Molecular Cell

Let’s wrap things up: open and closed hypernucleosomes in Asgard archaea

--Manuscript Draft--

Manuscript Number:	MOLECULAR-CELL-D-25-02586R1
Full Title:	Let’s wrap things up: open and closed hypernucleosomes in Asgard archaea
Article Type:	Preview
Keywords:	Chromatin; histones; archaea
Corresponding Author:	Daniela Barilla, PhD University of York York, UNITED KINGDOM
First Author:	Daniela Barilla, PhD
Order of Authors:	Daniela Barilla, PhD
Abstract:	Asgard archaea are widely considered the closest living relatives of Eukaryotes. In this issue of Molecular Cell, Ranawat et al.1 report high-resolution structures of hypernucleosomes formed by the hodarchaeal HHoB histone, disclosing open and closed chromatin conformations.
Additional Information:	
Question	**Response**

Previews

Let's wrap things up: open and closed hypernucleosomes in Asgard archaea

Daniela Barillà[1,*]
[1]Department of Biology, University of York, York YO10 5DD, UK
*Correspondence: daniela.barilla@york.ac.uk

Asgard archaea are widely considered the closest living relatives of Eukaryotes. In this issue of *Molecular Cell*, Ranawat et al.[1] report high-resolution structures of hypernucleosomes formed by the hodarchaeal HHoB histone, disclosing open and closed chromatin conformations.

Every cell faces the challenge of packaging a large volume of DNA within a miniscule compartment. Plant cells, which harbour exceptionally large genomes, provide extreme examples: for instance, *Viscum album* (mistletoe) cells possess 95 billion base pairs (bp) of DNA, whose length in a single cell amounts to 63 m, if fully elongated. An even more astounding case is the Japanese canopy plant, *Paris japonica,* with a genome size of ~149 billion bp that corresponds to a bewildering length of 100 m of DNA in a single cell.[2] This sesquipedalian DNA river must fit into a nucleus with a diameter of just a few microns. Eukaryotes and most Archaea overcome this spatial problem by deploying histone proteins that form a core around which the DNA wraps, thereby building a tightly packed structure denoted the *nucleosome*, the basic unit of chromatin.[3] The eukaryotic nucleosome consists of a histone octamer that includes two copies of core histones H2A, H2B, H3 and H4, wrapped by a 147bp long DNA fragment.[4] Histones assemble and function as heterodimers: H2A associates with H2B and H3 associates with H4. The canonical histone structural fold consists of three α-helices connected by two short loops. Eukaryotic core histones exhibit a N-terminal flexible tail that is subjected to post-translational modifications. Archaeal histones also display the typical histone fold, but most do not contain disordered tails and assemble into extended DNA-protein complexes, termed *hypernucleosomes*. Within the hypernucleosome complex, 30bp of DNA wraps around each histone dimer and dimers multimerize, forming an extended solenoid of variable length.[4]

Currently, most experimental studies of archaeal chromatin have been performed on two model systems from the Euryarchaeota phylum, *Methanothermus fervidus* and *Thermococcus kodakarensis*. Structural and biophysical investigations show that histones from these species bind DNA, assembling into hypernucleosome complexes with a DNA footprint of 90bp or more.[5-7] However, novel archaea phyla have been identified in the last decade, paving the way for a more systematic exploration of chromatin organization in this branch of life. Among the

new arrivals, the Asgard superphylum has assumed a centre-stage role in the debate on the origin of eukaryotes, as Asgard archaea encode eukaryotic signature proteins and are widely considered the closest living relatives of eukaryotes.[8] Bioinformatic analyses of Asgard genomes find that certain lineages encode multiple histone variants.[9,10] Hodarchaeales represent the Asgard clade that is phylogenetically closest to eukaryotes. The genome of Hodarchaeales LC3 encodes ten histones, two with extended tails and eight that lack tails. [9,10] How these intriguing histones wrap the DNA and assemble into chromatin complexes is unknown. Given the close phylogenetic relationship between Hodarchaeales and eukaryotes, the investigation of Hodarchaeales LC3 histones may shed light on the evolutionary journey of histone proteins from archaea to eukaryotes.

