The *Drosophila* inhibitor of apoptosis D-IAP1 suppresses cell death induced by the caspase drICE

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Abstract Many members of the Inhibitor of Apoptosis (IAP) family inhibit cell death and existing data suggest at least two mechanisms of action. *Drosophila* IAPs (D-IAP1 and D-IAP2) and a baculovirus-derived IAP, Op-IAP, physically interact with and inhibit the anti-apoptotic activity of Reaper, HID, and Grim, three genetically defined inducers of apoptosis in *Drosophila*, while human IAPs, c-IAP1, c-IAP2, and X-IAP interact with a number of different proteins including specific members of the caspase family of cysteine proteases which are crucial in the execution of cell death. We have examined whether insect-active IAPs can inhibit apoptosis induced by selected caspases, *Drosophila* drICE, Sf-caspase-1, and mammalian caspase-3, in insect SF-21 cells. D-IAP1 inhibited apoptosis induced by the active forms of all three caspases tested and physically interacted with the active, but not the proform of drICE. MIHA, the mouse homolog of X-IAP and an effective inhibitor of caspase-3, also interacted with and blocked apoptosis induced by active drICE but was relatively ineffective in blocking Sf-caspase-1. Op-IAP and D-IAP2 were unable to inhibit effectively any of the active caspases tested and failed to interact with drICE. The *Drosophila* IAPs and Op-IAP, but not MIHA, blocked HID-initiated activation of pro-drICE. We conclude that D-IAP1 is capable of inhibiting the activation of drICE as well as inhibiting apoptosis induced by the active form of drICE. In contrast, D-IAP2 and Op-IAP are more limited in their inhibitory targets and may be limited to inhibiting the activation of caspases.

Key words: Inhibitor of Apoptosis (IAP); Head Involution Defective (HID); Baculovirus IAP repeat (BIR); Baculovirus *Drosophila*; *Spodoptera frugiperda*

1. Introduction

The evolutionarily conserved process of apoptosis plays an important role in removing cells during development and in maintaining tissue homeostasis, but the dysregulation of apoptosis, leading to too much or too little cell death, can contribute to a number of diseases including neurodegenerative disorders and cancer [1]. Designing therapeutic solutions requires that we understand the interplay between positive and negative regulators of the apoptotic program.

Members of the *inhibitor of apoptosis* (*iap*) gene family act as antagonists of cell death in metazoans ranging from *Drosophila* to humans [2]. IAPs were first discovered in baculoviruses by their ability to block apoptosis induced during viral infection [3]. IAPs have since been found in nematodes, insects, birds, and mammals [4–11], underscoring the recurrent theme that the components of the cell death program have been conserved throughout metazoan radiation. Members of the IAP family are characterized by the presence of at least one and usually two or three tandem baculovirus IAP repeat (BIR) motifs located in the amino-terminal and central portions of the protein [12], and most of them have a carboxy-terminal RING finger motif. The ability of IAPs of animal origin to regulate cell death was genetically established in *Drosophila* where deletion of the chromosomal region encoding *Drosophila* IAP1 (D-IAP1) was found to enhance cell death induced by expression of the pro-apoptotic gene *reaper* [13]. Overexpression of either D-IAP1 or D-IAP2 in the *Drosophila* eye suppresses normal cell death induced by expression of the pro-apoptotic genes *reaper* or *hid* [13]. *Reaper*, *hid*, and another *Drosophila* pro-apoptotic gene, *grim*, are all located in the 75C1–2 chromosomal region, share a short stretch of sequence similarity at their N-termini and have been genetically identified as inducers of apoptosis in *Drosophila* embryos [14–16]. The precise mechanism by which these three proteins interact with the cell death machinery is not understood, but they must induce apoptosis through the activation of caspases, since p35, a known caspase (cysteine-dependent aspartate-specific proteases) inhibitor [17], can block cell death induced by the expression of Reaper, HID, or Grim [15,16,18–25].

The activation of caspases is a crucial step in apoptotic signaling and is required to execute the cell death program [26]. Caspases are activated by cleavage at internal aspartate residues. Two of the proteolytic fragments form heterodimers which assemble to generate the mature, active enzyme that subsequently uses its precursor form and other caspases as substrates [27]. The cascade of caspase activations amplifies the death signal, thereby committing the cell to apoptosis. drICE is one of three known caspases in *Drosophila melanogaster* [25]. Transcripts of drICE can be detected throughout fly development [25]. Overexpression of Reaper triggers the activation of endogenous drICE and immunodepletion studies suggest that drICE is essential for apoptosis in *Drosophila* S2 cells [28].

