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Review

Structure and function of the AAA+ nucleotide binding pocket ☆

Petra Wendler *, Susanne Ciniawsky, Malte Kock, Sebastian Kube

Gene Center, Ludwig-Maximilians-Universität München, Feodor Lynen Strasse 25, 81377 München, Germany

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ABSTRACT

Members of the diverse superfamily of AAA+ proteins are molecular machines responsible for a wide range of essential cellular processes. In this review we summarise structural and functional data surrounding the nucleotide binding pocket of these versatile complexes. Protein Data Bank (PDB) structures of closely related AAA+ ATPase are overlaid and biologically relevant motifs are displayed. Interactions between protomers are illustrated on the basis of oligomeric structures of each AAA+ subgroup. The possible role of conserved motifs in the nucleotide binding pocket is assessed with regard to ATP binding and hydrolysis, oligomerisation and inter-subunit communication. Our comparison indicates that in particular the roles of the arginine finger and sensor 2 residues differ subtly between AAA+ subgroups, potentially providing a means for functional diversification. This article is part of a Special Issue entitled: AAA ATPases: structure and function.

1. Many AAA+ proteins are p-loop containing molecular motors

A large range of molecular motors use the energy of ATP binding and hydrolysis to perform mechanical work. Included in this group are the dynein, myosin and kinesin superfamilies and a heterogeneous collection of ASCE (*a*dditional *s*trand *c*onserved *E* family; formerly RecA like) proteins, such as the AAA+ superfamily (extended *A*TPases *a*ssociated with various cellular *a*ctivities), the ABC (*A*TP *b*inding *c*assette) superfamily and helicase superfamilies I, II and III. The unifying structural motif shared by all these ATPases and some related GTPases is the p-loop (phosphate loop; [1]), which coordinates the β and γ phosphates of the nucleotide during hydrolysis. Yet, there is no overall sequence similarity between members of the superfamilies and their biological functions are very diverse as they range from actin and microtubule based motility to fusion of and transport across membranes, chromatin maintenance, coordinated proteolysis and disaggregation. Substrates of the ATPases are either oligonucleotides or proteins, which are moved relative to the stationary enzyme or are used to move the enzyme along a path. In general, interconnected dimers or oligomeric assemblies rather than just one ATPase domain perform the task. Whilst kinesin and myosin form a dimer of more or less indirectly interacting heads (reviewed in [2]), many ASCE proteins operate by direct interaction of several nucleotide binding domains. These multiple ATPase domains can either be on the same or different polypeptide chains. Examples of proteins that act as a dimer of two covalently linked ASCE domains are UvrD, PcrA and RecB in helicase superfamily I, RecG, eIF4A and NS3 in helicase superfamily II (reviewed in [3] and [4]) and the ABCA and ABCC subfamilies of the ABC ATPases [5]. In the case of the dynein and Rea1/midasin motor domains six ASCE domains are arrayed in one polypeptide chain, forming a hexameric ring of active and inactive ATPase domains [6,7]. However, many ASCE proteins function as homo- or heterooligomers. These motor complexes can be composed of two proteins such as the ABCD and ABCG subfamilies of the ABC ATPases [5], five proteins such as the clamp loaders [8], six proteins such as most classical AAA proteins [9] or seven proteins such as the *Aquifex aeolicus* NtrC1 σ^{54} activator [10]. In addition, hexameric motor complexes like Clp/Hsp100 proteins, p97 or NSF assemble as protomers of two covalently linked ASCE domains, bringing together 12 ATPase domains to form one active protein complex. In this review we focus on the hexameric AAA+ proteins and analyse the structures in the PDB with regard to conserved nucleotide binding motifs and oligomer contacts. We also provide a summary of relevant mutational analyses, without claiming a complete coverage of the available biochemical data. A good survey and visualisation of the evolutionary classification of ASCE proteins can be found elsewhere [11–13].

