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Mechanisms of Muscle Growth and Atrophy in Mammals and *Drosophila*

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Abstract

The loss of skeletal muscle mass (atrophy) that accompanies disuse and systemic diseases is highly debilitating. Although the pathogenesis of this condition has been primarily studied in mammals, *Drosophila* is emerging as an attractive system to investigate some of the mechanisms involved in muscle growth and atrophy. In this review, we highlight the outstanding unsolved questions that may benefit from a combination of studies in both flies and mammals. In particular, we discuss how different environmental stimuli and signaling pathways influence muscle mass and strength and how a variety of disease states can cause muscle wasting.

Keywords

skeletal muscle growth; muscle atrophy; animal models of muscle wasting; proteostasis

INTRODUCTION

Skeletal muscle accounts for approximately 40%–50% of the body mass in humans. Maintenance of muscle mass and strength through proper nutrition and exercise is critical to maintain full activity, prevent obesity and decrease the risk of heart disease, diabetes, and cancer (Pate et al., 1995). Lack of contractile activity and a variety of systemic diseases, as well as inadequate nutrition, all lead to fiber atrophy (i.e. loss of cell proteins), and consequently, a decrease in functional capacity. In addition, there is a progressive loss of muscle mass and strength in the aged, often termed "sarcopenia" (Nair, 2005), which is a major contributor to the frailty in the elderly and increases the risk of age-related diseases, injury and death. General wasting of all muscles occurs with fasting and disuse and is an integral feature of a number of systemic diseases, including many cancers, cardiac failure, renal failure, sepsis, AIDS, as well as burns and traumatic injury, and muscle loss correlates with a poor prognosis. In these conditions, the skeletal muscles are inherently normal, and the loss of mass is a response to physiological or pathological stimuli (e.g. fasting or cancer), rather than to any intrinsic genetic defect. By contrast, there are a variety of primary muscle diseases (termed myopathies) that result from inherent structural or enzymatic

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defects (e.g. muscular dystrophies) or from inflammatory diseases (e.g. myosites; (Askanas and Engel, 2002). Many mutations that perturb muscle function by affecting the contractile apparatus, energy metabolism or membrane integrity can lead to skeletal and cardiac myopathies.

Extensive studies in vertebrates have shown that skeletal muscle is a highly adaptive tissue that responds to exercise, nutrient supply, and endocrine factors with alterations in fiber composition and size (Figure 1). Similar observations have been made in *Drosophila* where muscles account for 40 to 50% of the body mass, and muscle growth depends on nutrient supply sensed via the Insulin/Akt/TOR pathway (as in mammals). For example, under normal nutrient conditions, *Drosophila* muscles during larval development undergo a striking ~50-fold increase in fiber size in only 5 days (Demontis and Perrimon, 2009; Figure 2A).

Similarities between vertebrate and *Drosophila* muscles are both structural and functional. Both are composed of tandem arrays of sarcomeres containing the thin and thick filaments, which, in a typical muscle twitch, slide past each other in response to calcium release from the sarcoplasmic reticulum (SR, the specialized endoplasmic reticulum (ER) of muscles), resulting in force generation (Taylor, 2006). In addition, in both insects and mammals, muscle fibers can be either glycolytic or oxidative. For example, *Drosophila* direct and indirect flight muscles, which promote wing motion indirectly by compressing the thorax, can function for extended periods during flight and are primarily oxidative. By contrast, body wall muscles of the larva and leg muscles of adult flies, which are used only intermittently, rely mainly on glycolysis (Taylor, 2006). These distinct patterns of energy metabolism resemble the differences between type I and type IIb fibers in mammalian muscles. Type I slow fibers are non-fatiguing, primarily burn fatty acids and glucose oxidatively, and are dark in color because they are rich in mitochondria, myoglobin, and blood supply. By contrast, the easily fatigued, fast type IIb fibers are primarily glycolytic, and have a low mitochondrial content and capillarity density (Taylor, 2006). Most mammalian muscles, especially in humans, are composed of mixtures of fiber types that are recruited in an ordered fashion, but overall fiber composition is adapted to the specific functions of the muscle. For example, in rodents the antigravity *soleus* muscle, which is continually used in standing, is composed primarily of oxidative fibers and is quite resistant to fatigue. In typical mixed muscles, the slower oxidative fibers are used in all contractions, but the easily fatigued larger glycolytic fibers are recruited only with maximal efforts (Brooke and Kaiser, 1970)

Here in this review, we discuss how *Drosophila* with its extensive genetic toolkit and short life cycle provides a powerful experimental system to address some of the outstanding unsolved questions about muscle atrophy. Specifically, we review the mechanisms of skeletal muscle atrophy and hypertrophy that may be similar in *Drosophila* and mammals, and discuss emerging insights and outstanding questions that may benefit from studies in both species (see Tables I and II).

MODULATION OF MUSCLE MASS BY ENVIRONMENTAL STIMULI AND TRANSCRIPTION FACTORS

In mammals, muscles are the major protein reservoir in the body, and during fasting, amino acids generated by net protein degradation are released into the venous blood to provide substrates for hepatic gluconeogenesis. Thus, under starvation conditions, muscle proteolysis is critical for maintaining the supply of glucose, in particular to the brain, and of amino acids essential for continued protein synthesis. Myofibrillar proteins comprise about two thirds of muscle dry weight, and changes in muscle size are due primarily to changes in

the content of the contractile apparatus (Cohen et al., 2009; Solomon and Goldberg, 1996). In most types of muscle atrophy, the loss of mass is driven by an increase in protein degradation and to a lesser extent by a decrease in protein synthesis (Figure 1). Protein degradation occurs via both the ubiquitin proteasome system (UPS) and the autophagy/lysosome pathway, while protein synthesis is regulated primarily by Insulin/IGF-1 (Insulin-like Growth Factor 1) acting through the Akt/TOR/FoxO pathway (Glass, 2010; Figure 3). During atrophy, both UPS and autophagy/lysosome degradative systems are activated, and key components are induced by transcription factors of the FoxO family (Sandri et al., 2004; Zhao et al., 2007) and NF-κB (Cai et al., 2004; Hunter et al., 2002).

In mammals, systemic muscle wasting is induced in response to fasting or diseases (i.e. untreated diabetes, AIDS, cancer and heart failure) and by various circulating hormones, cytokines, and excess of glucocorticoids (Figure 1). In addition, atrophy of specific muscles occurs upon decreased usage (e.g. with nerve injury or immobilization in a cast). These very different physiological stimuli may involve some common signaling mechanisms. Contractile activity causes release of IGF-1 (Insulin-like Growth Factor 1), which functions as an important autocrine growth factor. IGF-1 is also a circulating hormone released from the liver in response to pituitary growth hormone. The autocrine production of IGF-1 by muscle falls with disuse and following loss of innervation (Zeman et al., 2009), in animals lacking pituitary growth hormone or during chronic heart failure (Schulze and Spate, 2005). So the loss of IGF-1, through both endocrine and autocrine mechanisms, contributes to the atrophy process mainly by attenuating signaling by the IGF-1/Akt pathway in both systemic catabolic states and locally with disuse. Reduced Akt signaling decreases protein synthesis in muscle as in other cells and leads to enhanced muscle proteolysis via activation of FoxO transcription factors (Glass, 2010; Figure 3). Similarly, upon fasting when circulating insulin and IGF-1 levels fall, autocrine production of IGF-1 by muscle also decreases. As a result, the IGF-1/Akt pathway is markedly depressed leading to rapid protein loss. Furthermore, in fasting and other catabolic states, the release of glucocorticoids by the adrenal glands increases and further promotes proteolysis and inhibits muscle protein synthesis.

