Current Biology, Vol. 12, R366–R371, May 14, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)00862-X

Cdc48–Ufd1–Npl4: Stuck in the Middle with Ub

Review

Nathan W. Bays[1] and Randolph Y. Hampton[2]

[1]Exelixis, Inc., 170 Harbor Way, South San Francisco, California 94080, USA. E-mail: nbays@exelixis.com

[2]Division of Biology, Section of Cell and Developmental Biology, University of California, San Diego, 9500 Gilman Drive, 0347, La Jolla, California 92093-0347, USA. E-mail: rhampton@ucsd.edu

The ubiquitin–proteasome pathway has a well-defined beginning and end. Target proteins are initially recognized by upstream components and tagged with polyubiquitin chains. The 26S proteasome then degrades these polyubiquitinated proteins. Until recently, it was not known what, if any, steps occurred between the initial polyubiquitination of target proteins and their final degradation. Several new papers investigating the function of the Cdc48–Ufd1–Npl4 complex indicate that there is indeed a middle to the ubiquitin–proteasome pathway. The Cdc48–Ufd1–Npl4 complex functions in the recognition of several polyubiquitin-tagged proteins and facilitates their presentation to the 26S proteasome for processive degradation or even more specific processing. The elucidation of Cdc48, Ufd1 and Npl4 action not only provides long-sought functions for these specific proteins, but illuminates a poorly understood part of the ubiquitin–proteasome pathway.

Introduction

Geneticists hunt for mutants by casting very large nets, and it is often not obvious which genes will ultimately prove to be prize-winning catches, nor who has been fishing in the same waters. *NPL4* and *CDC48* were netted in separate expeditions to understand nuclear transport and the cell cycle. However, recent studies have shown these two distinct trophies to be intimately involved in a poorly understood part of the ubiquitin–proteasome pathway of degradation. In this pathway, multiple copies of the small protein (8 kDa) ubiquitin are covalently attached to a target protein, creating a polyubiquitin chain that serves as a signal for processive degradation, or even specific cleavage, by the 26S proteasome. Although the machinery of ubiquitination and the 26S proteasome are under intense scrutiny [1–3], much less is known about what happens *in vivo* between the actual ubiquitination of a protein and its processing by the 26S proteasome.

From elegant *in vitro* experiments, it is clear that highly purified 26S proteasomes can alone recognize ubiquitinated proteins and degrade them [4–6]. But is this sufficient *in vivo*? In the cell, there are many possible impediments to 26S degradation of ubiquitinated substrates. These can include removal from membranes or compartments, dissociation from multiprotein complexes, or disassembly of a targeted substrates. This review discusses recent studies that suggest there can be a 'middle' to the ubiquitin–proteasome pathway and that the proteins coded by *NPL4, UFD1* and *CDC48* participate in this stage of the pathway by recognizing a variety of ubiquitinated proteins and presenting them to the 26S proteasome.

NPL4: Out of the Pore House, and into the ER

NPL4 is an essential gene that was first identified in a selection for mutants defective in nuclear import [7]. At a restrictive temperature, *npl4* mutants fail to import proteins bearing a nuclear localization sequence (NLS). They have gross defects in nuclear morphology and membrane herniations similar to those seen when some actual pore proteins, such as Nup116, are missing [7,8]. Although these phenotypes are consistent with a role in nuclear transport, Npl4 has never been identified as a *bona fide* component of the nuclear pore despite exhaustive and highly effective efforts to identify all components of this complex in yeast [9–12]. Recent data from an entirely different genetic screen clearly implicates Npl4 in the ubiquitin–proteasome degradation pathway.

In isolating *hrd* mutants that fail to degrade the enzyme HMG-CoA reductase, Bays *et al.* [13] found an allele of *NPL4*, *hrd4-1,* broadly deficient in ER-associated degradation, stabilizing both misfolded and normal proteins degraded by this pathway. The original *npl4-1* and *npl4-2* alleles isolated in the *npl* selection were also strongly deficient in ER-associated degradation at a temperature permissive for nuclear import and export, suggesting that a defect in ER-associated degradation was a primary phenotype in *npl4/hrd4* mutants [13], and not a secondary result of deficient nuclear transport. This was further supported by the observation that ER-associated degradation proceeds normally when nuclear import and export are blocked in a *nup116*Δ mutant — a null in a gene encoding an actual component of the pore with nuclear phenotypes strikingly similar to those of *npl4* mutants [7,8,13]. As it had been previously noted that deficiencies in the ubiquitin–proteasome pathway can compromise nuclear import and export [14,15], the finding that *npl4/hrd4* mutant cells suffer primarily from a block in ubiquitin-mediated degradation was not completely shocking.

