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Review

Ubiquitin–protein ligases in muscle wasting

Pei Rang Cao, Hannah J. Kim, Stewart H. Lecker*

Renal Unit, DA517, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA

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Abstract

Muscle wasting occurs when rates of protein degradation outstrip rates of protein synthesis. Accelerated rates of protein degradation develop in atrophying muscle largely through activation of the ubiquitin-proteasome pathway. The complexity of the ubiquitination process, however, has hampered our understanding of how this pathway is activated in atrophying muscles and which enzymes of the ubiquitin conjugation system are responsible. Recent studies demonstrate that two ubiquitin–protein ligases (E3s), atrogin-1/MAFbx and MuRF1 are critical in the development of muscle atrophy. Other experiments implicate $E2_{14k}$ and E3α, of the N-end rule pathway, as important players in the process. It seems likely that multiple pathways of ubiquitin conjugation are activated in parallel in atrophying muscle, perhaps to target for degradation specific classes of muscle proteins. The emerging challenge will be to define the protein targets for, as well as to develop inhibitors of, these E3s.

Keywords: Atrogin-1; MAFbx; MuRF1; UBR1; Ubiquitin; E3

Contents

1. Introduction

Muscle wasting and fiber atrophy are seen frequently as the result of poor nutritional states and diverse human diseases, including cancer, uncontrolled diabetes, uremia, and sepsis. Beyond its contractile function, muscle serves as a major protein reservoir that can be mobilized in stressful states as a source of amino acids for energy. The reduction in function and

* Corresponding author. Tel.: +1 617 667 2147; fax: +1 617 667 5276.
E-mail address: slecker@bidmc.harvard.edu (S.H. Lecker).

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strength of muscle that results from atrophy is a major factor contributing to the morbidity characteristic of these disease states. Recent work has demonstrated that the rapid atrophy in experimental models of these various diseases is due primarily to enhanced degradation of muscle protein (Lecker, Solomon, Mitch, & Goldberg, 1999; Lecker, Solomon, Price et al., 1999). In all types of atrophying muscle, a common set of transcriptional adaptations and biochemical changes enhance the cell's capacity for protein breakdown (Hasselgren & Fischer, 2001; Lecker, Solomon, Mitch et al., 1999; Lecker, Solomon, Price et al., 1999; Lecker et al., 2004; Mitch & Goldberg, 1996). Clearly, a better understanding of the cellular pathways of protein degradation that are activated during muscle atrophy is not only of scientific interest, but may also help direct us towards rational preventative measures and treatments. Towards this goal, a number of major advances have recently been made suggesting that multiple protein degradation enzymes are activated during muscle wasting.

In muscle as in all other tissues, the vast majority of intracellular proteolysis occurs through the ubiquitin-proteasome system (Rock et al., 1994). Moreover, it is now well-established that this pathway is of primary importance in the enhanced protein degradation that occurs in muscle as it atrophies (Lecker, Solomon, Mitch et al., 1999; Lecker, Solomon, Price et al., 1999). This system is characterized by the concerted action of enzymes that link chains of the polypeptide cofactor ubiquitin onto proteins to mark them for degradation (Glickman & Ciechanover, 2002) (Fig. 1). This tagging process leads to their recognition by the 26S proteasome, a large multisubunit, multicatalytic protease complex that degrades ubiquitinated proteins to small peptides (Baumeister, Walz, Zuhl, & Seemuller, 1998). Three enzymatic components are required to link chains of ubiquitin onto proteins destined for degradation. E1 (Ub-activating enzyme) and E2s (Ub-carrier or conjugating proteins) prepare ubiquitin for conjugation but the key enzyme in the process is the E3 (Ub–protein ligase), since it couples the activated ubiquitin to the protein substrate (Fig. 1). The E3, then, is the component of the conjugation apparatus that confers specificity to the system. While only a single E1 protein and a few dozen E2 proteins exist in cells, there are more than a thousand E3s. Generally, E3s fall into two broad categories: they either contain HECT (*h*omologous to *E*6-AP *c*arboxy-*t*ermimus) domains or RING fingers (Jackson et al., 2000). Like E2s, HECT-domain E3s directly bind activated ubiquitin and thus are actual components of the enzymatic conjugation cascade (Scheffner, Nuber, & Huibregtse, 1995). RING-containing E3s catalyze ubiquitin conjugation by a less direct mechanism, probably by creating an appropriate microenvironment for transfer of activated ubiquitin (on the E2) to a lysine residue on the protein target (Lorick et al., 1999; Seol et al., 1999). The HECT-domain family is the smaller of the two groups, first identified in E6-AP (Huibregtse, Scheffner, Beaudenon, & Howley, 1995) that targets the viral E6 protein for degradation. More recent work also has found HECT domains in Nedd4 that ubiquitinates the epithelial sodium channel (Staub et al., 1996) and KIAA10 (You & Pickart, 2001) (see below).

The vast majority of E3s, however, contain RING finger domains. These 40–60 residue zinc-binding motifs contain core cysteine and histidine amino acids arrayed in a C3HC4 ("RING") or C3H2C3 ("RING-H2") pattern (Deshaies, 1999). RING fingers are typically found in a small protein that forms one subunit of a larger complex that together bears ubiquitin ligase activity. The prototype of such an E3 is the SCF (*S*kp1-*C*ullin-*F*box) complex (see below, Fig. 2) (Cardozo & Pagano, 2004; Deshaies, 1999). RING fingers have also been found, however, in many single subunit E3s such as E3α/UBR1, MuRF1 (*Mu*scle-specific *R*ING *F*inger protein 1) and Mdm2 (see below, Fig. 2) (Elenbaas, Dobbelstein, Roth, Shenk, & Levine, 1996; Kwon et al., 1998; Spencer, Eliazer, Ilaria, Richardson & Olson, 2000). Recently, a novel group of enzymes with ubiquitin ligase activity have been identified. These U-box domain proteins, such as UFD2 and CHIP, contain atypical RING fingers which lack the canonical cysteine residue usually required for Zn^{2+} coordination (Hatakeyama & Nakayama, 2003). These proteins are sometimes referred to as E4s, and may in some cases act in association with other E3s to enhance the number and rate of ubiquitin residues attached to substrates (Koegl et al., 1999).

