Chapter 5
Skeletal Muscle Homeostasis and Aging in *Drosophila*

Melissa J. Puppa and Fabio Demontis

Abstract A debilitating feature of aging in humans is the progressive loss of skeletal muscle mass and function termed sarcopenia. A variety of intrinsic and extrinsic factors that are induced by aging contribute to sarcopenia, which in turn is a risk factor for many other age-related diseases. While widely studied in human and rodent models, sarcopenia has been identified also in the common fruit fly *Drosophila melanogaster*. *Drosophila* is emerging as a powerful system to study the mechanisms underlying sarcopenia, as it shares many of the same skeletal muscle characteristics as mammalian models. Decreased protein homeostasis, mitochondrial dysfunction, increased apoptosis, and alterations in transcription are just a few of the features of sarcopenia that are shared between mammals and *Drosophila*. Given its short life span compared to mammals and the ease in conducting genetic manipulations, including genome-wide muscle-specific transgenic screens, *Drosophila* offers unique advantages for studying the fundamental mechanisms of skeletal muscle aging and may provide potential therapeutic targets to combat sarcopenia in humans.

Keywords *Drosophila* · Skeletal muscle · Sarcopenia · Protein homeostasis · Mitochondrial dysfunction · Apoptosis · Aging

5.1 Introduction

During their life span many animal species, including nematodes, flies, rodents, and primates, develop sarcopenia, the progressive loss of skeletal muscle mass and strength with age (Herndon et al. 2002; Wolkow 2006; Augustin and Partridge 2009; Demontis et al. 2013a, b). Sarcopenia can be attributed to muscle-intrinsic and extrinsic defects that lead to a gradual decrease in the capacity to maintain

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skeletal muscle function and mass during aging. While sarcopenia is progressive and it is not fully reversible, muscle atrophy that occurs in response to catabolic stimuli such as fasting is rapid (a matter of days), generally reversible, and typically it does not entail intrinsic defects. There are many models available to study skeletal muscle agingand *Drosophila* has become a useful one to examine the cellular processes and genetic pathways responsible for functional decrements of skeletal muscle with aging (Augustin and Partridge 2009; Demontis et al. 2013a, b).

The organization and metabolic properties of skeletal muscle fibers of *Drosophila melanogaster* are similar to those of mammals (Sink 2006). Both *Drosophila* and mammalian skeletal muscles are composed of tandem arrays of sarcomeres containing thin filaments, composed of actin, and thick filaments, composed of myosin (Sink 2006). Release of calcium from the sarcoplasmic reticulum results in binding of the myosin head to the actin filament, which leads to generating the force of contraction. After the calcium is reabsorbed by the sarcoplasmic reticulum, the muscle is prepared for another contraction. Alternatively, some skeletal muscle in *Drosophila,* such as the indirect flight muscles, do not rely on extensive calcium recycling to maintain contractions. Instead, mechanical stimuli, through stretching and shortening of the muscle, are used to maintain the high frequency of contractions needed for flight (Dickinson 2006; Vigoreaux 2006; Tregear 2011).

Similar to mammals, *Drosophila* skeletal myofibers appear to be either glycolytic or oxidative (Sink 2006). The flight muscles, both direct and indirect, have been proposed to have an oxidative phenotype, as they are fatigue resistant, similar to the *soleus* muscle in mammals. Conversely, the leg muscles of adult flies and the body wall muscles of larvae, which are used intermittently, are thought to be glycolytic, similar to the *tibialis anterior* muscle in mammals (Sink 2006).

The developmental origin of *Drosophila* skeletal muscle is similar to vertebrates, as the somatic, visceral and cardiac musclesarise from the mesoderm. The commonality in structure and developmental origin makes *Drosophila* an excellent model for studying skeletal muscle differentiation, growth, aging, and disease. During the embryonic stage muscle progenitor cells differentiate and fuse to form individual myofibers. Embryonic muscle development is completed within one day (Fig. 5.1). *Drosophila* embryonic development provides a useful system for the examination of the cellular mechanisms and genetic pathways regulating myoblast fusion and myofiber differentiation, which may be relevant for understanding muscle regeneration and satellite cell function during aging in mammals. Subsequently, during the ~5 days of larval development (Fig. 5.1), larval muscles (each composed by a single myofiber) grow 50-fold in size (Demontis and Perrimon 2009). The profound changes in muscle mass observed during larval development provide a sensitized setting for the identification of the cellular mechanisms and genetic pathways regulating muscle growth and atrophy.

