Chapter 8
Roles of Cdc48 in Regulated Protein Degradation in Yeast

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Abstract The chaperone-related, ubiquitin-selective AAA (ATPase associated with a variety of cellular activities) protein Cdc48 (also known as TER94, p97 and VCP) is a key regulator of intracellular proteolysis in eukaryotes. It uses the energy derived from ATP hydrolysis to segregate ubiquitylated proteins from stable assemblies with proteins, membranes and chromatin. Originally characterized as essential factor in proteasomal degradation pathways, Cdc48 was recently found to control lysosomal protein degradation as well. Moreover, impaired lysosomal proteolysis due to mutational inactivation of Cdc48 causes protein aggregation diseases in humans. This review introduces the major systems of intracellular proteolysis in eukaryotes and the role of protein ubiquitylation. It then discusses in detail structure, mechanism and cellular functions of Cdc48 with an emphasis on protein degradation pathways in yeast.

Introduction

Regulated intracellular proteolysis is essential for many aspects of cell biology: It takes center stage in protein quality control by eliminating aggregation-prone, potentially toxic conformers including aborted translational products, misfolded and damaged proteins; controls metabolic pathways, signal transduction and cell division through the degradation of key enzymes, transcription factors and signaling proteins; and ensures supply with amino acids and intermediary metabolites, in particular under conditions of nutrient deprivation. Eukaryotic cells possess two

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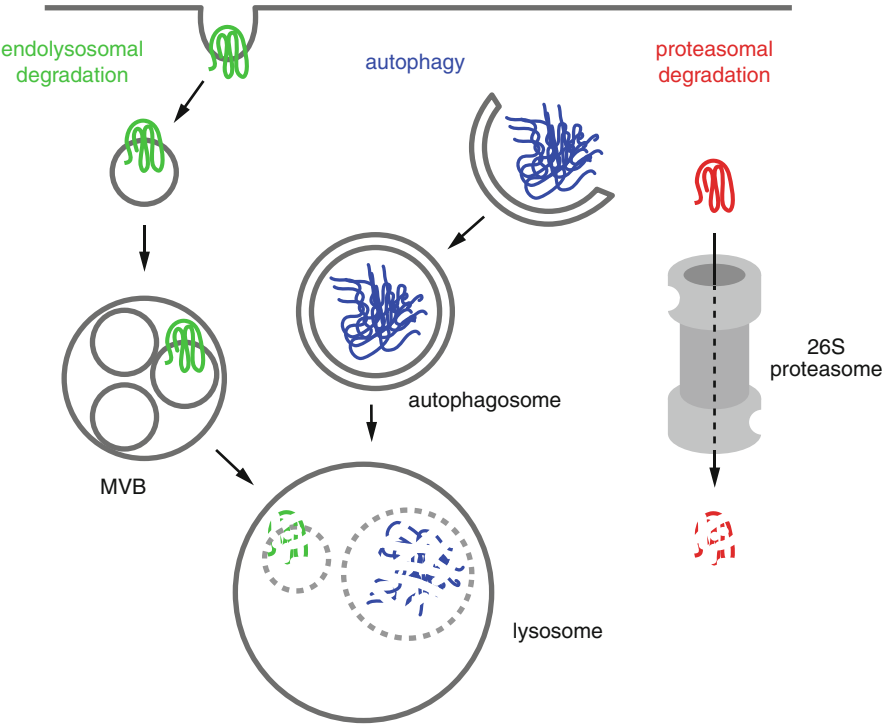


Fig. 8.1 Major pathways of intracellular proteolysis in eukaryotes. Most soluble proteins are degraded by the 26S proteasome (*red pathway*). Cytosolic structures incompatible with proteasomal degradation, including protein aggregates, large stable protein complexes and organelles, are degraded by autophagy (*blue pathway*). They are engulfed by a double membrane forming autophagosomes and delivered to lysosomes. Plasma membrane proteins are eliminated by endolysosomal degradation (*green pathway*). They are endocytosed, sorted into the luminal vesicles of multivesicular bodies (MVBs), and delivered to lysosomes. The acidic lysosomal lumen harbours various hydrolases that degrade cargo delivered by autophagosomes and MVBs

major proteolytic systems: the proteasome and the lysosomal compartment. While most soluble proteins are degraded by the proteasome, the lysosome is able to hydrolyze insoluble protein aggregates, vesicle-embedded plasma membrane proteins, and even entire organelles, viruses and bacteria (Fig. 8.1).

The proteasome is a large, barrel-shaped protease complex consisting of four stacked rings of seven structurally related subunits [1]. Its active sites are deeply buried in a central chamber formed by the two inner rings, and substrate proteins have to pass a central channel gated by the distal rings in order to be degraded. This 20S proteasomal core particle is sufficient for the degradation of polypeptides and some unfolded proteins, but most substrates require the additional presence of a 19S regulatory particle controlling substrate specificity, unfolding and access to the central channel. Together, the 20S and 19S subcomplexes form the eukaryotic 26S proteasome [2].

The lysosome is an acidic organelle harbouring various hydrolases, among them acid-optimal proteases of the cathepsin family [3]. It is the end-point of two proteolytic pathways delivering membrane-confined cargo: the endolysosomal pathway for the degradation of predominantly plasma membrane-derived proteins, and autophagy pathways for the degradation of cytosolic content including soluble proteins, insoluble protein aggregates, and organelles (Fig. 8.1) [4]. In the endolysosomal pathway, endocytosed proteins that evade recycling to the plasma membrane are sorted into luminal vesicles of multivesicular bodies (MVBs), membrane-bounded structures derived from sorting endosomes. MVBs fuse with lysosomes and release their luminal cargo for lysosomal degradation [5]. In autophagy, diverse cytosolic cargo is first engulfed during the *de novo* formation of a double membrane-bound compartment termed the autophagosome. Similar to MVBs, autophagosomes then fuse with lysosomes to form autolysosomes degrading the autophagic cargo [6]. While autophagy of cytosolic content is considered to be relatively non-specific, in particular under starvation conditions, there is clear evidence for the existence of selective autophagy pathways targeting protein complexes/aggregates and organelles [7, 8].

Intriguingly, all three major pathways for regulated proteolysis in eukaryotic cells are controlled by the covalent modification of target proteins with ubiquitin, a small, highly conserved protein of 76 amino acid residues [4]. This process (termed "ubiquitylation", "ubiquitination" or "ubiquitinylation") involves the formation of an isopeptide bond between the carboxy terminus of ubiquitin and the ε amino group of lysine residues on target proteins (Fig. 8.2). Ubiquitylation requires a catalytic cascade of three enzymatic activities E1, E2, and E3 [9]. The E1 (ubiquitin activating enzyme) activates ubiquitin by C-terminal adenylation, followed by formation of an energy-rich thioester with its active site cysteine thiol. Activated ubiquitin is then transferred in a transesterification reaction to the active site cysteine of the E2 (ubiquitin conjugating enzyme), followed by conjugation to target lysine residues mediated by the E3 (ubiquitin protein ligase). The substrate specificity of the system is primarily determined by the large number (dozens to hundreds) of different E3 ligases found in all eukaryotes. Ubiquitin itself can be ubiquitylated on either one of seven lysine residues or its amino terminus, allowing for target protein modification by ubiquitin chains of different length, linkage type and complexity [10–12]. Moreover, ubiquitin chains are also subject to editing by deubiquitylating enzymes (DUBs) [13], further increasing the plasticity of the ubiquitin signal. Importantly, different ubiquitin chains mark substrates for distinct downstream processes (Fig. 8.2) [11, 14]. Chains linked via lysine residue 48 of ubiquitin ("K48-linked chains") are prototypical signals for targeting to, and degradation by, the 26S proteasome [9, 15]. Chains linked via residues K11, K29, and perhaps also K6, K27 and K33, can also constitute proteasomal degradation signals [11, 16]. By contrast, K63-linked chains, "linear" chains linked via the amino terminus of ubiquitin, and single ubiquitin moieties ("mono-ubiquitylation") constitute signals for non-proteasomal fates of substrate proteins, for example in sorting processes, signal transduction, and DNA damage repair [12, 14, 17, 18]. In the endolysosomal pathway, ubiquitylation serves as a crucial sorting signal at two distinct steps.

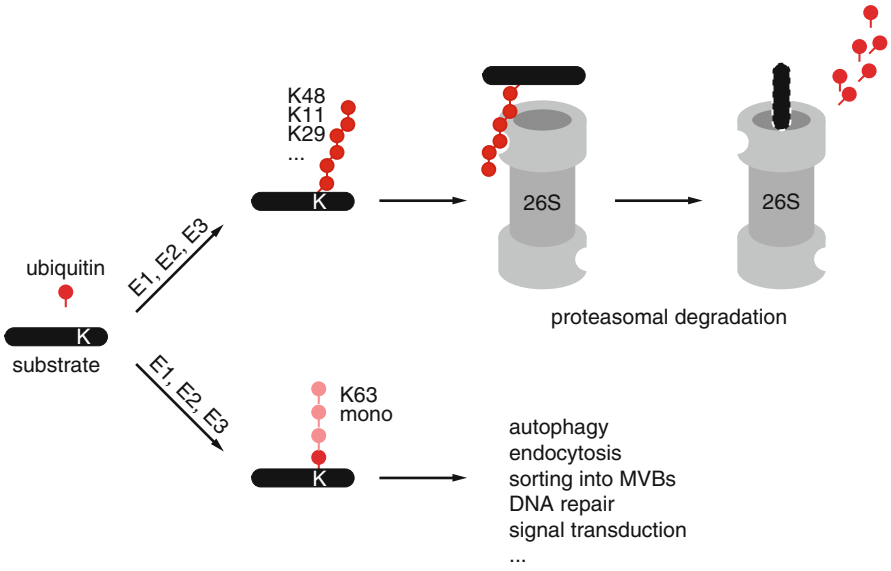


Fig. 8.2 The ubiquitin-proteasome system. Substrate proteins are covalently modified with ubiquitin through the formation of an isopeptide bond with lysine residues (K) in a reaction catalyzed by three enzymatic activities (E1, E2, E3). Polyubiquitin chains linked through ubiquitin residues K48, K11, K29, and perhaps also K6, K27 and K33, target substrate proteins for proteasomal degradation; K63-linked chains and mono-ubiquitin constitute signals in various non-proteasomal processes, among them sorting during autophagy and endolysososmal degradation

Mono-ubiquitylation and K63-linked chains mark plasma membrane proteins for internalization through primary endocytic vesicles [17]. During subsequent endosomal maturation, mono-ubiquitylation acts as a signal for the sorting of cargo into MVB vesicles [5, 19]. In autophagy, selective engulfment of ubiquitylated, cytosolic cargo by autophagosomes is mediated by a conserved family of adaptor proteins [7, 8].

