ANNALS OF SURGERY
Vol. 225, No. 3, 307–316

The Ubiquitin–Proteasome Pathway

Review of a Novel Intracellular Mechanism of Muscle Protein Breakdown During Sepsis and Other Catabolic Conditions

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Summary Background Data
Patients with sepsis and other catabolic conditions, such as severe trauma, cancer, and fasting, suffer significant loss of body protein, the majority of which originates from skeletal muscle. Recent evidence suggests that muscle protein breakdown during sepsis is caused by upregulated activity in the ubiquitin–proteasome pathway and is associated with increased expression of the ubiquitin gene.

Purpose
The purpose of the study was to review the role of the ubiquitin–proteasome pathway in the regulation of muscle proteolysis during sepsis and other catabolic conditions.

Review
Proteins that are degraded by the ubiquitin–proteasome mechanism are first conjugated to ubiquitin, a 76-amino-acid, highly conserved residue. Ubiquitinated proteins are recognized by the 26S proteasome, which is a large proteolytic complex consisting of the 19S cap complex and the 20S proteasome. The 20S proteasome is a cylindrical particle composed of four stacked rings, making it look like a barrel. The rings form a "tunnel" in which the target proteins are hydrolyzed, after which ubiquitin is released to be reused in the proteolytic pathway. A unique feature of the ubiquitin–proteasome proteolytic pathway is its energy dependency.

Conclusions
An understanding of the molecular regulation of protein metabolism in patients with sepsis and other catabolic conditions is important because it may form the basis for improved treatment in the future.

Patients with septic complications after surgery or major trauma suffer significant loss of body protein. The majority of this protein originates from skeletal muscle as evidenced by net release of amino acids from muscle tissue[1] and urinary excretion of 3-methylhistidine, a marker of myofibrillar protein breakdown.[2] Muscle breakdown during sepsis mainly is caused by increased protein degradation, in particular degradation of myofibrillar proteins, although inhibited protein synthesis may contribute to sepsis-induced muscle catabolism. The catabolic response in skeletal muscle results in muscle fatigue and wasting, which may have severe consequences for the

Supported in part by NIH grant DK 37908 and grants #9603 and #8610 from Shriners of North America.
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Accepted for publication April 16, 1996.

Table 1. DIFFERENT INTRACELLULAR PROTEOLYTIC PATHWAYS AND SOME OF THEIR REGULATORY ENZYMES

Pathway	Enzyme
Lysosomal	Cathepsin B,D,H,L
Nonlysosomal	
Energy dependent	
Ub dependent	26S protease
Ub independent	600-kDa protease
Energy independent	
Ca dependent	Calpain I, II

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recovery in patients with sepsis. An understanding of the mechanisms regulating protein metabolism in patients with sepsis and other catabolic conditions is important because it may form the basis for improved treatment of these patients in the future.

Most body proteins constantly are being synthesized and degraded, allowing for the rapid removal of abnormal proteins, modification of cellular levels of enzymes and other regulatory proteins, and an ongoing remodeling of cellular structures. This dynamic state of cellular proteins was described more than half a century ago.[3] Regulation of the breakdown of cellular proteins plays an important role in the modulation of tissue protein levels. The mechanisms regulating protein breakdown are highly specific. Some proteins have a half-life of minutes, whereas other proteins are turned over much more slowly with half-lives in the range of days. There is evidence that specific proteolytic mechanisms are involved in the degradation of different classes of proteins.

The hydrolysis of peptide bonds is an exergonic process, and protein breakdown by traditional proteases does not require energy.[4] It is therefore somewhat surprising that the selective degradation of many proteins in eukaryotic cells is energy dependent. The importance of an energy-dependent proteolytic pathway, both for the degradation of normal and abnormal proteins under physiologic and pathophysiologic conditions, has become increasingly recognized over the past decade.[5] In particular, an energy-dependent proteolytic pathway that degrades proteins conjugated to ubiquitin plays an important role in the regulation of muscle protein breakdown in various catabolic conditions as well as in the regulation of other important intracellular functions.[6–8]

Different intracellular proteolytic pathways and some of their proteolytic enzymes were reviewed recently[9] and are listed in Table 1. It should be emphasized that this is a highly simplified scheme because there is evidence that individual proteins can be degraded by different pathways and that there is an interaction between the different mechanisms. Of the different proteolytic pathways listed in Table 1, the lysosomal and the energy–ubiquitin-dependent mechanisms are the most important systems for regulation of protein breakdown in various physiologic and pathophysiologic conditions. In particular, the energy–ubiquitin-dependent pathway plays an important role in the regulation of muscle protein breakdown during sepsis and other catabolic conditions. The purpose of this review is to describe recent knowledge about the energy–ubiquitin-dependent proteolytic pathway and its role in the regulation of muscle protein breakdown in sepsis and other catabolic conditions. The involvement of the ubiquitin system in other intracellular functions also will be discussed briefly.

