The dynamic organization of prokaryotic genomes: DNA bridging and wrapping proteins across the tree of life

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Chapter 1

Introduction

Every organism in the tree of life faces the same challenge: the effective volume of its genome far exceeds the volume of the cell or cellular compartment in which it is contained. Therefore, strategies have evolved to ensure proper genome compaction and organization. At the same time, the DNA must be accessible for genomic transactions such as transcription and replication. The main factors contributing to these processes are DNA supercoiling, macromolecular crowding and binding of chromatin proteins with architectural properties (1–4). These chromatin-associated proteins can be classified based on their architectural properties: DNA wrapping, bending, bridging, or formation of a nucleoprotein filament. Proteins from the different classes can exhibit structural interplay in either a synergistic or antagonistic manner (2–4).

The transcription process is, to a large extent, conserved throughout the tree of life. Bacteria, archaea and eukaryotes all express RNA polymerase (RNAP) to transcribe DNA into mRNA. Transcription initiation in bacteria occurs when a σ factor binds to the promoter -10 element and guides the RNAP to the DNA (5). In archaea and eukaryotes, the TATA-box and the B recognition element (BRE) are necessary for transcription to initiate (6, 7). The TATA-binding protein (TBP) and Transcription factor B (TFB) bind to the respective DNA elements and recruit RNAP (8). Transcription elongation and, therefore, correct, uninterrupted mRNA synthesis is aided by the transcription elongation factor called Spt5 (in archaea and eukaryotes) or NusG (in bacteria) (9). Chromatin proteins, such as histones and other nucleoid-associated proteins (NAPs), can interfere with these processes in several ways and thereby regulate gene expression. They can bind to the transcription initiation sites and exclude RNAP from binding, they can trap RNAP by loop formation, or interfere with transcription elongation by binding across the coding region (10, 11). In general, chromatin proteins are relatively small and basic proteins. They can bend, wrap, stiffen or bridge DNA duplexes upon binding to the DNA. In eukaryotes, histones are the main chromatin proteins, while bacteria encode a plethora of NAPs. For instance, at least 12 proteins have been classified as NAPs in *Escherichia coli* (12). They are mainly defined as proteins that combine a function in transcriptional regulation and genome structure with limited sequence specificity, in contrast to transcription factors with a sequence-specific regulatory function. Archaea encode both histone proteins and NAPs (table 1.1). At the time of discovery, archaea were defined as a third branch of the tree of life, next to bacteria and eukaryotes. However, with advances in the phylogenetic description of archaea, a two-domain model of the tree of life was adopted, in which eukaryotes are a sister group of the archaea. This implies that archaea were at the basis of eukaryogenesis, and study of archaeal evolution could provide insight into this important evolutionary event.

In this thesis, I will focus on chromatin proteins throughout the tree of life and their DNA binding properties. In this chapter, I will introduce the different chromatin proteins based on their architectural effect on DNA and explore their distribution among species. In chapter 2, I will describe the known characteristics and functions of bacterial DNA bridging proteins in more depth. In chapters 3-6, I will describe my experimental studies on bacterial and archaeal DNA bridging proteins and archaeal histones.

DNA wrappers

Eukaryotic histones

The best-known architectural proteins, prototypical DNA wrappers, are eukaryotic histones. Conserved throughout the eukaryotic domain of life, they share the characteristic histone fold of three α-helices connected by two loops (13). They form obligatory H3-H4 and H2A-H2B heterodimers. Two H3-H4 dimers interact to form a tetramer with which two H2A-H2B dimers can associate. The result is an octameric protein core with around 147 bp DNA wrapped around it (figure 1.1A). Depending on the length of DNA between nucleosomes and other factors such as the linker histone H1, nucleosomes can be arranged in higher-order structures such as the 30 nm-fiber (14, 15). Nucleosomes are generally associated with repression of transcription by excluding other factors from binding to the DNA (16).

The N-terminal tails of histones can be post-translationally modified, which affects the accessibility of the chromatin and, as a consequence, gene transcription (17). Another mechanism of modulating nucleosome (and therewith chromatin) structure is exchange of histones with histone variants. The H2A-H2B dimers are generally more exchangeable than the H3-H4 tetramer (18). Histone variants, each with their own specific role in genome organization and gene regulation, exist of all core histones with (in number) a bias towards H2A and H3 (19).

Archaeal histones

Most archaeal genomes encode histones that resemble their eukaryotic counterparts (table 1.1). Histones are present at least in a minority of available genomes in every superphylum, phylum and class. Only in *Ca. Marsarchaeota* no histone genes have been found to date. Crenarchaeota, which have been considered histone-free for a long time, encode histones in a minority of genomes (20, 21). Due to the relatively low amount of available genomes for the three histone-free phyla, histone genes may be discovered later. Also, as they all still have the *Candidatus* status, advances in culturing

Archaea could provide more insight into their genome organization and the presence of histones.

