

# 15

## Apoptosis

Kerr, Wyllie, and Currie coined the term *apoptosis* in an article that appeared in the *British Journal of Cancer* in 1972. The term was taken from the Greek and has the meaning of “falling off” as in the dropping of petals from flowers, and leaves from trees, which occur in a programmed manner every autumn. Apoptosis, or programmed cell death, is widespread during animal development where it is used to sculpt and refine tissues and organs. Apoptosis makes possible the regression of tadpole tails, permitting the emergence of an adult tailless form. It is used to remove larval organs in insects, and to sculpt digits—fingers and toes—from undelineated limb buds in mammals.

Apoptosis is widely encountered during development of the nervous system. Some neurons are only transiently formed, and once their task is completed they are removed. In many parts of the nervous system, cells are initially overproduced. This overproduction ensures that there is adequate input to target neurons during the initial phase of the nervous system connection. Excess cells are removed by numerically matching pre- and post-synaptic structures. The initial set of neural connections is refined, and cells that are inappropriately wired are removed. In some neural populations, most of the cells are removed in order to produce a set of precise neural connections.

Apoptosis is an essential ingredient in the operation of the immune system. Cells of the immune system such as B cells die off as they age and when they are no longer needed. Virally infected cells, and damaged cells that cannot be repaired, are targeted for apoptosis to prevent their harming undamaged cells in the tissue or organ where they reside. The same strategy is used by the immune system to rid the body of cancer cells.

Apoptosis is different from necrosis. The plasma membrane does not rupture in apoptosis as it does in necrosis, but instead cellular components are degraded and packaged, and then digested. During apoptosis cells undergo an orderly sequence of morphological changes. These changes include cell shrinkage, chromatin condensation, DNA fragmentation, and membrane blebbing, in which membrane-wrapped pieces of cell boil off of

the cell surface as apoptotic bodies containing fragments of DNA and other macromolecules.

Malfunctions in the cellular machinery that controls apoptosis are encountered in many disease situations. Excessive apoptotic cell death occurs in Alzheimer's disease, Parkinson's disease, Huntington's disease, and ALS (Lou Gehrig's disease). Too little cell death is a hallmark of cancer. In B-cell leukemia, for example, key regulators of the decision circuitry that determines whether a cell survives or dies are overexpressed. Apoptosis is suppressed and because population control is lost leukemia develops.

## 15.1 Caspases and Bcl-2 Proteins Are Key Mediators of Apoptosis

Apoptosis is largely carried out by *caspases*, proteolytic enzymes that catalyze the cleavage of specific molecules and groups of molecules in response to activating signals. Caspases target critical repair, splicing, and replication components, they cut up membranes and cytoskeleton regulators, and they destroy cellular DNA. They also stimulate the expression of markers on the cell surface that tag the cell for orderly destruction and engulfment by neighboring cells. This orderly disassembly of a cell occurs in a way that prevents damage due to leakage.

*Bcl-2 proteins* are a second group of proteins intimately involved in apoptosis. They function as sensors and regulators of the apoptosis program. They were first identified in B-cell lymphomas and, since then, mutated forms have been found in a variety of cancers. These proteins are characterized by the presence of one or more Bcl-2 homology (BH) domains, the ability of some to form pores in internal membranes, and their propensity to either promote or inhibit the release of apoptotic signals and agents from the internal membranes.

Apoptosis can be initiated by death signals sent into the cell from other cells and by stress signals generated within the cell. Signals sent by other cells instructing a cell to undergo apoptosis are received by death receptors belonging to the tumor necrosis factor (TNF) family. When a death ligand binds the death receptors, a death inducing signaling complex is formed that initiates the apoptosis process. Death signals are also triggered by cellular stresses detected internally in organelles such as the endoplasmic reticulum, Golgi, nucleus, and mitochondria. Apoptosis signaling is sent in response to conditions such as irrevocable DNA damage in the nucleus, unfolded protein stresses in the ER, and oxidative stresses in the mitochondria. Two loci, one within the mitochondria and the other just outside that organelle, serve as the main control points for the launching of apoptotic responses.

## 15.2 Caspases Are Proteolytic Enzymes Synthesized as Inactive Zymogens

The activity of enzymes that chop up and digest molecules is tightly controlled in the cell. One common strategy for controlling proteolytic enzymes, or *proteases*, is to synthesize them in an inactive form that requires further processing for their activation. One common kind of inactive form is a *zymogen*, a proenzyme containing a prodomain that must be removed in order to create an active form of the enzyme. Zymogens are converted to catalytically active forms by their proteolytic cleavage into two or three pieces followed by assembly of the catalytic subunits into complexes. Examples of proteases synthesized as zymogens include digestive enzymes that reside in the stomach (pepsin) and pancreas (trypsin), and also include blood-clotting enzymes (thrombin).

Caspases are cysteine aspartate-specific proteases. They break peptide bonds after Asp residues, i.e., at Asp-X sites, and possess a highly conserved cysteine residue in their catalytic site. Caspases are synthesized as zymogens. They contain a prodomain in the amino terminal region that regulates the proenzyme, followed by a large domain, approximately 20kDa in size, and then a small domain, about 10kDa in size. The proenzyme is activated by proteolytic cleavage at two Asp-X sites, one situated at the end of the prodomain and the other separating the large and small domains (Figure 15.1).

The tetramer is the functional (active) form of the caspase. It is constructed in several stages. In the first stage, two zymogens associate to form a zymogen homodimer. Adjacent large and small subunits (left hand pair and right hand pair) are part of the same polypeptide chain with the small units placed on the inside and the large subunits on the outside. In the next stage, each of the polypeptide chains making up the caspase zymogen dimer is cleaved at the Asp-X sites. These cuts induce conformational changes in the subunits that are part of the caspase activation process because they bring the caspases closer to conformation supporting catalysis. The similarity in conformations can be seen in Figure 15.2 where zymogen and caspase homodimers are compared side-by-side. Differences in the crucial loops L1

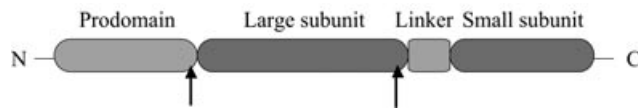


FIGURE 15.1. Caspase domain structure: The N-terminal prodomain is followed by a large subunit, a linker, and a small subunit. The location of the two Asp-X cleavage sites is indicated in the figure by arrows. Following cleavage, two large and two small subunits associate to form a caspase tetramer with the two small subunits inside and two large subunits on the outside.

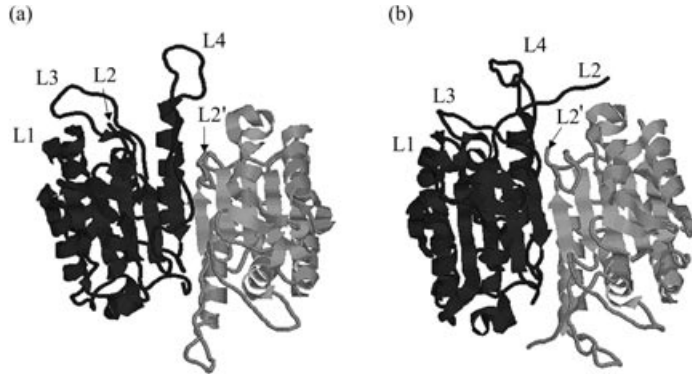


FIGURE 15.2. Structure of caspase-7 homodimers as determined by means of X-ray diffraction measurements: (a) Procaspase-7 zymogen homodimer, and (b) Caspase-7 homodimer. The figures were prepared using Protein Explorer using atomic coordinates deposited in the Brookhaven Protein Data Bank under accession codes 1K88 (a) and 1K86 (b).

to L4 that determine the catalytic cleft are clearly visible in the left and right hand panels of the figure. The caspases are still not fully activated in the more open caspase form, but a final set of changes occurring during substrate binding renders the caspases fully active.

