p97, a protein coping with multiple identities

Philip G. Woodman

School of Biological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK
(e-mail: philip.woodman@man.ac.uk)

Journal of Cell Science 116, 4283-4290 © 2003 The Company of Biologists Ltd
doi:10.1242/jcs.00817

Summary

A topic that is keeping cell biologists across several fields occupied is how the AAA ATPase p97 can have so many apparently unrelated functions. A recent model that proposed sets of adaptors for p97 selected according to the type of p97 activity seemed to afford a simple solution. For example, one known adaptor, the Ufd1–Npl4 complex, has been implicated in ubiquitin-dependent proteolysis whereas another, p47, is an essential co-factor for membrane fusion. However, further investigation has revealed that the situation is more complicated. Both Ufd1–Npl4 and p47 adaptors bind ubiquitin, and so their activities may be more closely related than first thought. A role for ubiquitin in p97-dependent membrane fusion is a particularly surprising development with no obvious explanation. However, some clues may be found from looking at the role of ubiquitin and the AAA ATPase Vps4 during sorting on the endocytic pathway.

Key words: AAA ATPase, Cdc48, Proteasome, Ubiquitin, SNARE

Structure and function of AAA proteins

Members of the AAA protein (ATPases associated with a variety of cellular activities) superfamily are involved in functions as diverse as proteolysis, cell cycle regulation, transcriptional activation, recombination, organelle biogenesis and vesicular transport (Confalonieri and Duguet, 1995; Ogura and Wilkinson, 2001; Patel and Latterich, 1998). A unifying theme is that they couple ATP hydrolysis to the unwinding, disassembly, unfolding or extraction of substrates. Hence, the primary signature of an AAA protein is the presence of one, or tandem copies, of the AAA ATPase domain. In addition, many AAA proteins have an N-terminal domain that contributes to the binding of the ATPase to its target(s)/effector(s).

AAA proteins are highly co-operative ATPases that form oligomeric structures (Hanson et al., 1997; Whiteheart et al., 1994). In the case of type II family members, which have two AAA domains, these oligomers are stable units; oligomeric assembly of type I members such as Vps4p (Babst et al., 1998) may be linked to nucleotide binding and substrate interaction. Among the most closely studied AAA proteins are the mammalian protein p97 [also called VCP (valosin-containing peptide)], its yeast homologue Cdc48p and its archebacterial homologue VAT (VCP-like ATPase) (Beuron et al., 2003; Dalal and Hanson, 2001; Rockel et al., 2002; Rouiller et al., 2000; Rouiller et al., 2002; Zhang et al., 2000). These form hexameric barrels in which there is six-fold symmetry and the two ATPase domains of each subunit are stacked. Structural information obtained from studies using different nucleotides in the solvent indicates that p97 undergoes rearrangements upon nucleotide binding and between partial reactions in the ATPase cycle. These include rotation of the AAA domains with respect to each other, closure and relaxation of the central cavity, and changes in the conformation and relative position of the N-terminal domain. How these structural changes are transmitted to alterations in the structure of p97 targets is not known and must probably await structural and biochemical studies of p97 in the presence of substrates.

The various activities of p97

The AAA family collectively has an enormous variety of targets, but p97 stands out by virtue of being associated with a range of functions itself (see Fig. 1). Only a few of these have been verified according to the strict criteria that the substrate has been identified and that a p97-dependent change in substrate status has been defined. This is important given that p97 is an abundant protein and that alterations in p97 activity might interfere with cellular ubiquitin homeostasis (see below) and have indirect consequences. Nevertheless, one may assume that, given time, most of the putative activities of p97 will be confirmed.

Proteolysis

Ubiquitin/proteasome-dependent proteolysis involves the covalent attachment of an 8 kDa polypeptide, ubiquitin, to lysine residues on the substrate protein (Hershko and Ciechanover, 1998; Hochstrasser, 1996; Weissman, 2001). The reaction is mediated by ubiquitin ligases. There are many of these within the cell, each having a narrow specificity for its substrates that is determined by structure or cellular location. Once conjugated, ubiquitin is often elaborated by further ubiquitylation. Proteins that have multi-ubiquitin chains are recognised by the 19S proteasome cap. Having bound to the proteasome, they are deubiquitylated prior to being unfolded by AAA proteins in the cap and then degraded in the 20S proteasome catalytic core (Hartmann-Petersen et al., 2002; Weissman, 2001).

Several observations link p97/Cdc48p with the ubiquitin/proteasome pathway, although the precise activity of p97 remains elusive. The link was established by Varshavsky and colleagues, who developed a genetic screen to identify components required for the degradation of yeast proteins with an artificial N-terminal ubiquitin fusion and isolated a set of ubiquitin fusion degradation mutants (*ufd1–ufd5*) (Ghislain et al., 1996). One of the corresponding genes, *UFD*3, encodes a

