DNA polymerase η (Polη, xeroderma pigmentosum variant, or Rad30) plays an important role in an error-free response to unrepairable UV damage during replication. It faithfully synthesizes DNA opposite a thymine-thymine cis-syn-cyclobutane dimer. We have purified the yeast Polη and studied its lesion bypass activity in vitro with various types of DNA damage. The yeast Polη lacked a nuclease or a proofreading activity. It efficiently bypassed 8-oxoguanine, incorporating C, A, and G opposite the lesion with a relative efficiency of ~100:36:14, respectively. The yeast Polη efficiently incorporated a C opposite an acetylaminofluorene-modified G, and efficiently inserted a G or less frequently an A opposite an apurinic/apyrimidinic (AP) site but was unable to extend the DNA synthesis further in both cases. However, some continued DNA synthesis was observed in the presence of the yeast Polζ following the Polη action opposite an AP site, achieving true lesion bypass. In contrast, the yeast Polα was able to bypass efficiently a template AP site, predominantly incorporating an A residue opposite the lesion. These results suggest that other than UV damage, Polη may also play a role in bypassing additional DNA lesions, some of which can be error-prone.

During replication, a variety of DNA lesions can block replicative DNA polymerases. Cells contain specialized proteins to overcome such replication blockage. These proteins can support DNA synthesis across from the damaged template, achieving true-lesion synthesis or lesion bypass, thus allowing normal replication to continue downstream of the damage. Since DNA lesions may alter the coding property of the modified base or render the base noncoding, trans-lesion synthesis is often results in mutations. One mechanism of lesion bypass is the damage-induced mutagenesis. In *Escherichia coli*, the damage-induced mutagenesis pathway is under the control of the SOS response and requires at least RecA, UmuC, and UmuD proteins (1–3). In the eukaryotic model organism *Saccharomyces cerevisiae*, the damage-induced mutagenesis pathway involves at least the following proteins: Rad6, Rad18, Rev1, Rev3, Rev6, Rev7, and Nmg2 (4–11).

**Specificity of DNA Lesion Bypass by the Yeast DNA Polymerase η***

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Yeast Rev3 forms a protein complex with Rev7 and is known as DNA polymerase (Pol)2 ζ (12). This polymerase is capable of limited trans-lesion synthesis opposite a template TT dimer in vitro (12). The Rev1 protein is a dCMP transferase that is able to efficiently insert a C residue opposite a template G or an apurinic/apyrimidinic (AP) site (13). In vitro, the combined activities of the Rev1 transerase and Polζ can effectively bypass a template AP site (13). Genetic analyses have demonstrated the importance of this mutagenesis pathway in error-prone lesion bypass of UV-induced damage and AP sites in DNA (7, 9, 14).

Recently, it became clear that the Rev1 protein belongs to a large protein family known as the UmuC family (15, 16). This family also includes the *E. coli* DinB protein (15, 16) and the yeast Rad30 gene product (17, 18). Genetic analyses of rad30 mutant cells indicate that its wild-type protein is involved in an error-free response to UV radiation but is independent of the Rad5 error-free mechanism (17). Biochemical studies of the Rad30 protein revealed that it is a DNA polymerase capable of error-free trans-lesion synthesis opposite a template cis-syn-TT dimer (19). This seventh eukaryotic DNA polymerase is thus referred to as DNA Polη (19). In *E. coli*, DinB is DNA polymerase IV (20), and UmuD2C complex is DNA polymerase V (21).

Most recently, it was found that the human xeroderma pigmentosum variant (XPV) is both a structural and functional homologue of the yeast Polη (22–24). Mutations in the XPV gene are responsible for the rare human hereditary disease xeroderma pigmentosum variant (1, 25). But unlike the other xeroderma pigmentosum genes, it has long been recognized that XPV is not involved in nucleotide excision repair (1, 25). UV sensitivity and elevated UV mutagenesis of the human XPV cells are now clearly explained by the activities of Polη in response to UV radiation. Intriguingly, humans contain another Rad30 homologue, whose gene is designated RAD30B (16). Additionally, a third human homologue more related to DinB has been identified (26). Apparently, proteins of the UmuC family are involved in different mechanisms of lesion bypass in response to unrepairable DNA damage during replication. The importance of lesion bypass is underscored by the fact that the fundamental mechanisms have been conserved from *E. coli* to humans.

