

Protein Degradation by the Ubiquitin–Proteasome Pathway in Normal and Disease States

Stewart H. Lecker,* Alfred L. Goldberg,[†] and William E. Mitch[‡]

**Nephrology Division, Beth Israel Deaconess, and [†]Department of Cell Biology, Harvard Medical School, Boston, Massachusetts; and [‡]Nephrology Division, Baylor College of Medicine, Houston, Texas*

J Am Soc Nephrol 17: 1807–1819, 2006. doi: 10.1681/ASN.2006010083

All intracellular proteins and many extracellular proteins are continually “turning over;” *i.e.*, they are being hydrolyzed to their constituent amino acids and replaced by new synthesis. Although the continual destruction of cell proteins might seem wasteful, this process serves several important homeostatic functions. Individual proteins in the nucleus and cytosol, as well as in the endoplasmic reticulum (ER) and mitochondria, are degraded at widely differing rates that vary from minutes for some regulatory enzymes to days or weeks for proteins such as actin and myosin in skeletal muscle or months for hemoglobin in the red cell. Cells contain multiple proteolytic systems to carry out the degradation process and complex regulatory mechanisms to ensure that the continual proteolytic processes are highly selective. Therefore, excessive breakdown of cell constituents is prevented. Overall, the rates of protein synthesis and degradation in each cell must be balanced precisely because even a small decrease in synthesis or a small acceleration of degradation, if sustained, can result in a marked loss of mass in the organism (1).

In all tissues, the majority of intracellular proteins are degraded by the ubiquitin (Ub)–proteasome pathway (UPP) (2). However, extracellular proteins and some cell surface proteins are taken up by endocytosis and degraded within lysosomes. These organelles contain several acid-optimal proteases, including cathepsins B, H, and D, and many other acid hydrolases. Some cytosolic proteins are degraded in lysosomes after being engulfed in autophagic vacuoles that fuse with lysosomes (3,4). In most cells, this process is accelerated by the lack of insulin or essential amino acids and in liver by glucagon (5). There are other cytosolic proteolytic systems in mammalian cells. The Ca²⁺-activated (ATP-independent) proteolytic process involves the cysteine proteases termed calpains. These proteases seem to be activated when cells are injured and cytosolic Ca²⁺ rises, so they may play an important role in tissue injury, necrosis, and autolysis (6). Another important family of cytosolic proteases is the caspases that cleave proteins

after aspartic acid residues. These enzymes, which are cysteine proteases, are critical in destruction of cell constituents during apoptosis (7).

Ubiquitin Proteasome Pathway

During the past two decades, the UPP has taken center stage in our understanding of the control of protein turnover (Figure 1). The UPP consists of concerted actions of enzymes that link chains of the polypeptide co-factor, Ub, onto proteins to mark them for degradation (8,9). This tagging process leads to their recognition by the 26S proteasome, a very large multicatalytic protease complex that degrades ubiquitinated proteins to small peptides (10). Three enzymatic components are required to link chains of Ub onto proteins that are destined for degradation. E1 (Ub-activating enzyme) and E2s (Ub-carrier or conjugating proteins) prepare Ub for conjugation, but the key enzyme in the process is the E3 (Ub-protein ligase), because it recognizes a specific protein substrate and catalyzes the transfer of activated Ub to it (Figure 2). The discovery of Ub and the biochemistry of its conjugation to substrate proteins culminated in the awarding of the Nobel Prize in Chemistry in 2004 to Avram Hershko, Aaron Ciechanover, and Irwin Rose (<http://nobelprize.org/chemistry/laureates/2004/>).

Since the initial description of the UPP as a protein tagging and destruction mechanism, knowledge in this area has exploded, with thousands of proteins shown to be degraded by the system and additional novel functions for Ub conjugation being uncovered. The major functions of the pathway are described next.

Rapid Removal of Proteins

Unlike most regulatory mechanisms, protein degradation is inherently irreversible. Destruction of a protein can lead to a complete, rapid, and sustained termination of the process involving the protein as well as a change in cell composition. The rapid degradation of specific proteins permits adaptation to new physiologic conditions.

Regulation of Gene Transcription

Ub conjugation affects transcription by multiple mechanisms (11). Many transcription factors are ubiquitinated and degraded by the proteasome. In fact, in many cases, transcriptional activation domains and signals for Ub conjugation di-

Received January 26, 2006. Accepted April 13, 2006.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. William E. Mitch, Nephrology Division M/S: BCM 285, Baylor College of Medicine, One Baylor Plaza, Alkek N-520, Houston, TX 77030. Phone: 713-798-8350; Fax: 713-798-5010; E-mail: mitch@bcm.edu

Ub Is Linked to Substrates through an Enzymatic Cascade

Ub is composed of 76 amino acids. Its C-terminus is a critical glycine that is required for its conjugation to other Ub molecules and substrates, and it contains internal lysine residues that are used in the creation of polyubiquitin chains. Ub is not expressed directly as free Ub but rather as linear fusions either to itself or to certain ribosomal protein subunits. These highly unusual Ub-fusion precursors are processed rapidly by deubiquitinating enzymes, yielding monomeric, Ub moieties; this is one way in which Ub is produced rapidly in times of cellular stress. Ub conjugation to cellular proteins also can be reversed by the many deubiquitinating enzymes in cells. Therefore, these enzymes free Ub from precursor fusion proteins; they also catalyze the disassembly of Ub chains. Presumably, they function to recycle monomeric Ub for use in new conjugation reactions and prevent the accumulation of polyubiquitin chains that could compete with the binding of ubiquitinated substrates to the proteasome.

The initial step in conjugation of Ub onto proteins is activation of Ub at its C-terminus by the Ub-activating enzyme E1 (Figure 2). This abundant 110-kD enzyme uses ATP to generate a Ub thiolester, a highly reactive form of Ub (19). In mammalian organisms, a single, functional E1 enzyme has been found, unlike the large number of E2s and E3s in cells (20). Once activated, the Ub that is bound to E1 *via* the thiolester linkage is transferred to a sulfhydryl group of one of the 30 to 40 Ub carrier proteins or E2s (21). The E2s generally are small proteins that share a conserved 16-kD core that contains the cysteine that forms a thiolester linkage with the activated Ub (21). The large number of E2s helps to generate the specificity of the ubiquitination system, because specific E2s function in the degradation of various types of substrates, and they can conjugate with various E3s.

E3s Recognize the Cellular Proteins That Undergo Ub Conjugation

The main specificity factor in the UPP is the E3 enzyme. There are >1000 E3s in cells that link Ub to proteins in a highly regulated manner. E3s catalyze the transfer of the activated Ub from an E2 initially to a lysine in the target protein and subsequently to lysines that are present in Ub, yielding a substrate-anchored chain of Ub molecules. Generally, E3s fall into two broad structural classes: They are either homologous to HECT (homologous to E6-AP carboxy-terminus) domains or RING fingers (22).

HECT domain proteins are large monomeric E3s that consist of two functionally distinct domains (23). The C-terminal HECT domain (350 amino acids) accepts the activated Ub from the E2s by forming a thiolester linkage with Ub, enabling it to be transferred to the substrate. HECT-domain E3s directly bind activated Ub and are actual components of the enzymatic conjugation cascade (24). The prototypical member of this family is the E6-associated protein (E6-AP) (25). Lack of this enzyme causes Angelman's syndrome, an inherited neurologic disorder (26). Nedd4, another HECT-E3, targets the epithelial sodium

channel for internalization and degradation (27) by recognizing specific residues in the channel's cytoplasmic tails. When these proteins cannot interact, either as a result of mutations in the sodium channel or the E3, the channel is more stable, resulting in increased sodium reabsorption, hypervolemia metabolic alkalosis, and hypertension, a genetic defect known as Liddle's syndrome (28).

The vast majority of E3s contain RING finger domains. These 40- to 60-residue zinc-binding motifs contain core amino acids, cysteine, and histidine (22,29). RING finger E3s can be monomeric enzymes or multisubunit complexes. As a whole, they seem to serve as scaffolds that bring the substrate and the E2 into close proximity, an optimal condition for Ub conjugation (29–31). Monomeric RING finger E3s include the oncoprotein Mdm2, a physiologic regulator of p53 stability in normal cells (32), and c-Cbl, which catalyzes ubiquitination of certain cell surface receptors. Two E3s that are important in the processes of muscle atrophy, muscle ring finger-1 (MuRF-1), and E3 α belong to this group; E3 α was among the first of the E3s to be biochemically identified. It recognizes protein substrates on the basis of their N-terminal amino acid. Proteins that begin with large basic or hydrophobic residues are targeted for ubiquitination and degradation by E3 α (32a). This “N-end rule” pathway seems to be important in the destruction of cohesions (33), certain signaling molecules (*e.g.*, regulator of G-protein signaling 4 [RGS4] [34]), and the enhanced protein degradation that occurs in atrophying muscle (see section titled Mechanisms That Cause Loss of Muscle Protein in Uremia).

