Nucleotide incorporation fidelity, mismatch extension, and translesion DNA synthesis efficiencies were determined using SOS-induced Escherichia coli DNA polymerases (pol) II, IV, and V to copy 10R and 10S isomers of trans-opened benzo[a]pyrene-7,8-diol 9,10-epoxide (BaP DE) A and G adducts. A-BaP DE adducts were bypassed by pol V with moderate accuracy and considerably higher efficiency than by pol II or IV. Error-prone pol V copied G-BaP DE-adducted DNA poorly, forming A-G-BaP DE-S and -R mismatches over C-G-BaP DE-S and -R correct matches by factors of ~350- and 130-fold, respectively, even favoring G-G-BaP DE mismatches over correct matches by factors of ~2–4-fold. In contrast, pol IV bypassed G-BaP DE adducts with the highest efficiency and fidelity, making misincorporations with a frequency of $10^{-2}$ to $10^{-4}$ depending on sequence context. G-BaP DE-S-adducted M13 DNA yielded 4-fold fewer plaques when transfected into SOS-induced ∆dinB (pol IV-deficient) mutant cells compared with the isogenic wild-type E. coli strain, consistent with the in vitro data showing that pol IV was most effective by far at copying the G-BaP DE-S adduct. SOS polymerases are adept at copying a variety of lesions, but the relative contribution of each SOS polymerase to copying damaged DNA appears to be determined by the lesion’s identity.

Recent studies suggest that in addition to DNA polymerases (pol)I–III, which are prototypes of the A-, B-, and C-polymerase families, respectively (1, 2), Escherichia coli possesses two members of the recently described Y-family of DNA polymerases (3). This new polymerase family is typified by the UmuD and UmuC mutants that the principal role of pol V is to bypass template bases that effectively block normal pol III-catalyzed DNA replication because deletion of the umuC operon or missense mutations in either umuD or umuC effectively renders E. coli cells non-mutable, despite being exposed to a variety of mutagens (8–10). Translesion DNA synthesis catalyzed by pol V results in mutations targeted directly opposite DNA damage sites. However, in addition to its primary role in replicating across from damaged bases, pol V also causes untargeted base substitution mutations in the absence of DNA damage (11).

pol IV is responsible for generating frameshift mutations on undamaged DNA, causing adaptive mutations in non-dividing cells (12, 13), mutations that are kept in check by the presence of pol II (14). It is possible, however, that the primary role of pol IV may be to rescue stalled replication forks, which seem to occur at least once per round of replication (15), possibly by extending slipped mispairs that are refractory to proofreading. Genetic experiments also implicate pol IV in translesion synthesis (TLS) of certain types of lesion such as acetylaminofluorene-modified G residues as well as benzo[a]pyrene-7,8-diol 9,10-epoxide (BaP DE) G-adducted DNA (16).

pol II, an SOS-induced DNA polymerase (17–19) that is the prototype for the B-family of polymerases (18), is a high fidelity enzyme containing an associated 3’-exonuclease proofreading activity (20). Although the main cellular role of pol II is believed to be in “error-free” replication restart (21), it also can copy specific DNA lesions in vivo (16, 22).

In this study, we have investigated the ability of the five E. coli DNA polymerases to copy bulky adducts derived from trans-ring opening of bay region 7,8-diol 9,10-epoxide metabolites of the polycyclic aromatic hydrocarbon benzo[a]pyrene (BaP DE; see Fig. 1A). Two 7,8-diol 9,10-epoxide diastereomers, each consisting of a pair of enantiomers, are formed metabolically in mammals. These BaP DEs are mutagenic in bacterial and mammalian cells (23), and the predominant isomer formed metabolically, the (+)-enantiomer of the diastereomer (shown in Fig. 1A), whose benzylic hydroxyl group and epoxide oxygen are trans, is highly tumorigenic in mice (24). DNA damage by...
BaP DEs, which target the exocyclic amino groups of purines in DNA (see Fig. 1A) (25), is most likely an important initiating event in both mutagenesis and cell transformation. Replicative DNA polymerases are generally blocked by bulky adducts (26), and human pol α replicates at best very poorly opposite BaP DE adducts (27), as does the non-SOS-induced E. coli polymerase I Klenow fragment (28, 29). Thus, it is attractive to speculate that error-prone TLS by bypass polymerases may be highly significant in the observed induction of mutations by these adducts, especially in SOS-induced cells (30, 31). Two central concepts are then compared with in vivo data in δPol (pol II), ΔdinB (pol IV), and ΔumuDC (pol V) mutant backgrounds to address the extent to which the model in vitro measurements correlate with in vivo results.

**EXPERIMENTAL PROCEDURES**

**Materials—**Ultrapure ATP, dNTPs, E. coli single-stranded DNA-binding protein (SSB), and RecA protein were purchased from New England Biolabs Inc. ATP-S was purchased from Roche Molecular Biochemicals. E. coli DNA polymerase I Klenow fragment (KF) and its exonuclease derivative were purchased from New England Biolabs Inc. pol II, pol II exo−, pol III subunits, pol IV (DinB), and pol V (UmuD′C′) were purified as previously described (32–34); and all are native enzymes, except for pol IV, which is linked at its N terminus to a maltose-binding protein. BaP DE-containing oligonucleotides were made as previously described (29, 30). Three sequence contexts (one for A and two for G adducts) that had previously been used to examine the mutational consequences of these adducts in an E. coli M13 system were used (30). For each of these adducts, two diastereomers are possible, corresponding to an S or R configuration at the point of attachment of the hydrocarbon to the purine base (see Fig. 1A); and both isomers, the BaP DE-S and -R adducts, respectively, were examined. Linear BaP DE-containing DNA templates used in the lesion bypass assay and fidelity study were prepared by ligation of linearized single-stranded M13mp7 DNA with BaP DE-containing oligonucleotides and a synthetic 48-mer as previously described (35). BaP DE-containing circular M13mp7 DNA used in E. coli transfections was prepared as described (30).

