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Effect of Histone Acetylation on Nucleosome Dynamics Revealed by spFRET Microscopy

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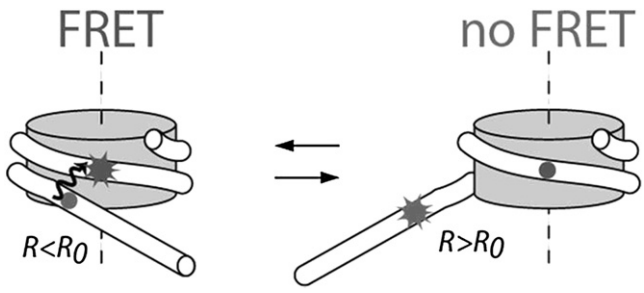
Nucleosomes form the basic unit of DNA compaction in eukaryotes. Besides condensing the DNA, nucleosomes play a crucial role in gene regulation by modulating access to the nucleosomal DNA for DNA-processing proteins. Accessibility of DNA within the nucleosome can be realized by spontaneous unwrapping (DNA breathing), and by ATP-dependent remodeling enzymes. Both mechanisms are regulated by specific post-translational modifications to the nucleosome histones. We applied single-pair FRET measurements to characterize the effect of specific histone modifications on DNA breathing dynamics in individual nucleosomes. DNA labeled with a FRET pair was used to reconstitute nucleosomes with three types of histones: native chicken erythrocyte histones, recombinant unmodified histones, and recombinant histones acetylated at H3K56. Using alternating laser excitation to select for the correct label stoichiometry, in combination with both widefield TIRF microscopy on immobilized nucleosomes and FCS on freely diffusing nucleosomes, we were able to quantify DNA breathing dynamics at timescales ranging from milliseconds to minutes. Uncovering the effect of histone modifications on the dynamic behavior of single nucleosomes provides insight in the physical mechanisms underlying gene regulation.



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Computer simulations of chromatin fibers

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In the nucleus of higher organisms the DNA is organized by histone proteins into a nucleoprotein complex termed chromatin. This packing controls DNA accessibility and is therefore an important factor for the control of gene expression. We developed a new coarse-grained computer model to represent different types of chromatin fibers. Based on model structures at atomic resolution, the common two-angle nucleosome geometry was enhanced by four additional angles. The nucleosomes are modeled as spherocylinders described by an S-functions expansion and are connected by cylindrical DNA segments. Harmonic potentials for stretching, bending, and torsion represent the elastic properties of the DNA. The negative charge of the DNA is described by a Debye-Hückel-approximation. This model was used to investigate the influence of the local nucleosome geometry and the internucleosomal interaction on the chromatin fiber conformation by Monte Carlo (MC) simulations [1,2]. Three fiber types derived from experimental data of native and reconstituted chromatin were systematically analyzed. For all investigated fiber types, the simulations revealed the large impact of the nucleosome repeat length on the stability of the fiber formation. A model was proposed, in which changes of the chromatin fiber conformation induced by linker histone H1 binding as predicted from high resolution model structures are reproduced by relatively small changes of the local nucleosome geometry. Furthermore, key factors for the control of the compaction and higher order folding of the chromatin fiber were identified. We have further developed this approach and are applying it to the analysis of the conformational space of the chromatin fiber, fiber force spectroscopy experiments and atomic force microscopy imaging of chromatin fibers.

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Nanomanipulation Of Single Chromatin Fiber With Magnetic Tweezers

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The nucleosome core particle is the basic subunit of chromatin structure. It consists of ~146 DNA bp coiled leftward around an octamer that contains pairs of histones H2A, H2B, H3 and H4. Magnetic tweezers were used to study the mechanical response under torsion of single chromatin fibers. Nucleosome arrays are reconstituted on tandem repeats of 601 positioning sequences. These fibers show higher torsional plasticity than naked DNA. Such a behavior can be explained by a dynamic equilibrium between three conformations of the nucleosome, corresponding to different crossing statuses of the entry/exit DNAs : negative, null or positive. Moreover after extensive positive supercoiling these chromatin fibers display an hysteretic behavior in their mechanical response to torsion. The fibers remain more extended when they are returning to negative supercoiling values. This hysteresis is the consequence of the trapping of one positive turn per nucleosome. The results suggest a rearrangement of the nucleosome structure which can be related with the previously documented chiral transition of the tetrasome (the H3-H4 tetramer with its bound DNA). As the energy of the altered form, named reversome, is ~6 kT, these abilities to endorse torsion may be related to physiological processes such as trancription elongation since RNA polymerases generate positive supercoiling downstream. As eukaryotic chromatin contains a high proportion of linker histone we investigate the effect of H5 (avian erythrocytes variant) on the mechanical response of an array. First we produced regular fiber containing this histone in stoechiometric amount. Then we showed that even if these kind of fiber are more condensed, it still displays a high torsional plasticity and the ability to form a reversome fiber.

