Emerging functions of the VCP/p97 AAA-ATPase in the ubiquitin system

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The ATP-driven chaperone valosin-containing protein (VCP)/p97 governs critical steps in ubiquitin-dependent protein quality control and intracellular signalling pathways. It cooperates with diverse partner proteins to help process ubiquitin-labelled proteins for recycling or degradation by the proteasome in many cellular contexts. Recent studies have uncovered unexpected cellular functions for p97 in autophagy, endosomal sorting and regulating protein degradation at the outer mitochondrial membrane, and elucidated a role for p97 in key chromatin-associated processes. These findings extend the functional relevance of p97 to lysosomal degradation and reveal a surprising dual role in protecting cells from protein stress and ensuring genome stability during proliferation.

VCP/p97 (also called Cdc48, CDC-48 or TER94 in other organisms) was originally discovered in vertebrates because of its sheer abundance, which foretells its broad cellular role[1]. Although the first identified activities for p97 in organelle membrane dynamics and ubiquitin-mediated protein degradation initially seemed contradictory, p97 is now known to be involved in a large number of independent cellular processes[2,3]. Through its ability to structurally remodel ubiquitinated client proteins and edit ubiquitin modification with the help of associated cofactors, it introduces an important additional level of regulation and plasticity to ubiquitin-mediated processes[3–5]. These activities regulate a myriad of pathways governing cellular physiology and are relevant in disorders as diverse as cancer and degenerative diseases[6,7]. In this review, we will discuss basic principles of the p97 system and then highlight emerging cellular roles that illustrate its relevance in health and disease.

A protein-remodelling machine and a hierarchical system of cofactors

p97 belongs to the hexameric AAA (ATPases associated with diverse cellular activities) family of proteins with two ATPase domains, D1 and D2 (refs 5,8,9; Fig. 1a). As such, it can convert the energy of ATP hydrolysis to structurally remodel or unfold client proteins[10]. Structural models of how it may achieve this are discussed in detail elsewhere[8,9]. ATP hydrolysis in D2 seems to generate the main driving force, because mutations that abrogate ATP binding or hydrolysis result in dominant-negative variants that bind but cannot release substrates[8,9]. A globular N-domain that resides at the periphery of D1 is essential for substrate binding. It can stabilize unfolded proteins, may regulate ATP hydrolysis and even couple substrate and adaptor binding to ATP hydrolysis[8,9].

p97 associates with a large number of interaction partners and protein cofactors, and these interactions are key for its activity and functional diversity[11,12]. The largest family of cofactors are proteins containing a ubiquitin-X (UBX) domain or UBX-like domain[11]. These domains assume a ubiquitin fold and interact with the N-domain of p97 (refs 11,12). However, other domains or shorter interaction motifs have been discovered that bind to the C-terminal tail or the N-domain, respectively[11,12] (Fig. 1b). So far, it is unclear if and how a particular type of interaction relates to the specific function of a cofactor.

It was initially suggested that p97 might employ a distinct cofactor for each reaction[13,14]. This model was extended to a hierarchical system, where p97 forms core complexes with mutually exclusive major cofactors, including the Ufd1 (ubiquitin fusion degradation 1)–Npl4 (nuclear protein localization homolog 4) heterodimer, p47 or UBXD1 (refs 11,15–20). Major cofactors have been shown to modulate p97 activity or govern assembly of additional cofactors[21,22]. Each core complex can then act in several pathways by associating with alternative sets of accessory proteins that determine localization or provide additional enzymatic activities[11,12]. Consistently, a recent mass spectrometry approach has identified additional interaction partners that bind either Ufd1–Npl4 or p47, or form independent complexes with p97 (ref. 19). Many of these cofactors contain distinct dedicated ubiquitin-binding domains, suggesting that they function as ubiquitin adaptors for p97 (refs 11,12,23,24; Fig. 1). In addition, p97 itself also has ubiquitin-binding activity and interacts with unmodified stretches of the substrate[25–27]. Thus, substrate interaction (and the contribution and specificity of ubiquitin-binding activities in substrate recognition and processing) is likely to be complex.

