1392-Pos
Salt Concentration and Force Affect HU-DNA Interaction
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HU is one of the most abundant proteins in bacterial nucleoid and participates in nucleoid compaction and regulation. We used magnetic tweezers to study the dependence of DNA condensation by HU on force, salt and HU concentration. DNA bending exhibited only flexible hinge behavior at 150 mM and 200 mM NaCl, which may be considered physiological levels. No binding was observed at 300 mM NaCl. We tracked the disassociation of HU-DNA complexes in real time and found HU binding to be fully reversible in salt concentration above 100 mM NaCl. The 90% disassociation lifetime, $t_{0.9}$, extended when the initial HU concentration in which the complexes formed was increased. If the salt concentration was raised while keeping the initial HU concentration and pulling force fixed, however, the $t_{0.9}$ decreased. Taking 150 nM HU and 0.08 pN force for example, the average $t_{0.9}$ was 233.0 minutes for 100 mM NaCl, 41.6 minutes for 150 mM, and 6.1 minutes for 200 mM. In addition, if the pulling force was increased from 0.08 pN to 0.28 pN, the $t_{0.9}$ decreased by an amount dependent on the initial HU concentration. Our results suggested that HU-DNA association and disassociation can be regulated by a combination of mechanical tension, salt and HU concentration.

1393-Pos
Molecular Properties of Telomeric TRF1/TRF2 - DNA Systems
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Telomeres are nucleoprotein complexes that protect the ends of linear eukaryotic chromosomes from degradation and fusion. Human telomeric DNA contains tandem arrays of double stranded TTAGGG repeats. Telomeric DNA forms specific complexes with many different proteins (shelterins), among which TRF1 and TRF2 are the most essential for the maintenance of telomere structure and function. TRF1 is a negative regulator of telomere length whereas TRF2 is involved in formation of telomeric higher order structures (t-loops), and functions more related to capping the DNA end. Both proteins bind to DNA as pre-formed homodimers. Although cellular functions of both these proteins are different, their structures are very similar. Both TRF1 and TRF2 contain two conserved sequence motives which form specific domains, namely homodimerisation and Myb-DNA binding domains.
In order to reveal the molecular properties of both proteins and also differences between binding modes of TRF1 and TRF2 to telomeric DNA, detailed studies of both binding domains have been performed. We carried out molecular dynamic simulations of TRF1 and TRF2 binding domains and their complexes with DNA. Starting models of studied systems were based on X-ray structures of TRF1 and TRF2 Myb-DNA binding domains [1]. The results have revealed structural differences between bound proteins and structural differences of their binding patterns with DNA. Additionally, we provide experimental evidence that interaction of both shelterins and DNA can be specifically perturbed by small molecular weight ligands. These results support the idea that TRF1/TRF2 - DNA systems are potential new targets for anticancer therapy.
1. R. Court, L. Chapman, L. Fairall, D. Rhodes, EMBO Reports 6 (2005) 39-45.

1394-Pos
Nucleic Acid Chaperone Activity of the Yeast Ty3 Retrotransposon Nucleocapsid Protein
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Reverse transcription in retroviruses and retrotransposons requires nucleic acid chaperones, which facilitate the rearrangement of nucleic acid secondary structure. The nucleic acid chaperone properties of the human immunodeficiency virus type-1 (HIV-1) nucleocapsid protein (NC) have been extensively studied, and nucleic acid aggregation, duplex destabilization, and rapid protein binding kinetics have been identified as major components of its activity. However, the properties of other nucleic acid chaperone proteins, such as retrotransposon Ty3 NC, a likely ancestor of HIV-1 NC, are not well understood. We used single molecule DNA stretching as a method for detailed characterization of Ty3 NC chaperone activity. Wild type Ty3 NC strongly aggregates both double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA), and melted DNA exhibits rapid reannealing in its presence. We also studied several Ty3 NC mutants to identify the roles of functional regions of the protein. We found that the N-terminal basic residues contribute to duplex stabilization, while the zinc finger at the C-terminus counteracts this effect. The mutants examined lack the rapid kinetics of wild type Ty3 NC, indicating that both the basic residues and the zinc finger are required for optimum chaperone activity, which is consistent with previous biochemical experiments. Ty3 NC therefore has a chaperone mechanism similar to that of HIV-1 NC. Although Ty3 NC does not exhibit the strong duplex destabilization of HIV-1 NC, this is consistent with the weaker secondary structure of the Ty3 long-terminal repeat region, which suggests that strong duplex destabilization is not needed for NC to facilitate minus-strand transfer during reverse transcription. This research was supported in part by funding from INSERM and ANRS (France).

