Published in final edited form as:

Semin Cell Dev Biol. 2007 December ; 18(6): 770–779.

Ubiquitin Ligases, Critical Mediators of Endoplasmic Reticulum-Associated Degradation

Zlatka Kostova, Yien Che Tsai, and Allan M. Weissman

Laboratory of Protein Dynamics and Signaling, Bldg. 560 Rm. 22-103 Center for Cancer Research, National Cancer Institute at Frederick, National Institutes of Health, Maryland 21702

Abstract

Endoplasmic reticulum-associated degradation (ERAD) represents the primary means of quality control within the secretory pathway. Critical to this process are ubiquitin protein ligases (E3s) which, together with ubiquitin conjugating enzymes (E2s), mediate the ubiquitylation of proteins targeted for degradation from the ER. In this chapter we review our knowledge of both *Saccharomyces cerevisiae* and mammalian ERAD ubiquitin ligases. We focus on recent insights into these E3s, their associated proteins and potential mechanisms of action.

Introduction

Ubiquitylation plays an essential regulatory role in all critical cellular processes in eukaryotes. ER-associated degradation (ERAD) is no exception. Ubiquitylation occurs as the result of a multi-enzyme process involving proteins referred to as ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin ligases (E3). Ubiquitylation begins with the ATP-dependent activation of the C-terminus of ubiquitin by the cellular E1. Ubiquitin is then transferred to the conserved active site cysteine of an E2 through a transesterification reaction. There are 12 ubiquitin E2s in *S. cerevisiae* and over 30 members of this family in humans. Substrate specificity in ubiquitylation is conferred by ubiquitin ligases. E3s mediate the final step in ubiquitylation. This entails the transfer of ubiquitin to a substrate or to the growing end of a substrate-bound polyubiquitin chain (For Reviews, see [1–3]).

The large majority of known E3s belong to two major classes. HECT domain E3s, like E1 and E2 enzymes, serve as catalytic intermediates in the transfer of ubiquitin to target proteins. These E3s form a transient thioester linkage between their active site cysteines and ubiquitin as a consequence of transesterification from E2. HECT E3s then catalyze the transfer of ubiquitin to substrates or to nascent polyubiquitin chains. There are six known HECT E3s in *S. cerevisiae* and approximately 27 encoded in the human genome. The large majority of the 60–100 E3s in *S. cerevisiae* and of the over 500 E3s in humans fall into the overall category of RING and RING finger-like E3s. The latter category includes PHD/LAP proteins and U-box proteins. RING finger E3s conform in a manner that recognizes E2s through the requisite coordination of two zinc ions. PHD/LAP E3s form E2 recognition sites in a similar manner. U-box proteins, on the other hand, form a similar platform for E2 binding but do so independent of metal coordination. All of the E3s with primary function in ERAD described in this chapter are members of the RING finger family.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The most well known function of ubiquitylation is targeting of proteins for proteasomal degradation, as is the case in ERAD. It is generally accepted that such targeting results from the generation of polyubiquitin chains of four or more ubiquitins linked through lysine 48 of ubiquitin (K48). These chains are generated on the ϵ -amino group of lysines within target proteins, although the N termini of proteins can also be modified [4] and the potential for other residues to be covalently modified with ubiquitin cannot be excluded [5]. Ubiquitin plays other critical roles within the cell in processes including DNA repair, kinase activation and trafficking of proteins within the secretory and endocytic pathways. In these cases either modification with a single ubiquitin or formation of chains linked through other lysines, particularly K63, seem to represent the preferred targeting signals.

In this chapter we provide an overview of the ubiquitin ligases implicated in ERAD. We begin with *S. cerevisiae*, where most of the initial characterization of ERAD components occurred. Yeast genetic screens have been a rich source of information about ERAD, which have been increasingly complemented by biochemical analyses. The two known yeast ERAD E3 complexes are discussed in detail. We then move on to mammals where less of the details have been fleshed out and the situation is far more complex, with at least three times as many E3s thus far implicated.

***S. cerevisiae* ERAD E3s**

There are two *bona fide* ERAD ubiquitin ligases that have been described in some detail. These are the RING finger E3s Hrd1/Der3 and Doa10. Hrd1/Der3 catalyzes the polyubiquitylation of substrates through the ubiquitin conjugating enzymes Ubc7, Ubc1 and, less frequently, Ubc6 [6]. Doa10, on the other hand, depends only on Ubc7 and Ubc6 [7]. Hrd1/Der3 and Doa10 each exist separately as a membrane protein complex, referred to as HRD1 and DOA10 (Figure 1). Similarity in composition is expected given their overall common function in ERAD. Differences arise, as we currently understand it, because of the need to differently recognize substrates with distinct characteristics. Hrd1/Der3 is responsible for the ubiquitylation of ER luminal and membrane proteins. Doa10, on the other hand, has a much broader range of substrates ranging from ER/nuclear soluble and membrane-bound proteins to cytoplasmic substrates. Cells lacking both Hrd1/Der3 and Doa10 exhibit a strong Unfolded Protein Response (UPR), while loss of only one E3 results in a modest induction [8]. This reflects the parallel functions of these E3s in ubiquitylation pathways with different substrate specificities. In addition to the overall structure and localization of substrates, the position of the misfolded domain in a substrate seems to be important in the choice of E3 and has led to the subdivision of ERAD into an ERAD-luminal (ERAD-L) and ERAD-cytosolic (ERAD-C) pathway. In this model, Doa10 recognizes degradation determinants on the cytosolic side of the ER membrane or in the nucleus (ERAD-C), while Hrd1/Der3 responds to lumenally exposed signals (ERAD-L) [9]. A subset of ER membrane substrates with intra-membrane lesions that depend on the Hrd1/Der3 ligase but exhibit differences in the requirement of other HRD1 ligase complex components are proposed to form yet another branch referred to as ERAD-membrane (ERAD-M) [10]. However, a mutation in one region may alter/influence the structure of another domain, crossing the topological barrier of the ER membrane. Thus, the determinant that leads to degradation may not necessarily be a confined signal. Alternatively, proteins may have lesions in multiple locations, in which case the degradation signals may operate in parallel or one may be dominant over the other, further complicating our understanding of the choice of ERAD components utilized. Gaps in knowledge notwithstanding, it is clear that the HRD1 and DOA10 complexes, which will be discussed in the following sections, lay at the heart of the multi-step and multi-component ERAD pathway that spans both sides of the ER membrane.

HRD1 complex

The HRD1 complex is composed of core components that include the ubiquitin ligase Hrd1/Der3 and its partner Hrd3, the cytoplasmic ubiquitin conjugating enzyme Ubc7 bound to Cue1, transmembrane Der1 and its recruitment factor Usa1, as well as associated proteins such as the cytosolic Cdc48 complex and its membrane anchor Ubx2, and the ER luminal Hsp70 chaperone Kar2 and the lectin Yos9 (Figure 1) [10–13].

