2455-Pos
Atomic Force Microscope Imaging of Chromatin Assembled in *Xenopus* Laevis Egg Extract
Hongxia Fu[1], Benjamin Freedman[2], Chwee Teck Lim[3], Rebecca Heald[2], Jie Yan[1].
[1]Research Centre of Excellence in Mechanobiology; Department of Physics, National University of Singapore, Singapore, Singapore, [2]Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA, [3]Research Centre of Excellence in Mechanobiology; Division of Bioengineering, Department of Mechanical Engineering, National University of Singapore, Singapore, Singapore.
Chromatin assembly in cellular extracts provides a very promising way to mimic chromatin assembly in vivo. In recent single-molecule manipulation experiments, DNA folding in *Xenopus* Laevis Egg Extract was found stepwise and had a characteristic step size around 50 nm, which is in consistent with the expectation that the folding is dominated by formation of nucleosomes. However, imaging of chromatins assembled in extracts has not been systemically investigated; likely due to the difficulty in getting clean imaging resulted from the complex components of the extracts. In this research, we developed a method that enabled us to image the chromatins assembled in the extracts using atomic force microscope. Based on this method, we observed "bead-on-a-string" structures. We studied the effects of ionic concentration and the effects of dilution of extracts on the overall conformations of these structures. We also investigated the hierarchical structures of high-order chromatin. The chromatin shows several levels of folding structures with the typical widths of 15 nm, 25 nm, 50 nm, 100 nm and above. Due to the capability of controlling the conditions of chromatin assembly, we believe this method has wide potential applications in studies chromatins assembled in the extracts.

2456-Pos
Unwrapping of Nucleosomes Detected by Time-Lapse AFM
Luda S. Shlyakhtenko, Alexander Lushnikov, **Yuri L. Lyubchenko**.
University of Nebraska Medical Center, Omaha, NE, USA.
The dynamics of chromatin provide the access to DNA within nucleosomes and therefore, this process is critically involved into the regulation of chromatin function. The questions, such as the range of opening of the nucleosome, and the mechanism whereby the opening occurs and propagates, remain unknown. Here we applied single molecule time lapse AFM imaging to directly visualize the dynamics of nucleosomes and identify the mechanism of the large range DNA exposure. With this technique, we are able to observe the process of unwrapping of nucleosomes. The unwrapping of nucleosomes proceeds from the ends of the particles, allowing for the unwrapping of DNA regions as large as dozens of base pairs. This process may lead to a complete unfolding of nucleosomes and dissociation of the histone core from the complex. The unwrapping occurs in the absence of proteins involved in the chromatin remodeling that require ATP hydrolysis for their function. This suggests that the inherent dynamics of nucleosomes can contribute to the chromatin unwrapping process. There is an electrostatic interaction of DNA with positively charged histone core and the AFM substrate; therefore a balance between these interactions is a driving force for unwrapping. Transiently unwrapped DNA segments can be trapped by electrostatic interactions with the surface increasing the probability for the next unwrapping step. We speculate that interaction of chromatin with surfaces within the cell including the surfaces of remodeling proteins involved into the interaction with chromatin can contribute to the chromatin dynamics facilitating unwrapping of the chromatin. Therefore, APS-mica can play a role of a model system for elucidating of the role of electrostatic interactions of chromatin with intracellular surfaces in regulation of the chromatin dynamics and genes activity.
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2457-Pos
RSC is an Efficient Nucleosome Randomizer: An AFM Quantitative Study on Oligo-Nucleosomal Templates
Fabien Montel[1], Martin Castelnovo[1], Hervé Menoni[1], Dimitar Angelov[1], Stefan Dimitrov[2], **Cendrine Faivre-Moskalenko**[1].
[1]Ecole Normale Superieure de Lyon, Lyon, France, [2]Institut Albert Bonniot, Grenoble, France.
The fundamental unit of chromatin, the nucleosome, constitutes a barrier for several processes including transcription, repair and replication. One of the main tools that the cell uses to overcome this barrier is the recruitment of chromatin remodeling factors. The yeast RSC remodeling complex, a sophisticated nanomachine belonging to the SWI/SNF family, is able both to alter the histone-DNA interactions and to relocate nucleosomes. How RSC or the other complexes from the SWI/SNF family act on polynucleosomal templates is not fully understood and few quantitative data are available. Nevertheless, such quantification is required for both the understanding of the mechanism of action of RSC and the key role that it plays in the numerous vital processes for the cell.
In order to gap this lack of quantitative information, we have used Atomic Force Microscopy (AFM) to visualize directly and at the single molecule level, the result of RSC action on oligo-nucleosomes. In parallel, we developed numerical simulations of the RSC sliding action, which quantitatively reproduce our experimental data. We demonstrate that RSC acts as an isotropic, processive and sequence-independent nucleosome randomizer. This multidisciplinary approach is very suitable to extend to other remodelers exhibiting different modes of action.

