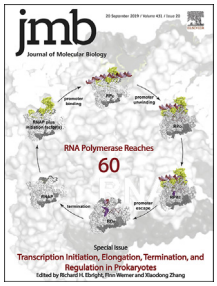
Review



The Role of Archaeal Chromatin in Transcription

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https://doi.org/10.1016/j.jmb.2019.05.006

Abstract

Genomic organization impacts accessibility and movement of information processing systems along DNA. DNA-bound proteins dynamically dictate gene expression and provide regulatory potential to tune transcription rates to match ever-changing environmental conditions. Archaeal genomes are typically small, circular, gene dense, and organized either by histone proteins that are homologous to their eukaryotic counterparts, or small basic proteins that function analogously to bacterial nucleoid proteins. We review here how archaeal genomes are organized and how such organization impacts archaeal gene expression, focusing on conserved DNA-binding proteins within the clade and the factors that are known to impact transcription initiation and elongation within protein-bound genomes.

Introduction

The regulation imposed on gene expression by chromatin or nucleoid structures in Eukarya and Bacteria, respectively, has a long and rich history [1–10]. Organization of the genome can facilitate or impair the ability of the transcription apparatus to recognize promoter elements, to form an open complex and to transition into stable elongation. Once transcription elongation complexes (TECs) are established, they must traverse a protein-bound template [11–14]. The dynamic associations of DNA-bound proteins and the resultant larger structures formed by cooperative interactions of such hinder translocation. Both bacterial nucleoid and eukaryotic chromatin structures involve the formation of loops, connecting spatially distant locations on the genome via protein–DNA interactions [15,16], and the formation and stability of such topologically constrained regions can be controlled to alter expression of single loci or very large regions of the genome. Regulation of gene expression through alteration of genomic architecture offers the potential to tailor gene expression to maximize fitness gains in changing environments.

The role of genomic architecture in modulating gene expression in archaeal species has only more recently been investigated with the scrutiny applied to bacterial and eukaryotic systems. Archaeal genomes are typically circular, small (<5 Mbp), gene dense (~80%–90% coding sequence), and many genes are organized within operons [17–21]. Despite sharing many hallmarks of typical bacterial genomes, archaeal genomes are expressed with a single RNA polymerase (RNAP) that shares more similarity in overall structure, subunit composition, and basal-transcription factor requirements with eukaryotic RNAPs, in particular Pol II [22–34]. The archaeal RNAP lacks the C-terminal repeats found on Pol II and is not known to be post-translationally modified, but the archaeal RNAP is still directed to the transcription start site in a manner comparable to Pol II. The archaeal transcription system is a component simplified version of the Pol II apparatus, requiring only interactions with transcription factor B (TFB; TFIIB in Eukarya), transcription factor E (TFE; TFIIE in Eukarya), and TATA binding protein (TBP) to recognize core archaeal promoter elements—TATA box and BRE—that share sequence conservation with eukaryotic promoter elements [34–38]. Minimal evidence for long-range interactions between transcription factors and promoter elements is known, and substantial evidence has instead emerged that demonstrates that most

archaeal promoters are regulated by bacterial-like repressors or activators that bind immediately adjacent to or overlapping core promoter elements [39–49]. Studies suggest that core promoters are generally devoid of organized chromatin structures [50,51], and that when present, the binding affinity of transcription regulators outcompetes the binding of histones or nucleoid-associated proteins (NAPs) to permit regulation within an organized and protein-bound genome [40,43,47,52].

Following transcription initiation, TECs must stably associate with and transcribe the template for long periods (e.g., minutes or hours at ~40 nt/s), necessarily displacing DNA-bound proteins that impede translocation. Transcription initiation and elongation in eukaryotes is facilitated by the combinatorial activities of transcription factors and chromatin remodeling and modification machinery. Given the absence of obvious chromatin remodeling and modification machinery in archaeal genomes, transcription factors likely play the dominant role in aiding archaeal transcription during initiation and elongation. The rates of elongation and pausing of archaeal transcription are regulated by conserved archaeal–eukaryotic factors Spt4/Spt5 and TFS (TFIIS in eukaryotes) [13]. Spt5, homologous to bacterial NusG, is the only universally conserved transcription factor. Spt5–RNAP interactions facilitate formation of the closed-clamp configuration of RNAP that aids in processive elongation. Pausing is inevitable, and when collisions with DNA-bound proteins stalls forward translocation of RNAP, reverse translocation can inactivate RNAP. The cleavage-stimulatory activity of TFS/TFIIS [53]—analogous to the cleavage stimulatory activities of GreA and GreB in Bacteria [54,55]—helps rescue such backtracked complexes, and the activity of TFS is essential for archaeal species [13].

