

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

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UV light-induced DNA lesions block the normal replication machinery. Eukaryotic cells possess DNA polymerase η (Pol η), which has the ability to replicate past a *cis-syn* thymine-thymine (TT) dimer efficiently and accurately, and mutations in human Pol η result in the cancer-prone syndrome, the variant form of xeroderma pigmentosum. Here, we test Pol η 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 Pol η preferentially insert a G residue, but they are unable to extend from the inserted nucleotide. DNA Pol ζ , essential for UV induced mutagenesis, efficiently extends from the G residue inserted opposite the 3' T of the (6-4) TT lesion by Pol η , and Pol ζ 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 Pol η and Pol ζ , wherein Pol η inserts a nucleotide opposite the 3' T of the lesion and Pol ζ 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 (Pol η) 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 Pol η 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 Pol η results in UV hypermutability (26, 29), and defects in Pol η 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.

Pol η 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, Pol η replicates through an 8-oxoguanine (8oxoG) (11) or an *O*⁶-methylguanine (m⁶G) lesion (10). Although the template strand is highly distorted in the vicinity of the lesion in the 8oxoG · C base pair, yeast Pol η (yPol η) efficiently replicates through this lesion by inserting a C opposite the lesion and then extending from the resulting base pair (11), and Pol η bypasses an m⁶G lesion by inserting a C or T opposite the lesion (10). The ability of Pol η 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 yPol η and human Pol η (hPol η) 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 Pol η to replicate through a (6-4) TT lesion. We find that although Pol η does not bypass this lesion, it is nevertheless able to insert a G residue opposite the 3' T of the lesion. DNA Pol ζ , 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 Pol η 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 Pol η 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 Pol η . All subsequent purification steps were done as described elsewhere (13, 15).

yPol ζ 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 NaPO₄ [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 TGCT AAGAGTTCG TATATGCC TACTGGA GTACCGGAG CAT

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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 non-damaged 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 μ l) was used; the mixture contained 25 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 1 mM dithiothreitol, 100 μ g of bovine serum albumin/ml, 10% glycerol, 100 μ M each deoxynucleoside triphosphate (dNTP) (dGTP, dATP, dTTP, and dCTP), 10 nM 5'-³²P-labeled oligonucleotide primer annealed to an oligonucleotide DNA template, 1 nM yeast or human Pol η , and 1.8 nM yPol ζ . 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 Pol η 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 hPol η or yPol η 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: $v_{\text{obs}} = (V_{\text{max}} \times [\text{dNTP}]) / (K_m + [\text{dNTP}])$. 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:

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

To determine the efficiency of nucleotide incorporation at the 5' T of the (6-4) TT photoproduct or an undamaged TT sequence by Pol ζ , following a correctly base-paired or mismatched primer terminus, the standard DNA polymerase assay was used except that reaction mixtures contained 40 mM Tris-HCl (pH 7.5), 5 nM Pol ζ , 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 μ M for dATP and from 0 to 500 μ M for dGTP, dTTP, or dCTP.

RESULTS

Bypass of a (6-4) TT photoproduct by the combined action of Pol η and Pol ζ . 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'-³²P-labeled 44-nt oligomer. As shown in Fig. 1, neither yeast or human Pol η 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 Pol ζ in the mutagenic bypass of DNA lesions, including those induced by UV light. Pol ζ , 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, how-