Knowledge about the ubiquitin system is important from a clinical standpoint for several reasons. Muscle catabolism commonly is encountered in different groups of surgical patients in addition to those with sepsis (*e.g.*, trauma, cancer, and malnutrition). There is evidence that muscle proteolysis is regulated by the energy–ubiquitin-dependent pathway in most of these conditions. The ubiquitin system also is important for several other essential cellular functions, such as antigen presentation, production of inflammatory mediators, and cell division and replication. Research in the ubiquitin field is fast expanding, and the time has come to bridge the gap between biochemists—molecular biologists and clinicians regarding the biologic and clinical implications of this system.

THE ENERGY–UBIQUITIN-DEPENDENT PROTEOLYTIC PATHWAY

Less than 20 years ago, Hershko et al.,[10] in a series of elegant experiments, discovered that a heat-stable polypeptide was required for the activity of an energy-dependent proteolytic system in reticulocytes.[10] This polypeptide subsequently was identified as ubiquitin, a 76-amino acid, 8500-d residue, highly conserved and present in all eukaryotic cells. Since then, a large number of studies have confirmed the importance of this proteolytic system for the breakdown of intracellular proteins during various pathophysiologic conditions. The great interest in the ubiquitin field is evidenced by the large number of recent reviews discussing the molecular regulation, genetics, various biologic roles, biochemistry, enzymology, and involvement in pathologic conditions of the ubiquitin pathway.[6–8,11,12]

A simplified scheme of the ubiquitin proteolytic pathway is shown in Figure 1. Proteins that are targeted for degradation by this mechanism are conjugated to polyubiquitin, which enables them to be recognized by the proteolytic 26S proteasome (the "tagging hypothesis"). The first step in the ubiquitin system is activation of the carboxyl terminal glycine residue of ubiquitin by the ubiquitin-activating enzyme E_1. This step is energy (adenosine

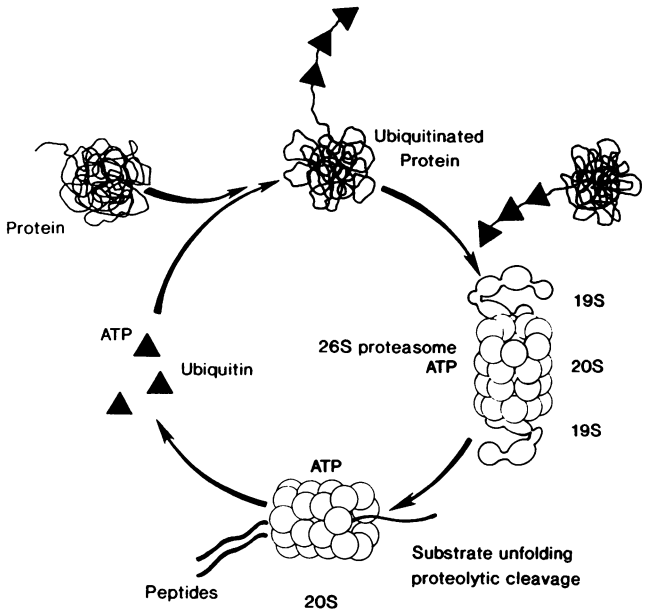

Figure 1. Simplified scheme of the ubiquitin-dependent proteolytic pathway. Refer to text for explanation of the different steps involved in the proteolytic pathway. The three steps that require adenosine triphosphate are indicated in the figure: 1) activation of ubiquitin; 2) the assembly of the 26S proteasome; and 3) the degradation of the ubiquitinated protein.

triphosphate) dependent. Next, activated ubiquitin is transferred to a family of ubiquitin carrier proteins (ubiquitin-conjugating enzymes, E_2). Conjugation of ubiquitin to the protein that is to be degraded can occur by direct transfer of ubiquitin from E_2 or by a process in which target proteins are first bound to specific sites of ubiquitin protein ligases, E_3, before ubiquitin is transferred from E_2. Proteins ligated to multiple ubiquitin units then are degraded by the 26S proteasome. This step, which results in the release of peptides and free ubiquitin, also is energy dependent. The number of ubiquitin molecules conjugated to each protein is specific for certain proteins and also influences the rate by which the proteins are degraded.

An additional step that is energy-requiring in the ubiquitin pathway is the assembly of the large 26S proteasome. This complex, which has a molecular weight of 1000 to 1500 kd, is formed by the assembly of three components (*i.e.*, the 20S proteasome and two 19S molecules). The 20S proteasome is the "catalytic core" of the 26S proteasome. The structure and function of the proteasome will be discussed in greater detail below. After proteolysis, ubiquitin is released from the polyubiquitin chain and can be reused in the proteolytic pathway. Thus, adenosine triphosphate is required for at least three, and possibly more, different steps in the ubiquitin system.

