Cellular control of protein degradation[1]

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Running head: UBIQUITIN-PROTEASOME-DEPENDENT PROTEOLYSIS

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ABSTRACT: This review focuses on recent advances in our understanding of the ubiquitin-proteasome-dependent pathway, which plays a major role in skeletal muscle proteolysis and is involved in the control of many major biological functions. The ubiquitination/deubiquitination system is a complex machinery responsible for the specific tagging and proofreading of substrates degraded by the 26S proteasome, but ubiquitination itself also serves other functions. The formation of a polyubiquitin degradation signal is usually required for proteasome-dependent proteolysis. Hierarchical families of enzymes, which may comprise dozens of members to achieve high selectivity, control this process. The substrates tagged by ubiquitin are then recognized by the 26S proteasome and degraded into peptides. However, the 26S proteasome also recognizes and degrades some non-ubiquitinated proteins, and several proteasome populations participate to protein breakdown. Thus, mammalian cells contain multiple ubiquitin- and/or proteasome-dependent pathways. These systems can degrade single proteins by alternative mechanisms and may also interfere or cooperate with other proteolytic pathways.

Key words: Protein Breakdown, Ubiquitin, Proteasome, Calpain, Cathepsin, Skeletal Muscle

Introduction

Protein breakdown not only regulates the amount of intracellular proteins, but is also involved in their quality control. However, proteolysis has been considered for years as a non-selective process responsible for basal protein turnover, the elimination of abnormal proteins, and the regulation of some key enzymes by unclear mechanisms. An exponential amount of information on the characterization and regulation of ubiquitin-proteasome-mediated proteolysis has been obtained during the last two decades. This pathway is the major non-lysosomal process responsible for the breakdown of most short- and long-lived proteins in mammalian cells (Rock et al., 1994). For example, in skeletal muscle, the system is responsible for the breakdown of the major contractile proteins, actin and myosins (for recent reviews see Attaix and Taillandier, 1998; Lecker et al., 1999; Hasselgren and Fischer, 2001). In addition, the pathway also controls various major biological events such as cell-cycle progression, oncogenesis, transcriptional control, development and differentiation, signal transduction, receptor down-regulation and antigen processing, via the breakdown of specific proteins (Peters et al., 1998; Hershko et al., 2000). In this review we first discuss the selectivity of the system, which is the most elaborate protein degradation machinery known. We also describe alternative ubiquitin- and/or proteasome-dependent proteolytic routes and their possible connections with other proteolytic systems.

Ubiquitination

There are two main steps in the pathway: (1) covalent attachment of a polyubiquitin chain to the substrate, and (2) specific recognition of this signal, and degradation of the tagged protein by the 26S proteasome (Fig. 1).

Ubiquitination is a multiple step process (Hershko and Ciechanover, 1998; Scheffner et al., 1998; Ciechanover et al. 2000). In brief, ubiquitin is initially activated in the presence of ATP to a high-energy thiol ester intermediate by the ubiquitin-activating enzyme (E1). E1 then transfers ubiquitin to one of the ubiquitin-conjugating enzymes (E2s), which also forms a thiol ester linkage between the active site cysteine and ubiquitin. E2s and/or ubiquitin-protein ligases (E3s), which play a role in the selection of proteins for conjugation, bind the first ubiquitin molecule to protein substrates via an isopeptide bond between the activated C-terminal glycine residue of ubiquitin and the ε-amino group of a lysine residue of the substrate.

The resulting monoubiquitinated protein is usually not targeted for degradation by the proteasome. Typical monoubiquitinated conjugates are receptors or basic proteins such as histones. Alternatively, E2s and/or E3s catalyze the formation of polyubiquitinated conjugates. This is usually achieved by transfer of additional activated ubiquitin moieties to Lys_{48} of the preceding conjugated ubiquitin molecule. Finally, a fourth enzyme called E4, which catalyzes the efficient polymerization of very long polyubiquitin chains, has been characterized in yeast (Koegl et al., 1999).

The ubiquitin-conjugating system is hierarchical. In mammals there is a single E1 (Hershko and Ciechanover, 1998; Pickart, 2001), at least 20-30 E2s (Scheffner et al., 1998), and several dozens of E3s (Wilkinson, 2000; Pickart, 2001).

Ubiquitin-activating enzyme (E1)

E1 exists as two isoforms of 110- and 117-kDa, which derive from a single gene and are found in both the nucleus and cytosol (Haas and Siepmann, 1997). The reaction starts with the binding of ATP-Mg^{2+}, and then of ubiquitin, leading to the formation of a ubiquitin adenylate intermediate that serves as the donor of ubiquitin to the critical cysteine residue in the E1 active site. When fully loaded, the E1 carries two molecules of activated ubiquitin (as a thiol ester and as an adenylate, respectively), so that the thiol-linked ubiquitin is transferred to one of the E2s. This reaction is very efficient and allows the production of activated ubiquitin for the entire downstream ubiquitin conjugation pathway. Thus, and not surprisingly, deletion of E1 is lethal in yeast.

Ubiquitin-conjugating enzymes (E2s)

E2s are a superfamily of related proteins, with a molecular weight range of ~14- to 35-kDa. There are 4 classes of E2s that share a central catalytic domain of ~150 amino acids with the active site cysteine, and some variable N- and/or C-terminal extensions that may play a role in substrate specificity. There are eleven E2s in yeast (Pickart, 2001), and 20-30 E2s in mammals (Scheffner et al., 1998). Despite their structural similarities, E2s are responsible for distinct biological functions so that only a limited number of E2s (e.g. three E2s in yeast) play a role in the formation of the polyubiquitin degradation signal. This signal is catalyzed by only the E1 and some E2s, and more generally by the E1, one E2 and one E3. A given E2 can interact with a

limited number of E3s (and conversely), which in turn recognize their specific protein substrates (Pickart, 2001). Moreover, a given protein substrate can be ubiquitinated by different combinations of E2s and E3s (Gonen et al., 1996). This results in a wide range of ubiquitination pathways, which are presumably specific for a given protein or a class of substrates.

Ubiquitin-protein ligases (E3s)

E3s play a key role in the ubiquitin pathway, as they are responsible for the selective recognition of protein substrates. Very few E3s that shared poor apparent structural similarities were first described (Hershko and Ciechanover, 1998). Recently, our knowledge of these enzymes has been rapidly growing. All known E3s are HECT domain E3s or RING finger E3s (Pickart, 2001).

