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The Sulfolobus solfataricus AAA protein Sso0909, a homologue of the eukaryotic ESCRT Vps4 ATPase

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Abstract
Sso0909 is a protein of the thermo-acidophilic crenarchaeon Sulfolobus solfataricus, annotated as a p60 katin-like ATPase. We present here results supporting the hypothesis that Sso0909 is an orthologue of the eukaryotic ESCRT (endosomal sorting complex required for transport) ATPase Vps4 (vacuolar protein sorting 4). The spectrum of Sso0909 homologues is limited to several orders of Crenarchaea and to three euryarchaeal Thermoplasma species, where they were presumably acquired by lateral gene transfer. Almost invariably, Sso0909 homologues occur in the genomic vicinity of homologues of eukaryotic ESCRT-III components, which are the targets of disassembly by Vps4, as well as with a crenarchaeal-specific coiled-coil protein. S. solfataricus sso0909 is constitutively expressed under normal growth conditions and appears to be essential, as judged by the failure to obtain stable deletion mutants. We expressed Sso0909 in Escherichia coli and S. solfataricus, but have not obtained preparations with ATPase activity so far.

Background
AAA (ATPase associated with various cellular activities) proteins are a large and diverse family involved in a wide range of cellular functions. Their common activity appears to be the disassembly and unfolding of proteins in an energy-dependent manner. All of the AAA proteins that have been characterized form hexameric rings, although, in a few cases, hexamerization occurs only under certain conditions. AAA proteins consist of an N-terminal domain, thought to mediate substrate recognition, which varies between family members, followed by one or two AAA domains (named D1 and D2) [1]. Although the mechanism of nucleotide hydrolysis in this family is understood in its main aspects, the mechanism and substrate specificity of the respective N-terminal domains remain largely conjectural (see [2] for a review).

The phylogeny of AAA proteins has been analysed repeatedly over the years, most recently by Frickey and Lupas [1]. One of the major, consistently identified, clades of AAA domains, loosely defined as the ‘meiotic’ group, comprises katanins [3], spastins [4] and Vps4 (vacuolar protein sorting 4)/SKD1 (suppressor of potassium transport growth defect 1) [5]. Unlike other AAA proteins, members of this group occur as monomers or dimers in solution and oligomerize in a substrate-dependent manner [1,3]. Morphologically, they are characterized by the presence of one or several MIT (microtubule-interacting) domains at their N-terminal ends. Proteins of the meiotic clade are almost exclusively eukaryotic, with a small number of archaeal proteins branching off deeply within the clade [1,6]. Among the latter is Sso0909 from Sulfolobus solfataricus, one of six AAA proteins in this organism. Although annotated as p60-katin [7], we have developed the hypothesis that it may actually be an orthologue of Vps4, based in part on its genomic vicinity to a homologue of the components in the final endosome sorting complex of eukaryotes, ESCRT (endosomal sorting complex required for transport)-III. In eukaryotes, Vps4 is involved in protein sorting at the surface of vacuoles (yeast) or endosomes (humans) [8–12] by recruiting the components of ESCRT-III [13,14]. This relationship was also noted by Williams and co-workers [15], who recently determined the structure of the MIT domain of Sso0909.

As the Archaea share similarities to many cellular complexes with eukaryotes, albeit in a simpler and more robust form, they have been proven to yield excellent model systems [16–18]. We are aiming to develop Sso0909 into a model system for studying the mechanism of Vps4-dependent ESCRT-III disassembly. In the present paper, we report the results of a first characterization of Sso0909, involving bioinformatics, gene expression analysis by quantitative RT–PCR, heterologous protein expression in Escherichia coli, as well as homologous protein expression and targeted gene disruption in S. solfataricus.

Sequence database analysis
Owing to its basal position in phylogenies, Sso0909 could not be clearly related to any of the major eukaryotic clades of meiotic AAA proteins. We therefore compared its sequence with that of other meiotic ATPases, such as katanin [3], spastin [4], and Vps4 [1], using profile HMMs (Hidden Markov models). Sso0909 appeared to be the most closely related to Vps4,
which we also found when including secondary structure in the analysis using the HHpred server [19]. Alignment of Sso0909 and human VPS4B reveals the presence of a major gap in the C-terminal region, corresponding to the β-domain protrusion (Figure 1). This domain is a Vps4-specific elaboration on the helical sub-domain of AAA proteins, which makes contacts to auxiliary components of ESCRT-III. The absence of this domain in Sso0909, and indeed throughout Archaea, appears to correlate with the absence of homologues for these auxiliary factors in these organisms.

Searches for Sso0909 homologues in Archaea yielded a spectrum essentially limited to Crenarchaea: the thermophilic and hyperthermophilic orders Sulfolobales and Desulfurococcales, the mesophilic sponge symbiont Cenarchaeum symbiosum and the ammonia-oxidizing Nitrosopumilus maritimus. The only euryarchaeal orthologues were found in the Thermoplasma Thermoplasma and Ferroplasma (but not in Picrophilus), where they were presumably acquired by lateral gene transfer; extensive transfer between Thermoplasma and Sulfolobus has been clearly documented previously [20]. All crenarchaeal homologues exhibit very high sequence conservation (Figure 2), but only limited similarity to the euryarchaeal sequences, which is particularly low in the MIT domain. All crenarchaeal sequences, except for that of Cenarchaeum, are preceded in their operon by a homologue of ESCRT-III subunits (Sso0910 in S. solfataricus), and one to three further homologues are found elsewhere in the genome (Sso0451, Sso0881 and Sso0619 in S. solfataricus). The Vps4 and ESCRT-III homologues are also adjacent in Thermoplasma volcanium, but not in Thermoplasma acidophilum, and ESCRT-III homologues are entirely absent from the Ferroplasma genome. A third protein, Sso0911 in S. solfataricus, occurs in all Crenarchaea containing a Vps4 homologue (but not in the Thermoplasmata) and is generally encoded as the first gene in the operon. Sso0911 contains a large amount of coiled-coil structure and appears to be unrelated to other proteins in the database.

