A Novel Study for Elucidating the Molecular Mechanism of Flap Endonuclease-1

Wali, Rozan

Flap Endonuclease-1 (FEN1) is a key protein involved in propagation and maintenance of the integrity of DNA. FEN1 mutations or abnormal expression has been linked to diseases in human and animal models. This enzyme belongs to a large family called 5’ nucleases that play an essential role in DNA replication, recombination and repair. FEN1 is a structure-specific protein that binds to DNA double-flap structures, bends the DNA then cleaves the flap to maintain the integrity of genomic DNA. Replication Protein A (RPA) affects FEN1’s affinity to its DNA substrate, however the exact mechanism remains unknown. This study analyzes the role of DNA bending in the regulatory effect of RPA on FEN1. Here, RPA effect on increasing concentrations of FEN1 was measured in different lengths of 5’ flap using single molecule FRET in three replicate experiments. FRET changes, which reflect the bending/unbending state of the double flap substrate, were recorded upon addition of RPA and/or different concentrations of FEN1. The data confirmed that RPA inhibits FEN1 from bending the DNA when the 5’ flap is large, which prevents flap threading through Capped-Helical gateway. In conclusion, this study reveals for the first time that 1) DNA bending is a key step in the regulation of RPA/FEN1 interaction. 2) DNA bending is a required step for flap threading through Capped-Helical gateway. This work elucidates that RPA regulation of FEN1 is dependent on DNA bending and flap–length. Further understanding of FEN1 function contributes to the overall understanding of molecular mechanisms of multiple diseases.

Awards Won: