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By design or by chance: cell death during *Drosophila* embryogenesis

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Abstract

Cell death plays an essential role during *Drosophila* embryogenesis. However, it remains an enigma as to what mechanisms determine (or select) the specific cells to be eliminated at a particular developmental stage. Is it mostly dependent on the lineage of the cell, signifying genetic predetermination, or is it due to the failure of a cell to compete for growth factors, which is more or less by chance? Recent developments in studying the molecular mechanism of cell death during *Drosophila* embryogenesis has provided much insight into our understanding of the relative importance of, and the interaction between, these two mechanisms in shaping the embryo.

Keywords

Drosophila; Embryogenesis; Trophic factor

Patterns of cell death during embryogenesis

Cell death has long been observed for embryos mutated for genes which govern differentiation and development [1,2]. Systematic analysis of cell death during *Drosophila* embryogenesis in wild type embryos was first carried out by Abrams et al. [3]. Using a combination of vital dyes and electron microscopy, they showed that most cell death during *Drosophila* embryogenesis share the canonic characteristics of apoptosis. The vital dye Acridine Orange (AO) was found especially useful in labeling apoptotic cells in *Drosophila* embryos, although it seems to preferentially label cells in later stage of apoptosis. Often, it also labels the apoptotic bodies phagocytosed by migrating macrophages [3]. AO-positive cells first appear at embryonic stage 11 (about 7 h after egg laying (AEL)) in the precephalic region. However, the AO-labeling pattern quickly spreads to the other segments and reaches a peak level at stages 12 and 13 (8–10 h AEL), when nearly all segments have AO-positive cells. The level of cell death wanes after stage 14, and becomes mainly restricted to the ventral nerve cord at the end stage of embryogenesis (Stage 16–17, after 15 h AEL).

The overall pattern of cell death, as revealed by AO staining or TUNEL, is very dynamic throughout the course of *Drosophila* embryogenesis after 7 h AEL [3,4]. Although the general pattern associated with a particular developmental stage is highly reproducible, the exact number and positions of dying cells at a given point may vary significantly. For instance, the pattern of AO or TUNEL-positive cells in the ventral epidermis between stages 12–14 shows a rough segmentally repeated pattern associated with segment boundaries [4]. However, the

positions and numbers of dying/dead cells are only partially symmetrical on the two sides of the midline.

A genetic screen identified that the genomic region deleted in the H99 deficiency mutant is required for almost all developmental cell death in *Drosophila* embryogenesis [5]. Three genes in this region, *reaper* [5], *hid* [6], and *grim* [7], encode pro-apoptotic proteins that function as IAP (Inhibitor of Apoptosis)-antagonists. These proteins share an IAP-binding motif (IBM), which can bind to IAP and relieve its inhibition on caspases. A 4th IAP-antagonist, *sickle*, reside just upstream of *reaper*, but was not deleted in the H99 deficiency [8–10]. The four IAP-antagonists reside in a ~350 kb region that is highly conserved as a synteny in the sequenced *Drosophila* genomes. With the exception of *hid*, expression of the IAP-antagonist genes appears to be limited to cells destined to die during embryogenesis. The pro-apoptotic function of Hid can be suppressed by the MAP kinase pathway and *hid* is the only one of the four whose mRNA can be detected in cells that do not die (Fig. 1).

Diap1 is essential for the survival of embryonic cells. All cells die in a synchronous manner in embryos lacking functional Diap1 [11–13]. The function of regulatory caspase *dronc* [14, 15] and effector caspases *drice* and *dcp-1* [16] is also required for proper programmed cell death during embryogenesis. However, since both *diap1* and the three caspases are expressed ubiquitously in the embryo, it is unlikely that they play an important role in selecting which cell to die. Similar conclusion may be drawn for *ark/hac-1*, the *Drosophila* ortholog of mammalian Apaf-1 [17–19]. Hac-1 is expressed ubiquitously in the developing embryo with an elevated level of expression in the procephalic region around stage 10–11 [17], which may explain the enlarged brain observed for a hypomorph mutant allele [17,19].

