p97 and close encounters of every kind: a brief review

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Abstract

The AAA (ATPase associated with various cellular activities) ATPase, p97, is a hexameric protein of chaperone-like function, which has been reported to interact with a number of proteins of seemingly unrelated functions. For the first time, we report a classification of these proteins and aim to elucidate any common structural or functional features they may share. The interactors are grouped into those containing ubiquitin regulatory X domains, which presumably bind to p97 in the same way as the p47 adaptor, and into non-ubiquitin regulatory X domain proteins of different functional subgroups that may employ a different mode of interaction (assuming they also bind directly to p97 and are not experimental artifacts). Future studies will show whether interacting proteins direct p97 to different cellular pathways or a common one and structural elucidation of these interactions will be crucial in understanding these underlying functions.

Introduction

p97 is a member of the AAA (ATPase associated with various cellular activities) ATPase family and is an essential protein, conserved throughout evolution from mammals to archaea. p97 may be thought of as a motor protein that binds to adaptor proteins and transfers energy from ATP binding and hydrolysis through the adaptor to participate in cellular functions such as Golgi reassembly [1], ubiquitin proteasome degradation [2] and spindle disassembly at the end of mitosis [3]. The mechanism by which p97 and its adaptors perform these tasks is poorly understood.

p97 is a homohexamer with subunits arranged in a ring with a pore in the centre (Figure 1) [4–8]. Each subunit is composed of an N-terminal domain (double ψ barrel/β barrel) and two AAA domains (α/β subunit and α-helical subunit) that are responsible for nucleotide binding and hydrolysis; the structure of its C-terminal extension remains elusive. A plethora of proteins have been reported to associate with p97 (Figures 2 and 3). How p97 can interact with so many different proteins is perplexing. It is important to note that the yeast homologue of p97, Cdc48, can recognize denatured proteins and this could give rise to non-specific binding in biochemical interaction experiments [9]. For the archaeal p97 homologue VAT, no interacting proteins have been reported to date. Only one adaptor interaction, that of p97–p47, has been studied structurally [10–13] and much information is still required to reveal how p97 associates with different adaptors and functions in so many different activities. We aim, in this review, to collate the information available regarding these interactions. Also we report a summary of what is known structurally of these adaptors to observe any common features that may be present (Figure 3).

Key words: adaptor, Cdc48, interactions, p97, valosin-containing protein (VCP).

Abbreviations used: BRCA1, Breast cancer type 1 susceptibility protein; UBX, ubiquitin regulatory X domain; Cui, Cdc48p UBX-containing interactor; DUF, DNA unwinding factor; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; polyQ, polyglutamine repeats; ex-polyQ, expanded polyQ; HDAC-6, histone deacetylase 6; SVIP, small VCP interacting protein; SYT, synaptotagmin; TB-RBP, testis brain RNA-binding protein; UBA, ubiquitin-associated domain; UN, ubiquitin fusion degradation 1 (Ufd1)–nuclear protein localization 4 (Npl4); VCIP135, valosin-containing protein [VCP][p97]/p47 complex-interacting protein, p135; WRN, Werner syndrome helicase.

