

AAA Proteins: Lords of the Ring

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AAA ATPases (those associated with various cellular activities)¹ play important roles in numerous cellular activities including proteolysis, protein folding, membrane trafficking, cytoskeletal regulation, organelle biogenesis, DNA replication, and intracellular motility. Recent structural and enzymatic studies are providing clues into the properties of the conserved AAA domain that defines this large protein superfamily. In many cases, AAA domains assemble into hexameric rings that are likely to change their shape during the ATPase cycle. This nucleotide-dependent conformational switch may apply tension to bound proteins and thereby allow AAA proteins to unfold polypeptides, dissociate protein-protein interactions, or generate unidirectional motion along a track. Thus, AAA proteins may represent a broad class of mechanoenzymes that have evolved unique ways of using a fundamentally similar conformational change in many different biological settings.

Introduction

AAA ATPases are not yet household words in the scientific community, although this situation is bound to change. AAA proteins are found in eukaryotes, prokaryotes, and archaeobacteria, revealing their ancient origin and central role in virtually all life forms. The utility of AAA proteins also is evident by their abundant genomic representation. Budding yeast, for example, contains ~50 AAA proteins (Neuwald et al., 1999), which exceeds by ~5-fold the number of mechanochemical ATPases belonging to the kinesin and myosin superfamilies.

The unifying feature of the AAA superfamily is an ATPase domain of ~220 amino acids (aa) whose structural fold has been solved recently by x-ray crystallography (Guenther et al., 1997; Lenzen et al., 1998; Yu et al., 1998; Bochtler et al., 2000). The classical AAA proteins are easily recognized by their strong sequence conservation in this domain (~30% identity). The activities executed by these well-conserved AAA proteins are numerous and include membrane trafficking (e.g., NSF, the best known member

of AAA superfamily), proteasome function, organelle biogenesis, and microtubule regulation (biological functions reviewed in Confalonieri and Duguet, 1995; Patel and Latterich, 1998). However, new sequence alignments and structural information have revealed that the superfamily is much broader than first appreciated (Neuwald et al., 1999). For example, although their sequences are more divergent compared with the classical AAA proteins, clamp loading subunits for DNA polymerase (Guenther et al., 1997), the Clp/Hsp100 family of ATPases (Bochtler et al., 1999), and most likely the microtubule-based motor dynein (Neuwald et al., 1999) all contain an AAA structural fold.

While AAA protein sequences continue to flood data bases, it has been more elusive to resolve their functions. The nomenclature of this superfamily (AAA, ATPase associated with various cellular activities, also sometimes called CAD, conserved ATPase domain), underscores the fact that their actions are not yet well understood. In particular, it has been difficult to pinpoint a common mechanism that unifies members of this ATPase superfamily, especially since their biological functions are so enormously diverse (Confalonieri and Duguet, 1995; Patel and Latterich, 1998; Neuwald, et al., 1999). However, the structural and sequence conservation of the AAA module throughout three phylogenetic kingdoms implies that some similarity in enzyme function must exist. This core enzyme mechanism, however, must also be extremely modular, thereby allowing AAA proteins to occupy so many niches in biology.

This review will first describe how AAA protein can be used to unfold or fold polypeptides, dissociate protein-protein interactions, and generate unidirectional motion along tracks. Then, recent structural and enzymatic data that sheds light into the mechanism of the AAA module will be presented. More complete discussions of the biological roles of AAA proteins are reviewed elsewhere (Confalonieri and Duguet, 1995; Patel and Latterich, 1998; see also an informative AAA Web site: <http://yeamob.pci.chemie.uni-tuebingen.de/AAA/>).

A Dazzling Array of Functions

Unfolding Proteins: Preparing Proteins for Proteolysis and Assisting Protein Folding

A fascinating group of AAA proteins recognize specific

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¹Abbreviations used in this paper: AAA, ATPases associated with various cellular activities; aa, amino acids; GFP, green fluorescence protein; NSF, N-ethylmaleimide sensitive factor; SNAP, NSF attachment protein; SNARE, soluble SNAP receptors.

