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Faithful to the Marseille tradition: Unique and intriguing—that's how Marseillevirus packs its DNA

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Not only does Marseillevirus bear the name of the city where it was identified, it also encompasses its values and what makes Marseille a wonderful city. Marseillevirus is unique and intriguing. As such, Bryson et al.[1] in this issue of *Molecular Cell* reveal how virion-associated Marseillevirus DNA is packed with nucleosomes.

Genomic DNA in eukaryotic cells is packaged and accommodated in the nucleus by the formation of chromatin with nucleosomes as the repeating unit. The nucleosome is composed of a histone octamer containing two of each of the histones H2A, H2B, H3, and H4 and approximately 150 base pairs (bp) of DNA wrapped around the histone octamer.[2] Genomic DNA of Marseillevirus,[3] and also Archaea, is packed with nucleosome-like particles composed of histone-like proteins, and the overall structure in the histone-mediated DNA geometry and the principles of histone-DNA interactions are well conserved.[4–6] In this issue of *Molecular Cell*, Bryson et al. reveal that viral DNA in Marseillevirus virions is tightly packed with nucleosomes wrapped around 121 bp of DNA, without linker DNA or phase differences along the gene. The dense packing of nucleosomes, rather than "beads-on-a-string" with genic punctuation, is a novel mode of DNA packaging by histones.

Nucleosomes, connected by linker DNA, form a fibrous beads-on-a-string structure and maintain an open and dynamic chromatin conformation in actively transcribed genomic loci.[7] Interestingly, archaeal nucleosome-like particles assemble in a unique polymeric structure that is continuous and superhelical.[4,8] Similarly, human telomere chromatin fibers are closely stacked columnar nucleosomes, with unusually short nucleosome repeat lengths of approximately 132 bp of DNA wrapped around a histone octamer in a continuous superhelix, that maintain susceptibility to both DNA damage and DNA damage responses.[9] In the Marseillevirus nucleosome, 121 bp of well-ordered DNA wraps around the viral histone doublets composed of Hβ-Hα and Hδ-Hγ, although it was reconstituted with 147 and 207 bp on the Widom 601 artificial positioning sequence.[5,6] As the Marseillevirus doublet was not fully wrapped by DNA, it has been proposed that Marseillevirus nucleosomes are inherently unstable, either to facilitate expression early in infection or for gene regulation.[5] However, the conformation of nucleosomes in Marseillevirus virions was unclear because previous studies used strong positioning sequences for the reconstitution of nucleosomes *in vitro* and crosslinking for cryoelectron microscopy (cryo-EM) analysis.[5,6] It was also shown that histone doublets of Marseillevirus accumulate in viral factories and are essential for the production of infectious viruses in amoeba.[5] However, it remained an enigma whether nucleosome-like structures are formed in the Marseillevirus virion and how the genomic DNA of Marseillevirus is packed within virions. Bryson et al. succeeded in extracting chromatin from Marseillevirus virions and assessed the loading of viral-like histones and the nucleosome structure and architecture of viral DNA. Extraction of virion-associated chromatin was achieved by breaching the capsid in low pH conditions and permeabilizing the lipid membrane, followed by either enzymatic digestion (micrococcal nuclease [MNase]) or chemical digestion (methidiumpropyl-EDTA-Fe(II) [MPE(FeII)]). The former resulted in highly efficient solubilization of viral chromatin. MNase gel analysis using MNase-treated Marseillevirus chromatin shows the DNA ladder corresponding to mono-, di-, and tri-nucleosomes with clearly shorter repeat lengths than that of *Drosophila*. Interestingly, the average size difference between di- and tri-nucleosomes was 120 bp, indicating that the genome in the Marseillevirus virion is tightly packed with nucleosomes without linker DNA. In addition, the protein composition of Marseillevirus chromatin was ascertained by mass spectrometry assays using solubilized viral chromatin. High amounts of Hβ-Hα and Hδ-Hγ peptides were found in viral chromatin, confirming that Hβ-Hα and Hδ-Hγ are loaded onto Marseillevirus DNA. MNase-treated fragments were then analyzed by paired-end DNA sequencing to determine the genomic status of Marseillevirus. Consistent with the gel analysis of MNase-treated fragments, a dominant mono-nucleosome-derived peak from 121 bp and a di-nucleosome-derived peak of less than 250 bp were observed. These results confirm the structure of the Marseillevirus nucleosome previously observed by cryo-EM.[5,6] MNase is an endo/exo-nuclease that preferentially digests AT-rich sequences; in fact, approximately 90% of the sites digested by MNase were between A/T base pairs and AT-rich regions.[1] To avoid artifacts, the authors performed MPE sequencing (MPE-seq) using viral chromatin cleaved with a small chemical compound,

