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Atomistic Studies Support a Detailed Model of RecA Mediated Homology Recognition and Strand Exchange

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The bacterial recombinase RecA mediates homologous recombination and recombinational DNA repair through homology search and strand exchange. During homologous recombination, RecA binds an incoming single-stranded DNA (ssDNA) in its primary binding site, site I, to form a nucleoprotein filament whose crystal structure is known. The structure of the final post-stand exchange structure with double stranded DNA (dsDNA) bound in site I is also known; however, despite enormous effort, the details of homology testing have remained uncertain, partly because of the dearth of information about the structure of DNA during homology testing. Recent work has suggested that homology testing begins with the dsDNA binding to the secondary RecA binding site, site II. With detailed examination of the crystal structure, we found that an intermediate structure must exist such that homology recognition does not simply proceed from the searching state to the final post-strand exchange state, suggesting that homology recognition is governed by transitions to and from the intermediate structure that differs significantly from its final position. In this work, we present evidence that ~ 9-15 dsDNA base pairs can bind to site II in a metastable conformation where single complementary strand bases can flip and stably pair with corresponding sequence matched incoming strand bases. That stable pairing deepens the dsDNA binding, allowing time for base flipping and strand exchange which in turn allows more dsDNA bases to bind to site II. In the absence of pairing between the complementary and incoming strands, a series of transitions drives dsDNA unbinding, with little opportunity for additional base flipping. Such a system offers very extremely rapid rejection of almost all mismatched pairings, which allows bacterial genomes to be searched on biologically relevant timescales.

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Investigation of the Drug Like Molecule Binding to DNA Single Strand Breaks using Improved Hydroxyl Radical Cleavage Methodology

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Cancer cells can have greater replication rate as well as defective DNA repair. The defective repair can lead to the accumulation of damaged DNA. One alternative approach to treat cancer cells is to hide the damaged cells from the repair machinery leading to fatal errors. The aim of this research is to discover drug-like molecules that could mask the DNA damaged sites. Various drug-like molecules have been tested for this purpose. The duplex DNA shown below is being used:
3'-CCGTGGTCCAGCCGCTCGGAGTGAGACTGTTTTTTTTTTT −5' (A)
5'-GGCACCAGGTCGGCGAGCCTCACTCTGAC-3' (B) An improved hydroxyl radical cleavage protocol is used that includes the use of a dT tail attached with the 5' end, which allows the co-precipitating the cleavage products with poly(A). The last 5' end T of dT tail is covalently attached to the fluorescent label Oregon Green 514. The dye avoids the limitations due to the lifetime of a 32P label. Preliminary results will be presented.

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Global and Local Conformation of Mismatched Duplex DNA Upon MSH2-MSH6 Binding Studied by Steady-State and Time-Resolved Fluorescence

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The DNA mismatch repair (MMR) system guards the integrity of genetic material by scanning and correcting errors in a post-replicative manner. In eukaryotic cells, the initiation of MMR is achieved by recognition of biosynthetic errors by Msh proteins (MutS homologs). The recognition of single base mismatches and small insertion/deletion loops (IDL) by the Msh2-Msh6 heterodimer is an important initial event in the MMR pathway. Several studies have suggested that the prokaryotic MutS homodimer and its eukaryotic counterpart Msh2-Msh6 bind duplex DNA containing a mismatch or IDL with a higher affinity than homoduplex DNA. However, the exact mechanism by which Msh2-Msh6 distinguishes different types of mismatched base pairs from a large excess of canonical Watson-Crick base pairs is still unknown. In this study, we are utilizing DNA duplexes containing 6-methylisoxanthopterin (6-MI) a fluorescent nucleoside analog to measure the binding affinity of S. cerevisiae Msh2-Msh6 to different single base pair mismatches. For greater sensitivity this analog is incorporated into a pentamer sequence ATFAA (F = 6-MI), which exhibits enhanced fluorescence. Fluorescence anisotropy measurements performed with these intrinsically labeled duplexes reveal the following order for Msh2-Msh6 binding affinity to DNA mismatches: G:T > +T ≈ G:A > G:C. This order is consistent with previously reported affinities measured in the gel. We have further investigated DNA dynamics upon Msh2-Msh6 binding using time-resolved fluorescence spectroscopy. Specific placement of the probe at the mismatch site or adjacent to it reveals significant local motion prior to protein binding. We observe that those mismatches with the highest affinity exhibit the greatest amount of motion. Protein binding stabilizes mismatch local motion, which is potentially consistent with Phe intercalation at the site, as observed in Msh2-Msh6-DNA co-crystal structures.

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Structurally Distinct Complexes of Ubiquitin and Sumo-Modified PCNA Lead to Distinct DNA Damage Response Pathways

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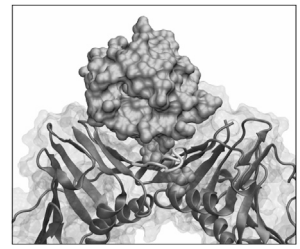
Ubiquitination of proliferating cell nuclear antigen (PCNA) in response to DNA damage leads to the recruitment of specialized translesion polymerases to the damage locus. This constitutes the initial step in translesion synthesis (TLS) - a critical pathway for cell survival and genome stability. By contrast, PCNA sumoylation leads to suppression of homologous recombination through recruitment of the antirecombinogenic helicase SRS2. How do these similar posttranslational modifications effect such vastly different functional outcomes? We modeled PCNA covalently modified by Ub and SUMO using a multi-scale protocol (conjugated docking with the Rosetta package, molecular dynamics and minimal ensemble searches). Our models rationalized the differences in solution scattering data (SAXS) from the PCNA-K164Ub, PCNA-K107Ub and PCNA-K164SUMO complexes. Our results suggest a structural basis for the different functional outcomes of Ub vs. SUMO modification of PCNA.



3502-Pos Board B230

Investigating Cre-Recombinase-Mediated DNA Looping using FRET

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The Cre recombination system has become an important tool for genetic manipulation of higher organisms and a model for site-specific DNA-recombination mechanisms employed by the λ-Int superfamily of recombinases. We report a novel quantitative approach for characterizing the probability of DNA-loop formation in solution by using time-dependent ensemble FRET measurements of Cre-recombination kinetics. Because the mechanism of Cre recombinase does not conform to a simple kinetic scheme, we employ numerical methods to extract rate constants for fundamental steps that pertain to Cre-mediated loop closure. Cre recombination does not require accessory proteins, DNA supercoiling, or particular metal-ion cofactors and is thus a highly flexible system for quantitatively analyzing DNA-loop formation in vitro and in vivo.

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DNA Bending and Discrimination of Mismatches by MutS and Human Homologs

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Post-replication repair of mismatch-containing DNA is a conserved process for ensuring DNA fidelity in prokaryotic and eukaryotic organisms. In this process, the MutS enzyme is responsible for initially recognizing defective DNA before signaling for subsequent DNA repair to occur. Structures of MutS in complex with mismatch DNA show highly bent DNA suggesting that DNA bending plays a role in the recognition process. Here, we present results from molecular dynamics simulations that indicate that mismatch-containing DNA can be bent more easily than DNA with canonical DNA and support that DNA bending is a key step in recognizing defective DNA. Results from additional simulations of the eukaryotic homologs MSH2-MSH6 and MSH2-MSH3 that specialize on recognizing mismatch-DNA vs. insetion/deletion-containing DNA, respectively,