In recent years, the evolutionary conserved protein Cdc48 (also known as TER94 in *Drosophila* and as p97 and VCP in vertebrates) has emerged as an important motor and regulator for a number of proteasomal degradation pathways. Cdc48 is a chaperone-related member of the ATPase associated with various cellular activities (AAA) protein family [20]. It converts the chemical energy released by ATP hydrolysis into mechanical force believed to drive the "segregation" of ubiquitylated substrate proteins from stable protein complexes, membranes and chromatin, thereby facilitating their delivery to and degradation by the 26S proteasome [21–23]. Importantly, Cdc48 has more recently also been shown to be critical for autophagy and endolysosomal protein degradation [24–26]. Even though functional insights into the role of Cdc48 in the latter pathways are just beginning to emerge, it is intriguing to note that Cdc48 is involved in all three major routes of intracellular proteolysis in eukaryotes. Consistent with such a central role in protein degradation,

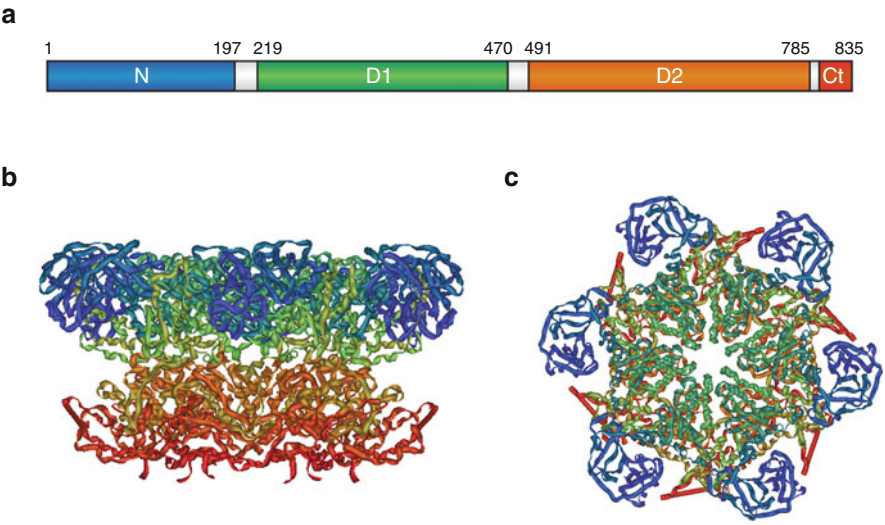


Fig. 8.3 Cdc48 structure. (**a**) Schematic view of Cdc48 domain organisation. The borders of the N, D1 and D2 domains as well of the carboxy-terminal tail (Ct) are indicated by residue numbers. Colours were chosen to closely match the colour scheme in (**b**) and (**c**). (**b**) Side view of the three-dimensional structure of murine p97 (PDB entry 1OZ4 [33]) in ribbon representation. Colour coding is from the amino-terminus (*top, blue*) to the carboxy-terminus (*bottom, red*). (**c**) Top view along the central axis

Cdc48 is essential in all organisms tested, and mutations in the human *VCP* gene cause proteinopathies including neurodegenerative, muscular and motor neuron diseases [24, 27–29].

This review discusses basic structural and functional properties of Cdc48, the complex control of distinct Cdc48 activities by regulatory cofactors, and the role of Cdc48 in various proteolysis pathways. The focus of the review will be on Cdc48-dependent degradation pathways in the Baker's yeast *Saccharomyces cerevisiae*, which serve as a paradigm for Cdc48 functions in higher eukaryotes.

Cdc48 Structure and Mechanism of Action

Three-Dimensional Structure and ATP-Dependent Conformational Changes

Like many other AAA enzymes, Cdc48 forms homohexameric, ring-shaped complexes [30, 31]. The protomer of Cdc48 consists of two Walker-type ATPase domains, D1 and D2, which are flanked by an amino-terminal N domain and a carboxy-terminal, unstructured tail (Fig. 8.3a) Whereas the ATPase domains provide the driving force for Cdc48 function, the N domain and the extreme C-terminus are

major docking sites for regulatory cofactors (see below). X-ray crystallography of the mammalian orthologue p97 (65% sequence identity to yeast Cdc48) provided detailed information about the architecture and structural organisation of the Cdc48/p97 hexamer (Fig. 8.3b, c) [32–34]. The D1 and D2 ATPase domains of the six protomers form two stacked rings in head-to-tail orientation, encircling a central, axial pore. The N domains are arranged radial to the D1 domains, whereas structure and orientation of the carboxy-terminal tail could not be solved, probably due to its high conformational flexibility.

Numerous studies employing complementary experimental approaches have provided compelling evidence for nucleotide-dependent conformational changes in Cdc48/p97 (for a detailed review, see [35]). Unfortunately, differences in the proteins studied, i.e. full-length p97 *versus* a ND1 fragment, the methodology, nucleotide and buffer conditions have so far precluded the formulation of a unifying model for the coupling of conformational states to the ATPase cycle [35]. Nevertheless, from a global view, the most pronounced nucleotide-dependent changes are (i) rigid body movements of the N domain relative to the D1 ring and (ii) widening/closing of the central pore of the D2 ring.

The biochemical characterization of Cdc48 orthologues from yeast, worm and mammals consistently revealed differences between the ATPase activities of the D1 and D2 domains [36–40]. ATP hydrolysis by D2 was found to contribute about 90% to the overall ATPase activity of Cdc48, whereas mutations in D1 compromising ATP hydrolysis had only moderate effects. In line with a slow turnover at the D1 active site inferred from these ATPase measurements, preparations of hexameric mammalian p97 contain up to six molecules of ADP stably bound to D1 that are in very slow exchange with free nucleotide (reviewed in [35]). It has therefore been proposed that the D1 nucleotide-binding site has a structural rather than catalytic function [41]. *In vivo*, ATP hydrolysis by both ATPase domains is essential for viability [37, 39], but for different reasons. While the ATPase activity of D2 is believed to be the main driving force for the segregase activity of Cdc48, hydrolysis at the D1 active site appears to merely be required to eliminate a yet ill-defined inhibitory effect of the D1 ATP state on Cdc48 function [39].

Models for Cdc48 Segregase Activity

Despite considerable knowledge about the three-dimensional structure of Cdc48/p97, the mechanism underlying its segregase activity has not been elucidated in molecular detail. Structurally related bacterial AAA+proteins, such as ClpA, ClpB, ClpX, FtsH and HslU from *E. coli* employ a “threading” mechanism of substrate unfolding [20, 42–44]. Instrumental to this mechanism are evolutionary highly conserved loops of the D1 and (when present) D2 domains that face the central pore and contain an aromatic residue next to a large hydrophobic or basic residue. These loops contact substrate proteins and are believed to transform conformational

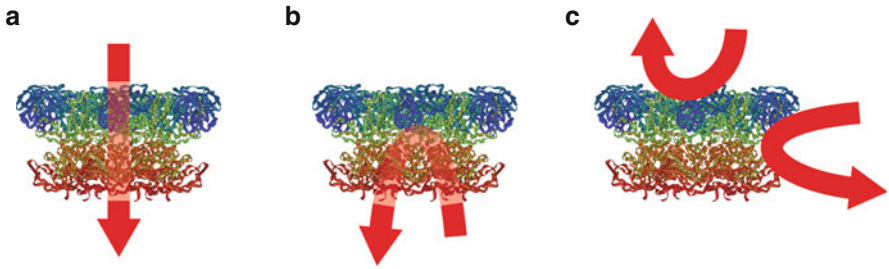


Fig. 8.4 Models for Cdc48 segregase activity. Side view of murine p97 as in Fig. 8.3b. *Red arrows* indicate the routes of substrate proteins according to the different models. (**a**) Threading. (**b**) D2 in – D2 out. (**c**) Surface only. See text for details

changes accompanying ATP binding and hydrolysis into ratchet-like movements, thereby pulling the substrate polypeptide chain through the central pore and unfolding it [42, 43].

In contrast to bacterial unfoldases, there exists no convincing experimental evidence that Cdc48 substrates are threaded through the entire length of the central pore (Fig. 8.4a). In fact, several lines of circumstantial evidence may argue against such a mechanism. First, Cdc48 substrates are typically marked by ubiquitin chains on one or several lysine residues. It is unclear how the resulting branched polypeptide could be efficiently threaded through the narrow central pore of Cdc48, aside from the fact that ubiquitin is remarkably stable thermodynamically and hence difficult to unfold. Based on studies of the Cdc48-dependent ER-associated protein degradation (ERAD) quality control pathway (see below), this conceptual problem was proposed to be solved by the presence of Cdc48-associated DUBs which could initially deubiquitylate substrates, followed by threading through the central pore and re-ubiquitylation for proteasomal targeting by Cdc48-associated E3 ligases [45, 46]. However, in disfavour of a critical role for DUBs in ERAD substrate turnover by Cdc48, siRNA-mediated depletion of Cdc48/p97-associated DUBs in mammalian cells does not, with the exception of the poorly characterized DUB Usp13, result in ERAD defects [47]. Similarly, deletion of known Cdc48-associated DUBs in yeast has not been reported to cause ERAD phenotypes. Second, the presence of the N domain appears to prevent threading of polypeptides. Interestingly, an archaeal homologue of Cdc48, VAT, was found to be capable of unfolding the ClpA and ClpX model substrate GFP-ssrA *in vitro* under certain experimental conditions [48]. Similar to the Clp-type AAA+proteins, this activity required the aromatic side chains in the conserved D1 and D2 loops facing the central pore. In addition, deletion of the VAT N domain enhanced its unfolding activity towards GFP-ssrA. In contrast to VAT and Clp-type AAA+proteins, however, Cdc48 orthologues lack the critical aromatic residue in the D1 loop [49] (P. Zwickl, personal communication). Consistently, wild type mammalian p97 was found to be unable to unfold GFP-ssrA *in vitro* and could only be converted into a GFP-ssrA unfoldase by introducing one or two aromatic D1 loop residues and simultaneously deleting its N domain [49].

Aromatic D1 loop residues, however, render Cdc48 inactive *in vivo* (our unpublished results), suggesting that a counter-selection against a Clp-like threading mechanism might have taken place during evolution of Cdc48 orthologues. Finally, known cellular functions of Cdc48 do not necessarily demand total unfolding of substrate proteins. ERAD substrates are presumably translocated to the cytosol in a largely unfolded state (see below), and other quality control substrates can be assumed to be at least partially unfolded as well. Of note, presentation of just an unstructured region(s) by proteasomal substrates is typically sufficient for their complete unfolding by the AAA subunits of the proteasomal 19S regulatory particle [50, 51], suggesting that the critical function of Cdc48 in protein quality control pathways may not be complete protein unfolding. Furthermore, Cdc48 substrates not destined for degradation by the 26S proteasome (see below) need not or must not be unfolded at all.

As an alternative mechanism to threading through the central pore of Cdc48, it was proposed that substrates enter and exit the pore at the D2 end (Fig. 8.4b) [38]. Interestingly, the D2 pore-facing loops of all Cdc48 orthologues possess the key aromatic residue absent in the D1 loop, allowing for a ratchet-like activity of the D2 ring similar to Clp-type unfoldases [49] (P. Zwickl, personal communication). Consistent with such a "D2 in – D2 out" model of Cdc48 activity, these aromatic residues are critical for Cdc48 function *in vitro* and *in vivo* [38] (M. Esaki and T. Ogura, cited as unpublished results in [39]). Furthermore, it has been hypothesized that the high local concentration of guanidyl groups present at a "denaturation collar" of arginine residues lining the D2 pore provides the denaturant for protein unfolding [38]. Even though direct evidence for such a denaturing property is missing, the "D2 in – D2 out" model is consistent with the hypothesis that local unfolding of weakly structured regions in substrate proteins rather than their complete unfolding is sufficient for subsequent global unfolding and degradation by the 26S proteasome [51].

A third model does not invoke substrate entry into the central pore at all. Instead, the pronounced domain movements of Cdc48 throughout its ATPase cycle, perhaps amplified by cofactor proteins acting as levers, could be sufficient to segregate or extract substrates from their cellular environments (Fig. 8.4c). Even though there is presently no experimental evidence to support such a "surface only" model, it is remarkable that the homotrimeric cofactor p47 (see below) was found to bind centrally on top of the hexameric p97 ring in a cryo-EM study [52]. On the basis of these data, it is difficult to imagine how p47-dependent substrates could enter the central pore of Cdc48 from either top or bottom.

It should be noted that the models depicted above need not be mutually exclusive, but could apply for distinct subsets of Cdc48 substrates. For instance, substrates destined for proteasomal degradation may be (partially) unfolded by threading or by the "D2 in – D2 out" mechanism, whereas p47-dependent substrates in membrane fusion processes may be prevented from entering the central pore and processed when bound to the Cdc48 surface. Clearly, all three models need to be scrutinized on the basis of additional structural and functional studies.

