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The roles of juvenile hormone, insulin/target of rapamycin, and ecydsone signaling in regulating body size in *Drosophila*

Christen Kerry Mirth[1,*] and Alexander William Shingleton[2,*]

[1]Development, Evolution, and the Environment Laboratory; Instituto Gulbenkian de Ciência; Oeiras, Portugal; [2]Department of Biology; Lake Forest College; Lake Forest, IL USA

Understanding how organisms regulate their body size has interested biologists for decades. Recent work has shown that both insulin/target of rapamycin (TOR) signaling and the steroid hormone ecdysone act to regulate rates of growth and the duration of the growth period in the fruit fly, *Drosophila melanogaster*. Our recent work has uncovered a third level of interaction, whereby juvenile hormone (JH) regulates levels of both ecdysone and insulin/TOR signaling to control growth rates. These studies highlight a complex network of interactions involved in regulating body and organ size.

Keywords: body size, growth rate, growth duration, juvenile hormone, ecdysone, insulin/insulin-like growth factor signalling

*Correspondence to: Christen Kerry Mirth; Email: christen@igc.gulbenkian.pt; Alexander William Shingleton; Email: shingleton@lakeforest.edu

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Most adult animals have characteristic body sizes: ants are generally millimeters in length whereas whales are many meters long. In most cases, adult body size results from growth during development. To regulate their final size, juvenile organisms control their rate of growth and the length of time they spend growing. The genetic and physiological mechanisms regulating growth rate and growth duration have been the focus of many recent studies, providing important new insight into body size control.

Over the past four decades, insects have served as study systems to understand the physiological underpinnings of size regulation. Classic studies in the tobacco hornworm, *Manduca sexta*, uncovered a role for the sesquiterpinoid hormone juvenile hormone (JH), and the steroid hormone ecdysone in regulating the duration of the growth period.[1] More recently, studies in *Drosophila* have helped identify the insulin/target of rapamycin (TOR) signaling-pathway as a regulator of growth rate not just in insects but virtually every other animal studied to date.[2]

Although initially growth rate and growth duration were thought to be separate processes regulated by separate signaling pathways, this vision has become increasingly muddied. Insulin/TOR signaling, known for regulating growth rate, is now known to control the timing and quantity of ecdysone synthesized[3-7] (**Fig. 1A**). Increasing insulin/TOR signaling specifically in the gland that synthesizes ecdysone, the prothoracic gland, accelerated the timing of ecdysone pulses, thereby shortening the time to metamorphosis.[3,5,6] In addition, increased insulin/TOR in the prothoracic gland increased the overall amount of ecdysone produced and this slowed growth rate by decreasing insulin/TOR signaling throughout the body.[3,5,6]

It is unclear, however, whether the interaction between the insulin/TOR and ecdysone signaling pathways is exceptional or represents a more general phenomenon whereby signaling pathways that canonically regulate growth rate crosstalk with those that canonically regulate growth duration. To address this we looked at a second hormone involved in the regulation of growth duration in insects, JH. In many insects, JH orchestrates the timing of metamorphosis.[1,8] However, because ectopic JH application did not appear to affect development in *Drosophila* larvae,[9] it was long thought that it did not play important roles in the development of this insect. Our recent study found that larvae that lacked the JH producing glands, the corpora allata (CA), produced adults of smaller than normal size.[10] This led our team to investigate the developmental

