

# Integration of Insulin receptor/Foxo signaling and dMyc activity during muscle growth regulates body size in *Drosophila*

Fabio Demontis<sup>1,\*</sup> and Norbert Perrimon<sup>1,2</sup>

*Drosophila* larval skeletal muscles are single, multinucleated cells of different sizes that undergo tremendous growth within a few days. The mechanisms underlying this growth in concert with overall body growth are unknown. We find that the size of individual muscles correlates with the number of nuclei per muscle cell and with increasing nuclear ploidy during development. Inhibition of Insulin receptor (InR; Insulin-like receptor) signaling in muscles autonomously reduces muscle size and systemically affects the size of other tissues, organs and indeed the entire body, most likely by regulating feeding behavior. In muscles, InR/Tor signaling, Foxo and dMyc (Diminutive) are key regulators of endoreplication, which is necessary but not sufficient to induce growth. Mechanistically, InR/Foxo signaling controls cell cycle progression by modulating *dmyc* expression and dMyc transcriptional activity. Thus, maximal dMyc transcriptional activity depends on InR to control muscle mass, which in turn induces a systemic behavioral response to allocate body size and proportions.

**KEY WORDS:** Foxo, InR/Tor signaling, Myc, Muscle growth, Endoreplication, Body size, Feeding behavior

## INTRODUCTION

Body and organ growth are among the most dramatic processes that a developing organism undergoes (Conlon and Raff, 1999), but an understanding of their inter-regulation remains elusive (Hafen and Stocker, 2003). The Insulin receptor/Target of rapamycin (InR/Tor) pathway regulates cell size and number and hence organ and body growth across evolution (Hafen and Stocker, 2003). Upon Insulin/IGF binding to the Insulin and IGF receptors, a signaling cascade of phosphorylation and docking events, antagonized by Pten, results in the activation of the Ser/Thr kinase Akt. Akt then controls cell survival, cell cycle, cell growth and metabolism through phosphorylation of a number of key substrates, including the Tsc1-Tsc2 complex, and the transcription factors of the Forkhead box O (FoxO) family. Phosphorylation and inhibition of the Tsc1-Tsc2 complex, which has an inhibitory effect on Tor, promotes protein synthesis (Manning and Cantley, 2007). By phosphorylating and sequestering Foxo in the cytoplasm, Akt further promotes cell growth and cell cycle progression (Accili and Arden, 2004; Greer and Brunet, 2008; Puig and Tjian, 2006).

Similar to InR/Tor signaling, Myc has an evolutionarily conserved function in promoting cell growth and proliferation (de la Cova and Johnston, 2006). Myc regulates gene expression by binding to Enhancer box sequences (E-boxes) in promoter regions of target genes, with its dimerization partner Max, but also independently (Steiger et al., 2008). Max also dimerizes with itself and with members of the Mad/Mnt family, opposing Myc-Max transcriptional activity (Eisenman, 2001; Gallant, 2006; Grandori et al., 2000).

Although InR/Tor signaling, Foxo and Myc have been causally associated with the growth of most cell types across species, how organ and body growth are in turn determined is still unclear. Possibly, body size is decided by stereotypical responses of each

organ to growth factors, which in turn regulate InR/Tor signaling and Myc activity. Alternatively, InR signaling in some sensor tissues might have a pivotal role in modifying body growth in response to the nutritional status of the organism. Consistent with this model, InR/Tor signaling in the *Drosophila* fat body, which corresponds to human liver and adipose tissue, and in endocrine glands regulates the growth of other unrelated tissues and, consequently, of the entire body, by modulating the actions of anabolic hormones (Edgar, 2006). However, it is unknown whether other tissues and mechanisms might contribute to the systemic regulation of growth.

Muscles have important metabolic functions, undergo dramatic growth during development, and are continually remodeled throughout life. Despite their importance, it is unclear how muscle growth occurs and whether it contributes to the overall control of body size.

In vertebrates, several stimuli, including those activating InR/Tor signaling and Myc, promote hypertrophy of skeletal muscles and cardiomyocytes by inducing protein synthesis (Glass, 2003b). Conversely, inhibition of InR signaling, which results in Foxo activation, promotes protein degradation and muscle atrophy (Sandri et al., 2004; Stitt et al., 2004). Other processes, in particular an increase in DNA content, either by increasing the number of nuclei or their ploidy, may be involved in muscle growth (Brodsky and Uryvaeva, 1977; Conlon and Raff, 1999). Consistently, satellite cells fuse to pre-existing skeletal muscles, increasing the number of nuclei and supporting hypertrophy (Buckingham, 2006). Further, cardiomyocytes increase their nuclear ploidy during the reparative growth that follows an ischemic injury (Herget et al., 1997; Meckert et al., 2005). However, it is unknown whether nuclear ploidy can sustain muscle growth, whether InR/Foxo signaling and Myc regulate these events, and whether they crosstalk during muscle growth. Studies in epithelial and hematopoietic cells have suggested that Myc might act either upstream (Bouchard et al., 2007), downstream (Bouchard et al., 2004), or in parallel with Foxo (Prober and Edgar, 2002). Thus, the interplay of InR/Foxo signaling and Myc might rely on the specific cellular context and needs to be analyzed in vivo to identify physiologically relevant interactions.

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Here, we have used *Drosophila* muscles to investigate: (1) how InR (Insulin-like receptor)/Tor signaling, Foxo and dMyc (Diminutive) interact in vivo during muscle growth; (2) whether they regulate the nuclear ploidy of muscle cells; (3) whether this is important for cell growth; and (4) whether muscle mass can in turn influence body size.

The *Drosophila* larval body wall muscles are skeletal muscles, each comprising a single, multinucleated syncytial cell (myofiber) that arises from the fusion of precursor cells (founder cells and fusion-competent myoblasts) during embryonic development. Different degrees of cell fusion account for different numbers of nuclei that are contained within distinct muscle cells (Bate et al., 1999; Beckett and Baylies, 2006). During larval development, body wall muscles (see Fig. 1A) grow dramatically in ~5 days, via sarcomere assembly and the addition of novel myofibrils, while the number of nuclei remains constant (Bai et al., 2007; Haas, 1950). Muscle growth may also rely on an increase in nuclear ploidy, as previously observed for other *Drosophila* tissues (Edgar and Orr-Weaver, 2001; Maines et al., 2004), via endoreplication (or endocycle), a modified cell cycle in which DNA replication is not accompanied by mitosis but rather by multiple G-S and S-G transitions (Edgar and Orr-Weaver, 2001).

Here, we find that dMyc and InR/Foxo signaling are key regulators of endoreplication that is necessary, but not sufficient, for muscle growth. Foxo has a pivotal role in this process by regulating *dmyc* expression and activity downstream of InR signaling. The functional interaction of the transcription factors Foxo and dMyc controls the final muscle mass, which in turn influences body size by regulating larval feeding behavior.

## MATERIALS AND METHODS

### *Drosophila* genetics and fly stocks

Fly stocks used are: *UAS-foxo* (Hwangbo et al., 2004); *UAS-InR*; *UAS-Pten*; *UAS-Tsc1*, *UAS-Tsc2* (Potter et al., 2001); *UAS-InR DN* (Bloomington #8253); *UAS-dmyc*, *UAS-dmyc* [second transgene, tr2 (Orlan et al., 2007)]; *UAS-CycE* (Bloomington #4781); *UAS-dmmt* (Loo et al., 2005); *chico<sup>1</sup>/CyO*, *act-GFP*; *dm<sup>4</sup>/FM7i*, *act-GFP* (Pierce et al., 2008); *Dmef2-Gal4* (Ranganayakulu et al., 1996); *Mhc-Gal4* (Schuster et al., 1996); *UAS-Dcr-2* (Dietzl et al., 2007); *UAS-dmyc hp* (CG10798, VDRC #2947); *UAS-Akt1 hp* (CG4006, DRSC TR00202A.1); *UAS-InR hairpin (hp)* [CG18402, DRSC TR00693A.1; courtesy of Dr Jianquan Ni (Ni et al., 2008)]; *Mhc-GFP (Wee-P26)* (Clyne et al., 2003); and *UAS-H2B-CFP* (from Dr Shu Kondo, Harvard Medical School, Boston, MA, USA).