proteins and prepare them for degradation by an associated protease. In bacteria, there are several AAA ATPases (Clp proteins) that work in conjunction with the ClpP protease; in eukaryotes, six AAA ATPases comprise the regulatory lid of the 20S proteasome (Baumeister et al., 1998). The architectures of both degradative systems are similar: the AAA ATPases form an oligomeric structure (in bacteria, they form a hexameric ring) that sits on top of stacked, 7-membered rings of protease subunits, thereby forming a continuous channel in the middle. Proteolysis occurs inside of the protease cavity, but the opening to the cavity (<15 Å) is only large enough to accommodate the entry of an unfolded and extended polypeptide. Given the location of the AAA ATPases, it seemed likely that these enzymes are used to unfold target proteins so that they could enter the protease cavity. This notion was recently confirmed by Weber-Ban et al. (1999) who showed that ClpA can unfold the stable β -barrel structure of a green fluorescence protein (GFP) that has a ClpA recognition sequence added to its COOH terminus. Moreover, during the unfolding reaction, GFP is directly inserted into the ClpP cavity without passing through a solution intermediate (Hoskins et al., 1998; Weber-Ban et al., 1999). Multiple rounds of ATP hydrolysis accompany the translocation and degradation of the target protein (Singh et al., 1999). Thus, Clp AAA ATPases appear to act as processive ratchets that both unfold and translocate an ill-fated target protein to its demise within the cavity of the associated protease.

In other instances, the AAA domain is joined directly to a protease domain on the same polypeptide (e.g., FtsH in the bacterial membrane and Yme1 in the mitochondrial inner-membrane). ATPase activity is necessary for proteolysis, and it is again thought that the AAA domains unravel proteins to make them more accessible to the adjoining protease domain. However, the structures and oligomeric states of these types of AAA proteins are not clear.

The AAA unfolding activity that makes proteins good substrates for proteolysis also may enable proteins to fold to their active conformation. The AAA Yta10-12 complex, for example, proteolyzes misfolded proteins in the mitochondrial inner membrane, but also functions in the assembly of the multisubunit ATP synthase (Arlt et al., 1996). Genetic experiments revealed that the latter function requires the AAA domain but not protease activity. Yme1, another proteolytic mitochondrial AAA protein (Leonhard et al., 1999), and the Clp/Hsp100 proteins also promote protein folding and reverse heat-induced protein aggregation *in vitro*. Thus, the same AAA chaperone activity that unfolds proteins for degradation may, in other circumstances, assist proteins in folding to their active state.

Disassembling Stable Protein-Protein Complexes

Many high affinity protein-protein interactions in cells must be dissociated using chemical energy derived from ATP hydrolysis. Motor proteins and G proteins, for example, use their built-in nucleotide hydrolysis cycle to switch between high and low affinity interaction states with their protein targets. Phosphoryl transfer from a protein kinase

is also used to modulate the affinity of a protein-protein interaction. In addition, AAA proteins can use energy derived from their ATPase cycle to dissociate stable protein-protein interactions.

The best-studied AAA protein-protein disassembler is NSF (*N*-ethylmaleimide sensitive factor), which dissociates a complex of SNAREs (soluble NSF attachment protein [SNAP] receptors). The necessity of an intervening ATPase is obvious, since the SNARE complex, which brings two membranes together to facilitate fusion in vesicle trafficking pathways, is stable at temperatures as high as 90°C (Fasshauer et al., 1997). Without NSF, vesicle trafficking comes to a grinding halt, since the SNARE complex can not be recycled for further rounds of membrane fusion. NSF requires an intermediate protein called α SNAP, which acts as a bridge between NSF and the helical SNARE complex (Barnard et al., 1997; Hohl et al., 1998; Rice and Brunger, 1999). While the details are not clear, it is believed that an ATP-driven conformational change in NSF is relayed to α SNAP, which in turn pries apart the SNAREs.

The AAA enzyme katanin breaks apart stable tubulin-tubulin interactions in the wall of a microtubule (Hartman et al., 1998). Since tubulin subunits are held in place through both longitudinal and lateral contacts, they dissociate very slowly from the microtubule wall (10^{-8} s^{-1} ; Dye et al., 1992). However, in the presence of ATP, katanin perturbs these tubulin-tubulin contacts and can sever and dismantle a taxol-stabilized microtubule within a couple of minutes. In the cell, katanin has been proposed to break connections between microtubules and their nucleating material at the centrosome, modulate microtubule dynamics during the cell cycle, and mediate flagellar excision (Quarmby, 2000).

