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The machinery of programmed cell death

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Abstract

Apoptosis or programmed cell death is an essential physiological process that plays a critical role in development and tissue homeostasis. However, apoptosis is also involved in a wide range of pathological conditions. Apoptotic cells may be characterized by specific morphological and biochemical changes, including cell shrinkage, chromatin condensation, and internucleosomal cleavage of genomic DNA. At the molecular level, apoptosis is tightly regulated and is mainly orchestrated by the activation of the aspartate-specific cysteine protease (caspase) cascade. There are two main pathways leading to the activation of caspases. The first of these depends upon the participation of mitochondria (receptor-independent) and the second involves the interaction of a death receptor with its ligand. Pro- and anti-apoptotic members of the Bcl-2 family regulate the mitochondrial pathway. Cellular stress induces pro-apoptotic Bcl-2 family members to translocate from the cytosol to the mitochondria, where they induce the release of cytochrome c, while the anti-apoptotic Bcl-2 proteins work to prevent cytochrome c release from mitochondria, and thereby preserve cell survival. Once in the cytoplasm, cytochrome c catalyzes the oligomerization of apoptotic protease activating factor-1, thereby promoting the activation of procaspase-9, which then activates procaspase-3. Alternatively, ligation of death receptors, like the tumor necrosis factor receptor-1 and the Fas receptor, causes the activation of procaspase-8. The mature caspase may now either directly activate procaspase-3 or cleave the pro-apoptotic Bcl-2 homology 3-only protein Bid, which then subsequently induces cytochrome c release. Nevertheless, the end result of either pathway is caspase activation and the cleavage of specific cellular substrates, resulting in the morphological and biochemical changes associated with the apoptotic phenotype. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Apoptosis; Caspases; Death receptor; Mitochondria

Abbreviations: Apaf-1, apoptotic protease activating factor-1; Asp, aspartic acid; BH, Bcl-2 homology; BIR, baculovirus IAP repeats; CAD, caspase-activated DNase; CARD, caspase-recruitment domain; caspase, cyteine aspartate-specific proteinase; CTL, cytotoxic T lymphocyte; DD, death domain; DED, death effector domain; DFF, DNA fragmentation factor; FADD, Fas-associated death domain; FasL, Fas-ligand; GFP, green fluorescent protein; IAP, inhibitor of apoptosis proteins; iCAD, inhibitor of caspase-activated DNase; ICE, interleukin-1 β converting enzyme; IL, interleukin; NF- κ B, nuclear factor- κ B; PS, phosphatidylserine; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TRADD, tumor necrosis factor receptor-1-associated death domain; TRAF, tumor necrosis factor receptor-associated factor; TRAIL, tumor necrosis factor-related apoptosis inducing-ligand; XIAP, X-linked inhibitor of apoptosis.

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1. Introduction

Apoptosis or programmed cell death is an essential physiological process that plays a critical role in controlling the number of cells in development and throughout an organism's life by removal of cells at the appropriate time. However, apoptosis is also involved in a wide range of pathological conditions, including acute neurological injuries, neurodegenerative diseases, cardiovascular diseases, immunological diseases, acquired immunodeficiency syndrome, and cancer. Therefore, knowledge of the mechanisms involved in apoptosis may prove important in the design of new therapeutic strategies. Apoptosis was first described by Kerr et al. in 1972, and is defined by the morphological appearance of the dying cell (Fig. 1), which includes blebbing, chromatin condensation, nuclear fragmentation, loss of adhesion and rounding (in adherent cells), and cell shrinkage. Biochemical features associated with apoptosis include internucleosomal cleavage of DNA, leading to an oligonucleosomal "ladder" (Cohen et al., 1994); phosphatidylserine (PS) externalization (Martin et al., 1995b); and proteolytic cleavage of a number of intracellular substrates (Martin & Green, 1995). As a result of

the plasma membrane changes, apoptotic cells are rapidly phagocytosed prior to the release of intracellular contents and without induction of an inflammatory response.

2. Executioner caspases and their substrates — the orchestration of death

The aspartate-specific cysteine protease (caspase) cascade now appears to be the main pathway by which cellular death is orchestrated (Martin & Green, 1995; Alnemri et al., 1996). Interleukin-1 β converting enzyme (ICE; caspase-1) is the prototypical caspase and initially, it was identified as the protease responsible for the maturation of pro-interleukin (IL)-1 β to its pro-inflammatory, biologically active form (Cerretti et al., 1992; Thornberry et al., 1992). At approximately the same time, a genetic pathway for cell death was defined in the nematode *Canorhabditis elegans* (Ellis et al., 1991). One of the genes identified, *ced-3*, encodes a protein that is essential for all 131 programmed cell deaths that occur during hermaphrodite development. When *ced-3* was cloned and sequenced, it was found to be a *C. elegans* homologue of mammalian ICE (Yuan et al., 1993; Xue et al.,

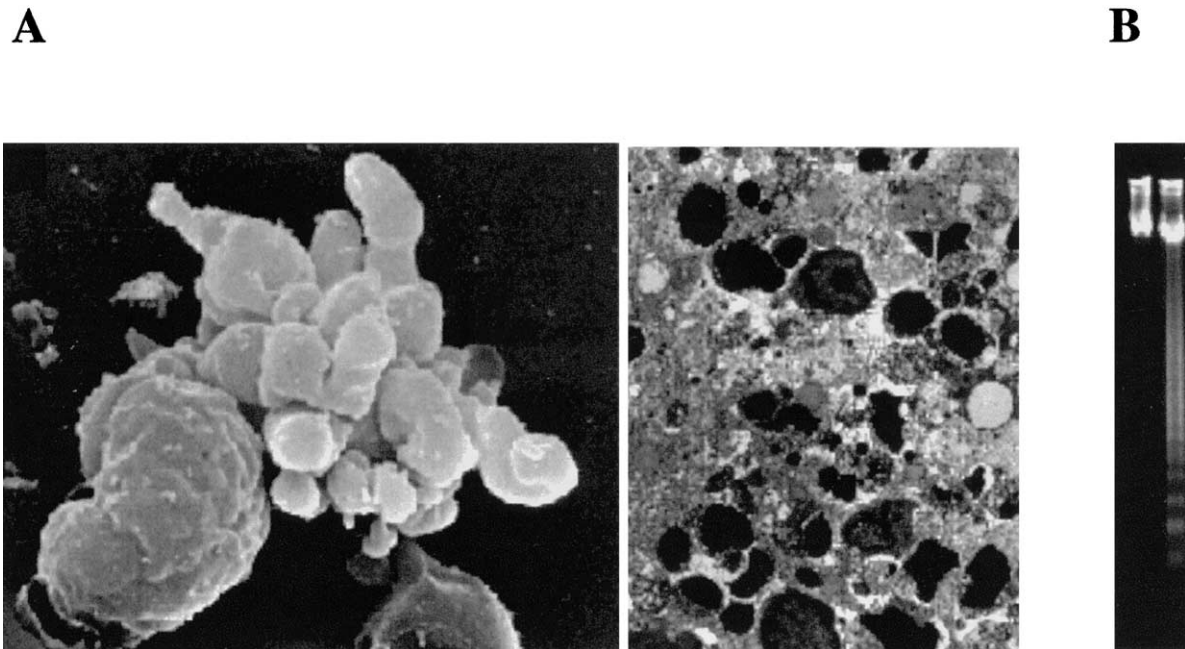


Fig. 1. Apoptotic changes. T-cells undergoing apoptosis in vitro and in the thymus after activation. A: Blebbing (left) and nuclear condensation (right). B: Oligonucleosomal DNA fragmentation in apoptotic T-cells is apparent in the right lane (left lane, DNA from untreated, viable cells).

