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Minireview

NSF and p97/VCP: similar at first, different at last

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Abstract ***N*-Ethylmaleimide sensitive factor (NSF) and p97/valosin-containing protein (VCP) are distantly related members of the ATPases associated with a variety of cellular activities (AAA) family of proteins. While both proteins have been implied in cellular morphology changes involving membrane compartments or vesicles, more recent evidence seems to imply that NSF is primarily involved in the soluble NSF attachment receptor (SNARE)-mediated vesicle fusion by disassembling the SNARE complex whereas p97/VCP is primarily involved in the extraction of membrane proteins. These functional differences are now corroborated by major structural differences based on recent crystallographic and cryo-electron microscopy studies. This review discusses these recent findings.**

Key words: AAA protein; Vesicle fusion; Membrane protein extraction; Endoplasmic reticulum-associated degradation

1. Introduction

When there is sufficient similarity in the primary sequence of two different proteins, it is often assumed that both proteins will carry out very similar tasks. Such was the rationale when similarities between two ATPases, p97/valosin-containing protein (VCP) and *N*-ethylmaleimide sensitive factor (NSF), were noted [1]. NSF, as its name implies, was discovered as a protein that is inactivated by *N*-ethylmaleimide [2]. It disassembles the soluble NSF attachment receptor (SNARE) complex during vesicle-membrane fusion [3–5]. p97/VCP was first discovered around the same time as the discovery of NSF, but in a completely different line of research. Valosin is a 25 amino acid peptide which had been isolated from pig intestine [6,7] and subsequently shown to affect gastro-intestinal activity [8]. The source of the valosin peptide was determined to be a gene that encoded the 806 amino acid protein dubbed VCP [9]. There were doubts in suggesting such a large protein as the pro-form for a 25 amino acid peptide, but nothing else was known about the function of VCP at the time. VCP was rediscovered [1] as an ubiquitous ATPase in *Xenopus* extracts and named p97 for its relative molecular mass in sucrose gradient sedimentation. Amino acid sequencing showed significant homology (35%–50%) between NSF and p97/VCP in regions near the ATP binding domains. Furthermore, both proteins had comparable domain organization: an N domain of similar length followed by tandem copies of a specific ATP binding domain, known as the AAA domain (Fig. 1). The only other suggestion for a biological role of p97/VCP arose from the significant primary sequence homology between VCP and a protein required for yeast cell maturation called Cdc48p [10].

The observed homologies between NSF and p97/VCP prompted an investigation of the effect of p97/VCP on Golgi body reformation, a process that occurs after cell division [11]. p97/VCP was demonstrated to have a qualitative effect on the reformed membranes. The studies used endogenous p97/VCP that had a consistent protein impurity with a M_r of 47 kDa. When this impurity protein, called p47, was isolated and added in a controlled manner to the Golgi body assays with p97/VCP, the effects on Golgi body reformation were more pronounced [12]. The requirement of a specific adapter protein for p97/VCP function was viewed as further evidence for functional similarity between p97/VCP and NSF, since the interaction of NSF with SNAREs requires the presence of adapter proteins, the soluble NSF attachment proteins (SNAPs). Furthermore, a possible link between p97/VCP·p47 and the SNARE complex was suggested by showing interactions using glutathione-*S*-transferase pulldown assays with the cytoplasmic domain of syntaxin 5 [13]. Subsequent investigations have shown that p97/VCP can interact with poorly folded proteins [14,15]. Since isolated syntaxin is only partially structured [16–18], the interaction between p97/VCP and syntaxin 5 does not necessarily imply that p97/VCP will interact with a fully formed SNARE complex.

Processes related to both NSF and p97/VCP are ATP dependent. Both proteins require additional protein factors to accomplish their tasks. And while p97/VCP does not appear to interact with an entire SNARE complex, it did appear to interact with one of its components. Thus it was reasonable at the time to classify p97/VCP and NSF as paralogs. It was proposed [11,13] that p97/VCP and NSF were partners in membrane fusion events such that p97/VCP was responsible for homotypic fusion events and NSF was responsible for heterotypic fusion events. We summarize herein recent functional and structural results that show that p97/VCP and NSF should be thought of as two different members of the AAA family.

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2. Functional differences

At approximately the same time that the effect on Golgi body reformation was discovered, it was found that there was a correlation between the phosphorylation of p97/VCP and the activation of T-cell receptors in hematopoietic cells [19]. The primary site of phosphorylation was established to be Tyr 805. This observation underscored a noted difference between p97/VCP and NSF. The former possesses a short, ~40 amino acid C-terminal extension that contains a conserved group of acidic residues as well as the phosphorylated Tyr 805. In contrast, NSF has been observed to undergo phosphorylation at Ser 237 [20]. NSF does not have the C-terminal extension, so the post-translational modification of these two proteins will necessarily be different.

Another functional dissimilarity arose from investigations of the relative importance of the tandem AAA domains in NSF and p97/VCP for overall ATPase activity. In NSF, the first (D1) domain is known to provide most, if not all, of the ATPase activity while the second (D2) is primarily responsible for hexamerization [21,22]. In contrast, the reverse is true for p97/VCP: the D2 domain provides the bulk of the ATPase activity whereas the D1 domain appears to be responsible for hexamerization [23–25]. Unlike NSF, nucleotide occupation of the inactive AAA domain is not required for hexamerization of p97/VCP, although it does accelerate hexamer formation. Regardless, given that the AAA domains comprise a large portion of each molecule, this observation was the first indication that the two proteins may perform their relative tasks with different mechanisms of action.