The central component of the complex is Hrd1/Der3. Hrd1/Der3 was identified in two independent genetic screens as a factor (Hrd1: HmgCoA reductase degradation) involved in the regulation of HMG-CoA reductase [14] and as a protein (Der3: degradation in the ER) involved in the degradation of misfolded CPY* and Sec61-2 [15] and Pdr5* [16]. Hrd1/Der3 is a RING-H2 domain ubiquitin ligase of 551 amino acids (~64 kDa) with six transmembrane spans and both N and C termini in the cytosol [17,18]. Hrd1/Der3 is not glycosylated and, although it appears to localize exclusively to the ER, it contains no recognizable ER retention signal. An intact cytoplasmic RING-H2 domain located between residues 349–399 is necessary for E3 activity [6,17]. *In vivo*, mutation of a critical zinc coordinating cysteine in the RING-H2 domain to a serine (Hrd1/Der3-C399S) abolishes the degradation of misfolded luminal (CPY*) and integral membrane (Sec61-2, Hmg2) proteins [6,19]. The cognate E2 for *in vivo* Hrd1/Der3-catalyzed polyubiquitylation is Ubc7, although Ubc1 is also utilized. The other ERAD E2 is Ubc6, which is anchored to the ER membrane through its C terminal hydrophobic tail. Ubc6 can function in the degradation of a number of Hrd1/Der3-dependent substrates, but is not specifically required for Hrd1/Der3 function [6]. The cytosolic E2 Ubc7 is recruited to the ER membrane by association to Cue1, a 203-residue (23 kDa) type I membrane protein [20]. Ubc7 and Ubc1 physically associate with Hrd1/Der3 in a RING finger-dependent manner [6]. The Hrd1/Der3-C399S mutant does not interact with Ubc7 or Ubc1 [6,17] but acts as a dominant negative in a wild type background in a dosage-dependent manner [19].

The ubiquitin ligase function of Hrd1/Der3 depends, indirectly, on the presence of Hrd3, a partner protein that interacts with the N-terminal transmembrane domain of Hrd1/Der3 in a 1:1 stoichiometry [18]. Hrd3 is a 95 kDa (833 residue) single pass ER-membrane protein with a large ER-luminal domain (767 residues), five glycosylation sites, and a short cytoplasmic tail (~40 residues) [14,21]. Originally identified as participating in the regulated degradation of Hmg2 [14], it was later shown to be involved in the degradation of CPY* [21]. In the absence of Hrd3, Hrd1/Der3 is unstable and is rapidly degraded by the ubiquitin-proteasome system [18,21]. The degradation of Hrd1/Der3 in the absence of Hrd3 requires Cue1, Ubc7 and an intact Hrd1/Der3 RING finger, suggestive of autoubiquitylation [21]. The ERAD deficiency of $\Delta hrd3$ cells can be partially suppressed by over-expression of Hrd1/Der3, indicating that the E3 can still associate with other substrate-recruiting factors to drive ERAD [18,21]. The luminal domain of Hrd3 (1–767) is responsible for the stability of Hrd1/Der3 indicating that regulatory information is transmitted across the ER membrane to the Hrd1/Der3 RING finger. However, Hrd3's role in ERAD extends beyond conferring stability to Hrd1/Der3 because stabilization and ERAD competence of Hrd1/Der3 can be uncoupled by using a Hrd3 mutant in which the N-terminal half of the luminal domain has been truncated. In these cells Hrd1/Der3 is stable but unable to carry out ERAD [18]. A glimpse into Hrd3's additional roles in ERAD was obtained from immunoprecipitation studies that found that Yos9 physically and mechanistically interacts with the Hrd1/Der3 ligase *via* Hrd3 [10–12]. Yos9 is a 61 kDa ER-luminal lectin implicated in the degradation of glycoprotein substrates [22–25]. It interacts with the yeast ER Hsp70 chaperone Kar2 and the luminal domain of Hrd3 to form a complex that is anchored to Hrd1/Der3 [11]. There appear to be two pools of the Hrd1/Der3-Hrd3 ligase complex: one containing Yos9, the other not [12]. It has been proposed that, in the case of glycosylated ERAD substrates, exposed hydrophobic patches and the glycosylation status of the protein are a bipartite signal to be recognized by the HRD1 ligase and that the initial

recognition of the misfolded protein occurs via recruitment to the luminal domain of Hrd3, which would then regulate substrate presentation to Hrd1/Der3. While recognition by Hrd3 seems to be the initial step in substrate targeting, it is the participation of the Yos9-Kar2 chaperones that represents the commitment to degradation via Hrd1/Der3. Hrd3 and Yos9 operate as a gating mechanism for substrate access to Hrd1/Der3: Hrd3 functions as a substrate receptor for the HRD1 ligase complex and Yos9 acts as a gate-keeper to prevent the unrestricted destruction of proteins that would otherwise not be committed to ERAD [11,12].

Biochemical approaches have determined that Hrd1/Der3 and Hrd3 independently interact with the ER membrane protein Der1 [13]. Der1 was one of the first yeast ERAD components identified in a screen for mutants defective in the degradation of the misfolded proteins CPY* and PrA* [26]. Der1 is a 211 amino acid (24 kDa) protein that spans the ER membrane four times with cytoplasmic N and C termini [27]. Although required for the degradation of a subclass of misfolded substrates, its function in yeast ERAD is largely unknown [9,28]. In mammals, Derlin-1, one of the mammalian homologs of Der1, has been proposed to function as a component of the retrotranslocation channel [29,30].

Another recently identified component of the HRD1 ligase complex is Usa1 [10]. The interaction of Der1 with Hrd1/Hrd3 appears to be mediated by this protein. Usa1 is a double-spanning membrane protein of 97 kDa with cytosolic N and C termini that, interestingly, includes an N-terminal UBL domain. Usa1 is necessary for the degradation of HRD1 substrates having misfolded luminal domains and it becomes essential under stress conditions in cells that cannot induce an UPR [10].

The Hrd1/Der3-Hrd3-Der1 ligase complex (HRD1) also associates with the Cdc48 complex [13]. This cytosolic complex serves an essential function in ERAD in binding to polyubiquitylated proteins and functioning in their dislocation from the ER membrane [31–33]. This complex is composed of a homo-hexamer of the AAA-ATPase Cdc48 and its co-factors Npl4 and Ufd1 [34,35]. Both Hrd1/Der3 and Hrd3 participate in the efficient binding to Cdc48, whereas Der1 displays weak binding on its own. Notably, the interaction of HRD1 with the Cdc48 complex requires active Hrd1/Der3 and Ubc7, indicating that a functional ubiquitin ligase is a prerequisite for optimal recruitment to the ER membrane [13]. The interaction between the HRD1 and Cdc48 complexes is facilitated by another newly identified protein: Ubx2/Sel1. Ubx2/Sel1 is a 67 kDa ER-membrane protein required for the degradation of diverse ERAD substrates. This protein has two membrane spans and both termini are cytoplasmic [36,37]. Ubx2/Sel1 contains an N-terminal UBA domain, which is important for ERAD, and a C-terminal ubiquitin-like UBX (ubiquitin-regulatory X) domain, which acts as a Cdc48 binding module [38]. Thus, it is likely that the Cdc48 complex can be recruited to the ERAD machinery both through interactions involving ubiquitin as well as through alternative binding sites that may bear structural similarity to ubiquitin. Based on this, as well as on studies in mammalian cells, it is probable that multiple factors facilitate recruitment of the Cdc48 complex to the ER membrane, including the polyubiquitylated substrates themselves [36,37, 39].

DOA10 complex

The DOA10 complex is relatively simple, consisting of the ubiquitin ligase Doa10, Ubc6, Ubc7, Cue1, Ubx2 and the Cdc48 complex. (Figure 1) [10].