Several AAA proteins are involved in regulating protein-DNA interactions (Neuwald et al., 1999). For example, the *E. coli* clamp loader complex opens the β sliding clamp ring and loads it onto DNA where it is used for the processive motion of DNA polymerase. The clamp loader contains two AAA proteins (γ and δ'), only one of which is enzymatically active (γ). When the AAA ATPase binds ATP or a nonhydrolyzable ATP analogue, it causes another subunit in the clamp loader complex (δ) to bind tightly to the β ring and open it up (Turner et al., 1999). While ATP hydrolysis is not necessary for ring opening, it is required for catalyzing the subsequent dissociation of the δ/β complex. This dissociation step allows β to close its ring and encircle the DNA and also releases the clamp loading machinery for further rounds of activity (Turner et al., 1999). Thus, the key role of the AAA ATPase activity in the clamp loader complex is in dissociating a stable protein-protein complex.

Molecular Motors

Certain microtubule- and DNA-based motor proteins also appear to be members of the AAA superfamily. The sequence alignments by Neuwald et al. (1999) resulted in the important discovery that the motor domain of dynein, a microtubule-based motor involved in chromosome motions, organelle transport, and ciliary/flagellar beating, most likely contains six AAA modules. Four of these AAA

modules have functional P-loops, which agrees with biochemical data showing the presence of four functional ATP binding sites (Mocz and Gibbons, 1996). However, the two COOH-terminal AAA modules are less well conserved, lack functional P-loops, and do not appear to be capable of binding nucleotide. In contrast to dynein, the other cytoskeletal motors kinesin and myosin have much smaller motor domains and contain only a single ATP binding site. Thus, while kinesin and myosin share a number of similar mechanistic features due to their common evolutionary origin (Vale and Milligan, 2000), dynein is likely to work by quite a different mechanism, and probably one that bears some similarity to other AAA enzymes. A group of DNA translocating helicases, such as RuvB and T antigen, contain AAA modules as well (Neuwald et al., 1999). Thus, dynein and some helicases exploit the AAA domain to generate unidirectional motion along a track.

Structures of AAA Proteins

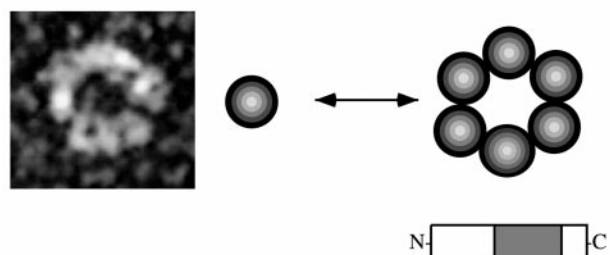
What features of AAA domains allow them to be used for activities as diverse as protein unfolding and physical motion along a track? As will be discussed below, AAA proteins function as oligomers, in most cases by forming hexameric rings. Atomic structures also reveal that the AAA core structure is highly conserved and that subunit-subunit interactions are likely to be important in the enzymatic mechanism.

Creating Rings out of AAA Modules

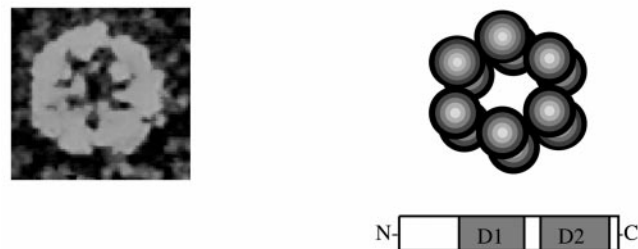
Symmetric Oligomers. Two proteins containing a single AAA domain (VPS4 [Babst et al., 1998] and katanin [Hartman and Vale, 1999]) exist in an equilibrium between monomers and oligomers. In the case of the katanin, the oligomeric state was shown to be a hexameric ring (Hartman and Vale, 1999; Fig. 1 A). Oligomerization of these proteins is promoted both by nonhydrolyzable ATP analogues (or by creating mutations that block ATP hydrolysis) and by substrate binding (microtubules for katanin or an unknown endosomal protein for VPS4).

