Biochemical and structural characterization of Cren7, a novel chromatin protein conserved among Crenarchaea

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Received October 30, 2007; Revised December 3, 2007; Accepted December 4, 2007

ABSTRACT

Archaea contain a variety of chromatin proteins consistent with the evolution of different genome packaging mechanisms. Among the two main kingdoms in the Archaea, Euryarchaeota synthesize histone homologs, whereas Crenarchaeota have not been shown to possess a chromatin protein conserved at the kingdom level. We report the identification of Cren7, a novel family of chromatin proteins highly conserved in the Crenarchaeota. A small, basic, methylated and abundant protein, Cren7 displays a higher affinity for double-stranded DNA than for single-stranded DNA, constrains negative DNA supercoils and is associated with genomic DNA in vivo. The solution structure and DNA-binding surface of Cren7 from the hyperthermophilic crenarchaeon Sulfolobus solfataricus were determined by NMR. The protein adopts an SH3-like fold. It interacts with duplex DNA through a β-sheet and a long flexible loop, presumably resulting in DNA distortions through intercalation of conserved hydrophobic residues into the DNA structure. These data suggest that the crenarchaeal kingdom in the Archaea shares a common strategy in chromatin organization.

INTRODUCTION

All cells must package their genomic DNA into a small space while providing controlled access to DNA for replication, recombination, repair and gene expression. Life appears to have evolved different strategies for genome packaging. In Eukarya, DNA is wrapped around the histone core of nucleosome, the basic structural unit for DNA packaging in the chromatin (1). In bacteria, chromosomal DNA is folded into a compact structure called nucleoid. Although the details of DNA packaging in bacterial cells are still lacking, the general organization of bacterial chromatin seems quite different from that of eukaryotic chromatin. An array of nucleoid-associated proteins (e.g. HU, IHF, H-NS, Fis and Lrp) have been identified, but their contributions to the overall structure of the bacterial nucleoid remain unclear (2,3).

The situation is intriguingly complex in Archaea. The Euryarchaeota contain archaeal histones that share a common ancestry with the histone fold regions of the eukaryotic nucleosome core histones (4,5). Extensive biochemical studies have revealed that archaeal histones form structures analogous to the eukaryotic H3/H4 tetrasome (5). Presumably, archaeal histones contribute to chromatin compaction and accessibility in vivo. Interestingly, histones are absent from the Crenarchaeota, with the exception of some species, e.g. psychrophilic Crenarchaeon from marine environments (6).

Efforts to understand DNA packaging in Crenarchaea have led to the isolation of a range of suspected chromatin proteins (e.g. Alba, CC1 and Sul7d) (7–9). However, all of these proteins, with the exception of Alba, appear to have a restricted phylogenetic distribution (4). Alba is highly conserved among both archaeal kingdoms (5). But, recent studies suggest that Alba binds to both DNA and RNA in vivo (10,11). So the physiological role of this protein family remains to be understood. CC1 denotes a family of DNA-binding proteins recently identified in the crenarchaeal orders Thermoproteales and Desulfoviococcales (9). Since CC1 binds equally well to both dsDNA and ssDNA, it is probably not involved in DNA packaging and may...
instead play a role in the protection of ssDNA in these organisms, which lack a canonical ssDNA-binding protein (SSB) (9). Sul7d is restricted to the order Sulfolobales. It includes a family of small, basic and abundant proteins that bind much more strongly to dsDNA than to ssDNA in vitro and are associated with the genomic DNA in vivo (11–13). These properties, together with the ability of the protein to constrain DNA supercoils and compact DNA, are consistent with a role for Sul7d in genome packaging. So far, no chromatin proteins have been found that are conserved among all phylogenetic branches of Crenarchaea. Whether Crenarchaea employ a conserved mechanism in chromatin organization is unknown. An answer to this question will not only shed light on the evolution of chromatin organization but also increase our knowledge about regulation of gene expression in Crenarchaea.