The expression patterns of the IAP-antagonist genes indicate that they play pivotal roles in selecting cells to die during embryogenesis. Essentially all development cell death during embryogenesis is blocked in embryos homozygous for the H99 deletion. The central nervous system (CNS) of the H99 mutant embryo is about 3–4 times larger than the wild type at the end of embryogenesis, indicating that approximately 70% of the cells in embryonic CNS die during embryogenesis [5]. A similar ratio was observed in monitoring the developmental cell death of the glia cells at the CNS midline [20,21]. Cell lineage-specific markers allowed monitoring of these cells during wild type embryogenesis as well as in the H99 mutant. While there are about 8–10 p{*slit1.0-lacZ*}-expressing midline glial (MG) cells per segment at early stage 12, only 3 cells remain there at the end of embryogenesis. The rest undergo cell death, which depends on the function of the three IAP-antagonist genes deleted in H99 mutant [21]. However, not every cell lineage undergoes cell death during embryogenesis. For instance, the number of ventral unpaired neurons in the CNS midline remains unchanged during both wild type and H99 embryogenesis [21].

Interestingly, many dying cells are quickly phagocytosed by migrating hemocytes/macrophages during embryogenesis [3,21–23]. It seems that dying cells are “pushed” from their original location towards the space through which hemocytes will migrate. The change of cell shape, and the dissociation with neighboring cells during early apoptosis are at least in part due to the cleavage of junction proteins Discs large and Bazooka by caspases, as demonstrated by Chandraratna et al. using Diap1 depleted embryos [11]. The display of phagocytotic signals on the dying cells also depends on the function of IAP-antagonists and caspase activation, as supernumerous cells in H99 mutant embryos can not be phagocytosed. On the macrophage side, *Croquemort* is required for recognizing the apoptotic cells [24,25]. However, the genesis of hemocytes in the cephalic mesoderm and their stereotypic migration in the embryo are independent of cell death, as both were largely normal in the H99 mutant background [21,23]. Reciprocally, cell death can proceed normally in embryos that lack macrophages [23].

The majority of cell death during embryogenesis occurs between stage 11 to stage 13. Most of the dying cells at this stage appear to be in the epidermis and the developing nervous system. In post stage 15 embryos, only discrete cells in the ventral nerve cord can be detected as AO or TUNEL-positive [3,26]. Most of these late-dying cells appear to be neuroblasts [27,28]. Unlike cell death at earlier stages, dying neuroblasts do not appear to be phagocytosed by macrophages.

Why are cells generated in the embryo eliminated just a few hours later? The reason may differ depending on the circumstance. For example, the midline glial cells guide the crossing over of the axons from each hemisphere, eventually forming the commissural axon tract. Interestingly, midline glial cell death concurs with the end of commissural axon formation and separation, suggesting that the MG cell death may be a mechanism to eliminate cells once they become obsolete [20]. On the other hand, cell death of the abdominal neuroblasts cells is timed to terminate their potential to proliferate [29,30]. In H99 deficiency mutant that lacks *reaper*, *hid*, and *grim*, the rescued neuroblasts continues to proliferate and generate supernumerary cells. This indicates that cell death also serves to prevent over-proliferation.

Toyama et al. also demonstrated that cell death in the aminerosa cells actually contribute to the movement of cell sheet during morphogenesis [31]. The delamination and extrusion of the apoptotic cell produces a force that is required for bringing the cell sheet together during dorsal closure. This was a rather surprising finding. Clearly, the functional significance of cell death may well surpass what we have already known or thought about so far.

Death by design: genetic programming of cell death in the embryo

The segmentally repeated pattern of embryonic cell death suggests that it may be under the control of the same genetic program governing embryonic pattern formation (Fig. 1). As we know, the positional information deposited in the egg during oogenesis is to be manifested by initiating in early embryogenesis a cascade of transcription factors and morphogens, the gradients of which specify individual cell fate based on the cell's position along the anterior-posterior and dorsal-ventral axes [32–34]. By stage 11 to 12, the segment polarity genes and homeotic genes have been induced and begin to take control of the development program. Segment polarity genes determine cell differentiation within each segment by marking the posterior and anterior borders of each segment, while homeotic genes define and maintain segment identity.

Cell death in the epidermis between stages 11–14 appears to be more concentrated at the border of each segment rather than in the middle [4]. In addition, the observed increase in cell death, when temperature sensitive *wingless* mutants are shifted to a non-permissive temperature at stage 11, appears to be concentrated at the anterior part of the segment. These results suggest that segmental polarity genes may have a direct impact on cell survival, although it is hard to rule out the possibility that the observed cell death is a consequence of abnormal differentiation.

