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Interaction of Loach DNA Polymerase δ with DNA Duplexes with Single-Strand Gaps

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Abstract—The interaction of DNA polymerase δ purified from eggs of the teleost fish *Misgurnus fossilis* (loach) with DNA duplexes with single-strand gaps of 1-13 nucleotides was studied. In the absence of template-restricting DNA, the enzyme elongated primers on single-stranded DNA templates in a distributive manner. However, in the presence of the proximal 5′-terminus restricting the template, the enzyme activity significantly increased. In this case, the enzyme was capable of processive synthesis by filling gaps of 5-9 nucleotides in DNA duplexes. These data indicate that DNA polymerase δ can interact with both the 3′- and 5′-termini located upstream and downstream from the gap. Analysis of the complexes formed by DNA polymerase δ and different DNA substrates by electrophoretic mobility shift assay confirmed the assumption that this enzyme can interact with the proximal 5′-terminus restricting the gap. DNA polymerase δ displayed much higher affinity in duplexes with gaps of approximately 10 nucleotides compared to the standard template–primer complexes. Maximal affinity was observed in experiments with DNA substrates containing unpaired 3′-tails in primers. The results of this study suggest that DNA polymerase δ exerts high activity in the cell nuclei during repair of DNA intermediates with single-strand gaps and unpaired 3′-termini.

Key words: replication, repair, DNA polymerases, DNA polymerase δ, oocytes, teleost fish

The first stage of base excision repair involves the removal of a DNA fragment at the damaged site through the coordinated action of specific endonucleases and exonucleases. The next step involves the filling of the gap by DNA polymerases. In eukaryotic cell nuclei, DNA repair is performed by DNA polymerases β, δ, and, possibly, ε [1-3]. Repair of damage of various chemical nature proceeds in alternative ways and is catalyzed by different enzymes. Using mutation analysis it was shown that DNA synthesis during excision base repair induced by nucleotide removal by a specific N-glycosidase is catalyzed by DNA polymerase β [4]. DNA polymerase β is the most effective (i.e., the $k_{cat}/K_{m,dNTP}$ ratio is maximal) in the case of one-nucleotide single-strand gaps [5, 6]. However, this enzyme is 500-fold less effective in six-nucleotide gaps and 2500-fold less effective in standard template–primer complexes. Thus, the major function of DNA polymerase β in the cell is, probably, filling of one-nucleotide gaps during short-patch base excision repair [7]. In extended single-strand templates, DNA polymerase β performs distributive polymerization by adding one nucleotide to the primer during one cycle of DNA substrate binding. However, when filling short gaps, DNA polymerase β is capable of processive synthesis by filling gaps of 4-6 nucleotides during one cycle [8]. Processive filling of short gaps shows that the 5′-terminus that restricts the gap stabilizes the complex of the enzyme with the DNA substrate. The binding site of the 5′-terminus of DNA in DNA polymerase β probably overlaps with the deoxyribophosphate lyase site [9-11]. The ability to do processive synthesis in short-patch gaps also allows DNA polymerase β to participate in long-patch base excision repair when several nucleotides are inserted into a gap that occurred as a result of excision of a damaged region [12, 13]. However, DNA polymerase δ and, possibly, ε seem to play the key role in base excision repair in long-patch gaps [14-21]. The main function of DNA polymerase δ is the highly processive synthesis of the leader DNA chain in the replication complex (holoenzyme) that comprises the processivity factor PCNA and replication factors RF-C and RP-A [22, 23]. The function of DNA polymerase δ in base excision repair has been studied in much less detail. In the absence of processivity factors, DNA polymerase δ exhibits low processivity in long-patch single-strand templates, and the question of whether this enzyme can independently perform repara-

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tive synthesis in short gaps in duplexes remains to be elucidated.

Earlier, we described the process of isolation of DNA polymerase δ from loach, which is classified with the teleost fish [24]. This enzyme seems to be the main repairing DNA polymerase in early embryos because the level of DNA polymerase β activity in loach eggs and embryos is abnormally low [25]. To analyze the mechanism of utilization of molecules with single-strand breaks and gaps by DNA polymerase δ, oligonucleotide substrates simulating DNA repair intermediates were synthesized. The experiments showed that DNA polymerase δ could effectively interact with DNA duplexes with short gaps and fill these gaps in a processive manner in the absence of processivity factors.

MATERIALS AND METHODS

Enzymes. DNA polymerase δ (fractions δ1 and δ2) from eggs of the teleost fish *Misgurnus fossilis* (loach) was obtained as described earlier [24]. The enzyme was stored at −20°C in buffer G containing 10 mM potassium phosphate buffer, 50% glycerol, 5 mM 2-mercaptoethanol, 1 mM EDTA, 10 mM $Na_2S_2O_5$, 0.2 mM PMSF, 1 μM leupeptin, pepstatin A (1 μg/ml), and aprotinin (1 μg/ml). Incorporation of 1 nmol of dNMP into activated DNA during 1-h incubation was taken as the DNA polymerase activity unit. Phage T4 polynucleotide kinase was obtained from Boehringer (Germany).

