

Comparison of nucleosome unfolding by yeast and human FACT: electron microscopy analysis

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Motivation and Aim: FACT (facilitates chromatin transcription) is a histone chaperone that participates in nucleosome removal and reassembly during transcription and replication. Previously we have shown that yeast FACT dramatically alters the nucleosome structure without ATP hydrolysis, but the extent of these alterations depends on the presence of HMGB domain-containing protein Nhp6 [1]. It was shown *in vitro* that hFACT drives nucleosome unfolding in presence DNA-intercalators curaxins; presumably curaxins destabilizes nucleosomes and thus facilitates FACT-nucleosome interaction [2]. Nevertheless, the detailed mechanism of this process is still unclear.

Methods and Algorithms: We used mononucleosomes assembled on the 603 Widom positioning sequence. Nucleosomes were assembled by histone octamer transfer to DNA templates after dialysis from 1M NaCl to 0.01M NaCl. SPT16/SSRP1 and SPT16/POB3 were prepared at concentration of 0.05 μ M. Complexes of FACT with the nucleosome were formed in the presence of 0.1 μ M FACT, 0.1 μ M core chicken nucleosomes, 0.5 nM fluorescently labeled core nucleosomes N35/112 and 5 μ M CBL0137 (for human FACT) or 10 μ M Nhp6 protein (for yeast FACT). For transmission electron microscopy (TEM) analysis samples were applied to the carbon-coated glow-discharged copper grid (Ted Pella, USA), subjected to glow-discharge using Emitech K100X device (Emitech Ltd., UK), stained for 30 sec with 1 % uranyl acetate, and air dried. Grids were studied in JEOL 2100 TEM (JEOL) microscope operated at 200 kV at low-dose conditions. Micrographs were captured by the Gatan Ultrascan camera with magnification x25,000, no tilt, with 4.1 Å pixel size using SerialEM software. Single particle images of FACT, complexes of FACT with the nucleosome and complexes of FACT with the nucleosome formed in the presence of CBL0137 were collected from the micrographs using a neural network provided by EMAN2.3 software. Single particles coordinates collected by the neural network were imported in RELION2.1 software; all further 2D-processing, analysis and CTF-correction were performed using RELION2.1 software. Extracted particles were used for iterative 2D-classification followed by the elimination of bad classes. Linear dimensions of the 2D-classes were measured with ImageJ.

Results: Here using TEM we studied human FACT (hFACT) and yeast FACT (yFACT) flexibility alone and in complexes with nucleosomes [3]. All studied complexes are highly flexible and adopt broad ranges of configurations. DNA-binding protein Nhp6 binds to the C-terminal tails of both yFACT subunits and induces formation of more open FACT complexes, thus altering the structures of FACT and the nucleosomes and facilitating nucleosome unfolding. Multiple closed and open conformations were also

demonstrated for nucleosome-free hFACT. The open conformations of FACT become predominant during curaxin-induced nucleosome unfolding involving multiple intermediates. We demonstrated that both yFACT and hFACT flexibility facilitates FACT-dependent nucleosome unfolding that occurs similarly for yFACT and hFACT, resulting in formation of nearly linear, extensively unfolded structure.

Conclusion: The data suggest that the process proceeds through a series of energetically similar intermediate structures, ultimately leading to an extensively unfolded form. We proposed FACT-dependent nucleosome unfolding pathway based on a large number of potential intermediates revealed by electron microscopy.

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