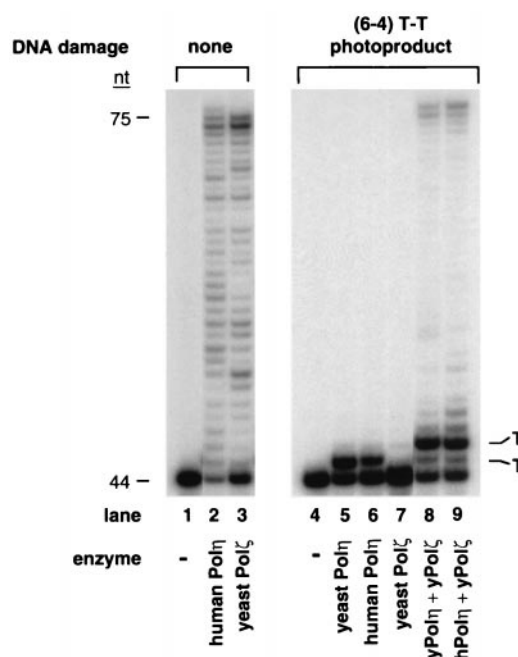


FIG. 1. Bypass of the (6-4) TT photoproduct by the combined action of Pol η and Pol ζ . Lanes 1 to 3, undamaged DNA; lanes 4 to 9, (6-4) TT photoproduct-containing DNA. Positions of the two T's in the undamaged or the (6-4) TT photoproduct-containing template are indicated on the right. hPol η (1 nM),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

ever, occurs when Pol η is combined with Pol ζ (Fig. 1, lanes 8 and 9).

Preferential incorporation of a G residue opposite the 3' T of the (6-4) TT photoproduct by human and yeast Pol η . To examine the efficiency (V_{max}/K_m) of nucleotide incorporation opposite the 3' T of the (6-4) lesion by human and yeast Pol η , we measured the kinetics of insertion for each deoxynucleotide under steady-state conditions. Figure 2 shows the incorporation pattern of each deoxynucleotide by human Pol η 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, hPol η inserts the correct A residue with a high efficiency (Table 1). Relative to the incorporation of A, hPol η 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 of the (6-4) TT lesion, hPol η 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. hPol η preferentially inserts a G opposite the 3' T of the (6-4) TT lesion, as hPol η 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, hPol η 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 hPol η has been reported, but in the absence of any kinetic analyses, the efficiency with which hPol η inserted this or other nucleotides could not be evaluated

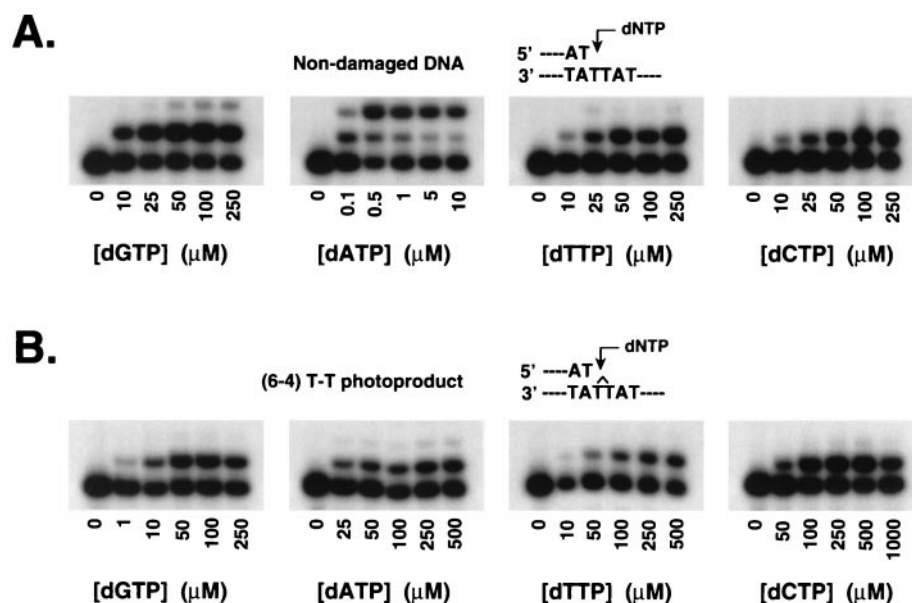


FIG. 2. Nucleotide incorporation by hPol η opposite the 3' T residue in a nondamaged template or a (6-4) TT photoproduct-containing template. (A) Incorporation of nucleotides opposite the nondamaged T residue; (B) incorporation opposite the equivalent 3' T residue of a (6-4) TT photoproduct. A portion of each primer:template substrate is shown at the top. hPol η (0.5 nM) was incubated with DNA substrate (10 nM) and the indicated concentrations of dNTPs for 5 min at 30°C.

