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Review

Developmentally programmed cell death in *Drosophila* ☆

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ABSTRACT

During the development of metazoans, programmed cell death (PCD) is essential for tissue patterning, removal of unwanted cells and maintaining homeostasis. In the past 20 years *Drosophila melanogaster* has been one of the systems of choice for studies involving developmental cell death, providing an ideal genetically tractable model of intermediary complexity between *Caenorhabditis elegans* and mammals. The lessons learned from studies using *Drosophila* indicate both the conserved nature of the many cell death pathways as well as novel and unexpected mechanisms. In this article we review the understanding of PCD during *Drosophila* development, highlighting the key mechanisms that are evolutionarily conserved as well as apparently unusual pathways, which indicate divergence, but provide evidence of complexity acquired during organismic evolution. This article is part of a Special Section entitled: Cell Death Pathways. Guest Editors: Frank Madeo and Slaven Stekovic.

1. Introduction

As discussed in various other articles in this special issue, programmed cell death (PCD), often mediated by a highly conserved pathway termed apoptosis, is essential for normal development of metazoans [1]. Generally, PCD is required for the removal of superfluous or damaged cells, thus not only protecting animals from the harmful effects of damaged cells, but also remodelling and sculpting tissues during morphogenesis [2]. The majority of developmental PCD in *Drosophila* appears to be dependent on canonical (as established from studies in *Caenorhabditis elegans*) caspase mediated apoptosis. However, other modes of PCD also function during *Drosophila* development, including autophagy [3]. Compared to our understanding of the molecular mechanisms regulating apoptosis, the mechanism and regulation of autophagy during cell death is only beginning to be uncovered. This review focuses on the molecular machinery of apoptosis and the regulation of specific cell death modalities during *Drosophila* development.

The *Drosophila* life cycle consists of four distinct stages, embryo, larva (first, second and third instar stages), pupa (prepupa and pupa stages) and adult. Cell death can be seen during early embryogenesis with apoptotic cells evident around 6 h following egg deposition and becoming more widespread throughout the embryo [4]. During larval stages, PCD can be seen in many tissues and is most evident in the central nervous system. Specific signalling pathways control cell death that is critical for the selective removal of cells during tissue morphogenesis [5]. Differentiation of the adult eye from the imaginal tissue during pupal development involves the precise patterning of interommatidial cells surrounding the photoreceptor clusters, and superfluous cells are eliminated by apoptosis [6–8]. Most dramatic cell death is evident during larval–pupal transition where many larval tissues undergo deletion that is primarily controlled by the steroid hormone ecdysone [9]. As discussed below, this massive cell death is transcriptionally controlled by nuclear hormone receptors and a set of transcription factors which impart spatial and temporal regulation. Furthermore, as large tissues are deleted cell death seems to involve mechanisms other than canonical apoptosis. Cell death also occurs during oogenesis where the nurse cells die and dump their contents into the developing oocyte, and in the removal of defective egg chambers. This type of cell death is also regulated by ecdysone signalling [10], but has distinct molecular features that are not seen in metamorphic cell death.

2. Components of the cell death pathway

The genetic basis of metazoan cell death was first discovered in *C. elegans* where four main genes/proteins, EGL-1, CED9, CED4 and CED3, constitute the cell death apparatus [11–14]. EGL-1, a BH3-only protein is required to antagonize CED9, a prosurvival Bcl-2-like protein. CED9 binds CED4, an adaptor protein required for the activation of caspase CED3. Upregulation of EGL-1 by death stimulus initiates sequestration of CED9, allowing CED4 to interact with CED3 and mediating apoptosome-dependent activation of CED3 [15–18]. This core machinery

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is conserved in mammals, with each of these proteins represented by one or more homologues and similarly centres around the activation of caspases.

While caspases (homologues of CED-3) and caspase activation mechanisms are fundamentally conserved in *Drosophila*, perhaps the most surprising difference is the lack of a BH3-like (EGL-1 homologue) protein involved in cell death (Fig. 1) [19]. Since BH3 proteins control caspase activation by inhibiting the functions of antiapoptotic Bcl-2 family members (CED-9 in *C. elegans*, Bcl-2, Mcl-1 and Bcl-xL in mammals) and mediating the activation of proapoptotic Bax and Bak in mammals, equivalent pathways appear to be absent in *Drosophila*. Consistent with this assumption, the two *Drosophila* Bcl-2-like proteins Debcl and Buffy play relatively minor roles in apoptosis, functioning only in specific context. On the other hand inhibitor of apoptosis proteins (IAPs), especially DIAP1 (an E3 ubiquitin ligase), play an essential role in suppression of caspase activation, and the removal of IAPs is critical in initiating caspase activation and apoptosis (described below).

2.1. *Drosophila* caspases and activation machinery

In *Drosophila* there are 7 caspases, three of which, Dronc, Dredd and Strica contain long prodomains, and four, Drice, Dcp-1, Decay and Damm lack a long prodomain [20–27] (Fig. 2). From genetic studies, it is now well established that Dronc, with a caspase activation and recruitment domain (CARD), is the primary initiator caspase (functionally similar to caspase-9 in mammals) [28]. Consistent with this, Dronc activation requires dimerization and the recruitment of Dark/Ark, a CED4/Apaf-1 like adaptor protein that assembles into a caspase activation platform, commonly known as the apoptosome [29,30].

Dronc is essential for all apoptotic cell death in *Drosophila*, including that occurring during embryogenesis, larval development, metamorphosis and in response to stress. As *dronc* is maternally deposited, *dronc* mutants die during the pupal stage with a dramatic reduction in PCD during development or following stress [31–35]. However, RNAi mediated knockdown of *dronc* or embryonic *dronc* mutant clones, leads to an almost complete block in apoptosis of embryonic cells [36]. Detailed studies of *dronc* null mutants demonstrate that *dronc* is required for cell death during larval stages, as well as salivary gland cells during larval–pupal metamorphosis [31,32]. Dronc function is also required for cell death in the ovary as a *dronc* mutation reverts the ovary degeneration defect in the *diap1* mutant, however this function is redundant to other caspases [34]. In addition to its proapoptotic functions, Dronc also has non-apoptotic roles in cell migration, spermatid individualization, compensatory cell proliferation, differentiation of sensory organ precursor cells, and tissue remodelling in dendrite pruning [37,38].

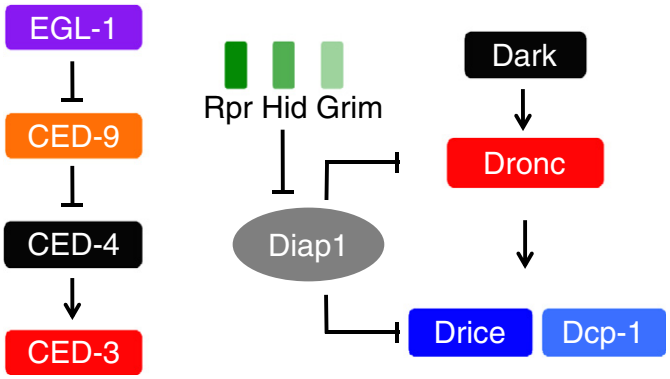

Fig. 1. The core components of the cell death machinery in *C. elegans* and *Drosophila*. The canonical apoptotic pathway is evolutionarily conserved and central to the regulation of apoptosis is the caspase family. In *C. elegans*, EGL-1 binds to CED-9, which enables CED-4 to activate CED-3 initiating apoptosis. In *Drosophila*, activation of the initiator caspase Dronc, by the Dark apoptosome, acts to transduce the death signal by cleavage and activation of effector caspases, Drice and Dcp-1. Activated effector caspases then cleave many cellular substrates causing ordered dismantling of the cell. Diap1 is essential for cell survival by binding to and inhibiting Dronc and Drice. During apoptosis, Rpr, Grim, and Hid bind Diap1, promoting its ubiquitination and degradation, thereby alleviating the block on caspase activation.

