

## Chemistry and Biology of Eukaryotic Translesion Synthesis

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### Abstract

Translesion synthesis (TLS), a mechanism utilized by cells to synthesize past DNA lesion, is evolutionarily conserved in organisms from prokaryotes to eukaryotes. Ubiquitylation of proliferating cell nuclear antigen (PCNA) plays an important role in eukaryotic TLS. The molecular details of how ubiquitylation of PCNA regulates TLS are poorly understood. To facilitate biochemical investigation of TLS, we developed a chemical approach for PCNA ubiquitylation. The chemically ubiquitylated PCNA is functionally equivalent to the native ubiquitylated PCNA in effecting polymerase switch. We also demonstrated the strict requirement of PCNA ubiquitylation for polymerase switch between Pol $\delta$  and Pol $\eta$ . Moreover, we investigated the various sites of ubiquitylation by preparing chemically ubiquitylated PCNAs that differ only in the position of modification. Together with a recent solution structure study of Ub-PCNA using SAXS, we revealed a high degree of mobility of ubiquitin moiety on PCNA. Another important aspect of ubiquitylation in TLS is how the specialized DNA polymerase is recruited to the lesion site. We investigated the ubiquitin-binding zinc finger (UBZ) domain in the C-terminal region of Pol $\eta$  and its role in recruiting the TLS polymerase. Our study suggested a novel mode of Ub binding that affords higher affinity interaction. We have recently targeted the human TLS to overcome cancer cells' resistance to DNA damaging drugs. Our quantitative high-throughput screening (qHTS) efforts have led to potent and selective inhibitors against the human deubiquitinating enzyme (DUB), USP1/UAF1. The reversible and noncompetitive USP1/UAF1 inhibitors were shown to be effective in reversing the chemoresistance of non-small cell lung cancer cells.