Regulation by Cofactors

The involvement of Cdc48 in fundamentally distinct cellular processes necessitates the tight control of its segregase activity. Indeed, a large number of cofactors regulate central aspects of Cdc48 function, including its subcellular localization and substrate specificity and fate [53]. The critical importance of regulatory cofactors for Cdc48 activity is underscored by the fact that yeast null mutants in major Cdc48 cofactors are either severely sick or non-viable [54–57]. Furthermore, human disease-associated p97 mutant proteins were found to exhibit significantly perturbed cofactor interactions [25, 58, 59]. According to their function, Cdc48 cofactors can be broadly classified as either substrate-recruiting or substrate-processing (Fig. 8.5) [53, 60]. Substrate-recruiting cofactors typically possess ubiquitin binding domains and function as adaptors between Cdc48 and specific substrates. Alternatively, or additionally, they can control Cdc48 recruitment to specific substrates/pathways by means of their subcellular localization, e.g. as integral ER membrane proteins. Substrate-processing cofactors, on the other hand, typically exhibit E3 ligase or DUB activity and modulate the ubiquitin signal on substrates in order to regulate their downstream fate [23, 60]. The two principal docking sites for regulatory cofactors are the N domain and the C-terminal tail of Cdc48 (Fig. 8.5). The majority of cofactors possess one or two defined modules for Cdc48 binding. These include the ubiquitin regulatory X (UBX) and UBX-like domains and the linear sequence motifs binding-site 1 (BS1), VCP-interacting motif (VIM), and VCP-binding motif (VBM), which all bind to partially overlapping regions of the N domain; and the peptide N-glycosidase/ubiquitin-associated (PUB) and PLAA, Ufd3 and Lub1 (PUL) domains interacting with the extreme carboxy-terminus of Cdc48 [23, 53, 61–63].

An efficient regulation of Cdc48 cellular functions requires that different cofactors do not randomly interact with the six N-terminal and six C-terminal cofactor binding sites present per Cdc48 hexamer. In accordance with this consideration, cofactor interactions are organized in a hierarchical manner dominated by the mutually exclusive binding of the major substrate-recruiting cofactors Ufd1-Npl4 and Shp1 (known as p47 in vertebrates) [53, 64]. Broadly speaking, the heterodimeric cofactor Ufd1-Npl4 recruits substrates of proteasomal degradation or processing pathways to Cdc48, whereas Shp1/p47 is believed to act primarily as an adaptor for non-proteasomal substrates of Cdc48. As discussed in the previous section, the two different cofactors may even dictate distinct mechanisms of Cdc48 substrate turnover. Importantly, the activities of the $Cdc48^{Ufd1\text{-}Npl4}$ and $Cdc48^{Shp1}$ complexes can be fine-tuned by additional substrate-recruiting cofactors [53]. For instance, the yeast cofactors Ubx2 and Ubx5 direct the $Cdc48^{Ufd1\text{-}Npl4}$ complex to the ER membrane for ERAD [65, 66] and to nuclear chromatin for DNA damage repair [67], respectively. Consistent with such a mechanism of multi-layered specificity control, only one Ufd1-Npl4 heterodimer binds one Cdc48 hexamer [68], leaving additional binding sites for Ubx2, Ubx5 and related cofactors. Conversely, the latter cofactors can only form stable complexes with Cdc48 in the presence of Ufd1-Npl4 [69].

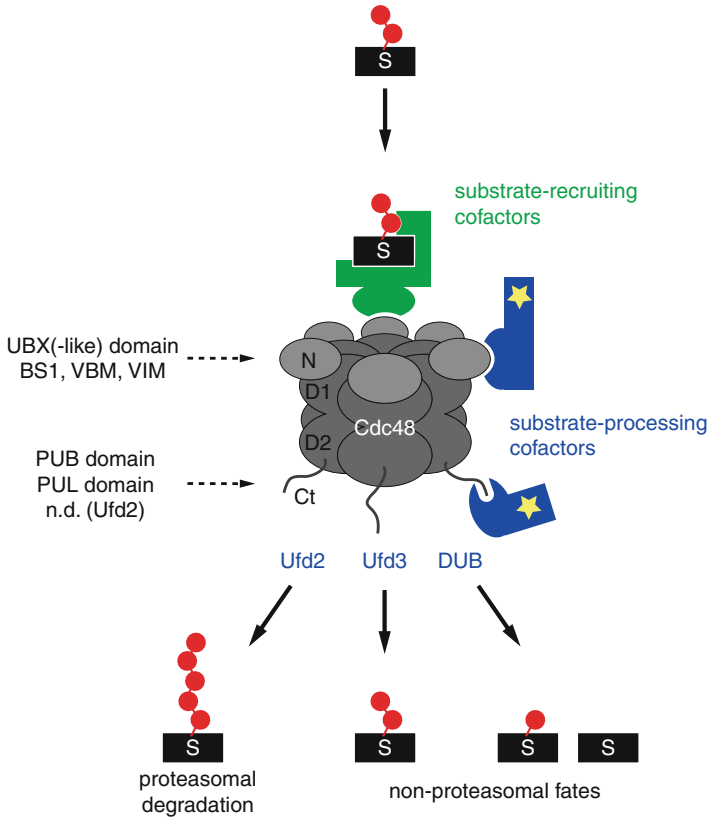


Fig. 8.5 Control of Cdc48 by regulatory cofactors. Substrate proteins (S) marked by short ubiquitin chains (*red circles*) are recognized by substrate-recruiting cofactors (*green*) and segregated from protein complexes, lipid membranes, or chromatin (not shown). Substrate-processing cofactors (*blue*) catalyze ubiquitin chain elongation, targeting the substrate for efficient proteasomal degradation (Ufd2); prevent chain elongation (Ufd3); or catalyze deubiquitylation (DUB, e.g. Otu1). The latter two activities prevent proteasomal targeting. Enzymatic activity of substrate-processing cofactors is indicated by a *yellow asterisk*. The domain organisation of Cdc48 according to Fig. 8.3a (N, D1, D2, Ct) and Cdc48 binding modules of cofactors (*left*) are indicated. Note that the Cdc48 binding site of Ufd2 has not been characterized in detail (n.d.) (Modified from [23])

Interestingly, there is evidence for Ufd1-independent functions of Npl4 in a recently discovered mitochondrial stress response pathway in yeast [70] and in certain mammalian ERAD pathways [71, 72]. While these findings raise the intriguing possibility that different binding partners of Npl4 define yet another layer of specificity control, this hypothesis still awaits a rigorous structural and biochemical characterization of the Cdc48-cofactor complexes involved.

Compared to the $Cdc48^{Ufd1\text{-}Npl4}$ complex, little is known about fine-tuning of the $Cdc48^{Shp1}$ complex by additional cofactors. The only well-established example so far is the role of mammalian VCIP135 in controlling the function of the related

$p97^{p47}$ and $p97^{p37}$ complexes in the homotypic fusion of Golgi and ER membrane vesicles [73–76]. VCIP135 is a large Cdc48/p97 cofactor possessing DUB activity [74], which initially suggested its classification as substrate-processing factor [23, 53]. However, recent results showed that the DUB activity of VCIP135 is exclusively required for the post-mitotic, $p97^{p47}$-mediated fusion of Golgi vesicles, but not for other VCIP135-controlled fusion events [75, 76]. Thus, VCIP135 may also be considered an additional substrate-recruiting factor directing $p97^{p47}$ and $p97^{p37}$ to ER and Golgi vesicles, analogous to the role of Ubx2 in recruiting $Cdc48^{Ufd1\text{-}Npl4}$ to the ER.

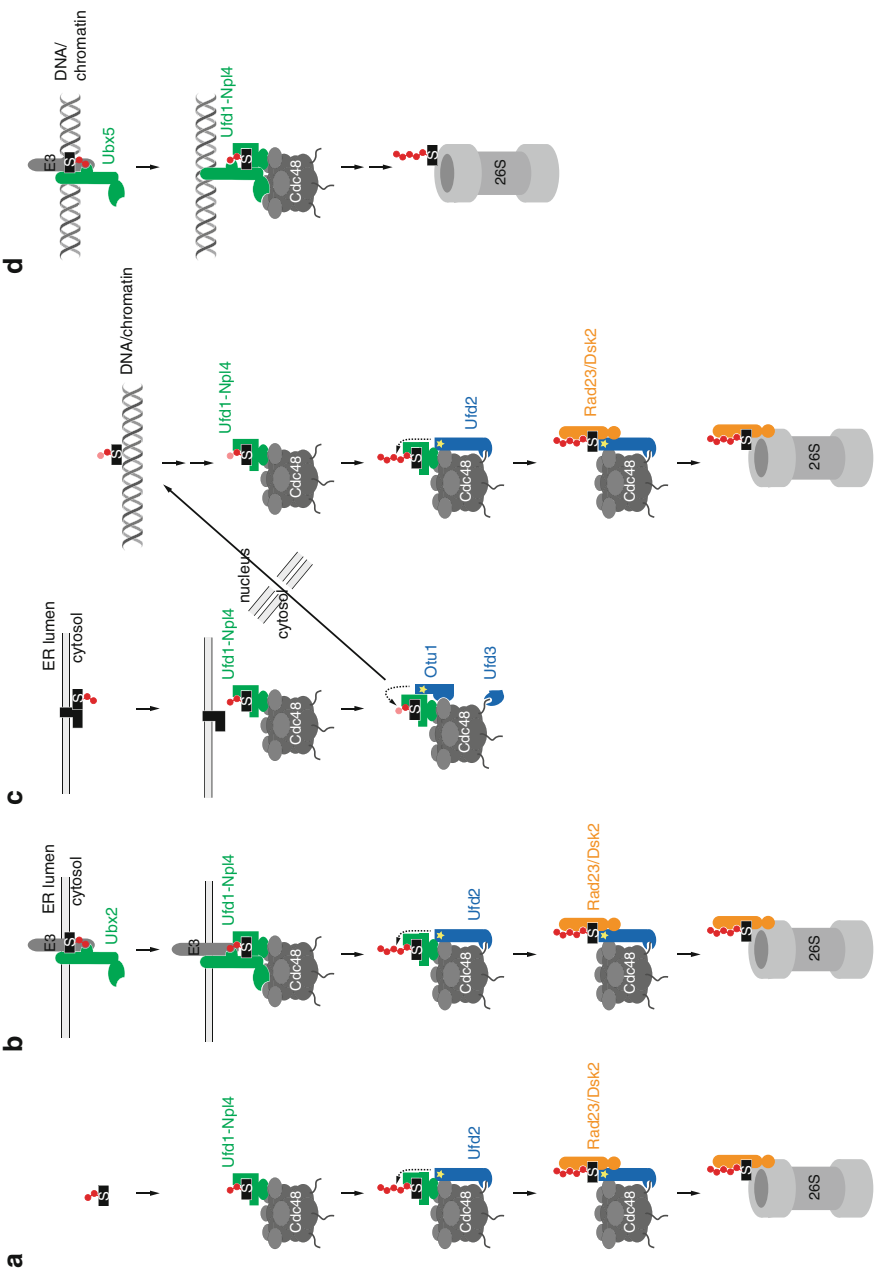
Recently, a potential third major Cdc48 cofactor was identified. UBXD1 interacts with both the N domain and the C-terminus of Cdc48/p97, by virtue of its VIM and PUB domains, respectively [77, 78]. Consistent with UBXD1 occupying both major cofactor binding regions of Cdc48 in a proposed stoichiometry of three molecules UBXD1 per hexamer [69], binding of UBXD1 appears to be mutually exclusive with the two other major Cdc48 cofactors, Ufd1-Npl4 and p47 [25, 77, 79]. Homologues of UBXD1 are found in all eukaryotes except fungi, suggesting that it may control a fundamental cellular function of Cdc48. Indeed, a recent study implicated the mammalian $p97^{UBXD1}$ complex in endolysosomal sorting, even though the precise role of UBXD1 in this process remains to be defined [25] (see below).

