The ubiquitin proteasome system in atrophying skeletal muscle: roles and regulation

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Bilodeau PA, Coyne ES, Wing SS. The ubiquitin proteasome system in atrophying skeletal muscle: roles and regulation. Am J Physiol Cell Physiol 311: C392–C403, 2016. First published August 10, 2016; doi:10.1152/ajpcell.00125.2016.—Muscle atrophy complicates many diseases as well as aging, and its presence predicts both decreased quality of life and survival. Much work has been conducted to define the molecular mechanisms involved in maintaining protein homeostasis in muscle. To date, the ubiquitin proteasome system (UPS) has been shown to play an important role in mediating muscle wasting. In this review, we have collated the enzymes in the UPS whose roles in muscle wasting have been confirmed through loss-of-function studies. We have integrated information on their mechanisms of action to create a model of how they work together to produce muscle atrophy. These enzymes are involved in promoting myofibrillar disassembly and degradation, activation of autophagy, inhibition of myogenesis as well as in modulating the signaling pathways that control these processes. Many anabolic and catabolic signaling pathways are involved in regulating these UPS genes, but none appear to coordinately regulate a large number of these genes. A number of catabolic signaling pathways appear to instead function by inhibition of the insulin/IGF-I/protein kinase B anabolic pathway. This pathway is a critical determinant of muscle mass, since it can suppress key ubiquitin ligases and autophagy, activate protein synthesis, and promote myogenesis through its downstream mediators such as forkhead box O, mammalian target of rapamycin, and GSK3β, respectively. Although much progress has been made, a more complete inventory of the UPS genes involved in mediating muscle atrophy, their mechanisms of action, and their regulation will be useful for identifying novel therapeutic approaches to this important clinical problem.

hormones; muscle atrophy

SKELETAL MUSCLE SERVES TWO essential functions, a contractile function for locomotion/maintenance of posture and a metabolic function as the protein reservoir of the body. The myofibers that make up the muscle consist primarily of myofibrillar proteins. The ability of the body to maintain posture or to move arises from the precise organization of myofibrillar proteins into a contractile unit called the sarcomere. The sarcomere consists of thick filaments containing primarily myosin that can slide over thin filaments containing primarily actin. Both types of filaments contain additional regulatory proteins and are linked to the α-actinin-containing Z disk, the thin filaments directly so and the thick filaments via titin. Vimentin and desmin are intermediate filaments that serve to anchor sarcomeres properly at the Z disks. These myofibrillar proteins, as well as the sarcoplasmic proteins, also serve as the protein reservoir of the body. Upon fasting, once hepatic glycogen stores are depleted, muscle protein degradation must be activated to provide amino acids to the liver for gluconeogenesis. These amino acids can also serve as building blocks for new protein synthesis in the body or be oxidized for energy production. Muscle protein degradation is also activated in response to illness. Although this response was evolutionarily beneficial as organisms were often unable to find food when ill, it came at the cost of loss of muscle mass, strength, and function. Advances in medicine have resulted in many new therapies that have converted previously acute and often fatal diseases into chronic diseases that are typically complicated by a persistent decrease in muscle mass. The associated weakness impairs not only quality of life but also survival. Atrophy of skeletal muscle has been demonstrated to be an independent risk factor predicting decreased survival in many common diseases such as cancer, chronic heart and lung disease, and aging (reviewed in Ref. 24). Thus, much attention has been devoted to elucidating the molecular mechanisms underlying this process.

The ability of nutrition, activity, and illness to affect muscle mass is mediated by a number of endocrine, paracrine, and autocrine signaling pathways. These pathways influence mus-