DNA substrates. To prepare DNA substrates, the following oligonucleotides were used: 17-mer (TGCCGGGATCATAGAAG), 21-mer (AGAGTGCCGGGATCATAGAAG), 25-mer (ACACAGAGTGCCGGGATCATAGAAG), 29-mer (AGCCACACAGAGTGCCGGGATCATAGAAG), standard primer 21-mer (AAGCGGAGTGTATGTGCAGTG), primer with unpaired 3′- tail (17+4)-mer (AAGCGGAGTGTATGTGCCTGA), and template 51-mer (CTTCTATGATCCCGGCACTCTGTGTGGCTACACTGCACATACACTCCGCTT). Some oligonucleotides were kindly provided by D. Mozzherin (University of New York at Stony Brook). Oligonucleotides (primers 21-mer, (17+4)-mer, or template 51-mer) were labeled at the 5′-terminus with [γ-^{32}P]ATP using phage T4 polynucleotide kinase. Labeled primers were hybridized with the template at a molar ratio of 1 : 1.2. Labeled template 51-mer was then hybridized with the primers at a ratio of 1 : 1.5. To obtain duplexes with gaps of one nucleotide (*21 : 51 : 29-mer), five nucleotides (*21 : 51 : 25-mer), nine nucleotides (*21 : 51 : 21-mer), and thirteen nucleotides (*21 : 51 : 17-mer), the labeled primer *21-mer was hybridized with the template 51-mer and the corresponding oligonucleotides at a ratio of 1 : 2 : 2.5. Duplexes with the primer (17+4), in which four residues at the 3′-end are not complementary to the template, were obtained in a similar way.

Elongation of primers by DNA polymerase δ. The ability of DNA polymerase δ for processive synthesis was determined in 20-μl samples containing 30 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, BSA (100 μg/ml), 2 mM dithiothreitol, 0.42 pmol of ^{32}P-labeled template–primer complex *21 : 51-mer (or of the duplex with the gap of five nucleotides (*21 : 51 : 25-mer) or nine nucleotides (*21 : 51 : 21-mer)), and dATP, dGTP, dCTP, and TTP (50 μM each). The reaction was started by adding 0.015 unit of DNA polymerase δ to the reaction medium at 30°C. After 1, 2, and 4 min of incubation, 5-μl aliquots were taken and mixed with 7 μl of stop solution containing formamide and the leader dyes. The preparations were analyzed by PAGE (10 or 12.5% polyacrylamide) in the presence of 8 M urea. The ability of DNA polymerase δ for strand displacement synthesis was determined in reaction medium (20 μl) that contained 0.8 pmol of gapped ^{32}P-labeled duplexes and the other components listed above. Aliquots of the reaction mixture were taken at 5, 12, and 30 min after the reaction was initiated by adding 0.15 unit of DNA polymerase δ.

Binding of DNA polymerase δ to DNA substrates. DNA polymerase δ (0.004-0.06 unit) was incubated with 0.005-0.04 pmol of ^{32}P-labeled DNA substrate in 10 μl of buffer containing 10 mM Tris-HCl, pH 8.0, BSA (100 μg/ml), and 2 mM dithiothreitol at 22-23°C for 10-15 min. The samples were then analyzed by PAGE in gels (60 × 100 × 0.75 mm) containing 6% acrylamide (acrylamide/bisacrylamide, 60 : 1) prepared in 20 mM HEPES, pH 8.0, and 0.1 mM EDTA. Electrophoresis was performed at 80 V in buffer containing 20 mM HEPES, pH 8.0, 0.1 mM EDTA, and 2 mM thioglycolic acid at room temperature. The gels were then transferred onto DE81 paper and dried under vacuum.

Determination of DNA polymerase δ activity in the gel. DNA polymerase δ (0.03 unit) was incubated with 0.04 pmol of the duplex 21 : 51 : 21-mer (nine-nucleotide gap) or the duplex 21 : 51 : 17-mer (thirteen-nucleotide gap) in 10 μl of the reaction mixture described above at 4°C for 20 min. Electrophoresis was performed at 4°C in buffer containing 20 mM HEPES, pH 8.0, 0.1 mM EDTA, and 2 mM thioglycolic acid. The gel was incubated in 15 ml of the buffer containing 10 mM Tris-HCl, pH 7.5, 8 mM $MgCl_2$, 2 mM dithiothreitol, BSA (100 μg/ml), dGTP, dCTP, TTP (5 μM each), and 50 pmol of [α-^{32}P]dATP (750 Ci/mmol) at 30°C for 10 min. The gel was then washed with 5% TCA supplemented with 1% sodium pyrophosphate, transferred onto chromatographic paper, and dried under vacuum.

RESULTS AND DISCUSSION

DNA polymerase δ-mediated elongation. To analyze the utilization of molecules with single-strand gaps by DNA polymerase δ, a set of oligonucleotide substrates

was constructed. Using hybridization of oligonucleotides comprised of 17-29 nucleotides with the 51-nucleotide template, we obtained duplexes containing single-strand gaps of one, five, nine, and thirteen nucleotides. Standard primer containing 21 nucleotides was radiolabeled at the 5′-terminus with radioactive phosphorus. Elongation of this primer by DNA polymerase δ in standard template–primer complex *21 : 51-mer and in gapped duplexes was examined.