A second type of AAA proteins (e.g., NSF/p97/CDC48 and Clp/HSP100) forms stable, two-tiered hexameric rings (Fig. 1 B), which are only prone to disassembly when nucleotide is removed. The two-tier architecture owes its existence to the presence of two AAA domains within the polypeptide chain which carry out different tasks: one AAA domain has high enzymatic activity and is principally involved in acting on the target protein, and the other binds ATP but hydrolyzes it slowly (or hardly at all in the case of the D2 domain of NSF) and serves a structural role in hexamer stability (Whiteheart et al., 1994; Singh et al., 1999). Since the ATP-bound state promotes oligomerization in the katanin and VPS4 enzymatic cycles, duplication and subsequent loss of hydrolytic activity in the second AAA domain may have been selected for in evolution as a means of maintaining AAA proteins in a stable ring configuration. In this way, the active AAA domains could loosen their interactions or even splay apart transiently during the enzymatic cycle, and yet still be held in close proximity by the second ring.

A Reversible Homo-Hexamer: Katanin



B Stable Homo-Hexamer: NSF



C Single Polypeptide Hexameric: Dynein

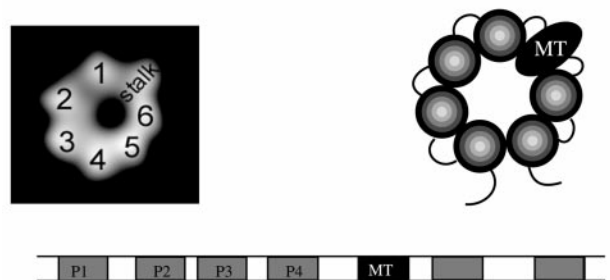


Figure 1. Three strategies for creating rings out of AAA domains. (A) Some single domain AAA proteins (katanin, VPS4) exist in an equilibrium between monomers and rings. Bound ATP (or nonhydrolyzable ATP analogues) as well as target protein binding favor ring formation. The NH₂-terminal domain (not shown on the ring structure) is involved in target protein binding. (B) A second class of AAA proteins (such as NSF) have two AAA domains, one of which serves as the main hydrolytic domain and a second which binds ATP, but hydrolyzes it very poorly, and is involved in creating a stable hexamer base. The NH₂-terminal domain of NSF also is involved in target protein (α SNAP) binding. (C) A third type of AAA protein (dynein) has six AAA domains within a single polypeptide chain as well as an additional microtubule binding domain. Four of these sites (P1-P4) bind ATP, and P1 acts as the main hydrolytic site. Electron micrographs of katanin and NSF were provided by J. Heuser, and the two-dimensional projection of the dynein motor domain (from single particle averaging of electron microscopic images) was provided by M. Samso and M. Koonce. The diameter of these AAA hexameric rings is ~ 15 nm.

Asymmetric Oligomers. Asymmetric rings also can be created by heterotypic interactions between AAA domains. In the case of dynein, Samso et al. (1998) discovered that the motor domain folds into a ring-like structure with a central cavity surrounded by approximately seven

lobes (Fig. 1 C). Extrapolating from the sequence alignment data of Neuwald et al. (1999), this ring is most likely created by interactions between the six different AAA domains contained within this large polypeptide chain; the seventh and more stalk-like lobe seen in the EM reconstructions may represent the microtubule binding domain (Gee et al., 1997; Koonce, 1997; Fig. 1 C). In contrast to the homotypic and symmetric AAA rings described above for katanin and NSF, the different AAA domains in dynein serve distinct functions. The first AAA module (termed the P1 site) is the main enzymatic site that accounts for dynein's rapid ATP turnover, while the next three sites (P2-P4) bind but do not rapidly hydrolyze nucleotide. They may serve a regulatory function, which conceivably could have a role in generating an unusual oscillating mechanical behavior described for dynein (Shingyoji et al., 1998). The last two AAA modules, which do not bind nucleotide, may serve a structural role in completing the ring by interacting with the other AAA modules and/or the microtubule binding domain.

Different AAA proteins can also come together to form hetero-oligomeric complexes. Examples include the two different polypeptides YTA10/YTA12 protease (Arlt et al., 1996), and the six AAA proteins that come together to form the regulatory particle of the proteasome (Rubin et al., 1998). These different AAA proteins appear to act cooperatively, since inactivating the ATPase of one AAA protein greatly diminishes the activity of the entire complex. As described earlier, the *E. coli* clamp loader also represents a mixed complex consisting of a AAA ATPase (γ), an AAA protein that has lost its ability to bind nucleotide (δ'), and three other proteins that may serve a transmission function in linking conformational changes in the AAA ATPase to the opening of the β clamp (Turner et al., 1999).