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cle size by regulating the rates of myofiber protein synthesis and protein degradation. Ongoing fusion of myoblasts with existing myoblasts may also play a role in maintenance or growth of the fiber. These signaling pathways can also influence the kinetics of maintenance of the muscle stem cell pool and its activation to myoblasts committed to myogenic differentiation. Chief among these signals is the insulin/IGF-I pathway, which promotes muscle growth. It is activated by nutrient-stimulated release of insulin in the circulation as well as by circulating and locally produced IGF-I. Other anabolic pathways include those activated by androgens and by bone morphogenetic proteins. Catabolic signaling pathways are numerous, consistent with the multitude of disorders that can cause muscle wasting. Myostatin, a TGF-β family member, is produced in skeletal muscle and can promote muscle atrophy in a paracrine/autocrine manner. Glucocorticoids are released in response to many illnesses and stresses and are therefore a common catabolic signal. Many inflammatory cytokines also activate signaling pathways in muscle to promote catabolism. Because activation of protein degradation in muscle has been observed in many catabolic conditions, much attention has been focused on delineating the molecular mechanisms by which it occurs. Both autophagic/lysosomal proteolysis and the ubiquitin proteasome system (UPS) are recognized to play important roles in the protein breakdown (reviewed in Refs. 93 and 118). However, therapeutic targeting of the UPS system is more attractive, since inhibition of autophagy leads to dystrophic muscle due to impaired clearance of damaged organelles and aggregated proteins (64). Ubiquitination can target proteins for degradation due to the ability of the 26S proteasome complex to recognize ubiquitin chains attached to proteins. The conjugation of ubiquitin to proteins is a finely regulated process mediated by the sequential action of three enzymes (reviewed in Ref. 46). First, a ubiquitin-activating enzyme (E1) activates the COOH-terminal end of ubiquitin by forming a highly reactive thiol ester between it and a cysteine residue in activatorizing in Ref. 46). First, a ubiquitin-activating enzyme (E1) activates the COOH-terminal end of ubiquitin by forming a highly reactive thiol ester between it and a cysteine residue in the active site of the enzyme. It then transfers the activated ubiquitin onto the active site cysteine residue of a ubiquitin-conjugating enzyme (E2). E2 then interacts with a ubiquitin ligase (E3) that binds the substrate. The E3 promotes transfer of the ubiquitin onto the substrate. Once recognized by the proteasome, the ubiquitin chains are removed by deubiquitinating enzymes to allow recycling of the ubiquitin for reuse in new conjugation reactions.

A hallmark feature of the UPS is its ability to target specific proteins for degradation in a temporally highly regulated manner. This ability arises from the large number of genes involved in regulating the ubiquitination state of proteins, ~35 E2s, ~750 E3s, ~90 deubiquitinating enzymes (37). In the past two decades, a large number of these genes have been implicated in muscle wasting. These genes, part of the “atrogenes” that are regulated in muscle atrophy, exert their effects by modulating or mediating the various processes that determine muscle mass (protein degradation, synthesis, and myogenesis) as well as the upstream regulatory pathways. In this review, we focus on those UPS genes whose roles in muscle wasting have been confirmed by loss-of-function studies (Table 1). We summarize current knowledge about their mechanisms of action to create a model of how these various components of the UPS can work together to mediate muscle atrophy. Current knowledge allows us to group these genes into three functions: 1) disassembly/degradation of myofibrillar proteins, 2) inhibition of myogenesis, and 3) modulation of autophagy. We then conclude the review with a discussion of how signaling pathways work together to coordinate the actions of these genes and how some UPS genes exert their effects on muscle wasting by regulating these signaling pathways.

The UPS Modulates Disassembly/Degradation of Myofibrillar Proteins

Myofibrillar proteins make up the bulk of muscle protein, and so their degradation is essential to induce muscle atrophy. The most abundant proteins are myosin and actin, the main

