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Cracking open cell death in the *Drosophila* ovary

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Abstract

The *Drosophila melanogaster* ovary is a powerful yet simple system with only a few cell types. Cell death in the ovary can be induced in response to multiple developmental and environmental signals. These cell deaths occur at distinct stages of oogenesis and involve unique mechanisms utilizing apoptotic, autophagic and perhaps necrotic processes. In this review, we summarize recent progress characterizing cell death mechanisms in the fly ovary.

Keywords

Drosophila; oogenesis; apoptosis; autophagy; caspase; insulin

Cell death pathways in *Drosophila melanogaster*

Programmed cell death (PCD) is an essential process in *Drosophila* development. Vast numbers of cells die during embryogenesis and imaginal disc differentiation, and entire structures are destroyed during pupal metamorphosis. Extensive cell death also occurs in the adult female during oogenesis as part of normal development and in response to poor environmental conditions.

The major forms of cell death are apoptosis, autophagic cell death, and necrosis [reviewed in 1]. Apoptosis is characterized by condensation and blebbing of nuclei and cytoplasm, whereas autophagic cell death is associated with autophagosomes, double membraned vesicles that surround cellular components. Necrosis is characterized by organelle swelling and lysis. All three forms of cell death have been reported in *Drosophila*. The majority of cell deaths during embryogenesis and imaginal disc differentiation occur by apoptosis, whereas some cell deaths during pupation are autophagic. To date, necrosis has only been described in mutant or pathological situations in *Drosophila* [2, 3].

Apoptosis in *Drosophila* is typically initiated by the expression of *reaper* (*rpr*), *head involution defective* (*hid*), *grim*, and/or *sickle* (*skl*), which encode inhibitor of apoptosis protein (IAP) binding proteins [reviewed in 4, 5]. These proteins inhibit DIAP1 (*Drosophila* IAP1, also known as Thread), which acts to suppress caspase activity in healthy cells. Once DIAP1 is inhibited, caspases are activated and apoptosis ensues. Analysis of caspase mutants has shown that the critical caspases during embryonic apoptosis are Dronc and Drice [reviewed in 4]. Dronc is an initiator caspase which interacts with the adaptor protein Ark, and Drice is an effector caspase that is activated by Dronc. Similarly, a Hid-Dronc-Drice cascade operates during eye differentiation later in development [6, 7].

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Autophagic cell death in *Drosophila* is best characterized in the salivary gland which is degraded during pupal metamorphosis. PCD of the salivary glands utilizes components of apoptosis, including Rpr, Hid, and caspases [8, 9]. Additionally, autophagosomes form during salivary gland cell death, and cell death is disrupted in mutants defective for autophagy [10]. Little is known about the genes involved in necrosis in *Drosophila*, however in other systems calcium signaling and lysosomal cathepsins have been shown to be important [reviewed in 11].

The *Drosophila* ovary

Most cell deaths in the *Drosophila* ovary occur by pathways distinct from those described in other tissues, indicating novel mechanisms of cell death in the ovary. Each *Drosophila* ovary is composed of approximately fifteen ovarioles, chains of developing egg chambers (Fig. 1) [reviewed in 12–14]. Egg chambers are sixteen-cell germline cysts surrounded by up to a thousand somatic follicle cells. Germline and somatic stem cells reside in the most anterior region of the ovariole, a region called the germarium (Fig. 1). Egg chambers move out of the germarium progressing through fourteen defined stages of oogenesis. Early in egg chamber development within the germarium, one of the germline cells is specified to differentiate as an oocyte, and the remaining fifteen cells develop as polyploid nurse cells. The nurse cells supply the oocyte with nutrients, organelles, mRNAs, and proteins needed throughout oogenesis and early embryonic development. The somatic follicle cells are required for proper axis specification of the oocyte and synthesis of yolk, vitelline membrane and chorion.

The first example of germline cell death occurs early in embryonic development when primordial germ cells (PGCs) that fail to coalesce into the gonad undergo PCD [reviewed in 15]. Interestingly, these “lost” PGCs undergo cell death independent of the major embryonic cell death regulators, *rpr*, *hid* and *grim* [16], similar to germline cell death in the adult (discussed below). Additionally, these cell deaths cannot be blocked by the expression of caspase inhibitors. Positive effectors of PGC death are *wunen* and *wunen-2* which encode lipid phosphate phosphatases, *p53* which is an ortholog of the mammalian tumor suppressor, and *outsiders* which encodes a putative monocarboxylate transporter [16, 17].

In the adult female fly, cell death occurs sporadically within the germarium and during mid-stages of oogenesis (stages 7–9) [reviewed in 18]. Cell death in these regions increases in response to poor nutrition, which can be induced experimentally by withholding yeast as a protein source. It is thought that these cell deaths are induced following a “checkpoint” where environmental and nutritional inputs determine whether an egg chamber will progress into vitellogenesis, the yolk deposition that occurs later in oogenesis [19]. Interestingly, chemical exposure or developmental insults induce cell death specifically in mid-oogenesis, suggesting that this stage is poised to undergo PCD. Cell death also occurs late in oogenesis as part of normal oocyte development, as the fifteen nurse cells and follicle cells are eliminated by PCD. These distinct examples of cell death are reviewed individually in the following sections, with a focus on recent findings.