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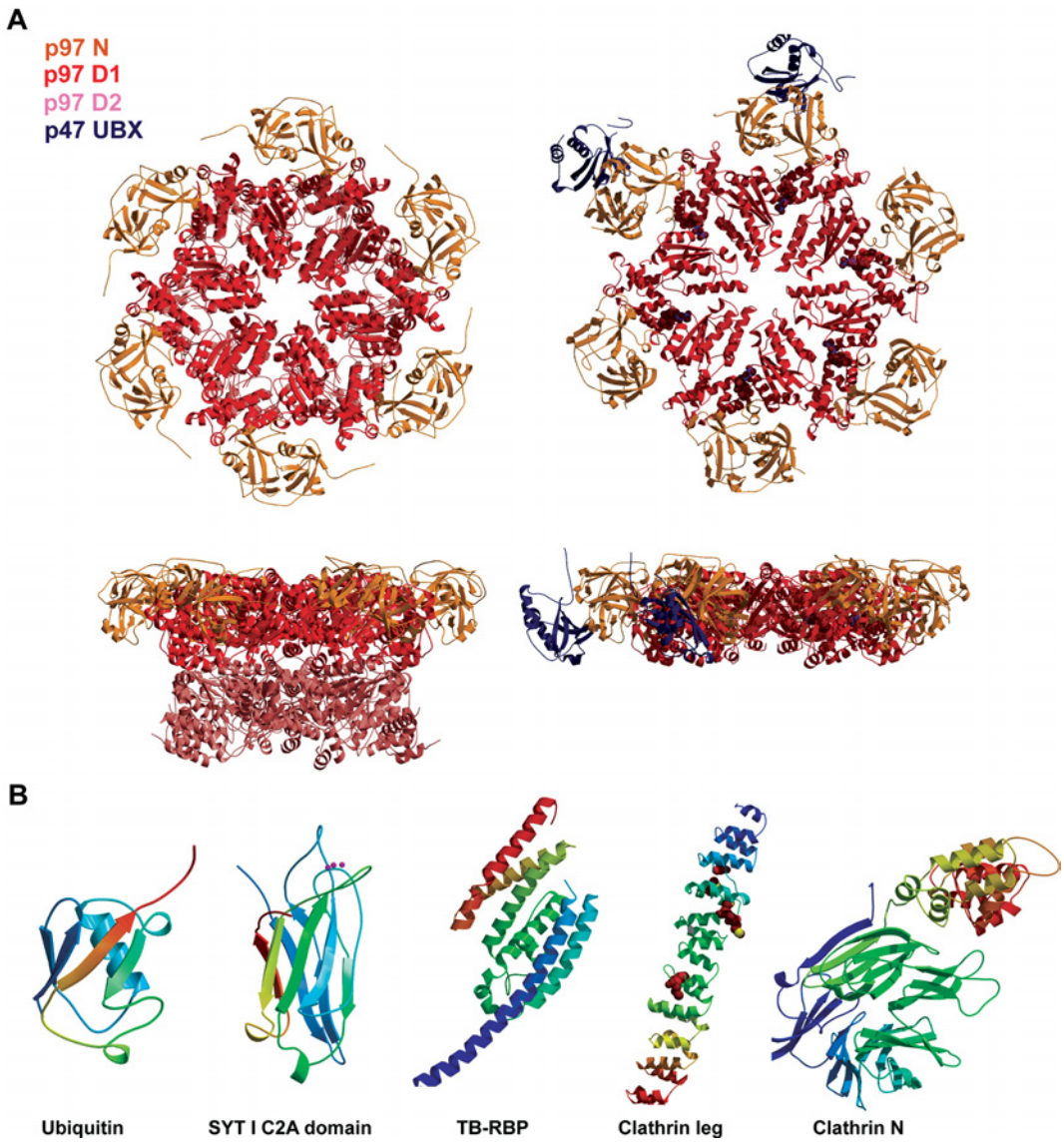
Adaptors containing a UBX (ubiquitin regulatory X) domain

The UBX is found in a variety of eukaryotic proteins and was suggested to function in ubiquitin-related processes [14]. A classification of different subfamilies of UBX-containing proteins has been proposed according to additional domains [14], but no common function has as yet emerged. From the analysis of the p97–p47-binding site, it was inferred that UBX, in general, could bind p97 [13].

