Translesion DNA Synthesis by Yeast DNA Polymerase η on Templates Containing N²-Guanine Adducts of 1,3-Butadiene Metabolites*

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Yeast DNA polymerase η can replicate through cis-syn cyclobutane pyrimidine dimers and 8-oxoguanine lesions with the same efficiency and accuracy as replication of an undamaged template. Previously, it has been shown that *Escherichia coli* DNA polymerases I, II, and III are incapable of bypassing DNA substrates containing N²-guanine adducts of stereoisomeric 1,3-butadiene metabolites. Here we showed that yeast polymerase η replicates DNA containing the monoadducts (S)-butadiene monoepoxide and (S,S)-butadiene diol epoxide N²-guanines albeit at a 200–300-fold lower efficiency relative to the control guanine. Interestingly, nucleotide incorporation opposite the (R)-butadiene monoepoxide and the (R,R)-butadiene diol epoxide N²-guanines was ~10-fold less efficient than incorporation opposite their S stereoisomers. Polymerase η preferentially incorporates the correct nucleotide opposite and downstream of all four adducts, except that it shows high misincorporation frequencies for elongation of C paired with (R)-butadiene monoepoxide N²-guanine. Additionally, polymerase η does not bypass the (R,R)- and (S,S)-butadiene diol epoxide N²-guanine-N²-guanine intrastrand cross-links, and replication is completely blocked just prior to the lesion. Collectively, these data suggest that polymerase η can tolerate the geometric distortions in DNA conferred by the N²-guanine butadiene monoadducts but not the intrastrand cross-links.

Various pathways exist in cells to overcome replication blockage caused by DNA lesions. One such pathway, translesion DNA synthesis, involves specialized polymerases that, unlike replicative polymerases, are able to perform DNA synthesis on a damaged DNA template (reviewed in Refs. 1–3). Translesion DNA synthesis can be error-free or error-prone, depending on the chemical structure of the lesion and the polymerase utilized for translesion replication. Among the eukaryotic DNA polymerases, yeast and human DNA polymerases η perform efficient and accurate replication past a cis-syn cyclobutane pyrimidine dimer, a predominant DNA lesion formed by ultraviolet irradiation (4–7). In the yeast *Saccharomyces cerevisiae*, deletion of RAD30, which encodes pol η, confers moderate sensitivity to UV irradiation and an increase in UV-induced mutagenesis (8).

Mutations in the human RAD30A gene, the counterpart of the yeast RAD30, cause the variant form of xeroderma pigmentosum (9, 10). Xeroderma pigmentosum variant cells are hypermutable in response to UV irradiation, and they exhibit a significantly reduced ability to bypass a T-T dimer (reviewed in Ref. 3). Consequently, xeroderma pigmentosum variant individuals suffer from a high incidence of sunlight-induced skin cancers. 7,8-Dihydro-8-oxoguanine is one of the lesions formed by oxidative damage to DNA. Yeast and human pol η both efficiently bypass the 7,8-dihydro-8-oxoguanine lesion. Whereas other polymerases insert A opposite this lesion, pol η preferentially inserts a C (11). Thus, pol η is unique among DNA polymerases in its ability to bypass a T-T dimer and a 7,8-dihydro-8-oxoguanine lesion efficiently and accurately.

Here we examined the ability of yeast pol η to carry out translesion synthesis on DNA substrates containing N²-guanine adducts of stereoisomeric 1,3-butadiene metabolites. 1,3-Butadiene is a potent carcinogen in mice, and to a lesser extent in rats (12) and has been classified as a probable human carcinogen. Butadiene-mediated carcinogenesis is initiated through its reactive metabolites: butadiene monoepoxide, butadiene diol epoxide, and butadiene diol epoxide. Each of these metabolites is represented by at least two stereoisomers. The mutagenicity of butadiene and its reactive metabolites has been observed in several biological systems, particularly in yeast (13, 14) and mammalian cells (15). Butadiene epoxides can react at numerous sites in DNA, forming a multitude of adducts that differ in their stereochemistry (16, 17). Butadiene epoxides are potent inhibitors of synthesis by DNA polymerases. Previously, it has been shown that *Escherichia coli* DNA polymerases I, II, and III are incapable of bypassing DNA substrates containing (R)- and (S)-BDO N²-guanines and (R,R)- and (S,S)-BDE N²-guanines (18) as well as (R,R)- and (S,S)-BDE N²-guanine-N²-guanine intrastrand cross-links (19). Here we examine the action of yeast pol η on these two types of the N²-guanine epoxide-containing DNA substrates.