In the present study, we report the identification of a family of small and basic DNA-binding proteins, denoted Cren7, that are highly conserved in Crenarchaea. Biochemical and structural analyses show that Cren7 is a chromatin protein. Our results suggest that the majority of Crenarchaea share a common strategy in chromatin organization.

MATERIALS AND METHODS

Preparation of native and recombinant Cren7 proteins

Native Cren7 was purified from Sulfolobus shibatae using the purification protocol described previously for Ssh10b (14) with modifications. Recombinant Cren7 was prepared by amplifying the gene encoding the protein (SSO6901) from Sulfolobus solfataricus by PCR, cloning it into pET30a. The recombinant protein was subsequently over-produced in Escherichia coli strain Rosetta 2 (DE3) plyS and purified. Detailed purification procedures for native and recombinant Cren7 proteins are in Supplementary Data.

Protein identification

Identification of Cren7 was carried out by subjecting the purified protein to SDS–PAGE. The protein was digested in-gel with trypsin, and tryptic fragments were analyzed by LC-MS, as described (15).

Quantitative immunoblotting

Samples were taken from a growing S. solfataricus culture at various cell densities and centrifuged. Intracellular levels of Cren7 were measured by immunoblotting using anti-Cren7 antibodies, as described (11). Anti-Cren7 antibodies were raised in rabbit using purified recombinant Cren7.

Electrophoretic mobility shift assays (EMSA)

Radiolabeling of DNA fragments and EMSAs were performed as described previously (11). A 60 bp dsDNA fragment (5′-GATCCCCCAATGCTTCTGGTCTGATCA CACACCCCAAAGCCTTCTGCTTTGAATGCTGCC) and a 60 nt ssDNA fragment [poly(GACT)15] were used in this study.

Thermal denaturation of dsDNA

Recombinant Cren7 was mixed with dsDNA (poly[dAdT]-poly[dAdT]) in 10 mM potassium phosphate (pH 7.0). Thermal denaturation of the DNA was measured at $A_{260}$ in a temperature range from 25°C to 90°C on a DU800 UV/Visible spectrophotometer (Beckman). The melting temperature ($T_m$) was obtained using DU800 software.

Association of Cren7 with nucleic acids in vivo

The nature of nucleic acids bound by Cren7 in the cell was determined by chromatin fractionation and by in vivo UV-crosslinking followed by co-immunoprecipitation, as described previously (10,11).

Nick closure assays

Plasmid pBR322 containing a single nick per circular molecule was prepared by digestion with the nicking enzyme Nb.Bpu10I (Fermentas). The nicked plasmid (0.5 μg) was mixed with recombinant Cren7 in 20 mM Tris–Cl (pH7.5), 1 mM DTT and 100 μg/ml BSA and ligated with T4 DNA ligase (Fermentas) for 5 min at 25°C in a final volume of 15 μL. Samples were deproteinized and analyzed by agarose gel electrophoresis in the presence or absence of chloroquine, as described previously (16).

NMR spectroscopy

Cren7 were uniformly labeled with $^{15}$N/$^{13}$C by growing the recombinant strain in M9-minimal medium containing $^{15}$NH$_4$Cl and $^{13}$C-glucose as the sole nitrogen and carbon sources, respectively. The labeled proteins were purified as described above. Samples for NMR measurements contained 1.0–2.0 mM Cren7, 90% H$_2$O/10% D$_2$O and 0.01% sodium 2,2-dimethylsilapentane-5-sulfonate (DSS) in 50-mM potassium phosphate buffer (pH 6.0).

