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Review

# Steroid regulation of programmed cell death during *Drosophila* development

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## Abstract

**Steroid hormones play an important role in the regulation of numerous physiological responses, but the mechanisms that enable these systemic signals to trigger specific cell changes remain poorly characterized. Recent studies of *Drosophila* illustrate several important features of steroid-regulated programmed cell death. A single steroid hormone activates both cell differentiation and cell death in different tissues and at multiple stages during development. While several steroid-regulated genes are required for cell execution, most of these genes function in both cell differentiation and cell death, and require more specific factors to kill cells. Genes that regulate apoptosis during *Drosophila* embryogenesis are induced by steroids in dying cells later in development. These apoptosis genes likely function downstream of hormone-induced factors to serve a more direct role in the death response. This article reviews the current knowledge of steroid signaling and the regulation of programmed cell death during development of *Drosophila*.** *Cell Death and Differentiation* (2000) **7,** 1057–1062.

**Keywords:** Steroid; ecdysone; development; metamorphosis; *Drosophila*

## Steroid hormones are important regulators of programmed cell death

Steroid hormones serve a critical role in the maintenance of homeostasis. Steroids regulate metabolism, reproduction, and development in animals that are as different as insects and humans. During animal development, steroids trigger distinct responses including cell differentiation and programmed cell death. These hormones have been linked to numerous human health problems, and defects in hormone-triggered programmed cell death may result in the survival of tumor cells.[1] In vertebrate organisms, steroids including androgens, estrogens, progesterone, and glucocorticoids regulate cell death.[2–5] Glucocorticoid regulation of programmed lymphocyte death has served as a paradigm for steroid activation of apoptosis, and this response is dependent on glucocorticoid receptor function.[6–10] In invertebrates, the steroid 20-hydroxyecdysone (ecdysone), and its receptor, have been implicated in the activation of programmed cell death during insect development.[11–14]

Steroids hormones appear to regulate programmed cell death by a variety of mechanisms. Most studies have reported that steroids serve as survival factors, and that hormone withdrawal results in the activation of programmed cell death. Examples of this mechanism include androgens in the prostate,[15] and ecdysteroids in the insect nervous system.[13] Alternatively, increases in steroids also activate programmed cell death. In *Drosophila*, increases in ecdysteroids trigger cell death in larval midguts and salivary glands.[16] Glucocorticoid regulation of thymocyte cell death is complex and has been reported to be under both positive and negative control by this hormone, but recent *in vivo* studies indicate that a decrease in steroid titer regulates thymocyte apoptosis.[17] While little is known about the steroid-regulated genes that control thymocyte and other vertebrate cell deaths, recent studies of *Drosophila* are providing insights into the genetic mechanisms underlying hormone-triggered programmed cell death.

## Steroid regulation of developmental changes at the onset of *Drosophila* metamorphosis

The *Drosophila* life-cycle consists of embryonic, three larval instar, prepupal, pupal, and adult developmental stages. Pulses of the steroid hormone ecdysone punctuate each of these life stages, and regulate important transitions in development.[18] During the onset of metamorphosis, fluctuations in ecdysone titer trigger dynamic cellular changes that are required to transform a larva into an adult (Figure 1). At the end of the third larval instar, an increase in ecdysone titer induces the formation of a prepupa.[19–21] The ecdysone titer then drops to a low level in the mid-prepupal stage,[20,21], and increases again 10–12 h following puparium formation.[20,22] This pulse of ecdysone triggers future adult head eversion, which marks the beginning of pupation.[20,22] The ecdysone titer then decreases at the onset of pupation before another large pulse of hormone occurs during pupal development.[21]

Metamorphosis of *Drosophila* involves the destruction of most of the larval tissues, and differentiation and morphogenesis of the tissues that form the adult fly (Figure 1). When the third instar larva turns into a prepupa, many tissues initiate metamorphic changes in