MATERIALS AND METHODS

DNA Substrates with Site-specific Lesions—The oligodeoxynucleotides containing butadiene epoxide N²-guanine adducts were prepared by the postoligomerization methodology developed by Harris et al. (20). A detailed description of the synthesis of the 11-mer oligonucleotides containing the (R)- and (S)-BDO and (R,R)- and (S,S)-BDE N²-guanines has been described previously (18). The 8-mer substrates containing the

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1. The abbreviations used are: pol η, polymerase η; pol, polymerase; T-T, cis-syn cyclobutane pyrimidine; BDO, butadiene monoepoxide; BDE, butadiene diol epoxide; nt, nucleotide(s).
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(R,R)- and (S,S)-intrastrand BDE \(N^2\)-guanine-N\(^2\)-guanine cross-links were synthesized as published previously (19).

To construct the templates for polymerase reactions, each adducted oligonucleotide was ligated by T4 DNA ligase (New England Biolabs Inc., Beverly, MA) with two flanking oligonucleotides in the presence of the complement 45-mer scaffold. The ligation products were purified via denaturing polyacrylamide gel electrophoresis. The sequences containing the BDO and BDE \(N^2\)-guanine lesions are identical: 5’-AGAATGGGAAAGATCTGAGGACCCATTCACCACATGTGACTGGGA-3’ where the adducted G is underlined. The sequence containing the BDE \(N^2\)-guanine-N\(^2\)-guanine cross-link is as follows: 5’-CTGAGAATGGGAAAGATCTGAGGACCCATTCACCACATGTGACTGGGA-3’ where the cross-linked guanines are underlined.

Oligodeoxynucleotides of anion-exchange grade purity were used as primers in the polymerase reactions and were obtained from the Midland Certified Reagent Co. (Midland, TX). Their sequences include 5’-ACGACATTGGCCAGACATTGGGAAAGATCTGAGGACCCATTCACCACATGTGACTGGGGA-3’ as the 5’-primer for the BDE \(N^2\)-guanine-N\(^2\)-guanine cross-link-containing substrates, being complementary from positions 3–24. 5’-ACATTGGCCAGACATTGGGAAAGATCTGAGGACCCATTCACCACATGTGACTGGGGA-3’ was used as the 3’-primer for the BDE \(N^2\)-guanine-N\(^2\)-guanine cross-link-containing substrates, and 5’-ATGCCCAGACCAGACATTGGGAAAGATCTGAGGACCCATTCACCACATGTGACTGGGGA-3’ served as the 3’-primer for the DNAs containing the BDO and BDE \(N^2\)-guanine lesions, 5’-TGCCCAGACATTGGGAAAGATCTGAGGACCCATTCACCACATGTGACTGGGGA-3’ served as the 3’-primer for the \(N^2\)-guanine substrates. Primer oligodeoxynucleotides were phosphorylated with T4 polynucleotide kinase (New England Biolabs Inc.) using \(\gamma\)-\[^{32}\]P\)ATP (PerkinElmer Life Sciences). The \(\gamma\)-\[^{32}\]P\)-labeled primers were mixed with the oligonucleotide substrates in a molar ratio of 1:2 in the presence of 50 mM Tris-HCl (pH 7.0) and 100 mM NaCl, heated at 90 °C for 2 min, and slow cooled to room temperature. The completeness of the primer annealing was confirmed by electrophoresis through a native 7.5% polyacrylamide gel.

**pol \(\eta\) Purification**—The glutathione S-transferase-pol \(\eta\) fusion protein was overexpressed and purified as described previously (4).

