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Strand Annealing and Terminal Transferase Activities of a B-family DNA Polymerase

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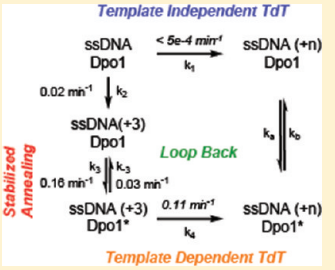
Supporting Information

ABSTRACT: DNA replication polymerases have the inherent ability to faithfully and rapidly copy a DNA template according to precise Watson−Crick base pairing. The primary B-family DNA replication polymerase (Dpo1) in the hyperthermophilic archaeon, *Sulfolobus solfataricus*, is shown here to possess a remarkable DNA stabilizing ability for maintaining weak base pairing interactions to facilitate primer extension. This thermal stabilization by Dpo1 allowed for template-directed synthesis at temperatures more than 30 °C above the melting temperature of naked DNA. Surprisingly, Dpo1 also displays a competing terminal deoxynucleotide transferase (TdT) activity unlike any other B-family DNA polymerase. Dpo1 is shown to elongate single-stranded DNA in template-dependent and template-independent manners. Experiments with different homopolymeric templates indicate that initial deoxyribonucleotide incorporation is complementary to the template. Rate-limiting steps that include looping back and annealing to the template allow for a unique template-dependent terminal transferase activity. The multiple activities of this unique B-family DNA polymerase make this enzyme an essential component for DNA replication and DNA repair for the maintenance of the archaeal genome at high temperatures.



A variety of DNA polymerases play individual roles in DNA replication and repair in all domains of life, to maintain the genomic integrity of the cell. In most respects, B-family DNA replication polymerases require a template to direct synthesis and incorporation of nucleotides into a growing strand. The stability of a primer on a template substrate allows for the faithful copying of the complementary strand. When DNA damage is encountered on the template strand, specialized Y-family lesion bypass DNA polymerases are recruited to transverse the damage.[1,2] Even these lesion bypass polymerases absolutely require the presence of a template strand to direct synthesis in a potentially mutagenic manner.[1] Some DNA polymerases, most notably Taq, can incorporate a single adenine base on the end of a double-stranded DNA (dsDNA) product in a single template-independent terminal transferase step that creates an A-tail.[3]

Within the DNA polymerase superfamily, a unique enzyme termed terminal deoxynucleotidyl transferase (TdT) that can extend single-stranded DNA (ssDNA) in a template-independent manner also exists.[4] Essentially, TdT can incorporate dNTPs onto the 3′-end of DNA indiscriminately. TdT was first identified in mammals[5] and is thought to play a role in the diversification of the vertebrate immune system through V(D)J recombination[6] or in specific aspects of DNA double-strand break repair.[7] V(D)J recombination is the rearrangement of antigen receptors to create unique antibody specificities to thwart biological attacks on the immune system.[8,9] Rearrangement requires the creation of double-strand breaks within immunoglobulin (Ig) genes, TdT activity to extend ssDNA, followed by reannealing of random gene regions to propagate immune diversity.[10,11] Eukaryotic X-family DNA polymerases (including TdT, pol μ, and pol λ) have also been shown to participate in nonhomologous end joining (NHEJ) by extending ssDNA at double-strand breaks as a template for annealing.[12]

Archaea are single-cell prokaryotic organisms and are not thought to need a V(D)J recombination system for diversification of the immune system, but growth at high temperatures would likely lead to accelerated DNA damage, including double-strand breaks (DSBs).[13] Interestingly, it has been noted that the mutational frequencies in the genome are equal to or lower than those found in mesophilc organisms, suggesting a highly active DNA repair system.[14] Homologous recombination (HR), not NHEJ, is thought to be the preferred mode for the repair of double-strand breaks in archaea.[15,16] The need for an X-family polymerase with TdT activity may not be necessary in archaea in light of the discovery that the eukaryotic-like primase (PriSL) has terminal transferase activity.[17] Interestingly, a crystal structure of the *Pyrococcus* PriS subunit has revealed unexpected structural homology with X-family DNA polymerases, prompting the authors to predict an unrecognized role for archaeal DNA primases in DNA repair.[17−19] Although archaea lack a putative X-family DNA polymerase, they have both a B-family DNA replication polymerase and a repair Y-family lesion bypass polymerase.[20,21] The genetic role, if any, for terminal transferase activity in archaea remains uncharacterized.

DNA replication and repair at high temperatures create significant thermodynamic problems for maintaining annealed double-stranded DNA templates. In fact, the small RNA primer required for initiation of DNA replication on both the leading

Received: March 21, 2011
Revised: April 29, 2011
Published: May 05, 2011

Table 1. DNA Sequences and Melting Temperatures

DNA NAME	Sequence	T_M (°C)[1] DNA	DNA/Dpo1	ΔT_M (°C)
DNA32	TTATG T ||| TTTACTTTTTTTTTT-5′	31.6 ± 1.3	37.0 ± 2.3	5.4
DNA37	TTTACG T |||| TTATGCTTTTTTTTTT-5′	37.0 ± 1.0	42.5 ± 1.4	5.8
DNA39	TTTTACG T |||| TTTATGCTTTTTTTTTT-5′	38.6 ± 1.9	44.5 ± 1.4	5.5
DNA88	TCGCCGGCCCGGG T ||||||||||||| TGCGGCCGGGCCCTTTTTTTTTT-5′	88	n/d[2]	n/d[2]

[1] Experimentally determined. [2] Not determined.

and lagging strand cannot be stably associated with the template at those high temperatures through thermodynamic interactions alone and would require help from other proteins to remain annealed.[22] It has been proposed previously that single-strand binding protein (SSB) or the processivity factor (PCNA) might stabilize short RNA primers before being captured by the DNA polymerase.[23,24] Enzymes such as topoisomerase[25] and the HEL112 helicase[26] in *Sso* have been shown to have strand annealing activities necessary for their respective functions in genomic maintenance, but they are not thought to stabilize RNA primers during DNA replication.

Classical B-family polymerases involved in DNA replication have been well characterized kinetically and structurally in *Sso*.[27−29] Recently, we have reported that the primary DNA polymerase in *Sso* (Dpo1) can form a trimer that activates the template-directed polymerization ability of the enzyme.[30] We now show that Dpo1 has a remarkable ability to stabilize weak base pairing interactions to replicate a template strand at high temperatures. This annealing activity is not recognized for either *Thermus aquaticus* pol I (Taq) or *Pyrococcus furiosus* B-family DNA polymerase (*Pfu*-Pol). In addition to stabilizing DNA templates, Dpo1 has a robust and unique terminal deoxynucleotidyl transferase activity that can extend ssDNA into extremely long products at high temperatures. We have confirmed that Dpo1's TdT activity can proceed by two mechanisms with differing kinetics: a traditional template-independent or a more unique template-dependent looping. The implications of strand annealing and terminal transferase activities for this multifaceted B-family DNA polymerase are discussed with regard to initiation of DNA replication and repair for the maintenance of the genome in archaea.

■ MATERIALS AND METHODS

Materials. [γ-^{32}P]ATP was purchased from MP Biomedicals (Solon, OH). Unlabeled deoxyribonucleotides and ribonucleotides were purchased from Invitrogen (Carlsbad, CA). All single-stranded DNA (ssDNA) was ordered from IDT (Coralville, IA) and gel purified as described previously.[30] DNA substrates are listed in Table 1 and the Supporting Information. Optikinase was purchased from USB (Cleveland, OH). Taq DNA polymerase was from Bioline (Taunton, MA), and *Pfu*Turbo DNA polymerase was from Stratagene (Santa Clara, CA). Wild-type (WT) Dpo1 and exonuclease deficient (exo$^-$) Dpo1 (D231A/D318A) were purified as described previously.[30] Restriction enzymes were purchased from New England Biolabs (Ipswich, MA). All other reagents were analytical grade or better.

Determination of Melting Temperatures. DNA melting temperature measurements were conducted on a Varian Cary 100 Bio UV−visible spectrophotometer. The UV absorbance at 260 nm was measured for 1.5 μM DNA with or without 1.5 μM Dpo1 exo$^-$ in assay buffer [50 mM glycine (pH 8) and 5 mM $Mg(CH_3COO)_2$] at every integral temperature point from 4 to 90 °C programmed at a rate of 1 °C/min. The melting temperature was determined from a plot of the UV absorbance versus temperature for multiple replicates and analyzed using the included software.

Strand Annealing and Terminal Transferase Assays. Unless specified otherwise, 1.5 μM Dpo1 (WT or exo$^-$) was incubated with approximately 80 nM ^{32}P 5′-end-labeled DNA and 100 μM specified nucleotides in glycine buffer [50 mM glycine (pH 8.0) and 5 mM $Mg(CH_3COO)_2$] in 10 μL reaction volumes at 70 °C for 60 min. ^{32}P 5′-end labeling was performed using Optikinase according to the manufacturer's directions. The reaction was stopped via addition of an equal volume of the quench solution (88% formamide, 10 mM EDTA, 1 mg/mL bromophenol blue, and 0.1% SDS). The quenched reaction samples were separated on a 20% denaturing polyacrylamide gel or a 0.8% alkaline agarose gel, imaged overnight, and scanned using a phosphorimager (Storm 20, GE Healthsciences). Quantification of the band intensities and lengths was performed using ImageQuant (version 5.0) compared with a ^{32}P-5′-end-labeled 1 kb ladder (Invitrogen).

