2466-Pos
Theoretical Modeling of Protein Accessibility to the Chromatin Fiber
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Gene expression is orchestrated by a host of regulatory proteins that coordinate the transcription of DNA to RNA. Regulatory proteins function by locating specific sequences of DNA and binding to these sequences to form the transcription initiation complex. The eukaryotic genome is tightly packaged into a dense chromatin fiber. This packaged structure acts both to store the massive genome and to facilitate the accessibility of the genome to regulatory proteins. The interplay between the packaging proteins and the regulatory proteins is critical in normal cellular function and plays a pivotal role in a number of human diseases. We present a theoretical study on the dynamic accessibility of the chromatin fiber, providing an overview of several approaches to this multi-faceted problem. Our modeling efforts address both the structural properties of packaged DNA and the dynamic processes involved in target-site localization of regulatory proteins. Modeling of the 30-nm fiber reveals the impact of local nucleosome configurational properties on the fiber geometry, and we predict the mechanical properties of the assemblies and the resulting dynamic accessibility. We also discuss the role of linker histones and variant histones in the fiber assembly. Using our 30-nm fiber models, we address large scale condensation by epigenetic factors and discuss the role of histone methylation in the determination of heterochromatin and euchromatin states. Upon establishing our chromatin model, we study the dynamic processes involved in the target-site search of regulatory proteins within the complex chromatin structure. This effort combines our structural models with our protein-transport models in order to provide a novel perspective on regulatory-protein function.

2467-Pos
Nucleosome Organization is Quantitatively Described by Statistical Positioning Up- and Downstream of Transcription Start Sites
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The positions of nucleosomes in eukaryotic genomes determine which parts of the DNA sequence are readily accessible for regulatory proteins and which parts are not. A salient feature in recent genome-wide nucleosome maps is that nucleosomes appear well-positioned around a nucleosome free region (NFR) just upstream from the transcription start site (TSS). What determines this nucleosome organization is not known. One scenario is that the majority of nucleosome positions near the TSS are directly encoded in the DNA sequence. The alternative ''statistical positioning'' scenario, is that a few local barriers on the genome strongly constrain the positions of closeby nucleosomes, purely on statistical grounds. We use a physical model for the latter scenario, based on the Tonks gas of statistical physics, to quantitatively analyze recent data for yeast. We find that although the typical patterns on the two sides of the TSS are different, they are both quantitatively described by the same physical model, with the same parameters, but different boundary conditions. The inferred boundary conditions suggest that the first nucleosome downstream from the NFR is typically directly positioned while the first nucleosome upstream is statistically positioned via a nucleosome-repelling DNA region.

2468-Pos
DNA Loop Formation in Nucleosomes
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Many key processes in the cell nucleus, such as replication, transcription or DNA-repair, require the physical accessibility of specific DNA sequences. In eukaryotes, DNA is wrapped in 1.67 left-handed superhelical turns around a histone protein core to form the nucleosome. Although the function of histones in eukaryotes remains elusive, it is possible that histone mobility on DNA is responsible for 'opening up' DNA surfaces at different periods of chromatin organization. One of the mechanisms of nucleosome repositioning on DNA involve formation of local defects in the form of a loop and diffusion of it over the stretch of DNA attached to the histone. DNA loop formation has so far been discussed in the context of the worm like chain (WLC) model [1]. However, atomic force microscopy experiments [2] suggest that large angle bending energetics of DNA does not follow a WLC. Here we describe the energetics of loop formation on nucleosomes using a model which offers softer bending potential for large deflections, namely the sub-elastic chain (SEC), and compare with WLC. Results show that SEC favors small loop (~ 10 bp) formation, WLC favors large loop formation. Different energetics of loop formation also leads to different nucleosome repositioning behaviors for WLC and SEC. Nucleosomes, initially positioned in the middle of a DNA segment, jumps to the extremities for WLC and to the nearest neighboring positions for SEC.
1. Kulic I. M. , Schiessel H. 2003. Nucleosome repositioning via loop formation. *Biophys J.*, 84(5):3197-211.
2. Wiggins et. al. 2006. High flexibility of DNA on short length scales probed by atomic force microscopy. *Nature Nanotechnology*.1.137.