In addition to FoxOs, other transcription factors (i.e. Smad 2 and 3, NF-κB, JunB and Myogenin) have been shown to influence muscle mass. For example, the Smad transcription factors mediate the catabolic effects of myostatin, a circulating TGF-family member that inhibits normal muscle growth (Lee, 2004). Myostatin binds to the Activin RIIB (ActRIIB) receptors and activates transcription by Smad 2 and 3. Conversely, inhibition of ActRIIB receptors or of Smad 2 and 3 causes muscle hypertrophy (Sartori et al., 2009; Figure 4). NF-κB has also been shown to be an important factor in muscle atrophy (Bonetto et al., 2011; Cai et al., 2004), although its exact mechanism appears unclear (Mourkioti et al., 2006). Endurance exercise induces the production of the transcriptional co-activator PGC-1α, which promotes mitochondrial gene expression leading to oxidative phosphorylation and subsequently differentiation of oxidative muscle fibers (Handschin and Spiegelman, 2008). In addition, JunB, which has long been known as a rapid-response gene that triggers cell proliferation, is also important in determining the size and growth rate of muscle fibers in adult mammals, which are postmitotic cells. JunB overproduction can both promote hypertrophy and inhibit muscle atrophy (Raffaello et al., 2010; Sandri et al., 2006; Wang et al., 2005) by inhibiting FoxO3, and perhaps other catabolic processes.

A number of other transcription factors (e.g. Myogenin, MyoD and MEF2) are important in embryonic differentiation of muscle, but are not critical in post-natal muscle growth. However, in the adult Myogenin participates in the induction of atrophy following denervation (Moresi et al., 2010). While multiple transcription factors can promote or inhibit muscle atrophy, their specific roles, modes of activation, and functional interactions occurring during different types of muscle atrophy remain unclear. This area represents an

important gap in our knowledge and possibly could lead to the development of rational interventions to combat muscle wasting.

The powerful genetic toolkit available in *Drosophila* may be of particular advantage to analyze the genetic interactions among transcription factors inducing muscle atrophy or hypertrophy and to identify novel regulators of muscle mass. In *Drosophila*, muscles undergo dramatic size increase during larval development, where fiber growth also relies heavily on nutrient sensing via the Insulin/Akt/TOR pathway (Demontis and Perrimon, 2009). In the larval muscles, the activities of the transcription factors FOXO, Myc and Mnt are integrated to achieve normal muscle growth. In particular, FOXO inhibits muscle growth at least in part by decreasing *Myc* (*diminutive*) gene expression and its transcriptional activity (Demontis and Perrimon, 2009). Conversely, overexpression of the Insulin receptor results in increased *Myc* gene expression, which promotes nucleolar biogenesis and primes muscle growth. In addition, RNAi-mediated knock-down of *Myc* inhibits muscle growth, as does overexpression of *Mnt* (Demontis and Perrimon, 2009), which antagonizes Myc activity by binding to the common interaction partner Max (Bellosta and Gallant, 2010). Finally, genome-wide RNAi analyses, as recently applied to the study of muscle morphogenesis (Schnorrer et al., 2010), may uncover new regulators of fiber size in *Drosophila*. Orthologs of these genes may also be important in human biology and thus represent new therapeutic targets.

DENERVATION

Like nutrient deprivation, loss of muscle stimulation by nerves, as occurs during inactivity, immobilization, paralysis, nerve injury and neuromuscular diseases, such as amyotrophic lateral sclerosis (ALS), cause profound muscle atrophy in mammals (Figure 1). Different muscle fiber types have distinct propensities to atrophy upon denervation, with type I oxidative fibers being more sensitive to denervation-induced atrophy than type II (Herbison et al., 1979) even though type II fibers are lost preferentially in various systemic wasting states, e.g. glucocorticoids treatment (Goldberg and Goodman, 1969), fasting (Li and Goldberg, 1976), sepsis (Tiao et al., 1997) or cancer (Acharyya et al., 2004; Baracos et al., 1995). Importantly, activation of the Insulin/Akt pathway is sufficient to counteract the loss of muscle mass associated with denervation (Bodine et al., 2001b). Acutely, the weight loss and transcriptional response to denervation and pure disuse (e.g. with spinal isolation) are very similar, but with time, denervated muscles show a more profound atrophy (Sacheck et al., 2007). So although the post-natal maintenance of muscle mass is clearly dependent on continual neuronal activity, which causes contractile activity, it remains possible that trophic factors released by innervating motor neurons may also be important in determining muscle properties. Interestingly, some muscles, such as the extraocular muscles of primates, are extremely resistant to denervation atrophy when compared to limb muscles (Porter et al., 1989). Differences in fiber-type composition and innervation pattern may explain the resistance of extraocular muscles to wasting following denervation.

Whether denervation influences muscle mass in the adult fly, and whether it can be counteracted by Insulin/Akt signaling has not yet been studied, probably because of the difficulties in altering physiological load and measuring the sizes of specific muscles in this organism. However, muscle-nerve interactions have been thoroughly examined during the developmental phase of metamorphosis in *Drosophila*, when these interactions are important to define proper muscle size and patterning. In particular, surgical severing of the mesothoracic nerve with microbeam lasers leads to denervation that can retard the formation of some muscle fibers, like the dorso-longitudinal flight muscles, by regulating the rate of proliferation of the pool of myoblasts, the muscle stem cells that are precursors of the adult musculature (Fernandes and Keshishian, 1998). Similarly, nerve-muscle interactions are

necessary for achieving proper muscle size in the moth *Manduca sexta*, where denervation during development also decreases the number of proliferating myoblasts (Bayline et al., 2001). In addition, denervation results in muscle patterning defects in *Drosophila*, with a failure to form dorsal ventral muscles, indicating that the differentiation of specific muscle fibers is perturbed (Fernandes and Keshishian, 1998). The interconnection between neuronal activity and muscle patterning has also been extensively studied in adult mammals, where motoneurons influence fiber type differentiation (Grinnell, 1995) even in the adult. Moreover, prolonged electrical stimulation of type II fibers causes the muscle to acquire many characteristics of type I fibers (Goldspink, 1985). Despite these observations, the transcriptional mechanisms underlying muscle-nerve interactions remain to be clarified. Knowledge gained from studies in *Drosophila* should help define the nature of muscle-nerve interactions, which are presumably related to the mechanisms functioning in mammalian adult differentiated muscles.

GLUCOCORTICOIDS, INFLAMMATION, AND OXIDATIVE STRESS

Among the major stimuli that can trigger muscle atrophy in mammals are the glucocorticoids (e.g. cortisol) or its synthetic analogs (e.g. dexamethasone), which at high pharmacological doses cause atrophy preferentially of type IIb glycolytic fibers (Dahlmann et al., 1986; Goldberg and Goodman, 1969). These steroids are released from the adrenal gland in stressful states and are essential for muscle wasting during fasting, renal failure, diabetes and sepsis (Menconi et al., 2007; Schakman et al., 2008). Glucocorticoids act to reduce muscle size by inhibiting amino acid transport into muscle, protein synthesis, and enhancing proteolysis. Consequently, they promote the release from muscles of amino acids that can be burned directly or serve as substrates in the liver for glucose production. Although not normally catabolic, normal or slightly increased levels of glucocorticoids can induce muscle wasting when there is a fall in Insulin/IGF-1 signaling, as occurs during fasting, diabetes and insulin-resistant states (most diseases; Hu et al., 2009). Because of their ability to also reduce inflammation, proliferation of white cells and immune responses, glucocorticoids are widely used to treat rheumatoid arthritis, allergic reactions, asthma, transplant rejection, lupus, and some forms of cancer. The induction of muscle (and bone) wasting is a serious adverse effect of high doses of these drugs and often limits their use in the clinic. Interestingly, lack of contractile activity enhances the tendency of muscles to atrophy in response to glucocorticoids (Goldberg and Goodman, 1969). For example, although the dark oxidative *soleus* is relatively resistant to these agents, the denervated *soleus* is highly susceptible to cortisone-induced atrophy, which helps explain the marked loss of body mass in the bed-ridden patients. Thus, an outstanding question is how the sensitivity of different muscles to glucocorticoids or other catabolic stimuli is modulated at the molecular level by contractile work or other anabolic stimuli.