The PG157-Gal4 line is a lethal insertion at position 12F7 that drives high transgene expression in ventral internal 1 muscle (VI1, also known as muscle 31 of abdominal segment 1) and muscles of the thoracic segment (see Fig. S5 in the supplementary material). PG157-Gal4 does not drive transgene expression in ventral longitudinal 3 and 4 muscles (VL3 and VL4, also known as muscles 6 and 7). *Dmef2-Gal4* and *Mhc-Gal4* drive transgene expression in all body wall muscles, but not in other endoreplicating tissues (see Fig. S3 in the supplementary material). For transgene expression with the Gal4-UAS system (Brand and Perrimon, 1993), flies were reared at 25°C (*Dmef2-Gal4*) or 31°C (*Mhc-Gal4*). Flies were reared at 29°C for hairpin expression and at 22°C in Fig. 1.

### Body size analysis

For analysis of body weight, groups of L3 wandering larvae were weighed on an analytical balance and the average body weight calculated. Larval staging was supported by analysis of mouth hook morphology. Larval and pupal length and diameter were measured manually using AxioVision v4.5 software (Zeiss). Larval and pupal volumes were calculated assuming a prolate spheroid geometry. For analysis of internal organs, dissected organs were stained in a micro-chamber with the lipophilic dye FM4-64 [Molecular Probes (Demontis and Dahmann, 2007)]. Images were acquired with an

epifluorescence microscope (Zeiss). Adult flies were analyzed according to Colombani et al. (Colombani et al., 2005), using the Measure Tools of the AxioVision software. Larval feeding behavior was estimated as described previously (Wu et al., 2005).

### Histology, laser-scanning confocal microscopy and image analysis

Larvae were dissected in ice-cold Ca<sup>2+</sup>-free saline buffer (128 mM NaCl, 2 mM KCl, 4 mM MgCl<sub>2</sub>, 1 mM EGTA, 35 mM sucrose, 5 mM HEPES pH 7.2) using dissection chambers (Budnik et al., 2006). Body wall muscles were fixed for 20–30 minutes in Ca<sup>2+</sup>-free saline buffer containing 4% paraformaldehyde and 0.1% Triton X-100. After washing, body wall muscles were incubated for 10 hours with DAPI (4',6-diamidino-2-phenylindole, 1 µg/ml) and Alexa633- or Alexa488-conjugated phalloidin (1:100) to visualize nuclei and F-actin, respectively. To examine biogenesis of nucleoli, an anti-Fibrillar antibody [EnCore Biotechnology #MCA-38F3 (Grewal et al., 2005)] was applied (1:100), followed by incubation with Alexa546-conjugated secondary antibodies (Molecular Probes). Muscles VL3 and VL4 of abdominal segments 2–5 were imaged using a Leica TCS SP2 confocal laser-scanning microscope. Confocal images were analyzed using the Measure Tools of the AxioVision software. Statistical analysis was performed using Student's *t*-test and Excel (Microsoft).

### Luciferase assays, RNAi treatment and plasmid DNAs

For Luciferase assays, 15 × 10<sup>4</sup> S2R+ cells/cm<sup>2</sup> were seeded in Schneider's medium (Gibco) containing 10% FCS, and transfected one day later using the Qiagen Effectene Transfection Kit. An *actin-Renilla* Luciferase reporter was co-transfected as a normalization control.

Double-stranded RNA (dsRNA) synthesis and RNAi treatment were performed according to the DRSC protocols (<http://flyRNAi.org>), using amplicons DRSC34258 (*dmyc*) and DRSC31746 (*foxo*). RNAi treatment was performed for 3 days. *foxo* and *dmyc* expression were induced 24 hours prior to Luciferase assay by addition of CuSO<sub>4</sub> directly to the culture medium to a final concentration of 500 µM. Serum starvation was also performed for 24 hours. The Luciferase assay was performed in quadruplicate using the Dual-Glo Luciferase Assay (Promega) according to the manufacturer's instructions. Luciferase activity refers to the ratio of firefly to *Renilla* Luciferase luminescence.

Plasmids used are *pMT-foxo* (Puig et al., 2003), *pMT-dmyc* (Orlan et al., 2003), *actin-Renilla* Luciferase, *CG4364*, *CG5033* and *CG5033 ΔE-box* Luciferase reporters (Hulf et al., 2005).

### Quantitative real-time RT-PCR

Total RNA was prepared from L3 wandering larvae using Trizol (Invitrogen), followed by RNA cleanup with the RNAeasy Kit (Qiagen). The RNA QuantiTect Reverse Transcription Kit (Qiagen) was used for cDNA synthesis, and quantitative real-time PCR was performed with the QuantiTect SYBR Green PCR Kit (Qiagen). *αTub84B* was used as normalization reference. Relative quantitation of mRNA levels was calculated using the comparative C<sub>T</sub> method.

### Immunoprecipitation and immunoblotting

For immunoprecipitation, S2R+ cells were washed with ice-cold PBS, lysed with lysis buffer (20 mM Tris pH 7.6, 150 mM NaCl, 2 mM EDTA, 10% glycerol, 1% Triton X-100, 1 mM DTT, 1 mM PMSF and Protease inhibitors), and centrifuged at 10,000 g for 10 minutes at 4°C. Equal amounts of supernatant were incubated with a monoclonal mouse anti-dMyc antibody (Prober and Edgar, 2000), and subsequently with an appropriate amount of protein A-agarose bead slurry (Amersham) in lysis buffer. Immunoprecipitates were washed three times with lysis buffer, boiled in sample buffer, resolved on 10% SDS-PAGE gels and transferred to nitrocellulose membranes. Western blotting was performed with a rabbit anti-Foxo (Puig and Tjian, 2005) or, after extensive membrane washing, with a rabbit anti-dMyc antiserum (Maines et al., 2004), and subsequently with anti-rabbit HRP-conjugated secondary antibodies (Amersham). Western blot and densitometric analysis were performed as previously described (Iurlaro et al., 2004; Schlichting et al., 2006).

## RESULTS

### Correlation of the number and size of nuclei with muscle size

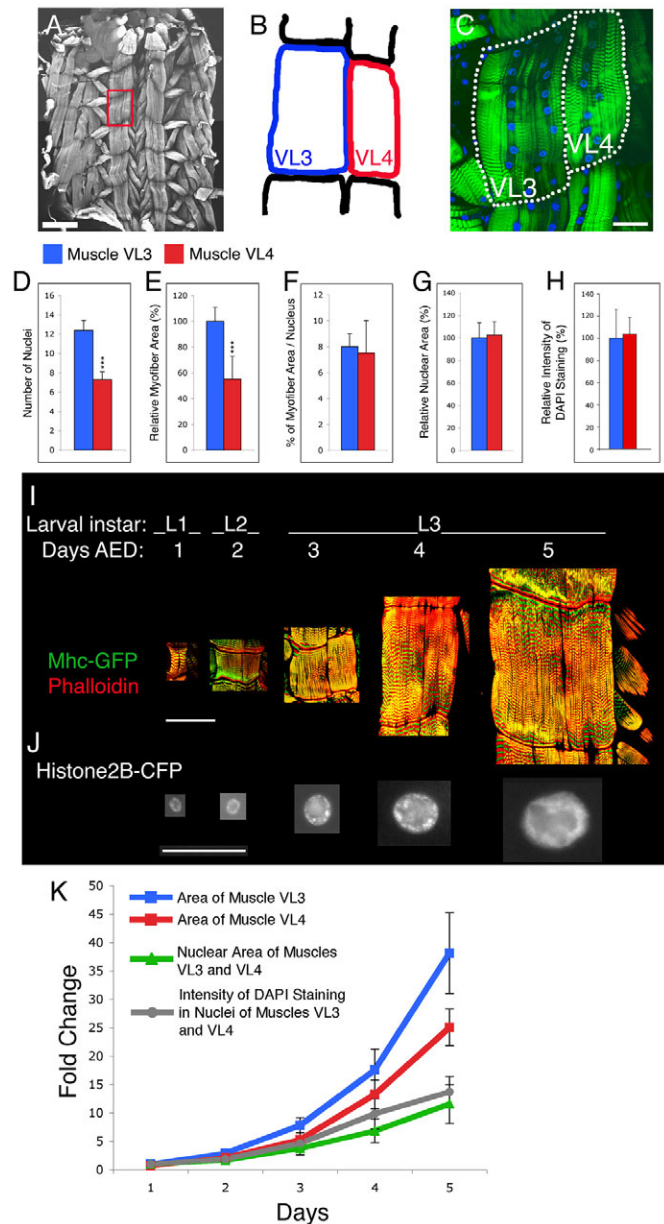
Examination of body wall muscles reveals extensive variability in the size of each muscle cell during larval stages (Bate et al., 1999) (Fig. 1A; note that in *Drosophila* larvae, one muscle is composed of a single multinucleated cell). Because the DNA content of a cell has been directly correlated with its size in a number of systems (Brodsky and Uryvaeva, 1977; Conlon and Raff, 1999), we have analyzed whether the differences observed in muscle sizes correlate with either the number of nuclei or their level of endoreplication (Edgar and Orr-Weaver, 2001).