In the first series, we assayed the ability of loach DNA polymerase δ in processive synthesis during elongation of primers in the template–primer complex *21 : 51-mer and in partial duplexes with gaps of five and nine nucleotides (duplexes *21 : 51 : 25-mer and *21 : 51 : 21-mer, respectively). The amount of the enzyme, concentration of the DNA substrate, and duration of the reaction were selected so that the reaction products evolved from a single act of binding of the enzyme to the DNA substrate (Fig. 1). DNA polymerase δ displayed low activity in standard template–primer complex, and the major reaction products were the primers elongated by 1 or 2 nucleotides, which under these conditions were masked by the excess of the original radiolabeled primer (lanes *1-3*). This is indicative of a distributive mode of synthesis performed by DNA polymerase δ, which is consistent with the previously obtained data [24]. Elongation observed in the absence of dCTP served as a control so as to provide termination of the synthesis at the first residue dGMP in the template (the fourth nucleotide of the template at the 3′-end). Exclusion of dCTP from the reaction mixture did not change the pattern of distribution of the elongation products in the gel (lanes *4-6*), this confirming the nonprocessive mode of elongation performed by DNA polymerase δ. However, when DNA duplex had a single-strand gap of 5 or 9 nucleotides, i.e., in the presence of oligonucleotide restricting the single-strand region in the template, elongation activity of the enzyme sharply increased. As a result, the enzyme gained the capacity for filling the gap in a processive mode (lanes *7-12*). Besides the primers elongated by 1 to 2 nucleotides, the major reaction products were primers elongated by 5 or 6 and 9 or 10 nucleotides in the case of duplexes with gaps of 5 and 9 nucleotides, respectively. Therefore, the enzyme was able to fill all gap in a processive mode and to terminate the synthesis near the 5′-terminal residue restricting this gap.

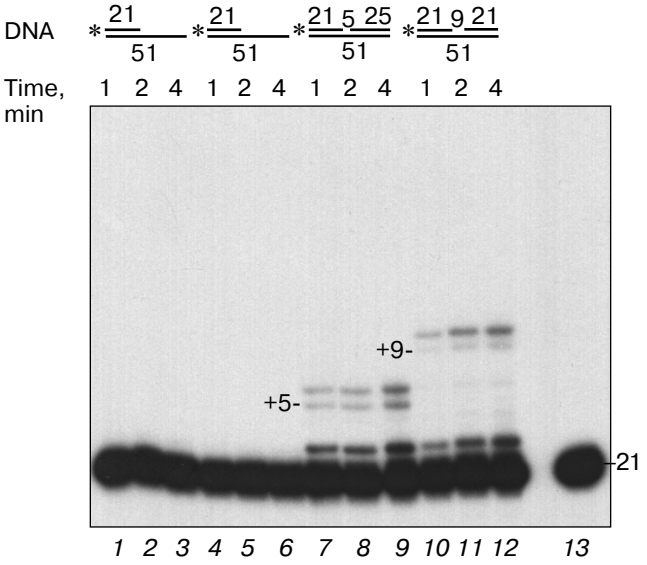

Fig. 1. DNA polymerase δ1-catalyzed polymerization of the primer [5′-^{32}P]21-mer in the template–matrix complex *21 : 51-mer (*1-6*), duplex *21 : 51 : 25-mer with a five-nucleotide gap (*7-9*), and duplex *21 : 51 : 21-mer with a nine-nucleotide gap (*10-12*). The reaction was conducted for 1, 2, and 4 min in a complete synthesis system (*1-3* and *7-12*) and in medium devoid of dCTP (*4-6*). The reaction products were analyzed by PAGE (10% acrylamide) in the presence of 8 M urea. Lane *13* shows migration of the original primer [5′-^{32}P]21-mer.

The results shown in Fig. 1 were obtained with fraction δ1. Given that the isolated preparation of loach DNA polymerase δ was heterogeneous [24], it was of interest to find out whether the mode of elongation described for the most purified fraction of DNA polymerase δ1 is characteristic of the other fractions of the enzyme. Figure 2 shows the results obtained in fraction δ2. In this experiment we used the standard template–primer complex *21 : 51-mer and the duplex *21 : 51 : 21-mer with a nine-nucleotide gap. We found that in the template–primer complex fraction δ2 performed synthesis in a distributive mode, whereas in the DNA duplex it filled the gap predominantly in the processive mode. Thus, similarly to fraction δ1, fraction δ2 can change from distributive elongation of primers in long-patch single-strand regions in the template to processive synthesis when the single-strand region of the template is restricted by the proximal 5′-terminus of the oligonucleotide.

The results of the experiments led us to the following conclusions on the mode of elongation performed by loach egg DNA polymerase δ. First, the enzyme performs distributive elongation of primers in the standard template–primer complexes when the template is not restricted by the 5′-terminal nucleotide residues hybridized with the template. Second, the enzyme displays substantially higher elongation activity in DNA duplexes with short single-strand gaps (five to nine nucleotides) than in standard template–primer complexes and is capable of processive synthesis over the whole gap region.