**DNA Polymerase Assays**—The pol \(\eta\) polymerase assays were carried out essentially as described by Johnson et al. (4). The reaction mixture (10 \(\mu\)l) contained 25 mM potassium phosphate buffer (pH 7.0), 5 mM MgCl\(_2\), 5 mM dithiothreitol, 100 \(\mu\)g/ml bovine serum albumin, 10% glycerol, 100 \(\mu\)M dNTPs (each of the four dNTPs or one, as indicated), 5 nM primer annealed to a template, and 2 nM glutathione S-transferase-pol \(\eta\). After incubation at room temperature for 20 min, reactions were terminated by the addition of a 10-fold excess loading buffer consisting of 95% (v/v) formamide, 20 mM EDTA, 0.02% (w/v) xylene cyanol, and 0.02% (w/v) bromophen blue. The pol I (Klenow fragment) polymerase reactions were performed basically under the same conditions as the pol \(\eta\) reactions but in the presence of the buffer provided by the enzyme supplier (New England Biolabs Inc.). The reaction products were resolved through a 15% polyacrylamide gel containing 8% urea. Bands were visualized by autoradiography of the wet gels using Hyperfilm MP x-ray film (Amersham Pharmacia Biotech). Quantitative analyses of the results were performed using a PhosphorImager screen and ImageQuant 5.0 software (Molecular Dynamics, Sunnyvale, CA).

**Steady State Kinetic Analysis**—Steady state kinetic assays were carried out under the same conditions as the DNA polymerase assays except that 1 nM pol \(\eta\) and 10 nM DNA substrates were used with various concentrations of one of the four nucleotides, and reactions were quenched after 5 min. DNA band intensities were quantitated using the PhosphorImager (Molecular Dynamics) and then used to calculate the rate of nucleotide incorporation as described previously (21). The rate of nucleotide incorporation was graphed as a function of nucleotide concentration, and \(k_{\text{on}}\) and \(k_{\text{off}}\) parameters were obtained from the best fit of the data to the Michaelis-Menten equation.

**RESULTS**

**Translesion DNA Synthesis by pol \(\eta\) on the (R)- and (S)-BDO and (R,R)- and (S,S)-BDE \(N^2\)-Guanine-adducted DNA Substrates**—The structures of the BDO and BDE \(N^2\)-guanine streisomers, which were examined in this study, are shown in Fig. 1. Among the butadiene epoxide guanine species that are formed as a result of the butadiene exposure, the \(N^2\)-guanine adducts are relatively stable (16). In *E. coli*, replication efficiencies past the BDO and BDE \(N^2\)-guanines are significantly reduced in vivo, and the presence of these lesions in DNA is a complete block to synthesis by *E. coli* pol I, II, and III in vitro (18).

Primer extension reactions were carried out to test the ability of yeast pol \(\eta\) to perform translesion DNA synthesis on the BDO and BDE \(N^2\)-guanine-adducted DNA substrates (Fig. 2A). Primers were designed that provided “running start” (−6 primer) and “standing start” (−1 primer) conditions. As shown in Fig. 2A, yeast pol \(\eta\) replicated through all four butadiene lesions, resulting in full-length products. However, pol \(\eta\) displays a strong stall site one nucleotide before the DNA lesion (lanes 3–6), suggesting an inhibition of nucleotide incorporation opposite the lesion. Interestingly, the bypass efficiency of pol \(\eta\) seems to show stereospecificity. On the BDO \(N^2\)-guanine-containing substrates, as well as on the BDE \(N^2\)-guanine adducts, translesion DNA synthesis was more efficient in the case of the S stereoisomers.

Primers extension reactions using *E. coli* pol I (Klenow fragment) were also carried out on substrates containing the BDO and BDE \(N^2\)-guanine adducts (Fig. 2B). These data confirm previous reports that BDO and BDE \(N^2\)-guanines block DNA replication by pol I (18). This polymerase incorporated one nucleotide opposite the lesion but in contrast to the yeast pol \(\eta\), failed to extend the primer further on all four damaged substrates tested. Additionally, heterogeneity in the mobility of the final products (26-mer in the running start assays and 21-mer in the standing start assays) suggested nucleotide misincorporation in these reactions (lanes 3–6 and 9–12).

Next, the specificity of nucleotide incorporation by pol \(\eta\) opposite and downstream of these lesions was examined. To identify the nucleotide that was incorporated by pol \(\eta\) opposite the adducted base, single-nucleotide incorporation experiments were carried out using the −1 primer (Fig. 3). On a nondamaged substrate, pol \(\eta\) predominantly incorporated a C opposite G, but some T was also incorporated. In the case of the BDO and BDE \(N^2\)-guanine-containing substrates, a C residue was the only base that was incorporated opposite the lesions. Taking into account the lower efficiency of primer extension by pol \(\eta\) on the adducted templates, the substrate to enzyme ratio in the reaction was changed from 5:2 to 1:4. Under these conditions, no extended primers were left in the reactions on all five substrates tested when dTTP was in the incubation mixture (data not shown). Again, in the presence of the dTTP, no primer extension was observed on any of the damaged DNA substrates, but primer extension occurred on the nondamaged template.

