Contribution ID: **75** Type: **Posters**

Structural Analysis of Condensed Metaphase Chromosomes by Synchrotron Small-Angle X-Ray Scattering

Tuesday, 16 June 2015 17:40 (1h 50m)

Each chromosome contains a single DNA molecule that is associated with histone proteins and forms a long filament containing many nucleosomes (the dimensions of the nucleosome core particle are shown in Fig. 1a). During mitosis, this chromatin filament is densely packed into metaphase chromosomes. TEM images of partially denatured chromosomes obtained using different procedures showed that bulk chromatin in chromosomes is organized forming multilayered plate-like structures (see example in Fig. 2b). This planar structure was studied using cryo-EM, electron tomography, AFM imaging in aqueous media, and AFM-based nanotribology and force spectroscopy (1). The results obtained suggested that nucleosomes in the plates are irregularly oriented, and that the successive layers are interdigitated (layer thickness 5-6 nm), presumably allowing face-to-face interactions between nucleosomes of adjacent layers (Fig. 1c). Multilayer plates (identical to those found in metaphase chromosomes) can be self-assembled from chromatin fragments obtained by micrococcal nuclease digestion of metaphase chromosomes (2), and it has been suggested (3-5) that metaphase chromosomes could be self-organizing liquid crystal structures formed by many stacked layers of chromatin oriented perpendicular to the chromosome axis (Fig. 1b). We have used the NCD beamline of ALBA Synchrotron to study the internal structure of native chromosomes. Sediments containing chromosomes from human (HeLa) cells under different conditions were placed in plastic capillaries and were exposed to X-rays for 20-80 s. The typical peaks at ~2.8 and ~3.7 nm corresponding to the internal nucleosome structure were observed in all samples. The peaks at ~11 and ~30 nm corresponding, respectively, to the distances between parallel nucleosome columns and laterally packed 30-nm fibers were absent or showed very low intensities. Under all conditions containing structuring cations, and in particular under metaphase ionic conditions (17 mM Mg, 120 mM K, 20 mM Na), a peak centered at 6 nm is prominent (Fig. 2c). This broad peak can be correlated with the short-range repetition of the ~6 nm distance between nucleosomes (face-to-face interactions) and between stacked layers.

References

1.Daban JR (2011) Electron microscopy and atomic force microscopy studies of chromatin and metaphase chromosome structure. Micron 42:733-750.

2.Milla M, Daban JR (2012) Self-assembly of thin plates from micrococcal nuclease-digested chromatin of metaphase chromosomes. Biophys J 103:567-575.

3.Gállego I, Castro-Hartmann P, Caravaca JM, Caño S, Daban JR (2009) Dense chromatin plates in metaphase chromosomes. Eur Biophys J 38:503-522.

4.Castro-Hartmann P, Milla M, Daban JR (2010) Irregular orientation of nucleosomes in the well-defined chromatin plates of metaphase chromosomes. Biochemistry 49:4043-4050.

5.Daban JR (2014) The energy components of stacked chromatin layers explain the morphology, dimensions and mechanical properties of metaphase chromosomes. J R Soc Interface 11:20131043.

Caption (s) - Add figures as attached files (2 fig. max)

Figure 1. Nucleosome core particle (a). In the thin-plate model (references 3-5) it is considered that the metaphase chromosome is formed by stacked chromatin layers (b) in which nucleosomes are interdigitated

(c). Figure 2. Native metaphase chromosomes (a) and plates emanated from soft-denatured chromosomes (b). Scattering curve of chromosomes in metaphase ionic conditions (c).

Primary author: Dr CROSAS, Eva (ALBA Synchrotron Light Source)

Co-authors: Dr SVENSSON, Agneta (ALBA Synchrotron); Ms CHICANO, Andrea (Universitat Autonoma de Barcelona); Dr KAMMA-LORGER, Christina (ALBA Synchrotron); Prof. DABAN, Joan-Ramon (Universitat Autònoma de Barcelona); MARTINEZ, Juan Carlos (ALBA); MALFOIS, Marc (ALBA-CELLS)

Presenter: Dr CROSAS, Eva (ALBA Synchrotron Light Source)

Session Classification: Special Session - Coffee and poster discussion: ALBA users and AUSE members

Track Classification: VII AUSE Congress