After the larval stage, developing *Drosophila* undergo pupal metamorphosis (Fig. 5.1) during which most of the larval muscles are degraded in a process called “histolysis” and replaced with adult muscles composed of multiple fibers similar to vertebrates (Patel et al. 2002). Analysis of the pupal stage of muscle development can further the understanding of myofiber degeneration and loss in

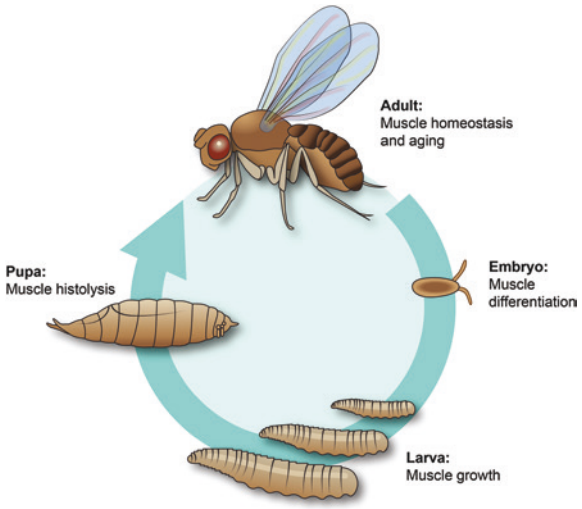

Fig. 5.1 The *Drosophila* life cycle: muscle differentiation, growth, remodeling, and aging. During embryonic development myoblasts fuse to form myofibers, which undergo differentiation. Subsequently, myofibers grow up to 50-fold in size during 4–5 days of larval development. During the pupal stage, most myofibers undergo rapid atrophy and degradation ("histolysis") while a few myofibers escape histolysis and form the template for the formation of the adult musculature by adult muscle precursor cells (AMPs). Finally in the adult fly, muscles must maintain their functional and structural integrity despite decreased homeostatic capacity and progressive incidence of muscle-extrinsic and muscle-intrinsic age-related defects

mammalian models of age-related diseases. Importantly, because some myofibers are spared and do not undergo histolysis, studies on muscle pupal development may shed light on the genetic and metabolic properties that can provide protection from different atrophic stimuli. For example, glycolytic muscles preferentially undergo atrophy during cachexia, sarcopenia and starvation in mammals (Li et al. 2007; Yu et al. 2008; Yamada et al. 2012; Wang and Pessin 2013), while oxidative muscles undergo wasting during disuse and immobilization in rodents and humans (Edstrom 1970; Appell 1990; Thomason and Booth 1990). However, the mechanistic basis for this differential sensitivity to atrophic stimuli is largely unknown and studies in *Drosophila* may provide some clues on the fundamental mechanisms involved.

After around 10 days of development, the adult flies eclose. Examination of skeletal muscle in the adult allows for the analysis of the role of organelle turnover and proteinhomeostasis in regulating skeletal muscle homeostasis and function during aging (Fig. 5.1). In fact, although there is currently no evidence for muscle mass loss in old flies, there is an abundance of literature demonstrating age-related muscle intrinsic defects leading to functional declines including decreased flight, climbing, and walking ability (Grotewiel et al. 2005; Dickinson 2006; Martinez et al. 2007; Miller et al. 2008). Here below we describe how *Drosophila* is emerging as a convenient model organism to study sarcopenia and related processes.

5.2 Developmental Skeletal Muscle Growth and Histolysis in Drosophila: A Model for Mammalian Muscle Atrophy and Hypertrophy

There are similarities in the mechanisms regulating skeletal muscle growth and atrophy in *Drosophila* and mammals. In mammals signaling from contraction, nutrients, and hormones through the IGF/Akt/TOR pathway leads to robust hypertrophy (Schiaffino and Mammucari 2011). Importantly, the hypertrophic response to nutrient and contraction stimuli can be attenuated in mice by inhibition of the TOR pathway through muscle specific knockouts or through activation/suppression of TOR regulators such as TSC1/2 and Raptor (Bentzinger et al. 2008; Schiaffino and Mammucari 2011; Bentzinger et al. 2013; Sandri et al. 2013). Similar to mammals, the rapid muscle growth during *Drosophila* larval development is heavily dependent on nutrient sensing through the insulin/Akt/TOR pathway. Signaling through the transcription factors FoxO, Myc, and Mnt are also required for normal muscle growth in *Drosophila*. Overexpression of *FoxO* can inhibit muscle growth at least in part through inhibition of Myc activity, and overexpression of *Mnt*, a Myc antagonist, also inhibits muscle growth (Demontis and Perrimon 2009). The extensive genetic resources available in *Drosophila* allow for rapidly testing the role of any given gene and signaling pathway in the process of muscle growth in the larva, providing comprehensive insight into signaling pathways that may regulate muscle hypertrophy in mammals.

