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RESEARCH ABSTRACT

Molecular Mechanisms of Hormone Action in *Drosophila*

Summary: Carl Thummel's laboratory uses the fruit fly, *Drosophila*, as a model system to study the molecular mechanisms of hormone signaling.

Small lipophilic hormones, including steroids, retinoids, and thyroid hormone, play a central role in the development and physiology of higher organisms. These signals are transduced by members of the nuclear receptor superfamily that act as hormone-dependent transcription factors, reprogramming gene expression within a target cell. Extensive studies in vertebrate systems have defined the molecular mechanisms by which nuclear receptors control promoter activity. In contrast, much less is known about the events that occur downstream from the receptor. It remains unclear how hormone-regulated target genes propagate the hormonal signal to direct appropriate biological responses during development.

Our laboratory is studying the mechanisms of hormone action in a simple model system—the fruit fly *Drosophila melanogaster*. Pulses of the steroid hormone ecdysone act as a critical temporal signal for the insect, triggering the major developmental transitions in the life cycle. These transitions include molting of the larval cuticle as the insect grows in size, and metamorphosis—the dramatic transformation of a crawling larva into a highly mobile and reproductively active adult fly. Metamorphosis is achieved by the ecdysone activation of two divergent genetic programs: the destruction of obsolete larval tissues and their replacement by developing adult tissues. We are studying the molecular basis of these two hormone-regulated pathways.

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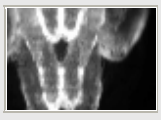

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The entire adult fly is carried within the larva in the form of small clusters of diploid adult progenitor cells. These cells are dispensable for larval growth and viability but are called into action at the onset of metamorphosis when sequential pulses of ecdysone trigger their morphogenesis and differentiation to form an adult animal. We have conducted several genetic screens for genes involved in formation of the adult leg as a model system for understanding how the hormone directs terminal differentiation. To date, 17 regions on the autosomes as

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well as mutations in 16 specific loci have been identified that are required for this response, defining a central role for the Rho1 small GTPase in controlling cell shape changes through effects on the actin cytoskeleton. A long-term goal in these studies is to identify the link between the ecdysone signal and Rho1 activation, providing insights into the molecular mechanisms by which a hormonal signal can drive tissue morphogenesis.

Unexpectedly, we have discovered that a parallel morphogenetic program occurs in response to ecdysone at an earlier stage in development—during embryogenesis. Ecdysone signaling at this stage is required for coordinated changes in cell shape that drive major morphogenetic movements, establishing the body plan of the first instar larva. Some of the mutations recovered in our genetic screens for adult leg development also affect these embryonic morphogenetic events, suggesting that the hormone acts through a common pathway to drive morphogenesis at different stages in the life cycle.

As the adult tissues grow and develop during metamorphosis, they replace larval tissues that are rapidly destroyed. We have shown that the destruction of the larval midgut and salivary glands occurs as a steroid-triggered programmed cell death response. Ecdysone coordinately induces two death-activator genes, *reaper* and *hid*, immediately before the onset of larval tissue cell death. Our functional studies indicate that these genes act together, in a partially redundant manner, to direct the death of larval tissues, and that premature activation of this pathway is prevented by the DIAP1 death inhibitor. In addition, we have shown that the ecdysone-receptor complex directly induces *reaper* transcription, providing a link between the steroid hormone and a programmed cell death response. We have also identified several key ecdysone-inducible transcription factors that direct appropriate *reaper* and *hid* expression in doomed salivary glands, defining a genetic cascade that leads to the destruction of this tissue. These studies provide insight into how the larval tissues are destroyed during metamorphosis, as well as a framework for understanding how steroid hormones control programmed cell death in other organisms.

Past studies of metamorphosis have required dissection or sectioning of animals to follow events that occur underneath the opaque pupal cuticle. We have circumvented this problem by expressing green fluorescent protein (GFP) in specific tissues and following, in time-lapse movies, the development of these tissues in living animals. This method has provided a foundation for open-ended genetic screens to dissect the molecular mechanisms of steroid-triggered programmed cell death. We are screening for mutants that show normal responses to ecdysone during development but retain persistent salivary glands that express GFP. A pilot screen identified eight genes in this pathway, including several transcription factors and signaling molecules. We are characterizing several of these loci in more detail, as well as conducting an open-ended EMS screen to saturate the third chromosome for genes in this pathway. This approach uses the animal for perhaps its greatest strength, as a genetic tool to unravel complex biological pathways that are likely to be conserved in other organisms—in this case, providing a foundation for understanding the molecular basis of steroid-triggered programmed cell death.

The *Drosophila* genome encodes 18 canonical members of the nuclear receptor superfamily, of which only one (EcR) has a known ligand that binds to its ligand-binding domain (LBD) and activates target gene transcription. This observation raises the question of whether the remaining orphan receptors have ligands and, if so, how these novel

hormones and their receptors contribute to growth and development. We are taking a genomic approach toward defining the regulation and function of the nuclear receptor gene family in *Drosophila*. To date, we have characterized the temporal expression patterns of all detectable nuclear receptors throughout the major ecdysone-triggered transitions in the life cycle. We are also performing genetic studies of cofactors that modulate receptor activity, as well as generating mutations in receptor genes as a step toward understanding their functions. Current efforts are focused on DHR4, DHR96, the *Drosophila* ortholog of vertebrate estrogen-related receptor, and the *Drosophila* ortholog of vertebrate HNF4. In addition, we are using transgenic animals that express a fusion of the yeast GAL4 DNA-binding domain with a nuclear receptor LBD, in combination with a GAL4-dependent reporter gene, to follow receptor activation during development. In collaboration with Henry Krause's lab (University of Toronto), we have characterized the activation patterns of GAL4-LBD constructs for all 18 *Drosophila* nuclear receptors during embryogenesis and the onset of metamorphosis. Our long-term goal in this study is to identify novel compounds that can activate these receptors, using the animal as a tool for ligand discovery. This method provides a new direction for defining the mechanisms of hormone action during development, as well as a means of determining how nuclear receptors can exert their multiple roles in the context of an intact developing animal.

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