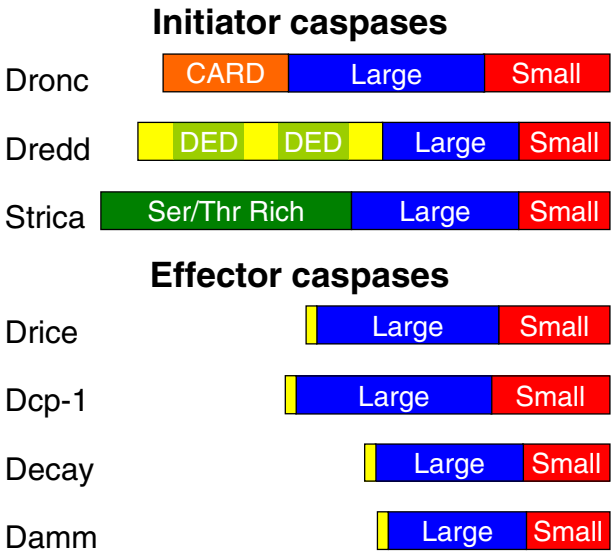

Fig. 2. *Drosophila* caspases. A schematic representation of the domain structure of the seven *Drosophila* caspases. Initiator caspases Dronc, Dredd and Strica contain a long prodomain at the amino-terminal, CARD (caspase activation and recruitment domain), DED (death effector domain) or Ser/The (serine/threonine rich domain) respectively. Effector caspases Drice, Dcp-1, Decay and Damm lack a long prodomain. The catalytic domains of caspases consisting of small and large subunits are indicated.

As stated above, Dronc is activated via a Dark/Ark-mediated apoptosome [29] and biochemical studies suggest that initial Dronc activation can occur by dimerisation without processing of the Dronc zymogen [39]. Following dimerisation Dronc autocleavage leads to stabilization of the dimer [39]. The apoptosome presumably allows the initial activation of Dronc by recruiting the zymogen. Recent structural studies suggest that the Dark apoptosome bound to Dronc forms a single ring structure made of 8 molecules of Dark where CARDs form a crown on the central hub of the Dark apoptosome [30]. While sharing overall similarity with the Apaf1 apoptosome, the unique feature of the Dark apoptosome is that it can form without the need for cytochrome c [30,40,41]. Consistent with a key role in Dronc activation, studies with null mutants indicate that loss of *dark* phenocopies the loss of *dronc*, showing tissue hyperplasia, reduced caspase activation and apoptosis [42–47]. Interestingly, as described below Dronc/Dark-dependent cell death is not required for the removal of some specific tissue [48].

Another long prodomain containing caspase is Dredd, which contains two death effector domains (DEDs) and is thus similar to mammalian caspase-8 [20]. The DED domains of Dredd are important for its interaction with the adaptor dFADD (Fas-associated death domain) [49]. Although initially suggested to play a role in apoptosis [20], subsequent studies indicate that the function of Dredd is mainly in the innate immune response in *Drosophila*. In this pathway Toll receptor activates Dredd that mediates proteolytic processing and activation of Relish, a NFκB-like transcription factor [50–52]. Additionally, Dredd has a role in sperm individualization [53].

Strica, the other potential upstream/initiator caspase in *Drosophila*, contains a unique Ser/Thr-rich prodomian of unknown function [23]. While *strica* mutants develop normally, this caspase appears to play context specific cell death functions. For example, the null mutants of

strica or *dronc*, on their own do not show any phenotype in oogenesis, while ovaries of *strica* and *dronc* double mutants contain defective mid-stage egg chambers and mature egg chambers with persisting nurse cell nuclei, suggesting redundant roles for these caspases in PCD during oogenesis [54]. This functional redundancy is also apparent in apoptosis of a group of interneurons (vCrz) that produce corazonin neuropeptide [55]. While vCrz apoptosis is suppressed in *dronc*-null mutants and not in *strica*-null mutants, the *dronc* and *strica* double mutants show a more severe phenotype than a *dronc* mutant alone, suggesting that Strica plays a small but significant role in vCrz apoptosis that partially overlaps with the function of Dronc [55].

Among the four caspases lacking a prodomain, Drice is the main effector caspase in *Drosophila* apoptosis pathway, being most abundant and is functionally analogous to caspase-3 in mammals [24,28]. *drice* null mutants show substantially reduced apoptosis in many tissues but are not as severe as *dronc* mutants [35,56,57]. The effector caspase Dcp-1 shares strong homology with Drice and caspase-3 and functions redundantly in apoptosis [35,54–57]. Despite its close similarity with Drice, Dcp-1 is not essential for apoptosis and null mutants do not show any overt defects but rather display defects in starvation-induced cell death during oogenesis [35,56–59]. However, *dcp-1* and *drice* double mutants show a much stronger apoptosis inhibition phenotype than either gene alone and these *dcp-1*/*drice* double mutants somewhat mimic the phenotype of *dronc* null animals [57]. These results suggest that Dronc is the essential initiator caspase, Drice the main effector caspase and Dcp-1 is required redundantly in some cells alongside Drice. Once activated, Dronc cleaves and activates Drice and Dcp-1, which then go on to cleave many of the caspase substrates in cells undergoing apoptosis [60].

The remaining two caspases lacking prodomains, Decay and Damm, appear to be dispensable for most developmental cell death. Decay is highly expressed in dying larval midguts [22,48], but *decay* null mutants and knockdown lines do not show any overt phenotype [35,61]. The role of the high level of Decay in larval midguts is unclear as knockdown or inhibition of this activity does not affect midgut removal [48]. There is some evidence suggesting that Decay may work redundantly with the canonical apoptotic machinery in specific context and overexpression of Damm in transgenic flies disrupts patterning of the eye [27,61]. However, the significance of these observations in developmental cell death remains to be established.

2.2. Drosophila inhibitor of apoptosis proteins

The prevention of inappropriate caspase activation during *Drosophila* development is important for viability and inhibitor of apoptosis proteins (IAPs) are the main regulators of cell survival. IAPs are conserved proteins, first discovered in Baculovirus [62]. They contain one or more BIR (baculovirus IAP repeat) motifs and a RING type ubiquitin ligase domain [63]. There are four IAPs in *Drosophila*, Diap1, Diap2, dBruce and Deterin [64]. Among the IAPs, Diap1 is most important and a critical regulator of cell survival. Binding of Diap1 via its BIR domain prevents Dronc activation and Diap1 removal alone is sufficient to induce rapid and spontaneous caspase activation and cell death [65,66]. Initially it was thought that Diap1 mediated ubiquitination and degradation was the main mechanism to control Dronc [67–69]. However Dronc is a long-lived protein and its turnover does not appear to be a mechanism of its regulation. Consistent with this prediction, recent data suggest that Diap1-mediated ubiquitination does not lead to proteasomal degradation of full-length Dronc, rather ubiquitination blocks processing and activation of Dronc [70]. In addition to binding and inhibiting Dronc, Diap1 also inhibits the activity of downsteam effector caspases Drice and Dcp-1 [71,72].

Another IAP, dBruce also appears to regulate caspase activation in specific contexts such as during spermatid individualization [73,74]. dBruce has an additional role in cell death during oogenesis [59,75]. Diap2 has a relatively minor function in apoptosis, and is probably more important in the *Drosophila* innate immune response pathway than in developmental cell death [76–79]. Finally, the function of Deterin in developmental cell death remains unknown [80].

2.3. IAP antagonists

The first cell death genes discovered in *Drosophila* by genetic analysis identified a chromosomal deficiency (*H99*) that resulted in a loss of developmental cell death. The H99 deficiency deletes three genes, *reaper (rpr)*, *head involution defect (hid*/*Wrinkled*) and *grim* [81–83]. These genes all encode IAP antagonists with amino-terminal RHG motifs (IAP-binding motif) [84] and cooperate to induce cell death in embryos and other tissues, but are not required for nurse cell death during oogenesis [85]. Additional RHG motif containing proteins have been identified including Jafrac2, Sickle and Omi/HtrA2 [86–92]. As well as binding caspases, the IAP BIR domains interact with the RHG motif in Rpr, Hid and Grim, leading to RING domain mediated ubiquitination. In cells initiated to undergo apoptosis, the proapoptotic proteins RHG bind Diap1 promoting its autoubiquitination and degradation, thus relieving its inhibitory effects on caspases [69,93–95].

2.4. The Bcl-2 homologues in Drosophila

Surprisingly, Bcl-2 family members in *Drosophila* do not seem to play a central role in apoptosis regulation. In a way this would be expected as the primary function of both prosurvival and proapoptotic Bcl-2 proteins is to control the release of cytochrome c from mitochondria, which is required for the formation of the mammalian Apaf-1 apoptosome, but not for Dark apoptosome [29,30]. BH3-only proteins such as Bim, Noxa and Puma mediate the signalling that leads to mitochondrial outer membrane permeabilization and cytochrome c release by directly activating pore forming Bax and Bak proteins and/or antagonising prosurvival members Bcl-2, Bcl-x and Mcl-1 [96]. As cytochrome c is not necessary for apoptosome formation in *Drosophila*, this function of the Bcl-2 members has probably become redundant [40]. Consistent with this hypothesis, there are no BH3-only proteins in *Drosophila*. The two Bcl-2-related proteins in *Drosophila* are Debcl/dBorg-1/dRob-1 and Buffy/dBorg-2 [97–101]. These proteins share the BH1, BH2, BH3 and carboxy-terminal transmembrane domains of the Bcl-2 family of proteins, but have no amino-terminal BH4 domain, which distinguishes the proapoptotic Bcl-2 family members from the prosurvival Bcl-2 proteins [102].

