Integrative Biosciences 9: 181-190, 2005

REVIEW

Integrative **Biosciences**

http://www.ibiosci.or.kr

BIR Containing Proteins (BIRPs): More Than Just Cell Death Inhibitors

Soon Ji Yoo*

Department of Biology, College of Sciences, Kyung Hee University, Seoul 130-701, Korea

Abstract: BIRPs (BIR containing Proteins) which contain one to three BIR domains constitute a highly conserved family from yeast to human. BIR domains mediate the interaction of BIRPs with various other proteins. Some of the members acquire a Ring domain which acts as an E3 ubiquitin ligase. The first member of BIRPs identified in the baculovirus was found as an inhibitor of apoptosis and most of the family members in the other species have been recognized to have the same function which bind to and inhibit caspases, thereby suppresses apoptotic cell death. But an increasing number of evidences indicate that BIRPs are involved in various cellular events such as cell division, control of cell cycle, signal transduction, cell migration, innate immunity as well as regulation of apoptosis. In this review, we summarize the structural and functional features of the BIRPs, especially focus on the various functions of BIRPs unrelated to regulation of apoptosis by the recent findings.

Key words: BIRP, Apoptosis, BIR, Ring

BIRPs Family - Identification and Synthesis

Members of BIR containing proteins

The first genes isolated among BIRPs were the baculovirus genes CpIAP (from *Cypia pomonella granulosis* virus, CpGV-inhibitor of apoptotic protein) and OpIAP (from *Orgyia pseudotsugata* nuclear polyhedrosis virus, OpNPV-inhibitor of apoptotic protein). They were found by virtue of their ability to compensate for loss of function of the caspase inhibitor protein p35 in *Autographa Californica* nuclear polyhedrosis virus (AcNPV) mutants (Crook et al., 1993). It is believed that these IAPs are used by baculoviruses to prevent a defensive apoptotic response of the host cells that would otherwise limit viral replication (Clem and Miller, 1993). That is why these were originally called as the Inhibitor of Apoptotic Protein (IAP).

These baculoviral IAPs share a common domain called BIR (Baculovirus IAP Repeat). The BIR is about 70-residue zinc-binding domain in which zinc ions are coordinated by three cysteine and one histidine residues in the order of CX2CX16HX6-8C (Hinds et al., 1999). The yeast carries two BIRPs - ScIAP in *S. cerevisiae*, SpIAP in *S. pombe* - and both of these proteins bear two BIR repeats. *C. elegans* has two BIRPs, of which one (CeBIR1) has two repeats and the other (CeBIR2) has a single repeat of BIR. The *Drosophila* genome encodes four BIRP genes-deterin, dBruce, diap1 and diap2. Only deterin gene contains a single BIR domain, the other genes acquire different domains in addition to BIR. Among mammalian BIRPs, Survivin is the most closely related to the type of BIRPs found in yeast and *C. elegans*. The survivin gene contains a single BIR domain. These only BIR-containing proteins have a slightly larger BIR and can form a distinct survivin type subfamily by sequence homology (Uren et al., 1998). In *C. elegans*, the deleted phenotype of ceBIR1 similarly to and partially rescued by Survivin (Speliotes et al., 2000). diap1 and diap2 genes in *Drosophila* contain a second type of zinc-binding motif known as a Ring domain (Fig. 1). This domain is also found in baculoviral IAPs and some of the mammalian BIRPs at the extreme carboxyl terminus of the protein. Among BIRPs, IAPs are a distinct subtype which contains BIR and Ring domains together. All genes encoding IAPs bear BIRs but not all BIRPs seem to have anti-apoptotic activity. The Ring domain is considered to function as an E3 ubiquitin ligase (Yang et al., 2000), which is preceded by ubiquitin-activating enzymes (E1) and ubiquitin-conjugating enzymes (E2) in the sequential reaction for protein ubiquitination and degradation by proteasome. In fact, diap1 is the first identified cellular BIRPs important in the regulation of apoptosis from the

*Tel: 82-2-961-0978; Fax: 82-2-961-0978
E-mail: yoosoonji@khu.ac.kr

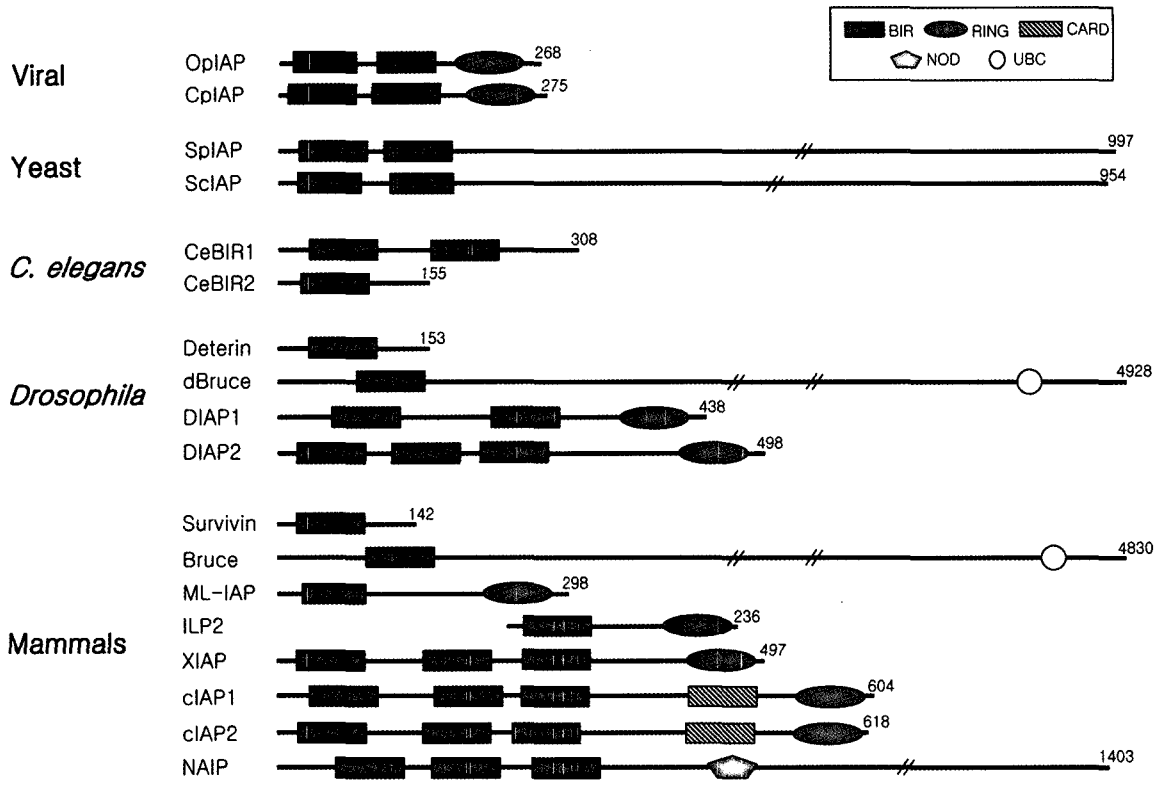

Fig. 1. The BIRP family members from virus to human. Viral BIRPs are from baculovirus. All the BIRPs in yeast and *C. elegans* have only BIR domains, no other domain. The *Drosophila* genome encodes four BIRPs. dBruce has a UBC domain as well as found in human Bruce. The human genome encodes eight BIRPs. Five of the BIRPs and two from *Drosophila* contain a Ring domain at the very C-terminal end. Human cIAP1 and cIAP2 have a CARD (caspase recruitment domain). Total protein amino acids lengths are indicated to the right. Filled square for BIR domains, oval for Ring domain, stripped square for CARD, pentagon for NOD and circle for UBC domain.

