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In vitro lesion bypass by human PrimPol

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Motivation and Aim: Several human DNA polymerases efficiently incorporate dNTPs opposite DNA lesions during the process called DNA translesion synthesis (TLS). Human PrimPol is a DNA primase with DNA polymerase activity which plays an important role in maintenance of genome integrity. The primary function of PrimPol in vertebrate cells is reinitiation of stalled DNA replication forks at the DNA damage sites and non-B DNA structures. Experiments suggest that PrimPol is capable of initiating *de novo* DNA synthesis downstream of DNA damage using the DNA primase activity. *In vitro* studies also show that human PrimPol is capable of bypassing some DNA lesions in a manner of TLS-polymerase. The TLS activity of PrimPol was demonstrated opposite 8-oxo-G, abasic site (AP-site) and photoproducts. However, the efficiency and accuracy of dNTP incorporation differ among studies and the activity of PrimPol on templates with other common DNA lesions is yet to be characterized.

Methods and Algorithms: In this work, we carried out analysis of the TLS activity of human PrimPol opposite a series of common DNA lesions: 8-oxo-G, thymine glycol, 5-formiluracil (5-fU), AP-site, O^6-me-G and 1,N^6-ethenoadenine (εA).

Results: We demonstrate that PrimPol possesses the properties of TLS polymerase and, in presence of Mg^{2+} ions as metal cofactor, PrimPol carries out very efficient and accurate synthesis past 8-oxo-G and 5-fU lesions caused by oxidative stress. The steady state kinetic analysis has shown that incorporation of complementary dCTP opposite 8-oxo-G is 8-fold higher than incorporation of non-complementary dATP. PrimPol incorporates correct dATP 20-fold more efficient then dGTP opposite 5-fU. In the presence of Mg^{2+} ions, PrimPol also bypasses AP-site and O^6-me-G with moderate efficiency but is blocked opposite thymine glycol and εA. PrimPol bypasses O^6-me-G in error-prone manner preferably incorporating non-complementary dTTP opposite the lesion. Mn^{2+} ions significantly stimulate the TLS activity of PrimPol: PrimPol carries out efficient synthesis opposite all tested DNA lesions including thymine glycol and εA but demonstrates very low accuracy of dNTPs incorporation. We also demonstrate that the accessory protein PolDIP2 stimulates the TLS activity of PrimPol *in vitro*.

Conclusion: These data suggest that the TLS activity of PrimPol have possible relevant functions *in vivo*. In particular, the combined primase and polymerase activities of PrimPol might facilitate replication of DNA with clustered damage.

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