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Vol. 4: Apoptosis

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1. CASPASE REGULATION AT THE MOLECULAR LEVEL

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1. Introduction

Apoptosis or programmed cell death is an essential process required for precise embryonic development and tissue homeostasis in adult species. Programmed cell death is controlled by sequential action of a specific set of proteins which are conserved throughout multicellular organisms and convert death-inducing signal(s) into cell-disassembling biochemical processes.

Molecular details of the apoptosis machinery first emerged from a genetic screen of the hermaphrodite nematode *Caenorhabditis elegans* which revealed the four global apoptosis regulators *ced*-3, *ced-4*, *ced-9* and *egl-1* (Metzstein *et al*., 1998). *ced-3* encodes a member of the cystein-containing aspartate-specific proteases family known as "caspases". *ced-3*-deficient *C. elegans* mutants were devoid of programmed cell death during development (Ellis and Horvitz 1986). Ced-4 is an activator of caspase-mediated cell death and nematodes lacking this global proapoptotic regulator exhibit superfluous cells (Ellis and Horvitz 1986). Conversely, Ced-4-mediated apoptosis induction could be blocked by direct interaction with the survival protein Ced-9 (Chen *et al*., 2000; del Peso *et al*., 2000; Parrish *et al*., 2000). The key role of Ced-9 in preventing apoptosis is exemplified by a gain-of-function mutation which constitutively activates Ced-9, resulting in sustained suppression of apoptosis in *C. elegans* while loss-of-function mutations are lethal at an embryonic stage (Hengartner *et al*., 1992). Egl-1 is produced in response to death signals and interacts with Ced-9 thus preventing Ced-4-mediated caspase activation (Conradt and Horvitz 1998).

This very basic regulatory network of pro- and antiapoptotic response regulators discovered in *C. elegans* has corresponding homologs in most mammalian cells where they often exist as multigene families. In this chapter we focus on describing

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the function and regulation of the mammalian caspase family of proteases (see Figure 1 for an overview of caspase-modulating proteins).

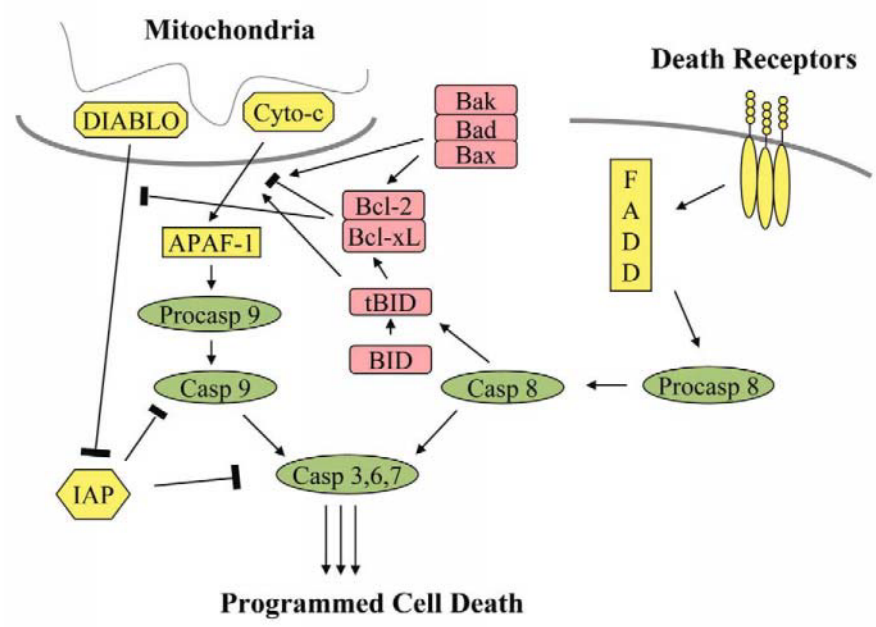

***Figure** 1.* Caspase regulation network. Activation of initiator caspases such as caspase-8 and -9 involves recruitment to large multiprotein complexes. IAP family members bind and inhibit active caspases and IAP antagonists such as DIABLO/Smac abolish this interaction once released from the mitochondria.

