Cell Death and Differentiation (2008) 15, 1132–1138

www.nature.com/cdd

Review

Regulation of apoptosis in *Drosophila*

H Steller*,[1]

Insects have made major contributions to understanding the regulation of cell death, dating back to the pioneering work of Lockshin and Williams on death of muscle cells during postembryonic development of *Manduca*. A physically smaller cousin of moths, the fruit fly *Drosophila melanogaster,* offers unique advantages for studying the regulation of cell death in response to different apoptotic stimuli *in situ.* Different signaling pathways converge in *Drosophila* to activate a common death program through transcriptional activation of *reaper, hid* and *grim.* Reaper-family proteins induce apoptosis by binding to and antagonizing inhibitor of apoptosis proteins (IAPs), which in turn inhibit caspases. This switch from life to death relies extensively on targeted degradation of cell death proteins by the ubiquitin–proteasome pathway. *Drosophila* IAP-1 (Diap1) functions as an E3-ubiquitin ligase to protect cells from unwanted death by promoting the degradation of the initiator caspase Dronc. However, in response to apoptotic signals, Reaper-family proteins are produced, which promote the auto-ubiquitination and degradation of Diap1, thereby removing the 'brakes on death' in cells that are doomed to die. More recently, several other ubiquitin pathway proteins were found to play important roles for caspase regulation, indicating that the control of cell survival and death relies extensively on targeted degradation by the ubiquitin–proteasome pathway.
Cell Death and Differentiation (2008) **15,** 1132–1138; doi:10.1038/cdd.2008.50; published online 25 April 2008

Drosophila offers unique opportunities to study the regulation of cell death in response to a wide range of different stimuli within the context of an intact organism, and the role that cell death plays for normal development, tissue homeostasis and in a variety of disease models. Similar to vertebrates, the regulation of apoptosis in *Drosophila* is highly plastic and involves a wide variety of stimuli originating from both within a cell, as well as from its environment.[1,2] These stimuli include many different developmental signals, as well as various forms of cellular stress or injury, such as DNA damage, unfolded proteins, ER-stress, reactive oxygen species and defects in cell specification or cell differentiation. Significantly, cell death can be studied in *Drosophila* at the level of individual cell types using powerful genetic and molecular biology techniques. This review focuses primarily on the role and regulation of ubiquitin-pathway proteins in controlling the activity of caspases. One general theme that emerges is that cells rely extensively on caspase inhibition to prevent unwanted death, and that a number of different E3-ubiquitin ligases are employed to degrade these inhibitors when cells need to activate cell death.

Brakes on Death: Caspase Inhibition by Inhibitor of Apoptosis Proteins

Historically most efforts for understanding the induction of apoptosis have focused on factors promoting the conversion of procaspase to the active enzyme.[3,4] Not surprisingly, *Drosophila* contains all canonical apoptosome proteins, including orthologs of Apaf-1, caspase-9 and cytochrome *c*, and appears to use them in a manner similar to what has been described for mammalian cells to activate downstream effector caspases.[5,6] However, an equally important layer of cell death control involves negative regulation of caspases.[7] Procaspases are widely expressed in living cells and have low but significant protease activity. Despite this potentially dangerous cargo, many cells can avoid the activation of a caspase cascade and death for many years (or even our lifetime, as in the case of many neurons). Therefore, efficient mechanisms must exist that prevent unwanted caspase activation. One important family of caspase inhibitors are the inhibitor of apoptosis proteins (IAPs), which can bind to and inhibit caspases.[8,9] IAPs were originally discovered in insect viruses,[10] but a family of related proteins were subsequently described in both insect and mammalian genomes.[8,9] IAPs are characterized by the presence of at least one BIR (baculovirus inhibitory repeat) domain, which can directly bind to and inhibit caspases.[8,9] In *Drosophila*, Diap1 is absolutely essential to prevent inappropriate caspase activation and ubiquitous apoptosis.[11–14] Furthermore, Diap1 functions as an E3-ubiquitin ligase, both to target the caspase-9 ortholog Dronc for degradation in living cells, and to promote self-conjugation and Diap1-degradation under apoptotic

[1]Howard Hughes Medical Institute, Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
*Correspondence: H Steller, Steller laboratory, Rockefeller University, Strang Laboratory of Cancer Research, 1230 York Avenue, New York, NY 10021, USA.
Tel: 212 327 7075; Fax: 212 327 7076;
E-mail: steller@rockefeller.edu
Keywords: apoptosis; ubiquitin; IAP; proteasome; reaper
Abbreviations: Apaf-1, apoptosis-activating factor-1; Diap1, *Drosophila* IAP-1; IAP, inhibitor of apoptosis protein; Hid, head involution defective gene; XIAP, X-linked inhibitor of apoptosis protein
Received 20.2.08; revised 17.3.08; accepted 17.3.08; Edited by G Melino; published online 25.4.08

conditions.[13,15,16] In the absence of Diap1-RING function, Diap1 is stabilized and its protein levels are increased, but the net outcome for most cells is still excessive cell death due to highly elevated Dronc levels.[13]

The best studied mammalian IAP is the X-linked inhibitor of apoptosis (XIAP) protein, which is considered the most potent caspase inhibitor *in vitro*.[17] Mammalian XIAP shares several properties with *Drosophila* Diap1, including the ability to bind to caspases, to Reaper-family proteins, and the ability to undergo auto-ubiquitination and proteasome-mediated degradation in response to apoptotic stimuli.[18] As IAPs are frequently overexpressed in human tumors and promote cancer cell survival, they have become major targets for developing new cancer therapeutics.[19] Although most of the attention has focused on the BIR domains, XIAP and many other mammalian IAPs also contain a RING motif and functions as E3-ubiquitin ligases.[18] However, as XIAP-deficient mice appear overall normal and no major cell death phenotypes have yet been reported, the physiological role of this gene *in vivo* remains to be defined.[20] Likewise, targeted gene disruption of cIAP1 and cIAP2 has not revealed any significant apoptotic phenotypes.[21,22] Although these results could be interpreted to indicate that IAPs do not play a major role for caspase regulation in mammals, a more likely alternative is that their physiological role is masked by functional redundancy. If relatively short-lived organisms such as fruit flies protect themselves against unwanted death using multiple caspase inhibitors (see below; Figure 1),[6,8–15,23–35] it would come as a great surprise if mammals would employ fewer safeguards to control caspase activity.