Although in reactions with the −1 primer no significant level of misincorporation opposite the lesion was observed, the smearing of bands was noted one nucleotide beyond the lesion, particularly in the case of the (R)-BDO \(N^2\)-guanine-adducted
substrate (Fig. 2A). To determine whether this smearing was attributable to nucleotide misincorporation past the lesion site, single-nucleotide incorporation studies were performed on (R)-BDO N2-guanine-adducted template using a 0 primer, which contains a C opposite the damaged G. On this template, pol h extended 93% of the 0 primer with dCTP, 23% with dTTP, 6% with dATP, and 2% with dGTP (Fig. 4). A low level of primer extension was also observed in the presence of dTTP on the (S)-BDO N2-guanine-containing template. On the (R,R)- and (S,S)-BDE N2-guanine-containing templates, pol h incorporated only the C residue.

To test whether any misincorporation occurred beyond one nucleotide downstream of the lesion site, we performed single-nucleotide incorporation experiments using a -1 primer (Fig. 5). No nucleotide misincorporation was observed on any of the DNA substrates (5 nM) were incubated for 20 min at 22 °C with each of the four dNTPs (— no nucleotide added, G = dGTP, A = dATP, T = dTTP, C = dCTP) and S. cerevisiae pol η (2 nM). The positions of the 20-nt primer and 22-nt products are indicated. *, position of the nondamaged G or the adducted G on the template strand.

Fig. 2. DNA polymerase activity of S. cerevisiae pol η (A) and E. coli pol I (Klenow fragment) (B) under standing start and running start conditions on the monoepoxide- and diolepoxide-guanine-adducted templates. Each of the templates (ND = nondamaged, (R)-BDO = (R)-BDO N2-guanine, (S)-BDO = (S)-BDO N2-guanine, (R,R)-BDE = (R,R)-BDE N2-guanine, (S,S)-BDE = (S,S)-BDE N2-guanine) was annealed to the -1 primer. The DNA substrates (5 nM) were incubated for 20 min at 22 °C in the presence of all four dNTPs and S. cerevisiae pol η (2 nM) or E. coli pol I (Klenow fragment) (1 unit, as defined by New England Biolabs Inc.). Incubation of the nondamaged substrate under the same conditions but without polymerase was used as a negative control reaction. The positions of the 20-nt primers and the 51-nt (running start reaction) and 46-nt (standing start reaction) full-length products are indicated. *, position of the nondamaged G or the adducted G on the template.
damaged substrates examined, and pol η synthesized nearly the same amount of DNA on different damaged substrates when all four dNTPs were added to reactions.

To quantify the efficiency of pol η-catalyzed synthesis past each of the BDO- and BDE-modified N²-guanines, steady state kinetic analyses were performed with both −1 and 0 primers. As shown in Table I, incorporation of dCTP opposite the (S)-BDO and (S,S)-BDE N²-guanines (−1 primer extension) was 200–300-fold less efficient than incorporation opposite the unmodified guanine, whereas incorporation opposite the R stereoisomers was 2000–3000-fold less efficient than incorporation opposite the unmodified guanine. The reduced efficiency for incorporating dCTP opposite BDO- and BDE-adducted N²-guanines is primarily a $k_{\text{cat}}$ effect, not a $K_m$ effect. Thus, there is a block to inserting dCTP opposite these lesions, as is also demonstrated by the pause site just prior to the adduct in Fig. 2A. The extent of the blockage depended on the stereochemistry of the adduct, and this result agrees with the data presented in Figs. 2A and 3. Interestingly, there is little block to extending from the C residue paired with the BDO- or BDE-modified N²-guanine (kinetics of the dCTP incorporation in reactions with 0 primer), as is also demonstrated by the lack of a pause site at the site of the adduct (Fig. 2A). In contrast to nucleotide incorporation opposite the lesion, no differences in efficiencies of elongation from the resulting base pair were observed. Thus, bypass efficiencies by pol η on the BDO- or BDE-modified N²-guanines are limited at the step of the nucleotide incorporation opposite the lesion but not at the extension step.