Role of Cdc48 in Intracellular Proteolysis

Proteasomal Degradation Pathways

The UFD Pathway

Cdc48 and its cofactors were first implicated in proteolysis on the basis of a genetic screen for yeast mutants defective in the proteasomal degradation of the ubiquitin fusion protein, ubiquitin-β-galactosidase (Ub-P-βGal) [54]. Despite the fact that the physiological relevance of this "ubiquitin fusion degradation" (UFD) pathway is still unclear, studies of the UFD pathway revealed important principles of Cdc48-mediated protein degradation. The UFD genetic screen [54] and subsequent candidate approaches [80–83] led to the identification of the E3 ligase specific for Ub-P-βGal (Ufd4) and of all proteins critical for Cdc48-dependent protein degradation by the 26S proteasome. Besides Cdc48 itself, these include Ufd1 and Npl4, forming the heterodimeric substrate-recruiting cofactor Ufd1-Npl4; the substrate-processing cofactor Ufd2; and the proteasomal targeting proteins Rad23 and Dsk2 (Fig. 8.6a). Ufd4 catalyzes the modification of Ub-P-βGal with one or few ubiquitin moieties [54, 84], which are insufficient for proteasomal targeting and degradation [85]. This "oligo-ubiquitylated" substrate is recognized by Ufd1-Npl4 and recruited to the $Cdc48^{Ufd1\text{-}Npl4}$ complex [86]. There, the substrate-processing cofactor Ufd2 by virtue of its "E4" ubiquitin ligase activity extends the oligo-ubiquitin tag to a long K48-linked chain representing an efficient proteasomal degradation signal [84, 87].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Cdc48
Ufd2
Otu1
Ufd3
Rad23/Dsk2
26S

Fig. 8.6 Cdc48-dependent proteasomal degradation pathways. (**a**) The UFD pathway. The substrate (S) Ub-P-βGal is oligo-ubiquitylated by the E3 ligase Ufd4 (not shown) and recruited to Cdc48 by Ufd1-Npl4. Ufd2 catalyzes the ubiquitin chain elongation (*dashed arrow*) and hands over the polyubiquitylated substrate to the proteasomal adaptor proteins Rad23 and Dsk2. (**b**) The ERAD pathway. The Cdc48 machinery is linked to the degradation of ER quality control substrates by Ubx2, an ER membrane-localized substrate-recruiting cofactor coordinating interactions of $Cdc48^{Ufd1\text{-}Npl4}$ with ERAD substrates and E3 ligases. (**c**) The OLE pathway. The processed, oligo-ubiquitylated p90 form of the transcription factor Spt23 is mobilized from stable associations with the unprocessed, ER membrane-tethered p120 precursor (*black*) by $Cdc48^{Ufd1\text{-}Npl4}$. It is unclear how the Cdc48 complex is recruited to the substrate. After mobilization, Ufd3 and Otu1 prevent Ufd2-catalyzed chain elongation and stabilize p90 (*dashed arrow*), enabling nuclear translocation and activation of target gene expression. Subsequently, in a mechanistically poorly understood process, p90 is presumably recruited by Ufd1-Npl4 to Cdc48 and proteasomally degraded in a Ufd2- and Rad23-/Dsk2-dependent manner. (**d**) Rpb1 degradation. In analogy to Ubx2 (panel b), $Cdc48^{Ufd1\text{-}Npl4}$ is recruited to chromatin-associated, ubiquitylated Rpb1 by the substrate-recruiting factor Ubx5 and segregates the substrate from chromatin. The 26S proteasome has been shown to interact with Cdc48, but a potential involvement of Ufd2 and Rad23/Dsk2 as well as the ubiquitylation state of the substrate have not been investigated

Polyubiquitylated Ub-P-βGal is then delivered to the 26S proteasome by the related proteasomal adaptor proteins Rad23 and Dsk2 [81, 83], which possess binding sites both for polyubiquitin chains and the proteasomal 19S regulatory particle [88].

Notably, not only Cdc48 and its cofactors, but also Ufd2 and Rad23/Dsk2 are linked by physical interactions [86, 89] (Fig. 8.6a), which led to the proposal that Cdc48 substrates are "escorted" to the 26S proteasome in order to ensure efficient degradation [86]. The importance of a substrate hand-over between Ufd2 and Rad23/Dsk2 was recently underscored by a study analyzing the $Cdc48^{Ufd1\text{-}Npl4}$-dependent degradation of a linear tetra-ubiquitin fusion protein of β-galactosidase (Ub_4-βGal) [90]. Consistent with tetra-ubiquitin being the minimal signal for proteasomal degradation [85], this longer ubiquitin fusion tag obliterated the need for polyubiquitylation by the combined activities of Ufd4 and Ufd2. However, a catalytically inactive truncated form of Ufd2 was still required for efficient proteasomal degradation of Ub_4-βGal, presumably in its capacity to escort the substrate from the Cdc48 complex to the proteasomal adaptors Rad23/Dsk2. Because binding of Rad23/Dsk2 to Ufd2 and the 19S complex is mutually exclusive [89], the relatively stable interaction between Ufd2 and Rad23 [91] must be abrogated after substrate hand-over to Rad23. Intriguingly, this process was recently found to require Cdc48 itself, raising the interesting possibility that the Ufd2-Rad23 complex is a (pseudo-)substrate for the Cdc48 segregase activity [92]. Thus, Cdc48 could be involved in three different steps of UFD substrate turnover: in (i) local destabilization/unfolding of the substrate [51]; (ii) Ufd2-catalyzed ubiquitin chain elongation [84, 86]; and (iii) breaking the Ufd2-Rad23 interaction [92]. Further studies are needed to establish the relative importance of these three functions for the UFD and other degradation pathways.

ERAD

The UFD pathway can be considered to represent a conserved "module" of $Cdc48^{Ufd1\text{-}Npl4}$ activity that can be integrated into various proteasomal degradation pathways. In ERAD, this module is placed downstream of a sophisticated quality control network of ER chaperones and lectins. This quality control network identifies irreparably damaged or misfolded ER proteins and directs them to dedicated E3 ligases at the ER membrane for ubiquitylation and subsequent proteasomal degradation [93] (Fig. 8.6b). In *S. cerevisisae*, the two principal ERAD E3s are the HRD (HMG-CoA reductase degradation) complex and Doa10 [94]. The HRD complex consists of a core and ancillary components. The core, composed of the catalytic subunit Hrd1 (also known as Der3) and the luminal substrate receptor Hrd3, is required for the degradation of all substrates, while the ancillary factors Der1, Usa1 and Yos9 are only involved in the degradation of certain subsets of HRD substrates [93, 94]. HRD directs the degradation of all luminal ERAD substrates and of ER membrane proteins exposing degradation signals in their luminal or transmembrane regions [95]. Doa10 is required for the degradation of ER membrane proteins exposing cytosolic degradation signals [95], as well as for the degradation

of several soluble non-ERAD substrates [96–98]. In order to be ubiquitylated by Hrd1, luminal substrates recognized by Hrd3 must be at least partially retro-translocated to the cytosolic face of the ER membrane *via* still intensely debated channel proteins and mechanisms (discussed in [99, 100]). In contrast, transmembrane substrates of HRD and Doa10 can presumably be ubiquitylated *in situ*.

Downstream of HRD- and Doa10-catalyzed substrate ubiquitylation, the different ERAD routes converge, as all substrates must be dislocated from the ER membrane by Cdc48 for subsequent delivery to the 26S proteasome (Fig. 8.6b). These steps require the same components as those in the UFD pathway, i.e. the $Cdc48^{Ufd1\text{-}Npl4}$ complex [101–105], Ufd2 [86, 106] and Rad23/Dsk2 [86, 107]. The essential function of $Cdc48^{Ufd1\text{-}Npl4}$ in ERAD is the ATP-dependent retro-translocation and dislocation of ubiquitylated substrates that are stably associated with or inserted into the ER membrane. Ufd2 and Rad23/Dsk2 then escort the dislocated substrates to the 26S proteasome, perhaps helping to suppress unwanted aggregation of non-native substrates in the cytosol. Importantly, the link between the "UFD module" and the ERAD pathways is provided by the substrate-recruiting cofactor Ubx2. Ubx2 is an integral ER membrane protein that serves as ER anchor for the $Cdc48^{Ufd1\text{-}Npl4}$ complex and coordinates interactions between $Cdc48^{Ufd1\text{-}Npl4}$, ERAD substrates, and the E3 ligases [65, 66] (Fig. 8.6b). In addition to Ubx2, which is critical for the degradation of most ERAD substrates, the Cdc48 cofactors Ubx4 [108] and Vms1 [109] play auxiliary roles in the degradation of certain ERAD substrates. Both appear to act independently of Ubx2 and probably downstream of Cdc48-driven substrate dislocation, but their precise role in ERAD remains to be defined. Of note, Ubx4 and Vms1 have also been implicated in DNA damage repair and mitochondrial stress response, respectively (see below), raising the possibility that they perform a more general function in Cdc48-mediated degradation pathways. Besides Ubx4 and Vms1, cytosolic chaperones, mainly from the Hsp70 and Hsp40 families, have also been demonstrated to assist in the degradation of some ERAD substrates [106, 110–112], presumably by preventing their aggregation after dislocation from the ER membrane.

In addition to its central role in protein quality control, the ERAD pathway is also used for regulatory proteolysis in the control of lipid metabolism [113, 114]. In yeast, the HMG-CoA reductase isoenzyme Hmg2 is degraded in a sterol-dependent manner via the canonical pathway for transmembrane substrates of the HRD complex [65, 105, 115–117]. In a negative feedback loop, high metabolic flux through Hmg2 is believed to result in altered fluidity of the ER membrane, causing conformational changes in the transmembrane domain of Hmg2 that lead to the exposure of degradation signals recognized by the HRD E3 ligase [114, 118, 119].

The OLE Pathway

The mobilization and degradation of the transcription factor Spt23 is another $Cdc48^{Ufd1\text{-}Npl4}$-mediated degradation pathway initiated at the ER membrane, but is distinct from the canonical ERAD pathways (Fig. 8.6c). In this so-called OLE

pathway, the inactive ER membrane-anchored form of Spt23 (p120) is mono- or oligo-ubiquitylated by the E3 ligase Rsp5 and processed by the 26S proteasome into its active form, p90 [120]. Ubiquitylated p90 is segregated from its stable association with unprocessed, ER membrane-anchored p120 precursors by the $Cdc48^{Ufd1\text{-}Npl4}$ complex [82] (Fig. 8.6c). In contrast to ERAD, it is still unclear how $Cdc48^{Ufd1\text{-}Npl4}$ is recruited to the ER membrane for the mobilization of p90, as this process does not appear to involve Ubx2 (our unpublished data). One possibility is that $Cdc48^{Ufd1\text{-}Npl4}$ is recruited directly by the 26S proteasome during proteasomal processing of p120, because Cdc48 has been identified as a proteasome interacting protein [121, 122]. Following its mobilization, p90 is transported to the nucleus, most likely in complex with $Cdc48^{Ufd1\text{-}Npl4}$, where it can activate expression of the key target gene, *OLE1* encoding Δ9 fatty acid desaturase [86]. The transcription factor activity of p90 is terminated by Ufd2-mediated polyubiquitylation, Rad23/Dsk2-mediated delivery to the 26S proteasome, and proteasomal degradation [86] (Fig. 8.6c). Notably, the homologous, functionally overlapping transcription factor Mga2 is controlled in a similar manner, even though mechanistic details appear to differ from Spt23 processing and mobilization [123, 124]. The OLE pathway provides another example for an involvement of Cdc48 in a negative feedback loop of lipid metabolism. Ole1 controls the concentration of unsaturated fatty acids which in turn influences ER membrane fluidity and thickness. These parameters, through regulation of Rsp5 access, are believed to control the balance between proteasomal processing of p120 into p90 and processive proteasomal degradation of p120, in a complex scenario involving casein kinase 2 and the prolyl isomerase Ess1 (Pin1 in mammals) [125].

