ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 11, pp. 1207-1213. © Pleiades Publishing, Ltd., 2008.
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Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM07-440, October 19, 2008.

DNA Polymerases β and λ as Potential Participants of TLS during Genomic DNA Replication on the Lagging Strand

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Received December 28, 2007
Revision received May 30, 2008

Abstract—The main strategy used by pro- and eukaryotic cells for replication of damaged DNA is translesion synthesis (TLS). Here, we investigate the TLS process catalyzed by DNA polymerases β and λ on DNA substrates using mono- or dinucleotide gaps opposite damage located in the template strand. An analog of a natural apurinic/apyrimidinic site, the 3-hydroxy-2-hydroxymetylthetrahydrofuran residue (THF), was used as damage. DNA was synthesized in the presence of either Mg^{2+} or Mn^{2+}. DNA polymerases β and λ were able to catalyze DNA synthesis across THF only in the presence of Mn^{2+}. Moreover, strand displacement synthesis was not observed. The primer was elongated by only one nucleotide. Another unusual aspect of the synthesis is that dTTP could not serve as a substrate in all cases. dATP was a preferential substrate for synthesis catalyzed by DNA polymerase β. As for DNA polymerase λ, dGMP was the only incorporated nucleotide out of four investigated. Modified on heterocyclic base photoreactive analogs of dCTP and dUTP showed substrate specificity for DNA polymerase β. In contrast, the dCTP analog modified on the exocyclic amino group was a substrate for DNA polymerase λ. We also observed that human replication protein A inhibited polymerase incorporation by both DNA polymerases β and λ on DNA templates containing damage.

DOI: 10.1134/S0006297908110060

Key words: DNA replication on lagging strand, translesion synthesis (TLS), AP-sites, DNA gaps, DNA polymerases β and λ, replication protein A (RPA)

Translesion synthesis (TLS) is the major process that provides for the replication of damaged DNA, and therefore it allows cells the chance to avoid death. This process is carried out by specialized DNA polymerases possessing one of the following properties: first, they are effective in coping with damaged DNA template; second, they have low fidelity for incorporation of complementary nucleotides during normal DNA synthesis (mutation rate estimated in range from 10^{-3} to 10^{-1}); third, they can catalyze polymerization using aberrant strand ends including uncomplementary base pairs and damaged DNA [1].

Recently, it was found that DNA polymerases β and λ from the structural X-family are able to carry out DNA synthesis across apurinic/apyrimidinic sites (AP-sites) [2, 3].

Abbreviations: AP-site) apurinic/apyrimidinic site; dRP) deoxyribose phosphate; hRPA) human replication protein A; THF) 3-hydroxy-2-hydroxymethyltetrahydrofuran; TLS) translesion synthesis.

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DNA polymerase β is a monomeric protein with a molecular mass of 39 kD (335 a.a.). This enzyme has two catalytic activities, DNA polymerase and dRP (deoxyribose phosphate)-lyase, but lacks 3′-5′ exonuclease activity [4]. This protein is the main enzyme of the base excision repair process (BER) [5, 6]. Additionally, it participates in meiosis and in neurogenesis [7, 8]. DNA polymerase β appears to be one of the major inaccurate eukaryotic enzymes with a mutation rate estimated to be higher than $\sim 10^{-3}$ [9]. This protein displays maximal accuracy with DNA substrates containing one-window gap with a phosphate group on the 5′-end. DNA polymerase β acts as a processive enzyme on DNA substrates containing a gap up to six nucleotides [10-12].

DNA polymerase λ is a recently identified DNA-dependent DNA polymerase in eukaryotic cells. Presumably, DNA polymerase λ participates in BER [13, 14], meiotic recombination [15], and non-homologous end joining process [16]. However, the biological role of this enzyme is not yet clear. DNA polymerase λ is a comparatively small monomeric protein of 67 kD (575 a.a.).

This enzyme has DNA polymerase activity and also lacks 3′-5′ exonuclease activity. Additionally, DNA polymerase λ is able to carry out template-independent synthesis utilizing single-stranded DNA [17]. This enzyme displays high efficacy with DNA substrates contained a one- or two-nucleotide gap, including a 5′-end phosphate, in contrast to extended DNA templates [14, 17].

