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.

3504-Pos Board B232

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.

3505-Pos Board B233

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.

3506-Pos Board B234

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

but cannot merge together to form a continuous filament. Since each Rad51 monomer covers three bases/basepairs, the naked DNA between two adjacent filament patches could be too short to accommodate another protein monomer. Moreover, dsDNA has no directionality, meaning that the filaments can grow in both directions, thus two filaments can grow in opposite directions resulting in them two being out-of-phase at their point of contact. Supporting our hypothesis, when filaments are formed at low protein concentration, which result a lower number of filaments per DNA, we see less rigid regions.
The results also demonstrate the general applicability of nanochannels for studies of DNA-protein complexes at extremely low forces not easily obtained using other techniques.

3509-Pos Board B237
A Single-Strand Annealing Protein Clamps DNA to Detect Homology
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DNA repair of double-strand breaks is essential for genome maintenance. Among other mechanisms, the repair is mediated by homologous recombination. The recombination pathway of single-strand annealing is promoted by proteins like the eukaryotic RAD52 or the related viral protein Redß, which are thought to act as multimeric ring-shaped complexes. However, it is unclear how a ring-like structure promotes annealing on the molecular level and recognizes homology. By single-molecule experiments, we show that annealing is driven by Redß monomers. Redß monomers weakly hold single DNA strands together. Dimerization of Redß on complementary DNA strands triggers the capture of the homologue and leads to nucleoprotein filament growth. Interestingly, this dimerization strongly clamps DNA, and subsequent filament formation is accompanied by a structural change of Redß. Due to biochemical, structural, and functional similarities of single-strand annealing proteins, our proposed DNA clamping may point at a general molecular recombination mechanism for genome maintenance.

3510-Pos Board B238
Kinetic Analysis of Interactions between MutS, MutL and DNA During Initiation of DNA Mismatch Repair
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Mismatch repair (MMR) is essential for correcting base-pairing errors in DNA. MutS protein recognizes mis-paired bases in DNA and recruits MutL to signal excision and re-synthesis of the mismatched strand. The mechanism of MMR is under active investigation_especially the transient events involved in mismatch recognition and initiation of repair. We are utilizing transient kinetic methods coupled with fluorescence spectroscopy to determine the mechanisms of action of MutS and MutL from Thermus aquaticus. We have built a kinetic model of the ATPase-linked actions of MutS on DNA whereby initial rapid encounter between the two establishes a weak binding equilibrium (KD1 = 5 μM), followed by slow formation of a mismatch-specific MutS-DNA complex with the DNA in bent conformation (kconf ~ 30 s-1 and KD2 = 5 nM). ATP binding to MutS-DNA complex (kON = 0.5 μM-1s-1) is followed by two slow steps involving conformational changes in MutS that correspond to unbending of DNA at the mismatch site (~ 3 s-1), and subsequent MutS release from the mismatch (~ 0.5 s-1).
To understand the mechanism of MMR during the transition between mismatch recognition and initiation of excision, we plan kinetic analysis of the concerted actions of MutS and MutL on DNA. We are developing fluorescence-based assays to monitor MutL interactions with DNA and with the MutS-DNA complex. Currently, we are testing the fluorescent nucleoside analog 6-methyl-isoxanthopterin (6-MI), paired with cytosine and located adjacent to a mismatch. This reporter can be used to analyze MutS dynamics at the mismatch site and its communication with MutL, thus enabling novel mechanistic insights into the mechanism of initiation of MMR.

