doi:10.1017/S1431927622005736
Microsc. Microanal. 28 (Suppl 1), 2022

FACT Unfolds Nucleosome into a Nearly Linear Protein-DNA Structure: Electron Microscopy Analysis

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Yeast FACT complex is a heterodimer containing Spt16 and Pob3 proteins (Figure 1a). yFACT dramatically alters the structure of a nucleosome without ATP hydrolysis, but the extent of these alterations depends on the concentration HMGB domain-containing protein Nhp6 [1-4].

To examine how yFACT affects the structure of intact nucleosomes, we attached fluorescent dyes to a 147-bp DNA fragment based on the Widom 603 positioning sequence [5] and assembled mononucleosomes using recombinant histones from Xenopus laevis. Cy3 and Cy5 labels were placed at positions 35 and 112 bp from the boundary of the nucleosomal DNA, bringing them close enough in the canonical nucleosome structure to provide efficient Förster resonance energy transfer (FRET) between the dyes [2] (Figure 1b). These nucleosomes were used to study the effects of FACT and Nhp6 on a nucleosome structure with single-particle FRET microscopy [2], in-gel FRET analysis [6] and transmission electron microscopy (TEM).

FACT:Nhp6 induces large-scale uncoiling of nucleosomal DNA, separating the DNA gyres carrying Cy3 and Cy5 dyes and reducing FRET efficiency; this uncoiling is reversible upon removal of FACT [2]. Uncoiling of nucleosomal DNA was detected in native gels as a change from orange (more efficient FRET) to green (less efficient FRET) color in FACT:Nhp6:nucleosome complexes (Figure 1c). Similarly spFRET microscopy of the complexes in solution revealed a typical transition from canonical to uncoiled form of nucleosomes (Figure 1d) [2].

Nhp6:nucleosome and FACT:Nhp6:nucleosome complexes were isolated from native gels, transferred to hydrophilized copper grids as described [7], stained with 1% uranyl acetate, and analyzed by TEM using a JEM-2100 electron microscope (JEOL, Japan) equipped with a 2K × 2K CCD camera Ultrascan 1000XP (Gatan, USA). The microscope was operated at 200 kV, with a magnification of 40,000× (2.5 Å/pixel). Images were acquired with SerialEM software [8] imported to the Eman2 suite [9, 10] and CTF-corrected. Particles of FACT-nucleosome complexes were exported to Relion2.0.5 [11] for 2D classification and analysis.

According to TEM analysis FACT alone produced three types of local electron densities; complexes with nucleosomes were typically characterized by 5-6 types of electron densities (Figure 2). The topologies of the complexes ranged from relatively compact conformations to the most elongated suggesting a stepwise nucleosome unfolding pathway (Figure 2). The longest particles were nearly

linear with a weak central density of ~5 nm in width. Over half of the nucleosome complexes with FACT:Nhp6 displayed an open conformation. The large number of configurations observed with TEM suggests that FACT:Nhp6 produces many structural intermediates of complexes with nucleosomes, not just canonical and unfolded forms.

Based on the obtained data, the stepwise model for nucleosome unfolding by FACT:Nhp6 was proposed (Figure 2). In this model, the negatively charged C-terminal tails of Spt16 and Pob3 initially bind to positively charged regions of the M domains. Nhp6 then binds to these tails, promoting formation of an open structure that exposes the histone-binding sites in both M domains. Other Nhp6 molecules bind to and trap the DNA as it releases from H2A/H2B sites transiently, stabilizing exposure of the binding sites for FACT's C-terminal tails. Once this configuration is populated, it can advance to further DNA uncoiling. Multiple, incremental steps lead to the formation of an extended, nearly linear structure (Figure 2).

In summary, here we used electron microscopy to study FACT:Nhp6:nucleosome complexes. These complexes produced a broad range of structures, revealing a large number of potential intermediates along a proposed unfolding pathway. Based on the obtained data, new model of nucleosome unfolding by FACT complex was proposed (Figure 2) [12].