Recent evidence has emerged that p97/VCP is responsible for membrane protein dislocation [26–28]. That is, it is capable of extracting ubiquitinated proteins from the endoplasmic reticulum (ER) and exporting them to the cytosol. Thus, p97/VCP is thought to play an essential role in ER-associated degradation of misfolded membrane proteins. It is possible that p97/VCP would also be capable of extracting proteins from Golgi and nuclear membranes as well. Such a function would be consistent with the observed effect of altering membranes in in vitro Golgi [11] and in vivo nuclear membrane assembly [29] without invoking p97/VCP to be necessarily involved in SNARE complex disassembly.

A final functional difference has arisen from studies of required adapter proteins or auxiliary protein factors. The highly conserved SNAP proteins are the adapter proteins associated with NSF and have a likely stoichiometry such that three SNAP proteins bind to the SNARE complex and then interact with one NSF hexamer [30]. The adapter protein for p97/VCP, p47, was initially thought to interact in a similar 3:6 stoichiometry. However, both averaged and un-averaged electron microscopy (EM) projections suggested a 6:6 stoichiometry and indicated that the association between p97/VCP and p47 is linked to the presence of nucleotide in p97/VCP [31]. Furthermore, several new and distinct adapter proteins have been identified for p97/VCP: Ufd1/Npl4 [32], VCIP135 [33], and SVIP (small VCP interacting protein) [34]. Except for VCIP135, all proteins interact with p97/VCP in a mutually exclusive manner. This has led to a hypothesis that the various adapter proteins direct the ubiquitous p97/VCP to different roles within the cell [32]. Regardless of whether this hypothesis will be validated by further evidence [35], no such variety of adapter protein has been observed for NSF up to this point. Indeed, the other known target of NSF function, the GluR2 C-terminal tail [36] appears to require the SNAP adapter proteins as well [37].

3. Structural differences from EM and X-ray studies

Recent structural investigations of both p97/VCP and NSF have reinforced the observed functional differences between these proteins. Both full-length NSF and p97/VCP have been studied by EM in projection and three-dimensional reconstructions [31,38–43]. Both the N [44,45] and the D2 domains [46,47] of NSF, and the N–D1 fragment [41] and full-length hexamer [48] of p97/VCP have been solved by X-ray crystallography.

3.1. Overall differences

A density map of NSF was recently obtained in complex with α-SNAP, the cytoplasmic portion of the neuronal SNARE complex, and a mixture of ADP and ATP by cryo-EM at 11 Å resolution [49]. This assembly is commonly referred to as the 20S complex for its sedimentation behavior. The appearance of this particular 20S complex is roughly similar to lower resolution images of a 20S complex that contains full-length SNAREs [42]. However, the latter 20S complex contains a pronounced rod-like feature that was presumably produced by the transmembrane domains of the SNAREs and a surrounding detergent micelle. Density maps of p97/VCP were obtained by cryo-EM images in a variety of nucleotide states [39–41]. It is difficult to make many detailed comparisons between proteins based only upon EM projections due to vastly different effective resolution ranges. Furthermore, a comparison of cryo-EM reconstructions of p97/VCP and NSF is hindered by the fact that it was impossible to obtain good images of NSF by itself for cryo-EM reconstruction [49]. One can, however, make comparisons regarding the overall motions of p97/VCP and NSF during the hydrolysis cycle. Lower resolution deep etch images of NSF [43] suggested that NSF is capable of large domain changes, in particular during ADP nucleotide release resulting in a 'splayed' structure. In contrast, higher resolution density maps of p97/VCP at 24 Å obtained by cryo-EM in a variety of nucleotide states showed relatively small domain motions [39]. Another distinct difference in the EM density maps of the two proteins is apparent at the interface between the two barrel-like D1 and D2 hexamers. p97/VCP exhibits six surface protrusions near this interface that show variable positions and size dependence upon nucleotide state. No such protrusions are visible in the EM density map of the 20S complex. Although the identity of these protrusions has been established as an extended region in the D2 AAA domain [48], their role in protein function remains speculative.

3.2. Comparison of N domains

The structural similarity of the N domains of NSF and p97/VCP is quite striking (Fig. 2), given that the sequence identity and similarity is only 9% and 19%, respectively. Other crystal structures of homologous N domains have been obtained for the archeal VAT protein [50] and the yeast sec18 [51], again showing similar structures. Currently, it is only known for p97/VCP how the N domain interacts with the adjacent D1 domain. The crystal structure of the N–D1 fragment was solved at 2.8 Å resolution, but required a fairly high ionic

strength solution of 4 M formate for crystal growth [41]. The full-length p97/VCP structure was solved at 4.7 Å resolution using crystals grown in conditions that are more representative of those found within the cell: a near-neutral pH and an ionic strength equivalent to 100–200 mM NaCl [48]. In both crystal structures, the N domains pack tightly up against the D1 domain, burying ~8% and ~6% of the solvent accessible surface area on the N and D1 domains, respectively [41]. The structures are similar for the N and D1 domains although a major difference is the presence of a Zn^{2+} in the center of the D1 pore of the full-length structure [48].

The N domains are only visible in EM density maps of p97/VCP complexed with the transition state analog ADP·AlF_x [39]. For this state, significant contiguous densities next to the D1 domains were visible. The agreement between the p97/VCP cryo-EM reconstructions and the N domains as determined from the crystal structure was not exact [48], a discrepancy that is likely related to N domain crystal packing contacts present in both the full-length and N–D1 fragment structure. The absence of density for the N domains in EM density maps of other nucleotide states (ATP, ADP, and apo) suggests that the N domains are highly mobile in all but the transition state. We have proposed a latch-type mechanism, whereby the D1 domains in the transition state analog provide several ionic interactions that lock the N domains into place [48]. The mobility of the N domains is in keeping with their proposed role for substrate interaction. Since p97/VCP has multiple substrates, one can envision that the N domains may have to take on slightly different conformations to interact with each particular substrate. For NSF, the mobility of the N domains has led to a proposed mechanism of action whereby once the SNARE complex is tightly bound to the N domains, the motion of these domains is able to pull the SNARE complex apart [43].