1395-Pos
5′-Single Stranded DNA Duplex Junctions Provide Specific Loading Sites for the *E. coli* UvrD Single Stranded DNA Translocase
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E. coli UvrD is a 3' to 5' SF1 helicase/ translocase involved in a variety of DNA metabolic processes. UvrD can function either as a helicase to unwind duplex DNA or simply as a single stranded (ss) DNA translocase. The switch between helicase and ss translocase activities *in vitro* is controlled by the UvrD oligomeric state, such that a UvrD monomer has only ssDNA translocase activity, whereas at least a dimeric form of UvrD is required to activate its helicase activity *in vitro*. A 3'-ssDNA partial duplex provides a high affinity site for UvrD monomer binding, however, the monomer is inhibited from initiating DNA unwinding. Here we show that a UvrD monomer also binds with specificity to duplex DNA junctions with a 5'-ssDNA flanking region, with nearly a 20-fold higher specificity than for ssDNA. Furthermore, the UvrD monomer can initiate 3' to 5' ssDNA translocation from this site. The higher specificity for the junction results in time courses that reflect the ssDNA translocation of two populations of UvrD monomers: I.) UvrD initially bound a the 5'-ss/dsDNA junction and II.) UvrD initially bound to random sites along the 5'-ssDNA tail. Our results suggest that the population of UvrD initially bound at the junction translocates with different translocation kinetic parameters. We hypothesize that a 5'-ss-duplex DNA junction may serve as a high affinity loading site for the monomeric UvrD translocase, and that this may facilitate its role as an anti-recombinase to disassemble RecA nucleoprotein filaments formed within a ssDNA gap or at arrested replication forks.

1396-Pos
Mechanisms of Nucleotide Cofactor Interactions with the RepA Protein of Plasmid RSF1010
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The dynamics of the nucleotide binding to a single, noninteracting nucleotide-binding site of the hexameric helicase RepA protein of plasmid RSF1010 has been examined, using the fluorescence stopped-flow method. The experiments have been performed with fluorescent analogs of ATP and ADP, TNP-ATP, and TNP-ADP. In the presence of Mg2+, the association of the cofactors proceeds as a sequential three-step process.
The sequential nature of the mechanism indicates the lack of significant conformational equilibria of the helicase prior to nucleotide binding. The major conformational change of the RepA helicase - nucleotide complex occurs in the formation of (H-N)2., which is characterized by a very high value of the partial equilibrium constant and large positive changes of the apparent enthalpy and entropy. Strong stabilizing interactions between subunits of the RepA hexamer contribute to the observed dynamics and energetics of the internal transitions of the formed complexes. Magnesium mediate the efficient and fast conformational transitions of the protein, independent of the structure of the cofactor phosphate group. The ssDNA bound to the enzyme preferentially selects a single intermediate of the RepA - ATP analog complex, (H-N)2, while the DNA has no effect on the intermediates of the RepA - ADP complex. Allosteric interactions between the nucleotide- and the DNA-binding site are established in the initial stages of the complex formation. In the presence of the ssDNA, all transitions in nucleotide binding become sensitive to the structure of the cofactor phosphate group.

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Dynamics of the ssDNA Recognition by the RepA Hexameric Helicase of Plasmid RSF1010. Analyses Using Fluorescence Stopped-Flow Intensity and Anisotropy Methods
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Kinetic mechanism of the ssDNA recognition by the RepA hexameric replicative helicase of the plasmid RSF1010 and the nature of formed intermediates, in the presence of the ATP nonhydrolyzable analog, AMP-PNP, have been examined, using the fluorescence intensity and anisotropy stopped-flow, and analytical ultracentrifugation methods. Association of the RepA hexamer with the ssDNA