The first major group of E3s corresponds to enzymes of the HECT (**H**omologous to **E**6-AP **C**-**T**erminus) domain family. E6-AP (E6-**A**ssociated **P**rotein) forms a complex with the papilloma virus E6 oncoprotein (that acts as an adapter protein) to ubiquitinate the tumor suppressor p53 protein. The final third of the E6-AP sequence, called the HECT domain, is approximately 350 amino acids in length (Fig. 2A). The HECT domain itself mediates E2 binding and ubiquitination of the target protein via thiol ester linkage formation with ubiquitin. The N-terminus region of every HECT E3 binds to specific substrate(s) (Fig. 2A). Twenty different human HECT E3s that interact with two classes of E2s have been described (Schwarz et al., 1998), but mammalian Genome sequencing projects have identified numerous potential uncharacterized HECT E3s (Pickart, 2001).

The RING finger structure is defined by eight cysteine and histidine residues that coordinate two zinc ions (Freemont, 2000; Pickart, 2001) (Fig. 2B). There are several hundred cDNAs encoding RING finger proteins in the GeneBank database, and many unrelated RING finger proteins with unknown functions behave *in vitro* as E3s (Lorick et al., 1999). This suggests that the number of E3s could be much larger than previously believed. The RING finger E3s are either monomeric proteins or multiple subunit complexes (Pickart, 2001).

The N-end rule enzyme E3α that binds to proteins bearing basic or bulky hydrophobic N-terminal amino acid residues (Fig. 2C) is the best known monomeric RING finger E3. E3α interacts with the 14-kDa E2.

The multiple subunit complexes of RING finger E3s comprise so far, at least three distinct E3 families called the cyclosome or APC (**A**naphase **P**romoting **C**omplex) (Hershko and Ciechanover, 1998), the SCF (**S**kp1-**C**dc53-**F**-box protein family), and the VCB-like **(V**on Hippel-Lindau tumor suppressor-Elongin**C**/**B**) E3s (Tyers and Willems, 1999; Lisztwan et al., 1999; Pickart, 2001). These complexes contain a catalytic core and substrate-specific adapter proteins (Tyers and Willems, 1999). For example, in SCF E3s the catalytic core is formed by three subunits: Cullin1, the RING finger subunit Rbx1 and an E2 (Fig. 2D). The adapter protein Skp1 recruits (via the F-box motif) F-box proteins, which themselves recruit specific protein substrates through protein-protein interaction domains such as leucine-rich repeats or WD-40 domains (Fig. 2D). F-box proteins represent an expanding family of eukaryotic proteins (Cenciarelli et al., 1999). Interestingly, several F-box proteins are themselves degraded in an ubiquitin- and proteasome-dependent manner *in vivo*, suggesting that their breakdown allow rapid switching among multiple E3 complexes (Galan and Peter, 1999).

Signals that target substrates for ubiquitination and proteolysis

Ubiquitination is an important and widespread post-translational modification of proteins, which resembles phosphorylation. Very importantly, ubiquitination is not only a degradation signal, but also directs proteins to a variety of fates which include roles in ribosomal function, in DNA repair, in protein translocation, and in modulation of structure or activity of the target proteins (Wilkinson, 2000; Pickart, 2001). For example, many monoubiquitinated proteins are targeted for endocytosis, ultimately resulting in proteolysis in the lysosome/yeast vacuole (Shih et al., 2000). In order to be efficiently degraded, the substrate must be bound to a polyubiquitin degradation signal that comprises at least four ubiquitin moieties (Thrower et al., 2000). These signals are usually determined by short regions in the primary sequence of the targeted protein (Pickart, 2001).

The nature of the N-terminal amino acid of a protein (N-end rule) may determine its rate of polyubiquitination and subsequent degradation (Varshavsky, 1996). However, the physiological role of the N-end rule is still unclear, because there are very few identified N-end rule substrates (Herskho & Ciechanover, 1998). So far, the N-end rule pathway is only known to be important for the increased breakdown of soluble muscle proteins (Solomon et al., 1998). However, it is now clear that this pathway plays a role in specific

biological functions such as the cell's capacity to import peptides (Turner et al., 2000) or chromosome stability (Rao et al., 2001), via the breakdown of specific protein substrates.

Phosphorylation is required for efficient polyubiquitination of several proteins, especially those recognized by the SCF E3s (Hershko and Ciechanover, 1998). The major recognition site of G1 cyclins is present within a 100-200 amino acid C-terminal region rich in Pro-Glu-Ser-Thr (PEST) motifs. PEST motifs, which are characteristic of rapidly degraded proteins (Rogers et al., 1986), are in fact minimum consensus phosphorylation sites for several protein kinases (Wilkinson, 2000). The ability of an E3 to recognize a phosphorylated signal may be due to the presence of phosphoamino acid binding motifs, such as WW or WD40 domains (Pickart, 2001). However and first, there is no clear pattern of phosphorylation that targets substrates for ubiquitination, as phosphorylation has been reported at single or multiple sites. Second, phosphorylation of some proteins (c-Fos, c-Jun) actually prevents their ubiquitination and degradation (Musti et al., 1997). Finally, recognition of substrates that depends on dephosphorylation has also been reported (Pickart, 2001).

By contrast, the destruction box (i.e. a very degenerate 9-amino acid motif) is a crucial signal for the ubiquitination and breakdown of mitotic cyclins and other cell-cycle regulators, both *in vivo* and *in vitro*. In this motif, only Arg and Leu are invariable amino acids in position 1 and 4, respectively, and are key determinants of specificity (Hershko and Ciechanover, 1998; Pickart, 2001). The destruction box itself is not an ubiquitination site, but is a transferable degradation signal. Reporter proteins containing such motifs are rapidly degraded in a cell-cycle-dependent manner. Recent data may suggest that a specific conformation of the destruction box is required for efficient E3 recognition (Pickart, 2001).

Deubiquitination

Eukaryotic cells also contain DUBs (**D**e**UB**iquitinating enzymes), which are encoded by the UCH (**U**biquitin **C**arboxyl-terminal **H**ydrolases) and the UBP (**UB**iquitin-specific **P**rocessing proteases) gene families (Chung and Baek, 1999). Genome sequencing projects have identified more than 90 DUBs (Chung and Baek, 1999). UCHs are relatively small proteins (< 40-kDa) and constitute a small family. Only one UCH exists in yeast, and only a couple of isoforms have been characterized in higher eukaryotes. UCHs mainly hydrolyze small amides and esters at the C-terminus of ubiquitin. In contrast, UBPs are 50-250-kDa

proteins and constitute a large family, as 16 UBPs are known in yeast. Deubiquitination can be compared to dephosphorylation, and not surprisingly UBPs are involved in several biological processes, including the control of growth, differentiation, and genome integrity. In proteasome-dependent proteolysis, the putative major roles of DUBs are (i) to maintain free ubiquitin levels, by processing ubiquitin precursors and polyubiquitin degradation signals into free monomers; (ii) to proof-read ubiquitination (e.g. to deubiquitinate substrates erroneously tagged for degradation); and (iii) to keep 26S proteasomes free of polyubiquitin chains that can interfere with the binding of another substrate.