Similar to hypertrophy, many different stimuli including disuse, starvation, denervation, aging, stress signals, and reactive oxygen species can induce atrophy in mammalian skeletal muscle. In *Drosophila*, muscle atrophy followed by cell death is observed during histolysis. Larval muscles undergo two fates during metamorphosis. While a few muscles change morphology and become the adult muscles, most are degraded and replaced with new muscles formed by adult muscle precursor cells (AMPs). One of the signals regulating the rapid degradation of muscle during histolysis is a class of steroid hormones, called ecdysteroids, which induce similar gene expression changes as glucocorticoids when applied to mammalian cells (Christopherson et al. 1992; Jindra 1993). These steroid molecules cause the rapid breakdown of myofibrils leading to severe atrophy. Similar to the mammalian response to glucocorticoids, not all muscles undergo histolysis when exposed to ecdysteroids (Goldberg and Goodman 1969; Hegstrom and Truman 1996). Interestingly, the muscles that do not undergo histolysis are used as scaffolding for the growth and development of the adult skeletal muscles (Chinta et al. 2012). Taken together, although muscle growth and atrophy in *Drosophila* is limited to specific stages of development, the vast similarities in the signaling pathways regulating muscle size in *Drosophila* and mammals makes flies a good model for examining the regulation of muscle hypertrophy and atrophy.

While there are no stem cells in the muscles of adult flies, there are some similarities between mammalian satellite cells and stem cell-like adult muscle precursors (AMPs) in *Drosophila*. The AMPs are responsible for the formation of

adult musculature during pupal metamorphosis (Figeac et al. 2007). AMPs, like quiescent satellite cells, can be regulated by epidermal growth factor (EGF) signaling and undergo fusion to promote muscle growth and new fiber formation. In *Drosophila*, over-expression of *EGFR* leads to increased number of AMPs in embryos (Bidet et al. 2003). Although different in many regards, AMPs can be a good model system for better understanding the cellular processes and signaling pathways regulating muscle stem cell behavior. Unlike mammals, there is no apparent regeneration of *Drosophila* muscles in adulthood. Therefore any muscle damage and intrinsic defects may rapidly lead to decreased muscle function given the lack of compensatory mechanisms.

In summary, different steps of *Drosophila* development allow for the investigation of the cellular and molecular mechanisms responsible for muscle growth and atrophy, regeneration, and muscle stem cell function, processes that are relevant for modulating muscle aging in mammals.

5.3 Age-Related Skeletal Muscle Functional Decay in Drosophila: A Model for Mammalian Sarcopenia

Sarcopenia has recently been defined as "an aging-related condition that normally manifests during or after the 4th decade of life where the overall quality of skeletal muscle decreases, ultimately leading to muscle weakness" (Brotto and Abreu 2012). Much attention has been given to the analysis of sarcopenia in mammals through the use of monkeys and rodents. The relatively long life span and the high costs associated with aging studies with these animal models as well as the inability to conduct large scale genetic screens suggests that simpler organisms may complement research in mammals and provide further insight into the mechanisms regulating sarcopenia.

Drosophila melanogaster undergoes dramatic age-related muscle deterioration, recalling the age-related decline in muscle function observed in humans (Doherty 2003). Defects in climbing, flight, and spontaneous movement are clearly discernable with aging in *Drosophila* over the course of its short life span of around 2–3 months (Grotewiel et al. 2005; Martinez et al. 2007; Miller et al. 2008). In *Drosophila* the decline in muscle function is largely due to a decrease in muscle strength. Decrements in muscle ultrastructure identified in old flies include mitochondrial degeneration, accumulation of misfolded protein and lysosomal dysfunction, and disorganization of the sarcoplasmic reticulum and sarcomeres (Takahashi et al. 1970; Hunt and Demontis 2013) (Fig. 5.2), defects that are also seen in mammalian models of sarcopenia (Tomonaga 1977).

The age-related decline in muscle function in humans correlates with an overall decrease in skeletal muscle mass, and muscle cross sectional area has been shown to decrease by 40 % between the ages of 20 and 60 years (Vandervoort 1994; Porter et al. 1995). Some of the loss in muscle mass could be attributed to disuse atrophy as the elderly display the lowest levels of physical activity of any age group (Nelson

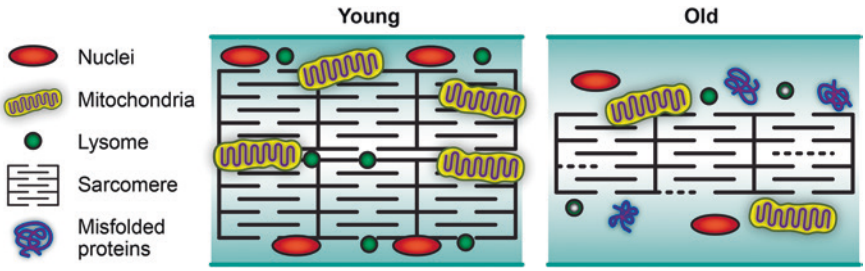

Fig. 5.2 Intracellular changes in *Drosophila* skeletal muscle during aging. During aging, skeletal muscle function declines. This is associated with accumulation of misfolded protein aggregates, dysfunctional organelles (including mitochondria and lysosomes), decreased number of nuclei (which are lost via syncytial apoptosis), decreased organization and contractile properties of sarcomeres, and many other degenerative changes in several cellular functions. Altogether, these changes contribute to the decreased muscle function observed during aging