The adaptor protein p47 plays an important role in homotypic membrane fusion events such as post-mitotic fusion of Golgi stacks [1] and the nuclear envelope growth [15]. The interaction between the t-SNARE syntaxinV and p97 is mediated by p47 [16] and interaction of its UBA (ubiquitin-associated domain) with ubiquitin has been reported [17]. p47 may also be involved in proteolytic ubiquitin-dependent processes [other than ERAD (endoplasmic reticulum-associated protein degradation)] [18], possibly delivering substrates to the 26 S proteasome. Atomic resolution structures of its three domains (UBA, Shp1-Eyc-p47 domain and UBX) have been solved [11,12] and the main p97-binding site for p47 has been well characterized [13]. p97 binding is mediated by a conserved loop region within the p47 UBX, which inserts into a hydrophobic pocket between the two subdomains of the N domain of p97. A second binding site N-terminal of the UBX was identified biochemically [19], which lies in a region of no apparent secondary structure in solution.

Figure 1 | Compilation of structures, p97 and p97-interacting proteins

(**A**) Ribbon representations of full-length p97 (pdb code, 1R7R) and p97 ND1 complexed with p47 UBX (pdb code, 1S3S) are shown in top and side views. (**B**) A gallery of protein structures that have been reported to interact with p97: ubiquitin (pdb code, 1UBI), SYT I C2A domain (pdb code, 1BYN), TB-RBP (pdb code, 1KEY) and clathrin heavy chain (Clathc) N-terminal domain and proximal leg (pdb codes, 1BPO and 1B89).