Although no hormones are known that function similarly to glucocorticoids upon stress in insects, a class of steroid hormones called ecdysteroids (including ecdysone) has been implicated in the complete breakdown and death of muscle cells (also termed histolysis) during pupal metamorphosis. Throughout this process, most larval muscles degenerate completely in response to ecdysone and presumably provide amino acids for the development of the adult tissues. Although glucocorticoids do not cause death of muscles in mammals, the rapid loss of muscle mass and breakdown of myofibrils in response to structurally related molecules (glucocorticoids and ecdysteroids) suggests some similar mechanisms of action. Accordingly, larval muscles undergo marked atrophy before apoptosis is evident in response to ecdysone (Bayline et al., 1998; Hegstrom and Truman, 1996). However, not all insect muscles undergo histolysis in response to ecdysteroids. An example of such diverse responses to ecdysteroids comes from pioneering studies in the moth *Manduca sexta*. During pupal metamorphosis, the larval muscles are either

maintained, modified, or degenerate (Bayline et al., 1998; Hegstrom and Truman, 1996). In particular, the large intersegmental muscles (ISMs) from *Manduca* initially atrophy and loose approximately 40% of their mass starting three days before adult eclosion, while at the same time, the flight muscles of the adult musculature are actively growing in mass. Subsequently, ISMs undergo cell death and are completely lost within 30 hours of adult life, while newly-formed flight muscles do not (Schwartz, 1992).

Studies in *Manduca* have benefited from the large sizes of these muscles, but the genetic analysis of this process is easier in *Drosophila*, where different muscles also respond in distinct fashion to ecdysone. By 8 hours after puparium formation, most muscles in the head and thoracic segments undergo histolysis while the abdominal muscles are preserved (Fernandes et al., 1991). Among the thoracic muscles, the larval oblique muscles escape histolysis and serve as template for the formation of the dorsal longitudinal muscles (DLMs), a group of indirect flight muscles (IFMs) of the adult (Farrell et al., 1996; Roy and VijayRaghavan, 1998; Dutta et al., 2004). Subsequently, most larval muscles in the abdomen undergo histolysis. For example, the dorsal external oblique muscles (DEOMs) degenerate in the late prepupal stage (Wasser et al., 2007). However, some other abdominal muscles initially persist, undergo atrophy (day 1 and 2 of pupal stage), and subsequently increase in mass (hypertrophy; late pupal stage) to form the temporary dorsal internal oblique muscles (DIOMs) of the adult. DIOMs are needed for eclosion and degenerate only after the adult has emerged (Kimura and Truman, 1990). Interestingly, the nuclear proteins EAST and Chromator have antagonistic functions and respectively accelerate and delay the atrophy of DIOMs during pupal metamorphosis. In addition, Chromator partially blocks the breakdown of DEOMs muscles (Wasser et al., 2007).

The mechanisms by which distinct insect muscles respond differently to ecdysone are still not understood, and thus this may represent a valuable model to better understand the specialized responses of distinct muscles to catabolic stimuli. Algorithms recently became available to facilitate the analysis of images from high-throughput RNAi screens for muscle histolysis (Chinta et al., 2012). In mammals, there are several sexually dimorphic muscles (e.g. the *levator anus* that is also involved in the control of the penis) that show much greater sensitivity to the anabolic actions of testosterone than typical muscles (Herbst and Bhasin, 2004). The precise basis for their greater sensitivity to these steroids is largely unknown.

In addition to glucocorticoids, pro-inflammatory cytokines including TNF-α (Tumor Necrosis Factor α), IL-1 (Interleukin 1), IL-6, activin, and myostatin have all been reported to contribute to muscle wasting (cachexia) in mammals during sepsis and in cancer-bearing animals at least in part by activating the NF-κB, STAT3 and/or the Smad transcription factors (Bonetto et al., 2011). Some of these cytokines and the pathways they activate are conserved in *Drosophila*. For example, *Drosophila eiger* (*CG12919*) is a TNF superfamily ligand that activates a canonical TNF signaling cascade (Moreno et al., 2002). In addition, the JAK/STAT pathway, which is activated in mammals by various members of the Interleukin family, is conserved in *Drosophila* where it is activated by the Unpaired family of cytokine-like ligands (*outstretched*, *unpaired 2*, and *unpaired 3*). Studies on the effects of these signaling pathways on *Drosophila* muscle growth and remodeling may provide useful models of inflammation-induced atrophy.

Another type of circulating factor that can increase muscle size or counteract atrophy are cathecolamines and their synthetic analogs, clenbuterol or β2 agonists, which activate β2 adrenergic receptors (whose *Drosophila* homolog is octopamine receptor 2). These agents cause production of cyclic-AMP and activation of PKA that can ultimately cause cardiac and skeletal muscle hypertrophy (Navegantes et al., 2001). In fact, clenbuterol has often been used illegally to induce growth of cattle and enhance food production but, because

residues of cathecolamines are retained in the meat, this practice is dangerous and is banned. Also, because of the side effects of cathecolamines (e.g. blood pressure and cardiac function), their ability to inhibit atrophy has not been exploited as a therapy for muscle wasting.

Increased production of reactive oxygen species (ROS) has been proposed to play a role in the muscle degeneration of the queen fire ant, *Solenopsis spp.*, where histolysis follows the mating flight and insemination (Davis et al., 1993). Similarly, in mammals there are multiple observations suggesting a possible role of oxidative stress in triggering muscle atrophy. First, the transcription factor ATF4, which promotes the expression of oxidative stress responsive genes, is upregulated in most types of atrophy (Lecker et al., 2004) as are the metallothioneins, heavy metal-binding components that can serve antioxidant roles (Sacheck et al., 2007). In addition, during disuse, ROS have been proposed to activate several transcription factors involved in muscle atrophy in mammals, including NF-κB and FoxO (Dodd et al., 2010), but other modes of activation have also been demonstrated. Genetic manipulations that enhance muscle ROS levels, such as knock-out of the cytoplasmic anti-oxidant enzyme Sod1 (superoxide dismutase 1) in mice leads to oxidative damage to proteins and muscle wasting (Jang and Van Remmen, 2011; Muller et al., 2006). On the other hand, mice devoid of the mitochondrial superoxide dismutase 2 in muscle have elevated oxidative stress, but do not exhibit muscle wasting (Kuwahara et al., 2010; Lustgarten et al., 2009). Thus, oxidative stress does not appear to be sufficient to induce atrophy, and it remains unclear if these signs of oxidative stress are a key factor in the atrophy process or an incidental factor.

PHYSICAL ACTIVITY AND INACTIVITY

Muscle wasting in adult mammals is generally viewed as a reversible process, but this reversibility has not been rigorously studied, e.g. at different ages. The loss of muscle on fasting is rapidly reversed by refeeding and can even lead to “overshoot” growth. Similarly, the loss of body mass with acute illness is rapidly reversed upon recovery, especially in children where it has been termed “catch-up” growth. In the elderly, this capacity to reverse wasting seems to be more limited but this impression and its possible cellular basis have not been systematically studied. The extent of muscle wasting induced by food deprivation in mammals can be counteracted by physical activity, especially isometric exercise which stimulates the IGF-1/Akt pathway and builds muscle mass and strength. In fact, in starving rats where there is rapid muscle wasting, generally increased activity of the soleus (e.g. induced by loss of a synergist) can induce hypertrophy (Goldberg, 1968). Both strength and endurance exercise increase the activity of the transcriptional co-activator PGC-1α (Gibala et al., 2009; Terada et al., 2002), which stimulates in turn mitochondrial biogenesis and oxidative metabolism characteristic of type I fibers but not hypertrophy (Handschin and Spiegelman, 2008). Type I aerobic fibers are relatively resistant to atrophy induced by various circulating catabolic factors, including fasting, glucocorticoids, cancer and sepsis, apparently due to the presence of PGC-1α. Its expression is decreased in many, perhaps all, types of atrophy (Sacheck et al., 2007; Sandri et al., 2006), and PGC-1α and its homolog PGC-1β, directly antagonize the induction of the atrophy gene program by both FoxO3 (Sandri et al., 2006) and NF-κB, and thus suppress the acceleration of protein degradation (Brault et al., 2010; Figure 3). Presumably, these effects help account for the ability of exercise to retard atrophy. Following disuse and upon restoration of activity, the increased PGC-1α levels and IGF-1/PI3K-Akt signaling inactivate FoxO leading to decreased expression of atrogenes. These genes are induced or suppressed coordinately during various types of atrophy due to uremia, diabetes, cancer, denervation, disuse and fasting and include components of both the UPS and autophagy/lysosome pathways (Zhao et al., 2007). Among these atrogenes, two muscle-specific ubiquitin ligases, MuRF1 and atrogin-1, are

dramatically induced by FoxO3 (Sandri et al., 2004) and are essential for muscle atrophy (Bodine et al., 2001a; Gomes et al., 2001). In addition to a role for PGC-1α in repressing FoxO activity, PGC-1α4 (a PGC-1α splice variant preferentially produced after resistance exercise) protects muscles from atrophy by inducing IGF-1 while repressing myostatin expression (Ruas et al., 2012).