We focused our analysis on two body wall muscles, VL3 and VL4, of third instar (L3) wandering larvae, as they are easy to analyze and have distinct sizes (Fig. 1A-C). VL3 muscles possess twice the number of nuclei as VL4 (Fig. 1D), which correlates with an approximate doubling in size (Fig. 1E) and a similar myofiber area/nucleus ratio (Fig. 1F). Further, the nuclear area (Fig. 1G) and the intensity of DAPI staining (Fig. 1H), which are indicators of nuclear ploidy (Maines et al., 2004; Ohlstein and Spradling, 2006; Sato et al., 2008; Shcherbata et al., 2004; Sun and Deng, 2007), did not significantly differ, suggesting that the amount of endoreplication in VL3 and VL4 nuclei is similar.

We next analyzed whether the ploidy of body wall muscle nuclei, as indicated by nuclear size and DNA content, correlates with muscle growth during larval development, similar to other *Drosophila* tissues (Edgar and Orr-Weaver, 2001; Maines et al., 2004). By scoring muscle size (Fig. 1I) and nuclear size (Fig. 1J) at various developmental stages, a high degree of correlation between these parameters and the intensity of DAPI staining was observed (Fig. 1K). To test whether endoreplication is necessary for growth, we overexpressed in muscles *Cyclin E*, which has been shown to block endoreplication when present at constant, but not oscillating, levels (Lilly and Spradling, 1996). *Dmef2 (Mef2)-Gal4 UAS-CycE* animals showed a severe reduction in nuclear size, intensity of DAPI staining and muscle size, demonstrating that muscle growth depends on endoreplication (see Fig. S1 in the supplementary material). Altogether, these results reveal that the number of nuclei is tightly coupled to the differential size of muscles, and that increasing nuclear ploidy is required for the overall growth of the muscles.

### Inhibition of InR signaling in muscles regulates muscle growth, body size and the size of unrelated tissues

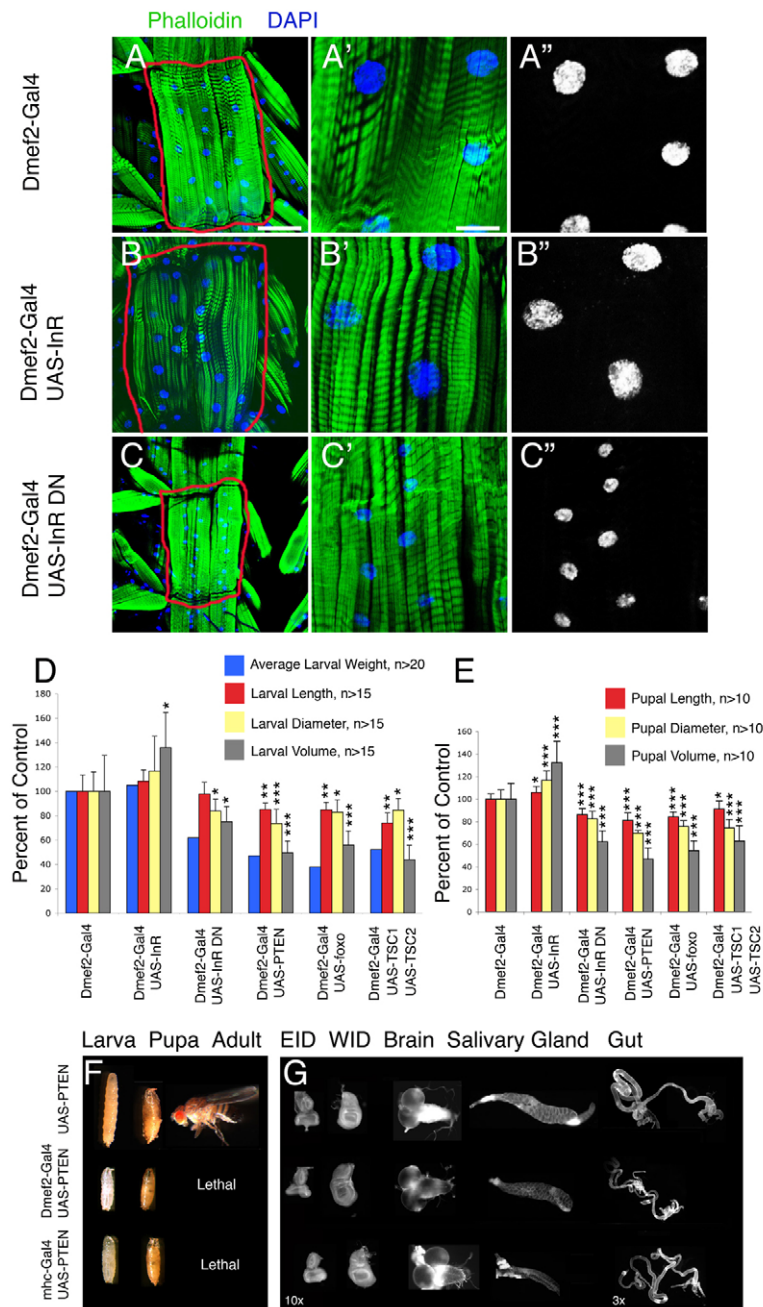
Since Insulin signaling is a major, evolutionarily conserved regulator of cell size (Hafen and Stocker, 2003), cell cycle progression and endoreplication (Burgering, 2008; Ho et al., 2008), we tested whether this pathway affects muscle growth. To modulate Insulin signaling in muscles, three different approaches were used. First, muscles of wild-type larvae were compared with those from larvae homozygous or heterozygous mutant for *chico* [also known as *Insulin receptor substrate (IRS)*] (see Fig. S1 in the supplementary material). Second, the levels of InR and Akt (Akt1) were reduced via RNAi knockdown in muscles using the Gal4-UAS system and *Dmef2-Gal4* (see Fig. S2 in the supplementary material), which drives transgene expression in muscles but not in other endoreplicating tissues (see Fig. S3 in the supplementary material). Third, we targeted the expression of activators (*InR*) (Fig. 2B) and inhibitors of InR signaling in muscles, including a dominant-negative form of *InR* (*InR DN*) (Fig. 2C). In all cases, inhibition of InR signaling resulted in decreased nuclear and muscle



**Fig. 1. Body wall muscles of *Drosophila* larvae consist of single, syncytial myofibers containing several polyploid nuclei.**

(A) Phalloidin staining of body wall muscles from a wild-type third instar (L3) larva. Several muscle cells (myofibers) of different sizes can be seen. The red box delineates VL3 and VL4 muscles of an abdominal segment. (B,C) The outline (B) and staining for F-actin (phalloidin, green) and nuclei (DAPI, blue) (C) of body wall muscles VL3 and VL4. Each muscle is composed of a single syncytial cell (myofiber), which differs in size and number of nuclei. (D-H) Quantification of (D) the number of nuclei, (E) myofiber area, (F) ratio of myofiber area/nucleus, (G) nuclear area, and (H) intensity of DAPI staining in VL3 and VL4 muscles. There is no significant difference in the nuclear area between body wall muscles VL3 and VL4, suggesting that ploidy is not a significant cause of differential growth.  $n(\text{muscles})=10$ ,  $n(\text{nuclei muscle VL3})=100$ ,  $n(\text{nuclei muscle VL4})=50$ ;  $***P<0.001$ . (I-K) Variation of (I) myofiber area and (J) nuclear area during larval growth, and (K) quantification of these results. Note the correlation between the extent of muscle growth, the increase in nuclear size, and the intensity of DAPI staining. For statistical analysis in K,  $n(\text{muscles})=9$ ,  $n(\text{nuclei})=100$  for nuclear size, and  $n(\text{nuclei})=10$  for intensity of DAPI staining. Error bars indicate s.d. Scale bars: 300  $\mu\text{m}$  in A; 47.5  $\mu\text{m}$  in C; 75  $\mu\text{m}$  in I; 22  $\mu\text{m}$  in J.