Ranawat et al.[1] report high-resolution structures of the nucleosome and hypernucleosome assembled by the HHoB histone encoded by Hodarchaeales LC3. HHoB is a short 68-residue histone without disordered tail. Reconstitution of the HHoB-DNA nucleosome using a 147bp DNA fragment, followed by cryo-electron microscopy (cryo-EM) single-particle analysis, reveals two different nucleosome conformations, designated as 'closed' and 'open'. The closed conformation features tightly packed histone dimers that wrap the DNA into a compact superhelix. In contrast, the open arrangement presents histone dimers that pack the DNA into a looser and elongated spiral structure, characterised by a larger distance between turns and lack of stacking interactions between non-adjacent dimers. Each nucleosome consists of a left-handed helix that contains four HHoB dimers bound to 120bp, corresponding to a 30bp footprint per dimer. The closed nucleosome conformation resembles that described for the euryarchaeal HMfB nucleosome[5], whereas the open geometry has not been previously observed in archaea. Mg^{2+} plays a role in shaping nucleosome structure, with prevalence of open nucleosomes at close-to-zero concentrations and predominance of closed nucleosomes from 60 nM Mg^{2+}. Nucleosomes stack to form hypernucleosomes at all Mg^{2+} concentrations and no such structures are observed in the absence of Mg^{2+}.

The closed HHoB hypernucleosome is similar to that described for HMfB[5] and exhibits dimers that continuously wrap the DNA into a compact left-handed superhelix with a pitch of ~25 Å and velcro-like stacking forces generated by electrostatic interactions between residues with opposite charges. Instead, the open hypernucleosome displays a looser superhelix with a pitch of 63 Å in which lateral interactions between histone dimers and DNA end-to-end contacts enable the formation of the helical structure, despite the absence of histone stacking interactions (Figure 1). Adjacent dimers are positioned at a steeper angle in the open conformation, resulting in a sharp rise in the superhelix leading to an elongated structure. The authors also draw a comparison between the open state observed in the HHoB nucleosome and that reported for the eukaryotic 'octasome', which is a complex formed by four H3-H4 heterodimers that shows an open conformation with a 53 Å helical pitch. This parallel is

attractive from an evolutionary perspective, as the octasome has been suggested to be an ancestral form of the eukaryotic nucleosome. Moreover, the comparison leads to establishing HHoB-Tyr44 as a key residue for open state stabilization. Lastly, biophysical investigations with optical tweezer force spectroscopy and tethered particle motion confirm the structural observations and provide further evidence that Mg^{2+} mediates the hypernucleosome's compact state.

The discovery of the open chromatin state in Asgard archaea is an exciting variation that represents a novel development in the histone field. Possessing an open chromatin may provide the cell with potential benefits, allowing faster exchange of histones, when required, and easier access of DNA polymerase, RNA polymerase and other proteins to DNA. Asgard archaea encode multiple histones. Whether a correlation exists between the presence of multiple histones and the prevalence of open chromatin conformation remains to be elucidated and will require surveying of further Asgard lineages. More broadly, is the chromatin open state a feature exclusive to Asgard archaea or a property shared with archaea lineages whose chromatin has not been examined? Asgard archaea have been isolated from diverse environments, including marine sediments, hot springs and deep-sea hydrothermal vents. Thus, members of different clades have diverse lifestyles, some being mesophilic and others thermophilic. If other Asgard archaea are found to contain the open chromatin state, it will be interesting to explore how at the macro-length scale the open conformation affects the physical fluidity and flexibility of the chromosome as a polymer within the cell. Moreover, the assortment of histones in different clades of the Asgard superphylum suggests that a functional specialization may have occurred at some point during the evolutionary journey, allowing different histones to assume specific roles. In this light, another future quest will be the investigation of histones that harbour tails to define the contribution of these flexible extensions to the function, regulation and dynamics of the nucleosome in Asgard archaea.