Archaeal histones share the characteristic histone fold with eukaryotic histones, but generally lack the N- and C-terminal tails (21) (figure 1.1B). Also, a preference for GC-rich sequences is shared between archaea and eukaryotes (22, 23). Due to the resemblance between the archaeal histone and the eukaryotic H3/H4 tetramer, it was suggested that eukaryotic histones evolved from the archaeal ones (24). The H2A and H2B histones likely later evolved from the H3/H4 tetramer (25). The expanding archaeal branch of the tree of life supports this evolutionary relationship. *Ca.* Asgardarchaeota are currently considered the closest living relatives to eukaryotes, and strikingly, some of their histones have an N-terminal tail (21, 26–28). As this tail also includes several lysine residues, a similar function and acetylation pattern to eukaryotic tails might be very well possible.

The archaeal histones HMfA and HMfB from *Methanothermus fervidus* and HTkA and HTkB from *Thermococcus kodakarensis* are the best studied. These model histones form homo- and heterodimers in solution. Micrococcal nuclease (MNase) digestion of *M. fervidus, Haloferax volcanii* and *Methanobacterium thermoautotrophicum* showed protection of ~60 bp of DNA, suggesting binding as tetramers (22, 29). High-affinity sites for HMfB found by systematic evolution of ligand by exponential enrichment (SELEX) were suggested to be bound by and wrapped around a tetrameric protein core, highlighting the importance of the tetrameric structure (30, 31). However, MNase digestion studies on chromatin of *T. kodakarensis* showed that the dimer is the basic unit when binding to the DNA. The size of protected DNA increased as multiples of 30 bp up until ~ 500 bp, suggesting that structures larger than the eukaryotic octamer are also relevant (32). Indeed, X-ray structures and single-molecule experiments on HMf show that a so-called hypernucleosome can be formed, which is stabilized by stacking interactions between dimers (33, 34) (figure 1.1B). Although these interactions are predicted to be widespread throughout the archaeal domain, several species encode multiple histone variants with different stacking propensities (21). The incorporation of different variants might be key to modulating the size and stability of hypernucleosomes by acting as capstones (35).

Little is known about the effects of archaeal histones and variants of these on transcription. A mildly repressive effect was found for HMf in *E. coli* cells without severe growth defects. In *T. kodakarensis,* gene expression patterns change depending on the presence of histones (un)able to form hypernucleosomes (36). This suggests that naturally occurring histone variants that are less likely to oligomerize have a function in modulating hypernucleosome size and structure, and therewith gene expression.

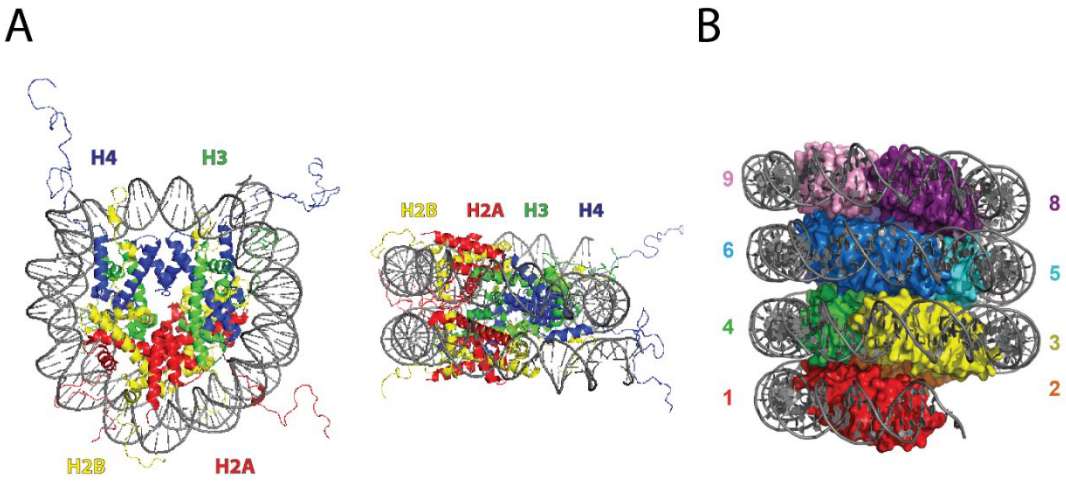

Figure 1.1 Eukaryotic and archaeal histones are examples of DNA wrappers. A) Two views of a eukaryotic nucleosome (PBD: 1KX5 (37)) consisting of a H3/H4 tetramer (H3: green, H4: blue) and two H2A/H2B dimers (H2A: red, H2B: yellow) that wrap 147 bp of DNA. B) A model of an archaeal hypernucleosome (PBD: 5T5K (33)) consisting of nine HMfB dimers that wrap around 270 bp of DNA. This image was reproduced from Henneman et al. 2018 (21).

Table 1.1 Distribution of chromatin proteins across the archaeal domain of life. For the archaeal histones, Alba and MC1, the entries from the NCBI protein database that were annotated as such and assigned to a superphylum and phylum were included. For Sso10a (CAH69222.2), Cren7 (P0C835.1), Sul7 (AAK42679.1) and CC1 (WP_ 053240420.1), BLAST with the reference sequences was performed. For SMC-proteins, the organisms found in Yoshinaga et al. are indicated (38). Presence of a protein in the genome of organisms of the phylum/class are indicated with Y and absence with N. Proteins indicated with Y* means that it was found only in the minority of the genomes of that specific class. Due to the incomplete picture of SMC-diversity in archaea, phyla without known SMC-proteins are left open instead of indicated with N. DPANN is an acronym for Diapherotrites, Parvarchaeaota, Aenigmarchaeota, Nanoarchaeota and Nanohaloarchaeota and TACK for Thaumarchaeota, Aigarchaeota, Crenarchaeota and Korarchaeota.