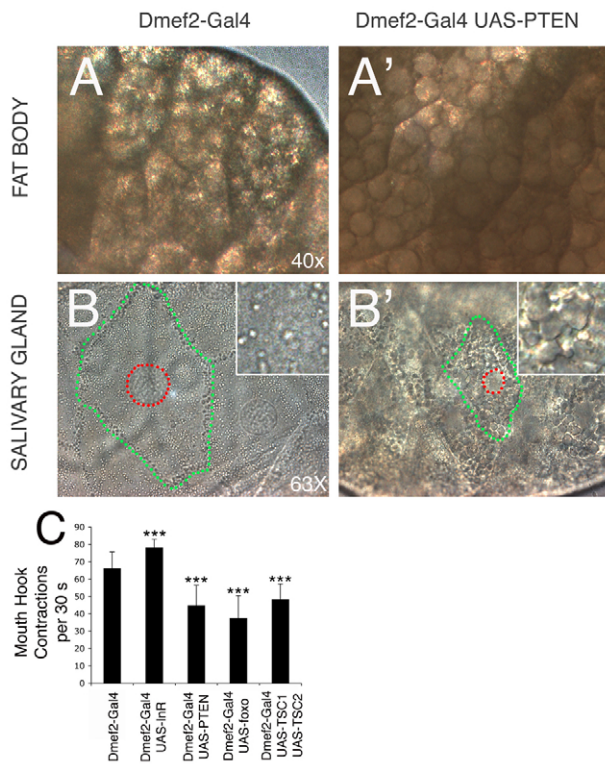


**Fig. 2. Inhibition of muscle growth affects the size of the entire body and of other tissues. (A-C'')** Staining of body wall muscles of L3 *Drosophila* larvae with phalloidin (green) and DAPI (blue). (A-A'') Matched controls (*Dmef2-Gal4*). (B-C'') Activators [B, *Insulin-like receptor (InR)*] and repressors [C, *Insulin-like receptor dominant-negative (InR DN)*] were overexpressed in muscles using the *Dmef2-Gal4* muscle driver. (B-B'') Activation of *InR* signaling results in a significant increase in the area of myofibers VL3 and VL4 (encircled in red) with a concomitant increase in nuclear area. (C-C'') Inhibition of *InR* signaling exerts converse effects. Scale bars: 75  $\mu$ m in A-C; 18.7  $\mu$ m in A'-C'. (D) Quantification of average larval weight ( $n>20$ ), larval length ( $n>15$ ), diameter ( $n>15$ ) and volume ( $n>15$ ) of larvae in which *InR*, *InR DN*, *Pten*, *foxo* or *Tsc1* and *Tsc2* have been overexpressed using *Dmef2-Gal4*. A decrease in muscle growth (see Fig. 5) always correlates with a reduction in larval body size. Consistent with these results, overexpression of *InR*, which promotes an increase in muscle growth (B), also increases larval body size. (E) Quantification of length ( $n>10$ ), diameter ( $n>10$ ) and volume ( $n>15$ ) of pupae arising from larvae in which *InR* signaling has been modulated in muscles (see Fig. 5). Error bars indicate s.d.; \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ . (F) Overexpression of *Pten* in muscles using *Dmef2-Gal4* (*Dmef2-Gal4 UAS-Pten*) or *Mhc-Gal4* (*Mhc-Gal4 UAS-Pten*) results in smaller larvae and pupae when compared with the control (*UAS-Pten*). (G) The size of internal tissues and organs, visualized with the lipophilic dye FM4-64, is also affected. Note, especially, the reduction in size of endoreplicating tissues. Magnification: 10 $\times$ , except for gut (3 $\times$ ). For a full description of genotypes, see Table S1 in the supplementary material.

size, as outlined by DAPI and phalloidin staining, respectively. Conversely, overexpression of wild-type *InR* resulted in a significant increase in myofiber size and nuclear size (Fig. 2B), suggesting that *InR* signaling controls muscle growth in part by modulating endoreplication.

Interestingly, we noticed that in addition to the autonomous effect on muscle growth, regulation of *InR* signaling in muscles exerted a systemic effect on body size. In all cases in which *InR* and *Tor* signaling were repressed, a significant decrease in weight, length, diameter and volume was observed in larvae (Fig. 2D,F) and pupae (Fig. 2E,F), without substantial developmental delay (not shown). By contrast, activation of *InR* signaling, following overexpression of *InR*, resulted in a significant increase in larval and pupal volumes (Fig. 2D,E).

To test whether the size of larval organs and tissues other than muscles are affected when *InR* signaling is repressed using the *Dmef2-Gal4* and *Mhc-Gal4* muscle drivers, we examined their size in L3 wandering larvae following staining with the lipophilic dye FM4-64 to outline their dimensions. In addition to a reduction in muscle size (Fig. 2C), the size of other endoreplicating organs, such as the salivary glands, gut, fat body and epidermis, was decreased (Fig. 2G; see Fig. S8 in the supplementary material; data not shown). However, the size of non-endoreplicating tissues, including the brain, wing and eye-antennal imaginal discs was less affected. Further, upon activation of *InR* signaling in muscles, an increase in muscle size (Fig. 2B) was accompanied by a parallel increase in the size of most other tissues (see Fig. S3 in the supplementary material; data not shown).

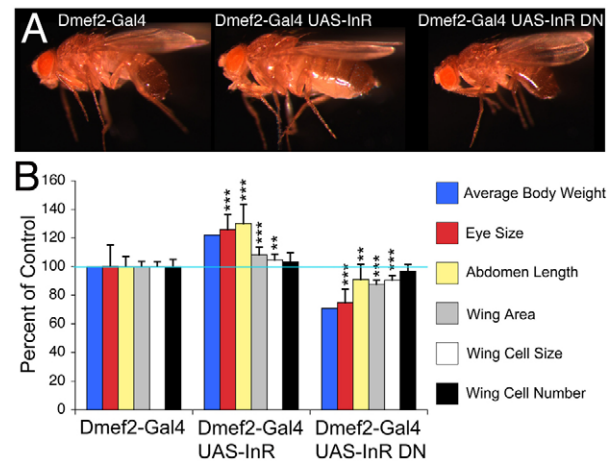


**Fig. 3. Inhibition of InR signaling in muscles induces catabolic programs in endoreplicating tissues and modulates larval feeding behavior.** (A-B') Transmitted light microscopy images of fat body and salivary gland cells from (A,B) control *Drosophila* larvae (*Dmef2-Gal4*) and (A',B') *Dmef2-Gal4 UAS-Pten* larvae. Lipid remobilization and catabolic events, possibly related to autophagy, are detected respectively in (A') fat body and (B') salivary gland cells of larvae in which *Pten* is overexpressed in muscles, in comparison with matched controls (A,B). Note the reduction in cell size (encircled in green) and nuclear size (indicative of nuclear ploidy, encircled in red) in salivary gland. Magnification: 40 $\times$  (fat body) and 63 $\times$  (salivary gland). (C) Modulation of InR signaling in muscles regulates larval feeding behavior. The number of mouth hook contractions every 30 seconds is significantly reduced in larvae that overexpress *Pten*, *Tsc1* and *Tsc2*, or *foxo* in muscles, and is increased upon *InR* overexpression.  $n>50$ ; error bars indicate s.d.; \*\*\* $P<0.001$ . A similar regulation of feeding behavior was observed in *Mhc-Gal4 UAS-Pten* larvae (not shown).

Altogether, perturbations of muscle growth through the InR and Tor signaling pathways not only regulate muscle size but also trigger a systemic response that affects body size.