2. Classification of AAA+ ATPases

The first suggestion that ATPases of different functions but similar peptide sequence group into a novel family of AAA proteins was published in 1991 [9]. At that time the family comprised four members. Over the past 20 years, at least 30.000 AAA+ proteins have been identified throughout all kingdoms of life (Pfam ID:

Abbreviations: aa, *Aquifex aeolicus*; af, *Archeoglobus fulgidus*; ap, *Aeropyrum pernix*; av, Adeno-associated virus-2; bs, *Bacillus subtilis*; cg, *Ceanothus griseus*; ec, *Escherichia coli*; hs, *Homo sapiens*; LTag, large T-antigen; mj, *Methanocaldococcus jannaschii*; mm, *Mus musculus*; pa, *Pyrobaculum aerophilum*; pf, *Pyrococcus furiosus*; rn, *Rattus norvegicus*; sc, *Saccharomyces cerevisiae*; st, *Salmonella typhimurium*; SV, Simian virus; ta, *Thermoplasma acidophilum*; tm, *Thermotoga maritima*; to, *Thermococcus onnurieneus*; tt, *Thermus thermophilus*

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* Corresponding author.
E-mail address: wendler@genzentrum.lmu.de (P. Wendler).

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PF00004; Interpro ID: IPR003959) with eukaryotic genomes typically encoding 50–100 different proteins of this superfamily (supfam.org; [14]). The divergent appearance of the superfamily and the ambiguous boundaries to other p-loop NTPases complicates classification into defined subgroups. However, with a growing number of sequences, structures and biochemical studies available, unifying and divergent features of AAA+ ATPases become more apparent. Classifications comprising sequence alignments and morphological traits suggest that the primary characteristic features of AAA+ proteins are the presence of a conserved ASCE core domain and an additional α-helical, C-terminal domain (Fig. 1A; [11,13]). Further subclassification based on the topology of the AAA+ domain or the C-terminal domain identified several higher-order groups or clades. At the core of the superfamily are the classic AAA ATPases (classical AAA clade or extended AAA group), including the NSF, CDC48, Pex, Bcs, proteasomal ATPase, katanin, Vps4, FtsH, Tip49 and Clp Domain 1 (D1) families with the latter three families diverging from the others. A potpourri of **p**rote**a**ses, **c**helatases, **t**ranscriptional activators and **t**ransport proteins, named PACTT or Pre-sensor I insert superclade, forms another major subgroup within the AAA+ ATPases. It includes the σ^{54} activator, Lon A, MCM, MoxR, midasin, dynein and Mg-chelatase families at its core and the peripherally connected HslU, ClpX, Clp D2 and Lon B families. A third group is formed by **hel**icases and **c**lamp loaders (HEC group or clamp loader clade) such as the RuvB, RFC and γ/δ′-Pol III families. However, the classification for some AAA+ families has not been conclusively determined. Proteins involved in replication initiation for example establish an independent lineage (DnaA, CDC6, ORC clade) in the classification of Iyer et al. [11] but are included in the HEC group in the study of Ammelburg et al. [13]. Ammelburg et al. also identified the **s**ignal **t**ransduction **A**TPases with **n**umerous **d**omains (STAND) family and the ExeA family as independent subfamilies of the AAA+ proteins and excluded the helicase III superfamily on the basis of an aberrant topology of the C-terminal domain. In this review we mainly discuss members of the classical AAA ATPase group, the PACTT group and the HEC group, but also refer to some superfamily III helicases.

3. The nucleotide binding domain of AAA+ ATPases

The defining feature of all AAA+ proteins is a ~230 amino acid ATPase module, composed of the nucleotide binding ASCE and the α-helical, C-terminal subdomains (Fig. 1). The ASCE core structure is characterised by the 51432 order of the central, parallel β-sheet and the presence of two acidic residues in the Walker B motif. The Walker A (p-loop) and Walker B motifs are located at the tip of β strands 1 and 3, respectively. They are crucially involved in nucleotide binding and hydrolysis by coordinating the β and γ phosphates of ATP and the water activating magnesium ion [15]. In contrast to other NTPases the two Walker motifs in ASCE ATPases are separated by the β4 strand insertion. The tip of this strand typically carries a polar residue in the sensor 1 motif that participates in hydrolysis by coordinating the attacking water in concert with the Walker B residues. Depending on the topology of each AAA+ subfamily, the substrate interacting loops are located in insertions in the α2 helix and/or the α3 helix (reviewed in [12]). In the oligomeric complex the loops are typically oriented towards the central pore of the ring shaped assembly. An analysis of side chain conformations in 50 AAA+ crystal structures showed that the conserved glutamate in the Walker B motif of most AAA+ ATPases can switch from an active to an inactive conformation upon ATP binding (glutamate switch; [16]). In the inactive, ATP bound state the glutamate interacts with a conserved asparagine on the β2 strand and is only released into the fully active ATPase configuration by substrate binding. This model provides a means to link ATP hydrolysis with substrate interaction and would explain why some ATPases are only