Relieving the Brakes: Induction of Apoptosis by Reaper-Family Proteins

Molecular genetic studies of apoptosis in *Drosophila* originally revealed the central importance of natural IAP antagonists, namely *reaper, grim* and *head involution defective (hid)* (reviewed in Kornbluth and White[7]). In the absence of all three genes, apoptosis is virtually completely blocked.[23] On the other hand, ectopic expression of these genes can lead to potent induction of apoptosis.[36,37] Although the proteins encoded by these genes share overall very little similarity, they all contain a short N-terminal peptide motif, termed IBM (IAP-Binding-Motif), which is required for IAP-binding and cell killing.[38] The *reaper* gene, and to some extent *grim*, *hid* and *sickle*, are transcriptionally activated in response to many different proapoptotic signals, including steroid hormones, a variety of developmental signals, radiation and various forms of cellular stress or injury.[24–26,36,39–41] These genes share a very large, complex regulatory region containing numerous enhancer (and silencer) elements that are the target for many different transcriptional regulators. Therefore, one major mechanism by which different signaling pathways converge in *Drosophila* is through transcriptional activation of *reaper, hid* and *grim.* In addition, the proapoptotic activity of the Hid protein is inhibited upon phosphorylation by MAP-kinase, and

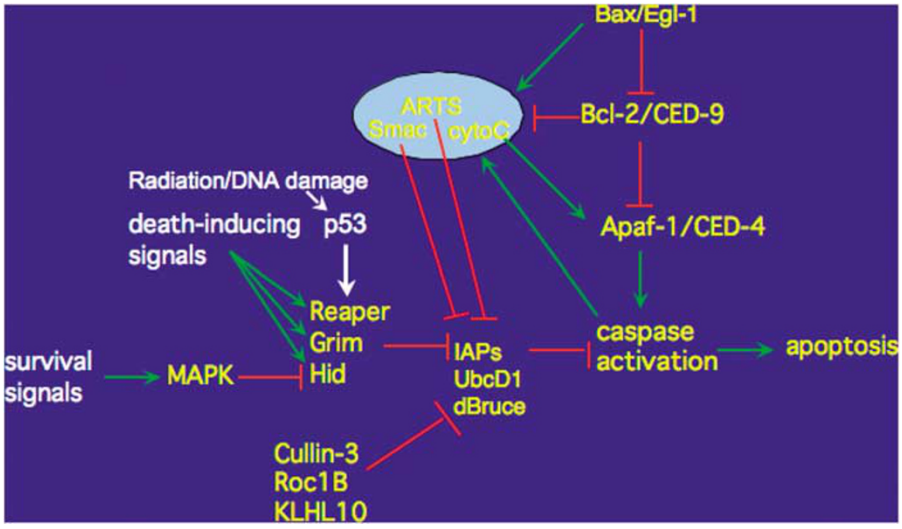

Figure 1 'Gas and Brake' model of apoptosis regulation. This model serves to illustrate some general concepts in the regulation of caspases by both activators ('gas') and inhibitors ('brakes') that broadly apply to caspase regulation in diverse organisms. However, it is neither intended to be comprehensive nor universal, as the use of and requirement for specific cell death proteins can vary significantly among different cell types. Genetic studies in *Drosophila* originally suggested that caspases are simultaneously controlled by opposing regulatory pathways.[35] Adapter proteins, such as Apaf-1/CED-4, promote caspase activation, whereas negative regulators, notably IAPs, inhibit caspases. IAPs were originally discovered in insect systems,[10] but a family of related proteins wa subsequently described in mammals.[8,9] In *Drosophila*, Diap1 is absolutely essential to prevent inappropriate caspase activation and ubiquitous apoptosis.[11–14] On the other hand, in order for cells to undergo apoptosis, inhibition of Diap1 by Reaper/Hid/Grim is essential, and virtually no apoptosis occurs in the absence of these genes.[23] Inhibition of Diap1 by Reaper-family proteins involves the direct stimulation of auto-ubiquitination and degradation of Diap1 protein.[15] *reaper, hid* and *grim* are transcriptionally induced in response to many apoptotic stimuli, and the proapoptotic activity of the Hid protein is blocked by phosphorylation by MAP-kinase.[24–28] The activity of both the 'gas' and 'brake' pathways is coordinated, at least in part, through the release of mitochondrial proapoptotic factors.[6] Active caspases have been shown to promote the release of proapoptotic mitochondrial factors in mammalian cells, thereby providing a feedback amplification loop. For example, in mammals the release of cytochrome *c* from mitochondria activates Apaf-1, whereas Smac/Diablo is thought to inhibit IAPs. In addition, the proapoptotic mitochondrial ARTS protein can also inhibit IAP function.[29–31] Caspase activation in *Drosophila* spermatids strictly requires cytochrome *c*[32,34] and also a cullin-3-based ubiquitin ligase complex that appears to target the giant IAP-like ubiquitin-conjugating enzyme dBruce.[33] Death receptors can promote caspase activation through a distinct signaling pathway. Stimulatory (→) and inhibitory interactions (—|) are indicated by green arrows and red T-bars, respectively

