

To die or not to die—a role for Fork head

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The precise determination of when and where cells undergo programmed cell death is critical for normal development and tissue homeostasis. Cao et al. (2007; see p. 843 of this issue) report that the Fork head (Fkh) transcription factor, which is essential for the early development and function of the larval salivary glands in *Drosophila melanogaster*, also contributes to its demise. These authors show that *fkh* expression in the salivary glands is normally lost at puparium formation, which is ~12 h before they undergo massive cell death triggered by the steroid hormone ecdysone, making room for their developing adult counterparts. The loss of Fkh eliminates its role in blocking cell death, allowing for subsequent ecdysone-induced *reaper* and *head involution defective* death activator expression and tissue destruction. This study provides new insights into the transcriptional regulation of programmed cell death and the mechanisms that underlie the precise spatial and temporal control of hormone responses during development.

Small lipophilic hormones represent one of the best understood signals for triggering a programmed cell death response, acting through members of the nuclear receptor family of ligand-regulated transcription factors. In frogs, thyroid hormone signals the destruction of the tadpole tail and the remodeling of the intestine as the animal progresses from a juvenile to adult form (Shi et al., 1996). Similarly, steroid hormones regulate mammalian cell death pathways, including the glucocorticoid-induced apoptosis of immature thymocytes and mature T cells (Winoto and Littman, 2002). Only in *Drosophila melanogaster*, however, has a regulatory network been defined that links the hormonal signal, the steroid ecdysone, to a cell death response—the stage-specific destruction of obsolete larval tissues during metamorphosis (Baehrecke, 2005; Yin and Thummel, 2005). A high-titer pulse of ecdysone at the end of larval development signals puparium formation and the destruction of the larval midgut, as an adult gut forms around the dying cells. A second ecdysone pulse, ~10 h after puparium formation, triggers adult head

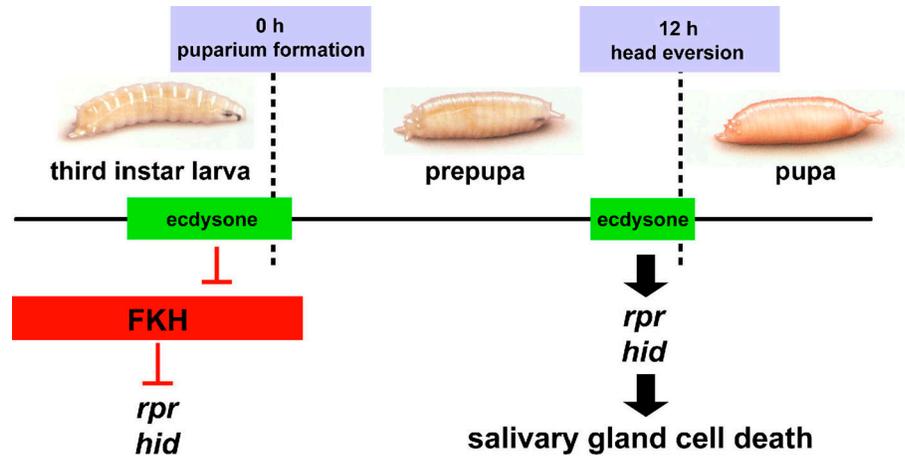
eversion, marking the prepupal–pupal transition and signaling the rapid elimination of the larval salivary glands (Fig. 1). Destruction of the larval tissues is accompanied by classic hallmarks of cell death, including TUNEL staining and caspase activation, although they undergo a distinct form of programmed cell death referred to as autophagy, which is characterized by the formation of intracellular autophagic vesicles (Jiang et al., 1997; Lee and Baehrecke, 2001; Baehrecke, 2005). *D. melanogaster* larval tissue cell death is dependent on the coordinate transcriptional induction of two key death activator genes, *reaper* (*rpr*) and *head involution defective* (*hid*; Yin and Thummel, 2004). Ecdysone directly induces *rpr* transcription in doomed larval salivary glands (Fig. 1; Jiang et al., 2000). This effect is augmented by the ecdysone induction of transcription factor–encoding genes, including the *Broad-Complex* (*BR-C*), *E74A*, and *E93*, which, in turn, are required for appropriate *rpr* and *hid* expression and salivary gland cell death (Jiang et al., 2000; Lee et al., 2000).

