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BREAKTHROUGHS AND VIEWS

Molecular Regulation of Muscle Cachexia: It May Be More Than the Proteasome

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Muscle cachexia induced by sepsis, severe injury, cancer, and a number of other catabolic conditions is mainly caused by increased protein degradation, in particular breakdown of myofibrillar proteins. Ubiquitin–proteasome-dependent proteolysis is the predominant mechanism of muscle protein loss in these conditions, but there is evidence that several other regulatory mechanisms may be important as well. Some of those mechanisms are reviewed in this article and they include pre-, para-, and postproteasomal mechanisms. Among preproteasomal mechanisms, mediators, receptor binding, signaling pathways, activation of transcription factors, and modification of proteins are important. Several paraproteasomal mechanisms may influence the trafficking of ubiquitinated proteins and their interaction with the proteasome, including the expression and activity of the COP9 signalosome, the carboxy terminus of heat shock protein 70-interacting protein (CHIP) and valosin-containing protein (VCP). Finally, because the proteasome does not degrade proteins completely into free amino acids but into peptides, postproteasomal degradation of peptides by the giant protease tripeptidyl peptidase II (TPP II) and various aminopeptidases is important in muscle catabolism. Thus, multiple mechanisms and regulatory steps may influence the breakdown of ubiquitinated muscle proteins by the 26S proteasome.

Key Words: **muscle; proteolysis; cachexia; ubiquitin; transcription factors; COP9 signalosome; tripeptidyl peptidase II.**

Muscle cachexia is a prominent metabolic consequence of a number of catabolic disease states, such as sepsis, AIDS, severe injury, including burn injury, cancer, renal failure and fasting (1). In most of these conditions, the catabolic response mainly reflects increased breakdown of muscle proteins, in particular myofibrillar proteins, although reduced protein synthesis and inhibited amino acid uptake contribute to the loss of muscle mass.

The catabolic response in skeletal muscle is important from a clinical standpoint for several reasons. Muscle is the major protein store in the body and depletion of muscle proteins is a significant component of whole body protein loss in catabolic patients. Increased myofibrillar proteolysis results in muscle weakness and fatigue that in turn delays or prevents ambulation. When respiratory muscles are involved (2), there is an increased risk for pulmonary complications and extended periods of ventilatory support. In patients with cancer, muscle cachexia is a contributory factor to morbidity and mortality. It has been estimated that almost one third of deaths in patients with cancer are related to muscle catabolism and weakness (3). Hence, a better understanding of the mechanisms regulating muscle proteolysis in catabolic patients has important clinical implications.

Several recent reports, both from our and other laboratories, reviewed the current understanding of the molecular regulation of muscle cachexia (1, 4–6), in particular, the role of the ubiquitin–proteasome pathway. In this report, we describe additional mechanisms related to the ubiquitin–proteasome pathway that may be important for the molecular regulation of muscle cachexia.

Although the 26S proteasome has a central role in the degradation of proteins in cachectic muscle, there is evidence that a number of other factors "surrounding" the proteasome may be important for the regula-

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TABLE 1

Molecular Mechanisms Potentially Involved in Muscle Cachexia

Preproteasomal
Mediators
Receptor binding
Signaling pathways
Transcription factors
Gene activation
Release of myofilaments
Substrate modification
Paraproteasomal
COP9 signalosome
CHIP
VCP
Proteasomal
Ubiquitination
Degradation by the 26S proteasome
Postproteasomal
TPP II
Aminopeptidases
Other exopeptidases

tion of muscle protein breakdown. From a systematic standpoint, keeping the essential role of the proteasome in mind, those factors can be divided into preproteasomal, paraproteasomal, and postproteasomal mechanisms (Table 1). Although this classification may be somewhat simplistic, it makes the discussion of the molecular regulation of muscle cachexia easier to understand. It should be noted that although there is data to support a role of several of the mechanisms listed in Table 1 in muscle cachexia, the involvement of some of the mechanisms in muscle protein breakdown is speculative at this point. Most of the discussion in this review applies to sepsis- and injury-induced muscle proteolysis (reflecting the main focus of this laboratory). There is evidence, however, that muscle cachexia in other catabolic conditions is regulated by similar (although not always identical) mechanisms and lessons learned from sepsis- and injury-induced muscle breakdown may apply to muscle cachexia in general.

PREPROTEASOMAL MECHANISMS

Among factors that regulate muscle protein breakdown even before ubiquitin–proteasome-dependent proteolysis occurs are the influence of mediators, receptor binding, signaling pathways and gene activation. In addition, substrate modification probably plays an important role in upregulation of proteasomal degradation of muscle proteins.

Mediators

Although muscle protein breakdown during sepsis and after injury is mediated by multiple factors, glucocorticoids are one of the most important factors regulating muscle proteolysis in these conditions. Circulating levels of glucocorticoids are increased in septic and injured patients, and the administration of glucocorticoids to humans or experimental animals results in muscle protein breakdown (7, 8). This probably reflects a direct effect of the hormone because treatment of cultured myotubes also induces protein breakdown (9). An additional strong support for the role of glucocorticoids is the observation that sepsis- and injury-induced muscle proteolysis can be prevented by treatment with the glucocorticoid receptor antagonist RU38486 (10). A more detailed review of the role of glucocorticoids in muscle cachexia was provided elsewhere (11).