### 15.3 Caspases Are Initiators and Executioners of Apoptosis Programs

More than a dozen different kinds of caspases have been identified in mammals. Those that have been found in humans have been placed in one of three groups in Table 15.1. Caspases belonging to Group I are associated with inflammatory responses. These caspases were first named for interleukin-1 $\beta$  converting enzyme (ICE), and then renamed caspases 1, 4, and 5. These enzymes process pro-inflammatory cytokines. The remaining two groups of caspases are specifically associated with apoptosis, either as initiators that convey signals through their proteolytic actions or as effectors that degrade cellular components. Group II caspases are effectors. They proteolytically degrade a variety of cellular components and are thus the executioners of the apoptosis program.

Group III caspases are apoptosis initiators. They act upstream of the effectors and activate them in response to proapoptotic signals and events. The four caspases appearing as Group III caspases all have large pro-domains in which there is either a DED (death effector domain) or a CARD (caspase recruitment domain) protein-protein interaction domain.

TABLE 15.1. Mammalian caspases and their roles in the cell: Group III initiator caspases act upstream of the Group II effector caspases. The executioners have small prodomains and require assistance of the initiators for their activation. Four element consensus sequences that the caspases recognize and cleave are presented in column 4. The most conserved residue in the consensus sequence is the Asp (D) residue proximal to the cleavage site while the residue in the fourth position mostly determines the substrate specificity.

Group	Caspase	Consensus sequence	Prodomain
I: ICE	1	(WL)EHD	Large, CARD
	4	(WL)EHD	Large, CARD
	5	(WL)EHD	Large
II: Effectors	3	DExD	Small
	6	(ILV)ExD	Small
	7	DExD	Small
III: Initiators	2	DExD	Large, CARD
	8	(ILV)ExD	Large, DED
	9	(ILV)ExD	Large, CARD
	10	(ILV)ExD	Large, DED

These regulatory sequences target the procaspases either to adapters bound to death receptors at the cell surface or to adapters positioned near mitochondria. At these locations the initiator caspases are positioned to respond to proapoptotic signals. Caspases 8 and 10 contain a pair of DEDs. Caspase 2 and 9 contain CARDS. The effector (Group II) caspases do not have a large prodomain in their N-terminus but instead possess a small N-terminal peptide. One other initiator caspase has been found—Caspase 12, a murine (mouse) caspase that is localized to the endoplasmic reticulum.

## 15.4 There Are Three Kinds of Bcl-2 Proteins

Bcl-2 proteins are central regulators of caspase activity and of the cell decision concerning whether or not to undergo apoptosis. Bcl-2 proteins can be grouped into three subfamilies according to their domain structure and their pro- or antiapoptotic activities (Table 15.2). The defining characteristic of the Bcl-2 proteins is the presence of one or more BH domains. The Bcl-2 and Bax subfamilies contain multiple BH domains while the Bad subfamily only possesses a BH3 domain.

There are four kinds of BH domains, designated as BH1 through BH4. A typical Bcl-2 subfamily member contains at least three of the BH domains, namely, BH1, BH2, and BH3. In addition, it has a hydrophobic tail that anchors the protein to the outer membrane of mitochondria, the endoplasmic reticular membrane, and the outer nuclear envelope. Members of the Bcl-2 subfamily inhibit apoptosis by restricting membrane permeabil-

TABLE 15.2. Bcl-2 family of apoptosis regulators: Listed are the numbers of amino acid residues in the proteins.

Bcl-2 subfamily:		Bax subfamily:		Bad subfamily:	
Antiapoptotic	size (aa)	Proapoptotic	size (aa)	Proapoptotic	size (aa)
A1	172	Bak	211	Bad	197
Bcl-2	239	Bax	192	Bid	195
Bcl-xL	233	Bcl-xS	170	Bik	160
Bcl-w	193	Bok	213	Bim	196
Boo	191			Blk	150
Mcl-1	350			Bmf	186
				Hrk	91
				Noxa	103
				Puma	193

ity through interactions with mitochondrial membrane components and by binding to and sequestering members of the proapoptotic Bax group.

The proapoptotic Bax subfamily consists of proteins that possess a BH3 domain, a hydrophobic transmembrane tail, and at least one other BH domain. Some have a BH4 domain; others have BH1 and BH2 domains and perhaps a BH4 domain. Their 3-D structure is similar to pore-forming bacterial toxins. The Bax proteins interact with the proteins embedded in the outer mitochondrial membrane to increase membrane permeability, and they can form pores by themselves in membranes when they oligomerize.

The pro- and antiapoptotic, multi-BH domain proteins have electrostatic and structural properties that enable them to not only operate in the cytoplasm but also insert into the membrane to make pores. Their polypeptide chains are organized into sets of eight  $\alpha$ -helices. There are three layers of two  $\alpha$ -helices and a pair of short capping helices at one end of the chain. The organization of the protein and the correspondence between helices and BH domains is presented in Figure 15.3. Several structural features support pore forming. The structure is fairly flexible and so can rearrange itself with little energy penalty. Two of the helices— $\alpha 5$  and  $\alpha 6$ —are able to span the membrane. There are several disordered, flexible regions, and there are three cavities. Charge-wise, the bottom of the protein is lined with basic (positively charged) residues that complement the acidic (negatively charged) membrane surface, and there is a pronounced hydrophobic cleft surrounded by basic residues. The picture that emerges from examinations of these structures is that of  $\alpha 5$  and  $\alpha 6$  along with the corresponding  $\alpha 5$  and  $\alpha 6$  helices from dimerization partners forming a pore, with  $\alpha 2$  to  $\alpha 4$  forming a binding groove, and the C-terminus forming an anchor.

The Bad subfamily is referred to as the *BH3-only family*. Some members of this group have a transmembrane anchor while others do not. When activated by proapoptotic stimuli, these proteins translocate to the mitochondria and stimulate apoptotic responses. BH3-only proteins function as cellular sentinels. In unstressed cells Bid, Bim, and Bmf are immobilized in

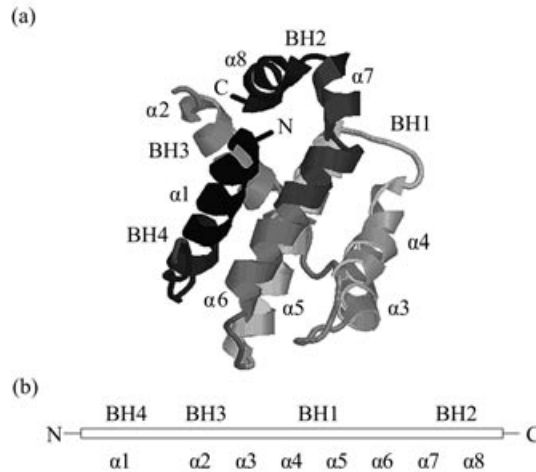


FIGURE 15.3. Structure of the antiapoptotic protein Bcl- $x_L$ : Shown in part (a) of the figure is a ribbon diagram of the portions of the protein whose structure could be determined through X-ray crystallography (i.e., highly disordered regions are not included in the model). Gray-scale shadings highlight the four BH regions. The correspondence between BH regions and the eight  $\alpha$ -helices is presented in part (b) of the figure. The figure was generated using Protein Explorer from Brookhaven Protein Data Bank entry 1AF3.

the cytoplasm. Bid is a sensor of death signals sent into the cell through the death receptors, and is localized in the vicinity of the death receptors. Bim is sequestered at microtubule associated myosin V motors where it awaits activation by cytokine and other stress signals. Bmf is also immobilized at myosin V motors where it responds to loss of cell attachment (*anoikis*) signals. Two other BH3-only proteins, Bik and Blk, function in the endoplasmic reticulum as sensors of cellular stress. The remaining BH3-only proteins are regulated at the transcriptional level. Noxa and Puma are transcribed in a p53-dependent manner and may be regarded as DNA damage sensors, while Hrk and Bim are upregulated in response to growth factor deprivation and cytokine withdrawal.