et al. 2007). However, the decrease in muscle strength is 3-fold greater than the loss in muscle mass indicating that the loss in mass alone is not sufficient to explain the decrements in muscle function (Goodpaster et al. 2006). Contrary to humans, disuse atrophy and age related decreases in muscle mass have not been described in the adult *Drosophila* (Piccirillo et al. 2014), whereby age-related changes in cellular homeostatic systems and organelles appear to be largely responsible for the decline in muscle function observed during aging. Alternatively, the lack of evidence for muscle loss with aging in *Drosophila* could be attributable to the small size of the muscle making it difficult to detect small changes in muscle mass that may occur with aging. Although this may be a potential difference in the occurrence of sarcopenia in *Drosophila* compared to mammals, there are many benefits to using *Drosophila* for investigating the intrinsic changes that are responsible for decreased muscle function during aging. Along with the wide array of genetic tools that allow for muscle specific and genome wide analysis of gene function in *Drosophila*, large cohorts of flies of a given age and/or genotype can be analyzed for environmental, pharmacological, and dietary interventions. Additionally, laboratory mice are typically housed in small cages that limit physical activity, a condition that clearly associates with decreased muscle mass and function, whereas flies have proportionally larger housing, which provides the possibility for being more active, and thus age in a manner closer to fly populations in the wild.

5.4 Protective Role of Balanced Protein Homeostasis

Skeletal muscle mass and function is maintained by constant turnover of proteins and organelles. A shift in the balance between protein synthesis and degradation can lead to severe myopathies and skeletal muscle atrophy. Protein degradation is vital to proper muscle growth and homeostasis, as the inhibition of protein degradation pathways such as autophagy leads to the accumulation of dysfunctional

proteins and organelles, ultimately leading to decreased function, atrophy, and apoptosis of myofibers (Masiero et al. 2009). The importance of muscle protein homeostasis is well established in humans and in rodent models of aging. During aging, multiple stress resistance pathways such as the unfolded protein response are activated to cope with the progressive accumulation of damaged proteins and organelle dysfunction (Haigis and Yankner 2010; Aoi and Sakuma 2011).

In *Drosophila*, increased gene expressions of the ubiquitin proteasome system components and antioxidant response pathways have been observed in aging skeletal muscle (Girardot et al. 2006). These responses include increased levels of heat shock protein 70 (Hsp70) (Wheeler et al. 1995), a cytosolic chaperone that aids in refolding proteins, as well as increased expression of JNK pathway components including *Jra* (the homologue of *Jun*) and factors involved in cytoskeletal rearrangements such as Myo31DF and Rac2 (Girardot et al. 2006). This response is also seen in the muscle of mice during aging (Clavel et al. 2006). While overexpression of *Hsp70* does not prevent age-induced accumulation of poly-ubiquitin aggregates in flies, Hsp70 does associate with these aggregates (Demontis and Perrimon 2010) presumably suppressing the proteotoxicity of the ubiquitinated misfolded proteins. In addition to the increase in cytosolic chaperones, mitochondrial chaperones Hsp22 and Hsp23 are also induced in aged *Drosophila* muscle (Wheeler et al. 1995). In mice, overexpression of *Hsp10*, another mitochondrial heat shock protein, attenuates both the decline in muscle mass and the decrease in muscle strength during aging (Kayani et al. 2010). In addition to heat shock proteins, the unfolded protein response in the endoplasmic reticulum (UPR^{ER}) is increased during aging in mouse skeletal muscle. The UPR^{ER} induces phosphorylation of eIF-2α leading to the suppression of muscle protein synthesis in mammals (Hasten et al. 2000) and *Drosophila* (Webster et al. 1980).

Although protein synthesis is a necessary component of the homeostatic balance that maintains skeletal muscle function during aging, its role is paradoxical. In humans, aged muscle is characterized by anabolic resistance, i.e. decreased protein synthesis in response to anabolic stimuli such as amino acid ingestion (Guillet et al. 2004; Cuthbertson et al. 2005). While dietary supplementation of amino acids and subsequent increases in protein synthesis may offset in the short-term some of the decreases in skeletal muscle mass observed with aging (Fujita and Volpi 2006), caloric restriction (which decreases protein synthesis) has been shown to delay age-associated muscle dysfunction in mammals and flies (Colman et al. 2008; Altun et al. 2010; Katewa et al. 2012). The suppression of protein synthesis during dietary restriction is mediated in part by the inhibition of IGF-1/TOR signaling (Mercken et al. 2013) and is associated with decreased muscle accrual of damaged proteins in mice (Lass et al. 1998) and preservation of muscle mass in rats (Altun et al. 2010). Moreover, activation of the protein synthesis machinery by insulin signaling and TOR activation has been associated with decreased longevity in both mammals and *Drosophila* (Garofalo 2002; Bartke 2008; Harrison et al. 2009), further indicating that a partial decrease in protein synthesis with age may be a protective mechanism elicited in the muscle. Recent studies have indeed suggested that moderate suppression of protein synthesis may in part explain the life span extension induced