1996). Although caspase-1 has no obvious role in cell death, it was the first identified member of a large family of proteases whose members have distinct roles in inflammation and apoptosis.

2.1. Members of the caspase family and their structures

Thus far, the caspase gene family contains 14 mammalian members, of which, 11 human enzymes are known (Fig. 2). Phylogenetic analysis indicates that this gene family is composed of two major subfamilies that are related to either ICE (caspase-1; inflammation group) or to the mammalian counterparts of ced-3 (apoptosis group). Caspases share similarities in amino acid sequence, structure, and substrate specificity (Nicholson & Thornberry, 1997). They are expressed as proenzymes (zymogens) that contain three domains: an N-terminal prodomain, a large subunit containing the active site cysteine within a conserved QACXG motif, and a C-terminal small subunit. Caspases are among the most specific of proteases, with an unusual and absolute requirement for cleavage after aspartic acid (Asp) residues (Stennicke & Salvesen, 1998). An aspartate cleavage site separates the prodomain from the large subunit, and an interdomain linker containing one or two aspartate cleavage sites separates the large and small subunits. The presence of Asp at the maturation cleavage sites is consistent with the ability of caspases to auto-activate or to be activated by other caspases as part of an amplification cascade.

Caspases are activated to fully functional proteases by two cleavage events. The first proteolytic cleavage divides the chain into large and small caspase subunits, and a second cleavage removes the N-terminal prodomain. The active caspase is a tetramer of two large and two small subunits, with two active sites (Wolf & Green, 1999). The individual caspases have two major structural differences. First, caspase prodomains vary in length and sequence. Long prodomain caspases function as signal integrators for apoptotic or pro-inflammatory signals, and contain sequence motifs that promote their interaction with activator molecules [caspase-recruitment domain (CARD) or death effector domain (DED)] (Hofmann et al., 1997; Ashkenazi & Dixit, 1998). The apoptotic initiators (caspase-2, -8, -9, and -10) generally act upstream of the small prodomain-containing apoptotic executioners (caspase-3, -6, and -7) (Nicholson & Thornberry, 1997; Ashkenazi & Dixit, 1998). Second, these proteases can be subdivided on the basis of their substrate specificities (defined using a positional scanning combinatorial substrate library) (Thornberry et al., 1997). The predicted S₂-S₄ substrate-binding sites vary significantly, resulting in varied substrate specificity in the P₂-P₄ position, despite an absolute requirement for aspartate in the P₁ position (Van de Craen et al., 1997; Humke et al., 1998; Cohen, 1997; Walker et al., 1994; Wilson et al., 1994; Rotonda et al., 1996; Mittl et al., 1997). Sequence preferences generally correlate with caspase

function as apoptotic initiators, apoptotic executioners, and cytokine processors.

2.2. Caspase substrates during apoptosis

The most prevalent caspase in the cell is caspase-3. It is the one ultimately responsible for the majority of the apoptotic effects, although it is supported by two others, caspase-6 and -7. Together, these three executioner caspases presumably cause the apoptotic phenotype by cleavage or degradation of several important substrates. For example, high- and low-molecular weight DNA fragmentation is caused by the action of caspase-3 on a complex of caspase-activated DNase (CAD)/DNA fragmentation factor (DFF)40, a nuclease, and iCAD/DFF45, its inhibitor (Enari et al., 1998; Liu et al., 1997). In non-apoptotic cells, CAD (caspase-activated deoxyribonuclease) is present as an inactive complex with iCAD. During apoptosis, caspase-3 cleaves the inhibitor, allowing the nuclease to cut the chromatin. Blebbing is orchestrated via the cleavage and activation of gelsolin (Kothakota et al., 1997), p21-activated kinase-2 (Lee et al., 1997; Rudel & Bokoch, 1997), and most likely through cleavage of fodrin (Martin et al., 1995a) to dissociate the plasma membrane from the cytoskeleton. The result is probably an effect of microfilament tension and local release, since depolymerization of actin prevents blebbing (Cotter et al., 1992). The externalization of PS during apoptosis is generally caspase-dependent (Martin et al., 1996), although in some cell types, PS externalization appears to be caspase independent (Vanags et al., 1996). The precise mechanisms have not been elucidated.

2.3. Inhibitors of caspases

Cells also contain natural inhibitors of the caspases. These inhibitors of apoptosis proteins (IAPs) were first identified in baculovirus, but subsequently were found in human cells. At least five different mammalian IAPs — X-linked inhibitor of apoptosis (XIAP, hIAP), c-IAP1 (HIAP2), c-IAP2 (HIAP1), neuronal IAP, and survivin — exhibit anti-apoptotic activity in cell culture (Roy et al., 1997; Deveraux et al., 1997, 1998; Liston et al., 1996; Uren et al., 1996; Tamm et al., 1998; Ambrosini et al., 1997). The spectrum of apoptotic stimuli that are blocked by mammalian IAPs is broad and includes ligands and transducers of the tumor necrosis factor (TNF) family of receptors, pro-apoptotic members of the ced-9/Bcl-2 family, cytochrome c, and chemotherapeutic agents (LaCasse et al., 1998; Deveraux & Reed, 1999). XIAP appears to have the broadest and strongest anti-apoptotic activity. XIAP, c-IAP1, and c-IAP2 are direct caspase inhibitors (Deveraux et al., 1997, 1999; Roy et al., 1997). They all bind to and inhibit active caspase-3 and -7, and also procaspase-9, but not caspase-1, -6, -8, nor -10. The binding and inhibition of caspases by IAPs is mediated by baculovirus IAP repeats (BIR) domain(s) present

