## Investigating the Role of Rev1 in Maintaining Accurate DNA Replication Past G4 Containing Regions in the Leading and Lagging Strands

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G-quadruplex structures (G4s) are one of many secondary structures that genomic DNA adopts, apart from its canonical B-form, and they are a vital part of cellular regulation. Sequences that are capable of forming these G4 structures in vitro are often sites of genomic instability in vivo. Rev1, is a human DNA polymerase that was shown to have a high binding affinity to G4 sequences. In order to perform G4 replication accurately, enzymes that are specialized for the function are needed. More specifically, helicases are needed for unwinding and polymerases, like Rev1, are needed to promote fork progression and prevent refolding of G4 DNA structures. In our earlier experiments, we used the supF forward mutagenesis assay to study the importance of Rev1 in G4 DNA replication. A significantly higher mutation frequency was observed in the Rev1-deficient cells, as compared to the Rev1-proficient, and this effect was further exacerbated in the presence of the G4-stabilizing chemical pyridostatin (PDS). These studies were done with the G4 motif placed on the lagging strand, with respect to the replisome. In addition, we investigated the positional effect of the G4 motif with respect to the supF gene. For this purpose, we used two variants of the pSP189 reporter plasmid; one where the Myc-G4 motif is placed upstream and on the same strand as the supF coding gene (lagging) as done earlier, and the other where it is placed on the opposite strand (leading). These plasmids were then replicated in Rev1-proficient or Rev1-deficient HEK293T cells, in combination with treatment with PDS. Measuring the mutation frequencies by blue-white colony counting allowed us to analyze whether the placement of G4 motif in one or the other orientation has a greater effect on replication accuracy.

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