Error-free bypass of 2-hydroxyadenine by human DNA polymerase \( \lambda \) with Proliferating Cell Nuclear Antigen and Replication Protein A in different sequence contexts

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ABSTRACT

1,2-dihydro-2-oxoadenine (2-OH-A), a common DNA lesion produced by reactive oxygen species, is a strong replicative block for several DNA polymerases (DNA pols). We have previously shown that various bases can be misincorporated opposite the 2-OH-A lesion and the type of mispairs varies with either the sequence context or the type of DNA pol tested. Here, we have analysed the ability of the human pol family X member DNA pol \( \lambda \), to bypass the 2-OH-A lesion. DNA pol \( \lambda \) can perform error-free bypass of 2-OH-A when this lesion is located in a random sequence, whereas in a repeated sequence context, even though bypass was also largely error-free, misincorporation of dGMP could be observed. The fidelity of translesion synthesis of 2-OH-A in a repeated sequence by DNA pol \( \lambda \) was enhanced by the auxiliary proteins Proliferating Cell Nuclear Antigen (PCNA) and Replication Protein A (RP-A). We also found that the DNA pol \( \lambda \) active site residue tyrosine 505 determined the nucleotide selectivity opposite 2-OH-A. Our data show, for the first time, that the 2-OH-A lesion can be efficiently and faithfully bypassed by a human DNA pol \( \lambda \) in combination with PCNA and RP-A.

INTRODUCTION

DNA is modified by many mutagens, including reactive oxygen species (ROS) (1). When ROS react with DNA, various kinds of modified base and/or sugar moieties are produced. The most common lesion is 7,8-dihydro-8-oxoguanine (8-oxo-G), which is highly mutagenic in bacterial and mammalian cells, due to its well-known miscoding potential leading to frequent G->T transversions (2). The 1,2-dihydro-2-oxoadenine (2-OH-A) is another common DNA lesion produced by ROS. 2-OH-A possesses significant mutagenic potential in living cells (3,4). Replication in bacteria or mammalian cells of shuttle vectors containing a single 2-OH-A produces a broad spectrum of mutations. Mutation analysis indicated that a significant fraction of the oxidation-related mutations occur at A:T base pairs. The mismatch repair (MMR) enzyme MYH, the MutY homolog which excises A incorporated opposite DNA 8-oxoG, also removes 2-OH-A from 2-OH-A:G base pairs. It has been previously shown that overexpression of hMTH1 in MMR-defective mouse and human cells reduces the level of DNA 8-oxoG and significantly attenuates their characteristic mutator phenotype (5). Mutation and microsatellite instability analysis indicated that a significant fraction of the oxidation-related mutations that were subject to correction by MMR occurred at A:T base pairs (5). In particular, AT->TA, AT->GC mutations and frameshifts in runs of As were all affected. Since hMTH1 acts on both 2-OH-dATP and 8-oxoGTP (6), its expression could influence mutation by either of the oxidized purines, suggesting that DNA 2-OH-A might make a significant contribution to the mutational burden.

No structural information for incorrect base pairs involving 2-OH-A are available, however thermodynamic analysis showed that 2-OH-A forms stable base pairs with T, C and G, and, to a lesser extent with A (3,7,8). Moreover, the presence of the 2-hydroxy and 1,2-dihydro-2-oxo tautomers, and the possible presence of the syn and anti conformers, may lead to various types of base pairs opposite 2-OH-A. Accordingly, when challenged with a 2-OH-A lesion on the template, DNA polymerases (DNA pols), beside correctly incorporating T, often misinsert G and C nucleotides, with various efficiencies depending upon the sequence context. So far only few DNA pols...
have been studied in detail with 2-OH-A (3,8–12). It has been shown that the replicative enzyme DNA pol α has greatly reduced incorporation efficiency opposite a 2-OH-A, suggesting that 2-OH-A, contrary to 8-oxo-G, might constitute a block for DNA replication, requiring some specialized DNA pol to be bypassed (8). Among translesion synthesis (TLS) DNA pols of the Y family, data are available only for human DNA pol η and for the archaeal enzyme Dpo4, a homologue to human DNA pol κ. Bypass of 2-OH-A by both enzymes was mutagenic, leading to AT->GC transitions and to AT->TA transitions, respectively (8). Finally, the major DNA repair enzyme DNA pol β of family X, was shown to catalyse significant error-prone TLS in the presence of 2-OH-A (11).