The mechanisms linking contractile activity to the action of these transcription factors in atrophy is still not clear even though this area is critical and determines whether a cell grows or atrophies. One well-studied exception is NF-AT, a key transcription factor that is activated by increases in Ca^{2+} levels. In skeletal muscle, it is involved in fiber type specification and influences PGC-1α expression (McCullagh et al., 2004) and in cardiac muscle it can trigger hypertrophy (van Rooij et al., 2002). An additional potential link between physical activity and protein turnover is the dihydropyridine receptor (DHPR) α1S subunit, which can serve as a molecular sensor of muscle activity (Pietri-Rouxel et al., 2010). DHPR α1S knock-down induces muscle atrophy via FoxO3A activation, which leads to upregulation of autophagy-related genes and the induction of autophagy. However, the loss of DHPR presumably represents a form of disuse since it should reduce contractile activity (i.e. disrupt excitation-contraction coupling).

Thus far, studies on the effects of muscular contractions have been lacking in insects. Although genetic studies in *Drosophila* in principle might help elucidate the interconnections between exercise and muscle mass, experimental approaches for exercising this organism and methods for measuring changes in muscle mass or metabolic adaptations have not been described, in contrast to the extensive literature on mammals. Recently, a mechanized platform for the physical training of flies has been described (Piazza et al., 2009), in which a repeated tapping of the container where the flies are housed activates the innate instinct of the flies to climb (negative geotaxis). This promising system may be useful to dissect the genetic basis underlying the effect of physical exercise on muscles. For example, an interaction between physical exercise and *spargel*, the PGC-1α homolog that promotes mitochondrial production in *Drosophila* (Tiefenbock et al., 2010), has been found with this system, highlighting that *spargel* is required for full exercise capacity *in Drosophila* and the induction of physiological effects deriving from exercise (Tinkerhess et al., 2012).

While endurance exercise clearly decreases the loss of muscle mass in mice and in humans, no morphological signs of muscle deterioration have been observed in flightless *Drosophila* mutants or in response to transient local paralysis of adult flies induced with the temperature-sensitive dynamin mutant *shibire* (which are depleted of synaptic vesicles; Deak, 1976). Such studies did not identify decreases in muscle mass of the kinds seen with disuse in small mammals where fiber diameters decrease by 10–40% within 1–2 weeks without any clear structural deterioration. This apparent lack of clear responses to disuse is important to define more rigorously since *Drosophila* muscles may differ radically from mammals and lack the capability to alter muscle mass in response to changes in contractile activity. While such an apparent difference may be due to insufficient studies, it is also possible that because of their brief lifespan and very different lifestyle, *Drosophila* may not have evolved similar regulatory mechanisms as mammals for mobilizing amino acids from muscle proteins. So disuse may not result in obvious muscle atrophy in flies.

ROLE OF PROTEIN SYNTHESIS AND DEGRADATION PATHWAYS IN MUSCLE ATROPHY

Despite the greater diversity of possible regulatory factors in mammals (ranging from hormones to cytokines), the final cellular mechanisms regulating muscle size appear similar

in *Drosophila* and mammals (Figures 1, 2 and 3). In *Drosophila*, the Insulin/Akt-responsive transcription factor FOXO mediates most of the gene expression changes induced by nutrient starvation in larval muscles (Teleman et al., 2008) and its activation is sufficient to stunt developmental muscle growth (Demontis and Perrimon, 2009; Figure 2B and C). Similarly, in mammals, various types of muscle atrophy share a common transcriptional program mainly driven by FoxO3 (Lecker et al., 2004; Sandri et al., 2004) and possibly other FoxO family members, like FoxO1. MuRF1 and atrogin-1 are key atrogenes induced by FoxO3 (Sandri et al., 2004) that are necessary for rapid muscle atrophy (Bodine et al., 2001a; Gomes et al., 2001). While proteins comprising the thick filaments of the myofibrils have been clearly identified as MuRF1 substrates (Cohen et al., 2009), how atrogin-1 causes muscle loss is less clear, but probably involves the degradation of growth-related proteins including the transcription factor MyoD, and the translation initiation factor eIF3-f, which in turn reduces protein synthesis (Lagirand-Cantaloube et al., 2008; Figure 3). Additional E3s are also important in muscle size control. These include TRAF6, responsible for activating many atrophy-related signaling cascades (Paul et al., 2010), TRIM32, involved in the degradation of thin filaments and the desmin cytoskeleton (Cohen et al., 2012; Kudryashova et al., 2005) and Cbl-b, which inhibits IGF-1 signaling by promoting the degradation of the Insulin Receptor Substrate IRS-1 (Nakao et al., 2009). Homologs of atrogin-1 (*Drosophila CG11658*), TRIM32 (*Drosophila abba*), TRAF6 (*Drosophila CG10961*) and Cbl-b (*Drosophila Cbl*) are encoded in the *Drosophila* genome, suggesting possible similarities in the pathways governing proteolysis and muscle atrophy in fruit flies and mammals. For example, *CG11658* expression levels increase in *muscle wasted* (*mute*) mutants, lacking a component of the histone locus body and characterized by a severe loss of muscle mass and integrity during development (Bulchand et al., 2010). Moreover, *Drosophila* TRIM32 (*abba*) is required for integrity of the costamere, a structure that connects the sarcomeres to the overlying sarcolemma providing stability during muscle contraction, and its mutation results in unbundling of myofibrils and progressive muscle wasting (LaBeau-DiMenna et al., 2012).

However, no homologs of MuRF1, a E3 ligase that ubiquitinates myofibrillar proteins, are found in flies. Very recently, the p97/VCP ATPase complex, which forms distinct complexes involving different adaptors (e.g. p47 and Ufd-1) and distinct components of the thick (i.e. Myosin Light chains) and thin (i.e. actin) filaments, has been shown to have a major role in multiple types of atrophy, where it seems to catalyze the extraction of ubiquitinated proteins from the myofibrils prior to proteasomal degradation (Piccirillo and Goldberg, 2012). Thus, *Drosophila* homologs of p97 (*Drosophila TER94*) and Ufd-1 (*Drosophila CG6233*) could participate to a conserved mechanism for muscle protein degradation, although p97 also functions in other disassociation and degradative processes (e.g. destruction of misfolded proteins in the ER). Interestingly, human mutations in p97 cause an inclusion body myopathy and all amino acid residues altered in the disease are perfectly conserved in *Drosophila* TER94 (Ritson et al., 2010). It will clearly be of interest to define the biochemical mechanisms for disassembly and degradation of the sarcomeric apparatus during pupal metamorphosis and if they involve the p97 complex.

Studies in *Manduca* have highlighted the involvement of the UPS in ecdysone-induced muscle histolysis. In particular, levels of ubiquitin conjugates dramatically increase at eclosion, during which loss of muscle proteins is maximal. Histolysis is also characterized by the coordinated induction of ubiquitin activating enzymes (E1), several ubiquitin conjugating enzymes (E2s), ubiquitin ligases (E3s), and ubiquitin itself (Haas et al., 1995; Schwartz et al., 1990) together with heightened expression in the abdominal intersegmental muscles (ISMs) of MS73/Rpt3 and S10b/Rpt4, which encode ATPase subunits of the 26S proteasome (Dawson et al., 1995; Hastings et al., 1999; Jones et al., 1995; Low et al., 1997). Thus, activation of the UPS seems to play a fundamental role in the loss of skeletal muscle

mass during insect metamorphosis similar to that in atrophy in mammals. These insect muscles shrink in size markedly (atrophy) before the onset of apoptosis, presumably due to increased proteolysis. It should be noted that the UPS is also required to maintain muscle mass and integrity during larval development (Haas et al., 2007) and for viability in mammals, due to its many critical roles in cellular quality control and regulating metabolism. In other words, different degrees of protein breakdown have beneficial or detrimental effects on muscle mass, depending on what proteins are digested.