Among cytokines that have been implicated in the regulation of muscle proteolysis in injury and sepsis, interleukin-1 (IL-1) and tumor necrosis factor (TNF) are most prominent. Interestingly, the effects of cytokines, at least those of TNF, are probably secondary to glucocorticoids (12). The role of IL-6 is more controversial. Thus, whereas some studies suggest that IL-6 stimulates muscle protein breakdown (13), other reports, including reports from our laboratory, cast doubt on the role of IL-6 in the regulation of muscle proteolysis (14, 15).

In addition to elevated glucocorticoid levels, another mechanism by which glucocorticoids may influence muscle protein breakdown is by increased expression and activity of the glucocorticoid receptor (GR). In recent experiments we found that sepsis in rats upregu-

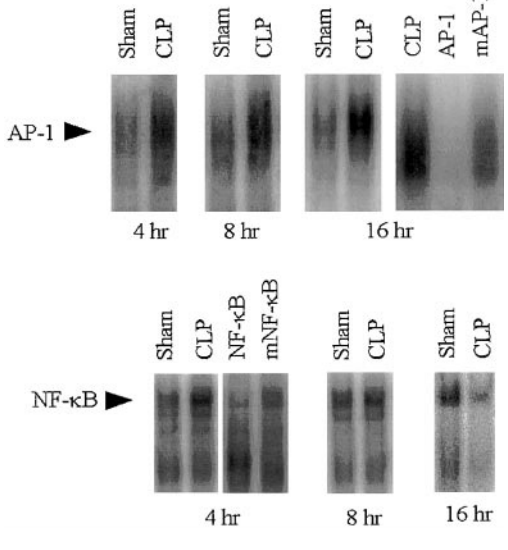

FIG. 1. DNA binding activity of the transcription factors AP-1 (upper panel) and NF-κB (lower panel) in rat extensor digitorum longus muscles at various time points after sham-operation or cecal ligation and puncture (CLP). Competition reactions were performed by adding an excess of unlabeled wild-type or mutant oligonucleotide to the reactions (lanes 8 and 9, upper panel, and lanes 3 and 4, lower panel). From Penner *et al.* (19) with permission.

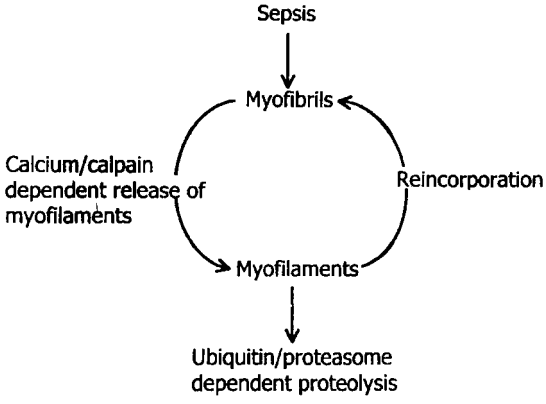

FIG. 2. Model of sepsis-induced muscle cachexia. In this model, sepsis results in calcium/calpain-dependent release of myofilaments from the sarcomere. The myofilaments are ubiquitinated in the N-end rule pathway and degraded by the 26S proteasome or reincorporated into the myofibrils. This model offers two levels of possible therapeutic intervention: inhibition of the calcium/calpain-dependent release of myofilaments (e.g., with dantrolene) or inhibition of the ubiquitin–proteasome pathway (e.g., with proteasome inhibitor). From Hasselgren and Fischer (1) with permission.

lated the amount of GR in skeletal muscle and that this response was associated with increased GR hormone binding activity (unpublished observations). Similar findings have been reported in other conditions characterized by stress and muscle cachexia (16).

In recent studies, Tisdale reported on the presence of a tumor-specific circulating factor that can induce muscle proteolysis (17). This mediator was called Proteolysis Inducing Factor (PIF), identical to the nomenclature used by Clowes *et al.* in their classical paper on a proteolysis-inducing factor in patients with injury and sepsis (18). Despite the identical names, however, the two substances are probably not the same. Although Tisdale = s PIF may be important in patients with certain cancers, it seems to be a factor that is not released by a number of other tumors. The role of a tumor-related circulating proteolysis-inducing factor awaits the confirmation from other laboratories.

Transcription Factors

Although some of the mediators of muscle cachexia have been defined and the expression of several genes involved in different proteolytic mechanisms is increased in cachectic muscle, surprisingly little information is available regarding signaling pathways and transcription factors in cachectic muscle. In a recent study, we found that sepsis was associated with altered DNA binding activity of the transcription factors NF-κB and AP-1 (19). In those experiments, AP-1 activity was upregulated throughout the septic course in rats after cecal ligation and puncture whereas NF-κB DNA binding activity was increased early during sepsis and was subsequently downregulated (Fig. 1). In other experiments, the DNA binding activity of C/EBP as well was increased in septic muscle (20). These observations are important because they suggest that multiple transcription factors that have been found previously to be involved in the inflammatory response in other tissues and cell types are activated in skeletal muscle during sepsis. The findings are pertinent to sepsis- and injury-induced muscle cachexia because in a recent analysis of published gene sequences we found potential binding sites for AP-1, NF-κB and C/EBP in the promoter regions of ubiquitin, ubiquitin-conjugating enzyme $E2_{14K}$, ubiquitin ligase E3 α, several proteasome subunits, and calpains, including the muscle specific calpain p94.