by caloric restriction or inhibition of TOR signaling through rapamycin administration in both vertebrates and invertebrates (Masoro 2005; Bjedov et al. 2010). Moreover, a recent paper has shown that overexpression of the transcription factor *Mnt* in skeletal muscle can reduce age-related muscle climbing defects and extend life span by reducing the function of the nucleolus, the site of rRNA transcription and ribosome biogenesis (Demontis et al. 2014) which determines the capacity for protein synthesis in the muscle. In addition to regulating protein synthesis, TOR activity has been linked to the inhibition of the autophagy pathway (Castets et al. 2013). Excessive activation of autophagy in mice has been associated with muscle wasting conditions in response to fasting and other catabolic stimuli (Bonaldo and Sandri 2013). However, activation of the autophagy pathway is necessary to maintain muscle mass and muscle quality (Masiero et al. 2009). Inhibition of autophagy through ablation of muscle Atg7 in mice results in abnormal mitochondria, accumulation of protein aggregates, and misaligned sarcomeres, which lead to decreased muscle function (Masiero et al. 2009). Muscle knockout of Atg7 in mice activates several stress responses including up-regulation of chaperones and ubiquitin proteasome system components such as the muscle E3 ligases atrogin-1 and MuRF1 (Masiero et al. 2009). Similar to the muscle ablation of Atg7, muscle Atg5 knockout mice accumulate protein aggregates leading to decreased muscle mass (Raben et al. 2008). A transcription factor regulating the expression of autophagy genes is FoxO. As demonstrated by Sandri et al. in mice and by Demontis et al. in *Drosophila*, overexpression of *FoxO* can lead to the induction of the autophagy pathway (Sandri et al. 2004; Demontis and Perrimon 2009). In *Drosophila*, muscle overexpression of *FoxO* delays the accumulation of poly-ubiquinated protein aggregates during muscle aging while *FoxO* null flies demonstrate accelerated accumulation of these aggregates. FoxO increases the expression of *Hsp70* as well as several genes regulating the autophagy/lysosomal pathway, such as *Atg* genes and *Lamp1*. Furthermore the knockdown of Atg7 in muscle with *FoxO* overexpression results in an increase in protein aggregate accumulation compared with age-matched *FoxO* overexpressing controls. These data demonstrate that overexpression of wild type *FoxO* in muscle of *Drosophila* prevents the age-related decrease in muscle protein homeostasis and maintains muscle function during aging by preserving the functionality of the autophagy pathway (Demontis and Perrimon 2010).

In addition to its role in autophagy, FoxO is also a mediator of protein degradation through the ubiquitin proteasome system (UPS). Activation of the UPS helps to maintain muscle integrity through selected degradation of poly-ubiquitinated proteins. The UPS requires the attachment of ubiquitin to targeted substrates. Ubiquitinated proteins are then preferentially degraded by the 26S proteolytic complex eliminating damaged and misfolded proteins and allowing for the recycling of amino acids under stress conditions. Overexpression of *FoxO* can increase expression of the E3 ubiquitin ligase *atrogin-1* in mice and in cultures of C2C12 myotubes (Sandri et al. 2004; Zhao et al. 2007). Sandri et al. demonstrated FoxO-mediated regulation of *atrogin-1* expression by examining mouse skeletal muscle transfected with constitutively active FoxO, which lead to a 20-fold increase in atrogin-1 promoter luciferase activity. Additionally, inhibition of *FoxO* through RNAi in mouse skeletal muscle prevented fasting-induced atrogin-1 promoter

luciferase activity. These data led to the identification of FoxO binding sites at the 5′ end of the *atrogin-1* gene that are necessary for its expression by FoxO (Sandri et al. 2004). In addition, constitutively active FoxO induces several autophagy-related genes including *LC3B*, *Gabarapl1* and *Atg12* in C2C12 myotubes through direct binging to their promoters (Zhao et al. 2007). While removal of damaged proteins is vital to the maintenance of muscle function with aging, over activation of protein degradation pathways including the UPS and autophagy is known to be deleterious and can lead to atrophy in mice and C2C12 myotubes.

In addition to its regulation through the ubiquitination of proteins, the proteasome is regulated in part by the composition of the 20S catalytic core as well as the 19S regulatory cap (Ciechanover 1994). The composition of the proteasome is altered during aging in mammals (Husom et al. 2004; Ferrington et al. 2005). Recently, a decrease in 26S proteasome activity and a decrease in proteasome assembly were reported in the course of *Drosophila* aging (Vernace et al. 2007). The decrease in proteasome assembly and activity was associated with an age-related reduction in ATP levels in the fly skeletal muscle, and given the ATP-dependent nature of the proteasome this may well contribute to proteasome dysfunction in aged muscle (Vernace et al. 2007). In rats, increased expression of the alternative version of the proteasome (the immunoproteasome) and increases in proteasome subunit oxidation may explain the overall decrease in proteasomal activity observed during aging in skeletal muscle (Husom et al. 2004; Ferrington et al. 2005). However, while some studies have demonstrated decreased activation of the proteasome in aged muscle (Low 2011), others reported increased function (Carrard et al. 2002; Altun et al. 2010). For example, proteasome peptidase activity was increased in the skeletal muscle of 30-month-old rats, indicating an increase in proteasome capacity, which was reduced by caloric restriction (Altun et al. 2010). These conflicting data on the regulation and role of the proteasome in skeletal muscle aging demonstrate the need for further research in this area. Although there is much debate over the role of the UPS in aging skeletal muscle, there is clear evidence that dysregulation of the systems controlling protein homeostasis can lead to accumulation of dysfunctional proteins and organelles as well as to unselective loss of muscle mass, ultimately leading to decreased muscle function.

