

Identification of an Intrinsic 5'-Deoxyribose-5-phosphate Lyase Activity in Human DNA Polymerase λ

A POSSIBLE ROLE IN BASE EXCISION REPAIR*

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Base excision repair (BER) is a major repair pathway in eukaryotic cells responsible for repair of lesions that give rise to abasic (AP) sites in DNA. Pivotal to this process is the 5'-deoxyribose-5-phosphate lyase (dRP lyase) activity of DNA polymerase β (Pol β). DNA polymerase λ (Pol λ) is a recently identified eukaryotic DNA polymerase that is homologous to Pol β . We show here that human Pol λ exhibits dRP lyase, but not AP lyase, activity *in vitro* and that this activity is consistent with a β -elimination mechanism. Accordingly, a single amino acid substitution (K310A) eliminated more than 90% of the wild-type dRP lyase activity, thus suggesting that Lys³¹⁰ of Pol λ is the main nucleophile involved in the reaction. The dRP lyase activity of Pol λ , in coordination with its polymerization activity, efficiently repaired uracil-containing DNA in an *in vitro* reconstituted BER reaction. These results suggest that Pol λ may participate in "single-nucleotide" base excision repair in mammalian cells.

DNA base damage is known to be one of the most challenging events to genomic stability. Either happening spontaneously, induced by radiation, alkylating mutagens, or as the result of enzymatic processes, the loss of a base in DNA must be efficiently repaired to sustain cellular function (1). Originally described as a process solving cytosine deamination (2), base excision repair (BER)¹ is the major pathway involved in the repair of DNA base lesions (for review, see Refs. 1 and 3–6). BER is a multistep process that is initiated with the removal of the modified base by a DNA glycosylase (7), followed by incision by an AP endonuclease (8–10). Subsequently, the remaining dRP residue can be either removed by a dRP lyase activity or displaced by DNA polymerase action, leading to "single-nucleotide" or "long patch" repair, respectively (3–6, 11, 12).

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¹ The abbreviations used are: BER, base excision repair; AP, apurinic/aprimidinic; Pol λ , DNA polymerase λ ; Pol β , DNA polymerase β ; Pol μ , DNA polymerase μ ; TdT, terminal deoxynucleotidyltransferase; dRP, 5'-deoxyribose-5-phosphate; hUDG, human uracil DNA glycosylase; hAPE, human AP endonuclease; hligase I, human DNA ligase I; PAGE, polyacrylamide gel electrophoresis.

DNA polymerase β (Pol β) is a small (39 kDa) mammalian nuclear DNA polymerase likely involved in gap-filling synthesis during DNA repair (13). In addition to DNA polymerization, Pol β was shown to contain an intrinsic dRP lyase activity (14). Compelling evidence indicates that Pol β is the main mammalian enzyme responsible for the removal of dRP groups (15, 16), a step that has proven to be limiting for efficient BER of lesions generated by monofunctional alkylating agents *in vivo* (16). Additionally, Pol β is thought to be almost fully responsible for DNA synthesis in single-nucleotide BER (17–20) and has recently been found crucial for the long patch pathway (21, 22). Thus, Pol β would contribute BER both with DNA synthesis (DNA polymerase activity) and dRP removal (dRP lyase).

The dRP lyase of Pol β resides in its N-terminal 8 kDa domain (14) and has been shown to proceed through a β -elimination mechanism that involves a Schiff-base intermediate (23). Extensive mutagenesis studies carried out with Pol β have identified several amino acid residues critical for the different functions inherent to the 8-kDa domain (24, 25). Furthermore, using mass spectrometry, Lys⁷² has been unequivocally determined as the nucleophile responsible for Schiff-base formation (26). Recently, a dRP lyase activity has been also reported in DNA polymerases γ (27, 28) and ι (29), but the functional significance of these enzymes in the BER process remains to be elucidated.