Both Debcl and Buffy are most related to the mammalian Bok proapoptotic protein [103], and as such overexpression of Debcl in transgenic flies and Buffy in *Drosophila* cell lines results in ectopic cell death [97–99,104]. Buffy also seems to have a prosurvival role in transgenic flies [101] and Debcl protects neurons from expanded polyglutamine-induced toxicity [105], suggesting that both Debcl and Buffy have prosurvival and proapoptotic activities. However, null mutants of *buffy* or *debcl*, or the double mutants lacking both, have no developmental phenotype suggesting that they are not essential for developmental cell death [106]. Interestingly though, data shows that DNA damage-induced apoptosis is suppressed by Buffy, and Debcl inhibits Buffy's prosurvival function [106]. Additionally, Debcl appears to be required for pruning cells in the developing central nervous system [107]. The alternative cell death that occurs during spermatogenesis requires Buffy and Debcl [108]. Finally, the loss of *buffy* has been shown to promote microchaete glial cell survival suggesting that Buffy has a proapoptotic function in developmental cell death in vivo [109]. Thus it appears that the *Drosophila* Bcl-2 members, while not essential for animal survival and development, have tissue and context specific functions.

3. Eiger-induced cell death

In mammals, caspase-dependent apoptosis is mediated by two main pathways, the death receptor (extrinsic) pathway and the

mitochondrial (intrinsic) pathway [110]. The death receptor pathway is induced by ligand-mediated activation of the tumour necrosis factor (TNF) family of receptors and involves FADD-mediated activation of caspase-8. The *Drosophila* genome encodes single orthologues of TNF and TNF receptor (TNFR) family proteins, Eiger (Egr) and Wengen (Wgn) respectively [111–114], suggesting that at least in part the extrinsic cell death pathway may be conserved. The expression of Egr promotes cell death through the receptor Wgn, by activating the JNK pathway; the JNKK kinase (dTAK1), the JNK kinase (Hep), and JNK (Bsk) [111,113]. While Eiger-induced JNK-dependent cell death can be prevented by DIAP1, there only appears to be a minimal contribution of caspase activity [111]. Blocking the activation of caspases by expression of dominant-negative Dronc or the baculovirus caspase inhibitor p35 only marginally suppresses Eiger-induced cell death [111]. Whereas, the expression of Diap1 strongly suppresses the Eiger-induced eye phenotype and reducing *diap1* enhances it [111]. Thus, even though Eiger-induced JNK-dependent cell death can be inhibited by Diap1, the roles of caspases in this process are yet to be fully understood. To gain an insight into the molecular basis of Eiger-induced cell death, a genetic screen identified components of metabolic pathways that act downstream of JNK specifically in Eiger-induced nonapoptotic cell death [115]. In this model, Eiger expression leads to the JNK-dependent production of ROS and further investigation into the consequence of this will be important.

4. Cell death during larval–pupal metamorphosis

During metamorphosis, obsolete larval tissues including the salivary glands and midgut are removed in response to changes in the concentration of steroid hormone ecdysone [116,117]. Salivary gland cell death requires both apoptosis and autophagy and the inhibition of both pathways completely block tissue degeneration [118]. Despite the high level of caspase activity present, the removal of larval midgut requires autophagy but not caspases [48].

4.1. Transcriptional regulation of salivary gland PCD

Correct transcriptional regulation of cell death genes is crucial for the spatio-temporal patterns of PCD during development. Together with various cofactors, the heterodimeric nuclear hormone receptor complex consisting of the ecdysone receptor (EcR) and ultraspiracle (USP) initiates transcriptional reprogramming during salivary gland PCD [119,120].

Prior to the transcriptional reprogramming for salivary gland PCD, preparation initiates during the larval stage. The ecdysone pulse during the third larval instar stage promotes the activation of EcR and its transcriptional co-activator CREB binding protein (CBP) that act to down-regulate the level of *diap1* [121]. The reduced levels of Diap1 are sufficient to prevent early cell death, while remaining sensitive enough to be overcome by the later induction of cell death genes. Salivary gland cell death is prevented during the larval stages due to Fork head (Fkh) mediated-repression of *rpr* and *hid*. In response to the subsequent ecdysone pulse at the larval–pupal transition activation of EcR/USP complex leads to the induction of Broad Complex (BR-C) releasing the transcriptional block on *rpr* and *hid* through the down-regulation of *fkh* [122]. Thus enabling the temporal induction of salivary gland PCD in response to the later prepupal pulse of ecdysone.

In response to the prepupal–pupal pulse of ecdysone, EcR/USP complex regulates the expression of primary-response genes, including those that encode transcription factors BR-C, a zinc finger transcription factor, E74 an ETS-domain transcription factor, and E93 a novel site-specific DNA binding protein [123–126]. These transcription factors, BR-C, E75 and E93, act to amplify the signal by directly regulating transcription of secondary-response genes, including *rpr*, *hid*, *dronc* and *dark* (Fig. 3) [127–129]. In addition, EcR/USP directly regulates cell death gene *rpr* and *dronc* [129–131].

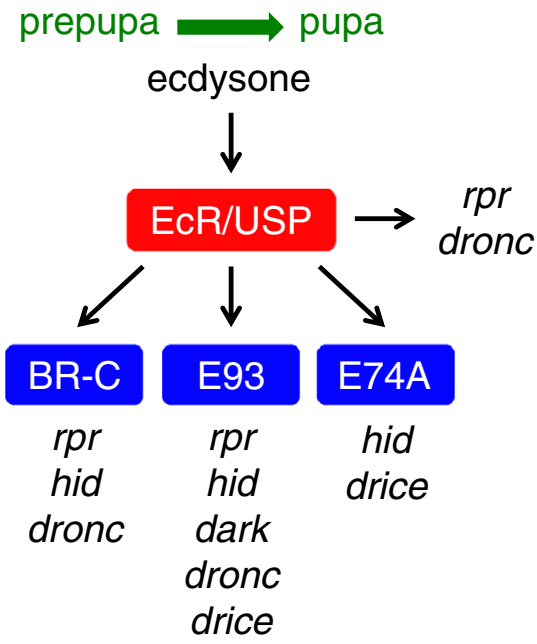

Fig. 3. Ecdysone induced gene hierarchy that controls larval salivary gland cell death. During metamorphosis the prepupal pulse of ecdysone triggers salivary gland PCD. Ecdysone binds to its heterodimeric receptor EcR/USP that regulates transcription of apoptotic genes both directly and indirectly through other transcription factors, BR-C, E93, and E74A.

EcR/USP also acts to repress expression of the orphan nuclear receptor βFTZ-F1 that acts as a competence factor during earlier stages to prime the salivary gland for PCD [132].

In addition to caspase activation, another prominent feature of salivary gland cell death is autophagy. Autophagy is a catabolic process that involves engulfment of cytoplasmic components and long-lived proteins into lysosomes for degradation [133]. An essential step is the formation and elongation of a double-membrane vesicle, the autophagosome, to sequester cytoplasmic material. This multistep process requires the products of numerous *autophagy specific (Atg)* genes. The loss of function mutations or gene specific knockdown of numerous *Atg* genes all result in a dramatic delay in the removal of the larval salivary glands [118]. Therefore the induction of autophagy is also important for salivary gland removal. Coinciding with the ecdysone pulse that triggers salivary gland PCD, there is an increase in the expression of many *Atg* genes such as *Atg2*, *Atg4*, *Atg5*, *Atg6*, *Atg7*, *Atg9* and *Atg12* [134,135]. Whether EcR and other transcription factors such as BR-C, E74 and E93 directly regulate these genes will need to be determined.