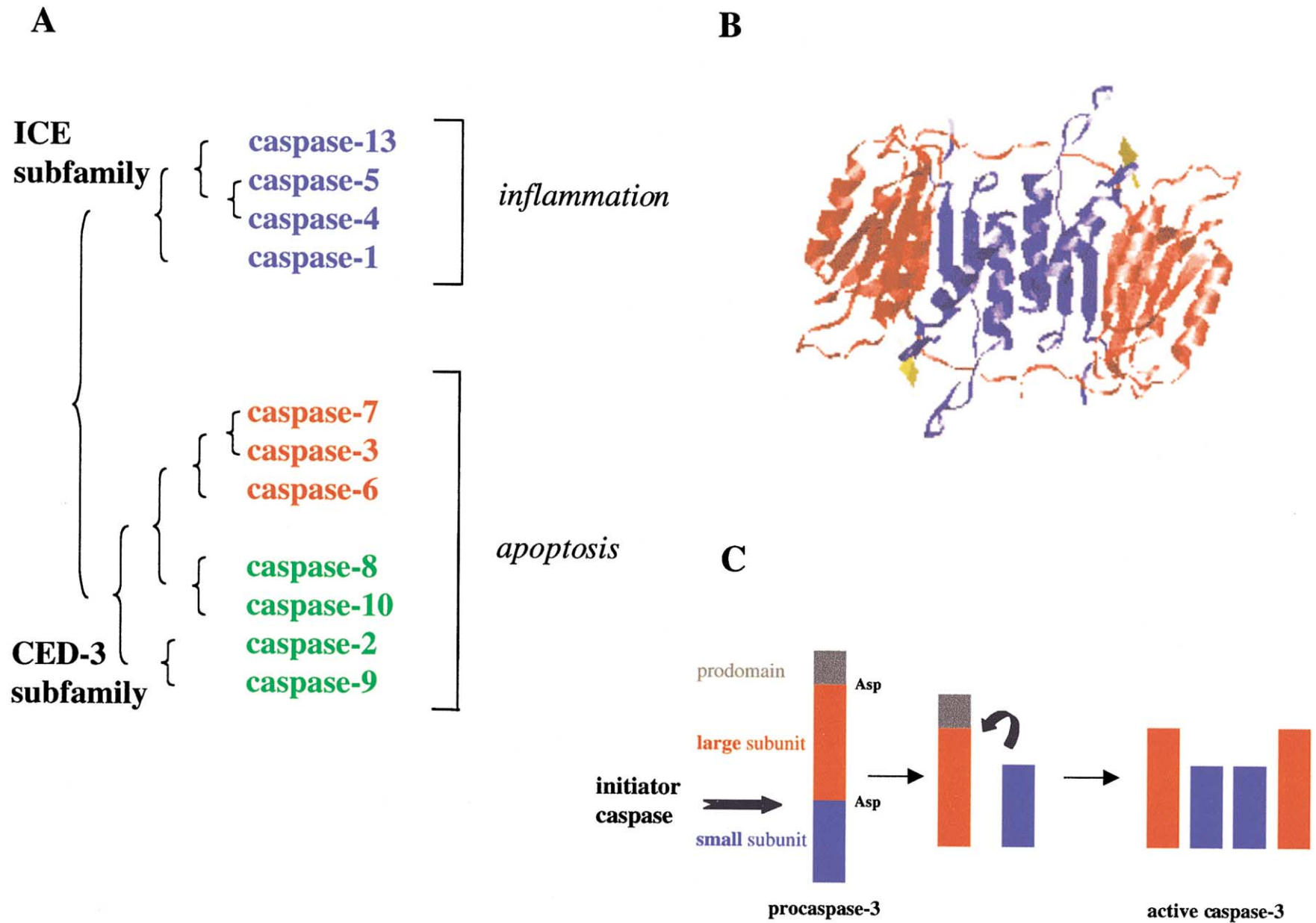


Fig. 2. The caspase family. **A:** Caspases segregate into two major phylogenetic subfamilies (ICE, CED-3). Based on their proteolytic specificities, caspases further divide into three groups: inflammatory caspases (blue) that mediate cytokine maturation, whereas the apoptotic caspases are either effectors of cell death (red) or upstream activators (green). **B:** Crystal structure of mature caspase-3 in complex with a tetrapeptide aldehyde inhibitor (yellow). The active caspase is a tetramer of a large (red) and a small (blue) subunit, each of which contributes amino acids to the active site. **C:** Activation of procaspase-3 by cleavage (see text).

within the IAP. BIR is a conserved sequence motif of ~ 70 amino acids that is repeated tandemly in a class of baculovirus proteins (e.g., Op-IAP). All mammalian IAPs have three BIR domains, except survivin, which has only a single BIR. Interestingly, in the case of XIAP, a 136 amino acid region spanning the middle BIR core was found to be necessary and sufficient for inhibition of caspase activity (Takahashi et al., 1998). Another region within the IAP, the RING domain, acts as a ubiquitin ligase, promoting the degradation of the IAP itself (Yang et al., 2000) and, presumably, any caspase bound to it. Near the RING finger of both c-IAP1 and c-IAP2 is a CARD domain, suggesting that these IAPs might directly or indirectly regulate the processing of caspases via CARD domain interactions. In this fashion, IAPs put the brakes on the apoptotic process by binding, inhibiting, and perhaps degrading caspases.

There are two general types of signaling pathways leading from a triggering event to the activation of an initiator caspase and from there, to apoptotic death. The first of these depends upon the participation of mitochondria, and the second involves death receptors, such as the TNF receptor (TNFR)-1 and the Fas molecule.

3. Mitochondria and the functions of the Bcl-2 family

There is accumulating evidence that mitochondria play an essential role in many forms of apoptosis (Green & Reed, 1998) by releasing apoptogenic factors, such as cytochrome c (Kluck et al., 1997; Yang et al., 1997) from the intermembrane space into the cytoplasm, which activates the downstream execution phase of apoptosis. In living cells, anti-apoptotic members of the Bcl-2 family of proteins predominantly prevent mitochondrial changes.

3.1. The Bcl-2 family of proteins

Bcl-2 was first discovered as a proto-oncogene in follicular B-cell lymphoma. Subsequently, it was identified as a mammalian homologue to the apoptosis repressor *ced-9* in *C. elegans*. Since then, at least 19 Bcl-2 family members have been identified in mammalian cells. These members possess at least one of four conserved motifs known as Bcl-2 homology domains (BH1–BH4). The Bcl-2 family members can be subdivided into three categories according to their function and structure (Fig. 3). (1) Anti-apoptotic members, such as Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, A1 (Bfl-1), NR-13, and Boo (Diva), all of which exert anti-cell death activity and contain at least BH1 and BH2 (those most similar to Bcl-2 contain all four BH domains). Viral members of this group include E1B-19K, BHRF1, KS-Bcl-2, ORF16, and LMW5-HL. (2) Pro-apoptotic members, such as Bax, Bak, and Bok (Mtd), which share sequence homology in BH1, BH2, and BH3, but not in BH4. (3) ‘BH3-only’ pro-apoptotic members, which

include Bid, Bad, Bim, Bik, Blk, Hrk (DP5), Bnip3, Bim_L, and Noxa, and possess only the central short BH3 domain.