2. $E2_{14k}$, E3α and the N-end rule pathway

The key role of the Ub-proteasome pathway in muscle wasting has helped shift research in the field to-

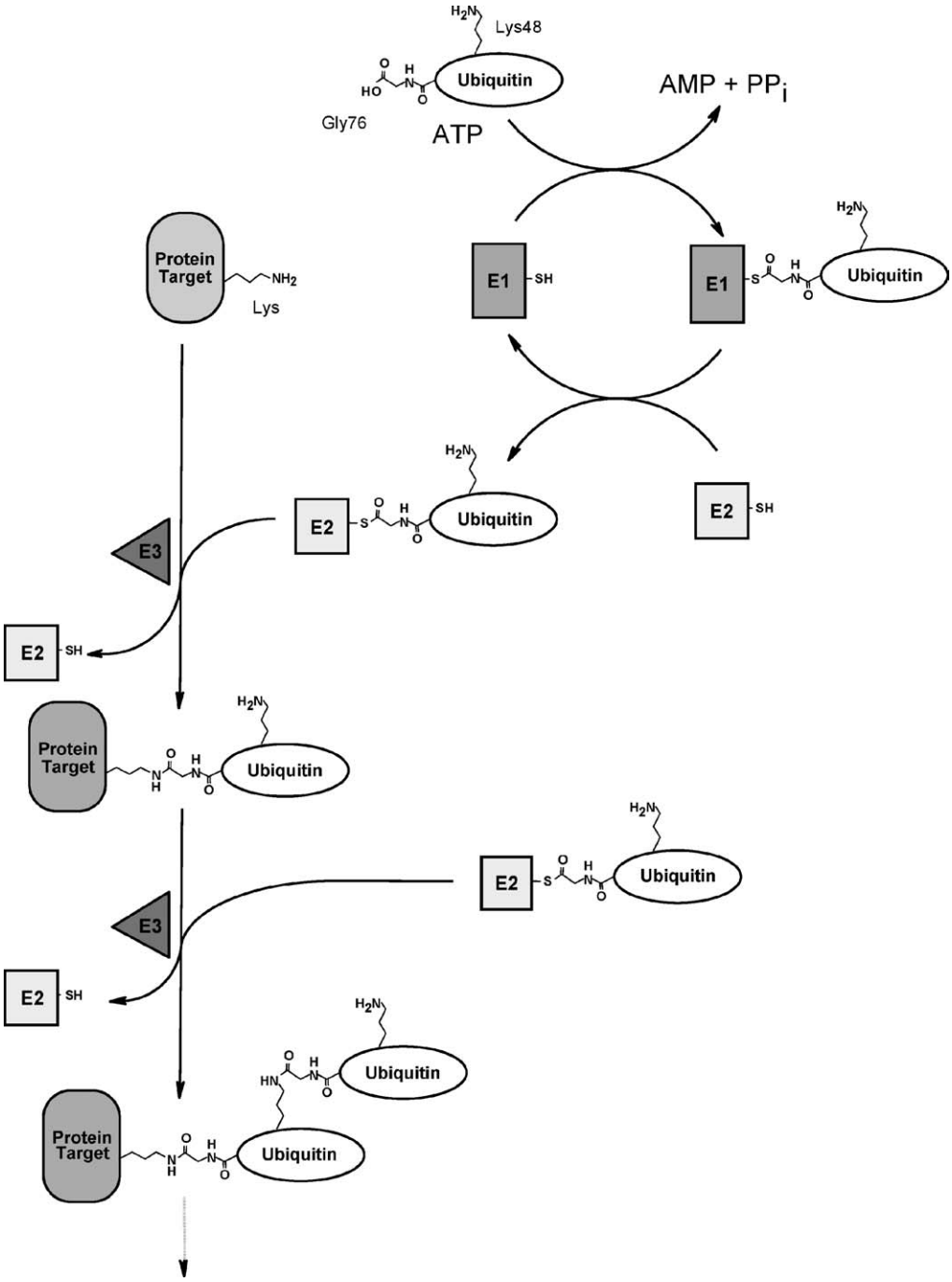

Fig. 1. Ubiquitin conjugation to protein substrates. Ubiquitin chains are conjugated to substrate proteins destined for destruction by a multistep process. First, the carboxyl terminal glycine residue of ubiquitin (Gly76) is activated by thiolester linkage to E1, in a reaction that consumes ATP, and releases AMP and pyrophosphate. The activated ubiquitin is then transferred to a sulfhydryl group on an E2 (ubiquitin carrier or conjugating protein). The activated ubiquitin is then conjugated to the ε-amino group of a lysine in the substrate protein, in a reaction catalyzed by an E3. Subsequent ubiquitin moieties are then conjugated to the prior ubiquitin in a similar cascade, usually at the ε-amino group of ubiquitin Lys48.