Two aspects of the $Cdc48^{Ufd1\text{-}Npl4}$ activity in the OLE pathway are worth mentioning. Firstly, this pathway provides a clear example of a temporal and spatial separation of Cdc48 segregase function at the ER membrane from Ufd2-triggered proteasomal degradation in the nucleus [82, 86] (Fig. 8.6c). This raises the interesting, but entirely speculative possibility that UFD and ERAD substrates are, at least partially, degraded in the nucleus as well by the significant nuclear subpopulation of 26S proteasomes [126, 127]. Secondly, in order to ensure proper expression of *OLE1*, it is vital for the OLE pathway to prevent the premature, Ufd2-catalyzed polyubiquitylation and degradation of p90. This is achieved by the combined action of two Cdc48 substrate-processing factors, Otu1 and Ufd3, which antagonize Ufd2 function by virtue of its DUB activity and by competing for Cdc48 binding, respectively [60, 78] (Fig. 8.6c). Importantly, the antagonistic effects of Ufd2 *versus* Ufd3 and Otu1 in the degradation of Cdc48 substrates led to the current model that the ubiquitylation status and, thus, the ultimate fate of Cdc48 substrates can be actively modulated and edited by substrate-processing cofactors of Cdc48 [23, 60, 128, 129] (Fig. 8.5, bottom).

Nuclear Substrates

The $Cdc48^{Ufd1\text{-}Npl4}$ complex is involved in the proteasomal degradation of a number of additional cellular proteins [67]. Recently, $Cdc48^{Ufd1\text{-}Npl4}$ was shown to be critical for the degradation of the largest subunit of RNA polymerase II, Rpb1 [67]. Upon

induction of DNA damage by UV radiation, RNA polymerase II becomes irreversibly stalled at the sites of DNA lesions and must be removed by proteasomal degradation in order to allow transcription to recommence [130, 131]. UV-induced Rpb1 degradation was found to depend on the $Cdc48^{Ufd1\text{-}Npl4}$ complex and, in addition, on the substrate-recruiting cofactor Ubx5 [67]. Moreover, ubiquitylated Rpb1 accumulated on chromatin in a *cdc48* mutant, and binding of Ubx5 to Rpb1 was greatly increased upon UV treatment [67]. Together, these data strongly suggest that Ubx5 recruits $Cdc48^{Ufd1\text{-}Npl4}$ to stalled, ubiquitylated Rpb1, for its extraction from chromatin and delivery to the proteasome (Fig. 8.6d). Thus, the function of Ubx5 bears analogy to the role of Ubx2 in recruiting $Cdc48^{Ufd1\text{-}Npl4}$ to the ER membrane in ERAD. Because potential roles of Ufd2 and Rad23/Dsk2 in Rpb1 degradation are yet to be investigated, it remains unclear if Rpb1 is escorted to the proteasome in a manner similar to UFD, ERAD and OLE pathway substrates. Contrary to this possibility, the finding that ubiquitylated Rpb1 interacts with the proteasome in a *cdc48* mutant was interpreted to indicate that Cdc48 does not act upstream of the proteasome, but rather exerts its activity on proteasome-bound substrates resistant to unfolding by the AAA subunits of the 19S complex. However, while further experimental work is clearly needed to elucidate the details of Rpb1 degradation, it should be noted that the specific molecular defect(s) caused by the conditional *cdc48* allele used are currently unknown and could, for instance, lead to the delivery of ubiquitylated substrates in a conformation incompatible with proteasomal degradation. Interestingly, Ubx4 is also involved in Rpb1 degradation, apparently in parallel to the pathway involving Ubx5 [67], but its specific role in this and other degradation pathways has yet to be identified.

Another transcription-related function of $Cdc48^{Ufd1\text{-}Npl4}$ is the extraction of the transcriptional repressor Matα2 from its DNA target sites during yeast mating type switching [132]. In this case however, in contrast to the proteasomal degradation of Spt23 p90 and Rpb1, the segregase activity of $Cdc48^{Ufd1\text{-}Npl4}$ appears to be uncoupled from proteasomal degradation. This finding raises the interesting possibility that $Cdc48^{Ufd1\text{-}Npl4}$ plays a more general role in transcriptional regulation by remodeling transcription factor complexes.

CDC48 was first identified in a yeast genetic screen for cell cycle defects, and conditional *cdc48* mutants arrest as large budded cells with 2n DNA content in metaphase [57, 133]. Studies in yeast and in higher eukaryotes revealed that Cdc48 acts on a number of substrates in different phases of the cell cycle. Even though the involvement of Ufd1-Npl4 is best established for cell cycle functions in metazoans, it is likely that Cdc48-dependent degradation of cell cycle substrates in yeast requires Ufd1-Npl4 as well. In *S. cerevisiae*, Cdc48 has been shown to be critical for the degradation of the G1 cyclin-dependent kinase inhibitor Far1 at the G1/S transition [134], and of the G1 cyclin Cln2 after entry into S phase [135]. During mitotic exit, Cdc48 has been implicated in spindle disassembly in yeast and in *Xenopus* egg extracts [136], although conflicting results exist at least for the latter system [137]. In yeast, the microtubule-binding protein Ase1 and the Polo-like kinase homologue Cdc5 were postulated to be critical targets of $Cdc48^{Ufd1\text{-}Npl4}$ that must either be degraded or sequestered from microtubules in order to allow spindle disassembly to

occur [136]. No further information on mechanisms and degradation pathways exists for any of the cell cycle substrates of Cdc48 in yeast. In addition, $Cdc48^{Ufd1\text{-}Npl4}$ has recently been implicated in cell wall integrity signaling and G1 progression [138], but Cdc48 substrate(s) critical for this function have yet to be identified.

Other Proteasomal Pathways

An interesting example for an involvement of $Cdc48^{Ufd1\text{-}Npl4}$ in the regulation of cellular metabolism is the degradation of Fructose-1,6,-bisphosphatase (FBPase), a key enzyme of gluconeogenesis. Upon shift from ethanol to glucose as carbon source, yeast cells switch metabolism from gluconeogenesis to glycolysis. This switching requires the rapid inactivation of FBPase, which is accomplished, in part, by proteasomal degradation [139, 140]. FBPase degradation depends on $Cdc48^{Ufd1\text{-}Npl4}$, Rad23/Dsk2, but not Ufd2 [141]. Ubx4 again appears to play an ancillary role in this process. Interestingly, the glucose-induced degradation of pyruvate carboxykinase, another gluconeogenetic enzyme, is also mediated by $Cdc48^{Ufd1\text{-}Npl4}$ [141], suggesting that Cdc48 may play a more general role in metabolic control than previously appreciated.

Cdc48-mediated proteasomal degradation was recently also implicated in a mitochondrial stress-responsive system, but here it appears to employ the substrate-recruiting cofactor Vms1 together with Npl4, not the canonical heterodimer Ufd1-Npl4 [70]. Upon mitochondrial stress, Vms1 relocates from the cytosol to mitochondria and recruits Cdc48 and Npl4, but not Ufd1. The $Cdc48^{Vms1\text{-}Npl4}$ complex is required for the degradation of the mitochondrial outer membrane protein Fzo1 and probably further mitochondrial substrates [70]. In metazoans, Cdc48/p97 is involved in the proteasomal degradation of the mitofusins Mfn1 and Mfn2 and of the anti-apoptotic Bcl-2 family member Mcl1 [142, 143]. While Vms1 is conserved from yeast to humans, a direct role in the Cdc48/p97-dependent degradation of mitochondrial proteins in metazoans has yet to be shown. Mechanistically, the role of Cdc48 in the degradation of outer mitochondrial membrane proteins may resemble the Cdc48-mediated dislocation of ERAD substrates, but this hypothesis requires further experimental support. Regardless of the exact mechanism of Cdc48 activity, the identified mitochondrial targets link Cdc48/p97 to mitophagy of damaged mitochondria and to apoptosis, and thus to central aspects of cellular stress response and survival [144, 145].

Autophagy

Besides its role in a growing number of proteasomal degradation pathways, Cdc48 is also involved in different forms of autophagy, both in yeast and in higher eukaryotes. In yeast, Cdc48 and its substrate-recruiting factor Shp1 were found to be required for general starvation-induced macroautophagy and for selective piecemeal microautophagy of the nucleus [146]. Intriguingly, these processes do not appear to involve

the ubiquitin-proteasome system. Instead, the ubiquitin-like modifier Atg8 was identified as a critical target of Shp1 in autophagy. Atg8 (LC3 in mammals) is anchored to membranes via reversible lipidation of its C-terminus. This is required for the formation and extension of the autophagosomal isolation membrane and for the incorporation of cargo into autophagosomes [6, 147]. In starved *shp1* mutant cells, neither GFP-Atg8-positive autophagosomes in the cytosol nor autophagic bodies in the vacuole (the yeast lysosome) could be detected, suggesting that Shp1 is critical for autophagosome biogenesis [146], but its exact role in this process and the significance of its physical interaction with Atg8 remain to be determined.

Nitrogen starvation induces the degradation of the yeast ribosomal 60S subunit in a selective autophagy pathway termed ribophagy [148]. Ribophagy depends on Cdc48, its substrate-processing cofactor Ufd3 (also known as Doa1) and the DUB complex Ubp3-Bre5 in a process which is still poorly understood [148, 149]. It has been proposed that ribophagy involves the dynamic ubiquitylation of ribosomes and/or the ribophagy machinery [26, 148]. Notably, in contrast to the study by Krick et al. [146], the study by Ossareh-Nazari et al. reported that general macroautophagy of GFP-Atg8 proceeds normally in a *cdc48* mutant [149]. Even though differences in the *cdc48* alleles and particular starvation conditions used may contribute to these conflicting results, additional experiments are required to clarify this critical point.

Mammalian cells depleted of Cdc48/p97 or expressing mutant forms of Cdc48/p97 associated with the familial proteinopathy termed Inclusion Body Myopathy, Paget's disease of the bone and Fronto-temporal Dementia (IBMPFD) accumulate nondegrading autophagosomes that fail to mature into autolysosomes in order to eliminate their cargo [150–152]. This finding suggests that impaired autophagy could be the critical cellular defect underlying the pathogenesis of IBMPFD and causing the characteristic accumulation of insoluble cytoplasmic inclusions positive for TDP-43 and ubiquitin. Of note, Cdc48/p97 appears to be required for a late step during autophagy in mammals, i.e. the fusion of autophagosomes and lysosomes into proteolytically active autolysosomes, whereas $Cdc48^{Shp1}$ in yeast has been implicated in some early step during autophagosome biogenesis [146]. It is presently unknown if yeast and mammalian autophagy have fundamentally different requirements for Cdc48 activity, or if Cdc48 possesses two distinct functions in autophagy that are evolutionary conserved from yeast to humans.

Endolysosomal Degradation

Cdc48 was very recently also implicated in the third major intracellular proteolytic pathway, endolysososmal degradation. Mammalian p97 and its cofactor UBXD1 were found to target mono-ubiquitylated forms of the caveolar scaffolding protein caveolin-1 on its endocytic route to endolysosomes [25]. Intriguingly, overexpression of IBMPFD-causing variants of Cdc48/p97 blocked endolysososmal sorting, suggesting that impaired endolysosomal degradation may contribute to, or aggravate, the autophagy defects observed in IBMPFD.

