cell* 88, 347–354.

Jiang X & Wang X (2004) Cytochrome C-mediated apoptosis. *Annu. Rev. Biochem.* 73, 87–106.

Kerr JF, Wyllie AH & Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Brit. J. Cancer* 26, 239–257.

Kumar S (2007) Caspase function in programmed cell death. *Cell Death Differ.* 14, 32–43.

Lavrik I, Golks A & Krammer PH (2005) Death receptor signaling. *J. Cell Sci.* 118, 265–267.

Lessene G, Czabotar PE & Colman PM (2008) BCL-2 family antagonists for cancer therapy. *Nat. Rev. Drug Discov.* 7, 989–1000.

Mace PD & Riedl SJ (2010) Molecular cell death platforms and assemblies. *Curr. Opin. Cell Biol.* 22, 828–836.

Nagata S (2005) DNA degradation in development and programmed cell death. *Annu. Rev. Immunol.* 23, 853–875.

Raff MC (1999) Cell suicide for beginners. *Nature* 396, 119–122.

Tait SW & Green DR (2013) Mitochondrial regulation of cell death. *Cold Spring Harb. Perspect. Biol.* 5, a008706.

Vanden Berghe T, Linkermann A, Jouan-Lanhouet S et al. (2014) Regulated necrosis: the expanding network of non-apoptotic cell death pathways. *Nat. Rev. Mol. Cell Biol.* 15, 135–147.

Vousden KH (2005) Apoptosis. p53 and PUMA: a deadly duo. *Science* 309, 1685–1686.

Willis SN & Adams JM (2005) Life in the balance: how BH3-only proteins induce apoptosis. *Curr. Opin. Cell Biol.* 17, 617–625.

Yuan S & Akey CW (2013) Apoptosome structure, assembly, and procaspase activation. *Structure* 21, 501–515.