# Apoptosis in *Drosophila*: neither fish nor fowl (nor man, nor worm)

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## Summary

**Studies in a wide variety of organisms have produced a general model for the induction of apoptosis in which multiple signaling pathways lead ultimately to activation of the caspase family of proteases. Once activated, these enzymes cleave key cellular substrates to promote the orderly dismantling of dying cells. A broad similarity exists in the cell death pathways operating in different organisms and there is a clear evolutionary conservation of apoptotic regulators such as caspases, Bcl-2 family members, inhibitor of apoptosis (IAP) proteins, IAP antagonists and caspase activators. Despite this, studies in *Caenorhabditis elegans*, *Drosophila* and vertebrates have revealed some apparent differences both in the way apoptosis is regulated and in the way individual molecules contribute to the propagation of the death signal. For example, whereas cytochrome *c* released from mitochondria clearly promotes caspase activation in vertebrates, there is no documented role for cytochrome *c* in *C. elegans* apoptosis and its role in *Drosophila* is highly controversial. In addition, the apoptotic potency of IAP antagonists appears to be greater in *Drosophila* than in vertebrates, indicating that IAPs may be of different relative importance in different organisms. Thus, although *Drosophila*, worms and humans share a host of apoptotic regulators, the way in which they function may not be identical.**

Key words: Apoptosis, *Drosophila*, Bcl-2 family, Inhibitor of apoptosis, IAP, Cytochrome *c*, Caspase, Apoptosome

## Introduction

Genetic model systems have provided crucial insight into the molecular mechanisms of apoptosis. Caspases, the critical effector proteases of apoptosis, were first identified in a screen for *C. elegans* mutants unable to undergo developmental programmed cell death (Yuan et al., 1993). The inhibitor of apoptosis (IAP) family of anti-apoptotic proteins were originally described in insect viruses, and later found to be essential regulators of apoptosis in *Drosophila* (Crook et al., 1993). Clearly, many of the elements of the apoptotic machinery are highly conserved throughout higher animals. However, there is also considerable controversy about how similar the pathways to execution are between mammals and invertebrates. Here, we highlight some of the mechanistic similarities and apparent differences between the activation and regulation of *C. elegans*, *Drosophila* and mammalian apoptotic pathways.

## Caspases are highly conserved and share common regulatory domains

The caspase proteases are the central executioners in most apoptotic cell deaths (Cryns and Yuan, 1998). Initiator caspases, usually distinguished by long regulatory pro-domains, are activated in response to a variety of apoptotic signals, including engagement of extracellular receptors by death ligands such as Fas, damage to cellular components, or breaches of mitochondrial integrity. Once active, the initiator caspases can cleave and thereby activate the effector caspases that go on to cleave a range of other targets, including structural proteins in the cell, pro-apoptotic proteins that are activated by cleavage, and anti-apoptotic proteins that are inactivated by cleavage.

The role of caspases in apoptosis was first identified in the nematode. The *C. elegans* Ced-3 caspase is required for all programmed somatic cell deaths in the worm (Yuan et al., 1993). It is present in an inactive pro-form in healthy cells and is activated through binding to the pro-apoptotic regulator Ced-4 (Chinnaiyan et al., 1997a; Seshagiri and Miller, 1997; Wu et al., 1997). Homotypic interactions between a caspase recruitment domain (CARD) in Ced-4 and a similar motif in the pro-domain of Ced-3 cause Ced-3 to oligomerize. This is thought to promote juxtaposition of adjacent Ced-3 molecules, consequent intermolecular cleavage and activation of the protease.

In *Drosophila*, the core effectors of the apoptotic process are also the caspases. There are seven identified in the fly genome: Dronc (also called Nc), Dredd, Strica, DrICE, Dcp-1, Decay and Damm (Fraser and Evan, 1997; Song et al., 1997; Chen et al., 1998; Dorstyn et al., 1999a; Dorstyn et al., 1999b; Doumanis et al., 2001; Harvey et al., 2001). Surprisingly, relatively little is known about most of these proteins. Dronc, Dredd and Strica are classified as initiator caspases because they have long pro-domains that appear to confer unique aspects of regulation on each of these proteins. The other four are classified as effector caspases.

Dronc is the best characterized of the fly caspases. Like mammalian caspase-9, and *C. elegans* Ced-3, it has a CARD

domain at its N-terminus (Dorstyn et al., 1999a). In all cases, these domains appear to serve as docking sites for adaptor proteins that promote the oligomerization and activation of the bound caspases. As mentioned above, in the case of Ced-3, this adaptor is Ced-4, by contrast, in the case of vertebrate caspase-9, the adaptor is a protein known as Apaf-1 (Li et al., 1997; Zou et al., 1997; Srinivasula et al., 1998). In both cases, the oligomerized adaptor-caspase complex is referred to as the apoptosome (Zou et al., 1999). In *Drosophila*, the fly Apaf-1 homolog Dark, also known as Hac-1 or dAPAF-1, binds to Dronc, and is required for its activation (Kanuka et al., 1999; Rodriguez et al., 1999; Zhou et al., 1999). However, as described below, the regulation of Dark may differ from that of mammalian Apaf-1.

Interestingly, Dronc shows unique substrate specificity, cleaving both after aspartate, like other caspases, and glutamate residues (Hawkins et al., 2000). Dronc autoprocesses itself by cleaving at a glutamate residue in a Dark-dependent manner (Muro et al., 2004).

During development, Dronc is transcriptionally regulated by the steroid hormone ecdysone and is expressed in tissues known to undergo apoptosis (Dorstyn et al., 1999a). Inhibition of Dronc activity by RNA interference (RNAi) or a dominant-negative Dronc mutant indicates that Dronc is important for a significant amount of developmental cell death in the animal (Quinn et al., 2000). Recent studies of Dronc-null flies confirm this (Chew et al., 2004; Daish et al., 2004). In these animals, developmental apoptosis in some tissues is blocked, whereas in other tissues apoptosis proceeds normally. Stress-induced apoptosis is dramatically reduced in the absence of Dronc. Similarly, caspase-9-knockout mice show reduced apoptosis in some tissues, although other developmental death appears normal (Hakem et al., 1998; Kuida et al., 1998). These data indicate that, both in mice and flies, the CARD-domain-containing caspases, and presumably the apoptosome in general, are required for some, but not all, developmental apoptosis.

Dredd, another long pro-domain caspase, resembles mammalian caspase-8 structurally and functionally (Chen et al., 1998; Leulier et al., 2000). Most evidence suggests that the major role of this caspase is in the innate immune response, in which it is required for the processing of an NF-κB-like transcription factor (Elrod-Erickson et al., 2000; Leulier et al., 2000; Stoven et al., 2000; Georgel et al., 2001). However, Dredd mutants also show defects in apoptosis (Chen et al., 1998; Leulier et al., 2000; Georgel et al., 2001). Surprisingly, Dredd is transcriptionally upregulated in response to cell death signals, unlike most other caspases (Chen et al., 1998).

The other putative initiator caspase, Strica, contains a long pro-domain that is rich in serine and threonine residues and displays no sequence similarity to any mammalian caspase pro-domains (Doumanis et al., 2001). Little is known about the role of Strica in developmental or stress-induced apoptosis, although it is expressed in tissues known to undergo apoptosis.

Dcp-1, Damm, DrICE and Decay are all classified as effector caspases because they do not contain a long pro-domain (Fraser and Evan, 1997; Song et al., 1997; Dorstyn et al., 1999b; Harvey et al., 2001). There is some evidence that these effector caspases do not have completely overlapping functions and can play essential roles in certain types of apoptosis. Dcp-1 is required for starvation-induced apoptosis during early stages of oogenesis (Laundrie et al., 2003), and depletion of DrICE inhibits apoptosis in S2 cell extracts (Fraser et al., 1997). The absence of loss-of-function analyses for these caspases precludes an accurate assessment of their roles in developmental apoptosis.

