DNA-Thumb Interactions and Processivity of T7 DNA Polymerase in Comparison to Yeast Polymerase η*

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The replicative polymerase of bacteriophage T7 is structurally and mechanistically well characterized. The crystal structure of T7 DNA polymerase or gene 5 protein complexed to its processivity factor, Escherichia coli thioredoxin, a primer-template, and a dideoxynucleotide reveals how this enzyme interacts with the 3′-end of the primer-template, but does not show how thioredoxin confers processivity to the polymerase. In the crystal structure highly conserved amino acids Asn335 and Ser338 of the thumb subdomain of T7 DNA polymerase are seen to interact with phosphates 7 and 8 of the DNA template strand. Results with a mutant T7 DNA polymerase in which aliphatic residues are substituted for these amino acids and experiments with different length and methylphosphonate-modified primer-templates demonstrate that these interactions are essential for processive synthesis and d(A/T)n tract bypass. Our data with methylphosphonate-modified DNA suggests that thioredoxin confers processivity to T7 DNA polymerase in part by causing an interaction with the phosphate backbone or minor groove of DNA. Residues Asn335 and Ser338 may also function with a nearby helix-loop-helix motif located at residues 339–372 to enclose the DNA during processive synthesis. Our results suggest that this structure must be held close to the DNA by ionic interactions to function. These interactions also allow for DNA sliding but physically block the passage of a 3T bulge in the template. In contrast, yeast polymerase η, a polymerase that non-mutagenically repairs cis-syn thymidine dimers, allows the same bulge to slide past its thumb subdomain during synthesis. A relaxed thumb interaction with the DNA could account for the notably low processivity of polymerase η.

An important biological process is the movement of proteins across DNA and RNA. For this to occur, the specific protein needs to maintain association with the polymer during translocation. Translocation by RNase II, an Escherichia coli 3′-5′ exonuclease, is an example of a simple processive reaction. A protein “anchor” holds the downstream RNA while the catalytic site sequentially releases 5′-monophosphates from the 3′-end of the RNA (1, 2). The more complex yeast RNA polymerase II stabilizes the elongation complex during catalytic site translocation at two positions. Jaws and clamp close on the downstream template while the nascent RNA binds to the underneath side of the protein (3–5). To achieve highly processive synthesis, replicative polymerases employ accessory proteins to maintain association with the DNA. The beta subunit of E. coli DNA polymerase III and proliferating cell nuclear antigen of human and yeast polymerase δ form sliding clamps around the DNA (6, 7). In contrast, the gap-filling DNA polymerase β binds to the 5′-phosphate located at the far end of a gap through an 8KΔ domain to anchor the polymerase in processive synthesis (8–10). How DNA polymerases bind to template primers is fundamental to eventually understanding how polymerases behave when they encounter DNA damage, such as DNA photoproducts.

The DNA polymerase of T7 bacteriophage (gene 5 protein) is a structurally and mechanistically well characterized enzyme that becomes highly processive upon binding to E. coli thioredoxin (11, 12). The crystal structure of the T7 DNA polymerase complexed to E. coli thioredoxin, a 22-mer primer/26-mer template, and a dideoxynucleotide triphosphate has been solved (13). Only the first 11 bp of the primer-template, along with the templating nucleotide and its 5′-neighbor, could be assigned coordinates. The rest of the primer-template could not be seen due to disorder. The thioredoxin is seen to bind to an extended loop between α-helices H and H1 (262–338), but, because of the disorder in the crystal, residues 294–318 cannot be located and it is thus not possible to determine how the thioredoxin confers processivity to T7 DNA polymerase. It has been suggested that the thioredoxin either enhances binding through electrostatic interactions or possibly swings across the primer template so as to encircle it (13) in a similar fashion to how proliferating cell nuclear antigen, the processivity factor for polymerase δ, forms a ring around the DNA (6, 14).

