Regulation of ER-associated degradation via p97/VCP-interacting motif

Petek Ballar*1 and Shengyun Fang†

*Ege University, School of Pharmacy, Biochemistry Department, Izmir 35100, Turkey, and †University of Maryland Biotechnology Institute, Medical Biotechnology Center, Baltimore, MD 21201, U.S.A.

Abstract

p97/VCP (valosin-containing protein) is a cytosolic AAA (ATPase associated with various cellular activities) essential for retrotranslocation of misfolded proteins during ERAD [endoplasmic reticulum-associated degradation]. gp78, an ERAD ubiquitin ligase, is one of the p97/VCP recruitment proteins localized to the ER membrane. A newly identified VIM (p97/VCP-interacting motif) in gp78 has brought about novel insights into mechanisms of ERAD, such as the presence of a p97/VCP-dependent but Ufd1-independent retrotranslocation during gp78-mediated ERAD. Additionally, SVIP (small p97/VCP-interacting protein), which contains a VIM in its N-terminal region, negatively regulates ERAD by uncoupling p97/VCP and Derlin1 from gp78. Thus SVIP may protect cells from damage by extravagant ERAD.

Introduction

The ER (endoplasmic reticulum) is the organelle for synthesis and maturation of proteins that are destined for the secretory and endocytic pathways. One of the major functions of the ER is to assist folding of the newly synthesized polypeptides to reach their functional native conformation and, if required, assemble into oligomeric complexes [1]. Proper folding is achieved by highly regulated processes driven by chaperones, oxidoreductases, and protein modification enzymes. However, the folding process is not faultless and many proteins fail to reach their native folded state. Such misfolded proteins are retained in the ER and eliminated by the ERAD (ER-associated degradation), ensuring misfolded proteins are not translocated to their functional sites. ERAD is not only essential for maintaining the homeostasis of the cell through degradation of misfolded proteins, but also important for the regulation of physiological processes. Thus it is not surprising that aberrant ERAD is involved in the pathogenesis of many diseases, such as cystic fibrosis, neurodegenerative diseases and diabetes [2–4]. ERAD is a complex process involving misfolded protein recognition, ubiquitination, dislocation to the cytosol, deglycosylation and proteasomal degradation. The ER membrane posts a physical barrier between misfolded protein within ER lumen and the proteasome, which is the degradation machinery in the cytosol. Therefore dislocation, also known as retrotranslocation, of misfolded proteins across the ER membrane to the cytosol is essential for ERAD [5].

Key words: endoplasmic reticulum (ER), endoplasmic-reticulum-associated degradation (ERAD), gp78, p97/VCP-interacting motif (VIM), small p97/VCP-interacting protein (SVIP).

Abbreviations used: ATZ, Z variant of α1-antitrypsin; CD44, cell division cycle 48; CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; ERAD, ER-associated degradation; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA, a key enzyme in cholesterol metabolism; INSIG1, the endurance-inducing gene; INSIG2, the endurance-inducing gene (mouse); INSIG3, the endurance-inducing gene (human); INSIGH, the endurance-inducing gene (human); INSIGH, really interesting new gene; INSIGH, T-cell receptor; gp78, valosin-containing protein; INSIGH, valosin-containing protein; INSIGH, really interesting new gene; INSIGH, valosin-containing protein; INSIGH, small p97/VCP-interacting protein; VIM, p97/VCP-binding motif; VIM, p97/VCP-interacting motif.

†To whom correspondence should be addressed (email petek.ballar@ege.edu.tr).

gp78

It is well established that ubiquitination is a prerequisite for dislocation of misfolded proteins [6]. gp78 is the first identified ER-membrane-spanning ubiquitin ligase [E3 (ubiquitin-protein isopeptide ligase)] of mammalian cells [7]. gp78 has been shown to ubiquitinate a variety of substrates, such as CD38 and TCRα, the unassembled members of the multisubunit cell-surface TCR (T-cell receptor); ATZ (Z variant of α1-antitrypsin), a secretory protein that is responsible for α1-antitrypsin deficiency; and mutant CFTR (cystic fibrosis transmembrane conductance regulator; CFTRΔF508), a sodium–chloride channel protein of plasma membrane that causes cystic fibrosis [7–10]. gp78 also regulates the abundance of some key proteins that are important for physiological and pathological processes. These include: (i) apolipoprotein B100, the essential protein component of atherogenic very-low-density and low-density lipoproteins; (ii) HMG-CoA reductase (3-hydroxy-3-methylglutaryl-CoA reductase), the key enzyme in cholesterol metabolism; (iii) the tumour metastasis suppressor protein KAI1; and (iv) the Ca2+ channel protein IP3R [Ins(1,4,5)P3 receptor] [11–14].