Superphylum	Phylum	Class	Histones	SMC	Alba	MC1	Sso10a	Cren7/Sul7/CC1
	Euryarchaeota	Archaeoglobi	Y	Y*	Y	N	Y*	N
		Hadesarchaea	Y		Y	N	N	N
		Halobacteria	Y	Y*	N	Y	N	N
		Hydrothermarchaeota	Y		Y	N	N	N
		Methanoatronarchaeia	Y		Y	N	N	N
		Methanobacteria	Y		Y	N	N	N
		Methanococci	Y		Y	N	Y*	N
		Ca. Methanofastidiosa	Y*		Y	N	N	N
		Ca. Methanoliparia	Y		Y	N	N	N
		Methanomicrobia	Y	Y*	Y	Y	Y*	N
		Methanopyri	Y*		Y*	N	N	N
		Nanohaloarchaeota	Y		Y	N	N	N
		Theionarchaea	Y	Y*	Y	N	N	N
		Thermococci	Y	Y*	Y	N	N	N
		Thermoplasmata	Y*	Y*	Y	Y	Y*	N
DPANN	Ca. Aenigmarchaeota		Y		Y	Y	N	N
	Ca. Altiarchaeota		Y		Y	Y	N	N
	Ca. Diapherotrites		Y*		Y	N	N	N
	Ca. Huberarchaeota		Y		N	Y	N	N
	Ca. Micrarchaeota		Y		Y	Y*	N	N
	Nanoarchaeota		Y		Y	N	N	N
	Ca. Pacearchaeota		Y*		Y	N	N	Y*
	Ca. Parvarchaeota		Y		N	N	N	N
	Ca. Woesearchaeota		Y		Y	Y*	N	Y*
TACK	Ca. Bathyarchaeota		Y	Y*	Y	N	Y*	N
	Crenarchaeota		Y*	Y*	Y	N	Y*	Y*
	Ca. Geothermarchaeota		Y		Y	N	N	N
	Ca. Korarchaeota		Y		Y	N	N	N
	Ca. Marsarchaeota		N		Y	N	N	N
	Ca. Nezhaararchaeota		Y		Y	N	N	N
	Thaumarchaeota		Y		Y	N	Y*	N
	Ca. Verstraetearchaeota		Y		Y	N	N	N
Asgard Archaea	Ca. Heimdallarchaeota		Y	Y*	Y	N	N	N
	Ca. Lokiarchaeota		Y		Y	N	N	N
	Ca. Odinarchaeota		Y		Y	N	N	N
	Ca. Thorarchaeota		Y		Y	N	N	N

DNA bridgers

SMC proteins – in all domains of life

The only architectural chromatin protein family that is conserved throughout the tree of life, is the structural maintenance of chromosomes (SMC) family of proteins (39). These proteins structure the chromosome by bridging two DNA strands followed by loop extrusion (40). This is an active process involving the hydrolysis of ATP (41, 42) (figure 1.2A). SMC proteins consist of a hinge dimerization domain, an ATPase head domain and an anti-parallel coiled-coil arm between the two domains (43, 44). The ATPase function was also found in the universally conserved Rad50, which has a function in DNA repair. Together they form an 'SMC-like' superfamily (39, 45). Eukaryotes encode for six SMC subfamilies (SMC1-6). Proteins belonging to these subfamilies form obligatory heterodimers called cohesin (SMC1/3), condensin (SMC2/4) and the SMC5/6 complex (46–49). Each heterodimeric complex is associated with distinct accessory proteins and functions in chromosome condensation during replication and DNA repair (50). In bacteria, several SMC-like proteins have been identified. For example, in *E. coli* the MukBEF complex has been shown to fulfill the SMC function (51) and in *B. subtilis* and *Caulobacter crescentus* this function is carried out by SMC-ScpAB (52–55). More widespread throughout the bacterial domain is the MksBEF complex (56).

In archaea, until recently, only a few SMC-like proteins were identified. Two SMC-like proteins in *Halobacterium salinarum* (Sph) were found (57) and Archadin-4 was identified in Thermoproteales archaea (58). More recently, coalescin (ClsN) in *Sulfolobus acidocaldarius* and *Sulfolobus islandicus* was shown to be involved in chromosome compartmentalization (59). SMC-like proteins, Sph, Archadin-4 and ClsN seemed to be restricted to specific lineages only. Considering the wide distribution of SMC-like proteins in bacteria and eukaryotes, this raised the question whether other SMC-like proteins are present in the archaeal domain. Yoshinaga et al. discovered a new, widespread group which they called Archaea-specific SMC-related proteins (ASRPs) (38). Although experimental validation is still lacking, this increased the potential diversity of SMC-like proteins in archaea. Because the diversity and distribution in the archaeal domain is a topic of ongoing investigation, SMC proteins could only partially be included in table 1.1.