Table 1. UPS genes in muscle wasting assessed by loss-of-function studies

<table>
<thead>
<tr>
<th>UPS Gene</th>
<th>Experimental Model</th>
<th>Phenotype of Loss of Function</th>
<th>Regulation</th>
</tr>
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<tbody>
<tr>
<td>Atrogin-1/MaBx/Fbxo32</td>
<td>Knockout</td>
<td>Protection from atrophy induced by denervation (6)</td>
<td>Upregulated in many catabolic conditions</td>
</tr>
<tr>
<td>Cbl-b</td>
<td>Knockout</td>
<td>Protection from unloading-induced atrophy (75)</td>
<td>Upregulated upon unloading (75)</td>
</tr>
<tr>
<td>Cul3-KLHL20</td>
<td>Skeletal muscle specific knockout</td>
<td>Increased diabetes-associated muscle atrophy (58)</td>
<td>Unknown</td>
</tr>
<tr>
<td>Fbxo21/SMART</td>
<td>Knockdown</td>
<td>Resistant to denervation-induced atrophy (70)</td>
<td>Upregulated in denervation and fasting (70)</td>
</tr>
<tr>
<td>Fbxo30/MUSA1</td>
<td>Knockdown</td>
<td>Resistant to Smad knockout induced atrophy (91)</td>
<td>Upregulated upon denervation (91) and fasting (70)</td>
</tr>
<tr>
<td>MuRF1/Trim63</td>
<td>Knockout</td>
<td>Protection from atrophy induced by denervation (6) or glucocorticoids (1)</td>
<td>Upregulated in many catabolic conditions</td>
</tr>
<tr>
<td>Nedd4–1</td>
<td>Knockout</td>
<td>Protection from atrophy induced by denervation (74)</td>
<td>Upregulated upon unloading or denervation (48)</td>
</tr>
<tr>
<td>SCF-Fbxo40</td>
<td>Knockdown</td>
<td>Thicker myofibers (95)</td>
<td>Upregulated upon denervation (122)</td>
</tr>
<tr>
<td>Traf6</td>
<td>Muscle-specific inactivation Knockdown</td>
<td>Protection from atrophy induced by cancer cachexia, denervation (83), fasting (82), or glucocorticoids (105)</td>
<td>Upregulated in many catabolic conditions</td>
</tr>
<tr>
<td>Trim32</td>
<td>Knockout shRNA knockdown</td>
<td>Premature senescence and sarcopenia (50)</td>
<td>Unknown</td>
</tr>
<tr>
<td>Trim72</td>
<td>Knockout</td>
<td>Protection from atrophy induced by fasting (15)</td>
<td>Unknown</td>
</tr>
<tr>
<td>USP19</td>
<td>Knockout Knockdown</td>
<td>Enhanced myogenesis (55)</td>
<td>Upregulated in many catabolic conditions</td>
</tr>
</tbody>
</table>
| UPS, ubiquitin proteasome system. See text for definitions.
components of the thick and thin filaments, respectively, of the myofibril. Fittingly, one of the first UPS genes shown by mouse knockout (KO) approaches to be essential for muscle wasting, the E3 ubiquitin ligase muscle ring finger (MuRF) 1/Trim63 has been implicated in the degradation of myofibrils (12, 25). Indeed, it binds to and ubiquitinates myosin heavy chain (13), myosin-binding protein C and myosin light chains 1 and 2 (13), and troponin I (43). The effect of MuRF1 on troponin I was observed in nonmuscle cell lines but could be relevant also in skeletal muscle. In support of a specific role for MuRF1 in targeting thick filaments for degradation, mice expressing a dominant negative mutant form of MuRF1 showed sparing of loss of thick filaments in response to denervation, but thin filaments were still degraded (13), suggesting that MuRF1 is not involved in targeting of thin filaments. In contrast, others have found that MuRF1 can target thin filaments in vitro in cultured cells, ubiquitinating actin in response to exogenous corticosteroids (85). This discrepancy is resolved by observations that purified monomeric actin is indeed ubiquitinated by MuRF1, but when present in myofibrils, the degradation of actin is independent of MuRF1 (13) (Table 2).

It appears that thin filament proteins are targeted for degradation by the Trim32 ubiquitin ligase. Silencing Trim32 expression in skeletal muscle fibers by electroporating short-hairpin RNA (shRNA) protects the myofibers from atrophy and spares thin filaments from degradation (15). Trim32 ubiquitini-
The above discussion has focused on the degradation of myofibrillar proteins. Inhibition of synthesis of these proteins can also contribute to muscle atrophy. To date, roles for UPS genes in modulating synthesis of myofibrillar proteins are less well established. The USP19 deubiquitinating enzyme may mediate muscle wasting partly through inhibition of protein synthesis. Silencing its expression in cultured myotubes promotes the transcription of myofibrillar proteins. This effect is due to increased levels of the myogenic transcription factor myogenin, but how USP19 modulates myogenin remains unknown (106). At a posttranscriptional level, atrogin-1/MafBx/Fbxo32 has been observed to target the initiation factor eIF3-f to proteasomal degradation in cultured muscle cells (54). Because this ligase is induced in atrophying muscles, this could be a mechanism by which activation of muscle protein degradation is linked to a concomitant inhibition of protein synthesis, but this remains to be proven.

The UPS Regulates Myogenesis

Myogenesis is the process whereby Pax7-positive muscle satellite stem cells are activated to become Myf5- and MyoD-positive proliferating myoblasts. These myoblasts subsequently undergo growth arrest and induce myogenin and MRF4 to fuse to form multinucleated myotubes (reviewed in Ref. 3). In postnatal life, there is ongoing fusion of myoblasts to existing myofibers, which is important for muscle growth in early life, but its role in modulation of muscle size in adulthood is more controversial. Under normal conditions, myogenesis is not required for myoblast maintenance, since inducible depletion of muscle satellite stem cells in young adult mice does not affect muscle mass upon subsequent aging (29). A study in which most satellite cells were destroyed by diphtheria toxin indicated that myogenesis is also not required for hypertrophy induced by mechanical loading (65). However, recent work has provided evidence that impaired myoblast fusion occurs in cancer cachexia and plays a significant role in the pathogenesis of the muscle wasting, suggesting that ongoing myoblast fusion is important in myoblast maintenance under catabolic conditions (35) (Table 3).