The mechanisms of IAP action are just beginning to be characterized, but since IAPs block apoptosis induced by a variety of stimuli, IAPs must target a central point or multiple points within apoptotic pathways [29]. The anti-apoptotic baculovirus IAPs (e.g. Op-IAP) block the activation of an endogenous caspase, Sf-caspase-1, which is normally activated during baculovirus infection of SF-21 insect cells and blocked by the caspases inhibitor p35 [30–32]. Unlike baculovirus p35 which is able to inhibit active Sf-caspase-1, the baculovirus

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IAPs are unable to block apoptosis induced by activated Sf-caspase-1 or by mammalian caspase-3 [30]. Although the mechanism by which Sf-caspase-1 is activated during infection is not known, Op-IAP as well as *Drosophila* IAPs (D-IAP1 and D-IAP2), physically interact with and block apoptosis induced by Reaper, HID, and Grim [24,33]. Baculovirus Op-IAP also physically interacts with and blocks apoptosis induced by Doom, a protein identified only in *Drosophila* to date [34].

Mammalian IAPs interact with a number of different proteins involved in apoptosis including caspases. Mammalian c-IAP1 and c-IAP2 are induced by NF-κB and, in conjunction with TRAF-1 and TRAF-2, are recruited to the tumor necrosis factor receptor (TNFR) and inhibit apoptosis induced by TNF-α [35–37]. The action of IAPs at proximal points in apoptotic signaling pathways is also suggested by the observation that c-IAP1 and c-IAP2 can interact with Reaper and Grim and block apoptosis initiated by their overexpression in mammalian cells [38]. However, direct involvement of mammalian IAPs in caspase regulation is supported by the observation that c-IAP1, c-IAP2 and X-IAP (hILP) bind to and inhibit the activated forms of caspase-3 and -7 and are also capable of binding to and inhibiting the activation of the proform of caspase-9 [39–42]. To date, no such caspase interaction or inhibition has been described for insect-active IAPs.

In this study, we examined the ability of selected IAPs to block apoptosis initiated by active caspases. We found that both D-IAP1 and the mouse X-IAP homolog, MIHA, but not D-IAP2 and Op-IAP, block apoptosis induced by expression of active drICE and caspase-3. D-IAP1 also efficiently suppressed apoptosis induced by active Sf-caspase-1, unlike the other IAPs tested. D-IAP1 and MIHA failed to bind the proform of drICE, but consistent with inhibition studies, D-IAP1 and MIHA physically interacted with activated drICE. HID initiated the processing of drICE, and this activity was blocked by Op-IAP and the *Drosophila* IAPs, but not by MIHA. Thus, our results further define the apoptotic pathway of *Drosophila* and establish caspase inhibition as an evolutionarily conserved mechanism by which at least some IAPs silence cell death signaling.

2. Materials and methods

2.1. Cell line and plasmid constructs

Spodoptera frugiperda (Lepidoptera: Noctuidae) IPLB-SF-21 (SF-21) cells were maintained at 27°C in TC-100 medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (Intergen, Purchase, NY) and 0.26% tryptose broth as previously described [43]. All plasmids used were derived from pHSP70PLVI+CAT, a plasmid expressing the *cat* gene under the control of the *Drosophila* hsp70 promoter [29]. Plasmids expressing non-tagged Op-*iap*, D-*iap1*, D-*iap2*, *p35*, *cat*, Sf-caspase-1(p19), caspase-3(p17) or *hid*, Flag-tagged Op-*iap*, *hid*, or *cat*, and HA.11-tagged (Epi) Op-*iap*, D-*iap1*, D-*iap1*-BIRs, D-*iap1*-RING, D-*iap2*, Sf-caspase-1-pro, Sf-caspase-1(p11), caspase-3(p12) or *cat* were all described previously [23,24,29,30,33]. Plasmids encoding the epitope-tagged and untagged versions of *miha* were constructed from pHSP70PLVI+CAT, pHSEpiOpIAPVI+, or pHSFlagD-IAP1VI+ by replacing *cat*, Op-*iap*, or D-*iap1* with the open reading frame of *miha*. The plasmid pHSdrICE-EpiHisVI+ contains the sequence encoding drICE fused to a carboxy-terminal HA.11-epitope tag and six consecutive histidines. The plasmid pHSdrICE(80–230)VI+ encodes amino acids 80–230 of drICE which corresponds to the large (p19) subunit of the active enzyme, and pHSdrICE(230–339)-EpiHisVI+ encodes the carboxy-terminal amino acids 230–339 of drICE fused to six histidines and the HA.11-epitope tag. This portion of drICE includes what probably constitutes the small subunit (p11) of the active form of drICE as well as an additional 13 amino acid residues. When transfected alone, this plasmid expressed a 12 kDa product, and when cotransfected with pHSdrICE(80–230)VI+, an 11 kDa product is generated from the 12 kDa product. For simplicity we refer to this plasmid as encoding the small (p11) subunit of drICE. The plasmid pHSP70PLVI+EpiD-IAP1-BIRs was digested with *Eco*RV and *Pst*I and the smallest fragments were subcloned into the *Psp*AI and *Pst*I sites of pHSEpiO-pIAPVI+ to generate pHSP70PLVI+EpiDIAP1BIR2 which expresses amino acids 97–342 of D-IAP1. The sequence encompassing the BIR1 of D-IAP1 (amino acids 2–186) were subcloned into pHSEpiOpBIR-VI+ by replacing the Op-*iap* sequence to generate pHSP70PLVI+EpiD-IAP1-BIR1. The amino acid numbers given for positions within D-IAP1 begin with the second methionine [44] of the published sequence [13].