The first checkpoint – Germarium

Ovaries from nutrient-deprived flies are significantly reduced in size compared to ovaries from flies that have been conditioned on food supplemented with yeast. This reduction in size is due to effects on cell proliferation as well as PCD [19]. Based on the TUNEL assay, nutrient-deprived flies show an increase in apoptosis in the germarium, primarily germline cells in region 2 where follicle cells begin to surround a germline cyst (Fig. 2a) [19]. Cell death is thought to serve as a mechanism to maintain the proper number of follicle cells that are needed to surround a germline cyst during oogenesis [19]. These findings imply that nutrient sensing pathways regulate the checkpoint in region 2 of the germarium.

A primary pathway for nutrient sensing is the insulin-mediated phosphoinositide kinase-3 (PI3K) pathway. In *Drosophila*, there are seven insulin-like peptides (Dilps) that interact with the insulin receptor (InR) [20]. Mutations in positive regulators of the *Drosophila* insulin-mediated PI3K pathway result in a reduction in body size and sterility [21–25]. This pathway may mediate nutrient responses in the germarium and thus negatively regulate cell death. Both InR and the insulin receptor substrate Chico have been shown to be required for follicle cell proliferation, although effects on cell death in the germarium have not been investigated [19, 26]. Target of Rapamycin (Tor) is a downstream target of the PI3K pathway, but may also be activated by other mechanisms [27]. Homozygous *tor* escapers are reduced in size compared to wild-type and have increased acridine orange positive puncta in germaria, indicating that cell death is increased [28]. The combined data from InR, Chico and Tor suggest that mutants in the insulin mediated PI3K pathway may mimic nutrient deprivation by affecting cell proliferation and cell death in germaria.

Recent findings indicate that nutrient deprivation leads to autophagy induction as well as cell death in the germarium, perhaps in a mechanism similar to the *Drosophila* salivary gland [9]. The genes required for autophagy were identified in yeast and are well-conserved in *Drosophila* and other metazoans [reviewed in 29]. The formation of autophagosomes and autolysosomes has been visualized in germaria by punctate staining of LysoTracker, or *Drosophila* Atg8a or the human ortholog LC3 fused to fluorescent tags [30, 31]. Germaria from well-fed flies have low levels of these markers in germaria [30], whereas nutrient-deprived flies display puncta in region 2 of many germaria [30]. Electron micrographs of degenerating germline cysts display numerous autophagosomes [31]. Taken together, these findings indicate that germline cyst cells undergo autophagic cell death in region 2. It is important to note that region 2 is also the area where apoptosis has been observed [19].

Caspases, proteases associated with apoptotic cell death, are required during salivary gland degradation along with the autophagy machinery [9] and a similar mechanism may be at work in the ovary. Active caspases can be detected in region 2 of the germarium using an anti-active caspase-3 antibody, and mutants lacking the effector caspase Dcp-1 display decreased levels of DNA fragmentation and autophagy in region 2 compared to wild-type [30]. Interestingly, mutants of autophagy genes *atg7* and *atg1* have decreased levels of DNA fragmentation in region 2 of the germarium compared to wild-type [30, 31]. These data indicate that autophagy and the caspase Dcp-1 are required for nutrient deprivation-induced PCD in the germarium.

The second checkpoint – mid-oogenesis

In addition to the germarium, mid-stage egg chambers from nutrient-deprived flies undergo cell death, characterized by nurse cell nuclear condensation and fragmentation, and engulfment by follicle cells (Fig. 2b,d) [reviewed in 18]. Numerous other stimuli can influence cell death at mid-oogenesis, including developmental abnormalities, chemical treatment, temperature, mating, and daylength [reviewed in 18]. Even modern hazards like cocaine exposure and cellular phone radiation can induce cell death in mid-oogenesis [32, 33].

A survey of known cell death genes has revealed a novel pathway in mid-oogenesis compared to other *Drosophila* tissues undergoing PCD. Cell death in most *Drosophila* tissues is highly regulated by the IAP binding proteins Rpr, Hid, Grim and Skl [reviewed in 4, 5]. Interestingly there is no apparent role for these cell death regulators in mid-oogenesis [34]. When *rpr*, *hid*, *grim* and *skl* were removed simultaneously during nutrient deprivation, mid-stage egg chambers still resembled wild-type animals treated under the same conditions [34]. Similarly, mutants of other cell death regulators *ark*, *debcl*, *p53*, and *eiger*, had no effect on mid-oogenesis cell death. This work implies that the known cell death regulators are not required for mid-oogenesis nutrient deprivation-induced cell death.

A role for the insulin-mediated PI3K pathway in the regulation of egg chamber survival at mid-oogenesis has been suggested by several studies. Heteroallelic mutant combinations of *InR* lead to immature ovaries and egg chambers that remain pre-vitellogenic [35, 36]. Similar phenotypes are seen in *InR* germline clones (GLCs), indicating that InR is required for germline development in a cell autonomous manner [26]. Additionally, *InR* GLCs produce abnormal egg chambers considered to be degenerating [26]. Homozygous mutant *chico* flies also produce abnormal egg chambers described as degenerating [19]. Similarly, egg chambers from *tor* homozygous escapers fail to develop to post-vitellogenic stages [28]. These findings suggest a negative regulation of apoptosis by the insulin pathway, but evidence that the terminal egg chambers in these mutants are apoptotic has yet to be shown.