In addition to the UPS, autophagy contributes to the histolysis of several tissues during metamorphosis in *Drosophila*, suggesting that it may be part of the catabolic response activated in muscles in response to ecdysone signaling (Baehrecke, 2003).

Many atrogenes induced by FoxO3 encode for proteins of the autophagy/lysosome system (e.g. Cathepsin L, LC3, GabarapL1; Lecker et al., 2004; Zhao et al., 2007), which contributes to muscle atrophy in mammals especially through the disposal of mitochondria (Romanello et al., 2010), as well as soluble cell proteins. Importantly, Mul1 is an E3 ubiquitin ligase recently implicated in FoxO induced-mitophagy (Lokireddy et al., 2012), the autophagic destruction of mitochondria, while the ubiquitin ligase Parkin is needed for mitophagy of damaged mitochondria (Yang and Yang, 2011). Both Mul1 and Parkin are conserved in *Drosophila* (*CG1134* and *parkin*, respectively) and may play a role in muscle wasting in this organism.

The loss of mitochondria must contribute importantly to the decreased endurance during atrophy. Nevertheless, the changes in mitochondrial numbers and functional capacity during different types of atrophy have surprisingly not been studied extensively. Because ubiquitination can also target larger structures to autophagic vacuoles, several ubiquitin-binding proteins (including p97, p62, NBR1, NDP52 and parkin) exist in cells that bind insoluble or organellar ubiquitinated proteins and facilitate their docking to the autophagic vacuole via LC3. Their functions in muscle merit further study to better understand the coordination between different protein degradation pathways during atrophy and their roles in clearing different cellular constituents (reviewed in Korolchuk et al., 2010), especially since certain components (p97 cofactors) are essential in proteolysis by both degradative systems.

In addition to the activation of protein degradation pathways, muscle wasting upon fasting, glucocorticoid treatment and other disease states result from an inhibition of protein synthesis that is triggered by decreased IGF-1/Akt/TOR signaling and inhibited by glucocorticoids (Hu et al., 2009). Interestingly, ecdysteroids inhibit Insulin signaling in *Drosophila* (Colombani et al., 2005), suggesting that muscle histolysis during metamorphosis may also rely on inhibition of Akt signaling and the activation of FOXO. However, mutant flies lacking the only *Drosophila foxo* gene are viable and have only minor developmental defects (Junger et al., 2003), suggesting that FOXO is not necessary for muscle histolysis during metamorphosis. By contrast, mammalian muscle contains three closely related FoxO genes and their respective roles are still uncertain. FoxO1, 3 and 4 are coordinately activated upon nutrient deprivation, but may have distinct roles in specific catabolic states and may regulate distinct genes (Moylan et al., 2008), although FoxO1 and 3 both induce atrogin-1, MuRF1 and Mul1. FoxO3a and FoxO4 knock-out mice (Hosaka et al., 2004) are viable and analysis of FoxOs-deficient mice (Paik et al., 2007) may clarify whether specific FoxO transcription factors are necessary for the induction of atrophy in response to different stimuli. Thus far, it is only known that overexpression of FoxO-dominant negative mutants, which block all FoxOs in adult muscles, can prevent multiple types of atrophy (Sandri et al., 2004). It would be interesting to test whether FOXO is able to alter the expression of the *Drosophila* homolog of *atrogin-1* as in mammals.

SYNCYTIAL APOPTOSIS

Additional mechanisms responsible for modifying muscle mass during development and adulthood include the poorly defined pathway of syncytial apoptosis. In this process, loss of individual nuclei within a fiber is more commonly observed than the death of the entire fiber, possibly via caspase-independent mechanisms acting via endonuclease-G (Primeau et al., 2002; Sandri and Carraro, 1999) or nucleophagy, the selective autophagic degradation of nuclear components (Park et al., 2009). In addition, segmental necrosis, the death of a portion of a fiber without the overall loss of the fiber, is a characteristic feature of many myopathies, especially Duchenne muscular dystrophy (Wallace and McNally, 2009).

The genetic regulation of syncytial apoptosis, and whether it differs from segmental necrosis is unclear and may benefit from genetic studies to elucidate the underlying regulatory pathways. In insects, syncytial apoptosis occurs during metamorphosis, when some nuclei within the same fiber degenerate and are lost, while those in proximity to the site of innervation are spared (Bayline et al., 1998; Hegstrom and Truman, 1996). Therefore, in insects the local induction of syncytial apoptosis may vary even within a single fiber depending on the proximity to the site of innervation.

While apoptosis occurs upon muscle histolysis during insect metamorphosis, fiber cell death has not been observed upon inhibition of larval muscle growth in *Drosophila* (Demontis and Perrimon, 2009) and during rapid atrophy in mammals (Bruusgaard and Gundersen, 2008). In animal models of uremia, fasting, diabetes and cancer, the pro-apoptotic gene *Bnip3* is induced but it also functions in mitophagy (Mammucari et al., 2007; Lecker et al., 2004). However, segmental apoptosis of fibers has been reported during aging in mammals and *Drosophila* (Marzetti et al., 2012; Zheng et al. 2005). Future studies in *Drosophila* and mammals should elucidate the pathways governing muscle syncytial apoptosis, and how this poorly understood process impacts on the loss of muscle mass and strength.

Importantly, other pathways of programmed cell death including necroptosis, which depends on the activity of the serine/threonine kinase RIP1, have not been examined in muscles. Notably, TNF-α, which helps trigger muscle wasting in inflammatory states (Glass, 2010), although perhaps indirectly, is able to induce necroptosis in other cell types (Galluzzi and Kroemer, 2008). The possible relevance of necroptosis during muscle wasting awaits further investigation.

ROLE OF MUSCLE STEM CELLS

In vertebrates, satellite cells are the small mononucleated muscle stem cells localized between the sarcolemma and the basal lamina of muscle fibers. In the adult, most stem cells are in a quiescent state, but can become dramatically activated upon injury or specific stimuli like IGF-1, which promotes their proliferation and differentiation during hypertrophy (Biressi and Rando, 2010). For example, after damage, satellite cells fuse to one another or to an undamaged fiber, promoting muscle regeneration, through a process that partially recapitulates myoblast fusion during development. Interestingly, apoptosis of satellite cells but not pre-existing fibers has been observed during rapid atrophy (Bruusgaard and Gundersen, 2008). Unfortunately, since satellite cells are heterogeneous, few in number (less than 2% of the nuclear content of muscle), and much less susceptible to *in vivo* electroporation than muscle fibers, their specific role in atrophy is still largely unknown. Although satellite cells are not needed for muscle re-growth following disuse-induced atrophy (Jackson et al., 2012), further studies are necessary to test whether satellite cells can possibly reverse or prevent muscle atrophy in other contexts, as has been recently suggested (Thornell, 2011).

Important roles in hypertrophy have often been postulated, especially in providing additional nuclei to prevent a fall in concentration of nuclei when fibers enlarge. However, it remains controversial whether stem cell proliferation is essential during hypertrophy as it clearly is upon regeneration (Zammit et al., 2002). New techniques have recently become available to dissect their possible contributions to muscle adaptations. These include the *ex vivo* transfection of muscle stem cells followed by intramuscular injection and the *in vivo* administration of adenoviral vectors where transgene expression is driven under muscle stem cell specific promoters of the *Pax3* and *Pax7* genes (Biressi and Rando, 2010).

Currently, there is no evidence of satellite cells in any *Drosophila* adult muscles, and it is not clear whether they do not exist or simply have not yet been identified. If present they may be related to the *twist*-expressing adult muscle precursors (AMPs), muscle progenitor cells that escape histolysis and form all adult muscles in *Drosophila* (Figeac et al., 2007). Interestingly, even if the *Drosophila* homologs of the mammalian satellite cells markers Pax3 and Pax7, *paired* and *gooseberry*, respectively, are not expressed in AMPs, these muscle-committed stem-like cells, like quiescent satellite cells, have high levels of Notch activation (Figeac et al., 2011). This finding suggests that studies on *twist*-expressing muscle progenitors in *Drosophila* may provide useful information on the regulation of satellite cell function in mammals.

