Autophagy in health and disease. 3. Involvement of autophagy in muscle atrophy

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Sandri M. Autophagy in health and disease. 3. Involvement of autophagy in muscle atrophy. Am J Physiol Cell Physiol 298: C1291–C1297, 2010. First published January 20, 2010; doi:10.1152/ajpcell.00531.2009.—Loss of muscle mass aggravates a variety of diseases, and understanding the molecular mechanisms that control muscle wasting is critical for developing new therapeutic approaches. Weakness is caused by loss of muscle proteins, and recent studies have underlined a major role for the autophagy-lysosome system in regulating muscle mass. Some key components of the autophagy machinery are transcriptionally upregulated during muscle wasting, and their induction precedes muscle loss. However, it is unclear whether autophagy is detrimental, causing atrophy, or beneficial, promoting survival during catabolic conditions. This review discusses recent findings on signaling pathways regulating autophagy.

skeletal muscle; ubiquitin; muscle wasting; protein breakdown

THE SIZE OF STABLE POSTMITOTIC tissues, such as neurons and skeletal and cardiac muscles, is regulated by protein turnover (44). In eukaryotic cells, most of the proteins are degraded via two proteolytic systems: the ubiquitin-proteasome and the autophagy-lysosome. In cardiac and skeletal muscles the two systems are coordinately regulated to preserve an almost normal composition of proteins and organelles in atrophying cells (29, 30, 56). These two systems are believed to serve distinct functions. Proteasomes degrade myofibrillar and most soluble short-lived proteins (4, 5, 9, 48), whereas autophagy-lysosomes are believed to control long-lived proteins and organelles (25, 34). Both of these proteolytic pathways are evolutionarily conserved and show similarities among yeast, worms, insects, plants, and mammals. In fact, both systems require energy to activate small molecules, i.e., ubiquitin and ubiquitin-like proteins. Different classes of enzymes catalyze the reaction of activation (E1 proteins) and the transfer of the small ubiquitins to the conjugation system (E2 proteins). In the ubiquitin-proteasome system, the final step, regulated by enzymes called E3 or ubiquitin ligases, is the transfer of ubiquitin from the conjugation system to the protein, leading to polyubiquitination and targeting to the proteasome for degradation (23). In the autophagy system, small ubiquitin-like molecules (LC3, GABARAP, GATE16, and Atg12) are transferred from the conjugation system to membranes for their growth and commitment to become a double-membrane vesicle (autophagosome) that engulfs portions of cytoplasm (25, 34) (Fig. 1). This reaction requires the recruitment and assembly of different components of the autophagy machinery on phospholipids, but only the ubiquitin-like components, LC3, GABARAP, and GATE16, are covalently bound to phosphatidylethanolamine (49, 50). This covalent bond occurs on both the outer and inner membranes of the autophagosome. Sequestered organelles and proteins are then docked to the lysosomes for their degradation. The fusion of the outer membrane of the autophagosome with the lysosomal membrane also determines the degradation of the inner membrane and of the proteins that are associated with it. Because of the transient nature of the autophagosomes, the life time of LC3 and its homologs is rather short. Thus, the main difference between the two systems is related to the fate of the ubiquitin and ubiquitin-like proteins. While the ubiquitin-proteasome pathway recycles ubiquitin molecules, the autophagy-lysosome system progressively loses the ubiquitin-like proteins, forcing the cell to replenish them to maintain the autphagic flux.

Skeletal Muscle as Protein Reservoir That Is Mobilized by Proteolytic Systems During Catabolism

During catabolic conditions, muscle proteins are mobilized to sustain gluconeogenesis in liver, to maintain protein synthesis, and to provide alternative energy substrates for the preservation of critical organs such as the heart, brain, lungs, and liver. However, excessive skeletal muscle protein degradation is highly detrimental for the economy of the human body and it can lead to death. For instance, excessive muscle atrophy of intercostals and diaphragm muscles can cause insufficient ventilation and hypoxia. Moreover, muscle loss aggravates catabolic conditions and impairs therapy of burn injuries, cancer, chronic heart failure, acquired immunodeficiency syndrome, sepsis, uremia, and many other pathological conditions, including sarcopenia in aging. Thus, muscle loss ultimately aggravates diseases and increases morbidity and mortality. Most of this increased degradation has been ascribed to an increase in ubiquitination and proteasomal degradation. Since the ubiquitin ligase is the rate-limiting enzyme in the ubiquitination reaction, an increase in its expression is sufficient to enhance proteasome-dependent protein breakdown (45). Indeed, all the conditions of muscle loss that have been studied show an induction of the muscle-specific and atrophy-related ubiquitin-ligases atrogin-1/muscle atrophy F-box (MAFbx) and muscle ring finger-1 (MuRF1) (43). Moreover, such conditions upregulate several subunits of the proteasome that are atrophy-related genes. The autophagy-lysosome system has been largely ignored despite the evidence that lysosomal degradation contributes to protein breakdown in atrophying muscles (10, 46). Indeed, some autophagy-related genes are upregulated in different conditions of muscle loss and belong to the atrophy-related genes or “atrogenes” (24, 43). However, upregulated autophagy genes are neither part of the E3 complex nor part of the proteolytic part of the system, the lysosome, but...
are instead the ubiquitin-like components that are lost during the fusion of the autophagosome with the lysosome. Different studies have shown that cathepsin L, a lysosomal protease, is upregulated in different models of muscle wasting (6, 24). The role of cathepsin L induction is still unclear, but recent evidence suggests that it may have a role in degradation that is independent of lysosomal function (8). Other components of the autophagy machinery are induced during muscle wasting and are involved in regulation of autophagy. Thus, the two major proteolytic pathways of the cell are coordinately regulated at the transcriptional level but the members that are induced play different roles in the two systems.

