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.
The work is supported by grants EPS-0701892, PHY-0615590 (both NSF) and GM062235 (NIH).

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

stacking is reversible and force dependent. We measure a stacking energy of $17k_BT$ and an unstacking distance that indicates the full exposure of the linker DNA. The time traces at constant force between 3.5 pN and 6.0 pN show that multiple nucleosome unstacking and restacking events take place simultaneously and non-cooperatively. The salt dependence of unstacking suggests competition between monovalent ions and divalent ions. These experiments provide the first single molecule data on nucleosome stacking and define a dynamic framework for chromatin organization in higher order structures.

2461-Pos

DNA Methylation Induces a More Compact and Rigid Nucleosome Structure

Sijie Wei, JuYeon Lee, Tae-Hee Lee.
The Pennsylvania State University, University Park, PA, USA.

Cytosine methylation in CpG dinucleotides is an important epigenetic modification in eukaryotes with roles in regulating a variety of genome transactions. There have been many studies of DNA methyltransferases and methyl-CpG binding proteins elucidating their roles in various genome activities. However, less is known about how methylated CpGs directly affect nucleosome structure. We implemented a single molecule FRET coupled with anisotropy that can simultaneously measure dynamic distance changes and flexibility of the two ends of a nucleosomal DNA. Using the method, we monitored effects of DNA methylation on the structure of mononucleosomes. In the absence of methylation, most nucleosomes displayed two low FRET states (FRET efficiency <0.5). When treated with a CpG methyltansferase, we observed a >40-fold increase in the number of nucleosomes that made excursions to a high FRET state (FRET efficiency >0.7). Moreover, based on the anisotropy measurements, a stronger association between the DNA ends and the histone octamer was observed from the nucleosomes in the high FRET state. The increased FRET and anisotropy after DNA methylation strongly suggest a more compact and rigid nucleosomal structure and provide a basic biophysical understanding of how DNA methylation may contribute to the formation of a repressive and transcriptionally inactive chromatin structure.

2462-Pos

Binding of the CHD4 PHD2 Finger to Histone H3 is Modulated by Covalent Modifications

Catherine A. Musselman[1], Robyn E. Mansfield[2], Adam L. Garske[3], Foteini Davrazou[1], Ann H. Kwan[2], Samuel S. Oliver[3], Heather O'Leary[1], John M. Denu[3], Joel P. Mackay[2], Tatiana G. Kutateladze[1].
[1]University of Colorado Denver School of Medicine, Aurora, CO, USA, [2]University of Sydney, Sydney, Australia, [3]University of Wisconsin, Madison, WI, USA.

CHD4 (chromodomain helicase DNA-binding protein 4) ATPase is a major subunit of the repressive NuRD (nucleosome remodeling and deacetylase) complex, which is involved in transcriptional regulation and development. CHD4 contains two plant homeodomain (PHD) fingers of unknown function. Here we show that the second PHD finger (PHD2) of CHD4 recognizes the amino-terminus of histone H3 and that this interaction is facilitated by acetylation or methylation of Lys9 (H3K9ac and H3K9me, respectively) but is inhibited by methylation of Lys4 (H3K4me) or acetylation of Ala1 (H3A1ac). An 18 μM binding affinity toward unmodified H3 rises to 0.6 μM for H3K9ac and to 0.9 μM for H3K9me3, while dropping to 2.0 mM for H3K4me3, as measured by tryptophan fluorescence and NMR. A peptide library screen further shows that phosphorylation of Thr3, Thr6 or Ser10 abolishes this interaction. A model of the PHD2-H3 complex, generated using a combination of NMR, data-driven docking and mutagenesis data, reveals an elongated site on the PHD2 surface where the H3 peptide is bound. Together our findings suggest that the PHD2 finger plays a role in targeting of the CHD4/NuRD complex to chromatin.

2463-Pos

Theoretical Model of HP1-Induced Heterochromatin Formation

Peter J. Mulligan, Elena F. Koslover, Andrew J. Spakowitz.
Stanford University, Stanford, CA, USA.