During processive filling short gaps in the duplexes, DNA polymerase δ can terminate synthesis at the 5′-terminal nucleotide of the fragment restricting the single-strand gap, thus displacing this residue from binding to the complementary nucleotide of the template (Figs. 1 and 2). However, in the aforementioned experiments

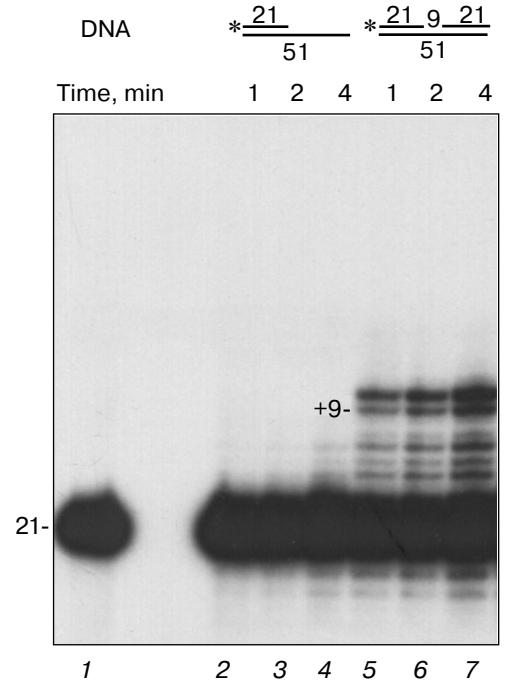

Fig. 2. DNA polymerase δ2-catalyzed polymerization of the primer [5′-^{32}P]21-mer in the template–matrix complex *21 : 51-mer (*2-4*) and duplex *21 : 51 : 21-mer with a nine-nucleotide gap (*5-7*). The reaction was conducted for 1, 2, and 4 min. The reaction products were analyzed by PAGE (12.5% acrylamide) in the presence of 8 M urea. Lane *1* shows migration of the primer [5′-^{32}P]21-mer.

elongation was performed in the presence of excess template–primer complexes so as to evaluate the real processivity of the enzyme. It remained unclear whether DNA polymerase δ can perform strand displacement DNA synthesis during long-term incubation under conditions that allow for multiple binding of the enzyme to the DNA substrate. To assess the ability of DNA polymerase δ for strand displacement DNA synthesis, we performed an experiment in which excess DNA polymerase δ1 was incubated for 30 min in reaction medium containing DNA duplexes with gaps of one, five, nine, and thirteen nucleotides (Fig. 3). It appeared that the labeled primers were utilized almost completely during the first five minutes of incubation. Irrespective of the gap size, the final reaction products were a mixture of oligonucleotides corresponding to the complete filling of the gap and an additional incorporation of usually one nucleotide. The absence of visible changes in the composition of elongation products during a 30-min incubation is indicative of a futile cycle when the enzyme adds one nucleotide to the oligonucleotide filling the gap and then removes this residue due to its 3′→5′-exonuclease activity. The pattern of distribution of the reaction products reflects the balance between the reactions of synthesis and exonuclease-mediated hydrolysis. On the whole, the experiment demonstrates the inability of DNA polymerase δ to perform DNA synthesis with displacement of the DNA strand that restricts the single-strand gap.

Binding of DNA polymerase δ to DNA. The above-mentioned change of DNA polymerase δ from distributive synthesis on single-strand templates to processive synthesis when filling short gaps in DNA duplexes implies an interaction of the enzyme with the 5′-terminus restricting the gap. The possibility of DNA polymerase δ binding to the 5′-terminus restricting the gap was studied by electrophoretic mobility shift assay. Labeled DNA substrates used before for elongation study were incubated with DNA polymerase δ. Then the complexes formed by the enzyme and labeled DNA were analyzed by native PAGE. The occurrence of radiolabeled complexes with lower mobility than the original DNA substrate is indicative of a stable binding of this substrate to the enzyme. At first we compared DNA polymerase δ binding to the standard template–primer complex *21 : 51-mer and duplexes with gaps of one, five, nine, and thirteen nucleotides (Fig. 4). DNA polymerase δ exerted low affinity for the

