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Ubiquitylation as a Quality Control System for Intracellular Proteins

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Quality control of intracellular proteins is essential for cellular homeostasis. Molecular chaperones recognize and contribute to the refolding of misfolded or unfolded proteins, whereas the ubiquitin-proteasome system mediates the degradation of such abnormal proteins. Ubiquitin-protein ligases (E3s) determine the substrate specificity for ubiquitylation and have been classified into HECT and RING-finger families. More recently, however, U-box proteins, which contain a domain (the U box) of about 70 amino acids that is conserved from yeast to humans, have been identified as a new type of E3. The prototype U-box protein, yeast Ufd2, was identified as a ubiquitin chain assembly factor (E4) that cooperates with a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and an E3 to catalyze the formation of a ubiquitin chain on artificial substrates. Yeast Ufd2 is functionally implicated in cell survival under stressful conditions. This review addresses recent progress in characterization of the role of E3 enzymes, especially that of U-box proteins, in quality control of intracellular proteins.

Key words: molecular chaperone, quality control, ubiquitin, ubiquitin ligase, U-box protein.

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; ERAD, endoplasmic reticulum–associated degradation; GR, glucocorticoid receptor; HMG, hydroxymethylglutaryl; MTOC, microtubule-organizing center; Nmnat, nicotinamide mononucleotide adenylyltransferase; TPR, tetratricopeptide repeat; UBC, ubiquitin-conjugating; UFD, ubiquitin fusion degradation.

The aggresome as a center for protein aggregation

The aggregation of cellular proteins results from a low solubility in aqueous solution that is attributable to a nonnative secondary structure. Aggregated proteins are sequestered in morphologically detectable intracellular foci known as inclusion bodies (*1*, *2*). Furthermore, inclusion bodies that form as a result of the retrograde transport of aggregated proteins by microtubules are known as "aggresomes" (Fig. 1) (*3*). Certain integral membrane proteins, such as the cystic fibrosis transmembrane conductance regulator (CFTR), presenilin 1, presenilin binding protein (PBP), peripheral myelin protein 22 (PMP22), and prion protein, when misfolded, appear to serve as nucleation sites for the formation of aggresomes that localize to the juxtanuclear region in the vicinity of the centrosome (*3*–*5*). The formation of aggresomes is inhibited by drugs such as nocodazole that induce depolymerization of microtubules as well as by overexpression of p50 (dynamitin), which triggers the dissociation of the dynein-dynactin complex, suggesting that the mictrotubule minus end–directed motor activity of cytoplasmic dynein is required for this process (*6*). Immunocytofluorescence analysis has indicated that, in addition to the aggregated seed proteins, aggresomes contain ubiquitin, proteasomes, and the heat shock proteins Hsc70 and Hsc40. Furthermore, intermediate filaments composed of vimentin, which are usually distributed throughout the cytoplasm, form a cagelike structure around aggresomes (*3*).

The formation of aggresomes in the pericentriolar region is triggered by treatment of cells with proteasome inhibitors. The colocalization of ubiquitin, proteasomes, and heat shock proteins with γ-tubulin of the microtubule-organizing center (MTOC) at the centrosome has led to the latter also being referred to as the proteolysis center (*7*). The pericentriolar colocalization of these proteins is apparent even in the absence of cellular stress. Centrosomes thus likely function as a center for the degradation of misfolded or unfolded proteins under both normal and stressful conditions. However, given that only about 1% of proteasomes are present at the MTOC under nonstress conditions, the significance of centrosomes in the elimination of aberrant cellular proteins remains to be demonstrated.

Cellular protective systems for the quality control of intracellular proteins

Protein aggregation is promoted both by conditions of chemical or physical stress, such as changes in pH, temperature, ionic strength, or redox status, that induce the partial unfolding of proteins as well as by the misfolding of proteins that results from gene mutation, RNA modification, or translational or other errors. In general, protein aggregates are insoluble and stable. Protein aggregation is responsible for the pathological lesions associated with protein accumulation or deposition diseases, which include certain viral infections, protein metabolic diseases such as systemic amyloidosis, and neuro-