The induction of apoptotic genes by ecdysone can occur through direct binding of EcR/USP to the conserved sequences found in the promoters. An ecdysone response element has been mapped for *dronc* and *rpr* genes [127,129,130]. Similarly, BR-C also binds to the *dronc* and *drice* promoter through BR-C binding sites [127,131,136]. In addition, the recruitment of nuclear receptor cofactors, including histone modifying enzymes, to ecdysone-regulated gene promoters plays an important role in regulating the timing of hormone-induced gene expression. The arginine–histone methyltransferase CARMER is recruited to EcR/USP and required for ecdysone-mediated apoptosis in cells [137]. This is antagonised by a novel cofactor, lysine ketoglutarate reductase/saccharopine dehydrogenase (dLKR/SDH) that inhibits CARMER mediated histone modification, and binds EcR/USP to regulate hormone-mediated transcription of cell death genes [138]. Similarly, the novel EcR coactivator dDOR is required for the correct timing of salivary gland degradation [139].

4.2. Autophagic cell death in the midgut

The larval midgut is removed in response to a pulse of ecdysone at the end of the third instar larval stage [140]. Similar to PCD of the salivary glands, cell death genes are up-regulated during midgut cell death, such as *rpr* and *hid* by BR-C and *dronc* by E93 [140]. However, there are distinct differences that have been identified. Interestingly, the high caspase activity observed in dying midgut is mainly contributed by Decay and it has been found to be dispensable for midgut removal [48]. Unlike salivary gland PCD that requires both caspase activity and autophagy for complete removal, in the midgut autophagy suppression alone through mutations or knockdown of *Atg1*, *Atg2*, and *Atg18* severely delays its PCD [48]. Consistent with this, expression of Atg1 is sufficient to induce premature midgut removal [141]. Although *Atg* genes such as *Atg1*, *Atg2*, *Atg3*, *Atg4*, *Atg7*, *Atg6*, *Atg8a*, *Atg12* and *Atg18* are found to be up-regulated just before midgut histolysis [48], direct regulation by EcR/USP complex is yet to be confirmed.

5. Regulation of germline cell death

5.1. Cell death during oogenesis

Distinct forms of cell death occur during oogenesis that requires overlapping functions of caspases however the mechanism of caspase activation is not clear as IAP antagonists are not required. Developmentally regulated PCD of nurse cells occurs late in oogenesis whereas during mid-oogenesis stress-induced cell death is required for the removal of defective egg chambers.

As a normal part of oocyte maturation, PCD in late oogenesis removes excess nurse cells and follicle cells [85,142,143]. This occurs by distinct PCD pathways that require the caspases *strica*, *dronc*, *drice*, and *dcp-1* in redundant roles but not *rpr*, *hid*, *grim*, *skl*, *debcl* and *Cyt-c* [54,58,85,144]. Consistent with this when Diap1 is ectopically expressed in the ovary, nurse cell death still occurs [145]. This developmental PCD also requires autophagy and removal of dBruce, as germline mutant clones of autophagy genes accumulate dBruce in late stage egg chambers with persisting nurse cells without fragmented DNA [146]. However, egg chamber defects have not been identified using alternative genetic analysis, and further studies are needed to understand the role of autophagy during oogenesis [147].

Two checkpoints act in response to stress, starvation or developmental defects at germarium and mid-stage oogenesis to induce cell death, requiring both autophagy and apoptosis to eliminate defective egg chambers [148]. These checkpoints require the effector caspase Dcp-1, as starved *dcp*-1 mutants have defective egg chambers with persisting uncondensed nurse cell nuclei and decreased autophagy [58,59,75]. The mechanism of Dcp-1 activation is unclear as double mutants lacking the initiator caspases *strica* and *dronc* only partially disrupt PCD in mid-stage oogenesis. Interestingly, Dcp-1 expression is sufficient to induce autophagy and egg chamber degeneration during mid-stage oogenesis [59]. However, autophagy appears to have a minor role during this stage. Autophagy-deficient egg chambers show normal chromatin condensation, but reduced levels of DNA fragmentation [59,75]. Under nutrient rich conditions dBruce acts to negatively regulate autophagy and cell death [59]. Similar to the late-stage cell death, IAP antagonists are not required for mid-stage cell death [144]. Thus PCD during oogenesis appears to utilize a novel pathway leading to caspase activation that requires autophagy.

5.2. Functions of caspase activity during spermatogenesis

The spontaneous elimination of approximately one-third of the differentiating male germ cells occurs prior to meiosis. Recent studies have shown that in *Drosophila*, this process involves a novel form of cell death that requires Dronc activity independent to the apoptosome and downstream effector caspases. The germ cells undergo death with morphological features of both apoptosis and necrosis [108]. This germ cell death requires lysosomal enzymes and mitochondrial proteins HtrA2/Omi, Pink1, Buffy, Debcl, and endonuclease G revealing an important context specific role of the *Drosophila* Bcl-2 proteins in developmental cell death [108].

In addition, localised caspase activation is also required during spermatid terminal differentiation called spermatid individualization. However, this activity has a non-apoptotic function to degrade the cytoplasmic remnants [53,149]. Caspase activation machinery is required as sperm individualization defects are observed in *dark* and *dronc* mutants [53,150]. This individualization involves the activity of multiple caspase activators, including Hid, Dark and Fadd, and the caspases Dronc, Dcp-1, Drice, and Dredd [53,56,149]. In addition, a testes specific cytochrome c gene *cyt-c-d* appears to be necessary for caspase activation [53,56,149,150]. The localised caspase activation is achieved due to a gradient of expression of Soti, an inhibitor of a Cullin-3 E3 ubiquitin ligase complex that is required for caspase activation by targeting dBruce for degradation. Soti competes with dBruce for binding to Cullin-3 E3 complex thus producing a gradient of dBruce that ultimately results in an inverse gradient of caspase activity. This results in spatially localised caspase activation to promote caspase-dependent differentiation while preventing apoptosis [74].

6. Conclusions

As reviewed here studies from *Drosophila* suggest conserved and nonconserved mechanisms of PCD during development. Not surprisingly most developmental PCD is through apoptotic mechanisms, even though some of the components of the canonical machinery in *C. elegans* and mammals, especially the Bcl-2 homologues appear not be critical for PCD in *Drosophila*. The nonapoptotic cell deaths during development, discovered through genetic studies in the fly are intriguing. Clearly, better understanding of these relatively poorly explored modalities, their regulation, origins and evolutionary conservation remain a priority.

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References

[1] J.F. Kerr, A.H. Wyllie, A.R. Currie, Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics, Br. J. Cancer 26 (1972) 239–257.
[2] E.H. Baehrecke, How death shapes life during development, Nat. Rev. Mol. Cell Biol. 3 (2002) 779–787.
[3] D. Denton, S. Nicolson, S. Kumar, Cell death by autophagy: facts and apparent artefacts, Cell Death Differ. 19 (2012) 87–95.
[4] J.M. Abrams, K. White, L.I. Fessler, H. Steller, Programmed cell death during *Drosophila* embryogenesis, Development 117 (1993) 29–43.
[5] J.C. Rusconi, R. Hays, R.L. Cagan, Programmed cell death and patterning in *Drosophila*, Cell Death Differ. 7 (2000) 1063–1070.
[6] C.B. Brachmann, R.L. Cagan, Patterning the fly eye: the role of apoptosis, Trends Genet. 19 (2003) 91–96.
[7] R.L. Cagan, D.F. Ready, The emergence of order in the *Drosophila* pupal retina, Dev. Biol. 136 (1989) 346–362.
[8] T. Wolff, D.F. Ready, Cell death in normal and rough eye mutants of *Drosophila*, Development 113 (1991) 825–839.
[9] E.H. Baehrecke, Steroid regulation of programmed cell death during *Drosophila* development, Cell Death Differ. 7 (2000) 1057–1062.
[10] M. Buszczak, L. Cooley, Eggs to die for: cell death during *Drosophila* oogenesis, Cell Death Differ. 7 (2000) 1071–1074.
[11] J. Yuan, S. Shaham, S. Ledoux, H.M. Ellis, H.R. Horvitz, The *C. elegans* cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme, Cell 75 (1993) 641–652.
[12] H.M. Ellis, H.R. Horvitz, Genetic control of programmed cell death in the nematode *C. elegans*, Cell 44 (1986) 817–829.