Other RING-finger E3s contain many subunits that serve as scaffolds to bring together the substrate and an E2 conjugated to an activated Ub. The largest (1.5 MDa), most complex E3 is the anaphase-promoting complex. It is important in ubiquitination of mitotic cyclins and other proteins that are involved in cell-cycle control. Cullin-RING Ub ligases form the largest group of E3s (29). The basic core of these E3s is the elongated, rigid cullin subunit. On end of these subunits bind the RING component (typically Rbx1/Roc1) and the E2, whereas at the other end, the substrate-interacting protein is bound, often through an additional adaptor protein. Because of the large number of cullins and substrate-binding subunits, the same organization using the same basic mechanism can recognize and ubiquitinate a large number of diverse proteins.

The best understood group of cullin-RING ligases are the Skp1-Cul1-F-box (SCF) complexes. The F-box protein is the subunit that contains the substrate-binding motif (see below). It binds to an adaptor, Skp1, through an approximately 45-amino acid F-box motif. Substrates of SCF complexes E3s are many key molecules that control inflammation and cell growth (*e.g.*, I κ B, NF- κ B, β -catenin) and cell cycle-induced proteins (*e.g.*, the cyclin-dependent kinase inhibitor p27^{Kip1}). In many cases, phosphorylation leads to binding of substrate to the F-box subunit and subsequent Ub conjugation. Regulated expression of F-box proteins can cause tissue- and disease-specific Ub conjugation of target proteins. For example, the F-box protein atrogin-1/MAFbx is expressed at high levels specifically in atrophying skeletal and cardiac muscle (35).

In addition to phosphorylation, other types of posttransla-

tional protein modifications can stimulate ubiquitination. For example, cellular oxygen levels are sensed by the Von Hippel-Lindau (VHL)-containing VHL-elongin BC (VBC) E3, which recognizes hydroxyproline (an oxygen-dependent protein modification). When oxygen levels are adequate in cells, the hypoxia-inducible factor 1 α (HIF-1 α) transcriptional activator undergoes prolyl hydroxylation and ubiquitination by this E3. When oxygen pressure falls, the unmodified HIF-1 α is not recognized by VHL and is not degraded, so it triggers transcription of genes for angiogenesis (vascular endothelial growth factor, erythropoietin, and glycolytic enzymes [36]). The VBC complex is another cullin-RING ligase, made up of Cul2 and a substrate-interacting domain that is made up of VHL and the adaptors elonginB and elonginC. VHL mutations are associated with highly vascular tumors in the kidney, presumably as a result at least in part of the presence of stable, active HIF-1 α . Other protein modifications that have been shown to recruit E3s include glycosylation, nitrosylation, and deacetylation. Substrate modification adds another layer of regulation to the UPP by integrating cell signaling and metabolic pathways with the conjugation-degradation machinery.

Recently, a novel group of enzymes with Ub ligase activity have been identified: The U-box domain proteins, such as Ub fusion degradation protein 2 (UFD2) and C-terminal of Hsp-70-interacting protein (CHIP). These E3 enzymes contain atypical RING finger motifs (37). CHIP is important for the removal of abnormal proteins such as the misfolded CFTR in cystic fibrosis and tau protein of polyglutamine repeat proteins that are present in several neurodegenerative diseases (38). Degradation of these proteins begins when they are bound by specific molecular chaperones followed by binding of the E3s. This leads to selective ubiquitination of the chaperone-bound substrate.

Most Cell Proteins Are Degraded by the 26S Proteasome

The rapid degradation of ubiquitinated proteins is catalyzed by the 26S proteasome. This structure is found in the nucleus and the cytosol of all cells and constitutes approximately 1 to 2% of cell mass (39). The 26S particle is composed of approximately 60 subunits and therefore is approximately 50 to 100 times larger than the typical proteases that function in the extracellular environment (*e.g.*, in digestion, blood clotting) and differs in critical ways. The most fundamental difference is that it is a proteolytic machine in which protein degradation is linked to ATP hydrolysis. The 26S complex is composed of a central barrel-shaped 20S proteasome with a 19S regulatory particle at either or both of its ends (Figure 3) (8,39,40). The 20S proteasome is a hollow cylinder that contains the mechanisms for protein digestion. It is composed of four stacked, hollow rings, each containing seven distinct but related subunits (39). The two outer α rings are identical, as are the two inner β rings. Three of the subunits in the β rings contain the proteolytic active sites that are positioned on the interior face of the cylinder. The outer α subunits of the 20S particle surround a narrow, central, and gated pore through which substrates enter and products exit (41). Substrate entry is a complex process that is

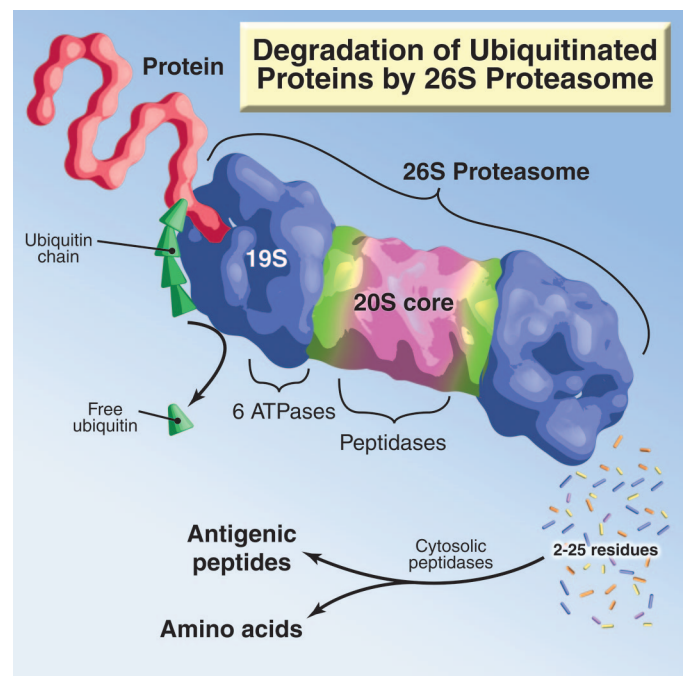


Figure 3. The 26S proteasome structure includes the 20S core that contains the unique proteolytic sites that break peptide bonds. The 19S caps cleave off the Ub chain and use ATPases to unfold the protein substrate and translocate it into the 20S core particle for degradation into peptides. Illustration by Josh Gramling—Gramling Medical Illustration.

catalyzed by the 19S particle. This complex architecture evolved to isolate proteolysis within a nano-sized compartment and prevents the nonspecific destruction of cell proteins. One can view protein ubiquitination and the functioning of the 19S particle as mechanisms that ensure proteolysis as an exquisitely selective process; only certain molecules get degraded within the 20S proteasome (42).

The 19S regulatory particles on the ends of the 20S proteasome are composed of at least 18 subunits (8,39). Its base contains six homologous ATPases in a ring and adjoins the outer ring of the 20S particle. These ATPases bind the proteins to be degraded and use ATP hydrolysis to unfold and translocate the protein into the 20S particle (43). The 19S's outer lid contains subunits that bind the polyubiquitin chains plus two deubiquitinating enzymes (also called isopeptidases) that disassemble the Ub chain so that the Ub can be reused in the degradation of other proteins (8). There is growing evidence that additional factors associate with the 19S particle and actually help deliver ubiquitinated proteins into the particle (44). Although the 26S complex catalyzes the degradation of ubiquitinated proteins, it can digest certain proteins without ubiquitination (especially damaged proteins); it remains uncertain how important this activity is *in vivo* (45).

The Proteasome's Multistep Mechanism

Much has been learned about the mechanisms by which a ubiquitinated protein is degraded. After it binds to the 19S component, the polyubiquitin chain is cleaved off the substrate

and disassembled. The protein is unfolded somehow by the six ATPases in the base of the particle (43). Linearization of the folded protein is essential for it to be translocated through the gated entry channel into the 20S particle because this pore, even in its open state, is too narrow for globular proteins (*i.e.*, most cell proteins) to enter (8,41). The ATPases also act as a “key in a lock” to cause opening of the gated, substrate entry channel of the 20S outer ring and into its central degradative chamber (46). Linearizing and delivering the substrate utilizes significant energy, perhaps one third as much ATP as the ribosome would use in synthesizing the protein, but it ensures the efficient and regulated elimination of the protein (43).