Direct evidence of homeotic genes controlling cell death came when Lohmann et al. showed that Deformed (Dfd) could activate the expression of *reaper* through directly binding to enhancer elements about 2–3 kb upstream of the promoter [35]. *Dfd*-mediated cell death in the head is required for maintaining the boundary of two distinct head segments. Expression of *reaper* is also observed prior to the death of a differentiated motorneuron in segments T3–A8 [36]. The segment-specific death of this neuron requires the function of homeotic gene *Ultrabithorax* (*Ubx*). The survival of this neuron in the T1 and T2 segments depend on the function of *Antennapedia* (*Antp*), another homeotic gene. It remains to be seen as to whether the two homeotic transcription factors, UBX and ANTP, directly regulate *reaper* expression, and whether any other H99 pro-apoptotic genes besides *reaper* are involved. A similar association between homeotic genes and cell death was also observed for the death of post-

embryonic neuro-blasts cells, which is initiated by a burst expression of *Abdominal-A* (*Abd-A*) in these cells [29]. These studies indicate that under many developmental contexts, homeotic genes can selectively activate cell-autonomous cell death and sculpt the tissues into their distinct shapes.

Death by chance: growth factor control of cell survival

In metazoans, cell proliferation, differentiation, and cell death are precisely regulated during normal development to generate and maintain the correct composition of different cell types in a given tissue. Extracellular growth factors play an essential role in this regulatory mechanism to ensure that each cell type is present in appropriate numbers. It was postulated that death is the default fate of every cells, and that survival requires the stimulation from “trophic” factors [37]. In both insects and mammals, varieties of growth factors are required for the survival of cells. Their withdrawal leads to apoptosis [38,39]. There are comprehensible advantages to relying on growth factors to determine a cell's survival or death. In addition to regulating cell number and organ size in developing organs to maintain tissue homeostasis, this mechanism could serve to remove misplaced cells formed by abnormal migration or injury. Moreover, competition for limiting amounts of growth factors could continuously select the most competitive cells that carry functional growth factor signal pathways [37].

The importance of growth factor in controlling cell death during embryogenesis was revealed by the cell viability defect in a variety of tissues in embryos mutated for *torpedo*, the *Drosophila* EGF receptor (DER) [40]. Specifically, it has been shown that the survival of the midline glia is dependent on the EGF signaling pathway. While there were about 3 *slit1.0-lacZ* expressing cells per segment in late stage wild type embryos, there was less than one per segment left in embryos mutated for *spitz* (*Drosophila* EGF), *rhomboid*, or *DER* [41,42].

The effect of the EGF signaling pathway on MG survival is mediated by the inhibition of HID pro-apoptotic activity and suppression of *hid* transcription [43,44]. Direct phosphorylation of HID by the MAP Kinase, a downstream component of the EGFR signaling pathway, inhibits its activity to induce cell death [43]. Using an elegant design, Bergmann et al. showed that the MG survival is directly correlated with MAPK activity levels [45]. Expression of dominant-negative EGFR or *spitz* null mutant leads to severely compromised MG survival. Expression of a membrane-linked Spitz precursor (mSpitz) in the MG did not rescue the cells, whereas mSpitz expressed from adjacent neurons effectively rescued MG. Moreover, overexpression of activated Spitz results in supernumerary MG, indicating that physiological levels of Spitz are limited and that is the determinant of MG survival. These data characterized a complete signaling pathway, in which extracellular growth factor Spitz, provided by the neuron, activates MG surface EGFR and the Ras/MAPK cascade to suppress Hid-dependent cell death.

The emerged model of MG cell survival can be summarized accordingly. Shortly after the formation of the commissural axons (stage 11), *hid* (and *reaper*) are expressed in a group of midline glia. These cells compete for the growth factor provided by neurons. The glial cells that have the closest (most) contact with the axons will survive while the others will die and become phagocytosed by migrating macrophages. All of this is completed by stage 15, roughly 6–7 h after stage 11. While the model seems rather stochastic, the result is amazingly precise. There are typically 3 *slit1.0-lacZ* expressing cells left per segment at the end of embryogenesis, with very little variation.

EGFR-mediated signaling is also responsible for controlling cell death and compartment size during *Drosophila* embryogenesis [46]. Each segment of the *Drosophila* embryonic epidermis can be divided into anterior (A) and posterior (P) compartments. Parker's work showed that cell survival and growth in the P compartment are stimulated by the release of Spitz from the neighboring A compartment cells. However, the Spitz secretion is limited, so that increasing

the number of cells within the P compartment causes a drop of per-cell Spitz level. This leads to compensatory apoptosis and cell-size reductions that preserve compartment dimension. In contrast, elevated EGFR signaling will promote cell survival and increase cell size.