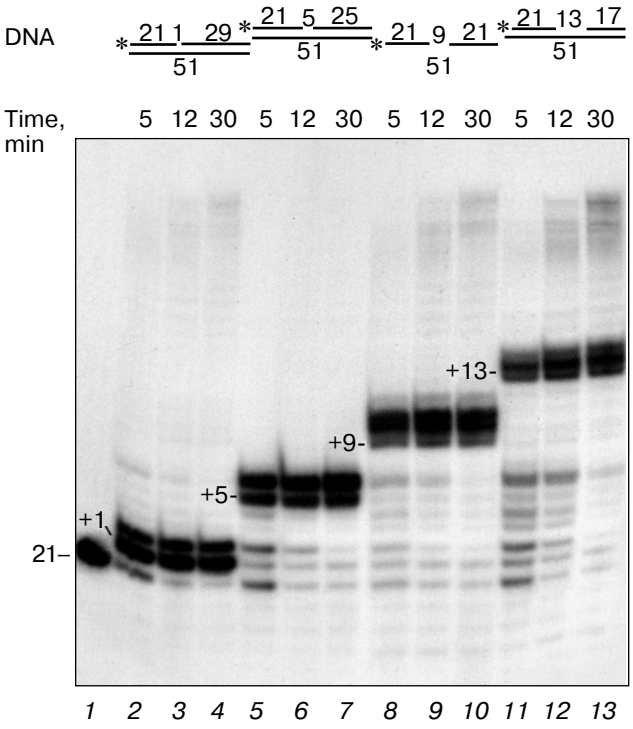

Fig. 3. DNA polymerase δ1-catalyzed polymerization of the primer [5′-^{32}P]21-mer in duplexes with gaps of one nucleotide (*21 : 51 : 29-mer) (*2-4*), five nucleotides (*21 : 51 : 25-mer) (*5-7*), nine nucleotides (*21 : 51 : 21-mer) (*8-10*), and thirteen nucleotides (*21 : 51 : 17-mer) (*11-13*). The reaction was conducted for 5, 12, and 30 min. The reaction products were analyzed by PAGE (10% acrylamide) in the presence of 8 M urea. Lane *1* shows migration of the original primer [5′-^{32}P]21-mer.

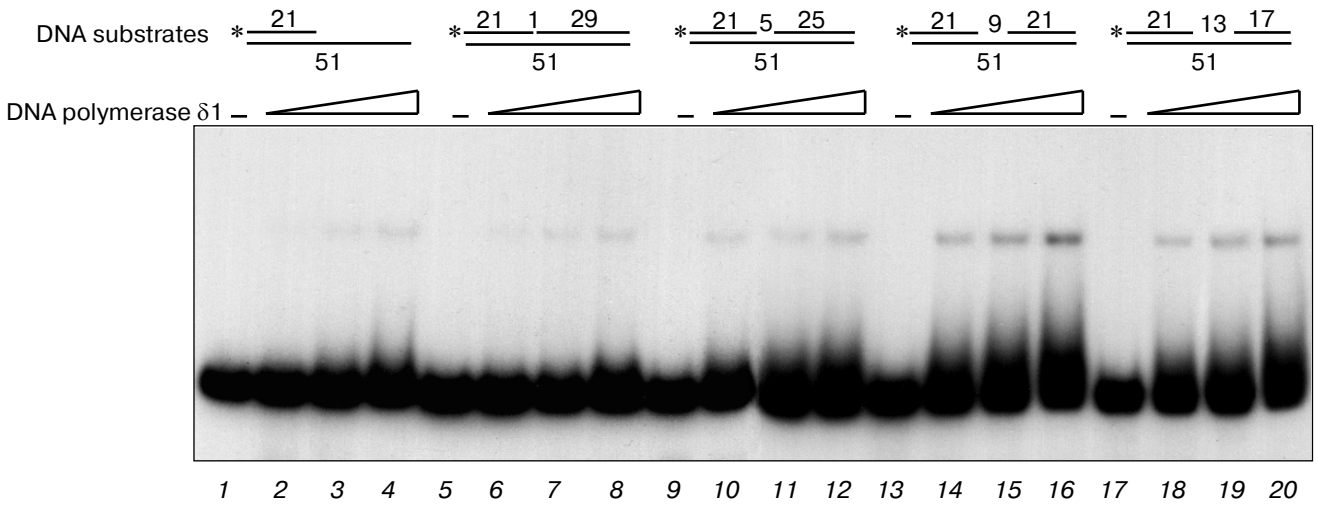

Fig. 4. Binding of DNA polymerase δ1 to template–primer complex *21 : 51-mer (*1-4*) and duplexes with gaps of 1 nucleotide (*21 : 51 : 29-mer) (*5-8*), 5 nucleotides (*21 : 51 : 25-mer) (*9-12*), 9 nucleotides (*21 : 51 : 21-mer) (*13-16*), and 13 nucleotides (*21 : 51 : 17-mer) (*17-20*). The samples (10 μl) contained 0.01 pmol of ^{32}P-labeled DNA substrates and 0.004 U (lanes *2*, *6*, *10*, *14*, and *18*), 0.01 U (lanes *3*, *7*, *11*, *15*, and *19*), or 0.03 U (lanes *4*, *8*, *12*, *16*, and *20*) of DNA polymerase δ1. Lanes *1*, *5*, *9*, *13*, and *17* contain no enzyme. The enzyme was incubated with the DNA substrates at room temperature for 15 min. The reaction products were analyzed by PAGE (6% acrylamide).

template–primer complex *21 : 51-mer and duplex with one-nucleotide gap. The affinity of the enzyme for five-nucleotide duplex was higher. Binding of the enzyme substantially increased with increase of the gap to 9 nucleotides and then somewhat decreased again as the gap grew to thirteen nucleotides. Thus, DNA polymerase δ exhibits much higher affinity for DNA duplexes with gaps of approximately ten nucleotides compared to standard template–primer complexes and duplexes with short gaps of one to five nucleotides.

