Genomic Instability Drives Cancer: Purification and Characterization of HLTF Helicase and Pol K Polymerase

Marupally, Tanvi (School: Arkansas School for Mathematics, Sciences and the Arts)

Besides the familiar double helix, DNA can form alternative structures such as G-quadruplexes (G4), guanine-rich four-stranded structures that arise during replication and repair. G4s are common in oncogene promoters, telomeres, and replication origins, where they can interfere with replication fork progression and threaten genome stability. This study investigated how the human helicase-like transcription factor (hHLTF) processes G4-containing replication intermediates. We hypothesized that HLTF would drive ATP-dependent fork regression on defined fork substrates under optimized conditions, regardless of substrate sequence or concentration. HLTF was expressed and purified using HisTrap FPLC chromatography, and fractions were confirmed by Western blot. Fluorescent helicase assays demonstrated robust ATP-dependent fork regression on synthetic fork substrates. HLTF maintained activity even at low DNA concentrations, and comparison of matched and mismatched forks showed no significant difference in regression efficiency, suggesting largely sequence-independent activity. To further examine replication fork processing, human DNA polymerase kappa (hPol ?), a translesion synthesis polymerase, was tested on control and G4-containing fork-extension substrates. Preliminary assays suggested potential issues with substrate preparation, highlighting the need for further optimization before accurately assessing hPol ? activity. Together, these results establish HLTF as an active fork-regressing helicase and lay the groundwork for investigating coordinated helicase–polymerase responses to G4-mediated replication stress relevant to cancer biology.

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