The Archaeal Sac10b Protein Family: Conserved Proteins with Divergent Functions

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Abstract: Here we review the present state of structural and functional studies of the Sac10b protein family, a class of highly conserved 10 kDa nucleic acid-binding proteins in archaea. Based on biochemical and structural studies, these proteins were originally assigned a role in the structural organization of chromatin; Sac10b proteins of hyperthermophilic archaea, for example, showed tight, unspecific DNA binding. More recently, however, Sac10b proteins of mesophilic archaea were found to interact preferentially with specific DNA sequences thereby affecting the expression of distinct genes. Furthermore, Sac10b proteins of hyperthermophilic, thermophilic and mesophilic archaea were also shown to bind to RNA with distinct affinities and specificities but functional consequences of RNA binding of these proteins, besides perhaps RNA stabilization, have not yet been observed. To better understand the physiological meaning of the various interactions of Sac10b proteins with nucleic acids, future work should concentrate on elucidating the molecular structures of complexes of Sac10b proteins of hyperthermophilic and mesophilic archaea with DNA and RNA. In addition, existing and new X-ray and NMR structures of individual hyperthermophilic Sac10b proteins may represent very good models for introducing thermostability especially in enzymes for industrial use.

Keywords: Archaea, Sac10b protein family, X-ray and NMR structures, DNA- and RNA-binding proteins, functional diversity.

1. INTRODUCTION

After their discovery archaea became an important research area caused by their special position in evolution and the unusual living conditions of most of their species. In all forms of cellular life - bacteria, archaea and eukaryota - DNA is packaged into highly condensed protein-DNA complexes by chromatin proteins [1] which are also involved in the regulation of gene expression [1, 2]. In eukaryotes, the chromatin proteins are the histones [2] whereas in bacteria DNA-binding proteins such as HU and H-NS seem to be the functional equivalents [3, 4]. The discovery of histone homologue Hmf in euryarchaea suggested that euryarchaea and eukaryotes may have similar chromatin structures [5]. There are up to six different histone homologues in euryarchaea which all lack the flexible tail extensions of their eukaryotic counterparts [6]. In some archaea, homologues of bacterial chromatin proteins were found in limited amounts [7]. In all archaea, small basic DNA-binding proteins were discovered in the 1980s [8], and many of them were assumed to be chromatin proteins. They were grouped into 7 kDa, 8 kDa, and 10 kDa proteins [8], and believed to be involved in packaging the genomic DNA. Interest in these proteins increased strongly after the first sequence of an archaeon genome, that of *Sulfolobus solfataricus* P2, had been determined [9].

The 7 kDa proteins, later called Sul7d family [7], were sequenced [10-12] and identified as chromatin proteins existing only in the order *Sulfolobales*. Their properties, structures and functions were reviewed by Edmondson and Shriver [13]. The 8 kDa proteins contain two groups, the Sac8a and Sac8b proteins. Sac8b itself may be ribosomal protein L14e with an SH3-like structure [14]. The recently discovered protein Cren7 may be a Sac8a family protein because it is an abundant, conserved chromatin protein in Crenarchaea with many properties similar to those of Sac8a, for example unusual CD spectra, a histidine- and tryptophan-containing sequence, and an apparent molecular weight of 8.6 kDa in solution [15-17]. The 10 kDa proteins can be divided into Sac10a and Sac10b proteins. The structure of Sso10a, a member of the Sac10a protein family, was elucidated by X-ray crystallography [18] and NMR spectroscopy [19]. Sso10a is a homodimer (95 residues per chain); the dimer interface consists of an antiparallel coiled-coil and each monomer carries at its N-terminus a winged helix DNA-binding domain. Sac10a itself, another homodimeric DNA-binding protein of the hyperthermophilic archaeon *Sulfolobus acidocaldaricus* with high affinity especially for duplex poly(dAdT) ($k_D = 5x10^{-10}$ M in 0.15 M KCl, pH 7), had no sequence similarity with the other *Sulfolobus* chromatin proteins [20]. In 1999, Lou *et al.* isolated Ssh12, an

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abundant DNA-binding protein of the archaeon *Sulfolobus shibatae* [21]. Later, Ssh12 was proved to be Ssh10b, a member of the Sac10b protein family [22, 23]. Identification of the gene encoding Ssh10b and finding homologues of Ssh10b occurred in all archaeal genomes sequenced at that time but not in bacteria and eukaryota [24], stimulated a large number of biophysical, biochemical, structural and physiological studies of these proteins.

