gp78: a Multifaceted Ubiquitin Ligase that Integrates a Unique Protein Degradation Pathway from the Endoplasmic Reticulum

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Abstract: The endoplasmic reticulum (ER) is the site for maturation of proteins destined for the secretory pathway. Failure in maturation leads to production of misfolded proteins that are eliminated through the ER-associated degradation (ERAD) pathway. ERAD is a complex process that includes misfolded protein recognition, retrotranslocation to the cytosol, ubiquitination and proteasomal degradation. gp78 is an E3 ubiquitin ligase that integrates these ERAD steps by nucleating a unique degradation machine, which uses the p97/VCP-Npl4 complex for retrotranslocation instead of the well-known p97/VCP-Ufd1-Npl4 complex. A growing list of substrates have been identified for gp78, which highlights the importance of gp78-mediated ERAD in essential physiological pathways and pathological processes.

Keywords: ubiquitination, retrotranslocation/dislocation, E3 ubiquitin ligase, gp78, Hrd1, p97/VCP, proteasome, endoplasmic reticulum, and ER-associated degradation

INTRODUCTION

Proteins destined for the secretory pathway are synthesized in the endoplasmic reticulum (ER) and then transported to their final functional destinations. Proper folding of newly synthesized polypeptides is required for transport and is achieved by enzymes that modify proteins and molecular chaperones that maintain polypeptide solubility and promote folding [1, 2]. The conformations of proteins are constantly monitored by the ER quality control (ERQC) system. Proteins that fail to achieve their native conformations are retained in the ER and eliminated by ER-associated degradation (ERAD) [3]. Thus, ERAD is a protective mechanism to prevent accumulation of misfolded proteins in the ER, and thereby safeguards the secretory pathway. When accumulation of misfolded proteins overwhelms the degradation capacity of ERAD, the unfolded protein response (UPR) is activated to restore ER homeostasis by limiting further loading of proteins to the ER, enhancing protein folding and elevating ERAD activity [4]. Prolonged UPR, however, triggers apoptosis, which has been implicated in the pathophysiology of many diseases, such as neurodegenerative diseases, cancer, cardiovascular diseases, muscle wasting and diabetes [5, 6].

Misfolded ER proteins localize either fully (luminal proteins) or partially (membrane proteins) in the lumen of the ER, but their degradation occurs in the cytosol by the proteasomes. Thus, retrotranslocation or dislocation of these misfolded ER proteins to the cytosol is an absolute requirement for their elimination [7, 8]. Moreover, retrotranslocation is intimately associated with recognition of misfolded proteins on the luminal side of the ER and ubiquitination and proteasomal targeting on the cytosolic side [3, 9]. It is well established that ER membrane-associated ubiquitin ligase complexes coordinate substrate recognition, retrotranslocation, ubiquitination and degradation during ERAD [10, 11]. gp78 is one of the ubiquitin ligases playing such a role [12]. Moreover, gp78 is unique among all known ERAD ubiquitin ligases in that it has multiple conserved domains that interact directly with components of both ubiquitination and retrotranslocation complexes. In this review, we will summarize the structure and function of gp78, and its importance in both physiological and pathological processes.

GP78-MEDIATED UBIQUITINATION

Ubiquitination is a process during which proteins are modified with a single ubiquitin or a chain of ubiquitin monomers. It occurs through a cascading action of E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and then E3 ubiquitin ligase [13] (Fig. **1A**). The complexity of ubiquitination is reflected by having two E1s, dozens of E2s and over a thousand E3s in mammalian cells. Moreover, ubiquitination can generate nine topologically different polymeric ubiquitin chains linked through one of the seven Lys residues (K6, K11, K27, K29, K33, K48, and K63), the amino terminus or a mixture of Lys residues of ubiquitin [14, 15]. Each E3 can interact with and ubiquitinate one or several substrate proteins, and by working with different E2s, each E3 can assemble different polyubiquitin chains on its substrates. In other words, E2 determines the linkage of polyubiquitination while E3 dictates substrate specificity [15, 16]. A number of proteins containing ubiquitin-binding domains (UBDs), such as the ubiquitin-interacting motif (UIM) and ubiquitin-associated (UBA) domains, recognize conjugated ubiquitin or polyubiquitin chains along with the downstream effector proteins of signaling pathways or degradation machinery. Through these interactions UBD-containing proteins transmit ubiquitin-dependent signals to the desired bio-

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logical function or proteasomal degradation [17, 18]. The nine topologically distinct polymeric ubiquitin chains achieve a remarkably diverse range functions in ubiquitin signaling, such as protein degradation, apoptosis, signal transduction, gene transcription, DNA repair, cell cycle progression, immune responses, virus budding, protein trafficking, and receptor and channel endocytosis [15-17]. Many of these functions control the life and death of cells. Accordingly, aberrant ubiquitination has been widely associated with development of malignancies, diabetes, cardiovascular diseases, inflammatory disorders and many neurodegenerative diseases [19, 20]. gp78 exemplifies the importance of E3 ubiquitin ligases in physiology and pathology.