In this review, we discuss recent advancements in archaeal chromatin and genome organization in the context of transcription regulation. We first examine the architectural mechanisms and regulatory implications of genome compaction dominated by archaeal histone proteins. Most archaeal clades encode histone proteins that generate DNA structures remarkably similar to eukaryotic nucleosomes, albeit with only the core histone-fold and often with only a single-histone isoform. We next identify and outline important advancements in the identification of transcription factors and basal transcription mechanisms that facilitate transcription in the context of an archaeal histone-based chromatin landscape. We then focus on the archaeal clades that lack histone proteins and instead encode a suite of small basic proteins that presumably function like bacterial NAPs to condense and organize the archaeal genome. Finally, we consider the major bottlenecks within the archaeal transcription field in the context of chromatin organized genomic architectures. We conclude with discussion of current debates within the field and highlight the future potential of studies investigating the influence of genomic architecture on archaeal gene expression.

Archaeal Histone-Based Chromatin

Structure of archaeal histone-based chromatin

Whole-genome sequencing of many cultured and many-more environmentally isolated, but not yet cultured Archaea suggests that most archaeal lineages encode one or more histone proteins (Fig. 1) [56–64]—six histone isoforms can be identified in *Methanocaldococcus jannaschii* [65]—that are likely to organize the genome into structures that mimic DNA organization by eukaryotic nucleosomes [56,66,67]. Although not universally encoded (typically to the exclusion of *Crenarchaeota* [56,64]), in archaeal species with histone proteins, a chromatin landscape presents barriers to initiation [42,52,65,68–70], elongation [12,13], and likely influences termination. Archaeal histones are composed of only the core-histone fold and lack the N- and C-terminal tails and extensions common to the canonical eukaryotic histones [66,67,71–75] (e.g., H2A, H2B, H3, and H4). Archaeal genomes do not encode obvious linker histones (e.g., H1) or chromatin-remodeling complexes that are abundant and essential for gene expression in eukaryotes. Unlike the mandatory eukaryotic histone heterodimer partnerships, archaeal chromatin can be spontaneously assembled with a single histone protein [51,66,67,72,76,77], and there is currently no evidence for post-translational modification (PTM) of archaeal histones.

Despite this minimalist approach to histone-based chromatin architecture, archaeal histone–DNA interactions align to the same nucleosome positioning code that was established for Eukarya [10,51,63], and the constrained structure of DNA bound by archaeal histones is nearly identical to the structure of DNA in the eukaryotic nucleosome (Fig. 2) [66,67,78,79]. The superhelically wrapped DNA shares the geometry, diameter, pitch, and writhe of the eukaryotic nucleosomal superhelix, and specific protein–DNA contacts that stabilize archaeal histone-based chromatin are conserved in eukaryotes [56,66,67,79]. The structure of archaeal histone-based chromatin suggests the architectural function of histones (i.e., the ability to bend DNA into the nucleosomal superhelix) was established long (>1 bya) ago, and that the "signaling functions" (i.e., addition of histone extensions and epigenetic modifications) were a secondary addition that came with the expansion to four canonical histones in eukaryotes [66,67].

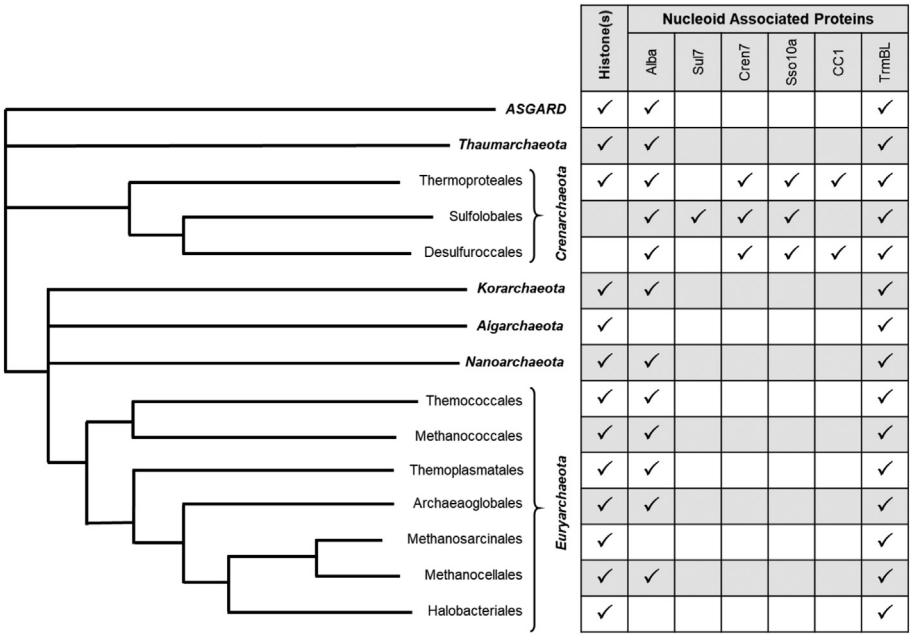

Fig. 1. Distribution of chromatin associated proteins identified across the Archaea. Histone proteins and NAPs (right) encoded in each phylum according to the schematic evolutionary tree of Archaea (left).