VCP/p97 as an additional layer in the ubiquitin system

Most studies link p97 to ubiquitin-dependent processes in which p97 complexes bind to ubiquitinated client proteins and then catalyse remodelling of the client structure to facilitate downstream steps[2–5] (Fig. 2a). At

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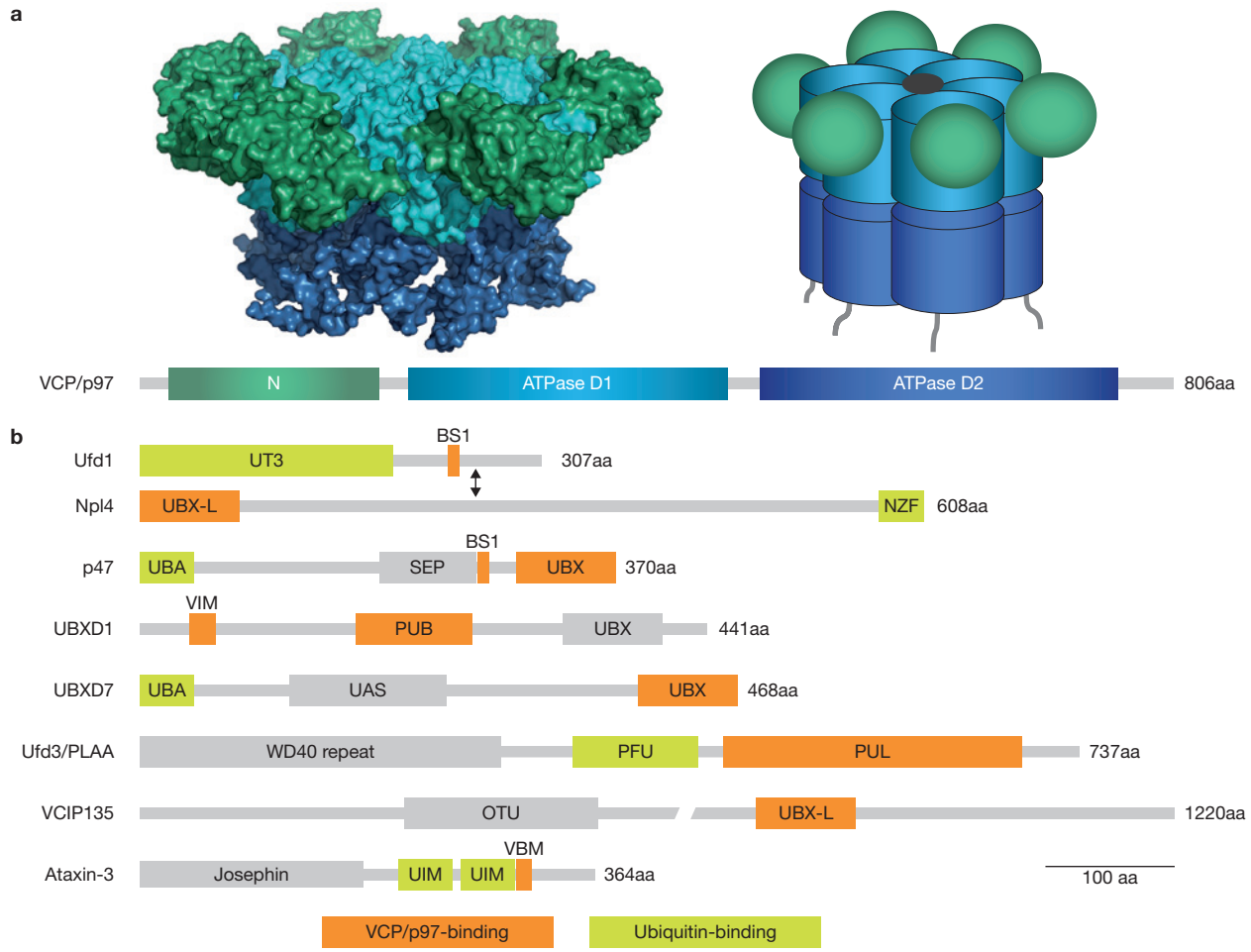

Figure 1 The hexameric AAA+ protein VCP/p97 and its cofactors. (**a**) Structure of VCP/p97. Each subunit consists of a globular N-terminal domain (green), the two AAA ATPase domains D1 (cyan) and D2 (blue), and a C-terminal tail (grey) D1 and D2 form two stacked hexameric rings. The N-terminal globular domain is positioned at the periphery of the D1 ring. ATP hydrolysis in D2 is believed to be the principal driving force to induce domain rearrangements in p97 that may be transduced to target substrates to promote their structural remodelling. Molecular visualization was generated by PyMOL software using the PDB id: 1R7R. (**b**) Domain structure of selected cofactors. UBX or UBX-like (UBX-L) domains, or short motifs including the binding site 1 motif (BS1), the VCP-interaction motif (VIM) or the VCP-binding motif (VBM), interact with the p97 N-domain. PUB (peptide N-glycosidase/ubiquitin-associated) or PUL (PLAA, Ufd3 and Lub1) domains bind the C-terminal tail. Ubiquitin-binding domains, such as the ubiquitin-associated (UBA), the Npl4 zinc finger (NZF) or the PLAA-family ubiquitin binding (PFU) domains are indicated. UT3, Ufd1 truncation 3 domain; SEP, Shp1-eyc–p47 domain; UAS, ubiquitin-associating. OTU (ovarian tumour) and Josephin domains have deubiquitinating activity. Numbers indicate amino acid positions.