All NMR experiments were carried out at 310 K on a Bruker DMX 600 MHz spectrometer with a triple resonance cryo-probe. NMR experiments for $^1$H, $^{15}$N, and $^{13}$C resonance assignments included: 2D $^1$H–$^1$H TOCSY, $^1$H–$^1$H NOESY, $^1$H–$^1$H DQF-COSY, $^1$H–$^{15}$N HSQC, $^1$H–$^{13}$C HSQC, 3D HNCA, CBCA(CO)NH, HNCO, HN(CA)CO, HBHA(CBCA)CONH, $^1$H–$^{15}$N TOCSY-HSQC, (H)CCH-TOSY and HC(H)COSY. Four NOE experiments: 2D $^1$H–$^1$H NOESY, 3D $^1$H–$^{15}$N NOESY-HSQC, (H)CCH-TOSY and HC(H)COSY. Four NOE experiments: 2D $^1$H–$^1$H NOESY, 3D $^1$H–$^{15}$N NOESY-HSQC and $^1$H–$^{13}$C NOESY-HSQC for aliphatic and aromatic regions with mixing times of 200 ms were carried out to obtain the distance constraints. Dihedral constraints were generated using TALOS (17). All NMR spectra were processed and analyzed using Felix software (Accelrys Inc.). Proton chemical shifts and $^{15}$N and $^{13}$C chemical shifts were referenced to internal DSS and indirectly to DSS, respectively (18).

Initial structures of Cren7 were generated using CANDID module of CYANA software (19). The NOE assignments given by CANDID were checked manually and the structures were refined in explicit water using CNS software (20) and RECOORDScript (21). 100 structures
were calculated, and 20 structures with lowest energies were selected for final analysis. The quality of the structures was analyzed with MOLMOL (22) and PROCHECK-NMR (23). Structure figures were prepared using MOLMOL. Structural similarity was searched using Dali server (24) and SSM server (25).

Binding of Cren7 to a DNA duplex (5'-GCGAATT CGC) in 50-mM potassium phosphate buffer (pH 6.0) was monitored by 1H-15N HSQC using the 15N-labeled protein (0.2 mM). The backbone assignments of the Cren7–dsDNA complex were obtained in CBCA(CO)NH and HNCA experiments, in which 15N/13C-labeled Cren7 (0.2 mM) was mixed with a slight excess amount of dsDNA.

Data deposition

Chemical shift assignments of *S. solfataricus* Cren7 have been deposited in the BioMagResBank under an accession number 15415. The atomic coordinates of *S. solfataricus* Cren7 and all restraints have been deposited in the Protein Data Bank with accession code 2JTM.

RESULTS

Identification of Cren7

In the process of purifying Ssh7, a member of the Sul7d family from *S. shibatae*, we co-purified a small, abundant protein (Figure 1A). LC-MS analysis identified this protein as a homolog of the product of an unknown gene (SSO6901) in the *S. solfataricus* P2 genome. Sequence comparison shows that this novel protein is highly conserved among the crenarchaeotal lineage of the Archaea (Figure 1B). All genome-sequenced Crenarchaea except for *Sulfolobus shibatae* and *Cenarchaeum symbiosum* encode a homolog of this protein. It is present in the orders *Sulfolobales*, *Thermoproteales* and *Desulfurococcales*. Since it was later shown to be a chromatin protein, we designate this novel protein family as Cren7 following the nomenclature used for Sul7d.

*Sulfolobus shibatae* Cren7 contains 60 amino acid residues (theoretical M_r: 6637) and is highly basic (calculated pI: 9.87). The protein has 22 charged residues including 12 lysines and 2 arginines. N-terminal microsequencing revealed the lack of the initiator methionine in the protein. The native protein appeared to be methylated at multiple sites since a cluster of peaks differing by 14 kDa between adjacent peaks were detected by MALDI-TOF-TOF in the protein sample (Figure 1C). Mass fingerprinting showed that the protein was methylated at these sites to various extents (Supplementary Figures S1 and S2 and Supplementary Table S1). Cren7 exists as a monomer in solution and is stable at high temperature (Supplementary Data, Supplementary Figure S3).

