

Abstract

RecQ helicases are a family of genome maintenance proteins present ubiquitously from bacteria to eukaryotes. Despite their conserved domain architecture, RecQ helicases exhibit considerable variation in domain orientation and substrate specificity. The Winged Helix (WH) domain, which is the DNA-binding domain of RecQ helicases, undergoes conformational rearrangement upon DNA binding in several bacterial and human RecQ helicases. Such conformational rearrangement is not found in human RECQ1. In order to address whether the variable WH domain architecture and conformation across RecQ family members influences the substrate selectivity of RecQ helicases, an *Ec-Hs* chimeric RecQ helicase was constructed by fusing the helicase and Zn-binding domains of *E. coli* RecQ with the WH domain of human RECQ1. The *Ec-Hs* chimeric RecQ enzyme exhibited markedly reduced DNA unwinding activity, ATPase activity, and DNA binding affinity than those of wild-type *E. coli* RecQ. *Ec-Hs* chimeric RecQ exhibited a substrate selectivity profile resembling mostly that of human RECQ1. These results indicated that the WH domain architecture dictates substrate recognition of RECQ1, and that domain swapping disrupts the inter-domain communication essential for coupling of DNA binding to ATP hydrolysis. Next, the mechanism of replication fork restoration by RECQ1 was investigated in the presence of Replication protein A (RPA). RPA, a key interacting partner of RECQ1, stimulates its DNA unwinding activity. RECQ1 promotes restoration of replication forks regressed by topoisomerase 1 (TOP1) inhibition. Previous work from our group (unpublished) identified that arginine residues at the N-terminus of RPA70 interact with acidic residues on the D2 domain of RECQ1. Mutations of these interacting residues on RECQ1 to alanine (RECQ1^{2A} mutant) diminished its interaction with RPA70. This study examined whether RECQ1 requires an association with RPA to facilitate fork restoration in MCF7 breast cancer cells. Expression of the interaction-deficient RECQ1^{2A} mutant in RECQ1-knockdown MCF7 cells was unable to rescue fork slowing and fork restoration defects subsequent to TOP1 or TOP2 inhibition. Consequently, there was an accumulation of double-strand breaks in response to genotoxic stress. Furthermore, PARP1 inhibition did not mitigate CPT-induced fork slowing in RECQ1^{2A}-expressing cells. It is hypothesized that RPA70 recruits RECQ1 and stimulates its branch migration activity to restore regressed forks in response to topoisomerase inhibition, thus safeguarding the stability of the genome.

Keywords: RECQ1, substrate selectivity, replication fork restoration, RECQ1-RPA interaction