As mentioned earlier, DNA polymerases β and λ are able to catalyze synthesis across AP-sites. Such damage is generated by the action of DNA glycosylases, and also occurs spontaneously. Every day up to 10,000 purine and 100,000 pyrimidine bases are lost [18]. The AP-site is the most dangerous lesion since the absence of a coding base can lead to unpredictable effects for the cell. Moreover, auxiliary replication proteins can influence the synthesis of DNA containing AP-sites. Hubscher and colleagues [19] analyzed the effect of human replication protein A (hRPA) on catalytic activity of different human DNA polymerases. When DNA substrates containing both protruding 5′-ends and AP-sites were used, hRPA had no influence on DNA synthesis catalyzed by DNA polymerase λ in the absence or in the presence of PCNA (proliferating cell nuclear antigen). Additionally, these two proteins had no influence on DNA polymerase β action under similar conditions [19].

Studies *in vitro* using various DNA lesions have shown that different TLS polymerases differ in their efficiency of dNTP insertion directly opposite the lesion, as well as in the processing of matched/mismatched termini. Until now, most research has been focused on the reconstitution of TLS on the leading strand. Primed DNA duplexes containing both damage and protruding 5′-ends were used for this goal. However, it is also possible for lesions to appear on the lagging strand, for example, during the synthesis of Okazaki fragments under oxidative stress [20]. In this case, damage can be located opposite the gap. Potentially, these DNA structures can be substrates for apurinic/apyrimidinic endonuclease 1 if an AP-site represents the lesion. Nevertheless, endonuclease activity of the enzyme will strongly depend on the secondary structure of DNA and on the reaction conditions [21]. Moreover, if cleavage near the AP-site occurs, there would be a double-strand break, which is a dangerous cell lesion [22].

Based on the published findings we can conclude that DNA polymerases β and λ of the structural X-family are potential participants of translesion synthesis during genomic replication on the leading strand. In this regard, it is important to examine in details the protein–protein and nucleic–protein interactions of DNA polymerases β and λ with TLS intermediates on the lagging strand.

The goal of the present investigation is kinetic study of the polymerase reaction catalyzed by DNA polymerases β and λ on DNA substrates containing gaps opposite the lesion. As damage, we used the synthetic analog of AP-site—3-hydroxy-2-hydroxymethyltetrahydrofuran (THF).

MATERIALS AND METHODS

The following materials and reagents were used: BSA (New England Biolabs, USA); phage T4 polynucleotide kinase (5000 U/ml; SibEnzyme, Russia); [γ-^{32}P]ATP with specific activity 5000 Ci/mmol (Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences); dNTP, reagents for electrophoresis and the main buffers components (Sigma, USA). Other reagents and buffer components were of analytically pure and extra pure grade of Russian production. Human recombinant DNA polymerase λ and human recombinant DNA polymerase β were purified from *Escherichia coli* BL21(DE3) RP cells as described in [23] and [24], correspondingly. Purified wild type hRPA was kindly provided by I. O. Petruseva (Institute of Chemical Biology and Fundamental Medicine). Photoreactive analogs of dCTP and dUTP (AL-FAB-dCTP, FAP-dCTP, and FAP-dUTP) (Fig. 1a) were synthesized in accordance with [25-27] and kindly supplied by S. V. Dezhurov. The following oligonucleotides (GenSet, Switzerland) were used for construction of DNA duplexes: template strand 5′-(d)*GGCTTCATCGTTGTCX*CAGACCTGGTGGATACCG-3′, where *X* is THF, 2′-deoxyguanosine, or 2′-deoxyadenosine residue; primer strands 5′-(d)CGGTATCCACCAGGTCTG-3′ (initial primer), 5′-*pGACAACGATGAAGCC*-3′, 5′-*pACAACGATGAAGCC*-3′.

