3436-Pos
Is RNA Self-Assembly a Mechanism Used by Cells to Influence Gene Expression?
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RNA molecules are important factors involved in different cellular processes and have a multitude of roles in the cell. These roles include serving as a temporary copy of genes used for protein synthesis or functions in translational machinery. Interestingly, RNA is so far the only biological molecule that serves both as a catalyst (like proteins) and as information storage (like DNA). However, in contrast to proteins well known to be able to self-associate, such polymers have never been reported for natural RNA. We present here evidence that such a polymer of a natural RNA, the DsrA RNA, exists in the bacterial cell. DsrA is a small noncoding RNA (87 nucleotides) of Escherichia coli that acts by base-pairing to mRNAs in order to control their translation and turnover. One of the best-characterized target of DsrA is the rpoS mRNA. This messenger is of primary importance in the bacteria because it encodes the sigma S subunit of RNA polymerase and thus is a major regulator of stress response: the production of sigma S results in dramatic changes in cellular morphology and physiology. The results we obtained about DsrA self-assembly and our assumption about the function of this assembly in the cell will be presented herein.

Protein-Nucleic Acid Interactions III

3437-Pos
ParA2, a Vibrio Cholerae Chromosome Partitioning Protein, Forms Helical Filaments on DNA
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Much less is known about how bacteria segregate DNA prior to replication than is known about such processes in eukaryotic cells. Most bacterial chromosomes contain homologs of plasmid partitioning (par) loci. These loci encode ATPases called ParA that are thought to contribute to the mechanical force required for chromosome and plasmid segregation, but the mechanisms by which these ATPases function in segregation are unknown. In Vibrio cholerae, the chromosome II (chrII) par locus is essential for chrII segregation. Electron microscopy and three-dimensional reconstruction revealed that ParA2 formed bipolar helical filaments on DNA in a sequence-independent manner. These filaments had a distinct change in pitch when ATP was present compared to when either ADP or no nucleotide was present. Fitting recently determined crystal structures of ParA proteins into our ParA2 nucleoprotein filament enabled us to model how ParA2 bound and coated the DNA. Our findings raise the possibility that ParA2-mediated changes in DNA topology could contribute to chrII segregation.

3438-Pos
Structure and Dynamics of the Cytidine Repressor DNA-Binding Domain
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The *E. coli* cytidine repressor (CytR) is a member of the LacR family of bacterial repressors that differentially regulates nine operons. The natural operators in CytR-regulated promoters are comprised of a pair of degenerate recognition half-sites arranged as inverted repeats and separated by a variable length spacer. Spacers range from 0 to 9 basepairs accounting for up to a 25Å translation and 310 degree rotation of the half-sites. Characterizing the interactions between the CytR DNA-binding domain (DBD) and DNA is critical to understanding the mechanism of differential gene regulation. Analysis of the DBD structure using NMR allowed us to assess both the structure and the dynamics of the DBD in relation to DNA sequence specificity. Here, we present the structure of a CytR DBD monomer bound specifically to one DNA half-site of the uridine phosphorylase (udp) operator. We find that the DBD exists as a three-helix bundle containing a canonical helix-turn-helix motif similar to other proteins that interact with DNA. The structure of the DBD in the presence of recognition site DNA reveals a departure in helical orientation from other members of the LacR family. In addition, the DBD structure differs when bound to nonspecific DNA and populates two distinct conformations when free. Nonspecific binding results in measurable changes in protein dynamics when compared to the protein specifically bound to the udp half-site substrate. Thus for CytR, the transition from nonspecific association to specific recognition results in changes in protein mobility that are coupled to structural rearrangements.

3439-Pos
Determining the Role DNA of Bending in Topology Simplification by Type II Topoisomerases
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Type II topoisomerases are essential and universally conserved enzymes that simplify the global topology of DNA by an ATP dependent mechanism that involves passing one double stranded segment (T segment) of DNA through a transient double stranded break in a second segment (G segment) of DNA. This core strand passage mechanism allows type II topoisomerases to decatenate DNA and relax supercoils to below equilibrium levels. Although much is known about the structure and function of these critical proteins, the specific mechanism that drives this topology simplification reaction remains an open question, though several compelling theories have been presented. Some of the more plausible theories postulate that type II topoisomerases achieve non-equilibrium topology simplification by inducing a sharp bend into the G segment. In this study we sought to determine if and to what extent type II topoisomerases impose a bend on DNA. We used Atomic Force Microscopy (AFM) to visualize protein-DNA complexes of three different type II topoisomerases that span the range of topology simplification activity. We directly measured the bend angles imposed on DNA by these proteins to determine if the bend angles could fully account for the observed topology simplification behavior. We found that type II topoisomerases bend DNA, but the measured bend angles were not in accordance with the relative non-equilibrium activity. These findings suggest that bending of DNA could be an important component of the mechanism of topology simplification by type II topoisomerases but it cannot completely account for the observed topology simplification behavior.