In the cell nucleus, certain regions of the genome form a condensed fiber structure called heterochromatin, while the rest is more loosely packed into euchromatin. Heterochromatin protein 1 (HP1) accumulates in the condensed heterochromatin regions, binding to nucleosomes that are methylated at lysine-9 of histone 3. We perform simulations on chromatin fibers to find their idealized fiber structure for different linker lengths, incorporating the local geometry of the nucleosomes. From these fiber structures, we look at optimal geometric arrangements to maximize connectivity between HP1 binding sites on adjacent fibers and study this connectivity in a three-dimensional lattice. Since HP1 is known to dimerize and is purported to form a network structure with other factors to stabilize the condensed state, we look at stability of HP1 binding to this three-dimensional lattice. In particular, we address whether these HP1 interactions and the connectivity are sufficient to generate a phase separation, whereby the HP1 accumulates in the condensed heterochromatin but is removed from binding in the euchromatin regions. We look at this phenomenon for different chromatin fiber configurations to address the role of local nucleosome arrangement on global chromatin condensation.

2464-Pos

Applications of VDNA from Basic Geometry to Chromatin Folding

Thomas C. Bishop.
Tulane University, New Orleans, LA, USA.

We recently introduced VDNA(1), a plugin for VMD, that allows users to construct arbitrarily complex models of double stranded DNA. VDNA is preinstalled in VMD 1.8.7 and has a number of predefined mathematical expressions that allow for investigation of the complex relations between the DNA base pair step parameters (Tilt, Roll, Twist, Shift, Slide, Rise) and spatial conformation. VDNA is capable of producing static images, ensembles and trajectories. Using VDNA we demonstrate some of the geometric relations between the DNA step parameters and Cartesian coordinates that do not readily yield to intuition. We also consider a number of biologically motivated models including: a model of thermal fluctuations in linear DNA, comparison of a Torsion Helix and a Shear Helix model of the nucleosome(2), and models for regular and irregularly folded chromatin. Finally we demonstrate how VDNA can be used to convert nucleosome footprints from a chromatin map into a three dimensional chromatin fold. Such folding provides an immediate answer as to whether or not the footprints yield a conformation that is sterically allowed or if extra-nucleosomal proteins, non-canonical nucleosome conformations or thermal effects should also be considered. The most up to date version of VDNA is available from http://dna.ccs.tulane.edu/vdna.

Acknowledgement:
This work supported in part by NIH R01-GM076356.

(1) T. C. Bishop. VDNA: The Virtual DNA plug-in for VMD, *Bioinformatics* (2009).
(2) T. C. Bishop. Geometry of the nucleosomal DNA superhelix, *Biophys. J.* 95, 1007-1017 (2008).

2465-Pos

Regulation of Nucleosome Conformational Dynamics by Post-Translational Histone Modifications Studied with Single-Pair FRET

Ruth Buning[1], Wiepke Koopmans[1], Heinz Neumann[2], Jason Chin[2], John van Noort[1].
[1]Physics of Life Processes, Leiden University, Leiden, Netherlands, [2]Laboratory of Molecular Biology, Medical Research Council, Cambridge, United Kingdom.

Nucleosomes form the basic unit of DNA compaction in eukaryotes. Not only do they condense the DNA, nucleosomes also play a crucial role in gene regulation: they modulate access to nucleosomal DNA for DNA-processing proteins. DNA within the nucleosome is made accessible via a combination of conformational changes caused by spontaneous fluctuations (DNA breathing), and by ATP-dependent remodeling enzymes. Both mechanisms are regulated by specific post-translational modifications to the nucleosome histones. Histone acetylation at H3K56, for example, has been shown to induce increased gene expression *in vivo*.

To characterize the effects of specific histone modifications on conformational dynamics of *individual nucleosomes*, we perform single-pair FRET (spFRET) measurements. We reconstitute nucleosomes from DNA labeled with a FRET pair and either modified or unmodified histones. The modified histones are obtained using a novel genetic code expansion technique that allows for genetically defined incorporation of modified amino acids. By placing FRET labels at different positions on the nucleosomal DNA, we show that transient DNA unwrapping occurs progressively from both nucleosome ends for up to at least 40 basepairs.

We follow DNA breathing dynamics of individual nucleosomes by combining spFRET and Alternating Laser Excitation (ALEX) with TIRF microscopy on immobilized nucleosomes. Alternatively, we combine spFRET and ALEX with gel electrophoresis and Fluorescence Correlation Spectroscopy (FCS) to diffusing nucleosomes. We show that a single acetylation at H3K56 increases DNA breathing of the first ~20 basepairs at least 2-fold. Furthermore, the initial state shifts to a more unwrapped conformation. Comparison with a simple model that assumes unwrapping of the DNA in ten-basepair steps indicates that acetylation at H3K56 causes the first DNA-histone contact point to break. Using these techniques we aim to further quantify epigenetic changes in chromatin at the single-molecule level.