Identification of SVIP as an Endogenous Inhibitor of Endoplasmic Reticulum-associated Degradation*

Received for publication, May 30, 2007, and in revised form, August 9, 2007. Published, JBC Papers in Press, September 14, 2007, DOI 10.1074/jbc.M704446200

Petek Ballar†‡§1, Yongwang Zhong†, Masami Nagahama§, Mitsuo Tagaya†, Yuxian Shen‡, and Shengyun Fang†§2

From the †University of Maryland Biotechnology Institute, Baltimore, Maryland 21201, ‡University of Tokushima, Tokushima 770-8506, Japan, §Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo 192-0392, Japan, and ¶Graduate Program in Molecular Medicine, University of Maryland School of Medicine, Baltimore, Maryland 21201

Misfolded proteins in the endoplasmic reticulum (ER) are eliminated by a process known as ER-associated degradation (ERAD), which starts with misfolded protein recognition, followed by ubiquitination, retrotranslocation to the cytosol, deglycosylation, and targeting to the proteasome for degradation. Actions of multisubunit protein machineries in the ER membrane integrate these steps. We hypothesized that regulation of the multisubunit machinery assembly is a mechanism by which ERAD activity is regulated. To test this hypothesis, we investigated the potential regulatory role of the small p97/VCP-interacting protein (SVIP) on the formation of the ERAD machinery that includes ubiquitin ligase gp78, AAA ATPase p97/VCP, and the putative channel Derlin1. We found that SVIP is anchored to microsomal membrane via myristoylation and co-fractionated with gp78, Derlin1, p97/VCP, and calnexin to the ER. Like gp78, SVIP also physically interacts with p97/VCP and Derlin1. Overexpression of SVIP blocks unassembled CD36 from association with gp78 and p97/VCP, which is accompanied by decreases in CD36 ubiquitination and degradation. Silencing SVIP expression markedly enhances the formation of gp78-p97/VCP-Derlin1 complex, which correlates with increased degradation of CD36 and misfolded Z variant of α-1-antitrypsin, established substrates of gp78. These results suggest that SVIP is an endogenous inhibitor of ERAD that acts through regulating the assembly of the gp78-p97/VCP-Derlin1 complex.

Degradation of misfolded or unassembled proteins from the endoplasmic reticulum (ER),3 a process known as ERAD, is essential for maintaining the homeostasis of the secretory pathway (1). Otherwise, delivery of misfolded proteins to their functional destinations or accumulation of misfolded proteins in the ER would have detrimental effects on cells (1, 2). In addition to its safeguard role, ERAD can directly regulate physiological processes that occur in the ER, for example, sterol-regulated degradation of HMG-CoA reductase, a rate-limiting enzyme in cholesterol synthesis, and inositol 1,4,5-trisphosphate (IP(3))-regulated degradation of IP(3) receptor that gates intracellular calcium release (1, 3–5). ERAD is also known to control the levels of normal serum and glucocorticoid-induced kinase 1 and Cox-2 proteins (6, 7). Furthermore, ERAD substrates have been extended to cytosolic and nuclear normal and/or misfolded proteins. For instance, ERAD ubiquitin ligase (E3) Hrd1 interacts with cytosolic p53 and misfolded Huntingtin protein and targets them to the proteasomes for degradation (8, 9).

Saccharomyces cerevisiae ERAD E3 Doa10 is spatially sorted to the inner nuclear membrane and targets nuclear-localized substrates for degradation (10).

Degradation of ER-localized misfolded proteins occurs in the cytosol by the proteasomes. Thus, luminal proteins have to be transported across the membrane, and membrane-integrated proteins have to be dislodged from the membrane to reach the proteasomes. These processes are collectively called retrotranslocation (1, 2, 11). Increasing evidence indicates that actions of large protein complexes in the ER integrate the processes of misfolded protein selection, retrotranslocation, ubiquitination, and degradation during ERAD (1, 2, 11). Indispensable to these complexes are the ER membrane-spanning RING finger E3s for ubiquitination and the AAA ATPase CDC48/p97/VCP for retrotranslocation (12). This is best characterized in S. cerevisiae in which distinct E3 complexes define different ERAD pathways (13, 14). Proteins with misfolded ER-luminal domains are targeted to the ERAD-L pathway. In this pathway, the Hrd1p/ Hrd3p ligase forms a core complex by binding to Der1p via the linker protein Usa1p. This core complex associates through Hrd3p with Yos9p and Kar2p, forming a luminal protein surveillance complex. Substrates with misfolded intramembrane domains are targeted to the ERAD-M pathway, which differs from ERAD-L by being independent of Usa1p and Der1p. Membrane proteins with misfolded cytosolic domains use the ERAD-C pathay and are directly targeted to the Doa10p E3. All three pathways converge at the Cdc48p ATPase complex. The general scheme of S. cerevisiae ERAD pathways is applicable to that in mammalian cells. So far, five ER membrane-spanning RING finger E3s, including gp78, Hrd1, TEB4, Rna1, and RFP2, have been identified in mammalian ERAD (15–19). Other components of S. cerevisiae ERAD pathways have also
been found in mammalian cells, such as p97/VCP (homologue of CDC48), Derlin1, Derlin2, and Derlin3 (homologues of Der1p), and Sel1L (homologue of Hrd3p) (12, 20–23). Unlike in S. cerevisiae, the ERAD pathways in mammalian cells are largely undefined. However, a link between ERAD E3s and p97/VCP ATPase has been demonstrated in mammalian cells and is essential for mammalian ERAD (12, 20, 24).

We have recently reported that gp78 directly interacts with p97/VCP through a newly identified p97/VCP-interacting motif (VIM) (25). This interaction is essential for degradation of ERAD substrates CD3δ and the Z variant of α-1-antitrypsin (ATZ). The putative retrotranslocation channel Derlin1 has been shown to be part of the gp78 and p97/VCP complex (12, 26). Interestingly, a highly conserved VIM is also found in the small p97/VCP-interacting protein (SVIP) (25). SVIP was isolated by a yeast two-hybrid screen using p97/VCP as bait (27). Overexpression of SVIP causes vacuolization of cells, but its physiological role is unknown (27). The sharing of VIM between gp78 and SVIP prompted us to evaluate a possible regulatory role of SVIP on gp78-mediated ERAD. We found that SVIP forms a complex with Derlin1 and p97/VCP. We provided evidence that SVIP regulates the formation of gp78-p97/VCP-Derlin1 complex, which leads to a change in substrate association with gp78 and p97/VCP and, eventually, changes in the efficacy of ERAD. These findings suggest that SVIP is an endogenous inhibitor of ERAD, acting through the inhibition of the assembly of the gp78-p97/VCP-Derlin1 complex. Thus, SVIP may act to prevent excessive ERAD that may cause damage to cells.

**EXPERIMENTAL PROCEDURES**

**Plasmids, siRNAs, Antibodies, and Cell Lines**

SVIP open reading frame was amplified by reverse transcription PCR from total RNA isolated from 293 cells. A His6 tag was added in-frame to the C terminus of SVIP during PCR amplification, and the PCR product was cloned into the mammalian expression vector pCIneo via EcoRI and SalI sites (pCIneo-SVIP). pCIneo-SVIP(G2A) was created by site-directed mutagenesis to mutate the PCR product was cloned into the mammalian expression vector pCIneo via EcoRI and SalI sites (pCIneo-SVIP). pCIneo-SVIP has been shown to be part of the gp78 and p97/VCP complex (12, 26). Stable Cell Lines

293 cells that stably express HA-CD3δ alone (clone-5) and HA-CD3δ together with gp78 (clone-20) have been previously reported (6). To generate cell lines that stably express ATZ, plasmid encoding hATZ was co-transfected with pBABE vector that confers puromycin resistance. 24 h after the transfection, 2.5 μg/ml puromycin was added to eliminate non-transfected cells. Positive clones were characterized by immunoblotting.

**Immunoblotting (IB) and Immunoprecipitation (IP)**

293 cells were seeded at 2.5 x 10^4/well in 6-well plates or 1.2 x 10^5/100-mm dish prior to the day of transfection with Lipofectamine 2000 (Invitrogen) (for plasmids and siRNAs) or by calcium phosphate precipitation (for plasmids only). Cells transfected with plasmids were collected 24 h after transfection, whereas those transfected with siRNA were harvested 72 h post-transfection. IB and IP were performed as we previously described (24).