Local histone binding is known to sterically compete with binding of transcription components and offers regulatory potential [12,40,42,43,80], and the extended structure of archaeal histone-based chromatin may also offer regulatory potential. Perhaps the most striking feature of the structure of archaeal histone-based chromatin is the continuous helical ramp of histone dimers and the close association of adjacent layers of the complex that result in a tightly packed 3D chromatin structure [66,67]. The extensions common to eukaryotic histones normally radiate into solution and facilitate nucleosome-nucleosome interactions. The absence of such extensions on archaeal histones in part permits the close association of adjacent layers of archaeal chromatin. The resultant superstructure places the L1 loops of histone-dimers 1 and 4 along the helical ramp in closest-proximity to each other. Apart from four helix-bundles that link the histone dimers, the only region of close contact between the adjacent layers of archaeal chromatin is where the L1 regions of dimers 1 and 4 meet. L1 sequences almost always retain a central glycine at the point of closest approach, and substitution of this glycine with larger side chains impedes tight packing of archaeal chromatin, impairs gene expression *in vivo*, and reduces overall fitness [67].

Extension of the structure by one additional histone-dimer extends the length of DNA protection by ~30 bp, resulting in extended polymers that protect DNA from minimally ~60 bp (two histone dimers) to ~480 bp, in 30-bp increments [81]. Comparisons of archaeal histone sequences with the atomic-resolution structure of archaeal chromatin reveal that most archaeal histones retain the residues that directly interact with the DNA backbone and use nearly identical residues to stabilize histone–histone and histone–DNA interactions, and that close association of chromatin gyres is likely possible due to minimal side chains in the L1–L1 interface. The eukaryotic nuclear RNAPs and the archaeal RNAP thus regularly encounter—and must overcome—nearly identical histone–DNA contacts that present barriers to transcription elongation [67,82–85].

In contrast to eukaryotic histones, there is no evidence of PTMs to archaeal histones. Although there are many acetyl- and methyl-transferases encoded throughout the Archaea, no activity toward histone proteins has been reported, and the bulk of characterized acetyl- and methyl-transferases are active on DNA or RNA [86–88]. A minority of archaeal organisms encode histones, which contain sequences beyond the core histone fold. Excluding single-histone isoforms that contain a fused second histone fold (effectively a histone-dimer within a single polypeptide) extended histone sequences are rarely observed [56]. Such extensions are not homologous to those found in eukaryotes but are "eukaryote-like" in being rich in charged residues, especially lysine. Investigation of one such extended archaeal histone variant, MJ1647, a C-terminal extension-containing histone in *M. jannaschii*, demonstrated that the C-terminal extension was critical for DNA binding and the formation of higher-order structures [89]. Modeling the C-terminal extension of MJ1647 into the atomic structure of archaeal histone-based chromatin suggests that the