3511-Pos Board B239
Building a Better Engine: Stimulation of Single Molecules of SGS1 by RPA and Top3-RMI1
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Sgs1 is the Bloom's Syndrome helicase (BLM) ortholog and the sole RecQ-family helicase in Saccharomyces cerevisae. Genetic defects in Sgs1 cause gross chromosomal rearrangements and in humans manifest as Bloom's Syndrome, a disease associated with accelerated onset of sporadic cancers. Early during homologous recombination-mediated repair of dsDNA breaks, Sgs1 stimulates long-range resection in coordination with Mre11/Rad50/Xrs2 and the helicase/endonuclease Dna2. Later during recombination, Sgs1 forms the core of the Holliday junction (HJ) dissolution complex with Top3 (type-I topoisomerase) and Rmi1. In contrast to other members of the RecQ-family, Sgs1 is a remarkably active helicase; however, the mechanism by which Sgs1 alternates between resection and HJ dissolution remain unclear–in part due to outstanding questions regarding properties of the core helicase activity. We combined optical trapping, microfluidics and fluorescence microscopy to visualize binding and translocation of Sgs1 on DNA. Sgs1 binds internally along dsDNA and association is enhanced by RPA and Top3-Rmi1. We observe complex translocation phenomenon including pauses, reversals and rate changes. In the presence of RPA, Sgs1 translocates at a rate of 200-350 bp/sec, traveling 1,200 bp, on average. Addition of Top3-Rmi1 doubles both the rate and translocation distance. To distinguish between DNA translocation and unwinding, we used TIRF microscopy to visualize the accumulation of fluorescent RPA on ssDNA. In the absence of salt, Sgs1 is a potent helicase, rapidly and completely unwinding lambda-DNA in minutes through the expansion and convergence of distributively initiated bubbles; however, under physiological salt concentrations, unwinding activity is severely attenuated, while translocation is paradoxically stimulated. We, therefore, propose a model in which Sgs1–in complex with RPA and Top3-Rmi1–translocates on dsDNA via bubble migration during HJ dissolution in a mode phenomenologically distinct from its end-dependent, Dna2-associated helicase activity.

DNA Structure and Dynamics III

3512-Pos Board B240
Construction and Characterization of Cy3- or Cy5-Conjugated Hairpin Pyrrole/Imidazole Polyamides Binding to DNA in the Nucleosome
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N-Methylpyrrole (Py)-N-methylimidazole-(Im) polyamides are small molecules that can recognize specific DNA sequences in the minor groove of B-form DNA with DNA recognition rules. Py favors the T, A, and C bases, excluding G and Im favors G. Lone pair of N3 in Im forms a hydrogen bond with 2 amino hydrogen of guanine. Anti-parallel pairings of Im/Py and Py/Im bind to the G•C and C•G sequence in DNA, respectively. Anti-parallel pairing of Py/Py binds to A•T and T•A degenerately. Aliphatic β-alanine (β) can be substituted for Py. Anti-parallel pairings of Py/β and β/Py bind to A•T and T•A degenerately, and anti-parallel pairings of Im/β and β/Im specify G•C and C•G, respectively. Recently, Py-Im polyamides have been conjugated with fluorophores and some of the fluorophores conjugate Py-Im polyamides could be used for specific DNA detection. In this study, we synthesized two Py-Im polyamide 1 and 2, which interact with the 145 bp DNA containing nucleosome positioning sequence 601. We conjugated cyanine dyes Cy3 or Cy5 with 1 or 2. In the absence of the target DNA, the fluorescent intensity from the fluorescence conjugate Py-Im polyamide diminished their fluorescence, compared with Cy3 or Cy5. In the presence of the target DNA or nucleosome, the fluorescent intensity from the fluorescence conjugate Py-Im polyamides increased. Furthermore, interestingly, FRET between Cy3-Py-Im polyamide and Cy5-Py-Im polyamide on nucleosome was observed. These results provide possibilities that the fluorescent conjugates of Py-Im polyamides can be used for characterization of the dynamic interactions within protein-DNA complexes.

3513-Pos Board B241
Extraction of Conventional Two-State Melting Temperature from DNA Oligomers with Significant Premelting Behavior
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The determination of some thermodynamic quantities of DNA, including melting temperature, may be confounded by premelt behavior. This is a significant problem for fluorescent observation of DNA melting and can be further exacerbated by the use of common dyes, which may actually increase deviation from the simple two-state melt model1. Using contact-quenched fluorescently labeled DNA oligomers2, we have developed a fitting function for fluorescent melting curves that may be used to extract the conventional 'two-state' melting temperature, even in the presence of significant premelt behavior of oligomers