genetic screen. Overexpression of diap1 gene was able to suppress developmental death as well as cell death caused by overexpression of the pro-apoptotic genes such as reaper or head involution defective genes (Hay et al., 1995) and diap1 loss-of-function mutants showed embryonic lethality (Wang et al., 1999). The human genome has eight genes that encode BIRPs including cIAP1, cIAP2, XIAP, ILP2, ML-IAP (livin or kIAP), ILP2 (TsIAP), bruce, survivin (Fig 1). The first mammalian IAP found, NAIP was identified as the product of a gene mutated in severe forms of spinal muscular atrophy, a disease in which motor neurons die prematurely (Roy et al., 1995). NAIP has the NOD domain which is also found in Apaf-1. Bruce found in *Drosophila* and mammals is a giant BIRP (528 kDa) having a single BIR and is the only BIRP containing a C-terminus UBC domain characteristic for ubiquitin-conjugating enzyme (Hauser et al., 1998). The BIRs are essential for the anti-apoptotic properties of the BIRPs and in several cases this has been directly attributed to the binding and inhibition of caspases, a family of cysteine proteases with a substrate preference for aspartic acid that are essential for the apoptotic process.

Regulation of synthesis and localization

Certain BIRPs expression is tightly controlled at the level of transcription. Survivin in mammals is regulated in a cell cycle-dependent manner and is mostly induced at the G2-M boundary (Ambrosini et al., 1997). cIAP1 and cIAP2 synthesis can be controlled at the transcriptional level by NFκB (Chu et al., 1997; Wang et al., 1998). The increase of anti-apoptotic proteins such as cIAP1 and cIAP2 may be correlated to the pro-survival effect of NFκB. The transcription of cIAP2 and XIAP was up-regulated by the PI3K/Akt pathway under ER stress-induced cell death (Hu et al., 2004). Ablation of these IAPs by RNAi sensitized cells to ER stress-induced death, which was reversed by the caspase inhibitor.

XIAP is controlled at the level of translation. Cap-dependent translational initiation for most of the cellular proteins was suppressed under a various cellular stress conditions and cap-independent translation continued to direct protein synthesis through the use of the Internal Ribosome Entry Site-IRES-(Clemens et al., 2000). Cellular IRES elements are relatively rare but found in a number of oncogenes and growth factors. XIAP mRNA contains a

functional IRES element at 5' untranslated region. Ionizing radiation has been shown to enhance translational efficiency of XIAP in an IRES-dependent mechanism (Holcik et al., 1999, 2000).

Many expression studies have revealed that elevated BIRPs levels in a wide variety of cancer cell lines and also primary tumor biopsy samples (Schimmer, 2004). XIAP levels are elevated in many cancer cell lines (Yang et al., 2003). ML-IAP was an IAP that is highly expressed in the majority of melanoma cell lines (Vucic et al., 2000). Survivin expression is normally limited to cells of the developing fetus and is not expressed in differentiated adult tissue but aberrant expression of survivin has been detected in a number of different cancers and lymphomas (Ambrosini et al., 1997). bruce gene is broadly expressed in various tissues, and is upregulated in certain tumors (Chen et al., 1999).

BIRPs have been expected to be localized mainly in cytoplasm because most of them bind to caspases which are believed to be in cytoplasm. However, the subcellular localization between members of BIRPs seems to be quite different. Bruce is associated with the Golgi compartment and the vesicular system (Hauser et al., 1998). XIAP in cytoplasm and Survivin, cIAP1, cIAP2 are mainly in nucleus although cIAP2 expression has been detected in mitochondrial fractions as well (Ekedahl et al., 2002). Some BIRPs are nucleocytoplasmic shuttling depending on the cellular situations. For example, Survivin in normal cells is predominantly a nuclear protein, expressed in cell cycle-dependent manner, but Survivin in most tumor cells is localized in nuclear and cytoplasm, which is cell cycle-independent (Reed, 2001). Also cIAP1 has been shown to be localized predominantly in nucleus but shuttled between nucleus and cytoplasm in dividing cells and exported to cytoplasm in apoptotic stimuli (Samuel et al., 2005a). XIAP is predominantly localized in cytoplasm but sequestered in nucleus in the presence of XAF1 (Liston et al., 2001). Interestingly, cIAP1 in nucleus translocates to the Golgi apparatus when monocytic cell lines undergo differentiation under phorbol ester exposure, but no translocation observed from nucleus whose differentiation is blocked (Plenchette et al., 2004). These data indicate that changes in subcellular localization may direct the functional differences. The mechanisms regulating the distribution of IAPs into various subcellular compartments and the correlation of their subcellular location with protein function require further clarification.

Various Cellular Functions

Regulation of apoptosis

Binding of BIRPs to caspases via BIR domain

Many of the BIRPs have been shown to directly bind to and suppress activity of caspases, thus serving as endogenous suppressors of the caspase-dependent cell death. Not all BIRPs are able to bind to and inhibit caspases. BIRPs in yeast and *C. elegans* regulate the cell division rather than cell death inhibition. In *Drosophila*, Diap1 binds to directly to and inhibits upstream and downstream caspases such as Dronc, Drice and Dcp-1 (Hawkins et al., 2000). In mammals, most of the BIRPs have been shown to inhibit the caspases activity in vitro. XIAP has been shown to directly suppress the enzymatic activity of caspase-3, caspase-7, caspase-9 in vitro and in intact cells (Deveraux et al., 1997). Crystal structure of XIAP interacting with casapse-3, caspase-7, caspase-9 revealed great detail of the molecular determinants necessary for these interactions. The linker region between BIR1 and BIR2 is important for binding to caspase-3 and caspase-7. BIR3 mediates the interaction with caspase-9 (Chai et al., 2001). These reports indicate that the caspase binding of the BIRPs seems to be selective, which means overexpression of a certain BIRP may block some apoptosis but not all. cIAP1 and cIAP2 can also inhibit caspase-9 by BIR3 although overall inhibitory activity of cIAP1 and cIAP2 is less potent than XIAP *in vitro*.