2. The Caspase Family of Proteins

Caspases are intracellular cysteine proteases that have specific substrate recognition sequences and cleave target proteins after aspartate residues (Thornberry *et al*., 1997). In healthy cells the majority of caspase molecules are present as monomeric zymogenes called procaspases. Nematodes harbor three caspases, the Drosophila genome encodes seven and 11 cystein proteases are known in humans (Stennicke *et al*., 2002). Although most caspases have evolved as key regulators of programmed cell death some members of this protease family including caspase-1, caspase-11 and presumably caspases-4 and -5 are involved in the enzymatic maturation of cytokines (Tingsborg *et al*., 1996; McAlindon *et al*., 1998; Wang *et al*., 1998; Furlan *et al*., 1999) (Table 1). Several extra- and intracellular signals are known to modulate activation of two classes of caspases during apoptosis: initiator and executioner caspases.

All caspases are produced as inactive zymogens (procaspases) which undergo proteolytic activation during apoptosis. While initiator caspases-2, -8, -9 and -10 undergo autocatalytic activation following recruitment of the pro-domain into multiprotein-complexes, executioner pro-caspases-3, -6 and -7 are processed by initiator caspases (Table 1). Cleavage of caspase substrates is believed to be the point of no return which commits a cell to apoptosis. While some activated caspases are directly involved in dismantling the cell by cleaving key structural and other proteins, others modulate the extent of caspase activation.

Table 1. Mammalian caspases. h, caspase only present in humans; m, caspase only present in mice; b, caspase only present in bovines; CARD, caspase recruitment domain; DED, death effector domain.

Caspase	Domains	Regulators	In vivo function
Initiator Caspases			
Caspase-2	CARD	RAIDD; RIP; TRADD	Initiation of apoptosis in germ cells; neurons and B-cells
Caspase-8	DED	FADD FLIP	Death receptor mediated apoptosis; developmental cell death
Caspase-9	CARD	APAF-1; IAPs; DIABLO/HtrA2	Neuronal cell death; apoptosis in thymocytes
hCaspase-10	DED	FADD FLIP	Death receptor mediated apoptosis
Executioner Caspases			
Caspase-3	-	Casp-9; IAPs; DIABLO/HtrA2	Neuronal cell death; required for apoptosis in thymocytes
Caspase-6	-	Casp-3 ?	Chromatin condensation; formation of apoptotic bodies?
Caspase-7	-	Casp-9; IAPs; DIABLO/HtrA2	Not known
Caspase-14	-	-	Not known
Cytokine processing Caspases			
Caspase-1	CARD	ASC; Ipaf	Activation of cytokines during inflammation
Caspase-4	CARD	Casp-8	Activation of cytokines during inflammation ?
Caspase-5	CARD	-	Activation of cytokines during inflammation ?
mCaspase-11	CARD	-	Activation of cytokines during inflammation
mCaspase-12	-	Casp-8	Not known
bCaspase-13	-	-	Not known

Caspase target sites contain at least four specific contiguous amino acids termed P4-P3-P2-P1. Cleavage occurs after the P1 residue that is typically an aparagine (Asp). For all caspases the preferred amino acid at position P3 is glutamine (Glu) whereas amino acids at positions P2 and P4 can vary considerably. Hence, the

consensus cleavage specificity of caspases is X-Glu-X-Asp (Thornberry *et al*., 1997). Various intracellular proteins have been identified as caspase targets, yet the relevance of caspase-mediated cleavage for apoptosis remains elusive in many cases, in particular those which occur at low efficiency and at late stages of programmed cell death.