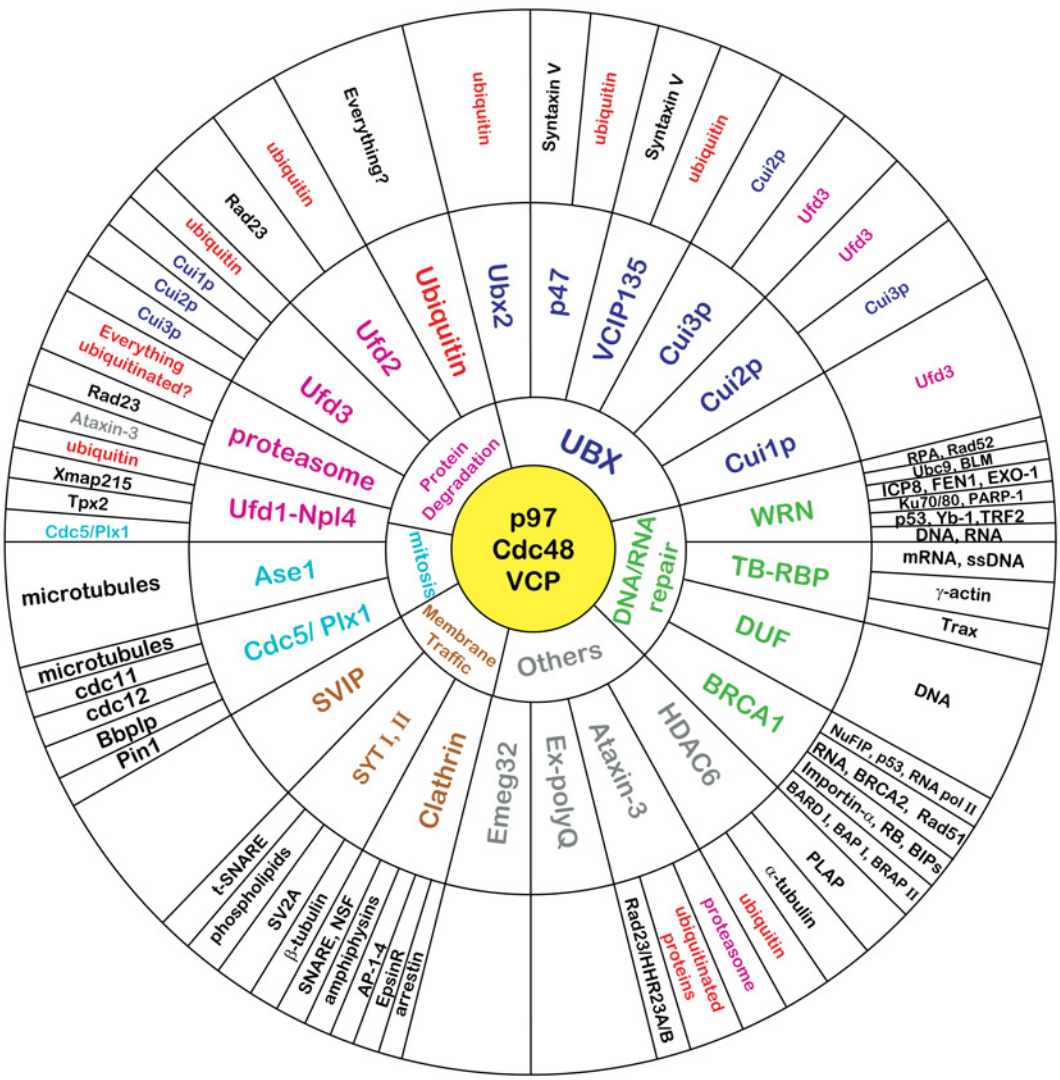


Another essential protein involved in the fusion events of organelles is VCIP135 (valosin-containing protein [VCP] [p97]–p47 complex-interacting protein, p135), this is believed to help disassemble the p97–p47–syntaxinV complex [19]. VCIP135 binds to p97 and syntaxinV and its activity was shown to be crucial for the reassembly of Golgi stacks after mitosis, which was independent of proteasome involvement [20]. It is a member of the otubain family of cysteine peptidases and acts as a deubiquitinating enzyme [20]. Sequence analysis predicts that it contains a UBX, which is suggested to be the main binding site for p97 [19].

Recently, four other proteins have been shown to interact with p97/Cdc48, representing examples from other UBX protein subfamilies. The yeast Cui (Cdc48p UBX-containing interactor) proteins, Cui1p, Cui2p and Cui3p, have been reported to interact with Cdc48 [21]. Cui2p and Cui3p are rather Y33K than p47-related. These proteins also interact with Ufd3 [21], a WD repeat protein associated with Cdc48p [22]. Diploid cells lacking these Cui proteins are defective in tetrad formation but their detailed function remains elusive. Lastly, Ubx2, found in yeast and humans, was identified to interact with Cdc48/p97 [18]. Cells lacking Ubx2 did not display any specific phenotype. Ubx2 displays some similarities with Fas-associated factor 1, as it is predicted to contain an N-terminal UBA, C-terminal UBX and a thioredoxin-like domain [18].

Figure 2 | Classification of p97 interacting proteins according to structural (UBX) or general functional criteria (protein degradation, mitosis, membrane traffic and DNA/RNA repair)

A non-exhaustive list of key interacting partners of these proteins has been added at the outer circle; where there is cross-interaction with one of the other p97 interactors the colour code has been maintained. Note the frequency of ubiquitin in the scheme.