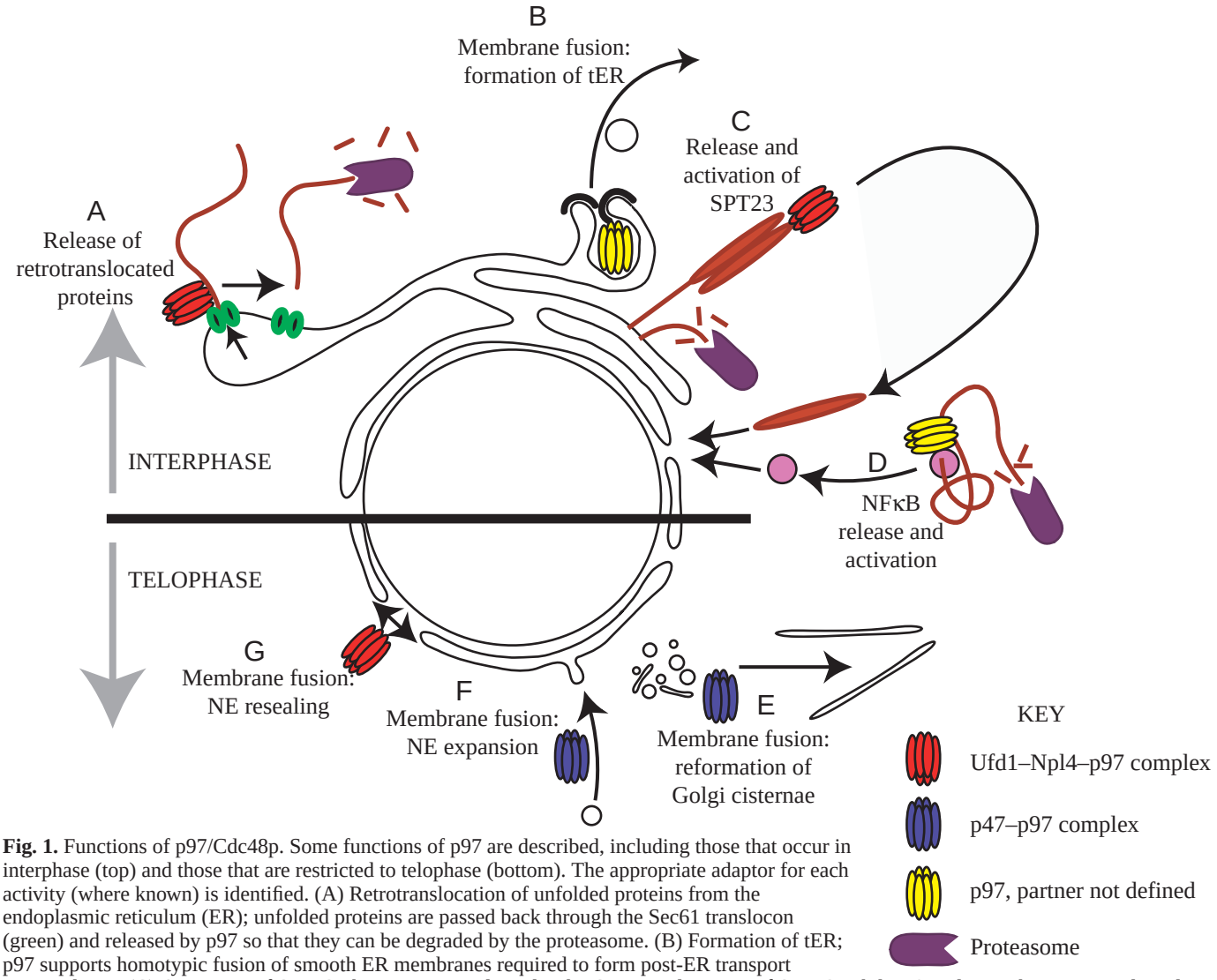

Fig. 1. Functions of p97/Cdc48p. Some functions of p97 are described, including those that occur in interphase (top) and those that are restricted to telophase (bottom). The appropriate adaptor for each activity (where known) is identified. (A) Retrotranslocation of unfolded proteins from the endoplasmic reticulum (ER); unfolded proteins are passed back through the Sec61 translocon (green) and released by p97 so that they can be degraded by the proteasome. (B) Formation of tER; p97 supports homotypic fusion of smooth ER membranes required to form post-ER transport intermediates. (C) Activation of SPT23; the proteasome degrades the C-terminal portion of SPT23, whilst p97 releases the N-terminal product from its full-length SPT23-binding partner so that it can be transported to the nucleus. (D) Nuclear factor κB (NFκB) activation; p97 cooperates in an unknown way with the proteasome to degrade the regulatory factor IκB, allowing NFκB (pink) to enter the nucleus. (E) Reformation of Golgi cisternae from tubulo-vesicular clusters occurs as the cell exits mitosis [note that another AAA protein, *N*-ethylmaleimide-sensitive fusion protein (NSF), is required in addition to p97 for full Golgi stack reassembly]. (F,G) Nuclear envelope reassembly; this occurs in two phases, resealing of the nuclear envelope (requiring Ufd1–Npl4–p97) and expansion (requiring p47–p97).

protein that interacts physically with Cdc48p. Moreover, a *cdc48* mutant has an impaired UFD pathway. Decisively, two further Ufd proteins bind to Cdc48p/p97 in both yeast and mammalian systems. One of these, Ufd2, enhances proteasomal degradation by elongating multi-ubiquitin conjugates (Koegl et al., 1999). The other, Ufd1, interacts stably with Npl4 (Meyer et al., 2000; Meyer et al., 2002; Rape et al., 2001), a protein previously implicated in maintaining nuclear envelope integrity (DeHoratius and Silver, 1996). It is the Ufd1–Npl4 complex that mediates many of the proteasome-related activities of p97 described below. Both Ufd1 and mammalian Npl4 bind to multi-ubiquitin chains, although the appropriate zinc finger domain within Npl4 is not conserved in yeast (Meyer et al., 2002; Wang et al., 2003; Ye et al., 2003).

Studies have confirmed the relationship between p97/Cdc48p and ubiquitin-dependent proteolysis of at least some cellular components. Firstly, p97 co-purifies with proteasomes (Dai et al., 1998), thus supporting a functional interaction. Secondly, p97 binds to and participates in the ubiquitin-dependent proteolysis of the transcription factor inhibitor IκB, thereby releasing and activating its binding partner, NFκB (Dai et al., 1998). It also influences the ubiquitin-dependent degradation of some other proteins (Dai and Li, 2001). Thirdly, the N-terminal domain of p97/Cdc48p binds ubiquitin directly, with a preference for multi-ubiquitin chains (Dai and Li, 2001; Rape et al., 2001; Ye et al., 2003). Collectively, these data suggest that p97 may be one of several factors that can target ubiquitylated proteins to the proteasome (Hartmann-Petersen et al., 2002). An unresolved question is whether p97 contributes to ubiquitin chain assembly itself by acting as a scaffold for, or regulating the activity of, components of the UFD pathway.