Some caspase substrates are activated by proteolytic processing. The most prominent examples include activation of executioner caspases, p21-activated kinase 2 (PAK2) (Rudel and Bokoch 1997) and protein kinase Cδ (PKCδ) (Emoto *et al*., 1995; Ghayur *et al.,* 1996). By contrast, caspase-mediated cleavage can inactivate some target proteins such as ICAD/DFF45, the inhibitor of the DNAse CAD, processing of which results in the release of active DNAse involved in DNA fragmentation (Enari *et al*., 1998; Halenbeck *et al*., 1998; Liu *et al.,* 1998).

The protease domain of caspases consists of a small and a large subunit. Caspase activation is believed to involve two distinct proteolytic steps: Firstly, the smaller C-terminal subunit of the protease domain is released, followed by removal of the prodomain from the large subunit of the protein. Crystallography studies suggest that active caspases are heterotetramers composed of two small and two large subunits (Walker *et al.,* 1994; Wilson *et al.,* 1994; Thornberry and Lazebnik 1998).

3. *In Vivo* Function of Caspases – Lessons from Knockout Mice

The central physiological role of caspases in modulating apoptosis and inflammation was revealed by phenotypic analysis of caspase-deficient/negative mouse mutants. Since the phenotypes of caspase-3- and caspase-9-deficient mice are remarkably similar and comparable to knockouts of the *ced-4* homologue Apaf-1 which activates caspase-9 suggested that all determinants impinge on the same developmental pathway. In fact, all abnormalities were found to be associated with brain development (Kuida *et al.,* 1996; Hakem *et al.,* 1998; Woo *et al.,* 1998). In caspase-$3^{-/-}$ and even more prominent in caspase-$9^{-/-}$ animals cell death in the proliferative neuroepithelium is drastically reduced which results in distorted anatomical structures (Hakem *et al.,* 1998; Kuida *et al.,* 1998). Some cell types in caspase-$9^{-/-}$ mice exhibited increased resistance to apoptosis when induced by toxic stimuli whereas the sensitivity to programmed cell death remains unchanged when stimulated by the tumor necrosis factor recptor family including Fas/Apo1/CD95 (also referred to as death receptors; see below).

Although caspase $2^{-/-}$ mice develop normally and are fertile, it has been reported that an increased number of germ cells accumulate in female mice, indicating that caspase-2 may be involved in the removal of excess oocytes (Bergeron *et al.,* 1998).

All phenotypes (lethality in early development, abnormal hearts, reduced hematopoietic precursor cell number and resistance of fibroblasts to receptor-mediated apoptosis induction) which are typically associated with caspase-$8^{-/-}$ mice suggest an essential role of the corresponding caspase in the differentiation of the

heart muscle and hematopoetic progenitor cells as well as in death receptor signaling in fibroblasts (Varfolomeev *et al.,* 1998).

While caspases-2, -3, -8 and -9 appear to play pivotal roles in apoptosis regulatory networks the phenotypes of caspase-1- and caspase-11-deficient mice rather suggest apoptosis-unrelated functions for these proteases *in vivo*. Caspase-$1^{-/-}$ mice lack mature interleukin-1β (IL-1β) and IL-18 (Kuida *et al.,* 1995; Li *et al.,* 1995; Ghayur *et al.,* 1997; Fantuzzi *et al.,* 1998). Furthermore, these mutant mice are unable to produce IL-1α, IL-6, tumor necrosis factor α (TNF-α) and interferon-γ (IF-γ) in response to polysaccharide (LPS) which may result from secondary effects associated with defective IL-1β and IL-18 processing. Caspase-$11^{-/-}$ mice showed a phenotypes comparable to caspase-$1^{-/-}$ mice which correlated with deficient caspase-1 activation (Wang *et al.,* 1998).

4. Caspase Structures

Full or partial structural information has been obtained for caspase-1 (Walker *et al.,* 1994; Wilson *et al,.* 1994), caspase-3 (Mittl *et al.,* 1997), caspase-7 (Chai, Shiozaki *et al.,* 2001), caspase-8 (Blanchard *et al.,* 1999; Watt *et al.,* 1999) and caspase-9 (Renatus *et al.,* 2001). All of these structural studies support a model by which active caspases are present as homodimers, with each monomer consisting of a large (20 kDa for caspase-1) and a small (10 kDa for caspase-1) subunit. Therefore, the active enzyme is best described as a heterotetramer.