gp78 is a polytypic RING (really interesting new gene) finger protein and is localized in the ER [12]. It contains five predicted transmembrane domains followed by a RING finger, an oligomerization site (OS), a coupling of ubiquitin to ER degradation (Cue) domain, a Ube2g2-binding region (G2BR) and a p97/VCP-interacting motif (VIM) [12, 21-23] (Fig. **2**). The RING finger defines a family of E3 ubiquitin ligases [24], which led to the identification of gp78 as an E3 ubiquitin ligase acting in the ERAD pathway [25]. Although the function of RING finger is to bind to ubiquitin-charged E2 to facilitate transfer of ubiquitin to a substrate, gp78 also binds Ube2g2 through G2BR in addition to its RING finger [12, 21]. It has been shown that its E3 activity requires the coordinated action of the RING finger, Cue domain, OS and G2BR. Mechanistically, binding to G2BR leads to conformational changes in Ube2g2 that affect ubiquitin loading and significantly enhance the affinity of Ube2g2 to the RING finger [26]. This unique dual binding mode of Ube2g2 to gp78 optimizes the efficiency of gp78-mediated ubiquitination of misfolded ER proteins. The mechanism by which gp78 cooperates with Ube2g2 to assemble polyubiquitin chains has been elegantly demonstrated [27] (Fig. **1B**). gp78/Ube2g2-mediated polyubiquitination involves preassembly of K48 polyubiquitin chains at the catalytic cysteine of Ube2g2. The extension of Ube2g2-anchored polyubiquitin chains is achieved by an aminolysis-based transfer reaction between two Ube2ge molecules that each carries a ubiquitin moiety on its active cysteine. gp78 oligomerization mediated by its OS leads to simultaneous binding of multiple Ube2g2 molecules in close proximity, which allows ubiquitin moieties to be transferred between neighboring Ube2g2s to form active site-linked polyubiquitin chains. These polyubiquitin chains are then transferred en bloc to substrate proteins [22].

gp78 appears to represent an example of convergent evolution with functions of both yeast ERAD E3 Hrd1p and its cofactor Cue1p found within a single molecule. Hrd1p is a polytypic RING finger E3 and Cue1p is a type III transmembrane protein [28-30]. Cue1p contains a Cue domain and a Ubc7p-binding region (U7BR) that is functionally analogous to G2BR [31]. Ubc7p is the yeast homolog of Ube2g2 and also interacts with the RING finger of Hrd1p [32]. Association of U7BR with Ubc7p activates the RING finger-dependent E3 activity of Hrd1p, and allows polyubiquitination of substrates [31]. Like Ube2g2, Ubc7p also assembles polyubiquitin chains at its active site and ubiquitinates substrates by en bloc transferring [33].

In addition to functioning as an E3, gp78 was reported to function as a polyubiquitin chain assembly factor (E4) to catalyze polyubiquitination of CFTRΔF508 [34]. In this case, another RING finger protein RMA1 acts as an E3 to ubiquitinate CFTRΔF508. gp78 then recognizes the ubiquitin that is already conjugated to CFTRΔF508 via its Cue domain and catalyzes polyubiquitination of CFTRΔF508. Whether this E4 function of gp78 is specific for CFTRΔF508 or general to all its substrates remains to be explored.

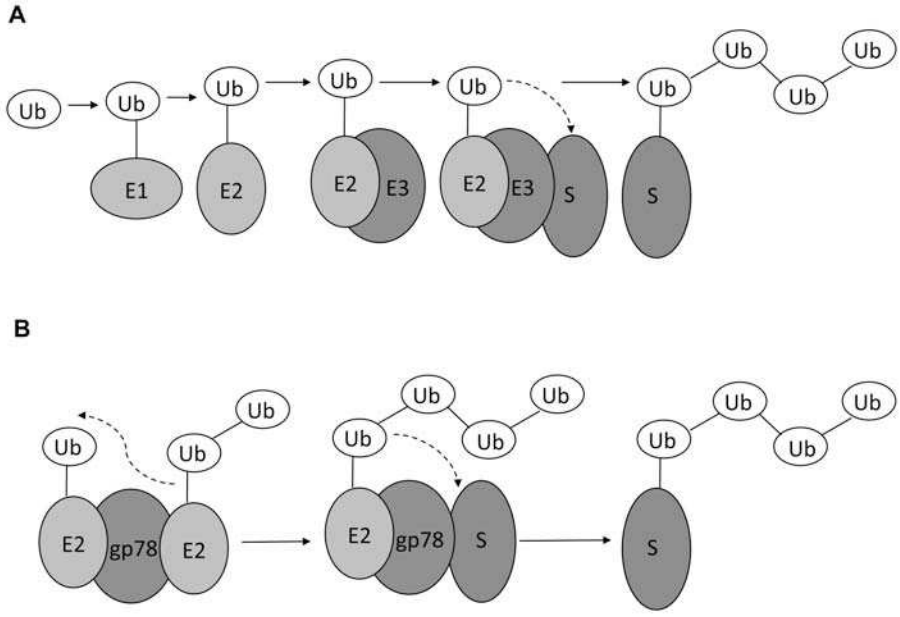

Fig. (1). (A) Schematic representation of RING finger E3 ubiquitin ligase-catalyzed ubiquitination. S: substrate protein. **(B)** gp78/Ube2g2-mediated substrate (S) ubiquitination. From the left: two E2s (Ube2g2s) preassemble a K48 ubiquitin chain on their active cysteines by aminolysis; the preassembled ubiquitin chain on the catalytic cysteine of the Ube2g2 is transferred to a substrate.