Degradation of non-ubiquitinated proteins

The 26S proteasome is not an absolute ubiquitin-dependent proteolytic enzyme, as it also degrades non-ubiquitinated substrates. The first discovered was ornithine decarboxylase (ODC) (Murakami et al., 1992). The 26S proteasome recognizes the C-terminal degradation signal of ODC exposed by attachment of antizyme (a protein that binds to ODC and substitutes for ubiquitin) (Murakami et al., 1999). Indeed, ubiquitination does not seem always a prerequisite for the breakdown of a growing number of substrates that include c-Jun (Jariel-Encontre et al., 1995), IκBα (Krappmann et al., 1996), the Cdk inhibitor p21Cip1 (Sheaff et al., 2000), denatured ovalbumin, and native forms of calmodulin or troponin C (Benaroudj et al., 2001). The 26S proteasome can recognize misfolded proteins that are not ubiquitinated (Strickland et al., 2000). This may account for the breakdown of denatured ovalbumin. In addition, hydrophobicity plays a major role in polyubiquitin chain recognition by the 26S proteasome (Thrower et al., 2000). Hydrophobic stretches of amino acids in the primary sequence of calmodulin and troponin C may substitute for ubiquitin and be sufficient for recognition by the 26S proteasome (Benaroudj et al., 2001).

The proteasomes

The second major step in the ubiquitin-proteasome pathways is the degradation of polyubiquitinated proteins by the 26S proteasome, which is formed by the binding of two 19S regulatory complexes with the 20S proteasome (Voges et al., 1999; DeMartino and Slaughter, 1999).

The 20S proteasome

The mammalian 20S proteasome is a cylindrical particle composed of four stacked rings of subunits, with each ring containing seven different subunits. The outer rings are composed of α-subunits, and the two inner rings of β-subunits, which contain the catalytic sites inside the particle (Fig. 3). Thus the proteasome is a self-compartmentalizing protease (Baumeister et al., 1998), as substrates must enter the catalytic chamber in order to be degraded into peptides. In eukaryotes, the 20S proteasome contains at least two chymotrypsin-like, two trypsin-like, and two caspase-like active sites (Kisselev et al., 1999). These activities are allosterically regulated and it has been suggested that there is an ordered, cyclical mechanism for protein degradation: the chymotrypsin-like site may initially cleave the polypeptide and stimulate the caspase-like sites; their activation accelerates further cleavage of the fragments, while the chymotrypsin-like activity is temporarily inhibited; when further caspase-like cleavages are impossible, the chymotryptic site is reactivated and the cycle repeated (Kisselev et al., 1999). The confinement of multiple active sites within a nano-compartment has another advantage. Proteasomes hydrolyze most peptide bonds and generate peptides that are typically 3 to 22 amino acids long and do not conserve biological properties, except for antigen presentation.

Substrates access the active sites by traversing a narrow opening in the α-ring that is blocked in the unliganded free 20S proteasome by N-terminal sequences of α-subunits (Whitby et al., 2000). The binding of 20S proteasomes to 11S or 19S regulator complexes induce conformational changes in α-subunits that open the gate separating the catalytic chamber of the 20S proteasome from the intracellular environment (Whitby et al., 2000; Groll et al. 2000) (Fig. 3). Thus, whether free 20S proteasomes may have any proteolytic activity in cells remains an open question. Oxidized proteins have been repeatedly reported to be degraded by 20S proteasomes and not by 26 proteasomes (Davies, 2001). However, it is totally unclear how oxidized proteins may gate the 20S proteasome channel.

It should be pointed out that the 20S proteasome is the proteolytic core of a modular system in which peptidase activities can be modulated by the binding of regulatory complexes (see below). Furthermore, there are immunoproteasomes in which three catalytic β subunits are replaced by three distinct β subunits, so that catalytic properties are also altered. These immunoproteasomes play a role in antigen presentation. Thus, there are different subtypes of 20S particles in a given tissue that differ by their catalytic properties. For example, the 20S proteasome population in skeletal muscle comprises six distinct subtypes, including constitutive

proteasomes, immunoproteasomes, and their intermediate forms (Dahlmann et al., 2001). Therefore, the properties of a 20S proteasome population isolated from a given tissue represent the average properties of the whole set of proteasomes subtypes.

The 19S complex

The 19S complex is an activator that stimulates both peptidase and proteolytic activities of the 20S proteasome. This complex contains at least 18 different subunits and can be topologically defined by two sub-complexes called the base and the lid (Glickman et al., 1998). The base contains six ATPases, and two non-ATPase subunits in yeast. The ATPases provide energy for the assembly of the 26S proteasome and the breakdown of ubiquitinated proteins into peptides, for the gating of the proteasome channel, and presumably the unfolding of protein substrates, and their injection into the catalytic chamber of the proteasome (Voges et al., 1999; Kohler et al. 2001). The binding of the 20S proteasome to the base alone supports ATP-dependent peptide hydrolysis. In contrast, both the base and the lid are required for ubiquitin-dependent proteolysis (Glickman et al., 1998). The lid contains at least eight non-ATPase subunits. Subunit S5a binds tightly to the polyubiquitin degradation signal (Deveraux et al., 1994). However, the yeast homolog of S5a is not essential for ubiquitin-dependent proteolysis (van Nocker et al., 1996). Thus, another subunit or several additional subunits may also act as a polyubiquitin-conjugate receptor.

Surprisingly, the base of the 19S complex has also chaperone-like activity and is able to refold a denatured protein *in vitro*, a function opposite to its presumed role in proteolysis (Braun et al., 1999). These findings suggest that the ultimate fate of a proteasome substrate (degradation or refolding) is determined by subsequent events (e.g. translocation into the proteolytic core) (Zwickl and Baumeister, 1999). Extensive experiments are currently in progress in several laboratories to elucidate the precise topology (Gorbea et al., 2000) and roles of subunits in both the base and the lid (Kohler et al., 2001). Such experiments should provide insights into the precise mechanisms that regulate the recognition, unfolding, and translocation of substrates into the proteasome, and contribute to elucidate the precise role(s) of ATP in proteolysis. For example, it has been recently reported that Rpt2, one of the six ATPases in the base of the 19S complex, gates the proteasome channel and controls both substrate entry and product release (Kohler et al., 2001).