**Subcellular Fractionation**

**Alkaline Extraction**—Microsomes were isolated as described (25) and then incubated with 0.1 M Na_2CO_3, pH 11, for 20 min at room temperature. After the incubation, microsomes were pelleted by centrifugation at 105,000 x g for 10 min at 4 °C. The resulting microsomes and supernatants were processed for IB.

**Proteasine K Digestion Assay**—Microsomes were incubated with 0, 3.1, 6.25, 12.5, 25, 50, 100 μg/ml proteinase K in 1X phosphate-buffered saline for 30 min at room temperature before being processed for IB.

**N-Myristoylation Inhibition**—1 mM 2-OHM (2-hydroxymyr-ristic acid) (Sigma) was delivered to 293 cells in a complex with fatty acid-free bovine serum albumin (Sigma) as previously described (30), and cells were incubated for 24 h. Because unmyristoylated cytosolic SVIP is not very stable, cells were treated with proteasome inhibitor for 5 h before being processed for IB.

**Gradient Fractionation**—293 cells were homogenized in buffer B (0.25 M sucrose, 1 mM EDTA, 10 mM HEPES-NaOH, pH 7.4) and then centrifuged at 3000 x g for 10 min to remove nuclei and broken cells. The post-nuclear supernatant was layered on top of a preformed 0–25% iodixanol gradient in buffer B and centrifuged at 200,000 x g for 2.5 h, after which fractions were collected from the bottom of the tube. Equal volume of each fraction was processed for IB.
SVIP Inhibits ERAD

FIGURE 1. SVIP is anchored to microsomal membrane, probably through myristoylation. A, alkaline extraction does not affect SVIP-microsome association. 293 cells were homogenized and the microsomes (m) were isolated from the post-nuclear homogenate as we previously described (25). For alkaline extraction, the microsomes were incubated in 0.1 M Na2CO3, pH 11, or in 1× phosphate-buffered saline as a control. After the incubation, the microsomes were pelleted, and the supernatants (s) were collected. These fractions were analyzed by IB using anti-SVIP, anti-Derlin1, and anti-p97/VCP antibodies. p97/VCP and Derlin1 were used as controls for peripheral protein and ER membrane-anchored protein, respectively. B, wild-type (Wt)-SVIP, but not myristoylation-deficient mutant SVIP(G2A), localizes to the microsomes. 293 cells transfected either with pCIneo, Wt-SVIP, or SVIP(G2A) were processed for IB—SVIP and SVIP(G2A) were processed for fractionation into microsomes (m) and cytosol (c). The localization of Wt-SVIP and SVIP-G2A was determined by blotting with anti-His6 antibody to detect their His6 tags. C, 2-hydroxymyristic acid (2-OHM) inhibits endogenous SVIP anchorage to membrane. 293 cells were treated with 2-OHM or its vehicle (ethanol: EtOH) followed by fractionation into microsomes (m) and cytosol (c). D, proteinase K digestion assay used to determine the membrane topology of SVIP. Microsomes prepared from 293 cells were incubated with increasing amounts of proteinase K (0–100 μg/ml) for 30 min before processing for IB with anti-SVIP and anti-calnexin (Cnx) antibodies.

RESULTS

SVIP Is a Membrane-anchored Protein—SVIP has been reported to be a membrane-anchored protein, but it possesses neither a transmembrane domain nor a signal peptide (27). How it associates with the membrane is not clear. To address this issue, we carried out alkaline extraction of the microsomes isolated from 293 cells. We found that Na2CO3 has no effect on SVIP-membrane association, as seen on the membrane-spanning protein Derlin1 (Fig. 1A). As a positive control, a significant amount of p97/VCP that is known to be peripheral associated with the microsomes is released into the cytosol (Fig. 1A, lane 2 versus 4). This result is consistent with the previous report that SVIP may be attached to membrane through myristoylation (27). To test this possibility, we mutated the putative myristoylation site glycine-2 to alanine and generated a mutant SVIP(SVIP(G2A)) that is defective in myristoylation. We found that SVIP(G2A) is exclusively localized in the cytosol (Fig. 1B, lanes 3, 4 versus 5, 6). The difference is not due to overexpression, since SVIP(G2A) was still in the cytosol when its expression was markedly reduced (lanes 7, 8 versus 9, 10). Furthermore, the membrane anchorange of SVIP can be abolished by the N-myristoylation inhibitor 2-hydroxymyristic acid (2-OHM). Treatment with 2-OHM slightly increased the mobility of SVIP and resulted in its cytosolic localization (Fig. 1C), which is consistent with inhibition of SVIP myristoylation. Thus, it is likely that SVIP is membrane-anchored through myristoylation on glycine 2. In agreement, we demonstrated that SVIP is attached on the cytosolic surface of microsomes, since proteinase K completely digested SVIP, while the intraluminal domain of calnexin remained protected by the microsomal membrane (Fig. 1D).