Four loops known as L1, L2, L3 and L4 form the active site which is conserved among all caspases. While L1 and L3 represent well-conserved structures, the amino acid composition and length of L2 and L4 vary substantially between different caspases (Shi 2002). The exact position of these four loops within a particular caspase determines its substrate specificity. The substrate recognition residues P1-4 bind to the substrate pockets S1, S2, S3 and S4 respectively, located between the base (L3) and the sides (L1 and L4) of the substrate binding groove (Shi 2002).

All caspases except caspase-9 appear to require proteolytic processing for full activation, and the recently resolved crystal structure of caspase-7 provides first insights into the molecular mechanism of caspase activation (Chai *et al.,* 2001; Riedl *et al.,* 2001). The caspase-7 zymogene forms homodimers in which the linker regions connecting the large and the small subunits block the groove of the cavity between the two monomers. This conformation prevents substrate binding and the formation of an active proteolytic center prior to this linker region being cleaved (Riedl *et al.,* 2001). Although the overall structures of procaspase-7 and active caspase-7 are almost identical (less then 0.8 A root-mean-square deviation for all aligned Cα atoms), drastic rearrangements take place within the active site following cleavage. In particular L2, which contains the catalytic residue Cys 186, is rotated by 90° in the active caspase-7 (Chai *et al.,* 2001).

Interestingly, proteolytic processing is not required for activation of procaspase-9 (Stennicke *et al.,* 2002). Structure-function analysis of caspase-9 and its mutants lacking the caspase recruitment domain (CARD) revealed the classic dimeric caspase conformation consisting of four catalytic domains. However, in contrast to all other caspases, caspase-9 dimers contain only one active catalytic site while the other remain inactive (Renatus *et al.,* 2001). This asymmetry of the caspase-9 dimer suggests a novel activation mechanism for this initiator caspase.

5. Activation of Caspases

5.1. THE DEATH RECEPTOR PATHWAY - ACTIVATION OF PROCASPASE-8 VIA FAS/APO1/CD95

Cytokines of the tumor necrosis factor (TNF) family play an essential role in development, function and homeostasis of the immune system, the bone as well as the mammary gland (Locksley *et al.,* 2001). Binding of TNF family members to their cognate receptors can lead to activation of initiator caspases and subsequent cell death (Baud and Karin 2001). Proteins of the TNF family of proteins contain a conserved C-terminal homologous to the TNF domain which forms homo- and less frequently heterotrimers interacting with cognate receptors of the TNF family (Bodmer *et al.,* 2002). Ligand-mediated oligomerization of TNF recpetors leads to their activation, and depending on the type of receptor, to either induction of pro- or antiapoptotis regulatory pathways (Wallach *et al.,* 1999). TNF-type of receptors that engage cell death pathways include Fas/Apo1/CD95 (specific for the Fas ligand (FasL)), type 1 tumor necrosis factor α (TNFα) receptor (TNFR1) (specific for TNFα), death receptor 3 (DR3; specific for the Apo3 ligand), and DR4 as well as DR5 (both of which bind Apo2/TRAIL). All of these receptors induce cell death by acting as scaffolds for caspase activation.

The best-characterized pathway of caspase activation is the processing of procaspase-8 to caspase-8 triggered following binding of FasL to the death receptor Fas/Apo1/CD95 (Figure 1). Fas/Apo1/CD95 exists as a preassociated complex which appears to be required for functional apoptosis signaling following FasL binding (Siegel *et al.,* 2000). When FasL binds to its cognate receptor Fas/Apo1/CD95, the death domain (DD), an intracellular portion of the Fas/Apo1/CD95 receptor of about 90 amino acids, interacts with the death domain of a bipartite adaptor molecule called FADD in a homotypic interaction (Boldin *et al.,* 1995; Chinnaiyan *et al.,* 1995). The rapid recruitment of FADD to form these receptor complexes is associated with the formation of microaggregates (Kischkel *et al.,* 1995; Kamitani *et al.,* 1997). The N-terminal death effector domain (DED) of FADD in turn interacts with the DED of procaspase-8 to form the so-called death-inducing signaling complex (DISC) (Medema *et al.,* 1997). Studies in cultured cells