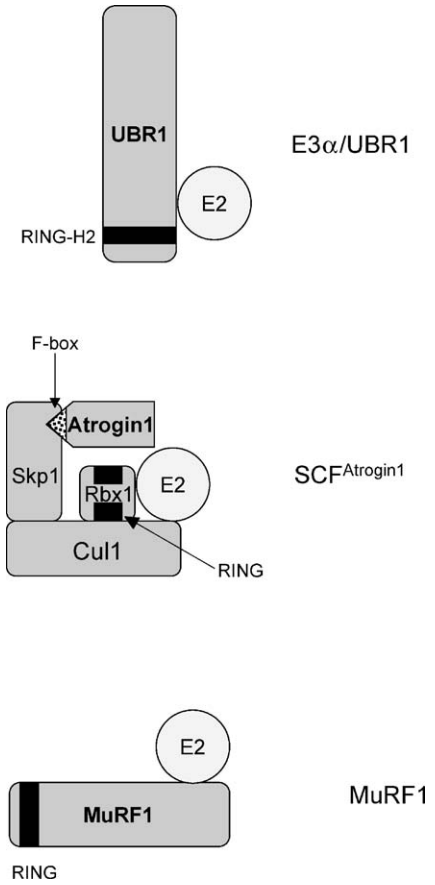

Fig. 2. Structure of E3s involved in muscle atrophy. E3 components are indicated by dark grey shading. Note that the $SCF^{Atrogin-1}$ E3 complex is made up of four components. Atrogin-1, the F-box component, presumably also binds to the protein substrate ubiquitinated by the complex. The RING finger motifs within each of these E3s are noted in black. E2: ubiquitin carrier protein.

wards identifying, from the very large number of enzymes involved in ubiquitin conjugation and specifically Ub–protein ligases (E3s) in muscle, the specific ones responsible for the activation of proteolysis during atrophy. Three candidates show promise as important players. E3α (also called UBR1) was the first to be implicated in the atrophy process (Solomon & Goldberg, 1996; Solomon, Baracos, Sarraf, & Goldberg, 1998; Solomon, Lecker, & Goldberg, 1998). This large, 200 kDa, single subunit ubiquitin–protein ligase contains a modified RING-H2 domain and acts together with the ubiquitin carrier protein, $E2_{14k}$. It is believed to recognize substrate proteins that begin with unblocked hydrophobic or basic amino acids (called the N-end rule pathway) (Kwon, Xia, Davydov, Lecker, & Varshavsky, 2001; Varshavsky, Turner, Du, & Xie, 2000; Xie & Varshavsky, 1999). The *S. cerevisiae* homolog of UBR1 also has been demonstrated to degrade cohesins in a step required for chromosome segregation (Rao, Uhlmann, Nasmyth, & Varshavsky, 2001). To complicate matters, in addition to recognizing proteins at their amino termini, *S. cerevisiae* UBR1 can also recognize substrates based on internal motifs, and can be allosterically activated by binding hydrophobic and basic amino acids (Du, Navarro-Garcia, Xia, Tasaki, & Varshavsky, 2002; Turner, Du, & Varshavsky, 2000).

The connection between E3α/UBR1 and muscle atrophy began with the finding that mRNA for $E2_{14k}$ and UBR1 are increased in atrophying muscles (Fischer, Sun, Gang, Pritts, & Hasselgren, 2000; Lecker, Solomon, Mitch, et al., 1999; Lecker, Solomon, Price, et al., 1999; Wing & Banville, 1994). In addition, experiments in cellular extracts prepared from atrophying muscles show increased rates of ubiquitin conjugation via this pathway, and inhibitors E3α/UBR1 reduce accelerated ubiquitin conjugation rates back to baseline levels (Lecker, Solomon, Mitch, et al., 1999; Lecker, Solomon, Price, et al., 1999; Solomon, Baracos, et al., 1998; Solomon, Lecker, et al., 1998). Because the vast majority of cellular proteins begin with methionine and are acetylated, the specific proteins ubiquitinated by E3α/UBR1 in muscle, and how they may be generated are quite unclear. The finding of an important role of this enzyme in the accelerated ubiquitin conjugation that occurs during atrophy suggests that there might be an undefined proteolytic enzyme that clips muscle proteins and generates free amino-terminal residues that can be recognized by this E3. Both calpains (Williams et al., 1999) and caspases are obvious candidates for such endoproteases and there may be emerging evidence that caspases are able to cleave muscle proteins and generate such fragments. dIAP1, a drosophila inhibitor of apoptosis, is cleaved by caspases leading to a short form of dIAP1 that is rapidly degraded via the N-end rule pathway (Ditzel et al., 2003). Furthermore, caspase 3 is known to be able to cleave myosin light chain (Moretti et al., 2002), and Mitch and colleagues (Du et al., 2004) recently demonstrated that caspase 3 can also cleave purified actomyosin complex in vitro to yield a 14 kDa actin fragment. This actin fragment was also found in "atrophying" cultured muscle cells treated with staurosporine (to activate cas-

pase 3) or deprived of serum (to mimic atrophy induced by food deprivation), as well as in atrophying muscles from diabetic and uremic rats. Interestingly, although this fragment seems to be further degraded by the Ub-proteasome pathway, the identity of its amino-terminal residue and whether E3α/UBR1 and the N-end rule pathway is involved in its ubiquitination is not known. These data have, however, led Mitch and coworkers to suggest that caspase 3 cleavage of actin might be an initial step in the enhanced proteolysis in muscle wasting conditions.

Most recently, a homologue of E3α, E3α-II, was identified by Han and coworkers (Kwak et al., 2004). This protein shares 60% overall sequence homology with E3α and was previously found by Varshavsky and his investigators, who named it UBR2 (Kwon et al., 2001). Han and colleagues found a dramatic up-regulation of E3α-II in atrophying muscles of tumor-bearing rodents. Interestingly, transfection of E3α but especially E3α-II into muscle cells in culture markedly increases rates of N-end rule ubiquitination (Kwak et al., 2004). Their data suggest that E3α-II or the combination of E3α and E3α-II may be responsible for the increased rates of ubiquitination by the N-end rule pathway found in atrophying muscle.