2.2. Viability assays

SF-21 cells plated at a density of 0.5×10^6 per 35 mm dish were transfected with recombinant plasmids. At 18 h post transfection, cells were heat shocked, and 8 h following heat shock, the medium was aspirated off each plate and replaced with 500 μl of phosphate buffered saline (PBS; pH 6.2) containing 0.04% trypan blue. Viable intact cells were counted from four different fields of view at 400× magnification. The percent of non-viable cells was calculated as the difference between the number of viable cells in the *cat*-only transfected control (100%) and the number of viable cells in the test transfected cells. Each datum plotted represents three experiments, ± standard deviation.

2.3. Immunoprecipitation and immunoblot analysis

Coimmunoprecipitations were performed essentially as described [24,33] except that the plasmid encoding p35 was included only where specifically indicated and that cells were harvested at 10 h following heat shock. At 4 h following induction of gene expression by heat shock, the medium of designated samples was replaced with medium containing actinomycin D at a concentration of 1 μg/ml.

3. Results and discussion

3.1. D-IAP1 inhibits apoptosis induced by the active form of drICE, caspase-3, and Sf-caspase-1

Preliminary studies showed that transfection of SF-21 cells with a plasmid expressing the proform of drICE did not induce apoptosis, but cotransfection with plasmids expressing the two subunits of active drICE, p19 and p11, induced apoptosis in approximately 50% of the cells (data not shown). To determine whether drICE-induced apoptosis can be blocked by members of the IAP family, we cotransfected plasmids expressing the two subunits of drICE along with plasmids expressing D-IAP1, D-IAP2, MIHA, Op-IAP, or the control chloramphenicol acetyl transferase (CAT) gene. Compared to a *cat*-only transfected control, drICE induced apoptosis in 46% of the cells (Fig. 1A). D-IAP2 and Op-IAP exhibited little or no ability to protect against drICE-induced apoptosis (Fig. 1A) even though they effectively block Reaper-induced apoptosis under identical conditions [23]. However, D-IAP1 reduced cell death by four-fold (Fig. 1A). MIHA was not as efficient as D-IAP1, but more than a two-fold decrease in apoptosis was observed (Fig. 1A). As previously reported [25], p35 potently blocked drICE (Fig. 1A). Thus, different IAPs display differing abilities to protect against drICE-induced apoptosis, and both MIHA and D-IAP1 can inhibit apoptosis induced by the active form of this caspase.

We have previously shown that baculovirus IAPs do not block apoptosis induced by the active form of mammalian caspase-3 or Sf-caspase-1 in SF-21 cells [30], but it was unknown whether the cellular IAPs D-IAP1, D-IAP2, and MIHA behave like the viral IAPs; therefore, we cotransfected

