Structural basis of gap-filling DNA synthesis in the nucleosome by DNA Polymerase β

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Genomic DNA is packaged into chromatin through a fundamental repeating unit known as the nucleosome. The nucleosome is a protein-nucleic acid complex composed of an octameric assembly of histone proteins wrapped by ~147 bp of nucleosomal DNA, which functions as a regulatory barrier that controls accessibility of genomic DNA. Within this chromatin context, genomic DNA is continuously exposed to endogenous and exogenous stress that leads to DNA damage. Importantly, this DNA damage must be effectively repaired to maintain genome stability. How DNA repair enzymes overcome the chromatin barrier to repair DNA damage remains an important unanswered question in the DNA repair field.

Base excision repair (BER) is a genome surveillance pathway responsible for repairing DNA base lesions that arise from alkylation, oxidation, and deamination events. DNA polymerase beta (Pol β) is the primary enzyme responsible for processing the 1-nt gap intermediate that arises during BER. Despite decades of research defining how Pol β function in the context of non-chromatinized DNA, how Pol β functions in chromatin has remained largely unexplored. To initially bridge this knowledge gap, we recently employed a combination of biochemical assays and cryogenic electron microscopy (cryo-EM) to unravel the mechanism used by Pol β to process 1-nt gaps embedded within the nucleosome (*Nature Communications*, 2025), providing initial mechanistic insight into Pol β function within chromatin.

We initially utilized enzyme kinetics to define how well DNA Pol β catalyzes 1-nt gap filling DNA synthesis within the nucleosome. We identified that Pol β can perform 1-nt gap filling within the nucleosome, though with a reduced efficiency compared to 1-nt gap filling in non-chromatinized DNA. To further understand the mechanistic basis for this observation, we determined a series of cryo-EM structures of Pol β bound to 1-nt gaps embedded at different locations within the nucleosome. From these structures, we identified that Pol β uses a global DNA sculpting mechanism for recognizing and processing 1-nt gaps within the nucleosome. This DNA sculpting mechanism is mediated by large structural changes in the configuration of the DNA within the nucleosome that includes ripping the DNA away from the histone octamer and bending the DNA ~90°, which enables Pol β to separate the two ends of the 1-nt gap into its active site for catalysis. We liken this process to a molecular tug-of-war, where the DNA Pol β and the histones are in a fierce competition for access to the damaged nucleosomal DNA (Figure 1), which provides a strong rationale for the reduction in Pol β 1-nt gap filling activity within chromatin. Ultimately, this work has provided foundational insight into how Pol β functions within the nucleosome and lays the groundwork for future collaborative work between the Freudenthal Lab and the recently established Weaver Lab in the Department of Biochemistry and Molecular Genetics at the University of Virginia School of Medicine.

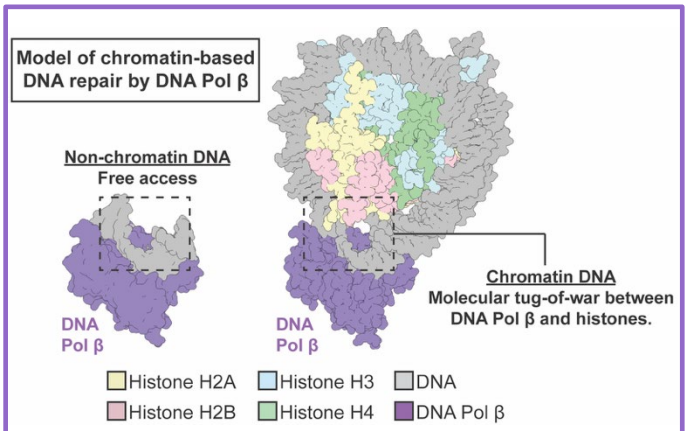

Figure 1: Model of the molecular tug-of-war between DNA Pol β and histones during chromatin-based DNA repair.

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