MOLECULAR AND CELLULAR BIOLOGY, May 2001, p. 3558–3563 Vol. 21, No. 10
0270-7306/01/$04.00+0 DOI: 10.1128/MCB.21.10.3558–3563.2001
Copyright © 2001, American Society for Microbiology. All Rights Reserved.

Role of DNA Polymerase η in the Bypass of a (6-4) TT Photoproduct

ROBERT E. JOHNSON, LAJOS HARACSKA, SATYA PRAKASH, AND LOUISE PRAKASH*

Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1061

Received 10 January 2001/Returned for modification 14 February 2001/Accepted 20 February 2001

UV light-induced DNA lesions block the normal replication machinery. Eukaryotic cells possess DNA polymerase η (Polη), which has the ability to replicate past a *cis-syn* thymine-thymine (TT) dimer efficiently and accurately, and mutations in human Polη result in the cancer-prone syndrome, the variant form of xeroderma pigmentosum. Here, we test Polη for its ability to bypass a (6-4) TT lesion which distorts the DNA helix to a much greater extent than a *cis-syn* TT dimer. Opposite the 3′ T of a (6-4) TT photoproduct, both yeast and human Polη preferentially insert a G residue, but they are unable to extend from the inserted nucleotide. DNA Polζ, essential for UV induced mutagenesis, efficiently extends from the G residue inserted opposite the 3′ T of the (6-4) TT lesion by Polη, and Polζ inserts the correct nucleotide A opposite the 5′ T of the lesion. Thus, the efficient bypass of the (6-4) TT photoproduct is achieved by the combined action of Polη and Polζ, wherein Polη inserts a nucleotide opposite the 3′ T of the lesion and Polζ extends from it. These biochemical observations are in concert with genetic studies in yeast indicating that mutations occur predominantly at the 3′ T of the (6-4) TT photoproduct and that these mutations frequently exhibit a 3′ T→C change that would result from the insertion of a G opposite the 3′ T of the (6-4) TT lesion.

The *RAD30* gene of *Saccharomyces cerevisiae* functions in error-free bypass of UV-damaged DNA, and *RAD30*-encoded DNA polymerase η (Polη) replicates through a *cis-syn* thymine-thymine (TT) dimer with the same efficiency and accuracy as through undamaged T's (13, 27). Both yeast and human Polη efficiently insert two A's opposite the two T's of the dimer and extend from the resulting primer (15, 27). In yeast as well as humans, inactivation of Polη results in UV hypermutability (26, 29), and defects in Polη in humans cause the variant form of xeroderma pigmentosum (XP-V) (12, 22). As a consequence of UV hypermutability, XP-V individuals suffer from a high incidence of skin cancers.

Polη is unique among eukaryotic DNA polymerases in its ability to replicate through lesions that distort the DNA helix. Thus, in addition to a *cis-syn* TT dimer, Polη replicates through an 8-oxoguanine (8oxoG) (11) or an O^6-methylguanine (m^6G) lesion (10). Although the template strand is highly distorted in the vicinity of the lesion in the 8oxoG · C base pair, yeast Polη (yPolη) efficiently replicates through this lesion by inserting a C opposite the lesion and then extending from the resulting base pair (11), and Polη bypasses an m^6G lesion by inserting a C or T opposite the lesion (10). The ability of Polη to bypass lesions which distort the DNA helix suggests an unusual tolerance of its active site for geometric distortions in DNA. Consistent with this idea, both yPolη and human Polη (hPolη) are low-fidelity enzymes, misincorporating nucleotides opposite nondamaged template bases with a frequency of 10^{-2} to 10^{-3} (15, 28).

In addition to *cis-syn* cyclobutane pyrimidine dimers, UV light elicits the formation of pyrimidine (6-4) pyrimidinone photoproducts. By contrast to a *cis-syn* TT dimer, which has only a modest effect on DNA structure and which does not affect the ability of the two T's in the dimer to base pair with A's, a (6-4) TT photoproduct induces a large structural distortion in DNA, and the 3′ T in the (6-4) lesion is oriented perpendicular to the 5′ T (17). Nuclear magnetic resonance studies have, however, indicated that the 3′ T in the (6-4) lesion can hydrogen bond with a G residue (20). Here we test the ability of Polη to replicate through a (6-4) TT lesion. We find that although Polη does not bypass this lesion, it is nevertheless able to insert a G residue opposite the 3′ T of the lesion. DNA Polζ, essential for the mutagenic bypass of DNA lesions, efficiently extends from the resulting base pair by incorporating the correct nucleotide A opposite the 5′ T of the lesion. Thus, the sequential action of DNA polymerases η and ζ coordinates the mutagenic bypass of a (6-4) TT lesion.