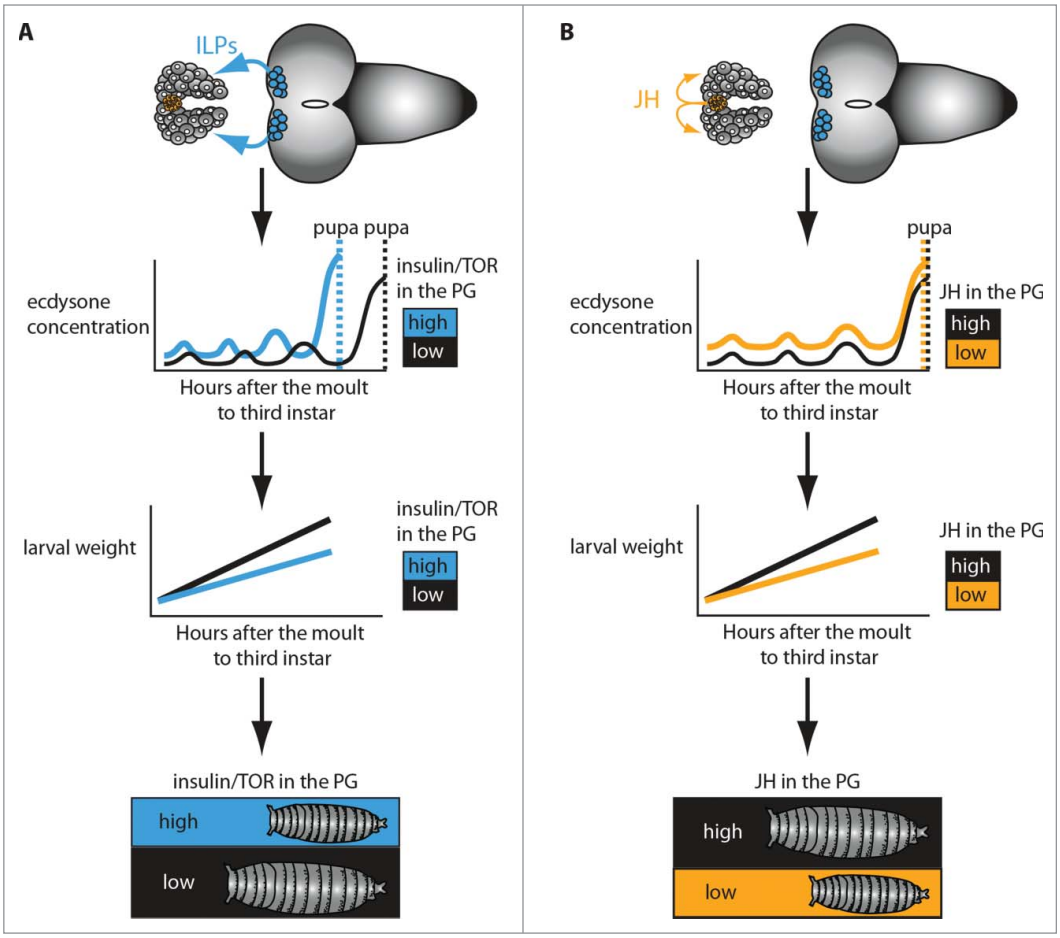

Figure 1. The effects of insulin/target of rapamycin (TOR) signaling and juvenile hormone (JH) signaling in the prothoracic gland during body size regulation. (**A**) Insulin-like peptides (ILPs) are secreted from neurosecretory cells in the brain. They act on the prothoracic gland to regulate the timing and amount of ecdysone synthesized by the prothoracic gland. By regulating the timing of ecdysone pulses, insulin/TOR affects developmental timing, hence the duration of the growth period. By affecting ecdysone concentrations, insulin/TOR regulates growth rates. (**B**) JH acts on the prothoracic gland to affect the concentration of ecdysone synthesized, but not the timing of ecdysone synthesis. In this way, JH does not affect developmental timing but rather regulates body size by controlling growth rates.

processes underlying this reduction in body size.

We found that, unlike many other insects, ablating the CA did not produce dramatic effects on the growth duration. This was perhaps not a surprising result given the lack of effect of ectopic JH application on *Drosophila* development.[11] What was wholly unexpected, however, was the discovery that ablation of the CA reduces growth rate. Even more surprisingly we found that JH appears to regulate growth rates by modulating both insulin/TOR signaling and ecdysone synthesis (**Fig. 1B**). Several lines of evidence support this hypothesis. First, the reduction in body size caused by ablation of the CA was eliminated in larvae also lacking the Forkhead Box class O (FOXO) transcription factor. FOXO is a major effector of the insulin/TOR signaling pathway and is a negative growth regulator that reduces growth rate when insulin/TOR signaling is low. The observation that CA-ablated larvae without FOXO are not small suggests that loss of JH reduces body size by reducing insulin/TOR signaling, a hypothesis further supported by the observation that CA-ablated larvae have elevated FOXO activity. Second, CA-ablated larvae also have elevated levels of circulating ecdysone and elevated levels of ecdysone signaling. Since ecdysone is a negative regulator of systemic insulin signaling, a compelling hypothesis is that CA-ablated larvae show reduced insulin signaling due to their elevated ecdysone levels. This hypothesis is supported by the observation that downregulating expression of the JH receptor in the prothoracic gland also reduces body size but does not alter developmental timing.

Taken together, our data suggest that JH acts to regulate insulin/TOR signaling by controlling ecdysone concentration but not the timing of ecdysone pulses (**Fig. 1B**). By regulating ecdysone concentration, JH may modulate insulin/TOR signaling throughout the body, thereby controlling growth rates without substantially interfering with developmental timing. Our data may also help to explain why JH appears to positively regulate the disproportional growth of secondary sexual characteristics in a number of insect species, including the eye stalks of stalk-eyed flies, and the enlarged mandibles of stag beetles and horned flour beetles.[12,13] Collectively, these results not only help us understand how body size is regulated, they also uncover an unexpected level of interaction between these three signaling systems that may be important in explaining how relative organ size is regulated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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