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Yeast DNA Polymerase ϵ Catalytic Core and Holoenzyme Have Comparable Catalytic Rates*

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Rais A. Ganai, Pia Osterman, and Erik Johansson[1]

From the Department of Medical Biochemistry and Biophysics, Umeå University, SE-901 87 Umeå, Sweden

Background: The catalytic rates for yeast Pol ϵ are unknown.
Results: The catalytic core and holoenzyme have comparable catalytic rates, but the loading onto primer termini differs.
Conclusion: The accessory subunits and C terminus of the catalytic subunit do not influence the catalytic rates.
Significance: The catalytic rates of Pol ϵ provide a benchmark for future mechanistic studies of leading strand synthesis.

The holoenzyme of yeast DNA polymerase ϵ (Pol ϵ) consists of four subunits: Pol2, Dpb2, Dpb3, and Dpb4. A protease-sensitive site results in an N-terminal proteolytic fragment of Pol2, called $Pol2_{core}$, that consists of the catalytic core of Pol ϵ and retains both polymerase and exonuclease activities. Pre-steady-state kinetics showed that the exonuclease rates on single-stranded, double-stranded, and mismatched DNA were comparable between Pol ϵ and $Pol2_{core}$. Single-turnover pre-steady-state kinetics also showed that the k_{pol} of Pol ϵ and $Pol2_{core}$ were comparable when preloading the polymerase onto the primer-template before adding Mg^{2+} and dTTP. However, a global fit of the data over six sequential nucleotide incorporations revealed that the overall polymerization rate and processivity were higher for Pol ϵ than for $Pol2_{core}$. The largest difference between Pol ϵ and $Pol2_{core}$ was observed when challenged for the formation of a ternary complex and incorporation of the first nucleotide. Pol ϵ needed less than 1 s to incorporate a nucleotide, but several seconds passed before $Pol2_{core}$ incorporated detectable levels of the first nucleotide. We conclude that the accessory subunits and the C terminus of Pol2 do not influence the catalytic rate of Pol ϵ but facilitate the loading and incorporation of the first nucleotide by Pol ϵ.

All eukaryotes have three replicative DNA polymerases (Pol[2] ϵ, Pol δ, and Pol α) that are responsible for the synthesis of the leading and lagging strands during DNA replication (1–3). Pol α and Pol δ are the major DNA polymerases that replicate the lagging strand. Pol α synthesizes short primers that are extended by Pol δ in a cyclical manner to produce stretches of DNA known as Okazaki fragments (4). Under normal conditions, Pol ϵ is primarily responsible for the synthesis of the leading strand (5). Both Pol δ and Pol ϵ have an exonuclease domain that provides a proofreading function and allows the polymerases to replicate DNA with high fidelity (6).

Kinetic studies of prokaryotic, archaeal, and eukaryotic DNA polymerases have shown that they all follow the same basic mechanism when incorporating nucleotides into the nascent DNA strand (7, 8). The first step involves binding of the enzyme to the DNA, and the second step is the binding of a dNTP and two metal ions into the active site of the enzyme. The third step involves a conformational change from an open to a closed state that aligns the incoming dNTP, the 3′-OH of the nascent DNA strand, and the metal ions in a precise arrangement to allow phosphodiester bond formation. Following the transfer of the phosphoryl group to the growing DNA chain, a second conformational change occurs that allows for the release of pyrophosphate. The length of the DNA strand has now increased by one nucleotide. The DNA polymerase can either remain bound to the DNA and continue synthesis (called "processive synthesis") or dissociate from the DNA and then bind again for the next nucleotide incorporation (called "distributive synthesis") (9).

The eukaryotic B-family polymerases are composed of several subunits. Yeast Pol ϵ consists of four subunits, Pol2, Dpb2, Dpb3, and Dpb4 (10, 11). Pol2 can be further divided into two domains, the N-terminal catalytic domain and a C-terminal domain that interacts with Dpb2, Dpb3, and Dpb4. The catalytic domain of Pol2 contains the polymerase site for synthesizing DNA and a 3′–5′ exonuclease site that is responsible for proofreading the newly synthesized DNA (12, 13). We will refer to the catalytic domain (amino acids 1–1228) as $Pol2_{core}$ and to the holoenzyme with all four subunits as Pol ϵ for the remainder of this work. The recently solved high resolution structure of $Pol2_{core}$ (amino acids 1–1228) revealed a domain that is not found among other DNA polymerases (13). This so-called P domain allows Pol ϵ to encircle double-stranded DNA as the DNA leaves the polymerase active site, and this domain was shown to be important for the processivity and polymerase activity of $Pol2_{core}$.

Pol ϵ replicates DNA with high fidelity. In general, it has been shown that the fidelity of replicative DNA polymerases is determined by three factors: the selection of correct nucleotides at the polymerase active site, the low likelihood to extend a mismatch, and the ability to excise the mismatch in the exonuclease site if incorporated. *In vitro* studies of Pol ϵ have suggested that the exonuclease activity increases the fidelity by

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[1] To whom correspondence should be addressed: Dept. of Medical Biochemistry and Biophysics, Umeå University, SE-901 87 Umeå, Sweden. Tel.: 46-90-7866638; E-mail: erik.johansson@medchem.umu.se.
[2] The abbreviations used are: Pol, polymerase; exo$^-$, exonuclease-deficient; (S_p)-dNTPαS, 2′-deoxynucleoside 5′-*O*-(1-thiotriphosphate); (S_p)-dTTPαS, 2′-deoxythymidine 5′-*O*-(1-thiotriphosphate); CMG, Cdc45-Mcm2–7-GINS.

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