SVIP Forms a Complex with p97/VCP and Derlin1—SVIP is localized to the ER and other membranes (27). To investigate whether the two known VIM-containing proteins, SVIP and gp78, are in the same membrane fraction, post-nuclear supernatants from 293 cells were fractionated on an iodixanol (Opti-prep) gradient. Indeed, SVIP colocalized with gp78 along with other ER membrane proteins such as Derlin1, Hrd1, and calnexin (Fig. 2A). As expected, a fraction of p97/VCP colocalized with SVIP and gp78 (Fig. 2A).

To determine whether SVIP interacts with ERAD components other than p97/VCP, we performed a co-immunoprecipitation assay. Among the additional five proteins examined, only Derlin1 strongly co-immunoprecipitated with SVIP (Fig. 2B, lane 2). As previously reported, VIM interacts with p97/VCP, Derlin1, and gp78 (Fig. 2A, lane 5), whereas p97/VCP co-immunoprecipitated with SVIP, Derlin1, gp78, Hrd1, and its cofactors Ufd1-Npl4 heterodimer (lane 4). Thus, SVIP and gp78 not only share a common motif, VIM, but also have common interacting proteins, p97/VCP and Derlin1. Next, we assessed how the SVIP-p97/VCP-Derlin1 complex is formed by inhibiting p97/VCP expression with RNA interference. Silencing p97/VCP expression markedly reduced the SVIP-Derlin1 interaction (Fig. 2C, lanes 5, 6), suggesting that the trimeric complex is formed through simultaneous binding of SVIP and Derlin1 to p97/VCP (illustrated in Fig. 2D). The same results were obtained by a GST-SVIP pulldown assay (supplemental Fig. S1A, lanes 5, 6). Interestingly, p97/VCP also appears to be essential for the formation of the gp78-p97/VCP-Derlin1 complex as demonstrated by GST-gp78VIM and GST fusion of the Derlin1 C-terminal tail (GST-Drlc) pulldown assays (supplemental Fig. S1A, lanes 3, 4, and S1B, lanes 3, 4). In addition, the transmembrane domains of gp78 interact with Derlin1 (supplemental Fig. S2), suggesting that p97/VCP interactions enhance the binding of gp78 and Derlin1. Considering that the SVIP-p97/VCP-Derlin1 complex does not contain gp78 and VIM is present in both gp78 and SVIP, it is likely that SVIP and gp78 form a mutually exclusive complex with p97/VCP-Derlin1.

SVIP Regulates gp78-mediated ERAD—Overexpression of SVIP induces cellular vacuolation (27), and electron microscopy revealed that the vacuoles represent dilated ER (27). We speculated that such vacuoles might be caused by accumulation of misfolded ER proteins and that SVIP might be an inhibitor of ERAD. To test this possibility, we determined the effects of SVIP on ERAD. Increasing amounts of SVIP were expressed in 293 cells that stably express CD36, a well known ERAD substrate (31). We found that SVIP causes a dose-dependent accumulation of CD36 (Fig. 3A). The accumulation was due to decreased degradation as shown by cycloheximide (CHX) chase analysis in 293 cells with approximately three times overexpression of SVIP (31) (Fig. 3B). When a similar exper-
SVIP inhibition of CD3\(\varepsilon\) degradation prompted us to determine the effect of gp78 on CD3\(\varepsilon\) degradation (24). The apparent opposite effect of SVIP on CD3\(\varepsilon\) degradation prompted us to determine the effect of
SVIP Inhibits ERAD

SVIP on CD3δ-p97/VCP association. Consistent with the observed decreased degradation in the previous experiment, overexpression of SVIP inhibits p97/VCP binding to CD3δ (Fig. 3E) and gp78 (Fig. 3F). Furthermore, in SVIP-overexpressing cells, gp78 did not interact with CD3δ, which correlates with a significant decrease in the level of ubiquitinated CD3δ (Fig. 3G). To further confirm this result, we immunoprecipitated HA-CD3δ in denatured cell lysates. Consistently, SVIP overexpression significantly decreased the levels of ubiquitinated CD3δ (supplemental Fig. S3). In addition, SVIP overexpression diminishes gp78-Derlin1 interaction (Fig. 3H). Collectively, these experiments indicate that SVIP inhibits the interactions of gp78 with CD3δ, p97/VCP, and Derlin1, resulting in inhibition of CD3δ ubiquitination and subsequent loading to p97/VCP for retrotranslocation.