The ER-associated degradation defect in *npl4* mutants is quite general. The *npl4* defect crosses several ubiquitin ligase boundaries in stabilizing both misfolded and regulated proteins. In order to determine where Npl4/Hrd4 acts in the ubiquitin-proteasome pathway, ubiquitination of HMG-CoA reductase (HMGR) was assayed in *hrd4-1* mutant cells. In these strains, HMGR is fully ubiquitinated, consistent with the defect being downstream of ubiquitination [13]. Although this would be consistent with a defect in

proteasome function, Npl4 has never been suggested to be a proteasome subunit. And general proteasome function is not deficient in *npl4* mutant strains as shown using several cytosolic test substrates [13].

The study of *npl4* and *hrd4* mutants makes two important points about Npl4 function. First, Npl4 shows an 'ER-centric' behavior in that it is broadly required for ER protein degradation, but does not seem to affect cytosolic protein degradation [13]. (Loss of Npl4 also induces an unfolded protein response [13].) Second, Npl4 appears to act at a post-ubiquitination but pre-proteasome step in ER-associated degradation as the ER membrane protein HMGR is fully ubiquitinated but not degraded in *npl4/hrd4* mutant cells. Npl4/Hrd4 therefore represents a previously uncharacterized step in ubiquitin-mediated protein degradation at the ER. The presence of this intermediate step is further indicated by study of the Cdc48/p97 protein and its binding partners.

Cdc48 Finally Finds a Function Free from Fusion

The AAA ATPase Cdc48 — also known as p97 or Valosin-containing protein, VCP — has been implicated in an even wider variety of cellular processes than Npl4, but like Npl4, recent data suggest that *cdc48* phenotypes are caused by a defect in the ubiquitin–proteasome pathway, and specifically at a step after ubiquitination. It has been known for some time that *CDC48* is required for the ubiquitin-mediated degradation of several short-lived proteins including N-end rule substrates and IκBα [16,17]. In the study of IκBα degradation and a large-scale study to identify proteasome-interacting proteins, Cdc48 was found to associate physically with the 26S proteasome in an ATP-dependent manner [17,18]. This raised the intriguing possibility that Cdc48 may act in the presentation of at least certain substrates to the 26S proteasome. Recent data support this idea and suggest that a Cdc48 complex acts broadly in the recognition and presentation of ubiquitinated proteins to the 26S proteasome.

Strikingly, Cdc48 can selectively recognize ubiquitinated proteins. In one study Dai and Li [19] find that several polyubiquitinated proteins copurify with VCP, the mammalian homologue of Cdc48, when tagged versions of VCP are affinity-purified from whole cell lysates. This copurification of polyubiquitinated proteins is inhibited by the addition of increasing amounts of tetra-ubiquitin chains. VCP preferentially binds tetra-ubiquitin chains in an ATP-dependent manner, with little affinity for mono-, di-, and tri-ubiquitin molecules *in vitro* [19]. This preference is especially interesting as tetra-ubiquitin is apparently a preferential unit in the recognition of polyubiquitin chains, and thus would be consistent with Cdc48 as a polyubiquitin chain binding protein [20,21].

The relevance of Cdc48's ability to recognize polyubiquitinated proteins is revealed by studies of virally-induced ER protein degradation. In an attempt to escape detection by the immune system, the cytomegalovirus (CMV) gene product US11 orchestrates retrograde translocation of MHC class I heavy chain proteins from the ER to the cytosol for rapid

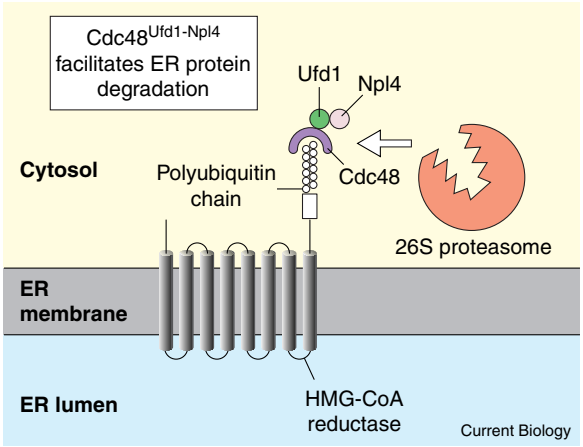

Figure 1. The Cdc48–Ufd1–Npl4 complex acts in ER-associated protein degradation.

Here, the ER membrane protein HMG-CoA reductase is tagged for proteasomal degradation by the addition of polyubiquitin chain(s). A Cdc48p–Ufd1p–Npl4p complex is then required for degradation by the 26S proteasome. A deficiency in any complex member results in HMG-CoA reductase or other ER degradation substrate remaining fully ubiquitinated but unable to be degraded by a fully functional 26S proteasome.

degradation by the 26S proteasome [22,23]. In a recent paper, Ye *et al.* [24] show that MHC retrotranslocation requires the mammalian Cdc48 homologue, p97. In semi-permeabilized cells expressing US11, addition of a dominant-negative version of p97 mutated in its conserved ATPase domain interferes with MHC retrotranslocation while an equal amount of wild-type p97 added to this *in vitro* system has no effect. This lack of wild-type effect may be due to the need for adding p97 complexes that include mammalian Npl4 and Ufd1 as well (see below).