## Is the apoptosome function less-highly conserved?

The term apoptosome refers to a protein complex that activates the initiator caspase(s) in response to an apoptotic signal. In its simplest form in the worm, it consists of a Ced-3–Ced-4 complex. The pro-apoptotic activity of this complex is normally held in check by sequestration of Ced-4 by Ced-9, an anti-apoptotic factor related to mammalian Bcl-2-family proteins (Hengartner and Horvitz, 1994; Chinnaiyan et al., 1997b; Spector et al., 1997; Wu et al., 1997). When cells receive apoptotic signals, Egl 1, a distant relative of the Bcl-2 family, is synthesized (Conradt and Horvitz, 1998). Binding of Egl-1 to Ced-9 releases Ced-4, thereby allowing it to activate Ced-3 (Yan et al., 2004). These changes are accompanied by re-localization of Ced-4 from a Ced-9-tethered position on the outside of the mitochondria to the outer nuclear membrane (Chen et al., 2000). Thus, the worm apoptosome appears to be directly regulated by Bcl-2-like proteins through a series of mutually exclusive binding interactions. This process is ultimately controlled at the transcriptional level, because production of Egl-1 sets it in motion.

The ability of Ced-4 to activate Ced-3 is constitutive but is blocked by the action of Ced-9. By contrast, the Ced-4-related Apaf-1 protein of vertebrates does not have this innate ability to activate caspase-9 in a resting cell (Li et al., 1997; Saleh et al., 1999). This difference arises, at least in part, because of a C-terminal extension on Apaf-1, absent from Ced-4, that contains a series of WD-40 repeats. Removal of these repeats yields a protein that has similar properties to Ced-4, and can oligomerize and activate caspase-9 in a constitutive fashion (Srinivasula et al., 1998; Hu et al., 1999). The WD-40 repeats are thought to regulate Apaf-1 function negatively through intramolecular interactions with N-terminal portions of the molecule to produce a closed, inactive conformation (Hu et al., 1999). Thus, unlike Ced-4, Apaf-1 is held inactive by self-inhibition, rather than by association with an anti-apoptotic Bcl-2-family member (Newmeyer et al., 2000). When Apaf-1 is in the inactive state, its CARD domain is apparently masked, which prevents recruitment, oligomerization and consequent activation of caspase-9.

In response to a variety of death signals, vertebrate cells release cytochrome *c* from the intermembrane space of the mitochondria into the cytoplasm (Liu et al., 1996; Kluck et al., 1997; Zou et al., 1997). Once cytoplasmic, cytochrome *c* interacts with Apaf-1 in a manner that is dependent on (though not exclusively through) the WD-40 repeats (Benedict et al., 2000). To do so, it must contain heme and, therefore, have come from mitochondria and not a newly synthesized cytoplasmic pool (Yang et al., 1997). Once bound to holocytochrome *c* (and dATP), Apaf-1 undergoes a conformational change, leading to exposure of the CARD domain and subsequent binding of caspase-9. Interestingly, the Apaf-1–caspase-9 complex appears to act as a holoenzyme: continued activation of caspase-9 depends upon its continued association with Apaf-1 rather than proteolytic processing

(Rodriguez and Lazebnik, 1999; Boatright et al., 2003). Thus, unlike effector caspases, caspase-9 does not require cleavage for activation, and an uncleavable caspase-9 retains enzymatic activity (Stennicke et al., 1999).

Cain et al. (Cain et al., 1999; Cain et al., 2000) have reported that productive, cytochrome-*c*-driven oligomerization of the Apaf-1–caspase-9 complex leads to formation of a 700 kDa complex containing Apaf-1, caspase-9 and the caspase-9-activated effector caspase-3 (and potentially other proteins, such as IAPs). In cell lysates supplemented with cytochrome *c* and dATP, Apaf-1 and caspase-9 are also detectable in a 1.4 MDa complex, although this complex is largely inactive in processing caspase-3 and may or may not exist in intact cells. Within cells, Apaf-1 does not appear to undergo any notable shift in subcellular localization upon activation (unlike Ced-4), but instead appears to be cytoplasmic in both healthy and dying cells (Chen et al., 2000; Hausmann et al., 2000).

The *Drosophila* Apaf-1 protein Dark also has an N-terminal CARD domain, a central domain homologous to Ced-4, and a series of WD-40 repeats. Given its WD-40 repeats, Dark might also be activated by cytochrome *c* released from mitochondria. Indeed, initial reports demonstrated that Dark can be immunoprecipitated in association with cytochrome *c* and that this association depends upon the WD-40 repeats (Kanuka et al., 1999; Rodriguez et al., 1999). Subsequent work consistent with this idea demonstrated that a shift in the gel filtration profile of the sole CARD-domain-containing initiator caspase in flies, Dronc, can be induced by addition of cytochrome *c* to cultured BG2 *Drosophila* cell lysates (Dorstyn et al., 2002).

Despite biochemical evidence implicating cytochrome *c* in Dark activation, functional evidence for this has been patchy, at best. Although purified cytochrome *c* from a variety of sources (including *Drosophila*) can activate caspases when added to cell lysates prepared from vertebrate cells, lysates from *Drosophila* cultured S2 cells are notably refractory to cytochrome *c* addition. Miura and co-workers have observed some degree (~2-fold increase) of caspase activation in embryonic fly lysates upon cytochrome *c* addition and, strikingly, this is absent from lysates prepared from Dark-deficient embryos (Kanuka et al., 1999). Stimulation of caspase activity by cytochrome *c* has also been reported in extracts primed for apoptosis (Dorstyn et al., 2004). However, although one group reported that apoptotic stimuli induce the exposure of a normally masked epitope on fly cytochrome *c* (Varkey et al., 1999), multiple labs have failed to observe any clear release of cytochrome *c* from the mitochondria into the cytoplasm on induction of apoptosis in S2 cells (Zimmermann et al., 2002; Dorstyn et al., 2004).

Additional doubts concerning the involvement of fly cytochrome *c* in apoptosis have arisen from reports that knocking down cytochrome *c* by RNAi in S2 cells does not alter apoptosis. Green and co-workers showed that double-stranded (ds)RNA directed against the *cyt-c-p* cytochrome *c* transcript (which apparently downregulates both of the cytochrome *c* proteins in *Drosophila*, Cyt-c-d and Cyt-c-p), abrogates neither stress nor macromolecular-synthesis-inhibitor-induced apoptosis, whereas dsRNA directed at Dark does protect SL2 cells from these death stimuli (Zimmermann et al., 2002). More recently, Kumar and co-workers have silenced both of the cytochrome *c* variants and found that neither cycloheximide-induced apoptosis in BG2 cells nor ecdysone-induced death of l(2)mbn cells is markedly affected (Dorstyn et al., 2004). By contrast, genetic data support a role for cytochrome *c* in developmental apoptosis in at least one tissue. A P-element insertion in one of the fly cytochrome *c* genes (*cyt-c-d*) results in a defect in caspase activation and a failure of caspase-dependent spermatid individualization (Arama et al., 2003). Further work is needed to determine whether this defect can be rescued by restoration of Cyt-c-d alone or whether effects on nearby genes contribute to the phenotype (Huh et al., 2004). Other studies have demonstrated that a 50% reduction in the gene dosage of one or both cytochrome *c* genes has no detectable affect on developmental apoptosis (Dorstyn et al., 2004). Zygotic deletion of both genes also has no obvious effect on embryonic apoptosis. Similarly, only relatively subtle defects in apoptosis are observed in embryos lacking Dark or Dronc (Kanuka et al., 1999; Rodriguez et al., 1999; Zhou et al., 1999; Chew et al., 2004). However, *Drosophila* embryos often have large amounts of maternally contributed gene products, and the lack of embryonic phenotypes in cytochrome *c*, Dark and Dronc zygotic mutants might simply reflect the maternal contribution. Given these issues and remaining gaps in our knowledge, we cannot yet say definitively that *Drosophila* and vertebrate Apaf-1 proteins operate in fundamentally different ways.