using the actin inhibitor Ltn A suggested that this step requires actin filaments (Algeciras-Schimnich *et al.,* 2002). In a final step, processed caspase-8 is freed from the DISC and starts to process its substrates such as caspase-3 or the proapoptotic Bcl-2 family member Bid (Li *et al.,* 1998; Luo *et al.,* 1998).

There is growing evidence that oligomerization of procaspase-8 within the DISC is essential for its proteolytic activation. Transfection of chimeric caspase-8 constructs in which the DED containing prodomain was replaced by a CD8 dimerization domain resulted in cell death induced by caspase-8 autoactivation (Martin *et al.,* 1998). Likewise, *in vitro* oligomerization of caspase-8 by engineered FK506 binding protein (FKBP) domains was shown to activate procaspase-8 (Yang *et al.,* 1998). Activity of the DISC can be regulated by the FADD-like inhibitor protein FLIP (Cryns and Yuan 1998). FLIP contains two DEDs enabling it to block recruitment of procaspase-8 to activated death receptor complexes by competing with its binding to FADD (Irmler *et al.,* 1997). Procaspase-10 also contains a DED and, like procaspase-8, can be recruited to TRAIL and Fas/Apo1/CD95 death receptor complexes via FADD (Sprick *et al.,* 2002). Although it could be shown that caspase-10 is proteolytically processed during FasL-induced apoptosis, its function appears to differ from that of caspase-8 since it was unable to complement defective apoptosis induction in caspase-8-deficient cells (Sprick *et al.,* 2002).

Mice which lack FADD die in an embryonic stage and FasL-induced apoptosis is completely blocked in T-cells derived from $FADD^{-/-}$ embryonic stem cells when transplanted into $RAG1^{-/-}$ hosts (Yeh *et al.,* 1998; Zhang *et al.,* 1998; Kabra *et al.,* 2001). These data indicate that there are no redundant Fas/Apo1/CD95-mediated apoptosis pathways.

5.2. CASPASE-9 ACTIVATION IN THE APOPTOSOME

Death stimuli such as UV irradiation lead to distinct changes in the integrity of mitochondria. At early stages of apoptosis transition of mitochondria from an orthodox to a condensed conformation associated with their intracellular redistribution into to perinuclear clusters has been observed (Mancini *et al.,* 1997; De Vos *et al.,* 1998). Furthermore, some apoptosis stimuli result in a reduction of the inner membrane potential, outer membrane discontinuities and release of cytochrome c into the cytoplasm (Desagher and Martinou 2000). Cell-free assays have demonstrated that cytochrome c interacts with apoptotic protease activating factor-1 (Apaf-1) to form a multiprotein complex containing active caspase-9 in the presence of dATP (Figure 1). This complex is also known as the apoptosome (Li *et al.,* 1997). The finding that caspase-9 enzymatic activity increases 1000-fold upon its association with the apoptosome led to the current model whereby the assembly of this scaffolding complex is the key molecular event in the activation of caspase-9 (Rodriguez and Lazebnik 1999). This Apaf-1/caspase-9 holoenzyme has been shown to activate executioner caspases such as caspase-3 (Rodriguez and Lazebnik 1999) (Figure 1).

