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.

1397-Pos

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

oligomers, which engage the total DNA-binding site and exclusively the strong DNA-binding subsite, is a minimum four-step sequential mechanism. Extreme stability of the RepA hexamer precludes any disintegration of its structure and the sequential character of the mechanism indicates that the enzyme exists in a predominantly single conformation prior to the association with the nucleic acid. Moreover, the hexameric helicase possesses a DNA-binding site located outside its cross channel. The reaction steps have dramatically different dynamics, with rate constants differing by two - three orders of magnitude. Such behavior indicates a very diverse nature of the observed transitions, which comprises binding steps and large conformational transitions of the helicase, including local opening of the hexameric structure. Steady-state fluorescence anisotropies of intermediates indicate that the entry of the DNA into the cross channel is initiated from the 5' end of the bound nucleic acid. The global structure of the tertiary, RepA - ssDNA - AMP-PNP complex is very different from the structure of the binary, RepA - AMP-PNP complex, indicating that, in equilibrium, the RepA hexamer - ssDNA - AMP-PNP complex exists as a mixture of partially open states.

1398-Pos

Energetics of the E. Coli Pria Helicase Interactions with the Double Stranded DNA

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Quantitative analyses of the interaction of the Escherichia coli monomeric PriA helicase with the double-stranded DNA (dsDNA) have been studied with fluorescent dsDNA oligomers, using quantitative fluorescence titrations, analytical ultracentrifugation, and fluorescence resonance energy transfer methods. The experiments have been performed with different dsDNA oligomers, long enough, to encompass the total DNA-binding site, as well as the DNA-binding site proper of the enzyme. Interactions with the dsDNA oligomers were examined as a function of different temperature, salts, and nucleotide cofactors. The stoichimetry of the PriA helicase - dsDNA is different from the stoichiometry of the analogous complexes with the ss conformation of the nucleic acid, indicating a very different orientation of the helicase on the dsDNA. Surprisingly, the intrinsic dsDNA-affinity of the enzyme is dramatically higher than the ssDNA affinity, indicating strong selectivity of the helicase for the dsDNA conformation of the nucleic acid. The intrinsic affinities are salt-dependent and the formation of the PriA helicase - dsDNA complex is accompanied by a net ion change. Moreover, the presence of nucleotide cofactors has a profound effect on the dsDNA interactions of the enzyme with the DNA. The interactions of the PriA helicase with the dsDNA are characterized by very weak, if any, cooperative interactions. The significance of these results on activities of the PriA helicase in the cell metabolism is discussed.

1399-Pos

Mechanistic Studies at the Single Molecule Level Reveal the Dynamics of HCV Polymerase Protein in Complex with RNA

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Viral RNA-dependent RNA polymerases (RdRp) that belong to the Flaviviridae family, including hepatitis C virus (HCV), are capable of initiating de novo RNA synthesis. The non-structural protein 5B (NS5B) in HCV shows RdRp activity that is required for viral replication. Due to its critical role in life cycle, understanding the mechanism of mRNA synthesis in HCV is fundamental for current drug discovery efforts.

We will present our results on studies on the enzymatic activity of the Hepatitis C Virus (HCV) RNA polymerase protein. Our studies, conducted with state-of-the-art fluorescence single molecule methodologies, aim to elucidate the dynamics of key protein/nucleic acid complexes.

Our work was conducted on DNA:RNA templates labeled with Cy5/Cy3 (Acceptor/Donor) fluorophores capable of undergoing Forster Resonance Energy Transfer (FRET). Binding of NS5B caused a significant increase in FRET. The SM-FRET studies on RNA-protein complexes revealed protein dynamics occurring with time scales of a few seconds. These dynamics change with the RNA template length, and with the presence of complementary DNA strands. Taken together, our single molecule studies provide for the first time direct evidence on the polymerase-substrate binding process and the effect of template length on protein dynamics.