For both NSF and p97/VCP, the N domains consist of two smaller subdomains, Na and Nb, and are joined by a non-conserved short linker region (Fig. 1). Na is a double-ψ β barrel fold while the Nb domain is an α/β roll. The ψ loops of the double-ψ β barrel comprising the Na subdomain have been implicated as a substrate recognition motif in other proteins [52]. These loops comprise a well-conserved groove in the N domain structure, an observation which would be consistent with substrate binding. It is interesting to note that the second of the two Na ψ loops of NSF but not p97/VCP comprises a short helical stretch (α1 helix, residues 14–17 in NSF from Chinese hamster ovary cells) (Fig. 2a). Both the adapter proteins (SNAP) and substrates (SNAREs) of NSF are comprised almost entirely of helical structure [53,54], whereas the C-terminal part of the adapter protein p47 of p97/VCP contains a mixture of α/β structure [55]. Subsequent circular dichroism studies of p47 indicated that there is an α/β structure before the C-terminal part [56]. Furthermore, the p97/VCP interacting protein tetra-ubiquitin has an α/β structure. If one assumes, in analogy with other double-ψ barrels, that the ψ loops are responsible for substrate interaction then another difference between NSF and p97/VCP emerges: the second ψ loop in the NSF N domain may be specialized for interactions with helical structures, whereas the second ψ loop in p97/VCP would be able to interact with a wider variety of substrates.

A handful of positively charged residues in the NSF N domain have been identified as important for SNAP/SNARE binding [57]. Of these residues, only NSF Arg 67 is conserved in p97/VCP as Arg 89. This arginine residue seems to be of particular importance, as it is the only residue that is strictly conserved in all known N domain sequences [41]. The arginine residue is located on a helix, α2, after the β5 strand following the second ψ loop. The location of the arginine was originally described [57] with an overly generous definition of the ψ loops; while substrate interactions outside of the ψ loops are indeed possible, neither the NSF R67, nor the VCP R89 residue are, strictly speaking, within the ψ loops. In both NSF and p97/VCP, the arginine points away from the putative substrate binding cleft. In the case of p97/VCP, where the interaction of the N domain with the rest of the protein has been observed directly, the Arg 89 residue interacts with one of two ionic residues, Glu 261 in the D1 domain [41] or Tyr 203 in the N–D1 linker [48]. This interaction suggests that Arg 89 maintains the orientation of the N domain with respect to the rest of the protein. Since the entire hexameric NSF protein is required to efficiently interact with adapter and substrate proteins [21], it is likely that the orientation of N with respect to the rest of the protein is important for adapter and substrate binding. Thus, the interactions disrupted by the afore-

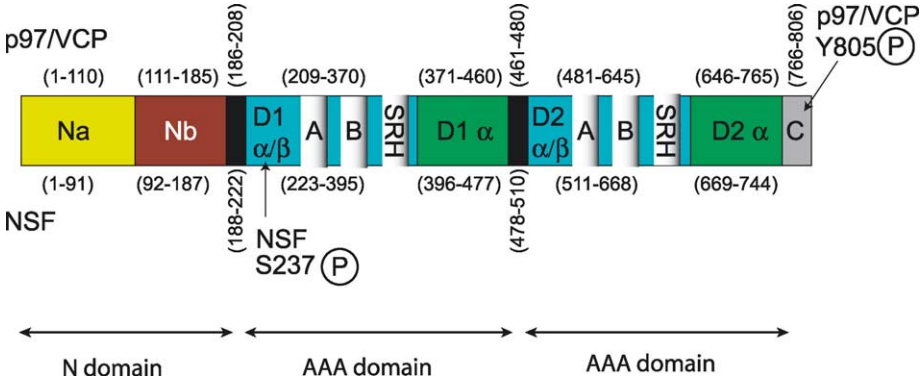

Fig. 1. Domain diagram of NSF and p97/VCP. Residue numbering for p97/VCP is on top, for NSF it is below the domain diagram. The color code is: Na subdomain = yellow, Nb subdomain = brown, interdomain linker = black, AAA α/β subdomain = teal, AAA conserved regions = white ('A', 'B', and 'SRH' are the Walker A, Walker B and second region of homology), AAA α-helical domain = green, C-terminal extension = gray. Note that NSF does not have a C-terminal extension. Residue assignments for the NSF N–D1 linker, D1–D2 linker, and D1 subdomain structure are speculative, based on structure/sequence alignment between the two proteins. Phosphorylation sites for each protein are noted.