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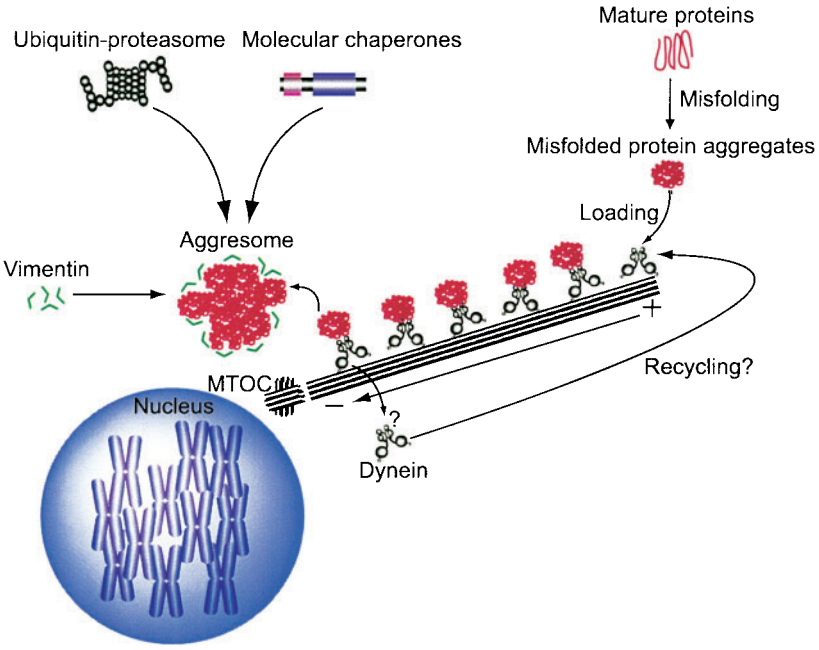

Fig. 1. **Proposed mechanism for the formation of aggresomes.** Unfolded or misfolded proteins aggregate, presumably through the interaction of hydrophobic surfaces, and the aggregated proteins are retrogradely transported by dynein along microtubules to the pericentriolar region, where they become surrounded by intermediate filaments. Protein aggregation is limited under normal conditions by molecular chaperones and the ubiquitin-proteasome system. MTOC, microtubule-organizing center.

degenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (*8*).

The intracellular aggregation of proteins is not usually apparent under physiological conditions, even though cells continuously produce large numbers of protein molecules. This observation suggests that cells possess quality control systems that repair (refold) or degrade misfolded proteins. Indeed, eukaryotic cells utilize two principal types of such defensive mechanisms: promotion of the correct folding of proteins by molecular chaperones, and the degradation of misfolded or unfolded proteins by the ubiquitin-proteasome system (Fig. 1). The failure of these quality control mechanisms may contribute to cellular pathology, especially in cells such as neurons that do not divide after differentiation. The consequent protein aggregation does not necessarily result in cell death, however, but rather may trigger cellular dysfunction. The extent of protein aggregation, determined in part by the activities of the cellular defense systems, likely determines cell fate.

Autophagy, the underlying biochemistry of which is thought to be similar to that of the ubiquitin-conjugating system, also contributes to the degradation of cytoplasmic components, including entire organelles (*9*). This phenomenon is characterized by the engulfment of the target entity by intracellular membranes, resulting in the formation of multilamellar autophagosomes. These structures subsequently fuse with lysosomes and their contents are thereby degraded. Multilamellar structures similar to autophagosomes have been observed in degenerating neurons and in cells exposed to proteasome inhibitors (*10–12*). Like molecular chaperones and the ubiquitin-proteasome system, autophagy may function to maintain quality control in the intracellular environment.

Ubiquitin is a heat-stable and heat shock protein

The ubiquitin-proteasome system mediates the selective degradation of many proteins that function in cellular activities such as cell cycle progression, the response to stress, antigen processing, signal transduction, transcriptional regulation, DNA repair, apoptosis, and organelle biogenesis (*13*, *14*). More recently, ubiquitin-mediated protein degradation has been linked to the pathogenesis of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and polyglutamine diseases (*15–18*).

Ubiquitin is a highly conserved 76–amino acid polypeptide that is present in all eukaryotes. Indeed, the amino acid sequence of ubiquitin is virtually identical in most organisms examined, with, for example, the yeast protein differing at only three residues from the human protein. Ubiquitin is also highly stable, being resistant to denaturation induced by heat or by exposure to acidic or alkaline conditions. These properties are thus consistent with a role for ubiquitin in cellular defense against the stress-induced accumulation of abnormal proteins. Most eukaryotes examined possess two types of ubiquitin gene. One of these genes is present in multiple copies arranged in tandem and encodes a precursor protein that is cleaved by a specific protease to yield mature ubiquitin (*19*). Although the structure of this gene is highly conserved, its copy number varies among organisms, with nine copies present in humans and six in yeast. It is not an essential gene, at least in yeast, but its transcription is induced by cellular stress such as heat shock (*20*, *21*). The stress-induced expression of this gene may thus contribute to the removal of unfolded proteins that accumulate under such conditions. The second type of ubiquitin gene actually encodes a fusion protein comprising both ubiquitin and a ribosomal protein. The ubiquitin component of this fusion protein is again released by the activity of a specific protease. This gene is an essential gene but it is not induced by stress (*22*).