Our finding that NF-κB activity was downregulated in muscle with established cachexia (i.e., 16 h after cecal ligation and puncture in rats) (19) is in line with a recent report by Du *et al.* (21). In that study, treatment of cultured myotubes with dexamethasone reduced NF-κB DNA binding activity and upregulated the transcription of the gene for the proteasome C3 subunit. The results were interpreted as indicating that NF-κB is a suppressor of the C3 gene and that the

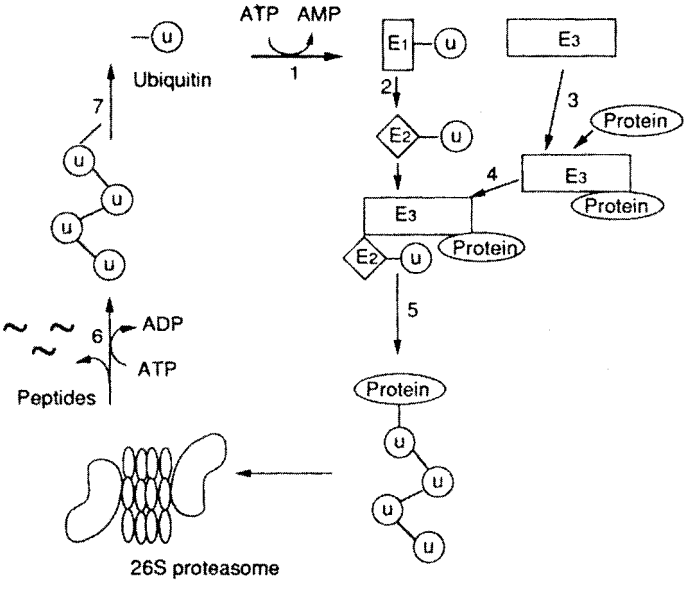

FIG. 3. Simplified scheme of the ubiquitin–proteasome proteolytic pathway. In this pathway, ubiquitinated proteins are recognized and degraded by the 26S proteasome. The steps involved in the breakdown of proteins by this mechanisms include (1) activation of ubiquitin by the ubiquitin activating enzyme E1; (2) transfer of ubiquitin to the ubiquitin conjugating enzyme E2; (3) interaction between the substrate protein and the ubiquitin ligase E3; (4) interaction between E2 and E3 resulting in (5) multiubiquitination of the substrate protein; (6) degradation of the ubiquitinated protein by the 26S proteasome; and (7) deubiquitination resulting in the release and reuse of ubiquitin in the pathway. Energy is required for multiple steps in the pathway, including activation of ubiquitin by E1 and the proteolytic activity in the 26S proteasome. From Hershko (1996) *Trends Biochem. Sci.* **21,** 445–449, with permission.

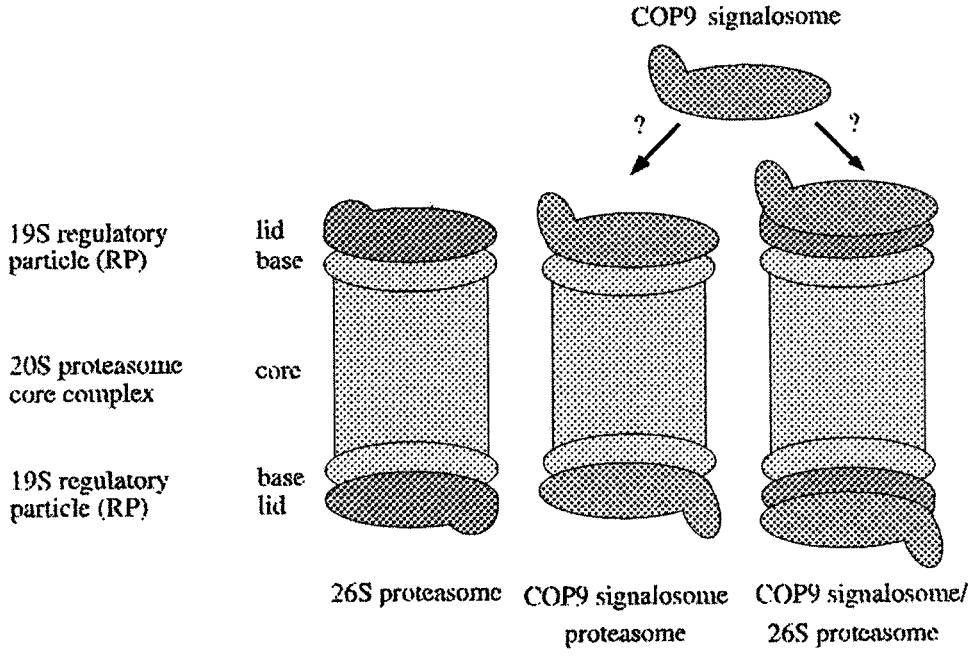

FIG. 4. Different models of potential interaction between the COP9 signalosome and the proteasome. The COP9 signalosome may replace the lid of the 19S regulatory protein (middle panel) or may interact with the normal lid subunit of the 19S complex to form a "super complex" consisting of the signalosome and the 26S proteasome. From Schwechheimer and Deng (36) with permission.

reduced NF-κB activity results in increased transcription of C3, ultimately leading to stimulated protein breakdown.