Survival beyond mid-oogenesis is also regulated by the hormones 20-hydroxyecdysone (20E) and juvenile hormone (JH) [reviewed in 18, 37]. Increased levels of 20E are seen following nutrient deprivation and ectopic 20E can induce egg chamber degeneration, suggesting that 20E induces PCD [38]. Paradoxically, GLCs of the ecdysone receptor, or its target E75, also degenerate in mid-oogenesis, indicating that signaling by 20E is required for egg chamber survival [39]. These findings can be reconciled by a model that a proper balance between JH and 20E is required for survival in mid-oogenesis [37]. Alternatively, a threshold level of 20E may determine the outcome in mid-oogenesis [38]. Known 20E target genes E74, E75 and BR-C show dynamic expression changes in mid-oogenesis, with some isoforms increasing and others decreasing expression in response to nutrient-deprivation [39–41]. These target genes also regulate each other and can be pro-or anti-apoptotic [40, 41]. To identify additional target genes of nutrient-deprivation and hormonal signaling in the ovary, gene expression profiling via microarray has been carried out [42]. Changes in expression were detected for cell death and hormone-related genes, as well as components of insulin-mediated PI3K signaling and stress response genes in the c-Jun N terminal kinase (JNK) pathway. InR is required for proper levels of JH and 20E [43], indicating that there is crosstalk between these pathways, as has been shown in other tissues [44].

Mid-stage egg chambers from nutrient-deprived flies display hallmarks of autophagy like those seen in the germarium. Degenerating mid-stage egg chambers accumulate autophagosomes, based on fluorescent markers (Fig. 2f) [30, 31]. Autophagosomes have also been observed in electron micrographs of degenerating mid-stage egg chambers from *Drosophila virilis* [45, 46]. Mutant *atg7* flies or *atg1* GLCs that have been nutrient-deprived show a decrease in LysoTracker staining at mid-oogenesis and reduced levels of TUNEL-positive staining, indicating a block in DNA fragmentation even though nurse cell chromatin condensed normally [30, 31]. These data suggest that autophagy may play a role in DNA fragmentation but not in chromatin condensation during PCD in mid-oogenesis.

During apoptosis, caspases play an essential role in the execution of cell death. Expression studies have revealed a high level of caspase activity in mid-oogenesis cell death (Fig. 2e) [47, 48]. There are three initiator and four effector caspases in *Drosophila* [4]. Homozygous mutants of the effector caspase *dcp-1* have a striking block in mid-oogenesis germline PCD in response to nutrient-deprivation (Fig. 2c) [49]. The ovaries of these mutants accumulate a number of egg chambers in which the follicle cells have died and the nurse cells remain intact, a phenotype referred to as “bald” or “peas without pods” (pwop) [48, 50]. Similar to the germarium, there appears to be a requirement for the caspase Dcp-1 for the induction of autophagy in mid-oogenesis. In *dcp-1* null mutants, LysoTracker and punctate *GFP-LC3* staining are decreased in pwop egg chambers compared to degenerating wild-type egg chambers [30]. Ectopic expression of *dcp-1* is sufficient to induce mid-stage degeneration accompanied by autophagy [30]. These results suggest that the caspase Dcp-1 acts to promote autophagic cell death of the germline in mid-oogenesis.

Further evidence for a caspase requirement in mid-oogenesis has come from studies of caspase inhibitors. Nutrient-deprived flies over-expressing the caspase inhibitors DIAP1 or p35, show the same phenotype as *dcp-1* mutants, suggesting that DIAP1 normally keeps Dcp-1 in check during mid-oogenesis [47, 48, 50]. DIAP1 shows dynamic expression changes in mido-ogenesis, suggesting that its levels may be carefully regulated during this stage. *diap1* mRNA and protein levels are reduced in mid-oogenesis even in healthy egg chambers [50, 51]. This down-regulation of *diap1* may be what makes mid-oogenesis highly susceptible to cell death stimuli.

Multiple caspases usually participate in cell death, with a typical initiator-effector caspase cascade [reviewed in 4]. To determine which caspases might act upstream of *dcp-1*, the three initiator caspases were investigated. Mid-oogenesis cell death occurred normally in all of the single initiator caspase mutants [50]. However, double *strica; dronc* mutants displayed a moderate pwop phenotype, indicating a redundant function for these two caspases in mid-oogenesis PCD. Unlike *dcp-1*, they did not show a complete block in cell death, suggesting that the effector caspase Dcp-1 can be activated by another mechanism. Taken together, these findings indicate that a novel caspase-dependent autophagic cell death pathway acts in mid-oogenesis PCD.

Developmental nurse cell death

In late oogenesis at stage 11, nurse cells transfer their cytoplasmic contents to the oocyte through cytoplasmic bridges called ring canals, in a process called “dumping” (Fig. 3a–c) [13]. Drastic cytoplasmic changes occur during dumping, including the formation of unique actin bundles that extend from the plasma membrane to the nuclear envelope [52]. After dumping, the nurse cell nuclei and other remnants are removed through cell death (Fig. 3d). Nurse cell nuclear breakdown is initiated about the same time as dumping [53], but it is not known if the cytoskeleton changes that occur concomitantly with the demise of the nurse cells are regulated by the same mechanism. The degradation of nurse cell components as cell death occurs would probably be detrimental to the survival of the adjacent oocyte, and it is unknown how the oocyte is protected. These characteristics make developmental nurse cell death a unique process.