The atrogin-1 ligase, in addition to its effect on intermediate filaments, ubiquinates and targets MyoD for degradation (53, 60), thereby impairing myoblast fusion and differentiation (78). This ubiquitination appears to be an important mediator of the atrogenic effect of atrogin-1, since mutant mice with a MyoD engineered to be resistant to atrogin-1 ubiquitination are significantly protected against muscle atrophy (53). Atrogin-1 is also capable of ubiquitinating myogenin, thereby inhibiting fusion and expression of myofibrillar proteins (40). These effects are likely limited to myoblasts, since in whole muscle both MyoD and myogenin are induced upon denervation (69, 110). This induced myogenin actually directly activates the promoters of MuRF1 and atrogin-1 and is required for atrophy (73). Trim32 is involved in myogenesis following disuse atrophy. The effect is thought to stem from its ubiquitination of the transcription factor NDRG2 (72). Absence of NDRG2 leads to upregulation of cell cycle inhibitors and markers of differentiation, indicating that it plays a role in exit from cell cycle and myogenesis, respectively. NDRG2 is believed to be phosphorylated by protein kinase B (Akt) (9) and may mediate the myogenic-promoting activity of insulin and IGF-I (27, 117).

USP19 has been shown to inhibit fusion of cultured muscle cells through suppression of a transient induction of the unfolded-protein response that is essential for myoblast fusion (116). Moreover, as mentioned earlier, USP19 downregulates myogenin, which plays an important role in myogenesis (106). Loss of Trim72 function promotes myogenesis (55) through its ability to modulate fusion and myogenin expression. It can target the focal adhesion kinase FAK for degradation (77), and FAK has been observed to promote the expression of the profusion genes caveolin-3 and β1D-integrin (86) as well as myogenin (61).

Finally, Nedd4-1 ubiquitinates Pax7, a transcription factor that can act both as a promoter of myogenesis and as a repressor of myogenesis, via its differential effects on MyoD (10). Therefore, it is believed that the Pax7-to-MyoD ratio plays a key role in regulation of myogenesis. By ubiquitinating Pax7, Nedd4-1 shifts the balance in favor of MyoD and promotes myogenesis. Interestingly, the TRAF6 ubiquitin ligase activates ERK1/2 and JNK1/2 in satellite cells, leading to activation of Jun and induction of Pax7, and the knockout of TRAF6 leads to increased Pax7 levels and impaired muscle regeneration (36). This mechanism, along with the observation that TRAF6 is involved in the p38/mitogen-activated protein kinases (MAPK) and Akt pathways, can provide a mechanistic explanation for the impaired myogenesis seen in mice with silenced TRAF6 (120).

The UPS Interacts with Autophagy

Autophagy is a major mechanism by which cytoplasmic contents and organelles are delivered to lysosomes for degradation. Activation of autophagy results in the sequestration of target material by a double membrane to form an autophagosome that then fuses with a lysosome. It is responsible for removal of protein aggregates, damaged mitochondria, in addition to contributing to protein folding quality control in the endoplasmic reticulum and to clearance of DNA damage (64). Autophagy proceeds in a stepwise fashion that starts with nucleation of the membrane, initiated by the Beclin-1/VPS34 complex, followed by elongation and maturation of the autophagosome, which is dependent upon conjugation of LC3 peptide to phosphatidylethanolamine (reviewed in Ref. 76). Silencing LC3 expression in myofibers by shRNA protects against forkhead box O (FoxO) 3-induced atrophy, supporting a role for autophagy in muscle wasting (62). However, complete/long-term inactivation of autophagy by knockout of the Atg7 gene leads to both atrophy and impaired muscle function, since the process plays a critical role in cell homeostasis through removal of dysfunctional mitochondria and protein aggregates (63). Therefore, both excessive autophagy, through

### Table 3. UPS genes involved in myogenesis

<table>
<thead>
<tr>
<th>UPS Gene</th>
<th>Mechanism of Action</th>
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<tbody>
<tr>
<td>Atrogin-1/MafBx/Fbxo32</td>
<td>Ubiquitinates MyoD, myogenin</td>
</tr>
<tr>
<td>Nedd4-1</td>
<td>Ubiquitinates Pax-7</td>
</tr>
<tr>
<td>Traf6</td>
<td>Indirectly induces Pax-7</td>
</tr>
<tr>
<td>Trim32</td>
<td>Ubiquitinates NDRG2</td>
</tr>
<tr>
<td>Trim72</td>
<td>Ubiquitinates IRS-1, FAK kinase</td>
</tr>
<tr>
<td>Usp19</td>
<td>Suppression of unfolded protein response during myogenesis</td>
</tr>
</tbody>
</table>

See text for definitions.
excessive catabolism, and insufficient autophagy, through accumulation of proteins, generation of oxidative stress, and apoptosis, can lead to muscle atrophy (Table 4).