Hid is the target for survival signaling by the EGF-receptor/Ras-pathway in *Drosophila*.[27,28] Finally, the more recently discovered Jafrac2 protein also contains an IBM and can inhibit IAPs upon its release from the endoplasmic reticulum into the cytosol upon apoptotic stimulation.[42] Therefore, it appears that Reaper-family proteins in *Drosophila* serve as links to connect many different signaling pathways with the core cell death program.[7]

In mammals, IBM-domain proteins such as Smac/DIABLO and Omi/HtrA2 have been identified as well.[8,9,38] Like in *Drosophila*, these proteins use their N-terminal IBM for IAP-binding and inhibition. However, unlike in *Drosophila*, all known mammalian IBM proteins are ubiquitously expressed, reside within mitochondria in living cells and are released into the cytosol at the onset of apoptosis. Furthermore, targeted gene disruption of either Smac/DIABLO, Omi/HtrA2 or both together in double-mutant mice did not cause increased resistance toward apoptosis.[43–45] Therefore, a physiological role of these proteins for regulating IAPs remains to be established, and it is likely that additional IAP-regulators remain to be discovered in mammals. One example is ARTS, which also localizes to mitochondria in living cells but represents a novel type of IAP-regulator.[29] ARTS binds to mammalian IAPs, such as XIAP, inhibits their anti-apoptotic activity and stimulates auto-ubiquitination of XIAP.[30] However, ARTS contains no detectable IBM motif and appears to use a distinct mechanism for IAP-binding and inhibition. Significantly, expression of ARTS is frequently lost in human leukemia and lymphoma, indicating that ARTS functions as a tumor suppressor.[46] Finally, mice deficient for the *Sept4*, which encodes ARTS, display elevated XIAP protein in certain tissues, have defects in the caspase-mediated elimination of bulk cytoplasm during spermiogenesis, and are predisposed to malignancies, in particular lymphoma.[31] These observations support a physiological role of ARTS in caspase regulation and tumor suppression. In evaluating these phenotypes, one should keep in mind that mammalian IAP antagonists are very likely to have redundant functions. Reaper-family proteins act in a partially redundant manner in *Drosophila*, and single-gene mutants have only rather mild cell death phenotypes.[37,47,48] Therefore, it is to be expected that a similar, if not greater redundancy exists for mammalian IAP-antagonists, and that appropriate double-mutant combinations are likely to have more severe cell death phenotypes.

Reaper-family proteins use a structurally conserved IBM to bind BIR domains and thereby prevent them from caspase inhibition.[7,38] In addition, Reaper can stimulate the intrinsic ubiquitin-ligase activity of IAPs to promote IAP auto-ubiquitination and degradation.[15] Significantly, mammalian IAPs are also undergoing self-conjugation and ubiquitin-mediated protein degradation in response to proapoptotic stimuli, but the precise mechanism by which this occurs is not known.[18] Interestingly, *Drosophila* Reaper, Hid and Grim can effectively bind to vertebrate IAPs, promote XIAP self-conjugation both *in vitro* and *in vivo* and potently induce apoptosis in certain mammalian cell types.[49–51] Finally, small molecule IAP-antagonists based on the IBM–IAP interaction are currently developed as anticancer therapeutics.[19,52,53] However, Reaper, Hid and Grim utilize additional domains besides the IBM for potent cell killing.[54,55] Therefore, insight into the precise mechanism by which Reaper-family proteins inactivate IAPs may lead to additional opportunities to target IAPs for therapeutic purposes.

Death by Degradation: Coordinate Regulation of Caspase and Intracellular Proteolysis by Ubiqutin-Proteasome Proteins

Diap1 is not the only ubiquitin pathway protein playing an important role in caspase regulation. The E1 enzyme Uba-1, the de-ubiquitinating enzyme fat facets *(faf),* the E2-ligases Morgue and dBruce, SkpA (the Skp1 component of *Drosophila* SCF ubiquitin ligases) and a cullin-3-based E3-ubiquitin ligase complex have all been implicated in apoptosis and/or caspase regulation in *Drosophila*.[32,33,56–58] This indicates a remarkable complexity in the use of the ubquitin-proteasome system for caspase regulation.

Proteasome function is often required together with caspase activity for efficient cell death, and proteasome activity mediates cellular atrophy both during normal development and in various diseases.[59,60] The coordinate activity of caspases and the ubiquitin-proteasome system is also employed for the terminal differentiation of spermatids in *Drosophila*. Developing spermatids eliminate the majority of their cytoplasm and organelles as they become highly elongated and severely reduce their overall cell volume. This phenomenon can be viewed as a form of natural, 'programmed atrophy' (i.e. marked loss of cellular proteins in the absence of cell death). Significantly, elimination of unwanted proteins and organelles in this system requires the activities of both canonical cell death proteins, including apoptotic effector caspases, as well as proteasome activity.[32,61] Although caspase activation in this system does not lead to death of the entire cell, spermatid differentiation resembles apoptosis in the sense that many cellular structures are degraded. Interestingly, caspase activation here strictly depends on a testis-specific cytochrome *c* gene and requires the activity of cullin-3-based E3-ubiquitin ligase complex.[33,34] As this system is both genetically and anatomically highly accessible, it promises to offer a powerful model to study how the potentially lethal activity of apoptotic effector caspases is restricted in time and space to only remove parts of a cell, and how localized intracellular degradation of proteins and organelles is achieved to dramatically alter the cyto-architecture and size of cells during normal development. A similar nonlethal use of apoptotic effector caspases is also seen during the differentiation of certain neurons, and in the pruning of neurites.[62–65]