EFFECTS OF MUSCLE MASS ON BODY METABOLISM IN *DROSOPHILA* AND MAMMALS

It is now clear that the skeletal muscle has major effects on the metabolism of the organism, its growth, aging, and resistance to disease (Demontis and Perrimon, 2010; Demontis et al., 2013). These organismal effects involve muscle-derived signaling factors but also arise from indirect effects of muscle mass and its high metabolic demand. Skeletal muscle consumes a major fraction of nutrients, and with intense exercise, its energetic demand increases dramatically. Consequently, multiple physiological mechanisms exist to provide glucose or fatty acids to muscle. For example, when muscle growth was induced in adult mice by increasing the expression of the Akt1 kinase in type IIb fibers, the transgenic mice displayed resistance to both diet-induced obesity and hepatic steatosis, at least in part via a stimulation of fatty acid oxidation in the liver (Izumiya et al., 2008). In addition, postnatal loss of the transcription factor *myogenin* in muscles results in smaller body size of mice, suggesting that Myogenin acts to influence the post-natal growth of muscles and also other tissues via un unknown mechanism (Knapp et al., 2006). As in mammals, in developing *Drosophila* larvae the extent of muscle growth influences whole-organism growth and metabolism. In particular, muscle-specific inhibition of the Insulin receptor/Akt/TOR pathway and of Myc activity decreases not only muscle growth, but also feeding behavior and the growth of non-muscle tissues, while muscle-specific activation of the Insulin receptor has the opposite effects (Demontis and Perrimon, 2009).

A remarkable and very promising intervention to increase muscle mass and reduce atrophy in mammals is through inhibition of the TGF-β family members, like Myostatin and Activin, all of which signal via the ActRIIB complex (Figure 4). Activin is produced normally in multiple cell types and also in cancerous cells and plays an important role in reproduction (Xia and Schneyer, 2009), while Myostatin is expressed predominantly in skeletal muscles (Zhou et al., 2010; Zimmers et al., 2002). Myostatin normally acts to limit prenatal and postnatal muscle growth and mutations in the *Myostatin* gene or its receptor result in increased muscle mass in mice, cattle, dogs and humans (Lee, 2004). Developmental inhibition of Myostatin results in both muscle hyperplasia and hypertrophy, while postnatal inhibition increases muscle mass via hypertrophy, with no accompanying hyperplasia in several species (McPherron 2010). Suppression of the Myostatin-based pathway (as in

Figure 4) has been also proven effective in counteracting muscle wasting in mice with chronic kidney disease (Zhou et al., 2010), following treatment with glucocorticoids (Gilson et al., 2007), as well as in cancer cachexia (Benny Klimek et al., 2010; Zhou et al., 2010). This effect on muscle size resulted at least in part from the inhibition of the FOXO-induced activation of atrogenes and ubiquitin-dependent proteolysis and perhaps also through the subsequent stimulation of muscle stem cell proliferation. Remarkably, inhibition of Activin and Myostatin signaling via blockade of the ActRIIB receptors not only dramatically reverses cancer-induced weight loss in certain cancer models, but also improves the survival of mice without slowing tumor growth (Zhou et al., 2010). Thus, improving muscle mass aside from enhancing the quality of life can strikingly influence cancer-related mortality, at least in mice.

PERSPECTIVES

In this review, we have highlighted a number of important similarities and differences between developmental and post-natal mechanisms regulating muscle size in *Drosophila* and mammals. The emerging evidence that multiple signaling pathways influence muscle size and are intricately interconnected should have important implications in understanding human diseases and applications in combating muscle wasting. Future studies in both *Drosophila* and mammals should further dissect the pathways governing muscle growth and atrophy in health and disease to discover novel drugs and therapeutic interventions.

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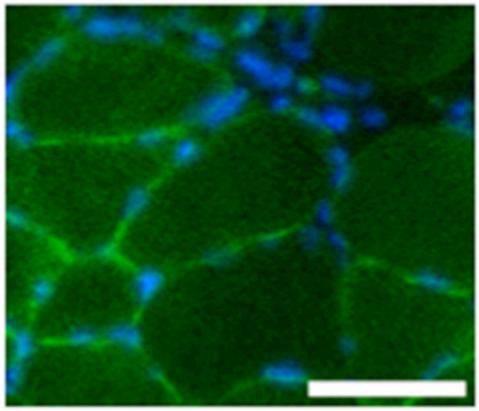
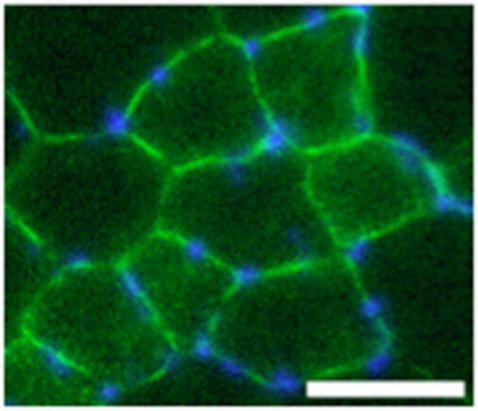


Fiber atrophy



MAMMALS

Nuclei Membrane

MAMMALS	ATROPHY	HYPERTROPHY
PHYSIOLOGICAL AND PATHOLOGICAL	Disuse, Denervation Fasting Aging Cancer Renal failure Cardiac failure Inflammation, Sepsis AIDS	Exercise (isometric work)
MEDIATORS	Excess glucocorticoids, low IGF-1/insulin inflammatory cytokines, myostatin, activin	IGF-1/Insulin Cathecolamine (β-agonists) Myostatin antagonists
MECHANISMS	↓ protein synthesis ↑ UPS: myofibrillar protein degradation ↑ Autophagy: loss of mitochondria	↑ protein synthesis ↓ protein degradation satellite cells activation
INSECTS	ATROPHY/HISTOLYSIS	HYPERTROPHY
MEDIATORS (DURING LARVAL DEVELOPMENT AND METAMORPHOSIS)	High levels of the hormone ecdysone Low activation of insulin-like signaling	insulin-like peptides
MECHANISMS	↑ UPS: protein degradation ↑ loss of organelles Syncytial apoptosis	↑ protein synthesis

Figure 1.

Environmental stimuli and signaling pathways increasing proteolysis and depressing protein synthesis and leading to muscle atrophy. Representative fields of a transverse section of muscle fibers from fed mice or weight-matched mice deprived of food for 48h are shown. Nuclei and membranes are indicated in blue (Hoechst) and green, respectively. Scale bar is 50 μm.