The findings in cell extracts from atrophying muscle, that rates of ubiquitin conjugation by $E2_{14k}$ and E3α/UBR1 are increased, have led to experiments in mice lacking these proteins to test whether such animals are resistant to the development of muscle atrophy. Both the $E2_{14k}$ (HR6B−/−) and E3α (UBR1−/−) knockout mice, however, develop muscle atrophy at the same rate as controls in response to food deprivation (Adegoke, Bedard, Roest, & Wing, 2002; Kwon et al., 1998, 2001; Roset et al., 1996). Furthermore, UBR2 null mice have normal muscles, though no studies have been performed to date to measure their response to catabolic stimuli (Kwon et al., 2003). These negative results are complicated by the fact that another isoform of $E2_{14k}$/HR6B exists (HR6A) (Roset et al., 1996) and at least one other protein with homology to UBR1 and UBR2 (i.e. UBR3) is present in mammalian muscle tissue (Kwon et al., 1998, 2001). The redundancy of enzymes in this pathway, then, makes it difficult to draw definitive conclusions regarding the role of the N-end rule pathway in muscle wasting. Clearly, animals with these multiple isoforms deleted will need to be generated to be able to definitively test the importance of the N-end rule pathway. This may be difficult or impossible to perform as it is already known that HR6A/B (Roset et al., 1996) and UBR1/2 (Kwon et al., 2001) double knockouts are not viable.

3. Atrogin-1/MAFbx

As an independent means to identify the components of the ubiquitin-proteasome pathway activated during muscle wasting, we have used cDNA microarrays to compare the transcriptional profile in atrophying muscles from fasted mice (Jagoe, Lecker, Gomes, & Goldberg, 2002), and more recently in rats with chronic renal failure, cancer cachexia and uncontrolled diabetes (Lecker et al., 2004). These analyses led to our identification of a transcript that is induced more than eight-fold in muscle from fasted mice (Gomes, Lecker, Jagoe, Navon, & Goldberg, 2001). The transcript is also strongly induced in muscles from the diabetic, uremic and tumor-bearing animals, as well as in atrophying human muscles (Jones et al., 2004). We cloned the gene, which we named atrogin-1. It contains an F-box domain that characterizes the class of Ub–protein ligases (E3s) called SCF complexes (Gomes et al., 2001). SCF complexes are modular E3s made up of Skp1, a cullin (Cul) protein, a RING finger protein and an F-box protein (Fig. 2) (Deshaies, 1999). The F-box protein, of which atrogin-1 is an example, is the component that links the protein substrate to be ubiquitinated with the rest of the E3 and ubiquitination machinery (Cardozo & Pagano, 2004). F-box proteins, in addition to the F-box domain that binds to Skp1, also contain a substrate recognition domain. While no clear motifs are present in atrogin-1 that may suggest which proteins in muscle it recognizes (e.g. leucine rich repeats and WD40 domains that typically recognize phosphorylated proteins), our initial characterization of atrogin-1 demonstrated that the 42 kDa protein does contain two other motifs which might suggest functional roles. The protein contains both a PDZ-binding domain, implicated in protein–protein interactions (Doyle et al., 1996), and a nuclear localization sequence. Indeed, data suggest that atrogin-1 can be found in the nuclei of muscle cells (Sandri et al., 2004, and our unpublished observations). The activity of SCF complexes can be further regulated by other protein–protein interactions. Nedd8, a ubiquitin-like protein can be conjugated to the Cul1

protein, enhancing its activity and helping to recruit the E2 to the SCF complex (Pan, Kentsis, Dias, Yamoah, & Wu, 2004). In addition, CAND1 (*C*ullin-*a*ssociated *N*edd8-*d*issociated) can also bind to cullin proteins and interrupt the Nedd8 modification and the formation of an active SCF complex (Liu, Furukawa, Matsumoto, & Xiong, 2002; Zheng et al., 2002). It is as yet unknown whether these modifications or interactions regulate the atrogin-1 SCF complex.

Our early studies indicated that expression of atrogin-1 is restricted to striated and cardiac muscle with induction at least 12 h before significant gastrocnemius muscle weight loss occurs (Gomes et al., 2001), strongly suggesting a role for atrogin-1 in the early phases of the atrophy process. Concomitant with our studies, Glass and colleagues at Regeneron Pharmaceuticals found the same gene (which they named MAFbx, for *M*uscle *A*trophy *F*-*B*o*x* protein) to be induced in muscles atrophying due to denervation, immobilization and hindlimb suspension (Bodine et al., 2001). Since these initial reports, increased atrogin-1 expression has also demonstrated in the muscle from the cecal ligation and puncture model of sepsis in rats (Wray, Mammen, Hershko, & Hasselgren, 2003) and by others studying muscle during fasting and diabetes (Dehoux et al., 2004). However, perhaps the most powerful data linking atrogin-1 with the generation of muscle wasting was obtained by the Regeneron group who demonstrated that mice lacking the MAFbx gene are resistant to denervation atrophy, with a 56% sparing of muscle weight loss after 14 days of denervation (Bodine et al., 2001). Conceptually, one could imagine atrogin-1 targeting for degradation key nuclear regulatory proteins or transcription factors that in turn might lead to a decrease in protein synthesis in atrophying muscle, or conversely, it could enhance bulk muscle protein degradation directly by ubiquitinating myofibrillar components. Clearly, identification of atrogin-1 targets is of primary importance in clarifying these issues. On the other hand, recent work in the oncology field found atrogin-1 induced in gastrointestinal tumors undergoing involution after Gleevec treatment (Frolov et al., 2003), raising some question as to the true tissue specificity of atrogin-1. In addition, a recent report showed that in cardiac muscle, atrogin-1 is able to ubiquitinate and target for degradation calcineurin A (Li et al., 2004). In the heart, transgenic overexpression of atrogin-1 was able to prevent the calcineurin-mediated cardiac hypertrophy that usually occurs following aortic banding (Li et al., 2004). It is not yet known if calcineurin A is a target of atrogin-1 in skeletal muscle, though calcineurin signaling pathways seem to affect fiber type more than fiber size in that tissue (Serrano et al., 2001), leading one to predict that other atrogin-1 substrates may be more critical in striated muscle. Finally, atrogin-1 has been reported to interact with and degrade myoD, a transcription factor critical in muscle development and differentiation (Tintignac et al., 2004). Since muscle atrophy occurs in fully differentiated adult muscle, and animals lacking atrogin-1 develop normal muscle, the physiological role of this interaction is not clear.