Apaf-1 contains at least three functional domains: (i) an N-terminal CARD that is required for binding caspase-9, (ii) a domain homologous to Ced-4 which mediates Apaf-1 self-organization and (iii) 12 or 13 C-terminal WD-40 repeats thought to be involved in protein-protein interactions (Zou *et al.,* 1997). Highly purified recombinant cytochrome c, Apaf-1 and caspase-9 proteins were demonstrated to form a 1.4 mDa complex as visualized by gel filtration experiments (Saleh *et al.,* 1999; Zou *et al.,* 1999). In agreement with these findings in cell-free assays, Apaf-1 was reported to be present in high molecular weight complexes in cell extracts upon treatment of lysates with dATP and incubation at 37°C (Cain *et al.,* 1999). In these experiments Apaf-1 was found to oligomerize in two distinct complexes, one in the order of 1.4 mDa the other around 700 kDa. The smaller complex appears to form more rapidly and has a higher caspase processing activity. Apoptosis induced in human tumor cells by etoposide or N-tosyl-phenylalanylchloromethyl ketone (TPCK) resulted in the formation of a 700 kDa complex suggesting a functional relevance *in vivo* (Cain *et al.,* 1999).

Recent studies provided further insights into the molecular mechanisms of caspase-9 activation within the apoptosome. In gel filtration experiments using dATP-activated cell lysates, caspase-3 was detected in apoptosome complexes, the recruitment and processing of which was dependent on the on caspase-9 (Bratton *et al.,* 2001). Interestingly, cleavage-resistant caspase-9 mutants can still recruit and activate caspase-3 leading to the conclusion that processing of caspase-9 is neither sufficient nor required for this process (Bratton *et al.,* 2001).

The three-dimensional structure of the apoptosome has been determined at a resolution of 27A using electron cryomicroscopy (Acehan, Jiang *et al.,* 2002). Assembly of purified Apaf-1, cytochrome c and dATP resulted in a wheel-like particle of 7-fold symmetry. Known high-resolution domain structures such as the Apaf-1 CARD or WD40 domains of other proteins enabled the determination of the domain architecture by positioning structural elements within the 3D structure. The spokes of the wheel contain the Ced4 nucleotide-binding domain and cytochrome c between two C-terminal subdomains containing WD40 repeats, while the central hub resembles oligomerised Apaf-1 CARD domains (Acehan *et al.,* 2002). In healthy cells, a mechanistic model for the assembly of the apoptosome has been proposed where the N-terminal CARD domain of Apaf-1 is bound to a Y-shaped structure formed by the C-terminal WD40 domains. Cytochrome c displaces the CARD domain and this change in Apaf-1 conformation enables oligimerization of CARD domains to form the wheel-like structure of the apoptosome.

6. Activation by Induced Proximity – A Common Theme for Activation of Initiator Caspases?

The aforementioned models for activation of caspase-8 and caspase-9 suggest a similar mechanism for induction of both initiator caspases. Initially, a model of

induced proximity was postulated for caspase-8 activation (Muzio *et al.,* 1998). This model was based on the findings that dimerization of chimeric Fpk3Caspase-8 induced their activation and that processing-incompetent caspase-8 mutants retain low but detectable enzymatic activity (Muzio *et al.,* 1998). Pore limit native PAGE experiments have shown that both caspases exist as monomers in their inactive zymogene form (Boatright *et al.,* 2003). Monomers of caspases-8 and -9 purified from bacteria exist in a slow equilibrium with their dimeric form and that enzymatic activity is restricted to the dimers (Renatus *et al.,* 2001; Boatright *et al.,* 2003; Donepudi *et al.,* 2003). The concentration dependency of the dissociation constant of these dimers further supports the induced proximity model (Donepudi *et al.,* 2003). Notably, dimer formation is still observed for non-cleavable mutants of both caspases, albeit with a much higher dissociation constant. As observed for wild-type caspases, cleavage activity is again restricted to dimers (Renatus *et al.,* 2001; Boatright *et al.,* 2003; Donepudi *et al.,* 2003). These findings suggest that the crucial initial step for activation of initiator caspases is the dimerization event and that autoprocessing is not required. Instead, processing of the procaspases may serve to stabilize the dimeric active forms.