Stimulation of Tissue Regeneration by Apoptotic Cells

Inactivation of *diap1* not only leads to caspase activation and cell death, but can also promote tissue regeneration. It has long been known that developing *Drosophila* tissues can compensate for the massive loss of cells in response to injury or stress, a phenomenon termed 'compensatory proliferation.'[66] A series of studies has shown that apoptotic cells actively promote tissue regeneration by secreting mitogens that stimulate proliferation of neighboring progenitor cells.[13,67,68] Doomed *Drosophila* imaginal disc cells produce

proteins with known mitogenic and morphogenic activities, such as Wnts and BMPs.[13,68] Furthermore, Wnt signaling is necessary and sufficient for cell proliferation in this system.[13] Significantly, the Wnt pathway has been implicated in regulating self-renewal of stem cells and tissue regeneration, both in vertebrates and insects.[69–72] Therefore, it is possible that a similar mechanism operates in vertebrates. Also, mitogenic signaling by doomed cells may contribute to the formation of neoplastic tumors. If doomed cells are prevented from executing apoptosis and are not rapidly cleared ('undead cells'), they can continue to release excessive amounts of mitogens and stimulate tissue overgrowth.[13,68] Many cancer cells have impaired caspase activity and may have properties of 'undead' cells,[73] and the abnormal activation of the Wnt pathway can contribute to oncogenesis.[74] Therefore, one so far overlooked aspect of how these pathways may contribute to oncogenesis is through compensatory proliferation.

The initiator caspase Dronc plays an important role in the induction of compensatory proliferation.[75] Dronc mutants, but not Drice mutants, suppress compensatory proliferation induced by γ-irradiation or by expression of apoptotic proteins. In addition, p53 is required for the induction of compensatory proliferation and is transcriptionally activated in 'undead cells' by a mechanism that requires Dronc function.[76] Furthermore, a recent report implicates Notch and Jak/STAT signaling in compensatory proliferation as well.[57] Finally, Fan and Bergmann have recently shown that a second mechanism of apoptosis-induced operates during retinal differentiation in *Drosophila*.[77] This mechanism involves the caspase-mediated activation of Hedgehog (Hh) signaling for compensatory proliferation. These studies reveal a remarkable degree of communication between apoptotic cells and their environment. Because defects in apoptosis or phagocytosis in mouse mutants can lead to hyperplasia and increased cell proliferation, it will be interesting to investigate whether this is due to excessive mitogenic signaling of 'undead' cells (for example, Cecconi *et al.*[78] and Li *et al.*[79]).

Conclusions

In hindsight, it is not difficult to see why degradation of key cell death regulators is highly suited for the commitment of cells to an irreversible fate, such as death. By redirecting the targets of IAP-mediated ubiquitilation, from caspases to self, cell fate can rapidly and radically switch from life to death, in particular if coupled with the enzymatic amplification of a caspase cascade. However, what is surprising is how many ubiquitin pathway proteins appear to participate in this switch, the extent to which this system is controlled by feedback loops, and how it is integrated with other cellular pathways, such as the production of mitogens in injured cells to promote tissue regeneration. As our understanding of cell death regulation in *Drosophila* has progressed, many new questions have emerged and it is clear that our knowledge of cell death