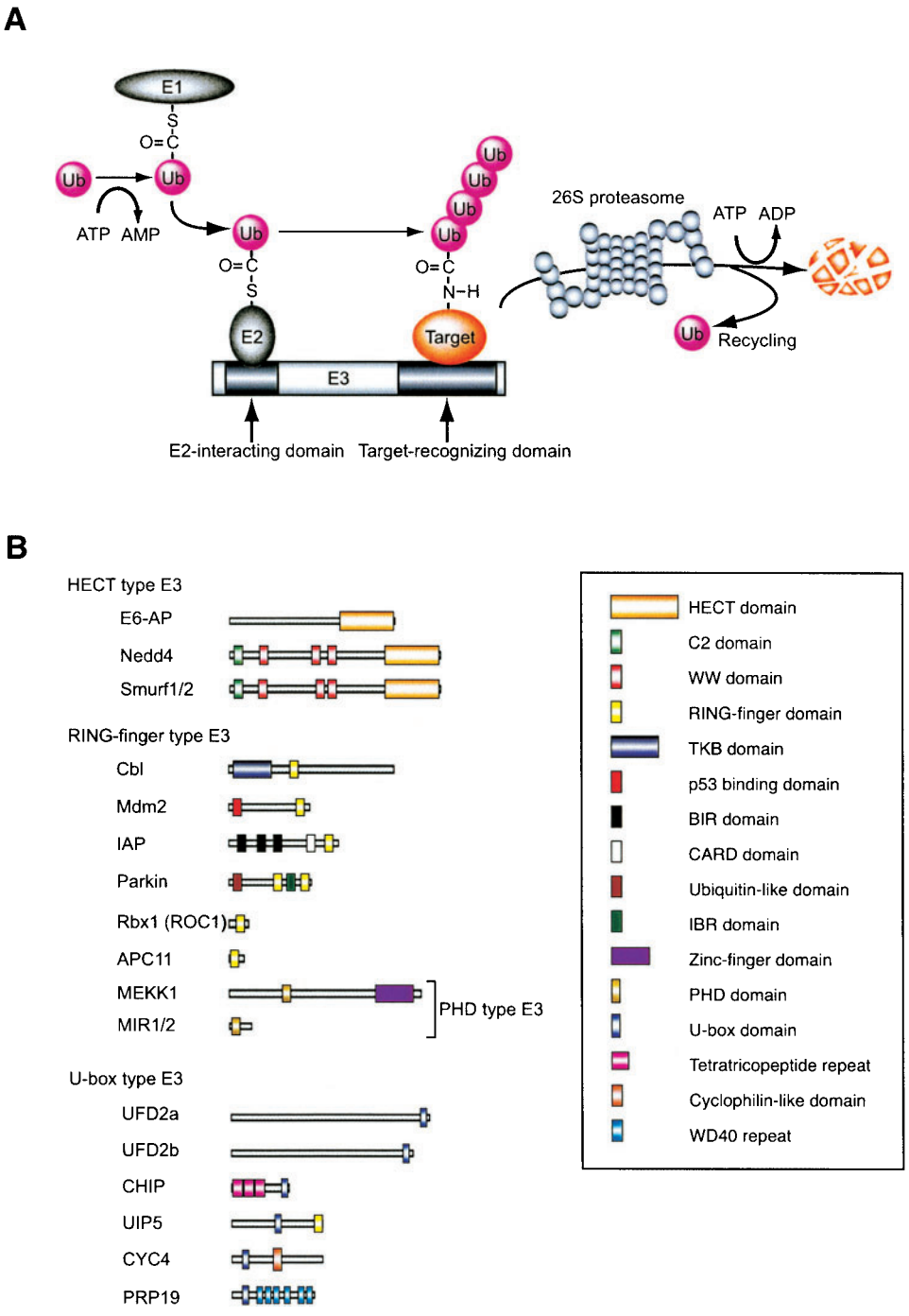

Fig. 2. **The ubiquitin-proteasome system and ubiquitin ligase families.** (A) The ubiquitylation pathway and its associated enzymes. E1 enzymes form a thiol-ester bond with ubiquitin (Ub) in an ATP-dependent manner. Ubiquitin is then transferred via an E2 and an E3 (all of which possess both an E2-interacting domain and a target-recognizing domain) to a lysine residue of the target protein, to which it is linked by an isopeptide bond. Polyubiquitylated target proteins are recognized by the S5a subunits of the 26S proteasome and degraded in an ATP-dependent manner. The ubiquitin moieties on the target are removed by deubiquitylating enzymes and recycled. (B) Three families of ubiquitin-protein ligases (E3s). HECT type and RING-finger type E3s have been identified at the molecular level. U-box proteins have been classified as E3s on the basis of their molecular structure and biochemical analyses. The WW domains of the HECT type E3s Nedd4, Smurf1, and Smurf2 appear to function in substrate recognition. Among the RING-finger type E3s, the TKB domain of Cbl is likely important for substrate recognition; the deletion of this domain results in persistent activation of intracellular signaling by the truncated protein in T cells. The ubiquitin-like domain of Parkin is also thought to play a role in substrate recognition; individuals with autosomal recessive juvenile parkinsonism have been shown to harbor mutations in the ubiquitin-like domain or RING-finger domain of this protein. Rbx1 is a component of SCF complexes, whereas APC11 is a subunit of the anaphase-promoting