least with the best-studied core complex p97–Ufd1–Npl4, remodelling triggers extraction of client proteins from complexes or cellular surfaces, often to facilitate degradation by the proteasome. Although this principle was first shown in the processing of membrane-anchored transcription factor precursors and then later for ER-associated degradation, where p97–Ufd1–Npl4 extracts polyubiquitinated substrates from the membrane into the cytosol for delivery to the proteasome[3,5,25,28] (Fig. 2b), it has now emerged as a unifying theme for p97 in degradation processes associated with chromatin or mitochondria[29–33] (Fig. 2c). In addition to substrate extraction, p97 has been proposed to assist the proteasome in unfolding a subset of substrates to facilitate their degradation[34]. Other core complexes that have been associated with monoubiquitination and proteasome-independent membrane trafficking events, including p97–p47 and p97–UBXD1, are proposed to catalyse slightly different remodelling reactions that involve membrane protein segregation rather than extraction[20,35] (Fig. 2d). The relevance of ATP-driven remodelling activity for a large population of substrates is illustrated by the cellular accumulation of ubiquitin conjugates after inhibition of p97 ATPase activity with either a D2 dominant-negative mutant or by chemical inhibition of p97 (refs 26,36).

The second function of p97 is to coordinate ubiquitination and downstream ubiquitin editing events (Fig. 2a). It binds several different E3 ubiquitin ligases directly or, in the case of cullin RING ligases (CRL), through the UBXD7/Ubx5 adaptor[19,37,38]. In addition, p97 recruits ubiquitin-chain editing factors for subsequent processing of the ubiquitin modification, including E4B/Ufd2, which can extend shorter ubiquitin chains[4]. Conversely, p97 binds a number of deubiquitinating enzymes, including VCIP135, Otu1, YOD1 or ataxin-3 (refs 15,39–43). Although the deubiquitinating enzymes are proposed to remove ubiquitin to promote substrate recycling[15,39], they can also act as positive regulators of protein degradation, possibly by shortening and removing branches to make the ubiquitin chains appropriate for proteasome recognition and subsequent degradation[40–42]. Although some studies suggest that p97 hands substrates over to the shuttling factors Rad23 and Dsk2 to deliver