Next, we asked whether the endogenous SVIP plays an inhibitory role in gp78-mediated ERAD. We again utilized 293 cells that stably express CD3δ. RNA interference was employed to silence endogenous SVIP expression. Degradation of CD3δ was assessed by CHX chase analysis. Inhibition of SVIP expression significantly increased CD3δ degradation (Fig. 4A). A similar experiment was performed on ATZ, a newly identified luminal substrate for gp78 (32). As predicted, silencing SVIP also decreased the intracellular levels of ATZ, which correlates with reduced ATZ secretion (Fig. 4B). These data further support the hypothesis that endogenous SVIP negatively regulates the function of gp78s. To determine whether endogenous SVIP inhibits gp78 interaction with p97/VCP and Derlin1, we silenced the expression of SVIP and evaluated changes in the formation of gp78-p97/VCP-Derlin1 complex by co-immunoprecipitation. Inhibition of SVIP expression markedly augmented the association of p97/VCP and Derlin1 with gp78, consistent with the fact that silencing SVIP enhances ERAD. To further substantiate gp78 as a target inhibited by SVIP, we studied the effects of SVIP silencing on CD3δ degradation in 293 cells that overexpress both gp78 and CD3δ. We predicted that overexpression of gp78 will overcome the inhibitory effect exerted by endogenous SVIP and that silencing SVIP would have no effect on CD3δ degradation in cells overexpressing gp78. This was indeed the case (Fig. 4D). Collectively, these results suggest that SVIP is an endogenous inhibitor for gp78-mediated ERAD that acts through its common motif shared with gp78 and uncouples gp78 from its substrates, p97/VCP and Derlin1.

Changes in the levels of SVIP and gp78 proteins appear to control the efficiency of ERAD (Fig. 3, A and B, and Fig. 4, A, B, and D). The next question is whether the expressions of SVIP and gp78 proteins are modulated under ER stress, a condition known to regulate the efficacy of ERAD. Interestingly, tunicamycin-induced ER stress inversely regulated the levels of SVIP and gp78 proteins in a time-dependent manner, which correlates well with previous reports that ER stress enhances, but prolonged ER stress (17 h) inhibits, ERAD (33, 34).

**DISCUSSION**

The central components of the ERAD pathways include the ER resident E3s and the cytosolic CDC48/p97/VCP ATPase (13, 14). Multiple ER membrane-anchored proteins interact with and link CDC48/p97/VCP to ERAD E3s, thereby promoting association of ubiquitinated ER proteins with the ATPase for retrotranslocation (12, 20). In this study, we found that myristoylation targets SVIP to the ER membrane where it competes with gp78 to bind to p97/VCP and Derlin1. As a result, SVIP reduces the association of ERAD substrates with gp78 and p97/VCP, thereby inhibiting sub-
strate ubiquitination and subsequent ERAD steps. This study identifies SVIP as the first endogenous inhibitor of ERAD that uses a novel mechanism through inhibiting the assembly of the gp78-p97/VCP-Derlin1 complex.

Previous studies have focused on the mechanisms of ERAD and how ERAD is enhanced under ER stress (1, 2, 11, 35). However, the control mechanism of ERAD activity is largely unknown. As a general mechanism, activated cellular events have to be turned off after having fulfilled their tasks, such as in the cases of the activated receptor tyrosine kinases and transcription factors (36, 37). Failure to control the durations of receptor tyrosine kinase signaling and gene transcription can cause devastating diseases. Therefore, it is conceivable that activated ERAD in response to accumulation of misfolded proteins in the ER has to be controlled once the misfolded proteins have been removed. A similar mechanism by which ERAD is controlled may be through degradation of key components of the ERAD machineries. For example, gp78 itself is degraded by ERAD (15). Importantly, gp78 degradation is inhibited under ER stress, which correlates with an increase in ERAD (24). Thus, when misfolded proteins are accumulated in the ER, gp78 is stabilized and it aids to remove misfolded proteins. Once the accumulated proteins in the ER have been eliminated, gp78 is degraded, thereby preventing excessive ERAD that may cause damage to cells. The present finding on the regulation of the assembly of ERAD machinery by SVIP represents another mechanism of control for ERAD activity.