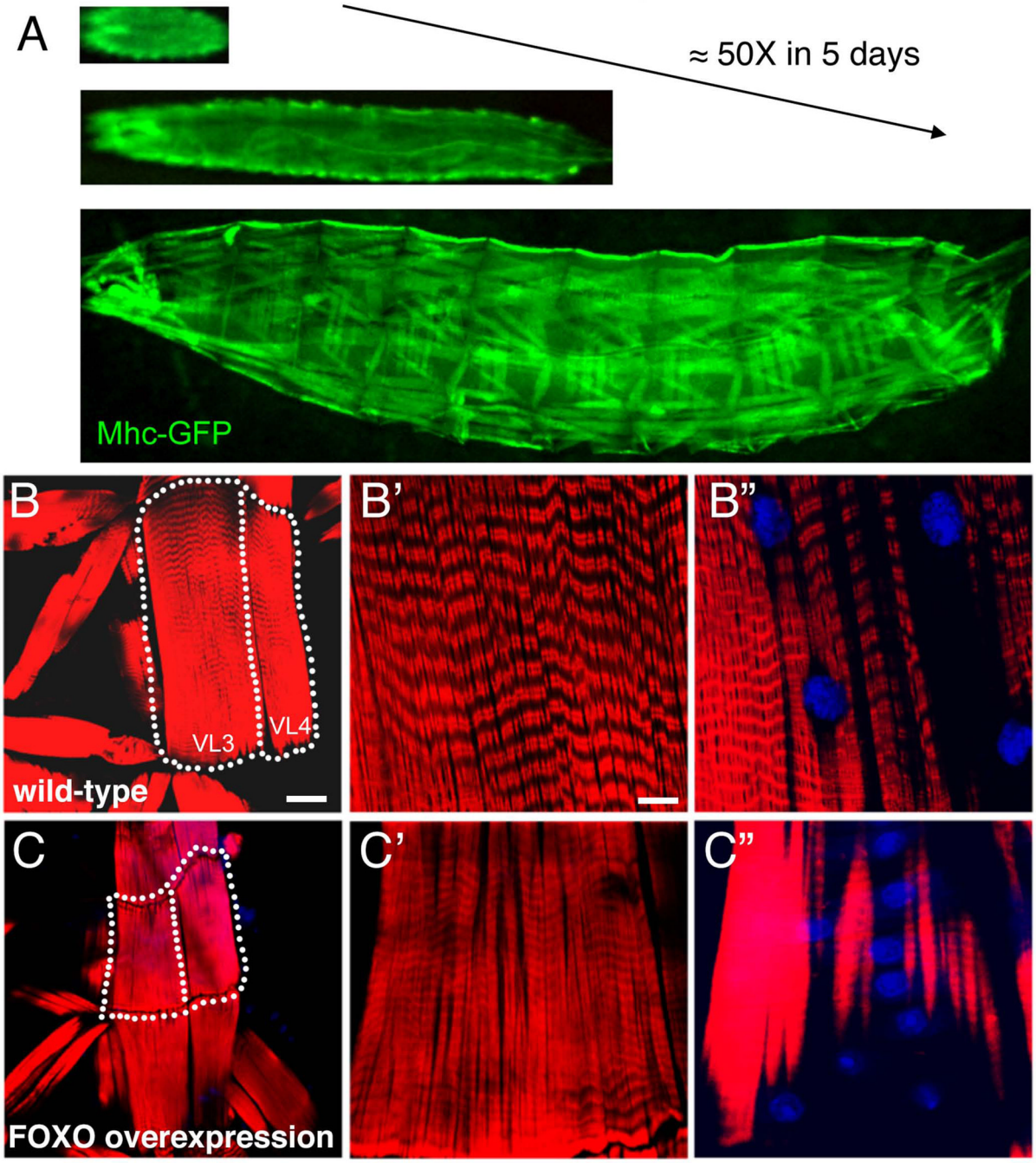


Figure 2.

Developmental growth of skeletal muscles in *Drosophila* larvae is inhibited by FOXO overexpression. A) Skeletal muscle size dramatically increases by 50-fold in the larval stage of development, which lasts 5 days. Muscle growth results from enhanced protein synthesis without any addition of muscle nuclei. Larvae expressing a GFP-tagged Myosin Heavy Chain (Mhc) protein in body wall muscles are shown. B) The size increase of body wall muscles is inhibited by overexpressing the transcription factor FOXO in muscles (C, *Dmef2-Gal4 UAS-foxo* versus *Dmef2-Gal4* in B). Ventral Longitudinal muscles 3 and 4 (VL3 and VL4) from animals at the end of larval development are outlined for comparison in B and C.

The dramatic increase in muscle mass observed during *Drosophila* larval development provides a sensitive setup for the identification of evolutionarily conserved genes regulating muscle mass. Micrographs in B'-B" and C'-C" outline sarcomeres and nuclei within muscle fibers (F-actin, red; Nuclei, blue). Scale bar is 40 μm in B-C and 10 μm in B'-B" and C'-C". See Demontis and Perrimon, 2009, for more information.

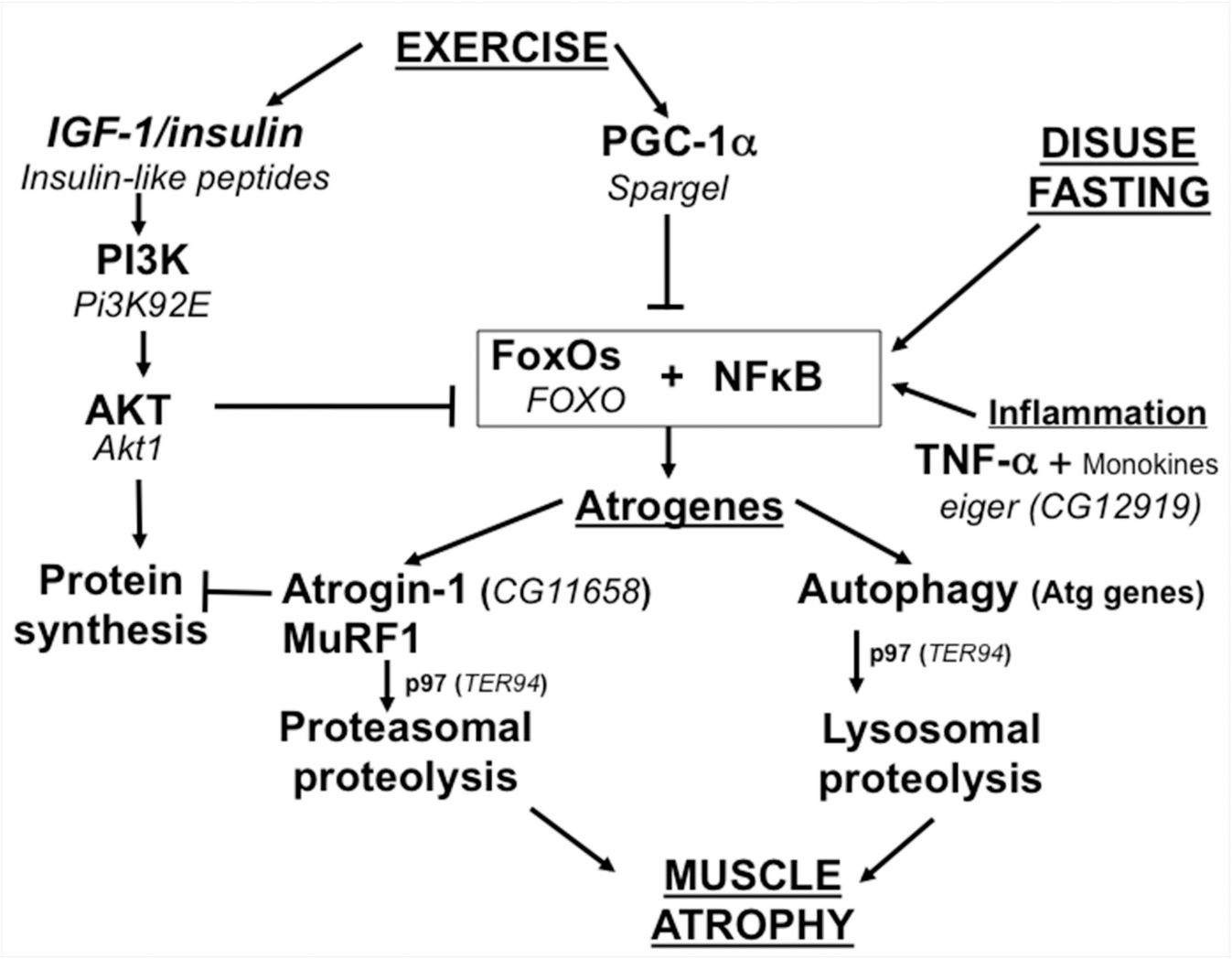


Figure 3.
Signaling pathways increasing proteolysis, depressing protein synthesis and leading to muscle atrophy. A comparison of the molecular players in *Drosophila* (indicated in *Italics*) and mammals is shown.