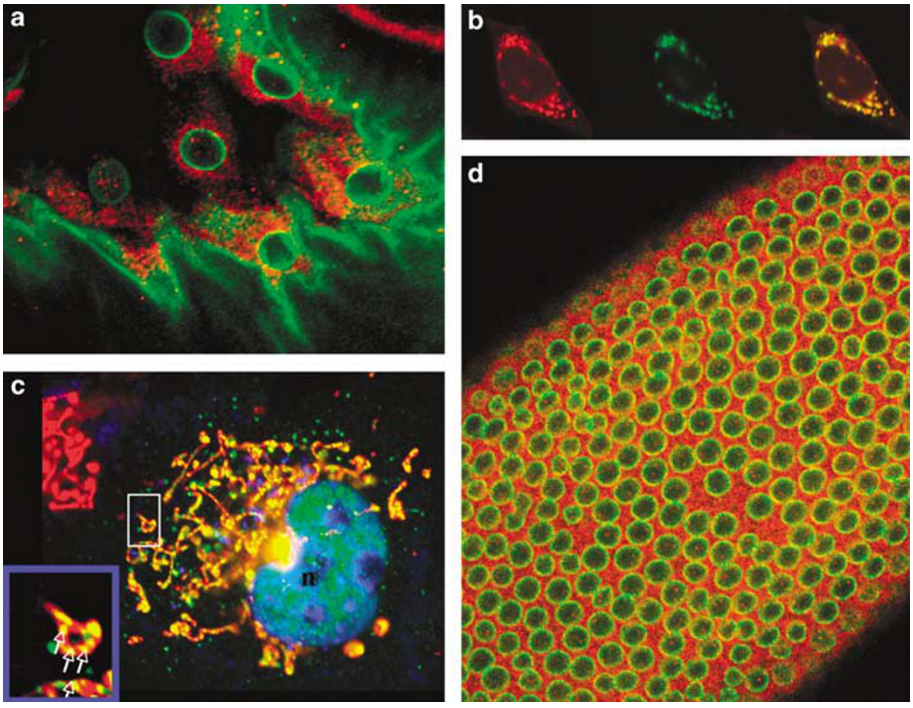

Figure 2 Many apoptotic proteins localize to mitochondria. The IAP-antagonist Hid localizes to the surface of mitochondria in *Drosophila* cells (**a**), and also upon expression in mammalian cultured cells (**b**).[49] Likewise, the mammalian IAP-antagonist ARTS localizes to mitochondria (**c**). Diap1, the target for cell killing by Reaper, Hid and Grim is ubiquitiously distributed in the cytoplasm of *Drosophila* embryos (**d**). (**a**) Cells of the larval epidermis in *Drosophila* stained with an antibody toward Hid (red staining); the nuclear membrane is labeled in green. (**b**) Hid localizes to mitochondria upon expression in mammalian cells. HeLa cells were transfected with an expression vector containing C-terminally HA-tagged Hid protein. Cells were labeled with CMXRos (Invitrogen, red staining) to label mitochondria, and with anti-HA antibody to visualize Hid-HA (green label). (**c**) COS-7 cells were transfected with AU5-ARTS as described in Gottfried *et al.*[30] ARTS expression (green) overlaps extensively with the mitochondrial marker CMXRos (red). The nucleus was visualized with Dap1 (blue). (**d**) Confocal micrograph of an early, preblastoderm *Drosophila* embryo stained with antibodies against Diap1 (red) and a nuclear membrane marker (green)

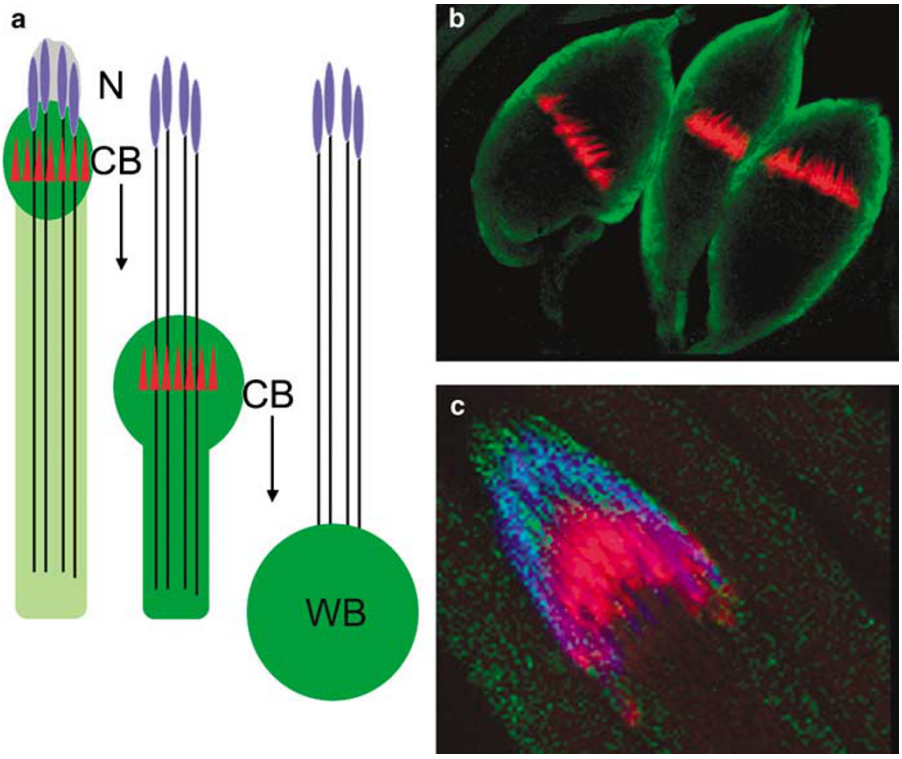

Figure 3 Apoptotic effector caspases are used to eliminate unwanted organelles and cytoplasm during sperm differentiation. (**a**) Schematic presentation of progressive stages during spermatid differentiation in *Drosophila*. During their terminal differentiation, spermatids eliminate the majority of their cytoplasm and organelles in a process termed 'individualization.' An actin-based 'individualization complex' (illustrated by red triangles) drives the expulsion of unwanted cellular material from a syncitium of 64 closely associated spermatids. The cellular waste is collected into 'bubble-like' structures termed 'cystic bulges' (CB), which grow in size as they collect increasingly more material during their travel away from the nuclei toward the tail. At the end of this journey, the CBs pinch off to form the 'waste bag' (WB), which is subsequently degraded. This process shares many features with apoptosis and requires canonical cell death proteins, including apoptotic effector caspases.[32] Interestingly, caspase activation in this system strictly requires a testis-specific cytochrome *c* gene.[34] As this system is highly accessible to a combination of anatomical, molecular and genetic analyses, it presents a powerful model to investigate how capases are activated, and how their potentially lethal is restricted in time and space to dramatically alter cell architecture. (**b**) Cystic bulges (CB) contain high levels of active caspase-3-like activity, as visualized with an antibody toward active caspase-3 (green label).[32] Actin filaments labeled with phalloidin (red staining) promote CB movement from the nuclear region to the tail of the developing spermatids. (**c**) Active caspase-3 staining (green) is initiated as cystic bulges form in the vicinity of the spermatid nuclei (Dapi, blue staining). Note the close proximity between the actin filaments (phalloidin, red staining), nuclei (blue) and active-caspase-3 staining (green)

pathways is still rudimentary. In particular, much remains to be learned about how specific cells are selected to undergo apoptosis *in situ*. This is an even greater enigma in vertebrates. One limitation stems from the technical difficulties to conduct cell death studies in the context of normal organismal development in vertebrates. Another challenge is how to deal with significant functional redundancy that is already seen in insect systems, and expected to pose even greater problems for genetic analyses in vertebrates. In an effort to reduce this complexity, most mammalian cell death study has been performed using cultured cells or simplified biochemical assays. However, as powerful as these approaches are to investigate the biochemical basis of apoptosis, they have obvious limitations for the discovery of new regulatory mechanisms that select cells for apoptosis *in situ.* Another common problem arises from the desire to develop 'universal' pathways for apoptosis in a given experimental system ('worms,' 'flies,' 'mouse'). This ignores that different cell types can show a strikingly different response to a given signal, and that even the requirement for 'core cell death proteins' (apoptosome proteins, caspases) can vary greatly among different cell types. Likewise, different caspases trigger distinct forms of compensatory proliferation in different cellular contexts during *Drosophila* development. This context-specificity of cell death signals is poorly understood at this point, but progress here will be extremely important to successfully manipulate apoptosis for therapeutic purposes. *Drosophila* offers a powerful model to advance our knowledge in this area, as regulation of cell death is considerably more plastic and complex than in *C. elegans,* yet it is accessible to systematic forward genetic screens. Many new concepts have already emerged from studying apoptosis in *Drosophila*, and it is likely that new insights will continue to provide an intellectual framework for a better understanding of mammalian apoptosis (Figures 2 and 3).

