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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE AND FUNCTIONS OF THE TRANSCRIPTION AND TRANSLATION APPARATUS OF THE CELL

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Abstract P-24: Microscopic Analyses of Liquid-Liquid Phase Separation Induced by Linker Histone H1.0

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Background: Liquid-liquid phase separation (LLPS) that leads to the formation of temporary functional domains in cells plays an important role in the processes of chromatin condensation and gene regulation. Earlier, it was demonstrated that histone H1.4 can form LLPS droplets with DNA. In the present work, LLPS was studied for histone H1.0, which is mainly expressed in differentiated and non-dividing cells. H1.0 is involved in cancer development: its amount decreases with the progression of tumor cells to malignancy.
Methods: LSM710 confocal microscope (Zeiss) equipped with the 40x/1.2W objective was used to image mixtures of H1.0 with Cy3/Cy5 labeled DNA or nucleosomes in fluorescent and transmitted-light channels at the excitation of 514 nm. The formation of condensates as a result of LLPS was confirmed by salt-jump and FRAP/FLIP experiments.
Results: Condensates were not observed when the ratio of negative to positive charges (N/P) in the samples was >1. At N/P~0.7, optically homogeneous droplet-like condensates were found. The appearance of condensates, their size and shape depended on concentrations of H1.0 and DNA. LLPS condensates but not aggregates disappeared by salt-jump to 650 mM NaCl. FRAP/FLIP experiments revealed a moderate rate of fluorescence recovery ($\tau_{1/2}$~22s) indicating moderate DNA mobility of the H1.0-mediated condensates. The appearance of condensates was also observed in the mixtures of H1.0, DNA and

Cy3/Cy5-labeled nucleosomes. Nucleosomes were involved in the condensate formation and found to be 2-fold more mobile ($\tau_{1/2}$~10 s) than DNA.

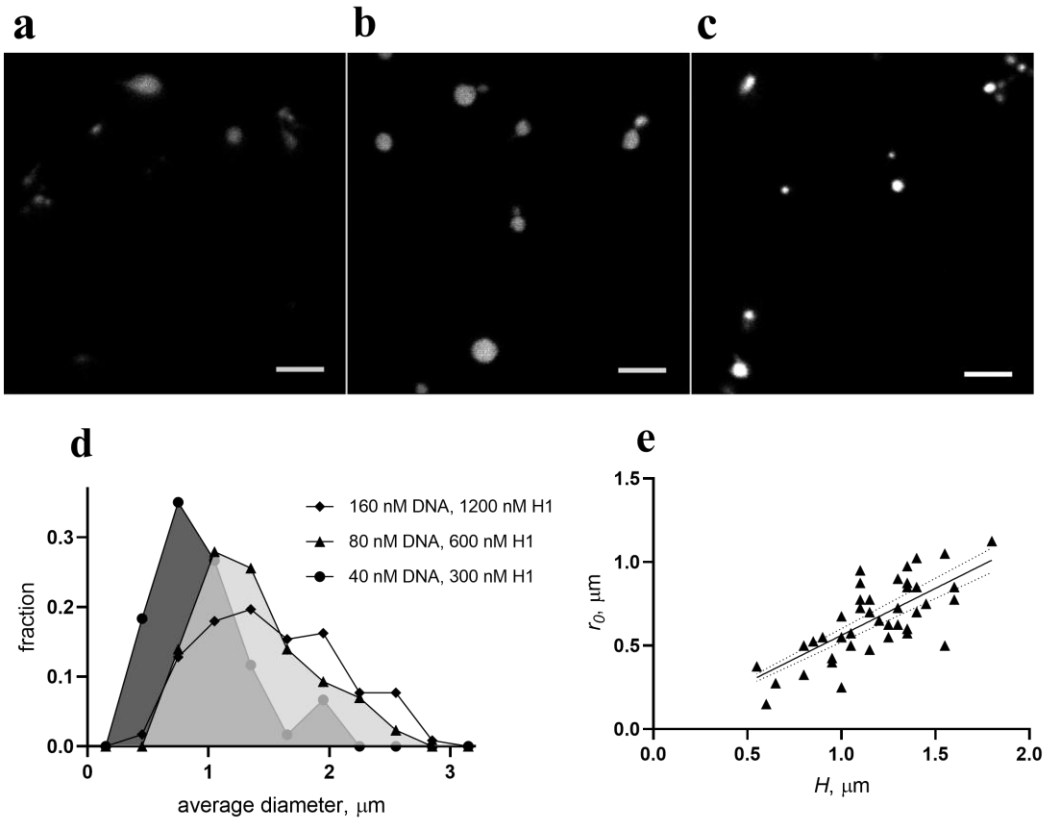

Figure. *Liquid-liquid phase separation of Cy3/Cy5-labelled DNA and nucleosomes induced by H1.0. Fluorescent images of condensates formed upon mixing: (a) 300 μM H1.0 and 40 nM DNA, (b) 1.2 μM H1 and 160 nM DNA, (c) 10 nM nucleosomes, 70 nM DNA, 600 nM H1.0. Bar size is 5 μm. (d) Distribution of condensates in specimens by size. (e) Graph of the relationship between the height (H) and the radius of a base (r0) of an immobilized condensate for a sample of condensates in specimen (b). Straight line - linear regression of data.*

Conclusion: LLPS-related properties of H1.0 were studied for DNA and nucleosomes *in vitro*. Comparison with H1.4 shows that H1.0 forms liquid condensates of approximately the same size. Our result also may indicate that chromatin retains pronounced dynamic properties in H1.0-induced droplets despite the fact that H1.0 induces the formation of more compact chromatin.

Key Words: LLPS • DNA • nucleosome • H1.0

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