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Rapid-Quench Mixing and Use of Fast Footprinting to Characterize DNA Opening in the Late Steps of Open Complex Formation at λPR by E. coli RNA Polymerase

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The precise mechanism by which multi-subunit, DNA-dependent RNA polymerases (RNAP) recognize promoter DNA and form stable, transcriptionally-competent open complexes (RPo) remains elusive. Chemical and enzymatic footprints of early intermediates and RPo provide detailed pictures of the DNA in these initial and final complexes in transcription initiation. We have recently discovered that the start site for transcription (+1) remains double-stranded when it is first loaded into the active site channel of *E. coli* RNAP at the λ phage P_R promoter (Davis et al. PNAS, 2007). These experiments reveal unambiguously that opening of the transcription bubble occurs subsequently in the ''jaws'' of RNAP as RNAP actively destabilizes the duplex. To probe whether opening of ~14 bp of DNA occurs in stages or is ''all or none'' we are using the perturbants urea and KCl to destabilize RPo and populate late transcription initiation intermediates. Analysis of the dissociation kinetics over a range of urea and KCl concentrations provides evidence for two transient intermediates in the DNA opening process. Using $KMnO_4$ footprinting we are probing the extent of DNA opening in these intermediates under these conditions. Results from these novel rapid (millisecond) footprinting experiments and the implications for the mechanism of promoter opening will be presented.

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Stress, Scrunching And Tethering: The Roles Of The Connecting Template Strand In Initiation By T7 RNA Polymerase

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T7 RNA polymerase is the most well characterized single subunit RNA polymerase. It possesses high activity in vitro and shows high specificity for its DNA promoter. It is also functionally similar to the multi subunit RNA polymerases, making it an ideal model to study the mechanistic aspects of RNA polymerase during its transition through the three phases of transcription; initiation, elongation and termination.

Like all DNA-dependent RNA polymerases, T7 RNA polymerase carries out de novo synthesis of RNA from a double stranded template. The enzyme melts open the duplex, initiates synthesis of a dinucleotide RNA, and moves along the template DNA extending and ultimately displacing the RNA transcript. In the initially transcribing phase, the bubble expands as the RNA:DNA hybrid grows. This phase is associated with large structural rearrangements in the complex

and is characterized by an instability leading to the release of short, abortive RNA products.
It was proposed many years ago that the release of abortive products results from a growing ''stress'' in the system. Recent crystal structures have suggested that ''scrunching'' of the template (single) strand connecting the initiation site to the duplex promoter region contributes substantially to this stress.
In order to probe the role of this connecting single strand, we have created various constructs in which regions of this single stranded DNA have been nicked, deleted (gapped) or overlapped and then have asked how this effects both 1) the initial positioning of the template DNA and 2) the stability of constructs stepping away from the promoter (abortive cycling). These results provide a direct test of the scrunching model for stress during the initially transcribing phase of transcription.

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Prevention Of Backtracking Alleviates Nucleosomal Barrier To Transcription
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Nucleosomes are fundamental repeating subunits of eukaryotic chromatin which help to package DNA tightly into the cell nucleus. During transcription, RNA polymerase (RNAP) must be able to transcribe the DNA associated with nucleosomes, yet nucleosomes are known to be major barriers to transcription. Here we use optical trapping techniques in combination with biochemical methods to study the mechanisms by which E.coli RNAP transcribes through nucleosomes. Although E. coli RNAP never encounters chromatin in vivo, its core enzyme is evolutionarily conserved and shares homology in sequence, structure and function with eukaryotic Pol II, suggesting that E. coli RNAP may be a simple system to study transcription through nucleosomes. We have constructed DNA templates, each containing a T7A1 promoter followed by a well-positioned mononucleosome. We use optical trapping to detect with high precision the position and structure of proteins before and after transcription. Our results show that RNA polymerase may backtrack when it encounters a nucleosome. Prevention of backtracking alleviates the nucleosomal barrier, promoting more efficient transcription through nucleosomes.