MATERIALS AND METHODS

Enzyme purification. The yeast *RAD30* and human *RAD30A* genes were cloned in frame with the glutathione *S*-transferase (GST) gene in the overexpression plasmid pBJ760 (14), generating plasmids pBJ763 and pBJ765, respectively. Yeast and human Polη were purified from *S. cerevisiae* BJ5464 harboring either pBJ763 or pBJ765 as described elsewhere (13, 15), except that protein was batch eluted from glutathione-Sepharose 4B by cleavage of the GST tag on Polη by treatment with thrombin for 4 h at 4°C. Cleavage of the GST portion by thrombin leaves an eight-amino-acid leader peptide on the N terminus of yeast and human Polη. All subsequent purification steps were done as described elsewhere (13, 15).

yPolζ was purified from *S. cerevisiae* Sc334 harboring plasmid pGST-REV3 and pREV7 as described previously (14) except that a Mini-Q column step was added. Protein eluted from glutathione-Sepharose 4B was dialyzed against buffer A (25 mM $NaPO_4$ [pH 7.4], 100 mM NaCl, 10% glycerol, 0.01% NP-40, 5 mM dithiothreitol, 0.5 mM EDTA), loaded onto a Mini-Q PC 2.3/3 column (Pharmacia), and washed with 20 column volumes buffer A before elution of the protein with a 2.4-ml 100 to 500 mM NaCl gradient in buffer A. The GST-Rev3/Rev7-containing fractions were pooled and concentrated in buffer A containing 200 mM NaCl and 50% glycerol and stored at −20°C.

DNA substrates. The 75-nucleotide (nt) template (5′-AGCAAGTCA CCAA TGTCT AAGAGTTCG TATTATGCC TACACTGGA GTACCGGAG CAT

* Corresponding author. Mailing address: Sealy Center for Molecular Science, University of Texas Medical Branch, 6.104 Medical Research Building, 11th & Mechanic St., Galveston, TX 77555-1061. Phone: (409) 747-8601. Fax: (409) 747-8608. E-mail: lprakash@scms.utmb.edu.

Downloaded from http://mcb.asm.org/ on October 28, 2020 by guest

CGTCGT GACTGGGAA AAC-3′) either containing or not containing a (6-4) TT photoproduct at the underlined position was derived from the 10-nt oligomer 5′ CGTATTATGC 3′ by ligation to flanking 25- and 40-nt oligomers. The (6-4) TT photoproduct was introduced into the 10-nt oligomer by irradiation with 254-nm-wavelength UV light and was purified by high-pressure liquid chromatography. The (6-4) TT photoproduct was identified by its unique absorption at 326-nm light (19). For bypass assays and for steady-state kinetic analyses of insertion of nucleotides opposite the 3′ T of the (6-4) photoproduct or a nondamaged T residue, the primer N4309 (5′ GTTTTCCCAG TCACGACGAT GCTCCGGTAC TCCAGTGTAG GCAT 3′) was annealed to the 75-nt template. For steady-state kinetic analyses of primer extension from an A or a G residue opposite the 3′ T of the (6-4) photoproduct in the template, oligomers 5′ GTTTTCCCAG TCACGACGAT GCTCCGGTAC TCCAGTGTAG GCATA 3′ and 5′ GTTTTCCCAG TCACGACGAT GCTCCGGTAC TCCAGTGTAG GCATG 3′ respectively, were used.

DNA polymerase assays. For synthesis assays on damaged and nondamaged DNAs (Fig. 1), the standard DNA polymerase reaction (5 μl) was used; the mixture contained 25 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol, 100 μg of bovine serum albumin/ml, 10% glycerol, 100 μM each deoxynucleoside triphosphate (dNTP) (dGTP, dATP, dTTP, and dCTP), 10 nM 5′-^{32}P-labeled oligonucleotide primer annealed to an oligonucleotide DNA template, 1 nM yeast or human Polη, and 1.8 nM yPolζ. Reactions were carried out at 37°C for 5 min and terminated by the addition of 50 mM EDTA. DNA products were precipitated with 6 volumes of ice-cold acetone, dried under vacuum, resuspended in loading buffer (95% formamide, 0.05% cyanol blue, 0.05% bromophenol blue), and then resolved on 10% polyacrylamide gels containing 8 M urea. Gels were dried before autoradiography at −70°C.

Steady-state kinetic analyses. To determine the efficiency and fidelity of deoxynucleotide incorporation by yeast and human Polη at the 3′ T of a (6-4) TT photoproduct or an undamaged T, the standard DNA polymerase assay was employed except that 0.5 nM hPolη or yPolη was used, and only a single deoxynucleotide was included at the concentrations indicated in the figures and figure legends. Reactions were carried out at 30°C for 5 min. Gel band intensities of the substrate and products of the deoxynucleotide incorporation reactions were quantitated using a PhosphorImager and ImageQuant software (Molecular Dynamics). The observed rate of deoxynucleotide incorporation, v_{obs}, was determined by dividing the amount of product formed by the reaction time. v_{obs} was plotted as a function of the deoxynucleotide concentration, and the data were fit to the Michaelis-Menten equation describing a hyperbola: $v_{obs} = (V_{max} \times [dNTP])/(K_m + [dNTP])$. From the best fit curve, the apparent K_m and V_{max} steady-state kinetic parameters for the incorporation of each deoxynucleotide were obtained and used to calculate the relative efficiency of deoxynucleotide incorporation, f_{inc}, using the following equation:

$$f_{inc} = (V_{max}/K_m)_{G,\ A,\ T,\ or\ C} / (V_{max}/K_m)_A \quad (7, 9, 24).$$

To determine the efficiency of nucleotide incorporation at the 5′ T of the (6-4) TT photoproduct or an undamaged TT sequence by Polζ, following a correctly base-paired or mispaired primer terminus, the standard DNA polymerase assay was used except that reaction mixtures contained 40 mM Tris-HCl (pH 7.5), 5 nM Polζ, and 20 nM DNA substrate, and the reactions were carried out for 3 min at 30°C. The dNTP concentration was varied from 0 to 25 μM for dATP and from 0 to 500 μM for dGTP, dTTP, or dCTP.