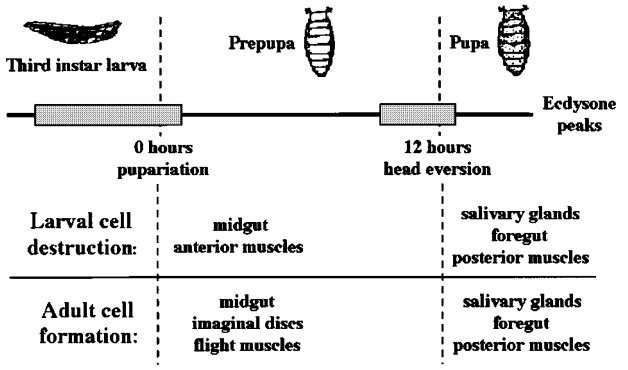


**Figure 1** Fluctuations in the ecdysone titer during the onset of metamorphosis trigger stage- and cell-specific biological responses. Consecutive increases in ecdysone at the end of the third larval instar and 12 h later trigger the formation of a prepupa and pupa. These pulses of hormone also coincide with numerous tissue rearrangements including the death of larval cells and the formation of adult cells

synchrony with the rise in ecdysone titer. Imaginal discs undergo morphogenesis to form future adult appendages, and adult flight muscles appear in the anterior region of the prepupa.[23,24] While these changes in adult structures occur, several tissues are destroyed including the anterior larval muscles and the larval midgut.[23,24] Similarly, numerous cell and tissue changes are also induced when the ecdysone titer rises at the prepupal-to-pupal transition. The adult appendages deposit procuticle,[25] the larval musculature completes its histolysis in the abdomen, the larval foregut epithelium is replaced by the adult foregut, and the larval salivary glands die while adult salivary glands initiate morphogenesis.[23,24] While many changes associated with the transformation of a larva into an adult fly occur during the prepupal stage, additional details of adult formation are elaborated during the 3 days between pupation and adult eclosion.

Ecdysone triggers stage- and cell-specific changes, indicating that fluctuations in this systemic signal alone are not sufficient to determine the nature of the cellular response. Analyses of the mechanisms underlying ecdysone-regulated responses have been restricted to a limited number of tissues. Studies of ecdysone-triggered imaginal disc evagination have served as a useful model for morphogenesis.[26,27] The nervous system, larval midgut, and larval salivary glands have been useful for studies of steroid regulation of cell death in *Drosophila*.[13,16] While most studies of cell death have emphasized the common features of apoptosis, it should be noted that at least three types of programmed cell death occur during development of evolutionarily diverse organisms.[28,29] During insect development, both apoptotic and autophagic cell death have been widely reported.[28] Comparison of lymphocyte apoptosis and insect intersegmental muscle autophagy indicate that these physiological cell deaths occur by distinct mechanisms,[30] but recent studies of *Drosophila* larval salivary glands suggest that these two types of cell death utilize some common mechanisms.

*Drosophila* salivary glands have been particularly useful as a model for steroid signaling, and possess several attributes making them an ideal system for studies of programmed cell death. Salivary gland cells die in a rapid and synchronous manner in response to the pulse of ecdysone that peaks 12 h following puparium formation.[16] These cells exhibit dynamic changes in the tubulin and actin cytoskeleton, and accumulate acid phosphatase activity preceding their demise which appears to be mediated by lysosome-derived autophagic vacuoles.[31,32] Markers of apoptosis including nuclear acridine orange staining and DNA fragmentation are detected by 14 h following puparium formation in salivary glands.[16] Furthermore, salivary glands that are cultured in a physiologically elevated level of 20-hydroxyecdysone undergo programmed cell death.[16]

## Steroids signal by triggering a genetic regulatory hierarchy

The mechanisms of steroid signaling have been extensively studied in *Drosophila* larval salivary glands because of the giant polytene chromosomes that form ecdysone-induced puffs reflecting a transcriptional regulatory hierarchy. Waves of chromosome puffs (decondensation of chromatin) accompany the late third instar larval and prepupal pulses of ecdysone. A series of elegant studies led to a model for genetic regulation of chromosome puffing.[33–35] According to this model, the ecdysone receptor complex directly induces a small set of early puff genes, and the protein products of these genes then repress their own activity and induce a large set of secondary late response genes.