The N-End Rule Pathway

Although there is evidence that the expression and activity of the ubiquitin–proteasome proteolytic pathway are upregulated in cachectic muscle, it is possible that alterations of protein substrates are equally important. Certain features of a protein can make it susceptible to degradation by the ubiquitin–proteasome mechanism. Such features have been called degradation signals, or degrons. The N-end rule, proposed by Varshavsky (22), suggests that there is a correlation between specific features of the N-terminal of a protein and the half-life of the protein. Certain N-terminal basic amino acids (Arg, Lys, His) and bulky hydrophobic amino acids (Phe, Leu, Trp, Tyr, Ile) make the proteins susceptible to breakdown in the ubiquitin–proteasome pathway. Ubiquitination of proteins in the N-end rule pathway in skeletal muscle is regulated by the ubiquitin-conjugating enzyme $E2_{14K}$ and the ubiquitin ligase E3 α. Evidence for a role of the N-end rule pathway in cachectic muscle was found in cell-free systems in which specific E3 α blockers inhibited protein breakdown (23). In other studies, increased gene expression of $E2_{14K}$ and E3 α provided further evidence for the involvement of the N-end rule pathway in cachectic muscle (24, 25). Because most proteins do not have a destabilizing N-terminal under basal conditions, proteins probably need to be modified before they undergo ubiquitinination and degradation by the 26S proteasome.

Calcium/Calpain-Dependent Release of Myofilaments

An additional observation supporting the concept that muscle proteins need to be modified before they under degradation by the proteasome is the finding that the proteasome does not degrade intact myofibrils (26). This may seem to contradict the ubiquitin–proteasome-dependent degradation of myofibrillar proteins in cachectic muscle. Recent studies from our laboratory suggest, however, that actin and myosin are released from the sarcomere by a calcium/calpain-dependent mechanism before ubiquitination (27) and it may be speculated that the released myofilaments have destabilizing N-terminals and therefore become substrates in the N-end rule pathway. In those experiments, we found both morphological and biochemical evidence that sepsis results in disintegration of the sarcomeric Z-bands with release of myofilaments. These changes were accompanied by upregulated gene expression of calpains and were prevented by dantrolene, a substance that blocks the release of calcium from intracellular stores. Taken together, these observations are consistent with a model in which calcium/calpain-dependent release of actin and myosin is an important component of preproteasomal mechanisms of muscle cachexia (Fig. 2).