Several UPS genes modulate autophagy, some positively, others negatively. Among genes that promote this process, the TRAF6 ligase forms K63-linked ubiquitin chains on Beclin-1, a gene essential for activation of autophagy. This ubiquitination does not target the protein for degradation but promotes the oligomerization of Beclin-1. TRAF6 may also interact with p62, a protein that plays a role in clearance of protein aggregates, and its inactivation in muscle leads to suppressed autophagy (83). Moreover, knocking out the deubiquitinating enzyme USP19 results in downregulation of autophagy-promoting genes in muscle, indicating that USP19 may promote autophagy (2). A recent report indicates that USP19 can deubiquitinate and stabilize Beclin-1, thereby promoting autophagy (39). These studies were carried out in nonmuscle cell lines, but, if relevant also in skeletal muscle, could be part of the mechanism by which USP19 promotes autophagy and muscle wasting.

Among negatively regulating genes, the ligase Cul3-KLHL20 ubiquitinates ULK1, the mammalian homolog of Atg1, a protein that is essential for initiation of autophagy, indirectly promotes the degradation of ATG13, another protein present in the same complex (58). It also ubiquitinates VPS34 and Beclin-1, necessary for phagophore formation, and indirectly promotes degradation of ATG14, a regulatory protein in the complex (58). Therefore, KHL20 contributes to autophagy termination. Depleting KLHL20 leads to defective autophagy and muscle atrophy. In nonmuscle cells, NEDD4-1 has been observed to target Beclin-1 for ubiquitination and degradation (84) (Fig. 2).

Integration of Signaling Pathways to Modulate UPS Function in Muscle Wasting

As described above, many genes in the UPS are involved in the regulation of muscle mass, raising the question as to how these genes can be regulated in a coordinated fashion to mediate their roles at temporally appropriate times. We now review briefly current knowledge about the signaling pathways that modulate these UPS genes (Fig. 3).

Insulin/IGF-1. The insulin/IGF-1 signaling pathway plays a central role in regulating muscle mass through its ability to modulate protein synthesis, autophagy, ubiquitin-mediated protein degradation, and myogenesis. Binding of insulin or IGF-I to its receptor activates the receptor’s tyrosine kinase activity, leading to phosphorylation of effector/adaptor proteins such as the insulin receptor substrate 1 (IRS-1). Phosphatidylinositol 3-kinase is recruited to phosphorylated IRS-1 and results in phosphorylation of membrane phospholipid and recruitment and activation of Akt kinase. Pathways downstream of Akt promote muscle growth through regulation of several processes. Akt activates mammalian target of rapamycin (mTOR), which in turn can activate S6K and 4E-binding protein 1 (4EBP1) to allow for initiation of translation and acceleration of protein synthesis. Activation of mTOR also suppresses autophagy by phosphorylating ULK1 and Atg13, thereby inactivating a complex that is important for initiation of autophagy. Importantly, Akt also phosphorylates FoxO transcription factors, resulting in their cytoplasmic sequestration and thereby inhibiting the transcription of their target genes. Prominent examples of these target genes are the ubiquitin ligases MuRF1, atrogin-1, Fbxo30/MUSA1, Fbxo21/

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**Table 4. UPS genes and autophagy**

<table>
<thead>
<tr>
<th>UPS Gene</th>
<th>Mechanism of Action</th>
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<tbody>
<tr>
<td>Cul3-KLHL20</td>
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</tr>
<tr>
<td>Nedd4-1</td>
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<tr>
<td>Traf6</td>
<td>Ubiquitinates Beclin-1, p62</td>
</tr>
<tr>
<td>Usp19</td>
<td>Deubiquitinates and stabilizes Beclin-1</td>
</tr>
</tbody>
</table>