Figure 1. Schematic showing open and closed hypernucleosomes present in archaeal lineages

The top diagram is a simplified representation of the archaea domain (light blue) that shows the archaeal clades discussed in the paper, Asgardarchaea (melon) and Euryarchaea (purple). Within the Asgardarchaea, the Hodarchaea phylum (orange) is shown. The bottom part illustrates open and closed hypernucleosomes, reporting the helical pitch for both states. The green arrow indicates how the Mg^{2+} concentration affects the transition from open to closed conformation.

ACKNOWLEDGMENTS

Work in D.B. laboratory is supported by the UKRI Biotechnology and Biological Sciences Research Council (grant BB/X00645X/1).

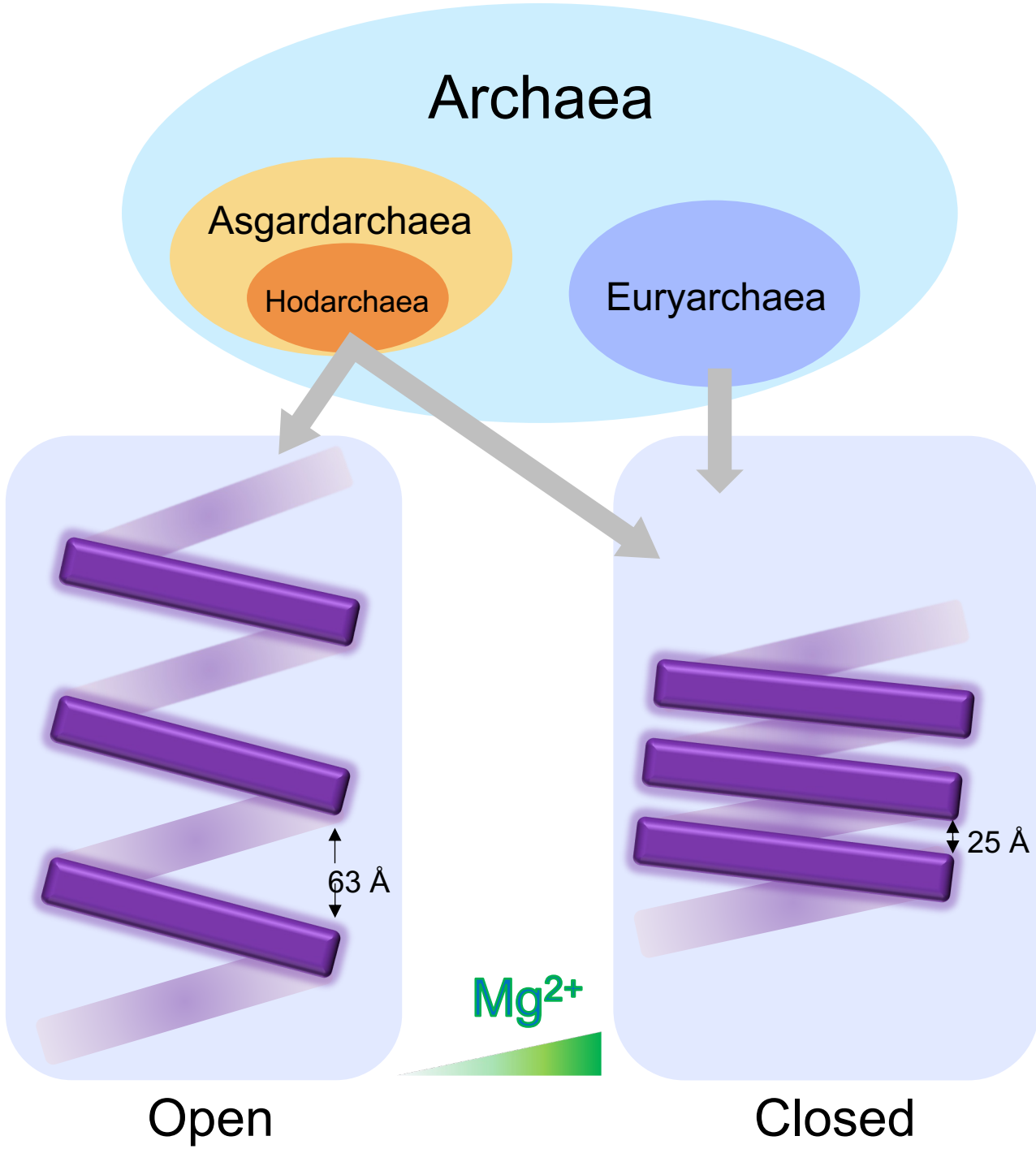
DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Ranawat, H. M., Cajili, M. K., Lopez-Barbosa, N., Quail, T., Dame, R. T., and Dodonova, S. O. (2025) Cryo-EM reveals open and closed Asgard chromatin assemblies. Mol. Cell XXXXX
2. Hidalgo, O., Pellicer, J., Christenhusz, M., Schneider, H., Leitch, A. R., and Leitch, I. J. (2017) Is there an upper limit to genome size? Trends Plant Sci. *22*, 567-573. http://dx.doi.org/10.1016/j.tplants.2017.04.005.
3. Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature *389*, 251-260. https://doi.org/10.1038/38444.
4. Schwab, S., Hu, Y., Alvarez, B. H., Alva, V., and Dame, R. T. (2025) Histone-mediated chromatin organization in prokaryotes and viruses. Trends Biochem. Sci. *50*, 695-706. https://doi.org/10.1016/j.tibs.2025.06.003.