Preparation of 5′-^{32}P-labeled primers. Radioactive label was incorporated into the 5′-end of the primer using phage T4 polynucleotide kinase as described in [28]. The reaction mixture (10 μl) contained 0.5 μM primer, 10 MBq [γ-^{32}P]ATP, and 5 U T4 polynucleotide kinase. The reaction was performed at 37°C for 30 min, then continued overnight at 4°C. Nucleotide material was resolved by 20% denatured PAGE, visualized by autoradiography, and isolated by electroelution on DE-81 in 50 mM Tris-borate buffer, pH 8.3. The product was eluted from DE-81 by five portions (20 μl) of 3 M $LiClO_4$, and then 1.2 ml cooled acetone (4°C) was added to the eluate. The probe was incubated at −40°C for 1 h. The precipitate was collected by centrifugation, washed twice with 1 ml cooled acetone (4°C), air dried, and dissolved in water to the required concentration.

Incorporation of dNMP or their photoactive analogs to the 3′-end of radioactive labeled primers using activity of DNA polymerases β and λ. dNMP and photoreactive analogs were incorporated into the 3′-end of primer by DNA polymerases β and λ. Reactions mixtures (10 μl) contained 0.01 μM 5′-^{32}P-labeled DNA substrate ($DNA_{THF}(1)$ or $DNA_{THF}(2)$), 0.05 μM DNA polymerase β or 0.15 μM DNA polymerase λ, 5 μM dNTP or 5 μM photoreactive analog, 5 mM $MgCl_2$ or 0.5 mM $MnCl_2$, and standard buffer components (TDB buffer: 50 mM Tris-HCl, pH 8.0, 0.5 mM dithiothreitol, 0.25 mg/ml BSA). All reactions were incubated at 37°C for 20 min.

The reactions were terminated by placement on ice. Reaction products were resolved by denaturing electrophoresis in 20% polyacrylamide gel as described in [29] and subjected to autoradiography using Molecular Imager FX (BioRad, USA). Analysis was performed with Quantity One (BioRad).

Michaelis constants and maximal rates of DNA synthesis for various DNA substrates were determined in two steps. In the first step, kinetics of dNMP incorporation into $DNA_{THF}(1)$ and $DNA_{THF}(2)$ by DNA polymerases β and λ were studied (Fig. 1b). The reaction was performed in 80 μl of a mixture containing 0.05 μM DNA polymerase β or 0.15 μM DNA polymerase λ, 5 μM dNTP or photoreactive analog, 0.01 μM 5′-^{32}P-labeled DNA substrate, and 0.5 mM $MnCl_2$ in TDB buffer at 37°C. Aliquots (10 μl) were taken after selected time periods in the range 0-40 min. The reaction was terminated by placement on ice. The reaction products were separated as described above. The data were collected using Quantity One software from BioRad and analyzed using OriginPro7.5 (Microcal Software, USA). To determine the Michaelis constants and the maximal rates of DNA synthesis catalyzed by DNA polymerases β and λ, in the second reaction step the dNTP concentration was varied from 0.01 to 10 μM and reaction products were analyzed as described above. The data were fitted according to the Michaelis–Menten kinetic equation.

The effect of hRPA on the polymerization reaction catalyzed by DNA polymerases β and λ was studied as follows. The reaction mixtures (10 μl) contained 0.05 μM DNA polymerase β and 5 μM dATP, or 0.15 μM DNA polymerase λ and 5 μM dGTP, 0.01 μM 5′-^{32}P-labeled DNA substrate, 0.5 mM $MnCl_2$, hRPA ranging from 0 to 500 nM, and TDB buffer. The reaction was performed at 37°C and terminated by placement on ice. The reaction products were analyzed as described above.

