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Review

Histone-mediated chromatin organization in prokaryotes and viruses

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Histones are fundamental chromatin-organizing proteins in eukaryotes and archaea, where they assemble into (hyper)nucleosomes that wrap DNA. Recent studies have expanded the known repertoire of histones, identifying new variants in both prokaryotes and large DNA viruses. In prokaryotes, histones exhibit a range of DNA-binding modes, including wrapping, bending, and bridging, rather than exclusively forming nucleosomes. Notably, large DNA viruses encode histone paralogs that structurally resemble eukaryotic core histones and assemble into nucleosome-like complexes. This review summarizes recent discoveries on canonical archaeal nucleosomal histones and newly identified histones in archaea, bacteria, and viruses, highlighting their structural and functional diversity in genome organization.

Highlights

Large-scale bioinformatics studies reveal a diverse and widespread distribution of histones in prokaryotes and viruses.

Unlike eukaryotic histones, newly identified prokaryotic histones appear not to form nucleosomes, but instead bridge, bend, and wrap DNA.

Asgard archaea, the closest known relatives of eukaryotes, encode histone proteins with eukaryotic-like N-terminal tails, whose function remains unknown.

Megaviruses encode eukaryotic-like histones that form nucleosomes and compact viral DNA.

Canonical histones: a preamble

Organisms from all domains of life compact and functionally organize their DNA to accommodate the genome within the confines of the cell. Among the first DNA-binding proteins to be studied were histones and protamines [1,2]. In eukaryotes, the core histones – H2A, H2B, H3, and H4 – serve as major organizers of the genome, playing additional roles in replication, repair, and gene expression. These histones are highly conserved across eukaryotes, and share the characteristic histone fold, a structural motif composed of three α-helices (termed α1, α2, and α3) connected by two short linkers. Core histones assemble into obligate heterodimers: H2A-H2B and H3-H4. In the presence of DNA, two H3-H4 heterodimers combine to form a tetramer, which then associates with two H2A-H2B heterodimers to form an octamer. This octamer wraps 147 base pairs of DNA, forming the nucleosome, the fundamental repeating unit of chromatin (Figure 1) [3]. A defining feature of eukaryotic core histones is their intrinsically disordered N-terminal tails. These tails undergo extensive post-translational modifications (PTMs) that dynamically regulate nucleosome behavior, thereby influencing genome compaction, organization, repair, and replication [4].

Eukaryotes are now understood to have originated from a symbiotic partnership between an archaeon and a bacterium, with the archaeal partner belonging to the **Asgard** lineage (see Glossary), the closest known prokaryotic relatives of eukaryotes [5,6]. Like eukaryotes, most archaea, except Crenarchaeota, employ histone proteins to organize their DNA. However, archaea also possess **nucleoid-associated proteins (NAPs)** (Box 1), a group of DNA-binding proteins that are widespread in both archaea and bacteria [7–10]. Although NAPs have historically been referred to as histone-like proteins, due to their role in DNA binding and organization, they fundamentally differ from histones as they lack the characteristic histone fold. The first archaeal histones were identified in *Methanothermus fervidus* in 1990 [11]. These histones assemble into nucleosome-like structures [12], reinforcing the evolutionary connection between archaeal and eukaryotic chromatin organization. Until recently, nucleosomal histones were the only well-characterized prokaryotic histone type. However, recent large-scale studies have uncovered

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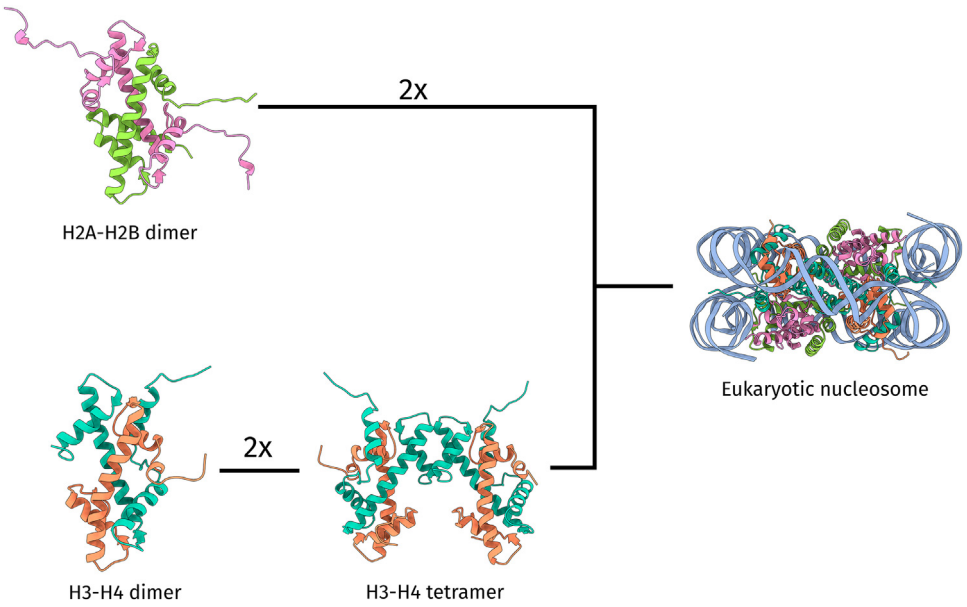

Figure 1. The eukaryotic nucleosome. The eukaryotic nucleosome (PDB: 1AOI) is formed by two H3-H4 heterodimers, which together form a tetramer, and two H2A-H2B heterodimers, which flank the tetramer on either side [3].

a broader diversity of prokaryotic and viral histones, including newly identified variants in bacteria and viruses, expanding our understanding of histone function and evolution.

