# 18

# Apoptosis

Cell death plays a crucially important part in animal and plant development, and it usually continues into adulthood. In a healthy adult human, billions of cells die in the bone marrow and intestine every hour. Our tissues do not shrink because, by unknown regulatory mechanisms, cell division exactly balances the cell death. We now know that these "normal" cell deaths are suicides, in which the cells activate an intracellular death program and kill themselves in a controlled way—a process known as **programmed cell death**. The idea that animal cells have a built-in death program was proposed in the 1970s, but its general acceptance took another 20 years and depended on genetic studies in the nematode *C. elegans* that identified the first genes dedicated to programmed cell death and its control.

Programmed cell death in animals usually, but not exclusively, occurs by **apoptosis** (from the Greek word meaning "falling off," as leaves from a tree). Although apoptosis is only one form of programmed cell death, it is by far the most common and best understood, and, confusingly, biologists often use the terms programmed cell death and apoptosis interchangeably. Cells dying by apoptosis undergo characteristic morphological changes. <GCCC> 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 blebs and, if the cell is large, often breaks up into membrane-enclosed fragments called *apoptotic bodies*. Most importantly, 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 23) 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 probably underestimate its extent.

By contrast to apoptosis and other less well characterized forms of programmed cell death (which implies the operation of an intracellular death program), animal cells that die accidentally 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).

Programmed cell death is not confined to animals. In plants, it occurs during development and in the senescence of flowers and leaves, as well as in the response to injury and infection. Programmed cell death even occurs in unicellular organisms, including yeasts and bacteria. The molecular mechanisms involved in these cases are distinct from those that mediate apoptosis in animal cells, and we shall not consider them. In this chapter, we discuss the functions of programmed cell death in animals, the molecular mechanism of apoptosis and its regulation, and how excessive or insufficient apoptosis can contribute to human disease.

## Programmed Cell Death Eliminates Unwanted Cells

The amount of programmed cell death that occurs in developing and adult animal tissues can be astonishing. In the developing vertebrate nervous system, for

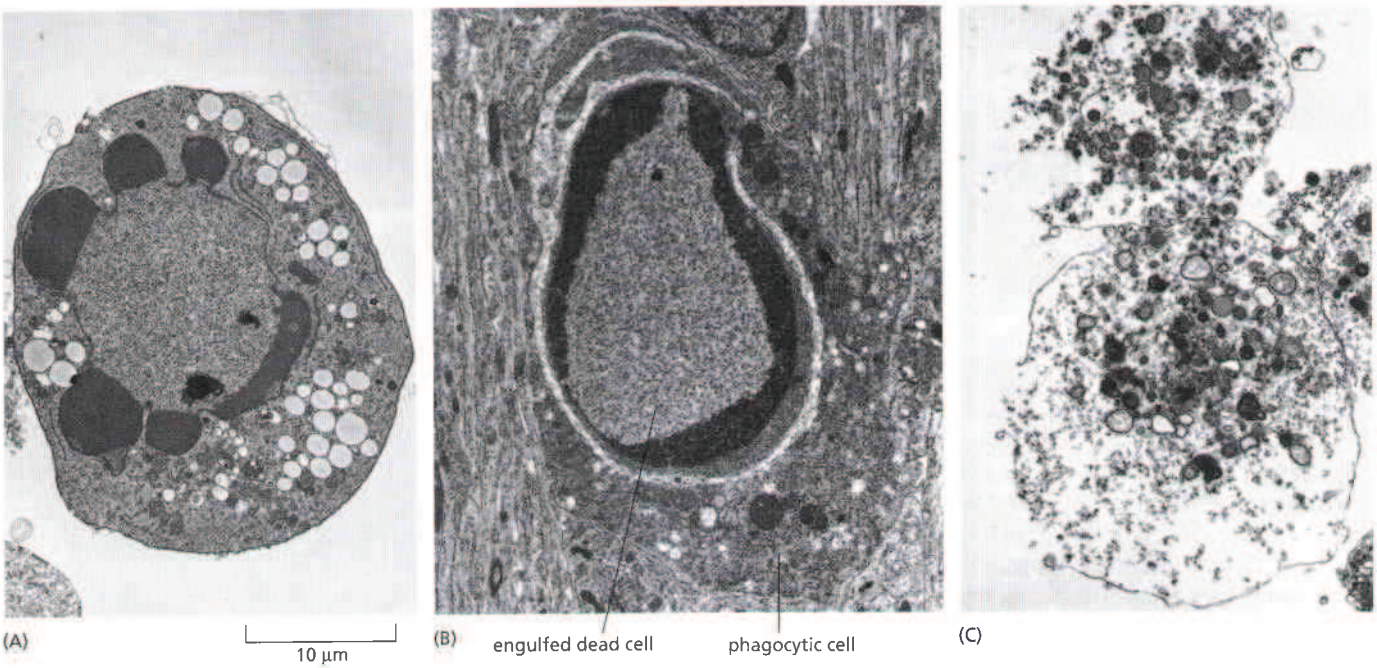



**Figure 18–1 Two distinct forms of cell death.** These electron micrographs show cells that have died by apoptosis (A and B) or by a type of accidental cell death called necrosis (C). The cells in (A) and (C) died in a culture dish, whereas the cell in (B) died in a developing tissue and has been engulfed 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.)

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?

In some cases, the answer is clear. In animal development, programmed cell death eliminates unwanted cells, usually by apoptosis. Cell death, for example, 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 (**Figure 18–3**). In many other cases, cell death helps regulate cell numbers. In the developing nervous system, for example, cell death adjusts the number of nerve cells to match the number of target cells that the nerve cells connect to, as we discuss later.

Programmed cell death 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; it also eliminates most of the lymphocytes activated by an infection, after they have helped destroy the responsible microbes (discussed in Chapter 25).

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

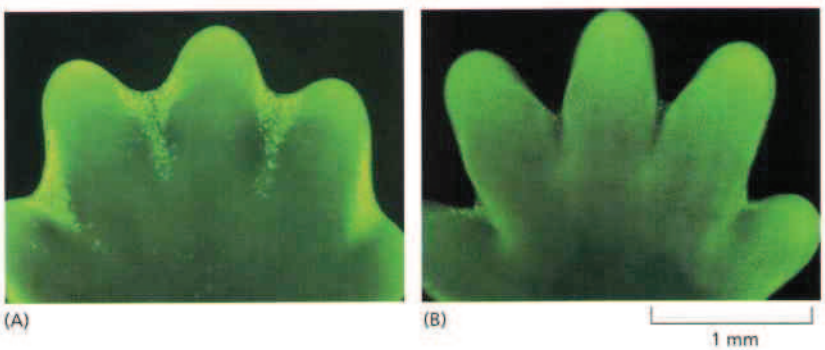



**Figure 18–2 Sculpting the digits in the developing mouse paw by apoptosis.** (A) The paw in this mouse fetus has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as *bright green* dots between the developing digits. (B) The interdigital 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.)