**Gene expression analysis**

Several studies have focused on gene expression changes in S. solfataricus following heat shock or UV treatment [21,22] and sso0909 has been found to be strongly down-regulated under these conditions. Little is known of the proteome content under standard growth conditions, so we tracked the expression of sso0909 by qPCR (quantitative PCR). We isolated total mRNA from cell aliquots taken at specific time points from an S. solfataricus culture growing at 76 °C with tryptone as carbon source [7]. Following RT of all mRNAs, we quantified the relative amount of sso0909-specific cDNAs and normalized the expression levels against the levels of the 23S rRNA gene (Figure 3). Gene expression of sso0909 appeared to be constant, whether cells were in the early-, mid- or late-exponential growth phase, suggesting constitutive expression. We conclude that Sso0909 is not involved in a cellular protection mechanism, but is required during normal cell growth.

**Biochemical analysis by heterologous and homologous expression**

We undertook the biochemical characterization of Sso0909 following standard heterologous expression in *E. coli* as a soluble GST (glutathione transferase)-fusion protein. After affinity chromatography and enzymatic GST removal, Sso0909 was purified by gel-sizing chromatography. The recombinant 42 kDa protein appeared to be folded using CD spectroscopy and behaved as a mixture of monomers and dimers in solution,
but, despite extensive efforts, neither ATP binding nor ATP hydrolysis could be observed under any conditions tested.

The recent development of genetic methods in *S. solfataricus* offer the possibility to (over)express exogenous proteins in this organism [23,24]. Consequently, we overexpressed the protein in *S. solfataricus* as a doubly tagged construct, Sso0909–His10–Strep, using the method of Albers et al. [23]. Overexpression resulted in poor growth and...
Figure 3 | Relative expression levels of the sso0909 gene according to the growth conditions of cells

\[D_{600}\] (line) and expression level (bars) data are the means ± S.D. of three replicas.

Figure 4 | Purification of the His10-tagged Sso0909 overexpressed in S. solfataricus

The Ni-NTA affinity chromatography yields an almost pure band (arrow). Lane M, molecular mass markers; lane 1, load; lane 2, flow-through; lanes 3 and 4, washing steps; lane 5, elution.

very low biomass yields, and the purification by Ni-NTA (Ni\(^{2+}\)-nitrilotriacetate) affinity chromatography gave low amounts of protein (Figure 4). Again, the protein exhibited no ATPase activity under any assay conditions, unlike other meiotic ATPases, which always show basal ATP hydrolysis levels even in the absence of substrate proteins [3,6].

Targeted gene disruption via genetic recombination

Albers and Driessen [25] recently proposed a greatly improved method for homologous recombination in S. solfataricus. The procedure disrupts the target locus, replacing it with a β-galactosidase (LacS) selection cassette in a lacS deletion mutant strain of S. solfataricus using homologous recombination. After several rounds of selection by growth on lactose as the sole carbon source, mutant colonies are identified by their colour upon X-Gal (5-bromo-4-chloroindol-3-yl β-D-galactopyranoside) treatment [25,26]. We repeatedly attempted to disrupt sso0909 by this method, but failed to obtain stable mutants. We tentatively conclude that the gene may be essential.

Discussion

We have described here the first steps in an ongoing investigation of the S. solfataricus protein Sso0909, following the hypothesis that this is an archaeal Vps4 homologue. Sso0909 has a spectrum largely limited to certain Crenarchaea, where it occurs in conjunction with homologues of ESCRT-III subunits and with a hitherto uncharacterized coiled-coil protein, suggesting that these are functionally coupled. In support of this notion, Williams and co-workers have shown an interaction between the Sso0909 MIT domain and a C-terminal fragment of Sso0910 [15]. The only other organisms containing an Sso0909 orthologue are Thermoplasma and Ferroplasma, and both of these lack the coiled-coil protein, while Ferroplasma also lacks an ESCRT-III subunit homologue. We conclude that the activity of the ATPase in these organisms may have changed substantially.

Sso0909 appears to be essential and constitutively expressed under normal growth conditions. Its mode of action is probably simpler than that of eukaryotic Vps4, shown for instance by the absence of the C-terminal β-domain. Unfortunately, the recombinant proteins we purified from E. coli or from their native environment, S. solfataricus were inactive under all assay conditions. We are unsure whether the proteins suffer from partial misfolding, for example in the N-terminal MIT domain, or whether we might be missing cofactors or interactors that regulate the activity. Our current efforts are focused on obtaining protein preparations with ATPase activity, on identifying binding partners and on clarifying the intracellular localization of the protein.

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References


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