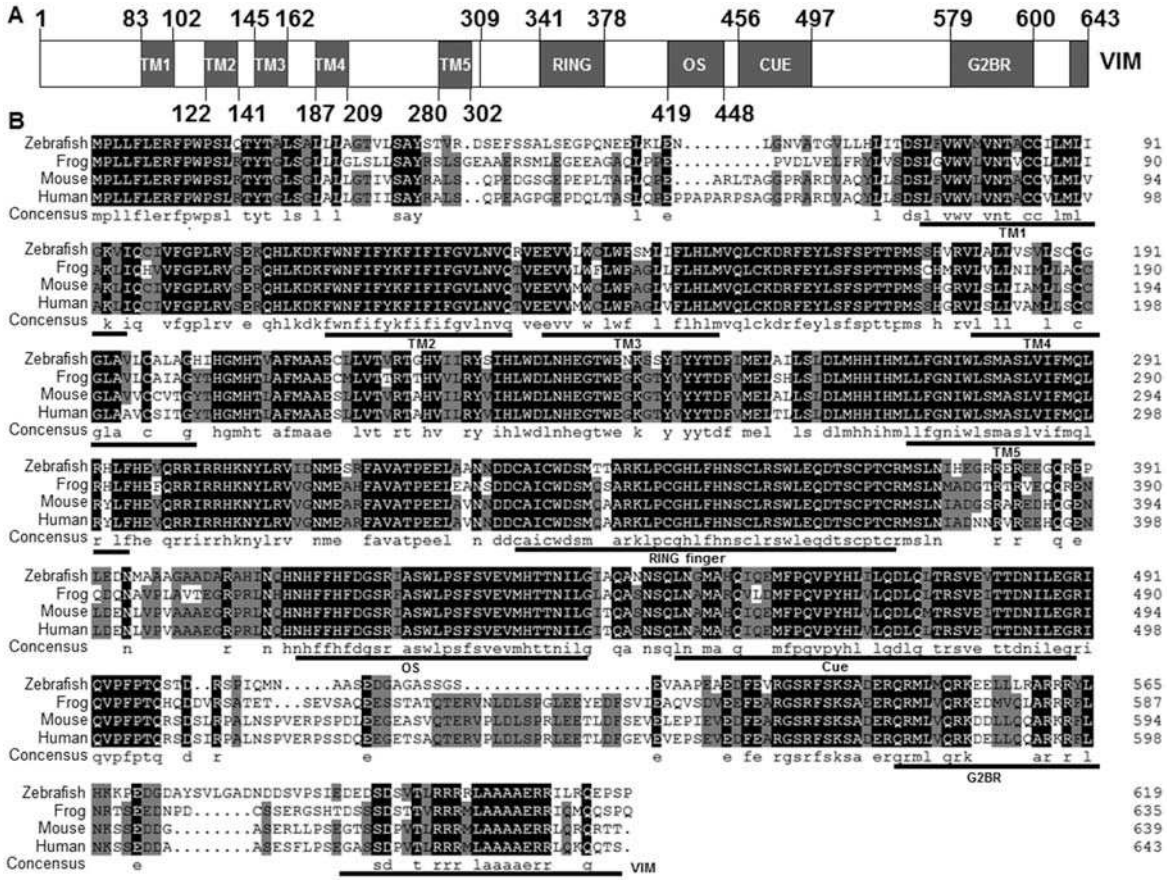

Fig. (2). (A) Diagram of the domain structure of human gp78. **(B)** Sequence alignment of gp78 from different species. Multiple sequence alignment was performed using DNAMAN. Conserved cytosolic domains are underlined. The TMs are predicted using TMHMM-2.0.

GP78 DIRECTLY LINKS UBIQUITINATION TO RETROTRANSLOCATION

The general scheme of ERAD has been well established. ERAD substrates are first recognized and delivered to the membrane-anchored E3 ubiquitin ligase complexes by ER luminal chaperones and lectins followed by retrotranslocation, ubiquitination and proteasomal degradation [3]. In budding yeast, two complexes, one composed of the Hrd1p ubiquitin ligase degrades substrates with lesions exposed to the ER lumen or transmembrane, namely ERAD-L and ERAD-M, whereas the other composed of Doa10p ubiquitin ligase disposes of substrates with lesions on the cytosolic side of the ER, namely ERAD-C [35, 36]. These ERAD complexes are essentially conserved in mammalian cells, but the three ERAD pathways described in yeast are not well defined in mammalian cells, which is especially true for gp78-mediated ERAD.

gp78 has been reported to degrade all types of substrates, for example, the luminal substrate, the Z variant of α-1-antitrypsin (ATZ), the membrane substrate HMG-CoA reductase, and the cytosolic substrate mutant SOD1 (see Table **1** for a list of substrates for gp78). How luminal substrates, such as ATZ, are targeted to gp78 is not known. As ATZ is a glycosylated substrate, mannose-trimming factors, such as ER Mannosidase I (ER ManI) or one or more ER degradation-enhancing-mannosidase-like proteins (EDEMs) [37, 38], must be involved in ATZ degradation mediated by gp78. BiP/grp78 and the ER lectin OS-9, whose function is to target substrates to the Hrd1 complexes, are associated with gp78 (Zhong, Y and Fang, S, unpublished data), suggesting that they may target substrates to the gp78 complex as well. In contrast, XTP3-B, another ER lectin for substrate targeting, does not associate with gp78, and thus is unlikely to function with gp78 [39]. gp78 appears to recognize membrane substrates via different adaptor proteins, such as Derlin1, insig-1 and SPFH1/SPFH2 that recruit CFTRΔF508, HMG-CoA reductase and possibly inositol 1,4,5-trisphosphate (IP(3)) receptors, respectively [40-45]. We do not know whether the transmembrane domains of gp78 directly recognize substrates. As expected, gp78 recognizes its cytosolic substrates, such as mutant huntingtin (htt) and SOD1 using its cytosolic tail [46, 47]. Therefore, the function of gp78 is not confined to any particular ERAD pathway as defined in yeast.