Some of the different enzymes that are involved in the regulation of the ubiquitin pathway and their functions are summarized in Table 2. The cloning of genes encoding ubiquitin, the various enzymes, and other proteins involved in the ubiquitin pathway, including the proteasome subunits, has made it possible to study regulation of protein degradation at the molecular level.[12,13]

Recent studies suggest that the deubiquitination of proteins (*i.e.*, removal of ubiquitin from the substrate) also is important for the regulation of protein breakdown rates.[7,14] Changing the rate of ubiquitin removal from the protein may influence the probability of the ubiquitinated substrate to be recognized by the 26S protease complex. Thus, the deubiquitinating enzymes (ubiquitin C-terminal hydrolases) may be as important for the regulation of the ubiquitin pathway as the E_1, E_2, and E_3 enzymes. Interestingly, several proteins implicated in tumorigenesis have been found to be deubiquitinating enzymes.[15]

THE PROTEASOMES

Approximately 25 years ago, electron microscopy studies showed the presence of 17- × 11-nm protein particles in extracts of human erythrocytes,[16] but the function and significance of these particles were not known at that time. Subsequent reports showed that these particles corresponded to the 20S proteasome, which is the proteolytic core in the 26S protease complex described above. The 20S proteasome has been given different names throughout the years, including the multicatalytic proteinase and the 20S cylinder particle. The 20S proteasome and its role in intracellular protein degradation have been the subject of intense recent research.[17–22] The crystal structure of the 20S proteasome particle was reported only recently.[23] The 20S proteasome is a cylindrical particle composed of four stacked rings,[24] making the particle

Table 2. ENZYMES OF THE UBIQUITIN SYSTEM

Enzyme	Function
Ubiquitin activating enzyme (E_1)	Initial activation of ubiquitin C-terminus
Ubiquitin-conjugating enzymes (E_2)	Conjugation of activated ubiquitin to substrate proteins
Ubiquitin-protein ligase (E_3)	Substrate recognition; catalysis of ubiquitin transfer from $E_2 \sim$ Ub to substrate
Ubiquitin C-terminal hydrolases (deubiquitinating enzymes)	Processing of ubiquitin precursors; regeneration of free ubiquitin from conjugation products
Ubiquitin-conjugate degrading enzyme (26S protease)	Degradation of ubiquitin-protein conjugates

From Hasselgren[9] with permission.

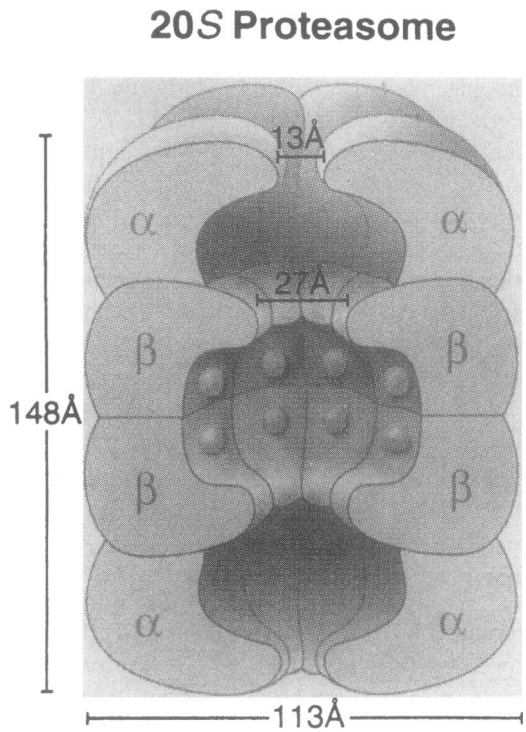

Figure 2. Cross section of the 20S proteasome. The 20S proteasome is composed of four stacked, seven-membered rings. The rings form a central channel with three chambers: two antechambers and a central chamber that houses the catalytic sites. The catalytic sites on the inner side of the β-subunits are indicated in the figure. From Weissman et al[24] with permission.

look like a barrel (Fig. 2). Each ring consists of seven polypeptide subunits with a molecular weight ranging from 19 to 36 kd. The subunits of the outer rings are called α-subunits, and those of the two inner rings are called β-subunits, and the overall structure of the 20S proteasome can therefore be written as $\alpha_7\beta_7\beta_7\alpha_7$. It generally is believed that the catalytic sites are located on the inner side of the β-subunits, whereas the α-subunits account for substrate discrimination. The stacked rings form a "tunnel" through which the protein that is to be degraded is funneled. The tunnel forms an inner chamber and two antechambers. A polypeptide has to pass through two narrow constrictions (13 Å and 27 Å wide) before it can be hydrolyzed (Fig. 2), in part explaining the preference of the 20S particle for unfolded substrates.

The activity of the 20S proteasome is regulated by a number of other proteins.[17] Some of these proteins inhibit and other proteins activate the 20S proteasome. The characteristics of several of these proteins have been defined recently and are summarized in Table 3. Among the regulatory proteins, the 19S cap complex is important for regulation of the breakdown of ubiquitin-conjugated proteins by the 20S proteasome. The 19S cap complex is the same protein that has been called PA700 activator (for 700-kd proteasome activator).[25] The 19S cap complexes and the 20S particles are found in cellular extracts and are in assembly–disassembly equilibrium with the 26S proteasome complex.

The function of the 19S cap complex is to recognize, transport, and unfold substrates on the 20S proteasome. One 19S cap complex attaches to each end of the cylindrical 20S particle, thereby forming the 26S proteasome complex (Fig. 3). The cap complexes are asymmetrically attached to the 20S proteasome and look V-shaped in projection images with the openings of the V pointing in opposite directions.[18] The ultrastructure of the 19S cap complex shows that there is a distinct protein mass intruding into the cleft of the V-shaped particle. Different regions of the 19S complex account for recognition and unfolding of the protein substrate (Fig. 3).