Atomic Structures and Enzymatic Cycles of AAA Proteins

Atomic structures have been solved for the clamp-loading subunit (δ') (Guenther et al., 1997), the D2 AAA domain of NSF (Lenzen et al., 1998; Yu et al., 1998), and HsIU (Bochtler et al., 2000), a member of the Clp/Hsp100 protease family. The structural folds of these distantly related AAA proteins superimpose closely with one another, which suggests that all AAA domains will share this basic core structure. The AAA core is composed of two parts: an NH_2 -terminal α/β subdomain that contains the nucleotide-binding pocket and a COOH-terminal four-helix bundle subdomain that lies on top of the nucleotide pocket. A nucleotide-dependent hinge motion between the two subdomains has been described for HsIU (Bochtler et al., 2000), which may be important for the mechanism of AAA enzymes.

The D2 AAA domains of NSF (Lenzen et al., 1998; Yu et al., 1998) and HsIU (Bochtler et al., 2000) were crystallized as hexameric rings, which has provided additional insight into subunit-subunit interactions in AAA oligomers (Fig. 2). Interestingly, the nucleotide is located near the interface between subunits, and the active site appears to contain important residues from the adjacent protomer. This is reminiscent of aspartate transcarbamoylase, whose

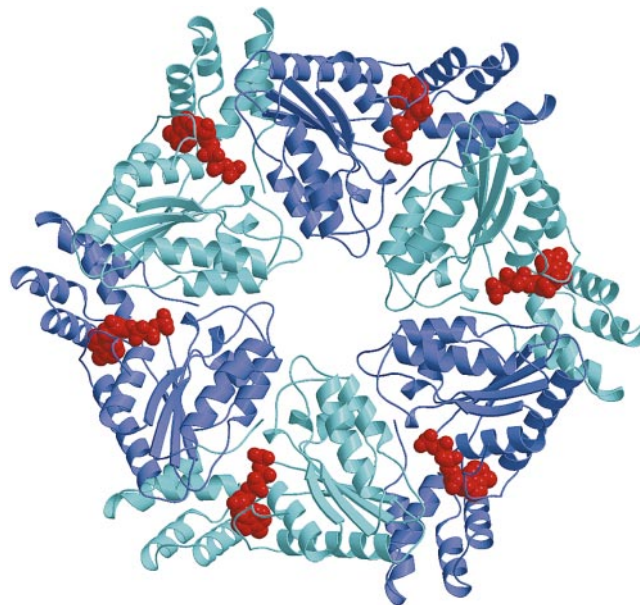


Figure 2. Atomic structure of the D2 domain of NSF in a hexameric ring. All subunits are identical, but adjacent subunits are colored in different shades of blue to illustrate the subunits interface. The nucleotide (AMPPNP shown as a red space-filling model) is located close to the interface between subunits, and subunit-subunit interactions are likely to affect the ATPase cycle. Atomic coordinates were provided by W. Weis (Stanford University).

active site is composed of residues from adjacent subunits in the ring-like trimer. These observations provide a potential structural explanation for the finding that oligomerization of katanin (Hartman and Vale, 1999), VPS4 (Babst et al., 1998), and NSF (Nagiec et al., 1995) accelerate ATP turnover. Mutagenesis of residues at the presumptive subunit-subunit interface of FtzH also significantly diminish ATPase activity (Karata et al., 1999). Target proteins (e.g., microtubules for dynein or α SNAP for NSF) also stimulate the ATPase activity of stable AAA hexamers, and perhaps this enzymatic activation also involves a rearrangement or tightening of connections between adjacent AAA domains. The enzymatic transition(s) that is accelerated by oligomerization or target protein binding, however, remains to be determined.

The nucleotide state of the AAA proteins also affects its affinity for its target protein. Although exceptions to this rule exist (e.g., ciliary dynein; Porter and Johnson, 1983), most AAA proteins bind their targets tightly in their ATP-bound state and weakly in their ADP-bound state. For example, nonhydrolyzable ATP analogues or blocking ATP hydrolysis by mutation (E to Q mutation in the Walker B [DExx] box) result in high-affinity interactions of NSF (Whiteheart et al., 1994), katanin (Hartman and Vale, 1999), VPS4 (Babst et al., 1998), ClpA (Pak et al., 1999; Singh et al., 1999), and YTA10-12 (Arlt et al., 1996) with their respective protein targets. These observations suggest that unknown protein targets of AAA proteins might be identified (even on a genome-wide scale) by creating E-Q mutations in the AAA Walker B box motif and determining which proteins uniquely coimmunoprecipitate with these ATP-locked AAA proteins.