SMC-like proteins are the only class of DNA bridging proteins in bacteria, archaea and eukaryotes. Below, the domain-specific DNA bridging proteins are discussed.

H-NS-like proteins – in bacteria

The histone-like nucleoid structuring protein (H-NS) is a key actor in both genome organization and transcription regulation in *E. coli.* H-NS regulates around 5-10% of the *E. coli* genes, especially genes acquired by horizontal gene transfer (60, 61). In contrast to the active, ATP-driven SMC proteins, H-NS is an example of a passive DNA bridger (figure 1.2B). Structurally, H-NS consists of an N-terminal oligomerization domain containing a dimerization and an oligomerization site, followed by a flexible linker and a C-terminal DNA binding domain (10, 62, 63). This last domain harbors an AT-hook-like motif to recognize the minor groove of the DNA. H-NS has two modes of DNA binding, resulting in DNA bridging or nucleofilament formation (64–66). The switch between these two modes is dependent on environmental conditions and interaction with protein partners (10, 67–69) (see also Chapter 2). Several functional and structural homologs of H-NS have been found in other bacteria, which are discussed in more depth in Chapter 2.

Alba – in archaea and some eukaryotes

The most widespread NAP in archaea is Alba (Acetylation lowers binding affinity). At least one copy of Alba is present in nearly every phylum and class (table 1.1). There are a few exceptions, namely Halobacteria (Euryarchaeota), *Ca.* Huberarchaeota and *Ca.* Parvarchaeota (both DPANN). In eukaryotes, RNA-binding proteins such as subunits from ribonucleoprotein complexes (RNase P/MRP) and ciliate macronuclear development protein 2 (Mdp2) are related to archaeal Alba (70). These proteins were mainly studied in protozoan parasites, such as the malaria parasite *Plasmodium falciparum* (71, 72), but similar proteins have been found in *Caenorhabditis elegans, Homo sapiens* and *Arabidopsis* (73). Most of the research on archaeal Alba was done in non-histone containing *Sulfolobus spp.* where Alba is the main chromatin protein. Alba is a 10 kDa protein and forms homodimers in solution (74). It contains a β-sheet arrangement and two α-helices and the dimer has a highly basic surface which functions as DNA binding interface (75, 76). Alba constitutes about 4% of the cellular protein of *S. shibatae* and binds DNA without apparent sequence selectivity (77). The DNA binding affinity of Alba was found to be dependent on its modification status. Originally it was thought that Alba was subject to lysine acetylation (hence its name), but further research identified trimethylation of Lys16 as the factor that lowers the DNA binding affinity (78, 79).

Alba binds to ssDNA and RNA with a similar affinity as to dsDNA *in vitro* (80). In many cases, such as the eukaryotic Alba proteins mentioned above, Alba domains are found in proteins related to RNA metabolism (70). Recently it was found that Alba

catalyzes RNA unwinding and unfolding, especially at elevated temperature, which is Alba's natural environment in hyperthermophilic *Sulfolobus spp.* Therefore, a role as RNA chaperone was proposed (81). This second function of Alba was shown to be dependent on Lys17, which is involved in RNA binding (82).

Two distinct DNA binding modes have been identified: at low protein:DNA ratio (about 1 dimer per 15 bp) Alba bridges two DNA duplexes (figure 1.2B), while at higher protein:DNA ratios (one dimer per 5 bp), it binds cooperatively along the DNA (75, 83, 84). The phenylalanine on position 60 has been shown to be important for dimer-dimer interactions (76), along with hydrophobic interactions between the two α1-helices (76, 85). Phe60 is responsible for side-by-side interactions and is, therefore necessary for cooperative binding along the DNA.

S. solfataricus, among many archaea, encode two Alba proteins: Alba1 and Alba2 (86), where Alba1 does have Phe60 while it is absent in Alba2. Alba1 is expressed at a higher level than Alba2. When Alba2 is present, obligate heterodimers are formed, resulting in a mixture of Alba1 homodimers and Alba1:Alba2 heterodimers (86). Alba1 homodimers can both bridge two DNA duplexes and form a stiffening filament along the DNA in a concentration-dependent manner (87). However, as Alba2 lacks the Phe60 for effective dimer-dimer interactions, Alba1:Alba2 heterodimers only bridge DNA due to a loss of cooperativity. Therefore, tuning the relative concentrations of Alba1 and Alba2 was proposed to be an effective way to regulate DNA bridging behavior and nucleoprotein filament formation, akin to H-NS and its interaction partners (87). Besides, Alba and H-NS share high cellular expression levels and a lack of sequence specificity. It is currently unknown, but well possible that Alba has a similar function in gene regulation and/or genome organization as H-NS-like proteins.