To further evaluate the accuracy of pol η replication through BDO and BDE N²-guanine adducts, kinetic analyses of nucleotide misinsertion were carried out, and frequencies of misincorporation were calculated as the ratio of $h_{\text{cat}}/K_m$ of the incorrect nucleotide to the correct nucleotide (21). In reactions with the −1 primer, frequencies of misincorporation were below the limit of detection under conditions used for all four damaged substrates. Thus, pol η incorporates the correct nucleotide C quite accurately opposite N²-guanine modified with BDO or BDE. In experiments utilizing the 0 primer, high frequencies of misincorporation were observed in extension from C base-paired with the (R)-BDO N²-guanine. On this substrate, the frequencies of misincorporation were 2.0 × 10⁻³ for a T misincorporation and 6.2 × 10⁻⁴ for an A misincorporation. In all other cases, nucleotide misincorporation was below the limit of detection, which was approximately 5 × 10⁻⁴. Thus, kinetic data confirmed the results of the single-nucleotide incorporation experiment (Fig. 4), indicating that pol η is less accurate in extension from the base paired with (R)-BDO N²-guanine than with (S)-BDO N²-guanine.

### Lack of Bypass of (R,R)- and (S,S)-BDE N²-Guanine-N²-Guanine Cross-links by pol η—Structures of the BDE N²-guanine-N²-guanine cross-links are shown in Fig. 6. Cross-linked adducts are believed to contribute to butadiene-mediated carcinogenesis (22, 23). Previously, in E. coli, both (R,R)- and (S,S)-BDE N²-guanine-N²-guanine cross-links were shown to be extremely inhibitory to replicative bypass in vivo, and E. coli DNA pol I, II, and III were shown to be completely blocked on the templates containing these cross-links in vitro (19). To examine whether yeast pol η can bypass these lesions, primer extension experiments were performed. As shown in Fig. 7, on the (R,R)- as well as on the (S,S)-BDE N²-guanine-N²-guanine cross-link-containing substrates, DNA synthesis by pol η was

### Table I

| Substrate                        | dNTP          | $k_{\text{cat}}$ | $K_m$       | $h_{\text{cat}}/K_m$ | Relative efficiency |
|----------------------------------|---------------|-----------------|-------------|----------------------|---------------------|
|                                 |               | min⁻¹           | μM          | min⁻¹ · μM⁻¹          |                     |
| −1 primer                        |               |                 |             |                      |                     |
| Nondamaged guanine               | dCTP          | 0.78 ± 0.02     | 0.17 ± 0.02 | 4.6                  | 1.0                 |
| (R)-BDO N²-guanine               | dCTP          | 0.12 ± 0.01     | 80 ± 12     | 1.5 × 10⁻³           | 3.3 × 10⁻³          |
| (S)-BDO N²-guanine               | dCTP          | 0.23 ± 0.04     | 9.5 ± 3.2   | 2.4 × 10⁻²           | 5.2 × 10⁻³          |
| (R,R)-BDE N²-guanine             | dCTP          | 0.32 ± 0.04     | 120 ± 30    | 2.7 × 10⁻³           | 5.9 × 10⁻⁴          |
| (S,S)-BDE N²-guanine             | dCTP          | 0.71 ± 0.04     | 79 ± 12     | 9.0 × 10⁻³           | 3.7 × 10⁻³          |
| 0 primer                         |               |                 |             |                      |                     |
| Nondamaged guanine               | dCTP          | 0.46 ± 0.02     | 0.36 ± 0.05 | 1.3                  | 1.0                 |
| (R)-BDO N²-guanine               | dCTP          | 0.85 ± 0.09     | 1.4 ± 0.3   | 0.61                 | 0.47                |
| (S)-BDO N²-guanine               | dCTP          | 0.34 ± 0.04     | 0.66 ± 0.19 | 0.52                 | 0.40                |
| (R,R)-BDE N²-guanine             | dCTP          | 0.20 ± 0.02     | 0.86 ± 0.22 | 0.21                 | 0.16                |
| (S,S)-BDE N²-guanine             | dCTP          | 0.34 ± 0.02     | 0.62 ± 0.12 | 0.55                 | 0.42                |