Expression of the IAP-antagonist genes

The fact that almost all developmental cell death is blocked in H99 mutant embryos underscores the importance of the IAP-antagonists in regulating cell death during embryogenesis. In the developing embryo, both caspases and DIAP1 are ubiquitously expressed. It seems that in all of the analyzed embryonic systems, expression of the IAP-antagonists *reaper*, *hid*, or *grim* is required for inducing cell death. It has recently been shown that HOW (*held-out wing*), an RNA-binding protein that can interact with the 3'untranslated region of *diap1* mRNA, and suppresses DIAP1 levels [47]. HOW mutants have more MG cells per segment than wild type, and have lower levels of activated caspase-3. While reduction of DIAP1 by HOW can increase cellular sensitivity to apoptotic signals, there is no evidence that HOW expression alone is sufficient to induce cell death. Thus the expression of the IAP-antagonists appears to be the main mechanism of developmental cell death in the embryo.

The pattern of *reaper*, *grim*, and *sickle* in post-stage 11 embryos corresponds well with the cell death pattern, indicating that these genes are specifically expressed in cells destined to die. HID is the only one of the four IAP-antagonists that is expressed in cells that do not die at the end of embryogenesis, which is most likely due to the fact that its pro-apoptotic activity can be suppressed by MAPK. In the case of the MG cells, both *hid* and *reaper* are expressed in these cells to mediate cell death in a synergistic fashion [48]. Removing the function of one of the genes will result in a partial rescue compared to the total blocking of cell death in H99 mutants [48]. Coordinated expression of *reaper* and *hid* has also been observed in several other systems, such as ecdysone-induced degeneration of the midgut and salivary gland during metamorphosis [49]. In these systems, removing the function of one of the IAP-antagonists often has only a mild or minor effect [50,51]. Although all four IAP-antagonists induce cell death mainly through their IAP-binding motif, their functions are not merely redundant [52, 53]. In addition, RPR, GRIM, and SICKLE also have a C-terminal motif that could induce cell death at least in over-expression settings [54–57].

Interestingly, coordinated expression of *reaper* and *hid* is also observed when embryos at or before stage 11 are irradiated with ionizing irradiation. This coordinated induction is mediated by enhancers located upstream of *reaper* [26]. When this enhancer region is deleted, both *reaper* and *hid* lose their responsiveness to irradiation, suggesting that the same set of enhancers can regulate the expression of both genes. The responsiveness of *reaper* and *hid* to irradiation-induced DNA damage is developmental stage specific—both genes became irresponsive to irradiation in embryos after stage 12. This sensitive-to-resistant transition is due to epigenetic regulation of the irradiation responsive enhancer region (IRER). Around stage 12, this region forms a heterochromatin-like structure that is inaccessible to DNase I, accompanied with the enrichment of repressive chromatin marks, such as H3K27me3 and H3K9me3, and the binding of Heterochromatin Protein 1 and Polycomb group proteins [26]. This epigenetic regulation is specific to the enhancer region. The transcribed region of *reaper* remains open at later stage of embryogenesis. Nonetheless, it is notable that epigenetic blocking of IRER corresponds with a general decline of *reaper/hid* expression and cell death in the embryo. It is possible that IRER is responsible for mediating *reaper/hid* expression in response to cellular stress such as limitation of growth factors, a hypothesis that still awaits experimental evidence.

It seems that understanding the transcriptional regulation of the IAP-antagonists is crucial to understand how cells are specified to die during embryogenesis. Even though the EGFR pathway is responsible for determining the number of MG cells that can survive, the specific

expression of *hid* (and *reaper*) in these cells is the prerequisite for cell death in this lineage (Fig. 2). The full characterization of the transcriptional regulation of the IAP-antagonists will not be a simple task. The four genes are located in a 350 kb region that is conserved as a syntenic region in all of the sequenced *Drosophila* genomes (Fig. 3). All four genes are transcribed in the same direction (telomere of Chr. 3L). Remarkably long intergenic regions (99 and 40 kb, respectively) flank the *reaper* transcribed region. These two long intergenic regions are highly conserved in distantly related *Drosophila* species and are enriched for Highly Conserved Non-coding Elements (HCNE). HCNEs are 50–150 bp genomic sequences that are over 95–98% identical in different species. The clustering of HCNEs, such as the pattern in the IAP-antagonists' region, is often associated with syntenies that form a genomic regulatory block (GRB) in both insects and vertebrates [58,59]. GRBs often have several genes that are coordinately regulated by the enhancers located in the HCNEs. It seems clear that the four IAP-antagonists are in a genomic regulatory block. However, identifying all of the enhancers and interactions between/among the enhancers and promoters will be a challenge that demands dedicated effort.