Figure 18–3 **Apoptosis during the metamorphosis of a tadpole into a frog.** As a tadpole changes into a frog, the cells in the tadpole tail are induced to undergo apoptosis; as a consequence, the tail is lost. An increase in thyroid hormone in the blood stimulates all the changes that occur during metamorphosis, including apoptosis in the tail.

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, although the control mechanisms responsible for such regulation are largely unknown.

Apoptosis occurs at a staggeringly high rate in the adult human bone marrow, where most blood cells are produced. Here, for example, *neutrophils* (a type of white blood cell discussed in Chapter 23) are produced continuously in very large numbers, but the vast majority die by apoptosis in the bone marrow within a few days without ever functioning. This apparently futile cycle of production and destruction serves to maintain a ready supply of short-lived neutrophils that can be rapidly mobilized to fight infection wherever it occurs in the body. Compared with the life of the organism, cells are evidently cheap.

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, if they cannot repair it, they often kill themselves by undergoing apoptosis.

## Apoptotic Cells Are Biochemically Recognizable

Cells undergoing apoptosis not only have a characteristic morphology but also display characteristic biochemical changes, which can be used to identify apoptotic cells. During apoptosis, for example, an endonuclease cleaves the chromosomal DNA into fragments of distinctive sizes; because the cleavages occur in the linker regions between nucleosomes, the fragments separate into a characteristic ladder pattern when analyzed by gel electrophoresis (**Figure 18–4**A). Moreover, the cleavage of DNA generates many new DNA ends, which can be marked in apoptotic nuclei by using a labeled nucleotide in the so-called TUNEL technique (Figure 18–4B).

An especially important change occurs in the plasma membrane of apoptotic cells. The negatively charged phospholipid *phosphatidylserine* is normally exclusively located in the inner leaflet of the lipid bilayer of the plasma membrane (see Figures 10–3 and 10–16), but it flips to the outer leaflet in apoptotic cells, where it can serve as a marker of these cells. The phosphatidylserine on the surface of apoptotic cells can be visualized with a labeled form of the *Annexin V* protein, which specifically binds to this phospholipid. The cell-surface phosphatidylserine is more than a convenient marker of apoptosis for biologists; it helps signal to neighboring cells and macrophages to phagocytose the dying cell. In addition to serving as an "eat me" signal, it also blocks the inflammation often associated with phagocytosis: the phosphatidylserine-dependent engulfment of apoptotic cells inhibits the production of inflammation-inducing signal proteins (cytokines) by the phagocytic cell.

Macrophages will phagocytose most types of small particles, including oil droplets and glass beads, but they do not phagocytose any healthy cells in the animal, presumably because healthy cells express "don't eat me" signal molecules on their surface. Thus, in addition to expressing cell-surface "eat me" signals such as phosphatidylserine that stimulate phagocytosis, apoptotic cells must lose or inactivate their "don't eat me" signals in order for macrophages to ingest them.

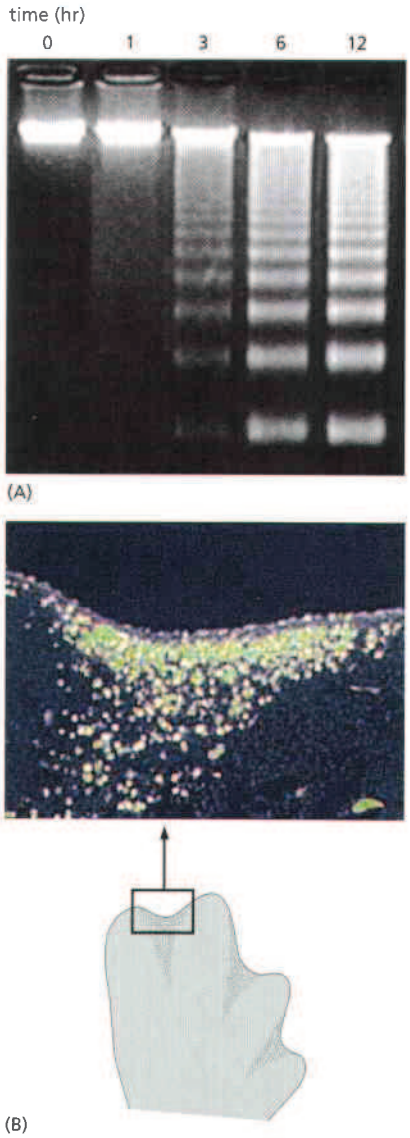



**Figure 18–4 Markers of apoptosis.** (A) Cleavage of nuclear DNA into a characteristic ladder pattern of fragments. Mouse thymus lymphocytes were treated with an antibody against the cell-surface death receptor Fas (discussed later), inducing the cells to undergo apoptosis. After various times (indicated in hours at the top of the figure), DNA was extracted, and the fragments were separated by size by electrophoresis in an agarose gel and stained with ethidium bromide. (B) The TUNEL technique was used to label the cut ends of DNA fragments in the nuclei of apoptotic cells 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. (A, from D. McIlroy et al., *Genes Dev.* 14:549–558, 2000. With permisison from Cold Spring Harbor Laboratory Press; B, from V. Zuzarte-Luís and J.M. Hurlé, *Int. J. Dev. Biol.* 46:871–876, 2002. With permission from UBC Press.)

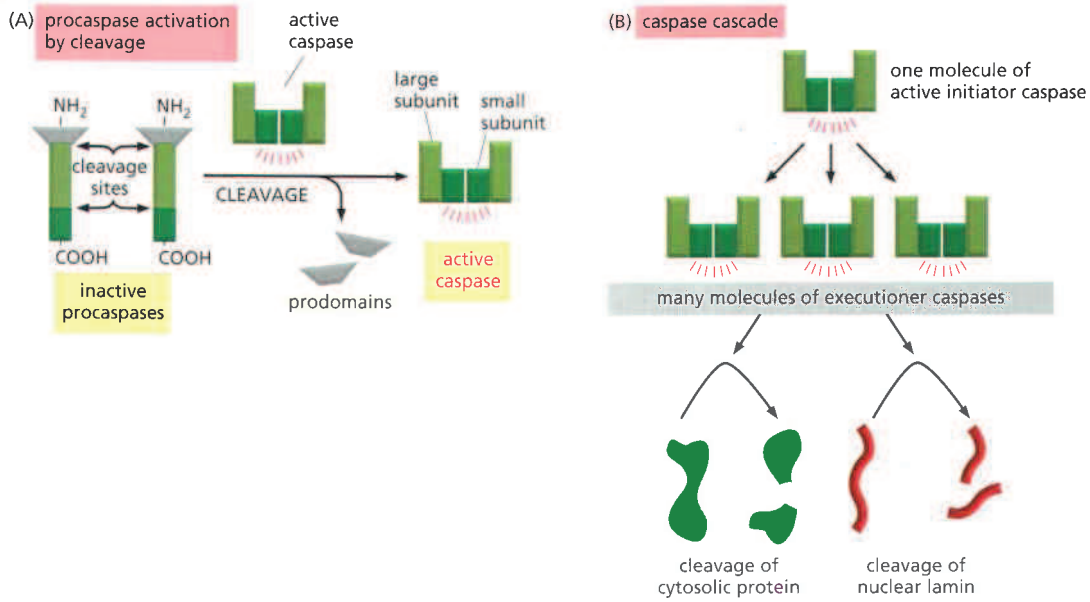
Cells undergoing apoptosis often lose the electrical potential that normally exists across the inner membrane of their mitochondria (discussed in Chapter 14). This membrane potential can be measured by the use of positively charged fluorescent dyes that accumulate in mitochondria, driven by the negative charge on the inside of the inner membrane. A decrease in the labeling of mitochondria with these dyes helps to identify cells that are undergoing apoptosis. As we discuss later, proteins such as *cytochrome c* are usually released from the space between the inner and outer membrane (the *intermembrane space*) of mitochondria during apoptosis, and the relocation of cytochrome *c* from mitochondria to the cytosol can be used as another marker of apoptosis (see Figure 18–7).