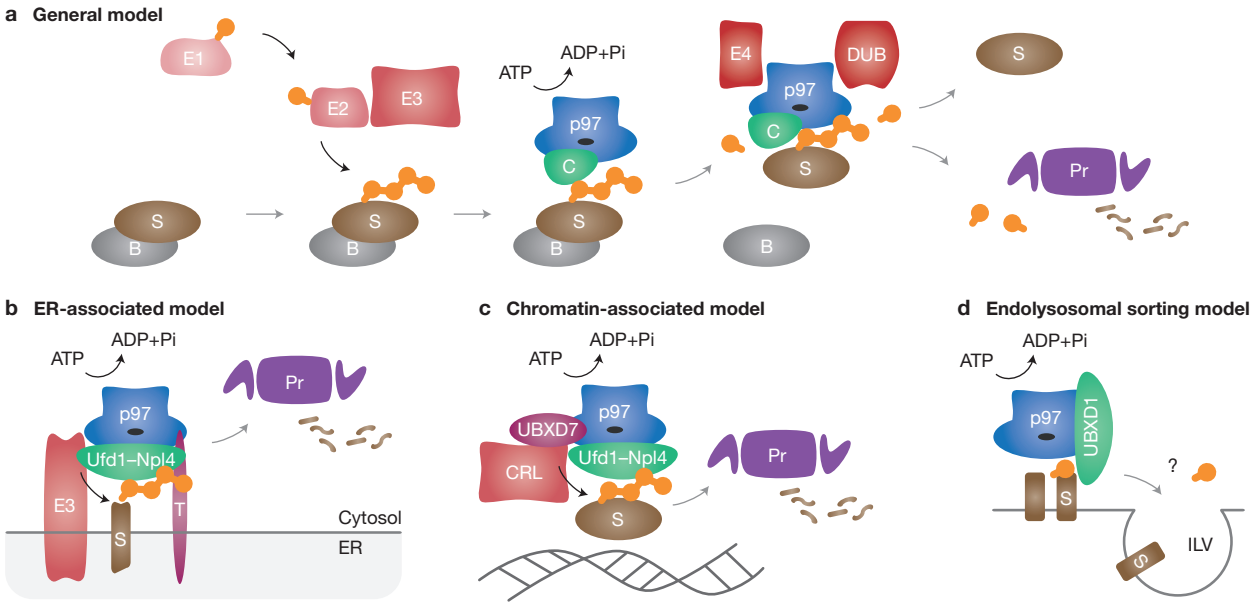

Figure 2 Models for p97 activity in the ubiquitin system. (**a**) General model for p97 in substrate segregation and ubiquitin chain editing. A target substrate (S) is post-translationally modified with ubiquitin (orange) by a cascade of the ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a specific ubiquitin ligase (E3). p97 binds to the ubiquitinated substrate with the help of ubiquitin-binding cofactors (C). p97 then converts the energy of ATP hydrolysis to structurally remodel the target in order to segregate it from binding partners (B) or cellular surfaces. Interactions with ubiquitin-editing factors, such as an E4 chain extension factor or deubiquitinating enzymes (DUBs), may help the ubiquitin modification process to either recycle the substrate or direct it to the proteasome (Pr) for degradation. (**b**) Function of p97 during ER-associated degradation. A substrate is ubiquitinated by an E3 ligase on the cytosolic side of the ER membrane. p97–Ufd1–Npl4 is recruited to the membrane by interactions with targeting factors (T). It then binds the substrate through the Ufd1-Npl4 cofactor and extracts it for degradation by the proteasome (Pr) in the cytosol. (**c**) Model for chromatin-associated degradation. A cullin-RING ligase (CRL) ubiquitinates a chromatin-bound substrate (S). The p97–Ufd1–Npl4 complex is recruited to the CRL by the UBXD7 adaptor and extracts the substrate from chromatin to allow degradation by the proteasome (Pr). (**d**) Speculative model for the role of p97 segregase activity during endolysosomal sorting. p97–UBXD1 is recruited to mono-ubiquitinated substrate oligomers (S) on endosomal membranes. It segregates the oligomers to allow sorting into forming intraluminal vesicles (ILV) for transport to the lysosome.

them to the proteasome[44], others suggest that p97 is required for substrate remodelling at the proteasome rather than delivery[30].

Protein quality control extending to the outer mitochondrial membrane

As a central element of the ubiquitin-proteasome system, p97 protects cells from the cytotoxic effects of damaged or misfolded proteins. As such, it is essential for cellular homeostasis, especially under stress conditions. p97 has a key role in endoplasmic-reticulum-associated degradation (ERAD) as reviewed in detail elsewhere[3,5,45]. Importantly, degradation of both ER luminal and membrane proteins requires p97, whose function in extracting substrates from the ER is essential for their delivery to the proteasome. Consequently, p97 inactivation elicits the unfolded protein response[46], which can trigger ER-stress-induced apoptosis. Although it is well-established that p97 facilitates degradation of individual proteins in the nucleus and cytosol in regulatory processes, much less is known about its relevance for protein homeostasis in these compartments. p97 was genetically shown to protect against the toxic effects of aggregation-prone protein species, including those with extended poly-glutamine (polyQ) stretches, and was found to localize to different types of cytosolic protein aggregates[47–50]. This suggests that p97 may help to disassemble aggregates and thus facilitate proteasomal degradation. However, the link could also be indirect, as polyQ aggregates induce the unfolded protein response in the ER, whereas ER stress aggravates the polyQ phenotype[51].

Although mitochondria have unique machinery to eliminate misfolded and unwanted proteins, proteins at the cytosolic side of the outer mitochondrial membrane are subject to ubiquitination and proteasomal degradation[52]. Recent reports now point to a critical role of p97 for the degradation of two unrelated outer membrane proteins, the anti-apoptotic factor Mcl1 and fusion protein mitofusin-1 (ref. 32). Similarly to ERAD, p97 binds the ubiquitinated membrane proteins and transports them to the cytosol for degradation. In yeast and *Caenorhabditis elegans*, this process is regulated by the cofactor Vms1 (ref. 33), which binds Cdc48 directly and forms a complex with Npl4. The hypothesis that the Vms1–Npl4 heterodimer is a mitochondria-specific cofactor explains its topologically similar function in this different compartment, although further confirmation is required. Tanaka and colleagues have also shown in mammalian cells that ubiquitination and extraction of Mfn1 by p97 prevents fusion of damaged and intact mitochondria, and thus facilitates packaging and disposal by autophagy[53,54]. Together, these findings suggest that p97 is central to protein quality control at the outer mitochondrial membrane, and also suggest that p97-mediated degradation may have a regulatory role in mitochondrial membrane fusion and removal of mitochondria through mitophagy.