After the substrate enters the 20S's central chamber, the polypeptide is cleaved by its six proteolytic sites on the inner face of the changer, forming small peptides that range from three to 25 residues in length (47). Unlike traditional proteases, which cut a protein once and release the fragments, the proteasome digests the substrates all the way to small peptides that exit the particle. Peptides that are released by the proteasome only exist in the cell for seconds, because they are quickly digested into amino acids by the abundant cytosolic endopeptidases and aminopeptidases. The amino acids can be reutilized to synthesize new proteins or metabolized, yielding energy (48,49).

Although the proteasome principally catalyzes the complete hydrolysis of cell proteins, in a few cases, the 26S proteasome degrades proteins only partially, yielding a biologically active fragment. An example of the latter activity is the generation of a subunit of the transcription factor NF- κ B (50). For NF- κ B to be functional in inflammatory processes, the proteasome must digest an inactive precursor molecule and release one half, which functions in transcriptional regulation.

Proteasome Inhibitors and Cancer Therapy

In its central chamber, the proteasome contains six proteolytic sites: Two cleave preferentially after hydrophobic amino acids, two cleave preferentially after basic residues, and two cleave after acidic ones (39,40). The proteasome's active sites (like the set of proteases in the lysosome and pancreas) therefore have the capacity to degrade nearly all the various types of amino acid sequences that are found in proteins. One interesting exception that the proteasome's active site cannot cut is repeated sequences of glutamines. This exception seems to be important in the pathogenesis of certain neurodegenerative diseases (*e.g.*, Huntington's disease) that result from a genetically linked series of polyglutamines within certain proteins (51). Apparently, the glutamines are poorly degraded and accumulate as toxic, intracellular inclusions. The active sites in the proteasome cleave peptide bonds by a unique mechanism; peptide bonds are cleaved by the hydroxyl group on a critical threonine residue (45). Because the proteolytic mechanism is novel, highly specific inhibitors of the active sites have been synthesized (or microorganisms have produced them) (40). These inhibitors (*e.g.*, MG132, lactacystin, epoxymycin) have been used widely as research tools that have enabled investigators to discover many of the key functions of the UPP.

One such synthetic inhibitor, Bortezomib (Velcade, PS-341)

has emerged as an important new anticancer drug. Bortezomib has been approved by the US Food and Drug Administration and is widely used for the treatment of multiple myeloma; clinical trials against various other cancers are under active investigation (47,52). The proteasome inhibitors initially were synthesized in an attempt to develop agents that could block the excessive breakdown of muscle proteins in various cachectic states (see section titled Mechanisms That Cause Loss of Muscle Protein in Uremia), but it was discovered that they also could block the activation of NF- κ B (50), the critical transcription factor that mediates production of many inflammatory cytokines. NF- κ B also has important antiapoptotic roles that could block the death of cancer cells. However, inhibition of the proteasome was found to induce apoptosis, especially in neoplastic cells and transplanted tumors (40). With the backing of the National Cancer Institute, these agents went into human trials against various cancers, and the special sensitivity of myeloma cells became evident in phase I trials (52).

Surprising, these agent have therapeutic efficacy, even when protein degradation by the proteasome in cancer cells is only partially compromised. Apparently, the myeloma cells are particularly dependent on NF- κ B for production of essential growth factors (especially IL-6). In addition, malignant plasma cells produce exceptionally large amounts of abnormal Ig that are degraded by the ER-associated degradation–proteasome pathway (see section titled The Proteasome and Immune Surveillance). However, promising responses have been obtained in patients with other hematologic malignancies, and Bortezomib in combination with other chemotherapeutic agents is being tested against other malignancies in clinical trials. Our understanding of the mechanism by which proteasome inhibitors synergize with various cytotoxic agents is incomplete, but combating the antiapoptotic effects of NF- κ B, especially after there is DNA damage, is an attractive possibility. Surprising, proteasome inhibitors also have benefits in animal models of strokes. The compounds seem to reduce postischemia adhesion of cells and reperfusion injury. Again, the mechanism might involve combating the influence of NF- κ B. Human trials of the compounds in treatment of strokes soon will be undertaken. Notably, these applications initially were not recognized and probably could not have been predicted. In short, the development of proteasome inhibitors that exhibit several biologic properties emphasizes the enormous benefits that are emerging from basic biochemical research.

The Proteasome and Immune Surveillance

Besides essential roles in regulating cell growth and metabolism and in the elimination of misfolded proteins, the UPP serves a critical role as an information-gathering mechanism for the immune system (2,47,53). In higher vertebrates, the proteasome along with the cell's other proteolytic apparatus, the lysosome, generates antigenic peptides that are presented to the immune system. These peptides enable circulating lymphocytes to screen for foreign proteins in the extracellular and intracellular environments. Extracellular proteins that are taken up by endocytosis of antigen-presenting cells are degraded by the lysosome; this process provides the antigenic peptides that

are presented on MHC class II molecules to CD4⁺ lymphocytes. These lymphocytes are activated to produce antibodies (Figure 4).

The continual breakdown of intracellular proteins as may arise during viral or bacterial infection or with cancer allows the immune system to screen for non-native proteins within cells (2,53,54). Although the great majority of peptides that are generated by the proteasome during breakdown of intracellular proteins are digested rapidly to free amino acids (49,55), some escape this fate and are transported into the ER. In the ER, they bind to MHC class I molecules and are delivered to the cell surface for presentation to cytotoxic CD8⁺ lymphocytes (Figure 4) (2). When non-native epitopes (*e.g.*, ones derived from viruses) are presented on the cell surface, the presenting cells are killed quickly by cytotoxic T cells. This critical role of the proteasome in antigen presentation was demonstrated first through the use of proteasome inhibitors (54). These inhibitors proved invaluable in the elucidation of this immunologic process (2,56).

To bind to most MHC class I molecules, peptides have to be eight to nine residues long. Approximately 70 to 80% of proteasome products are too small to serve as antigens (56). The products that are used as antigenic precursors not only are the eight- to nine-residue peptides but also the longer ones with additional amino acids on their amino termini. They are taken up into the ER by a specific peptide transport system, called transporter associated with antigen processing (TAP). Within the ER is a newly discovered aminopeptidase, endoplasmic reticulum aminopeptidase 1 (ERAP1), which has the unusual capacity to trim extra amino acids off the longer precursors and then stops at eight to nine residues, the precise length for binding to MHC molecules (53,57,58).

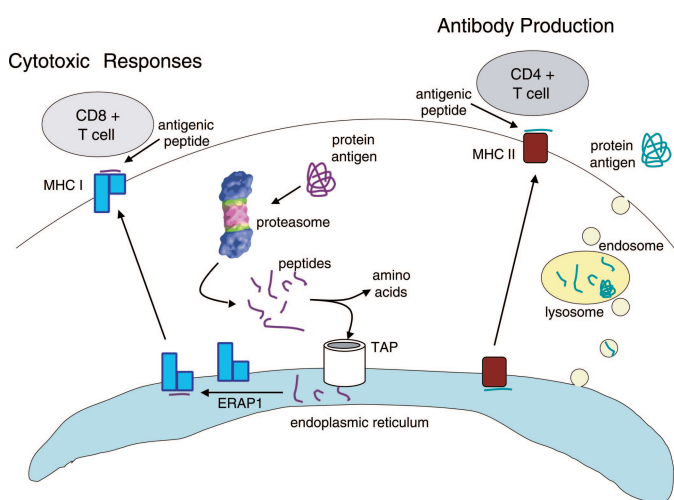


Figure 4. The generation and presentation of antigenic peptides. Intracellular proteins are degraded by the UPP and imported into the endoplasmic reticulum, where they are processed and lead to binding to MHC class I receptors. CD8-positive T cells respond to the antigen. Extracellular proteins that are endocytosed and degraded to peptides in lysosomes are processed to bind to MHC class II receptors. CD4-positive T cells respond to these antigens. Illustration by Josh Gramling—Gramling Medical Illustration.