Acknowledgements. We apologize for the omission of many relevant publications that could not be included here due to space constraints. I thank S Larisch for her comments on this paper and for contributing Figure 2c, and M Bader, A Kelkar, and HR Ryoo for providing photographs. HS is an investigator with the Howard Hughes Medical Institute. Part of this study was supported by NIH Grant RO1GM60124 to HS.

1. Steller H. Mechanisms and genes of cellular suicide. *Science* 1995; **267**: 1445–1449.
2. Domingos PM, Steller H. Regulation of apoptosis during patterning and development. *Curr Opin Genet Dev* 2007; **17**: 294–299.
3. Hengartner MO. The biochemistry of apoptosis. *Nature* 2000; **407**: 685–687.
4. Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science* 1998; **281**: 1312–1316.
5. Salvesen GS, Abrams JM. Caspase activation – stepping on the gas or releasing the brakes? Lessons from humans and flies. *Oncogene* 2004; **23**: 2774–2784.
6. Wang X. The expanding role of mitochondria in apoptosis. *Genes Dev* 2001; **15**: 2922–2933.
7. Kornbluth S, White K. Apoptosis in *Drosophila:* neither fish nor fowl (nor man, nor worm). *J Cell Sci* 2005; **118**: 1779–1787.
8. Salvesen GS, Duckett CS. IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol* 2002; **3**: 401–410.
9. Vaux DL, Silke J. IAPs, RINGs and ubiquitylation. *Nat Rev Mol Cell Biol* 2005; **4**: 287–297.
10. Crook NE, Clem RJ, Miller LK. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. *J Virol* 1993; **67**: 2168–2174.
11. Goyal L, McCall K, Agapite J, Hartwieg E, Steller H. Induction of apoptosis by *Drosophila* reaper, hid and grim through inhibition of IAP function. *EMBO J* 2000; **19**: 589–597.
12. Lisi S, Mazzon I, White K. Diverse domains of THREAD/DIAP1 are required to inhibit apoptosis induced by REAPER and HID in *Drosophila*. *Genetics* 2000; **154**: 669–678.
13. Ryoo HD, Gorenc T, Steller H. Apoptotic cells can induce compensatory cell proliferation through the JNK and the wingless signaling pathways. *Dev Cell* 2004; **7**: 491–501.
14. Wang SL, Hawkins CJ, Yoo SJ, Muller HA, Hay BA. The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. *Cell* 1999; **98**: 453–463.
15. Ryoo HD, Bergmann A, Gonen H, Ciechanover A, Steller H. Regulation of *Drosophila* IAP1 degradation and apoptosis by *reaper* and *ubcD1*. *Nat Cell Biol* 2002; **4**: 432–438.
16. Wilson R, Goyal L, Ditzel M, Zachariou A, Baker DA, Agapite J *et al.* The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis. *Nat Cell Biol* 2002; **4**: 445–450.
17. Eckelman BP, Salvesen GS. The human anti-apoptotic proteins cIAP1 and cIAP2 bind but do not inhibit caspases. *J Biol Chem* 2006; **281**: 3254–3260.
18. Yang Y, Fang S, Jensen JP, Weissman AM, Ashwell JD. Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli. *Science* 2000; **288**: 874–877.
19. Hunter AM, Lacasse EC, Korneluk RG. The inhibitors of apoptosis (IAPs) as cancer targets. *Apoptosis* 2007; **12**: 1543–1568.
20. Harlin H, Reffey SB, Duckett CS, Lindsten T, Thompson CB. Characterization of XIAP-deficient mice. *Mol Cell Biol* 2001; **21**: 3604–3608.
21. Conte D, Holcik M, Lefebvre CA, Lacasse E, Picketts DJ, Wright KE *et al.* Inhibitor of apoptosis protein cIAP2 is essential for lipopolysaccharide-induced macrophage survival. *Mol Cell Biol* 2006; **26**: 699–708.
22. Conze DB, Albert L, Ferrick DA, Goeddel DV, Yeh WC, Mak T *et al.* Posttranscriptional downregulation of c-IAP2 by the ubiquitin protein ligase c-IAP1 *in vivo*. *Mol Cell Biol* 2005; **25**: 3348–3356.
23. White K, Grether ME, Abrams JM, Young L, Farrell K, Steller H. Genetic control of programmed cell death in *Drosophila*. *Science* 1994; **264**: 677–683.
24. Brodsky MH, Nordstrom W, Tsang G, Kwan E, Rubin GM, Abrams JM. *Drosophila* p53 binds a damage response element at the reaper locus. *Cell* 2000; **101**: 103–113.
25. Jiang C, Lamblin AF, Steller H, Thummel CS. A steroid-triggered transcriptional hierarchy controls salivary gland cell death during *Drosophila* metamorphosis. *Mol Cell* 2000; **5**: 445–455.
26. Lohmann I, McGinnis N, Bodmer M, McGinnis W. The *Drosophila* Hox gene deformed sculpts head morphology via direct regulation of the apoptosis activator reaper. *Cell* 2002; **110**: 457–466.
27. Bergmann A, Agapite J, McCall K, Steller H. The *Drosophila* gene hid is a direct molecular target of Ras-dependent survival signaling. *Cell* 1998; **95**: 331–341.
28. Bergmann A, Tugentman M, Shilo BZ, Steller H. Regulation of cell number by MAPK-dependent control of apoptosis: a mechanism for trophic survival signaling. *Dev Cell* 2002; **2**: 159–170.
29. Larisch S, Yi Y, Lotan R, Kerner H, Eimerl S, Tony Parks W *et al.* A novel mitochondrial septin-like protein, ARTS, mediates apoptosis dependent on its P-loop motif. *Nat Cell Biol* 2000; **2**: 915–921.
30. Gottfried Y, Zivony Y, Steller H, Larisch S. The mitochondrial ARTS protein promotes apoptosis through targeting XIAP. *EMBO J* 2004; **23**: 1627–1635.
31. Kissel H, Hunnicut G, Georgescu M, Larisch S, Steller H. The *sept4* septin locus is required for sperm terminal differentiation in mice. *Dev Cell* 2005; **8**: 353–364.
32. Arama E, Agapite J, Steller H. Caspase activity and a specific cytochrome *c* are required for sperm differentiation in *Drosophila*. *Dev Cell* 2003; **4**: 687–697.
33. Arama E, Bader M, Gabrielle ERieckhof GE, Steller H. A ubiquitin ligase complex regulates caspase activation during sperm differentiation in Drosophila. *PLoS Biol* 2007; **18**: 5–10.
34. Arama E, Bader M, Steller H. The two *Drosophila* cytochrome *c* proteins can function in both respiration and caspase Activation. *EMBO J* 2006; **25**: 232–243.
35. Zhou L, Song Z, Tittel J, Steller H. HAC-1, a *Drosophila* homolog of APAF-1 and CED-4 functions in developmental and radiation-induced apoptosis. *Mol Cell* 1999; **4**: 745–755.