In this review, we explore the latest discoveries on prokaryotic and viral histones, highlighting their widespread distribution, structural diversity, and the diverse mechanisms by which they contribute to **DNA organization**.

Archaeal histones

Among prokaryotes, histones are most prevalent in archaea, where they function by either wrapping or bridging DNA [7,13,14]. The most common group is the nucleosomal histones, which form structures similar to eukaryotic nucleosomes. However, unlike their eukaryotic counterparts, which are restricted to an octameric core, canonical archaeal nucleosomal histones multimerize along DNA, assembling into an extended **hypernucleosome** (Figure 2, Key Figure) [7,12,15]. The best-characterized canonical archaeal histones include *M. fervidus* histones A (HMfA) and B (HMfB), and *Thermococcus kodakarensis* histones A (HTkA) and B (HTkB) (Table 1) [11,16–19]. Like most archaeal histones, these proteins contain a histone fold but lack tails [17,19,20]. Upon DNA binding, they assemble into hypernucleosomes as homo-oligomers [12,21,22]. Although heteromeric complexes remain poorly understood, HMfA and HMfB can form heterodimers [23], and other archaeal histones likely heterodimerize as well, though their

Box 1. What is a "histone"?

The term "histone" or "histone-like" has historically led to confusion in the prokaryotic field, as various DNA-binding proteins have been labeled histone-like, despite lacking significant similarity at the protein level. In this review, we define histones as proteins that contain a histone fold, providing a clear and biologically relevant framework for classification. This definition places prokaryotic histones in the same structural category as their eukaryotic counterparts, emphasizing shared architectural features rather than functional assumptions. Importantly, our definition does not require histones to form nucleosomes, as prokaryotic histones exhibit diverse modes of DNA interaction beyond nucleosomal organization.

Glossary

Asgard archaea: A phylum of archaea discovered at Loki's Castle at the bottom of the North Sea. Asgard archaea are the closest archaeal relatives to eukaryotes and encode various eukaryotic signature proteins.
Capstone: Histones that can bind at the extremities of hypernucleosome structures, but prevent further extension.
DNA organization: At the molecular level, proteins can structure and organize DNA by bending it, wrapping it, bridging separate DNA duplexes, or forming nucleoprotein filaments.
Hypernucleosome: A protein-DNA complex consisting of histones assembled into an endless helical protein core with DNA wrapping around it.
Nucleoid-associated proteins (NAPs): Small, positively charged proteins that bind and organize DNA in bacteria and archaea. Unlike histone proteins, NAPs do not share a common fold.
Pseudodimeric histone: A histone protein that contains two distinct histone fold domains within a single polypeptide chain. Although it is a monomer, the two folds interact intramolecularly in a manner analogous to a heterodimer of separate histone proteins, giving rise to a dimer-like structure within a single chain.
Roadblock: A protein that forms a physical barrier against the hypernucleosome on DNA, preventing the structure from extending beyond the roadblock.
Stacking interactions: Salt bridge interactions between individual layers of the hypernucleosome. Each histone dimer *i* in the hypernucleosome forms stacking interactions with dimer *i*+2 and *i*+3, which are positioned above histone *i*.
Systematic evolution of ligands by exponential enrichment (SELEX): SELEX involves the use of degenerate DNA libraries, from which target DNA sequences are enriched, sequenced, and classified based on relative affinity.