Sso10a – in Archaea

Most Crenarchaeota do not express histone proteins, but encode several NAPs instead. Sequences of the Sso10a family of proteins have been found in the TACK superphylum (Ca. Bathyarchaeota, Crenarchaeota and Thaumarchaeota) and Euryarchaeota (Methanomicrobia, Methanococci, Archaeoglobi and Thermoplasmata) (88) (table 1.1). The best-studied members of this family are from *Sulfolobus spp* (74, 89). Sso10a proteins are small 10 kDa proteins with a winged helix-turn-helix (wHTH) DNA binding domain and an anti-parallel coiled-coil structure as dimerization site (89–92). *S. solfataricus* expresses three Sso10a homologues: Sso10a1, Sso10a2 and Sso10a3. Both Sso10a1 and Sso10a2 were shown to bend DNA at low protein concentrations (92). At higher concentrations, however, the behaviour of the two proteins is different. Where Sso10a1 is able to bridge two DNA duplexes, Sso10a2 forms a

stiffening filament along the DNA. This difference in DNA binding properties might have a structural basis as Sso10a2 contains four extra residues in the wHTH domain, creating an extended loop with two additional charged residues compared to Sso10a1.This could lead to the formation of extra electrostatic interactions between Sso10a2 dimers, resulting in the formation of a nucleofilament (92). Therefore, the absence of DNA stiffening behavior for Sso10a1 is most likely the result of differences in the dimer-dimer interface.

The multiple effects on DNA conformations of the Sso10a proteins resemble the multiple architectural properties of bacterial NAPs. For instance, the bacterial chromatin protein HU is a DNA bender (see below), but also stiffens DNA at higher concentrations (93, 94). The DNA bridging behaviour of Sso10a1 resembles that of H-NS-like proteins (64, 65, 95–98). It is likely, based on sequence similarity, that Sso10a1 and Sso10a2 can heterodimerize, and possibly also form heterodimeric complexes with Sso10a3. By regulating the relative expression levels of the Sso10a proteins, the cell can potentially regulate genome architecture. Although it is currently unclear whether Sso10a proteins affect transcription, this feature might be relevant in gene expression.

H1 and BAF – in eukaryotes

Histone H1 and the barrier-to-autointegration factor (BAF) are two examples of passive DNA bridging proteins in eukaryotes. H1 binds at the entry/exit site of the nucleosome and influences the nucleosome repeat length (99). Structurally, H1 consists of a winged helix domain with an unstructured N-terminal tail and a highly basic, unstructured C-terminal domain, which is necessary for DNA binding *in vivo* (100) and nucleosome condensation in higher-order structures (101). The structured globular domain of H1 was found to bridge the nucleosome complex with linker DNA, thereby compacting the chromatin structure (102). There are multiple subtypes of H1 in eukaryotic cells with subtype-specific PTMs, and they play particular roles in the formation of chromatin structure (nicely reviewed by Hergeth and Schneider in 2015 (103)).

BAF was originally identified as a protein to prevent autointegration of retroviral DNA (104, 105), but it also has a function in repair of nuclei ruptures (106) and is involved in various diseases (107). It is a dimeric protein in solution and uses a helix-hairpin-helix DNA binding domain (108, 109). When bound to the DNA, BAF can bridge two DNA strands (110–112) by either forming a higher-order complex (a dodecamer) or binding as a dimer depending on the length of the DNA (113). Because BAF binds DNA without sequence specificity, a role in chromatin organization rather than transcription regulation was proposed (111). The binding of BAF to the DNA and its interaction partners depends on its phosphorylation status (114, 115). Interestingly, BAF interacts with histone H3 and the histone variant H1.1 and affects the modification status of histones, but the *in vivo* function of this interplay between BAF and histones is still unclear (116, 117)

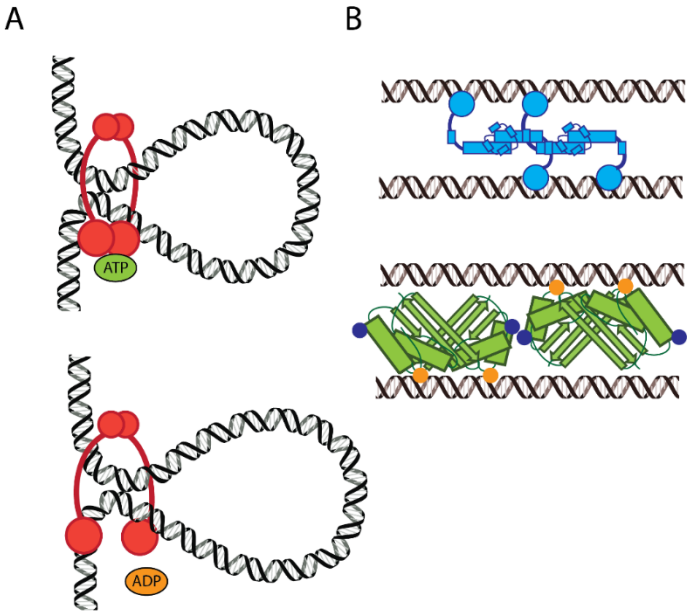

Figure 1.2 Active and passive DNA bridgers A) SMC proteins are examples of active DNA bridgers. When ATP is bound (top) the head domains dimerize and a ring structure is formed. Upon hydrolysis of ATP to ADP (bottom), the head domains release and DNA can be pulled through the ring. B) Bacterial H-NS (top) and archaeal Alba (bottom) are examples of passive DNA bridgers. H-NS dimerizes via a hand-shake topology and multimerizes using a helix-turn-helix interface. Alba forms dimers via a β-sheet arrangement and residue Phe60 (in blue) is used for multimerization. Residue Lys16 important for DNA binding is indicated in orange. Rectangles represent α-helices and arrows indicate β-sheets.