The 26S proteasome shows a clear selectivity for conjugated proteins, in particular for proteins conjugated to ubiquitin, although ubiquitin-independent degradation by the 26S complex recently has been shown for the short-lived enzyme ornithine decarboxylase.[26] Instead, ornithine decarboxylase is conjugated to another small protein called antizyme. It presently is unknown how this interaction leads to the destruction of ornithine decarboxylase while sparing antizyme.

Although the exact interaction between the 19S complex (PA700) and the 20S proteasome is not known presently, Hochstrasser[7] recently proposed the following sequence of events: the ubiquitin chain of multiubiquitinated proteins is bound to chain-binding subunits of the 19S complex; a series of adenosine triphosphate-dependent unfolding and translocation steps feed the unfolded polypeptide into the central channel of the 20S proteasome; substrate is cleaved into small peptides that are released through fenestrations in the proteasome wall; and, finally, the ubiquitin chains on the remaining proteolytic remnants are disassembled (deubiquitination), facilitating their release from the proteasome. Because the current model of the 26S proteasome suggests that the 20S proteasome is capped by two identical 19S particles on each side, it is reasonable to assume that ubiquitinated proteins can enter the proteolytic complex from either side, although it presently is not known if that can occur simultaneously.

Among the other protein regulators of the 20S proteasome (Table 3), PA28 also is a capping complex. Recent morphologic studies suggest that the PA28 activator protein exists as a six- or seven-member ring with a molecular weight of approximately 28 kd.[27] When the PA28 unit caps the 20S proteasome, the ring forms a domelike cap. There is evidence that the 20S proteasome is first capped on one end and subsequently on the other end to form PA28:proteasome:PA28 complexes. Capping of proteasomes by PA28 stimulates the hydrolysis of oligopeptide substrates but not the hydrolysis of large proteins.[28] The

Table 3. PROTEINS INTERACTING WITH THE 20S PROTEASOME

Name	M_r (kDa)	S Value	M_r of Subunits (kDa)	Interaction with 20S Proteasome		Source of Purification
				Physically	Enzymatically*	
CF1	600	ND	ND	Part of 26S complex	ND	ATP-depleted rabbit reticulocyte#
CF2	250	ND	40	Part of 26S complex	Inhibition	ATP-depleted rabbit reticulocytes
240 kDA inhibitor	240	ND	40/55§	Part of 26S complex	Inhibition	Human erythrocytes
20S ball/ATPase complex	ND	20	30–110	Part of 26S complex	ND	Rabbit erythrocytes
μ particle	ND	ND	32–110	Part of 26S complex	ND	Drosophilia embryos
PA700	700	ND	20–100	Part of 26S complex	Activation	Bovine erythrocytes
19S cap complex	ND	19	35–110	Part of 26S complex; capping	ND	Xenopus oocytes
PA28†	180	ND	28	Capping	Activation	Human erythrocytes
11S regulator†	200	11	30	Binding	Activation	Human erythrocytes
200 kDa inhibitor	200	ND	50	ND	Inhibition¶	Human erythrocytes
60 kDa inhibitor	60‡	ND	31	Binding‖	Inhibiton¶	Bovine erythrocytes

* The data refer to influence on peptidase activity if not otherwise stated.
† Identity of PA28 and 11S regulator appears likely but has not been reported yet.
‡ Forms of higher molecular weight have also been observed.
§ Ubiquitination can increase the M_r from 40 to 55 kDa. It is unknown whether the oligomeric state of the inhibitor or single subunits are incorporated into the 26S protease.
‖ Binding was concluded from kinetic analysis.
¶ Proteinase and some, but not all, peptidase activities are inhibited.
# CF1 has only been partially purified.
CF = conjugate breakdown factor; ND = not determined.
From Peters[17] with permission.

exact role of the 20S–PA28 complex in the ubiquitin pathway presently is unclear.

MUSCLE PROTEOLYSIS DURING SEPSIS

Regulation by the Energy–Ubiquitin-Dependent Proteolytic Pathway

One of the most significant metabolic consequences of sepsis is a loss of body proteins, reflected by increased urinary nitrogen excretion and negative nitrogen balance. The major source of proteins in this and other catabolic conditions is skeletal muscle resulting in muscle wasting and fatigue, which are well-recognized features of patients with sepsis. Muscle catabolism during sepsis mainly is caused by increased protein breakdown, in particular degradation of myofibrillar proteins,[2,29] but reduced protein synthesis and inhibited uptake of amino acids contribute to the catabolic response in skeletal muscle in this condition.[30] One of the consequences of muscle breakdown is peripheral release of amino acids, in particular glutamine and alanine. A large portion of these amino acids are taken up by the liver to support (and perhaps initiate and regulate) acute phase protein synthesis and gluconeogenesis. Glutamine is used by cells in the immune system[31] and by enterocytes.[32] Thus, the release of glutamine and other amino acids from catabolic muscle probably is beneficial to the organism, at least during the early phase of sepsis. In severe and protracted sepsis, however, the negative consequences of muscle breakdown will dominate with muscle weakness and fatigue, delaying recovery and increasing the risk for thromboembolic and pulmonary complications, in particular when ambulation of the patient is delayed and when respiratory muscles are affected.