MPE(FeII), which catalyzes H_2O_2-depending DNA cleavage without sequence bias. In contrast to fragments produced by MNase treatment, MPE cleavage of Marseillevirus chromatin produced mono-nucleosomal fragments of approximately the same size (~150 bp) as those of *Drosophila*. The authors concluded that 13 bp at both the entry and exit of the nucleosome are less ordered than the central 121 bp DNA in the Marseillevirus nucleosome reconstituted with 147-bp Widom 601 DNA,[5,6] and that the 13-bp ends are sensitive to MNase digestion but not MPE cleavage. In MPE-seq, the 10-bp periodic cleavage pattern was not observed in Marseillevirus chromatin in contrast to that of *Drosophila*. With respect to this result, the authors considered that Marseillevirus chromatin is inherently highly resistant to intranucleosomal cleavages by both MNase and MPE. Such resistance could not be due to the presence of non-histone proteins as no plausible candidates were detected by mass spectrometry. Interestingly, in MPE-seq, the chromatin-accessibility features found in the genomic viral DNA were not observed, suggesting that the Marseillevirus nucleosome has evolved only for viral genome packaging in the virion.

Bryson et al. propose that densely packed chromatin in Marseillevirus virions has a protective role during infection in the cytoplasm of its host, amoeba. The viral chromatin conformation described here differs from eukaryotic chromatin in which histones pack DNA but also regulate gene expression by controlling the accessibility of regulatory proteins to genomic DNA. Archaeal chromatin is also tightly packed; however, its fibers are flexible and can take on a "closed" or "open" accessibility status, suggesting that Marseillevirus chromatin architecture is different from that of Archaea and more likely resembles the recently published human telomeric chromatin.[4,8,9] During infection, viral chromatin must become accessible in the amoeba cytoplasm for expression of Marseillevirus-encoded genes, and chromatin remodeling must occur for transcription in viral factories. This begs the question of what the mechanisms and factors regulating the transition from a densely packed chromatin to a transcriptionally permissive state required for Marseillevirus replication are. So far, no Marseillevirus-encoded histone chaperones have been identified, and although host factors can localize to viral factories,[10] considering the differences in histone sequences, it remains unclear if host chaperones can interact with the viral nucleosomes. Post-translational modification of viral histones is likely to contribute to the remodeling of Marseillevirus chromatin. Additionally, a less abundant viral histone doublet, Hζ-Hε, when assembled into nucleosomal particles with Hδ-Hγ, showed less protection of DNA from MNAse cleavage relative to Hβ-Hα/Hδ-Hγ. The lower stability of Hζ-Hε/Hδ-Hγ resembles the incorporation of the H2AZ histone variant in labile nucleosomes. Further functional and structural studies are needed to elucidate the dynamics and regulation of the chromatin landscape of Marseillevirus.

Marseillevirus histone doublets are distant homologs of the four eukaryotic core histones that originated prior to the divergence of modern eukaryotes.[11] In the context of the viral karyogenesis hypothesis in which the eukaryotic nucleus evolved from viral infection events,[12] the study of viral chromatin will provide valuable contributions for our understanding of the evolution of eukaryotes and their viruses.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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