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Switching between polymerase and exonuclease sites in DNA polymerase ε

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ABSTRACT

The balance between exonuclease and polymerase activities promotes DNA synthesis over degradation when nucleotides are correctly added to the new strand by replicative B-family polymerases. Misincorporations shift the balance toward the exonuclease site, and the balance tips back in favor of DNA synthesis when the incorrect nucleotides have been removed. Most B-family DNA polymerases have an extended β-hairpin loop that appears to be important for switching from the exonuclease site to the polymerase site, a process that affects fidelity of the DNA polymerase. Here, we show that DNA polymerase ε can switch between the polymerase site and exonuclease site in a processive manner despite the absence of an extended β-hairpin loop. K967 and R988 are two conserved amino acids in the palm and thumb domain that interact with bases on the primer strand in the minor groove at positions *n*–2 and *n*–4/*n*–5, respectively. DNA polymerase ε depends on both K967 and R988 to stabilize the 3′-terminus of the DNA within the polymerase site and on R988 to processively switch between the exonuclease and polymerase sites. Based on a structural alignment with DNA polymerase δ, we propose that arginines corresponding to R988 might have a similar function in other B-family polymerases.

INTRODUCTION

The fidelity of DNA replication depends on the accuracy with which the DNA polymerase incorporates a nucleotide, the enzyme's built-in exonuclease activity that removes misincorporated nucleotides, and the mismatch repair system that corrects errors that elude the DNA polymerase (1). In eukaryotes, DNA polymerase ε (Pol ε) is considered to be responsible for leading strand synthesis and DNA polymerase δ (Pol δ) for lagging strand synthesis (2,3). Both enzymes have proofreading capacity with a built in 3′–5′ exonuclease activity, and the exonuclease and the polymerase sites are located approximately 40 Å apart (4,5).

The transfer of the 3′-terminus of the primer strand between the polymerase and exonuclease sites occurs either through an intermolecular mechanism that involves dissociation and reassociation events or through an intramolecular mechanism without dissociating from the template DNA. In general, DNA polymerases are non-exclusive between these options (6).

The ability of the replicative DNA polymerases to both synthesize and degrade DNA allows the polymerases to build DNA with very high fidelity. However, the partitioning between the polymerase and exonuclease sites not only influences the fidelity, it also determines whether the net result is synthesis of a new DNA strand. The concept of a 'balance' was used as a metaphor by Reha-Krantz to describe the relationship between the two activities (6). Under normal circumstances, the polymerase activity dominates over the exonuclease activity. However, there are substitutions in DNA polymerases that affect this balance by creating mutator polymerases or antimutator polymerases. Structural studies have revealed that a large change occurs in the positioning of the thumb domain so as to accommodate the transfer of the DNA primer terminus between the polymerase and exonuclease active sites (7). The separation of the primer strand from the template DNA is an energetically unfavorable process that requires assistance to occur.

The mechanism by which the 3′-terminus of the growing DNA strand is transferred between the polymerase site and exonuclease site is still unclear. Crystal structures have shown that at least three to four base-pairs are separated, and this allows the single-stranded DNA to interact with a groove within the exonuclease domain that leads to the exonuclease active site (8). Genetic studies of bacteriophage T4 revealed that mutations leading to substitutions in an extended β-hairpin loop influence the fidelity of the T4 DNA polymerase (9,10). Biochemical studies later showed that the extended β-hairpin loop influences the switch between the exonuclease site and polymerase site in the B-family DNA polymerase from bacteriophage RB69 (RB69 gp43) (11). Pol ε consists of four subunits, Pol2 (catalytic subunit), Dpb2 (interacts with the replicative polymerase), Dpb3 and Dpb4 (Dpb3 and Dpb4 interacts with double-

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