After delivery to the gp78 complex (Table **2**), luminal and probably some membrane substrates require retrotranslocation in order to be ubiquitinated. This is determined by the topology of gp78. As in all known ERAD E3s, the E3-active domain-the RING finger of gp78 is localized on the cytosolic surface of the ER [48]. Retrotranslocation enables access of luminal substrates to the E3 activity for ubiquitination. Recent studies suggest that cytosolic exposure of luminal substrates is promoted by a cooperative action of im-

Table 1. List of Identified Substrates for gp78

Substrate	E3 ubiquitin ligase(s)	Comments (substrate)	References
ApoB-100	gp78	A key protein component of LDL	[61, 81-83]
HMG-CoA reductase	gp78	A rate-limiting enzyme in cholesterol biosynthesis	[43, 84-86]
Insig1	gp78	Regulator of cholesterol synthesis	[43, 87]
CYP3A and CYPE21	gp78 and CHIP	Liver cytochrome P450 enzymes	[90, 91]
KAI1	gp78	Tumor metastasis suppressor	[98, 99]
CFTRΔF508	gp78, CHIP, Fbs1 and RMA1	The most common mutation in cystic fibrosis transmembrane conductance regulator (CFTR) causing cystic fibrosis	[34, 40, 102]
Mutant huntingtin	gp78 and Hrd1	The Huntington's disease protein	[46, 103]
Mutant neuroserpin	gp78 and Hrd1	A mutant serine protease inhibitor causing familial encephalopathy with neuroserpin inclusion bodies	[104]
Mutant SOD1	gp78	A mutant antioxidant enzyme causing familial amyotrophic lateral sclerosis	[47]
Ataxin-3	gp78	A mutant deubiquitinating enzyme causing Machado–Joseph disease/spinocerebellar ataxia type 3	[47]
ATZ	gp78	Z variant of α-1-antitrypsin (ATZ) causing deficiency in circulating α-1-antitrypsin	[72]
Cholera toxin (CT)	gp78 and Hrd1?	The virulence factor produced by *Vibrio cholera* requires retrotranslocation to exert its cytotoxicity	[111]

Table 2. Proteins in the gp78 Complex

Protein	Yeast Homolog	Validated	Direct vs. Indirect interaction with gp78	Function	References
gp78	Hrd1p	Yes	Direct, via OS	gp78 oligomerization required for gp78 E3 activity	[22]
Ube2g2	Ubc7p	Yes	Direct, with G2BR	Cognate E2 for gp78	[12]
Derlin1	Der1p	Yes	Unknown	Substrate recruitment	[51, 54]
p97/VCP	Cdc48p	Yes	Direct, with VIM	Retrotranslocation of substrates	[58]
Ufd1	Ufd1p	Yes	Direct	Cofactor for gp78 E3 activity towards HMG-CoA reductase	[86]
Npl4	Npl4p	Yes	Unknown	Forms a complex with p97/VCP in gp78-mediated ERAD	[62, 63, 77]
PNGase mHR23B	Png1p Rad23p	Yes	Indirect, via p97/VCP	ERAD substrate-processing factors	[70, 71]
Erasin	Ubx2p	Yes	Indirect, via p97/VCP and ubiquilin	Involved in recruiting p97/VCP and ubiquilin to ERAD complex	[56]
Ubiquilin	Dsk2p	Yes	Indirect, via p97/VCP	Binds the proteasome and delivers the misfolded protein to proteasome	[56]
Bag6	Unavailable	Yes	Unknown	Associates with gp78, maintains polypeptide solubility and may escort substrates to the proteasome	[73]
UbxD8	Unavailable	Yes	Unknown	Unknown	[57]
Herp	Usa1p	Yes	Unknown	Unknown	[55]
SPFH2	Unavailable	Yes	Unknown	Unknown	[44]
TMUB1	Unavailable	Yes	Unknown	Bridges SPFH2 to gp78 in ER membrane	[44]
VIMP	Unavailable	Yes	Unknown	Recruits p97/VCP to ER membrane	[52]

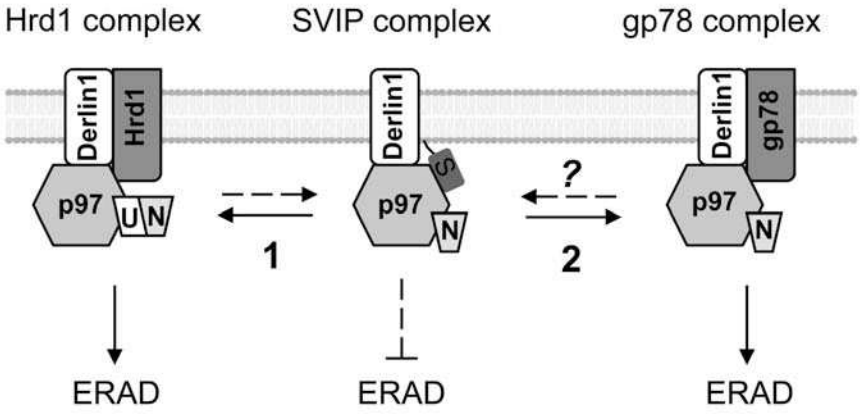

Fig. (3). A simplified view of gp78 and Hrd1-mediated ERAD pathways and their regulation by SVIP. Right: gp78 recruits p97/VCP-Npl4(N) to the cytosolic surface of the ER for coupling ubiquitination with retrotranslocation to enhance ERAD; middle: SVIP(S) is anchored to the membrane via myristoylation and sequesters Derlin1, p97/VCP and probably Npl4(N) away from gp78 leading to inhibition of ERAD; left: p97/VCP-Ufd1(U)-Npl4(N) complex is recruited to the Hrd1 complex to couple ubiquitination with retrotranslocation. SVIP may inhibit Hrd1-mediated ERAD by sequestering p97/VCP, Npl4 and Derlin1 away from Hrd1.

portin β and RanGDP [48], although the underlying mechanism is not known. Following ubiquitination, the cytosolic AAA ATPase (ATPase associated with various cellular activities) p97/VCP/Cdc48 and its cofactors Ufd1 and Npl4 are recruited to the cytosolic surface of the ER to extract polyubiquitinated substrates into the cytosol through hydrolysis of ATP [8, 49] (Fig. **3**). The role of these cofactors is to enhance the binding of p97/VCP with the polyubiquitinated substrates [50]. The polyubiquitin chain conjugated to the substrates provides a handle for the p97/VCP complex to pull the substrate from the ER. How p97/VCP along with Ufd1 and Npl4 are recruited to the ER remains unclear. Evidence suggests that the recruitment may be a concerted effort of several proteins in the ERAD complex. For example, p97/VCP/Cdc48 interacts with Hrd1, another well-established polytypic ERAD E3, and also several other proteins that interact with Hrd1, including Derlin1-3, VIMP, Erasin, UbxD8 and Herp [51-57]. Although we do not know whether these multiple interactions lead to recruitment of the p97/VCP-Ufd1-Npl4 complex, functional studies in both yeast and mammalian cells have shown that the p97/VCP/Cdc48-Ufd1-Npl4 complex is required for degradation of Hrd1 substrates [8, 49].

