

remodeling complex from *S. cerevisiae*. We monitored the double-stranded DNA translocation activity of RSC through its ability to actively displace streptavidin from biotin labeled double-stranded DNA. This displacement activity is ATP-dependent and is correlated with the translocation of the complex along the double-stranded DNA. We have also characterized the double-stranded DNA translocation of RSC through the use of a fluorescence-based stopped-flow assay in which the translocation of the complex is monitored through the interactions of the complex with fluorophores attached to the ends of the DNA. The kinetics of double-stranded DNA binding were also monitored using a fluorescence-based stopped-flow assay. The results indicate that double-stranded DNA binding occurs through a two-step mechanism, similar to what has been reported for other chromatin remodeling complexes and genetically related helicases.

1409-Pos

***E. coli* SSB Under Tension**

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E. coli SSBs (single-stranded DNA binding proteins) are essential accessory proteins in replication, recombination and during DNA repair. They not only protect ssDNA from chemical and nucleolytic attack, but also associate with many genome maintenance proteins. *E. coli* SSBs are tetrameric and have at least two binding modes: SSB₃₅ and SSB₆₅, which bind 35 and 65 nucleotides, respectively. Proteins in the SSB₃₅ mode bind cooperatively, interacting with each other and creating long nucleoprotein filaments. In this study, we present mechanical studies of SSB bound to ssDNA using dual trap, high-resolution optical tweezers. This assay allows us to probe the interaction of SSBs to ssDNA constructs of various lengths, in real time, with nanometer resolution. By directly detecting wrapping of ssDNA by a single protein, we are able to characterize the thermodynamics and kinetics of nucleoprotein complex formation. Mechanical pulling of our constructs in the presence of SSBs reveals that the protein condenses ssDNA in the force range 0-8 pN and that tension can be used to modulate the binding mode of SSBs. Binding kinetics further indicate that SSBs bind to ssDNA in two successive processes consisting of a loose binding step in which the protein associates weakly with its substrate, followed by a wrapping step in which ssDNA is condensed.

1410-Pos

Analysis of Nucleic Acid Conformations and Amino Acid Propensities in Single-Stranded Binding Proteins

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Single stranded DNA- or RNA-protein complexes appear in a number of biological pathways, particularly those relating to DNA replication, gene regulation, and chromosome stability. Protein-nucleic acid binding in these complexes has been studied for some time, and a number of structures of these complexes exist; however, few consistent rules for binding have been determined. In particular, DNA and RNA conformation appears to vary widely, even when bound to homologous proteins (e.g., telomere end-binding proteins). No systematic study of nucleic acid conformational has been done, so it is unknown how different these conformations actually are from each other or from double-stranded DNA and RNA. In addition, no comparison of amino acid propensity for single stranded versus double stranded nucleic acids has been done. To those ends, we have built a non-redundant database of single stranded binding proteins. We have calculated various base step conformational and find wide statistical distributions for these parameters, but with some significant differences from double stranded nucleic acids.

1411-Pos

Binding and Bending Parameters of Integration Host Factor to Four-Way Holliday Junction

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Binding and Bending Parameters of Integration Host Factor to Four-Way Holliday Junction

Integration host factor (IHF) is a small heterodimeric protein that sequence specifically binds the minor groove of DNA and facilitates a bend of nearly 180 degrees. This bending is crucial for cellular processes such as recombination, replication and transcription. Previous work in the Mukerji lab characterized the binding properties of the structurally similar but sequence non-specific DNA-binding protein HU, to duplex and Holliday junction DNA. This research demonstrated that HU binds to the central region of the junction with nanomolar affinity and prefers the stacked form of the Holliday junction. Given the similarities in structure and function of HU and IHF, we elected to study the

binding capability of IHF to the Holliday junction. We have compared the binding affinity of IHF for Holliday junction DNA with its binding affinity to duplex DNA with and without a consensus sequence. Binding measurements were performed using fluorescence intensity and anisotropy methods and confirmed with the gel mobility shift assay. All binding assays established that IHF binds to the Holliday junction lacking a consensus sequence with high affinity (~3 nanomolar K_d) similar to HU, suggesting that both proteins might recognize and bind similar structural aspects of the Holliday junction. Moreover, anisotropy affinity measurements demonstrated that IHF binds the Holliday junction with similar affinity as it does to duplex DNA containing its consensus sequence. The span of binding capabilities exhibited by IHF indicate that it can function both as a specific and non-sequence specific DNA binding protein.