## The Bcl-2 family as regulators of apoptosome function

In vertebrates, Bcl-2-family proteins do not directly regulate Apaf-1 but instead regulate release of cytochrome *c* from the mitochondria, either positively or negatively (Fig. 1) (Kluck et al., 1997; Yang et al., 1997). Anti-apoptotic members of the family, including Bcl-2, Bcl-$x_L$, Bcl-w and Boo/Diva, are able to heterodimerize with pro-apoptotic Bcl-2-family members. The latter can be divided into two subgroups: those containing multiple Bcl-2-homology (BH) domains (e.g. Bax, Bak, Bok) and those containing a single BH3 domain (the BH3-only proteins, such as Bid, Bad, Noxa, Puma and Bim). Bax and Bak can be activated by the BH3-only proteins either through direct binding (e.g. tBid binds to Bax) or by BH3-only proteins binding to and neutralizing anti-apoptotic family members that would otherwise hold the multi-domain Bax and Bak proteins in check (reviewed by Gross et al., 1999).

The anti-apoptotic action of Bcl-2-family members is exerted largely at the mitochondria or the endoplasmic reticulum, membrane association being mediated by a C-terminal hydrophobic tail (Chen-Levy and Cleary, 1990; Janiak et al., 1994). The membrane-anchoring domain of multi-domain pro-apoptotic family members can confer constitutive mitochondrial localization (as in the case of Bak) or be masked prior to receipt of an apoptotic signal (e.g. Bax) (Wolter et al., 1997; Nechushtan et al., 1999). Indeed, activation of Bax is associated with both a conformational change and translocation to mitochondria. Although Bak is also likely to undergo a conformational change and appears to oligomerize prior to induction of cytochrome *c* release, its activation is not accompanied by an overt shift in subcellular localization (Korsmeyer et al., 2000; Mikhailov et al., 2003; Wei et al., 2000).

In vertebrates, apoptosis can be controlled transcriptionally through induction of BH3-only proteins (e.g. Noxa and Puma)

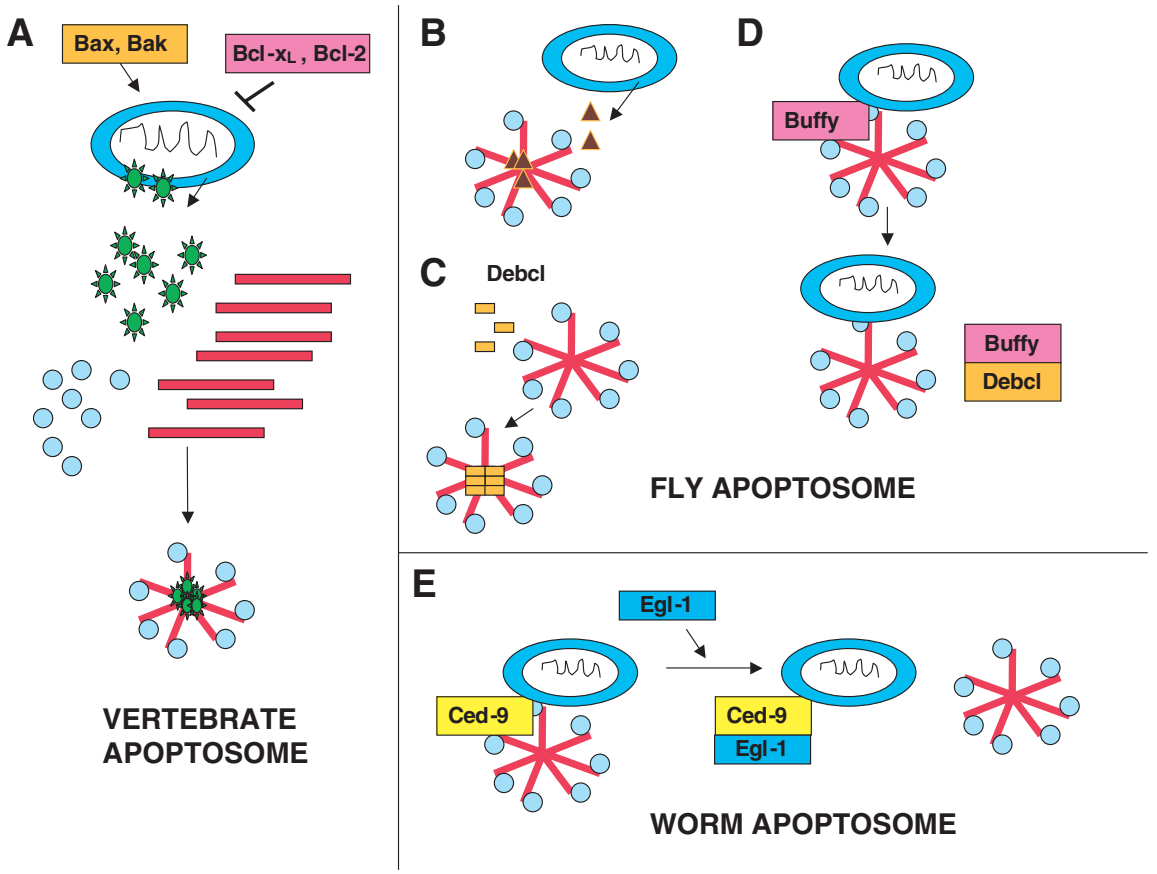


**Fig. 1.** Models of apoptosome regulation. (A) In vertebrate cells, a variety of apoptotic stimuli induce release of cytochrome *c* from the mitochondria. Once released, cytochrome *c* binds to Apaf-1, promoting a conformational change that leads to the exposure of the CARD domain and consequent recruitment of pro-caspase-9 into the active oligomerized apoptosome; caspase-9 can then cleave and activate effector caspases. Cytochrome *c* release from the mitochondria is promoted by pro-apoptotic Bcl-2-family members such as Bax and Bak and is antagonized by anti-apoptotic members of the family such as Bcl-2 and Bcl-$x_L$. Red rectangles, Apaf-1; light-blue circles, caspase-9; green stars, cytochrome *c*. (B) In *Drosophila* cells, it is possible that cytochrome *c*, or some other intramitochondrial factor (represented here by brown triangles) is released from mitochondria in response to apoptotic stimuli, to promote the activation of the Dark/Dronc apoptosome (red rectangles and blue circles, respectively). This might stimulate oligomerization or enhance activation of the already oligomerized structure. (C) If the fly Bcl-2-family members do not act at the level of cytochrome *c* release from the mitochondria as in vertebrates, it is possible that pro-apoptotic Debcl protein (yellow rectangles) interacts in some way with the apoptosome to promote its activation. (D) By analogy to the worm system, it is possible that the anti-apoptotic Bcl-2-family protein, Buffy, binds to and inhibits the apoptosome (either at the mitochondrial surface or elsewhere). One possible scenario is that pro-apoptotic Debcl facilitates apoptosome activation by dimerizing with and thereby removing Buffy from the apoptosome. (E) The *C. elegans* apoptosome is held in check by binding of Ced-9 to Ced-4. This repression is relieved following Egl-1 production and interaction with Ced-9. Red bars, Ced-4; light-blue circles, Ced-3.

(Oda et al., 2000; Nakano and Vousden, 2001). However, these proteins may also be sequestered intracellularly in inactive forms and liberated through a variety of post-translational mechanisms for promoting apoptosis (reviewed by Gross et al., 1999).

Thus far, two Bcl-2-family members have been identified in flies. These include the pro-apoptotic Debcl (also called Drob-1, dBorg-1 and dBok) and the Buffy/dBorg-2, which has been reported to have anti-apoptotic activity but lacks the BH4 domain characteristic of its anti-apoptotic mammalian counterparts (Brachmann et al., 2000; Colussi et al., 2000; Igaki et al., 2000; Zhang et al., 2000; Quinn et al., 2003). Characterization of these proteins has yielded somewhat conflicting results. Although some investigators report that overexpression of Debcl increases caspase activity, others have suggested that Debcl-induced cell death is caspase independent (it is unaffected by either p35 or zVAD inhibitors of caspases) (Colussi et al., 2000; Igaki et al., 2000; Zimmermann et al., 2002).