The gp78 complex contains similar membrane components to those of the Hrd1 complex. Therefore, proteins in the gp78 complex also make multiple contacts with p97/VCP. Evidence suggests that the gp78-p97/VCP interaction is most critical for coupling ubiquitination with retrotranslocation [58]. gp78 contains a p97/VCP-interacting motif (VIM) near its C-terminus [23]. The VIM has a high affinity towards p97/VCP and is sufficient to recruit p97/VCP to the ER surface [23, 59]. Deletion of the VIM from gp78 stabilizes CD3δ, a well-established gp78 substrate. Moreover, the stabilized CD3δ is highly ubiquitinated, suggesting that loss of VIM in gp78 fails to recruit p97/VCP, which in turn fails to extract ubiquitinated CD3δ [58]. VIM of gp78 interacts with the ND1 domain of p97/VCP that is also the binding site for Ufd1 [23]. In addition, Ufd1 bridges the interaction of the Ufd1-Npl4 dimer with p97/VCP [60]. Consistently, gp78 and the Ufd1-Npl4 dimer form mutually exclusive complexes with p97/VCP [23, 61]. Therefore, it is unlikely that gp78 can recruit p97/VCP along with the Ufd1-Npl4 dimer. Functional studies indeed show that gp78-mediated ERAD is independent of Ufd1, but surprisingly, requires Npl4 [23, 62]. Nevertheless, the interaction between gp78 and p97/VCP enhances p97/VCP-polyubiquitin binding [58], suggesting that Npl4 and the Cue domain of gp78 may play an analogous role to that of the Ufd1-Npl4 dimer. This Ufd1-independent retrotranslocation has recently been demonstrated for the human cytomegalovirus protein US2-mediated degradation of MHC class I heavy chain from the ER [63]. Reminiscent of ERAD, yeast Cdc48 is recruited to stressed mitochondria, retrotranslocates ubiquitinated proteins from the outer mitochondria membrane and delivers ubiquitinated proteins to the proteasome for degradation [64]. Interestingly, Cdc48 is recruited to mitochondria by the VIM of Vms1. Moreover, Vms1 recruits Cdc48-Npl4 complex to retrotranslocate proteins independent of Ufd1. Moreover, Vms1 does not directly interact with Npl4. The Vms1-Npl4 interaction is bridged by Cdc48 [64]. The mammalian homolog of Vms1**,** ANKZF1, although not evaluated, is likely to play the same role [61, 64]. Since gp78-mediated ERAD requires p97/VCP and Npl4 independent of Ufd1 and gp78 contains a VIM, we predict that gp78 recruits p97/VCP-Npl4 to the ER during ERAD (Fig. **3**).

Retrotranslocation is thought to occur through a proteinaceous channel. Although the identity of this channel remains elusive, it must be associated with the E3 ubiquitin ligase complexes and formed by transmembrane proteins. Previous studies suggest that the sec61 translocon may also serve as a channel for retrotranslocation during ERAD [65]. Other transmembrane proteins, such as Derlins, gp78 and Hrd1 have been suggested to be part of the retrotranslocation channel [66, 67]. The yeast homolog of Hrd1, Hrd1p, has indeed been shown to be the retrotranslocation channel in yeast [68]. It is not known, however, whether Hrd1 plays the

same role. Although Derlin1 was considered as the best candidate channel protein, recent studies indicate that Derlin1 is a rhomboid pseudoprotease that is unlikely to function as a retrotranslocation channel [69]. gp78 contains five transmembrane domains and can form large oligomers. It is tempting to speculate that its oligomerization may form the retrotranslocation channel.

It is known that at least some glycosylated substrates are degraded through the gp78-mediated pathway. Removal of glycans from substrates is an essential step required for degradation by the proteasomes. Indeed, gp78 is associated, via p97/VCP, with the peptide N-glycanase (PNGase), a cytosolic enzyme that deglycosylates misfolded glycoproteins, and mHR23B, a ubiquitin chaperone that delivers polyubiquitinated substrates to the proteasomes [70, 71]. Another important issue is how cells maintain the solubility of retrotranslocated substrates before they reach the proteasomes. Previous studies suggest that gp78 appears to play such a role [72]. It is now known that gp78 associates with a multiprotein complex comprising Bag6, Ubl4A and Trc35, which chaperones retrotranslocated polypeptides en route to the proteasome. Bag6 contains a chaperone-like activity capable of maintaining an aggregation-prone substrate in an unfolded yet soluble state [73].

REGULATION OF GP78-MEDIATED ERAD

The function of gp78 in ERAD is subject to a multilayered regulation. The levels of gp78 expression is subject to regulation. ERAD prevents protein accumulation through elimination of misfolded proteins from the ER. When misfolded proteins fail to be removed efficiently by ERAD, accumulation of them will result in ER stress, which activates UPR. UPR upregulates transcription of ERAD components including E3 ubiquitin ligases [74]. We have demonstrated that tunicamycin-induced UPR increases the expression of gp78 mRNA (Chen, Z, Du, S and Fang, S, unpublished data). In addition, acute ER stress enhances ERAD by stabilizing the gp78 protein [75]. This is achieved by suppressing gp78 autoubiquitination. Autoubiquitination of gp78 targets itself for degradation by the proteasomes. Inhibition of E3 autoubiquitination may be a general mechanism by which cells rapidly respond to acute accumulation of misfolded proteins in the ER. Hrd1 exhibits the same response to that of gp78. This posttranslational response to boost ERAD activity is not limited to E3 ubiquitin ligases. For example, it has been reported that UPR boosts glycoprotein ERAD by suppressing the proteolytic downregulation of ER ManI. Stabilization of ER ManI protein enhances mannose processing, thereby facilitating ERAD [76]. Thus, UPR enhances gp78-mediated ERAD at both the transcriptional and post-translational levels.