In inflammatory conditions, the immune cells (*e.g.*, spleen or thymus cells) develop several adaptive mechanism functions that enhance the efficiency of antigen presentation (54). In response to the immune modifier γ -IFN, immune cells express novel types of proteasomes, termed immunoproteasomes, which play a major role in enhancing MHC class I presentation. The immunoproteasomes differ because they contain three novel peptidase subunits in place of the proteases that normally are present. These specialized subunits exhibit different specificities that enable them to cleave proteins differently so that more of the products have the correct features for processing to peptides that are capable of binding to MHC class I molecules (54,59). In addition, γ -IFN induces a special proteasome-activating complex, PA28. It binds to one end of the 20S proteasome and forms a hybrid 26S particle that has a 19S complex at the other end (60). These hybrid particles degrade ubiquitinated proteins at normal rates but cleave them differently, generating an even higher fraction of peptides that are capable of serving as antigenic precursors. At the same time, γ -IFN can cause cells to express higher amounts of the TAP transporter, the ERAP1 peptide trimming enzyme, and MHC molecules (54,56,57). Together, these changes stimulate the host defense. Presumably, antigen processing by these systems normally functions well below maximal levels until activation of the host's defenses is necessary. However, a number of viruses have evolved sophisticated mechanisms to escape immune detection by inhibiting the uptake of proteasome products into the ER by the TAP transporter or by promoting the degradation of MHC class I molecules (55). This competition between immune defenses and viruses demonstrates that the immune systems in higher vertebrates have evolved in terms of modifications of the UPP that otherwise is highly conserved in organisms from yeast to human. These alterations in proteasome structure and the changes in the proteolytic pathway allow efficient antigen presentation and, therefore, the basal recognition of infected or neoplastic cells and their rapid elimination under a variety of conditions.

Maintenance of Tissue Protein Content in Chronic Kidney Disease

The maintenance of tissue mass and body protein stores in normal adults occurs when cells achieve a balance between the rates of synthesis and degradation of cell proteins. In uremic patients, protein stores frequently are depressed: Serum albumin is low, and there is weight loss largely as a result of loss of muscle mass (61,62). This decrease in body protein has been attributed to "malnutrition" (*i.e.*, an insufficient or imbalanced diet), but, in fact, it results from complex physiologic and biochemical adaptations (63,64). In experimental animals and humans with uremia, overall rates of protein synthesis generally are unchanged, whereas rates of protein degradation tend to increase (65,66). Because the rates of protein turnover in cells are very high (3.5 to 4.5 g protein/kg per d) (1), even a small increase in proteolysis, with time, will cause marked protein depletion. Most of this acceleration of protein degradation in muscle in disease states occurs *via* a programmed activation of the UPP (66,67).

Mechanisms That Cause Loss of Muscle Protein in Uremia

Recent studies in rodent models have established that accelerated muscle protein catabolism that is induced by uremia involves similar cellular mechanisms that cause muscle wasting in a variety of other catabolic conditions, such as cancer cachexia, starvation, insulin deficiency, or sepsis (1). Atrophying muscles from such animals show accelerated proteolysis *via* the UPP, higher levels of mRNA that encode certain components of this proteolytic system, and a similar pattern of changes (both increases or decreases) in the expression of approximately 100 atrophy-related genes, also termed atrogenes (35) (Table 1). In humans with these conditions, there also is evidence for activation of the UPP in muscle (*e.g.*, an increase in mRNA that encode Ub and proteasome subunits) (67–70). In these catabolic states, the increase in mRNA levels for these atrophy-related genes in muscle occurs through increased gene transcription (35,66,71,72). As part of this common transcriptional program, the expression of various growth-related genes decreases in the atrophying tissues (35). Therefore, multiple transcriptional factors seem to change coordinately to bring about the loss of muscle mass. The strongest evidence for activation of the UPP muscles of animals that undergo atrophy as a result of uremia (or other catabolic diseases) is that when studied *in vitro*, muscles from these models exhibit increased proteolysis that can be blocked by inhibitors of the proteasome (66,71,73).

For these reasons, muscle wasting represents a specific, carefully orchestrated response that is triggered by various stimuli in various pathologic conditions (*e.g.*, acidosis in renal failure, low insulin levels in fasting and diabetes, inactivity, glucocorticoids or cytokines in sepsis). In fasting and presumably in other disease states, an identifiable function of accelerated proteolysis is to mobilize amino acids from dispensable muscle proteins to provide the organism with precursors for hepatic gluconeogenesis or for new protein synthesis (1). However, these losses can have deleterious effects with time, especially in renal failure, in which disposal of nitrogenous waste is com-

promised, and they accumulate to cause symptoms and metabolic problems.

Because the UPP serves many essential functions in cell regulation and homeostasis, its activation in these states must be highly selective and precisely regulated to avoid the unwanted removal of muscle proteins that are essential for cell function in muscle and other organs. Whereas atrophy seems to affect all muscle cell components, contractile proteins are lost differentially. The specificity of the UPP in protein breakdown is determined by the cell's content of Ub ligases (E3s), which, as discussed, target specific sets of proteins for degradation. The cellular content of E3s varies among tissues and physiologic states. Two Ub ligases, atrogen-1 (also known as MAFbx) and MuRF-1, are specific constituents of muscle; their expression increases dramatically (eight- to 20-fold) in catabolic states, and they play a critical role in mediating the loss of muscle protein (Figure 5). In mice that lack the genes for atrogen-1 or MuRF-1, muscles grow normally, but if they are denervated, then the rate of muscle atrophy is reduced (74). Muscles of these knock-

Table 1. Biochemical adaptations found in atrophying skeletal muscle^a

Increased ATP-dependent proteolysis by the ubiquitin-proteasome pathway
Greater sensitivity to proteasome inhibitors
Associated with activation of caspase-3 which provides substrates for the ubiquitin-proteasome pathway
Increased conjugation of ubiquitin to muscle proteins
Activation of a common transcriptional program:
induction/suppression of a set of atrogenes
Upregulation of the muscle-specific Ub-protein ligases, atrogen-1/MAFbx and MuRF1
Suppression of the IGF-1/PI-3 kinase/Akt signaling pathway and activation of FoxO transcription factors

^aMuRF-1, muscle ring finger-1; PI3-K, phosphatidylinositol 3-kinase; Ub, ubiquitin; UPP, ubiquitin-proteasome pathway.

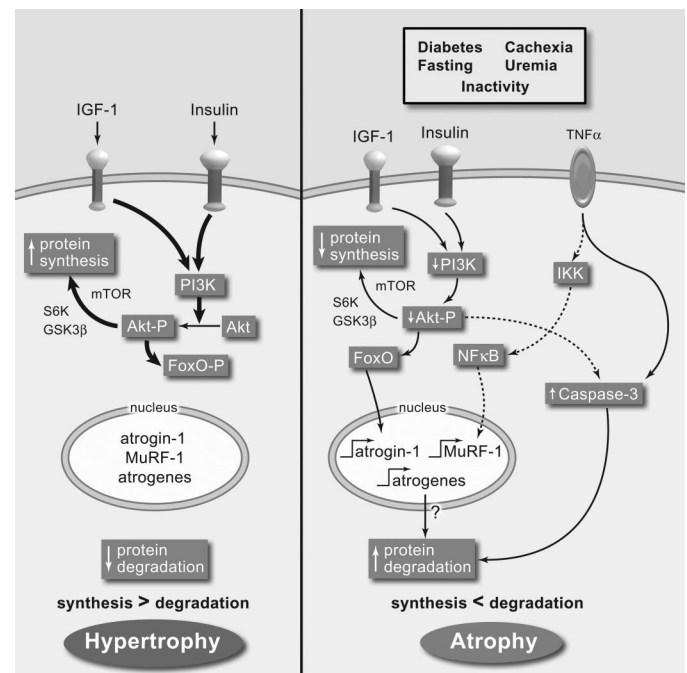


Figure 5. The balance between protein synthesis and degradation determines whether muscles hypertrophy or atrophy. With hypertrophy, protein synthesis exceeds degradation because the phosphatidylinositol 3 kinase (PI3-K)/Akt pathway stimulates protein synthesis and phosphorylates the FoxO transcription factors that stimulate protein degradative processes. In diseases that suppress insulin/IGF-1 signaling, the PI3-K/Akt pathway is depressed, leading to decreased protein synthesis and FoxO phosphorylation. This allows FoxO to stimulate the expression of the E3 enzymes atrogen-1 and muscle ring finger-1 (MuRF-1) and protein degradation. Another stimulus, inflammatory cytokines such as TNF- α , increases the activation of NF- κ B, leading to expression of the E3 enzyme MuRF-1 and protein degradation. Illustration by Josh Gramling—Gramling Medical Illustration.

out mice also show reduced atrophy upon fasting. In wild-type animals that are subjected to muscle denervation or disuse, expression of atrogin-1 and MuRF-1 rises quickly just when muscle atrophy is most rapid, and in cultured muscle cells, the content of atrogin-1 mRNA correlates tightly with rates of protein breakdown (75–77). Therefore, the muscle's content of atrogin-1 mRNA is an excellent biomarker for the rates of proteolysis and rapid wasting.