Polη is able to bypass a template TT dimer in an error-free manner (19, 23). However, it is not known whether Polη is also capable of bypassing other DNA lesions. Furthermore, it is not known whether the error-free lesion bypass by Polη is a unique feature confined to TT dimer or is more general to other DNA lesions. To address these questions, we have examined the...
response of the yeast Polζ to several other DNA lesions in vitro. In this report, we (i) show the properties of the yeast Polζ in response to an 8-oxoguanine, an AAF-modified guanine, and an AP site in the DNA template; and (ii) provide evidence that Polζ can be error-prone during DNA synthesis opposite some lesions.

**EXPERIMENTAL PROCEDURES**

**Materials**—A mouse monoclonal antibody against the Hisα tag was purchased from Qiagen. Alkaline phosphatase-conjugated anti-mouse IgG was from Sigma. The Pfu DNA polymerase was from Stratagene. The E. coli uracil-DNA glycosylase was from New England Biolabs. The yeast rad30 deletion mutant strain (MATa his3 leu2 met15 ura3) and its isogenic wild-type strain BY4741 were purchased from Research Genetics. N-Acetoxy-N-2-acetylaminofluorene (AAAF) was obtained from the Midwest Research Institute. Purified yeast DNA polymerase α (Polα) with associated primase activity was generously provided by David Hinkle, Department of Biology, University of Rochester. One unit of Polα incorporates 1 nmol of total nucleotide per 30 min at 30 °C, using activated salmon sperm DNA as the substrate.

To construct the Rad30 overexpression plasmid, the RAD30 gene was amplified by polymerase chain reaction from yeast genomic DNA using Pfu DNA polymerase and two primers, CATGGCAGTGCTCAATGCTGCTGACGAAGGCAGGATGGCATGCA GCTGAAGCATATAATGCTGC. The resulting polymerase chain reaction product was cloned into the XbaI and SalI sites of the vector pEGU6 (27) to yield pEGU6-Rad30. Its expression in yeast was under the control of the inducible GAL11GAL10 promoter and produced the Rad30 protein (Polζ) containing a Hisα tag at its N terminus.

**DNA Substrates with Site-specific Lesions**—A 49-mer DNA template containing a site-specific cis-syn TT dimer was prepared as described previously (28). Its sequence is 5′-AGCTACCATGCCTGCACGAATTA-AGCAATTCGTTACATGCTGCTAGTCC-3′, where the TT dimer is underlined. A 30-mer DNA template containing a site-specific 8-oxoguanine (8-oxoG) was synthesized from a DNA synthesizer by Operon. Its sequence is 5′-GGATGGCATGCATTAACCGGAGGCCGCGCCGCG-3′, where the position of the 8-oxoG residue is underlined. To prepare AAF-damaged DNA, 2 nmol of the oligonucleotide 5′-GGATGGCATGCAATACCGGAGGCCGCGCG-3′, where the position of the 8-oxoG residue is underlined, was annealed with 1 pmol of undamaged oligonucleotide by electrophoresis on a 20% denaturing polyacrylamide gel. Modified DNA migrated slower on the gel and was sliced out of the gel. The gel slices were soaked in 150 μl water at room temperature for 4 h. The AAF-damaged 30-mer DNA was recovered using GenElute DNA spin column (Supelco).

To prepare a 30-mer DNA template containing a site-specific AP site, the following oligonucleotide with a uracil residue at position 13, 5′-GGCCGGCCTCCGGTTA-3′, was then labeled with 32P at its 5′ end and annealed to the uracil-containing template. Finally, 10 pmol of the uracil-containing substrate was treated with 4 units of E. coli uracil-DNA glycosylase at 37 °C for 60 min. The resulting substrate was examined for the presence of the site-specific AP site by cleavage with the E. coli endonuclease III at 37 °C for 30 min, followed by electrophoresis on a 15% non-denaturing polyacrylamide gel as described previously (29).