See text for definitions.
SMART, Fbxo31, Ube4b, USP14, and the ubiquitin gene UBC, some proteasome subunits, as well as many autophagy genes (70). This pathway can also modulate myogenesis in a GSK3b-dependent mechanism (109) and regulate the expression of myogenin (26, 121). Interestingly, although many UPS genes are involved in muscle atrophy, to date the IGF-I has only been shown to clearly modulate the MuRF1 and atrogin1 ligases through the regulation of the FoxO transcription factors that induce these ligases (101). However, the recent identification of additional UPS genes downregulated in muscle of mice lacking FoxO1/3/4 (Fbxo30/MUSA1, Fbxo21/SMART, Fbxo31, Ube4b, USP14, UBC, some proteasome subunits) revealed that many genes involved in proteolysis are regulated by these transcription factors that induce these ligases (101). However, the recent identification of additional UPS genes downregulated in muscle of mice lacking FoxO1/3/4 (Fbxo30/MUSA1, Fbxo21/SMART, Fbxo31, Ube4b, USP14, UBC, some proteasome subunits) revealed that many genes involved in proteolysis are regulated by these transcription factors and therefore potentially regulated by insulin/IGF-I (70). The ones that, when inactivated, show some protection against myofiber atrophy are presently MuRF1, atrogin1, Fbxo21/SMART, and Fbxo30/MUSA1.

Steroid hormones. Glucocorticoid levels are increased under conditions of physiological stress as well as in many illnesses and are therefore important inducers of muscle wasting (reviewed in Ref. 92). Not surprisingly, glucocorticoids induce the expression of the MuRF1 and atrogin-1 in muscle (12) but do so through multiple mechanisms. Some of the effects may be direct, since the MuRF1 promoter contains a functional glucocorticoid response element that can be synergistically activated with FoxO1 (111), but a large part of the effect appears to be due to the induction of the transcription factor KLF15, which targets the promoters of not only MuRF1 and atrogin1 but also that of FoxO1 and branched-chain amino transferase 2 (BCAT2) (96). The latter metabolizes and thereby lowers the levels of branched-chain amino acids, which are potent activators of mTOR activity. Glucocorticoids also induce Ddit4/REDD1 (96), an inhibitor of mTOR, and so this effect, along with that of BCAT2, allows glucocorticoid signaling to be an important modulator of mTOR-mediated protein synthesis and autophagy. Furthermore, glucocorticoids induce expression of the miR1 micro-RNA, which targets 70-kDa heat shock protein (Hsp70) and thereby decreases Akt activity (52), which could also lead to decreased mTOR activity and increased FoxO activation. Glucocorticoids also induce the expression of the ligase TRAF6 and can thereby potentiate the effects of NF-κB signaling on muscle wasting (105). Thus glucocorticoids do modulate a number of UPS genes involved in atrophy.

Sex steroid hormones can also have important effects on muscle mass. Testosterone acts as an anabolic signal in muscle...
and appears to exert its effects by increasing levels of IGF-1 mRNA (42, 56, 119), Akt, Akt phosphorylation, and protein synthesis in muscle (114). Testosterone may also negatively regulate myostatin, since elevated myostatin levels are observed after castration in androgen-responsive muscle (68). The satellite cell appears to be an important target of testosterone action, since it is the predominant site of androgen receptor expression (98). Testosterone can stimulate satellite cell proliferation (41), but its effects on differentiation are still unclear (17, 20, 113). The roles of estrogen in skeletal muscle are more pleiotropic and complex. The loss of estrogen seen in ovariec-tomized mice leads to progressive muscle atrophy (45). Estrogens may help stabilize membranes (115), act as antioxidants (102, 103), and thereby protect from muscle damage and inflammation (47, 108). However, others have shown estrogens to be negative regulators of muscle mass. Estradiol, acting through ERα, induces the USP19 deubiquitinating enzyme, resulting in repression of myogenic differentiation (81) and decreased muscle mass (80). Estrogen, like testosterone, can exert effects by activating and increasing proliferation of muscle satellite cells (22, 88, 89).

TGF-β. Myostatin is a TGF-β family ligand that is a potent inducer of muscle atrophy (67, 126). It is expressed and secreted predominantly from skeletal muscle. It acts via activin type II receptors (ActRIIA and ActRIIB) and activin type I receptors (ALK4 and ALK5) to phosphorylate the transcription factors Smad2 and Smad3 (87). This allows for a transcriptional complex to be formed with Smad4 and the subsequent activation of target gene expression. However, to date, none of the ubiquitin genes that are clearly involved in muscle wasting have been shown to be direct targets of the SMAD complex. Myostatin does upregulate the expression of MuRF1 and atrogin-1, but does so in a FoxO-dependent manner (66), and this effect is probably due to the ability of myostatin signaling to inhibit Akt through mechanisms that are still unclear.