Are there other functions for atrogin-1/MAFbx? Studies in cultured skeletal muscle cells and in transgenic mice that over-express atrogin-1 in heart muscle have suggested that myoD and calcineurin A might be substrates for ubiquitination by atrogin-1 (38,78). It is unlikely that degradation of myoD by atrogin-1 will explain the mechanism of muscle atrophy, because myoD is critical for the differentiation of muscle cells during development, whereas atrogin-1 is expressed in fully differentiated muscle only during wasting. It is not known whether calcineurin A is a target of atrogin-1 in skeletal muscle, but in terms of maintaining muscle mass, calcineurin signaling pathways seem to affect fiber type more than fiber size (79). MuRF-1 was found to interact with glucocorticoid modulatory element binding protein-1, a glucocorticoid-modulated nuclear protein that regulates transcriptional responses (80). It is intriguing that glucocorticoids are potent activators of muscle protein breakdown and that MuRF-1 expression is increased in the presence of glucocorticoids. MuRF-1 has been found to associate with and conjugate Ub to troponin I in cardiac muscle; it is not known whether MuRF-1 serves a similar function in skeletal muscle (81).

As discussed below, the FoxO and NF- κ B transcription factors act on the promoters for atrogin-1 and MuRF-1 to stimulate their expression, respectively. When activated, FoxO and NF- κ B cause profound muscle wasting through induction of these E3s together with other atrogenes (75,82,83). Because muscle atrophy that is caused by uremia and other catabolic diseases requires activation of UPP generally and atrogin-1 and MuRF-1 specifically (35,82), efforts are being pursued to reduce the activities of the Ub proteasome pathway or to prevent the induction or activation of these E3s as means of slowing muscle wasting. It is not known how the evidence of inflammation that frequently is present in patients with kidney disease affects muscle protein degradation (64).

Recently, a homologue of E3 α , E3 α -II, was identified by Han and colleagues (84). They found a dramatic upregulation of E3 α -II in atrophying muscles of tumor-bearing rodents. Their results suggest that E3 α -II or a combination of E3 α and E3 α -II may be responsible for the increased rates of Ub conjugation to muscle proteins by the N-end rule pathway.

Initial Cleavage of Myofibrillar Proteins in Uremia by Caspase-3

Myofibrillar proteins, which comprise approximately two thirds of the protein in muscle, require additional mechanisms for their degradation in addition to the UPP. Other steps are necessary because the UPP readily degrades the main components of the myofibril (actin, myosin, troponin, or tropomyosin). These same proteins are digested only very slowly by the

UPP when they are present as complexes or in intact myofibrils (85). Recent findings suggest that these proteins initially are cleaved by cytosolic proteases, which promote myofibrillar disassembly to facilitate degradation of constituent proteins by the proteasome. Cleaved components of myofibrils may be recognized by E3 α , the N-end rule E3 that recognizes certain amino terminal amino acids (86–88). Because most proteins have N-terminal amino acids that are not recognized by E3 α , the initial cleavage of myofibrils might explain why activity of the N-end rule pathway in muscle extracts from catabolic animals is increased. Because another protease must breakdown the complex structure of muscle to provide substrates for the UPP, we tested whether caspases may play this critical initial role. Indeed, several catabolic states (sepsis, renal failure) are characterized by high circulating levels of TNF- α or insulin resistance, conditions that induce programmed cell death by activating the caspase cascade (89–91). We found that caspase-3 cleaves actomyosin *in vitro* and in cultured muscle cells to produce and yields substrates that are degraded rapidly by the UPP as well as a 14-kD C-terminal fragment of actin that accumulates in the insoluble fraction of the cell (92). The same processes occur in the muscle of animals with uremia, diabetes, and angiotensin II-induced hypertension, and in humans, a similar 14-kD actin fragment is found in muscle of patients with muscle atrophy as a result of disuse from the pain of osteodystrophy and related to either hemodialysis treatment or burn injury (92–94).

Another family of proteases, the calpains, also has been suggested as a mechanism that catalyzes the initial cleavage of myofibrillar proteins in atrophy. Calpains are calcium-dependent cysteine proteases that have been suggested to play roles in several disorders, especially muscular dystrophy. Evidence for calpain's activation has been reported in muscles of animals with sepsis (95). Although inhibition of the calcium-activated proteases in muscles from rodents with uremia or several other types of atrophy has not been found to block the increase in protein degradation or the degradation of myofibrillar proteins or the accumulation of the 14-kD actin fragment in muscle cells (92), the transgenic expression of calpastatin, an endogenous calpain inhibitor, can reduce denervation atrophy and progression of muscular dystrophy in mice (96). Additional studies are needed to determine whether different catabolic disorders stimulate caspase-3 and/or calpains and their specific roles of these proteases in the breakdown of different muscle proteins.

Signals That Trigger Muscle Atrophy in Kidney Disease and Other Catabolic States

Chronic kidney disease is associated with several physiologic complications that can trigger the UPP to breakdown muscle protein, metabolic acidosis, decreased insulin action, increased glucocorticoids, high levels of angiotensin II, and inflammation (66,71,93,97). These complications seem to be related to and function in concert to bring about protein loss. For example, metabolic acidosis stimulates muscle protein breakdown by the UPP, but it also increases glucocorticoid production and causes insulin resistance in muscle. Either of these clinical problems can activate the UPP and the breakdown of muscle protein (72,90,98–100). Glucocorticoids also suppress insulin sensitiv-

ity, and insulin deficiency or insulin resistance increases production of these hormones (72,100,101). Finally, the sensitivity of muscle to catabolic signals, such as glucocorticoids, rises with inactivity. Angiotensin II infusion into rodents causes both anorexia and muscle protein losses by mechanisms that depend on glucocorticoids (93). Last, inflammatory mediators suppress insulin responses, and various conditions that are associated with inflammation (*e.g.*, arthritis) can cause muscle wasting, but the link between the increase in inflammatory markers (*e.g.*, C-reactive protein and other acute-phase reactant proteins) in uremic patients and their loss of muscle mass has not been established (83,102).

As noted, activation of the UPP in various types of muscle wasting and the coordinated changes in the expression of a set of genes in muscle suggest that these catabolic states activate a common cellular signaling pathway (35). Recent studies have established that in fasting and, presumably, other insulin-deficient states, the fall in protein synthesis and the rise in proteolysis are linked events that occur through decreased signaling by the phosphatidylinositol 3-kinase/Akt (PI3-K/Akt) pathway, which in normal individuals is activated by insulin or IGF-1 (82,103,104). IGF-1 is released by the liver and mediates the anabolic effects of the growth hormone, but it also is an autocrine factor that is released by muscle after exercise. In fasting and disease, when insulin signaling is low, the activity of PI3-K is reduced and there is less production of phosphatidylinositol-3,4,5 phosphate, the active product of PI3-K; the decrease in this regulatory molecule decreases the phosphorylation and activity of the serine/threonine kinase Akt (Figure 5). Activated Akt is a major activator of growth-related processes, and, when overproduced, Akt causes muscle hypertrophy *via* phosphorylation of the downstream kinases glycogen synthase kinase β (GSK3 β) and mammalian target of rapamycin (mTOR)/S6kinase, which enhance protein synthesis by ribosomes. In many types of atrophy, the reduction in signaling by the PI3-K/Akt pathway leads to reduced mRNA translation and protein production. Rapid atrophy, however, also requires enhanced proteolysis and induction of the E3 atrogin-1 and MuRF-1, leading somehow to enhanced protein degradation in muscle cells (75,76,82). These recent results show that activation of protein degradation and induction of these E3 enzymes in atrophying muscles also result from a decrease in activated Akt (*i.e.*, phosphorylated Akt). One of the targets of activated Akt is the forkhead family of the transcription factors (FoxO1, 3, and 4); when they are not phosphorylated, they migrate into the nucleus and catalyze the transcription of atrogin-1 (75,76,82). Conversely, insulin and IGF-1, by activating PI3-K and Akt, phosphorylate FoxO and suppress expression of atrogin-1 (Figure 5).

In addition to activating atrogin-1/MAFbx, the activation of FoxO will stimulate the cell's other proteolytic system, the autophagic-lysosomal pathway. This pathway seems to catalyze the degradation of particular cellular components, especially mitochondria. The influence of Akt on the other critical E3 Ub ligase, MuRF-1, is somewhat less clear, but decreased phosphorylation of Akt was found to increase MuRF-1 and atrogin-1 transcription after glucocorticoid treatment or dener-

vation (76). There also is emerging evidence that an unrelated transcription factor, NF- κ B, can cause overproduction of MuRF-1 and muscle atrophy. Like FoxO3, NF- κ B, if overproduced, can cause dramatic muscle atrophy and also seems to be required for muscle denervation or disuse atrophy and cancer cachexia (83,105). In the various conditions that are associated with muscle atrophy, both transcription factors seem to contribute to muscle wasting, but their relative importance remains to be resolved.

