Single Molecule Studies of Eukaryotic Replisomes in Xenopus Egg Extracts
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fluorescence assays and include the fingers-closing transition that has been characterized in structural studies. Using DNA polymerase I (Klenow fragment) with both donor and acceptor fluorophores, we have employed single-molecule fluorescence resonance energy transfer (smFRET) to study the polymerase conformational transitions that precede nucleotide addition. Our experiments clearly distinguish the open and closed conformations that predominate in Pol-DNA and Pol-DNA-dNTP complexes, respectively; minor conformations (corresponding to the closed conformation in the Pol-DNA complex, and the open conformation in the Pol-DNA-dNTP) are also present. By contrast, the unliganded polymerase shows a broad distribution of FRET values, indicating a high degree of conformational flexibility in the protein in the absence of its substrates; such flexibility was not anticipated on the basis of the available crystallographic structures. Real-time observation of conformational dynamics showed that most of the unliganded polymerase molecules sample the open and closed conformations in the millisecond timescale. Termination dynamics showed that most of the mismatched dNTPs that result from the mismatched dNTPs are used. This study uses the photoswitchable fluorescent protein mKikGR. The aim of this work is to study the conformational dynamics of the DNA substrate (bending and unbinding) and the conformational dynamics of the DNA substrate (bending and unbinding) and to study the conformational dynamics of the DNA substrate (bending and unbinding) and the conformational dynamics of the DNA substrate (bending and unbinding) and to study the conformational dynamics of the DNA substrate (bending and unbinding). The DNA substrate was labeled with fluorescent dyes that constitute a fluorescence resonant energy transfer (FRET) pair. Experiments at the single molecule level allow us to follow the conformational dynamics of the DNA substrates by determining the substrate’s end-to-end distance. We were able to determine the binding and dissociation rates of the proteins from the substrates as well as the conformational state of the substrates under different conditions, including studies with FRET and ADP under both hydrolytic and non-hydrolytic conditions. In particular we discuss the role of the substrate’s intrinsic dynamics for binding of hMutS-hMsh3 to DNA hairpins and DNA 3-way junctions.

2259-Plat DNA Conformational Dynamics in Mismatch Recognition Julie Coats1, Walter H. Lang2, Yuyen Lin1, Cynthia T. McMurray2,3, Ivan Rasin1

1Emory University, Atlanta, GA, USA, 2Lawrence Berkeley Laboratories, Berkeley, CA, USA, 3Mayo Foundation, Rochester, MN, USA. DNA mismatch recognition is done by the homodimer MutS in prokaryotes and by its homologues: heterodimers Msh2-Msh3 and Msh2-Msh6 in eukaryotes. Msh2-Msh6 binds preferentially to single insertion/deletions. Msh2-Msh3 has been shown to bind to DNA hairpins. It has been suggested that the conformational dynamics of the DNA substrate (bending and unbinding) plays a fundamental role in the recognition process. Mismatch recognition allows identifying a single mismatched DNA pair among thousands of matched basepairs. The process is ATP dependent and different models for DNA discrimination have been proposed based in biochemical evidence as well as AFM studies. In this work we study the conformational dynamics of several DNA substrates and its complexes with the human MutS homologs. The DNA substrates were labeled with fluorescent dyes that constitute a fluorescence resonant energy transfer (FRET) pair. Experiments at the single molecule level allow us to follow the conformational dynamics of the DNA substrates by determining the substrate’s end-to-end distance. We were able to determine the binding and dissociation rates of the proteins from the substrates as well as the conformational state of the substrates under different conditions, including studies with FRET and ADP under both hydrolytic and non-hydrolytic conditions. In particular we discuss the role of the substrate’s intrinsic dynamics for binding of hMutS-hMsh3 to DNA hairpins and DNA 3-way junctions.

2260-Plat The Dance of Chromosomes during DNA Repair Judith Mine-Hattab, Rodney J. Rothstein.

Columbia university, New York, NY, USA. DNA repair is an essential process for preserving genome integrity. Among the various forms of DNA damage, double-strand breaks (DSBs) are the most toxic and genotoxic. To repair them, eukaryotic organisms use homologous recombination (HR): it consists of exchanging DNA strands between the broken DNA and an intact homologous DNA and it is choreographed by multi-protein complexes (1). During HR, the search for an intact homologous sequence among the whole genome is the most enigmatic stage (2). How can two homologous needles find each other in the genomic haystack? Is search the result of diffusion and chance encounters, or is there a search apparatus dedicated to bringing the homologous sequences together? To explore the choreography of the DNA and the recombination proteins during homologous search, we developed an in vivo 3-colors assay in diploid yeast cells where 2 homologous chromosomes are fluorescently marked at the same locus (with GFP-Lac and RFP-Tet arrays), as well as recombination factors (FEP-tagged proteins). Using deconvolution microscopy, we tracked the movement of the two chromosomes in 3-dimensions in the absence and in the presence of a unique DSB induced near one of the marked chromosomes. In the absence of DSB, we found that homologous chromosomes undergo a constrain Brownian motion with a diffusion coefficient of 4.10^-8 μm²/s inside a small area of 300 nm. When a DSB is induced, the two homologous DNA become highly dynamic and homologous pairing occurs within one hour. This work is the first attempt to visualize simultaneously the movement of two homologous sequences in vivo into and out of repair centres. 1. Lisby, M., Barlow, J.H., Burgess, R.C. and Rothstein R. Cell: 118, 699-713, 2004. 2. Barzel, A. and Kupiec, M. Nature: 9, 27-37, 2007.

2261-Plat Single-Molecule Measurements of Synthesis by DNA Polymerase with Base-Pair Resolution Thomas Christian, Lou Romano, David Rueda.

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