We have previously developed an in vitro system that allows quantitative evaluation of the fidelity of transcription during synthesis on a natural template in the presence of all four nucleotides. Here, we have employed this system using a TAA ochre codon reversion assay to examine the fidelity of transcription by T7 RNA polymerase past an adenine residue adducted at the N6-position with (-)-anti-trans- or (+)-anti-trans-benzo[a]pyrene diol epoxide (BPDE). T7 RNAP was capable of transcribing past either BPDE isomer to generate full-length run-off transcripts. The extent of bypass was found to be 32% for the (-)-anti-trans-isomer and 18% for the (+)-anti-trans-isomer. Transcription past both adducts was highly mutagenic. The reversion frequency of bypass synthesis of the (-)-anti-trans-isomer was elevated 11,000-fold and that of the (+)-anti-trans-isomer 6000-fold, relative to the reversion frequency of transcription on unadducted template. Adenine was misinserted preferentially, followed by guanine, opposite the adenine adducted with either BPDE isomer. Although base substitution errors were by far the most frequent events in in vitro transcription on unadducted template, Adenine was misinserted preferentially, followed by guanine, opposite the adenine adducted with either BPDE isomer. Although base substitution errors were by far the most frequent events.

It is well documented that genomic instability can result from errors made during DNA replication, repair, or recombination. However, less is known concerning the effects of inaccurate transcription and/or translation on the integrity of the genetic information. Transcriptional and translational errors may lead to production of mutant proteins. If the defective protein is involved in DNA replication or repair, then even its transient presence may result in permanent changes in the cell’s DNA. In fact, a recent paper by Slupska et al. (1) suggests that transcriptional miscopying may result in a mutator phenotype in Escherichia coli due to production of mutant DNA polymerases with dysfunctional proofreading activity. A likely source of transcriptional errors is damage to the DNA template and/or ribonucleotide pools. Some DNA lesions, such as UV light-induced cyclobutane dimers, block the progression of RNA polymerase (2). Such lesions have been shown to be preferentially repaired, relative to other parts of the genome, when present in the template strand of an actively transcribed gene (3–4). However, lesions that are bypassed by the polymerase may result in erroneous transcripts that, if translated, will give rise to mutant proteins. The biological consequences of transcriptional mutagenesis may be particularly significant in nondividing cells in which some mutant proteins may accumulate over time. Evidence presented by van Leeuwen et al. (5) points to transcription errors as a possible source of a mutant form of β-amyloid precursor protein found in neurons of Alzheimer’s and Down’s syndrome patients. Deposition of this mutant protein in neuritic plaque is probably involved in neuron degeneration.

A recent study indicates that oxidative damage to ribonucleotide pools may result, through transcriptional miscoding, in production of mutant proteins. Taddei et al. (6) observed an increase in phenotypic suppression of the Lac− phenotype in MutT− E. coli relative to MutT+ as a result of 8-oxo-GTP misincorporation during transcription. The E. coli MutT protein, which is known to degrade 8-oxo-dGTP (7), the product of dGTP oxidation, was shown to also degrade 8-oxo-GTP. The finding that MutT is responsible for cleansing the rNTP pools raises the question of whether other mechanisms exist, such as RNA-specific repair processes, to control the production of mutant proteins.

Our knowledge of how RNA polymerases react to various spontaneous or induced DNA lesions is limited. In particular, little is known about the mutagenic potential of transcriptional damage bypass synthesis. Several recent studies have examined the behavior of SP6 and/or T7 RNAP1 at small, nondistorting DNA lesions such as abasic sites, 8-oxoguanine, and dihydouracil. These results indicate that all three lesions are easily bypassed and that bypass involves a misincorporation event, most often insertion of an A opposite the lesion (8–10). Unfortunately, the methods used in these studies were not quantitative and only sensitive enough to detect high frequency events.

