

## Cell Death Regulation in *Drosophila*: Conservation of Mechanism and Unique Insights<sup>Ⓢ</sup>

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Programmed cell death, or apoptosis, is a genetically encoded form of cell suicide that results in the orderly death and phagocytic removal of excess, damaged, or dangerous cells during normal development and in the adult. The cellular machinery required to carry out apoptosis is present in most, if not all cells, but is only activated in cells instructed to die (for review see Jacobson et al., 1997). Here, we review cell death regulation in the fly in the context of a first pass look at the complete *Drosophila* genome and what is known about death regulation in other organisms, particularly worms and vertebrates.

### *Caspases: The Core of the Cell Death Machine*

The caspase family of cysteine proteases is central to apoptotic signaling and cell execution in all animals that have been studied, including worms, flies, and vertebrates (Thornberry and Lazebnik, 1998). As with many proteases, caspases are synthesized as inactive zymogens, known as procaspases, and are generally thought to be present in all cells at levels sufficient to induce apoptosis when activated. Death stimuli lead to one or more cleavages COOH-terminal to specific aspartate residues. These cleavage events separate the large and small subunits that make up the active caspase. Two sets of these subunits assemble to form the active caspase heterotetramer, which has two active sites. Frequently an NH<sub>2</sub>-terminal prodomain is also removed during caspase processing. An important point is that the sites cleaved to produce an active caspase often correspond to caspase target sites. Thus, once activated, caspases can participate in proteolytic cascades.

Caspases play two roles in bringing about the death of the cell. They transduce death signals that are generated in specific cellular compartments, and they cleave a number of cellular proteins, resulting in the activation of some and the inactivation of others. These latter cleavage events are thought to lead, through a number of mechanisms, to many of the biochemical and morphological changes associated with apoptosis. Caspases that act as signal transducers (known as apical or upstream caspases) have long pro-

domains. These regions contain specific sequence motifs (known as death effector domains [DEDs]<sup>1</sup> or caspase recruitment domains [CARDs]) that are thought to mediate procaspase recruitment into complexes in which caspase activation occurs in response to forced oligomerization (Budihardjo et al., 1999). Some caspases may also become activated as a consequence of prodomain-dependent homodimerization (Kumar and Colussi, 1999). Once activated, long prodomain caspases are thought to cleave and activate short prodomain caspases (known as downstream or executioner caspases) that rely on cleavage by other caspases for activation. This review focuses on caspases as cell death regulators. However, it is important to note that, in mammals and flies, mutant phenotypes suggest caspases can also play important nonapoptotic roles (Song et al., 1997; Zheng and Flavell, 2000), and the functions of a number of caspases are still unclear.

For much of our analysis of the *Drosophila* genome we used the BLAST search programs available through the Berkeley *Drosophila* Genome Project (<http://www.fruitfly.org/>). Motif search programs were also sometimes used. Instances in which use of these latter programs resulted in the identification of proteins that were not identified using the standard BLAST server are indicated in the text. *Drosophila* encodes three long prodomain caspases, *dcp-2/dredd* (Inohara et al., 1997; Chen et al., 1998), *dronc* (Dorstyn et al., 1999a), and *dream* (accession No. AF275814), as well as four caspases with short prodomains, *dcp-1* (Song et al., 1997), *drICE* (Fraser and Evan, 1997), *decay* (Dorstyn et al., 1999b), and *daydream* (accession No. AF281077). An eighth *Drosophila* caspase, a head-to-head partial duplication of *daydream*, is likely to be nonfunctional because of numerous mutations (including premature stop codons and deletions). There is also good evidence that cell death in the fly is caspase-dependent (for review see Abrams, 1999). The *Caenorhabditis elegans* genome encodes three caspases, the known apoptosis inducer *ced-3* (Yuan et al., 1993), and *csp-1* and *csp-2* (Shaham, 1998), all of which have long prodomains. 14 caspases have been identified in mammals, 10 of which have long prodomains (Budihardjo et al., 1999).

<sup>Ⓢ</sup>The online version of this article contains supplemental material.