Other proteasome activators

The 11S regulator is another regulatory particle of the 20S proteasome. The 11S regulator (an hexamer or heptamer of subunits PA28α and β) binds to both ends of the 20S proteasome in an ATP-independent fashion to form a PA28 particle. The binding of the 11S regulator to the 20S proteasome only modulates its peptidase activities. PA28 particles play a role in antigen presentation, by generating peptides for MHC class I molecules, possibly in connection with 26S immunoproteasomes (Rechsteiner et al., 2000).

Subunit PA28γ is closely related to subunits PA28α and β. PA28γ is able to form a homopolymer that also binds to 20S proteasomes. The resulting proteasome-PA28γ seems to be involved in growth control (Murata et al., 1999).

Finally, the existence of hybrid proteasomes, in which one 11S regulator and one 19S complex bind simultaneously to a 20S proteasome has been demonstrated (Hendil et al., 1998). Such complexes are induced by interferon-γ and play a role both in antigen presentation and in the breakdown of some proteins (Tanahashi et al., 2000, and see below).

The ubiquitination and the proteasome systems are part of a huge proteolytic machinery

The mechanism that facilitates the translocation of a substrate to the proteasome *in vivo* is poorly understood. However, a physical association between several E2s and the 26S proteasome has been reported (Tongaonkar et al., 2000) and E2s bind tightly to the ubiquitin-loaded E1 (Pickart et al., 2001). Various E3s also interact with specific subunits of the 19S complex (Xie and Varshavsky, 2000). Thus, the whole ubiquitination system may directly and physically participate in the delivery of tagged substrates to the 26S proteasome.

The 26S proteasome degrades proteins only into peptides. Except when presented on MHC Class I molecules, these peptides must undergo further hydrolysis into free amino acids. In *Thermoplasma acidophilum* the proteasome generated peptides are then cleaved into smaller di- to tetra-peptides by a huge (14.6-MDa) self-compartmentalizing proteolytic complex called the tricorn protease; finally, these di- to tetra-peptides are sequentially hydrolyzed into free amino acids by at least three additional aminopeptidase-interacting factors (Tamura et al., 1998). The tricorn protease has no homolog in higher organisms. However,

several giant proteases that may act downstream of the proteasome and partially substitute for its functions have been recently characterized in eukaryotic cells (Yao and Cohen, 1999).

Conversely, other proteases may act upstream of the proteasome. Specific interactions between the myofibrillar proteins appear to protect them from ubiquitin-dependent degradation, and the rate-limiting step in their degradation is probably their dissociation from the myofibril (Solomon and Goldberg, 1996). Calpains play key roles in the disassembly of sarcomeric proteins (Huang and Forsberg, 1998) and in Z-band disintegration, resulting in the release of myofilaments (Williams et al., 1999). These data suggest that calpains are acting upstream of the proteasome. However it remains to be demonstrated whether there is a functional connection between the two proteolytic systems. Similarly, a putative cooperative role of calpain and proteasome has been reported in the breakdown of the retinoblastoma family protein p107 (Jang and Choi, 1999).

Degradation of substrates of the ubiquitin-proteasome pathway by alternative proteolytic routes

ODC, the first discovered non-ubiquitinated substrate of 26S proteasomes (see above) can also be degraded by hybrid proteasomes (Tanahashi et al., 2000). Thus, several proteasome-dependent pathways can be involved in the breakdown of a single protein substrate.

Another non-ubiquitinated substrate of the 26S proteasome is the protooncogene c-Jun (Jariel-Encontre et al., 1995). However, c-Jun can also be ubiquitinated (Treier et al., 1994), and both ubiquitin-dependent and ubiquitin-independent proteasome pathways degrade the protein. Similar situations are known for the *in vivo* proteasomal breakdown of the IκBα transcription factor inhibitor (Krappmann et al., 1996) and of the Cdk inhibitor p21Cip1 (Sheaff et al., 2000). At least in the case of IκBα, these alternative ubiquitin-dependent and ubiquitin-independent, but proteasome-dependent pathways dramatically affect the protein half-life, and thus tightly control IκBα activity (Krappmann et al., 1996).

Finally, the tumor suppressor p53 protein (Salvat et al., 1999) and the retinoblastoma family protein p107 (Jang et al., 1999) are degraded *in vivo* by distinct proteolytic systems, the proteasome and the calpains. The proteasome, calpain (Salvat et al., 1999) and lysosomal (Knecht et al., 1998) systems can even degrade the protooncogene c-Fos. All these observations suggest that a given protein substrate is targeted for

degradation by many alternative proteolytic routes. Assuming that various signaling pathways dictate the routes, this may clearly explain how the cell rapidly modulates the half-lives of various proteins, in response to the cell environment, and accounts for the fine tuning of individual protein levels.

Implications

The ubiquitin-proteasome-dependent pathway is the most elaborate protein-degradation machinery known. The precise mechanisms that regulate the breakdown of some key proteins (e.g. cyclins, transcription factors) have started to be elucidated. In contrast, how the ubiquitin-proteasome-pathway degrades muscle proteins, and more particularly contractile proteins, remains largely unknown. Information on the E2s and E3s that operate in muscle is very scarce, and neither the signals that target myofibrillar proteins for breakdown, nor the precise substrates of the pathway have been identified. Studies that aim to explore the role of deubiquitinating enzyme and the regulation of proteolytic/peptidase activities of muscle proteasomes are also clearly needed. Finally, studies on the possible relationships of the ubiquitin-proteasome-pathway with the cathepsins and calpains should also contribute to provide valuable information on skeletal muscle proteolysis. However, the complexity of the ubiquitin pathway will clearly impede the identification of the precise mechanisms that are important in the control of muscle proteolysis.

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Literature Cited

Attaix, D., and D. Taillandier. 1998. The critical role of the ubiquitin-proteasome pathway in muscle wasting in comparison to lysosomal and Ca^{2+}-dependent systems. In: E. E. Bittar, and A. J. Rivett (eds.). Intracellular protein degradation, p. 235. JAI Press Inc., Greenwich, CT.

Baumeister, W., J. Walz, F. Zühl, and E. Seemüller. 1998. The proteasome: paradigm of a self-compartmentalizing protease. Cell 92:367-380.