**In Vitro Translesion-Synthesis Analysis—**28-mer and 120-mer labeled 28-mer primers were annealed to the BaP DE-containing template so that the 3’-end of the primer was situated either 2 or 48 nucleotides before the lesion site. The reaction mixtures contained 20 mM Tris-HCl (pH 7.5), 8 mM MgCl₂, 5 mM dithiothreitol, 0.1 mM EDTA, 25 mM sodium glucose-maltose, 40 μg/ml bovine serum albumin, 4% glycerol, 2 mM primer-template, and four dNTPs at 0.5 mM each. pol I KF and pol I KF exo− were used. reactions pol II and pol II exo− were present in the reactions at 20 nm. The pol III α-subunit, pol IV, and pol V were present at 10, 50, and 200 nm, respectively. For pol II, pol II exo−, pol IIIα, pol IV, and pol V, βγ complex, SSB, and ATP or ATP-S (for pol V) were included in the reaction at 40 nm, 10 nm, 300 nm, and 1 nm, respectively. Reactions with pol V also included 1 μM RecA. Reactions were carried out at 37 °C for 10 min and quenched by addition of an equal volume of 40 mM EDTA and 95% formamide. Primer extension products were heat-denatured and resolved on a denaturing polyacrylamide gel. Integrated gel band intensities were measured with a PhosphorImager using ImageQuant software (Molecular Dynamics, Inc.). The bypass efficiencies were measured as the fraction of primers extended beyond the lesion site X: \[ \text{X} = \frac{\text{IG}_{1} + \text{IG}_{2} + \text{I}_{1} + \text{I}_{2}}{\text{P}_{x}}, \] where \( \text{IG}_{1} \) and \( \text{IG}_{2} \) are the integrated gel band intensities measured directly opposite the lesion site X and 1 base prior to the lesion site X − 1, respectively.

**Nucleotide Misincorporation and Mismatch Extension Analysis—**A standard gel kinetic assay was used to measure the misincorporation and mismatch extension fidelity as previously described (36, 37). The concentration of primer-template DNA in the reactions was 2 nm. The reaction conditions were the same as those used in the translesion synthesis analysis except that different sets of primers were annealed to the template, and incorporation of individual dNTP substrates was measured as a function of concentration to determine apparent Vₘₐₓ and Kₘ values for each substrate. Standing-start primer extension reactions (38) were performed with incubation times chosen to satisfy single-completed-hit conditions, i.e. <20% of the input primer molecules were extended (36, 38). Apparent Vₘₐₓ and Kₘ values were determined by plotting primer extension velocity versus dNTP concentration, fitting the data to a rectangular hyperbola with nonlinear least-squares regression using SigmaPlot. The misincorporation efficiency (fₘ) was determined as the ratio of apparent Vₘₐₓ/Kₘ for incorrect incorporation to apparent Vₘₐₓ/Kₘ for correct incorporation. The intrinsic mismatch extension efficiency (fₑ) was determined as the ratio of apparent Vₑ/Kₑ for mismatch extension to apparent Vₑ/Kₑ for correct match extension (36, 37).

**BaP DE-containing M13 Transfection and Analysis of Progeny Bacteriophage—**The transfection procedure was the same as previously described (30) with the following modifications. AB1157 (F') was used in the wild-type strain, with CHS50 (F') added as the indicator prior to plating. STL1336 (AB1157 with δpol::B::TII and SR1157U (AB1157 with ΔumuDC::cat) were used as pol II-deficient and pol V-deficient strains, respectively. The pol IV-deficient strain was constructed by P1 transduction into AB1157 using a lysate from RW625 (AB1157 with dnaE486 dpm502::Tn10 δpol::B::TII ΔdinB::cat), which is inserted for the associated kanamycin resistance of the ΔdinB allele. M13 DNAs containing A-BaP DE-S and G-BaP DE-S adducts in contexts I and IV (see Fig. 1) and unadded control DNAs were used in parallel for comparison. pol II, IV, and V were induced by UV irradiation as noted in Table IV. Analysis of progeny bacteriophage was carried out by a combination of probe hybridization and sequencing following the protocol referred to as “Experiment 1” in Ref. 30.

**RESULTS**

**BaP DE Lesion Bypass in Vitro Using the Five E. coli DNA Polymerases—**All five E. coli DNA polymerases were used to copy each of the two diastereomeric (10S and 10R) transopened A- and G-BaP DE adducts. The A adducts were examined in context I, and the G adducts in contexts III and IV (Fig. 1). The notation for the oligonucleotide sequence contexts was adopted from a previous in vivo study (30). Two running-start primer-template DNAs were used, one with a primer 3′-end located 2 nucleotides from the lesion (Fig. 2, upper panels) and the other with the primer end 48 nucleotides upstream from the lesion (Fig. 2, lower panels). TLS efficiencies for the three SOS polymerases (pol II, IV, and V) are calculated in Table I.