With a few exceptions, Sac10b proteins are ubiquitous in archaea. Several species contain two subgroups of these proteins, called Sac10b1 and Sac10b2 [7], which differ in their DNA binding properties. The DNA binding of one member of the Sac10b protein family, Sso10b or Alba, is regulated by acetylation (Alba: acetylation lowers binding affinity); the acetyltransferase Pat specifically acetylates Alba on Lys16 thereby lowering the affinity for DNA [25] whereas removal of the acetyl group is catalyzed by an archaeal homologue of the deacetylase Sir2 resulting in transcriptional repression [26]. Alba was the first Sac10b family protein whose high-resolution structure was determined by X-ray crystallography and for which a model of its interaction with DNA was proposed [27]. Meanwhile the structures of further hyperthermophilic archaeal proteins of the Sac10b family were solved by X-ray crystallography and/or NMR spectroscopy [28-35]. Another member of the Sac10b protein family, the hyperthermophilic Ssh10b, served as a model to explain the folding and the extreme stability of hyperthermophilic proteins [33, 36-40] and was found to bind not only to double-stranded and single-stranded DNA but also to RNA in a sequence-independent manner [41]. The nucleic acid binding properties of Sac10b proteins of hyperthermophilic and mesophilic organisms can differ. Mma10b for example, a Sac10b protein of mesophilic *Methanococcus maripaludis*, bound preferentially specific DNA sequences [42]. These studies suggest that Sac10b proteins might have multiple and divergent functions in different species.

In this review we discuss the properties, structures and functions of major members of the Sac10b protein family and point out directions for future work to advance the field.

2. BIOPHYSICAL AND BIOCHEMICAL PROPERTIES OF THE SAC10B PROTEINS

The Sac10b protein family is widely distributed in archaea. Sac10b itself was the first DNA-binding protein of the hyperthermophile *Sulfolobus acidocaldaricus* studied in detail, therefore its name was used as the family name [10]. Sac10b homologues have 87 to 102 amino acid residues and share ~35 to 66 % identity and 55 to 82 % similarity at the amino acid sequence level [22]. Multiple alignment of the Sac10b proteins shows that they possess a positive charge density of ~15 to16 % and share several fairly conserved charged positions [43]. The residues lining the dimer interface of the Sac10b proteins are also fairly well conserved implying a conserved quaternary structure of the dimer. The majority of these proteins, however, differs in certain sequence motifs such as GXKP in the loop between strand 1 and helix 1 and [KR]AVD in helix 2 [43]. The high overall conservation including sequences, charge densities and lengths of the cores of the secondary structural elements suggests that the basic biochemical properties are also conserved throughout this protein family.

Most of Sac10b homologues with basic isoelectric points (for example: 10.5 for Sac10b and Ssh10b, 9.7 for Mja10b, 9.4 for Sso10b, 8.0 for Mth10b, and 7.9 for Mvo 10b) show cooperative and more or less strong DNA binding without apparent sequence specificity [27, 42, 44] (pIs were calculated from the amino acid compositions of the corresponding proteins). However, some mesophilic members of the Sac10b protein family have a slightly acidic pI. The pI of Mma10b, for example, is 5.8 indicating a biological function (see Introduction, [42]) different from that of Sac10b proteins of hyperthermophilic archaea.

Early electron microscopic studies had shown that Sac10b bound both double-stranded and single-stranded DNA [44]. Double-stranded (ds) DNA was enveloped by Sac10b and the complexes had linear, flexible structures. The nature of the complexes formed with double-stranded closed-circular, open-circular or linear DNA was identical. At low protein:DNA ratios, Sac10b bound randomly to single-stranded ΦX 174 DNA to give small irregular protein clusters; with increasing protein:DNA ratios, partially circular nucleoprotein complexes were formed.

Sac10b and Ssh10b, a Sac10b protein from hyperthermophilic *Sulfolobus shibatae*, have similar affinities for ds and ssDNA but differ in their DNA binding patterns [22]. Ssh10b covers approximately 12 bp and has the ability to constrain negative DNA supercoiling [22], a property which is enhanced with rising temperatures up to 80°C. Ssh10b may thus affect the topology of chromosomal DNA at the growth temperature of thermophilic archaea. Ssh10b binds dsDNA with $k_D \sim 2x10^{-6}$ M [22].

The affinities for DNA of the Sac10b proteins Mja10b (hyperthermophilic) and Mth10b (thermophilic) were similar to that of Ssh10b showing a low-binding-density and a high-binding-density phase [42]. At low protein concentrations they formed a series of low-molecular-weight, separable protein-DNA complexes whereas at high protein concentrations aggregates were produced presumably resulting from uncharacterized protein-protein and/or protein-DNA interactions. On the other hand, Mma10b (mesophilic) was shown by DNase I footprinting to bind preferentially to specific DNA sequences with k_D in the 100 nM range [42]; its DNA-binding motif covered 18 bp including highly conserved A and T in the 5th and 14th positions, respectively. The sequence-dependent association of Mma10b was not affected by the structure of the DNA; Mma10b bound equally well to supercoiled plasmid as well as linear DNA [42] but had only low affinity for DNA lacking specific binding sites. The fact that Mma10b constitutes only ~ 0.01 % of the total cellular protein and affects the expression levels of several genes underlines its sequence-specific interaction with DNA.

