Muscle atrophy occurs as the result of changes in the balance between anabolic and catabolic processes, with the result being a loss of muscle mass when protein breakdown exceeds protein synthesis. At the end of the 20th century, a primary driver of muscle atrophy, under most conditions, was thought to be an increase in protein degradation (108). In 1969, Goldberg (49) demonstrated that an increase in protein degradation contributed to the loss of muscle mass and myofibrillar proteins following denervation and glucocorticoid treatment. Subsequently, it was shown that increases in proteolysis occur in a variety of muscle-wasting conditions, including sepsis, cancer, burns, diabetes, and starvation, and that the primary pathway responsible for the increase in muscle proteolysis under these catabolic conditions was the ATP-dependent, ubiquitin-proteasome pathway (109, 140, 183). What remained a mystery, however, was the molecular mediators of protein degradation and the atrophy process. Moreover, it was unknown whether...
muscle atrophy, caused by different stressors, was regulated by a common signaling pathway.

In 2001, two reports that identified two novel E3 ubiquitin ligases as key regulators of muscle atrophy were published (15, 51). In Bodine et al. (15), a differential display approach was used to identify MuRF1 (Trim63) and MAFbx (FBX032) as genes that are similarly altered under disparate atrophy conditions, including immobilization, denervation, hindlimb unloading, dexamethasone treatment, and interleukin-1-induced cachexia. The approach taken was designed to identify a common trigger for atrophy and measured differentially expressed genes in the rat medial gastrocnemius after 3 days of ankle immobilization and then determined whether a subset of genes was universally modified in other types of muscle atrophy. This approach revealed two genes whose expression was relatively low in resting skeletal muscle but increased significantly within 24 h of inactivity or decreased loading. Sequence analysis of the genes revealed that one gene was a RING finger protein previously identified as MuRF1 in cardiac tissue but not previously associated with skeletal muscle atrophy (24). The other gene was novel and contained an F-box domain, which is previously associated with skeletal muscle atrophy (24). The approach revealed two genes whose expression was relatively low in resting skeletal muscle but increased significantly within 24 h of inactivity or decreased loading. Sequence analysis of the genes revealed that one gene was a RING finger protein previously identified as MuRF1 in cardiac tissue but not previously associated with skeletal muscle atrophy (24). The other gene was novel and contained an F-box domain, which is previously associated with skeletal muscle atrophy (24). The approach revealed two genes whose expression was relatively low in resting skeletal muscle but increased significantly within 24 h of inactivity or decreased loading. Sequence analysis of the genes revealed that one gene was a RING finger protein previously identified as MuRF1 in cardiac tissue but not previously associated with skeletal muscle atrophy (24). The other gene was novel and contained an F-box domain, which is previously associated with skeletal muscle atrophy (24).

The FBX032 gene was discovered simultaneously by another group using a similar approach but different atrophy models and given the name atrogin-1 (51). In pursuit of key factors that regulate muscle proteolysis under catabolic states, Gomes et al. (51) used an Affymetrix microarray to identify MuRF1 and MAFbx as key regulators of muscle atrophy were published (15, 51). In Bodine et al. (15), a differential display approach was used to identify MuRF1 (Trim63) and MAFbx (FBX032) as genes that are similarly altered under disparate atrophy conditions, including immobilization, denervation, hindlimb unloading, dexamethasone treatment, and interleukin-1-induced cachexia. The approach taken was designed to identify a common trigger for atrophy and measured differentially expressed genes in the rat medial gastrocnemius after 3 days of ankle immobilization and then determined whether a subset of genes was universally modified in other types of muscle atrophy. This approach revealed two genes whose expression was relatively low in resting skeletal muscle but increased significantly within 24 h of inactivity or decreased loading. Sequence analysis of the genes revealed that one gene was a RING finger protein previously identified as MuRF1 in cardiac tissue but not previously associated with skeletal muscle atrophy (24). The other gene was novel and contained an F-box domain, which is previously associated with skeletal muscle atrophy (24).

MuRF1 and MAFbx are E3 Ubiquitin Ligases

MuRF1 was first identified as a novel muscle-specific RING finger protein that bound to the kinase domain of the giant sarcomeric protein titin (24). Upon identification of MuRF1, additional yeast two-hybrid screens were performed using heart cDNA libraries and MuRF1 as bait, leading to the identification of two additional RING finger proteins with high homology to MuRF1, with MuRF2 having 62% homology to MuRF1 and MuRF3 having 77% homology to MuRF1 (24). MuRF1, -2, and -3 are encoded by distinct genes and represent a subgroup of the TRIM superfamily of multidomain ubiquitin E3 ligases characterized by the presence of the NH2-terminal tripartite motif: RING domain, zinc-finger B-box domain, and leucine-rich coiled-coil domain (Fig. 1) (24, 104, 165). Sequence comparison of the NH2-terminal region (140 residues) encoding the RING domain, a zinc-finger B-box domain, and a MuRF family-specific conserved box shows 82–85% homology between MuRF1, -2, and -3 (24). Within the muscle fiber, MuRF1, -2, and -3 have been shown to localize at the M-line.

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of the sarcomere. Additionally, MuRF1 and MuRF3 have been found at the Z-lines (24, 104), whereas both MuRF1 and MuRF2 have been detected in the nucleus (86, 104, 135). The first MuRF protein to be characterized was MuRF3, which was discovered to be essential for muscle differentiation during development and microtubule stability (165). Further studies have revealed that all three MuRFs are tightly regulated during development, with MuRF2 acting predominantly at embryonic stages, whereas MuRF1 and MuRF3 expression increases after birth (131). Similar to MuRF3, MuRF2 has been shown to be important for microtubule stability along with other M-line proteins (105, 135). MuRF1 is the only family member shown to be associated with muscle atrophy and to result in the attenuation of muscle loss when deleted (7, 15, 43, 82).