The crosstalk between E3 ubiquitin ligases has been shown to be involved in regulation of the ubiquitination activity of gp78 during ERAD [77, 78]. gp78 is a substrate for the Hrd1 ubiquitin ligase [77, 78]. Autoubiquitination of gp78 requires its functional RING finger while the ubiquitination of gp78 by Hrd1 is solely dependent on the RING finger activity of Hrd1 but not on that of gp78 [78]. The regulation of gp78 by Hrd1 is underscored by the observation that gp78 is stabilized in Hrd1 knockdown cells and embryonic fibroblasts of Hrd1 homozygous knockout mice ($Syvn^{-/-}$) [78]. An interesting question is what determines when and to what extent gp78 is regulated by autoubiquitination or Hrd1.

The function of gp78 is also regulated at the step of retrotranslocation. This regulation is mediated by the small VCP/p97-interacting protein (SVIP) [79]. SVIP does not have a transmembrane domain and is localized to the ER membrane through myristoylation. SVIP contains a well-conserved VIM that competes with gp78 for binding to p97/VCP, leading to interruption of the gp78-p97/VCP interaction. Moreover, SVIP in fact sequesters p97/VCP and Derlin1 away from gp78. Derlin1 is a substrate-recruiting protein for gp78. As expected, SVIP also inhibits the ubiquitination of the gp78 substrate, CD3δ. Thus, dependent on the relative levels of gp78 and SVIP, p97/VCP and Derlin1 can either form an ERAD-inhibitory complex with SVIP or an ERAD-active complex with gp78. Therefore, SVIP regulates gp78-mediated ERAD by regulating the assembly of the gp78-Derlin1-p97/VCP complex. This regulation might not be limited to gp78-mediated ERAD, since p97/VCP is probably a converging point for all ERAD pathways (Fig. **3**).

In addition to its role in ERAD, SVIP is also a regulator of the autophagy pathway. As an ERAD inhibitor, SVIP facilitates autophagy by promoting LC3 lipidation, enhancing p62 expression, sequestration of polyubiquitinated proteins to autophagosomes and increasing starvation-induced degradation of LC3II and p62 proteins [80]. The opposite roles of SVIP in ERAD and autophagy may be important mechanisms by which cells handle ER stress. It was shown that ER stress causes an early downregulation of the SVIP protein, and prolonged ER stress markedly increases SVIP protein levels. We speculate that when SVIP is downregulated, gp78 is upregulated, which leads to increases in ERAD activity. Prolonged ER stress causes a significant accumulation and aggregation of misfolded proteins in the ER, and ERAD is not expected to be effective under these conditions. Thus, prolonged ER stress upregulates SVIP to enhance autophagic removal of aggregated proteins from the ER. Therefore, SVIP may be a switch from ERAD to autophagy during the course of ER stress.

REGULATION OF PHYSIOLOGICAL FUNCTIONS BY GP78-MEDIATED ERAD

Increasing evidence indicates that gp78-mediated ERAD plays an important role in the regulation of physiological processes. Apolipoprotein B-100 (ApoB-100), an essential protein for the assembly and secretion of very low-density lipoproteins (VLDL) from the liver, is the first physiological substrate identified for gp78 [81, 82] (Table **1**). ApoB-100 is degraded by ERAD when lipid availability limits the assembly of VLDL [82]. This is part of the quality control mechanism that eliminates orphan subunits of protein complexes. gp78 and p97/VCP have been implicated in the proteasomal degradation of ApoB-100 [81-83]. Overexpression of gp78 is sufficient to increase ubiquitination and proteasomal degradation of ApoB-100, with reduced secretion of ApoB-100 in HepG2 cells [81]. By contrast, knockdown of gp78 expression decreased ApoB-100 ubiquitination and retrotranslocation. Concomitantly, VLDL assembly is enhanced, and tria-

cylglycerol secretion is increased. gp78-mediated ubiquitination commits ApoB-100 to p97/VCP-mediated retrotranslocation [82]. Therefore, gp78 plays an important regulatory role in VLDL assembly through ubiquitination of ApoB-100.

gp78 also has an established role in the regulated degradation of HMG-CoA reductase (HMGCR), a key enzyme that catalyzes the conversion of HMG-CoA to mevalonate in the rate-limiting step of cholesterol biosynthesis [84]. The conversion is under strict feedback regulation mediated by sterol and nonsterol metabolites of mevalonate [84]. One mechanism for the feedback regulation involves rapid degradation of HMGCR through ERAD, according to studies in cultured cells [85]. Accumulation of sterols in the ER membrane triggers binding of the ER membrane proteins Insig-1 and Insig-2 to the sterol-sensing domain of HMGCR. Insig-1 in turn interacts with the N-terminal transmembrane domains of gp78, thereby targeting HMGCR to the gp78 complex. gp78 then catalyzes polyubiquitination of HMGCR through its interaction with the E2 Ube2g2 [43, 84]. Ufd1 acts as a cofactor for gp78 to promote HMGCR ubiquitination [86]. The ubiquitinated HMGCR is extracted from the ER by p97/VCP and then delivered to the proteasomes for degradation [43]. Taken together, gp78 binds to HMGCR in an Insig1-dependent and sterol regulated manner. When cells are depleted of sterols, gp78 targets Insig-1 for degradation leading to increases in sterol synthesis by HMGCR [87]. It is worth noting that Hrd1p is the E3 ubiquitin ligase involved in ERAD of HMGCR in yeast [88], while Hrd1, the mammalian homolog of Hrd1p, is not involved in the regulation of HMGCR degradation in mammalian cells [43, 89].