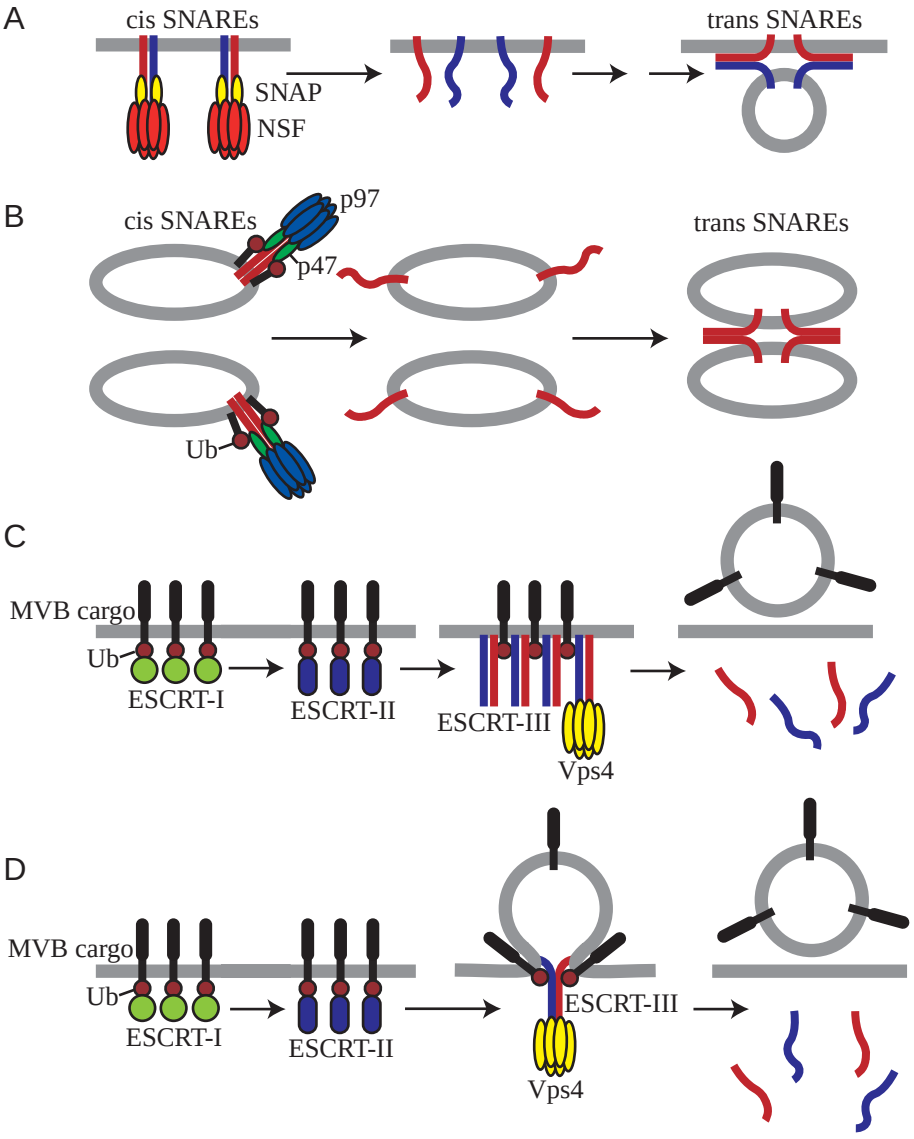

Fig. 2. AAA proteins involved in membrane fusion. (A) NSF (*N*-ethylmaleimide-sensitive fusion protein) acts to separate cis SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein proteins) complexes formed as a consequence of membrane fusion. The freed v-SNAREs (blue) are recycled so that they can be incorporated into new transport vesicles and thereby eventually form trans SNARE complexes with t-SNAREs (red) in the target membrane. (B) p97 separates t-SNARE complexes on post-mitotic membranes, allowing subsequent trans SNARE pairing and membrane fusion. Ubiquitinated cofactors may regulate SNARE disassembly by helping to recruit p47–p97. (C,D) Two possible modes of ubiquitin-dependent multivesicular body (MVB) formation. In C, ubiquitinated cargo recruits ESCRT-I, which recruits/activates ESCRT-II and ESCRT-III complexes, forming a domain in the endosome membrane that restricts cargo and selects it for incorporation into internal vesicles (ESCRT-III recruitment is linked to deubiquitination). The ESCRT-III complex is recycled by VPS4. In D, recruited ESCRT-III may act directly during internal vesicle invagination in an analogous way to SNARE-mediated membrane fusion. Again, VPS4 is required for ESCRT-III recycling.

Whether or not p97 contributes to the unfolding of proteasomal substrates in general is still unclear. However, one set of substrates that certainly are acted upon by p97 prior to proteasomal degradation are proteins that are retrotranslocated across the endoplasmic reticulum (ER) membrane. Polypeptide precursors to secretory and integral membrane proteins are translocated via the Sec61 translocon into the ER (Stirling et al., 1992), where they encounter a quality-control apparatus that ensures they are folded appropriately (Ellgaard and Helenius, 2001). Those that fail to fold are retrotranslocated to the cytosol, probably also via Sec61 (Tsai et al., 2002). Here, they are polyubiquitylated and targeted to the proteasome (Tsai et al., 2002). Removal of functional p97/Cdc48p stabilises misfolded luminal ER proteins (Bays et al., 2001; Braun et al., 2002; Jarosch et al., 2002; Rabinovich et al., 2002; Ye et al., 2001). Specifically, Ufd1–Npl4–p97 binds to the peptide as it emerges from the translocon and utilises ATP hydrolysis to extract it from the membrane (Ye et al., 2001; Ye et al., 2003). Presumably, this involves separating the peptide from the translocon or translocon-associated protein(s). This process may also be coupled to extension of the ubiquitin chains on the substrate, which would enhance their proteasomal degradation (Ye et al., 2001), although this is not yet fully resolved (Jarosch et al., 2002).

Jentsch and colleagues have delineated an additional processing pathway in yeast that resembles NFκB activation and may pertain to p97-mediated proteasomal degradation in other contexts (Hoppe et al., 2000; Rape et al., 2001). The transcriptional activator SPT23 is localised to the ER as a C-terminally anchored precursor and is processed by the proteasome, which selectively degrades the C-terminal portion. The remaining N-terminal 90 kDa product is released so that it can translocate to the nucleus. Intriguingly, processing of SPT23 requires its dimerisation. This may constrain the proteasome to ensure that processing of a single SPT23 chain is initiated from within an internal loop, so that only the C-terminal portion is degraded. The 90 kDa product remains ubiquitylated and is released from its full-length binding partner by the action of Ufd1p–Npl4p–Cdc48p. Hence, Cdc48p is required for coupling the degradation of one portion of the molecule with release of the other. The Ufd1–Npl4–p97/Cdc48p complex could therefore be more widely responsible for regulating the turnover of proteins that need to be released from larger complexes/structures.