### Reduction of muscle size by InR signaling non-autonomously regulates the size of other organs and affects feeding behavior

To characterize how changes in InR signaling in muscles affect the size of other tissues, we analyzed the morphological changes induced in fat body and salivary glands following *Pten* overexpression in muscles. Strikingly, reduction of Insulin signaling in muscles was accompanied by a reduction of cell size in endoreplicating tissues, via lipid remobilization in fat body cells (Fig. 3A,A'), and activation of catabolic programs possibly related to autophagy in salivary glands (Fig. 3B,B'). Because lipid remobilization and activation of catabolic programs in endoreplicating tissues are common events in response to improper feeding behavior and metabolic regulation (Colombani et



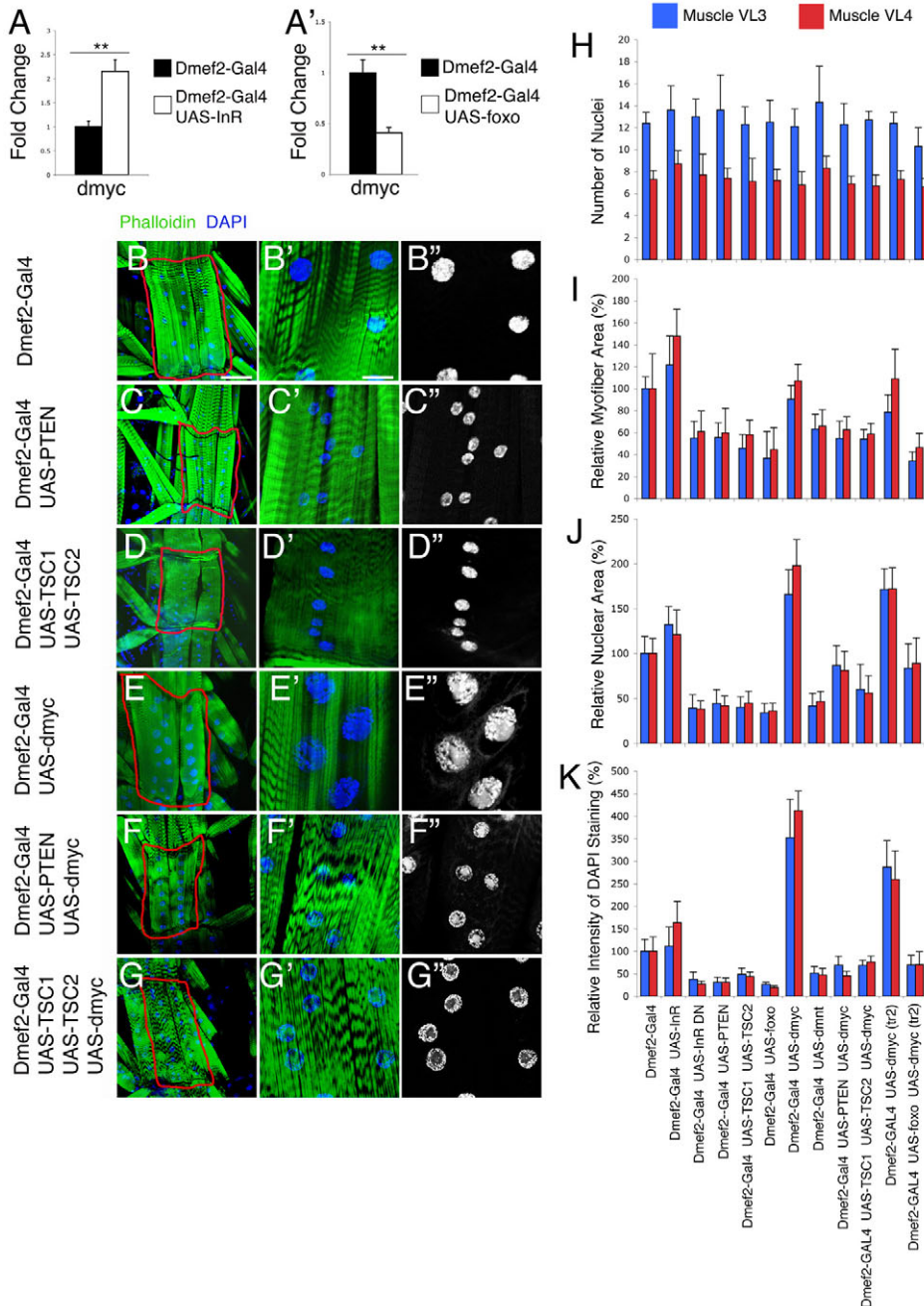
**Fig. 4. Modulation of InR signaling during muscle growth affects the final size of adult flies.** (A) Adult flies in which muscle growth has been altered by either activating (InR) or inhibiting (InR DN) InR signaling. (B) Significant changes are correspondingly observed in fly body weight, eye size, abdomen length and wing area. Changes in wing area result from an increase (InR) or decrease (InR DN) in cell size and possibly also cell number. Note that because the growth of distinct body parts is differentially affected, body proportions are also altered. Error bars indicate s.d.; \*\* $P<0.01$ , \*\*\* $P<0.001$ ;  $n(\text{weight})>20$ ,  $n(\text{eye})>12$ ,  $n(\text{abdomen})>22$ ,  $n(\text{wing})=10$ .

al., 2003), we tested whether feeding was affected in larvae with either repressed or activated InR signaling in muscles. Feeding behavior is under strict control in *Drosophila* and other organisms, as nutrient uptake is crucial for appropriate developmental growth (Saper et al., 2002). To monitor feeding activity, the number of mouth hook contractions, which has been shown to be an indicator of this behavior (Wu et al., 2005), was scored. Interestingly, overexpression of the InR antagonists *Pten*, *Tsc1* and *Tsc2* (*gigas*) or of *foxo* in muscles resulted in a significant decrease in larval feeding, whereas *InR* overexpression promoted this behavior (Fig. 3C). Thus, we propose that the levels of InR signaling in muscles somehow modulate larval feeding behavior, which in turn influences body size and tissue growth.

To test whether this systemic effect reflects a direct role of InR pathway activity or, rather, a general reduction in muscle mass, we inhibited muscle growth by means distinct from InR signaling, such as by *Cyclin E* overexpression (see Fig. S1 in the supplementary material). Similar to inhibition of InR signaling, both the larval feeding behavior and the size of most internal organs were affected in *Dmef2-Gal4 UAS-CycE* larvae (see Fig. S8 in the supplementary material), suggesting that non-autonomous effects might rely principally on muscle size, rather than on InR signaling per se. Consistent with this notion, concomitant overexpression of *InR* and *CycE* in muscles was not sufficient to rescue the developmental growth defects associated with *CycE* overexpression alone (not shown).

Although most manipulations of InR signaling during larval muscle growth result in pupal lethality, we recovered *Dmef2-Gal4 UAS-InR* and *Dmef2-Gal4 UAS-InR DN* adult flies, in which InR signaling was activated and inhibited, respectively. As expected, whereas activation of InR during muscle growth resulted in larger flies, smaller flies arose upon inhibition of this pathway (Fig. 4A). To test whether developmental muscle growth regulates, in turn, the size of body parts in adults, we scored the weight, eye size, abdomen length and wing area of these recovered flies. As expected, all these





