Less Steroids Make Bigger Flies

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The simple observation that higher organisms achieve a final body size that is characteristic of their species raises the profound biological question of how that final size is achieved. Detailed studies over the past decade have provided part of the answer, demonstrating that insulin signaling plays a central role in directing animal growth. However, it remains unclear why growth is largely restricted to juvenile stages and how it is terminated upon sexual maturation. A report by Colombani et al. (1) on page 667 of this issue provides important new insights into the coordination of growth and maturation, using the fly Drosophila melanogaster as a model. The study shows that the steroid hormone ecdysone, which directs insect maturation, suppresses growth by antagonizing insulin activity.

Insulin-like peptides and the insulin receptor drive organismal growth, acting through a cellular signaling cascade that includes phosphatidylinositol 3-kinase (PI3K). Superimposed on insulin-mediated growth is temporal control by hormones that direct the juvenile-to-adult transition. In insects, this temporal control is provided by pulses of the steroid hormone ecdysone that are released from the prothoracic gland in response to neuropeptide signaling. Ecdysone pulses trigger two larval molts to accommodate the ~200-fold increase in mass that occurs as the larva feeds. Increases in ecdysone at the end of the last larval stage terminate feeding and initiate maturation via metamorphosis. The rate of larval growth and the duration of feeding both contribute to final body size, with no further growth occurring after puparium formation.

Colombani et al. exploited earlier studies showing that ectopic expression of PI3K accelerates cell growth, whereas expression of a dominant negative form (PI3KDN), which inhibits PI3K) retards cell growth (2). Expressing these insulin regulators specifically in the prothoracic gland affected the size of the gland as expected but, remarkably, had the opposite effect on overall body size. Activated insulin signaling in the prothoracic gland created smaller animals, whereas insulin inhibition created larger animals. Enlarging the prothoracic gland by expressing insulin-independent growth regulators had no effect on body size, indicating that gland size per se is not the culprit. Rather, changes in insulin signaling within the prothoracic gland affect overall body size (see the figure). Given that the primary function of this organ is to produce ecdysone, the authors used various strategies to measure ecdysone levels in animals that express either PI3K or PI3KDN in their prothoracic glands. They found that larvae with smaller glands produced less ecdysone, whereas those with enlarged glands produced more. This suggests that the effects of prothoracic gland insulin activity on body size are mediated by changes in ecdysone levels (see the figure). This proposal is reminiscent of the effects of insulin on insect ovaries, where it promotes ecdysone production (3, 4). Feeding ecdysone throughout larval stages or inactivating the ecdysone receptor resulted in reduced or increased body weight, respectively, further defining a role for ecdysone in insect growth.

How do changes in ecdysone levels affect final body size? One possibility arises from the role of the hormone in determining the duration of larval feeding. Changes in ecdysone levels could direct shorter or longer feeding periods. Alternatively, ecdysone could affect larval growth rates, allowing animals to achieve different sizes over the same time interval. Colombani et al. favor the latter model and show that the growth rate is enhanced in larvae that express PI3KDN in their prothoracic gland, but is reduced in larvae that express PI3K, with little or no effect on the timing of ecdysone pulses triggering two larval molts to accommodate the increase in mass that occurs as the larva feeds.

Coordination of organism growth through insulin and ecdysone signaling. (Top) The four major stages of the Drosophila life cycle are depicted: embryonic, larval, pupal, and adult. Growth occurs during larval stages in response to insulin signaling and basal levels of the steroid hormone ecdysone. This is followed by sexual maturation during metamorphosis. (Bottom) The prothoracic gland releases ecdysone that activates the ecdysone receptor in fat body cells, producing an unknown factor X. This factor may suppress growth by inhibiting the release of insulin-like peptides from insulin-producing cells. Insulin-like peptides activate the insulin receptor and PI3K signaling pathway that blocks nuclear translocation of the transcription factor dFOXO. The ecdysone receptor may also induce expression of a factor Y that directs nuclear translocation of dFOXO, activating genes that inhibit growth, including that which encodes the 4E-BP protein translation inhibitor.

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larval molts or puparium formation. In addition, they found that feeding ecdysone to larvae that express P3K<sup>DN</sup> in their prothoracic gland slowed the enhanced growth of these animals, suggesting that the increased growth rate is indeed due to reduced ecdysone titers.