The DNA-protein interactions most remote from the active site of T7 DNA polymerase that can be located in the crystal structure are between highly conserved amino acids Asn335 and Ser338 (15), located between beta strand 6b and α-helix H1 of the polymerase thumb and template phosphates 7 and 8. These two amino acids are also in close proximity to a nearby helix-loop-helix (HLH)1 motif (amino acids 339–372, domains H1–6c–6d-H2). Similarly, the Klenow editing complex contains 50 amino acid residues between helices H and I of the thumb subdomain, which include Asn335 and Ser338, which are close to template phosphates 7 and 8 and also a nearby HLH motif (16). A deletion of the entire loop of the HLH (amino acids 590–613) limits processive synthesis and leads to frameshift mutations (17). The authors concluded that the deleted peptide somehow tracks the minor groove during processive synthesis.

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1 The abbreviations used are: HLH, helix-loop-helix; IPTG, isopropyl-1-thio-β-D-galactopyranoside; DTT, dithiothreitol; Mes, 4-morpholinethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; MGBT, minor groove binding track; pol, polymerase; ODN, oligodeoxynucleotide.
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Consistent with this finding, the p66 helix H of the HIV-1 reverse transcriptase thumb subdomain has been linked to processivity, and the amino acid residues interacting with the DNA have been designated a “minor groove binding track” (MGBT). Mutations within the MGBT effect processivity and increase the possibility of frameshift mutations (18–23). DNA mapping shows that HIV-1 reverse transcriptase protects the same number of nucleotides after limited synthesis (24). Based on this observation, it was suggested that the events in forming a single phosphodiesterase bond strip the MGBT from the DNA, allowing it to translocate one position upstream (25).

In contrast to T7 DNA polymerase, which belongs to the family of replicative polymerases, which have high processivity and fidelity, polymerase η belongs to a group of DNA damage repair enzymes that have low processivity and fidelity and have been the subject of intensive study since its discovery (26). Despite its low processivity, polymerase η can efficiently and non-mutagenically synthesize past cis-syn thymine dimers, and a condition called xeroderma pigmentosum occurs when this activity is missing in humans (27). T7 DNA polymerase, on the other hand, bypasses the thymine dimer very inefficiently (28–30) and only in the presence of the thioredoxin processivity factor and in the absence of its exonuclease activity. The crystal structure of the catalytic core of yeast pol η has been solved (31) and contains a small thumb subdomain that consists of only 90 residues and alpha helices L, M, N, O, P, and Q, which bear no structural similarity to any polymerase in the pol A family of which T7 DNA polymerase is a member.

Herein we show that the T7 DNA polymerase thumb residues Asn235 and Ser238 interact with the primer-template stem and are critical for processive synthesis. These interactions may function with a nearby HLH motif to enclose the DNA. Our data suggest this structure must be drawn close to the DNA by ionic interactions to function. These interactions allow free sliding of the DNA during processive synthesis but physically block the passage of a 3T bulge. Our results also support the idea that thioredoxin confers processivity by enhancing the interactions between the phosphate backbone of the DNA, or possibly by causing the polymerase to track the minor groove of DNA, rather than by encircling the DNA. In contrast, the catalytic core of yeast pol η allows easy passage of a 3T bulge past its thumb subdomain, which could be explained by a relaxed thumb-DNA interaction, which could also account for its low processivity.

**EXPERIMENTAL PROCEDURES**

**Materials**—Thioredoxin crystals were from U. S. Biochemical Corp. and taken up in 50% glycerin with 20 mM Tris-HCl at pH 7.5 and stored at −20 °C at 5 mg/ml. DNase I, in crystal form, was obtained from Sigma, and was resuspended and stored in 50% glycerin with 10 mM Tris-HCl, pH 7.5, and 50% glycerin. Polymerase η could be stored without NaCl at pH 5.8 in 10 mM Mes buffer stored under the same conditions. Alternatively, the T7 DNA polymerase could be stored without NaCl at pH 5.8 in 10 mM Mes buffer containing 5 mM EDTA and 50% glycerin.