[13] M.O. Hengartner, R.E. Ellis, H.R. Horvitz, *Caenorhabditis elegans* gene ced-9 protects cells from programmed cell death, Nature 356 (1992) 494–499.
[14] J. Yuan, H.R. Horvitz, The *Caenorhabditis elegans* cell death gene ced-4 encodes a novel protein and is expressed during the period of extensive programmed cell death, Development 116 (1992) 309–320.
[15] B. Conradt, H.R. Horvitz, The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9, Cell 93 (1998) 519–529.
[16] L. del Peso, V.M. Gonzalez, G. Nunez, *Caenorhabditis elegans* EGL-1 disrupts the interaction of CED-9 with CED-4 and promotes CED-3 activation, J. Biol. Chem. 273 (1998) 33495–33500.
[17] L. del Peso, V.M. Gonzalez, N. Inohara, R.E. Ellis, G. Nunez, Disruption of the CED-9.CED-4 complex by EGL-1 is a critical step for programmed cell death in *Caenorhabditis elegans*, J. Biol. Chem. 275 (2000) 27205–27211.
[18] X. Yang, H.Y. Chang, D. Baltimore, Essential role of CED-4 oligomerization in CED-3 activation and apoptosis, Science 281 (1998) 1355–1357.
[19] P. Chen, J.M. Abrams, Drosophila apoptosis and Bcl-2 genes: outliers fly in, J. Cell Biol. 148 (2000) 625–627.
[20] P. Chen, A. Rodriguez, R. Erskine, T. Thach, J.M. Abrams, Dredd, a novel effector of the apoptosis activators reaper, grim, and hid in Drosophila, Dev. Biol. 201 (1998) 202–216.
[21] L. Dorstyn, P.A. Colussi, L.M. Quinn, H. Richardson, S. Kumar, DRONC, an ecdysone-inducible *Drosophila* caspase, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4307–4312.
[22] L. Dorstyn, S.H. Read, L.M. Quinn, H. Richardson, S. Kumar, DECAY, a novel *Drosophila* caspase related to mammalian caspase-3 and caspase-7, J. Biol. Chem. 274 (1999) 30778–30783.
[23] J. Doumanis, L. Quinn, H. Richardson, S. Kumar, STRICA, a novel *Drosophila melanogaster* caspase with an unusual serine/threonine-rich prodomain, interacts with DIAP1 and DIAP2, Cell Death Differ. 8 (2001) 387–394.
[24] A.G. Fraser, G.I. Evan, Identification of a *Drosophila melanogaster* ICE/CED-3-related protease, drICE, EMBO J. 16 (1997) 2805–2813.
[25] N. Inohara, T. Koseki, Y. Hu, S. Chen, G. Nunez, CLARP, a death effector domain-containing protein interacts with caspase-8 and regulates apoptosis, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 10717–10722.
[26] Z. Song, K. McCall, H. Steller, DCP-1, a *Drosophila* cell death protease essential for development, Science 275 (1997) 536–540.
[27] N.L. Harvey, T. Daish, K. Mills, L. Dorstyn, L.M. Quinn, S.H. Read, H. Richardson, S. Kumar, Characterization of the *Drosophila* caspase, DAMM, J. Biol. Chem. 276 (2001) 25342–25350.
[28] S. Kumar, J. Doumanis, The fly caspases, Cell Death Differ. 7 (2000) 1039–1044.
[29] X. Yu, L. Wang, D. Acehan, X. Wang, C.W. Akey, Three-dimensional structure of a double apoptosome formed by the *Drosophila* Apaf-1 related killer, J. Mol. Biol. 355 (2006) 577–589.
[30] S. Yuan, X. Yu, M. Topf, L. Dorstyn, S. Kumar, S.J. Ludtke, C.W. Akey, Structure of the Drosophila apoptosome at 6.9 a resolution, Structure 19 (2011) 128–140.
[31] S.K. Chew, F. Akdemir, P. Chen, W.J. Lu, K. Mills, T. Daish, S. Kumar, A. Rodriguez, J.M. Abrams, The apical caspase dronc governs programmed and unprogrammed cell death in Drosophila, Dev. Cell 7 (2004) 897–907.
[32] T.J. Daish, K. Mills, S. Kumar, Drosophila caspase DRONC is required for specific developmental cell death pathways and stress-induced apoptosis, Dev. Cell 7 (2004) 909–915.
[33] M. Waldhuber, K. Emoto, C. Petritsch, The Drosophila caspase DRONC is required for metamorphosis and cell death in response to irradiation and developmental signals, Mech. Dev. 122 (2005) 914–927.
[34] D. Xu, Y. Li, M. Arcaro, M. Lackey, A. Bergmann, The CARD-carrying caspase Dronc is essential for most, but not all, developmental cell death in *Drosophila*, Development 132 (2005) 2125–2134.
[35] S. Kondo, N. Senoo-Matsuda, Y. Hiromi, M. Miura, DRONC coordinates cell death and compensatory proliferation, Mol. Cell Biol. 26 (2006) 7258–7268.
[36] L.M. Quinn, L. Dorstyn, K. Mills, P.A. Colussi, P. Chen, M. Coombe, J. Abrams, S. Kumar, H. Richardson, An essential role for the caspase dronc in developmentally programmed cell death in *Drosophila*, J. Biol. Chem. 275 (2000) 40416–40424.
[37] E. Kuranaga, Beyond apoptosis: caspase regulatory mechanisms and functions in vivo, Genes Cells 17 (2012) 83–97.
[38] C.H. Yi, J. Yuan, The Jekyll and Hyde functions of caspases, Dev. Cell 16 (2009) 21–34.
[39] L. Dorstyn, S. Kumar, A biochemical analysis of the activation of the *Drosophila* caspase DRONC, Cell Death Differ. 15 (2008) 461–470.
[40] L. Dorstyn, K. Mills, Y. Lazebnik, S. Kumar, The two cytochrome c species, DC3 and DC4, are not required for caspase activation and apoptosis in Drosophila cells, J. Cell Biol. 167 (2004) 405–410.
[41] L. Dorstyn, S. Kumar, A cytochrome c-free fly apoptosome, Cell Death Differ. 13 (2006) 1049–1051.
[42] H. Kanuka, K. Sawamoto, N. Inohara, K. Matsuno, H. Okano, M. Miura, Control of the cell death pathway by Dapaf-1, a *Drosophila* Apaf-1/CED-4-related caspase activator, Mol. Cell 4 (1999) 757–769.
[43] A. Rodriguez, H. Oliver, H. Zou, P. Chen, X. Wang, J.M. Abrams, Dark is a *Drosophila* homologue of Apaf-1/CED-4 and functions in an evolutionarily conserved death pathway, Nat. Cell Biol. 1 (1999) 272–279.
[44] L. Zhou, Z. Song, J. Tittel, H. Steller, HAC-1, a *Drosophila* homolog of APAF-1 and CED-4 functions in developmental and radiation-induced apoptosis, Mol. Cell 4 (1999) 745–755.
[45] F. Akdemir, R. Farkas, P. Chen, G. Juhasz, L. Medved'ova, M. Sass, L. Wang, X. Wang, S. Chittaranjan, S.M. Gorski, A. Rodriguez, J.M. Abrams, Autophagy occurs upstream or parallel to the apoptosome during histolytic cell death, Development 133 (2006) 1457–1465.
[46] K. Mills, T. Daish, K.F. Harvey, C.M. Pfleger, I.K. Hariharan, S. Kumar, The *Drosophila melanogaster* Apaf-1 homologue ARK is required for most, but not all, programmed cell death, J. Cell Biol. 172 (2006) 809–815.
[47] M. Srivastava, H. Scherr, M. Lackey, D. Xu, Z. Chen, J. Lu, A. Bergmann, ARK, the Apaf-1 related killer in *Drosophila*, requires diverse domains for its apoptotic activity, Cell Death Differ. 14 (2007) 92–102.
[48] D. Denton, B. Shravage, R. Simin, K. Mills, D.L. Berry, E.H. Baehrecke, S. Kumar, Autophagy, not apoptosis, is essential for midgut cell death in *Drosophila*, Curr. Biol. 19 (2009) 1741–1746.
[49] S. Hu, X. Yang, dFADD, a novel death domain-containing adapter protein for the *Drosophila* caspase DREDD, J. Biol. Chem. 275 (2000) 30761–30764.
[50] F. Leulier, A. Rodriguez, R.S. Khush, J.M. Abrams, B. Lemaitre, The *Drosophila* caspase Dredd is required to resist gram-negative bacterial infection, EMBO Rep. 1 (2000) 353–358.
[51] S. Stoven, I. Ando, L. Kadalayil, Y. Engstrom, D. Hultmark, Activation of the *Drosophila* NF-kappaB factor Relish by rapid endoproteolytic cleavage, EMBO Rep. 1 (2000) 347–352.
[52] S. Stoven, N. Silverman, A. Junell, M. Hedengren-Olcott, D. Erturk, Y. Engstrom, T. Maniatis, D. Hultmark, Caspase-mediated processing of the *Drosophila* NF-kappaB factor Relish, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 5991–5996.
[53] J.R. Huh, S.Y. Vernooy, H. Yu, N. Yan, Y. Shi, M. Guo, B.A. Hay, Multiple apoptotic caspase cascades are required in nonapoptotic roles for *Drosophila* spermatid individualization, PLoS Biol. 2 (2004) E15.
[54] J.S. Baum, E. Arama, H. Steller, K. McCall, The *Drosophila* caspases Strica and Dronc function redundantly in programmed cell death during oogenesis, Cell Death Differ. 14 (2007) 1508–1517.
[55] G. Lee, Z. Wang, R. Sehgal, C.H. Chen, K. Kikuno, B. Hay, J.H. Park, *Drosophila* caspases involved in developmentally regulated programmed cell death of peptidergic neurons during early metamorphosis, J. Comp. Neurol. 519 (2011) 34–48.
[56] I. Muro, D.L. Berry, J.R. Huh, C.H. Chen, H. Huang, S.J. Yoo, M. Guo, E.H. Baehrecke, B.A. Hay, The *Drosophila* caspase Ice is important for many apoptotic cell deaths and for spermatid individualization, a nonapoptotic process, Development 133 (2006) 3305–3315.
[57] D. Xu, Y. Wang, R. Willecke, Z. Chen, T. Ding, A. Bergmann, The effector caspases drICE and dcp-1 have partially overlapping functions in the apoptotic pathway in *Drosophila*, Cell Death Differ. 13 (2006) 1697–1706.
[58] B. Laundrie, J.S. Peterson, J.S. Baum, J.C. Chang, D. Fileppo, S.R. Thompson, K. McCall, Germline cell death is inhibited by P-element insertions disrupting the dcp-1/pita nested gene pair in *Drosophila*, Genetics 165 (2003) 1881–1888.
[59] Y.C. Hou, S. Chittaranjan, S.G. Barbosa, K. McCall, S.M. Gorski, Effector caspase Dcp-1 and IAP protein Bruce regulate starvation-induced autophagy during *Drosophila melanogaster* oogenesis, J. Cell Biol. 182 (2008) 1127–1139.
[60] C.J. Hawkins, S.J. Yoo, E.P. Peterson, S.L. Wang, S.Y. Vernooy, B.A. Hay, The *Drosophila* caspase DRONC cleaves following glutamate or aspartate and is regulated by DIAP1, HID, and GRIM, J. Biol. Chem. 275 (2000) 27084–27093.
[61] F. Leulier, P.S. Ribeiro, E. Palmer, T. Tenev, K. Takahashi, D. Robertson, A. Zachariou, F. Pichaud, R. Ueda, P. Meier, Systematic in vivo RNAi analysis of putative components of the *Drosophila* cell death machinery, Cell Death Differ. 13 (2006) 1663–1674.
[62] N.E. Crook, R.J. Clem, L.K. Miller, An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif, J. Virol. 67 (1993) 2168–2174.
[63] D.L. Vaux, J. Silke, IAPs, RINGs and ubiquitylation, Nat. Rev. Mol. Cell Biol. 6 (2005) 287–297.
[64] M. Orme, P. Meier, Inhibitor of apoptosis proteins in *Drosophila*: gatekeepers of death, Apoptosis 14 (2009) 950–960.
[65] S.L. Wang, C.J. Hawkins, S.J. Yoo, H.A. Muller, B.A. Hay, The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID, Cell 98 (1999) 453–463.
[66] P. Meier, J. Silke, S.J. Leevers, G.I. Evan, The *Drosophila* caspase DRONC is regulated by DIAP1, EMBO J. 19 (2000) 598–611.
[67] I. Muro, B.A. Hay, R.J. Clem, The *Drosophila* DIAP1 protein is required to prevent accumulation of a continuously generated, processed form of the apical caspase DRONC, J. Biol. Chem. 277 (2002) 49644–49650.
[68] J. Chai, N. Yan, J.R. Huh, J.W. Wu, W. Li, B.A. Hay, Y. Shi, Molecular mechanism of Reaper–Grim–Hid-mediated suppression of DIAP1-dependent Dronc ubiquitination, Nat. Struct. Biol. 10 (2003) 892–898.
[69] R. Wilson, L. Goyal, M. Ditzel, A. Zachariou, D.A. Baker, J. Agapite, H. Steller, P. Meier, The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis, Nat. Cell Biol. 4 (2002) 445–450.
[70] T.V. Lee, Y. Fan, S. Wang, M. Srivastava, M. Broemer, P. Meier, A. Bergmann, *Drosophila* IAP1-mediated ubiquitylation controls activation of the initiator caspase DRONC independent of protein degradation, PLoS Genet. 7 (2011) e1002261.
[71] M. Ditzel, M. Broemer, T. Tenev, C. Bolduc, T.V. Lee, K.T. Rigbolt, R. Elliott, M. Zvelebil, B. Blagoev, A. Bergmann, P. Meier, Inactivation of effector caspases through nondegradative polyubiquitylation, Mol. Cell 32 (2008) 540–553.
[72] X. Li, J. Wang, Y. Shi, Structural mechanisms of DIAP1 auto-inhibition and DIAP1-mediated inhibition of drICE, Nat. Commun. 2 (2011) 408.
[73] E. Arama, M. Bader, G.E. Rieckhof, H. Steller, A ubiquitin ligase complex regulates caspase activation during sperm differentiation in *Drosophila*, PLoS Biol. 5 (2007) e251.