**Purification of the Yeast DNA Polζ**—DNA Polζ was expressed in yeast AMY32 (9) cells from the Rev3 and Rev7 overexpression plasmids pEGU6-Rev3 and pEGU6-Rev7, respectively. Their expressions in yeast were under the control of the inducible GAL11GAL10 promoter and produced both the Rev3 and the Rev7 proteins containing a Hisα tag at their N termini. Overexpression of the plasmids was achieved as described above for the yeast Polζ. Cell extracts were processed and purified through a Hitrap desalting column charged with NISO4, followed by liquid chromatography on an FPLC Mono S HR5/5 column essentially as described above for the yeast Polζ, except that the buffers used were Ni-P buffer A (200 mM phosphate buffer, pH 7.4, 0.5 mM NaCl, 10% glycerol, 5 mM β-mercaptoethanol, and protease inhibitors) and P buffer (20 mM phosphate buffer, pH 7.4, 1 mM EDTA, 10% glycerol, 5 mM β-mercaptoethanol, and protease inhibitors) used for the Ni column and the Mono S column, respectively. The desalted Mono S sample (20 ml) was loaded onto an FPLC Mono Q HR5/5 column (Amersham Pharmacia Biotech). Bound proteins were eluted with a 30-ml linear gradient of 0–500 mM KCl in the P buffer. The Rev3 and Rev7 proteins copurified and were identified by Western blot analyses using a mouse monoclonal antibody against the N-terminal Hisα tag.

**DNA Lesion Bypass Assays**—DNA polymerase assays were performed essentially as described by Johnson et al. (19). The reaction mixture (10 μl) contained 25 mM potassium phosphate, pH 7.0, 5 mM MgCl₂, 5 mM dithiothreitol, 100 μg/ml bovine serum albumin, 100 μM dNTPs (dATP, dCTP, dTTP, and dGTP individually or together as indicated), 100 μM of the 32P-labeled DNA substrate as indicated, and ~10 ng of the purified Polζ. After incubation at 37 °C for 10 min, reactions were terminated with 7 μl of a stop solution (20 mM EDTA, 95% formamide, 0.05% bromphenol blue, and 0.05% xylene cyanol). The reaction products were resolved on a 20% polyacrylamide gel containing 8 μM urea and visualized by autoradiography. Primer extension was quantified by scanning densitometry of the autoradiogram using the SigmaGel software (Sigma) for analysis.

![Western blots using a mouse monoclonal anti-His antibody.](http://www.jbc.org/)

**Fig. 1. Analyses of the purified yeast Polζ.** A, the purified Polζ (200 ng) was analyzed by electrophoresis on a 10% SDS-polyacrylamide gel and visualized by silver staining of the gel. Protein size markers in kDa (lane M) are indicated on the left. B, the purified Polζ (200 ng) was analyzed by a Western blot using a mouse monoclonal antibody against the Hisα tag. Protein size markers in kDa (lane M) are indicated on the right. C, DNA polymerase assays were performed without (lane 1) or with (lane 2) the purified Polζ (Rad30, 10 ng), using the 30-mer template DNA, 5′-GGATGGCATGCAATACCGGAGGCCGCGCG-3′, annealed with the 5′-32P-labeled primer, 5′-GGCCGGCCTCCGGTTA-3′. DNA size markers in nt are indicated on the right.
**RESULTS**

**Purification of the Yeast Polh**—To facilitate protein purification, we tagged the yeast Polh with 6 histidine residues at its N terminus. The His-tagged Polh (coded by the RAD30 gene) fully complemented the rad30 mutant for UV sensitivity (data not shown), indicating that the His tag did not affect its function. The most pure fraction of the His-tagged yeast Polh was analyzed by electrophoresis on a 10% SDS-polyacrylamide gel followed by silver staining of the gel (Fig. 1A). Two major bands and two faster migrating bands were evident on the SDS-polyacrylamide gel (Fig. 1A). Western blot analyses using a monoclonal antibody against the His tag confirmed that these bands were Polh (Fig. 1B). At present, it is not known whether they are modified Polh or partially degraded Polh or both. Thus, our Polh preparation is pure without detectable contamination by other proteins. The purified Polh migrated at ~66 kDa on a 10% SDS-polyacrylamide gel (Fig. 1A), consistent with its calculated molecular mass of 71 kDa. As expected, the pure Polh possesses a DNA polymerase activity on a primed 30-mer DNA template (Fig. 1C). Furthermore, Polh was able to bypass a cis-syn-TT dimer on a 50-mer DNA template (data not shown). Thus, our purified Polh is biochemically active. DNA polymerase activity of the yeast Polh requires Mg2+, and the polymerase was active with 1–20 mM MgCl2 and 0–80 mM KCl. Higher concentrations of MgCl2 or KCl and over 1 mM EDTA significantly inhibited the yeast Polh activity.