DNA pol λ belongs to the DNA pol family X, together with DNA pol β, μ and TdT (13). We have recently shown that DNA pol λ is very efficient in performing error-free TLS past an 8-oxo-G, and its fidelity and efficiency is enhanced several-fold by the auxiliary proteins PCNA and RP-A, both for normal and translesion synthesis (14–18). These results led us to hypothesize that DNA pol λ might be the principal enzyme involved in error-free bypass of oxidized bases. In this work we have analysed the 2-OH-A bypass in either a random sequence or in a A-run, in the presence of DNA pol λ. Polypurine tracts have been shown to reduce the intrinsic fidelity of DNA pol λ (17) as well as to constitute hotspots for oxidative lesions and genomic instability (19). We therefore, assessed the fidelity of the 2-OH-A bypass by DNA pol λ in this highly mutagenic context. Our results suggested that DNA pol λ can perform error-free bypass of 2-OH-A. A specific role of the DNA pol λ active site residue Tyr 505 in determining nucleotide selectivity opposite the lesion was found. Finally, PCNA and RP-A specifically enhanced the fidelity of TLS by DNA pol λ even on ‘difficult’ sequence contexts such as A-runs.

MATERIALS AND METHODS

Oligonucleotide synthesis

Oligonucleotides were synthesized by MWG-Biotech AG. 6-Carboxyfluorescein (6-FAM) labelled and 2-OH-A containing oligonucleotides were synthesized by the Eurogentec S.A. All oligonucleotides were further purified by denaturing polyacrylamide gels (PAGE).

The sequence of the oligonucleotides used was:

A*-31mer 5’GCAAGAACTTATAGATTGACACACAGAGG3’

6A*-36mer 5’GCAAGAACTTATAGAAAAATTGAGCACACAGAGG3’

Bold letters indicate the position of the 2-OH-A lesion. Underlined sequences represent primer annealing sites.

Proteins

Recombinant human DNA pol β was from Trevigen; recombinant human his-tagged DNA pol λ wild type and the Tyr505Ala mutant were cloned, expressed and purified as described (20). Recombinant human PCNA and human RP-A were expressed and purified as described (17). After purification, all the proteins were >90% homogenous, as judged by SDS–PAGE and Coomassie staining.

DNA polymerase assays

For denaturing gel analysis of DNA synthesis products, the reaction mixtures contained 50 mM Tris–HCl (pH 7.0), 0.25 mg/ml BSA, 1 mM DTT and 20 nM (0.2 pmol of 3′ OH ends) of the 6-FAM-labelled primer/template (unless otherwise stated). Concentrations of DNA pol λ, PCNA, RP-A, dNTPs and Mg++ were as indicated in the Figure Legends. Reactions were incubated 10 min at 37°C and then stopped by addition of standard denaturing gel loading buffer (95% formamide, 10 mM EDTA, xylene cyanol and bromophenol blue), heated at 95°C for 3 min and loaded on a 7 M urea/14% polyacrylamide gel.

Fluorescent bands were visualized by Typhoon 9200 Gel Imager (GE Healthcare) and quantitated by ImageQuant TL software.

Kinetic analysis

Experiments were performed under the conditions described earlier using the following nucleotide concentrations (dATP or dGTP or dTTP): 0.1, 0.25, 0.5, 2, 5, 10, 25, 100 μM, respectively. Data points were derived from the analysis of the intensities of the products bands. The values of integrated gel band intensities in dependence of the nucleotide substrate concentrations ([dNTP]) were fitted to the Equation (1):

\[ \sum I_T/I_{T-1} = V_{max}[dNTP]/(K_m + [dNTP]) \]

where \( T \) is the target site, the template position of interest; \( \sum I_T \) = the sum of the integrated intensities at positions \( T, T+1 \ldots T+n \).

Before being inserted in Equation (1), the intensities of the single bands of interest were first normalized by dividing for the total intensity of the lane. This reduced the variability due to manual gel loading. An empty portion of the gel was scanned and the resulting value was subtracted as background.

\( k_{cat} \) values were derived from the relationship: \( V_{max} = k_{cat} [E]_0 \), where [E]₀ is the input enzyme concentration.