## 15.5 How Caspases Are Activated

Caspases are activated by external suicide instructions and internal stress signals. Cells receive death instructions from other cells. These messages are conveyed by cell-to-cell messengers called *death ligands*. The messages are received by death receptors embedded in the plasma membrane. The death messages are transduced into the cell interior through a multiprotein signaling complex formed by the activated death receptors. This complex is

called the *death-inducing signaling complex*, or DISC. The DISC is the control point for external signal activation of initiator Caspases 8 and 10 and signal to the BH3-only sensor protein Bid.

The counterpart to DISC for internal stress signals is a signaling complex called the *apoptosome*. This multiprotein signaling complex is formed just outside the mitochondria in response to internal stress signals. Initiator caspase 9 is activated at this control point in response to the stress-induced release of proapoptotic factors from the mitochondria. The release of the mitochondrial factors is triggered by activity at the mitochondrial control point called the *permeability transition pore complex*, or PTPC, where multidomain Bcl-2 proteins are localized and BH3-only sensor proteins converge when activated.

Several families of positive and negative regulators control the caspase machinery. The regulatory proteins ensure that apoptosis is not triggered inappropriately in response to random perturbations and aberrant signals. External and internal signals are integrated together, and both contribute to the live or die decision. If strong signals are sent into the cell instructing it to undergo apoptosis, and strong stress signals are also present within the cell, the decision is fairly simple—caspases will be activated and the cell will die. If, as is normally the case, neither external nor internal death signals are present the cell will live. All other situations are more complex. Cellular context comes into play through adjustments in the expression levels of the positive and negative regulators that determine the set, balance, or commitment point for apoptosis. The remainder of the chapter is devoted to exploring how the apoptosis control system works.

## 15.6 Cell-to-Cell Signals Stimulate Formation of the DISC

The death receptors that transduce the death messages into the cell belong to the tumor necrosis factor (TNF) superfamily. The TNF superfamily in humans includes 19 Type II ligands, single-pass transmembrane proteins with cytoplasmic N-terminals, and at least 29 receptors, mostly Type I (extracellular N-terminals). Recall from Chapter 9 that these receptors are widely expressed in the immune system where they respond to variety of growth, proliferation, and death signals. Their extracellular region is characterized by the presence of from 2 to 5 repeats of a cysteine-rich motif containing a number of disulfide bridges. Their cytoplasmic portions contain docking sites for several different kinds of adapters that mediate the recruitment of key signaling elements to the receptors.

The death receptor family includes the TNF- $\alpha$ , Fas/Apo-1, and TNF-related apoptosis-inducing ligand (TRAIL) receptors (Table 15.3). The receptors and ligands operate as homotrimeric proteins. Signaling begins when a trio of ligands binds to a trio of receptors. This event triggers the



TABLE 15.3. Members of the TNF family of receptors containing death domains in their cytoplasmic region: Abbreviations—Death receptor (DR); TNF-related apoptosis inducing ligand (TRAIL).

Death receptor	Alternative name(s)
Fas	Apo-1, CD95
TNF R1	CD120a
DR3	Apo-3
TRAIL R1	Apo-2, DR4
TRAIL R2	DR5
DR6	

recruitment of the adapters to the cytoplasmic portion of the receptors leading to the assembly of a DISC. In forming a DISC, the TNF-associated death domain (DD) proteins are first recruited to the cytoplasmic portion of the TNF receptors and bind by means of their death domains. Proteins with death effector domains (DEDs) bind next, and other binding events follow. In this manner, a DISC is formed with FADD, TRAF2, TRADD adapters serving as a starting point for three pathways—Caspase 8, NF- $\kappa$ B, and MAP kinase, respectively.

## 15.7 Death Signals Are Conveyed by the Caspase 8 Pathway

The Caspase 8 pathway (Figure 15.4) begins when the Fas-associated death domain (FADD) protein is recruited to the nascent DISC. Zymogens with large prodomains such as Caspase 2, Caspase 8, and Caspase 10 are brought into close proximity with one another at the FADDs and as a result can form zymogen dimers and act on themselves to remove their prodomains. Several caspase molecules can enter, become activated, and leave, one after the other. Once activated these enzymes make contact with and activate the effector caspases such as Caspase-3.

One of the Caspase-3 substrates is the caspase-activated deoxyribonuclease (CAD) protein and its inhibitor ICAD. The CAD and ICAD proteins are the catalytic and regulatory subunits of a protein referred to as the *DNA fragmentation factor*, or DFF. The ICAD subunit remains bound to the CAD subunit in the absence of Caspase 3 activities and inhibits its enzymatic actions. Caspase 3 cleaves the ICAD thereby freeing the CAD DNase and allowing it to move into the nucleus where it cleaves chromatin.

The BH3-only protein, Bid, is sited at the DISC. It functions as a sensor and as part of the circuitry that integrates externally and internally generated signals. As indicated in Figure 15.4, Caspase 8 cleaves the 22-kDa Bid sensor protein to create the 15-kDa tBid. Once formed, the tBids translo-

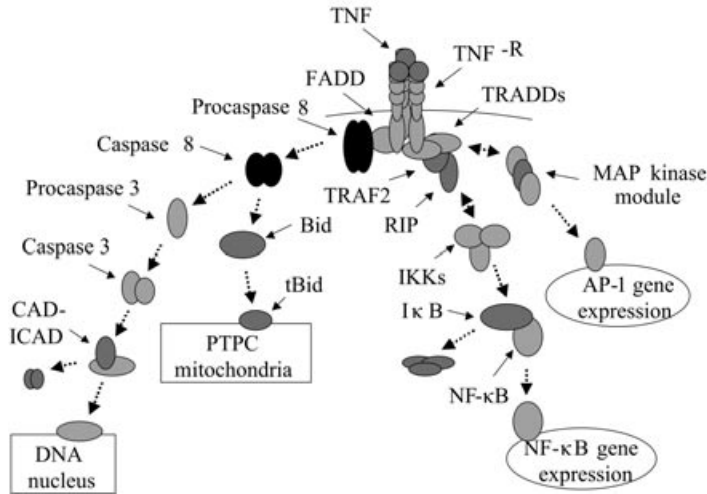


FIGURE 15.4. Signaling through the DISC located at the plasma membrane: Depicted are the main positive-regulating signaling elements. Homotrimeric TNF ligands bind homotrimeric TNF receptors. In response, several adapter molecules are recruited to the cytoplasmic portion of the receptors. The FADD adapter mediates recruitment and activation of the Caspase 8 pathway. The TRAF2 and RIP proteins mediate signaling and activation of the IKKs, which disinhibit NF- $\kappa$ B from the IKKs. The TRADDs also signal to the JNK MAP kinase cascade.

cate to the mitochondrial PTPC where they promote the activities of proapoptotic Bcl-2 proteins. If the overall mix of BH proteins at the PTPC favors apoptosis, proapoptotic factors are released from the mitochondria leading to stimulation of the apoptosome and the sequential activation of Caspase 3 and then Caspase 6, which further stimulates Caspase 8 activities in situations where the externally driven stimulation is weak.