Non-UBX interactors

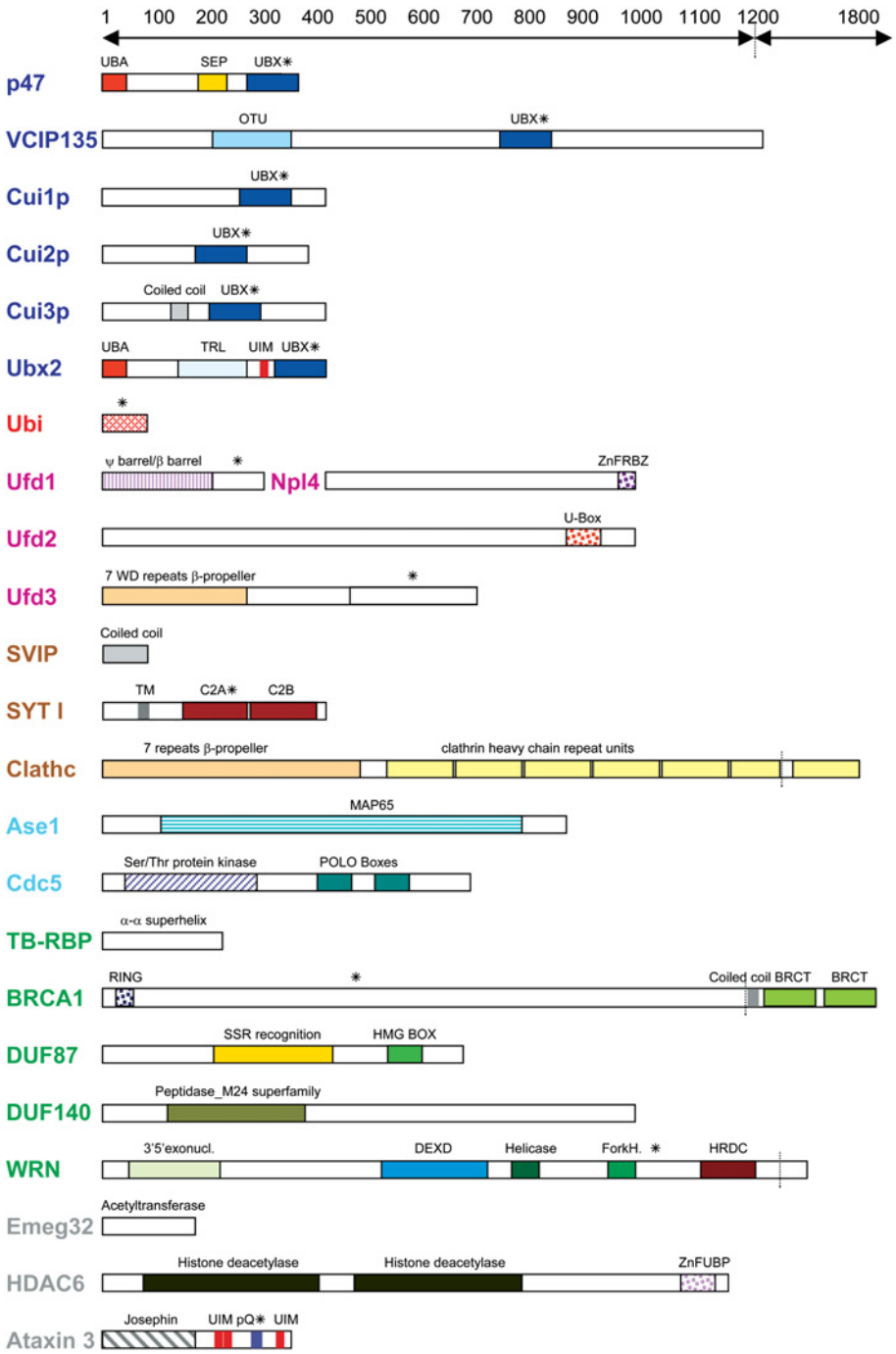
Ubiquitin fusion degradation pathways

It is essential for cells to degrade misfolded and misassembled proteins for normal growth and metabolism. Misfolded and unassembled polypeptides that cannot reach their native conformation in the ER are translocated into the cytosol where they are covalently modified with a poly-ubiquitin chain that targets them for degradation by the proteasome (ERAD). p97 has been shown to fraternize with various proteins involved in protein degradation.

One of the most studied adaptors of p97 is the Ufd1–Npl4 (UN) complex (where Ufd1 stands for ubiquitin fusion degradation 1 and Npl4 for nuclear protein localization 4) [2,15,17,23]. p97-UN is essential for the translocation of poly-ubiquitinated peptides from the ER to the cytosol [23]. p97-UN has also been shown to be involved in regulating spindle disassembly at the end of mitosis [3], assembly of nuclear envelopes [15] and dissociation of a membrane-tethered transcription factor from its binding partner that is also tethered on the membrane [24,25]. Both Ufd1 and Npl4 bind to ubiquitin [17,23]. Cdc48p also interacts with Ufd2 and Ufd3 [22,26]. Ufd2 belongs to the U-box family of ubiquitin ligases and probably functions as both an E3 and E4. Cdc48-Ufd2 has been suggested to function in the proteolysis pathway subsequent to the multi-ubiquitination

Figure 3 | Representation of the different subgroups of p97/Cdc48 interactors as cartoons showing their predicted and/or known domain structures

A star denotes the region of the protein that is mapped to interact with p97/Cdc48. Domain names and structural descriptions are mostly in agreement with the SMART database [50] nomenclature or else with the literature.



reaction [26,27]. Ufd3 has been reported to bind to 29-linked poly-ubiquitin chains [28] and is involved in controlling the cellular ubiquitin concentration [29].