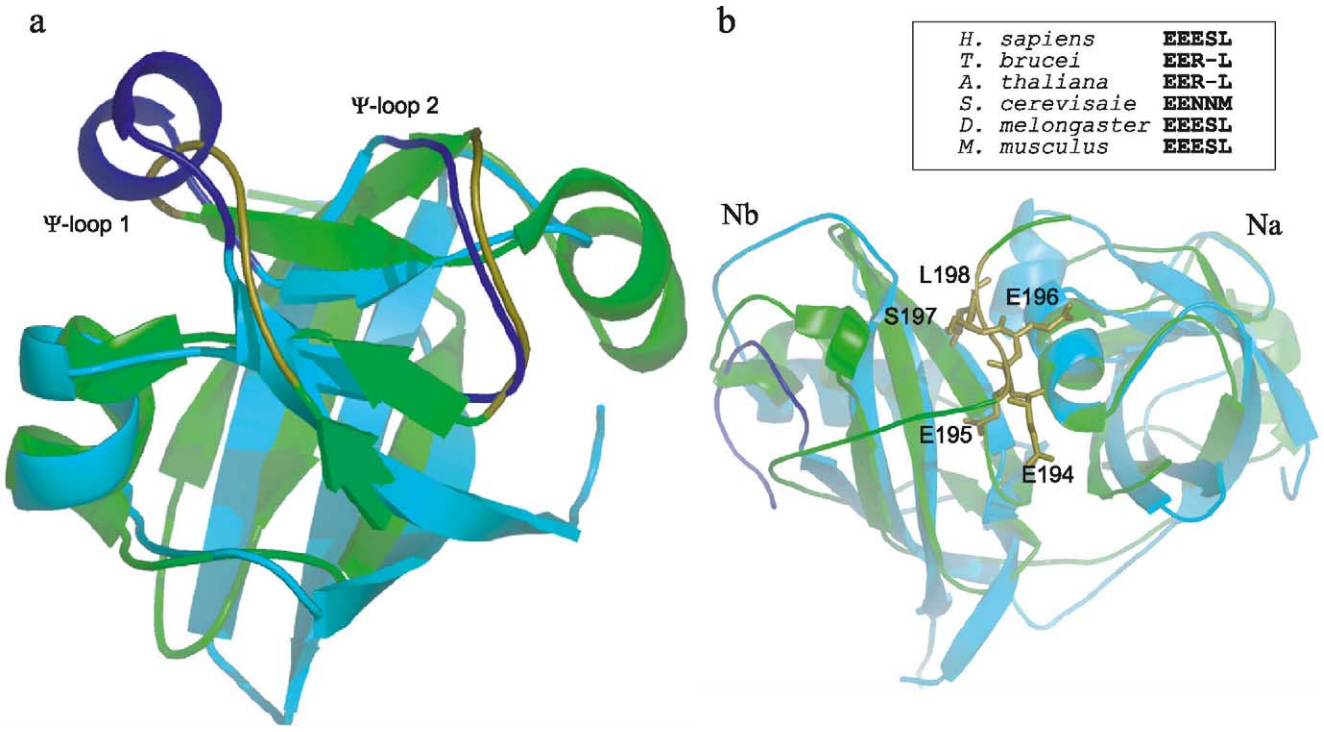

Fig. 2. Comparison of the N domains of NSF and p97/VCP. a: Overlay of the Na subdomain structures from NSF (cyan, residues 1–91) and p97/VCP (green, residues 27–110) showing the ψ loops in blue and olive for NSF and p97/VCP, respectively. b: Overlay of NSF and p97/VCP showing the putative substrate or adapter protein binding cleft between the Na and Nb subdomains. Residues from the N–D1 linker region of p97/VCP interacting with the cleft are shown. The inset shows an alignment and consensus between p97/VCP from a variety of organisms. The overall protein sequence identity among these six species is $>80\%$ for p97/VCP.

mentioned mutations [57] do not necessarily reflect direct interactions between the ATPase, adapters, and substrate, but could be considered more of an overall structural perturbation to the dynamics of the system during nucleotide hydrolysis.

Another proposed site for substrate and adapter binding is the cleft between Na and Nb. In the p97/VCP crystal structure, this cleft is occupied by the N–D1 linker, specifically residues 194–199 (Fig. 2b). These residues form a consensus sequence of negative, negative, charged, non-conserved, and small hydrophobic residues among the homologs of p97/VCP (see inset in Fig. 2b). No such consensus between p97/VCP and NSF exists anywhere within the region from the end of the conserved structure (β6′/β7′) in the respective N domains to the beginning of the well-conserved Walker A domains of the respective D1 domain. If one assumes that the N–D1 linker region is representative of interactions between the N domain and substrate or adapter proteins, then it is likely that p97/VCP and NSF do not have similar substrate interactions. Since there is no conservation of residues between NSF and p97/VCP in the putative substrate binding regions in the N domains, as well as no obvious conservation between adapter proteins for the respective ATPases, we propose that the two proteins are interacting in very different ways with their respective substrates.

3.3. Comparison of AAA domains

Overall, the primary sequence alignment is slightly better between the NSF D2 and the p97/VCP D1 domains rather than between the respective D2 domains, consistent with the reversed role of the nucleotide domains in both proteins [48]. The AAA domain can be thought to consist of as two subdomains (Fig. 1): the α/β subdomain contains the Walker A and B motifs and AAA homology region [58,59] while the α-helical subdomain has less primary conservation but is typically comprised of α-helical secondary structure. The structures of the α/β subdomains of the D1 and D2 domains from VCP are similar both to one another and to the D2 domain of NSF. The α-helical subdomains show large differences [48]. Even within p97/VCP they are different from one another, with the helices and intervening loops of the D2 α-helical subdomain exhibiting a more extended conformation than that observed for the D1 domain of p97/VCP. The NSF D2 helical subdomain consists of a four-helix bundle and is more compact than either of the p97/VCP α-helical subdomains.