Key figure

Histones in prokaryotes and viruses

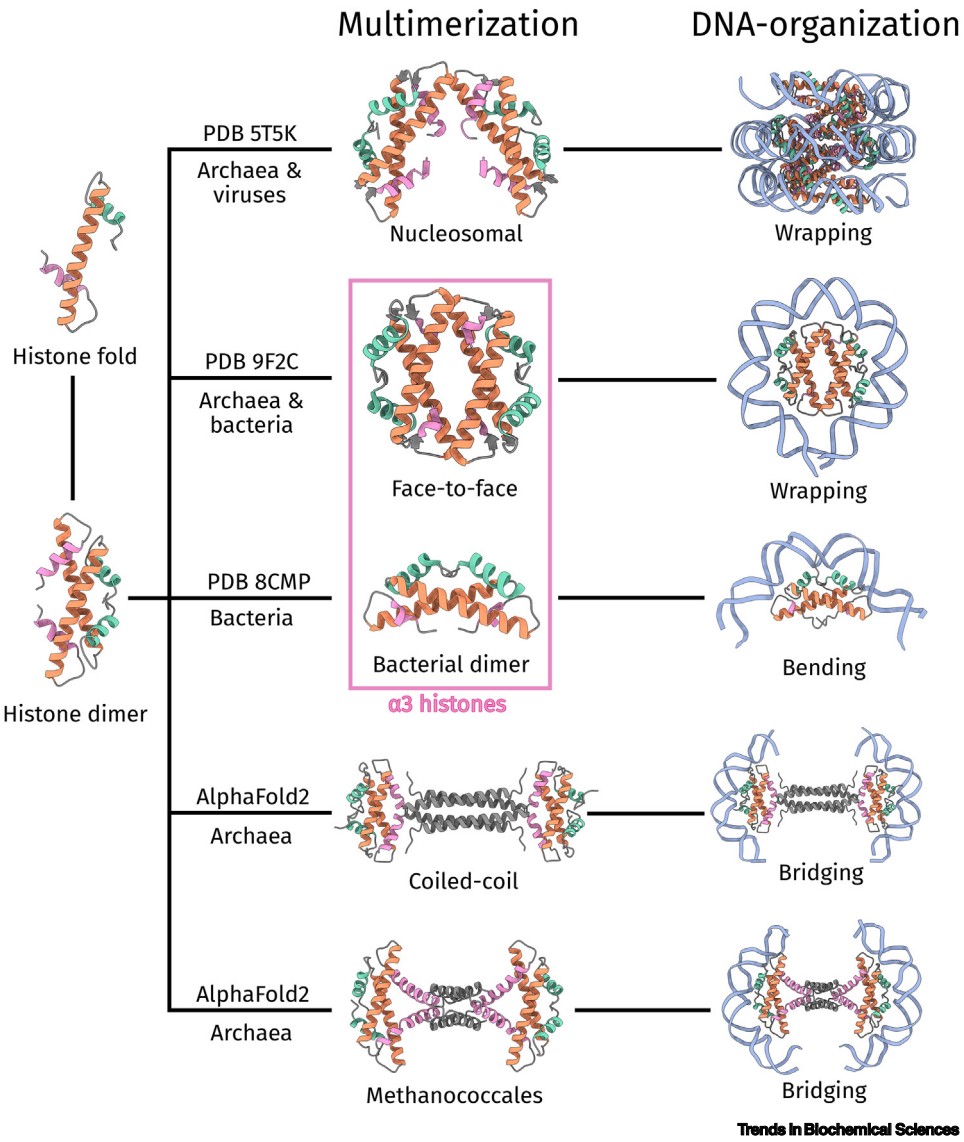

Figure 2. Overview of characterized histone types, their unique forms of multimerization, and the manner in which they organize DNA. The bacterial dimer and face-to-face histones are part of the α3 histone family. The protein-DNA complexes of the bacterial dimer and face-to-face histones were obtained from MD simulations [48,50].

DNA-binding properties have yet to be characterized. A distinct hypothetical variant of nucleosomal histones, termed **"capstone" histones** [24], has been proposed as a mechanism for limiting hypernucleosome size by featuring an unstable dimer-dimer interface. These capstone histones are thought to function similarly to eukaryotic H2A/H2B heterodimers, which flank nucleosomes at their boundaries and prevent further oligomerization beyond the H3/H4 tetramer. Several

Table 1. Prokaryotes and viruses of interest and their histones

Organisms	Histone type	UniProt ID	Gene,locus
Methanothermus fervidus	Nucleosomal	E3GZI5	hmfA
		E3GWR6	hmfB
	Coiled-coil	E3GZL0	hmfC,Mfer_0945
Thermococcus kodakarensis	Nucleosomal	Q9Y8I1	htkA,TK1413
		Q9Y8I2	htkB,TK2289
	Face-to-face	Q5JDW7	htkC,TK1040
	DUF1931	Q5JHD0	TK0750
Haloferax volcanii	Face-to-face	D4GZE0	HVO_0196
		D4GQ55	HVO_A0023
	Halo	D4GS56	hstA,HVO_0520
	RdgC	D4GVY1	HVO_2265
Bdellovibrio bacteriovorus	Bacterial dimer	Q6MRM1	hbb,Bd0055
	ZZ	Q6MIV3	Bd3044
Methanocaldococcus jannaschii	Nucleosomal	Q57632	MJ0168
		Q58342	MJ0932
		Q58655	MJ1258
		Q60264	MJECL29
	Methanococcales	Q59041	hmvA,MJ1647
	DUF1931	Q58904	MJ1509
Heimdallarchaeota LC3	Nucleosomal	A0A1Q9N418	HeimC3_51210
		A0A1Q9NAM9	HeimC3_47090
		A0A1Q9NIV9	HeimC3_33200
		A0A1Q9NJR1	HeimC3_31270
		A0A1Q9NJV8	HeimC3_31310
		A0A1Q9NR84	HeimC3_18970
		A0A1Q9NRJ0	HeimC3_18960
		A0A1Q9NRY6	HeimC3_17480
		A0A1Q9NVH8	HeimC3_10830
		A0A1Q9NVH8	HeimC3_10830
	Dimer	A0A1Q9N8N6	HeimC3_49130
Melbournevirus	H2A-H2B	A0A097I2B5	MEL_369
	H3-H4	A0A097I2D0	MEL_368
	miniH2A-H2B	A0A097I1R9	MEL_149

potential capstone histones have been identified computationally in Methanobacteriales, but their functional roles remain experimentally uncharacterized [25].