The liver cytochrome P450 (CYP) enzymes have recently joined the list of gp78 substrates. Specifically, CYP3A4 and CYPE21 have been shown to be substrates for gp78 [90, 91]. CYP3A4 is responsible for the metabolism of the majority of xenobiotics including anticancer agents. The levels of CYP3A4 expression have been proposed as a factor responsible for the variability in clinical response to chemotherapy. gp78 regulates the levels of CYP3A4 by ubiquitinating CYP3A4, leading to its degradation by the proteasomes. This finding has important clinical implications, because most anticancer agents have very narrow therapeutic windows, thus even slight changes in CYP3A4 levels could alter the exposure of the drug and result in either insufficient efficacy or toxicity [92]. Liver CYP2E1 is responsible for the biotransformation of clinically relevant drugs, low molecular weight xenobiotics, carcinogens and endogenous ketones. gp78 is able to ubiquitinate and target CYP2E1 for proteasomal degradation [91]. Phosphorylation of CYP2E1 and CYP3A4 may serve to engage the gp78/Ube2g2 complex to enhance their ubiquitination. The hepatic function of gp78 *in vivo* is further highlighted by its high level of expression in mouse liver compared with other organs (Ballar, P and Fang, S, unpublished data). Thus, gp78 may play important roles in the regulation of drug metabolism in liver.

DISEASES ASSOCIATED WITH GP78-MEDIATED ERAD

The significance of gp78-mediated ERAD is underscored by its association with not only physiological proteins but also proteins that are linked to human diseases, such as KAI1, ATZ, and CFTRΔF508, and mutant huntingtin (htt), neuroserpin, ataxin-3 and SOD1.

gp78 was originally identified as a 78-kDa glycoprotein that promotes tumor metastasis [93]. Subsequently, it was shown to be the tumor autocrine motility factor (AMF) receptor (AMFR) [94, 95]. Consistently, gp78 has been shown to be highly expressed in various cancers, such as bladder cancer, colorectal cancer, esophageal cancer, gastric cancer and hepatocelllular carcinoma, and its elevated expression is correlated with metastasis [96]. This is in accordance with the observation that patients with increased expression of gp78 have significantly worse disease-free survival rates [97]. The correlation of elevated expression in tumors and increased metastasis has been solely attributed to the function of gp78 as AMFR. However, gp78 also promotes metastasis through the ERAD pathway [98]. gp78 associates with and targets the transmembrane metastasis suppressor, KAI1 (also known as CD82), for degradation. Reduction of gp78 expression increases the abundance of KAI1 and reduces the metastatic potential of tumor cells, an effect that is largely abrogated by concomitant suppression of KAI1. This inverse relationship between these proteins was revealed in a human sarcoma tissue microarray [98]. When overexpressed in mammary glands, gp78 promotes cell proliferation and non-tumorigenic ductal outgrowth mediated by the metastasis suppressor KAI1 [99]. Therefore, gp78 may promote tumor cell proliferation, invasion and metastasis by more than one mechanism.

Another pathogenic role of gp78 is in cystic fibrosis (CF). CF is a common autosomal recessive disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), an epithelial anion channel [100]. Deletion of phenylalanine 508 (CFTRΔF508) is the most common CF-associated mutation, which accounts for about 70% of CF alleles [101]. CFTRΔF508 is retained in the ER and rapidly degraded through the ERAD pathway, which prevents its trafficking to the plasma membrane [101]. RMA1, an ER-anchored RING finger E3, is involved in ERAD of CFTRΔF508 [40]. gp78 may act as an E4 to extend the polyubiquitin chain that has been conjugated to CFTRΔF508 by RMA1 [34]. gp78 also enhances the interaction of CFTRΔF508 with p97/VCP, presumably to increase CFTRΔF508 retrotranslocation. Harnessing gp78-mediated ERAD via knockdown of p97/VCP or overexpression of gp78 dominant negative mutant rescues CFTRΔF508 from ERAD and increases its trafficking to the cell surface and partially restores its channel function [102]. By contrast, Hrd1 inhibits CFTRΔF508 degradation by acting as an E3 for gp78 [77]. Knockdown of Hrd1 results in stabilization of gp78, and consequently increases in CFTRΔF508 degradation [77]. Both p97/VCP and Derlin1 are critical components of the CFTRΔF508 degradation machinery. SVIP is known to sequester p97/VCP and Derlin1 away from gp78 to form an ERAD-inactive complex [79] (Fig. **3**). It was shown that overexpression of SVIP leads to accumulation of CFTRΔF508 [77], supporting the idea that gp78 targets CFTRΔF508 for degradation.

gp78 is widely involved in degradation of neurodegenerative disease proteins. This function is unlikely to be specific for gp78, since Hrd1 also acts in the same spectrum of

neurodegenerative disease proteins [46, 47, 103, 104]. Therefore, gp78 and Hrd1 probably recognize these mutant proteins by a quality control mechanism, although the mechanism of substrate recognition by these two E3s may be different. Polyglutamine expansion in htt protein induces Huntington's disease (HD), although the mechanism remains uncertain. Some insights into the mechanism come from the discovery that mutant htt interacts with gp78 [46]. The HEAT repeats 2&3 of htt interact with the Cue domain of gp78. The interaction competitively reduces polyubiquitinated protein binding to gp78 and also sterically blocks gp78 interaction with p97/VCP. These effects of htt negatively regulate the function of gp78 in ERAD and are aggravated by polyglutamine expansion. Paradoxically, gp78 is still able to ubiquitinate and facilitate degradation of htt proteins with expanded polyglutamines. When mutant htt accumulates and aggregates, it also impairs the function of p97/VCP-Ufd1-Npl4 in ERAD by sequestering them to its aggregates [105]. Therefore, it is not surprising that the impairment of ERAD by mutant htt proteins is associated with induction of ER stress [46, 105]. We speculate that mutant htt accumulates and gradually aggregates in neurons during HD progression, probably because the rate of mutant htt degradation is slower than the rate of its production/accumulation. The inefficiency in degradation of mutant htt proteins would preoccupy E3 proteins like gp78 and Hrd1 that might typically operate in ERAD in a futile effort toward degrading mutant htt proteins. This nonproductive interaction would lead to an accumulation of misfolded proteins in the ER leading to ER stress.