Restriction Digest Assays. Dpo1 exo$^-$ (1.0 μM) was incubated with 100 μM dNTPs and approximately 280 nM ^{32}P 5′-end-labeled DNA with a restriction site close to the 5′-end in assay buffer for 60 min at 70 °C. Three microliters of the reaction mixture was used in a 10 μL restriction digest reaction mixture containing 0 or 25 units of the restriction enzyme and the specific buffer. Digestion reactions were conducted at optimal temperatures as specified by the manufacturer for 2 h. The reactions were stopped and analyzed by denaturing polyacrylamide gel electrophoresis as described above.

DNA Polymerase and Transferase Kinetics. Time courses for transferase and polymerase assays were analyzed by calculating the

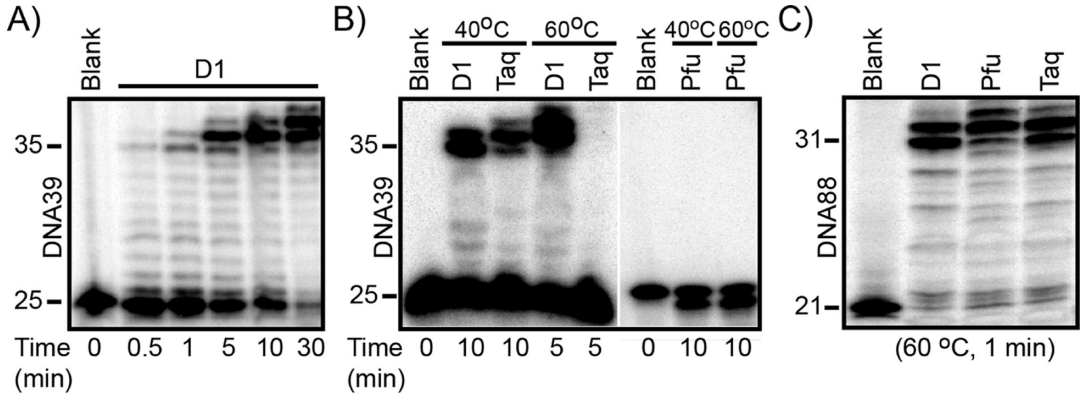


Figure 1. (A) Dpo1 (D1) and 5′-end-labeled DNA39 were incubated in a standard polymerase reaction mixture as described in Materials and Methods at 60 °C for the indicated times. (B) Dpo1 (1 μM), *Taq* DNA polymerase (2.5 units), or *Pfu*Turbo DNA polymerase (1.25 units) was incubated with ^{32}P 5′-end-labeled DNA39 at 40 or 60 °C for the indicated times. (C) A thermally stable hairpin, DNA88, was used as a positive control for each DNA polymerase under standard reaction conditions for 1 min at 60 °C.

fraction of product (+1, +2, +3, or total) to substrate. Data from reactions with $(dT)_{20}$AAA were fit to a single-exponential equation using Kaleidagraph (Synergy Software) according to eq 1:

$$[\text{product}] = A[1 - \exp(-k_{obs}t)] \quad (1)$$

where A represents the amplitude and k_{obs} is the observed rate constant. Data from reactions with $(dT)_{20}$ were fit and modeled according to the minimal kinetic scheme outlined in Figure 8E using a kinetic simulation with Berkeley Madonna (University of California, Berkeley, CA) (see the Supporting Information for a full description of the parameters).

RESULTS

Thermal Stabilization and Annealing Activity of Dpo1. We designed four hairpin DNA substrates (named according to their melting temperatures, T_M) with different T_M values that place the annealed bases at the 3′-end, leaving 10 thymine bases in the template strand. A stretch of thymidines in the template region was included to limit any hairpin formation to the designed site. The T_M values of the DNA substrates without and with Dpo1 were experimentally measured as described in Materials and Methods (Table 1). In all cases, addition of Dpo1 increased the T_M and stabilized weak base pairing interactions for the DNA hairpins. There was an average increase of 5.5 °C in the hairpin T_M when Dpo1 was included.

Remarkably, we have also found that Dpo1 is able to rapidly incorporate 10 bases onto the 3′-end of DNA39 at a temperature (60 °C) 25−30 °C higher than the T_M (Figure 1A). Although we primarily used trimeric concentrations of Dpo1 (1 μM),[30] lower monomeric concentrations (150 nM) had very similar activity (data not shown). We compared these associated annealing and polymerase activities of Dpo1 to those of other thermophilic polymerases, Taq and *Pfu*-Pol, and found that this DNA stabilization and annealing activity is unique to Dpo1. At 40 °C, Dpo1 and Taq can polymerize DNA39, but only Dpo1 can function at 60 °C (Figure 1B). At 60 °C, there is no polymerase product seen for Taq, while Pfu has only DNA exonuclease activity. As a positive control, all three polymerases can rapidly polymerize DNA88 at 60 °C (Figure 1C).

To further explore the DNA annealing activity, we monitored a time course for polymerase activity of Dpo1 on DNA32, DNA37, and DNA39 at 50 °C (Figure 2). Dpo1 has polymerization activity on all three DNA hairpins, although the rate is much faster on DNA37 and DNA39 (Figure 2D). At longer times, there are polymerase products when using DNA32, but the low T_M of this substrate limits Dpo1's activity. We also examined the annealing activity for Dpo1 at two other temperatures (30 and 60 °C) for each DNA substrate (Figure S1 of the Supporting Information). At 30 °C, the rate of synthesis is more equivalent for the three DNA substrates. The results at 60 °C are more similar to those at 50 °C where polymerization on DNA37 and DNA39 is significantly faster than on DNA32. Therefore, the efficiency of extension by Dpo1 is dependent on the reaction temperature and T_M of the DNA substrate. Surprising still is Dpo1's ability to polymerize any product at a temperature that is 30 °C above the melting temperature of the DNA primer/template.

Template Specific Terminal Transferase Activities. Noticeably, Dpo1 begins to incorporate additional bases to the 3′-end of the fully polymerized hairpin at higher temperatures (Figure 1 and Figure S1F,G of the Supporting Information). All of the DNA hairpins used included 10 thymidines in the template to limit hairpin formation to a single site. It is possible that template fluidity in these regions and resulting polymerase slippage could account for longer products. To test this, we monitored polymerase products using either a DNA template with a homopolymeric 5′-overhang (DNA39) or a template that contained three interspaced cytosines in the 5′-overhang (DNA39C) (Figure 3). Reactions with DNA39 resulted in fast extension to a 36- or 37-base product. This is consistent with the addition of one or two extra bases to the end of the template in a terminal transferase reaction or the result of template-dependent polymerase slippage. When DNA39C was used instead at 60 °C, the major product included 35 bases with a significant decrease in the level of longer products. At 70 °C though, a limited amount of longer products was detected with DNA39C. Therefore, the composition of the template sequence and the reaction temperature may result in the insertion of additional bases during DNA replication by Dpo1 in a polymerase slipping mechanism.

To verify whether direct terminal transferase activity is also possible for Dpo1, we switched to linear homopolymeric DNA substrates [$(dT)_{20}$, $(dC)_{20}$, $(dA)_{20}$, and $(dG)_{20}$] to remove any possible interfering Dpo1-directed annealing or hairpin formation. Homopolymeric DNA substrates with >10 bases were required for detectable TdT activity (data not shown).

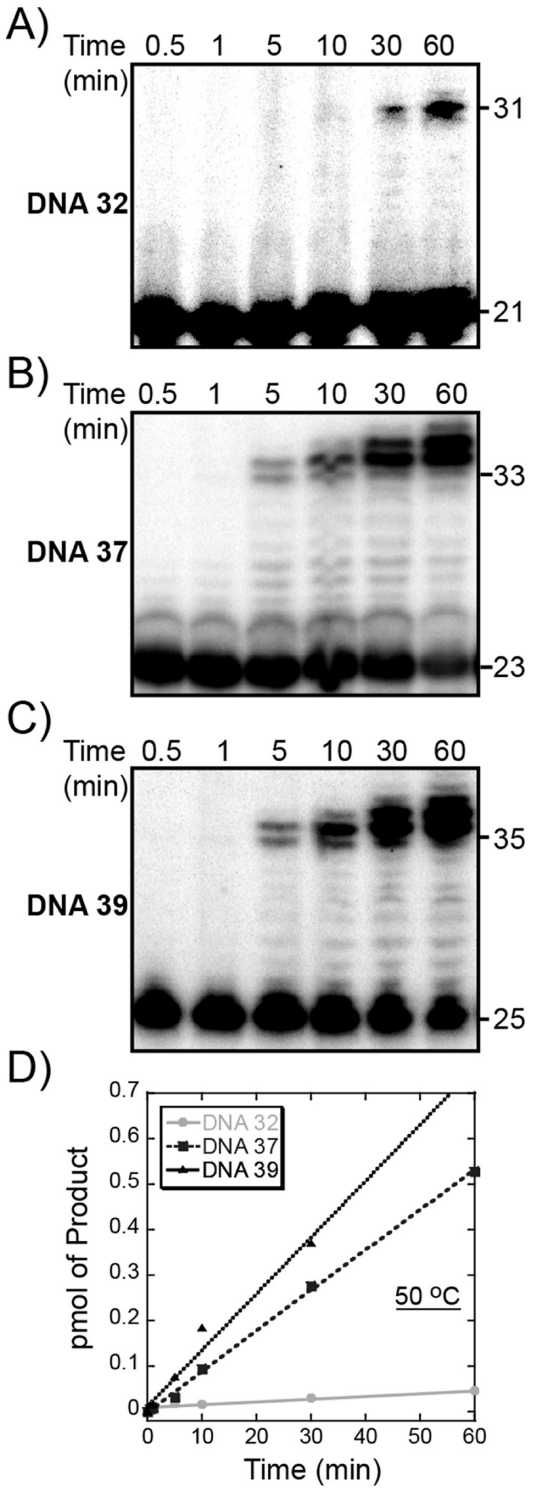


Figure 2. Time course for polymerase activity on DNA hairpin substrates (A) DNA32, (B) DNA37, and (C) DNA39 at 50 °C. Concentrations of Dpo1 and DNA were held constant at 1 μM and 80 nM, respectively. The size of the polymerase products is shown to the right of the gel figures. (D) Rate of polymerase activity quantified and compared for DNA32 (●), DNA37 (■), and DNA39 (▲) at 50 °C. The rates are 0.6 ± 0.1, 8.9 ± 0.1, and 12.4 ± 0.1 fmol/min for DNA32, DNA37, and DNA39, respectively.