UBXD1 is not conserved in yeast, and there exists no direct evidence for an involvement of yeast Cdc48 in endolysosomal degradation so far. Interestingly, however, the substrate-processing cofactor Ufd3 has been shown to be critical for sorting ubiquitylated membrane proteins into MVBs on their biosynthetic route from the *trans*-Golgi network to the vacuole [153]. The same study also provided evidence for a role of Ufd3 in the sorting of endocytic cargo into MVBs [153], but this possibility has not been directly addressed yet. While results obtained using a Ufd3-binding deficient *cdc48* mutant strain suggested that the function of Ufd3 in MVB sorting is independent of Cdc48 [154], this issue deserves further attention in light of the new findings for mammalian Cdc48/p97.

Other Functions of Cdc48

While the focus of this review is the role of yeast Cdc48 in intracellular proteolysis, a number of additional proteolytic substrates and pathways involving Cdc48/p97 have been identified in metazoans. Exciting examples include the proteasomal degradation of the hypoxia-inducible transcription factor 1α [79], the myosin chaperone Unc45 [155], and the replication licensing factor Cdt1 [156, 157], as well as roles of Cdc48/p97 in DNA double strand break repair [158, 159] and lifespan control [129]. For a recent review focusing on Cdc48 functions in metazoans, see [160, 161].

In addition to its activities in various proteolytic pathways, Cdc48 also possesses functions in several non-proteolytic processes that are beyond the scope of this review. For example, the substrate-recruiting cofactor Shp1/p47 controls Cdc48/p97 activities in membrane fusion processes that do not appear to involve the degradation of Cdc48 substrates (reviewed in [53, 162–164]). In yeast, *shp1* mutants exhibit vacuolar fragmentation as well as defective V-ATPase targeting and/or activity [165, 166]. It is presently unclear if and how these phenotypes may be related to the autophagy function of Shp1 discussed above. Interestingly, Shp1 was identified as a positive regulator of the catalytic subunit of yeast protein phosphatase 1, Glc7 [55], although the underlying regulatory mechanism is unknown. In agreement with such a function, Cdc48 and Shp1 were recently shown to be important for the balance between the antagonistic Glc7 phosphatase and Ipl1 (Aurora B) kinase activities on elusive nuclear substrates [57]. Consistently, loss of Shp1 function causes hyperphosphorylation of Ipl1 substrates, defective bipolar attachment of kinetochores to spindle microtubules, and chromosomal mispositioning [57]. However, these phenotypes are most likely secondary to a partial loss of mitotic Glc7 function(s), and so far direct mitotic substrates of $Cdc48^{Shp1}$ have not been identified. In metazoans, by contrast, Cdc48/p97 together with Ufd1-Npl4 has an established function as a direct negative regulator of Aurora B [167, 168]. In *Xenopus* egg extracts, $p97^{Ufd1\text{-}Npl4}$ was shown to remove ubiquitylated Aurora B from chromosomes at the end of mitosis, in order to allow for chromosome decondensation and nucleus formation [167]. In HeLa cells, $p97^{Ufd1\text{-}Npl4}$ negatively

regulates Aurora B earlier in mitosis, at the metaphase/anaphase transition, to allow chromosome segregation to occur properly [168]. Interestingly, after its extraction from chromatin, Aurora B is stable in *Xenopus* egg extracts, while it is apparently subject to proteasomal degradation in HeLa cells [168]. It is presently unclear if the respective activities of $Cdc48^{Shp1}$ and $p97^{Ufd1\text{-}Npl4}$ in the negative regulation of yeast and metazoan Aurora B are mechanistically related, or if they rather reflect independent solutions for the general requirement of tight spatiotemporal regulation of Aurora B during mitosis.

Outlook

The Baker's yeast *S. cerevisiae* has proven a valuable model organism for the genetic, biochemical and cellular analysis of Cdc48. A wealth of studies performed in the yeast system formed the basis for our detailed understanding of Cdc48 cellular functions, in particular in proteasomal protein degradation. While the yeast system will continue to deepen our knowledge on fundamental cellular functions of Cdc48, metazoan models including worm, fly and mouse are rapidly closing the gap and provide valuable insights into exciting additional Cdc48 functions in the complexity of multicellular organisms. Ultimately, these complementary approaches and models should help to better understand the defects caused by Cdc48 malfunction in human disease in the near future.

References

1. Baumeister W, Walz J, Zuhl F, Seemuller E (1998) The proteasome: paradigm of a self-compartmentalizing protease. Cell 92(3):367–380
2. Lasker K, Förster F, Bohn S, Walzthoeni T (2012) Molecular architecture of the 26S proteasome holocomplex determined by an integrative approach. Proc Natl Acad Sci USA 109(5):1380–1387
3. Kornfeld S, Mellman I (1989) The biogenesis of lysosomes. Annu Rev Cell Biol 5:483–525
4. Clague MJ, Urbe S (2010) Ubiquitin: same molecule, different degradation pathways. Cell 143(5):682–685
5. Katzmann DJ, Odorizzi G, Emr SD (2002) Receptor downregulation and multivesicular-body sorting. Nat Rev Mol Cell Biol 3(12):893–905
6. Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol 10(7):458–467
7. Kirkin V, McEwan DG, Novak I, Dikic I (2009) A role for ubiquitin in selective autophagy. Mol Cell 34(3):259–269
8. Kraft C, Peter M, Hofmann K (2010) Selective autophagy: ubiquitin-mediated recognition and beyond. Nat Cell Biol 12(9):836–841
9. Hershko A, Ciechanover A (1998) The ubiquitin system. Annu Rev Biochem 67:425–479
10. Ikeda F, Dikic I (2008) Atypical ubiquitin chains: new molecular signals. 'Protein modifications: beyond the usual suspects' review series. EMBO Rep 9(6):536–542
11. Komander D (2009) The emerging complexity of protein ubiquitination. Biochem Soc Trans 37(Pt 5):937–953

12. Behrends C, Harper JW (2011) Constructing and decoding unconventional ubiquitin chains. Nat Struct Mol Biol 18(5):520–528
13. Komander D, Clague MJ, Urbe S (2009) Breaking the chains: structure and function of the deubiquitinases. Nat Rev Mol Cell Biol 10(8):550–563
14. Pickart CM, Fushman D (2004) Polyubiquitin chains: polymeric protein signals. Curr Opin Chem Biol 8(6):610–616
15. Hochstrasser M (1996) Ubiquitin-dependent protein degradation. Annu Rev Genet 30:405–439
16. Xu P, Duong DM, Seyfried NT, Cheng D et al (2009) Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. Cell 137(1):133–145
17. Hicke L, Dunn R (2003) Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annu Rev Cell Dev Biol 19:141–172
18. Chen ZJ (2005) Ubiquitin signalling in the NF-[kappa]B pathway. Nat Cell Biol 7(8):758–765
19. Piper RC, Katzmann DJ (2007) Biogenesis and function of multivesicular bodies. Annu Rev Cell Dev Biol 23:519–547
20. Hanson PI, Whiteheart SW (2005) AAA+ proteins: have engine, will work. Nat Rev Mol Cell Biol 6(7):519–529
21. Bays NW, Hampton RY (2002) Cdc48-Ufd1-Npl4: stuck in the middle with Ub. Curr Biol 12(10):R366–R371
22. Elsasser S, Finley D (2005) Delivery of ubiquitinated substrates to protein-unfolding machines. Nat Cell Biol 7(8):742–749
23. Buchberger A (2010) Control of ubiquitin conjugation by Cdc48 and its cofactors. Subcell Biochem 54:17–30
24. Ju JS, Weihl CC (2010) Inclusion body myopathy, Paget's disease of the bone and frontotemporal dementia: a disorder of autophagy. Hum Mol Genet 19(R1):R38–R45
25. Ritz D, Vuk M, Kirchner P, Bug M et al (2011) Endolysosomal sorting of ubiquitylated caveolin-1 is regulated by VCP and UBXD1 and impaired by VCP disease mutations. Nat Cell Biol 13(9):1116–1123
26. Dargemont C, Ossareh-Nazari B (2012) Cdc48/p97, a key actor in the interplay between autophagy and ubiquitin/proteasome catabolic pathways. Biochim Biophys Acta 1823(1): 138–144
27. Watts GD, Wymer J, Kovach MJ, Mehta SG et al (2004) Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 36(4):377–381
28. Weihl CC, Pestronk A, Kimonis VE (2009) Valosin-containing protein disease: inclusion body myopathy with Paget's disease of the bone and fronto-temporal dementia. Neuromuscul Disord 19(5):308–315
29. Johnson JO, Mandrioli J, Benatar M, Abramzon Y et al (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. Neuron 68(5):857–864
30. Rouiller I, Butel VM, Latterich M, Milligan RA et al (2000) A major conformational change in p97 AAA ATPase upon ATP binding. Mol Cell 6(6):1485–1490
31. Zhang X, Shaw A, Bates PA, Newman RH et al (2000) Structure of the AAA ATPase p97. Mol Cell 6(6):1473–1484
32. DeLaBarre B, Brunger AT (2005) Nucleotide dependent motion and mechanism of action of p97/VCP. J Mol Biol 347(2):437–452
33. DeLaBarre B, Brunger AT (2003) Complete structure of p97/valosin-containing protein reveals communication between nucleotide domains. Nat Struct Biol 10(10):856–863
34. Beuron F, Flynn TC, Ma J, Kondo H et al (2003) Motions and negative cooperativity between p97 domains revealed by cryo-electron microscopy and quantised elastic deformational model. J Mol Biol 327(3):619–629
35. Pye VE, Dreveny I, Briggs LC, Sands C et al (2006) Going through the motions: the ATPase cycle of p97. J Struct Biol 156(1):12–28