Each of the five polymerases was able to catalyze nucleotide incorporation, at least to some extent, directly opposite A-BaP DE adducts with both 10S and 10R configurations (Fig. 2). However, only two of the polymerases, pol I KF and pol V, were able to replicate beyond the lesion (Fig. 2, lanes 1 and 6). Bypass of either A adduct isomer by KF was weak (~3–5%) efficiency even in the presence of a large enough excess of KF to extend almost all of the input primer (Fig. 2, lower left panel, lane 1). The pol V-catalyzed TLS required RecA + SSB and was stimulated in the presence of βγ processivity factors (data not shown). Lesion bypass by pol V was processive and much more robust than poorly hydrolyzable ATP-S was used in place of ATP for assembling the RecA nucleoprotein filament (Fig. 2, compare lanes 5 and 6; and Table I). Contact between pol V and the 3′-tip of the RecA nucleoprotein filament is required both for TLS to occur and for pol V to remain bound at a primer 3′-end (39–41). Disassembly of the filament in the 5′ to 3′ direction occurred in the presence of ATP, but not ATP-S (42, 43), causing termination of pol V synthesis roughly 1–4 bases downstream from the lesion (Fig. 2, upper left panel, lane 5 for both the S and R isomers). pol V showed a somewhat greater TLS efficiency when replicating beyond the R rather than the S isomer (Table I, ATP-S column).

With each of the two diastereomeric G adducts in both sequence contexts, all five polymerases could incorporate a base opposite the adduct. Incorporation at the lesion site was most easily seen when the enzymes were given a long running start (primer 2) (Fig. 2, lower panels). The efficiencies of nucleotide incorporation at the lesion site varied, as did the efficiencies with which further primer extension (TLS) occurred beyond this site. Both pol IV and V carried out TLS past G-BaP DE-R
moieties, with pol IV being much more efficient. Thus, pol IV is by far the best at primer extension past the G-BaP DE adducts and is clearly better than pol V, which was most efficient for TLS at A adducts (Table I). Of the four G lesions (two isomers in two sequence contexts), three were bypassed by pol IV with similar high efficiencies (∼70%), and the fourth (G-BaP DE-R in context III) was bypassed about half as well. pol V exhibited a modest diastereomer preference for bypassing the G-BaP DE-R isomer in both sequence contexts, and the only apparent effect of sequence context on pol V-catalyzed TLS was that the “favored” G-BaP DE-R isomer in context IV was bypassed to a significant extent (7%) even when the RecA filament was assembled in the presence of ATP (Table I).

In summary, pol IV bypassed only G-BaP DE adducts, favoring the R over the S isomer in one context but not in the other, whereas pol V bypassed both A- and G-BaP DE lesions to about

![Diagram](image.png)

**Fig. 1.** Structures of trans-opened G- and A-BaP DE adducts. A, structures of the adducts in DNA and their relationship to the parent BaP DE diastereomer, whose C-7 hydroxyl group and epoxide oxygen are trans. The configuration at the benzylic epoxide carbon (C-10) inverts upon opening the ring by the nucleic acid base. B, diagram of BaP DE-containing DNA templates and primers used for in vitro translesion synthesis assay. Single-stranded M13mp7 DNA was linearized and ligated with a BaP DE-containing 16-mer and a 48-mer. The 16-mer sequence and BaP DE adduct configuration are varied as shown. Two 28-mers (primers 1 and 2) were synthesized as the running-start primers for BaP DE translesion synthesis assay. Primer 1 is close to the BaP DE site, with its 3′-end situated 2 nucleotides upstream of the BaP DE site when annealed to the template, whereas the 3′-end of primer 2 is situated 48 nucleotides upstream of the BaP DE site.
the same extent, favoring the R over the S isomer in a sequence context-independent manner. The replicative pol III carried out limited synthesis (5.1%) past the G-BaP DE-R isomer in context IV, but no significant synthesis past G-BaP DE-S (Fig. 2, lower panels). No detectable synthesis beyond either G-BaP DE isomer was observed for pol III in context III.

Fidelity of Nucleotide Incorporation and Extension at BaP DE-adducted Template Sites—The induction of the SOS response increases both phage survival and mutations in M13 phage containing BaP DE adducts. Because both survival and mutagenesis are likely to be impacted by the absence or presence of one or more of the SOS-induced polymerases, we measured the efficiency of translesion synthesis catalyzed by SOS DNA polymerases.