Similar to MuRF1, MAFbx expression is selective to striated muscle. The identification of an F-box domain within its sequence suggested that MAFbx might function in a SCF-ubiquitin ligase complex (15, 51). Further study using yeast-two hybrids and full-length MAFbx as bait revealed that MAFbx can bind both Skp1 and Cullin1 and that this interaction is dependent on the F-box domain (15). The F-box protein provides specificity for substrate binding and through interactions with other subunits delivers those substrates to an E2 enzyme for ubiquitination (21). In mammals, there are three classes of F-box proteins based on the structure of the substrate-binding domain. MAFbx is part of the FBX class because it lacks WD40 repeats and leucine-rich regions, which are common structural motifs found in the other two classes. Instead, MAFbx contains a PDZ motif and a cytochrome c family heme-binding site in the carboxyl terminus (Fig. 1) (51). Additional characterization of MAFbx has revealed that it contains a leucine zipper domain and a leucine-charged residue-rich domain near its amino terminus that increases the number of substrates that MAFbx can potentially recognize (171). Interestingly, MAFbx also contains two nuclear localization signals, suggesting that it may target transcription factors or other nuclear proteins for ubiquitination.
produces K48 chains on the substrate (72). An important unanswered question is the following: What E2s pair with MuRF1 and MAFbx in skeletal muscle under conditions that induce muscle loss? It is quite possible that different E2s are upregulated in response to different stressors, which would affect the E2-E3 pairings and substrate binding and ubiquitin linkages under divergent atrophy-inducing conditions.

**MuRF1 and MAFbx are Atrophy-Associated Genes**

The discovery of MuRF1 and MAFbx yielded two genes having characteristics of key regulators of skeletal muscle atrophy: 1) both genes are selectively expressed in striated muscle, 2) both genes are expressed at relatively low levels in resting skeletal muscle, and 3) the expression of both genes increases rapidly upon the onset of a variety of stressors and prior to the onset of muscle loss. Since their initial discovery, MuRF1 and MAFbx mRNA expression has been reported to be elevated in a wide range of atrophy-inducing conditions, and thus the two genes have become recognized as key markers of muscle atrophy (Table 1). One major gap in our understanding of MuRF1 and MAFbx, however, is the lack of reliable protein expression data. Although we know much about the time course of mRNA expression of both genes under a variety of atrophy conditions, we know almost nothing about the kinetics of protein translation or decay of these ligases. In this regard, a major limitation has been the availability of selective antibodies that do not cross-react with other proteins.

Demonstrations that MuRF1 and MAFbx are expressed in human muscle and are elevated during conditions that elicit muscle atrophy were critical findings that opened up the use of MuRF1 and MAFbx as markers for muscle atrophy. However, the lack of reliable protein expression data has limited our understanding of the molecular mechanisms involved in muscle atrophy.

**Fig. 2. The ubiquitin (Ub)-proteasome Pathway.** The process of ubiquitination is controlled by the Ub-activating enzymes (E1), Ub-conjugating enzymes (E2), and Ub-protein ligases (E3). Both MuRF1 and MAFbx are E3 Ub ligases that control the ubiquitination of specific substrates. Ub can be added to a substrate as a single monomer on one (monoubiquitination) or more lysines (multibuquitination) or as a chain of ubiquitins of variable length on a single lysine (polyubiquitination). The model of ubiquitination can lead to different substrate fates. Polyubiquitination of a substrate with a ubiquitin chain using K11 or K48 linkages generally results in proteasomal degradation, whereas Ub chains using K63 linkages can lead to alterations in signaling or endocytosis. RBX, RING finger protein.
MuRF1. muscle RING finger 1; MAFbx, muscle atrophy F-box; COPD, chronic obstructive pulmonary disease. List of atrophy models in which MuRF1 and/or MAFbx have been shown to be upregulated.

possibility that both genes could be targets for drug development (Table 1). However, instances have been found in humans where expression of MuRF1 and MAFbx was not found to be elevated during muscle atrophy (69, 122, 147). For the most part, these discrepancies have come from human biopsy samples taken during disuse muscle atrophy induced by limb immobilization (69) or bed rest (122, 147), and often the muscle samples were taken after an extended period of time (14–60 days) following the initial unloading. The mRNA expression pattern of MuRF1 and MAFbx in rodent models of unloading and inactivity (i.e., immobilization, hindlimb suspension, and denervation) is typically a rapid rise within the first 48 h following the trigger, followed by sustained elevation for 7–10 days and then a gradual decrease to baseline by ~14 days (15, 58, 95, 193). In humans, no time course studies comparable with the rodent experiments have been done. One study did report an increase in MuRF1, but not MAFbx, after 10 days of immobilization in the vastus lateralis, followed by a decrease in expression of both genes from 10 to 21 days (35), which is similar to what happens in rodents. Other human immobilization studies found no increase in MuRF1 or MAFbx after 48 h (173) but significant increases in both genes at 14 days in the vastus lateralis (69). The inconsistencies between human and rodent immobilization studies may relate to the degree of neural inactivity and joint restriction between the models, which would result in varying degrees of muscle atrophy. For example, Bodine et al. (15) induced immobilization by pinning the ankle joint, which caused complete joint restriction, some neural inactivity, and rapid muscle loss (~10% loss at 3 days and 30% at 7 days). In contrast, muscle loss in human immobilization studies often occurs at a significantly slower rate (5–10% over 7 days), which could explain the slower time course and lower expression levels of MuRF1 and MAFbx in human muscle. In contrast to immobilization, mRNA expression of MuRF1 and MAFbx increases significantly within 48 h following spinal cord injury in humans (172) and is at resting levels or reduced following chronic spinal cord injury (2 mo to 30 yr) (90), which is an expression profile that closely parallels what is observed following denervation (13) and spinal cord injury (146, 193) in rodents.

The animal and human data suggest that under conditions of disuse, MuRF1 and MAFbx RNA expression rapidly increases for a relatively short period of time, and thus the inability to measure elevated levels of MuRF1 and MAFbx after extended periods of unloading should not be interpreted to mean that these genes have not had a significant impact on the atrophy process. In addition to disuse conditions (i.e., unloading and inactivity), MuRF1 and MAFbx expression is elevated by inflammation, metabolic stress, and glucocorticoids (Table 1). To date, MuRF1 and MAFbx mRNA expression have been shown to be elevated at some point during every condition that induces skeletal muscle atrophy. In addition, MuRF1 and MAFbx are transiently expressed at the onset of reloading following disuse atrophy (161) and following eccentric contractions (101, 190), suggesting that they could be involved in the remodeling process. In this regard, caution should be used when referring to the genes as “atrogenes”, a term that is commonly used to describe the set of genes that are upregulated and responsible for the atrophy process.

