# Flies on steroids: The interplay between ecdysone and insulin signaling

**In the fruit fly *Drosophila melanogaster*, the insulin and ecdysone signaling pathways have long been known to regulate growth and developmental timing, respectively. Recent findings reveal that crosstalk between these pathways allows coordination of growth and developmental timing and thus determines final body size.**

Despite significant advances in our understanding of how growth is regulated in vivo, little is known about how the cessation of growth, and hence final body size, is determined. *Drosophila* has emerged as a useful model organism for studying the control of growth. No growth occurs in the adult fly, so final body size is determined by the size of the larva when metamorphosis occurs. This is affected by two factors: the rate at which the larva grows and the length of the larval growth period. These are controlled respectively by insulin signaling and a signaling pathway mediated by the steroid hormone 20-hydroxyecdysone (20E), the active metabolite of ecdysone. Three recent papers provide exciting and unexpected new insights into the interplay between insulin and ecdysone signaling, which ultimately regulates fly size (Caldwell et al., 2005; Colombani et al., 2005; Mirth et al., 2005).

*Drosophila* feed and grow throughout the larval period, moulting twice, until they reach the so-called "critical weight"—the weight at which they become committed to undergo metamorphosis (Robertson, 1963). Before the critical weight is attained, modulating the growth rate, for example by reducing nutrition, can result in a compensatory extension of the developmental time, so that final body size is unaltered. However, in the window of time after the critical size is attained but before pupation, lowered nutrition and growth rates do not delay metamorphosis, so manipulations during this period impact upon the final body size (Shingleton et al., 2005). Pulses of 20E in the presence of juvenile hormone (JH) trigger moulting between larval stages, and a final 20E pulse in the absence of JH initiates pupation (Nijhout, 2003). 20E is produced mainly by the prothoracic gland (PG), part of a composite organ called the ring gland.

At the cellular level, growth rate is regulated by the insulin/phosphatidylinositol 3′-kinase (PI3K) signaling pathway (Leevers and Hafen, 2004). Activation of PI3K increases levels of phosphatidyinositol-(3,4,5)-trisphosphate ($PIP_3$) at the membrane, which in turn stimulates a signaling cascade that increases cellular growth. This effect is mediated in part by the resulting cytoplasmic retention of the transcription factor dFOXO. Insulin signaling is activated by *Drosophila* insulin-like peptides (dILPs), and the key sites of dILP production are seven median neurosecretory cells (mNSCs) in the brain. As in mammals, production of these insulin homologs is modulated by sugar levels. Thus *Drosophila* larval growth is fast when there is abundant food and slow in times of starvation. The fat body, a major larval tissue analogous to the mammalian liver, has been shown to sense amino acid levels and to modulate insulin signaling (and thus growth) in other tissues accordingly (Colombani et al., 2003).

In new work reported in *Science*, Colombani et al. investigate a possible role for 20E in the regulation of fly growth (Colombani et al., 2005). By overexpressing the catalytic subunit of PI3K (Dp110) in the PG, they were able to increase the size of this gland, with a concomitant increase in systemic levels of 20E and its transcriptional targets. Increased 20E signaling throughout the body might be predicted to result in premature metamorphosis, thus giving rise to small flies, and small flies were indeed the outcome. Surprisingly however, the flies were not small because they had pupated early—the timing of larval development was unaltered. Instead the flies were small because of reduced growth rates. Conversely, decreasing insulin signaling in the PG via expression of dominant-negative Dp110 resulted in small PGs, reduced 20E signaling, and large flies due to increased larval growth rates. These data indicate that in addition to its well-characterized role in controlling developmental timing, 20E can affect larval growth rates directly.

Two independent groups have reported similar findings in *Current Biology* (Caldwell et al., 2005; Mirth et al., 2005): they also see that increasing insulin signaling in the PG produces small flies, while decreasing insulin signaling in the PG produces large flies. However, in both cases, the change in size was found to be due not just to differences in the growth rate but also changes in developmental timing. The reason for this discrepancy is not clear, though it is probably at least partly due to differences in the way in which PG insulin signaling was manipulated, and the nutritional content of the food in the various laboratories.

Does the PG act as a size sensor for the whole body, so that increasing the size of the PG causes the larva to overestimate its size, triggering a compensatory reduction in growth rate? Colombani and colleagues tested this hypothesis by increasing PG size through overexpression of either dMyc or cyclinD/Cdk4, both of which induce growth through mechanisms distinct from those used by PI3K. If the PG is indeed a general size sensor, any means of increasing its size should result in small flies; however, enlarging the PG by these alternative treatments had no effect on adult fly size. Excitingly, this means that the ability of the PG to act as a body size sensor may be specifically linked to the regulation of its growth by dILPs, and hence to the nutrient status and growth of the rest of the larva.