**Fig. 5. InR/Tor signaling regulates muscle growth and nuclear ploidy by inhibiting dMyc function.**

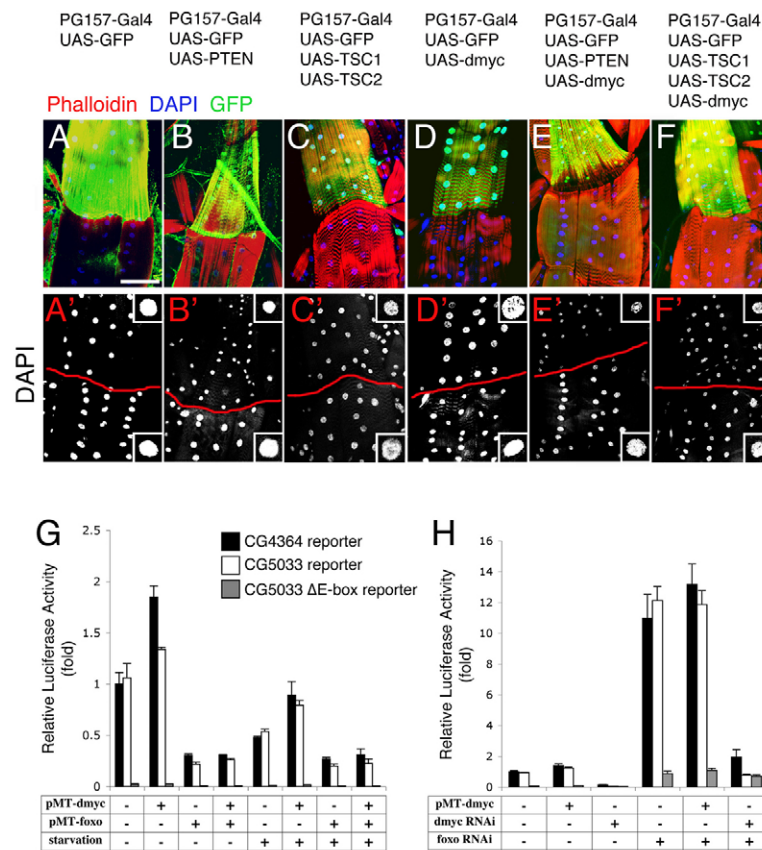
(A, A') Expression of *dmyc* is promoted by InR and antagonized by Foxo. qRT-PCR analysis of *dmyc* transcript levels in *Drosophila* L3 larvae in which either *InR* or *foxo* is overexpressed in body wall muscles. (A) A 2-fold increase in *dmyc* levels is observed upon *InR* overexpression in muscles, whereas (A') *foxo* activation results in a significant 2.5-fold reduction of *dmyc* transcripts. Error bars indicate s.d. ( $n=4$ );  $**P<0.01$ . (B-G'') Staining of body wall muscles of L3 larvae with phalloidin (green) and DAPI (blue). (B-B'') *Dmf2-Gal4*. Overexpression of the InR signaling negative regulators (C) *Pten* and (D) *Tsc1* and *Tsc2* in muscles using *Dmf2-Gal4*. (B-D'') Repression of InR signaling results in all cases in a significant decrease in the area of muscles VL3 and VL4 (encircled in red) with a concomitant reduction in nuclear area. (E) Overexpression of *dmyc* results in a significant increase in nuclear area without a proportional increase in myofiber area. (F) Co-expression of *dmyc* with *Pten*, or (G) with *Tsc1* and *Tsc2*, is sufficient to suppress dMyc-driven polyploidization, indicating that Insulin signaling antagonizes dMyc. For additional examples of muscle phenotypes, generated by overexpressing *dmnt* and *foxo*, see Fig. S4 in the supplementary material. Scale bars: 75  $\mu$ m in B-G; 18.7  $\mu$ m in B'-G'. (H-K) Quantification of (H) the number of nuclei, (I) myofiber area, (J) nuclear area and (K) intensity of DAPI staining in muscles VL3 (blue) and VL4 (red). Modulation of InR/Tor signaling in muscles is sufficient to promote significant changes in muscle size, which parallel variation in nuclear area and intensity of DAPI staining, but not in the number of nuclei. For statistical analysis,  $n(\text{myofibers})=10$ ,  $n(\text{nuclei muscle VL3})=100$ ,  $n(\text{nuclei muscle VL4})=50$ ;  $n(\text{nuclei})=10$  for intensity of DAPI staining; error bars indicate s.d.

parameters were respectively either increased or decreased upon activation or inhibition of InR signaling in muscles. Changes in tissue and whole-body size occurred by modulating cell size, as observed in the wings of adult flies, whereas cell number barely varied (Fig. 4B). Thus, several tissues, deriving from both endoreplicating and non-endoreplicating tissues, are affected to different extents upon developmental modulation of muscle mass.

**dMyc is necessary and sufficient to regulate endoreplication in muscles**

Similar to components of InR signaling, the transcription factor dMyc has been implicated in growth events in *Drosophila*, in part via the induction of endoreplication (Maines et al., 2004; Pierce et al., 2004). To test whether dMyc plays a role in muscle growth, we modulated its

function in several ways. First, muscles from wild-type larvae were compared with those from larvae that were homozygous or heterozygous mutant for *dmyc* (see Fig. S1 in the supplementary material). Second, levels of dMyc were reduced via RNAi knockdown in muscles (see Fig. S2 in the supplementary material). Third, we targeted expression of *dmyc* in muscles (Fig. 5E), as well as the expression of its inhibitor *dmnt* (*Mnt*) (see Fig. S4 in the supplementary material). In all cases, inhibition of dMyc activity resulted in smaller muscles and nuclei and decreased body size. dMyc overexpression was associated with an increase in nuclear size and DAPI staining that was, however, accompanied by only a slight increase in muscle size (Fig. 5E,H-K). Thus, during muscle growth, dMyc is both necessary and sufficient to regulate endoreplication. However, although dMyc and endoreplication are necessary, they are not sufficient to sustain extensive growth.



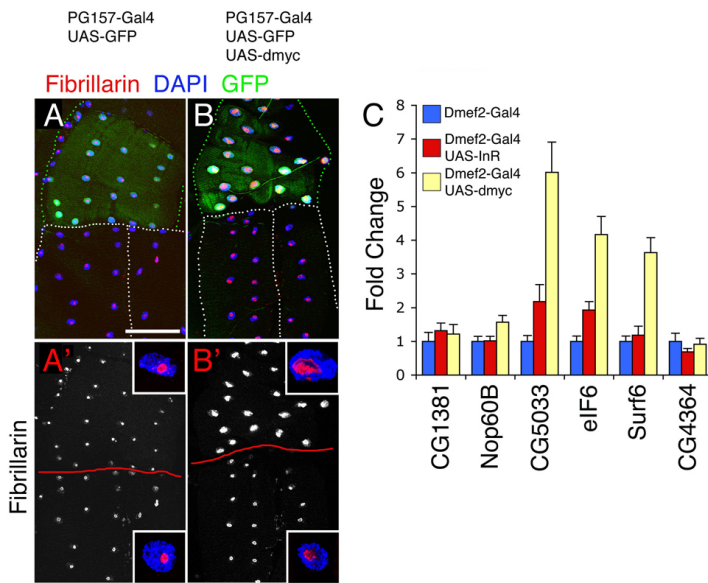
**Fig. 6. dMyc is autonomously antagonized by InR/Tor signaling.** (A-F') Mosaic analysis of *Drosophila* body wall muscles. (A) PG157-Gal4 drives the expression of transgenes in only a subset of body wall muscles (green for concurrent GFP expression; yellow in merge). Body wall muscles with no GFP expression (red) serve as control. DAPI (blue) and phalloidin (red) staining are used to outline nuclei and muscles, respectively. In A'-F', only DAPI staining is shown (white) and the red line demarcates transgene-expressing (above) from non-expressing (below) muscles. (A,A') Expression of GFP alone does not alter muscle and nuclear area. (B,B') Expression of GFP concomitantly with *Pten*, or (C,C') *Tsc1* and *Tsc2*, results in a marked decrease in muscle growth (see Fig. S6 in the supplementary material) and nuclear size (green, above in micrographs), in comparison with neighboring VL3 and VL4 muscles in which no transgene expression occurs (red, below in micrographs). (D) *dmyc* expression results in a marked increase in nuclear size, in comparison with neighboring control muscles. (E,E') Concomitant expression of *dmyc* with *Pten*, or (F,F') with *Tsc1* and *Tsc2*, results in decreased muscle and nuclear size, indicating that dMyc function is autonomously controlled by *Pten* and *Tsc*. Representative nuclei are shown in the insets. Scale bar: 37.5  $\mu$ m. (G) Foxo inhibits dMyc transcriptional activity. Luciferase assays performed using three different dMyc transcriptional reporters (CG4364, CG5033 and CG5033  $\Delta$ E-box) and overexpression of *dmyc* and *foxo*. Transfection of S2R+ cells, with or without subsequent serum starvation, was performed with *dmyc* (pMT-dmyc), *foxo* (pMT-foxo), or both in combination. Activation of endogenous Foxo by serum starvation, or following *foxo* overexpression, suppresses transcription of the dMyc Luciferase reporters. (H) Luciferase assays using dMyc reporters in *dmyc* and *foxo* RNAi-treated cells. *dmyc* RNAi suppresses, whereas *foxo* RNAi promotes, Luciferase expression. However, in combination with *dmyc* RNAi, *foxo* RNAi does not bring about a similar transcriptional regulation. Relative Luciferase activity corresponds to the firefly:*Renilla* luminescence ratio. The s.e.m. is indicated ( $n=4$ ).

### InR, Tor and Foxo are required for optimal dMyc function during muscle growth

Since InR signaling and dMyc loss-of-function elicit similar effects on the control of muscle growth and body size, we further investigated the mechanisms by which InR signaling and dMyc interact. InR overexpression resulted in a 2-fold increase in *dmyc* expression (Fig. 5A), as estimated by qRT-PCR. Consistent with a previous report (Teleman et al., 2008), overexpression of *foxo* in muscles resulted in a significant, 2.5-fold reduction in *dmyc* transcript levels (Fig. 5A').