*The ratio of $h_{\text{cat}}/K_m$ of the adduct-containing substrate versus the nondamaged substrate.*
Based on the ability of pol η to bypass a T-T dimer efficiently and accurately, it has been suggested that its active site is flexible enough to tolerate the distortion of the Watson-Crick geometry caused by the T-T dimer (6, 7, 24). However, such a flexibility of the polymerase active site should decrease its overall fidelity. Indeed, steady state kinetics assays of nucleotide incorporation have shown that pol η misincorporates nucleotides on undamaged DNA with frequencies of approximately 10^{-2} to 10^{-3} (6, 24, 25). Interestingly, the accuracy of replication by yeast as well as by the human pol η opposite a T-T dimer does not differ from that opposite nondamaged DNA (6, 7). The fact that both yeast (4, 26) and human (25) pol η do not possess any intrinsic proofreading exonuclease activity could explain in part the low fidelity of these polymerases. However, pol η has a lower fidelity than the other 3′→5′ exonuclease-deficient DNA polymerases (6, 24, 25), suggesting that its low fidelity derives from the relaxed requirement of its active site for correct base-pairing geometry. A flexible active site should enable pol η to bypass DNA lesions other than the T-T dimer. In agreement with this, both yeast and human pol η also bypass a 7,8-dihydro-8-oxoguanine lesion efficiently, and they do so by predominantly inserting a C opposite the lesion (11). In addition, both yeast (26) and human (27) pol η preferentially insert the correct nucleotide (C) opposite an N2-acetylaminofluorene-guanine. However, yeast pol η is unable to further extend DNA synthesis beyond the lesion (26). Human pol η can incorporate relatively efficiently one more nucleotide beyond the lesion, but only when the modified guanine is primed with a C (27).

Here it has been shown that yeast pol η can bypass (S)-BDO N2-guanine as well as (S,S)-BDE N2-guanine with 200–300-fold less efficient nucleotide insertion opposite the lesion relative to the nondamaged guanine. pol η can also bypass the (R)-BDO N2-guanine and (R,R)-BDE N2-guanine adducts, but these lesions pose an approximately 10-fold greater block to replication by pol η than their S stereoisomers. Thus, the efficiency of translesion DNA synthesis by yeast pol η is stereoisomer-specific. Blockage of the pol η-catalyzed replication through the BDO and BDE N2-guanines occurs at the step of the nucleotide insertion opposite the lesion, not at the extension step. In its ability to effectively extend synthesis past the BDO and BDE N2-guanine adducts, yeast pol η differs from E. coli pol I, which fails to continue DNA synthesis beyond the lesion. Single-nucleotide incorporation experiments on BDO- and BDE N2-guanine-containing substrates and steady state kinetic data indicate that lesion bypass by pol η can be error-prone at the step of postlesion replication and that the accuracy of translesion DNA synthesis at this step can also be stereoisomer-specific. On three out of four substrates tested, namely on (S)-BDO, (R,R)-BDE, and (S,S)-BDE N2-guanine DNA adducts, nucleotide insertion opposite the lesion as well as elongation from the resulting base pair appeared to be quite accurate. On the (R)-BDO N2-guanine-containing substrate, pol η inserted the correct nucleotide opposite the lesion, but it...
showed a tendency for nucleotide misincorporation in elongation from the resulting base pair.

Stereoisomeric BDE $N^2$-guanine-$N^2$-guanine intrastrand cross-links were also examined in this study. However, these lesions were a complete block to synthesis by yeast pol $\eta$, and in this case, synthesis terminated one base prior to the first adducted guanine. Interestingly, it has been recently demonstrated that human pol $\eta$ is capable of inserting a C opposite the first G of a cisplatin-GG intrastrand cross-link, but incorporation of the second C was highly inefficient, even using higher concentrations of pol $\eta$ in the reaction. When the cisplatin cross-link was primed with a CC opposite the lesion, bypass was achieved (27).

The $N^2$-guanine adducts of stereoisomeric 1,3-butadiene metabolites are a complete block to synthesis by E. coli DNA polymerases I, II, and III. In contrast, yeast pol $\eta$ can insert nucleotides opposite these lesions and is able to efficiently extend from the resulting base pair. The ability of yeast pol $\eta$ to bypass $N^2$-guanine butadiene adducts provides further support to the hypothesis (6, 7, 24) that in general, the pol $\eta$ active site tolerates geometric distortions within DNA caused by these and other DNA-damaging agents. However, the inability of pol $\eta$ to bypass an $N^2$-guanine-$N^2$-guanine intrastrand cross-link suggests that its active site is not flexible enough to adapt to the rather severe distortion imposed upon DNA by the cross-link.

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