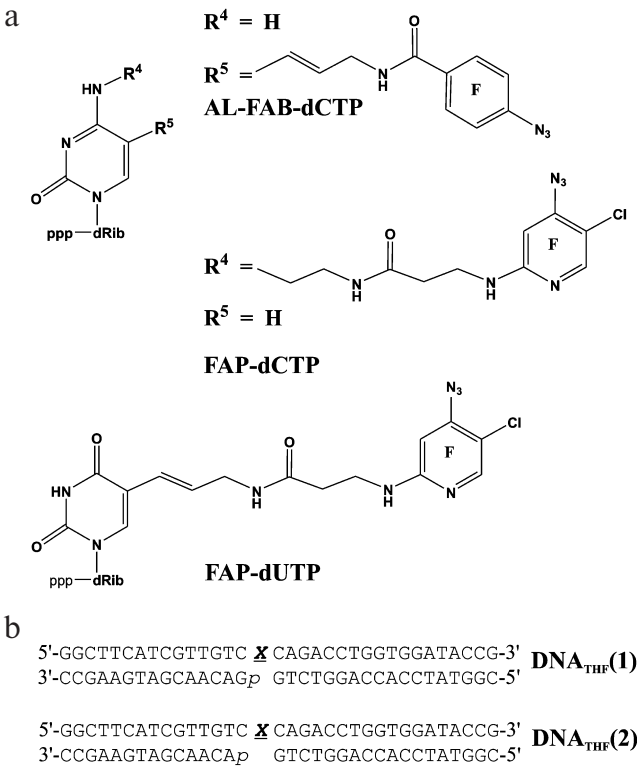

Fig. 1. Structures of photoreactive analogs of dNTP (a) and DNAs (b) used in the present study. AL-FAB-dCTP, 5-[3-(4-azido-2,3,5,6-tetrafluorobenzamido)-trans-propenyl-1]-2′-deoxycytidine-5′-triphosphate; FAP-dCTP, exo-N-[2-N-(N-(4-azido-2,5-difluoro-3-chlorpyridin-6-yl)-3-aminopropionyl)-aminoethyl]-2′-deoxycytidine-5′-triphosphate; FAP-dUTP, 5-{N-[N-(4-azido-2,5-difluoro-3-chlorpyridin-6-yl)-β-alanyl]-trans-3-aminopropenyl-1}-2′-deoxyuridine-5′-triphosphate.

RESULTS AND DISCUSSION

In spite of intensive investigation of translesion synthesis, there are a number of unresolved problems. Key questions include defining how the exchange between the arrested replication machinery and specialized TLS DNA polymerases is organized, and which DNA polymerases and associated factors act in TLS across specific DNA damages? It is also important to determine if there is a difference in the list of proteins bypassing the lesion on the leading and on the lagging strands. Photoaffinity labeling is a very favorable strategy to provide relevant information about translesion synthesis. This technique utilizes specific DNA substrates that bear photoreactive groups. These groups can be introduced into DNA probes using the catalytic activity of DNA polymerases. A number of different photoreactive dNTP derivatives bearing arylazido groups attached to the base can be used to introduce a photoreactive nucleotide moiety at the 3′-end of DNA substrates for analysis.

Here, we investigated the TLS process catalyzed by DNA polymerases β and λ on DNA substrates using mono- or dinucleotide gaps opposite the damage located in the template strand. These DNA structures imitate the intermediates of the replication process on the lagging strand of damaged DNA. As a lesion, we used a synthetic analog of AP-site—THF. Control experiments were done on undamaged DNA substrates. Kinetic approaches were performed for study of DNA synthesis.

Principal advantage of incorporating dNTPs and their photoreactive analogs into damaged DNA substrates by DNA polymerases β and λ. DNA substrates were constructed as partial DNA duplexes with either a mono-($DNA_{THF}(1)$) or dinucleotide ($DNA_{THF}(2)$) gap containing 3′-hydroxyl and 5′-phosphate groups. The lesion was located in the +1 position of the template strand relative to the 3′-end of the primer (Fig. 1b). Reactions were carried out in the presence of either Mg^{2+} or Mn^{2+} ions, i.e. in optimal polymerization conditions or to support high efficacy but low fidelity of enzymatic synthesis, correspond-

ingly. dNTPs and their photoreactive analogs were used as substrates. Photoderivatives of dCTP (AL-FAB-dCTP) and dUTP (FAP-dUTP) were modified in the C5 position of the base, and the dCTP (FAP-dCTP) analog bore an active group attached to the exocyclic amino group (Fig. 1a). Use of photoreactive analogs provides a good perspective since DNA substrate, which contains such dNTP derivatives, can be applied to analyze the composition of the TLS system *in vitro* by the photoaffinity labeling approach. After incubation of examined proteins with photoreactive DNA and following UV-induced linkage, the DNA–protein adducts can be resolved by denaturing PAGE. In the next stage, protein subunits crosslinked to DNA can be identified by the immunochemical methods or by the MALDI-MS technique. The photoaffinity labeling approach allows disclosing and characterizing stable nucleic–protein complexes as well as complexes organized by weak interactions [30]. Because many points in damage bypassing are not clear, revelation of such complexes is a very important question in the terms of studying TLS in eukaryotic nuclear or cellular extracts.