The differences in the α-helical subdomains between NSF and p97/VCP point towards a much larger difference between these two proteins: the orientation of the domains with respect to each other. The crystal structure of full-length VCP corrected a model from a previous study [41] which proposed that the orientation of the D1 and D2 domains were oriented such that their ATP binding pockets faced outward from each other, i.e. in an anti-parallel, tail-to-tail fashion. In the AAA module, the α-helical domain is considered to be the 'head'; the 'tail' corresponds to the N-terminal edge of the β-sheet in the nucleotide binding domain. The full-length crystal structure clearly showed that the D1 and D2 domains of p97/VCP are actually arranged in a parallel, head-to-tail fashion [48]. For NSF, the most recent EM density map of the 20S complex [49] suggests an entirely different arrangement for the AAA domains in NSF. They are arranged in an anti-parallel, head-to-head orientation such that in NSF the D2 domain is inverted from its orientation in p97/VCP (Fig. 3). Being mind-

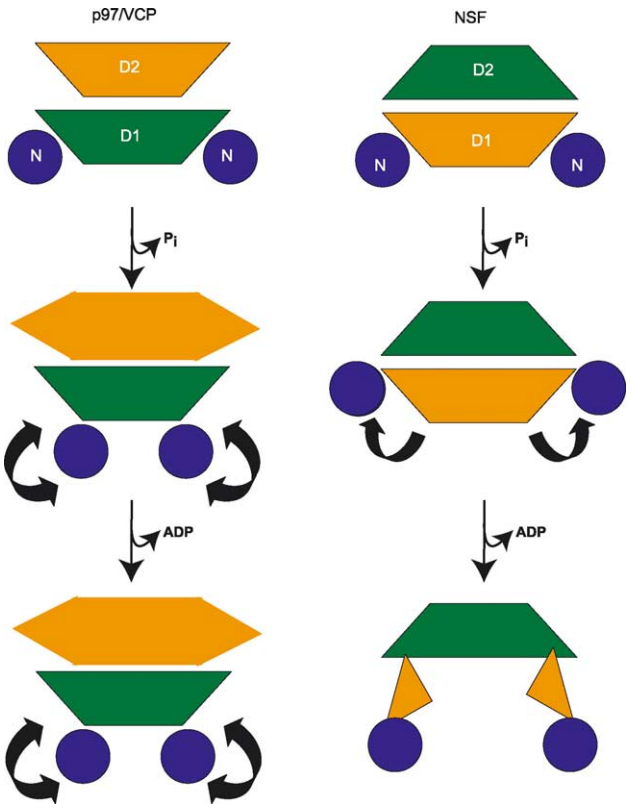

Fig. 3. Different mechanisms of action for p97/VCP and NSF. The AAA domains are depicted as rhombohedra, with the narrow face as the 'tail' and the wide face as the 'head'. Note the difference in orientation between the D2 domains of the respective proteins. The AAA domain with predominant ATPase activity for the respective proteins is shown in orange, and the less active or invariant domain in green. The N domains are indicated as blue circles. The change in nucleotide state that results in the largest change for p97/VCP is the loss of γ-phosphate from the nucleotide binding site. The N domains become mobile and there are changes in the D2 domain. In contrast, the most drastic conformational changes for NSF appear to occur when ADP leaves the nucleotide binding site. One can consider the less active AAA domain within the respective molecules as a fulcrum for conformational change. Since the fulcrum is at the end of the molecule in NSF, relatively large conformational changes are observed. The fulcrum is in the middle of p97/VCP, so smaller conformational changes are observed and a mechanism for communicating the nucleotide state throughout the entire molecule is required. The different location of the fulcrum in both molecules illuminates what appears to be a large mechanistic difference between these proteins.
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ful to avoid the previous fitting error with p97/VCP [41], significantly higher resolution data for the EM density map of the 20S complex was obtained than for p97/VCP. There was excellent agreement between the fitted D2 domain crystal structure and the density map at 11 Å resolution including secondary structure details [49]. Furthermore a 'model-free' analysis was carried out to establish the relative orientation of the two nucleotide domains. Finally, a detailed sequence comparison reveals the presence of a long region of random coil between the D1 and D2 domains has very little homology in terms of either primary sequence or tertiary structure with

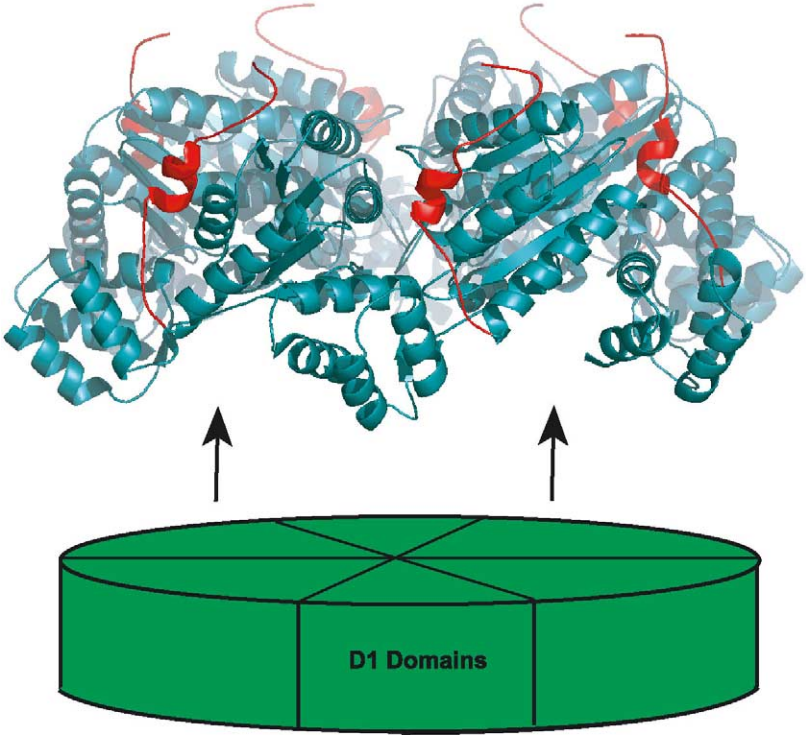

Fig. 4. Orientation of the NSF D2 domain relative to the D1 domain. The orientation of D2 with respect to D1 was established by the optimal fit to the 11 Å cryo-EM density map [49], with the α-helical domain of D2 pointing towards D1 in a head-to-head orientation. Part of the putative D1–D2 linker region, shown in red, is folded in inside of the isolated NSF D2 hexameric domain and points away from the D1 domain. Thus, the conformation of the linker is probably an artifact of crystallizing the truncated D2 domain.