The isolation and characterization of the ecdysone receptor and ecdysone-regulated puff genes have provided substantial support for the model proposed based on chromosome puffing.[36,37] The *EcR*[38] and *usp*[39–41] genes both encode members of the nuclear hormone receptor family of proteins, and heterodimerize to form the ecdysone receptor.[42,43] This receptor complex binds to DNA, and activates transcription of early puff genes, as early puffs and the genes encoded by these genetic loci are not properly induced in *EcR* and *usp* mutants.[44,45] The characterization of the *BR-C, E74*, and *E75* early puff genes provided further support of the puffing model for ecdysone signaling.[46–48] These early puff genes are complicated and encode multiple isoforms of transcription factor proteins by alternative promoter usage and splicing. *BR-C* encodes zinc finger proteins, *E74* encodes members of the ETS family of DNA binding proteins, and *E75* encodes nuclear hormone receptor family member zinc finger proteins. E74 and E75 proteins bind to both early and late puff chromosome loci.[49,50] Late puff genes have not been extensively characterized, but the isolation of the *L71* late genes[51,52] have been useful for testing the tenets of the steroid signaling model that was based on chromosome puffing. *BR-C* and *E74* mutations impact transcription of late target genes.[53] Furthermore, BR-C and E74 proteins bind to glue and *L71* gene regulatory elements, providing a direct link between these DNA binding proteins and the regulation of target gene transcription.[54–56]

The steroid regulatory hierarchy is activated by different pulses of ecdysone during development (Figure 2). The

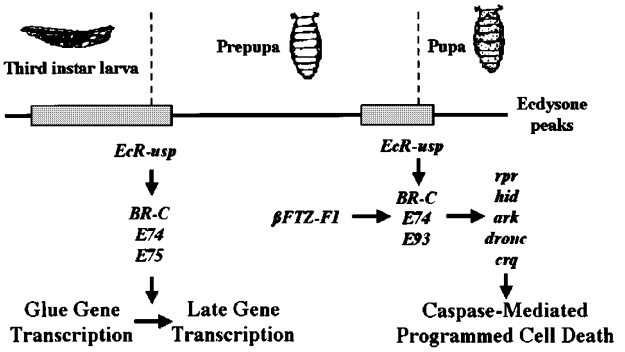


**Figure 2** Steroid genetic regulatory hierarchy in *Drosophila* larval salivary glands. The rise of ecdysone at the end of the third laval instar triggers a change in salivary gland glue to late gene transcription, and this change is mediated by the ecdysone receptor (encoded by the *EcR* and *usp* genes) and the *BR-C, E74*, and *E75* early puff genes. The subsequent increase in ecdysone titer at the end of prepupal development triggers caspase-mediated programmed cell death. This steroid-triggered cell death is regulated by the ecdysone receptor complex and *βFTZ-F1*, which enable ecdysone induction of the *BR-C, E74*, and *E93* genes. These regulatory factors are required for proper induction of the cell death genes *rpr, hid, ark, dronc*, and *crq* in salivary glands

increase in ecdysone titer at the end of the third larval instar regulates the transcription of glue and late genes in the salivary gland.[51,52,57,58] The ecdysone titer then drops to a low level in midprepupae, enabling the induction of the nuclear hormone receptor *βFTZ-F1*.[59] *βFTZ-F1* serves as competence factor that enables the reinduction of the *BR-C* and *E74* early genes, and the stage-specific induction of *E93* by the pulse of ecdysone 12 h after puparium formation in salivary glands.[59,60] While late puffs are observed at this stage of development,[61] none of these late genes have been identified based on puffing. However, targets of the early genes that are induced at this stage have been identified.[62,63]

## Steroid-regulated genes function in programmed cell death

Several ecdysone-regulated genes function in programmed cell death (Figure 2). The *EcR, usp*, *βFTZ-F1, BR-C, E74*, and *E93* genes have been implicated in the regulation of programmed cell death in a variety of tissues including the midgut, salivary glands, and nervous system.[13,14,45,60,62–64] *EcR, usp*, *βFTZ-F1, BR-C*, and *E74* are pleiotropic, however, and function in cell responses other than death including the proper formation of adult cells.[44,60,65] Significantly, *E93* appears to function more specifically in the destruction of larval tissues.[63] *E93* encodes a novel nuclear protein that is expressed in larval midgut and salivary gland cells immediately prior to ecdysone induction of their death. Furthermore, *E93* mutants have defects in larval salivary gland cell destruction, and expression of E93 is sufficient to induce programmed cell death. If one reconsiders the steroid regulatory hierarchy in salivary glands, a cell death signaling hierarchy emerges (Figure 2). The ecdysone receptor complex and *βFTZ-F1* regulate *BR-C, E74*, and *E93* transcription in larval salivary glands immediately prior to the initiation of cell destruction.[59,60] *E93* mutants possess decreased transcription of the *BR-C* and *E74* genes, and each of these early genes impact the transcription of programmed cell death genes prior to the stage that larval salivary glands initiate destruction.[62,63]