Benaroudj, N., E. Tarcsa, P. Cascio, and A. L. Goldberg. 2001. The unfolding of substrates and ubiquitin-independent protein degradation by proteasomes. Biochimie 83:311-318.275:875-882.

Braun, B. C., M. Glickman, R. Kraft, B. Dahlmann, P. M. Kloetzel, D. Finley, and M. Schmidt. 1999. The base of the proteasome regulatory particle exhibits chaperone-like activity. Nat. Cell Biol. 1:221-226.

Cenciarelli, C., D. S. Chiaur, D. Guardavaccaro, W. Parks, M. Vidal, and M. Pagano. 1999. Identification of a family of human F-box proteins. Curr. Biol. 9:1177-1179.

Chung, C. H., and S. H. Baek. 1999. Deubiquitinating enzymes: their diversity and emerging roles. Biochem. Biophys. Res. Commun. 266:633-640.

Ciechanover, A., A. Orian, and A. L. Schwartz. 2000. Ubiquitin-mediated proteolysis: biological regulation via destruction. Bioessays 22:442-451.

Dahlmann, B., T. Ruppert, P. M. Kloetzel, and L. Kuehn. 2001. Subtypes of 20S proteasomes from skeletal muscle. Biochimie 83:295-299.

Davies, K. J. 2001. Degradation of oxidized proteins by the 20S proteasome. Biochimie 83:301-310.

DeMartino, G. N., and C. A. Slaughter. 1999. The proteasome, a novel protease regulated by multiple mechanisms. J. Biol. Chem. 274:22123-22126.

Deveraux, Q., V. Ustrell, C. Pickart, and M. Rechsteiner. 1994. A 26S protease subunit that binds ubiquitin conjugates. J. Biol. Chem. 269:7059-7061.

Freemont, P.S. 2000. RING for destruction? Curr. Biol. 10:R84-R87.

Galan, J. M., and M. Peter. 1999. Ubiquitin-dependent degradation of multiple F-box proteins by an autocatalytic mechanism. Proc. Natl. Acad. Sci. USA 96:9124-9129.

Glickman, M. H., D. M. Rubin, O. Coux, I. Wefes, G. Pfeifer, Z. Cjeka, W. Baumeister, V. A. Fried, and D. Finley. 1998. A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3. Cell 94:615-623.

Gonen, H., I. Stancovski, D. Shkedy, T. Hadari, B. Bercovich, E. Bengal, S. Mesilati, O. Abu-Atoum, A.L. Schwartz, and A. Ciechanover, 1996. Isolation, characterization, and partial purification of a novel ubiquitin-protein ligase, E3. J. Biol. Chem. 271:302-310.

Gorbea, C., D. Taillandier, and M. Rechsteiner. 2000. Mapping subunit contacts in the regulatory complex of the 26 S proteasome. S2 and S5b form a tetramer with ATPase subunits S4 and S7. J. Biol. Chem. 275:875-882.

Groll, M., M. Bajorek, A. Kohler, L. Moroder, D. M. Rubin, R. Huber, M. H. Glickman, and D. Finley. 2000. A gated channel into the proteasome core particle. Nat. Struct. Biol. 7:1062-1067.

Haas, A.L., and T.J. Siepmann. 1997. Pathways of ubiquitin conjugation. FASEB J. 11:1257-1268.

Hasselgren, P. O., and J. E. Fischer. 2001. Muscle cachexia: current concepts of intracellular mechanisms and molecular regulation. Ann. Surg. 233:9-17.

Hendil, K. B., S. Khan, and K. Tanaka. 1998. Simultaneous binding of PA28 and PA700 activators to 20S proteasomes. Biochem. J. 332:749-754.

Hershko, A., and A. Ciechanover. 1998. The ubiquitin system. Annu. Rev. Biochem. 67:425-479.

Hershko, A., A. Ciechanover, and A. Varshavsky. 2000. Basic Medical Research Award. The ubiquitin system. Nat. Med. 6:1073-1081.

Huang, J., and N. E. Forsberg. 1998. Role of calpain in skeletal-muscle protein degradation. Proc. Natl. Acad. Sci. USA 95:12100-12105.

Jang, J. S., and Y. H. Choi. 1999. Proteolytic degradation of the retinoblastoma family protein p107: a putative cooperative role of calpain and proteasome. Int. J. Mol. Med. 4:487-492.

Jariel-Encontre, I., M. Pariat, F. Martin, S. Carillo, C. Salvat, and M. Piechaczyk 1995. Ubiquitinylation is not an absolute requirement for degradation of c-Jun protein by the 26S proteasome. J. Biol. Chem. 270:11623-11627.

Kisselev, A. F., T. N. Akopian, V. Castillo, and A. L. Goldberg. 1999. Proteasome active sites allosterically regulate each other, suggesting a cyclical bite-chew mechanism for protein breakdown. Mol. Cell 4:395-402.

Knecht, E., J. J. M. de Llano, E. J. Andreu, and I. M. Miralles. 1998. Pathways for the degradation of intracellular proteins within lysosomes in higher eukaryotes. In: E. E. Bittar, and A. J. Rivett (eds.). Intracellular protein degradation, p. 201. JAI Press Inc., Greenwich, CT.

Koegl, M., T. Hoppe, S. Schlenker, H. D. Ulrich, T. U. Mayer, and S. Jentsch. 1999. A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. Cell 96:635-644.

Kohler, A., P. Cascio, D. S. Leggett, K. M. Woo, A. L. Goldberg, and D. Finley. 2001. The axial channel of the proteasome core particle is gated by the rpt2 atpase and controls both substrate entry and product release. Mol. Cell 7:1143-52.

Krappmann, D., F. G. Wulczyn, and C. Scheidereit. 1996. Different mechanisms control signal-induced degradation and basal turnover of the NF-κB inhibitor IκBα in vivo. EMBO J. 15:6716-6726.

Lecker, S. H., V. Solomon, W. E. Mitch, and A. L. Goldberg. 1999. Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. J. Nutr. 129(1S Suppl):227S-237S.

Lisztwan, J., G. Imbert, C. Wirbelauer, M. Gstaiger, and W. Krek. 1999. The von Hippel-Lindau tumor suppressor protein is a component of an E3 ubiquitin-protein ligase activity. Genes Dev. 13:1822-1833.

Lorick, K. L., J. P. Jensen, S. Fang, A. M. Ong, S. Hatakeyama, and A. M. Weissman. 1999. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proc Natl Acad Sci USA 96:11364-11369.