When expressed heterologously in mammalian cells, Debcl can induce cytochrome *c* release from mitochondria, but it has not been reported to do so in fly cells (Zhang et al., 2000). Moreover, its C-terminal hydrophobic tail, which presumably confers mitochondrial localization, appears to be important for its pro-apoptotic activity (Igaki et al., 2000). Thus, Debcl might kill cells by compromising mitochondrial function, entirely bypassing any Dark/caspase pathway. In support of this, Zimmerman et al. reported that knocking down Dark has no marked effect on Debcl-induced cell death (Zimmermann et al., 2002). By contrast, Colussi et al. reported that the rough-eye phenotype resulting from UAS-Debcl expression in the eye is suppressed in Dark hypomorphic flies (Colussi et al., 2000)

and by the caspase inhibitor p35. These differences might well reflect different complements of co-factors in cultured S2/BG2 cells and the whole animal. However, they do suggest that Debcl can activate both caspase-dependent and caspase-independent modes of cell death. One possible scenario is that the caspase-dependent pathway involves Debcl-induced activation of Dark, which might occur either by release of a Dark-activating factor from the mitochondria (if not cytochrome *c*, then perhaps some other factor), through a direct interaction with Dark or through an indirect interaction (see below).

## IAPs as critical caspase inhibitors

Virtually all *Drosophila* cells undergo rapid apoptosis in the absence of the DIAP1 protein (Goyal et al., 2000; Lisi et al., 2000; Yoo et al., 2002). DIAP1 is a member of the IAP family of proteins first identified in insect viruses as critical inhibitors of host cell apoptosis (Crook et al., 1993). In flies and mammals, endogenous IAPs bind to active caspases and inhibit their function (reviewed by Salvesen and Duckett, 2002), but, in *C. elegans*, no IAP-like protein has been found to play a role in regulating apoptosis (Fraser et al., 1999). IAPs can inhibit caspase function by binding of their conserved baculoviral IAP repeat (BIR) domain to the caspase active site, by promoting the degradation of active caspases, or by sequestering the caspase away from its substrates (Tenev et al., 2004). IAP BIR domains also bind to a short IAP-binding motif (IBM) at the processed N-terminus of several pro-apoptotic proteins (Vucic et al., 1997; Vucic et al., 1998). In flies, these pro-apoptotic proteins, Reaper, Grim, Hid and Sickle, act as critical inhibitors of IAP activity (Goyal et al., 2000; Lisi et al., 2000; Yoo et al., 2002). Binding of the IBM proteins to the BIR domain prevents caspase inhibition by competing for caspase binding and by promoting IAP degradation (Chai et al., 2003; Holley et al., 2002; Ryoo et al., 2002; Yoo et al., 2002; Tenev et al., 2004; Yokokura et al., 2004) (Fig. 2).

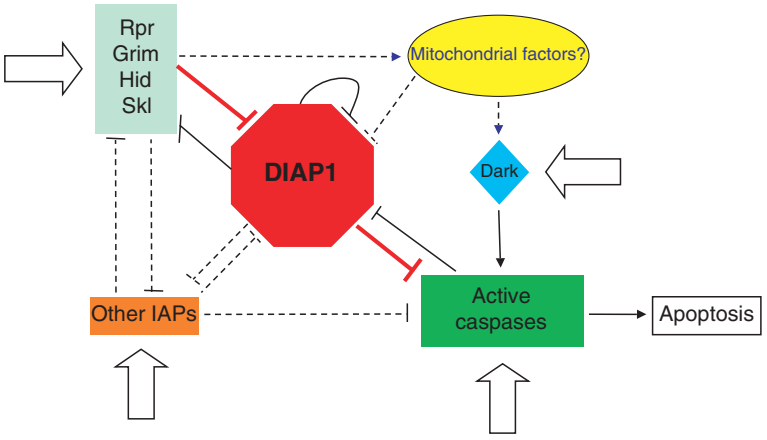


**Fig. 2.** Apoptosis through the fly's eye. In our current understanding of apoptosis in *Drosophila*, DIAP1 acts as a central brake on apoptosis, inhibiting activated caspases (reviewed by Hay, 2000). Caspase binding to DIAP1 can result in sequestration of the caspase away from its targets or in caspase degradation. The IBM proteins Rpr, Grim, Hid and Skl compete with caspases for binding to DIAP1 and promote apoptosis. Rpr and Grim can also suppress DIAP1 translation (Holley et al., 2002; Yoo et al., 2002). This is the fundamental mechanism of apoptosis induction in fly development, as well as in response to stress such as DNA damage (red bars) (White et al., 1994; Nordstrom et al., 1996; Brodsky et al., 2004). DIAP1 also has other functions: it can promote its own degradation and the degradation of IBM proteins (Hay, 2000). DIAP1 may also regulate the levels of other IAPs (broken arrows and bars are speculative). Caspase activity also contributes to DIAP1 degradation (Ditzel et al., 2003; Yokokura et al., 2004). No role for mitochondrial factors has been definitively demonstrated in *Drosophila* apoptosis, although Rpr and Grim can release cytochrome *c* in heterologous systems (Thress et al., 1999; Claveria et al., 2004). Dark is required to activate the apical caspase Dronc (Kanuka et al., 1999; Rodriguez et al., 1999; Zhou et al., 1999), and could be regulated by cytochrome *c*, although this has not been demonstrated. It is clear that the other IAP-like proteins in flies, DIAP2, deterin and dBRUCE, can suppress apoptosis, but their role and mechanism of regulation has yet to be fully explored (Hay et al., 1995; Jones et al., 2000; Vernooy et al., 2002; Arama et al., 2003). Developmental and other environmental inputs (open arrows) can influence the apoptotic pathway at several points, including the transcription and activity of the IBM proteins, the synthesis of Dark and some of the caspases, and the synthesis of some of the IAPs (White et al., 1994; Zhou et al., 1999; Dorstyn et al., 1999a; Jiang et al., 2000).

In mammalian systems, the best characterized of the IBM-containing proteins are SMAC/DIABLO and OMI/HTRA2 (Du et al., 2000; Verhagen et al., 2000; Suzuki et al., 2001; Hegde et al., 2002). These proteins are normally sequestered in mitochondria and are released only in apoptotic cells that have compromised mitochondrial integrity. Mammalian cells also contain non-IBM IAP inhibitors, such as ARTS (Gottfried et al., 2004). Unlike Reaper, Grim, Hid and Sickle, the known mammalian IBM-containing proteins do not appear to have potent autonomous apoptotic activity. Rather, upon release from mitochondria, they enhance the action of cytochrome *c* to achieve maximal caspase activation. Thus, these proteins act quite far downstream in the induction of apoptosis. This is in marked contrast to the central regulatory role of the IBM proteins in flies. In the absence of the *reaper*, *grim* and *hid* genes, developmental apoptosis is not initiated in most tissues (White et al., 1994). The production of these IBM proteins is subject to exquisite transcriptional regulation during *Drosophila* development. Reaper, for example, appears to be expressed in almost all of the cells fated to die during embryonic development and is not expressed in most of the cells fated to survive (White et al., 1994). Grim and Sickle show similar regulation, although exhibit slightly more-limited expression, whereas Hid is more broadly expressed and is regulated at both the transcriptional and post-translational levels (Grether et al., 1995; Chen et al., 1996; Bergmann et al., 1998; Kurada and White 1998; Wing et al., 2002).

Inhibition of DIAP1 by IBM proteins or by knocking out or knocking down the gene encoding DIAP1 is sufficient to initiate apoptosis (Goyal et al., 2000; Lisi et al., 2000; Yoo et al., 2002; Zimmermann et al., 2002). This suggests that caspases are already activated in fly cells and are only held in check by IAP binding, indicating that apoptosome-mediated activation of caspases is not an essential regulatory step in *Drosophila* apoptosis. Clearly, Dark is important for activating caspases. The massive apoptosis in the absence of DIAP1 is partially inhibited in the absence of Dark (Rodriguez et al., 2002; Zimmermann et al., 2002). The most parsimonious explanation

for this is that Dark is normally constitutively active, producing active caspases that are then inhibited by DIAP1.

Mammalian cells seem to be less sensitive to the levels of IAPs than are insect cells. Knocking out XIAP, the most potent anti-apoptotic mammalian IAP, has little or no effect on apoptosis (Harlin et al., 2001). However, there is a clear upregulation of cIAP1 and cIAP2 in the XIAP-null animals that may explain the lack of a phenotype. Most data support a role for mammalian IAPs as inhibitors of apoptosis acting downstream of the apoptosis-initiating event. In support of this, XIAP loss makes cells more sensitive to the induction of apoptosis (Potts et al., 2003). Also, the known mammalian IBM proteins show weak activity in triggering apoptosis in some cells but potentiate other death signals (e.g. Arnt et al., 2002; Li et al., 2004).