Hrd1 and gp78 are also involved in ubiquitination and degradation of mutant neuroserpin [104], a secreted glycoprotein and a serine protease inhibitor of serpin family predominantly expressed in the neurons of the central nervous system (CNS) [106]. The role of neuroserpin is largely unknown, but it has been suggested that neuroserpin plays a neuroprotective role and may be involved in regulation of the morphology of neuroendocrine cells and neurite outgrowth [107]. Point mutations in the neuroserpin gene result in its misfolding, accumulation and formation of neuroserpin inclusion bodies in the ER, which causes familial encephalopathy with neuroserpin inclusion bodies [108]. Recent studies demonstrate that overexpression of Hrd1 and gp78 reduces the mutant neuroserpin levels, whereas knockdown of either E3 stabilizes it [104]. Impairment of p97/VCP function also stabilizes neuroserpin and increases its aggregation. These results suggest that mutant neuroserpin is a bona fide ERAD substrate for both gp78 and Hrd1 [104]. Therefore, gp78 and Hrd1 may play a protective role against mutant neuroserpin-induced neuronal degeneration. Similarly, gp78 has been shown to promote degradation of mutant SOD1 and ataxin-3, two neurodegenerative disease proteins, associated with familial amyotrophic lateral sclerosis and Machado–Joseph disease/spinocerebellar ataxia type 3, respectively [47]. The common pathological feature of these neurodegenerative disease proteins is their accumulation and aggregation in neurons during disease progression. gp78 and Hrd1 act as quality control E3s for these mutant proteins, which is another common feature. These commonalities may explain why ER stress has been increasingly recognized as a common pathogenic factor in various neurodegenerative diseases [109]. It is likely that gp78 and Hrd1 protect neurons at the early stage of the disease when disease proteins are not in aggregates. As the disease progresses, production of mutant proteins exceeds the degradation capacity of gp78 and Hrd1, which leads to accumulation and aggregation of the mutant proteins. The disease protein aggregates interact with gp78 and Hrd1 as well as p97/VCP and impair their functions in ERAD leading to ER stress. The findings that gp78 and Hrd1 mediate degradation of cytosolic misfolded proteins, such as mutant htt, SOD1 and ataxin-3, extend the territory of the role of gp78 and Hrd1 in quality control to cytosolic proteins.

Other substrates of gp78 include ATZ and cholera toxin (CT). Mutations of α-1-antitrypsin (AAT) lead to AAT protein retention in the ER and deficiency of circulating AAT. Accumulation of mutant AAT in the ER causes severe liver injuries, such as neonatal hepatitis, juvenile cirrhosis and hepatocellular carcinoma [110]. gp78 was shown to ubiquitinate and facilitate degradation of ATZ, the classic deficiency variant of circulating AAT having a Z mutation (Glu 342 Lys) [72]. Cholera toxin (CT) is the virulence factor produced by *Vibrio cholera*. It is transported from the cell surface to the ER lumen where the catalytic CTA1 subunit is retrotranslocated to the cytosol to induce pathological water secretion. Although CTA1 is not degraded after retrotranslocation, gp78 and Hrd1 were shown to cooperate with Derlin1 and the ER luminal chaperone protein disulfide isomerase (PDI) to facilitate CTA1 retrotranslocation, suggesting that ubiquitination may be involved in CTA1 retrotranslocation [111].

CONCLUDING REMARKS

We have summarized the structure and function of gp78 in ERAD. Through ERAD gp78 regulates several important physiological processes and is involved in pathogenesis of many human diseases. However, many questions remain to be answered. For example, gp78-mediated ERAD is independent of Ufd1 and dependent on Npl4. This is in contrast to the demonstrated requirement for p97/VCP-Ufd1-Npl4 in other ERAD pathways. We do not know how gp78, p97/VCP and Npl4 act together in ERAD. gp78 contains a complex domain structure that mediates its own oligomerization or interacts with other proteins to promote ERAD. The list of proteins that have been isolated as gp78-interacting proteins is growing, but some of the interactions remain to be validated. Moreover, gp78 is involved in disposing of all types of substrate proteins, including luminal, membrane and cytosolic proteins, but it is not known whether different subcomplexes of gp78 are formed to handle different types of substrates. We still do not have any information on luminal substrates targeting to the gp78 complex and do not known the identity of the retrotranslocation channel, if any. How the domains unique to gp78, including G2BR, Cue and VIM coordinate ubiquitination and retrotranslocation of substrates requires further investigation. Most of the conserved domains in the cytosolic tail of gp78 have been or have started to be elucidated, but the transmembrane domains are well conserved and should have important functions, but this remains unexplored. It would be interesting to know if the transmembrane domains of gp78 participate in the formation of a retrotranslocation channel and/or are involved in interac-

tions with other membrane components of the gp78 complex. gp78-mediated ERAD targets both physiological and pathological proteins for degradation, reflecting its significance in regulation of cellular processes and removal of unwanted proteins during ERAD. However, most of these studies were performed in cellular systems. It would be important to validate the roles of gp78 in animals and animal models of human diseases.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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