Human DNA polymerase η was used to copy four stereoisomeric deoxyguanosine (dG) adducts derived from benzo[a]pyrene 7,8-diol 9,10-epoxide (diastereomer with the 7-hydroxyl group and epoxide oxygen trans (BaP DE-2)). The adducts, formed by either cis or trans epoxide ring opening of each enantiomer of BaP DE-2 by N\textsuperscript{2} of dG, were placed at the fourth nucleotide from the 5’-end in two 16-mer sequence contexts, 5’-CG\textsuperscript{A}- and 5’-GG\textsuperscript{T}. pol\textsuperscript{η} was remarkably error prone at all four diol epoxide adducts, preferring to misincorporate G and A at frequencies 3- to more than 50-fold greater than the frequencies for T or the correct C, although the highest rates were 60-fold below the rate of incorporation of C opposite a non-adducted G. Antis to syn rotation of the adducted base, consistent with previous NMR data for a BaP DE-2 dG adduct placed just prior to a primer terminus, provides a rationale for preferring purine misincorporation. Extension of purine misincorporations occurred preferentially, but extension beyond the adduct site was weak with V\textsubscript{max}/K\textsubscript{m} values generally 10-fold less than for misincorporation. Mostly A was incorporated opposite (+)-BaP DE-2 dG adducts, which correlates with published observations that G → T is the most common type of mutation that (+)-BaP DE-2 induces in mammalian cells.

Somatic mutation of proto-oncogenes and tumor suppressor genes is a key component in the initiation of cancer (1). Any DNA damage that escapes repair could lead to mutations. However, replicative DNA polymerases are blocked in a potentially lethal manner when they encounter bulky adducts (2). Thus, they are unable to convert adducts to mutations. Recently, a growing number of DNA polymerases capable of conducting translesion DNA synthesis have been discovered (3–11); these likely hold the key to mutagenesis and the initiation of cancer induced by bulky adducts. One of these lesion-bypassing DNA polymerases, human DNA polymerase eta (pol\textsuperscript{η})\textsuperscript{1} (12, 13) is a member of the UmuC/DinB/Rev1/Rad30 superfamily (now called the Y family (14)) of DNA polymerases. In humans it is a product of the XPV skin cancer susceptibility gene; inactivation of pol\textsuperscript{η} results in a variant form of xeroderma pigmentosum (12, 15). pol\textsuperscript{η} incorporates mostly correctly at cis-syn T-T cyclobutane dimers (16, 17), N-(deoxyguanosin-8-yl)-acetylaminofluorene (18), and cis-Pt G-G adducts (18, 19); small amounts of incorrect nucleotides are also incorporated, mostly at the level seen with undamaged DNA. With (6-4) T-T photoproducts, however, a single G is preferentially incorporated (20).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental carcinogens (21). Bay-region diol epoxides, formed on an angular benzo-ring, have been shown to be the ultimate carcinogenic metabolites of the PAHs (21–25). Four optically active bay-region diol epoxide isomers (enantiomers of a pair of diastereomers) are formed metabolically from a given hydrocarbon. In the DE-1 (“syn”) diastereomer, the epoxide oxygen and benzylic 7-hydroxyl groups are cis, whereas in the DE-2 (“anti”) diastereomer these groups are trans (see Fig. 1A below). For bay-region diol epoxides, the DE-2 isomer with R,S,S,R absolute configuration exhibits by far the greatest carcinogenicity compared with the other three optically active isomers, whereas for fjord-region diol epoxides significant carcinogenic activity is not limited to this isomer (26).

The exocyclic amino groups of purine bases (N\textsuperscript{2} of G and N\textsuperscript{6} of A) in DNA are the principal targets of PAH diol epoxides (27). Cis and trans addition of the amino group to the benzylic epoxide position of the diol epoxide produces adducts that have either retained or inverted configuration, respectively, at the point of attachment (C-10 for benzo[a]pyrene) to the hydrocarbon moiety. Either fig. 1B shows how cis addition at C-10 of (+)-7R,8S,9S,10R)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (reflects -BaP DE-2) results in retention of configuration at C-10 (10R → 10R) whereas trans addition results in inversion of configuration (10R → 10S).