Doa10 (degradation of α 2) was identified in a screen for genes complementing the growth phenotype of a *Deg1*-fusion protein [8]. *Deg1* is a complex targeting signal that resides within the first 67 residues of the Mat α 2 transcriptional regulator and mediates its ubiquitin-proteasome dependent proteolysis [7,40]. The *in vivo* degradation of Mat α 2 is carried out by two distinct pathways involving the E2 pairs Ubc4/Ubc5 and Ubc6/Ubc7 [7]. Doa10 functions

specifically in the *Deg1*-mediated Ubc6/Ubc7 pathway of Mat α 2 degradation [8]. Doa10 is a 151 kDa (1319 amino acid) ER/nuclear envelope protein with 14 transmembrane domains. The majority of its sequence, including its N and C termini, is cytoplasmic [41]. As with Hrd1/Der3, its activity is dependent on an intact RING-CH finger [8]. Homology to mammals is concentrated to the N-terminal region spanning the RING-CH finger domain and an internal ~130 residue block, called the TD (TEB4-DOA10) domain, uniquely conserved among the Doa10-homologous proteins, including mammalian TEB4. In addition, it contains a WW domain consensus sequence at residues 775–807, which is a protein interaction motif found in the yeast HECT domain E3, Rsp5, and its mammalian orthologs [3,8]. This region, however, falls mostly in a predicted transmembrane domain of Doa10, making its significance unclear [8,41]. Doa10 levels are limiting for ER degradation *in vivo* and, similar to what was observed with Hrd1/Der3 [15,18], overexpression of the E3 leads to an increased rate of degradation of its substrates [8].

Together with Ubc6 and Ubc7, Doa10 targets a broad range of substrates including naturally short lived [8] or misfolded polytopic ER proteins [9,42–44], mutated nuclear envelope [43] or soluble nuclear proteins [8,43], and synthetic cytoplasmic degron-protein fusions [43,45]. A recent genetic screen of non-essential yeast genes identified Doa10, Ubc6, Ubc7 and Cue1 as the only nonredundant factors for *Deg1*-dependent degradation [43].

The requirement for the Cdc48 complex seems to be restricted to the degradation of the membrane protein substrates but not for soluble cytoplasmic substrates of Doa10 [43]. Similar to Hrd1/Der3, the interaction between Doa10 and the Cdc48 complex is mediated by Ubx2/Sel1 [36]. However, the physical interactions between the Cdc48 complex components (Cdc48, Ufd1, and Npl4) and those between the ubiquitin-conjugation machinery (Ubc6, Ubc7-Cue1) and Doa10 are not affected by the absence of Ubx2 [36].

Substrate specificity in ubiquitylation is attained through the interplay between the various E2s and E3s. Both E2 and E3s often localize to specific regions of the cell. Such compartmentalization is undoubtedly a key regulator of their specificity. The identification of the nuclear protein Mat α 2 as a Doa10 substrate [8] raised the question as to whether the nuclear substrate reached ER-localized Doa10 or whether Doa10 was transported to the inner nuclear membrane (INM). A combination of imaging and biochemical approaches determined that Doa10 is localized to both sides of the nuclear envelope (ER/NE). Ubc6, Ubc7, and Cue1 co-localize with Doa10 and localization to the INM is required for nuclear substrate degradation [46]. Doa10 does not carry an identifiable INM-targeting or retention signal, suggesting that it reaches the INM through lateral channels in the nuclear pore complex. The wider distribution of Doa10 along the ER/NE continuum, without specific targeting to the INM, is consistent with the requirement for Doa10 in the degradation of ER proteins. It has been speculated that Doa10's ability to diffuse into the INM depends partially on the relatively small size of its cytoplasmic loops (~24 kDa and less) and partially on the nature of its transmembrane domains [46].

ERAD E3s and Retrotranslocation

Given the diverse set of Doa10 substrates, its 14 membrane domains and accompanying cytosolic loops are most probably necessary to create an interaction platform for the binding of both substrates and soluble cofactors. In addition, the TD (TEB4-DOA10) domain of Doa10, which spans three transmembrane helices (5–7), is highly conserved among the family members in comparison to the other transmembrane segments, raising the likelihood of a specific core function for this domain [41]. Another, much discussed possibility is that the transmembrane segments may form or contribute to the formation of a protein retrotranslocation channel [8,41], a suggestion that has also been raised for Hrd1/Der3. Originally, based on genetic evidence, the ER protein import channel Sec61 also was postulated

to participate in retrograde protein transport [21]. However, biochemical approaches have yet to verify this hypothesis. Recently, a mammalian Der1 homolog, Derlin-1, has been proposed to be involved in the formation of the retrotranslocation channel, based on the finding of its association with retrotranslocating substrate molecules in mammalian cells [29,30]. Neither Sec61 [42,47], Der1 nor the Der1 homolog, Dfm1 [9,41,47], are required for the degradation of two well-studied Doa10 substrates, Ubc6 and Ste6-166, reinforcing the hypothesis that Doa10 may, indeed, participate in channel formation itself. Notably, none of the proposed retrotranslocon proteins are absolutely essential for ERAD.

So far, the only common ERAD components that have emerged from analyses undertaken to define the organization of the HRD1 and DOA10 complexes are Ubc6, Ubc7, Cue1, Ubx2 and the Cdc48 complex. Given the infinite variety of ERAD substrates, ranging from mutant/misfolded to naturally short lived/regulated and from soluble luminal to polytopic membrane proteins with transmembrane and/or cytoplasmic lesions, we should be open to the possibility that multiple retrotranslocons may exist.

Mammalian ERAD E3s

It is now clear that the number of ERAD E3s in mammals is substantially greater than that in yeast. Thus, much remains to be discovered even about basic aspects of ERAD. As expected for a conserved cellular system, yeast and mammalian ERAD pathways share common features. There are two mammalian homologs for each of the yeast ubiquitin-conjugating enzymes Ubc6 and Ubc7 (Table 1). The mammalian homologs of Ubc6, Ube2j1 and Ube2j2, have hydrophobic sequences at their C termini that mediate post-translational insertion into the ER membrane. The mammalian homologs of Ubc7, Ube2g1 and Ube2g2, on the other hand, are cytosolic proteins. Ube2g2 is strongly implicated in ERAD, functioning with multiple ERAD E3s [48–50]; there is also evidence supporting roles for Ube2j1 and Ube2j2 [51–53]. To date, there is little evidence implicating Ube2g1 in ERAD. The most studied mammalian ERAD E3s are discussed in the following sections.

gp78/RNF45/AMFR

The first mammalian ubiquitin ligase integral to the ER membrane shown to function in ERAD was gp78/RNF45/AMFR [54]. Knockdown of gp78 by siRNA abolishes ERAD of several mammalian ERAD substrates. These include the extensively utilized test substrate T-cell receptor subunits (CD3- δ and TCR- α), Apolipoprotein B-100, Z variant of α 1-antitrypsin, Insig-1 and HMG-CoA reductase [48,55–57]. Notably, gp78 is involved in the sterol-regulated degradation of both Insig-1 and HMG-CoA reductase, suggesting that it plays an important role in cholesterol metabolism [56,57]. gp78 is an integral membrane protein with multiple transmembrane spans with a topology similar to Hrd1/Der3 (Figure 2). gp78 and the human homolog of Hrd1/Der3 (Synoviolin/HsHrd1) share over 50% homology in their transmembrane regions. Computer algorithms predict 5 or 6 transmembrane spans in gp78. The single putative N-glycosylation site (N599) is in the cytosolic domain in a novel region involved in Ube2g2 recruitment (described below). Thus, it is not evident that gp78/RNF45 is a glycoprotein (gp) [48,54]. Interestingly, the C-terminus of gp78, in addition to a RING finger, contains an extended area of homology to the cytoplasmic domain of yeast Cue1, which recruits Ubc7 to the ER membrane as part of the HRD1 and DOA10 ligase complexes. Contained within this region of gp78 is a CUE domain and a binding site that specifically recruits Ube2g2, which is referred to as G2BR (Ube2g2 binding region). Indeed, overexpression of G2BR alone in cells is sufficient to inhibit ERAD due to sequestration of Ube2g2. The Ube2g2 binding site is required for gp78's ubiquitin ligase activity in cells [48]. Curiously, gp78 also requires its CUE domain for function *in vivo* [48]. CUE domains are ~40 amino acid regions that fold in a three-helix bundle and interact with ubiquitin [58,59]. An interesting dichotomy is that while the CUE domains of gp78 and several other proteins bind ubiquitin, the analogous region in