Is this apparent difference in the requirement for IAPs in insect and mammalian systems simply a reflection of the increased activation of *Drosophila* caspases by constitutively active Dark? This seems unlikely, because DIAP1 is required for viability even in the absence of Dark. Perhaps fly cells are primed in multiple ways to undergo apoptosis more easily than mammalian cells. This would be compatible with the need for precise spatial and temporal control of apoptosis during the rapid development of the organism. Alternatively, perhaps the apparent differences are due to a weakness in our ability to assess the role of IAPs in mammalian development, owing to their redundancy and cross-regulation. Not only do the functions of the 8 mammalian IAP-like proteins appear to overlap, but there is evidence that IAPs can regulate the levels of other IAPs at the level of protein degradation, creating the potential for compensatory activity of other IAPs when one or more is knocked out (Harlin et al., 2001) (J. Silke and D. Vaux, personal communication; T. Yokokura and K.W., unpublished).

## Conclusions and Perspectives: is the fly a worm with wings?

Despite our efforts to shoehorn the fly into a worm or a vertebrate model, perhaps the fly is neither flying nematode nor human in terms of its apoptotic regulation. The major areas of controversy appear to be the regulation of apoptosome activity by cytochrome *c* and the role of the Bcl-2-family proteins.

Because the bulk of the data are primarily negative, the issue of whether cytochrome *c* is involved in fly cell apoptosis may not be entirely closed. Scrutiny of each line of evidence raises some questions. For example, in experiments where purified cytochrome *c* failed to elicit caspase activation in fly cell lysates, were levels of the IAPs sufficiently high to 'mop up' and neutralize any incipiently activated caspases (and could IAP levels be much higher in fly cell lysates than in vertebrate cell lysates)? Perhaps the ability of cytochrome *c* to elicit increased caspase responses in lysates primed for apoptosis (Dorstyn et al., 2004) is due to decreased levels of DIAP1. If the affinity of Dark for cytochrome *c* is high (as is the case for Apaf-1), can knocking it down by RNAi reduce cytochrome *c* levels sufficiently to disrupt caspase activation? Similarly, might maternal pools of cytochrome *c* remaining in the zygotic cytochrome *c* mutants be enough to support caspase activation? What if cytochrome *c* is released from the intermembrane space of the mitochondria in response to apoptotic stimuli but remains tightly tethered to the outer membrane, rather than being released to the cytoplasm? Because Dark and Dronc can both reportedly associate with mitochondria, it is plausible that an apoptosome is nucleated by membrane-tethered cytochrome *c*. Perhaps Dark is even constitutively bound to cytochrome *c* in fly cells (which would be consistent with the observation that some cytochrome *c* is extra-mitochondrial even in living cells) (Dorstyn et al., 2004) and a conformational change occurs in cytochrome *c* to allow apoptotic activation of Dark, explaining the observed alteration in cytochrome *c* immunoreactivity (Varkey et al., 1999). Under these circumstances, Dark-bound cytochrome *c* might be refractory to the turnover necessary to rid cells of cytochrome *c* following dsRNA treatment (or loss of maternal pools in the mutant embryos). Because an active fly apoptosome assembled from purified Dark and Dronc has not yet been reported, the possibility that the isolated apoptosome (in the absence of DIAP or other cellular factors) exhibits increased activity in the presence of cytochrome *c* cannot be ruled out.

The presence of WD-40 repeats on Dark led many to assume that Dark is more similar in function to Apaf-1 than to Ced-4. It is interesting to note that one group has detected a truncated form of Dark in embryos (Kanuka et al., 1999). This form lacks the WD repeats and would be expected to behave more similarly to Ced-4. However, if cytochrome *c* does not activate Dark, it is attractive to consider the possibility that Dark has the potential to be constitutively active but is, like Ced-4, restrained by an anti-apoptotic Bcl-2-family protein. Alternatively, a pro-apoptotic Bcl-2-family member might somehow serve as a direct Dark activator (Fig. 1).

With regard to the possibility that the fly apoptosome more precisely parallels the worm apoptosome, it is significant that Dark, Dronc and Buffy have all been reported to localize to mitochondria, which is analogous to the behavior of the worm Ced-9–Ced-4–Ced-3 complex (Dorstyn et al., 2002b; Quinn et al., 2003). However, this analogy breaks somewhat if one considers the fact that Buffy does not look precisely like an anti-apoptotic Bcl-2-family member and that at least one group has observed pro-apoptotic, rather than anti-apoptotic, effects of Buffy overexpression (Igaki and Miura, 2004). Furthermore, no BH3-only protein has been discovered in the *Drosophila* genome. Yet, it was only through very careful scrutiny (and genetic implication) that Egl-1 was deduced to contain a BH3 domain. Moreover, the presumably pro-apoptotic Debcl [one group has also seen pro-survival activity from this molecule (Brachmann et al., 2000)] can heterodimerize with Buffy (Brachmann et al., 2000; Quinn et al., 2003). This raises the possibility that Buffy holds Dark/Dronc in check and full activation is achieved by Debcl-induced removal of Buffy from the complex.

It is possible that differences in apoptotic signaling in *Drosophila* and vertebrates are only apparent, reflecting the incomplete state of our knowledge of these pathways. Thus, further genetic and biochemical analysis will be required to determine exactly where flies land in the spectrum of apoptotic regulation.

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## Note added in proof

New analysis of embryos lacking both maternal and zygotic Dronc shows that Dronc is required for almost all apoptosis in the Drosophila embryo (Xu et al., 2005).

## References


**Arama, E., Agapite, J. and Steller, H.** (2003). Caspase activity and a specific Cytochrome C are required for sperm differentiation in *Drosophila*. *Dev. Cell* **4**, 687-697.

**Arnt, C. R., Chiorean, M. V., Heldebrant, M. P., Gores, G. J. and Kaufmann, S. H.** (2002). Synthetic Smac/DIABLO peptides enhance the effects of chemotherapeutic agents by binding XIAP and cIAP1 in situ. *J. Biol. Chem.* **277**, 44236-44243.

**Benedict, M. A., Hu, Y., Inohara, N. and Nunez, G.** (2000). Expression and functional analysis of Apaf-1 isoforms. Extra WD-40 repeat is required for cytochrome *c* binding and regulated activation of procaspase-9. *J. Biol. Chem.* **275**, 8461-8468.

**Bergmann, A., Agapite, J., McCall, K. and Steller, H.** (1998). The *Drosophila* gene *hid* is a direct molecular target of Ras-dependent survival signaling. *Cell* **95**, 331-341.

**Boatright, K. M., Renatus, M., Scott, F. L., Sperandio, S., Shin, H., Pedersen, I. M., Ricci, J. E., Edris, W. A., Sutherlin, D. P., Green, D. R. et al.** (2003). A unified model for apical caspase activation. *Mol. Cell* **11**, 529-541.

**Brachmann, C. B., Jassim, O. W., Wachsmuth, B. D. and Cagan, R. L.** (2000). The *Drosophila* bcl-2 family member dBorg-1 functions in the apoptotic response to UV-irradiation. *Curr. Biol.* **10**, 547-550.

**Brodsky, M. H., Weinert, B. T., Tsang, G., Rong, Y. S., McGinnis, N. M., Golic, K. G., Rio, D. C. and Rubin, G. M.** (2004). *Drosophila melanogaster* MNK/Chk2 and p53 regulate multiple DNA repair and apoptotic pathways following DNA damage. *Mol. Cell Biol.* **24**, 1219-1231.

**Cain, K., Brown, D. G., Langlais, C. and Cohen, G. M.** (1999). Caspase activation involves the formation of the apoptosome, a large (approximately 700 kDa) caspase-activating complex. *J. Biol. Chem.* **274**, 22686-22692.