If proteasome activity is inhibited in US11-expressing or CMV-infected cells, ubiquitinated heavy chain proteins can be found in the cytosol as degradation fails to follow retrotranslocation [23]. Importantly, Ye *et al.* [24] showed this was not the case when a dominant-negative p97 protein was present in the permeabilized assay. The p97 dominant-negative resulted in accumulation of ubiquitinated heavy chain proteins in a microsomal cell fraction rather than a soluble, cytosolic fraction as occurred when an equal amount of wild-type p97 was added under the same assay conditions [24]. Thus, blocking Cdc48/p97 activity had no apparent effect on the actual ubiquitination of heavy chain proteins, just as blocking Npl4 function in yeast also left ER-localized proteins fully ubiquitinated. In the case of US11-induced heavy chain degradation, this block occurs at the actual step of retrotranslocation of heavy chain proteins to the cytosol.

Unfortunately, no dominant-negative mutants of Npl4 are currently available to test whether blocking Npl4 activity in the Ye *et al.* experiments would also block retrotranslocation of heavy chain proteins, but there is very good reason to suspect that Npl4 acts with Cdc48 in US11-mediated retrotranslocation — with the first hints of guilt occurring by association. Cdc48 physically associates with an impressive array of proteins in the

cell, and one Cdc48 complex is composed of Cdc48, Npl4 and Ufd1. Both Bays *et al.* [13] and Ye *et al.* [24] report that each member of this complex is required for ER-associated degradation and loss of each one results in a broad defect in this process — even at each mutant's permissive temperature (all three genes are essential). A requirement for Cdc48 in ER-associated degradation has also been described by Rabinovich *et al.* [25].

Experiments in the Ye *et al.* paper [24] suggest that the entire Cdc48–Ufd1–Npl4 complex does indeed act at the same step in ER-associated degradation (illustrated in Figure 1). These experiments exploit the observation that the protein p47 competes with Ufd1–Npl4 for binding of Cdc48. When Ye *et al.* [24] added excess p47 to permeabilized cells to displace Ufd1–Npl4, retrotranslocation of heavy chains was blocked, consistent with a requirement for Npl4–Ufd1 binding to Cdc48 in this action. Unfortunately, p47 is not a benign Cdc48 binding partner, and p47 even decreases Cdc48 ATPase activity — an activity required for Cdc48 to function in heavy chain retrotranslocation.

An even more recent paper by Jarosch *et al.* [26] studying the mutant, misfolded form of carboxypeptidase Y, CPY*, shows that loss of Ufd1 also blocks ER-associated degradation at a post-ubiquitination but pre-proteasome step. In order to be degraded by the cytosolic 26S proteasome, CPY* must be retrotranslocated from the ER lumen to the cytosol. Jarosch *et al.* [26] show that polyubiquitination by the ubiquitin-protein ligase Hrd1 and its associated ubiquitin-conjugating enzymes Ubc7 and Ubc1 is required for this retrotranslocation. While polyubiquitination of CPY* is blocked by *hrd1*Δ or *ubc1*Δ/ *ubc7*Δ mutations, CPY* is fully ubiquitinated in wild-type and *ufd1-1* mutant cells.

Intriguingly, CPY* is also fully ubiquitinated in proteasome mutant strains, but in these strains ubiquitinated CPY* can be found in both cytosolic and ER membrane fractions showing that a fraction of CPY* is being retrotranslocated but not degraded when proteasome activity is compromised. This differs from wild-type and *ufd1-1* strains where polyubiquitinated CPY* is found exclusively in ER membrane fractions. Therefore, *ufd1-1* and proteasome mutant strains are blocked in CPY* degradation at a step following ubiquitination but only *ufd1-1* strains are blocked in CPY* retrotranslocation, consistent with a role for the Cdc48–Ufd1–Npl4 complex in the retrotranslocation of substrates for ER-associated degradation.

Cdc48–Ufd1–Npl4 Picks at the Remains of a Transcription Factor

The model that the entire Cdc48–Ufd1–Npl4 complex acts in the ubiquitin–proteasome pathway is further supported by studies of a novel role for the ubiquitin–proteasome pathway in the processing of an ER-bound transcription factor. The activities of transcription factors are regulated in diverse ways. One of the more recently discovered modes involves the regulated cleavage of transcription factors from transmembrane anchors, allowing the active transcription factor domain to enter the nucleus and regulate gene expression. The first such transcription factor to be characterized was SREBP (sterol regulatory element binding protein), required for transcription of sterol synthetic genes. SREBP is cleaved by the action of specific proteases to release a soluble, active transcription factor [27,28].

