Structural dynamics of human histone chaperone FACT

P-01.1-35

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Multifunctional histone chaperon FACT is involved in nucleosome dis- and reassembly during transcription, replication and repair of DNA, and has a relatively conserved structure. Human FACT (hFACT) is a heterodimer of SPT16 and SSRP1 subunits that moderately stabilizes nucleosomes. Here we studied human FACT structure using single particle electron microscopy after negative staining. We evaluated a set of conformational states and proposed a hypothesis describing the structural dynamics of human FACT. It has been shown that nucleosome-free hFACT is a dynamic structure: several states reflect its conformational flexibility. The“closed” complex is characterized by four compact domains; “intermediate” state represented by three domains having compact structure and more disordered fourth domain, and the “open” complex, represented by three domains forming almost linear structure. Based on results a mechanism of conformational flexibility of human FACT has been proposed. It has been shown that hFACT domains are connected to each other through flexible linkers and SPT16 and SSRP1 dimerization domains (DDs) form the “joint”-like connection between the two subunits. In the “closed” conformation the DNA-binding surface of FACT is covered by its two C-terminal and middle domains (MDs). We propose that during conversion to the “open” complexes SPT16 N-terminal domain (NTD) is moving away from the other subunits leading to formation of the first intermediate state with the NTD domain poorly resolved or not resolved, while less mobile DDs and MDs maintain more compact structure and the DNA-binding site is still protected by the CTDs. In the “open” state SPT16/SSRP1 visible MDs and DDs form almost linear structure, unmasking the DNA-binding sites and making them accessible for the interaction with a nucleosome.
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.

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