1400-Pos

Single-Molecule Visualization of the Oligomeric form of *Escherichia Coli* UvrD Helicase *In Vitro*

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Escherichia coli UvrD protein is a superfamily 1 DNA helicase which plays a crucial role in nucleotide excision repair and methyl-directed mismatch repair. There is a general consensus that the enzyme unwinds a duplex DNA from a 3′ end single-stranded DNA (ssDNA) tail, a gap or a nick. However, conflicting models for the unwinding mechanism have been proposed. Concerning its stoichiometry, some biochemical studies have suggested that the enzyme has optimal activity as an oligomeric form. However, a structural study has indicated that the enzyme functions as a monomer deduced from structural analysis of UvrD-DNA complexes. To address this issue, we attempted to unravel the number of UvrD molecules bound to DNA in the presence and absence of nucleotide by single-molecule fluorescence microscopy. We performed single-molecule visualization of a Cy5-labeled Cys-Ala mutant (Cy5-UvrDC640A), in which Cys52 was labeled with high specificity, bound to 18-bp duplex DNA having a 12, 20 or 40-nt ssDNA tail under several Cy5-UvrDC640A concentrations (0.5, 1.0 and 2.0 nM). We analyzed the number of Cy5 photobleaching steps to quantify the number of UvrD molecules bound to the DNA in the absence and presence of an ATP analog, ATPγS. All the distributions of the number agreed well with the predicted distributions which support the model that UvrD protein is bound to the DNA as an oligomeric form. In the presence of ATP, inefficient DNA unwinding in the absence of free Cy5-UvrDC640A in solution and higher fluorescence intensity of Cy5-UvrDC640A compared to that non-specifically attached on the surface were observed. These results indicate that an oligomer of UvrD is the active form of the helicase.

1401-Pos

Mechanism of DNA-Dependent Enzymatic Activation of E. Coli RecQ Helicase

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RecQ family helicases, which are widespread from humans to bacteria, play essential roles in homologous recombination mediated DNA double strand break repair. Escherichia coli RecQ helicase suppresses illegitimate recombination, initiates homologous recombination, and stabilizes stalled replication forks as part of the recF pathway. Many aspects of RecQ function have been investigated in detail. However, a detailed understanding of the mechanoenzymatic mechanism of RecQ (or other superfamily-2 helicases) is still missing. Here we present a detailed quantitative model of the DNA-dependent ATPase mechanism of Escherichia coli RecQ helicase, based on steady-state, rapid transient kinetic and fluorescence spectroscopic data. We show that the binding of DNA to RecQ does not influence the rapid and reversible process of nucleotide (ATP, ADP) binding. The interaction of RecQ with DNA, however, is important for ATP hydrolysis, which is unfavorable in the absence of DNA. The high DNA-binding affinity of the post-hydrolysis state of RecQ indicates that this step may be coupled to translocation on DNA. Our data suggest that the rate-limiting step of the cycle is the hydrolysis step in the absence of DNA, whereas in DNA bound state the reversible hydrolysis and the irreversible phosphate release together determine the rate of the reaction. Translocation along single-stranded DNA enhances the ATPase activity of RecQ. Our data show that, once bound to ssDNA oligonucleotides, RecQ performs processive translocation until it reaches the 5' end, from which it rapidly dissociates to avoid futile cycling. These mechanistic findings will lead to a deeper understanding of superfamily-2 helicase function.

1402-Pos

E. Coli RecBC Helicase Actively Translocates on Both Strands during DNA Unwinding

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E. coli RecBCD is a bipolar DNA helicase consisting of two superfamily-1 motor subunits: RecB (3' to 5' directionality) and RecD (5' to 3' directionality). Although these subunits have opposite translocation polarities, they function in unison and unwind duplex DNA in the same net direction by acting on opposite ends of the nucleic acid. We have investigated previously the mechanism of initiation of DNA unwinding by the single motor RecBC helicase, which lacks the RecD motor subunit. In order to understand the relationship between single stranded (ss) DNA translocation and DNA unwinding, we compared the ssDNA translocation mechanisms of the RecB monomer and the RecBC heterodimer using stopped-flow fluorescence approaches. The RecB monomer translocates 3' to 5' along the linear ssDNA lattice with a macroscopic rate of 803 ± 13 nt/sec. This is about two times faster than the rate determined previously for RecBC unwinding ($mk_{obs} = 348 \pm 5$ bp/sec). RecBC can also translocate on ssDNA in the 3' to 5' direction with a similar rate ($mk_t = 920 \pm 6$ nt/sec). Remarkably, we also find that RecBC is able to translocate along ssDNA with the opposite directionality (5' to 3'). These results suggest an allosteric communication between the RecB motor domain and another region of the RecBC enzyme that allows RecBC to actively translocate along both DNA strands during DNA unwinding, without the aid of the RecD subunit. (supported by NIH GM045948).