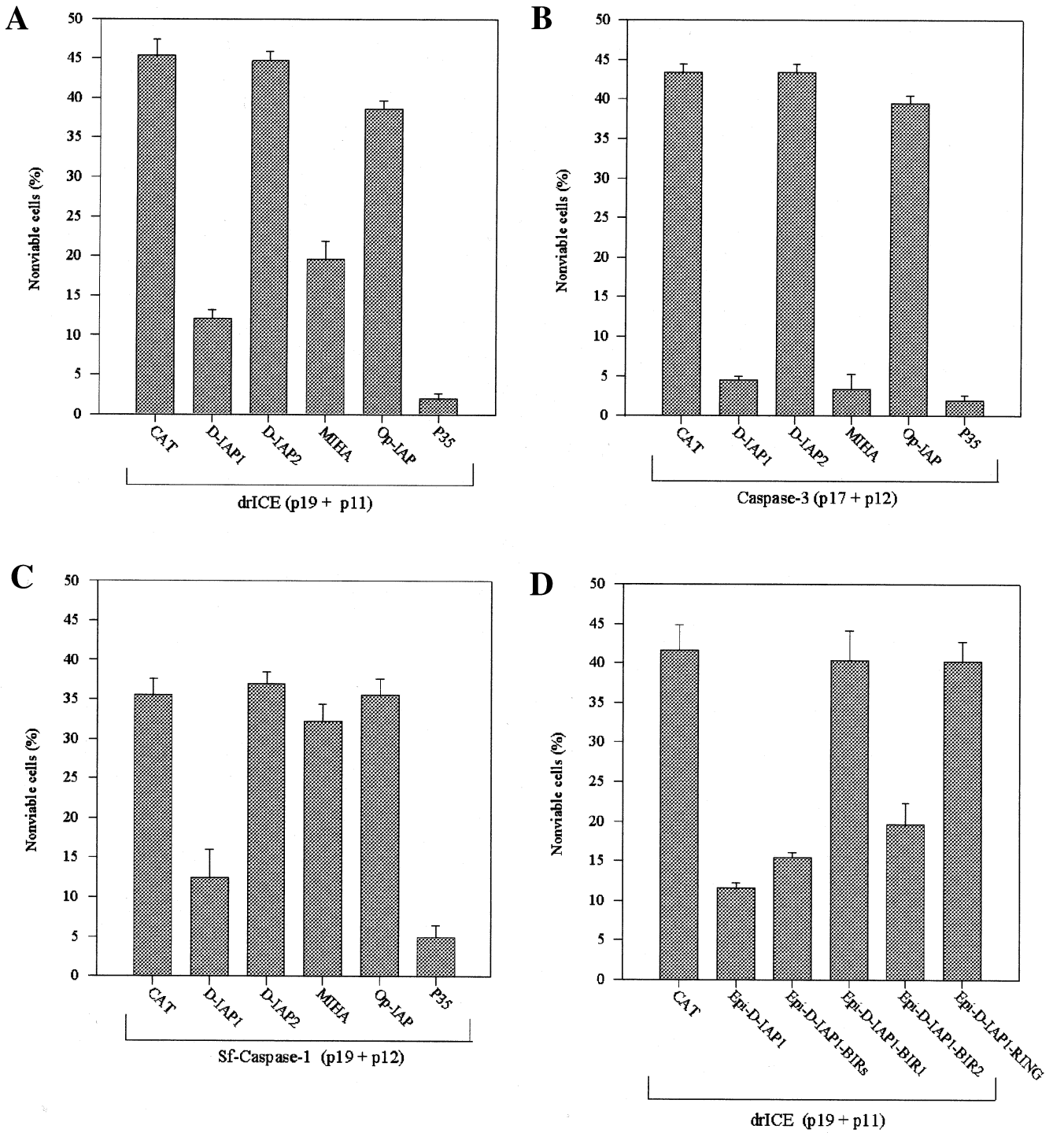

Fig. 1. D-IAP1 inhibits active drICE, caspase-3, and Sf-caspase-1. SF-21 cells were cotransfected with 1.25 μg of each plasmid expressing the subunits of active drICE (p19+p11) (A), caspase-3 (p17+p12) (B), or Sf-caspase-1 (p19+p12) (C) and 2.5 μg of plasmids expressing *cat*, D-*iap1*, D-*iap2*, *miha*, Op-*iap*, or *p35*. D: SF-21 cells were cotransfected with 0.5 μg of each plasmid expressing the subunits of active drICE and 4 μg of plasmids expressing the HA.11 epitope-tagged D-IAP1 constructs Epi-D-IAP1 (amino acids 2–401), Epi-D-IAP1-BIRs (2–342), Epi-D-IAP1-BIR1 (2–186), Epi-D-IAP1-BIR2 (97–342), or Epi-D-IAP1-RING (275–401). Cell viability was determined by trypan blue exclusion 8 h following induction of gene expression by heat shocking the cells for 30 min at 42°C. The results shown are relative to the viability of *cat*-only transfected cells (100%) and represent at least three independent experiments for each cotransfected combination. Standard deviations are indicated by error bars.

