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).