5. Mattiroli, F., Bhattacharyya, S., Dyer, P. N., White, A. E., Sandman, K., Burkhart, B. W., Byrne, K. R., Lee, T., Ahn, N. G., and Santangelo, T. J., Reeve, J. N., and Luger, K. (2017) Structure of histone-based chromatin in Archaea. Science *357*, 609-612. http://doi.org/10.1126/science.aaj1849.
6. Maruyama, H., Harwood, J. C., Moore, K. M., Paszkiewicz, K., Durley, S. C., Fukushima, H., Atomi, H., Takeyasu, K., and Kent, N. A. (2013) An alternative beads-on-a-string chromatin architecture in *Thermococcus kodakarensis.* EMBO Rep. *14*, 711-717. https://doi.org/10.1038/embor.2013.94.
7. Bowerman, S., Wereszcynski, J., and Luger, K. (2021) Archaeal chromatin 'slinkie' are inherently dynamic complexes with deflected DNA wrapping pathways. eLife 10:e65587. https://doi.org/10.7554/eLife.65587.
8. Zaremba-Niedzwiedzka, K., Caceres, E. F., Saw, J. H., Bäckström, D., Juzokaite, L., Vancaester, E., Seitz, K. W., Anantharaman, K., Starnawski, P., Kjeldsen, K. U., et al. (2017) Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature *541*, 353–358. https://doi.org/10.1038/nature21031.
9. Henneman, B., van Emmerik, C., van Ingen, H., and Dame, R. T. (2018) Structure and function of archaeal histones. PLOS Genet. *14*, e1007582. https://doi.org/10.1371/journal.pgen.1007582.
10. Hocher, A., and Warnecke, T. (2024) Nucleosome at the Dawn of Eukaryotes. Genome Biol. Evol. *16*, evae029. https://doi.org/10.1093/gbe/evae029.

Figure


Archaea
Asgardarchaea
Hodarchaea
Euryarchaea
63 Å
25 Å
Mg2+
Open
Closed