Table 1. Atrophy models that have shown MuRF1 and MAFbx to be upregulated

<table>
<thead>
<tr>
<th>Model</th>
<th>Gene</th>
<th>Species</th>
<th>Ref. Nos.</th>
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<tbody>
<tr>
<td>Fasting</td>
<td>MAFbx</td>
<td>Mouse</td>
<td>36, 51, 66</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>MuRF1 and MAFbx</td>
<td>Mouse</td>
<td>3, 87, 111</td>
</tr>
<tr>
<td>Denervation</td>
<td>MuRF1 and MAFbx</td>
<td>Mouse</td>
<td>7, 15, 176</td>
</tr>
<tr>
<td>Spinal cord transection</td>
<td>MuRF1 and MAFbx</td>
<td>Mouse</td>
<td>4, 187</td>
</tr>
<tr>
<td>Bed rest</td>
<td>MuRF1 and MAFbx</td>
<td>Mouse</td>
<td>15, 111, 146</td>
</tr>
<tr>
<td>ICU mimic</td>
<td>MuRF1 and MAFbx</td>
<td>Mouse</td>
<td>35</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>MuRF1 and MAFbx</td>
<td>Mouse</td>
<td>69</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>MuRF1 and MAFbx</td>
<td>Mouse</td>
<td>25, 56</td>
</tr>
<tr>
<td>Smoking</td>
<td>MAFbx</td>
<td>Human</td>
<td>122</td>
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<tr>
<td>COPD</td>
<td>MAFbx</td>
<td>Human</td>
<td>197</td>
</tr>
<tr>
<td>Aging</td>
<td>MuRF1 and MAFbx</td>
<td>Human</td>
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</tr>
<tr>
<td>ALS</td>
<td>MAFbx</td>
<td>Human</td>
<td>142</td>
</tr>
<tr>
<td>Alcohol</td>
<td>MuRF1 and MAFbx</td>
<td>Rat</td>
<td>125, 175</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>MuRF1 and MAFbx</td>
<td>Mouse</td>
<td>19</td>
</tr>
<tr>
<td>Space flight</td>
<td>MAFbx</td>
<td>Mouse</td>
<td>2</td>
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<tr>
<td>Thermal injury</td>
<td>MuRF1 and MAFbx</td>
<td>Rat</td>
<td>85, 156</td>
</tr>
<tr>
<td>Inflammatory cytokines</td>
<td>MAFbx</td>
<td>Mouse</td>
<td>1</td>
</tr>
<tr>
<td>Smoking</td>
<td>MAFbx</td>
<td>Human</td>
<td>133</td>
</tr>
<tr>
<td>Myositis</td>
<td>MAFbx</td>
<td>Human</td>
<td>88</td>
</tr>
<tr>
<td>Acute lung injury</td>
<td>MAFbx</td>
<td>Mouse</td>
<td>43</td>
</tr>
<tr>
<td>Statins</td>
<td>MAFbx</td>
<td>Humans</td>
<td>57</td>
</tr>
<tr>
<td>Arthritis</td>
<td>MuRF1 and MAFbx</td>
<td>Rat</td>
<td>55</td>
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<tr>
<td>Pulmonary arterial hypertension</td>
<td>MuRF1 and MAFbx</td>
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<td>14</td>
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<tr>
<td>Hypoxia</td>
<td>MuRF1 and MAFbx</td>
<td>Mouse</td>
<td>38a</td>
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<tr>
<td>Knee arthroplasty</td>
<td>MAFbx</td>
<td>Human</td>
<td>9</td>
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Identification of MuRF1 and MAFbx Targets

Identification of the cellular targets of MuRF1 and MAFbx has been a difficult task and remains an active area of research. The literature suggests that a given E3 can pair with different E2s, depending on the tissue (e.g., skeletal vs. cardiac) and the cellular environment, making it possible that the substrates targeted for ubiquitylation by MuRF1 and MAFbx vary as a function of the atrophy-inducing conditions (e.g., denervation vs. unloading vs. glucocorticoids). The putative substrates for MuRF1 and MAFbx have been identified primarily through binding studies and in vitro ubiquitin ligase assays (71, 83, 94, 171, 184), and many have not been validated in vivo. The targets identified to date have varied depending on whether in vitro or in vivo systems have been used, the tissues examined (skeletal or cardiac), and the methods used to increase the expression of the E3 ligases (29, 31, 68, 145).

In skeletal muscle, potential targets of MuRF1 have been based primarily on yeast two-hybrid screens using MuRF1 as bait or in vitro ubiquitination assays using different E2 partners. Using a yeast two-hybrid screen of a skeletal muscle cDNA library, Witt et al. (184) identified two major classes of proteins as potential MuRF1 targets: proteins involved in ATP generation and myofibrillar proteins. A number of the myofibrillar proteins that were found to interact with MuRF1 (i.e., nebulin, titin, MLC-2, and cTNI) were determined not to be primary MuRF1 targets since they had similar expression and ubiquitination levels in wild-type (WT) and MuRF1-knockout (KO) mice (184). Examination of protein expression in mice selectively overexpressing MuRF1 in skeletal muscle has also suggested that the myofibrillar proteins are not the primary targets of MuRF1 since the muscles of the transgenic mice are not atrophied and the expression levels of myofibrillar proteins are not decreased relative to WT mice (60, 103). Instead, a comparison of the proteasomes and transcriptomes of MuRF1-transgenic and WT mice revealed significant differences in enzymes involved in ATP generation, especially those involved in glycolysis, suggesting that MuRF1 may play a role in metabolic regulation (60).

A primary role for MuRF1 in the degradation of myofibrillar proteins has been suggested by studies using the MuRF1-KO mice and knockdown of MuRF1 in C2C12 myotubes (29). Cohen et al. (31) compared the atrophy response in WT mice and mice expressing the RING deletion mutant MuRF1 and reported that following denervation and fasting the loss of thick filament proteins, such as myosin-binding protein C (MyBP-C) and myosin light chains 1 and 2 (MyLC1 and MyLC2), occurred prior to the loss of myosin heavy chain (MyHC) and was dependent on MuRF1. Furthermore, it was concluded that upon disassembly of the myofibrils, the ultimate ubiquitination and degradation of MyHC, but not actin or other thin filament proteins, was MuRF1 dependent. The selective degradation of thick filament proteins by MuRF1 is based on the observation that the content of MyLC1, MyLC2, MyBP-C, and MyHC was higher in the MuRF1 mutant mice than in WT mice after 14 days of denervation, whereas the content of actin and other thin filament proteins was decreased to a similar extent in both mutant and WT mice. Of note is that this selective sparing was observed only after 14 days of denervation and not at earlier time points. Furthermore, it is curious that under resting and growth conditions the turnover of myofilament proteins appears to be normal in mice with a null deletion of MuRF1, which suggests the involvement of other ligases in the ubiquitination and turnover of both thick and thin filament proteins, at least under resting conditions. With respect to actin degradation, multiple E3 ligases have been implicated in its ubiquitination and targeted destruction (32, 79, 137). At this time, it is clear that myofibrillar proteins can be ubiquitinylated by MuRF1 in vitro; however, under in vivo conditions of atrophy it is not clear whether the myofibrillar proteins are the primary targets of MuRF1-dependent ubiquitination and degradation or are spared as a consequence of changes in other signaling pathways.