DNA polymerase λ (Pol λ) is a recently described eukaryotic DNA polymerase belonging to Pol X family. It is the closest homologue to Pol β , sharing 32% identity at the protein level (30). Based on three-dimensional structure modeling, Pol λ is predicted to have a Pol β -like core formed by two domains: a 31-kDa polymerase domain (with three subdomains, named fingers, palm, and thumb) and the 8-kDa domain (30). However, unlike Pol β , Pol λ contains additional N-terminal domains, including a BRCT domain that could mediate protein-protein interactions (30–34). The predominant expression of Pol λ in testis has led to the hypothesis that Pol λ participates in meiotic recombination. This is further supported by immunolocalization of Pol λ in the nuclei of pachytene spermatocytes (30). However, Pol λ mRNA expression has been detected in all tissues examined, suggesting a general role for this enzyme (30, 33, 34). Recent evidence² demonstrates that Pol λ polymerization capacity is similar to that of Pol β , what is consistent with a possible role of Pol λ in nuclear DNA repair. We address here if Pol λ , as Pol β , is endowed with a dRP lyase activity, an activity essential for "short patch" BER in eukaryotic cells.

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EXPERIMENTAL PROCEDURES

Materials—Synthetic oligonucleotides purified by PAGE were obtained from Life Technologies, Inc. Ultrapure deoxynucleoside triphosphates, [α - 32 P]dCTP, and [α - 32 P]ddATP (3000 Ci/mmol) were from Amersham Pharmacia Biotech. Terminal deoxynucleotidyltransferase was from Promega. Human recombinant Pol λ and Pol λ K310A mutant were overexpressed in *Escherichia coli* and purified nearly to homogeneity. The purified fractions were devoid of detectable nuclease activity.² Purified human Pol β , hUDG, hAPE, and hligase I were generous gifts of Dr. S. H. Wilson (NIEHS, Research Triangle Park, NC).

Preparation of the Substrate for dRP Lyase Activity Assay—A 34-mer oligonucleotide containing uracil at position 16 (P6: 5' CTGCAGCT-GATGCGCUGTACGGATCCCCGGGTAC 3') was labeled at the 3'-end by terminal deoxynucleotidyltransferase using [α - 32 P]ddATP and annealed to its complementary oligonucleotide (T4: 5' GTACCCGGG-GATCCGTACGGCGCATCAGCTGCAG 3'). This labeled double-stranded substrate (300 nM) was treated with hUDG (100 nM) for 20 min at 37 °C in 20 μ l of buffer containing 50 mM Hepes, pH 7.5, 20 mM KCl, and 2 mM DTT to remove the uracil. After incubation, the mixture was supplemented with 10 mM MgCl₂ and 40 nM hAPE, thus generating the substrate for dRP lyase activity (Fig. 1B).

Preparation of the Reduced Substrate—The labeled P6 was annealed to T4 oligonucleotide and treated with hUDG as described above. Following hUDG treatment, 340 mM NaBH₄ was added, and the mixture was incubated on ice for 20 min. The reduced DNA was then purified by ethanol precipitation and preincubated with hAPE prior to use.

dRP Lyase Activity Assay—Reaction mixtures (10 μ l) contained 50 mM Hepes, pH 7.5, 10 mM MgCl₂, 20 mM KCl, 2 mM dithiothreitol, and 30 nM concentration of the labeled substrate described in the previous section. The reaction was initiated by adding different amounts of Pol λ or Pol β and incubated at 37 °C as indicated. After incubation, NaBH₄ was added to a final concentration of 340 mM, and the reactions were kept for 20 min on ice. Stabilized (reduced) DNA products were ethanol-precipitated in the presence of 0.1 μ g/ml of tRNA, resuspended in water, and analyzed by electrophoresis in a 16% polyacrylamide gel and visualized with a Storm 860 PhosphorImager (Molecular Dynamics, Inc.). Alternatively, normal AP DNA was substituted by equal amounts of a reduced substrate.

AP Lyase Activity Assay—Reactions were performed essentially as described for dRP lyase activity assays, but using a substrate that had not been preincubated by AP endonuclease. After incubation, the samples were processed and analyzed as described above.

NaBH₄ Trapping Assay—10- μ l reaction mixtures contained 50 mM Hepes, pH 7.5, 10 mM MgCl₂, 20 mM KCl, 2 mM dithiothreitol, and 100 nM concentration of the labeled substrate used for dRP lyase activity assays. Reactions were initiated by adding the indicated amounts of Pol λ or Pol β and 20 mM NaBH₄ or 20 mM NaCl. After 30 min on ice, reactions were run on a 10% SDS-PAGE gel, and the trapped polymerase/DNA complex was visualized in a Storm 860 PhosphorImager.