Our laboratory has been involved in studies on the regulation of muscle proteolysis during sepsis over the past decade. Results from these studies suggest that sepsis mainly stimulates the breakdown of the myofibrillar proteins actin and myosin[29] and that the proinflammatory cytokines interleukin-1 and tumor necrosis factor as well as glucocorticoids are important mediators of sepsis-induced muscle breakdown.[33–35]

In more recent studies, we have explored the intracellular mechanisms of sepsis-induced muscle proteolysis. Results in those studies suggest that sepsis selectively stimulates an energy-dependent proteolytic pathway with no or only minor increase in other proteolytic mechanisms, including the lysosomal and calcium-dependent proteolytic pathways.[36] The energy-dependent component of

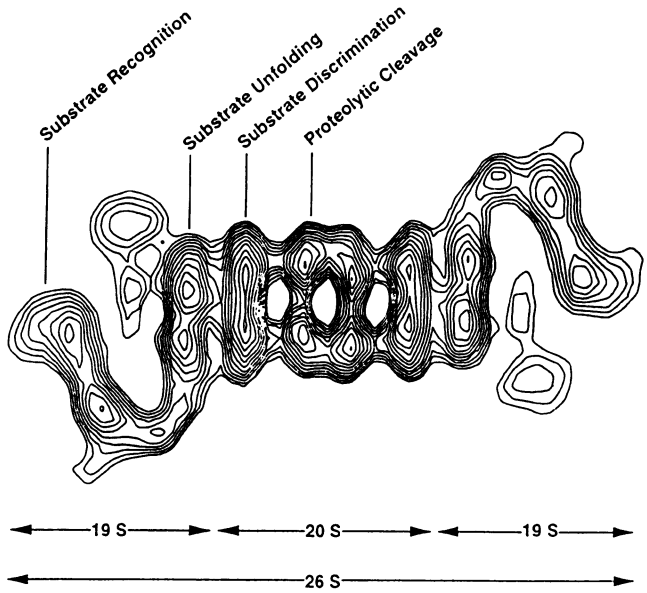

Figure 3. Schematic drawing of a cross section of the 26S proteasome and putative functions assigned to the different components. From Koster et al[18] with permission.

sepsis-induced muscle proteolysis was determined by measuring the difference in proteolytic rates in muscles incubated under normal conditions or in energy-depleting medium (containing 2,4-dinitrophenol and 2-deoxyglucose). Determined in this way, the sepsis-induced increase in both total and myofibrillar protein breakdown was almost exclusively caused by an increase in energy-dependent protein breakdown (Fig. 4).

Concomitantly with the increase in energy-dependent muscle proteolysis, evidence for increased expression of the ubiquitin system was found. Thus, mRNA levels for ubiquitin in muscle from septic rats, determined by Northern blot analysis, were increased several-fold above the levels seen in muscles from control rats (Fig. 5). In the same muscles, the total amount of free ubiquitin was increased as determined by Western blot analysis (although to a less extent than the ubiquitin mRNA levels) and the ubiquitination of certain fractions of the myofibrillar proteins was particularly enhanced.[36]

In addition to increased expression of the ubiquitin gene, we have found evidence of upregulated expression of the 20S proteasome. Thus, mRNA levels for some (but not all) of the 20S proteasome subunits were increased in skeletal muscle from septic rats[37] (Fig. 6). Further, and perhaps the most compelling, evidence that sepsis-induced muscle proteolysis is caused by upregulated activity in the ubiquitin system was found in recent experiments in which the increase in protein breakdown in septic muscle was blocked by the 20S proteasome inhibitor acetyl-leu-leu-norleu-aldehyde (LLnL).[37]

The experiments described above were performed in septic rats. In more recent studies, we have found evidence that the ubiquitin system is involved in the regulation of muscle protein breakdown in patients with sepsis as well. Ubiquitin mRNA levels were increased in muscle tissue of patients with sepsis, and intracellular levels of phenylalanine, tyrosine, and 3-methylhistidine (indicators of increased total and myofibrillar protein breakdown) were elevated in the same muscles (unpublished data). Thus, upregulated expression of the ubiquitin system probably is important for regulation of muscle proteolysis during sepsis both in experimental animals and patients.

OTHER CATABOLIC CONDITIONS IN WHICH THE UBIQUITIN SYSTEM IS UPREGULATED IN SKELETAL MUSCLE

Stimulation of the energy–ubiquitin-dependent proteolytic pathway in skeletal muscle is not unique to sepsis