The present study utilizes N\textsuperscript{2} dG adducts derived from the carcinogenic (+)-BaP DE-2 and the weakly carcinogenic or non-carcinogenic (26) (−)-7S,8R,9R,10S)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (−)-BaP DE-2. A recent paper is available on line at http://www.jbc.org
Misincorporation of Purines Opposite BaP DE-2 dG Adducts by polη

**Materials and Methods**

Oligonucleotides—Oligonucleotides containing a single specific cis or trans BaP DE-2 dG adduct placed on the fourth nucleotide from the 5′-end in either sequence context III(G) (5′-TTCG∗AATCCITCCCCC-3′) or IV(G) (5′-GGGG∗TTCCGGAGCGGC-3′) were synthesized as described previously (29, 30). The basis for selection of these sequences has been described previously (30). Polyacrylamide gel-purified, non-adducted oligomers were purchased from either Lofstrand Laboratories Limited (Gaithersburg, MD) or Midland Certified Reagent Co. (Midland, TX) and were purified further by reverse-phase high performance liquid chromatography if necessary.

DNA Polymerase η Reactions—His-tagged recombinant DNA polymerase η was produced as described previously (18). Polymerase reactions contained final concentrations of 40 mM Tris-HCl (pH 8.0), 1 mM MgCl₂, various concentrations of the four dNTPs, 10 mM dithiothreitol, 250 μg/ml bovine serum albumin, 60 mM KCl, 2.5% glycerol, 40 mM 5′-32P-labeled primer previously annealed to 60 nt 16-mer template (by heating at 95 °C for 5 min, and slowly cooling), and 2 mM DNA polymerase η in a 10-μl volume. Reactions proceeded at 37 °C for 3–15 min. Reactions were stopped by addition of 10 μl of 90% aqueous formamide containing EDTA (25 mM) and gel sequencing dye solution, followed by heating in a boiling water bath.

Kinetic Analysis of Incorporation—Reaction products were subjected to electrophoresis on 20% polyacrylamide-7.5 urea gels run at 60 watts. After drying the gels, the extent of incorporation was quantitated with a Fujifilm Fluorescent Image Analyzer and Image Gauge V3.12 software. Steady-state kinetic experiments followed the procedures of Creighton et al. (31) whereby maximum incorporation is kept below 20%. Rectangular hyperbolic fits yielding apparent Vₘₐₓ and Kₘₚ values were calculated using SigmaPlot 2000. Relative values of Vₘₐₓ/Kₘₚ for incorrect versus correct nucleotide incorporation were used to calculate the misincorporation efficiency (fₘᵢₓ).

**Results**

Misincorporation on the Non-adducted Sequence Context III(G) DNA Template—Previous studies of polη have used templates that ranged from 30 to 75 bases in length (17, 18, 28, 32). We first examined our shorter context III(G) 16-mer template to determine its efficacy as a substrate. In standing start assays with a 12-mer primer, non-adducted 16-mer template of context III(G) DNA (12/16 DNA) and dCTP at 1 mM concentration, the primer was mostly extended by one nucleotide (Fig. 2A). When all four dNTPs were present at 1 mM further extension occurred, with limited procession to the end of the template (Fig. 2A). Misincorporation efficiencies (fₘᵢₓ) were measured in standing start, steady-state kinetic assays. Table I shows similar misincorporation efficiencies of around 2 × 10⁻³ for all three incorrect nucleotides. These misincorporation efficiencies are somewhat lower than those reported by Johnson et al. (17) for the three nucleotides and Matsuda et al. (32) for G. Matching fₘᵢₓ values are not expected because of differences in assay conditions and templates. However, observation of misincorporation frequencies that fall within the range of 10⁻² to 10⁻³ previously observed for polη increases our confidence that correct fₘᵢₓ values are likely to be obtained from kinetic studies with 12/16 oligomers containing adducts at the fourth nucleotide (dG) from the 5′-end of the 16-mer template.

**Patterns of Incorporation in Sequence Context III(G) with BaP DE-2 dG Adducts—** Fig. 2B shows dNTP incorporation across from each of the four stereoisomeric BaP DE-2 dG adducts, compared with the non-adducted template. Assays were...
performed with 0.2 μm dNTP concentrations. It is clear that when any one of the stereoisomeric BaP DE-2 adducts is present, purine nucleotides are incorporated far more than either T or the correct C. Misincorporation of A is most extensive with the trans S adduct.