Cren7 is a chromatin protein

We first investigated the interaction of Cren7 with DNA by EMSA. Cren7–DNA complexes initially generated...
resolvable shifts and, after a binding ratio of ∼10 bp per protein molecule was reached, formed increasingly slow-migrating smears as protein concentration increased (Figure 2A). Therefore, the gel retardation pattern of Cren7 was biphasic, with a low-binding density phase and a high-binding density one. Cren7 probably bound to DNA with a binding site size of 10 bp in the low-binding density phase, but switched to a new binding mode in the high-binding density phase. This new binding mode presumably involved a change in protein–DNA and/or protein–protein contacts. The inability to resolve large Cren7–DNA complexes formed at high protein concentrations by EMSA prevented an accurate determination, based on the relationship between the maximum number of shifts and the size of the dsDNA fragment, of the binding site size of the protein in the high-density-binding phase. However, judging from the lowest protein/DNA ratio at which plasmid pBR322 was maximally retarded by Cren7 at the following protein/DNA mass ratios: 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.375 and 0.5, respectively. N, nicked circular plasmid; L, linear plasmid; S, supercoiled plasmid. (D) Plot of the linking number change of nick-closed plasmid against the Cren7/DNA mass ratio. The linking change of pBR322 ligated in the presence or absence of Cren7 was measured by resolving topoisomers on agarose gels in the presence or absence of chloroquine and band counting.

Like Sul7d, Cren7 bound only weakly to ssDNA (apparent K_D = ∼2.5 μM) in low salt, and generated no resolvable shifts on a labeled ssDNA (Figure 2A) (12,13), indicating that Cren7 binds preferentially to dsDNA over ssDNA. This observation suggests that Cren7 might affect the T_m of dsDNA. Indeed, we found that the T_m of poly[dAdT]–poly[dAdT] was raised by 23.3 ± 8°C and 28.8 ± 8°C, respectively, in the presence of Cren7 at protein/DNA mass ratios of 1 and 5 (Figure 2B). So, binding by Cren7 significantly increased the stability of dsDNA against thermal denaturation.
We then determined if binding by Cren7 affected the geometry of DNA using a nick closure assay (16). A single-nicked plasmid became supercoiled when ligated at 25°C in the presence of the protein (Figure 2C). Comparison of the mobilities of plasmid topoisomers in agarose gels in the presence and absence of chloroquine indicated that the protein constrained the DNA in negative supercoils (data not shown). The number of constrained supercoils was proportional to the protein/DNA mass ratio in the tested range (Figure 2D). Binding of ∼10 protein molecules resulted in one negative supercoil being constrained. The ability of the protein to constrain negative DNA supercoils at 45°C, 60°C and 80°C did not differ measurably from that at 25°C (data not shown). Taken together, Cren7 is about twice as efficient as, but otherwise resembles, Sul7d in constraining DNA supercoils (16,27).

As a small, basic and methylated protein capable of efficient binding to dsDNA and constraining negatively DNA supercoils, Cren7 may be a chromatin protein in vivo. To verify this possibility, we examined the association of Cren7 with nucleic acids in the S. solfataricus cell by using two approaches. In the first approach, we performed chromatin fractionation as described previously (10). As shown in Figure 3A, the bulk of Cren7 was in the insoluble chromatin-containing fraction. Treatment of the pellet with DNase I, but not RNase A, released a significant proportion of Cren7, indicating that the protein was associated with chromosomal DNA in vivo. In the second approach, we irradiated S. solfataricus cells with UV light to induce the formation of protein–nucleic acid cross-links, lysed the cells and subjected the lysates to immunoprecipitation with antibodies against Cren7. Nucleic acids co-immunoprecipitated with Cren7 were treated with either DNase I or RNase A + T1, and electrophoresed in agarose. These data indicate that Cren7 is a chromatin DNA-binding protein.