Intriguingly, another class of TGF-β family members, bone morphogenic proteins (BMPs), has been shown to be hyper-trophic for muscle. BMPs signal through specific receptors to phosphorylate BMP-specific Smad1/5/8. Similarly to Smad2/3, Smad1/5/8 form a transcriptional complex with Smad4. Inhibition of BMP signaling causes muscle atrophy in mice (91). Interestingly, a novel E3 ubiquitin ligase, Fbxo30 or MUSA1, is negatively regulated by BMP signaling, and its overexpression in myofibers promotes atrophy (91). The substrates of Fbxo30/MUSA1 are unknown (91).

Cytokines. Inflammation is a common trigger for muscle wasting, and many proinflammatory cytokines are potent inducers of muscle atrophy. Many cytokines have been implicated in muscle wasting, including TNF-α, TNF-like weak inducer of apoptosis (TWEAK), IL-6, IL-1β, and IFNγ. The cytokine TNF-α, by binding to its cognate receptor, activates the classical NF-κB pathway. NF-κB signaling has been implicated in muscle wasting, since the muscle-specific overexpression of IKKβ (leading to the activation of NF-κB) causes severe atrophy by upregulating MuRF1 but not atrogin-1 (11). TWEAK is another cytokine that promotes muscle atrophy by acting through the Fn14 receptor, which is upregulated in atrophying muscle (19). Additionally, TWEAK can stimulate myoblast proliferation and inhibit differentiation (23). This signaling can also lead to NF-κB activation. TNF-α and TWEAK induce MuRF1 expression through a TRAF6, NF-κB-dependent pathway. TRAF6 promotes signaling through its K63 autoubiquitination and ubiquitination of NEMO, which serve to recruit the downstream kinase TAK1. Muscle-specific inactivation of this ligase protects from muscle atrophy (82, 83). This ligase can also regulate other signaling pathways, including JNK, p38MAPK, and Akt, allowing cytokine signaling to cross talk with other pathways to promote atrophy (82, 83). BMPs signal through specific receptors to phosphorylate FoxO3 on non-Akt sites, thereby increasing its transcriptional activity (31). Downstream targets of BMP-activated FoxO3 include MuRF1 and autophagy-related genes that contribute to the pathophysiology of muscle wasting (38, 90).

Elevated IL-6 levels are associated with diseases such as cancer cachexia and can induce muscle atrophy by acting through the Jak/Stat3 pathway. Stat3 is an important promoter of muscle atrophy, since it is sufficient to induce the expression of the ubiquitin ligase atrogin-1 (7). However, there are conflicting reports in vivo about whether atrogin-1 is induced by the treatment of muscle with IL-6 (5, 34). Pharmaceutical inhibition of Stat3 downstream of IL-6 can reduce muscle atrophy (7). In addition, Stat3 also indirectly induces myostatin, MuRF-1, and atrogin-1 expression by upregulating the transcription factor C/EBPβ (125).

Other pathways that regulate the catabolic program include signaling through MAPK. MAPKs are activated by a wide variety of stimuli, but it has been established that ERK1/2 are generally activated by mitogenic growth stimuli, whereas p38 and JNK are activated by stress stimuli. In catabolic states, stress stimuli such as cytokines are high while growth stimuli are typically low, thereby permitting a preferential activation of p38 and JNK pathways. Stress signaling through p38 has been shown to upregulate the levels of the ubiquitin ligase atrogin-1 (57) and the deubiquitinating enzyme USP19 (59). Interestingly, altered p38 signaling disrupts satellite cell self-renewal (4).

Angiotensin II. Angiotensin II (ANG II) signaling can also promote skeletal muscle atrophy and is important in conditions such as cardiac cachexia and chronic kidney disease where its circulating levels are elevated. ANG II signals through AT1 and AT2 receptors. ANG II can activate the UPS and muscle proteolysis by increasing the expression of atrogin-1 and MuRF1 (100, 124). Recent evidence indicates that this upregulation is dependent on activation of the transcription factor TFB1 (21). Additionally, infusion of ANG II can negatively regulate insulin/IGF-I signaling by decreasing the levels of circulating and muscle IGF-I (8) and increasing serine phosphorylation of IRS-1 (28, 100), which decreases activation of Akt.