For each dNTP, more incorporation is seen with the trans-ring opened adducts compared with the cis adducts with the same R or S configuration at C-10. Less purine misincorporation occurs at cis adducts than trans adducts, particularly the cis S adduct, but incorporation of the correct C is also lower. Incorporation at cis R adducts formed from the carcinogenic (+)-BaP DE-2 is greater than incorporation at cis S adducts formed from the non-carcinogenic (−)-BaP DE-2.

The results shown in Fig. 2B for the BaP DE-2 trans S adduct agree with those obtained by Zhang et al. (28), who examined only this adduct, in that A is incorporated the best, but differ significantly in that we observed much higher G incorporation and much lower T incorporation. Differences between the assays, such as DNA sequence context or assay buffer composition and pH, could underlie the differences.

**Kinetics of Misincorporation at BaP DE-2 dG Adducts in Sequence Context III(G)**—Table II shows $V_{\text{max}}/K_m$ data for incorporation of individual nucleotides by pol$\eta$ opposite BaP DE-2 dG adducts in sequence context III(G). In the presence of these adducts, the highest $V_{\text{max}}/K_m$ values observed are about two orders of magnitude lower than the $V_{\text{max}}/K_m$ for incorporation of the correct C at non-adducted G (Table I). The adducts constitute a fairly strong block to the enzymatic activity of pol$\eta$.

Notably, however, the adducts have a blocking effect only on incorporation of a correct C, whereas incorrect base incorporation is either changed very little or actually enhanced in the presence of adducts. Thus, the presence of an adduct lowers $V_{\text{max}}/K_m$ for C incorporation by factors of 1600 to more than 18,000 whereas $V_{\text{max}}/K_m$ for T incorporation decreases by 2.2- to 16-fold, G incorporation rises up to 3-fold and A incorporation rises 1.5- to 7.4-fold.

Typical kinetic data for the trans S dG adduct are shown in Fig. 3. For all four adducts, incorporation of the correct C is the lowest of the four dNTPs. In the assays, there is evidence for inhibition by dNTP concentrations greater than 2 μm, which limits the amount of dCTP that can be used to increase incorporation. This makes it difficult to determine a precise $V_{\text{max}}/K_m$ value for C incorporation at cis S adducts and precise $f_{\text{inc}}$ values for G, A, and T misincorporation. Still, it is clear that incorporation of purines far exceeds that of pyrimidines opposite all four adducts in sequence context III(G). The preference for G misincorporation over correct incorporation of C ranges from 5.5-fold to more than 50-fold, whereas the preference for A over C ranges from 20-fold to more than 50-fold.

With all four dNTPs, $V_{\text{max}}/K_m$ values for purine and T misincorporation are generally greater for trans ring-opened adducts than cis ring-opened adducts, whether comparing the pairs trans R versus cis R or trans S versus cis S (members of each pair derived from opposite enantiomers of the diol epoxide) or the pairs trans R versus cis S or trans S versus cis R (members of each pair derived from the same diol epoxide enantiomer so that the configuration differs only at C-10). $V_{\text{max}}/K_m$ values for A, T, and C incorporation are greatest at trans S adducts, whereas $V_{\text{max}}/K_m$ for G incorporation is greatest at trans R adducts. The overall rate of incorporation (sum of $V_{\text{max}}/K_m$ values) follows the pattern trans R >> trans S ≈ cis R >> cis S. Values of $f_{\text{inc}}$ for A and G, however, are much greater for cis adducts than trans adducts, because of far lower incorporation of C at cis adducts. Values of $f_{\text{inc}}$ for G versus A are similar with all of the adducts except trans S, whose $f_{\text{inc}}$ for A is four times larger than $f_{\text{inc}}$ for G.

**Kinetic Comparisons of Incorporation Across from BaP DE-2 dG Adducts in Sequence Contexts III(G) and IV(G)**—Preferential incorporation of purine nucleotides over pyrimidine nucleotides is seen with both sequence contexts III(G) (5′-CG*A–) (Table II) and IV(G) (5′–GG*T–) (Table III). But, except for the trans S isomer, the $V_{\text{max}}/K_m$ for misincorporation is far lower and $V_{\text{max}}/K_m$ for C is unchanged or higher, with the result that $f_{\text{inc}}$ values are far lower in context IV(G). This is particularly so with the cis isomers. With the trans R, cis R and cis S isomers, the decreased preference for purine misincorporation is due to the decline in $V_{\text{max}}/K_m$ values in context IV(G) compared with context III(G) being greater for the purines than the pyrimidines. With the change in sequence context, the $V_{\text{max}}/K_m$ values for purine misincorporation drop an order of magnitude for trans R and cis R adducts and 3- to 5-fold for cis S adducts. With trans S adducts, $V_{\text{max}}/K_m$ values are greater in sequence context IV(G), substantially so with G and T incorporation. At the same time, when the sequence is changed to IV(G), C incorporation efficiency declines only 4-fold for trans R adducts and either stays about the same or rises for the other three adducts. Consequently, A incorporation at trans S adducts has the highest efficiency of misincorporation ($f_{\text{inc}}$) of all.