Although originally identified as DNA-binding proteins, Sac10b proteins were found to interact also with RNA. As mentioned above, Ssh10b showed similar affinities for ds and ssDNA as well as for RNA *in vitro* [41]. In *Sulfolobus shibatae*, however, Ssh10b bound exclusively to RNA *in vivo* which prompted the authors to postulate that RNA was the physiological binding target of the Sac10b protein family [41]. The Sac10b proteins Mja10b, Mth10b, Mma10b, and Mvo10b also formed distinct complexes with RNA *in vitro*. Whereas the hyperthermophilic proteins Mja10b and Ssh10b

bound RNA strongly and formed high-molecular-weight aggregates at high protein concentrations, the RNA binding of the thermophilic and mesophilic proteins Mth10b, Mma10b, and Mvo10b was clearly weaker [42]. The protein concentrations required to retard half of the input RNA (20~30 nM) in electrophoretic mobility shift assays were approximately 2.5 nM Mja10b, 10 nM Ssh10b, 150 nM Mma10b, 150 nM Mth10b, and 600 nM Mvo10b in the presence of 0.4 M KCl. Interestingly, high salt concentrations weakened the DNA binding of Mth10b, Mma10b, and Mvo10b strongly but affected their RNA binding only slightly indicating that, in high salt, these methanococcal Sac10b proteins had higher affinity for oligoribonucleotides than for oligodeoxyribonucleotides *in vitro*.

3. STRUCTURES OF SAC10B FAMILY PROTEINS

Up to now, the structures of five Sac10b(1) and two Sac10b2 proteins of six archaeal species have been solved by X-ray crystallography and /or NMR spectroscopy (Table **1**). The six species include three crenarchaea and three euryarchaea. The seven proteins have very similar structures (Fig. (**1a**)); they are homodimers and each monomer contains four β-strands (β1 to β4) and two α-helices (α1 and α2) with β1-α1-β2-α2-β3-β4 connectivity. The dimer interface is formed by helix α2 and strands β3 and β4 of both monomers whereby the two β-strands form a long β-hairpin arm resulting in an X-shaped quaternary structure of the dimer. The root mean square deviation between the X-ray structures of these proteins is ~1.0 Å, the relative flexible β-hairpin arms contributing the major part of the conformational differences.

The Sac10b protein family shows significant structural similarity to several bacterial and eukaryotic proteins including the C-terminal domain of bacterial initiation factor IF3, *Escherichia coli* protein Yhhp implicated in cell division, *Arabidopsis* protein At2g34160, the N-terminal DNA-binding domain of DNase I [34], and bacterial stage V sporolation protein S (SpoVS) [45]. These similarities indicate that an ancestral protein was retained by bacteria, archaea, and eukarya and evolved in all three domains of life into different protein families involved in the structural and functional regulation of DNA and RNA.

Taking into account the Sac10b proteins' surface charge distribution and structural similarity to part of DNase I allowed to predict the location of the DNA-binding surface of these proteins [27] (Fig. (**1b**)). The surface comprised mainly the loop β1α1, the N-terminal portion of α1, the C-terminal portion of β2, the loop β2α2, the N-terminal portion of α2, and the long β-hairpin arm formed by β3 and β4. Based on the components of the binding surface, a model of the Sso10b dimer - dsDNA complex was proposed [27] (Fig. (**1b**)) in which the two arms of the Sso10b dimer reached into the minor groove of dsDNA occupying two binding sites that were separated by a complete turn of B-form DNA whereas the central protrusion of the dimer contacted the intervening part of the major groove. Lys16, proposed to be involved in the regulation of DNA binding of Sso10b [25, 26], was located at the tip of the β3-β4 hairpin loops (Fig. (**1b**)). This model showed also that continuous tight packing of Sso10b dimers around dsDNA resulted in a stoichiometry of ~5 bp per dimer which was supported by physicochemical data [27] and further X-ray structural analyses [28, 29]. The model does not explain how Sac10b proteins constrain negatively supercoiled DNA. The tight packing of Sso10b around dsDNA had been visualized by electron microscopy [44]. Upon binding to dsDNA, excess Sso10b formed extended helical fibres, most likely through continuous association of Sso10b dimers *via* dimer-dimer interfaces as shown in (Fig. (**1c**)). The dimer-dimer interfaces observed in most of the crystal structures of Sac10b family proteins [27-29] are formed between the strongly conserved hydrophobic residues of the α1 helix and the C-terminal tip of the α2 helix of one subunit each of the dimers. NMR studies of Sso10b by Jelinska *et al*. [46] demonstrated the importance of the two phenylalanine residues of the interface (drawn as sticks in (Fig. (**1c**)) for dimer-dimer formation and DNA binding; replacement of the phenylalanine by site-directed mutagenesis resulted in weaker DNA binding. *In vivo*, the association of Sso10b and other Sac10b protein dimers *via* dimer-dimer