The His6-tagged proteins were purified from BL21(DE3)IysS cells by affinity chromatography using a nickel-nitrilotriacetic acid-agarose column (Qiagen). Cells were lysed in 0.5 mM salt by sonication on ice (5 times 20 s). The sizing column was equilibrated and run with 0.5 mM NaCl, and both ion exchange columns were equilibrated and loaded in 0.25 mM NaCl. The enzyme was eluted from the Q-Sepharose column in a step gradient at 0.7 mM NaCl and batch-eluted from the S-Sepharose column in 1 mM NaCl. The enzyme (1 mg/ml) was stored at −20 °C with 0.3 M NaCl and 5 mM EDTA with 0.5 mM thioredoxin. The reactions were conducted at 4 °C unless stated otherwise. 5 pmol of T7 DNA polymerase or polymerase η were added and allowed to equilibrate with the primer-template. After a 10-min preincubation, 15 μl of 500 μM dNTPs containing 100 μg of calf thymus DNA as a trap was added. The reaction was allowed to proceed for 30 min.
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We were interested in determining what structural features and interactions of T7 DNA polymerase were important in conferring processivity in DNA synthesis. T7 DNA polymerase becomes highly processive when complexed to E. coli thioredoxin. Only the first 11 of the 21 bp of the primer-template stem can interact and are written 3′-end of the primer or the corresponding position in the template. To determine which of these interactions might be important in conferring processivity, we decided to make use of changes in primer-template length and sequence (Fig. 1), salt concentration, methyphosphonate substitution, bulge-containing templates, and site-directed mutagenesis, along with binding constant determinations and DNase I footprinting. To separate the effects of the processivity factor, E. coli thioredoxin, we prepared the exo− T7 polymerase by a previously described method from thioredoxin-deficient E. coli strain to which we could then add thioredoxin. An exo− mutant was used to eliminate degradation of the primer terminus, which would complicate analysis of the processivity of the enzyme. The D5A, E7A mutant was chosen because it causes the minimum alteration of the exonuclease domain, and its kinetic properties have been thoroughly characterized (32). We also decided to compare the properties of T7 DNA polymerase with that of yeast pol η, which as a member of the DNA damage bypass family, and is much less processive and more error-prone.

Effect of Primer-template Length on Processivity of T7 DNA Polymerase—We first wanted to see if we could localize the sites of important interactions on T7 DNA polymerase in the absence of thioredoxin by determining the effect of stem length on the processivity. We compared single round synthesis at 4°C with different length primer-template stems (Fig. 2) carried out by pre-annealing the primer-template and polymerase followed by addition of dNTPs and calf thymus DNA as a trap. Complete details are given under “Experimental Procedures.” Methylphosphate-modified nucleotides are in lowercase. Lanes designated T7 (bottom) are the synthetic bursts with the T7 DNA polymerase in the absence of thioredoxin, and the ones designated C are control lanes in which the protein was omitted from the reaction. T7M is the mutant T7 DNA polymerase with thioredoxin described under “Experimental Procedures.” g, a native acrylamide gel run at 4°C with the 32P-labeled primer-templates P7/T17 and P10/T20 in lanes 1 and 3, respectively, or the primers run separately (P7 in lane 2 and P10 in lane 4).

RESULTS

Determinants of Processivity of T7 DNA Polymerase—We proceed for 10 s before quenching with 80 μl of formamide containing xylene cyanol dye, 250 mM EDTA, and 100 pmol of cold primer. The trap prior to the polymerase and dNTPs. The samples were heated to 100°C for 5 min before loading 5 μl of each sample on a 40-cm sequencing acrylamide gel. The gel was then exposed to a PhosphorImager screen and scanned with a Bio-Rad FX PhosphorImager, and the primer extension was quantified by the Bio-Rad Quantity One program. The processivity index (M/(N−M)) was calculated for each nucleotide insertion in single round synthesis, where

\[ \text{processivity index} = \frac{M}{N-M} \]

\( M = \) the integrated volume of band \( N = \) the summation of the volume integral of all subsequent bands.