Patterns emerge from trans versus cis comparisons of $V_{\text{max}}/K_m$ values for incorporation in sequence context IV(G) that are like the ones seen in sequence context III(G). $V_{\text{max}}/K_m$ values for purine misincorporation are appreciably larger for trans adducts. However, because C incorporation is so different at context IV(G) adducts, $f_{\text{inc}}$ values for G and A for trans adducts are no longer much smaller than for cis adducts, as

### Table I

| Nucleotide incorporated | $V_{\text{max}}/K_m$ | $f_{\text{inc}}$ | $V_{\text{max}}/K_m$ | $f_{\text{inc}}$ |
|-------------------------|--------------------|---------------|--------------------|---------------|
|                         | × 10^6             |               | × 10^6             |               |
| G                       | 2.8 ± 0.90         | 3.0 × 10^-3   | 4.8 × 10^-3        | 11.0 × 10^-3  |
| A                       | 1.6 ± 0.16         | 1.8 × 10^-3   | 8.8 × 10^-3        |               |
| T                       | 1.3 ± 0.18         | 2.0 × 10^-3   | 2.9 × 10^-3        |               |
| C                       | 910 ± 180          | 1             | 1                  |               |

- In units of micromolar primer extended/μm dNTP/min; means and standard deviations generated from at least three kinetic assays.
- Template G at position 23 (from 3′) in 53-mer with local sequence 5′-AGT.
- Template G at position 21 (from 3′) in 30-mer with local sequence 5′-TGG.

### Table II

| Adduct isomer | Parent DE isomer | Nucleotide incorporated | $V_{\text{max}}/K_m$ | $f_{\text{inc}}$ |
|--------------|------------------|-------------------------|--------------------|---------------|
| Trans R      | 7S,8R,9R,10S     | G                       | 8.7 ± 2.5          | 20             |
|              |                  | A                       | 8.7 ± 2.0          | 20             |
|              |                  | T                       | 0.54 ± 0.17        | 1.2            |
|              |                  | A                       | 0.44 ± 0.12        | 1.5            |
| Trans S      | 7R,8S,9S,10R     | G                       | 5.1 ± 0.63         | 5.5            |
|              |                  | A                       | 12 ± 4.6           |               |
|              |                  | T                       | 0.84 ± 0.17        | 1.5            |
|              |                  | C                       | 0.56 ± 0.13        | 1.5            |
| Cis R        | 7R,8S,9S,10R     | G                       | 7.5 ± 3.0          | 48             |
|              |                  | A                       | 5.1 ± 1.6          | 33             |
|              |                  | T                       | 0.26 ± 0.13        | 1.7            |
|              |                  | C                       | 0.16 ± 0.054       | 1.53           |
| Cis S        | 7S,8R,9S,10R     | G                       | 2.5 ± 1.1          | > 49           |
|              |                  | A                       | 2.6 ± 0.55         | > 51           |
|              |                  | T                       | 0.12 ± 0.054       | > 2.4          |
|              |                  | C                       | ND (<0.05)         |                |

- In units of micromolar primer extended/μm dNTP/min; means and standard deviations generated from at least three kinetic assays.
- ND, not determined.
was seen in context III(G). Patterns based on R versus S comparisons in sequence context IV(G) are not evident. The effect of changing the sequence from III(G) to IV(G) on T misincorporation parallels the effect seen with the purines. $V_{\text{max}}/K_m$ for T misincorporation is higher at trans S adducts, and lower at the other three. Combined with the aforementioned changes in C incorporation, the result is a decrease in $f_{\text{inc}}$ for T at cis adducts, little change at trans R adducts and a rise at trans S adducts.