plasmids expressing the subunits of caspase-3 or Sf-caspase-1 with CAT, D-IAP1, D-IAP2, MIHA, and Op-IAP. D-IAP1 and MIHA blocked caspase-3-induced apoptosis almost as completely as p35, but as observed for drICE-induced apoptosis, D-IAP2 and Op-IAP had little effect (Fig. 1B). D-IAP1 also suppressed apoptosis induced by active Sf-caspase-1, whereas Op-IAP and D-IAP2 did not (Fig. 1C). MIHA, which blocked both drICE and caspase-3, only slightly dimin-

ished cell death initiated by Sf-caspase-1. Thus, the ability of an IAP to block active caspases depends on the IAP as well as the caspase. Although the viral Op-IAP is unable to block apoptosis induced by any of the active caspases tested, D-IAP1 is a potent inhibitor of active caspases from dipteran, lepidopteran, and mammalian sources.

To better define the region of D-IAP1 responsible for inhibiting drICE, a series of N-terminally HA.11 epitope-tagged (Epi) D-IAP1 truncations were tested for anti-apoptotic function. Epi-D-IAP1-RING, which contained the RING finger and most of the linker region between the BIRs and RING finger, did not block apoptosis induced by drICE, but the N-terminal region of D-IAP1 containing BIRs 1 and 2 (Epi-D-IAP1-BIRs) retained the ability to suppress cell death (Fig. 1D). Epi-D-IAP1-BIR1, which expressed BIR1 and most of the linker region between the two BIRs, was unable to block drICE, but a construct expressing BIR2 and the flanking spacer regions (Epi-D-IAP1-BIR2) retained the ability to block apoptosis (Fig. 1D). Thus, the region encompassing the BIR2 of D-IAP1 is necessary and sufficient for blocking drICE-induced apoptosis. A single BIR of X-IAP is also known to possess anti-apoptotic activity [41].

3.2. D-IAP1 physically interacts with active drICE

Because D-IAP1 protected SF-21 cells from drICE-induced cell death and because mammalian IAPs are known to physically interact with and inhibit caspases, we investigated the possibility that D-IAP1 physically associates with drICE. We transiently coexpressed Flag-tagged versions of CAT, D-IAP1, D-IAP2, MIHA, or Op-IAP with the carboxy-terminal HA-tagged drICE (drICE-pro-Epi) and determined if anti-Flag antibody could coprecipitate the HA-tagged drICE protein. In the absence of an apoptotic stimulus, drICE remained primarily in its proform (drICE-pro-Epi; Fig. 2, middle panel, lanes 1, 3, 5, 7, and 9) and failed to coprecipitate with the Flag-CAT negative control or any of the Flag-tagged IAPs (Fig. 2, top panel, lanes 1, 3, 5, 7, and 9) even though all Flag-tagged constructs were expressed (Fig. 2, bottom panel, lanes 1, 3, 5, 7, 9). Thus, none of the IAPs tested appear to interact efficiently with the proform of drICE.

Since mammalian IAPs interact with the active but not the proform of some caspases, we also investigated the possibility that IAPs interact with the active form of drICE. Although we did not detect interactions when IAPs were cotransfected with the two drICE subunits (data not shown); an interaction was observed when the proform of drICE was activated by addition of actinomycin D, a potent inducer of apoptosis in SF-21 cells [29,44]. The processing of drICE, as evidenced by the presence of the p11-Epi subunit (Fig. 2, middle panel, lanes 2, 4, 6, 8, and 10), was observed in all actinomycin D treated samples including those expressing Flag-D-IAP1 and Flag-Op-IAP. Although D-IAP1 and Op-IAP inhibit actinomycin D-induced apoptosis in SF-21 cells [44], the Flag-tagged versions of these IAPs are only partially effective at inhibiting this potent stimulus and extensive apoptosis was observed in all treated samples (data not shown). Flag-D-IAP1 strongly coprecipitated the p11-Epi subunit, indicating that D-IAP1 physically interacts with active drICE (Fig. 2, top panel, lane 4). MIHA also coprecipitated the p11 subunit of drICE (Fig. 2, top panel, lane 8). The drICE-p11 interaction with MIHA was less pronounced than with D-IAP1 probably because MIHA expression levels were not as high