The processivity index approaches 1 (most processive) as \( N \) approaches 0.

Effect of Primer-template Length on Processivity of T7 DNA Polymerase—We first wanted to see if we could localize the sites of important interactions on T7 DNA polymerase in the absence of thioredoxin by determining the effect of stem length on the processivity. We compared single round synthesis at 4°C with different length primer-template stems (Fig. 2, a−d) by preincubating the T7 DNA polymerase with the primer templates and initiating reaction with a mixture of dNTPs and many-fold excess calf thymus DNA to act as a trap for dissoci-
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Effect of Primer-template Length on T7 DNA Polymerase Binding—Dissociation constants for the T7 DNA polymerase were calculated from synthetic bursts with constant primer-template over a range of polymerase concentrations at 4 °C (Fig. 3, b and c). Polymerase η shows about the same $K_d$ for both the 7- and 10-mer primer-templates ($307 \pm 52$ and $240 \pm 26$ nM, respectively) indicating that its recognition is within 7 nucleotides. In contrast, the $K_d$ for the His-tagged T7 DNA polymerase with no added thioredoxin for the 7-mer was 4.5 times higher than for the 10-mer primer-template ($299 \pm 60$ versus $63 \pm 10$ nM, respectively) showing that the primer-template stem beyond 7 nucleotides contributes significantly to binding. The $K_d$ for the 10-mer primer-template is similar to that of 65 nM reported for oligo(dT)$_{20}$poly(dA)$_{300}$ (12).

Effect of Methylphosphonate Substitutions on Processivity of T7 DNA Polymerase—Methyl phosphonates neutralize the negative charge in DNA and can be used to identify electrostatic interactions between DNA and protein (35), although as a secondary effect methylphosphonates narrow the minor groove of DNA (36) and can bend DNA (37). Because we had found that the processivity of synthesis dropped when the primer template was less than 8 bp, we decided to determine what the effect of changing phosphates 7 and 8 in both primer and template to methyl phosphonates would be on the processivity of T7 DNA polymerase in the absence of thioredoxin. We found that synthesis with P22m2/T46m2 is nearly distributive (Fig. 2e) and similar to the synthesis with the 7-mer primer-template stem (Fig. 2b).

Effect of Salt on T7 DNA Polymerase Processivity—Given that many of the interactions between the T7 polymerase and the primer-template involve phosphates, we wanted to see to what extent the processivity of DNA synthesis on different templates is modulated by salt. Increasing salt concentration has been previously shown to decrease the binding constant of the T7 DNA polymerase-thioredoxin complex for poly(dA)-oligo(dT) (12). Fig. 4 shows single round synthesis with T7 DNA polymerase in the presence of thioredoxin on the 10-mer primer-template stem (P10/T20) with increasing salt concentration. As the salt approaches 500 mM, the reaction becomes almost completely distributive, similar to the reaction with the 7-mer primer-template (Fig. 2b). We then examined the processivity of T7 polymerase complexed to thioredoxin on a series of 22-mer primer-template stems with different template sequences for comparison. Processivity also decreased with ionic strength with a mixed sequence DNA template (Fig. 4b) but even more drastically for a poly(dA) or poly(T) template (Fig. 4c and d). In each case, synthesis terminated between 7 and 9 nucleotides. Surprisingly, synthesis on a poly(dC) template was highly processive and virtually insensitive to salt (Fig. 4e).