RESULTS

Bypass of a (6-4) TT photoproduct by the combined action of Polη and Polζ. The bypass of a (6-4) TT photoproduct was examined in standing start reactions using a 75-nt template containing the lesion 45 nt from the 3′ end and primed with a 5′-^{32}P-labeled 44-nt oligomer. As shown in Fig. 1, neither yeast or human Polη could replicate through the (6-4) TT lesion. Both polymerases, could insert a deoxynucleotide opposite the 3′ T of the lesion (Fig. 1, lanes 5 and 6), but neither could extend past this site. Extensive genetic studies in yeast have indicated the requirement of Polζ in the mutagenic bypass of DNA lesions, including those induced by UV light. Polζ, however, does not bypass the (6-4) TT photoproduct; moreover, it does not even insert a nucleotide opposite the 3′ T of the lesion (Fig. 1, lane 7). Efficient bypass of the (6-4) TT lesion, however, occurs when Polη is combined with Polζ (Fig. 1, lanes 8 and 9).

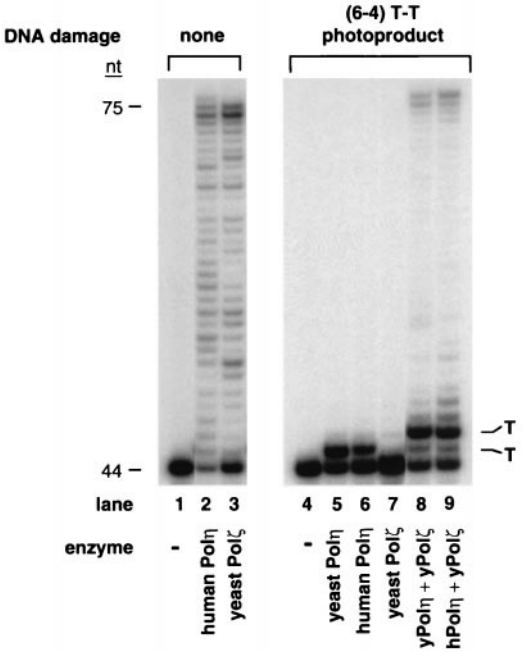

FIG. 1. Bypass of the (6-4) TT photoproduct by the combined action of Polη and Polζ. Lanes 1 to 3, undamaged DNA; lanes 4 to 9, (6-4) TT photoproduct-containing DNA. Positions of the two T's in the undamaged or the (6-4) TT photoproduct-containing template are indicated on the right. hPolη (1 nM), yPolη (1 nM), yPolζ (1.8 nM), or either yeast or human Polη combined with yPolζ was incubated with the DNA substrate for 5 min at 37°C in the presence of 100 μM each of the four dNTPs.

Preferential incorporation of a G residue opposite the 3′ T of the (6-4) TT photoproduct by human and yeast Polη. To examine the efficiency (V_{max}/K_m) of nucleotide incorporation opposite the 3′ T of the (6-4) lesion by human and yeast Polη, we measured the kinetics of insertion for each deoxynucleotide under steady-state conditions. Figure 2 shows the incorporation pattern of each deoxnucleotide by human Polη opposite a nondamaged T residue (Fig. 2A) and opposite the 3′ T of the (6-4) TT photoproduct (Fig. 2B). Opposite the nondamaged T residue, hPolη inserts the correct A residue with a high efficiency (Table 1). Relative to the incorporation of A, hPolη misincorporates a G, a T, or a C opposite the nondamaged T template with frequencies of $\sim 3 \times 10^{-3}$ to 1×10^{-2}. However, opposite the 3′ T the (6-4) TT lesion, hPolη is almost 450-fold less efficient at incorporating an A than on the equivalent T residue in the nondamaged template, and T and C are incorporated even less well than an A opposite the 3′ T of the lesion. hPolη preferentially inserts a G opposite the 3′ T of the (6-4) TT lesion, as hPolη is eightfold more efficient at inserting a G opposite the 3′ T of the (6-4) TT lesion than it is at inserting an A opposite this site (Table 1). Relative to the insertion of a G or an A opposite the nondamaged T template, hPolη inserts a G opposite the 3′ T of the (6-4) TT lesion about 2-fold better or 55-fold less well, respectively (Table 1). The insertion of G opposite the 3′ T of this lesion by hPolη has been reported, but in the absence of any kinetic analyses, the efficiency with which hPolη inserted this or other nucleotides could not be evaluated