Similar to the DNA hairpins described above, the terminal transferase activity on $(dT)_{20}$ not only increased with temperature but also seemed to be activated at physiological temperatures greater than 60 °C (Figure S2 of the Supporting Information). Dpo1 was incubated at 70 °C with each homopolymer labeled at the 5′-end with ^{32}P for the indicated times (Figure 4). Dpo1 shows strong TdT activity on both $(dT)_{20}$ and $(dA)_{20}$ but very weak activity on $(dG)_{20}$ and $(dC)_{20}$. We have noticed that at times greater than 60 min, there is a decrease in the amount of product that remains in the wells. We attribute this loss to precipitation or the inability of extremely large DNA molecules to enter an acrylamide gel. TdT activity is detected for both wild-type and exonuclease mutant (Dpo1 exo$^-$) forms of Dpo1 of $(dT)_{20}$, although the rate is accelerated roughly 2-fold for Dpo1 exo$^-$ (Figure S4 of the Supporting Information). To eliminate any competing nuclease proofreading activity and to more accurately measure transferase activity, we used Dpo1 exo$^-$ in all subsequent experiments.

To better visualize longer TdT products, we analyzed the reactions on denaturing agarose gels. After a significant lag phase of >10 min, abundant TdT products with more than several thousand bases are seen (Figure 5). There is a wide distribution of product length during the reaction times. The average rate of transferase activity over 20−40 min on $(dT)_{20}$ was 115 ± 4 bases/min, while the maximal rate was 310 ± 27 bases/min.

Nucleotide Specificity for TdT Activity. We then examined the incorporation of individual nucleotides on $(dT)_{20}$, $(dA)_{20}$, $(dC)_{20}$, and $(dG)_{20}$. Dpo1 transferase reaction mixtures were incubated with individual nucleotides and the reactions quenched after 60 min. Dpo1 was unable to incorporate ribonucleotides (rNTPs) into any DNA substrate we tested (data not shown). No visible elongation of $(dT)_{20}$ was observed with dGTP or dCTP (Figure 6A). dAMP was clearly incorporated repetitively on the 3′-end of $(dT)_{20}$ to create long terminal transferase products. Reduced TdT activity was also noted with dTTP on a $(dT)_{20}$ substrate. A time course of this reaction showed a slow increase in product length with time (data not shown). When only dATP is used as the substrate, there is a faster addition of three bases followed by a slower extension of the template. This is indicated in lanes 2, 6, 8, and 9 in Figure 6A, where there is a buildup of the +1, +2, and +3 products (also seen in Figure S4A of the Supporting Information). Experiments with dual combinations of each dNTP and constant dATP indicate that inclusion of dTTP stimulates the transferase rate equivalent to that when all dNTPs are utilized (Figure 6A). There is a disappearance in the +1, +2, and +3 products indicating that subsequent steps are no longer rate-limiting when dTTP is included with dATP. Inclusion of dGTP or dCTP with dATP did not increase the transferase activity over that of dATP alone.

In contrast, dTTP is required to efficiently elongate $(dA)_{20}$ by Dpo1, while dATP alone resulted in a slow extension of TdT products. Neither dGTP nor dCTP resulted in any detectable products over the time course. Again, there is a buildup of the +1, +2, and +3 products with dTTP, indicating an initial fast addition followed by slower extension to generate products greater than +4. Inclusion of dATP with dTTP or all dNTPs elevates the terminal transferase activity to a value similar to a rate seen with $(dT)_{20}$ above (Figure 6B). Interestingly, although the extent of incorporation of dNTPs onto $(dC)_{20}$ or $(dG)_{20}$ is greatly reduced, the preference is for the complementary base to the template to be added to the 3′-end (data not shown) similar to that detected for $(dT)_{20}$ and $(dA)_{20}$.

Loop-Back Annealing Model for Transferase Activity. The efficient incorporation of complementary bases into the

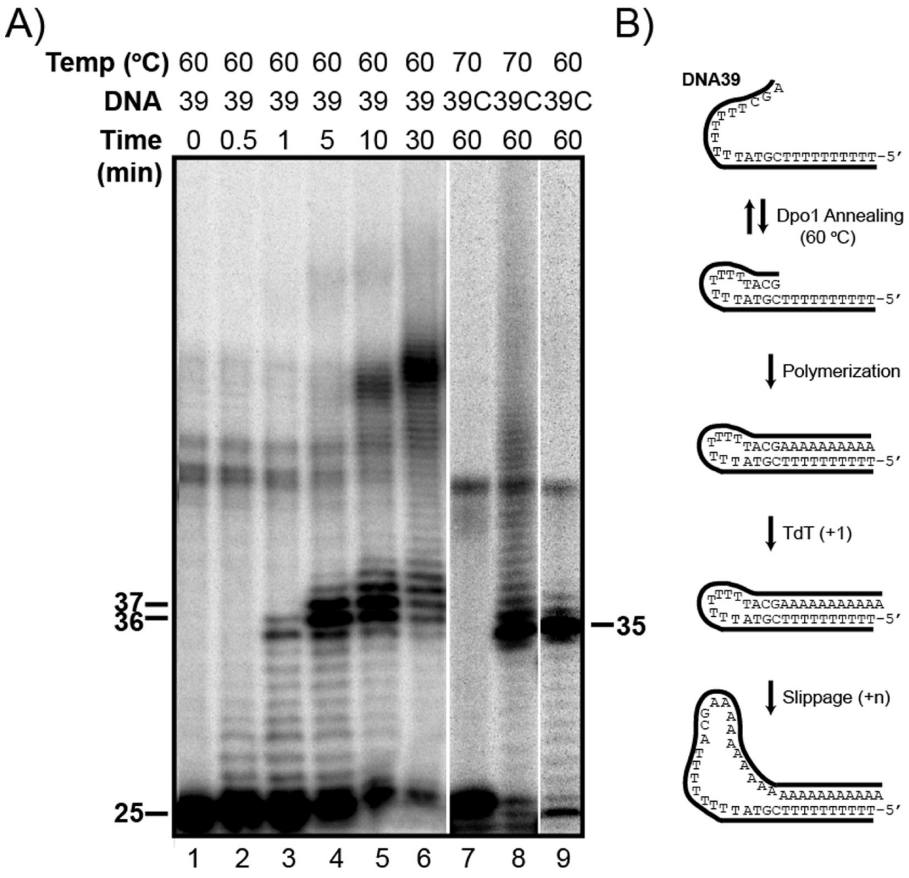


Figure 3. Evidence of a polymerase slippage mechanism on hairpin DNA39. (A) The reactions were conducted as described in Materials and Methods with the indicated temperatures, DNA substrates, and time periods. Lanes 8 and 9 show that insertion of three cytosines on the 5′-overhang or template (DNA39C) of the hairpin reduces the level of production of long products (>36 bases) observed for the DNA39 hairpin that only includes 10 thymidines in the 5′-overhang (lanes 2−7). Lanes 1 and 7 show DNA only for DNA39 and DNA39C, respectively. (B) Proposed model for the slippage of a DNA template during polymerization by Dpo1.

homopolymeric templates suggests that Dpo1 is promoting annealing as a mechanism to extend a ssDNA template. To test this, we designed four ssDNA substrates that included restriction sequences near the 5′-end. The templates had at least 15 thymidines at the 3′-end determined to be an optimal template for Dpo1's TdT activity. If after addition of a few complementary bases to the 3′-end Dpo1 utilized the ssDNA as a template for a loop-back polymerization reaction, then dsDNA would be synthesized across the restriction site, and subsequent restriction digestion would liberate a small ^{32}P-labeled ssDNA product (Figure 7A). Terminal transferase reactions were performed as described above at 70 °C with each of the four DNA templates containing distinct restriction sites. After the TdT reactions, each of the four restriction enzymes was added and incubated under standard conditions. The appearance of a seven- to nine-base product after restriction digestion (Figure 7B, lanes 4, 8, 12, and 16) is indicative of looping back and synthesizing DNA in a template-dependent manner across the restriction site. Templates with restriction sites, *Eco*RV and *Swa*I, resulted in eight-bases products, while the *Hin*dIII and *Apa*I templates resulted in seven- and nine-base products, respectively. Control reactions with no restriction enzyme added did not exhibit any degradation (Figure 7B, lanes, 3, 7, 11, and 15). Interestingly, not all of the transferase products were digested even after reaction for 2 h. These products can be attributed to either a template-independent transferase activity or severe template-dependent slippage precluding replication across the restriction site.