Because the regulation of *dmyc* gene expression by InR/Foxo might only in part account for the regulation of dMyc activity, we tested whether InR and Tor signaling regulate dMyc protein function, as estimated by their ability to control dMyc-driven phenotypes in

muscles. When either *Pten*, or *Tsc1* and *Tsc2* were co-expressed together with *dmyc*, dMyc activity was inhibited, resulting in defective myofiber growth and endoreplication (Fig. 5F,G), similar to the expression of *Pten* (Fig. 5C) or *Tsc1* and *Tsc2* alone (Fig. 5D). Consistent with being regulated by InR signaling, *foxo* overexpression also impaired dMyc activity (see Fig. S4 in the supplementary material; Fig. 5H-K). Quantification of muscle phenotypes indicated that significant changes in myofiber area (Fig. 5I), nuclear size (Fig. 5J) and the intensity of DAPI staining (Fig. 5K) occur in concert, without any change in the number of nuclei (Fig. 5H). Thus, maximal dMyc activity relies on optimal InR/Tor signaling and inhibition of Foxo activity. Furthermore, and contrary to previous analyses in fat body cells (Pierce et al., 2004), dMyc overexpression in muscles promoted endoreplication without a proportional increase in cell size.



**Fig. 7. dMyc primes muscle cells for growth.** dMyc promotes biogenesis of nucleoli and expression of genes required for protein synthesis. (A) Fibrillarlin immunoreactivity (red) stains the nucleoli of transgene-expressing muscles (green for concurrent *GFP* expression, above in micrographs; nuclei identified by DAPI staining, blue) and of neighboring control myofibers [outlined in white, based on phalloidin staining (not shown), below in micrographs]. (B) *dmyc* overexpression promotes nucleolus biogenesis that is, however, insufficient to drive muscle growth. (A', B') Fibrillarlin immunoreactivity, together with representative nuclei (blue) and nucleoli (red; insets). Scale bar: 37.5  $\mu$ m. (C) qRT-PCR analysis of dMyc target genes involved in growth. Significant induction of gene expression is observed upon *dmyc* overexpression and, to a lesser extent, upon overexpression of *InR*. Error bars indicate s.d. ( $n=4$ ).

### dMyc is autonomously modulated by InR/Tor signaling

Because muscle growth impacts body growth (Fig. 2), to exclude any potential non-autonomous effect that might bias this analysis, we created genetic mosaics to further test the functional relationship between *Pten*, *Tsc1/2* and dMyc. To this purpose, we used the PG157-Gal4 driver, a previously uncharacterized Gal4 line that we find can drive expression in only a subset of larval muscles (see Fig. S5 in the supplementary material; Fig. 6A; and Materials and methods). Using this method, the expression of *Pten*, *Tsc1/2* and *dmyc* alone, and *dmyc* together with *Pten* or *Tsc1/2*, was driven in a subset of larval muscles. By examining DAPI staining in adjacent control myofibers, in which no transgene expression is driven, significant decreases or increases in intensity of DAPI staining (see Fig. S6 in the supplementary material) and nuclear size in *GFP*-expressing myofibers were detected upon *Pten* (Fig. 6B, B'), *Tsc1/2* (Fig. 6C, C') or *dmyc* (Fig. 6D, D') expression. Co-expression of *dmyc* together with *Pten* (Fig. 6E, E') or *Tsc1/2* (Fig. 6F, F') resulted in impaired dMyc-mediated endoreplication. Thus, *Pten* and *Tsc1/2* autonomously control cellular events that are induced by dMyc activity in muscles.

### Foxo inhibits dMyc transcriptional activity

dMyc acts primarily via inducing a transcriptional response, suggesting that InR signaling might regulate dMyc function by modulating its transcriptional activity. Similar to *Pten* and *Tsc*, Foxo can regulate dMyc protein function in vivo (see Fig. S4 in the supplementary material; Fig. 5H-K). To test whether Foxo inhibits dMyc by regulating its transcriptional activity, Luciferase assays were performed using *CG4364* and *CG5033* transcriptional reporters, previously described to be directly regulated by dMyc (Hulf et al., 2005) but not directly regulated by Foxo. The *CG5033*  $\Delta$ E-box reporter is devoid of E-boxes, the dMyc-responsive sequences, and is therefore refractory to dMyc transcriptional regulation.

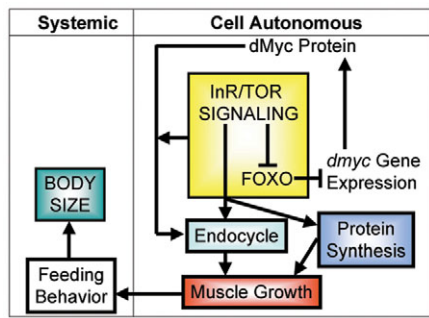
In S2R+ cells, Luciferase activity of *CG4364* and *CG5033* reporters was detected in response to endogenous dMyc and was increased by overexpression of *dmyc* (*pMT-dmyc*) (for characterization of overexpression, see Fig. S7 in the supplementary material). However, overexpression of wild-type *foxo* (*pMT-foxo*)

(see Fig. S7 in the supplementary material) or serum starvation, which activates endogenous Foxo, decreased the Luciferase activity of the *CG4364* and *CG5033* reporters (Fig. 6G) and suppressed the transcriptional response induced by *dmyc* overexpression. Further, without E-boxes, no substantial Luciferase activity was detected, indicating that it depends on dMyc. Consistently, RNAi treatment against *dmyc* and *foxo* respectively attenuated and increased Luciferase activity of the *CG4364* and *CG5033* reporters (Fig. 6H; see Fig. S7 in the supplementary material), but not of the *CG5033*  $\Delta$ E-box reporter. The increase in Luciferase activity upon *foxo* RNAi is likely to reflect its ability to inhibit both *dmyc* gene expression and dMyc protein function. However, upon RNAi treatment of *foxo* and *dmyc*, no increase in Luciferase activity was observed (Fig. 6H), further confirming that Foxo regulates transcription of the *CG4364* and *CG5033* reporters via dMyc. Therefore, Foxo tightly controls dMyc function by modulating its expression (Fig. 5) and its transcriptional activity.

### dMyc primes muscle growth via nucleolus biogenesis and expression of growth-promoting genes

Although dMyc promotes endoreplication, no substantial muscle growth results. To further dissect the role of dMyc in muscle growth, we tested whether dMyc induces (1) nucleolus biogenesis, which is necessary for rRNA transcription and ribosome subunit assembly (Prieto and McStay, 2005), and (2) the expression of genes required for protein translation; both events are necessary for cell growth. By staining with an anti-Fibrillarlin antiserum, which outlines nucleoli (Grewal et al., 2005), we observed a dramatic increase in the size of the nucleolus upon *dmyc* overexpression with the PG157-Gal4 driver, in comparison with controls (Fig. 7A, B). Further, *dmyc* overexpression with *Dmef2-Gal4* increased, to different extents (Fig. 7C), the expression of some dMyc target genes (Grewal et al., 2005; Hulf et al., 2005) that are involved in rRNA processing (*Nop60B*), ribosome assembly and biogenesis (*CG1381*, *CG5033*, *Surf6*), and translational control (*eIF6*), but not cell proliferation (*CG4364*). *InR* overexpression promoted a modest increase in the expression of dMyc target genes involved in growth (*CG1381*, *CG5033*, *eIF6*, *Surf6*). Thus, dMyc primes muscles for growth by promoting endoreplication, nucleolus biogenesis and the expression





**Fig. 8. Interplay of growth signals during muscle growth regulates *Drosophila* body size.** Integration of growth signals controls endoreplication, muscle growth and body size. Inhibition of InR/Tor signaling is accompanied by activation of Foxo, which inhibits transcription of *dmyc*. In turn, dMyc protein requires input from InR/Tor signaling for its maximal function. dMyc promotes endoreplication, biogenesis of nucleoli and expression of genes required for protein synthesis. However, dMyc is necessary, but not sufficient, to sustain extensive muscle growth, which also requires concurrent activation of InR/Tor signaling to drive protein synthesis and other anabolic processes. A decrease or increase in muscle mass in turn perturbs the growth of other tissues and, indeed, the whole body, at least in part by regulating the feeding behavior of the larva.

of some genes necessary for protein translation. However, gene expression programs governed by dMyc require concomitant InR/Tor signaling to drive substantial muscle growth (Fig. 8).

