(P/T) junction have a significant impact on the dynamics of PolBI, indicating the translocation along the single-stranded DNA as the mechanism for the spontaneous motion revealed by FRET. In addition, we have found PCNA not only improves the binding affinity of its cognate polymerase, but suppresses the frequent movement of PolBI from the P/T junction. In summary, many of the dynamics discussed here are reported for the first time and will provide a new perspective for understanding the orchestration of replication-related processes in archaea.

339-Pos

The Dynamic DNA Damage Inducible Protein UmuD Inhibits Replication

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All organisms experience DNA damage from myriad sources. When bacterial cells experience DNA damage, the SOS response is induced, leading to upregulation of at least 57 genes in *E. coli*. The SOS regulated genes include those involved in DNA repair and cell cycle regulation. Also induced as part of the SOS response are Y family DNA polymerases, which have the specialized ability to copy damaged DNA. This specialized ability comes at a potentially mutagenic cost as Y family DNA polymerases replicate undamaged DNA in an error-prone manner. Multiple layers of regulation control the activity of these potentially mutagenic Y family polymerases. UmuD, a small manager protein, and its cleaved form, UmuD', directly interact with both Y family polymerases as well as the beta processivity clamp and the replicative DNA polymerase. We find that UmuD, but not UmuD′, inhibits primer extension by the DNA polymerase III alpha subunit. We probed the conformation and dynamics of the *umuD* gene products. Thermal shift experiments show that UmuD undergoes two melting transitions, one likely due to the dissociation of the N-terminal arms and the other due to unfolding of the globular domain. We used hydrogen-deuterium exchange mass spectrometry (HXMS) to probe the conformations of UmuD and UmuD'. In HXMS, backbone amide hydrogens become labeled with deuterium over time. Our HXMS results reveal that the N-terminal arm of UmuD, which is not present in the cleaved form UmuD', is highly dynamic. Residues that are likely to contact the N-terminal arm show more protection from exchange in UmuD than UmuD′. Additionally, there are regions of both proteins that are less dynamic. Our observations are consistent with the proposed model of UmuD and the finding that UmuD is relatively unstructured.

340-Pos

A Three Pool Model of DNA Digest Gels

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The purpose of the project is to determine the effectiveness and to clarify the mechanism of action of potential antibiotic compounds to interfere with the mechanisms of DNA repair in bacteria. After treating the bacteria with the compounds of interest (norfloxacin, novobiocin, and a novel antibiotic, peptide wrwycr), the DNA, now broken into many fragments, was separated based on size using pulse field gel electrophoresis. Preliminary analysis of the gels reveals three pools of DNA fragments: (1) unbroken, (2) broken at a few random spots into fragments larger than about 30 kb and described by a Poisson distribution, and (3) digested into fragments smaller than 30 kb, probably with help of the exonuclease RecBCD. Fits to these three pools are presented and the implications for antibiotic activity are discussed.

341-Pos

Direct Visualization of Fluorescent SSB on Single Molecules of ssDNA as a Mechanistic Probe in the Early Stages of Homologous Recombination

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In all organisms homologous recombination (HR) is essential for the efficient and error-free repair of DNA lesions. Defects in HR result in genomic instability, which often manifests in humans as a genetic disposition to cancer. Central to the process of homologous recombination is the strand exchange activity of the RecA/Rad51 class of proteins. Through the formation of a pre-synaptic filament on single stranded DNA (ssDNA), RecA/Rad51 aligns a broken chromosome with an intact one (a process called synapsis), allowing for a subsequent array of potential repair pathways. During the presynaptic stage, filament formation is inhibited by the diffusion-limited association of ssDNA with the high-affinity single stranded DNA binding protein, SSB/RPA. A class of positive regulators called mediators facilitate filament formation by alleviating this biochemical inhibition. These mediators include RecF/O/R (E. coli), Rad52 (S. cerevisiae and H. sapiens), and BRCA2 (H. sapiens). We have fluorescently modified several of the key proteins involved in pre-synaptic filament formation in E. coli, specifically SSB and RecA. Here we present their characterization and utility as fluorescent biochemical sensors for single-stranded DNA in single molecule assays designed to mechanistically probe the early, pre-synaptic stage of homologous recombination.

342-Pos

Analysis of Dynamic Properties of DNA Repair Protein MutS and DNA Complexes Using Molecular Dynamics Simulations

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DNA mismatch repair (MMR) maintains genome stability by repairing mismatches that arise through DNA replication errors and during recombination. Defects in MMR result in a significant increase in the spontaneous mutation rate and predispose humans to cancer.

In *E. coli*, the proteins MutS, MutL and MutH are responsible for the MMR. MMR is initiated by MutS, which functions in the homodimer form. MutS recognizes and efficiently binds to mispaired bases and unpaired bases in DNA duplexes. It is thought that the ATPase activity of MutS plays a role in proofreading to verify mismatch binding and authorize the following downstream excision in which MutL and MutS are involved.