the corresponding region in VCP. In the crystal structure of the NSF D2 domain, a part of this region, comprising residues 480–510, packs inside of the D2 hexamer and interacts with the α/β domains (Fig. 4). An examination of the structure reveals that it is unlikely that the position of this loop is required for the hexameric structure of the NSF D2 domain, and quite possible that the conformation of this loop is an artifact of crystallizing the truncated NSF D2 domain.

3.4. Comparison of interprotomer interactions

A short helix that mediates interprotomer interactions in both D1 and D2 of p97/VCP but that is not present in the known structure of the NSF D2 domain illustrates another key difference between these molecules. Helix α5/α5′ (residues 227–223 in D1, 498–501 in D2) wraps around helix α12/α12′ in the adjacent protomer (Fig. 5). The helical element in the D2 domain is shorter, consistent with the larger motions observed for this AAA domain in p97/VCP [39] (Fig. 3). An interprotomer interaction that is structurally conserved between NSF and p97/VCP is the presence of a basic residue that interacts with the phosphate of the nucleotide in the binding pocket of the adjacent protomer. In NSF D2 it is Lys 631, whereas in p97/VCP D1 this is Arg 359. The corresponding residue in the active p97/VCP D2 domain is disordered; such an arrangement is consistent with the observed different activities and motions of the two AAA domains of p97/VCP.

4. Mechanistic differences

The nucleotide hydrolysis cycle in p97/VCP results in changes throughout the entire molecule [39]. The observed motions in p97/VCP upon nucleotide hydrolysis make it likely that there are interactions with either substrate or adapter proteins on the outer regions of the 'barrel'. Because the N domain mobility appears to be correlated with the nucleotide state and the catalytically most active domain (D2) in p97/VCP is also the most remote from the N domains, we

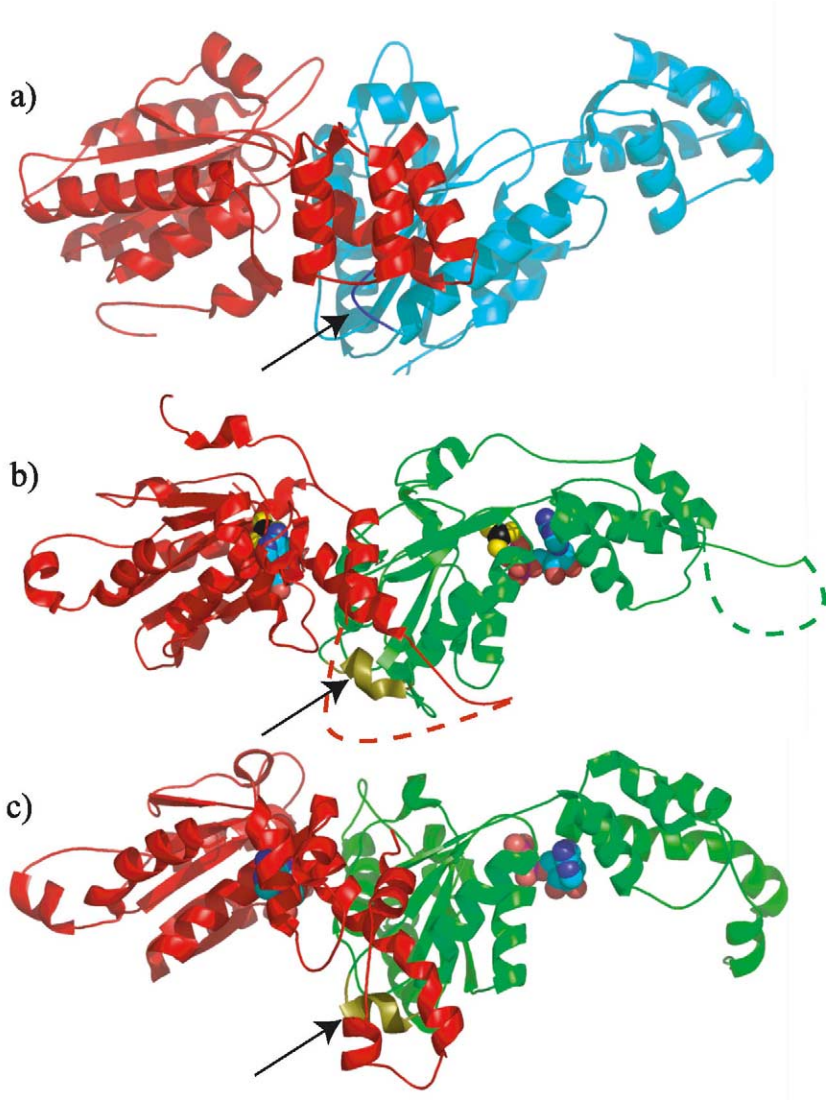

Fig. 5. Interprotomer interactions. The adjacent protomer is colored red in all panels. NSF is colored cyan, and p97/VCP is colored green. The arrow indicates the olive-colored α5/α5′ helix that is found in p97/VCP but not in NSF. The corresponding loop in NSF is colored blue. a: NSF D2–D2 interaction (residues 489–735). b: p97/VCP D2–D2 interaction (residues 481–765). Dashed lines indicate disordered loops in the crystal structure. c: p97/VCP D1–D1 interaction (residues 209–370).

proposed a communication mechanism from D2 to D1 through the linker region to regulate the position of the D1 α-helical domain [48]. The D1 α-helical domain may serve as a latch, regulating the position of the N domains. Such a communication mechanism would not be required for NSF, since the N domains are most likely positioned adjacent to the catalytically active D1 domain. The immediate proximity of the N domains to the active AAA domain would avoid the requirement of distributing conformational changes throughout the entire molecule and thus may result in more of the energy from ATP hydrolysis being focused on changes in the N domains.