We have developed an in vitro system that allows quantitative assessment of the fidelity of transcription.2 Here, this system is employed to examine transcription by T7 RNAP past an adenine residue adducted with (-)-anti-trans- or (+)-anti-trans- benzo[a]pyrene diol epoxide (BPDE). BPDE is a metabolite of the common environmental pollutant, benzo[a]pyrene. The stereochemistry of BPDE has an influence on its potent mutagenic and carcinogenic properties (11–14). It has been shown that BPDE-adducted DNA blocks synthesis by a variety
of DNA polymerases (15–19). Several studies have also demonstrated that BPDE adducts in DNA impede transcription (20–27). The primary product formed when BPDE reacts with DNA is deoxyguanosine adducted at the N7 position. Adenine adducts at the N6-position are also formed but in smaller amounts (11). Studies have shown that at high, nonphysiological concentrations, BPDE induces mutations primarily at dG. However, at lower, more physiological doses, mutations at dA become much more significant (28–30).

In this study, we demonstrate that T7 RNAP is found capable of transcribing past BPDE-adducted adenine and report on the fidelity of this bypass synthesis.

**EXPERIMENTAL PROCEDURES**

**Materials**—E. coli strains, bacteriophage, and gapped substrate preparation have been described previously (31). RNase H-deficient Moloney murine leukemia virus reverse transcriptase (SuperScript II) and T4 DNA ligase were obtained from Life Technologies, Inc. T7 RNA polymerase was obtained from Promega (Madison, WI). T4 polynucleotide kinase was obtained from New England Biolabs (Beverly, MA), and Sequenase version 2.0 was obtained from Amersham Pharmacia Biotech. AmpliTaq DNA polymerase was purchased from PerkinElmer.

**Synthesis of Stereochemically Defined N6 Deoxyadenosine Adducts of BPDE**—The two stereospecific BPDE adducts were constructed on adenine N6 at position 10 within a 16-base deoxyligouronucleotide. The 16-mers, 5′-GATAAATCAAGCCAG-3′ (lacZa, positions +46 to +75), were modified by the postligomerization methodology previously described (32–34) except that purification was performed on a reversed phase column (YMC-ODS-AQ (4.6 × 250 mm) using a linear gradient of 100 mM ammonium formate, pH 6.5, containing 9.5–11% CH3CN over 25 min at a flow rate of 1.25 ml/min; the elution times for the 10R and the 10S isomers were 17.69 min and 19.40 min, respectively. The collected peaks were lyophilized, redissolved, and desalted on OPA™ cartridges according to the manufacturer's directions (Applied Biosystems, Foster City, CA). Peak 1 (10R isomer) base composition based on mass spectrometry (electrospray) was 5200.49; observed ions 1299.2 (M+H)/6z, 865.8 (M−H)/6z, representing a measured mass of 5199.66. Peak 2 (10S isomer) enzyme digestion was as follows: dC (3.0); dG (3.0); T (3.0); dA (6.0); dAN6(10S)-BPDE (1.0); theory: dC (3.0); dG (3.0); T (2.7); dA (5.8); dAN6(10S)-BPDE (1.2); (2); T (3.0); dA (6.0); dAN6(10S)-BPDE (1.0). Mass spectrometry (electrospray) calculated M+ for the unadducted template generated an average of 510 ng of full-length transcript.

**Transcription Reactions**—Transcription reactions (50 μl) were performed as described previously (37) and contained 10 mM Tris-HCl, pH 7.5; 7.5 mM MgCl2; 2 mM spermidine; 10 mM NaCl; 10 mM dithiothreitol; 50 units of ribonuclease inhibitor (RNasin, Promega); 200 ng of modified or unmodified, linearized M13mp2 DNA; a 500 μM concentration of each of ATP, CTP, GTP, and UTP; 10 μCi of [α-32P]GTP (3000 Ci/mmol); and 15 units of T7 RNA polymerase. Reaction components were mixed at room temperature and then incubated at 37 °C for 2 h. Template DNA was digested with RNase-free DNase (Ambion, 1 unit/μg DNA) for 15 min at 37 °C. The RNA was purified by phenol extraction and ethanol precipitation and resuspended in RNase-free water. An aliquot of the transcript was analyzed on a denaturing 4% polyacrylamide gel and visualized with a PhosphorImager (Molecular Dynamics STORM 8600). The average yield of transcription products, including full-length and truncated ones, was 200 and 170 ng from (−)-anti-trans- and (+)-anti-trans-BPDE-adducted templates, respectively. Transcription with the unadducted template generated an average of 510 ng of full-length transcript.