Lysosomal degradation through autophagy and endolysosomal sorting

Although the key role of p97 in proteasomal degradation of a large number of client proteins has long been established, recent findings now link it to

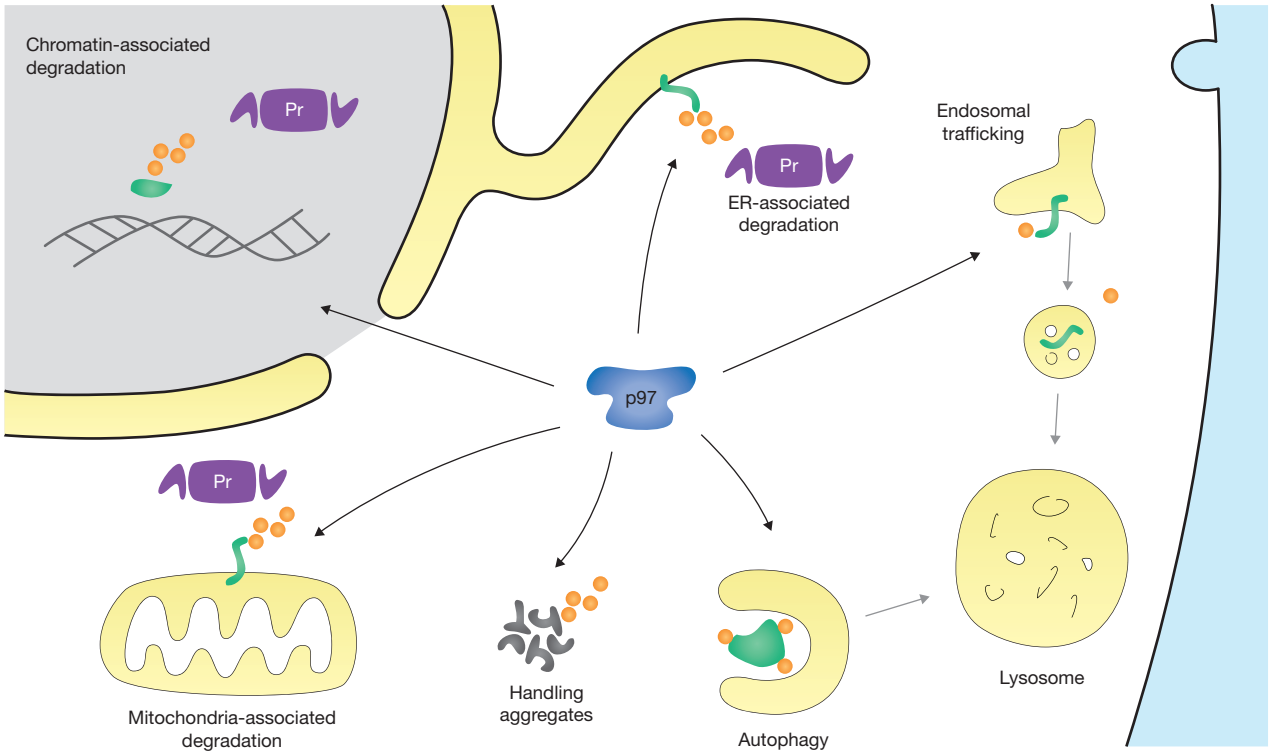

Figure 3 Emerging cellular roles of p97 in interphase. In addition to ER-associated degradation, p97 governs proteasome-mediated degradation of outer mitochondrial membrane proteins, as well as proteins associated with chromatin. It also helps degrade certain cytosolic proteins and handles protein aggregates. These activities help remove misfolded proteins at different locations to protect the cell from protein stress. In addition, they help eliminate regulatory proteins in various intracellular signalling pathways, including those that govern chromatin remodelling and DNA replication and repair. In parallel, p97 facilitates proteasome-independent degradation in the lysosome through controlling protein sorting in the endocytic pathway and by regulating autophagy. Not all cofactors or substrates in the individual pathways have been identified. Green, target substrates; Pr, proteasome; orange, ubiquitin.