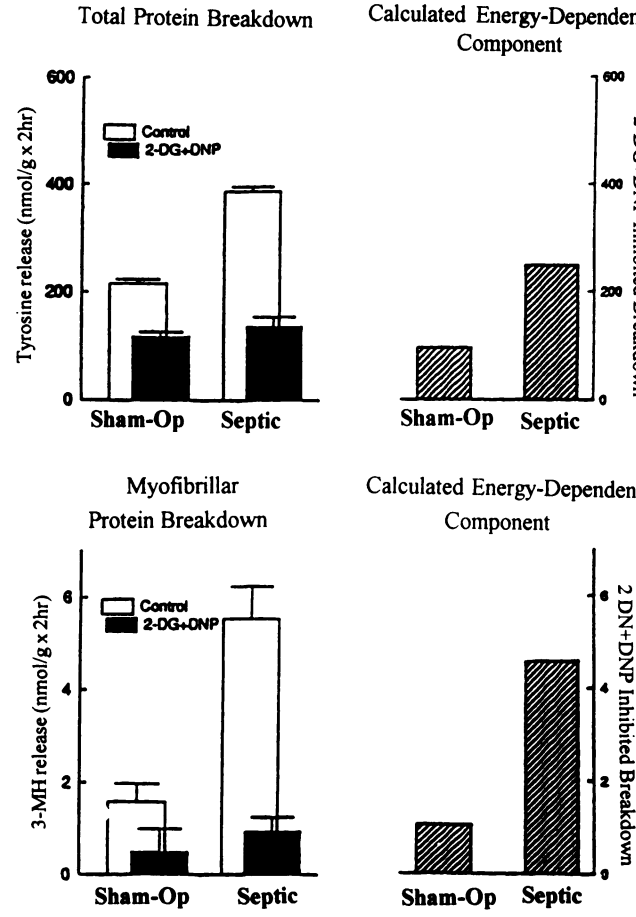

Figure 4. Total (upper panel) and myofibrillar (lower panel) protein breakdown rates in muscles from sham-operated and septic rats (16 hours after induction of sepsis by cecal ligation and puncture, CLP). Muscles were incubated in control medium (open bars) or in energy-depleting medium (filled bars) containing 5-mmol/L 2-deoxyglucose and 0.2-mmol/L 2,4-dinitrophenol. The energy-dependent component (hatched bars) was calculated as the difference between muscles incubated under the different conditions. Based on data in Tiao et al.[36]

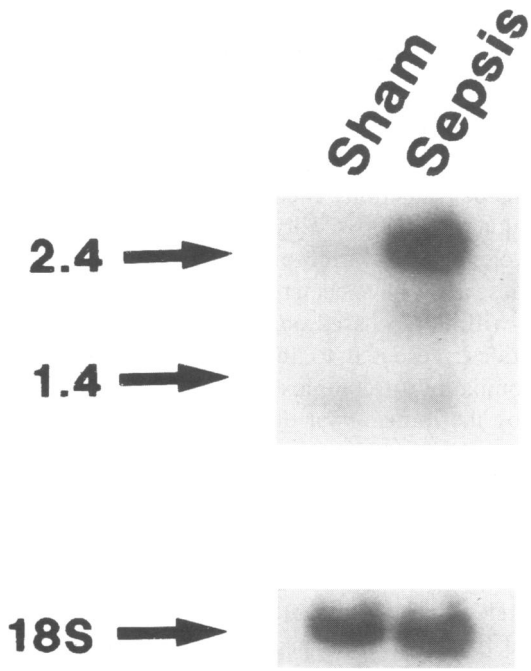

Figure 5. Ubiquitin mRNA levels in extensor digitorum longus (EDL) muscles of sham-operated and septic (cecal ligation and puncture) rats as determined by Northern blot. The blot was probed for an 18S polyribosome oligonucleotide to control for equal loading of the lanes. From Tiao et al[36] with permission.

but may play an important role in other catabolic conditions as well. In addition to sepsis, burn injury is one of the conditions that is associated with the most pronounced increase in muscle protein breakdown. In recent studies, we found that burn-induced muscle proteolysis reflects stimulated energy-dependent total and myofibrillar protein breakdown, and this increase in energy-dependent proteolysis was associated with increased muscle levels of ubiquitin mRNA.[38] In contrast to the situation in septic muscle, protein breakdown after burn injury does not seem to reflect a selective increase in ubiquitin-dependent protein breakdown because the lysosomal component of muscle breakdown also was increased in muscle from burned rats. Thus, although both sepsis and burn result in increased muscle proteolysis and both conditions stimulate the energy–ubiquitin-dependent proteolytic pathway, the regulation of muscle protein breakdown does not seem to be identical in the two conditions, possibly reflecting damage to different classes of proteins after burn and sepsis.

Additional conditions in which muscle proteolysis has been associated with increased activity in the ubiquitin system include cancer, fasting, metabolic acidosis, denervation, and treatment with cytokines. A more-than five-fold increase in the ubiquitin gene expression in skeletal muscle concomitant with a marked increase in free and conjugated ubiquitin levels was reported recently in rats with an experimental tumor (AH-130 Yoshida ascites hepatoma).[39] In the same experiments, muscle weight was reduced and protein breakdown rates were increased in tumor-bearing rats. In a more recent study from the same laboratory, muscle wasting effectively was antagonized by the β-2-adrenergic agonist clenbuterol, and this effect was caused by an almost complete abolishment of the increase in the ubiquitin gene expression.[40]

Further support for a role of the energy–ubiquitin-dependent proteolytic pathway in cancer-related muscle cachexia was found in studies by Baracos et al.[41] using the same AH-130 Yoshida ascites hepatoma tumor model. In those experiments, energy-dependent muscle protein breakdown was accelerated, and mRNA levels for ubiquitin were increased sixfold to ninefold concomitant with increased concentrations of ubiquitin-conjugated proteins. In addition, the expression of mRNA for several of the 20S proteasome subunits (C-2, C-3, C-8, and C-9) was increased in muscles from tumor-bearing rats. The results were interpreted as indicating that accelerated muscle proteolysis and muscle wasting in tumor-bearing rats result primarily from activation of the energy-dependent pathway involving ubiquitin and the 20S proteasome.