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Monte Carlo Simulation of Transcriptional Control in 6kbp DNA
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Eukaryotic genes are regulated by gene-control regions such as enhancers and insulators. Enhancers are regulatory DNA sequences that can activate transcription of genes located at large distance. Insulators are regulatory DNA sequences that can inhibit transcription when they are located between an enhancer and a promoter. Recently, it has been reported that the enhancer is in physical proximity to the active genes in vivo with the intervening DNA looping out [1]. It has also been indicated that insulators may modulate the enhancer-promoter interactions by interacting with each other and facilitating the formation of chromatin or the DNA loop domains [2, 3]. In this way, importance of the DNA or chromatin conformation at transcriptional control has begun to be recognized. However, by only using molecular biological techniques, we can hardly see the relationship between the atomic level information of DNA and the transcriptional control. Thus, in order to investigate effects of atomistic and thermodynamic properties of DNA on the enhancer-blocking activity, we performed Monte Carlo simulation with the wormlike chain model of DNA. Results are consistent with the experimental data of Ameres et al. [3], showing that the amount of gene expression is proportional to the relative proximity probability between an enhancer and a promoter. Furthermore, our results suggest that the proximity probability between these two elements is reduced because the stretching and bending energies increase when the formation of DNA loop domains modulates the enhancer-promoter interactions.
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Investigating The Structural Dynamics Of The Lict Transcriptional Antiterminator And Its RNA Target By Single Molecule FRET
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LicT belongs to a family of bacterial transcriptional anti-terminators that regulates the expression of sugar metabolizing operons through a riboswitch mechanism. When activated, these proteins bind to a short RNA hairpin in the 5′UTR of their target mRNAs and thereby prevent the formation of an overlapping transcriptional terminator. Previous structural studies of conformational changes have revealed the structural basis of RNA recognition by the N-terminal RNA-binding domain and the phosphorylatable regulation domain upon activation (1). The current model of the LicT regulation mechanism posits that the inactive protein oscillates between several open dimeric conformations. Activation via phosphorylation (or by constitutive mutations) locks the LicT dimer in a unique closed conformation which can efficiently bind and stabilize the anti-terminator RNA hairpin. We investigated the conformational dynamics of LicT and its target RNA using single molecule Forster Resonance Energy Transfer (smFRET) with Alternating Laser EXcitation (ALEX). By this technique, we obtain quantitative information on the magnitude of the structural changes undergone by the LicT protein domains and the RNA molecule in the active/inactive and free/bound states. We observe different mutations to quantify how they affect the thermodynamic equilibrium and dynamics.
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Characterization of The Open Complex of Yeast Mitochondrial RNA Polymerase
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The mitochondrial RNA polymerase (mtRNAP) of Saccharomyces cerevisiae, consisting of a complex of Rpo41 and Mtf1, is homologous to the phage single polypeptide T7/T3/SP6 RNA polymerase and mammalian mitochondrial RNA polymerases. The yeast mtRNAP recognizes a conserved nonanucleotide sequence to initiate specific transcription. However, there is little clue about how mtRNAP recognizes the promoter, melts the double-stranded DNA and forms a transcription-competent open complex, which are steps essential to initiating RNA synthesis. In this work, we have defined the region of the nonanucleotide melted by the mtRNAP using 2-aminopurine fluorescence changes that are sensitive to changes in DNA base stacking interactions. We have also characterized DNA bending of the promoter induced by mtRNAP by fluorescence resonance energy transfer (FRET) measurements. We show that DNA melting requires the simultaneous presence of Rpo41 and Mtf1, whereas DNA bending can be induced by Rpo41 itself and regulated by MtF1. By monitoring DNA melting and bending under various conditions, we are able to dissect roles of Rpo41 and MtF1 in DNA recognition and forming the open complex. These results shed new insights into mechanisms of transcription initiation of mitochondrial RNA polymerases.

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Characterizing the Effects of Highly Bent DNA on Transcription
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It is well-established that many transcriptional repressors such as the loop-forming lactose or galactose repressors tightly bend DNA. However, it remains unclear whether this bending directly affects the activity of RNA polymerase. Characterizing the effects of repressor-bound, highly stressed DNA on transcription is complicated by the inability to decouple the mechanical state of the DNA template from the kinetics of repressor binding, which itself will be influenced by the energy required for the repressor to strain the DNA. To our knowledge there currently exists no established assay capable of quantifying transcription by RNA polymerase from highly bent DNA templates which mimic the bending induced by loop-forming repressors in the absence of other DNA-binding proteins. We have developed a fluorescence-based *in vitro* assay capable of addressing this experimental limitation by exploiting the ability of DNA minicircles to impose both varying degrees of bending and twist. We are now applying this assay to characterize the rates of transcription by T7 RNA polymerase from highly stressed templates. We hypothesize that the activity of T7 RNA polymerase will be reduced on templates that are bent with degrees of curvature comparable to the loops generated by loop-forming repressor proteins. Contrary to our initial hypothesis, preliminary data suggests that at least one such highly bent DNA template is in fact readily transcribed by T7 RNA polymerase. However, the degree to which the minicircle templates are twisted beyond the torsionally relaxed helical structure may play a greater-than-expected role on the activity of RNA polymerase, and must therefore be addressed. Conclusions resulting from these measurements will shed light on the mechanism by which loop-forming repressors affect transcription, and could reveal a more significant role for the mechanical state of the DNA than was previously thought.