**TABLE I**

Table 1: BaP DE translesion synthesis efficiencies catalyzed by SOS DNA polymerases

| BaP DE adduct | pol II | pol IV | pol V   |
|---------------|--------|--------|---------|
|               | %      | %      | ATP     | ATPγS   |
| Context I     |        |        |         |         |
| A-BaP DE-S    | ND     | ND     | 1.2     | 8.2     |
| A-BaP DE-R    | ND     | ND     | 1.4     | 22.9    |
| Context III   |        |        |         |         |
| G-BaP DE-S    | ND     | ND     | 36.3    | 13.8    |
| G-BaP DE-R    | ND     | ND     | 71.3    | 23.5    |
| Context IV    |        |        |         |         |
| G-BaP DE-S    | 1.4    | 70.9   | 7.3     |         |
| G-BaP DE-R    | 12.5   | 66.8   | 24.7    |         |

*Lesion bypass experiments for a BaP DE-G adduct in context III were carried out using proofreading-deficient pol II exo− in place of wild-type pol II.*

**FIG. 2. In vitro translesion synthesis on BaP DE-adducted DNAs by E. coli DNA polymerases.** ³²P-Labeled primer extension was carried out on BaP DE-containing DNA templates as described under “Experimental Procedures.” Extension from primers 1 and 2 is shown in the upper and lower panels respectively. The positions corresponding to the unextended primer, extension to the BaP DE site, and full extension to the end of the template are marked on the left. E. coli DNA polymerases applied in each lane were as follows: lane 1, pol I KF (lane 1*, context III, pol I KF exo−); lane 2, pol II (lane 2*, context III, pol II exo−) + β-γ complex, SSB, and ATP; lane 3, pol IIIα + β-γ complex, SSB, and ATP; lane 4, pol IV + β-γ complex, SSB, and ATP; lane 5, pol V + β-γ complex, SSB, RecA, and ATP; lane 6, pol V + β-γ complex, SSB, RecA, and ATPγS.
ured the relative nucleotide incorporation and primer extension efficiencies (Tables II and III) for the three SOS polymerases (pol II, IV, and V) at the A-BaP DE-S and -R and G-BaP DE-S and -R adducts using the sequence contexts shown in Fig. 1. The nucleotide incorporation specificities for the three SOS-induced polymerases are unusual, with major differences in pol V, IV, and pol II incorporation and extension fidelities depending on sequence context, adduct configuration at C-10 (R or S), and whether the adduct is at A or G.

**pol V Nucleotide Incorporation and Extension Fidelity at BaP DE Template Adducts**—pol V catalyzed misincorporation of A opposite G-BaP DE-S in context III by a factor of 350-fold greater than the “correct” incorporation of C (Table II). An anomalously high misincorporation specificity was also observed with G-BaP DE-R, where formation of an A-G-BaP DE-R mispair was favored by a factor of 132-fold compared with a C-G-BaP DE-R correct pair (Table II). Thus, pol V strongly favors making G-C to T-A transversions in this sequence context. Both A-G-BaP DE mispairs were also extended with higher efficiency than their correctly paired counterparts: 23-fold greater for the A-G-BaP DE-S isomer compared with C-G-BaP DE-S and 3.9-fold greater for the corresponding matched and mismatched G-BaP DE-R isomers in context III (Table III).

An important point is that the A-G-BaP DE misincorporation errors in context III are unlikely to result from a simple template slippage event (44, 45), with the lesion out of the helical plane, because the template base immediately downstream from the adduct is not a T, but is instead a C. The favored misincorporation of A opposite G-BaP DE also cannot be caused by backward slippage of the primer relative to the template strand. This type of misalignment event is precluded in context III because an incoming dATP substrate is unlikely to be incorporated readily opposite the As on the template strand immediately upstream from the lesion (Fig. 1). In contrast, backward slippage events should be more likely to result in misincorporation of A opposite the G adduct in context IV because in this sequence, two Ts are just upstream from the lesion. Remarkably, however, A-G-BaP DE-S and -R mispairs have f_{inc} values of “only” 0.78 and 0.18, respectively, in context IV, compared with 350 and 132 in context III, a result clearly inconsistent with backward slippage of the primer. The lower frequency of A-G-BaP DE mispairs relative to correct pairs in sequence context IV, might, however, result from a loop-out of the adducted G that could enable pol V to use an unmodified G downstream in this sequence as a template for incorporating a correct C.

G-G mispairs in context III, which are favored over C-G base pairs by factors of 2- and 3.9-fold for S and R isomers, respectively, could have resulted from a similar primer-template misalignment with the modified base looped out of the helical plane, allowing incorporation of G opposite the downstream template C. However, we think it is much more likely that G is incorporated directly opposite the G-BaP DE adduct because the very high apparent K_m values (0.74 nM for the R isomer and >1 nM for the S isomer) (data not shown) are characteristic of direct misincorporations (46, 47). Much smaller apparent K_m values intermediate in magnitude between Watson-Crick base pair formation and non-Watson-Crick nucleotide misincorporation are a hallmark of correct incorporations on misaligned primer-template DNA (46, 47). Once formed, both G-BaP DE-S and -R mispairs were extended more efficiently than the corresponding correctly matched C-G-BaP DE pairs: 2.2-fold for the R isomer and 1.8-fold for the S isomer (Table III).

pol V is the only SOS polymerase that could bypass the A-BaP DE adducts efficiently (Fig. 2). The major errors observed when pol V copied A-BaP DE were A-A-BaP DE-R mispairs (f_{inc} = 0.23) and A-A-BaP DE-S mispairs (f_{inc} = 0.08). Thus, the primary mutational event when pol V copies an A-BaP DE moiety is expected to be an A-T to T-A transversion.