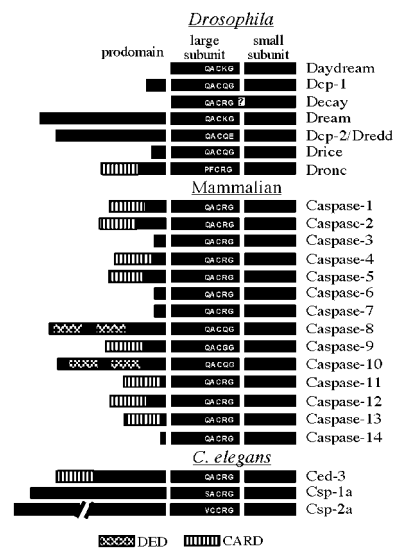
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<sup>1</sup>Abbreviations used in this paper: AIF, apoptosis-inducing factor; BIR, baculovirus IAP repeat; BIRP, BIR repeat-containing protein; CAD, caspase-activated DNase; CARD, caspases recruitment domain; DD, death domain; DED, death effector domain; FADD, FAS-associated death domain; IAP, inhibitor of apoptosis; ICAD, inhibitor of CAD.

All long prodomain caspases, identified to date, in mammals contain either CARD or DED sequences. In contrast, both *Drosophila* and *C. elegans* encode caspases that have long prodomains with unique sequences, as well as a single caspase with a CARD (Fig. 1 and Figure S1 [available at <http://www.jcb.org/cgi/content/full/150/2/F69>]). The unique prodomain sequences in these caspases may promote death-inducing caspase activation in response to unknown stimuli. Alternatively, they may regulate caspase activation in contexts other than cell death. Several *Drosophila* and *C. elegans* caspases, Dronc and Csp-1a and Csp-2a, respectively, are unique in a second way as well. Caspases are described as being specific for cleavage after aspartate and typically have an active site that conforms to the consensus QAC(R/Q/G)(G/E) (catalytic cysteine is underlined). Dronc, Csp-1a, and Csp-2a have active sites that differ in the first two positions. Because the glutamine at the first position of the active site pentapeptide QACRG is part of the substrate binding pocket, it is likely that caspases with different amino acids at this position will have unique cleavage preferences. In support of this hypothesis Dronc, which has the active site sequence PFCRG, cleaves itself after glutamate rather than aspartate, and cleaves tetrapeptide substrates after glutamate as well as aspartate (Hawkins et al., 2000). Cleavage specificity data for Csp-1 and Csp-2 have not been reported. Why might these caspases have altered cleavage specificity? All are long prodomain caspases, suggesting that they act to transduce signals. One possibility is simply that these proteins have unique substrates (which may or may not be death related) that require an altered cleavage specificity. The altered cleavage specificity may also have evolved to be able to efficiently cleave the sequences present between their large and small caspase subunits, which contain sequences predicted to be very poor target sites for traditional caspases. An altered cleavage specificity, in conjunction with an absence of good target sites for other caspases in the linker region, may also serve as a way of making the activation of these caspases more strictly dependent on oligomerization rather than activation by other caspases.

### Activating the Caspase Cascade

In mammals three pathways have been described that lead to caspase activation. In one pathway, which will not be discussed further, a serine protease, granzyme B, is delivered directly into the cytoplasm of target cells from cytotoxic T cells, where it activates executioner caspases (Trapani et al., 2000). In the other two pathways, cytoplasmic adaptor proteins link a cell death signal transducer to a long prodomain caspase through homophilic receptor-adaptor and adaptor-caspase interactions leading to caspase activation (Hofmann, 1999). In one pathway, initiating at the plasma membrane, caspase recruitment is initiated by the binding of ligands to receptors of the tumor necrosis factor/nerve growth factor receptor superfamily. The cytoplasmic region of these receptors contains a region known as the death domain (DD). Ligand-dependent receptor multimerization results in the recruitment of DD-containing cytoplasmic adaptors such as Fas-associated death domain (FADD) through homophilic DD interac-



**Figure 1.** Schematic representation of caspases with relative prodomain sizes is shown to highlight prodomain structure. The prodomain of Csp-2a, shown truncated, is about twice as long as that of Csp-1a. Csp-1 and Csp-2 also encode other splice products, which are not shown. Catalytic subunits are less variable in size, and are not shown exactly to scale. Active site sequences are indicated. Many caspases have a small linker sequence removed between the large and small subunits; linkers are not shown. Where prodomain cleavage sites were not known, they were chosen based on homology to caspases with known cleavage sites. A lack of aspartate residues for caspase cleavage in decay is indicated by a question mark. Caspase-1 through Caspase-10, Caspase-13 and Caspase-14 are human caspases. Caspase-11 and Caspase-12 are mouse caspases. Full sequence alignment of the caspases is available at <http://www.jcb.org/cgi/content/full/150/2/F69> and corresponds to Figure S1.

