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Molecular functional analysis of DNA polymerase I in base excision repair

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Many types of genomic DNA damage occur frequently in all organisms. These damages include deamination, methylation and oxidation of bases, misincorporation during DNA replication, and DNA strand breaks. To counteract deleterious effects of DNA damage, organisms have evolved several strategies to repair damaged DNA. Base excision repair (BER) is an efficient system which excises and replaces a damaged base. BER is initiated with excision of a damaged base by DNA glycosylase, which cleaves the N-glycosidic bond of the damaged base. AP-endonuclease (APE) cleaves the DNA strand at the resulting AP site in the second step, generating 3'-OH and 5'-deoxyribose phosphate (5'-dRP) termini. In eukaryotes, BER further proceeds by two sub-pathways distinguished by their patch size. In single nucleotide BER (SN-BER), 5'-dRP residue is enzymatically removed to generate 5'-phosphate group and after single nucleotide incorporation the resulting nick is sealed by DNA ligase in the final step. In contrast, long patch BER (LP-BER) proceeds when AP sites is further oxidized by ROS, or the resulting 5' blocking groups after cleavage of the DNA strand by APE cannot be removed. In LP-BER, two to eight nucleotides are incorporated into the repaired DNA strand by DNA polymerase and the resulting flap structure produced during primer extension is cleaved by flap endonuclease (FEN). Bacteria have no FEN homologue and thus it is unclear how LP-BER proceeds in bacterial cells. DNA polymerase I (Pol I) contains a 5' exonuclease domain with a limited homology to FEN. This domain has similar structural organization to FEN. This raised the possibility that Pol I works as FEN in bacterial LP-BER. In this study, we characterized the nuclease activity of *Thermus thermophilus* Pol I (ttPolI). We comfirmed ttPolI had flap structure-specific endonuclease activity towards flap and Y structured DNA. In contrast, ttPolI exhibited low gap-filling activity. We further examined whether the 5' exonuclease activity of ttPolI is involved in LP-BER.