## Apoptosis genes are regulated by steroid-induced genes prior to programmed cell death

*Drosophila* possesses the programmed cell death pathway components that have been conserved in organisms as different as nematodes and humans.[66] Caspases including DCP-1, Dredd, DrICE, Dronc, and Decay,[67–72] the CED4/APAF-1 homolog Ark,[73–75] the CED-9/Bcl-2 family member Drob-1/Debcl/dBorg-1,[76–78] and the inhibitors of apoptosis DIAP1 and DIAP2[79] have been identified. In addition, the novel *rpr, hid*, and *grim* cell death genes have been isolated and molecularly characterized.[80–82]

Several apoptosis genes have been implicated in ecdysone regulated programmed cell death (Figure 2). An increase in *rpr* and *grim* transcription foreshadows ecdysone induction of neuronal cell death,[83] and mutations that remove these genes prevent these neurons from dying.[84] In larval midguts and salivary glands, *rpr* and *hid* transcription increase prior to ecdysone-regulated programmed cell death.[16] The core cell death machinery also appears to be involved in ecdysone-regulated programmed cell death. Inhibition of caspases by expression of the baculovirus inhibitor p35 blocks midgut and salivary gland cell death.[16] In addition, *ark* and *dronc* transcription increase immediately prior to programmed cell death salivary glands.[63]

Ecdysone-regulated genes are required for proper transcription of apoptosis genes in salivary glands. *rpr* transcription is directly regulated by the ecdysone receptor complex, but *BR-C* function is also required for maximum levels of *rpr* mRNA transcript in dying larval salivary glands.[62] While mutations in the *E74A* gene do not impact *rpr* transcription, both *BR-C* and *E74A* are required for proper transcription of *hid* in dying salivary glands.[62] *E93* mutants also impact the levels of several important apoptosis genes in salivary glands.[63] Mutations in *E93* result in decreased levels *rpr, hid, ark, dronc*, and *crq* RNA transcription. While *E93* mutants do not impact the transcription of *EcR* or *βFTZ-F1*, these mutants do impact transcription of *BR-C* and *E74A*. E93 protein binds to sites in the salivary gland polytene chromosomes that contain both steroid-regulated genes and programmed cell death genes.[63] These data suggest that *E93* regulates the apoptosis genes by either directly impacting their transcription, or indirectly by impacting early genes such as *BR-C* and *E74* that in turn regulate cell death gene expression.

## Concluding remarks

The regulation of programmed cell death plays a critical role during animal development by functioning in the destruction of unneeded cells and tissues.[85,86] Proper implementation of a cell death response is also important for the removal of

abnormal cells during development including tumor cells.[1] Studies of *Drosophila* larval salivary glands have been emphasized in this review because of the utility of this tissue for studies of steroid signaling. However, steroids are only one of many developmental signals that activate programmed cell death in *Drosophila* and other organisms.[87] Furthermore, many possible regulatory pathways could modulate cell death following the initial activation of death signaling. While cell death is regulated at the post-transcriptional level in *Drosophila*,[88–90] emphasis has been placed on transcriptional control of cell death in this review, since steroid activity is mediated by nuclear receptor DNA binding proteins.

Larval tissues are destroyed by programmed cell death during *Drosophila* metamorphosis. The coordination of ecdysone induction of both cell death and cell differentiation during metamorphosis indicates that fluctuations in this systemic signal alone can't be responsible for the complexity of cell responses. Rather, expression of the proper combinations of regulatory proteins appears to be critical for the appropriate activation of genes that play a more direct role in the cell response. Some of these regulatory proteins, such as *EcR, usp*, *βFTZ-F1, BR-C*, and *E74*, regulate both cell differentiation and cell death, indicating that other more specific factors must specify the type of cell response to steroid. The *E93* gene appears to specify ecdysone induction of cell death during metamorphosis, but it also appears to require other regulatory factors to properly activate the programmed cell death response. Many of the genes that function in the regulation of apoptosis during *Drosophila* embryogenesis are also involved in steroid activation of larval salivary gland cell death. Clearly, one of the most difficult challenges is to identify the novel components of the signaling pathways that lead to cell death, and integrate this knowledge into the development of the organism. The conservation of steroid signaling and apoptosis pathways in diverse organisms indicates that future genetic studies of *Drosophila* should lead to advances in understanding the mechanisms of programmed cell death and its regulation in higher organisms.

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