tions. FADD and related adaptors also contain a second motif known as DED, copies of which are also present in the prodomains of caspase-8 and caspase-10. Homophilic interactions between the DEDs present in receptor-bound adaptors and procaspases leads to caspase oligomerization and subsequent autoactivation. Other adaptors that include DD and CARD domains may also couple activated receptors to CARD domain-containing caspases.

We used the programs PFSCAN ([http://www.isrec.isb-sib.ch/software/PFSCAN\\_form.html](http://www.isrec.isb-sib.ch/software/PFSCAN_form.html)) and Pfam (<http://www.sanger.ac.uk/Pfam/>) to search for candidate death receptors (predicted type 1 transmembrane proteins containing intracellular DDs) in the fly genome. We found a number of proteins or predicted proteins with DD homology, including the kinase pelle (accession No. AA540441), a *Drosophila* netrin receptor (accession No. AAF7419), a protein with a number of ankyrin repeats (accession No. CG7462), and three other proteins that lack significant similarity to other proteins (accession No. CG2031, AF22205, and AF22206). (CG numbers refer to genes predicted by Celera Genomics.) However, none of these also shows DED or CARD homology. The prodomain of Dcp-2/Dredd does share weak homology with that of caspase-8 (Chen et al., 1998), but the Dcp-2/Dredd prodomain is not itself identified in searches for *Drosophila* proteins with DEDs using PFSCAN or Pfam. In fact, no *Drosophila*

proteins with significant DED homology were identified in similar searches. These observations suggest several possibilities. One is that *Drosophila* lacks death receptor signaling pathways. A second possibility is that *Drosophila* has a death receptor pathway analogous to that found in mammals, but that the level of homology of these proteins with their mammalian counterparts is very low. Finally, *Drosophila* death receptors may incorporate a distinct set of oligomerization motifs. In the context of this possibility, it will be interesting to identify proteins that interact with the Dream and Dcp-2/Dredd prodomains.

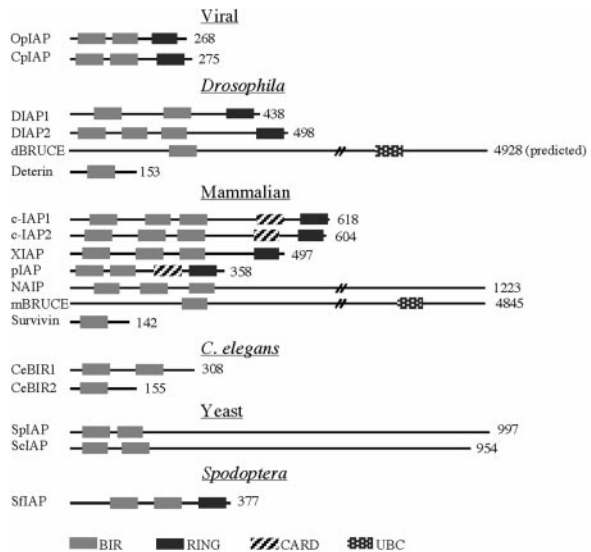
In a second major pathway of apical caspase activation in mammals, cellular stress of various sorts leads to the release of mitochondrial cytochrome *c* (cyto-*c*), which in conjunction with the cytosolic adapter protein Apaf-1, promotes caspase-9 activation (for review see Budihardjo et al., 1999). Apaf-1 shows large regions of homology with the *C. elegans* apoptosis inducer, Ced-4. In both organisms, caspase-activating adapter-caspase interactions are dependent on homophilic interactions between the two proteins, mediated at least, in part, by CARDS present at the NH<sub>2</sub> terminus of Ced-4/Apaf-1 and in the caspase prodomain. In the case of worms, caspase activation by Ced-4 requires disruption of an association between Ced-4 and the apoptosis inhibitor and Bcl-2 family member Ced-9 by Egl-1, which is a second Bcl-2 family member that acts as an apoptosis inducer. Activation of Apaf-1 in mammals in vitro requires cyto-*c*, which stably interacts with WD-40 repeats present at the COOH terminus of Apaf-1 but absent in Ced-4. The Apaf-1 WD-40 repeats inhibit its function, and this inhibition is relieved after cyto-*c* binding in the presence of ATP/dATP, allowing the formation of a multimeric Apaf-1/cyto-*c* complex. Procaspase-9 is recruited to this complex and activated through autocatalysis (for review see Budihardjo et al., 1999). Recently, several Apaf-1-like genes have been identified in vertebrates (Ceconi, 1999). The proteins encoded by these genes contain distinct NH<sub>2</sub>- and COOH-terminal sequences, suggesting that they may activate other caspases through different upstream signaling pathways.