3440-Pos
Single-Molecule Study of Human Topoisomerase II
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Topoisomerase II is an essential enzyme that maintains genomic integrity by simplifying global DNA topology through supercoil relaxation, decatenation of linked chromosomal DNA, and DNA unknoting. This is achieved by generating a transient double-stranded break in one DNA segment through which a second double-stranded segment is passed prior to religation of the break. Failure or prevention of DNA religation leads to double stranded DNA breaks and fragmentation of the genome. Thus, the DNA cleavage reaction of human topoisomerase II (hTopo II) is one of the most successful targets for anti-cancer drugs. In order to develop effective anti-cancer drugs, it is critical to understand the detailed mechanism of DNA supercoil relaxation driven by hTopo II. One of the two isoforms of human Topoisomerase II, hTopo IIα, preferentially relaxes positive supercoils, a feature it shares with only one other type II topoisomerase; *E. coli* topoisomerase IV. Here, we have investigated the mechanism of DNA supercoil relaxation by hTopo IIα using single molecule magnetic tweezers experiments. We measured the DNA relaxation rate of positive and negative supercoils and found that positive supercoil relaxation was ~2 times faster than negative supercoil relaxation. This result indicates that the relaxation mechanism of hTopo IIα is different from that of topoisomerase IV, which displays an almost absolute preference for positively supercoiled DNA in single-molecule experiments. Our data further suggest that there is a small, ~30%, difference in the processivity of hTopo IIα relaxing positive versus negative supercoils. These data, together with twist dependent relaxation rate measurements, allow a detailed comparison with the mechanism of chiral discrimination by *E. coli* topoisomerase IV, and suggest possible mechanisms of chiral discrimination by type II topoisomerases.

3441-Pos
Dynamics of DNA-Bending in Binding Site Recognition by IHF
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We present recent progress in monitoring the DNA bending dynamics in site-specific recognition by IHF, an architectural protein from *E. coli* that recognizes several sites on phage λ DNA, primarily by indirect readout. IHF bends the DNA at its cognate site by nearly 180° over ~35 bp, creating two kinks in

DNA stabilized by intercalation of conserved proline residues located on two β-ribbon arms that wrap around the DNA.
Previous stopped-flow and laser T-jump measurements on IHF binding to its cognate H'site revealed that DNA bending in the complex occurs on ~1-10 ms, similar to the time-scales for thermal disruption of a single base pair in B-DNA. Here we find that inserted mismatches that increase the DNA flexibility at the site of the kinks accelerate the bending rates by nearly the same factor as the corresponding increase in binding affinity. On the other hand, modifications in DNA away from the site of the kink, as well as mutations in IHF, designed to perturb specific protein-DNA contacts, leave the bending rates unchanged despite a ~60-100-fold decrease in the binding affinity. These results support our earlier conclusion that in the transition state ensemble separating the nonspecific from the specific complex the DNA is bent/kinked, but protein-DNA interactions that stabilize the complex have not yet been made.
Our measurements also reveal a rapid (~100 microseconds) phase in the bending kinetics. In contrast to the relaxation rates for the slow phase, which are affected by modifications in the DNA at the site of the kinks, the relaxation rates for the fast phase appear to be unaffected. This rapid phase may correspond to the wrapping/unwrapping of the β-arms of the protein in a nonspecific binding mode, as IHF scans potential binding sites on genomic DNA.

3442-Pos
Parallel Single-Molecule Study of DNA Repressor Kinetics
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In a bottom up approach to understanding molecular motors, a synthetic protein-based molecular motor, the ''tumbleweed'', is being designed and constructed [1]. This design uses three ligand dependent DNA repressor proteins to rectify diffusive motion of the construct along a DNA track. These proteins are MetJQ44K, TrpR, and DtxR. To predict the behavior of this artificial motor one needs to understand the binding and unbinding kinetics of the repressor proteins at a single-molecule level. An assay, similar to tethered particle motions assays [2], is used to measure the unbinding rates of these three DNA repressor proteins. In this assay the repressor is immobilized to a surface in a microchamber. Long DNA with the correct recognition sequence for one of the repressors is attached to a streptavidin-coated microsphere. As the DNA-microsphere construct diffuses through the microchamber it will sometimes bind to the repressor protein. Using brightfield microscopy and a CCD camera the diffusive motion of the microsphere can be characterized and bound and unbound states can be differentiated. On the order of ten microspheres can be easily visualized at one time allowing single-molecule measurements to be done in parallel. The resulting kinetic measurements are compared to bulk binding kinetics measured in a QCM device.
[1] EHC Bromley et al. 2009. The Tumbleweed: Towards a synthetic protein motor. HFSP Journal. 3:204-212.
[2] F Vanzi et al. 2006. Lac Repressor hinge flexibility and DNA looping: single molecule kinetics by tethered particle motion. Nuc. Acids. Res. 34:3409-3420.