DNA benders

DNA wrapping, with histones as prototypical examples, can be considered an extended form of DNA bending. Bacteria encode several DNA bending proteins, but mostly lack histones and other DNA wrappers. Recently, a first indication that bacteria also encode histones was published, but their DNA binding mode might be distinct from eukaryotic and archaeal histones (118). Clustered binding of DNA bending proteins would result in a structure comparable to a DNA wrap (12, 119–121). Several archaeal phyla, most notably the histone-lacking Crenarchaeota, also encode unique DNA bending proteins (122–125). In other archaeal phyla that lack Alba proteins, another DNA bender is encoded called MC1 (table 1.1).

HU, IHF and Fis

The histone-like protein from *E. coli* strain U93 (HU) is a widely conserved NAP among bacteria (126). Most bacteria encode one HU protein, while *E. coli* expresses HUα and HUβ that can heterodimerize (126). HU functions in many cellular processes such as DNA organization, gene expression and protection of DNA against various stresses (127–130). Due to being an abundant protein that binds DNA without sequence specificity, HU binds throughout the bacterial genome. However, HU does have a higher affinity for already bent and/or distorted DNA (12, 127, 131).

HU consists of an α-helical body with two β-ribbon arms (121) (figure 1.3A). Proline residues at the end of the arms intercalate in the minor groove of the DNA and three lysine residues facilitate DNA binding (120, 127, 132). The bending angle caused by HU binding is flexible between 105° and 140°, suggesting that HU acts as a flexible hinge (93, 121). Next to the DNA bending mode of HU, a second DNA binding mode has been described, where HU forms nucleofilaments along the DNA (93). The switch between the two binding modes is dependent on local HU concentration.

Next to bacteria, the archaeon *Thermoplasma acidophilum* encodes a HU homolog called HTa (133). Despite sharing its primary, and predicted secondary to quaternary structure with bacterial HU, HTa evolved to behave like an archaeal histone protein in terms of DNA binding preferences and oligomerization behavior.

With 40% sequence identity, integration host factor (IHF) is similar to HU in many respects (126). However, for IHF a consensus sequence has been found (134) and IHF binding induces a DNA bend of 160° (120). IHF is only present in Gram-negative bacteria in contrast to HU (126). IHF fulfills a more specific role than the general HU protein in transcriptional regulation, replication and integration of phage DNA (135–137).

Fis is another DNA bending protein across the bacterial domain of life. It binds DNA as a dimer using a helix-turn-helix motif (138). The binding of Fis induces a bend in

the DNA between 50-90°(139). It recognizes a 15 bp palindromic sequence, mainly by the sequence-dependent width of the minor groove (138). Fis is mainly present in intergenic regions of the genome, but it also acts as a transcription regulator (140, 141). As Fis can often be found at overlapping and branched DNA strands, an architectural role was proposed next to its regulatory function (142). The binding profiles of H-NS, IHF and Fis partly overlap and they work together to repress certain genes (140, 143). This highlights the structural and functional interplay between different NAPs.

MC1

Some archaea lack genes encoding for Alba proteins, but express Methanogen Chromosomal protein 1 (MC1), a small monomeric protein of 93 amino acids (144), instead. These are mainly Halobacteria (Euryarchaeota) and *Ca.* Huberarchaeota (DPANN). However, some phyla encode both Alba and MC1, which are Methanomicrobia (Euryarchaeota), *Ca*. Aenigmarchaeota and *Ca.* Altiarchaeota (both DPANN). So far, no MC1 sequences have been found in the TACK and Asgard superphyla. Most organisms have only one copy of the gene encoding MC1, but two or more have also been found. For instance, *Halococcus thailandensis JCM 13552* harbors nine copies of this gene. To what extent these MC1 paralogues are all expressed in the cell and have similar or different DNA binding characteristics remains to be determined.

Structurally, MC1 consists of five β-sheets and one α-helix leading to the formation of a pseudo-barrel connected to a long flexible arm (145, 146) (figure 1.3B). The residues identified as important for DNA binding are Arg25, Trp74 and Lys86, which are conserved among the MC1 containing species (147). MC1 binds to the DNA in a noncooperative manner as monomer with a binding site size of around 11 bp (148). MC1s affinity for double-stranded DNA is high (K_D<100 nM). It preferentially binds to the following DNA sequence only consisting of adenines and cytosines: [AAAAACACAC(A/C)CCC(C/A)] (149). Furthermore, MC1 binds strongly to bent DNA, such as four-way junctions (150).

Upon binding of MC1, the DNA undergoes a significant conformational change caused by two bends of 55° and 75° resulting in a V-turn (151, 152). On longer DNA, MC1 can stabilize multiple V-turn conformations, leading to a structure which resembles a DNA wrap. This observation lead to the hypothesis that MC1 has two different DNA binding modes: DNA bending at lower concentration and DNA wrapping at higher concentrations (152). This might relate to the two observed effects MC1 has on transcription *in vitro*, which is activated at low MC1/DNA ratio (DNA bending), but repressed at higher ratios (DNA wrapping), but this is still an open question (144). Next to regulation by tuning the expression level, methylation on Lys37 of MC1 was found

(153), but what effect this has on the DNA binding properties of MC1 is currently unknown.