36. Chen P, Nordstrom W, Gish B, Abrams JM. Grim, a novel cell death gene in *Drosophila*. *Genes Dev* 1996; **10**: 1773–1782.
37. Grether ME, Abrams JM, Agapite J, White K, Steller H. The head involution defective gene of *Drosophila melanogaster* functions in programmed cell death. *Genes Dev* 1995; **9**: 1694–1708.
38. Shi Y. A conserved tetrapeptide motif: potentiating apoptosis through IAP-binding. *Cell Death Differ* 2002; **9**: 93–95.
39. Christich A, Kauppila S, Chen P, Sogame N, Ho SI, Abrams JM. The damage-responsive *Drosophila* gene sickle encodes a novel IAP binding protein similar to but distinct from reaper, grim, and hid. *Curr Biol* 2002; **12**: 137–140.
40. Nordstrom W, Chen P, Steller H, Abrams JM. Activation of the reaper gene during ectopic cell killing in *Drosophila*. *Dev Biol* 1996; **180**: 213–226.
41. Robinow S, Draizen TA, Truman JW. Genes that induce apoptosis: transcriptional regulation in identified, doomed neurons of the *Drosophila* CNS. *Dev Biol* 1997; **190**: 206–213.
42. Tenev T, Zachariou A, Wilson R, Paul A, Meier P. Jafrac2 is an IAP antagonist that promotes cell death by liberating Dronc from DIAP1. *EMBO J* 2002; **21**: 5118–5129.
43. Jones JM, Datta P, Srinivasula SM, Ji W, Gupta S, Zhang Z *et al.* Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice. *Nature* 2003; **425**: 721–727.
44. Martins LM, Morrison A, Klupsch K, Fedele V, Moisoi N, Teismann P *et al.* Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. *Mol Cell Biol* 2004; **24**: 9848–9862.
45. Okada H, Suh WK, Jin J, Woo M, Du C, Elia A *et al.* Generation and characterization of Smac/DIABLO-deficient mice. *Mol Cell Biol* 2002; **22**: 3509–3517.
46. Elhasid R, Sahar D, Merling A, Zivony Y, Rotem A, Ben-Arush M *et al.* Mitochondrial pro-apoptotic ARTS protein is lost in the majority of acute lymphoblastic leukemia patients. *Oncogene* 2004; **23**: 9450.
47. Peterson C, Carney GE, Taylor BJ, White K. Reaper is required for neuroblast apoptosis during *Drosophila* development. *Development* 2002; **129**: 1467–1476.
48. Zhou L, Schnitzler A, Agapite J, Schwartz LM, Steller H, Nambu JR. Cooperative functions of the reaper and head involution defective genes in the programmed cell death of *Drosophila* central nervous system midline cells. *Proc Natl Acad Sci USA* 1997; **94**: 5131–5136.
49. Haining WN, Carboy-Newcomb C, Wei CL, Steller H. The proapoptotic function of *Drosophila* Hid is conserved in mammalian cells. *Proc Natl Acad Sci USA* 1999; **96**: 4936–4941.
50. McCarthy JV, Dixit VM. Apoptosis induced by *Drosophila* reaper and grim in a human system. Attenuation by inhibitor of apoptosis proteins (cIAPs). *J Biol Chem* 1998; **273**: 24009–24015.
51. Olson MR, Holley CL, Gan EC, Colón-Ramos DA, Kaplan B, Kornbluth SA. GH3-like domain in reaper is required for mitochondrial localization and induction of IAP degradation. *J Biol Chem* 2003; **278**: 44758–44768.
52. Varfolomeev E, Blankenship JW, Wayson SM, Fedorova AV, Kayagaki N, Garg P *et al.* IAP antagonists induce autoubiquitination of c-IAPs, NF-kB activation, and TNFa-dependent apoptosis. *Cell* 2007; **131**: 669–681.
53. Vince JE, Wong WW, Khan N, Feltham R, Chau D, Ahmed AU *et al.* IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. *Cell* 2007; **131**: 682–693.
54. Abdelwahid E, Yokokura T, Krieser RJ, Balasundaram S, Fowle WH, White K. Mitochondrial disruption in *Drosophila* apoptosis. *Dev Cell* 2007; **12**: 793–806.
55. Thomenius M, Kornbluth S. Multifunctional reaper: sixty-five amino acids of fury. *Cell Death Differ* 2006; **13**: 1305–1309.
56. Hays R, Wickline L, Cagan R. Morgue mediates apoptosis in the *Drosophila melanogaster* retina by promoting degradation of DIAP1. *Nat Cell Biol* 2002; **4**: 425–431.
57. Lee TV, Ding T, Chen Z, Rajendran V, Scherr H, Lackey M *et al.* The E1 ubiquitin-activating enzyme Uba1 in *Drosophila* controls apoptosis autonomously and tissue growth non-autonomously. *Development* 2008; **135**: 43–52.
58. Wing JP, Schreader BA, Yokokura T, Wang Y, Andrews PS, Huseinovic N *et al. Drosophila* Morgue is an F box/ubiquitin conjugase domain protein important for grim-reaper mediated apoptosis. *Nat Cell Biol* 2002; **4**: 451–456.
59. Grimm LM, Goldberg AL, Poirier GG, Schwartz LM, Osborne BA. Proteasomes play an essential role in thymocyte apoptosis. *EMBO J* 1996; **15**: 3835–3844.
60. Nury D, Doucet C, Coux O. Roles and potential therapeutic targets of the ubiquitin proteasome system in muscle wasting. *BMC Biochem* 2007; **8** (Suppl 1): S7.
61. Zhong L, Belote JM. The testis-specific proteasome subunit Prosalpha6T of *D melanogaster* is required for individualization and nuclear maturation during spermatogenesis. *Development* 2007; **134**: 3517–3525.
62. Awasaki T, Tatsumi R, Takahashi K, Arai K, Nakanishi Y, Ueda R *et al.* Essential role of the apoptotic cell engulfment genes draper and ced-6 in programmed axon pruning during *Drosophila* metamorphosis. *Neuron* 2006; **50**: 855–867.
63. Mistry H, Wilson BA, Roberts IJ, O'Kane CJ, Skeath JB. Cullin-3 regulates pattern formation, external sensory organ development and cell survival during *Drosophila* development. *Mech Dev* 2004; **121**: 1495–1507.
64. Kuranaga E, Miura M. Nonapoptotic functions of caspases: caspases as regulatory molecules for immunity and cell-fate determination. *Trends Cell Biol* 2007; **17**: 135–144.
65. Williams DW, Kondo S, Krzyzanowska A, Hiromi Y, Truman JW. Local caspase activity directs engulfment of dendrites during pruning. *Nat Neurosci* 2006; **9**: 1234–1236.
66. Haynie JL, Bryant PJ. The effects of X-rays on the proliferation dynamics of cells in the imaginal wing disc of *Drosophila melanogaster*. *Wilhelm Roux's Arch* 1977; **183**: 85–100.