Although p97/VCP binds gp78 and SVIP in a mutually exclusive manner, both p97/VCP-gp78 and p97/VCP-SVIP can associate with Derlin1. This type of interaction is reminiscent of the Hrd1-p97/VCP-Derlin1 complex, in which p97/VCP simultaneously binds Derlin1 and Hrd1 (12). Our study also revealed an interaction between the transmembrane domains of gp78 and Derlin1, supporting the possibility that gp78 and Derlin1 may be part of the protein retrotranslocation channel as previously suggested (12, 24). Derlin1 has two homologues, Derlin2 and Derlin3. Derlin2 also forms complex with p97/VCP and Hrd1 (20). It would be important to know whether SVIP also interacts with Derlin2 and Derlin3. If it does, SVIP may play a more general inhibitory role on ERAD. CDC48/p97/VCP ATPase is the converging point of probably all the characterized ERAD pathways. Inhibition of this ATPase is expected to disrupt the ERAD process. The question is why SVIP also uncouples Derlin1 from gp78. Derlin1 has been proposed as the long sought after channel for retrotranslocation (12). Thus, simultaneous sequestration of p97/VCP and Derlin1 by SVIP would inhibit the targeting of misfolded proteins to the retrotranslocation channel and subsequent retrotranslocation. Additionally, SVIP diminished the gp78-CD3δ interaction, which results in stabilization of non-ubiquitinated CD3δ. By doing so, SVIP may enhance protein trafficking through the ER/Golgi. In support of this possibility, we found that silencing SVIP expression increases ATZ degradation and decreases ATZ secretion (Fig. 4B).

Our data presented strongly suggest that p97/VCP and Derlin1 can form a complex with either gp78 or SVIP. The gp78 complex facilitates ERAD, whereas the SVIP complex inhibits ERAD; the stoichiometry between these two complexes dictates the efficacy of gp78-mediated ERAD (Fig. 5). Importantly, the stoichiometry is apparently regulated under ER stress (Fig. 4E).

Acknowledgments—We thank Dr. Yihong Ye for providing antibodies and plasmids and Drs. Russell A. DeBose-Boyd, Richard N. Sifers, and Michael Seeger for providing plasmid constructs. We thank Drs. Martin F. Flajnik and Robert Cohen for critical review of the manuscript.

REFERENCES

1. Hampton, R. Y. (2002) Curr. Opin. Cell Biol. 14, 476–482
2. Tsai, B., Ye, Y., and Rapoport, T. A. (2002) Nat. Rev. Mol. Cell Biol. 3, 246–255
3. Song, B. L., Sever, N., and DeBose-Boyd, R. A. (2005) Mol. Cell 19, 829–840
4. Goldstein, J. L., DeBose-Boyd, R. A., and Brown, M. S. (2006) Cell 124, 35–46
5. Alzayady, K. J., Panning, M. M., Kelley, G. G., and Wojcikiewicz, R. J. (2005) J. Biol. Chem. 280, 34530–34537
6. Arteaga, M. F., Wang, L., Ravid, T., Hochstrasser, M., and Canessa, C. M. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 11178–11183
7. Mbonve, U. R., Wada, M., Rieke, C. J., Tang, H. Y., Dewitt, D. L., and Smith, W. L. (2006) J. Biol. Chem. 281, 35770–35778
8. 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, H., Aratani, S., Eguchi, K., Komiyama, 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., and Nakajima, T. (2007) EMBO J. 26, 113–122
9. Yang, H., Zhong, X., Ballar, P., Luo, S., Shen, Y., Rubinsztein, D. C., Monteiro, M. J., and Fang, S. (2007) Exp. Cell Res. 313, 538–550
10. Deng, M., and Hochstrasser, M. (2006) Nature 443, 827–831
11. Meusser, B., Hirsch, C., Jarosch, E., and Sommer, T. (2005) Nat. Cell Biol. 7, 766–772
12. Ye, Y., Shibata, Y., Kikkert, M., van Vooroen, S., Wiertz, E., and Rapoport, T. A. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 14132–14138
13. Carvalho, P., Goder, V., and Rapoport, T. A. (2006) Cell 126, 361–373
14. Denic, V., Quan, E. M., and Weissman, J. S. (2006) Cell 126, 349–359
15. Fang, S., Ferrone, M., Yang, C., Jensen, J. P., Tiwari, S., and Weissman, J. S. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 11178–11183
16. Kikkert, M., Doolman, R., Dai, M., Avner, R., Hassink, G., van Vooroen, S., Thanedar, S., Roitelman, J., Chau, V., and Wiertz, E. (2005) J. Biol. Chem. 279, 3525–3534
17. Hassink, G., Kikkert, M., Vooroen, S., Lee, S. J., Spaapen, R., Laar, T., Coleman, C. S., Bartee, E., Fruh, K., Chau, V., and Wiertz, E. (2005) Biochem. J. 388, 647–655
18. Younger, J. M., Chen, L., Ren, H. Y., Rosser, M. F., Turnbull, E. L., Fan, C. Y., Patterson, C., and Cyr, D. M. (2006) Cell 126, 571–582
SVIP Inhibits ERAD