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TABLE II

| Template | dNTP | pol II exo | pol IV | pol V |
|----------|------|-----------|--------|--------|
|          |      | V_{max/K_m} | f_{inc} | V_{max/K_m} | f_{inc} | V_{max/K_m} | f_{inc} |
|          |      | % min^{-1} μM^{-1} |        | % min^{-1} μM^{-1} |        | % min^{-1} μM^{-1} |        |
| Context I |      |             |        |             |        |             |        |
| A-BaP DE-S | dCTP | A* 2.3 × 10^{-3} | 1 | 2.6 × 10^{-2} | 1 | 1 × 10^{-3} | 1 |
|           | A* 1.8 × 10^{-4} | 8.0 × 10^{-2} |        | 9.3 × 10^{-3} | 3.6 × 10^{-2} |        | 9.3 × 10^{-3} | 1.2 × 10^{-1} |
|           | dCTP | A* 1.3 × 10^{-4} | 5.7 × 10^{-2} |        | 3.1 × 10^{-3} | 1.2 × 10^{-1} |        | 5.0 × 10^{-5} | 5.6 × 10^{-3} |
| A-BaP DE-R | dCTP | A* 1.6 × 10^{-2} | 1 | 1.3 × 10^{-3} | 1 | 1.0 × 10^{-3} | 1 |
|           | A* 1.0 × 10^{-4} | 6.3 × 10^{-3} |        | 2.1 × 10^{-5} | 1.6 × 10^{-2} |        | 5.5 × 10^{-4} | 2.3 × 10^{-1} |
|           | dCTP | A* 6.7 × 10^{-4} | 4.1 × 10^{-2} |        | 1.5 × 10^{-5} | 1.1 × 10^{-2} |        | 6.2 × 10^{-5} | 2.6 × 10^{-2} |

| Context III |      |             |        |             |        |             |        |
| G-BaP DE-S | dCTP | G* 1.1 × 10^{-5} | 1 | 3.8 × 10^{-1} | 1 | 6.9 × 10^{-6} | 1 |
|           | G* 4.2 × 10^{-3} | 380 |        | 1.5 × 10^{-5} | 2.1 |        | 2.4 × 10^{-3} | 350 |
|           | G* ND | ND |        | ND | ND |        | ND | ND |
| G-BaP DE-R | dCTP | G* 9.6 × 10^{-6} | 1 | 6.6 × 10^{-5} | 1.8 × 10^{-4} |        | 5.2 × 10^{-5} | 1 |
|           | G* 3.5 × 10^{-4} | 36 |        | 2.9 × 10^{-1} | 1 |        | 2.0 × 10^{-4} | 3.9 |
|           | G* 1.8 × 10^{-4} | 19 |        | 4.0 × 10^{-5} | 1.4 × 10^{-4} |        | 6.9 × 10^{-3} | 132 |
|           | G* 6.0 × 10^{-5} | 6.3 |        | 2.8 × 10^{-3} | 9.5 × 10^{-3} |        | 1.8 × 10^{-5} | 3.5 × 10^{-1} |

| Context IV |      |             |        |             |        |             |        |
| G-BaP DE-S | dCTP | G* 2.0 × 10^{-3} | 1 | 1.1 × 10^{-1} | 1 | 3.3 × 10^{-5} | 1 |
|           | G* 7.7 × 10^{-5} | 3.8 × 10^{-2} |        | 8.6 × 10^{-5} | 7.9 × 10^{-4} |        | 7.8 × 10^{-1} | ND |
|           | G* 1.4 × 10^{-4} | 6.9 × 10^{-2} |        | 1.7 × 10^{-3} | 1.6 × 10^{-2} |        | 2.6 × 10^{-5} | ND |
|           | G* 2.9 × 10^{-4} | 1.4 × 10^{-1} |        | ND | ND |        | ND | ND |
| G-BaP DE-R | dCTP | G* 2.9 × 10^{-3} | 1 | 3.0 × 10^{-2} | 1 | 3.8 × 10^{-3} | 1 |
|           | G* 1.7 × 10^{-4} | 5.8 × 10^{-2} |        | 1.5 × 10^{-3} | 5.1 × 10^{-2} |        | 4.3 × 10^{-5} | 1.2 × 10^{-2} |
|           | G* 1.1 × 10^{-4} | 3.9 × 10^{-2} |        | 5.9 × 10^{-4} | 2.0 × 10^{-2} |        | 6.6 × 10^{-4} | 1.8 × 10^{-1} |
|           | G* 1.4 × 10^{-4} | 4.9 × 10^{-2} |        | 6.7 × 10^{-5} | 2.3 × 10^{-3} |        | 3.5 × 10^{-4} | 9.1 × 10^{-2} |

* A* and G* represent A- and G-BaP DE-adducted sites on the template.
Copying BaP DE Adducts with SOS-induced Polymerases

**TABLE III**

Intrinsic mismatch extension efficiencies \( f_{\text{ext}} \) (intrinsic mismatch extension efficiencies) were calculated as the ratio of apparent \( V_{\text{max}}/K_m \) for extension of incorrect to correctly paired primer 3'-ends (see "Experimental Procedures"). The standard errors for \( f_{\text{ext}} \) are approximately ±30%, ND, not detectable.

