Available online at www.sciencedirect.com
ScienceDirect
Journal of Structural Biology 156 (2006) 29–40
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

Review

Diverse functions with a common regulator: Ubiquitin takes command of an AAA ATPase

Yihong Ye *

NIDDK, National Institutes of Health, Laboratory of Molecular Biology, Bethesda, MD 20892-0540, USA

Received 5 December 2005; received in revised form 15 January 2006; accepted 19 January 2006
Available online 20 February 2006

Abstract

Cdc48/p97, a member of the AAA (ATPase associated with various cellular activities) ATPase family, participates in various cellular pathways including membrane fusion, protein folding/unfolding, proteolysis-dependent transcriptional control, protein degradation, and spindle disassembly. How Cdc48/p97 can perform such diverse functions is unclear, but the recently established connection between components of the ubiquitination system and various p97 activities suggests that these seemingly unrelated processes mediated by Cdc48/p97 may all be governed by ubiquitin.
Published by Elsevier Inc.

Keywords: Ubiquitin; Cdc48/p97/VCP; AAA ATPase/chaperone; Retro-translocation/ERAD; The proteasome

1. Introduction

Cdc48 was discovered more than two decades ago in a genetic screen for mutants that cause cell cycle arrest in *Saccharomyces cerevisiae* (Moir et al., 1982). The mammalian homolog of Cdc48 was initially characterized as a 97-kDa precursor protein for valosin, a small peptide isolated from pig intestine. It was therefore named valosin-containing protein (VCP). However, further work showed that valosin is an artifact of purification, and is unrelated to the identified 97-kDa protein (Koller and Brownstein, 1987). For this reason, we will refer to the protein as p97, a name that was given on the basis of its molecular weight (Peters et al., 1990). As a member of the AAA ATPase family, Cdc48/p97 performs a variety of cellular functions in eukaryotic cells. The recent identification of a Cdc48/p97-like protein in archaea suggests that the function of the ATPase may not be limited to the eukaryotic kingdom (Golbik et al., 1999). Moreover, p97 has drawn much attention in recent years because it has been implicated in the pathogenesis of many human diseases such as breast cancer, the Paget's disease of bone, and several types of neurodegenerative diseases (Higashiyama et al., 2002; Watts et al., 2004; Yamamoto et al., 2004b; Yamanaka et al., 2004).

As one of the most abundant proteins in cells (~1% of the cytosolic protein mass), Cdc48/p97 is ubiquitously expressed in all eukaryotic cells. In humans, its expression level was found to be elevated in certain types of cancer tissues (Yamamoto et al., 2004a, 2005). The protein is mainly localized in the cytosol, but a significant fraction is associated with membranes of various organelles such as the endoplasmic reticulum (ER) and the Golgi (Acharya et al., 1995; Latterich et al., 1995; Rabouille et al., 1995). A small amount of Cdc48 was also found in the nucleus (Madeo et al., 1998).

Cdc48/p97 can undergo phosphorylation at certain tyrosine residues, but the physiological relevance of this modification is unclear. Tyrosine phosphorylation of p97 was found to be associated with the activation of T cells and the capacitation of sperm (Egerton et al., 1992; Ficarro et al., 2003). Phosphorylation of Cdc48/p97 may offer a mechanism to regulate its subcellular localization, as was proposed for Cdc48, which appears to enter the nucleus in the late G1 phase in a phosphorylation-dependent manner (Madeo et al., 1998).

* Fax: +1 301 496 0201.
E-mail address: yihongy@mail.nih.gov.

1047-8477/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.jsb.2006.01.005

Similar to other type II AAA ATPases, Cdc48/p97 contains two ATPase domains (D1 and D2) connected by a short linker, and an additional N-terminal domain (N-domain) (Ogura and Wilkinson, 2001). Electron microscopy (EM) and crystallography studies showed that Cdc48/p97 assembles into a stable homo-hexameric barrel structure with a small central channel (DeLaBarre and Brunger, 2003; Huyton et al., 2003; Rouiller et al., 2002; Zhang et al., 2000b). The ATPase was found to undergo drastic conformational changes during its nucleotide hydrolysis cycle (Beuron et al., 2003; DeLaBarre and Brunger, 2005; Rouiller et al., 2002; Wang et al., 2003). It is believed that the ATP-dependent conformational changes in Cdc48/p97 generate mechanical force which is used, depending on different cellular contexts, to either disassemble large protein complexes or segregate polypeptides from certain intracellular structures such as the ER membrane (Fig. 1).

A large number of Cdc48/p97-associated proteins (co-factor) have been identified in the past decade (Fig. 2), which partially explains the functional diversity of this enzyme. p97 co-factors may recruit the ATPase to different subcellular localizations, allowing it to act on various substrates. In many cases, co-factors of p97 also contain domains with certain enzymatic activities, which may facilitate the processing of substrates bound by p97 (Fig. 2). All p97 co-factors known so far, with the exception of Ufd2 (Richly et al., 2005), binds the ATPase via its N-domain. Based on the observation that two co-factors of p97, which function in different cellular pathways, bind the ATPase in a mutually exclusive manner, Meyer and colleagues proposed that the vast number of p97-associated proteins may define a set of distinct Cdc48/p97 complexes, which confer different cellular activities (Meyer et al., 2000). However, it is currently unclear how many independent p97 complexes exist in cells.

Many substrates of p97 are eventually degraded by the 26S proteasome, although the p97 ATPase was also found to be involved in certain non-proteolysis-dependent processes. Substrates of the proteasome are usually conjugated with chains of a small polypeptide called ubiquitin through the sequential actions of three types of enzymes, namely ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2), and ubiquitin ligase (E3). Ubiquitin chains can be formed through one of the seven lysine residues on an ubiquitin molecule, but the proteasome apparently prefers lysine^{48}-linked ubiquitin chains. Poly-ubiquitinated substrates are delivered to the proteasome via certain ubiquitin receptors that recognize both ubiquitin-conjugates on a substrate and the proteasome (Elsasser and Finley, 2005). Intriguingly, many co-factors of Cdc48/p97 also bind ubiquitin conjugates (Elsasser and Finley, 2005), and it becomes evident now that various activities of Cdc48/p97, regardless of whether they are associated with proteolysis or not, all seem to implicate ubiquitin as a common regulator.

2. The diverse functions of Cdc48/p97

2.1. Cell cycle regulation

Despite the fact that *cdc48* was identified as a cell cycle mutant more than two decades ago, its role in cell cycle remains poorly understood. In *S. cerevisiae*, *cdc48* mutants arrest as large budded cells with microtubules spreading aberrantly throughout the cytoplasm from a single spindle plaque (Frohlich et al., 1991). This cell cycle arrest phenotype was once attributed to a defect in fusion of the endoplasmic reticulum (ER) membrane (Latterich et al., 1995), a process that may be involved in the maintenance of the ER integrity during cell cycle progression. However, the recent discovery of the proteolysis-associated activity of Cdc48/p97 has prompted investigations on the involvement of Cdc48/p97 in the degradation of critical cell cycle regulators. Indeed, several lines of evidence suggest that Cdc48/p97 may regulate the stability of cyclin E (Dai and Li, 2001), the G1-cyclin-dependent kinase inhibitor Far1 (Fu et al., 2003), and proteins that control chromosome segregation during mitosis such as securin and separase (Yuasa et al., 2004). Altered stability of these cell cycle regulators in *cdc48* mutants may explain some of the cell cycle defects associated with reduced Cdc48/p97 activity. However, it is worth noting that since *cdc48* mutants have severe growth defects, the change in

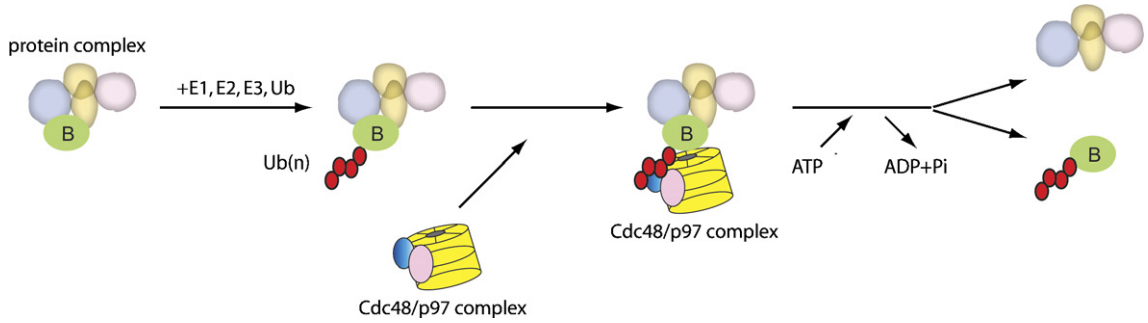

Fig. 1. The proposed function of Cdc48/p97. Disassembly of protein complex by Cdc48/p97. Selective removal of one polypeptide subunit from a protein complex may be mediated by Cdc48/p97. The target protein is first modified by poly-ubiquitination by the sequential action of E1, E2, and E3 enzymes. The Cdc48/p97 complex is recruited to segregate the target protein from the rest of the complex. During retro-translocation, the p97–Ufd1-Npl4 complex releases ubiquitinated proteins from the ER membrane in an analogous manner. Many substrates released by Cdc48/p97 are delivered to the downstream proteasome for degradation.