## 15.8 How Pro- and Antiapoptotic Signals Are Relayed

Pro- and antiapoptotic signals are relayed to the nucleus by NF- $\kappa$ B proteins and MAP kinases. The two other pathways activated by death ligand relay signals via NF- $\kappa$ B proteins and MAP kinase modules as is usual for other members of the TNF superfamily. These pathways were discussed earlier in Chapter 9. Downstream signaling proteins establish contact with receptor interacting proteins (RIPs) and tumor necrosis factor receptor associated factor (TRAFs) that are recruited into the DISC. These pathways promote the expression of both pro- and antiapoptotic genes. The NF- $\kappa$ B module usually, but not always, acts to promote survival by raising the threshold for

apoptosis. The IKKs are the key point of convergence of a variety of regulatory signals triggered by cellular stresses. The IKKs are activated when recruited to and phosphorylated at the DISC. They, in turn, phosphorylate the I $\kappa$ Bs, resulting in the activation of NF- $\kappa$ B. In their prosurvival mode, the NF- $\kappa$ B dimers translocate to the nucleus where they stimulate transcription of negative regulators of not only DISC signaling but also of mitochondrial proapoptotic signaling elements. As a consequence, the balance between pro- and antiapoptotic factors is shifted in favor of the antiapoptotic ones and apoptosis is prevented.

Recall from Chapter 9 that MAP signaling pathways convey stress (JNK and p38) and growth (ERK) signals from the plasma membrane to the nucleus where they influence transcription of a different sets of target genes. As shown in Figures 9.4 and 15.4, the JNK pathway begins in the DISC, where the TRADDs recruit and activate MEKK1, the first of the kinases in the MAP kinase cascade. The last kinase in the cascade is JNK. Once activated this kinase translocates to the nucleus where it phosphorylates members of the AP-1 family of transcription factors. A similar set of signaling steps occurs in the p38 pathway.

Transcription factors such as AP-1 family members and NF- $\kappa$ B reflect cellular conditions and prior signaling events in their transcriptional activities. Depending on the specific mix of coactivators and corepressors present, subunit composition, and the set of residues that have been phosphorylated (and acetylated), these transcription factors will either promote or inhibit apoptosis. For instance, the c-Jun transcription factor, an AP-1 family member activated at the end of the MAP kinase cascade, usually functions as a transcription activator, but can also function as transcription repressors when associated with corepressors. In NF- $\kappa$ B signaling, flexibility of response is provided by variations in subunit composition. Depending on Rel subunit composition, NF- $\kappa$ B will either promote apoptosis by expressing TRAIL receptors or inhibit apoptosis by expressing antiapoptotic survival factors.

## 15.9 Bcl-2 Proteins Regulate Mitochondrial Membrane Permeability

Mitochondria occupy a central place in internal stress-induced apoptosis. As noted earlier in the chapter, the PTPC located in the mitochondria serves as a key control point for internal stress responses. The PTPC, or alternatively, the permeability transition pore (PTP), is formed at points of contact between the inner and outer mitochondrial membranes. These complexes are a conduit for the passage of agents such as Cytochrome c and Smac/DIABLO that trigger apoptosome assembly and activation of Caspase 9 (Figure 15.5). The PTPC encompasses the crucial inner membrane (IM) and outer membrane (OM) proteins along with key constituents

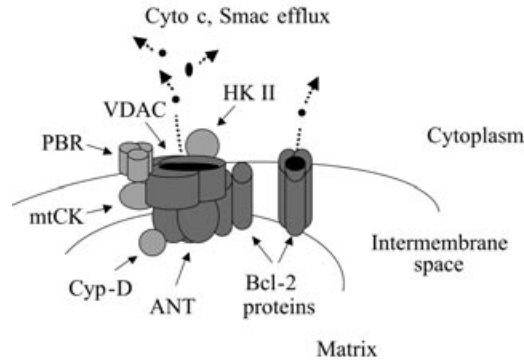


FIGURE 15.5. Central components of the mitochondrial PTPC: Depicted in the figure are the central elements—the ANT located in the inner membrane and the VDAC situated in the outer membrane. They form a pore through which Cyto c, Smac/DIABLO, and a number of different effector molecules can diffuse through and into the cytoplasm. Also shown are a variety of key Bcl-2 family members. A proapoptotic Bcl-2 protein, for example, Bax, is depicted bound to the ANT/VDAC, having displaced an antiapoptotic Bcl-2 protein, while several other Bax/Baks form a pore.

of the intermembrane space and the matrix. It is composed of voltage-dependent anion channels (VDACs) located in the outer mitochondrial membrane and the adenosine nucleotide translocator (ANT) situated in the inner membrane. The PTPC also includes the peripheral benzodiazepine receptors, creatine kinases, hexokinase II, and cyclophilin D.

The mitochondrial PTPC is the main target of Bcl-2 signaling and is the main site of their pro- and antiapoptotic actions. If a molecule can cross a membrane by means of simple passive diffusion, driven only by a concentration gradient, then that membrane is permeable to that molecule. In apoptosis, the Bcl-2 proteins regulate the permeability of the mitochondrial membrane to the apoptosis-promoting molecules. Bcl-2 proteins act as sensors of stress signals and as regulators of mitochondrial membrane permeability. They regulate membrane permeability by binding to and altering the pores formed by the ANT and VDAC, and by oligomerizing and forming pores by themselves.

BH3-only proteins stimulate the release from mitochondria of Cytochrome c and other apoptosis-promoting factors. The BH3-only protein tBid, for example, promotes the permeability-increasing activities of Bax and Bak proteins, stimulates the remodeling of the mitochondrial cristae, and triggers the release of Cytochrome c. Members of the anti-apoptotic branch of the family inhibit apoptosis by sequestering BH3-only proteins thereby preventing their stimulation of Bax and Bak. There are two kinds of BH3 protein actions. Bid-like BH3 proteins facilitate the oligomeriza-

tion of Bax and Bak, while Bad-like BH3 proteins bind Bcl-2s and displace Bid-like peptides from them.

## 15.10 Mitochondria Release Cytochrome c in Response to Oxidative Stresses

Oxidative phosphorylation is carried out in mitochondria. One of the key agents in this process is Cytochrome c, an evolutionary ancient electron carrier. It is a small protein, consisting of 104 amino acid residues and a covalently attached heme group. Cytochrome c is part of the machinery that transfers electrons through several protein complexes located in the inner mitochondrial membrane. It carries electrons from the cytochrome reductase complex to the cytochrome oxidase complex. The electron transport activity leads to the pumping of protons from the matrix side to the cytoplasmic side of the inner mitochondrial membrane. The biochemical process, oxidative phosphorylation, generates ATP by utilizing the energy released during the electron transfer from NADH or FADH<sub>2</sub> to O<sub>2</sub>.

Recall that atoms in stable molecules are tied together by bonds consisting of pairs of electrons, one with spin up and the other with spin down. When bonds are broken the molecules may end up with one or more unpaired electrons. Molecules possessing unpaired electrons are referred to as *free radicals*. The presence of an unpaired electron renders the molecules highly reactive. Because of the presence of an electron the molecule has a propensity to “steal” electrons from other molecules, in many cases breaking bonds to acquire the electrons. Chain reactions can be produced in the cell, and free radicals are highly dangerous. Most commonly encountered free radicals in the cell involve oxygen and these are called *reactive oxygen species* (ROS).

Mitochondria are the major cellular source of reactive oxygen species. In mitochondria, a small fraction, perhaps 2 to 5%, of the molecular oxygen being reduced by the respiratory electron transport chain is converted to superoxide (O<sub>2</sub><sup>-</sup>) and then to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) or to the hydroxyl radical (OH<sup>-</sup>). The ROS cause cellular (oxidative) stresses because they can oxidize DNA, proteins, and lipids. The presence of ROS influences cellular physiology in many ways and triggers protective reactions involving the upregulation of antioxidants and detoxifying enzymes. Antioxidants are free radical scavengers. They supply electrons to the free radicals, allowing them to form bonds and converting them nonreactive forms.