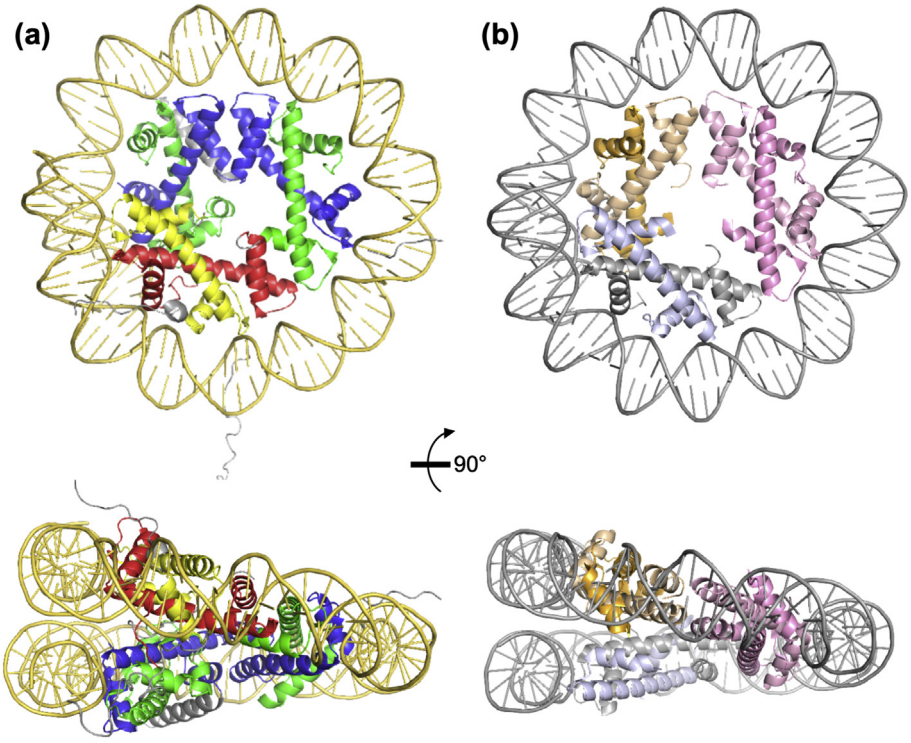

Fig. 2. The structure of histone-based chromatin in Archaea mirrors that of the eukaryotic nucleosome. (a) The eukaryotic nucleosome hexamer containing two H3–H4 dimers (blue and green respectively) and one H2A–H2B dimer (yellow and red respectively) with wrapped DNA (gold) from a top-down and side view. N and C terminal extensions, specific to eukaryotic histones, are shown in gray. (b) Histone-based chromatin in Archaea can form from varied numbers of histone dimers (three dimers are shown here for comparison to the eukaryotic hexasome), with wrapped DNA (silver) from a top-down and side view. The archaeal histone-based chromatin structure formed with three histone dimers is almost identical to the eukaryotic hexasome without the N- and C-terminal extensions.

C-terminal extension might impair continued polymerization and impact the global structure of archaeal chromatin. The discovery of new histone variants and histone proteins with extensions in the *Heimdallarchaeota* and *ASGARD* archaeal clades hints at the expansion to four canonical histones, the exchange of a histone-polymer for discrete nucleosome particles, and the regulation imposed by PTMs of the histone proteins in all Eukarya [56,57,61,66,67]. Structural modeling of *Heimdall LC 3* histones, which contain tails similar in length and sequence composition to extensions on eukaryotic H4, suggests that the extended archaeal histone-based chromatin structure will not be impacted by inclusion of such tails [56]. It will now be important to elucidate the expression, abundance, and function of these archaeal histone variants, included extended-histone variants, in controlling genomic architecture and gene expression.

Global regulation of transcription by archaeal histone-based chromatin

A consensus surrounding the role of archaeal histones in transcription regulation is dubious. This is highlighted by the varying essentiality of histone proteins across archaeal species. Controversy on the role of archaeal histones in controlling gene expression exists at the total transcriptome level when genetically accessible archaeal species have their histone-encoding loci deleted or modified. In the euryarchaeaon *Thermococcus kodakarensis,* two histone variants are encoded, and while each individually is not essential, attempts at deleting both histones have been unsuccessful indicating histone-based chromatin is critical for regulation of cellular processes. The importance of regulated genomic architecture was revealed by changes—up to ~10-fold—in the expression of ~5% of genes upon deletion of either histone isoform [90]. The importance of tightly packed 3D archaeal histone-based chromatin was demonstrated by introduction of histone variants with specific mutations to residues in the L1–L1 interface [67]. Replacing G17 with bulkier amino acid residues does not disrupt local DNA binding but does disrupt extended chromatin structures that in turn impact gene expression. Disruption of extended histone-based chromatin structures also abrogates adaptive gene expression necessary to respond to changing environmental conditions.

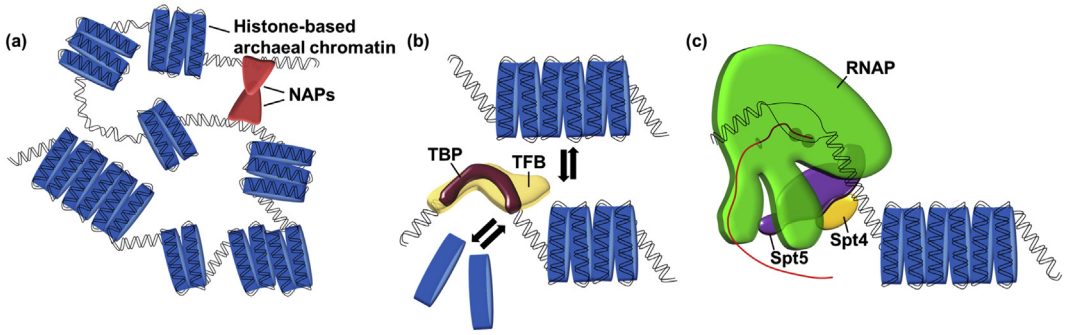

Fig 3. The archaeal chromatin landscape is dynamic. (a) Wrapping of DNA by archaeal histones forms various sizes of extended histone-based chromatin structures. The regulation and depositions of these structures is unknown, but NAPs may play a role in both looping of DNA and size restriction of extended histone polymers. (b) Transcription initiation factors TFB and TBP compete with histone proteins for the promoter element in archaea allowing transcription initiation upstream of a chromatinized gene body. (c) RNAP must traverse a chromatinized gene body. Spt4–Spt5 permits the transition from initiation to early elongation by displacing TFE and facilitating processive elongation through a chromatin landscape.