In contrast, single BIR-containing BIRPs in mammal have a broad specificity. Survivin inhibits caspase-3 and caspase-7 (Shin et al., 2001) and Livin with the single BIR domain has been reported to inhibit caspase-3, caspase-7, caspase-9 (Vucic et al., 2000). Apoptotic functions of Survivin have been related to its interaction with cofactors such as HBXIP, which facilitates interactions with procaspase-9 (Marusawa et al., 2003) and to interactions with other IAPs that directly bind to caspases (Dohi et al., 2004). Molecular antagonists of Survivin, including siRNA or dominant-negative mutants caused caspase-dependent cell death and the enhancement of apoptotic stimuli (Li et al.,1999; Beltrami et al., 2004).

Recently, Bruce has been reported that it inhibits caspase and apoptosis depending on its BIR domain by binding to especially caspase-3 (Bartke et al., 2004). In *Drosophila*, dBruce can suppress caspase-dependent Reaper and Grim dependent cell death but not hid-dependent cell death.

Negative regulation of BIRPs by endogenous antagonists

On apoptotic signals, caspases have to be released from the BIRP and carry out the apoptotic processes. Several endogenous antagonists of the BIRPs help to keep these apoptotic suppressors in check and eventually promote apoptosis. In *Drosophila*, four endogenous BIRP antagonists have been identified, including Reaper, Grim, Hid (RGH) and Sickle, (Goyal et al., 2000; Srinivasula et al., 2002). These genes are closely linked in a single genomic region and encode pro-apoptotic proteins. The genomic deletion in these region, Df(3L)H99, prevents nearly all occurring cell

death in fly. Also Jafrac2 has been reported as an endogenous antagonist from ER to cytoplasm on apoptotic stimuli (Tenev et al., 2002). These proteins were shown to bind and inhibit the apoptotic function of Diap1. In mouse and human, Smac (second mitochondria-derived activator of caspases or also called Diablo), Omi (also called HtrA2) (Du et al., 2000; Verhagen et al., 2000) are identified as the negative regulators of certain BIRPs (Liston et al., 2001). Smac and Omi are localized to the inter-membrane space of mitochondria in non-apoptotic cells. During apoptosis Smac and Omi can be released from mitochondria into the cytoplasm, where they bind to BIR3 that is responsible for XIAP interaction with caspase-9 (Wu et al., 2000). These antagonists including RGH in *Drosophila* have a common mode of binding to the BIR domains of BIRPs, which prevents BIRPs from binding and inhibiting caspases. The IBM (IAP Binding Motif) is the tetrapeptides found in amino-terminal of these antagonists. The sequences of tetrapeptides are A, (I/T/V), (A/P), (F/Y/I/S). All mammalian IBM containing proteins that have been discovered so far undergo proteolytic processing to expose IBMs. The *Drosophila* RGH are located immediately C-terminal to the initiation methionine, which is presumably removed by methionine amino-peptidase activity *in vivo*.

Not all BIRPs are negatively regulated by IBM containing proteins. FAX1 (XIAP associated factor) binds to XIAP without IBM and directs the nuclear sequestration of XIAP (Liston et al., 2001). Recently, NAIP has been shown to bind to caspase-9 in the presence of ATP. LRR domain in NAIP in the absence of ATP negatively regulates the BIR function against casapse-9 (Davoodi et al., 2004). Smac fails to interact with the NAIP BIR domains and a peptide containing IBM does not compete for the binding of caspase-9 to NAIP. These suggest that NAIP inhibit caspase-9 in a distinct mechanism compared to other BIRPs.

Ubiquitination of pro-apoptotic proteins by E3 activity of Ring domain

C-terminal of several BIRPs (IAPs) contain a highly conserved Ring domain. The Ring domain can function as an E3 ubiquitin ligase. E3 provides specificity for the transfer of ubiquitin moieties onto the target proteins. It means that the interacting proteins including caspases which are already known to bind to BIRPs through BIR domains can be a substrate for a Ring E3 ubiquitin ligase activity. And also IAPs themselves can be a substrate by auto-ubiquitination. It has been shown that apoptotic stimuli resulted in the rapid degradation of cIAP1 and XIAP, which can be blocked by proteasome inhibitors (Yang et al., 2000). The decision on cell death or survival can be made depending on the kinds of the substrates, although intracellular circumstances are not that simple. For example, if IAPs predominantly ubiquitinate caspases or even antagonists of BIRPs, apoptosis will be apparently suppressed but if auto-ubiquitination of IAPs is preferred in some situation, apoptosis will occur. A single BIRPs family protein such as cIAP1 can direct its E3 ligase activity towards different substrates and can alter the cellular functions of different protein targets in accordance with differences in the specificity of individual BIR domains (Samuel et al., 2005b).

Caspase degradation

In *Drosophila*, Diap1 has been shown to mediate the ubiquitination of Dronc, a homolog of caspase-9 in a Ring-dependent manner (Wilson et al., 2002). A recent data showing Dronc levels are higher in Diap1 Ring mutant cells (Ryoo et al., 2004) suggests that Diap1-mediated ubiquitination and degradation are important for regulation of Dronc activity rather than inhibition by BIR domains. In mammal, XIAP has been reported to induce the ubiquitination of caspase-3 and proteasomal degradation in Fas-induced cell death (Suzuki et al., 2001). But in other study, caspase-3 and caspase-7 has shown to be mono-ubiquitinated by cIAP2 (Huang et al., 2000). Therefore it needs more conclusive evidence whether the ubiquitination and subsequent degradation of caspases by BIRPs occur in physiological condition especially in mammals.

Antagonist degradation

cIAP1, cIAP2 and XIAP can mediate the ubiquitination of Smac/DIABLO, an antagonist of IAPs (MacFarlane et al., 2002; Hu and Yang, 2003). Actually, there are quite different lines of reports on ubiquitination of Smac. Smac can be a substrate for XIAP-mediated ubiquitination in vitro (Wilkinson et al., 2004) but the ubiquitination of Smac does not result in Smac degradation, which means Smac is either mono-ubiquitinated or Non-K48 ubiquitinated. But in contrast, Smac ubiquitination by cIAP1 and cIAP2 not by XIAP results in its degradation. Interestingly, Bruce which does not have a Ring domain has been reported to ubiquitinate Smac and caspase-9 possibly by its UBC domains. One group reported that Bruce induced Smac-polyubiquitination and degradation by proteasome (Hao et al., 2004) but the other group reported only monoubiquitination of Smac by Bruce (Bartke et al., 2004) though. In *Drosophila*, IAP-mediated poly-ubiquitination and degradation of Reaper has been described in vitro and a mutant which cannot be ubiquitinated is more effective than the wild-type at inducing cell death in vitro (Olson et al., 2003).