Membrane fusion

Among the first activities ascribed to p97 was involvement in membrane fusion, where it is thought to perform a role

analogous to that of another AAA ATPase, NSF (*N*-ethylmaleimide-sensitive fusion protein). Fusion of transport vesicles with their appropriate target membrane is brought about by the binding of membrane-anchored v-SNAREs on the transport vesicle with corresponding t-SNAREs on the target membrane (McNew et al., 2000; Rothman, 1994). Each type of transport vesicle and each target membrane has a unique complement of v-SNAREs and t-SNAREs, respectively. The specificity of membrane fusion can thus be explained in part by the ability of appropriate combinations of SNAREs to form highly stable complexes (McNew et al., 2000; Weber et al., 1998) (since organelles contain both v- and t-SNAREs, homotypic fusion can be explained by the same model). SNARE complexes have a common structure, consisting of a parallel four-helix coiled-coil bundle that is stabilised by hydrophobic interactions between residues from each helix at every turn (layer) of the coil (Sutton et al., 1998). A polar layer at the centre of the bundle that contains three asparagine residues and an arginine residue, each provided by one SNARE helix, interrupts this pattern. Hence, SNARE partnerships conform to the 3 asparagine (Q) SNARE : 1 arginine (R) SNARE principle (Fasshauer et al., 1998). The formation of an arrangement of SNARE complexes between apposing membranes (trans complexes) may provide sufficient energy to drive fusion (Hu et al., 2003; Weber et al., 1998), and a host of chaperones and specificity factors act collectively to bring this about.

The role of NSF here is to use ATP hydrolysis to break apart SNARE complexes within the same membrane (cis complexes), which are naturally produced as a consequence of membrane fusion (Fig. 2A). This allows v-SNAREs and t-SNAREs to be reused for further rounds of transport (Otto et al., 1997; Söllner et al., 1993a; Söllner et al., 1993b; Swanton et al., 1998). NSF, which binds to SNARE complexes through its cofactor SNAP (soluble NSF attachment protein) (Clary et al., 1990), is virtually ubiquitous in the range of SNARE complexes it acts upon. p97 appears to have a similar role in disruption of SNARE complexes but, in contrast, acts only in a restricted set of homotypic fusion pathways, including a subset of fusion reactions required for the post-mitotic reassembly of the Golgi complex (Rabouille et al., 1995; Shorter and Warren, 2002) (Fig. 2B). In yeast, Cdc48p participates in homotypic fusion of the ER (Latterich et al., 1995). For both Golgi reassembly and yeast ER fusion, the probable ATPase target is a t-SNARE–t-SNARE pairing (syntaxin 5 and Ufe1p, respectively) (Patel et al., 1998; Rabouille et al., 1998). Neither syntaxin 5 nor Ufe1p homodimers fit the 3Q:1R rule. Hence, the precise make-up of these SNARE complexes, including the possible contribution of additional partners in vivo, remains unclear but might explain why they are not substrates for NSF.

Syntaxin 5 and p97 also contribute to the morphogenesis of a further component of the early secretory pathway. Both p97 (Zhang et al., 1994) and a low-molecular-mass form of syntaxin 5 (Hui et al., 1997; Rowe et al., 1998) are localised within the transitional elements of the ER (tER) and have been implicated in the formation of tubulo-vesicular ER–Golgi transport intermediates. Recent data suggest that p97 specifically acts in the homotypic fusion of smooth membrane elements within the tER (Roy et al., 2000).

Like NSF, p97 does not act alone upon its target during Golgi membrane fusion. An additional factor, p47, co-purifies with p97 (Kondo et al., 1997). It binds to the N-terminal domain of p97 through two contiguous sites and modulates the ATPase activity of p97 (Kondo et al., 1997; Meyer et al., 1998; Uchiyama et al., 2002; Yuan et al., 2001). Unlike the action of SNAP on NSF (Morgan et al., 1994), however, the effect of p47 on p97 is to inhibit its ATPase activity (Meyer et al., 1998). Consistent with it being essential for p97-dependent membrane fusion is the finding that p47 mediates the interaction of p97 with syntaxin 5 (Rabouille et al., 1998). Surprisingly, however, this complex is stable even in the presence of ATP, which suggests that additional factor(s) are required for disassembly. One such factor has now been identified: VCIP135 (VCP[p97]–p47 complex-interacting protein, p135) (Uchiyama et al., 2002). VCIP135 binds to syntaxin 5 but also competes with one of the p47-binding sites at the N-terminus of p97. It thereby participates in a displacement reaction that reduces the stability of the p47–p97 complex bound to syntaxin 5. Whether this releases the p47-mediated block on p97 ATPase activity is unresolved but is an attractive possibility. Whatever the case, the metastable complex can now release syntaxin 5 in an ATP hydrolysis-dependent reaction. A change in syntaxin 5 conformation is indicated by its inability to re-bind p47.

Kondo and co-workers have now identified a further way by which p47 regulates the membrane fusion activity of p97 to ensure that it acts preferentially post-mitotically (Uchiyama et al., 2003). Both p47 and p97 localise to the cytoplasm and nucleus, but p47 contains two functional nuclear localisation signals and the bulk of p47 is found in the nucleus of interphase cells. Hence, the concentration of p47–p97 available to modulate Golgi membrane dynamics is low (some p47 can be detected on the interphase Golgi, although this is apparently not essential for maintaining Golgi structure). When the cell enters mitosis, the nuclear envelope breaks down and p47 is released into the cytosol. Since the Golgi complex disassembles during mitosis it is essential that the p97-mediated fusion pathway does not operate. This is achieved by mitotic phosphorylation of p47, which can then no longer bind to syntaxin 5. Strikingly, microinjection of a non-phosphorylatable version of p47 prevents complete dispersal of the Golgi complex during mitosis and allows it to retain its stack organisation, underlining the contribution that mitotic phosphorylation of p47 makes to structural changes within the Golgi complex. Dephosphorylation of p47 during telophase permits Golgi reassembly, before p47 is returned to the interphase nucleus.

Two distinct steps in reformation of the nuclear envelope during exit from mitosis in higher eukaryotes also require p97 (Hetzer et al., 2001). Nuclear envelope reassembly occurs in several phases (Burke and Ellenberg, 2002). First, an ER-like network forms upon the surface of uncondensed chromatin. This is then remodelled to form a sealed nuclear envelope around the chromatin, a process that requires sheets of membrane to join by ‘annular’ fusion, which removes any holes in the double membrane. Finally, the nuclear pore expands by fusion of further ER-derived vesicles with the sealed sheet of nuclear envelope. Both the annular fusion and subsequent conventional fusion events require p97, although the former uses Ufd1–Npl4 and the latter p47. In neither case is the substrate for p97 clear.

And more…

Many additional functions for p97/Cdc48p have been described. These are not understood in detail, and some may be related to the activities described above. For example, *Cdc48* was first isolated as a cold-sensitive cell cycle mutant that fails to undergo nuclear division (Fröhlich et al., 1991). p97 is involved in lymphocyte stimulation, during which it is tyrosine phosphorylated (Egerton et al., 1992), and in signal transducer and activator of transcription (STAT)-mediated cell cycle progression (Shirogane et al., 1999). It may function in DNA replication, since it binds to a DNA-unwinding protein (Yamada et al., 2000). It also contributes to the formation of vacuoles in polyglutamine repeat-associated disorders (Hirabayashi et al., 2001).