In summary, the specificity and activity of protein degradation pathways needs to be tightly controlled for ensuring the maintenance of skeletal muscle mass and function during aging.

5.5 Systemic Aging and Life Span Determination in Response to Exercise and Signals from Skeletal Muscle

The human body is comprised by nearly 50 % skeletal muscle that mediate the body's movements. There is extensive epidemiological evidence indicating that muscle contraction leads to organism-wide responses following different exercise

regimens, at least in part due to the high nutrient/metabolic demand of contracting muscle. A common example of muscle's systemic effects is the observation of cellular and metabolic responses following muscle contraction. For example, exercise can reduce whole body glucose levels as well as increase lipolysis in the adipose tissue. Exercise also protects from neurodegeneration (Ahlskog 2011; Revilla et al. 2014), reduces the risk of developing many types of cancers (Brown et al. 2012), improves cardiac and endothelial function (Shephard and Balady 1999), and potentially increases life span (Piazza et al. 2009).

Similar to mammals, studies in *Drosophila* also demonstrate muscle adaptations to exercise. For example, climbing (negative geotaxis) exercise increases mitochondrial function and preserves motor capacity in flies (Piazza et al. 2009) at least in part via the PGC-1α/β homolog *spargel* (Tinkerhess et al. 2012). While exercise has potent health benefits, muscle disuse and physical inactivity have been linked with increased health risk and mortality. Interestingly, studies in both flies and mammals have shown an increase in physical activity that is associated with caloric restriction (Holloszy and Schechtman 1991; Giustina et al. 1997; Weed et al. 1997; Katewa et al. 2012). Moreover, disuse of skeletal muscle induced through wing ablation can prevent the life span extension caused by caloric restriction in *Drosophila* (Katewa et al. 2012). These data demonstrate that the protective effects of some life span-extending interventions such as caloric restriction appear to be directly tied to skeletal muscle function and physical activity.

The complex interplay between exercise, metabolic homeostasis, and life span clearly requires further studies. A recent avenue of investigation suggests that skeletal muscle is an important endocrine tissue with the capacity to influence whole-organism metabolism via the secretion of muscle-derived cytokines and growth factors known as "myokines" (Pedersen and Febbraio 2008; Pratesi et al. 2013). The release of myokines from muscle may explain how exercise and perturbations in skeletal muscle signaling can lead to alterations in organism-wide physiological homeostasis and aging. Specifically, there is evidence for myokine-based crosstalk of skeletal muscle with several organs and tissues such as the liver, endothelium, pancreas, adipose tissue, and perhaps the brain during both healthy and disease states (Pedersen and Hojman 2012).

A notable recent case is irisin, a myokine that is secreted by skeletal muscle during endurance exercise following the cleavage of its transmembrane precursor (FNDC5, fibronectin type III domain containing 5). Once released, irisin can then induce the browning of adipose tissue making it more metabolically active than the white adipose tissue. The browning of the adipose tissue may indeed result in increased metabolic substrate utilization and may thus mimic (phenocopy) caloric restriction. The health benefits of irisin are still under investigation; however, irisin is closely linked to metabolic homeostasis and is emerging as a possible therapeutic target for the treatment of obesity and diabetes (Elbelt et al. 2013). Treatment of C2C12 myotubes with irisin can lead to an increase in mitochondrial biogenesis and increased expression of Glut4 glucose transporter (Vaughan et al. 2014). Additionally, irisin has been shown to increase IGF-1 and suppress myostatin in primary human myocytes (Huh et al. 2014). A recent study suggested that irisin

may also play a role in the aging process as elevated plasma irisin in humans was associated with increased telomere length, which declines during cellular aging (Rana et al. 2014).