complex or cyclosome (APC/C). MEKK1 as well as MIR1 and MIR2 each possess a PHD domain, which is defined as a modified RING-finger domain. Among the U-box type E3s, CHIP contains three TPR domains that interact with the COOH-terminus of Hsc70 or Hsp90, both of which recognize unfolded proteins. UIP5 contains both a U-box domain and a RING-finger domain, the former of which possesses E3 activity. CYC4 has a cyclophilin-like domain that possesses peptidyl-prolyl cis-trans isomerase activity. PRP19 contains six WD40 repeats that may mediate interaction with target proteins.

Biochemistry of the ubiquitin-proteasome pathway of protein degradation

Individual ubiquitin molecules are linked to target proteins by isopeptide bonds between the COOH-terminal glycine of ubiquitin and the ε-amino group of a lysine residue in the substrate. Additional ubiquitin monomers are added to substrate-bound ubiquitin moieties in a sequential manner, resulting in the formation of a polyubiquitin chain through linkage between lysine-48 of the last ubiquitin in the chain and the COOH-terminus of the new ubiquitin molecule. Protein ubiquitylation is mediated by a multienzyme cascade that involves at least three distinct types of enzyme (Fig. 2A): a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-protein ligase (E3). E1 activates ubiquitin in an ATP-dependent manner by catalyzing the formation of a thiol ester between a reactive cysteine in E1 and the COOH-terminus of ubiquitin. Ubiquitin is then transferred from E1 first to a catalytic cysteine residue within a conserved ubiquitin-conjugating (UBC) domain of E2