**Construction of the Ochre Codon-containing Transcription Template**—The DNA substrate for the ochre codon-based transcription assay was constructed as follows. Site-directed mutagenesis was performed (35) using M13mp2SV (36) to replace the ninth codon of the lacZ RI site at positions 53 to 56 by restriction with Fsp I and contained 40 mM Tris-HCl, pH 8.0, 10 mM NaCl, 15 mM dithiotreitol, 100 μM of each dNTP, 10 μCi of [α-32P]dCTP (900 Ci/mmol), 50 units of RNasin, 10–50 ng of RNA transcript, 2 pmol of a 21-mer DNA oligonucleotide primer, and 0.5 pmol of RNase H-deficient Moloney murine leukemia virus reverse transcriptase. The RNA was heated to 65 °C for 5 min and cooled on ice before adding to the other reagents. Reactions were incubated at 37 °C for 1 h and then terminated by the addition of EDTA to 15 mM. The RNA from hybridized were denatured at 80 °C and cooled on ice, and the RNA was digested with 50 units of RNase A and 800 units of RNase T1 (Amersham Pharmacia Biotech) at 37 °C for 1 h. The cDNA was precipitated with isopropyl alcohol, using glycerol as a carrier, and resuspended in sterile water. An aliquot of the cDNA was analyzed by electrophoresis on a 4% denaturing polyacrylamide gel and visualized with a PhosphorImager. The amount of cDNA synthesis was estimated by quantitative autoradiography of the cDNA bands found on gel and determining the radioactivity in a 5-ml scintillation cocktail in an LS7800 scintillation counter (Beckman). The 5′-end of the cDNA was phosphorylated in a 50-μl reaction that contained 50 mM Tris-HCl, pH 8.0, 10 mM MgCl2, 1.5 mM spermidine, 1 mM ATP, and 100 units of T4 polynucleotide kinase per pmol of cDNA. Phosphorylation reactions were conducted at 37 °C for 1 h and terminated by heating at 70 °C for 5 min.

**Hybridization of cDNA Fragment to Gapped M13mp2 DNA**—The cDNA fragment was hybridized to a double-stranded circular M13mp2 substrate that contained a 442-base single-stranded gap. The gap spanned the sequence in the lacZa gene complementary to the cDNA (lacZ positions −216 to +195). A molar excess of cDNA was mixed with gapped M13mp2 DNA in 300 mM NaCl, 30 mM sodium citrate, heated to 70 °C, and slowly cooled to room temperature. The annealed product was analyzed by electrophoresis in an 0.8% agarose gel and translocated into an E. coli α-complementation host, and mutations were scored, all as described previously (31).

**RESULTS**

**Construction of Transcription Templates**—Templates for transcription were constructed that contained the lacZa mutational target and the T7 RNA polymerase promoter sequence as described under “Experimental Procedures.” The target for measuring transcriptional fidelity of errors, a TATAA codon, was engineered within the (+)-trans-strand of lacZ and contained lacZ sequences on the nontranscribed (+)-strand. Opposite the T of the ochre codon, the transcribed strand contained either (+)-anti-trans-N6- BPDE-adducted adenine, (−)-anti-trans-N6-BPDE-adducted adenine, or nonadducted adenine (Fig. 1). To ensure that the BPDE-adducted transcription templates produced by primer extension reactions actually contained restriction endonuclease A/III were added to the reactions containing BPDE-adducted DNA in order to cleave any unadducted DNA molecules. Incubation was continued 1 h, after which reactions were terminated with EDTA (15 mM) and resolved by 0.8% agarose gel electrophoresis in the presence of 0.5 μg/ml ethidium bromide. Gel slices containing the covalently closed circular (RF I) DNA of each of the three primer extension reactions were cut from the gel; the DNA was then eluted using an Elutrap device (Schleicher and Schuell), concentrated, and equilibrated in dH2O. Prior to use in transcription reactions, templates were linearized at position +195 by restriction with FspI.
BPDE-adducted adenine, a unique recognition site for the restriction endonuclease AflII, CTGTAAG, was created by changing the alanine codon preceding the ochre codon from GCC to GCT, which also codes for alanine. Accordingly, the BPDE-adducted adenine was predicted to reside in the center of the AflII recognition sequence (5’-CTTAAG-3’, where A* represents BPDE-adducted adenine) and to inhibit restriction of an adducted template. This was indeed the case, as shown in Fig. 1C. Templates containing BPDE-adducted adenine were resistant to AflII restriction (Fig. 1C, lanes 4 and 5), whereas the unadducted template was completely cleaved (Fig. 1C, lane 6). Thus, by treating the BPDE-adducted templates with AflII following completion of the primer extension reactions and prior to gel purification, a homogeneous, fully adducted transcription template was assured.

