technology could be applied to map the dynamics of a unique combination of 14 loci tagged in chromosome III, IV, VI, XII and XIV at the single cell level in yeast. The spatio-temporal dynamics of these loci, as inferred from their mean square displacement, appeared to be similar over a very large time domain from 10 ms to 50 s. Moreover, anomalous sub-diffusive behaviors were systematically detected, and the anomaly parameters were consistent with the polymer model of reptation that describes polymers as snakes randomly crawling in grass. This model is characterized by distinct dynamics at short and long time scales, Rouse and reptation behaviors, respectively, that were both observed experimentally. The quantitative analysis of our results unravels that two parameters of chromatin, namely its persistence length and its viscous friction, suffice to determine its dynamics. Finally, we are currently analyzing the dynamics of a variety of yeast mutants, in which chromatin structural proteins have been depleted, to explore how chromatin compaction is regulated in vivo and its effect on chromatin dynamics. Taken together, our study sheds new light on chromatin structure and dynamics built on physical models.

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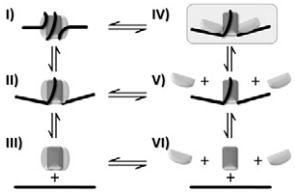
Nucleosome Dynamics Studied by Single Molecule FRET

Vera Böhm, Aaron Hieb, Andrew Andrews, Alexander Gansen, Andrea Rocker, Katalin Tóth, Karolin Luger, **Jörg Langowski**.

We studied the mechanism of nucleosome opening to allow DNA access, using single molecule FRET. Here we show evidence for a previously uncharacterized intermediate structural state that occurs before H2A-H2B dimer release, and is characterized by an increased distance between H2B and the nucleosomal dyad. Our data suggest that the first step in nucleosome disassembly is the opening of the (H3-H4)2 tetramer/(H2A-H2B) dimer interface, followed by H2A-H2B dimer release from the DNA and, lastly, (H3-H4)2 tetramer removal (see Figure). We estimate that the open intermediate state is populated at 0.2 - 3 % under physiological conditions, and could have significant in vivo implications for factor-mediated histone removal and exchange, as well as for regulating DNA accessibility to the transcription and replication machinery.



Additionally, histone variants and histone modifications could substantially change the stability of the nucleosome. Our most recent spFRET data indicate that such effects may occur both at the level of DNA-histone and histone-histone interactions.

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Discrete Mechanisms Govern the Modulation of Chromatin Structure by Ubiquitylation and Acetylation

Beat Fierz, Champak Chatterjee, Robert K. McGinty, Maya Bar-Dagan, Daniel P. Raleigh, Tom W. Muir.

Regulation of chromatin structure involves histone post-translational modifications which can modulate intrinsic properties of the chromatin fiber to change the chromatin state. In humans, attachment of ubiquitin to lysine 120 in histone H2B (uH2B) (1) is associated with active transcription and occurs at promoters and within gene coding regions (2). A structural role for uH2B in rendering chromatin permissive to transcription has been suggested but experimental proof remained elusive (3). We use chemically defined nucleosome arrays to demonstrate that uH2B interferes with chromatin folding and leads to an open and biochemically accessible fiber conformation. Importantly, these effects are specific for ubiquitin, as chromatin modified with a similar ubiquitin sized protein, Hub1, can compact unhindered. Applying a fluorescence homotransfer based method we find that uH2B acts through a mechanism distinct from H4 tail acetylation (acH4), a modification known to disrupt chromatin folding (4, 5). Finally, incorporation of both uH2B and acH4 in nucleosomes results in synergistic inhibition of higher order chromatin structure formation, possibly a result of their distinct mode of action. We therefore establish a novel function of uH2B, which by locally disrupting chromatin fiber structure facilitates biological processes that require access to the histone proteins and the underlying DNA.

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Depletion Effects Massively Change Chromatin Properties and Influence Genome Folding

Philipp M. Diesinger.

We present a Monte Carlo model for genome folding at the 30-nm scale with focus on linker-histone and nucleosome depletion effects. We find that parameter distributions from experimental data do not lead to one specific chromatin fiber structure, but instead to a distribution of structures in the chromatin phase diagram. Depletion of linker histones and nucleosomes affects, massively, the flexibility and the extension of chromatin fibers. Increasing the amount of nucleosome skips (i.e., nucleosome depletion) can lead either to a collapse or to a swelling of chromatin fibers. These opposing effects are discussed and we show that depletion effects may even contribute to chromatin compaction. Furthermore, we find that predictions from experimental data for the average nucleosome skip rate lie exactly in the regime of maximum chromatin compaction. Finally, we determine the pair distribution function of chromatin. This function reflects the structure of the fiber, and its Fourier-transform can be measured experimentally. Our calculations show that even in the case of fibers with depletion effects, the main dominant peaks (characterizing the structure and the length scales) can still be identified.

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Consequences of Enantiomorphy in Chromatids

Arthur Cole.

Mirror-image symmetry (enantiomorphy) occurs when morphology on one side of a bisecting plane is reflected to the other side. My stereo electron microscope studies of isolated and intact mammalian cell structures show sister chromatids in metaphase cells have mirror-image morphologies across the bisecting plane. Chromatid morphology is determined by an internal eight-stranded planar ribbon running the length of the chromatid. This 180 nm-wide primary ribbon has polarities with a beginning and end and top and bottom strands. Lateral extensions, incorporating DNA loops, project outward as secondary ribbons spaced every 60 nm along the primary ribbon. Lateral extensions are of two types. Short ones extend 400 nm and are stabilized by (lipo)proteins that support outgoing and returning DNA strands as a single 7nm-wide fiber. Long extensions have the DNA wound on nucleosomes. Sister chromatids are held together, at the bisecting plane, by associations between ends of inner-facing short extensions. The stated enantiomorphic relationships are anticipated assuming a side-by-side replication of the backbone and its related structures.

I propose that components of the mitotic apparatus also display polarity and enantiomorphy. Thus, left-handed centrioles associate with left-handed 4-stranded ribbons of microtubules that connect to left-handed kinetochores of left-handed chromatids. Right-handed mitotic components connect to right-handed chromatids. This assures correct segregation of chromatids. In meiosis, left-handed chromatid segments may need to pair with homologous right-handed chromatid segments for crossing-over to occur. I propose this pairing is made at ends of short lateral extensions, as for sister chromatid attachments. To accommodate such pairings, chromatids from egg and sperm must replicate before pairing to assure availability of both left and right-handed homologous pairing segments. Thus enantiomorphy provides an understanding for the generation of tetrads in meiosis.

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Polymer Model of the Spatial Organization of Interphase Chromosomes in Yeast

Baris Avsaroglu, Susannah Gordon-Messer, James E. Haber, Jane' Kondev.

The spatial organization of chromosomes in cells plays an important role in the biological functions they perform, such as gene regulation and DNA recombination. Recent experiments have measured chromosome organization in yeast during interphase in quantitative detail yielding cell-to-cell distributions of the positions of different genetic loci within the nucleus.

These experiments pose a challenge to theoretical models of chromosomes. Using a simple, analytically tractable, polymer model that take into account nuclear confinement and tethering we compute the distributions of telomere positions along the nuclear periphery (ref. 1), and the location of the HML locus on chromosome III (ref. 2), and find good agreements with experimental data. Furthermore, we investigate theoretically the effect of nuclear size, telomere positioning, and chromosome flexibility on the spatial distributions of genetic loci suggesting new experiments for testing polymer models of yeast interphase chromosomes.

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