DNA synthesis was carried out in the presence of either one or all four dNTPs to determine which dNMP could be incorporated. As shown on Fig. 2, DNA polymerases β and λ were able to catalyze DNA synthesis across a THF-fragment only in the presence of Mn^{2+}. However, even in this case we observed the elongation over the range of the gap (Fig. 2, lanes *5*). Moreover, the primer was elongated by only one nucleotide, i.e. strand displacement synthesis was not observed, except in the case of DNA synthesis catalyzed by DNA polymerase β on $DNA_{THF}(2)$ substrate (Fig. 2a, lanes *5*). These data are consistent with reports that the presence of a phosphate group on the 5′-end of a gap restricts the typical ability for DNA polymerase λ to maintain strand displacement synthesis [17].

Another unusual aspect of the synthesis is that dTTP could not serve as a substrate in all cases (Fig. 2, a and b, lanes *2*). It should be noted that (i) dGTP was not utilized as a substrate for synthesis on $DNA_{THF}(1)$ catalyzed by DNA polymerase β (Fig. 2a, lanes *3*), and (ii) dGMP was the only incorporated nucleotide out of four investigated in the case of DNA polymerase λ (Fig. 2b, lanes *3*).

These data are in agreement with previous reports. At excessive dNTP concentrations, DNA polymerases β and λ preferentially incorporate the nucleotide that is the complement to the nucleotide on the 5′-side of the damage on the template strand. Such a mechanism of poly-