Downloaded from http://mcb.asm.org/ on October 28, 2020 by guest

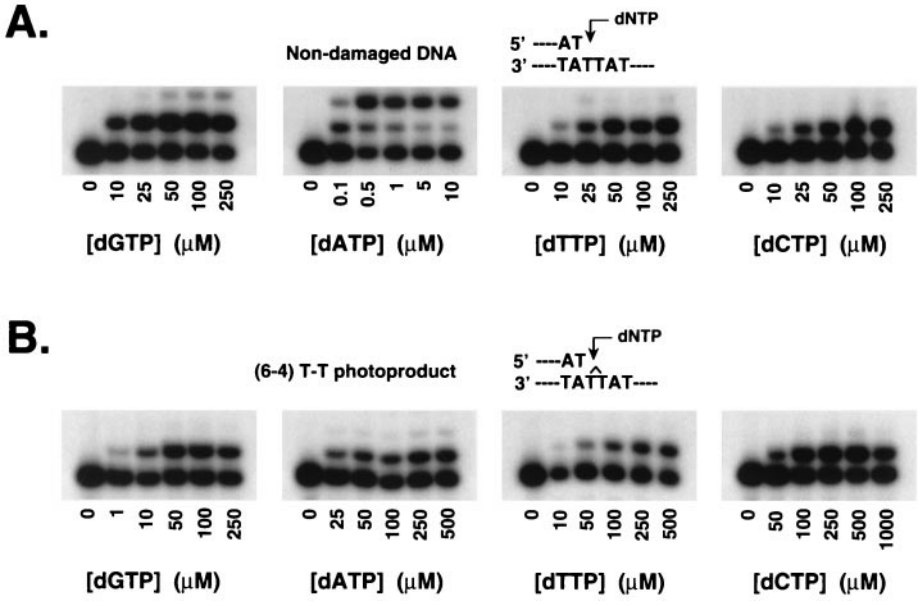

FIG. 2. Nucleotide incorporation by hPolη opposite the 3′ T residue in a nondamaged template or a (6-4) TT photoproduct-containing template. (A) Incorporation of nucleotides opposite the nondamaged T residue; (B) incorporation opposite the equivalent 3′ T residue of a (6-4) TT photoproduct. A portion of each primer:template substrate is shown at the top. hPolη (0.5 nM) was incubated with DNA substrate (10 nM) and the indicated concentrations of dNTPs for 5 min at 30°C.

(31). yPolη is also highly inefficient at inserting an A opposite the 3′ T of the (6-4) TT lesion, and compared to the insertion of an A opposite nondamaged T, yPolη incorporates an A opposite the 3′ T of the (6-4) lesion over 700-fold less well (Table 2). yPolη also preferentially inserts a G residue opposite the 3′T of the 6-4 lesion, and the yeast enzyme is 1.5-fold more or ~100-fold less efficient at inserting a G opposite the lesion than it is at inserting a G or an A opposite the nondamaged T, respectively (Table 2).

Nucleotide incorporation opposite the 5′ T of the (6-4) TT photoproduct by Polζ. Since Polζ is able to extend from the nucleotide inserted by Polη opposite the 3′ T of the (6-4) TT lesion (Fig. 1, lanes 8 and 9), we next examined the relative efficiency of each nucleotide incorporation by Polζ opposite the 5′ T of the nondamaged or (6-4) TT photoproduct using steady-state kinetic assays. Since Polη preferentially inserts a G residue opposite the 3′ T of the (6-4) TT lesion, we compared the efficiency of incorporation of nucleotides following a primer in which a G (Fig. 3A) or an A (Fig. 3B) is paired with the 3′ T of the lesion. When extending from the A · T or the G · T base pair on nondamaged TT sequence, Polζ incorporates the correct A opposite the 5′ T with high efficiency and misincorporates nucleotides with a frequency of ~10^{-3} to 10^{-4} (Table 3). Polζ, however, is somewhat more accurate in inserting nucleotides opposite the 5′ T of the (6-4) TT photoproduct when extending from a G opposite the 3′ T of the lesion than when extending from an A opposite the 3′ T of the lesion (Table 3). Furthermore, Polζ is about threefold more efficient at incorporating an A when G is paired with the 3′ T of the (6-4) lesion than when an A is paired with the 3′ T of the lesion and is almost fourfold more efficient at incorporating an A following the G opposite the (6-4) lesion than from the G · T base pair in the nondamaged template (Table 3).

DISCUSSION

Biochemical studies with yeast and human Polη have indicated a role for this enzyme in the error-free bypass of a *cis-syn* TT dimer. Both enzymes replicate through this lesion by inserting As opposite the two T's of the dimer, and they do so

TABLE 1. Steady-state kinetic parameters for nucleotide incorporation opposite the 3′T of the (6-4) TT photoproduct by hPolη

Template	Template nucleotide	Incoming residue	V_{max} (nM/min)	K_m (μM)	V_{max}/K_m	f_{inc}
Nondamaged	T	G	1.4 ± 0.06	9.4 ± 2.1	0.15	9.5×10^{-3}
		A	1.1 ± 0.05	0.07 ± 0.02	15.7	1
		T	1.1 ± 0.09	26 ± 7	0.042	2.7×10^{-3}
		C	1.3 ± 0.07	53 ± 8	0.025	1.6×10^{-3}
(6-4) TT	3′ T of (6-4) TT photoproduct	G	0.9 ± 0.02	3.2 ± 0.5	0.28	7.8
		A	0.9 ± 0.005	25 ± 0.6	0.036	1
		T	1.0 ± 0.05	84 ± 12	0.012	0.33
		C	1.3 ± 0.2	89 ± 37	0.015	0.42