1412-Pos

Inhibition of Eukaryotic RNase P

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Ribonuclease P (RNase P) is an essential RNA enzyme found in all phylogenetic domains that is best known for catalyzing the 5' endonucleolytic cleavage of precursor transfer RNAs (pre-tRNAs). In bacteria, the enzyme consists of a single, catalytic RNA subunit and one small protein, while the archaeal and eukaryotic enzymes have 4-10 proteins in addition to a similar RNA subunit. The RNA has been shown to act as a ribozyme at high salt *in vitro*; however the added protein optimizes kinetics and makes specific contacts with the pre-tRNA substrate. The bacterial protein subunit also appears to be required for the processing of non-tRNA substrates by broadening substrate recognition tolerance. In addition, the immense increase in protein content in the eukaryotic enzyme suggests substantially enlarged capacity for recognition of additional substrates. Recently, intron-encoded box C/D snoRNAs and HRA1 RNA were shown to be likely substrates for yeast RNase P. In addition, yeast RNase P seems to be inhibited by single stranded RNA. This inhibition was shown to be specific to *S. cerevisiae* RNase P but not bacterial RNase P with poly-ribonucleic acid homopolymers having varying levels of inhibition (polyG>polyU>>polyA>>>polyC). This inhibition is also size dependent with larger RNA being inhibitory. In addition, the sequence specificity seen with the small homoribopolymers is lost, as larger RNA of various sequences show relatively high levels of inhibition. To further characterize the extent of RNase P inhibition by these various inhibitors, the location of the inhibitor contact is being mapped by crosslinking. Individually 6xHis tagged strains are being used to enable isolation of crosslinks induced by UV light. The characterization of the eukaryotic specific inhibitor interaction with yeast RNase P will provide further information about the evolution of the essential activity of tRNA processing.

1413-Pos

Finding the Right 'Mis' Match: Millisecond Conformational Dynamics of MutS-DNA Complex During DNA Damage Recognition

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Errors in replication and recombination cause base-pair mismatches and insertion-deletion loops (IDLs) in DNA, which, if unchecked, lead to mutations implicated in cancer and heart disease. Thus, repair pathways that detect such errors and initiate their repair are vital to preserving DNA integrity. This study focuses on prokaryotic *T. aquaticus* (Taq) MutS and its eukaryotic homolog *S. cerevisiae* MutS_{sc}, both proteins that recognize mispairs and IDLs in DNA and recruit downstream proteins to rectify the damage. Structural studies have shown that MutS-bound DNA is bent at the mispair/IDL site. However, the underlying mechanism of recognition of these target sites is not well understood. In particular, the role of DNA flexibility at the target site in the recognition mechanism remains unclear. We employ nanosecond laser temperature jump (T-jump) to perturb the MutS-DNA complex, and monitor the conformational relaxation dynamics on microsecond-to-milliseconds time-scales relevant to the formation of the initial recognition complex that triggers DNA repair. We have carried out equilibrium and kinetic measurements on DNA labeled with 2-aminopurine (2AP) adjoining a T-bulge, in complex with Taq MutS and MutS_{sc}. Equilibrium fluorescence measurements as a function of increasing temperature reveal a sharp increase in 2AP fluorescence at ~40°C for MutS_{sc}, and at ~70°C for Taq MutS, indicating a significant conformational change in the MutS-DNA complex near their optimal physiological temperatures. Relaxation kinetics monitored with 2AP fluorescence in response to a rapid T-jump show relaxation kinetics in Taq MutS near 70°C, occurring with a time constant of a ~10 milliseconds. This rapid phase has not been observed in previous dynamics studies, and we propose that it may correspond to the transition from a non-specific MutS-DNA complex to a specific complex that signals initiation of repair.