Degradation of BIRP themselves by E3 activity of Ring domain

Antagonists of BIRPs can accelerate auto-ubiquitination of BIRPs and subsequent degradation by proteasome. In *Drosophila*, Hid has been shown to stimulate the

degradation of Diap1 in a Ring-dependent manner (Yoo et al., 2002) and Reaper has been shown to stimulation of auto-ubiquitination of Diap1 in the presence of UbcD1 (Ryoo et al., 2002). Reaper and Grim were able to induce XIAP degradation in heterologous systems like *Xenopus* extract or mammalian cell culture. It seems to be complicated but interestingly, at least three events can possibly happen by the antagonists of BIRPs; the activity which stimulates the auto-ubiquitination and subsequent degradation of BIRPs is pro-apoptotic, the ubiquitination of the antagonists by BIRPs is anti-apoptotic and the action of the antagonists of BIRPs which compete with caspases is pro-apoptotic.

BIRPs can control the other BIRPs protein levels through the interaction between different BIRPs via a Ring domain. The Ring domain of cIAP1 allows it to bind directly to the Ring of XIAP, causing its ubiquitination and degradation by the proteasome. Expression of a construct containing the Ring only of cIAP1 was able to deplete endogenous XIAP of melanoma cells, promoted apoptosis. Thus BIRPs are able to regulate their abundance by Ring-dependent interaction and degradation (Silke et al., 2005). The XIAP knockout mice are not predisposed to apoptosis (Harlin et al., 2001). Compensatory expression of other BIRPs such as cIAP1 has been reported in embryonic fibroblasts derived from these animals. This could explain why cIAP1 protein levels are elevated in XIAP-deleted mice and why XIAP-deleted mice have such a minor phenotype. But in terms of the caspase inhibitory activity, XIAP is a much potent inhibitor of caspases than cIAP1, which suggests the non-redundant roles for these proteins. The other group has shown that cIAP2 is ubiquitinated by cIAP1 and subsequently degraded and TRAF2 is able to potentiate this interaction (Conze et al., 2005). The cIAP1 knockout mice are viable and have no obvious sensitization to pro-apoptotic stimuli. However, markedly elevated levels of cIAP2 protein is expressed in the absence of increased cIAP2 mRNA (Conze et al., 2005). Therefore this mechanism could achieve homeostatic regulation of IAP levels and explain why it has been difficult to observe the distinct phenotype from the deletion mutants and to achieve stable high-level expression of BIRPs in cells.

The other piece of the data has been reported that XIAP and Survivin form a hetero-complex in response to cell death stimulation in vitro cell culture (Dohi et al., 2004). This interaction promotes cell survival by enhancing the stability of XIAP against proteasomal destruction and by synergistically antagonizing apoptosome-mediated cell death, in a pathway abolished in XIAP-/- cells. Formation of a Survivin-XIAP complex resulted in increased stability of XIAP against poly-ubiquitination and proteasomal degradation *in vitro* and *in vivo*, potentially by excluding the ubiquitin-conjugating enzyme. Although the mechanisms of Survivin cytoprotection have long remained elusive, these data suggest a model of intermolecular cooperation in which Survivin enhances the anti-apoptotic activity of XIAP to suppress the upstream initiation of mitochondrial cell death. It is not clear whether post-translational modification in either Survivin or XIAP or both is necessary on apoptotic stimuli.

Modulation of signaling pathway

In addition to regulation of apoptosis, several BIRPs have been shown to be involved in the signaling pathways. XIAP can bind to the type I BMP receptor and TAB1. The complex can recruit TAK1, which leads to activation of JNK (c-Jun-NH_2-terminal kinase) and NFκB sequentially (Sanna et al., 2002). It has been shown that XIAP can affect BMP-regulated dorsal-ventral polarity in a *Xenopus* developmental model (Yamaguchi et al., 1999). XIAP can also bind to TGFβ, receptor, which can activate NFκB, JNK, and Smad-dependent transcription. Interestingly an association between XIAP and the type I TGF-β, receptor results in synergistic activation of TGFβ,-dependent transcription (Birkey Reffey et al., 2001). Recently, it has been shown in vitro that apoptotic activity and signaling activity of XIAP can be separated (Lewis et al., 2004). E3 ubiquitin ligase function of the Ring is required for the activation for NFκB activation suggesting that this effect occurs as a result of ubiquitination of a regulator of the NFκB signaling pathway. Interestingly, none of the BIR domains of XIAP individually or in various combinations possessed any signaling activity over background.

Recently, XIAP has been shown to regulate intracellular copper levels. Murr1 is a factor to regulate intracellular copper homeostasis and it was found to be a target of E3 ubiquitination ligase activity of XIAP, which resulted in decreased level of intracellular copper. Murr1 protein level was not changed in XIAP-deficient mice compared to wild type, which suggests that other molecules are also involved in Murr1 regulation as well as XIAP (Burstein et al., 2004). cIAP1 and cIAP2 can bind to Ring containing TRAF2 and decrease of TRAF2 following TNFR2 signaling is mediated by E3 activity of cIAP1 (Li et al., 2002). TNF signaling is extremely complex, resulting in either pro-survival or cell death depending on the cell type and environmental cues. TNFR2 related signaling favors the activation of pro-survival transcription factor NFκB. Resulting activated NFκB can stimulate the transcription of pro-survival genes including cIAP1 and cIAP2. In *Drosophila*, Diap1 shows the same role as cIAP1 does for dTRAF (Kuranaga et al., 2002).

Regulation of mitosis

The yeast genome does not encode an obvious caspase and yeast is not known to undergo apoptosis. Yeast lack

caspases but have BIRP homologs that contain BIR domain only. Targeting of BIR-proteins in yeast produced defects in chromosomal segregation and cytokinesis (Uren et al., 1999) and inefficient spore formation (Vaux et al., 1999). *C. elegans* CeBIR1, CeBIR2 seem to regulate chromosome segregation and cytokinesis during mitosis or meiosis, rather than apoptosis (Fraser et al., 1999). In mammals, a number of studies indicate that Survivin has important roles in mitosis, particularly in microtubule organization and cytokinesis, and possibly in localizing members of the Aurora-like kinase family (Altieri, 2003). Mouse survivin gene knockout resulted in embryonic lethality caused by defects in mitosis and mitotic-spindle formation (Uren et al., 2000). CeBIR1 functions in *C. elegans* similarly to and partially rescued by Survivin (Speliotes et al., 2000), which indicates that Survivin is the true homolog of the BIRPs in yeast and *C. elegans*. Collectively, the function of these Survivin type BIRPs seems to regulate cell division rather than inhibiting apoptosis. Actually, there is no supportive data which the BIRPs in yeast and *C. elegans* can bind to caspases.

Recently, cIAP1 has been reported to play a role in regulation of cell division and cytokinesis (Samuel et al., 2005a). cIAP1 is almost exclusively localized in nucleus but in dividing cells cIAP1 is released to cytoplasm early in mitosis, then re-accumulated in nucleus in late anaphase and in telophase. Interestingly cIAP1 and Survivin both are associated with the midbody and interact with each other. cIAP1-overexpressing cells also exhibit cytokinesis defects over 10 times more than control cells and display a mitotic checkpoint abnormality with production of polyploidy cells when exposed to microtubule-targeting drugs nocodazole and taxol. This can be the evidence that the subcellualr localization is important for the function of the specific BIRPs.