Cytochrome c is loosely bound to the inner mitochondrial membrane by cardiolipin and other anionic lipids. When ROS levels rise it disrupts the binding of Cytochrome c thereby making it easier to release. Once Cytochrome c is released it stimulates assembly of the apoptosome leading to activation of Caspase 9 and Caspase 3. Thus, oxidative stress conditions arising when ROS activity levels in mitochondria become excessive and are no longer controlled by antioxidant scavengers are signaled by Cytochrome

c release from the mitochondria. The Cytochrome c molecules act as local signaling molecules that convey stress messages from the mitochondria to the apoptosome.

### 15.11 Mitochondria Release Apoptosis-Promoting Agents

Several different kinds of molecules are sent out through the PTPC, as indicated in Table 15.4. Cytochrome c and deoxyadenosine triphosphate (dATP) convey signals that are required for assembly of the apoptosome and the activation of Caspase 9. Two other regulators of the apoptosome, Smac/DIABLO and Omi/HtrA2 are also released from the mitochondria. A number of apoptotic effectors are released in addition to the activators and regulators of apoptosome and Caspase 9 functions. These include Apoptosis inducing factor (AIF) and Endonuclease G, which target nuclear chromatin and degrades it.

Chromatin condensation and fragmentation of nuclear DNA is an important part of apoptosis. A number of proteins fragment DNA and target chromatin in response to proapoptotic signals. One of these, the CAD DNase, was discussed in Section 15.7. Two apoptotic enzymes are released by mitochondria that target chromatin and fragment DNA. One of the DNA chopping proteins, or *DNases*, is Endonuclease G. This enzyme is released from mitochondria in response to stimulation by proapoptotic proteins. Once it is released into the cytosol it translocates to the nucleus where it fragments DNA into nucleosomal-sized fragments. In the first nuclear steps of apoptosis, DNA repair is halted and chromatin condensation occurs, which turns off DNA transcription. Another apoptosis promoting agent sent out from the mitochondria is Apoptosis-inducing factor. This protein is released from mitochondria at an early stage in the apoptosis process. It is responsible for peripheral chromatin condensation and for large-scale chromatin fragmentation.

TABLE 15.4. Agents released by mitochondria: Abbreviations—Second mitochondrial activator of caspases (Smac); direct IAP binding protein with low pI (DIABLO).

Agent	Action
Cytochrome c	Required for assembly of the apoptosome
dATP	Required for assembly of the apoptosome
Smac/DIABLO	Proapoptotic regulator
Omi/HtrA2	Proapoptotic regulator
AIF	Early acting nuclear factor
Endonuclease G	Later acting nuclear factor

## 15.12 Role of Apoptosome in (Mitochondrial Pathway to) Apoptosis

The apoptosome is the main control point in the mitochondrial pathway to apoptosis. When released from the mitochondria, Cytochrome *c* interacts with a 130-kDa adapter protein called Apaf-1 located in the cytoplasm just outside the mitochondria. This adapter contains three domains (Figure 15.6a). It has an N-terminal caspase recruitment domain (CARD), a central domain that binds dADP/ATP, and a C-terminal domain containing a series of WD-40 repeats. When Cytochrome *c* binds to the WD-40 repeats to override the autoinhibition, and deoxyadenosine triphosphate (dATP) hydrolysis occurs, Apaf-1 is able to form oligomers leading to the formation of a 1-MDa apoptosome.

The apoptosome is a sevenfold symmetric platform. It is wheel-shaped with seven spokes radiating out from a central hub (Figure 15.6b), and functions to activate Procaspase 9 when these zymogens are incorporated into it. When fully saturated, seven Procaspase 9 molecules are bound to the seven Apaf-1 CARD domains and seven Cytochrome *c* molecules are bound to the Y domains. The formation of an apoptosome containing activated Caspase 9 molecules triggers the apoptosis process by interacting with and activating Caspase 3.

The apoptosome is a major control point where proapoptotic agents released by the mitochondria converge and where Caspase 9 and Caspase 3 interact and activate one another. At the apoptosome, Caspase 9 processes and activates Caspase 3. A feedback loop in which processed Caspase 3 cleaves and activates Caspase 9 amplifies the production of activated caspases. Several other proteins are present, and form complexes with the caspases. Among these are caspase inhibitors and caspase counterinhibitors.

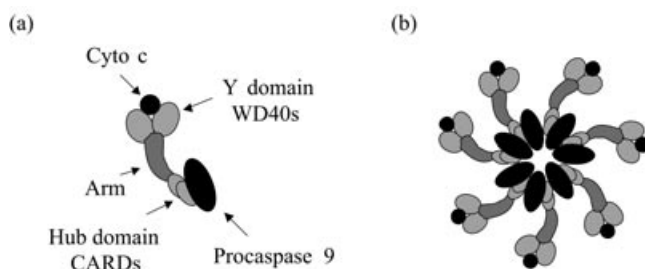


FIGURE 15.6. Apaf-1 and the apoptosome: (a) Apaf-1 molecule bound to Cytochrome *c* and Procaspase 9—Shown are a pair of WD-40 repeats in the C-terminal Y domain that bind Procaspase 9, and a pair of CARD domains in the N-terminal that bind Cyto *c*. A flexible arm containing a CED4 homology motif that binds dATP/ADP connects the N- and C-terminal regions to one another. (b) Fully assembled apoptosome illustrating how the individual Apaf-1 molecules associate into a sevenfold symmetric platform.



### 15.13 Inhibitors of Apoptosis Proteins Regulate Caspase Activity

Inhibitor of apoptosis proteins (IAPs) bind to and inhibit the activities of caspases once they have been converted by proteolysis from zymogens to caspase monomers and homodimers. The presence of these regulators (and their counterregulators) establishes a second tier of control over the catalytic activities of caspases. The defining feature of the IAPs is the presence of one or more BIR (baculoviral IAP repeat) domains (Figure 15.7). These are found in the eight mammalian IAPs discovered to date. A second prominent feature of the IAPs is the presence of a RING domain in the C-terminal of some but not all IAPs.

Caspases must form homodimers to become catalytically active. The homodimerization partner supplies a sequence crucial for catalysis, a sequence that that monomeric form of the caspases lacks. Crystal structure studies of Caspase 9 in complex with the BIR3 domain of an X-linked AIP (XIAP) protein show how the IAP proteins inhibit the catalytic activities of the caspases. The BIR3 domain binds to the homodimerization domain of monomeric caspase, thereby preventing formation of caspase homodimers. This mechanism differs from that used by the IAPs to inhibit effector caspases such as Caspase 3 and Caspase 7. Rather than preventing formation of the homodimers, XIAP uses a sequence just forward of its BIR2 domain to block the active site of the effector caspases.

Inhibitor of apoptosis proteins possess a ubiquitin ligase activity. As shown in Figure 15.7, IAPs such as XIAP and c-IAP1 and c-IAP2 possess a RING finger domain in their C-terminus. This motif is often encountered in E3 ubiquitin ligases. In the case of IAPs bearing this motif, there seem

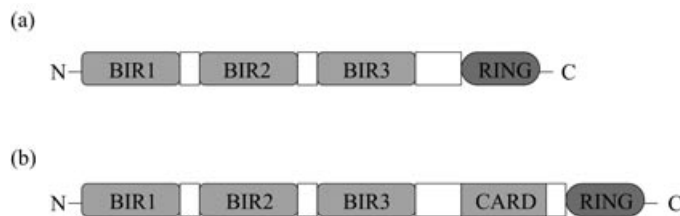


FIGURE 15.7. Domain organization of the inhibitor of apoptosis proteins (IAP): (a) Structure of XIAP. (b) Structure of c-IAP1 and c-IAP2. ILP-2 and Livin resemble XIAP except that they only have one BIR domain (BIR3 in the case of ILP-2 and BIR2 in the case of Livin). The other three mammalian AIP proteins—NAIP, Survivin, and Apollon/Bruce—have from one to three BIR domains but lack a RING finger domain. Abbreviations: X-linked IAP (XIAP); IAP-like protein (ILP); neuronal inhibitory apoptosis protein (NAIP); baculoviral IAP repeat (BIR); caspase recruitment domain (CARD).



to be two choices of substrates. In response to proapoptotic signals, the IAPs trigger their own ubiquitination. Alternatively, in the absence of proapoptotic signals, the IAPs act in an antiapoptotic capacity by promoting the ubiquitination of activated Caspases 3 and 7.