Cren7, Sul7 and CC1

Next to DNA bridging proteins Alba and Sso10a, several Crenarchaeota-specific DNA benders were identified in the search for proteins with a different architectural effect on the DNA in these organisms. Cren7 and Sul7 are both small, monomeric proteins, which are not related in terms of amino acid sequence, but their tertiary structure and biochemical properties are similar (122, 123, 125, 154, 155). They contain two antiparallel beta-sheets with either an extended loop in between (Cren7, figure 1.3C) or an additional C-terminal α-helix (Sul7) (125, 155). Crystal structures with DNA show binding to the minor groove of the DNA and an induced bend of 50-60° (156–159) . The binding site size for Cren7 is 6-7 bp, binding as a monomer to the DNA in a head-to-tail manner. Cren7 has a slight preference for AT-rich DNA, while no sequence preference was found for Sul7 (157). Lysine methylation was found on five positions for Cren7 and on seven and nine positions for the two Sul7 proteins, respectively in *S. islandicus*, but their relative occurrence and function *in vivo* remain to be investigated (160, 161).

In the search for a second chromatin protein next to Alba in the crenarchaea *Pyrobaculum aerophilum* and *Thermoproteus tenax,* another small (6 kDa) protein was identified: Crenarchaeal Chromatin Protein 1 (CC1). This protein has further homologues in *Aeropyrum pernix* (124) and a few sequences were found in *Ca.* Pacearchaeota and *Ca*. Woesearchaeota (both DPANN) (table 1.1). Secondary structure prediction suggested mainly β-sheet organization, similar to Cren7 and Sul7. However, CC1 binds both ss and dsDNA, while Cren7 and Sul7 do not bind ssDNA (162).

HMG-box proteins

Known DNA-bending proteins in eukaryotes are the high motility group (HMG)-box proteins. The global fold of a HMG-box consists of three α-helices in an L-shape (163). They bind the DNA using a hydrophobic interface and introduce a substantial bend in the DNA (164–166). Often, HMG-box proteins have an N- or C-terminal extension that stabilizes their binding to the DNA and the introduced bend (167, 168). They recognize DNA independently of DNA sequence but mainly on the basis of structure, for instance distorted DNA or four-way junctions (169, 170). Functionally, they have been associated with both activation and repression of transcription *in vitro*. This could be related to interactions of HMG-box proteins with transcription factors and the basal transcription machinery (171–178). Also, a more architectural role has been proposed where HMG-box proteins ‘pre-bend’ the DNA for other chromatin proteins to form nucleoprotein

complexes (179, 180). In *Saccharomyces cerevisiae,* loss of a HMG-box protein resulted in higher susceptibility for micrococcal nuclease (MNase) of the DNA, suggesting a structural and protecting function (163).

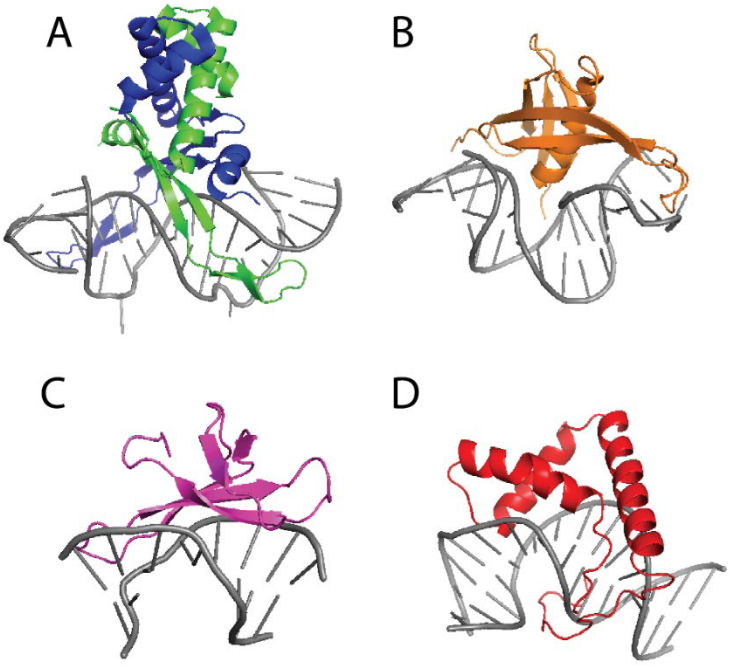

Figure 1.3 DNA bending proteins A) Bacterial HU protein dimer (PDB: 1P78 (121)) using a β-sheet "clamp" to bind the DNA and α-helices to dimerize. B-C) The archaeal monomeric DNA bending proteins MC1 (B) (PDB: 2NBJ (146)) and Cren7 (C) (PDB: 5K17 (181)). D) Eukaryotic HMG-box domain from the LEF-1 protein from mouse (PDB: 2LEF (165)). Note that HU and MC1 bend the DNA in the opposite direction compared to Cren7 and the HMG-box domain.