| Template | Primer 3'-end · template | \( V_{\text{max}}/K_m \) | \( f_{\text{ext}}^0 \) | \( V_{\text{max}}/K_m \) | \( f_{\text{ext}}^0 \) | \( V_{\text{max}}/K_m \) | \( f_{\text{ext}}^0 \) |
|----------|---------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| A-BaP DE-S | T · A*                     | 3.5 × 10^{-2}          | 1                      | 8.0 × 10^{-3}          | 1                      | 1.2 × 10^{-2}          | 1                      |
|           | A*                        | 1.8 × 10^{-2}          | 5.1 × 10^{-3}          | 9.9 × 10^{-3}          | 1.2                    | 1.5 × 10^{-3}          | 1.3                    |
|           | G · A*                    | 2.5 × 10^{-3}          | 7.1 × 10^{-4}          | 4.4 × 10^{-3}          | 5.5 × 10^{-3}          | 4.4 × 10^{-5}          | 3.7 × 10^{-3}          |
|           | C · A*                    | 1.2 × 10^{-3}          | 3.4 × 10^{-4}          | 3.9 × 10^{-4}          | 4.9 × 10^{-4}          | 1.4 × 10^{-2}          | 1.2                    |
| A-BaP DE-R | T · A*                     | 2.8 × 10^{-2}          | 1                      | 7.0 × 10^{-5}          | 1                      | 1.3 × 10^{-2}          | 1                      |
|           | A*                        | 2.7 × 10^{-3}          | 9.6 × 10^{-4}          | 3.2 × 10^{-3}          | 46                     | 4.6 × 10^{-3}          | 3.5 × 10^{-1}          |
|           | G · A*                    | 4.3 × 10^{-5}          | 1.5 × 10^{-2}          | 8.3 × 10^{-4}          | 12                     | 4.3 × 10^{-3}          | 3.3 × 10^{-1}          |
|           | C · A*                    | 3.1 × 10^{-3}          | 1.1 × 10^{-4}          | ND                     | ND                     | 5.6 × 10^{-4}          | 4.3 × 10^{-3}          |
| G-BaP DE-S | C · G*                    | ND                     | ND                     | 6.6 × 10^{-1}          | 1                      | 1.3 × 10^{-3}          | 1                      |
|           | G*                        | ND                     | ND                     | 2.2 × 10^{-5}          | 3.3 × 10^{-5}          | 2.3 × 10^{-3}          | 1.8                    |
|           | A*                        | ND                     | ND                     | 1.2 × 10^{-3}          | 1.8 × 10^{-3}          | 3.0 × 10^{-3}          | 23                    |
|           | T · G*                    | ND                     | ND                     | 2.1 × 10^{-3}          | 3.2 × 10^{-3}          | 8.3 × 10^{-3}          | 6.4                    |
| G-BaP DE-R | C · G*                    | 1.0 × 10^{-4}          | 1                      | 20                     | 1                      | 1.0 × 10^{-2}          | 1                      |
|           | G*                        | 1.9 × 10^{-4}          | 1.9                    | 3.0 × 10^{-5}          | 1.5 × 10^{-4}          | 2.2 × 10^{-2}          | 2.2                    |
|           | A · G*                    | 2.7 × 10^{-3}          | 27                     | 2.0 × 10^{-2}          | 1.0 × 10^{-3}          | 3.9 × 10^{-2}          | 3.9                    |
|           | T · G*                    | ND                     | ND                     | 2.5 × 10^{-2}          | 1.3 × 10^{-3}          | 8.2 × 10^{-3}          | 8.2 × 10^{-1}          |
| G-BaP DE-S | C · A*                    | 1.8 × 10^{-5}          | 1                      | 2.0 × 10^{-2}          | 1                      | 5.1 × 10^{-4}          | 1                      |
|           | G*                        | ND                     | ND                     | 5.1 × 10^{-5}          | 2.5 × 10^{-3}          | 5.0 × 10^{-5}          | 9.8 × 10^{-2}          |
|           | A*                        | ND                     | ND                     | 1.4 × 10^{-4}          | 7.1 × 10^{-3}          | 1.1 × 10^{-3}          | 2.1                    |
|           | T · G*                    | ND                     | ND                     | 1.4 × 10^{-4}          | 7.1 × 10^{-3}          | ND                     | ND                     |
| G-BaP DE-R | C · G*                    | 4.9 × 10^{-2}          | 1                      | 9.1 × 10^{-5}          | 1                      | 3.5 × 10^{-3}          | 1                      |
|           | G*                        | 9.5 × 10^{-5}          | 1.9 × 10^{-4}          | 2.6 × 10^{-4}          | 3.2 × 10^{-4}          | 4.8 × 10^{-5}          | 1.4 × 10^{-2}          |
|           | A · G*                    | 6.5 × 10^{-6}          | 1.3 × 10^{-3}          | 2.4 × 10^{-3}          | 3.0 × 10^{-3}          | 2.2 × 10^{-3}          | 6.3 × 10^{-3}          |
|           | T · G*                    | 5.8 × 10^{-3}          | 1.2 × 10^{-4}          | 6.7 × 10^{-4}          | 8.3 × 10^{-4}          | 3.7 × 10^{-5}          | 1.1 × 10^{-2}          |

\( A^* \) and \( G^* \) represent A- and G-BaP DE-adducted sites on the template.
TABLE IV
Survival of BaP DE-adducted DNA transfected into wild-type and SOS polymerase-null mutant backgrounds in the presence and absence of SOS induction

| Context I | Wild-type | ΔpolB | ΔdinB | ΔumuDC |
|-----------|-----------|-------|-------|--------|
| Non-adducted control DNA | 580\(^a\) | 855 | 372 | 424 |
| A-BaP DE-S-adducted DNA | 4 | 49 | 1 | 26 |
| Survival (%) | 0.7\(^b\) | 5.7 | 0.3 | 6.1 |

| Context IV | Wild-type | ΔpolB | ΔdinB | ΔumuDC |
|-----------|-----------|-------|-------|--------|
| Non-adducted control DNA | 1300 | 1090 | 486 | 684 |
| G-BaP DE-S-adducted DNA | 113 | 206 | 2 | 138 |
| Survival (%) | 8.7 | 18.9 | 0.4 | 20.2 |

\(^a\) Numbers are the M13 progeny plaques formed following transfection. These values represent the average from three independent experiments.