3.2. The mitochondrial pathway

Subcellular localization studies have shown that Bcl-2 and Bcl-X_L reside on the mitochondrial outer membrane, while the pro-apoptotic family members may be either cytosolic or present on the mitochondrial membrane. Although they are also found elsewhere (endoplasmic reticulum and nuclear envelope), their major effect appears to be on the mitochondria. During apoptosis, the pro-apoptotic Bcl-2 family members are activated; presumably undergo a conformational change (Desagher et al., 1999), leading to the exposure of the pro-apoptotic BH3 domain [may occur via several mechanisms, including dephosphorylation (e.g., Bad) (Zha et al., 1996) and proteolytic cleavage by caspases (e.g., Bid) (Li et al., 1998)]; and translocate to the mitochondria (Fig. 4). Bax translocation to the mitochondria involves homo-oligomerization (Gross et al., 1998). The translocation of Bax, Bid, or Bad to the mitochondria can then induce a dramatic event — these organelles release the proteins contained within the intermembrane space, including one key protein, cytochrome c. Cytochrome c is encoded by a nuclear gene, but when it is imported into the mitochondria, it is coupled with a heme group to become holocytochrome c, and it is only this form that functions to induce caspase activation (Yang et al., 1997).

While the anti-apoptotic proteins Bcl-2 and Bcl-X_L work to prevent cytochrome c release from mitochondria, and thereby preserve cell survival (Kluck et al., 1997; Yang et al., 1997), the pro-apoptotic members play a major role in initiating cytochrome c release in certain settings of apoptosis. For example, Bid is implicated in Fas-mediated apoptosis, Bax in DNA damage-induced apoptosis and neurotrophin deprivation-induced death, and Bad in lymphokine deprivation-induced cell death of certain neuronal cells.

Time-lapse confocal microscopy using HeLa cells expressing cytochrome c-green fluorescent protein (GFP) has greatly extended our knowledge of the mechanism of cytochrome c release in cells (Goldstein et al., 2000). These studies revealed that cytochrome c release is an early event during apoptosis, occurring hours before PS exposure and loss of plasma membrane integrity. These studies also showed that although cytochrome c-GFP release is an early event, mitochondria retain cytochrome c-GFP for many hours after the initial apoptosis-inducing stimulus. However, once initiated, all mitochondria release cytochrome c-GFP in ~ 5 min, regardless of the nature of the initial stimulus. The relatively invariable duration of cytochrome c-GFP release indicates that a universal pathway of cytochrome c release may be used during cytotoxic drug-induced and TNF-induced apoptosis, or that the mechanisms of release are remarkably similar.

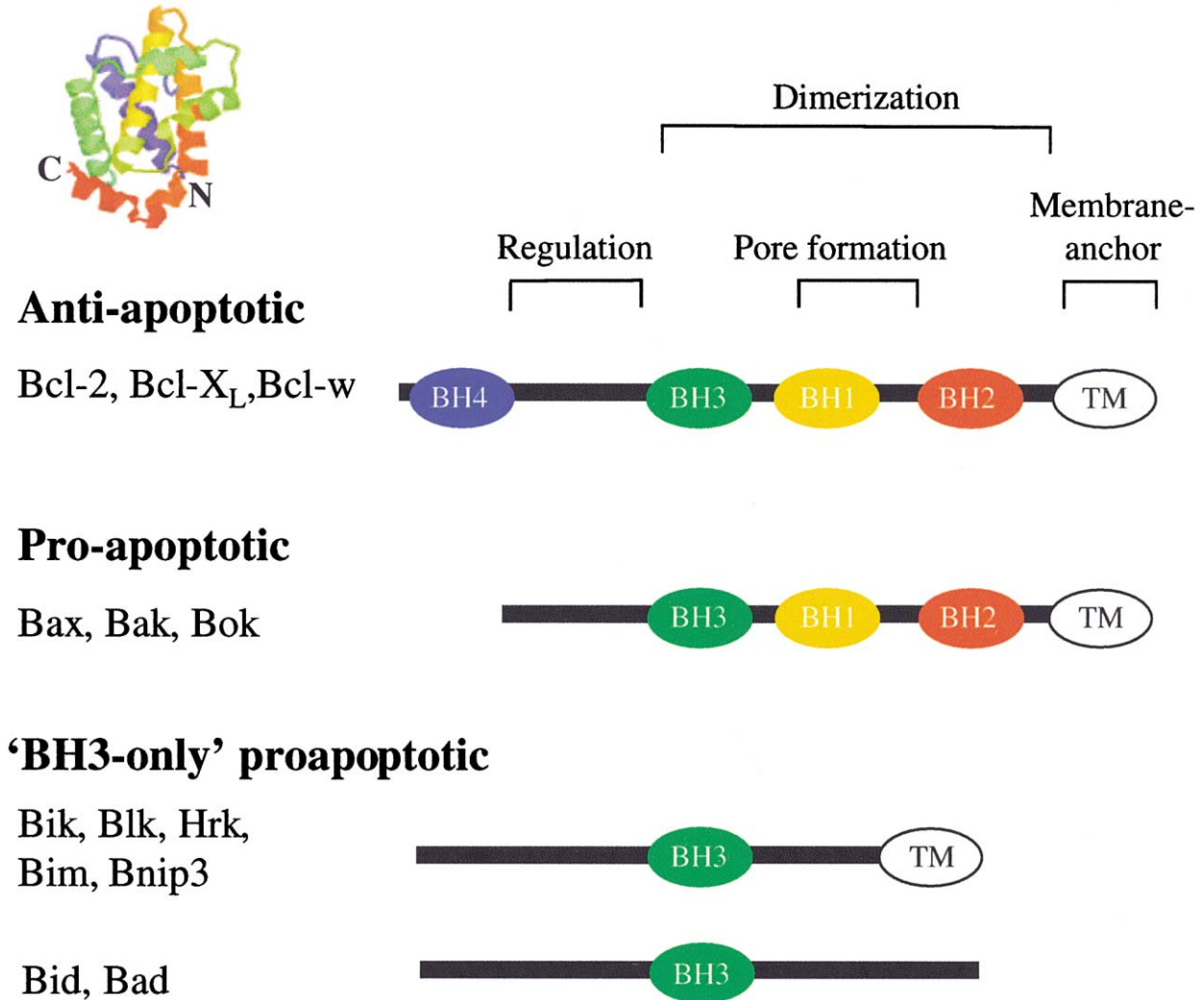


Fig. 3. The members of the Bcl-2 family. Three subfamilies are indicated: the anti-apoptotic Bcl-2 members promote cell survival, whereas pro-apoptotic and BH3-only members facilitate apoptosis. BH1-BH4 are conserved sequence motifs. Several functional domains of Bcl-2 are shown. A membrane-anchoring domain (TM) is not carried by all members of the family.