**T7 RNA Polymerase Transcription from Templates with a BPDE-adducted or Unadducted Adenine**—Run-off transcription from FspI-linearized templates is predicted to generate a transcript 313 nucleotides in length (from position −118 through +195). Reactions performed using either an unadducted template or a template in which adenine at position +66 was adducted with (+)-anti-trans-BPDE and a template in which adenine at position +65 was adducted with (−)-anti-trans-BPDE generated 313-nucleotide transcripts, as shown in Fig. 2A. In each reaction with an adducted template, several shorter bands were observed, including one major band. These truncated transcripts indicate that the polymerase had stalled or dissociated. We have estimated that the major band representing the truncated product is approximately 3 nucleotides shorter than a transcript generated from an adducted template, which is predicted to yield a 186-nucleotide transcript. If transcription was aborted after insertion of a nucleotide directly opposite the adduct, the RNA would be 184 nucleotides in length. Therefore, we deduce that the transcript is probably truncated one base 5’ of the adduct, at position +65. Based on the amount of full-length transcript relative to the total amount of products in the lane, there was 32% bypass for (−)-anti-trans-BPDE and an 18% bypass for (+)-anti-trans-BPDE (Fig. 2B). The amount of full-length transcript in reactions with the (−)-anti-trans-BPDE-adducted template was significantly greater than in reactions with the (+)-anti-trans-BPDE-adducted template. This was verified by the Wilcoxon rank sum test, which gave a p value of 0.05.

As is indicated by the presence of truncated transcripts, the BPDE adducts did impede transcription; however, T7 RNAP was able to bypass both adducts to generate full-length transcripts.

**Fidelity of Transcription Past a BPDE Adduct**—The observation that T7 RNAP was capable of transcription past the BPDE adducts allowed us to examine the fidelity of bypass synthesis. Transcriptional fidelity measurements were obtained using an ochre codon reversion assay based on complementation of β-galactosidase activity by the α-peptide portion of the enzyme. This assay is a modification of a method that we used previously to examine the fidelity of reverse transcription (37). Briefly, transcription was carried out from the T7 promoter, which was located 5’ to the lacZ gene. The run-off transcript was primed with a DNA oligonucleotide and cDNA synthesis performed. The cDNA was hybridized to a double-stranded, circular M13mp2 substrate containing a single-stranded gap that spanned the lacZ gene complementary to the cDNA. The products of cDNA hybridization reactions were transfected into an E. coli α-complementation host, which yielded rescued errors as mutant plaques. This assay focuses specifically on single-base substitution errors at the three-base mutational target, the TAA ochre codon in the lacZ sequence. Accurate transcription of the ochre codon-containing template results in a white plaque. Of the nine possible single-base substitutions at the TAA codon, seven are predicted to restore complementation and result in a blue lacZ gene phenotype, while two result in nonsense codons and do not yield a phenotypically detectable change in plaque color (38).

Transcripts generated from the two BPDE-adducted templates and the control, unadducted template were used as templates for cDNA synthesis. The cDNAs were then hybridized to a gapped M13mp2 substrate containing the TAA ochre codon at positions +66 to +68 within the single-stranded region (see “Experimental Procedures”). We specifically selected
full-length or nearly full-length transcripts as templates for cDNA products by using a 21-mer oligonucleotide cDNA primer complementary to the 3′ terminal region of a full-length, run-off transcript (primer extends from lac position +172 to +191).