67. Huh JR, Guo M, Hay BA. Compensatory proliferation induced by cell death in the *Drosophila* wing disc requires activity of the apical cell death caspase Dronc in a nonapoptotic role. *Curr Biol* 2004; **14**: 1262–1266.
68. Perez-Garijo A, Martin FA, Morata G. Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in *Drosophila*. *Development* 2004; **131**: 5591–5598.
69. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer and cancer stem cells. *Science* 2001; **414**: 105–109.
70. Spradling A, Drummond-Barbosa D, Kai T. Stem cells find their niche. *Nature* 2001; **414**: 98–104.
71. Yamashita Y, Fuller M, Jones DL. Signaling in stem cell niches: lessons from the *Drosophila* germline. *J Cell Sci* 2005; **118**: 665–672.
72. Stoick-Cooper CL, Weidinger G, Riehle KJ, Hubbert C, Major MB, Fausto N *et al.* Distinct Wnt signaling pathways have opposing roles in appendage regeneration. *Development* 1997; **134**: 479–489.
73. Fennell DA. Caspase regulation in non-small cell lung cancer and its potential for therapeutic exploitation. *Clin Cancer Res* 2005; **11**: 2097–2105.
74. Bienz M, Clevers H. Linking colorectal cancer to Wnt signaling. *Cell* 2000; **103**: 311–320.
75. Kondo S, Senoo-Matsuda N, Hiromi Y, Miura M. DRONC coordinates cell death and compensatory proliferation. *Mol Cell Biol* 2006; **26**: 7258–7268.
76. Wells BS, Yoshida E, Johnston LA. Compensatory proliferation in *Drosophila* imaginal discs requires Dronc-dependent p53 activity. *Curr Biol* 2006; **16**: 1606–1615.
77. Fan Y, Bergmann A. Distinct mechanisms of apoptosis-induced compensatory proliferation in proliferating and differentiating tissues in the *Drosophila* eye. *Dev Cell* 2008; **14**: 399–410.
78. Cecconi F, Alvarez-Bolado G, Meyer BI, Roth KA, PGruss P. Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development. *Cell* 1998; **94**: 727–737.
79. Li MO, Sarkisian MR, Mehal WZ, Rakic P, Flavell RA. Phosphatidylserine receptor is required for clearance of apoptotic cells. *Science* 2003; **302**: 1560–1563.