Downloaded from http://mcb.asm.org/ on October 28, 2020 by guest

TABLE 2. Steady-state kinetic parameters for nucleotide incorporation opposite the 3′ T of the (6-4) TT photoproduct by yPolη

Template	Template nucleotide	Incoming residue	V_{max} (nM/min)	K_m (μM)	V_{max}/K_m	f_{inc}
Nondamaged	T	G	1.5 ± 0.06	70 ± 7	0.021	7.2×10^{-3}
		A	1.4 ± 0.1	0.48 ± 0.2	2.9	1
(6-4) TT	3′ T of (6-4) T-T photoproduct	G	1.4 ± 0.03	47 ± 4	0.03	7.5
		A	0.8 ± 0.08	180 ± 42	0.004	1

with the same efficiency and fidelity as when replicating through undamaged T's. In addition to the TT dimer, UV light also induces the formation of cyclobutane dimers at the CC and TC sites. Because of the rapid deamination of C to U, in vitro bypass studies with these lesions are difficult to perform; genetic studies in yeast, however, have implicated Polη in the error-free bypass of cyclobutane dimers at the CC and TC sites as well (30).

By contrast to a *cis-syn* cyclobutane pyrimidine dimer, which has only a modest effect on the DNA structure, a (6-4) TT photoproduct induces a large structural distortion, leading to a 44° bend in the DNA helix; moreover, the 3′ T in the (6-4) lesion is held perpendicular to the 5′ T (16). Nuclear magnetic resonance studies have shown that the O_2 carbonyl of the 3′ T in the (6-4) TT lesion cannot hydrogen bond with the amino proton of an opposed A residue (17). The O_2 carbonyl of the 3′ T residue, however, can form hydrogen bonds with the imino and amino protons of the opposed G residue (20). The 5′ T at the (6-4) lesion maintains normal hydrogen bonding interactions with the A residue in the complementary strand (20).

In UV-irradiated DNA, the (6-4) TT photoproduct is formed much less frequently than the *cis-syn* TT dimer; the (6-4) lesion, however, is more mutagenic than the dimer (1, 2, 4, 5). Experiments in *S. cerevisiae* with single-stranded or gapped duplex vectors that carried a *cis-syn* TT dimer or a (6-4) TT photoproduct at a unique site have indicated that by contrast to a *cis-syn* dimer, which is replicated very accurately (0.4% targeted mutations), a (6-4) TT photoproduct induces mutations in 30 to 40% of the replicated plasmid molecules, and as many as 50% of these mutations are 3′ T→C substitu-

Downloaded from http://mcb.asm.org/ on October 28, 2020 by guest

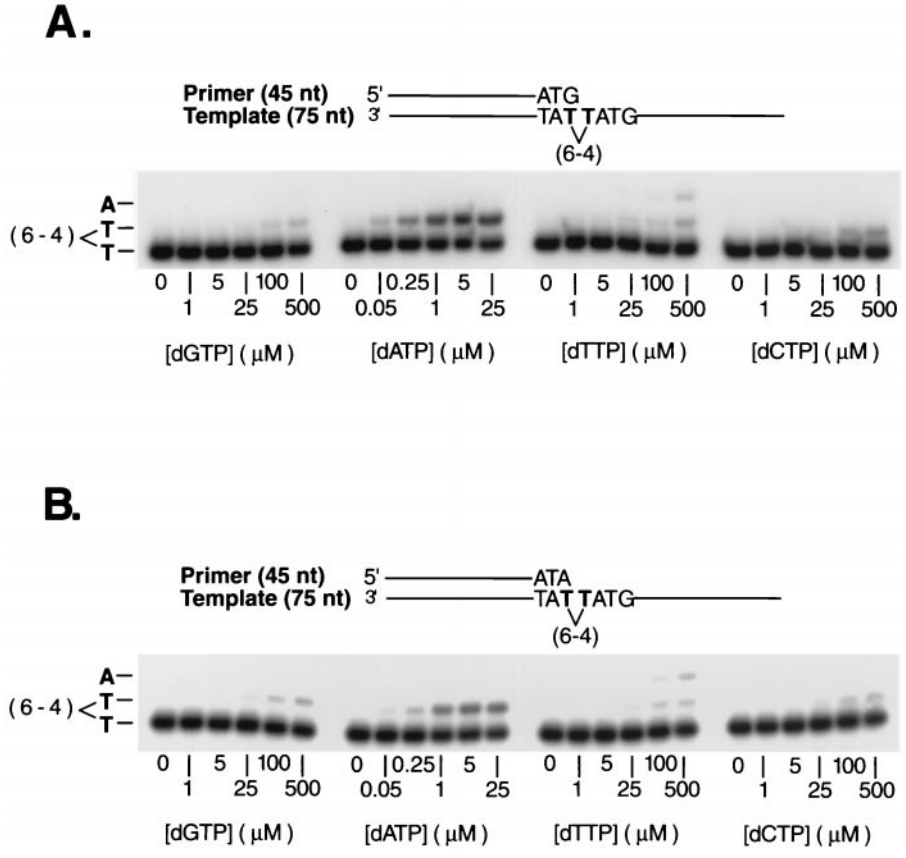

FIG. 3. Nucleotide incorporation opposite the 5′ T of the (6-4) TT photoproduct by yPolζ. (A) Nucleotide incorporation following a G residue opposite the 3′ T of the (6-4) photoproduct; (B) nucleotide incorporation following an A residue opposite the 3′ T of a (6-4) photoproduct. yPolζ (5 nM) was incubated for 3 min at 30°C with the primer:template DNA substrate (20 nM) and with the indicated concentrations of dNTPs.