Table 1. Sac10b Proteins with Known Three-Dimensional Structures

PDB Number	Protein	Species	Methods	Reference
1H0X, 1H0Y	Sso10b	*Sulfolobus solfataricus* P2 (CA)	X-ray	[27]
1NFH, 1NFJ	Afu10b	*Archaeoglobus fulgidus* (EA)	X-ray	[30]
1NH9	Mja10b	*Methanocaldococcus jannaschii* (EA)	X-ray	[29]
1Y9X	Ssh10b (P62A mutant)	*Sulfolobus shibatae* (CA)	NMR	[33]
2Z7C	Pho10b	*Pyrococcus horikoshii* (EA)	X-ray	[34]
2BKY	Sso10b, Sso10b2 heterodimer	*Sulfolobus solfataricus* P2 (CA)	X-ray	[32]
1UDV	Sso10b2	*Sulfolobus solfataricus* P2 (CA)	X-ray	[28]
2A2Y	Sso10b2	*Sulfolobus solfataricus* P2 (CA)	NMR	[31]
2H9U	Ape10b2	*Aeropyrum pernix* K1 (CA)	X-ray	[35]

CA, Crenarchaea; EA, Euryarchaea.

interfaces may facilitate the packaging of dsDNA by bending [30] and/or bridging [1]. The NMR experiments of Jelinska *et al*. [46] suggested also that, contrary to the model of the Sso10b-dsDNA complex proposed by Wardleworth *et al*. [27] (Fig. (**1b**)), the two β3-β4 hairpins of the Sso10b dimer do not bind in minor groove segments of dsDNA because in the complex the chemical shifts in the loop regions do not change and the loops remain flexible.

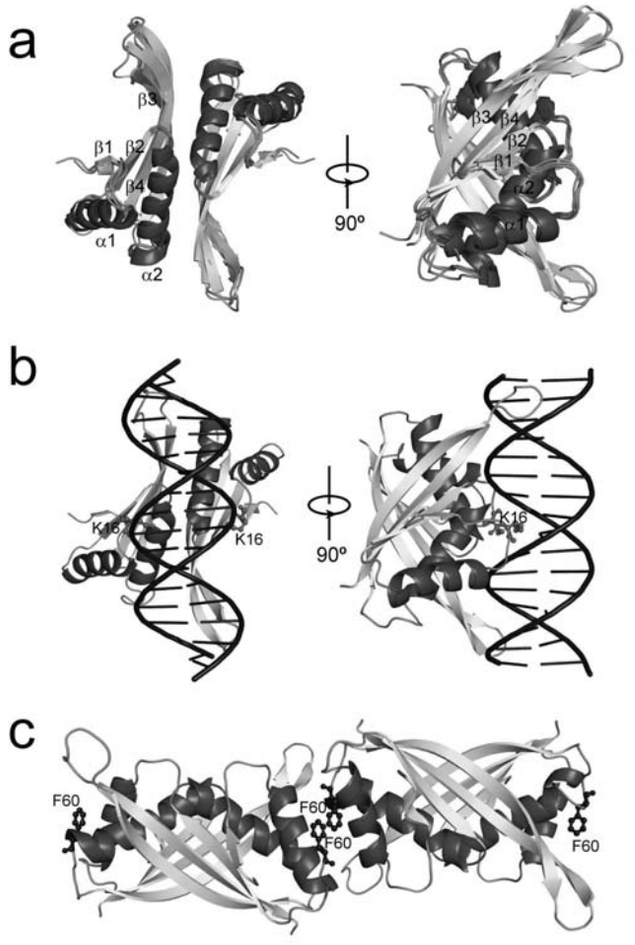

Fig. (1). X-ray structures of Sac10b family proteins. (**a**) Superposition of the structures of four Sac10b family proteins: Sso10b, Afu10b, Pho10b, and Mja10b (PDB 1H0X, 1NFH, 2Z7C, and 1NH9, respectively) [27, 29-30, 34]. (**b**) Model of an Sso10b-dsDNA complex. Lys16 of Sso10b which could be acetylated to regulate the DNA binding of Sso10b [26-27] is shown as ball-and-stick model. (**c**) The dimer-dimer interface of Sso10b in the crystal structure [46]. Residue Phe60 believed to play a key role in the dimer-dimer interactions is shown as ball-and-stick model.