**Cain, K., Bratton, S. B., Langlais, C., Walker, G., Brown, D. G., Sun, X. M. and Cohen, G. M.** (2000). Apaf-1 oligomerizes into biologically active approximately 700-kDa and inactive approximately 1.4-MDa apoptosome complexes. *J. Biol. Chem.* **275**, 6067-6070.

**Chai, J., Yan, N., Huh, J. R., Wu, J. W., Li, W., Hay, B. A. and Shi, Y.** (2003). Molecular mechanism of Reaper-Grim-Hid-mediated suppression of DIAP1-dependent Dronc ubiquitination. *Nat. Struct. Biol.* **10**, 892-898.

**Chen, F., Hersh, B. M., Conradt, B., Zhou, Z., Riemer, D., Gruenbaum, Y. and Horvitz, H. R.** (2000). Translocation of *C. elegans* CED-4 to nuclear membranes during programmed cell death. *Science* **287**, 1485-1489.

**Chen, P., Nordstrom, W., Gish, B. and Abrams, J. M.** (1996). *grim*, a novel cell death gene in *Drosophila*. *Genes Dev.* **10**, 1773-1782.

**Chen, P., Rodriguez, A., Erskine, R., Thach, T. and Abrams, J.** (1998). Dredd, a novel effector of the apoptosis activators *reaper*, *grim*, and *hid* in *Drosophila*. *Dev. Biol.* **201**, 202-216.

**Chen-Levy, Z. and Cleary, M. L.** (1990). Membrane topology of the Bcl-2 proto-oncogenic protein demonstrated in vitro. *J. Biol. Chem.* **265**, 4929-4933.

**Chew, S. K., Akdemir, F., Chen, P., Lu, W. J., Mills, K., Daish, T., Kumar, S., Rodriguez, A. and Abrams, J. M.** (2004). The apical caspase dronc governs programmed and unprogrammed cell death in *Drosophila*. *Dev. Cell* **7**, 897-907.

**Chinnaiyan, A. M., Chaudhary, D., O'Rourke, K., Koonin, E. V. and Dixit, V. M.** (1997a). Role of CED-4 in the activation of CED-3. *Nature* **388**, 728-729.

**Chinnaiyan, A. M., O'Rourke, K., Lane, B. R. and Dixit, V. M.** (1997b). Interaction of CED-4 with CED-3 and CED-9: a molecular framework for cell death. *Science* **275**, 1122-1126.

**Claveria, C., Martinez, A. C. and Torres, M.** (2004). A Bax/Bak-independent mitochondrial death pathway triggered by *Drosophila* Grim GH3 domain in mammalian cells. *J. Biol. Chem.* **279**, 1368-1375.

**Colussi, P. A., Quinn, L. M., Huang, D. C., Coombe, M., Read, S. H., Richardson, H. and Kumar, S.** (2000). Debcl, a proapoptotic Bcl-2 homologue, is a component of the *Drosophila melanogaste*r cell death machinery. *J. Cell Biol.* **148**, 703-714.

**Conradt, B. and Horvitz, H. R.** (1998). The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. *Cell* **93**, 519-529.

**Crook, N. E., Clem, R. J. and Miller, L. K.** (1993). An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. *J. Virol.* **67**, 2168-2174.

**Cryns, V. and Yuan, J.** (1998). Proteases to die for. *Genes Dev.* **12**, 1551-1570.

**Daish, T. J., Mills, K. and Kumar, S.** (2004). *Drosophila* caspase DRONC is required for specific developmental cell death pathways and stress-induced apoptosis. *Dev. Cell* **7**, 909-915.

**Ditzel, M., Wilson, R., Tenev, T., Zachariou, A., Paul, A., Deas, E. and Meier, P.** (2003). Degradation of DIAP1 by the N-end rule pathway is essential for regulating apoptosis. *Nat. Cell Biol.* **5**, 467-473.

**Dorstyn, L., Colussi, P., Quinn, L., Richardson, H. and Kumar, S.** (1999a). DRONC, an ecdysone-inducible *Drosophila* caspase. *Proc. Natl. Acad. Sci. USA* **96**, 4307-4312.

**Dorstyn, L., Read, S., Quinn, L., Richardson, H. and Kumar, S.** (1999b). DECAY, a novel *Drosophila* caspase related to mammalian caspase-3 and caspase-7. *J. Biol. Chem.* **274**, 30778-30783.

**Dorstyn, L., Read, S., Cakouros, D., Huh, J. R., Hay, B. A. and Kumar, S.** (2002). The role of cytochrome *c* in caspase activation in *Drosophila melanogaster* cells. *J. Cell Biol.* **156**, 1089-1098.

**Dorstyn, L., Mills, K., Lazebnik, Y. and Kumar, S.** (2004). The two cytochrome *c* species, DC3 and DC4, are not required for caspase activation and apoptosis in *Drosophila* cells. *J. Cell Biol.* **167**, 405-410.

**Doumanis, J., Quinn, L., Richardson, H. and Kumar, S.** (2001). STRICA, a novel *Drosophila melanogaster* caspase with an unusual serine/threonine-rich prodomain, interacts with DIAP1 and DIAP2. *Cell Death Differ.* **8**, 387-394.

**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.

**Elrod-Erickson, M., Mishra, S. and Schneider, D.** (2000). Interactions between the cellular and humoral immune responses in *Drosophila*. *Curr. Biol.* **10**, 781-784.

**Fraser, A. and Evan, G.** (1997). Identification of a *Drosophila melanogaster* ICE/CED-3-related protease, drICE. *EMBO J.* **16**, 2805-2813.

**Fraser, A. G., McCarthy, N. J. and Evan, G. I.** (1997). drICE is an essential caspase required for apoptotic activity in *Drosophila* cells. *EMBO J.* **16**, 6192-6199.

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

**Georgel, P., Naitza, S., Kappler, C., Ferrandon, D., Zachary, D., Swimmer, C., Kopczynski, C., Duyk, G., Reichhart, J. M. and Hoffmann, J. A.** (2001). *Drosophila* immune deficiency (IMD) is a death domain protein that activates antibacterial defense and can promote apoptosis. *Dev. Cell* **1**, 503-514.

**Gottfried, Y., Rotem, A., Lotan, R., Steller, H. and Larisch, S.** (2004). The mitochondrial ARTS protein promotes apoptosis through targeting XIAP. *EMBO J.* **23**, 1627-1635.

**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.

**Grether, M. E., Abrams, J. M., Agapite, J., White, K. and Steller, H.** (1995). The *head involution defective* gene of *Drosophila melanogaster* functions in programmed cell death. *Genes Dev.* **9**, 1694-1708.

**Gross, A., McDonnell, J. M. and Korsmeyer, S. J.** (1999). Bcl-2 family members and the mitochondria in apoptosis. *Genes Dev*. **13**, 1899-1911.

**Hakem, R., Hakem, A., Duncan, G. S., Henderson, J. T., Woo, M., Soengas, M. S., Elia, A., de la Pompa, J. L., Kagi, D., Khoo, W. et al.** (1998). Differential requirement for caspase 9 in apoptotic pathways in vivo. *Cell* **94**, 339-352.

**Harlin, H., Reffey, S. B., Duckett, C. S., Lindsten, T. and Thompson, C. B.** (2001). Characterization of XIAP-deficient mice. *Mol. Cell Biol.* **21**, 3604-3608.

**Harvey, N. L., Daish, T., Mills, K., Dorstyn, L., Quinn, L. M., Read, S. H., Richardson, H. and Kumar, S.** (2001). Characterization of the *Drosophila* caspase, damm. *J. Biol. Chem.* **276**, 25342-25350.

**Hausmann, G., O'Reilly, L. A., van Driel, R., Beaumont, J. G., Strasser, A., Adams, J. M. and Huang, D. C.** (2000). Pro-apoptotic apoptosis protease-activating factor 1 (Apaf-1) has a cytoplasmic localization distinct from Bcl-2 or Bcl-x(L). *J. Cell Biol.* **149**, 623-634.

**Hawkins, C. J., Yoo, S. J., Peterson, E. P., Wang, S. L., Vernooy, S. Y. and Hay, B. A.** (2000). The *Drosophila* caspase DRONC cleaves following glutamate or aspartate and is regulated by DIAP1, HID, and GRIM. *J. Biol. Chem.* **275**, 27084-27093.