Finally, the authors show that expression of P3K in the prothoracic gland and changes in ecdysone signaling affect components of the insulin signaling pathway in other tissues in a manner that is consistent with the effects on growth. Expression of P3K in the prothoracic gland resulted in increased translocation of the transcription factor dFOXO into nuclei of fat body cells. This consequently increased expression of a direct target of dFOXO, the 4E-BP protein synthesis inhibitor. These are all indicators of decreased insulin signaling and reduced growth (see the figure). A similar effect was seen by feeding ecdysone to normal (wild-type) larvae. Conversely, inactivating the ecdysone receptor in the fat body decreased nuclear levels of dFOXO and reduced 4E-BP expression, further suggesting that ecdysone regulates organismal growth through effects on the insulin signaling pathway. The observation that a mutant fly lacking functional dFOXO does not exhibit the growth defect caused by expressing P3K in the prothoracic gland also supports this model. Moreover, Colombani et al. show that reducing ecdysone receptor activity exclusively in the fat body is sufficient to produce larger animals, indicating that this tissue (the insect equivalent of mammalian liver and adipose tissue) plays a central role in relaying systemic information regarding final overall body size, albeit through an unknown signal (see the figure).

Several important questions remain. First, is insulin signaling in the prothoracic gland a natural means of regulating ecdysone titers? It will be interesting to determine whether tissue-specific loss-of-function mutations in insulin signaling components in the prothoracic gland have the predicted effects on ecdysone titers and body size. A second related question is whether changes in insulin signaling in the prothoracic gland affect body size solely through changes in ecdysone levels. Partial reduction in the activity of key enzymes in the ecdysone biosynthetic pathway would address the question of whether the corresponding changes in ecdysone levels are sufficient to alter body size. Further studies will also have to examine how insulin balances its normal growth-promoting effects with its proposed growth-inhibitory effects through ecdysone synthesis (see the figure).

Another critical question is whether changes in larval growth rates alone explain the observed effects on body size. This question is highlighted by two recent studies (5, 6) that use similar strategies to modulate prothoracic gland insulin activity and report comparable effects on body size and larval growth rates. In contrast to the work of Colombani et al., however, these studies find that the larval stages are shorter for small animals and prolonged for larger animals, indicating that the duration of the larval growth phase contributes to final body size. The fact that each study uses different transgenic tools to modulate prothoracic gland insulin activity may provide one reason for the observed differences in developmental timing. In addition, small differences in the duration of larval development can have a significant effect on overall body size, given that Drosophila larvae gain on average 7% of their weight per hour. Moreover, as shown by Mirth et al. (3), nutrition and photoperiod can affect the degree of overall growth directed by insulin signaling in the prothoracic gland. Clearly, more work is required to resolve this discrepancy.

Our current understanding of ecdysone action is derived largely from studies of higher-titer ecdysone pulses in directing developmental transitions during the insect life cycle. However, these three new reports draw our attention back to basal ecdysone levels and their roles in insect physiology. Although relatively few studies have addressed this issue, basal ecdysone levels maintain cell proliferation in the eye priomium of the moth Manduca sexta, with higher hormone titers arresting proliferation and promoting eye maturation (7). In addition, studies of the wing imaginal discs of the butterfly Precis coenia demonstrate a requirement for both ecdysone and bombyxin (a lepidopteran insulin-like peptide) for growth (8). The molecular basis of these effects, however, remains unclear. The results reported by Colombani et al., along with the related studies by Mirth et al. (5) and Caldwell et al. (6), provide insights into how steroid and insulin signaling are integrated to coordinate growth and maturation, and establish new directions for future studies of growth regulation in higher organisms.

**References**


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**PHYSICS**

The Observation of Matter Wave Fluctuations

Peter L. Knight

This month’s Nobel Prize in Physics recognizes Roy Glauber for his work in quantum optics, and especially for his role in distinguishing the different kinds of fluctuations or correlations that exist in natural light from thermal sources such as the Sun, which are quite different from those of the unnatural radiation from a laser. On page 648 of this issue, we see a further chapter opening in this story, in which Schellekens et al. (1) report on observations of analogus fluctuations in matter waves, those formed from cold atoms. Using a clever microchannel plate detection scheme, they have been able to demonstrate the transition from the fluctuations in a thermal cloud of atoms to the lack of fluctuations in a coherent Bose condensate as their atom cloud cooled.

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The study of fluctuations in thermal light was initiated in the 1950s by the British astronomers R. Hanbury Brown and R. Q. Twiss (2), who were eager to develop a new method to determine the size of stars that improved on Michelson’s stellar interferometer (see the figure). Hanbury Brown had a curious initiation into the study of fluctuating signals: Almost kidnapped from the student labs by the then-rector of Imperial College, Henry Tizard, he was press-ganged into joining the nascent British radar project at Bawdsey, and after the war joined Lovell’s group setting up the Jodrell Bank Observatory in Cheshire. He and Twiss were determined to show that an intensity interferometer would generate the improvements they sought over the Michelson interferometer, and started by demonstrating the effect in a laboratory experiment with thermal light from a spectral lamp (this later led to the successful