Proteolysis by the Ubiquitin-Proteasome System and Kidney Disease

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ABSTRACT
Proteins in all cells turnover continuously such that rigorous control of proteolysis is required to govern levels of proteins with vastly different half-lives and actions including those regulating transcription, metabolic pathways, or the breakdown of muscle proteins to amino acids used in gluconeogenesis or the synthesis of new proteins. Critical cellular functions would be disrupted without precise regulation of protein degradation. Thus, it is surprising that the bulk of protein in all cells is degraded by the ATP-dependent, ubiquitin-proteasome system. The system achieves remarkable specificity by selective conjugation of ubiquitin (Ub) to a doomed protein in a process catalyzed by >1000 ubiquitin ligases that recognize individual substrate proteins. Because the pathogenesis of certain kidney diseases and their complications are linked to the function of the ubiquitin-proteasome system, understanding its mechanisms could lead to novel therapies.


Intracellular proteins turn over continually in all organ tissues. They do this through a process of degradation and replacement. Protein breakdown must be highly selective because a degraded protein is rapidly and irreversibly lost, completely terminating its actions. Cellular degradation systems were initially considered only as a way to remove damaged proteins, but it is now clear these systems are much more complex. Protein degradation for the purposes of altering function or reclaiming amino acids is tightly regulated to avoid widespread breakdown of cellular constituents that would cripple stability, integrity, and signaling. For example, proteins regulating transcription or metabolic pathways have vastly different half-lives, and unless the timing of their removal is precise, survival of the organism would be in jeopardy.

It is somewhat surprising, therefore, that most intracellular proteins are degraded by the same ATP-dependent system, the ubiquitin (Ub)-proteasome system (UPS). The UPS achieves remarkable precision in degrading specific proteins because its activity is regulated at several steps, beginning by marking proteins destined for degradation (Figure 1). Initially, the small protein, Ub, is conjugated to doomed proteins. This ATP-dependent process requires three enzymatic reactions: the first two are catalyzed by a single, E1 Ub-activating enzyme and one of about 20 E2 or Ub-carrier proteins yielding an activated Ub. The third reaction, catalyzed by an E3 (ubiquitin-conjugating enzyme/ligase), transfers the activated Ub to a lysine in the substrate protein (or to lysines present in Ub, forming a Ub chain). This marking step enables Ub-conjugated proteins to be recognized by the 26S proteasome. Because each E3 recognizes specific proteins destined for destruction, there are >1000 E3 ligases, making this step the major mechanism ensuring specificity toward substrate proteins. Structures of E3 ligases generally contain HECT or CHIP domains or RING fingers that are required for their activity.

Substrate degradation occurs within the 26S proteasome, a complex composed of >60 subunits, making the complex 50 to 100 times larger than extracellular proteases. The 26S complex, in turn, is composed of a central, 20S, barrel-shaped particle with 19S regulatory particles at either or both of its ends. The 19S regulatory particles contain ≥19 subunits that exhibit different properties including the ability to recognize polyubiquitin chains bound to proteins. After recognition, the 19S particles cleave the polyubiquitin chain from the substrate protein and unfold the doomed protein. This step permits translocation of the substrate into the 20S particle for protein degradation. There also are de-ubiquitinating enzymes that disassemble polyubiquitin chains, enabling Ub re-utilization. Many of these activities occurring in the 19S particles are ATP dependent.

The 20S proteasome is a cylinder composed of four stacked rings, each
The UPS of protein degradation. Proteins destined for degradation are conjugated to Ub by an ATP-dependent process that involves three enzymes: E1, E2, and E3. Selectivity of the protein substrates for degradation principally depends on recognition of the protein by specific E3 ligases. When a chain of five Ub proteins is attached to the protein substrate, the complex can be recognized by the 26S proteasome. This leads to release of the Ub chain, which is cleaved to individual Ub s, and unfolding of the protein substrate for injection into the proteasome and digestion to peptides. Peptidases in the cytoplasm degrade peptides into amino acids. This sequence of events occurs when ENaC or HIF-1α is degraded. In catabolic states, a similar process degrades muscle proteins, but they are specifically degraded because of the activation of a specific E3 ligase, Atrogin-1/MAFbx. Muscle proteolysis is augmented by caspase-3, which cleaves muscle proteins, thereby increasing the availability of substrates for the UPS. Caspase-3 also stimulates proteolytic activity in the proteasome. In the presence of IFN-γ, antigen presentation is augmented by the creation of immunoproteasomes, which trim antigens to a size that maximizes interactions with MCH class I molecules.

Figure 1. The UPS of protein degradation. Proteins destined for degradation are conjugated to Ub by an ATP-dependent process that involves three enzymes: E1, E2, and E3. Selectivity of the protein substrates for degradation principally depends on recognition of the protein by specific E3 ligases. When a chain of five Ub proteins is attached to the protein substrate, the complex can be recognized by the 26S proteasome. This leads to release of the Ub chain, which is cleaved to individual Ub s, and unfolding of the protein substrate for injection into the proteasome and digestion to peptides. Peptidases in the cytoplasm degrade peptides into amino acids. This sequence of events occurs when ENaC or HIF-1α is degraded. In catabolic states, a similar process degrades muscle proteins, but they are specifically degraded because of the activation of a specific E3 ligase, Atrogin-1/MAFbx. Muscle proteolysis is augmented by caspase-3, which cleaves muscle proteins, thereby increasing the availability of substrates for the UPS. Caspase-3 also stimulates proteolytic activity in the proteasome. In the presence of IFN-γ, antigen presentation is augmented by the creation of immunoproteasomes, which trim antigens to a size that maximizes interactions with MCH class I molecules.