The *Drosophila* genome has one Ced-4/Apaf-1 homologue, variously known as *dapaf-1* (Kanuka et al., 1999), *dark* (Rodriguez et al., 1999), or *hak-1* (Zhou et al., 1999). Here, we refer to this gene as *apaf-1-related killer* (*ark*), its designation in the *Drosophila* online database (<http://flybase.bio.indiana.edu/genes/>). This gene encodes two splice forms. The long form most closely resembles Apaf-1, in that it contains a series of COOH-terminal WD-40 repeats that presumably mediate regulation by cyto-*c*. The short form most closely resembles CED-4, which lacks these repeats, and would thus be predicted to be constitutively active. Genetic evidence indicates that Ark is important for cell death induction in the fly (as well as other processes such as specification of photoreceptor number), and biochemical data point toward interactions between Ark, cyto-*c*, and *Drosophila* caspases. Mitochondrial cyto-*c* is at least shifted in localization (Varkey et al., 1999), and perhaps released into the cytoplasm during apoptosis (Kanuka et al., 1999). Thus, the weight of evidence suggests that in *Drosophila*, as in vertebrates, cyto-*c* functions to transduce apoptotic signals through Apaf-1.

## Keeping Caspases in Their Place: The IAP Family of Cell Death Inhibitors

Since proteolysis is irreversible, and caspases have the potential to engage in amplifying cascades of proteolysis, caspase activation and activity must be carefully regulated in cells that normally live. The only known cellular caspase inhibitors are members of the inhibitor of apoptosis (IAP) family (for review see Deveraux and Reed, 1999; Miller, 1999). Genetic and biochemical evidence from *Drosophila* argues that IAP-dependent inhibition of caspase activity is essential for cell survival, and that one mechanism for cell death activation involves inhibition of IAP function (Wang et al., 1999; Goyal et al., 2000; Lisi et al., 2000).

IAPs were first identified as baculovirus-encoded cell death inhibitors. These proteins contain several NH<sub>2</sub>-terminal repeats of an ~70-amino acid motif known as a baculovirus IAP repeat (BIR) as well as a COOH-terminal RING finger domain (for review see Miller, 1999). RING fingers have since been found in proteins that function in a number of different contexts. For a number of proteins this domain confers E3 ubiquitin protein ligase activity (for review see Freemont, 2000). A number of cellular proteins that share homology with the viral IAPs, based on the presence of one or more BIR repeats (referred to as BIR repeat-containing proteins, or BIRPs) have now been identified in organisms ranging from yeast to humans (Uren et al., 1998; Fig. 2 and Figures S1 and S2 [available



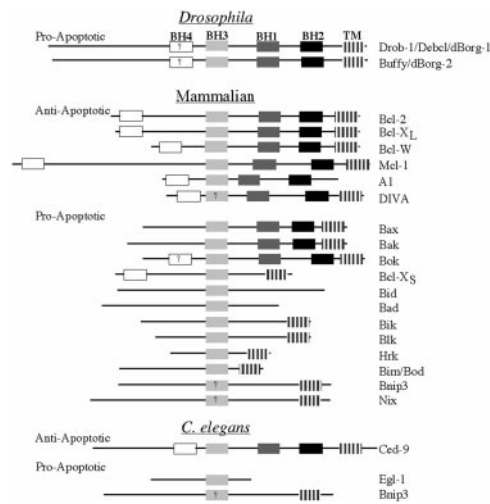
**Figure 2.** Schematic representation of the structures of selected viral and cellular BIR-containing proteins. The approximate positions of BIR, RING, CARD, and UBC domains are shown, with the total amino acid length shown to the right of each protein. In some proteins, RING domains confer E3 ubiquitin protein ligase activity on proteins that contain them; UBC refers to the ubiquitin-conjugating domain predicted to have E2 activity. The presence of both of these domains in components of the apoptotic machinery suggests a link between apoptosis and protein degradation. The length of the *Drosophila* BRUCE homologue is not known, as it derives solely from predicted sequence. Sequence alignment of the BIR repeat-containing proteins is available at <http://www.jcb.org/cgi/content/full/150/2/F69> and corresponds to Figures S2 and S3.