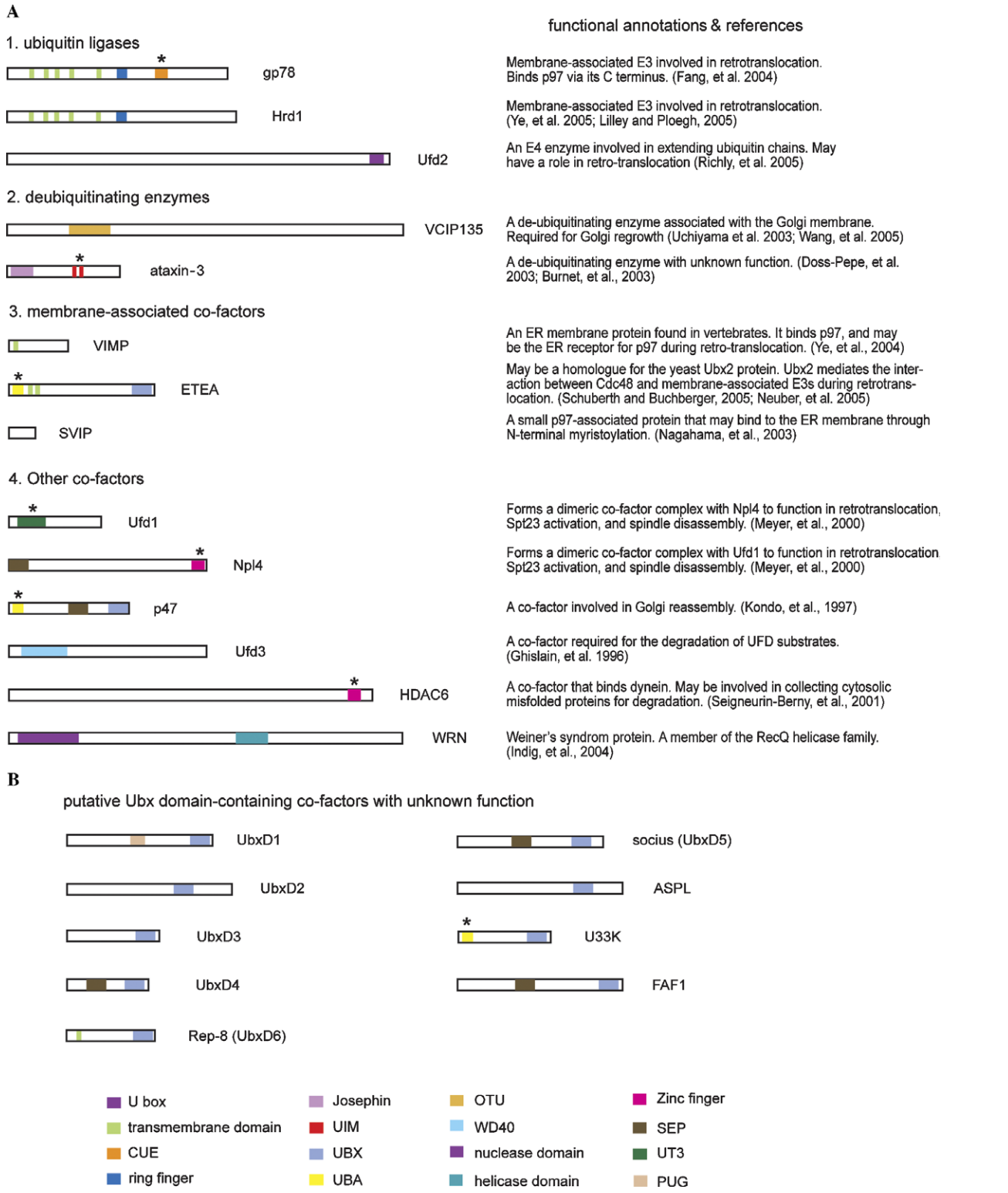

Fig. 2. Known and predicted interacting partners of mammalian p97. (A) Proteins known to bind p97. (B) Uncharacterized proteins bearing UBX domains of *Homo sapiens*. These proteins likely bind p97 because all seven UBX domain-containing proteins of *S. cerevisiae* interact with Cdc48 (Schuberth et al., 2004). Domain structures were analyzed by the SMART program, and the nomenclature of various domains is based on that determined by SMART. The proteins in (A) are artificially divided into four groups on the basis of their functions. Proteins with a known ubiquitin binding domain were marked with an asterisk.

the stability of these cell cycle regulators may not be a direct consequence of loss of Cdc48 activity. Alternatively, the cell cycle defects of the cdc48-mutants may be due to a more general inhibition of the ubiquitin–proteasome system, as mitosis is known to be sensitive to treatment by proteasome inhibitors.

More recently, Cao and colleagues have reported a novel connection between Cdc48/p97 and mitosis. They found that Cdc48/p97 and its co-factors Ufd1 and Npl4 are required for spindle disassembly during mitotic exit (Cao et al., 2003). In *Xenopus* egg extracts, addition of dominant negative p97 or of antibodies to Ufd1 leads to dramatic defect in spindle and chromosome structure at the exit of mitosis: chromosomes fail to decondense, and the spindle remains elongated and attached to the chromosomes. In yeast mutants of *cdc48*, *ufd1*, and *npl4*, the spindle also fails to disassemble following the segregation of chromosomes. The mechanism by which the Cdc48/p97 complex regulates spindle dynamics in mitosis is unclear. In *Xenopus* extracts, p97 was found to associate with several microtubule binding proteins including XMAP215 and TPX2. In budding yeast, Cdc48 appears to control the stability of Ase1, another microtubule binding protein involved in the maintenance of spindle structure. It was proposed that spindle disassembly may require the removal of certain microtubule binding proteins from the spindle, and the Cdc48/p97–Ufd1-Npl4 complex may participate in this process by either promoting the degradation of these factors or facilitating their dissociation from the microtubule.

The discovery by Cao et al. has added a new layer of complexity to the role of Cdc48/p97 in cell cycle regulation. The work does not exclude the possibility that Cdc48/p97 also acts on cell cycle regulators other than microtubule binding proteins. In fact, it is possible that the ATPase complex is required at multiple points during cell cycle progression. An important unsolved issue is to establish the exact substrate relationship between Cdc48/p97 and various cell cycle regulators, which will require reconstituting Cdc48/p97-dependent cell cycle events in vitro using purified components.

2.2. Membrane fusion

Cdc48/p97 is closely related to NSF (*N*-ethylmaleimide sensitive factor), an AAA ATPase involved in the fusion of vesicles with their target membranes in the secretory pathway (Brunger and DeLaBarre, 2003; Meyer, 2005). This fusion reaction is catalyzed by the formation of a highly stable, four-helix, coiled-coil bundle between the v- and the corresponding t-SNAREs (soluble NSF attachment protein receptor), localized in the vesicles and the target membrane, respectively (McNew et al., 2000; Weber et al., 1998). NSF, which binds the SNARE complex through its co-factor SNAP, disassembles the SNARE complex once fusion is completed. This allows the disassembled SNARE molecules to participate in further rounds of fusion (Mayer et al., 1996; Sollner et al., 1993a,b). The sequence homology between NSF and Cdc48/p97 suggests that the latter may have a similar function in membrane fusion. Indeed, early work showed that membranes isolated from *cdc48* mutant yeast strains are defective for ER-membrane fusion in vitro (Latterich et al., 1995). In addition, Cdc48 interacts with Ufe1, an ER resident t-SNARE candidate, which is required for the fusion of the ER membrane in yeast (Patel et al., 1998). Along the same lines, p97, together with its associated proteins, Ufd1-Npl4, and p47, is involved in the reassembly of the nuclear envelope during mitosis, which requires fusion of vesicles docked on the chromatin (Hetzer et al., 2001).

The p97 ATPase also mediates the reformation of the Golgi complex following its mitotic disassembly (Acharya et al., 1995; Rabouille et al., 1995). At the onset of mitosis, the Golgi apparatus undergoes extensive fragmentation. In telophase, fragmented Golgi vesicles rapidly reassemble to form a new Golgi organelle in each daughter cell (Warren, 1993). In an in vitro system that recapitulates this process, the assembly of post-mitotic Golgi fragments into stacked cisternae requires p97 and its co-factor p47 in addition to the NSF-SNAP complex (Kondo et al., 1997; Rabouille et al., 1995). p47, which cycles on and off the Golgi membrane in a phosphorylation-dependent manner, regulates the binding between p97 and a Golgi t-SNARE, syntaxin 5 (Rabouille et al., 1998; Uchiyama and Kondo, 2005). The analogy between p47 and the NSF co-factor, SNAP, makes it an attractive possibility that p97 may act similarly to NSF to disentangle the syntaxin 5-containing SNARE complex following the fusion of the Golgi membrane. While the validity of this model still awaits proof, recent work has uncovered a novel twist for the role of p97 in Golgi reassembly, which involves a cycle of ubiquitination and de-ubiquitination.

