Nucleosome conformation and local order explored by cryo-electron tomography in relationship with chromatin functional compartmentation

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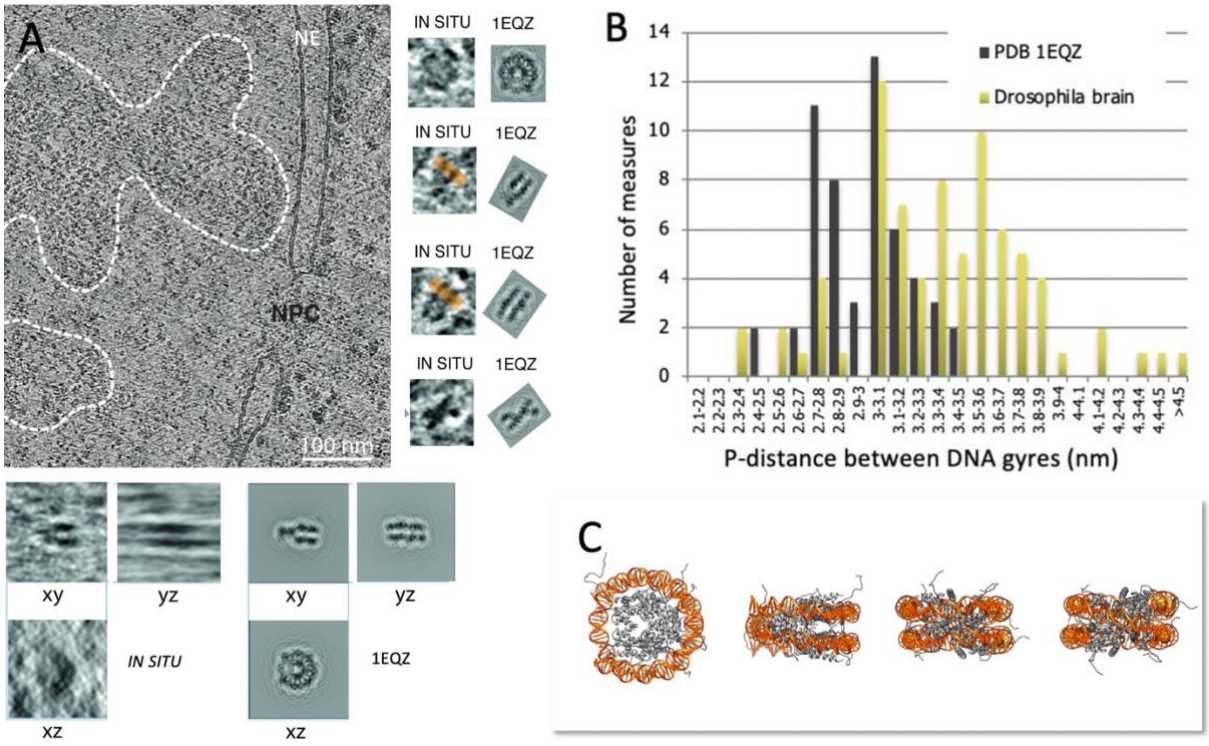
Eukaryotic chromosomes are complex polymorphic and dynamic objects. Centimeters to meters of DNA condense into a micron-scale nucleus though multiple levels of structural organisation, while interacting with a multitude of factors driving functional compartmentalization. DNA is regularly associated with histones to form the so-called « bead-on-string » filament. Its building block, the nucleosome core particle is formed by a segment of 146 bp of DNA wrapped into 1.75 turn of a left-handed superhelix around a histone octamer (two copies of the four histones H2A, H2B, H3, H4). Chromatin is not uniform in space and time. It can be described as a hetero-polymer with structurally and functionally defined domains: the less compact, gene-rich and transcriptionally active euchromatin (EuC), and more compact and little transcribed heterochromatin domains, including constitutive heterochromatin (cHC), gene-poor and enriched in repetitive sequences. Despite spectacular advances in the knowledge of chromosome dynamics and large-scale organisation, at the nucleosome level, structural information *in situ* is dramatically lacking.