The abrupt termination observed on the poly(dA) or poly(T) templates is probably due to the formation of a d(A(dT)$_n$) tract. That the termination is due to the specific structure that the d(A(dT)$_n$) tract adopts rather than the stability of its duplex form is indicated by the inability of the randomized template sequence d(TAATTATAT) (Fig. 4g) to cause termination. In this later case, termination occurs about 3 to 5 nucleotides later within the poly(dA) template. Likewise, when a primer ending

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**Fig. 3.** Processivity index and binding constants for the primer-templates. a, the processivity index for extension of the primer corresponding to each band shown in Fig. 2 (a–d). Extension from primers P10, P8, P7, and P6 are indicated by circles, diamonds, x’s, and squares, respectively. Synthetic burst amplitudes at 4 °C were quantified for different concentrations of His-tagged T7 DNA polymerase (b) or His-tagged polymerase η (c) with 100 nM primer-templates P7/T17 and P10/T20 (circles and squares, respectively) used to calculate dissociation constants ($K_d$ values). Each data point was normalized to the maximum amplitude determined at the highest enzyme concentration and plotted as a fraction of that amount.
in 4 Ts is used opposite the poly(dA) template, synthesis by T7 DNA polymerase terminates after the insertion of only 3 – 5 nucleotides (Fig. 4f), and with a template containing 5 dCs before the dA template, synthesis terminates 7 – 9 nucleotides after entering the poly(dA) template (Fig. 4h).

**Effect of Mutating Asn335 and Ser338 of T7 DNA Polymerase on Processivity**—The crystal structure of T7 DNA polymerase-thioredoxin complex with DNA indicates that Asn335 and Ser338 of T7 gene 5 protein interact with template phosphates 7 and 8 (13). To investigate the role of these amino acids in processivity we constructed a His-tagged T7 polymerase mutant in which these residues were mutated. We decided to change 5 residues between 334 – 338 from FNPSS to GLPIA to incorporate a BamH1 site into the DNA for screening purposes. The alteration also eliminates Ser337, which might otherwise be able to compensate for the loss of Ser338, although this residue appears distant from the DNA in the crystal structure. The proline in the sequence was retained to maintain the peptide conformation, and modeling of the mutant suggested that the mutations would not cause any significant structural distortions. Because the expression vector for the mutant T7 M DNA polymerase did not contain the appropriate antibiotic resistance gene for expression in the thioredoxin-deficient host used to prepare the unmodified T7 DNA polymerase, we had to use a host that was not deficient in thioredoxin. As a result, we could not directly test the effect of thioredoxin on the processivity of the mutant T7 M DNA polymerase, but we could compare the processivity of T7 DNA polymerase and the mutant on longer primer templates in the presence of thioredoxin.

The mutant His-tagged T7 M DNA polymerase was nearly distributive with the 10-mer primer-template stem in the absence of added thioredoxin (Fig. 2f) giving results similar to the 7-mer primer-template stem and the methylphosphonate substituted primer-template (Fig. 1e). The processivity of the mutated His-tagged T7 M DNA polymerase on a 22-mer primer template in the presence of excess thioredoxin and low salt were similar to those observed for the His-tagged T7 DNA polymerase in the presence of excess thioredoxin under high salt conditions (Fig. 5). Processivity was very high with the dC template, but much lower with the mixed template. With the dA or dT templates, synthesis terminated sharply after 4 or 5 nucleotides. The dissociation constants for the P22/T46 primer-template in the presence of excess thioredoxin at 23 °C were found to be 0.9 ± 0.3 nM for the His-tagged T7 DNA polymerase and 3 ± 1.5 nM for the mutant polymerase. A similar 4-fold decrease in binding affinity for the P10/T20 template at 23 °C was found for the His-tagged T7 DNA polymerase (3.3 ± 1.5 nM) the mutant His-tagged T7 M DNA polymerase (12 ± 4.6 nM) in the absence of added thioredoxin.