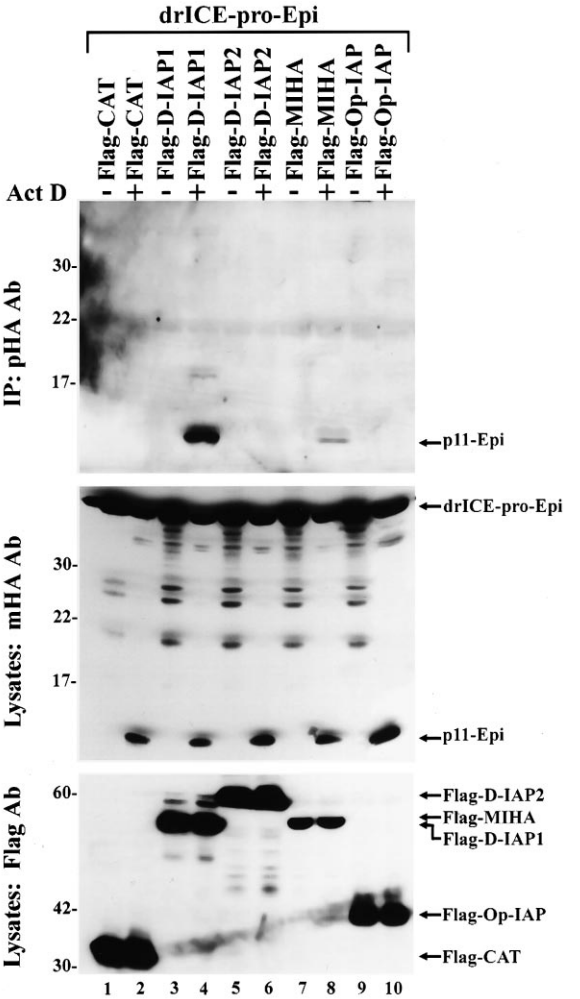

Fig. 2. D-IAP1 physically interacts with active drICE. SF-21 cells were transiently transfected with plasmids expressing C-terminally HA.11 epitope-tagged (Epi) drICE and the indicated Flag epitope-tagged constructs. At 4 h following heat shock, the medium of the indicated samples was replaced with medium containing actinomycin D (Act D+). Ten hours following heat shock, aliquots of cell lysates were incubated overnight with M2 anti-Flag monoclonal antibody resin. Coprecipitated HA.11 epitope-tagged constructs were detected by immunoblot analysis with the rabbit anti-HA.11 polyclonal antiserum (IP). Expression of HA.11-tagged (mHA Ab) and Flag-tagged proteins (Flag Ab) were confirmed. Molecular mass markers (in kDa) are shown on the left.

as for D-IAP1 (Fig. 2, bottom panel, compare lanes 4 and 8). Thus, the two IAPs which are able to effectively block drICE-induced apoptosis also physically interacted with the active, not the proform, of drICE in SF-21 cells. Op-IAP and D-IAP2, which were ineffective in blocking drICE-induced apoptosis, and the control CAT failed to interact with the proform or the active form of drICE (Fig. 2, top panel, lanes 2, 6, and 10).

3.3. Drosophila and baculovirus IAPs block HID-induced processing of drICE

We have previously demonstrated that HID-induces apoptosis in SF-21 cells and that Op-IAP as well as D-IAP1 and D-IAP2 are able to block HID-induced apoptosis [24]. To determine if drICE is processed into its active form during HID-induced apoptosis, we cotransfected SF-21 cells with

