ON THE ROLE OF PROOFREADING EXONUCLEASE IN BYPASS OF A BULKY 1,2 D(GPG) CISPLATIN ADDUCT BY THE HERPES SIMPLEX VIRUS-1 DNA POLYMERASE

Mercedes E. Arana¹, Liping Song², Nicolas Tanguy Le Gac³, Deborah S. Parris², Giuseppe Villani³ and Paul E. Boehmer¹*: ¹Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL 33101, ²Department of Molecular Virology, Immunology and Medical Genetics, Ohio State University, Columbus, OH 43210, ³Institut de Pharmacologie et de Biologie Structurale, CNRS-UMR, 5089, 205 route de Narbonne, 31077 Toulouse cedex 4, France
*Corresponding author: pboehmer@molbio.med.miami.edu

INTRODUCTION: Translesion synthesis provides a mechanism whereby DNA replication can be completed in the absence of DNA repair often at the expense of fidelity. We previously used a 60:17-mer template primer containing a site-specific 1,2 d(GpG) cisplatin adduct to examine the capacity of the replicative DNA polymerase of herpes simplex virus type-1 (UL30) to perform translesion synthesis in vitro (1). We found that when Mg²⁺ was substituted with Mn²⁺, UL30 efficiently catalyzed bypass of the lesion. Moreover, substitution of Mg²⁺ with Mn²⁺ reduced the 3'-5' exonuclease activity of UL30 and elicited a conformational change in the enzyme. A long-standing view has been that polymerases with Exo activity engage in a futile cycle of incorporation and excision (idling) at sites of DNA damage, since any incorporation is viewed by the Pol as a misincorporation event (2). Consequently, inhibition of Exo activity may facilitate TLS by this class of enzyme. We predicted that an exonuclease-deficient UL30 (UL30D368A) would perform translesion synthesis.

METHOD: Primer extension assays reactions were performed as previously described (1) for the times indicated and the indicated concentrations of undamaged or damaged template primer, UL30 and UL42. Samples were resolved by electrophoresis through 15% polyacrylamide-7 M urea-30% formamide gels in 1X glycerol tolerant buffer (United States Biochemicals). Lesion-dependent turnover reactions were examined by measuring ³²P dAMP production by UL30. Following incubation at 37 °C for 60 min, reactions were analyzed by chromatography on silica gel 60 F₂₅₄ thin layer plates (EM Science). For both assays, reaction products were visualized and quantified by storage phosphor analysis.

RESULTS: Here, we show that UL30, the herpes simplex virus type-1 DNA polymerase, stalls at the base preceding a cisplatin crosslinked 1,2 d(GpG) dinucleotide and engages in a futile cycle of incorporation and excision by virtue of its 3'-5' exonuclease (Fig. 1). Our data show that UL30D368A did not perform complete translesion synthesis but incorporated one nucleotide opposite the first base of the adduct. dATP was sufficient for incorporation indicating that it is error-prone. This addition also required a running start of at least one nucleotide and was affected by the propensity of the enzyme to dissociate from the damaged template. Consequently, addition of the polymerase processivity factor, UL42, increased nucleotide incorporation opposite the
lesion. The inability of UL30D368A to extend beyond the first nucleotide opposite the lesion may be attributed to the finding that the primer terminus opposite the crosslinked d(GpG) dinucleotide and at least 3 bases downstream of the lesion is unpaired and not utilized by the enzyme. These data provide new insight into the role of proofreading exonuclease activity in translesion synthesis of a bulky adduct by a replicative polymerase.

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