A number of mutants that disrupt nurse cell cytoplasm transfer have been described. Many of these “dumpless” mutants (Fig. 3f) disrupt cytoskeletal genes, which do not affect the initiation of nurse cell nuclear breakdown, although final DNA fragmentation of nurse cell nuclei is delayed [51, 53, 54]. Pathways that control dumping upstream of the cytoskeletal proteins are less clear. Genetic analysis over a decade ago implicated the BMP receptor Saxophone in nurse cell dumping [55], but further analysis has not been done. A “dumpless” phenotype was initially attributed to mutants of *dcp-1*, demonstrating a potential link between dumping and cell death, but this phenotype is now known to be caused by disruption of the neighboring gene *pita* [49, 56]. In subsequent studies, *dcp-1* mutants were found to show a complete block in mid-oogenesis cell death but only a mild block in nurse cell nuclear clearance in late oogenesis [49, 50]. *pita*, also known as *spotted-dick*, encodes a Zn-finger transcription factor required for DNA replication [57], potentially implicating cell cycle regulation in the control of dumping. Consistent with this hypothesis, GLCs of the cell cycle regulator *E2F* produce a dumpless phenotype [58, 59]. However, both *E2F* and *pita* GLCs show additional defects, suggesting that their effects on dumping could be indirect.

The cell death mechanism that removes the nurse cells is different from canonical cell death mechanisms in the fly. Similar to mid-oogenesis cell death, the IAP binding proteins are not required for late oogenesis nurse cell death [34, 51]. Surprisingly, the requirement for caspases in nurse cell death appears to be minimal. In a wild-type fly, a small percentage of mature stage

14 egg chambers show the persistence of any nurse cell nuclei, whereas in flies overexpressing the caspase inhibitors DIAP1 or p35, up to a third of stage 14 egg chambers have some persisting nurse cell nuclei (Fig. 3e) [50]. Similar frequencies of persisting nuclei have been observed in certain caspase mutant combinations as well as GLCs of *ark* [34, 50]. Contradictory results were obtained with a caspase peptide inhibitor [60], however these inhibitors are known to have off-target effects [61]. Overall, these findings suggest that degradation of the nurse cells can occur largely independently of caspases and other known apoptosis genes [50]. In general, mutants in the apoptotic cascade result in only a mild disruption to nurse cell PCD, suggesting that other cell death mechanisms are acting in conjunction with apoptosis, or compensating when apoptosis is inhibited.

The minor requirement for the caspases suggests there are other players that have not been identified. *longitudinals lacking* (*lola*), which encodes a BTB protein previously reported to be involved in axon guidance, was identified in a forward genetics screen for effectors of late oogenesis cell death [62]. *lola* GLCs show a block in nurse cell chromatin condensation and DNA fragmentation, as well as effects on dumping. *lola* has been shown to interact with JIL-1, a chromosomal kinase, which affects the nuclear lamina [63, 64]. Mutants of *lola* or *jil-1* show abnormal nuclear lamin morphology, suggesting a role for *lola*, *jil-1*, and nuclear lamins in chromatin condensation during developmental nurse cell death [62]. *lola* GLCs also show defects in chromatin condensation and DNA fragmentation during mid-oogenesis PCD, suggesting that *lola* affects mechanisms common to both mid- and late oogenesis PCD.

Following chromatin condensation of nurse cell nuclei in late oogenesis, DNA fragmentation occurs [51, 56, 64, 65]. DNA fragmentation is generally thought to be a two step process during apoptotic cell death [66–68]. Caspases activate CAD (caspase activated DNase) by cleaving its inhibitor, ICAD. CAD then localizes to the nucleus and cleaves DNA between nucleosomes. DNase II, acting within engulfing cells, subsequently breaks down DNA into nucleotides. DNase II is an acidic DNase with the highest activity in acidic environments such as lysosomes. Disruption of *Drosophila* CAD blocks nucleosomal fragmentation but has no apparent effect on clearance of nurse cell nuclei in late oogenesis [69]. However, *DNase II* mutants have a persisting nurse cell nuclei phenotype in late oogenesis [69] and recent findings indicate that DNase II is required cell-autonomously in the dying nurse cells [70]. This suggests that the two step model of DNA fragmentation can probably apply in nurse cell death, with a slight twist. Caspases may activate CAD to cleave chromatin between nucleosomes, followed by DNase II activity in the dying nurse cell. Considering that DNase II is an acid nuclease, the cell autonomous role for DNase II suggests a role for lysosomes or acidic conditions within the dying nurse cells.