How can the functions of p97 be resolved and how might these relate to other AAA proteins?

How can a single protein participate in processes as different as membrane fusion and proteasome-dependent proteolysis, not to mention additional, unrelated activities? Warren and colleagues have proposed a satisfyingly simple solution. They demonstrated that the binding of p47 and Ufd1–Npl4 to p97 is mutually exclusive. Moreover, they observed that Ufd1–Npl4 blocks Golgi reassembly in vitro (Meyer et al., 2000), whereas p47 inhibits retrotranslocation of proteins from the ER (Ye et al., 2001). Hence, it seems that p97 could exist in entirely different functional pools according to which 'adaptor' is bound to it. By extension, additional unrelated pathways might require novel adaptors, and this idea is supported by the recent isolation of SVIP (small VCP/p97-interacting protein), a protein involved in an additional, not fully characterised, ER function (Nagahama et al., 2003). The situation becomes more interesting, however, because it transpires that the p47 and Ufd1–Npl4 pathways are not as distinct as first thought.

Firstly, the p47 pathway requires a ubiquitin-like interaction. Although the primary sequences of p47 and VCIP135 are different, both contain ubiquitin-like UBX domains, which resemble the structure of ubiquitin and the ubiquitin-related proteins SUMO-1 and GATE-16 (Uchiyama et al., 2002; Yuan et al., 2001). The UBX domains are required for binding to p97, which presumably binds multi-ubiquitin chains through the same site. Note, incidentally, that GATE-16 binds to NSF, in combination with the v-SNARE GOS-28 (Sagiv et al., 2000). Hence, there may be further links between AAA proteins and ubiquitin-related pathways.

The similarities between the different p97 pathways are brought even closer, however, by the finding that p47 possesses a UBA (ubiquitin-pathway-associated) domain (Bertolaet et al., 2001) and binds ubiquitin (Meyer et al., 2002). Unlike Ufd1 and mammalian Npl4, p47 shows a marked preference for mono-ubiquitin, no doubt providing an important distinction between the p47 and Ufd1–Npl4 pathways. UBA domains are present in many proteins involved in ubiquitin-dependent processes, including two putative regulators of the metaphase–anaphase transition, Rad23p and Ddi1p (Bertolaet et al., 2001). These regulate the stability of the anaphase inhibitor Psd1p/securin, probably by preventing its multi-ubiquitylation and consequent degradation (Clarke et al., 2001). Like p47, both of these also have ubiquitin-like domains. In fact, Ddi1p was originally identified as a direct effector of the yeast v-SNAREs Snc1 and Snc2p (Lustgarten and Gerst, 1999). This association may have relevance to the p47–syntaxin 5 interaction.

The UBA domain of p47 is essential for Golgi reassembly in vitro (Meyer et al., 2002), and nuclear envelope reassembly requires both Ufd1–Npl4–p97 and p47–p97. This begs the question: how might ubiquitylation be involved in p97-mediated membrane fusion? One answer could be that p97 acts in fusion indirectly. Given the established role for the ubiquitin/proteasome system in releasing cells from mitosis (Peters, 2002), it can be speculated that p97 acts during post-mitotic fusion by ubiquitin-dependent processing/translocation of a regulatory factor(s). This is certainly an attractive possibility for explaining Ufd–Npl4–p97-dependent annular fusion during nuclear envelope reassembly, given the function of the Ufd1–Npl4 complex in other contexts. The p97–p47 complex could act in a similar fashion on distinct substrate(s). Alternatively, it might protect positive regulator(s) from p97–Ufd1–Npl4-dependent proteolysis, which would resemble the activity of Rad23 and Ddi1 in protecting Psd1/securin. In this context, it is interesting that p47 prevents multi-ubiquitylation of a substrate protein in vitro (Meyer et al., 2002).

Such an indirect involvement in fusion seems unlikely in the case of the p47–p97 complex, however, given the evidence for its direct interactions with syntaxin 5 and the close parallels between NSF and p97 function. The answer may instead lie in the role of ubiquitin or ubiquitin-like proteins in myriad non-proteasomal pathways, many of which involve monoubiquitylated substrates (Aguilar and Wendland, 2003; Hicke, 2001). Ubiquitin is used in many cellular signalling processes as a means to attach a recruitment domain transiently to a substrate in a regulated fashion. It is conceivable that some of these signalling events involve the recruitment of AAA ATPases.

A case in point may be endocytic sorting, which is one of the most actively studied areas of ubiquitin-dependent signalling (Hicke, 2001). Mono-ubiquitin or short ubiquitin chains provide signals for receptor internalisation (Hicke, 1997) and for sorting from the early endosome to the multivesicular body (MVB) pathway (Katzmann et al., 2002). The cargo itself is ubiquitylated, as are several accessory proteins (Haglund et al., 2002; Polo et al., 2002). Consequently, several soluble protein complexes implicated in MVB sorting recognise ubiquitin and are recruited to the endosome membrane during cargo transport. These include a complex containing Vps27p and Hse1p (Hrs and STAM in mammals), both of which contain UIMs (ubiquitin-interacting motifs) (Bilodeau et al., 2002; Polo et al., 2002). An additional complex termed ESCRT-I contains Vps23p (TSG101 in mammals), which binds to ubiquitin through an unrelated fold (Bishop et al., 2002; Garrus et al., 2001; Katzmann et al., 2001; Pornillos et al., 2002). ESCRT-I activates another peripheral membrane complex, ESCRT-II (Babst et al., 2002b). Interestingly, the yeast ESCRT-II component Vps36p (but not its mammalian homologue) possesses an Npl4 zinc finger domain (Meyer et al., 2002; Wang et al., 2003), which suggests that ubiquitin also recruits this complex. Finally, a further complex (ESCRT-III) composed of four proteins with extensive coiled-coil-forming domains is built on the endosome membrane and presumably plays a direct role in forming the internal vesicles of MVB from the limiting

membrane of the endosome (Babst et al., 2002a). This could provide a cytoplasmic coat to the endosome that delineates regions for internal vesicle formation (Babst et al., 2002a) (Fig. 2C). Alternatively, the ESCRT-III components might form complexes with each other analogous to the trans SNARE complexes required for vesicle–target membrane fusion. In conjunction with activities that mediate the inward deformation of membrane, they might thereby drive the capture of internal vesicles (Fig. 2D).