Unlike sequence-specific DNA-binding proteins, nucleosomal histones do not exhibit strict sequence specificity, since they lack direct nucleobase contacts. However, in *T. kodakarensis*, HTkA and HTkB are underrepresented in poly dA:dT tracts, yet enriched in sequences conducive to DNA wrapping, such as poly dG:dC tracts or phased helical repeats of AA/TT/AT/TA and CC/GG/CG/GC dinucleotides [26,27]. Consistent with these observations, an artificial high-affinity sequence known as Clone20, obtained via **SELEX** [28,29], consists of periodically spaced

alternating A/T- and G/C-rich regions, although its similarity to natural archaeal binding patterns is limited. HMfA and HMfB bind Clone20 as tetramers [30], yielding tetrasomes – self-limiting structures that prevent further hypernucleosome formation. The AT/GC dinucleotide periodicity found in the Clone20 sequence is also present in some archaeal genomes, such as those of *Methanothermobacter thermautotrophicus* and *T. kodakarensis* [26,27] However, sequences with high sequence identity to Clone20 have not been identified in archaeal genomes.

The best-characterized histones in vivo are HTkA and HTkB from *T. kodakarensis*. Both histones are important for viability: deletion of either histone individually is tolerated, but loss of both is lethal [31]. Genome-wide mapping shows that HTkA and HTkB are depleted immediately upstream of start codons, particularly in promoter regions [26], suggesting a regulatory mechanism that preserves accessibility for transcription initiation. Mutations in, or deletions of, HTkA or HTkB also alter the transcriptome and transcription elongation rates [32,33]. HTkA-based chromatin reduces elongation rates by 20% compared to histone-free DNA. Disrupting the histone-based chromatin by mutating HTkA further reduces the elongation rates, in some cases by 55%. The transcriptional changes are genome-wide, affecting gene expression both positively and negatively, with some mutants altering the expression of more than ≥11% of the transcriptome [32]. HTkA and HTkB undergo PTMs, notably acetylation of lysines within the histone fold, rather than the histone tails as seen in eukaryotes [34]. These lysines are thought to participate in **stacking interactions** between histone dimers within the hypernucleosome (Figure 3). As a consequence, the hypernucleosome might be destabilized or become more “breathable”. It is plausible that the cell actively controls introduction or removal of PTMs as their frequency changes depending on growth phase [34].

Recently, diverse histone variants have been discovered across archaea, differing from canonical nucleosomal histones in DNA organization and multimer structure [14,35]. For instance, MJ1647 from *Methanocaldococcus jannaschii* structures DNA by bridging DNA duplexes rather than wrapping DNA around itself (Figure 2) [36]. MJ1647’s C-terminal α-helices facilitate tetramerization, crucial for DNA bridging. Removal of these helices abolishes DNA-bridging activity, underscoring the tetramer as the functional unit. AlphaFold2 predicts that these C-terminal helices engage in a ’handshake’ arrangement within the tetramer, orienting the histone folds outward to bind separate DNA duplexes. Similar DNA-bridging tetramers occur in other histone families, such as HMfC from *M. fervidus*, which belongs to a family called coiled-coil histones [14]. Unlike MJ1647, which is limited to Methanococcales, coiled-coil histones are widely distributed among archaea. Despite only 25% sequence identity with MJ1647, HMfC is predicted to form similar tetramers, and experimental evidence confirms its DNA-bridging activity (Figure 2) [14].

Archaeal genomes exhibit varied histone usage patterns, as evidenced by findings in two main model organisms (Table 1). In *T. kodakarensis*, canonical nucleosomal histones are the most abundant [37]. In contrast, *Haloferax volcanii* employs a distinct histone variant, HstA, which has been extensively studied as a potential global genome organizer [38]. Unlike canonical histones, HstA contains two histone folds within a single polypeptide chain, allowing it to function as a **pseudodimer**. HstA-like histones are highly conserved across Haloarchaea [14]. As pseudodimers, they function similarly to heteromeric histones and are predicted to form a nucleosomal tetramer as a dimer of dimers, but lack the capacity to assemble into nucleosomal structures [14]. However, HstA and its homolog HpyA from *Halobacterium salinarum* are expressed at very low levels, likely too low to facilitate genome-wide packaging [38–40]. Instead, HpyA binds selectively to 59 genomic sites, including both coding and non-coding regions, under low salt conditions, and to only five sites under optimal salt conditions, suggesting a regulatory role in osmotic stress response [39]. Deletion of HpyA leads to differential expression of 122 genes in *H.*

