Mechanism of mismatch recognition by MutS in linear and circular DNA

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Post-replication DNA mismatch repair is initiated by MutS protein, which recognizes single mismatches/insertion-deletion errors. Many studies suggest that MutS senses altered local flexibility at the mismatch. However, all mismatches are not recognized equally efficiently, which remains puzzling. We compared intrinsic DNA flexibility/dynamics for efficiently- (T-bulge and G.T) and inefficiently- (T.T and T.C) repaired mismatches using fluorescent nucleotide analog 6-MI placed adjacent to the mismatch (Li et al. (2019), Int. J. Mol. Sci. 20:4271-4298). Fluorescence lifetime studies on 61-bp DNA revealed a narrow range of accessible conformations for T.T and T.C, indistinguishable from the matched (A.T) counterpart. In contrast, T-bulge toggled between two distinct conformations while G.T appeared partially unstacked. With laser T-jump, we measured nearly identical millisecond dynamics on low-specificity T.C and nonspecific A.T, while T-bulge exhibited much faster relaxation kinetics (<10-μs resolution of our T-jump). Our studies validate recent MD simulations results that showed rapid fluctuations in T-bulge DNA on the 15-μs MD timescales (Jayaraj et al. (2023), Biophys. J. 122:3031-3043). These rapidly fluctuating sites likely help stall a diffusing MutS to facilitate interrogation and represent mismatched sites more amenable to being kinked to form the recognition complex.

Most studies of mismatch recognition have focused on torsionally relaxed, linear DNA oligomers. However, DNA structure and deformability are strongly influenced by DNA topology (looping and supercoiling), which in turn should impact mismatch recognition. We examined the effect of DNA looping by incorporating a T-bulge into a 126-bp DNA minicircle. The fluorescence lifetime distribution at the T-bulge site for minicircle alone resembled that of the MutS-bound complex, and MutS binding affinity for the minicircle increased nearly 10-fold compared with linear DNA. These studies imply that the bending strain deforms the T-bulge site to a conformation more readily recognized by MutS.