DEFINING THE ROLE OF DNA POLYMERASE η IN TOLERATING REPLICATION STRESS

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Transcription–replication conflicts (TRCs) and RNA:DNA hybrids (R-loops) are major sources of replication stress and genome instability, particularly under hydroxyurea (HU)-induced nucleotide depletion. Here we describe a translesion synthesis (TLS)-independent function of DNA polymerase η (pol η) in *Saccharomyces cerevisiae* during HU stress. Pol η localizes to a subset of replication origins and to regions adjacent to highly transcribed genes, accumulating at TRC sites independently of collision orientation, consistent with a general role in limiting transcription–replication interference. Genetic and biochemical evidence indicates that pol η promotes TRC bypass by incorporating RNA into DNA and elongating nascent RNA transcripts. These RNA:DNA tracts require RNase H for resolution; otherwise, they elicit DNA damage checkpoint activation, persistent stress, and lethality. The toxic effect of pol η is suppressed by overexpression of Sen1 or RNase H1, or by inhibition of transcriptional elongation, supporting its direct role in RNA-mediated replication stress. We propose that pol η facilitates TRC bypass by stabilizing or remodelling RNA:DNA hybrids at stalled forks. This work identifies an unexpected genome maintenance role for pol η, dependent on its RNA-extension activity.

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