We have quantitatively assessed each individual step of the Rad51-ssDNA interaction (i.e. nucleation, filament extension and disassembly). Moreover, we investigate the mechanical coupling between the ssDNA template and the reaction kinetics of filament by varying the tension on the DNA molecule. Hence, we have obtained new insight into the reaction pathway of this essential biological system.

334-Pos

Isothermal Amplification and Quantification of Nucleic Acids Using Intrinsic Fluorescence of Primers

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Real-time polymerase chain reaction (RT-PCR) is widely used to amplify, detect and quantify nucleic acids. Current RT-PCR specific probes (Molecular Beacons, TaqMan, Scorpions) use complicated mechanisms based on fluorescence resonance energy transfer, and require costly synthesis and considerable effort to achieve optimal sensitivity. Typically, a fluorophore-quencher pair is attached to the ends of a probe oligonucleotide, which doesn't fluoresce when free in solution. Upon probe hybridization to a target sequence, the fluorophore is separated from the quencher and a signal is released. Temperature cycling is another limitation of PCR since it requires expensive instrumentation for thermocycling and complicates rapid detection of pathogens in the field and at point-of-care.

We developed a new method, quadruplex priming amplification (QPA), which uses intrinsic fluorescence of primers for quantification of DNA products and can proceed under isothermal conditions. A key feature of QPA is that after polymerase elongation, the specifically designed guanine-rich primers are capable of forming a quadruplex structure with significantly more favorable thermodynamics than the corresponding DNA duplexes. As a result, target sequences are accessible for the next round of priming since their complementary strands are trapped in a quadruplex conformation and DNA amplification proceeds under isothermal conditions. In addition, 2-aminopurine (2Ap), which is part of the primers and quenched before polymerase elongation, regains its maximum emission upon quadruplex formation, which allows simple and accurate detection of product DNA. The advantages of QPA over traditional quantification methods and its thermodynamic bases will be discussed.

335-Pos

Binding Affinity and Displacement Synthesis Activity of Pol I DNA Polymerase on Different Gapped DNAs

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Gapped DNAs, intermediates in excision repair, can be filled by DNA polymerase I (Pol I) and sealed by ligase. Understanding the binding preferences of Pol I for different gaps and how their binding affinity correlates with displacement synthesis is helpful for investigating repair mechanisms *in vivo*. The roles of the 5′- or 3′-phosphate, and of magnesium, in the binding of Klenow and Klentaq polymerases to gapped DNAs, differing in the size of the ssDNA gap (0, 2, and 10 nt), were examined using a fluorescence anisotropy binding assay. 5 mM Mg^{2+} does not significantly alter the binding of gapped DNAs to Klenow, but Mg^{2+} weakens the binding of gapped DNAs to Klentaq. For Klentaq, but not Klenow, a 5′-terminal phosphate increasingly weakens the binding as the gap size increases. Under the same conditions, $Mg^{2+}$and 5′-phosphate do not alter the displacement synthesis ability of Klenow with gap2 and gap10 substrates, but do alter the activity of Klentaq. Conversely, a 3′-phosphate in the gap significantly weakens the binding of Klenow, but not Klentaq. We hypothesize that Klentaq can bind either the 5′- or 3′- end of the gap, while Klenow binds preferentially at 3′- end of the gap due to the 3′-end being pulled into the editing site. The binding affinity of Klenow, but not Klentaq, to different gaps increases as the size of the gap increases, and this correlates with the displacement synthesis ability of Klenow on gaps versus nicks. Klenow binds primed-template DNA substrates with 2-3 kcal/mol tighter affinity than gap0 or gap2 substrates, while Klentaq shows only a slight preference (0.7 kcal/mol) for primed-template over gapped DNAs, indicating that Klenow more significantly prefers replication over repair substrates.