In a control experiment, we obtained a formal confirmation that the discovered complexes of labeled DNA substrates with the enzyme contain functionally active DNA polymerase δ. Duplexes containing gaps of nine and thirteen nucleotides were used as DNA probes. In the first and second series, the enzyme was bound to duplexes labeled with radioactive phosphorus or unlabeled duplexes, respectively. To determine the position of the complexes of the enzyme with DNA in the gel, the radioactive portion of the gel after electrophoresis was exposed to a Roentgen film (Fig. 5, lanes *1-4*). To reveal DNA polymerase δ activity *in situ*, the portion of the gel containing unlabeled DNA probes was incubated in reaction mixture for DNA synthesis which contained [α-^{32}P] dATP (lanes *5-8*). Complexes exerting DNA polymerase δ activity were present in the samples that contained DNA substrates and the enzyme (lanes *6* and *7*). A coincidence between the position of functional complexes of DNA polymerase δ with DNA substrates in the gel and the position of the complexes of radiolabeled probes with the enzyme was observed (lanes *2* and *4*). Therefore, the

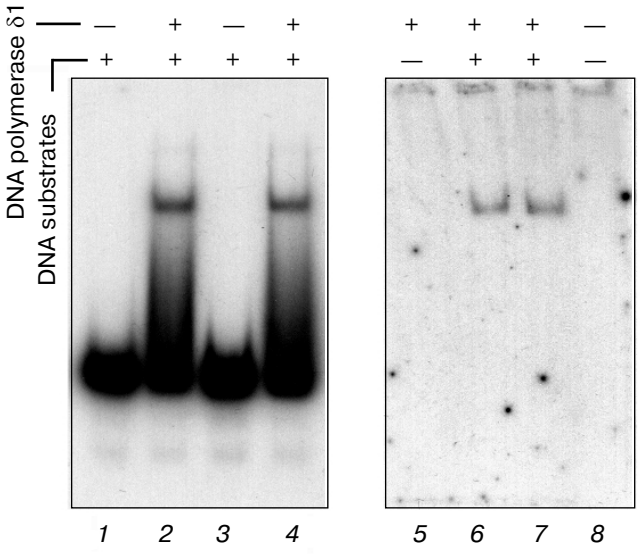

Fig. 5. Manifestation of DNA polymerase δ1 activity in polyacrylamide gel *in situ*. The samples (10 μl) contained 0.01 pmol of ^{32}P-labeled duplexes with gaps of 9 nucleotides (*21 : 51 : 21-mer) (lanes *1* and *2*) and 13 nucleotides (*21 : 51 : 17-mer) (lanes *3* and *4*) or 0.04 pmol of unlabeled duplexes with gaps of 9 nucleotides (21 : 51 : 21-mer) (lane *6*) and 13 nucleotides (21 : 51 : 17-mer) (lane *7*). After a 20-min incubation at 4°C with 0.03 U of DNA polymerase δ1 (lanes *2*, *4*, *5-7*) or without enzyme (lanes *1*, *3*, and *8*), the reaction products were analyzed by PAGE (6% acrylamide). The portion of the gel containing radiolabeled DNA substrates (lanes *1-4*) was transferred onto DE81 paper and dried under vacuum. Part of the gel with non-radioactive DNA substrates (lanes *5-8*) was incubated in the reaction mixture containing [α-^{32}P]dATP to reveal DNA polymerase activity in the gel as described in "Materials and Methods".