2469-Pos
The Third Level of Genome Functioning: Chromatin Folding
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Recent experiments have provided us with extensive amount of data which all suggest that chromatin folding is achieved by formation of chromatin-chromatin loops. This observed looping is highly dynamic and linked to cell differentiation. We propose that the chromatin folding constitutes a third level of genome functioning on top of the individual genes and their epigenetic control. Our interest is to study the principles of chromatin folding in the cell nucleus, above all how does chromatin folding relate to gene expression. In this we focus on the molecular mechanisms of formation of loops, dynamics and ergodicity of looping as well as to relation of dynamic looping to genome function. Our experimental approach relies on three-dimensional cell imaging to map the in vivo folding state of chromatin. For the interpretation of our data we use polymer-physics models. The most recent work of our group shows that chromatin folding status is different in genomic regions with higher transcription levels and in transcriptionally silent regions. In both cases the data can be fitted to a "random loop" polymer model(1).
1. Mateos-Langerak, J., Bohn, M., de Leeuw, W., Giromus, O., Manders, E.M.M., Verschure, P. J., Indemanns, M.H.G., Gierman, H.J., Heermann, D.W., van Driel, R., Goetze, S. 2009. Spatially confined folding of chromatin in the interphase nucleus. Proc Natl Acad Sci U S A. 106:3812-7.

2470-Pos
The Chromatin-Remodeling Complex ACF Functions as a Dimeric Motor to Space Nucleosomes
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The chromatin structure at a given locus is a key determinant of its transcriptional state. Evenly spaced nucleosomes directly correlate with condensed chromatin structures and gene silencing. The ATP-dependent chromatin assembly factor (ACF) generates such structures in vitro and is required for transcriptional silencing in vivo. ACF generates and dynamically maintains nucleosome spacing by constantly moving a nucleosome towards the longer flanking DNA faster than the shorter flanking DNA. But how the enzyme rapidly moves back and forth between both sides of a nucleosome to accomplish such bidirectional movement is not known. Using FRET to follow disruption of histone-DNA interactions in real time we show that nucleosome movement depends cooperatively on two ACF molecules, suggesting that ACF functions as a dimer of ATPases. Employing Electron Paramagnetic Resonance (EPR) to resolve different populations of the nucleosome-ATPase complex, we find that the nucleotide state determines whether the dimer closely engages one vs. both sides of the nucleosome. Furthermore three-dimensional reconstruction by single particle electron microscopy of the ATPase-nucleosome complex in an activated ATP state reveals a dimer architecture in which the two ATPases bind facing each other. Our results suggest a model in which the two ATPases work in a coordinated manner, taking turns to engage either side of a nucleosome. Such a mechanism would allow rapid sampling of both sides of the nucleosome and allow bidirectional movement without dissociation. This novel dimeric motor mechanism differs from that of other dimeric motors such as kinesin and dimeric helicases that processively translocate in one direction and reflects the unique challenges faced by motors that move nucleosomes.

2471-Pos
Role of DNA Fluctuations in RNA Polymerase Translocation through a Single Nucleosome
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We analyze two aspects of the physical behavior of a single nucleosome: the response of a single nucleosome core particle to tension and the translocation

of RNA polymerase through the nucleosome. We develop a statistical-mechanics model of a nucleosome as a wormlike chain bound to a spool, incorporating fluctuations in the number of bases bound, the spool orientation, and the conformations of the unbound polymer segments. With the resulting free-energy surface, we perform dynamic simulations that permit a direct comparison with single-molecule experiments on a single nucleosome. This simple approach demonstrates that the experimentally observed structural states at nonzero tension are a consequence of the tension. Therefore, our model plays an important role in extrapolating the behavior to zero tension. This mechanism would arise in any system where the tether molecule is deformed in the transition state under the influence of tension.
Using our statistical-mechanics model, we also study the translocation of RNA polymerase through a nucleosome. We consider RNA polymerase as a Brownian ratchet and model the translocation process using dynamic Monte Carlo simulation. We incorporate the effect of DNA elasticity on protein movement by considering the probability of RNA polymerase going into the pause state due to the force being applied by the bent DNA. Our theory suggests that RNA polymerase translocation is in pseudo-equilibrium with local DNA fluctuations and is not rate-limiting. Our theory predicts a very small change in translocation velocity of RNA polymerase in presence of TFIIS, suggesting that RNA polymerase generates sufficient forces to unravel the nucleosome in the absence of TFIIS.

2472-Pos
A Magnetic Force Micropiston for Analysis of Chromosome Expansive and Compressive Forces and their Effects on Structure and Function
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Dynamic changes in the level of chromatin compaction occur throughout the cell cycle, leading to consideration of the potential effects of expansion. Such effects include the fact that when expansion is constrained (by surrounding objects, an external cage, or internal tethers) they may generate pushing forces (compressive stress) on those constraining features. We have developed a Magnetic Force Micropiston, a device that combines magnetic bead manipulation with confinement created by microfluidic channels, to probe the magnitude of, basis for and effects of the forces generated by DNA/chromatin/chromosome expansion and/or compression. This device accommodates objects whose size scales are of the order of bacterial cells and yeast nuclei (1um) and mammalian nuclei (4.5um) and, for some appications, even smaller scales (~150nm). Objects and changes of interest can be monitored by phase or fluorescence microscopy (presently epifluorescence or TIRF), with effects assessed as a function of time and with real-time monitoring of the effects of changes in the ionic or molecular/biochemical composition of the buffer. Measurable quantities include expansion force, volume changes, mobility of individual loci, hydrodynamic properties of the solvent, changes in the affinities of chromosomal components, and alterations in the organization and order of the confined chromatin. Applications of this system to mammalian and E.coli chromatin will be presented. The device can also be used, without application of force, for multiple parallel single cell analyses, e.g. of vegetative growth and meiosis in budding yeast and of E.coli, as will be illustrated.