**The Yeast Polh Does Not Possess A Nuclease Activity**—In addition to DNA polymerizing activity, some DNA polymerases also possess 5’ → 3’ or 3’ → 5’ nuclease activity or both. To examine whether the yeast Polh possesses a nuclease activity, we incubated the polymerase with a 32P-labeled 18-mer single-stranded DNA (Fig. 2A, primer P2). As shown in Fig. 2B, this DNA remains intact after incubation with Polh. To examine nuclease activities on a double-stranded DNA, we labeled several primers (Fig. 2A) at their 5’ ends with 32P and annealed them to the 30-mer DNA template. These DNA substrates contained either a TC mismatch (Fig. 2A, primer P2), a TT mismatch (Fig. 2A, primer P3), a TG mismatch (Fig. 2A, primer P4), or a complementary TA base pair (Fig. 2A, primer P1) at the primer end. Then we incubated these DNA substrates with the yeast Polh without dNTPs and analyzed by electrophoresis on a 20% denaturing gel. As shown in Fig. 2C, the labeled primers were not degraded regardless of whether they contained a terminal mismatch or not. To determine whether the unlabeled DNA template strand was degraded by Polh, we analyzed the incubation products by electrophoresis on a 15% native polyacrylamide gel. Again, a nuclease activity was not detected (Fig. 2D). These results show that the yeast Polh does not possess a nuclease or a proofreading activity.

**Trans-lesion Synthesis of Template 8-Oxoguanine by the Yeast Polh**—8-Oxoguanine is a major form of oxidative damage in DNA. To examine whether Polh can bypass this DNA lesion, we synthesized a 30-mer DNA template containing a site-specific 8-oxoguanine residue (Fig. 3A). A 32P-labeled 17-mer primer was annealed to the template, right before the 8-oxoguanine residue. The yeast Polh efficiently bypassed the template 8-oxoguanine and extended the primer to the end of the template (Fig. 3B, lanes 5 and 6). DNA synthesis by Polh is similarly efficient using either the undamaged DNA template or the 8-oxoguanine-containing template (Fig. 3B, compare lanes 1 and 2 with lanes 4 and 5). These results indicate that 8-oxoguanine in DNA does not block the yeast Polh and does not significantly affect the polymerase activity of the enzyme.

8-Oxoguanine is a miscoding DNA lesion. It is capable of
base pairing with either a C or an A residue (31). To identify the base incorporated opposite the 8-oxoguanine residue by the yeast Polη, we performed DNA synthesis assays with only one deoxyribonucleoside triphosphate: dATP, dCTP, dGTP, or dTTP individually. As shown in Fig. 3C, under identical reaction conditions, except different deoxyribonucleoside triphosphates, the yeast Polη extended near 100% of the primers using dCTP, 56% of the primers with dATP, and 14% of the primers with dGTP opposite the template 8-oxoguanine. Primer extension was not detected with dTTP (Fig. 3C). These results suggest that the yeast Polη predominantly incorporates a C residue opposite a template 8-oxoguanine but can also incorporate an A residue with a lower efficiency and a G residue with the least efficiency. Hence, we conclude that when encountered with 8-oxoguanine residues in DNA, the yeast Polη could cause mutagenic trans-lesion synthesis.

Error-free Nucleotide Insertion Opposite AAF-adducted Guanine by the Yeast Polη—In contrast to the 8-oxoguanine, an AAF-adducted guanine in the template DNA blocks many DNA polymerases (32). Since the yeast Polη can efficiently bypass a TT dimer that is considered to be a bulky lesion, we asked whether this polymerase is able to bypass the bulky AAF-guanine lesion. A 32P-labeled 17-mer primer was annealed to a 30-mer DNA template right before the site-specific template AAF-guanine (Fig. 4A). The yeast Polη effectively incorporated one nucleotide opposite the template AAF-guanine (one nucleotide extension) but failed to extend the primer further (Fig. 4B, lane 2). To reveal the identity of the nucleotide incorporated opposite the AAF-guanine, we did lesion bypass assays with only one deoxyribonucleoside triphosphate in the reaction mixture. As show in Fig. 4B (lanes 3–6), the yeast Polη exclusively incorporated a C residue opposite the template AAF-guanine. In contrast to Polη, the yeast Polα was unable to insert any nucleotide opposite the template AAF-guanine, even when excess amount of the polymerase was used (Fig. 4C, lane 3). In comparison, the purified Polα was able to use the same DNA template without the AAF adduct for DNA synthesis in the presence of all four dNTPs (Fig. 4C, lane 1) or for one nucleotide extension of the primer in the presence of dCTP alone (Fig. 4C, lane 2). These results show that the yeast Polη is able to perform error-free nucleotide insertion opposite a template AAF-guanine but is unable to extend the DNA synthesis further.