The structural basis of how AAA proteins interact with their targets is still poorly understood, although recent progress has been made in this area. A structure of two HsIU hexamers bound to its dodecamer protease partner (HsIV) was solved by x-ray crystallography (Bochtler et al., 2000). Solving a structure of this holoenzyme protease with its target protein should provide insight into how proteins are translocated into the proteolytic cavity. Atomic structures of several components of the NSF complex (α SNAP; Rice and Brunger, 1999), the α SNAP-binding domain of NSF (Babor and Fass, 1999; May et al., 1999; Yu et al., 1999), and a tSNARE-vSNARE complex (Sutton et al., 1998) also were solved recently. Further progress in this area awaits more complete NSF structures in different nucleotide states as well as cocrystallization of NSF with α SNAP/SNARES.

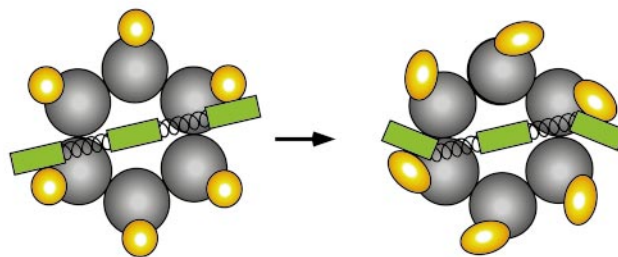
Using Rings as Molecular Crowbars

The structural and enzymatic studies described above suggest the following model for AAA proteins. ATP binding induces structural rearrangements at the interface region which increases interactions between adjacent AAA domains as well as between the AAA protein and its target. Borrowing a term used for allosteric enzymes, this creates a tense state of the AAA–target protein complex. The tighter subunit–subunit interactions in turn accelerate a step(s) in the ATPase reaction. Once the AAA modules are in an ADP state, the complex reverts to a relaxed configuration in which interactions between AAA domains and the target protein both weaken.

What is the utility of forming rings in the AAA enzyme mechanism? One advantage of this architecture is that it potentially allows all subunits to switch between tense and relaxed states in a concerted manner. Precedence for such a mechanism is found in other oligomeric ring enzymes such as aspartate transcarbamoylase, F1 ATPase, and GroEl, all of which undergo concerted conformational changes. Evidence suggesting that AAA proteins may employ a similar strategy comes from observations that incorporation of an inactive subunit in NSF (Whiteheart et al., 1994), ClpA (Singh et al., 1999), or the proteasome lid (Rubin et al., 1998) poisons the activity of the entire complex.

Rings also provide a framework for binding target proteins at multiple sites. If the ring-binding sites change their positions during the ATPase cycle, then tension could be applied to a bound protein (Fig. 3 A). Indeed, nucleotide-dependent changes in the NSF ring were observed using electron microscopy by Hanson et al. (1997). These authors speculated that “a common mode of action of these ATPases in dissociating protein complexes may involve their symmetrical distribution of multiple substrate binding sites and their ability to rearrange these sites during ATP hydrolysis, almost like mini-muscles.” Precedence for this type of model again can be found in GroEl. Within the cavity of this enzyme, polypeptides are bound at multiple points (Farr et al., 2000) and ATP-driven conformational changes in the ring can stretch the tethered polypeptides (Shtilerman et al., 1999). Thus, conformational changes in a protein ring may act like medieval rack that stretches bound substrates.