(31). yPol η 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, yPol η incorporates an A opposite the 3' T of the (6-4) lesion over 700-fold less well (Table 2). yPol η 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 \sim 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 Pol ζ . Since Pol ζ is able to extend from the nucleotide inserted by Pol η 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 Pol ζ opposite the 5' T of the nondamaged or (6-4) TT photoproduct using steady-state kinetic assays. Since Pol η 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, Pol ζ incorporates the correct A opposite the 5' T with high efficiency and misincorporates nucleotides with a frequency of $\sim 10^{-3}$ to 10^{-4} (Table 3). Pol ζ , 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, Pol ζ 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 Pol η 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 Ts 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 hPol η

| 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 |

TABLE 2. Steady-state kinetic parameters for nucleotide incorporation opposite the 3' T of the (6-4) TT photoproduct by yPol η

| Template | Template nucleotide | Incoming residue | V_{\max} (nM/min) | K_m (μ M) | 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 Pol η 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 O₂ 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 O₂ 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-

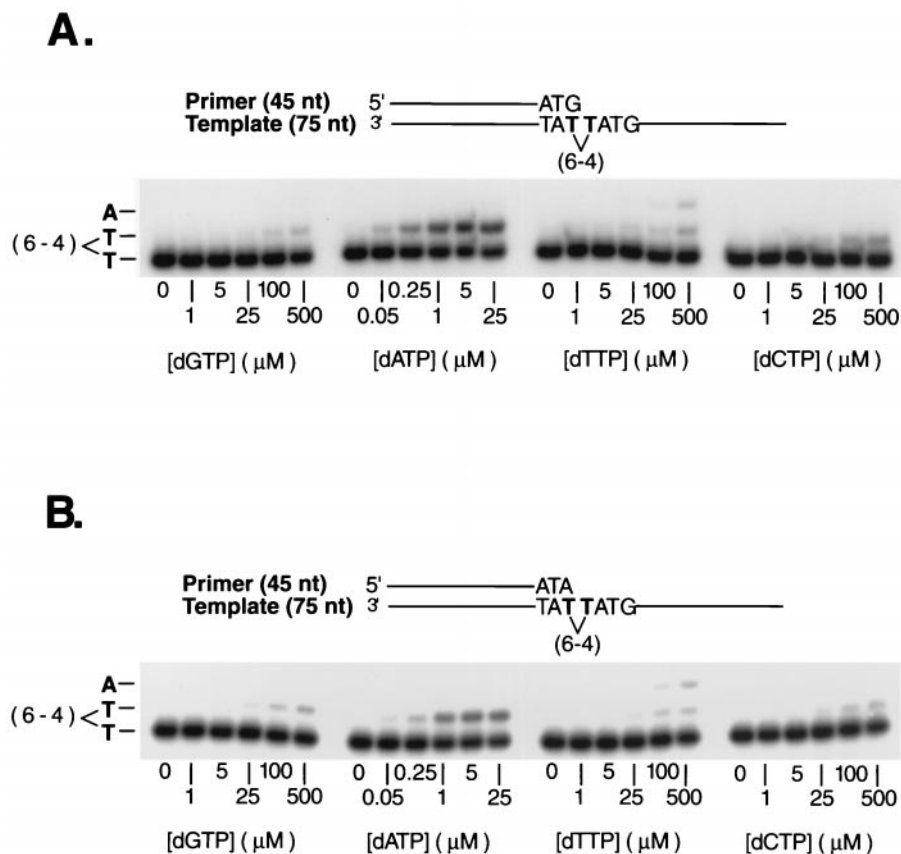


FIG. 3. Nucleotide incorporation opposite the 5' T of the (6-4) TT photoproduct by yPol ζ . (A) Nucleotide incorporation following a G residue opposite the 3' T of the (6-4) photoproduct; (B) nucleotide incorporation following an A residue opposite the 3' T of a (6-4) photoproduct. yPol ζ (5 nM) was 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 yPol ζ