Ubiquitin is an omnipresent feature in the interactions of p97 (Figure 2). p97 has been shown to bind mono-ubiquitin [25] but has a higher affinity for multi-ubiquitin chains [30]. When p97 is unable to function in the cell, there is an inhibition of ubiquitin–proteasome-mediated degradation that results in an accumulation of ubiquitinated proteins [30]. It is intriguing that both the N-terminal domains of p97 and Ufd1 are predicted to have a similar structure and bind to ubiquitin; perhaps this is achieved by a similar interaction.

The 26 S proteasome, the multisubunit complex that is crucial for the degradation of unfolded and misassembled peptides, is composed of a 20 S multicatalytic proteinase core and a 19 S cap-like regulatory complex. Dai et al. [31] propose that p97 is a component of the 19 S complex, but exact interactions of this association of p97 with the 26 S proteasome have not yet been reported.

Mitotic spindle disassembly

Recently, a role for p97 and its UN cofactor in the spindle disassembly process that accompanies the mitosis-to-interphase transition has been identified [3]. The absence of p97-UN severely affected spindle morphology. A number of microtubule-binding proteins transiently associate with p97-UN during this cell-cycle changeover. p97-UN sequesters the spindle assembly factors XMAP215, TPX2, Plx1/Cdc5, Ase1 and probably others, possibly in a ubiquitin-dependent fashion, inhibiting their interactions with microtubules and resulting in the spindle morphology changes observed at mitotic exit.

Membrane trafficking

In addition to p47 and VCIP135, four other proteins have been reported to interact with p97 that are predicted to be important in membrane trafficking events. SVIP (small VCP interacting protein) is associated with membranes presumably through its lipid modifications [32]. No specific function has been assigned to SVIP, although overexpression results in cell vacuolization, and its localization with ER and Golgi membranes suggests that it may participate in ERAD and/or Golgi reassembly [32]. p97 has been shown to interact with clathrin, which is the structural protein of coated membranes involved in receptor-mediated endocytosis and aspects of Golgi sorting in eukaryotic cells [33]. Also, SYT (synaptotagmin) I and II have been found to bind p97 in high Ca^{2+} concentrations [34]. SYTs are thought to function in membrane trafficking and the C_2 domains mediate SYT's functions by binding to specific targets. SYT I is required in Ca^{2+} triggering of exocytosis.

Nucleic acid repair and replication

p97 has been found to be associated with four nucleic acid-binding proteins, although a direct function with p97 is yet to be defined. p97 interacts with DUF (DNA unwinding factor, DUF140 and DUF87 subunits) in somatic cells and in *Xenopus* egg extracts [35]. DUF unwinds duplex DNA and is involved in DNA replication. TB-RBP (testis brain RNA-binding protein) interacts with p97 [36] and is expressed in brain and testis. TB-RBP recognizes specific mRNA sequence elements and ssDNA [37], and is implicated in DNA recombination and repair events. The nucleolar physical interaction between WRN (Werner syndrome helicase; gene responsible for Werner's syndrome encoding a protein homologous with *Escherichia coli* RecQ) and p97 has been reported [38,39]. Werner's syndrome is a rare autosomal recessive disorder characterized by premature aging. WRN is an ATP-dependent helicase, with exonuclease and DNA and RNA unwinding activities; it has been suggested that p97 may play a role in modulating the nuclear–nucleolar trafficking of WRN. Finally, a screen for interacting partners of BRCA1 (Breast cancer type 1 susceptibility protein) revealed p97 to co-immunoprecipitate [40]. The BRCA1 gene encodes a protein supposed to participate in DNA repair, transcription and transcription-coupled DNA repair and exhibits ubiquitin conjugation activity [41]. Mutations within the BRCA1 gene are found to coincide with a high proportion of cases of breast cancer.