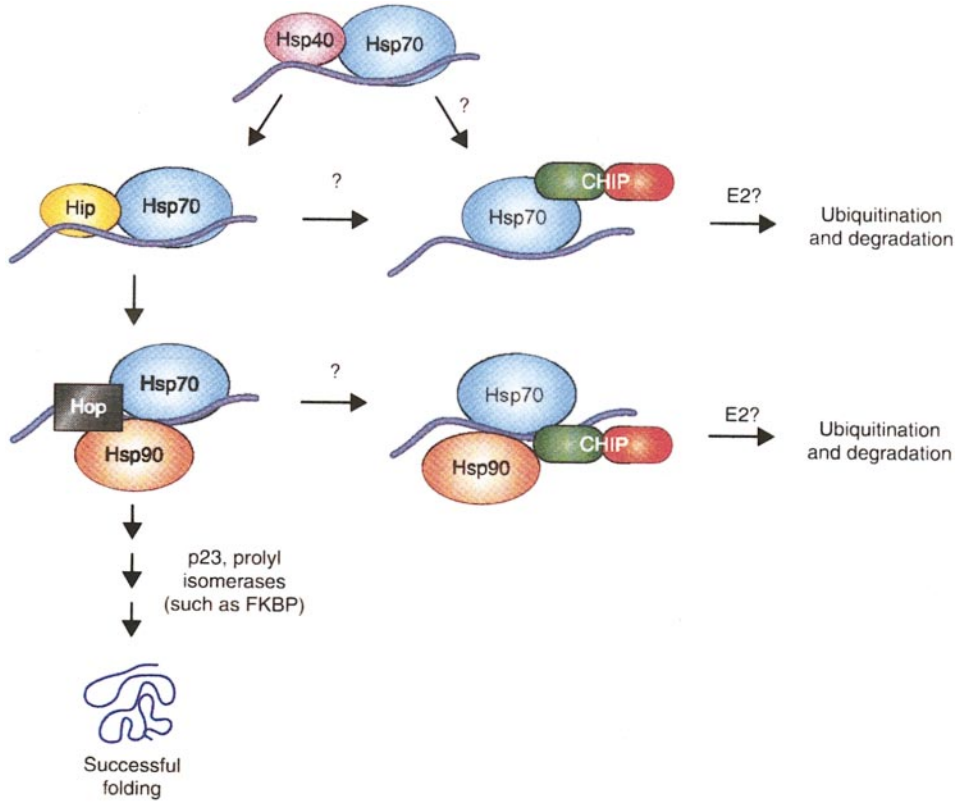

FIG. 5. Model for CHIP-mediated degradation and Hip/Hop-regulated refolding of proteins. In this model, interaction between CHIP and the chaperones Hsp 70 and Hsp 90 initiates the pathway to degradation, whereas Hip and Hop direct the protein toward refolding. From McClellan and Frydman (42) with permission.

PROTEASOMAL PROTEOLYSIS

The ubiquitin–proteasome proteolytic pathway and its involvement in muscle cachexia during sepsis and other catabolic conditions were reviewed in detail recently (1, 4–6) and is only described briefly here. Proteins degraded by this mechanism are first ubiquitinated, a process that is regulated by at least three different sets of enzymes, i.e., ubiquitin activating enzyme, E1, ubiquitin-conjugating enzyme, E2, and ubiquitin ligase, E3 (Fig. 3). Polyubiquitinated proteins are recognized and degraded by the large proteolytic 26S proteasome. The catalytic core of the 26S proteasome is the 20S proteasome, a barrel-shaped particle consisting of four stacked rings with seven subunits in each ring (28). The 19S capping protein, attached to each end of the 20S proteasome, recognizes, binds, and unfolds ubiquitinated proteins that are subsequently funneled through the central core of the 20S proteasome and hydrolyzed. A characteristic feature of the proteasomal degradation of proteins is the energy requirement. ATP hydrolysis is needed not only for the unfolding of ubiquitinated proteins and their translocation into the 20S proteolytic compartment, but also for the activation of some of the enzymes regulating ubiquitination of the proteins.

There are multiple lines of evidence supporting the concept that muscle cachexia during sepsis, severe injury, cancer and a number of other catabolic conditions mainly reflects ubiquitin–proteasome-dependent proteolysis. When muscles from rats with sepsis and other catabolic conditions were incubated in energy-depleting medium, results showed that the increase in protein breakdown in cachectic muscle reflected energy-dependent proteolysis (29). The gene expression of several components in the ubiquitin–proteasome pathway, including ubiquitin, $E2_{14K}$, E3 α, and several of the 20S proteasome subunits, is increased in cachectic muscle both from experimental animals and patients (reviewed in Ref. 1). The rate of protein ubiquitination was increased in cell-free systems prepared from cachectic muscles (23). In other experiments, the activity of the 20S proteasome was upregulated in muscle from septic (30) and burned (31) rats, and specific proteasome inhibitors reduced protein breakdown in cachectic muscle both *in vitro* and *in vivo* (32, 33).

PARAPROTEASOMAL MECHANISMS

Although mechanisms involved in the ubiquitination of proteins and the subsequent degradation by the 26S

proteasome have been extensively studied in cachectic muscle, there are a number of important questions that remain to be answered. One such question relates to the mechanisms that account for the step(s) between ubiquitination of the protein and the processing of the ubiquitinated protein by the proteasome. Although the 19S subunit S5a has been identified as a multiubiquitin chain-binding protein, or "ubiquitin receptor" (28), there is recent evidence that other mechanisms may be involved as well. Related to the question how ubiquitinated proteins are recognized and bound to the proteasome is the question how these proteins find their way to the proteasome. An additional mechanism that needs to be considered when the regulation of muscle cachexia is studied is that of triage of proteins. For example, what are the cellular components and mechanisms that mediate the decision to commit a misfolded or unfolded protein (or otherwise abnormal protein, for example one displaying a destabilizing N-end) to ubiquitination and degradation rather than to refolding? Some of these questions were addressed in recent studies and although they were not performed in muscle cells, there is reason to believe that similar mechanisms may be involved in muscle protein breakdown as well and they are therefore discussed briefly here. Because these mechanisms regulate the handling and trafficking of proteins that are already ubiquitinated or are just about to be ubiquitinated and then delivered to the proteasome, we propose to call these mechanisms paraproteasomal (or juxtaproteasomal).

The COP9 Signalosome

The COP9 signalosome is a multiprotein complex with a molecular weight of approximately 450 kDa (reviewed in Refs. 34 and 35). The complex consists of eight subunits termed CSN1–CSN8 in order of descending size. The COP9 signalosome was first isolated from plants as an essential regulator of light-mediated development (COP, constitutive photomorphogenesis) but has subsequently been found to be essential for both plant and animal development.