Murakami, Y., S. Matsufuji, T. Kameji, S. Hayashi, K. Igarashi, T. Tamura, K. Tanaka, and A. Ichihara. 1992. Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. Nature 360:597-599.

Murakami, Y., S. Matsufuji, S. I. Hayashi, N. Tanahashi, and K. Tanaka. 1999. ATP-dependent inactivation and sequestration of ornithine decarboxylase by the 26S proteasome are prerequisites for degradation. Mol. Cell. Biol. 19:7216-7227.

Murata, S., H. Kawahara, S. Tohma, K. Yamamoto, M. Kasahara, Y. Nabeshima, K. Tanaka, and T. Chiba. 1999. Growth retardation in mice lacking the proteasome activator PA28γ. J. Biol. Chem. 274 : 38211-38215.

Musti, A. M., M. Treier, and D. Bohmann. 1997. Reduced ubiquitin-dependent degradation of c-Jun after phosphorylation by MAP kinases. Science 275:400-402.

Peters, J.-M., J. R. Harris, and D. Finley (editors). 1998. Ubiquitin and the Biology of the Cell. Plenum Press, New York.

Pickart, C. M. 2001. Mechanisms underlying ubiquitination. Annu. Rev. Biochem. 70:503-533.

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

Rechsteiner, M., C. Realini, and V. Ustrell. 2000. The proteasome activator 11S REG (PA28) and class I antigen presentation. Biochem. J. 345:1-15.

Rock, K. L., C. Gramm, L. Rothstein, K. Clark, R. Stein, L. Dick, D. Hwang, and A. L. Goldberg. 1994. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. Cell 78:761-771.

Rogers, S., R. Wells, and M. Rechsteiner. 1986. Amino acids sequences common to rapidly degraded proteins: the PEST hypothesis. Science 234:364-368.

Salvat, C., C. Acquaviva, I. Jariel-Encontre, P. Ferrara, M. Pariat, A.-M. Steff, S. Carillo, and M. Piechaczyk. 1999. Are there multiple proteolytic pathways contributing to c-Fos, c-Jun and p53 protein degradation in vivo? Mol. Biol. Rep. 26:45-51.

Scheffner, M., S. Smith, and S. Jentsch. 1998. The ubiquitin-conjugation system. In: J.-M. Peters, J. R. Harris, and D. Finley (eds.) Ubiquitin and the Biology of the Cell, p. 65. Plenum Press, New York.

Schwarz, S. E., J. L. Rosa, and M. Scheffner. 1998. Characterization of human hect domain family members and their interaction with UbcH5 and UbcH7. J. Biol. Chem. 273:12148-12154.

Sheaff, R. J., J. D. Singer, J. Swanger, M. Smitherman, J. M. Roberts, and B. E. Clurman. 2000. Proteasomal turnover of p21Cip1 does not require p21Cip1 ubiquitination. Mol. Cell 5:403-410.

Shih, S. C., K. E. Sloper-Mould, and L. Hicke. 2000. Monoubiquitin carries a novel internalization signal that is appended to activated receptors. EMBO J. 19:187-198.

Solomon, V., V. Baracos, P. Sarraf, and A. L. Goldberg. 1998. Rates of ubiquitin conjugation increase when muscles atrophy, largely through activation of the N-end rule pathway. Proc. Natl. Acad. Sci. USA 95:12602-12607.

Solomon, V., and A. L. Goldberg. 1996. Importance of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. J. Biol. Chem. 271:26690-26697.

Strickland, E., K. Hakala, P. J. Thomas, and G. N. DeMartino. 2000. Recognition of misfolding proteins by PA700, the regulatory subcomplex of the 26 S proteasome. J. Biol. Chem. 275:5565-5572.

Tamura, N., F. Lottspeich, W. Baumeister, and T. Tamura. 1998. The role of tricorn protease and its aminopeptidase-interacting factors in cellular protein degradation. Cell 95:637-648.

Tanahashi, N., Y. Murakami, Y. Minami , N. Shimbara, K. B. Hendil, and K. Tanaka. 2000. Hybrid proteasomes. Induction by interferon-gamma and contribution to ATP-dependent proteolysis. J. Biol. Chem. 275:14336-14345.

Thrower, J. S., L. Hoffman, M. Rechsteiner, and C. M. Pickart. 2000. Recognition of the polyubiquitin proteolytic signal. EMBO J. 19:94-102.

Tongaonkar, P., L. Chen, D. Lambertson, B. Ko, and K. Madura. 2000. Evidence for an interaction between ubiquitin-conjugating enzymes and the 26S proteasome. Mol. Cell. Biol. 20:4691-4698.

Treier, M., L. M. Staszewski, and D. Bohmann. 1994. Ubiquitin-dependent c-Jun degradation in vivo is mediated by the delta domain. Cell 78:787-798.

Turner, G. C., F. Du, and A. Varshavsky. 2000. Peptides accelerate their uptake by activating a ubiquitin-dependent proteolytic pathway. Nature 405:579-583.

Tyers, M.., and A. R. Willems. 1999. One ring to rule a superfamily of E3 ubiquitin ligases. Science 284:601-604.

van Nocker, S., S. Sadis, D. M. Rubin, M. Glickman, H. Fu, O. Coux, I. Wefes, D. Finley, and R. D. Vierstra. 1996. The multiubiquitin-chain-binding protein Mcb1 is a component of the 26S proteasome in Saccharomyces cerevisiae and plays a nonessential, substrate-specific role in protein turnover. Mol. Cell. Biol. 11:6020-6028.

Varshavsky, A. 1996. The N-end rule: functions, mysteries, uses. Proc. Natl. Acad. Sci. USA 93:12142-12149.

Voges, D., P. Zwickl, and W. Baumeister. 1999. The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu. Rev. Biochem. 68:1015-1068.

Wilkinson, K. D. 2000. Ubiquitination and deubiquitination: targeting of proteins for degradation by the proteasome. Semin. Cell. Dev. Biol. 11:141-148.

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

Whitby, F. G., E. I. Masters, L. Kramer, J. R. Knowlton, Y. Yao, C. C. Wang, and C. P. Hill. 2000. Structural basis for the activation of 20S proteasomes by 11S regulators. Nature 408:115-120.

Xie, Y., and A. Varshavsky. 2000. Physical association of ubiquitin ligases and the 26S proteasome. Proc. Natl. Acad. Sci. USA 97:2497-2502.

Yao, T., and R. E. Cohen. 1999. Giant proteases: beyond the proteasome. Curr. Biol. 9:R551-R553.