Regulation of cell cycle

Transient overexpression of XIAP arrests cells in the G0-G1 phase of the cell cycle, and this growth arrest is associated with down-regulation of cyclin A and D1 and induction of the cyclin-dependent kinase inhibitors p21 and p27 (Levkau et al., 2001). Recently, XIAP has been reported to form a complex with the checkpoint kinase Chk1 in association with condensed chromosomes aligned at the metaphase plate during mitosis (Galvan et al., 2004). Chk1 has the N-terminal IBM to interact with BIR3 of XIAP. The interaction of Chk1 and XIAP appears to be restricted to mitosis and occurs after the disruption of the nuclear envelope at prometaphase. The physiological meaning of this interaction is not clear but the interaction between Chk1 and BIRPs might be the cross-talk between control of cell cycle progression and apoptosis during mitosis.

Survivin is also implicated in the regulation of cell cycle. The protein level of Survivin is correlated to cell cycle, undetectable in G1-phase, but almost 40 fold increase in S- and G2-M phase (Li et al., 1998). Additionally Survivin and procaspase-9 reportedly co-localize in mitotic cells (O'Connor et al., 2000), suggesting a role for Survivin in controlling a cell cycle checkpoint that results in cell suicide if improperly executed.

Function in cell migration: Inhibition of non-apoptotic caspase activity

In genetic screen to identify the genes which on overexpression suppress the border cell migration defect caused by dominant-negative Rac in the *Drosophila* ovary, overexpression of Diap1 turned out to suppress the migration defect (Geisbrecht and Montell, 2004). Loss-of-function mutations in *th* (diap1 gene) caused migration defects but, surprisingly, did not cause apoptosis. The lack of apoptosis in *th* mutant follicle cell clones was striking since at other stages of *Drosophila* development, cells fail to survive in the absence of Diap1 (Yoo et al., 2002). Inhibition of initiator caspase Dronc activity can rescue border cell migration defects, which suggests that the diap1 effects result from inhibition of Dronc activity. BIR1 and BIR2 are related to defective phenotype of border cell migration rather than a Ring domain. The substrates of non-apoptotic activity of Dronc are not known yet, but Rac or Profilin can be a substrate for Dronc. It seems that the function of Diap1 on border cell migration may be mediated by inhibiting Dronc activity and the Dronc activity in ovary seems to be not related to apoptosis. In fact, caspases have been shown to function in cell proliferation and differentiation in a variety of the cell types rather than their better known role in promoting apoptosis (Schwerk and Schulze-Osthoff, 2003). For example, caspase activity is required for terminal differentiation that resembles incomplete apoptosis, for example *Drosophila* sperm requires caspase activity (Huh et al., 2004). Similarly, non-apoptotic activities of caspases are required for differentiation of mammalian lens cells and erythrocytes. Therefore, some unknown mechanism that makes caspases more selective for the substrates must exist in such cells and in border cells, so that unwanted apoptosis does not occur. BIRPs such as Diap1 may be involved in the selection process or simply be the inhibitor of non-apoptotic caspases. Diap1 has been also shown to be involved in Rac-dependent actin polymerization. Overexpression of diap1 could enhance activated Rac's effect on the actin cytoskeleton in cultured cells and F-actin is reduced in *th* mutant cells. Many growth factors activate cell growth and increase the cell motility through the signal transduction and Diap1 might be a new molecular link between survival and migration.

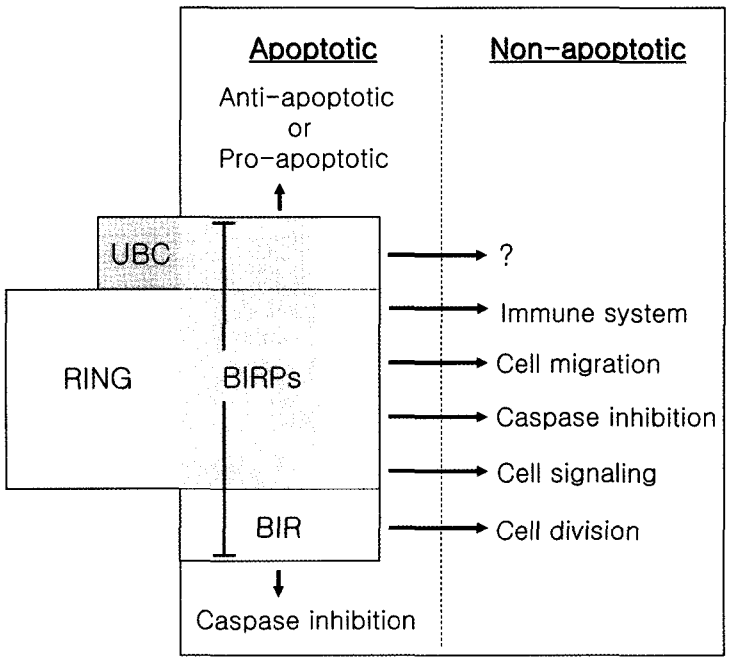

Fig. 2. The various functions of BIRPs family. Note that apoptotic functions and non-apoptotic functions are separated in the figure although overall function of the BIRPs is towards cytoprotection. Among BIRPs, BIR only Survivin subtype proteins are involved in cell division. BIR domain is able to bind to and inhibit caspases. Certain BIRPs (IAPs) which acquire a Ring domain can be the critical factors to decide either cell death (pro-apoptotic) or cell survival (anti-apoptotic). Collectively, BIRPs have the various non-apoptotic functions such as signal transduction, non-apoptotic caspase inhibition, cell migration, innate immunity as well as inhibition of apoptosis.

Regulation of innate immunity

In *Drosophila*, there are not much things known about the function of Diap2 compared to Diap1 for a decade. Recently, Diap2 has been reported as a component of *Drosophila* Imd pathway from the RNAi screen to identify novel components of the Imd pathway in S2 cells (Kelino et al., 2005; Gesellchen et al., 2005). *Drosophila* immunity, which is devoid of an adaptive response, relies on two pathways (Toll and Imd pathway) to activate NFκB signaling. The Imd pathway in *Drosophila* regulates the production of antimicrobial peptides following microbial challenge. Diap2 and TAB (TAK1 binding protein) which is another novel component of Imd pathway appear to be involved in nuclear localization of Relish (*Drosophila* NFκB homolog). Diap2 is not required for the inhibition of Dredd which is the caspase involved in cleavage of Relish. Diap2 is not essential for cell viability in haemocyte-like cells. This is actually consistent with the previous report indicating RNAi of Diap2 only sensitized S2 cells to stress-induced apoptosis, not directly induced apoptosis (Zimmermann et al., 2002). The molecular mechanism on the innate immunity pathway involving Diap2 is not fully understood yet. But the evidence which the ubiquitination is required for the activation of Tak1 and IKK complex in *Drosophila* (Zhou et al., 2005) suggest that Diap2 may have a role in ubiquitinating Tak1 by a Ring domain. Diap2 and TAB both have mammalian homologs, which indicate that the pathway including Diap2 and TAB in innate immunity can be possibly conserved in mammalian system. It remains to be established whether certain mammalian BIRPs have the same role in innate immune response.