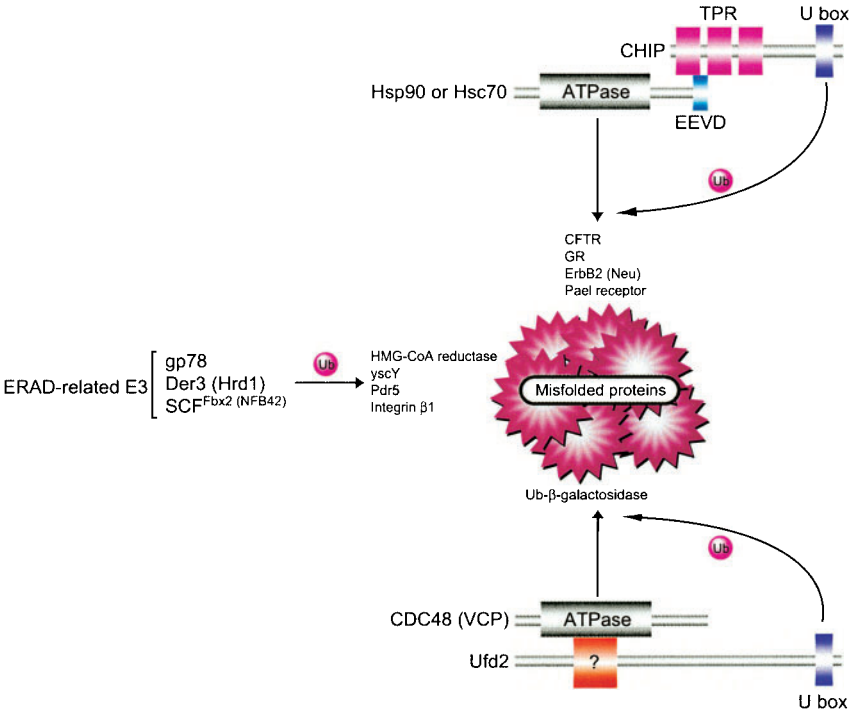

Fig. 3. **Role of E3s in the ubiquitylation of misfolded proteins.** Misfolded or unfolded proteins are recognized by molecular chaperones such as Hsc70, Hsp90, or Cdc48 (or VCP), and are then either refolded in an ATP-dependent manner or ubiquitylated by a U-box protein, such as CHIP or Ufd2 (or UFD2a), that interacts with the molecular chaperone. Some U-box proteins themselves possess chaperone activity. To date, misfolded forms of CFTR, GR, ErbB2 (Neu), and the Pael receptor have been identified as substrates for CHIP (the TPR domains of which interact with the EEVD motif of Hsp90 or Hsc70), whereas a ubiquitin–β-galactosidase fusion protein is a target of Ufd2. Der3 (Hrd1), gp78, and SCF^{Fbx2} recognize and ubiquitylate substrates that fail to fold correctly in the endoplasmic reticulum. These various E3s and molecular chaperones may thus contribute to quality control systems that prevent the impairment of cellular functions induced by the accumulation of abnormal proteins in the cytosol or endoplasmic reticulum.

and then to E3 (*23*). E3 enzymes catalyze the final step in the ubiquitylation pathway, the formation of a stable isopeptide linkage between the COOH-terminal glycine of ubiquitin and the ε-amino group of a lysine residue in the target protein. To date, E3 enzymes have been classified on the basis of their primary structure into three families: the HECT, RING-finger, and U-box families (*13*, *24*) (Fig. 2B).

The best-characterized function of polyubiquitylation is to provide a tag that marks target proteins for ATP-dependent proteolysis by the 26S proteasome (*25*, *26*) (Fig. 2A). The proteolytic core of the proteasome is a 20S multicatalytic complex that contains four alternating heptameric rings of α or β subunits in a barrel-shaped arrangement (*27*, *28*). The 26S structure is formed by the addition of one or two multisubunit 19S complexes, known as the proteasome activator 700 (PA700) or μ particle, to the ends of the 20S assembly. These 19S structures are caplike in appearance and constitute the regulatory components of the proteasome that recognize, deubiquitylate, and unfold the target protein to facilitate its entry into the hollow core of the 20S proteasome.

Ufd2 as a ubiquitin chain assembly factor (E4)

An artificial fusion protein containing an NH_2-terminal ubiquitin moiety resistant to ubiquitin-specific hydrolase activity was shown to be highly unstable and to be rapidly degraded in yeast cells (*29*). The proteolytic system responsible for the degradation of this fusion protein, designated the UFD (ubiquitin fusion degradation) pathway, has been characterized in detail by analysis of the stability of a ubiquitin–β-galactosidase fusion construct. Genetic screening in yeast led to the identification of five genes whose products (designated Ufd1 to Ufd5) contribute to the UFD pathway. One of these proteins, Ufd4 is a HECT type E3, Ufd3 contributes to regulation of the cellular concentration of ubiquitin, and both Ufd1 and Ufd5 appear to function downstream of the ubiquitylation step in the UFD pathway. Ufd5 was found to be identical to the previously identified SON1, which was isolated as the product of an extragenic suppressor of *sec63* alleles that impair protein transport into the nucleus. Ufd5 is essential for the activities of both the UFD and N-end rule pathways, the latter of which mediates the degradation of proteins with specific NH_2-terminal residues.

Ufd2 contributes to polyubiquitin chain elongation in a specific manner (*30*). This new type of ubiquitylation enzyme was designated E4 and shown to be required, together with E1 (Uba1), E2 (Ubc4), and a HECT type E3 (Ufd4), for the assembly of a polyubiquitin chain on the ubiquitin–β-galactosidase fusion protein. In yeast, Ufd2 is implicated in cell survival under stressful conditions and is associated with Cdc48, which belongs to the large family of AAA type ATPases that are thought to catalyze protein folding. Ufd2 and its homologs in other eukaryotes share a conserved domain of about 70 amino acids that has been designated the U box (Fig. 2B). The U box of Ufd2 mediates functional interaction of this protein with ubiquitin-conjugated targets. This domain therefore appears to be an essential functional unit of E4 enzymes, at least in yeast.

Database analysis has revealed that Ufd2 homologs are highly conserved from yeast to humans. *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Encephalitozoon cuniculi*, *Caenorhabditis elegans*, *Dictyostelium discoideum*, and *Arabidopsis thaliana* each possess a single Ufd2 gene, whereas *Drosophila melanogaster*, *Mus musculus*, and *Homo sapiens* each have two related Ufd2 (UFD2a and UFD2b) genes (*24*).