at <http://www.jcb.org/cgi/content/full/150/2/F69>). The *Drosophila* genome encodes four BIRPs, including *DIAP1*, the product of the *thread* locus (Hay et al., 1995), *DIAP2* (Hay et al., 1995; Duckett et al., 1996; Liston et al., 1996; Uren et al., 1996), *deterin*, a homologue of Survivin (Jones et al., 2000), and *dBRUCE*, a homologue of BRUCE (accession No. CG6303). A number of the cellular BIRPs, including XIAP, cIAP-1, cIAP-2, NAIP, and Survivin in mammals, and *DIAP1*, *DIAP2*, and *Deterin* in *Drosophila*, have been tested and shown to act as cell death inhibitors. Notable exceptions are BIRPs from *C. elegans* and yeast, which regulate cell division (Fraser et al., 1999; Uren et al., 1999). Thus, whereas all IAPs contain BIR repeats by definition, not all proteins with BIRs are IAPs. Many of the death-inhibiting BIRPs, including XIAP, cIAP-1, cIAP-2, Survivin, and *DIAP1*, have been shown to directly inhibit caspase activation or activity (for review see Deveraux and Reed, 1999). However, IAPs have been found to associate with a number of different proteins, and may have multiple mechanisms of action. This is particularly suggested in the case of those proteins that contain domains associated with ubiquitin conjugation.

### Mitochondrial Regulation of Cell Death

Mitochondria are necessary for cellular energy production, and, thus, are essential for cell survival. In vertebrates (and probably *Drosophila*) the mitochondria are an important site of integration for cell death and survival signals. As discussed above, the decision to release cyto-c constitutes one proapoptotic output of this calculation. A second proapoptotic protein released from mitochondria is apoptosis-inducing factor (AIF), which in mammals translocates from the mitochondria to the nucleus upon receipt of a death signal and causes large-scale fragmentation of the DNA (Susin et al., 1999). *Drosophila*, but not *C. elegans*, encodes a clear AIF homologue (accession No. CG7263).

In some cells undergoing apoptosis, caspase inhibitors are unable to prevent cell death. One cause of this caspase-independent death is thought to be due to mitochondrial damage that occurs upstream of caspase activation (for review see Vander Heiden and Thompson, 1999). The Bcl-2 family of proteins constitutes a major family of cell death regulators, and many of their pro- and antiapoptotic functions in vertebrates can be traced to their effects on mitochondrial function. Currently 19 distinct vertebrate Bcl-2 family members have been identified that share up to four Bcl-2 homology domains (BH1-4). Some also have a hydrophobic COOH terminus that targets them to membranes. An important aspect of Bcl-2 family member function is that pro- and antiapoptotic proteins can heterodimerize (though this is not always required for function), and a large body of evidence argues that they titrate each other's function. However, exactly how these proteins regulate cell death is still unclear. *Drosophila* encodes two clear Bcl-2 family members. The first is known variously as *drob-1* (Igaki et al., 2000), *dBorg-1* (Brachmann et al., 2000), *debcl* (Colussi et al., 2000), or *dbok* (Zhang et al., 2000). The second gene is known as *buffy* (Colussi et al., 2000; accession No. AF237864) or *dBorg-2* (Brachmann et al., 2000). Both proteins have BH1, BH2,