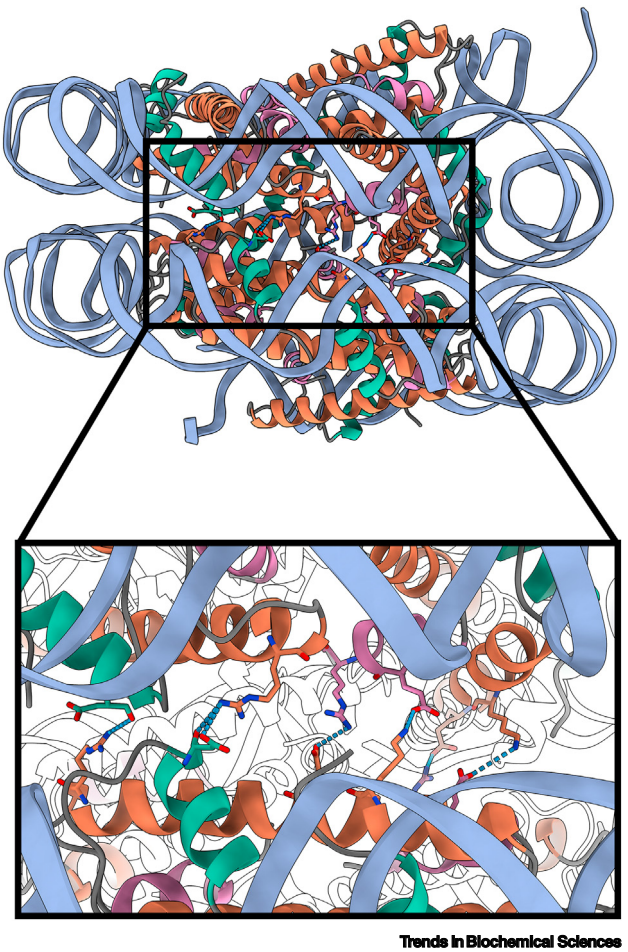

Figure 3. Stacking interactions in the hypernucleosome formed by HMfB. A 12-mer of hypernucleosome histone HMfB (PDB: 5T5K) and a zoom-in highlighting the stacking interactions [12]. The interactions from left to right are D14-R48, R48-D14, E34-R65, E61-K30, R65-E34, and K30-E61.

salinarum, notably upregulating genes involved in iron uptake and cellular homeostasis under low ionic strength conditions [39]. Although HstA and HpyA function more like transcription factors than chromatin organizers, *H. volcanii* chromatin exhibits several hallmarks of eukaryotic chromatin, including -1 and +1 promoter nucleosome occupancy, nucleosome-depleted transcriptional start sites, and MNase-protected genomic regions [41]. A recently identified histone, D4GZE0 (*HVO_0196*), is among the most highly expressed genes in *H. volcanii* and may play a major role in its genome organization [14]. The encoded protein belongs to the widespread face-to-face (FtF) histone family, which is highly conserved across Haloarchaea and found in nearly all archaeal lineages, including Asgard archaea and the model archaeon *T. kodakarensis*. The FtF histone from *T. kodakarensis*, HTkC, forms tetramers through face-to-face interactions between dimers, creating a torus-like structure (Figure 2) [14]. While their precise DNA-binding mode remains unclear, FtF histones likely wrap DNA around their tetrameric assemblies.

Bacterial histones

Bacteria are generally considered to lack histones, relying instead on NAPs for genome organization. However, recent studies have challenged this view. Initial indications of bacterial histones emerged nearly two decades ago when structural genomics efforts identified homologs in *Thermus thermophilus* (PDB: 1WWI) and *Aquifex aeolicus* (PDB: 1R4V) [42]. These proteins, belonging to the PFAM DUF1931 family, form pseudodimers but lack the DNA-binding residues

required for nucleosome formation. Their significance remained unclear, and bacterial histones were long thought to be rare exceptions.

Only much later did large-scale bioinformatics analyses reveal their broader distribution. In 2019, over 500 bacterial histone homologs were identified across diverse bacterial phyla, including Actinomycetota, Cyanobacteriota, and Deinococcota [43]. These newly identified histones share $\leq 25\%$ sequence identity with canonical archaeal and eukaryotic histones. Analysis of this expanded bacterial histone sequence dataset revealed that, beyond pseudodimeric histones, bacteria also encode single histone fold proteins, many of which retain key DNA-binding residues typical of archaeal and eukaryotic histones. Subsequent studies further broadened the repertoire of bacterial histones and, in parallel, uncovered homologs of these proteins in archaea [14,44]. AlphaFold2-based structural predictions strongly suggest that these proteins adopt the characteristic histone fold [14].