336-Pos

Conformational Dynamics of a DNA Polymerase At the Single-Molecule Level

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DNA polymerases are essential components of the cellular machinery necessary for survival of an organism through the accurate replication of the genetic material. The replication pathway of DNA polymerases has been extensively studied to elucidate the structures and dynamics of the polymerizing and proofreading modes, but currently only a static collection of individual states has been extracted. With the advancements in modern single-molecule fluorescence technology, the potential now exists to examine all enzymatic processes and transitions during real time dynamic measurements. By employing the E. coli DNA polymerase I Klenow fragment (KF) as a model system, along with single-pair FRET labeling, we designed an experimental system to examine conformational dynamics during both nucleotide selection and proofreading steps. In the absence of nucleotides, the bound KF complex was observed to cycle repeatedly between two distinct conformations (open and closed). In contrast, just a single conformation (closed) was populated in the presence of a correct incoming nucleotide. In addition, with the presence of mismatches at the primer-template junction, the previously hypothesized intermolecular and intramolecular pathways were directly observed for transfer of a DNA substrate between the polymerase and exonuclease sites of KF. The evolution and continuous advancement of single-molecule FRET methodology has provided the opportunity to witness events and intermediates previously unobservable in standard bulk studies, leading to a more complete view of the enzymatic pathway. Supported by NIH grant GM44060.

337-Pos

Topoisomerase IB Activity Investigated By Single Molecule Magnetic Tweezers: Mechanisms of Cytotoxicity

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Magnetic Tweezers (MT) are a powerful tool to investigate how single topoisomerase IB (topIB) molecules relax DNA supercoils [1,2]. MT studies have revealed that topIB activity is dramatically affected by the presence of camptothecin-class (CPT) inhibitors, used clinically as anti-cancer drugs [3]. In the presence of CPT, topIB remains covalently bound to DNA for much longer than in the absence of the drug (>100 s vs. ~ 2 s) and the rate of supercoil removal is significantly reduced, in particular for positive supercoils. The CPT-induced asymmetry in the rate of supercoil removal between positive and negative supercoils leads to an accumulation of positive supercoils in the G1 and S-phases in yeast cells in vivo [3].

Here, we present results on the G365C topIB point mutant, which exhibits CPT resistance and shows no accumulation of positive supercoils in vivo. In the MT assay in the absence of CPT, the G365C mutant shows activity similar to wt topIB. In the presence of CPT, G365C exhibits long-lived DNA-topIB complexes and slow supercoil removal for positive supercoils, similar to the wt enzyme. Surprisingly, for negative supercoils we found similarly long-lived complexes and slow supercoil removal for the G365C mutant. In contrast to the wt enzyme, the G365C mutant removes positive and negative supercoils with similar (slow) velocities in the presence of CPT. These results suggest that CPT cytotoxicity might be more strongly dependent on the asymmetry of the rate of positive vs. negative positive supercoil removal and the corresponding accumulation of positive supercoils than on the lifetime of the covalent DNA-topIB complex.

[1] Koster, et al. Nature 2005
[2] Lipfert, et al. Meth. Mol. Biol. 2009
[3] Koster, et al. Nature 2007

338-Pos

Dynamics of An Archael DNA Polymerase Revealed By Single Molecule Fret

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In the archaeon *Methanosarcina acetivorans*, DNA replication is done by DNA polymerase BI. The processivity of this enzyme is greatly enhanced by a conserved cofactor known as PCNA, which plays a crucial role in orchestrating many replication-related processes. To understand the dynamics of these proteins, we have used single molecule FRET to examine the behavior of PolBI labeled with FRET donor on various DNA structures labeled with FRET acceptor and the effect of PCNA on the dynamics of PolBI. The binding of PolBI to DNA was observed in the low nanomolar concentration range as expected. Interestingly, this polymerase is highly mobile on the DNA structures with two nonadjacent primer strands that are complementary to two different regions in the template strand, 20 nucleotides apart, as evidenced by the frequent transitions between two long-lived FRET states exhibited in single molecule trajectories. To explore the nature of this spontaneous motion, we considered several possible mechanisms including translocations along single- or double-stranded DNA, polymerase binding orientation flipping, and polymerase active site switching. We observed that changes made downstream of the primer/template

(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