Recent studies have elucidated one of the major signaling pathways responsible for activation of atrogin-1 during muscle atrophy (Lee et al., 2004; Sandri et al., 2004; Stitt et al., 2004). In conditions of muscle atrophy, signaling through the IGF-1/PI3K/Akt pathway is suppressed, which in turn maintains FoxO transcription factors in a dephosphorylated (active) state. FoxO factors are potent activators of atrogin-1 transcription, and bind directly to multiple sites within the atrogin-1 promoter (Sandri et al., 2004).

4. MuRF1

The genomic approach used by the workers at Regeneron also identified another E3, MuRF1, in their models of atrophying muscle (Bodine et al., 2001). Like atrogin-1, MuRF1 is also induced in atrophying muscles from fasted, diabetic, tumor-bearing and uremic animals as well as atrophying human muscles, suggesting that it plays a fundamental role in the development of muscle wasting (Jones et al., 2004; Lecker et al., 2004). Mice lacking MuRF1 are also protected from denervation-induced muscle atrophy, though to a somewhat lesser degree than mice lacking atrogin-1 (Bodine et al., 2001). MuRF1 is a 40 kDa protein which contains a RING domain at its amino-terminal end, as well as two coiled-coil domains in its central region (Centner et al., 2001; Spencer et al., 2000). The RING domain of MuRF1 imparts ubiquitin conjugating activity, as was demonstrated in vitro by Bodine et al. (2001), though the targets of this activity are not yet known. Interestingly, MuRF1 and other members of the MuRF family have been found in association with

the myofibrillar component, titin, at the M-line, raising the possibility that they might target components of the myofibrillar apparatus for degradation in atrophying muscle (Centner et al., 2001; McElhinny, Perry, Witt, Labeit, & Gregorio, 2004). On the other hand, there are also indications that MuRF1 can be found in the nucleus of muscle cells (McElhinny, Kakinuma, Sorimachi, Labeit, & Gregorio, 2002), though its location in atrophying muscle is not known. Recently, MuRF1 was also found to interact with glucocorticoid modulatory element binding protein-1 (GMEB-1), a nuclear protein that regulates transcription in response to glucocorticoids (McElhinny et al., 2002). It is intriguing that glucocorticoids are potent activators of muscle protein breakdown and that MuRF1 expression is increased in the presence of glucocorticoids (Bodine et al., 2001). Finally, MuRF1 has also been shown to degrade troponin I in cardiomyocytes (Kedar et al., 2004) The interaction between MuuRF1 and this myofibrillar protein in skeletal muscle and its potential role in the development of muscle atrophy is yet to be established.

5. Other muscle specific enzymes involved in ubiquitin conjugation

In addition to atrogin-1 and MuRF1, Kandarian and coworkers have evidence that mRNA for Nedd4, a HECT-domain E3 is increased in muscles atrophying from disuse (Jackman & Kandarian, 2004), though activation of this E3 has not been seen in other types of atrophying muscle. Pickart and coworkers have also identified a HECT-domain ubiquitin–protein ligase called KIAA10 that is highly expressed in skeletal muscle (You & Pickart, 2001). KIAA10 targets TIP120B, a muscle-specific TATA-box interacting protein, for ubiquitination and degradation by the 26S proteasome (You, Wang, Aoki, Tamura, & Pickart, 2003). The role of KIAA10 in normal or atrophying muscle is, however not yet known. Additionally, Ozz, a muscle specific RING finger ubiquitin ligase (Nastasi et al., 2004), has recently been shown to ubiquitinate membrane-bound beta-catenin and may play an important role in sarcomere organization and possibly dystrophic states (Wu, Liu, & He, 2004). U box-containing E3s, such as UFD2 and CHN-1, the *C. elegans* orthologue of CHIP, form an E3/E4 complex that enhances the ubiquitination of UNC-45, a myosin chaperone that is involved in myosin assembly (Hoppe et al., 2004). These ubiquitination enzymes could also potentially play roles in muscle protein breakdown in atrophying muscle.

Two other Ub-carrier proteins (E2s) also demonstrate muscle specific patterns of expression. Chrysis and Underwood showed that transcripts of E2G, first identified by Watanabe et al. (1996), and a UBC4/UBC5 isoform are increased in muscles of rats treated with glucocorticoids (Chrysis & Underwood, 1999). Li et al. (2003) have recently demonstrated that UbcH2 is also predominantly expressed in muscle, and that its levels rise in response to TNFα, another mediator of muscle atrophy. Whether these E2s act in combination with atrogin-1 or MuRF1 or, alternatively, with some as yet undefined additional E3 is not known.

6. Conclusions

One concept that seems to be emerging is that multiple distinct ubiquitin conjugation pathways, and possibly other proteolytic systems, are activated in muscle as it atrophies. This coordinate regulation of proteolytic enzymes suggests that in atrophy each functions to remove distinct cellular components. Such parallel pathways acting on different muscle proteins might explain why the gene knockouts of E3α/UBR1 and $E2_{14k}$ in mice are still able to undergo atrophy, and why atrogin-1/MAFbx and MuRF1 knockouts are only partially effective at preventing the development of atrophy. Mating these animals to test if the effects of deleting individual enzymes are additive in diminishing atrophy should help to clarify this important issue. It is also possible that the function of one or more proteolytic systems are linked. For instance, a caspase or a member of the calpain family could be the elusive protease able to generate proteins with the novel N-terminal amino groups recognized by E3α (UBR1/UBR2). In such a model, atrophy of muscle would be a multi-step proteolytic process. Conclusive evidence for this model will require careful biochemical studies and identification of the critical proteins degraded by these enzymes. Until the activities and cellular substrates of these proteases and enzymes of the ubiquitin conjugating system are more fully characterized, it is difficult to predict if these various proteolytic systems work in series or in parallel. Although much work lies ahead, the major

players in this excessive proteolysis now appear to be coming into focus.