Conclusions

BIR-domain containing proteins are found in organisms from yeast to human. BIRPs (originally called IAPs) have been considered as an inhibitor of apoptosis. However, an increasing number of studies collectively suggest that BIRPs play important roles in various intracellular events such as regulation of cell division, cell cycle, cell migration, modulating cell signaling and innate immune signaling (Fig. 2). In fact, the BIRPs in yeast and *C. elegans* play a role in cell division rather than inhibition of apoptosis, which suggests that BIR domain seems to have arisen in evolution prior to the apoptotic machinery established. BIR domains seem to mediate the protein-protein interaction and they interact with various other types of proteins including caspases and endogenous antagonists of BIRPs. And certain members of the BIRPs family have acquired the other catalytic domain such as a Ring, enabling them to interact with different target proteins and regulate those protein levels including themselves. In addition, subcellular localization of BIRPs might be the critical point of regulation. The number of targets of BIRPs-mediated ubiquitination is

increasing and recent results indicate that outcomes following ubiquitination are enormously complex. As summarized, several members of BIRPs are nucleocytoplasmic shuttling depending on intracellular environmental cues, which may allow them to direct the various functions. The mechanisms regulating the distribution of IAPs into various subcellular compartments and the correlation of their subcellular location with protein function require further clarification. It is elusive at this point, why these various functions are integrated into a single molecule (each member of BIRPs family) through the evolution. Many vigorous studies will answer this question in near future.

References

Altieri DC (2003) Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene* 22: 8581-8589.

Ambrosini G, Adida C, and Altieri DC (1997) A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* 3: 917-921.

Bartke T, Pohl C, Pyrowolakis G, and Jentsch S (2004) Dual role of Bruce as an antiapoptotic IAP and a chimeric E2/E3 ubiquitin ligase. *Mol Cell* 14: 801-811.

Beltrami E, Plescia J, Wilkinson JC, Duckett CS, and Altieri DC (2004) Acute ablation of survivin uncovers p53-dependent mitotic checkpoint functions and control of mitochondrial apoptosis. *J Biol Chem* 279: 2077-2084.

Birkey Reffey S, Wurthner JU, Parks WT, Roberts AB, and Duckett CS (2001) X-linked inhibitor of apoptosis protein functions as a cofactor in transforming growth factor-beta signaling. *J Biol Chem* 276: 26542-26549.

Burstein E, Ganesh L, Dick RD, van De Sluis B, Wilkinson JC, Klomp LW, Wijmenga C, Brewer GJ, Nabel GJ, and Duckett CS (2004) A novel role for XIAP in copper homeostasis through regulation of MURR1. *EMBO J* 23: 244-254.

Chai J, Shiozaki E, Srinivasula SM, Wu Q, Dataa P, Alnemri ES, and Shi Y (2001) Structural basis of caspase-7 inhibition by XIAP. *Cell* 104: 769-780.

Chen Z, Naito M, Hori S, Mashima T, Yamori T, and Tsuruo T (1999) A human IAP-family gene, Apollon, expressed in human brain cancer cells. *Biochem Biophys Res Commun* 264: 847-854.

Chu Z-L, McKinsey TA, Liu L, Gentry JJ, Malim MH, and Ballard DW (1997) Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis cIAP2 is under NFkB control. *Proc Natl Acad Sci USA* 94: 10057-10062.

Clem RJ and Miller LK (1993) Apoptosis reduces both the in vitro replication and the in vivo infectivity of a baculovirus. *J Virol* 67: 3730-3738.

Clemens MJ, Bushell M, Jeffrey IW, Pain VM, and Morley SJ (2000) Translation initiation factor modifications and the regulation of protein synthesis in apoptotic cells. *Cell Death Differ* 7: 603-615.

Conze DB, Albert L, Ferrick DA, Goeddel DV, Yeh W-C, Mak T, and Ashwell JD (2005) Posttranscriptional downregulation of cIAP2 by the ubiquitin protein ligase cIAP1 in vivo. *Mol Cell Biol* 25: 3348-3356.

Crook NE, Clem RJ, and Miller LK (1993) An apoptosis inhibiting baculovirus gene with a zinc finger like motif. *J Virol* 67: 2168-2174.

Davoodi J, Lin L, Kelly J, Liston P, and MacKenzie AE (2004) Neuronal apoptosis-inhibitory protein does not interact with Smac and requires ATP to bind caspase-9. *J Biol Chem* 279: 40622-40628.

Deveraux QL, Takahashi R, Salvesen GS, and Reed JC (1997) X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 388: 300-304.

Dohi T, Okada K, Xia F, Wilford CE, Samuel T, Welsh K, Marusawa H, Zou H, Armstrong R, Matsuzawa S, Salvesen GS, Reed JC, and Altieri DC (2004) An IAP-IAP complex inhibits apoptosis. *J Biol Chem* 279: 34087-34090.

Du C, Fang M, Li Y, Li L, and Wang X (2000) SMAC, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* 102: 33-42.

Ekedahl J, Joseph B, Grigoriev MY, Muller M, Magnusson C, Lewensohn R, and Zhivotovsky B (2002) Expression of inhibitor of apoptosis proteins in small- and non-small-cell lung carcinoma cells. *Exp Cell Res* 279: 277-290.

Fraser A, James C, Evan G, and Hengartner M (1999) *C. elegans* inhibitor of apoptosis protein (IAP homologue BIR-1 plays a conserved role in cytokinesis. *Curr Biol* 9: 292-301.

Galvan V, Kurakin AV, and Bredesen DE (2004) Interaction of checkpoint kinase 1 and the X-linked inhibitor of apoptosis during mitosis. *FEBS Lett* 558: 57-62.

Geisbrecht ER and Montell DJ (2004) A role for *Drosophila* IAP1-mediated caspase inhibition in Rac-dependent cell migration. *Cell* 118: 111-125.

Gesellchen V, Kuttenkeuler D, Steckel M, Pelte N, and Boutros M (2005) An RNA interference screen identifies inhibitor of apoptosis protein 2 as a regulator of innate immune signaling in *Drosophila*. *EMBO Rep* 6: 979-984

Goyal L, McCall K, Agapite J, Hartwieg E, and Steller H (2000) Induction of apoptosis by Drosophila reaper, hid and grim through inhibition of IAP function. *EMBO J* 19: 589-597.