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

The intracellular machinery responsible for apoptosis is similar in all animal cells. It depends on a family of proteases that 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, or **procaspases**, which are typically activated by proteolytic cleavage. Procaspase cleavage occurs at one or two specific aspartic acids and is catalyzed by other (already active) caspases; the procaspase is split into a large and a small subunit that form a heterodimer, and two such dimers assemble to form the active tetramer (**Figure 18–5**A). Once activated, caspases cleave, and thereby activate, other procaspases, resulting in an amplifying proteolytic cascade (Figure 18–5B).

Not all caspases mediate apoptosis. Indeed, the first caspase identified was a human protein called *interleukin-1-converting enzyme (ICE)*, which is concerned with inflammatory responses rather than with cell death; ICE cuts out the inflammation-inducing cytokine *interleukin-1 (IL1)* from a larger precursor protein. Subsequent to the discovery of ICE, a gene required for apoptosis in *C. elegans* was shown to encode a protein that is structurally and functionally similar to ICE, providing the first evidence that proteolysis and caspases are involved in apoptosis. It is now clear that, whereas several human caspases are involved in inflammatory and immune responses, most are involved in apoptosis (**Table 18–1**).

As shown in Figure 18–5B and Table 18–1, some of the procaspases that operate in apoptosis act at the start of the proteolytic cascade and are called **initiator procaspases**; when activated, they cleave and activate downstream **executioner procaspases**, which, then cleave and activate other executioner procaspases, as well as specific *target proteins* in the cell. Among the many target proteins cleaved by executioner caspases are the nuclear lamins (see Figure 18–5B), the cleavage of which causes the irreversible breakdown of the nuclear lamina (discussed in Chapter 16). Another target is a protein that normally holds




the DNA-degrading enzyme mentioned earlier (an endonuclease) in an inactive form; its cleavage frees the endonuclease to cut up the DNA in the cell nucleus. 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 healthy 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 reaches a critical point along the path to destruction, it cannot turn back.

The caspases required for apoptosis vary depending on the cell type and stimulus. Inactivation of the mouse gene encoding caspase-3, an executioner caspase, for example, reduces normal apoptosis in the developing brain. As a result, the mouse often dies around birth with a deformed brain that contains too many cells. Apoptosis occurs normally, however, in many other organs of such mice.

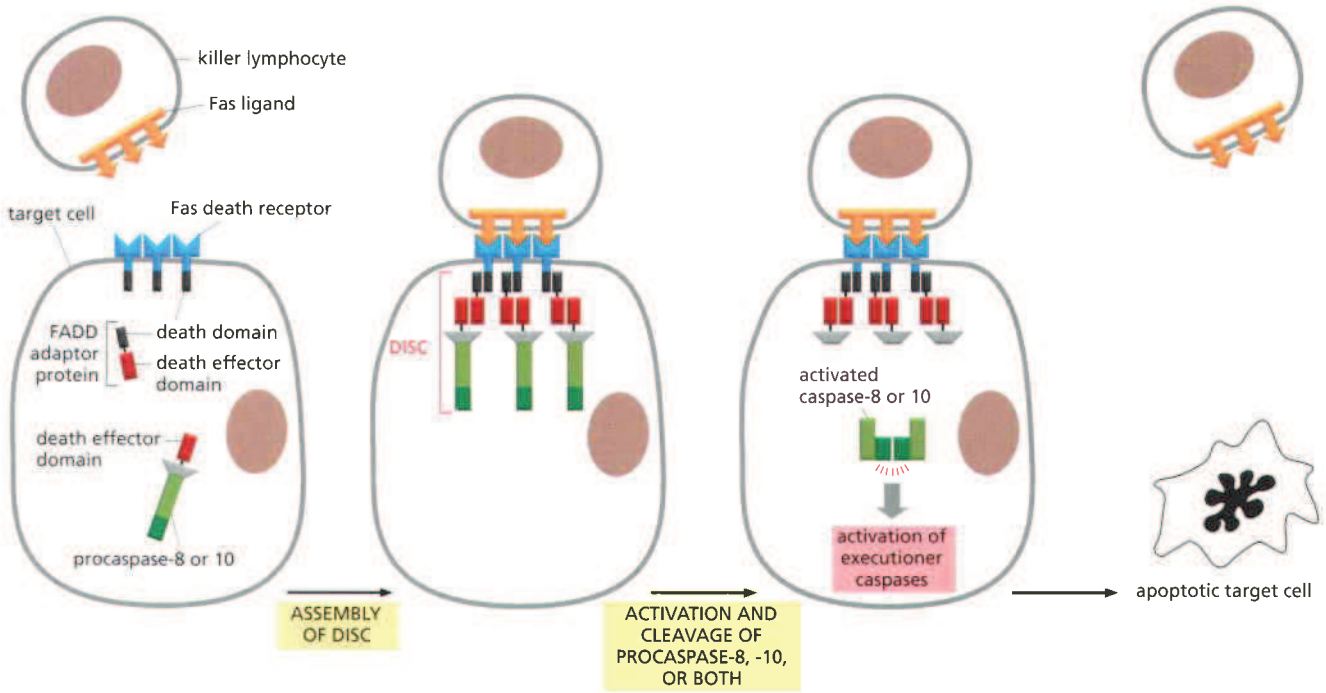
From the earliest stages of an animal's development, healthy cells continuously make the procaspases and other proteins required for apoptosis. Thus, the apoptosis machinery is always in place; all that is needed is a trigger to activate it. How, then, is a caspase cascade initiated? In particular, how is the first procaspase in the cascade activated? Initiator procaspases have a long *prodomain*, which contains a *caspase recruitment domain (CARD)* that enables them to assemble with adaptor proteins into *activation complexes* when the cell receives a signal to undergo apoptosis. Once incorporated into such a complex, the initiator procaspases are brought into close proximity, which is sufficient to activate them; they then cleave each other to make the process irreversible. The activated initiator caspases then cleave and activate executioner procaspases, thereby initiating a proteolytic caspase cascade, which amplifies the death signal and spreads it throughout the cell.

