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Monitoring the Bending Stiffness of DNA CHONGLI YUAN, XIONGWEN LOU, ELIZABETH RHOADES, HUIMIN CHEN, LYNDEN ARCHER, Cornell University — In eukaryotic cells, the accessibility of genomic sequences provides an inherent regulation mechanism for gene expression through variations in bending stiffness encoded by the nucleic acid sequence. Cyclization of dsDNA is the prevailing method for determining DNA bending stiffness. Recent cyclization data for short dsDNA raises several fundamental questions about the soundness of the cyclization method, particularly in cases where the probability of highly bent DNA conformations is low. We herein evaluate the role of T4 DNA ligase in the cyclization reaction by inserting an environmental sensitive base analogue, 2-amino purine, to the DNA molecule. By monitoring the 2-AP fluorescence under standard cyclization conditions, it is found that in addition to trapping highly-bent cyclic DNA conformations, T4 DNA ligase enhances the apparent base pair flip out rate, thus exaggerating the measured flexibility. This result is further confirmed using fluorescence anisotropy experiments. We show that fluorescence resonance energy transfer (FRET) measurements on suitably labeled dsDNA provides an alternative approach for quantifying the bending stiffness of short fragments. DNA bending stiffness results obtained using FRET are compared with literature values.

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