The two most widely acknowledged targets of MAFbx in skeletal muscle are MyoD, a myogenic regulatory factor, and eukaryotic translation initiation factor 3 subunit f (eIF3-f) (83, 171). More recently, MyHC and other sarcomeric proteins, such as the intermediate filament proteins vimentin and desmin, have been identified as potential MAFbx substrates (97, 99). All of the putative targets of MAFbx have been identified using C2C12 cells and require further in vivo validation. In developing C2C12 cells, there is strong support for a direct link between MyoD and MAFbx. First, MyoD degradation is mediated by the ubiquitin proteasome system, and MAFbx was found to interact with MyoD and mediate its ubiquitination in vitro through a lysine-dependent pathway (171). Second, MAFbx and MyoD expression was inversely related during the course of C2C12 differentiation, and overexpression of MAFbx suppressed MyoD-induced differentiation and inhibited myotube formation (171). Additional support for the conclusion that high levels of MAFbx are incompatible with proper myoblast fusion and differentiation comes from a study by Nicolas et al. (118), who found elevated levels of MAFbx, satellite cell deregulation, and failure of postnatal growth in transgenic mice expressing a truncated dominant-negative form of PW1. However, other ligases must be able to mediate MyoD degradation since myogenesis and postnatal growth are normal in mice with a null deletion of MAFbx.

The identification of MyoD and eIF3-f as MAFbx targets has led many to suggest that MAFbx controls protein synthesis, whereas MuRF1 controls protein degradation. Although overexpression of eIF3-f can induce hypertrophy and inhibition can cause atrophy in myotubes, there is no direct data to show that eIF3-f expression levels in adult muscle fibers are controlled by MAFbx expression. Furthermore, the extent and importance of MAFbx-mediated MyoD degradation in adult muscle fibers during atrophy is not clear. In fact, mice with genetic ablation of MAFbx do not have larger-than-normal myofibers, nor do they hypertrophy to a greater extent than WT mice in response to functional overload (8). To the contrary, mice with a null deletion of MuRF1 have been shown to have elevated levels of protein synthesis under certain atrophy conditions (7, 76) and to maintain the ability to hypertrophy in response to loading as they age (62). To date, both MuRF1 and MAFbx have been reported to have the ability to bind MyHC and other myofibrillar proteins and to mediate the ubiquitination of these proteins in vitro. It is possible that the two E3 ligases have overlapping sets of substrates; however, it is more likely the case that they have distinct sets of substrates, especially given their different phenotypes when challenged in vivo (Table 2). Clearly, additional studies are required to identify the primary in vivo substrates of these atrophy-associated muscle-specific
Aging (HIF-1α) skeletal muscle in MuRF1- and MAFbx-KO mice at various life stages and in response to various atrophy and hypertrophy challenges. Atrophy models - muscle sparing Denervation (15, 50), unloading (82), glucocorticoids (7), and Adult (2–18 mo) Elevated HIF-1α receptor to increase MuRF1 and MAFbx expression (180). Although the induction of MuRF1 and MAFbx expression in responsive transcription factors such as the FOXO transcription factors is often associated with increased expression of other glucocorticoid-responsive transcription factors is often associated with increased expression of other glucocorticoid-responsive transcription factors which could vary depending on the atrophy conditions.

Transcriptional Regulation of MuRF1 and MAFbx

The finding that MuRF1 and MAFbx are transcriptionally upregulated together under most atrophy-inducing conditions suggests that the two E3 ligases are under the regulation of similar sets of transcription factors. The first transcription factors shown to regulate transcription of both E3 ligases were the class O-type forkhead transcription factors (FOXO), which include FOXO1, FOXO3a, and FOXO4. All of the FOXO transcription factors are expressed in skeletal muscle, and expression of FOXO1 and FOXO3a increases during certain forms of atrophy (7, 27, 47, 87, 146, 148, 154). In 2004, two reports demonstrating the ability of the FOXO transcription factors to activate MuRF1 and/or MAFbx were published (148, 167). In Stitt et al. (167), it was revealed that activated FOXO1 was necessary but not sufficient to increase MuRF1 and MAFbx gene expression in cultured myotubes and that IGF-I was able to block the transcriptional activation of both genes in response to dexamethasone. In contrast, Sandri et al. (148) concentrated their investigation on FOXO3a and demonstrated that constitutively activated FOXO3a was able to activate the MAFbx promoter in vitro and in vivo and induce muscle atrophy. Following these reports, Waddell et al. (177) provided further evidence that the FOXO transcription factors can directly bind to the MuRF1 and MAFbx promoter; however, it was observed that not all FOXO family members equally activate the FOXO binding motif in the promoters. Moreover, it was demonstrated that the MuRF1 promoter has a perfect palindromic glucocorticoid response element (GRE) in the proximal region of the promoter that directly binds the homodimerized glucocorticoid receptor (177). Although expression of both MuRF1 and MAFbx increases in response to the synthetic glucocorticoid dexamethasone, the activation of MAFbx is indirect since the promoter activity is not increased by an activated glucocorticoid receptor alone, and thus increased expression of MAFbx in response to glucocorticoids is likely related to increased expression of other glucocorticoid-responsive transcription factors such as the FOXO transcription factors (7) or KLF-15 (kruppel-like factor-15) (157). Although the induction of MuRF1 and MAFbx expression in response to exogenous glucocorticoids is dependent on the glucocorticoid receptor, other atrophy-inducing stimuli, such as denervation, do not require an activated glucocorticoid receptor to increase MuRF1 and MAFbx expression (180).

