such as apparent bending and twisting stiffnesses and degrees of compaction. We have also studied how fluctuations in nucleosome positioning can affect chromatin. We have found that minor changes of the order of a few base pairs in the locations of nucleosomes along DNA can greatly alter the local and global properties of chromatin. Our work shows that the synergy between DNA and nucleosomes in chromatin gives rise to a highly versatile biomolecule, which is able to accommodate tight packing while maintaining the accessibility necessary for specific expression.

407-Pos Board B162

Modeling Effects of Nucleosome Positioning in Short and Long Chromatin Fibers

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In eukaryotes DNA is associated with proteins in a complex structure termed chromatin. The basic packaging unit of chromatin is the nucleosome in which DNA is wrapped around a histone octamer. The mechanisms of the folding of DNA into chromatin are still under debate. Experiments indicate that chromatin has different packaging conditions connected to distinct activation states. Experimental evidence showed that packaging and activation states are closely linked to positions of nucleosomes on the DNA which are actively regulated. To improve the understanding of the interplay between nucleosome positions and chromatin structure we applied computer simulations of a coarse-grained chromatin model including fundamental physical properties such as elasticity, electrostatics and nucleosome interactions. We calculated the effect of nucleosome positioning on the structure of polynucleosomes of different length scales, up to the size of a gene locus. We compared chromatin models based on synthetic positions with models based on experimentally derived nucleosome positions from cells at different stages of cell differentiation. Simulation results revealed a significant influence of nucleosome positions on the three dimensional structure of chromatin.

408-Pos Board B163

The Physics of DNA in Confinement

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Throughout all kingdoms of life, DNA is found to form compact structures. DNA is neatly wound inside a viral capsid, DNA forms hierarchical structures in cell nuclei, DNA could even be woven into complex 3D structures known as DNA origami. Such a ubiquitous compaction is surprising, as it contradicts, at the first look, the very basic physical properties of DNA: the high electrostatic charge and resistance to bending at the scale of 50 nm or less. Experimental work has shown that counterions surrounding DNA can considerably alter its properties, for example, turning electrostatic self-repulsion into attraction. Yet, elucidating the precise microscopic structure and mechanism of DNA-DNA interaction in confined environment remains beyond the experimental capability. Here, we report the results of all-atom molecular dynamics simulations that investigated the microscopic structure of dense DNA assemblies and the physics of interactions that makes such assemblies possible. First, we show that a refined parameterization of ion-DNA interaction [1] permits the all-atom MD method to quantitatively reproduce experimentally known properties [2,3] of dense DNA arrays. Next we characterize the microscopic structure of the arrays, elucidating their ionic atmosphere, preferred azimuthal orientation of DNA molecules, the pair-wise additivity of DNA-DNA forces, the longitudinal friction forces between DNA molecules, and the role of solvation force. Our study demonstrates the ability of all-atom molecular dynamics simulations to provide quantitative, accurate information about dense DNA systems, opening exciting opportunities for future work in the area of synthetic DNA nanostructures, DNA packaging in viral capsids and cell nuclei.

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[3] Biophys J 94:4775-82.

409-Pos Board B164

Mechanism of Nucleosome Remodeling by INO80 from S. Cerevisiae

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Chromatin remodeling enzymes use the energy of ATP to alter chromatin structure by disrupting DNA-histone contacts. These enzymes play a major role in the regulation of all nuclear processes, including transcription, replication, and DNA repair. A major goal in chromatin biology is to understand how the basic biochemical mechanisms of these chromatin remodelers relate to, or even dictate their regulatory roles in vivo. INO80 is a 15-subunit chromatin remodeling complex from S. cerevisiae. INO80 is involved in the transcription of over 1000 yeast genes, and has additional roles in processes that promote genomic stability such as DNA repair. Unlike most remodeling enzymes INO80 contains several active ATPases. In addition to the canonical remodeling ATPase, Ino80p, the complex also contains two hexameric AAA^+ ATPases, Rvb1 and Rvb2. Our work seeks to understand the mechanism of nucleosome remodeling by INO80 as well as the roles that Rvb1 and Rvb2 have in this process. We employ several types of remodeling and ATPase assays to gather information about the kinetics of the different steps in remodeling. We have discovered that nucleosome movement by INO80 involves a fast step that is detectable by FRET, followed by a slow step that is detectable by a change in migration of the nucleosome on a native gel. Both steps are greatly affected by the length of extra-nucleosomal DNA. Interestingly, this length sensing behavior by INO80 is not strongly coupled to ATPase activity of the complex.

410-Pos Board B165

Dynamic Regulation of Transcription Factors by Nucleosome Remodeling

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In eukaryotic cells, transcription activation and repression are regulated by transcription factors (TFs) and nucleosomes. Although it has been demonstrated that a TF and a nucleosome can directly compete for binding to the same region of DNA, it is unclear whether and how such a competition may continue after a TF has been bound and a nucleosome has been assembled. In this study, we have addressed an aspect of this question by investigating whether a nucleosome under remodeling can displace a bound TF. We determined the locations of both a nucleosome and a TF on DNA by mechanically unzipping the DNA molecule. We found that under the action of ISWI remodeling, a nucleosome was not repositioned past a TF. In contrast, under the action of SWI/SNF remodeling, a nucleosome was repositioned past a TF with concurrent eviction of the TF from the DNA. Our results demonstrate a novel mechanism for how TFs may be regulated via dynamic repositioning of nucleosomes.

411-Pos Board B166

Higher-Order Chromatin Organisation by Insulator Proteins Revealed Using Super-Resolution Microscopy

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Insulators are regulatory DNA elements demarcating the boundaries between differentially expressed genomic regions. Five insulator binding proteins (IBPs), BEAF-32, dCTCF, Su(Hw), GAF and Zw5, and two co-factors, CP190 and Mod(mdg4)67.2, have been described in *Drosophila melanogaster*. These factors have been reported to maintain physical interactions between distant genomic sites. Such evidence predicts that insulator proteins should form multi-protein clusters that regroup specific sequences, by establishing long-range chromatin contacts, which ultimately help define gene expression programs.

Despite the thorough mapping of protein binding sites and genome-wide interactions, the clustering of IBPs has been inferred from biochemical experiments, in which heterogeneity and dynamics are intrinsically averaged out. Direct monitoring of the spatial organization, dynamics and distribution of IBPs and their partners require single-cell microscopy approaches. However, these measurements have been so far hampered by the size of IBP clusters, the high local protein density, and the intrinsic resolution limit of conventional microscopies. Here, we implemented 3D Structured Illumination (SIM), multicolor and 3D localization-based (PALM/STORM) microscopies, with lateral resolutions of ~100 and ~20 nm respectively, to explore IBP clustering and its implication in gene expression regulation.

We find that under normal growth conditions, BEAF-32, CP190, and dCTCF form 100-300 nano-clusters per nucleus, with a mean size of ~40 nm, both in transfected and immuno-stained *Drosophila* S2 cells. The number and distribution of nano-clusters display cell-to-cell variability, consistent with IBP roles in maintaining chromatin organization throughout cell divisions. Two-color SIM and STORM showed different classes of IBPs and co-factors co-localize with characteristic patterns. Furthermore, different IBP clusters specifically associate with gene activity markers like RNA Pol2 and epigenetic histone modifications. Our study introduces a highly sensitive approach to functionally investigate the complex interplay between chromatin folding and gene regulation, with single-molecule resolution.