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Single Molecule Study on Incorporation Efficiency of DPO4 and Klenow Fragment to BPDE Adduct LU SONG, UC Davis, LLNL, YIN YEH, ROD BALHORN, MONIQUE COSMAN, UC Davis — DNA synthesis involving high fidelity A-family polymerases such as Klenow fragment is blocked by DNA adducts, while Y-family DNA polymerases such as Dpo4 can bypass the DNA adducts to resume DNA synthesis. So understanding the functional relationship between A-family and Y-family DNA polymerases in DNA replication and the mechanism of bypassing DNA adducts is of great help to explain the cause of mutagenesis. We introduce a flow cell on modified surface to study the incorporation efficiency of Dpo4 and Klenow fragments to benzo[a]pyrene-diol-epoxide (BPDE) adduct at single molecule level. Specifically, we anchor the labeled DNA onto the modified surface with adduct site open for nucleotide incorporation and flow the polymerases and labeled nucleotides into flow cell. With Total Internal Reflection Fluorescence Microscopy (TIRFM) we identify the incorporation of the nucleotides onto the anchored DNA template by identifying the co-localization of the template position and that of the labeled nucleotide. 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. We can also identify, on the specific adduct site, which nucleotide, if any, is incorporated by each of the two polymerases.

Lu Song
University of California at Davis, Lawrence Livermore National Lab

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