Error-prone Nucleotide Insertion Opposite a Template AP Site by the Yeast Polη—For UV-induced cyclobutane pyrimidine dimers in DNA, Polη bypasses the lesions in an error-free mode (19, 23). However, an AP site would pose a challenge for any DNA polymerase that can bypass it. Since an AP site can act as a bulky lesion, we asked whether this polymerase is able to bypass the bulky AP site. A 32P-labeled 17-mer primer was annealed to a 30-mer DNA template right before the site-specific template AP-guanine (Fig. 4A). The yeast Polη effectively incorporated one nucleotide opposite the template AP-guanine (one nucleotide extension) but was unable to continue DNA synthesis downstream of the template AP site. Under identical conditions, the corresponding undamaged template supported efficient primer extension by the yeast Polη up to the end of the template (Fig. 4B, lane 2). Since this undamaged control template contained a uracil residue, we further identified that A was exclusively incorporated opposite the template U (data not shown). Thus, the yeast Polη efficiently recognized the template U as a coding base.

To identify the nucleotide incorporated opposite the template AP site, we performed similar AP site lesion bypass assays with only one deoxyribonucleoside triphosphate. Both G (Fig. 5B, lane 6) and A (Fig. 5B, lane 3) were significantly incorporated opposite the template AP site, supporting 67 and 38% 1-nt primer extensions, respectively. To a much less extent, C and T were also incorporated opposite the template AP site by the yeast Polη, supporting 6 and 5% 1-nt primer extensions, respectively (Fig. 5B, lanes 4 and 5). These results show that the yeast Polη predominantly incorporates a G and less frequently
an A opposite the template AP site but by itself cannot continue DNA synthesis further after the template AP site.

**AP Site Bypass by Combined Activities of the DNA Polymerases**

Rev1 is another protein that can efficiently insert a nucleotide opposite a template AP site (13). However, unlike Polh, the Rev1 activity specifically inserts a C residue opposite the AP site (13). Following the Rev1 action, DNA Polz (the Rev3-Rev7 protein complex) can then continue DNA synthesis, achieving error-prone bypass of the AP site (13). Thus, we asked if Polz could also continue DNA synthesis following nucleotide insertion opposite the AP site by Polh. By using the AP site-containing template (Fig. 5A), we performed lesion bypass assays. As shown in Fig. 6 (lane 2), the yeast Polz, contained in a partially purified fraction, was unable to extend the 17-mer primer annealed right before the template AP site, consistent with the observation of Nelson et al. (13). The purified yeast Polz incorporated one nucleotide opposite the template AP site, extending the 17-mer primer to an 18-mer (Fig. 6, lane 1). However, upon subsequent addition of the yeast Polz, ~13% of the 18-mer products were further extended to the end of the template (Fig. 6, lane 3). These results suggest that the combined actions of the yeast Polh and Polz can result in error-prone bypass of the template AP site.

**AP Site Bypass by the Yeast DNA Polα**—As a control for the yeast Polh trans-lesion synthesis experiments, we performed AP site bypass assays with the purified yeast Polα using the AP site-containing substrate (Fig. 5A). Surprisingly, we found that the yeast Polz alone was able to bypass efficiently the template AP site (Fig. 7A, lanes 3–5), in contrast to the yeast Polh activity on this substrate (Fig. 7A, lane 2). To reveal...
the base identity opposite the template AP site, we performed the bypass assay in the presence of only one deoxyribonucleoside triphosphate. As shown in Fig. 7B (compare lane 1 with lanes 2–4), an A residue was predominantly incorporated opposite the template AP site. Hence, we conclude that the yeast Polz itself can cause a specific mutagenic trans-lesion synthesis opposite a template AP site.

**DISCUSSION**

The recently described DNA Polη is encoded by the RAD30 gene in yeast and the XPF gene in humans (17, 18, 22, 24). The role of DNA Polη in response to UV radiation has largely been defined. It functions as a DNA polymerase bypassing cyclobutane pyrimidine dimers in an error-free manner (19, 23). Thus, inactivation of the Polη gene results in enhanced UV sensitivity and UV-induced mutagenesis (17, 18, 33). Such defects in humans can lead to the hereditary disease xeroderma pigmentosum characterized by photosensitivity and a predisposition to skin cancer.