The products of the hybridization reactions were analyzed by electrophoresis in a 0.8% agarose gel. The mobility of these products was compared with that of gapped M13mp2 and double-stranded, nicked M13mp2 RF II DNAs. The products of all three hybridization reactions co-migrated with the double-stranded, nicked M13mp2 RF II DNAs. The products of all hybridization reactions were analyzed by DNA sequencing.

The number of dark blue plaques, which represent expression of the minus strand, 47 and 22% minus strand expression was observed for AAA and GAA codon-containing heteroduplexes, respectively, each being an average of two independent determinations. While 47% expression is similar to the average minus strand expression values previously observed (37, 39), the 22% expression is roughly 2-fold lower. This difference (47 versus 22%) is not inconsistent with previous results (Ref. 39) indicating that the expression of the minus strand for different heteroduplexes may vary up to 2-fold. We have used the values of 47 and 22% to calculate the error rate of AAA and GAA revertants, respectively. For calculation of the error rate of other events and the overall base substitution error rate, we have used the mean of AAA and GAA expression efficiency values, 35%.

Competent E. coli cells were transfected with the products of the hybridization reactions. Reactions containing cDNAs generated from transcripts of the unadducted template yielded an average overall reversion frequency of $3 \times 10^{-4}$ (Table I). In contrast, products of hybridization reactions containing cDNAs generated from transcription of the (−)-anti-trans- and (+)-anti-trans-BPDE-adducted templates yielded reversion frequencies of $3300 \times 10^{-4}$ and $1800 \times 10^{-4}$, respectively (Table I). Both transcription and reverse transcription may contribute to these reversion frequencies. However, the reversion frequency resulting from reverse transcription errors is at most $0.3 \times 10^{-4}$, assuming that all errors observed from reactions with the unadducted template were introduced by the reverse transcriptase. Thus, the observed 11,000- and 6000-fold increase in reversion frequency for reactions with the (−)-anti-trans- and (+)-anti-trans-BPDE-adducted templates, respectively, relative to reactions with the unadducted template, is clearly due to transcription errors. We therefore conclude that transcriptional bypass of BPDE-adducted adenine is highly mutagenic.

To determine the specificity of transcriptional errors during BPDE bypass, revertants were analyzed by DNA sequencing. This analysis included 13 revertants from two experiments with the unadducted template, 19 revertants from experiment one, 25 revertants from experiment two, 24 revertants from experiment three with the (−)-anti-trans-BPDE-adducted template, and finally 18 and 24 revertants from experiments one and two, respectively, with the (−)-anti-trans-BPDE-adducted template (Table I). The analysis revealed that the increase in reversion frequency observed in experiments with both adducted templates was due primarily to base substitutions (Table II). The overall base substitution error rates were approximately 1 in 3 and 1 in 6 errors per incorporated nucleotide for transcription of the (−)-anti-trans- and (+)-anti-trans-BPDE adducted templates, respectively. These error rates are 3–4 orders of magnitude higher than the base substitution error rate for transcription of the unadducted template (Table II). All base substitutions from reactions with the adducted templates are at the T, the first position of the ochre codon (Table II). The most frequent change observed in reactions with both BPDE-adducted templates is a T to A transversion, followed by a T to G transversion. With the (−)-anti-trans-BPDE-adducted template, the T to A transversions were only 1.5-fold more frequent, whereas in reactions with the (+)-anti-trans-BPDE adducted template, they were 9-fold more frequent than T to G substitutions. Only one revertant with a T to C change was recovered from reaction with the (+)-anti-trans-BPDE-adducted template.

3 K. M. Remington, S. E. Bennett, C. M. Harris, T. M. Harris, and K. Bebenek, unpublished observations.
The T to A and T to G substitutions at the first position of the ochre codon were also found to be the most frequent changes in revertants from reactions with the unadducted template (Table II). In this case, the error rates for both substitutions were similar and were 3–4 orders of magnitude lower than error rates for the same substitutions resulting from transcription with the adducted templates.