A Protein Unfolding



B Motor Activity

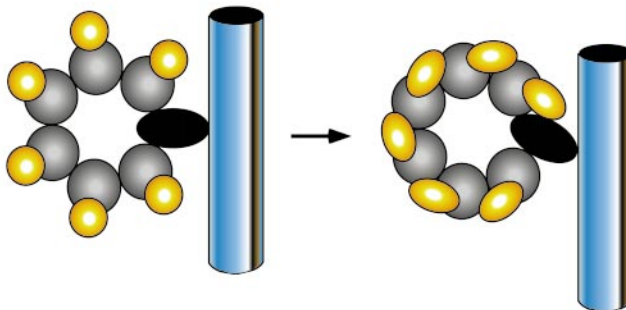


Figure 3. Speculative models for conformational changes of AAA proteins. (A) Target binding domains undergo a concerted, twisting conformational change. If a protein or protein complex is bound at two or more sites, it will be subject to mechanical strain by the conformational change. (B) A model for dynein-based motility based upon a conformational change in the ring. In this case, the shift in the AAA ring alters the angle of an embedded microtubule binding domain, which in turn produces a force that moves a microtubule. The dark gray spheres are the AAA domains and the smaller yellow sphere represent elements outside of the AAA domains (relative sizes are not necessarily accurate). Although not shown here, a conformational change may also have a vectoral component directed towards the center of the ring, as discussed in the text.

Although there is currently little information in this area, it is tempting to speculate that AAA motor proteins might also harness ring conformational changes to produce unidirectional motion. In dynein, for example, the main enzymatic AAA domain (P1) may induce a conformational changes that affects the adjacent AAA modules. These less enzymatically active AAA modules (P2–P4) may act as regulatory gates that affect the propagation of the conformational change. Ultimately, these nucleotide-dependent changes could change the shape or angle of the microtubule binding domain, which is inserted in the midst of the ring structure (Fig. 3 B), and this could generate a power stroke that moves dynein along the microtubule (Burgess, 1995; Gee et al., 1997; Goodenough and Heuser, 1982). This model for dynein has parallels with the one proposed for NSF. In NSF, ring conformational changes are thought to alter the conformation of the intermediate protein α SNAP, which in turn pries apart the SNARE complex like a crowbar.

Mysteries of the Mechanism

The studies described above provide a first glimpse into

how AAA might work. However, details of the mechanism are still very murky, and many unresolved issues remain, a few of which are highlighted below.

What Is the Nature of AAA Conformational Change?

Very little is known about how AAA proteins change conformation during the ATPase cycle and how these changes affect bound target proteins. Moreover, it is not clear whether the main action takes place entirely on the outside of the ring (as drawn in Fig. 3) or whether the cavity of the AAA ring also plays a role. Since the Clp proteins unfold and may translocate an extended polypeptide through its central channel, perhaps the AAA conformational change has a vectorial component directed towards its internal pore (in contrast to that shown in Fig. 3). This type of conformational change would act like a centripetal ratchet that draws bound objects towards the cavity. Loosely supporting this possibility are electron micrographs that show that the SNAP/SNARE complex is positioned centrally over the NSF pore (Hohl et al., 1998) as well as ones showing a potential contraction of dynein's microtubule binding domain (Goodenough and Heuser, 1982; Burgess, 1995). Even though the AAA pore is too small to accommodate a large protein complex, it may accept a piece of unfolded polypeptide that is generated during a protein-protein dissociation reaction, as discussed below.

When in the Cycle Does the Key Conformational Change Occur?

While ATP hydrolysis is required for AAA proteins to function, the enzymatic transition that triggers the major conformational change is not known. It is possible that ATP binding triggers the conformational strain between the AAA protein and its substrate, and ATP hydrolysis and phosphate release is used to dissociate the strained protein substrate from the AAA binding site. Alternatively, the major conformational change and strain could occur during the hydrolysis or phosphate release step. Further work is needed to resolve this issue as well as to determine the rate constants that govern the transitions in the enzymatic cycles of AAA proteins.

Does Protein Unfolding Play a Role in Dissociating Protein-Protein Interactions?

Protein unfolding, disassembly of protein-protein interactions, and motor activity appear to be disparate activities. However, perhaps there are some common features, in particular between unfolding and protein-protein disassembly reactions. Specifically, it is conceivable that AAA proteins break apart protein-protein complexes by unfolding or partially unfolding a structural element at or near the binding interface. Evidence suggesting this possibility comes from the finding that ClpA/ClpP can carry out concurrent chaperone activities of dissociating Rep A dimers to active monomers as well as unfolding and translocating some of the RepA into the protease for degradation (Pak et al., 1999).

In conclusion, the mechanisms of AAA enzymes work are still poorly understood, especially in comparison to

other mechanoenzymes such as myosin, kinesin, GroEL, and the F1 ATPase. Certainly, these enzymes cry out for more enzymatic, structural, and biophysical characterization. Such information not only will resolve how AAA proteins work, but it should also produce such a clearer picture of how these proteins function in various biological processes.

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