### 15.14 Smac/DIABLO and Omi/HtrA2 Regulate IAPs

Smac/DIABLO is an IAP counterregulator. It is released from mitochondria in response to proapoptosis signals, and once released disrupts the ability of IAPs to inhibit the caspases. The IAP family member XIAP is one of its main targets. In the absence of Smac/DIABLO, XIAP binds to the small subunit of Caspase 9. It does not bind to Caspase 9 in its procaspase form, but only to the cleaved and assembled Caspase 9 monomer. Smac/DIABLO disrupts the ability of XIAP to inhibit Caspase 9 by binding to its BIR3 domain. A sequence of four residues in the N-terminal recognizes and binds a surface groove in the BIR3 domain.

The protein Omi/HtrA2 is another IAP counterregulator released by mitochondria in response to proapoptotic signaling. Like Smac/DIABLO it promotes apoptosis by antagonizing the IAPs ability to bind to and inhibit activation of Caspases 3, 7, and 9. Upon release from the mitochondria Omi/HtrA2 forms complexes with XIAP and disrupts the latter's caspase-inhibiting functions. Although Omi/HtrA2 binds to the IAP in a manner resembling that of Smac/DIABLO, its manner of action is different. Unlike Smac/DIABLO, Omi/HtrA2 is a serine protease and may act in a proteolytic manner to degrade and thus limit the inhibitory activities of the IAPs.

### 15.15 Feedback Loops Coordinate Actions at Various Control Points

The apoptosome and the mitochondrial PTPC are major control points for apoptosis. These control points communicate with each other and with the DISC in order to arrive at live or die decisions. The communication between PTPC and apoptosome is summarized in Figure 15.8. In the figure, a stress-generating perturbation causes the loss of inner mitochondrial transmembrane potential  $\Psi_m$ , produces ROS, and/or elevates the free calcium concentration  $[Ca^{2+}]_m$  in the mitochondrial matrix. In response, the mitochondrial membranes become more permeable to Cytochrome c and other proapoptotic agents. These agents leave the mitochondria. Some (cytochrome c and dATP) trigger formation of the apoptosome and others (Smac/DIABLO and Omi/HtrA2) activate the caspases by binding and inhibiting the IAPs. Caspase 3 activated at the apoptosome diffuses to and enters the mitochondria. It then cleaves a number of components of the mitochondrial electron transport chain. These feedback operations trigger the

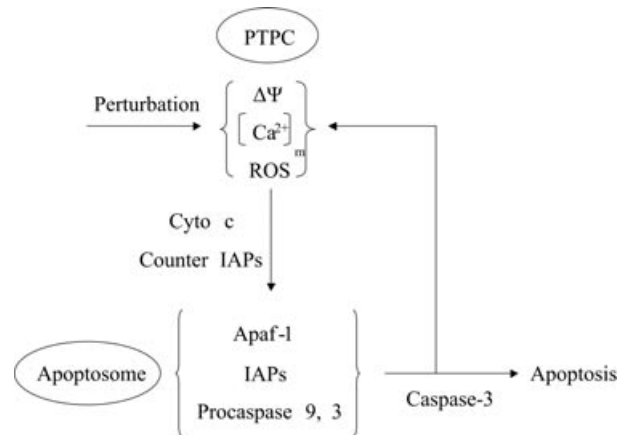


FIGURE 15.8. Mitochondrial PTPC—Apoptosome circuit: Perturbations of the mitochondrial permeability transition pore complex (PTPC) stimulates the release of apoptosis-promoting factors such as Cytochrome c and counter inhibitor of apoptosis proteins (IAPs). These act at the apoptosome to activate Caspases 3 and 9.

stepped-up production of ROS and efflux of Cytochrome c, which then acts at the apoptosome to amplify the amount of activated Caspase 9 and Caspase 3.

Another feedback loop connects events at the apoptosome with those occurring at the DISC. The connecting link is Caspase 6. Caspase 3 cleaves and activates Caspase 6, which then migrates to the DISC where it stimulates Caspase 8, thereby amplifying the strength of the apoptotic signal at the cell surface as was discussed in Section 15.7. The BH3-only protein Bid completes the circuit by connecting actions taking place at the DISC with those occurring at the PTPC.

Apoptosis is controlled by a plethora of positive feedback loops that ensure that once the appropriate thresholds are passed there will be a firm commitment to apoptosis. The presence of thresholds ensures that random excursions and perturbations do not unnecessarily commit the cell to apoptosis when it ought not to. An equally important ensemble of negative feedback loops generates the threshold dependences.

### 15.16 Cells Can Produce Several Different Kinds of Calcium Signals

Calcium is an important signaling intermediary, or second messenger, and was introduced as such in Chapter 8. Calcium can also function as a first messenger. Calcium is well suited to function as a signaling molecule. It is able to accommodate from 4 to 12 oxygen atoms in their primary coordination sphere. This property allows the calcium ions to contact multiple

partners and trigger large conformational changes when binding a protein. In addition, calcium does not diffuse very far because of the presence of a large number of calcium buffers in the cytosol.

The normal intracellular calcium ion concentration is about 100 nM. This level is several orders of magnitude lower than the calcium ion concentrations in the extracellular spaces, which is roughly 2 mM. To maintain the intracellular calcium concentrations at the resting levels, the  $Na^+/Ca^{2+}$  ion exchanger and pumps such as the plasma membrane calcium ATPase (PMCA), remove calcium from the cell. In addition, the sarco-endoplasmic reticulum calcium ATPase (SERCA) embedded in the SR/ER ships calcium from the cytosol into the intracellular stores. Because of the presence of cellular machinery keeping calcium levels low, transient local increases in calcium concentration can be produced easily and serve as a signal.

Lipid second messengers relay signals from the plasma membrane to the intracellular stores. These molecules trigger the release of calcium when they bind inositol (1,4,5) triphosphate receptors ( $InsP_3Rs$ ). A second kind of calcium release channel, the ryanodine receptor (RyR), releases calcium from the intracellular stores, as well. Several different kinds of calcium signals can be produced. One kind of calcium signal, the calcium “wave,” is a global signal that propagates over large distances across the cytosol. The propagation of these waves over large intracellular distances is made possible by positive feedback in which calcium released from stores in one locale diffuses to and triggers the release of calcium from nearby stores that sets off further releases, thereby generating a wave of calcium. Calcium can be released from stores in a more localized manner. The local releases of calcium from intracellular stores are called “puffs” when produced by  $InsP_3Rs$  and “sparks” when facilitated by RyRs.

## 15.17 Excessive $[Ca^{2+}]$ in Mitochondria Can Trigger Apoptosis

Calcium signals are sent to the mitochondria under normal conditions and also under abnormal conditions. Calcium signals are sent to the mitochondria in response to increased signaling at the plasma membrane in order to spur increases in metabolic activity to support the elevated signaling load. This coupling of metabolism and calcium is made possible by the presence in the mitochondrial matrix of a number of calcium sensitive metabolic enzymes. Increased calcium signaling to the mitochondria also occurs under abnormal conditions of cytosolic calcium overload (i.e., disruptions in calcium homeostasis) and ER stresses. In these situations, the changes in mitochondrial physiology drive the cell towards apoptosis.

Calcium ions are cycled between the SR/ER and mitochondria. The membranes of these organelles are in close proximity to one another and

highly local and directional signaling similar to that occurring at chemical synapses takes place. One of the early events taking place in stress-generated apoptosis is an increased permeability of the PTPC to calcium entry. This event leads to increases in permeability to cytochrome c, resulting in its increased efflux from the mitochondria.