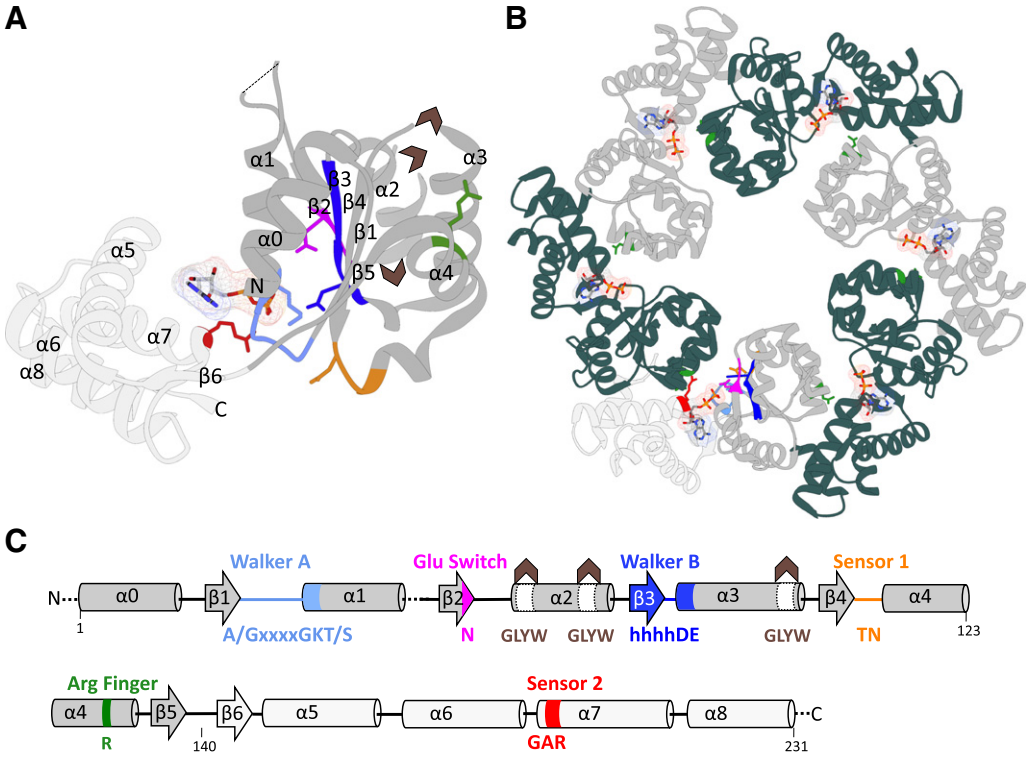

Fig. 1. Structure and oligomeric arrangement of the AAA+ domain. The AAA+ ATPase module (A) and its hexameric assembly (B) as seen in many crystal structures are depicted using the example of *to*Lon bound to ADP (PDB ID: 3K1J). Only the core ATPase domains of *to*Lon are shown. In (A) the ASCE domain is coloured in dark grey and the C-terminal domain in light grey. All structural elements are highlighted according to the colour code in the secondary structure annotation (C). Consensus sequences for each motif are given. Brown arrows indicate the position of pore loop insertions present in different AAA+ subgroups. Visible secondary structural elements are numbered in the order of appearance from N- to C-terminus. Protomers of the hexameric assembly in (B) are coloured alternating in dark green and grey. One protomer is coloured as shown in (A). Arginine fingers are coloured in green and the nucleotide is shown as stick and surface representation in all protomers.