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References

1. Magrassi L, Lawrence PA. The pattern of cell death in fushi tarazu, a segmentation gene of *Drosophila*. *Development* 1988;104(3):447–451. [PubMed: 3256471]
2. Smouse D, Goodman C, Mahowald A, Perrimon N. Polyhomeotic: a gene required for the embryonic development of axon pathways in the central nervous system of *Drosophila*. *Genes Dev* 1988;2(7):830–842. doi:10.1101/gad.2.7.830. [PubMed: 2905316]
3. Abrams JM, White K, Fessler LI, Steller H. Programmed cell death during *Drosophila* embryogenesis. *Development* 1993;117(1):29–43. [PubMed: 8223253]
4. Pazdera TM, Janardhan P, Minden JS. Patterned epidermal cell death in wild-type and segment polarity mutant *Drosophila* embryos. *Development* 1998;125(17):3427–3436. [PubMed: 9693146]
5. White K, Grether ME, Abrams JM, Young L, Farrell K, Steller H. Genetic control of programmed cell death in *Drosophila*. *Science* 1994;264(5159):677–683. doi:10.1126/science.8171319. [PubMed: 8171319]
6. Grether ME, Abrams JM, Agapite J, White K, Steller H. The head involution defective gene of *Drosophila melanogaster* functions in programmed cell death. *Genes Dev* 1995;9:1694–1708. doi:10.1101/gad.9.14.1694. [PubMed: 7622034]
7. Chen P, Nordstrom W, Gish B, Abrams JM. grim, a novel cell death gene in *Drosophila*. *Genes Dev* 1996;10:1773–1782. doi:10.1101/gad.10.14.1773. [PubMed: 8698237]
8. Christich A, Kaupila S, Chen P, Sogame N, Ho SI, Abrams JM. The damage-responsive *Drosophila* gene sickle encodes a novel IAP binding protein similar to but distinct from reaper, grim, and hid. *Curr Biol* 2002;12(2):137–140. doi:10.1016/S0960-9822(01)00658-3. [PubMed: 11818065]
9. Srinivasula SM, Datta P, Kobayashi M, Wu JW, Fujioka M, Hegde R, Zhang Z, Mukattash R, Fernandes-Alnemri T, Shi Y, Jaynes JB, Alnemri ES. Sickel, a novel *Drosophila* death gene in the reaper/hid/grim region, encodes an IAP-inhibitory protein. *Curr Biol* 2002;12(2):125–130. doi:10.1016/S0960-9822(01)00657-1. [PubMed: 11818063]
10. Wing JP, Karres JS, Ogdahl JL, Zhou L, Schwartz LM, Nambu JR. *Drosophila* sickle is a novel grim-reaper cell death activator. *Curr Biol* 2002;12(2):131–135. doi:10.1016/S0960-9822(01)00664-9. [PubMed: 11818064]
11. Chandraratna D, Lawrence N, Welchman DP, Sanson B. An in vivo model of apoptosis: linking cell behaviours and caspase substrates in embryos lacking DIAP1. *J Cell Sci* 2007;120(Pt 15):2594–2608. doi:10.1242/jcs.03472. [PubMed: 17636001]