Nucleoprotein filament formation

Several proteins mentioned above exhibit a second DNA binding mode, where they form a filament along the DNA, effectively stiffening it. Such binding is often observed for passive DNA bridgers, such as H-NS-like proteins, Alba and Sso10a (66, 87, 92, 182–184). Also, the DNA bender HU exhibits DNA stiffening behavior (93). For H-NS-like proteins, it has been a topic of discussion which DNA binding mode is relevant *in vivo*. It is possible that the change between DNA bridging and the formation of nucleofilaments functions as a switch between a repressive state and a state permissive of transcription (69, 185). Multimerization of proteins is necessary for nucleoprotein filament formation, which can result from either high local protein concentration, as for HU, or cooperative interactions, as for Alba and Sso10a, or both (87, 92, 93). However, as *in vivo* data is lacking for these proteins, the likelihood and functional relevance of nucleoprotein filament formation remains an open question.

Discussion

Across the tree of life, architectural chromatin proteins act in concert to organize the genome and regulate gene expression. Conserved structural effects of these proteins on DNA, such as wrapping or bridging, can be found in every domain of life. It has been hypothesized that archaea need at least two chromatin proteins (most likely two that execute a different structural effect) (186), but there is very little data about the interplay between archaeal chromatin proteins. In *S. solfataricus,* the interplay between the chromatin proteins Alba and Cren7 was investigated *in vitro*. It was found that Cren7 disrupts larger structures formed by Alba (187), which could result in more open chromatin. The effects of this interplay might be relevant *in vivo*.

The lack of standard laboratory strains of most phyla currently limits research in the archaeal domain of life. Most data is either from Euryarchaeaota, such as *M. fervidus* and *T. kodakarensis,* or Crenarchaeota, such as *Sulfolobus spp.* Recent advances in culturing two Lokiarchaeota from the Asgard archaea could help to establish more standard laboratory strains across different phyla to study chromatin proteins *in vivo* by, for instance, Chromosome Conformation Capture (3C)-based techniques and Chromatin Immunoprecipitation Sequencing (ChIP-seq). Close monitoring of genomic changes in newly cultured organisms is necessary as studies of a laboratory strain of *Methanothermobacter thermautotrophicus* revealed that two chromatin proteins (the histone HMtB and MtAlba) had lost their ability to bind DNA by specific arginine to isoleucine mutations after several passages. Histone HMtB could likely still interact with the DNA-binding histones HMtA1 and HMtA2 and fusion proteins were able to compact

DNA (188). What evolutionary pressure drove these mutations is unknown, but *M. thermautotrophicus* was able to alter the DNA binding properties of chromatin proteins without effects on growth rate under laboratory conditions.

In contrast to the archaea they evolved from, eukaryotes use histones and SMC proteins as their main, nearly exclusive chromatin proteins. As all Asgard archaea found so far contain genes for histones and Alba (table 1.1), it is likely that the first eukaryotic cells also had both. Several RNA-binding proteins in eukaryotes do contain an Alba domain (see above), but Alba, as a separate protein, is mostly lost. The hypothesis that archaea need at least two chromatin proteins was based on extended polymerization on DNA observed for reconstituted DNA-protein complexes *in vitro* (186). A second protein would be necessary to prevent unlimited polymerization of the first protein. An outstanding difference between archaeal and eukaryotic histones is the size of the 'nucleosomal complex'. Association of archaeal histones yields a theoretically endless hypernucleosome, while eukaryotic histones do not yield nucleosomes with more than eight histone subunits, although larger structures have been recently observed at telomers (189). The loss of 'endless' polymerizing histones might have resulted in a partial loss of Alba's cellular function. Instead, other regulatory mechanisms, such as PTMs on the histone tails, might have taken over as main regulatory mechanism in eukaryotes.

Thesis outline

In this thesis I describe studies on the structural properties of chromatin proteins from bacteria and archaea. I study several DNA bridging proteins across the tree of life and discuss the DNA binding properties of some archaeal histones. In Chapter 2, I discuss the structural and functional characteristics of a family of bacterial DNA bridging proteins, H-NS-like proteins. Also, I propose in this chapter that the protein charge distribution is an good predictor of the responsiveness of a protein to physico-chemical cues. In Chapter 3, I demonstrate that Rok is an atypical H-NS-like protein. I investigate the DNA structuring properties of Rok and demonstrate that the binding of the protein is not affected by environmental conditions. Also, I investigated its, naturally occurring, truncated derivative sRok and the interplay between the two proteins. We identify differences in the DNA binding characteristics of the two proteins and demonstrate that this translates in different regulons in B. subtilis. In Chapter 4, I describe studies on the binding of archaeal histones HMfA and HMfB from *M. fervidus* to a specific DNA sequence. This is relevant in the context of nucleosome-positioning *in vivo*, with possible impact on transcription. In Chapter 5 we investigate histones from *M. jannaschii*. Specifically, we show that one of these histones, MJ1647, is a novel atypical histone capable of DNA bridging. The ability to bridge is attributed to the presence of a C-terminal which promotes tetramerization. In Chapter 6, I discuss a toolbox for the biological expression and synthesis of archaeal histones with HA and HB from Heimdallarchaeota as example. Chapter 7 is a general discussion.

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