Investigating the Interplay between PCNA and FEN-1 in Genome Maintenance at the Single Molecule Level

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Malignant tumors and neurological diseases are amongst the many maladies caused by genome instability. The defects from genome instability are caused by significant changes in flap endonuclease-1 (FEN-1) protein concentrations, which create alterations in the DNA base sequence due to altered maturation of the Okazaki Fragments and the formation of Okazaki Flaps. The project's goal is to determine, in a novel way, the role of FEN-1 on double Okazaki flaps removal using the Single Molecular Tracking (SMT) method. Experiments were conducted using in-vitro lambda (?) phage DNA. Methods include tracking movement of PCNA and FEN-1 as well as the reliability of FEN-1 on PCNA during Okazaki flap elimination. Two experiments were conducted; FEN-1 tracking using Cy-333 fluorophore in the absence and presence of PCNA and tracking of FEN-1 interaction with Biotinylated lambda DNA bound with PEG-biotin/Neutravidin. This was accomplished using Total Internal Reflection Fluorescence (TIRF) microscope to illuminate tagged molecules. Nine trials of both experiments were conducted. Results indicate an increase of untethered double-flaps in the solution containing larger concentration of FEN-1. Furthermore, FEN-1 exhibited a greater movement in the presence of PCNA, suggesting the interaction of PCNA is involved in Okazaki elimination and ultimately genome instability increasing the diffusion rate from 2 ?m to 5 ?m in the absence of PCNA. In conclusion, the direct relationship of double-flap removal and FEN-1 could improve the knowledge of genome instability. The molecular basis of this interaction will be essential for limiting the generation of mutations and pursuing treatments for genetic diseases.

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