The α3 histone family, which is also present in archaea, represents the dominant bacterial histone class (Figure 2). It is characterized by a shorter α2 helix and a truncated α3 helix and comprises four subfamilies: FtF histones, bacterial dimer histones, ZZ histones, and phage histones. FtF histones, the most widespread, are found in the phyla Spirochaetota, Planctomycetota, Bdellovibrionota (class Bacteriovoracia), and Myxococcota, where they are predicted to form tetramers [14]. Bacterial dimer histones, present in the phyla Bdellovibrionota (class Bdellovibrionia), Elusimicrobiota, Spirochaetota (class Spirochaetia), Planctomycetota, Myxococcota_A, and Chlamydiota, are predicted to form dimers [14]. ZZ histones, found mainly in Proteobacteria, contain an N-terminal ZZ-type zinc finger domain, which is associated with protein-protein and protein-DNA interactions in eukaryotic chromatin [45–47]. These histones are predicted to form dimers. Phage histones, identified in bacterial and prokaryotic dsDNA virus metagenomes, contain a C-terminal α-helical domain, which may influence oligomerization or interactions with viral or bacterial DNA. They are predicted to form tetramers [14].

Bacterial histones are rare, present in fewer than 2% of bacterial genomes, whereas, HU, the most widespread NAP is found in 92% of genomes [14,44,48]. Despite their rarity, most bacterial histones contain DNA-binding residues typical of archaeal and eukaryotic histones, suggesting a potential role in genome organization. Two recent studies have characterized HBb (Bd0055), a representative of the bacterial dimer histone family in *Bdellovibrio bacteriovorus*, demonstrating that it is highly abundant in the nucleoid, binds DNA in a sequence-independent manner, and is essential for survival. These findings underscore its critical role in genome organization and gene regulation [44,48]. Crystal structures of DNA-bound HBb have led to two differing interpretations of its DNA-binding mode. The first study proposed that HBb binds DNA end-on, forming a sheath of dimers that encase straight DNA rather than wrapping around it, a mechanism distinct from eukaryotic and archaeal histones [44]. However, a subsequent study suggested that HBb dimers instead bend DNA upon binding, facilitated by interaction interfaces reminiscent of those in eukaryotic and archaeal histones [48]. This bending mechanism likely contributes to genome compaction and chromatin organization in *B. bacteriovorus*. In addition to HBb, *B. bacteriovorus* encodes another histone, Bd3044, a ZZ-type histone (Table 1). While HBb is strongly expressed during the bacterium's growth phase, Bd3044 is generally less abundant and cannot functionally compensate for HBb, suggesting distinct roles for these histones in genome organization [44,48,49].

Another experimentally characterized bacterial histone group is the FtF histones of the *Leptospira* genus. FtF histones are the only α3-type histone shared between bacteria and archaea. In the pathogenic species *Leptospira interrogans*, the FtF histone has been shown to be indispensable

for viability [44], while its homolog, HLp, in *Leptospira perolatii*, binds DNA non-specifically and alters the nucleoid structure of *Escherichia coli* when heterologously expressed in vivo [50]. Crystal structures of the HLp-DNA complex revealed that HLp assembles into a tetramer that wraps and compacts DNA in a manner similar to eukaryotic and archaeal nucleosomal histones [50]. Within the tetramer, two dimers interact via the α3-helices and parts of the α2 helices, resembling nucleosomal histones. Uniquely, however, both sides of each dimer participate in interactions, forming a toroidal structure.

Collectively, these studies suggest that bacterial histones like HBb and HLp play significant roles in genome organization, employing mechanisms distinct from their eukaryotic and archaeal counterparts. Histone types with representatives in both bacteria and archaea, such as the FtF histones HLp (bacterial) and HTkC (archaeal, from *T. kodakarensis*), are expected to function similarly. While some bacterial histones, such as DUF1931 histones, may have lost DNA-binding ability, others—such as those with additional domains—may have evolved alternative functions, including regulatory interactions, genome remodeling, or structural roles in bacterial nucleoid organization.

Viral histones

Like bacteria, viruses generally do not encode histones but often interact with host histones to regulate their life cycles. Many DNA viruses, including *Herpesviridae* [51] and *Papillomaviridae* [52,53], hijack host histones to wrap their genomes, modulating transcription and evading immune detection. Retroviruses such as human immunodeficiency virus integrate into the host genome, where histone modifications regulate viral latency and reactivation [54]. Other viruses, including Epstein-Barr virus and human cytomegalovirus, actively remodel chromatin, altering histone methylation and acetylation to suppress immune-related genes while maintaining viral gene expression [55].

