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DEPARTMENT OF GENETICS

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Dear Editors:

Please find enclosed our manuscript titled “The chromatin landscape of the euryarchaeon *Haloferax volcanii*”, which we would like to submit for publication as an Article in *Nature Microbiology*.

Many of the defining features of the physical and regulatory organization of eukaryotic genomes trace their roots to the archaeal origins of eukaryotes, chief among them being the nucleosomal packaging of chromatin, which first arose in archaea. However, the chromatin organization of archaeal genomes has only been studied with functional genomic tools to a limited extent. Indeed, some work in this direction was recently published in *Nature Microbiology* (Badel et al 2022), wherein the 1D and 3D chromatin organization of *Sulfolobus* archaea was mapped. While this work begins to shed light on archaeal chromatin organization, unlike the majority of archaea, *Sulfolobus* lacks nucleosomes, and thus it does not provide insight into the ancestral state of nucleosome organization. Therefore, we still cannot relate strategies that archaea use to organize nucleosomal chromatin to the more derived eukaryotic state.

In this work, we map for the first time chromatin accessibility (adapting and optimizing both ATAC-seq and single-molecule methylation-based footprinting methods to the archaeal context) as well as the active transcriptional landscape by mapping single-stranded DNA primarily associated with RNA polymerase binding (using KAS-seq) in *Haloferax volcanii*, an euryarchaeote with nucleosomal chromatin. We find that *H. volcanii* promoters exist in increased accessibility states and all accessibility is concentrated at them, similar to the situation in eukaryotes with small and compact genomes, and that in aggregate, archaeal nucleosomal chromatin is comparably accessible, if not slightly more accessible than that of eukaryotes. However, unlike the typical situation in eukaryotes, transcriptional activity is not associated with corresponding changes in promoter accessibility. These eukaryote-like properties of euryarchaeotes are unlike the characteristics found in non-nucleosomal archaeal species, which lack similarly strong promoter accessibility peaks and exhibit large-scale domains of elevated or diminished accessibility analogous to those seen in bacteria. Instead of observing the expected relatively homogenous occupancy of polymerases across operons, we observe a striking and widespread heterogeneity in polymerase occupancy between individual genes within a given operon. These observations suggest a number of interesting possibilities regarding regulatory mechanisms in *Haloferax*, including the possibility that genes within the “operon” support independent transcriptional initiation, or that polymerase pausing, perhaps coupled with translation, is a major feature of the transcriptional process. Finally, we observe entirely unexpected chromatin properties of CRISPR arrays in *Haloferax* (which happens to be one of the original organisms in which CRISPR arrays were first discovered). For one of the three annotated CRISPR arrays we observe highly abundant ssDNA structure dwarfing other sources of ssDNA (including signal from polymerases), and points to potentially interesting and unexplored mechanisms by which CRISPR arrays function.

Overall, this work represents the first window into the dynamic functional chromatin organization of an archaeal genome that possesses nucleosomes, and we thus believe that our manuscript will be of great interest to readers of *Nature Microbiology*.

We confirm that our manuscript has not been published elsewhere and is not under consideration for publication in other journals.

Sincerely,

A handwritten signature in black ink that reads "William James Greenleaf". The signature is written in a cursive style with a large, sweeping initial 'W'.

Professor
Department of Genetics and, by courtesy, Applied Physics
Stanford University School of Medicine