An interesting feature of Ssh10b is that it affects DNA supercoiling in a temperature-dependent fashion *in vitro* [22]. NMR and mutagenesis revealed that this could be attributed to *cis-trans* isomerization of the Leu61-Pro62 peptide bond [47]. This isomerization results in significant conformational heterogeneity at low temperature; approximately one third of the residues display two sets of peaks in the ^{1}H, ^{15}N-HSQC (heteronuclear single quantum coherence) spectrum. The two forms, called T- and C-form corresponding to *trans*- and *cis*-peptide bond, were almost equally populated at 10°C in solution. With increasing temperature the C-form decreased to less than 20 % of the total amount at 57°C. The Pro62Ala mutant of Ssh10b, however, had only *trans* conformation indicating that *cis-trans* isomerization of the Leu61-Pro62 peptide bond was the structural origin of the temperature-dependent interactions between Ssh10b and DNA. The fact that Pro62 is not located at the DNA-binding surface of Ssh10b suggests that negative DNA supercoiling *in vitro* is at least partially determined by allosterically controlled conformational changes in the Ssh10b dimer. If conformational flexibility of Ssh10b existed also at 80°C, the optimal growth temperature of *S. shibatae*, it may allow the organism to survive at varying temperature. Several Sac10b proteins of mesophilic archaea also have a proline residue in analogous position, so it would be interesting to know if *cis-trans* isomerizations occur at the corresponding Xxx-Pro peptide bond of these proteins resulting in conformational heterogeneity and variable strength of protein-DNA interactions. A recent study of Mth1483, a Sac10b homolog from *Methanobacterium thermoautotrophicum* ΔH, reported a similar NMR spectrum with two sets of peaks, and Mth1483 contains an Ile-Pro peptide bond at the corresponding position to the Leu61-Pro62 peptide bond of Ssh10b [48], suggesting the *cis-trans* isomerizations occur widely in the Sac10b proteins (In the reference of 59, Mth1483 from *Methanobacterium thermoautotrophicum* ΔH was termed as Mth10b, but the protein Mth10b in this paper was referred to the Sac10b protein from *Methanothermococcus thermolithotrophicus*.).

Several structures of Sac10b2 group proteins have also been determined [28, 31, 35]. The overall structures, including the dimer-dimer interactions, of Sso10b2 and Ape10b2 were similar to those of Sac10b1 (Sac10b) group proteins but differed from the latter in the length of their β3-β4 hairpin arms: Sso10b2 had a much shorter, Ape10b2 had a much longer arm than the Sac10b1 group proteins. Interestingly, Sso10b1 and Sso10b2 can form a heterodimer in solution at physiological concentration [32]. The crystal structure of the heterodimer was similar to the crystal structures of the corresponding homodimers but DNA packaging by the two kinds of dimer differed noticeably. The reason for this difference in DNA binding could be that the crystal structure of the heterodimer lacked those dimer-dimer interactions that were conserved in the Sac10b1 crystal structures [27]. The formation of different dimers may be a means of the fine-regulation of DNA condensation to higher-order chromatin structures.

4. THERMOSTABILITY

As hyperthermophilic and mesophilic proteins typically show 40-85 % sequence similarity, they are very useful models to study the structural principles of protein folding and thermostability [49, 50]. Folding and thermostability of the dimeric hyperthermophilic protein Ssh10b have been particularly well studied [51]. Because unfolding by heat and denaturants was fully reversible, Ssh10b served as an additional good model for unfolding/folding studies of dimeric proteins. Using stopped-flow circular dichroism, Ge *et al*. [39] showed that in the presence of guanidinium chloride

native dimeric Ssh10b was rapidly converted to fully unfolded monomers without detectable kinetic intermediates. Upon refolding, two unfolded monomers acquired secondary structure and formed a dimeric intermediate in a burst phase followed by a slower, first-order process to give the native conformation of the dimer.

The X-ray structure of Sso10b had revealed that each monomer contained a stable α/β hydrophobic core, four salt bridges two of which were highly conserved in the Sac10b protein family (Glu36/Lys68 and Glu54/Arg57), and an ion-pair network (Lys40/Glu91/Arg71/Glu69) [27]. Various investigations showed that salt bridges and hydrophobic interactions contributed also strongly to the thermostability of Ssh10b. Compared to corresponding double mutants, the coupling free energies of the two highly conserved salt bridges remained almost constant between 25°C and 80°C (2.4 and 2.2 kJ/mole, respectively, for Glu54/Arg57; and 6.0 and 5.9 kJ/mole, respectively, for Glu36/Lys68) demonstrating the extreme resistance of these salt bridges to high temperatures [37]. The hyperthermostability of the Pro62Ala mutant of Ssh10b (i.e. stable *trans* configuration at the Leu61-Ala62 amide bond [47]) originated also in the presence of a stable α/β hydrophobic core as revealed by NMR and H/D exchange [33]. Furthermore, CD studies of three heat- and guanidinium chloride-denatured mutants of the C-terminal lysine residue (Lys97) of Pro62Ala Ssh10b suggested that Lys97 contributed also to the hyperthermostability of this protein through electrostatic interactions with closely located acidic residues (Asp63 and/or Glu66) and through preserving the local tight packing of the side chains [38]. Structure-based protein sequence alignment showed that salt bridge Asp63-Lys97 may especially contribute to hyperthermostability because it is conserved only in hyperthermophilic proteins [38]. Sequence comparison showed further that the thermophilic protein Mth10b and the mesophilic proteins Mma10b and Mvo10b have a glutamic acid residue in position 53 whereas in hyperthermophilic Pro62Ala Ssh10b this position is occupied by a valine residue which is buried in the hydrophobic core of the protein [33]. To clarify the influence of the nature of the amino acid residue in position 53 on thermostability, valine 53 of Pro62Ala Ssh10b was replaced by glutamic acid and heat- and denaturant-induced unfolding of "wild-type" (Val53) and Glu53 variant was studied. Compared to Pro62Ala Ssh10b having valine in position 53, the free energy of unfolding and the melting temperature of the Val53Glu variant had dropped from 24.3 kcal/mole to 19.5 kcal/mole and from 77.4°C to 37.3°C, respectively [33]. This result clearly demonstrates the importance of the intactness of the hydrophobic core for the hyperthermostability of Pro62Ala Ssh10b but the structural changes caused by the Val to Glu exchange could also have hampered stabilizing salt bridges and together these effects may have caused the strong decline of the thermodynamic parameters. Further X-ray and NMR studies may reveal the detailed structural changes caused by the Val53Glu mutation in Pro62Ala Ssh10b and, more generally, visualize the stepwise structural development from mesophilic to thermophilic and finally hyperthermophilic Sac10b proteins. However, the development of temperature-adjusted protein structures may have gone as well in the opposite direction (i.e. from hyperthermophilic to mesophilic) considering the climatic conditions on the primeval earth.