Nucleotide incorporation efficiencies were defined as the \( k_{cat}/k_m \) ratio. Under single nucleotide incorporation conditions \( k_{cat} = k_{pol} k_{off}/(k_{pol} + k_{off}) \) and \( K_m = K_k k_{off}/ (k_{pol} + k_{off}) \), where \( k_{pol} \) is the true polymerization rate, \( k_{off} \) is the dissociation rate of the enzyme–primer complex and \( K_k \) is the true Micheli constant for nucleotide binding. Thus, \( k_{cat}/K_m \) values are equal to \( k_{pol}/K_k \).

The goodness of fit of the interpolated curve was assessed by computer-aided calculation of the sum of squares of errors SSE and the correlation coefficient \( R^2 \). Interpolation, SSE, \( R^2 \) and standard errors determination were done with the computer program GraphPadPrism.

RESULTS

DNA pol λ catalyses error-free bypass of 2-OH-A

DNA pol λ was tested on the A’31-mer template containing a single 2-OH-A lesion in the presence of
Next, the 2-OH-A lesion was placed in the middle of a six-mer template (lanes 2–5). The major DNA repair enzyme, DNA pol β, which is closely related to DNA pol λ, catalysed high dGTP misincorporation in front of the lesion (Figure 1D, lane 8). Interestingly, the lesion did not affect the elongation at all by DNA pol β in the presence of all four dNTPs (Figure 1D, compare lane 2 with lane 7). Also the prototypic Y-family enzyme DNA pol η, showed significant misincorporation of dGTP opposite the lesion (Figure 1E, lane 3). In contrast, however, under the same conditions, DNA pol λ did not show misincorporation (Figure 1A). Thus, DNA pol λ can catalyse the error-free dTTP incorporation opposite 2-OH-A, albeit with reduced overall efficiency. On the opposite, DNA pol β catalytic efficiency was not affected by the lesion, but the bypass reaction was error-prone due to the significant dGTP misincorporation.

The fidelity of the 2-OH-A bypass by DNA pol λ is influenced by the sequence context

Next, the 2-OH-A lesion was placed in the middle of a six-A run, a sequence context known to be extremely mutagenic in vitro (21) and in vivo (22). On this 6A36-mer template, in the presence of 1 mM Mg++, DNA pol λ misincorporated dGTP both in front of the lesion and in front of an undamaged template (Figure 2A, lanes 3 and 8). In addition, slippage products of +4 and +5 were observed 6A36-mer templates (lanes 6 and 11). dGTP titration experiments, however, indicated that the efficiency of dGTP misincorporation was comparable on both the control (Figure 2B, lanes 2–5) and damaged (Figure 2B, lanes 7–10) templates. Thus, this sequence context could induce dGTP misincorporation by DNA pol λ also in the presence of the lesion. Moreover, a reduced elongation efficiency of damaged versus undamaged template was observed on this template (Figure 2B, compare lane 1 with lane 6). The relative incorporation efficiencies for DNA pol λ in the different sequence contexts are summarized in Table 1.

The error-free bypass of 2-OH-A placed in an A-run by DNA pol λ is enhanced by PCNA and RP-A

The data so far indicated that DNA pol λ was intrinsically able to perform error-free dTTP incorporation opposite 2-OH-A, but at the expense of a reduced catalytic efficiency. Moreover, when the lesion was placed in an A-run, the overall fidelity of DNA pol λ decreased, leading to significant error-prone translesion synthesis. We have previously shown that the auxiliary proteins PCNA and RP-A are able to increase DNA pol λ catalytic efficiency, fidelity and translesion synthesis (14–18). Thus, PCNA and RP-A effects on the 2-OH-A bypass by DNA pol λ were investigated in the highly mutagenic sequence context of the 6A36-mer template. As shown in Figure 3A, RP-A alone was able to inhibit dGTP incorporation opposite 2-OH-A by DNA pol λ, albeit only partially (compare lanes 1, 2 with lanes 5, 6), whereas dTTP incorporation was not affected (lanes 7–12). When tested alone, PCNA showed a similar effect (Figure 3B), with a slight inhibition of dGTP incorporation (compare lane 1 with lanes 2–4). Most interestingly, however, when PCNA was tested together with RP-A, a strong reduction of error-prone dGTP incorporation (Figure 3B, lanes 5 and 6), but not of the faithful dTTP incorporation (Figure 3C, lanes 2–6) opposite 2-OH-A was observed. Titration experiments confirmed the high selectivity for dTTP (Figure 3D, lanes 1–4) versus dGTP (Figure 3D, lanes 5–8) incorporation opposite the lesion in the presence of PCNA and RP-A. As summarized in Table 1, in the presence of PCNA and RP-A, dTTP incorporation efficiency (k_{cat}/K_{m}) opposite 2-OH-A was increased 2.2-fold, whereas dGTP incorporation was reduced 3.2-fold. As a consequence the bias for dTTP versus dGTP incorporation by DNA pol λ on the 6A36-mer template raised from 23.1 in the absence, to 166 in the presence of PCNA and RP-A (Table 1). Elongation past the lesion by DNA pol λ in the presence of all four dNTPs was also enhanced by PCNA and RP-A (Figure 3D, compare lanes 1, 2 with lanes 6, 7), resulting in a 3.3-fold increase in the corresponding catalytic efficiency (k_{cat}/K_{m}, see Table 1).