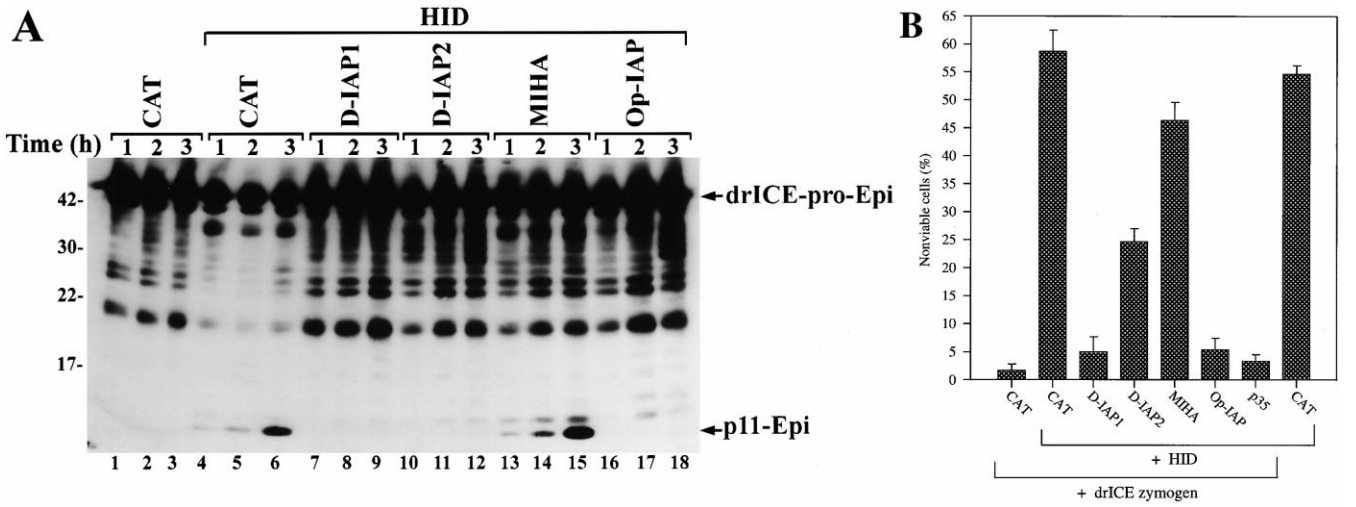

Fig. 3. D-IAP1, D-IAP2, and Op-IAP block HID-initiated processing of drICE. A: SF-21 cells were transfected with plasmids expressing drICE-pro-Epi and one of the following: CAT, D-IAP1, D-IAP2, MIHA, or Op-IAP. HID was also cotransfected in lanes 4–18. Plasmid concentrations were balanced for all samples with addition of CAT-expressing plasmid. Cells were collected and lysed at the indicated times following heat shock. Immunoblot analysis of C-terminally HA.11-tagged drICE using anti-HA.11 monoclonal antibody revealed the drICE zymogen (drICE-pro-Epi) and processed p11 (p11-Epi) subunit. B: SF-21 cells were transfected with plasmids expressing the indicated proteins. Plasmid concentrations were balanced for all samples with addition of CAT-expressing plasmid. Cell viability was determined by trypan blue exclusion 8 h following induction of gene expression. The results shown are relative to the viability of *cat*-only transfected cells (100%) and represent at least three independent experiments for each cotransfected combination. Standard deviations are indicated by error bars.

plasmids expressing drICE-pro-Epi and either CAT (Fig. 3A, lanes 1–3) or HID (Fig. 3A, lanes 4–6). Following induction of gene expression, samples were collected at 1h intervals. Immunoblot analysis revealed that HID initiates drICE-pro-Epi processing as indicated by the appearance of the small active p11-Epi subunit (Fig. 3A). The p11-Epi subunit was not detected in samples cotransfected with CAT and drICE-pro-Epi. To determine the effect of IAPs on HID-activation of drICE, we cotransfected plasmids expressing drICE-pro-Epi and HID, with plasmids expressing D-IAP1, D-IAP2, MIHA, or Op-IAP. Proteolytic processing was not observed in samples cotransfected with the D-IAP1, D-IAP2, or Op-IAP (Fig. 3A, lanes 7–12 and 16–18), but MIHA did not block processing (Fig. 3A, lanes 13–15).

All IAPs tested were able to reduce the level of apoptosis induced by HID (Fig. 3B), but MIHA was not as effective as the other IAPs. The presence of drICE cleavage products observed in cotransfections of HID and MIHA correlates with the high levels of apoptosis (Fig. 3). D-IAP1, D-IAP2, and Op-IAP all interact with HID [24], but we did not detect interaction between MIHA and HID (data not shown). Thus, there is a correlation between the ability of the IAPs to bind to HID, to block HID-induced apoptosis, and to block drICE activation. Although it is possible that D-IAP1, D-IAP2, and Op-IAP act by binding to the caspase(s) which may activate drICE in SF-21 cells, only D-IAP1 is capable of blocking Sf-Caspase-1. Thus D-IAP2 and Op-IAP must either block HID-induced apoptosis by their interaction with another Sf-caspase or with HID itself. Until we know more about how HID initiates caspase activation, it will be difficult to distinguish between these two possible mechanisms. The mechanisms may also be related if there is competition between HID and active caspases for D-IAP1 binding; through competitive binding to IAPs, the presence of pro-apoptotic proteins could result in the release of active caspases from D-IAP1 inhibition.