yeast Cue1 shows no significant binding. One possible role for the gp78 CUE domain that we have considered is that it functions to recruit substrates ubiquitylated by other E3s, allowing gp78 to function as an 'E4' [60] in the ubiquitylation cascade. However, in the case of the T-cell receptor subunit CD3- δ , recognition by gp78 does not depend on the CUE domain, although ubiquitylation of CD3- δ in cells requires this domain. Similarly, the capacity of gp78 to target itself for ubiquitylation and degradation also requires an intact CUE domain [48]. Another possibility is that it functions to facilitate the assembly of polyubiquitin chains either on substrate or on E2 for transfer of pre-assembled ubiquitin chains to substrate. While this is yet to be proven, a recent study suggests the potential to build such chains on Ube2g2 [61].

Depletion of gp78 results in the accumulation of CD3- δ in the ER membrane, suggesting the gp78-mediated ubiquitylation is an early event preceding retrotranslocation of substrates into the cytosol [48]. The presence of a CUE domain in gp78 has also led us to consider the possibility that gp78 may act as a functional homolog of yeast Cue1 by recruiting Ube2g2 to the ER membrane, allowing it to also function with other ERAD ubiquitin ligases such as HsHrd1/Synoviolin. To date there is no evidence for this hypothesis. On the contrary, expression of mutant forms of gp78 that lack ubiquitin ligase activity, but have an intact G2BR, result in a dominant inhibition of ERAD of CD3- δ due to sequestration of Ube2g2 [48,62]. While the CUE domain and Ube2g2 binding site are required for gp78 activity *in vivo*, the C-terminal p97/VCP (mammalian homolog of Cdc48)-binding region of gp78 appears to be largely dispensable for ERAD [48,62]. However, there are a number of other ways in which p97/VCP can be recruited to the ERAD machinery including direct recruitment by polyubiquitylated substrates or by proteins associated with gp78. In this regard, recent studies have shown that gp78 co-purifies with Derlin-1 and UbxD2 in a complex involved in ERAD of CD3- δ [63]. UbxD2 is a mammalian UBX domain containing protein that interacts with p97/VCP via its UBX domain. Whether UbxD2 facilitates p97/VCP recruitment to the ERAD machinery, as is the case for Ubx2 in yeast is currently unknown.

There is an extensive literature on gp78 that preceded its identification as an E3. The protein originally referred to as gp78 and identified as a receptor for the autocrine motility factor (AMF) (aka glucose-6-phosphate isomerase) was isolated by phage expression screening using a monoclonal antibody (3F3A) that stimulates tumor cell motility *in vitro* [64]. This 323 amino acid protein, now referred to as AMFR isoform 1, was predicted to have a single transmembrane segment [64]. The discrepancy between the predicted size of the recombinant protein (34–35 kDa) and the migration of the cellular species recognized by 3F3A (78–80 kDa) was attributed to glycosylation, hence the name gp78. Subsequently, a transcript was identified encoding a 643 amino acid protein with a predicted molecular weight of 73 kDa derived from the same gene as isoform 1. This is referred to as AMFR isoform 2 [65] and is the protein referred to herein as gp78/RNF45 and commonly referred to as AMFR. Based on computer algorithms and effects of pertussis toxin on AMF-stimulated motility, isoform 2 was predicted to be a G protein-coupled receptor. However, this topology is inconsistent with its ubiquitin ligase domain structure. Comparison of the open reading frames from isoforms 1 and 2 reveal only a single stretch of 48 predicted amino acids conserved between the two cDNAs. This region is proposed to be in the N-terminal luminal/extracellular domain of isoform 1 but corresponds to the C-terminal region that includes the cytosolic binding sites for Ube2g2 and p97/VCP in isoform 2. These discrepancies raise question as to whether 3F3A, which recognizes a cell surface epitope, actually recognizes gp78/RNF45/AMFR isoform 2.

AMFR is considered to be a metastasis factor because of a correlation between tumor grades and AMFR levels in several cancers, as assessed by immunoreactivity to 3F3A monoclonal antibody [66–69]. However, as the epitope recognized by 3F3A, which was raised against tumor cell membrane fractions, has not been defined, the relationship between the two isoforms and cell migration and metastasis is unclear. Using antibodies raised against peptides based on

the sequence of isoform 2 we have found an inverse correlation between gp78 levels and survival in canine osteosarcomas (unpublished observation). However, it remains unresolved whether gp78/RNF45 is the same protein as AMFR characterized by 3F3A monoclonal antibody.

Synoviolin/HsHrd1

Another ER resident E3 implicated in ERAD is Synoviolin, also known as HsHrd1, because of its similarity to *S. cerevisiae* Hrd1/Der3. Synoviolin has been described as an ERAD E3 for TCR- α and CD3- δ as well as for the Parkin-associated endothelin-like receptor (Pael-R), which is a misfolded G-protein coupled receptor implicated in PARK2-related Parkinson's disease [50,70,71]. Cytosolic serum- and glucocorticoid-induced kinase 1 (Sgk1), which regulates cell survival under stress conditions, is targeted to the ER via a N-terminal hydrophobic sequence leading to its rapid degradation by an ERAD pathway involving Synoviolin [52]. More recently, Synoviolin has also been reported to ubiquitylate cytosolic p53 [72]. Synoviolin functions with Ube2g2 *in vitro*, although its cognate E2 in cells has not been identified [50, 70,71]. In contrast to Hrd1/Der3, there is no evidence that Synoviolin is involved in the sterol-regulated degradation of HMG-CoA reductase in mammalian cells, although it can constitutively target HMG-CoA reductase for degradation [50,70].

Synoviolin has been implicated in the pathogenesis of rheumatoid arthritis [73]. Overexpression in mice causes arthropathy with synovial hyperplasia [73]. Homozygous deletion of Synoviolin results in embryonic lethality whereas heterozygous knockdown increases apoptosis of synovial cells and confers resistance to collagen-induced arthritis in mice [73,74]. Consistent with these observations, proliferating synovial cells in rheumatoid arthritis overexpress Synoviolin and acquire resistance to ER stress-induced apoptosis *ex vivo* [73]. Supporting its role as an anti-apoptotic protein, Synoviolin reduction by siRNA sensitizes rheumatoid synovial cells to ER stress-induced cell death and inhibits their *ex vivo* proliferation [75].

Trc8

Trc8 was originally identified as a tumor suppressor that was lost as a result of chromosomal translocation t(3;8)(p14.2;q24.1) associated with hereditary renal cell carcinoma [76]. Trc8 is a 664 amino acid protein localized to the ER and contains multiple membrane spanning domains including a sterol-sensing domain [77]. Overexpression in kidney cells suppresses growth *in vitro* and tumor formation in xenograft models in a RING-finger dependent manner [78]. This is due to a G2/M arrest and increased apoptosis. Overexpression of Trc8 represses genes involved in cholesterol and fatty acid biosynthesis regulated by sterol response element binding proteins (SREBPs). However, the mechanism by which Trc8 regulates SREBPs remains to be determined [78].

