is the dominant pathway of telomere protection against RPA binding. TPP1 enhances POT1's ability to compete against RPA in both pathways. Thus, the synergistic effect of GQ formation and POT1/TPP1 binding protects telomeric overhangs against DNA damage signals.

396-Pos Board B165

Energy Landscapes of Triplet Repeat DNA Bulge Loops: Implications for DNA Expansion and Disease States

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Triplet repeat DNA sequences are prone to undergo DNA expansion events that correlate with a range of debilitating neurological diseases such as Huntington's and myotonic dystrophy type 1 (DM1). Repeat DNA expansion occurs in response to error prone DNA replication, DNA recombination, and DNA repair events. It is commonly believed that the propensity of repeat DNAs to adopt (meta-)stable self-structures plays a critical role in the processes that lead to the erroneous expansion events. In particular, repeat DNA sequence can form slipped out bulge-loop structures via out-of-phase alignment between the two different DNA strands. Such slipped out bulge loop structures may be difficult targets to process by the DNA replication, DNA recombination, and/or DNA repair machinery, thereby contributing to expansion events. To better understand such altered substrate structures, we have been mapping the energy landscape of select models of repeat bulge loops using differential scanning calorimetry and temperature dependent spectroscopy. Our data reveal novel insights into the thermal and thermodynamic features of such slipped-out repeat DNA bulge loop structures, including characteristics that may contribute to their differential recognition and processing that lead to expansion events, the genotypical signature that correlates with neurological disorders.

397-Pos Board B166

WT UV-DDB Performs a 3D Search on DNA whereas the XP-E Mutant (K244E DDB2) Mutant Slides

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The DNA damage binding protein complex (UV-DDB) recognizes ultraviolet light (UV) induced lesions such as 6-4 photoproducts (6-4PPs) and cyclobutane pyrimidine dimers (CPDs) in DNA and initiates human nucleotide excision repair in chromatin. Crystallographic studies have revealed that UV-DDB binds to damaged DNA as a heterodimer of DDB1 and DDB2 on short DNA substrates; however, its oligomeric state on longer, physiologically relevant substrates and on nucleosomes remains undetermined. Additionally, the question of how UV-DDB searches a sea of undamaged chromatin for UV-induced lesions remains unresolved. We assayed purified UV-DDB for binding to a 517 bp UV-irradiated, PCR fragment using atomic force microscopy (AFM). Volume analysis revealed that UV-DDB binds primarily as a dimer of heterodimers to UV damaged DNA, with 19% of these UV-DDB dimers binding simultaneously to two DNA molecules In order to study damage recognition in real time, we used a His-tag on UV-DDB (either DDB1 or DDB2) to conjugate quantum dots(QDs). QD-UV-DDB retained DNA damage binding activity, as assayed by electrophoretic mobility shift assays. To identify the search mode, we have employed an oblique angle fluorescence microscopy setup to track single molecules of QD tagged UV-DDB on UV-damaged DNA tightropes. We have identified that WT UV-DDB employs a 3D search to identify DNA damage, with a long residence time when bound to sites of damage. Consistent with several salt-bridges observed in the co-crystal structure, we have found that the mobility of UV-DDB on DNA is salt dependent. Further, we have assayed the disease causing K244E mutant of DDB2 and observed that the mutant retains DNA binding activity, but slides on DNA compared to the WT. Our results reveal the stoichiometry and search mechanism of UV-DDB in damage surveillance.

398-Pos Board B167

Formation and Stability of E.Coli RecA Nucleoprotein Filament Regulated by Single-Stranded DNA Binding Protein

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In Escherichia coli, the RecA nucleoprotein filament formed on single-stranded DNA (ssDNA) is essential for homologous recombinational DNA damage repair. In vivo, formation and stability of the RecA nucleoprotein filament is regulated by the single-stranded DNA binding protein (SSB). However, current understanding of RecA-ssDNA interaction has been mainly based on experiments in the absence of SSB. In this study, direct knowledge of the influence of SSB on the RecA nucleation, polymerization, and stability is obtained by single ssDNA manipulation method using magnetic tweezers. Our results reveal an SSB, force, and ATP hydrolysis dependent regulation of the RecA nucleoprotein filament formation and stability.

399-Pos Board B168

RecA Filament Migration on a Single-Stranded DNA

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RecA protein lies at the core of DNA repair mechanism. RecA forms a helical-structured filament on a single-strand DNA (ssDNA) as an intermediate of homologous recombination. Recent crystal structure of RecA filament with embedded ssDNA found that each RecA monomer in the filament is bound to a group of three nucleotides, simultaneously forming a gap between neighboring groups. Thus, there are three possibilities (phases) in the binding of RecA according to the relative position of the gap in the substrate ssDNA. We developed a single-molecule fluorescence based assay to identify the phase of individual RecA filament. On a polythymine substrate, RecA filaments were formed randomly in the three phases. Each RecA filament changed its phase via migration along the substrate ssDNA for which the energy from ATP hydrolysis was required. On the other hand, the RecA filament was arrested into a single phase when the poly thymine was replaced by TGG repeats. Our results provide a new insight into the molecular mechanism of sequence specific RecA filament dynamics.

400-Pos Board B169

***In Vivo* Visualization of Mismatch Repair in *Bacillus Subtilis* using Single-Molecule Fluorescence Microscopy**

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The high fidelity of DNA replication is vital to genomic integrity and ultimately to the survival of species. Errors occurring during DNA replication can have severe consequences such as producing certain types of cancers, and the correction of these errors is carried out by various DNA repair systems, one of which is the mismatch repair (MMR) system that exists in both prokaryotes and eukaryotes. Mismatched nucleotides are recognized by MutS, an MMR system protein that first binds to the mismatched DNA and then recruits other MMR proteins responsible for the downstream repair process. Despite its crucial role in this process, little is known about how MutS first locates mismatched base pairs. Two competing models have been proposed in the literature: the replisome-associated model argues that MutS is bound to and travels with the replication fork and can bind mismatches immediately as they are produced, while the scanning model states that MutS binds and scans the DNA strands on its own to search for mistakes long after the DNA polymerase complex has synthesized DNA. To address this question, we apply single-molecule fluorescence microscopy to reveal the *in vivo* distributions and dynamics of MutS and the replisome in live *Bacillus subtilis* bacterial cells. Based on photoactivated localization microscopy (PALM), PAmCherry-labeled MutS and mCitrine-labeled DnaX (a subunit of the replisome) are tracked with nanometer-scale precision to reveal the spatial relationship between these two as well as their motional correlations. Our preliminary results suggest transient yet significant co-localization between MutS-PAmCherry and DnaX-mCitrine and thus support the replisome-associated model.

401-Pos Board B170

Quadruplex Priming Amplification of DNA Coupled with Nicking Endonuclease Activity

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Identification, amplification and quantification of nucleic acids for detection of pathogenic organisms and genetic diseases is one of the most important field in biomedical research. Polymerase chain reaction (PCR) is commonly used for nucleic acid diagnostics. However, temperature cycling, limited yield of product DNA, the need for specialized instrumentation and expensive detection probes are not compatible with the goals of point-of-care diagnostics. To address these limitations we have developed quadruplex priming amplification (QPA), which relies on specifically designed guanine-rich primers. After polymerase elongation, the primers are capable of spontaneous dissociation from