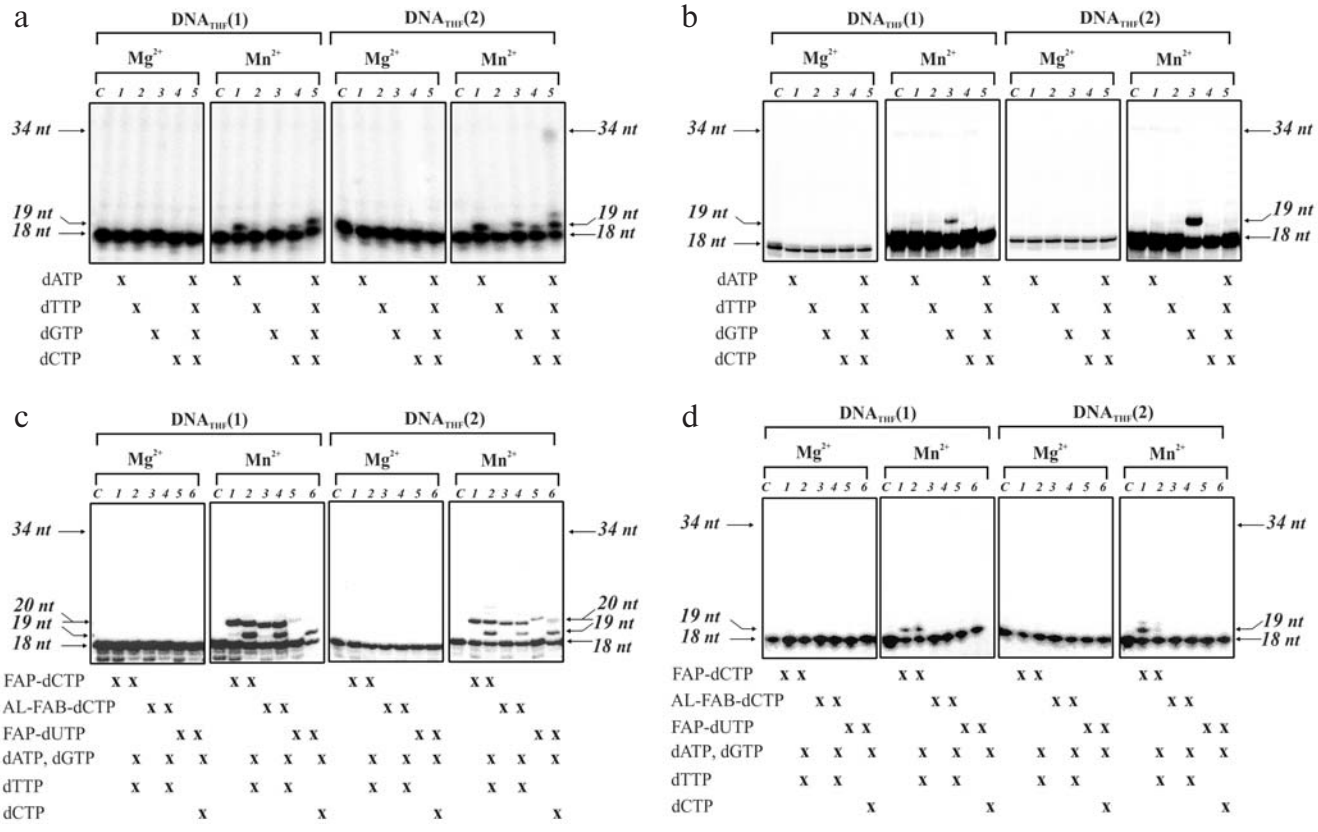

Fig. 2. Estimation of the opportunity for incorporation of dNMP and their photoreactive analogs into DNA substrates by DNA polymerases β and λ. a) dNMP incorporation by DNA polymerase β; b) dNMP incorporation by DNA polymerase λ; c) incorporation of photoreactive dNMP by DNA polymerase β; d) incorporation of photoreactive dNMP by DNA polymerase λ. Lane *C*, initial position of the 5′-^{32}P-labeled DNA substrate (18 nt); 34 nt, the length of full-size product. Reactions were performed as described in "Materials and Methods".

merization is known as "template slippage" [31]. In our case, dGTP acted as such a nucleotide. The fact that dGTP did not display substrate specificity during the synthesis catalyzed by DNA polymerase β on $DNA_{THF}(1)$ can be explained by the higher stability of the secondary structure of $DNA_{THF}(1)$. This prevents template curvature at the active site, which is needed for "template slippage" to occur. Additionally, DNA polymerase β demonstrates the "A-rule" as an alternative mechanism of synthesis. Studies of purified prokaryotic and viral DNA polymerases showed that the frequency of dAMP insertion opposite the AP-site is about 10-fold higher in comparison with dGMP, and up to 50-fold higher in comparison with either dCMP or dTMP [29]. NMR studies have shown that when dAMP is opposite an abasic lesion, it stacks in an intrahelical configuration causing virtually no distortion of the DNA helix, providing a physical explanation for this preferential incorporation of dAMP opposite the AP-site.

All photoreactive analogs used in this study were substrates for DNA polymerase β (Fig. 2c), whereas DNA polymerase λ was able to catalyze DNA synthesis only in the presence of FAP-dCTP (Fig. 2d, lanes *1* and *2*).

Therefore, use of DNA polymerases β and λ for the construction and application of photoreactive DNA structures is possible and perspective in the investigation of the TLS process in eukaryotic nuclear or cellular extracts.

Determination of kinetic parameters of DNA synthesis catalyzed by DNA polymerases β and λ. The data presented in Tables 1 and 2 illustrate the selectivity for incor-

Table 1. Kinetic parameters of dNMP incorporation into $DNA_{THF}(1)$ and $DNA_{THF}(2)$ catalyzed by DNA polymerase β in the presence of Mn^{2+}

Substrate	K_m, μM	V_{max}, pM/sec	$k_{cat} \times 10^3$, sec^{-1}	k_{cat}/K_m, $M^{-1} \cdot sec^{-1}$
		$DNA_{THF}(1)$		
dATP	1.18 ± 0.07	550 ± 11	10.9 ± 0.2	9237
dCTP	1.71 ± 0.15	262 ± 12	5.20 ± 0.18	3041
FAP-dCTP	9.41 ± 0.63	429 ± 19	8.68 ± 0.34	922
AL-FAB-dCTP	0.85 ± 0.13	60 ± 0.3	1.10 ± 0.06	1294
FAP-dUTP	9.12 ± 1.37	42 ± 0.3	0.74 ± 0.06	81
		$DNA_{THF}(2)$		
dATP	2.34 ± 0.25	311 ± 13	6.40 ± 0.24	2735
dGTP	3.83 ± 0.58	304 ± 23	5.92 ± 0.38	1546
dCTP	4.55 ± 0.77	296 ± 27	5.76 ± 0.44	1266
FAP-dCTP	11.3 ± 0.44	294 ± 13	5.86 ± 0.12	519
AL-FAB-dCTP	1.83 ± 0.23	45 ± 0.2	0.84 ± 0.04	459
FAP-dUTP	6.03 ± 0.55	31 ± 0.1	0.64 ± 0.02	106