Combinations of proapoptotic conditions such as oxidative stresses and calcium signaling between the ER and mitochondria can initiate apoptotic responses under condition where one or the other condition by itself cannot. The cooperation between the two is mediated by a positive feedback loop in which ROS generated in the mitochondria promote increased  $\text{Ca}^{2+}$  release from the ER. The calcium accumulates in the mitochondria and triggers further increases in ROS production. This process generates the progressive depolarization of the inner mitochondrial membrane and increases membrane permeation leading to cell death.

The PTPC is not the only control point where Bcl-2 family proteins exert their regulatory influences. They also act at the endoplasmic reticulum where they influence the release of Cytochrome c occurs through their modulation of calcium signaling. They help maintain homeostatic control over movement of calcium into and between the two organelles (the ER and the mitochondria), and mobilize calcium release. Proapoptotic Bax and Bak proteins localize to both the endoplasmic reticulum and mitochondria where they control calcium trafficking between the two organelles. In the ER, Bax and Bak help initiate the apoptosis process by contributing to Caspase 12 activation, and in the mitochondria they help activate Caspase 7.

### 15.18 p53 Promotes Cell Death in Response to Irreparable DNA Damage

In response to DNA damage signals, the p53 protein will either halt the cell cycle to allow for repairs or promote apoptosis if the damage is irreparable. The signaling proteins that convey messages to p53 do so through phosphorylations and acetylations of specific residues. The specific mix of phosphorylations and acetylations determines in large measure the response that will be made by p53. For example, phosphorylation of p53 on Ser46 stimulates transcription of apoptosis-promoting genes, but does not stimulate transcription of cell cycle genes. A second factor that guides the p53 response is the mix of cofactors present at the promoter sites. As is the case for other transcription factors, the cofactors help determine which genes get transcribed and which ones do not. In the case of p53 two other p53 family members—p63 and p73—work together with p53 to transcribe proapoptosis genes.

The p53 protein can shift the balance of pro- and antiapoptosis factors in at least two distinct ways. First, in its role as a transcription factor, the p53 proteins can step up the transcription of proapoptotic genes. As

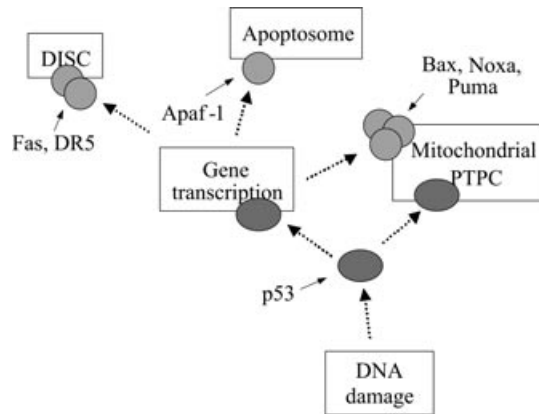


FIGURE 15.9. Regulation of apoptosis by p53: DNA damage signals are conveyed to p53 in the form of posttranslational modifications of specific residues such as phosphorylation on Ser46. In response, p53 (along with p63 and p73, not shown) stimulates transcription of apoptosis-promoting proteins acting at the DISC, PTPC, and apoptosome. It also diffuses to the mitochondria where it interacts with antiapoptotic Bcl-2 family members to increase membrane permeability.

depicted in Figure 15.9, p53 can increase the numbers of propapoptotic proteins. These include death ligands and receptors at the DISC, the Apaf-1 scaffold proteins at the PTPC, and proapoptotic multidomain Bax, and BH3-only proteins, at the apoptosome. Second, p53 can translocate to the mitochondria where it can interact with the antiapoptotic Bcl-x<sub>L</sub> and Bcl-2 proteins to increase the efflux of Cytochrome c from the mitochondria. Recall from the last chapter that some of the most lethal mutations to p53 occur in its DNA binding domain. This domain contains the binding site for attachment to the Bcl-2 proteins so these mutations not only destroy p53's ability to bind DNA in the nucleus but also negate its ability to interact with the Bcl-x<sub>L</sub> proteins at the mitochondria.

## 15.19 Anti-Cancer Drugs Target the Cell's Apoptosis Machinery

The machinery involved in regulating apoptosis is a major target of anti-cancer therapies. The goal of these therapeutic strategies is to induce apoptosis in the malignant tissue while leaving healthy tissue alone. Components of the key control loci—DISC, apoptosome, and PTPC—are the primary targets of many of these strategies. One set of strategies revolve about causing apoptosis-inducing damage to DNA and other cellular components, while another set of approaches focuses on causing oxidative damage in the mitochondria in a way that stimulates the release of proapoptotic factors.

The majority of chemotherapeutic approaches to killing tumors is based on the idea of selectively inducing apoptosis in the diseased cells. Many anti-cancer drugs target the mitochondria with the aim of producing a decrease in membrane potential leading to the efflux of pro-apoptotic molecules and the activation of the apoptosome/Caspase9 pathway. There are a number of ways that a decrease in mitochondrial membrane potential may be induced. One way is to supply ligands for either the ANT or the VDAC. Alternatively, drugs may be used that trigger increases in ROS leading to an increase in membrane permeability.

All of the major components of the apoptosis machinery are tightly controlled. Negative feedback loops ensure that small perturbations and random releases of Cytochrome c or Smac/DIABLO from the mitochondria do not set off apoptosis. Restorative processes in the form of antioxidant scavenger systems prevent ROS generated within the mitochondria from causing excessive damage. Calcium homeostasis within the cytosol and organelles such as the ER and mitochondria are under negative feedback control. Finally, the inner mitochondrial transmembrane potential  $\Psi_m$  is a quantity with an important role in apoptosis and endowed with restorative properties. In order to be effective, therapeutic approaches that produce stresses and perturb the mitochondria must overcome the plethora of protective measures used by the cell to deal with such stresses. These issues compound the difficulties in dealing with machinery that doesn't work quite right because of mutations in genes that encode its key elements.

## *References and Further Reading*

### *General References*

- Igney FH, and Krammer PH [2002]. Death and anti-death: Tumor resistance to apoptosis. *Nature Rev. Cancer*, 2: 277–288.
- Johnstone RW, Ruefli AA, and Lowe SW [2002]. Apoptosis: A link between cancer genetics and chemotherapy. *Cell*, 108: 153–164.
- Mattson MP [2000]. Apoptosis in neurodegenerative disorders. *Nature Rev. Mol. Cell Biol.*, 1: 120–129.
- Meier P, Finch A, and Evan G [2000]. Apoptosis in development. *Nature*, 407: 796–801.
- Vila M, and Przedborski S [2003]. Targeting programmed cell death in neurodegenerative diseases. *Nature Rev. Neurosci.*, 4: 1–11.

### *Caspases*

- Boatright KM, et al. [2003]. A unified model for apical caspase activation. *Mol. Cell*, 11: 529–541.
- Chai JJ, et al. [2001]. Crystal structure of a procaspase-7 zymogen: Mechanisms of activation and substrate binding. *Cell*, 107: 399–407.
- Chang DW, et al. [2003]. Interdimer processing mechanism of procaspase-8 activation. *EMBO J.*, 22: 4132–4142.

Shi YG [2002]. Mechanisms of caspase activation and inhibition during apoptosis. *Mol. Cell*, 9: 459–479.

Stennicke HR, and Salvesen GS [2000]. Caspases—Controlling intracellular signals by protease zymogen activation. *Biochim. Biophys. Acta*, 1477: 299–306.

### *Bcl-2 Proteins*

Cheng EHYA, et al. [2001]. Bcl-2, Bcl<sub>xL</sub> sequester BH3 domain only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. *Mol. Cell*, 8: 705–711.