Besides controlling the expression of E3 Ub ligases, activation of the insulin/IGF-1—PI3-K/Akt pathway suppresses activation of caspase-3. This would decrease the breakdown of the complex structure of muscle proteins. In insulin-deficient rats that exhibit accelerated muscle protein degradation, we found that the proapoptotic factor Bax is activated, leading to the release of cytochrome C from mitochondria, which in turn activates caspase-3 and increases production of the 14-kD actin fragment (82). Similar changes were seen in cultured muscle cells with genetic or pharmacologic inhibition of PI3-K activity. Together, these recent developments provide evidence that the activation of muscle protein loss in kidney disease and other catabolic conditions occurs through a common signaling pathway that alters transcription of key enzymes that modulate protein synthesis and degradation in complementary ways to cause muscle wasting.

One of the key endocrine factors that are essential for these catabolic responses is glucocorticoids. Administration of high doses of these hormones to rodents, like Cushing's syndrome in humans, stimulates protein degradation in muscle *via* atrogin-1, and this response requires FoxO activation (77,106,107). *In vivo*, glucocorticoids can inhibit insulin/IGF-1 responses, by complex mechanisms. These hormones also exert permissive effects that activate the UPP when other catabolic signals are present. For example, activation of muscle proteolysis does not occur in adrenalectomized animals that are starved or treated with NH₄Cl to induce metabolic acidosis or made insulin deficient unless the animals are given a physiologic dose of glucocorticoids (72,108,109). Similarly, the increase in muscle proteolysis that is induced by angiotensin II or sepsis can be blocked by inhibiting the glucocorticoid receptor (93). It is important to note that in these experiments, the same physiologic levels of cortisol did not stimulate muscle protein degradation unless the animals also were acidotic or made insulin deficient; normal animals require higher, pharmacologic doses to cause muscle wasting. These complex regulatory interactions actually make "physiologic sense" because glucocorticoids evolved to integrate stress responses in various tissues. When glucose is needed, an increase in glucocorticoids mobilizes amino acids from muscle protein. At the same time, these hormones induce gluconeogenic enzymes in liver that catalyze conversion of the amino acids to glucose and urea.

Conclusion

In this brief review of the UPP, we have discussed how the complex series of biochemical reactions within this pathway act to tag and degrade proteins. Because the UPP is responsible for the turnover of so many different cellular proteins, there are

critical mechanisms that regulate its function precisely. We have emphasized the importance of the UPP in the turnover of transport proteins, in presenting antigens to the immune system, and how uremia activates the UPP to cause muscle wasting because these functions are of special interest to nephrology. However, the UPP also plays important roles in the regulation of other cellular functions, ranging from the control of the cell cycle to activities that promote cancer. Indeed, inhibitors of proteasome activity, the final component of the pathway, have emerged as novel chemotherapeutic agents. Involvement in such a wide range of functions explains why the 2004 Nobel Prize in Chemistry was awarded for the discovery of Ub and its role in orchestrating cellular protein turnover.

Acknowledgments

This study was supported by National Institutes of Health grants R01 DK62307 (S.H.L.), R01 GM51923, R01 GM46147 (A.L.G.), and DK37175, and P50 DK64233 (W.E.M.).

References

- Mitch WE, Goldberg AL: Mechanisms of muscle wasting: The role of the ubiquitin-proteasome system. *N Engl J Med* 335: 1897–1905, 1996
- Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, Hwang D, Goldberg AL: 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, 1994
- Baehrecke EH: Autophagy: Dual roles in life and death? *Nat Rev Mol Cell Biol* 6: 505–510, 2005
- Lardeux BR, Mortimore GE: Amino acid and hormonal control of macromolecular turnover in perfused rat liver. Evidence for selective autophagy. *J Biol Chem* 262: 14514–14519, 1987
- Gronostajski RM, Goldberg AL, Pardee AB: The role of increased proteolysis in the atrophy and arrest of cell division in fibroblasts following serum deprivation. *J Cell Biol* 121: 189–198, 1984
- Goll DE, Thompson VF, Li H, Wei W, Cong J: The calpain system. *Physiol Rev* 83: 731–801, 2003
- Salvesen GS, Dixit VM: Caspases: Intracellular signaling by proteolysis. *Cell* 91: 443–446, 1997
- Glickman MH, Ciechanover A: The ubiquitin-proteasome proteolytic pathway: Destruction for the sake of construction. *Physiol Rev* 82: 373–428, 2002
- Pickart CM: Back to the future with ubiquitin. *Cell* 116: 181–190, 2004
- Baumeister W, Walz J, Zuhl F, Seemuller E: The proteasome: Paradigm of a self-compartmentalizing protease. *Cell* 92: 367–380, 1998
- Muratani M, Tansey WP: How the ubiquitin-proteasome system controls transcription. *Nat Rev Mol Cell Biol* 4: 192–201, 2003
- Lipford JR, Smith GT, Chi Y, Deshaies RJ: A putative stimulatory role for activator turnover in gene expression. *Nature* 438: 113–116, 2005
- Karin M, Ben-Neriah Y: Phosphorylation meets ubiquitination: The control of NF-kappaB activity. *Annu Rev Immunol* 18: 621–663, 2000
- Jensen TJ, Loo MA, Pind S, Williams DB, Goldberg AL, Riordan JR: Multiple proteolytic systems, including the proteasome contribute CFTR processing. *Cell* 83: 129–135, 1995
- Ward CL, Omura S, Kopito RR: Degradation of CFTR by the ubiquitin-proteasome pathway. *Cell* 83: 121–127, 1995
- Meusser B, Hirsch C, Jarosch E, Sommer T: ERAD: The long road to destruction. *Nat Cell Biol* 7: 766–772, 2005
- Hicke L, Dunn R: Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. *Annu Rev Cell Dev Biol* 19: 141–172, 2003
- Sigismund S, Polo S, Di Fiore PP: Signaling through monoubiquitination. *Curr Top Microbiol Immunol* 286: 149–185, 2004
- Haas AL, Rose IA: The mechanism of ubiquitin activating enzyme. A kinetic and equilibrium analysis. *J Biol Chem* 257: 10329–10337, 1982
- Handley PM, Mueckler M, Siegel NR, Ciechanover A, Schwartz AL: Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme E1. *Proc Natl Acad Sci U S A* 88: 258–262, 1991
- Jentsch S: The ubiquitin-conjugation system. *Annu Rev Genet* 26: 179–207, 1992
- Jackson PK, Eldridge AG, Freed E, Furstenthal L, Hsu JY, Kaiser BK, Reimann JD: The lore of the RINGs: Substrate recognition and catalysis by ubiquitin ligases. *Trends Cell Biol* 10: 429–439, 2000
- Ingham RJ, Gish G, Pawson T: The Nedd4 family of E3 ubiquitin ligases: Functional diversity within a common modular architecture. *Oncogene* 23: 1972–1984, 2004
- Scheffner M, Nuber U, Huibregtse JM: Protein ubiquitination involving an E1–E2–E3 enzyme ubiquitin thioester cascade. *Nature* 373: 81–83, 1995
- Huibregtse JM, Scheffner M, Beaudenon S, Howley PM: A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. *Proc Natl Acad Sci U S A* 92: 5249, 1995
- Albrecht U, Sutcliffe JS, Cattanauch BM, Beechey CV, Armstrong D, Eichele G, Beaudet AL: Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. *Nat Genet* 17: 75–78, 1997
- Malik B, Schlanger L, Al-Khalili O, Bao H-F, Yue G, Price SR, Mitch WE, Eaton DC: ENaC degradation in A6 cells by the ubiquitin-proteasome proteolytic pathway. *J Biol Chem* 276: 12903–12910, 2001
- Kamynina E, Debonneville C, Hirt RP, Staub O: Liddle's syndrome: A novel mouse Nedd4 isoform regulates the activity of the epithelial Na(+) channel. *Kidney Int* 60: 466–471, 2001
- Petroski MD, Deshaies RJ: Function and regulation of cullin-RING ubiquitin ligases. *Nat Rev Mol Cell Biol* 6: 9–20, 2005
- Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM: RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc Natl Acad Sci U S A* 96: 11364–11369, 1999
- Seol JH, Feldman RM, Zachariae W, Shevchenko A, Correll CC, Lyapina S, Chi Y, Galova M, Claypool J, Sandmeyer S, Nasmyth K, Deshaies RJ, Shevchenko A, Deshaies RJ: Cdc53/cullin and the essential Hrt1 RING-H2 subunit of SCF define a ubiquitin ligase module that activates the E2 enzyme Cdc34. *Genes Dev* 13: 1614–1626, 1999

