nature structural & molecular biology

Structural basis for processive DNA synthesis by yeast DNA polymerase ε

Matthew Hogg[1], Pia Osterman[1], Göran O Bylund[1], Rais A Ganai[1], Else-Britt Lundström[1], A Elisabeth Sauer-Eriksson[2] & Erik Johansson[1]

DNA polymerase ε (Pol ε) is a high-fidelity polymerase that has been shown to participate in leading-strand synthesis during DNA replication in eukaryotic cells. We present here a ternary structure of the catalytic core of Pol ε (142 kDa) from *Saccharomyces cerevisiae* in complex with DNA and an incoming nucleotide. This structure provides information about the selection of the correct nucleotide and the positions of amino acids that might be critical for proofreading activity. Pol ε has the highest fidelity among B-family polymerases despite the absence of an extended β-hairpin loop that is required for high-fidelity replication by other B-family polymerases. Moreover, the catalytic core has a new domain that allows Pol ε to encircle the nascent double-stranded DNA. Altogether, the structure provides an explanation for the high processivity and high fidelity of leading-strand DNA synthesis in eukaryotes.

DNA replication requires the coordinated activities of numerous proteins to unpack, copy and repackage the long strands of DNA. At the core of the replicative machinery are DNA polymerases that read the single-stranded DNA (ssDNA) template and insert complementary nucleotides from the cellular nucleotide pool. In eukaryotes, DNA replication is carried out by three distinct DNA polymerases that belong to the B family of DNA polymerases[1].

DNA polymerase α (Pol α) contains a primase domain that initiates replication by laying down a short RNA primer onto both the leading and lagging strands of the unwound DNA. Pol α then switches to DNA-polymerizing mode and extends these RNA primers by about 20 dNTPs and is subsequently replaced by DNA polymerase δ (Pol δ) or Pol ε. Accumulating evidence suggests that Pol δ is responsible for the replication of the lagging strand[2–4] in a process that occurs in short stretches known as Okazaki fragments and requires continuous release and rebinding by Pol δ. Pol ε is believed to be responsible for the largely processive and continuous replication of the leading strand[2,5].

The Pol ε holoenzyme consists of four polypeptides[6]: Pol2, which contains both polymerase and 3′-5′–exonuclease domains, and three accessory subunits, Dpb2, Dpb3 and Dpb4. The *DPB2* gene is essential in yeast, but the Dpb2 protein is not required for the polymerase activity[7,8]. *DPB3* and *DPB4* are nonessential genes in yeast[9,10], but biochemical studies have shown that Dpb3 and Dpb4 are important for the interaction between Pol ε and double-stranded DNA (dsDNA) to increase Pol ε processivity[11,12].

In contrast to Pol α and Pol δ, Pol ε is a highly processive enzyme[13–15]. Pol δ becomes processive only when bound to proliferating cell nuclear antigen (PCNA). The high fidelity of both Pol ε and Pol δ is in part conferred by their 3′-5′–exonuclease domains. It was recently shown that a substitution (L424V) in the human *POLE* gene (encoding Pol ε) impairs proofreading and is associated with a predisposition for colorectal cancer[16]. Somatic mutations in the Pol ε exonuclease domain are also cancer associated[17,18].

X-ray crystal structures of bacteriophage and archaeal B-family polymerases have been solved, and ternary-complex structures are available for the polymerase domains of eukaryotic Pol δ and Pol α (refs. 19–21). The structures of Pol δ and Pol α are highly similar to that of the DNA polymerase from bacteriophage RB69 (RB69 gene product 43 (gp43)) despite their low sequence identity[20,22]. Structural data for eukaryotic Pol ε, however, is limited to a low-resolution cryo-EM structure of yeast Pol ε, an NMR solution structure of a domain of Dpb2 and a crystal structure of Dpb4 in complex with a chromatin-remodeling complex[23–25].

To gain a more detailed understanding of the molecular basis of Pol ε function, we have solved a 2.2-Å ternary-complex crystal structure of the catalytic core of yeast Pol ε (residues 31–1184) in complex with a primer-template DNA and an incoming dATP. The structure provides insights into the similarities and differences between Pol ε and other B-family polymerases and suggests possible mechanisms responsible for the high processivity and fidelity of Pol ε.

RESULTS

Crystal structure of Pol ε

We crystallized the 142-kDa catalytic domain of Pol2 (residues 1–1228), which corresponds to the catalytic subunits of Pol δ (985 residues)[20] and RB69 gp43 (903 residues)[22]. Both Pol δ and RB69 gp43 are typical B-family polymerases with fingers, palm, thumb, exonuclease and N-terminal domains[19]. We postulated that these domains would be structurally conserved in Pol ε despite the low sequence identity between

[1]Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden. [2]Department of Chemistry, Umeå University, Umeå, Sweden. Correspondence should be addressed to E.J. (erik.johansson@medchem.umu.se) or A.E.S.-E. (elisabeth.sauer-eriksson@chem.umu.se).

Received 3 June; accepted 7 October; published online 1 December 2013; doi:10.1038/nsmb.2712