primosome disassembly, thereby increasing primase processivity. In contrast, priming in bacteriophage T7 involves discrete pausing of the primosome, and in Escherichia coli it appears to be associated primarily with dissociation of the primase from the helicase. Thus nature appears to use several strategies to couple the disparate helicase and primase activities within primosomes.

356-Pos

Single Molecule Study on Incorporation Efficiency of DPO4 and Klenow Fragment in the Presence of BPDE Adduct

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It's well known that the binding of DNA adducts such as benzo[a]pyrene-diol-epoxide (BPDE) to DNA template strand can impede or block DNA synthesis during the process of DNA replication. While DNA synthesis involving high fidelity replicative A-family polymerases such as Klenow fragment are blocked by DNA adducts, members of Y-family DNA polymerases such as Sulfolobus solfataricus P2 DNA polymerase IV (DPO4) can bypass the DNA adducts and resume the DNA synthesis. Understanding the functional differences between A-family and Y-family DNA polymerases in the process of DNA replication and the mechanism of bypassing DNA adducts is of great value to explain the cause of mutagenesis. We introduce an assay by anchoring DNA molecules to the modified surface to study the incorporation efficiency of DPO4 and Klenow fragment with the presence of BPDE adduct at single molecule level. Specifically, we anchor fluorescent labeled DNA template onto this surface with the adduct site open for nucleotide incorporation, photobleach the labels and flow the polymerases and labeled nucleotides into the hybridization cell. Using Total Internal Reflection Fluorescence Microscopy (TIRFM) we identify the time sequence incorporation of the nucleotides onto the anchored DNA template by identifying the location of the labeled nucleotide from TIRF images. We further quantify the signal densities of the images obtained from the two different polymerases, thus examining whether incorporation reactions have been executed and quantifying the incorporation efficiency of the polymerases.

357-Pos

DNA Polymerization in Optical Tweezers

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Molecular motors associated with nucleic acids ensure replication, maintenance and expression of genetic information, which is crucial for life. Biochemical and biological studies have generated considerable knowledge about these molecular motors. However, these studies assess only sum reactions/effects due to ensemble averaging. Single molecule (SM) measurements overcome this shortcoming. Such measurements allow learning about individual motor functions, such as processivity, fidelity, step size, template dependence and generated forces. Transient intermediate states and rarely occurring events can also be observed.

We are developing an assay based on bacteriophage phi29 polymerase to study DNA/RNA polymerases in action using high resolution optical tweezers (OT) combined with fluorescence imaging (OT-SMF). We use an optically levitated 'dumbbell' assay: the nucleic acid (NA) construct features biotinylated and digixigeninated ends that tether two different kinds of microspheres (coated with streptavidin and anti-digoxigenin); the protein of interest is attached directly to the NA tether. Since in our double OT instrument one trap is stable whereas the other mobile, we can manipulate the tethers, detect changes in tether length and stiffness, apply different forces and simultaneously observe the fluorescently stained template/protein. We present preliminary results on DNA replication by phi29 polymerase.

358-Pos

Conformational Dynamics of Mismatch Recognition By E. coli Muts

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DNA mismatch repair protects the genome from spontaneous mutations by recognizing and repairing DNA synthesis errors in a pathway that is highly conserved. The MutS family of proteins initiate DNA mismatch repair by specifically binding mismatched or extrahelical bases and communicating the presence of damage to downstream repair proteins in an ATP-dependent manner. Previous structural studies have implied that MutS-induced conformational changes on DNA are central to damage recognition. Because the conformational changes occur on the timescale of seconds, it is difficult to obtain kinetic information on this highly dynamic process with traditional ensemble techniques. In this work, we use single molecule fluorescence resonance energy transfer (smFRET) to investigate the conformational dynamics of DNA in the presence of MutS from *E. coli*. FRET can measure changes in the relative distances between two fluorophores that are sufficiently close together (20-80 Å). In our experiments, DNA conformational dynamics are observed by detecting changes in the end to end distance of fluorescently labeled oligonucleotides with milliseconds time resolution. We present quantitative kinetic information on the rates of MutS binding and dissociation and the effect of nucleotides on the conformational dynamics of the mismatched DNA-protein complexes. Our results are discussed in the context of current models for DNA mismatch recognition.

359-Pos

Direct Visualization Of Joining of DNA Fragments By LigIIIβ Using Atomic Force Microscopy

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DNA ligaseIII is one of three mammalian DNA ligases (LigI, LigIII and Lig IV). LigIII is distinguished from the other ligases by the presence of a Zinc finger(ZnF) that improves ligation efficiency. Previously, it was demonstrated that LigIII has two different DNA binding modules (ZnF-DBD and NTase-OB) and a jack-knife model has been proposed to explain nick recognition and joining of double-strand breaks. However, the oligomeric state of LigIII in solution and during ligation, and the role of ZnF in end-end ligation are unknown. Using atomic force microscopy, we directly visualized a germ cell-specific form of DNA LigIII, LigIIIb and a delZnF mutant, their interactions with DNA and ligation products. We found no evidence for oligomerization of WT and delZnF LigIIIb in solution or when complexed to DNA. Importantly, WT and delZnF proteins exist in three distinct conformational states: closed, semi-extended, and extended conformations. While WTLigIIIb protein accesses all three conformational states significantly, delZnF LigIIIb occupies primarily the closed and semi-extended states, suggesting that ZnF is part of one wing as proposed in the jack-knife model. Furthermore, binding and ligation studies on nicked and non-nicked blunt-end DNA and DNA with 5′ overhang indicate that in addition to tandem joining of two linear DNA molecules, LigIIIb can mediate the ligation of a variety of higher order structures including three way junctions. In addition, with 5′ overhang DNA, compared to WT protein, delZnF promotes a small but significant occurrence of three-way junctions, lassoes and knots in the presence of MgCl2. These data suggest that monomeric DNA LigIIIb is capable of binding two DNA molecules simultaneously. Currently, we are testing the hypothesis that the ZnF in LigIIIb may be involved in quality control ensuring only tandem end-end ligation.

Transcription

360-Pos

Synergistic Action of RNA Polymerases in Overcoming the Nucleosomal Barrier

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During gene expression, RNA polymerase (RNAP) encounters a major barrier at a nucleosome and yet it must access the nucleosomal DNA. Various lines of in vivo evidence suggest that multiple RNAPs might increase transcription efficiency through nucleosomal DNA. Here we have quantitatively investigated this hypothesis by using E. coli RNAP as a model system and directly monitoring its location on the DNA via a single molecule DNA unzipping technique. When a single RNAP encountered a nucleosome, it paused with a distinctive 10-bp periodicity and was backtracked by an average distance of ~10-15 bp. When two RNAPs were elongating in close proximity, the trailing RNAP exerted an assisting force on the leading RNAP, reducing its backtracking and enhancing its transcription through a nucleosome ~5-fold. Taken together, our data indicate that histone-DNA interactions within a nucleosome dictate RNAP pausing behavior, and that alleviation of nucleosome-induced backtracking by multiple polymerases is a likely mechanism for overcoming the nucleosomal barrier in vivo.