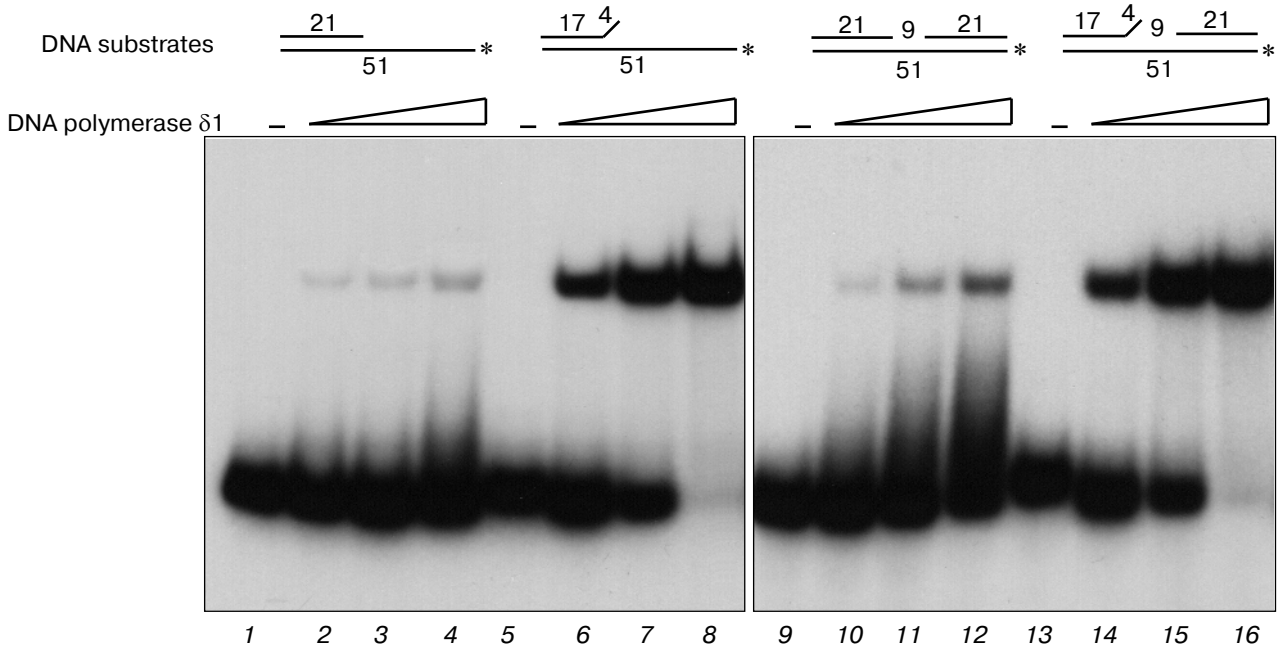

Fig. 6. Binding of DNA polymerase δ to template–matrix complexes containing primers with unpaired 3′-terminal tails. The samples (10 μl) contained 0.005 pmol of ^{32}P-labeled DNA substrates: 21 : *51-mer (lanes *1-4*), (17+4) : *51-mer (lanes *5-8*), 21* : 51 : 21-mer (lanes *9-12*), and (17+4) : *51 : 21-mer (lanes *13-16*). DNA polymerase δ1 was added in the amount of 0.004 U (lanes *2*, *6*, *10*, and *14*), 0.01 U (lanes *3*, *7*, *11*, and *15*), or 0.03 U (lanes *4*, *8*, *12*, and *16*). Lanes *1*, *5*, *9*, and *13* contain no enzyme. The enzyme was incubated with the DNA substrates for 15 min at room temperature. The reaction products were analyzed by PAGE (6% acrylamide).

previously discovered complexes of DNA probes with the enzyme are in fact complexes of DNA with DNA polymerase δ.

The fact that DNA polymerase δ possesses 3′→5′-exonuclease activity specific towards single-strand DNA implies the possibility of binding of the enzyme to unpaired 3′-termini in DNA. To evaluate the affinity of DNA polymerase δ for unpaired DNA regions, we synthesized partial duplexes containing four unpaired nucleotide residues at the 3′-terminus of the primer comprised of 21 nucleotides. Binding of DNA polymerase δ to duplexes containing the standard primer and those with unpaired 3′-tails was compared by electrophoretic mobility shift assay as described above. As shown in Fig. 6, DNA polymerase δ displays high affinity for the substrates with unpaired 3′-tails both in standard template–primer complexes and duplexes with single-strand gaps. Tight binding of DNA polymerase δ to the unpaired 3′-tails suggests that the enzyme also exhibits high activity in similar structures in damaged DNA in cell nuclei.

Because the DNA polymerase δ preparation was heterogeneous [24], it was of interest to determine whether characteristics of the enzyme binding to DNA substrates described for the most purified fraction δ1 can be extrapolated from the less purified fraction δ2. Incubation of fraction δ2 with labeled DNA probes yielded complexes that had similar (although somewhat lower) mobility in the gel as fraction δ1 (Fig. 7). Thus, the mode of DNA binding by fraction δ2 is similar to that of fraction δ1. Besides the major area of slowly migrating complexes, fraction δ2 contained a minor area with higher mobility that coincided with the mobility of the complexes formed by δ1 (lanes *7* and *8*). A similar pattern was observed in the case of binding of template–primer complex to DNA polymerase δ from calf thymus (Fig. 6 in paper [24]). With regard for different molecular weights of immunoreactive polypeptides contained in fractions δ1 and δ2 (120 and 130 kD, respectively [24]), it can be suggested that the occurrence of close but different in mobility complexes of the enzyme with DNA probes can result from partial proteolysis of the catalytic subunit of DNA polymerase δ. The results suggest that partial proteolysis does not significantly change the mode of binding of the enzyme to DNA substrates and the mode of elongation of primers during DNA synthesis.

DISCUSSION

It is well known that in the absence of processivity factors (PCNA, RF-C, and ATP) DNA polymerase δ performs distributive polymerization [26]. In this work we

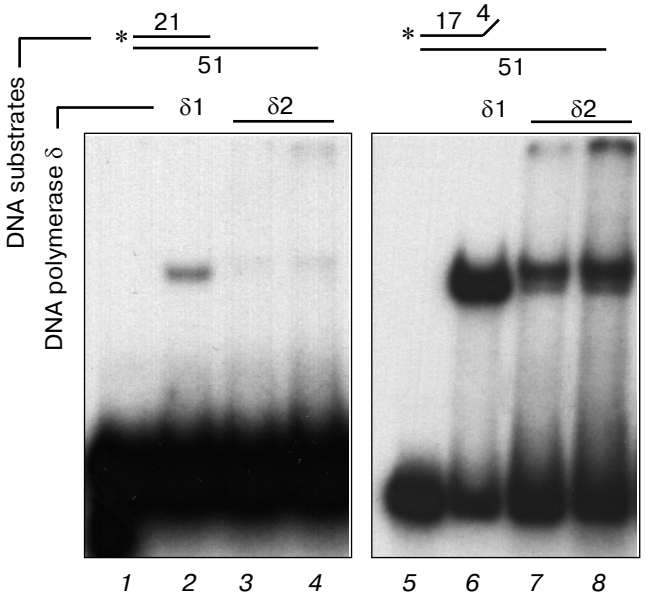

Fig. 7. Comparison of electrophoretic mobility of the complexes of DNA substrates with DNA polymerases δ1 and δ2. The samples (10 μl) contained 0.01 pmol of ^{32}P-labeled DNA substrates: *21 : 51-mer (lanes *1-4*) or (17+4) : 51-mer (lanes *5-8*). The samples contained 0.017 U of DNA polymerase δ1 (lanes *2* and *6*) or DNA polymerase δ2: 0.006 U (lane *3*), 0.015 U (lanes *4* and *7*), and 0.045 U (lane *8*). Lanes *1* and *5* contain no enzyme. The enzyme was incubated with the DNA substrates at room temperature for 15 min. The reaction products were analyzed by PAGE (6% acrylamide).

