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Identification of the NTD in hFACT Complex by Electron Microscopy

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The multifunctional histone chaperone FACT ("FAcilitates Chromatin Transcription") is involved in the disassembly and reassembly of nucleosomes during transcription, replication, and DNA repair. [1]. It has a rather conservative structure, which in human FACT (hFACT) is represented by a heterodimer of SPT16/SSRP1 subunits, which is able to moderately stabilize nucleosomes. [2]. Human FACT (hFACT) is overexpressed in various types of cancer, making hFACT a promising target for anticancer drugs [3-5]. Particularly curaxin CBL0137 cause FACT redistribution and trapping in chromatin of cancer cells [6], leading to a large scale nucleosome unfolding that modulates the accessibility of nucleosomal DNA.

The structure of hFACT in complex with intact and partially assembled subnucleosomes was studied using cryo-EM [7, 8]. Within these structures hFACT SSRP1-NTD/DD-SPT16-DD, SSRP1-MD and SPT16-MD domains were identified. However, neither SPT16-NTD nor structural features of the chaperone FACT alone have been studied in detail yet.

In present work in order to localize hFACT SPT16-NTD domain we studied human FACT in the absence of nucleosomes using single particle electron microscopy with negative staining. We analyzed hSPT16/SSRP1 wild type and hSPT16-DNTD/SSRP1-munatnt using JEOL 2100 TEM operated at 200 kV at low-dose conditions. Micrographs were captured by the Gatan Ultrascan camera with 25k magnification, with 4.1 Å pixel size. EM images pre-processing and single particles collection were performed in EMAN2.3, followed by 2D-particles analysis and further 3D-recunstruction in RELION3.0.

Previously we have shown that nucleosome-free hFACT is a dynamic structure taking "closed" and "open" states [9]. Based on 2D-classes comparison we see complete hFACT in compact "closed" conformation that is characterized by four domains (Figure 1A), while SPT16ΔNTD truncated mutant in the compact state lacks one of the four densities (Figure 1B). SPT16-NTD has molecular weight of ~50 kDa, which is the biggest one comparing to the others domains (SSRP1-NTD/DD-SPT16-DD, SSRP1-MD and SPT16-MD), which have comparable molecular weights of 42, 28 and 33 kDa, respectively. This fourth electron density is 5-6 nm in diameter on 2D projections (Figure 1A) and matches nicely with crystal structure of SPT16-NTD in terms of shape (Figure 1C).

To further evaluate localization of the SPT16-NTD domain, we built and aligned low-resolution 3D-maps of full-length hFACT in closed conformation (Figure 2, grey mesh) with hFACT- SPT16ΔNTD mutant (Figure 2, pink surface) in UCSF Chimera. An extra-density on the front-side of the wild-type hFACT 3D-map was identified (Figure 2, grey arrows). Rigid fitting of crystal structure of NTD-SPT16 (PDB ID 5E5B, Figure 2 yellow atomic structure) into the density resulted in correlation coefficient 0.91, suggesting good fitting results that testify in favor of the domain localization hypothesis at a given location.

In summary, here we identified and localized SPT16-NTD domain of human FACT for first time. This localization is in a good agreement with previously proposed one based on confirmation flexibility of hFACT [9]. During the act of the conformational change the flexible SPT16 N-terminal domain (NTD) moves away from the other subunits and therefore is not resolved in more open conformational states of hFACT, while less mobile DDs and MDs maintain more compact structure. The structural flexibility of hFACT is likely important for its interaction with the nucleosome [10].

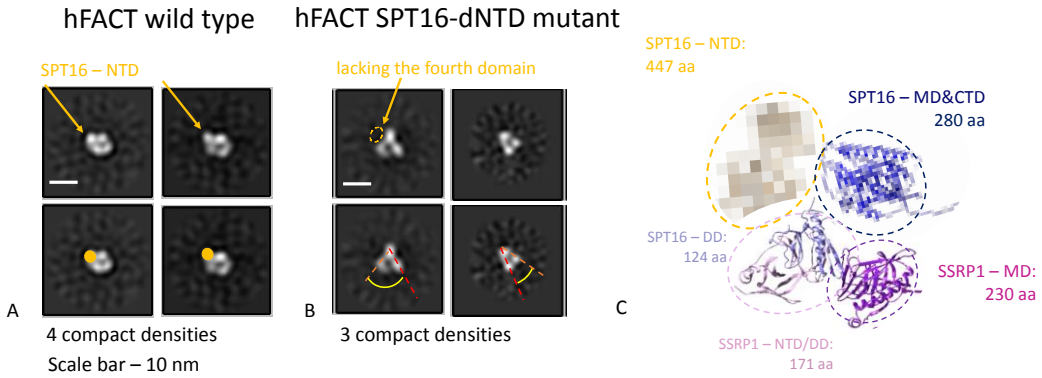

Figure 1. A -2D class-averages of hFACT wild type, B - 2D class-averages hFACT-SPT16ΔNTD truncated mutant. Yellow arrows point to position of SPT16-NTD in wild type FACT, and corresponding empty space in truncated FACT. Scale bar – 10 nm. C - Schematic model of hFACT domains mapping in compact state. SPT16 and SSRP1 DDs and MDs domains localization is based on cryo-EM data from [8], SPT16 NTD mapping is described in text.

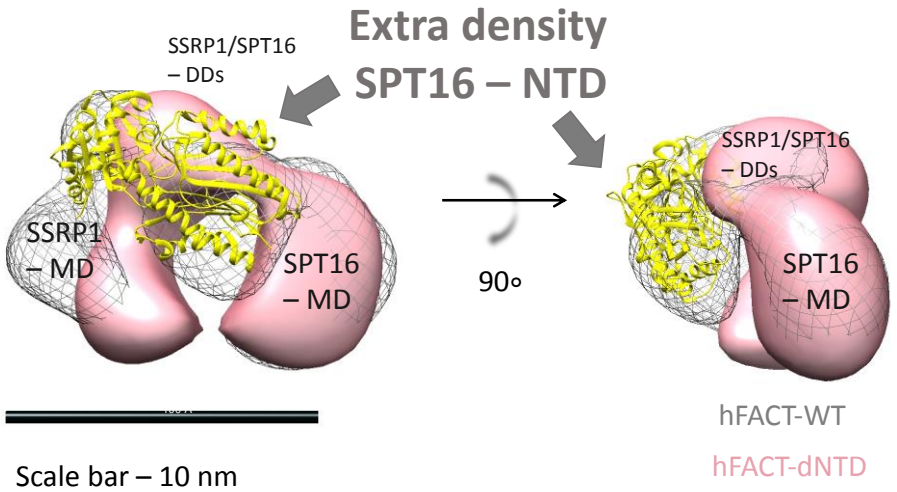

Figure 2. Alignment of 3D maps of hFACT (grey mesh) and hFACT-SPT16ΔNTD mutant (pink surface) in the closed state. Area of differential density was identified and docked with SPT16-NTD (yellow atomic model).

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