the other major cellular degradative system, the lysosome. Interestingly, p97 functions in both ubiquitin-controlled entry gateways to the lysosome: the autophagy pathway and endolysosomal sorting. In general, autophagy is mediated by specialized membranes that engulf cytoplasmic components and subsequently fuse with the lysosome for degradation[55]. An important element in this process is lipidation of the ubiquitin-like modifier LC3/Atg8 (microtubule-associated protein 1 light chain 3α in humans/autophagy-related protein 8 in yeast), which decorates autophagosomal membranes. Adaptor molecules such as p62 and NBR1 then link LC3 to ubiquitin-labelled target structures to recruit autophagosomal membranes and promote engulfment. The first evidence for p97 in autophagy came from studies on the pathogenesis of inclusion body myopathy with Paget's disease of bone and frontotemporal dementia (IBMPFD), a disease associated with missense mutations in p97 (refs 56,57). The dominant feature in affected patient muscle tissue is LC3-enriched 'rimmed vacuoles' that are thought to represent aberrant autophagosomes[57]. This phenotype was reproduced in transgenic mice that overexpress a disease mutant of p97 (refs 57–59). Consistently, overexpression of p97 disease-associated mutants in cultured cells caused accumulation of p62 and lipidated LC3, and induced LC3-positive vacuoles that failed to mature[56,57]. These findings were confirmed by short interfering RNA (siRNA)-mediated depletion or pharmacological inhibition of p97 (refs 56,57,60). The underlying mechanism for these roles is unclear. In yeast, Cdc48 was found to cooperate with Ufd3 and Ubp3 in the autophagy of ribosomes[61] and to promote sealing of autophagosomal membranes around the target structures in cooperation with Shp1/p47 and Atg8 (ref 62).

The other main gateway into the lysosome is through the sorting of membrane proteins from the endocytic compartments. This is particularly relevant for ubiquitinated signalling receptors that are endocytosed from the plasma membrane, sorted at the endosome and packaged into intraluminal vesicles of multivesicular bodies by a series of ESCRT complexes, leading to subsequent lysosomal degradation. p97 has been associated with the early steps of this process, as it binds the endocytic coat protein clathrin and the endosomal sorting factor EEA1 (early endosome antigen 1)[63,64]. Inhibition of p97 affects the oligomerization state of EEA1, suggesting that it structurally remodels EEA1 oligomers to regulate endosome size and sorting[64]. Evidence from yeast implicated the Cdc48 cofactor Ufd3 further downstream in the trafficking of lysosomal cargo[65]. In contrast, a p97 core complex with the UBXD1 cofactor was found to be critical in mammals[20]. p97–UBXD1 is recruited to endosomes through ubiquitination of at least one cargo, the membrane protein caveolin-1, and then governs subsequent sorting of caveolin-1 to endolysosomes. Inactivation of p97 by dominant-negative mutations or pharmacological inhibition, or depletion of UBXD1, led to accumulation of caveolin-1 at the limiting membrane and inhibition of multivesicular body formation[20]. It is therefore tempting to speculate that p97–UBXD1 segregates larger caveolin-1 oligomers to allow packaging into intraluminal vesicles (Fig. 2d). Importantly, other endosomal cargo proteins (including transferrin receptor and epidermal growth factor receptor), as well as antigen cross-presentation through the mannose receptor, are affected by p97 inhibition, suggesting a more general role for p97 in endolysosomal trafficking[20,64,66].

Similarly to autophagy, endolysosomal sorting is strongly affected by p97 mutations associated with IBMPFD, which abrogate p97 binding to UBXD1 and caveolin-1 and impair multivesicular body formation[20]. It is even possible that the defects in autophagy and endosomal sorting are connected, because impaired endosomal sorting affects autophagy, possibly indirectly[67]. In contrast, two studies found no evidence that the mutations impaired ER-associated degradation[20,56]. Although still controversial, this may suggest that impairment of lysosomal degradation, rather than the proteasome, may be most relevant for the disease pathogenesis. Indeed, disease-affected muscle exhibits severe mislocalization of caveolin, which may explain the pathogenesis of the myopathy in IBMPFD. It is also noteworthy that, like p97 mutations, mutations in the ESCRT component Chmp2B cause frontotemporal dementia with ubiquitin inclusions (FTD-U)[68], raising the possibility that impaired sorting in the endocytic pathway may also contribute to neuronal symptoms in IBMPFD patients.