Several reports by Goldberg and coauthors[42–44] have provided evidence that the ubiquitin system is important for the regulation of muscle protein breakdown induced by fasting or denervation. More important, sepsis and cancer frequently are associated with reduced food intake. Thus, the starvation or semistarvation in these conditions may contribute to the stimulation of the ubiquitin pathway in skeletal muscle.

An additional condition that is associated with muscle

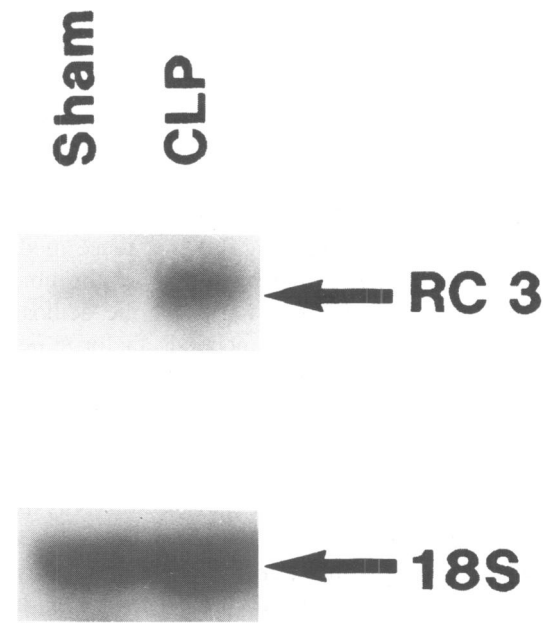

Figure 6. mRNA levels for the 20S proteasome subunit RC3 in muscle from sham-operated and septic (cecal ligation and puncture) rats as determined by Northern blot analysis. From Tiao et al[37] with permission.

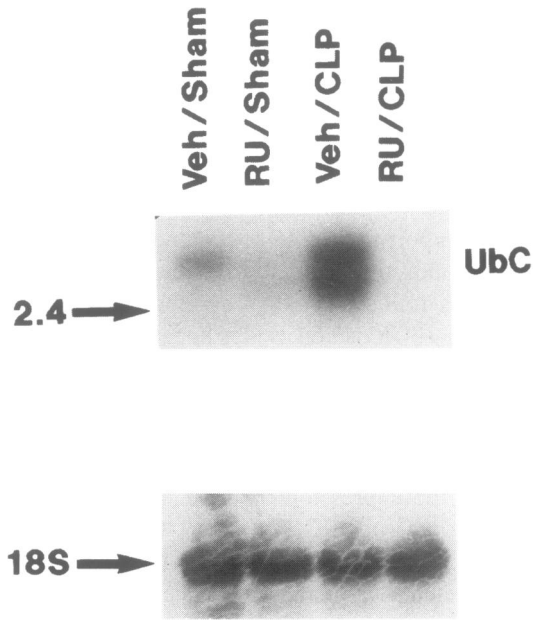

Figure 7. Ubiquitin mRNA levels in muscles from sham-operated and septic (cecal ligation and puncture) rats pretreated with 10 mg/kg of the glucocorticoid receptor antagonist RU38486 or vehicle. The sepsis-induced increase in ubiquitin mRNA was almost completely abolished in septic rats pretreated with RU38486, suggesting that glucocorticoids play an important role in regulating the ubiquitin gene expression in skeletal muscle during sepsis. From Tiao et al[48] with permission.

catabolism is metabolic acidosis, such as seen in renal insufficiency. In a series of reports, Mitch et al.[45] and Price et al.[46] have found evidence that the ubiquitin system is important for the regulation of muscle protein breakdown in this condition as well. Thus, increased muscle protein breakdown rates were associated with upregulated expression of the ubiquitin gene and several of the 20S proteasome subunit genes during metabolic acidosis in rats induced by treatment with ammonium chloride or by a chronic renal failure model.

The role of cytokines and glucocorticoids in the induction of muscle proteolysis during sepsis and cancer is well established. Recent studies suggest that these mediators stimulate muscle proteolysis by activating the ubiquitin system, both during fasting and metabolic acidosis.[44,46] Treatment of rats with tumor necrosis factor-α resulted in a time-dependent increase in the levels of both free and conjugated ubiquitin in skeletal muscle.[47] In recent studies, we found that treatment of septic rats with the glucocorticoid receptor antagonist RU 38486 blocked the increase in energy-dependent muscle proteolysis and abolished the upregulation of the ubiquitin gene expression (Fig. 7), supporting a role of glucocorticoids as a mediator of sepsis-induced activation of the ubiquitin system.[48] There is evidence to suggest that glucocorticoids may be important for the regulation of the ubiquitin pathway after burn injury as well.[38,49]

THE UBIQUITIN SYSTEM AND ANTIGEN PRESENTATION

In addition to regulating muscle proteolysis during sepsis and other catabolic conditions, the ubiquitin–proteasome system plays an important role in a number of other essential cell functions, including antigen presentation by the major histocompatibility complex I, regulation of the cell cycle, and activation of the transcription factor NF-κB. These areas have been reviewed in detail elsewhere[50–56] and will be discussed only briefly here.