\(^b\) Survival percent was calculated as the ratio of the plaque number from BaP DE-adducted DNA to that from the corresponding control DNA. Equal concentrations of non-adducted DNA were used in the control experiments.

...2-fold better than the correct C-G-BaP DE-R (Table III). Unexpectedly, an A-G-BaP DE-R mismatch, which was formed 19-fold more efficiently than C-G-BaP DE-R by direct misincorporation of A (Km = 1 mM) (data not shown), was extended 27-fold better than either G-G- or C-G-BaP DE-R (Table I). Yet, the incorporation of A opposite the G-BaP DE-S isomer was not detectable, nor was a preformed A-G-BaP DE-S mispair extendable in sequence context III (Tables II and III). Proofreading-defective pol II copied A-BaP DE adducts somewhat accurately, with the highest error rates being 8% for incorporation of A opposite A-BaP DE-S and 5.7 and 4.1% for incorporation of G opposite the A-BaP DE-S and -R isomers, respectively (Tables II and III). Extension of the correct pair was favored over extension of the three mispairs for both isomers (Tables II and III).

The most striking results are the 4-fold reduction in survival when replicating a G-BaP DE-S adduct in SOS-induced cells lacking pol IV and the loss of ability to copy this same adduct when replicating a G-BaP DE-S adduct in SOS-non-induced cells when any one of the SOS polymerases was missing (Fig. 3B and Table IV).

DISCUSSION

The related UmuC/DinB/RevI/Rad30 polymerases, recently renamed the Y-family of DNA polymerases (3), appear to be earmarked for use in specialized DNA synthesis reactions, including copying damaged DNA, rescuing stalled replication forks, meiosis-associated DNA repair, and somatic hypermutation (6, 49, 50). The relationship between the in vivo function and biochemical properties of pol V has been investigated extensively with respect to its role during SOS mutagenesis in E. coli (33, 35, 39, 41). pol V, acting in conjunction with RecA, SSB, and β-γ processivity proteins, is primarily responsible for copying UV-damaged DNA such as TT cis-syn photodimers and TT (6-4) photoproducts (33) and abasic template lesions (33, 39, 41, 51), with fidelities corresponding to in vivo mutational spectra for these three lesions (35). There are two additional SOS-induced DNA polymerases, pol IV, another Y-family member encoded by dinB (52), and pol II (17–19). pol IV causes frameshift errors in λ phage (53) and is responsible for generating "adaptive" frameshift mutations in non-dividing E. coli (12, 13), whereas pol II plays a pivotal role in error-free replication restart in UV-irradiated cells (21) and also acts to reduce adaptive mutation levels (14, 54). Thus, pol IV and II are believed to carry out specialized reactions principally involving undamaged DNA, whereas the role of pol V seems restricted to copying damaged DNA.
It is crucial, however, to avoid “pigeonholing” the SOS polymerases into categories of those that copy either damaged or undamaged DNA, especially because so little is known concerning their actual cellular occupations. Although the genetic (55–57) and biochemical (35) data support a primary, perhaps exclusive role for pol V in copying UV-damaged DNA and abasic lesions, different polymerases might well be used to copy other types of damaged or modified template bases. Previous examples that support this point in *E. coli* include the use of pol II to copy abasic sites in the absence of heat shock proteins (22) and the involvement of both pol II and V (but not pol IV) in copying an N-acetyl-2-aminofluorene G adduct (16). In a reversal of logic, it turns out that the high fidelity pol II generates frameshifts during TLS of acetylaminofluorene G, whereas the low fidelity pol V is responsible for error-free lesion bypass (16). Most closely related to our experiments are data showing that...
pol IV and V carry out error-free and ~1 frameshift TLS, respectively, when copying a G-BaP DE adducted template in vivo (16).

SOS Polymerase-catalyzed BaP DE Adduct Bypass Efficiencies—G-BaP DE adducted DNA were bypassed most efficiently by pol IV in vitro, irrespective of isomer and sequence context, with TLS efficiencies ~3–10-fold higher than those for pol V (Fig. 3A and Table I). pol II was able to bypass G-BaP DE, albeit with poor efficiency, in just one of the two sequence contexts (context IV) (Fig. 3A and Table I). The TLS efficiency reflects the fraction of primer molecules that are extended beyond the lesion. The biological relevance of these in vitro observations is supported by the observation that when transfected into pol IV (AdinB)-null mutant cells induced for SOS, G-BaP DE adducted M13 DNA gave rise to 4-fold fewer plagues relative to wild-type cells or compared with null mutants in either pol II (ΔpolB) or pol V (ΔmutDC) (Fig. 3B and Table IV).