In Vitro Reconstitution of Base Excision Repair—A 34-mer double-stranded DNA substrate was used that contained a G opposite a uracil at position 16. Reactions (10 μ l) contained 250 nM substrate, 50 mM Hepes, pH 7.5, 10 mM MgCl₂, 20 mM KCl, 2 mM dithiothreitol, 1 mM ATP, 2 μ M dCTP or dTTP, 10 nM hUDG, 40 nM hAPE, 200 nM hLigase I, and 0.3 μ M [α - 32 P]dCTP. Reactions were initiated by addition of different amounts of Pol λ or Pol β (100 nM and 5 nM, respectively) and incubated for the indicated times at 37 °C. Reactions were terminated by addition of 20 mM EDTA, ethanol-precipitated in the presence of 0.1 μ g/ml tRNA, analyzed by electrophoresis in a 10% polyacrylamide gel, and visualized with a Storm 860 PhosphorImager.

RESULTS AND DISCUSSION

The 8-kDa Domain of Pol λ Conserves the Critical Residues for dRP Lyase Activity—Amino acid residues 240–575 of human Pol λ , the closest homologue of human Pol β , are predicted to form a Pol β -like fold, formed by two different domains: the 8-kDa domain and a 31-kDa polymerization domain, constituted by the “fingers,” “palm,” and “thumb” subdomains (13).

The 8-kDa domain of Pol β is responsible for single-stranded DNA binding, 5'-phosphate recognition and binding, and dRP lyase activity (for review, see Ref. 13). With the exception of African Swine Fever Virus PolX (35), this domain can be predicted by sequence comparison in all Pol X family enzymes, even though some of these proteins are known or presumed to lack dRP lyase activity (*i.e.* Pol μ , see below). Therefore, con-

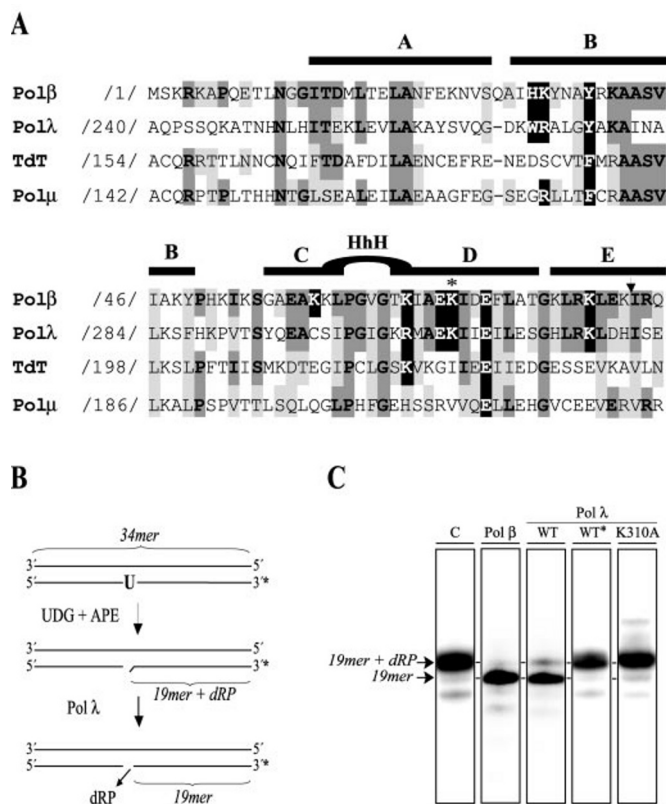


FIG. 1. A, multiple amino acid alignment of the 8-kDa domain of human Pol λ with the other human family X DNA polymerases. Residues described to be relevant to the 8-kDa domain function of Pol β (His³⁴, Lys³⁵, Tyr³⁹, Lys⁶⁰, Lys⁶⁸, Glu⁷¹, Lys⁷², Glu⁷⁵, and Lys⁸⁴) are indicated in white letters over a black background. The position of Lys³¹⁰ (Pol β residue Lys⁷²) is indicated with an asterisk. Invariant (bold type) and conservative substitutions referred to Pol β residues are boxed in dark and light gray, respectively. The arrow indicates the position of proteolytic cleavage for Pol β . B, schematic representation of a dRP lyase reaction. A 34-mer double-stranded oligonucleotide containing a uracil residue at position 16 in one strand is treated with hUDG and hAPE to release a dRP-containing substrate. This dRP moiety will be cleaved by dRP lyase activity. C, autoradiogram illustrating Pol λ dRP lyase activity. 10-Min reactions were performed as described under “Experimental Procedures,” using 50 nM Pol β , 80 nM (wild-type) or 100 nM (K310A mutant) Pol λ . Asterisk indicates the use of a reduced AP substrate.

servation of this domain suggests a more general function, perhaps related to its DNA binding capacity. Indeed, structural (36, 37), cross-linking (38) and kinetic studies (38) indicate that the 8-kDa domain plays a major role in the positioning of the enzyme in gapped or nicked DNA templates.