The two best understood signaling pathways that can activate a caspase cascade leading to apoptosis in mammalian cells are called the *extrinsic pathway* and the *intrinsic pathway*. Each uses its own initiator procaspases and activation complex, as we now discuss.

**Figure 18–5 Procaspase activation during apoptosis.** (A) Each caspase is initially made as an inactive proenzyme (procaspase). Some procaspases are activated by proteolytic cleavage by an activated caspase: two cleaved fragments from each of two procaspase molecules associate to form an active caspase, which is a tetramer of two small and two large subunits; the prodomains are usually discarded, as indicated. (B) The first procaspases activated are called *initiator procaspases*, which then cleave and activate many *executioner procaspase* molecules, producing an amplifying chain reaction (a proteolytic caspase cascade). The executioner caspases then cleave a variety of key proteins in the cell, including specific cytosolic proteins and nuclear lamins, as shown here, leading to the controlled death of the cell. Although not shown, the initiator procaspases are activated by adaptor proteins that bring the procaspases together in close proximity within an activation complex; although the initiator procaspases cleave each other within the complex, the cleavage serves only to stabilize the active protease.

**Table 18–1 Some Human Caspases**

| | |
|---|---|
| Caspases involved in inflammation | caspases 1 (ICE), 4, 5 |
| Caspases involved in apoptosis | |
| Initiator caspases | caspases 2, 8, 9, 10 |
| Executioner caspases | caspases 3, 6, 7 |




## 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 (discussed in Chapter 15) 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 (discussed in Chapter 25). When activated by the binding of Fas ligand, the death domains on the cytosolic tails of the Fas death receptors recruit intracellular adaptor proteins, which in turn recruit initiator procaspases (*procaspase-8, procaspase-10,* or both), forming a **death-inducing signaling complex** (**DISC**). Once activated in the DISC, the initiator caspases activate downstream executioner procaspases to induce apoptosis (**Figure 18–6**). As we discuss later, in some cells the extrinsic pathway must recruit the intrinsic apoptotic pathway to amplify the caspase cascade in order to kill the cell.

Many cells produce inhibitory proteins that act either extracellularly or intracellularly to restrain the extrinsic pathway. For example, some produce cell-surface *decoy receptors,* which have a ligand-binding domain but not a death domain; because they can bind a death ligand but cannot activate apoptosis, the decoys competitively inhibit the death receptors. Cells can also produce intracellular blocking proteins such as *FLIP,* which resembles an initiator procaspase but lacks the proteolytic domain; it competes with procaspase-8 and procaspase-10 for binding sites in the DISC and thereby inhibits the activation of these initiator procaspases. Such inhibitory mechanisms help prevent the inappropriate activation of the extrinsic pathway of apoptosis.

In some circumstances, death receptors activate other intracellular signaling pathways that do not lead to apoptosis. TNF receptors, for example, can also activate the NFκB pathway (discussed in Chapter 15), which can promote cell

**Figure 18–6 The extrinsic pathway of apoptosis activated through Fas death receptors.** Fas ligand on the surface of a killer lymphocyte activates Fas death receptors on the surface of the target cell. Both the ligand and receptor are homotrimers. The cytosolic tail of Fas then recruits the adaptor protein FADD via the death domain on each protein (FADD stands for Fas-associated death domain). Each FADD protein then recruits an initiator procaspase (procaspase-8, procaspase-10, or both) via a death effector domain on both FADD and the procaspase, forming a death-inducing signaling complex (DISC). Within the DISC, the initiator procaspase molecules are brought into close proximity, which activates them; the activated procaspases then cleave one another to stabilize the activated protease, which is now a caspase. Activated caspase-8 and caspase-10 then cleave and activate executioner procaspases, producing a caspase cascade, which leads to apoptosis.

survival and activate genes involved in inflammatory responses. Which responses dominate depends on the type of cell and the other signals acting on it.

## The Intrinsic Pathway of Apoptosis Depends on Mitochondria

Cells can also activate their apoptosis program from inside the cell, usually in response to injury or other stresses, such as DNA damage or lack of oxygen, nutrients, or extracellular survival signals (discussed later). In vertebrate cells, such intracellular activation of the apoptotic death program occurs via the **intrinsic 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 12–21A). Some of the released proteins activate a caspase proteolytic cascade in the cytoplasm, leading to apoptosis.

A crucial protein released from mitochondria in the intrinsic pathway is **cytochrome *c***, a water-soluble component of the mitochondrial electron-transport chain. When released into the cytosol (**Figure 18–7**), it has an entirely different function: it binds to a procaspase-activating 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 procaspase proteins *(procaspase-9)*, which are activated by proximity in the apoptosome, just as procaspase-8 and -10 proteins are activated in the DISC. The activated caspase-9 molecules then activate downstream executioner procaspases to induce apoptosis (**Figure 18–8**).

As mentioned earlier, in some cells, the extrinsic pathway must recruit the intrinsic pathway to amplify the apoptotic signal to kill the cell. It does so by activating a member of the *Bcl2* family of proteins, which we now discuss.

## 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 apoptosis 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 *C. elegans*.

