

The Steroid Hormone Ecdysone Controls Systemic Growth by Repressing dMyc Function in *Drosophila* Fat Cells

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SUMMARY

How steroid hormones shape animal growth remains poorly understood. In *Drosophila*, the main steroid hormone, ecdysone, limits systemic growth during juvenile development. Here we show that ecdysone controls animal growth rate by specifically acting on the fat body, an organ that retains endocrine and storage functions of the vertebrate liver and fat. We demonstrate that fat body-targeted loss of function of the Ecdysone receptor (EcR) increases dMyc expression and its cellular functions such as ribosome biogenesis. Moreover, changing dMyc levels in this tissue is sufficient to affect animal growth rate. Finally, the growth increase induced by silencing EcR in the fat body is suppressed by co-silencing dMyc. In conclusion, the present work reveals an unexpected function of dMyc in the systemic control of growth in response to steroid hormone signaling.

INTRODUCTION

Growth is a discontinuous process that needs to be coordinated with the developmental program. In many species, growth is restricted to the juvenile period. Passage into adulthood is accompanied by the acquisition of sexual maturity (maturation) and rapid changes in growth control, leading to a systemic arrest of body growth. The relationships between the onset of maturation and the regulation of growth remain poorly understood.

In holometabolous insects, growth and maturation are temporally distinct. Growth is mainly restricted to the larval period and maturation occurs during metamorphosis or pupal development. Tissue growth relies on the insulin/IGF (insulin-like growth factor) signaling pathway (IIS), a highly conserved pathway that couples nutrition with growth. *Drosophila* has a conserved IIS system with seven insulin-like peptides named Dilps, a unique insulin receptor (dInR), and a conserved cascade of intracellular effectors (Géminard et al., 2006). In parallel to the canonical InR pathway, the target of rapamycin (TOR) pathway promotes cell growth through its action on translational initiation, ribosome biogenesis, nutrient storage, endocytosis, and autophagy (reviewed in Arsham and Neufeld, 2006; Wullschleger et al.,

2006; Guertin and Sabatini, 2007). Multiple molecular crosstalks have been established between the TOR and InR signaling pathways, ensuring that cells integrate both systemic and local cues in their growth program. Other growth inducers have been identified in multicellular organisms, among them the Myc family of transcription factors (reviewed in Eilers and Eisenman, 2008). In mammalian cells, activity of the Myc proteins is associated with cell growth and proliferation, inhibition of terminal differentiation, and apoptosis (Grandori et al., 2000). Consistent with its role in growth control, Myc is a potent regulator of many components of the translation apparatus. It activates RNA polymerase III, rRNA synthesis, ribosome biosynthesis genes, and translation initiation factors. In line with their common action on ribosome biosynthesis and translation initiation, recent data suggest a link between the IIS/TOR network and Myc. In specific tissues, expression of the *Drosophila* Myc ortholog dMyc is repressed by dFoxO, a transcription factor that is inhibited by the activation of IIS. In addition, TOR signaling regulates dMyc protein levels and the activation of dMyc target genes (Teleman et al., 2008).

In parallel to the molecular machinery that promotes larval tissue growth, a humoral clock times the different developmental stages. In both vertebrates and insects, maturation is triggered by steroid hormones. In *Drosophila*, larvae progress through a series of molts, the duration of which is determined by a pulse of the steroid hormone 20-hydroxyecdysone (20E, also referred to as ecdysone; reviewed by Thummel, 1996). At the end of larval development, a strong induction of ecdysone production promotes growth arrest and the transition to pupal development. Like vertebrate steroids, ecdysone acts through members of the nuclear receptor superfamily that function as ligand-regulated transcription factors. The ecdysone receptor is a heterodimer consisting of two nuclear receptors, EcR and Ultraspiracle (Usp) (reviewed by King-Jones and Thummel, 2005). Ecdysone rapidly induces early response genes such as those encoding the nuclear receptors E74, E75, and Broad Complex (BR-C), a family of transcription factors. Subsequently, late genes that control the biological responses to each ecdysone pulse are induced, leading to the morphological changes specific to each developmental stage.

Many studies suggest that growth and developmental timing are interconnected to establish final body size. Suppression of the prothoracicotrophic hormone (PTTH, the hormone that stimulates ecdysone production in the larva) results in a delayed transition to pupal development. As a consequence, animals benefit from a longer larval growth period and eclose as larger