The transcription factor KLF-15 is a glucocorticoid-responsive gene that is upregulated in skeletal muscle by dexamethasone. Furthermore, forced overexpression of KLF-15 in muscle is capable of increasing the expression of both MuRF1 and MAFbx (157). Examination of the MuRF1 and MAFbx proximal promoter regions revealed that both genes have multiple KLF-15-binding sites, some of which are in close proximity to the FOXO-binding sites and the GRE (157). Interestingly, KLF-15 also increases the expression of FOXO1 and FOXO3a, and thus KLF-15 has the ability to activate transcription of MuRF1 and MAFbx directly and through the induction of the FOXO transcription factors. Indeed, activation of the MuRF1 and MAFbx promoters in L6 myoblasts and in the tibialis anterior was significantly greater in response to a combination of FOXO1 and KLF-15 compared with the individual effects. This was a second demonstration of cooperative activation of the MuRF1 promoter, since Waddell et al. (177) demonstrated synergy between FOXO1 and the activated glucocorticoid receptor. In this instance, it was shown that the GR-FOXO1 synergy was dependent on the interaction between the GRE and the adjacent FOXO-binding element. Of potential physiological relevance was the finding that this interaction enabled low doses of glucocorticoids to fully activate the MuRF1 promoter in the presence of FOXO1. The MuRF1 and MAFbx promoters have putative binding sites for other transcription factors such as the NF-κB transcription factors CCAAT/enhancer-binding protein-β (C/EBPβ) and Smad3, which can activate either MuRF1 or MAFbx individually or in combination with other factors. Numerous studies have demonstrated a role for the NF-κB transcription factors (p65, c-Rel, RelB, p52, p50) in the induction of muscle atrophy under conditions such as disuse (70, 174), denervation (112), aging (12), and cachexia (20, 144). Muscle atrophy induced by elevated levels of the NF-κB transcription factors is often associated with increased expression of the E3 ligases MuRF1 and MAFbx (20, 186), and in recent reports it was demonstrated that both genes are direct targets of p50 and Bcl-3 and have conserved κB sites either upstream or downstream of the transcriptional start site (65, 186).

Activation of MAFbx, but not MuRF1, also appears to be under the control of the C/EBPβ transcription factor (194). Tumor necrosis factor-α (TNFα) increases MAFbx expression both in vitro and in vivo via a mechanism dependent on FOXO4 expression, but not FOXO1/3a, and activation of p38/MAPK (96, 113). More recently, it was demonstrated that

<table>
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<th>Table 2. Phenotype of MuRF1- and MAFbx-KO mice</th>
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<tr>
<td>MuRF1-KO (Ref. Nos.)</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Postnatal (0–2 mo)</td>
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<tr>
<td>Adult (2–18 mo)</td>
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<tr>
<td>Aging (&gt;18 mo)</td>
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<tr>
<td>Atrophy models - muscle sparing</td>
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<td>Load-induced hypertrophy</td>
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KO, knockout; HIF-1α, hypoxia-inducible factor-1α; GS, glycogen synthase; PFK, phosphofructokinase; PDH, pyruvate dehydrogenase. Characteristics of skeletal muscle in MuRF1- and MAFbx-KO mice at various life stages and in response to various atrophy and hypertrophy challenges.
increased p38 α/β-MAPK activity in myotubes led to phosphorylation and activation of C/EBPβ, which was capable of binding to and activating the MAFbx promoter (195).

Another example of diversity in the activation of MuRF1 and MAFbx is through myostatin, a TGFβ family member, which when elevated in vitro results in an increase in the expression of MAFbx, and to a lesser extent MuRF1 (97, 99). Activation of the TGFβ pathway in vivo through overexpression of constitutively active activin receptor-like kinase 5 was shown to induce muscle atrophy through a mechanism that involved the increased expression of MAFbx but not MuRF1 (149). More recently, it was shown that overexpression of Smad3, a transcription factor activated by myostatin, in myofibers resulted in a 1.8-fold induction of MAFbx promoter activity and no change in MuRF1 activity, suggesting a mechanism by which myostatin could induce MAFbx expression (54). However, in this report it was unclear whether Smad3 activation of MAFbx was direct or indirect through the induction of another factor such as the transcription factor FOXO3a.

Recently, Bollinger et al. (16) examined the ability of Smad3 and FOXO3a to increase both MuRF1 and MAFbx expression (16). It was found that coexpression of Smad3 and FOXO3a (or FOXO1) increased MuRF1 and MAFbx transcriptional activity to a greater extent than FOXO expression alone. This report differed from previous publications in that they found that Smad3 overexpression alone did not increase the expression of either MuRF1 or MAFbx. Examination of the proximal promoter regions of both MuRF1 and MAFbx revealed multiple conserved FoxO-responsive elements adjacent to SMAD-binding elements in the MuRF1 promoter. Mutation of the proximal SMAD-binding site eliminated the synergistic activation of the MuRF1 promoter by FOXO3a and Smad3 (16).

These authors proposed a mechanism by which Smad3 regulates MuRF1 expression by increasing binding of the FOXO transcription factors to the proximal promoter region and also by increasing FOXO3a protein content through an increase in FOXO3a transcription. As noted earlier, cooperative regulation of the MuRF1 promoter by the FOXO transcription factors and other factors such as the glucocorticoid receptor (177) and KLF-15 (157) has been observed.

The literature suggests that transcriptional control of MuRF1 and MAFbx is complex. The discovery that multiple transcription factors can activate MuRF1 and MAFbx may explain the fact that these genes are activated under such a variety of atrophy conditions. Furthermore, the ability of multiple transcription factors to cooperate in the activation of these genes may explain the variable expression patterns observed under different atrophy conditions.

The Role of MuRF1 and MAFbx in the Regulation of Muscle Mass

The generation of mice with a null deletion of either MuRF1 (Trim63) or MAFbx (Fbxo32) has allowed for the examination of the physiological function and importance of these two E3 ligases in the regulation of skeletal muscle mass (15). Table 2 describes the phenotype of each knockout mouse under resting conditions and in response to various challenges. Interestingly, at birth and during the first 2 mo of postnatal development, neither mouse strain exhibits a phenotype that differs from WT mice. With age, the two strains begin to show different phenotypes, particularly with respect to the heart, suggesting that MuRF1 and MAFbx control different sets of substrates (63, 192).