Following cytochrome *c* release, caspases are activated and the cell undergoes apoptosis (Fig. 4). This occurs through the formation of an “apoptosome” [consisting of cytochrome *c*, apoptotic protease activating factor-1 (Apaf-1), and procaspase-9], dependent on either ATP or dATP in the cell. Apaf-1 (Zou et al., 1997, 1999) is a protein contained within the cytosol, and cytochrome *c* binds and induces it to oligomerize. This, then, recruits an initiator caspase, procaspase-9 (Li et al., 1997; Zou et al., 1999). Unlike other caspases, procaspase-9 does not appear to be activated simply by cleavage, but, instead, must be bound to Apaf-1 to be activated (Rodriguez & Lazebnik, 1999; Stennicke et al., 1999). These proteins interact via CARD-CARD interactions. The apoptosome can then recruit procaspase-3, which is cleaved and activated by the active caspase-9, and can release it to mediate apoptosis.

3.3. A second mitochondrial activator of caspases

Recently, a new protein with the dual name Smac/DIABLO (Du et al., 2000; Verhagen et al., 2000) (Fig. 5) has been discovered, which is released from the mitochondria along with cytochrome *c* during apoptosis. This protein functions to promote caspase activation by associating with the apoptosome and by inhibiting IAPs. It has been shown that cytochrome *c* and Smac/DIABLO are released coordinately. While cytochrome *c* activates Apaf-1, Smac/DIABLO relieves the inhibition on caspases by binding to the IAPs (this has been shown for the murine XIAP/MIHA) and disrupting its association with processed caspase-9, thereby allowing caspase-9 to activate caspase-3, resulting in apoptosis (Ekert et al., 2001). In some cellular systems, cytochrome *c* is necessary, but not sufficient, for cell death. In these systems, Smac/DIABLO may be the second factor

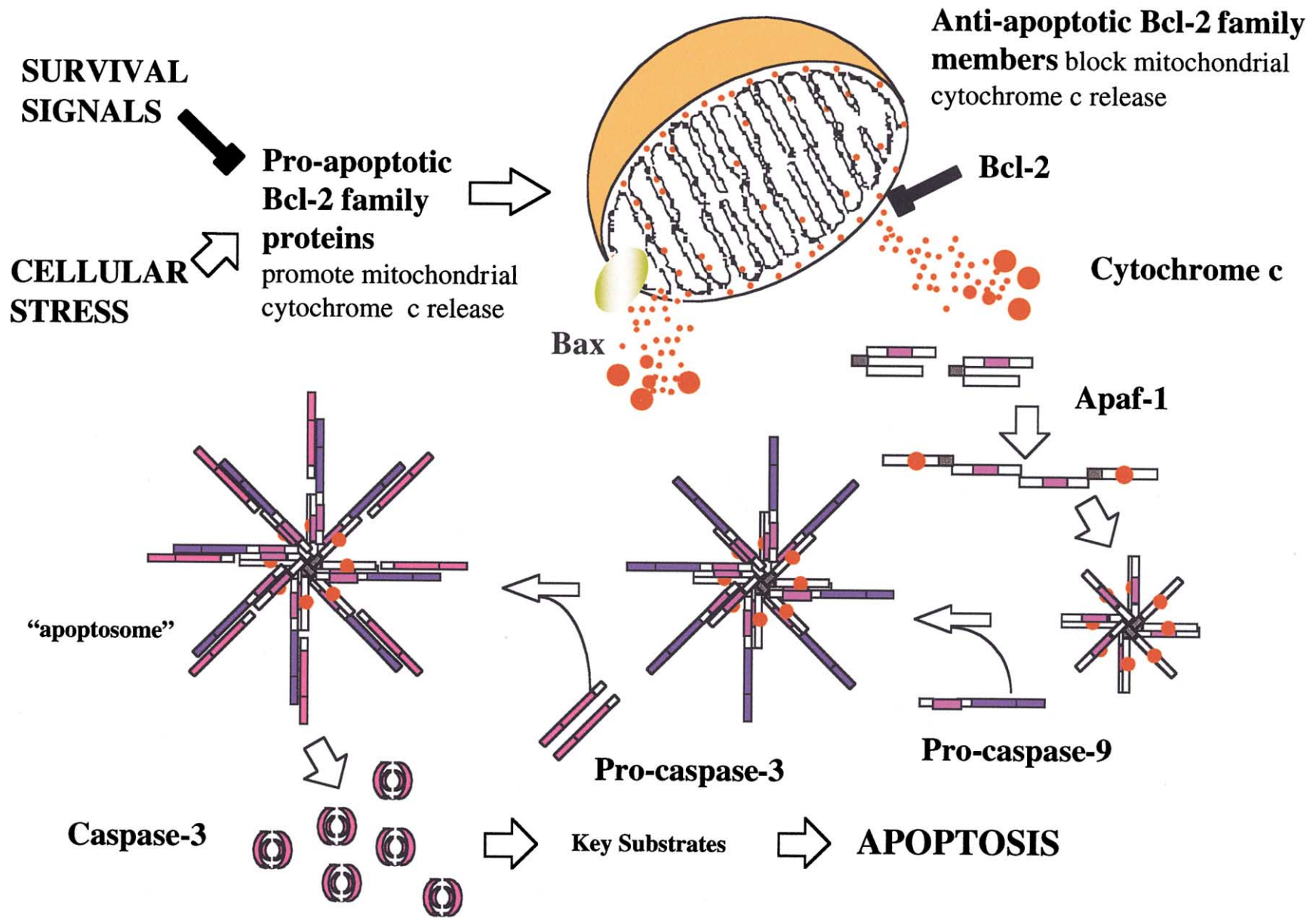


Fig. 4. Induction of apoptosis via the mitochondrial pathway. Cellular stress induces pro-apoptotic Bcl-2 family members to translocate from the cytosol to the mitochondria, where they induce the release of cytochrome c. Cytochrome c catalyzes the oligomerization of Apaf-1, which recruits and promotes the activation of procaspase-9. This, in turn, activates procaspase-3, leading to apoptosis.