32. Elenbaas B, Dobbelstein M, Roth J, Shenk T, Levine AJ: The MDM2 oncoprotein binds specifically to RNA through its RING finger domain. *Mol Med* 2: 439–451, 1996
- 32a. Varshavsky A, Turner G, Du F, Xie Y: Felix Hoppe-Seyler Lecture 2000: The ubiquitin system and the N-end rule pathway. *Biol Chem* 381: 779–789, 2000
33. Rao H, Uhlmann F, Nasmyth K, Varshavsky A: Degradation of a cohesin subunit by the N-end rule pathway is essential for chromosome stability. *Nature* 410: 955–959, 2001
34. Hu RG, Sheng J, Qi X, Xu Z, Takahashi TT, Varshavsky A: The N-end rule pathway as a nitric oxide sensor controlling the levels of multiple regulators. *Nature* 437: 981–986, 2005
35. Lecker SH, Jagoe RT, Gomes M, Baracos V, Bailey JL, Price SR, Mitch WE, Goldberg AL: Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J* 18: 39–51, 2004
36. Kaelin WG Jr: How oxygen makes its presence felt. *Genes Dev* 16: 1441–1445, 2002
37. Hatakeyama S, Nakayama KI: U-box proteins as a new family of ubiquitin ligases. *Biochem Biophys Res Commun* 302: 635–645, 2003
38. Li HH, Kedar V, Zhang C, McDonough H, Arya R, Wang DZ, Patterson C: Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex. *J Clin Invest* 114: 1058–1071, 2004
39. Voges D, Zwickl P, Baumeister W: The 26S proteasome: A molecular machine designed for controlled proteolysis. *Annu Rev Biochem* 68: 1015–1068, 2000
40. Kisselev AF, Goldberg AL: Proteasome inhibitors: From research tools to drug candidates. *Chem Biol* 8: 739–758, 2001
41. Groll M, Bajorek M, Kohler A, Moroder L, Rubin DM, Huber R, Glickman MH, Finley D: A gated channel into the proteasome core particle. *Nat Struct Biol* 7: 1062–1067, 2000
42. Goldberg AL: Nobel committee tags ubiquitin for distinction. *Neuron* 45: 339–344, 2005
43. Benaroudj N, Zwickl P, Seemuller E, Baumeister W, Goldberg AL: ATP hydrolysis by the proteasome regulatory complex PAN serves multiple functions in protein degradation. *Mol Cell* 11: 69–78, 2003
44. Schmidt M, Hanna J, Elsassner S, Finley D: Proteasome-associated proteins: Regulation of a proteolytic machine. *Biol Chem* 386: 725–737, 2005
45. Goldberg AL: Protein degradation and protection against misfolded or damaged proteins. *Nature* 426: 895–899, 2003
46. Smith DM, Kafri G, Cheng Y, Ng D, Walz T, Goldberg AL: ATP binding to PAN or the 26S ATPases causes association with the 20S proteasome, gate opening, and translocation of unfolded proteins. *Mol Cell* 20: 687–698, 2005
47. Kisselev AF, Akopian TN, Woo KM, Goldberg AL: The sizes of peptides generated from protein by mammalian 26 and 20 S proteasomes. Implications for understanding the degradative mechanism and antigen presentation. *J Biol Chem* 274: 3363–3371, 1999
48. Reits E, Neijssen J, Herberts C, Benckhuijsen W, Janssen L, Drijfhout JW, Neefjes J: A major role for TPPII in trimming proteasomal degradation products for MHC class I antigen presentation. *Immunity* 20: 495–506, 2004
49. Saric T, Graef CI, Goldberg AL: Pathway for degradation of peptides generated by proteasomes: A key role for thimet oligopeptidase and other metallopeptidases. *J Biol Chem* 279: 46723–46732, 2004
50. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T: The ubiquitin-proteasome pathway is required for processing the NF-kappaB1 precursor protein and the activation of NF-kappaB. *Cell* 78: 773–785, 1994
51. Venkatraman P, Wetzel R, Tanaka M, Nukina N, Goldberg AL: Eukaryotic proteasomes cannot digest polyglutamine sequences and release them during degradation of polyglutamine-containing proteins. *Mol Cell* 14: 95–104, 2004
52. Adams J: The proteasome: A suitable antineoplastic target. *Nat Rev Cancer* 4: 349–360, 2004
53. Shastri N, Schwab S, Serwold T: Producing nature's gene-chips: The generation of peptides for display by MHC class I molecules. *Annu Rev Immunol* 20: 463–493, 2002
54. Rock KL, York IA, Saric T, Goldberg AL: Protein degradation and the generation of MHC class I-presented peptides. *Adv Immunol* 80: 1–70, 2002
55. Tortorella D, Gewurz BE, Furman MH, Schust DJ, Ploegh HL: Viral subversion of the immune system. *Annu Rev Immunol* 18: 861–926, 2000
56. Rock KL, York IA, Goldberg AL: Post-proteasomal antigen processing for major histocompatibility complex class I presentation. *Nat Immunol* 5: 670–677, 2004
57. Saric T, Chang SC, Hattori A, York IA, Markant S, Rock KL, Tsujimoto M, Goldberg AL: An IFN-gamma-induced aminopeptidase in the ER, ERAP1, trims precursors to MHC class I-presented peptides. *Nat Immunol* 3: 1169–1176, 2002
58. York IA, Chang SC, Saric T, Keys JA, Favreau JM, Goldberg AL, Rock KL: The ER aminopeptidase ERAP1 enhances or limits antigen presentation by trimming epitopes to 8–9 residues. *Nat Immunol* 3: 1177–1184, 2002
59. Gaczynska M, Rock KL, Spies T, Goldberg AL: Peptidase activities of proteasomes are differentially regulated by the MHC-encoded genes LMP2 and LMP7. *Proc Natl Acad Sci U S A* 91: 9213–9217, 1994
60. Cascio P, Call M, Petre BM, Walz T, Goldberg AL: Properties of the hybrid form of the 26S proteasome containing both 19S and PA28 complexes. *EMBO J* 21: 2636–2645, 2002
61. Qureshi AR, Alvestrand A, Danielsson A, Divino-Filho JC, Gutierrez A, Lindholm B, Bergstrom J: Factors predicting malnutrition in hemodialysis patients: A cross-sectional study. *Kidney Int* 53: 773–782, 1998
62. Kopple JD: National Kidney Foundation K/DOQI clinical practice guidelines for nutrition in chronic renal failure. *Am J Kidney Dis* 37: S66–S70, 2001
63. Mitch WE: Malnutrition: A frequent misdiagnosis for hemodialysis patients. *J Clin Invest* 110: 437–439, 2002
64. Kaysen GA, Dubin JA, Muller H-G, Rosales L, Levin NW, Mitch WE: Inflammation and reduced albumin synthesis associated with stable decline in serum albumin in hemodialysis patients. *Kidney Int* 65: 1408–1415, 2004
65. Lim VS, Kopple JD: Protein metabolism in patients with chronic renal failure: Role of uremia and dialysis. *Kidney Int* 58: 1–10, 2000
66. Bailey JL, Wang X, England BK, Price SR, Ding X, Mitch WE: The acidosis of chronic renal failure activates muscle proteolysis in rats by augmenting transcription of genes encoding proteins of the ATP-dependent, ubiquitin-proteasome pathway. *J Clin Invest* 97: 1447–1453, 1996
67. Pickering WP, Price SR, Bircher G, Marinovic AC, Mitch