Kinetic Mechanism for TdT Activity. Kinetic TdT assays were performed at 70 °C on $(dT)_{20}$ or $(dT)_{20}$AAA with either dATP or dATP/dTTP as a function of time at 70 °C. The kinetic time course with $(dT)_{20}$ involved a lag phase followed by robust TdT activity with either dATP or dATP/dTTP (Figure 8A,C and Figure S4 of the Supporting Information). During this lag, there is an obvious buildup of +1 to +3 products with $(dT)_{20}$ and dATP. The lag phase can be reproduced at different temperatures and combinations of nucleotides. Preincubation of enzyme with the $(dT)_{20}$ template at 70 °C and initiation with dNTPs show the same lag as when the reaction is initiated by the addition of enzyme (data not shown). Although the lag persists, the +2 and +3 products disappear when dTTP is included (Figure 8C), indicating that formation of +2 or +3 products is no longer rate-limiting. Therefore, the lag is most likely due to a slow step in the rearrangement of Dpo1 and/or the DNA template into the correct conformation for rapid template-dependent extension at the 3′-OH end.

Because the incorporation of dNTPs on the 3′-end seemed to be most active when the complementary base was used (Figure 6) and we noted the buildup of a +3 product, we also tested TdT activity on $(dT)_{20}$AAA with either dATP or dATP/dTTP (Figure 8B,D). Interestingly, the lag phase and any +2 or

dx.doi.org/10.1021/bi200421g |*Biochemistry* 2011, 50, 5379–5390

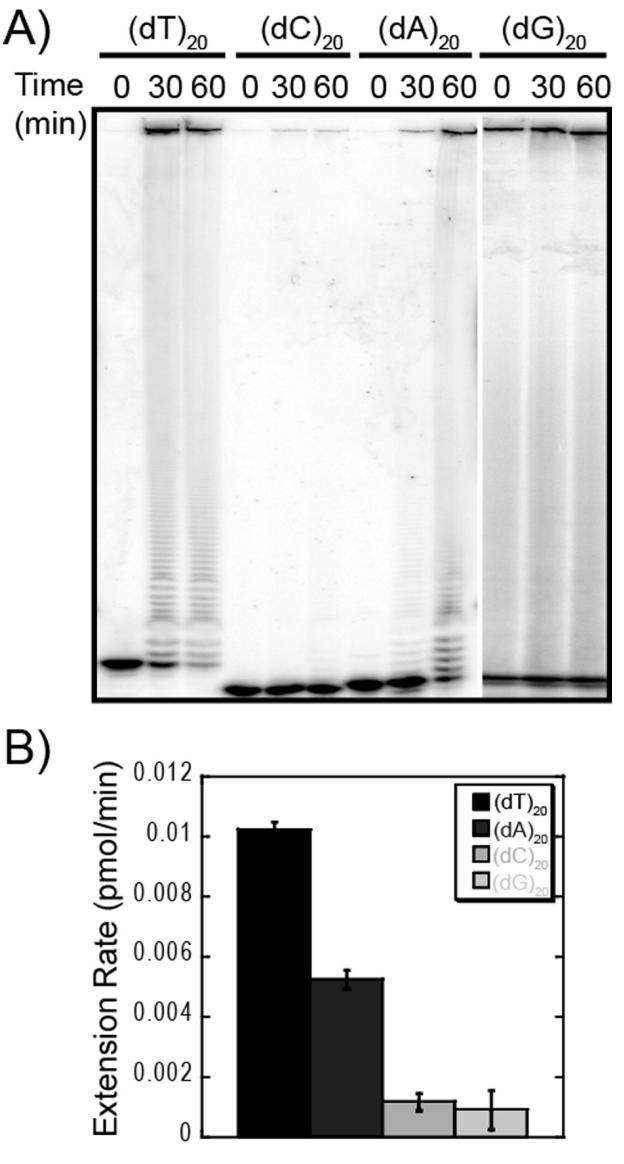


Figure 4. (A) Terminal transferase activity of Dpo1 measured using homopolymeric oligos $(dT)_{20}$, $(dC)_{20}$, $(dA)_{20}$, and $(dG)_{20}$ tested in standard assay reactions for 30 or 60 min. (B) Quantification of the transferase rates of 10.2 ± 0.2 fmol/min for $(dT)_{20}$, 5.3 ± 0.3 fmol/min for $(dA)_{20}$, 1.2 ± 0.3 fmol/min for $(dC)_{20}$, and 0.9 ± 0.7 fmol/min for $(dG)_{20}$.

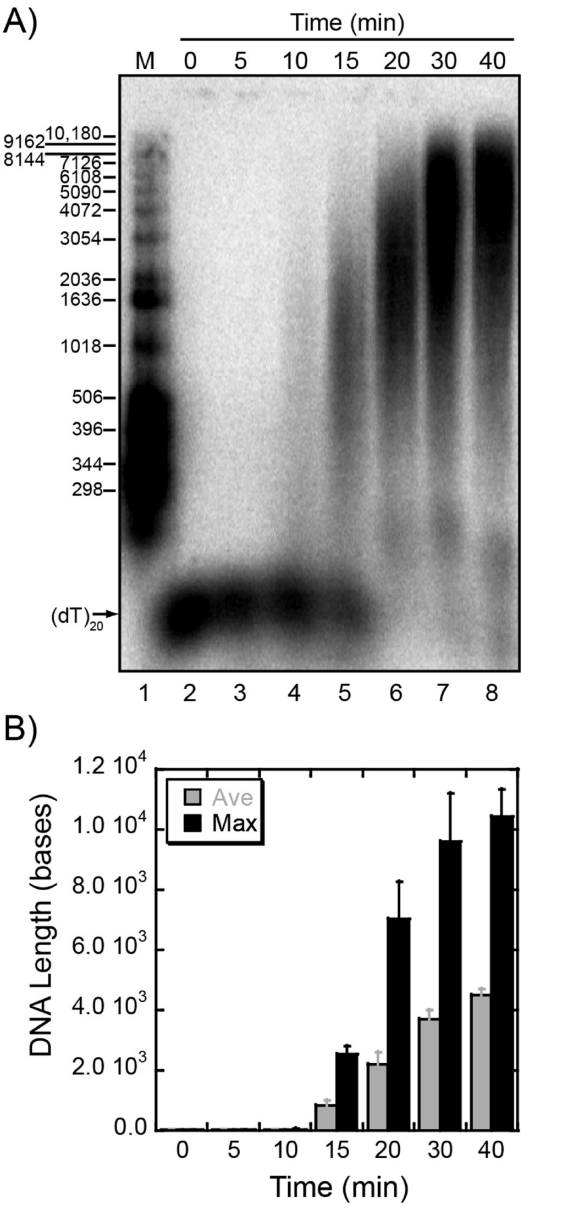


Figure 5. (A) Product size distribution of TdT activity on $(dT)_{20}$ at 70 °C by Dpo1 over a 40 min time course resolved using a 0.8% alkaline agaose gel. The ^{32}P-labeled DNA size markers are indicated on the left of the gel. (B) Maximal size (black) and average size (gray) of the TdT product at each time point calculated from the DNA size markers for at least three independent experiments using ImageQuant.

+3 products disappeared, and the results fit easily to a single-exponential equation consistent with a single observed rate constant with dATP (0.027 ± 0.003 min^{-1}) or dATP/dTTP (0.090 ± 0.005 min^{-1}). The observed rate with dATP/dTTP is 3-fold faster than that with dATP alone most likely because of the ability to perform template-directed synthesis equally across from template thymidines and adenines or newly incorporated bases complementary to the template $(dT)_{20}$AAA. The addition of three adenines at the 3′-end of the ssDNA template also allows for the possible preorganization of the loop-back annealing, eliminating the lag and resulting in faster template-dependent transferase activity than with $(dT)_{20}$ alone. Interestingly, these reactions are performed at 70 °C, which is well above the melting temperature for formation of a hairpin with $(dT)_{20}$AAA, so Dpo1 again is able to stabilize weak base pairing interactions for rapid extension in a template-dependent manner.