**DISCUSSION**

**A Mechanism for Preferential Purine Nucleotide Misincorporation Across from BaP DE-2 dG Adducts**—All four BaP DE-2 dG adducts represent significant blocks to C incorporation. In

**TABLE III**

Fidelity of pol$\eta$ at BaP DE-2 adducts in sequence context IV(G)
For template sequence see Figure 1C.

| Adduct isomer | Parent DE isomer | Nucleotide incorporated | $V_{\text{max}}/K_m$ $^{a}$ | $f_{\text{inc}}$ |
|---------------|------------------|-------------------------|-----------------------------|------------|
| Trans R       | 7S,8R,9R,10S     | G                       | 0.91 ± 0.30                 | 7.6        |
|               |                  | A                       | 0.84 ± 0.17                 | 7.0        |
|               |                  | T                       | 0.17 ± 0.025                | 1.4        |
|               |                  | C                       | 0.12 ± 0.030                | 1          |
| Trans S       | 7R,8S,9R,10R     | G                       | 0.81 ± 1.8                  | 8.3        |
|               |                  | A                       | 15 ± 2.8                    | 15         |
|               |                  | T                       | 2.7 ± 0.66                  | 2.8        |
|               |                  | C                       | 0.98 ± 0.19                 | 1          |
| Cis R         | 7R,8S,9R,10R     | G                       | 0.46 ± 0.08                 | 3.5        |
|               |                  | A                       | 0.43 ± 0.02                 | 3.3        |
|               |                  | T                       | 0.045 ± 0.020               | 0.3        |
|               |                  | C                       | 0.13 ± 0.011                | 1          |
| Cis S         | 7S,8R,9R,10S     | G                       | 0.72 ± 0.25                 | 8.4        |
|               |                  | A                       | 0.51 ± 0.078                | 5.9        |
|               |                  | T                       | 0.049 ± 0.011               | 0.6        |
|               |                  | C                       | 0.086 ± 0.038               | 1          |

$^{a}$ In units of micromolar primer extended/μM dNTP/min; means and standard deviations generated from at least three kinetic assays.
sequence context III(G), incorporation of the correct C drops as much as 20,000-fold relative to the corresponding rate in the absence of adduct. In contrast, the rates of incorporation of the purine nucleotides rise slightly (less than 10-fold compared with their rates in the absence of adduct). Consequently, the efficiency ($f_{\text{ext}}$) of A misincorporation at BaP-DE-2-dG adducts in sequence context III(G) (Table II), relative to A misincorporation in normal DNA (Table I), jumps over four orders of magnitude from $1.8 \times 10^{-3}$ to a range of 20 to over 50 times C incorporation (Table II), and the efficiency of G misincorporation jumps nearly as much.

We propose the following mechanism for preferential purine misincorporation opposite the BaP-DE-2-dG adducts: 1) Stacking of the hydrocarbon with the preceding base pair causes the adducted dG to assume the unusual syn rather than the usual anti torsion angle, with the stacked hydrocarbon and the syn dG conformation being accommodated by the open polη active site; 2) the enzyme misincorporates purine nucleotides opposite this syn dG adduct by Topal and Fresco (33) purine-purine base pairing, and 3) after purine misincorporation the adduct remains intercalated in the DNA. Evidence supporting this mechanism comes from the structural information that follows. polη exhibits unusually high misincorporation frequencies of $10^{-2}$ to $10^{-3}$ in normal, non-adducted DNA (17, 32). This indicates an active site whose discrimination in favor of Watson-Crick and other base pairs with similar geometry is considerably relaxed compared with many other non-proofreading polymerases but is still 100- to 1000-fold. Crystal structures for members of the Y family of DNA polymerases (34–37), including the catalytic core of polη from yeast (35), have been reported. Although Y family polymerases have a classic DNA polymerase palm structure, the finger and thumb structures are smaller than usual. An additional structure called a polymerase-associated domain (35) or little finger (36) is present. Despite the latter, the Y-family DNA polymerase active site is more open where the incoming deoxyribonucleotide bind, and the degree of interaction with the primer-template complex is less than what is seen with high fidelity DNA polymerases. These features, which are inferred by homology modeling with T7 DNA polymerase in two studies (34, 35) and are observed directly in a third (36), could underlie the relative lack of fidelity exhibited toward non-adducted DNA, and the ability to accommodate bulky adducts attached to syn-rotated dG and incorporate a nucleotide across from such lesions (34–37).