Histone proteins are not encoded in all species (Fig. 1), and histones are not essential for some extant histone-encoding archaeal species. The sole histone encoded in the methanogen *Methanosarcina mazei* is dispensable but deletion results in reduced growth, increased sensitivity to DNA damaging agents, reduced overall transcription for many genes, and an altered overall transcriptome [91]. Thus, although non-essential, deletion of the histone-encoding locus, and thus the presumptive loss of histone-based genomic organization—does significantly impact global transcription. Changes to global gene expression and growth were restored upon complementation of *M. mazei* strains with exogenously produced histone protein, suggesting that histone-based genomic architecture is important, but not essential in some archaeal species. A potentially different view of the role of archaeal histones emerges from studies of halophilic (e.g., salt-loving) archaea. *Halobacterium salinarum* encodes just one histone protein with several unique attributes. Unlike the typical basic pI of most histone proteins, the high-intracellular salt concentrations of halophiles (~4 M) has likely resulted in retention of a histone with an acidic p*I*. The halophilic histone proteins are also typically a single polypeptide containing two tandemly repeated histone folds. The single *H. salinarum* histone, like the single *M. mazei* histone, is dispensable and deletion results in globally significant, but mild fold-changes in gene expression [91]. Interestingly, these mild changes are growth-phase dependent and, although often small at the transcriptome level, result in significant changes in overall cell morphology. These results were interpreted as indicating a transcription factor-like function of histone proteins in *Halobacterium*, with global architecture imparted by histone proteins as largely unimportant to regulating transcriptome-wide expression but select loci with critical histone-binding positions displaying differential expression due to loss of histone production in deletion strains.

Regulation of transcription initiation and elongation with archaeal histone-based chromatin

Genome-wide impacts of archaeal histone-based chromatin on regulation of gene expression imply that histones are important, often essential, and that changes in histone expression, or histone-induced genomic architecture, impact cellular fitness [9]. To determine how the histone-based landscape directly impacts gene expression, most studies have taken advantage of purified transcription systems and the capacity of archaeal histones to spontaneously bind DNAs *in vitro* at the same positions utilized *in vivo* and to form structures that match *in vivo* 3D chromatin architectures. Early *in vitro* transcription experiments using components from *Methanothermobacter thermautotrophicus* demonstrated a repressive effect of histone-addition on transcript production, with complete inhibition of transcription when histone proteins were provided at levels that would theoretically saturate DNA binding (~1 histone dimer per 30 bp of DNA) [12,52,72,92]. These *in vitro* results were later extended and confirmed using components from *Pyrococcus furiosus* [69].

Transcription regulation must normally occur within a chromatin landscape (Fig. 3). Most archaeal transcription regulators mimic bacterial transcription regulators and bind within or immediately adjacent to core promoter elements to impact formation of initiation complexes. DNA-binding positions upstream of the *rb2* gene in *M. jannaschii* were shown to act as histone-nucleating sites, localizing histones whose binding reduces transcription by blocking formation of pre-initiation complexes [65]. Histones are non-specific DNA-binding proteins, and unsurprisingly, precision *in vitro* hydroxyl radical footprinting revealed that the site-specific

DNA-binding transcription factor Ptr2 effectively competes with localized histone binding—even at saturating histone levels—to activate transcription.

Transcription elongation is also affected by archaeal histone-based chromatin. *In vitro* transcription assays have been used to establish that the archaeal RNAP is unable to achieve elongation rates that are physiologically relevant through an archaeal chromatin barrier [12,13]. Using DNA templates capable of binding *M. thermautotrophicus* histone proteins, the *M. thermautotrophicus* RNAP transcribed template DNA at a rate of ~20 nt/s in the absence of histone, but just ~2–5 nt/s when archaeal histones were added to template. The initial collision between the TEC and the histone-barrier results in the greatest obstacle, causing RNAP to pause and likely backtrack. The duration of the initial pause is much greater than subsequent pauses which occur every ~10–15 bp after the TEC escapes the initial collision. The rate-limiting step of transcription through these archaeal histone-based barriers is translocation through the initial DNA–histone contacts.

The first data supporting factors that facilitate elongation through chromatin barriers are supportive of the congruent nature of the simplified archaeal transcription system and the more component complex Pol II apparatus [13]. *In vitro* transcription experiments, using factors purified from *T. kodakarensis*, demonstrate that the activities of the conserved transcription factor TFS (TFIIS in Eukarya), and an Spt4/5 complex (also termed Spt4/5 in Eukarya) accelerate the archaeal transcription apparatus through histone-bound templates. The archaeal RNAP often backtracks due to downstream chromatin barriers, and archaeal TFS stimulated endonucleolytic cleavage of transcripts within backtracked complexes results in formation of a new RNA 3′-OH in the active center of RNAP [93–96]. Reactivation of backtracked TECs permits elongation restart and another opportunity for the TEC to transcribe up to and through a downstream chromatin barrier. The Spt4/5 complex, but neither factor individually, also aided *in vitro* transcription through archaeal histone-based chromatin, presumably due to their stabilizing effects of a closed-clamp configuration of the TEC in aiding proper alignment and retention of the 3′-OH in the RNAP active center [32,95,97–99].