Recent work in the Jentsch and Silver labs has shown that the 26S proteasome can also participate in the regulated cleavage of transcription factors from their transmembrane anchors. Hoppe *et al.* [29] and Hitchcock *et al.* [30] show that the Cdc48–Ufd1–Npl4 complex plays a central role in this proteasome-dependent processing. Both of these studies [29,30] investigated the mechanism of Spt23 and Mga2 regulation. These two transcription factors are synthesized as integral ER membrane proteins and are required for the transcription of *OLE1* mRNAs coding for Ole1, an essential Δ9-fatty acid desaturase in *Saccharomyces cerevisiae* [30,31]. In order to activate transcription of *OLE1*, the transcriptionally active portions of Spt23 and Mga2 must be cleaved from their transmembrane domains. This cleavage requires ubiquitination and the 26S proteasome [29].

Hitchcock *et al.* [30] reported that *CDC48, UFD1* and *NPL4* are each required for the efficient processing of Spt23 and Mga2. When *cdc48, ufd1* and *npl4* mutant cells are shifted to a restrictive temperature, Spt23 and Mga2 are less efficiently cleaved from their transmembrane domains and the majority of Spt23 and Mga2 remains as full-length protein at the ER [30]. Consistent with a deficiency in processing, these same mutants show reduced expression of *OLE1* mRNA [30].

In a related paper, Rape *et al.* [33] study the role of Cdc48–Ufd1–Npl4 in Spt23 and Mga2 processing, but focus on events that occur after the proteasomal cleavage of Spt23 and Mga2. They show that when Spt23 is cleaved by the 26S proteasome, the active transcription factor domain (p90) can remain physically associated with the larger, unprocessed p120. Their studies implicate the Cdc48–Ufd1–Npl4 complex in the dissociation of processed p90 from the membrane anchored p120. When membrane fractions were isolated from yeast cells, both p120 and p90 were detected, as p90 remains associated with p120. Addition of Cdc48-expressing insect cell extracts caused the release of p90 from p120 and its appearance in the soluble fraction. Insect cell extract lacking Cdc48 did not cause this same mobilization of p90. Furthermore, addition of apyrase (to remove ATP) and NEM (to inhibit Cdc48 activity) blocked the ability of Cdc48-containing extract to mobilize p90 [33,34].

When p120 and p90 membrane fractions were isolated from *npl4-1* cells, Cdc48 was not able to cause the dissociation of p90 from p120 despite the lower levels of p90 produced due to the less efficient cleavage of p120 in *npl4-1* strains. From these *in vitro* studies, it appears that Cdc48 is indeed acting as a chaperone in dismantling the protein–protein interaction between p90 and p120. What is striking about this Cdc48 chaperone action is that Cdc48 selectively

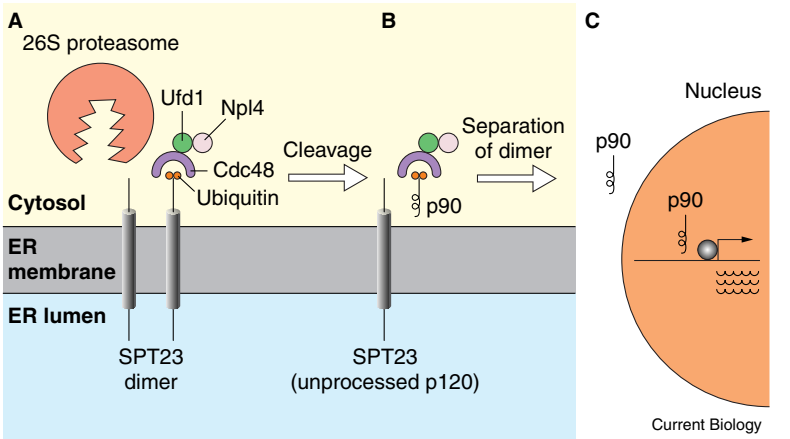

Figure 2. The Cdc48–Ufd1–Npl4p complex acts at several stages of transcription factor Spt23 processing.
(A) The complex is first required for the proteasome-dependent cleavage of the Spt23 transcription factor domain (p90) from its transmembrane anchor. (B) After cleavage, p90 remains associated with full-length Spt23p (p120). (C) Cdc48p-Ufd1p-Npl4p is also required for the separation of this dimer, allowing p90 to enter the nucleus and promote the transcription of OLE1 mRNA.

binds the ubiquitinated protein in this complex. The term 'segregase' has been suggested for this action of Cdc48 [35].