Kinetic modeling of the data from panels C and D of Figure 8 with the minimal reaction scheme shown Figure 8E resulted in the fit of rate constants for each of the competing steps. The observed rate constant for template-independent base additions (k_1) was modeled from the average of the data from Figure 6A (lane 3) and Figure 6B (lane 2) and a kinetic time course (data not shown) following the incorporation of dT or dA on a $(dT)_{20}$ or $(dA)_{20}$ template, respectively, where no base pairing is possible. In the presence of all nucleotides, terminal transferase

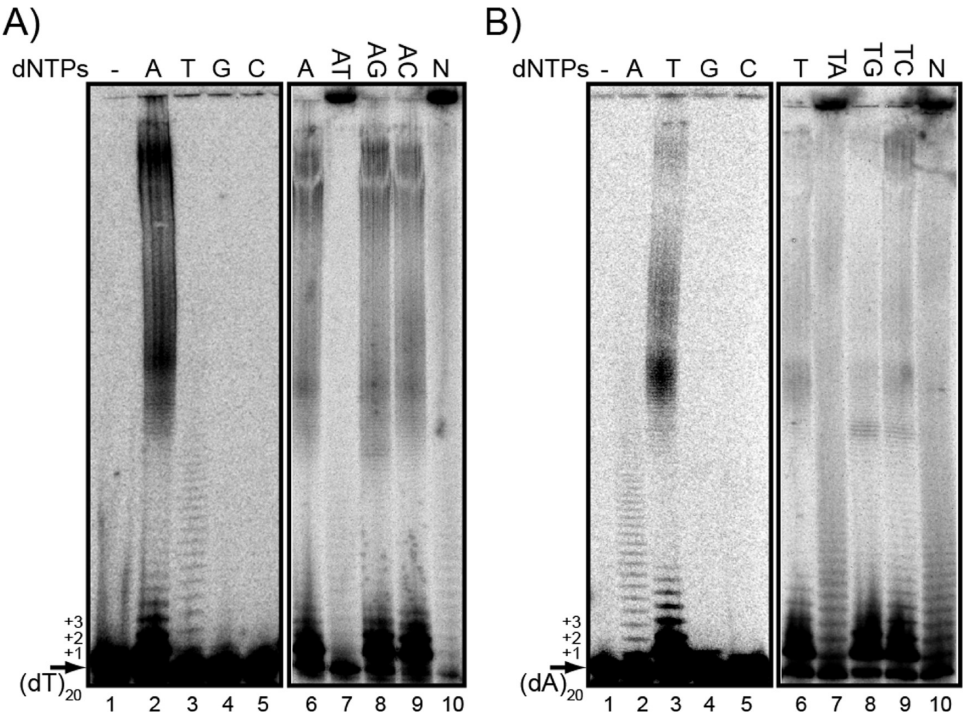


Figure 6. Test of individual deoxyribonucleotides or a combination of them on the terminal transferase activity of (A) $(dT)_{20}$ and (B) $(dA)_{20}$ in standard assay reactions for 60 min at 70 °C as described in Materials and Methods. Various combinations of dNTPs were used in the reaction as indicated. N represents the inclusion of all dNTPs. Concentrations of dNTPs were held constant at 0.1 μM. +1, +2, and +3 note the location of individual TdT products.

activity for the first few base additions is slightly faster (k_2). On the basis of the results from Figure 6, this initial transferase activity is dependent on the formation of a quasi-stable state that incorporates nucelotides complementary to the template but not stable enough to iteratively incorporate nucleotides opposite the ssDNA template without melting.

The rate constants for annealing (k_3 and k_{-3}) were derived from a fit of the data in Figure 8C for $(dT)_{20}$ and dATP/dTTP while independently modeling each rate constant (k_1, k_2, and k_4) based on initial values described above (see the Supporting Information for details). Inclusion of the slower quasi-stable step for +1 to +3 additions (k_2) was necessary to improve the fit of the simulation. On the basis of the simulation results, a reversible step of annealing was fit with a forward rate constant (k_3) of 0.16 min^{-1} and a reverse rate constant (k_{-3}) of 0.03 min^{-1} for annealing of the +3 product. The rate constant for this equilibrium step is modeled from data using $(dT)_{20}$ and will likely increase if more thermodynamically stable annealing intermediates are available (either k_a or k_b). The rate constant for rapid extension (k_4) was fit to 0.11 min^{-1} and compares well with the observed rate constant for extension of $(dT)_{20}$AAA (Figure 8D). On the basis of our results described above for a template of this type, Dpo1 is most likely extending ssDNA in a template-dependent manner that involves slippage of the synthesized polyA strand on a polyT template. Therefore, k_4 includes individual components of template-dependent DNA synthesis and DNA template slippage. Inclusion and simulation of reverse rate constants for k_1, k_2, and k_4 did not improve the fits and were removed for the sake of simplicity. As the rate constant for template-dependent synthesis for Dpo1 was measured previously to be 189 s^{-1} at 56 °C,[27] k_4 most likely represents the slower polymerization slippage rate.

DISCUSSION

DNA-dependent DNA polymerases coordinate the positioning of the incoming nucleotide with the template strand for optimal catalysis. Upon completion of polymerization opposite a template, further nucleotide addition is disfavored when no templating base is available. Some DNA polymerases that lack a proofreading exonuclease domain can inefficiently incorporate a single (+1) nucleotide addition.[31−33] Other polymerases from the X-family such as calf thymus TdT, Pol μ, and Pol λ have the surprising ability to incorporate successive nucleotide additions in a template-independent manner. Only X-family DNA polymerases have been shown to have consecutive nucleotide additions on single-stranded DNA,[7,12,34] and Pol μ and Pol λ are the only DNA polymerase known to possess both template-dependent and -independent activities.[35,36] Neither of these X-family DNA polymerases has a proofreading exonuclease domain that can limit the terminal transferase activity.

Sulfolobus solfataricus is a hyperthermophilic aerobic archaeon that optimally grows at 80 °C. How this organism survives and propagates under such extreme conditions is not fully understood. Severe destabilizing thermodynamic forces that persist at high temperatures would make maintaining annealed dsDNA templates difficult. It is thought dsDNA is stabilized by specific DNA binding proteins, which is necessary to protect the integrity of the genome at high temperatures. *Sso*Dpo1 is classified as a model B-family DNA polymerase because of sequence and functional homology.[37] It has rapid polymerization kinetics,[27] efficient exonuclease proofreading,[38] high processivity when bound to PCNA,[39] and structural homology to other B-family members.[28] In this study, we provide evidence that Dpo1 has the inherent ability to stabilize thermodynamically weak base pairing

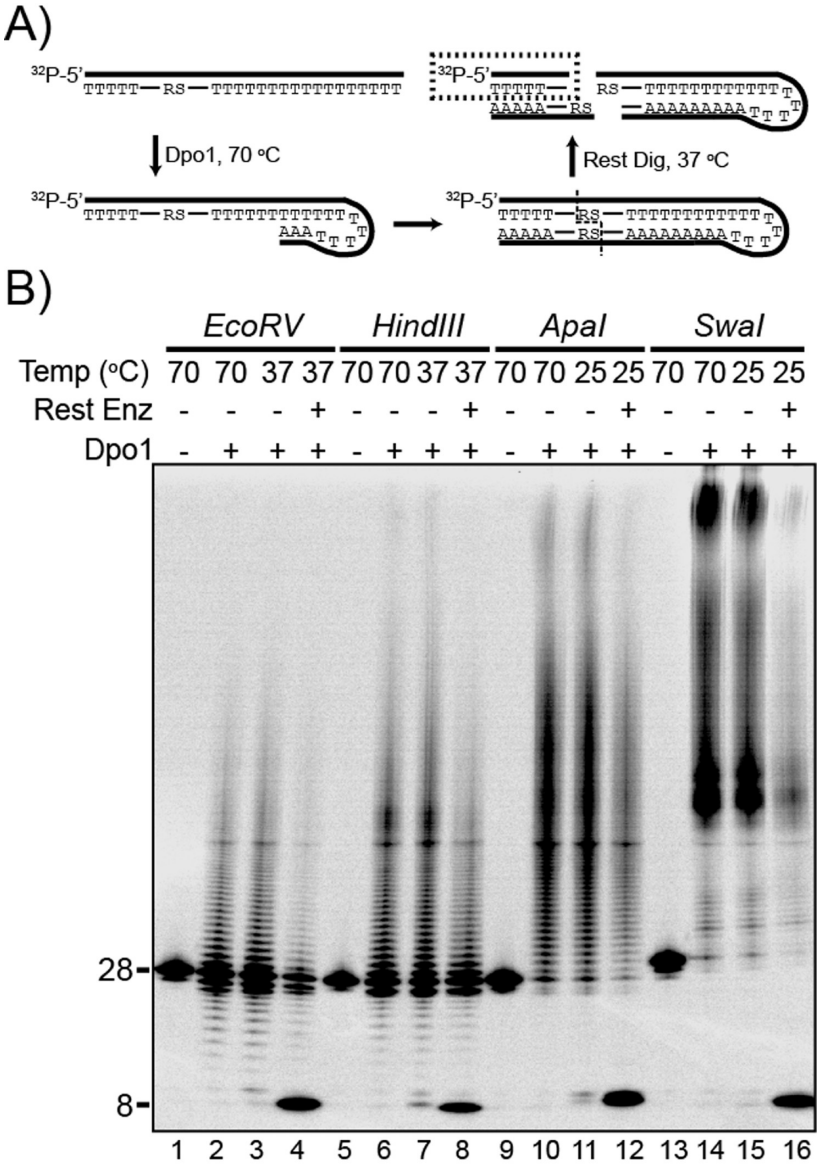


Figure 7. (A) Model of hairpin DNA with a restriction site close to the 5′-end. A loop-back mechanism that involves template-dependent polymerization would create a competent restriction site. Cleavage of the resulting product with a specific restriction enzyme would liberate a short (seven- to nine-base) ^{32}P-labeled ssDNA at the 5′-end. (B) Restriction digestion analysis of Dpo1 transferase products. Lanes 1, 5, 9, and 13 are blanks consisting of DNA only for transferase reactions of lanes 2, 6, 10, and 14 that include Dpo1. Lanes 3, 7, 11, and 15 are control reactions for restriction digestion that do not include restriction enzymes. Lanes 4, 8, 12, and 16 are the restriction-digested products from the Dpo1 transferase assays incubated with specific restriction enzymes resulting in eight-, seven-, nine-, and eight-base products, respectively.

interactions to facilitate template-dependent DNA polymerization. Surprisingly, this B-family DNA polymerase (Dpo1) is also found to have robust terminal transferase activity that proceeds by two independent mechanisms: a loop-back annealing template-dependent polymerase activity and a slower template-independent TdT activity. These annealing and transferase activities have not been noted for any other DNA replication polymerase within this family and may provide a mechanism for efficient replication and repair at high temperatures.