Given the observations of archaeal histone-based chromatin controlling the initiation and elongation aspects of transcription, it is likely that the local chromatin environment also plays a role in termination and proper 3′ end formation of transcripts.

NAPs in Archaea

The regulation imposed by genomic architecture in archaeal species that do not encode histone proteins has also been investigated in diverse clades. Perhaps the best-studied protein is the well-conserved Alba (Sac10b homologues), but abundant small basic proteins are encoded in both histone- and non-histone encoding archaea that likely impact genomic architectures. We focus first on Alba, then on more recently identified and emerging NAPs in diverse species.

Alba, a conserved chromatin protein, with controversial roles in genomic architecture

Substantial and contentious debate surrounds the Sac10b family of proteins, commonly termed Alba for "*a*cetylation *l*owers *b*inding *a*ffinity," which dominates studies of the non-histone-based organization and regulation of archaeal genomes [100]. Sac10b is a general nucleic-acid binding protein, with affinity for both single-stranded and double-stranded RNA and DNA. Evidence for Sac10b-mediated roles in DNA compaction and organization is recognized, although near equal evidence supports a role for Sac10b in RNA metabolism and binding. A contentious debate surrounds Sac10b, its role in DNA *versus* RNA binding, and whether acetylation or methylation is the PTM that may impact function of Sac10b proteins *in vivo*. The focus of many studies was the modification of lysine 16, a well-conserved residue in Sac10b homologues, and identification of proteins that could add or remove a reported acetyl group to impact Sac10b activity. Post-translational modification of K16 within Sac10b proteins was initially described as an acetylation event, hence the common Alba acronym (*a*cetylation *l*owers *b*inding *a*ffinity), but this modification has more recently been identified as a trimethylation [101]. Due to the limited research regarding other nucleoid associated proteins, examination of this paradox is presented here from a historical perspective in the context of newer findings and argues for the further examination of other potential chromatin protein targets.

The Sac10b family of nucleic acid-binding proteins are highly conserved within Archaea, especially species that thrive in (hyper)thermophilic environments. Sac10b family members are encoded in both histone-encoding and non-histone-encoding archaea and are thought to play a major structural role in archaeal chromatin. Most research has focused within the *Crenarchaeota*, specifically the *Sulfolobales*. Much of the initial biochemical analyses focused on Alba–DNA interactions. The Sac10b homologue from *Sulfolobus shibatae* (Ssh10b) is a highly abundant protein (~4% of total protein), was shown to bind dsDNA and influence DNA topology at physiological temperatures [102]. Both electron microscopy and atomic force microscopy experiments revealed an Alba concentration-dependent compaction of archaeal DNA [103–105].

Sac10b proteins are typically encoded in archaeal genomes in the form of Alba1, but some species

encode an additional paralog (Alba2) that is typically expressed at lower steady-state protein levels [103]. More detailed investigations detailed that Sac10b bound DNA as a homodimer, and when Alba2 isoforms were present, that Alba heterodimers could also bind and compact DNA [103–105]; Alba2 forms obligate heterodimers with Alba1 and is found exclusively associated with Alba1 *in vivo*. At lower Alba:DNA ratios, Alba1 homodimers bridge DNA duplexes, slightly compacting DNA by promoting the formation of loop structures [104,105]. At higher concentrations, Alba1 homodimers form rigid protein-bound DNA structures [105]. Much like Alba1 homodimers at low concentration, Alba1/Alba2 heterodimers form looped, slightly contracted DNA structures [103]. However, at higher Alba:DNA ratios, the Alba1/Alba2 heterodimers induced highly compacted DNA structures that differed significantly from the rigidified linear chromatin structure of Alba1 homodimers [105]. Crystal structures of Sac10b protein homologues from *Aeropyrum pernix* K1, *Sulfolobus solfataricus,* and *Pyrococcus horikoshii* OT3 all confirm a dimeric mode of nucleic acid interaction [106–111].

In addition to forming distinct protein:DNA complexes that impact DNA topology based on concentration and dimeric partnerships, Sac10b proteins were shown to have high affinity for RNA [112,113]. In Eukarya, Alba-like proteins have diverse RNA metabolism roles [112], suggesting that Sac10b proteins may be involved in RNA stability or degradation pathways. Localization of Sac10b to the cytoplasm with no observable association with the nucleoid suggested interaction with RNA rather than DNA *in vivo* [114]. This suggestion was corroborated by *in vivo* cross-linking studies with Ssh10b that resulted in the co-purification of primarily ribosomal RNA and mRNA over DNA [113]. Finally, addition of Ssh10b was demonstrated to directly destabilize RNA secondary structure *in vitro* [115]. The *in vitro* binding affinity of Sac10b is comparable between RNA, ssDNA, and dsDNA and Sac10b can protect both RNA and DNA from RNase and DNase digestion.