| Template | Primer:template base pair | Incoming residue | V_{\max} (nM/min) | K_m (μ M) | V_{\max}/K_m | Relative efficiency ^a |
|------------|---------------------------|------------------|---------------------|------------------|----------------|----------------------------------|
| Nondamaged | G·T | G | 0.11 \pm 0.01 | 170 \pm 33 | 0.0006 | 6.0 \times 10 ⁻⁴ |
| | | A | 0.70 \pm 0.05 | 0.67 \pm 0.06 | 1.0 | 1 |
| | | T | 0.38 \pm 0.04 | 130 \pm 8 | 0.0029 | 2.9 \times 10 ⁻³ |
| | | C | 0.12 \pm 0.07 | 250 \pm 56 | 0.0005 | 5.0 \times 10 ⁻⁴ |
| | A·T | G | 0.13 \pm 0.01 | 140 \pm 11 | 0.0009 | 1.0 \times 10 ⁻⁴ |
| | | A | 1.06 \pm 0.07 | 0.12 \pm 0.07 | 8.8 | 1 |
| | | T | 0.42 \pm 0.02 | 94 \pm 14 | 0.0045 | 5.1 \times 10 ⁻⁴ |
| | | C | 0.34 \pm 0.05 | 140 \pm 22 | 0.0024 | 2.7 \times 10 ⁻⁴ |
| (6-4) TT | G·T | G | 0.13 \pm 0.01 | 130 \pm 17 | 0.001 | 2.6 \times 10 ⁻⁴ |
| | | A | 1.2 \pm 0.03 | 0.32 \pm 0.05 | 3.8 | 1 |
| | | T | 0.34 \pm 0.08 | 180 \pm 32 | 0.0019 | 5.0 \times 10 ⁻⁴ |
| | | C | 0.27 \pm 0.03 | 160 \pm 24 | 0.0017 | 4.5 \times 10 ⁻⁴ |
| | A·T | G | 0.24 \pm 0.02 | 57 \pm 10 | 0.0042 | 3.0 \times 10 ⁻³ |
| | | A | 0.89 \pm 0.04 | 0.63 \pm 0.11 | 1.4 | 1 |
| | | T | 0.43 \pm 0.02 | 130 \pm 10 | 0.0033 | 2.4 \times 10 ⁻³ |
| | | C | 0.28 \pm 0.03 | 200 \pm 34 | 0.0014 | 1.0 \times 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 Pol η and Pol ζ , wherein Pol η inserts a G opposite the 3' T of the (6-4) lesion and Pol ζ extends from the resulting base pair.

Our steady-state kinetic analyses indicate that both yeast and human Pol η 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, Pol η is able to insert a G opposite the 3' T of the lesion; Pol η , however, does not extend from the ensuing base pair. The ability of Pol η to preferentially insert a G rather than an A opposite the 3' T of the lesion supports the view that although Pol η 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 Pol η 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·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 Pol ζ is essential for the mutagenic bypass of UV induced DNA lesions. By itself, Pol ζ bypasses a *cis-syn* TT dimer quite poorly, and it does not bypass a (6-4) TT lesion. This is because Pol ζ is highly inefficient at inserting nucleotides opposite the 3' T of either of these lesions; Pol ζ , however, is very adept at extending from nucleotides inserted opposite the 3' T of either lesion by another DNA polymerase (14). Interestingly, Pol ζ extends from a G opposite the 3' T of the (6-4) TT lesion fourfold more efficiently than it extends from a G op-

posite the nondamaged T; importantly, Pol ζ 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 Pol ζ 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 Pol η . The accurate insertion of an A opposite the 5' T of the (6-4) lesion by Pol ζ 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 Pol η accounts for the 3' T \rightarrow 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 Pol η 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, Pol η 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 Pol η 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 Pol η 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 O₂ 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 Pol η , followed by extension by the incorporation of the correct nucleotide opposite the 5' nucle-

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

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