As shown in Fig. 1A, the 8-kDa domain of human Pol λ (residues 240–325) bears a significant amino acid similarity (30% identity) with that of Pol β (residues 1–87). Moreover, the 8-kDa domain of human Pol β can be aligned with the same region of the other two human DNA polymerases belonging to the Pol X family: TdT (residues 154–239) and the recently described Pol μ (residues 142–227; Ref. 39). However, in the region aligned, both TdT and Pol μ share only a 22% identity with Pol β .

Extensive mutational analysis of the 8-kDa domain of Pol β has identified several amino acid residues critical for its different activities (24, 25). Strikingly, all Pol β residues proposed to play a role in dRP lyase catalysis (His³⁴, Tyr³⁹, Lys⁶⁰, Lys⁶⁸, Glu⁷¹, Lys⁷², and Glu⁸⁴; shown in black boxes in Fig. 1A) are identical or conserved in Pol λ , with the exception of Lys⁶⁰. Most of these residues are absent from TdT or Pol μ . Particularly, Lys⁷², identified as the nucleophile in Pol β responsible for Schiff-base formation during β -elimination of the dRP moi-

ety (26), is present in Pol λ (Lys³¹⁰) but is lacking in the other two Pol X enzymes. Interestingly, Tyr³⁹, suggested to play a role in both DNA binding, through base-stacking interactions (25), and dRP lyase activity through the action of its hydroxyl residue (40), is conserved in Pol λ but substituted by a phenylalanine residue in TdT (Phe¹⁹¹) and Pol μ (Phe¹⁷⁹). This is consistent with the lack of dRP lyase activity in these two enzymes and supports previous work by Maciejewski *et al.* (40). Therefore, although an overall sequence similarity along the 8-kDa domain is shared by all human DNA polymerases of the Pol X family, the conservation of the identified Pol β dRP lyase catalytic residues only in Pol λ suggests a close functional relatedness between these two proteins.

dRP Lyase Activity Associated to Pol λ —To test the ability of Pol λ to remove a dRP group, we used a 34-mer double-stranded

oligonucleotide containing a uracil residue at position 16. As described under “Experimental Procedures,” the uracil-containing strand was 3'-end-labeled with [α -³²P]ddATP, annealed to its complementary strand, treated with hUDG to remove the uracil residue, and finally incubated with hAPE1 to release a dRP containing substrate (see scheme in Fig. 1B). This substrate was incubated in the absence (control) or in the presence of either Pol β or Pol λ . As shown in Fig. 1C, both enzymes removed the dRP moiety, as detected by the reduction in size of the labeled substrate. Under these conditions, as indicated by an initial time course, the rate of dRP excision by Pol λ was ~25% of the rate catalyzed by Pol β (data not shown). As predicted from the alignment shown in Fig. 1A, Pol μ lacked significant dRP lyase activity ($\leq 3\%$ of the activity of Pol β , data not shown).

Excision of dRP Groups by Pol λ through a β -Elimination Mechanism—The dRP lyase activity of Pol β proceeds through β -elimination, a mechanism that involves generation of a Schiff-base intermediate (23). To elucidate whether this was also the case with Pol λ , we took advantage of the fact that sodium borohydride is able to reduce a Schiff-base intermediate to form a covalent protein-DNA complex. Therefore, if the mechanism of catalysis of Pol λ involves a Schiff-base intermediate, addition of sodium borohydride in a dRP lyase assay should allow trapping of a DNA-protein complex that should be detected by autoradiography after separation by SDS-PAGE. Fig. 2A shows the result of a borohydride trapping experiment, where a single labeled band of the expected size can clearly be seen migrating slower than the free DNA and presenting a different mobility for each polymerase-DNA complex. The appearance of this band was dependent on both the presence of either Pol β or Pol λ and on the presence of NaBH₄. As a control of specificity, and in agreement with its lack of dRP lyase activity, no polymerase-DNA complex was seen when using Pol μ . These results suggest that the dRP removal activity of Pol λ proceeds through β -elimination. Therefore, it is very likely that Pol λ shares a common mechanism of catalysis with Pol β and other dRP lyases described. Further supporting this idea, Pol λ was unable to excise a reduced dRP residue, a substrate that is resistant to β -elimination (Fig. 1C).