Signalling and cell cycle regulation

The activity of p97 in the ubiquitin system is also important for controlling key intracellular signalling pathways[6,7]. Recent evidence has shown that in mammalian cells, p97 governs degradation of the HIF1α transcription factor in concert with the CRL complex CUL2–VHL and the UBXD7 adaptor to regulate the hypoxia response[19]. In *C. elegans*, CDC-48 cooperates with the CUL2 ligase in regulating levels of the transcription factor TRA-1 to control sex determination[69], and modulates longevity in cooperation with the ataxin-3 deubiquitinating enzyme[40]. p97 has also been connected to the regulation of cell proliferation and survival[6,7]. One possible link may be regulation of apoptosis through degradation of key apoptotic factors, such as Mcl1 or DIAP1 (refs 32,70). Consistently, pharmacological inhibition of p97 activates the intrinsic (rather than the extrinsic) apoptotic pathway within hours[60,71].

In addition, p97 is required at several stages of the cell cycle in different organisms. In budding yeast, Cdc48 helps to degrade the G1-CDK inhibitor Far1 to launch the cell cycle[72], whereas in *C. elegans*, CDC-48 and Ufd1–Npl4 are essential for efficient replication as depletion of the complex leads to activation of the DNA damage checkpoint that delays entry into mitosis[73]. During mitosis, p97 is required for faithful chromosomal segregation in HeLa cells and budding yeast, and consequently its inactivation leads to the spindle assembly checkpoint[74–78]. At least one pivotal target is the kinase Aurora B/Ipl1, which ensures bipolar attachment of chromatid pairs to the mitotic spindle early in mitosis. Although initial findings in HeLa cells suggested that the p97 cofactor Ufd1 was required for Aurora B recruitment to chromosomes[77], more recent work in HeLa cells and yeast indicate that p97/Cdc48 antagonizes Aurora B/Ipl1 and balances its kinase activity in this critical phase[75,76]. The observation of accumulation and persistence of Aurora B on chromosomes after Ufd1–Npl4 depletion in HeLa cells suggests the molecular mechanism is based on the p97-mediated removal of the kinase from chromatin. Indeed, ubiquitin-dependent extraction of Aurora B from chromatin was demonstrated *in vitro* in *Xenopus* egg extracts during later stages of mitosis, where p97 and Ufd1–Npl4 antagonizes the kinase to allow chromatin decondensation and nuclear envelope formation[29,79,80]. Of note, depletion of the CUL3 ligase or its Aurora-B-specific adaptors — similarly to Ufd1–Npl4 inactivation — causes persistence of Aurora B on chromatin and segregation defects in HeLa cells. This suggests that p97 and CUL3 cooperate to regulate Aurora B during mitosis[81].

Chromatin-associated functions

The extraction of Aurora B from mitotic chromosomes exemplifies a more general role for the p97–Ufd1–Npl4 core complex in chromatin-associated functions. This is suggested by previous reports on p97 interactions with DNA-repair factors BRCA1 (ref. 82), the Werner syndrome protein WRN (ref. 83) and DNA unwinding factor DUF (ref.84), and the fact that p97 is phosphorylated after DNA damage[85]. More recently, Cdc48–Ufd1–Npl4 was shown in yeast to remove the ubiquitinated transcriptional repressor α2 complex from promoter elements in a proteasome-independent manner during mating-type transition[31]. In addition, Cdc48 mediates removal of the RNA polymerase (Pol) II complex when it is stalled at UV-induced DNA lesions[30]. In this case, a Cul3-based ubiquitin ligase modifies Rpb1, the main subunit of Pol II. Cdc48–Ufd1–Npl4 is then recruited to Cul3 by the Ubx5 adaptor and cooperates with Ubx4 to extract Rpb1 for degradation by the proteasome.

Two other reports revealed links to the regulation of DNA replication through the control of the replication licensing factor Cdt1 (refs 86,87). The first study applied a genome-wide screen for factors involved in DNA-damage-induced degradation of Cdt1 in HeLa cells, and identified p97–Ufd1–Npl4 in addition to the CRL ligase complex CUL4–DDB1–CDT2 (ref. 86). Critically, Cdt1 degradation by the CRL4 and p97 complexes depended on interaction of Cdt1 with the DNA clamp PCNA (proliferating cell nuclear antigen) on chromatin both in HeLa cells and *in vitro*, highlighting the role of p97–Ufd1–Npl4 in extracting factors bound to chromatin rather than promoting degradation in solution. Interestingly, p97 depletion stabilized Cdt1 in untreated cells, suggesting that p97–Ufd1–Npl4 governs Cdt1 degradation not only to block replication after DNA damage but also in S phase to prevent re-licensing and over-replication[86]. Intriguingly, the second study found no evidence for CDC-48 and Ufd1–Npl4 involvement in S-phase degradation of CDT-1/Cdt1 in *C. elegans* embryos[87]. Instead, experiments both in *C. elegans* and *Xenopus* egg extract suggest that CDC-48/p97 helps to remove ubiquitinated Cdt1 from mitotic chromatin. Cdt1 removal and the disassembly of the licensing complex (including CDC-45 and the GINS complex) triggers elongation of the replication fork, and thus provides an explanation for the requirement of CDC-48 and Ufd1–Npl4 for replication in *C. elegans*. Both studies differ in the timing of p97-mediated degradation of Cdt1 and its proposed relevance. This may be due to differences in organisms or developmental stages, as Ufd1–Npl4 depletion also does not block replication in HeLa cells[76]. Importantly, however, both studies concur on a key role of p97–Ufd1–Npl4 in extracting Cdt1 from chromatin.