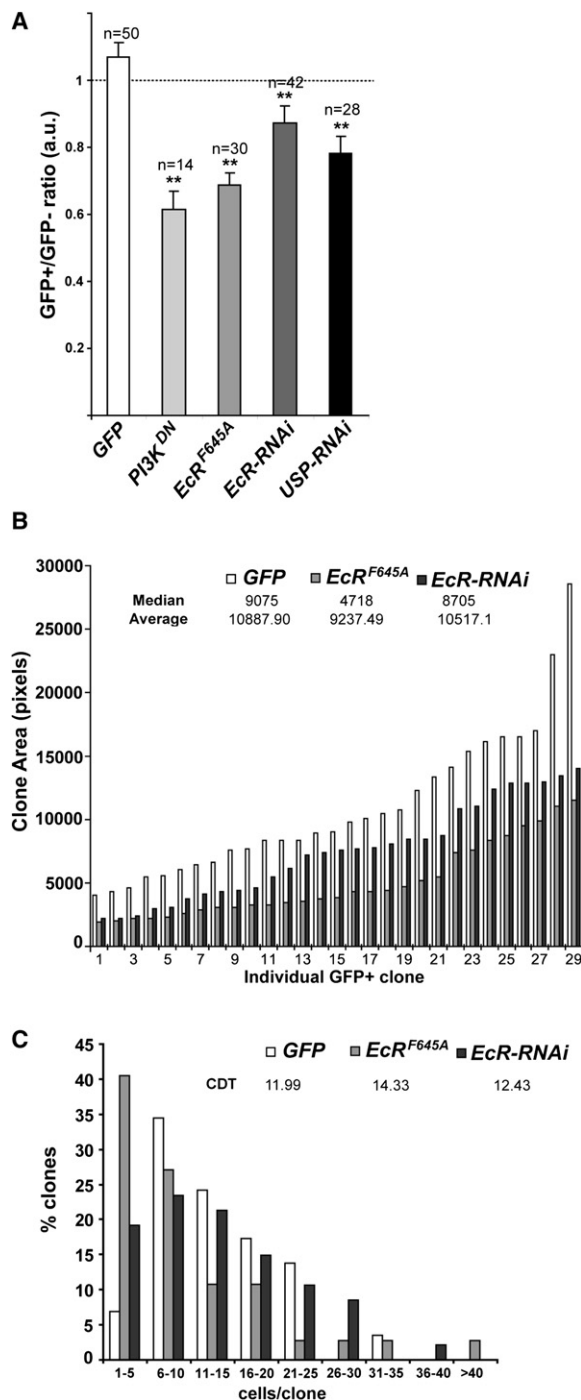


Figure 1. EcR Signaling Is Required for Tissue Growth and Cell Proliferation

(A) Ratios between the areas of clonal cells overexpressing the indicated transgenes (GFP+) versus the wild-type cells (GFP-) using the flip-out Gal4 technique. In the fat body, EcR loss of function (*EcR^{F645A}*, *EcR-RNAi*, and *USP-RNAi*) significantly decreases the cell size compared to control (GFP overexpression). Overexpression of a dominant-negative form of PI3K (*PI3K^{DN}*) gives a similar effect. n, number of GFP+ cells measured. Error bars represent standard error of the mean (SEM); **p < 0.01 versus control (GFP-expressing cells).

(B) Clones were induced at 48 hr AED. Discs were fixed at wandering stages (115 hr AED). Twenty-nine random clones of each genotype were measured and

flies (McBrayer et al., 2007). A manipulation of the ras/raf/MAPK signaling pathway in the prothoracic gland (PG, the larval site for ecdysone synthesis) modifies the timing of the ecdysone peak, the timing of the larval/pupal transition, and the size of the adults (Caldwell et al., 2005). Drastic changes in PI3-kinase activity in this tissue lead to similar effects (Mirth et al., 2005).

Last, besides its ability to time developmental transitions, the molting hormone ecdysone has also been shown to specifically control the speed at which animals grow, or growth rate. Indeed, changes in circulating ecdysone levels during larval development affect IIS activity in larval tissues and influence final body size (Colombani et al., 2005). This indicates that IIS and ecdysone signaling pathways carry antagonistic actions that establish the animal growth rate. The physiology and the molecular aspects of the interaction between these two major pathways remain unknown.

In the present study, we show that the fat body, the functional homolog of vertebrate liver and adipose tissue, acts as a unique relay tissue for the control of larval growth by circulating ecdysone. The repression of IIS by ecdysone signaling is not required in fat cells to mediate ecdysone-dependent growth inhibition. RNA profiling of dissected fat bodies revealed that EcR signaling represses the *Drosophila dMyc* gene and its downstream targets. Furthermore, the downregulation of *dMyc* in fat cells is critical for growth inhibition by ecdysone. We propose a model whereby the rise of ecdysone levels at the end of the juvenile period represses *dMyc* expression in the fat body. This inhibition restricts ribosome biosynthesis and translation efficiency in fat cells, and induces a general pause in the growth program that precedes entry into metamorphosis.

RESULTS

Cell Proliferation and Tissue Growth Are Reduced upon EcR Loss of Function

We previously showed that circulating ecdysone impedes the larval growth rate through an antagonistic interaction with IIS (Colombani et al., 2005). Because IIS is an important regulator of cell growth, we tested the cell-autonomous effects of reducing EcR signaling on cell size. Surprisingly, clonal silencing of *EcR* and *Usp* or clonal expression of a dominant-negative form of EcR (*EcR^{F645A}*) in the fat body led to a reduction of cell size, despite higher levels of IIS (Figure 1A; see Figures S1A and S1B, available online, for the characterization of the *UAS-EcR-RNAi* line). The same analysis made in imaginal tissues also showed reduced growth in clones upon *EcR* loss of function (Figure 1B). More specifically, the cell-doubling time of imaginal cells was strongly increased in clones expressing *EcR^{F645A}* and moderately affected upon *EcR* silencing (Figure 1C). These results demonstrate that despite an antagonistic action on IIS,

plotted individually in order of increasing size. Controls are GFP-expressing clones. Values for median and average are indicated.

(C) Clones were induced at 72 hr AED and analyzed after fixation at wandering stages. The distribution of the number of cells per clone as a percentage of the total number of clones for each genotype is shown. The median cell-doubling time (CDT) is shown.

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