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ATOMIC FORCE MICROSCOPY STUDIES OF INITIATION STEPS OF DNA MISMATCH REPAIR

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The ability to replicate DNA with high fidelity is essential to all organisms. Replication of a DNA genome is a complex process involving a large number of enzymes, including enzymes that are responsible for the maintenance of fidelity. DNA mismatch repair (MMR) is the mechanism by which DNA synthesis errors are corrected post-replicatively, and it is central to the survival of organisms. MutS and MutL homologs which involved in the initiation step of MMR are well conserved from bacterials to eukaryotics. It is well established that MutS homologs recognize the mismatch or small insertion deletion loops, while MutL homologs function as a match-maker. The interaction between MutS and MutL homologs activate the downstream events in MMR. But the details of mechanism of repair initiation is still under hot debate. We are investigating the protein-protein, protein-DNA interactions involved in the initiation steps of DNA MMR in three systems, *Escherichia coli* (*E. coli*), thermophile *Thermus aquaticus* (*Taq*) and yeast. Our long-term goal is to use atomic force microscopy to elucidate the mechanism of initiation of MMR in each organism, as well as underline the differences between these three representative organisms. To accomplish this end, we begin with the study of the interactions between DNA and the individual proteins. In this presentation, I will discuss the preliminary data on the unique DNA-protein interactions, which have been identified by AFM.

MutL homologs have been known to bind DNA nonspecifically. We found that yeast MutL homolog Mlh1-Pms1 appears to bind to dsDNA nonspecifically and cooperatively. These complexes were observed visually as long continuous tracts of Mlh-Pms1 protein associated with DNA. In addition, Mlh1-Pms1 binds to two strands of dsDNA simultaneously, indicating that Mlh1-Pms1 has at least two distinct DNA binding sites[1]. Figure 1 shows a representative AFM image of DNA-Mlh1-Pms1 complexes. Later experiments mapped the DNA binding sites to amino terminal fragments of Mlh1 and Pms1. The potential role of two DNA binding sites and cooperative DNA binding by yeast Mlh1-Pms1 in the DNA MMR or other DNA transaction events are under further investigation.

E. coli MutS and *Taq* MutS crystal structures showed that they recognize the mismatch by the interaction between DNA and proteins, which include an aromatic-ring stack with Phe39 (in *Taq*) or Phe36 (in *E. coli*) and hydrogen bonds between DNA phosphate backbones and side chains of proteins[2-3]. As a result, the DNA is sharply kinked. Using AFM, we found that upon binding of *E. coli* MutS in the absence of any nucleotide, there are two populations, bent and unbent DNA molecules (Fig. 2). This result is seen both at mismatches and at homoduplex sites. These results demonstrate the dynamic characteristics of mismatch recognition by MutS homologs, which have not been identified by other techniques.

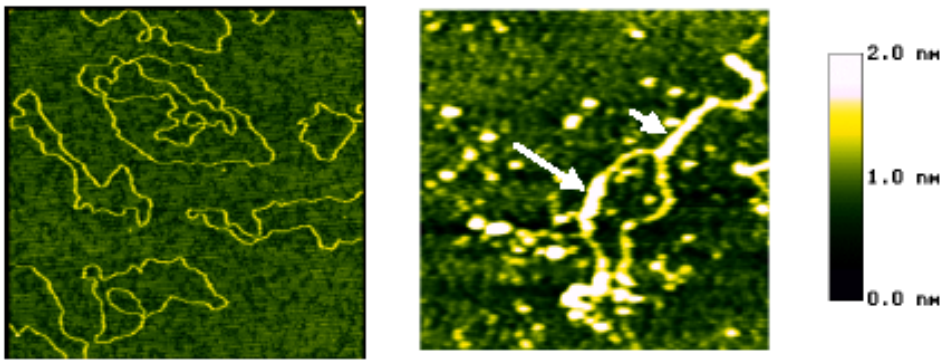

Fig. 1: Cooperative DNA binding by yeast Mlh1-Pms1: A) M13mp2 RFI DNA alone. The DNA concentration was 8.7 µM (nucleotide). B) 8.7 µM (nucleotide) M13mp2 RFI DNA in the presence of 35 nM yeast Mlh1-Pms1. The long arrow indicates a tract of cooperatively bound Mlh1-Pms1 associated with a single dsDNA region. The short arrow indicates a tract of cooperatively bound Mlh1-Pms1 associated with two dsDNA region of one M13 molecule. The image sizes are 1500 nm.

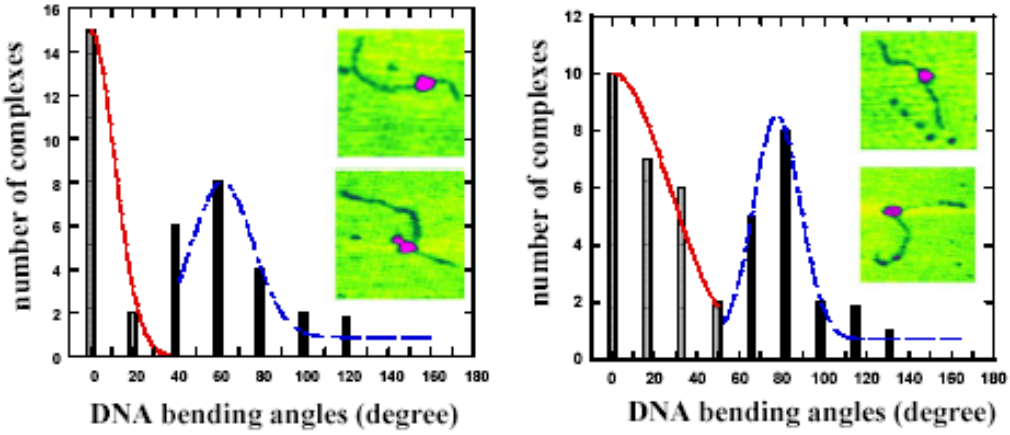

Fig. 2: Histograms showing the measured DNA bending angles induced by *E. coli* MutS binding: A) at mismatches, B) at homoduplex sites. The insets show AFM images of individual MutS-DNA complexes. The image sizes are 180 nm. Light is low and dark is high. The DNA substrates used are 762 bp long linear dsDNA and have a one T bulge 192 bp from one end. The protein and DNA concentrations used for sample deposition are 5 nM (dimmer) and 2.5 nM respectively.

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