Whereas Cdt1 and Rpb1 degradation is induced by DNA damage to control replication and transcription, recent data show that the p97–Ufd1–Npl4 core complex has an integral role in the repair of radiation-induced DNA double-strand breaks[88]. Damage signalling involves extensive ubiquitination at break sites that is initiated by the ubiquitin ligase RNF8 and extended by RNF168-generated lysine-63-linked chains further downstream[89]. The p97–Ufd1–Npl4 complex is recruited to double-strand breaks, but not by RNF168 and lysine-63 ubiquitin chains[88]. Instead, p97 is recruited to previously unappreciated lysine-48 conjugates that are generated in an RNF8-dependent manner, probably by additional ligases[88,89]. p97 then mediates removal of the lysine-48-conjugated substrate proteins to allow proper assembly of downstream signalling factors, including Rad51, BRCA1 and 53BP1 (ref. 88). Although the modified proteins and other critical contributing ligases await identification, the data have established that p97- and

ubiquitin-mediated remodelling of signalling complexes at the damage site is essential for subsequent repair of the double-strand breaks and cellular survival after gamma irradiation. Together, these new findings reveal roles of p97–Ufd1–Npl4 in fundamental chromatin-associated processes that ensure proper transcription, replication and stability of the genome, and indicate that there are probably more to be discovered.

VCP/p97 in disease

The wide-ranging functions of p97 suggest a diverse involvement in disease. Augmented p97 expression correlates with poor prognosis of certain cancers[6,7], whereas p97 also prevents aneuploidy and genomic instability[75–77], which are hallmarks of cancer cells. In addition, p97 has also been associated with various aggregation diseases, as it attenuates the cytotoxic effects of misfolded proteins or amyloids and regulates protein homeostasis[6,7]. As stated above, p97 missense mutations cause the degenerative late-onset disorder IBMPFD that affects various tissues[90], but a subset of mutations are also associated with 1–2% of amyotrophic lateral sclerosis (ALS) cases[91]. The pathological and clinical aspects are reviewed in detail elsewhere [57,92,93]. On the molecular level, it is noteworthy that the mutated residues are located at the interface between N and D1 domains[94], which might lead to deregulated ATPase activity[95,96], although alternative explanations are also possible[97,98]. Disease-associated mutations in p97 specifically abolish binding to only a subset of cofactors[20,98], consistent with late onset of the disease and the notion that essential functions mediated by Ufd1–Npl4, for example, are not affected. Although some studies report a defect in proteasomal degradation[97,99], others suggest that lysosomal degradation, rather than proteasomal degradation, is impaired by mutant p97 and that this contributes to the pathogenesis[20,56,100].

Conclusions and outlook

A vast body of work has established p97 as a principal and versatile element of the ubiquitin system. p97 helps to deliver protein substrates to the proteasome in quality control and regulatory pathways, but also governs proteasome-independent processes (Fig. 3). Indeed, recent findings have extended its involvement to lysosomal degradation and regulation of chromatin-related events such as DNA repair. This work cements p97 as a fundamental element of cellular physiology that maintains cellular homeostasis, contributes to genomic stability and governs important signalling pathways. Extraction and segregation of ubiquitinated proteins has emerged as a common theme in p97-mediated reactions, but much remains to be learned about how p97 converts the energy of ATP hydrolysis within the hexamer and transduces it, possibly though cofactors, onto client proteins. At the cellular level, identifying the whole range of p97 substrates will promote understanding of how this system is relevant for cellular physiology. Similarly, characterizing the distinct p97–cofactor complexes, and understanding how their hierarchical assembly and activity is regulated in different cell states and subcellular locations, will be essential for a comprehensive view of p97 function. It is also important to understand the activities and significance of p97 in different developmental stages and cell types, particularly cancer cells. Its central role in signalling, DNA repair and cell-cycle control make it a prime target for drug development, but the full value of p97 as a drug target depends on the development of additional pharmacological tools to modulate p97 activity, as well as a fuller understanding of its myriad cellular functions.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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