As a protein serving a structural role in chromatin usually exists in abundance. So we measured the cellular level of S. solfataricus Cren7 by quantitative immunoblotting. Samples taken from various stages during the growth of S. solfataricus appeared to contain similar amounts of Cren7 (Figure 3C). The protein constituted ∼1% of the total cellular protein. Based on this estimate, the mass ratio of Cren7 to genomic DNA was ∼0.8 in vivo (16). This number translates into one Cren7 molecule per 12 bp of DNA. In other words, Cren7 binds ∼1/4 of the genomic DNA in the cell. By comparison, Ssh7 amounts to 4.8% of the cellular protein in S. shibatae (16), and HmfA/HmfB are ∼1% of the cellular protein in Methanothermus fervidus (28).

### Solution structure and DNA-binding surface of Cren7

The solution structure of Cren7 was determined on the basis of 1623 NOE-derived distance restraints, 72 dihedral constraints and 30 hydrogen bond restraints derived from multidimensional NMR spectroscopy (Supplementary Table S2). It has a β-barrel structure including two anti-parallel β-sheets. β-sheet 1 is composed of two β-strands (β1: 8–11 and β2: 17–20) while β-sheet 2 contains three
β-strands (β3: 24–28, β4: 36–42, and β5: 49–53) (Figure 4A). The side-chains of hydrophobic residues V8, V10, A18, L20, V25, F41 and F50 of the five β-strands make a tight packing within the ‘β-barrel’. The C-terminal region (L54–I60) also participates in the hydrophobic packing through residues L54 and Y58 and, therefore, is structurally ordered. On the other hand, the N-terminal region (M1–K6) of the protein is unstructured. Among the four loops linking the five β-strands, loop Lβ3β4 (A29-G35) is most flexible. These structural features of the protein are supported by dynamic data (Supplementary Data and Supplementary Figure S4). Side chains of L28 in β3, V36 and I38 in β4 and P30 in loop Lβ3β4 form a notable solvent-exposed hydrophobic region (Figure 4B). Side chains of K11, K24, K48, R51 and K53 from the β-strands, and K31 and R33 from loop Lβ3β4 face outside of the ‘β-barrel’, forming a positively charged region on the electrostatic surface of the protein (Figure 4B). Most of these residues are highly conserved, indicating that proteins of the Cren7 family have similar structures as well as surface charge distributions (Figure 1B).

DNA-binding surface of Cren7 was determined by monitoring signal changes in two-dimensional $^{1}H-{^{15}N}$ HSQC spectra following titration of the protein with a 10-bp duplex DNA (Supplementary Figure S5). As the DNA was titrated into the mixture, changes were observed for the cross peaks of residues involved in DNA binding. No further changes in the HSQC spectra occurred at DNA/protein molar ratios $>1$, suggesting a stoichiometry of one for the Cren7–dsDNA complex (Supplementary Data). Approximately one-third of the residues in Cren7 showed significant chemical shift changes in the HSQC spectra during dsDNA titration, indicating the presence of a large DNA-binding surface on the protein. In addition to the chemical shift changes, two cross peaks for side chains of R33 (on loop Lβ3β4) and R51 (in β5), which were not observed in the HSQC spectrum of free protein because of amide-water proton exchange, appeared in the spectrum at the saturating level of dsDNA. Therefore, the two Arg residues are located at the binding interface of the protein–DNA interaction, and the amide-water proton exchange was prevented by bound dsDNA. The binding surface, as obtained by mapping these residues on the structure of Cren7, spans residues on strands β3, β4, β5, the C-terminus of β1 and loop Lβ3β4 (Figure 5A–C). It consists mainly of the positively charged surface region and the solvent-exposed hydrophobic region. It was noted that the conformational flexibility of loop Lβ3β4 was significantly reduced upon binding of the protein to DNA, as evidenced by a significant increase in $^{1}H-{^{15}N}$ NOE value (Supplementary Figure S4).