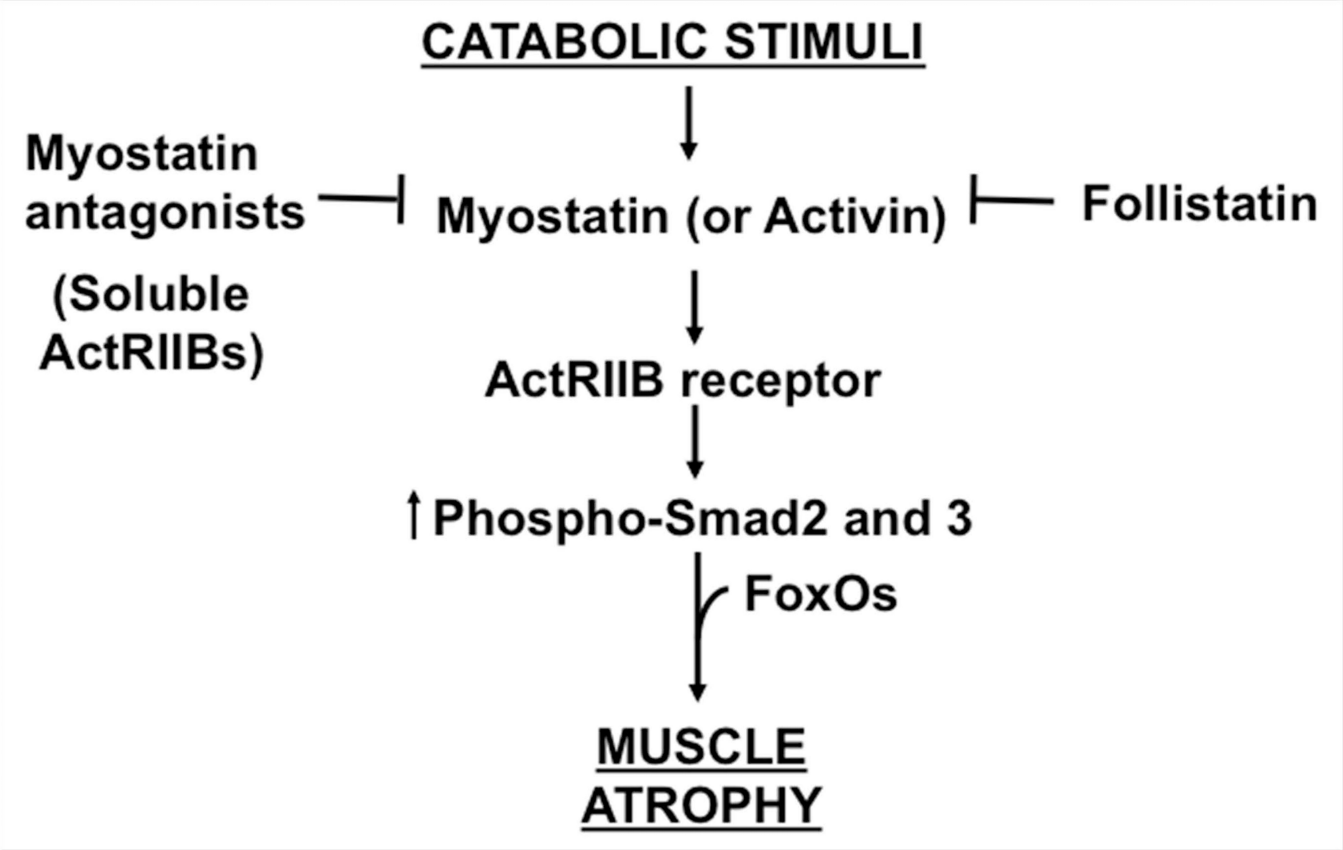


Figure 4.
Myostatin signaling pathway as a basis to counteract muscle wasting. Molecules to inhibit this pathway are currently in human clinical trials to cure the muscle wasting associated with Duchenne Muscular Dystrophy.

Table I

A list of prominent yet unsolved questions for future research on muscle atrophy in *Drosophila* and mammals.

OUTSTANDING QUESTIONS FOR FUTURE RESEARCH
IN *DROSOPHILA* • WHICH ARE THE MECHANISMS OF DEVELOPMENTAL MUSCLE GROWTH, ATROPHY, AND SEGMENTAL APOPTOSIS IN *DROSOPHILA*? • WHAT DETERMINES THE DISTINCT SENSITIVITY TO HISTOLYSIS OF DIFFERENT INSECT MUSCLES DURING PUPAL METAMORPHOSIS? • ARE *DROSOPHILA* MUSCLE PRECURSOR CELLS SIMILARLY REGULATED AS MAMMALIAN SATELLITE CELLS? • IS THERE TURNOVER OF THE CONTRCTILE APPARATUS IN NORMAL MUSCLES?
IN MAMMALS • WHAT IS THE BASIS FOR THE DIFFERENTIAL RESPONSE OF DIFFERENT MAMMALIAN FIBER TYPES TO CATABOLIC STIMULI? • HOW ARE COMPONENTS OF THE MYOFIBRIL ASSEMBLED AND DEGRADED BOTH NORMALLY AND AT INCREASED RATES DURING ATROPHY, WHILE PRESERVING CONTRACTILE FUNCTION? • IN ATROPHYING MUSCLES, HOW ARE THE INCREASES IN PROTEOLYSIS BY THE UBIQUITIN PROTEASOME PATHWAY AND BY AUTOPHAGY AND THE DECREASES IN PROTEIN SYNTHESIS COORDINATED? • DO SYNCYTIAL APOPTOSIS AND NECROPTOSIS CONTRIBUTE TO SARCOPENIA? • WHAT IS THE PRECISE CONTRIBUTION, IF ANY, OF SATELLITE STEM CELLS TO MUSCLE ATROPHY AND HYPERTROPHY?
IN BOTH • HOW DO VARIOUS SIGNALING PATHWAYS AND TRANSCRIPTION FACTORS (NFκB, Junβ, etc.) INTERACT WITH FoxOs TO CAUSE (OR COMBAT) MUSCLE ATROPHY IN MAMMALS AND *DROSOPHILA*?

Table II

Similarities and differences in muscle atrophy and hypertrophy in insects and mammals. Future research will benefit from studies in both species because of their complementary advantages.

COMPARING ADAPTIVE PROPERTIES OF MUSCLES IN MAMMALS AND *DROSOPHILA*

SIMILARITIES	**MAJOR DIFFERENCES**
• FIBER COMPOSITION DIFFERS IN MUSCLES WITH DISTINCT PHYSIOLOGICAL ROLES (CONTINUALLY ACTIVE OXIDATIVE VS. GLYCOLYTIC MUSCLES) • DISTINCT MUSCLES DIFFER IN SENSITIVITY TO ATROPHIC/HISTOLYTIC STIMULI (i.e. STEROIDS) • FoxOs CAUSE MUSCLE ATROPHY IN MAMMALS AND INHIBITS GROWTH OF LARVAL MUSCLES IN FLIES • THE UPS PLAYS A ROLE IN MUSCLE ATROPHY IN MAMMALS AS WELL AS IN MUSCLE LOSS DURING INSECT METAMORPHOSIS	• FIBER ATROPHY AND HYPERTROPHY OCCUR IN ADULT MAMMALS BUT MAINLY DURING DEVELOPMENT *DROSOPHILA* • MUSCLE STEM CELLS HAVE NOT BEEN OBSERVED IN FLIES • *DROSOPHILA* LACKS A MAJOR UBIQUITIN LIGASE IMPLICATED IN MYOFIBRILLAR TURNOVER (MURF1)
ADVANTAGES FOR STUDYING MUSCLE ATROPHY AND HYPERTROPHY	
IN *DROSOPHILA*	IN MAMMALS
• FLIES HAVE A SHORTER LIFE CYCLE THAN MICE • FLIES ARE EASILY AMENABLE TO VARIOUS GENETIC MANIPULATIONS (e.g. GENOME-WIDE RNAi SCREENS) • LARGE GROUPS OF FLIES CAN BE ANALYZED FOR A GIVEN INTERVENTION • THE 50-FOLD GROWTH DURING LARVAL DEVELOPMENT IS VALUABLE FOR STUDIES OF MYOFIBRILLAR ASSEMBLY AND GROWTH MECHANISMS	• RODENTS LOSE MUSCLE MASS SIMILARLY TO HUMANS DURING DIFFERENT KINDS OF ATROPHY • MUSCLES ARE ACCESSIBLE FOR PHYSIOLOGICAL AND BIOCHEMICAL STUDY • ENDOCRINE SIGNALS REGULATING ATROPHY HAVE BEEN CHARACTERIZED IN MAMMALS • ENERGY METABOLISM AND PHYSIOLOGICAL REGULATION OF MUSCLE MASS ARE WELL-CHARACTERIZED