Lysosomes are critical for autophagy, and the presence of autophagosomes during late oogenesis has been revealed by transmission electron microscopy of *Drosophila virilis* late stage egg chambers [46], suggesting that developmental nurse cell PCD occurs by autophagic cell death. Characterization of the autophagic machinery has not yet been reported in developmental nurse cell PCD, however mutants of the lysosomal gene *spinster* have a significant disruption to nurse cell PCD [71]. It is important to note that lysosomes have been shown to be involved in necrosis as well as autophagic cell death [11]. Necrosis has always been thought of as an accidental death that occurs when a cell is injured, and had been characterized more as a series of catastrophic events rather than an organized process [reviewed in 11]. In recent years, however, evidence for programmed necrosis is emerging. Examples in *C. elegans*, mammalian cell lines, primate ischemia models, and *Dictyostelium* have shown that necrosis follows a common set of events [11]. These events include an influx of ions or misregulation of ion homeostasis, mitochondrial uncoupling leading to ROS generation and ATP depletion, mitochondrial swelling and perinuclear clustering, lysosomal rupture, and activation of non-caspase proteases such as calpains and lysosomal cathepsins [11].

Interestingly, some of these cellular events have been shown to occur during late oogenesis. There is a release of calcium from nuclear stores early in the dumping process [72] and the transfer of nurse cell mitochondria to the oocyte would be expected to leave the nurse cells largely devoid of an intracellular source of ATP [73]. Further studies are necessary to determine whether developmental nurse cell PCD occurs by necrotic or autophagic PCD or a distinct mechanism.

Follicle Cell Death

In addition to germline cell death, cell death occurs in the somatic follicle cells. There are three types of follicle cells: stalk cells, polar cells and epithelial follicle cells [reviewed in 12]. Compared to germline cell death, follicle cell death is largely uncharacterized, with the exception of the polar cells. The polar cells are clusters of 2–5 follicle cells that are located at the most anterior and posterior region of each egg chamber. Early in oogenesis, excess numbers of polar cells undergo PCD, leaving precisely two polar cells at each end of the oocyte during the later stages of oogenesis [74]. If the polar cells do not undergo PCD, anterior follicle cells that cover the nurse cells fail to thin and stretch properly. Additionally, a specialized group of epithelial follicle cells called the border cells fail to migrate towards the oocyte properly, which would be expected to lead to defective formation of the micropyle, the site of sperm entry [75]. Death of the polar cells is mediated by a canonical apoptosis cascade utilizing Hid, Dronc and Drice [76]. Interestingly, to date this is the only type of cell death in the ovary shown to use this cascade.

The epithelial follicle cells “disappear” following germline cell death in mid-oogenesis and after chorion deposition in late oogenesis. In late oogenesis, only a small subset of anterior follicle cells show any initiation of cell death while still in contact with the oocyte [77]. These follicle cells have condensed chromatin and stain positively for acridine orange, although they do not display caspase-3 activity [78]. These dying follicle cells also contain autophagosomes and autolysosomes. Remnants of the remaining follicle cells are found at the base of the ovary at the entrance of the lateral oviduct [77]. These cells stain positively for acridine orange and show condensed chromatin, and ultimately appear to be engulfed by epithelial cells and macrophages at the oviduct entrance. Taken together, these findings suggest that follicle cell death could occur by an autophagic mechanism that does not utilize caspases, unlike the death of nurse cells in mid-oogenesis.

The genetic control of follicle cell death is largely unknown. A recent paper reports that a specific isoform of the ecdysone receptor (EcR-B1) is required for follicle cell survival in mid-oogenesis [79]. RNAi knockdown of EcR-B1 leads to caspase activation and decreased levels of DIAP1. Given that caspase activation is not thought to occur during normal follicle cell death, these findings indicate that ecdysone signaling may act normally to prevent the apoptosis of follicle cells. EcR-B1-deficient follicle cells also show disruptions to the organization of the epithelial follicle cell monolayer, suggesting the effects on apoptosis could be indirect.

Insight into the novel mechanisms of cell death in both follicle cells and nurse cells comes from a recent study on endocycling cells [80]. During oogenesis, both nurse cells and follicle cells exit mitosis and enter an endocycle where DNA replication continues in the absence of cell division, leading to polyploidy. Work from the Calvi group has shown that ectopic expression of Double-parked (Dup) activates DNA re-replication and apoptosis in most *Drosophila* cells. Follicle cells expressing Dup in early oogenesis undergo apoptosis, however later stage follicle cells that have entered the endocycle fail to undergo apoptosis despite experiencing re-replication and DNA damage. Subsequent analysis revealed that *rpr*, *hid*, *grim* and *skl* could not be induced in endocycling cells, suggesting that these loci are specifically repressed [80]. Further support for this hypothesis comes from a recent study

showing that these loci are epigenetically silenced by Polycomb-mediated repression in late embryogenesis [81]. Taken together these findings suggest that expression of these IAP-binding proteins could be transcriptionally repressed by Polycomb in endocycling cells such as nurse cells and follicle cells. Thus the canonical cell death pathway that utilizes these IAP-binding proteins would not be initiated during PCD of endocycling nurse cells and follicle cells. These findings help to explain why follicle cells and nurse cells use alternative PCD pathways. However, *reaper* and *hid* transcripts have been detected in nurse cells at stage 10 [51], indicating that repression of these genes is at least partially reversible in late stage nurse cells. Perhaps the level of *rpr* and *hid* expression is insufficient to drive cell death of nurse cells, or other downstream targets of the *rpr-hid* cascade are not adequately expressed in late oogenesis.

