STRUCTURE OF CENTROMERE CHROMATIN REVEALED BY SUPER-RESOLUTION MICROSCOPY AND ADVANCED DATA PROCESSING

Leonid Andronov, Khalid Ouararhni, Isabelle Stoll, Jonathan Michalon, Yves Lutz, Igor Orlov, Jean-Luc Vonesch, Ali Hamiche, Bruno Klaholz

Centre for Integrative Biology (CBI); Institute of Genetics, Molecular and Cellular Biology (IGBMC); Centre National de la Recherche Scientifique (CNRS); University of Strasbourg. 1 rue Laurent Fries, 67404 Illkirch Cedex, France.
E-mail: andronov@igbmc.fr

KEY WORDS: chromatin structure, centromeres, super-resolution microscopy, STORM, SMLM, STED, image processing, cluster analysis

The detailed structure of chromatin *in situ* is so far poorly understood. In this study [1], we focus with super-resolution microscopy on the centromere regions of chromatin that are epigenetically defined by CENP-A, an essential histone H3 variant. Using our recently developed pipeline for SMLM data processing (correction of drift and chromatic aberrations, visualization etc.) [2], and robust Voronoi-diagram-based cluster analysis methods in 2D [3] and 3D [4], we discover that CENP-A nucleosomes form characteristic clusters in the centromere regions of chromosomes. Using timepoint analysis, we show that during the early G1 phase of the cell cycle, these clusters exhibit a globular rosette-like structure, which evolves into a more compact cloud-like structure in late G1. The clusters contain numerous CENP-A molecules and form a large supra-molecular structure of ~250–300 nm diameter with similar shapes for different centromeres, both in human and mouse (Fig. 1A). Dual-color super-resolution microscopy and colocalization analysis show that HJURP, the CENP-A chaperone, is located in the center of the rosette and serves as a nucleation point for the CENP-A deposition in early G1 (Fig. 1B-C). The discovery of an HJURP-mediated CENP-A nucleation and its structural description provide important insights into the mechanism of CENP-A deposition and the organization of centromere chromatin.

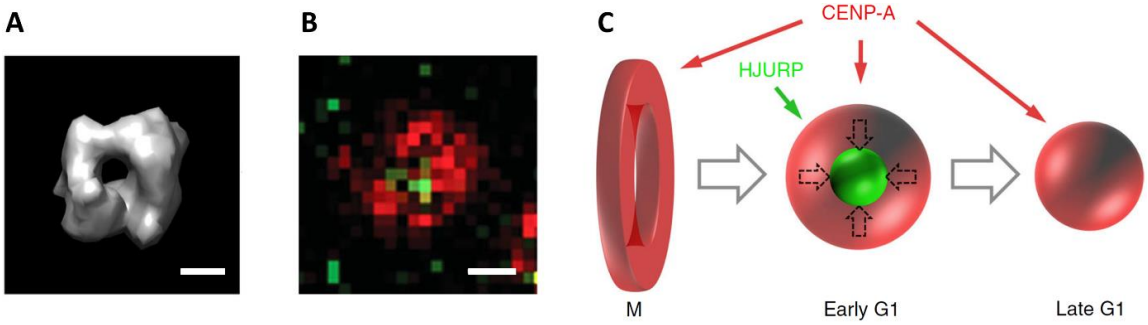

Figure 1: A. Globular rosette-like clustering of CENP-A, obtained with 3D SMLM. **B**. Localization of HJURP (green) in the center of the CENP-A cluster (red) in early G1, revealed by STED microscopy. **C**. Evolution of the shape of CENP-A clusters during the cell cycle. Scale bars, 100 nm.

[1] L. Andronov; K. Ouararhni; I. Stoll; B.P. Klaholz, and A. Hamiche, "CENP-A nucleosome clusters form rosette-like structures around HJURP during G1," *Nat. Commun.* **10**, 4436 (2019).
[2] L. Andronov; Y. Lutz; J.-L. Vonesch, and B.P. Klaholz, *Bioinformatics* **32**, 2239-2241 (2016). https://github.com/andronovl/SharpViSu
[3] L. Andronov, I. Orlov, Y. Lutz, J.-L. Vonesch, B.P. Klaholz, *Sci. Rep.* **6**, 24084 (2016).
[4] L. Andronov; J. Michalon; K. Ouararhni; I. Orlov; A. Hamiche; J.-L. Vonesch, and B.P. Klaholz, *Bioinformatics* **34**, 3004-3012 (2018). https://cbi-dev.igbmc.fr/cbi/voronoi3D