Hao Y, Sekine K, Kawabata A, Nakamura H, Ishioka T, Ohata H, Katayama R, Hashimoto C, Zhang X, Noda T, Tsuruo T, and Naito M (2004) Apollon ubiquitinates Smac and caspase-9, and has an essential cytoprotection function. *Nat Cell Biol* 6: 849-860.

Harlin H, Reffey SB, Duckett CS, Lindsten T, and Thompson CB (2001) Characterization of XIAP-deficient mice. *Mol Cell Biol* 21: 3604-3608.

Hauser H-P, Bardroff M, Pyrowolakis G, and Jentsch S (1998) A giant ubiquitin-conjugating enzyme related to IAP apoptosis inhibitors. *J Cell Biol* 141: 1415-1422.

Hawkins CJ, Wang SL, and Hay BA (2000) A cloning method to identify caspases and their regulators in yeast: identification of *Drosophila* IAP1 as an inhibitor of the Drosophila caspase DCP-1. *Proc Natl Acad Sci USA* 96: 2885-2890.

Hay BA, Wassarman DA, and Rubin GM (1995) *Drosophila* homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. *Cell* 83: 1253-1262.

Hinds MG, Norton RS, Vaux DL, and Day CL (1999) Solution structure of a baculoviral inhibitor of apoptosis (IAP) repeat. *Nat Struct Biol* 6: 648-651.

Holcik M, Lefebvre C, Yeh C, Chow T, and Korneluk RG (1999) A new internal ribosome entry site motif potentiates XIAP-mediated cytoprotection. *Nat Cell Biol* 1: 190-192.

Holcik M, Sonenberg N, and Korneluk RG (2000) Internal ribosome initiation of translation and the control of cell death. *Trends Genet* 16: 469-473.

Hu P, Han Z, Couvillon AD, and Exton JH (2004) Critical role of endogenous Akt/IAPs and MEK1/ERK pathways in counteracting endoplasmic reticulum stress-induced cell death. *J Biol Chem* 279: 49420-49429.

Hu S and Yang X (2003) Cellular inhibitor of apoptosis 1 and 2 are ubiquitin ligases for the apoptosis inducer Smac/ DIABLO. *J Biol Chem* 278: 10055-10060.

Huang H, Joazeiro CA, Bonfoco E, Kamada S, Leverson JD, and Hunter T (2000) The inhibitor of apoptosis, cIAP2, functions as a ubiquitin-protein ligase and promotes in vitro mono-ubiquitination of caspase 3 and 7. *J Biol Chem* 275: 26661-26664.

Huh JR, Vernooy SY, Yu H, Yan N, Shi Y, Guo M, and Hay BA (2004) Multiple apoptotic caspase cascades are required in nonapoptotic roles for *Drosophila* spermatid individualization. *PLoS Biol* 2: 43-53.

Kelino A, Valanne S, Ulvila J, Kallio J, Myllymaki H, Enwald H, Stoven S, Poidevin m, Ueda R, Hultmark D, Lemaitre B, and Ramet M (2005) Inhibitor of apoptosis 2 and TAK1-binding protein are components of the *Drosophila* Imd pathway. *EMBO J* 24: 3423-3434.

Kuranaga E, Kanuka H, Igaki T, Sawamoto K, Ichijo H, Okano H, and Miura M (2002) Reaper-mediated inhibition of Diap1-induced DTRAF degradation results in activation of JNK in *Drosophila*. *Nat Cell Biol* 4: 705-710.

Levkau B, Garton KJ, Ferri N, Kloke k, Nofer JR, Baba HA, Raines EW, and Breithardt G (2001) XIAP induces cell-cycle arrest and activates nuclear factor-kappaB: new survival pathways diabled by caspase-mediated cleavage during apoptosis of human endothelial cells. *Circ Res* 88: 282-290.

Lewis j, Burstein E, Reffey SB, Bratton SB, Roberts AB, and Duckett CS (2004) Uncoupling of the signaling and caspase-inhibitory properties of X-linked inhibitor of apoptosis. *J Biol Chem* 279: 9023-9029.

Li F, Ambrosini G, Chu EY, Marchisio PC, and Altieri DC (1998) Cell cycle control of apoptosis and mitotic spindle checkpoint by survivin. *Nature* 396: 580-584.

Li T, Ackermann EJ, Bennett CV, Rothermel AL, Plescia J, Tognin S, Villa A, Marchisio PC, and Altieri DC (1999) Pleiotropic cell-division defects and apoptosis induced by interference with survivin function. *Nat Cell Biol* 1: 461-466.

Li X, Yang Y, and Ashwell JD (2002) TNF-RII and cIAP1 mediate ubiquitination and degradation of TRAF2. *Nature* 416: 345-347.

Liston P, Fong WG, Kelly NL, Toji S, Miyazaki T, Conte D, Tamai K, Craig CG, McBurney MW, and Korneluk RG (2001) Identification of XAF1 as an antagonist of XIAP anti-caspase activity. *Nat Cell Biol* 3: 128-133.

MacFarlane M, Merrison W, Bratton SB, and Cohen GM (2002) Proteasome-mediated degradation of Smac during apoptosis: XIAP promotes Smac ubiquitination in vitro. *J Biol Chem* 277: 36611-36616.

Marusawa H, Mat suzawa S-I, Welsh K, Zou H, Armstrong R, Tamm I, and Reed JC (2003) HBXIP functions as a cofactor of survivin in apoptosis suppression. *EMBO J* 22: 2729-2740.

O'Connor DS, Grossman D, Li F, Zhang H, Villa A, Tognin S, Marchisio PC, and Altieri DC (2000) Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. *Proc Natl Acad Sci USA* 97: 13107-13107.

Olson MR, Holley CL, Gan EC, Colon-Ramos DA, Kaplan B, and Kornbluth S (2003) Reaper is regulated by IAP-mediated ubiquitination. *J Biol Chem* 278: 4028-4034.

Plenchette S, Cathelin S, Rebe C, Launay S, Ladoire S, Sordet O, Ponnelle T, Debili N, Phan T-H, Padua R-A, Dubrez-Daloz L, and Solary E (2004) Translocation of the inhibitor of apoptosis protein cIAP1 from the nucleus to the Golgi in hematopoietic cells undergoing differentiation: a nuclear export signal-mediated event. *Blood* 104: 2035-2043.

Reed JC (2001) The survivin saga goes in vivo. *J Clin Invest* 108: 965-969.

Rothe M, Pan M-G, Henzel WJ, Ayres TM, and Goeddel DV (1995) The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. *Cell* 83: 1243-1252.

Roy N, Mahadevan MS, Mclean M, Shutler G, Yaraghi Z, Farahani R, Baird S, Besnerjohnston A, Lefebvre C, and Kang XL (1995) The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. *Cell* 80: 167-178.