To characterize processing more fully, Rape *et al.* [33] compared p90 generated naturally or from a coding region lacking the transmembrane region and only expressing the processed p90 fragment, referred to as Spt23pΔTM. They found that p90 derived from full-length Spt23 is ubiquitinated whereas the independently produced p90 is not. Thus, the ubiquitination of p90 comes about by its release from full-length Spt23. Importantly, only the ubiquitinated p90 physically associates with Cdc48 while nonubiquitinated Spt23pΔTM does not. Rape *et al.* [33] also showed that Cdc48 associates with the unstable and ubiquitinated N-end rule/ubiquitin fusion degradation (UFD) pathway substrate Ub–Pro-β galactosidase while Cdc48 does not associate with the stable and nonubiquitinated Ub–Met-β galactosidase. When added to other data in the paper, these results strongly suggest that Cdc48 can act as a chaperone with specificity for ubiquitinated proteins.

A comparison of the Hitchcock *et al.* [30] and Rape *et al.* [33] papers illustrates important features of the Cdc48–Ufd1–Npl4 complex. These two papers join Bays *et al.* [13] and Ye *et al.* [24] in showing instances where loss of function in the Cdc48–Ufd1–Npl4 complex results in ER proteins being fully ubiquitinated yet unprocessed by the 26S proteasome. Hitchcock [30] and Rape [33] both show the accumulation of ubiquitinated but unprocessed p120 in *cdc48*, *npl4*, and *ufd1* mutants. Likewise, Bays *et al.,* [13] Ye *et al.* [24] and Jarosch *et al.* [26] show ubiquitinated HMG-CoA reductase, ubiquitinated MHC heavy chains, and ubiquitinated CPY* are all stuck at the ER membrane, escaping the processive degradation and even retrotranslocation seen in wild-type cells.

The Hitchcock [30] and Rape [33] papers also emphasize two different functions for the Cdc48–Ufd1–Npl4 complex in the same OLE pathway (Figure 2). Hitchcock *et al.* [30] show that the efficiency of Spt23 and Mga2 processing is reduced when components of the Cdc48–Ufd1–Npl4 complex are compromised. Because cells must produce *OLE1* transcript in order to live, processing is not completely absent in the *cdc48, npl4* and *ufd1* mutants tested, but it is clearly less efficient. Rape *et al.* [33] show that the Cdc48–Ufd1–Npl4 complex is not just required for processing of Spt23, but that the complex is also required to separate the processed p90 protein from the p120 protein — a process that does *not* require the 26S proteasome. Here, in the same pathway, there are different uses for the ability of Cdc48–Ufd1–Npl4 to recognize selectively ubiquitinated proteins, and at least in one case to act as a molecular chaperone.

The study of the OLE pathway also provides yet another example of Cdc48–Ufd1–Npl4 action in ER-associated degradation. In an additional Jentsch lab paper, Braun *et al.* [35] show that the membrane bound Ole1 protein itself is unstable and undergoes ER-associated degradation. Degradation of Ole1 requires each member of the Cdc48–Ufd1–Npl4 complex: *cdc48, ufd1* and *npl4* mutants were each deficient in the degradation of Ole1.

Cdc48–Ufd1–Npl4 : Resolving Con-Fusion About Mutants

These recent data regarding the function of the Cdc48–Ufd1–Npl4 complex serve to make sense of diverse and seemingly disparate phenotypes, especially those originating from *cdc48* and *npl4* mutants. They also present compelling avenues of investigation. It is now a reasonable hypothesis that all *npl4* and *cdc48* phenotypes involve, somehow, loss of function in ubiquitin- or proteasome-mediated pathways. Therefore, processes involving the Cdc48–Ufd1–Npl4 complex, but with no known ubiquitin/proteasome connection, now elicit an investigation into whether ubiquitin/proteasome is involved. One example of such a process is membrane fusion. Cdc48 has been implicated for some time in homotypic ER membrane fusion [36] and Golgi vesicle fusion [37,38], but its role has not been extensively characterized. A recent report by Hetzer *et al.* [39] shows that Cdc48–Ufd1–Npl4 is required for membrane fusion, specifically to form a closed nuclear envelope at the end of mitosis in larger eukaryotes. Given this requirement for Cdc48–Ufd1–Npl4, it may be informative to seek directly roles for ubiquitin/proteasome in nuclear envelope formation specifically and in membrane fusion generally.

Investigation of other processes requiring a functional Cdc48–Ufd1–Npl4 complex may well begin with models involving ubiquitin/proteasome. For instance, the mammalian homologue of Cdc48/VCP is currently being studied in the case of neural degeneration caused by polyglutamine-containing proteins. In work by the Kakizuka lab [40,41], VCP has been implicated as a factor that modifies the progress of this important and general pathway of neural death. Several models implicate the ubiquitin–proteasome pathway in the dying efforts of a neural cell to clear damaged proteins, and Cdc48/VCP may further strengthen this connection.