**Is Autophagy the Devil or the Guardian Angel of the Myofiber?**

The ubiquitin-proteasome system in skeletal muscle seems to control the half-life of sarcomeric proteins, and its inhibition has been reported to have some beneficial effect on muscle mass during catabolic conditions (27). On the other hand, autophagy-lysosome substrates in skeletal muscle are not known and the role of autophagy in skeletal muscle homeostasis has been only recently studied. Activation of autophagy in skeletal muscle following denervation was described several years ago (46). However, only recently several tools have been developed to monitor autophagy, allowing us to unravel its role in skeletal muscle (19). As mentioned above, LC3 is the mammalian homolog of the yeast Atg8 gene and is critical for membrane engulfment of organelles, cytoplasm, glycogen, and protein aggregates. Mizushima et al. (35) generated transgenic mice expressing LC3 fused with green fluorescent protein. This animal model was extremely useful to determine the level of formation of autophagosomes in different tissues under basal conditions and during fasting. Indeed, the morphological analyses of different organs of these mice revealed that skeletal muscle was one of the tissues with the highest rates of vesicle formation during fasting. Another important observation from Mizushima and colleagues was a higher level of autophagosomes in fast glycolytic muscles than in slow oxidative muscle. However, it is worth noting that slow soleus muscle also displayed both a basal and a fasted rate of vesicle formation during fasting. Another important observation from Mizushima and colleagues was a higher level of autophagosomes in fast glycolytic muscles than in slow oxidative muscle. However, it is worth noting that slow soleus muscle also displayed both a basal and a fasted rate of vesicle formation so autophagic flux is also present in β-oxidative fibers. Together, these observations suggest that autophagy can be modulated and may play a role in muscle mass maintenance, but whether this function is beneficial or detrimental remains unclear.

**The devil.** Several recent findings suggest that activation of autophagy can aggravate muscle loss during catabolic conditions. Denervation is able to induce autophagy in skeletal...
muscle, although at a slower rate than fasting. This effect is mediated by Runx1, which is upregulated during denervation and is required to preserve muscle mass. Lack of Runx1 results in myofibrillar disorganization and excessive autophagy in denervated muscles and leads to atrophy (53). Runx1-knockout mice show double- or multimembrane vacuoles, which enclose mitochondria and membranes. This finding indicates that excessive autophagy promotes severe wasting during denervation and needs to be reduced by Runx1. Indeed, we have recently shown that the autophagy-lysosome and ubiquitin-proteasome systems are coordinately regulated during muscle wasting (29, 56). In fact, some critical autophagy-related genes are among the atrogenes and are under the control of Forkhead box, class O (FoxO3). Expression of FoxO3 is sufficient and necessary for activation of lysosomal-dependent protein breakdown in cell culture and in vivo. Indeed, when we reduced the level of the ubiquitin-like LC3 in atrophying muscle by an RNA interference (RNAi) approach, we partially prevented muscle loss. Similarly, specific expression of mutant SOD1G93A in skeletal muscle caused muscle atrophy and weakness mainly via autophagy activation. Moreover, reduction of autophagic flux by knocking down LC3 spared muscle mass (7). The maintenance of high levels of PGC1α expression in skeletal muscles during aging ameliorates sarcopenia, the excessive loss of muscle mass that occurs in elderly people, and reduces the number of autophagosomes (54). Recently, some genetic disorders of muscle have been reported to be related to increased autophagy. Mutations that inactivate Jumpy, a phosphatase that counteracts the action of VPS34 for autophagosome formation and reduces autophagy flux, is associated with centronuclear myopathy (52). Together, these findings strongly suggest that excessive autophagy, similar to the ubiquitin-proteasome system, contributes to muscle loss.