TABLE 3. Steady-state kinetic parameters for nucleotide incorporation opposite the 5′ T of the (6-4) TT photoproduct by yPolζ

Template	Primer:template base pair	Incoming residue	V_{max} (nM/min)	K_m (μM)	V_{max}/K_m	Relative efficiency[a]
Nondamaged	G·T	G	0.11 ± 0.01	170 ± 33	0.0006	6.0×10^{-4}
		A	0.70 ± 0.05	0.67 ± 0.06	1.0	1
		T	0.38 ± 0.04	130 ± 8	0.0029	2.9×10^{-3}
		C	0.12 ± 0.07	250 ± 56	0.0005	5.0×10^{-4}
	A·T	G	0.13 ± 0.01	140 ± 11	0.0009	1.0×10^{-4}
		A	1.06 ± 0.07	0.12 ± 0.07	8.8	1
		T	0.42 ± 0.02	94 ± 14	0.0045	5.1×10^{-4}
		C	0.34 ± 0.05	140 ± 22	0.0024	2.7×10^{-4}
(6-4) TT	G·T	G	0.13 ± 0.01	130 ± 17	0.001	2.6×10^{-4}
		A	1.2 ± 0.03	0.32 ± 0.05	3.8	1
		T	0.34 ± 0.08	180 ± 32	0.0019	5.0×10^{-4}
		C	0.27 ± 0.03	160 ± 24	0.0017	4.5×10^{-4}
	A·T	G	0.24 ± 0.02	57 ± 10	0.0042	3.0×10^{-3}
		A	0.89 ± 0.04	0.63 ± 0.11	1.4	1
		T	0.43 ± 0.02	130 ± 10	0.0033	2.4×10^{-3}
		C	0.28 ± 0.03	200 ± 34	0.0014	1.0×10^{-3}

[a] For each DNA substrate, the relative efficiency of nucleotide incorporation was determined by dividing the efficiency (V_{max}/K_m) for incorporating each nucleotide by the efficiency (V_{max}/K_m) for incorporating the correct nucleotide, A.

tions (8). Such mutations would arise from the incorporation of a G opposite the 3′ T of the (6-4) TT lesion. In concurrence with these genetic observations, we show here that the bypass of a (6-4) TT lesion is accomplished by the combined action of Polη and Polζ, wherein Polη inserts a G opposite the 3′ T of the (6-4) lesion and Polζ extends from the resulting base pair.

Our steady-state kinetic analyses indicate that both yeast and human Polη incorporate a G opposite the 3′ T of the (6-4) TT lesion about eightfold more efficiently than an A. Thus, in spite of the large distortion of the DNA duplex, Polη is able to insert a G opposite the 3′ T of the lesion; Polη, however, does not extend from the ensuing base pair. The ability of Polη to preferentially insert a G rather than an A opposite the 3′ T of the lesion supports the view that although Polη is rather insensitive even to a major geometric distortion such as that conferred upon DNA by the (6-4) lesion, it prefers to insert nucleotides opposite DNA lesions where some base pairing is possible. Our previous observations that Polη inserts A's opposite the two T's in the dimer which form correct base pairs, and that it inserts a C rather than an A opposite an 8-oxoG lesion, also concur with this view. Thus, although the 8-oxoG · A base pair has the correct Watson-Crick geometry, this base pairing involves the same two hydrogen bonds as in the T · A base pair, whereas in the 8-oxoG · C base pair, in spite of the very considerable distortion of the template, the base pairing involves the same three hydrogen bonds as in the G · C base pair (18, 21, 23, 25).

DNA Polζ is essential for the mutagenic bypass of UV induced DNA lesions. By itself, Polζ bypasses a *cis-syn* TT dimer quite poorly, and it does not bypass a (6-4) TT lesion. This is because Polζ is highly inefficient at inserting nucleotides opposite the 3′ T of either of these lesions; Polζ, however, is very adept at extending from nucleotides inserted opposite the 3′ T of either lesion by another DNA polymerase (14). Interestingly, Polζ extends from a G opposite the 3′ T of the (6-4) TT lesion fourfold more efficiently than it extends from a G opposite the nondamaged T; importantly, Polζ incorporates the correct nucleotide A opposite the 5′ T of the (6-4) lesion, whereas the wrong nucleotides are incorporated very poorly, with a frequency of $\sim 5 \times 10^{-4}$. Thus, although Polζ is quite accurate in inserting the correct nucleotide opposite the 5′ T of the lesion, its contribution to mutagenesis emanating from the bypass of (6-4) TT lesions would derive from its ability to efficiently extend from the G nucleotide inserted opposite the 3′ T by Polη. The accurate insertion of an A opposite the 5′ T of the (6-4) lesion by Polζ explains the genetic observation that mutations occur predominantly at the 3′ site of the (6-4) TT lesion and not at the 5′ site, and the insertion of a G opposite the 3′ T of the (6-4) TT lesion by Polη accounts for the 3′ T→C substitutions that occur at this lesion site (8).