Although the identification of this regulatory cascade has provided a framework for understanding how steroids control a programmed cell death response, it also raises the critical question of how temporal specificity is achieved. The destruction of the larval salivary glands is preceded by repeated systemic pulses of ecdysone during the life cycle of *D. melanogaster*, some of which result in *BR-C* and *E74A* induction. Yet, *rpr* and *hid* are only expressed in response to the prepupal pulse of ecdysone (Fig. 1). What are the molecular mechanisms that determine the temporal specificity of *rpr* and *hid* expression, and hence, the appropriate timing of steroid-triggered cell death?

In their article, Cao et al. (2007) provide an answer to this question. They show that Fork head (Fkh), which is the defining member of the Forkhead box family of transcription factors (Friedman and Kaestner, 2006), plays a critical role in determining when salivary gland cell death can occur. Fkh is among the earliest expressed factors in the larval salivary glands and is required for internalization of the secretory cells, as well as later salivary gland functions, including *Sgs* glue gene expression (Kuo et al., 1996; Myat and Andrew, 2000; Renault et al., 2001). Interestingly, *fkh* is also required to prevent salivary gland apoptosis in embryos, accompanied by *rpr* and *hid* induction and is dependent on genes encoded within the *H99* deficiency, which is an interval that spans the *rpr* and *hid* loci (Myat and Andrew, 2000). However, the observation that >20% of chromosomal deficiencies tested in *D. melanogaster* result in increased apoptosis during embryogenesis makes it difficult to interpret the significance of this phenotype (White et al., 1994).

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Abbreviation used in this paper: *hid*, *head involution defective*.

Figure 1. **A model for the temporal specification of salivary gland cell death by Fkh.** The Fkh transcription factor is expressed throughout larval stages in the salivary glands (red box), effectively blocking *reaper* (*rpr*) and *hid* death activator expression. It is only after puparium formation, apparently in response to the late-larval ecdysone pulse (left, green box), that Fkh is down-regulated. In the absence of Fkh, the prepupal ecdysone pulse (right, green box) can induce *rpr* and *hid*, triggering salivary gland cell death.



Fkh is normally expressed in the salivary glands throughout the larval stages, and then down-regulated at puparium formation, in synchrony with the late-larval ecdysone pulse (Fig. 1; Renault et al., 2001). Cao et al. (2007) show that this down-regulation of *fkh* is essential for the proper timing of cell death. Ectopic *fkh* expression in mid-prepupae results in a complete block in salivary gland cell death 6 h after the wild-type glands are destroyed. Consistent with this result, *rpr* mRNA levels are reduced, and *hid* transcripts are not detectable in salivary glands isolated from these animals. Moreover, microarray analysis revealed that other key cell death genes are down-regulated by ectopic *fkh*, including *Jafra2*, *dark*, and *dronc*, demonstrating widespread effects on the death pathway. Interestingly, however, ectopic *fkh* expression in prepupal salivary glands results in elevated levels of *BR-C*, *E74A*, and *E93*, key transcriptional inducers of *rpr* and *hid*. It will be interesting to determine the mechanisms by which Fkh exerts its effects on *rpr* and *hid* expression independently of these ecdysone-induced transcription factors. Moreover, some salivary glands that fail to undergo cell death in the presence of ectopic *fkh* display large, vacuole-like structures, indicating that some aspects of cell death are underway in these tissues. A possibility to be explored is whether this represents the progression of autophagic cell death (Lee and Baehrecke, 2001; Baehrecke, 2005).

To confirm and extend these observations, Cao et al. (2007) prematurely removed *fkh* function from third instar larvae using RNAi. Although *E74A* is expressed normally under these conditions, both *rpr* and *hid* are prematurely induced at puparium formation in larval salivary glands. As might be expected, the expression of these key death activators has catastrophic consequences for the salivary glands, directing their early entry into cell death. Interestingly, the final stages of tissue destruction are not properly executed under these conditions. Further studies are required to determine whether other key death effectors fail to be prematurely expressed, resulting in an incomplete death response in these animals.

Finally, Cao et al. (2007) provide evidence that *fkh* might be responsible for the defects in salivary gland cell death observed in *BR-C* mutant salivary glands. They show that *fkh* expression is maintained in these mutant tissues after puparium formation, similar to the effects of ectopic *fkh* expression in prepupae.

It will be interesting to determine if removal of *fkh* function by RNAi in *BR-C* mutant salivary glands is sufficient to restore the normal death response in these tissues.