19. Lerner, M., Corcoran, M., Cepeda, D., Nielsen, M. L., Zubarev, R., Ponten, F., Uhlen, M., Hober, S., Grander, D., and Sangfelt, O. (2007) Mol. Biol. Cell 18, 1670–1682
20. Lilley, B. N., and Ploegh, H. L. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 14296–14301
21. Oda, Y., Okada, T., Yoshida, H., Kaufman, R. J., Nagata, K., and Mori, K. (2006) J. Cell Biol. 172, 383–393
22. Hampton, R. Y., Gardner, R. G., and Rine, J. (1996) Mol. Biol. Cell 7, 2029–2044
23. Mueller, B., Lilley, B. N., and Ploegh, H. L. (2006) J. Cell Biol. 175, 261–270
24. Zhong, X., Shen, Y., Ballar, P., Apostolou, A., Agami, R., and Fang, S. (2004) J. Biol. Chem. 279, 45676–45684
25. Ballar, P., Shen, Y., Yang, H., and Fang, S. (2006) J. Biol. Chem. 281, 35359–35368
26. Ye, Y., Shibata, Y., Yun, C., Ron, D., and Rapoport, T. A. (2004) Nature 429, 841–847
27. Nagahama, M., Suzuki, M., Hamada, Y., Hatsuzawa, K., Tani, K., Yamamoto, A., and Tagaya, M. (2003) Mol. Biol. Cell 14, 262–273
28. Wu, Y., Swulius, M. T., Moremen, K. W., and Sifers, R. N. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 8229–8234
29. Schulze, A., Standera, S., Buerger, E., Kikker, M., van Voorden, S., Wiertz, E., Koning, F., Kloetzel, P. M., and Seeger, M. (2005) J. Mol. Biol. 354, 1021–1027
30. Nadler, M. J., Harrison, M. L., Ashendel, C. L., Cassady, J. M., and Geahlen, R. L. (1993) Biochemistry 32, 9250–9255
31. Chen, B., Mariano, J., Tsai, Y. C., Chan, A. H., Cohen, M., and Weissman, A. M. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 341–346
32. Shen, Y., Ballar, P., and Fang, S. (2006) Biochem. Biophys. Res. Commun. 349, 1285–1293
33. Shen, Y., Ballar, P., Apostolou, A., Doong, H., and Fang, S. (2007) Biochem. Biophys. Res. Commun. 352, 919–924
34. Menendez-Benito, V., Verhoef, L. G., Masucci, M. G., and Dantuma, N. P. (2005) Hum. Mol. Genet. 14, 2787–2799
35. Travers, K. J., Patel, C. K., Wodicka, L., Lockhart, D. J., Weissman, J. S., and Walter, P. (2000) Cell 101, 249–258
36. Haglund, K., Sigismund, S., Polo, S., Szymkiewicz, I., Di Fiore, P. P., and Dikic, I. (2003) Nat. Cell Biol. 5, 461–466
37. Muratani, M., and Tansey, W. P. (2003) Nat. Rev. Mol. Cell. Biol. 4, 192–201