The ochre codon reversion assay was designed primarily to detect base substitution errors. Interestingly, DNA sequence analysis revealed that among the revertants recovered from reactions with adducted templates some were the result of small deletion mutations or complex changes. The phenotype of these revertants, light blue, was quite distinct from that of base substitution revertants. Deletion errors included three- and six-base deletions spanning the TAA codon. Complex changes involved both a base substitution and a 3–6-base deletion. Among 149 revertants generated in reactions with the (-)-anti-trans-BPDE-adducted template, five had a lighter blue plaque phenotype (three contained a six-base deletion, and two contained a complex change). A total of 124 revertants from reactions with the (+)-anti-trans-BPDE-adducted template were examined. Seven light blue plaques were recovered, three with a three-base deletion and four with a six-base deletion. We conclude that if “white” deletion mutants were in fact produced, the error rate for this class of mutation is not higher than that for the deletions that result in a change of plaque phenotype.

Error rates (ER) are based on the reversion frequencies in Table I and the data from the sequence analysis. Error rates were calculated by dividing the number of revertants recovered in a given class by the total number of plaques sequenced. The analysis involved 36 phage DNAs from each reaction; no changes at or surrounding the TAA codon were detected. To test this possibility, phage DNA from white plaques generated upon transfection of reaction products with both adducted templates, was sequenced. The analysis involved 36 phage DNAs from each reaction; no changes at or surrounding the TAA codon were detected. We conclude that if “white” deletion mutants were in fact produced, the error rate for this class of mutation is not higher than that for the deletions that result in a change of plaque phenotype.

**DISCUSSION**

The data presented here demonstrate that transcriptional bypass of a BPDE-adducted template is highly mutagenic. Misincorporations occurred at a rate of 1 in 3 errors per nucleotide incorporated for the (-)-anti-trans-isomer and 1 in 6 for the (+)-anti-trans-isomer. This study is the first quantitative analysis of the fidelity of site-specific damage bypass synthesis during transcription.

Although transcription by T7 RNAP was stalled by the (-)-anti-trans- and (+)-anti-trans-BPDE-adducted adenine, generation of full-length transcripts in reactions with the adducted templates indicated that the polymerase was capable of synthesizing past both adducts (Fig. 2A). However, the amount of...
bypass synthesis appears to depend on the stereochemistry of the adduct. The observation that on average 40% less full-length transcript is produced from (+)-anti-trans-BPDE adenine templates, relative to (-)-anti-trans-BPDE adenine templates suggests that the (+)-BPDE adenine adduct is more inhibitory to transcription than the (-)-BPDE adduct. This result is consistent with the report that the stereochemistry of BPDE-guanine adds affects the degree of inhibition of T7 RNAP-dependent transcription (26).

According to NMR studies of duplex oligodeoxyribonucleotides containing site-specific BPDE-adenine adduct, the pyrenyl moiety of the (-)-anti-trans-isomer is directed toward the 5'-end of the modified strand (40–42). The (+)-anti-trans-adduct is oriented mainly toward the 3'-end of the adducted strand, although it may exist in more than one conformation. A 3'-oriented adduct may sterically hinder an RNA polymerase moving in a 5' to 3' direction and thus would be more difficult to bypass. Our observation that the (+)-anti-trans-BPDE adenine adduct was a stronger block to T7 RNAP than the (-)-anti-trans-BPDE adduct is therefore consistent with this hypothesis. However, the actual conformation of these adducts at single-strand template-duplex junctions may be different from that in duplex oligodeoxyribonucleotides.

Full-length transcripts generated with the adducted templates contained a high level of misincorporation events. The reversion frequency of the TAA codon was elevated 11,000- and 6000-fold due to transcription errors on the (+)-anti-trans- and (+)-anti-trans-BPDE-adducted templates, respectively, relative to reversion frequency in the reaction with the unadducted template. Thus, it appears that T7 RNAP synthesis past the (+)-anti-trans-BPDE-adducted adenine was almost twice as mutagenic as synthesis past the (+)-anti-trans-adduct. How the efficiency of BPDE bypass and the miscoding potential of the lesion's stereochemistry are related is an intriguing question.