Denervation and unloading. Loss of neural activity (as occurs upon denervation) or simple inactivity (as occurs upon hind limb unloading) are also frequent contributing factors in human muscle wasting. Many of the classic anabolic and catabolic pathways are affected in response to denervation and inactivity. Myostatin expression is increased (94) and activation of Akt is decreased (104) in inactive skeletal muscle. Recently, some novel mechanisms have been implicated in denervation and unloading-induced
atrophy. Denervation was observed to increase phosphorylation of AMPKα2 and decrease phosphorylation of FoxO3, whereas ablation of AMPKα2 protects against wasting and reversed the effect on FoxO3 (32). Another study found that Smad2/3 protein levels but not mRNA levels are increased in denervation independent of myostatin levels and that muscle-specific ablation of Smad2/3 is protective against atrophy (107). During disuse, mitochondrial reactive oxygen species (ROS) production leads to mitochondrial dysfunction and muscle atrophy (71). ROS can regulate NF-κB and FoxO signaling, thereby leading to the activation of atrogenes (18). Decreased sympathetic stimulation of muscle may also play a role in inactivity-related muscle wasting. Such sympathetic activity appears important for maintenance of the neuromuscular junction (44). In addition, β-adrenergic receptor signaling through cAMP and activation of PKA can inhibit expression of MuRF1 and atrogin-1 in muscle via mechanisms that are yet to be delineated (97).

**The UPS Regulates Signaling Pathways**

As described above, many signaling pathways, both anabolic and catabolic, regulate the expression of important UPS genes required for muscle wasting. Interestingly, signaling through these same pathways is also regulated by components of the UPS (Fig. 4 and Table 5).

### Table 5. UPS genes and signaling pathways

<table>
<thead>
<tr>
<th>UPS Gene</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cbl-b</td>
<td>Ubiquinates IRS-1 for degradation</td>
</tr>
<tr>
<td>Nedd4-1</td>
<td>Ubiquinates PTEN</td>
</tr>
<tr>
<td>SCF-Fbxo40</td>
<td>Ubiquinates IRS-1</td>
</tr>
<tr>
<td>Traf6</td>
<td>K63-linked polyubiquitination of TAK1</td>
</tr>
<tr>
<td>Trim32</td>
<td>Decreases Akt activity by dissociating phosphatidylinositol 3-kinase from plakoglobin</td>
</tr>
<tr>
<td>Trim72</td>
<td>Ubiquinates IRS-1, insulin receptor</td>
</tr>
</tbody>
</table>

See text for definitions.
Several UPS genes modulate the insulin/IGF-I pathway. Cbl-b is a RING-type E3 ligase that ubiquitinates IRS-1, targeting it for degradation. (75). Transducing into muscle the phosphopentapeptide DGpYMP, which interferes with Cbl-IRS1 interaction, protects from glucocorticoid-induced myofiber atrophy (79). Another ubiquitin ligase targeting IRS-1 is SCF-Fbxo40, which, upon IGF-I stimulation, promotes the degradation of IRS-1 (95). It is also upregulated following denervation-induced atrophy (122), and its knockdown in mice using small-interfering RNA (siRNA) leads to thicker myotubes (95). Recently, Trim72 has been shown to ubiquitinate IRS-1 and the insulin receptor (99, 123). Knockout of Trim72 in mice leads to increased Akt activation and increased myogenesis (55, 123). Another ligase, Trim32, negatively regulates Akt signaling by causing the dissociation between plakoglobin and phosphatidylinositol 3-kinase (14). Finally, Nedd4-1 has also been observed, in nonmuscle cell lines, to ubiquitinate PTEN (112), a phosphatase that inhibits activation of Akt by hydrolyzing phosphatidylinositol 3,4,5-trisphosphate, providing still another potential mechanism for Nedd4-1’s atrophic effects.

**Concluding Perspectives**

Although the current literature offers the above important insights into how UPS genes mediate muscle wasting, the picture is still incomplete. There are many UPS components that are implicated in muscle wasting (e.g., by their expression) but have not yet been shown to be critically involved through loss-of-function studies. The tools for such discovery are largely at hand, so we can expect more refined models of the mechanisms of muscle wasting in the near future. Some genes such as the ligases Fbxo21/SMART (70) and Fbxo30/MUSA (91) are clearly involved based on loss-of-function studies, but their mechanisms of action remain to be reported. Although some signaling pathways, such as that activated by glucocorticoids, can regulate several UPS genes, there is no apparent mechanism that coordinates all or most of the currently known essential genes involved in this process. In most conditions of muscle wasting, there is activation of several catabolic pathways, and this can lead to regulation of several of the UPS genes. Finally, many catabolic pathways can cross talk with and inhibit Akt signaling and in this way modulate protein synthesis, ubiquitination, autophagy, and myogenesis in a coordinate fashion and thereby overall protein content.

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No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

P.A.B., E.S.C., and S.S.W. conception and design of research; P.A.B., E.S.C., and S.S.W. analyzed data; P.A.B. and E.S.C. prepared figures; P.A.B., E.S.C., and S.S.W. approved final version of manuscript.

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