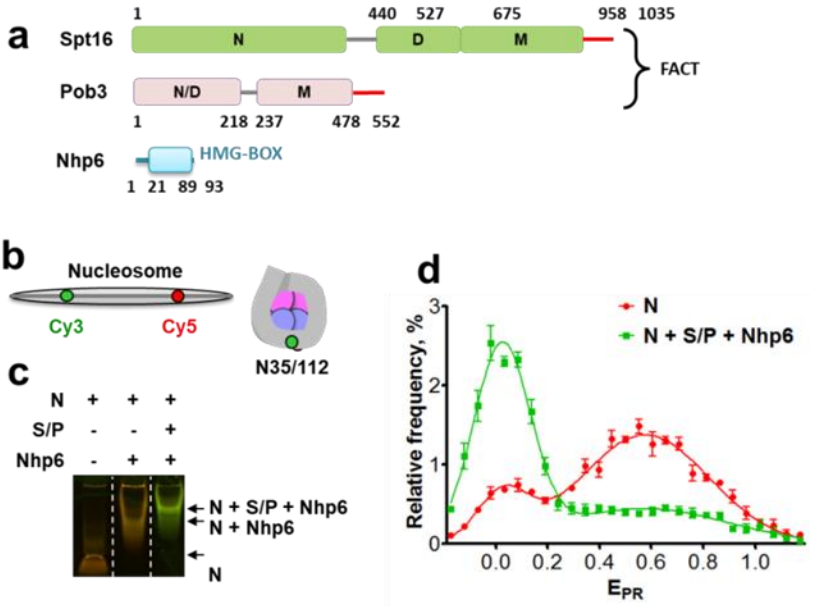

Figure 1. a) FACT and Nhp6 domain structures. FACT is a dimer of Spt16 and Pob3 subunits and requires Nhp6 protein for nucleosome unfolding. b) Scheme of the fluorescently labeled nucleosome (Cy3 at 35 bp and Cy5 at 112 bp from the nucleosome boundary). c) Characterization of FACT:Nhp6 complexes by in-gel FRET. d) Typical frequency distributions of nucleosomes by the FRET efficiency (Epr) in the absence (red) or in the presence of Nhp6 and FACT (green). Figure was adapted from [7].

https://doi.org/10.1017/S1431927622005736 Published online by Cambridge University Press

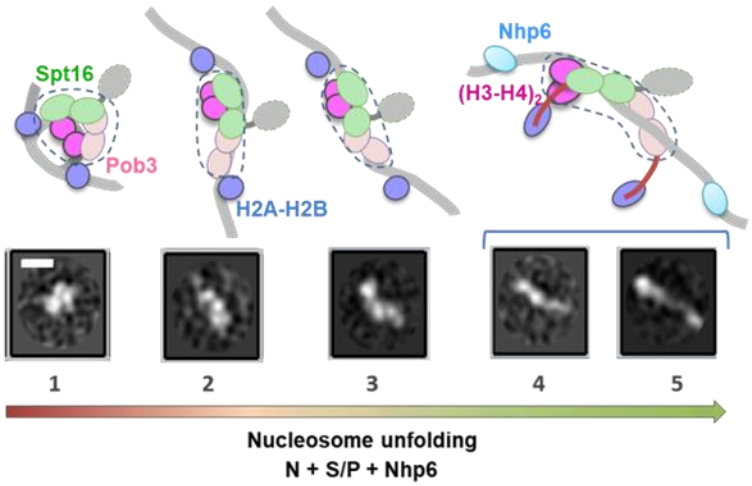

Figure 2. Representative 2D class averages of FACT:Nhp6:nucleosome complexes with different distances between edges of the complex are arranged to show the proposed sequence of events during nucleosome unfolding by FACT:Nhp6. Scale bar: 10 nm. Figure was adapted from [7].

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[12] This work was supported by the Russian Science Foundation (#19-74-30003). Electron microscopy was performed on the Unique equipment setup “3D-EMC” of Moscow State University, Department of Biology. SA, KM, and VM have equal authorship.