Lys³¹⁰ Is Likely the Main Nucleophile Involved in Schiff-base Formation—Amino acid sequence comparison between Pol λ

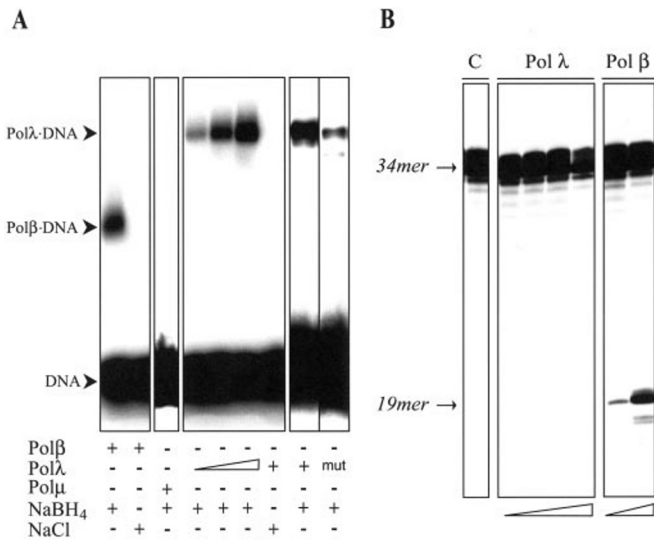
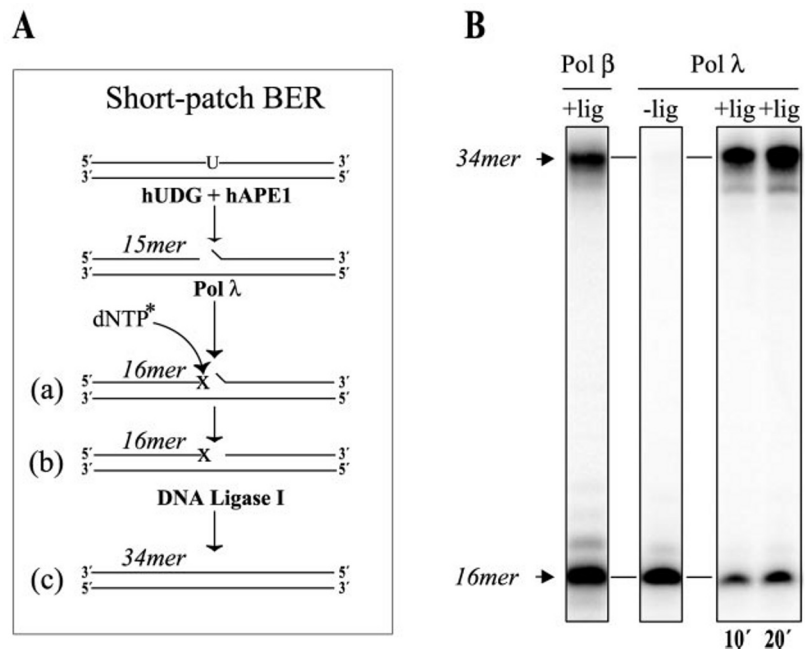


FIG. 2. **A**, trapping of a Pol λ -DNA complex using sodium borohydride. Reactions were performed as described under “Experimental Procedures,” using 50 nM (+) Pol β , 50 nM (+) Pol μ , 20, 50 (+), and 100 nM concentration of wild-type Pol λ or 50 nM K310A Pol λ mutant (*mut*), and in the presence of either 20 mM NaBH₄ or NaCl (as indicated). **B**, AP lyase activity. 20-Min reactions were carried out as described under “Experimental Procedures,” using 20 and 200 nM Pol β or 20, 40, 80, and 400 nM wild-type Pol λ .