5. *IN VIVO* FUNCTIONS - CHROMATIN PROTEINS

It is a universal problem for all organisms that they have to package a long genomic DNA into a small space while maintaining access to the DNA for the protein complexes of replication and gene expression. This problem is solved by the presence of small, basic and abundant DNA-binding proteins, the so-called chromatin proteins. In archaea, the Sac10b proteins are widely distributed and possess the typical properties of chromatin proteins, especially their high abundance in cells; Sac10b is the second most abundant protein in *S. acidocaldaricus* [10] and Ssh10b constitutes 1.7 % of the total cellular protein in *S. shibatae* [22, 41]. Ssh10b forms structurally unspecific oligomers that bind to dsDNA without sequence specificity.

In 1986 [44], electron microscopic studies of complexes of Sac10b with dsDNA did not detect significant compaction of DNA *in vitro*. Later [22] it was shown that Ssh10b, like eukaryal histones, bacterial HU, archaeal histones and 7-kD proteins, introduced negative supercoiling in plasmids *in vitro*. This ability which is weak at 25°C but increases substantially with rising temperature up to 80°C [22] suggested that Sac10b proteins may also be involved in chromosomal organization and accessibility *in vivo*. That this is indeed the case was demonstrated by Marsh *et al*. [25]. These authors treated *S. solfataricus* with formaldehyde to introduce intracellular protein-DNA cross-links. Addition of an anti-Sso10b antibody resulted in chromatin immunoprecipitation. In a control experiment, excess recombinant Sso10b was added as competitor. The experiments showed that eight randomly chosen regions of the *S. solfataricus* genome obtained by PCR amplification co-precipitated with Sso10b and the anti-Sso10b antibody and that in the presence of excess Sso10b (control) the amounts of immunoprecipitated Sso10b-DNA complexes were reduced demonstrating the specificity of the immunoprecipitation. The fact that Sso10b could be obtained through DNase I treatment of the insoluble chromatin-containing fraction of an *S. solfataricus* chromatin fractionation [25] provided further evidence for association of Sso10b with chromosomal DNA *in vivo*. Comparison of recombinant Sso10b with Sso10b isolated from an *S. solfataricus* cell lysate showed that the native protein was acetylated at the N-terminus and at Lys16 [26]. It was found later that, *in vivo*, Lys16 of Sso10b was specifically acetylated by an *S. solfataricus* homologue of the acetyltransferase Pat [25]. Vice versa, an *S. solfataricus* homologue of the conserved NAD-dependent protein deacetylase Sir2, ssSir2, interacted specifically with and deacetylated Sso10b [26]. (In eukaryotes, Sir2 is an NAD-dependent histone deacetylase [52, 53].) This Sso10b acetylation/deacetylation switch determines the affinity of Sso10b for DNA. Compared to recombinant Sso10b (no acetyl groups), acetylated Sso10b had an approximately three-fold reduced affinity for DNA. The decrease in DNA binding was reversed through Sir2-catalyzed deacetylation of the protein which resulted in a quantitatively corresponding transcriptional silencing *in vitro* [25, 26]. Reversible acetylation is thus a means of regulating the DNA binding of chromosomal proteins in eukaryotes and in archaea.