The nucleotide selectivity by DNA pol λ during bypass of 2-OH-A is dependent on the residue Tyr505

We have previously shown that the DNA pol λ residue Tyr505 is important for nucleotide discrimination (20), being involved in interactions with the incoming dNTP. We therefore tested the mutant Tyr505Ala in the presence of the 2-OH-A lesion. As shown in Figure 4A, the Tyr505Ala mutant with the A31-mer template showed both dGTP and dATP misincorporation opposite the lesion (lanes 8 and 10). When the same enzyme was tested with the damaged A36-mer template (Figure 4B), dGTP and dATP misincorporations were detected, on both the control and damaged template (Figure 4B, lanes 3, 5 and 8, 10). In addition, on this template slippage products were also observed both in the absence and in the presence of the lesion (Figure 4B, lanes 6 and 11). As shown in Figure 4C, dATP misincorporation could be detected exclusively in front of the lesion with the A31-mer template (lanes 9 and 10). Similar experiments on the same template were carried out for dGTP misincorporation. As shown in Figure 4D, again dGTP misincorporation could be only observed in front of the lesion by the Tyr505Ala (lanes 10 and 11). These data indicated that misincorporation was not due to a reduction in the overall fidelity of DNA pol λ by the Tyr505Ala mutation, since error-free incorporation was observed on the undamaged
Figure 1. DNA pol λ catalyses the error-free bypass of 2-OH-A. Experiments were performed as described in 'Materials and Methods' section in the presence of the A‘31-mer template. A* indicates the position of the labelled 15-mer primer. The sequence of the template strand is indicated on the right side of the panels. (A) Incorporation by DNA pol λ of 10μM of each dNTPs (lane 2) and each 10μM of dGTP (lanes 3, 8); dCTP (lanes 4, 9), dATP (lanes 5, 10) or dTTP (lanes 6, 11) opposite A (lanes 2–6) or 2-OH-A (lanes 7–10). Lane 1, control reaction in the absence of nucleotides. (B) Titration of dTTP in the presence of DNA pol λ on the undamaged (lanes 2–6) or 2-OH-A damaged (lanes 7–10) template. Lane 1, control reaction in the absence of nucleotides. (C) Titration of dNTPs in the presence of DNA pol λ on the 2-OH-A damaged (lanes 2–5) or undamaged (lanes 6–9) template. Lane 1, control reaction in the absence of nucleotides. (D) Incorporation in the presence of 0.5 pmols DNA pol β of each of 100μM dNTPs (lanes 2, 7) and each 100μM of dGTP (lanes 3, 8); dCTP (lanes 4, 9), dATP (lanes 5, 10) or dTTP (lanes 6, 11) opposite A (lanes 2–6) or 2-OH-A (lanes 7–11). Lane 1, control reaction in the absence of nucleotides. (E) Incorporation in the presence of 0.5 pmols DNA pol η of 10μM each of dATP, lane 1, dCTP, lane 2, dGTP, lane 3 or dTTP, lane 4, opposite 2-OH-A. Lane 5, control reaction in the absence of nucleotides.
The presence of the oxidized base 8-oxo-G in the replicating strand has been shown to easily misdirect templates in both cases. Under these conditions, wild-type DNA Pol did not misincorporate either dGTP or dATP (Figure 1A). Thus, the Tyr505Ala mutant showed significantly reduced fidelity specifically for 2-OH-A bypass on the A/C3 template, indicating a key role for this residue in determining nucleotide selection for incorporation opposite this lesion, suggesting an important role of this active site residue in 2-OH-A translesion synthesis.

