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RESEARCH ABSTRACT FORM

TITLE: Bypass Mechanism of a DNA Lesion Caused by an Environmental Pollutant 1-Nitropyrene

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An environmental pollutant 1-nitropyrene at the molecular level reacts predominantly with deoxyguanine in DNA forming deoxyguanine with a 1-aminopyrene (1-AP) adduct at the C8 position. These 1-AP adducts are thought to be bypassed by the Y-family DNA polymerases, which perform translesion synthesis to replicate damaged DNA. In this project, *Sulfolobus solfataricus* DNA Polymerase IV (Dpo4), a Y-family polymerase, was chosen to study the bypass of the 1-AP adduct because Dpo4 is a good structural and biochemical model for human Y family polymerases. In order to characterize the mechanism by which Dpo4 bypasses the 1-AP adduct, pre-steady state kinetic assays were utilized. The kinetic results from these assays were compared to previously performed kinetic assays of undamaged DNA to elucidate the effect of the 1-AP adduct on DNA replication. Although these assays demonstrated the bypass of the lesion, Dpo4 paused upon the incorporation of nucleotides directly opposite and one position downstream from the 1-AP adduct. When investigating the cause and nature of these pausing events, a decrease in the catalytic efficiency and fidelity of incorporation were found. In addition, the binding affinity of the DNA duplex was found to be altered. The strong pause sites have the potential to be mutagenic hot spots, which would confirm the International Agency of Research on Cancer's classification of 1-nitropyrene as a mutagen and possible carcinogen. As a Y-family representative, the mechanism associated with Dpo4's bypass of the 1-AP adduct is tantamount to the bypass that would be performed by human Polymerases eta and kappa. Thus, the mechanism elucidated from this study will have implications for the understanding of TLS processes in humans following exposure to environmental pollutants.