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.