Buchberger and colleagues reported that the p97 co-factor p47 contains an ubiquitin-like segment (UBX) and an ubiquitin binding motif (UBA) (Buchberger et al., 2001). The UBX domain of p47 mediates its association with p97, whereas its UBA domain can recognize ubiquitin conjugates, and is required for the fusion activity of p47 (Meyer et al., 2002; Yuan et al., 2004). These observations suggest that the reassembly of the Golgi may involve a function of ubiquitin. Although ubiquitin was initially characterized as a key regulator for protein degradation, it has become clear now that certain type of ubiquitin conjugates such as mono-ubiquitinated proteins or ubiquitin chains linked by lysine^{63} are associated with non-proteolysis functions such as endocytic trafficking and DNA repair (Hicke and Dunn, 2003; Hoege et al., 2002; Ulrich, 2004). The reassembly of the Golgi mediated by the p97–p47 complex seems to require a non-proteolytic activity of ubiquitin, because (1) The p97–p47 complex preferentially binds mono-ubiquitinated proteins (Meyer et al., 2002); (2) Addition of an ubiquitin variant (UbI44A) defective in recognition by ubiquitin binding proteins to the Golgi reassembly reaction inhibits Golgi reformation. In contrast, addition of a proteasome inhibitor has no effect (Wang et al., 2004b). Interestingly, the mutant ubiquitin needs to be added before Golgi fragmentation is completed, suggesting that certain unknown factors are ubiquitinated during Golgi disassembly, and ubiquitin conjugates are recognized and processed by the p97–p47 complex during the subsequent reassembly process. Moreover, the reassembly of the Golgi also requires another co-factor of p97, termed as

VCIP135 (Uchiyama et al., 2002), which contains deubiquitinating activity (Wang et al., 2004b). It seems that ubiquitin conjugates also need to be removed by VCIP135 during p97–p47-mediated reformation of the Golgi complex. Although the nature of the ubiquitinated proteins as well as how the p97–p47-VCIP135 complex acts on these molecules during Golgi reassembly remains to be determined, the new findings have revealed a novel regulation that implicates a cycle of ubiquitination and de-ubiquitination in p97-mediated Golgi reassembly (Meyer, 2005).

2.3. Protein degradation

An implication of Cdc48/p97 in ubiquitin-dependent protein degradation was initially suggested by Varshavsky and colleagues when they found that Cdc48 is required for the degradation of an unstable model substrate containing an amino-terminal ubiquitin fusion (Ghislain et al., 1996). Genetic dissection of components required for the degradation of this substrate in yeast uncovered five genes named Ufd1–Ufd5, which defines a special proteolytic pathway termed the ubiquitin fusion degradation (UFD) pathway (Johnson et al., 1995). Intriguingly, Cdc48/p97 interacts with Ufd1, Ufd2, and Ufd3 (Ghislain et al., 1996; Koegl et al., 1999; Meyer et al., 2000), suggesting that it is a central component of the UFD pathway. These observations, together with the fact that many p97 co-factors recognize ubiquitin (Fig. 2), raise the possibility that p97 may shuttle a subset of ubiquitinated substrates to the proteasome for degradation.

The role of Cdc48/p97 in proteolysis is best understood for the degradation of misfolded or incompletely assembled ER proteins. These unwanted ER proteins are selectively exported from the ER by a pathway termed retro-translocation or dislocation, and are subsequently degraded by the ubiquitin–proteasome system in the cytosol (Hampton, 2002; Kostova and Wolf, 2003; Meusser et al., 2005; Ye, 2005). Interestingly, the retro-translocation pathway may be co-opted by certain viruses to degrade normally folded cellular proteins required for immune defense, allowing the virus to evade host immune surveillance (Tortorella et al., 2000). For example, the human cytomegalovirus (HCMV) encoded proteins, US2 and US11, can efficiently down-regulate newly synthesized MHC class I heavy chains by the retro-translocation pathway (Wiertz et al., 1996).

The first step of retro-translocation is substrate recognition and targeting to the ER membrane. Misfolded or unassembled polypeptides are selected by ER chaperones, whereas viral factors associate with their specific "clients" (e.g., US2 and US11 are ER resident proteins that specifically bind MHC class I heavy chain), and deliver them to the site of translocation. Subsequently, retro-translocation is initiated when a segment of a substrate is inserted into a protein-conducting channel. The nature of the protein-conducting channel for retro-translocation is an issue of debate. It was initially proposed that the Sec61 complex, which mediates the translocation of polypeptides into the ER lumen or the integration of membrane proteins into the lipid bilayer, might also export misfolded proteins during retro-translocation (Wiertz et al., 1996). However, the degradation of certain misfolded model substrate is not affected by Sec61 mutants (Walter et al., 2001). In fact, recent studies raise the possibility that the Sec61 complex may only be required for the translocation of a few substrates that do not involve poly-ubiquitination (Kalies et al., 2005; Lee et al., 2004). Consistent with this notion, components of the Sec61 complex seem not to interact with the cytosolic retro-translocation machineries such as ER-associated ubiquitin ligases and the p97 ATPase (see below) (Y. Y., unpublished results). In contrast, a recently identified multi-spanning membrane protein, Derlin-1, provides a link between substrate recognition in the ER lumen and the p97-mediated polypeptide dislocation in the cytosol, and thus may be part of an alternative channel for a subset of substrates (Lilley and Ploegh, 2004; Ye et al., 2004).

In the next step, polypeptides emerging into the cytosol from a putative translocation channel undergo poly-ubiquitination at the ER membrane, and the modified polypeptides are then released into the cytosol for proteasomal degradation (Tsai et al., 2002). Intriguingly, the dislocation of most polypeptides into the cytosol requires both poly-ubiquitination (Biederer et al., 1997; de Virgilio et al., 1998; Kikkert et al., 2001; Shamu et al., 2001) and the function of the Cdc48/p97–Ufd1-Npl4 complex (Bays et al., 2001; Jarosch et al., 2002; Ye et al., 2001). Accordingly, it was found that the Cdc48/p97 complex contains several ubiquitin binding sites that are essential for its function in retro-translocation (Park et al., 2005; Ye et al., 2003). Nonetheless, ubiquitin binding is not involved in the initial substrate recognition. When poly-ubiquitination is abolished, the p97 ATPase can still associate with polypeptides undergoing retro-translocation at the ER membrane, but the associated substrates cannot be dislocated into the cytosol. In addition, the p97 complex binds retro-translocation substrates modified by poly-GST-tagged-ubiquitin with a similar affinity to those containing untagged ubiquitin conjugates. Unlike normal ubiquitin chains, conjugates containing GST-tagged ubiquitin molecules are not recognized by either p97 or its co-factor Ufd1. Thus, the association between the ATPase complex and poly-GST-ubiquitinated substrates presumably occurs through a non-modified segment of a retro-translocation substrate (Flierman et al., 2003). Based on these observations, a dual recognition model was proposed, in which the p97/Cdc48 complex first joins a retro-translocation complex containing a non-modified, presumably unfolded substrate, emerging from the ER lumen. Once ubiquitin chains are attached to the substrate, the conjugates can be recognized by both the ATPase and Ufd1 (Fig. 3). Since interfering with ubiquitin recognition leads to a similar defect as that caused by a mutant p97 lacking ATPase activity, ubiquitin binding by the p97 complex may somehow activate the ATPase, leading to the dislocation of substrates into the cytosol.

The function of Cdc48/p97 in retro-translocation appears to be similar to that of AAA proteases in bacteria