Dali and SSM searches show that the structure of Cren7 is a SH3-like fold. Although this fold was originally identified in proteins that bind poly-proline peptides, it has since been found in proteins with a number of other functions (29,30). A typical SH3 fold contains five β-strands and a short 3_10-helix between β4 and β5. Cren7 lacks both the C-terminal β5 and the 3_10-helix, but has a structured loop at the C-terminus. The other β-strands of Cren7 are similar to SH3, except that the β2 found in a typical SH3 fold is split into two sequential β-strands in Cren7. Interestingly, Sac7d, a Sul7d protein from Sulfolobus acidocaldarius, is the most relevant hit among those identified SH3-like proteins in the searches although the two proteins share no significant similarity at the amino-acid sequence level. The RMSD between Cren7 and Sac7d aligned by Dali is 2.6 Å (with a Z-score of 2.8), indicating a high structural similarity between the two proteins. Among 60 residues in Cren7 and 66 residues in Sac7d, 44 are structurally aligned by Dali. The ‘β-barrel’ parts of the two proteins are aligned best (Figures 4C and 5A). The two proteins also have similar solvent-exposed hydrophobic regions and positively charged regions on β-sheet 2, which form the DNA-binding surfaces (Figure 4B and C). A number of residues on the DNA-binding surfaces of the two proteins are conserved or similar. These include positively charged residues (K11, K24 and R51), hydrophobic residues (L28, V36 and L40) and a tryptophan residue (W26) in Cren7 (Figure 5D and E). However, several notable structural differences exist between Cren7 and Sac7d. At the DNA-binding surface, Cren7 has a long and highly flexible loop (Lβ3β4), whereas only a small hinge exists at the corresponding position in Sac7d. In addition, the solvent-exposed hydrophobic region of Cren7 (L28, P30, V36, I38) is larger than that of Sul7d (V26, M29). Cren7 has a flexible N-terminal

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Overall solution structure of *S. solfataricus* Cren7. (A) Ribbon representation of 20 Cren7 solution structures. The backbone of five β-strands are shown in green and labeled. (B) Molecular surface of Cren7 colored by electrostatic potential. Blue, positively charged; red, negatively charged; white, neutral. Some charged or hydrophobic residues on the molecular surface are labeled. A ribbon representation is also shown for reference. (C) Structure alignment of Cren7 (in green) and Sac7d (in red, PDB code: 1AZP) by SSM. A ribbon representation is also shown for reference. (D) Molecular surface of Sac7d colored by electrostatic potential. For comparison, the Sac7d protein is aligned in the same orientation as Cren7 in Figure 4B.
loop which is longer than that of Sac7d. On the other hand, the C-terminal segment of Sac7d is 15 residues longer than that of Cren7 and forms a helix which is absent in Cren7.

**DISCUSSION**

In contrast to the wide distribution of archaeal histones in the Euryarchaeota, no chromatin proteins conserved at the kingdom level in and unique to the Crenarchaeota have been found. The discrepancy between the two main archaeal lineages in this regard is puzzling. Identification of Cren7, a highly conserved protein family in Crenarchaeota, has offered important clues to the issue. Biochemical and structural studies reported in the present work suggest that Cren7 is a chromatin protein. Crenarchaeota whose genome has been sequenced so far, encode Cren7 homologs with only two known exceptions. The conservation of Cren7 among Crenarchaeota points strongly to the possibility that this archaeal lineage shares a common feature in genome packaging and/or regulation of gene expression. The Crenarchaeota known to lack this protein are *T. pendens* Hrk5 and *C. symbiosum*. Intriguingly and, probably not incidentally, both of these organisms encode an archaeal histone (5,31). Therefore, it is tempting to speculate that Cren7 plays a role in Crenarchaeota similar to that of archaeal histones in Euryarchaeota. Clearly, this hypothesis needs to be tested with more crenarchaeal genomes. It is also of interest to find out if there are Archaea that contain neither Cren7 nor histones.