References

Adegoke, O. A., Bedard, N., Roest, H. P., & Wing, S. S. (2002). Ubiquitin-conjugating enzyme E214k/HR6B is dispensable for increased protein catabolism in muscle of fasted mice. *American Journal of Physiology-Endocrinology and Metabolism*, *283*, E482–E489.

Baumeister, W., Walz, J., Zuhl, F., & Seemuller, E. (1998). The proteasome: Paradigm of a self-compartmentalizing protease. *Cell*, *92*, 367–380.

Bodine, S. C., Latres, E., Baumhueter, S., Lai, V. K., Nunez, L., Clarke, B. A., et al. (2001). Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science*, *294*, 1704–1708.

Cardozo, T., & Pagano, M. (2004). The SCF ubiquitin ligase: Insights into a molecular machine. *Nature Reviews Molecular Cell Biology*, *5*, 739–751.

Centner, T., Yano, J., Kimura, E., McElhinny, A. S., Pelin, K., Witt, C. C., et al. (2001). Identification of muscle specific ring finger proteins as potential regulators of the titin kinase domain. *Journal of Molecular Biology*, *306*, 717–726.

Chrysis, D., & Underwood, L. E. (1999). Regulation of components of the ubiquitin system by insulin-like growth factor I and growth hormone in skeletal muscle of rats made catabolic with dexamethasone. *Endocrinology*, *140*, 5635–5641.

Dehoux, M., Van Beneden, R., Pasko, N., Lause, P., Verniers, J., Underwood, L., et al. (2004). Role of the IGF-I decline in the induction of Atrogin-1/MAFbx during fasting and diabetes. *Endocrinology*.

Deshaies, R. J. (1999). SCF and Cullin/Ring H2-based ubiquitin ligases. *Annual Review of Cell and Developmental Biology*, *15*, 435–467.

Ditzel, M., Wilson, R., Tenev, T., Zachariou, A., Paul, A., Deas, E., et al. (2003). Degradation of DIAP1 by the N-end rule pathway is essential for regulating apoptosis. *Nature Cell Biology*, *5*, 467–473.

Doyle, D. A., Lee, A., Lewis, J., Kim, E., Sheng, M., & MacKinnon, R. (1996). Crystal structures of a complexed and peptide-free membrane protein-binding domain: Molecular basis of peptide recognition by PDZ. *Cell*, *85*, 1067–1076.

Du, F., Navarro-Garcia, F., Xia, Z., Tasaki, T., & Varshavsky, A. (2002). Pairs of dipeptides synergistically activate the binding of substrate by ubiquitin ligase through dissociation of its autoinhibitory domain. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 14110–14115.

Du, J., Wang, X., Miereles, C., Bailey, J. L., Debigare, R., Zheng, B., et al. (2004). Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *Journal of Clinical Investigation*, *113*, 115–123.

Elenbaas, B., Dobbelstein, M., Roth, J., Shenk, T., & Levine, A. J. (1996). The MDM2 oncoprotein binds specifically to RNA through its RING finger domain. *Molecular Medicine*, *2*, 439–441.

Fischer, D., Sun, X., Gang, G., Pritts, T., & Hasselgren, P. O. (2000). The gene expression of ubiquitin ligase E3alpha is upregulated in skeletal muscle during sepsis in rats-potential role of glucocorticoids. *Biochemical and Biophysical Research Communications*, *267*, 504–508.

Frolov, A., Chahwan, S., Ochs, M., Arnoletti, J. P., Pan, Z. Z., Favorova, O., et al. (2003). Response markers and the molecular mechanisms of action of Gleevec in gastrointestinal stromal tumors. *Molecular Cancer Therapeutics*, *2*, 699–709.

Glickman, M. H., & Ciechanover, A. (2002). The ubiquitin-proteasome proteolytic pathway: Destruction for the sake of construction. *Physiological Reviews*, *82*, 373–428.

Gomes, M. D., Lecker, S. H., Jagoe, R. T., Navon, A., & Goldberg, A. L. (2001). Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 14440–14445.

Hasselgren, P. O., & Fischer, J. E. (2001). Muscle cachexia: Current concepts of intracellular mechanisms and molecular regulation. *Annals of Surgery*, *233*, 9–17.

Hatakeyama, S., & Nakayama, K. I. (2003). U-box proteins as a new family of ubiquitin ligases. *Biochemical and Biophysical Research Communications*, *302*, 635–645.

Hoppe, T., Cassata, G., Barral, J. M., Springer, W., Hutagalung, A. H., Epstein, H. F., et al. (2004). Regulation of the myosin-directed chaperone UNC-45 by a novel E3/E4-multiubiquitylation complex in *C. elegans*. *Cell*, *118*, 337–349.

Huibregtse, J. M., Scheffner, M., Beaudenon, S., & Howley, P. M. (1995). A family of proteins structurally and functionally related to the E6-AP ubiquitin protein ligase. *Proceedings of the National Academy of Sciences of the United States of America*, *92*, 2563–2567.

Jackman, R. W., & Kandarian, S. C. (2004). The molecular basis of skeletal muscle atrophy. *American Journal of Physiology-Cell Physiology*, *287*, 834–843.

Jackson, P. K., Eldridge, A. G., Freed, E., Furstenthal, L., Hsu, J. Y., Kaiser, B. K., et al. (2000). The lore of the RINGs: Substrate recognition and catalysis by ubiquitin ligases. *Trends in Cell Biology*, *10*, 429–439.