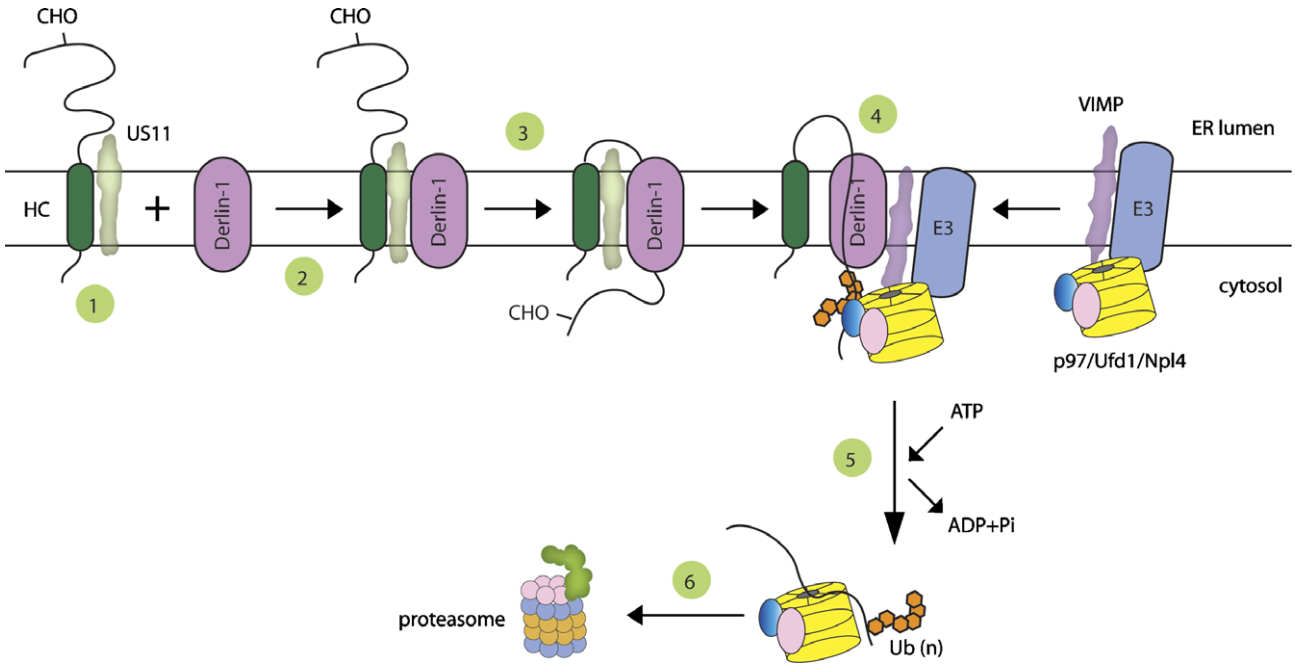

Fig. 3. The US11-dependent retro-translocation of MHC class I heavy chain. In step 1, the viral protein, US11 recognizes MHC class I heavy chain (HC), a glycoprotein with a single carbohydrate chain (CHO). In step 2, heavy chain is targeted to a multi-spanning membrane protein called Derlin-1. In step 3, retro-translocation is initiated when heavy chain is partially moved out of the ER through a protein-conducting channel postulated to contain Derlin-1. In step 4, the emerging polypeptide is captured by the cytosolic ATPase p97, which may lead to the assembly of a complex containing the p97 cofactor dimer (Ufd1-Npl4), the single spanning membrane protein, VIMP, and an unknown ubiquitin ligase. Heavy chain undergoes poly-ubiquitination, while a cytosolic *N*-glycanase removes the attached carbohydrate chain. In the next step, the poly-ubiquitin chains are recognized by both p97 and Ufd1, which may trigger the ATP hydrolysis by p97, leading to the release of heavy chains from the ER membrane. It is possible that the ATPase may accompany heavy chains to enter the cytosol. In step 6, poly-ubiquitinated heavy chain is delivered to the proteasome for degradation.

and mitochondria (Ito and Akiyama, 2005). These AAA proteases contain an amino-terminal transmembrane segment, followed by an AAA ATPase domain, and a protease domain. Similarly to Cdc48/p97, both FtsH in the inner membrane of *Escherichia coli*, and Yta10/Yta12 and Yme1 that face the two sides of the inner membrane of mitochondria, form hexameric rings. They are capable of binding to a misfolded membrane protein at the membrane, and of using the energy generated by their ATPase domain to processively pull the substrate out for subsequent degradation by their protease domain. For Cdc48/p97, its membrane localization requires an association with a membrane protein since neither the ATPase nor its co-factors contain a transmembrane segment. In yeast, a UBA–UBX domain-containing protein, Ubx2, facilitates the membrane association of Cdc48 by mediating its interaction with several ER-localized ubiquitin ligases and Der1, the yeast homologue of Derlin-1 (Neuber et al., 2005; Schuberth and Buchberger, 2005). In vertebrates, a single-spanning membrane protein, VIMP (VCP-interacting membrane protein), appears to recruit p97 to the site of retro-translocation, at which it interacts with both Derlin-1 and ubiquitin ligases (E3) required for retro-translocation (Lilley and Ploegh, 2004, 2005; Ye et al., 2005, 2004). The mammalian homologue of Ubx2 may also contribute to the membrane association of p97. Cdc48/p97 also needs to communicate with the downstream proteasome to deliver substrates to the degradation machinery. AAA proteases such as FtsH appear to combine the function of Cdc48/p97, its membrane receptor, and the proteasome in one polypeptide.

2.4. Transcriptional control

Several lines of evidence suggest that Cdc48/p97 may also function in transcriptional regulation. First of all, Dai et al. reported that p97 interacts with IκBα, an inhibitory factor of the NFκB signaling pathway. The association appears to require both phosphorylation and ubiquitination of IκBα, two post-translational modifications that precede the degradation of IκBα during NFκB activation (Dai et al., 1998). These data suggest that p97 may regulate the NFκB-mediated gene expression by controlling the stability of IκBα. Secondly, Cdc48 was found to regulate a similar processing pathway that controls the expression of genes involved in fatty acid metabolism in *S. cerevisiae* (Hitchcock et al., 2001; Hoppe et al., 2000; Rape et al., 2001; Shcherbik et al., 2003). The central players, Spt23 and Mga2, are both synthesized as dormant transcription factor precursors (p120) that are associated through their carboxy-terminal transmembrane domains with the ER membrane. Spt23 and Mga2 form a homo-dimer, which is processed in an unusual way by the 26S proteasome, leading to the selective degradation of the carboxy-terminal segment of one subunit (Rape and Jentsch, 2004). The remaining amino-terminal portion (p90), albeit lacking any transmembrane anchor, is still associated with the ER

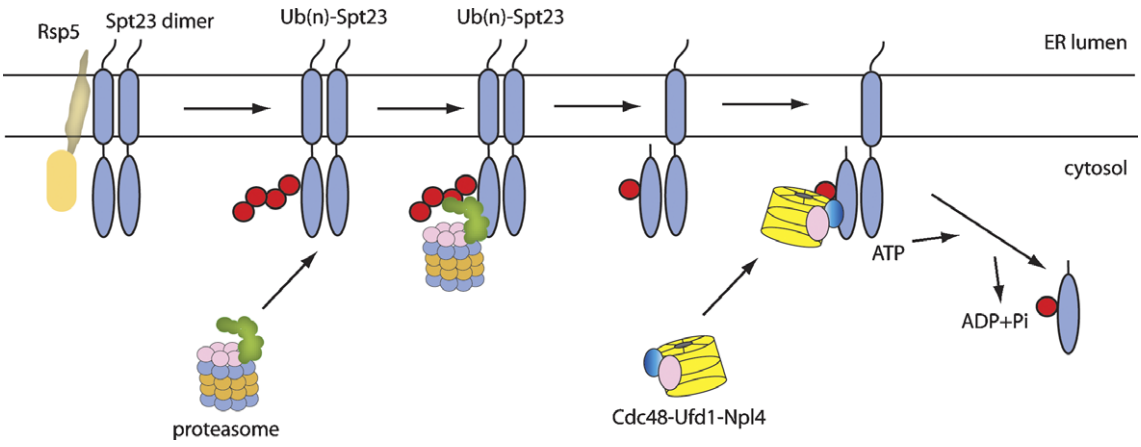

Fig. 4. Cdc48-dependent activation of Spt23 in *S. cerevisiae.* Spt23 is synthesized as an inactive precursor that forms a homodimer at the ER membrane. It then undergoes ubiquitination by a membrane-associated ubiquitin ligase, Rsp5. This modification appears to be regulated by the level of unsaturated fatty acid in the ER membrane. Subsequently, the proteasome is recruited, leading to the partial degradation of one Spt23 subunit. The processed Spt23 apparently still carries some ubiquitin conjugates, which may be the signal to recruit the Cdc48–Ufd1-Npl4 complex. The latter may apply the force generated by ATP hydrolysis to release the processed Spt23 subunit from its partner, allowing its entry to the nucleus to regulate gene expression.

membrane via an interaction with the intact p120 subunit. The p90 subunit, which still retains several ubiquitin moieties, is recognized by the Cdc48–Ufd1-Npl4 complex. The ATPase complex then releases the p90 subunit from the membrane, allowing its translocation into the nucleus to regulate gene expression. Thus, the Cdc48/p97-dependent mobilization of Spt23 (Fig. 4) seems to be analogous to the extraction of misfolded proteins during retro-translocaiton. Interestingly, the release of the processed Mga2 subunit from the ER membrane appears to follow a different mechanism that also requires Cdc48. Specifically, the mobilization of the processed Mga2 may be a consequence of the Cdc48-mediated degradation of the unprocessed p120 subunit of Mga2 (Shcherbik et al., 2003).