There are several lines of evidence suggesting that the COP9 signalosome may regulate ubiquitin–proteasome-dependent proteolysis. The COP9 signalosome copurifies with the 26S proteasome in biochemical purification suggesting that the two complexes interact physically. Interestingly, the eight subunits of the COP9 signalosome are identical to the eight polypeptides which form the lid subcomplex of the 19S capping protein. Based on that observation, different models have been proposed for the interaction between the COP9 signalosome and the proteasome (Ref. 36 and Fig. 4). Thus, the COP9 signalosome may interact with the base of the 19S complex, replacing the normal lid, or may interact with the lid, forming a supercomplex consisting of the signalosome and the 26S proteasome. It is possible that the COP9 signalosome recognizes ubiquitinated proteins and delivers them to the proteasome for degradation and therefore acts as a specialized proteasome regulator.

The function of some of the signalosome subunits further supports the role of this complex in ubiquitin–proteasome-dependent proteolysis. For example, CSN5 causes translocation of the nuclear cell cycle inhibitor $p27^{Kip1}$ to the cytoplasm where it is rapidly degraded by the 26S proteasome (35). Interestingly, CSN5 acts as a coactivator of the transcription factor AP-1 by interacting with c-Jun and Jun-D. CSN5 is also called JAB1 (Jun-activation domain-binding protein 1). CSN5/JAB1 is the only COP9 signalosome subunit that is present not only as a signalosome subunit, but also as a monomer. The AP-1 activating properties of CSN5/JAB1 are particularly interesting in light of the strong upregulation of AP-1 activity in cachectic muscle discussed above.

Recent studies provided evidence for a novel mechanism by which the COP9 signalosome may influence protein degradation (37). In those studies, COP9 increased removal of the tagging protein NEDD8 from one of the subunits of a ubiquitin ligase; this "deneddylation" of ubiquitin ligase resulted in increased protein degradation. Other recent studies suggest that the COP9 signalosome may phosphorylate certain proteins, targeting them for degradation by the ubiquitin system. Further evidence that the COP9 signalosome is important in regulating ubiquitin ligase-mediated responses was provided by Schwechheimer *et al.* (38). Thus, the signalosome may influence protein degradation by a number of different mechanisms. A commentary by Marx (39) concluded that the COP9 signalosome "and its activities in neddylation and elsewhere should give cell biologists plenty to work on in the next few years." The potential role of the COP9 signalosome in muscle cachexia should be part of such work.

CHIP

In recent experiments, using the GR as a model substrate, evidence was found that CHIP (carboxyl terminus of Hsp70-interacting protein) interacted directly with the GR-bound heat shock protein 90 (hsp 90) and induced ubiquitination of the GR and degradation through the proteasome (40). Similar results were observed in experiments in which the cystic-fibrosis transmembrane-conductance regulator (CFTR) was used as a model protein (41). In that study, CHIP functioned with hsp 70 to sense the folded state of CFTR and targeted aberrant forms for proteasomal degradation by promoting their ubiquitination. When CHIP levels were elevated, the balance between CFTR folding and degradation was shifted markedly toward degradation. The results were interpreted as indicating that CHIP can function as a ubiquitination factor. In addition, there is evidence that CHIP can interact