U-box proteins as a new E3 family

The predicted three-dimensional structure of the U box is similar to that of the RING finger, even though the former lacks the hallmark metal-chelating residues of the latter (*31*). This observation prompted us to investigate the possibility that U-box proteins in general are able to function as E3s in E2-dependent ubiquitylation. We isolated six mammalian U-box proteins (UFD2a, UFD2b, CHIP, UIP5, CYC4, and PRP19) and showed that they all mediate ubiquitylation in conjunction with E1 and E2 and in the absence of other E3 components (*32*). Deletion of the U-box domain or point mutation of conserved amino acids within this domain resulted in the loss of E3 activity. The U-box proteins thus define a third family of E3 enzymes, in addition to the HECT and RING-finger families (Fig. 2B). We propose that E4 activity is a specialized type of E3 activity that targets oligoubiquitylated artificial fusion proteins as substrates. Whether, like yeast Ufd2, any of the mammalian U-box proteins identified to date also possess E4 activity remains to be determined.

Unusual ubiquitin chain conjugation mediated by U-box type E3s

The formation of a polyubiquitin chain by linkage of the COOH-terminus of a new ubiquitin molecule to lysine-48 of the last ubiquitin moiety of the growing chain is thought to mark target proteins for proteolysis by the 26S proteasome. However, recent observations indicate that polyubiquitin chains are also assembled by conjugation to lysine residues of ubiquitin other than lysine-48, and the resulting chains appear to function in distinct biological processes. A short chain of lysine-29–linked ubiquitins is thus thought to constitute a signal for the recruitment of E4 before proteasome-mediated proteolysis (*30*), and a polyubiquitin chain composed of lysine-63–linked ubiquitin moieties is implicated in the cellular response to stress, DNA repair, ribosomal function, inheritance of mitochondrial DNA, and endocytosis of certain plasma membrane proteins (*33–37*). TRAF6, a RING-finger type E3, in conjunction with the E2 Ubc13 and the Ubc-like protein Uev1A, targets lysine-63 of ubiquitin conjugated to TAK1 and plays an important role in the phosphorylation of IκB in the NF-κB signaling pathway (*38*, *39*).

UFD2a, CHIP, and UIP5 conjugate ubiquitin not only to lysine-48 of the terminal moiety of ubiquitin chains, as do almost all HECT and RING-finger type E3s, but also to lysines at other positions (*32*, *40*). It is thus possible that polyubiquitylation of target proteins by U-box type E3s results in the formation of heterogeneous or multiply branched structures with biological functions distinct from the provision of a marker for proteolysis.

Biological aspects of UFD2 function

Loss of heterozygosity of the distal portion of chromosome 1, which is thought to harbor tumor suppressor genes, is frequently observed in many types of human cancer, including neuroblastoma. The human UFD2a gene is located in this region of chromosome 1. A 500-kb deletion at chromosome 1p36.2-p36.3 in a human neuroblastoma cell line has been shown to encompass at least six genes, including those for DFF45 (ICAD), PGD, CORT, KIF1B-β, PEX14, and UFD2a (HDNB1) (*41*). Although it remains unclear which of these genes is responsible for tumorigenicity, the role of Ufd2 together with Cdc48 in the cellular response to stress in *S. cerevisiae* suggests that the human UFD2a gene may be a tumor suppressor.

The C57/*Wld*^{s} mouse harbors a dominant mutation that delays Wallerian degeneration in the distal stump of an injured axon (*42*). Exons of three related genes have recently been identified within the 85-kb genomic region that is tandemly triplicated in the C57/*Wld*^{s} mouse. The gene for UFD2a and a previously undescribed gene (*D4cole1e*) that was subsequently found to encode a nicotinamide mononucleotide adenylyltransferase (Nmnat) were shown to span the proximal and distal boundaries of the repeat unit, respectively (*43*). A chimeric mRNA that encodes an in-frame fusion protein (Wld protein) consisting of the NH_2-terminal 70 amino acids of UFD2a separated by an aspartic acid residue from the COOH-terminal 302 amino acids of D4cole1e is abundant in the nervous system of C57/*Wld*^{s} mice. The distal axons of stumps of neurons in wild-type mice transfected *in situ* with a vector encoding the Wld protein survived for 2 weeks after axotomy, compared with a survival time of only 2 to 3 days in control animals. Expression of this chimeric protein also protected neuromuscular junctions from injury-induced degeneration. The Wld protein localizes predominantly to the nucleus of neurons, suggesting that its protective action is indirect. Nmnat activity, but not NAD^+ content, is increased fourfold in the tissues of C57/*Wld*^{s} mice. Axon protection thus likely results from altered ubiquitylation or pyridine nucleotide metabolism.