However, little is known of the relationship between the recognition of DNA and the ATP hydrolysis by MutS at the atomic level. In order to investigate how the binding of MutS to the DNA and ATP hydrolysis are coordinated, molecular dynamics (MD) simulations of the wild-type and mutant MutS in water with mismatched and undamaged DNA were performed. Including the water molecules, each system comprised about 200,000 atoms. The MD simulations were carried out at a constant pressure of one bar and a temperature of 300 K for several tens of nanoseconds in total. The binding free energies were calculated using the MM-GBSA method.

It was found that the interaction between MutS and DNA changes significantly according to the different kinds of mismatch base pair or different kinds of mutation in MutS. It was shown that the electrostatic energy significantly contributed to the binding free energies. Moreover, a correlation between the binding free energies and the functional movement of MutS was observed.

343-Pos

Base Pair-Position-Specific DNA 'Breathing' At the Replication Fork Junction Regulates Helicase Access

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Thermal fluctuations induce transient opening of base pairs in dsDNA constructs. In previous studies with DNA constructs of conserved sequence containing 2-AminoPurine (2-AP) probes, we showed that position-specific base-pair (bp) fraying that depends on proximity to the ss/ds junction can be observed in forked DNA constructs of conserved sequence, and that significant (>1%) thermal fraying of base-pairs at helix ends extends 2-3 bps into the dsDNA. Here we build on these results to study the initial steps of DNA helicases at replication forks. Proteins that bind preferentially to ssDNA can capture thermally frayed bps without the expenditure of chemical (NTP-dependent) free energy. The bacteriophage T4 DNA replication complex provides a favorable model system to study basic helicase mechanisms. The T4 helicase-primase (gp41-gp61) sub-assembly forms a tight-binding helicase that unwinds dsDNA and translocates processively along ssDNA lattices, driven by NTP binding and hydrolysis. We use fluorescence and low energy CD spectral signals of site-specifically placed 2-AP probes to monitor the initial steps of helicase activity at a forked DNA construct. We find, on binding a helicase-primase complex to the DNA construct in the presence of non-hydrolysable NTP, that the first bp on the duplex side of the fork opens and additional destabilization penetrates to ~ the 3rd bp. This is consistent with a largely passive mechanism for helicase-dependent DNA unwinding, with the helicase complex binding on the 5′→3′ loading strand at the fork and trapping the first adjacent bp as it is opened by thermal fluctuations.

344-Pos

Single-Molecule Studies of the ssDNA Binding Activity of E. Coli MutL

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MutL stimulates the DNA duplex unwinding activity of UvrD in methyl-directed DNA mismatch repair (MMR) via their physical interactions. However, the molecular functions of MutL associated with the DNA binding and UvrD helicase have been partially understood. We present the kinetic characteristics of the single-stranded DNA (ssDNA) binding activity of MutL in the absence or the presence of UvrD helicases using the single-molecule techniques. The lengthening of the ssDNA due to the ssDNA binding of MutL allows us to observe association and dissociation of MutL from the ssDNA in real-time. In this

study, we demonstrate that the nonspecific ssDNA binding of MutL can be involved in subsequent loading of UvrD helicases onto the ssDNA in a manner independent of ATP hydrolysis of MutL.

345-Pos

Extremely-Low-Frequency Magnetic Field Induces DNA Double Strand Breaks in Human Cells

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For several decades, we have been exposed to chronic environmental extremely-low-frequency magnetic fields (ELF-MF) from various electric appliances that range the power frequencies of 50-60 Hz. In this study, we aimed to investigate the potential effect and genotoxicity of ELF-MF on human cells. When human cervical cancer cell line (HeLa) and human fibroblast cells (IMR90) were exposed to a 60 Hz magnetic field at intensities of 7-35 militesla (mT) continuously or intermittently, there was no change in cell viability by MTT assays. However, we observed severe double strand breaks (DSBs) in chromosomes of HeLa and IMR90 cells exposed to 60 Hz MF of 7 mT for 10-30 min. The phosphorylated H2AX (γ-H2AX), an obvious DNA double strand break marker, was detected in the chromosomes of these cells by immunofluorescence microscopy and western blots. In addition, ATM and Chk1 kinases in the DNA damage checkpoint pathway were activated in these cells. These results strongly suggest that continuous exposures of human cells to 60-Hz ELF-MF cause genomic instability that may lead to carcinogenesis. This possibility could produce human health issues associated with exposure to ELF-MFs in the occupational and public environments.

346-Pos

Specificity of *E.coli* SSB Protein Binding To the Chi Subunit of DNA Pol III He and Pria Helicase in the Presence and Absence of ssDNA

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The homotetrameric E.coli single stranded DNA binding (SSB) protein, is a key protein involved in replication, recombination and repair. Its unstructured C-terminal domains (SSB-Ct), which are not required for ssDNA binding, provide the binding site for at least 14 accessory proteins and serve to target these proteins to regions of DNA, where they function (Shereda et al., 2008, *Crit Rev Biochem Mol Biol*, **43**, 289). Here, we present a thermodynamic study of SSB interactions with two such proteins, the Chi subunit of DNA Pol III holoenzyme and the PriA helicase, using Isothermal Titration Calorimetry (ITC). Both proteins interact with SSB via the last 9 amino acids of SSB-Ct with similar moderate affinities and stoichiometries of approximately 4 molecules per SSB tetramer. However, these affinities are somewhat weaker than for PriA and Chi interactions with the corresponding Ct peptide. We ascribe this to an inhibitory effect of the SSB core, which may compete for the binding to SSB-Ct. We find that dT_{70} prebound to the SSB core (forming 1:1 complex) eliminates this inhibitory effect for Chi protein. However for PriA, a much greater binding enhancement (>10 fold) is observed. We discuss a possible origin of this specificity for PriA and the role it may play at initial stages of DNA processing (supported by NIH Grant GM30498).