The first atrophy model to be tested using the knockout mice was denervation, where it was demonstrated that deletion of each gene individually resulted in significant sparing of mass in both the tibialis anterior and gastrocnemius muscles; MAFbx-KO showed sparing at both 7 and 14 days, and the MuRF1-KO showed sparing at 14 days (15). In followup studies with an extended period of denervation (28 days), it was shown that the mass of both the gastrocnemius and tibialis anterior muscles continued to be spared in MuRF1- and MAFbx-KO mice (50). Histological analysis, however, revealed a significant difference in the response of muscles from MuRF1- and MAFbx-KO mice to denervation, with muscles in the MAFbx-KO mice showing an increase in the percentage of vacuole-containing fibers and a greater incidence of muscle fiber necrosis (50). The phenotype observed in the MAFbx-KO mice following extended denervation is reminiscent of the phenotype observed following denervation in mice with a muscle-specific deletion of Runx1 (runt-related transcription factor 1) (178) and might suggest that some level of MAFbx expression is required to prevent rapid degeneration of muscle fibers following the loss of innervation. It could be that preventing the ubiquitination of MAFbx targets prevents their degradation, leading to their accumulation, which ultimately initiates fiber necrosis. This theory requires further investigation; however, what is known is that complete suppression of MAFbx is not required to achieve muscle sparing since MAFbx expression levels are not suppressed but maintained at elevated levels in the MuRF1-KO mice following denervation (50).

Besides denervation, the deletion of MuRF1 has been shown to lead to functional sparing of muscle mass following hindlimb unloading (82), treatment with excess synthetic glucocorticoids (7), and acute lung injury (43). In contrast, deletion of MAFbx does not lead to muscle sparing following glucocorticoid treatment or acute lung injury. Furthermore, neither deletion of MuRF1 nor MAFbx results in the sparing of muscle mass in response to nutritional deprivation (7). The role of MuRF1 and MAFbx in age-related muscle loss has been controversial since no change (48), decreased expression (42), and increased expression (30) of both E3 ligases have been reported in aging rodents. We recently examined the expression of both MuRF1 and MAFbx in aging WT (C57BL6 background) and MuRF1-KO mice and found no significant increase in either gene in WT mice ≤24 mo of age; however, we did find a significant increase in MAFbx expression in the MuRF1-KO mice (62). Interestingly, we found that deletion of MuRF1 resulted in the maintenance of muscle mass with age and in a maintained ability to hypertrophy in response to increased loading with age (62). Age-related muscle loss was not examined in the MAFbx-KO mice since these mice develop congestive heart failure and die prematurely between 16 and 18 mo of age (102, 192).

In general, the experiments on the MuRF1- and MAFbx-KO mice suggest that MuRF1 would be a better candidate than MAFbx for drug development targeted to treat muscle atrophy because the deletion of MuRF1 results in muscle sparing under more conditions than the deletion of MAFbx. Moreover, the mass that is spared in response to MuRF1 deletion appears to be functional in that force output is proportional to muscle mass.
mass. The only instance where MuRF1 deletion did not lead to functional sparing was in aged (24 mo old) mice; however, further studies are needed to rule out changes in innervation or synaptic instability in the MuRF1-KO mice with age. In the case of MAFbx, it may be that suppression (as opposed to complete inhibition) of MAFbx, along with complete inhibition of MuRF1, could be a good strategy for many forms of muscle atrophy. A question that remains to be answered and will likely require pharmaceutical agents to block MuRF1 activity is, when and for how long does MuRF1 need to be inhibited? The timing and duration of inhibition likely depends on the type of atrophy. For example, in response to inactivity or unloading, MuRF1 expression increases rapidly and then returns to baseline within 2 wk. Interestingly, the muscle-sparing effect in the MuRF1-null mice does not occur until after the first week of inactivity when MuRF1 expression is on its decline (15). Given this observation, one might propose that MuRF1 inhibition does not need to occur until after the first week of inactivity; however, that may be too late since the critical interactions between MuRF1 and its primary targets may occur early, soon after the onset of the atrophy stimulus, setting off a cascade of events having significant ramifications at later time points. Although targeting MuRF1 early may be necessary for inactivity models of atrophy, it may be that under chronic metabolic or inflammatory diseases, suppression of MuRF1 would be advantageous at any time during the course of the disease. In this regard, a better understanding of the kinetics of MuRF1 and MAFbx protein expression is needed.

The ability of MuRF1 inhibition to produce functional muscle sparing in a variety of atrophy models suggests that the deletion of MuRF1 may target key regulatory proteins involved in the activation of critical signaling cascades that drive muscle atrophy. It is still unclear whether the primary pathways affected by upregulation of MuRF1 expression are those involved in protein degradation, protein synthesis, or energy production. Comparisons of WT and MuRF1-KO mice under a variety of challenges have revealed that different pathways are affected by the deletion of MuRF1, depending on the model under study (Fig. 3); however, the suppression of cellular stress may be a common theme in a number of the models, which requires further investigation.

We recently compared the effect of denervation on the activation of a number of proteolytic pathways in WT and MuRF1-KO mice and found that deletion of MuRF1 did result in suppression of some components of the ubiquitin proteasome pathway, such as Nedd4 (neural precursor cell expressed developmentally downregulated protein 4), USP19, USP14, and UCHL1, but did not suppress the overall capacity for proteolysis in that the activity of calcium-dependent calpain I and II proteases and the lysosomal enzyme cathepsin L was similar between WT and KO mice (46, 50). Moreover, it was found that both 20S and 26S proteasome activities were elevated to a greater extent in KO compared with WT mice following denervation and that MAFbx expression remained elevated at 14 days in the MuRF1-KO mice (50). Interestingly, we found that the fractional synthesis rate was elevated in both WT and KO mice at 14 days of denervation (50); however, what remains to be determined is whether the same set of proteins is being translated in the WT and MuRF1-KO mice following denervation.

Differential regulation of protein synthesis in MuRF1-KO relative to WT mice has been observed in response to glucocorticoid treatment (7) and during load-induced growth in aged mice (62). In response to glucocorticoids, muscle sparing in the MuRF1-KO mice was related primarily to the early maintenance of protein synthesis and the overall suppression of FOXO1 expression (7). More recently, we also observed that the deletion of MuRF1 prevents, or at least delays, the development of anabolic resistance in older mice. Muscle growth in response to functional overload is significantly reduced in old (18 mo) relative to young (6 mo) WT mice (62). In contrast, there is no decrease in load-induced growth in old MuRF1-KO mice. The maintained growth response in MuRF1-KO mice was associated with less endoplasmic reticulum stress and greater activation of the Akt/mTOR signaling pathways, suggesting an increase in protein synthesis (62). One mechanism by which the deletion of MuRF1 results in muscle sparing under a variety of disparate conditions may be related to an increased ability to decrease cellular stress and maintain global protein synthesis. This could be mediated in part through the ubiquitin proteasome system and the removal of misfolded or modified proteins. Overall, we have found no evidence that MuRF1 and MAFbx directly control other proteolytic pathways such as the calcium-dependent calpains, lysosomal cathepsins, caspase-3, or autophagy.