Peptides presented to the cell surface by the major histocompatibility complex (MHC) I molecules are derived by the intracellular degradation of antigens by the proteasome mechanism after ubiquitination.[50,51] Support for a role of the ubiquitin pathway and the proteasome in the antigen presentation was found in a recent study in which inhibitors of the proteasome blocked the generation of peptides presented on MHC class I molecules.[52] The MHC class I molecules are important for peptides derived from intracellular antigens, such as those introduced into the cell by viral infection. In contrast, MHC class II molecules obtain their peptides predominantly from extracellular sources by endocytosis.[57] These antigens are degraded by lysosomes and the ubiquitin–energy-dependent pathway is not believed to play a role in the MHC class II restricted antigen processing.

UBIQUITIN AND REGULATION OF THE CELL CYCLE

An additional, extremely important, biologic role of the ubiquitin pathway is the regulation of the cell cycle. Recent research has provided evidence that ubiquitination is essential for the degradation of cyclins. Cyclins are intracellular proteins that activate the cyclin-dependent kinase, which in turn triggers mitosis. The mitosis is stopped after ubiquitination of cyclin and breakdown of the ubiquitin–cyclin complex, thereby inactivating the cyclin-dependent kinase. There is evidence that the cyclin–cyclin-dependent kinase complex can be inactivated by the attachment of a specific inhibitor.[54] The breakdown of this inhibitor also is regulated by the ubiquitin pathway. Degradation of the kinase inhibitor activates the cyclin–cyclin-dependent kinase complex, whereby the S phase begins, further moving the cell cycle forward. It is obvious, then, that regulation of the ubiquitin system may have vast implications, not only for the regulation of the normal cell cycle, but also for playing an important role in cancer. The role of ubiquitin in the regulation of the cell cycle was reviewed recently.[53]

UBIQUITIN AND THE TRANSCRIPTION FACTOR NF-κB

The activation of genes is regulated by DNA-binding proteins, so-called transcription factors. In general, tran-

scription factors contain two functional domains, one for DNA binding and one for transcriptional activation. NF-κB is a multisubunit transcription factor that can activate readily the expression of genes that are involved in inflammatory, immune, and acute phase responses.[58] NF-κB was first described as a nuclear factor necessary for immunoglobulin-M gene expression in mature B cells,[59] hence its name. The protein, however, is found in many and perhaps all cell types and tissues. After translocation to the nucleus, the activated NF-κB binds to a specific site of a number of genes that are involved in immune and inflammatory responses, including the immunoglobulin-M light chain, tumor necrosis factor-α, interleukin-2, interleukin-6, serum amyloid A, and IFN-β genes. It has been proposed recently that NF-κB is a transcription factor that has specialized to induce the rapid synthesis of defense and signaling proteins on exposure of cells to a variety of pathogenic agents.[58]

In most cells, NF-κB is present in an inactive, non-DNA-binding form in the cytoplasm. This complex is composed of three subunits: 1) a DNA-binding 48- to 55-kd protein (p50), 2) a DNA-binding 65- to 68-kd protein (p65), and 3) a third inhibitor subunit, called IκB, which is bound to p65. Phosphorylation and subsequent degradation of the inhibitory factor IκB result in the active form of the transcription factor NF-κB. The breakdown of IκB (and thereby the activation of NF-κB) requires ubiquitin and the 26S protease complex.[55,56] Thus, this is another example of an extremely important biologic function of the ubiquitin system and illustrates that this proteolytic pathway can catalyze the signal-induced breakdown of specific cellular proteins. The role of the ubiquitin system in the activation of NF-κB was reviewed recently.[55,56]

CLINICAL IMPLICATIONS AND OUTLOOK

The discovery of the ubiquitin-dependent proteolytic pathway has made it possible to begin to understand the molecular regulation of muscle protein breakdown during sepsis as well as other catabolic conditions. The involvement of the ubiquitin–proteasome system in other important cell functions further illustrates the essential biologic role of this system in health and disease. The clinical implications of this intracellular mechanism only have begun to be appreciated. It may be speculated that in the future, manipulation of the molecular regulation of muscle proteolysis, antigen presentation, cell division, and gene transcription will be part of the therapeutic armamentarium for patients with sepsis, injury, cancer, and malnutrition. A detailed knowledge of the mechanisms of muscle protein breakdown also will provide means to monitor the effects of different treatments administered to reduce muscle catabolism. Because most studies that have been performed so far have been in experimental animals, it will be important in the future to examine the role of the ubiquitin pathway in patients with sepsis and other catabolic conditions. Indeed, recent studies (unpublished data) in our laboratory suggest that ubiquitin-dependent muscle proteolysis is increased in patients with sepsis, and similar studies presently are being performed in patients with burn injury.

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