Published online 20 June 2016

Nucleic Acids Research, 2016, Vol. 44, No. 17 ***8229–8240***
doi: 10.1093/nar/gkw556

Strand displacement synthesis by yeast DNA polymerase ε

Rais A. Ganai[1,2], Xiao-Ping Zhang[3], Wolf-Dietrich Heyer[3] and Erik Johansson[1,*]

[1]Department of Medical Biochemistry and Biophysics, Umeå University, SE-90187 Umeå, Sweden, [2]Howard Hughes Medical Institute, Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, NY 10016, USA and [3]Department of Microbiology and Molecular Genetics, University of California, Davis, CA 95616-8665, USA

Received March 18, 2016; Revised June 08, 2016; Accepted June 09, 2016

ABSTRACT

DNA polymerase ε (Pol ε) is a replicative DNA polymerase with an associated 3′–5′ exonuclease activity. Here, we explored the capacity of Pol ε to perform strand displacement synthesis, a process that influences many DNA transactions *in vivo*. We found that Pol ε is unable to carry out extended strand displacement synthesis unless its 3′–5′ exonuclease activity is removed. However, the wild-type Pol ε holoenzyme efficiently displaced one nucleotide when encountering double-stranded DNA after filling a gap or nicked DNA. A flap, mimicking a D-loop or a hairpin structure, on the 5′ end of the blocking primer inhibited Pol ε from synthesizing DNA up to the fork junction. This inhibition was observed for Pol ε but not with Pol δ, RB69 gp43 or Pol η. Neither was Pol ε able to extend a D-loop in reconstitution experiments. Finally, we show that the observed strand displacement synthesis by exonuclease-deficient Pol ε is distributive. Our results suggest that Pol ε is unable to extend the invading strand in D-loops during homologous recombination or to add more than two nucleotides during long-patch base excision repair. Our results support the hypothesis that Pol ε participates in short-patch base excision repair and ribonucleotide excision repair.

INTRODUCTION

DNA polymerase ε (Pol ε) is a replicative polymerase that carries out leading-strand synthesis at the eukaryotic replication fork (1–7). Pol ε is a high-fidelity polymerase with high nucleotide selectivity and an associated 3′–5′ exonuclease activity (8). In addition to its role at the replication fork, Pol ε is engaged in many different cellular functions such as the initiation of DNA replication, S-phase checkpoint activation and DNA repair (9). Participation in DNA repair processes often requires that a DNA polymerase is able to extend 3′-OH ends on nicked substrates, fill in short gaps and/or carry out strand displacement synthesis. Strand displacement synthesis is a process in which the DNA polymerase simultaneously synthesizes DNA and separates the two strands ahead of the DNA polymerase. A single-stranded DNA flap is created after the DNA polymerase has dissociated, and the flap must be removed by a nuclease before a ligase can seal the remaining nick. The commercially available φ29 DNA polymerase has a very high strand displacement activity that is utilized to create PCR-free templates for DNA sequencing (10). However, replicative DNA polymerases are in general very inefficient in strand displacement synthesis. It was recently shown that the regression pressure imposed by the upstream double-stranded DNA shifts the primer terminus from the polymerase active site to the exonuclease active site (11), thus suppressing strand displacement synthesis. It has also recently been shown in the T4 and T7 bacteriophage replication systems that there is a functional coupling between the helicase and polymerase that promotes strand displacement synthesis. In one of the studies, the authors showed that the helicase relieves the regression pressure of the downstream DNA and thus promotes strand displacement synthesis (12). In another study, it was shown that T7 DNA polymerase opens up the double-stranded DNA, and the helicase translocates along and traps the unwound bases (13). In the T4 and T7 bacteriophage, the helicase resides on the lagging strand and thus the leading strand polymerase is on the opposite strand. However, the properties of the leading strand DNA polymerase in eukaryotes might be different because in eukaryotes both Pol ε and the helicase reside on the same strand, the leading strand (3).

Previous studies where strand displacement synthesis by Pol ε were discussed suggested that Pol ε was very inefficient in performing strand displacement synthesis (3,14,15). Here, we show that the strand displacement synthesis by Pol ε is limited to only one nucleotide. We further examined the ability of Pol ε to carry out strand displacement synthesis on different model substrates resembling the intermediate

*To whom correspondence should be addressed. Tel: +46 90 7866638; Fax: +46 90 7869795; Email: erik.tm.johansson@umu.se