The guardian angel. The fact that autophagy is activated in atrophying muscle implies the potential use of autophagy inhibitors as a therapeutic approach to combat muscle loss. However, this concept must be contrasted with the side effects of lysosome inhibitors, such as chloroquine, particularly on muscle function: lysosome impairment can induce myopathy (e.g., chloroquine myopathy). We have recently unraveled the role of autophagy in skeletal muscle by generating muscle-specific autophagy-knockout mice (31). Deletion of the unique E1 enzyme, Atg7, of the autophagy machinery led to a complete inhibition of vesicle formation in skeletal muscle. The blockade of autophagy was also studied in an inducible muscle-specific knockout animal model used to study the role of acute inhibition of autophagy in adulthood. The effects were similar independent of the time of inhibition of autophagy. Suppression of autophagy was not beneficial and instead triggered atrophy, weakness, and several features of myopathy. Deletion of Atg7 genes caused accumulation of protein aggregates, appearance of abnormal mitochondria, induction of oxidative stress, and activation of unfolded protein response that together led to myofiber degeneration (31) (Fig. 2). The morphological features of myofiber degeneration are very similar to several myopathies that are characterized by protein aggregates and inclusions, such as the sporadic inclusion body myositis (16, 38), or by persistence of dysfunctional mitochondria, as occurs in Ullrich dystrophy (1). Another interesting aspect was the important accumulation of polyubiquitinated proteins in detergent-soluble and -insoluble fractions of autophagy-null muscles. This increase of ubiquitinated proteins was not caused by a failure/decrease of proteasome function, since the in vivo proteasome activity was not impaired. These data were confirmed in other tissue-specific autophagy-knockout mice which showed an increase of polyubiquitinated pro-

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**Fig. 2.** Scheme of the pathway induced by autophagy inhibition. During physiological conditions, autophagosomes remove damaged mitochondria, altered proteins, and unfolded proteins that are prone to aggregate; control the quality of the folding process in the endoplasmic reticulum, and affect DNA stability. When autophagy is deficient, abnormal mitochondria start to accumulate, release proapoptotic factors, and produce reactive oxygen species (ROS) that damage proteins and DNA. Damaged, misfolded, or unfolded proteins can have two fates, being recognized and removed by the proteasome or docked to the autophagosome. When autophagy is blocked, the chains of polyubiquitins interact with the ubiquitin-binding domains of p62 protein that oligomerizes, resulting in ubiquitin-positive protein aggregates. Accumulation of aggregates can be toxic for mitochondria, endoplasmic reticulum, and nuclear functions. Autophagy deficiency affects the quality control of protein synthesis and induces an unfolded protein response. Together, these abnormalities induce different signaling pathways that suppress protein synthesis and activate protein degradation, leading to atrophy and degeneration. Dotted lines depict potential toxic actions of aggregates whose molecular mechanisms and role in adult skeletal muscle have yet to be completely defined. Ub, ubiquitin.
teins (11, 20, 22, 37). We now know that ubiquitinated proteins can be delivered to autophagosomes via p62/SQSTM1 protein that binds the polyubiquitin chains and LC3 (12, 13, 18, 21, 40) (Fig. 2).

Taken together, these findings suggest that failure or alteration of autophagy might be a pathogenetic mechanism of several myopathies and dystrophies and that a subset of ubiquitinated proteins are delivered to the lysosomes for their degradation rather than to the proteasome. The last concept implies that ubiquitin is also lost during the fusion of autophagosomes with lysosomes in atrophying muscles and may explain why there is a transcriptional upregulation of ubiquitin during muscle loss (24).

Conclusion: devil or guardian angel? Understanding whether reduction of autophagy is helpful during muscle loss or instead whether enhancement of autophagosome flux is beneficial for the clearance of dangerous organelles or toxic proteins is important from a therapeutic perspective. Autophagic flux appears to be a double-edged sword that is critical for muscle function: too much induces atrophy (Fig. 3) whereas too little causes weakness and degeneration (Fig. 2). Theoretically, there may be differences in the timing in which these opposing functions are manifest. In fact, the pathological consequences of inhibition of autophagy become symptomatic only after a series of damaged proteins and dysfunctional organelles accumulate within myofibers, thereby impairing basal homeostatic functions. This critical situation might only take place months or years after a reduction in autophagy. Conversely, an acute exacerbation of autophagic flux can trigger muscle loss in a much shorter time, for example in days or weeks. Therefore, the critical question is, what happens to myofibers when autophagy is blocked in catabolic conditions to spare muscle mass? The possibility that aggregate-prone proteins and abnormal organelles accumulate and activate/ generate deleterious signals after a period of blockade of autophagy should be considered as an important focus for research in the coming years. In conclusion, more data are needed and it is premature to reach a conclusion regarding the role of autophagy in muscle disease.

Is Autophagy Regulation Peculiar to Skeletal Muscle?