The observed increase in reversion frequency was due mainly to base substitution errors. The most frequently recovered errors from reactions with both adducted templates were T to A transversions followed by T to G transversions. This reversion specificity is consistent with misinsertions opposite the adducted adenine and indicates that T7 RNAP most readily misincorporates A opposite either of the adducts. Interestingly, adenine was also the most commonly inserted base opposite an abasic site by T7, SP6, and E. coli RNA polymerases (8, 9). The second most frequently inserted nucleotide was G. Guanine appeared to be incorporated 1.5- and 9-fold less frequently, relative to adenine, opposite (-)-anti-trans-BPDE and (+)-anti-trans-BPDE-adducted adenine, respectively. The fact that we see different proportions of adenine and guanine opposite the two adducts would argue that the stereochemistry of the adduct plays an important role in determining the nature of the misinserted nucleotide.

It has been shown that the stability of a (+)-anti-trans-BPDE-adducted adenine mismatched with dG is significantly higher than the stability of the adducted adenine paired with the correct dT (41, 43). This suggests that the differences in stability between the incorrect and correct base pairs containing the different diastereomers may play a role in BPDE-induced mutagenesis. The difference in the proportion of errors resulting from G misincorporation relative to A misincorporation opposite the two adducts may also reflect inequality in the efficiency of extension of mispairs containing the (+)-anti-trans- or the (-)-anti-trans-adducted adenine.

In contrast to the results presented here, bypass of BPDE-adducted guanine by T7 RNAP was reported to be accurate (26). Misinsertions opposite the adducted guanine, mainly of A and to a lesser extent of G, resulted in termination and a truncated transcript. The difference in mutagenicity of bypass between adducted adenine and adducted guanine may be due to the difference in orientation of the adduct within the DNA.

When adducted to guanine, the BPDE moiety lies in the minor groove, either in a 3' or 5' orientation, depending on the diastereomer (41). When adducted to adenine, BPDE is partially intercalated in the major groove, 5' for (-)-isomers and 3' for (+)-isomers (40–42). The steric interaction between the hydroxyls of the pyrene moiety and DNA nucleotides is reduced when the adduct is intercalated in the major groove, as it is for adenine adducts. In contrast, guanine adducts lie in the minor groove (41). The adduct-directed DNA distortion may stabilize an A:A or A:G mispair, thereby increasing significantly the probability of a misinsertion event. In addition, normal base pairing between the adducted adenine and the correct nucleotide may be destabilized. As mentioned above, the thermostability of a (+)-anti-trans-BPDE-adenine:guanine mismatch is higher than that of a correct base pair (41), indicating that the adduct alters even normal base pair stability. In the case of an adducted guanine template, insertion of the correct nucleotide may be the only means by which T7 RNAP can accommodate the lesion and synthesize past the adduct. On the other hand, it is possible that some mutagenic bypass of the BPDE-adducted guanine takes place. However, this bypass may not have been detected due to the relatively low sensitivity of the method used for detection of misincorporations (26).

We observed a significant increase in three- and six-base deletions spanning the adducted site, relative to reactions with the nonadducted template. These results suggest that adenine-adducted BPDE may induce not only base misincorporations but also small deletion mutations during transcription by T7 RNAP. However, in order to evaluate the level and specificity of these mutations, a substrate designed to detect deletion mutations should be used.

In summary, we have shown that BPDE-adenine adducts in the template strand can be bypassed by T7 RNAP and that the bypass is highly mutagenic. Misinsertion of adenine and to a lesser extent of guanine opposite the adduct occurred at a very high frequency. These results suggest that if bypass transcrip-
tion of unrepaired BPDE-adducted adenine does occur in vivo, it may result in transcripts that contain missense mutations and, hence, code for mutant proteins. Such proteins may not only have cytotoxic effects; by interfering with DNA metabolism, they could alter the stability of genetic information. The potential biological consequences of transcriptional mutagenesis in higher organisms merits further investigation.

Acknowledgments—We thank Miriam Sander and Roel M. Schaaper for helpful discussions and for critical evaluation of the manuscript. We gratefully acknowledge the help of Pamela Horton (Vanderbilt University) in the synthesis and purification of the BP-adducted oligonucleotides.

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