36. Song C, Wang Q, Li CC (2003) ATPase activity of p97-valosin-containing protein (VCP). D2 mediates the major enzyme activity, and D1 contributes to the heat-induced activity. J Biol Chem 278(6):3648–3655
37. Ye Y, Meyer HH, Rapoport TA (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(1):71–84
38. DeLaBarre B, Christianson JC, Kopito RR, Brunger AT (2006) Central pore residues mediate the p97/VCP activity required for ERAD. Mol Cell 22(4):451–462
39. Esaki M, Ogura T (2010) ATP-bound form of the D1 AAA domain inhibits an essential function of Cdc48p/p97. Biochem Cell Biol 88(1):109–117
40. Nishikori S, Esaki M, Yamanaka K, Sugimoto S et al (2011) Positive cooperativity of the p97 AAA ATPase is critical for essential functions. J Biol Chem 286(18):15815–15820
41. Wang Q, Song C, Li CC (2003) Hexamerization of p97-VCP is promoted by ATP binding to the D1 domain and required for ATPase and biological activities. Biochem Biophys Res Commun 300(2):253–260
42. Zolkiewski M (2006) A camel passes through the eye of a needle: protein unfolding activity of Clp ATPases. Mol Microbiol 61(5):1094–1100
43. Sauer RT, Baker TA (2011) AAA+ proteases: ATP-fueled machines of protein destruction. Annu Rev Biochem 80:587–612
44. Gur E, Ottofuelling R, Dougan DA (2013) Machines of destruction – AAA+proteases and the adaptors that control them. In: Dougan DA (ed) Regulated proteolysis in microorganisms. Springer, Subcell Biochem 66:3–33
45. Ernst R, Claessen JH, Mueller B, Sanyal S et al (2011) Enzymatic blockade of the ubiquitin-proteasome pathway. PLoS Biol 8(3):e1000605
46. Claessen JH, Kundrat L, Ploegh HL (2011) Protein quality control in the ER: balancing the ubiquitin checkbook. Trends Cell Biol 22(1):22–32
47. Sowa ME, Bennett EJ, Gygi SP, Harper JW (2009) Defining the human deubiquitinating enzyme interaction landscape. Cell 138(2):389–403
48. Gerega A, Rockel B, Peters J, Tamura T et al (2005) VAT, the thermoplasma homolog of mammalian p97/VCP, is an N domain-regulated protein unfoldase. J Biol Chem 280(52): 42856–42862
49. Rothballer A, Tzvetkov N, Zwickl P (2007) Mutations in p97/VCP induce unfolding activity. FEBS Lett 581(6):1197–1201
50. Prakash S, Tian L, Ratliff KS, Lehotzky RE et al (2004) An unstructured initiation site is required for efficient proteasome-mediated degradation. Nat Struct Mol Biol 11(9):830–837
51. Beskow A, Grimberg KB, Bott LC, Salomons FA et al (2009) A conserved unfoldase activity for the p97 AAA-ATPase in proteasomal degradation. J Mol Biol 394(4):732–746
52. Beuron F, Dreveny I, Yuan X, Pye VE et al (2006) Conformational changes in the AAA ATPase p97-p47 adaptor complex. EMBO J 25(9):1967–1976
53. Schuberth C, Buchberger A (2008) UBX domain proteins: major regulators of the AAA ATPase Cdc48/p97. Cell Mol Life Sci 65(15):2360–2371
54. Johnson ES, Ma PC, Ota IM, Varshavsky A (1995) A proteolytic pathway that recognizes ubiquitin as a degradation signal. J Biol Chem 270(29):17442–17456
55. Zhang S, Guha S, Volkert FC (1995) The *Saccharomyces* SHP1 gene, which encodes a regulator of phosphoprotein phosphatase 1 with differential effects on glycogen metabolism, meiotic differentiation, and mitotic cell cycle progression. Mol Cell Biol 15(4): 2037–2050
56. 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(8):818–824
57. Cheng YL, Chen RH (2010) The AAA-ATPase Cdc48 and cofactor Shp1 promote chromosome bi-orientation by balancing Aurora B activity. J Cell Sci 123(Pt 12):2025–2034
58. Fernandez-Saiz V, Buchberger A (2010) Imbalances in p97 co-factor interactions in human proteinopathy. EMBO Rep 11(6):479–485

59. Manno A, Noguchi M, Fukushi J, Motohashi Y et al (2010) Enhanced ATPase activities as a primary defect of mutant valosin-containing proteins that cause inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia. Genes Cells 15(8):911–922
60. Rumpf S, Jentsch S (2006) Functional division of substrate processing cofactors of the ubiquitin-selective Cdc48 chaperone. Mol Cell 21(2):261–269
61. Stolz A, Hilt W, Buchberger A, Wolf DH (2011) Cdc48: a power machine in protein degradation. Trends Biochem Sci 36(10):515–523
62. Bohm S, Lamberti G, Fernandez-Saiz V, Stapf C et al (2011) Cellular functions of Ufd2 and Ufd3 in proteasomal protein degradation depend on Cdc48 binding. Mol Cell Biol 31(7):1528–1539
63. Yeung HO, Kloppsteck P, Niwa H, Isaacson RL et al (2008) Insights into adaptor binding to the AAA protein p97. Biochem Soc Trans 36(Pt 1):62–67
64. Meyer HH, Shorter JG, Seemann J, Pappin D et al (2000) A complex of mammalian ufd1 and npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. EMBO J 19(10):2181–2192
65. 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(10):999–1006
66. Neuber O, Jarosch E, Volkwein C, Walter J et al (2005) Ubx2 links the Cdc48 complex to ER-associated protein degradation. Nat Cell Biol 7(10):993–998
67. Verma R, Oania R, Fang R, Smith GT et al (2011) Cdc48/p97 mediates UV-dependent turnover of RNA Pol II. Mol Cell 41(1):82–92
68. Pye VE, Beuron F, Keetch CA, McKeown C et al (2007) Structural insights into the p97-Ufd1-Npl4 complex. Proc Natl Acad Sci USA 104(2):467–472
69. Hanzelmann P, Buchberger A, Schindelin H (2011) Hierarchical binding of cofactors to the AAA ATPase p97. Structure 19(6):833–843
70. Heo JM, Livnat-Levanon N, Taylor EB, Jones KT et al (2010) A stress-responsive system for mitochondrial protein degradation. Mol Cell 40(3):465–480
71. Ballar P, Pabuccuoglu A, Kose FA (2011) Different p97/VCP complexes function in retrotranslocation step of mammalian ER-associated degradation (ERAD). Int J Biochem Cell Biol 43(4):613–621
72. Ballar P, Shen Y, Yang H, Fang S (2006) The role of a novel p97/valosin-containing protein-interacting motif of gp78 in endoplasmic reticulum-associated degradation. J Biol Chem 281(46):35359–35368
73. Uchiyama K, Jokitalo E, Kano F, Murata M et al (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(5):855–866
74. Wang Y, Satoh A, Warren G, Meyer HH (2004) VCIP135 acts as a deubiquitinating enzyme during p97-p47-mediated reassembly of mitotic Golgi fragments. J Cell Biol 164(7):973–978
75. Uchiyama K, Totsukawa G, Puhka M, Kaneko Y et al (2006) p37 is a p97 adaptor required for Golgi and ER biogenesis in interphase and at the end of mitosis. Dev Cell 11(6):803–816
76. Totsukawa G, Kaneko Y, Uchiyama K, Toh H et al (2011) VCIP135 deubiquitinase and its binding protein, WAC, in p97ATPase-mediated membrane fusion. EMBO J 30(17): 3581–3593
77. Kern M, Fernandez-Saiz V, Schafer Z, Buchberger A (2009) UBXD1 binds p97 through two independent binding sites. Biochem Biophys Res Commun 380(2):303–307
78. Stapf C, Cartwright E, Bycroft M, Hofmann K et al (2011) The general definition of the p97/valosin-containing protein (VCP)-interacting motif (VIM) delineates a new family of p97 cofactors. J Biol Chem 286(44):38670–38678
79. Alexandru G, Graumann J, Smith GT, Kolawa NJ et al (2008) UBXD7 binds multiple ubiquitin ligases and implicates p97 in HIF1alpha turnover. Cell 134(5):804–816
80. Ghislain M, Dohmen RJ, Levy F, Varshavsky A (1996) Cdc48p interacts with Ufd3p, a WD repeat protein required for ubiquitin-mediated proteolysis in *Saccharomyces cerevisiae*. EMBO J 15(18):4884–4899

81. Lambertson D, Chen L, Madura K (1999) Pleiotropic defects caused by loss of the proteasome-interacting factors Rad23 and Rpn10 of Saccharomyces cerevisiae. Genetics 153(1):69–79
82. Rape M, Hoppe T, Gorr I, Kalocay M et al (2001) Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48(UFD1/NPL4), a ubiquitin-selective chaperone. Cell 107(5):667–677
83. Rao H, Sastry A (2002) Recognition of specific ubiquitin conjugates is important for the proteolytic functions of the ubiquitin-associated domain proteins Dsk2 and Rad23. J Biol Chem 277(14):11691–11695
84. Koegl M, Hoppe T, Schlenker S, Ulrich HD et al (1999) A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. Cell 96(5):635–644
85. Thrower JS, Hoffman L, Rechsteiner M, Pickart CM (2000) Recognition of the polyubiquitin proteolytic signal. EMBO J 19(1):94–102
86. Richly H, Rape M, Braun S, Rumpf S et al (2005) A series of ubiquitin binding factors connects CDC48/p97 to substrate multiubiquitylation and proteasomal targeting. Cell 120(1):73–84
87. Saeki Y, Tayama Y, Toh-e A, Yokosawa H (2004) Definitive evidence for Ufd2-catalyzed elongation of the ubiquitin chain through Lys48 linkage. Biochem Biophys Res Commun 320(3):840–845
88. Buchberger A (2002) From UBA to UBX: new words in the ubiquitin vocabulary. Trends Cell Biol 12(5):216–221
89. Kim I, Mi K, Rao H (2004) Multiple interactions of rad23 suggest a mechanism for ubiquitylated substrate delivery important in proteolysis. Mol Biol Cell 15(7):3357–3365
90. Zhao S, Ulrich HD (2010) Distinct consequences of posttranslational modification by linear versus K63-linked polyubiquitin chains. Proc Natl Acad Sci U S A 107(17): 7704–7709
91. Hanzelmann P, Stingele J, Hofmann K, Schindelin H et al (2010) The yeast E4 ubiquitin ligase Ufd2 interacts with the ubiquitin-like domains of Rad23 and Dsk2 via a novel and distinct ubiquitin-like binding domain. J Biol Chem 285(26):20390–20398
92. Baek GH, Kim I, Rao H (2011) The Cdc48 ATPase modulates the interaction between two proteolytic factors Ufd2 and Rad23. Proc Natl Acad Sci USA 108(33):13558–13563
93. Buchberger A, Bukau B, Sommer T (2010) Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. Mol Cell 40(2):238–252
94. Ismail N, Ng DT (2006) Have you HRD? Understanding ERAD is DOAble! Cell 126(2):237–239
95. Vashist S, Ng DT (2004) Misfolded proteins are sorted by a sequential checkpoint mechanism of ER quality control. J Cell Biol 165(1):41–52
96. Ravid T, Kreft SG, Hochstrasser M (2006) Membrane and soluble substrates of the Doa10 ubiquitin ligase are degraded by distinct pathways. EMBO J 25(3):533–543
97. Swanson R, Locher M, Hochstrasser M (2001) A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matalpha2 repressor degradation. Genes Dev 15(20):2660–2674
98. Hwang CS, Shemorry A, Varshavsky A (2010) N-terminal acetylation of cellular proteins creates specific degradation signals. Science 327(5968):973–977
99. Bagola K, Mehnert M, Jarosch E, Sommer T (2011) Protein dislocation from the ER. Biochim Biophys Acta 1808(3):925–936
100. Claessen JH, Kundrat L, Ploegh HL (2012) Protein quality control in the ER: balancing the ubiquitin checkbook. Trends Cell Biol 22(1):22–32
101. Bays NW, Gardner RG, Seelig LP, Joazeiro CA et al (2001) Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. Nat Cell Biol 3(1):24–29
102. Ye Y, Meyer HH, Rapoport TA (2001) The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. Nature 414(6864):652–656
103. Braun S, Matuschewski K, Rape M, Thoms S et al (2002) Role of the ubiquitin-selective CDC48(UFD1/NPL4) chaperone (segregase) in ERAD of OLE1 and other substrates. EMBO J 21(4):615–621