Role of DNA Polymerase Strand Annealing and Stabilization in DNA Replication. During the initiation of DNA replication, a small RNA primer is synthesized by a DNA primase as a template for the DNA polymerase. The length of this RNA primer ranges from 4 to 14 nucleotides depending on the species.[22,40,41] The stability of the primer/template in hyperthermophilic organisms such as archaea is a significant thermodynamic issue that is thought to be overcome primarily through protein binding and stabilization. Even mesophilic organisms require the stabilization of this short RNA primer before a DNA polymerase can be loaded at the priming site. In *Escherichia coli* and bacteriophage T4, this is accomplished through interactions and stabilization by SSB and PCNA.[23,24] In this report, we can show that the B-family DNA polymerase in *Sso* (Dpo1) is able to stabilize weak base pairing interactions of a primer/template to extend the primer in a template-dependent manner. This activity may be required to efficiently extend a short RNA primer during DNA replication at high temperatures.

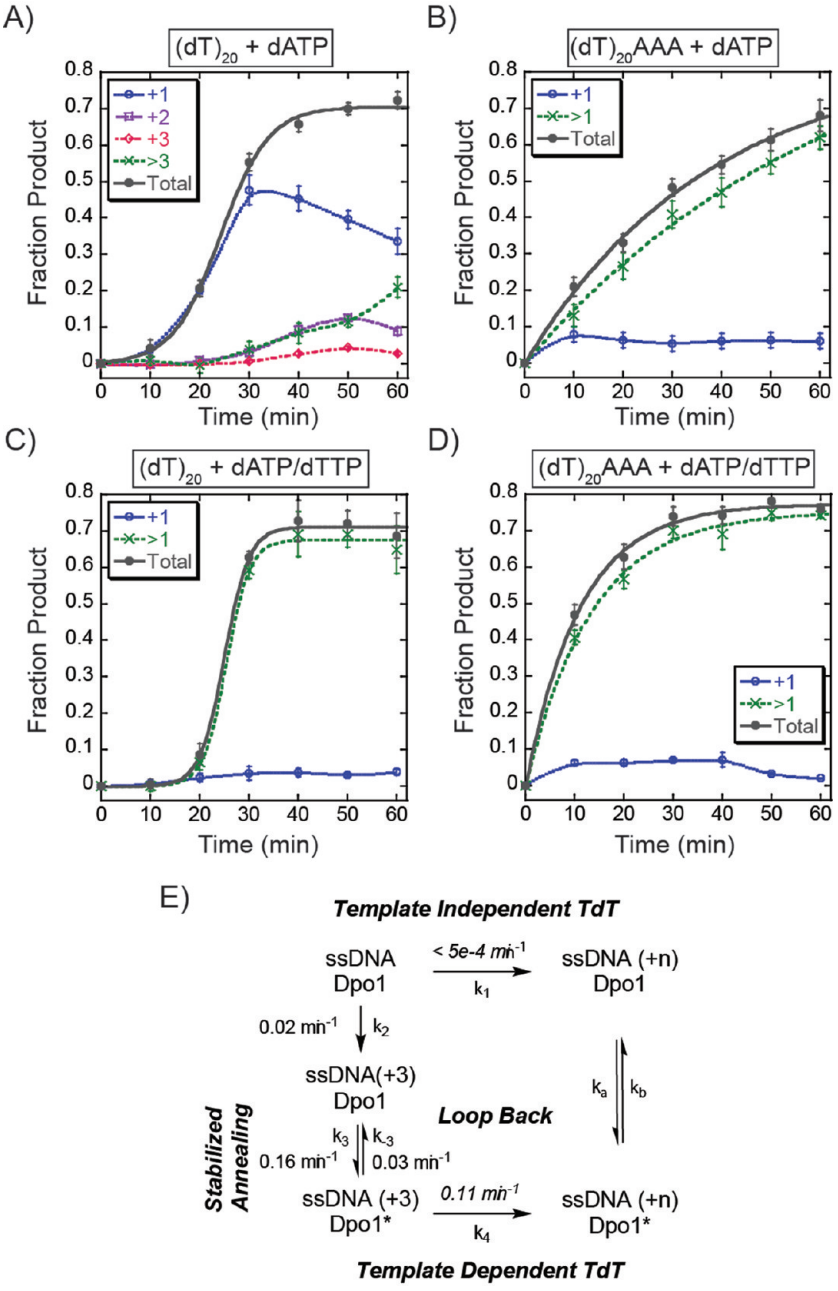


Figure 8. Comparison of time courses for $(dT)_{20}$ and $(dT)_{20}AAA$ using (A and B) dATP and (C and D) dATP/dTTP as the nucleotide substrate. The reactions were conducted as described in Materials and Methods. All values of data points for +1, +2, +3, >1, >3, and total were averaged from at least three independent experiments. The data points (>1 and total) for $(dT)_{20}AAA$ were fit to a single-exponential equation (eq 1) described in Materials and Methods to extract observed rate constants for dATP (0.031 ± 0.001 min^{-1}) and dATP/dTTP (0.089 ± 0.006 min^{-1}). (E) Kinetic reaction pathway simulated and modeled on the basis of the experimental data from panels B and D as well as Figure 6 as described in Materials and Methods and the Supporting Information. k_1 is an upper estimate of the template-independent transferase rate. k_2 represents the initial formation of +1, +2, and +3 products required for stable loop-back annealing. k_2 may or may not be necessary and will depend on the sequence of the DNA and available probability of hairpin formation [$(dT)_{20}$ vs $(dT)_{20}AAA$]. k_3 and k_{-3} represent the loop-back step required for the template-dependent transferase mechanism resulting in a starred (*) ssDNA(+3)/Dpo1* species. k_4 represents the rate constant for template-dependent terminal transferase activity calculated from the fit of the data for $(T_{20})AAA$ and in panel D. k_a and k_b represent subsequent annealing equilibrium steps for longer terminal transferase products dependent on DNA sequence and temperature.

We have been able to directly measure this increase in the level of stabilization in DNA melting temperature assays that include Dpo1. Although there is a significant increase in the T_m when Dpo1 is included, the value does not approach the reaction temperature. Therefore, there is still a dynamic equilibrium between the annealed hairpin and free DNA at 70 °C. Dpo1 must capture the annealed conformation and in the presence of dNTPs extend the hairpin in a template-dependent manner. Unlike Dpo1, neither Taq nor *Pfu*-Pol could extend primers at 20 °C above the T_M of the DNA. The polymerization of DNA

dx.doi.org/10.1021/bi200421g |*Biochemistry* 2011, 50, 5379–5390

with this template (to 35 bases) does not result in a thermodynamically stable duplex at 70 °C even with Dpo1 bound, and an equilibrium between melted and annealed hairpin products would persist. Therefore, there is a balance between the stability of the primer template (T_M) and the reaction temperature that determines the efficiency of extension for Dpo1.

Both Template-Independent and -Dependent Terminal Transferase Activities. Terminal transferase activity is characterized by the incorporation of nucleotides onto the 3′-end of a single-stranded DNA in a template-independent manner. Biochemical studies have shown that calf thymus TdT requires ssDNA as an initiator that is at least three nucleotides long.[42] The elongation of a homopolymer by calf thymus TdT requires at least six nucleotides for poly(dA) and five nucleotides for poly(dT).[42] Dpo1 requires a slightly longer homopolymeric template (10−15 bases) for efficient TdT activity. This is most likely due to the larger site size of Dpo1 for binding DNA[30] compared with that of calf thymus TdT.

Dpo1 is able to preferentially extend $(dT)_{20}$ and $(dA)_{20}$ versus $(dG)_{20}$ and $(dC)_{20}$. From a molecular point of view, this variation of TdT efficiency on different ssDNA initiators may be the result of optimal DNA conformations or favorable interactions between the template and the active site amino acids that direct the ssDNA elongation. This mechanism may resemble that shown by Pol μ in which a conserved His orients an incoming nucleotide toward a productive complex in the absence of a templating base for TdT activity.[12] Additionally, both $(dG)_{20}$ and $(dC)_{20}$ have increased propensities to form significant secondary structures, including quartets and quadraplexes,[43] that would preclude Dpo1 binding and terminal transferase activity.

The studies on nucleotide selections of TdT enzymes have exhibited diverse rules. Other DNA polymerases have been shown to place an adenine opposite an abasic site during DNA translesion repair [44] or at the end of a polymerized product in the +1 position.[3] The short isoform of murine TdT was reported to indiscriminately incorporate both dNTPs and rNTPs onto the 3′-end of a $(dA)_{10}$ initiator with a higher efficiency for dNTPs resulting in longer products (∼18 bases added to the initiator).[45] The preference to incorporate complementary bases to the end of the homopolymer DNA initiators by Dpo1 has similarities to the selection by human polymerase λ. Pol λ was found to incorporate nucleotides on the 3′-end either independently, resulting in +1 or +2 products, or following a loop-back model that utilized the template strand to initiate elongation resulting in longer +15 to +17 products.[36] Interestingly, Pol λ is classified as a terminal transferase enzyme even though the primary mechanism for extension is template-dependent.