12. Goyal L, McCall K, Agapite J, Hartwig E, Steller H. Induction of apoptosis by *Drosophila* reaper, hid and grim through inhibition of IAP function. *EMBO J* 2000;19(4):589–597. doi:10.1093/emboj/19.4.589. [PubMed: 10675328]
13. Wang SL, Hawkins CJ, Yoo SJ, Muller HA, Hay BA. The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. *Cell* 1999;98(4):453–463. doi:10.1016/S0092-8674(00)81974-1. [PubMed: 10481910]
14. Quinn LM, Dorstyn L, Mills K, Colussi PA, Chen P, Coombe M, Abrams J, Kumar S, Richardson H. An essential role for the caspase dronc in developmentally programmed cell death in *Drosophila*. *J Biol Chem* 2000;275(51):40416–40424. doi:10.1074/jbc.M002935200. [PubMed: 10984473]
15. Xu D, Li Y, Arcaro M, Lackey M, Bergmann A. The CARD-carrying caspase Dronc is essential for most, but not all, developmental cell death in *Drosophila*. *Development* 2005;132(9):2125–2134. doi:10.1242/dev.01790. [PubMed: 15800001]
16. Xu D, Wang Y, Willecke R, Chen Z, Ding T, Bergmann A. The effector caspases drICE and dcp-1 have partially overlapping functions in the apoptotic pathway in *Drosophila*. *Cell Death Differ* 2006;13(10):1697–1706. doi:10.1038/sj.cdd.4401920. [PubMed: 16645642]
17. Zhou L, Song Z, Tittel J, Steller H. HAC-1, a *Drosophila* homolog of APAF-1 and CED-4 functions in developmental and radiation-induced apoptosis. *Mol Cell* 1999;4(5):745–755. doi:10.1016/S1097-2765(00)80385-8. [PubMed: 10619022]
18. Rodriguez A, Oliver H, Zou H, Chen P, Wang X, Abrams JM. Dark is a *Drosophila* homologue of Apaf-1/CED-4 and functions in an evolutionarily conserved death pathway. *Nat Cell Biol* 1999;1(5):272–279. doi:10.1038/12984. [PubMed: 10559939]
19. Kanuka H, Sawamoto K, Inohara N, Matsuno K, Okano H, Miura M. Control of the cell death pathway by Dapaf-1, a *Drosophila* Apaf-1/CED-4-related caspase activator. *Mol Cell* 1999;4(5):757–769. doi:10.1016/S1097-2765(00)80386-X. [PubMed: 10619023]
20. Sonnenfeld MJ, Jacobs JR. Apoptosis of the midline glia during *Drosophila* embryogenesis: a correlation with axon contact. *Development* 1995;121(2):569–578. [PubMed: 7768193]
21. Zhou L, Hashimi H, Schwartz LM, Nambu JR. Programmed cell death in the *Drosophila* central nervous system midline. *Curr Biol* 1995;5(7):784–790. doi:10.1016/S0960-9822(95)00155-2. [PubMed: 7583125]
22. Sonnenfeld MJ, Jacobs JR. Macrophages and glia participate in the removal of apoptotic neurons from the *Drosophila* embryonic nervous system. *J Comp Neurol* 1995;359(4):644–652. doi:10.1002/cne.903590410. [PubMed: 7499553]
23. Tepass U, Fessler LI, Aziz A, Hartenstein V. Embryonic origin of hemocytes and their relationship to cell death in *Drosophila*. *Development* 1994;120(7):1829–1837. [PubMed: 7924990]
24. Franc NC, Dimarcq JL, Lagueux M, Hoffmann J, Ezekowitz RA. Croquemort, a novel *Drosophila* hemocyte/macrophage receptor that recognizes apoptotic cells. *Immunity* 1996;4(5):431–443. doi:10.1016/S1074-7613(00)80410-0. [PubMed: 8630729]
25. Franc NC, Heitzler P, Ezekowitz RAB, White K. Requirement for Croquemort in phagocytosis of apoptotic cells in *Drosophila*. *Science* 1999;284(5422):1991–1994. [PubMed: 10373118]
26. Zhang Y, Lin N, Carroll P, Chan G, Guan B, Xiao H, Yao B, Wu S, Zhou L. Epigenetic blocking of an enhancer region controls irradiation-induced proapoptotic gene expression in *Drosophila* embryos. *Dev Cell* 2008;14:481–493. doi:10.1016/j.devcel.2008.01.018. [PubMed: 18410726]
27. Karcavich R, Doe CQ. *Drosophila* neuroblast 7–3 cell lineage: a model system for studying programmed cell death, Notch/Numb signaling, and sequential specification of ganglion mother cell identity. *J Comp Neurol* 2005;481(3):240–251. doi:10.1002/cne.20371. [PubMed: 15593370]
28. Rogulja-Ortmann A, Luer K, Seibert J, Rickert C, Technau GM. Programmed cell death in the embryonic central nervous system of *Drosophila melanogaster*. *Development* 2007;134(1):105–116. doi:10.1242/dev.02707. [PubMed: 17164416]
29. Bello BC, Hirth F, Gould AP. A pulse of the *Drosophila* Hox protein abdominal-A schedules the end of neural proliferation via neuroblast apoptosis. *Neuron* 2003;37(2):209–219. doi:10.1016/S0896-6273(02)01181-9. [PubMed: 12546817]