Jagoe, R. T., Lecker, S. H., Gomes, M., & Goldberg, A. L. (2002). Patterns of gene expression in atrophying skeletal muscles: The response to food deprivation. *FASEB Journal*, *16*, 1697–1712.

Jones, S. W., Hill, R. J., Krasney, P. A., O'Conner, B., Peirce, N., & Greenhaff, P. L. (2004). Disuse atrophy and exercise rehabilitation in humans profoundly affects the expression of genes associated with the regulation of skeletal muscle mass. *FASEB Journal*, *18*, 1025–1027.

Kedar, V., McDonough, H., Arya, R., Li, H., Rockman, H.A., Patterson, C. (2004). Muscle-specific RING fingger 1 is a bona fide ubiquitin ligase that degrades troponin I. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 18135–18140.

Koegl, M., Hoppe, T., Schlenker, A., Ulrich, H. D., Mayer, T. U., & Jentsch, S. (1999). A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell*, *96*, 635–644.

Kwak, K. S., Zhou, X., Solomon, V., Baracos, V. E., Davis, J., Bannon, A. W., et al. (2004). Regulation of protein catabolism

by muscle-specific and cytokine-inducible ubiquitin ligase E3a-II during cancer cachexia. *Cancer Research*, 64, 8193–8198.

Kwon, Y. T., Reiss, Y., Fried, V. A., Hershko, A., Yoon, J. K., Gonda, D. K., et al. (1998). The mouse and human genes encoding the recognition component of the N-end rule pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 7898–7903.

Kwon, Y. T., Xia, Z., An, J. Y., Tasaki, T., Davydov, I. V., Seo, J. W., et al. (2003). Female lethality and apoptosis of spermatocytes in mice lacking the UBR2 ubiquitin ligase of the N-end rule pathway. *Molecular and Cellular Biology*, *23*, 8255–8271.

Kwon, Y. T., Xia, Z., Davydov, I. V., Lecker, S. H., & Varshavsky, A. (2001). Construction and analysis of mouse strains lacking the ubiquitin ligase UBR1 (E3alpha) of the N-End Rule pathway. *Molecular and Cellular Biology*, *21*, 8007–8021.

Lecker, S. H., Jagoe, R. T., Gilbert, A., Gomes, M., Baracos, V., Bailey, J., et al. (2004). Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB Journal*, *18*, 39–51.

Lecker, S. H., Solomon, V., Mitch, W. E., & Goldberg, A. L. (1999). Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. *Journal of Nutrition*, *129*, 227S–237S.

Lecker, S. H., Solomon, V., Price, S. R., Kwon, Y. T., Mitch, W. E., & Goldberg, A. L. (1999). Ubiquitin conjugation by the N-end rule pathway and mRNAs for its components increase in muscles of diabetic rats. *Journal of Clinical Investigation*, *104*, 1411–1420.

Lee, S. W., Dai, G., Hu, Z., Wang, X., Du, J., & Mitch, W. E. (2004). Regulation of muscle protein degradation: Coordinated control of apoptotic and ubiquitin-proteasome systems by phosphatidylinositol 3 kinase. *Journal of the American Society of Nephrology*, *15*, 1537–1545.

Li, H. H., Kedar, V., Zhang, C., McDonough, H., Arya, R., Wang, D. Z., et al. (2004). Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex. *Journal of Clinical Investigation*, *114*, 1058–1071.

Li, Y. P., Lecker, S. H., Chen, Y., Waddell, I. D., Goldberg, A. L., & Reid, M. B. (2003). TNF-alpha increases ubiquitin-conjugating activity in skeletal muscle by up-regulating UbcH2/E220k. *FASEB Journal*, *17*, 1048–1057.

Liu, J., Furukawa, M., Matsumoto, T., & Xiong, Y. (2002). NEDD8 modification of CUL1 dissociates p120(CAND1), an inhibitor of CUL1-SKP1 binding and SCF ligases. *Molecular Cell*, *10*, 1511–1518.

Lorick, K. L., Jensen, J. P., Fang, S., Ong, A. M., Hatakeyama, S., & Weissman, A. M. (1999). RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 11364–11369.

McElhinny, A. S., Kakinuma, K., Sorimachi, H., Labeit, S., & Gregorio, C. C. (2002). Muscle-specific RING finger-1 interacts with titin to regulate sarcomeric M-line and thick filament structure and may have nuclear functions via its interaction with glucocorticoid modulatory element binding protein-1. *Journal of Cell Biology*, *157*, 125–136.

McElhinny, A. S., Perry, C. N., Witt, C. C., Labeit, S., & Gregorio, C. C. (2004). Muscle-specific RING finger-2 (MURF-2) is important for microtubule, intermediate filament and sarcomeric M-line maintenance in striated muscle development. *Journal of Cell Science*, *117*, 3175–3188.

Mitch, W. E., & Goldberg, A. L. (1996). Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. *New England Journal of Medicine*, *335*, 1897–1905.

Moretti, A., Weig, H. J., Ott, T., Seyfarth, M., Holthoff, H. P., Grewe, D., et al. (2002). Essential myosin light chain as a target for caspase-3 in failing myocardium. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 11860–11865.

Nastasi, T., Bongiovanni, A., Campos, Y., Mann, L., Toy, J. N., Bostrom, J., et al. (2004). Ozz-E3, a muscle-specific ubiquitin ligase, regulates beta-catenin degradation during myogenesis. *Developmental Cell*, *6*, 269–282.

Pan, Z. Q., Kentsis, A., Dias, D. C., Yamoah, K., & Wu, K. (2004). Nedd8 on cullin: Building an expressway to protein destruction. *Oncogene*, *23*, 1985–1997.

Rao, H., Uhlmann, F., Nasmyth, K., & Varshavsky, A. (2001). Degradation of a cohesin subunit by the N-end rule pathway is essential for chromosome stability. *Nature*, *410*, 955–959.