Even though addition of a complementary base is favorable, Dpo1 can incorporate nucleotides identical to the homopolymeric template at a slower rate in a template-independent manner. For example, incorporation of dA on $(dA)_{20}$ and dT on $(dT)_{20}$ is clearly seen. This template-independent terminal transferase activity has not been detected for any other B-family DNA polymerase. Therefore, Dpo1 has the unique ability to extend ssDNA with at least two terminal transferase mechanisms.

Looping Back as a Mechanism for Terminal Transferase Activity. The transferase activity of Dpo1 is optimal at higher temperatures (>50 °C), resulting in extremely long products. Using homo-oligomeric DNA substrates, we detect a lag phase in which Dpo1 extends and rearranges the ssDNA template into a productive complex before template-dependent terminal transferase activity of Dpo1 is activated. This lag phase can be reduced with DNA substrates that include three residues on the end $[(dT)_{20}AAA]$ that can participate in the formation of a weakly stable hairpin. Because of this result, we speculate that Dpo1 can facilitate the looping back and stabilization of weak base pairing interactions to extend ssDNA in a template-dependent manner.

Upon modeling and simulation of the kinetics of terminal transferase activity for Dpo1, it is clear that there are multiple kinetic routes. The preferred pathway involves the stabilization of weak base pairing by Dpo1 to form a hairpin where extension occurs in a template-dependent manner. The rate-limiting step for this pathway involves the template-independent extension of ssDNA to produce a favorable loop-back annealing intermediate. The annealing step is reversible and will be dependent on the thermodynamics of the resultant DNA hairpin. If a short complementary sequence can be readily stabilized by Dpo1, then template-dependent terminal transferase is the preferred mechanism. If no complementary region can be stabilized, then Dpo1 can extend DNA in a template-independent manner at a much reduced rate before possible switching to a template-dependent mechanism.

When dATP/dTTP was included in a reaction with either $(dT)_{20}$ or $(dA)_{20}$, the rate of transferase activity was significantly increased over that with the single nucleotide. This extension rate with dATP/dTTP is equivalent to that for the case in which all nucleotides are included. This provides evidence of an increasingly complicated loop-back mechanism for transferase activity. When only dATP is available, looping back occurs and slippage is restricted to the $(dT)_{20}$ region because dATP is the only available substrate complementary to the T template. However, when both dATP and dTTP are available, the slippage reaction is expanded from the $(dT)_{20}$ region to the newly synthesized poly(dA) region. Homopolymeric stretches or repeats of DNA sequence are typically difficult to replicate accurately without template slipping that results in errors, including insertions or deletions,[46−48] and are even associated with a variety of diseases.[49] The ability of one strand to slide relative to the other in these stretches of DNA is improved at higher temperatures. The availability of both complementary nucleotides would eliminate any restrictions on slippage and increase the transferase rate and product length. For $(dT)_{20}$ when dATP is used, initial complementary extension is preferred, resulting in a buildup of +1, +2, and +3 products. Our results suggest that initial +3 extension exists in a quasi-stable state that fluctuates between annealed and melted states. Once three bases of complementarity are available, rapid template-dependent extension occurs as indicated by the results with $(dT)_{20}AAA$.

The length of terminal transferase products synthesized by Dpo1 routinely exceeds 5000 bases. These extreme TdT product lengths synthesized by Dpo1 are much greater than the lengths of 10−20 bases of TdT products produced by any other DNA polymerase (pol μ, TdT, or pol λ). Interestingly, analysis of the crystal structure identified a small extra α-helical region within Dpo1 that is not homologous to other polymerase members within the B-family.[28] This region makes contact with the fingers domain that undergoes large conformational changes during nucleotide selection. The uniqueness of this region may provide for the conformational transition required for the synthesis of TdT products by Dpo1.

Role of Terminal Transferase Activity in Archaea. In addition to the suggested DNA priming role of archaeal PriSL, this enzyme also has a promiscuous terminal nucleotidyl transferase

activity.[17] In contrast to Dpo1, the terminal transferase activity of PriSL was greater for incorporation of NTPs versus dNTPs on a DNA template. PriSL also displayed complex template and nucleotide specificities that gave products ranging from 2 to 7000 bases. Interestingly, the optimal temperature for PriSL transferase activity (~50 °C) was lower than the physiological temperature for *Sso*, while the RNA primer products (2−14 bases) were more abundant at 70 °C. Dpo1, on the other hand, has an absolute preference for dNTPs and is active at physiological temperatures. It is conceivable that the terminal transferase activity of PriSL is vestigial and therefore active only at lower temperatures, and its primary role is DNA priming in *Sso*.

Terminal transferase activity has been shown to be required for both V(D)J recombination and NHEJ repair in eukaryotes. As there is no obvious need for V(D)J recombination in archaea, the terminal transferase activity of Dpo1 would most likely participate in the repair of double-strand breaks. There are two pathways of double-strand break (DSB) repair in eukaryotes. Homologous recombination (HR) is the more conserved and precise method for repairing DSBs. Within *P. furiosus*, four HR genes have been identified and reconstituted biochemically to efficiently process the DNA for homologous strand exchange in vitro.[50] Further experiments have examined the transcriptional response to double-strand breaks in archaea and have determined that the homologous recombination genes, RadA and associated HR genes, were upregulated.[13] Although HR seems to be the preferred pathway for double-strand break repair in archaea, a 3′-phosphoesterase module within the NHEJ DNA ligase that performs 3′-healing reactions at DSBs has been shown to be conserved in some archaeal species, giving some credence to this mode of repair as well.[51] Further genomic and biochemical characterizations will be needed to determine if NHEJ is a functional repair pathway in archaea, and if the terminal transferase activity of Dpo1 shown here is involved.

■ ASSOCIATED CONTENT

Supporting Information. DNA sequences, kinetic simulation material, kinetic polymerization on other DNA hairpins, temperature-dependent TdT activity, comparison of wild-type Dpo1 to exo$^-$ TdT activity, and gels of TdT time courses with different nucleotides and templates. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding Sources

Funding was provided by startup funds from the University of Pittsburgh, Department of Chemistry, and a Research Scholar Grant (RSG-11-049−01-DMC) from the American Cancer Society (M.A.T.).

■ ACKNOWLEDGMENT

We thank Linda Jen-Jacobson for allowing access to her UV spectrometer for the melting temperature measurements.

■ ABBREVIATIONS

Sso, *S. solfataricus*; Dpo1, *Sso* DNA polymerase I; TdT, terminal deoxynucleotide transferase; Dpo1 exo$^-$, exonuclease deficient Dpo1; PCNA, proliferating cell nuclear antigen; PriSL, archaeal DNA primase; NHEJ, nonhomologous end joining; DSB, double-strand break; Taq, *T. aquaticus* pol I; *Pfu*-Pol, *P. furiosus* B-family polymerase; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; WT, wild type; HR, homologous recombination.

■ REFERENCES

(1) Prakash, S., Johnson, R. E., and Prakash, L. (2005) Eukaryotic translesion synthesis DNA polymerases: Specificity of structure and function. *Annu. Rev. Biochem. 74*, 317–353.

(2) Goodman, M. F. (2002) Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annu. Rev. Biochem. 71*, 17–50.

(3) Forrest, R., Zhou, H., and Hickford, J. G. (2000) 3′overhangs influence PCR-SSCP patterns. *BioTechniques 29* (958−960), 962.

(4) Motea, E. A., and Berdis, A. J. (2010) Terminal deoxynucleotidyl transferase: The story of a misguided DNA polymerase. *Biochim. Biophys. Acta 1804*, 1151–1166.

(5) Bollum, F. J. (1959) Thermal conversion of nonpriming deoxyribonucleic acid to primer. *J. Biol. Chem. 234*, 2733–2734.

(6) Desiderio, S. V., Yancopoulos, G. D., Paskind, M., Thomas, E., Boss, M. A., Landau, N., Alt, F. W., and Baltimore, D. (1984) Insertion of N regions into heavy-chain genes is correlated with expression of terminal deoxytransferase in B cells. *Nature 311*, 752–755.

(7) Moon, A. F., Garcia-Diaz, M., Batra, V. K., Beard, W. A., Bebenek, K., Kunkel, T. A., Wilson, S. H., and Pedersen, L. C. (2007) The X family portrait: Structural insights into biological functions of X family polymerases. *DNA Repair 6*, 1709–1725.

(8) Tonegawa, S. (1983) Somatic generation of antibody diversity. *Nature 302*, 575–581.

(9) Lieber, M. R., Lu, H., Gu, J., and Schwarz, K. (2008) Flexibility in the order of action and in the enzymology of the nuclease, polymerases, and ligase of vertebrate non-homologous DNA end joining: relevance to cancer, aging, and the immune system. *Cell Res. 18*, 125–133.

(10) Nick McElhinny, S. A., Havener, J. M., Garcia-Diaz, M., Juarez, R., Bebenek, K., Kee, B. L., Blanco, L., Kunkel, T. A., and Ramsden, D. A. (2005) A gradient of template dependence defines distinct biological roles for family X polymerases in nonhomologous end joining. *Mol. Cell 19*, 357–366.

(11) Bertocci, B., De, S. A., Berek, C., Weill, J. C., and Reynaud, C. A. (2003) Immunoglobulin κ light chain gene rearrangement is impaired in mice deficient for DNA polymerase μ. *Immunity 19*, 203–211.

(12) Andrade, P., Martin, M. J., Juarez, R., Lopez de Saro, F., and Blanco, L. (2009) Limited terminal transferase in human DNA polymerase μ defines the required balance between accuracy and efficiency in NHEJ. *Proc. Natl. Acad. Sci. U.S.A. 106*, 16203–16208.