Like SNAREs, ESCRT-III components must be reused in order to drive further rounds of MVB formation. This recycling reaction is mediated by Vps4p, an AAA ATPase (Babst et al., 1998). Although the ESCRT-III proteins are the most likely substrates of Vps4p, ubiquitin is involved at least indirectly in the recruitment of this AAA ATPase by recruiting the ESCRT-I and ESCRT-II complexes. The primary role of ubiquitin here must therefore be to organise the structured assembly of a protein scaffold that drives MVB formation concomitant with the transit of cargo receptors. A passing consequence of this could be that it optimises the configuration of these components so that they are suitable substrates for Vps4p. It is a matter of conjecture whether ESCRT-II or other ubiquitin-interacting proteins could act as 'adaptors' for Vps4p to further enhance its recruitment.

In a parallel manner, ubiquitin (or perhaps a structurally related protein) could act as a positive regulator of syntaxin 5-mediated fusion and in doing so aid the recruitment of p97 through p47 (of course, this would not necessarily require that syntaxin 5 itself is ubiquitinated). Binding of p47 to ubiquitin would enhance the recruitment of the p47–p97 complex to syntaxin 5 and perhaps ensure its alignment with its substrate. Given that ubiquitylation plays an essential role in releasing cells from mitosis, it is by no means unreasonable to suggest that post-mitotic membrane fusion is regulated at some level by protein ubiquitylation. Involvement of ubiquitin in other fusion events is more speculative. However, ubiquitylation of factors required for tER formation could be related to the flux of cargo. In the case of p97-dependent homotypic ER fusion, ubiquitylation might be linked to remodelling of the ER during stress. Indeed, several ubiquitin ligases have been localised to the ER and are linked to stress responses (Haynes et al., 2002).

Conclusions

Links between ubiquitylated proteins and AAA ATPases were first established for components of the 19S proteasome. Recent developments in the p97 field have provided further mechanistic insights into this link, although outstanding issues remain. How is the ATPase cycle linked to substrate translocation/folding? To what extent does p97 participate in the ubiquitylation of substrates? How can p97 act upstream of the proteasome in some cases (e.g. retrotranslocation) and downstream of it in others (e.g. SPT23 processing)? Does the localisation of adaptors contribute more widely to the regulation of p97 function, as is the case for p47 regulation of Golgi membrane dynamics? The known roles of p97 are widespread and likely to expand. Further investigation of each pathway is likely to throw new light on the other activities of p97 and may offer exciting clues about the functions of other AAA ATPases in other ubiquitin-dependent pathways.

I would like to thank Viki Allan and Martin Lowe, University of Manchester, and Hemmo Meyer, University of Yale, for their helpful comments during the preparation of this article.

References

Aguilar, R. C. and Wendland, B. (2003). Ubiquitin: not just for proteasomes anymore. *Curr. Opin. Cell Biol.* **15**, 184-190.

Babst, M., Wendland, B., Estapa, E. J. and Emr, S. D. (1998). The Vps4p AAA ATPase regulates membrane association of a Vps protein complex required for normal endosome function. *EMBO J.* **17**, 2982-2993.

Babst, M., Katzmann, D. J., Estepa-Sabal, E. J., Meerloo, T. and Emr, S. D. (2002a). ESCRT-III: an endosome-associated heterooligomeric protein complex required for MVB sorting. *Dev. Cell* **3**, 271-282.

Babst, M., Katzmann, D. J., Synder, W. B., Wendland, B. and Emr, S. D. (2002b). Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body. *Dev. Cell* **3**, 283-289.

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

Bertolaet, B. L., Clarke, D. J., Wolff, M., Watson, M. H., Henze, M., Divita, G. and Reed, S. I. (2001). UBA domains of DNA damage-inducible proteins interact with ubiquitin. *Nat. Struct. Biol.* **8**, 417-422.

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

Bilodeau, P. S., Urbanowski, J. L., Winistorfer, S. C. and Piper, R. C. (2002). The Vps27p-Hse1p complex binds ubiquitin and mediates endosomal protein sorting. *Nat. Cell Biol.* **4**, 534-539.

Bishop, N., Horman, A. and Woodman, P. (2002). Mammalian class E vps proteins recognize ubiquitin and act in the removal of endosomal protein-ubiquitin conjugates. *J. Cell Biol.* **157**, 91-102.

Braun, S., Matuschewski, K., Rape, M., Thoms, S. and Jentsch, S. (2002). Role of the ubiquitin-selective CDC48UFD1/NPL4 chaperone (segregase) in ERAD of OLE1 and other substrates. *EMBO J.* **21**, 615-621.

Burke, B. and Ellenberg, J. (2002). Remodelling the walls of the nucleus. *Nat. Rev. Mol. Cell. Biol.* **3**, 487-497.

Clarke, D. J., Mondesert, G., Segal, M., Bertolaet, B. L., Jensen, S., Wolff, M., Henze, M. and Reed, S. I. (2001). Dosage suppressors of pds1 implicate ubiquitin-associated domains in checkpoint control. *Mol. Cell. Biol.* **21**, 1997-2007.

Clary, D. O., Griff, I. C. and Rothman, J. E. (1990). SNAPs, a family of NSF attachment proteins involved in intracellular membrane fusion in animals and yeast. *Cell* **61**, 709-721.

Confalonieri, F. and Duguet, M. (1995). A 200-amino acid ATPase module in search of a basic function. *Bioessays* **17**, 639-650.

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

Dai, R.-M., Chen, E., Longo, D. L., Gorbea, C. M. and Li, C.-C. H. (1998). Involvement of valosin-containing protein, an ATPase co-purified with Ikappa Balpha and 26S Proteasome, in ubiquitin-proteasome-mediated degradation of Ikappa Balpha. *J. Biol. Chem.* **273**, 3562-3573.

Dalal, S. and Hanson, P. I. (2001). Membrane traffic: what drives the AAA motor? *Cell* **104**, 5-8.

DeHoratius, C. and Silver, P. (1996). Nuclear transport defects and nuclear envelope alterations are associated with mutation of the *Saccharomyces cerevisiae NPL4* gene. *Mol. Biol. Cell* **7**, 1835-1855.

Egerton, M., Ashe, O. R., Chen, D., Druker, B. J., Burgess, W. H. and Samelson, L. E. (1992). VCP, the mammalian homolog of cdc48, is tyrosine phosphorylated in response to T cell antigen receptor activation. *EMBO J.* **11**, 3533-3540.

Ellgaard, L. and Helenius, A. (2001). ER quality control: towards an understanding at the molecular level. *Curr. Opin. Cell Biol.* **13**, 431-437.