**Processivity of T7 DNA Polymerase in the Absence of Thioredoxin on Long Template Primers**—Early termination in dA or T templates was also observed for T7 DNA polymerase in the absence of thioredoxin under low salt conditions with the 22-mer primer template stem (Fig. 6). The processivity

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**Fig. 4. Effect of salt concentration on processivity.** Single round synthesis with His-tagged T7 DNA polymerase with excess thioredoxin (0.2 nM) at different salt concentrations with the dA template at 4 °C. In this experiment the His-tagged T7 DNA polymerase was 20-fold excess over primer-template to ensure complete binding of the gene 5 protein-thioredoxin complex even in the presence of salt, which interferes with thioredoxin-DNA binding. The NaCl concentration (in millimolar) is indicated above the lanes and is in addition to ~30 mM that comes from the polymerase stock solution. Lane C is a control lane in which the polymerase was omitted from the reaction.
index for T7 DNA polymerase under these conditions remained constant at about 0.8 with the mixed template for about 19 nucleotides until it neared the end of the template. In contrast, synthesis on the poly(dA) and poly(T) templates (T46 and T46A) terminated sharply at 7 and 6 nucleotides, respectively, and behaved in a very similar manner to the P10/T20 template (Fig. 3a). As expected, synthesis on the template beginning in d(TAATTATAT), T46AT, stopped after 13 nucleotides, which is 4 nucleotides within the poly(dA) template. Unexpectedly, synthesis on the C template, which was highly processive with both T7 DNA polymerase and the mutant T7 M DNA polymerase in the presence of thioredoxin, was almost completely distributive with the T7 DNA polymerase in the absence of thioredoxin.

Effect of a 3T Bulge on Processivity—Fig. 7 compares synthesis with two primer-templates, one containing a 3T bulge (T47T3) in the template strand located 3 nucleotides from the site of Asn335-DNA interaction in the T7 DNA polymerase, and one without (T47). His-tagged T7 DNA polymerase synthesis in the presence of excess thioredoxin (0.2 mM) with this primer-template progressed only two nucleotides (Fig. 7a), which is what one would expect if the Asn335-DNA interaction, or physical interaction with the thumb structure, blocks the passage of the extrahelical bulge. In contrast, synthesis with polymerase η did not appear to be affected by the template bulge (Fig. 7b).

Effect of Methylphosphonates on Thioredoxin-mediated Processivity—We tested the effectiveness of thioredoxin as a processivity factor for T7 DNA polymerase with a primer-template containing methylphosphonates. Fig 8a shows that thioredoxin does not promote processive synthesis with a 10-bp primer-template stem, whereas Fig. 8b shows that thioredoxin can act as a processivity agent with a longer primer-template stem of 22 nucleotides. On the other hand, a 22-bp primer-template stem containing 22 methylphosphonates distributed throughout the putative thioredoxin binding site (13) did not promote processive synthesis and behaved like an unmodified 10-bp primer-template stem, which is thioredoxin-insensitive. A time-course experiment with the modified primer-template shows only short synthetic products until 80% of the initial substrate is extended, after which only full-length product is seen (Fig. 8c). The modified primer migrated as a fuzzy band in acrylamide electrophoresis, which might be due to the presence of so many diastereomeric methylphosphonates. We therefore performed single round synthesis with an unmodified primer and modified template, P22/T46m11 (Fig. 8d), and obtained a synthetic burst, which was the same as seen for the unmodified 10-bp primer-template stem (Fig. 8a). Although this reduction in processivity might be due to interference with protein-phos-
Panel c is a time course with faint band at 28 nucleotides and more intense bands at 27 and P46 primer shows a different cleavage pattern consisting of a 26 bp (38). Footprinting with the primer-template stem was 25–26 bp (38), which concluded that the optimal primer-template stem was 25–26 bp (38). Footprinting with the P46 primer shows a different cleavage pattern consisting of a faint band at 28 nucleotides and more intense bands at 27 and 30 nucleotides reflecting the positional change of 4 nucleotides in the primer. In contrast, polymerase η only protects 6 or 7 nucleotides of the P42/T51 primer-template stem at the highest DNase I concentrations. The strong 13-mer cleavage band found in all lanes corresponds to cleavage in the template that corresponds to a position that is 4 nucleotides from the 3'-end of the primer and results from the presence of unbound primer-template.