3443-Pos
Determination of Protein Sliding and Hopping Kinetics Along DNA using Brownian Dynamics Simulations
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Recent single-molecule tracking experiments have observed diffusion of a protein, such as LacI, along DNA and reported its 1D diffusion coefficient (D_1) in the range of 10^2 nm^2/s to 10^5 nm^2/s in the timescale of seconds. These studies, however, lack the temporal resolution to capture the protein's sliding and hopping kinetics since the sliding or dissociation time has been estimated to be on the order of several milliseconds. A protein's dissociation time from nonspecific DNA and its sliding diffusion coefficient ($D_{sliding}$) are important parameters for understanding the mechanisms governing protein interaction with non-specific DNA and quantification of facilitated diffusion. Here we report on simulations, on the timescale of seconds, of proteins interacting with nonspecific DNA subject to alternating 1D (sliding) and 3D (hopping) diffusive processes with initial rates taken from literature. Comparison with the aforementioned experimental results (D_1 in the timescale of seconds) permits calculation of an upper bound for the dissociation time and $D_{sliding}$ by analyzing the mean square displacements of the simulated individual trajectories.

3444-Pos
Single Molecule Analysis of Yeast Rrp44 Exonuclease Reveals a Spring-Loaded Mechanism of RNA Unwinding
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The eukaryotic exosome catalyzes a series of reactions such as RNA processing and decay. Rrp44 is a key catalytic subunit of the yeast exosome complex and enables multi-enzymatic activities, including endoribonuclease, exoribonuclease and duplex unwinding. Its exoribonuclease and unwinding activities are indispensable for the complete degradation of mRNA that forms a variety of secondary structures. It is, however, unknown how the unwinding activity is coordinated with the exonuclease one. We used single-molecule fluorescence techniques to investigate the mechanism of unwinding by Rrp44 in real time. Surprisingly, we found that Rrp44 does not unwind the RNA duplex each time it digests a single nucleotide off the 3'end of the single stranded RNA tail. Instead, it accumulates elastic energy during multiple steps of RNA digestion and unwinds several basepairs simultaneously. The kinetic analysis of each unwinding step shows that RNA unwinding, not RNA digestion, determines the overall RNA degradation rate. A series of control experiments varying the RNA sequence, salt concentrations and temperature, and the use of hybrid RNA/DNA duplex demonstrate that the unwinding step size is determined by the physical properties of the enzyme itself, not by the duplex stability. Our studies represent the first example of a multiple hierarchy of stepping for an exonuclease.

3445-Pos
Design and Construction of a DNA Nanostructure for Direct and Dynamic Measurement of DNA Bending by DNA-Binding Proteins
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We report the design and construction of a nanostructure that makes it feasible to measure bend angles of short DNAs using conventional fluorescence microscopy. The nanostructure is comprised of a double-stranded DNA linker ~50 bp long, with a pair of non-complementary 5 nt sequences at each end, which bind to and bridge between two DNA nanotubes in a specific way. The DNA nanotubes are 10 double-helices in circumference and ~5um long. The resulting structure is thus a very stiff polymer with only one flexible point at the linker position. When electrostatically adsorbed onto a supported lipid bilayer, the nanostructures were effectively constrained to diffuse two dimensions, allowing direct visualization and measurement of the bend angle of the linker DNA. Our data show that this is a very promising approach for direct and dynamic measurements of DNA bending induced by DNA-binding proteins.

3446-Pos
Single Molecule FRET Observations of Pre-MRNA Splicing
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The spliceosome is the snRNA-protein machine that is responsible for the precise excision of introns from pre-mRNAs in eukaryotes. The steps required for the catalytic activation of the spliceosome require a dynamic set of ATP dependent RNA-RNA and RNA-protein interactions. To begin to dissect the kinetic and conformational requirements for pre-mRNA positioning during spliceosome assembly, we have developed a single molecule FRET (smFRET) splicing assay in which donor and acceptor fluorophores are placed at various positions within the pre-mRNA. By using continuous excitation of the donor fluorophore to obtain short high resolution FRET trajectories of single pre-mRNAs in splicing extract we have been able to observe time- and ATP- dependent conformational dynamics during spliceosome assembly. In a complimentary approach we have used pulsed excitation of the donor fluorophore to limit the effects of photobleaching on our observation window. Using this complimentary approach we are now able to visualize a single pre-mRNA in the time scales required for an in-vitro splicing assay. We have seen that the pre-mRNA is taken through a series of largely reversible conformational steps throughout every step of the splicing reactions. Labeling spliceosomal proteins and RNAs in extract will give us dynamic information regarding their assembly and regulation.