DISCUSSION

The presence of the oxidized base 8-oxo-G in the replicating strand has been shown to easily misdirect templates in both cases. Under these conditions, wild-type DNA Pol did not misincorporate either dGTP or dATP (Figure 1A). Thus, the Tyr505Ala mutant showed significantly reduced fidelity specifically for 2-OH-A bypass on the A/C3 template, indicating a key role for this residue in determining nucleotide selection for incorporation opposite this lesion, suggesting an important role of this active site residue in 2-OH-A translesion synthesis.

Table 1. Kinetic parameters for Mg²⁺-dependent 2-OH-A bypass by DNA Pol λ

| Template | Control | 2-OH-A |
|----------|---------|--------|
| Nucleotide | K_m (μM) | k_cat (s⁻¹) | k_cat/K_m M⁻¹×s⁻¹ | K_m (μM) | k_cat (s⁻¹) | k_cat/K_m M⁻¹×s⁻¹ |
| dTTP | 0.8±0.1 | 0.06±0.01 | 7.5×10⁴ | 2±0.5 | 0.03±0.003 | 1.5×10⁴ | 0.6±0.1 | 0.04±0.007 | 6.6×10⁴ |
| dGTP | n.a. | n.a. | n.a. | 1.5±0.3 | 0.02±0.002 | 1.3×10⁴ | 0.7±0.1 | 0.05±0.006 | 7.1×10⁴ |
| dNTPs | 0.7±0.1 | 0.04±0.006 | 5.7×10⁴ | 7±0.1 | 0.01 | 10⁴ | 0.6±0.08 | 0.05±0.001 | 8.3×10⁴ |
| dTTP+PCNA/RP-A | 12±1 | 0.06±0.001 | 2×10⁴ | 0.5±0.06 | 0.05±0.007 | 10⁴ |

*Kinetic parameters were derived as described in ‘Materials and Methods’ section. Values are the means of three independent replicates ± SD values.

b n.a., not applicable. No dGTP incorporation was detected in the presence of Mg + + on this template.

cCalculated as the ratio (k_cat/K_m) dTTP/(k_cat/K_m) dGTP.
Figure 3. PCNA and RP-A allow the error-free bypass of 2-OH-A by DNA pol λ. Experiments were performed as described in 'Materials and Methods' section in the presence of the 6A*36-mer template. 6A* indicates the position of the labelled 18-mer primer. The sequence of the template strand is indicated on the right side of the panels. (A) Incorporation opposite 2-OH-A by 0.75 pmols of DNA pol λ of dGTP (lanes 1–6) or dTTP (lanes 7–12), in the absence (lanes 1, 2, 7 and 8) or in the presence (lanes 3–6, 9–12) of RP-A. C, control reaction in the absence of nucleotides. (B) Incorporation opposite 2-OH-A by 0.75 pmols of DNA pol λ of 10 μM dGTP, in the absence (lane 1) or in the presence of PCNA alone (lanes 2–4) or in combination with RP-A (lanes 5, 6). Lane C, control reaction in the absence of nucleotides. (C) Incorporation opposite 2-OH-A by 0.75 pmols of DNA pol λ of 2 μM dTTP, in the absence (lane 1) or in the presence of PCNA alone (lanes 2–4) or in combination with RP-A (lanes 5, 6). Lane C, control reaction in the absence of nucleotides. (D) Incorporation of dTTP (lanes 1–4) or dGTP (lanes 5–8) by 0.75 pmols DNA pol λ in the presence of PCNA and RP-A, opposite 2-OH-A. Lane C, control reaction in the absence of nucleotides. (E) Incorporation of dNTPs by 0.75 pmols DNA pol λ opposite 2-OH-A, in the presence (lanes 1–4) or in the absence (lanes 6–9) of PCNA and RP-A.
nucleotide incorporation by replicative DNA pols, and frequent misincorporation of A opposite 8-oxo-G is likely to occur (2). Another oxidized base, 2-OH-A, is also a potential source of errors by different DNA pols, with A and G being the most frequently misincorporated nucleotides (3,4). A major difference between the two oxidized purines is however that 8-oxo-G lesion does not constitute a block for replicative (or repair) DNA pols, while 2-OH-A causes a serious reduction in the catalytic efficiency of several DNA pols, including the replicative enzyme DNA pol α (8). Thus, this lesion might sensibly slow down replication fork progression and this might constitute the signal to recruit a specialized TLS DNA pol (23). Recent data, also from our laboratories, suggested that the most accurate DNA pols in dealing with 8-oxo-G are DNA pol λ and DNA pol η, in combination with the auxiliary proteins PCNA and RP-A (18,24).

