

To Molt or Not to Molt: Growth Control in Flies and Mosquitoes

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Growth in insects occurs during larval life punctuated by molting to acquire a larger cuticular exoskeleton to support the increased size. Usually metamorphosis occurs when the final species-specific size is attained. Molting and metamorphosis are governed by two hormones, ecdysone and juvenile hormone (JH), with ecdysone causing molting and JH preserving the “status quo” at the molt. In Lepidoptera, exposure to suboptimal food resources delays the onset of metamorphosis until they reach a certain threshold size by prolonging the presence of JH. By contrast, mosquito larvae are adapted for rapid larval growth to take advantage of ephemeral habitats and some metamorphose at smaller sizes when faced with dwindling food resources. Thus, their larval growth is adapted for speed rather than size.

In *Drosophila* larval growth is dependent on nutritional factors that act through the insulin signaling pathway. There are 7 *Drosophila* insulin-like peptides (dILPs) that appear at different times in embryonic and larval development and are variously controlled by nutrient intake, but there appears to be only one insulin receptor (InR) gene. Although final size at metamorphosis is clearly regulated by this pathway, the mechanism whereby the final critical size is communicated to the insect’s endocrine system to initiate metamorphosis is not well understood. One possibility is that the size of the imaginal discs that proliferate throughout larval life is the signal; another is that a “sentinel” tissue communicates this signal.

In *Drosophila* normally the critical size for metamorphosis is attained after approximately 6 hrs of feeding in the final instar. However, metamorphosis can occur at a smaller size after the penultimate instar when the normal ecdysone signaling pathway for the molt is disrupted. When the ecdysone-induced transcription factor Broad (BR)-Z3 was overexpressed by heat shock during the first 2-5 hr of the second instar, the feeding period was prolonged and second instar puparia were produced. The critical size for metamorphosis was reduced in these larvae. Feeding 20-hydroxyecdysone (20E) after the heat shock caused the formation of pharate third instar larvae at the normal time, indicating that the prolonged second instar was due to a deficit of 20E during the 12-15 hrs that Broad was present. Expression of the isoforms of Broad in the various neuroendocrine cells and the ring gland using different Gal4 drivers showed that expression in the prothoracic glands but not in the PTH cells or in the corpora allata was responsible for the block in ecdysone secretion in the larvae. Sustained expression of Broad eventually caused degeneration of the glands in an isoform-specific manner. Normally Broad only appears in the prothoracic glands at metamorphosis and signals the onset of their slow, progressive cell death.

To study possible interactions of the insulin-signaling pathway and the endocrine signaling pathway, we have used two lines: UAS-PTEN flies carry a constitutively active 3’ lipid phosphatase (dephosphorylates PIP3) that results in undersized cells; UAS-Dp110 flies carry a constitutively active PI3 kinase that results in giant cells. Both act in a strictly cell-autonomous manner. When either of these was expressed in the neuroendocrine cells controlling the ring gland or in the ring gland itself, neither size nor viability was affected. In contrast, when expressed in the fat body, the UAS-Dp110 line produced about 30% larger final stage larvae whereas the UAS-PTEN line initiated metamorphosis after the 2nd larval stage, rather than the 3rd. The latter phenotype is significant because all other cases of 2nd instar puparia have been caused by whole animal expression of genes involved in either ecdysone or JH signaling such as E75A. Thus, the state of the fat body may be a key player in signaling entry to metamorphosis.

In *Anopheles gambiae* there are only 5 ILPs, but nothing is known about their regulation. In *Aedes aegypti*, an InR is involved in activation of ecdysteroidogenesis in the follicle cells of the ovary after a blood meal and thus is necessary for vitellogenesis (Riehle and Brown, 2002, 2003). Interestingly, this InR is only found in the adult. One of the key enzymes in the insulin signaling pathway, the serine/threonine kinase Akt, is also present in the *Aedes* ovaries and in early embryos but is not expressed during larval life or at metamorphosis. Further studies are necessary to determine whether the insulin signaling pathway is involved in growth regulation in mosquitoes.

Riehle, MA and M Brown (2002). *Cell Tiss.Res.* **308**: 409-420.

Riehle, MA and M Brown (2003). *Insect Mol. Biol.* **12**: 225-232.