It is likely that the interaction between D-IAP1 and drICE results in inhibition of drICE proteolytic activity although out data are restricted to showing that D-IAP1 can inhibit drICE-induced apoptosis in vivo. drICE may activate apoptosis in SF-21 cells directly or by activation of another caspase such as Sf-caspase-1. The striking correlation between the ability of IAPs to bind drICE and inhibit drICE-induced apoptosis suggests that IAP interaction results in inhibition of drICE proteolysis. Although Op-IAP is an effective inhibitor of a variety of apoptotic inducers, it fails to interact with or block drICE-induced apoptosis in SF-21 cells.

The observation that D-IAP1 interacts with caspases as well as apoptotic inducers such as Reaper, HID and Grim, places D-IAP1 in a potentially pivotal position to regulate apoptosis. If a significant portion of caspases are present in the cell in their mature form but are bound to and inhibited by D-IAP1, then expression of an inducer that competitively binds D-IAP1 would be sufficient to release caspases from inhibition and initiate a proteolytic cascade. The role of other IAPs such as D-IAP2 and Op-IAP, which do not appear to interact with and block caspases, would be to serve as buffers, competing for D-IAP1 binding. This model would provide a mechanism for Reaper, HID, and Grim action in which D-IAP1 inhibition of caspase activity plays a central, decisive role in governing cell death. It remains possible, however, that inducers such as Reaper, HID, and Grim have a more direct role in caspase activation. In this case, the fact that D-IAP1 also binds caspases can be viewed as one more pro-apoptotic protein that IAPs are able to interact with in their role as sensors and enumerators of intracellular apoptotic inducing signals.

The emerging picture is that IAPs are broadly functional inhibitors of apoptosis and that their levels relative to that of apoptotic inducers, determines cell survival or cell death (see Fig. 4). Thus, the level of IAPs in a cell acts as an apopstat: when IAP levels are high, caspases are inhibited, and the cell is relatively resistant to apoptosis, but when the levels of IAP-interacting inducers rise above the level of available unbound

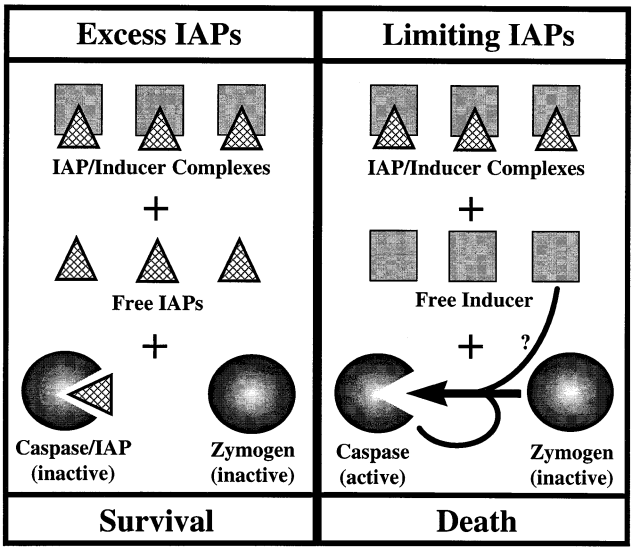

Fig. 4. The IAP apopstat model for regulating cell death. IAPs (triangles) interact with pro-apoptotic inducers such as Reaper, HID, Grim, and Doom (squares) and, in some cases, with caspases (circles, zymogen form a closed circle, mature form a sectored circle). When IAP levels exceed those of inducers and mature caspases, the cell is relatively resistant to apoptotic signals and survives. When inducer levels rise, IAPs are bound in complexes with inducers, possibly releasing mature caspases from IAP inhibition. The free, unbound inducers or mature caspases may further activate zymogen processing, thereby triggering a caspase cascade resulting in cell death.

IAPs, caspase processing is initiated, whether by the inducer itself or by caspases released from IAP inhibition by inducer competition for IAP binding.

In summary, we have found that D-IAP1, but not D-IAP2 or Op-IAP, inhibits apoptosis induced by active caspases of dipteran, lepidopteran, and mammalian origin. D-IAP1 physically interacts with the active but not the zymogen form of drICE suggesting that direct caspase inhibition is an evolutionarily conserved mechanism by which some IAPs can silence cell death signaling. D-IAP1, D-IAP2, and Op-IAP block HID-initiated processing of the drICE zymogen and this activity correlates with their ability to interact with HID. We propose a model in which the levels of IAPs relative to inducers govern cell death.

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