Ryoo HD, Bergmann A, Gonen H, Ciechanover A, and Steller H (2002) Regulation of *Drosophila* IAP1 degradation and apoptosis by reaper and ubcD1. *Nat Cell Biol* 4: 432-438.

Ryoo HD, Gorenc T, and Steller H (2004) Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. *Dev Cell* 7: 491-501.

Samuel T, Okada K, Hyer M, Welsh K, Zapata JM, and Reed JC (2005a) cIAP1 localizes to the nuclear compartment and modulates the cell cycle. *Cancer Res* 65: 210-218.

Samuel T, Welsh K, Lober T, Togo SH, Zapata JM, and Reed JC (2005b) Distinct BIR domains of cIAP1 mediate binding to and ubiquitination of TRAF2 and Smac. *J Biol Chem* Nov doi:10.1074/jbc.M509381200: in press.

Sanna MG, da Silva Correia J, Ducrey O, Lee J, Nomoto K, Schrantz N, Deveraux QL, and Ulevitch RJ (2002) IAP suppression of apoptosis involves distinct mechanisms: the TAK1/JNK1 signaling cascade and caspase inhibition. *Mol Cell Biol* 22: 1754-1766.

Schimmer AD (2004) Inhibitor of apoptosis proteins: translating basic knowledge into clinical practice. *Cancer Res* 64: 7183-7190.

Schwerk C and Schulze-Osthoff K (2003) Non-apoptotic functions of caspases in cellular proliferation and differentiation. *Biochem Pharmacol* 66: 1453-1458.

Shin S, Sung BJ, Cho YS, Kim HJ, Ha NC, Hwang JL, Chung CW, Jung YK, and Oh BH (2001) An anti-apoptotic protein human survivin is a direct inhibitor of caspase-3 and -7. *Biochemistry* 40: 1117-1123.

Silke J, Kratina T, Chu E, Ekert PG, Day CL, Pajusch M, Huang DCS, and Vaux DL (2005) Determination of cell survival by Ring-mediated regulation of inhibitor of apoptosis (IAP) protein abundance. *Proc Natl Acad Sci USA* 102: 16182-16187.

Speliotes EK, Uren A, Vaux D, and Horvitz HR (2000) The survivin-like *C. elegans* BIR-1 protein acts with the Aurora-like kinase AIR-2 to affect chromosomes and the spindle midzone. *Mol Cell* 6: 211-223.

Srinivasula SM, Datta P, Kobayashi M, Wu J-W, Fujioka M, Hedge R, Zhang Z, Makattash R, Fernandes-Alnemri T, Shi Y, Jaynes JB, and Alnemri ES (2002) Sickle, a novel

Drosophila death gene in the reaper/hid/grim region, encodes an IAP-inhibitory protein. *Curr Biol* 12: 125-130.

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

Tenev T, Zachariou A, Wilson R, Paul A, and Meier P (2002) Jafrac2 is an IAP antagonist that promotes cell death by liberating Dronc from DIAP1. *EMBO J* 21: 6118-5129.

Uren AG, Coulson EJ, and Vaux DL (1998) Conservation of baculovirus inhibitor of apoptosis repeat proteins (BIRPs) in viruses, nematodes, vertebrates and yeasts. *Trends Biochem Sci* 5: 159-162.

Uren AG, Beiharz T, O'Connell MJ, and Lithgow T (1999) Role for yeast inhibitor of apoptosis (IAP)-like proteins in cell division. *Proc Natl Acad Sci USA* 96: 10170-10175.

Uren AG, Wong L, Pakusch M, Fowler KJ, Burrows FJ, Vaux DL, and Choo KH (2000) Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype. *Curr Biol* 10: 1319-1328.

Vaux DL and Korsmeyer SJ (1999) Cell death in development. *Cell* 96: 245-254.

Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ, and Vaux DL (2000) Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* 102: 43-53.

Vischioni B, Giaccone G, Span SW, Kruyt FAE, and Rodriguez JA (2004) Nuclear shuttling and TRAF2-mediated retention in the cytoplasm regulate the subcellular localization of cIAP1 and cIAP2. *Exp Cell Res* 298: 535-548.

Vucic D, Stennicke HR, Pisabarro MT, Salvesen GS, and Dixit VM (2000) ML-IAP, a novel inhibitor of apoptosis that is preferentially expressed in human melanomas. *Curr Biol* 10: 1359-1366.

Wang C-Y, Mayo MW, Korneluk RG, Goeddel DV, and Baldwin AS (1998) NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and cIAP1 and cIAP2 to suppress casapse-8 activation. *Science* 281: 1680-1683.

Wang SL, Hawkins CJ, Yoo SJ, Muller HA, and Hay BA (1999) The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. *Cell* 98: 453-463.

Wilkinson JC, Wilkinson AS, Scott FL, Csomos RA, Salvesen GS, and Duckett CS (2004) Neutralization of Smac/Diablo by inhibitors of apoptosis (IAPs): a caspase-independent mechanism for apoptotic inhibition. *J Biol Chem* 279: 51082-51090.

Wilson R, Goyal L, Ditzel M, Zachariou A, Baker DA, Agapite J, Steller H, and Meier P (2002) The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis. *Nat Cell Biol* 4: 445-450.

Wu G, Chai J, Suber TL, Wu JW, Du C, Wang X, and Shi Y (2000) Structural basis of IAP recognition by Smac/DIABLO. *Nature* 408: 1008-1012.

Yamaguchi K, Nagai S, Ninomiya-Tsuji J, Nishita M, Tamai K, Irie K, Ueno N, Nishida E, Shibuya H, and Matsumoto K (1999) XIAP, a cellular member of the inhibitor of apoptosis protein family, links the receptors to TAB1-TAK1 in the BMP signaling pathway. *EMBO J* 18: 179-187.

Yang L, Cao X, Yan H, and Wood WC (2003) Coexistence of high levels of apoptotic signaling and inhibitor of apoptosis proteins in human tumor cells: implication for cancer specific therapy. *Cancer Res* 63: 6815-6824.

Yang QH, Church-Hajduk R, Ren J, Newton ML, and Du C (2003) Omi/HtrA2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. *Genes Dev* 17: 1487-1496.

Yang Y, Fang S, Jensen JP, Wissman AM, and Ashwell JD (2000) Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli. *Science* 288: 874-877.

Yoo SJ, Huh JR, Muro I, Yu H, Wang L, Wang SL, Feldman RM, Clem RJ, Muller HA, and Hay BA (2002) Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms. *Nat Cell Biol* 4: 416-24.

Zhou R, Silverman N, Hong M, Liao DS, Chung Y, Chen ZJ, and Maniatis T (2005) The role of ubiquitination in *Drosophila* innate immunity. *J Biol Chem* 280: 34048-34055.

Zimmermann KC, Ricci J-E, Droin NM, and Green DR (2002) The role of ARK in stress-induced apoptosis in *Drosophila* cells. *J Cell Biol* 156: 1077-1087.

[Received October 24, 2005]