Mammalian target of rapamycin (mTOR) is a critical kinase downstream of insulin and nutrient-sensitive pathways that is required for the cell growth. Muscle hypertrophy requires the activation of mTOR since treatment with rapamycin, an mTOR inhibitor, completely blocks muscle growth of adult or regenerating myofibers (2, 39). The role of mTOR in protein breakdown and in the regulation of autophagy has been investigated in cell culture systems and in organisms such as Drosophila and Caenorhabditis elegans. Genetic and pharmacological studies have shown that inhibition of mTOR triggers activation of autophagy (32). However, the translation of these findings to skeletal muscle did not result in similar conclusions. Muscle cell culture confirmed that the autophagy-lysosome system is the major proteolytic pathway implicated in nutrient-dependent proteolysis (36). Further experiments lent insight into the signaling pathways involved and identified an mTOR-independent but phosphatidylinositol 3-kinase (PI3K)III-beclin-dependent control of the autophagic system in myotubes (36, 51). We and others have recently confirmed that mTOR signaling is not the major regulator of autophagic flux in adult muscles (29, 30, 56). Detailed biochemical studies have determined that rapamycin-mediated mTOR inhibition only barely (~10%) increases protein breakdown in differentiated myotubes. This response was much smaller than the 50% increase (P < 0.01) in this process induced by Akt inhibition (56). Studies of adult muscles revealed similar findings. Inhibition of mTOR by rapamycin or RNAi was not sufficient to trigger vesicle formation in vivo (29). Moreover, deletion of S6K1, a down-
stream target of mTOR, and of S6K2 did not affect the autophagic flux in cultured myotubes (33). Therefore, the inhibition of the IGFl-Akt pathway during fasting must stimulate autophagy by mTOR-independent mechanism. Indeed, we found that FoxO3 was necessary and sufficient to activate autophagy in myotubes and in adult myofibers. In fact, some critical autophagy-related genes (LC3b, GABARAP1, and Bnip3) are among the atrogenes and are under FoxO3 control (29, 56). Thus we conclude that a reduction in the activity of the PI3K/Akt signaling pathway can activate autophagy by two mechanisms: a rapid transcription-independent mechanism through mTOR that slightly induces autophagosomes, and a slower but more robust mechanism that is independent of mTOR and requires gene expression, via FoxO3 (29, 30, 56, 57). The critical question is whether this transcriptional-dependent mechanism is peculiar to skeletal muscles or not. Recent reports described a FoxO-dependent transcriptional upregulation of several autophagy-related genes in Drosophila larval fat body (15), in mammalian cardiomyocytes (17, 47), in hepatocytes (26), in colorectal cancer cells (3), and during cellular senescence (55). Thus, autophagy can be regulated by a transcription-dependent program in several different cell types. It is still unclear how much general upregulation of autophagy genes is necessary to sustain autophagy for periods longer than a few hours.

**Lyososome Function and Autophagy Activation**

A final important, but unresolved, issue is understanding cross talk between the autophagy pathway and the lysosome system. Autophagosomes occur in many myopathies and are the major features of a group of muscle disorders named autophagic vacuolar myopathies (28). This group of muscle disorders is characterized by an alteration in lyosomal function. Pompe disease is caused by a defect in lysosomal acid α-glucosidase (14). Danon disease is due to a defect in lysosome-associated membrane protein 2 (LAMP-2) (28), and X-linked myopathy with excessive autophagy (XMEA) (42) is triggered by mutations in an essential assembly chaperone of the vacuolar-type H+-ATPase, the principal mammalian proton pump complex (42). The pathogenetic mechanism of these myopathies has been attributed to the functional impairment of lysosomes. This view has been recently challenged by a new hypothesis which proposes that the massive accumulation of autophagosomes is the primary event that causes myofibrillar disorganization and alteration in endocytic trafficking. However, even this view does not explain all the features of these myopathies. In fact, inhibition of the autophagy system in the animal model of Pompe disease does not ameliorate the muscle phenotype (41). Thus, we speculate that the accumulation of autophagosomes, while detrimental to normal sarcomeric structure and altering muscle signaling/trafficking, may also have a beneficial function. In fact, autophagosomes can protect myofibers from the toxic action of the damaged proteins/organelles that are sequestered in the vesicles.

**Conclusions**

There is growing evidence that the autophagic and the ubiquitin-proteasome systems are closely interrelated and that their coordinated activation contributes to muscle loss. These two pathways present novel drug targets for therapeutic approaches to prevent muscle loss. However, long-term autophagy inhibition/failure is detrimental for myofiber survival and its reduction or exhaustion may be a principal factor that leads to late-onset muscle disorders. The key question that requires answers in the coming years is whether one must block the degradation pathways during atrophy or whether activation of the proteolytic systems in normal muscles is necessary to prevent atrophy, weakness, and degeneration.

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**DISCLOSURES**

No conflicts of interest are declared by the author.

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Themes

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