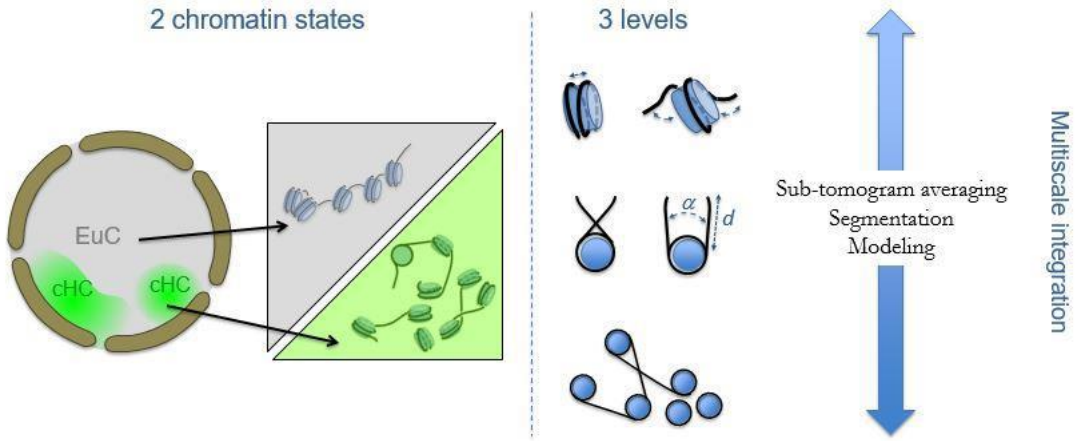
Using cryo electron microscopy (cryo-EM) and tomography (cryo-ET) of vitreous sections, we have recently succeeded, for the first time, in imaging nucleosomes in their native nuclear context at a level of detail sufficient to follow the DNA molecule wrapped around and analyse their conformation by comparison with available crystallographic structures (see Figure, Eltsov et al, 2018).



Cryo-ET of vitreous sections of *Drosophila* embryonic brain interphase nucleus. (A) Virtual section (5 nm) in a tomogram showing the nuclear envelope (NE) and a nuclear pore complex (NPC). Zooming into chromatin areas reveal nucleosomes variously oriented, here in top and side views. The different patterns are analyzed by comparison with images simulated from the crystallographic structure PDBID 1EQZ shown in (C). Perpendicular views of a nucleosome are compared with the corresponding views of the crystallographic structure. The horizontal smearing visible in the YZ view is a missing wedge artefact of tomography. (B) Evidence of open conformations: the distance between DNA gyres of the nucleosome measured on particles observed *in situ* by comparison with the crystallographic structure.

Our objective is now to determine the nucleosome conformation and local chromatin fold inside the interphase cell nucleus, in relationship with the functional state of chromatin.

For this purpose, we use *Drosophila* embryonic brain as a model. This model presents many practical advantages, in particular a distinct segregation of large cHC domains from EuC. We will implement a cryo-correlative light and electron microscopy (cryo-CLEM) workflow to target GFP-lebelled heterochromatin 1 proteins (HP1) in cHC. We will thus explore, in relationship with chromatin functional compartmentalization: i) the nucleosome conformation and its structural variability landscape, ii), the local chromatin fold, and iii) the interplay between nucleosome conformation and chromatin fold.



Multiscale structural analysis of eukaryotic chromatin *in situ* in relationship with chromatin functional state

This experimental M2 internship will be devoted to specimen preparation (vitrification, cryo-sectionning), cryo-EM/ET data acquisition/reconstruction, and test of cryo-CLEM devices, including access to high end cryo-EM facility at IGBMC (Strasbourg).

This project is part of an ANR-funded collaborative project including specialists in image analysis and polymer physics.

References

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