**Hay, B. A.** (2000). Understanding IAP function and regulation: a view from *Drosophila*. *Cell Death. Differ.* **7**, 1045-1056.

**Hay, B. A., Wassarman, D. A. and Rubin, G. M.** (1995). *Drosophila* homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. *Cell* **83**, 1253-1262.

**Hegde, R., Srinivasula, S. M., Zhang, Z., Wassell, R., Mukattash, R., Cilenti, L., DuBois, G., Lazebnik, Y., Zervos, A. S., Fernandes-Alnemri, T. et al.** (2002). Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction. *J. Biol. Chem.* **277**, 432-438.

**Hengartner, M. O. and Horvitz, H. R.** (1994). *C. elegans* cell survival gene ced-9 encodes a functional homolog of the mammalian proto-oncogene bcl-2. *Cell* **76**, 665-676.

**Holley, C. L., Olson, M. R., Colon-Ramos, D. A. and Kornbluth, S.** (2002). Reaper eliminates IAP proteins through stimulated IAP degradation and generalized translational inhibition. *Nat. Cell Biol.* **4**, 439-444.

**Hu, Y., Benedict, M. A., Ding, L. and Nunez, G.** (1999). Role of cytochrome *c* and dATP/ATP hydrolysis in Apaf-1-mediated caspase-9 activation and apoptosis. *EMBO J.* **18**, 3586-3595.

**Huh, J. R., Vernooy, S. Y., Yu, H., Yan, N., Shi, Y., Guo, M. and Hay, B. A.** (2004). Multiple apoptotic caspase cascades are required in nonapoptotic roles for *Drosophila* spermatid individualization. *PLoS Biol.* **2**, E15.

**Igaki, T. and Miura, M.** (2004). Role of Bcl-2 family members in invertebrates. *Biochim. Biophys. Acta* **1644**, 73-81.

**Igaki, T., Kanuka, H., Inohara, N., Sawamoto, K., Nunez, G., Okano, H. and Miura, M.** (2000). Drob-1, a *Drosophila* member of the Bcl-2/CED-9 family that promotes cell death. *Proc. Natl. Acad. Sci. USA* **97**, 662-667.

**Janiak, F., Leber, B. and Andrews, D. W.** (1994). Assembly of Bcl-2 into microsomal and outer mitochondrial membranes. *J. Biol. Chem.* **269**, 9842-9849.

**Jiang, C. A., Lamblin, A. F. J., Steller, H. and Thummel, C. S.** (2000). A steroid-triggered transcriptional hierarchy controls salivary gland cell death during *Drosophila* metamorphosis. *Mol. Cell* **5**, 445-455.

**Jones, G., Jones, D., Zhou, L., Steller, H. and Chu, Y.** (2000). Deterin, a new inhibitor of apoptosis from *Drosophila melanogaster*. *J. Biol. Chem.* **275**, 22157-22165.

**Kanuka, H., Sawamoto, K., Inohara, N., Matsuno, K., Okano, H. and Miura, M.** (1999). Control of the cell death pathway by Dapaf-1, a *Drosophila.* Apaf-1/CED-4-related caspase activator. *Mol. Cell* **4**, 757-769.

**Kluck, R. M., Bossy-Wetzel, E., Green, D. R. and Newmeyer, D. D.** (1997). The release of Cytochrome C from mitochondria: a primary site for bcl-2 regulation of apoptosis. *Science* **275**, 1132-1136.

**Korsmeyer, S. J., Wei, M. C., Saito, M., Weiler, S., Oh, K. J. and Schlesinger, P. H.** (2000). Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. *Cell Death Differ.* **7**, 1166-1173.

**Kuida, K., Haydar, T., Kuan, C.-Y., Gu, Y., Taya, C., Karasuyama, H., Su, M. S.-S., Rakic, P. and Flavell, R.** (1998). Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell* **94**, 325-337.

**Kurada, P. and White, K.** (1998). Ras promotes cell survival in *Drosophila* by downregulating *hid* expression. *Cell* **95**, 319-329.

**Laundrie, B., Peterson, J. S., Baum, J. S., Chang, J. C., Fileppo, D., Thompson, S. R. and McCall, K.** (2003). Germline cell death is inhibited by P-element insertions disrupting the dcp-1/pita nested gene pair in *Drosophila*. *Genetics* **165**, 1881-1888.

**Leulier, F., Rodriguez, A., Khush, R. S., Abrams, J. M. and Lemaitre, B.** (2000). The *Drosophila* caspase Dredd is required to resist gram-negative bacterial infection. *EMBO Rep.* **1**, 353-358**.**

**Li, L., Thomas, R. M., Suzuki, H., de Brabander, J. K., Wang, X. and Harran, P. G.** (2004). A small molecule Smac mimic potentiates TRAIL- and TNFalpha-mediated cell death. *Science* **305**, 1471-1474.

**Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S., Ahmad, M., Alnemri, E. and Wang, X.** (1997). Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* **91**, 479-489.

**Lisi, S., Mazzon, I. and White, K.** (2000). Diverse domains of THREAD/DIAP1 are required to inhibit apoptosis induced by REAPER and HID in *Drosophila*. *Genetics* **154**, 669-678.

**Liu, X., Kim, C. N., Yang, J., Jemmerson, R. and Wang, X.** (1996). Induction of apoptotic program in cell-free extracts: requirement for dATP and Cytochrome C. *Cell* **86**, 147-157.

**Mikhailov, V., Mikhailova, M., Degenhardt, K., Venkatachalam, M. A., White, E. and Saikumar, P.** (2003). Association of Bax and Bak homo-oligomers in mitochondria. Bax requirement for Bak reorganization and cytochrome *c* release. *J. Biol. Chem.* **278**, 5367-5376.

**Muro, I., Monser, K. and Clem, R. J.** (2004). Mechanism of Dronc activation in *Drosophila* cells. *J. Cell Sci.* **117**, 5035-5041.

**Nakano, K. and Vousden, K. H.** (2001). PUMA, a novel proapoptotic gene, is induced by p53. *Mol. Cell* **7**, 683-694.

**Nechushtan, A., Smith, C. L., Hsu, Y. T. and Youle, R. J.** (1999). Conformation of the Bax C-terminus regulates subcellular location and cell death. *EMBO J.* **18**, 2330-2341.

**Newmeyer, D. D., Bossy-Wetzel, E., Kluck, R. M., Wolf, B. B., Beere, H. M. and Green, D. R.** (2000). Bcl-xL does not inhibit the function of Apaf-1. *Cell Death Differ.* **7**, 402-407.

**Nordstrom, W., Chen, P., Steller, H. and Abrams, J. M.** (1996). Activation of the reaper gene during ectopic cell killing in *Drosophila*. *Dev. Biol.* **180**, 213-226.

**Oda, E., Ohki, R., Murasawa, H., Nemoto, J., Shibue, T., Yamashita, T., Tokino, T., Taniguchi, T. and Tanaka, N.** (2000). Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* **288**, 1053-1058.

**Potts, P. R., Singh, S., Knezek, M., Thompson, C. B. and Deshmukh, M.** (2003). Critical function of endogenous XIAP in regulating caspase activation during sympathetic neuronal apoptosis. *J. Cell Biol.* **163**, 789-799.

**Quinn, L. M., Dorstyn, L., Mills, K., Colussi, P. A., Chen, P., Coombe, M., Abrams, J., Kumar, S. and Richardson, H.** (2000). An essential role for the caspase dronc in developmentally programmed cell death in *Drosophila*. *J. Biol. Chem.* **275**, 40416-40424.

**Quinn, L., Coombe, M., Mills, K., Daish, T., Colussi, P., Kumar, S. and Richardson, H.** (2003). Buffy, a *Drosophila* Bcl-2 protein, has anti-apoptotic and cell cycle inhibitory functions. *EMBO J.* **22**, 3568-3579.

**Rodriguez, A., Oliver, H., Zou, H., Chen, P., Wang, X. and Abrams, J.** (1999). Dark, a *Drosophila* homolog of Apaf-1/ced-4, functions in an evolutionarily conserved death pathway. *Nat. Cell Biol.* **1**, 272-279.