2.5. Other activities

In addition to the cellular activities described above, recent work has linked Cdc48/p97 to many other cellular processes such as protein folding/unfolding, DNA repair, and apoptosis. The archaeal Cdc48/p97-like protein, VAT, was shown to contain both protein folding and unfolding activities depending on salt concentration (Gerega et al., 2005; Golbik et al., 1999). The yeast Cdc48 is capable of recognizing unfolded polypeptides to suppress their aggregation in vitro. Surprisingly, this does not require the ATPase activity of Cdc48 (Thoms, 2002). A connection between p97 and DNA repair was suggested when Zhang and colleagues found that p97 interacts with BRCA1 (Zhang et al., 2000a). Consistent with this notion, p97 was recently found to undergo phosphorylation at a serine residue in response to DNA damage (Livingstone et al., 2005). The ATPase also binds to a DNA unwinding helicase, and thus may function in DNA replication (Yamada et al., 2000). A role for p97 in apoptosis was suggested to be critical for the mechanism underlying the pathogenesis of several neurodegenerative diseases (Higashiyama et al., 2002; Yamanaka et al., 2004). However, given that p97 is an abundant essential protein, any perturbation of its function may ultimately cause cell death.

3. Ubiquitin commands the AAA ATPase

At first glance, it seems that all the p97-regulated processes described above are unrelated. However, a close examination reveals that the diverse p97 functions are all connected, in one way or the other, to the ubiquitin pathway. First of all, p97 itself contains an intrinsic ubiquitin binding site in its N domain, although its affinity is low (Dai and Li, 2001; Rape et al., 2001; Ye et al., 2003). Along the same lines, many co-factors of p97 also bear ubiquitin binding domains (Fig. 2). These additional ubiquitin binding sites may either enhance the affinity of the p97 complex for ubiquitin conjugates (e.g., the p97 and its co-factor Ufd1 can interact with ubiquitin chains linked by lysine^{48} in a synergistic manner) (Ye et al., 2003), or alter the specificity of ubiquitin recognition (e.g., p97 by itself prefers poly-ubiquitin conjugates, whereas the p97–p47 complex appears to interact selectively with mono-ubiquitinated substrates) (Meyer et al., 2002). Curiously, not all p97 co-factors identified so far contains an ubiquitin binding motif. However, since p97 forms a homo-hexamer that can bind up to six co-factors directly, co-factors without ubiquitin binding activity may team up with an ubiquitin binding co-factor, which would allow every p97-containing complex to act on ubiquitin conjugated substrates with different chain linkages and chain lengths.

In addition to ubiquitin conjugates, p97 also associates with several ubiquitin ligases (E3). In the degradation of misfolded ER proteins, p97 interacts with Hrd1 and gp78, two ER-localized ubiquitin ligases, to facilitate the recruitment of these enzymes to the site of retro-translocation (Lilley and Ploegh, 2005; Ye et al., 2005; Zhong et al., 2004). p97 also interacts with a class of U-Box-containing ubiqui-

tin ligases (Hatakeyama et al., 2004). An interaction between p97 and ubiquitin ligases raises the possibility that certain ubiquitin conjugates may be synthesized while substrates are bound by p97. In this context, the ATPase may even regulate the ubiquitination reaction per se. Indeed, recent work showed that Cdc48 can bind and modulate the activity of Ufd2, an ubiquitin extending enzyme (E4) that normally extends ubiquitin chains to a length containing more than 20 units. The association of Cdc48 with Ufd2 restricts the chains to a length of four to six ubiquitins (Richly et al., 2005).

Intriguingly, certain p97-interacting proteins also contain deubiquitinating activity. In addition to VCIP135 which was described above, another p97 co-factor with deubiquitinating activity is ataxin-3, a poly-glutamine (Q) containing protein that has been implicated in the pathogenesis of spinocerebellar ataxia, a neurodegenerative diseases caused by poly-Q expansion (Burnett et al., 2003; Chow et al., 2004; Warrick et al., 2005). Ataxin-3 binds p97 in a poly-Q-dependent manner (Matsumoto et al., 2004). Its deubiquitinating activity is conferred by the amino-terminal Josephin domain, which adopts a papain-like fold commonly found in many other deubiquitinating enzymes (Mao et al., 2005). In addition, Ataxin-3 also contains 2–3 ubiquitin interacting motifs (UIM) that are critical for its activity (Warrick et al., 2005). The physiological function of ataxin-3 is unclear, but its association with p97 suggests that the function of Cdc48/p97 may generally be associated with a deubiquitinating event.

The close connection between Cdc48/p97 and the ubiquitin pathway is striking, considering that archaea contain a Cdc48-like protein, VAT, but lack ubiquitin. How can VAT function in the absence of ubiquitin, whereas ubiquitin seems to be indispensable for the function of Cdc48/p97 in eukaryotic cells? It is also known that archaea do not contain the intracellular membrane system present in eukaryotic cells. Interestingly, Cdc48/p97 appears to be particularly well adapted to act on membrane substrates. This suggests that the core function of VAT/Cdc48/p97 is ubiquitin-independent chaperone activity, and the vast number of Cdc48/p97 co-factors may appear later during evolution to allow the ATPase to acquire novel activities in various membrane-associated events. Given the fact that ubiquitin is implicated in almost every aspect of eukaryotic cell biology, the innovation of a large number of co-factors that are capable of recognizing ubiquitin certainly expands the functional repertoire of Cdc48/p97. The close interplay between Cdc48/p97 and ubiquitin suggests that the latter is an important regulator for various p97-dependent activities.

4. Does the ring matter: a tunnel view?

Structural studies showed that p97 forms a homo-hexameric ring with a central channel (DeLaBarre and Brunger, 2003; Huyton et al., 2003; Zhang et al., 2000b). This structure resembles the AAA ATPase ring connected to the ClpP protease in *E. coli* or the base of the 19S regulatory particle in the eukaryotic proteasome (Pickart and Cohen, 2004). Another AAA ATPase homologous to Cdc48/p97 is ClpB in bacteria (also called Hsp104 in yeast) (Lee et al., 2003). All these proteins exhibit canonical chaperone activities, and can unfold polypeptides by threading them through a central channel (Pickart and Cohen, 2004; Weibezahn et al., 2004). In the case of the proteasome or its bacterial counterparts, polypeptide unfolding is coupled to its translocation into the degradation chamber that is physically connected to the ATPase ring. In contrast, ClpB/Hsp104 is not associated with any proteolytic complex. Polypeptides processed by ClpB/Hsp104 are released into the cytoplasm, where they can refold with the help of other chaperones (Glover and Lindquist, 1998). Since Cdc48/p97 can mediate both proteolytic and non-proteolytic functions, it must be able to cooperate with the proteasome in one case, but act on its own in another. When both the proteasome and Cdc48/p97 are involved, the connection between the two remains unclear. Dai et al. reported a weak association between p97 and the 26S proteasome, although the physiological specificity of this interaction is unclear (Dai et al., 1998). Recent work demonstrated that at least some ubiquitinated substrates are shuttled from Cdc48 to the proteasome by certain ubiquitin receptors that associate alternately with Cdc48 and the proteasome (Richly et al., 2005). Although the generality of this finding remains to be tested, it seems to suggest that no physical association between Cdc48 and the proteasome is necessary for the transfer of at least some substrates from Cdc48/p97 to the degradation machinery. For Cdc48/p97-mediated non-proteolytic functions, the identity and the fate of its substrates are even more mysterious.

The structural and functional similarity between Cdc48/p97 and other ring-shaped AAA ATPases such as ClpB/Hsp104 raises the possibility that Cdc48/p97 may also thread polypeptides through its central channel. In Cdc48/p97-mediated proteolytic function, substrates emerging from its central pore would subsequently be delivered to the proteasome for degradation. Alternatively, a substrate may be released into the cytoplasm, leading to its separation from the membranes or certain protein complexes. Consistent with this view, recent biochemical studies of VAT suggest that the residues forming the central pore of this p97-like protein are indeed mechanistically important for its unfolding activity (Gerega et al., 2005).

Nonetheless, caveat associated with a complete threading model is apparent, which raises the possibility that p97 may act by a mechanism that does not engage its central channel. As described above, substrates of Cdc48/p97 usually contain bulky ubiquitin conjugates, which seem to exceed the size limit of its pore. Although the current structural studies do not provide any accurate information on the flexibility of the pore, a significant increase in the pore size during nucleotide cycle to accommodate folded ubiquitin conjugates seems to be unlikely. An alternative solution is that the ATPase may unfold polypeptides including the

attached ubiquitin chains when moving them through its central pore. However, since ubiquitin are also required downstream of p97 for substrate delivery to the proteasome, the unfolded ubiquitin chains have to be refolded to avoid premature removal of the proteasomal targeting signal. In addition, many substrates of p97 are fully functional after being processed by the ATPase (e.g., Spt23), it is difficult to imagine how complete unfolding required by threading polypeptides through the narrow channel of p97 would not interfere with their function unless these substrates are able to refold rapidly.

Another equally attractive model is that p97 may function as a "molecular switch" to generate mechanical force. Structural studies showed that the N-terminal domain of p97 is flexible (Brunger and DeLaBarre, 2003). Since the N-domain is required for p97 to interact with its co-factors as well as substrates, it is possible that conformational rearrangement of this domain associated with nucleotide binding and hydrolysis may generate sufficient motion to separate the bound substrates from membranes or other proteins. It is worth noting that the NSF, a p97-related protein that forms a similar hexameric ring structure, is believed to act in this manner (Owen and Schiavo, 1999).