**Figure 3.** Schematic diagram of *Drosophila* Debcl/Drob-1/DBorg-1/DBok and Buffy/DBorg-2 and members of the mammalian and *C. elegans* Bcl-2 structural families. DBok initiates 86 residues COOH-terminal to the start codon that is predicted for Debcl/Drob/DBorg-1. BH1, BH2, BH3, BH4, and transmembrane domains are represented. The presence of the question mark in some BH3 and BH4 domains indicates predicted BH domains based on weak sequence similarity. Debcl/Drob-1/DBorg-1/DBok and Buffy/DBorg-2 both contain BH1-4 domains as well as a transmembrane domain. Sequence alignment of the *Drosophila* Bcl-2 family members with selected mammalian and *C. elegans* members is available at <http://www.jcb.org/cgi/content/full/150/2/F69> and corresponds to Figure S4.

and BH3 domains. Weak BH4 domain homology may also be present (Fig. 3 and Figure S4 [available at <http://www.jcb.org/cgi/content/full/150/2/F69>]). They show the greatest overall homology to the mammalian proapoptotic protein Bok/Mtd, and have proapoptotic function. Genes encoding candidate prosurvival Bcl-2 proteins are not apparent in the fly genome. One possibility is that prosurvival Bcl-2 proteins do not exist. Alternatively, prosurvival members may exist, but have such low homology that we were unable to identify them. Finally, prosurvival Bcl-2 function may be obtained from posttranslational conversion of one or both of these proteins into an antiapoptotic form (Brachmann et al., 2000).

### Cell Death in the Nucleus

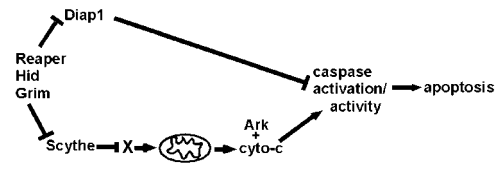
A common feature of apoptotic cell death is nuclear condensation and extensive DNA degradation. Apoptotic DNA degradation involves at least several steps. In vertebrates, the initial degradation of DNA is triggered by the caspase-dependent activation of a 40-kD nuclease known as CPAN/CAD/DFF. This protein is synthesized complexed to a specific chaperone/inhibitor known as DFF45/ICAD. Caspase cleavage of DFF45/ICAD by caspase-3 releases CPAN/DFF40/CAD, which moves to the nucleus and cleaves DNA (for review see Nagata, 2000). Both DFF45/ICAD and CPAN/DFF40/CAD, as well as several other vertebrate proteins, contain a motif known as a CIDE domain. Experimental observations suggest that CIDE-CIDE interactions are important for regulation of

CPAN/DFF40/CAD activity (Lugovskoy et al., 1999). Degradation of DNA after cell death also occurs in *Drosophila* and *C. elegans*. The fly genome encodes functional homologues of caspase-activated DNase (CAD) and CAD inhibitor (ICAD), as well as several other predicted proteins that have CIDE domains (Inohara et al., 1998; Inohara and Nunez, 1999; Yokoyama et al., 2000). CAD-like DNases or other proteins with CIDE domains have not been identified in the *C. elegans* genome. However, DNA fragmentation occurs cell autonomously in a CED-3-dependent manner in dying cells, suggesting that a CAD-like activity is present (Wu et al., 2000). In a second step in apoptotic DNA degradation, which involves the participation of cells that engulf the dying cell, DNA is further processed by an acidic endonuclease. In mammals, this activity is probably an acid lysosomal DNase, either DNase II or a DNase II-like enzyme (McIlroy et al., 2000), and in *C. elegans* it is the product of the nuc-1 gene (Wu et al., 2000). *Drosophila* also encodes a DNase II-like protein (accession No. CG7780), and it seems likely that this form of DNA degradation occurs in flies as well.

Two other mammalian proteins that promote nuclear apoptotic events are AIF and acinus. AIF translocates from the mitochondria to cause chromatin condensation and large-scale DNA fragmentation (Susin et al., 1999). Acinus, a DNA-condensing factor with no nuclease activity, localizes to the nucleus, and is activated during apoptosis by combined caspase and serine protease cleavage (Sahara et al., 1999). *Drosophila*, but not *C. elegans*, encodes clear homologues of both these proteins (Acinus, accession No. CG10437; AIF, accession No. CG7263).