30. Maurange C, Cheng L, Gould AP. Temporal transcription factors and their targets schedule the end of neural proliferation in *Drosophila*. *Cell* 2008;133(5):891–902. doi:10.1016/j.cell.2008.03.034. [PubMed: 18510932]
31. Toyama Y, Peralta XG, Wells AR, Kiehart DP, Edwards GS. Apoptotic force and tissue dynamics during *Drosophila* embryogenesis. *Science* 2008;321(5896):1683–1686. doi:10.1126/science.1157052. [PubMed: 18802000]
32. Nusslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. *Nature* 1980;287(5785):795–801. doi:10.1038/287795a0. [PubMed: 6776413]
33. Wolpert L. Positional information and pattern formation in development. *Dev Genet* 1994;15(6):485–490. doi:10.1002/dvg.1020150607. [PubMed: 7834908]
34. Ingham PW. The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* 1988;335(6185):25–34. doi:10.1038/335025a0. [PubMed: 2901040]
35. Lohmann I, McGinnis N, Bodmer M, McGinnis W. The *Drosophila* Hox gene deformed sculpts head morphology via direct regulation of the apoptosis activator reaper. *Cell* 2002;110(4):457–466. doi:10.1016/S0092-8674(02)00871-1. [PubMed: 12202035]
36. Rogulja-Ortmann A, Renner S, Technau GM. Antagonistic roles for Ultrabithorax and Antennapedia in regulating segment-specific apoptosis of differentiated motoneurons in the *Drosophila* embryonic central nervous system. *Development* 2008;135(20):3435–3445. [PubMed: 18799545]
37. Raff MC. Social controls on cell survival and cell death. *Nature* 1992;356(6368):397–400. [PubMed: 1557121]
38. Barres BA, Schmid R, Sendtner M, Raff MC. Multiple extracellular signals are required for long-term oligodendrocyte survival. *Development* 1993;118(1):283–295. [PubMed: 8375338]
39. Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD. Programmed cell death and the control of cell survival: lessons from the nervous system. *Science* 1993;262(5134):695–700. doi:10.1126/science.8235590. [PubMed: 8235590]
40. Clifford R, Schupbach T. The torpedo (DER) receptor tyrosine kinase is required at multiple times during *Drosophila* embryogenesis. *Development* 1992;115(3):853–872. [PubMed: 1425358]
41. Stemerink C, Jacobs JR. Argos and Spitz group genes function to regulate midline glial cell number in *Drosophila* embryos. *Development* 1997;124(19):3787–3796. [PubMed: 9367434]
42. Jacobs JR. The midline glia of *Drosophila*: a molecular genetic model for the developmental functions of glia. *Prog Neurobiol* 2000;62(5):475–508. doi:10.1016/S0301-0082(00)00016-2. [PubMed: 10869780]
43. Bergmann A, Agapite J, McCall K, Steller H. The *Drosophila* gene hid is a direct molecular target of Ras-dependent survival signaling. *Cell* 1998;95(3):331–341. doi:10.1016/S0092-8674(00)81765-1. [PubMed: 9814704]
44. Kurada P, White K. Ras promotes cell survival in *Drosophila* by downregulating hid expression. *Cell* 1998;95(3):319–329. doi:10.1016/S0092-8674(00)81764-X. [PubMed: 9814703]
45. Bergmann A, Tugentman M, Shilo BZ, Steller H. Regulation of cell number by MAPK-dependent control of apoptosis: a mechanism for trophic survival signaling. *Dev Cell* 2002;2(2):159–170. doi:10.1016/S1534-5807(02)00116-8. [PubMed: 11832242]
46. Parker J. Control of compartment size by an EGF ligand from neighboring cells. *Curr Biol* 2006;16(20):2058–2065. doi:10.1016/j.cub.2006.08.092. [PubMed: 17055987]
47. Reuveny A, Elhanany H, Volk T. Enhanced sensitivity of midline glial cells to apoptosis is achieved by HOW(L)-dependent repression of Diap1. *Mech Dev* 2009;126(1–2):30–41. doi:10.1016/j.mod.2008.10.004. [PubMed: 18984040]
48. Zhou L, Schnitzler A, Agapite J, Schwartz LM, Steller H, Nambu JR. Cooperative functions of the reaper and head involution defective genes in the programmed cell death of *Drosophila* central nervous system midline cells. *Proc Natl Acad Sci USA* 1997;94(10):5131–5136. doi:10.1073/pnas.94.10.5131. [PubMed: 9144202]
49. Jiang C, Baehrecke EH, Thummel CS. Steroid regulated programmed cell death during *Drosophila* metamorphosis. *Development* 1997;124(22):4673–4683. [PubMed: 9409683]
50. Yin VP, Thummel CS. A balance between the diap1 death inhibitor and reaper and hid death inducers controls steroid-triggered cell death in *Drosophila*. *Proc Natl Acad Sci USA* 2004;101(21):8022–8027. doi:10.1073/pnas.0402647101. [PubMed: 15150408]