104. Jarosch E, Taxis C, Volkwein C, Bordallo J et al (2002) Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48. Nat Cell Biol 4(2):134–139
105. Rabinovich E, Kerem A, Frohlich KU, Diamant N et al (2002) AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. Mol Cell Biol 22(2):626–634
106. Nakatsukasa K, Huyer G, Michaelis S, Brodsky JL (2008) Dissecting the ER-associated degradation of a misfolded polytopic membrane protein. Cell 132(1):101–112
107. Medicherla B, Kostova Z, Schaefer A, Wolf DH (2004) A genomic screen identifies Dsk2p and Rad23p as essential components of ER-associated degradation. EMBO Rep 5(7):692–697
108. Alberts SM, Sonntag C, Schafer A, Wolf DH (2009) Ubx4 modulates cdc48 activity and influences degradation of misfolded proteins of the endoplasmic reticulum. J Biol Chem 284(24):16082–16089
109. Tran JR, Tomsic LR, Brodsky JL (2011) A Cdc48p-associated factor modulates endoplasmic reticulum-associated degradation, cell stress, and ubiquitinated protein homeostasis. J Biol Chem 286(7):5744–5755
110. Zhang Y, Nijbroek G, Sullivan ML, McCracken AA et al (2001) Hsp70 molecular chaperone facilitates endoplasmic reticulum-associated protein degradation of cystic fibrosis transmembrane conductance regulator in yeast. Mol Biol Cell 12(5):1303–1314
111. Han S, Liu Y, Chang A (2007) Cytoplasmic Hsp70 promotes ubiquitination for endoplasmic reticulum-associated degradation of a misfolded mutant of the yeast plasma membrane ATPase, PMA1. J Biol Chem 282(36):26140–26149
112. Taxis C, Hitt R, Park SH, Deak PM et al (2003) Use of modular substrates demonstrates mechanistic diversity and reveals differences in chaperone requirement of ERAD. J Biol Chem 278(38):35903–35913
113. Hampton RY (2002) Proteolysis and sterol regulation. Annu Rev Cell Dev Biol 18:345–378
114. Hampton RY, Garza RM (2009) Protein quality control as a strategy for cellular regulation: lessons from ubiquitin-mediated regulation of the sterol pathway. Chem Rev 109(4):1561–1574
115. Hampton RY, Gardner RG, Rine J (1996) Role of 26S proteasome and HRD genes in the degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, an integral endoplasmic reticulum membrane protein. Mol Biol Cell 7(12):2029–2044
116. Bays NW, Wilhovsky SK, Goradia A, Hodgkiss-Harlow K et al (2001) HRD4/NPL4 is required for the proteasomal processing of ubiquitinated ER proteins. Mol Biol Cell 12(12):4114–4128
117. Carroll SM, Hampton RY (2010) Usa1p is required for optimal function and regulation of the Hrd1p endoplasmic reticulum-associated degradation ubiquitin ligase. J Biol Chem 285(8):5146–5156
118. Gardner RG, Shearer AG, Hampton RY (2001) In vivo action of the HRD ubiquitin ligase complex: mechanisms of endoplasmic reticulum quality control and sterol regulation. Mol Cell Biol 21(13):4276–4291
119. Sato BK, Schulz D, Do PH, Hampton RY (2009) Misfolded membrane proteins are specifically recognized by the transmembrane domain of the Hrd1p ubiquitin ligase. Mol Cell 34(2):212–222
120. Hoppe T, Matuschewski K, Rape M, Schlenker S et al (2000) Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. Cell 102(5):577–586
121. Verma R, Chen S, Feldman R, Schieltz D et al (2000) Proteasomal proteomics: identification of nucleotide-sensitive proteasome-interacting proteins by mass spectrometric analysis of affinity-purified proteasomes. Mol Biol Cell 11(10):3425–3439
122. Isakov E, Stanhill A (2011) Stalled proteasomes are directly relieved by P97 recruitment. J Biol Chem 286(35):30274–30283
123. Shcherbik N, Zoladek T, Nickels JT, Haines DS (2003) Rsp5p is required for ER bound Mga2p120 polyubiquitination and release of the processed/tethered transactivator Mga2p90. Curr Biol 13(14):1227–1233
124. Shcherbik N, Haines DS (2007) Cdc48p(Npl4p/Ufd1p) binds and segregates membrane-anchored/tethered complexes via a polyubiquitin signal present on the anchors. Mol Cell 25(3):385–397

125. Siepe D, Jentsch S (2009) Prolyl isomerase Pin1 acts as a switch to control the degree of substrate ubiquitylation. Nat Cell Biol 11(8):967–972
126. Russell SJ, Steger KA, Johnston SA (1999) Subcellular localization, stoichiometry, and protein levels of 26 S proteasome subunits in yeast. J Biol Chem 274(31):21943–21952
127. Laporte D, Salin B, Daignan-Fornier B, Sagot I (2008) Reversible cytoplasmic localization of the proteasome in quiescent yeast cells. J Cell Biol 181(5):737–745
128. Jentsch S, Rumpf S (2007) Cdc48 (p97): a "molecular gearbox" in the ubiquitin pathway? Trends Biochem Sci 32(1):6–11
129. Kuhlbrodt K, Janiesch PC, Kevei E, Segref A et al (2011) The Machado-Joseph disease deubiquitylase ATX-3 couples longevity and proteostasis. Nat Cell Biol 13(3):273–281
130. Beaudenon SL, Huacani MR, Wang G, McDonnell DP et al (1999) Rsp5 ubiquitin-protein ligase mediates DNA damage-induced degradation of the large subunit of RNA polymerase II in *Saccharomyces cerevisiae*. Mol Cell Biol 19(10):6972–6979
131. Woudstra EC, Gilbert C, Fellows J, Jansen L et al (2002) A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. Nature 415(6874):929–933
132. Wilcox AJ, Laney JD (2009) A ubiquitin-selective AAA-ATPase mediates transcriptional switching by remodelling a repressor-promoter DNA complex. Nat Cell Biol 11(12): 1481–1486
133. Moir D, Stewart SE, Osmond BC, Botstein D (1982) Cold-sensitive cell-division-cycle mutants of yeast: isolation, properties, and pseudoreversion studies. Genetics 100(4): 547–563
134. 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(1):21–26
135. Archambault V, Chang EJ, Drapkin BJ, Cross FR et al (2004) Targeted proteomic study of the cyclin-Cdk module. Mol Cell 14(6):699–711
136. Cao K, Nakajima R, Meyer HH, Zheng Y (2003) The AAA-ATPase Cdc48/p97 regulates spindle disassembly at the end of mitosis. Cell 115(3):355–367
137. Heubes S, Stemmann O (2007) The AAA-ATPase p97-Ufd1-Npl4 is required for ERAD but not for spindle disassembly in Xenopus egg extracts. J Cell Sci 120(Pt 8):1325–1329
138. Hsieh MT, Chen RH (2011) Cdc48 and cofactors Npl4-Ufd1 are important for G1 progression during heat stress by maintaining cell wall integrity in Saccharomyces cerevisiae. PLoS One 6(4):e18988
139. Schork SM, Bee G, Thumm M, Wolf DH (1994) Catabolite inactivation of fructose-1, 6-bisphosphatase in yeast is mediated by the proteasome. FEBS Lett 349(2):270–274
140. Santt O, Pfirrmann T, Braun B, Juretschke J et al (2008) The yeast GID complex, a novel ubiquitin ligase (E3) involved in the regulation of carbohydrate metabolism. Mol Biol Cell 19(8):3323–3333
141. Barbin L, Eisele F, Santt O, Wolf DH (2010) The Cdc48-Ufd1-Npl4 complex is central in ubiquitin-proteasome triggered catabolite degradation of fructose-1,6-bisphosphatase. Biochem Biophys Res Commun 394(2):335–341
142. Tanaka A, Cleland MM, Xu S, Narendra DP et al (2010) Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J Cell Biol 191(7):1367–1380
143. Xu S, Peng G, Wang Y, Fang S et al (2011) The AAA-ATPase p97 is essential for outer mitochondrial membrane protein turnover. Mol Biol Cell 22(3):291–300
144. Karbowski M, Youle RJ (2011) Regulating mitochondrial outer membrane proteins by ubiquitination and proteasomal degradation. Curr Opin Cell Biol 23(4):476–482
145. Braun RJ, Zischka H (2008) Mechanisms of Cdc48/VCP-mediated cell death: from yeast apoptosis to human disease. Biochim Biophys Acta 1783(7):1418–1435
146. Krick R, Bremer S, Welter E, Schlotterhose P et al (2010) Cdc48/p97 and Shp1/p47 regulate autophagosome biogenesis in concert with ubiquitin-like Atg8. J Cell Biol 190(6):965–973
147. Ohsumi Y (2001) Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol Cell Biol 2(3):211–216
148. Kraft C, Deplazes A, Sohrmann M, Peter M (2008) Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. Nat Cell Biol 10(5):602–610

149. Ossareh-Nazari B, Bonizec M, Cohen M, Dokudovskaya S et al (2010) Cdc48 and Ufd3, new partners of the ubiquitin protease Ubp3, are required for ribophagy. EMBO Rep 11(7):548–554
150. Ju JS, Fuentealba RA, Miller SE, Jackson E et al (2009) Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. J Cell Biol 187(6):875–888
151. Tresse E, Salomons FA, Vesa J, Bott LC et al (2010) VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBMPFD. Autophagy 6(2)
152. Vesa J, Su H, Watts GD, Krause S et al (2009) Valosin containing protein associated inclusion body myopathy: abnormal vacuolization, autophagy and cell fusion in myoblasts. Neuromuscul Disord 19(11):766–772
153. Ren J, Pashkova N, Winistorfer S, Piper RC (2008) DOA1/UFD3 plays a role in sorting ubiquitinated membrane proteins into multivesicular bodies. J Biol Chem 283(31):21599–21611
154. Qiu L, Pashkova N, Walker JR, Winistorfer S et al (2010) Structure and function of the PLAA/Ufd3-p97/Cdc48 complex. J Biol Chem 285(1):365–372
155. Janiesch PC, Kim J, Mouysset J, Barikbin R et al (2007) The ubiquitin-selective chaperone CDC-48/p97 links myosin assembly to human myopathy. Nat Cell Biol 9(4):379–390
156. Raman M, Havens CG, Walter JC, Harper JW (2011) A genome-wide screen identifies p97 as an essential regulator of DNA damage-dependent CDT1 destruction. Mol Cell 44(1):72–84
157. Franz A, Orth M, Pirson PA, Sonneville R et al (2011) CDC-48/p97 coordinates CDT-1 degradation with GINS chromatin dissociation to ensure faithful DNA replication. Mol Cell 44(1):85–96
158. Meerang M, Ritz D, Paliwal S, Garajova Z et al (2011) The ubiquitin-selective segregase VCP/p97 orchestrates the response to DNA double-strand breaks. Nat Cell Biol 13(11):1376–1382
159. Acs K, Luijsterburg MS, Ackermann L, Salomons FA et al (2011) The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. Nat Struct Mol Biol 18(12):1345–1350
160. Yamanaka K, Sasagawa Y, Ogura T (2012) Recent advances in p97/VCP/Cdc48 cellular functions. Biochim Biophys Acta 1823(1):130–137
161. Meyer H, Bug M, Bremer S (2012) Emerging functions of the VCP/p97 AAA-ATPase in the ubiquitin system. Nat Cell Biol 14(2):117–123
162. Uchiyama K, Kondo H (2005) p97/p47-mediated biogenesis of Golgi and ER. J Biochem 137(2):115–119
163. Meyer HH (2005) Golgi reassembly after mitosis: the AAA family meets the ubiquitin family. Biochim Biophys Acta 1744(3):481–492
164. Buchberger A (2006) Cdc48 (p97) and its cofactors. In: Mayer RJ, Ciechanover AJ, Rechsteiner M (eds) Protein degradation, vol 3: cell biology of the ubiquitin-proteasome system. Wiley-VCH, Weinheim, pp 194–211
165. Seeley ES, Kato M, Margolis N, Wickner W et al (2002) Genomic analysis of homotypic vacuole fusion. Mol Biol Cell 13(3):782–794
166. Sambade M, Alba M, Smardon AM, West RW et al (2005) A genomic screen for yeast vacuolar membrane ATPase mutants. Genetics 170(4):1539–1551
167. Ramadan K, Bruderer R, Spiga FM, Popp O et al (2007) Cdc48/p97 promotes reformation of the nucleus by extracting the kinase Aurora B from chromatin. Nature 450(7173):1258–1262
168. Dobrynin G, Popp O, Romer T, Bremer S et al (2011) Cdc48/p97-Ufd1-Npl4 antagonizes Aurora B during chromosome segregation in HeLa cells. J Cell Sci 124(Pt 9):1571–1580