So, how do altered 20E levels have this direct and unexpected effect on larval tissue growth? Using various measures of insulin signaling activity, including dFOXO localization, Colombani et al. went on to demonstrate that levels of 20E were inversely correlated with levels of insulin signaling in the fat body. In addition, they used ubiquitous RNAi-mediated depletion of the receptor for 20E (EcR) to show that 20E acts via its receptor to modulate insu-

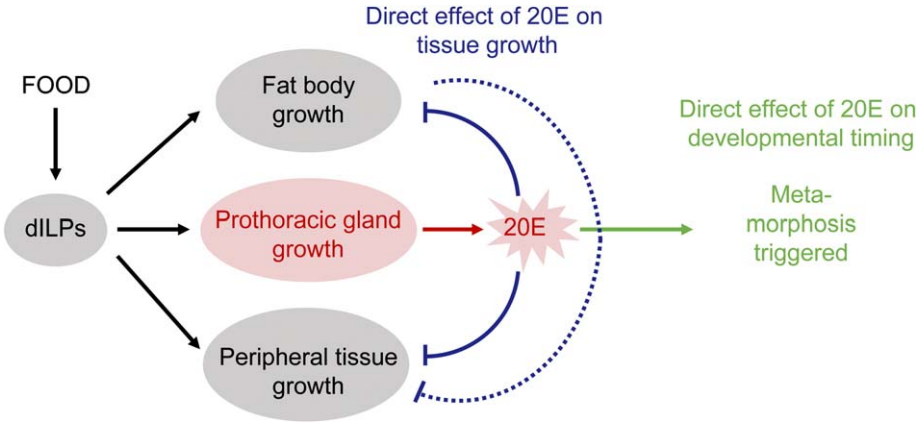


**Figure 1**. A schematic depicting possible interplay between insulin and 20E signaling in the control of *Drosophila* growth and developmental timing

Nutrition promotes release of dILPs from the mNSC into the hemolymph. Thus the growth of the fat body and other peripheral tissues, including the PG, is increased. The amount of dILP-stimulated growth of the PG dictates the amount of 20E it releases. 20E directly inhibits fat body growth by reducing dILP-mediated $PIP_3$ production and affects the growth of other peripheral tissues. In addition, 20E can signal that the larval critical weight has been reached and trigger the onset of metamorphosis.

lin signaling and thus organismal growth rate. So it seems that 20E is able to antagonize the insulin signaling pathway in larval tissues and therefore reduce growth. Furthermore, depleting EcR specifically in the fat body was sufficient to phenocopy inhibition of insulin signaling in the PG, indicating that the fat body is an important target of 20E signaling, which may be able to relay a growth-inhibitory signal to other larval tissues.

Together, these papers show that there is significant and complex crosstalk between the insulin and 20E signaling pathways. Insulin signaling can increase the levels of 20E produced by the PG; 20E in turn can act both to directly inhibit insulin signaling and growth in the fat body (and likely other peripheral tissues) and to promote developmental progression and hence larval wandering and metamorphosis (Figure 1). What is the functional significance of these links? They may provide a way of coupling growth rate and developmental timing, such that the same molecule (20E) can limit tissue growth and induce metamorphosis. It may also ensure that in times of low nutrition, development can be delayed and growth increased.

It remains to be seen exactly how the crosstalk between ecdysone and insulin signaling occurs at a molecular level. First, how do dILPs affect 20E levels? Do they promote the synthesis, processing, secretion, or stability of 20E in the PG? Colombani et al. showed that elevated insulin signaling in the PG increases the levels of at least two enzymes that are required for ecdysteroid synthesis, suggesting that the former hypothesis may be true. Second, how does 20E inhibit insulin signaling in the fat body and other larval tissues? The use of the tGPH reporter, which binds to $PIP_3$, indicates that the non-cell-autonomous effect mediated by 20E acts upstream in the insulin pathway and can modulate $PIP_3$ levels in the fat body cell membrane. The proposed model predicts that systemic insulin signaling fluctuates in response to changing 20E levels during normal development. Indeed, it has been shown that during the last larval stage, the level of insulin signaling starts off high but gradually decreases as development progresses and 20E levels rise (Rusten et al., 2004). It will be interesting to know whether such fluctuations also occur earlier in development and in other tissues.

No doubt further complexities in the interplay between insulin and 20E signaling in the whole organism will be revealed by future work. For the time being though, the papers discussed here take our knowledge further and reveal an elegant mechanism for coupling the control of growth and developmental progression. It will also be intriguing to see whether similar links between control of growth and developmental progression exist in other organisms.

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