[74] Y. Kaplan, L. Gibbs-Bar, Y. Kalifa, Y. Feinstein-Rotkopf, E. Arama, Gradients of a ubiquitin E3 ligase inhibitor and a caspase inhibitor determine differentiation or death in spermatids, Dev. Cell 19 (2010) 160–173.
[75] I.P. Nezis, T. Lamark, A.D. Velentzas, T.E. Rusten, G. Bjorkoy, T. Johansen, I.S. Papassideri, D.J. Stravopodis, L.H. Margaritis, H. Stenmark, A. Brech, Cell death during *Drosophila melanogaster* early oogenesis is mediated through autophagy, Autophagy 5 (2009) 298–302.
[76] J.R. Huh, I. Foe, I. Muro, C.H. Chen, J.H. Seol, S.J. Yoo, M. Guo, J.M. Park, B.A. Hay, The *Drosophila* inhibitor of apoptosis (IAP) DIAP2 is dispensable for cell survival, required for the innate immune response to gram-negative bacterial infection, and can be negatively regulated by the reaper/hid/grim family of IAP-binding apoptosis inducers, J. Biol. Chem. 282 (2007) 2056–2068.
[77] V. Gesellchen, D. Kuttenkeuler, M. Steckel, N. Pelte, M. Boutros, An RNA interference screen identifies Inhibitor of Apoptosis Protein 2 as a regulator of innate immune signalling in *Drosophila*, EMBO Rep. 6 (2005) 979–984.
[78] F. Leulier, N. Lhocine, B. Lemaitre, P. Meier, The *Drosophila* inhibitor of apoptosis protein DIAP2 functions in innate immunity and is essential to resist gram-negative bacterial infection, Mol. Cell Biol. 26 (2006) 7821–7831.
[79] A. Kleino, S. Valanne, J. Ulvila, J. Kallio, H. Myllymaki, H. Enwald, S. Stoven, M. Poidevin, R. Ueda, D. Hultmark, B. Lemaitre, M. Ramet, Inhibitor of apoptosis 2 and TAK1-binding protein are components of the *Drosophila* Imd pathway, EMBO J. 24 (2005) 3423–3434.
[80] G. Jones, D. Jones, L. Zhou, H. Steller, Y. Chu, Deterin, a new inhibitor of apoptosis from *Drosophila melanogaster*, J. Biol. Chem. 275 (2000) 22157–22165.
[81] K. White, M.E. Grether, J.M. Abrams, L. Young, K. Farrell, H. Steller, Genetic control of programmed cell death in *Drosophila*, Science 264 (1994) 677–683.
[82] P. Chen, W. Nordstrom, B. Gish, J.M. Abrams, Grim, a novel cell death gene in *Drosophila*, Genes Dev. 10 (1996) 1773–1782.
[83] M.E. Grether, J.M. Abrams, J. Agapite, K. White, H. Steller, The head involution defective gene of *Drosophila melanogaster* functions in programmed cell death, Genes Dev. 9 (1995) 1694–1708.
[84] J.P. Wing, L.M. Schwartz, J.R. Nambu, The RHG motifs of *Drosophila* Reaper and Grim are important for their distinct cell death-inducing abilities, Mech. Dev. 102 (2001) 193–203.
[85] K. Foley, L. Cooley, Apoptosis in late stage *Drosophila* nurse cells does not require genes within the H99 deficiency, Development 125 (1998) 1075–1082.
[86] T. Tenev, A. Zachariou, R. Wilson, A. Paul, P. Meier, Jafrac2 is an IAP antagonist that promotes cell death by liberating Dronc from DIAP1, EMBO J. 21 (2002) 5118–5129.
[87] A. Christich, S. Kauppila, P. Chen, N. Sogame, S.I. Ho, J.M. Abrams, The damage-responsive *Drosophila* gene sickle encodes a novel IAP binding protein similar to but distinct from reaper, grim, and hid, Curr. Biol. 12 (2002) 137–140.
[88] J.P. Wing, J.S. Karres, J.L. Ogdahl, L. Zhou, L.M. Schwartz, J.R. Nambu, *Drosophila* sickle is a novel grim-reaper cell death activator, Curr. Biol. 12 (2002) 131–135.
[89] M. Challa, S. Malladi, B.J. Pellock, D. Dresnek, S. Varadarajan, Y.W. Yin, K. White, S.B. Bratton, Drosophila Omi, a mitochondrial-localized IAP antagonist and proapoptotic serine protease, EMBO J. 26 (2007) 3144–3156.
[90] T. Igaki, Y. Suzuki, N. Tokushige, H. Aonuma, R. Takahashi, M. Miura, Evolution of mitochondrial cell death pathway: Proapoptotic role of HtrA2/Omi in *Drosophila*, Biochem. Biophys. Res. Commun. 356 (2007) 993–997.
[91] F.S. Khan, M. Fujioka, P. Datta, T. Fernandes-Alnemri, J.B. Jaynes, E.S. Alnemri, The interaction of DIAP1 with dOmi/HtrA2 regulates cell death in *Drosophila*, Cell Death Differ. 15 (2008) 1073–1083.
[92] S.M. Srinivasula, P. Datta, M. Kobayashi, J.W. Wu, M. Fujioka, R. Hegde, Z. Zhang, R. Mukattash, T. Fernandes-Alnemri, Y. Shi, J.B. Jaynes, E.S. Alnemri, Sickle, a novel *Drosophila* death gene in the reaper/hid/grim region, encodes an IAP-inhibitory protein, Curr. Biol. 12 (2002) 125–130.
[93] R. Hays, L. Wickline, R. Cagan, Morgue mediates apoptosis in the *Drosophila melanogaster* retina by promoting degradation of DIAP1, Nat. Cell Biol. 4 (2002) 425–431.
[94] H.D. Ryoo, A. Bergmann, H. Gonen, A. Ciechanover, H. Steller, Regulation of *Drosophila* IAP1 degradation and apoptosis by reaper and ubcD1, Nat. Cell Biol. 4 (2002) 432–438.
[95] S.J. Yoo, J.R. Huh, I. Muro, H. Yu, L. Wang, S.L. Wang, R.M. Feldman, R.J. Clem, H.A. Muller, B.A. Hay, Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms, Nat. Cell Biol. 4 (2002) 416–424.
[96] S.N. Willis, J.M. Adams, Life in the balance: how BH3-only proteins induce apoptosis, Curr. Opin. Cell Biol. 17 (2005) 617–625.
[97] T. Igaki, H. Kanuka, N. Inohara, K. Sawamoto, G. Nunez, H. Okano, M. Miura, Drob-1, a *Drosophila* member of the Bcl-2/CED-9 family that promotes cell death, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 662–667.
[98] P.A. Colussi, L.M. Quinn, D.C. Huang, M. Coombe, S.H. Read, H. Richardson, S. Kumar, Debcl, a proapoptotic Bcl-2 homologue, is a component of the *Drosophila melanogaster* cell death machinery, J. Cell Biol. 148 (2000) 703–714.
[99] C.B. Brachmann, O.W. Jassim, B.D. Wachsmuth, R.L. Cagan, The *Drosophila* bcl-2 family member dBorg-1 functions in the apoptotic response to UV-irradiation, Curr. Biol. 10 (2000) 547–550.
[100] H. Zhang, Q. Huang, N. Ke, S. Matsuyama, B. Hammock, A. Godzik, J.C. Reed, *Drosophila* pro-apoptotic Bcl-2/Bax homologue reveals evolutionary conservation of cell death mechanisms, J. Biol. Chem. 275 (2000) 27303–27306.