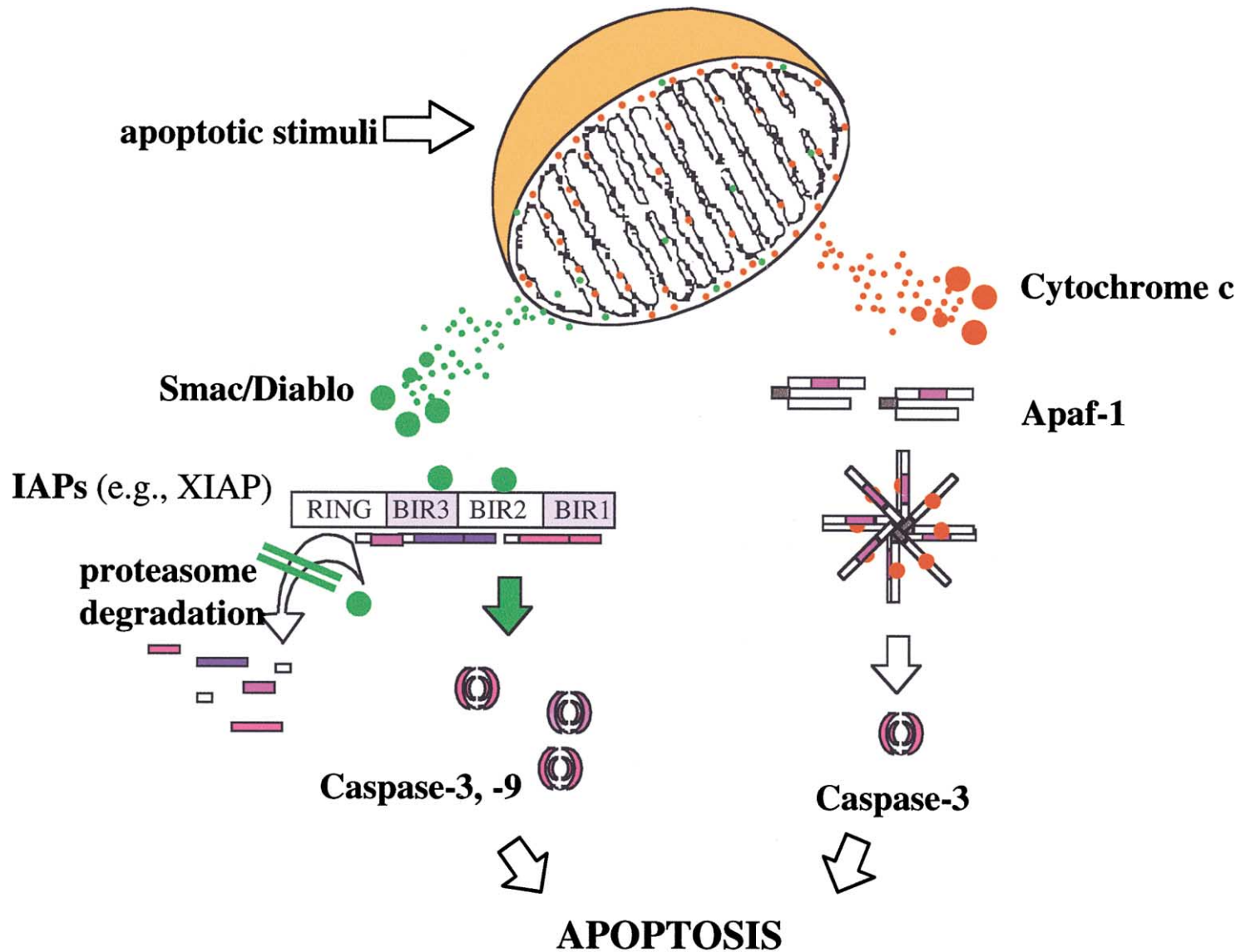


Fig. 5. A second mitochondrial activator of caspases (Smac/DIABLO). Cytochrome c and Smac/DIABLO are released coordinately from the mitochondria upon an apoptotic stimuli. While cytochrome c activates Apaf-1, Smac/DIABLO relieves the inhibition on caspases by binding to the IAPs. Thereby, Smac/DIABLO disrupts the association of IAPs with processed caspase-9, allowing caspase-9 to activate caspase-3, leading to apoptosis.

required for the so-called competence to die (Deshmukh & Johnson, 1998; Green, 2000; De Laurenzi & Melino, 2000).

4. Death ligands and death receptors

The “death receptors” of the TNFR family include TNFR1, Fas (CD95), DR3/WSL, and the TNF-related apoptosis-inducing ligand (TRAIL)/Apo-2L receptors (TRAIL-R1/DR4, TRAIL-R2/DR5). Members of this family are characterized by two to five copies of cysteine-rich extracellular repeats. Death receptors also have an intracellular amino acid stretch within the carboxy terminus of the receptor called the “death domain” (DD). When these death receptors are bound by ligand (TNF or lymphotoxin, Fas-ligand (FasL), the ligand for DR3, or TRAIL/Apo-2L, respectively), apoptosis can occur as a consequence.

4.1. The Fas receptor signaling pathway

Fas is a glycosylated cell surface molecule of ~ 45 – 52 kDa (355 amino acids). It is a Type I transmembrane receptor that can also be found in a soluble form (generated via alternative splicing) (Oehm et al., 1992; Cheng et al., 1994). Fas is expressed on several different cell types (mainly in the thymus, activated T and B lymphocytes, macrophages, liver, spleen, lung, testis, brain, intestines, heart, and ovaries), and its expression can be augmented by cytokines, such as interferon- γ and TNF, and also by lymphocyte activation. In contrast, expression of FasL is more tightly regulated, often only inducible under specific conditions. FasL expression is restricted to immune cells, including T and B lymphocytes, macrophages, and natural killer cells, and to non-immune sites, such as the testis, kidney, lung, intestine, and the eye (Watanabe-Fukunaga et al., 1992; Suda et al., 1993; Nagata & Golstein, 1995). (In the case of immune-privileged sites, such as the eye and testis, FasL expression is constitutive.)

Ligation of death receptors causes the rapid formation of a death-inducing signaling complex, through the receptors DD. This domain is responsible for coupling the death receptor either to a cascade of caspases, leading to induction of apoptosis, or to the activation of kinase signaling pathways, resulting in gene expression through nuclear factor- κ B (NF- κ B) and/or activator protein-1. Interestingly, it recently has been suggested that there is an additional family of related decoy receptors lacking the DD signaling motif that seems to function by sequestering the appropriate ligands, thus seeming to operate as inhibitors of apoptosis (Ashkenazi & Dixit, 1999).

The Fas-FasL interaction is perhaps the simplest scenario to understand the signaling pathway of death receptor-induced apoptosis (Fig. 6). Ligation of Fas (by FasL or stimulatory antibodies) causes rapid death-inducing signaling complex formation. The Fas-associated DD (FADD) protein is recruited to the receptor's DD (Ashkenazi & Dixit,

1998). (FADD itself has a DD, and these domains bind via a DD-DD interaction.) FADD also contains a DED, and this, in turn, recruits procaspase-8, which binds via its DED. Via aggregation of two or more procaspase-8 molecules, procaspase-8 becomes activated. In general, procaspase-8 has a low level of activity, but when in close proximity to one another, two procaspase-8 molecules can process each other to the mature, active form. Active caspase-8 can then go on to trigger the apoptotic caspase cascade.