2458-Pos
ATP Dependent Nucleosome Remodelling - Mechanistic Insights from Single Molecule Experiments
Jens Michaelis[1], Wolfgang Kügel[1], Barbara Treutlein[1], Josef Exler[2], Gernot ängst[2].
[1]Ludwigs Maximilians University, Munich, Germany, [2]University of Regensburg, Regensburg, Germany.
DNA in eukaryotes is highly compacted. However, it is essential that proteins can have controlled access to the DNA during a number of fundamental cellular processes such as replication, transcription or repair. A large family of protein complexes commonly referred to as Swi2/Snf2 complexes allows for the transient remodelling of the essential compaction unit, the nucleosome and thus assures the access to, or repression of certain segments of DNA. In spite of a large number of research efforts using bio-chemical, structural and theoretical approaches the molecular mechanism of the ATP dependent nucleosome remodelling is currently not well understood. We therefore performed single molecule FRET experiments aimed to unravel details of the remodelling kinetics and pathway.
We present remodelling data obtained by the remodelling complex ACF from drosophila on mono-nucleosomal constructs that use the 601- localisation sequence in combination with a biochemically well characterised linker DNA. The remodelling data was obtained, both, on nucleosomes immobilised onto surfaces of micro-fluidic chambers as well as from solution measurements using pulsed interleaved excitation and multi-parameter fluorescence detection.

2459-Pos
Role of DNA Elasticity and Nucleosome Geometry in Hierarchical Packaging of Chromatin
Elena F. Koslover, Colin Fuller, Aaron F. Straight, Andrew J. Spakowitz.
Stanford University, Stanford, CA, USA.
Eukaryotic cells face the daunting task of packaging meters of DNA into micrometer-sized nuclei while retaining the accessibility of the genome to transcriptional machinery. We use a combination of analytical theory and simulations to address the question of how DNA elasticity determines the structure and dynamics of chromatin fibers. Treating the DNA as a worm-like chain, we study the size and fluctuations of extended nucleosome arrays in solution. In addition, we investigate the role of local nucleosomal geometry in dictating global chromatin structure. Specifically, we map out the elastically preferred structures of compact chromatin fibers with different linker lengths and consider the effect of nucleosomal modifications on these structures. We find that the altered nucleosome geometry arising from introduction of histone variants significantly affects the energetics of linker DNA in different compact structures. Our model provides a set of verifiable predictions that allow for direct connections with experimental data. The results highlight the key role played by DNA elasticity and local geometry in tight hierarchical packaging of the genome into chromatin.

2460-Pos
Quantification of Nucleosome Stacking in Single 30 Nm Chromatin Fibers
Fan-Tso Chien[1], Maarten Kruithof[1], Andrew Routh[2], Daniela Rhodes[2], John van Noort[1].
[1]Leiden Institute of Physics, Leiden, Netherlands, [2]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.
DNA and core histones form dense 30 nm chromatin fibers in vitro. The order of nucleosome stacking drives the folding of 30 nm fibers and may control enzymatic accessibility of the linker DNA in vivo. The energy and dynamics of nucleosome stacking are not well quantified. Here, we investigated nucleosome stacking by pulling on reconstituted chromatin fibers with magnetic tweezers. The force extension traces of fibers are well described as transition between a Hookean spring representing the 30 nm fiber and a worm like chain representing a bead-on-a string conformation. The results show that nucleosome