However, some members of the Nucleocytoviricota (NCVs), a group of large DNA viruses with genome sizes ranging from 100 kilobases up to 2.5 megabases [56], encode their own histones, which exhibit distinct structural and functional features [57–62]. For example, *Medusavirus* encodes genes for all four core histones (H2A, H2B, H3, and H4), as well as linker histone H1, forming a complete histone complement [59]. A comprehensive analysis of NCV genomes and metagenomes identified 258 histone genes across 168 viruses, revealing that histone-encoding viruses are more widespread than previously recognized [63]. Many of these viruses encode multiple histones, often assembling a full histone complement through diverse pairings. For example, several viruses feature an H2B-H2A-H3 triplet alongside a separate H4 singlet, suggesting a flexible assembly strategy that may serve distinct functional roles in viral genome regulation [64]. Notably, the structural organization of viral histones differs from their eukaryotic counterparts. Instead of existing as separate proteins, many viral histones contain multiple histone repeats within a single polypeptide chain, extending their size and complexity. These repeats predominantly form doublets (H2A-H2B, H2B-H2A), triplets (H2B-H2A-H3), or quadruplets (H2B-H2A-H3-H4, H2B-H2A-H4-H3, H4-H3-H2B-H2A). Furthermore, viral histones exhibit a highly ordered domain organization, with H2A/H2B and H3/H4 typically appearing in tandem, mirroring the conserved histone pairings found in eukaryotic nucleosomes. For instance, members of the *Marseilleviridae* family contain genes for obligate H2B-H2A and H4-H3 pseudodimers [57]. Their distribution varies across viral lineages, with histone repeats being prevalent in early-branching viral superclades, such as *Marseillevirus*, *Iridovirus*, and *Medusavirus*, whereas deeper-branching lineages predominantly encode histone singlets. This pattern suggests that histone repeats may have an ancient origin, with histone singlets potentially arising from more recent horizontal gene transfer events from eukaryotes.

Melbournevirus, a member of the *Marseilleviridae* family primarily found in the amoeba *Acanthamoeba castellanii*, is the first giant DNA virus whose histones have been shown to assemble into nucleosome-like structures (Figure 2) [60–62]. These viruses encode fused histone doublets (H2B-H2A and H4-H3), which share high structural similarity with eukaryotic histones but exhibit low sequence identity (<30%) (Table 1). Additionally, *Melbournevirus* histones possess extensions resembling eukaryotic histone tails, which are thought to modulate nucleosome structure, though no evidence for PTMs has been found. Unlike eukaryotic nucleosomes, which typically wrap 147 base pairs of DNA, *Melbournevirus* nucleosome-like particles compact shorter DNA fragments (121 base pairs). They lack linker DNA and regular phasing along genes, resulting in a densely packed chromatin structure. Thermal stability assays demonstrate that these viral nucleosomes are less stable than their eukaryotic counterparts, likely due to differences in histone-histone and histone-DNA interactions [65]. Despite this, viral histones play essential roles in genome organization, DNA packaging, protection within virions, and localization to cytoplasmic viral factories during infection.

Interestingly, *Melbournevirus* also encodes a second, shorter H2B-H2A doublet, which, although significantly less abundant, is essential for viral fitness [66]. Recent cryo-EM studies of reconstituted *Melbournevirus* nucleosomes incorporating this second H2B-H2A doublet revealed that these particles wrap only 90 base pairs of DNA and are less stable than the primary nucleosome-like structures. This instability has been proposed to facilitate rapid genome unpacking, potentially accelerating the onset of viral gene expression upon infection.

Recently, *Medusavirus medusae* (MM), a giant virus distantly related to *Melbournevirus*, was also found to assemble nucleosome-like particles with structural similarities to eukaryotic nucleosomes but with distinct adaptations [65]. Unlike *Melbournevirus*, MM is one of the few viruses that encodes all four core histones on separate genes and possesses the linker histone H1. These histones assemble into octamers with DNA, forming tri-nucleosome arrays featuring elongated loops and tails. A particularly notable feature of medusavirus histones is the unique structure of its H1 linker histone. Unlike eukaryotic H1, which contains a single winged-helix domain, medusavirus H1 has two winged-helix domains. However, rather than promoting chromatin compaction, as in eukaryotes, medusavirus H1 appears to have a virus-specific function, possibly in reshaping host transcription patterns. Functionally, medusavirus nucleosomes are thought to compact the viral genome during replication and packaging, while also modulating host-virus interactions during infection.

These findings suggest that viral histones play a critical role in genome organization, replication, and infection, enabling viruses to mimic or repurpose eukaryotic histone-based regulatory mechanisms. Their highly ordered domain architecture and functional integration into the viral life cycle highlight an evolutionary adaptation that enhances viral genome stability, replication efficiency, and host interaction strategies.

Concluding remarks

Over the past decade, histone research has expanded significantly beyond eukaryotes to encompass all domains of life. Across these domains, histone proteins function primarily by binding and organizing DNA. While histone dimers share a common structural core, the specific interaction between these dimers dictate how they organize DNA. Dimers that interact with their histone folds positioned far away bridge DNA; nucleosomal histones wrap DNA; FtF histones wrap DNA; and simpler histone dimers bend DNA. These findings suggest that histones are fundamentally simple DNA-binding proteins, versatile enough to evolve distinct mechanisms that support complex genome architectures across life.