There exists the possibility that some of the targets of MuRF1-dependent ubiquitination are transcription factors. MuRF1 has been reported to be localized at the M-line of the sarcomere (24) but also to translocate to the nucleus of muscle fibers following inactivity and unloading (121). Furthermore, in vitro, MuRF1 has been reported to interact with known transcription factors such as serum response factor (182) and glucocorticoid modulatory element-binding protein-1 (104). The ability of MuRF1 to influence gene expression was recently examined and compared in two models of atrophy, denervation and glucocorticoid treatment (46). Evaluation of
gene expression patterns in WT and MuRF1-KO mice following 3 and 14 days of denervation or dexamethasone treatment revealed that MuRF1 influenced distinct gene networks in these two distinct atrophy models. Overall, denervation resulted in the differential expression of a much larger and different set of genes than dexamethasone treatment, with only a small set of regulated genes being common to both models: MuRF1, MAFbx, FOXO1, E1F4EBP1, ANKR1D1 (mCARP), CDKN1A (p21), and TNFRSF12A (TWEAK) (46). Following denervation, mice lacking MuRF1 showed suppression of genes associated with the neuromuscular junction, which correlated with blunted histone deacetylase 4 (HDAC4) upregulation (46).

Examination of muscle atrophy in MuRF1-KO mice, and to a lesser degree MAFbx-KO mice, has demonstrated the importance of these E3 ubiquitin ligases in mediating muscle atrophy in response to divergent triggers. Sparing of muscle mass has been achieved through the manipulation of other molecules such as the FOXO and NF-κB transcription factors; however, in the majority of these studies suppression of both MuRF1 and MAFbx has been reported to occur and may be playing a role in the muscle-sparing effect. For example, direct inhibition of FOXO transcriptional activity leads to the attenuation of cachexia and disuse-induced muscle atrophy through a mechanism that likely includes the suppression of MuRF1 and MAFbx expression (143). Nearly complete sparing of immobilization-induced atrophy was found with overexpression of heat shock protein 70, which suppressed the transcriptional activities of both FOXO3a and NF-κB and reduced the expression of MuRF1 and MAFbx (155). Muscle sparing and suppression of MuRF1 and MAFbx expression have also been reported following hindlimb unloading in mice lacking the Nfkbi gene (which encodes the NF-κB transcription factor p50) or Bcl-3 (186). A role for MuRF1 in NF-κB-mediated loss of muscle mass was demonstrated in Cai et al. (20), where NF-κB signaling was activated through muscle-specific expression of activated IκB kinase-β (MIKK mice) and shown to induce muscle atrophy (20). Increased expression of MuRF1, but not MAFbx, was observed in the MIKK mice, and subsequent breeding of MIKK × MuRF1/−/− mice resulted in an intermediate phenotype with an ~50% increase in body weight in the MIKK × MuRF1/−/− mice relative to the MIKK mice.

Several pharmaceutical treatments have been shown to result in muscle sparing, with variable effects on MuRF1 and MAFbx expression. Clenbuterol, a β2-adrenergic agonist, has been shown to induce muscle hypertrophy in young and old rodents in an mTOR-dependent manner and to attenuate the loss of muscle mass following denervation and hindlimb unloading through both mTOR-dependent and -independent mechanisms (59, 74, 162). What is interesting is that, under multiple conditions (resting, growth, and atrophy-inducing conditions), clenbuterol has been shown to suppress the expression levels of both MuRF1 and MAFbx (52, 53, 74). Other compounds that mediate their anti-atrophy responses through G protein-coupled receptors are acetylated and unacetylated ghrelin (138). Recently, both acetylated and unacetylated ghrelin have been shown to spare muscle mass under conditions of fasting and denervation through a mechanism that appears to involve the suppression of MAFbx and possibly MuRF1 (138). Furthermore, exogenous ghrelin has been shown to inhibit protein breakdown and significantly suppress the upregulation of both MuRF1 and MAFbx mRNA and inflammatory cytokine expression in lower-limb muscles following a third-degree burn injury to the back affecting 30% of the total body surface area (10). These studies provide additional support for the conclusion that MuRF1 and MAFbx are critical downstream regulators of the atrophy process, but they highlight the fact that other signaling pathways are involved. As noted in the original publication of the MuRF1- and MAFbx-KO mice (15), deletion of each gene individually attenuates muscle atrophy but does not prevent muscle atrophy. Furthermore, the sparing of muscle mass does not always occur immediately in either knockout mouse. Of particular note is the fact that forced overexpression of MuRF1 or MAFbx alone does not induce muscle atrophy. This may suggest that the substrates for MuRF1 and MAFbx are not expressed in resting muscle and/or that MuRF1 and MAFbx require the interaction with other signaling pathways to induce muscle loss.

Signaling pathways, which are independent of MuRF1 and MAFbx expression, are clearly involved in muscle atrophy. Potential pathways that are involved in muscle atrophy and independent of MuRF1/MAFbx expression are those regulated by ATF4 (activating transcription factor 4) and the tumor suppressor p53 (18, 41, 44). Both transcription factors are upregulated under a variety of atrophy conditions, and forced overexpression of each gene induces muscle loss (41, 44). Interestingly, mice with a muscle-specific ATF4 deletion are protected from immobilization-induced atrophy without the suppression of MuRF1 or MAFbx; however, the sparing of muscle mass occurs only during the first 4 days of disuse and is not evident after 7 days (41). Furthermore, muscle-specific ATF-KO mice do not exhibit muscle sparing following denervation (44). Muscle atrophy induced by p53 appears to be independent of both ATF4 and MuRF1/MAFbx, and mice with muscle- specific deletion of p53 are partially protected from immobilization-induced atrophy (44). Of particular interest is the observation that muscle-specific deletion of both ATF4 and p53 results in complete sparing of muscle fiber cross-sectional area following immobilization for at least 3 days (44). These recent data, coupled with the findings from the MuRF1-KO mice, show that multiple signaling pathways are involved in muscle atrophy and suggest that different signaling pathways may be responsible for muscle loss at specific time points throughout the process. If this is the case, then multiple therapeutic agents may be required to target multiple proteolytic pathways to prevent muscle atrophy.