The solution NMR structure of a BaP-DE-2 trans S dG adduct bound to the fourth base from the 5’-end of a 13-mer template (5’-AACG*C-3’) adjacent to the terminus of a 9-mer primer strand provides a model for a partially extended DNA duplex as “seen” by a polymerase. This structure shows that prior to insertion of the complementary nucleotide, the adducted dG is rotated anti to syn to allow the hydrocarbon moiety to stack with the base pair formed by the 3’-terminal primer base and the base immediately 3’ to the modified dG on the template strand (38). After a complementary C is incorporated opposite the BaP-DE-2 trans S dG adduct, the adducted dG assumes the normal anti conformation and the adduct swings out into the minor groove (39). This groove-bound, anti conformation of the adduct is maintained upon further extension of the primer chain beyond the adduct site, as is shown by the structure of a fully complementary 11-mer duplex (40) with the same local –CG*C– sequence around the adduct.

There are no NMR data for mismatched duplex structures corresponding to incorrect purine nucleotide insertion opposite BaP-dG adducts; however, substantial UV red shifts are observed for the long-wavelength pyrene band of duplexes containing purine mismatches opposite BaP trans R and trans S dG adducts in the same –CG*C– context (41), from 346 nm (when C is paired with the adducted G) to around 350 nm in the G and A mispaired duplexes. These shifts are indicative of base stacking, and suggest that the hydrocarbon remains intercalated (41), with the adducted dG presumably still in the syn conformation, after an incorrect purine nucleotide is inserted opposite the adduct and the primer is further extended.

If the adducted dG is in the syn conformation before and after polymerization then presumably it is also syn when the incoming nucleotide binds and is incorporated. A syn glycidosic torsion angle of the adducted dG in the template allows purine-purine base pairing of the type proposed by Topal and Fresco (33). Molecular modeling (42) of the BaP-DE-2 trans S adduct in a 13-mer template, 10-mer primer structure (the 13-mer template and 9-mer primer described above with the primer extended to include A across from the adduct) suggests that the size and shape of the (syn-adducted-G)-(anti-A) pair very much resembles that of a normal T-A pair. The 100- to 1000-fold preference for base pairs having the approximate size and shape of a Watson-Crick base pair could also cause the enzyme to select syn-adducted G paired with anti-A over pairing with the correct C. The observed red shifts (41) increase (more favorable base stacking) in the same order as the present $V_{\text{max}}/K_m$ values for polη misincorporation of purines at trans adducts.

Even though an NMR structure could not be determined (38) when the trans S dG adduct above is replaced by a trans R dG adduct, it is likely that the polη preference for forming (adducted-G)-(purine) mispairs with the BaP trans R adduct can be explained by a mechanism similar to that described above for the trans S adduct. UV spectra of oligonucleotide duplexes containing the trans R dG adduct mispaired with purines show significant red shifts of the long wavelength absorbance (41), consistent with base stacking and syn glycidosic rotation, and are indicative of a structure analogous to that observed with the trans S dG adduct.

Structural analysis of either a cis R or cis S BaP-DE-2 dG
adduct placed in the same position as the trans adducts just described, either before or after a correct or incorrect incorporation of nucleotide has yet to be reported. In fully duplex DNA, the guanine base bearing either a cis R (43) or a cis S (44) BaP adduct is displaced out of the DNA helix as is its complementary C, and neither NMR nor UV studies with purine-purine mispairs of cis adducts are available. Thus no structural information exists that might explain preferential purine incorporation versus C incorporation at the present cis adducts; however, as in the case of the trans adducts, the observed preference for purine misincorporation presumably could also result from anti to syn rotation of the adducted dG and Topal and Fresco (33) base pairing.

8-Oxo-dG forms stable GA pairs in DNA with the G rotated anti to syn (45), resulting in G to T transversions (48). pol\(\eta\) misincorporation at the trans adduct combination and is 18-fold greater than for A misincorporation from these two types of C-8 G adducts (16, 49), suggestive that the 5'-nearest neighbor is A in sequence context III(G) and is slightly higher (83%), and G 3'-nearest neighbor to the adducted G is C (see above). Small differences in stacking geometry between the adduct and different 3'-nearest neighbor bases and their complements could account for the observed differences in misincorporation frequency between templates III(G) and IV(G). Specific effects of the 5'-nearest neighbor are also possible, such as template slippage leading to enhanced correct insertion of C with template IV(G) as mentioned above or changes in stacking depending on the identity of the 5'-base. Our observed dependence of misincorporation on sequence suggests that it will be fruitful to focus bypass studies of misincorporation on specific proto-oncogene and tumor suppressor sequences.