Evolutionary comparisons

There are intriguing similarities between mammalian and *Drosophila* ovarian cell death. As in flies, mammalian ovarian cell death occurs at multiple yet specific stages of oocyte development [reviewed in 82]. Lost primordial germ cells undergo PCD in mammals as they do in flies. Numerous follicles are destroyed in adult mammals by follicular atresia, a process comparable to mid-oogenesis PCD in flies. The structure of the insect egg chamber with the germline-derived nurse cells is not found in mammals, however mammalian oocytes develop in interconnected cysts early in oocyte development, and this stage is correlated with extensive germ cell death. Analogies have also been made between nurse cells and somatic granulosa cells in mammals [82]. Similar to nurse cells, granulosa cells initiate the cell death process during follicular atresia and have been found to deliver cellular organelles to developing oocytes in some species. Germ cells are also lost at the “pachytene checkpoint” in both *C. elegans* and mammals [82, 83]. Interestingly, in *Drosophila*, pachytene occurs between stages 2 and 3 within the germarium [84], the same region found to exhibit sporadic PCD. Whether any of the PCD observed in the germarium is due to improper synapsis at pachytene remains to be shown. Excessive ovarian cell death in humans is associated with fertility disorders, chemotherapy-induced premature menopause, and poor outcome in assisted reproduction technologies, and is therefore of high clinical significance [reviewed in 82].

As in the *Drosophila ovary*, a balance between survival and death signals regulates follicular atresia in the mammalian ovary [reviewed in 82]. Cell death is induced by withdrawal of survival signals in conjunction with activation of pro-apoptotic signals. Interestingly, follicular atresia is influenced by hormones and the insulin/IGF signaling pathways in both flies and mammals [85]. Similar to *Drosophila*, autophagic cell death and apoptosis may act cooperatively during follicle atresia in the rat and quail [82, 86]. Granulosa cell apoptosis during follicular atresia is mediated by both death receptors and the Bcl-2 proteins [reviewed in 82]. The involvement of multiple mechanisms for the selection of the healthiest oocytes is consistent with findings in the *Drosophila* ovary. Germline cell death is found in many, if not all, animals [18, 87], suggesting that it may play evolutionarily conserved roles in both development and germ cell selection.

Conclusions and future directions

The *Drosophila* ovary provides unique opportunities for the study of cell death. Cell death occurs at distinct stages and in response to diverse stimuli, but in only a small number of cell types. Most intriguingly, these cell deaths occur predominantly by unusual and still largely uncharacterized mechanisms. Genetic control over germline cell death in the germarium and follicle cell death is for the most part a black box, and systematic surveys of cell death mutants could be informative for these examples of PCD. Additionally, very little is known about how

the follicle cells and other cells carry out engulfment of nurse cell and follicle cell remnants after PCD.

Cell death in the germarium and at mid-oogenesis is regulated by nutrient availability. A challenge for the future is to determine how the insulin and ecdysone signaling pathways might interface with the cell death and autophagic machinery in the ovary. In mid-oogenesis, the effector caspase Dcp-1 is essential for germline PCD and DIAP1 levels decrease in dying egg chambers [49, 50]. Interestingly, *Drosophila* salivary gland death utilizes both apoptosis and autophagy, and is also regulated by the PI3K signaling pathway and ecdysone, suggesting that there may be more parallels between ovarian and salivary gland cell death. In the case of the salivary gland, a decrease in PI3K signaling leads to a growth arrest that is necessary for the onset of cell death [10]. It is unknown if such a link exists in the ovary.

However, unlike the salivary gland and other tissues in *Drosophila*, the ovary does not require initiator caspases or the cell death genes *rpr*, *hid*, *grim* and *skl* to bring about cell death [18, 34]. This implies that there are other mechanisms by which Dcp-1 and DIAP1 are regulated in mid-oogenesis. The insulin-mediated PI3K signaling pathway has been shown to regulate cell death in mammalian systems by direct phosphorylation and suppression of Bcl-2 pro-apoptotic family members and caspases [88, 89]. Perhaps a similar mechanism could regulate the activity of Dcp-1 or DIAP1. Another open question is how Dcp-1 regulates autophagy during cell death in the germarium and in mid-oogenesis. Dcp-1 may cleave and activate a component of the autophagic machinery directly, or activate a signaling protein that in turn triggers autophagy.

Developmental nurse cell death in late oogenesis has many unique and interesting characteristics. The nurse cells have a highly specialized function to provide essential components to the oocyte. Once the nurse cells have transferred their contents to the oocyte, their remnants, predominantly the large nurse cell nuclei, are removed. This presents a unique situation that may utilize distinct cell death mechanisms to reach this goal without damaging the oocyte. Canonical cell death components including caspases are only partially involved. Evidence for autophagic and necrotic mechanisms has also emerged but a big question remains as to whether these mechanisms are acting redundantly or in parallel to bring about the destruction of the nurse cell.

Finally, the upstream activators in developmental nurse cell death are still unknown. Several signaling pathways are present at the right time and place to be good candidates, and careful genetic dissection of these pathways may be enlightening. Alternatively, developmental nurse cell death may occur because of the loss of protective factors during the dumping process. In such a situation, the nurse cell nuclei die by extreme neglect, having lost most of their nutrients, proteins and organelles to the oocyte. Further study of cell death in the *Drosophila* ovary will provide insight into the diverse ways that cells die, potentially utilizing all three forms of cell death, apoptosis, autophagy and necrosis.