Other E3 Ligases in Muscle

In addition to MuRF1 and MAFbx, a number of other E3 ubiquitin ligases have been identified in skeletal muscle and associated with the regulation of skeletal muscle under atrophy-inducing conditions. A new F-box protein (Fbxo30) belonging to the SCF complex family of E3 ubiquitin ligases was recently identified in skeletal muscle and shown to be upregulated following denervation and to undergo autoubiquitination, similar to other E3 ligases. Fbxo30, named MUSA1 (muscle ubiquitin ligase of SCF complex in atrophy-1), appears to be inhibited by the bone morphogenetic protein (BMP) pathway under normal conditions based on the findings that MUSA1 is more highly expressed in denervated muscles of Smad4−/− mice than WT mice and is elevated in muscles overexpressing.
the BMP inhibitor noggin (150). Knockdown of MUSA1 protein expression in the tibialis anterior using in vivo electroporation of shRNA resulted in sparing of muscle mass following denervation and prevented the excessive denervation-induced atrophy observed in Smad4−/− mice (150). These data suggest that MUSA1 is an important regulator of skeletal muscle mass, especially under extended durations of inactivity. Interestingly, MuRF1 and MAFbx expression were not affected by manipulation of BMP signaling (150).

Nedd4−1 is a HECT domain ubiquitin ligase that shows sustained long-term increased expression in skeletal muscle following denervation (13, 75), hindlimb unloading (75), and severe chronic obstructive pulmonary disease (136). In a recent study, a skeletal muscle-specific Nedd4-KO mouse was generated and shown to have muscle sparing following both 7 and 14 days of denervation, which differs from the MuRF1-KO that shows sparing only after 7 days of denervation (115). Interestingly, Nedd4 expression is selectively suppressed in the MuRF1-KO mice following denervation (50). It is unknown whether MuRF1 and/or MAFbx expression was affected in the Nedd4-KO mice, since their expression was not reported.

Trim32 is another E3 ubiquitin ligase belonging to the tripartite motif family, like MuRF1 (Trim63). Unlike MuRF1, Trim32 is expressed ubiquitously, with expression levels being 100-fold higher in brain than in skeletal muscle (80). Mutations in Trim32 have been linked to a mild form of dystrophy, limb girdle muscular dystrophy type 2H, and sarcotubular myopathy (79, 152). Mice with a null deletion of Trim32 develop a mild myopathy and premature aging, yet they demonstrate a normal rate and amount of muscle loss in response to fasting and hindlimb unloading (80). The observation that deletion of Trim32 does not affect muscle atrophy contrasts with another study reporting that suppression of Trim32 expression, using in vivo electroporation of shRNA, selectively blocked the loss of thin filament proteins and α-actinin under fasting conditions (32). Cohen et al. (32) proposed that fasting leads to the phosphorylation of desmin, which leads to its ubiquitylation by Trim32 and subsequent degradation. The loss of desmin subsequently leads to the degradation of α-actinin and the loosening of the thin filaments, making them susceptible to ubiquitylation by Trim32. This model differs greatly from that proposed by Kudryashova et al. (80), who suggested that Trim32 plays an important role in muscle growth through the regulation of satellite cell proliferation and differentiation since Trim32-KO mice were found to have impaired growth following disuse induced by hindlimb unloading and demonstrate premature aging of skeletal muscle. Trim32 is not upregulated under all forms of atrophy, and its precise role in the regulation of skeletal muscle size, especially muscle loss, remains to be determined.

TNF receptor adaptor protein 6 (TRAF6) is a member of the TRAF family of conserved adaptor proteins that has been shown to be involved in the activation of various signaling cascades, including NF-κB, MAPK, and phosphatidylinositol 3-kinase/Akt (84, 129, 189). TRAF6 is distinct among the TRAF family members in that it has been shown to have E3 ubiquitin ligase activity and to be upregulated in skeletal muscle in response to denervation, starvation, and cancer cachexia (128, 129). Skeletal muscle-specific deletion of TRAF6 in mice results in partial sparing of muscle mass following denervation (129) and starvation (128) and prevention of cancer cachexia induced by Lewis lung carcinoma cells (129). The protective effects of TRAF6 deletion were related to suppression of NF-κB, AMPK, JNK, and p38 MAPK activation as well as a suppression of MuRF1 and MAFbx expression (129). Interestingly, there was little suppression of MuRF1 and MAFbx expression in the TRAF6-KO mice following starvation; however, this is consistent with the finding that muscle loss in response to nutritional deprivation is not spared in either the MuRF1 or MAFbx-KO mice (7, 129). In TRAF6-KO mice, muscle sparing is much greater in the cancer cachexia than denervation, which also correlates to the level of suppression of MuRF1 (129). The data suggest that TRAF6 is upstream of MuRF1 and MAFbx and is an important regulator of muscle mass under conditions involving the activation of proinflammatory pathways (81).

Conclusions

MuRF1 and MAFbx represent just two of many E3 ubiquitin ligases that are expressed in skeletal muscle. However, they are significant because they are expressed primarily in muscle tissues, and their expression level in skeletal muscle is significantly increased in response to multiple stressors that induce skeletal muscle atrophy. More importantly, suppression or complete inhibition of their expression results in muscle sparing in multiple atrophy models. In the case of MAFbx, complete inhibition of its expression during atrophy conditions appears to be deleterious. Because the expression of MuRF1 and MAFbx increases during multiple atrophy-inducing conditions, they are often referred to as “atrogenes.” However, the expression of MuRF1 and MAFbx is also transiently increased in response to mechanical load and thus could be associated with the remodeling process.

The distinct phenotypes of the MuRF1- and MAFbx-KO mice suggest that these two E3 ligases have distinct sets of substrates. However, the mechanisms by which suppression of MuRF1 in particular leads to sparing of muscle atrophy remain unclear. The primary substrates for MuRF1 have been suggested to be proteins associated with the thick myofilament; however, the broad effects of MuRF1 deletion on multiple cellular pathways suggest that the primary in vivo substrates of MuRF1 have yet to be identified. One pathway that appears to be affected by MuRF1 deletion in multiple atrophy models is endoplasmic reticulum stress, but further research is needed to determine the mechanism by which MuRF1 regulates cellular stress within the myofiber. In conclusion, MuRF1 and MAFbx are important muscle-specific E3 ligases; however, much remains to be understood about their function in regulating muscle mass and other cellular functions in skeletal muscle.

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