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Two-domain HMGB proteins HMGB1 and HMO1 have different effects on the structure of nucleosomes and chromatosomes

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Motivation and Aim: HMGB (high mobility group B) is an abundant family of architectural non-histone proteins characterized by the presence of DNA-binding domain called HMG-box, which also has DNA bending activity. In cells these proteins are actively involved in DNA repair, transcription, replication and recombination. Homologous proteins HMO1 (yeast) and HMGB1 (mammalian) are members of this family. Both proteins contain two DNA binding boxes A and B, as well as a disordered C-terminal region. It is assumed that, despite their structural similarity, the roles of these proteins in the organization of chromatin are different: HMO1 stabilizes chromatin by acting as a linker histone [1]; HMGB1 promotes destabilization of the nucleosome structure and thereby facilitates the binding of chromatin remodelers and transcription factors to the nucleosomal DNA [2]. However, these hypotheses about the effect of HMO1 and HMGB1 on the structure of nucleosomes and chromatosomes need to be experimentally evaluated.

Methods and Algorithms: Mononucleosomes were assembled on fluorescently labeled DNA containing the 603 Widom nucleosome positioning sequence. Donor (Cy3) and acceptor (Cy5) fluorophores were situated at neighboring gyres of nucleosomal DNA or at linkers. Chromatosomes were formed by incubating nucleosomes with linker histone H1.0. Formation of the complex between HMGB protein (HMO1/HMGB1) and nucleosomes was studied by the electrophoretic mobility shift assay. Structural changes in nucleosomes and chromatosomes were investigated using single particle Förster resonance energy transfer (spFRET) microscopy [3].

Results: It is found that several HMO1 molecules bind to a nucleosome, affect conformation of nucleosomal DNA and increase a distance between the helices of linker DNA [4]. It was revealed that HMO1 counteracts structural changes in the region of linker DNA caused by the linker histone H1.0 during the chromatosome formation. At the same time, H1.0 and HMO1 appear to bind to independent sites in the nucleosome, so H1.0 remains bound in the presence of bound HMO1 [4].

HMGB1 is found to bind to the nucleosome in a 1:1 stoichiometry, and the mode of binding is independent of the presence or absence of linker DNA. HMGB1 binding does not affect the conformation of nucleosomal DNA, but decreases the distance between the linker DNA helices. HMGB1 partially destabilizes chromatosomes by releasing linker DNA from the complex with H1.0 histone.

Conclusion: HMO1 and HMGB1 differ significantly in their effect on the structure of nucleosomes and chromatosomes that can be a reason of their differences in the functional activity *in vivo*.

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