Note: Here and further, the results are presented as the mean of three independent experiments (± standard error). Experiments were performed as described in "Materials and Methods".

Table 2. Kinetic parameters of dNMP incorporation into $DNA_{THF}(1)$ and $DNA_{THF}(2)$ catalyzed by DNA polymerase λ in the presence of Mn^{2+}

Substrate	K_m, μM	V_{max}, pM/sec	$k_{cat} \times 10^3$, sec^{-1}	k_{cat}/K_m, $M^{-1} \cdot sec^{-1}$
		$DNA_{THF}(1)$		
dGTP	1.84 ± 0.27	44 ± 0.2	0.29 ± 0.03	158
FAP-dCTP	0.34 ± 0.05	23 ± 1.1	0.11 ± 0.01	324
		$DNA_{THF}(2)$		
dGTP	0.36 ± 0.06	41 ± 0.1	0.23 ± 0.01	639
FAP-dCTP	0.83 ± 0.16	12 ± 0.1	0.07 ± 0.01	84

Table 3. Kinetic parameters of dNMP incorporation into $DNA_A(1)$, (2) and $DNA_G(1)$, (2) catalyzed by DNA polymerase β and λ in the presence of Mn^{2+}

Substrate		K_m, nM	V_{max}, nM/sec	k_{cat}, sec^{-1}	$(k_{cat}/K_m) \times 10^5$, $M^{-1} \cdot sec^{-1}$
		DNA polymerase β			
DNA_A	(1)	740 ± 121	1.16 ± 0.12	0.23 ± 0.01	3.1
	(2)	181 ± 32	3.72 ± 0.12	0.74 ± 0.04	40.7
DNA_G	(1)	263 ± 31	21.0 ± 4.88	4.21 ± 0.18	163
	(2)	202 ± 35	2.05 ± 0.07	0.41 ± 0.02	20.1
		DNA polymerase λ			
DNA_A	(1)	85.1 ± 10.0	1.35 ± 0.03	0.14 ± 0.01	16.5
	(2)	97.3 ± 9.5	1.94 ± 0.03	0.20 ± 0.01	20.5
DNA_G	(1)	92.8 ± 13.1	0.24 ± 0.01	0.05 ± 0.002	5.4
	(2)	50.3 ± 13.2	0.35 ± 0.01	0.07 ± 0.003	13.9

poration of native dNTPs and their photoreactive analogs opposite a THF-fragment by DNA polymerases β and λ. The control experiments were done with the native DNA substrates $DNA_A(1)$, (2) and $DNA_G(1)$, (2) (X = dA or dG) (Table 3).

As shown in Table 1, dATP was a preferential substrate for DNA synthesis catalyzed by DNA polymerase β. dCTP and dGTP were incorporated with similar efficacy into $DNA_{THF}(2)$. FAP-dCTP was the most efficient substrate of all investigated. It should be noted that DNA polymerase β catalyzed synthesis on $DNA_{THF}(1)$ was carried out with higher efficacy in comparison with the synthesis on $DNA_{THF}(2)$ in all of the cases.

As for the activity for DNA polymerase λ, dGTP was inserted more efficiently into $DNA_{THF}(2)$ than into $DNA_{THF}(1)$ (Table 2). Furthermore, only the photoreactive analog FAP-dCTP could be incorporated by DNA polymerase λ into both DNA substrates. In all of the cases, the yield of DNA synthesis products was higher using $DNA_{THF}(2)$ as a template.