6. FUNCTIONAL DIVERGENCE - RNA-BINDING PROTEINS

In 1994, Bohrmann *et al*. [54] made the interesting observation using immunogold electron microscopy that, *in vivo*, two histone-like proteins of *S. acidocaldaricus* (named HSNP-A and HSNP-C') were colocalized with DNA in the ribosome-free nucleoid whereas a third one (DBNP-B, now called Sso10b), which *in vitro* bound to ds and ssDNA and to RNA, was located exclusively in the ribosome-containing cytoplasm and therefore did not seem to be involved in DNA condensation. Later, Guo *et al*. [41] found that Ssh10b bound with similar affinities to double-stranded DNA, single-stranded DNA, and RNA *in vitro* but bound exclusively to RNA in *S. shibatae* cells as demonstrated by *in vivo* UV cross-linking followed by co-immunoprecipitation with anti-Ssh10b antibodies. Ssh10b was also co-purified with ribosomes and became cross-linked to 16S rRNA, 23S rRNA and mRNAs upon UV irradiation of *S. shibatae* cells; at the time it remained unclear whether Ssh10b bound also to 5S rRNA and tRNAs *in vivo*. These results indicated that several if not all RNA species are physiological binding targets of Ssh10b.

Marsh *et al*. [25] found that Sso10b associates with both DNA and RNA *in vivo*. They treated the insoluble chromatin-containing fraction, obtained during chromatin fractionation, with DNase I and RNase A, respectively, and found that Sso10b was liberated in both cases. Furthermore, structural comparison had shown that Sac10b members shared their mixed α/β-fold with IF3-C, the C-terminal domain of translation initiation factor IF3 [27], which had the full biological activity of the entire protein molecule [55] and interacted with 16S rRNA and $tRNA^{fMet}$ in the 30S ribosomal subunit detected by a combination of hydroxyl radical footprinting and directed hydroxyl radical probing with Fe(II)-derivatized IF3 [56].

Using comparative genomics and sequence profile analysis, Aravind *et al*. [43] pointed out that the Sac10b proteins were structurally probably closest to the YhbY proteins which are involved in prokaryotic translation and in intron splicing in plants [57]. Other proteins unified by these authors with the Sac10b protein family were the Rpp20 proteins from yeast and vertebrates and Rpp25 from vertebrates [43] which constitute essential RNA-binding protein subunits of RNase P, the precursor-tRNA-processing ribozyme.

More recently, Hada *et al*. [34] determined the crystal structure of *Pho*Alba, a Sac10b homologue of the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3. Not unexpectedly, this structure was similar to that of archaeal homologues and RNA-binding proteins including *B. stearothermophilus* IF3-C and *E. coli* YhhP. Gel shift assays showed that *Pho*Alba interacted with the RNA of RNase P and with pre-$tRNA^{Tyr}$. However, addition of *Pho*Alba to RNase P reconstituted from the RNA and known protein components had little effect on the catalytic activity of the *P. horikoshii* enzyme.

Ellis *et al*. [58] examined whether Sac10b (Alba) is a component of RNase P holoenzyme in the archaeon *Methanothermobacter thermoautotrophicus*. The authors found that the Sac10b homologue Mth1483p of this organism did not co-purify with the RNase P activity and anti-Mth1483p antibodies did not immunoprecipitate RNase P on an agarose-protein A-antibody column [58]. This indicated strongly that Mth1483p did not interact with the RNase P holoenzyme and, consequently, inclusion of Mth1483p in RNase P reconstitution assays did not enhance the enzymatic activity.

The results obtained so far about RNA binding of the archaeal hyperthermophilic Sac10b proteins are still sparse and not uniform. Based on the structural similarities between Sac10b and prokaryotic and eukaryotic RNA-binding proteins, Aravind *et al*. [43] had predicted that the Sac10b family probably originated as RNA-binding proteins, which would have been realistic in a primordial RNA world, and that at least some of the family members may play a role in RNA metabolism. However, the latter does not seem to be the case for the RNA-binding Sac10b proteins [34, 41, 58]. *Pho*Alba, for example, interacted with the RNA of RNase P but was not essential for the catalytic activity of this ribozyme [34]. In general, functions triggered by RNA binding of any of the Sac10b proteins have not (yet) been detected and the Sac10b homologue Mth1483p of *M. thermoautotrophicus* did not even bind to RNase P [58].

7. FUNCTIONAL DIVERGENCE - FUNCTIONS OF SAC10B FAMILY MEMBERS IN MESOPHILIC ARCHAEA

As shown in this review, the Sac10b family proteins studied best are those of hyperthermophilic archaea. But Sac10b proteins exist also in many thermophilic and mesophilic archaea and it was noticed that some of the functions of mesophilic Sac10b proteins differed from those of their thermophilic and hyperthermophilic homologues. Mvo10b, for example, is a Sac10b chromatin protein from the mesophilic methanogenic archaeon *Methanococcus voltae* with an optimal growth temperature of ~37°C. Deletion of the *mvo10b* gene did not affect viability and growth of the mutant archaeon but changed the protein expression pattern relative to that of the wild-type as revealed by 2D gel electrophoresis of cell extracts [59]. Recently [60], we cloned and expressed recombinant Mvo10b in *E. coli*. To purify the recombinant mesophilic protein we had to develop a new protocol because procedures used for the purification of hyperthermophilic Sac10b proteins failed to remove trace amounts of nucleic acids. This indicated differences in DNA binding by mesophilic Mvo10b, thermophilic Mth10b and the hyperthermophilic Sac10b proteins.