5. Perspectives

The field of AAA ATPases has been evolving rapidly in recent years. Among all the AAA ATPase family members, Cdc48/p97 has particularly drawn much attention, not only because of its diverse cellular functions, but also because of its enigmatic mechanism of action. The rapid unfolding of different stories associated with various Cdc48/p97 activities is exciting, but it has not added much to our understanding of how this impressive machinery works in the context of different substrates. Instead, more questions have been generated. For example, the precise role of ubiquitin in p97-mediated cellular processes is still unknown. How the ATPase hydrolyze ATP remains as a controversial issue (Wang et al., 2004a). Several lines of evidence indicate that the two ATPase domains (D1 and D2) may alternate in ATP hydrolysis, whereas other data suggest that all the ATPase activity can be attributed to the D2 domain. Evidence accumulated so far suggest that the central pore of Cdc48/p97 may be mechanistically essential, but whether substrates can be threaded through the pore remains to be demonstrated. How the ATPase activity of Cdc48/p97 is regulated is another question. The only co-factor that was found to modulate the activity of p97 is p47. Unexpectedly, p47 inhibits the p97 activity rather than increasing it (Meyer et al., 1998), but the physiological relevance of this regulation is unclear. Measuring the ATP hydrolysis of p97 in the context of a defined substrate will provide valuable information on this issue. Another interesting question is how p97 generates force upon nucleotide hydrolysis. Recent structural studies on p97 have started to reveal conformational changes associated with nucleotide hydrolysis cycle. Finally, the search for novel p97 co-factors and functions has to be matched by the identification and characterization of various p97 substrates. A thorough understanding of p97 function would require a study of its activity using purified components including a well defined substrate. With so many challenging questions, Cdc48/p97 will certainly offer more excitement than a demanding cell biologist can absorb.

Acknowledgments

I thank H. Bernstein, T. Rapoport, and M. Gellert for critical reading of the manuscript, and apologize for not being able to cite all the papers relevant to the development of this field due to space constraints. This Research was supported in part by the Intramural Research Program of the NIH, NIDDK.

References

Acharya, U., Jacobs, R., Peters, J.M., Watson, N., Farquhar, M.G., Malhotra, V., 1995. The formation of Golgi stacks from vesiculated Golgi membranes requires two distinct fusion events. Cell 82, 895–904.

Bays, N.W., Wilhovsky, S.K., Goradia, A., Hodgkiss-Harlow, K., Hampton, R.Y., 2001. HRD4/NPL4 is required for the proteasomal processing of ubiquitinated ER proteins. Mol. Biol. Cell 12, 4114–4128.

Beuron, F., Flynn, T.C., Ma, J., Kondo, H., Zhang, X., Freemont, P.S., 2003. Motions and negative cooperativity between p97 domains revealed by cryo-electron microscopy and quantised elastic deformational model. J. Mol. Biol. 327, 619–629.

Biederer, T., Volkwein, C., Sommer, T., 1997. Role of Cue1p in ubiquitination and degradation at the ER surface. Science 278, 1806–1809.

Brunger, A.T., DeLaBarre, B., 2003. NSF and p97/VCP: similar at first, different at last. FEBS Lett. 555, 126–133.

Buchberger, A., Howard, M.J., Proctor, M., Bycroft, M., 2001. The UBX domain: a widespread ubiquitin-like module. J. Mol. Biol. 307, 17–24.

Burnett, B., Li, F., Pittman, R.N., 2003. The polyglutamine neurodegenerative protein ataxin-3 binds polyubiquitylated proteins and has ubiquitin protease activity. Hum. Mol. Genet. 12, 3195–3205.

Cao, K., Nakajima, R., Meyer, H.H., Zheng, Y., 2003. The AAA-ATPase Cdc48/p97 regulates spindle disassembly at the end of mitosis. Cell 115, 355–367.

Chow, M.K., Mackay, J.P., Whisstock, J.C., Scanlon, M.J., Bottomley, S.P., 2004. Structural and functional analysis of the Josephin domain of the polyglutamine protein ataxin-3. Biochem. Biophys. Res. Commun. 322, 387–394.

Dai, R.M., Chen, E., Longo, D.L., Gorbea, C.M., Li, C.C., 1998. Involvement of valosin-containing protein, an ATPase Co-purified with IkappaBalpha and 26 S proteasome, in ubiquitin–proteasome-mediated degradation of IkappaBalpha. J. Biol. Chem. 273, 3562–3573.

Dai, R.M., Li, C.C., 2001. Valosin-containing protein is a multi-ubiquitin chain-targeting factor required in ubiquitin–proteasome degradation. Nat. Cell Biol. 3, 740–744.

de Virgilio, M., Weninger, H., Ivessa, N.E., 1998. Ubiquitination is required for the retro-translocation of a short-lived luminal endoplasmic reticulum glycoprotein to the cytosol for degradation by the proteasome. J. Biol. Chem. 273, 9734–9743.

DeLaBarre, B., Brunger, A.T., 2003. Complete structure of p97/valosin-containing protein reveals communication between nucleotide domains. Nat. Struct. Biol. 10, 856–863.

DeLaBarre, B., Brunger, A.T., 2005. Nucleotide dependent motion and mechanism of action of p97/VCP. J. Mol. Biol. 347, 437–452.

Egerton, M., Ashe, O.R., Chen, D., Druker, B.J., Burgess, W.H., Samelson, L.E., 1992. VCP, the mammalian homolog of cdc48, is tyrosine phos-