## DISCUSSION

We have examined the mechanisms regulating the extensive muscle growth that occurs during *Drosophila* larval development. We found that interplay between InR/Tor signaling, Foxo and dMyc activity regulates this process, in part via the induction of endoreplication. Interestingly, the extent of muscle growth is sensed systemically, regulates feeding behavior and, in turn, influences the size of other tissues and indeed the whole body. Thus, the growth of a single tissue is sensed systemically via modulating a whole-organism behavior (Fig. 8).

### Foxo regulates endoreplication and dMyc transcriptional activity

We found that dMyc, as well as activation of InR signaling, can promote endoreplication in muscles, whereas Foxo and inhibitors of dMyc and of InR/Tor have the opposite effect. dMyc is likely to regulate the expression of genes required for multiple G–S and S–G transitions during endoreplication (Edgar and Orr-Weaver, 2001; Lilly and Duronio, 2005), similar to vertebrate Myc, which regulates key cell-cycle regulators including cyclin D2, cyclin E, and the cyclin kinase inhibitors p21 and p27 (Cdkn1a and Cdkn1b, respectively) (Grandori et al., 2000). Indeed, aberrant levels of Cyclin E (Lilly and Spradling, 1996) block muscle growth (see Fig. S1 in the supplementary material), indicating that proper muscle growth requires tight control of the expression and activity of endoreplication genes. Further, we found that endoreplication is also modulated by Foxo, which is activated in conditions of nutrient starvation, impaired InR/Tor signaling and by other cell stressors (Arden, 2008). Foxo presumably regulates cell cycle progression at least in part by modulating the expression of evolutionarily conserved Foxo/Myc-target genes, such as *dacapo* (the *Drosophila* p21/p27 homolog) and *Cyclin E*, that regulate the G1–S transition,

as previously reported in mammalian systems (Grandori et al., 2000; Salih and Brunet, 2008). Interestingly, Foxo and Myc might control different steps in the activation of common target genes (Bouchard et al., 2004).

In addition, we found that active Foxo can also inhibit dMyc protein activity and regulates *dmyc* gene expression. Mechanistically, Foxo could influence dMyc activity in several ways. First, it might physically interact with dMyc, although we found no evidence to support this notion (see Fig. S7 in the supplementary material). Second, Foxo could regulate the expression of genes that target dMyc for proteasomal degradation, including several ubiquitin E3 ligases that are induced by Foxo during muscle atrophy in mice and humans (Sandri et al., 2004; Stitt et al., 2004). However, by analyzing dMyc protein levels by western blot, we did not detect significant dMyc protein instability upon Foxo overexpression (see Fig. S7 in the supplementary material). Third, Foxo might promote the expression of transcriptional regulators that oppose dMyc function, including Mad/Mnt (Delpuech et al., 2007), although no substantial increase in *dmnt* mRNA levels was detected upon Foxo activation in muscles (not shown). Possibly, the expression of other dMyc regulators might be affected by Foxo. Future experiments will be needed to dissect the Foxo-dMyc interaction.

Finally, by manipulating muscle growth and/or endoreplication, we found that in muscles the ratio of cell size to nuclear size is not constant, and increased nuclear size and DNA content, indicative of ploidy, is necessary but not sufficient to drive growth. Usually, an increase in cell size is matched by an increase in nuclear size (Neumann and Nurse, 2007), which commonly parallels increases in nuclear ploidy (Maines et al., 2004; Ohlstein and Spradling, 2006; Sato et al., 2008; Shcherbata et al., 2004; Sun and Deng, 2007). However, our findings indicate that in muscles, dMyc-driven variation in nuclear size and ploidy is permissive but not sufficient for substantial growth, even in the presence of increased biogenesis of nucleoli and expression of genes involved in protein translation. This is different from fat body cells, in which *dmyc* overexpression induces endoreplication and proportional cell growth (Pierce et al., 2004). Thus, additional instructive signals, possibly modulating protein synthesis, mitochondriogenesis, ribosome biogenesis (Teleman et al., 2008), sarcomere assembly (Bai et al., 2007; Haas, 1950), and other anabolic responses must be concomitantly received to promote maximal muscle growth. Therefore, increases in cell size and nuclear ploidy are surprisingly uncoupled during muscle growth.

### Muscle size regulates systemic growth

Little is known about the mechanisms that control and coordinate cell, organ and body size (Edgar, 2006; Mirth and Riddiford, 2007), and in particular how muscle growth is matched with the growth of other tissues and of the entire organism. We found that inhibition of InR/Tor signaling and dMyc activity in muscles impairs, in addition to muscle mass, the size of the entire body and of other internal organs. Similarly, overexpression of *Cyclin E* in muscles also resulted in autonomous and systemic growth defects (see Figs S1 and S8 in the supplementary material), indicating that, at least in some cases, modulation of muscle growth by means independent from InR signaling can be sensed systemically. In the larva, endoreplicating tissues and organs (gut, salivary glands, epidermis, fat body) were severely affected, whereas non-endoreplicating tissues (brain and imaginal discs) were less affected, indicating distinct tissue responsiveness to this regulation. Similarly, inhibition of Tor signaling in the fat body also primarily affects the size of endoreplicating tissues (Britton et al., 2002; Colombani et al., 2003).

Non-autonomous regulation of tissue size may rely on humoral factors (e.g. hormone-binding proteins, hormones, metabolites) produced by muscles in response to achieving a certain mass (Gamer et al., 2003). However, alternative models are possible. In particular, we observed decreased and increased larval feeding, respectively, upon inhibition and activation of InR signaling in muscles. This whole-organism behavioral adaptation is possibly due to decreased and increased efficiency of smaller and bigger muscles, respectively, and to regulated expression of neuropeptides that hormonally control feeding behavior. As a consequence of the regulation of feeding behavior, nutrient uptake is decreased and larval growth is blocked in the cells of endoreplicating tissues, which are extremely sensitive to poor nutritional conditions, and to a lesser extent in non-endoreplicating tissues, which are more resistant to limited nutritional supply (Bradley and Leever, 2003; Colombani et al., 2003). In turn, increased or decreased size of non-muscle tissues arise as a consequence of abnormal feeding. Thus, muscle size coordinates with the size of other organs and of the entire body, at least in part via a systemic, behavioral response. Distinct tissues are differently sensitive to this regulation, resulting in altered body proportions.

### ***Drosophila* as a disease model of muscle atrophy and hypertrophy**

Understanding the mechanisms regulating muscle mass is of special interest because they underline the etiology of several human diseases (Glass, 2003a). Directly relevant to our studies, both MYC and InR (INSR) signaling have been found to regulate muscle growth and maintenance in humans (Sandri et al., 2004; Southgate et al., 2007; Stitt et al., 2004; Zhong et al., 2006). Further, muscle atrophy is triggered by FOXO activation in several pathological conditions (Glass, 2003b; Sandri et al., 2004; Stitt et al., 2004). In addition, MYC function has been implicated in heart hypertrophy (Bello Roufai et al., 2007; Xiao et al., 2001; Zhong et al., 2006), a process that is conversely regulated by FOXO (Evans-Anderson et al., 2008; Skurk et al., 2005).

Our findings that Foxo functionally antagonizes dMyc during the growth of *Drosophila* muscles suggest that these factors might also interact similarly in humans. Consistent with this hypothesis, FOXO and MYC regulate, in opposite fashions, the atrophic and hypertrophic programs in human skeletal muscles and cardiomyocytes, and display complementary gene expression and activity in these contexts (Lecker et al., 2004; Mahoney et al., 2008; Sandri et al., 2004; Spruill et al., 2008; Stitt et al., 2004).

Finally, our finding that during larval development, inhibition of InR signaling in muscles has profound systemic effects might also reflect physiological conditions found in humans. Indeed, defective responsiveness of muscles to Insulin during type II diabetes has autonomous effects on muscle maintenance that are associated with systemic effects on the metabolism of the entire organism, contributing to the improper control of glycemia and the development of metabolic syndrome (Wells et al., 2008). Here, we have identified feeding behavior as part of the systemic response that in *Drosophila* senses perturbations in muscle mass. These findings might help further elucidate the signals involved in metabolic and growth homeostasis, which may be conserved across evolution.

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#### **Supplementary material**

Supplementary material for this article is available at <http://dev.biologists.org/cgi/content/full/136/6/983/DC1>

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