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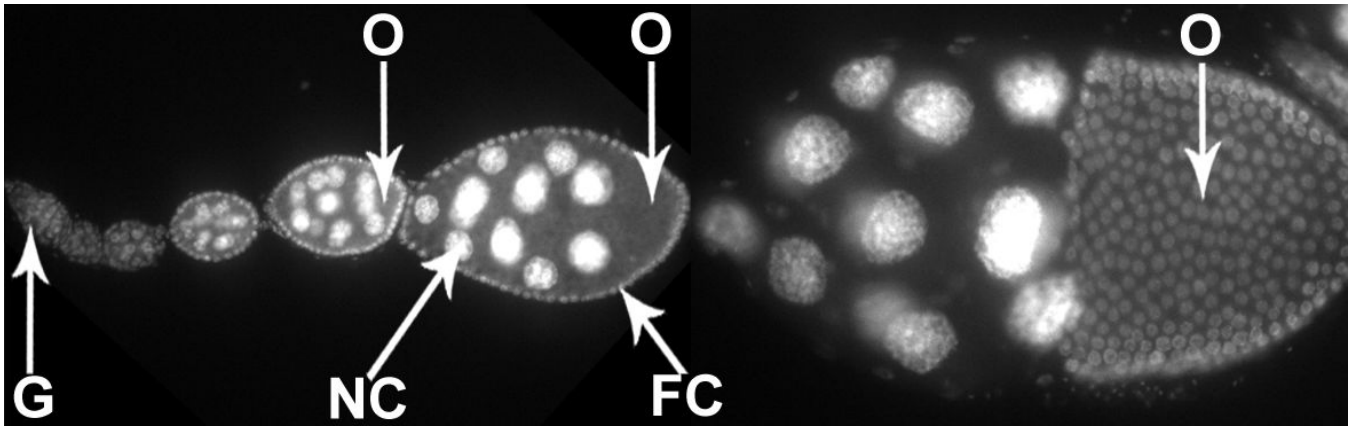

Fig. 1.

Stages of *Drosophila* Oogenesis. Egg chambers stained with 4',6 diamidino-2-phenylindole (DAPI) to label DNA. Anterior portion of ovariole (left) shows early stages starting at the germarium (G). Nurse cells (NC) and follicle cells (FC) are labeled in a stage 8 egg chamber. A stage 10 egg chamber (right) shows that the FC have migrated. The oocyte (O) increases in size as the egg chamber develops. In the most posterior stage 10 egg chamber the oocyte (O) is under the follicle cell layer. All egg chambers at the same magnification.

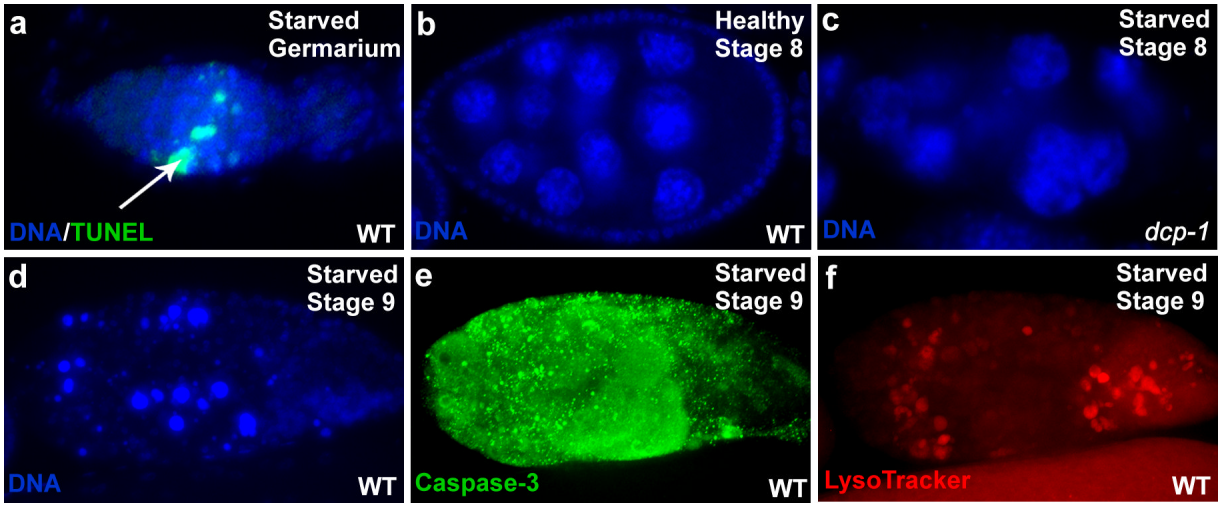

Fig. 2.

Programmed cell death events in early and mid-oogenesis. (a–d) Egg chambers stained with DAPI (blue) to label DNA. (a) A germarium from a nutrient-deprived (Starved) wild-type fly stained with TUNEL (green) to label dying cells. Arrow indicates cells dying in region 2, the central portion of the germarium. (b) Stage 8 healthy egg chamber from a wild-type fly. (c) Abnormal egg chamber from a homozygous *dcp-1*^{prev1} nutrient-deprived fly [49]. The follicle cells have died while the nurse cells remain intact. (d) Degenerating wild-type stage 8 egg chamber shows fragmented and condensed DNA. (e) Caspase activity (green) is noted in the same egg chamber, labeled with an anti-cleaved caspase-3 antibody (Cell Signaling). (f) An induction in autophagy is also shown in the same egg chamber by an increase in LysoTracker (red, Invitrogen). All images at the same magnification.