FIG. 3. **In vitro** reconstitution of single-nucleotide BER. **A**, schematic representation of a reconstituted BER reaction, indicating the different products formed. **B**, autoradiogram illustrating the products observed during an *in vitro* BER reaction with Pol β or Pol λ . Reactions were carried out as described under “Experimental Procedures.”



and Pol β (Fig. 1A) suggests that the Pol λ residue Lys³¹⁰ may be the nucleophile responsible for the Schiff-base formation during dRP lyase catalysis. Consequently, a single mutation at Lys³¹⁰ (K310A) of Pol λ , although not affecting its DNA polymerization capacity (not shown), reduced dRP lyase activity to less than 10% of that observed with wild-type Pol λ (Fig. 1C). As it has been suggested previously (24), Schiff-base formation likely involves a preferred nucleophile that, albeit with lower efficiency, could be substituted by other nearby alternate nucleophiles, thus accounting for the residual activity of the mutant. Accordingly, as described for the Pol β K72A mutant (23), Schiff-base intermediate of the Pol λ K310A mutant was trapped with very low efficiency compared with the wild-type (Fig. 2A). These results implicate Lys³¹⁰ as the preferred Pol λ nucleophile involved in Schiff-base formation.

Pol λ Exhibits No AP Lyase Activity—Pol β has an intrinsic AP lyase activity, *i.e.* it is able to incise DNA on the 3' side of an unincised AP site (41). AP lyase has been proposed to involve the same reaction mechanism as dRP lyase, suggesting involvement of the same catalytic residues (42). Therefore, we tested Pol λ for AP lyase activity. Fig. 2B shows that, unlike Pol β , Pol λ did not exhibit AP lyase activity. However, similar amounts of enzyme were proficient in both DNA polymerization and dRP excision (data not shown). Given the structural and functional similarities between Pol λ and Pol β , the presence or absence of AP lyase activity can be considered as a feature distinguishing these enzymes.

In Vitro BER Reconstitution with Pol λ —Removal of a dRP residue is an essential step for completion of single-nucleotide BER. Pol β is able to efficiently promote *in vitro* BER of a uracil-containing duplex DNA in the presence of hUDG, hAPE, and DNA ligase I (43, 44). After hUDG and hAPE action, the substrate is accessible for a DNA repair polymerase. Thus, as shown in Fig. 3, two main products are observed in a Pol β -based reconstituted BER reaction. 1) A 16-mer product is generated by a single nucleotide insertion (labeled) at the 3' terminus of the 5'-incised AP site without ligation. This product can correspond to two different repair intermediates (Fig. 3), in which the dRP residue is not (*a*) or is (*b*) excised. 2) A 34-mer product is also generated that corresponds to the complete repair of the DNA strand (*c*). This product, that originates from the 16-mer (*b*) product, requires that the dRP group be excised and strictly depends on DNA ligase addition (data not shown). We examined whether Pol λ could substitute for Pol β to promote repair of a BER intermediate. As shown in Fig. 3B, addition of Pol λ and DNA ligase I also produced the same 16-mer- and 34-mer-labeled products. Therefore, Pol λ was able to coordinate both gap-filling and dRP excision steps of repair preceding DNA ligase action. These data are consistent with a role for human Pol λ in BER.

Although Pol β is a crucial enzyme for single-nucleotide BER, it is well known that in its absence, single-base repair activity can be observed (18, 45). This implies that proteins other than Pol β exist in mammalian cells that are able to conduct single-nucleotide BER. As it has been shown recently, the dRP lyase activity of Pol β , and not its DNA polymerization capacity, determines the sensitivity of Pol β -null fibroblasts to monofunctional DNA-methylating agents (16). This highlights the importance of the dRP removal step *in vivo* and suggests that a dRP lyase-containing enzyme like Pol λ could participate to some extent in single-nucleotide BER *in vivo*.

Implications—This work describes a novel dRP lyase activity associated with DNA Pol λ , a β -like polymerase. Pol λ is the fourth polymerase in human cells, along with DNA polymerases β , γ , and ι , to exhibit this activity. Furthermore, as demonstrated for Pol γ (27, 28) and ι (29), Pol λ can also

substitute for Pol β in an *in vitro* BER reaction. These results suggest that Pol λ could participate in some form of BER *in vivo*. DNA polymerase β is the main DNA polymerase involved in BER of lesions generated by monofunctional alkylating agents in nuclear DNA in eukaryotic cells (17). The three other polymerases may be involved in other types of BER that are differentiated by lesion or location in the cell. For example, the biochemical properties of pol ι suggest that it could be involved in repairing uracil present in DNA due to incorporation of dUTP during DNA replication or in maintaining the stability of G-C base pairs upon deamination of 5-methyl-C (29). DNA polymerase γ is the only DNA polymerase found in mitochondria, suggesting that it may conduct BER of damaged mitochondrial DNA (27, 28). It is also possible that the relative contribution to BER of DNA polymerases with intrinsic dRP lyase activities may depend on their expression levels, perhaps related to different stages of the cell cycle or particular developmental processes or cell types. For example, Pol λ may play an active role in DNA repair synthesis in spermatogenic cells (30), where high levels of BER activity have been described (46, 47). Based on unique features such as its BRCT domain that likely mediates specific protein-protein interactions, Pol λ may be recruited to specific BER subpathways that are not accessible to Pol β .