phorylated in response to T cell antigen receptor activation. EMBO J. 11, 3533–3540.
Elsasser, S., Finley, D., 2005. Delivery of ubiquitinated substrates to protein-unfolding machines. Nat. Cell Biol. 7, 742–749.
Ficarro, S., Chertihin, O., Westbrook, V.A., White, F., Jayes, F., Kalab, P., Marto, J.A., Shabanowitz, J., Herr, J.C., Hunt, D.F., Visconti, P.E., 2003. Phosphoproteome analysis of capacitated human sperm. Evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-containing protein/p97 during capacitation. J. Biol. Chem. 278, 11579–11589.
Flierman, D., Ye, Y., Dai, M., Chau, V., Rapoport, T.A., 2003. Polyubiquitin serves as a recognition signal, rather than a ratcheting molecule, during retrotranslocation of proteins across the endoplasmic reticulum membrane. J. Biol. Chem. 278, 34774–34782.
Frohlich, K.U., Fries, H.W., Rudiger, M., Erdmann, R., Botstein, D., Mecke, D., 1991. Yeast cell cycle protein CDC48p shows full-length homology to the mammalian protein VCP and is a member of a protein family involved in secretion, peroxisome formation, and gene expression. J. Cell Biol. 114, 443–453.
Fu, X., Ng, C., Feng, D., Liang, C., 2003. Cdc48p is required for the cell cycle commitment point at Start via degradation of the G1-CDK inhibitor Far1p. J. Cell Biol. 163, 21–26.
Gerega, A., Rockel, B., Peters, J., Tamura, T., Baumeister, W., Zwickl, P., 2005. VAT, the thermoplasma homolog of mammalian p97/VCP, is an N domain-regulated protein unfoldase. J. Biol. Chem. 280, 42856–42862.
Ghislain, M., Dohmen, R.J., Levy, F., Varshavsky, A., 1996. Cdc48p interacts with Ufd3p, a WD repeat protein required for ubiquitin-mediated proteolysis in *Saccharomyces cerevisiae*. EMBO J. 15, 4884–4899.
Glover, J.R., Lindquist, S., 1998. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell 94, 73–82.
Golbik, R., Lupas, A.N., Koretke, K.K., Baumeister, W., Peters, J., 1999. The Janus face of the archaeal Cdc48/p97 homologue VAT: protein folding versus unfolding. Biol. Chem. 380, 1049–1062.
Hampton, R.Y., 2002. ER-associated degradation in protein quality control and cellular regulation. Curr. Opin. Cell Biol. 14, 476–482.
Hatakeyama, S., Matsumoto, M., Yada, M., Nakayama, K.I., 2004. Interaction of U-box-type ubiquitin-protein ligases (E3s) with molecular chaperones. Genes Cells 9, 533–548.
Hetzer, M., Meyer, H.H., Walther, T.C., Bilbao-Cortes, D., Warren, G., Mattaj, I.W., 2001. Distinct AAA-ATPase p97 complexes function in discrete steps of nuclear assembly. Nat. Cell Biol. 3, 1086–1091.
Hicke, L., Dunn, R., 2003. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annu. Rev. Cell Dev. Biol. 19, 141–172.
Higashiyama, H., Hirose, F., Yamaguchi, M., Inoue, Y.H., Fujikake, N., Matsukage, A., Kakizuka, A., 2002. Identification of ter94, Drosophila VCP, as a modulator of polyglutamine-induced neurodegeneration. Cell Death Differ. 9, 264–273.
Hitchcock, A.L., Krebber, H., Frietze, S., Lin, A., Latterich, M., Silver, P.A., 2001. The conserved npl4 protein complex mediates proteasome-dependent membrane-bound transcription factor activation. Mol. Biol. Cell 12, 3226–3241.
Hoege, C., Pfander, B., Moldovan, G.L., Pyrowolakis, G., Jentsch, S., 2002. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419, 135–141.
Hoppe, T., Matuschewski, K., Rape, M., Schlenker, S., Ulrich, H.D., Jentsch, S., 2000. Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. Cell 102, 577–586.
Huyton, T., Pye, V.E., Briggs, L.C., Flynn, T.C., Beuron, F., Kondo, H., Ma, J., Zhang, X., Freemont, P.S., 2003. The crystal structure of murine p97/VCP at 3.6 Å. J. Struct. Biol. 144, 337–348.
Ito, K., Akiyama, Y., 2005. Cellular functions, mechanism of action, and regulation of ftsh protease. Annu. Rev. Microbiol. 59, 211–231.
Jarosch, E., Geiss-Friedlander, R., Meusser, B., Walter, J., Sommer, T., 2002. Protein dislocation from the endoplasmic reticulum-pulling out the suspect. Traffic 3, 530–536.
Johnson, E.S., Ma, P.C., Ota, I.M., Varshavsky, A., 1995. A proteolytic pathway that recognizes ubiquitin as a degradation signal. J. Biol. Chem. 270, 17442–17456.
Kalies, K.U., Allan, S., Sergeyenko, T., Kroger, H., Romisch, K., 2005. The protein translocation channel binds proteasomes to the endoplasmic reticulum membrane. EMBO J. 24, 2284–2293.
Kikkert, M., Hassink, G., Barel, M., Hirsch, C., van der Wal, F.J., Wiertz, E., 2001. Ubiquitination is essential for human cytomegalovirus US11-mediated dislocation of MHC class I molecules from the endoplasmic reticulum to the cytosol. Biochem. J. 358, 369–377.
Koegl, M., Hoppe, T., Schlenker, S., Ulrich, H.D., Mayer, T.U., Jentsch, S., 1999. A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. Cell 96, 635–644.
Koller, K.J., Brownstein, M.J., 1987. Use of a cDNA clone to identify a supposed precursor protein containing valosin. Nature 325, 542–545.
Kondo, H., Rabouille, C., Newman, R., Levine, T.P., Pappin, D., Freemont, P., Warren, G., 1997. p47 is a cofactor for p97-mediated membrane fusion. Nature 388, 75–78.
Kostova, Z., Wolf, D.H., 2003. For whom the bell tolls: protein quality control of the endoplasmic reticulum and the ubiquitin–proteasome connection. EMBO J. 22, 2309–2317.
Latterich, M., Frohlich, K.U., Schekman, R., 1995. Membrane fusion and the cell cycle: Cdc48p participates in the fusion of ER membranes. Cell 82, 885–893.
Lee, R.J., Liu, C.W., Harty, C., McCracken, A.A., Latterich, M., Romisch, K., DeMartino, G.N., Thomas, P.J., Brodsky, J.L., 2004. Uncoupling retro-translocation and degradation in the ER-associated degradation of a soluble protein. EMBO J. 23, 2206–2215.
Lee, S., Sowa, M.E., Watanabe, Y.H., Sigler, P.B., Chiu, W., Yoshida, M., Tsai, F.T., 2003. The structure of ClpB: a molecular chaperone that rescues proteins from an aggregated state. Cell 115, 229–240.
Lilley, B.N., Ploegh, H.L., 2004. A membrane protein required for dislocation of misfolded proteins from the ER. Nature 429, 834–840.
Lilley, B.N., Ploegh, H.L., 2005. Multiprotein complexes that link dislocation, ubiquitination, and extraction of misfolded proteins from the endoplasmic reticulum membrane. Proc. Natl. Acad. Sci. USA 102, 14296–14301.
Livingstone, M., Ruan, H., Weiner, J., Clauser, K.R., Strack, P., Jin, S., Williams, A., Greulich, H., Gardner, J., Venere, M., et al., 2005. Valosin-containing protein phosphorylation at Ser784 in response to DNA damage. Cancer Res. 65, 7533–7540.
Madeo, F., Schlauer, J., Zischka, H., Mecke, D., Frohlich, K.U., 1998. Tyrosine phosphorylation regulates cell cycle-dependent nuclear localization of Cdc48p. Mol. Biol. Cell 9, 131–141.
Mao, Y., Senic-Matuglia, F., Di Fiore, P.P., Polo, S., Hodsdon, M.E., De Camilli, P., 2005. Deubiquitinating function of ataxin-3: insights from the solution structure of the Josephin domain. Proc. Natl. Acad. Sci. USA 102, 12700–12705.
Matsumoto, M., Yada, M., Hatakeyama, S., Ishimoto, H., Tanimura, T., Tsuji, S., Kakizuka, A., Kitagawa, M., Nakayama, K.I., 2004. Molecular clearance of ataxin-3 is regulated by a mammalian E4. EMBO J. 23, 659–669.
Mayer, A., Wickner, W., Haas, A., 1996. Sec18p (NSF)-driven release of Sec17p (alpha-SNAP) can precede docking and fusion of yeast vacuoles. Cell 85, 83–94.
McNew, J.A., Parlati, F., Fukuda, R., Johnston, R.J., Paz, K., Paumet, F., Sollner, T.H., Rothman, J.E., 2000. Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. Nature 407, 153–159.
Meusser, B., Hirsch, C., Jarosch, E., Sommer, T., 2005. ERAD: the long road to destruction. Nat. Cell Biol. 7, 766–772.
Meyer, H.H., 2005. Golgi reassembly after mitosis: the AAA family meets the ubiquitin family. Biochim. Biophys. Acta 1744, 481–492.
Meyer, H.H., Kondo, H., Warren, G., 1998. The p47 co-factor regulates the ATPase activity of the membrane fusion protein, p97. FEBS Lett. 437, 255–257.
Meyer, H.H., Shorter, J.G., Seemann, J., Pappin, D., Warren, G., 2000. A complex of mammalian ufd1 and npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. EMBO J. 19, 2181–2192.

Meyer, H.H., Wang, Y., Warren, G., 2002. Direct binding of ubiquitin conjugates by the mammalian p97 adaptor complexes, p47 and Ufd1-Npl4. EMBO J. 21, 5645–5652.

Moir, D., Stewart, S.E., Osmond, B.C., Botstein, D., 1982. Cold-sensitive cell-division-cycle mutants of yeast: isolation, properties, and pseudo-reversion studies. Genetics 100, 547–563.

Neuber, O., Jarosch, E., Volkwein, C., Walter, J., Sommer, T., 2005. Ubx2 links the Cdc48 complex to ER-associated protein degradation. Nat. Cell Biol. 7, 993–998.

Ogura, T., Wilkinson, A.J., 2001. AAA+ superfamily ATPases: common structure-diverse function. Genes Cells 6, 575–597.

Owen, D.J., Schiavo, G., 1999. A handle on NSF. Nat. Cell Biol. 1, E127–E128.

Park, S., Isaacson, R., Kim, H.T., Silver, P.A., Wagner, G., 2005. Ufd1 exhibits the AAA-ATPase fold with two distinct ubiquitin interaction sites. Structure (Camb) 13, 995–1005.

Patel, S.K., Indig, F.E., Olivieri, N., Levine, N.D., Latterich, M., 1998. Organelle membrane fusion: a novel function for the syntaxin homolog Ufe1p in ER membrane fusion. Cell 92, 611–620.

Peters, J.M., Walsh, M.J., Franke, W.W., 1990. An abundant and ubiquitous homo-oligomeric ring-shaped ATPase particle related to the putative vesicle fusion proteins Sec18p and NSF. EMBO J. 9, 1757–1767.

Pickart, C.M., Cohen, R.E., 2004. Proteasomes and their kin: proteases in the machine age. Nat. Rev. Mol. Cell Biol. 5, 177–187.

Rabouille, C., Kondo, H., Newman, R., Hui, N., Freemont, P., Warren, G., 1998. Syntaxin 5 is a common component of the NSF- and p97-mediated reassembly pathways of Golgi cisternae from mitotic Golgi fragments in vitro. Cell 92, 603–610.

Rabouille, C., Levine, T.P., Peters, J.M., Warren, G., 1995. An NSF-like ATPase, p97, and NSF mediate cisternal regrowth from mitotic Golgi fragments. Cell 82, 905–914.

Rape, M., Hoppe, T., Gorr, I., Kalocay, M., Richly, H., Jentsch, S., 2001. Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48(UFD1/NPL4), a ubiquitin-selective chaperone. Cell 107, 667–677.

Rape, M., Jentsch, S., 2004. Productive RUPture: activation of transcription factors by proteasomal processing. Biochim. Biophys. Acta 1695, 209–213.

Richly, H., Rape, M., Braun, S., Rumpf, S., Hoege, C., Jentsch, S., 2005. A series of ubiquitin binding factors connects CDC48/p97 to substrate multiubiquitylation and proteasomal targeting. Cell 120, 73–84.

