The Effect of a Wedge Residue on the DNA Scanning Behavior of MutY Homologs using Single Molecule Methods

Proper repair of damaged DNA bases is important for genomic stability and cancer prevention. One pathway that has evolved to remove oxidative DNA damages is called the base excision repair pathway. The first step in this pathway utilizes enzymes called DNA glycosylases, which are responsible for finding, recognizing, and removing damaged bases. Mutations in one glycosylase, MUTYH, have been found to be responsible for a particular type of colon cancer in humans. One of these mutations is a single amino acid substitution in a "wedge" residue that crystal structures show is inserted into the DNA adjacent to the damage. Determining the DNA scanning behavior of the wedge variant will elucidate the mechanism behind this particular tumorigenesis.

In this project, we have examined the DNA scanning behavior of the wild-type (WT) and wedge variant using an *E. coli* homolog, MutY, and a *M. musculus* homolog, mMYH, on undamaged DNA and DNA containing the catalytic product, apurinic/apyrimidinic (AP) sites. We show that the wedge residue variants (Y82C and Y150C, respectively) scan faster than their wild-type counterparts on DNA. These data demonstrate the tyrosine wedge is critical for lesion recognition. Additionally, the wedge variants scan slightly slower in the presence of AP sites, indicating that the cysteine wedge contains low damage recognition activity. Overall, this gives a better understanding of the mechanism behind DNA repair and insight into MYH associated cancer.