for the first time showed that DNA polymerase δ is capable of processive filling of short gaps in DNA in the absence of any processivity factors. Apparently, the enzyme can interact with the 5′-terminal region of a DNA strand restricting the single-strand gap, and this interaction stabilizes the complex of the enzyme with the template–primer. A similar phenomenon was described earlier for DNA polymerase β [8]. In this case binding of the enzyme to the 5′-terminal nucleotide restricting the gap is due to the presence of a deoxyribophosphate lyase (dRp- or AP-lyase) site in the DNA polymerase β molecule [9-11]. AP-lyase activity was also discovered in the A-type DNA polymerase family that comprises DNA polymerase I from *E. coli* and mitochondrial DNA polymerase γ [27]. AP-lyase site in DNA polymerase γ from *Xenopus* oocytes probably determines the ability of this enzyme to bind to the 5′-terminal region of a strand that restricts a short gap (Mikhailov, Pinz, and Bogenhagen, unpublished data). However, data on AP-lyase activity in DNA polymerase δ are absent. It should be noted that some DNA polymerases, DNA polymerase I (Kornberg fragment) from *E. coli* in particular, possess 5′→3′-exonuclease activity specific towards double-stranded DNA. These fragments bind to the 5′-terminal region of the strand that restricts the gap owing to the presence of the 5′→3′-exonuclease site [28]. Although eukaryotic DNA polymerases do not possess 5′→3′-exonuclease activity, they may have retained the ability to interact with the 5′-terminal DNA regions during evolution.

The assumption that DNA polymerase δ can interact with the 5′-end that restricts the gap was confirmed using the electrophoretic mobility shift assay. A low affinity of loach DNA polymerase δ for standard template primers (Fig. 4) is consistent with data published in the literature. It was shown that the formation of a tight complex between the enzyme and DNA requires a structural "clamp" consisting of three PCNA molecules, which is formed by the RF-C protein complex in the presence of ATP [22, 26]. An addition of oligonucleotide restricting the template region to the template–primer increased the amount of the enzyme bound in the complex with DNA. This increase was insignificant in the case of short gaps of one to five nucleotides but relatively pronounced in the case of nine-nucleotide gaps. Further increase in the gap size from nine to thirteen nucleotides resulted in some decrease in binding. Thus, the optimal distance between the 5′- and 3′-termini restricting the gap that provides for DNA polymerase δ binding should be about ten nucleotides. Such a distance approximately corresponds to the size of template region that is bound to different DNA polymerases in complexes with template primers [29, 30].

The results of experiments on binding of the enzyme to duplexes with single-strand gaps account for the ability of DNA polymerase δ to fill short gaps in DNA duplexes in a processive mode. When filling the gap, DNA polymerase δ interacts not only with the primer and template, but also with the proximal 5′-terminus downstream from the gap. Binding to the 5′-terminus additionally stabilizes the productive complex of the enzyme with the DNA substrate and confines the enzyme in this complex after a successive addition of a nucleotide residue to the growing primer, thus allowing the enzyme to perform processive polymerization. In the absence of proximal 5′-terminus downstream from the gap, binding of DNA polymerase δ with the template primer is apparently insufficient for confinement of the enzyme in the productive complex after the nucleotidyl transferase reaction. For this reason, the enzyme dissociates from the DNA substrate after the addition of a successive nucleotide residue to the primer. In this case, polymerization proceeds according to the distributive mechanism. During processive synthesis, loach DNA polymerase δ filled the gap completely and either terminated synthesis directly before the 5′-terminal nucleotide downstream from the gap or added one more nucleotide to the growing strand (Figs. 1 and 2). As shown in Fig. 3, even when present in excessive amount, DNA polymerase δ did not perform strand displacement DNA

synthesis. Thus, DNA polymerase δ meets the requirements for an enzyme that fills gaps during DNA repair.

Tight binding of DNA polymerase δ to duplexes containing unpaired 3′-tails in primers (Fig. 6) possibly reflects the participation of the exonuclease site of the enzyme in DNA substrate binding. In this binding type, the 5′-terminus downstream from the gap does not significantly affect the affinity of the enzyme for DNA substrate. High affinity of DNA polymerase δ for duplexes containing unpaired 3′-termini implies that DNA with such structural lesions represents an effective substrate for DNA polymerase δ. Thus, unpaired 3′-termini and single-strand gaps, which are conventional intermediates during DNA repair, can serve as targets for excision repair activity of DNA polymerase δ in the cell nucleus. It is believed that participation of DNA polymerase δ and ε in DNA excision repair is associated with the PCNA-dependent pathway of restoring intact DNA structure [14-21]. The processive mode of short gap filling by DNA polymerase δ in the absence of processivity factors, as described in this work, suggests that this enzyme can function on its own in damaged DNA molecules with single-strand gaps.

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