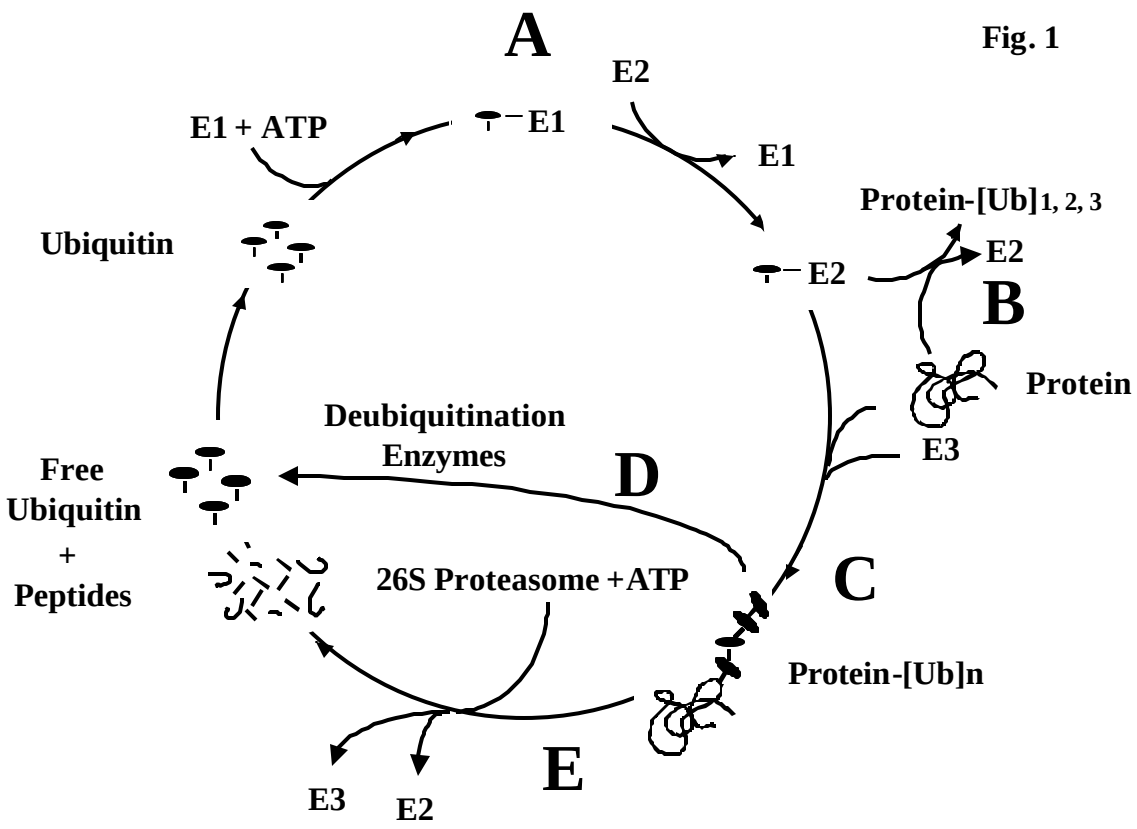
Zwickl, P., and W. Baumeister. 1999. AAA-ATPases at the crossroads of protein life and death. Nat. Cell Biol. 1:E97-E98.

Figure Legends.

Figure 1. A. Ubiquitin is first activated by the ubiquitin-activating enzyme (E1) and transferred on one ubiquitin-conjugating enzyme (E2). B. The E2 with or without an ubiquitin-protein ligase (E3), mono- di or tri-ubiquitinates the substrate (Protein-$[Ub]_{1,2,3}$), which is not targeted for breakdown. C. In constrast, when a polyubiquitin degradation signal is formed (Protein-$[Ub]_n$), the substrate can be deubiquitinated (D) or is recognized and degraded into peptides by the 26S proteasome (E) .

Figure 2. Schematic representation of E3s. A. General structure of the HECT domain E3s. N and C denotes the N-terminus and C-terminus, respectively, of the HECT E3. AA, amino acid; Cyst, cysteine residue, Ub, ubiquitin. B. Schematic representation of the RING finger motif. C and H denotes cysteine and histidine residues, respectively. C. Schematic representation of the monomeric RING finger E3α. Type I and II denotes the binding site for basic and hydrophobic N-terminal amino acid of the substrate, respectively. D. Schematic representation of a multisubunit RING finger SCF E3. The three subunits in the catalytic core are boxed in white, and Rbx1 is the RING finger subunit. Cul, cullin1. Adapter proteins are boxed in grey (see text).

Figure 3. Schematic representation of the gating of the channel of the 20S proteasome by the 19S or 11S regulatory complex. Left panel: in the free 20S proteasome particle (α and β denotes α and β subunits, respectively) the channel (black oval within the upper α-ring) is blocked by N-terminal sequences of α-subunits. Substrate entry and peptide release occur at a very low rate, if any. Right panel: the binding of one regulatory complex to the α-ring gates the 20S proteasome channel, so that both substrate entry and peptide release are increased.


