Results All experiments were performed with HCT-116-RAD21-mAID-mClover cells, where degradation of cohesin is achieved by auxin-induced proteolysis of its subunit RAD21 6. In live cell studies we demonstrated the appearance of mitotic cells up to ~16h after cohesin degradation with a strongly prolongated mitotic phase and final transition into one multilobulated nucleus. Typical RD patterns, achieved by replication-scratch labeling, persisted after cohesin degradation at least up to 46h and were transmitted over mitosis irrespective of highly abnormal nuclear morphologies. No significant difference of RD patterns and size of individual RDs was noted at the resolution level of 3D-SIM between control cells and cells, which were replication-labeled before cohesin degradation. By contrast, when replication-labeling was performed after cohesin degradation, we noted a reduced number and coarsening of RD structures. Conclusions Our microscopic observations provided information on the effects of a cohesin degradation in individual cells, which were not recognized in Hi-C studies of entire cultures. These findings exemplify the importance to combine Hi-C with advanced 3D and 4D microscopy. Single cell Hi-C studies of individual interphase and mitotic cells identified by microscopy could help to further study connections between the level of chromatin loops and higher order chromatin arrangements in interphase and mitosis following cohesin depletion.

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Workshop I: Nuclear structure – chromatin II

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Determinants of nucleosome stability

Laszlo Imre, Peter Nanasi, Rosevalentine Bosire, Erfaneh Firouzi Niaki and Gabor Szabo

Dept. Biophysics and Cell Biology, University of Debrecen, Hungary
szabog@med.unideb.hu

Nucleosome structure is, in general, repressive; hence, stability of nucleosomes is of regulatory importance in eukaryotes. Recently, we have developed a quantitative, laser scanning cytometric approach (1) to characterize nucleosome stability in situ, in a cell cycle-, post-translational modification-, and histone variant-specific manner. This approach has allowed us to assess how the above actors, as well as superhelical density, the presence of certain reader proteins and other molecular associations regulate this central player of transcriptional regulation. Through the spectacles of nucleosome stability, a simple link can be established between the superimposed levels of chromatin

organization. Our recent findings on site-specific strand-discontinuities in the genomic DNA of eukaryotes (2) also fit such a scenario.

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(1) Imre at al., Sci Rep. 7(1):12734, 2017 (2) Hegedus et al., Nucleic Acids Res. 46(20):10649-10668, 2018

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The nucleosome: from structure to function through physics

Andrew Fenley, Ramu Anandakrishnan, Yared Kidane, David Adamas, Alexey V. Onufriev.

Virginia Tech, USA.
alexey@cs.vt.edu

Charge-altering PTMs in the globular histone core—including acetylation, phosphorylation, crotonylation, propionylation, butyrylation, formylation, and citrullination—can alter the strong electrostatic interactions between the oppositely charged nucleosomal DNA and the histone proteins and thus modulate accessibility of the nucleosomal DNA, affecting processes that depend on access to the genetic information, such as transcription. However, direct experimental investigation of the effects of these PTMs is very difficult. The goal is to make a step towards a unified theoretical model that connects post-translational modifications in the nucleosome with transcription in-vivo. Methods: Theory, Atomistic Simulations. Results: A physics-based framework is proposed that predicts the effect of charge-altering PTMs in the histone core, for most types of lysine charge-neutralizing PTMs including acetylation, and for phosphorylation. The predicted effect of charge-altering PTMs on DNA accessibility can vary dramatically, from virtually none to a strong, region-dependent increase in accessibility of the nucleosomal DNA; in some cases, e.g., H4K44, H2AK75, and H2BK57, the effect is significantly stronger than that of the extensively studied acetylation sites such H3K56, H3K115 or H3K122. Proximity to the DNA is suggestive of the strength of the PTM effect, but there are many exceptions. For the vast majority of charge-altering PTMs, the predicted increase in the DNA accessibility should be large enough to result in a measurable modulation of transcription. However, a few possible PTMs, such as acetylation of H4K77, counterintuitively decrease the DNA accessibility, suggestive of the repressed chromatin. A structural explanation for the phenomenon is provided. Conclusions: Charge-altering post-translational modifications in the relatively unexplored globular histone core may provide a precision mechanism for controlling accessibility to the nucleosomal DNA.