pol IV, which bypassed G-BaP DE adducts with efficiencies between 36 and 71%, did not significantly bypass A-BaP DE adducts, nor did pol II (Fig. 3A and Table I), pol V, however, bypassed both A- and G-BaP DE adducts with roughly similar efficiencies, which were lower than that of pol IV for the G adducts (Fig. 3A and Table I). The A-BaP DE-S adduct was bypassed ~3-fold less efficiently than G-BaP DE-S in SOS-induced cells in vivo (Fig. 3B and Table IV). However, no difference was observed in the “survival” of A-BaP DE-ad-duced M13 DNA in any of the wild-type or polymerase-deficient mutant backgrounds (Fig. 3B and Table IV). Consequently, no clear conclusion can be drawn concerning which polymerase or polymerases might be involved in replicating past A-BaP DE-S. pol I showed a weak ability to bypass the A-BaP DE-S adduct, so perhaps it might play a role in vivo, even though it was not as active in bypassing this lesion compared with pol V in vitro (Fig. 2, lanes 1 and 6). It has previously been shown that proofreading-proficient and -deficient forms of pol I KF exhibit different patterns of TLS and misincorporations when copying A-BaP DE adducts depending on the stereochemistry at C-10 (58). pol III holoenzyme was essentially unable to bypass any of the BaP DE adducts (Fig. 2, lane 3).

SOS Polymerase Misincorporation and Extension Fidelity at BaP DE Adducts—Not only did pol IV copy G-BaP DE adducts more efficiently compared with pol V, but it did so much more accurately. Thus, pol IV favors incorporation of C opposite R and S isomers of BaP DE by 2 and 4 orders of magnitude, whereas pol V favors formation of A-G-BaP DE mispairs by factors of 130–350 (Table II). The extension efficiencies for the two enzymes mirror the incorporation efficiencies. pol IV favored extension of C-G-BaP DE correct base pairs by 1000–10,000-fold over any of the mispairs (Table III). True to form, pol V favored extension of the favored A-G-BaP DE mispairs over the C-G-BaP DE correct pairs (Table III).

The bottom line is that the action of pol V on G-BaP DE adducts would be expected to generate G-C to T-A transversions, whereas few base substitution mutations would be expected to occur when G-BaP DE adducts are copied by pol IV. We made a preliminary estimate of the types and frequency of mutations by sequencing a subset of M13 progeny that were SOS-induced. The in vitro kinetic data and in vivo mutational data agree with respect to the “strongest” prediction: the frequency of G-C to T-A mutations increases by ~7-fold in a pol IV-null mutant background. With the G-BaP DE-S adducts, G-C to T-A mutations occur with a frequency of ~1% in the SOS-induced wild-type strain (two mutants/176 DNA molecules sequenced) compared with 6.8% in the absence of pol IV (three mutants/44 DNA molecules sequenced).

Although these numbers are admittedly small, the conclusions are supported by data in the pol II-null background, where the G to T transversion frequency is again 1% (one mutant/104 DNA molecules sequenced). G to T error frequencies have an intermediate value of ~3.1% in the pol V-null background (3/98). These errors are perhaps made by pol II, which forms AG-BaP DE-S mispaired intermediates at an elevated frequency of ~7% in context IV (although the favored pol II mispair is with T at a frequency of ~14%) (Table II).

Because of the small numbers and the obvious complexities of attempting to make a direct comparison of the in vitro and in vivo data, e.g., the inability to take into account the effects of proofreading and post-replication mismatch repair (acting on double-stranded M13 replication intermediates), no strong conclusions can be drawn concerning which of the polymerases is responsible for causing specific types of mutations in vivo. Despite this caveat, however, several conclusions can be drawn from the data. The most important conclusion, from a biological perspective, is that the three SOS polymerases (pol II, IV, and V) are each likely to play some role in bypassing BaP DE adducts. Based on both the in vivo and in vitro kinetic data, pol IV copies and bypasses G-BaP DE adducts with highest efficiency and accuracy. In contrast, the errant pol V copies G-BaP DE adducts with remarkable inaccuracy and furthermore favors extension of the resultant mispairs over correct pairs. The most exceptional errors are A-G-BaP DE-S mispair, favored by 350-fold over a C-G-BaP DE-S correct pair, and an A-G-BaP DE-R mispair, favored by 132-fold over its correctly paired counterpart. Both of these mispairs are selectively extended by pol V with efficiencies of 23- and 3.9-fold over the corresponding correct pairs. In contrast to its ability to catalyze G-BaP DE TLS efficiently, pol IV is essentially unable to bypass R and S isomers of A-BaP DE. On the other hand, pol V copies A-BaP DE adducts efficiently and relatively “accurately,” making A-A-BaP DE-R mispairs with a frequency of only 23% and A-A-BaP DE-S mispairs with a frequency of 8.4%. Extension of both of these mispairs by pol V occurs with efficiencies similar to those of extension of TA-BaP DE correct pairs. Thus, the kinetic data support a growing body of evidence that although each of the SOS polymerases appears to have a primary role to play in the cell (pol V for error-prone TLS on heavily damaged DNA, pol IV for relieving blocked replication forks on undamaged DNA, and pol II for catalyzing error-free replication restart), each enzyme can also play a role in copying various types of DNA template damage.

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