**Fig. 8. Effect of methylphosphonate substitutions on processive synthesis.** Reactions were carried out with T7 DNA polymerase alone (−) or in a complex with excess thioredoxin (+) with the indicated primer templates (Fig. 1). Panels a, b, and d are single round reactions. Panel c is a time course with lanes 1–6 as 0, 10, 20, 30, 60, and 90 s, respectively. Lane C is a control lane in which the polymerase was omitted from the reaction.

**DISCUSSION**

According to the crystal structure, Asn\(^{335}\) and Ser\(^{338}\) of the thumb domain of T7 DNA polymerase interact with template phosphates 7 and 8 of the primer-template stem (13). Results with different sizes or methylphosphonate-modified primer-templates demonstrate that these interactions are essential for processive synthesis (Figs. 2–4). These interactions may also function with a nearby helix-loop-helix motif located at residues 339–372 to enclose the DNA. It has been proposed that the analogous structure found in the Klenow fragment limits DNA dissociation during processive synthesis (17). The thumb structure must be held in close proximity to the DNA by weak ionic interactions. Salt interferes with these interactions, and amino acid substitutions of non-polar aliphatic residues for Asn\(^{335}\) and Ser\(^{338}\) render the structure non-functional except in a complex with excess thioredoxin (37). DNase I footprinting was used to map the pol η and T7 DNA polymerase-thioredoxin-DNA complexes using two different primers opposite the same 5'-end labeled template, T51. The P46 primer corresponds to the P42 primer extended by 4 nucleotides and was used to see if the footprint tracked with the position of the primer along the template. Fig. 9a, lane C, shows a limit digest of 13 nucleotides or less with the P42/T primer-template in the absence of polymerase. This same primer-template with T7 DNA polymerase-thioredoxin shows an intense band located 28 nucleotides from the 5'-end of the template. This corresponds to cleavage 19 nucleotides from the 3'-end of the primer and probably defines the outer border of the T7 DNA polymerase-thioredoxin interaction with the DNA and is consistent with the crystal structure (13). This footprint is smaller than what was determined by burst experiments on synthetic primer-template, which concluded that the optimal primer-template stem was 25–26 bp (38). Footprinting with the P46 primer shows a different cleavage pattern consisting of a faint band at 28 nucleotides and more intense bands at 27 and 30 nucleotides.
beginning with T tracts (40). Similarly, T7 DNA polymerase alone or with thioredoxin but missing Asn$^{335}$ and Ser$^{338}$ interactions terminates synthesis 4–9 nucleotides within the poly(dA) or poly(T) templates (Figs. 4 and 5). Termination must be caused by the specific structure formed by d(A-T)$_n$ tracts, because termination does not occur within a randomized poly-(dA,T) template (Fig. 4g).

Thioredoxin does not promote processive synthesis with a primer-template containing methylphosphonates distributed throughout the putative thioredoxin binding site (Fig. 5). In contrast, poly(dA) and poly(T) (Fig. 5). What was completely unexpected, however, is that synthesis opposite the modified T7 DNA polymerase in the absence of thioredoxin terminates synthesis with the poly(dC) template after inserting only 3 nucleotides (Fig. 6). The explanation for this unusual behavior might have to do with the specific structure of poly(dC) (41) and the spatial positioning of its backbone phosphate and/or base stacking and its interactions with the polymerase.

In conclusion, we have shown that Asn$^{335}$ and Ser$^{338}$ of the thumb domain of T7 DNA polymerase are involved in binding to the primer-template and may function with a nearby HLH motif (residues 339–372) to enhance processive synthesis. The T7 DNA polymerase thumb domain also interacts with thioredoxin, and we have shown this complex interacts directly with the DNA. It seems clear that thioredoxin, Asn$^{335}$, and Ser$^{338}$ interactions with DNA are required for efficient bypass of d(A-T)$_n$ tracts and even for optimal processive synthesis with mixed sequence DNA. In contrast, polymerase $\eta$ only binds to a 7-bp primer-template stem, and its weak hold on DNA is consistent with a loosely interacting thumb domain.

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