An interesting finding of the present study is that Cren7 bears significant resemblance in structure to Sul7d, whose structure has been extensively investigated (12,26,32,33), although the two proteins are unrelated at the amino acid sequence level. Comparative analyses of the similarities and the differences between the two proteins in amino acid sequence, structure and DNA-binding properties may offer valuable insight into the mechanisms of DNA binding and distortion by Cren7. It has been shown that binding by Sac7d produces, through intercalation of the side chains of V26 and M29 (26,34), a sharp single-step kink (≈60°) associated with helix unwinding in dsDNA, which induces negative DNA supercoiling (32,35).
The two intercalating amino acid residues are substituted for by two conserved hydrophobic residues (L28 and V36) in the Cren7 family (Figure 1B), which appear to be properly positioned in the protein structure (Figure 5D) to serve a role similar to that of V26 and M29 in Sac7d. So it is likely that Cren7 also causes a sharp kink in bound dsDNA. Notably, however, Cren7 differed from Sac7d in the ability to induce DNA distortions. In our topological assays, Cren7 was significantly more efficient than Sul7d in constraining negative DNA supercoils, suggesting that the former is probably able to induce greater helix unwinding and larger kinks in DNA than the latter. This difference may be due to the presence of a longer flexible loop (Lb3a4) and a larger solvent-exposed hydrophobic patch at the DNA-binding surface in Cren7 than in Sac7d.

Additional major structural differences between Cren7 and Sul7d are present in the terminal regions of the two proteins. Cren7 lacks the C-terminal α-helix present in Sac7d but possesses a flexible N-terminal loop absent in Sac7d. Both of these terminal regions are located opposite to the DNA-binding face of the protein and, therefore, are likely involved in protein–protein interactions. This notion is consistent with the finding that Cren7 was capable of forming large complexes with duplex DNA fragments at higher protein concentrations.

The presence of long and flexible loop Lb3a4 and N-terminal tail in Cren7, but not in Sac7d, also suggests that the interaction of the two proteins with DNA may be regulated differently. Residue K31 on loop Lb3a4 in Cren7 is partially methylated, and the N-terminal tail of Cren7 contains two serine and two lysine residues, which are often sites of phosphorylation and methylation or acetylation, respectively, in eukaryotic histones (36–38). Taken together, the ability of Cren7 to constrain negative supercoils, form large complexes with dsDNA and undergo potential regulatory modifications is consistent with a role for the protein in chromosomal organization.

Archaea of the genus *Sulfolobus* synthesize copious amounts of both Cren7 and Sul7d. It seems unlikely that either protein is an evolutionary fossil. So a question arises as to how the two proteins are evolutionarily and functionally related. Presumably, both proteins serve important roles in chromosomal organization and their functions are not entirely redundant. The differences between the two proteins in function may have led to the loss in sequence similarity between them. Given its wide distribution, Cren7 is probably the best candidate for a major chromatin protein in Crenarchaeae. The remarkable ability of the protein to distort DNA conformation is consistent with a role in DNA supercoiling and compaction. So our results reinforce the suggestion that Archaea employ two major strategies in chromatin compaction (26,33): one involves archaean histones and the other uses the minor-groove binding proteins such as Cren7. While the histone-based strategy is primarily employed by the Euryarchaeota, the Cren7-based strategy may predominate in the Crenarchaeota. In *Sulfolobus* species, Cren7 and Sul7d (and, possibly, other unidentified chromatin proteins) may participate in chromosomal organization by forming functional complexes and/or interacting in a coordinated manner with DNA. The role of Sul7d is probably served by functional equivalents in other Crenarchaeae.

**SUPPLEMENTARY DATA**

Supplementary Data are available at NAR Online.

**ACKNOWLEDGEMENTS**

We thank Danxia Yu for help in recombinant protein preparation. This work was supported by National Basic Research Program of China (2004CB719603 to L.H.); National Natural Science Foundation of China (30470025 and 30621005 to L.H. 30570375 to J.W.); Chinese Academy of Sciences (KSCX2-YW-G-023 to L.H., KSCX1-SW-17 to J.W.). Funding to pay the Open Access publication charges for this article was provided by National Natural Science Foundation of China (30470025).

**Conflict of interest statement.** None declared.

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