TEB4

TEB4 or MARCH VI is a mammalian homolog of *S. cerevisiae* Doa10 [79] and has a similar membrane topology to its yeast counterpart [41,79]. TEB4 catalyzes its own autoubiquitylation with K48-linked polyubiquitin chains in the presence of Ube2g2 and promotes its own degradation [79]. Based on these properties, it has been postulated that TEB4 functions as an ERAD E3 although no cellular substrates have yet been described for this ubiquitin ligase.

Non-ER Ubiquitin Ligases involved in ERAD

In addition to ER-resident ubiquitin ligases, some cytosolic E3s also function in mammalian ERAD. Parkin, which belongs to a family of ubiquitin ligases that include two RING fingers and a cysteine-rich In-Between-RING (IBR) region, has been identified as an ubiquitin ligase

for Pael-R [49]. In addition, it also recognizes non-ER substrates, possibly as a consequence of its interaction with the cytosolic chaperone Hsp70 [80,81]. Parkin is one of the most commonly mutated proteins in hereditary Parkinson's disease. Overexpression of Pael-R in mouse dopaminergic neurons induces an unfolded protein response and apoptosis [82]. Parkin also suppresses the toxicity of Pael-R in *Drosophila* models [83]. These results are consistent with its ability to promote the ubiquitylation and degradation of Pael-R *in vitro* [49,84]. The recent finding that Synoviolin also promotes the degradation of Pael-R raises the interesting possibility that Synoviolin and Parkin may function in the same pathway and help explain how a cytosolic E3 can influence the degradation of an ER substrate [71].

CHIP is a cytosolic E3 that is also implicated in the ERAD of CFTR Δ F508 [53]. CHIP is a U-box ubiquitin ligase that plays a role in protein quality control in collaboration with cytosolic chaperones. CHIP has also been suggested to function together with Parkin and in Pael-R degradation [84]. More recently, RNF5 and CHIP have been shown to function sequentially in recognizing the folding defect of the CFTR Δ F508 mutant and targeting this protein for degradation [53]. Sequential or cooperative utilization of ER-resident and soluble E3s has not been reported in *S. cerevisiae*. Whether this represents a widespread adaptation in mammalian ERAD remains to be determined. CHIP is also involved in the degradation of glycoproteins including the NMDA receptor, which is recognized by the F-box protein Fbx2 [85]. In addition, CHIP has been implicated in antigen processing of OVA for presentation by MHC Class I in a murine dendritic cell line through a process similar to ERAD [86].

In addition to Parkin and CHIP, other cytosolic E3s have been implicated in ERAD. The Skp1-Cullin-F-box (SCF) E3 complexes, for example, have the capacity to recognize and target some glycoprotein substrates for degradation [87,88].

Summary

Ten years ago the ER quality control was thought to be intrinsic to the ER lumen. It is now clear that this process crosses topological barriers and that the cytosolic ubiquitin-proteasome system is essential to this process. Despite dramatic advances over the last decade, many questions remain, particularly in mammals. Analogous to yeast, there are an increasing number of proteins that are implicated in either functionally or physically interacting with gp78 or Synoviolin, including Ube2g2, UbxD2, Derlin1-VIMP, p97/VCP, PNG1, ataxin-3 and Herp, which is the mammalian ortholog of Usa1 [48,62,63,89,90]. The extent to which these two major ligases have overlapping associated components or use distinct mechanisms to recognize proteins and to transit them out of the secretory pathway remains to be determined. It is also almost certain that the E3s described here do not constitute the whole story. Rfp2, for example, is a RING finger E3 and putative tumor suppressor localized to the ER through a C-terminal transmembrane domain that appears to have the capacity to mediate degradation of CD3- δ [91]. There are more than 50 uncharacterized RING finger proteins with predicted transmembrane segments. It is likely that some of these will also be implicated in ERAD.

Another outstanding issue relates to how substrate specificity is determined. It is reasonable to speculate that substrates are differentially recognized by ligase complexes both due to specific determinants (glycans, hydrophobic patches, etc.) and as a consequence of their localization within the ER. Sterol-regulated degradation of Insig-1 and HMG-CoA reductase involves gp78 but not Synoviolin [56,57], while the opposite is the case for Sgk1 [52]. On the other hand, at least three E3s have been implicated in degradation of TCR subunits. Whether this is telling us something about redundancy or cooperation between E3s, or whether some of what is being assessed are indirect effects of perturbation of the ER by loss of a critical element, are among the questions that now must be addressed.

Acknowledgements

This work was supported by the intramural research program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research.

References

1. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* 2002;82:373–428. [PubMed: 11917093]
2. Pickart CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 2001;70:503–33. [PubMed: 11395416]
3. Weissman AM. Themes and variations on ubiquitylation. *Nat Rev Mol Cell Biol* 2001;2:169–78. [PubMed: 11265246]
4. Ciechanover A, Ben-Saadon R. N-terminal ubiquitination: more protein substrates join in. *Trends Cell Biol* 2004;14:103–6. [PubMed: 15055197]
5. Wang X, Herr RA, Chua WJ, Lybarger L, Wiertz EJ, Hansen TH. Ubiquitination of serine, threonine, or lysine residues on the cytoplasmic tail can induce ERAD of MHC-I by viral E3 ligase mK3. *J Cell Biol* 2007;177:613–24. [PubMed: 17502423]
6. Bays NW, Gardner RG, Seelig LP, Joazeiro CA, Hampton RY. Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. *Nat Cell Biol* 2001;3:24–9. [PubMed: 11146622]
7. Chen P, Johnson P, Sommer T, Jentsch S, Hochstrasser M. Multiple ubiquitin-conjugating enzymes participate in the in vivo degradation of the yeast MAT alpha 2 repressor. *Cell* 1993;74:357–69. [PubMed: 8393731]
8. Swanson R, Locher M, Hochstrasser M. A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matalpha2 repressor degradation. *Genes Dev* 2001;15:2660–74. [PubMed: 11641273]
9. Vashist S, Ng DT. Misfolded proteins are sorted by a sequential checkpoint mechanism of ER quality control. *J Cell Biol* 2004;165:41–52. [PubMed: 15078901]
10. Carvalho P, Goder V, Rapoport TA. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. *Cell* 2006;126:361–73. [PubMed: 16873066]
11. Denic V, Quan EM, Weissman JS. A luminal surveillance complex that selects misfolded glycoproteins for ER-associated degradation. *Cell* 2006;126:349–59. [PubMed: 16873065]
12. Gauss R, Jarosch E, Sommer T, Hirsch C. A complex of Yos9p and the HRD ligase integrates endoplasmic reticulum quality control into the degradation machinery. *Nat Cell Biol* 2006;8:849–54. [PubMed: 16845381]
13. Gauss R, Sommer T, Jarosch E. The Hrd1p ligase complex forms a linchpin between ER-luminal substrate selection and Cdc48p recruitment. *Embo J* 2006;25:1827–35. [PubMed: 16619026]
14. Hampton RY, Gardner RG, Rine J. Role of 26S proteasome and HRD genes in the degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, an integral endoplasmic reticulum membrane protein. *Mol Biol Cell* 1996;7:2029–44. [PubMed: 8970163]
15. Bordallo J, Plemper RK, Finger A, Wolf DH. Der3p/Hrd1p is required for endoplasmic reticulum-associated degradation of misfolded luminal and integral membrane proteins. *Mol Biol Cell* 1998;9:209–22. [PubMed: 9437001]
16. Plemper RK, Egner R, Kuchler K, Wolf DH. Endoplasmic reticulum degradation of a mutated ATP-binding cassette transporter Pdr5 proceeds in a concerted action of Sec61 and the proteasome. *J Biol Chem* 1998;273:32848–56. [PubMed: 9830032]
17. Deak PM, Wolf DH. Membrane topology and function of Der3/Hrd1p as a ubiquitin-protein ligase (E3) involved in endoplasmic reticulum degradation. *J Biol Chem* 2001;276:10663–9. [PubMed: 11139575]
18. Gardner RG, Swarbrick GM, Bays NW, Cronin SR, Wilhovsky S, Seelig L, Kim C, Hampton RY. Endoplasmic reticulum degradation requires lumen to cytosol signaling. Transmembrane control of Hrd1p by Hrd3p. *J Cell Biol* 2000;151:69–82. [PubMed: 11018054]

