that folding of the fibers depends on the length of the linker DNA between the nucleosomes, and that a 50 bp linker length results in a helical folding. Here we used magnetic tweezers to probe the mechanical response of a single 30 nm fiber under torsion, and found that it is folded in a left handed helix. Applying negative twist stabilizes a fiber against rupture for forces up to 5 pN. Positive twist can compensate the buildup of negative supercoiling when nucleosomes unwrap their DNA at forces above 3 pN and therefore positive twist destabilizes the fiber. These experiments have revealed for the first time the topology of a 30nm chromatin fiber and its response to torque. The results have important implications for the mechanism of DNA based molecular motors that operate in a chromatin context, like the ones involved in chromatin remodeling, transcription and replication.

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Chromatin Structure and Dynamics in Response to External Forces and Torques

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Chromatin is subjected to myriad forces and torques during DNA repair, transcription, recombination, and replication. Despite the growing realization that such forces and torques play important roles in chromatin function, our understanding of how these perturbations affect chromatin structure and dynamics remains poor. I will describe recent efforts from our group in modeling chromatin structure and dynamics subjected to external torques and forces.

First, I will describe Monte Carlo simulations of a mesoscale model of chromatin to study the propagation of DNA twist across nucleosomes [1,2]. The magnitude and sign of the imposed and induced twist on contiguous linker DNAs is found to depend strongly on their relative orientation. Interestingly, the relative sign of the induced and applied twist becomes inverted for a subset of linker orientations. We have characterized twist inversion as a function of linker orientation in a phase diagram and explained its key features using a geometrical model. We also reveal rapid flipping of nucleosomes in response to applied twist, which allows for rapid changes in the overall twist and writhe of nucleosome arrays. Second, I will describe Brownian dynamics simulations of a mesoscale model of the nucleosome to elucidate the dynamics of force-induced unwrapping of DNA from histone octamers [3]. We demonstrate why the first turn of DNA unwraps reversibly from the octamers and the second turn wraps irreversibly, as observed in single-molecule experiments. We also reveal the complex flipping and rocking motions of the octamer accompanying nucleosome unraveling and the role of the strong histone/DNA interactions at the dyad and ~35 bp from the entry/exit site.

1. Grigoryev et al., PNAS 106, 13317 (2009)
2. Dobrovolskaia et al., Biophys. J. 99, 3355 (2010)
3. Dobrovolskaia & Arya, to be submitted

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Looping and Long-Distance Communication on Chromatin

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Although the genetic messages in DNA are stored in a linear sequence of base pairs, the genomes of living species are highly packed and organized three-dimensional systems. Gene expression is regulated by DNA sites that often lie far apart along the genomic sequence and hence depends on cooperation between tight packing and protein-induced deformations of DNA. For example, eukaryotic histones, which wrap and package DNA into chromatin, are known to facilitate the communication between distant transcription factors by promoting the formation of chromatin loops. In order to clarify the role of histones and DNA deformation in chromatin looping, we have developed a structurally-based model of chromatin at the resolution of a single base pair and performed Monte-Carlo simulations. Our model successfully reproduces experimental measurements of gene expression induced by proteins bound to distant genomic sites on DNA fragments decorated by arrays of nucleosomes. We found in our simulations that changes, of the order of a few base pairs, in the spacing between nucleosomes give rise to a great diversity of fiber structures and looping probabilities. We are using our model to investigate the role of histone tails on nucleosome-nucleosome interactions and chromatin looping. We also study the interplay between local chromatin architecture and chromatin loop formation by simulating fibers with nucleosome-depleted regions and different spacing between the nucleosomes. In addition, we are able to model the presence of the protein assemblies used in the experiments to induce gene expression (RNA polymerase and NtrC) and to characterize their influence on chromatin structure and looping. The combination of simulations and experiments gives us the ability to relate local changes in nucleosome composition and chromatin architecture to the looping propensities of fluctuating chromatin fibers.

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Computer Simulation of Chromatin: Interdependency Between Nucleosome Positions and Chromatin Structure

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The three-dimensional structure of chromatin is a key factor for controlling DNA accessibility, replication and repair. Despite numerous experimental efforts many details of the spatial organization and structural regulation mechanisms of chromatin remain unclear.

Most theoretical models of chromatin proposed in literature imply a periodical positioning and uniform occupancy of the fiber nucleosomes. However, recent studies suggest a dynamic rather than static nucleosome positioning, which is both actively regulated by chromatin-remodeling complexes (CRCs) and passively influenced by thermal fluctuations. These processes have been subject to intensive scientific work, yielding new insights into the function of CRCs and the biophysical properties of the histone-DNA interface. However, nucleosome positions are also influenced by energetic effects imposed by structural constraints inherent in the chromatin fiber, and, vice versa, nucleosome positioning impacts chromatin fiber structure as well.

To investigate the effects of nucleosome repositioning, we carried out Monte Carlo simulations with a coarse-grained chromatin model incorporating elastic fiber properties as well as a detailed description of the electrostatic and internucleosomal interactions. We created computational fiber conformations based on experimental results. These fiber conformations were modified by repositioning nucleosomes by a range of base pair steps. After simulation, the chromatin energy landscape and shape were analyzed. We observed a significant energy barrier against nucleosome repositioning which is larger than thermal fluctuations but within the range of ATP-dependent biological processes. Moreover, analysis of fiber shape data revealed an increased kinking susceptibility of the fiber within the region proximate to a repositioned nucleosome. This behavior is accompanied by increased fiber flexibility within the same region.

These findings facilitate a deeper understanding of the relation between nucleosome positions and chromatin fiber structure.

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Chromatin Architecture Reconstruction

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Three-dimensional genomic architecture is increasingly being found to play an important role in the regulation of gene expression through direct physical interactions of distant genomic sites. Conformation Capture Techniques (CCTs) attempt to resolve the conformation of a genetic sequence or entire genome by measuring mean interaction frequencies between pairs of genomic loci in a population of fixed cells. Essential to the interpretation of these measurements is the use of a molecular model to infer genomic conformation from measured interaction frequencies.

Unlike macromolecules that exhibit a single, dominant conformation in their native state, chromatin exhibits conformational polymorphism at multiple scales that renders optimization techniques that solve for a single, dominant conformation in the structure inference process suboptimal for the interpretation of CCT data.

As an alternative, we present a computational procedure that solves for the unique, maximum entropy structural ensemble that is consistent with experimentally measured interaction frequencies. By modeling genomic topology and treating the full conformational ensemble in the fitting process, ensemble average structural quantities including correlations in distances between distinct pairs of genomic sites are obtained in addition to mean interaction frequencies between all genomic sites.

Application of the procedure to the human HoxA cluster suggests that it is organized into multiple chromatin loops in differentiated cells. The present approach that is founded on the principle of maximum entropy is equally applicable to fluorescence-based data as obtained, for example, from fluorescence in situ hybridization as it is to CCT-based measurements.