(13) Rolfsmeier, M. L., Laughery, M. F., and Haseltine, C. A. (2010) Repair of DNA double-strand breaks following UV damage in three *Sulfolobus solfataricus* strains. *J. Bacteriol. 192*, 4954–4962.

(14) Zhang, L., Lou, H., Guo, L., Zhan, Z., Duan, Z., Guo, X., and Huang, L. (2010) Accurate DNA synthesis by *Sulfolobus solfataricus* DNA polymerase B1 at high temperature. *Extremophiles 14*, 107–117.

(15) Grogan, D. W. (2000) The question of DNA repair in hyperthermophilic archaea. *Trends Microbiol. 8*, 180–185.

(16) White, M. F. (2011) Homologous recombination in the archaea: The means justify the ends. *Biochem. Soc. Trans. 39*, 15–19.

(17) Lao-Sirieix, S. H., and Bell, S. D. (2004) The heterodimeric primase of the hyperthermophilic archaeon *Sulfolobus solfataricus* possesses DNA and RNA primase, polymerase and 3′-terminal nucleotidyl transferase activities. *J. Mol. Biol. 344*, 1251–1263.

(18) Augustin, M. A., Huber, R., and Kaiser, J. T. (2001) Crystal structure of a DNA-dependent RNA polymerase (DNA primase). *Nat. Struct. Biol. 8*, 57–61.

(19) Ito, N., Nureki, O., Shirouzu, M., Yokoyama, S., and Hanaoka, F. (2003) Crystal structure of the *Pyrococcus horikoshii* DNA primase-UTP complex: Implications for the mechanism of primer synthesis. *Genes Cells 8*, 913–923.

(20) Grabowski, B., and Kelman, Z. (2003) Archeal DNA replication: Eukaryal proteins in a bacterial context. *Annu. Rev. Microbiol. 57*, 487–516.

(21) Barry, E. R., and Bell, S. D. (2006) DNA replication in the archaea. *Microbiol. Mol. Biol. Rev. 70*, 876–887.

(22) Zuo, Z., Rodgers, C. J., Mikheikin, A. L., and Trakselis, M. A. (2010) Characterization of a functional DnaG-type primase in archaea: Implications for a dual-primase system. *J. Mol. Biol. 397*, 664–676.

(23) Yuzhakov, A., Kelman, Z., and O'Donnell, M. (1999) Trading places on DNA: A three-point switch underlies primer handoff from primase to the replicative DNA polymerase. *Cell 96*, 153–163.

(24) Nelson, S. W., Kumar, R., and Benkovic, S. J. (2008) RNA primer handoff in bacteriophage T4 DNA replication: The role of single-stranded DNA-binding protein and polymerase accessory proteins. *J. Biol. Chem. 283*, 22838–22846.

(25) Chen, L., and Huang, L. (2006) Oligonucleotide cleavage and rejoining by topoisomerase III from the hyperthermophilic archaeon *Sulfolobus solfataricus*: Temperature dependence and strand annealing-promoted DNA religation. *Mol. Microbiol. 60*, 783–794.

(26) De Felice, M., Aria, V., Esposito, L., De Falco, M., Pucci, B., Rossi, M., and Pisani, F. M. (2007) A novel DNA helicase with strand-annealing activity from the crenarchaeon *Sulfolobus solfataricus*. *Biochem. J. 408*, 87–95.

(27) Brown, J. A., and Suo, Z. (2009) Elucidating the Kinetic Mechanism of DNA Polymerization Catalyzed by *Sulfolobus solfataricus* P2 DNA Polymerase B1. *Biochemistry 48*, 7502–7511.

(28) Savino, C., Federici, L., Johnson, K. A., Vallone, B., Nastopoulos, V., Rossi, M., Pisani, F. M., and Tsernoglou, D. (2004) Insights into DNA replication: The crystal structure of DNA polymerase B1 from the archaeon *Sulfolobus solfataricus*. *Structure 12*, 2001–2008.

(29) Pisani, F. M., De, F. M., Manco, G., and Rossi, M. (1998) Domain organization and biochemical features of *Sulfolobus solfataricus* DNA polymerase. *Extremophiles 2*, 171–177.

(30) Mikheikin, A. L., Lin, H. K., Mehta, P., Jen-Jacobson, L., and Trakselis, M. A. (2009) A trimeric DNA polymerase complex increases the native replication processivity. *Nucleic Acids Res. 37*, 7194–7205.

(31) Clark, J. M., Joyce, C. M., and Beardsley, G. P. (1987) Novel blunt-end addition reactions catalyzed by DNA polymerase I of *Escherichia coli*. *J. Mol. Biol. 198*, 123–127.

(32) Clark, J. M. (1988) Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerases. *Nucleic Acids Res. 16*, 9677–9686.

(33) Golinelli, M. P., and Hughes, S. H. (2002) Nontemplated base addition by HIV-1 RT can induce nonspecific strand transfer in vitro. *Virology 294*, 122–134.

(34) Delarue, M., Boule, J. B., Lescar, J., Expert-Bezancon, N., Jourdan, N., Sukumar, N., Rougeon, F., and Papanicolaou, C. (2002) Crystal structures of a template-independent DNA polymerase: Murine terminal deoxynucleotidyltransferase. *EMBO J. 21*, 427–439.

(35) Dominguez, O., Ruiz, J. F., Lain de Lera, T., Garcia-Diaz, M., Gonzalez, M. A., Kirchhoff, T., Martinez-A, C., Bernad, A., and Blanco, L. (2000) DNA polymerase μ (Pol μ), homologous to TdT, could act as a DNA mutator in eukaryotic cells. *EMBO J. 19*, 1731–1742.

(36) Maga, G., Ramadan, K., Locatelli, G. A., Shevelev, I., Spadari, S., and Hubscher, U. (2005) DNA elongation by the human DNA polymerase λ polymerase and terminal transferase activities are differentially coordinated by proliferating cell nuclear antigen and replication protein A. *J. Biol. Chem. 280*, 1971–1981.

(37) Pisani, F. M., Manco, G., Carratore, V., and Rossi, M. (1996) Domain organization and DNA-induced conformational changes of an archaeal family B DNA polymerase. *Biochemistry 35*, 9158–9166.

(38) Pisani, F. M., De Felice, M., and Rossi, M. (1998) Amino acid residues involved in determining the processivity of the 3′-5′ exonuclease activity in a family B DNA polymerase from the thermoacidophilic archaeon *Sulfolobus solfataricus*. *Biochemistry 37*, 15005–15012.

(39) Dionne, I., Nookala, R. K., Jackson, S. P., Doherty, A. J., and Bell, S. D. (2003) A heterotrimeric PCNA in the hyperthermophilic archaeon *Sulfolobus solfataricus*. *Mol. Cell 11*, 275–282.

(40) Frick, D. N., and Richardson, C. C. (2001) DNA primases. *Annu. Rev. Biochem. 70*, 39–80.

(41) Kuchta, R. D., and Stengel, G. (2010) Mechanism and evolution of DNA primases. *Biochim. Biophys. Acta 1804*, 1180–1189.

(42) Kato, K. I., Goncalves, J. M., Houts, G. E., and Bollum, F. J. (1967) Deoxynucleotide-polymerizing enzymes of calf thymus gland. II. Properties of the terminal deoxynucleotidyltransferase. *J. Biol. Chem. 242*, 2780–2789.

(43) Neidle, S., and Parkinson, G. N. (2003) The structure of telomeric DNA. *Curr. Opin. Struct. Biol. 13*, 275–283.

(44) Zhang, L., Brown, J. A., Newmister, S. A., and Suo, Z. (2009) Polymerization Fidelity of a Replicative DNA Polymerase from the Hyperthermophilic Archaeon *Sulfolobus solfataricus* P2. *Biochemistry 48*, 7492–7501.

(45) Boule, J. B., Rougeon, F., and Papanicolaou, C. (2001) Terminal deoxynucleotidyl transferase indiscriminately incorporates ribonucleotides and deoxyribonucleotides. *J. Biol. Chem. 276*, 31388–31393.

(46) Schlotterer, C., and Tautz, D. (1992) Slippage synthesis of simple sequence DNA. *Nucleic Acids Res. 20*, 211–215.

(47) Shinde, D., Lai, Y., Sun, F., and Arnheim, N. (2003) Taq DNA polymerase slippage mutation rates measured by PCR and quasi-likelihood analysis: $(CA/GT)_n$ and $(A/T)_n$ microsatellites. *Nucleic Acids Res. 31*, 974–980.

(48) Kuhner, F., Morfill, J., Neher, R. A., Blank, K., and Gaub, H. E. (2007) Force-induced DNA slippage. *Biophys. J. 92*, 2491–2497.

(49) Richard, G. F., Kerrest, A., and Dujon, B. (2008) Comparative genomics and molecular dynamics of DNA repeats in eukaryotes. *Microbiol. Mol. Biol. Rev. 72*, 686–727.

(50) Hopkins, B. B., and Paull, T. T. (2008) The *P. furiosus* mre11/rad50 complex promotes 5′ strand resection at a DNA double-strand break. *Cell 135*, 250–260.

(51) Nair, P. A., Smith, P., and Shuman, S. (2010) Structure of bacterial LigD 3′-phosphoesterase unveils a DNA repair superfamily. *Proc. Natl. Acad. Sci. U.S.A. 107*, 12822–12827.