19. Bordallo J, Wolf DH. A RING-H2 finger motif is essential for the function of Der3/Hrd1 in endoplasmic reticulum associated protein degradation in the yeast *Saccharomyces cerevisiae*. *FEBS Lett* 1999;448:244–8. [PubMed: 10218484]
20. Biederer T, Volkwein C, Sommer T. Role of Cue1p in ubiquitination and degradation at the ER surface. *Science* 1997;278:1806–9. [PubMed: 9388185]
21. Plemper RK, Bordallo J, Deak PM, Taxis C, Hitt R, Wolf DH. Genetic interactions of Hrd3p and Der3p/Hrd1p with Sec61p suggest a retro-translocation complex mediating protein transport for ER degradation. *J Cell Sci* 1999;112(Pt 22):4123–34. [PubMed: 10547371]
22. Bhamidipati A, Denic V, Quan EM, Weissman JS. Exploration of the topological requirements of ERAD identifies Yos9p as a lectin sensor of misfolded glycoproteins in the ER lumen. *Mol Cell* 2005;19:741–51. [PubMed: 16168370]
23. Buschhorn BA, Kostova Z, Medicherla B, Wolf DH. A genome-wide screen identifies Yos9p as essential for ER-associated degradation of glycoproteins. *FEBS Lett* 2004;577:422–6. [PubMed: 15556621]
24. Kim W, Spear ED, Ng DT. Yos9p detects and targets misfolded glycoproteins for ER-associated degradation. *Mol Cell* 2005;19:753–64. [PubMed: 16168371]
25. Szathmary R, Biemann R, Nita-Lazar M, Burda P, Jakob CA. Yos9 protein is essential for degradation of misfolded glycoproteins and may function as lectin in ERAD. *Mol Cell* 2005;19:765–75. [PubMed: 16168372]
26. Knop M, Finger A, Braun T, Hellmuth K, Wolf DH. Der1, a novel protein specifically required for endoplasmic reticulum degradation in yeast. *Embo J* 1996;15:753–63. [PubMed: 8631297]
27. Hitt R, Wolf DH. Der1p, a protein required for degradation of malformed soluble proteins of the endoplasmic reticulum: topology and Der1-like proteins. *FEMS Yeast Res* 2004;4:721–9. [PubMed: 15093775]
28. Taxis C, Hitt R, Park SH, Deak PM, Kostova Z, Wolf DH. Use of modular substrates demonstrates mechanistic diversity and reveals differences in chaperone requirement of ERAD. *J Biol Chem* 2003;278:35903–13. [PubMed: 12847107]
29. Lilley BN, Ploegh HL. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* 2004;429:834–40. [PubMed: 15215855]
30. Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. *Nature* 2004;429:841–7. [PubMed: 15215856]
31. Jarosch E, Taxis C, Volkwein C, Bordallo J, Finley D, Wolf DH, Sommer T. Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48. *Nat Cell Biol* 2002;4:134–9. [PubMed: 11813000]
32. Rabinovich E, Kerem A, Frohlich KU, Diamant N, Bar-Nun S. AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Mol Cell Biol* 2002;22:626–34. [PubMed: 11756557]
33. Ye Y, Meyer HH, Rapoport TA. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 2001;414:652–6. [PubMed: 11740563]
34. Meyer HH, Shorter JG, Seemann J, Pappin D, Warren G. A complex of mammalian ufd1 and npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. *Embo J* 2000;19:2181–92. [PubMed: 10811609]
35. Pye VE, Beuron F, Keetch CA, McKeown C, Robinson CV, Meyer HH, Zhang X, Freemont PS. Structural insights into the p97-Ufd1-Npl4 complex. *Proc Natl Acad Sci U S A* 2007;104:467–72. [PubMed: 17202270]
36. Neuber O, Jarosch E, Volkwein C, Walter J, Sommer T. Ubx2 links the Cdc48 complex to ER-associated protein degradation. *Nat Cell Biol* 2005;7:993–8. [PubMed: 16179953]
37. Schubert C, Buchberger A. Membrane-bound Ubx2 recruits Cdc48 to ubiquitin ligases and their substrates to ensure efficient ER-associated protein degradation. *Nat Cell Biol* 2005;7:999–1006. [PubMed: 16179952]
38. Schubert C, Richly H, Rumpf S, Buchberger A. Shp1 and Ubx2 are adaptors of Cdc48 involved in ubiquitin-dependent protein degradation. *EMBO Rep* 2004;5:818–24. [PubMed: 15258615]