The (6-4) photoproduct is formed much more frequently at the TC and CC sequences than at the TT site (1–6). At TC, the (6-4) lesion is formed almost as frequently as the cyclobutane dimer, whereas at CC, dimer formation predominates over the (6-4) lesion. At TT sites, the (6-4) lesion is formed even much less frequently than at CC sites. Genetic studies in yeast indicating a role of Polη in the error-free bypass of UV lesions at the TC and CC sites (30) have raised the possibility that in addition to its role in the error-free bypass of *cis-syn* CC and TC dimers, Polη contributes also to the error-free bypass of (6-4) lesions at these sites.

Similar to the bypass of a *cis-syn* TT dimer, we presume that Polη bypasses a *cis-syn* CC or TC dimer by incorporating the correct nucleotides opposite the two residues of the dimer. Opposite the CC or TC (6-4) lesion, we expect Polη to insert a G opposite the 3′ C of the lesion because the (6-4) lesion at these sites is structurally very similar to that at the TT site, and the O_2 carbonyl of the 3′ C in the lesion is expected to form hydrogen bonds with a G. The insertion of a G opposite the 3′ C of the (6-4) lesion by Polη, followed by extension by the incorporation of the correct nucleotide opposite the 5′ nucle-

Downloaded from http://mcb.asm.org/ on October 28, 2020 by guest

otide of the lesion, would then promote error-free bypass of (6-4) TC and CC lesions.

ACKNOWLEDGMENTS

We thank M. T. Washington for helpful discussions.

This work was supported by National Institutes of Health grant GM19261. The (6-4) TT photoproduct containing DNA was constructed in the Synthetic Organic Chemistry Core Laboratory, supported by NIEHS Center grant P30-ESO6676, and we are grateful to Richard Hodge for providing this DNA.