A molecule with a number of different names, which is often referred to as c-FLIP (also FLAME, I-FLICE, Casper, CASH, usurpin, MRIT, CLARP) can block Fas-mediated apoptosis (Irmeler et al., 1997). c-FLIP resembles procaspase-8, but does not have the active proteinase site. Therefore, although it can be recruited to the ligated Fas signaling complex, it can not convey a death signal. IL-2 has been shown to down-regulate c-FLIP expression (Van Parijs et al., 1999), and this may explain why IL-2 can sensitize activated T-cells to Fas. However, other studies have shown that c-FLIP is not recruited to the signaling complex in resistant T-cells (Scaffidi et al., 1999), suggesting that other regulators may be important.

4.2. Other death receptor pathways

DR3 also binds FADD, and requires this adapter for induction of apoptosis (Chinnaiyan et al., 1996; Yeh et al., 1998). It is likely that DR3 signals similarly to Fas. In contrast, TRAIL receptor signaling for apoptosis can occur in the absence of FADD (Yeh et al., 1998), although the receptor does appear to bind this adapter (Schneider et al., 1997). Signaling from TNFR1 is more complex than signaling through the aforementioned receptors due to the need for another adapter protein, TNFR-1-associated DD (TRADD). TRADD binds ligated TNFR1 via a DD-DD interaction. TRADD can then recruit FADD to the complex, resulting in caspase-8 recruitment and activation.

As mentioned in Section 4.1, ligation of a death receptor does not necessarily lead to caspase-8 activation and death. TNFR1 can also activate NF- κ B, and in cells that are resistant to TNFR1-mediated apoptosis, inhibition of this transcription factor can sensitize the cells to this form of death (Van Antwerp et al., 1998). One way NF- κ B works is to induce the expression of two TNFR1-associated factors (TRAF-1 and -2) and an IAP (c-IAP). Once bound to the receptor, the TRAFs are capable of recruiting c-IAP to the complex, and by this, apoptotic signaling is inhibited (Wang et al., 1998). Most likely, there are several other anti-apoptotic functions of NF- κ B.

4.3. Fas and the immune system

Fas and FasL have important functions in the immune system. Perhaps the best understood role for Fas in the immune system is in T lymphocytes, specifically CD4⁺ T lymphocytes, undergoing activation-induced cell death

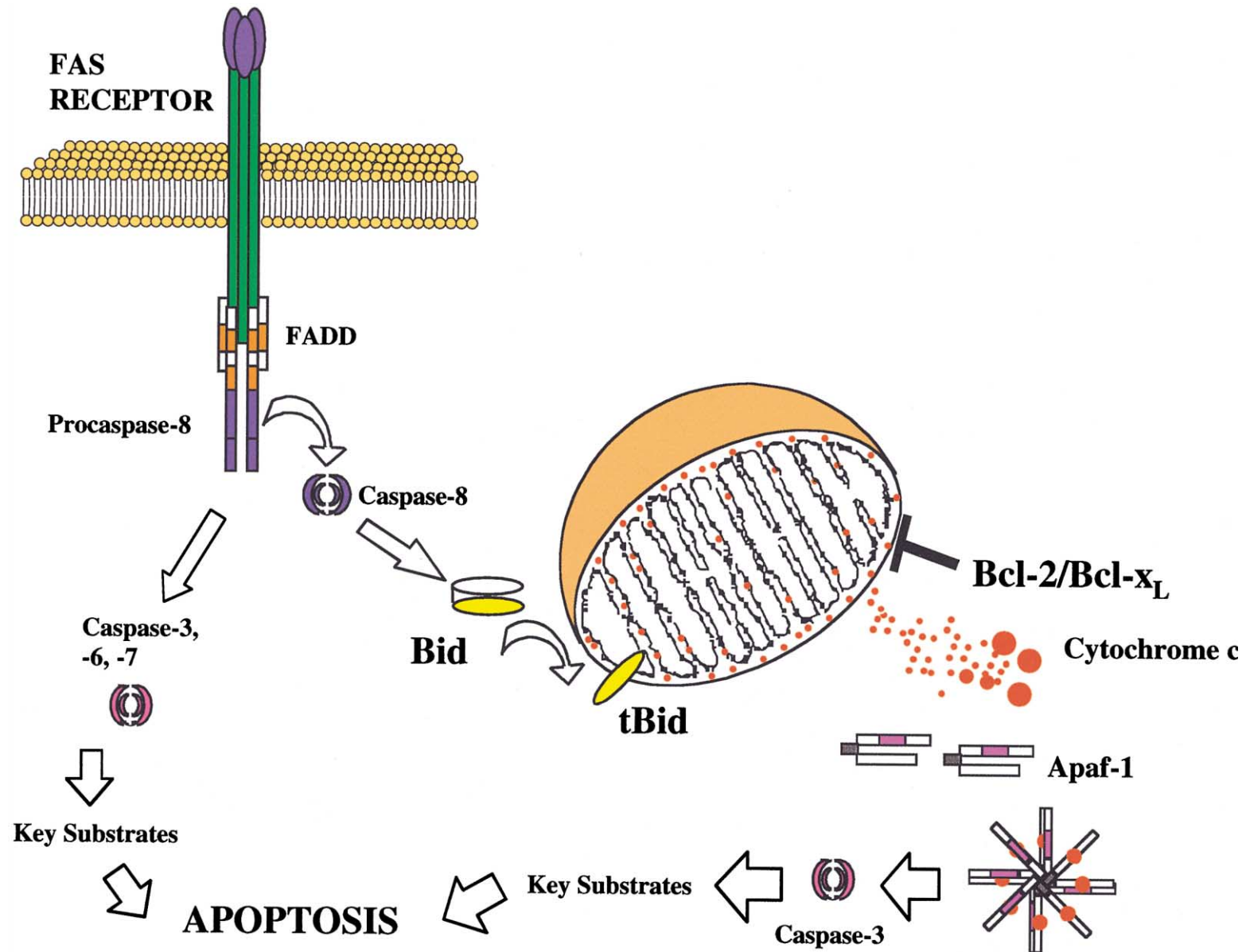


Fig. 6. Signaling from a death receptor. The activation of caspase-8 by ligation of Fas is illustrated. FasL recruits FADD to the intracellular region, which, in turn, recruits procaspase-8. The procaspase-8 transactivates, and the mature caspase now can cleave and activate procaspase-3, leading to apoptosis. Signaling from the Fas receptor to mitochondria involves cleavage of the BH3-only protein Bid by caspase-8. Bid subsequently induces cytochrome c release and downstream apoptotic events.