Other myokines such as Myostatin (MSTN) and interleukin-6 (IL-6) can also regulate whole body metabolism. Myostatin is a negative regulator of muscle mass; however, it has recently been shown to act on non-muscle tissues such as the adipose and other tissues, as reviewed by Argiles et al. (2012). For example, exposure of mesenchymal stem cells to MSTN leads to the differentiation of immature adipocytes that protect from obesity and metabolic diseases (Feldman et al. 2006). Interestingly Myoglianin, the *Drosophila* homolog of human Myostatin and GDF11, has been recently shown to extend life span and delay systemic aging by acting on muscle, adipocytes, and possibly other tissues (Demontis et al. 2014). These effects were not due to feeding or changes in muscle mass (Demontis et al. 2014), suggesting that *Drosophila* may be a convenient system for testing the direct signaling roles of GDF11/Myostatin signaling without the indirect confounding effects deriving from the increased muscle mass observed in Myostatin (MSTN) knock-out mice. In fact, MSTN knock-out mice have increased insulin sensitivity and glucose oxidation and decreased whole body adiposity at least in part due to the higher metabolic demand deriving from the doubling in muscle mass, which leads to higher nutrient utilization in muscle and reduced nutrient availability for other tissues (Guo et al. 2009).

Another prominent myokine is Interleukin-6, IL-6, which can induce lipolysis and increase insulin sensitivity when elevated for a short term, such as during exercise. While acute elevations in IL-6 appear beneficial, long-term increases in IL-6 levels are associated with muscle wasting (Haddad et al. 2005). Although paradoxical, IL-6 is a myokine with the potential to affect the aging process through inter-tissue crosstalk. For example, IL-6 increases glucose uptake in the muscle, it also signals for the secretion of insulin from the pancreas (Ellingsgaard et al. 2011).

Although little is known on myokine signaling in *Drosophila*, many putative evolutionarily conserved myokines are encoded by the *Drosophila* genome. Several studies indicate systemic regulation of agingfollowing muscle-specific genetic interventions in *Drosophila* (Demontis et al. 2013b) and signals released by muscle (such as myokines) may play a role. For example, muscle-specific *FoxO* overexpression increases autophagy/lysosomal activities locally in the muscle but also systemically in the brain, retina, and adipose tissue via the organism-wide induction of FoxO/4E-BP signaling (Demontis and Perrimon 2010). Interestingly, muscle specific overexpression of FoxO increases life span and also preserves muscle function, decreases feeding behavior, and lowers glycemia (Demontis and Perrimon 2010). FoxO activity in muscle can improve muscle function and systemic proteostasis at least in part by decreasing the expression of *dawdle*, an activin-related secreted factor which is a direct FoxO target gene (Bai et al. 2013). Thus, FoxO-regulated myokines released by the muscle may be responsible at least in part for the regulation of systemic aging and life span.

A recent study has described a transgenic RNAi screen for myokines regulating life span in *Drosophila* (Demontis et al. 2014). Among the myokines identified,

there was Myoglianin, the *Drosophila* homolog of human Myostatin and the related factor GDF11. Overexpression of *myoglianin* in muscle extended life span and reduced the number of flies that displayed climbing defects in old age (Demontis et al. 2014). Conversely, *myoglianin* RNAi in the muscle lead to accelerated muscle aging and shorter life span. Myoglianin regulates aging by reducing the activity of the nucleolus (which is the key site for ribosome biogenesis and thus protein synthesis) and by activating p38 MAPK (Demontis et al. 2014), a regulator of aging in multiple species and a signal transduction component of non-canonical TGF-beta signaling in vertebrates. Thus, myokine signaling appears to be an important determinant of systemic aging and life span. *Drosophila* may be a valuable system for studying how muscle-specific genetic interventions can regulate life span, the role of myokines in mediating the crosstalk between muscle and other tissues, and the cellular and molecular responses induced in distant tissues.

Other studies have emphasized a protective role of skeletal muscle against whole body oxidative stress. Muscle specific suppression of super-oxide dismutase-2 (Sod-2), p38 MAPK, and AMPK expression can reduce the resistance of the organism to oxidative stress while overexpression of *p38 MAPK* in muscle can increase life span and stress resistance in *Drosophila* (Vrailas-Mortimer et al. 2011). Additionally, p38 has been recently implicated in the regulation of skeletal muscle protein translation through its interaction with the scaffold protein Receptor of activated protein kinase C-1 (Rack1) (Belozerov et al. 2014). Taken together, these data demonstrate that muscle-specific activation of signaling pathways can alter organism-wide aging and stress resistance through modulation of systemic metabolism, myokine signaling, and perhaps also neuronal interactions (Fig. 5.3).

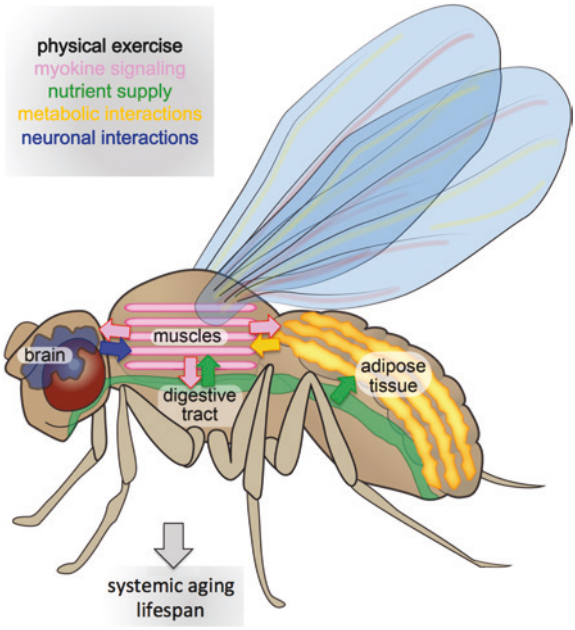

Fig. 5.3 Systemic regulation of aging and lifespan in response to signals from skeletal muscle. Skeletal muscle can interact with a host of tissues and organs to regulate systemic aging and lifespan. Myokine signaling, nutrient demand of contracting muscle, metabolic homeostasis, and crosstalk with neuronal circuits all intersect with skeletal muscle and may impact lifespan and systemic aging in *Drosophila*