REFERENCES

1. **Armstrong, J. D., and B. A. Kunz.** 1990. Site and strand specificity of UVB mutagenesis in the *SUP4-o* gene of yeast. Proc. Natl Acad. Sci. USA **87:** 9005–9009.
2. **Bourre, F., G. Renault, and A. Sarasin.** 1987. Sequence effect on alkali-sensitive sites in UV-irradiated SV40 DNA. Nucleic Acids Res. **15:**8861–8875.
3. **Brash, D. E.** 1997. Sunlight and the onset of skin cancer. Trends Genet. **13:**410–414.
4. **Brash, D. E., and W. A. Haseltine.** 1982. UV-induced mutation hotspots occur at DNA damage hotspots. Nature **298:**189–192.
5. **Brash, D. E., S. Seetharam, K. H. Kraemer, M. M. Seidman, and A. Bredberg.** 1987. Photoproduct frequency is not the major determinant of UV base substitution hot spots or cold spots in human cells. Proc. Natl. Acad. Sci. USA **84:**3782–3786.
6. **Canella, K. A., and M. M. Seidman.** 2000. Mutation spectra in supF: approaches to elucidating sequence context effects. Mutat. Res. **450:**61–73.
7. **Creighton, S., L. B. Bloom, and M. F. Goodman.** 1995. Gel fidelity assay measuring nucleotide misinsertion, exonucleolytic proofreading, and lesion bypass efficiencies. Methods Enzymal. **262:**232–256.
8. **Gibbs, P. E. M., A. Borden, and C. W. Lawrence.** 1995. The T-T pyrimidine (6-4) pyrimidinone UV photoproduct is much less mutagenic in yeast than in *Escherichia coli*. Nucleic Acids Res. **23:**1919–1922.
9. **Goodman, M. F., S. Creighton, L. B. Bloom, and J. Petruska.** 1993. Biochemical basis of DNA replication fidelity. Crit. Rev. Biochemi. Mol. Biol. **28:**83–126.
10. **Haracska, L., S. Prakash, and L. Prakash.** 2000. Replication past O^6-methylguanine by yeast and human DNA polymerase η. Mol. Cell. Biol. **20:**8001–8007.
11. **Haracska, L., S.-L. Yu, R. E. Johnson, L. Prakash, and S. Prakash.** 2000. Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase η. Nat. Genet. **25:**458–461.
12. **Johnson, R. E., C. M. Kondratick, S. Prakash, and L. Prakash.** 1999. *hRAD30* mutations in the variant form of xeroderm pigmentosum. Science **285:**263–265.
13. **Johnson, R. E., S. Prakash, and L. Prakash.** 1999. Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Polη. Science **283:**1001–1004.
14. **Johnson, R. E., M. T. Washington, L. Haracska, S. Prakash, and L. Prakash.** 2000. Eukaryotic polymerases ι and ζ act sequentially to bypass DNA lesions. Nature **406:**1015–1019.
15. **Johnson, R. E., M. T. Washington, S. Prakash, and L. Prakash.** 2000. Fidelity of human DNA polymerase η. J. Biol. Chem. **275:**7447–7450.
16. **Kim, J.-K., and B.-S. Choi.** 1995. The solution structure of DNA duplex-decamer containing the (6-4) photoproduct of thymidyl(3′→5′)thymidine by NMR and relaxation matrix refinement. Eur. J. Biochem. **228:**849–854.
17. **Kim, J.-K., D. Patel, and B.-S. Choi.** 1995. Contrasting structural impacts induced by *cis-syn* cyclobutane dimer and (6-4) adduct in DNA duplex decamers: implication in mutagenesis and repair activity. Photochem. Photobiol. **62:**44–50.
18. **Kouchakdjian, M., V. Bodepudi, S. Shibutani, M. Eisenberg, F. Johnson, A. P. Grollman, and D. J. Patel.** 1991. NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (-8-oxo-7*H*-dG) opposite deoxyadenosine in a DNA duplex. 8-oxo-7*H*-dG(*syn*).dA(*anti*) alignment at lesion site. Biochemistry **30:**1403–1412.
19. **LeClerc, J. E., A. Borden, and C. W. Lawrence.** 1991. The thymine-thymine pyrimidine-pyrimidone (6-4) ultraviolet light photoproduct is highly mutagenic and specially induces 3′ thymine-to-cytosine transitions in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **88:**9685–9689.
20. **Lee, J.-H., G.-S. Hwang, and B.-S. Choi.** 1999. Solution structure of a DNA decamer duplex containing the stable 3′ T · G base pair of the pyrimidine(6-4)pyrimidone photoproduct [(6-4) adduct]: implications for the highly specific 3′ T → C transition of the (6-4) adduct. Proc. Natl. Acad. Sci. USA **96:**6632–6636.
21. **Lipscomb, L. A., M. E. Peek, M. L. Morningstar, S. M. Verghis, E. M. Miller, A. Rich, J. M. Essignman, and L. D. Williams.** 1995. X-ray structure of a DNA decamer containing 7,8-dihydro-8-oxoguanine. Proc. Natl. Acad. Sci. USA **92:**719–723.
22. **Masutani, C., R. Kusumoto, A. Yamada, N. Dohmae, M. Yokoi, M. Yuasa, M. Araki, S. Iwai, K. Takio, and F. Hanaoka.** 1999. The *XPV* (xeroderma pigmentosum variant) gene encodes human DNA polymerase η. Nature **399:**700–704.
23. **McAuley-Hecht, K. E., G. A. Leonard, N. J. Gibson, J. B. Thomson, W. P. Watson, W. N. Hunter, and T. Brown.** 1994. Crystal structure of a DNA duplex containing 8-hydroxydeoxyguanine-adenine base pairs. Biochemistry **33:**10266–10270.
24. **Mendelman, L. V., J. Petruska, and M. F. Goodman.** 1990. Base mispair extension kinetics. Comparison of DNA polymerase α and reverse transcriptase. J. Biol. Chem. **265:**2338–2346.
25. **Oda, Y., S. Uesugi, M. Ikehara, S. Nishimura, Y. Kawase, H. Ishikawa, H. Inoue, and E. Ohtsuka.** 1991. NMR studies of a DNA containing 8-hydroxydeoxyguanosine. Nucleic Acids Res. **19:**1407–1412.
26. **Wang, Y.-C., V. M. Maher, D. L. Mitchell, and J. J. McCormick.** 1993. Evidence from mutation spectra that the UV hypermutability of xeroderma pigmentosum variant cells reflects abnormal, error-prone replication on a template containing photoproducts. Mol. Cell. Biol. **13:**4276–4283.
27. **Washington, M. T., R. E. Johnson, S. Prakash, and L. Prakash.** 2000. Accuracy of thymine-thymine dimer bypass by *Saccharomyces cerevisiae* DNA polymerase η. Proc. Nat. Acad. Sci. USA **97:**3094–3099.
28. **Washington, M. T., R. E. Johnson, S. Prakash, and L. Prakash.** 1999. Fidelity and processivity of *Saccharomyces cerevisiae* DNA polymerase η. J. Biol. Chem. **274:**36835–36838.
29. **Waters, H. L., S. Seetharam, M. M. Seidman, and K. H. Kraemer.** 1993. Ultraviolet hypermutability of a shuttle vector propagated in xeroderma pigmentosum variant cells. J. Investig. Dermatol. **101:**744–748.
30. **Yu, S.-L., R. E. Johnson, S. Prakash, and L. Prakash.** 2001. Requirement of DNA polymerase η for error-free bypass of UV-induced CC and TC photoproducts. Mol. Cell. Biol. **21:**185–188.
31. **Zhang, Y., F. Yuan, X. Wu, O. Rechkoblit, J.-S. Taylor, M. E. Geachintov, and Z. Wang.** 2000. Error-prone lesion bypass by human DNA polymerase η. Nucleic Acids Res. **28:**4717–4724.

Downloaded from http://mcb.asm.org/ on October 28, 2020 by guest