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Abstract

The roles of TIMELESS in the suppression of single-stranded DNA gaps and mutagenesis

By

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DNA replication is a vital biological process often challenged by replication stressors that contribute to replication stress. Replication fork integrity is maintained by the fork protection complex, a member of which, TIMELESS (TIM) localizes ahead of the helicase to scaffold replisome architecture by preventing helicase and polymerase uncoupling. While this structural role of TIM is well-appreciated, the underlying basis by which TIM coordinates key replicative activities like coordinating leading and lagging strand synthesis whilst suppressing mutagenesis remains elusive.

Okazaki fragments (OFs) generated during lagging strand synthesis are canonically processed by FEN1 and LIG1. OF processing defects cause replication-associated ssDNA gaps, activating the single-stranded break repair activity of PARP1 and its downstream effectors, XRCC1 and LIG3. Here, we demonstrate the stable interaction of TIM and PARP1 at replication forks. Acute TIM depletion via auxin-inducible degradation revealed ssDNA gap accumulation and decreased PARP1 catalytic activity, which was phenocopied when the TIM-PARP1 interaction was physically disrupted by the expression of a polypeptide that competed with TIM for PARP1 binding. Additionally, both TIM loss and disruption of the TIM-PARP1 interaction when combined with canonical OF processing inhibition resulted in decreased PARP1 and XRCC1 recruitment to replication-associated ssDNA gaps, increased DNA damage, and decreased cell viability and colony formation. Taken together, our results indicate that TIM through its interaction with PARP1 maintains genomic integrity by suppressing replication-associated ssDNA gaps by modulating PARP1-mediated OF processing.

TIM's protective role in genome maintenance may extend to preventing the aberrant engagement of mutagenic DNA damage tolerance mechanisms, translesion synthesis (TLS), and PRIMPOL-mediated repriming. Following TIM loss, we observed increased mutagenesis, possibly stemming from aberrant TLS engagement and increased *PRIMPOL* mRNA levels, potentially resulting in increased repriming. Furthermore, bulk RNA-sequencing revealed the signature of apoptotic cell death and inflammatory signaling when TIM loss is combined with the inhibition of ATR activity, underscoring the role of TIM in preventing DNA replication problems and thus limiting engagement of the DNA replication checkpoint. Overall, these fundamental discoveries will inform strategies to target TIM or the TIM-PARP1 interaction to exploit the replication vulnerabilities of cancer cells for therapy.

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