2473-Pos
Nucleosome Dynamics Studied by Free Solution Single Molecule FRET
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To help understanding the mechanism of nucleosome opening and closing, we studied the disassembly of mononucleosomes by quantitative single-molecule FRET, using the SELEX-generated "Widom 601" positioning sequence labeled with donor and acceptor fluorophores. Reversible dissociation was induced by increasing NaCl concentration. At least 3 species with different FRET were identified and assigned to structures: (i) the most stable high-FRET species corresponding to the intact nucleosome, (ii) a less stable mid-FRET species that we attribute to a first intermediate with a partially unwrapped DNA and less histones, and (iii) a low-FRET species characterized by a very broad FRET distribution, representing highly unwrapped structures and free DNA formed at the expense of the other 2 species. Selective FCS analysis indicates that even in the low-FRET state, some histones are still bound to the DNA. The interdye distance of 54.0 Å measured for the high-FRET species corresponds to a compact conformation close to the known crystallographic structure. The coexistence and interconversion of these species is first demonstrated under non-invasive conditions. A geometric model of the DNA unwinding predicts the presence of the observed FRET species. The different structures of these species in the disassembly pathway map the energy landscape indicating major barriers for 10-bp and minor ones for 5-bp DNA unwinding steps. Further information about stepwise dissociation, and exchange of histones between nucleosomes is obtained by FRET between DNA and labeled histones.

2474-Pos
Histone Post-Translational Modifications Buried within the Nucleosome DNA-Histone Interface Facilitate Nucleosome Disassembly
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A number of histone post-translational modifications are located in the nucleosome DNA-histone interface. These modifications include the acetylation of histone H3 at K115 and K122, which are located near the dyad symmetry axis. While these modifications are implicated in DNA repair, the molecular mechanisms by which these modifications are involved in DNA repair processes are not well-understood. We are investigating the hypothesis that these modifications biophysically alter the nucleosome to facilitate DNA repair. We introduced these modifications into histone H3 by protein ligation and incorporated them into single nucleosomes and nucleosome arrays. We employed a combination of ensemble and single molecule methods to determine that these modifications reduce DNA-histone binding free energy and enhance nucleosome disassembly induced by the mechanical unwrapping of nucleosomal DNA, or by the human mismatch repair complex hMSH2-hMSH6. These studies suggest that modifications in the nucleosome DNA-histone interface function to reduce DNA-histone interactions to facilitate nucleosome disassembly for DNA repair.
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2475-Pos
DNA-Protamine Toroids Pull on their Attachment Points
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The fundamental unit of sperm chromatin is the DNA-protamine toroid which contains 50 kbp of DNA and is shaped like a donut with an outer diameter of 100 nm. This unique form of chromatin occurs when histones are displaced from nucleosomes and replaced by protamines during the differentiation of the sperm cell, known as spermiogenesis. This condenses the sperm genome into a much smaller volume, at the physical limit of packing, to protect it from exogenous insult and inactivate it during its journey to the egg. To determine if biomechanical forces participate in the formation of toroids we decided to examine this process at the single molecule level. Using dual optical traps to expose a single lambda phage DNA molecule to mammalian protamine in a multichannel flow cell we were able to show that toroids exert significant forces on their attachment points. Elasticity measurements of the toroids revealed that they are relatively inflexible. We will discuss what these measurements imply about the architecture of chromatin within the mature sperm cell.

2476-Pos
Single Molecular Torque Measurements of Chromatin Fibers
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Chromatin structure and properties modulates the activity of the transcription machinery. The torsional properties of chromatin are critical since during translocation polymerase twists and applies torque to the molecule. We have measured torsional properties of chromatin using a new single molecule technique. In particular, these experiments show that the torsional rigidity of decondensed chromatin molecules is significantly lower than the torsional rigidity of bare DNA, suggesting that this property may be controlled *in vivo* to modulate transcription activity. We use Monte Carlo simulations and a coarse grain model of chromatin to explain experimental measurements. The model considers DNA as a flexible polymer that can unwrap from histones proteins. Histones and DNA electrostatic interactions are included. We exclude configurations in which DNA or histones cross into each other. The model successfully fit the torque measurements and molecule extension as a function of turns. We use this model to predict the torque required to twist compact chromatin fiber in which histones proteins are in close proximity. We find that compact chromatin has torsional rigidity higher than decondensed chromatin.