UPS in kidney-related diseases

Several kidney-related conditions influenced by Ub conjugation and proteasomal degradation illustrate some of the myriad functions of UPS. For example, Ub-like protein, FAT10, mediates NF-κB activation in tubular cells to promote tubulointerstitial inflammation in chronic kidney disease, and Trsp1, the downstream effector of BMP7, regulates the E3 ubiquitin ligase, Arkadia, in renal fibrogenesis. Ub conjugation also efficiently removes key cellular proteins that regulate erythropoietin production. Specifically, erythropoietin production depends on the HIF-1α transcriptional activator, whose level is controlled by the UPS. In the presence of sufficient O₂, HIF-1α is first hydroxylated on specific prolines, followed by Ub-conjugation by the VHL-containing E3 ligase, and finally degraded. With hypoxia or if VHL is mutated (Von Hippel Lindau disease), HIF-1α is not degraded and the transcription of genes for erythropoietin, angiogenesis (VEGF), and glycolytic enzymes proceeds unabated. Levels of the epithelial sodium channel (ENaC) are also regulated by the UPS. The cytoplasmic tails of the β and γ subunits of ENaC are ubiquitinated by the E3 ligase, Nedd4, leading to ENaC subunit degradation. When this process is disrupted as in Liddle Syndrome, ENaC expression on the luminal membrane is increased, leading to enhanced sodium reabsorption in the distal nephron and hypertension.

Immune surveillance involving the presentation of antigenic peptides to immune modulatory cells is critically dependent on proteasome function and is relevant to kidney transplantation. Proteins resulting from viral or bacterial infections or even cancer cells are converted to antigenic peptides by the proteasome. Some of these peptides are
transported into the endoplasmic reticulum and bound to MHC class I molecules before being transferred to the cell membrane for presentation to cytotoxic CD8+ lymphocytes. These UPS-mediated events are enhanced by two changes in UPS activity. First, because peptides of eight to nine amino acids are most efficiently bound to most MHC class I molecules, longer peptides are trimmed to the desired eight to nine residue size. This occurs following peptide uptake into the endoplasmic reticulum by a specific peptide transporter and peptide cleavage by an aminopeptidase, ERAP1.15 Second, antigen presentation is boosted because the immune modifer, IFNγ, increases expression of both the peptide transporter and ERAP1 aminopeptidase while stimulating the production of specialized immunoproteasomes. These specialized immunoproteasomes are created when unique subunits are inserted and a proteasome-activating complex, PA28, is bound to one end of the 20S proteasome. The result is rapid generation of peptides of ideal length for MHC class I binding.16

A final example of how the UPS influences kidney diseases involves the regulation of muscle protein degradation that is stimulated by chronic kidney disease (CKD). Evidence for UPS-induced muscle proteolysis in CKD is an accompanying increase in the transcription of Ub and proteasome subunits. Similar responses occur in cancer, diabetes, or starvation, suggesting that conditions causing muscle wasting include a coordinated multigene response.17 The muscle atrophy stimulated by catabolic conditions includes selective degradation of contractile proteins; the specificity for contractile proteins is presumably determined by the cell’s content of two E3 ligases: atrogin-1/MAFbx and MuRF-1. In muscle, expression of these enzymes is correlated with the rate of muscle protein degradation; mice lacking these enzymes have reduced muscle atrophy in response to denervation.18 Recent findings indicate that the atrogin-1/MAFbx E3 ligase also mediates the muscle injury induced by statins.19 The mechanism stimulating expression of these E3 ligases in muscle involves both a decrease in insulin/IGF-1 signaling and an increase in glucocorticoids.20–22 Investigations into the complications of CKD indicate that the same factors activate Ub conjugation and ultimately, muscle protein degradation, in conditions such as inflammation, acidosis, and excess angiotensin II,20,23–25 which are partially offset by the activity of the X chromosome–linked inhibitor of apoptosis protein, XIAP.26 The impairment in insulin/IGF-1 signaling in catabolic conditions also occurs when there is interference with PI3K/Akt activity.27

Another mechanism, activation of caspase-3, stimulates muscle protein degradation by increasing activity of the UPS. First, caspase-3 cleaves muscle proteins, providing substrates for the UPS.28,29 This process is important because the UPS digests actomyosin or myofibrils slowly, although it rapidly degrades monomeric myosin or actin. Second, activated caspase-3 stimulates muscle protein degradation in the proteasome.30 Increased protein degradation in muscle results from a caspase-3–induced modification of the structure of the proteasome. The mechanism underlying this response was uncovered by incubating caspase-3 with isolated proteasomes and finding that proteasomal proteolysis is boosted when specific subunits of the 19S proteasome are cleaved. Presumably, changes in specific proteasome subunits yield greater access of protein substrates to the 20S proteasome and, hence, their degradation.

In summary, the UPS is a complex system that first tags and then degrades proteins. The process is complex because the UPS is capable of degrading so many proteins that are critical for cell function including transport proteins, transcription factors, proteins controlling metabolic functions, and muscle proteins. In the absence of specific regulation, uncontrolled proteolysis would be disastrous. Besides activities that are intrinsic to the system, degradation of protein by the UPS is coordinated with other cellular responses in a complex fashion like Bach’s Orchestral Suites. Learning more about the UPS will uncover new secrets about cell function, hopefully leading to insights, inhibitors, or novel chemotherapeutic agents. Critical function of the UPS explains why the 2004 Nobel Prize in Chemistry was awarded to Avram Hershko, Aaron Ciechanover, and Irwin Rose for discovering how the UPS causes cellular protein turnover.

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DISCLOSURES

None.

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