Rock, K. L., Gramm, C., Rothstein, L., Clark, K., Stein, R., Dick, L., et al. (1994). Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell*, *78*, 761–771.

Roset, H. P., van Klaveren, J., de Wit, J., van Gurp, C. G., Koken, M. H. M., Vermey, M., et al. (1996). Inactivation of the HR6B ubiquitin-conjugating DNA repair enzyme in mice causes male sterility associated with chromatin modification. *Cell*, *86*, 799–810.

Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., et al. (2004). Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell*, *117*, 399–412.

Scheffner, M., Nuber, U., & Huibregtse, J. M. (1995). Protein ubiquitination involving an E1–E2–E3 enzyme ubiquitin thioester cascade. *Nature*, *373*, 81–83.

Seol, J. H., Feldman, R. M., Zachariae, W., Shevchenko, A., Correll, C. C., Lyapina, S., et al. (1999). Cdc53/cullin and the essential Hrt1 RING-H2 subunit of SCF define a ubiquitin ligase module that activates the E2 enzyme Cdc34. *Genes and Development*, *13*, 1614–1626.

Serrano, A. L., Murgia, M., Pallafacchina, G., Calabria, E., Coniglio, P., Lomo, T., et al. (2001). Calcineurin controls nerve activity-dependent specification of slow skeletal muscle fibers but not muscle growth. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 13108–13113.

Solomon, V., & Goldberg, A. L. (1996). Importance of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. *Journal of Biological Chemistry*, *271*, 26690–26697.

Solomon, V., Baracos, V., Sarraf, P., & Goldberg, A. L. (1998). Rates of ubiquitin conjugation increase when muscles atrophy, largely through activation of the N-end rule pathway. *Proceedings of the*

National Academy of Sciences of the United States of America, *95*, 12602–12607.

Solomon, V., Lecker, S. H., & Goldberg, A. L. (1998). The N-end rule pathway catalyzes a major fraction of the protein degradation in skeletal muscle. *Journal of Biological Chemistry*, *273*, 25216–25222.

Spencer, J. A., Eliazer, S., Ilaria, R. L., Jr., Richardson, J. A., & Olson, E. N. (2000). Regulation of microtubule dynamics and myogenic differentiation by MURF, a striated muscle RING-finger protein. *Journal of Cell Biology*, *150*, 771–784.

Staub, O., Dho, S., Henry, P. C., Correa, J., Ishikawa, T., McGlade, J., et al. (1996). Ww domains of Nedd4 bind to the proline-rich Py motifs in the epithelial Na+ channel deleted in liddles syndrome. *EMBO Journal*, *15*, 2371–2380.

Stitt, T. N., Drujan, D., Clarke, B. A., Panaro, F., Timofeyva, Y., Kline, W. O., et al. (2004). The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Molecular Cell*, *14*, 395–403.

Tintignac, L.A., Lagirand, J., Batonnet, S., Sirri, V., Leibovich, M.P., & Leibovich, S.A., (2004). Degradation of MyoD mediated by the SCF (MAFbx) ubiquitin ligase. *Journal of Biological Chemistry*, M411346200.

Turner, G. C., Du, F., & Varshavsky, A. (2000). Peptides accelerate their uptake by activating a ubiquitin-dependent proteolytic pathway. *Nature*, *405*, 579–583.

Varshavsky, A., Turner, G., Du, F., & Xie, Y. (2000). The ubiquitin system and the N-end rule pathway. *Biological Chemistry*, *381*, 779–789.

Watanabe, T. K., Kawai, A., Fujiwara, T., Maekawa, H., Hirai, Y., Nakamura, Y., et al. (1996). Molecular cloning of UBE2G, encoding a human skeletal muscle-specific ubiquitin-conjugating enzyme homologous to UBC7 of *C. elegans*. *Cytogenetics and Cell Genetics*, *74*, 146–148.

Williams, A. B., Decourten-Myers, G. M., Fischer, J. E., Luo, G., Sun, X., & Hasselgren, P. O. (1999). Sepsis stimulates release of myofilaments in skeletal muscle by a calcium-dependent mechanism. *FASEB Journal*, *13*, 1435–1443.

Wing, S. S., & Banville, D. (1994). 14-kDa ubiquitin-conjugating enzyme: Structure of the rat gene and regulation upon fasting and by insulin. *American Journal of Physiology*, *267*, E39–E48.

Wray, C. J., Mammen, J. M., Hershko, D. D., & Hasselgren, P. O. (2003). Sepsis upregulates the gene expression of multiple ubiquitin ligases in skeletal muscle. *International Journal of Biochemistry and Cell Biology*, *35*, 698–705.

Wu, G., Liu, C., & He, X. (2004). Ozz; a new name on the long list of beta-catenin's nemeses. *Molecular Cell*, *13*, 451–453.

Xie, Y., & Varshavsky, A. (1999). The E2–E3 interaction in the N-end rule pathway: The RING-H2 finger of E3 is required for the synthesis of multiubiquitin chain. *EMBO Journal*, *18*, 6832–6844.

You, J., & Pickart, C. M. (2001). A HECT domain E3 enzyme assembles novel polyubiquitin chains. *Journal of Biological Chemistry*, *276*, 19871–19878.

You, J., Wang, M., Aoki, T., Tamura, T. A., & Pickart, C. M. (2003). Proteolytic targeting of transcriptional regulator TIP120B by a HECT domain E3 ligase. *Journal of Biological Chemistry*, *278*, 23369–23375.

Zheng, J., Yang, X., Harrell, J. M., Ryzhikov, S., Shim, E. H., Lykke-Andersen, K., et al. (2002). CAND1 binds to unneddylated CUL1 and regulates the formation of SCF ubiquitin E3 ligase complex. *Molecular Cell*, *10*, 1519–1526.