The inability of Polη to bypass (6-4) photoproducts in DNA (23) provided the first clue that properties of trans-leision synthesis by this polymerase may be lesion-specific. In this study, we have examined the response of the yeast Polη to the following three additional DNA lesions in vitro: 8-oxoguanine, AAF-modified guanine, and AP sites. It is known that 8-oxoguanine is a miscoding lesion that can direct either a C or an A base into the newly synthesized DNA strand (31). Consistent with this miscoding property of the 8-oxoguanine lesion, the yeast Polη also incorporated a C or an A base opposite the lesion. However, unlike other DNA polymerases tested (31), the yeast Polη additionally can incorporate a G base opposite the template 8-oxoguanine, although at a lower frequency than C or A incorporation. Comparing DNA synthesis on undamaged and 8-oxoguanine-containing templates indicates that this lesion does not significantly block the movement of the yeast Polη on the DNA template. Hence, when Polη encounters an 8-oxoguanine in the DNA template in vivo, trans-lesion synthesis by this polymerase will most likely occur resulting in error-prone lesion bypass.

In contrast to 8-oxoguanine and a cis-syn-TT dimer, the AAF adduct on a guanine effectively blocks the movement of the yeast Polη on the DNA template. However, the Polη is able to incorporate a C residue opposite the AAF-guanine lesion before aborting DNA synthesis. This activity appears to be specific to Polη, since the yeast Polα is completely blocked before the AAF damage. It remains to be determined if other DNA polymerases could utilize such a DNA primer to continue the DNA synthesis after the error-free nucleotide insertion by Polη. Our preliminary results indicate that the purified yeast Pol or the partially purified yeast Polζ alone is unable to do so. Nevertheless, it is conceivable that the combined actions of Polη and another DNA polymerase could lead to an effective bypass of AAF-guanine, achieving an error-free response to this lesion in cells.

AP sites are significant spontaneous and induced DNA lesions. They are considered as noninstructional lesions due to...
loss of the coding base. In yeast, the Rev1-Rev3 mutagenesis pathway is the major mechanism responsible for the error-prone bypass of AP sites (13, 14). An important role of the Rev1 protein is to insert a dCMP opposite the template AP site such that the Polξ activity encoded by the REV3 and REV7 genes could continue the primer extension further downstream of the AP site (13). This mechanism of AP site bypass is most likely operational in humans, since both the Rev1 and the Rev3 homologues have been identified in humans (29, 34, 35), and the human REV1 is also capable of inserting a dCMP opposite a template AP site (29). However, it is not clear whether other mechanisms may additionally contribute to the AP site bypass in eukaryotes. Our in vitro studies suggest such a possibility. The yeast Polξ is able to insert efficiently a nucleotide opposite the template AP site. Once incorporated into the newly synthesized DNA, the nucleotide opposite the template AP site cannot be removed by Polξ due to lack of a 3′ → 5′ proofreading nuclease activity. Thus, this Polξ DNA synthesis product may be subsequently extended by another DNA polymerase to achieve a complete AP site bypass. Indeed, we observed that a partially purified Polξ preparation is able to do so. If Polξ contributes to the bypass of some AP sites in vitro, such bypass would be error-prone due to the predominant incorporation of a G or less frequently an A base opposite the AP site by this polymerase. Since the Polξ is DNA damage-inducible (17, 18), the putative contribution of this polymerase to AP site bypass, if any, may only be important when cellular DNA is damaged by exogenous agents.

Unlike Polη and Polξ, the yeast DNA Polα is able to bypass efficiently a template AP site in our studies. Lack of AP site bypass by the yeast Polα was reported by Nelson et al. (13) at a different DNA sequence context, although the purified Polα preparations used in both studies were from the same laboratory. The apparent discrepancy is not clear at present. We further observed that an A residue is predominantly incorporated opposite the template AP site by the yeast Polα, consistent with the “A rule” (36). Thus, when encountering a template AP site during DNA replication, Polα is very likely to bypass the lesion and generate a mutation opposite the AP site. On the other hand, the yeast Polα is unable to bypass a TT dimer (12) or insert a nucleotide opposite a template AAF-guanine (Fig. 4C). Thus, the response of a DNA polymerase to DNA damage is lesion-specific and depends on the specific interaction between the lesion and the polymerase. The unique properties of Polη toward DNA lesions may be a result of the structural features of this polymerase, which can lead to an error-free or an error-prone consequence. Hence, Polη may not be generalized as an error-free DNA polymerase for trans-lesion DNA synthesis based only on its response to UV radiation.

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