These observations with neuroblastoma cells and C57/*Wld*^{s} mice implicate UFD2a in the proliferation and survival of cells of the neuronal lineage. UFD2a has also been shown to be cleaved during apoptosis induced by various stimuli, including ultraviolet B irradiation, Fas ligation, staurosporine treatment, and exposure to the granular contents of cytotoxic T lymphocytes (*44*). Both caspase-6 and granzyme B efficiently cleave UFD2a, and the E3 activity of full-length recombinant UFD2a *in vitro* was abolished by truncation at the cleavage site targeted by these proteases. Such cleavage and inactivation of UFD2a may thus play an important role in apoptotic signaling.

U-box proteins as partners of molecular chaperones

Through its interaction with the AAA type ATPase Cdc48, which possesses chaperone activity, Ufd2 is thought to contribute to cell survival under stressful conditions in yeast (*30*). Similarly, in mammals, UFD2a binds to VCP, an ortholog of Cdc48 (*45*). The chaperone activity of VCP or Cdc48 and the ability of UFD2a or Ufd2 to bind ubiquitin chains may be implicated in endoplasmic reticulum–associated degradation (ERAD); these proteins are thought to act at the cytosolic face of the endoplasmic reticulum to promote the translocation of ERAD substrates across the membrane of this organelle and to present them to the proteasome (*46–48*).

The U-box protein CHIP binds to the molecular chaperones Hsp90 or Hsc70 via tetratricopeptide repeat (TPR) domains and is thereby thought to contribute to

the cellular response to the accumulation of unfolded or misfolded proteins (Fig. 3). The combination of CHIP and Hsp90 mediates ubiquitylation of the glucocorticoid receptor (GR), and that of CHIP with Hsc70 targets the immature CFTR protein for proteasomal degradation (*49*, *50*). The folding of both the GR and CFTR is controlled by molecular chaperones under normal conditions. CHIP has been proposed to act as a cochaperone that determines the balance between the folding of these two proteins and their degradation by the ubiquitin-proteasome pathway. We and others have shown that CHIP itself possesses ubiquitin ligase activity (*32*, *40*). Indeed, CHIP is a *bona fide* E3 that interacts functionally with members of the UbcH5 family of E2s. It was shown to mediate the polyubiquitylation of firefly luciferase, used as an artificial misfolded substrate, in the presence of E1 and E2 (Ubc4 or UbcH5) *in vitro* only after the substrate was denatured and captured by Hsp90 or by the combination of Hsc70 and Hsp40 (*51*). A CHIP mutant lacking the U-box domain was devoid of ubiquitylation activity, indicating that this domain is required for E3 function. CHIP is thus thought to act as an E3 in the quality control of protein folding by catalyzing the selective ubiquitylation of unfolded proteins associated with molecular chaperones.

We have recently shown that CHIP and Hsp70 are also associated both with Parkin, a RING-finger type E3 that when mutated contributes to autosomal recessive juvenile parkinsonism, and with the Pael receptor, a substrate targeted by Parkin for degradation (*52*). These interactions are thought to be related to the ERAD pathway. During the cellular response to the accumulation of unfolded proteins, the expression of Hsp70 is up-regulated rapidly, whereas that of CHIP is increased more slowly. Hsp70 inhibits Parkin-mediated degradation of the Pael receptor to allow its refolding, whereas interaction of CHIP with Hsp70 promotes the release of the latter from its complex with the Pael receptor; CHIP then promotes the ubiquitylation of the Pael receptor by Parkin.

Other U-box proteins

The U-box protein UIP5 (UbcM4/UbcH7–interacting protein 5), also known as KIAA0860, was isolated by yeast two-hybrid screening with UbcM4 as the bait. In addition to its U-box domain, UIP5 contains a RING-finger domain at its COOH-terminus (*53*) (Fig. 2B). The E3 activity of UIP5 depends on its U-box domain, however, not on its RING-finger domain. Mutational analysis has also indicated that the U-box domain mediates the physical interaction of UIP5 with E2 enzymes. UIP5 is localized exclusively to the nucleus, where it exhibits a punctate distribution pattern. The proteins targeted by UIP5 for ubiquitylation remain to be identified.

One of the human cyclophilins, cyclophilin-60 (hCyp-60), which is also known as CYC4 and interacts with the proteinase inhibitor eglin c, contains a U-box domain in its NH_2-terminal region and a cyclophilin-like domain that possesses peptidyl-prolyl cis-trans isomerase activity in its COOH-terminal region (Fig. 2B). This protein exhibits E3 activity with the E2 Ubc3 (Cdc34) and is localized to the nucleus (*54*). Given that cyclophilins are thought to perform a molecular chaperone function, it is possible that hCyp-60 mediates the ubiquitylation of nuclear proteins and that its peptidyl-prolyl cis-trans isomerase activity contributes to the regulation of this process.