**Rodriguez, A., Chen, P., Oliver, H. and Abrams, J.** (2002). Unrestrained caspase-dependent cell death caused by loss of *Diap1* function requires the *Drosophila* Apaf-1 homolog, Dark. *EMBO J.* **21**, 2189-2197.

**Rodriguez, J. and Lazebnik, Y.** (1999). Caspase-9 and APAF-1 form an active holoenzyme. *Genes Dev.* **13**, 3179-3184.

**Ryoo, H. D., 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.

**Saleh, A., Srinivasula, S. M., Acharya, S., Fishel, R. and Alnemri, E. S.** (1999). Cytochrome *c* and dATP-mediated oligomerization of Apaf-1 is a prerequisite for procaspase-9 activation. *J. Biol. Chem.* **274**, 17941-17945.

**Salvesen, G. S. and Duckett, C. S.** (2002). IAP proteins: blocking the road to death's door. *Nat. Rev. Mol. Cell Biol.* **3**, 401-410.

**Seshagiri, S. and Miller, L. K.** (1997). *Caenorhabditis elegans* CED-4 stimulates CED-3 processing and CED-3-induced apoptosis. *Curr. Biol.* **7**, 455-460.

**Song, Z., McCall, K. and Steller, H.** (1997). DCP-1, a *Drosophila* cell death protease essential for development. *Science* **275**, 536-540.

**Spector, M. S., Desnoyers, S., Hoeppner, D. J. and Hengartner, M. O.** (1997). Interaction between the *C. elegans* cell-death regulators CED-9 and CED-4. *Nature* **385**, 653-656.

**Srinivasula, S., Ahmad, M., Fernandes-Alnemri, T. and Alnemri, E.** (1998). Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol. Cell* **1**, 949-957.

**Stennicke, H. R., Deveraux, Q. L., Humke, E. W., Reed, J. C., Dixit, V. M. and Salvesen, G. S.** (1999). Caspase-9 can be activated without proteolytic processing. *J. Biol. Chem.* **274**, 8359-8362.

**Stoven, S., Ando, I., Kadalayil, L., Engstrom, Y. and Hultmark, D.** (2000). Activation of the *Drosophila* NF-kappaB factor Relish by rapid endoproteolytic cleavage. *EMBO Rep.* **1**, 347-352.

**Suzuki, Y., Imai, Y., Nakayama, H., Takahashi, K., Takio, K. and Takahashi, R.** (2001). A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. *Mol. Cell* **8**, 613-621.

**Tenev, T., Zachariou, A., Wilson, R., Ditzel, M. and Meier, P.** (2004). IAPs are functionally non-equivalent and regulate effector caspases through distinct mechanisms. *Nat. Cell Biol.* **7**, 70-77.

**Thress, K., Evans, E. K. and Kornbluth, S.** (1999). Reaper-induced dissociation of a Scythe-sequestered Cytochrome C-releasing activity. *EMBO J.* **18**, 5486-5493.

**Varkey, J., Chen, P., Jemmerson, R. and Abrams, J. M.** (1999). Altered

Cytochrome C display precedes apoptotic cell death in *Drosophila*. *J. Cell Biol.* **144**, 701-710.

**Verhagen, A., Ekert, P., Pakusch, M., Silke, J., Connolly, L., Reid, G., Moritz, R., Simpson, R. and Vaux, D.** (2000). Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* **102**, 43-53.

**Vernooy, S. Y., Chow, V., Su, J., Verbrugghe, K., Yang, J., Cole, S., Olson, M. R. and Hay, B. A.** (2002). *Drosophila* Bruce can potently suppress Rpr- and Grim-dependent but not Hid-dependent cell death. *Curr. Biol.* **12**, 1164-1168.

**Vucic, D., Kaiser, W. J., Harvey, A. J. and Miller, L. K.** (1997). Inhibition of Reaper-induced apoptosis by interaction with inhibitor of apoptosis proteins (IAPs). *Proc. Natl. Acad. Sci. USA* **94**, 10183-10188.

**Vucic, D., Kaiser, W. J. and Miller, L. K.** (1998). Inhibitor of apoptosis proteins physically interact with and block apoptosis induced by *Drosophila* proteins HID and GRIM. *Mol. Cell Biol.* **18**, 3300-3309.

**Wei, M. C., Lindsten, T., Mootha, V. K., Weiler, S., Gross, A., Ashiya, M., Thompson, C. B. and Korsmeyer, S. J.** (2000). tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c. *Genes Dev.* **14**, 2060-2071.

**White, K., Grether, M. E., Abrams, J. M., Young, L., Farrell, K. and Steller, H.** (1994). Genetic control of programmed cell death in *Drosophila*. *Science* **264**, 677-683.

**Wing, J. P., Karres, J. S., Ogdahl, J. L., Zhou, L., Schwartz, L. M. and Nambu, J. R.** (2002). *Drosophila* sickle is a novel grim-reaper cell death activator. *Curr. Biol.* **12**, 131-135.

**Wolter, K. G., Hsu, Y. T., Smith, C. L., Nechushtan, A., Xi, X. G. and Youle, R. J.** (1997). Movement of Bax from the cytosol to mitochondria during apoptosis. *J. Cell Biol.* **139**, 1281-1292.

**Wu, D., Wallen, H. D., Inohara, N. and Nunez, G.** (1997). Interaction and regulation of the *Caenorhabditis elegans* death protease CED-3 by CED-4 and CED-9. *J. Biol. Chem.* **272**, 21449-21454.

**Xu, D., Li, Y., Arcano, M., Lackey, M. and Bergmann, A.** (2005). The CARD-carrying caspase Dronc is essential for most, but not all, developmental cell death in *Drosophila*. *Development* **132**, 2125-2134.

**Yan, N., Gu, L., Kokel, D., Chai, J., Li, W., Han, A., Chen, L., Xue, D. and Shi, Y.** (2004). Structural, biochemical, and functional analyses of CED-9 recognition by the proapoptotic proteins EGL-1 and CED-4. *Mol. Cell* **15**, 999-1006.

**Yang, J., Liu, X, Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T-I., Jones, D. P. and Wang, X.** (1997). Prevention of apoptosis by Bcl-2: release of cytochrome *c* from mitochondria blocked. *Science* **275**, 1129-1132.

**Yokokura, T., Dresnek, D., Huseinovic, N., Lisi, S., Abdelwahid, E., Bangs, P. and White, K.** (2004). Dissection of DIAP1 functional domains via a mutant replacement strategy. *J. Biol. Chem.* **279**, 52603-52612.

**Yoo, S. J., Huh, J. R., Muro, I., Yu, H., Wang, L., Wang, S. L., Feldman, R. M., Clem, R. J., Muller, H. A. and Hay, B. A.** (2002). Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms. *Nat. Cell Biol.* **4**, 416-424.

**Yuan, J., Shaham, S., Ellis, H. M. and Horvitz, H. R.** (1993). The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1b-converting enzyme. *Cell* **75**, 641-652.

**Zhang, H., Huang, Q., Ke, N., Matsuyama, S., Hammock, B., Godzik, A. and Reed, J. C.** (2000). *Drosophila* pro-apoptotic Bcl-2/Bax homologue reveals evolutionary conservation of cell death mechanisms. *J. Biol. Chem.* **275**, 27303-27306.

**Zhou, L., Song, Z., Tittel, J. and Steller, H.** (1999). HAC-1, a *Drosophila* homolog of APAF-1 and CED-4, functions in developmental and radiation-induced apoptosis. *Mol. Cell* **4**, 745-755.

**Zimmermann, K. C., Ricci, J. E., Droin, N. M. and Green, D. R.** (2002). The role of ARK in stress-induced apoptosis in *Drosophila* cells. *J. Cell Biol.* **156**, 1077-1087.

**Zou, H., Henzel, W., Liu, X., Lutschg, A. and Wang, X.** (1997). Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of Caspase-3. *Cell* **90**, 405-413.

**Zou, H., Li, Y., Liu, X. and Wang, X.** (1999). An APAF-1.cytochrome *c* multimeric complex is a functional apoptosome that activates procaspase-9. *J. Biol. Chem.* **274**, 11549-11556.

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