New structural observations suggest an alternative to the 'ubiquitin only' interpretation for some functions of the Cdc48 complex. As mentioned earlier, p47 is a binding partner of Cdc48 and appears to act with Cdc48p in mediating certain membrane dynamics. The carboxy-terminal portion of p47 has a ubiquitin-like UBX domain. NMR studies now indicate that, despite the limited homology to ubiquitin (~14% identity), the tertiary structure of the p47 UBX domain is essentially superimposable with authentic ubiquitin [42]. This raises the intriguing possibility that p47 substitutes partially or completely for ubiquitin in some actions of the Cdc48 complex, allowing the same nanomechanical actions to be performed on molecules that are not ubiquitinated, but can interact with this clever 'p47-in-ubiquitin's clothing'.

Further Study of the Cdc48–Ufd1–Npl4 Complex

Far more mechanistic information has been characterized for Cdc48 than for Npl4 or Ufd1. Data from the papers discussed here and elsewhere indicate that Cdc48 can act as an ATP-dependent chaperone, and Cdc48 can bind many different proteins — several of which are known to modulate directly its function [16,43–46]. Not much more is known about Ufd1 and Npl4 other than they are part of the long list of Cdc48-binding proteins and that they are both required for several ubiquitin- or proteasome-mediated processes.

To complicate the study of Cdc48–Ufd1–Npl4 further, the substrate range for Cdc48, Ufd1 and Npl4p implies that the three proteins do not always act together in ubiquitin-mediated protein degradation. While Cdc48 is required for the degradation of both N-end rule and UFD pathway substrates [16], Ufd1 is only required for the degradation of UFD substrates and not N-end rule substrates [47]. Npl4 is required for neither group of substrates [13]. (One experiment in Rape *et al.* [33] does show stabilization of Ub–Pro-β galactosidase in a *npl4-1* strain but the chase was performed after incubation at the restrictive temperature. The experiments in Bays *et al.* [13] testing cytosolic N-end rule, UFD and Deg1 substrates were all performed at the permissive temperature where *npl4/hrd4* mutants show a strong block in ER-associated degradation.) Discovering exactly how Ufd1 and Npl4 affect Cdc48 function will undoubtedly become a major focus in future studies of Cdc48–Ufd1–Npl4 function.

Cdc48–Ufd1–Npl4 has broader implications for the nature of the ubiquitin–proteasome system. The phenotypes resulting from loss of this complex strongly suggest that there can be intermediate steps following ubiquitination that must take place in order for a ubiquitinated protein to be recognized and processed by the 26S proteasome. Now studies will focus on how general the requirement for an intermediate step is, what events are actually occurring in such intermediate steps, and how many different proteins can act as an intermediary between ubiquitinated proteins and the 26S proteasome. All of these studies will produce exciting and illuminating insights into the ubiquitin–proteasome pathway.