Studies in yeast suggest that there are specialized ERAD pathways consisting of different E3 protein complexes [15,16]. Such protein complexes facilitate degradation of proteins with different folding lesions. Depending on the position of the misfolded region, the recognition could occur on the luminal side (ERAD-L), cytosolic side (ERAD-C) or intramembrane side (ERAD-M) [15,16]. Interestingly, gp78 appears to target all types of substrates for degradation, including; substrates with luminal (ATZ), transmembrane (HMG-CoA reductase and CD38), and cytosolic (CFTRΔF508) misfolded regions [7,9,10,12]. Therefore gp78 may form a master complex that disposes of all types of substrates or might be organized into different complexes that are specialized for the elimination of certain types of substrates.
gp78 is a unique E3 that has several substrates with important contributions to normal and disease physiology. Therefore further studies identifying the details of gp78-mediated ERAD are essential. A recently identified VIM (p97/VCP (valosin-containing protein)-interacting motif) has brought about such novel insights into the mechanism of gp78-mediated ERAD and its regulation [17,18].

**The role of VIM in gp78**

It is evident now that gp78 functions not only as an ERAD E3, but also as a scaffold protein to assemble a complex that couples ubiquitination, retrotranslocation and deglycosylation [19–21]. gp78 fulfills these functions mostly by its multifunctional cytosolic tail that conveys E3 activity [RING (really interesting new gene) domain], polyubiquitin-binding (Cue domain) and E2 (ubiquitin-conjugating enzyme) interaction [G2BR (Ube2g2-binding region) domain] [7,8,19]. In addition, the C-terminal cytosolic tail of gp78 interacts directly with p97/VCP [19]. This interaction was also shown using a proteomic approach, which identified p97/VCP-binding proteins [22]. With the co-operative function of all these domains, gp78 is capable of coupling ubiquitination with retrotranslocation as well as subsequent events during ERAD.

Our group has demonstrated that that the C-terminal 17 amino acid residues of gp78 are sufficient to bind to p97/VCP (Figures 1A and 1B) [17]. This sequence, namely VIM, is highly conserved among vertebrates and predicted to form an α-helix (Figure 1A) [17]. In addition to gp78, another p97/VCP-interacting protein, namely SVIP (small p97/VCP-interacting protein), also contains a VIM in its N-terminal region (Figures 1A and 1B) [17]. Subcellular fractionation and immunofluorescence assays have revealed that gp78 via its VIM recruits p97/VCP from the cytosol to the ER membrane, and this recruitment is through direct interaction and is independent of E3 activity of gp78 [17]. This is different from the yeast Hrd1/Der3p interaction with Cdc48 (cell division cycle 48), the homologue of p97/VCP. Hrd1/Der3p requires its E3 activity and Hrd3p to interact with Cdc48 [23]. Furthermore, the interaction between Hrd1/Der3p and Cdc48 has been suggested to be bridged by ubiquitinated substrates rather than by direct interaction [23].

Despite the importance of gp78–p97/VCP interaction in ERAD, gp78 is known to form a complex with Derlin1, Derlin2, VIMP (VCP-interacting membrane protein) and Hrd1, all of which interact with p97/VCP [24,25]. The mechanisms by which these multiple interactions are involved in dislocation remain to be determined. Because most of these proteins do not contain an identifiable p97/VCP-interacting domain, the mechanisms of their interactions with p97/VCP could be different. Boeddrich et al. [26] identified another novel VIM: VBM (p97/VCP-binding motif) in Ataxin-3, which is a deubiquitinating enzyme. Following their work, Yeung et al. [27] identified a VBM in Hrd1 by using bioinformatics approaches. The VBM of Hrd1 appears to weakly interact with p97/VCP and the fusion of the VIM of gp78 to the C-terminus of Hrd1 significantly enhances Hrd1 interaction with p97/VCP [17]. Therefore it is conceivable that VIM is a transferable functional unit.

