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Combination of chirality and electrostatic effects in the supramolecular organization of nucleosome core particles assemblies

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The nucleosome core particle consists of a protein octamer, with two copies of the four histones (H2A, H2B, H3, H4) and 146bp of DNA wrapped around in a left-handed helix [1]. DNA overcharges the protein core and the resulting net charge is about 150 e^-. This complex forms a flat rigid cylinder, 110Å in diameter and 57Å high from which the flexible and basic terminal histone parts, usually called the « tails », may extend. In the living cell, these particles, connected to each other by linker DNA fragments, are concentrated up to 100-500 mg/ml but their supramolecular ordering remains unknown. A few years ago, we began a systematic analysis of the phases formed by the isolated NCPs under concentrations that are biologically relevant. These dense phases can be prepared experimentally in two different ways : i) in the presence of monovalent salt (3.5 to 160mM NaCl) under controlled osmotic stress [2] or ii) by addition of multivalent cations (Mg^{2+}, spermidine^{3+}, spermine^{4+}) that induce the aggregation of NCP [3-4]. Although this experimental system is extremely simplified and does not reproduce all details of chromatin in the living cell, these phases constitute a library of structures that may possibly exist *in vivo* since they were found under biologically relevant salt and NCP concentration ranges.

We showed how slight changes in the ionic concentration may have tremendous effects on the interactions between particles, and produce large changes in their supramolecular organization. We will focus here on the phases observed under low monovalent salt conditions. By controlling the osmotic pressure applied to the sample, we progressively increased the NCP concentration and followed the evolution from the lamello-columnar phase (Figure 1) to the inverse hexagonal phase. Multiple textures are observed in optical microscopy: simple concentric tubes, complex tubes with budding ends and concentric spherulites. This evolution of the textures reveals a combination of chiral and electrostatic interactions between the NCPs that we investigated mainly by cryoelectron microscopy of thin sections of the vitrified material. We show how and why NCP tilt in the columns and how the columns of stacked NCP, first aligned in parallel when the distance separating the layers is large enough, tilt with respect to each other when the NCP concentration is increased (and the distance between the layers is reduced). Details of this chiral NCP phase is given in Figure 2. Above a critical concentration, this chiral lamello-columnar phase transforms into an inverse hexagonal phase, in which columns of nucleosomes stay aligned in parallel.

References

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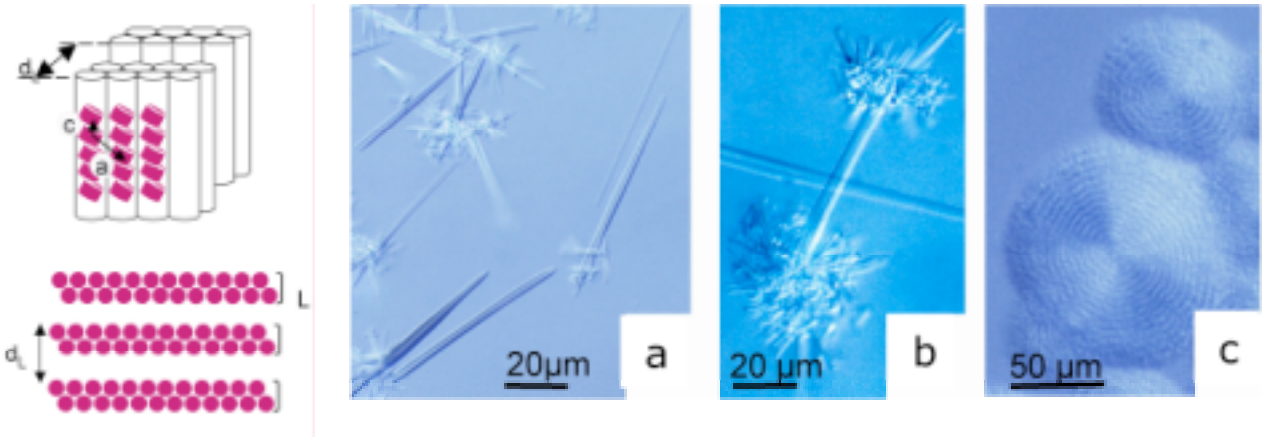

FIG. 1. Schematic drawing of the lamello-columnar phase observed in side view and in top view. The lemallar periodicity d_L corresponds to the thickness of one bilayer of columns (L) plus the layer of solvant. Textures of this phase observed in Nomarski phase contrast: tubes (a,b) and spherulites (c).

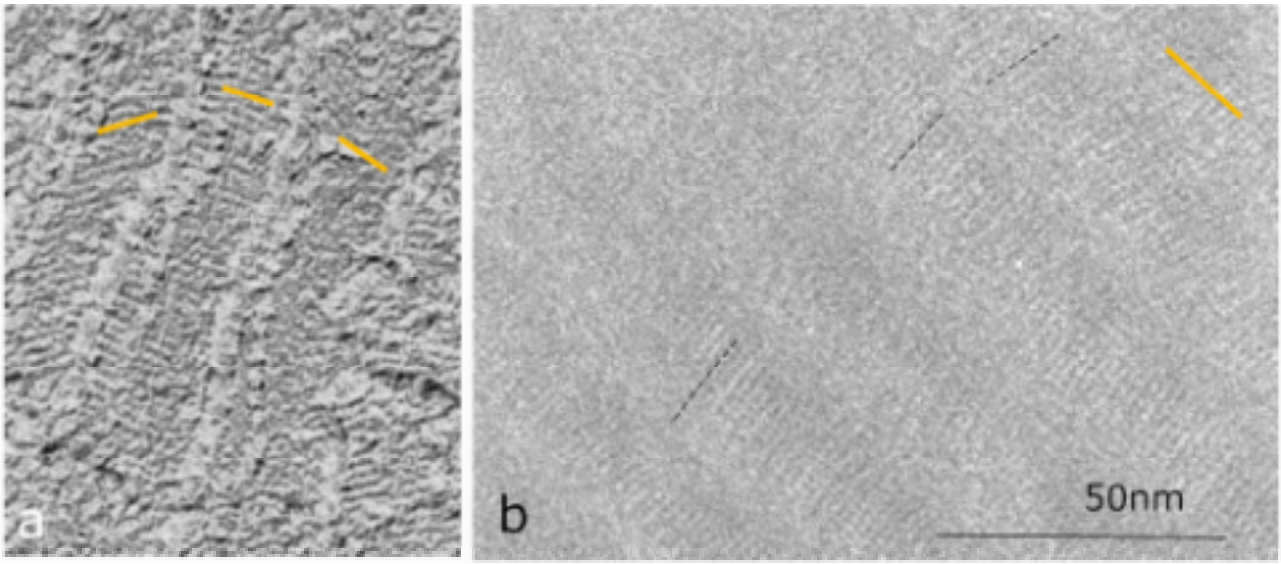

FIG. 2. Chiral lamello-columnar phase of nucleosomes observed in electron microscopy after freeze-fracturing (a) and in cryoTEM of vitreous sections (b). In both cases the observation plane is oblique with respect to the lamellar structure, allowing us to observe several consecutive bilayers. In (a) the fracture plane jumps from one bilayer to the next and the orientation of the colums (continuous yellow line) rotates from plane to plane. In (b), the direction of the columns is more difficult to follow, but the DNA strands of NCP can be seen and their orientation changes from one layer to the next.

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