Similarly, disruption of *mma10b*, the gene encoding the mesophilic Sac10b homologue of the euryarchaeote *Methanococcus maripaludis*, Mma10b (optimal growth temperature, 37°C), resulted also in altered expression levels of several genes which is in line with sequence-specific DNA binding of Mma10b as shown by electrophoretic mobility shifts and DNase I footprinting [42]. Liu *et al.* reported systematical and detailed studies on Sac10b members from methanogenic archaea including hyperthermophilic, thermophilic, and mesophilic species [42]. These proteins include Mja10b, Mth10b, Mvo10b and Mma10b, from *Methanocaldococcus jannaschii* (optimal growth temperature, 80°C),

Methanothermococcus thermolithotrophicus (optimal growth temperature, 63°C), *Methanococcus voltae* (optimal growth temperature, 37°C), and *Methanococcus maripaludis* (optimal growth temperature, 37°C), respectively. It is interesting that the expression levels of these proteins are positively correlated with methanococcal optimum growth temperatures. The fact that both Mma10b and Mvo10b constituted only ~0.01 % each of the total cellular protein suggested that these mesophilic proteins participated in the regulation of distinct genes but it cannot be excluded that they are also involved in the regulation of chromatin structure [42, 59] and that the two functions overlap.

A Sac10b protein, Mth1438, from *Methanobacterium thermoautotrophicum* ΔH (optimal growth temperature, 65°C) was recently reported to bind neither DNA nor RNA *in vitro* [48]. The low isoelectric point (4.56) of Mth1483 was used to explain the loss of nucleic acid binding ability. However, the *in vitro* assays in the study used a plasmid and *E. coli* total RNA to detect the nucleic acid binding, which could not exclude the possible specific binding to DNA or RNA. For example, Mma10b, an acidic Sac10b protein with the isoelectric point 5.8, has specific DNA-binding activity [42]. Nevertheless, the different properties of the acidic Sac10b proteins indicate the different physiological functions of these proteins from those of the basic Sac10b proteins with the isoelectric point greater than 7.0.

In conclusion, the data presently available show that, during evolution, the archaeal Sac10b proteins have acquired divergent functions. There are also archaeal species such as *Halobacteria* that do not contain Sac10b family proteins [7] with possible reasons being loss or switch off of the corresponding genes.

8. THE SAC10B PROTEINS - PRESENT KNOWLEDGE AND FUTURE WORK

Sac10b proteins are highly conserved in archaea indicating that they are physiologically important for this kingdom of cellular life. Most members of this protein family are DNA- and RNA-binding but there is at least one Sac10b protein, i.e. Ssh10b, which was found to bind exclusively to RNA *in vivo* [41]. The DNA binding properties of these proteins seemingly depend on the optimal growth temperature of the corresponding organisms; of the Sac10b proteins studied so far, those of hyperthermophilic organisms show unspecific DNA binding whereas Mma10b, one Sac10b protein of mesophilic organism, interacts with specific DNA sequences.

The differences in the mode of DNA binding suggest that Sac10b proteins exert different functions: unspecific interaction of these proteins with DNA hints to a major role in chromatin organization whereas sequence-specific binding is more characteristic of a regulatory role in gene expression. Functional consequences of the binding of Sac10b proteins to RNA [25, 34, 41, 54] still have to be discovered.

To date, only structures of hyperthermophilic Sac10b proteins (Table **1**) and only one study of the binding of a Sac10b protein to DNA and RNA using NMR have been published [46]. In order to answer the major open questions concerning the physicochemical properties and physiological activities of the Sac10b proteins, more X-ray and NMR structures of individual hyperthermophilic, thermophilic and mesophilic Sac10b proteins and of various Sac10b protein - nucleic acid complexes must be available. Structural studies of complexes are expected to reveal how Sac10b proteins, perhaps in cooperation with other chromatin proteins such as histones and Cren7, constrain negatively supercoiled DNA. It would also be interesting to compare the molecular structures of Lys16-acetylated and Lys16-deacetylated Sso10b (Alba) - DNA complexes; it seems that the acetylation/deacetylation cycle would have to be coupled to some kind of sequence-specific Sso10b - DNA binding, perhaps supported by other protein factors. If not, this cycle should have a noticeable effect on chromatin structure.

As Sac10b family proteins occur in hyperthermophilic, thermophilic and mesophilic archaea and show high sequence similarity, the determination of their 3-D structures will also be an excellent means to study protein folding and evolution of protein stability, and it is conceivable that the knowledge gained from these studies can be applied to engineer enzymes with enhanced stability for use in industrial processes or to design proteins with novel functions. An increasing number of structural data of the Sac10b proteins will also allow following the evolution and functional divergence of this family of nucleic acid-binding proteins in the three kingdoms of cellular life.

CONFLICT OF INTEREST

None declared.

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