Does this model, in which the most apical caspases of an apoptotic cascade are activated by an increase in their local concentration through scaffolding to large multiprotein complexes, apply to other known initiator caspases such as caspase-2? Indeed, a recent study suggests that caspase-2 is recruited to high molecular weight complexes upon incubation of cell lysates at 37°C by a mechanism that requires its prodomain (Read *et al.,* 2002). Interestingly, these complexes still form in cell extracts lacking cytochrome c or Apaf-1 (Read *et al.,* 2002).

7. Caspase Inhibition

Does activation of initiator and effector caspases represent an ultimate commitment to apoptosis? It appears that some proteins can act as a last-line-of-defense, modulating or preventing apoptosis by binding and inactivating caspases. These proteins include viral caspase inhibitors such as p35 and CrmA and the inhibitor of apoptosis protein (IAP) family.

7.1. THE VIRAL CASPASE INHIBITORS P35 AND CRMA

Many viruses have evolved mechanisms to prevent apoptosis of their host cell in order to enable sustained viral replication (O'Brien 1998; Roulston *et al.,* 1999). The p35 gene of the baculovirus *Autographa california* multiply embedded nuclear polyhedrosis virus (AcMNPV) encodes a potent and broad-acting caspase inhibitor that can inhibit caspase activity and cell death in nematode, insect and mammalian systems (Birnbaum *et al.,* 1994). Infection of SF21 cells derived from the *Spodoptera frugiperda* (Order Lepidoptera) by a p35-deficient baculovirus causes

cell death and prevents viral replication (Clem *et al.,* 1991; Bump *et al.,* 1995). In addition to blocking apoptosis induced by viral infection, p35 can inhibit insect cell death caused by overexpression of activated insect SF-caspase-1 or mammalian caspase-3 (Seshagiri and Miller 1997). Overexpression of p35 can protect mammalian cells from various forms of apoptosis (Rabizadeh *et al.,* 1993; Beidler *et al.,* 1995). Expression of p35 in transgenic nematodes, flies and mice produces phenotypes characterized by excessive developmental cell death (Sugimoto *et al*., 1994; Hay *et al.,* 1994; Izquierdo *et al.,* 1999).

The mechanism by which p35 neutralizes caspase activity involves its proteolytic cleavage (Seshagiri and Miller 1997). Upon binding to caspases, p35 is cleaved at the caspase cleavage sequence DQMD↓G and the cleavage product remains tightly associated with the caspase (Bump *et al.,* 1995). Mutation of the P1 aspartate to a glutamate or an alanine prevents cleavage of p35 and supports the requirement of precise p35 processing for caspase inactivation (Bertin *et al.,* 1996). The inhibited caspase is trapped as a covalent adduct through the binding of the catalytic cysteine by the P1 aspartate, presumably by formation of a thiolester bond (Riedl *et al.,* 2001).

Cowpox virus expresses the protein CrmA (cytokine response modifier A) in order to avoid inflammatory and apoptotic responses following host cell infection (Zhou and Salvesen 2000). CrmA targets members of the caspase family of proteases that either initiate apoptosis pathways (caspases-8 and -10) or trigger activation of the pro-inflammatory cytokines interleukin-1 and interleukin-18 (via caspase-1, see above) (Komiyama *et al.,* 1994; Zhou *et al.,* 1997). Similar to p35, CrmA inhibits proteases by acting as a pseudosubstrate (Ray *et al.,* 1992; Stennicke *et al.,* 2002). However, structural analysis showed that CrmA represents a true member of the serpin family of protease inhibitors (Renatus *et al.,* 2000; Simonovic *et al.,* 2000). CrmA can also inhibit the serin protease Granzyme B and therefore represents a cross-class inhibitor of proteases (Stennicke *et al.,* 2002).

7.2. IAPs

IAPs are an evolutionarily conserved group of proteins distinguished by the presence of the baculovirus IAP repeat (BIR) motif (Crook *et al.,* 1993) (Figure 2). This BIR domain consists of around 70 amino acids and has been identified in baculoviral protein Op-IAP which harbors a conserved core structure of three cysteines and one histidine containing a zinc ion (Crook *et al.,* 1993; Hinds *et al.,* 1999; Sun *et al.,* 1999).