[101] L. Quinn, M. Coombe, K. Mills, T. Daish, P. Colussi, S. Kumar, H. Richardson, Buffy, a *Drosophila* Bcl-2 protein, has anti-apoptotic and cell cycle inhibitory functions, EMBO J. 22 (2003) 3568–3579.
[102] J.M. Adams, S. Cory, The Bcl-2 protein family: arbiters of cell survival, Science 281 (1998) 1322–1326.
[103] H. Zhang, W. Holzgreve, C. De Geyter, Evolutionarily conserved Bok proteins in the Bcl-2 family, FEBS Lett. 480 (2000) 311–313.
[104] J. Doumanis, L. Dorstyn, S. Kumar, Molecular determinants of the subcellular localization of the *Drosophila* Bcl-2 homologues DEBCL and BUFFY, Cell Death Differ. 14 (2007) 907–915.
[105] N. Senoo-Matsuda, T. Igaki, M. Miura, Bax-like protein Drob-1 protects neurons from expanded polyglutamine-induced toxicity in *Drosophila*, EMBO J. 24 (2005) 2700–2713.
[106] E.A. Sevrioukov, J. Burr, E.W. Huang, H.H. Assi, J.P. Monserrate, D.C. Purves, J.N. Wu, E.J. Song, C.B. Brachmann, *Drosophila* Bcl-2 proteins participate in stress-induced apoptosis, but are not required for normal development, Genesis 45 (2007) 184–193.
[107] K.A. Galindo, W.J. Lu, J.H. Park, J.M. Abrams, The Bax/Bak ortholog in *Drosophila*, Debcl, exerts limited control over programmed cell death, Development 136 (2009) 275–283.
[108] K. Yacobi-Sharon, Y. Namdar, E. Arama, Alternative germ cell death pathway in *Drosophila* involves HtrA2/Omi, lysosomes, and a caspase-9 counterpart, Dev. Cell 25 (2013) 29–42.
[109] J.N. Wu, N. Nguyen, M. Aghazarian, Y. Tan, E.A. Sevrioukov, M. Mabuchi, W. Tang, J.P. Monserrate, K. White, C.B. Brachmann, grim promotes programmed cell death of *Drosophila* microchaete glial cells, Mech. Dev. 127 (2010) 407–417.
[110] L. Galluzzi, I. Vitale, J.M. Abrams, E.S. Alnemri, E.H. Baehrecke, M.V. Blagosklonny, T.M. Dawson, V.L. Dawson, W.S. El-Deiry, S. Fulda, E. Gottlieb, D.R. Green, M.O. Hengartner, O. Kepp, R.A. Knight, S. Kumar, S.A. Lipton, X. Lu, F. Madeo, W. Malorni, P. Mehlen, G. Nunez, M.E. Peter, M. Piacentini, D.C. Rubinsztein, Y. Shi, H.U. Simon, P. Vandenabeele, E. White, J. Yuan, B. Zhivotovsky, G. Melino, G. Kroemer, Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012, Cell Death Differ. 19 (2012) 107–120.
[111] T. Igaki, H. Kanda, Y. Yamamoto-Goto, H. Kanuka, E. Kuranaga, T. Aigaki, M. Miura, Eiger, a TNF superfamily ligand that triggers the *Drosophila* JNK pathway, EMBO J. 21 (2002) 3009–3018.
[112] H. Kanda, T. Igaki, H. Kanuka, T. Yagi, M. Miura, Wengen, a member of the *Drosophila* tumor necrosis factor receptor superfamily, is required for Eiger signaling, J. Biol. Chem. 277 (2002) 28372–28375.
[113] E. Moreno, M. Yan, K. Basler, Evolution of TNF signaling mechanisms: JNK-dependent apoptosis triggered by Eiger, the *Drosophila* homolog of the TNF superfamily, Curr. Biol. 12 (2002) 1263–1268.
[114] S. Kauppila, W.S. Maaty, P. Chen, R.S. Tomar, M.T. Eby, J. Chapo, S. Chew, N. Rathore, S. Zachariah, S.K. Sinha, J.M. Abrams, P.M. Chaudhary, Eiger and its receptor, Wengen, comprise a TNF-like system in *Drosophila*, Oncogene 22 (2003) 4860–4867.
[115] H. Kanda, T. Igaki, H. Okano, M. Miura, Conserved metabolic energy production pathways govern Eiger/TNF-induced nonapoptotic cell death, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 18977–18982.
[116] C.Y. Lee, E.H. Baehrecke, Genetic regulation of programmed cell death in *Drosophila*, Cell Res. 10 (2000) 193–204.
[117] C. Jiang, E.H. Baehrecke, C.S. Thummel, Steroid regulated programmed cell death during *Drosophila* metamorphosis, Development 124 (1997) 4673–4683.
[118] D.L. Berry, E.H. Baehrecke, Growth arrest and autophagy are required for salivary gland cell degradation in *Drosophila*, Cell 131 (2007) 1137–1148.
[119] H.E. Thomas, H.G. Stunnenberg, A.F. Stewart, Heterodimerization of the *Drosophila* ecdysone receptor with retinoid X receptor and ultraspiracle, Nature 362 (1993) 471–475.
[120] T.P. Yao, W.A. Segraves, A.E. Oro, M. McKeown, R.M. Evans, *Drosophila* ultraspiracle modulates ecdysone receptor function via heterodimer formation, Cell 71 (1992) 63–72.
[121] V.P. Yin, C.S. Thummel, A. Bashirullah, Down-regulation of inhibitor of apoptosis levels provides competence for steroid-triggered cell death, J. Cell Biol. 178 (2007) 85–92.
[122] C. Cao, Y. Liu, M. Lehmann, Fork head controls the timing and tissue selectivity of steroid-induced developmental cell death, J. Cell Biol. 176 (2007) 843–852.
[123] E.H. Baehrecke, C.S. Thummel, The *Drosophila* E93 gene from the 93F early puff displays stage- and tissue-specific regulation by 20-hydroxyecdysone, Dev. Biol. 171 (1995) 85–97.
[124] K.C. Burtis, C.S. Thummel, C.W. Jones, F.D. Karim, D.S. Hogness, The *Drosophila* 74EF early puff contains E74, a complex ecdysone-inducible gene that encodes two ets-related proteins, Cell 61 (1990) 85–99.
[125] C.S. Thummel, K.C. Burtis, D.S. Hogness, Spatial and temporal patterns of E74 transcription during *Drosophila* development, Cell 61 (1990) 101–111.
[126] P.R. DiBello, D.A. Withers, C.A. Bayer, J.W. Fristrom, G.M. Guild, The *Drosophila* Broad-Complex encodes a family of related proteins containing zinc fingers, Genetics 129 (1991) 385–397.
[127] D. Cakouros, T. Daish, D. Martin, E.H. Baehrecke, S. Kumar, Ecdysone-induced expression of the caspase DRONC during hormone-dependent programmed cell death in *Drosophila* is regulated by Broad-Complex, J. Cell Biol. 157 (2002) 985–995.
[128] C.Y. Lee, D.P. Wendel, P. Reid, G. Lam, C.S. Thummel, E.H. Baehrecke, E93 directs steroid-triggered programmed cell death in *Drosophila*, Mol. Cell 6 (2000) 433–443.
[129] C. Jiang, A.F. Lamblin, H. Steller, C.S. Thummel, A steroid-triggered transcriptional hierarchy controls salivary gland cell death during *Drosophila* metamorphosis, Mol. Cell 5 (2000) 445–455.

