In and out of replication stress: PCNA/RPA1-based dynamics of fork stalling and restart in the same cell

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Abstract:

Replication forks encounter various impediments which induce fork stalling and threaten genome stability, yet the precise dynamics of fork stalling and restart at the single-cell level remain elusive. Herein, we devise a live-cell microscopy-based approach to follow hydroxyurea-induced fork stalling and subsequent restart at 30-s resolution. We measure two distinct processes during fork stalling. One is rapid PCNA removal, which reflects the drop in DNA synthesis. The other is gradual RPA1 accumulation on up to 2400 nt of ssDNA per fork despite an active intra-S checkpoint. Restoring the nucleotide pool enables prompt restart without post-replicative ssDNA and a smooth cell cycle progression. ATR, but not ATM inhibition, accelerates hydroxyurea-induced RPA1 accumulation nine-fold, leading to RPA1 exhaustion within 20 min. Fork restart under ATR inhibition led to the persistence of ~600 nt ssDNA per fork after S-phase, which reached 2500 nt under ATR/ATM co-inhibition, with both scenarios leading to mitotic catastrophe. MRE11 inhibition had no effect on PCNA/RPA1 dynamics regardless of ATR activity. Our results shed light on fork dynamics during nucleotide depletion and provide a valuable tool for interrogating the effects of replication stress-inducing anti-cancer agents.