Rouiller, I., DeLaBarre, B., May, A.P., Weis, W.I., Brunger, A.T., Milligan, R.A., Wilson-Kubalek, E.M., 2002. Conformational changes of the multifunction p97 AAA ATPase during its ATPase cycle. Nat. Struct. Biol. 9, 950–957.

Schuberth, C., Buchberger, A., 2005. Membrane-bound Ubx2 recruits Cdc48 to ubiquitin ligases and their substrates to ensure efficient ER-associated protein degradation. Nat. Cell Biol. 7, 999–1006.

Schuberth, C., Richly, H., Rumpf, S., Buchberger, A., 2004. Shp1 and Ubx2 are adaptors of Cdc48 involved in ubiquitin-dependent protein degradation. EMBO Rep. 5, 818–824.

Shamu, C.E., Flierman, D., Ploegh, H.L., Rapoport, T.A., Chau, V., 2001. Polyubiquitination is required for US11-dependent movement of MHC class I heavy chain from endoplasmic reticulum into cytosol. Mol. Biol. Cell 12, 2546–2555.

Shcherbik, N., Zoladek, T., Nickels, J.T., Haines, D.S., 2003. Rsp5p is required for ER bound Mga2p120 polyubiquitination and release of the processed/tethered transactivator Mga2p90. Curr. Biol. 13, 1227–1233.

Sollner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H., Rothman, J.E., 1993a. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75, 409–418.

Sollner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., Rothman, J.E., 1993b. SNAP receptors implicated in vesicle targeting and fusion. Nature 362, 318–324.

Thoms, S., 2002. Cdc48 can distinguish between native and non-native proteins in the absence of cofactors. FEBS Lett. 520, 107–110.

Tortorella, D., Gewurz, B.E., Furman, M.H., Schust, D.J., Ploegh, H.L., 2000. Viral subversion of the immune system. Annu. Rev. Immunol. 18, 861–926.

Tsai, B., Ye, Y., Rapoport, T.A., 2002. Retro-translocation of proteins from the endoplasmic reticulum into the cytosol. Nat. Rev. Mol. Cell Biol. 3, 246–255.

Uchiyama, K., Jokitalo, E., Kano, F., Murata, M., Zhang, X., Canas, B., Newman, R., Rabouille, C., Pappin, D., Freemont, P., Kondo, H., 2002. VCIP135, a novel essential factor for p97/p47-mediated membrane fusion, is required for Golgi and ER assembly in vivo. J. Cell Biol. 159, 855–866.

Uchiyama, K., Kondo, H., 2005. p97/p47-Mediated biogenesis of Golgi and ER. J. Biochem. (Tokyo) 137, 115–119.

Ulrich, H.D., 2004. How to activate a damage-tolerant polymerase: consequences of PCNA modifications by ubiquitin and SUMO. Cell Cycle 3, 15–18.

Walter, J., Urban, J., Volkwein, C., Sommer, T., 2001. Sec61p-independent degradation of the tail-anchored ER membrane protein Ubc6p. EMBO J. 20, 3124–3131.

Wang, Q., Song, C., Li, C.C., 2004a. Molecular perspectives on p97-VCP: progress in understanding its structure and diverse biological functions. J. Struct. Biol. 146, 44–57.

Wang, Q., Song, C., Yang, X., Li, C.C., 2003. D1 ring is stable and nucleotide-independent, whereas D2 ring undergoes major conformational changes during the ATPase cycle of p97-VCP. J. Biol. Chem. 278, 32784–32793.

Wang, Y., Satoh, A., Warren, G., Meyer, H.H., 2004b. VCIP135 acts as a deubiquitinating enzyme during p97-p47-mediated reassembly of mitotic Golgi fragments. J. Cell Biol. 164, 973–978.

Warren, G., 1993. Membrane partitioning during cell division. Annu. Rev. Biochem. 62, 323–348.

Warrick, J.M., Morabito, L.M., Bilen, J., Gordesky-Gold, B., Faust, L.Z., Paulson, H.L., Bonini, N.M., 2005. Ataxin-3 suppresses polyglutamine neurodegeneration in Drosophila by a ubiquitin-associated mechanism. Mol. Cell 18, 37–48.

Watts, G.D., Wymer, J., Kovach, M.J., Mehta, S.G., Mumm, S., Darvish, D., Pestronk, A., Whyte, M.P., Kimonis, V.E., 2004. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat. Genet. 36, 377–381.

Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B., Gmachl, M., Parlati, F., Sollner, T.H., Rothman, J.E., 1998. SNAREpins: minimal machinery for membrane fusion. Cell 92, 759–772.

Weibezahn, J., Tessarz, P., Schlieker, C., Zahn, R., Maglica, Z., Lee, S., Zentgraf, H., Weber-Ban, E.U., Dougan, D.A., Tsai, F.T., et al., 2004. Thermotolerance requires refolding of aggregated proteins by substrate translocation through the central pore of ClpB. Cell 119, 653–665.

Wiertz, E.J., Tortorella, D., Bogyo, M., Yu, J., Mothes, W., Jones, T.R., Rapoport, T.A., Ploegh, H.L., 1996. Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature 384, 432–438.

Yamada, T., Okuhara, K., Iwamatsu, A., Seo, H., Ohta, K., Shibata, T., Murofushi, H., 2000. p97 ATPase, an ATPase involved in membrane fusion, interacts with DNA unwinding factor (DUF) that functions in DNA replication. FEBS Lett. 466, 287–291.

Yamamoto, S., Tomita, Y., Hoshida, Y., Sakon, M., Kameyama, M., Imaoka, S., Sekimoto, M., Nakamori, S., Monden, M., Aozasa, K., 2004a. Expression of valosin-containing protein in colorectal carcinomas as a predictor for disease recurrence and prognosis. Clin. Cancer Res. 10, 651–657.

Yamamoto, S., Tomita, Y., Nakamori, S., Hoshida, Y., Iizuka, N., Okami, J., Nagano, H., Dono, K., Umeshita, K., Sakon, M., et al., 2004b. Valosin-containing protein (p97) and Ki-67 expression is a useful marker in detecting malignant behavior of pancreatic endocrine neoplasms. Oncology 66, 468–475.

Yamamoto, S., Tomita, Y., Uruno, T., Hoshida, Y., Qiu, Y., Iizuka, N., Nakamichi, I., Miyauchi, A., Aozasa, K., 2005. Increased expression of valosin-containing protein (p97) is correlated with disease recurrence in follicular thyroid cancer. Ann. Surg. Oncol. 12, 925–934.

Yamanaka, K., Okubo, Y., Suzaki, T., Ogura, T., 2004. Analysis of the two p97/VCP/Cdc48p proteins of Caenorhabditis elegans and their suppression of polyglutamine-induced protein aggregation. J. Struct. Biol. 146, 242–250.

Ye, Y., 2005. The role of the ubiquitin–proteasome system in ER quality control. Essays Biochem. 41, 99–112.

Ye, Y., Meyer, H.H., Rapoport, T.A., 2001. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. Nature 414, 652–656.

Ye, Y., Meyer, H.H., Rapoport, T.A., 2003. Function of the p97-Ufd1-Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains. J. Cell Biol. 162, 71–84.

Ye, Y., Shibata, Y., Kikkert, M., van Voorden, S., Wiertz, E., Rapoport, T.A., 2005. Inaugural article: recruitment of the p97 ATPase and ubiquitin ligases to the site of retrotranslocation at the endoplasmic reticulum membrane. Proc. Natl. Acad. Sci. USA 102, 14132–14138.

Ye, Y., Shibata, Y., Yun, C., Ron, D., Rapoport, T.A., 2004. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. Nature 429, 841–847.

Yuan, X., Simpson, P., McKeown, C., Kondo, H., Uchiyama, K., Wallis, R., Dreveny, I., Keetch, C., Zhang, X., Robinson, C., et al., 2004. Structure, dynamics and interactions of p47, a major adaptor of the AAA ATPase, p97. EMBO J. 23, 1463–1473.

Yuasa, T., Hayashi, T., Ikai, N., Katayama, T., Aoki, K., Obara, T., Toyoda, Y., Maruyama, T., Kitagawa, D., Takahashi, K., et al., 2004. An interactive gene network for securin-separase, condensin, cohesin, Dis1/Mtc1 and histones constructed by mass transformation. Genes Cells 9, 1069–1082.

Zhang, H., Wang, Q., Kajino, K., Greene, M.I., 2000a. VCP, a weak ATPase involved in multiple cellular events, interacts physically with BRCA1 in the nucleus of living cells. DNA Cell Biol. 19, 253–263.

Zhang, X., Shaw, A., Bates, P.A., Newman, R.H., Gowen, B., Orlova, E., Gorman, M.A., Kondo, H., Dokurno, P., Lally, J., et al., 2000b. Structure of the AAA ATPase p97. Mol. Cell 6, 1473–1484.

Zhong, X., Shen, Y., Ballar, P., Apostolou, A., Agami, R., Fang, S., 2004. AAA ATPase p97/valosin-containing protein interacts with gp78, a ubiquitin ligase for endoplasmic reticulum-associated degradation. J. Biol. Chem. 279, 45676–45684.