5.6 Methods for Studying Skeletal Muscle Homeostasis and Aging in *Drosophila*

Drosophila melanogaster has been a model organism for developmental research for over a century. Recently it has emerged as a promising model for the study of skeletal muscle homeostasis during aging because of its short life span, the evolutionary conservation of signaling pathways, and the wide array of genetic and molecular interventions available. In particular, the ability to quickly generate muscle-specific genetic mutations that encompass all of the skeletal muscle has allowed for the analysis of muscle signaling on organismal wide aging and homeostasis. While techniques to induce genetic mutations in the muscle of rodents are available through electroporation of overexpression and knockdown plasmids, typically only few myofibers are successfully transfected, thus limiting the experimental tissue available for analysis. It is well known that electroporation allows for the monitoring of transgene expression only for a few weeks rather than over many months, as it would be required for the analysis of sarcopenia progression. Alternative methods, such as the Cre-LoxP system, allow for the modulation of transgene expression in the entire tissue and for a longer period of time. However, the breeding and development of tissue specific transgenic mice can be expensive and slow. Additionally, variation in the genetic background in mice can be a confounder in life span and aging studies. For example, Liao et al. demonstrated that caloric restriction only extended life span in 9 mouse strains out of 42, and that caloric restriction even shortened life span in four strains (Liao et al. 2010). These findings demonstrate the inconsistency across different mouse strains and the need for careful control of the genetic background in aging studies. Given the short lifecycle of *Drosophila*, fly strains carrying different transgenes or classical mutations can be easily isogenized by backcrossing them over many generations against the same genetic background to avoid any confounding effects due to background mutations.

In addition to minimization of variation deriving from differences in the genetic background, gene knockdown through RNA interference (RNAi) has allowed for genome-wide screens to be conducted in the adult bypassing developmental effects (Duffy 2002), such as gene mutations causing embryonic and larval lethality. In *Drosophila* RNAi is cell autonomous (Van Roessel et al. 2002). When used in combination with the GAL4/UAS expression system, tissue/cell specific gene function can be studied (Brand and Perrimon 1993). For example several GAL4 drivers are available that are specific to skeletal muscle including Mef2-GAL4 and Mhc-GAL4. Additionally, the availability of drug- and temperature-controlled GAL4 systems allow for gene inactivation and transgene expression during specific stages of the lifecycle and tissue-specifically (Duffy 2002). Importantly, the availability of multiple genome wide transgenic RNAi libraries has made it possible to conduct systematic RNAi screens in specific tissue types during different stages of development (Dietzl et al. 2007). Additionally, clustered regularly interspaced short palindromic repeats (CRISPR) and TALEN technologies are emerging as

complementary approaches for the generation of *Drosophila* lines containing novel mutations in targeted genes (Bassett and Liu 2014).

In addition to a vast array of technologies for modulating gene expression and function during aging, multiple tests can be used in *Drosophila* to examine muscle activity. Spontaneous locomotion, climbing, jumping and flight assays have all been used to analyze the functional decrements of different muscle subsets during aging and thus provide a physiological readout of any given genetic intervention. Climbing assays utilize the flies' negative geotaxis behavior, e.g. the innate instinct of the flies to move away from the earth, while flight and jumping assays probe the function of other muscle groups upon stimulation. Analysis of single muscle fibers from *Drosophila* can also be conducted to determine alterations in muscle tension, power output, and calcium levels in the muscle (Miller et al. 2008). Along with the host of genetic and functional assays available, *Drosophila* is also amenable to many routine molecular and cellular assays used in mammals for the investigation of gene function in muscle and for probing the effect of drugs and dietary regimens and for assessing their interaction with genetic interventions.

5.7 Conclusion

Drosophila melanogaster is a promising model for the study of skeletal muscle homeostasis and aging. During aging, *Drosophila* muscle display profound deterioration and dysfunction, a key characteristic of mammalian sarcopenia. In addition to age-associated muscle dysfunction, systemic aging is evident in *Drosophila* and the examination of muscle-specific genetic alterations has lead to striking findings on the role of muscle in regulating aging in other tissues and life span. Further work is still necessary to determine the signaling factors released by muscle and regulating organismal aging and to dissect the fundamental muscle-intrinsic mechanisms responsible for sarcopenia.

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