39. Ye Y, Shibata Y, Kikkert M, van Voorden S, Wiertz E, Rapoport TA. Inaugural Article: Recruitment of the p97 ATPase and ubiquitin ligases to the site of retrotranslocation at the endoplasmic reticulum membrane. *Proc Natl Acad Sci U S A* 2005;102:14132–8. [PubMed: 16186510]
40. Johnson PR, Swanson R, Rakhilina L, Hochstrasser M. Degradation signal masking by heterodimerization of MATalpha2 and MATa1 blocks their mutual destruction by the ubiquitin-proteasome pathway. *Cell* 1998;94:217–27. [PubMed: 9695950]
41. Kreft SG, Wang L, Hochstrasser M. Membrane topology of the yeast endoplasmic reticulum-localized ubiquitin ligase Doa10 and comparison with its human ortholog TEB4 (MARCH-VI). *J Biol Chem* 2006;281:4646–53. [PubMed: 16373356]
42. Huyer G, Piluek WF, Fansler Z, Kreft SG, Hochstrasser M, Brodsky JL, Michaelis S. Distinct machinery is required in *Saccharomyces cerevisiae* for the endoplasmic reticulum-associated degradation of a multispanning membrane protein and a soluble luminal protein. *J Biol Chem* 2004;279:38369–78. [PubMed: 15252059]
43. Ravid T, Kreft SG, Hochstrasser M. Membrane and soluble substrates of the Doa10 ubiquitin ligase are degraded by distinct pathways. *Embo J* 2006;25:533–43. [PubMed: 16437165]
44. Wang Q, Chang A. Substrate recognition in ER-associated degradation mediated by Eps1, a member of the protein disulfide isomerase family. *Embo J* 2003;22:3792–802. [PubMed: 12881414]
45. Gilon T, Chomsky O, Kulka RG. Degradation signals recognized by the Ubc6p-Ubc7p ubiquitin-conjugating enzyme pair. *Mol Cell Biol* 2000;20:7214–9. [PubMed: 10982838]
46. Deng M, Hochstrasser M. Spatially regulated ubiquitin ligation by an ER/nuclear membrane ligase. *Nature* 2006;443:827–31. [PubMed: 17051211]
47. Walter J, Urban J, Volkwein C, Sommer T. Sec61p-independent degradation of the tail-anchored ER membrane protein Ubc6p. *Embo J* 2001;20:3124–31. [PubMed: 11406589]
48. Chen B, Mariano J, Tsai YC, Chan AH, Cohen M, Weissman AM. The activity of a human endoplasmic reticulum-associated degradation E3, gp78, requires its Cue domain, RING finger, and an E2-binding site. *Proc Natl Acad Sci U S A* 2006;103:341–6. [PubMed: 16407162]
49. Imai Y, Soda M, Inoue H, Hattori N, Mizuno Y, Takahashi R. An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. *Cell* 2001;105:891–902. [PubMed: 11439185]
50. Kikkert M, Doolman R, Dai M, Avner R, Hassink G, van Voorden S, Thanedar S, Roitelman J, Chau V, Wiertz E. Human HRD1 is an E3 ubiquitin ligase involved in degradation of proteins from the endoplasmic reticulum. *J Biol Chem* 2004;279:3525–34. [PubMed: 14593114]
51. Lenk U, Yu H, Walter J, Gelman MS, Hartmann E, Kopito RR, Sommer T. A role for mammalian Ubc6 homologues in ER-associated protein degradation. *J Cell Sci* 2002;115:3007–14. [PubMed: 12082160]
52. Arteaga MF, Wang L, Ravid T, Hochstrasser M, Canessa CM. An amphipathic helix targets serum and glucocorticoid-induced kinase 1 to the endoplasmic reticulum-associated ubiquitin-conjugation machinery. *Proc Natl Acad Sci U S A* 2006;103:11178–83. [PubMed: 16847254]
53. Younger JM, Chen L, Ren HY, Rosser MF, Turnbull EL, Fan CY, Patterson C, Cyr DM. Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator. *Cell* 2006;126:571–82. [PubMed: 16901789]
54. Fang S, Ferrone M, Yang C, Jensen JP, Tiwari S, Weissman AM. The tumor autocrine motility factor receptor, gp78, is a ubiquitin protein ligase implicated in degradation from the endoplasmic reticulum. *Proc Natl Acad Sci U S A* 2001;98:14422–7. [PubMed: 11724934]
55. Shen Y, Ballar P, Fang S. Ubiquitin ligase gp78 increases solubility and facilitates degradation of the Z variant of alpha-1-antitrypsin. *Biochem Biophys Res Commun* 2006;349:1285–93. [PubMed: 16979136]
56. Song BL, Sever N, DeBose-Boyd RA. Gp78, a membrane-anchored ubiquitin ligase, associates with Insig-1 and couples sterol-regulated ubiquitination to degradation of HMG CoA reductase. *Mol Cell* 2005;19:829–40. [PubMed: 16168377]
57. Lee JN, Song B, DeBose-Boyd RA, Ye J. Sterol-regulated degradation of Insig-1 mediated by the membrane-bound ubiquitin ligase gp78. *J Biol Chem* 2006;281:39308–15. [PubMed: 17043353]

58. Kang RS, Daniels CM, Francis SA, Shih SC, Salerno WJ, Hicke L, Radhakrishnan I. Solution structure of a CUE-ubiquitin complex reveals a conserved mode of ubiquitin binding. *Cell* 2003;113:621–30. [PubMed: 12787503]
59. Prag G, Misra S, Jones EA, Ghirlando R, Davies BA, Horazdovsky BF, Hurley JH. Mechanism of ubiquitin recognition by the CUE domain of Vps9p. *Cell* 2003;113:609–20. [PubMed: 12787502]
60. Koegl M, Hoppe T, Schlenker S, Ulrich HD, Mayer TU, Jentsch S. A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell* 1999;96:635–44. [PubMed: 10089879]
61. Li W, Tu D, Brunger AT, Ye Y. A ubiquitin ligase transfers preformed polyubiquitin chains from a conjugating enzyme to a substrate. *Nature* 2007;446:333–7. [PubMed: 17310145]
62. Zhong X, Shen Y, Ballar P, Apostolou A, Agami R, Fang S. AAA ATPase p97/valosin-containing protein interacts with gp78, a ubiquitin ligase for endoplasmic reticulum-associated degradation. *J Biol Chem* 2004;279:45676–84. [PubMed: 15331598]
63. Liang J, Yin C, Doong H, Fang S, Peterhoff C, Nixon RA, Monteiro MJ. Characterization of erasin (UBXD2): a new ER protein that promotes ER-associated protein degradation. *J Cell Sci* 2006;119:4011–24. [PubMed: 16968747]
64. Watanabe H, Carmi P, Hogan V, Raz T, Silletti S, Nabi IR, Raz A. Purification of human tumor cell autocrine motility factor and molecular cloning of its receptor. *J Biol Chem* 1991;266:13442–8. [PubMed: 1649192]
65. Shimizu K, Tani M, Watanabe H, Nagamachi Y, Niinaka Y, Shiroishi T, Ohwada S, Raz A, Yokota J. The autocrine motility factor receptor gene encodes a novel type of seven transmembrane protein. *FEBS Lett* 1999;456:295–300. [PubMed: 10456327]
66. Otto T, Bex A, Schmidt U, Raz A, Rubben H. Improved prognosis assessment for patients with bladder carcinoma. *Am J Pathol* 1997;150:1919–23. [PubMed: 9176385]
67. Onishi Y, Tsukada K, Yokota J, Raz A. Overexpression of autocrine motility factor receptor (AMFR) in NIH3T3 fibroblasts induces cell transformation. *Clin Exp Metastasis* 2003;20:51–8. [PubMed: 12650607]
68. Nakamori S, Watanabe H, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, Kabuto T, Raz A. Expression of autocrine motility factor receptor in colorectal cancer as a predictor for disease recurrence. *Cancer* 1994;74:1855–62. [PubMed: 8082090]
69. Jiang WG, Raz A, Douglas-Jones A, Mansel RE. Expression of autocrine motility factor (AMF) and its receptor, AMFR, in human breast cancer. *J Histochem Cytochem* 2006;54:231–41. [PubMed: 16204225]
70. Nadav E, Shmueli A, Barr H, Gonen H, Ciechanover A, Reiss Y. A novel mammalian endoplasmic reticulum ubiquitin ligase homologous to the yeast Hrd1. *Biochem Biophys Res Commun* 2003;303:91–7. [PubMed: 12646171]
71. Omura T, Kaneko M, Okuma Y, Orba Y, Nagashima K, Takahashi R, Fujitani N, Matsumura S, Hata A, Kubota K, Murahashi K, Uehara T, Nomura Y. A ubiquitin ligase HRD1 promotes the degradation of Pael receptor, a substrate of Parkin. *J Neurochem* 2006;99:1456–69. [PubMed: 17059562]
72. Yamasaki S, Yagishita N, Sasaki T, Nakazawa M, Kato Y, Yamadera T, Bae E, Toriyama S, Ikeda R, Zhang L, Fujitani K, Yoo E, Tsuchimochi K, Ohta T, Araya N, Fujita H, Aratani S, Eguchi K, Komiya S, Maruyama I, Higashi N, Sato M, Senoo H, Ochi T, Yokoyama S, Amano T, Kim J, Gay S, Fukamizu A, Nishioka K, Tanaka K, Nakajima T. Cytoplasmic destruction of p53 by the endoplasmic reticulum-resident ubiquitin ligase ‘Synoviolin’. *Embo J* 2007;26:113–22. [PubMed: 17170702]
73. Amano T, Yamasaki S, Yagishita N, Tsuchimochi K, Shin H, Kawahara K, Aratani S, Fujita H, Zhang L, Ikeda R, Fujii R, Miura N, Komiya S, Nishioka K, Maruyama I, Fukamizu A, Nakajima T. Synoviolin/Hrd1, an E3 ubiquitin ligase, as a novel pathogenic factor for arthropathy. *Genes Dev* 2003;17:2436–49. [PubMed: 12975321]
74. Yagishita N, Ohneda K, Amano T, Yamasaki S, Sugiura A, Tsuchimochi K, Shin H, Kawahara K, Ohneda O, Ohta T, Tanaka S, Yamamoto M, Maruyama I, Nishioka K, Fukamizu A, Nakajima T. Essential role of synoviolin in embryogenesis. *J Biol Chem* 2005;280:7909–16. [PubMed: 15611074]
75. Yamasaki S, Yagishita N, Tsuchimochi K, Kato Y, Sasaki T, Amano T, Beppu M, Aoki H, Nakamura H, Nishioka K, Nakajima T. Resistance to endoplasmic reticulum stress is an acquired cellular characteristic of rheumatoid synovial cells. *Int J Mol Med* 2006;18:113–7. [PubMed: 16786162]