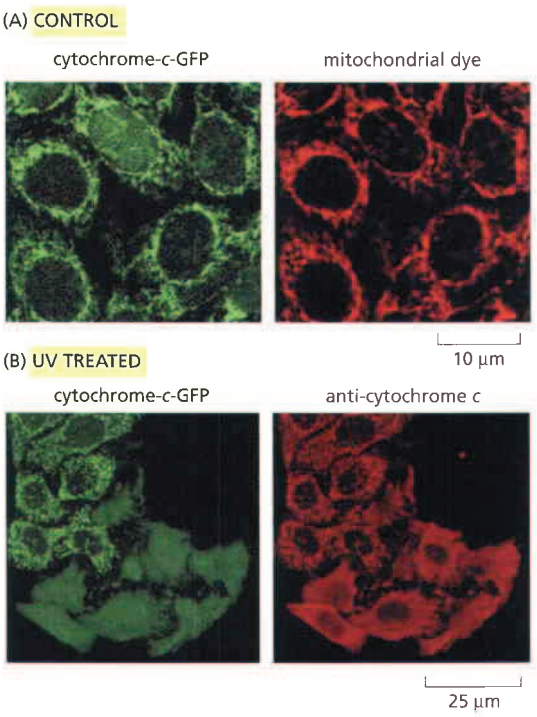



**Figure 18–7 Release of cytochrome *c* from mitochondria during apoptosis.** Fluorescence micrographs of human cancer cells in culture. (A) The control cells were transfected with a gene encoding a fusion protein consisting of cytochrome *c* linked to green fluorescent protein (cytochrome-*c*-GFP); they were also treated with a positively charged red dye that accumulates in mitochondria. The overlapping distribution of the *green* and *red* indicate that the cytochrome-*c*-GFP is located in mitochondria. (B) Cells expressing cytochrome-*c*-GFP were irradiated with ultraviolet light to induce apoptosis, and after 5 hours they were stained with antibodies (in *red*) against cytochrome *c*; the cytochrome-*c*-GFP is also shown (in *green*). The six cells in the bottom half of the micrographs in B have released their cytochrome *c* from mitochondria into the cytosol, whereas the cells in the upper half of the micrographs have not yet done so. (From J.C. Goldstein et al., *Nat. Cell Biol.* 2:156–162, 2000. With permission from Macmillan Publishers Ltd.)

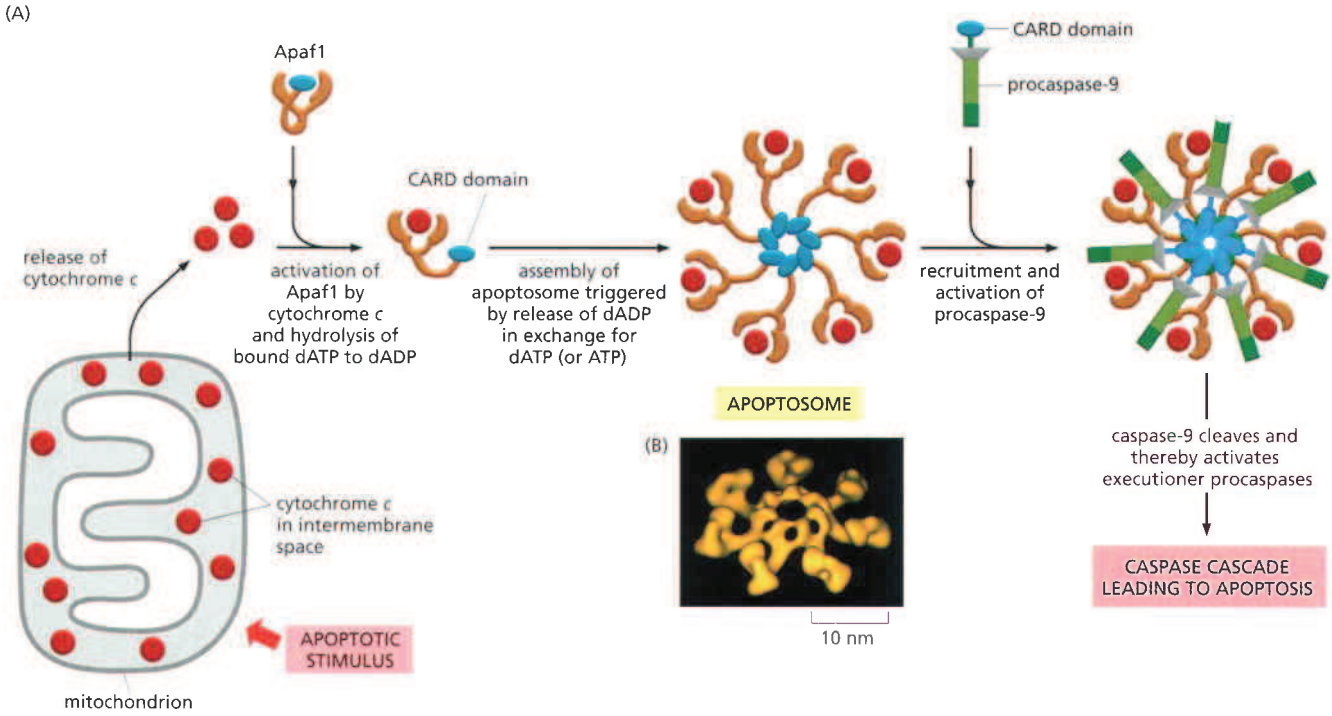



**Figure 18–8 The intrinsic pathway of apoptosis.** (A) A schematic drawing of how cytochrome *c* released from mitochondria activates Apaf1. The binding of cytochrome *c* causes the Apaf1 to hydrolyze its bound dATP to dADP (not shown). The replacement of the dADP with dATP or ATP (not shown) then induces the complex of Apaf1 and cytochrome *c* to aggregate to form a large, heptameric apoptosome, which then recruits procaspase-9 through a caspase recruitment domain (CARD) in each protein. The procaspase-9 molecules are activated within the apoptosome and are now able to cleave and activate downstream executioner procaspases, which leads to the cleavage and activation of these molecules in a caspase cascade. Other proteins released from the mitochondrial intermembrane space are not shown. (B) A model of the three-dimensional structure of an apoptosome. Note that some scientists use the term "apoptosome" to refer to the complex containing procaspase-9. (B, from D. Aceham et al., *Mol. Cell* 9:423–432, 2002. With permission from Elsevier.)

Mammalian Bcl2 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 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 Bcl2 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 proteins largely determines whether a mammalian cell lives or dies by the intrinsic pathway of apoptosis.

As illustrated in **Figure 18–9**, the anti-apoptotic Bcl2 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 proteins consist of two subfamilies—the *BH123* proteins and the *BH3-only* proteins. The main BH123 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 (see Figure 18–9).

When an apoptotic stimulus triggers the intrinsic pathway, the pro-apoptotic **BH123 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 18–10**). In mammalian cells, **Bax** and **Bak** are the main BH123 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 tightly 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. Both Bax and Bak also operate on the surface of the endoplasmic reticulum (ER) and nuclear membranes; when activated in response to ER stress, they are thought to release $Ca^{2+}$ into the cytosol, which helps activate the mitochondrial-dependent intrinsic pathway of apoptosis by a poorly understood mechanism.