(Brunner et al., 1995; Dhein et al., 1995; Ju et al., 1995). In response to antigenic stimulation, peripheral T lymphocytes proliferate and expand to produce a population of clonally related cells that react against a certain antigen. However, once an immune response is developed, it must also be controlled, as some of the cytokines produced will trigger inflammation and lead to tissue damage. Therefore, with the exception of a few that will survive to become memory cells, the antigen-specific T lymphocytes generated must be eliminated. Upon repeated antigenic stimulation through the T-cell receptor, peripheral T lymphocytes rapidly up-regulate expression of Fas (Owen-Schaub et al., 1992). Along with the up-regulation of Fas expression, activated peripheral T lymphocytes are also induced to up-regulate FasL expression (this effect is enhanced in the presence of IL-2) (Alderson et al., 1995). In this fashion, these cells can eliminate neighboring cells expressing Fas, hence the term “activation-induced cell death” (Kawabe & Ochi, 1991).

Another role for the Fas-FasL interaction in the immune system can be found in apoptosis induced by cytotoxic T lymphocytes (CTLs). CTLs expressing FasL may exert their killing effects on target cells, such as virally infected cells, which express Fas. (Alternatively, CTLs may use perforin and granzyme B to activate caspases and eliminate target cells.) This mechanism of CTL killing has also been implicated in the phenomenon of graft-versus-host disease (Via et al., 1996).

Immunologic privilege provides another instance of the Fas-FasL interaction at work in the immune system. Some sites in the body are devoid of cell-mediated immune responses, and, thus, are considered privileged sites. A prime example of this can be found in the eye. If an immunological response is induced via injection of virus into the anterior chamber of the eye, the infiltrating cells die by apoptosis (Griffith et al., 1999). However, injection of virus into the eye of a Fas- or FasL-defective animal results in a massive and destructive inflammatory response, presumably due to the failure of the infiltrating cells to undergo apoptosis. The clearance of immune cells after viral injection was shown to be dependent on the expression of Fas on the infiltrating cells and FasL on the cells of the eye tissue (Griffith et al., 1999; Griffith et al., 1996). Additionally, there are other tissues, such as the liver and small intestine, that normally do not constitutively express FasL, but up-regulate it only during potent immune responses in the presence of activated T lymphocytes (Bonfoco et al., 1998). It is thought that this may represent a form of “inducible immune privilege.”

The role of Fas and FasL in T lymphocyte development still remains unclear. Early studies investigating negative selection (the process by which self-reactive immature T lymphocytes are deleted) in *lpr/lpr* mice suggested that the Fas-FasL interaction is not involved in this process (Matsumoto et al., 1991; Zhou et al., 1991). However, further studies have shown that this may

not be the case (Castro et al., 1996; Adachi et al., 1996; Kishimoto et al., 1998). It has been demonstrated that negative selection may be dependent upon Fas and FasL, but only in the case of antigens that are expressed at high levels (the previous studies were performed under conditions of relatively low-level antigenic expression) (Kishimoto et al., 1998).

Although this story remains unresolved, as more and more data are uncovered regarding the role(s) of Fas and FasL, it does become clear that these molecules are essential for molding and shaping the immune response and immune homeostasis.

5. Death receptors and mitochondria — a bid farewell

The mitochondrial pathway involving Bcl-2 family members, mitochondria, cytochrome c, Apaf-1, and caspase-9, is fundamentally distinct from that of the death receptors. Cells lacking caspase-8 (Varfolomeev et al., 1998) or FADD (Yeh et al., 1998) do not respond to death ligands, but undergo apoptosis induced by other agents, such as cellular stress. On the other hand, cells lacking caspase-9 (Hakem et al., 1998; Kuida et al., 1998) or Apaf-1 (Cecconi et al., 1998; Yoshida et al., 1998) are incapable of undergoing apoptosis induced by such stressors, but readily die in response to death ligands.

A controversy arises, however, when the anti-apoptotic effects of Bcl-2 on Fas signaling are considered. Some investigators have found that Bcl-2 can prevent Fas-mediated apoptosis in certain cell types, while in others, it cannot (Scaffidi et al., 1998). However, others contend that Bcl-2 family members never interfere with Fas-mediated apoptosis (Huang et al., 1999). Therefore, if Bcl-2 can sometimes interfere with death mediated through Fas, is this through a new function of the anti-apoptotic protein or its role in the mitochondrial pathway? Evidence suggests the latter. One of the BH3-only proteins, Bid, is cleaved by caspase-8 (activated by ligation of the death receptors) and translocates to the mitochondria, where it triggers cytochrome c release (Luo et al., 1998; Gross et al., 1999; Li et al., 1998), most likely through interaction with Bax (Desagher et al., 1999; Eskes et al., 2000) or Bak. Bcl-2 can prevent this Bid-mediated effect. In cells, expression of Bid can sensitize for TNF-induced apoptosis (Luo et al., 1998). In vitro, the ability of caspase-8 to activate caspase-3 is greatly facilitated by the presence of Bid and mitochondria (Bossy-Wetzel & Green, 1999). These observations suggest that when caspase-8 is limiting, the action of Bid on mitochondria can determine whether a cell will undergo apoptosis (via cytochrome c release and the activation of caspase-9). In support of this, Bid-deficient mice are relatively resistant to the lethal effects of anti-Fas antibody in vivo, and their hepatocytes do not readily undergo Fas-mediated death (Yin et al., 1999). In contrast, lymphoid cells from these mice are fully sensitive to death via Fas ligation.

6. Conclusions

In this review, we have sketched out the major molecular pathways for the induction and control of caspase activation and apoptosis following a variety of stimuli for death. It is possible that these are the only pathways that exist in mammalian cells, but this seems unlikely. Apoptosis induced by UV light and other stimuli can proceed in animals lacking caspase-9 (Hakem et al., 1998), and this suggests at least one alternative to the cytochrome c-Apaf-1-procaspase 9 apoptosome. However, stress can trigger expression of death ligands, such as FasL (Faris et al., 1998). Perhaps this accounts for such an alternative pathway as Fas triggers caspase activation independently of caspase-9. It has also been noted that in forms of apoptosis that proceed slowly, such as those triggered by growth factor withdrawal or other means, apoptosis apparently can proceed without obvious processing of caspase-3 or its substrates (Ruiz-Vela et al., 1999). Again, is an alternative pathway operating here? Currently, while there are hints that such pathways do exist, we do not have any incontrovertible evidence or ways in which to engage such a pathway consistently. Of course, we do not know how such a pathway would work. The prospects, though, are exciting, and the hunt goes on for all of the machineries of death.

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