76. Gemmill RM, West JD, Boldog F, Tanaka N, Robinson LJ, Smith DI, Li F, Drabkin HA. The hereditary renal cell carcinoma 3;8 translocation fuses FHIT to a patched-related gene, TRC8. *Proc Natl Acad Sci U S A* 1998;95:9572–7. [PubMed: 9689122]
77. Gemmill RM, Bemis LT, Lee JP, Sozen MA, Baron A, Zeng C, Erickson PF, Hooper JE, Drabkin HA. The TRC8 hereditary kidney cancer gene suppresses growth and functions with VHL in a common pathway. *Oncogene* 2002;21:3507–16. [PubMed: 12032852]
78. Brauweiler A, Lorick KL, Lee JP, Tsai YC, Chan D, Weissman AM, Drabkin HA, Gemmill RM. RING-dependent tumor suppression and G2/M arrest induced by the TRC8 hereditary kidney cancer gene. *Oncogene*. 2006
79. Hassink G, Kikkert M, van Voorden S, Lee SJ, Spaapen R, van Laar T, Coleman CS, Bartee E, Fruh K, Chau V, Wiertz E. TEB4 is a C4HC3 RING finger-containing ubiquitin ligase of the endoplasmic reticulum. *Biochem J* 2005;388:647–55. [PubMed: 15673284]
80. Cookson MR. The biochemistry of Parkinson's disease. *Annu Rev Biochem* 2005;74:29–52. [PubMed: 15952880]
81. Tsai YC, Fishman PS, Thakor NV, Oyler GA. Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. *J Biol Chem* 2003;278:22044–55. [PubMed: 12676955]
82. Kitao Y, Imai Y, Ozawa K, Kataoka A, Ikeda T, Soda M, Nakimawa K, Kiyama H, Stern DM, Hori O, Wakamatsu K, Ito S, Itohara S, Takahashi R, Ogawa S. Pael receptor induces death of dopaminergic neurons in the substantia nigra via endoplasmic reticulum stress and dopamine toxicity, which is enhanced under condition of parkin inactivation. *Hum Mol Genet* 2007;16:50–60. [PubMed: 17116640]
83. Yang QH, Church-Hajduk R, Ren J, Newton ML, Du C. Omi/HtrA2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. *Genes Dev* 2003;17:1487–96. [PubMed: 12815069]
84. Imai Y, Soda M, Hatakeyama S, Akagi T, Hashikawa T, Nakayama KI, Takahashi R. CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. *Mol Cell* 2002;10:55–67. [PubMed: 12150907]
85. Nelson RF, Glenn KA, Miller VM, Wen H, Paulson HL. A novel route for F-box protein-mediated ubiquitination links CHIP to glycoprotein quality control. *J Biol Chem* 2006;281:20242–51. [PubMed: 16682404]
86. Imai J, Hasegawa H, Maruya M, Koyasu S, Yahara I. Exogenous antigens are processed through the endoplasmic reticulum-associated degradation (ERAD) in cross-presentation by dendritic cells. *Int Immunol* 2005;17:45–53. [PubMed: 15546887]
87. Yoshida Y, Adachi E, Fukiya K, Iwai K, Tanaka K. Glycoprotein-specific ubiquitin ligases recognize N-glycans in unfolded substrates. *EMBO Rep* 2005;6:239–44. [PubMed: 15723043]
88. Yoshida Y, Tokunaga F, Chiba T, Iwai K, Tanaka K, Tai T. Fbs2 is a new member of the E3 ubiquitin ligase family that recognizes sugar chains. *J Biol Chem* 2003;278:43877–84. [PubMed: 12939278]
89. Zhong X, Pittman RN. Ataxin-3 binds VCP/p97 and regulates retrotranslocation of ERAD substrates. *Hum Mol Genet* 2006;15:2409–20. [PubMed: 16822850]
90. Schulze A, Standera S, Buerger E, Kikkert M, van Voorden S, Wiertz E, Koning F, Kloetzel PM, Seeger M. The ubiquitin-domain protein HERP forms a complex with components of the endoplasmic reticulum associated degradation pathway. *J Mol Biol* 2005;354:1021–7. [PubMed: 16289116]
91. Lerner M, Corcoran M, Cepeda D, Nielsen ML, Zubarev R, Ponten F, Uhlen M, Hober S, Grandér D, Sangfelt O. The RBCC Gene RFP2 (Leu5) Encodes a Novel Transmembrane E3 Ubiquitin Ligase Involved in ERAD. *Mol Biol Cell*. 2007

Figure 1.

The *S. cerevisiae* ERAD ubiquitin ligase complexes HRD1 (upper panel) and DOA10 (lower panel). The HRD1 ligase complex includes the luminal components Kar2 and Yos9. Kar2 is required to retain the substrate in a soluble conformation. Yos9, together with Hrd3, is involved in the initial steps of substrate recruitment. The DOA10 complex appears to be composed of fewer members and shares components of the ubiquitylation machinery and the cytosolic Cdc48 complex with the HRD1 ligase. It is anticipated that additional components of the DOA10 complex will be elucidated as this ligase is studied in greater detail.

Figure 2.

Schematic representation of the mammalian ERAD E3, gp78. gp78 is predicted to span the ER membrane 5–6 times. gp78 interacts with the ERAD E2 Ube2g2 (MmUbc7) via the G2BR (Ube2g2 binding region) which is located between residues 579–600. One model is that this interaction facilitates the interaction between the E2 and the RING domain (341–379). The ubiquitin binding CUE domain is located between 459–497 and is required for the function of gp78 in cells. The RING finger, G2BR and CUE domain are all required for the cellular function of gp78 while the C-terminal p97/VCP binding site appears to be largely dispensable.

Table 1

Nomenclature of mammalian and *S. cerevisiae* ERAD E2s. The yeast ERAD E2s Ubc6 and Ubc7 each have two mammalian homologs. This table lists the various correct names for each E2 and their gene ID numbers.

Name	Gene ID/Location	Alternative names	<i>S. cerevisiae</i> homolog
Ube2i1	51465/6q15	NCUBE1, Ubc6e	Ubc6
Ube2j2	118424/1p36.33	NCUBE2, MmUbc6	
Ube2g1	7326/17p13.2	-	Ubc7
Ube2g2	7327/21q22.3	MmUbc7	