Fasshauer, D., Sutton, R. B., Brunger, A. T. and Jahn, R. (1998). Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. *Proc. Natl. Acad. Sci. USA* **95**, 15781-15786.

Fröhlich, K.-U., Fries, H.-W., Rüdiger, M., Erdmann, R., Botstein, D. and Mecke, D. (1991). Yeast cell cycle protein CDC48p shows full-length homology to the mammalian protein VCP and is a member of a protein

family involved in secretion, peroxisome formation, and gene expression. *J. Cell Biol.* **114**, 443-453.

Garrus, J. E., von Schwedler, U. K., Pornillos, O. W., Morham, S. G., Zavitz, K. H., Wang, H. E., Wettstein, D. A., Stray, K. M., Côté, M., Rich, R. L. et al. (2001). Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* **107**, 1-20.

Ghislain, M., Dohmen, R. J., Lévy, F. and Varshavsky, A. (1996). Cdc48p interacts with Ufd3p, a WD repeat protein required for ubiquitin-mediated proteolysis in *Saccharomyces cerevisiae*. *EMBO J.* **15**, 4884-4899.

Haglund, K., Shimokawa, N., Szymkiewicz, I. and Dikic, I. (2002). Cbl-directed monoubiquitination of CIN85 is involved in regulation of ligand-induced degradation of EGF receptors. *Proc. Natl. Acad. Sci. USA* **99**, 12191-12196.

Hanson, P. I., Roth, R., Morisaki, R., Jahn, R. and Heuser, J. E. (1997). Structure and conformational changes in NSF and its membrane receptor complexes visualized by quick-freeze/deep-etch electron microscopy. *Cell* **90**, 523-536.

Hartmann-Petersen, R., Seeger, M. and Gordon, C. (2002). Transferring substrates to the 26S proteasome. *Trends Biochem. Sci.* **28**, 26-31.

Haynes, C. M., Caldwell, S. and Cooper, A. A. (2002). An HRD/DER-independent ER quality control mechanism involves Rsp5p-dependent ubiquitination and ER-Golgi transport. *J. Cell Biol.* **158**, 91-102.

Hershko, A. and Ciechanover, A. (1998). The ubiquitin system. *Annu. Rev. Biochem.* **67**, 425-479.

Hetzer, M., Meyer, H. H., Walther, T. C., Bilbao-Cortes, D., Warren, G. and Mattaj, I. W. (2001). Distinct AAA-ATPase p97 complexes function in discrete steps of nuclear assembly. *Nat. Cell Biol.* **3**, 1086-1091.

Hicke, L. (1997). Ubiquitin-dependent internalization and down-regulation of plasma membrane proteins. *FASEB J.* **11**, 1215-1226.

Hicke, L. (2001). Ubiquitin and proteasomes. Protein regulation by monoubiquitin. *Nat. Rev. Mol. Cell. Biol.* **2**, 195-201.

Hirabayashi, M., Inoue, K., Tanaka, K., Nakadate, K., Ohsawa, Y., Kamei, Y., Popiel, A. H., Sinohara, A., Iwamatsu, A., Kimura, Y. et al. (2001). VCP/p97 in abnormal protein aggregates, cytoplasmic vacuoles, and cell death, phenotypes relevant to neurodegeneration. *Cell Death Diff.* **8**, 977-984.

Hochstrasser, M. (1996). Ubiquitin-dependent protein degradation. *Annu. Rev. Genet.* **30**, 405-439.

Hoppe, T., Matuschewski, K., Rape, M., Schlenker, S., Ulrich, H. D. and Jentsch, S. (2000). Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. *Cell* **102**, 577-586.

Hu, C., Ahmed, M., Melia, T. J., Söllner, T., Mayer, T. and Rothman, J. E. (2003). Fusion of cells by flipped SNAREs. *Science* **300**, 1745-1749.

Hui, N., Nakamura, N., Sönnichsen, B., Shima, D. T., Nilsson, T. and Warren, G. (1997). An isoform of the Golgi t-SNARE, syntaxin 5, with an endoplasmic reticulum retrieval signal. *Mol. Biol. Cell* **8**, 1777-1787.

Jarosch, E., Taxis, C., Volkwein, C., Bordallo, J., Finley, D., Wolf, D. H. and Sommer, T. (2002). Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48. *Nat. Cell Biol.* **4**, 134-139.

Katzmann, D. J., Babst, M. and Emr, S. D. (2001). Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* **106**, 145-155.

Katzmann, D. J., Odorizzi, G. and Emr, S. D. (2002). Receptor downregulation and multivesicular-body sorting. *Nat. Rev. Cell Mol. Biol.* **3**, 893-905.

Koegl, M., Hoppe, T., Schlenker, S., Ulrich, H. D., Mayer, T. U. and Jentsch, S. (1999). A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell* **96**, 635-644.

Kondo, H., Rabouille, C., Newman, R., Levine, T. P., Pappin, D., Freemont, P. and Warren, G. (1997). p47 is a cofactor for p97-mediated membrane fusion. *Nature* **388**, 75-78.

Latterich, M., Frölich, K.-U. and Schekman, R. (1995). Membrane fusion and the cell cycle: Cdc48p participates in the fusion of ER membranes. *Cell* **82**, 885-893.

Lustgarten, V. and Gerst, J. E. (1999). Yeast VSM1 encodes a v-SNARE binding protein that may act as a negative regulator of constitutive exocytosis. *Mol. Cell. Biol.* **19**, 4480-4494.

McNew, J. A., Parlati, F., Fukuda, R., Johnston, R. J., Paz, K., Paumet, F., Söllner, T. H. and Rothman, J. E. (2000). Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. *Nature* **407**, 153-159.

Meyer, H. H., Kondo, H. and Warren, G. (1998). The p47 co-factor regulates the ATPase activity of the membrane fusion protein, p97. *FEBS Lett.* **437**, 255-257.

Meyer, H. H., Shorter, J. G., Seemann, J., Pappin, D. and Warren, G. (2000). A complex of mammalian Ufd1 and Npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. *EMBO J.* **19**, 2181-2192.

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

Morgan, A., Dimaline, R. and Burgoyne, R. D. (1994). The ATPase activity of N-ethylmaleimide-sensitive fusion protein (NSF) is regulated by soluble NSF attachment proteins. *J. Biol. Chem.* **269**, 29347-29350.

Nagahama, M., Suzuki, M., Hamada, Y., Hatsuzawa, K., Tani, K., Yamamoto, A. and Tagaya, M. (2003). SVIP is a novel VCP/p97-interacting protein whose expression causes cell vacuolation. *Mol. Biol. Cell* **14**, 262-273.