The products of the *prp* genes of *S. cerevisiae* are required for the splicing of nuclear precursor mRNAs (*55*). The yeast *prp19* mutant is defective in spliceosome assembly. The human PRP19 protein contains a U-box domain and six WD40 repeats (Fig. 2B), which are thought to mediate protein-protein interaction. We have shown that PRP19 possesses E3 activity in the presence of either Ubc2B or Ubc3 as an E2. These observations suggest that PRP19 may play a role in the regulation of RNA splicing by mediating the ubiquitylation and degradation of spliceosomal components.

In addition to the U-box proteins identified in mammals and yeast, at least 37 genes encoding such proteins have been detected in *A. thaliana* by genetic screening or database searches (*56*). The functions of these plant proteins, however, remain to be determined.

Ubiquitin ligases and protein quality control

The prevention of protein aggregation by molecular chaperones and the degradation of misfolded or unfolded proteins by the ubiquitin-proteasome system are two important aspects of the cellular response to stressful conditions. It has been unclear, however, how unfolded or misfolded polypeptides are recognized by the ubiquitin-proteasome system. Although molecular chaperones are thought to contribute to this process, direct evidence of a link between molecular chaperones and the ubiquitylation system has been elusive. To date, four distinct E3 enzymes have been shown to contribute to the quality control of protein folding in the endoplasmic reticulum or cytosol.

First, the RING-finger type E3 Der3 (Hrd1) functions as a ubiquitin ligase in ERAD (*57*) (Fig. 3). This protein thus exhibits a preference for misfolded proteins associated with molecular chaperones in the endoplasmic reticulum. Indeed, Der3 is required for the ubiquitylation and degradation of hydroxymethylglutaryl (HMG)–CoA reductase, the carboxypeptidase yscY, and the plasma membrane protein Pdr5 after their retrograde transport from the endoplasmic reticulum into the cytosol, presumably mediated by the Sec61 channel.

Second, the RING-finger type E3 gp78, which is also known as the tumor autocrine motility factor receptor and contains a transmembrane region important in tumor metastasis, is implicated, together with Ubc7, in ERAD (*58*) (Fig. 3). Full-length gp78 was shown to mediate the degradation of the δ subunit of CD3, a well-characterized ERAD substrate, whereas expression of gp78 mutants lacking an intact RING finger resulted in an increase in the stability of CD3-δ. It is thus possible that gp78 links ubiquitylation, ERAD, and metastasis.

Third, N-glycosylation of proteins in the endoplasmic reticulum plays an important role in protein quality control, with N-glycans serving as a signal for degradation by the SCF^{Fbx2} ubiquitin ligase complex (*59*) (Fig. 3). Fbx2 (NFB42) binds specifically to proteins with N-linked high-mannose oligosaccharides and thereby mediates their ubiquitylation. The integrin β1 preprotein is a target of Fbx2. SCF^{Fbx2} is thus thought to catalyze the ubiq-

uitylation of N-glycosylated proteins translocated from the endoplasmic reticulum to the cytosol.

Fourth, as described above, the U-box type E3 CHIP plays an important role in ERAD by mediating the ubiquitylation of unfolded or misfolded CFTR, and Pael receptor (Fig. 3). Other U-box type E3s, including UFD2a, UFD2b, and CYC4, also interact with molecular chaperones or contain chaperone-like motifs (Hatakeyama, S. *et al*., in preparation), and UFD2a likely regulates the stability of proteins related to certain types of neurodegenerative disease (Matsumoto, M. *et al*., in preparation). U-box proteins thus likely play a general role in the ubiquitylation of misfolded or unfolded proteins, especially under conditions of cellular stress. They may serve as a link between the processes of protein folding by molecular chaperones and protein degradation by the ubiquitin-proteasome pathway.

Conclusions and perspectives

A quality control system for cellular proteins is essential for maintenance of the intracellular environment. The accumulation of unfolded or misfolded proteins is likely to impair cellular functions and to result eventually in cell death. Cells have thus developed molecular chaperones and the ubiquitin-proteasome pathway to deal with this problem. Certain U-box type proteins with E3 activity associate with molecular chaperones that mediate the recognition of target proteins for ubiquitylation. These U-box type E3s and their associated chaperones are thus likely important participants in protein quality control.

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