[130] T.J. Daish, D. Cakouros, S. Kumar, Distinct promoter regions regulate spatial and temporal expression of the *Drosophila* caspase dronc, Cell Death Differ. 10 (2003) 1348–1356.
[131] D. Cakouros, T.J. Daish, S. Kumar, Ecdysone receptor directly binds the promoter of the *Drosophila* caspase dronc, regulating its expression in specific tissues, J. Cell Biol. 165 (2004) 631–640.
[132] J. Broadus, J.R. McCabe, B. Endrizzi, C.S. Thummel, C.T. Woodard, The *Drosophila* beta FTZ-F1 orphan nuclear receptor provides competence for stage-specific responses to the steroid hormone ecdysone, Mol. Cell 3 (1999) 143–149.
[133] Z. Yang, D.J. Klionsky, Eaten alive: a history of macroautophagy, Nat. Cell Biol. 12 (2010) 814–822.
[134] C.Y. Lee, E.A. Clough, P. Yellon, T.M. Teslovich, D.A. Stephan, E.H. Baehrecke, Genome-wide analyses of steroid- and radiation-triggered programmed cell death in *Drosophila*, Curr. Biol. 13 (2003) 350–357.
[135] S.M. Gorski, S. Chittaranjan, E.D. Pleasance, J.D. Freeman, C.L. Anderson, R.J. Varhol, S.M. Coughlin, S.D. Zuyderduyn, S.J. Jones, M.A. Marra, A SAGE approach to discovery of genes involved in autophagic cell death, Curr. Biol. 13 (2003) 358–363.
[136] Z.E. Kilpatrick, D. Cakouros, S. Kumar, Ecdysone-mediated up-regulation of the effector caspase DRICE is required for hormone-dependent apoptosis in *Drosophila* cells, J. Biol. Chem. 280 (2005) 11981–11986.
[137] D. Cakouros, T.J. Daish, K. Mills, S. Kumar, An arginine-histone methyltransferase, CARMER, coordinates ecdysone-mediated apoptosis in *Drosophila* cells, J. Biol. Chem. 279 (2004) 18467–18471.
[138] D. Cakouros, K. Mills, D. Denton, A. Paterson, T. Daish, S. Kumar, dLKR/SDH regulates hormone-mediated histone arginine methylation and transcription of cell death genes, J. Cell Biol. 182 (2008) 481–495.
[139] V.A. Francis, A. Zorzano, A.A. Teleman, dDOR is an EcR coactivator that forms a feed-forward loop connecting insulin and ecdysone signaling, Curr. Biol. 20 (2010) 1799–1808.
[140] C.Y. Lee, B.A. Cooksey, E.H. Baehrecke, Steroid regulation of midgut cell death during *Drosophila* development, Dev. Biol. 250 (2002) 101–111.
[141] D. Denton, T.K. Chang, S. Nicolson, B. Shravage, R. Simin, E.H. Baehrecke, S. Kumar, Relationship between growth arrest and autophagy in midgut programmed cell death in *Drosophila*, Cell Death Differ. 19 (2012) 1299–1307.
[142] V. Cavaliere, C. Taddei, G. Gargiulo, Apoptosis of nurse cells at the late stages of oogenesis of *Drosophila melanogaster*, Dev. Genes Evol. 208 (1998) 106–112.
[143] I.P. Nezis, D.J. Stravopodis, I. Papassideri, M. Robert-Nicoud, L.H. Margaritis, Stage-specific apoptotic patterns during *Drosophila* oogenesis, Eur. J. Cell Biol. 79 (2000) 610–620.
[144] J.S. Peterson, B.P. Bass, D. Jue, A. Rodriguez, J.M. Abrams, K. McCall, Noncanonical cell death pathways act during *Drosophila* oogenesis, Genesis 45 (2007) 396–404.
[145] J.S. Peterson, M. Barkett, K. McCall, Stage-specific regulation of caspase activity in drosophila oogenesis, Dev. Biol. 260 (2003) 113–123.
[146] I.P. Nezis, B.V. Shravage, A.P. Sagona, T. Lamark, G. Bjorkoy, T. Johansen, T.E. Rusten, A. Brech, E.H. Baehrecke, H. Stenmark, Autophagic degradation of dBruce controls DNA fragmentation in nurse cells during late *Drosophila melanogaster* oogenesis, J. Cell Biol. 190 (2010) 523–531.
[147] J.M. Barth, J. Szabad, E. Hafen, K. Kohler, Autophagy in *Drosophila* ovaries is induced by starvation and is required for oogenesis, Cell Death Differ. 18 (2011) 915–924.
[148] T.L. Pritchett, E.A. Tanner, K. McCall, Cracking open cell death in the *Drosophila* ovary, Apoptosis 14 (2009) 969–979.
[149] E. Arama, J. Agapite, H. Steller, Caspase activity and a specific cytochrome C are required for sperm differentiation in *Drosophila*, Dev. Cell 4 (2003) 687–697.
[150] E. Arama, M. Bader, M. Srivastava, A. Bergmann, H. Steller, The two *Drosophila* cytochrome C proteins can function in both respiration and caspase activation, EMBO J. 25 (2006) 232–243.