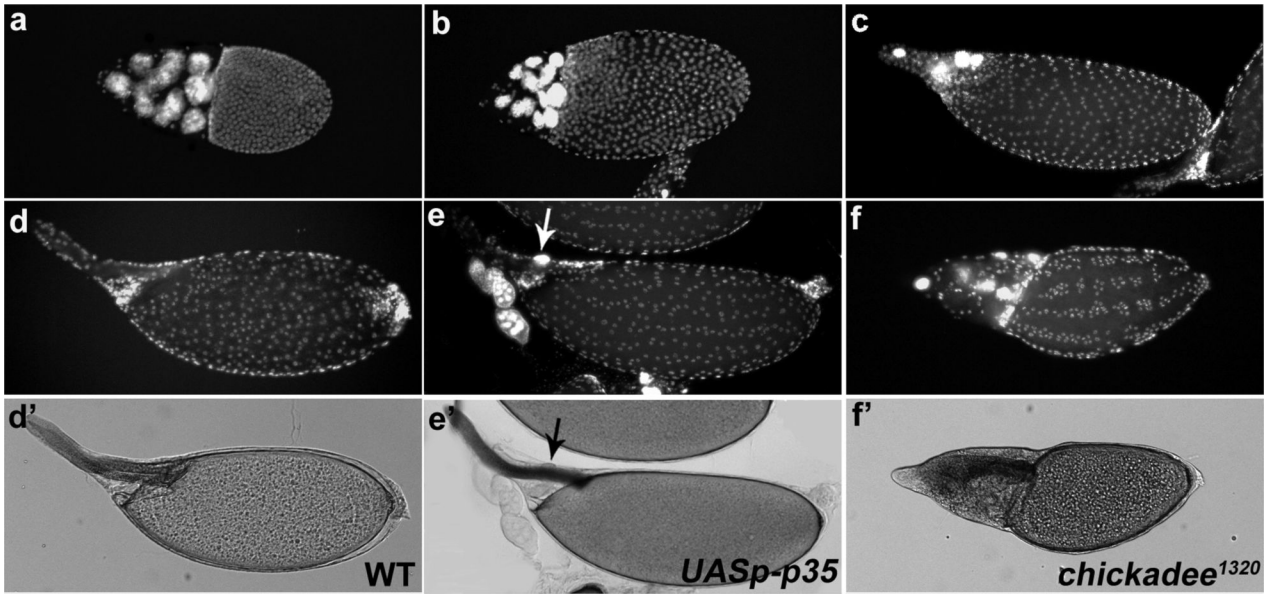

Fig. 3.

Progression and disruption in developmental nurse cell death of late oogenesis. (a–f) Egg chambers stained with DAPI to visualize nurse cell nuclear morphology. (d'–f') DIC images of the same egg chambers in (d–f). (a) Wild-type stage 10 egg chamber prior to dumping. (b) Wild-type stage 11 egg chamber in the process of transferring nurse cell cytoplasm to the oocyte. (c) Wild-type stage 13 egg chamber in which the cytoplasmic transfer is complete, the dorsal appendages are clearly visible but not fully formed, and the nurse cells remnants are dying. (d–d') Wild-type stage 14 egg chamber with no remaining nurse cell remnants and dorsal appendages fully formed. (e–e') *NGT/+;UASp-p35/nosGAL4* [50] stage 14 egg chamber with persisting nurse cell nuclei indicated by arrows. NGT and nosGAL4 are germline-specific GAL4 drivers [90]. (f–f') *chickadee*^{1320} [53] dumpless egg chamber in which nurse cell dumping has not occurred but oocyte development is complete as indicated by fully formed dorsal appendages. All images at the same magnification.

Table 1

Cell death genes and ovarian phenotypes

Mechanism	Gene	Germarium	Mid-oogenesis	Late oogenesis	Mammalian Homology
DNA Fragmentation	*lola*	ND	Strong	Strong	ZBTB3
	dICAD	ND	Moderate	Weak	ICAD
	DNaseII	ND	Moderate	Moderate	DNase II
Apoptosis	*rpr*	ND	wt	wt	SMAC, OMI
	hid	ND	wt	wt	SMAC, OMI
	grim	ND	wt	wt	SMAC, OMI
	skl	ND	wt	wt	SMAC, OMI
	diap1 (over-expression)	ND	Strong	Weak	IAP
	dronc	ND	Weak	Weak	Caspase-9
	drice	ND	Weak	Weak	Caspase-3
	strica	ND	Weak	Weak	Caspase
	dcp-1	Moderate	Strong	Weak	Caspase-3
	ark	ND	wt	wt	Apaf-1
	eiger	ND	wt	wt	TNF family
Insulin-Mediated PI3K Pathway	*InR*	ND	Moderate	N/A	IGFR
	chico	ND	Moderate	N/A	IRS
	tor	Moderate	Moderate	N/A	mTOR
Autophagy	*spinster*	ND	ND	Strong	Spns
	atg1	Moderate	Weak	ND	Ulk
	atg7	Moderate	Weak	ND	ATG7

See text for details. ND not determined; N/A not applicable (ovaries fail to develop); wt, wild-type