It has been shown that the VIM of gp78 binds to ND1 domain of p97/VCP, which is the mutually exclusive binding site for the several p97/VCP-interacting proteins, including SVIP, Ufd1–Npl4 and p47 [17,28]. Of these p97/VCP-interacting proteins, Ufd1–Npl4 dimer is known to enhance the interaction of Cdc48/p97/VCP with the ubiquitinated ERAD substrates [29]. The Cdc48/p97/VCP–Ufd1–Npl4 complex has been proposed to form a ring-like structure around the substrates, thereby facilitating dislocation [30,31]. Consequently, retrotranslocation of the ubiquitinated ERAD substrates from the ER to the cytosol requires the Cdc48/p97/VCP–Ufd1–Npl4 complex [32–34] (Figure 2, right). Because the VIM of gp78 interacts with ND1 domain of p97/VCP, which is also the binding site for Ufd1 [17], it raises the possibility that gp78-mediated ERAD is independent of Ufd1 and Npl4. One such pathway has been identified based on several lines of evidence (Figure 2, left). First, the VIM of gp78 mediates the formation of the gp78–p97/VCP complex from which Ufd1 is physically excluded. Secondly, when gp78 is overexpressed in the CD3δ-expressing cells, Ufd1 knockdown does not cause accumulation of the substrate. This suggests that when gp78 is overexpressed, Ufd1 is dispensable for CD3δ degradation. Thirdly, simultaneous knockdown of gp78 and Ufd1 results in significant additive effect on CD3δ stabilization, which is possible only if gp78 and Ufd1 act in parallel independent pathways. Finally, Ufd1 does not affect the gp78-mediated degradation of ATZ [17].

The role of Ufd1–Npl4 dimer is to enhance p97/VCP binding to polyubiquitin [5,35]. At this point, the mechanism by which p97/VCP binds to polyubiquitin without the presence of Ufd1–Npl4 dimer is not clear. However, it is plausible that the Cue domain of gp78 could substitute for Ufd1–Npl4 dimer [7,18]; thus gp78 itself fulfills the pronounced cofactor function of Ufd1–Npl4 dimer for p97/VCP.

Later, Cao et al. [36] showed that Ufd1 directly interacts with gp78 and p97/VCP does not mediate gp78–Ufd1

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**Figure 1** | Sequence alignments and position of the VIM of gp78 and SVIP

(A) Sequence alignment of the VIM motif, with conserved binding residues shaded in grey. Multiple sequence alignment was prepared using ClustalW. (B) Position of VIM in human gp78 and human SVIP.
Figure 2 | Schematic representation of the three complexes working on mammalian ERAD

Derlin1 and p97/VCP are the common members of all gp78 recruits p97/VCP via its VIM and physically excludes Ufd1–Npl4 (left). The p97/VCP–Ufd1–Npl4 complex indeed exists in mammalian ERAD. This Ufd1-dependent pathway is possibly centred through another ER-resident E3, such as Hrd1 (right). Sharing the VIM, SVIP uncouples Derlin1 and p97/VCP from gp78 (middle). While the shift of the complex to gp78 facilitates ERAD, its shift towards SVIP inhibits ERAD (1). Considering the fact that p97/VCP and Derlin1 are centred in these ERAD pathways, SVIP may play a general role in controlling ERAD (2). Abbreviations: D, Derlin1; G, gp78; S, SVIP; H, Hrd1; U, Ufd1; N, Npl4; p97, p97/VCP.

interaction. This is due to the fact that a gp78 mutant without VIM can still bind to Ufd1. Moreover, the Ufd1-binding region of gp78 is mapped between residues 383 and 497, which is different from the p97/VCP-binding site, VIM [36]. One question that remains to be answered is the function of Ufd1 during gp78-mediated ERAD when it is not in complex with p97/VCP. It has been shown that the mono-ubiquitin-binding ability of Ufd1 significantly enhances gp78-mediated polyubiquitination of HMG-CoA reductase [36], disclosing an unappreciated function of Ufd1 as a cofactor of gp78. However, Ufd1 as a cofactor for gp78’s E3 activity is not substantiated by other studies [7,37]. gp78 is able to catalyse polyubiquitination efficiently without the need of Ufd1 in vitro [7]. Li et al. [37] showed that gp78 first catalyses the formation of polyubiquitin chain on its E2, Ube2g2 (human homologue of Ubc7p), and then transfers the preassembled chain to the substrate. This robust polyubiquitination catalysed by gp78 in vitro was seen under the conditions lacking Ufd1 [37]. Additionally, degradation of ERAD substrate such as ATZ in cells is not affected when the expression of Ufd1 is markedly reduced by RNA interference, suggesting that Ufd1 is dispensable for the gp78-mediated degradation of ATZ [17]. Although we do not understand the cause for the discrepancy in the requirement of Ufd1 for the gp78-mediated ERAD, one possibility is that Ufd1 could be specifically required for HMG-CoA reductase ubiquitination.