Interestingly, we have recently observed that Pol λ performs a limited but significant strand displacement synthesis on gapped DNA substrates,² a capacity that would be essential to allow the participation of Pol λ in long patch BER. This issue, however, remains to be ascertained. Being or not restricted to certain tissues or specific transactions, the participation of Pol λ during BER should be further investigated and considered when trying to get a full picture of DNA repair.

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REFERENCES

- Lindahl, T., and Wood, R. D. (1998) *Science* **286**, 1897–1905
- Lindahl, T. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 3649–3653
- Krokan, H. E., Nilsen, H., Skorpen, F., Otterlei, M., and Slupphaug, G. (2000) *FEBS Lett.* **476**, 73–77
- Memisoglu, A., and Samson, L. (2000) *Mutat. Res.* **451**, 39–51
- Norbury, C. J., and Hickson, I. D. (2001) *Annu. Rev. Pharmacol. Toxicol.* **41**, 367–401
- Hoelijmakers, J. H. (2001) *Nature* **411**, 366–374
- McCullough, A. K., Dodson, M. L., and Lloyd, R. S. (1999) *Annu. Rev. Biochem.* **68**, 255–285
- Kane, C. M., and Linn, S. (1981) *J. Biol. Chem.* **256**, 3405–3414
- Demple, B., Herman, T., and Chen, D. S. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 11450–11454
- Robson, C. N., and Hickson, I. D. (1991) *Nucleic Acids Res.* **19**, 5519–5523
- Matsumoto, Y., Kim, K., and Bogenhagen, D. F. (1994) *Mol. Cell. Biol.* **14**, 6187–6197
- Frosina, G., Fortini, P., Rossi, O., Carrozzino, F., Raspaglio, G., Cox, L. S., Lane, D. P., Abbondandolo, A., and Dogliotti, E. (1996) *J. Biol. Chem.* **271**, 9573–9578
- Beard, W. A., and Wilson, S. H. (2000) *Mutat. Res.* **460**, 231–244
- Matsumoto, Y., and Kim, K. (1995) *Science* **269**, 699–702
- Podlutzky, A. J., Dianova, I. I., Wilson, S. H., Bohr, V. A., and Dianov, G. L. (2001) *Biochemistry* **40**, 809–813
- Sobol, R., Prasad, R., Evenski, A., Baker, A., Yang, X., Horton, J., and Wilson, S. H. (2000) *Nature* **405**, 807–810
- Sobol, R. W., Horton, J. K., Kühn, R., Gu, H., Singhal, R. K., Prasad, R., Rajewsky, K., and Wilson, S. H. (1996) *Nature* **379**, 183–186
- Fortini, P., Pascucci, B., Parlanti, E., Sobol, R. W., Wilson, S. H., and Dogliotti, E. (1998) *Biochemistry* **37**, 3575–3580
- Ochs, K., Sobol, R. W., Wilson, S. H., and Kaina, B. (1999) *Cancer Res.* **59**, 1544–1551
- Nealon, K., Nicholl, I. D., Kenny, M. K. (1996) *Nucleic Acids Res.* **24**, 3763–3770
- Dianov, G. L., Prasad, R., Wilson, S. H., and Bohr, V. A. (1999) *J. Biol. Chem.* **274**, 13741–13743
- Podlutzky, A. J., Dianova, I. I., Podust, V. N., Bohr, V. A., and Dianov, G. L. (2001) *EMBO J.* **20**, 1477–1482
- Pierson, C. E., Prasad, R., Wilson, S. H., and Lloyd, R. S. (1996) *J. Biol. Chem.* **271**, 17811–17815