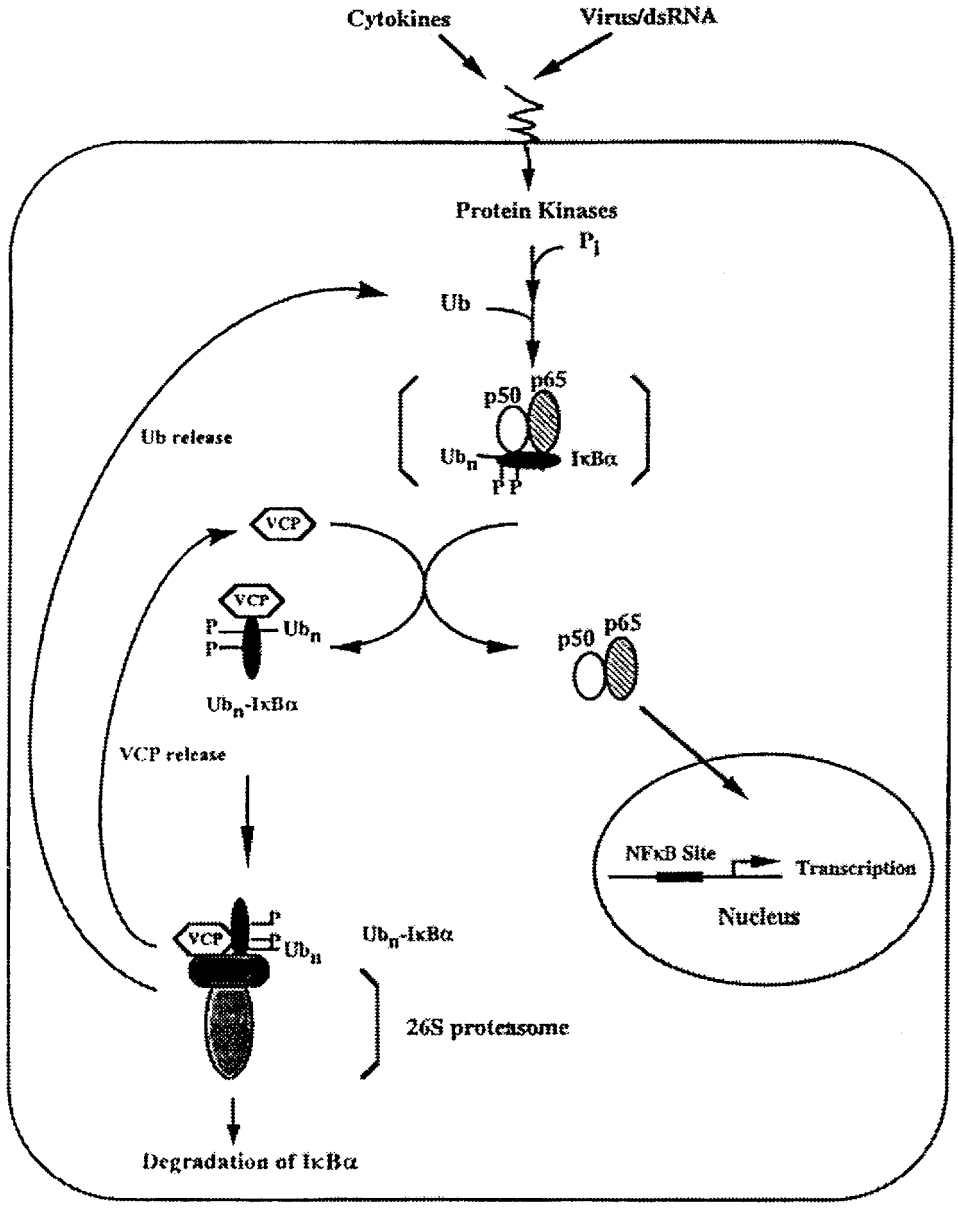

FIG. 6. A model illustrating the role of VCP in the proteasomal degradation of ubiquitinated IκBα. In this model, VCP physically associates with ubiquitinated IκBα and directs the complex to the 26S proteasome for degradation. The VCP and ubiquitin molecules are released and recycled. There is evidence that this model of VCP interaction is not unique for IκBα, but may be needed for most ubiquitinated proteins (43). From Dai *et al.* (44) with permission.

directly with the proteasome subunit C8 and with the "ubiquitin receptor" S5a of the 19S capping protein (40, 41).

The results described here illustrate the important role of molecular chaperones in the intracellular processing of unfolded or misfolded proteins and suggest that CHIP is a co-chaperone that regulates protein triage decisions mediated by heat shock proteins. It should be noted that whereas CHIP is important for diverting proteins to ubiquitination and degradation by the proteasome, other proteins may be equally important in diverting proteins away from degradation and toward refolding. The process by which proteins are refolded to regain their native state is regulated by chaperone cofactors such as Hip (hsp 70 interacting protein) and Hop (hsp 70–hsp 90-organizing protein) (42). The relationship between CHIP, Hip and Hop in the triage of cellular proteins is illustrated in Fig. 5. It will be important in future studies to examine the role of these mechanisms in cachectic muscle.

Valosin-Containing Protein

Valosin-containing protein (VCP), the mammalian homolog of the cell division cycle protein Cdc48p in

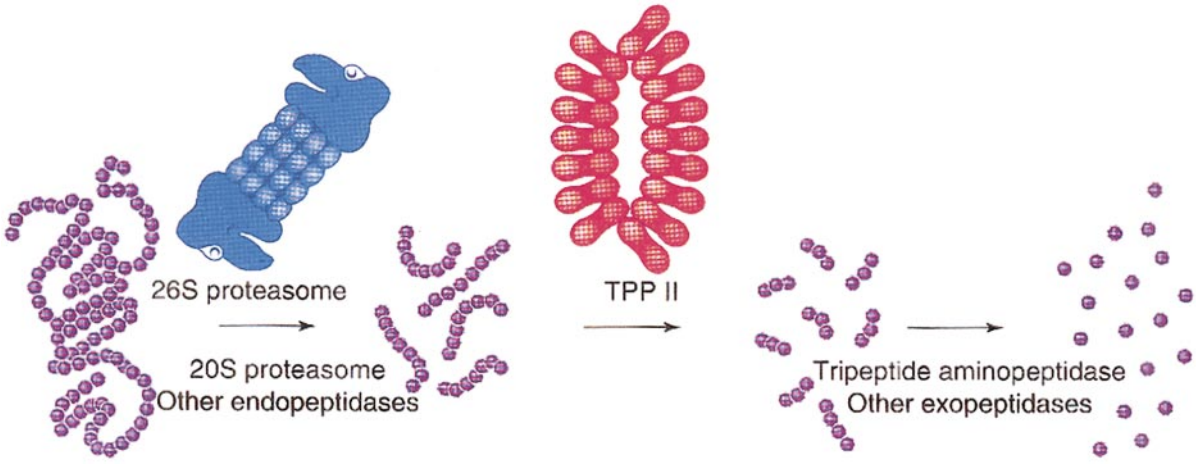

FIG. 7. Proposed scheme for the involvement of TPP II in the degradation of peptides generated by the 26S proteasome. Note that there may be multiple steps involved in the complete hydrolysis of the postproteasomal peptides into free amino acids. TPP II is a "giant protease," similar to the 26S proteasome. Modified from Tompkinson (46) with permission.

yeast and p97 in Xenopus, is a member of the so-called AAA (ATPases associated with different cellular activities) family (43). VCP forms a hexameric, barrel-shaped structure and can be divided into three distinct domains, i.e., the N domain consisting of 200 amino-terminal residues, and the D1 and D2 domains that contain the first and second ATPase molecules, respectively.