On the other hand, when Ufd1 was silenced in the cells with physiological levels of gp78, the CD38 accumulated [17]. This suggests the presence of a Ufd1–Npl4-dependent pathway in CD38 degradation. Although CD38 is accumulated under this condition, its degradation still occurs at a lower rate, possibly by the Ufd1–Npl4-independent pathway [17]. Hence, it can be concluded that the p97/VCP- and Ufd1–Npl4-dependent pathway works in parallel to a p97/VCP-dependent, Ufd1-independent gp78-mediated pathway during CD38 degradation [17]. The Ufd1-dependent pathway is probably mediated through other ERAD E3s (Figure 2, right). For instance, at least four more ER-resident E3s including Hrd1 (HMG-CoA reductase degradation 1), TEB4 (March VI, membrane-associated RING finger 6), Rma1 (RING finger protein with a membrane anchor), RFP2 (Ret finger protein 2) and a cytosolic ERAD E3 {CHIP [C-terminus of the Hsc (heat-shock cognate) 70-interacting protein]} have been reported [38–41]. It is likely that one or more of these E3s may couple with the p97/VCP–Ufd1–Npl4 complex during ERAD. The expression levels of different E3s may dictate the predominant pathway (i.e. Ufd1-dependent or -independent) in eliminating misfolded ER proteins.

The role of VIM in SVIP

The second VIM-containing protein, SVIP, was isolated by a yeast two-hybrid system using p97/VCP as bait [28]. The sharing of VIM between gp78 and SVIP suggests a regulatory role for SVIP in the function of gp78 [17]. Indeed, overexpression of SVIP causes vacuolization of cells [28,42]. These vacuoles might be caused by accumulation of misfolded proteins, suggesting that SVIP might negatively regulate ERAD. Our understanding of the mechanisms of ERAD and how ERAD is enhanced under ER stress conditions has advanced significantly in recent years. However, at present little is known about how the ERAD activity is controlled. As in the case of many other cellular processes, ERAD should be turned down or off when cells surpass the condition that required hyperactive ERAD. In other words, hyperactive ERAD must be controlled once the misfolded proteins have been removed. There are several reasons to hypothesize that SVIP is one potential negative regulator of ERAD. First, p97/VCP is a converging point of probably all ERAD pathways. Secondly, gp78 has a broad range of substrates. Thirdly, the presence of VIM in both gp78 and SVIP suggests that they might be competitors of p97/VCP binding.

Recently, our group has demonstrated that SVIP is localized to the ER membrane through myristoylation [18]. It was also shown that SVIP strongly interacts with two major ERAD components: p97/VCP and Derlin1, which interact with gp78 as well (Figure 2, left and middle) [18]. As anticipated, through VIM, SVIP uncouples p97/VCP from gp78, resulting in inhibition of the retrotranslocation and subsequent proteosomal degradation of the ERAD substrates during gp78-mediated ERAD [18]. Additionally, SVIP uncouples Derlin1 from gp78 [18]. It has been proposed that Derlin1 is not only the long sought after channel for retrotranslocation [24], but a substrate selector for Rma1-mediated CFTRΔF508 degradation [40,43]. Therefore sequestering Derlin1 from gp78 by SVIP might inhibit both the selection and targeting of misfolding proteins to the retrotranslocation channel. In support of this notion, overexpression of SVIP has been shown to inhibit the interaction between gp78 and its substrate [18]. The inhibition of substrate–E3 binding in turn results in a dramatic decrease in the ubiquitination of
the substrate [18]. Therefore the accumulation of gp78’s substrates observed with SVIP overexpression is caused by inhibition of their interaction with gp78, ubiquitination, and retrotranslocation.

The physiological importance of SVIP is reflected by the inverse regulation of expression between SVIP and gp78 expressions during the course of ER stress. At early stages of ER stress, the misfolded proteins need to be degraded. Therefore, to activate ERAD, gp78 is up-regulated, whereas SVIP is down-regulated (Figure 2, left and middle). Under the condition of prolonged ER stress (17 h), ERAD is inhibited [44,45] accompanied by up-regulation of SVIP and down-regulation of gp78 [18]. This suggests that once the accumulated proteins are eliminated, gp78 is down-regulated to prevent excessive ERAD that may cause damage to cells.

Conclusions

ERAD is an important and complicated cellular process. It consists of multiple steps, including recognition of substrate, ubiquitination, retrotranslocation, deglycosylation and chaperoning to the proteasome for degradation. Although different ERAD pathways have been reported, the converging point of these pathways is the Cdc48/p97/VCP-dependent and Ufd1-independent retrotranslocation. Identification of the VIM in converging point of these pathways is the Cdc48/p97/VCP-mediated retrotranslocation. Identification of the VIM in retrotranslocation and by inhibiting substrate targeting exists, and is coupled with gp78-mediated ubiquitination. SVIP negatively regulates ERAD by uncoupling p97/VCP from retrotranslocation and by inhibiting substrate targeting to gp78 by Derlin1. Therefore SVIP may protect cells from damage by excessive ERAD. In addition, SVIP may play a general role in controlling ERAD considering the fact that p97/VCP is centred in all known ERAD pathways.

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