51. Peterson C, Carney GE, Taylor BJ, White K. Reaper is required for neuroblast apoptosis during *Drosophila* development. *Development* 2002;129(6):1467–1476. [PubMed: 11880355]
52. Wing JP, Schwartz LM, Nambu JR. The RHG motifs of *Drosophila* Reaper and Grim are important for their distinct cell death-inducing abilities. *Mech Dev* 2001;102(1–2):193–203. doi:10.1016/S0925-4773(01)00316-1. [PubMed: 11287192]
53. Zachariou A, Tenev T, Goyal L, Agapite J, Steller H, Meier P. IAP-antagonists exhibit non-redundant modes of action through differential DIAP1 binding. *EMBO J* 2003;22(24):6642–6652. doi:10.1093/emboj/cdg617. [PubMed: 14657035]
54. Claveria C, Caminero E, Martinez AC, Campuzano S, Torres M. GH3, a novel proapoptotic domain in *Drosophila* Grim, promotes a mitochondrial death pathway. *EMBO J* 2002;21(13):3327–3336. doi:10.1093/emboj/cdf354. [PubMed: 12093734]
55. Claveria C, Martinez AC, Torres M. A Bax/Bak-independent mitochondrial death pathway triggered by *Drosophila* Grim GH3 domain in mammalian cells. *J Biol Chem* 2004;279(2):1368–1375. doi:10.1074/jbc.M309819200. [PubMed: 14551193]
56. Freel CD, Richardson DA, Thomenius MJ, Gan EC, Horn SR, Olson MR, Kornbluth S. Mitochondrial localization of Reaper to promote inhibitors of apoptosis protein degradation conferred by GH3 domain-lipid interactions. *J Biol Chem* 2008;283(1):367–379. doi:10.1074/jbc.M708931200. [PubMed: 17998202]
57. Olson MR, Holley CL, Gan EC, Colon-Ramos DA, Kaplan B, Kornbluth S. A GH3-like domain in reaper required for mitochondrial localization and induction of IAP degradation. *J Biol Chem* 2003;278(1):13–23.
58. Engstrom PG, Ho Sui SJ, Drivenes O, Becker TS, Lenhard B. Genomic regulatory blocks underlie extensive micro-synteny conservation in insects. *Genome Res* 2007;17(12):1898–1908. doi:10.1101/gr.6669607. [PubMed: 17989259]
59. Kikuta H, Laplante M, Navratilova P, Komisarczuk AZ, Engstrom PG, Fredman D, Akalin A, Caccamo M, Sealy I, Howe K, Ghislain J, Pezeron G, Mourrain P, Ellingsen S, Oates AC, Thisse C, Thisse B, Foucher I, Adolf B, Geling A, Lenhard B, Becker TS. Genomic regulatory blocks encompass multiple neighboring genes and maintain conserved synteny in vertebrates. *Genome Res* 2007;17(5):545–555. doi:10.1101/gr.6086307. [PubMed: 17387144]

Fig. 1. Dynamic expression pattern of *reaper* and *hid*. The distribution of *reaper* (**A, B**) and *hid* (**C, D**) mRNA in embryos at different stages was revealed via in situ hybridization. (**A**) Sagittal view of stage 11 embryo, *reaper* is expressed in a segmentally repetitive pattern in the epidermis. However there are significant variation among segments. At later stage (**B**), *reaper* is only expressed in discrete cells in the ventral nerve cord (*arrow*). At stage 11–12 (**C**), *hid* is expressed in the epidermis as well as the CNS midline (*arrow head*). Some of these *hid*-expressing MG cells remain alive at the end of embryogenesis (**D**)

Fig. 2.

Summary of mechanisms that controls cell death during *Drosophila* embryogenesis. Developmental cues control the expression of IAP-antagonists through specific transcription factors and through epigenetic regulation of the enhancer regions. The EGFR pathway suppresses the expression of *hid* and inhibits the pro-apoptotic activity of HID protein, thus determines the number of cells that can survive

Fig. 3.

Genomic regulatory block of the IAP-antagonists (figure generated in <http://ancora.genereg.net/>). The four pro-apoptotic genes, *hid* (h), *grim* (g), *reaper* (r), and *sickle* (s), are in the same synteny that has high density of HCNE in the middle. The blue dotted lines indicated the minimum syntenic region that is conserved in all sequenced *Drosophila* genomes. The green dotted lines indicate the non-coding genomic regions surrounding *reaper*, which is enriched with HCNE (coordinates based on genome release 5.0)