Among the multiple functions ascribed to AAA family members, recent studies suggest that VCP regulates ubiquitin–proteasome-dependent protein degradation, and, at least in some cell types, may even be necessary for protein degradation. Three different mechanisms by which VCP influences protein degradation have been proposed; (i) VCP may act as a chaperone, transferring ubiquitinated proteins to the 26S proteasome; (ii) VCP may be a component of the 26S proteasome; and (iii) VCP may have unfoldase/disassembly activity, participating in the unfolding of ubiquitinated proteins before they are transferred into the proteasome.

The requirement for VCP in the proteolytic process has been shown for a number of different proteins. Dai *et al.* (44) found evidence that VCP is necessary for the degradation of IκBα, the inhibiting protein of NFκB. Based on their observations, they proposed a model in which VCP regulates the ubiquitin–proteasome-dependent IκBα degradation by at least two mechanisms, i.e., by acting as a chaperone transferring the ubiquitin-IκBα complex to the 26S proteasome (Fig. 6) and by being a physical component of the 26S proteasome. In subsequent studies from the same group, evidence was found that VCP regulates the ubiquitin–proteasome-dependent degradation of the receptors for IL-9, IL-2, and erythropoietin (45) and may indeed be needed for the degradation of most ubiquitin–proteasome substrates (43).

Although a role for VCP, COP9, or CHIP in muscle cachexia has not yet been established, it is tempting to speculate that these mechanisms are involved in muscle catabolism. The involvement of these mechanisms in muscle cachexia certainly deserves exploration.

POSTPROTEASOMAL MECHANISMS

The proteasome degrades ubiquitinated proteins into peptides, rather than free amino acids and further proteolytic activity "beyond the proteasome" is needed for complete proteolysis into free amino acids. A frequently employed method to measure protein breakdown in muscle tissue is to determine the net release from muscle of an amino acid that is not metabolized by the tissue, e.g., tyrosine or phenylalanine. Therefore, when such methods are employed, results reflect not only proteasome-dependent degradation, but postproteasomal mechanisms as well.

The enzyme tripeptidyl peptidase II (TPP II) may be an important component of the degradation of peptides generated by proteolysis in the proteasome (46). Although TPP II was discovered almost 20 years ago (47), it is only recently that its potential relationship with the proteasome has been suggested. Like the COP9 signalosome and the 26S proteasome, TPP II is a "giant" complex, consisting of at least eight subunits each with a molecular weight of 138 kDa (46, 48). There is evidence that the complete assembly of this complex is needed for its full proteolytic capacity, but the mechanisms regulating the assembly of TPP II are poorly understood at present. The role of TPP II in the postproteasomal degradation of peptides is illustrated in Fig. 7. This illustration also suggests that other enzymes, including various aminopeptidases, are important for the complete processing of peptides into free amino acids.

TPP II cleaves peptides generated by the 26S proteasome into tripeptides and has therefore been characterized as an enzyme that "can count to three" (46). Interestingly, recent studies suggest that this complex, which forms an even larger particle than the 26S proteasome, can actually substitute for some functions of the proteasome under certain conditions (49). The role of TPP II expression and activity in cachectic muscle has not been reported. In recent experiments in our laboratory, however, we have found evidence that both the expression and activity of TPP II are increased in muscle from septic rats (unpublished observations). Although TPP II activity and other postproteasomal mechanisms may not be rate-limiting for the ubiquitin–proteasome-dependent breakdown of muscle proteins, their activities are probably important because accumulation of abnormal peptides may be injurious to the cell. It will be important in continued experiments to characterize the role of TPP II (and other peptidases) for the complete hydrolysis of proteins in cachectic muscle.

CONCLUSIONS

Muscle cachexia following severe injury, during sepsis, and in patients with cancer is characterized by ubiquitin–proteasome-dependent breakdown of proteins, in particular myofibrillar proteins. Although the ubiquitin–proteasome pathway plays a central role in the development of muscle cachexia, a number of additional mechanisms are probably involved as well. Such mechanisms may be preproteasomal, paraproteasomal, or postproteasomal. Among preproteasomal mechanisms, mediators, receptor-binding, signaling pathways, and activation of specific transcription factors are important. Among para(juxta)proteasomal mechanisms, the potential involvement of the COP9 signalosome, CHIP, and VCP remains to be established. Because the proteasome does not degrade proteins completely, mechanisms "beyond the proteasome" hydrolyzing peptides into free amino acids may also be important. Such a mechanism may be the processing of peptides by TPP II and possibly other peptidases as well.

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