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

Otto, H., Hanson, P. I. and Jahn, R. (1997). Assembly and disassembly of a ternary complex of synaptobrevin, syntaxin, and SNAP-25 in the membrane of synaptic vesicles. *Proc. Natl. Acad. Sci. USA* **94**, 6197-6120.

Patel, S. and Latterich, M. (1998). The AAA team: related ATPases with diverse functions. *Trends Cell Biol.* **8**, 65-71.

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

Peters, J. M. (2002). The anaphase-promoting complex: proteolysis in mitosis and beyond. *Mol. Cell* **9**, 931-943.

Polo, S., Sigismund, S., Faretta, M., Guidi, M., Capua, M. R., Bossi, G., Chen, H., Camilli, P. D. and Fiore, P. P. D. (2002). A single motif responsible for ubiquitin recognition and monoubiquitination in endocytic proteins. *Nature* **416**, 451-455.

Pornillos, O., Alam, S. L., Rich, R. L., Myszka, D. G., Davis, D. R. and Sundquist, W. I. (2002). Structure and functional interactions of the Tsg101 UEV domain. *EMBO J.* **21**, 2397-2406.

Rabinovich, E., Kerem, A., Frohlich, K.-U., Diamant, N. and Bar-Nun, S. (2002). AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Mol. Cell. Biol.* **22**, 626-634.

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

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

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

Rockel, B., Jakana, J., Chiu, W. and Baumeister, W. (2002). Electron cryo-microscopy of VAT, the Archaeal p97/CDC48 homologue from *Thermoplasma acidophilum*. *J. Mol. Biol.* **317**, 673-681.

Rothman, J. E. (1994). Mechanism of intracellular protein transport. *Nature* **372**, 55-63.

Rouiller, I., Butel, V. M., Latterich, M., Milligan, R. A. and Wilson-Kubalek, E. M. (2000). A major conformational change in p97 AAA ATPase upon ATP binding. *Mol. Cell* **6**, 1485-1490.

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

Rowe, A., Dascher, C., Bannykh, S., Plutner, H. and Balch, W. E. (1998). Role of vesicle-associated syntaxin 5 in the assembly of pre-Golgi intermediates. *Science* **279**, 696-700.

Roy, L., Bergeron, J. J. M., Lavoie, C., Hendriks, R., Gushue, J., Fazel, A., Pelletier, A., Morre, D. J., Subramaniam, V. N., Hong, W. et al. (2000). Role of p97 and syntaxin 5 in the assembly of transitional endoplasmic reticulum. *Mol. Biol. Cell* **11**, 2529-2542.

Sagiv, Y., Legesse-Miller, A., Porat, A. and Elazar, Z. (2000). GATE-16, a membrane transport modulator, interacts with NSF and the Golgi v-SNARE GOS-28. *EMBO J.* **19**, 1494-1504.

Shirogane, T., Fukada, T., Müller, J. M., Shima, D. T., Hibi, M. and Hirano, T. (1999). Synergistic roles for Pim-1 and c-Myc in STAT3-mediated cell cycle progression and antiapoptosis. *Immunity* **11**, 709-719.

Shorter, J. and Warren, G. (2002). Golgi architecture and inheritance. *Ann. Rev. Cell Dev. Biol.* **18**, 379-420.

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

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

Stirling, C., Rothblatt, J., Hosobuchi, M., Deshaies, R. and Schekman, R. (1992). Protein translocation mutants defective in the insertion of integral membrane proteins into the endoplasmic reticulum. *Mol. Biol. Cell* **3**, 129-142.

Sutton, R. B., Fasshauer, D., Jahn, R. and Brünger, A. T. (1998). Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4Å resolution. *Nature* **395**, 347-353.

Swanton, E., Sheehan, J., Bishop, N., High, S. and Woodman, P. (1998). Formation and turnover of NSF- and SNAP-containing “fusion” complexes occur on undocked, clathrin-coated vesicle-derived membranes. *Mol. Biol. Cell* **9**, 1633-1647.

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

Uchiyama, K., Jokitalo, E., Kano, F., Murata, M., Zhang, X., Canas, B., Newman, R., Rabouille, C., Pappin, D., Freemont, P. 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**, 855-866.

Uchiyama, K., Jokitalo, E., Lindman, M., Jackman, M., Kano, F., Murata, M., Zhang, X. and Kondo, H. (2003). The localization and phosphorylation of p47 are important for Golgi disassembly-assembly during the cell cycle. *J. Cell Biol.* **161**, 1067-1079.

Wang, B., Alam, S. L., Meyer, H. H., Payne, M., Stemmler, T. L., Davis, D. R. and Sundquist, W. I. (2003). Structure and ubiquitin interactions of the conserved zinc finger domain of Npl4. *J. Biol. Chem.* **278**, 20225-20234.

Weber, T., Zemelman, B. V., McNew, J. A., Westermann, B., Gmachl, M., Parlati, F., Söllner, T. H. and Rothman, J. E. (1998). SNAREpins: minimal machinery for membrane fusion. *Cell* **92**, 759-772.

Weissman, A. M. (2001). Ubiquitin and proteasomes: themes and variations on ubiquitylation. *Nat. Rev. Mol. Cell. Biol.* **2**, 169-178.

Whiteheart, S. W., Rossnagel, K., Buhrow, S. A., Brunner, M., Jaenicke, R. and Rothman, J. E. (1994). N-ethylmaleimide-sensitive fusion protein: a trimeric ATPase whose hydrolysis of ATP is required for membrane fusion. *J. Cell Biol.* **126**, 945-954.

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

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

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

Yuan, X., Shaw, A., Zhang, X., Kondo, H., Lally, J., Freemont, P. S. and Matthews, S. (2001). Solution structure and interaction surface of the C-terminal domain from p47: a major p97-cofactor involved in SNARE disassembly. *J. Mol. Biol.* **311**, 255-263.

Zhang, L., Ashendel, C. L., Becker, G. W. and Morré, D. J. (1994). Isolation and characterisation of the principal ATPase associated with transitional endoplasmic reticulum of rat liver. *J. Cell Biol.* **127**, 1871-1883.

Zhang, X., Shaw, A., Bates, P. A., Newman, R. H., Shaw, A., Bates, P. A., Newman, R. H., Gowen, B., Orlova, E., Gorman, M. A. et al., (2000). Structure of the AAA ATPase p97. *Mol. Cell* **6**, 1473-1484.