The **anti-apoptotic Bcl2 proteins** such as **Bcl2** itself and **Bcl-$X_L$** are also mainly located on the cytosolic surface of the outer mitochondrial membrane, the ER, and the nuclear envelope, where they help preserve the integrity of the

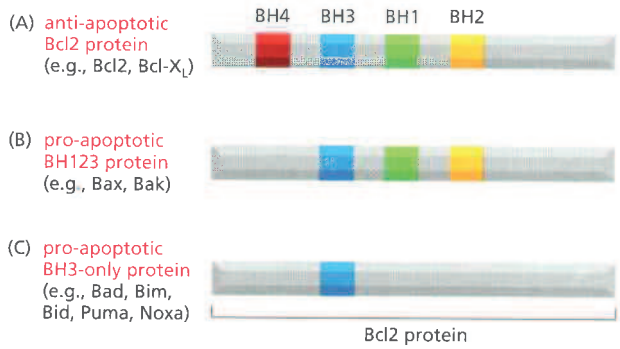



**Figure 18–9 The three classes of Bcl2 proteins.** Note that the BH3 domain is the only BH domain shared by all Bcl2 family members; it mediates the direct interactions between pro-apoptotic and anti-apoptotic family members.

membrane—preventing, for example, inappropriate release of intermembrane proteins from mitochondria and of $Ca^{2+}$ from the ER. These proteins inhibit apoptosis mainly by binding to and inhibiting pro-apoptotic Bcl2 proteins—either on these membranes 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 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 proteins. Their BH3 domain binds to a long hydrophobic groove on anti-apoptotic Bcl2 proteins, neutralizing their activity. By a poorly understood mechanism, 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 18–11**). Some BH3-only proteins may bind directly to Bax and Bak to help trigger the activation and aggregation of these BH123 pro-apoptotic proteins on mitochondria and thereby help release the intermembrane proteins.

BH3-only proteins provide the crucial link between apoptotic stimuli and the intrinsic pathway of apoptosis, with different stimuli activating different BH3-only proteins. When some cells are deprived of extracellular survival signals, for example, an intracellular signaling pathway that depends on the MAP kinase *JNK* activates the transcription of the gene encoding the BH3-only protein *Bim*, which then triggers the intrinsic pathway. 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.

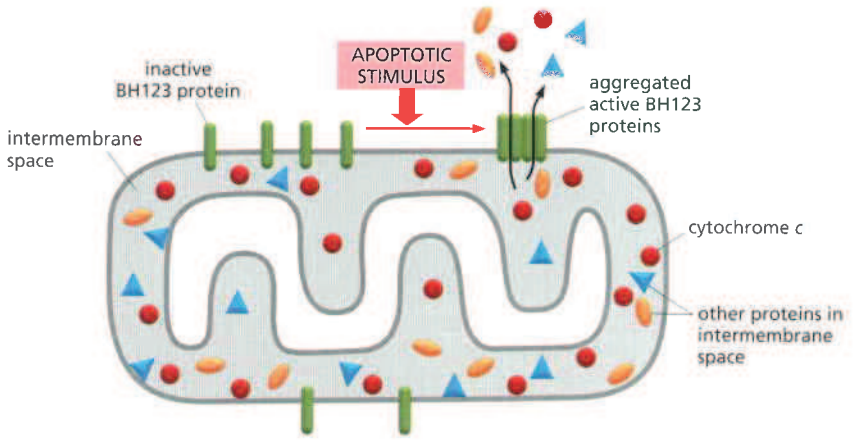



**Figure 18–10 The role of BH123 pro-apoptotic Bcl2 proteins (mainly Bax and Bak) in the release of mitochondrial intermembrane proteins in the intrinsic pathway of apoptosis.** When activated by an apoptotic stimulus, the BH123 proteins aggregate on the outer mitochondrial membrane and release cytochrome *c* and other proteins from the intermembrane space into the cytosol by an unknown mechanism.

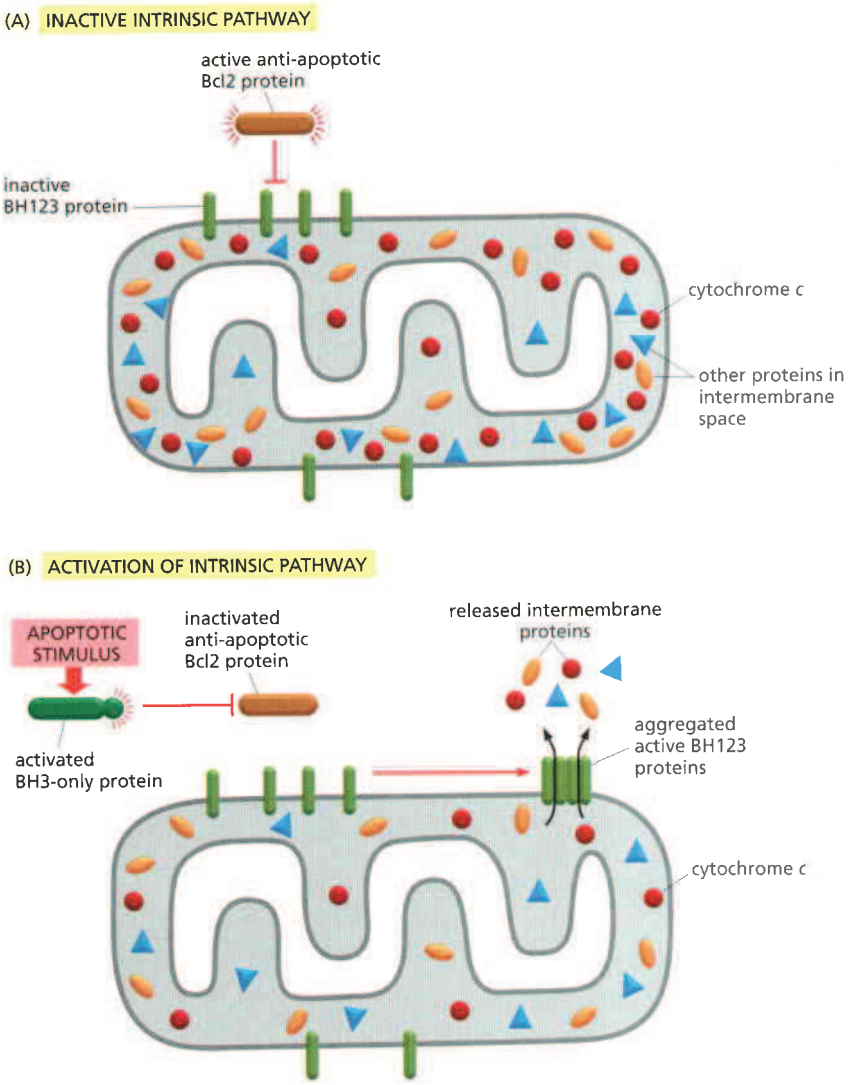



**Figure 18–11 How pro-apoptotic BH3-only and anti-apoptotic Bcl2 proteins regulate the intrinsic pathway of apoptosis.** (A) In the absence of an apoptotic stimulus, anti-apoptotic Bcl2 proteins bind to and inhibit the BH123 proteins on the mitochondrial outer membrane (and in the cytosol—not shown). (B) In the presence of an apoptotic stimulus, BH3-only proteins are activated and bind to the anti-apoptotic Bcl2 proteins so that they can no longer inhibit the BH123 proteins, which now become activated and 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 BH123 proteins. Although not shown, the anti-apoptotic Bcl2 proteins are bound to the mitochondrial surface.