A
E2
E1 + ATP
E1
E1
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Ubiquitin
E2
E2
B
Protein
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Deubiquitination
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Free
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Peptides
D
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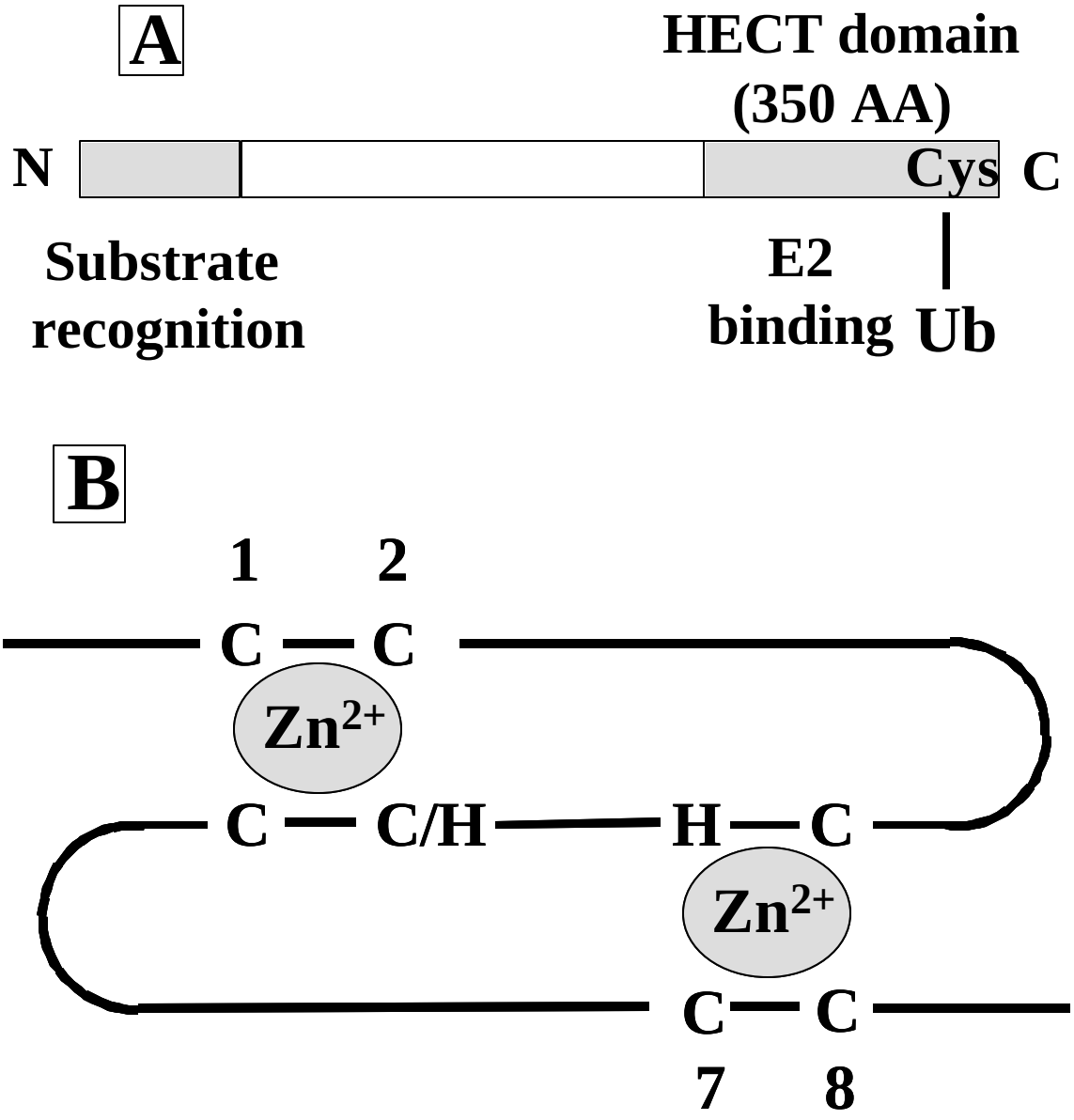
Fig. 1


A
HECT domain
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N
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Substrate
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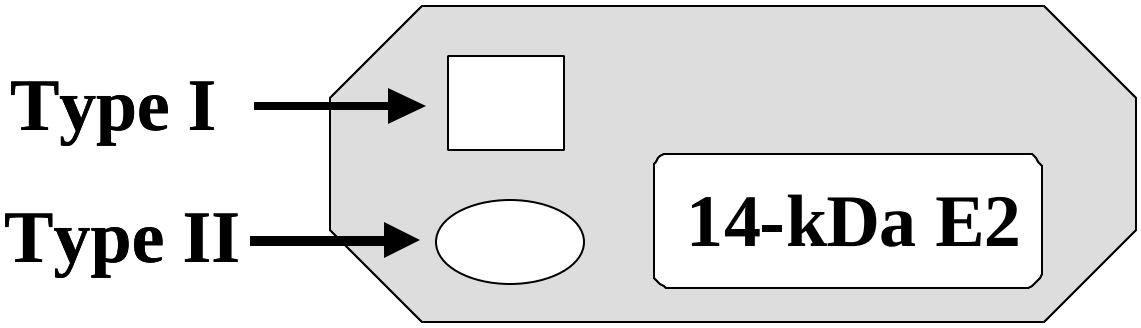

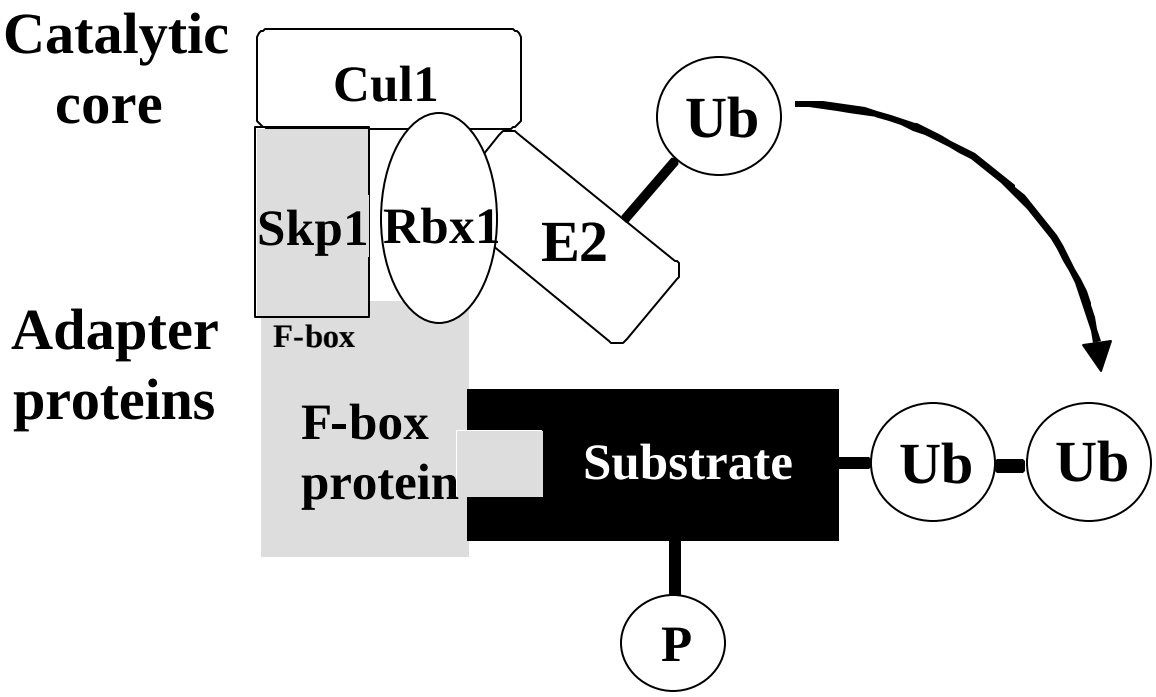
Catalytic core
Cul1
Ub
Skp1
Rbx1
E2
Adapter proteins
F-box
F-box protein
Substrate
Ub
Ub
P

Fig. 3