furthermore suggest that MutS and its homologs are exquisitely tuned to bend DNA just enough to discriminate mismatches from canonical DNA. Moreover, the recognition of insertion/deletion-containing DNA also appears to require bending but at an even greater extent than mismatch-containing DNA. The simulation results are further discussed in the context of the overall mechanism of post-replication DNA repair.

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Single Molecule Dynamics Governing the Initiation of V(D)J Recombination

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The recombination activating genes (RAG)1 and RAG2 perform V(D)J recombination by rearranging conserved recombination signal sequences (RSSs) to generate antigen-receptors during lymphopoiesis. However the orchestration of V(D)J recombination on biologically relevant (long) length scales has resisted experimental investigation. Here we develop single-molecule assays to watch in real time as RAG1/2 and its co-factor HMGB1 carry out V(D)J recombination from start (RSS binding) to finish (hairpin formation) on long DNA molecules. We capture various intermediate states preceding hairpin formation, show how RAG1/2 and HMGB1 form bends on the DNA, demonstrate how the identity of the recombination signal sequence modulates bending with single bp resolution and show HMGB1 must compact DNA flanking RSSs to form hairpins. Our results provide single-molecule mechanistic insight into the orchestration of V(D)J recombination.

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Substrate Interactions of a Human DNA Alkyltransferase

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Human cells contain DNA alkyltransferases that protect genomic integrity under normal conditions but also defend tumor cells against chemotherapeutic alkylating agents. Here we explore how structural features of the DNA substrate affect the binding and repair activities of the human O6-alkylguanine-DNA alkyltransferase (AGT).

To perform its repair functions, AGT partitions between adduct-containing sites and adduct-free genomic DNA. Cooperative binding results in an all-or-nothing association pattern on short templates. The apparent binding site size S(app) oscillates with template length. Oscillations in cooperativity factor ω have the same frequency but are of opposite phase to S(app) so the most stable complexes occur at the highest packing densities. At high binding densities the site size (~4 bp/protein) is smaller than the contour length (~8 bp) occupied in crystalline complexes. A protein-overlap model has been proposed; this predicts that optimal protein-protein contacts will occur when the DNA is torsionally relaxed. Binding competition and topoisomerase assays support this prediction and predict that AGT will partition in favor of torsionally-relaxed, relatively protein-free DNA structures like those near replication forks.

AGT must also function at telomeres, where G-rich sequences have the potential to form quadruplex structures and where methylation at the O6 position of guanines interferes with quadruplex formation. AGT binding to quadruplex DNA is characterized by reduced binding stoichiometries, affinities and O6-methyl G repair activities when compared to linear DNAs. Thus, AGT may function best at telomeres when quadruplex formation is inhibited by helicases or other telomere-binding proteins. This work was supported by NIH grant GM070662 to MGF.

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Watching Aid Scanning Single Stranded and Transcribed DNA with Single Molecule Resolution

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The activation-induced deoxycytidine deaminase (AID) is a member the Apobec family of enzymes that catalyzes dC to dU deamination on ssDNA trinucleotide motifs. In B cells, it is required to generate antibody diversity by initiating somatic hypermutation (SHM) in the variable region of immunoglobulin genes and class-switch recombination (CSR) in immunoglobulin switch regions. In turn, SHM and CSR are required to generate high-affinity antibodies that bind and neutralize invading antigens. Thus, AID plays an indispensable role in causing mutational diversity to enhance fitness and optimize the immune response.

Here, we have used single-molecule fluorescence resonance energy transfer (smFRET) to visualize co-transcriptional scanning of AID. Our data show that AID can follow an active RNA polymerase directionally and processively with speeds upwards of 200 nt/s. However, transcription-stalling leads to bidirectional scanning in the transcription bubble, which in turn, provides AID the necessary time window to carry out deaminations. In bear ssDNA, AID scanning is slow (~1 s-1), random and bi-directional. The enzyme remains bound to the ssDNA for ~250 s on average. During this time, it can scan large (>70 nt) ssDNA regions, and it exhibits 'quasi-localization' near favorable deamination motifs. AID also creases the ssDNA during scanning in a sequence dependant manner.

3507-Pos Board B235

Nicking Single DNA Molecules to Study Initiation of Mismatch Repair

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In E. coli the error rate of replication (10-6) is lowered another thousand-fold by the MutS, MutL and MutH system of proteins responsible for directing repair of base insertions, deletions, and mismatches. We provide here a study using magnetic trapping of single DNA molecules to analyze the interactions between these proteins and DNA containing a single indel mutation. MutS, albeit at relatively high concentrations, can be observed to stabilize small thermal loops of DNA on an indel-containing substrate, and this in an ATP-dependent manner; addition of MutL reduces the concentration of MutS required for looping to be observed. Loop size distributes roughly according to a J-factor distribution, and decreases as force increases, consistent with thermal loop stabilization by MutSL complexes. The reaction is allowed to proceed to the stage of DNA incision by the addition of MutH. With the further addition of T4 DNA ligase the steady-state rate of incision can be studied as a function of different parameters including DNA supercoiling, extending force, length of substrate DNA, or relative positioning between indel and incision sites. We compare these results to biophysical models for the protein-DNA interactions involved as well as stochastic simulations of the incision reaction occurring on linear DNA with a centrally-located indel and two symmetric proximal incision sites. Experimental results indicate that DNA incision can occur even in the absence of looping, and comparison to simulations supports the view that communication from DNA mismatch to DNA incision site takes place via thermal diffusion.

3508-Pos Board B236

Using Nanofluidic Channels to Probe the Dynamics of Rad51-DNA Filaments

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Rad51 is a key protein involved in the strand exchange reaction, a reaction where genetic material is transferred between two homologous DNA strands. Strand exchange is initiated by Rad51 forming a helical filament around single-stranded DNA (ssDNA), and the strand exchange is thereafter executed with a homologous double-stranded DNA (dsDNA). The structure of Rad51-DNA filaments, and also the activity of the strand exchange reaction, is dependent on the presence of ATP and dications, where Ca^{2+} has been shown to promote a higher degree of strand exchange than Mg^{2+}.

In the present study we have investigated the dynamic behavior of single Rad51-DNA filaments formed with Rad51, dsDNA/ssDNA and Ca^{2+}/Mg^{2+} using nanofluidic channels and fluorescence microscopy. Nanofluidic channels allow us to probe the filaments at a different force regime than that traditionally obtained in for example optical tweezers experiments. We note that the formed Rad51-DNA filaments have both rigid and flexible sections. We speculate that the rigid regions stem from when two adjacent filament patches meet on a DNA