This paper by Cao et al. (2007) addresses a central question of ecdysone-triggered salivary gland cell death, showing that *fkh* contributes to the proper timing of *rpr* and *hid* transcriptional induction. The expression of *fkh* in this tissue throughout larval stages effectively blocks the death response, maintaining normal salivary gland function. At the onset of metamorphosis, however, *fkh* down-regulation provides competence for salivary gland cell death, allowing the death cascade to be triggered by the subsequent prepupal pulse of hormone (Fig. 1). It is interesting to note that the same tissue-specific factor that plays a critical role in early salivary gland development and maintains salivary gland function during larval stages also blocks its destruction. In this way, Fkh links tissue specification and identity to the prevention of tissue destruction, defining Fkh as a survival factor and ensuring that the salivary glands can provide their normal functions for the larva. Moreover, the observation that Fkh orthologues can directly facilitate steroid-regulated transcription in vertebrates suggests that their role in regulating steroid-triggered cell death may be conserved through evolution (Friedman and Kaestner, 2006). Finally, this study casts a new light on work by Myat and Andrew (2000), which showed that *fkh* is required to suppress *rpr* and *hid* expression and salivary gland apoptosis during *D. melanogaster* embryogenesis. Interestingly, the timing of premature *rpr* and *hid* expression in *fkh* mutant embryos is coincident with the leading edge of the embryonic ecdysone pulse and activation of the ecdysone receptor (Kozlova and Thummel, 2003). This raises the possibility that *fkh* silences ecdysone-triggered salivary gland cell death throughout the life cycle, and that *fkh* down-regulation is a key step that allows the larval gland to meet its ultimate fate, cell death, in response to the next pulse of ecdysone.

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References

- Baehrecke, E.H. 2005. Autophagy: dual roles in life and death? *Nat. Rev. Mol. Cell Biol.* 6:505–510.

- Cao, C., Y. Liu, and M. Lehmann. 2007. Fork head controls the timing and tissue selectivity of steroid-induced developmental cell death. *J. Cell Biol.* 176:843–852.
- Friedman, J.R., and K.H. Kaestner. 2006. The Foxa family of transcription factors in development and metabolism. *Cell. Mol. Life Sci.* 63:2317–2328.
- Jiang, C., E.H. Baehrecke, and C.S. Thummel. 1997. Steroid regulated programmed cell death during *Drosophila* metamorphosis. *Development.* 124:4673–4683.
- Jiang, C., A.-F. Lamblin, H. Steller, and C.S. Thummel. 2000. A steroid-triggered transcriptional hierarchy controls salivary gland cell death during *Drosophila* metamorphosis. *Mol. Cell.* 5:445–455.
- Kozlova, T., and C.S. Thummel. 2003. Essential roles for ecdysone signaling during *Drosophila* mid-embryonic development. *Science.* 301:1911–1914.
- Kuo, Y.M., N. Jones, B. Zhou, S. Panzer, V. Larson, and S.K. Beckendorf. 1996. Salivary duct determination in *Drosophila*: roles of the EGF receptor signalling pathway and the transcription factors fork head and trachealess. *Development.* 122:1909–1917.
- Lee, C.Y., and E.H. Baehrecke. 2001. Steroid regulation of autophagic programmed cell death during development. *Development.* 128:1443–1455.
- Lee, C.Y., D.P. Wendel, P. Reid, G. Lam, C.S. Thummel, and E.H. Baehrecke. 2000. *E93* directs steroid-triggered programmed cell death in *Drosophila*. *Mol. Cell.* 6:433–443.
- Myat, M.M., and D.J. Andrew. 2000. Fork head prevents apoptosis and promotes cell shape change during formation of the *Drosophila* salivary glands. *Development.* 127:4217–4226.
- Renault, N., K. King-Jones, and M. Lehmann. 2001. Downregulation of the tissue-specific transcription factor Fork head by Broad-Complex mediates a stage-specific hormone response. *Development.* 128:3729–3737.
- Shi, Y.B., J. Wong, M. Puzianowska-Kuznicka, and M.A. Stolow. 1996. Tadpole competence and tissue-specific temporal regulation of amphibian metamorphosis: roles of thyroid hormone and its receptors. *Bioessays.* 18:391–399.
- White, K., M.E. Grether, J.M. Abrams, L. Young, K. Farrell, and H. Steller. 1994. Genetic control of programmed cell death in *Drosophila*. *Science.* 264:677–683.
- Winoto, A., and D.R. Littman. 2002. Nuclear hormone receptors in T lymphocytes. *Cell.* 109:S57–S66.
- Yin, V.P., and C.S. Thummel. 2004. 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.* 101:8022–8027.
- Yin, V.P., and C.S. Thummel. 2005. Mechanisms of steroid-triggered programmed cell death in *Drosophila*. *Semin. Cell Dev. Biol.* 16:237–243.