References

1. Haas, A.L. and Siepmann, T.J. (1997). Pathways of ubiquitin conjugation. FASEB J. *11,* 1257–1268.
2. Zwickl, P., Voges, D. and Baumeister, W. (1999). The proteasome: a macromolecular assembly designed for controlled proteolysis. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *354,* 1501–1511.
3. Weissman, A.M. (2001). Themes and variations on ubiquitylation. Nat. Rev. Mol. Cell Biol. *2,* 169–178.
4. van Nocker, S., Sadis, S., Rubin, D.M., Glickman, M., Fu, H., Coux, O., Wefes, I., Finley, D. and Viestra, R.D. (1996). The multiubiquitin-chain-binding protein Mcb1 is a component of the 26S proteasome in *Saccharomyces cerevisiae* and plays a nonessential, substrate-specific role in protein turnover. Mol. Cell. Biol. *16,* 6020–6028.
5. Fu, H., Sadis, S., Rubin, D.M., Glickman, M., Van Nocker, S., Finley, D. and Vierstra, R.D. (1998). Multiubiquitin chain binding and protein degradation are mediated by distinct domains within the 26 S proteasome subunit Mcb1. J. Biol. Chem. *273,* 1970–1981.
6. Ugai, S., Tamura, T., Tanahashi, N., Takai, S., Komi, N., Chung, C.H., Tanaka, K. and Ichihara, A. (1993). Purification and characterization of the 26S proteasome complex catalyzing ATP-dependent breakdown of ubiquitin-ligated proteins from rat liver. J. Biochem. (Tokyo). *113,* 754–768.
7. DeHoratius, C. and Silver, P.A. (1996). Nuclear transport defects and nuclear envelope alterations are associated with mutation of the *Saccharomyces cerevisiae NPL4* gene. Mol. Biol. Cell *7,* 1835–1855.
8. Wente, S.R. and Blobel, G. (1993). A temperature-sensitive *NUP116* null mutant forms a nuclear envelope seal over the yeast nuclear pore complex thereby blocking nucleocytoplasmic traffic. J. Cell Biol. *123,* 275–284.
9. Rout, M.P., Aitchison, J.D., Suprapto, A., Hjertaas, K., Zhao, Y. and Chait, B.T. (2000). The yeast nuclear pore complex: composition, architecture, and transport mechanism. J. Cell Biol. *148,* 635–651.
10. Corbett, A., Ferrigno, P., Henry, M., Kahana, J., Koepp, D., Lee, M., Nguyen, L., Schlenstedt, G., Seedorf, M., Shen, E., Taura, T., Wong, D. and Silver, P. (1996). Genetic analysis of macromolecular transport across the nuclear envelope. Exp. Cell Res. *229,* 212–216.
11. Ryan, K.J. and Wente, S.R. (2000). The nuclear pore complex: a protein machine bridging the nucleus and cytoplasm. Curr. Opin. Cell Biol. *12,* 361–371.
12. Vasu, S.K. and Forbes, D.J. (2001). Nuclear pores and nuclear assembly. Curr. Opin. Cell Biol. *13,* 363–375.
13. Bays, N.W., Wilhovsky, S.K., Goradia, A., Hodgkiss-Harlow, K. and Hampton, R.Y. (2001). *HRD4/NPL4* Is required for the proteasomal processing of ubiquitinated ER proteins. Mol. Biol. Cell *12,* 4114–4128.
14. Yokota, K.Y., Kagawa, S., Shimizu, Y., Akioka, H., Tsurumi, C., Noda, C., Fujimuro, M., Yokosawa, H., Fujiwara, T., Takahashi, E.I., *et al.* (1996). cDNA cloning of p112, the largest regulatory subunit of the human 26S proteasome, and functional analysis of its yeast homologue, Sen3p. Mol. Biol. Cell *7,* 853–870.
15. Utsugi, T., Hirata, A., Sekiguchi, Y., Sasaki, T., Toh-e, A. and Kikuchi, Y. (1999). Yeast *tom1* mutant exhibits pleiotropic defects in nuclear division, maintenance of nuclear structure and nucleocytoplasmic transport at high temperatures. Gene *234,* 285–295.
16. Ghislain, M., Dohmen, R.J., Levy, F. and Varshavsky, A. (1996). Cdc48p interacts with Ufd3p, a WD repeat protein required for ubiquitin-mediated proteolysis in *Saccharomyces cerevisiae.* EMBO J. *15,* 4884–4899.
17. Dai, R.M., Chen, E., Longo, D.L., Gorbea, C.M. and Li, C.C.H. (1998). Involvement of valosin-containing protein, an ATPase co-purified with I-kappa-B-alpha and 26 S proteasome, in ubiquitin-proteasome-mediated degradation of I-kappa-B-alpha. J. Biol. Chem. *273,* 3562–3573.
18. Verma, R., Chen, S., Feldman, R., Schieltz, D., Yates, J., Dohmen, J. and Deshaies, R.J. (2000). Proteasomal proteomics: identification of nucleotide-sensitive proteasome-interacting proteins by mass spectrometric analysis of affinity-purified proteasomes. Mol. Biol. Cell *11,* 3425–3439.