The mammalian proteins XIAP, cIAP-1 and c-IAP2 are direct caspase inhibitors capable of blocking activities of caspases-3, -7 and -9 but not of caspases-1, -6, -8 or -10 (Deveraux *et al.,* 1997; Roy *et al.,* 1997). Amongst these proteins, XIAP is the most potent inhibitor showing an inhibition constant comparable to p35 (K_i of 0.7 nM) (Deveraux *et al.,* 1997). In contrast to XIAP, c-IAP-1 and c-IAP-2 are 50-

10'000 less efficient in binding and inhibiting their target caspases (Roy *et al.,* 1997). Cell death induced by a wide variety of stimuli including death receptor activation as well as irradiation can be efficiently blocked in mammalian cells by heterologous expression of IAPs (Figure 1).

Unlike some IAPs, many BIR domain-containing proteins (BIRPs) show no apoptosis-inhibiting activities. For example, the yeast genome lacks any caspase-encoding genes but harbors BIRP family members such as the *Schizosaccharomyces pombe* protein Bir1p. Bir1p regulates cell division during mitosis and was found to be essential for chromosome condensation as well as spindle elongation (Rajagopalan and Balasubramanian 2002). Genetic studies in *C. elegans* revealed that the nematode BIRP BIR-1 is a complex showing Aurora-like kinase AIR-2 activities involved in regulating mitosis, a characteristic which seems to be conserved in the human proteins survivin and Aurora kinase (Skoufias *et al.,* 2000; Speliotes *et al.,* 2000; Uren *et al.,* 2000) (Figure 2).

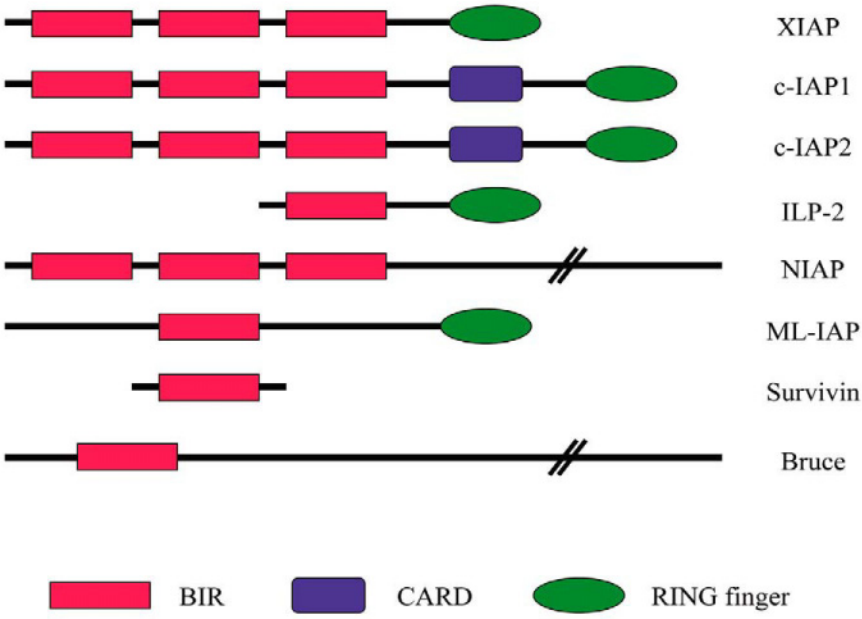

***Figure** 2.* Mammalian IAP family members.Mammalian IAPs such as XIAP, cIAP1 and cIAP2 bind an inactivate caspases. Other family members such as survivin appear to function in cell division. BIR, baculovirus IAP repeat; CARD, caspase-recruitment domain.

Genetic studies in *Drosophila* provide evidence that IAPs may be essential for apoptosis regulation, at least in *Drosophila*. Homozygous mutants lacking D-IAP1 expression exhibit embryonic lethality, presumably due to excessive cell death during development (Wang *et al.,* 1999) The fact that cell death in these embryos is