Here, our results strongly suggested that DNA pol λ might also be a prime candidate in correctly coping the 2-OH-A lesion. DNA pol λ is intrinsically able to perform error-free TLS past a 2-OH-A lesion, although with a slight reduction in catalytic efficiency. This ability is not shared by other DNA pols and 2-OH-A produced high error rates when replicated by DNA pols belonging to the B or Y-family.

The structural information on 2-OH-A-containing duplexes indicates that 2-OH-A can assume keto or enol tautomeric forms in variable proportion depending on solvent polarity and neighbouring bases (25). Thus the polarity of the microenvironment within the active site of various polymerases might influence the tautomeric equilibrium of 2-OH-A (26). In particular the fraction of 2-OH-A enol tautomers, which closely resembles A, is favoured by a decrease in solvent polarity.
The active site of DNA pol λ is known to assume a closed conformation, even prior to dNTP binding (27,28), and this less polar microenvironment might increase the enol fraction of 2-OH-A. In addition DNA pol λ shows limited interactions with the template strand and strict geometric requirements confined to the terminal and nascent base pairs. These factors together with the preferred enolic form of 2-OH-A might be responsible for an equivalent use of the oxidized and unmodified A in the pairing with dTTP in a random sequence.

In an attempt to clarify the molecular basis for the high selectivity shown by DNA pol λ for the T:2-OH-A base pair we have analysed the contribution of the critical residue Tyr 505, present in the DNA pol λ nucleotide-binding pocket. Kinetic and structural studies have shown that this residue Tyr 505 plays an important role in dNTP binding (27–30). In the DNA-DNA pol λ binary complex, Tyr 505 obstructs the nucleotide-binding pocket through an hydrogen bond with the N1 of a templating adenine (27,28). In the precatalytic ternary complex, dNTP binding causes a rotation of the Tyr505 side chain, which repositions itself in order to take interactions with the base at the primer terminus. Such an interaction plays a crucial role in nucleotide selectivity, allowing the enzyme to assess proper base pair geometry. We have also shown that Tyr505 acts as a ‘steric gate’ checking the correct size of the nascent base pair (29,30). In this study, we showed that mutant DNA pol λ carrying an Ala instead of a Tyr at position 505, was able to misincorporate both dATP and dGTP opposite a 2-OH-A lesion, at variance with the wild type enzyme, which exclusively incorporated dTTP. These results suggest that the Tyr505 residue in DNA pol λ is the major determinant preventing 2-OH-A-mediated misincorporation. Thus it is possible that the shorter side chain of Ala does not block 2-OH-A in the suitable position for correct base pairing and is unable to sense the altered geometry of the nascent A:2-OH-A or G:2-OH-A mispairs. On particular sequence contexts such as A-runs a reduction in DNA pol λ fidelity has been already reported (17). The presence of 2-OH-A in a repeated sequence does not further worsen the error rate of DNA pol λ. However, due to the highly mutagenic properties of the A-run, significant dGTP misincorporation could be observed by DNA pol λ alone opposite the lesion. The auxiliary proteins PCNA and RP-A efficiently remove these drawbacks, ensuring efficient error-free TLS of a 2-OH-A lesion even in such a ‘difficult’ sequence context. In summary, our findings indicated that DNA pol λ couples flexible substrate recognition properties (being able to bypass a 2-OH-A lesion) to high intrinsic fidelity in TLS, and PCNA and RP-A auxiliary proteins enhance these properties. Both these proteins play essential roles in DNA replication and DNA repair. Moreover, while RP-A is an important ‘sensor’ protein for DNA replication fork stalling (31,32), PCNA has been proposed to coordinate the switching from replicative DNA pols to specialized TLS enzymes (33). DNA pol λ interacts both functionally and physically with PCNA and its activity is modulated in various way by RP-A. DNA pol λ has been implicated in various pathways such as abasic site bypass, base excision repair (34–36) and non-homologous end joining (37,38). The present data show, for the first time, that the 2-OH-A lesion can be efficiently and faithfully bypassed by human DNA pol λ in combination with PCNA and RP-A, suggesting a role of this enzyme in multiple pathways, including the translesion bypass of 2-OH-A.

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