- WE, Walls J: Nutrition in CAPD: Serum bicarbonate and the ubiquitin-proteasome system in muscle. *Kidney Int* 61: 1286–1292, 2002
68. Mansoor O, Beaufre Y, Boirie Y, Ralliere C, Taillandier D, Aurousseau E, Schoeffler P, Arnal M, Attaix D: Increased mRNA levels for components of the lysosomal, Ca^{++} -activated and ATP-ubiquitin-dependent proteolytic pathways in skeletal muscle from head trauma patients. *Proc Natl Acad Sci U S A* 93: 2714–2718, 1996
69. Tiao G, Hobler S, Wang JJ, Meyer TA, Luchette FA, Fischer JE, Hasselgren P-O: Sepsis is associated with increased mRNAs of the ubiquitin-proteasome proteolytic pathway in human skeletal muscle. *J Clin Invest* 99: 163–168, 1997
70. Williams AB, Sun X, Fischer JE, Hasselgren P-O: The expression of genes in the ubiquitin-proteasome proteolytic pathway is increased in skeletal muscle from patients with cancer. *Surgery* 126: 744–749, 1999
71. Price SR, Bailey JL, Wang X, Jurkovitz C, England BK, Ding X, Phillips LS, Mitch WE: Muscle wasting in insulinopenic rats results from activation of the ATP-dependent, ubiquitin-proteasome pathway by a mechanism including gene transcription. *J Clin Invest* 98: 1703–1708, 1996
72. Mitch WE, Bailey JL, Wang X, Jurkovitz C, Newby D, Price SR: Evaluation of signals activating ubiquitin-proteasome proteolysis in a model of muscle wasting. *Am J Physiol* 276: C1132–C1138, 1999
73. Tawa NE, Odessey R, Goldberg AL: Inhibitors of the proteasome reduce the accelerated proteolysis in atrophying rat skeletal muscles. *J Clin Invest* 100: 197–203, 1997
74. Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clark BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuel DM, DeChiara TM, Stitt TN, Yancopoulos GD, Glass DJ: Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294: 1704–1708, 2001
75. Sandri M, Sandri C, Gilbert A, Skuck C, Calabria E, Picard A, Walsh K, Schiaffino S, Lecker SH, Goldberg AL: Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 117: 399–412, 2004
76. Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, Klinenber JR, Gonzalez M, Yancopoulos GD, Glass DJ: The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell* 14: 395–403, 2004
77. Sackey JM, Ohtsuka A, McLary SC, Goldberg AL: IGF-1 stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. *Am J Physiol* 287: E591–E601, 2004
78. Tintignac LA, Lagirand J, Battonnet S, Sirri V, Leibovitch MP, Leibovitch SA: Degradation of MyoD mediated by the SCF (MAFbx) ubiquitin ligase. *J Biol Chem* 280: 2847–2856, 2005
79. Serrano AL, Murgia M, Pallafacchina G, Calabria E, Coniglio P, Lomo T, Schiaffino S: Calcineurin controls nerve activity-dependent specification of slow skeletal muscle fibers but not muscle growth. *Proc Natl Acad Sci U S A* 98: 13108–13113, 2001
80. McElhinny AS, Kakinuma K, Sorimachi H, Labeit S, Gregorio CC: 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. *J Cell Biol* 157: 125–136, 2002
81. Kedar V, McDonough H, Arya R, Li HH, Rockman HA, Patterson C: Muscle-specific RING finger 1 is a bona fide ubiquitin ligase that degrades cardiac troponin 1. *Proc Natl Acad Sci U S A* 101: 18135–18140, 2004
82. Lee SW, Dai G, Hu Z, Wang X, Du J, Mitch WE: Regulation of muscle protein degradation: Coordinated control of apoptotic and ubiquitin-proteasome systems by phosphatidylinositol 3 kinase. *J Am Soc Nephrol* 15: 1537–1545, 2004
83. Cai D, Frantz JD, Tawa NE, Melendez PA, Oh BC, Lidov HG, Hasselgren PO, Frontera WR, Lee J, Glass DJ, Shoelson SE: IKK β /NF- κ B activation causes severe muscle wasting in mice. *Cell* 119: 285–298, 2004
84. Kwak KS, Zhou X, Solomon V, Baracos VE, Davis J, Bannon AW, Boyle WJ, Lacey DL, Han HQ: Regulation of protein catabolism by muscle-specific and cytokine-inducible ubiquitin ligase E3 α -II during cancer cachexia. *Cancer Res* 64: 8193–8198, 2004
85. Solomon V, Goldberg AL: Importance of the ATP-ubiquitin-proteasome pathway in degradation of soluble and myofibrillar proteins in rabbit muscle extracts. *J Biol Chem* 271: 26690–26697, 1996
86. Varshavsky A: The N-end rule: Functions, mysteries, uses. *Proc Natl Acad Sci U S A* 93: 12142–12149, 1996
87. Solomon V, Baracos V, Sarraf P, Goldberg AL: Rates of ubiquitin conjugation increase when muscles atrophy, largely through activation of the N-end rule pathway. *Proc Natl Acad Sci U S A* 95: 12602–12607, 1998
88. Lecker SH, Solomon V, Price SR, Kwon YT, Mitch WE, Goldberg AL: Ubiquitin conjugation by the N-end rule pathway and mRNAs for its components increase in muscles of diabetic rats. *J Clin Invest* 104: 1411–1420, 1999
89. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF- α - and obesity-induced insulin resistance. *Science* 271: 665–668, 1996
90. DeFronzo RA, Beckles AD: Glucose intolerance following chronic metabolic acidosis in man. *Am J Physiol* 236: E328–E334, 1979
91. Kimmel PL, Phillips TM, Simmens SJ, Peterson RA, Weihs KL, Alleyne S, Cruz I, Yanovski JA, Veis JH: Immunologic function and survival in hemodialysis patients. *Kidney Int* 54: 236–244, 1998
92. Du J, Wang X, Meireles CL, Bailey JL, Debigare R, Zheng B, Price SR, Mitch WE: Activation of caspase 3 is an initial step triggering muscle proteolysis in catabolic conditions. *J Clin Invest* 113: 115–123, 2004
93. Song Y-H, Li Y, Du J, Mitch WE, Rosenthal N, Delafontaine P: Muscle-specific expression of insulin-like growth factor-1 blocks angiotensin II-induced skeletal muscle wasting. *J Clin Invest* 115: 451–458, 2005
94. Workeneh BT, Rondon-Berrios H, Ayehu G, Hu Z, Ferrando A, Wang H, Kopple JD, Du J, Mitch WE: Development of a diagnostic method for detecting increased muscle protein degradation in patients with catabolic conditions, including kidney failure [Abstract]. *J Am Soc Nephrol* 16: 713A, 2005
95. Wei W, Fareed MU, Evenson A, Menconi MJ, Yang H,

- Petkova V, Hasselgren PO: Sepsis stimulates calpain activity in skeletal muscle by decreasing calpastatin activity but does not activate caspase-3. *Am J Physiol Regul Integr Comp Physiol* 288: R580–R590, 2005
96. Tidball JG, Spencer MJ: Expression of a calpastatin transgene slows muscle wasting and obviates changes in myosin isoform expression during murine muscle disuse. *J Physiol* 545: 819–828, 2002
97. Stenvinkel P, Heimbürger O, Paulsen F, Diczfalussy U, Wang T, Berglund L, Jørgensen T: Strong association between malnutrition, inflammation and atherosclerosis in chronic kidney failure. *Kidney Int* 55: 1899–1911, 1999
98. Mitch WE, Medina R, Greiber S, May RC, England BK, Price SR, Bailey JL, Goldberg AL: Metabolic acidosis stimulates muscle protein degradation by activating the ATP-dependent pathway involving ubiquitin and proteasomes. *J Clin Invest* 93: 2127–2133, 1994
99. May RC, Bailey JL, Mitch WE, Masud T, England BK: Glucocorticoids and acidosis stimulate protein and amino acid catabolism in vivo. *Kidney Int* 49: 679–683, 1996
100. Wang XH, Hu JP, Hu Z, Du J, Mitch WE: Diabetes in db/db mice causes muscle atrophy via the ubiquitin-proteasome system and is reversed by rosiglitazone administration [Abstract]. *J Am Soc Nephrol* 16: 632A, 2005
101. Saad MJA, Folli F, Kahn JA, Kahn CR: Modulation of insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of dexamethasone-treated rats. *J Clin Invest* 92: 2065–2072, 1993
102. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE: Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 11: 183–190, 2005
103. Bailey JL, Price SR, Xiang B, Mitch WE: Chronic kidney disease causes defects in signaling through the insulin receptor substrate/phosphatidylinositol 3-kinase/Akt pathway: Implications for muscle atrophy. *J Am Soc Nephrol* 17: 1388–1394, 2006
104. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ, Yancopoulos GD: Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* 3: 1014–1019, 2001
105. Hunter RB, Kandarian SC: Disruption of either the NFKB1 or the Bcl3 gene inhibits skeletal muscle atrophy. *J Clin Invest* 114: 1504–1511, 2004
106. Tran H, Brunet A, Griffith EC, Greenberg ME: The many forks in FOXO's road. *Sci STKE* 2003: RE5, 2003
107. Dardevet DD, Sornet C, Taillandier D, Savary I, Attaix D, Grizard J: Sensitivity and protein turnover response to glucocorticoids are different in skeletal muscle from adult and old rats: Lack of regulation of the ubiquitin-proteasome proteolytic pathway in aging. *J Clin Invest* 96: 2113–2119, 1995
108. Wing SS, Goldberg AL: Glucocorticoids activate the ATP-ubiquitin-dependent proteolytic system in skeletal muscle during fasting. *Am J Physiol* 264: E668–E676, 1993
109. Price SR, England BK, Bailey JL, Van Vreede K, Mitch WE: Acidosis and glucocorticoids concomitantly increase ubiquitin and proteasome subunit mRNAs in rat muscle. *Am J Physiol* 267: C955–C960, 1994

Access to UpToDate on-line is available for additional clinical information
at <http://www.jasn.org/>