Another potential difference between NSF and p97/VCP is the putative sites of substrate and adapter binding. The protrusions found at the interface between the D1 and D2 domains in p97/VCP, but not NSF, consist of a loop-like extension of a helix in the D2 domain that contacts the adenine base of the bound nucleotide [48]. This helix comprises a previously noted conserved sequence motif [60] among AAA proteins that is not found within NSF. Thus, the helix-loop protrusion is a likely site for substrate or adapter protein interactions and a distinct difference between NSF and p97/VCP. Underscoring this difference is the presence of a Zn^{2+} in the central pore in p97/VCP, completely closing the pore ~15 Å from the opening viewed from the flat N–D1 surface of the molecule [48]. It is unknown if a similar ion exists in the central pore of NSF.

5. Concluding remark

One final difference to note is the location of the above-mentioned interprotomer basic residue (NSF Lys 631, p97/VCP Arg 359) in the primary amino acid sequence. This structurally conserved residue follows what has been dubbed the AAA helix in p97/VCP, whereas it precedes the equivalent helix (α5) in NSF. This suggests an instance of convergent evolution between p97/VCP and NSF. The two ATPases have evolved to interact with proteins on the membrane, but in very different ways. NSF acts specifically on SNARE complexes, directing the energy of ATP hydrolysis towards conformational change in order to disassemble the complex. In contrast, p97/VCP acts more generally on ubiquitinated proteins in a manner that extracts them from the membrane. So, while this pair of AAA molecular machines could be considered as members of a class of proteins that work on membrane-bound proteins, the details of their structure, function, and mechanism makes them very distinct from one another.