Phyla-specific modes of action have also been observed for Sac10b homologues, and particular notice should be taken to studies in mesophilic species *versus* (hyper)thermophilic archaea. Current evidence suggests that the biological role of Sac10b proteins may have diverged between mesophilic and thermophilic archaea. In contrast to the abundance of Sac10b in (hyper)thermophiles, studies of the Sac10b protein homolog Mmo10b in the mesophilic species *Methanococcus maripaludis* revealed that Mmo10b is present only in low abundance and bound specific DNA sequences rather than displaying general DNA affinity [116,117]. Deletion of a Sac10b homolog from *Methanococcus voltae* resulted in changes to protein expression patterns that overlapped with a histone B deletion in the same species [116] and in *T. kodakarensis* deletion of histone B resulted in altered Sac10b homolog expression [90]. Taken together, these results suggest that Sac10b homologues may share an overlapping regulatory role with histones in archaea, and that the presence of histones may reduce the impact of Sac10b regulation of genomic architecture.

Post-translational modification of Alba may impact genomic architecture and gene expression *in vivo*

The PTM of Sac10b was shown to impact DNA-binding affinity and was extrapolated to suggest that PTM of Alba provided regulation akin to PTMs of histone residues common in eukaryotes [100,107]. Recombinant preparations of Alba lacking PTMs displayed greater affinity for DNA than natively purified, PTM-Alba populations. The increased affinity of unmodified Alba also impeded transcription elongation to a greater extent than native, PTM-Alba preparations, consistent with Alba-mediated regulation of genomic structure based on PTM of Alba.

Initial MALDI-TOF mass spectrometry analysis identified lysine 16 (K16) in the Sac10b protein from *S. solfataricus* P2 as the primary site of acetylation. *In vitro* acetylation by *p*rotein *a*cetyl*t*ransferase 1 (Pat1) and *in vitro* deacetylation by the *s*ilent *i*nformation *r*egulator (Sir2) were shown to modify Sac10b imparting a mechanism of Sac10b binding control [100,108,118]. However, K16 is not well conserved in Sac10b homologues [117], and the initial identification of K16 as the site of modification and even the PTM itself are now in question. More recent studies have identified Sac10b as a target for both methylation and N-terminal acetylation, but not K16 acetylation [119]. Post-translational modification of the N-terminus of Sac10b by *N*-acetyl transferase has been demonstrated *in vitro* and is proposed to be the primary site of Sac10b acetylation *in vivo*. Recent mass-spectrometry (NanoLC-MS-MS) data of a Sac10b homologue from *Sulfolobus islandicus* have revealed methylation, acetylation, and deamination of this protein [101]. Strikingly, K16 was trimethylated, not acetylated. The improvements in mass spectrometry and identification of K16 trimethylation challenge the core assertion of Sac10b:DNA interactions being controlled by acetylation at K16. Taken together, the conflicting information on the PTM status of K16, the likely role of Sac10b homologues in binding DNA and RNA, and the differential abundance and importance of Sac10b homologues in diverse species argues that PTM(s) of Sac10b members may also be diverse and likely impact aspects of both RNA and DNA binding.

Variety in archaeal NAPs may shape genomes in diverse environments

While the biological importance of mechanisms governing Sac10b nucleic acid interactions is heavily

debated, it is important to consider the roles of the many other NAPs encoded in archaeal genomes. In addition to Sac10b, most crenarchaea encode small ~7-kDa proteins, with Sul7 and Cren7 dominating the literature. Cren7 is a 7-kDa, basic protein that has been found associated with DNA *in vivo*. The abundant and basic Cren7 protein has high affinity for double-stranded DNA, suggesting a primary role in genomic organization [120]. Although no obvious relationship is present at the primary amino acid level, Sul7 is structurally homologous to Cren7, and both are known to induce DNA compaction *in vitro* [121]. Crenarchaeal species such as *Pyrobaculum aerophilum* and *Thermoproteus tenax* lack obvious Cren7 or Sul7 homologues and instead encode the chromatin protein CC1. Like Sac10b proteins, CC1 is able to bind double stranded and single stranded DNA, suggesting a role in chromatin organization [122].