Cory S, and Adams JM [2002]. The Bcl-2 family: Regulators of the cellular life-or-death switch. *Nature Rev. Cancer*, 2: 647–656.

Gross A, McDonnell JM, and Korsmeyer SJ [1999]. Bcl-2 family members and the mitochondria in apoptosis. *Genes Dev.*, 13: 1899–1911.

Letai A, et al. [2002]. Distinct BH3 only domain either sensitize or activate mitochondrial apoptosis, serving as prototypes cancer therapeutics. *Cancer Cell*, 2: 183–192.

Moreau C, et al. [2003]. Minimal BH3 peptides promote cell death by antagonizing anti-apoptotic proteins. *J. Biol. Chem.*, 278: 19426–19435.

Scorrano L, et al. [2002]. A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. *Dev. Cell*, 2: 55–67.

### *DISC Signaling*

Enari M, et al. [1998]. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature*, 391: 43–50.

Nagata S [1997]. Apoptosis by death factor. *Cell*, 88: 355–365.

Scaffidi C, et al. [1998]. Two CD95(APO-1/Fas) signaling pathways. *EMBO J.*, 17: 1675–1687.

Weber CH, and Vincenz C [2001]. The death domain superfamily: A tale of two interfaces? *Trends Biochem. Sci.*, 26: 475–481.

### *Regulation of the Apoptotic Signaling Pathways by NF-κB*

Karin M, and Lin A [2002]. NF-κB at the crossroads of life and death. *Nature Immunology*, 3: 221–227.

Mayo CY, et al. [1998]. NF-κB antiapoptosis: Induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase 8 activation. *Science*, 281: 1680–1683.

### *Permeability Transition Pore Complex*

Crompton M [1999]. The mitochondrial permeability transition pore and its role in cell death. *Biochem. J.*, 341, pt. 2: 233–249.

Ott M, et al. [2002]. Cytochrome c release from mitochondria proceeds by a two-step process. *Proc. Natl. Acad. Sci. USA*, 99: 1259–1263.

Susin SA, Zamzami N, and Kroemer G [1998]. Mitochondria as regulators of apoptosis: Doubt no more. *Biochim. Biophys. Acta.*, 1366: 151–165.

### *Apoptosome Assembly*

Acehan D, et al. [2002]. Three-dimensional structure of the apoptosome: Implications for assembly, pro-caspase-9 binding, and activation. *Mol. Cell*, 9: 423–432.



Bratton SB, et al. [2001]. Recruitment, activation and retention of caspase-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes. *EMBO J.*, 20: 998–1009.

### *Inhibitor of Apoptosis Proteins*

Huang HK, et al. [2000]. The inhibitor of apoptosis, cIAP2, functions as a ubiquitin-protein ligase and promotes in vitro monoubiquitination of caspases 3 and 7. *J. Biol. Chem.*, 275: 26661–26664.

Salvesen GS, and Duckett CS [2002]. IAP proteins: Blocking the road to death's door. *Nature Rev. Mol. Cell Biol.*, 3: 401–410.

Suzuki Y, Nakabayashi Y, and Takahashi R [2001]. Ubiquitin-protein ligase activity of X-linked inhibitor of apoptosis protein promotes proteosomal degradation and caspase-3 and enhances its anti-apoptotic effect in Fas-induced cell death. *Proc. Natl. Acad. Sci. USA*, 98: 8662–8667.

Yang Y, et al. [2000]. Ubiquitin protein ligase activity of IAPs and their degradation in proteosomes in response to apoptotic stimuli. *Science*, 288: 874–877.

### *IAP Counterregulators Smac/DIABLO and Omi/HtrA2*

Holley CL, et al. [2002]. Reaper eliminates IAP proteins through stimulated IAP degradation and generalized translational inhibition. *Nature Cell Biol.*, 4: 439–444.

Nicholson DW [2002]. Baiting death inhibitors. *Nature*, 410: 33–34.

Srinivasula SM, et al. [2001]. A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. *Nature*, 410: 112–116.

Yoo SJ, et al. [2002]. Hid, Ror and Grim negatively regulate DIAP1 levels through distinct mechanisms. *Nature Cell Biol.*, 4: 416–424.

### *Mitochondrial Physiology*

Duchen MR [2000]. Mitochondria and calcium: From cell signaling to cell death. *J. Physiol.*, 529: 57–68.

Jacobson J, and Duchen MR [2002]. Mitochondrial oxidative stress and cell death in astrocytes—Requirement for stored  $\text{Ca}^{2+}$  and sustained opening of the permeability transition pore. *J. Cell Sci.*, 115: 1175–1188.

Kowaltowski AJ, Castilho RF, and Vercesi AE [2001]. Mitochondrial permeability transition and oxidative stress. *FEBS Lett.*, 495: 12–15.

Marchetti P [1997]. Redox regulation of apoptosis: Impact of thiol oxidation status on mitochondrial function. *Eur. J. Immunol.*, 27: 289–296.

Ricci JE, Gottlieb RA, and Green DR [2003]. Caspase-mediated loss of mitochondrial function and generation of reactive oxygen species during apoptosis. *J. Cell Biol.*, 160: 65–75.

### *ER and Calcium Signals to the Mitochondria*

Boehning D, et al. [2003]. Cytochrome c binds to inositol (1,4,5) triphosphate receptors, amplifying calcium-dependent apoptosis. *Nature Cell Biol.*, 5: 1051–1061.

Li C, et al. [2002]. Bcl-x<sub>L</sub> affects  $\text{Ca}^{2+}$  homeostasis by altering expression of inositol 1,4,5-triphosphate receptors. *Proc. Natl. Acad. Sci. USA*, 99: 9830–9835.

Nutt LK, et al. [2002]. Bax-mediated  $\text{Ca}^{2+}$  mobilization promotes cytochrome c release during apoptosis. *J. Biol. Chem.*, 277: 20301–20308.



- Orrenius S, Zhivotovsky B, and Nicotera P [2003]. Regulation of cell death: The calcium-apoptosis link. *Nature Rev. Mol. Cell Biol.*, 4: 552–565.
- Scorrano L, et al. [2003]. BAX and BAK regulation of endoplasmic reticulum  $\text{Ca}^{2+}$ : A control point for apoptosis. *Science*, 300: 135–139.

### *p53 Regulation*

- Flores ER [2002]. p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. *Nature*, 416: 560–564.
- Mihara M, et al. [2003]. p53 has a direct apoptogenic role at the mitochondria. *Mol. Cell*, 11: 577–590.
- Vousden KH, and Lu X [2002]. Live or let die: The cell's response to p53. *Nature Rev. Cancer*, 2: 594–604.

### *Cancer Therapy*

- Herr I, and Debatin KM [2001]. Cellular stress response and apoptosis in cancer therapy. *Blood*, 98: 2603–2614.
- Johnstone RW, Ruefli AA, and Lowe SW [2002]. Apoptosis: A link between cancer genetics and chemotherapy. *Cell*, 108: 153–164.
- Kaufmann SH, and Earnshaw WC [2000]. Induction of apoptosis by cancer chemotherapy. *Exp. Cell Res.*, 256: 42–49.
- Nicholson DW [2000]. From bench to clinic with apoptosis-based therapeutic agents. *Nature*, 407: 810–816.

## *Problem*

- 15.1 Some cell types are more susceptible to apoptosis than others. What classes of cells are especially responsive to apoptosis signals? What kinds of cells send out proapoptosis messages as part of their normal functions? Briefly describe some of the ways a cell has of regulating its own sensitivity to apoptosis.





<http://www.springer.com/978-0-387-22130-4>

Molecular and Cellular Signaling

Beckerman, M.

2005, XXXIV, 582 p. 227 illus., Hardcover

ISBN: 978-0-387-22130-4