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. When death receptors activate the extrinsic pathway in these cells, the initiator caspase, caspase-8, cleaves Bid, producing a truncated form of Bid called *tBid*. tBid translocates to mitochondria, where it inhibits anti-apoptotic Bcl2 proteins and triggers the aggregation of pro-apoptotic BH123 proteins to release cytochrome *c* and other intermembrane proteins, thereby amplifying the death signal.

The BH3-only proteins Bid, Bim, and Puma (see Figure 18–9) can inhibit all of the anti-apoptotic Bcl2 proteins, whereas the other BH3-only proteins can inhibit only a small subset of the anti-apoptotic proteins. Thus, Bid, Bim, and Puma are the most potent activators of apoptosis in the BH3-only subfamily of Bcl2 proteins.

Bcl2 proteins are not the only intracellular regulators of apoptosis. The IAP (inhibitor of apoptosis) proteins also play an important part in suppressing apoptosis, especially in *Drosophila*.

## IAPs Inhibit Caspases

**Inhibitors of apoptosis** (**IAPs**) 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. (Virus-infected animal cells frequently kill themselves to prevent the virus from replicating and infecting other cells.) 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 activated caspases must overcome to trigger apoptosis.

In *Drosophila* at least, this inhibitory barrier provided by IAPs can be neutralized by **anti-IAP** proteins, which are produced in response to various apoptotic stimuli. There are five 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 anti-IAP proteins in apoptosis is more controversial. As illustrated in **Figure 18–12**, 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. When, however, the genes encoding two known mammalian anti-IAPs called *Smac* (also called *DIABLO*) and *Omi* are inactivated in mouse cells, apoptosis is apparently unaffected—hence the controversy about their normal roles in regulating apoptosis.

In summary, the combined activities of the Bcl2 proteins, IAPs, and anti-IAPs determine the sensitivity of an animal cell to apoptosis-inducing stimuli, with IAPs and anti-IAPs dominant in flies and Bcl2 proteins dominant in mammals.

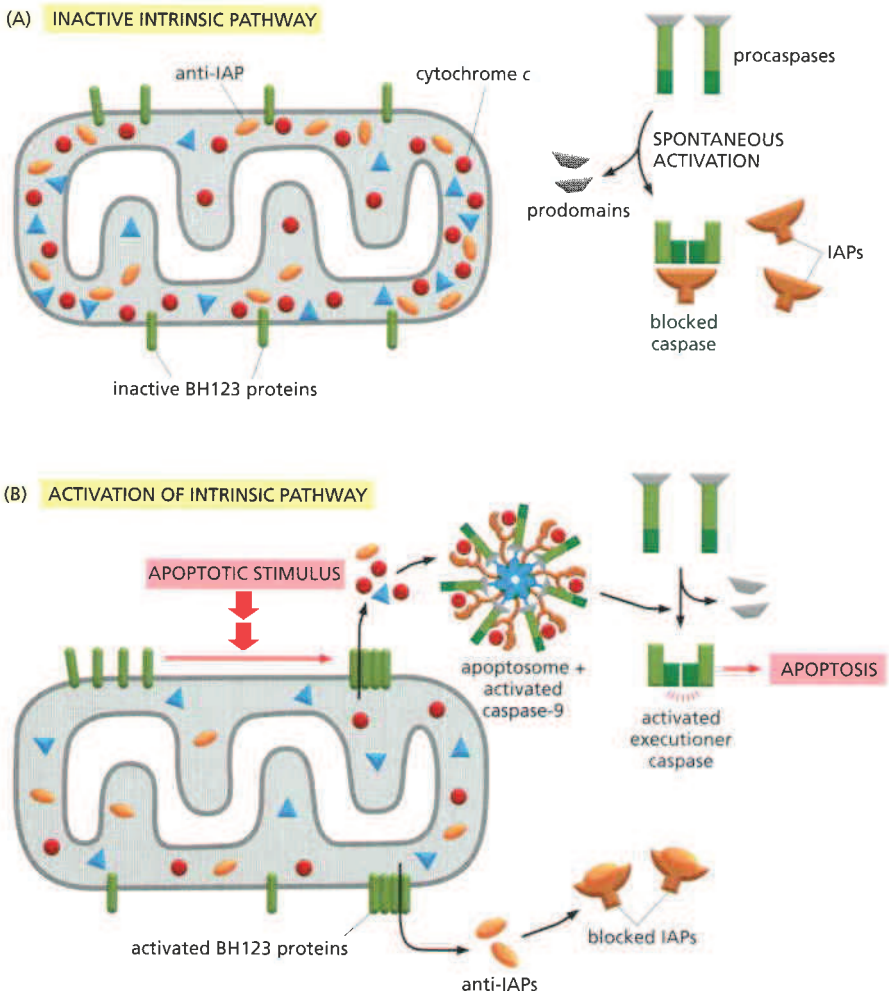



**Figure 18–12 A proposed model for the roles of IAPs and anti-IAPs in the control of apoptosis in mammalian cells.** (A) In the absence of an apoptotic stimulus, IAPs prevent accidental apoptosis caused by the spontaneous activation of procaspases. The IAPs are located in the cytosol and bind to and inhibit any spontaneously activated caspases. Some IAPs are also ubiquitin ligases that ubiquitylate the caspases they bind to, marking them for degradation in proteasomes (not shown). (B) When an apoptotic stimulus activates the intrinsic pathway, among the proteins released from the mitochondrial intermembrane space are anti-IAP proteins, which bind to and block the inhibitory activity of the IAPs. At the same time, the released cytochrome *c* triggers the assembly of apoptosomes, which can now activate a caspase cascade, leading to apoptosis.

## Extracellular Survival Factors Inhibit Apoptosis in Various Ways

As discussed in Chapter 15, 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 animal development: a surge of thyroid hormone in the bloodstream, for example, signals cells in the tadpole tail to undergo apoptosis at metamorphosis (see Figure 18–3), while locally produced bone morphogenic proteins (BMPs, discussed in Chapters 15 and 22) 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. Nerve cells that receive enough of the appropriate type of survival signal 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 18–13**). 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.

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 an increased production of anti-apoptotic Bcl2 proteins such as Bcl2 itself or Bcl-$X_L$ (**Figure 18–14**A). Others act by inhibiting the function of BH3-only pro-apoptotic Bcl2 proteins such as *Bad* (Figure 18–14B). In *Drosophila*, some survival factors act by phosphorylating and inactivating anti-IAP proteins, thereby enabling IAP proteins to suppress apoptosis (Figure 18–14C).

When mammalian cells are deprived of survival factors, they kill themselves by producing and activating pro-apoptotic BH3-only proteins, which activate the intrinsic pathway of apoptosis by overriding the anti-apoptotic Bcl2 proteins that are required to keep the cells alive. Mouse cells that lack both Bax and Bak are unable to activate the intrinsic pathway and can therefore live for weeks in culture in the absence of survival factors; without survival signals, however, the cells cannot efficiently import nutrients. Such cells fuel their metabolic needs through *autophagy*, in which the cell sequesters organelles and bits of its cytoplasm within autophagosomes, which then fuse with lysosomes (discussed in Chapter 13). The cells eventually die from starvation, but not by apoptosis.