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References

[1] Peters, J.M., Walsh, M.J. and Franke, W.W. (1990) EMBO J. 9, 1757–1767.
[2] Malhotra, V., Orci, L., Glick, B.S., Block, M.R. and Rothman, J.E. (1988) Cell 54, 221–227.
[3] Söllner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H. and Rothman, J.E. (1993) Cell 75, 409–418.
[4] Hanson, P.I., Heuser, J.E. and Jahn, R. (1997) Curr. Opin. Neurobiol. 7, 310–315.
[5] May, A.P., Whiteheart, S.W. and Weis, W.I. (2001) J. Biol. Chem. 276, 21991–21994.
[6] Schmidt, W.E., Mutt, V., Carlquist, M., Kratzin, H., Conlon, J.M. and Creutzfeldt, W. (1985) FEBS Lett. 191, 264–268.
[7] Gill, J.S., Ghatei, M.A., Domin, J. and Bloom, S.R. (1989) Life Sci. 44, 483–491.
[8] Konturek, S.J., Schmidt, W.E., Mutt, V., Konturek, J.W. and Creutzfeldt, W. (1987) Gastroenterology 92, 1181–1186.
[9] Koller, K.J. and Brownstein, M.J. (1987) Nature 325, 542–545.
[10] Frohlich, K.U., Fries, H.W., Rudiger, M., Erdmann, R., Botstein, D. and Mecke, D. (1991) J. Cell Biol. 114, 443–453.
[11] Rabouille, C., Levine, T.P., Peters, J.M. and Warren, G. (1995) Cell 82, 905–914.
[12] Kondo, H., Rabouille, C., Newman, R., Levine, T.P., Pappin, D., Freemont, P. and Warren, G. (1997) Nature 388, 75–78.
[13] Rabouille, C., Kondo, H., Newman, R., Hui, N., Freemont, P. and Warren, G. (1998) Cell 92, 603–610.
[14] Sugita, S. and Sudhof, T.C. (2000) Biochemistry 39, 2940–2949.
[15] Ye, Y., Meyer, H.H. and Rapoport, T.A. (2003) J. Cell Biol. 162, 71–84.
[16] Fasshauer, D., Otto, H., Eliason, W.K., Jahn, R. and Brunger, A.T. (1997) J. Biol. Chem. 272, 28036–28041.
[17] Fasshauer, D., Bruns, D., Shen, B., Jahn, R. and Brunger, A.T. (1997) J. Biol. Chem. 272, 4582–4590.
[18] Fiebig, K.M., Rice, L.M., Pollock, E. and Brunger, A.T. (1999) Nat. Struct. Biol. 6, 117–123.
[19] Egerton, M., Ashe, O.R., Chen, D., Druker, B.J., Burgess, W.H. and Samelson, L.E. (1992) EMBO J. 11, 3533–3540.
[20] Matveeva, E.A., Whiteheart, S.W., Vanaman, T.C. and Slevin, J.T. (2001) J. Biol. Chem. 276, 12174–12181.
[21] Nagiec, E.E., Bernstein, A. and Whiteheart, S.W. (1995) J. Biol. Chem. 270, 29182–29188.
[22] Whiteheart, S.W., Rossnagel, K., Buhrow, S.A., Brunner, M., Jaenicke, R. and Rothman, J.E. (1994) J. Cell Biol. 126, 945–954.
[23] Song, C., Wang, Q. and Li, C.C. (2003) J. Biol. Chem. 278, 3648–3655.
[24] Wang, Q., Song, C. and Li, C.C. (2003) Biochem. Biophys. Res. Commun. 300, 253–260.
[25] Wang, Q., Song, C., Yang, X. and Li, C.C. (2003) J. Biol. Chem. 13, 13.
[26] Braun, S., Matuschewski, K., Rape, M., Thoms, S. and Jentsch, S. (2002) EMBO J. 21, 615–621.
[27] Rabinovich, E., Kerem, A., Frohlich, K.U., Diamant, N. and Bar-Nun, S. (2002) Mol. Cell. Biol. 22, 626–634.
[28] Ye, Y., Meyer, H.H. and Rapoport, T.A. (2001) Nature 414, 652–656.
[29] Hetzer, M., Meyer, H.H., Walther, T.C., Bilbao-Cortes, D., Warren, G. and Mattaj, I.W. (2001) Nat. Cell Biol. 3, 1086–1091.
[30] Wimmer, C., Hohl, T.M., Hughes, C.A., Muller, S.A., Söllner, T.H., Engel, A. and Rothman, J.E. (2001) J. Biol. Chem. 276, 29091–29097.
[31] Rouiller, I., Butel, V.M., Latterich, M., Milligan, R.A. and Wilson-Kubalek, E.M. (2000) Mol. Cell 6, 1485–1490.
[32] Meyer, H.H., Shorter, J.G., Seemann, J., Pappin, D. and Warren, G. (2000) EMBO J. 19, 2181–2192.
[33] Uchiyama, K. et al. (2002) J. Cell Biol. 159, 855–866.
[34] Nagahama, M., Suzuki, M., Hamada, Y., Hatsuzawa, K., Tani, K., Yamamoto, A. and Tagaya, M. (2003) Mol. Biol. Cell 14, 262–273.
[35] Meyer, H.H., Wang, Y. and Warren, G. (2002) EMBO J. 21, 5645–5652.
[36] Jahn, R. (1998) Curr. Biol. 8, R856–R858.
[37] Hanley, J.G., Khatri, L., Hanson, P.I. and Ziff, E.B. (2002) Neuron 34, 53–67.
[38] Peters, J.M., Harris, J.R., Lustig, A., Muller, S., Engel, A., Volker, S. and Franke, W.W. (1992) J. Mol. Biol. 223, 557–571.
[39] Rouiller, I., DeLaBarre, B., May, A.P., Weis, W.I., Brunger, A.T., Milligan, R.A. and Wilson-Kubalek, E.M. (2002) Nat. Struct. Biol. 9, 950–957.
[40] Beuron, F., Flynn, T.C., Ma, J., Kondo, H., Zhang, X. and Freemont, P.S. (2003) J. Mol. Biol. 327, 619–629.
[41] Zhang, X. et al. (2000) Mol. Cell 6, 1473–1484.
[42] Hohl, T.M., Parlati, F., Wimmer, C., Rothman, J.E., Söllner, T.H. and Engelhardt, H. (1998) Mol. Cell 2, 539–548.

[43] Hanson, P.I., Roth, R., Morisaki, H., Jahn, R. and Heuser, J.E. (1997) Cell 90, 523–535.
[44] Yu, R.C., Jahn, R. and Brunger, A.T. (1999) Mol. Cell 4, 97–107.
[45] May, A.P., Misura, K.M., Whiteheart, S.W. and Weis, W.I. (1999) Nat. Cell Biol. 1, 175–182.
[46] Yu, R.C., Hanson, P.I., Jahn, R. and Brunger, A.T. (1998) Nat. Struct. Biol. 5, 803–911.
[47] Lenzen, C.U., Steinmann, D., Whiteheart, S.W. and Weis, W.I. (1998) Cell 94, 525–536.
[48] DeLaBarre, B. and Brunger, A.T. (2003) Nat. Struct. Biol. 10, 856–863.
[49] Furst, J., Sutton, R.B., Chen, J., Brunger, A.T. and Grigorieff, N. (2003) EMBO J. 22, 4365–4374.
[50] Coles, M. et al. (1999) Curr. Biol. 9, 1158–1168.
[51] Babor, S.M. and Fass, D. (1999) Proc. Natl. Acad. Sci. USA 96, 14759–14764.
[52] Castillo, R.M., Mizuguchi, K., Dhanaraj, V., Albert, A., Blundell, T.L. and Murzin, A.G. (1999) Struct. Fold Des. 7, 227–236.
[53] Sutton, R.B., Fasshauer, D., Jahn, R. and Brunger, A.T. (1998) Nature 395, 347–353.
[54] Rice, L.M. and Brunger, A.T. (1999) Mol. Cell 4, 85–95.
[55] Yuan, X., Shaw, A., Zhang, X., Kondo, H., Lally, J., Freemont, P.S. and Matthews, S. (2001) J. Mol. Biol. 311, 255–263.
[56] DeLaBarre, B., unpublished results.
[57] Matveeva, E.A., May, A.P., He, P. and Whiteheart, S.W. (2002) Biochemistry 41, 530–536.
[58] Neuwald, A.F., Aravind, L., Spouge, J.L. and Koonin, E.V. (1999) Genome Res. 9, 27–43.
[59] Lupas, A.N. and Martin, J. (2002) Curr. Opin. Struct. Biol. 12, 746–753.
[60] Beyer, A. (1997) Protein Sci. 6, 2043–2058.