Others

p97 has been found to be associated with a number of other proteins which cannot easily be grouped, although there still may be a link as they play a role in neurodegenerative diseases. Huntington's, Machado-Joseph disease and spinocerebral ataxias are characterized by the aggregation of certain polyglutamine proteins [naturally occurring polyQ (polyglutamine repeats) are expanded in certain proteins, ex-polyQ (expanded polyQ)] in nuclear inclusions, leading to neuronal cell death. Ubiquitin, proteasomal components and p97 have been found within ex-polyQ aggregates. Pull-down assays showed that p97 alone specifically bound to the ex-polyQ repeat of ataxin 3 (Machado-Joseph disease protein) with binding affinity directly proportional to the length of polyQ expansion [42]. Ataxin-3 probably serves as a proteasome-associated factor that mediates the degradation of ubiquitinated proteins [43].

Ex-polyQ has been shown to sequester several histone acetyltransferases and HDAC (histone deacetylase) inhibitors ameliorated aggregate formation [44]. p97 also binds to mouse HDAC-6 [45]. HDAC-6 is a nucleo-cytoplasmic shuttling, ubiquitin-binding protein which deacetylates lysine residues [45] and is found in the aggresome [46]. Emeg32 has glucosamine-6-phosphate acetyltransferase activity and associates with p97 localizing at the cytoplasmic side of Golgi and other intracellular membranes [47].

Conclusions

Although there are still many missing pieces in the p97 puzzle, such efforts to consolidate the current knowledge yield striking common denominators. On a structural basis, the p97 interactors can be grouped into UBX and non-UBX containing proteins. On the functional side, UBX and non-UBX proteins loosely belong to four groups (Figures 2 and 3) according to their proposed and established functions, namely protein degradation (UN, Ufd2, Ufd3, ubiquitin, proteasome, Cui1–3 and p47), mitotic spindle disassembly (UN, Ase1 and Cdc5), membrane traffic and fusion (SVIP, clathrin, SYTs, p47 and VCIP135) and proteins that are involved in either nucleic acid repair or replication (BRCA1, DUF, TB-RBP and WRN). Apart from the UBX-binding mode, there is little information on the structural basis of the other interactions, consistently though binding seems to be mostly independent of ATP and involves the p97 N domains. It should be noted that some of the interactions reported could be due to p97 binding unfolded proteins in general

or due to indirect binding, also proteins classified here as non-UBX may contain a similar fold which has not yet been confidently predicted or determined.

The diversity of additional domains in UBX-containing proteins suggests that there might not be one common function for UBX, but that it rather functions as a p97-binding module. p47 alone seems to be involved in membrane fusion and protein degradation. p97 and many other p97-interacting proteins associate with ubiquitin (Figure 2), which apart from its well-known role in proteasomal targeting can also serve as a signal for various other processes [48]. Ubiquitin has the same fold as the UBX [11,14] but residues that are important for p97 binding are poorly conserved or lacking in ubiquitin, which may result in a different binding mode [13]. There seems to be a tendency for p97 to interact with helical structures (TB-RBP, UFD3 and SVIP). This could represent another major structural adaptor motif for p97 binding and could be parallel to the interaction of the p97 homologue, NSF, with its α-helical adaptor α-SNAP (see [49] for a review). In addition, p97 also binds polyQ sequences (ataxin 3) and hydrophobic stretches of unfolded proteins [9]. Further studies will show whether the hydrophobic pocket between p97 N subdomains involved in p47 binding also plays a role in the latter interaction. Further functional and structural information is essential to elucidate all of p97 functions in cellular activities.

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