Interestingly, DNA synthesis catalyzed by both enzymes was more effective on undamaged templates than templates with an AP-site regardless of DNA structure (Table 3). However, the main regularities were conservative: DNA substrate with a one-window gap was more optimal for DNA polymerase β whereas DNA duplex containing two-window gap was more preferential for DNA polymerase λ.

These findings are consistent with previously published results. Based on these results, we propose that the "template slippage" mechanism is supported by both DNA polymerases β and λ. Additionally, DNA polymerase β catalyzed TLS across the AP-site according to the "A-rule" [32]. The incorporation of dCTP into both $DNA_{THF}(1)$ and $DNA_{THF}(2)$ by activity of DNA polymerase β cannot be explained by any of the "rules". Presumably, it is linked to the low fidelity of DNA synthesis catalyzed by DNA polymerase β [33].

Our results suggest that DNA polymerases β and λ are candidates for participation in translesion synthesis across the AP-site during genomic replication on the lagging strand *in vivo*.

Influence of hRPA on TLS catalyzed by DNA polymerase β and λ. One participant in DNA replication is replication protein A, hRPA. Hubscher and colleagues found that RPA influences the fidelity of DNA polymerase λ on undamaged templates [34, 35] as well as on DNA containing 8-oxoguanine or 2-hydroxyadenine as a

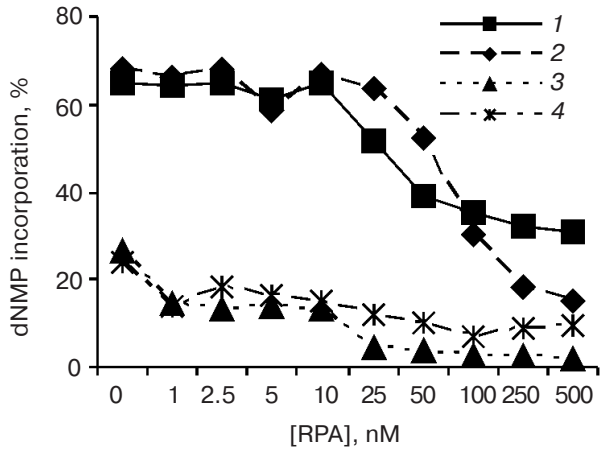

Fig. 3. Effect of hRPA on the incorporation of dAMP into $DNA_{THF}(1)$ (*1*) and $DNA_{THF}(2)$ (*2*) catalyzed by DNA polymerase β and on the incorporation of dGMP into $DNA_{THF}(1)$ (*3*) and $DNA_{THF}(2)$ (*4*) catalyzed by DNA polymerase λ. The final concentration of hRPA in the reaction mixture is shown on the X-axis. Amount of product, in percentages, i.e. amount of radioactive reaction product with respect to initial radioactivity of DNA substrate indicated in percentages, is shown on the Y-axis.

lesion [19, 36]. These results were obtained using DNA structures with protruding 5′-ends. Moreover, it is known that hRPA influences DNA synthesis catalyzed by DNA polymerase λ only if the replication factor forms a complex with the enzyme with defined stoichiometry [35, 37]. For this reason it was interesting to estimate the effect of hRPA on synthesis catalyzed by DNA polymerases β and λ on DNA substrates containing gaps.

This was done by analyzing the change in the polymerization reaction rate. The reaction progression catalyzed by DNA polymerases β and λ with DNA substrates $DNA_{THF}(1)$ and $DNA_{THF}(2)$ as a function of hRPA concentration is shown in Fig. 3. It was found that hRPA inhibited the incorporation of dAMP by DNA polymerase β and of dGMP by DNA polymerase λ opposite the THF-residue with a one-window gap-containing substrate. Additionally, hRPA displayed an inhibiting effect upon increase in the gap length. Moreover, it was shown that hRPA also inhibited the incorporation dCMP by both DNA polymerases opposite the 8-oxoguanine residue in both one- and two-nucleotide gaps. At the same time, hRPA had no effect on reaction rate using undamaged DNA templates (data not shown). This indicates that effect of hRPA is modulated by the presence and type of damage.

This work was supported by a grant from the Russian Foundation for Basic Research (No. 06-04-48612-a), by INTAS YSF (Ref. Nr. 06-10000014-6307), and by a grant from CRDF-BRHE (RNP.2.2.2.3.10031).

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