Outstanding questions

When did histones emerge? Were histones already present in LUCA or in LACA? Did bacteria inherit histones from LUCA or acquire them via horizontal gene transfer? Did histones emerge multiple times independently through convergent evolution?

What are the functions of the N-terminal tails in the Asgard archaea histones? Are these tails subject to PTMs?

What is the in vivo role of hypernucleosomes?

How are hypernucleosomes regulated in vivo (e.g., through PTMs, capstones, other NAPs, physico-chemical condition, transcriptional activity)? Are these regulatory mechanisms conserved across archaea?

To what extent are heterodimers important for hypernucleosome function in vivo? Do certain histones, particularly those from Asgard archaea, form obligatory heterodimers?

What roles do bacterial histones, particularly those with additional domains (e.g., ZZ-type zinc fingers), play in genome organization or regulation in vivo?

Are bacterial histones subject to PTMs, and do these modifications impact their biological functions?

What biological functions do histone-DNA complexes serve in archaea and bacteria beyond DNA structuring?

What are the biological functions of histone-fold proteins lacking predicted DNA-binding capabilities?

An unresolved question is precisely when histones emerged during evolution (see Outstanding questions). Histones were likely already present in the last archaeal common ancestor (LACA), as both nucleosomal and FtF histones are widespread and broadly distributed throughout archaea. However, whether histones existed in the last universal common ancestor (LUCA) remains uncertain. The scattered presence of histones in deeply branching bacterial lineages supports the hypothesis that LUCA may have already possessed histones. Yet, the rarity of histones in bacteria overall lends support to the alternative hypothesis that bacterial histones resulted from horizontal gene transfer events from archaea. Clarifying this evolutionary history is challenging due to the low sequence identity among histone groups across domains.

Eukaryotic histones likely originated from nucleosomal histones of archaea. With the discovery of the Asgard archaea, the closest known archaeal relatives of eukaryotes, characterizing Asgard histones has become an important goal. A distinctive feature of some Asgard histones is their disordered N-terminal tails, which are largely absent in other archaeal nucleosomal histones [7]. These tails are similar in amino acid composition to eukaryotic histone tails, raising questions about their functional role, potential for PTMs, and evolutionary relationship to eukaryotic histone tails [7,35]. However, the N-terminal tails of Asgard histones lack discernible sequence similarity to those of eukaryotic histones, leaving open whether they represent the ancestral form of eukaryotic histone tails [35]. It also remains unclear whether tail-less Asgard histones function similarly to well-studied archaeal histones such as HMfA, HMfB, HTkA, and HTkB, or exhibit distinct modes of DNA organization. Biochemical and biophysical characterization of Asgard histones, with and without their N-terminal tails, will clarify whether these proteins assemble into hypernucleosomes and how the tails influence their structural and functional properties. With the increasing availability of Asgard (meta)genomes, an exciting research direction is the characterization of histones from Heimdallarchaeota, currently recognized as the archaeal lineage closest to eukaryotes.

While the nucleosomal histones from *T. kodakarensis* and *M. fervidus* have been extensively studied in vitro [11,12,16–19,21,22,30], relatively little is known about their behavior in vivo. Open questions include whether PTMs are a common feature of hypernucleosome-forming histones, how these modifications influence their function, and to what extent hypernucleosome structures respond to physio-chemical conditions, such as divalent ion concentrations. Additionally, the mechanisms regulating hypernucleosome length remain unknown. Addressing these questions will require further in vivo studies of *T. kodakarensis*, one of the few archaeal species amenable to genetic and biochemical manipulation. Techniques, such as proteomics could identify PTMs on archaeal histones, while ChIP-seq could determine their genomic positioning and identify potential histones or NAPs that function as capstones or **roadblocks**. Furthermore, in vitro biophysical and biochemical approaches, including tethered particle motion or single particle cryo-EM, could help elucidate how hypernucleosome compaction and size are regulated under different physico-chemical conditions, such as varying magnesium concentrations.

Compared to archaeal histones, the in vivo functions of bacterial and viral histones remain largely unexplored. Many bacterial histone variants – especially those containing additional domains, such as ZZ-type zinc fingers or other predicted DNA-binding or protein-interaction domains – are yet to be characterized. However, studying these bacterial histones is challenging, since most are found in deeply branching lineages that are difficult to culture or genetically manipulate. Future research will therefore require developing genetic and biochemical tools for more tractable bacterial species, such as *B. bacteriovorus* and *L. interrogans*. Similarly, the structural and functional diversity of viral histones is still poorly understood. Future research efforts should focus on characterizing histone variants from giant DNA viruses, using biochemical, biophysical, and

structural assays to determine how viral histone diversity contributes to genome packaging, host-virus interactions, and the regulation of infection. Such investigations will significantly advance our understanding of the complex biology underlying bacterial and viral histones.

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Declaration of interests

The authors declare no competing interests.

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