19. Dai, R.M. and 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.
20. Thrower, J.S., Hoffman, L., Rechsteiner, M. and Pickart, C.M. (2000). Recognition of the polyubiquitin proteolytic signal. EMBO J. *19,* 94–102.
21. Beal, R.E., Toscano-Cantaffa, D., Young, P., Rechsteiner, M. and Pickart, C.M. (1998). The hydrophobic effect contributes to polyubiquitin chain recognition. Biochemistry (Mosc). *37,* 2925–2934.
22. Wiertz, E.J.H.J., Jones, T.R., Sun, L., Bogyo, M., Geuze, H.J. and Ploegh, H.L. (1996). The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. Cell *84,* 769–779.
23. Shamu, C.E., Story, C.M., Rapoport, T.A. and Ploegh, H.L. (1999). The pathway of US11-dependent degradation of MHC class I heavy chains involves a ubiquitin-conjugated intermediate. J. Cell Biol. *147,* 45–58.
24. Ye, Y., Meyer, H.H. and Rapoport, T.A. (2001). The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. Nature *414,* 652–656.
25. Rabinovich, E., Kerem, A., Frohlich, K.U., Diamant, N. and Bar-Nun, S. (2002). AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. Mol. Cell. Biol. *22,* 626–634.
26. Jarosch, E., Taxis, C., Volkwein, C., Bordallo, J., Finley, D., Wolf, D.H., and Sommer, T. (2002) Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48. Nat Cell Biol. *4,* 134-139.
27. Hua, X., Nohturfft, A., Goldstein, J.L. and Brown, M.S. (1996). Sterol resistance in CHO cells traced to point mutation in SREBP cleavage-activating protein. Cell *87,* 415-426.
28. Hampton, R.Y. (2000). Cholesterol homeostasis: ESCAPe from the ER. Curr. Biol. *10,* R298–R301.
29. Hoppe, T., Matuschewski, K., Rape, M., Schlenker, S., Ulrich, H.D. and Jentsch, S. (2000). Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. Cell *102,* 577–586.
30. Hitchcock, A.L., Krebber, H., Frietze, S., Lin, A., Latterich, M. and Silver, P.A. (2001). The conserved Npl4 protein complex mediates proteasome-dependent membrane-bound transcription factor activation. Mol. Biol. Cell *12,* 3226–3241.
31. Zhang, S., Skalsky, Y. and Garfinkel, D.J. (1999). MGA2 or SPT23 is required for transcription of the delta9 fatty acid desaturase gene, OLE1, and nuclear membrane integrity in Saccharomyces cerevisiae. Genetics *151,* 473–483.
32. Stukey, J.E., McDonough, V.M. and Martin, C.E. (1990). The *OLE1* gene of *Saccharomyces cerevisiae* encodes the delta 9 fatty acid desaturase and can be functionally replaced by the rat stearoyl-CoA desaturase gene. J. Biol. Chem. *265,* 20144–20149.
33. Rape, M., Hoppe, T., Gorr, I., Kalocay, M., Richly, H. and Jentsch, S. (2001). Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48(UFD1/NPL4), a ubiquitin-selective chaperone. Cell *107,* 667–677.
34. Frohlich, K.U., Fries, H.W., Peters, J.M. and Mecke, D. (1995). The ATPase activity of purified CDC48p from Saccharomyces cerevisiae shows complex dependence on ATP-, ADP- and NADH-concentrations and is completely inhibited by NEM. Biochim. Biophys. Acta *1253,* 25–32.
35. Braun, S., Matuschewski, K., Rape, M., Thoms, S. and Jentsch, S. (2002). Role of the ubiquitin-selective CDC48(UFD1/NPL4)chaperone (segregase) in ERAD of OLE1 and other substrates. EMBO J. *21,* 615–621.
36. Latterich, M., Frohlich, K.U. and Schekman, R. (1995). Membrane fusion and the cell cycle: Cdc48p participates in the fusion of ER membranes. Cell *82,* 885–893.
37. Acharya, U., Jacobs, R., Peters, J.M., Watson, N., Farquhar, M.G. and Malhotra, V. (1995). The formation of Golgi stacks from vesiculated Golgi membranes requires two distinct fusion events. Cell *82,* 895–904.
38. Rabouille, C., Levine, T.P., Peters, J.M. and Warren, G. (1995). An NSF-like ATPase, p97, and NSF mediate cisternal regrowth from mitotic Golgi fragments. Cell *82,* 905–914.
39. Hetzer, M., Meyer, H.H., Walther, T.C., Bilbao-Cortes, D., Warren, G. and Mattaj, I.W. (2001). Distinct AAA-ATPase p97 complexes function in discrete steps of nuclear assembly. Nat. Cell Biol. *3,* 1086–1091.
40. Higashiyama, H., Hirose, F., Yamaguchi, M., Inoue, Y.H., Fujikake, N., Matsukage, A. and Kakizuka, A. (2002). Identification of ter94, Drosophila VCP, as a modulator of polyglutamine-induced neurodegeneration. Cell Death Differ. *9,* 264–273.
41. Hirabayashi, M., Inoue, K., Tanaka, K., Nakadate, K., Ohsawa, Y., Kamei, Y., Popiel, A.H., Sinohara, A., Iwamatsu, A., Kimura, Y. *et al.* (2001). VCP/p97 in abnormal protein aggregates, cytoplasmic vacuoles, and cell death, phenotypes relevant to neurodegeneration. Cell Death Differ. *8,* 977–984.
42. Yuan, X., Shaw, A., Zhang, X., Kondo, H., Lally, J., Freemont, P.S., Matthews, S. (2002) Solution structure and interaction surface of the C-terminal domain from p47: a major p97-cofactor involved in SNARE disassembly. J. Mol. Biol. *311*, 255-263.
43. Meyer, H.H., Kondo, H. and Warren, G. (1998). The p47 co-factor regulates the ATPase activity of the membrane fusion protein, p97. FEBS Lett. *437,* 255–257.
44. Kondo, H., Rabouille, C., Newman, R., Levine, T.P., Pappin, D., Freemont, P. and Warren, G. (1997). p47 is a cofactor for p97-mediated membrane fusion. Nature *388,* 75–78.
45. Patel, S.K., Indig, F.E., Olivieri, N., Levine, N.D. and Latterich, M. (1998). Organelle membrane fusion: a novel function for the syntaxin homolog Ufe1p in ER membrane fusion. Cell *92,* 611–620.
46. Meyer, H.H., Shorter, J.G., Seemann, J., Pappin, D. and 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.
47. Johnson, E.S., Ma, P.C.M., Ota, I.M. and Varshavsky, A. (1995). A proteolytic pathway that recognizes ubiquitin as a degradation signal. J. Biol. Chem. *270,* 17442–17456.