In the euryarchaeal *Thermococcales*, the TrmBL2 family is an abundant DNA-associated protein [123]. At likely physiological salt concentrations (~300 mM KCl) TrmBL2 binds DNA in a site-specific manner, while displaying non-specific DNA binding at lower salt concentrations. Non-specific DNA binding results in a filamentous structure that can compete with histone binding [124]. In *T. kodakarensis*, the abundance of TrmBL2 changes with the growth phase, and the interplay/competition between histones and TrmBL2 may offer an additional path to regulate genomic architecture and thus gene expression in response to environmental conditions. TrmBL2 occupancy of promoter regions can impact transcription, whereas TrmBL2 minimally impacts transcription elongation [125]. Deletion of TrmBL2 is possible and results in reduced condensation of chromatin and altered expression of approximately the same percentage of genes as deletion of a histone isoform [125].

Conclusions and Future Perspectives

Archaea are ecologically and metabolically diverse, and thus, it is perhaps not surprising that substantial differences in genomic architecture and regulation are imposed in different clades. Most species encode proteins with the core histone-fold, and archaeal chromatin thus dominates the landscape of regulation in archaeal species. The overall structural similarities between archaeal histone-based chromatin and eukaryotic chromatin are obvious, but the regulatory potential of the latter far exceeds the potential of the former. Archaeal chromatin is often formed with only one histone isoform, and given the absence of identifiable PTMs, it is likely that archaeal histones are not subject to repositioning or changes in DNA affinity that could increase or decrease transcription levels at specific loci. Significant questions remain for species that encode multiple histone isoforms and whether regulated assembly or binding of unique heterodimers impacts genomic architecture and thus gene regulation. The identification of PTMs or factors that could impact the normally tight association of adjacent gyres of archaeal histone-based chromatin may provide a route to regulate chromatin structure and transcriptional output. Identification of any such factors may help reveal the evolutionary origin of remodeling and modification machineries found ubiquitously in eukaryotes.

The identification of archaeal species that encode extensions on the core histone fold is an exciting new revelation in the context of histone-based regulation of gene expression. The expansion beyond the core histone-fold and the retention of discrete histone isoforms in many archaeal species provide tantalizing evidence in support of the expansion that must have occurred to provide all extant eukaryotes with the canonical four histones. The length and stability of extended nucleosome-like structures formed with archaeal histones are likely impacted by histone isoforms and the presence of extensions beyond the core histone-fold. Given that disrupting the tight association of archaeal histone-based chromatin results in massive fitness defects, it is plausible to predict that more fine-tuned and regulated mechanisms may exist to control and adjust chromatin formation or limit the length of the extended histone-based polymers to control gene expression *in vivo*. The timing of and expansion to defined heterodimeric histone partnerships that lead to the transition from an extended histone-based polymer structure to the discrete particles that define the eukaryotic nucleosome is a major outstanding question.

In addition to chemical modification machinery, eukaryotes encode a wealth of complexes to reposition nucleosomes. Repositioning nucleosomes or altering histone–DNA affinity may help or hinder transcription in eukaryotic cells. It remains possible that archaeal encoded modification or repositioning complexes exist, but current evidence suggests instead that archaeal TECs are reliant on conserved transcription factors to aid in overcoming histone-induced barriers to transcription elongation. To fully illustrate the evolution of the transcription apparatus, the roles of other conserved and potentially novel transcription factors and effectors will need to be characterized. The noted effects of Spt4/5 and TFS suggest that direct modification of the transcription apparatus may suffice for unmodified and relatively uniform histone-based chromatin structures, but that more powerful chromatin remodeling complexes and modification machinery are required for the diverse landscape of extant eukaryotic chromatin landscapes.

Despite many archaea encoding both NAPs and histone proteins, only limited information is available regarding the combinatorial regulation provided by the interplay of architectures produced by binding of both

classes of proteins [125]. It is logical to predict that the length and stability of extended histone-based structures may be regulated by NAP binding or NAP-mediated formation of DNA loops that impact overall topology and DNA flexibility. While minimally conserved at an amino acid sequence level, the structural conservation and functionality of archaeal NAPs suggests a conserved strategy for organizing DNA structure [126]. Clarity surrounding the role of the nearly ubiquitous Sac10b family of proteins with respect to RNA *versus* DNA binding—and clarification of the locations, identity, and impacts of potential PTMs—should illuminate the role of this often-abundant protein in organizing and providing dynamic regulation of archaeal genomes.

Acknowledgments

We thank members of the Santangelo laboratory for assistance with manuscript preparation and editing. Studies in the Santangelo laboratory are supported with funding from the National Institutes of Health (GM100329) and the Department of Energy (DE-SC0014597).

Received 26 February 2019;
Received in revised form 2 May 2019;
Accepted 4 May 2019
Available online 11 May 2019

Keywords:
RNA polymerase;
histone;
Alba;
archaea;
transcription regulation

These authors contributed equally to this work.

Abbreviations used:
TEC, transcription elongation complex; RNAP, RNA polymerase; NAP, nucleoid-associated protein; PTM, post-translational modification.

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