347-Pos

Conformational Dynamics of Single RecBCD Molecules

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RecBCD is a multifunctional enzyme possessing both helicase and nuclease activities. It harnesses the energy of ATP hydrolysis to processively unwind DNA. We used an optical-trapping assay featuring one base-pair stability to investigate the mechanism of RecBCD unwinding. Records of RecBCD motion at 6 pN of applied load showed fluctuations [4.1 $\pm$ 0.1 bp, (mean $\pm$ std. err.; freq. bandwidth = 0.1-10 Hz)] substantially above the control records with DNA alone. These fluctuations persisted when the enzyme's forward motion was stopped by removing ATP. Records of RecBCD bound to blunt-end DNA in the absence of ATP showed reduced dynamics (2.4 $\pm$ 0.2 bp), indicating the primary origin of the fluctuations was not due to anchoring via RecBCD. Prior biochemical studies showed that unwinding activity is preceded by an initiation phase consisting of several kinetic steps that generates a 10-nt, 5′-tailed substrate inside the RecBCD-DNA complex that engages RecD's helicase domain. This work also showed that binding to a forked 3′-$(dT)_6$ and 5′-$(dT)_6$ DNA substrate is kinetically equivalent to binding to a blunt-end DNA, while a 3′-$(dT)_6$ and 5′-$(dT)_{10}$substrate bypasses initiation. We found that records of RecBCD bound to these tailed DNA substrates showed fluctuations that quantitatively mirrored our records of RecBCD bound to blunt-end DNA and stopped within a long DNA substrate, respectively. Thus, the onset of large fluctuations in the RecBCD-DNA complex was coincident with that of unwinding activity. The magnitude and frequency of fluctuations increased when the DNA sequence immediately in front of the forked substrate was changed from GC to AT base pairs, consistent with RecBCD transiently translocating along the DNA without ATP hydrolysis. A tightly bound state with reduced dynamics (2.7 $\pm$ 0.1 bp) was observed with ADP-BeF_2. These findings support a ratchet model for RecBCD movement.

348-Pos

Biochemical Analysis of RuvA-RuvB Complex Formation During Branch Migration of Holliday Junction DNA

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Escherichia coli RuvA-RuvB protein complex promotes Holliday junction branch migration during homologous recombination and recombination repair. RuvA forms tetramer and the two tetramers sandwich planer Holliday junction. RuvB is a member of AAA+ ATPase superfamily and forms a hexameric ring, which acts as a motor protein. The two rings flank the junction by interacting RuvA octameric core and promote branch migration by pumping out DNA duplex through their central cavities. Two models are conceived to explain how the DNA double helices are pulled out through the cavities of the rings. (i) RuvB hexameric rings rotate against RuvA octameric core and the duplexes are moved by interacting with inner surfaces of the rotating RuvB rings. (ii) RuvB hexamric rings are fixed to the RuvA octameric core and the duplexes are moved by interaction with RuvB subunits which undergo sequential conformational changes. Previously, we showed that I150T-RuvB mutant was defective in interaction with RuvA. Here, we show the detailed analysis of the heterooligomer composed of wild type and the mutant I150T RuvB proteins in vitro to clarify which mechanism is employed for the RuvA-RuvB directed branch migration of Holliday junction. In this study, we would like to discuss how RuvA-RuvB promote branch migration of Holliday junction.

349-Pos

Atomic Force Microscopy Shows that Chi Sequences and SSB Proteins Prevent DNA Reannealing Behind the Translocating AddAB Helicase-Nuclease

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Recombinational repair of DNA breaks requires processing of a DNA end to a 3′-ssDNA overhang. In B.subtilis, this task is done by the helicase-nuclease AddAB which generates ssDNA overhangs terminated at a recombination hotspot (Chi) sequence. This is a substrate for the formation of a RecA nucleoprotein filament that searches for a homologous donor molecule and catalyses DNA strand exchange to promote repair. In this study, we have used AFM to visualize the products of reactions including AddAB and double-stranded DNA molecules. AFM images consistently showed a remaining population of apparently unprocessed dsDNA molecules. The fraction of unprocessed molecules dropped upon addition of increasing concentrations of SSB protein or larger amounts of AddAB protein. Moreover, a larger fraction of DNA molecules were processed to ssDNA when DNA substrates contained the regulatory Chi sequence. Our results are consistent with a model in which the DNA strands reanneal behind the translocating AddAB enzyme. This effect is suppressed by destabilizing the interaction between DNA strands via binding of SSB or multiple AddAB motors, or by the interaction between AddAB and Chi during translocation.