### **REAPER, HID, and GRIM. Insect-specific Death Regulators or Conserved Prophets of Death?**

One of the reasons for working with a model system such as the fly is the hope of being able to get a different perspective that will afford unique insight into a conserved, but complex process such as apoptosis. *Drosophila* has arguably been in this position for some time. An early genetic screen identified a genomic region at 75C that contained genes required for essentially all normally occurring cell deaths during *Drosophila* embryogenesis (White et al., 1994). Three genes within this region, *reaper* (*rpr*; White et al., 1994), *head involution defective* (*hid*; Grether et al., 1995), and *grim* (Chen et al., 1996), mediate this proapoptotic requirement. A large body of evidence argues that they act to integrate and transduce many different cell death signals that, ultimately, lead to the activation of caspase-dependent cell death (for review see Abrams, 1999). *Rpr*, *Hid*, and *Grim* have only very limited homology with each other (a short stretch of roughly 14 amino acids near their NH<sub>2</sub> termini), and sequence homologues have not been identified in other organisms. However, recent observations argue that the mechanisms of action defined by these genes are likely to be conserved. First, each of these proteins induces apoptosis in mammalian cells, strongly suggesting that some aspect of their function is evolutionarily conserved (for review see Abrams, 1999). Second, despite their very low level of homology with each



**Figure 4.** Mechanisms by which *Rpr*, *Hid*, and *Grim* are proposed to induce apoptosis. In one pathway *Rpr*, *Hid*, and *Grim* bind to *Diap1*, suppressing its ability to inhibit caspase activation and/or activity. *Diap2* also inhibits *Rpr*, *Hid*, and *Grim*-dependent cell death but has not been shown to act as a caspase inhibitor. Therefore, it has not been included in this diagram. In a second pathway, *RPR*, *HID*, and *GRIM* promote the release of a *Scythe*-bound factor that promotes the release of apoptosis inducers such as *cyto-c*, which is necessary for the activation of one form of the *Drosophila* *Apaf-1* homologue, *Ark*. See text (under *REAPER*, *HID*, and *GRIM*) for details.

other, they each interact with several different conserved death regulators (Fig. 4). This suggests that putative mammalian homologues may also be quite divergent in sequence. For example, they each bind the *Drosophila* caspase inhibitor *DIAP1* through interactions that require their NH<sub>2</sub> termini (Vucic et al., 1997, 1998), and genetic and biochemical data argue that one way they promote apoptosis is by inhibiting *DIAP1*'s ability to prevent death-inducing caspase activity (Wang et al., 1999; Goyal et al., 2000). Since IAPs and caspases also function to regulate death in vertebrates, it seems reasonable that *Rpr*, *Hid*, and *Grim* orthologues exist that perform a similar death-promoting function (see Note Added in Proof). *Rpr*, *Hid*, and *Grim* also bind a *Xenopus* protein, *Scythe*, in an interaction that does not require their NH<sub>2</sub> termini (Thress et al., 1999). In the case of at least *Rpr* this interaction leads to release of a *Scythe*-bound proapoptotic factor that promotes *cyto-c* release. *Drosophila* encodes a *Scythe* homologue (accession No. CG7546), suggesting that a similar pathway may exist in flies as well.

### **Cell Death in the 21st Century: Why the Fly?**

This review has discussed a number of core cell death regulators found in worms, flies, and vertebrates. However, we have only scratched the surface in terms of discussing all the genes and pathways that have been shown to regulate cell death in various systems. In particular, we have not dealt with the extensive literature on survival factors, many of which lead to the activation of the Akt/PkB kinase (Datta et al., 1999). What is clear from our analysis is that *Drosophila* shares many of the molecules and pathways that are used by vertebrates to control cell death. A description of the full complement of *Drosophila* coding regions, in conjunction with mass spectroscopic analysis of protein complexes, will provide an important new approach to understanding how pieces of the death machine talk to each other, as will the use of DNA microarrays. However, *Drosophila* is likely to have its biggest impact on the cell death field in the 21st century, as in the 20th, through the continued use of genetics to carry out function-based screens. This is because the genetic approach to identifying cell death regulators makes few assumptions about the kinds of molecules and mechanisms that regu-

late this process and, thus, is well positioned to uncover new molecules and mechanisms. The completed genomic sequence provides an invaluable resource for this work because it tells us where the homologues of known death regulators are, and it greatly speeds the identification of novel genes identified in these screens.

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*Note Added in Proof.* A mammalian protein called Smac/DIABLO which appears to play such a role has recently been described (*Cell* 2000. 102: 33-42; *Cell* 2000. 102:43-53).

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