**Mutational Spectra of BaP DE-2 dG Adducts in Mammalian DNA**—It is of considerable interest to establish what role pol\(\eta\) plays in determining the mutational spectrum that has been observed for PAHs in mammalian cells. The preponderant products of *in vitro* reaction of BaP diol epoxides with dG in DNA are trans ring-opened adducts formed at the exocyclic nitrogen of dG. Upon reaction of (−)-BaP DE-2 with calf thymus DNA *in vitro*, trans R and cis S dG adducts are formed in the ratio of ~6:1 (50). At both types of adduct, pol\(\eta\) strongly favored misincorporation of purines, with both G and A being misincorporated about equally and 5 to 20 times the rate for T misincorporation. Once purine misincorporation occurred, such mispairs were extended somewhat better than the correctly paired primers for three out of the four adducts studied in sequence III(G). However, the extension of these mispairs was still quite inefficient (\(V_{\text{max}}/K_{\text{m}}\) generally about 10- to 20-fold smaller than \(V_{\text{max}}/K_{\text{m}}\) for purine incorporation; Tables II and IV), suggesting that if purine misincorporation by pol\(\eta\) plays a role in mutagenesis induced by BaP DE adducts, an additional polymerase or polymerases are likely to be required for full extension of the damaged DNA to give an observed mutagenic event. Precedent for such a mechanism is provided by the observation that pol\(\alpha\) and pol\(\eta\) act sequentially to bypass abasic sites as well as the (6-4) T-T photoproduct (51).

The relative lack of T misincorporation by pol\(\eta\) contrasts with limited data (52) on the mutational spectrum of (−)-BaP DE-2 in the *HPRT* gene in Chinese hamster V-79 cells, in which G → C, G → T, and G → A mutations (misincorporation of G, A, and T, respectively) occurred to similar extents (1.5: 1:0.9:8). Far more information is available about the spectrum of mutations at G produced by (−)-BaP DE-2 in mammalian cells. In the *HPRT* gene in V-79 cells, the relative proportions of the three possible base substitutions at G are virtually independent of dose (53). At three different doses of (−)-BaP DE-2, G → T transversions accounted for 67–75%, G → C transversions for 18–21%, and G → A transitions for 7–11% of the total mutations (53). The use of repair-deficient Chinese hamster V-H1 cells (54) resulted in little change in the relative mutational frequencies at two different doses: 60–63% of G mutations were G → T transversions, 20–23% were G → C transversions, and 15–19% were G → A transitions. These frequencies of misincorporation are consistent with our pol\(\eta\) results described below.

The spectrum of mutations produced in the ras proto-oncogene was determined (55) in mice whose skins were painted with the parent hydrocarbon, BaP. When forming bay-region diol epoxides from the (−) and (−)-enantiomers of BaP 7,8-dihydridiol, liver microsomes from 3-methylcholanthrene-treated rats produce predominantly (−)-BaP DE-2 and much less of the other three diol epoxide isomers (56). Despite the different cell types and the overlay of BaP metabolism in the skin experiments, the mutational pattern is quite similar for BaP on mouse skin and (−)-BaP DE-2 in hamster cells in culture. In mouse skin, the proportion of G → T transversions is slightly higher (83%), and G → C transversions (13%) and G → A transitions (4%) are correspondingly lower than what was seen in the cultured cell studies.

Misincorporation by pol\(\eta\) at (−)-BaP DE-2 adducts fits the above mutational spectra well. *In vitro* reaction of (−)-BaP DE-2 with calf thymus DNA gives trans S and cis R adducts in the ratio of ~40:1 (50). Therefore, it is reasonable to assume that mutations arising at G in mammalian cells are likely to arise largely from the trans S adducts formed in multiple sites...
in the DNA, and the effect of cis R adducts may be ignored. Misincorporation of A, G, and T by pol η at trans S adducts would give rise to G → T, G → C, and G → A substitutions, respectively. The proportions of A, G, and T misincorporation in sequence contexts III(G) and IV(G) (calculated from the data shown in Tables II and III, respectively) are A, 76% and 58%; G, 19% and 31%; and T, 5% and 10%. When extension of the appropriate mispairs is also taken into account by comparison of the products (\(V_{\text{max}}/K_{\text{m}}\),loc (\(V_{\text{max}}/K_{\text{m}}\),ext), the proportions of A, G, and T mispairs that are formed and also extended by one DNA lesions.

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