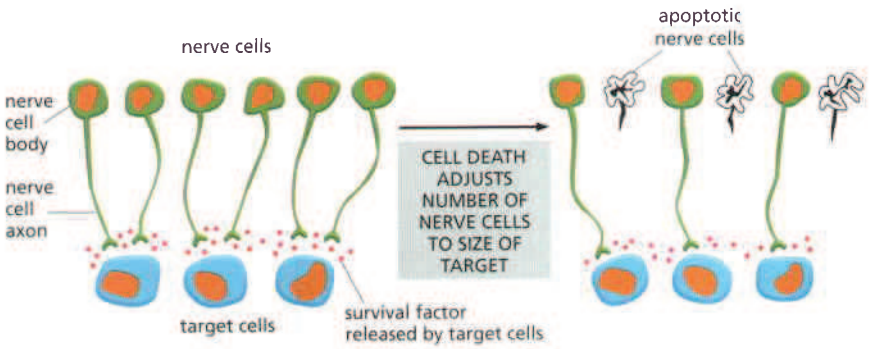



**Figure 18–13 The role of survival factors and cell death in adjusting the number of developing nerve cells to the amount of target tissue.** More nerve cells are produced than can be supported by the limited amount of survival factors released by the target cells. Therefore, some cells receive an insufficient amount of survival factors to avoid apoptosis. This strategy of overproduction followed by culling ensures that all target cells are contacted by nerve cells and that the extra nerve cells are automatically eliminated.

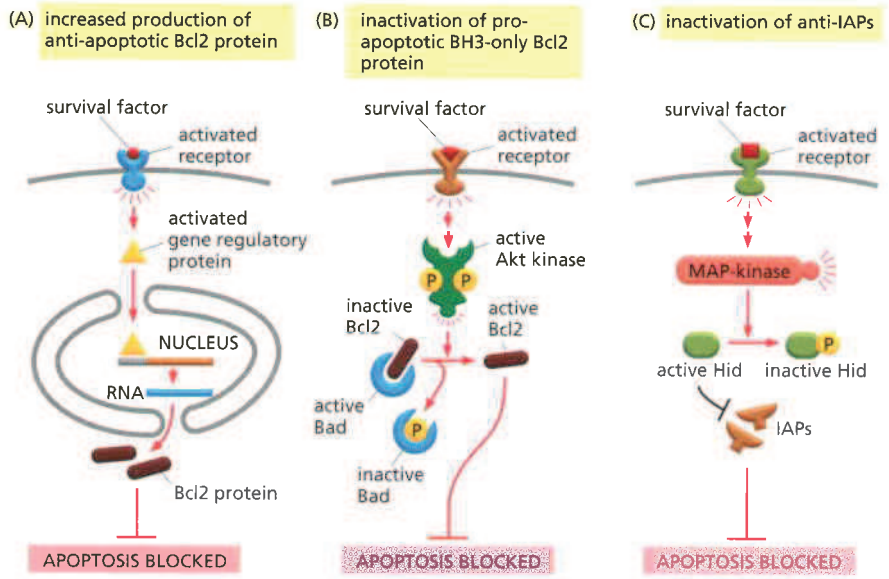



**Figure 18–14 Three ways that extracellular survival factors can inhibit apoptosis.** (A) Some survival factors suppress apoptosis by stimulating the transcription of genes that encode anti-apoptotic Bcl2 proteins such as Bcl2 itself or Bcl-$X_L$. (B) Many others activate the serine/threonine protein kinase Akt, which, among many other targets, phosphorylates and inactivates the BH3-only pro-apoptotic Bcl2 protein Bad (see Figure 15–64). 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 gene regulatory proteins of the Forkhead family 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.

## 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 such as caspase inhibitors that block apoptosis will prove useful in saving cells in these conditions.

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 the apoptotic program abnormally. <TGAA> 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 cell survival and increasing cell numbers; 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 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.

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 their behavior, including a decreased ability to adhere to the extracellular matrix and to adhere 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

*Cells can activate an intracellular death program and kill themselves in a controlled way—a process called programmed cell death. In this way, animal cells that are irreversibly damaged, no longer needed, or are a threat to the organism can be eliminated quickly and neatly. 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 depends on 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 called procaspases. Initiator procaspases are activated when brought into proximity in activation complexes: once activated, they cleave and activate downstream executioner procaspases, which activate other executioner procaspases (and various other target proteins in the cell), producing an amplifying, irreversible proteolytic cascade.*

*Cells use at least two distinct pathways to activate initiator procaspases 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 procaspases, which are activated in distinct activation complexes, called the DISC and the apoptosome, respectively. In the extrinsic pathway, the death receptors recruit procaspases-8 and 10 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 procaspase-9.*

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

# 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 UV light.

### Discuss the following problems.

**18–3** One important role of Fas and Fas ligand is to mediate 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 programmed cell death. Classical genetic experiments in *C. elegans* isolated mutants that identified the first genes involved in apoptosis. Of the many mutant genes 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 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?

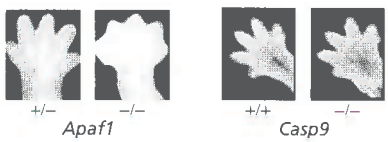



**Figure Q18–1** Appearance of paws in *Apaf1*$^{-/-}$ and *Casp9*$^{-/-}$ newborn mice relative to normal newborn mice (Problem 18–6). (From H. Yoshida et al., *Cell* 94:739–750, 1998. With permission from Elsevier.)

**18–7** When human cancer (HeLa) cells are exposed to 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.

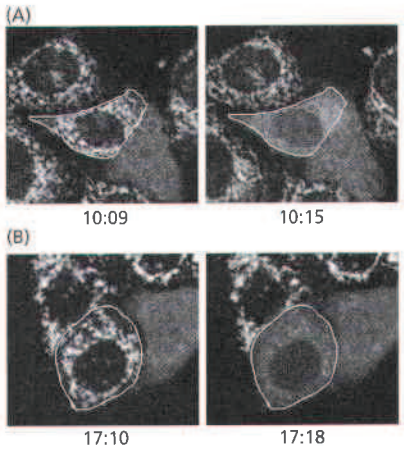



**Figure Q18–2** Time-lapse video, fluorescence microscopic analysis of cytochrome *c*–GFP release from mitochondria of individual cells (Problem 18–7). (A) Cells observed for 8 minutes, 10 hours after UV irradiation. (B) Cells observed for 6 minutes, 17 hours after UV irradiation. One cell in (A) and 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.)

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