24. Prasad, R., Beard, W. A., Chyan, J. Y., Maciejewski, M. W., Mullen, G. P., and Wilson, S. H. (1998) *J. Biol. Chem.* **273**, 11121–11126
25. Matsumoto, Y., Kim, K., Katz, D. S., and Feng, J. (1998) *Biochemistry* **37**, 6456–6464
26. Deterding, L. J., Prasad, R., Mullen, G. P., Wilson, S. H., and Tomer, K. B. (2000) *J. Biol. Chem.* **275**, 10463–10471
27. Longley, M. J., Prasad, R., Srivastava, D. K., Wilson, S. H., and Copeland, W. C. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 12244–12248
28. Pinz, K. G., and Bogenhagen, D. F. (2000) *J. Biol. Chem.* **275**, 12509–12514
29. Bebenek, K., Tissier, A., Frank, E. G., McDonald, J. P., Prasad, R., Wilson, S. H., Woodgate, R., and Kunkel, T. (2001) *Science* **291**, 2156–2159
30. García-Díaz, M., Domínguez, O., López-Fernández, L., Lain de Lera, T., Saniger, M. L., Ruiz, J. F., Párraga, M., García-Ortiz, M. J., Kirchhoff, T., del Mazo, J., Bernad, A., and Blanco, L. (2000) *J. Mol. Biol.* **301**, 851–867
31. Bork, P., Hofmann, K., Bucher, P., Neuwald, A. F., Altschul, S. F., and Koonin, E. V. (1997) *FASEB J.* **11**, 68–76
32. Zhang, X. D., Morera, S., Bates, P. A., Whitehead, P. C., Coffey, A. I., Hainbucher, K., Nash, R. A., Sternberg, M. J. E., Lindahl, T., and Freemont, P. S. (1998) *EMBO J.* **17**, 6404–6411
33. Aoufouchi, S., Flatter, E., Dahan, A., Faili, A., Bertocci, B., Storek, S., Delbos, F., Cocea, L., Gupta, N., Weill, J. C., and Reynaud, C. A. (2000) *Nucleic Acids Res.* **28**, 3684–3693
34. Nagasawa, K., Kitamura, K., Yasui, A., Nimura, Y., Ikeda, K., Hirai, M., Matsukage, A., and Nakanishi, M. (2000) *J. Biol. Chem.* **275**, 31233–31238
35. Oliveros, M., Yañez, R. J., Salas, M. L., Salas, J., Viñuela, E., and Blanco, L. (1997) *J. Biol. Chem.* **272**, 30899–30910
36. Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., and Kraut, J. (1994) *Science* **264**, 1891–1903
37. Sawaya, M. R., Prasad, R., Wilson, S. H., Kraut, J., and Pelletier, H. (1997) *Biochemistry* **36**, 11205–11215
38. Prasad, R., Beard, W. A., and Wilson, S. H. (1994) *J. Biol. Chem.* **269**, 18096–18101
39. Domínguez, O., Ruiz, J. F., Lain de Lera, T., García-Díaz, M., González, M. A., Kirchhoff, T., Martínez-A, C., Bernad, A., and Blanco, L. (2000) *EMBO J.* **19**, 1731–1742
40. Maciejewski, M. W., Liu, D., Prasad, R., Wilson, S. H., and Mullen, G. P. (2000) *J. Mol. Biol.* **296**, 229–253
41. Prasad, R., Beard, W. A., Strauss, P. R., and Wilson, S. H. (1998) *J. Biol. Chem.* **273**, 15263–15270
42. Piersen, C. E., McCullough, A. K., and Lloyd, R. S. (2000) *Mutat. Res.* **459**, 43–53
43. Nicholl, I. D., Nealon, K., and Kenny, M. (1997) *Biochemistry* **36**, 7557–7566
44. Srivastava, D. K., Vande Berg, B. J., Prasad, R., Molina, J. T., Beard, W. A., Tomkinson, A. E., and Wilson, S. H. (1999) *J. Biol. Chem.* **273**, 21203–21209
45. Stucki, M., Pascucci, B., Parlanti, E., Fortini, P., Wilson, S. H., Hübscher, U., and Dogliotti, E. (1998) *Oncogene* **17**, 835–843
46. Intano, G. W., McMahan, C. A., Walter, R. B., McCarrey, J. R., and Walter, C. A. (2001) *Nucleic Acids Res.* **29**, 1366–1372
47. Olsen, A. K., Bjortuft, H., Wiger, R., Holme, J., Seeberg, E., Bjaras, M., and Brunborg, G. (2001) *Nucleic Acids Res.* **29**, 1781–1790