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The DNA replication licensing factor Tah11 (Cdt1) functions to protect cells from camptothecin and replication fork arrest

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Abstract

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Camptothecin (CPT) targets DNA topoisomerase I by reversibly stabilizing a covalent enzyme-DNA intermediate. The cytotoxic activity of the drug, however, is S-phase dependent and results from the collision of advancing replication forks with the ternary CPT-Top1-DNA complexes. The response of S-phase and DNA damage checkpoints to CPT-induced lesions is consistent with this cytotoxic mechanism. Yet, the molecular interactions required to elicit these cytotoxic lesions during S-phase and the nature of the lesion(s) induced remain largely unknown. Using a self-poisoning Top1T722A mutant enzyme that mimics the action of CPT in stabilizing the Top1-DNA complex, a conditional yeast *tah11-10* mutant was isolated, which exhibits enhanced sensitivity to Top1 poisons at high temperature. In all eukaryotes, Cdc6 and Tah11(Cdt1) function in G1 phase of the cell cycle to license origins of replication to fire during S-phase. This process is tightly regulated to ensure that the activity of Cdc6 and Tah11(Cdt1) is restricted to G1-phase so that a given origin will fire once and only once per cell cycle. In *tah11-10*, a single Gly233 to Glu substitution alters the activity of Tah11 such that the cells are viable at 36°C in the absence of DNA damage, but are inviable in the presence of Top1 poisons or hydroxyurea (HU). HU induces replication checkpoint activation by inhibiting ribonucleotide reductase and depleting nucleotide pools. At 36°C, *tah11-10* cell viability was also dependent on the presence of Sic1, the inhibitor of Cdc28-Clb kinase activity that functions in the G1-S phase transition. These phenotypes are consistent with the known origin licensing function of Tah11(Cdt1). However, the inability of the S-phase checkpoint to abolish the synthetic lethality of *tah11-10,sic1*Δ strains at 36°C coupled with the enhanced rate of plasmid loss observed in *tah11-10* cells in the presence of Top1 poisons suggest that Tah11 also functions during S-phase. To determine whether the Gly233 to Glu mutation alters the function/localization of Tah11 or simply decreases the stability of the active protein, two independent genetic approaches were pursued. First in a screen for rta mutants, suppressor mutations in genes other than *TAH11* were isolated that restored *tah11-10* resistance to HU. In a second screen, dosage suppressors of *tah11-10* were isolated, where increased copies of a given gene restores cellular resistance to Top1 poisons or HU. Surprisingly, both screens yielded suppressors that were able to restore *tah11-10* resistance to HU, yet failed to complement *tah11-10* hypersensitivity to Top1 poisons. These results suggest distinct alterations in Tah11 function exacerbate the cytotoxic activity of Top1T722A, independent of the S-phase checkpoint signaling pathways induced by HU. This work was supported by NIH grant CA58755 and ALSAC.

Footnotes

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